

Appendix for:

ABCA12 regulates insulin secretion from β -cells

Gloria M. Ursino^{*1}, Ying Fu^{*2}, Denny L. Cottle¹, Nigora Mukhamedova², Lynelle K. Jones¹, Hann Low², Ming Shen Tham¹, Wan Jun Gan³, Natalie A. Mellett², Partha P. Das¹, Jacquelyn M. Weir², Michael Ditiatkovski², Stacey Fynch⁴, Peter Thorn³, Helen E. Thomas⁴, Peter J. Meikle², Helena C. Parkington⁵, Ian M. Smyth^{^#1}, Dmitri Sviridov^{^2}.

Page 2:

Appendix Figure S1: Immunostaining of pancreatic islets, mouse targeting strategy and pituitary analysis.

Page 3:

Appendix Figure S2: Assessment of beta cell mass.

Page 4:

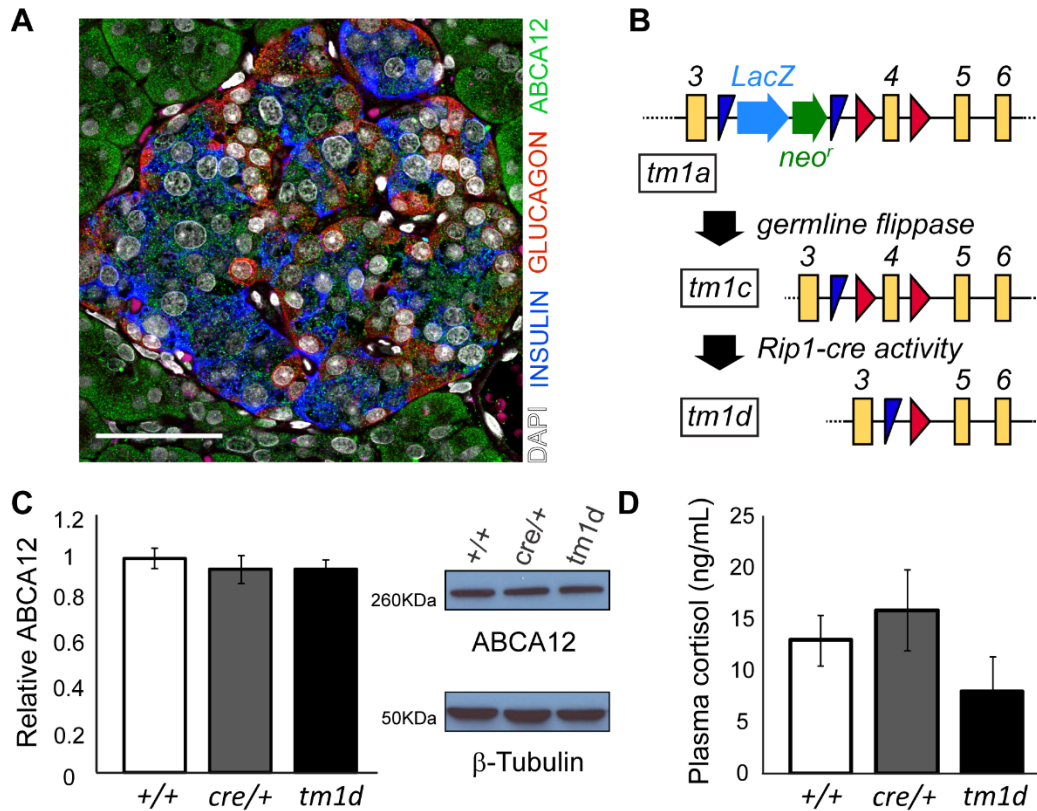
Appendix Figure S3: Gene expression and ceramide homeostasis in beta cells.

Page 5:

Appendix Figure S4: Gene expression changes in Abca12tm1d islets.

