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2019

Seishima, R., & Barker, N. (2019). A contemporary snapshot of intestinal stem cells and their regulation. *Differentiation*, 108, 3-7. doi:10.1016/j.diff.2019.01.004

<https://hdl.handle.net/10356/145297>

<https://doi.org/10.1016/j.diff.2019.01.004>

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A contemporary snapshot of intestinal stem cells and their regulation

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Running title: Intestinal stem cells and their regulation

Key words: intestinal stem cell, Wnt signaling, stem cell niche

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Abstract

Intestinal stem cells (ISCs) marked by Lgr5 are located at the bottom of the epithelial crypt compartment. Canonical Wnt signaling, activated by Wnt/Rspo ligands, determines the stem cell identity of Lgr5⁺ ISCs and is strictly regulated by the ISC niche. Emerging evidence indicates that both epithelial and stromal compartments provide the requisite Wnt/Rspo ligands, confining the ISC niche to the lower crypt regions of the intestine. Recent studies have also shown that the ISC niche can reprogram differentiated cells to replenish lost ISCs following tissue injury, accounting for the epithelial cell plasticity within the crypt compartment. Here we review these recent advances and discuss the role of canonical Wnt signaling in maintaining homeostasis and effecting cell plasticity following tissue injury in the intestine, which could reveal potential novel therapeutic opportunities in the clinic.

1. Intestinal stem cells

The intestine plays important roles in absorbing nutrients from foods and serves as an essential barrier against the numerous potential pathogens present in the intestinal lumen. Optimal functionality of the intestinal epithelium is critical for achieving these tasks and is implemented by a rapid and continuous turnover of epithelial cells, served by rapidly dividing stem cells (Leblond and Walker, 1956; Potten and Loeffler, 1990). The intestine also displays a remarkable regeneration capacity following injury, driven by quiescent reserve stem cell populations and functional plasticity within more differentiated populations (Beumer and Clevers, 2016). The homeostatic intestinal stem cells (ISCs) are marked by *Lgr5* and reside at the crypt base in close association with immune responsive Paneth cells. ISCs divide every 24 hours (Barker et al., 2007) to generate a new population of transit-amplifying (TA) cells, which gradually migrate towards the top of the villi whilst differentiating into new secretory, enteroendocrine or absorptive cell lineages. These differentiated cells then undergo apoptosis and are finally shed into the lumen. Using fate-mapping approaches in mice, these self-renewing ISCs have been shown to generate all types of differentiated cells in the villi. *Lgr5*, the marker of ISCs, is one of three highly related 7-transmembrane domain receptors which bind secreted R-spondins (*Rsp*) with high affinity and serves to amplify canonical Wnt signaling, underscoring the importance of Wnt signaling in ISC maintenance (Figure 1) (Carmon et al., 2011; de Lau et al., 2011; Glinka et al., 2011). Functional heterogeneity exists within the *Lgr5*⁺ cell compartment, with some cells behaving as dedicated Paneth cell & enteroendocrine cell precursors (Buczaki et al, Nature 2013), likely dictated by local differences in niche signals (Snippert et al., 2010; Kozar et al., 2013).

2. Regulation of ISCs by Wnt signaling

Abrogation of canonical Wnt signaling in the intestine through genetic ablation of either the major Wnt effector protein, *Tcf4*, or transgenic overexpression of the secreted Wnt antagonist *Dickkopf-1* in adult mice severely impairs ISC-driven epithelial renewal and secretory cell specification, highlighting the critical role of Wnt signaling in ISC function and lineage commitment *in vivo* (Korinek et al., 1998; van Es et al. 2012; Pinto et al., 2003; Kuhnert et al., 2004). Purified *Lgr5*⁺ cells isolated from adult crypts exhibit robust expression of numerous Wnt target genes including *Sox9*, *Ascl2*, *EphB2*, *Troy* and *Axin2*, further underscoring the

robust Wnt signaling that exists within the stem cell zone at the crypt base (Muñoz et al., 2012; Van der Flier et al., 2007).

