

Supplemental Information

The Polycomb-Dependent Epigenome

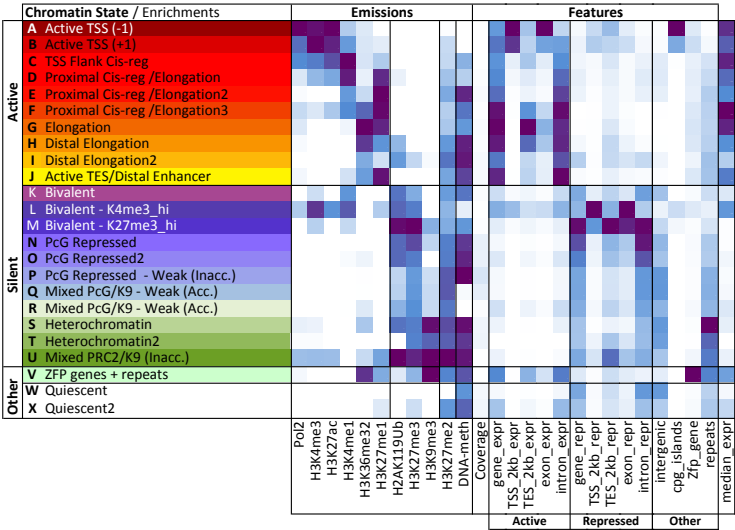
Controls β Cell Dysfunction,

Dedifferentiation, and Diabetes

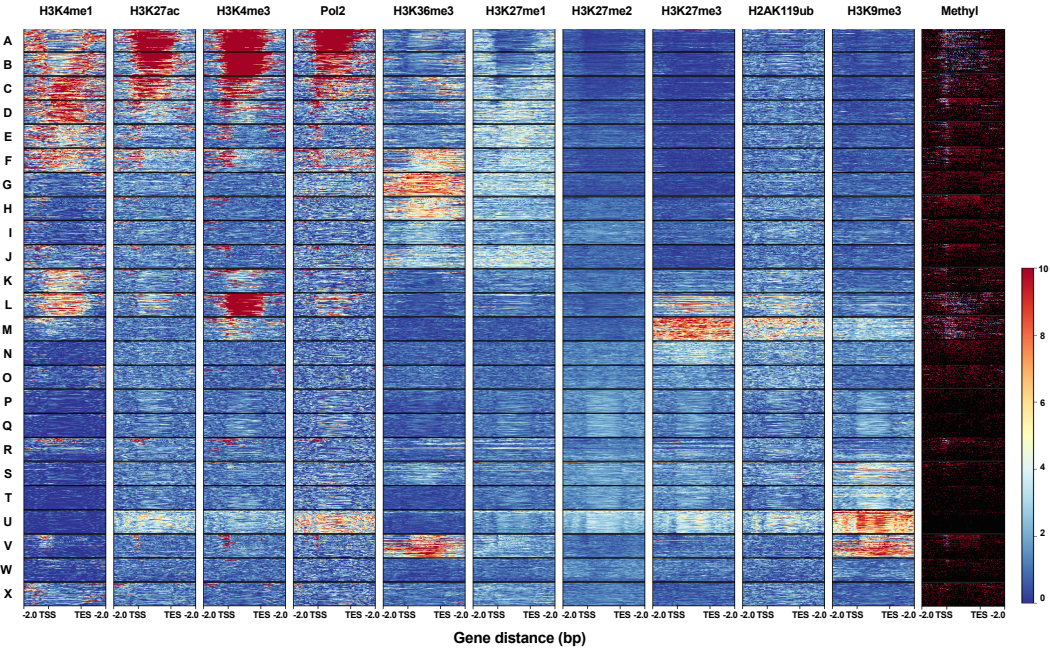
Tess Tsai-Hsiu Lu, Steffen Heyne, Erez Dror, Eduard Casas, Laura Leonhardt, Thorina Boenke, Chih-Hsiang Yang, Sagar, Laura Arrigoni, Kevin Dalgaard, Raffaele Teperino, Lennart Enders, Madhan Selvaraj, Marius Ruf, Sunil J. Raja, Huafeng Xie, Ulrike Boenisch, Stuart H. Orkin, Francis C. Lynn, Brad G. Hoffman, Dominic Grün, Tanya Vavouri, Adelheid M. Lempradl, and J. Andrew Pospisilik

