The functional significance of Talin1 in dendritic cells

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**NANYANG
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UNIVERSITY**

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**The Functional Significance of Talin1 in
Dendritic Cells**

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SCHOOL OF BIOLOGICAL SCIENCES

2018

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雨に打たれ 風に吹かれ でも諦めないから
(Beaten by the rain, blown by the wind, nonetheless we will not give up)

みんな、ありがとう そして頑張ってください!
(Everyone. Thank you and do your very best!)

II. Abbreviations

2- or 3-D	2- or 3-Dimension
ABS	Actin Binding Site
ACD	Allergic Contact Dermatitis
AP1	Activator Protein-1
APC	Antigen-Presenting Cell
ARP2/3	Actin Related Protein 2/3
AU	Arbitrary Units
BM	Bone Marrow
BMDc	Bone Marrow-derived Dendritic Cell
BMDM	Bone Marrow-derived Macrophage
BMMC	Bone Marrow-derived Mast Cell
BrdU	Bromodeoxyuridine
cDNA	Complementary Deoxyribonucleic Acid (DNA)
Cbl	Casitas B-Lineage Lymphoma
CCL	Chemokine C-C Motif Ligand
CCR	C-C Motif Chemokine Receptor
CDK5	Cyclin-dependent Kinase 5
CHS	Contact Hypersensitivity
CR	Complement Receptor
CREB	Cyclic AMP-Responsive Element-Binding Protein
CXCL	Chemokine C-X-C Motif Ligand
CXCR	C-X-C Motif Chemokine Receptor
DAPI	4,6-diamidino-2-phenylindole
DC	Dendritic Cell
DD	Death Domain
dDC	Dermal Dendritic Cell
DH	Dimerization Domain
DMEM	Dulbecco Modified Eagle Medium
DNTB	2,4-dinitrothiocyanobenzene
DNFB	2,4-dinitrofluorobenzene
DNP	Dinitrophenyl
DUSP1	Dual Specificity Phosphatase 1
ECM	Extracellular Matrix
EpCAM	Epithelial Cell Adhesion Molecule
ERK1/2	Extracellular Signal-Regulated Kinase 1/2
EZH2	Enhancer of Zeste Homolog-2
FACS	Fluorescence-Activated Cell Sorting
FCS	Fetal Calf Serum
FERM	Four-point-one, Ezrin, Radixin, Moesin
Foxo1	Forkhead Box protein O1
FL	Full-Length
Flt3L	FMS-like tyrosine kinase 3 ligand
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GEF	Guanine Nucleotide Exchange Factor
GFP	Green Fluorescent Protein
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
GTPase	Guanosine Triphosphate (GTP) Hydrolase
IBS	Integrin Binding Site
ICAM	Intercellular Adhesion Molecule
ICOS	Inducible T Cell Costimulator
IFN	Interferon
I κ B	Inhibitor of κ B
IKK	Inhibitor of κ B Kinase
IL	Interleukin
IL-1R	Interleukin-1 Receptor
IL-1RA	Interleukin-1 Receptor Antagonist
IL-18R	Interleukin-18 Receptor

IMDM	Iscove's Modified Dulbecco's Medium
IRAK	Interleukin-1 Receptor-Associated Kinase
IRF	Interferon Regulatory Factor
ITAM	Immunoreceptor Tyrosine-based Activation
IVT	<i>In Vitro</i> Transcription/Translation
JNK	c-Jun N-Terminal Kinase
K2454me3	Tri-methylation of Lys2454
LBP	LPS-Binding Protein
LC	Langerhans Cell
LFA-1	Leukocyte Functional Antigen-1
LN	Lymph Node
LPS	Lipopolysaccharide
Ly96	Lymphocyte Antigen 96
MAL	MyD88 Adaptor Like
MAP	Mitogen Activated Protein
MAPK(KK)	MAP Kinase (Kinase Kinase)
MD	Membrane Distal
mDC	Migratory DC
mdDC	Migratory Dermal DC
MFI	Mean Fluorescence Intensity
MHC	Major Histocompatibility Complex
mLC	Migratory LC
MMPs	Matrix Metalloproteinases
MP	Membrane Proximal
MPR	Membrane Proximal Region
mRNA	Messenger Ribonucleic Acid
mTORC2	Mechanistic Target of Rapamycin Complex 2
MyD88	Myeloid Differentiation Primary Response 88
MZ	Marginal Zone
NF κ B	Nuclear Factor κ B
PBS	Phosphate Buffered Saline
PDLIM2	PDZ and LIM Domain Protein 2
PDK1	Phosphoinositide-Dependent Kinase 1
PFA	Paraformaldehyde
PH	Pleckstrin Homology
PIC	Protease Inhibitor Cocktail
PKC	Protein Kinase C
Poly(I:C)	Polyinosinic:polycytidylic acid (TLR3 agonist)
Poly(I)•poly(C)	Polyinosinic acid•polycytidylic acid (to induce gene deletion in <i>Tln1^{fl/fl}Mx-Cre</i> mice)
PIP5K1C	Phosphatidylinositol-4-phosphate 5-kinase type-1 gamma
PIPK γ	Phosphatidylinositol Phosphate Kinase γ
PIP5K	Phosphatidylinositol-4-phosphate 5-kinases
PI(3)K	Phosphoinositide 3-Kinase
PI(4)P	Phosphatidylinositol-4-Phosphate
PI(4,5)P2	Phosphatidylinositol (4,5)-Bisphosphates
PI(3,4,5)P3	Phosphatidylinositol (3,4,5)-Trisphosphates
PRR	Pattern Recognition Receptor
PTB	Phosphotyrosine-Binding
PTEN	Phosphatase and Tensin Homolog
PTM	Post-Translational Modification
PVDF	Polyvinylidene Difluoride
Rap1A	Ras Related Protein-1A
RIAM	Rap1-GTP-Interacting Adaptor Molecule
RING	Really Interesting New Gene
RIPK1	Receptor-Interacting Serine/Threonine Protein-1 Kinase
RPMI	Roswell Park Memorial Institute Medium
RTq-PCR	Real Time quantitative-Polymerase Chain Reaction
RT	Room Temperature
SARM	Sterile Alpha- and Armadillo-Motif-Containing Protein

SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
SD	Standard Deviation
sdLN	Skin-draining LN
SEM	Standard Error of the Mean
SMOC	Supramolecular Organizing Centres
SOCS	Suppressor of Cytokine Signaling
Syk	Spleen-Associated Tyrosine Kinase
TAB	TAK1-Associated Binding Protein
TAK1	TGF β Activated Kinase 1
TANK	TRAF Family Member-Associated NF-kappa-B Activator
TBK1	TANK-binding Kinase 1
TCR	T Cell Receptor
TF	Transcription Factor
TGF β	Transforming Growth Factor- β
TIAM1	T Lymphoma Invasion and Metastasis-inducing 1
TICAM	TIR Domain-Containing Adaptor Molecule
TIMP	Tissue Inhibitor of Metalloproteinase
TIR	Toll/Interleukin-1 Receptor
TIRAP	TIR Domain-Containing Adaptor Protein
TLR	Toll-Like Receptor
TNF α	Tumor Necrosis Factor α
TRAF	Tumor Necrosis Factor Receptor-Associated Factor
TRAM	TRIF-Related Adaptor Molecule
TRIF	TIR domain-containing adaptor-Inducing Interferon- β
UBC13	Ubiquitin-Conjugating Enzyme E2 13
UEV1A	Ubiquitin-Conjugating Enzyme E2 Variant 1A
UV	Ultraviolet
VBS	Vinculin Binding Site
VCAM	Vascular Cell Adhesion Molecule
VLA	Very Late Antigen

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VI. Abstract

Skin inflammation caused by foreign pathogens or allergens promotes the activation of skin dendritic cells (DCs) and induces their chemokine-guided migration to the draining lymph nodes. We discovered that talin1, a well-established integrin co-activator, not only regulates the integrin-dependent migration of Langerhans Cells across the epidermal basement membrane, it also regulates the activation of DCs. DC activation was compromised in cells depleted of talin1, resulting in an impeded NF κ B activation and a reduction in the expression levels of various pro-inflammatory cytokines. Biochemical analyses reveal that the integrin-binding and activating properties of talin1 are essential for the assembly of TLR4 signalosome, where the interaction between talin1 and MyD88 bridges integrin heterodimers to TLR4. Conversely, TRIF-dependent TLR3 signaling cascade is not affected by the lack of talin1, suggesting that talin1 regulates the MyD88-dependent pathway exclusively. Taken together, our data highlights a novel role of talin1 in TLR4 signaling pathways.

1. Introduction

1.1 The Toll-Like Receptor (TLR) superfamily

Toll-Like Receptors (TLRs) are a subset of Pattern Recognition Receptors (PRRs) conserved in vertebrates¹. As biological sensors of the cell, TLRs are critical for the detection of tissue damages or invading microorganisms^{1, 2, 3, 4}. They are classified as type I transmembrane receptors with a carboxyl (C)-terminal cytosolic Toll/Interleukin-1 Receptor (TIR) domain, a single transmembrane spanning segment and an amino (N)-terminal extracellular horseshoe-like ligand-binding domain^{2, 3, 4}. Current research has identified 10 human and 12 mouse TLRs⁴. TLRs are further categorized according to their spatial localization: the plasma membrane (extracellular) or the endo-lysosomal (intracellular) compartment of the cell. Extracellular TLRs such as TLR1, 2, 4, 5 and 6 are activated by microbial-derived membrane lipids and proteins while intracellular TLRs including TLR3, 7, 8, 9 and 13 are predominantly activated by non-self, microbial-derived nucleic acids^{2, 3, 4}.

1.2 Ligand-induced receptor dimerization

Receptor dimerization of extracellular TLRs takes place upon ligand engagement to the ectodomain². Intracellular TLRs on the other hand, exist as preformed dimers^{2, 3, 5, 6}. TLR dimerization induces conformational changes within the receptor TIR domains, stabilizing the complex and promoting the recruitment of cytosolic TIR domain-containing adaptors: Myeloid Differentiation Primary Response Protein 88 (MyD88), TIR Domain-Containing Adaptor Protein (TIRAP) (also known as MyD88 Adaptor Like (MAL)), TIR Domain-Containing Adaptor-Inducing Interferon- β (TRIF), TRIF-Related Adaptor Molecule (TRAM) and Sterile Alpha- and Armadillo-Motif-Containing Protein (SARM)^{2, 3, 4}. The recruitment of adaptor proteins promotes the assembly of high-ordered complexes or Supramolecular Organizing Centres (SMOCs) known as the MyD88-associated 'Myddosome' and TRIF-associated 'Trifosome'^{2, 3, 7, 8, 9, 10}. Typically, the assembly of the Myddosome complex drives the MyD88-dependent activation of Nuclear Factor- κ B (NF κ B) and the induction of pro-inflammatory cytokines such as Interleukin-6 (IL-6), IL-23 and Tumor Necrosis Factor α (TNF α)^{2, 3, 4}. The Trifosome complex on the other hand, induces the production of type I Interferons (IFNs) via the activation of IFN-Regulatory Factors (IRFs)^{2, 3, 4}. Interleukin-1 (IL-1) family of receptors including IL-1R and IL-18R also initiate similar signal transduction cascades upon activation via their respective TIR domains¹¹.

1.3 TLR signaling cascades

1.3.1 MyD88-dependent pathway

Upon ligand-receptor engagement, MyD88 associates with Interleukin-1 Receptor-Associated Kinase (IRAK) family members at their respective Death Domains (DDs) to form ordered complexes referred to as the Myddosome^{2, 3, 7, 9, 10}. Intrinsic auto-

phosphorylations of IRAKs (IRAK1, IRAK2 and IRAK4) promote the recruitment of TNF Receptor-Associated Factor 6 (TRAF6)⁹. TRAF6, together with Ubiquitin-Conjugating Enzyme E2 Variant 1A (UEV1A) and Ubiquitin-Conjugating Enzyme E2 13 (UBC13), mediate K63-linked polyubiquitination of Transforming Growth Factor- β (TGF β) Activated Kinase 1 (TAK1) protein kinase complex^{12, 13, 14, 15, 16}.

The TAK1 complex consists of TAK1 and regulatory subunits TAK1-Associated Binding Protein 1 (TAB1), TAB2 and TAB3, held together by polyubiquitin chains^{12, 17}. K63-linked polyubiquitination promotes the activation of TAK1 complex, triggering the concomitant activation of Mitogen Activated Protein Kinase (MAPK) pathway and the Inhibitor of κ B Kinase (IKK) complex-NF κ B pathway^{4, 12}. TAK1-mediated MAPK pathway activates downstream MAPK family members such as Extracellular Signal-Regulated Kinase 1/2 (ERK1/2), p38 and c-Jun N-Terminal Kinase (JNK), leading to the eventual activation of Activator Protein-1 (AP-1) family of transcription factors (TFs) to up-regulate gene transcription and the stabilization of mRNA products^{4, 18, 19, 20}. Concurrently, TAK1 complex also phosphorylates and activates IKK β , the catalytic subunit of the IKK complex. Activated IKK complex subsequently phosphorylates Inhibitor of κ B α (I κ B α), which undergoes proteasome degradation²¹. Nuclear translocation of NF κ B takes place upon degradation of I κ B α to induce the expression of pro-inflammatory genes²². Notably, all TLRs require engagement of MyD88 to initiate downstream signaling pathways with the exception of TLR3^{2, 3, 4, 7, 9, 18, 19, 23} (**Fig. 1**).

1.3.2 TRIF-dependent pathway (MyD88-independent pathway)

TLRs also trigger a second TRIF-dependent pathway via the adaptor, TRIF. With the exception of TLR3 which interacts directly with TRIF, all other TLRs require TRIF-Related Adaptor Molecule (TRAM) as the bridging molecule to initiate the signaling cascade^{7, 23, 24}. TRIF-dependent signaling is initiated upon the translocation of TLRs into endosomal compartments, thereby promoting the interaction between adaptors TRIF, TRAF3 and Receptor-Interacting Serine/Threonine-Protein Kinase 1 (RIPK1)^{25, 26}. Assembly of the TRIF, TRAF3 and RIPK1 complex facilitates the activation of the TAK1 complex and the downstream NF κ B and MAPK signaling pathways^{4, 7, 12, 18, 19}. The interaction between TRIF and TRAF3 also recruits TRAF Family Member-Associated NF κ B Activator (TANK)-Binding Kinase 1 (TBK1) and the phosphorylation of IRF3^{27, 28}. Phosphorylation promotes the dimerization and nuclear translocation of IRF3, which induces the expression of type I IFNs^{18, 19, 20} (**Fig. 1**).

Figure 1. TLR signaling pathways

Schematic of TLR signaling pathways. Extracellular TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11 localizes to the plasma membrane, whereas intracellular TLR3, TLR7, TLR8, TLR9 and TLR13 are found in endosomes. TLR4 localizes to the plasma membrane and the endosomes. Initiation of TLR signaling occurs upon ligand binding, leading to the dimerization of receptors. Next, TIR domain-containing adaptor proteins MyD88 and TIRAP (or MAL), followed by TRIF and TRAM will be recruited to the TIR domain of TLRs, with the exception of TLR3 which signals through TRIF exclusively. Endocytosis of TLR4 promotes the transition from MyD88- to the TRIF-dependent pathway. The assembly of IRAKs and TRAFs promotes the activation of MAP kinases ERK1/2 (not depicted), p38 and JNK, the activation of transcription factors (TFs) such as NFκB, IRFs, CREB and AP1, eventually inducing the gene expression of pro-inflammatory cytokines and type I IFNs. Toll-Like Receptor (TLR); MyD88 Adaptor Like (MAL); Toll/Interleukin-1 Receptor Domain-Containing Adapter Protein (TIRAP); TIR-Domain-Containing Adapter-Inducing Interferon-β (TRIF); TRIF-Related Adapter Molecule (TRAM); Toll/Interleukin-1 Receptor (TIR); Interleukin-1 Receptor-Associated Kinase (IRAK); TNF Receptor-Associated Factor (TRAF); Mitogen-Activated Protein kinase (MAP kinase); Extracellular Signal-Regulated Kinase 1/2 (ERK1/2); c-Jun N-Terminal Kinase (JNK); Nuclear Factor κB (NFκB); Interferon Regulatory Factor (IRF); Cyclic AMP-Response Element-Binding Protein (CREB); Activator Protein-1 (AP1); Interferon (IFN); TGFβ-Activated Kinase 1 (TAK1); TAK1-Binding Protein (TAB); MAP Kinase Kinase (MKK); Inhibitor of NFκB Kinase (IKK); Receptor-Interacting Protein 1 (RIP1); TRAF Family Member-Associated NFκB Activator (TANK)-Binding Protein 1 (TBK1); double-stranded RNA (dsRNA); Lipopolysaccharide (LPS); ribosomal RNA (rRNA); single-stranded RNA (ssRNA). Taken from O'Neill, L.A. *et al.* The history of Toll-like receptors - redefining innate immunity. *Nat. Rev. Immunol.* **13**, 453–460 (2013).

1.4 Regulation of TLR signaling pathways

Spatial and temporal regulation of TLR signaling pathways are essential to limit the inflammatory response. Negative regulators target key signal transducers of the signaling pathways through various mechanisms: degradation of signal transducers, inhibition of complex assembly and the suppression of NFκB activation.

Protein degradation through proteasome-dependent pathways plays an essential role in the regulation of TLR signaling. Suppressor of Cytokine Signaling (SOCS) proteins such as SOCS1^{29, 30, 31} and SOCS3³² negatively regulate TLR signaling pathways by promoting the degradation of TIRAP^{29, 30, 31} and TRAF6³² through the process of ubiquitination via their intrinsic E3 ligase activity. SOCS1 knockout mice, much like SOCS-1-deficient macrophages are highly sensitive to the endotoxin LPS^{29, 30, 31}. Deubiquitinating Enzyme A (DUBA) also suppresses TLR-induced type I IFN production by mediating the proteolytic degradation of K63-polyubiquitinated TRAF3³³. In addition, phosphorylation of TRIF and MyD88 by activated Syk kinase promotes adaptor degradation in a Casitas B-Lineage Lymphoma (Cbl)-b-dependent manner in macrophages³⁴.

Assembly of the TLR signalosome can be inhibited by negative regulators. SARM suppresses TLR signaling by binding to MyD88 and TRIF at their respective TIR domain, preventing the assembly of the Myddosome and Trifosome^{35, 36}. Other examples of inhibitory proteins include the kinase-deficient IRAK-M (or IRAK3) and protein phosphatase Src Homology 2 Domain-Containing Protein Tyrosine Phosphatase-2 (SHP-2)^{37, 38}. IRAK-M antagonizes TLR signaling by inhibiting the interaction between TRAF6 and IRAKs³⁸ while SHP-2 negatively regulates TRIF-dependent type I IFN production by binding to the kinase domain of TBK1³⁷, thereby inhibiting TBK1-dependent NFκB activation and cytokine production³⁷. TRAF6, a molecule intrinsic to both MyD88- and TRIF-dependent pathways is also heavily regulated by deubiquitinating enzymes (DUBs) and protein phosphatases^{36, 39, 40}. DUBs enzymatically cleave polyubiquitin chains from TRAF6, thus inhibiting the signal transduction cascade downstream of TRAF6 and blocking the activation of NFκB and MAPK³⁶.

Inhibitors can also suppress TLR signaling pathways directly by targeting NFκB family members. For example, the E3 ubiquitin ligase PDZ and LIM Domain Protein 2 (PDLIM2) negatively regulates TLR-induced NFκB activation in DCs by promoting the degradation of p65, a member of the NFκB family of proteins^{36, 41}. In addition, Dual Specificity Phosphatase 1 (DUSP1) limits the inflammatory response by inhibiting p38 and JNK in a mechanism not well understood^{36, 42}.

1.5 The TLR4 signal transduction pathway

TLR4 is without a doubt, the best-studied member of the TLR family. Two sequential signaling events – MyD88-dependent and TRIF-dependent pathways – take place upon TLR4-Lipopolysaccharide (LPS) engagement. Working cooperatively with TLR4, MD2 (Lymphocyte Antigen 96 (Ly96)), CD14 and LPS-Binding Protein (LBP) induce receptor dimerization (**Fig. 2**) and the aggregation of adaptor molecules^{2, 3, 9} (**Fig. 1 and 2**). Initiation of the MyD88-dependent pathway at the membrane triggers a series of signaling events to promote the activation of MAPKs, the NFκB and AP-1 signaling pathways^{2, 3, 9} (**Fig. 2**). Meanwhile, the transition from MyD88- to the TRIF-dependent pathway is triggered upon the internalization of TLR4 (**Fig. 2**), which promotes the activation of IRF3 and the production of type I IFNs^{25, 43}. The degradation of TLR4 within endo-lysosomal compartments terminates signaling events⁴⁴.

Figure 2. The canonical TLR4 signaling pathway

Soluble LBP binds to the endotoxin LPS and transfers it to CD14 on the membrane (depicted) or soluble form (not depicted). The LPS-LBP-CD14 complex is subsequently recruited to TLR4-MD2 complex, where MD2 (Ly96) is required for the dimerization of TLR4. Receptor dimerization is a pre-requisite for the activation of the MyD88-dependent pathway (represented by the Myddosome). Internalization of TLR4 dimers into endosomes takes place upon Myddosome assembly. TLR4 endocytosis is a key transitional event preceding the assembly of the Trifosome. Both Myddosome and Trifosome activates NFκB and AP-1 signaling pathways. The TRIF-dependent pathway also activates IRF3. Activated Protein 1 (AP-1); Interferon Regulatory Factor 3 (IRF3); LPS-Binding Protein (LBP); Lipopolysaccharide (LPS); Lymphocyte Antigen 96 (Ly96); Nuclear Factor κB (NFκB); Toll-Like Receptor 4 (TLR4). Taken from Kieser, K.J. and Kagan, J.C. Multi-receptor detection of individual bacterial products by the innate immune system. *Nat. Rev. Immunol.* **17**, 376–390 (2017).

1.6 Dendritic cells (DCs) of the skin

Mouse skin consists of epidermis-restricted Langerhans cells (LCs; Langerin⁺ CD103⁻ CD11b⁺) and at least 3 subsets of dermal DCs (Langerin⁺ CD103⁺ CD11b⁻; Langerin⁻ CD103⁻ CD11b⁺ and Langerin⁻ CD103⁻ CD11b⁻) in the dermis^{45, 46}. Dermal DCs are consistently replenished by bone marrow-derived progenitor cells while epidermal LCs proliferate *in situ* without further input from the bone marrow in the steady state^{45, 47}. The localization of epidermal LCs and dermal DCs in the skin reveals unique compartmentalization of DC functions in skin immunity. They express unique surface receptors^{45, 48}, thereby select DC subsets can be activated by various antigens or Pathogen Associated Molecular Products (PAMPs) either in the epidermis or dermis depending on the abundance and penetrance of antigens.

1.6.1 Dendritic cell-mediated immune response in the skin

Skin DCs are crucial players in the immune response against foreign pathogens^{45, 48, 49, 50, 51}. They express a variety of PRRs such as TLR1, TLR2, TLR3, TLR4 and TLR6 essential for the detection of PAMPs^{52, 53} derived from skin commensals and opportunistic pathogens such as *Candida Albicans* and *Staphylococcus Aureus*. Skin infections caused by *C. Albicans* and *S. Aureus* are common, but when left unchecked, these pathogens can penetrate deep into the subcutaneous layers of the skin and eventually enter the bloodstream, causing widespread systemic infections, sepsis and death.

Epicutaneous *C. Albicans* infection are detected by receptors such as dectin-1, dectin-2, macrophage mannose receptor (MMR), TLR2 and TLR4 expressed on the surface of skin DCs against fungal PAMPs such as glucans, mannans, chitin and curdlan^{45, 48, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67}. Signals transduced from ligand-engaged receptors induce the activation and migration of skin DCs to the skin-draining lymph nodes (sdLNs) to drive the Th17, Th1 and cytotoxic T cell responses^{48, 54, 67}. Interleukin-6-derived from epidermal LCs drives the differentiation of Th17 cells while dermal DCs promote the Th1 and cytotoxic T cell response^{48, 67}. However, CD103⁻ CD11b⁺ dermal DCs can also induce the differentiation of Th17 cells in the absence of LCs and CD103⁺ CD11b⁻ dermal DCs^{48, 54}. Compartmentalized DC functions can also be observed in the mouse model of epicutaneous *S. Aureus* infection⁵⁴.

Besides their role in pathogen clearance, DCs are also involved in the pathologies of skin inflammatory diseases such as contact dermatitis^{67, 68}. Skin DCs are the main drivers of contact dermatitis^{69, 70, 71, 72, 73}, however, several factors such as the spatial localization of DCs (LCs reside in the epidermis while dermal DCs reside in the dermis), the availability and penetrance of antigens^{70, 71, 72, 73, 74} and the type of

antigen (hapten or protein)^{70, 71, 72} collectively influences the functional compartmentalization of skin DCs in the pathology of contact dermatitis. Besides the assigned roles of skin DCs in epicutaneous candidiasis, functional redundancy of skin DCs can also be observed in other skin conditions such as the induction of immunological tolerance against self or environmental antigens (e.g. non-allergic hapten 2,4-dinitrothiocyanobenzene (DNTB)), whereby both LCs and dermal DC subsets can promote immunosuppressive or tolerogenic responses via the activation of Foxp3⁺ regulatory T cells^{73, 75, 76}. The functional role of unique skin DC subsets becomes critical in situations of low or limited antigen exposure.

1.7 TLR signaling pathways in dendritic cells (DCs)

DCs are professional Antigen-Presenting Cells (APCs)^{77, 78}. They bridge the gap between innate and adaptive immunity by presenting foreign antigens to T and B lymphocytes so as to generate an adaptive immune response^{77, 78}. DCs can be found in various primary and secondary lymphoid organs, and peripheral organs such as the skin, lungs, liver, kidneys and the gastrointestinal tract^{77, 78, 79, 80, 81, 82, 83}. As a result of their strategic localization, tissue-resident DCs have the extraordinary ability to respond dynamically to environmental cues^{77, 78, 79, 80, 81, 82, 83}. The ligation of PAMPs to TLRs promote DC activation, which is a highly specialized process characterized by complex changes in effector functions and cellular morphology^{77, 78}. These changes include the up-regulation of costimulatory molecules and antigen processing machinery, enhanced production of pro-inflammatory cytokines, altered cell motility and the suppression of phagocytosis^{84, 85}.

1.7.1 TLR4 signaling pathway induces DC maturation and enhances DC motility

Optimal DC activation is dependent on dual signaling events mediated by MyD88 and TRIF, two core adaptor molecules downstream of TLR4⁸⁶. DC migration is transiently arrested upon activation as a result of modifications to the actin cytoskeleton^{87, 88, 89}, a phenomenon linked to the sequestration of the motor protein myosin II by Major Histocompatibility Complex Class II (MHCII)-associated Invariant Chain (CD74 or Ii) in the endosomes⁸⁸. The entrapment of myosin II by MHCII-Ii complex affects the localization and activation (phosphorylation) of myosin II, two critical parameters governing the functional role of myosin II in actomyosin contraction and cell motility⁸⁸. The transient inhibition of DC motility will be relieved upon enzymatic cleavage of Ii and the presentation of antigen-bound MHCII complex⁸⁸. Furthermore, transient episodes of reduced motility in conjunction with enhanced phagocytosis and antigen uptake have been observed in dermal DCs upon exposure to LPS⁸⁹. Motility of activated dermal DCs resumed 6 to 8 hours post LPS exposure⁸⁹. These observations implied that antigen capture and DC migration are mutually exclusive. The distribution of F-actin in immature and mature (LPS-activated) DCs, as regulated by actin

cytoskeleton modifiers such as Actin Related Protein 2/3 (Arp2/3) and mDia-1 (a family member of Formins), are distinctly different⁸⁵. Arp2/3-dependent nucleation of F-actin at the front of immature DCs is essential for antigen uptake while mDia-1-dependent assembly of F-actin at the rear of activated DCs controls cell motility⁸⁵. Taken together, TLR4 is involved in the regulation of DC maturation and motility.

1.8 The role of talin in cell migration

Cell migration is essential for biological processes such as the immune response, wound healing and embryonic development^{90, 91}. A common feature of cell migration – integrin-dependent or integrin-independent – is the generation of traction forces to promote the displacement of cells relative to their surroundings. Talin1 is critical for integrin-dependent migration on 2-dimensional (2-D) surfaces but is dispensable for integrin-independent migration in 3-dimensional (3-D) environments⁹².

1.8.1 A brief history of Talin

Discovered in 1983, talin was found to be enriched at membrane ruffles and cell adhesion sites^{93, 94, 95, 96, 97, 98, 99, 100}. There are two talin genes – *Tln1* and *Tln2* – in vertebrates¹⁰¹. Talin1 is ubiquitously expressed and it plays a prominent role in embryogenesis^{93, 94, 95, 96, 97, 98, 99}. Talin2 is the dominant isoform expressed in muscle cells, but various organs such as the lungs, brain and kidneys also express talin2^{96, 97, 98}. Hematopoietic lineage cells only express *Tln1*¹⁰¹. Even though both talin1 and talin2 share approximate 75% identity, neither of them are able to fully compensate for each other functionally^{93, 94, 95, 96, 97, 98, 99}.