Rspo proteins that are known to amplify Wnt signaling through direct binding with Lgr5 (Kim et al., 2005; de Lau et al., 2011; Ruffner et al., 2012), have also been shown to be critical for intestinal homeostasis. *In vivo* administration of exogenous Rspo1 drives expansion of ISC numbers and enhances proliferation within the stem cell/TA cell compartments (Kim et al., 2005). Rspo1 is also an essential factor for establishing and maintaining *in vitro* organoids, which are near-physiological 3D primary epithelial structures (Sato et al., 2009). Importantly, *in vitro* Wnt signaling levels are also critical for regulating the balance between expansion and terminal differentiation of intestinal organoids. Robust Wnt signaling driven by additional of Wnt3a promotes a highly proliferative, stem-like organoid phenotype, whereas the absence of Wnt3a directs to epithelial cell differentiation and spontaneous reorganization of the organoids into crypt-like buds connected by villus-like domains (Sato and Clevers, 2013). These *in vitro* observations suggest that Wnt signaling also plays a key role in organizing stem cells in the intestinal epithelium. *In vivo* anti-Rspo2/3 treatment using neutralizing antibodies does not impact intestinal homeostasis or crypt structure, but robustly impairs epithelial regeneration following irradiation, highlighting the role of Rspo2/3 in intestinal repair following injury (Storm et al., 2016). Recently, Rspos have been reported to be a stronger modulator of ISC proliferation than Wnt ligands (Yan et al., 2017). Wnts are postulated to act as priming factors that fine-tune the expression levels of Rspo receptors, whereas the actual proliferation of ISCs and the epithelial turnover rate are primarily determined by Rspos. Thus, exploring the source of Rspos in the epithelium/stroma of stem cell zone at the bottom of the crypt may have greater functional importance than previously appreciated.

3. Paneth cells as ISC niche

In the small intestine, Paneth cells serve as an epithelial niche component for ISCs by providing many of the requisite stem cell factors, such as Wnt3 or EGF (Sato et al., 2011). When ISCs are co-cultured with Paneth cells, their organoid formation capacity is sustained even without exogenous Wnt. Importantly, when Wnt secretion from the Paneth cells is pharmacologically inhibited, organoid outgrowth is prevented, confirming the Paneth cells as a critical epithelial source of Wnts. Although Paneth cells do not exist in colon, CD24+ cells and C-Kit+

colonic goblet cells, which reside adjacent to the ISCs, have been identified as essential niche components in the colon (Rothenberg et al., 2012).

Despite the critical role for Paneth cells as stem cell niche components *in vitro*, conditional depletion of Paneth cells *in vivo* does not show a major effect on intestinal homeostasis or epithelial regeneration following injury. (Kim et al., 2012; Durand et al., 2012). In addition, conditional *in vivo* deletion of *Wnt3* (Farin et al., 2012) or the general blockade of Wnt secretion by *Porcn* or *Wntless* ablation (Kabiri et al., 2014; San Roman et al., 2014; Valenta et al., 2016) in the intestinal epithelium has no major influence on either epithelial homeostasis or injury repair. These observations collectively indicate that Paneth cells are an important epithelial niche component, but are redundant with other, non-epithelial, sources of essential stem cell factors.

4. Pericryptal mesenchymal cells as ISC niche components

Recently, pericryptal mesenchymal cells, which reside within the stroma adjacent to the intestinal crypts, have been identified as an additional source of Wnt ligands (Table 1). Indeed, several Wnts such as Wnt2b, Wnt4 and Wnt5a are endogenously expressed in the intestinal stroma (Gregorieff et al., 2005). In particular, Wnt2b is a ligand for Fzd7, which is highly expressed in Lgr5⁺ ISCs and is required for their maintenance (Flanagan et al., 2015). The local stroma is also considered to be an important source of Rspodin proteins, with endogenous Rspo1–3 expression being largely confined to the intestinal stromal fraction *in vivo* (Kabiri et al., 2014). The emerging importance of stromal-resident mesenchymal populations as endogenous sources of critical growth factors has fuelled intensive research efforts to decipher their contribution to ISC-driven epithelial homeostasis, regeneration and disease in recent years.

A subpopulation of pericryptal mesenchymal cells marked by Foxl1 was shown to express Wnt2b and Rspo3, as well as the BMP inhibitors Gremlin 1/2 (Aoki et al., 2016). Conditional ablation of these Foxl1⁺ mesenchymal cells in mice impairs the proliferation of intestinal epithelial cells with a loss of active Wnt signaling and a reduced expression of the stem cell marker Olfm4 (Aoki et al., 2016). Similarly, CD34⁺Gp38⁺ stromal cells, which also express Foxl1, were found to express Wnt2b and Rspo1, and contribute to the growth and maintenance of stemness in intestinal organoid culture (Stzepourginski et al., 2017). The Foxl1⁺ and CD34⁺Gp38⁺ stromal populations likely overlap since CD34⁺Gp38⁺ stromal cells also express Foxl1 (Stzepourginski et al., 2017).