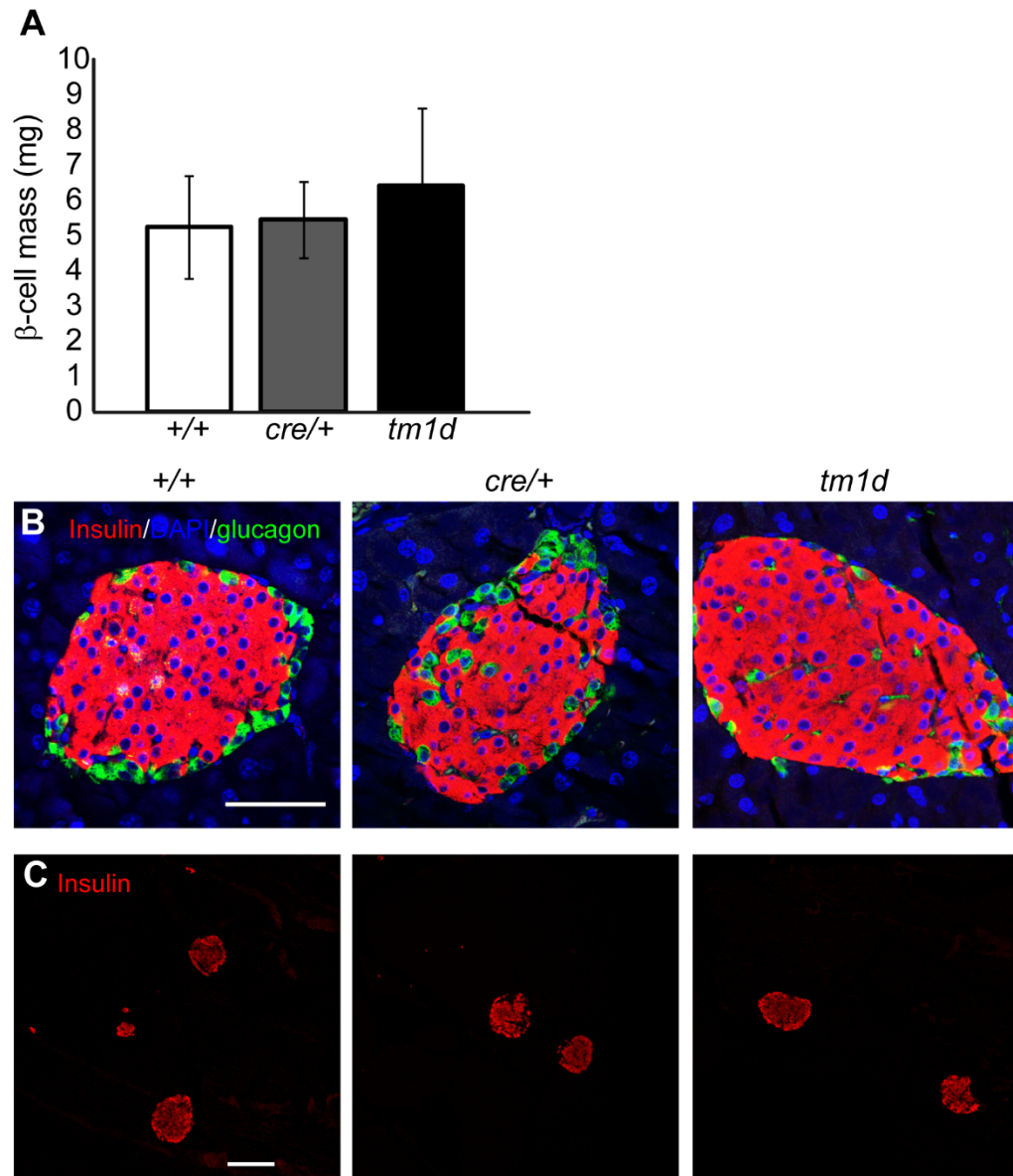
Page 6:

Appendix Figure S5: Quantitation of fatty acids in Abca12tm1d islets.