1.8.2 Structure and binding partners of Talin

Talin1 is a large 270kDa cytosolic scaffold protein that comprises of 2541 amino acids^{93, 94, 96, 97, 102, 103}. The N-terminal globular head domain consists of an atypical Four-point-one, Ezrin, Radixin, Moesin (FERM) domain with 4 subdomains: F0, F1, F2, and F3^{93, 94, 96, 97, 103}. Ubiquitin-like folds between F0 and F1 subdomains have been documented¹⁰⁴. The interaction of β integrin tails to the Phosphotyrosine-Binding (PTB)-like motif within the F3 subdomain (where integrin Binding Site-1 (IBS-1) is located) is essential for integrin activation^{93, 94, 96}. Besides integrins, the head domain also interacts with phosphoinositides^{93, 94, 96, 105, 106, 107}, Phosphatidylinositol Phosphate Kinase γ or Phosphatidylinositol-4-phosphate 5-kinase type-1 gamma (PIPK γ or PIP5K1C)^{93, 96, 105, 106, 107}, F-actin^{93, 96, 108, 109, 110}, Layilin^{93, 96, 111}, Focal Adhesion Kinase (FAK)^{93, 96, 112}, Ras Related Protein-1A (Rap1A)^{113, 114, 115} and Smurf1, an E3 ubiquitin ligase¹¹⁶ (**Fig. 3**).

An unstructured linker sequence of approximately 80 amino acid residues links the head domain to the much larger C-terminal helical rod domain^{93, 96}. The rod domain

consists of a second IBS^{117, 118, 119, 120}, two F-actin binding sites^{108, 110}, four Rap1-GTP-Interacting Adaptor Molecule (RIAM) binding sites¹²¹ and up to eleven Vinculin binding sites^{110, 121, 122, 123}. The rod domain also contains a single helical Dimerization Domain (DH) at the C-terminus^{93, 96} (**Fig. 3**).

Figure 3. Structure of talin and its defined domains

Interaction sites of talin1-binding proteins and domain boundaries of talin1 are as indicated. Talin1 head domain comprises of an atypical Four-point-one, Ezrin, Radixin, Moesin (FERM) domain containing four subdomains F0, F1, F2 and F3. The head domain is joined by an unstructured linker sequence (~80 amino acids) to the rod domain. The rod domain is made up of α-helices that are organized into thirteen 4- or 5-helix bundles (R1–R13) and a single helical Dimerization Domain (DH) (α-helix 62) at the C-terminus. Binding sites are colour coded: Integrin Binding Site 1 (IBS-1) in orange, Actin Binding Sites (ABS) in red and Vinculin Binding Sites (VBS) in purple. α-helix 50 depicted in blue with purple stripes consists of IBS-2 and a VBS. Deleted in Liver Cancer (DLC); Focal Adhesion Kinase (FAK); Ras-Related Protein 1 (Rap1); Rap1-GTP-Interacting Adaptor Molecule (RIAM); Phosphatidylinositol-4,5-Bisphosphate (PI(4,5)P2); Phosphatidylinositol 4-Phosphate 5-Kinase Type Iγ (PIPKIγ); T Lymphoma Invasion and Metastasis-Inducing 1 (TIAM). Taken from Klapholz, B. and Brown, N.H. Talin – the master of integrin adhesions. *J. Cell Sci.* **130**, 2435–2446 (2017).

1.8.3 Post-translational modifications (PTMs) of talin1

PTMs of talin1 such as methylation¹²⁴, phosphorylation^{116, 125, 126} and arginylation¹²⁷ further refine talin1-dependent effector functions. For instance, tri-methylation of talin1 at Lys2454 (K2454me3) by cytosolic Ezh2 promotes the dissociation of F-actin from talin1 while enhancing calpain II-dependent talin1 cleavage, thereby facilitating the turnover of adhesion structures (protein complexes that connect the actin cytoskeleton of a cell to the Extracellular Matrix (ECM)) in various cell types¹²⁴. Mass spectroscopic analysis also reveal multiple phosphorylation sites on talin1¹²⁵. For example, the phosphorylation of Ser425 (S425) mediated by Cyclin-dependent Kinase 5 (CDK5) prevents the ubiquitination and degradation of the head domain by inhibiting the binding of the E3 ubiquitin ligase Smurf1¹¹⁶. Phosphorylations at T144, T150 and S446 also regulate calpain II-dependent cleavage of talin1¹²⁶.

1.8.4 Functional regulation of talin1

The distribution of talin1 in the cytosol of resting cells is stochastic and diffused^{93, 96}. Electron microscopic and biochemical data reveal that talin1 undergoes auto-inhibition, whereby the rod domain structurally restrains the head domain and prevents it from associating with integrins and the plasma membrane^{93, 96, 128, 129}. Various proteins and molecules such as Rap1A^{113, 114, 115}, RIAM^{113, 114, 115, 121}, PIPKly or PIP5K1C^{106, 130} and Phosphatidylinositol-4,5-Bisphosphate (PI(4,5)P2)^{128, 131, 132} are involved in the activation of talin1. The recruitment of RIAM/Rap1A complex to the talin1 head domain promotes the activation of talin1 by disengaging the rod domain-mediated auto-inhibition of talin1^{113, 114, 115, 121} while recruiting talin1 to PI(4,5)P2-enriched region of the cell membrane, whereby RIAM and talin1 via their respective Pleckstrin Homology (PH) domain will dock^{115, 132, 133}. Talin1 also interacts with PIPKly (or PIP5K1C), an enzyme that mediates the synthesis of PI(4,5)P2, to enhance the local concentration of PI(4,5)P2 to further promote the recruitment of talin1 molecules¹³². The association of RIAM/Rap1A complex and PI(4,5)P2 with talin1 exposes integrin binding site-1 (IBS-1) of talin1^{93, 96, 133, 134, 135, 136}, promoting the interaction between talin1 and integrins. Recently, kank2¹³⁷ and Rap1B¹³⁸ (isoform of Rap1) were identified as novel regulators of talin. Integrin heterodimers upon activation by talin1 will bind to their respective ligands (e.g. constituents of the ECM) to initiate integrin outside-in signaling and assembly of focal adhesions^{93, 96, 133, 134, 135, 136}.

1.8.5 Activation of integrins by talin

Integrins are heterophilic cell adhesion molecules consisting of two non-covalently linked α and β subunits^{139, 140, 141} (**Fig. 4a**). They maintain cellular interactions with the external environment by binding to ECM components such as laminin, collagen and fibronectin^{139, 140, 141}. At least 24 distinct integrin heterodimers can be identified from unique pairings between 8 β integrin and 18 α integrin subunits^{139, 140, 141}.

One model of integrin activation proposes the initial recruitment of kindlin to the Membrane Distal (MD) NxXY/F motif of the β integrin tail^{142, 143}. The binding of kindlin via its PH domain to PI(4,5)P2-enriched membrane reinforces the kindlin-integrin interaction¹⁴⁴ (**Fig. 4b**) and promotes the subsequent recruitment of talin to the Membrane Proximal Region (MPR) and the Membrane Proximal (MP) NPXY/F motif of the same β integrin tail^{94, 142, 143, 145} (**Fig. 4c**). Leu325 (L325)¹¹⁸ and Tryp359 (W359)^{118, 146} are critical residues mediating the interaction between talin1 and the MPR and MP NPXY/F motif of the β integrin tail respectively (**Fig. 4c**). The binding of talin to the β integrin tail is further stabilized by ionic interactions between the F2 subdomain of talin and PI(4,5)P2 on the cell membrane¹³² (**Fig. 4c**). The association of talin to the MPR of β integrin tail disrupts with the ionic bonds between α and β integrin subunits, unhinges the integrin tail and facilitates the activation of the integrin

heterodimer^{147, 148, 149, 150} (**Fig. 4d**). Integrins in the extended, high-affinity conformation have greater affinity (more than 1000-fold) for their respective ligands compared to integrins in the bent, low-affinity state¹⁵¹.

Figure 4. The proposed model of integrin activation

(a) Localization of inactive integrin heterodimers on the cell membrane. (b) The recruitment of kindlin to the Membrane Distal (MD) NxxY/F motif of β integrin tail. The interaction of kindlin via its PH domain to PI(4,5)P2 (red bars) stabilizes the kindlin-integrin complex. (c) The binding of kindlin to integrins promotes the recruitment of talin to the same β integrin tail. Tryp359 (W359) and Leu325 (L325) are critical residues that bind to the Membrane Proximal (MP) NPxY/F motif and the Membrane Proximal Region (MPR) respectively. The binding of PI(4,5)P2 (red bars) to the F2 subdomain of talin stabilizes the interaction between talin and integrin. (d) The interaction of Talin1-L325 to the MPR of the β integrin tail unhinges the α and β integrin subunits, activating the integrin heterodimer. Phosphatidylinositol-4,5-Bisphosphate (PI(4,5)P2); Pleckstrin Homology (PH). Integrin: α subunit in red, β subunit in blue. Adapted from Bennet, J.S. A little bit of kindlin goes a long way. *Blood*. **126**, 2535–2536 (2015).

1.9 The role of integrins in cell migration

1.9.1 Integrin-dependent migration on 2-dimensional surfaces

Migration of leukocytes within the interstitial environment can be integrin-independent (adhesion-independent) or integrin-dependent (adhesion-dependent) depending on the density and constituents of the ECM. During integrin-dependent migration, adhesion structures will assemble and disassemble rapidly^{90, 113, 152, 153, 154, 155, 156, 157, 158}. The velocity and trajectory of migratory cells are regulated by the turnover kinetics of adhesion structures and cell polarity^{113, 152, 153, 154, 155}. In brief, cell movement is tightly controlled by both actin polymerization and actin-bound myosin II contraction^{154, 155, 156} (**Fig. 5**). To generate a net forward displacement, ECM-engaged integrins resist the retrograde flow of actin filaments, forming a molecular clutch^{90, 154}. With talin or the 'clutch' in place, actin polymerization-driven protrusions (**Fig. 5**) at the tip of the cell pushes actin filaments towards the cell body where the network of actin is continuously disassembled in a process known as treadmilling^{90, 152, 153, 154, 158}. Next, myosin II-dependent contraction pulls the actin cortex backwards to support the polymerization of actin at the leading edge of the migrating cell^{90, 154}. Collectively, both engines – actin polymerization and myosin II contraction – generate traction forces which propel the leading edge of the cell forward while integrin-anchored F-actin via talin pulls on the ECM, resulting in a forward displacement of the cell. Rho GTPases also regulate adhesion dynamics by facilitating rapid focal contact formation and the generation of traction forces^{152, 153, 154, 158}. Acting in unison, this mechanism is collectively known as the 'molecular clutch'^{90, 152, 153, 154, 158} (**Fig. 6a**).

Membrane blebbing or bleb-driven cell migration (**Fig. 6b**) can occur concurrently with myosin II-dependent actomyosin contraction as described above. Blebbing occurs when the plasma membrane disengages from the actin cortex due to a localized increase in hydrostatic pressure within the cell as generated by the contraction of the actomyosin cortex^{90, 152, 158} (**Fig. 6b**). To relieve the build-up in pressure, the plasma membrane detaches from the underlying actin cortex, permitting the influx of cytoplasmic material into the newly formed bleb⁹⁰. Newly synthesized cortical actin, together with numerous motor proteins coat the inner surface of blebs as they mature⁹⁰. As mature blebs contract, traction forces generated by integrins linked to the ECM and the actin cytoskeleton pushes the cell forward⁹⁰ (**Fig. 6b**).

The importance of integrins in 2-D cell migration was highlighted in several reports using various cell types^{92, 116, 124, 152, 157, 159, 160, 161, 162, 163, 164}. In particular, Lämmermann and colleagues showed that integrin-null DCs are not able to adhere or migrate on 2-D substrates⁹², suggesting that integrin-dependent responses play vital roles in the function of leukocytes. Two prominent examples of integrin-dependent leukocyte migration are the recruitment of neutrophils from blood to inflamed

tissues^{165, 166, 167} and the migration of epidermal LCs across the basement membrane in an $\alpha 6$ integrin-dependent manner¹⁶⁸.

Figure 5. The molecular clutch model

(a) Actin polymerization-driven protrusions at the leading edge of the cell. Incorporation of new actin molecules (light blue) on existing F-actin structures (dark blue). Binding of integrin heterodimers (green and purple) to the ECM. (b) The continuous actin polymerization enhances actin retrograde flow in the absence of clutch (yellow) engagement with the actin cytoskeleton and integrin heterodimers. Slippage occurs when the clutch is not engaged. (c) Engagement of the clutch with polymerized F-actin and integrin heterodimers resist the retrograde flow of actin while actin polymerization-driven protrusions at the leading edge pushes the cell forward by mechanical forces. Talin functions as the clutch (yellow). Taken from Case, L. B. and Waterman, C. M. Integration of actin dynamics and cell adhesion by a three-dimensional, mechanosensitive molecular clutch. *Nat. Cell Biol.* **17**, 955–963 (2015).

1.9.1 Integrin-independent migration in 3-dimensional space

Besides integrin-mediated migratory mechanisms (**Fig. 6a,b**), leukocytes also utilize integrin-independent migration to manoeuvre the interstitial environment^{90, 92, 152, 158}. DCs particularly, are able to switch between integrin-dependent and integrin-independent mechanisms depending on the nature (adhesiveness and density) of the substratum without altering the velocity or directionality of migration^{90, 152, 158}.

Enhanced actin polymerization independent of integrins and talin is sufficient to counteract actin slippage and retrograde flow (**Fig. 5b**), while generating adequate traction to displace migratory DCs (mDCs) confined in a dense 3-D environment rich in ECM constituents¹⁵² (**Fig. 6c,d**). The density of the ECM determines the need for

myosin II-mediated contraction of the region around the nucleus of the migrating cell to permit efficient cell migration^{90, 158} (**Fig. 6c**). Myosin II-dependent contraction deforms the nucleus of the migrating cell, allow it to squeeze through tight spaces^{90, 158}. Membrane blebbing, similar to the mechanism as describe earlier (1.9.1) can also be utilized by mDCs in a 3-D environment^{90, 158} (**Fig. 6e**). Deformation-based migration or ‘chimneying’ are employed by leukocytes, cancer cells and cells in the developing embryo^{90, 158, 160}.

Overall, leukocytes rapidly adapt to the ECM by altering their migratory machinery and cell shape through a process known as plasticity, enabling them to effectively transverse interstitial spaces unimpeded^{92, 152, 158, 159, 160, 169}. It is likely that leukocytes employ a combination of amoeboid movement – adhesion-dependent or deformation-based migration – interchangeably to navigate the diverse 3-D network of ECM.

Figure 6. Modes of cell migration in 2-D or 3-D environment

(a) The molecular clutch mechanism: cells migrating on 2-D surfaces utilize combined traction forces generated by both actin polymerization and myosin II-mediated contraction to push itself forward. (b) Locomotion driven by membrane blebbing on a 2-D surface. (c,d) Locomotion by actin polymerization: polymerizing actin pushes the leading edge of the confined cell against boundaries of the (c) high- or (d) low-density 3-D environment. Density of the ECM determines the need for (c) myosin II-dependent contraction of the region around the nucleus to permit efficient cell migration. (e) Locomotion driven by membrane blebbing in a 3-D environment. Cell migration in a 3-D environment can be a combination of both integrin-dependent and integrin-independent mechanism. Adapted from Lämmermann, T. and Sixt, M. Mechanical modes of ‘amoeboid’ cell migration. *Curr. Opin. Cell Biol.* **21**, 636–644 (2009).

1.10 Migration of skin DCs

1.10.1 Transmigration of LCs across the basement membrane

Migration of epidermal LCs across the basement membrane at the dermo-epidermal junction is a prerequisite for the homing of LCs to the sdLNs^{170, 171, 172, 173}. Rich in structural proteins such as type IV collagen and laminin, the basement membrane separates the epidermis from the dermis. External stimuli such as Ultraviolet (UV) radiation and inflammatory cytokines including Interleukin-1 β (IL-1 β) and TNF α induce the maturation of epidermal LCs by promoting the down-regulation of surface E-cadherin, effectively releasing LCs from the surrounding keratinocytes and permitting their descent towards the basement membrane^{170, 171, 172}. TNF α also induces the up-regulation of integrin $\alpha 6 \beta 1$ (Very Late Antigen-6 (VLA-6)) on LCs, whereby the binding of VLA-6 to laminin, a ligand abundant on the epidermal basement membrane initiates LC transmigration^{45, 168, 171, 172, 173}. In contrast, Transforming Growth Factor- β (TGF β) derived from epidermal LCs inhibits the activation and migration of LCs in an autocrine or paracrine feedback loop^{170, 171, 172, 174, 175}. Typically secreted in the inactive form, the latent TGF β complex upon activation by integrin $\alpha 8 \beta 1$ ¹⁷⁶, $\alpha \nu \beta 3$ ¹⁷⁷, $\alpha \nu \beta 5$ ^{178, 179}, $\alpha \nu \beta 6$ ¹⁸⁰ or $\alpha \nu \beta 8$ ¹⁸¹, releases active TGF β which will then bind to and initiate signaling events via TGF β RI or TGF β RII to suppress the activation and migration of epidermal LCs^{45, 170, 171, 172, 173, 174, 175}. Mechanical force is required for the integrin-dependent activation of TGF β ^{182, 183, 184}, whether talin1 plays a role in this process requires further investigation. C-X-C Motif Chemokine Receptor type 4 (CXCR4) and its respective ligand Chemokine C-X-C Motif Ligand 12 (CXCL12) also regulate the emigration of LCs from the epidermis into the dermis¹⁸⁵.

1.10.1 Homing of migratory LCs and dermal DCs towards the draining lymph nodes

Upon transversing the basement membrane, migratory LCs (mLCs) together with *in situ* dermal DCs (dDCs) navigate the dermis (3-D environment) via integrin-independent mechanisms^{45, 171, 172, 173}. Chemokine C-C Motif Ligand 19 (CCL19) and CCL21 expressed by endothelial cells lining the dermal lymphatics guide mDCs (both mLCs and dDCs) toward the sdLNs in a C-C Motif Chemokine Receptor 7 (CCR7)-dependent manner^{45, 171, 172, 173}. Intracellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1) are adhesion molecules prominently expressed on the surface of endothelial cells lining the lymphatic endothelium¹⁸⁶. ICAM-1 is a ligand of Leukocyte Functional Antigen-1 (LFA-1 or integrin $\alpha L \beta 2$) while VCAM-1 is a ligand of VLA-4 (integrin $\alpha 4 \beta 1$) and integrin $\alpha 4 \beta 7$ ¹⁸⁶. The engagement of DC-associated integrins (LFA-1 and VLA-4) with endothelial cell-bound adhesion molecules (ICAM-1 and VCAM-1), under the guidance of chemokines CCL19 and CCL21, directs the migration of skin DCs towards the sdLNs.

2. Agenda

2.1 Research gap

Collaborative signaling between integrins and PRRs^{34, 187, 188, 189} has been proposed by several groups but the mechanism of action has **yet to be resolved** in DCs. Hence, we are keen to explore the interdependent relationship between integrins and PRRs. The knowledge gained from this study will allow us to better understand receptor crosstalk between integrins and TLRs and to seek potential treatments to life-threatening diseases associated with aberrant signaling events downstream of these molecules.

2.2 Objectives

Having identified the research gap, the objectives of the study are as follows:

2.2.1 Objective 1

Since the transmigration of epidermal LCs across the basement membrane is dependent on the interaction between integrin $\alpha 6 \beta 1$ (VLA-6) and laminin, we expect the transmigration of talin1-deficient epidermal LCs across the basement membrane to be impaired. As such, the **first objective** of the project is to verify the functional significance of talin1 in the migration of skin DCs using conditional talin1 knockout mice with specific deletion of *Tln1* gene in DCs.

2.2.2 Objective 2

Besides regulating the activation of integrins and cell migration, our data strongly suggest that talin1 also plays a functional role in TLR signaling pathway. With this observation, our **second objective** is to elucidate the role of talin1 in TLR signaling cascade. A combination of gene knockout, biochemical, immunological and microscopic techniques will be employed to unravel the functional significance of talin1 in TLR signaling pathways of DCs.

3. Materials and Methods

3.1 Materials and reagents

All general reagents and chemicals were purchased from Roche (Basel, Switzerland), Sigma-aldrich (St Louis, MO, USA), Merck Millipore (Darmstadt, Germany), Bio-Rad Laboratories (Hercules, CA, USA), Gibco (Waltham, MA, USA), Thermo Fisher Scientific (Waltham, MA, USA), Invitrogen (Carlsbad, CA, USA), GE Healthcare (Piscataway, NJ, USA) or Stemcell Technologies (Vancouver, BC, Canada) unless stated otherwise. All recombinant cytokines were purchased from Peprotech (Rocky Hill, NJ, USA) and Miltenyi Biotec (Bergisch Gladbach, Germany). Low endotoxin Fetal Calf Serum (FCS) was purchased from Biowest (Riverside, MO, USA). All culture dishes were purchased from Corning (Corning, NY, USA) or Thermo Fisher Scientific. Imaging slides and chemotaxis chambers were purchased from Ibidi (Matrinsried, Germany). TLR agonists were purchased from InvivoGen (San Diego, CA, USA).

3.2 Animals

CD11c-Cre mice¹⁹⁰ were crossed with *Tln1*^{ff} mice^{99, 191, 192} to generate offsprings with talin1-deficient DC. *Mx*-Cre mice¹⁹³ were crossed with *Tln1*^{ff} mice to generate offsprings with Cre-recombinase under the control of the *Mx1* promoter. Deletion of talin1 in *Tln1*^{ff}*Mx*-Cre mice was induced by intraperitoneal (*i.p.*) injection of polyinosinic acid•polycytidylic acid (poly(I)•poly(C)) (GE Healthcare). In general, poly(I)•poly(C) was administered 3 times in 2-day intervals and animals were analysed 2 or 3 weeks (or before fatality as a result of impaired erythropoiesis) after last injection. Poly(I)•poly(C) was reconstituted in sterile PBS (Gibco) at 50°C for 3 hrs (constant shaking), to a final concentration of 1 mg/ml before allowing to cool down to RT overnight and aliquoted the next day. Stocks were kept frozen at -20°C. All mice were bred and maintained under specific pathogen-free conditions at the animal facility of the School of Biological Sciences and Lee Kong Chian School of Medicine, Nanyang Technological University. All mice were at least 8 to 10-weeks old at time of study. All animal experiments were performed in compliance with protocols approved by Nanyang Technological University - Institutional Animal Care and Use Committee (NTU-IACUC) (Nanyang Technological University, Singapore).

3.3 Antibodies

Antibodies and matching isotype controls were all purchased from BioLegend (San Diego, CA, USA), eBioscience (San Diego, CA, USA), BD Biosciences (San Jose, CA, USA), BD PharMingen (San Jose, CA, USA), Miltenyi Biotec, Cell Signaling Technology (Danvers, MA, USA), Santa Cruz Biotechnology (Santa Cruz, CA, USA), Bio-vision (Milpitas, CA, USA), R&D Biosystems (Minneapolis, MN, USA), Abcam (Cambridge, UK), Genetex (Irvine, CA, USA), Jackson ImmunoResearch (West Grove, PA, USA), Invitrogen and Sigma-aldrich.

Table 1. List of antibodies used for Fluorescence-Activated Cell Sorting (FACS) and flow cytometry analysis

Antibody	Clone	Source
anti-BrdU	-	BD PharMingen
anti-CCR7	4B12	Biolegend
anti-CD3 ϵ	145-2C11	Biolegend
anti-CD4	GK1.5, RM4-5	Biolegend
anti-CD8 α	53-6.7	Biolegend
anti-CD11b	M1/70	Biolegend, BD Bioscience
anti-CD11c	N418	Biolegend
anti-CD25	PC61	Biolegend
anti-CD29	HM β 1-1	Miltenyi biotec
anti-CD43	1B11	Biolegend
anti-CD44	IM7	Biolegend
anti-CD45	30-F11	Biolegend
anti-CD45.1	A20	Biolegend
anti-CD45.2	104	Biolegend
anti-CD86	GL-1	Biolegend
anti-CD103	2E7	Biolegend
anti-CD206 (MMR)	C068C2	Biolegend
anti-CD207 (Langerin)	eBioL31	eBioscience
anti-CD282 (TLR2)	CB225	Biolegend
anti-CD284 (TLR4)	SA15-21	Biolegend
Anti-317 (pDCA-1)	eBio927	eBioscience
anti-CD324 (E-cadherin)	36/E-cadherin	BD Bioscience
anti-CD326 (EpCAM)	G8.8	Biolegend
anti-CD369 (dectin-1)	bg1fpj	eBioscience
anti-CXCR4	L276F12	Biolegend
anti-Foxp3	FJK-16s	eBioscience
anti-F4/80	BM8, REA126	Biolegend, Miltenyi biotec
anti-Gr-1	RB6-8C5	Biolegend
anti-I-A/I-E (MHCII)	M5/114.15.2	Biolegend
anti-ICOS	E7.17G9	Biolegend
anti-IFN γ	XMG1.2	Biolegend
anti-IgG (Isotype)	RTK2071, RTK2758, R2a_21B2	Biolegend
anti-Ly6C	HK1.4	Biolegend
anti-Ly6G	1A8	Biolegend
anti-MHCII ^b	AF6_120.10	Biolegend

anti-phospho AKT S473	REA359	Miltenyi biotec
anti-phospho ERK	REA152	Miltenyi biotec
Streptavidin or anti-biotin	-	Biolegend, BD Bioscience

Table 2. List of primary antibodies

Antibody	Catalogue	Source
anti-MyD88 (D80F5)	4283	Cell Signaling Technology
anti-MyD88 (E-11)	sc-74532	Santa Cruz Biotechnology
anti-MyD88 (B-1)	sc-136970	Santa Cruz Biotechnology
anti-MyD88	3244R	Bio-vision
anti-TIRAP (D6M9Z)	13077	Cell Signaling Technology
anti-TRAM	AF4348	R&D Biosystems
anti-TRIF	4596	Cell Signaling Technology
anti-GAPDH (6C5)	AM4300	Invitrogen
anti-Vav1	2502	Cell Signaling Technology
anti-Vav1 (D45G3)	4657	Cell Signaling Technology
anti-Vav1 (C-14)	sc-132	Santa Cruz Biotechnology
anti-PI(3)K p85 (19H8)	4257	Cell Signaling Technology
anti-Talin (8D4)	T3287	Sigma-aldrich
anti-CD11b (M1/70)	ab8878	Abcam
anti-IRAK4 (12H2L6)	700026	Invitrogen
anti-IRAK4	4364	Cell Signaling Technology
anti-TRAF6 (N2C2)	GTX113029	Genetex
anti-TRAF3	4729	Cell Signaling Technology
anti-TLR4 (D8L5W)	14358	Cell Signaling Technology
anti-TLR4 (25)	sc-293072	Santa Cruz Biotechnology
anti-TLR4	48-2300	Invitrogen
anti-TLR3 (D10F10)	6961	Cell Signaling Technology
anti-TLR3 (40C1285)	sc-52961	Santa Cruz Biotechnology
anti-Na/K ATPase (C454.6)	sc-21712	Santa Cruz Biotechnology
anti-phospho ERK1/2 T202/Y204 (D13.14.4E)	4370	Cell Signaling Technology
anti-ERK1/2 (137F5)	4695	Cell Signaling Technology
anti-phospho AKT T308 (D25E6)	13038	Cell Signaling Technology
anti-phospho AKT S473 (D9E)	4060	Cell Signaling Technology
anti-AKT (C-20)	sc-1618	Santa Cruz Biotechnology
anti-phospho JNK1/2 T183/Y185 (G9)	9255	Cell Signaling Technology

anti-JNK1/2 (D-2)	sc-7345	Santa Cruz Biotechnology
anti-phospho p38 T180/Y182 (D3F9)	4511	Cell Signaling Technology
anti-p38 (A-12)	sc-7972	Santa Cruz Biotechnology
anti-phospho IRF3 S396 (4D4G)	4947	Cell Signaling Technology
anti-IRF3 (D-3)	sc-376455	Santa Cruz Biotechnology
anti-Lamin B1 (B-10)	sc-374015	Santa Cruz Biotechnology
anti-phospho p65 S536 (93H1)	3033	Cell Signaling Technology
anti-phospho p65 S276	ab106129	Abcam
anti-p65 (A)	sc-109	Santa Cruz Biotechnology
anti-GFP (B-2)	sc-9996	Santa Cruz Biotechnology
anti-GFP (FL)	sc-8334	Santa Cruz Biotechnology
anti-FLAG® M2	F3165	Sigma-aldrich
anti-FLAG® M2	F7425	Sigma-aldrich
anti-CD29 (9EG7)	550531	BD Bioscience
anti-CD29 (M-106)	sc-8978	Santa Cruz Biotechnology

Table 3. List of secondary antibodies

Antibody	Source
donkey anti-mouse HRP conjugate	Sigma-aldrich
goat anti-rabbit HRP conjugate	Sigma-aldrich
rabbit anti-goat HRP conjugate	Sigma-aldrich
protein G HRP conjugate	Invitrogen
AffiniPure F(ab') ₂ donkey anti-rabbit AF647 conjugate	Jackson ImmunoResearch
AffiniPure F(ab') ₂ donkey anti-rabbit rhodamine conjugate	Jackson ImmunoResearch
AffiniPure F(ab') ₂ goat anti-mouse FITC conjugate	Jackson ImmunoResearch
AffiniPure F(ab') ₂ goat anti-mouse rhodamine conjugate	Jackson ImmunoResearch
anti-mouse IgG (H+L), highly cross adsorbed, CF™ 350 antibody produced in donkey	Sigma-aldrich

3.4 Media, buffers and solutions

Table 4. Media, buffers and solutions

Name	Composition
Triton-X buffer	20 mM HEPES, 1% Triton X-100, 150 mM NaCl, 1.5 mM

	MgCl ₂ , 2 mM EDTA
RIPA buffer	10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 140 mM NaCl, 1% Triton-X, 0.1% SDS, 0.1% DOC
Ponceau Red	0.5% (w/v) Ponceau Red in 1% Acetic acid
TBS / TBST	10 mM Tris, 150 mM NaCl + (0.1% Tween-20)
Blocking buffer	3% or 5% BSA or non-fat dry milk in TBS or TBST
Stripping buffer	0.1 M Glycine pH 2.2, 1% Tween-20. 0.1% SDS
PBAS	2% BSA, 0.05% Saponin, 0.01% NaN ₃ in PBS
PBA	2% BSA, 0.01% NaN ₃ in PBS
Complete RPMI (Gibco)	10% FCS, 100 U/ml penicillin-streptomycin, 2 mM L-Glutamine and 0.1 mM 2-mercaptoethanol
Complete DMEM (GE Hyclone)	10% FCS, 100 U/ml penicillin-streptomycin, 2 mM L-Glutamine and 0.1 mM 2-mercaptoethanol
Reduced serum IMDM (GE Hyclone)	2% FCS, 100 U/ml penicillin-streptomycin, 2 mM L-Glutamine and 0.1 mM 2-mercaptoethanol
Balanced Salt Solution (BSS)	20x stock 1: 111 mM Dextrose, 8.8 mM KH ₂ PO ₄ , 26.7 mM Na ₂ HPO ₄ , 40 mM 0.5% Phenol Red 20x stock 2: 25.8 mM CaCl ₂ , 107 mM KCl, 2.73 M NaCl, 19.6 mM MgCl ₂ .6H ₂ O, 16.6 mM MgSO ₄
Erylysis Buffer	17 mM Tris-base pH 7.4, 140 mM NH ₄ Cl

3.5 Animal work

3.5.1 Contact hypersensitivity and tolerance induction model

Mice were sensitized epicutaneously by painting shaved back skin with 25 µl of 0.5% DNFB (Sigma-aldrich) (or as indicated) in a vehicle consisting of acetone (Merck Millipore) and olive oil (Borges, Tàrrega, Spain) (AOO) (4:1, v/v) and challenged 5 days later by topical application onto both sides of the left and right ears with 4 µl of 0.1% DNFB in AOO. Ear swelling post 0.1% DNFB challenge was measured using a micrometer (Mitutoyo, Kanagawa, Japan). Inguinal LNs were harvested and cells stained for migratory DCs (mDCs) and cytotoxic T cells (re-stimulated with PBDu and A23187) 2 and 5 days post DNFB (various doses) sensitization respectively.