Mesenchymal cells marked by either Gli1 or Acta2 also express high levels of Wnt2b and Wnt5a (Valenta et al., 2016). Despite these Wnt-secreting cells being present in the pericryptal region, they lacked Foxl1 expression, highlighting the phenotypic heterogeneity of the Wnt-secreting mesenchyme around the crypts (Valenta et al., 2016). Body-wide blockade of Wnt secretion through conditional ablation of Wntless, a protein essential for secretion of palmitoylated Wnt ligands, severely impaired intestinal homeostasis (Valenta et al., 2016). In contrast, epithelial homeostasis was maintained following epithelial-specific ablation of Wntless, highlighting the essential role of the mesenchymal Wnt source *in vivo* (Valenta et al., 2016). Acta2 is a marker for subepithelial myofibroblasts that support the growth of intestinal organoids as a feeder of Wnt/Rspo ligands when co-cultured with ISCs (Lahar et al., 2011; Kabiri et al., 2014; Lei et al., 2014). Similarly, Gli1 expressing cells also contribute to the stem cell niche by producing various Wnts and Rspo3 in both the colon and the small intestine (Degirmenci et al., 2018).

PDGFR α is another marker for pericryptal myofibroblasts that secrete various Wnts and Rspo3 (Greicius et al., 2018). Subepithelial telocytes, characterized as PDGFR α /Foxl1-positive cells were also shown to express Wnt2b, Wnt5a and Rspo3 (Shoshkes-Carmel et al., 2018). PDGFR α positive cells are not restricted to the pericryptal region, but there is a gradation of Wnt and Rspo expression in these cells, with highest levels found in the pericryptal region.

In contrast to the observations of Valenta et al, an independent study targeting *in vivo* ablation of Porcn-dependent Wnt secretion within both the epithelial and mesenchymal compartments found no significant impact on intestinal homeostasis (San Roman et al., 2014). These apparently contradictory results likely reflect the efficacy of the different Cre drivers used, with RosaCreERT2 achieving more uniform ablation of Wnt secretion in the mesenchymal compartment than Myh11-CreERT2. Overall, the various studies discussed here do support a major role for the mesenchymal compartment as an *in vivo* Wnt source for the ISC's and further highlight their phenotypic heterogeneity and likely functional redundancy.

Collectively, studies in this field suggest that there are multiple types of cells acting as ISC niche components by regulating Wnt signaling. Redundant Wnt signals both from various pericryptal mesenchymal cells and crypt base resident epithelial cells support the ISCs (Figure 2).

5. Intestinal cell plasticity

The intestine displays an impressive inherent ability to regenerate following major injury due to local infection or cytotoxic cancer therapies. Studies have shown that the resident stem cells readily succumb to such damage agents, raising the question of how the intestinal epithelium is subsequently able to effect a rapid renewal process. Recent studies have identified epithelial plasticity as being a critical component of this survival mechanism in the gut. Dll1+ secretory progenitor cells, for example, are typically short-lived and confined to secretory fates. However, once crypt-resident ISCs are depleted, Dll1 positive secretory progenitor cells can regain Lgr5+ stem cell status in response to injury (vans Es et al., 2012). Similarly, Paneth cells (Schmitt et al., 2018; Yu et al., 2018), enteroendocrine cells (Yan et al., 2017) and absorptive cells (Tetteh et al., 2016) have all been shown to possess at least some potential to restore the crypt stem cell pool following damage or loss of the endogenous ISC population. The +4 cells, defined as bordering the Paneth cell compartment at the crypt base, have been proposed as a dedicated reserve stem cell population independent of the active Lgr5-expressing ISCs (Dekaney et al., 2009). These cells, marked by Bmi1 (Sangiorgi and Capecchi, 2008; Tian et al., 2011), Lrig1 (Powell et al., 2012; Yan et al., 2012), Hopx (Takeda et al., 2011), or Tert (Montgomery et al., 2011) are relatively slow-cycling and generally do not contribute to homeostatic epithelial renewal (Barker et al., 2012). However, following injury-driven loss of Lgr5+ ISCs, these 'reserve stem cells' can be activated to proliferate and function, as multipotent ISCs to maintain epithelial integrity (Montgomery et al., 2011; Tian et al., 2011; Powell et al., 2012). The precise functional relationship of Lgr5+ ISCs and the various +4 populations during intestinal homeostasis and regeneration remains unclear. However, these two populations likely overlap to some extent due to the endogenous plasticity within the crypt base compartment (Itzkovitz et al., 2012; Muñoz et al., 2012). Indeed, a subset of Lgr5+ cells are quiescent secretory precursors and co-express +4 cell markers (Buczacki et al., 2013). Following tissue damage, these precursor cells that are typically confined to secretory fates can be dedifferentiated to multipotent stem cell status (Buczacki et al., 2013). Thus, it appears that the remarkable ability of the crypts to survive major injury is largely due to the inherent plasticity bestowed on non-stem cell epithelial cells by the local microenvironment that facilitates their rapid conversion into active ISC's to ensure the restoration of epithelial renewal.