Figure S1 (Related to Figure 1)

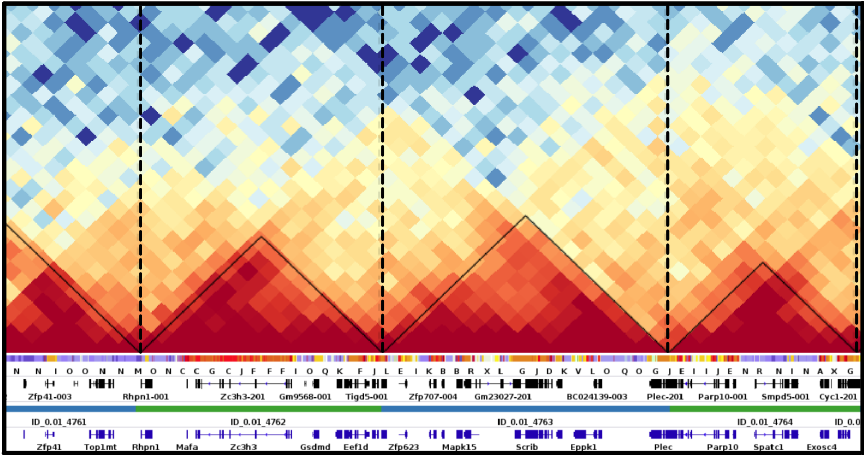
A



B



C



Supplementary Figure S1.

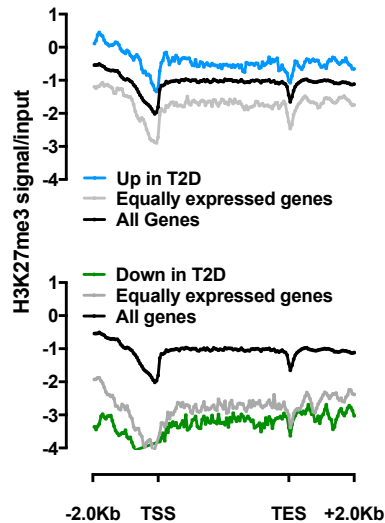
A.Histone mark characteristics, gene expression levels and genomic features of healthy islets. EpiCSeq chromatin state segmentation and manual annotation according to emissions and annotations in D. (Inacc=Inaccessible; +1 or -1= \pm nucleosome position around TSS, expr = expressed, repr= repressed, median_expr= Median expression)

B.Heatmap profiles of average normalized read coverage from ChIP-seq signals for various histone marks, Pol2 and CpG methylation signal of 100 genes from each chromatin state.

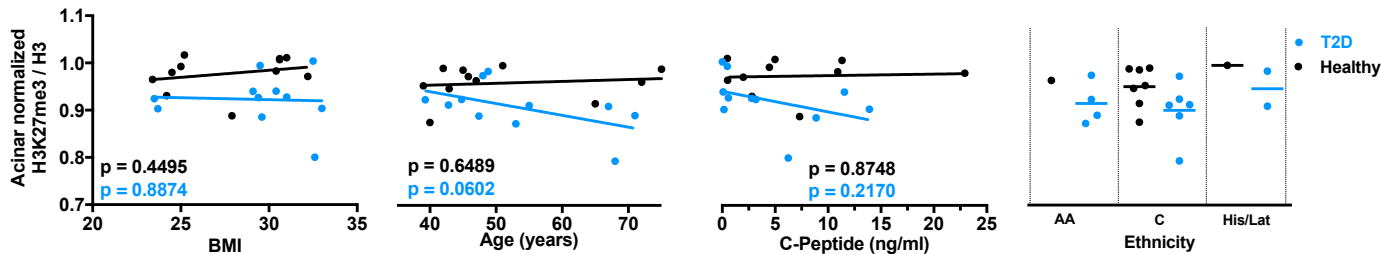
C.Example region showing Hi-C TAD boundaries aligning with chromatin state segmentation boundaries. Dash lines indicates TAD boundaries. (Hi-C image taken from <http://chorogenome.ie-freiburg.mpg.de>; dataset mouse CH12; GEO accession GSE63525)