For induction of cutaneous tolerance, 100 µl of 1.0% DNTB (Sigma-aldrich) in AOO was applied onto shaved abdominal skin 7 days before sensitization with 0.5% DNFB on the shaved back skin and challenged 5 days later with 0.1% DNFB (as described above). Ear swelling post 0.1% DNFB challenge was measured using a micrometer (Mitutoyo, Kanagawa, Japan). Inguinal LNs were harvested and cells stained for mDCs and activated regulatory T cells 3 and 5 days post DNTB painting respectively.

Inguinal LNs were harvested and cells stained for cytotoxic T cells (re-stimulated with PBDu and A23187) 5 days after 0.5% DNFB sensitization.

3.5.2 Intradermal (*i.d.*) injection of mouse ear and skin

Briefly, mice were anesthetized with isoflurane. Five micro-litres and 200 μ l of 10 μ g/ml O111:B4 LPS (Sigma-aldrich) were *i.d.* injected into the ear and trunk skin respectively. The tip of the 31G BD Ultrafine II insulin syringe (BD Bioscience) was bent to facilitate *i.d.* injection of the ear. A 29G insulin syringe (BD Bioscience) was used for trunk skin injection. Ears and sdLNs (defined as axillary, brachial and inguinal LNs) were harvested 24 hrs and 48 – 60 hrs post LPS injection respectively. Single cell suspensions were generated and cells stained for flow cytometry analysis. Epidermal sheets were also isolated and stained for IF.

3.5.3 Generation of bone marrow chimeric mice

Bone marrow (BM) was harvested from hipbones, femurs and tibiae of 8-week old donor mice. Total BM cells (1×10^7) was injected *i.v.* into lethally-irradiated (5.5Gy x 2) (Eckert & Ziegler, Berlin, Germany) 8-week old recipient mice. Recipient mice were fed with drinking water supplemented with antibiotics (neomycin, kanamycin and gentamycin) (all from Sigma-aldrich) for at least 2 weeks. BM chimeras were analysed 8 weeks after BM transplant.

3.5.4 *In vivo* Bromodeoxyuridine (BrdU) labelling assay

Mice were *i.p.* injected with 1 mg Bromodeoxyuridine (BrdU) (Sigma-aldrich) and fed with drinking water supplemented with 0.8 mg/ml BrdU for 7 days. Epidermal cell suspensions were generated and processed using the FITC BrdU Flow Kit (BD PharMingen).

3.6 Cell culture

3.6.1 Cell lines

HEK-293T (ATCC, Manassas, VA, USA) cells were cultured in complete DMEM (GE Hyclone). Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)-producing hybridoma cells (Gift from Christiane Ruedl) were cultured in reduced serum IMDM (GE Hyclone).

3.6.2 Generation of Bone Marrow-derived Dendritic Cells (BMDCs)

BMDCs were generated with a standard protocol¹²⁴. Briefly, hipbones, femurs and tibiae from poly(I)•poly(C)-treated control or *Tln1^{ff}Mx*-Cre mice were flushed with complete RPMI. Two million BM cells/well were seeded into 24-well plates (Thermo Fisher Scientific) in complete RPMI (Gibco) supplemented with 1% Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) conditioned media.

Alternatively, BM cells were cultured with recombinant GM-CSF (20 ng/ml) and IL-4 (10 ng/ml) for the first 2 days, followed by GM-CSF (10 ng/ml) without IL-4 for the subsequent days (Both from Peprotech). On day 2 of BMDC culture (both GM-CSF or GM-CSF/IL-4 methods), BM cells were gently pipetted to remove suspension cells and half of the existing media replaced with fresh GM-CSF-containing complete RPMI (1% GM-CSF conditioned media or 10 ng/ml GM-CSF). BM Cells were further cultured for 6 more days with fresh GM-CSF-containing complete RPMI replaced every 2 days or as required. For the generation of mature BMDCs, immature cells were stimulated with 100 ng/ml O111:B4 LPS overnight. BMDCs from control mice are defined as *Tln1^{fl/fl}* while BMDCs from *Tln1^{fl/fl}Mx-Cre* mice are defined as *Tln1^{Δ/Δ}*.

3.6.3 Stimulation of BMDCs

BMDCs were stimulated with 100 ng/ml O111:B4 LPS, 300 ng/ml Pam3CSK4 (InvivoGen), 100 ng/ml MALP-2 (InvivoGen), 100 ng/ml flagellin (kind gift from Christiane Ruedl) or transfected (Lipofectamine 2000, Invitrogen) with 10 μg/ml high molecular weight poly(I:C) (InvivoGen) or 1 μg/ml CpG ODN 2395 (InvivoGen) for the indicated times. Following stimulation, cells were quenched with ice-cold PBS (Gibco) and lysed on the plate with RIPA lysis buffer supplemented with 10 mM NaF, 10 mM Na₃VO₄, 1 mM DTT, 2% mammalian Protease Inhibitor Cocktail (PIC) and 2 mM PMSF (all from Sigma-aldrich). Samples were resolved on Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), transferred to a 0.2 μm or 0.45 μm Polyvinylidene difluoride (PVDF) (Merck Millipore) membrane and probed with the indicated antibodies (Immunoblot).

To study the activation of integrins *in vitro*, BMDC were seeded onto fibronectin (10 μg/ml, Sigma-aldrich)-coated culture dishes (Corning) or microscopy slides (Ibidi), or in suspension (control) for 2 hrs at 37°C. Non-adherent cells were removed while adherent cells were subsequently harvested and stained with 9EG7 (in the presence of 5 mM MnCl₂) and other indicated antibodies for flow cytometry analyses.

3.6.4 Retroviral transduction of BMDCs

Retroviral supernatant supplemented with 5 μg/ml Polybrene (Sigma-aldrich) were added to BMDCs 3 days from initial seeding. Room temperature (RT) spin transduction at 1000 G for 1.5 hrs (maximum acceleration, minimum deceleration) was performed. Viral supernatant was removed 1 hr after spin transduction and replaced with fresh media. Spin transduction was also performed at day 4 of BMDC culture. Transduced BMDCs were harvested and processed on day 8.

3.6.5 *In vitro* re-stimulation of T cells

To determine the cytokine secretion potential of T cells, 5×10^4 cells from sdLNs were cultured in 96-well culture plate (Corning, Corning, NY, USA) and re-stimulated with 10 ng/ml Phorbol 12,13-dibutyrate (PBDu) and 500 ng/ml calcium ionophore A23187 (both from Sigma-aldrich) in complete RPMI (Gibco) supplemented with 10 μ g/ml Brefeldin A (Sigma-aldrich) for 5 hrs at 37°C. After re-stimulation, cells were harvested and stained for flow cytometry analysis.

3.6.6 Ear explant culture

Mice were sacrificed, and ears were cut off at the base. Ear skin was split into dorsal and ventral halves and cultured in complete RPMI (Gibco) for the indicated time-points. For epidermal ear sheet cultures, ear sheets were split into dorsal and ventral halves and digested with 1.2 mg/ml Dispase I (Roche) in 10% FCS/RPMI for 1 – 2 hrs at 37°C. After digestion, epidermal ear sheets were separated from the dermis and cultured. After 4 or 24 hrs, epidermal ear sheets were fixed in ice-cold acetone (Merck Millipore) and stained for epidermal LCs using MHCII.

To sort for epidermal LCs, ear or trunk skin of control and *Tln1^{fl/fl}CD11c-Cre* mice were harvested. Epidermal cell suspensions were generated from the epidermis and stained. Cell sorting was performed using BD Aria (BD Bioscience).

3.6.7 Production of GM-CSF conditioned media

GM-CSF-producing hybridoma cells were cultured in reduced serum IMDM (GE Hyclone) for 10 to 14 days. Cells were pelleted and conditioned media collected, pooled, sterile filtered and kept frozen at -80°C.

3.7 Molecular techniques

3.7.1 Isolation of cells from lymphoid and non-lymphoid organs

LNs were disrupted with forceps and digested in a solution of 0.42 U/ml Liberase TM (Roche) and 0.1 – 0.5 mg/ml DNase I (Roche or Sigma-aldrich) in 10% FCS/RPMI for 30 min at 37°C. Digested tissues were titrated 8 – 10 times and filtered using a 100- μ m cell strainer (BD Bioscience). Skin-draining LNs were defined as axillary, brachial and inguinal LN unless stated otherwise. Alternatively, LNs were harvested and cell suspensions generated in chilled Balanced Salt Solution (BSS) supplemented with 2% FCS (Biowest) if Liberase treatment is not required.

To isolate cells from the skin, mice ears were split into dorsal and ventral halves with forceps. Epidermis and dermis were separated by 1.2 mg/ml Dispase I (Roche). Epidermis and dermis were further digested in a solution of 0.1 mg/ml collagenase IV (Sigma-aldrich) and 0.1 – 0.5 mg/ml DNase I (Roche or Sigma-aldrich). After

digestion, both epidermal and dermal cell suspensions were titrated, and filtered to remove debris. Cells from suspensions were counted and stained for FACS or flow cytometry analysis.

To isolate epidermal cells from the trunk skin, mice hair was removed by shaving before excision of trunk skin. Subcutaneous fat from the skin was removed using a scalpel (Swann-Morton, Sheffield, England, UK). Skin was subsequently cut into smaller pieces (2 cm x 2 cm) before Trypsin (0.25%, Gibco) digestion for 1 hr at 37°C. Epidermis from the trunk skin was scraped off using a scalpel before further digestion in a solution of collagenase IV and DNase I as described above. Dermis was discarded. After digestion, epidermal cell suspensions were titrated, and filtered to remove debris. Cells from suspensions were counted and stained for FACS or flow cytometry analysis.

3.7.2 Cloning

Cloning was performed using KAPA HiFi PCR kit (Roche, Basel, Switzerland) and Takara PrimeSTAR™ HS DNA polymerase kit (Clontech, Mountain View, CA, USA). Primers were designed accordingly and synthesized (IDT, Singapore). Mutations were verified by restriction enzyme digestion (New England Biolabs, Ipswich, MA, USA) and sequencing (First Base, Singapore). Transformation at 42°C for 45 sec was performed using commercial or homemade TOP10 (Thermo Fisher Scientific), XL-Blue or XL-Gold (Both from Agilent, Santa Clara, CA, USA) competent cells and plated onto ampicillin or kanamycin LB agar plates (All from Sigma-aldrich). LB plates were incubated at 37°C overnight and colonies were propagated at 30°C to 37°C. Miniprep (Geneaid, New Taipei City, Taiwan) or Midiprep (NucleoBond, Macherey-Nagel, Düren, Germany) was performed to isolate plasmid DNA for sequencing or further experiments.

3.7.3 Flow cytometry

Extracellular staining was performed according to standard protocol. Intracellular staining was performed according to manufacturer's instructions (eBioscience or BD Biosciences). FcBlock (CD16/32) (Biolegend) was used in select stainings. Acquisition of flow cytometry data was performed using BD LSRFortessa X-20 5 Laser, BD LSRFortessa X-20 3 Laser or LSRII 4 Laser (BD Biosciences). Flow cytometry data analysis was performed using FlowJo software (Tree Star Inc., Ashland, OR, USA).

3.7.4 Phospho-flow cytometry

To assess the activation or phosphorylation of proteins using flow cytometry, cells were harvested with ice-cold PBS (Gibco) and transferred into pre-chilled eppendorf or falcon tubes (Greiner Bio-one, Kremsmünster, Austria). Cells were aliquoted into

pre-chilled 96-well plates, pelleted and fixed in 4% PFA (BD Biosciences) for 15 min at 37°C. Fixed cells were permeabilized with ice-cold methanol (7 parts methanol (Merck) : 3 parts sterile PBS) on ice for 15 mins, rehydrated with sterile PBS, pelleted, followed by blocking with FcBlock (CD16/32). Permeabilized cells were stained with fluorochrome*-conjugated antibodies and other primary antibodies in PBA for 1 hr at RT. Primary antibodies were further stained with secondary antibodies in PBA for 1 hr at RT. Acquisition of flow cytometry data was performed using BD LSRFortessa X-20 5 Laser, BD LSRFortessa X-20 3 Laser or LSR II 4 Laser (BD Biosciences). Flow cytometry data analysis was performed using FlowJo software (Tree Star Inc., Ashland, OR, USA).

*Certain fluorochromes such as PE and APC and their derivatives including tandem dyes, and select antibody clones are not compatible with methanol fixation. Optimize prior.

3.7.5 Calcium flux assay

BMDCs were harvested, resuspended to a density of 5×10^6 cells/ml and loaded with 5 μ M Indo-1 AM (Invitrogen) in 0.1% FCS/RPMI (Gibco) for 45 min at 37°C. Cell suspensions were agitated at 10-min intervals to ensure even loading of Indo-1 AM. Cells were quenched with RT 0.1% FCS/RPMI and pelleted. Cells can be further stained with extracellular antibodies (optional) before flow cytometric analysis of samples. Pre-warm indo-1-loaded cells to 37°C before acquisition. Record all events (500 – 600 events / sec) for 30 sec (baseline) before addition of stimulus (5 – 10 sec downtime), followed by uninterrupted acquisition for the remaining time. Recorded data was presented as a ratio of bound (405 nm) / free (485 nm) calcium ions, as a function of time (sec) by FlowJo software (Tree Star Inc., Ashland, OR, USA).

3.7.6 Calcium phosphate transfection

HEK-293T cells (4×10^5 cells) were seeded onto 6 cm culture dishes (Corning, Corning, NY, USA) and transfected the next day using standard calcium phosphate transfection protocol. Transfected cells were lysed 48 hrs later with supplemented Triton-X buffer and immunoprecipitation of MyD88 (Flag IP) or talin1 (Green fluorescent protein (GFP) IP) was performed. To produce retroviral supernatant for BMDC transduction, HEK-293T (3×10^6 cells seeded onto 10 cm culture dishes) were transfected with 10 μ g MIG-EV, MIG-GFP-talin1, MIG-GFP-talin1-W359A or MIG-GFP-talin1-L325R with 10 μ g pCL-Eco packaging plasmid (all vectors). Virus containing supernatant were collected at 48 and 72 hrs respectively and filtered (0.45 micron unit) (Pall, Port Washington, NY, USA).

3.7.7 Enzyme-linked immunosorbent assay (ELISA)

BMDCs were stimulated with 100 ng/ml LPS, 10 ng/ml IL-1 β or 10 ng/ml TNF α (or in various combination) for 1 hr before exogenous stimuli-containing supernatants were replaced with fresh complete RPMI. Duration of stimulation starts upon addition of exogenous stimuli. Supernatants from stimulated BMDCs were collected at 12 and 24 hrs respectively and stored at -20°C until sample collection was completed. 96-well plates (Thermo Fisher Scientific or Corning, Corning, NY, USA) were prepared for ELISA according to manufacturer's instructions (Biolegend). Absorbance was measured using the Tecan Infinite M200 spectrophotometer (Tecan, Männedorf, Switzerland).

3.7.8 Immunoprecipitation

BMDCs were stimulated with 100 ng/ml O111:B4 LPS (TLR4 IP) for 30 min, or transfected with 10 μ g/ml high molecular weight poly(I:C) (TLR3 IP) for 45 min. Following stimulation, cells were quenched with ice-cold PBS (Gibco) and harvested by scraping (VWR, Randor, PA, USA). Harvested cells were lysed in supplemented Triton-X lysis buffer or fractionated into membrane and cytosol fractions (Cell fractionation kit, Cell Signaling Technology) according to manufacturer's instructions. Fractions and samples were pre-cleared with protein G sepharose beads (GE Healthcare) for 30 to 60 min at 4°C. TLR4 (25, sc-293072) or TLR3 (40C1285, sc-52961) antibody was added and samples were further incubated overnight at 4°C. Protein G sepharose beads were added and samples were incubated for 3 – 4 hrs at 4°C. Subsequently, antibody-bound beads were washed 3 times with Triton-X buffer and boiled in 2x Laemmli buffer (Bio-Rad). Samples were resolved on SDS-PAGE and immunoblot was performed using the indicated antibodies.

3.7.9 *In vitro* Transcription/Translation (IVT) assay

IVT or cell free protein expression assays (Invitrogen) were performed according to manufacturer's instructions. Translated proteins were incubated in a one-to-one volume ratio overnight before immunoprecipitation was performed using anti-MyD88, anti-talin1 or anti-Vav1 antibodies. Subsequently, antibody-bound beads were washed 3 times with Triton-X buffer and boiled in 2x Laemmli buffer (Bio-Rad). Samples were resolved on SDS-PAGE and immunoblot was performed using the indicated antibodies.

3.7.10 Real Time quantitative-Polymerase Chain Reaction (RTq-PCR)

Total RNA from cells and tissues was extracted using TRIzol (Invitrogen) and complementary DNA (cDNA) reversed transcribed using Superscript Reverse Transcriptase (Invitrogen). Primers were designed or retrieved from PrimerBank (Harvard Medical School, Boston, MA, USA) to amplify specific genes. Messenger

RNA (mRNA) were quantitated by RTq-PCR using IQ SYBR Green Supermix (Bio-Rad) according to manufacturer's instructions. Data were normalized to *Gapdh* and expressed as fold change relative to control.

Table 5. List of primers

Primer	Sequence
<i>Tln1</i> F	CCTGCCGCATGATTCTGTGA
<i>Tln1</i> R	TCGGAGCATGTAGTAGTCCAAA
<i>Fermt3</i> F	ATGGCGGGTATGAAGACAGC
<i>Fermt3</i> R	CACCAATGTGCGACTCCCC
<i>Gapdh</i> F	TGGATTTGGACGCATTGGTC
<i>Gapdh</i> R	TTTGCACTGGTACGTGTTGAT
<i>Il1b</i> F	GCAACTGTTCTGAAGTCAACT
<i>Il1b</i> R	ATCTTTTGGGGTCCGTCAACT
<i>Il6</i> F	CCAAGAGGTGAGTGCTTCCC
<i>Il6</i> R	CTGTTGTTTCAGACTCTCTCCCT
<i>Il23a</i> F	ATGCTGGATTGCAGAGCAGTA
<i>Il23a</i> R	ACGGGGCACATTATTTTATAGTCT
<i>Tnf</i> F	CCCTCACACTCAGATCATCTTCT
<i>Tnf</i> R	GCTACGACGTGGGCTACAG
<i>Ifnb1</i> F	CAGCTCCAAGAAAGGACGAAC
<i>Ifnb1</i> R	GGCAGTGTAAGTCTTCTGCAT
<i>Mmp2</i> F	CAAGTTCCCCGGCGATGTC
<i>Mmp2</i> R	TTCTGGTCAAGGTCACCTGTC
<i>Mmp9</i> F	CTGGACAGCCAGACACTAAAG
<i>Mmp9</i> R	CTCGCGGCAAGTCTTCAGAG

3.8 Microscopic techniques

3.8.1 Epidermal sheet microscopy

Mice ears were split into dorsal and ventral halves with forceps. Split ears were floated on 0.5 M ammonium thiocyanate (Sigma-aldrich) in PBS at 37°C for 40 min. Isolated sheets were then fixed in ice-cold acetone (Merck Millipore) for 10 min, rinsed with PBS (Gibco), blocked in PBA (table 2) for 1 hr at RT and stained with anti-I-A/I-E antibody (Biolegend) overnight. After staining, sheets were mounted with Fluoromount™ aqueous mounting medium (Sigma-aldrich) or ProLong Gold AntiFade mountant (Invitrogen) containing DAPI (Invitrogen) and images acquired with Nikon fluorescence microscope using Metamorph (Molecular Devices, San Jose, CA, USA). Captured images were analysed and processed with Fiji¹⁹⁴.

3.8.2 Confocal microscopy

BMDCs were seeded onto μ -slide IV^{0.4} (Ibidi), stimulated with 100 ng/ml O111:B4 LPS (Sigma-aldrich) for 30 min and fixed with 2% PFA (Sigma-aldrich) for 15 min at RT or 37°C. Fixed cells were incubated with 0.5% ice-cold Triton-X (Sigma-aldrich) on ice for 5 min and washed with PBAS (table 2). If required, F-actin was stained with Phalloidin (Invitrogen) for 1 hr at RT and washed with PBAS. Washed cells were

blocked with 5% donkey and 5% goat serum (Sigma-aldrich) in PBAS for 1 hr at RT and stained overnight with anti-talin1 (Sigma-aldrich) and anti-MyD88 (Bio-vision, Milpitas, CA, USA) in 0.5% donkey and 0.5% goat serum in PBAS. Primary antibody-stained cells were washed three times with PBS followed by further incubated with secondary antibodies (Jackson ImmunoResearch) in 1% donkey and goat serum in PBAS for 1 – 2 hrs at RT. Secondary antibody-stained cells were washed three times with PBS and subsequently mounted with Fluoromount™ aqueous mounting media (Sigma-aldrich) containing DAPI (Thermo Fisher Scientific) and visualized with LSM 710 SIM (Zeiss, Oberkochen, Germany). Captured images were processed with ZEN microscope software (Zeiss, Oberkochen, Germany) and/or with Fiji¹⁹⁴.

To visualize the localizations of talin1 and MyD88, non-fluorescent 0.3 µm latex beads (washed with PBS for 5 times) (Sigma-aldrich) were added to BMDCs pre-seeded onto µ-slide 8 well chambers (Ibidi), in a ratio of 10 beads : 1 cell. Mixture of beads and BMDCs were incubated at 37°C for 30 min before 2% PFA fixation. BMDCs were further processed as described earlier. Colocalization analyses were performed using Coloc 2¹⁹⁴ and Plot Profile plugin (Fiji). In brief, a line was drawn over the region of interest on overlaid (merged) images and data-points plotted (GraphPad Prism 6 or 7) to illustrate the overlapping signals expressed in fluorescence intensity (Arbitrary Units, AU).

3.8.3 Live-cell imaging of BMDC migration in 3-D collagen matrices

BMDCs were embedded into bovine collagen type I (final concentration of 1.6 mg/ml) (Advanced BioMatrix, San Diego, CA, USA) matrix and subsequently introduced into µ-slide chemotaxis chamber (Ibidi) according to manufacturer's instructions. After the collagen matrix has solidified (37°C for 45 min), various concentrations (10 ng/ml, 50 ng/ml, 100 ng/ml and 600 ng/ml) of the chemokine CCL19 (Peprotech) in 2% FCS/RPMI were added to the chamber and live-cell imaging (5% CO₂, 37°C) was performed immediately using Nikon microscope. Time-lapse images (2 to 3 min intervals between images) were acquired using Metamorph (Molecular Devices). Captured images were processed (Fiji) using the chemotaxis plugin¹⁹⁴.

3.9 Statistics

Statistical significance was determined using Student's *t*-test and defined as follows: *0.01 < *P* ≤ 0.05, **0.005 < *P* ≤ 0.01, ***0.001 < *P* ≤ 0.005. Bar graphs show average ± Standard Deviation (SD) from combined experiments and group sizes as indicated in the figure legends. Standard Error of the Mean (SEM) was depicted for measurement of ear thickness. Figures were prepared using GraphPad Prism 6 or 7 (GraphPad Software, San Diego, CA, USA) and Adobe Illustrator (Adobe, Mountain View, CA, USA).

4. Results

4.1 Talin1 is essential for the migration of epidermal Langerhans Cells

Since the migration of Langerhans Cells (LCs) across the collagen and laminin-rich epidermal basement membrane is mediated by integrin $\alpha 6 \beta 1$ ¹⁶⁸, talin1 is likely to be essential for LC migration *in vivo*. To address the role of talin1 in LC migration, epidermal ear sheets were isolated from control and *Tln1^{fl/fl}CD11c-Cre* mice in the steady state and stained for MHCII to visualize epidermal LCs. We observed a significant increase in the number of talin1-deficient LCs compared to wildtype LCs (**Fig. 7a,b**). The increased number of talin1-deficient LCs persisted even under several inflammatory conditions; intradermal (*i.d.*) injection of bacterial-derived LPS, painting of the non-allergic hapten 2,4-dinitrothiocyanobenzene (DNTB) or artificially induced via ear explant culture¹⁹⁵ (**Fig. 7a,b**). A concomitant reduction in the number of wildtype LCs can be detected upon induction of skin inflammation due to the migration of LCs out of the epidermis upon activation.

Up-regulation of chemokine receptors CCR7 and CXCR4, and integrin subunits CD49f (integrin $\alpha 6$) and CD29 (integrin $\beta 1$; dimerizes with integrin $\alpha 6$ to form integrin $\alpha 6 \beta 1$ or VLA-6) were detected on both wildtype and talin1-deficient LCs under inflammatory conditions (**Fig. 7c**). Activated CD29, as determined by 9EG7 antibody, was detected on wildtype epidermal LCs (**Fig. 7c**). However, activated CD29 was substantially reduced on talin1-deficient LCs under similar conditions, which is consistent with the role of talin1 as an integrin activator^{93, 96, 104, 117, 119, 120, 162} (**Fig. 7c**).

Next, we performed BrdU incorporation assay to rule out that the increased number of talin1-deficient LCs in epidermis is caused by differential rates of LC self-renewal *in situ*. We did not observe any difference in the proliferative capacity between wildtype and talin1-deficient LCs (**Fig. 7d,e**). Enzymatic digestion of the basement membrane with dispase, an enzyme that selectively cleaves type IV collagen and fibronectin¹⁹⁶ facilitated the exit of both wildtype and talin1-deficient LCs (**Fig. 7f,g**). These data suggest that talin1 is essential for the integrin-dependent migration of LCs across the basement membrane in the steady state and under inflammatory conditions.

Lastly, we examined the expression of the integrin co-activator kindlin3 (*Fermt3*, an isoform of kindlin unique to hematopoietic lineage cells) and talin2 (*Tln2*, an isoform of Talin). Up-regulation of talin2 has been observed in fibroblasts depleted of talin1, indicating that talin2 is able to compensate for the loss of talin1 in non-hematopoietic cell types¹⁹⁷. Since cells of the hematopoietic lineage do not express talin2^{93, 96, 101, 198, 199}, as expected we did not detect *Tln2* expression in wildtype LCs (**Fig. 7h,i**), using heart muscle as a control for *Tln2* expression. Compensatory up-regulation of *Tln2* was not observed in talin1-deficient LCs as well (**Fig. 7i**). Moreover, the expression of

kindlin3 (*Fermt3*) also remained unaltered in talin1-deficient LCs (**Fig. 7i**), indicating that talin1 deficiency is not compensated by talin2 and kindlin3. In addition, up-regulation of *Mmp2* and *Mmp9*, two metalloproteinases involved in the disruption of the basement membrane²⁰⁰ can be detected in talin1-deficient LCs (**Fig. 7j**), which could be a compensatory measure adapted by talin1-deficient LCs to overcome the defective integrin-dependent migration across the basement membrane.