6. Regulation of cell plasticity

In contrast to Lgr5+ ISCs, +4 cells exhibit a relatively low level of canonical Wnt signaling. Based on transcriptional profiling, Wnt target gene expression is significantly lower in Bmi1+ cells or Hopx+ cells compared with Lgr5+ ISCs. (Takeda et al., 2011; Li et al., 2014). Bmi1+ cells have also been postulated to be unresponsive to the activation or inhibition of Wnt signaling (Yan et al., 2012), although sorted +4 cells generate organoids *in vitro* using the same Wnt/Rspo+ conditions used for Lgr5+ cells, which suggests a reliance on Wnt signaling *in vitro* (Tian et al., 2011; Powell et al., 2012; Yan et al., 2012). Moreover, it has been shown that Wnt/ β catenin activation is indispensable for the exit from quiescence in Tert+ +4 cells after irradiation (Suh et al., 2017).

During the process of dedifferentiation and acquisition of multipotency in Paneth cells following ISC loss, Wnt signaling is activated through SCF/c-kit signaling (Schmitt et al., 2018). Notch signaling is also reported to be indispensable for Paneth cells to dedifferentiate after irradiation injury (Yu et al., 2018). Similarly, secretory progenitor cells express high level of *Ascl2*, one of the Wnt target genes, during the dedifferentiation process, indicating the activation of Wnt signaling during this process (Buczacki et al., 2013; Schuijers et al., 2015).

Collectively, although epithelial cell plasticity within the crypt compartment has been recently established, it is still largely unknown how differentiated cells undergo dedifferentiation or the precise role of Wnt signaling in this process.

7. Conclusions

Emerging evidence indicates that the *in vivo* source of the various Wnts required for maintenance of the ISC compartment is likely to be confined to the lower crypt regions of the intestine. A better understanding of how pericryptal mesenchymal cells regulate ISCs during homeostasis will help us decipher the mechanisms of both normal intestinal regeneration and dysregulation associated with various intestinal diseases. This knowledge will also be instrumental in helping to develop novel therapies targeting activation of endogenous ISCs to effect more efficient epithelial regeneration following chemotherapy or disease. Given the critical function of Rspos in ensuring maintenance of the ISC pool, further exploration of the *in vivo* source and biology of Rspos should further enhance our understanding of ISC regulation during homeostasis, regeneration and disease. Indeed, exogenous administration of Rspo1 improves recovery

from injury after chemoradiation in mice (Kim et al., 2005), indicating a potential clinical application. In addition, the role of Wnt signaling in effecting cell plasticity following tissue injury in the intestine demands further exploration to reveal potentially novel therapeutic opportunities in the clinic.

Accepted manuscript

References

- Aoki R, Shoshkes-Carmel M, Gao N, Shin S, May CL, Golson ML, Zahm AM, Ray M, Wiser CL, Wright CV, Kaestner KH., 2015. Foxl1-expressing mesenchymal cells constitute the intestinal stem cell niche. *Cell Mol Gastroenterol Hepatol.* 2(2), 175–188.
- Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ, Clevers H., 2007. Identification of stem cells in small intestine and colon by marker gene *Lgr5*. *Nature* 449, 1003–1007.
- Barker N, van Oudenaarden A, Clevers H., 2012. Identifying the stem cell of the intestinal crypt: strategies and pitfalls. *Cell Stem Cell* 11, 452–460.
- Beumer J, Clevers H., 2016. Regulation and plasticity of intestinal stem cells during homeostasis and regeneration. *Development* 143, 3639–3649.
- Buczacki SJ, Zecchini HI, Nicholson AM, Russell R, Vermeulen L, Kemp R, Winton DJ., 2013. Intestinal label-retaining cells are secretory precursors expressing *Lgr5*. *Nature* 495, 65–69.
- Carmon KS, Gong X, Lin Q, Thomas A, Liu Q., 2011. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. *Proc Natl Acad Sci U S A.* 108, 11452–7.
- Degirmenci B, Valenta T, Dimitrieva S, Hausmann G, Basler K. 2018. GLI1-expressing mesenchymal cells form the essential Wnt-secreting niche for colon stem cells. *Nature* 558, 449–453.
- Dekaney CM, Gulati AS, Garrison AP, Helmrath MA, Henning SJ., 2009. Regeneration of intestinal stem/progenitor cells following doxorubicin treatment of mice. *Am J Physiol Gastrointest Liver Physiol.* 297, G461–470.
- de Lau W, Barker N, Low TY, Koo BK, Li VS, Teunissen H, Kujala P, Haegebarth A, Peters PJ, van de Wetering M, Stange DE, van Es JE, Guardavaccaro D, Schasfoort RB, Mohri Y, Nishimori K, Mohammed S, Heck AJ, Clevers H. 2011. *Lgr5* homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature.* 476, 293–297.
- Durand A, Donahue B, Peignon G, Letourneur F, Cagnard N, Slomianny C, Perret C, Shroyer NF, Romagnolo B. 2012. Functional intestinal stem cells