Figure S2 (Related to Figure 2)

A



B

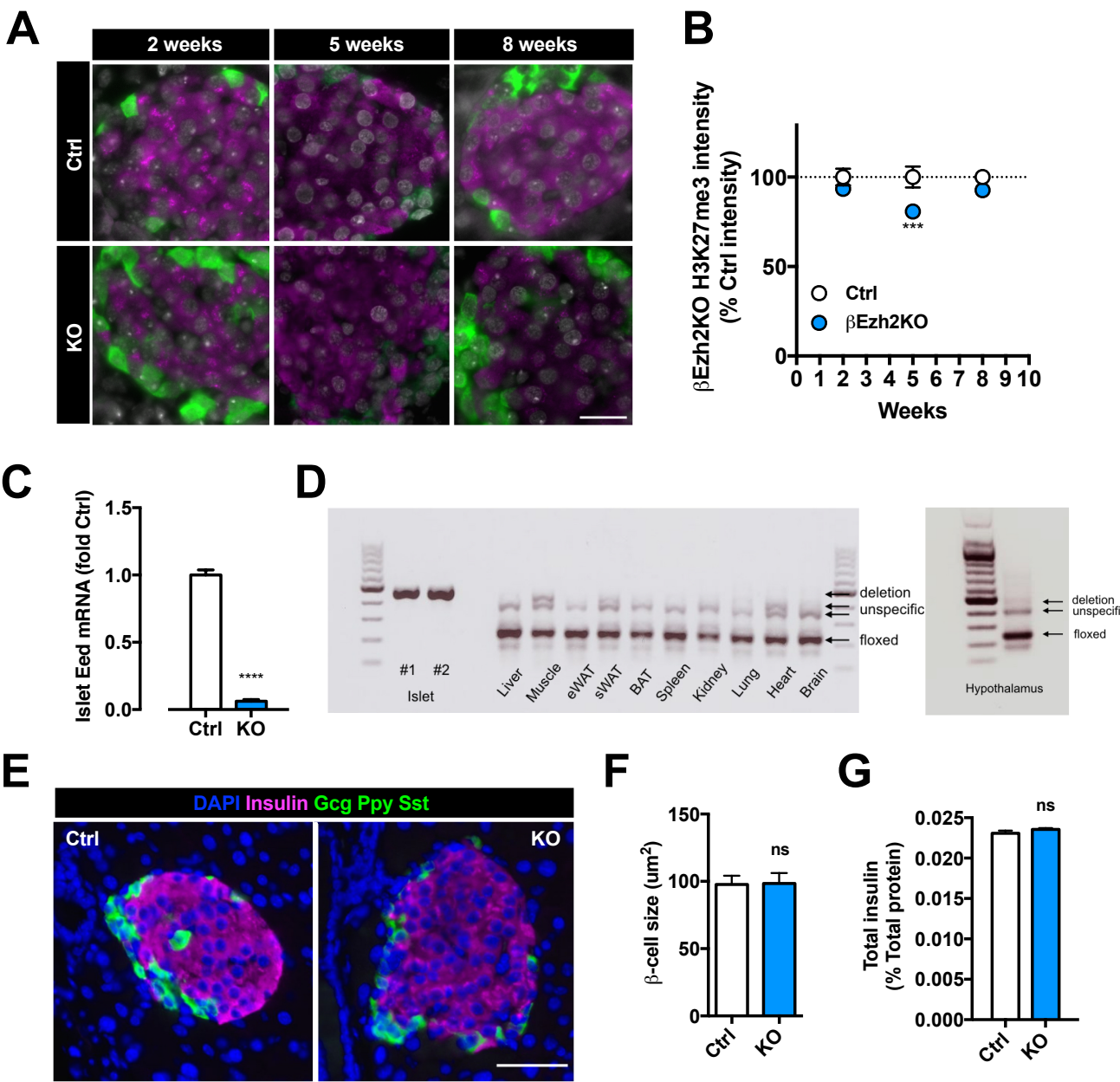


Supplementary Figure S2.

A. Average H3K27me3 signals at genes up or down-regulated in T2D and equally expressed genes. TSS: transcription start site, TES: transcription end.

B. H3K27me3 immunostaining intensity in β -cells of pancreatic sections from T2D healthy (black) and diabetic (blue) donors vs BMI, age, C-peptide levels or ethnicity. Solid lines = linear regression analysis of groups. $P < 0.05$ indicates regression slopes being significantly non-zero.

Supplementary Figure 3 (Related to Figure 3)



Supplementary Figure S3

A. Representative images of immunostaining for H3K27me3 (grey), insulin (magenta) and glucagon (green) in Ctrl (top) and β Ezh2KO (bottom) islets at 2, 5 and 8 weeks of age.

B. Quantification of relative intensity of H3K27me3 immunostainings of β EedKO and control islet β -cells at different ages. All data points were normalized to alpha-cell H3K27me3 levels in the same islets prior to comparison.

C. Quantitative RT-PCR showing loss of *Eed* mRNA in β EedKO islets.

D. PCR validation of *Eed* allele deletion in islets and other tissues of β EedKO animals. One animal is represented here for all tissues except for islets where two animals are shown

E. Immunofluorescence staining of islet hormones in Ctrl and β EedKO islets at 8 weeks of age.

F-G. Quantification of total β -cell mass (left) and insulin area (right) per islet shows no significant difference between Ctrl and β EedKO animals at 8 weeks of age.

Data represents mean \pm SEM. Scale bar = 25 μ m. *** $p < 0.001$, **** $p < 0.0001$, ns = $p > 0.05$.

