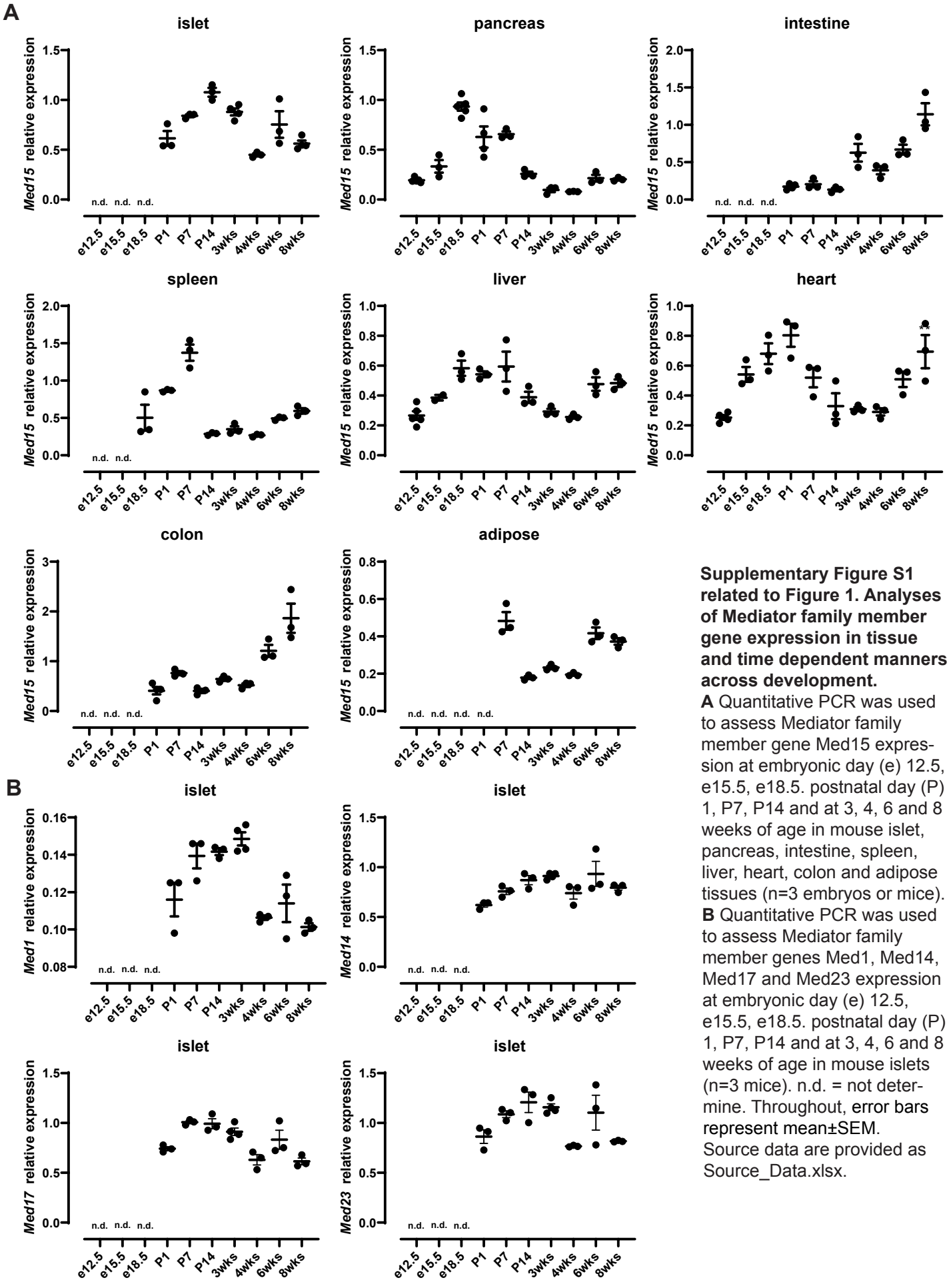
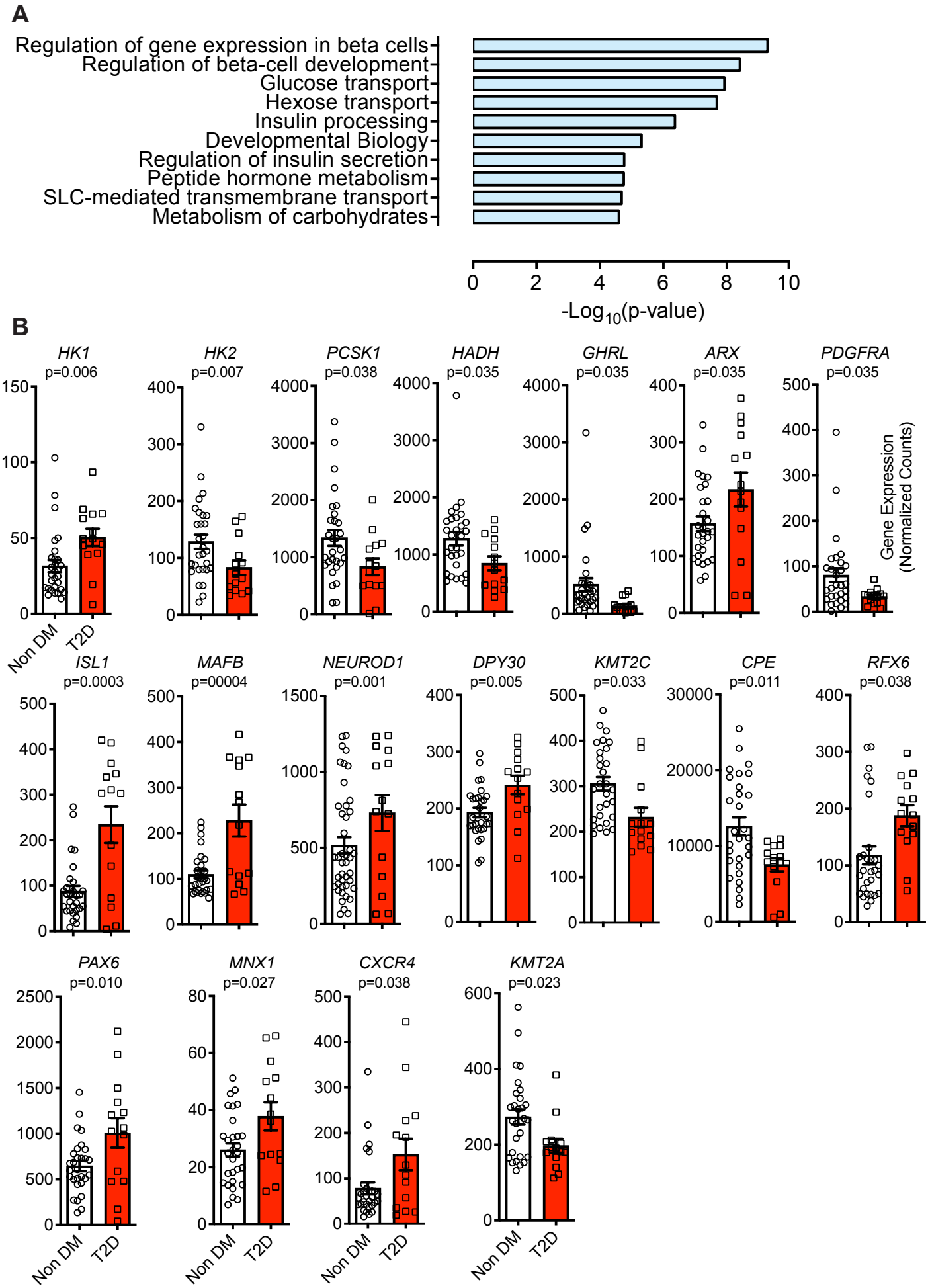


