

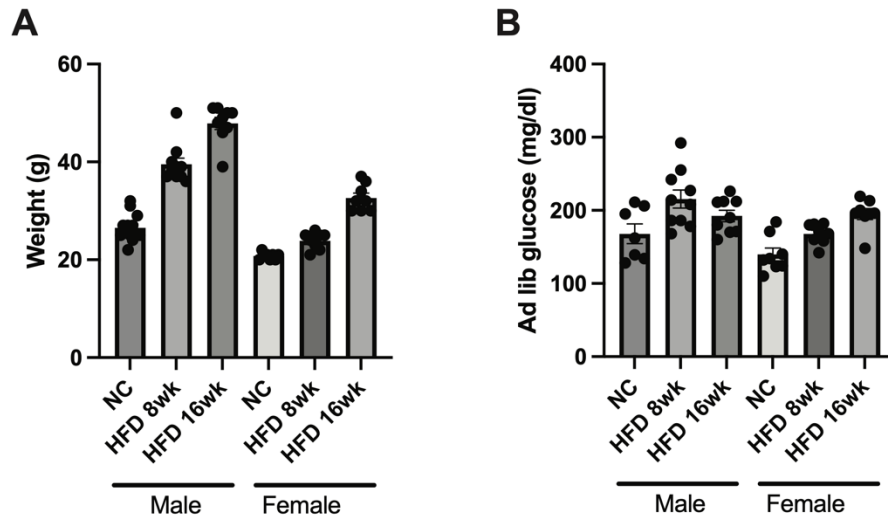


Figure S1. Body weight (A) and ad lib glucose (B) in NC and HFD mice. Number of mice included in (A): male NC: 13, HFD 8wk: 10, HFD 16wk: 9, female: 8 in all groups. No. of mice included in (B): male NC: 7, HFD 8wk: 10, HFD 16wk: 9. Female: 8 in all groups.

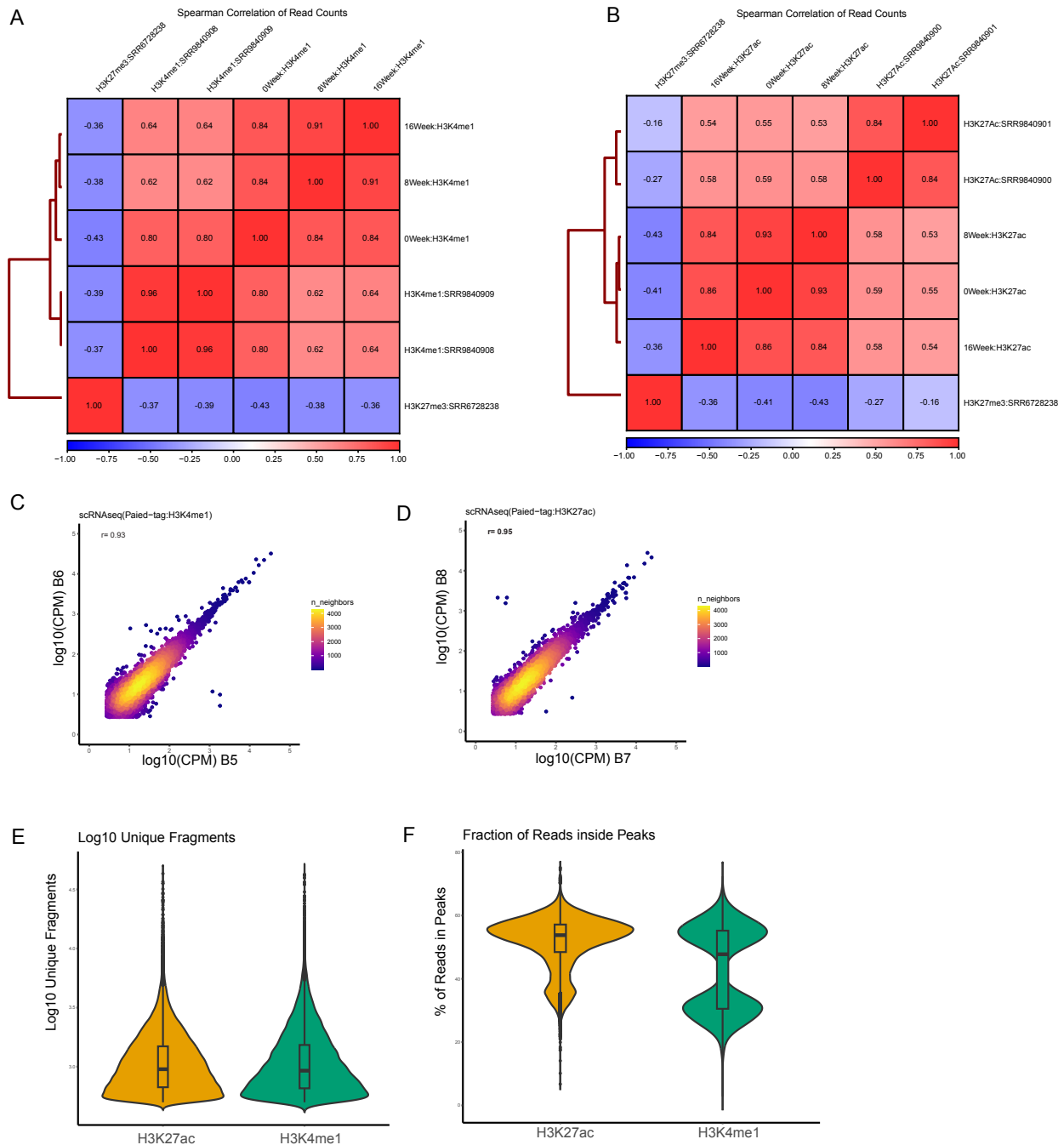


Figure S2. Quality control metrics of mouse pancreatic islets Paired-Tag.