A

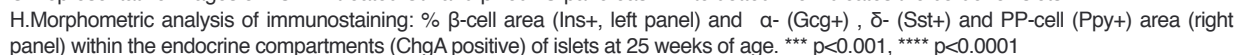
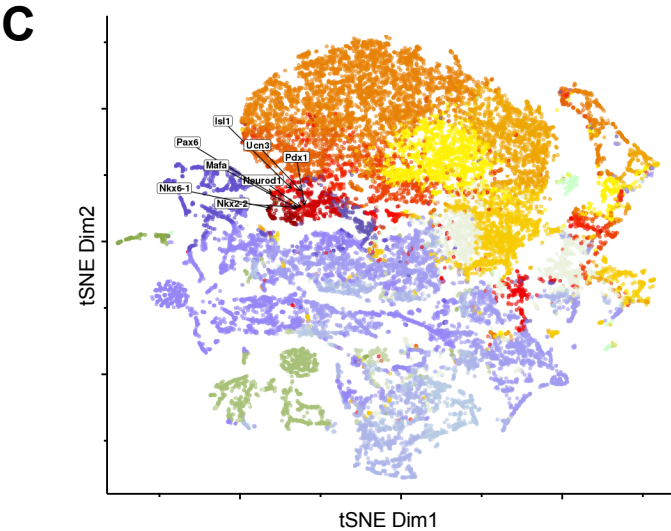
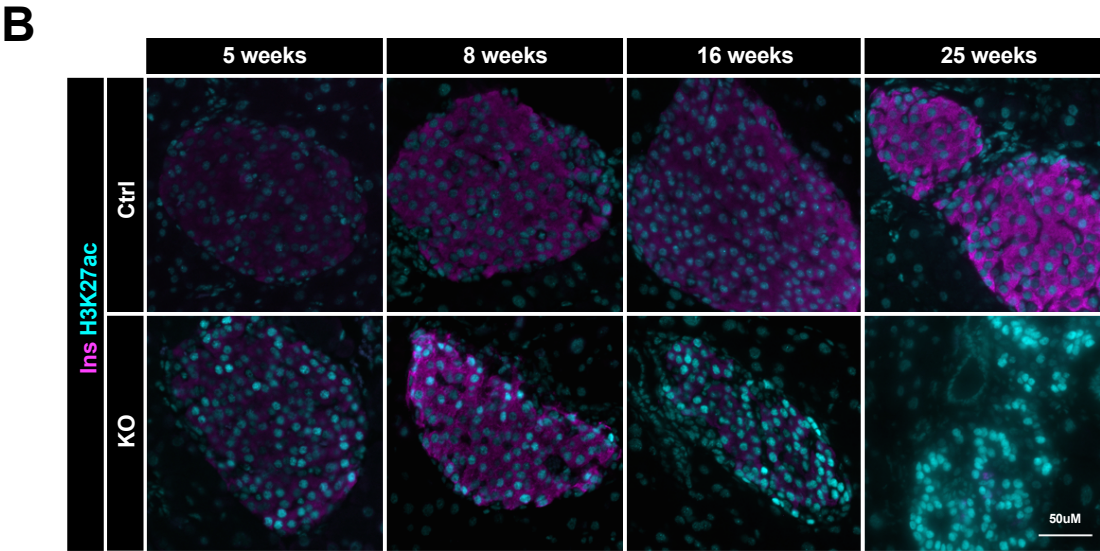
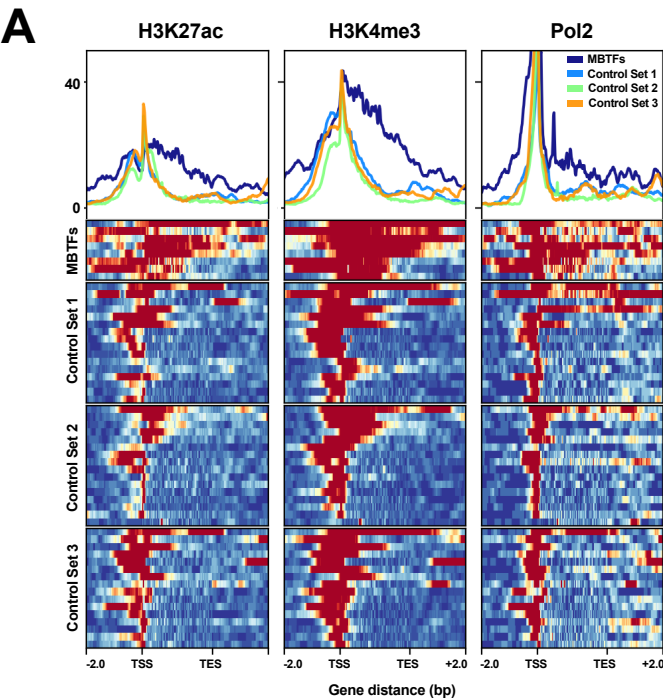


Figure S5 (Related to Figure 5)



Supplementary Figure S5.

A. Profiles of average normalized read coverage from ChIP-seq signals for H3K27ac, H3K4me3 and PolII for MβTFs and control gene sets of similar gene expression in mouse β-cells (top). Profiles are presented as heatmaps and sorted according to H3K4me3 signals (bottom).

B. Representative immunostaining images for H3K27ac (cyan) and insulin (magenta) in Ctrl and βEedKO islets at 5, 8, 16, and 25 weeks of age.

C. tSNE representation of all genes colored according to chromatin state (color coded based on Figure 1E). MβTFs are highlighted.