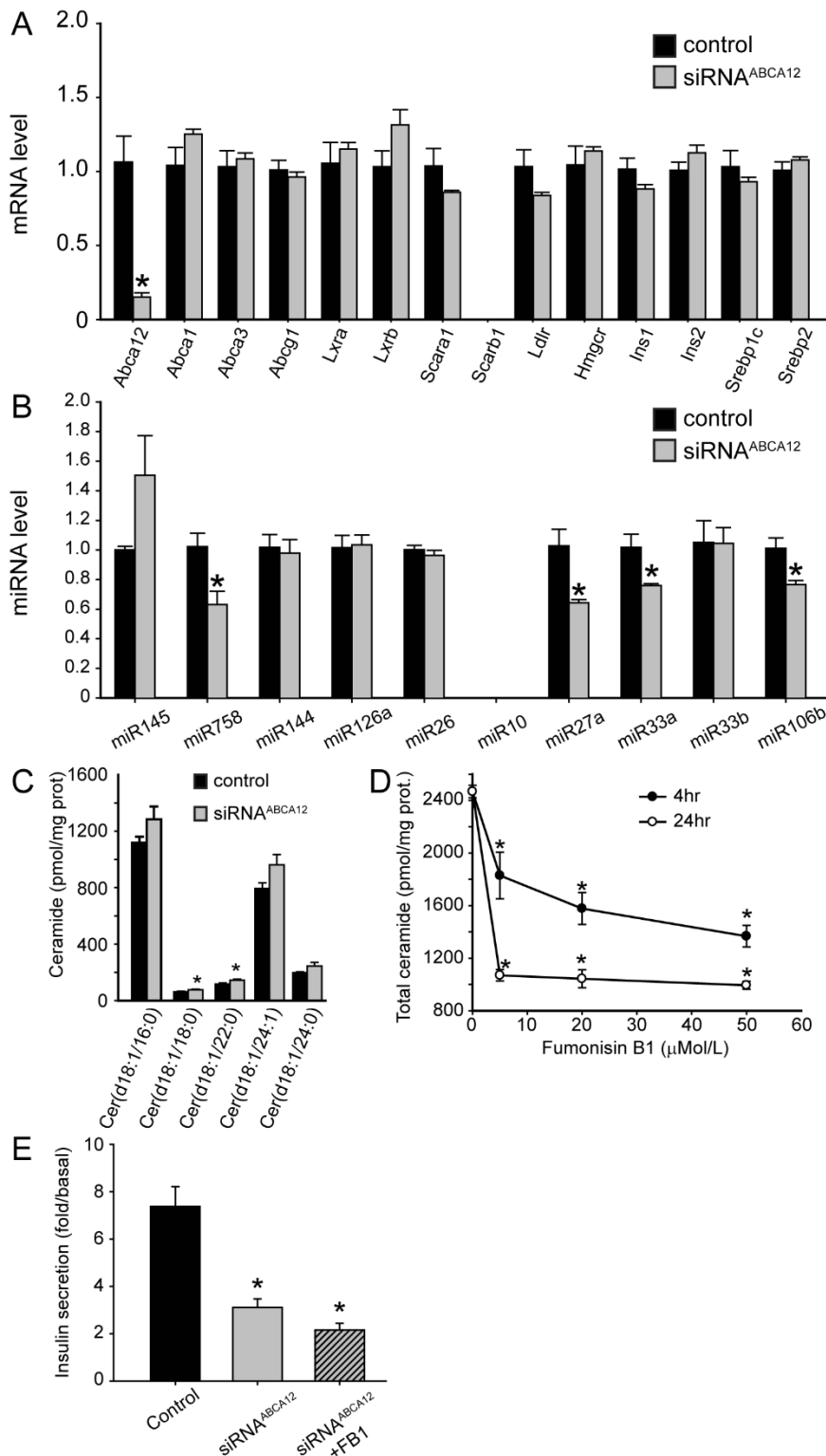
Appendix Figure S1 Immunostaining of pancreatic islets, mouse targeting strategy and pituitary analysis.

(A) Immunostaining of human pancreatic islets with antibodies to ABCA12, α -GLUCAGON and INSULIN, showing expression of ABCA12 in α and β cells (bar = 50 μ m). (B) Details of the genomic structure of the EUCOMM ES cells used to generate “knockout-first” and subsequent mouse alleles. (C) Western blot and densitometry for ABCA12 from pituitary lysate of wild type, *cre/+* and *Abca12^{tm1d}* mice (n=3 biological replicates, mean \pm SEM, no significant difference in values by Students t-test). (D) Plasma cortisol levels of wild type, *cre/+* and *Abca12^{tm1d}* mice. All blood samples for cortisol measurements were taken at 1pm (n=3-8 animals per genotype, mean \pm SEM, no significant difference in values by Students t-test).



Appendix Figure S2: Assessment of beta cell mass

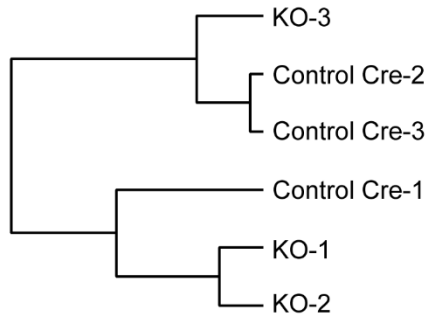
(A) β -cell mass in 16 week mice lacking β -cell ABCA12 (determined by histomorphometry from 10 evenly spaced pancreatic sections in $n=3$ mice of each genotype; mean \pm SEM). (B) Representative immunofluorescence images for insulin (red), glucagon (green) and DAPI (blue) from 16 week wild type, *cre/+* and *Abca12^{tm1d}* mice (scale bar = 50 μ m). (C) Low power view of pancreata from wild type, *cre/+* and *Abca12^{tm1d}* mice showing immunofluorescence for insulin (red) (scale bar = 100 μ m).