Figure 7. Talin1 regulates the migration of LCs across the basement membrane

(a) Immunofluorescence analyses of epidermal ear sheets isolated from control (*Tln1^{fl/fl}*) and *Tln1^{fl/fl}CD11c-Cre* mice painted with 1% DNTB, *i.d.* injected with LPS or explants cultured for 24 and 48 hrs. LCs were identified by MHCII staining. At least 5 random images per ear were acquired from each mouse. Scale bar, 20 μ m. (b) Quantification of LCs (scatter plot) on epidermal ear sheets. Each data point in scatter bar graph indicates the cell number from one image. Data from 4 independent experiments are summarized (n = 4, total $\geq$ 30 fields per group). (c) Histograms as determined by flow cytometry show the expression levels of indicated molecules on the surface of LCs isolated from the epidermis of treated mouse ears as indicated or ear explants cultured for 24 hrs. (d) Flow cytometric dot plots and (e) percentage of BrdU⁺ LCs (scatter plot) isolated from the epidermis of mice fed with BrdU for 7 days. Each data point in scatter bar graph indicates the cell number from one experiment. Data from 4 independent experiments are summarized (n = 4) (e). Data were pre-gated on singlet, live cells. CD45⁺ MHCII⁺ EpCAM⁺ CD11b⁺ LCs were further analysed for the expression of MHCII, CD49f (integrin α 6), CD29 (integrin β 1), activated CD29 (as indicated by 9EG7), Talin1, CCR7, CXCR4 and BrdU (c,d). Histograms (c) and dot plots (d) are

representative at least 3 independent experiments ($n \geq 3$). **(f)** Immunofluorescence analyses of dispase-treated epidermal ear sheets from control (*Tln1^{fl/fl}*) and *Tln1^{fl/fl}CD11c-Cre* mice. Dispace-treated ear sheets were further cultured for 4 and 24 hrs before MHCII staining of LC. At least 5 random images per ear were acquired from each mouse. Scale bar, 40 μ m. **(g)** Quantification of LCs (scatter plot) on epidermal ear sheets. Each data point in scatter bar graph indicates the cell number from one image. Data from 4 independent experiments were summarized ($n = 4$, total ≥ 30 fields per group). **(h)** Flow cytometric analyses indicating the purity of EpCAM⁺ MHCII⁺ LCs pre- and post-FACS sorting. Data were pre-gated on singlet, live, CD45⁺ cells. **(i,j)** RTq-PCR analyses (represented in fold change) of mRNA transcript levels of **(i)** *Tln2* and *Fermt3* and **(j)** *Mmp2* and *Mmp9* from **(h)** FACS-sorted LCs. Transcript levels were normalized to *gapdh*. Data from 2 (*Tln2*, *Mmp2* and *Mmp9*, $n = 2$) or 3 (*Fermt3*, $n = 3$) independent experiments are summarized. Intradermal (*i.d.*); Fluorescence-Activated Cell Sorting (FACS); Langerhans Cell (LC); Real Time quantitative-Polymerase Chain Reaction (RTq-PCR). Statistical analysis was performed using two-tailed Student's *t*-test. NS, not significant; * $0.01 < P \leq 0.05$, ** $0.005 < P \leq 0.01$.

4.2 Talin1 regulates the migration of dermal DCs to the draining lymph nodes

Besides the accumulation of talin1-deficient LCs in the epidermis (**Fig. 7a,b and 8a,b**), our data also revealed a small but significant increase in the abundance of talin1-deficient dermal DC (dDC) subsets in the dermis of *Tln1^{ff}CD11c*-Cre mice compared to control mice in the steady state (**Fig. 8c,d**). The accumulation of talin1-deficient skin DCs in both epidermal and dermal compartments may indicate that these cells cannot migrate to the skin-draining LNs (sdLNs), so we decided to examine the abundance of migratory DCs (mDCs) in the sdLNs of *Tln1^{ff}CD11c*-Cre mice. As expected, we detected a collective reduction in the cell counts of various talin1-deficient mDC subsets in the sdLNs of *Tln1^{ff}CD11c*-Cre mice compared to control mice (**Fig. 8e,f**). The reduced number of talin1-deficient migratory LCs (mLCs) in the sdLNs is most likely to be caused by a defect in LC migration across the basement membrane because we did not observe any significant difference in the proliferative capacity between wildtype and talin1-deficient LCs (**Fig. 7d,e**) and the disruption of the basement membrane restored the emigration of talin1-deficient LCs out of the epidermis in explant culture (**Fig. 7f,g**). Dermal DCs, unlike their epidermal counterparts, do not proliferate *in situ*; instead they are repopulated by lineage-restricted progenitors from the bone marrow^{81, 201}. The reduced number of talin1-deficient migratory dDCs (mdDCs) in the sdLNs suggests a defect in integrin-independent migration or responsiveness to chemotactic recruitments.

In addition, we also detected a significant reduction in the number of talin1-deficient resident DCs in the sdLNs (**Fig. 8g**), suggesting that talin1 or integrins activated by talin1 influences the homing of DC progenitors into the LNs to differentiate into resident DCs.

Figure 8. Accumulation of talin1-deficient DCs in the skin

Flow cytometric analyses (a,c,e) and the absolute numbers (b,d,f,g, scatter plots) of the DC subsets in the epidermis (a,b), dermis (c,d) and sdLNs (e,f,g) of control ($Tln1^{ff}$) and $Tln1^{ff}CD11c-Cre$ mice in the steady state. Data were pre-gated on singlet, live, CD45⁺ MHCII⁺ cells (a,c). LCs (in the epidermis) and migratory LCs (mLCs, in the dermis) are defined as Langerin⁺ CD11b⁺ (a-d). Dermal DCs (dDCs) are defined as Langerin⁺ CD11b⁻, Langerin⁻ CD11b⁺ and Langerin⁻ CD11b⁻ (c,d). Data were pre-gated on singlet, live cells. CD11c⁺ MHCII⁺ migratory DCs (mDCs) in the sdLNs were further analyzed for EpCAM⁺ CD11b⁺ mLCs, CD103⁺ CD11b⁻ migratory dermal DCs (mdDCs), CD103⁻ CD11b⁺ mdDCs and CD103⁻ CD11b⁻ mdDCs (e,f). (g) Absolute number of CD11c^{hi} MHCII⁺ resident DCs in the lymph nodes. Each data point in scatter bar graph indicates the cell number from one experiment (b,d,f,g). Dendritic Cell (DC); Langerhans Cell (LC); Skin-draining Lymph Nodes (sdLNs). All data shown in this figure are representative or summary of 5 independent experiments ($n \geq 5$). Statistical analysis was performed using two-tailed Student's *t*-test. * $0.01 < P \leq 0.05$, ** $0.005 < P \leq 0.01$.

4.3 Talin1 is required for integrin-independent migration

The accumulation of talin1-deficient dDCs in the dermis was unexpected because the migration of DCs in a three-dimensional (3-D) environment such as the dermis or in a collagen matrix is not known to be dependent on integrins⁹². To address this unexpected observation, we derived dendritic cells from the bone marrow (BMDCs) of poly(I)•poly(C)-treated control (*Tln1^{fl/fl}*) and *Tln1^{fl/fl}*Mx-Cre mice. Comparable numbers of wildtype (*Tln1^{fl/fl}*) and talin1-deficient (*Tln1^{Δ/Δ}*) BMDCs can be generated *in vitro* (**Fig. 9a**), with near complete depletion of talin1 protein in talin1-deficient BMDCs (**Fig. 9b**). Spreading of wildtype BMDCs on fibronectin-coated slides was observed, with an average cell area of 115 μm^2 (**Fig. 9c**). Talin1-deficient BMDCs however, remained rounded with an average cell area of 27 μm^2 (**Fig. 9c**), supporting the critical role of talin1 in cell adhesion and spreading. We observed the activation of CD29 (integrin $\beta 1$) as defined by 9EG7 antibody (detects activated, high-affinity CD29) in wildtype BMDCs seeded on fibronectin-coated slides (**Fig. 9d**). Activated CD29 was barely detectable in talin1-deficient BMDCs (**Fig. 9d**). These experiments revealed a functional role of talin1 as an integrin co-activator in BMDCs.

To investigate DC migration *in vitro*, we embedded wildtype and talin1-deficient BMDCs in 3-D collagen matrices. We observed a significant reduction in the migration capacity of LPS-stimulated talin1-deficient BMDCs compared to wildtype cells towards titrated amounts of the chemokine CCL19 in 3-D collagen matrices (**Fig. 9e**). The reduced propensity of talin1-deficient BMDCs to migrate can be reflected in the lowered speed (**Fig. 9f**), reduced distance travelled (**Fig. 9g**) and directional persistency (**Fig. 9h**) compared to wildtype BMDCs, suggesting that talin1 deficiency could affect integrin-independent migration.

The chemokine receptor CCR7 is indispensable for the migration of DCs towards CCL19 or CCL21^{92, 152, 159, 202, 203}. Pronounced up-regulation of CCR7 was observed in both wildtype and talin1-deficient BMDCs upon LPS stimulation (**Fig. 9i**), indicating that the reduced migratory capacity of talin1-deficient DCs is not contributed by the inability to detect CCL19 or CCL21. The up-regulation of CCR7 can also be detected on wildtype and talin1-deficient epidermal LCs (**Fig. 7c, CCR7 histogram**), indicating that talin1 deficiency does not impair chemokine sensing through CCR7.

Figure 9. The role of talin1 in integrin-independent migration of BMDCs

(a) Flow cytometric dot plots of unstimulated wildtype and talin1-deficient DCs derived from bone marrow cells of poly(I)•poly(C)-injected control and *Tln1^{fl/fl}Mx-Cre* mice. BMDCs were cultured in the presence of GM-CSF (details see methods). Data were pre-gated on singlet, live cells. BMDCs were defined as CD11c⁺ MHCII⁺. (b) Immunoblot analysis of talin1 in wildtype and talin1-deficient BMDCs. (c) Quantification of the cell area (left, scatter plot) and representative brightfield images (right) of wildtype (n = 127) and talin1-deficient (n = 128) BMDCs seeded on fibronectin-coated slides. Scale bar, 4 μm. Each data point in scatter bar graph indicates the cell area of one cell. (d) Histograms as determined from flow cytometric analyses of total CD29 and activated CD29 (indicated by 9EG7) in wildtype and talin1-deficient BMDCs seeded on fibronectin-coated culture dish or in suspension for 2 hrs. Data were pre-gated on singlet, live CD11c⁺ MHCII⁺ BMDCs. (e) Single cell tracks of migrating wildtype and talin1-deficient BMDCs towards various doses of CCL19 in 1.6 mg/ml 3-D collagen matrices. Migration of BMDCs was visualized using live-cell imaging. Cells were

tracked for 4 – 5 hrs with images acquired at every 2 – 3 min intervals. Black tracks indicate forward net migration towards chemoattractant, red tracks indicate net reverse migration. Green triangles indicate the gradient of CCL19. **(f-h)** Scatter plot analyses of **(f)** cell speed, **(g)** distance travelled and **(h)** directional persistence of chemotaxing wildtype and talin1-deficient BMDCs in collagen matrices. Each data point in scatter bar graph indicates the behaviour of one cell **(f-h)**. At least 30 wildtype or talin1-deficient BMDCs were tracked in each experiment **(e-h)**. **(i)** Histograms as determined from flow cytometric analyses of CCR7 in unstimulated (PBS) and LPS-stimulated (100 ng/ml LPS, overnight) wildtype and talin1-deficient BMDCs. Data were pre-gated on singlet, live CD11c⁺ MHCII⁺ BMDCs. Analyses were performed using unstimulated **(a-c)**, LPS-stimulated (100 ng/ml LPS) BMDCs **(e-i)** or BMDCs plated on fibronectin (10 µg/ml) dishes **(d)**. Wildtype (*Tln1^{ff}*); Talin1-deficient (*Tln1^{ΔΔ}*); Bone Marrow-derived Dendritic Cell (BMDC); C-C Motif Chemokine Ligand 19 (CCL19); Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF); 3-Dimensional (3-D). All data shown in this figure are representative or summary of **(c,d,i)** 3 (n = 3), **(e-h)** 4 to 6 (n ≥ 4) or 6 **(a,b)**, n = 6) independent experiments. Statistical analysis was performed using two-tailed Student's *t*-test. *0.01 < *P* ≤ 0.05.

4.4 Attenuated tolerance induction in mice with Talin1-deficient LCs

Contact Hypersensitivity (CHS), a mouse model for the human disease Allergic Contact Dermatitis (ACD) is a cytotoxic T cell-mediated skin inflammation induced by exposure to contact sensitizers such as strong hapten 2,4-dinitrofluorobenzene (DNFB), imiquimod (TLR7 agonist) and oxazolone²⁰⁴. These low-molecular weight sensitizers often cross-react with surrounding peptides or carrier proteins, forming complexes to facilitate efficient uptake by skin DCs^{205, 206, 207}. CHS/ACD occurs in two – afferent (sensitization) and efferent (challenge) – phases²⁰⁸. Upon first contact with the hapten, skin DCs will phagocytose the hapten-carrier protein complex, migrate to the sdLNs to prime hapten-specific cytotoxic T cells (afferent phase). Subsequent exposure to the same hapten elicits the recruitment of hapten-carrier T cells to the site of contact, resulting in skin inflammation due to the cytotoxic activities of hapten-specific T cells (efferent phase)²⁰⁴. ACD/CHS is largely mediated by dermal DCs^{72, 74, 173, 209, 210}.

LCs on the other hand are able to activate Foxp3⁺ regulatory T cells in the sdLNs to suppress the activation of CD8 cytotoxic T cells in the mouse model of ACD²¹¹. We inferred that migration-incompetent talin1-deficient epidermal LCs will not be able to cross the basement membrane to exert tolerogenic effects on T cells in ACD. Here, we employed an experimental hapten mouse model to determine whether talin1 expression in skin DCs plays a critical role in skin inflammation²¹¹. We induced CHS to a strong hapten 2,4-dinitrofluorobenzene (DNFB) by first sensitizing shaved skin on the back of the mice with DNFB, then challenged the sensitized-mice by applying DNFB onto the ear 5 days later (**Fig. 10a, experimental outline**). Here, we were able to induce strong immunogenic response in both control and *Tln1^{ff}CD11c*-Cre mice as determined by ear swelling (**Fig. 10a**). Since the innocuous weak/tolerogenic hapten 2,4-dinitrothiocyanobenzene (DNTB) generates dinitrophenyl–self peptide (DNP–self peptide) motifs similar to those of the strong hapten DNFB, we induced immunotolerance against DNFB by applying DNTB onto shaved abdomen skin of both control and *Tln1^{ff}CD11c*-Cre mice 7 days prior to DNFB sensitization²¹¹ (**Fig. 10a, experimental outline**). As expected, ear swelling was not observed in DNTB-tolerized control mice (**Fig. 10a**), which correlated with the activation of repressive ICOS⁺ Foxp3⁺ regulatory T cells by wildtype LCs (**Fig. 10b,c**) and the significantly reduced cell count of IFN- γ -producing CD8 cytotoxic T cells (**Fig. 10d,e**). In contrast, robust ear swelling responses were recorded in DNTB-painted *Tln1^{ff}CD11c*-Cre mice, with maximal ear swelling peaking at day 2 post DNFB ear challenge (**Fig. 10a**). Correspondingly, the modest increase in the percentage and number of ICOS⁺ Foxp3⁺ regulatory T cells in DNTB-painted *Tln1^{ff}CD11c*-Cre mice was not sufficient to suppress the priming of IFN- γ -producing CD8 cytotoxic T cells (**Fig. 10b,c**). The percentage and number of activated CD8 cytotoxic T cells in DNTB-painted *Tln1^{ff}CD11c*-Cre mice were

comparable to those in control and *Tln1^{ff}CD11c*-Cre mice during CHS response (**Fig. 10d,e**).

In addition to the reduced mLCs in the sdLNs, similar to our earlier observations in the steady state (**Fig. 8**), we also found significantly lower number of talin1-deficient mdDCs in the sdLNs of *Tln1^{ff}CD11c*-Cre mice upon DNTB painting compared to control mice (**Fig. 10f**). To determine if the defective talin1-deficient LC motility is the main contributor to the failed tolerance induction against the strong hapten DNFB, we exploited the radio-resistant properties of LCs by generating bone marrow-chimeric mice with wildtype epidermal LCs and talin1-deficient dDCs (wt LC, mt dDC) or with talin1-deficient epidermal LCs and wildtype dDCs (mt LC, wt dDC). We repeated the tolerance induction experiment using these chimeric mice. Ear swelling was not observed in DNTB-tolerized chimeras with wildtype LCs and talin1-deficient dDCs (**Fig. 10g, top, wt LC, mt dDC**) while strong ear swelling responses were recorded in DNTB-painted chimeras with talin1-deficient epidermal LCs and wildtype dDCs (**Fig. 10g, top, mt LC, wt dDC**). CHS responses upon DNFB painting were not affected in both chimeric mice (**Fig. 10g, bottom**). These data suggest that talin1 expression in epidermal LCs is essential for tolerance induction.

Figure 10. Attenuated tolerance induction in mice with migration-incompetent talin1-deficient LCs

(a) Mice were tolerized with 1% DNTB on shaved abdomen skin. Seven days later, mice were sensitized with 0.5% DNFB on shaved back skin and ear challenged 5 days later with 0.1% DNFB (top, experimental outline). Ear swelling response (bottom, mean $\pm$ SEM) of painted mice was determined over a period of 7 days post-challenge. (b) Flow cytometric analyses and (c) absolute numbers (scatter plot) of ICOS⁺ CD4⁺ Foxp3⁺ regulatory T cells in the sdLNs of *Tln1^{ff}* and *Tln1^{ff}CD11c-Cre* mice 5 days after vehicle or DNTB painting. Data were pre-gated on singlet, live CD3⁺ CD4⁺ Foxp3 Treg cells. Inguinal LNs were harvested 5 days after DNTB sensitization. (d) Flow cytometric analyses and (e) absolute numbers (scatter plot) of IFN- γ -producing CD8 T cells in the sdLNs of vehicle or DNTB-painted *Tln1^{ff}* and *Tln1^{ff}CD11c-Cre* mice followed by DNFB sensitization. Inguinal LNs were harvested 5 days after DNFB sensitization. Single cell suspensions were generated from LNs and T cells re-stimulated (5 hrs) to detect IFN- γ production. Data were pre-gated on singlet, live CD3⁺ CD8⁺ T cells. (f) Absolute numbers (scatter plot) of CD11c⁺ MHCII⁺ mDCs in the sdLNs 3 days after DNTB painting. CD11c⁺ MHCII⁺ migratory DCs (gating strategy as described in Fig. 8e) in the sdLNs were further analysed for EpCAM⁺ CD11b⁺ mLCs, CD103⁺ CD11b⁻ mdDCs, CD103⁻ CD11b⁺ mdDCs and CD103⁻ CD11b⁻ mdDCs. (g) Ear

swelling response (mean $\pm$ SEM) of treated chimeric mice as described in (a, experimental outline). Chimeric mice were generated by adoptive transfer of total BM cells from *Tln1^{fl/fl}CD11c-Cre* mice into *Tln1^{fl/fl}* recipient mice or vice versa. Talin1-deficient cells are defined as mutant (mt), talin1-sufficient cells are defined as wt (wildtype). Each data point in scatter bar graph indicates the cell number from one experiment (**c,e,f**). Standard error of the mean (SEM); Interferon- γ (IFN- γ); Bone Marrow (BM); migratory DC (mDC); migratory LC (mLC); migratory dermal DC (mdDC). Data shown are summarized from 3 ($n \geq 8$) (**a,g**) or 4 independent experiments ($n \geq 4$) (**c,e,f**) or representative of 4 independent experiments (**b,d**). Statistical analysis was performed using two-tailed Student's *t*-test. * $0.01 < P \leq 0.05$, ** $0.005 < P \leq 0.01$.

4.5 Compromised responsiveness of talin1-deficient dermal DCs to various inflammatory stimulations

Even though dDCs are known to play crucial roles in specific ACD models^{72, 74, 76, 173, 209, 210} and mice with talin1-deficient DCs exhibited comparable CHS response as control mice (**Fig. 10a**), we observed that talin-deficient dDCs accumulated in the dermis in steady state (**Fig. 8c,d**) and failed to be recruited to sdLNs even upon DNTB treatment (**Fig. 10f**). Talin1 deficiency in DCs may compromise the responsiveness to weak or low-dose of strong haptens. Hence, we sensitized both control and *Tln1^{ff}CD11c-Cre* mice with titrated doses of the strong hapten DNFB followed by ear challenge 5 days later with DNFB. Comparable ear swelling responses of control and *Tln1^{ff}CD11c-Cre* mice were observed upon sensitization with 0.5% DNFB, 0.125% DNFB and 0.03125% DNFB, with maximal ear swelling observed at day 2 post DNFB ear challenge (**Fig. 11a**). Both control and *Tln1^{ff}CD11c-Cre* mice treated with 0.5% DNFB exhibited robust ear swelling with greatest numbers of IFN- γ -producing CD8 cytotoxic T cells in the sdLNs (**Fig. 11b**). A significant increase in the number of wildtype (CD103⁺ CD11b⁻ and CD103⁻ CD11b⁺) and talin1-deficient (CD103⁻ CD11b⁺) mdDCs can also be detected in the sdLNs of 0.5% DNFB-sensitized mice compared to vehicle-painted littermates (**Fig. 11c,d**). Even though the number of talin1-deficient CD103⁻ CD11b⁺ mDCs was lower compared to wildtype counterparts in the sdLNs, this number of talin1-deficient mDCs is probably sufficient to mount an immunogenic response (ear swelling) (**Fig. 11d**).

Interestingly, significant ear swelling was only detectable in control mice sensitized with the lowest dose of DNFB (0.008% DNFB) but not in *Tln1^{ff}CD11c-Cre* mice (**Fig. 11a**). Ear swelling response of 0.008% DNFB-sensitized control mice corresponded with an increase in the number of IFN- γ -producing CD8 cytotoxic T cells (**Fig. 11b**) and wildtype CD103⁻ CD11b⁺ mdDCs (**Fig. 11c,d**) in the sdLNs. In contrast, the number of IFN- γ -producing CD8 cytotoxic T cells (**Fig. 11b**) and talin1-deficient mDC subsets (**Fig. 11c,d**) in the sdLNs of *Tln1^{ff}CD11c-Cre* mice painted with the lowest dose of DNFB remained comparable to vehicle-painted (unsensitized) control and *Tln1^{ff}CD11c-Cre* mice.

Skin painting of strong haptens such as DNFB induces the enzymatic degradation of hyaluronic acid (component of the ECM), whereby fragments of degraded hyaluronic acid products can activate TLR2 and TLR4 signaling pathways^{212, 213, 214, 215, 216, 217}. Therefore, we intradermally injected LPS into the skin of control and *Tln1^{ff}CD11c-Cre* mice to study the effects of talin1 deficiency in TLR4-mediated DC activation *in vivo*. LPS elicited an influx of wildtype and talin1-deficient mDCs into the sdLNs (**Fig. 11e**). The number of wildtype mdDCs in the sdLNs were significantly higher than that of talin1-deficient cells (**Fig. 11e**). Since *i.d.* LPS injection and DNFB painting create an inflammatory environment as a result of the production of pro-inflammatory cytokines by

keratinocytes, the activation of TLR4 signaling pathways directly or through the by-products of hyaluronic acid degradation^{212, 213, 214, 215, 216, 217}, we speculate that talin1-deficient DCs are less sensitive or responsive to the inflammatory environment (e.g. the abundance of pro-inflammatory cytokines) or LPS in the skin.

Figure 11. Reduced responsiveness of talin1-deficient mdDCs to DNFB or LPS stimulation

(a) Ear swelling response (bottom, mean $\pm$ SEM) of mice sensitized with various doses (0.5%, 0.125%, 0.03125% and 0.008%) of the strong hapten DNFB and ear challenged 5 days later with 0.1% DNFB (top, experimental outline). CHS response was induced as described in Fig. 10a without DNTB treatment. (b) Absolute numbers (scatter plot) of IFN- γ -producing

CD8 T cells in the sdLNs of *Tln1^{ff}* and *Tln1^{ff}CD11c-Cre* mice sensitized with various doses of DNFB. Inguinal LNs were harvested 5 days after first dose of DNFB. Single cell suspensions were generated from LNs and T cells re-stimulated (5 hrs) to detect IFN- γ production. **(c)** Flow cytometric analyses and **(d)** absolute numbers (scatter plot) of CD11c⁺ MHCII⁺ mDCs (individual subsets) in the sdLNs of *Tln1^{ff}* and *Tln1^{ff}CD11c-Cre* mice 2 days after DNFB sensitization. **(e)** Absolute numbers (scatter plot) of CD11c⁺ MHCII⁺ mDCs in the sdLNs of *Tln1^{ff}* and *Tln1^{ff}CD11c-Cre* mice 48 – 60 hrs after *i.d.* LPS injection. Data were pre-gated on singlet, live cells. CD11c⁺ MHCII⁺ mDCs in the sdLNs were further analysed for EpCAM⁺ CD11b⁺ mLCs, CD103⁺ CD11b⁻ mdDCs, CD103⁻ CD11b⁺ mdDCs and CD103⁻ CD11b⁻ mdDCs. Each data point in scatter bar graph indicates the cell number from one experiment **(b,d,e)**. Standard error of the mean (SEM); Contact hypersensitivity (CHS); Intradermal (*i.d.*); migratory DC (mDC); migratory LC (mLC); migratory dermal DC (mdDC); Interferon- γ (IFN- γ). Data shown are representative of 6 **(c)**, summary of 3 (n = 6) **(a)** or 6 (n = 6) **(b-e)** independent experiments. Statistical analysis was performed using two-tailed Student's *t*-test. NS, not significant; *0.01 < *P* ≤ 0.05, **0.005 < *P* ≤ 0.01.

4.6 Talin1 is essential for the activation and maturation of epidermal LCs and dermal DCs

We have demonstrated the importance of talin1 expression in skin DCs using the mouse model of human ACD (**Fig. 10, 11**). Given the fundamental role of DC activation in its effector functions, we decided to examine the significance of talin1 in DC activation. Down-regulation of surface E-cadherin is an indicator of epidermal LC activation^{45, 215}. Meanwhile, up-regulation of MHCII and the co-stimulatory molecule CD86 are also commonly used to study DC (LCs and dDCs) activation²¹⁸. By intradermally injecting LPS or painting the shaved skin of control or *Tln1^{ff}CD11c-Cre* mice with the weak (tolerogenic) hapten DNTB, we observed a down-regulation of E-cadherin on the surface of wildtype LCs, but not on talin1-deficient LCs (**Fig. 12a**). Down-regulation of E-cadherin also took place on the surface of wildtype LCs isolated from 24hr skin explant culture (**Fig. 12a**). In contrast, surface expression of E-cadherin remained unchanged in talin1-deficient LCs under similar experimental conditions (**Fig. 12a**). Total levels of E-cadherin (intracellular and extracellular) were comparable between wildtype and talin1-deficient LCs, indicating that the differences in the down-regulation of E-cadherin is not due to the intrinsic expression of E-cadherin (**Fig. 12a**). Analysis of CD86 and MHCII also revealed a similar trend. Significant up-regulation of CD86 (**Fig. 12b**) and surface MHCII (**Fig. 12c**) can be detected in wildtype LCs under various inflammatory conditions, whereas CD86 (**Fig. 12b**) and MHCII (**Fig. 12c**) remained unchanged or weakly up-regulated in talin1-deficient LCs. In fact, impaired down-regulation of E-cadherin (**Fig. 12a**), in conjunction with the reduced emigration of talin1-deficient LCs out of the dispase-treated epidermal ear sheets (**Fig. 7f,g, 4hr explant culture**) suggest that talin1-deficient LCs could still be in close contact with the surrounding keratinocytes via homotypic E-cadherin interactions and that talin1 regulates the activation of LCs.

We were unable to successfully isolate sufficient numbers of viable dDCs from the dermis of LPS-injected skin due to the overwhelming inflammation (thickening of the dermis) induced by the current dose of LPS. As such, we decided to once again utilize skin explant culture to study the activation of dDCs. Instead of 24 hours (for epidermal LCs), we cultured skin explants for 15 hours to preserve the viability of dDCs. Majority of wildtype and talin1-deficient dDCs have yet to migrate out of the dermis after 15 hours in culture, offering us a window to study the activation status of these cells. While wildtype dDCs steadily up-regulated surface MHCII, talin1-deficient dDCs did not do so efficiently (**Fig. 12d**), suggesting that talin1-deficient dDCs, much like talin1-deficient LCs were not able to become activated under inflammatory conditions.

Figure 12. Talin1 regulates the activation of skin DCs

(a-c) Flow cytometric analyses of LCs isolated from the epidermis of *Tln1^{ff}* and *Tln1^{ff}CD11c-Cre* mice *i.d.* injected with LPS, painted with 1% DNTB or cultured for 24 hrs and stained for (a) E-cadherin, (b) CD86 and (c, top) MHCII. (c, bottom) Summarized MFI of MHCII expression of epidermal LCs as described in (a). (d) Flow cytometric analyses (top) of dDCs isolated from the dermis of 15hr cultured skin explant of *Tln1^{ff}* and *Tln1^{ff}CD11c-Cre* mice. Summarized MFI (bottom) of MHCII expression of dDCs. Data were pre-gated on singlet, live cells. CD45⁺ MHCII⁺ EpCAM⁺ CD11b⁺ LCs (a-c) or CD45⁺ MHCII⁺ EpCAM⁻ CD11b^{+/-} dDCs (d) were further analysed for the expression of indicated molecules. Each data point in scatter bar graph indicates the MFI of cells from one experiment (c,d). Intradermal (*i.d.*); Mean Fluorescence Index (MFI). Data shown are representative of 3 – 5 (a-d), summary of 5 (n = 5) (a) or 3 (n ≥ 3) (b-d) independent experiments. Statistical analysis was performed using two-tailed Student's *t*-test. *0.01 < *P* ≤ 0.05.

