

# Elucidating the role of fat-inducing transmembrane protein 2 (Fit2) in insulin-producing beta-cells ( $\beta$ -cells)

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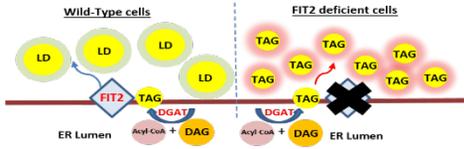
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**INTRODUCTION**

Fat-Inducing Transmembrane protein 2 (Fit2/Fitm2) is an evolutionarily conserved transmembrane protein. Located on the membrane of the endoplasmic reticulum(ER), Fit2 has been shown to play an important role [1] in the packaging of triglycerides into lipid droplets(LD) within adipocytes (Fig.1). Pancreatic beta-cells ( $\beta$ -cells) secrete insulin. In diabetes  $\beta$ -cell dysfunction is driven partly by lipid stress (lipotoxicity). Fit2 is expressed in  $\beta$ -cells but its function remains elusive.



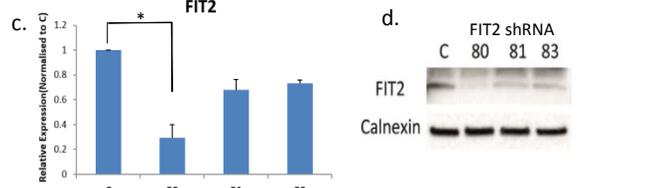
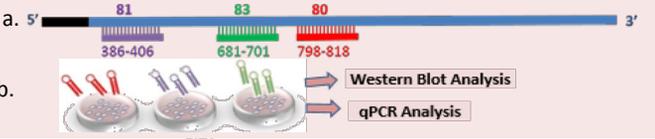
**Figure 1:** Illustration of Fat-Inducing Transmembrane Protein 2(Fit2) role in sequestering lipids into LD (lipid droplets). TAG: triglyceride, DAG: diacylglycerol, DGAT: diacylglycerol transferase, Acyl-CoA: Acyl-coenzyme A, ER: Endoplasmic Reticulum

**OBJECTIVES**

We hypothesise that Fit2 plays a protective role in  $\beta$ -cells by sequestering lipids, thereby reducing  $\beta$ -cell lipotoxicity. To test our hypothesis, we aim to knockdown(KD) Fit2 expression in  $\beta$ -cells to determine its effect on 1) lipid droplet packaging, 2) cell death and 3) cellular lipid metabolism.

**METHODS AND RESULTS**

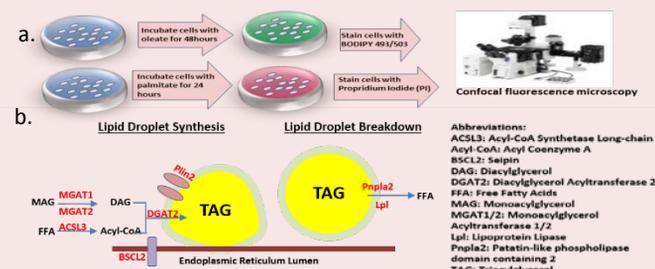
**I. Evaluating the efficiency of different FIT2 KD constructs in  $\beta$ -cells.** Three commercially available Fit2 shRNA sequences (80, 81 and 83) targeting Fit2 mRNA were tested in Min6 cells ( $\beta$ -cell line) after infection with the respective shRNA-containing lentiviruses.



**Figure 2. Knockdown of Fit2 in Min6 cells.** (a) Respective regions targeted by the 3 shRNA constructs on FIT2 transcript with 1643 bases in total (exon 1 in black, exon 2 in blue). (b) Min6 cells were infected with shRNA-containing lentiviruses for 18hours prior to harvesting. Efficiency of KD is confirmed by qPCR analysis and (d) western blot. Calnexin is used as loading control. Data represent mean  $\pm$ SEM, N=3-4, \*P<0.05