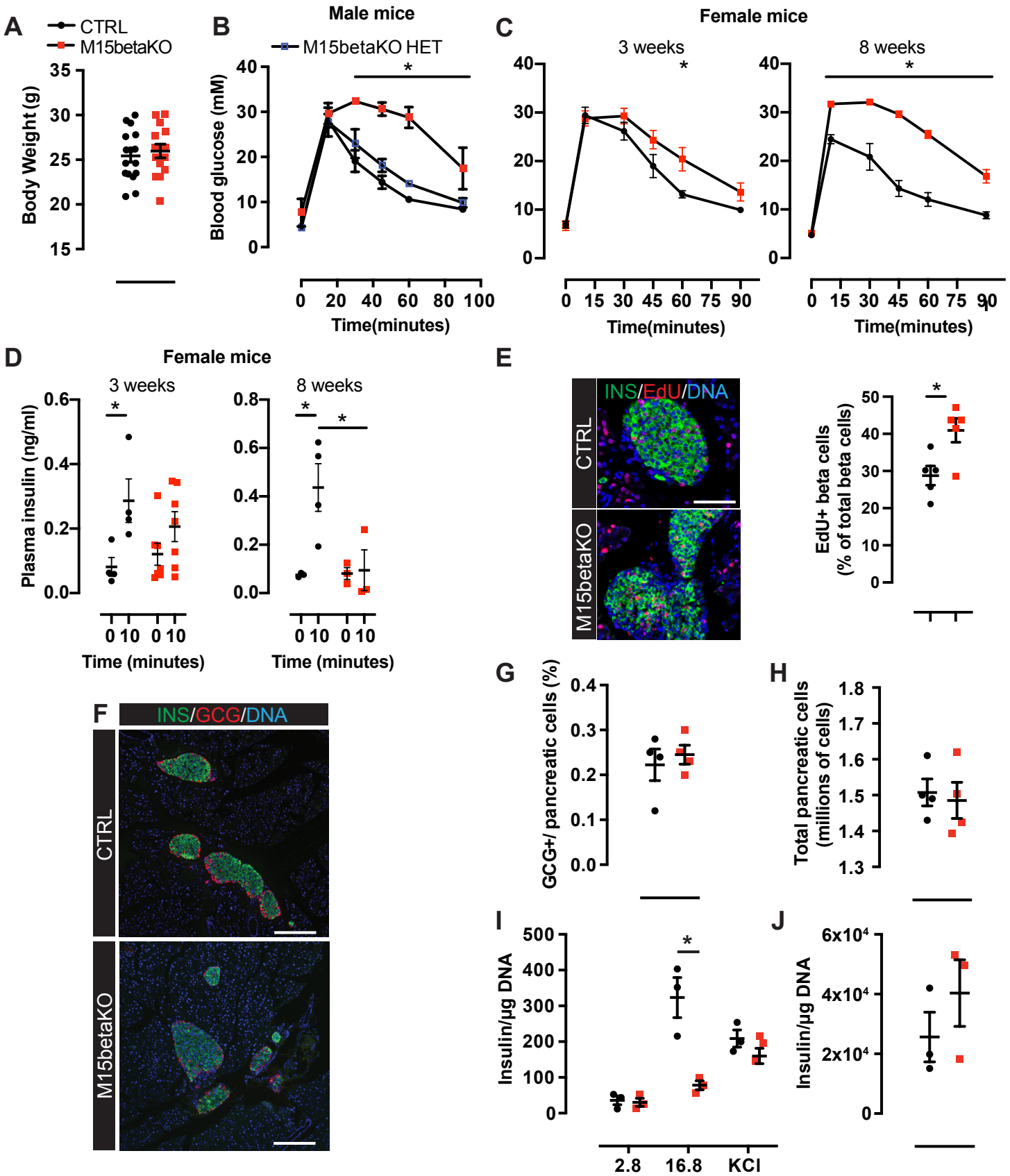
Supplementary Figure S1 related to Figure 1. Analyses of Mediator family member gene expression in tissue and time dependent manners across development.