4.7 Talin1 is required for optimal cytokine production in epidermal LCs

To investigate the consequences of talin1 deficiency in LC activation, we FACS-sorted wildtype or talin1-deficient epidermal LCs from cultured skin explants to determine the expression level of pro-inflammatory cytokines. Consistently low transcript levels of *Il1b*, *Il6*, *Il23a* and *Tnfa* were detected in talin1-deficient LCs, reinforcing the hypothesis that talin1 deficiency compromises the activation of LCs (**Fig. 13**).

Figure 13. Reduced cytokine production of talin1-deficient LCs

RTq-PCR analyses (represented in fold change) of pro-inflammatory cytokines from FACS-sorted epidermal LCs isolated from cultured skin explants (6, 12 and 24hr) using a gating strategy as described in Fig. 7h. Transcript levels were normalized to *gapdh*. Real Time quantitative-Polymerase Chain Reaction (RTq-PCR). Data shown are summarized from 4 independent experiments (n = 4). Statistical analysis was performed using two-tailed Student's *t*-test. *0.01 < *P* ≤ 0.05, **0.005 < *P* ≤ 0.01.

4.8 Talin1 is required for optimal cytokine production in BMDCs

To study the significance of talin1 in DC activation, we stimulated wildtype and talin1-deficient BMDCs with recombinant IL-1 β and TNF α , and the endotoxin LPS. Reduced mRNA transcript levels of pro-inflammatory cytokines such as *Il1b*, *Il6*, *Il23a* and *Ifnb1* were detected in LPS or IL-1 β -stimulated talin1-deficient BMDCs compared to wildtype BMDCs under similar conditions (**Fig. 14a**). However, comparable mRNA transcript levels of pro-inflammatory cytokines (*Il1b*, *Il6*, *Il23a*, *Tnfa* and *Ifnb1*) in TNF α -stimulated wildtype and talin1-deficient BMDCs, in addition to the similar *Tnfa* transcript profile in LPS, IL-1 β or TNF α -stimulated wildtype and talin1-deficient BMDCs suggest that talin1 does not regulate the TNFR signaling pathway nor the transcription of *Tnfa*.

Next, we moved on to study the production of pro-inflammatory cytokines in response to LPS and/or IL-1 β stimulation in BMDCs. Talin1-deficient BMDCs produced significantly lower amounts of pro-inflammatory cytokines than wildtype BMDCs in the presence of one or both stimuli (**Fig. 14b**), suggesting that talin1 deficiency predominantly affects TLR/IL-1R signaling pathway. In addition, surface expression of MHCII in LPS-stimulated talin1-deficient BMDCs was lower than wildtype cells while the total amount of MHCII molecules (extracellular and intracellular) remained comparable (**Fig. 14c**). These observations in talin1-deficient BMDCs reflect (**Fig. 14**) a similar phenotype as talin1-deficient epidermal LCs (**Fig. 12c**) and dDCs *in vivo* (**Fig. 12d**). Together, these data support earlier observations that talin1 deficiency compromises DC activation. These data also permit the usage of BMDCs as a tool to study the functional role of talin1 in DC activation.

Figure 14. Reduced cytokine production of talin1-deficient BMDCs

(a) RTq-PCR analyses (represented in fold change) and (b) ELISA of pro-inflammatory cytokines produced by stimulated (100 ng/ml LPS, 10 ng/ml IL-1β or 10 ng/ml TNFα (a) or 100 ng/ml LPS and/or 10 ng/ml IL-1β (b)) wildtype and talin1-deficient BMDCs for 6 hrs (a) or 12 and 24 hrs (b). Transcript levels were normalized to *gapdh* (a). (c) Histograms obtained from flow cytometric analyses of LPS-stimulated (100 ng/ml LPS, overnight) wildtype and talin1-deficient BMDCs and further analysed for (c) surface (left) and total (right, extracellular and intracellular) MHCII molecules. Data were pre-gated on singlet, live CD11c⁺ MHCII⁺ BMDCs. BMDCs generated from poly(I)•poly(C)-treated *Tln1^{ff}* and *Tln1^{ff}Mx-Cre* mice as described in Fig. 9a. Wildtype (*Tln1^{ff}*); talin1-deficient (*Tln1^{ΔΔ}*); Real Time quantitative-Polymerase Chain Reaction (RTq-PCR); Bone Marrow-derived Dendritic Cell (BMDC). Data shown are representative and summary of 3 independent experiments (n = 3). Statistical analysis was performed using two-tailed Student's *t*-test. *0.01 < *P* ≤ 0.05, **0.005 < *P* ≤ 0.01.

4.9 Talin1 is required for optimal NF κ B activation in DCs

Since the signal transduction cascade downstream of TLR and IL-1R is highly conserved³, we decided to study the functional role of talin1 in TLR signaling pathway in DCs. We stimulated both wildtype and talin1-deficient BMDCs with LPS to examine the activation of various protein kinases downstream of TLR4. NF κ B activation as indicated by the nuclear translocation of p65 upon LPS stimulation was significantly impeded in talin1-deficient BMDCs (**Fig. 15a**). Moreover, site-specific phosphorylation of p65 at S276 and S536, mediated by ERK and Phosphoinositide 3-Kinase/AKT (PI(3)K/AKT) pathways respectively²¹⁹, were also reduced in talin1-deficient BMDCs (**Fig. 15a**), although less so for the ERK-mediated phosphorylation of p65 at S276. Both phosphorylation events are thought to enhance the transactivation activities of p65, i.e. the recruitment of transcriptional co-regulators²²⁰. These observations indicate a defect in the signal transduction cascade upstream of NF κ B activation.

Indeed, our data revealed a collective reduction in the activities (phosphorylation) of various kinases upstream of NF κ B such as ERK1/2, p38 and AKT, except JNK (**Fig. 15b**). The reduced activities of ERK1/2, p38 and AKT (S473 and T308) correlated with the diminished phosphorylation-dependent degradation of I κ B (S32) in talin1-deficient BMDCs compared to wildtype BMDCs at 15 and 30 mins post LPS stimulation (**Fig. 15b**). Defective I κ B degradation could greatly contribute to the compromised activation of NF κ B in talin1-deficient DCs^{21, 220}. MyD88-dependent pathways are critical for the production of pro-inflammatory cytokines such as IL-1 β , IL-6, IL-23 and TNF α through the activation of NF κ B^{86, 221, 222, 223, 224, 225}. Our data suggest that talin1 may regulate MyD88-dependent pathways in BMDCs.

Furthermore, our data also revealed reduced IRF3 activation (phosphorylation at S396) in LPS-stimulated talin1-deficient BMDCs (**Fig. 15b**), and consequently a reduction of *Irfn1* transcription and IFN β secretion (**Fig. 14a,b**), which is generally regulated through the TRIF (MyD88-independent) pathway^{86, 221, 222, 223, 224, 225}. Collectively, talin1 appears to regulate MyD88- and TRIF-dependent pathways upstream of MAPK and AKT signaling cascades in BMDCs.

Figure 15. Talin1 is required for optimal NFκB activation in DCs

Immunoblot analyses of LPS-stimulated (100 ng/ml LPS) wildtype and talin1-deficient BMDCs generated from poly(I)•poly(C)-treated *Tln1^{ff}* and *Tln1^{ff}Mx-Cre* mice as described in Fig. 9a. **(a)** BMDCs were stimulated with LPS for 0, 30 and 60 min followed by cellular fractionation into cytosol and nuclear fractions. **(b)** BMDCs were stimulated with LPS for 0, 5, 15, 30, 60 min. Wildtype (*Tln1^{ff}*); talin1-deficient (*Tln1^{ΔΔ}*). GAPDH (total lysate or cytosol fraction) and Lamin B (nuclear fraction) as loading control. Data shown are representative of 3 independent experiments (n = 3).

4.10 Talin1 regulates the assembly of TLR4 signalosome in DCs

LPS signaling through TLR4 induces the assembly of large protein complexes – the Myddosome and the Trifosome – known collectively as Supramolecular Organizing Centres (SMOC)^{7, 10}, we postulate here that talin1 may be involved in the assembly of these high-ordered complexes. To address this question, we separated unstimulated and LPS-stimulated wildtype and talin1-deficient BMDCs into cytosol and membrane fractions followed by TLR4 immunoprecipitation. Components of the MyD88-dependent pathway such as TIRAP, MyD88 and IRAK4 and molecules unique to the TRIF-dependent pathway TRIF, TRAM and TRAF3 were sequentially recruited to TLR4 (both membrane-bound and cytosolic TLR4) within 30 min of TLR4-LPS ligation in wildtype BMDCs (**Fig. 16a**). Interestingly, we observed the formation of a pre-formed complex consisting of talin1 with TLR4/TIRAP/MyD88 in unstimulated wildtype BMDC (**Fig. 16a**). The pre-formed complex was not observed in Bone Marrow-derived Macrophages (BMDMs)²²⁶. Even though the assembly of TLR4 signalosome (IRAK4 from Myddosome and components of Trifosome) coincided with the dissociation of talin1 from TLR4 in wildtype BMDCs, the aforementioned complex was not detectable in the absence of talin1 (**Fig. 16a**), suggesting that the pre-formed talin1/TLR4 complex is required for the assembly of high-ordered complexes upon TLR4-LPS ligation.

Moreover, preassembled TLR4-associated regulatory proteins such as p85²²⁷, the regulatory subunit of PI(3)K complex, Vav1-Guanine Nucleotide Exchange Factor²²⁸ (Vav1-GEF), CD11b¹⁸⁷ (integrin α M) and CD29 (integrin β 1) were detected in wildtype BMDCs (**Fig. 16a**). These proteins were largely absent or greatly reduced in talin1-deficient cells (**Fig. 16a**). Similar to talin1, these regulatory proteins also dissociated from TLR4 upon LPS ligation (**Fig. 16a**). Together, these results suggest that talin1 is required for the steady state recruitment of p85, Vav1, CD11b and CD29 to TLR4 in unstimulated BMDCs, and the subsequent assembly of the Myddosome and Trifosome components upon TLR4-LPS ligation.

To further examine the dissociation of talin1 from TLR4 upon TLR4-LPS engagement, we introduced an earlier time-point of 10 mins post LPS stimulation of wildtype BMDCs. Vav1 dissociated completely from TLR4 while partial dissociation of talin1, integrins CD11b and CD29 and p85 were observed 10 mins post LPS stimulation, during which the core components of MyD88- and TRIF-dependent pathways were recruited to the TLR4/MyD88 complex (**Fig. 16b**). Further dissociation of talin1, integrins and p85 occurred 30 mins post LPS stimulation (**Fig. 16b**). These observations suggest that the preassembled complex of talin1, integrins, p85, Vav1, MyD88, TIRAP and TLR4 is a pre-requisite for the assembly of the TLR4 signalosome. The dissociation of the aforementioned regulatory proteins from TLR4 upon TLR4-LPS ligation permits the concomitant recruitment of TLR4 signaling proteins.

Endocytosis of TLR4 is a pre-requisite for the transition from MyD88-dependent pathway to the TRIF-signaling pathway in BMDCs¹⁸⁷, thus we decided to examine the down-regulation of TLR4 by flow cytometry. Sequential internalization of surface TLR4 can be observed in LPS-stimulated wildtype BMDCs while the down-regulation of TLR4 in talin1-deficient BMDCs was only marginal (**Fig. 16c**). Reduced endocytosis of surface TLR4 could affect the assembly of the core components of the TRIF-dependent pathway in talin1-deficient BMDCs (**Fig. 16a**). The differences in TLR4 internalization is not due to the differential expression of TLR4 between wildtype and talin1-deficient BMDCs (**Fig. 16d**). Together, our data suggest that talin1 also regulates the endocytosis of TLR4 in BMDC.

Figure 16. Talin1 regulates the assembly of the TLR4 signalosome in DCs

(a) Immunoprecipitation (IP) of TLR4 from the membrane (left) and cytosol (right) fractions of unstimulated and LPS-stimulated (100 ng/ml LPS, 30 min) wildtype and talin1-deficient BMDCs, followed by immunoblot analysis of the indicated molecules. 1/10 input or 10% of the cell lysate used for IP. (b) IP of TLR4 from unstimulated and LPS-stimulated (100 ng/ml LPS, 10 and 30 min) wildtype BMDCs, followed by immunoblot analysis of the indicated molecules. Black arrows indicate Talin1 protein. IgG IP as control. 1/10 input or 10% of the cell lysate used for IP. (c,d) Histograms as determined by flow cytometry show the expression levels of (c) surface and (d) total (intracellular and extracellular) TLR4 in unstimulated and LPS-stimulated wildtype and talin1-deficient CD11c⁺ MHCII⁺ BMDCs (100 ng/ml LPS, 30 and 90 min). Unstimulated wildtype and talin1-deficient BMDCs were used as isotype controls. Data were pre-gated on singlet, live cells. CD11c⁺ MHCII⁺ BMDCs were

further analysed for the expression of TLR4. Histograms are representative of 3 independent experiments. Wildtype (*Tln1^{fl/fl}*); talin1-deficient (*Tln1^{Δ/Δ}*). GAPDH (total lysate or cytosol fraction) and Na/K ATPase (membrane fraction) as loading control. **(a,b)**. BMDCs were generated as described in Fig. 9a. Data shown are representative of 3 independent experiments (n = 3).

4.11 Integrin-dependent TLR4 signalosome assembly

Since membrane-localized talin1 is usually in an active configuration and binds to integrin heterodimers while cytosolic talin1 is predominantly in the auto-inhibited state¹³², talin1 associated with membrane proximal TLR4/MyD88 complex is likely to be functionally active. Leu325 (L325) and Tryp359 (W359) (**Fig. 4**), located within the F3 region (**Fig. 3**) of the talin head domain regulate the integrin-activating and integrin-binding properties of talin respectively^{118, 146, 191, 192, 229, 230}. W359A mutation abolishes the interaction between talin and the Membrane Proximal (MP) NPxY/F motif of the β integrin tail^{118, 192, 230}. The anchorage of talin to the MP NPxY/F motif is crucial for talin-integrin binding^{118, 191, 230}. L325R mutation on the other hand, does not affect the binding of talin1 to the MP NPxY/F motif but drastically impairs the association of talin1 to the Membrane Proximal Region (MPR) of the β integrin tail^{96, 117, 118}, the final step in promoting the activation of the integrin heterodimer. The sequential engagement of talin1 to both high-affinities MP NPxY/F motif and MPR of the same β integrin tail is critical for talin1-mediated integrin activation^{118, 191} (**Fig. 4**). To investigate the significance of the integrin-binding and activating properties of talin1 in TLR4 signaling, we expressed talin1-W359A (integrin-binding mutant) or talin1-L325R (integrin-activating mutant) in talin1-deficient BMDCs via retroviral transduction, followed by TLR4 immunoprecipitation.

First, we validated the importance of both residues (L325 and W359) by pulling down talin1 from BMDCs expressing wildtype talin1, talin1-L325R or talin1-W359A. As expected, the integrin-binding property of talin1-W359A was severely abolished as indicated by the lack of co-immunoprecipitated integrins (CD11b and CD29) (**Fig. 17a**), whereas the integrin-binding property of talin1-L325R was retained (**Fig. 17a**). Talin1-L325R-bound integrins however, were not activated (**Fig. 17b**) because the L325R mutation abolishes talin1-integrin interaction at the MPR of the integrin subunit¹⁹². The binding of FAK to talin1-W359A and talin1-L325R was not impeded by both mutations (**Fig. 17a**). Assembly of the TLR4-associated regulatory molecules (CD11b, CD29 and talin1) and the signalosome (MyD88, IRAK4 and TRIF) before and after stimulation were only detected in BMDCs expressing wildtype talin1 (**Fig. 17c**), suggesting that both integrin-binding (W359) and activating (L325) properties of talin1 are critical for TLR4 signaling in DCs.

We also performed reciprocal immunoprecipitation of talin1, CD11b and CD29 from wildtype and talin1-deficient BMDCs to examine the association of TLR4 signalosome with talin1 or integrins. Neither TLR4 nor its downstream signaling molecules (e.g. MyD88, IRAK4 and TRIF) were associated with CD11b or CD29 in talin1-deficient BMDCs (**Fig. 17d**). In contrast, TLR4, MyD88 and talin1 co-immunoprecipitated with CD11b or CD29 in unstimulated wildtype BMDCs (**Fig. 17d**), suggesting that talin1-

bound active integrins are in the proximity of TLR4 in BMDCs. Indeed, we observed that the amount of TLR4, MyD88 and talin1 associated with active integrins (pulled down using 9EG7 antibody against active integrin β 1/CD29) is similar to the immunoprecipitation profile of total CD29 (**Fig. 17d, CD29 IP**), even though the amount of active CD29 enriched in 9EG7 pull-down was much reduced (**Fig. 17d, 9EG7 IP**), suggesting that most of the TLR4-associated integrins are in the active configuration. A significant portion of TLR4 dissociated from talin1, CD11b or CD29 upon TLR4-LPS ligation, which is in agreement with our earlier observations that talin1 dissociates from TLR4 upon LPS stimulation in wildtype BMDCs (**Fig. 16a,b**). Collectively, our data suggest that talin1 and integrins activated by talin1 are required for the pre-assembly of TLR4 with MyD88 and the recruitment of downstream signaling molecules upon TLR4-LPS ligation. Talin1 is likely to function as a scaffold protein that activates integrins and helps to recruit TLR4 downstream signaling molecules to the pre-assembled TLR4/MyD88 complex upon LPS stimulation. The dissociation of talin1 and active integrins from the progressively assembling TLR4 signalosome could be a mechanism to negatively regulate the signaling pathway.

Figure 17. Integrins activated by talin1 promote TLR4 signalosome assembly

(a) Immunoprecipitation (IP) of talin1 from total cell lysate of talin1-deficient BMDCs (generated as described in Fig. 9a) transduced with control retrovirus (EV) or viruses to express wildtype talin1 (WT), talin1-W359A or talin1-L325R (details see materials and methods), followed by immunoblot analysis of the indicated molecules. 1/10 input or 10% of the cell lysate used for IP. (b) Histograms as determined by flow cytometry show the activation of CD29 (determined by 9EG7 antibody against activated integrin $\beta 1$ /CD29) of talin1-deficient BMDCs (EV) and talin1-deficient BMDCs reconstituted with wildtype talin1 (WT), talin1-W359A or talin1-L325R. Talin1-deficient BMDCs reconstituted with wildtype talin1 (WT) was used as isotype control. Data were pre-gated on singlet, live cells. CD11c⁺ MHCII⁺ BMDCs were further analysed for the expression of CD29 (integrin $\beta 1$) and activated CD29 (as indicated by 9EG7). (c) IP of TLR4 from the membrane (left) and cytosol (right) fractions of wildtype and LPS-stimulated (100 ng/ml LPS, 30 min) talin1-deficient BMDCs (EV) or talin1-deficient BMDCs reconstituted with wildtype talin1 (WT), talin1-W359A or talin1-L325R, followed by immunoblot analysis of the indicated molecules. 1/10 input or 10% of the cell lysate used for IP. (d) IP of talin1, CD11b, CD29 and activated CD29 (9EG7) from total cell lysate of unstimulated and LPS-stimulated (100 ng/ml LPS, 30

min) wildtype and talin1-deficient BMDCs, followed by immunoblot analysis of the indicated molecules. 1/10 input or 10% of the cell lysate used for IP. GAPDH (total lysate or cytosol fraction) and Na/K ATPase (membrane fraction) as loading control. Empty Vector (EV); Wildtype (WT). Data shown are representative of 3 independent experiments (n = 3).

4.12 Talin1 colocalizes with MyD88 in BMDCs

While talin and integrins are largely associated with focal adhesion complexes and cell migration^{93, 96, 100, 103, 106, 117, 119, 120, 121, 123, 146, 162, 197, 231, 232, 233, 234, 235, 236}, TLR4 is predominantly involved in LPS-induced inflammatory responses^{1, 2, 7, 8, 9, 10, 25}. These talin1-associated integrin-dependent pathways and the TLR4 signal transduction cascade are seemingly unrelated to each other, thus we are keen to seek more evidence supporting the link between talin1 and the TLR4 signaling pathway.

TLR4 is a transmembrane protein with a prominent leucine-rich extracellular domain connected by a transmembrane segment to a short (relative to the extracellular domain) intracellular TIR domain²³⁷. TIR domain interactions are predominantly homotypic (e.g. MyD88/MyD88 interaction) or heterotypic²³⁸ (e.g. MyD88/TIRAP interaction), thereby making the short intracellular TIR domain of TLR4 an unattractive and unlikely interacting target of talin1. Besides, talin1 does not have a defined TIR domain. Instead of TLR4, we turned our attention to TIR domain-containing adaptors (e.g. MyD88), which could be recruited to TLR4.

With these considerations in mind, we proceeded to visualize the localizations of talin1 and MyD88 in wildtype BMDCs. Indeed, talin1 colocalized with MyD88 in wildtype BMDCs (**Fig. 18a,b L1**), which is in agreement with the immunoprecipitation data depicting the interaction of talin1-TLR4-MyD88 (**Fig. 16a,b**). Colocalization between talin1 and MyD88 was enriched in select regions (**Fig. 18b, L3**) of the cell with an average correlation coefficient (r) of 0.8852, while barely detectable in other parts of the same cell (**Fig. 18b, L2**, $r \approx 0.03$). Talin1-deficient BMDCs on the other hand, failed to spread under similar conditions (**Fig. 18a, *Tln1*^{Δ/Δ} BMDC and 9c**). Besides, the congregation of talin1 and MyD88 around non-fluorescent beads (**Fig. 18d,e**) in wildtype BMDC also aligned with earlier immunoprecipitation (**Fig. 16a,b**) and confocal imaging data (**Fig. 18a-c**).

Figure 18. Talin1 colocalizes with MyD88 in BMDCs

(a) Confocal microscopy of unstimulated wildtype and talin1-deficient BMDCs (generated as described in Fig. 9a) stained with MyD88 (red), talin1 (green) and DAPI (blue). Scale bar, 10 μm. Colocalization analyses of wildtype BMDCs represented using (b) fluorescence intensity profiles and expressed in (c) Pearson's correlation coefficient (r, scatter plot) of select regions indicated as L1 (r ≈ 0.4209; total length of a cell), L2 (r ≈ 0.03; region without colocalization) and L3 (r ≈ 0.8852; region enriched with MyD88 and talin1 colocalization). Each data point in scatter bar graph indicates the correlation coefficient, r, of each cell. (d) Confocal microscopy and (e) colocalization analysis of wildtype BMDCs in the presence of non-fluorescent beads (indicated by bars), stained with MyD88 (red), talin1 (green) and DAPI (blue). Scale bar, 8 μm. Colocalization analyses performed using Plot Profile plugin (Fiji) and represented in fluorescence intensity profiles (Arbitrary Units, AU) show the region of the line drawn in overlaid (merged) images (b,e). At least 16 images of BMDCs were acquired for each genotype (a,d). Wildtype (*Tln1*^{+/+}); talin1-deficient (*Tln1*^{Δ/Δ}). Data shown are representative and summary of 3 independent experiments (n = 3).

4.13 Talin1 interacts with MyD88

Since talin1 colocalized with MyD88 in wildtype BMDCs (**Fig. 18**), we decided to further determine if talin1 interacts directly with MyD88. Meanwhile, we also investigated the relationship between talin1 and Vav1 because Vav1 is an important regulator of the TLR4 signaling cascade²²⁸ and we have reported earlier that Vav1 is essential for Ezh2-mediated talin1 tri-methylation at Lys2454, which is involved in adhesion turnover^{124, 239}. To investigate if talin1 interacts with Vav1 or MyD88 directly, we synthesized recombinant talin1, Vav1 and MyD88 using cell-free protein expression system followed by reciprocal immunoprecipitation of the protein of interest. We did not detect a direct interaction between talin1 and Vav1 (**Fig. 19a**). The interaction between talin1 and Vav1^{124, 239, 240} is likely to be mediated by one or several unidentified intermediate protein(s). However, reciprocal immunoprecipitation of recombinant talin1 and MyD88 revealed a direct interaction between these two proteins (**Fig. 19b**). To identify the domains responsible for talin1-MyD88 interaction, we generated three MyD88 fragments: TIR-domain, intermediate domain and the Death domain (DD), as well as two talin1 fragments – head and the rod domain. Full-length MyD88 or talin1, together with their respective truncated fragments were transiently overexpressed in HEK-293T cells followed by immunoprecipitation. To avoid pulling down endogenous talin (HEK-293T cells express both talin1 and talin2 isoforms) and MyD88, we fused GFP and Flag-tag to the N-terminus of talin1 (GFP-talin1) and the N-terminus of MyD88 (Flag-MyD88) respectively. Reciprocal immunoprecipitation revealed an interaction between the rod domain of talin1 with the intermediate domain of MyD88 (**Fig. 19c,d**). It is likely that the interaction between talin1 and MyD88 bridges integrins to TLR4 while promoting the assembly of downstream signaling molecules of the MyD88-dependent pathway.

Figure 19. Direct interaction between talin1 and MyD88

Immunoprecipitation (IP) of recombinant (a) talin1 and Vav1 or (b) talin1 and MyD88 using the indicated antibodies. Talin1, Vav1 and MyD88 proteins were synthesized using cell-free IVT systems. Black arrow indicates talin1 (a). (c,d) IP of full-length or truncated (c) MyD88 (top) or (d) talin1 (top) fragments overexpressed in HEK-293T cells. Schematic representation and length (in amino acids) of full-length and truncated (c) MyD88 (bottom) or (d) talin1 (bottom) fragments. Immunoprecipitated proteins were analysed by immunoblot with the indicated antibodies. 1/10 input or 10% (a,b,d) or 1/20 input or 5% (c) of the cell lysate used for IP. *In Vitro* Transcription/Translation (IVT); Empty Vector (EV); Full-Length (FL); Death Domain (DD); Intermediate domain (Int); Toll/Interleukin-1 Receptor domain (TIR); Green Fluorescent Protein (GFP). Data shown are representative of 3 independent experiments (n = 3).

4.14 Talin1 is not required for MyD88-independent TLR3 signaling pathway

In TLR4 signaling cascade, the MyD88-dependent pathway is a prerequisite for TRIF-dependent events^{2, 3, 9, 44}. Even though our data showed that the recruitment of molecules unique to the TRIF-dependent pathway were also reduced in the absence of talin1 (**Fig. 16a and 17c,d**), it is not clear if talin1 regulates TRIF-dependent pathway per se. We therefore examined the role of talin1 in TLR3 signaling pathway, which is dependent on TRIF exclusively³. Immunoprecipitation of cytosolic TLR3 from poly(I:C)-stimulated wildtype and talin1-deficient BMDCs revealed that the assembly of TLR3 signalosome is not dependent on talin1 (**Fig. 20**). These data suggest that talin1 is dispensable in TLR3 signaling.

Figure 20. Assembly of TLR3 signalosome is not dependent on talin1

Immunoprecipitation (IP) of TLR3 from unstimulated and poly(I:C)-stimulated (10 µg/ml poly(I:C), 45 min) wildtype (*Tln1^{+/+}*) and talin1-deficient (*Tln1^{Δ/Δ}*) BMDCs (generated as described in Fig. 9a), followed by immunoblot analysis of the indicated molecules. 1/10 input or 10% of the cell lysate used for IP. Data shown are representative of 3 independent experiments (n = 3).

4.15 Talin1 regulates MyD88-dependent pathway downstream of TLRs

To determine if talin1 regulates the MyD88-dependent pathway downstream of various TLRs, we stimulated wildtype and talin1-deficient BMDCs with different TLR agonists such as Pam3CSK4 (TLR1/2), MALP-2 (TLR2/6), flagellin (TLR5) and CpG (TLR9). Reduced activation (phosphorylation) of AKT (S473 and T308) and diminished phosphorylation-dependent degradation of I κ B (S32) can be observed in talin1-deficient BMDCs upon exposure to Pam3CSK4 (**Fig. 21a**), MALP-2 (**Fig. 21b**), flagellin (**Fig. 21c**) and CpG (**Fig. 21d**). These data suggest that talin1 regulates the MyD88-dependent pathway in general and not restricted to specific types of ligand-receptor engagement.

Figure 21. Talin1 regulates MyD88-dependent pathway downstream of TLRs

Immunoblot analyses of TLR agonist-stimulated wildtype and talin1-deficient BMDCs generated from poly(I)•poly(C)-treated *Tln1^{ff}* and *Tln1^{ff}Mx-Cre* mice as described in Fig. 9a. (**a-d**) BMDCs were stimulated with (**a**) Pam3CSK4, (**b**) MALP-2, (**c**) flagellin and (**d**) CpG (transfected with lipofectamine 2000) for 0, 5, 15, 30, 60 min. Wildtype (*Tln1^{ff}*); talin1-deficient (*Tln1^{Δ/Δ}*). GAPDH as loading control. Data shown are representative of 2 independent experiments.

5. Discussion

5.1 Talin1 is essential for integrin-dependent LC migration across the basement membrane

In our report, we observed an accumulation of talin1-deficient LCs in the epidermis in the steady state and under inflammatory conditions. Enzymatic disruption of the basement membrane by dispase facilitated the exit of both wildtype and talin1-deficient LCs, supporting the role of integrins in the transmigration of LCs across the basement membrane. If the transmigration of LCs across the basement membrane is solely dependent on integrins, we would not expect to detect any talin1-deficient LCs in the sdLNs of *Tln1^{fl/fl}CD11c-Cre* mice. This is not the case because a small number of talin1-deficient LCs were still present in the sdLNs. We reasoned that LCs preferentially utilize integrin-dependent migration to cross the basement membrane. However, LCs deprived of talin1 may utilize compensatory mechanisms to transverse the basement membrane.