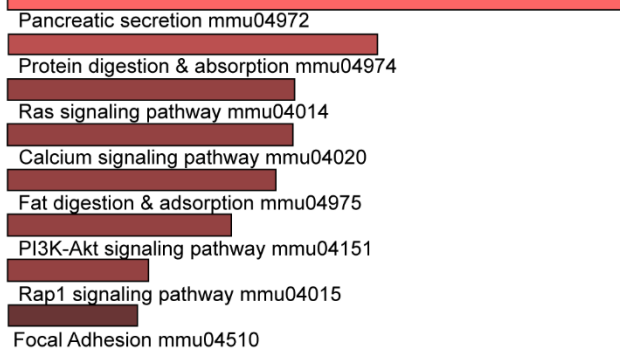
Appendix Figure S3: Gene expression and ceramide homeostasis in beta cells

(A) The effects of siRNA mediated knockdown of *Abca12* in MIN6 cells on genes known to be associated with the regulation of cholesterol metabolism (mean±SEM; n=4 biological replicates; *p<0.001 versus control, Students t-test). (B) The effects of siRNA mediated knockdown of *Abca12* in MIN6 cells on the expression of microRNA's known to be associated with alterations in cholesterol metabolism (mean±SEM; n=4 biological replicates; *p<0.05 versus control, Students t-test). (C) Mass spectroscopy analysis of levels of ceramides in MIN6 cells transfected with indicated siRNAs. (D) Reduction in cellular ceramide content by treatment of MIN6 cells with Fumonisin B1 (mean±SEM; n=4 biological replicates; *p<0.001 versus control, Students t-test). (E) The impact of fumosin treatment on glucose stimulated insulin secretion (20μMol/L treatment for 24hr, mean±SEM, n=4 biological replicates, *p<0.01, students t-test).

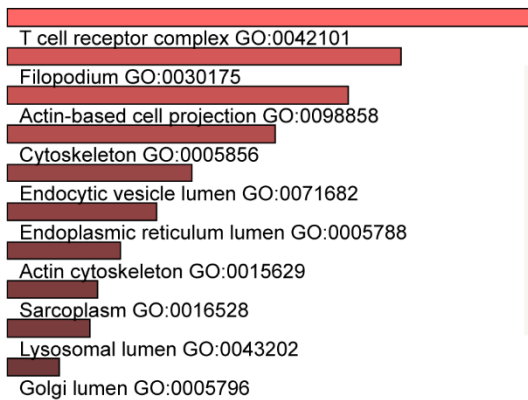
A 5wk islets (All probes)



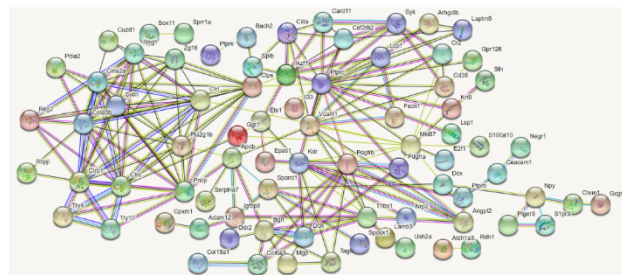
B KEGG pathways (Filtered DE gene list)



C GO_Cell.Comp. terms (DE gene list)