A Quantitative PCR was used to assess Mediator family member gene Med15 expression at embryonic day (e) 12.5, e15.5, e18.5. postnatal day (P) 1, P7, P14 and at 3, 4, 6 and 8 weeks of age in mouse islet, pancreas, intestine, spleen, liver, heart, colon and adipose tissues (n=3 embryos or mice). **B** Quantitative PCR was used to assess Mediator family member genes Med1, Med14, Med17 and Med23 expression at embryonic day (e) 12.5, e15.5, e18.5. postnatal day (P) 1, P7, P14 and at 3, 4, 6 and 8 weeks of age in mouse islets (n=3 mice). n.d. = not determine. Throughout, error bars represent mean±SEM. Source data are provided as Source_Data.xlsx.



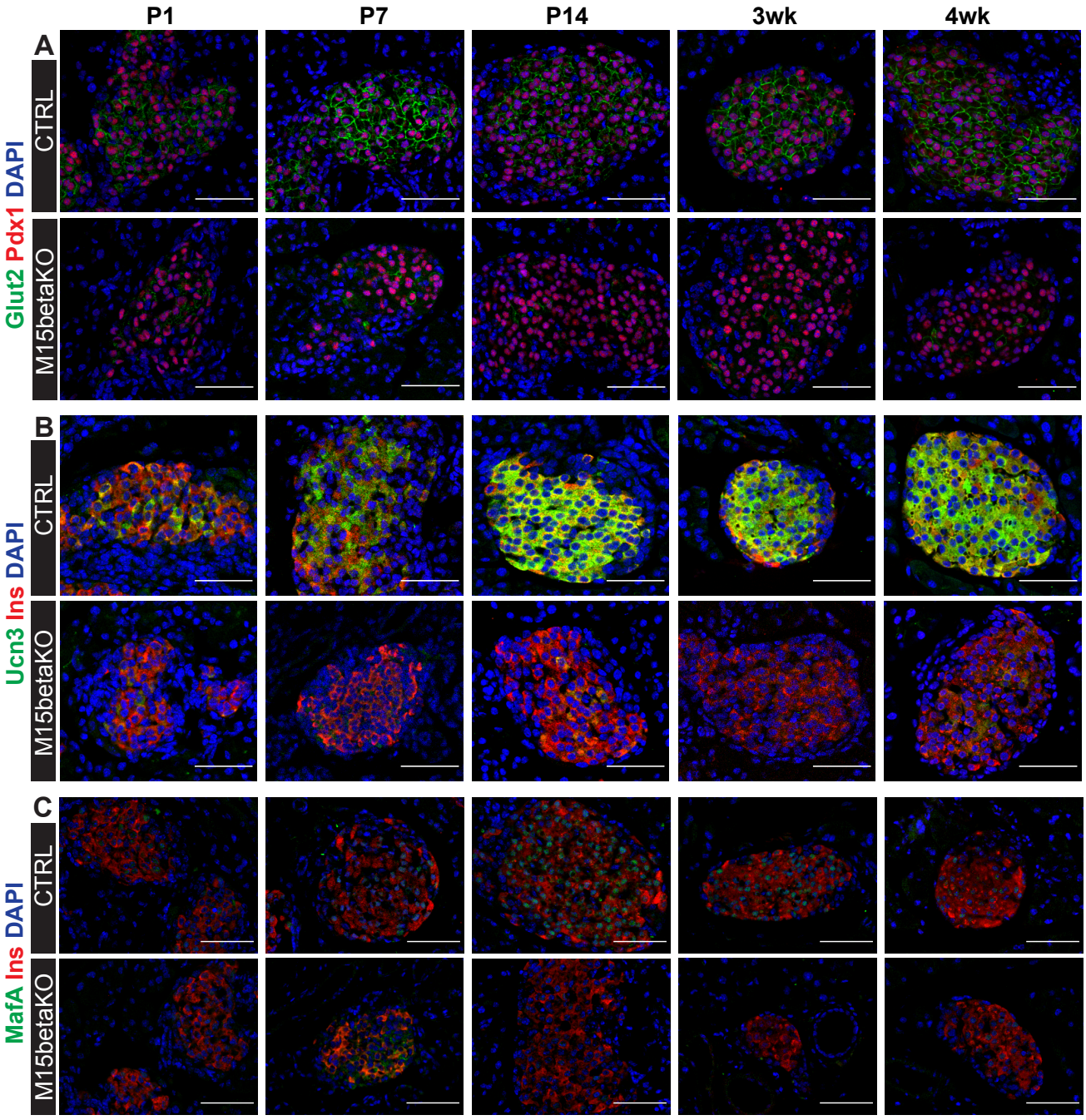
Supplementary Figure S2, related to Figure 1. Differential gene expression in islets from those living with type 2 diabetes.