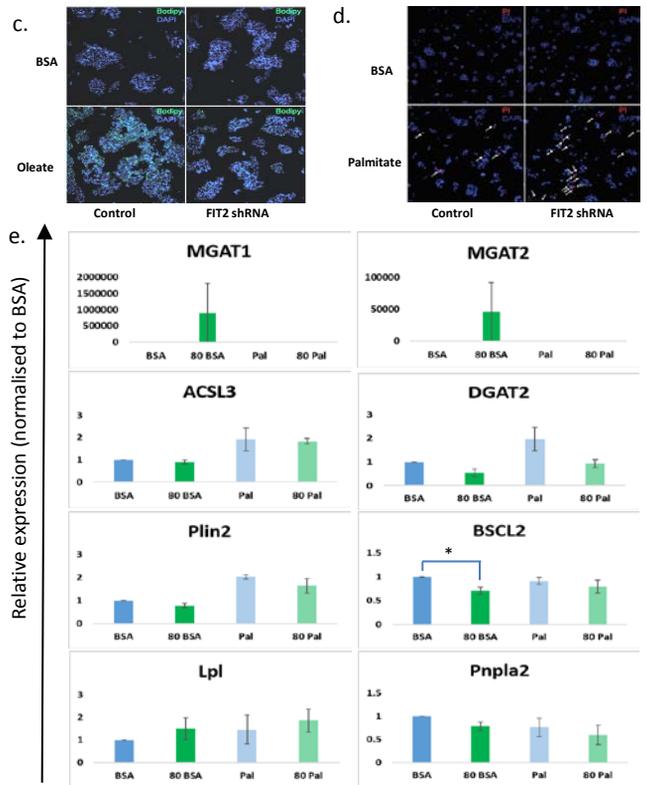
**II. Effect of FIT2 KD on LD formation, survival and gene expression of fat metabolism-related genes in Min6 cells**

FIT2 KD cells were treated with oleate to determine effect on LD formation. Palmitate unlike oleate induces cell death. Cells were treated with palmitate to determine effect of FIT2 KD on cell survival. Changes in expression of fat metabolism-related genes[2] were measured following palmitate exposure.



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**METHODS AND RESULTS (cont'd)**



**Figure 3. Effect of Fit2 KD on LD formation, cell death, lipogenesis- and lipolysis-gene expression.** (a) Cells treated with oleate and palmitate were stained and imaged using confocal fluorescence microscopy. (b) Sub-cellular localisation and function of selected lipid regulation proteins (in red). (c) Representative images showing LD formation (BODIPY 493/503-green) in control and FIT2 silenced Min6 cells after 48h oleate treatment. DAPI (purple) marks the nuclei. (d) Representative images showing cell death (Propidium Iodide(PI)-red, white arrows) after 24h palmitate treatment. DAPI (purple) marks nuclei. (e) Expression of different lipogenesis and lipolysis genes, relative to  $\beta$ -actin and normalised to wild-type cells treated with BSA (BSA). 80 BSA: FIT2 KD cells treated with BSA; Pal: wild-type cells treated with palmitate(Pal); 80Pal: FIT2 KD cells treated with palmitate. Data represent mean  $\pm$ SEM, N=4, \*P<0.05

**CONCLUSION & FUTURE WORK**

Among the three KD constructs tested, 80 was most efficient in silencing Fit2 expression in Min6 cells. For subsequent experiments, only the 80 Min6 stable line was used. Silencing of Fit2 decreases LD formation but increases cell death in Min6 cells, suggesting the Fit2-mediated LD formation is protective in  $\beta$ -cells.

Surprisingly, reduction of Fit2 resulted in a significant change in Bsc12 gene expression in Min6 cells. Further experiments are required to understand if Fit2 directly affects Bsc12 expression. In addition, a Fit2 rescue experiment is needed to determine possible off-target effects of shRNA clone 80.

**REFERENCES**

- Miranda et al. (2014). Fat storage-Inducing transmembrane protein 2 is required for normal fat storage in adipose tissue. *Journal of Biological Chemistry*, 289(14), 9560-9572
- Pol, A. et al. (2014) Biogenesis of the multifunctional lipid droplet: Lipids, proteins, and sites. *J. Cell Biol.* 204:635–646.