- after Paneth cell ablation induced by the loss of transcription factor Math1 (Atoh1). *Proc Natl Acad Sci U S A.* 109, 8965–70.
- Farin HF, Van Es JH, Clevers H., 2012. Gastroenterology. Redundant sources of Wnt regulate intestinal stem cells and promote formation of Paneth cells. 143, 1518–1529.
- Flanagan DJ, Pheesse TJ, Barker N, Schwab RH, Amin N, Malaterre J, Stange DE, Nowell CJ, Currie SA, Saw JT, Beuchert E, Ramsay RG, Sansom OJ, Ernst M, Clevers H, Vincan E. 2015. Frizzled7 functions as a Wnt receptor in intestinal epithelial Lgr5(+) stem cells. *Stem Cell Reports* 4, 759–767.
- Glinka A, Dolde C, Kirsch N, Huang YL, Kazanskaya O, Ingelfinger D, Boutros M, Cruciat CM, Niehrs C., 2011. LGR4 and LGR5 are R-spondin receptors mediating Wnt/ β -catenin and Wnt/PCP signalling. *EMBO Rep.* 12, 1055–1061.
- Gregorieff A, Pinto D, Begthel H, Destree O, Kielman M, Clevers H., 2005. Expression pattern of Wnt signaling components in the adult intestine. *Gastroenterology* 129, 626–638.
- Greicius G, Kabiri Z, Sigmundsson K, Liang C, Bunte R, Singh MK, Virshup DM., 2018. *PDGFR α* ₊ pericryptal stromal cells are the critical source of Wnts and RSPO3 for murine intestinal stem cells in vivo. *Proc Natl Acad Sci U S A.* 115, E3173–E3181.
- Hong YK, Carrington J, Breault DT, Epstein J, Houchen CW, Lynch JP, Martin MG, Plevritis SK, Curtis C, Ji HP, Li L, Henning SJ, Wong MH, Kuo CJ., 2017. Intestinal Enteroendocrine Lineage Cells Possess Homeostatic and Injury-Inducible Stem Cell Activity. *Cell Stem Cell* 21, 78–90.
- Itzkovitz S, Blat IC, Jacks T, Clevers H, van Oudenaarden A., 2012. Optimality in the development of intestinal crypts. *Cell* 148, 608–619.
- Kabiri Z, Greicius G, Madan B, Biechele S, Zhong Z, Zaribafzadeh H, Edison, Aliyev J, Wu Y, Bunte R, Williams BO, Rossant J, Virshup DM., 2014. Stroma provides an intestinal stem cell niche in the absence of epithelial Wnts. *Development.* 141, 2206–2215.
- Kim KA, Kakitani M, Zhao J, Oshima T, Tang T, Binnerts M, Liu Y, Boyle B, Park E, Emtage P, Funk WD, Tomizuka K., 2005. Mitogenic influence of human R-spondin1 on the intestinal epithelium. *Science* 309, 1256–1259.