A Reactome pathway terms for all significantly decreased genes in Figure 1D. **B** Normalized expression levels of selected significantly differentially-expressed genes in islets isolated from healthy individuals (white bars; n=29) or those living with T2D (red bars; n=14). Exact p values indicated under gene name by two-tailed Student's t-test; error bars represent mean±SEM. Source data are provided as Source_Data.xlsx.

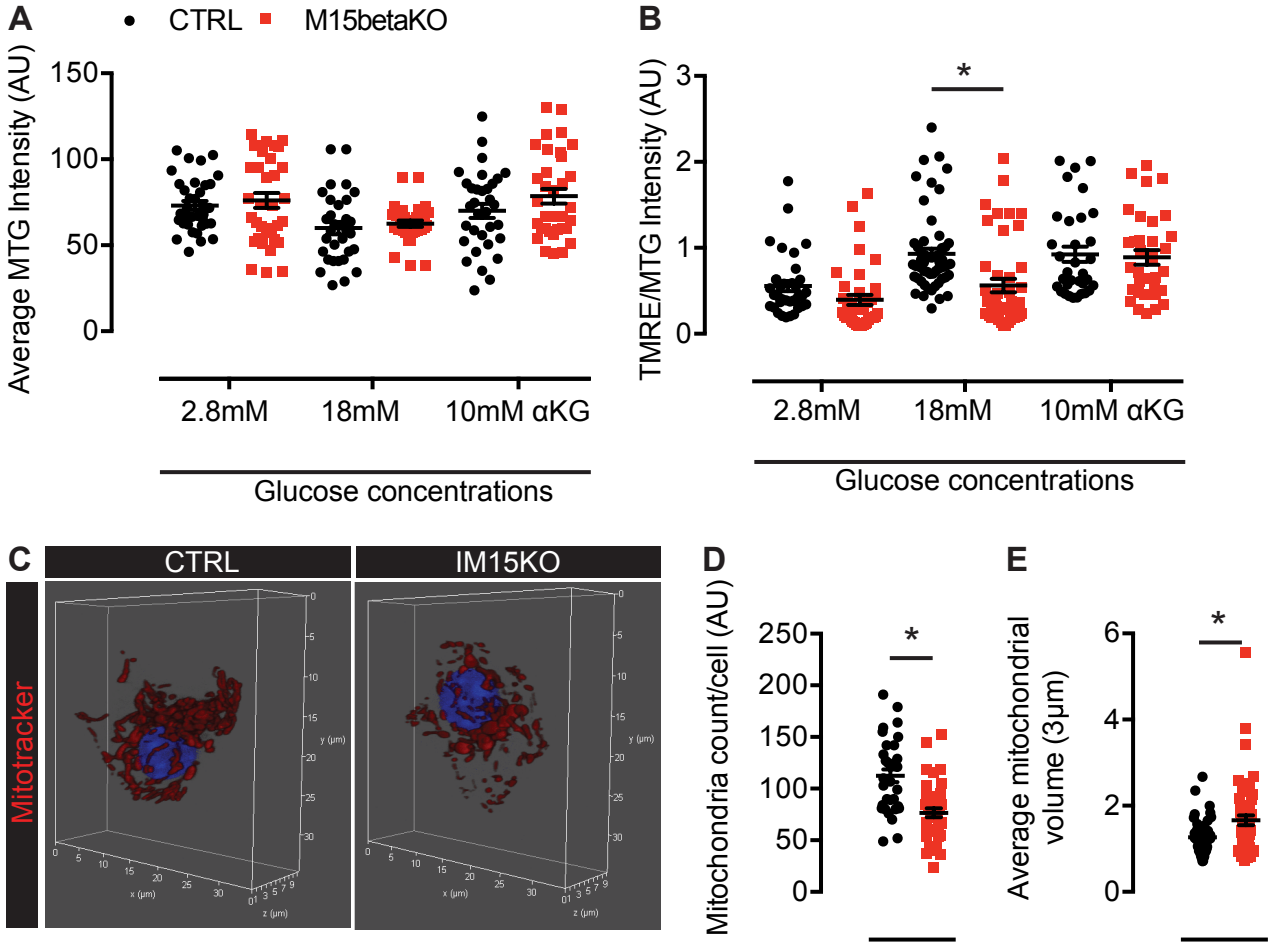


Supplementary Figure S3 related to Figure 2. Analysis of M15betaKO mouse physiology, islet morphology, and insulin secretion.