During the process of transmigration, epidermal LCs express matrix metalloproteinases (MMPs) to support proximal degradation of ECM proteins without disrupting the integrity of the basement membrane²⁰⁰. Indeed, we found enhanced expression of *Mmp2* and *Mmp9* in talin1-deficient LCs. The compensatory up-regulation of MMPs could in theory, create fissures in the basement membrane large enough for LCs to squeeze through in an integrin-independent manner, hence a small number of talin1-deficient mLCs is present in the dermis and in the sdLNs in the steady state. Even though increased transcript levels of *Mmp2* and *Mmp9* can be detected in talin1-deficient LCs, functional experiments have to be performed in the future to examine the protein level and activities of LC-derived MMP2 and MMP9. Synthetic or natural inhibitors of MMPs such as Tissue Inhibitor of Metalloproteinase (TIMPs) can be administered subcutaneously to study the effects of MMP inhibition in the migration of talin1-deficient LCs.

LCs could also compensate for the loss of talin1 by up-regulating isoform protein talin2 (*Tln2*) or coactivator kindlin3 (*Fermt3*). Transcript levels of *Fermt3* remained unchanged while the expression of *Tln2* was not detectable in control and talin1-deficient LCs, suggesting that neither kindlin3 nor talin2 compensated for the loss of talin1 in LCs. The failure of kindlin3 to compensate for talin1 deficiency could be due to the differences in the interaction between kindlin3 (MD NxxY/F motif) and talin1 (MPR and MP NPxY/F motif) with β integrin tail^{118, 146}. Talin2 on the other hand, is largely expressed in non-hematopoietic lineage cells such as muscle cells, epithelial cells and fibroblasts^{241, 242, 243} while hematopoietic lineage cells only express talin1¹⁰¹.

In addition, Nagao and colleagues recently characterized a unique migration mechanism adopted by infiltrating inflammatory monocytes into the epidermis via hair

follicles, under inflammatory conditions²⁴⁴. These monocytes will differentiate into short-term LCs with a life span of 2 to 3 weeks^{45, 77, 215, 245, 246}, which are different from the fetal origin, self-renewing epidermal LCs^{47, 81}. The ingress of inflammatory monocytes via hair follicles however raises the possibility that a small portion of epidermal LCs may be able to migrate into the dermis using the same route but in the opposing direction. Nevertheless, our data support that the talin1-mediated, integrin-dependent migration of LCs across the basement membrane as the dominant strategy adopted by transmigrating wildtype epidermal LCs. More work is required to examine if the elevation of MMPs activity or the unconventional migration route contributes to the low number of talin1-deficient mLCs found in the sdLNs.

5.2 Talin1 regulates DC activation and motility upon LPS-stimulation

Exposure to external stimuli like pro-inflammatory cytokines and endotoxins trigger DC activation^{77, 78}. Activated DCs exhibit enhanced cell motility, in stark contrast to immature DCs which are less mobile but have greater propensity for antigen uptake^{77, 78, 84, 85}. Since talin1-deprived skin DCs and BMDCs were less activated as reflected by the reduced production of pro-inflammatory cytokines and lowered MHCII expression levels, we speculate that talin1 critically regulates DC activation. Indeed, we are able to show that talin1 controlled the assembly of the TLR4 signalosome upon TLR4-LPS engagement. The absence of talin1 compromised LPS-mediated DC activation that is also likely to negatively impact DC effector functions such as migration and MHCII up-regulation for antigen presentation. Besides, talin1 deficiency could also compromise the generation of cell polarity^{163, 231, 247} as revealed by reduced directional persistency of talin1-deficient BMDCs. Taken together, the loss of talin1 affects TLR-mediated DC activation and its downstream effector functions.

Intriguingly, up-regulation of the chemokine receptor CCR7 was not impaired by talin1 deficiency in activated LCs and BMDCs. CCR7 is transcriptionally regulated by transcription factors (TFs) Forkhead box protein O1 (FOXO1)²⁴⁸, AP-1 and NFκB family members^{249, 250}, all of which can be activated via TLR-dependent pathways. Unlike FOXO1, transcriptional regulation of CCR7 by AP-1 and NFκB family members have yet to be demonstrated in DCs, although the promoter region of CCR7 does contain binding sites for these TFs^{249, 250, 251}. Activation of the TLR4 signaling cascade as indicated by the assembly of the TLR4 signalosome, the activation of downstream kinases and nuclear translocation of p65 could still be detected in LPS-stimulated talin1-deficient BMDCs, albeit at much reduced levels compared to LPS-stimulated wildtype BMDCs, suggesting that minimal TLR4 signal transduction can still occur in the absence of talin1 in BMDCs. The reduced signaling capacity is likely to be sufficient to promote the up-regulation of CCR7 in talin1-deficient LCs and BMDCs, but not strong enough to promote additional effector functions such as the production of pro-

inflammatory cytokines, MHCII up-regulation and cell migration. Besides, IFN- β secreted by LPS-stimulated BMDCs can also negatively regulate the gene transcription of *Ccr7* in an autocrine fashion²⁵². Since LPS-stimulated talin1-deficient BMDCs produced reduced amounts of IFN- β than their wildtype counterparts, we reasoned that the inhibitory effect of IFN- β will be less pronounced in talin1-deficient BMDCs compared to wildtype BMDCs. In other words, it is also likely that the reduced TLR4 signaling strength in talin1-deficient DCs resulted in decreased *Ccr7* transcription but coupled to the diminished IFN β -mediated inhibition of *Ccr7* (due to lowered secretion of IFN β), the overall CCR7 expression levels in both LPS-stimulated control and talin1-deficient DCs remained comparable.

5.3 Differential regulation of TLR signaling in DCs and macrophages

TLR signal transduction cascades have been extensively studied in macrophages due to the relative ease of generating Bone Marrow-derived Macrophages (BMDMs) *in vitro*. The recruitment of MyD88 to TLR4²²⁶ upon TLR4-LPS ligation is thought to be conserved across immune cell types^{2, 3, 9}. However, here we observed a constitutive association of MyD88 with TLR4 in unstimulated BMDCs. Talin1 and its ability to bind/activate integrins was required for this interaction between MyD88 and TLR4. In the absence of talin1, not only did the pre-assembly of TLR4/MyD88 complex not occur efficiently, the subsequent formation of the Myddosome and Trifosome also did not take place upon LPS stimulation. Moreover, we demonstrated that talin1 interacted directly with MyD88 through the rod and intermediate domains of talin1 and MyD88 respectively. As sentinels of the immune system, DCs are usually the first line of defence against foreign pathogens, as such the preformed TLR4/MyD88 complex could enable rapid DC activation to initiate host defence mechanisms. In contrast, macrophages are primarily involved in the clearance of pathogens and apoptotic cells^{253, 254}. The preassembled TLR4/MyD88 complex may not be necessary for macrophages.

Differential regulation of the TLR signaling pathway between DCs and macrophages have also been reported by several groups. CD11b (integrin α M) is required for the endocytosis of TLR4 in DCs to initiate the cytosolic TRIF-dependent pathway, but is dispensable in macrophages¹⁸⁷. The same integrin subunit however, negatively regulates TLR4 signaling by inducing the activation of Syk and promoting Cbl-b-dependent degradation of MyD88 and TRIF in macrophages³⁴. Diminished assembly of the preformed TLR4/MyD88 complex including CD11b, in conjunction with reduced TLR4 internalization in talin1-deficient BMDCs suggest that talin1-bound CD11b is required for TLR4 endocytosis and the transition from MyD88- to TRIF-dependent pathway in DCs.

Besides DCs and macrophage-specific TLR regulations, mast cell-specific regulation of TLR signaling were also documented²⁵⁵. For example, the TRIF-dependent pathway does not occur in Bone Marrow-derived Mast Cells (BMMCs) because of the low expression level of the adaptor TRAM²⁵⁵. Taken together, current evidence indicates differential regulation of TLR signaling in various immune cell types.

5.4 The functional role of talin1 in TLR4 signaling cascades

5.4.1 Talin1 specifically regulates MyD88-dependent TLR4 pathways

The signal transduction cascade downstream of TLR4 is dependent on adaptors MyD88 and TRIF while TLR3 signals through TRIF exclusively^{2, 3, 7, 8, 9, 25}. Our report demonstrated a functional significance of talin1 in TLR4 signaling cascade, whereby talin1 deficiency impaired the assembly of both Myddosome and Trifosome. Since the assembly of TLR3 signaling components were unaffected by talin1 deficiency in poly(I:C)-stimulated BMDCs, the destructive effect on the endosomal TRIF pathway in LPS-stimulated talin1-deficient BMDC, downstream of the MyD88-dependent pathway in TLR4 signaling cascade is likely to be a secondary spill-over effect from the impaired recruitment of MyD88 and its associated signal molecules upon LPS stimulation. In addition, stimulation of BMDCs through other MyD88-dependent TLRs also showed diminished AKT activation and phosphorylation-dependent degradation of I κ B, suggesting that talin1 regulates MyD88-dependent inflammatory responses in DCs.

5.4.2 Talin1 promotes the functional role of MyD88

Here, we reported the novel interaction between the rod domain of talin1 and intermediate domain of MyD88. MyD88 consists of three domains: the death domain (DD), the TIR domain and the lesser-studied intermediate domain. The functional roles of the DD^{10, 256} and TIR domain^{3, 257, 258} have been extensively studied over the years. The short unstructured intermediate domain^{259, 260, 261, 262} however, remained comparatively elusive although it is thought to be a positive regulator of the TLR signaling pathway^{259, 260, 261, 262}. MyD88s, an isoform lacking the intermediate domain, does not interact with IRAK4, but its interaction with IRAK1 is unperturbed²⁶². As a consequence, IRAK4 activation by means of autophosphorylation²⁶³ is inhibited, thus impairing the activation of downstream kinase IRAK1 and the TLR signaling cascade^{259, 260, 261, 262}. Besides, transfection of synthetic peptides encoding the intermediate domain of MyD88 into RAW264.7 (mouse macrophage cell line) cells inhibited downstream signaling responses of TLR2, 4, 5 and 9²⁵⁹. These peptides selectively bound to IRAK4²⁵⁹. Taken together, these experiments suggest that the short intermediate domain of MyD88, on top of the DD, is likely to be essential for the interaction and activation of IRAK4²⁶². We propose that talin1 promotes TLR signaling by recruiting MyD88 to TLR4 while concurrently priming the receptor to initiate rapid

signal transduction upon TLR4-LPS ligation. The engagement between the rod domain of talin1 to the intermediate domain of MyD88 is likely to prevent the intermediate domain-mediated activation (autophosphorylation) of IRAK4²⁶² and the indiscriminate activation of TLR4 signaling pathway in the absence of LPS.

5.4.3 The significance of talin1 in the pre-assembled TLR4 complex in DCs

Our results showed that talin1-mediated integrin activation is critical for TLR4 signaling because the assembly of the TLR4 signalosome (indicated by MyD88, IRAK4 and TRIF) was disrupted in BMDCs reconstituted with talin1-W359A (integrin-binding mutant) or talin1-L325R (integrin-activating mutant). Talin1-bound integrins associated with TLR4 are likely to be in the high-affinity, active conformation^{93, 96, 132, 192}. These integrins are not as expected to be enriched at focal adhesions^{92, 93, 96, 124, 152, 158, 159}, but in the proximity of TLR4. Pulling down activated CD29, together with the core components of the TLR4 signalosome from LPS-stimulated wildtype BMDCs further reinforced the importance of activated integrins in TLR4 signaling pathway.

Interestingly, epitope mapping revealed two LPS-binding sites within the ligand-binding domains of integrin $\beta 2$ (CD18)²⁶⁴. Since ligand-binding domains across β integrin subunits are structurally and functionally conserved²⁶⁵, we predict that most if not all β integrin subunits can bind to LPS. LPS binding could be enhanced in activated integrins (open, high-affinity ligand-binding conformation) as a result of reduced steric hindrance compared to inactive integrins. These integrins adjacent to TLR4 could work cooperatively with LPS-Binding Protein (LBP) and CD14 to recruit LPS to TLR4 so as to activate the TLR4 signaling pathway.

CD11b is essential for the endocytosis of TLR4 in order to initiate TRIF-dependent signaling in DCs¹⁸⁷. We were able to co-immunoprecipitate CD11b with TLR4 and talin1 in unstimulated wildtype BMDCs. Since talin1 is not required for TLR3 signaling cascade, it is not likely to directly regulate the endosomal TRIF pathway upon TLR4 activation but rather controls the assembly of MyD88-dependent signaling complex preceding the internalization of TLR4. Besides, the loss of CD11b could be functionally compensated for by alternative α integrin subunits such as CD49d (integrin $\alpha 4$), CD103 (integrin αE), CD49f (integrin $\alpha 6$), CD11c (integrin αX) or CD11a (integrin αL), which dimerizes with CD18 (integrin $\beta 2$), CD29 (integrin $\beta 1$) or ITGB7 (integrin $\beta 7$)¹⁴⁰. Here we showed that both CD11b and CD29 co-immunoprecipitated with TLR4 in wildtype BMDCs, but not with each other. Both CD11b and CD29 integrin subunits dissociated from TLR4 upon LPS stimulation in a similar fashion, while the recruitment of the TLR4 signalosome was initiated. Taken together, our data implied that talin1-bound active integrins associated with TLR4 is critical for the assembly of the TLR4 signalosome.

5.4.4 Collaborative signaling between CD11b and TLR4

Collaborative signaling between integrins and TLR4 have been observed in various immune cells^{34, 187, 188, 189} but the mechanism has not been described in DCs. CD11b regulates the TLR4 signaling cascade and promotes the internalization of TLR4 in DCs but not in macrophages¹⁸⁷. On the other hand, Cao and colleagues showed that CD11b promotes the degradation of MyD88 in a Syk-dependent manner upon TLR4-LPS engagement in macrophages³⁴. We did not observe significant changes in the expression level of MyD88 in DCs upon TLR4-LPS ligation, suggesting that the aforementioned mechanism³⁴ is cell-type specific and not likely to be conserved in DCs. Alternatively, the reported functions of CD11b in macrophages³⁴ may not be associated with talin1. Here, we demonstrate that the direct interaction between talin1 and MyD88 supported the assembly of the TLR4 signalosome and this interaction is likely to be conserved in most if not all MyD88-dependent signaling cascades downstream of TLRs with the exception of TLR3. In addition, our data also revealed that integrins activated by talin1 proximal of TLR4 is critical to the signaling process whereby talin1 functions as a bridge between both integrin and TLR4 signaling pathways in DCs.

5.4.5 Talin1 recruits TLR4-associated regulatory molecules

Basal recruitment of TLR4-associated regulatory molecules such as p85 and Vav1 were significantly reduced in unstimulated talin1-deficient BMDC. The recruitment of PI(3)K (p85/p110 complex) to TLR4 is likely to be dependent on the talin1-MyD88 interaction. Since the regulatory subunit p85 of the PI(3)K complex associates with the TIR domain of MyD88²²⁷, it is likely that the recruitment of p85/MyD88 complex to TLR4 is enhanced by talin1 in wildtype BMDCs. P85 dimerizes with the catalytic subunit p110 to form a functional Class I PI(3)K complex^{266, 267}. Class I PI(3)K complexes, one of the 3 classes of PI(3)K complexes in immune cells, are involved in the synthesis of Phosphatidylinositol (3,4,5)-Trisphosphates (PI(3,4,5)P3) that serve as docking sites for numerous proteins such as Phosphoinositide-Dependent Kinase 1 (PDK1) and AKT^{266, 267}. The reduced activation (phosphorylation) at T308 (mediated by PDK1) and S473 (mediated by numerous kinases) of AKT, a downstream effector kinase of the PI(3)K signaling pathway, is likely to be a consequence of impaired PI(3)K recruitment to TLR4 in talin1-deficient. P110 δ , an isoform of the catalytic subunit of PI(3)K complex can also regulate the internalization of TLR4 and calpain-mediated TIRAP degradation in DCs²⁶⁸, two processes essential for the initiation of the endosomal TRIF pathway²⁶⁸. The dissociation of talin1 from TLR4 could be regulated by calpain cleavage because talin1²³³, like TIRAP²⁶⁸, is also a substrate of the protease calpain. Since p85 and p110 usually existing in PI(3)K heterodimers²⁶⁹, the reduced recruitment of p85 to TLR4 in talin1-deficient BMDCs could diminish the

activity of PI(3)K and consequently affects the downstream AKT signaling pathway, the internalization of TLR4 and the TRIF-dependent pathway.

Reduced activation of kinases such as p38 and ERK1/2, in conjunction with decreased production of pro-inflammatory cytokines were observed in LPS-stimulated talin1-deficient BMDCs. The phenotype of talin1-deficient BMDCs mirrors Vav1-deficient macrophages²²⁸. In fact, impaired AKT activation is apparent in various Vav1-deficient immune cells^{228, 270, 271}, suggesting that Vav1 could be involved in the recruitment of PI(3)K to TLR4. Our data also revealed that talin1 and Vav1 co-immunoprecipitated with TLR4 in unstimulated wildtype BMDCs, which subsequently dissociated from TLR4 upon TLR4-LPS ligation. Vav1 has not been shown to interact with TLR4 or its associated downstream effector molecules. In addition, the interaction between talin1 and Vav1^{124, 239} is probably mediated by one or several unidentified protein(s), thus it will be interesting to identify the elusive bridging molecule(s) between talin1 and Vav1^{124, 239}. These experiments may offer greater insight into the mechanisms of Ezh2-mediated talin1 methylation^{124, 239} and talin1-dependent assembly of the TLR4 signalosome in DCs.

5.5 The functional role of talin1 in immune cells

Conditional deletion of talin1 in B cells by CD19 promoter-driven Cre-recombinase did not impact B cell development in the bone marrow²⁷². Despite the loss of the integrin activator talin1, immature B cells are able to migrate into the splenic white pulp in an integrin-dependent manner to further their maturation^{273, 274}. This could be due to the accumulation of a reservoir of talin1 molecules in the peri-nuclear region of the cell^{124, 275, 276, 282}. In addition, the loss of talin1 in B cells could also be partially compensated by paxillin, a regulator of VLA-4 (integrin $\alpha 4 \beta 1$) whereby it promotes the anchorage of VLA-4 to its ligand VCAM-1 independent of chemokine signaling-mediated integrin inside-out signaling to enhance both integrin binding affinity and avidity to its respective ligand^{272, 277, 278, 279, 280}, under shear flow.

Immature B cells would mature into follicular B cells or Marginal Zone (MZ) B cells in the spleen²⁸¹. The retention of MZ B cells in the spleen is compromised in the absence of talin1²⁷² or in the presence of blocking antibodies against integrins VLA-4 and LFA-1^{273, 274}. Integrins are required to anchor B cells in the MZ, a region of the spleen that lies between the white pulp and the vast splenic circulatory network (red pulp), so as to resist the shear flow of blood through the spleen^{273, 274}, a process similar to the integrin-dependent extravasation of neutrophils into the peritoneal cavity²⁸². Furthermore, talin1-deficient mature B cells are also unable to enter the LNs from the blood and the lymphatic vascular network²⁷², suggesting that integrin inside-out signaling mediated by talin1 and the CXCR4/CXCL12, CCR7/CCL19 and CCR7/CCL21 chemokine axes²⁸³

are necessary for the homing process. Finally, homing and retention of mature talin1-deficient B cells into bone marrow niches are also impaired²⁸³. It is likely that both processes, similar to the migration of mature B cells to the LNs²⁸³, are also dependent on the CXCR4/CXCL12 chemokine axis²⁸⁴ and chemokine receptor-mediated integrin inside-out signaling.

The loss of talin1 did not affect the development of CD4 T cells in the thymus but talin1-deficient CD4 T cells failed to home to the LNs²⁸⁵. This could be due to the defective chemokine receptor-mediated integrin inside-out signaling, similar to that observed in talin1-deficient B cells²⁷². Besides, T cell proliferation and cytokine production were also affected in talin1-deficient CD4 T cells due to reduced stable interactions with APCs, suggesting that talin1-mediated activation of LFA-1 within the immunological synapse is required to stabilize contacts between T cells and APCs^{285, 286}. NK cell degranulation and polarization are impaired in cells lacking talin1, suggesting that integrins activated by talin1 are required for both processes to take place²⁴⁷.

Recently, our lab described the significance of talin1 methylation in the integrin-dependent migration of DC^{73, 124} and neutrophils²⁸², whereby the tri-methylation of talin1 at Lys2454 (K2454me3) by cytosolic Ezh2 regulates the turnover of focal adhesions¹²⁴. Besides, mice with talin1-deficient neutrophils or neutrophils expressing un-methylatable talin1 (K2454A and K2454Q) variants are less susceptible to LPS endotoxemia-induced mortality compared to mice with wildtype neutrophils or neutrophils expressing methyl-mimicking talin1 (K2454F)²⁸². Enhanced survivability of mice can be explained by the diminished infiltration of talin1-deficient neutrophils or neutrophils with un-methylatable talin1 variants into organs such as the kidneys, liver and lungs, protecting these mice from the cytotoxic effects of neutrophils and multi-organ damage²⁸². However, with the present data at hand, it is possible that the TLR4 signal transduction cascade in talin1-deficient neutrophils, similar to talin1-deficient DCs are also compromised by the depletion of talin1. The effect of talin1 methylation on TLR4 signaling pathway warrants future investigation.

6. Summary — Talin1 is a novel regulator of TLR4 signaling pathways

Here, we show that conditional deletion of talin1 in DCs not only impaired integrin-dependent migration of the epidermal LCs across basement membrane, but also compromised DC activation *in vivo*, which led to the accumulation of talin1-deficient DCs in the skin and reduced numbers of mDCs in the sdLNs. Furthermore, we demonstrated that the loss of talin1 in BMDCs impaired the pre-assembly of a novel TLR4-associated complex and the recruitment of the TLR4 signalosome upon TLR4-LPS ligation. As a result, effector functions of LPS-stimulated talin1-deficient BMDCs and LCs such as the production of pro-inflammatory cytokines, MHCII up-regulation and cell motility were perturbed. Moreover, we demonstrated that talin1 directly interacted with MyD88 and its integrin binding/activating capacity was essential for the pre-assembly of TLR4-associated complex as well as the TLR4 signalosome formation upon LPS stimulation. Collectively, we provide comprehensive data suggesting talin1 as a novel regulator of the TLR4 signaling pathway in DCs (**Fig. 22**).

Figure 22. Talin1, a novel regulator of TLR4 signaling pathway

Chronological schematic depicting the role of talin1 in TLR4 signaling. **1)** The association of talin1, integrins, MyD88 and TLR-associated regulatory molecules (e.g. PI(3)K) with TLR4 in the absence of LPS. **2)** Upon TLR4-LPS engagement, talin1, integrins and TLR-associated regulatory molecules dissociate from TLR4, followed by the recruitment of the core components of the MyD88-dependent pathway. The proposed sequence of events are likely to occur within minutes upon TLR4-LPS engagement. **3)** Assembly of the core components of the TRIF-dependent pathway (endosome) upon the internalization of TLR4. **4)** Activation of the MAP Kinase pathway downstream of MyD88- and TRIF-dependent pathways followed by **5)** the transcription of pro-inflammatory cytokines.

7. Future directions

7.1 Aim 1: To further characterize talin1-MyD88 interaction and its possible implications other than controlling TLR signaling

Background / Rationale

We identified talin1 as a novel regulator of TLR4 signal transduction cascade, whereby it interacts with the intermediate domain of MyD88 directly to support the assembly of the TLR4 signalosome. However, the talin1 rod domain consists of approximately 2000 amino acid residues with domains that interact with numerous partners⁹⁶. Hence, we are keen to determine if the talin1-MyD88 interaction also influences other cellular events in DCs.

Methodology

Talin1 rod domain will be truncated into shorter fragments to screen for the region of interest that promotes the interaction between talin1 and MyD88. Structural studies will also be performed to better understand the molecular interactions between talin1 and MyD88.

Expected results

Identification of the region of interest on talin1 rod domain which mediates the interaction between talin1 and MyD88.

Limitations

Talin1 rod domain contains numerous protein-protein interaction sites⁹⁶. Furthermore, deleting the region of interest responsible for the interaction with MyD88 may potentially disrupt proximal protein-protein interaction sites on the talin1 rod domain or alter the intrinsic function of talin1.

7.2 Aim 2: To determine whether talin1 promotes TLR signaling via recruitment of PIP5K

Background / Rationale

Modification of inositol-containing phospholipids (phosphoinositides) by proteins such as Phosphatidylinositol-4-phosphate 5-kinases (PIP5K), Phosphatase and Tensin Homolog (PTEN), PI(3)K complex and 1-Phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma 1 (PLCγ1) are essential for the recruitment of membrane-bound signal transducers and actin cytoskeleton modifiers^{132, 287, 288} and the activation of downstream calcium signaling and Protein Kinase C (PKC) pathways¹⁸⁹.

Furthermore, the recruitment of TIRAP, an adaptor of the MyD88-dependent pathway is dependent on the availability of PI(4,5)P2, a product of the enzyme PIP5K¹⁸⁸.

Since the head domain of talin1 contains a PIP5K binding site¹⁰⁶ and the pre-formed TLR4/MyD88/Talin1 complex consists of TIRAP, we hypothesized that the lack of talin1 in the proximity of TLR4 can negatively affect the abundance of PI(4,5)P2 and TIRAP recruitment to the proximity of TLR4.

Methodology

Immunoprecipitation of TLR4, talin1 and activated integrins will be performed to examine the association of PIP5K with these proteins. Immunofluorescence microscopy and flow cytometry will be performed to study the abundance of phosphoinositides in the proximity of talin1, TLR4, TIRAP and MyD88.

Expected results

Reduced localization of phosphoinositides such as PI(4,5)P2 and PI(3,4,5)P3 in the vicinity of TLR4 in talin1-deficient BMDCs due to the lack of talin1-bound PIP5K¹⁰⁶ and TLR4-associated PI(3)K (p85/p110). Preliminary data revealed a reduction in the strength of calcium flux downstream of TLR4 upon LPS ligation in talin1-deficient BMDCs, suggesting an altered abundance of phosphoinositides (PI(4)P, PI(4,5)P2 and PI(3,4,5)P3) in the proximity of TLR4.

Limitations

Since we do not expect global changes in the amount of PI(4,5)P2 and PI(3,4,5)P3 within the cell and that there are no commercially available antibodies that can detect specific classes of phosphoinositides with minimal cross reactivity, overexpression of truncated domains of membrane-bound peripheral proteins²⁸⁷ that recognize and bind to PI(4,5)P2 and PI(3,4,5)P3 is the only way to detect localized changes in the abundance of phosphoinositides in wildtype and talin1-deficient BMDCs.

7.3 Aim 3: To investigate the implications of talin1 expression in DCs in disease pathogenesis

Background / Rationale

Skin DCs play dominant roles in the immune response of epicutaneous *C. Albicans* infection, whereby epidermal LC-derived IL-6 is critical for the Th17 response while dermal DCs are responsible for the Th1 and effector CD8 T cells responses^{45, 48, 49, 54, 289}. The defined roles of skin DCs in the pathogenesis of *C. Albicans* in epicutaneous infection permit us to examine the functional role of talin1 in skin DCs during inflammation *in vivo*.

Methodology

Mice will be epicutaneously infected with *C. Albicans*⁴⁸ followed by immune profiling of the cells in the sdLNs 6 days later.

Expected results

Reduced activation and migration of talin1-deficient skin DCs to the sdLNs to raise an effective Th1 and Th17 responses.

Limitations

Epicutaneous *C. Albicans* infection requires hair removal on the back of the mice and the substratum corneum scrapped (sandpaper) prior to infection. Hair and substratum corneum removal treatments could potentially introduce excessive chemical burns and physical damage to the skin of the mice, which can alter the immune response towards the *C. Albicans* infection.

Heterogeneity of fungal PAMPs present in live *C. Albicans* such as glycans, mannans, chitin and other cell wall proteins could be recognized by a variety of receptors such as dectin-1, dectin-2, mannose receptor, TLR2 and TLR4^{55, 56, 57, 58, 61, 63, 64, 65, 66, 290}. Fungal PAMPs could activate a variety of downstream signaling events that could interfere with each other, as such it is important to clarify the implications of talin1 deficiency in the signal transduction pathway of other receptors besides TLRs.