- Kim TH, Escudero S, Shivdasani RA., 2012. Intact function of Lgr5 receptor-expressing intestinal stem cells in the absence of Paneth cells. *Proc Natl Acad Sci U S A*. 109, 3932–7.
- Korinek V, Barker N, Moerer P, van Donselaar E, Huls G, Peters PJ, Clevers H., 1998. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat Genet*. 19, 379–83.
- Kozar S, Morrissey E, Nicholson AM, van der Heijden M, Zecchini HI, Kemp R, Tavaré S, Vermeulen L, and Winton DJ., 2013. Continuous clonal labeling reveals small numbers of functional stem cells in intestinal crypts and adenomas. *Cell Stem Cell* 13, 626–633.
- Kuhnert F, Davis CR, Wang HT, Chu P, Lee M, Yuan J, Nusse R, Kuo CJ., 2004. Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. *Proc Natl Acad Sci U S A*. 101, 266–271.
- Lahar N, Lei NY, Wang J, Jabaji Z, Tung SC, Joshi V, Lewis M, Stelzner M, Martín MG, Dunn JC., 2011. Intestinal subepithelial myofibroblasts support in vitro and in vivo growth of human small intestinal epithelium. *PLoS One*. 6, e26898.
- Leblond, C.P., Walker, B.E., 1956. Renewal of cell populations. *Physiol. Rev*. 36, 255–276.
- Lei NY, Jabaji Z, Wang J, Joshi VS, Brinkley GJ, Khalil H, Wang F, Jaroszewicz A, Pellegrini M, Li L, Lewis M, Stelzner M, Dunn JC, Martín MG., 2014. Intestinal subepithelial myofibroblasts support the growth of intestinal epithelial stem cells. *PLoS One*. 9, e84651.
- Li N, Yousefi M, Nakauka-Ddamba A, Jain R, Tobias J, Epstein JA, Jensen ST, Lengner CJ., 2014. Single-cell analysis of proxy reporter allele-marked epithelial cells establishes intestinal stem cell hierarchy. *Stem Cell Reports* 3, 876–891.
- Montgomery RK, Carlone DL, Richmond CA, Farilla L, Kranendonk ME, Henderson DE, Baffour-Awuah NY, Ambruzs DM, Fogli LK, Algra S, Breault DT., 2011. Mouse telomerase reverse transcriptase (mTert) expression marks slowly cycling intestinal stem cells. *Proc Natl Acad Sci U S A*. 108, 179–184.
- Muñoz J, Stange DE, Schepers AG, van de Wetering M, Koo BK, Itzkovitz S, Volckmann R, Kung KS, Koster J, Radulescu S, Myant K, Versteeg R, Sansom

OJ, van Es JH, Barker N, van Oudenaarden A, Mohammed S, Heck AJ, Clevers H., 2012. The Lgr5 intestinal stem cell signature: robust expression of proposed quiescent '+4' cell markers. *EMBO J.* 31, 3079–3091.

Pinto D, Gregorieff A, Begthel H, Clevers H., 2003. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. *Genes Dev.* 17, 1709–1713.

Potten, C.S., Loeffler, M., 1990. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. *Development* 110, 1001–1020.

Powell AE, Wang Y, Li Y, Poulin EJ, Means AL, Washington MK, Higginbotham JN, Juchheim A, Prasad N, Levy SE, Guo Y, Shyr Y, Aronow BJ, Haigis KM, Franklin JL, Coffey RJ., 2012. The pan-ErbB negative regulator Lrig1 is an intestinal stem cell marker that functions as a tumor suppressor. *Cell* 149, 146–158.

Rothenberg ME, Nusse Y, Kalisky T, Lee JJ, Dalerba P, Scheeren F, Lobo N, Kulkarni S, Sim S, Qian D, Beachy PA, Pasricha PJ, Quake SR, Clarke MF., 2012. Identification of a cKit(+) colonic crypt base secretory cell that supports Lgr5(+) stem cells in mice. *Gastroenterology* 142, 1195–1205.

Ruffner H, Sprunger J, Charlat O, Leighton-Davies J, Grosshans B, Salathe A, Zietzling S, Beck V, Therier M, Isken A, Xie Y, Zhang Y, Hao H, Shi X, Liu D, Song Q, Clay I, Hintzen G, Tchorz J, Bouchez LC, Michaud G, Finan P, Myer VE, Bouwmeester T, Porter J, Hild M, Bassilana F, Parker CN, Cong F., 2012. R-Spondin potentiates Wnt/ β -catenin signaling through orphan receptors LGR4 and LGR5. *PloS One* 7, e40976.

Sangiorgi E, Capecchi MR., 2008. Bmi1 is expressed in vivo in intestinal stem cells. *Nat Genet.* 40, 915–920.

San Roman AK, Jayewickreme CD, Murtaugh LC, Shivdasani RA., Stem Cell Reports. 2014. Wnt secretion from epithelial cells and subepithelial myofibroblasts is not required in the mouse intestinal stem cell niche in vivo. 2, 127–134.

Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, Clevers H., 2009. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 459, 262–265.

Sato T, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, Barker N, Shroyer NF, van de Wetering M, Clevers H., 2011. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature*. 469, 415–418.

Sato T, Clevers H., 2013. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. *Science* 340, 1190–1194.

Schmitt M, Schewe M, Sacchetti A, Feijtel D, van de Geer WS, Teeuwssen M, Sleddens HF, Joosten R, van Royen ME, van de Werken HJG, van Es J, Clevers H, Fodde R., 2018. Paneth Cells Respond to Inflammation and Contribute to Tissue Regeneration by Acquiring Stem-like Features through SCF/c-Kit Signaling. *Cell Rep*. 24, 2312–2328.

Schuijers J, Junker JP, Mokry M, Hatzis P, Koo BK, Sasselli V, van der Flier LG, Cuppen E, van Oudenaarden A, Clevers H., 2015. Ascl2 acts as an R-spondin/Wnt-responsive switch to control stemness in intestinal crypts. *Cell Stem Cell* 16, 158–170.

Shoshkes-Carmel M, Wang YJ, Wangenstein KJ, Tóth B, Kondo A, Massasa EE, Itzkovitz S, Kaestner KH., 2018. Subepithelial telocytes are an important source of Wnts that supports intestinal crypts. *Nature* 557, 242–246.

Snippert HJ, van der Flier LG, Sato T, van Es JH, van den Born M, Kroon-Veenboer C, Barker N, Klein AM, van Rheenen J, Simons BD, Clevers H., 2010. Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. *Cell* 143,134–144.

Storm EE, Durinck S, de Sousa EMF, Tremayne J, Kljavin N, Tan C, Ye X, Chiu C, Pham T, Hongo JA, Bainbridge T, Firestein R, Blackwood E, Metcalfe C, Stawiski EW, Yauch RL, Wu Y & de Sauvage FJ., 2016. Targeting PTPRK-RSPO3 colon tumours promotes differentiation and loss of stem-cell function. *Nature* 529, 97–100.

Stzepourginski I, Nigro G, Jacob JM, Dulauroy S, Sansonetti PJ, Eberl G, Peduto L., 2017. CD34+ mesenchymal cells are a major component of the intestinal stem cells niche at homeostasis and after injury. *Proc Natl Acad Sci U S A*. 114, E506–E513.

Suh HN, Kim MJ, Jung YS, Lien EM, Jun S, Park JI., 2017. Quiescence Exit of Tert⁺ Stem Cells by Wnt/ β -Catenin Is Indispensable for Intestinal Regeneration. *Cell Rep*. 21, 2571–2584.

- Takeda N, Jain R, LeBoeuf MR, Wang Q, Lu MM, Epstein JA., 2011. Interconversion between intestinal stem cell populations in distinct niches. *Science* 334, 1420–1424.
- Tetteh PW, Basak O, Farin HF, Wiebrands K, Kretschmar K, Begthel H, van den Born M, Korving J, de Sauvage F, van Es JH, van Oudenaarden A, Clevers H., 2016. Replacement of Lost Lgr5-Positive Stem Cells through Plasticity of Their Enterocyte-Lineage Daughters. *Cell Stem Cell* 18, 203–213.
- Tian H, Biehs B, Warming S, Leong KG, Rangell L, Klein OD, de Sauvage FJ., 2011. A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. *Nature* 478, 255–259.
- Valenta T, Degirmenci B, Moor AE, Herr P, Zimmerli D, Moor MB, Hausmann G, Cantù C, Aguet M, Basler K., 2016. Wnt Ligands Secreted by Subepithelial Mesenchymal Cells Are Essential for the Survival of Intestinal Stem Cells and Gut Homeostasis. *Cell Rep.* 15, 911–918.
- Van der Flier LG, Sabates-Bellver J, Oving I, Haegebarth A, De Palo M, Anti M, Van Gijn ME, Suijkerbuijk S, Van de Wetering M, Marra G, Clevers H., 2007. The Intestinal Wnt/TCF Signature. *Gastroenterology*. 5, 1263–1275.
- van Es JH, Haegebarth A, Kujala P, Itzkovitz S, Koo BK, Boj SF, Korving J, van den Born M, van Oudenaarden A, Robine S, Clevers H. 2012. A critical role for the Wnt effector Tcf4 in adult intestinal homeostatic self-renewal. *Mol Cell Biol.* 32, 1918-1927.
- van Es JH, Sato T, van de Wetering M, Lyubimova A, Yee Nee AN, Gregorieff A, Sasaki N, Zeinstra L, van den Born M, Korving J, Martens ACM, Barker N, van Oudenaarden A, Clevers H., 2012. Dll1+ secretory progenitor cells revert to stem cells upon crypt damage. *Nat Cell Biol.* 14, 1099–1104.
- Yan KS, Chia LA, Li X, Ootani A, Su J, Lee JY, Su N, Luo Y, Heilshorn SC, Amieva MR, Sangiorgi E, Capecchi MR, Kuo CJ., 2012. The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. *Proc Natl Acad Sci U S A.* 109, 466–471.
- Yan KS, Gevaert O, Zheng GXY, Anchang B, Probert CS, Larkin KA, Davies PS, Cheng ZF, Kaddis JS, Han A, Roelf K, Calderon RI, Cynn E, Hu X, Mandleywala K, Wilhelmy J, Grimes SM, Corney DC, Boutet SC, Terry JM, Belgrader P, Ziraldo SB, Mikkelsen TS, Wang F, von Furstenberg RJ, Smith NR,