A Body weight of male and female combined 8-week old control (CTRL; black circles) and M15betaKO (red squares) mice (n=15 mice). **B** Intraperitoneal glucose tolerance test in 6-week old CTRL (black circles; n=3), heterozygous (blue squares; n=4), and M15betaKO mice (red squares; n=3); * = p≤0.05 by unpaired two-tailed Student's t-test. **C** Intraperitoneal glucose tolerance tests in 3- and 8-week old female CTRL (black circles; n=4, n=5 respectively) and M15betaKO (red squares; n=7) mice; * = p≤0.05 by ANOVA with Sidak post-hoc test. **D** Plasma insulin during glucose tolerance in 3- and 8-week old female CTRL (black circles; n= 4) and M15betaKO (red squares; n=7, n=3 respectively) mice; * = p≤0.05 by ANOVA with Sidak post-hoc test. **E** Beta cell (INS; green) proliferation assessed by EdU (red) incorporation into nuclei (blue; DAPI) of 3 week old CTRL (black circles) M15betaKO (red squares) mice (n=5; * = p≤0.05 by unpaired two-tailed Student's t-test; scale bars 50µM. **F** Immunofluorescence for insulin (INS, green), glucagon (GCG, red) and DNA (blue) in 8-week control and M15beta-KO mice (representative of n=4); scale bars 100µM. **G** Quantification of GCG+ α-cells/DAPI+ pancreatic cells in CTRL (black circles) and M15betaKO (red squares; n=4 mice). **H** Quantification of DAPI+ pancreatic cells in CTRL (black circles) and M15betaKO (red squares; n=4 mice). **I** Glucose-stimulated insulin secretion from 8-week CTRL (black circles) and IM15KO (red squares) islets (n=3); * = p≤0.05 by two-tailed unpaired Student's t-test). **J** Total insulin quantification from (I) (n=3). Throughout, error bars represent mean±SEM. Source data are provided as Source_Data.xlsx.

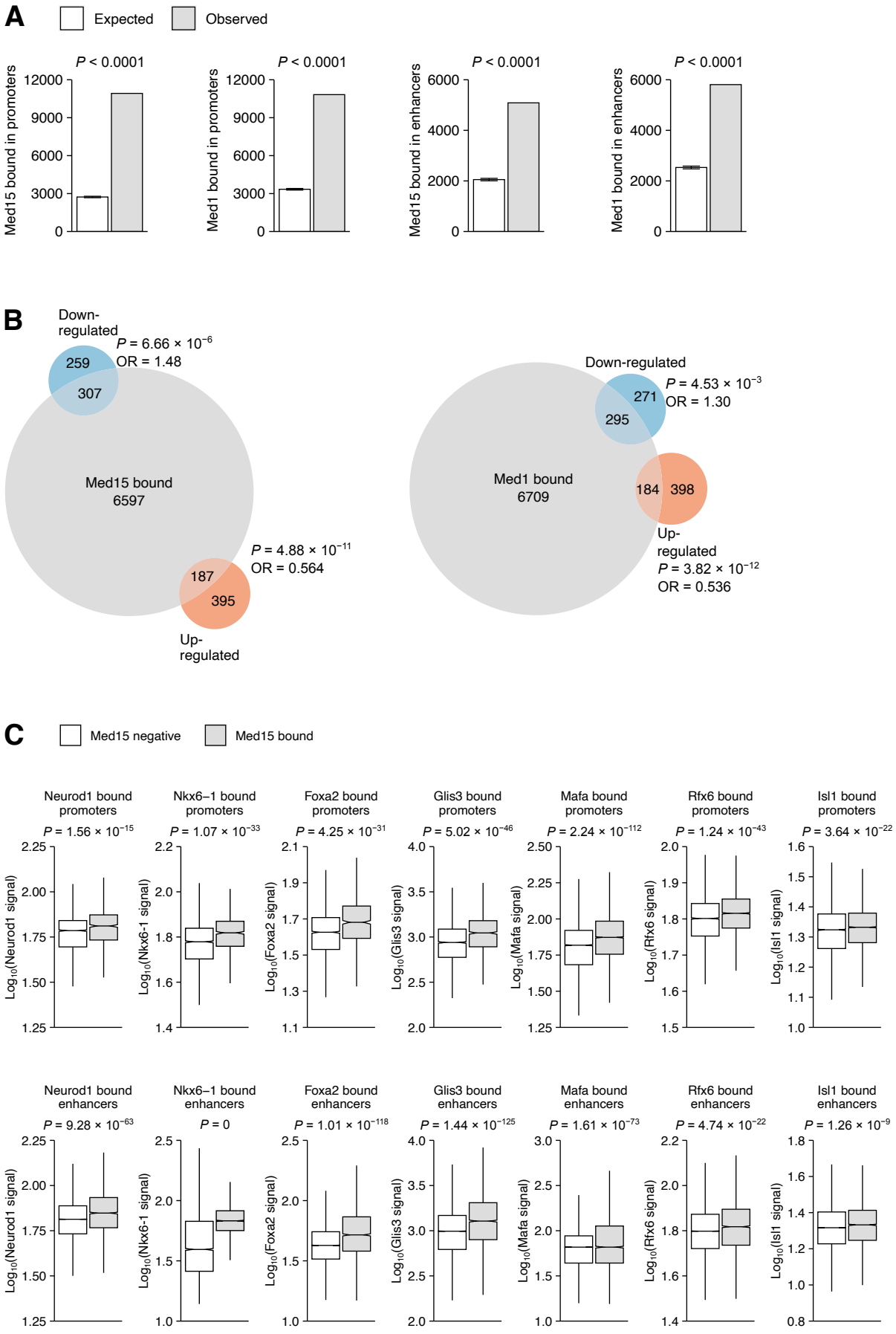


Supplementary Figure S4 related to Figure 3. Immunostaining for islet maturation markers in Control (CTRL) and M15betaKO mice at postnatal day 1 (P1), P7, P14, 3 weeks and 4 weeks of age
A Co-immunostaining for Glut2 (green), Pdx1 (red) in CTRL and M15betaKO pancreatic sections (representative of n=3). **B** Co-immunostaining for Ucn3 (green), Ins (red) in CTRL and M15betaKO pancreatic sections (representative of n=3). **C** Co-immunostaining for MafA (green), Ins (red) in CTRL)and M15betKO pancreatic sections (representative of n=3).Throughout, DNA is labelled with DAPI (blue) and scale bars = 50µm.