8. References

1. Roach, J.C. *et al.* The evolution of vertebrate Toll-like receptors. *Proc. Natl. Acad. Sci. USA* **102**, 9577-9582 (2005).
2. Gay, N.J., Gangloff, M. & Weber, A.N.R. Toll-like receptors as molecular switches. *Nat. Rev. Immunol.* **6**, 693-698 (2006).
3. O'Neill, L.A.J. & Bowie, A.G. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat. Rev. Immunol.* **7**, 353-364 (2007).
4. Kawasaki, T. & Kawai, T. Toll-like receptor signaling pathways. *Front. Immunol.* **5**, 461 (2014).
5. Latz, E. *et al.* Ligand-induced conformational changes allosterically activate Toll-like receptor 9. *Nat. Immunol.* **8**, 772-779 (2007).
6. Tanji, H., Ohto, U., Shibata, T., Miyake, K. & Shimizu, T. Structural Reorganization of the Toll-Like Receptor 8 Dimer Induced by Agonistic Ligands. *Science* **339**, 1426-1429 (2013).
7. Kagan, J.C., Magupalli, V.G. & Wu, H. SMOCs: supramolecular organizing centres that control innate immunity. *Nat. Rev. Immunol.* **14**, 821-826 (2014).
8. Kieser, K.J. & Kagan, J.C. Multi-receptor detection of individual bacterial products by the innate immune system. *Nat. Rev. Immunol.* **17**, 376-390 (2017).
9. Gay, N.J., Symmons, M.F., Gangloff, M. & Bryant, C.E. Assembly and localization of Toll-like receptor signalling complexes. *Nat. Rev. Immunol.* **14**, 546-558 (2014).
10. Lin, S.-C., Lo, Y.-C. & Wu, H. Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling. *Nature* **465**, 885-890 (2010).
11. Garlanda, C., Riva, F., Bonavita, E., Gentile, S. & Mantovani, A. Decoys and regulatory "receptors" of the IL-1/Toll-like receptor superfamily. *Front. Immunol.* **4**, 180 (2013).
12. Ajibade, A.A., Wang, H.Y. & Wang, R.-F. Cell type-specific function of TAK1 in innate immune signaling. *Trends Immunol.* **34**, 307-316 (2013).
13. Alagbala Ajibade, A. *et al.* TAK1 negatively regulates NF- κ B and p38 MAP Kinase Activation in Gr-1+CD11b+ neutrophils. *Immunity* **36**, 43-54 (2012).
14. Fan, Y., Yu, Y., Mao, R., Zhang, H. & Yang, J. TAK1 Lys-158 but not Lys-209 is required for IL-1 β -induced Lys63-linked TAK1 polyubiquitination and IKK/NF- κ B activation. *Cell. Signal.* **23**, 660-665 (2011).
15. Fan, Y. *et al.* Lysine 63-linked Polyubiquitination of TAK1 at Lysine 158 Is Required for Tumor Necrosis Factor α - and Interleukin-1 β -induced IKK/NF- κ B and JNK/AP-1 Activation. *J. Biol. Chem.* **285**, 5347-5360 (2010).
16. Mao, R. *et al.* TAK1 lysine 158 is required for TGF- β -induced TRAF6-mediated Smad-independent IKK/NF- κ B and JNK/AP-1 activation. *Cell. Signal.* **23**, 222-227 (2011).
17. Chen, I.T., Hsu, P.-H., Hsu, W.-C., Chen, N.-J. & Tseng, P.-H. Polyubiquitination of Transforming Growth Factor β -activated Kinase 1 (TAK1) at Lysine 562 residue regulates TLR4-mediated JNK and p38 MAPK activation. *Sci. Rep.* **5**, 12300 (2015).
18. Kawai, T. & Akira, S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* **34**, 637-650 (2011).
19. Kawai, T. & Akira, S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat. Immunol.* **11**, 373 (2010).
20. Akira, S., Uematsu, S. & Takeuchi, O. Pathogen Recognition and Innate Immunity. *Cell* **124**, 783-801 (2006).
21. Viatour, P., Merville, M.-P., Bours, V. & Chariot, A. Phosphorylation of NF- κ B and I κ B proteins: implications in cancer and inflammation. *Trends Biochem. Sci.* **30**, 43-52 (2005).
22. Napetschnig, J. & Wu, H. Molecular basis of NF- κ B signaling. *Annu. Rev. Biophys.* **42**, 443-468 (2013).
23. Yamamoto, M. *et al.* Role of Adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science* **301**, 640-643 (2003).
24. Yamamoto, M. *et al.* TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. *Nat. Immunol.* **4**, 1144-1150 (2003).
25. Kagan, J.C. *et al.* TRAM couples endocytosis of Toll-like receptor 4 to the induction of interferon- β . *Nat. Immunol.* **9**, 361-368 (2008).
26. Rajaiah, R., Perkins, D.J., Ireland, D.D.C. & Vogel, S.N. CD14 dependence of TLR4 endocytosis and TRIF signaling displays ligand specificity and is dissociable in endotoxin tolerance. *Proc. Natl. Acad. Sci. USA* **112**, 8391-8396 (2015).
27. Sato, S. *et al.* Toll/IL-1 Receptor Domain-Containing Adaptor Inducing IFN- β (TRIF) associates with TNF Receptor-Associated Factor 6 and TANK-Binding Kinase 1, and activates two distinct transcription factors, NF- κ B and IFN-Regulatory Factor-3, in the Toll-Like Receptor signaling. *J. Immunol.* **171**, 4304 (2003).
28. Gohda, J., Matsumura, T. & Inoue, J. Cutting Edge: TNFR-Associated Factor (TRAF) 6 is essential for MyD88-dependent pathway but not Toll/IL-1 Receptor Domain-Containing Adaptor-Inducing IFN- β (TRIF)-dependent pathway in TLR signaling. *J. Immunol.* **173**, 2913-2917 (2004).
29. Mansell, A. *et al.* Suppressor of cytokine signaling 1 negatively regulates Toll-like receptor signaling by mediating Mal degradation. *Nat. Immunol.* **7**, 148-155 (2006).
30. Nakagawa, R. *et al.* SOCS-1 participates in negative regulation of LPS responses. *Immunity* **17**, 677-687 (2002).
31. Kinjyo, I. *et al.* SOCS1/JAB is a negative regulator of LPS-induced macrophage activation. *Immunity* **17**, 583-591 (2002).

32. Frøbøse, H. *et al.* Suppressor of cytokine Signaling-3 inhibits interleukin-1 signaling by targeting the TRAF-6/TAK1 complex. *Mol. Endocrinol.* **20**, 1587-1596 (2006).
33. Kayagaki, N. *et al.* DUBA: a deubiquitinase that regulates type I interferon production. *Science* **318**, 1628-1632 (2007).
34. Han, C. *et al.* Integrin CD11b negatively regulates TLR-triggered inflammatory responses by activating Syk and promoting degradation of MyD88 and TRIF via Cbl-b. *Nat. Immunol.* **11**, 734-742 (2010).
35. Carty, M. *et al.* The human adaptor SARM negatively regulates adaptor protein TRIF-dependent Toll-like receptor signaling. *Nat. Immunol.* **7**, 1074-1081 (2006).
36. Kondo, T., Kawai, T. & Akira, S. Dissecting negative regulation of Toll-like receptor signaling. *Trends Immunol.* **33**, 449-458 (2012).
37. An, H. *et al.* SHP-2 phosphatase negatively regulates the TRIF adaptor protein-dependent type I interferon and proinflammatory cytokine production. *Immunity* **25**, 919-928 (2006).
38. Kobayashi, K. *et al.* IRAK-M is a negative regulator of Toll-like Receptor signaling. *Cell* **110**, 191-202 (2002).
39. Skaug, B. *et al.* Direct, Non-catalytic Mechanism of IKK Inhibition by A20. *Mol. Cell* **44**, 559-571 (2011).
40. Yuk, J.-M. *et al.* The orphan nuclear receptor SHP acts as a negative regulator in inflammatory signaling triggered by Toll-like receptors. *Nat. Immunol.* **12**, 742-751 (2011).
41. Tanaka, T., Grusby, M.J. & Kaisho, T. PDLIM2-mediated termination of transcription factor NF- κ B activation by intranuclear sequestration and degradation of the p65 subunit. *Nat. Immunol.* **8**, 584-591 (2007).
42. Reyskens, K.M.S.E. & Arthur, J.S.C. Emerging roles of the mitogen and stress activated kinases MSK1 and MSK2. *Front. Cell Dev. Biol.* **4**, 56 (2016).
43. Fitzgerald, K.A. *et al.* LPS-TLR4 Signaling to IRF-3/7 and NF- κ B Involves the Toll Adaptors TRAM and TRIF. *J. Exp. Med.* **198**, 1043-1055 (2003).
44. McGettrick, A.F. & O'Neill, L.A.J. Localisation and trafficking of Toll-like receptors: an important mode of regulation. *Curr. Opin. Immunol.* **22**, 20-27 (2010).
45. Kaplan, D.H. Ontogeny and function of murine epidermal Langerhans cells. *Nat. Immunol.* **18**, 1068-1075 (2017).
46. Merad, M. *et al.* Langerhans cells renew in the skin throughout life under steady-state conditions. *Nat. Immunol.* **3**, 1135-1141 (2002).
47. Hoeffel, G. *et al.* Adult Langerhans cells derive predominantly from embryonic fetal liver monocytes with a minor contribution of yolk sac-derived macrophages. *J. Exp. Med.* **209**, 1167-1181 (2012).
48. Kashem, S.W. *et al.* *Candida albicans* morphology and dendritic cell subsets determine T helper cell differentiation. *Immunity* **42**, 356-366 (2015).
49. Haley, K. *et al.* Langerhans cells require MyD88-dependent signals for *Candida albicans* response but not for contact hypersensitivity or migration. *J. Immunol.* **188**, 4334-4339 (2012).
50. Voorhees, T. *et al.* Dendritic cells produce inflammatory cytokines in response to bacterial products from *Staphylococcus aureus*-infected atopic dermatitis lesions. *Cell. Immunol.* **267**, 17-22 (2011).
51. Schindler, D. *et al.* Dendritic cells are central coordinators of the host immune response to *Staphylococcus aureus* bloodstream infection. *Am. J. Pathol.* **181**, 1327-1337 (2012).
52. Mitsui, H. *et al.* Differential expression and function of Toll-like Receptors in Langerhans cells: comparison with splenic dendritic cells. *J. Invest. Dermatol.* **122**, 95-102 (2004).
53. Fujita, H., Asahina, A., Mitsui, H. & Tamaki, K. Langerhans cells exhibit low responsiveness to double-stranded RNA. *Biochem. Biophys. Res. Commun.* **319**, 832-839 (2004).
54. Igyarto, B.Z. *et al.* Skin-resident murine dendritic cell subsets promote distinct and opposing antigen-specific T helper cell responses. *Immunity* **35**, 260-272 (2011).
55. Netea, M.G. *et al.* Immune sensing of *Candida albicans* requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors. *J. Clin. Invest.* **116**, 1642-1650 (2006).
56. Netea, M.G. *et al.* Toll-Like Receptor 2 suppresses immunity against *Candida albicans* through Induction of IL-10 and regulatory T cells. *J. Immunol.* **172**, 3712-3718 (2004).
57. Netea, M.G., Van der Graaf, C., Van der Meer, J.W.M. & Kullberg, B.J. Toll-like receptors and the host defense against microbial pathogens: bringing specificity to the innate-immune system. *J. Leukoc. Biol.* **75**, 749-755 (2004).
58. Netea, M.G. *et al.* The role of Toll-like Receptor (TLR) 2 and TLR4 in the host defense against disseminated candidiasis. *The J. Infect. Dis.* **185**, 1483-1489 (2002).
59. Gantner, B.N., Simmons, R.M. & Underhill, D.M. Dectin-1 mediates macrophage recognition of *Candida albicans* yeast but not filaments. *EMBO J.* **24**, 1277-1286 (2005).
60. Gantner, B.N., Simmons, R.M., Canavera, S.J., Akira, S. & Underhill, D.M. Collaborative induction of inflammatory responses by Dectin-1 and Toll-like Receptor 2. *J. Exp. Med.* **197**, 1107-1117 (2003).
61. Villamón, E. *et al.* Toll-like receptor-2 is essential in murine defenses against *Candida albicans* infections. *Microbes Infect.* **6**, 1-7 (2004).
62. Hernández-Santos, N. & Gaffen, Sarah L. Th17 Cells in Immunity to *Candida albicans*. *Cell Host Microbe* **11**, 425-435 (2012).
63. Saijo, S. & Iwakura, Y. Dectin-1 and Dectin-2 in innate immunity against fungi. *Int. Immunol.* **23**, 467-472 (2011).

64. Hise, A.G. *et al.* An essential role for the NLRP3 inflammasome in host defense against the human fungal pathogen *Candida albicans*. *Cell Host Microbe* **5**, 487-497 (2009).
65. Ferwerda, B. *et al.* Functional consequences of toll-like receptor 4 polymorphisms. *Mol. Med.* **14**, 346-352 (2008).
66. Vautier, S., Sousa, M.d.G. & Brown, G.D. C-type lectins, fungi and Th17 responses. *Cytokine Growth Factor Rev.* **21**, 405-412 (2010).
67. Kashem, S.W., Haniffa, M. & Kaplan, D.H. Antigen-Presenting cells in the skin. *Annu. Rev. Immunol.* **35**, 469-499 (2017).
68. Kabashima, K. & Nomura, T. Revisiting murine models for atopic dermatitis and psoriasis with multipolar cytokine axes. *Curr. Opin. Immunol.* **48**, 99-107 (2017).
69. Honda, T. *et al.* Compensatory role of Langerhans cells and langerin-positive dermal dendritic cells in the sensitization phase of murine contact hypersensitivity. *J. Allergy Clin. Immunol.* **125**, 1154-1156 (2010).
70. Romani, N., Brunner, P.M. & Stingl, G. Changing views of the role of Langerhans cells. *J. Invest. Dermatol.* **132**, 872-881 (2012).
71. Romani, N., Clausen, B.E. & Stoitzner, P. Langerhans cells and more: langerin-expressing dendritic cell subsets in the skin. *Immunol. Rev.* **234**, 120-141 (2010).
72. Kissenpfennig, A. *et al.* Dynamics and function of Langerhans cells in vivo. *Immunity* **22**, 643-654 (2005).
73. Loh, J.T. *et al.* Ezh2 controls skin tolerance through distinct mechanisms in different subsets of skin dendritic cells. *iScience* **10**, 23-39 (2018).
74. Noordegraaf, M., Flacher, V., Stoitzner, P. & Clausen, B.E. Functional redundancy of Langerhans cells and Langerin+ dermal dendritic cells in contact hypersensitivity. *J. Invest. Dermatol.* **130**, 2752-2759 (2010).
75. Idoyaga, J. *et al.* Specialized role of migratory dendritic cells in peripheral tolerance induction. *J. Clin. Invest.* **123**, 844-854 (2013).
76. Bennett, C.L. *et al.* Inducible ablation of mouse Langerhans cells diminishes but fails to abrogate contact hypersensitivity. *J. Cell Biol.* **169**, 569-576 (2005).
77. Ginhoux, F. & Jung, S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat. Rev. Immunol.* **14**, 392-404 (2014).
78. Guillems, M. *et al.* Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat. Rev. Immunol.* **14**, 571-578 (2014).
79. Gordon, S. Alternative activation of macrophages. *Nat. Rev. Immunol.* **3**, 23-25 (2003).
80. Gordon, S., Plüddemann, A. & Martinez Estrada, F. Macrophage heterogeneity in tissues: phenotypic diversity and functions. *Immunol. Rev.* **262**, 36-55 (2014).
81. Merad, M., Sathe, P., Helft, J., Miller, J. & Mortha, A. The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu. Rev. Immunol.* **31**, 563-604 (2013).
82. Worbs, T., Hammerschmidt, S.I. & Förster, R. Dendritic cell migration in health and disease. *Nat. Rev. Immunol.* **17**, 30-48 (2016).
83. Mildner, A. & Jung, S. Development and function of dendritic cell subsets. *Immunity* **40**, 642-656 (2014).
84. Watts, C., West, M.A. & Zaru, R. TLR signalling regulated antigen presentation in dendritic cells. *Curr. Opin. Immunol.* **22**, 124-130 (2010).
85. Vargas, P. *et al.* Innate control of actin nucleation determines two distinct migration behaviours in dendritic cells. *Nat. Cell Biol.* **18**, 43-53 (2015).
86. Shen, H., Tesar, B.M., Walker, W.E. & Goldstein, D.R. Dual signaling of MyD88 and TRIF is critical for maximal TLR4-induced dendritic cell maturation. *J. Immunol.* **181**, 1849-1858 (2008).
87. West, M.A. *et al.* TLR ligand-induced podosome disassembly in dendritic cells is ADAM17 dependent. *J. Cell Biol.* **182**, 993-1005 (2008).
88. Faure-André, G. *et al.* Regulation of dendritic cell migration by CD74, the MHC class II-associated invariant chain. *Science* **322**, 1705-1710 (2008).
89. Ng, L.G. *et al.* Migratory dermal dendritic cells act as rapid sensors of protozoan parasites. *PLoS Pathog.* **4**, e1000222 (2008).
90. Charras, G. & Paluch, E. Blebs lead the way: how to migrate without lamellipodia. *Nat. Rev. Mol. Cell Biol.* **9**, 730-736 (2008).
91. Narumiya, S., Tanji, M. & Ishizaki, T. Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion. *Cancer Metastasis Rev.* **28**, 65-76 (2009).
92. Lammernann, T. *et al.* Rapid leukocyte migration by integrin-independent flowing and squeezing. *Nature* **453**, 51-55 (2008).
93. Critchley, D.R. Biochemical and structural properties of the integrin-associated cytoskeletal protein Talin. *Annu. Rev. Biophys.* **38**, 235-254 (2009).
94. Das, M., Subbayya Ithychanda, S., Qin, J. & Plow, E.F. Mechanisms of talin-dependent integrin signaling and crosstalk. *Biochim. Biophys. Acta* **1838**, 579-588 (2014).
95. Monkley Susan, J. *et al.* Disruption of the talin gene arrests mouse development at the gastrulation stage. *Dev. Dyn.* **219**, 560-574 (2000).
96. Calderwood, D.A., Campbell, I.D. & Critchley, D.R. Talins and kindlins: partners in integrin-mediated adhesion. *Nat. Rev. Mol. Cell Biol.* **14**, 503-517 (2013).
97. Conti, F.J., Monkley, S.J., Wood, M.R., Critchley, D.R. & Muller, U. Talin 1 and 2 are required for myoblast fusion, sarcomere assembly and the maintenance of myotendinous junctions. *Development* **136**, 3597-3606 (2009).

98. Senetar, M.A., Moncman, C.L. & McCann, R.O. Talin2 is induced during striated muscle differentiation and is targeted to stable adhesion complexes in mature muscle. *Cell Motil. Cytoskeleton* **64**, 157-173 (2007).
99. Monkley, S.J. *et al.* Endothelial cell talin1 is essential for embryonic angiogenesis. *Dev. Biol.* **349**, 494-502 (2011).
100. Liu, J. *et al.* Talin determines the nanoscale architecture of focal adhesions. *Proc. Natl. Acad. Sci. USA* **112**, E4864-E4873 (2015).
101. Monkley, S.J., Pritchard, C.A. & Critchley, D.R. Analysis of the Mammalian Talin2 Gene TLN2. *Biochem. Biophys. Res. Commun.* **286**, 880-885 (2001).
102. Nieswandt, B. *et al.* Loss of talin1 in platelets abrogates integrin activation, platelet aggregation, and thrombus formation in vitro and in vivo. *J. Exp. Med.* **204**, 3113-3118 (2007).
103. Elliott, P.R. *et al.* The structure of the talin head reveals a novel extended conformation of the FERM domain. *Structure* **18**, 1289-1299 (2010).
104. Goult, B.T. *et al.* Structure of a double ubiquitin-like domain in the talin head: a role in integrin activation. *EMBO J.* **29**, 1069-1080 (2010).
105. Di Paolo, G. *et al.* Recruitment and regulation of phosphatidylinositol phosphate kinase type 1 gamma by the FERM domain of talin. *Nature* **420**, 85-89 (2002).
106. Barsukov, I.L. *et al.* Phosphatidylinositol phosphate kinase type 1gamma and beta1-integrin cytoplasmic domain bind to the same region in the talin FERM domain. *J. Biol. Chem.* **278**, 31202-31209 (2003).
107. Ling, K., Doughman, R.L., Firestone, A.J., Bunce, M.W. & Anderson, R.A. Type Iy phosphatidylinositol phosphate kinase targets and regulates focal adhesions. *Nature* **420**, 89 (2002).
108. Hemmings, L. *et al.* Talin contains three actin-binding sites each of which is adjacent to a vinculin-binding site. *J. Cell Sci.* **109**, 2715 (1996).
109. Gingras, A.R. *et al.* The structure of the C-terminal actin-binding domain of talin. *EMBO J.* **27**, 458-469 (2008).
110. Gingras, A.R. *et al.* Central region of talin has a unique fold that binds vinculin and actin. *J. Biol. Chem.* **285**, 29577-29587 (2010).
111. Borowsky, M.L. & Hynes, R.O. Layilin, A novel talin-binding transmembrane protein homologous with C-type lectins, is localized in membrane ruffles. *J. Cell Biol.* **143**, 429-442 (1998).
112. Chen, H.-C. *et al.* Interaction of Focal Adhesion Kinase with Cytoskeletal Protein Talin. *J. Biol. Chem.* **270**, 16995-16999 (1995).
113. Lagarrigue, F. *et al.* A RIAM/lamellipodin-talin-integrin complex forms the tip of sticky fingers that guide cell migration. *Nat. Commun.* **6**, 8492 (2015).
114. Lee, H.S., Lim, C.J., Puzon-McLaughlin, W., Shattil, S.J. & Ginsberg, M.H. RIAM activates integrins by linking talin to ras GTPase membrane-targeting sequences. *J. Biol. Chem.* **284**, 5119-5127 (2009).
115. Yang, J. *et al.* Conformational activation of talin by RIAM triggers integrin-mediated cell adhesion. *Nat. Commun.* **5**, 5880 (2014).
116. Huang, C. *et al.* Talin phosphorylation by Cdk5 regulates Smurf1-mediated talin head ubiquitylation and cell migration. *Nat. Cell Biol.* **11**, 624-630 (2009).
117. Anthis, N.J., Wegener, K.L., Critchley, D.R. & Campbell, I.D. Structural diversity in integrin/talin interactions. *Structure* **18**, 1654-1666 (2010).
118. Wegener, K.L. *et al.* Structural basis of integrin activation by talin. *Cell* **128**, 171-182 (2007).
119. Moes, M. *et al.* The integrin binding site 2 (IBS2) in the talin rod domain is essential for linking integrin beta subunits to the cytoskeleton. *J. Biol. Chem.* **282**, 17280-17288 (2007).
120. Gingras, A.R. *et al.* Structural determinants of integrin binding to the talin rod. *J. Biol. Chem.* **284**, 8866-8876 (2009).
121. Goult, B.T. *et al.* RIAM and vinculin binding to talin are mutually exclusive and regulate adhesion assembly and turnover. *J. Biol. Chem.* **288**, 8238-8249 (2013).
122. Gingras, A.R. *et al.* Structural and dynamic characterization of a vinculin binding site in the talin rod. *Biochemistry* **45**, 1805-1817 (2006).
123. Gingras, A.R. *et al.* Mapping and consensus sequence identification for multiple vinculin binding sites within the talin rod. *J. Biol. Chem.* **280**, 37217-37224 (2005).
124. Gunawan, M. *et al.* The methyltransferase Ezh2 controls cell adhesion and migration through direct methylation of the extranuclear regulatory protein talin. *Nat. Immunol.* **16**, 505-516 (2015).
125. Ratnikov, B. *et al.* Talin phosphorylation sites mapped by mass spectrometry. *J. Cell Sci.* **118**, 4921-4923 (2005).
126. Li, Y. *et al.* High stoichiometry phosphorylation of talin at T144/T150 or S446 produces contrasting effects on calpain-mediated talin cleavage and cell migration. *J. Cancer* **7**, 1645-1652 (2016).
127. Zhang, F., Saha, S. & Kashina, A. Arginylation-dependent regulation of a proteolytic product of talin is essential for cell-cell adhesion. *J. Cell Biol.* **197**, 819-836 (2012).
128. Goksoy, E. *et al.* Structural basis for the autoinhibition of talin in regulating integrin activation. *Mol. Cell* **31**, 124-133 (2008).
129. Song, X. *et al.* A novel membrane-dependent on/off switch mechanism of talin FERM domain at sites of cell adhesion. *Cell Res.* **22**, 1533-1545 (2012).
130. de Pereda, J.M. *et al.* Structural basis for phosphatidylinositol phosphate kinase type Iy binding to talin at focal adhesions. *J. Biol. Chem.* **280**, 8381-8386 (2005).

131. Martel, V. *et al.* Conformation, localization, and integrin binding of talin depend on its interaction with phosphoinositides. *J. Biol. Chem.* **276**, 21217-21227 (2001).
132. Ye, X., McLean, M.A. & Sligar, S.G. PIP2 modulates the affinity of talin-1 for phospholipid bilayers and activates its auto-inhibited form. *Biochemistry* **55**, 5038-5048 (2016).
133. Bertagnoli, M.E., Locke, S.J., Hensler, M.E., Bray, P.F. & Beckerle, M.C. Talin distribution and phosphorylation in thrombin-activated platelets. *J. Cell Sci.* **106**, 1189-1199 (1993).
134. Han, J. *et al.* Reconstructing and deconstructing agonist-induced activation of integrin α IIb β 3. *Curr. Biol.* **16**, 1796-1806 (2006).
135. Legate, K.R. *et al.* Integrin adhesion and force coupling are independently regulated by localized PtdIns(4,5)2 synthesis. *EMBO J.* **30**, 4539-4553 (2011).
136. Saltel, F. *et al.* New PI(4,5)P2- and membrane proximal integrin-binding motifs in the talin head control β 3-integrin clustering. *J. Cell Biol.* **187**, 715-731 (2009).
137. Sun, Z. *et al.* Kank2 activates talin, reduces force transduction across integrins and induces central adhesion formation. *Nat. Cell Biol.* **18**, 941 (2016).
138. Zhu, L. *et al.* Structure of Rap1b bound to talin reveals a pathway for triggering integrin activation. *Nat. Commun.* **8**, 1744 (2017).
139. Humphries, J.D., Byron, A. & Humphries, M.J. Integrin ligands at a glance. *J. Cell Sci.* **119**, 3901-3903 (2006).
140. Hynes, R.O. Integrins. *Cell* **110**, 673-687 (2002).
141. Shattil, S.J., Kim, C. & Ginsberg, M.H. The final steps of integrin activation: the end game. *Nat. Rev. Mol. Cell Biol.* **11**, 288-300 (2010).
142. Pinon, P. *et al.* Talin-bound NPLY motif recruits integrin-signaling adapters to regulate cell spreading and mechanosensing. *J. Cell Biol.* **205**, 265-281 (2014).
143. Gough Rosemarie, E. & Goult Benjamin, T. The tale of two talins – two isoforms to fine-tune integrin signalling. *FEBS Lett.* **592**, 2108-2125 (2018).
144. Ni, T. *et al.* Structure and lipid-binding properties of the kindlin-3 pleckstrin homology domain. *Biochem. J.* **474**, 539-556 (2017).
145. Ajuebor, M.N. *et al.* Role of resident peritoneal macrophages and mast cells in chemokine production and neutrophil migration in acute inflammation: evidence for an inhibitory loop involving endogenous IL-10. *J. Immunol.* **162**, 1685-1691 (1999).
146. García-Alvarez, B. *et al.* Structural determinants of integrin recognition by talin. *Mol. Cell* **11**, 49-58 (2003).
147. Kim, C., Ye, F. & Ginsberg, M.H. Regulation of integrin activation. *Annu. Rev. Cell Dev. Biol.* **27**, 321-345 (2011).
148. Kim, C., Ye, F., Hu, X. & Ginsberg, M.H. Talin activates integrins by altering the topology of the beta transmembrane domain. *J. Cell Biol.* **197**, 605-611 (2012).
149. Lau, T.L., Kim, C., Ginsberg, M.H. & Ulmer, T.S. The structure of the integrin α IIb β 3 transmembrane complex explains integrin transmembrane signalling. *EMBO J.* **28**, 1351-1361 (2009).
150. Kurtz, L., Kao, L., Newman, D., Kurtz, I. & Zhu, Q. Integrin α IIb β 3 inside-out activation: an in situ conformational analysis reveals a new mechanism. *J. Biol. Chem.* **287**, 23255-23265 (2012).
151. Pepinsky, R.B. *et al.* Comparative Assessment of the Ligand and Metal Ion Binding Properties of Integrins α 9 β 1 and α 4 β 1. *Biochemistry* **41**, 7125-7141 (2002).
152. Renkawitz, J. *et al.* Adaptive force transmission in amoeboid cell migration. *Nat. Cell Biol.* **11**, 1438-1443 (2009).
153. Plotnikov, S.V. & Waterman, C.M. Guiding cell migration by tugging. *Curr. Opin. Cell Biol.* **25**, 619-626 (2013).
154. Gardel, M.L., Schneider, I.C., Aratyn-Schaus, Y. & Waterman, C.M. Mechanical integration of actin and adhesion dynamics in cell migration. *Annu. Rev. Cell Dev. Biol.* **26**, 315-333 (2010).
155. Huttenlocher, A., Sandborg, R.R. & Horwitz, A.F. Adhesion in cell migration. *Curr. Opin. Cell Biol.* **7**, 697-706 (1995).
156. Beningo, K.A., Hamao, K., Dembo, M., Wang, Y.-I. & Hosoya, H. Traction forces of fibroblasts are regulated by the Rho-dependent kinase but not by the myosin light chain kinase. *Arch. Biochem. Biophys.* **456**, 224-231 (2006).
157. Rabinovitz, I. & Mercurio, A.M. The integrin α 6 β 4 functions in carcinoma cell migration on laminin-1 by mediating the formation and stabilization of actin-containing motility structures. *J. Cell Biol.* **139**, 1873 (1997).
158. Renkawitz, J. & Sixt, M. Mechanisms of force generation and force transmission during interstitial leukocyte migration. *EMBO Rep.* **11**, 744-750 (2010).
159. Lämmermann, T. *et al.* Cdc42-dependent leading edge coordination is essential for interstitial dendritic cell migration. *Blood* **113**, 5703-5710 (2009).
160. Lämmermann, T. & Sixt, M. Mechanical modes of 'amoeboid' cell migration. *Curr. Opin. Cell Biol.* **21**, 636-644 (2009).
161. Jin, J.-K. *et al.* Talin1 phosphorylation activates β 1 integrins: a novel mechanism to promote prostate cancer bone metastasis. *Oncogene* **34**, 1811-1821 (2015).
162. Lefort, C.T. & Ley, K. Neutrophil arrest by LFA-1 activation. *Front. Immunol.* **3**, 157 (2012).
163. Mathew, S. *et al.* Talin regulates integrin β 1-dependent and -independent cell functions in ureteric bud development. *Development* **144**, 4148 (2017).
164. Mitra, S.K. & Schlaepfer, D.D. Integrin-regulated FAK-Src signaling in normal and cancer cells. *Curr. Opin. Cell Biol.* **18**, 516-523 (2006).