Chandrakesan P, May R, Chrissy MAS, Jain R, Cartwright CA, Niland JC, Hong YK, Carrington J, Breault DT, Epstein J, Houchen CW, Lynch JP, Martin MG, Plevritis SK, Curtis C, Ji HP, Li L, Henning SJ, Wong MH, Kuo CJ., 2017, Intestinal Enteroendocrine Lineage Cells Possess Homeostatic and Injury-Inducible Stem Cell Activity. *Cell Stem Cell* 21, 78–90.

Yan KS, Janda CY, Chang J, Zheng GXY, Larkin KA, Luca VC, Chia LA, Mah AT, Han A, Terry JM, Ootani A, Roelf K, Lee M, Yuan J, Li X, Bolen CR, Wilhelmy J, Davies PS, Ueno H, von Furstenberg RJ, Belgrader P, Ziraldo SB, Ordonez H, Henning SJ, Wong MH, Snyder MP, Weissman IL, Hsueh AJ, Mikkelsen TS, Garcia KC, Kuo CJ., 2017. Non-equivalence of Wnt and R-spondin ligands during Lgr5⁺intestinal stem-cell self-renewal. *Nature* 545, 238–242.

Yu J, Virshup DM., 2014. Updating the Wnt pathways. *Biosci. Rep.* 34, 593–607.

Yu S, Tong K, Zhao Y, Balasubramanian I, Yap GS, Ferraris RP, Bonder EM, Verzi MP, Gao N., 2018. Paneth Cell Multipotency Induced by Notch Activation following Injury. *Cell Stem Cell* 23, 46–59.

Figure legends

Figure 1

A schematic of Wnt signaling activation by Lgr5/Rspo (Yu et al., 2014).

In the absence of Rspo, E3 ubiquitin ligases Rnf43/Znrf3 ubiquitinate Wnt receptors for degradation. When Rspo binds with Lgr5, this ligand-receptor pair directly inhibits Rnf43/Znrf3 to ubiquitinate Wnt receptors, and as a result, Wnt signaling is activated.

Rspo; R-spondin Rnf; Ring finger Znrf; Zinc and ring finger Fzd; Frizzled Lrp; Lipoprotein receptor-related protein Ub; ubiquitin

Figure 2

A schematic of intestinal crypt with subepithelial mesenchymal cells.

Intestinal stem cells are supported by both epithelial cells (Paneth cells) and various pericryptal mesenchymal cells which secrete Wnts/Rspos.

Table 1

The markers of reported pericryptal mesenchymal cells and their producing niche factors.

	Marker	Producing niche factors
Lei et al., 2014	Vimentin, α -SMA	Rspo2
Kabiri et al., 2014	Vimentin, α -SMA	Wnt3, Rspo3
Aoki et al., 2016	Foxl1	Rspo3, Wnt2b, Wnt4, Wnt5a, Grem1/2
Valenta et al., 2016	Gli1 and/or Foxl1	Wnt2b
Stzepourginski et al., 2017	CD34/Gp38	Wnt2b, Rspo1, Grem1
Degirmenci et al., 2018	Gli1	Wnt2, Wnt2b, Wnt4, Rspo3
Greicius et al., 2018	PDGFR α	Rspo3
Shoshkes-Carmel et al., 2018	PDGFR α /Foxl1	Wnt2b, Wnt5a, Rspo3