Supplementary Figure S5 related to Figure 3. RNA-Seq GO-term analysis, mitochondrial function and morphology in IM15KO mouse islets

A Mitotracker green FM (MTG) intensity in control (CTRL; black circles) and M15betaKO (red squares) islets stimulated with 18mM glucose or 2.8mM Glucose + 10mM αKetoglutarate (αKG) (30 cells from n=4 islet preps). **B** Normalized mitochondrial function, as measured by TMRE/MTG intensity in 8-week CTRL (black circles) and M15betaKO (red squares) dispersed islets when stimulated with 18mM glucose or 10mM αKetoglutarate (32-45 cells from n=4 islet preps) * = p≤0.05 by two-way ANOVA with Sidak multiple-comparison test. **C** Three-dimensional reconstruction of mitochondrial network in dispersed 8-week CTRL and IM15KO islets (representative of n=3). **D** Mitochondrial count per cell measured in (**B**), each dot represents one mitochondrion (43-53 cells from n=3 mice, * = p≤0.05 by unpaired two-tailed Student's t-test. **E** Mitochondrial volume measured in (**B**); each dot represents one mitochondrion (43-53 cells from n=3 mice), * = p≤0.05 by unpaired two-tailed Student's t-test. Throughout, error bars represent mean±SEM. Source data are provided as Source_Data.xlsx.



Supplementary Figure S6, related to Figure 4: Med15 co-operates with mature beta cell transcription factors to regulate gene expression.

A Bar plots showing the number of annotated promoters and enhancers bound by Med15 or Med1 (grey bars) and the numbers expected (white bars) by chance. P-values calculated using permutation tests. **B** Euler diagrams showing overlap between Med15 or Med1 bound gene promoters (grey circles) with genes that are downregulated (blue) or upregulated (orange) in IM15KO islets. P-values and odds ratios were calculated using two-sided Fisher's exact tests with Benajmini-Hochberg correction; OR, odds ratio. **C** Box and whisker plots showing the ChIP-seq signal of transcription factors in promoters and enhancers bound by the transcription factor that are also bound by Med15 (grey bars) or not (white bars). p-values calculated using Man-Whitney U tests with Benjamini-Hochberg correction. Source data are provided as Source_Data.xlsx.