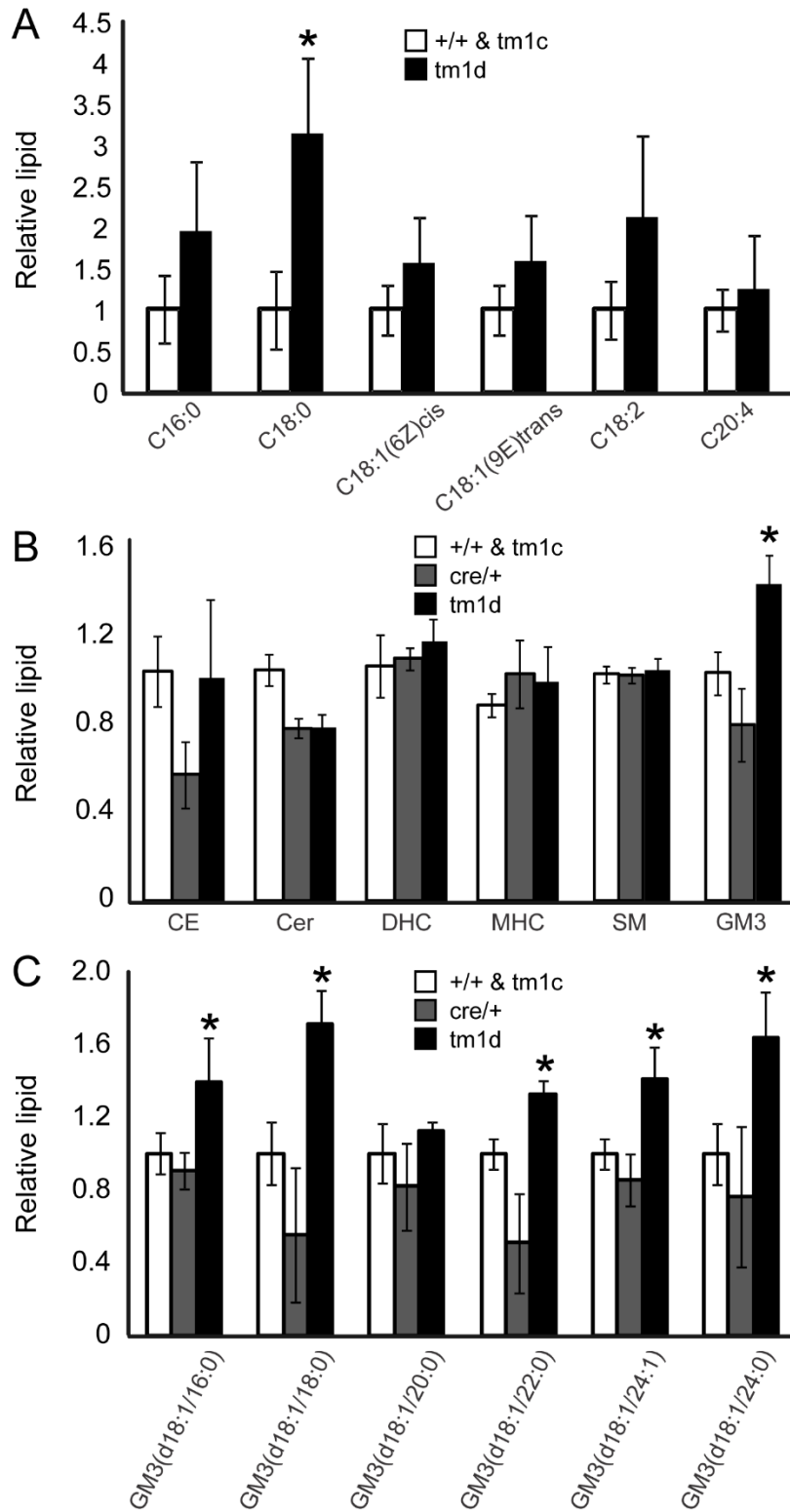


D String network within DE gene list



Appendix Figure S4: Gene expression changes in *Abca12tm1d* islets

(A) Analysis of global gene expression from islets isolated from 5 week old *Abca12^{tm1d}* mice (KO) versus *cre* control islets showing clustering of RNA seq datasets at 5 weeks of age. (B) Analysis of filtered differentially expressed genes analysed by KEGG pathways. (C) Clustering of differentially expressed genes based on GO terms. (D) Network of relationships between differentially expressed genes identified by STRING analysis.



Appendix Figure S5: Quantitation of fatty acids in *Abca12tm1d* islets

(A) Saturated Fatty Acid levels from 16 week *Abca12tm1d* islets and *cre* controls were analysed by Gas-Chromatography Mass Spectrometry (GC-MS). Control samples (pool of *Abca12+/+* and *Abca12tm1c/+*) are defined as 1; *Abca12tm1d* samples are shown relative to this baseline (n=3-4, error bars = SEM, *p<0.05 (Students t-test)). (B,C) Mass spectroscopy analysis of total levels of different lipid families (B) and of different GM3 species (C) in purified islets of 24 week old mice of the indicated genotypes (n= 3-9 mice per genotype, error bars +/- SEM, *p<0.05 (Students t-test)).