165. Kolaczowska, E. & Kubes, P. Neutrophil recruitment and function in health and inflammation. *Nat. Rev. Immunol.* **13**, 159-175 (2013).
166. Ley, K., Laudanna, C., Cybulsky, M.I. & Nourshargh, S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat. Rev. Immunol.* **7**, 678-689 (2007).
167. Zarbock, A., Ley, K., McEver, R.P. & Hidalgo, A. Leukocyte ligands for endothelial selectins: specialized glycoconjugates that mediate rolling and signaling under flow. *Blood* **118**, 6743-6751 (2011).
168. Price, A.A., Cumberbatch, M., Kimber, I. & Ager, A. $\alpha 6$ Integrins are required for langerhans cell migration from the epidermis. *J. Exp. Med.* **186**, 1725-1735 (1997).
169. Weninger, W., Biro, M. & Jain, R. Leukocyte migration in the interstitial space of non-lymphoid organs. *Nat. Rev. Immunol.* **14**, 232-246 (2014).
170. Villablanca, E.J. & Mora, J.R. A two-step model for Langerhans cell migration to skin-draining LN. *Eur. J. Immunol.* **38**, 2975-2980 (2008).
171. Randolph, G.J., Angeli, V. & Swartz, M.A. Dendritic-cell trafficking to lymph nodes through lymphatic vessels. *Nat. Rev. Immunol.* **5**, 617-628 (2005).
172. Randolph, G.J., Ochando, J. & Partida-Sánchez, S. Migration of Dendritic Cell Subsets and their Precursors. *Annu. Rev. Immunol.* **26**, 293-316 (2008).
173. Kaplan, D.H., Jenison, M.C., Saeland, S., Shlomchik, W.D. & Shlomchik, M.J. Epidermal langerhans cell-deficient mice develop enhanced contact hypersensitivity. *Immunity* **23**, 611-620 (2005).
174. Kel, J.M., Girard-Madoux, M.J.H., Reizis, B. & Clausen, B.E. TGF- β is required to maintain the pool of immature langerhans cells in the epidermis. *J. Immunol.* **185**, 3248-3255 (2010).
175. Bobr, A. *et al.* Autocrine/paracrine TGF- β 1 inhibits Langerhans cell migration. *Proc. Natl. Acad. Sci. USA* **109**, 10492-10497 (2012).
176. Lu, M. *et al.* Integrin $\alpha 8 \beta 1$ mediates adhesion to LAP-TGF β 1. *J. Cell Sci.* **115**, 4641-4648 (2002).
177. Asano, Y. *et al.* Increased expression of integrin $\alpha \nu \beta 3$ contributes to the establishment of autocrine TGF- β signaling in scleroderma fibroblasts. *J. Immunol.* **175**, 7708-7718 (2005).
178. Asano, Y., Ihn, H., Jinnin, M., Mimura, Y. & Tamaki, K. Involvement of $\alpha \nu \beta 5$ integrin in the establishment of autocrine TGF- β signaling in dermal fibroblasts derived from localized scleroderma. *J. Invest. Dermatol.* **126**, 1761-1769 (2006).
179. Asano, Y. *et al.* Involvement of $\alpha \nu \beta 5$ integrin-mediated activation of latent transforming growth factor β 1 in autocrine transforming growth factor β signaling in systemic sclerosis fibroblasts. *Arthritis Rheum.* **52**, 2897-2905 (2005).
180. Munger, J.S. *et al.* A mechanism for regulating pulmonary inflammation and fibrosis: the integrin $\alpha \nu \beta 6$ binds and activates latent TGF β 1. *Cell* **96**, 319-328 (1999).
181. Mu, D. *et al.* The integrin $\alpha \nu \beta 8$ mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF- β 1. *J. Cell Biol.* **157**, 493 (2002).
182. Abram, C.L. & Lowell, C.A. The ins and outs of leukocyte integrin signaling. *Annu. Rev. Immunol.* **27**, 339-362 (2009).
183. Buscemi, L. *et al.* The single-molecule mechanics of the latent TGF- β 1 complex. *Curr. Biol.* **21**, 2046-2054 (2011).
184. Shi, M. *et al.* Latent TGF- β structure and activation. *Nature* **474**, 343 (2011).
185. Ouwehand, K. *et al.* CXCL12 is essential for migration of activated Langerhans cells from epidermis to dermis. *Eur. J. Immunol.* **38**, 3050-3059 (2008).
186. Johnson, L.A. *et al.* An inflammation-induced mechanism for leukocyte transmigration across lymphatic vessel endothelium. *J. Exp. Med.* **203**, 2763-2777 (2006).
187. Ling, G.S. *et al.* Integrin CD11b positively regulates TLR4-induced signalling pathways in dendritic cells but not in macrophages. *Nat. Commun.* **5**, 3039 (2014).
188. Kagan, J.C. & Medzhitov, R. Phosphoinositide-mediated adaptor recruitment controls toll-like receptor signaling. *Cell* **125**, 943-955 (2006).
189. Zanoni, I. *et al.* CD14 Controls the LPS-induced endocytosis of toll-like receptor 4. *Cell* **147**, 868-880 (2011).
190. Caton, M.L., Smith-Raska, M.R. & Reizis, B. Notch-RBP-J signaling controls the homeostasis of CD8- dendritic cells in the spleen. *J. Exp. Med.* **204**, 1653-1664 (2007).
191. Haling, J.R., Monkley, S.J., Critchley, D.R. & Petrich, B.G. Talin-dependent integrin activation is required for fibrin clot retraction by platelets. *Blood* **117**, 1719-1722 (2011).
192. Yago, T. *et al.* Blocking neutrophil integrin activation prevents ischemia-reperfusion injury. *J. Exp. Med.* **212**, 1267-1281 (2015).
193. Kuhn, R., Schwenk, F., Aguet, M. & Rajewsky, K. Inducible gene targeting in mice. *Science* **269**, 1427-1429 (1995).
194. Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**, 676-682 (2012).
195. Larsen, C.P. *et al.* Migration and maturation of Langerhans cells in skin transplants and explants. *J. Exp. Med.* **172**, 1483-1493 (1990).
196. Stenn, K., Link, R., Moellmann, G., Madri, J. & Kuklinska, E. *Dispase, a neutral protease from bacillus polymyxa, is a powerful fibronectinase and type iv collagenase*, vol. 93, 1989.
197. Zhang, X. *et al.* Talin depletion reveals independence of initial cell spreading from integrin activation and traction. *Nat. Cell Biol.* **10**, 1062-1068 (2008).

198. Debrand, E. *et al.* Mice carrying a complete deletion of the talin2 coding sequence are viable and fertile. *Biochem. Biophys. Res. Commun.* **426**, 190-195 (2012).
199. Senetar, M.A. & McCann, R.O. Gene duplication and functional divergence during evolution of the cytoskeletal linker protein talin. *Gene* **362**, 141-152 (2005).
200. Ratzinger, G. *et al.* Matrix metalloproteinases 9 and 2 are necessary for the migration of langerhans cells and dermal dendritic cells from human and murine skin. *J. Immunol.* **168**, 4361-4371 (2002).
201. Naik, S.H. *et al.* Intrasplenic steady-state dendritic cell precursors that are distinct from monocytes. *Nat. Immunol.* **7**, 663-671 (2006).
202. Song, J.-H. *et al.* CCR7-CCL19/CCL21-regulated dendritic cells are responsible for effectiveness of sublingual vaccination. *J. Immunol.* **182**, 6851-6860 (2009).
203. Haessler, U., Pisano, M., Wu, M. & Swartz, M.A. Dendritic cell chemotaxis in 3D under defined chemokine gradients reveals differential response to ligands CCL21 and CCL19. *Proc. Natl. Acad. Sci. USA* **108**, 5614-5619 (2011).
204. Gaspari Anthony, A., Katz Stephen, I. & Martin Stefan, F. Contact hypersensitivity. *Curr. Protoc. Immunol.* **113**, 4.2.1-4.2.7 (2016).
205. Aiba, S. Dendritic cells—importance in allergy—. *Allergol. Int.* **56**, 201-208 (2007).
206. Lemus, R. & Karol, M.H. Conjugation of Haptens. In: Jones, M.G. & Lympary, P. (eds). *Allergy Methods and Protocols*. Humana Press: Totowa, NJ, 2008, pp 167-182.h
207. Gefen, T. *et al.* The effect of haptens on protein-carrier immunogenicity. *Immunology* **144**, 116-126 (2015).
208. Saint-Mezard, P. *et al.* Afferent and efferent phases of Allergic Contact Dermatitis (ACD) can be induced after a single skin contact with haptens: evidence using a mouse model of primary ACD. *J. Invest. Dermatol.* **120**, 641-647 (2003).
209. Clausen, B.E. & Stoitzner, P. Functional specialization of skin dendritic cell subsets in regulating t cell responses. *Front. Immunol.* **6**, 534 (2015).
210. Bennett, C.L., Noordegraaf, M., Martina, C.A.E. & Clausen, B.E. Langerhans cells are required for efficient presentation of topically applied hapten to t cells. *J. Immunol.* **179**, 6830-6835 (2007).
211. Gomez de Agüero, M. *et al.* Langerhans cells protect from allergic contact dermatitis in mice by tolerizing CD8(+) T cells and activating Foxp3(+) regulatory T cells. *J. Clin. Invest.* **122**, 1700-1711 (2012).
212. Esser, P.R. *et al.* Contact sensitizers induce skin inflammation via ros production and hyaluronic acid degradation. *PLOS ONE* **7**, e41340 (2012).
213. Shimada, M. *et al.* Hyaluronan fragments generated by sperm-secreted hyaluronidase stimulate cytokine/chemokine production via the TLR2 and TLR4 pathway in cumulus cells of ovulated COCs, which may enhance fertilization. *Development* **135**, 2001 (2008).
214. Scheibner, K.A. *et al.* Hyaluronan fragments act as an endogenous danger signal by engaging TLR2. *J. Immunol.* **177**, 1272 (2006).
215. Kaplan, D.H., Igyártó, B.Z. & Gaspari, A.A. Early events in the induction of allergic contact dermatitis. *Nat. Rev. Immunol.* **12**, 114-124 (2012).
216. Martin, S.F. *et al.* Toll-like receptor and IL-12 signaling control susceptibility to contact hypersensitivity. *J. Exp. Med.* **205**, 2151-2162 (2008).
217. Weber, F.C. *et al.* Lack of the purinergic receptor P2X(7) results in resistance to contact hypersensitivity. *J. Exp. Med.* **207**, 2609-2619 (2010).
218. Price, J.G. *et al.* CDKN1A regulates Langerhans cell survival and promotes Treg cell generation upon exposure to ionizing irradiation. *Nat. Immunol.* **16**, 1060 (2015).
219. Kwon, H.-J. *et al.* Stepwise phosphorylation of p65 promotes NF- κ B activation and NK cell responses during target cell recognition. *Nat. Commun.* **7**, 11686 (2016).
220. Christian, F., Smith, E.L. & Carmody, R.J. The regulation of nf- κ b subunits by phosphorylation. *Cells* **5**, 12 (2016).
221. Kaisho, T. & Akira, S. Dendritic-cell function in Toll-like receptor- and MyD88-knockout mice. *Trends Immunol.* **22**, 78-83 (2001).
222. Macedo, G.C. *et al.* Central role of MyD88-dependent dendritic cell maturation and proinflammatory cytokine production to control *brucella abortus* infection. *J. Immunol.* **180**, 1080-1087 (2008).
223. Klaska Izabela, P., Muckersie, E., Martin-Granados, C., Christofi, M. & Forrester John, V. Lipopolysaccharide-primed heterotolerant dendritic cells suppress experimental autoimmune uveoretinitis by multiple mechanisms. *Immunology* **150**, 364-377 (2016).
224. Siegemund, S. *et al.* Conventional bone marrow-derived dendritic cells contribute to toll-like receptor-independent production of alpha/beta interferon in response to inactivated parapoxvirus ovis. *J. Virol.* **83**, 9411-9422 (2009).
225. Krummen, M. *et al.* Release of IL-12 by dendritic cells activated by TLR ligation is dependent on MyD88 signaling, whereas TRIF signaling is indispensable for TLR synergy. *J. Leukoc. Biol.* **88**, 189-199 (2010).
226. Tseng, P.-H. *et al.* Different modes of TRAF3 ubiquitination selectively activate type I interferon and proinflammatory cytokine expression. *Nat. Immunol.* **11**, 70-75 (2010).
227. Laird, M.H.W. *et al.* TLR4/MyD88/PI3K interactions regulate TLR4 signaling. *J. Leukoc. Biol.* **85**, 966-977 (2009).
228. Miletic, A.V. *et al.* Vav proteins control MyD88-dependent oxidative burst. *Blood* **109**, 3360 (2007).
229. Tadokoro, S. *et al.* Talin binding to integrin β tails: a final common step in integrin activation. *Science* **302**, 103 (2003).

230. Stefanini, L. *et al.* A talin mutant that impairs talin-integrin binding in platelets decelerates $\alpha\text{IIb}\beta 3$ activation without pathological bleeding. *Blood* **123**, 2722-2731 (2014).
231. Atherton, P. *et al.* Vinculin controls talin engagement with the actomyosin machinery. *Nat. Commun.* **6**, 10038 (2015).
232. Bate, N. *et al.* Talin contains a C-terminal calpain2 cleavage site important in focal adhesion dynamics. *PLoS One* **7**, e34461 (2012).
233. Franco, S.J. *et al.* Calpain-mediated proteolysis of talin regulates adhesion dynamics. *Nat. Cell Biol.* **6**, 977-983 (2004).
234. Giannone, G., Jiang, G., Sutton, D.H., Critchley, D.R. & Sheetz, M.P. Talin1 is critical for force-dependent reinforcement of initial integrin-cytoskeleton bonds but not tyrosine kinase activation. *J. Cell Biol.* **163**, 409-419 (2003).
235. Goult, B.T. *et al.* Structural studies on full-length talin1 reveal a compact auto-inhibited dimer: implications for talin activation. *J. Struct. Biol.* **184**, 21-32 (2013).
236. Petrich, B.G. *et al.* Talin is required for integrin-mediated platelet function in hemostasis and thrombosis. *J. Exp. Med.* **204**, 3103-3111 (2007).
237. Kim, H.M. *et al.* Crystal structure of the TLR4-MD-2 complex with bound endotoxin antagonist Eritoran. *Cell* **130**, 906-917 (2007).
238. Ve, T., Williams, S.J. & Kobe, B. Structure and function of Toll/interleukin-1 receptor/resistance protein (TIR) domains. *Apoptosis* **20**, 250-261 (2015).
239. Venkatesan, N. *et al.* EZH2 promotes neoplastic transformation through VAV interaction-dependent extranuclear mechanisms. *Oncogene* **37**, 461-477 (2018).
240. García-Bernal, D. *et al.* Chemokine-induced zap70 kinase-mediated dissociation of the vav1-talin complex activates $\alpha 4\beta 1$ integrin for t cell adhesion. *Immunity* **31**, 953-964 (2009).
241. Manso, A.M. *et al.* Talin1 has unique expression versus talin 2 in the heart and modifies the hypertrophic response to pressure overload. *J. Biol. Chem.* **288**, 4252-4264 (2013).
242. Debrand, E. *et al.* Mice carrying a complete deletion of the talin2 coding sequence are viable and fertile. *Biochem. Biophys. Res. Commun.* **426**, 190-195 (2012).
243. Sun, Z., Guo, S.S. & Fässler, R. Integrin-mediated mechanotransduction. *J. Cell Biol.* **215**, 445-456 (2016).
244. Nagao, K. *et al.* Stress-induced production of chemokines by hair follicles regulates the trafficking of dendritic cells in skin. *Nat. Immunol.* **13**, 744-752 (2012).
245. Ginhoux, F. *et al.* Langerhans cells arise from monocytes in vivo. *Nat. Immunol.* **7**, 265-273 (2006).
246. Seré, K. *et al.* Two distinct types of langerhans cells populate the skin during steady state and inflammation. *Immunity* **37**, 905-916 (2012).
247. Mace, E.M., Monkley, S.J., Critchley, D.R. & Takei, F. A dual role for talin in NK cell cytotoxicity: activation of LFA-1-mediated cell adhesion and polarization of NK Cells. *J. Immunol.* **182**, 948 (2009).
248. Dong, G. *et al.* FOXO1 regulates dendritic cell activity through ICAM-1 and CCR7. *J. Immunol.* **194**, 3745-3755 (2015).
249. Mathas, S. *et al.* Aberrantly expressed c-Jun and JunB are a hallmark of Hodgkin lymphoma cells, stimulate proliferation and synergize with NF- κ B. *EMBO J.* **21**, 4104-4113 (2002).
250. Höpken, U.E. *et al.* Up-regulation of the chemokine receptor CCR7 in classical but not in lymphocyte-predominant Hodgkin disease correlates with distinct dissemination of neoplastic cells in lymphoid organs. *Blood* **99**, 1109-1116 (2002).
251. Sánchez-Sánchez, N., Riol-Blanco, L. & Rodríguez-Fernández, J.L. The multiple personalities of the chemokine receptor ccr7 in dendritic cells. *J. Immunol.* **176**, 5153-5159 (2006).
252. Yen, J.-H., Kong, W. & Ganea, D. Interferon beta inhibits dendritic cell migration through stat-1 mediated transcriptional suppression of CCR7 and metalloproteinase 9. *J. Immunol.* **184**, 3478-3486 (2010).
253. Murray, P.J. & Wynn, T.A. Protective and pathogenic functions of macrophage subsets. *Nat. Rev. Immunol.* **11**, 723-737 (2011).
254. Roberts, A.W. *et al.* Tissue-resident macrophages are locally programmed for silent clearance of apoptotic cells. *Immunity* **47**, 913-927 (2017).
255. Keck, S. *et al.* Absence of TRIF signaling in lipopolysaccharide-stimulated murine mast cells. *J. Immunol.* **186**, 5478-5488 (2011).
256. Loiarro, M. *et al.* Identification of critical residues of the MyD88 death domain involved in the recruitment of downstream kinases. *J. Biol. Chem.* **284**, 28093-28103 (2009).
257. Guven-Maiorov, E. *et al.* The architecture of the tir domain signalosome in the toll-like receptor-4 signaling Pathway. *Sci. Rep.* **5**, 13128 (2015).
258. Ohnishi, H. *et al.* Structural basis for the multiple interactions of the MyD88 TIR domain in TLR4 signaling. *Proc. Natl. Acad. Sci. USA* **106**, 10260-10265 (2009).
259. Avbelj, M., Horvat, S. & Jerala, R. The role of intermediary domain of MyD88 in cell activation and therapeutic inhibition of TLRs. *J. Immunol.* **187**, 2394-2404 (2011).
260. Janssens, S., Burns, K., Vercammen, E., Tschopp, J. & Beyaert, R. MyD88S, a splice variant of MyD88, differentially modulates NF- κ B- and AP-1-dependent gene expression. *FEBS Lett.* **548**, 103-107 (2003).
261. Janssens, S., Burns, K., Tschopp, J. & Beyaert, R. Regulation of interleukin-1- and lipopolysaccharide-induced NF- κ B activation by alternative splicing of MyD88. *Curr. Biol.* **12**, 467-471 (2002).
262. Burns, K. *et al.* Inhibition of interleukin 1 receptor/toll-like receptor signaling through the alternatively spliced, short form of MyD88 is due to its failure to recruit IRAK-4. *J. Exp. Med.* **197**, 263-268 (2003).

263. Ferrao, R. *et al.* IRAK4 dimerization and *trans*-autophosphorylation are induced by myddosome assembly. *Mol. Cell* **55**, 891-903 (2014).
264. Wong, K.-F., Luk, J.M., Cheng, R.H., Klickstein, L.B. & Fan, S.-T. Characterization of two novel LPS-binding sites in leukocyte integrin β A domain. *FASEB J.* **21**, 3231-3239 (2007).
265. Pan, Y. *et al.* Cation- π interaction regulates ligand-binding affinity and signaling of integrin α 4 β 7. *Proc. Natl. Acad. Sci. USA* **107**, 21388-21393 (2010).
266. Mayer, I.A. & Arteaga, C.L. The PI3K/AKT pathway as a target for cancer treatment. *Annu. Rev. Med.* **67**, 11-28 (2016).
267. Manning, B.D. & Toker, A. AKT/PKB Signaling: navigating the network. *Cell* **169**, 381-405 (2017).
268. Aksoy, E. *et al.* The p110 δ isoform of the kinase PI(3)K controls the subcellular compartmentalization of TLR4 signaling and protects from endotoxic shock. *Nat. Immunol.* **13**, 1045-1054 (2012).
269. Geering, B., Cutillas, P.R., Nock, G., Gharbi, S.I. & Vanhaesebroeck, B. Class IA phosphoinositide 3-kinases are obligate p85-p110 heterodimers. *Proc. Natl. Acad. Sci. USA* **104**, 7809 (2007).
270. Reynolds, L.F. *et al.* Vav1 Transduces T Cell Receptor Signals to the Activation of the Ras/ERK Pathway via LAT, Sos, and RasGRP1. *J. Biol. Chem.* **279**, 18239-18246 (2004).
271. Reynolds, L.F. *et al.* Vav1 transduces t cell receptor signals to the activation of phospholipase c- γ 1 via phosphoinositide 3-kinase-dependent and -independent pathways. *J. Exp. Med.* **195**, 1103-1114 (2002).
272. Manevich-Mendelson, E. *et al.* Talin1 is required for integrin-dependent B lymphocyte homing to lymph nodes and the bone marrow but not for follicular B-cell maturation in the spleen. *Blood* **116**, 5907-5918 (2010).
273. Lu, T.T. & Cyster, J.G. Integrin-mediated long-term b cell retention in the splenic marginal zone. *Science* **297**, 409-412 (2002).
274. Lo, C.G., Lu, T.T. & Cyster, J.G. Integrin-dependence of lymphocyte entry into the splenic white pulp. *J. Exp. Med.* **197**, 353-361 (2003).
275. Banno, A. *et al.* Subcellular localization of talin is regulated by inter-domain interactions. *J. Biol. Chem.* **287**, 13799-13812 (2012).
276. Boras, M. *et al.* Skap2 is required for β 2 integrin-mediated neutrophil recruitment and functions. *J. Exp. Med.* **214**, 851-874 (2017).
277. Manevich, E., Grabovsky, V., Feigelson, S.W. & Alon, R. Talin 1 and paxillin facilitate distinct steps in rapid v α 4-mediated adhesion strengthening to vascular cell adhesion molecule 1. *J. Biol. Chem.* **282**, 25338-25348 (2007).
278. Alon, R. *et al.* Alpha4beta1-dependent adhesion strengthening under mechanical strain is regulated by paxillin association with the alpha4-cytoplasmic domain. *J. Cell Biol.* **171**, 1073-1084 (2005).
279. Rose, D.M. The role of the α 4 integrin-paxillin interaction in regulating leukocyte trafficking. *Exp. Mol. Med.* **38**, 191 (2006).
280. Rose, D.M. *et al.* Paxillin binding to the α 4 integrin subunit stimulates LFA-1 (integrin α L β 2)-dependent T cell migration by augmenting the activation of focal adhesion kinase/proline-rich tyrosine kinase-2. *J. Immunol.* **170**, 5912 (2003).
281. Pillai, S. & Cariappa, A. The follicular versus marginal zone B lymphocyte cell fate decision. *Nat. Rev. Immunol.* **9**, 767-777 (2009).
282. Lim, T.J.F. & Su, I.H. Talin1 methylation is required for neutrophil infiltration and lipopolysaccharide-induced lethality. *J. Immunol.* **201**, 3651-3661 (2018).
283. Okada, T. *et al.* Chemokine requirements for B cell entry to lymph nodes and Peyer's patches. *J. Exp. Med.* **196**, 65-75 (2002).
284. Nie, Y. *et al.* The role of CXCR4 in maintaining peripheral B cell compartments and humoral immunity. *J. Exp. Med.* **200**, 1145-1156 (2004).
285. Wernimont, S.A. *et al.* Contact-dependent T cell activation and T cell stopping require talin1. *J. Immunol.* **187**, 6256-6267 (2011).
286. Simonson, W.T.N., Franco, S.J. & Huttenlocher, A. Talin1 regulates TCR-mediated LFA-1 function. *J. Immunol.* **177**, 7707-7714 (2006).
287. Gawden-Bone, C.M. *et al.* PIP5 kinases regulate membrane phosphoinositide and actin composition for targeted granule secretion by cytotoxic lymphocytes. *Immunity* **49**, 427-437 (2018).
288. Hammond, G.R.V. *et al.* PI4P and PI(4,5)P2 are essential but independent lipid determinants of membrane identity. *Science* **337**, 727-730 (2012).
289. Kagami, S., Rizzo, H.L., Kurtz, S.E., Miller, L.S. & Blauvelt, A. IL-23 and IL-17A, but not IL-12 and IL-22, are required for optimal skin host defense against *Candida albicans*. *J. Immunol.* **185**, 5453-5462 (2010).
290. Yuan, X. & Wilhelmus, K.R. Toll-like receptors involved in the pathogenesis of experimental *Candida albicans* keratitis. *Invest. Ophthalmol. Vis. Sci.* **51**, 2094-2100 (2010).

9. Author's publications

1. Neo, W. H., **Lim, J. F.**, Grumont, R., Gerondakis, S. and Su, I. C-rel regulates Ezh2 expression in activated lymphocytes and malignant lymphoid cells. *J. Biol. Chem.* **289**: 31693 – 31707 (2014).
2. **Lim, J. F.**, Berger, H. and Su, I. 2016. Purification and activation of murine lymphocytes. *J. Vis. Exp.* **116**: e54596, doi:10.3791/54596.
3. **Lim, T. J. F.** and Su, I. Talin1 methylation is required for neutrophil infiltration and lipopolysaccharide-induced lethality. *J. Immunol.* **201**, 3651 – 3661 (2018).
4. Loh, J. T., **Lim, T. J. F.**, Ikumi, K., Matoba, T., Janela, B., Gunawan, M., Toyama, T., Bunjamin, M., Ng, L.G., Poidinger, M., Morita, A., Ginhoux, F., Yamasaki, S., Lam, K.P. and Su, I. Ezh2 regulates host protection against allergic contact dermatitis through distinct mechanisms in DC subsets. *iScience* **10**, 23 – 39 (2018).
5. **Lim, T. J. F.**, Ruedl, C. and Su, I. A novel mechanism for talin1 to regulate inflammatory responses through TLR-signaling cascades. (Manuscript in preparation)

10. Posters and presentations

1. Lim, T. J. F. and Su, I. A novel mechanism for talin1 to regulate inflammatory responses through TLR-signaling cascades. Oral presentation. PhD Retreat (co-organized by NTU, NUS and SIgN), Singapore, November 2018.
2. Lim, T. J. F. and Su, I. Talin1-dependent assembly of TLR4 signaling complex is essential for the activation of dendritic cell and macrophage. Oral presentation. 10th Anniversary Symposium, Singapore, May 2018.
3. Lim, T. J. F. and Su, I. Talin1-dependent assembly of TLR4 signaling complex is essential for the activation of dendritic cell and macrophage. Poster presentation. CSH: Gene expression and signaling in the immune system, Cold Spring Harbor, New York, April 2018.
4. Lim, T. J. F. and Su, I. Talin1 regulates the migration of Langerhans cells from the skin to the lymph nodes. Poster presentation. 16th TNF Superfamily meeting, Singapore, April 2017.
5. Lim, T. J. F. and Su, I. Talin1 regulates the migration of Langerhans cells from the skin to the lymph nodes. Poster presentation. GRC: Directed Cell Migration, Galveston, Texas, January 2017.
6. Lim, T. J. F. and Su, I. Talin1 regulates the migration of Langerhans cells from the skin to the lymph nodes. Oral presentation. PhD Retreat (co-organized by NTU, NUS and SIgN), Singapore, November 2016.
7. Lim, T. J. F. and Su, I. Talin1 regulates the migration of Langerhans cells from the skin to the lymph nodes. Poster presentation. 8th International Singapore Symposium of Immunology, Singapore, May 2016.
8. Lim, T. J. F. and Su, I. Talin1 regulates the migration of Langerhans cells from the skin to the lymph nodes. Poster presentation. CSH: Gene expression and signaling in the immune system, Cold Spring Harbor, New York, April 2016.
9. Neo, W. H., Lim, T. J. F., Grumont, R., Gerondakis, S. and Su, I. C-rel regulates Ezh2 expression in activated lymphocytes and malignant lymphoid cells. Poster presentation. 6th Congress of FIMSA, Singapore, June 2015.