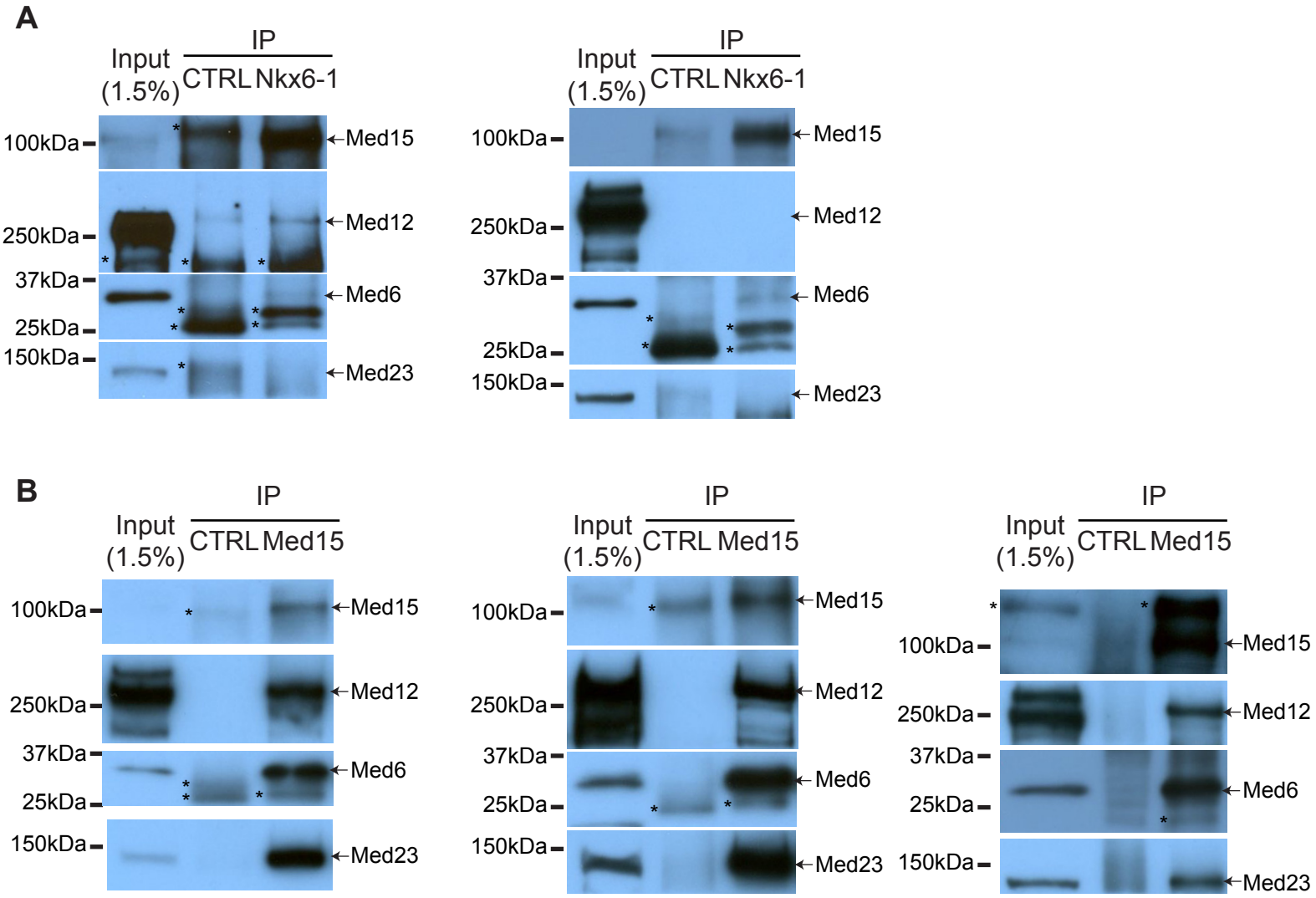
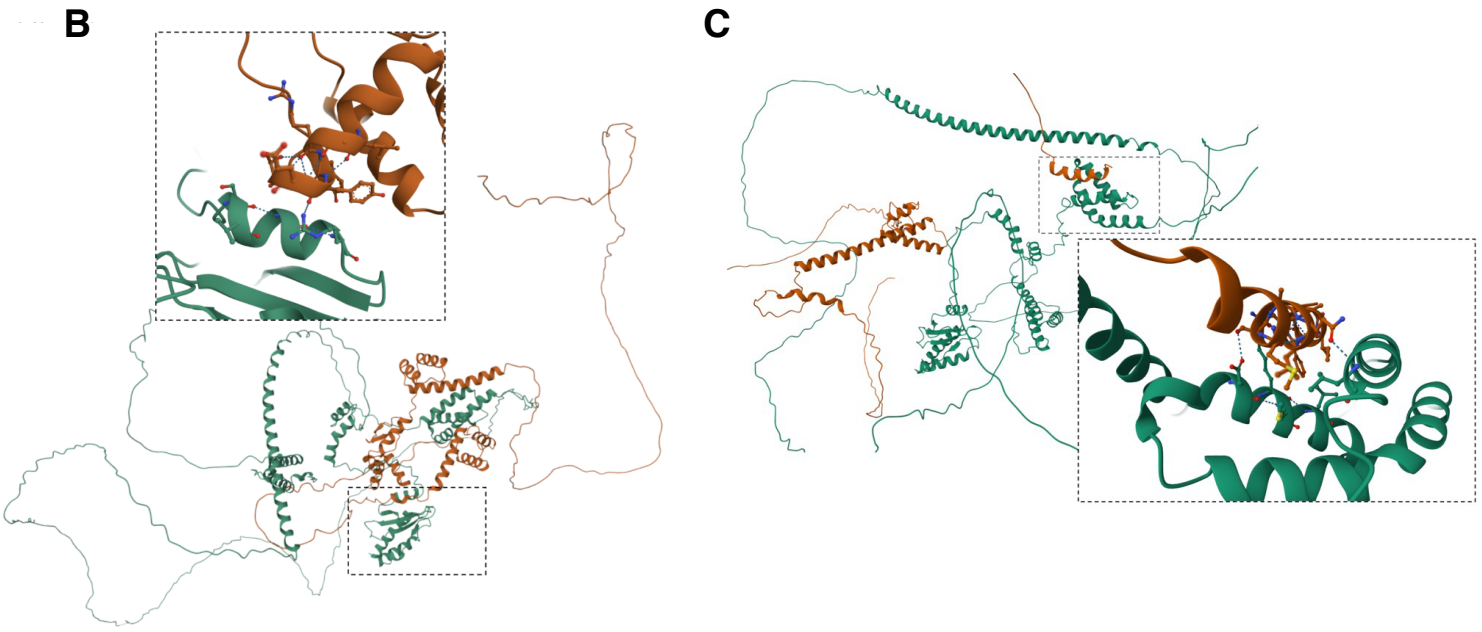


Figure S7: Med15 interacts with Nkx6-1 and co-purifies with Mediator complex subunits
A Co-immunoprecipitation of Med15 and other Mediator subunits with Nkx6-1 using mouse β-cell line (Min6) lysates. CTRL = non-specific IgG control using Min6 cell lysates (n=2 additional biological replicates supporting Fig 5). **B** Co-immunoprecipitation of Mediator complex subunits with Med15 using mouse β-cell line (Min6) lysates. CTRL = non-specific IgG control (n=3 biological replicates). In all panels, arrows = predicted molecular weight and * = non specific signal. Source data are provided as Source_Data.xlsx.

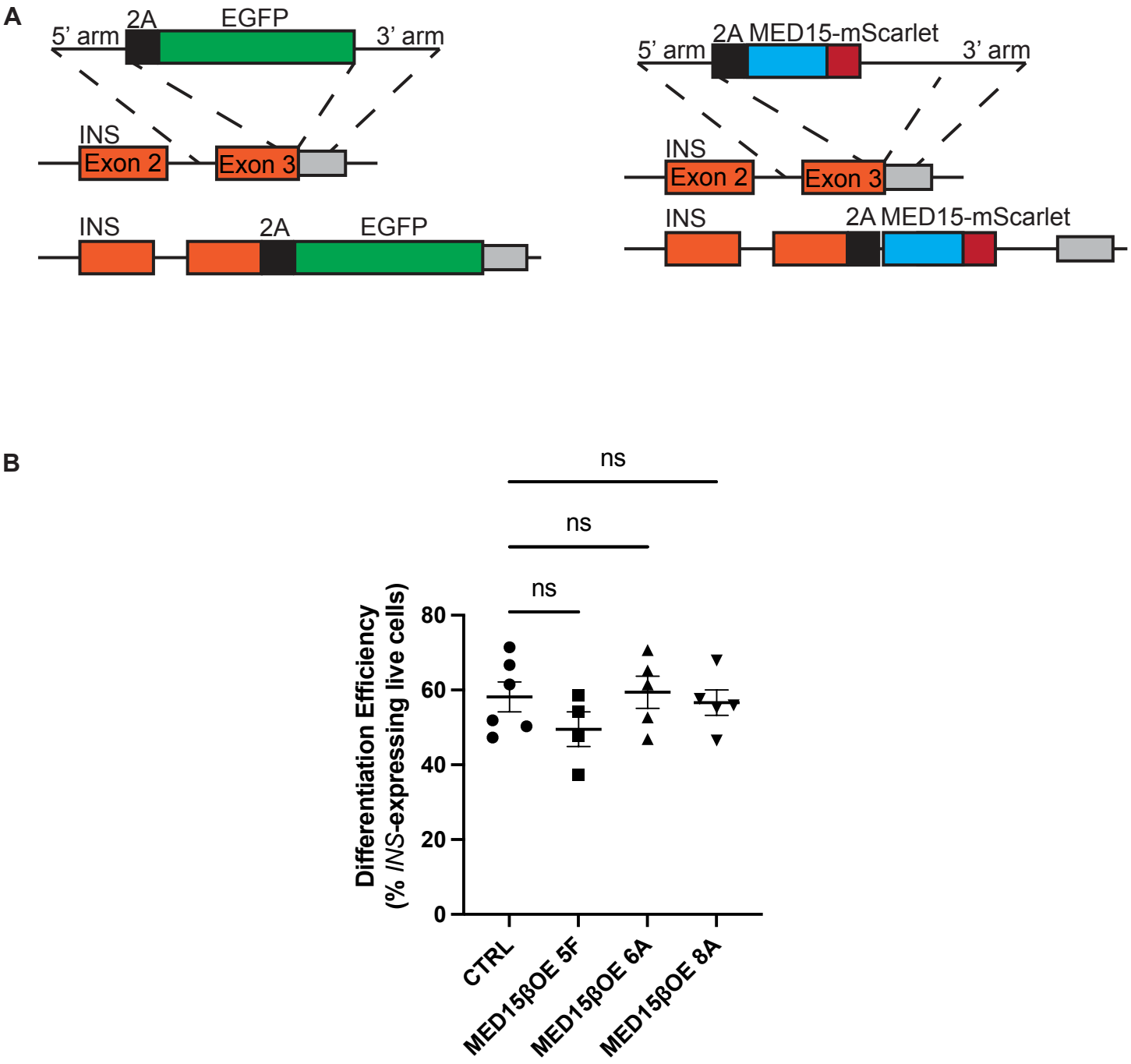
A

	Accession ID	Contact pairs	H-bonds	Pi_score	Iptm	DockQ
NKX6.1	P78426	33	4	-2	0.233	0.163
NEUROD1	Q13562	7	3	0.33	0.185	0.074
PAX6	P26367	111	17	-0.31	0.324	0.319
NKX2.2	O95096	27	10	-1.57	0.273	0.073
ERR-γ	P62508	NA	NA	NA	0.195	0.044
MAFA	Q8NHW3	NA	NA	NA	0.142	0.025
INSM1	Q01101	70	14	-0.27	0.199	0.090
MAFB	Q9Y5Q3	59	10	-0.27	0.137	0.074
SRBP1	P36956	NA	NA	NA	0.189	0.019



Source data are provided as Source_Data_Suppl_Figures.xlsx.

Supplementary Figure S8, related to Figure 5: MED15 is predicted to weakly interact with a number of transcription factors that are essential for β-cell maturation.
A Table of AlphaPullDown predicted interactions between selected transcription factors and MED15. NKX6-1, NEUROD1, PAX6, ERR-γ, MAFA, and INSM are all important for β-cell maturation. **B** Ribbon diagram showing domains of MED15 (green) that interact with PAX6 (orange). **C** Ribbon diagram showing domains of MED15 (green) that interact with NEUROD1(orange)



Supplementary Figure S9, related to Figure 6: β-cell MED15 overexpression does not impact differentiation efficiency in multiple clonal embryonic stem cell lines.
A Cartoon diagrams of the Control and MED15βOE insulin gene loci, cell lines were all heterzygous for the knock-in alleles. **B** Three separate genome edited clones (5F, squares, n=4; 6A, triangles, n=5; 8A, inverted triangles, n=5 differentiations) and control cells (circles, n=6) were differentiated until Day 30 prior to being analysed for the formation of insulin-expressing cells using flow cytometry for GFP (CTRL) or mScarlet (MED15βOE), no sigficance (ns) was found by two-way ANOVA with Dunnett's multiple comparisons test; error bars represent mean±SEM. Source data are provided as Source_Data.xlsx.