(A). Heatmap showing the Spearman's Correlation Coefficients of genome-wide reads distribution between H3K4me1 Paired-Tag and published ChIP-seq (H3K4me1 and H3K27ac). (B) Heatmaps showing the Spearman's Correlation Coefficients of genome-wide reads distribution between H3K27ac Paired-Tag datasets and ChIP-seq (H3K4me1 and H3K27ac). (C) Scatter plot showing the correlation between read counts of genes in Paired-Tag RNA B5 (H3K4me1 of NC female islets) and B6 (H3K4me1 of NC male islets). (D). Scatter plot showing

read counts of genes in H3K27ac Paired-Tag RNA dataset B7 (H3K27ac of NC female islets) and (H3K27ac of B8 NC male islets). **(E)** Violin plots showing the numbers of unique Fragments per nucleus for all sequenced DNA libraries. **(F)** Violin plots showing the % of reads located inside H3K27ac (left) or H3K4me1 (right) peaks.

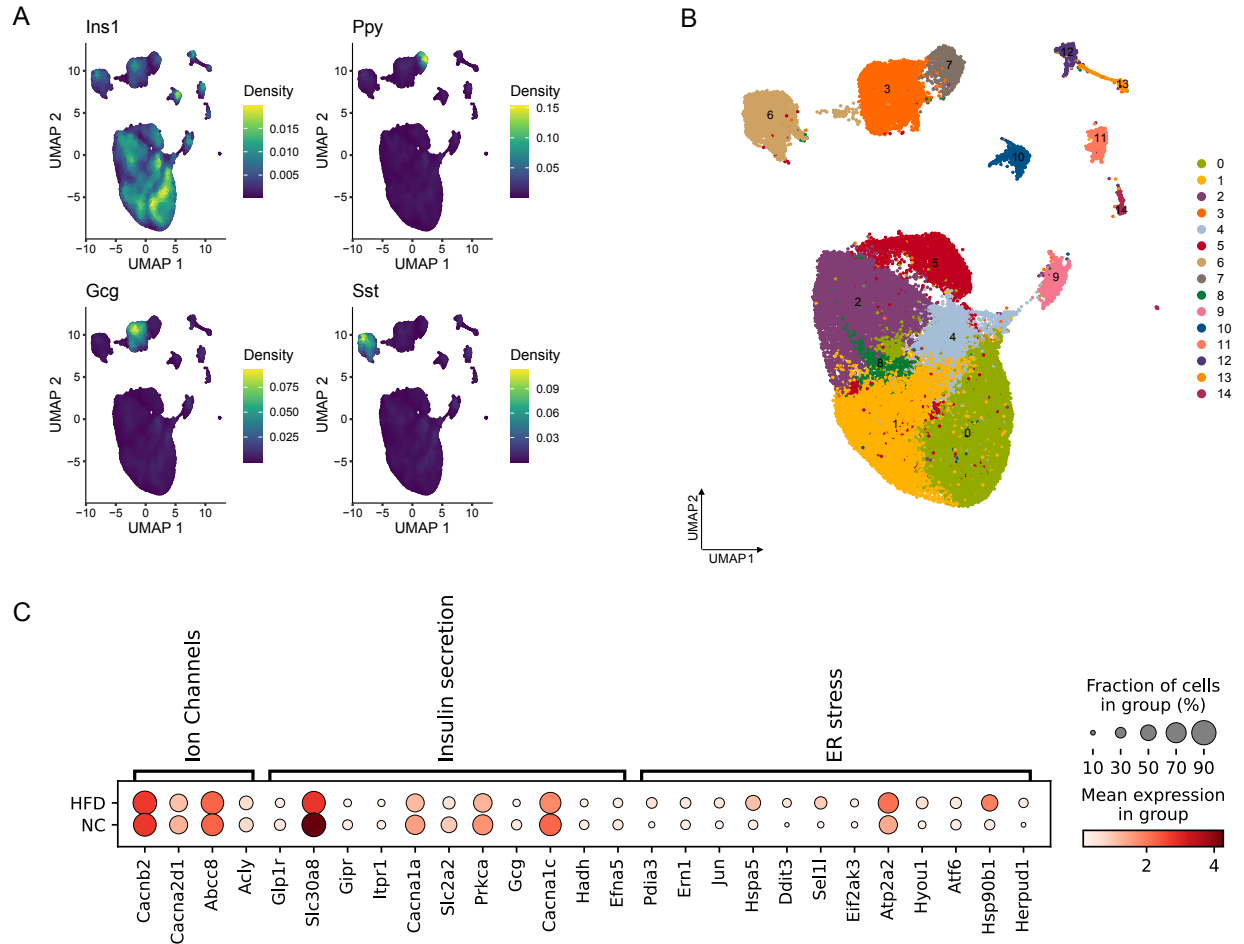


Figure S3. Gene expression signatures in mouse islets.

(A) Visualization of *Ins1*, *Ppy*, *Gcg*, and *Sst* expression patterns in UMAP space using the kernel density estimation by R package Nebulosa. (B). UMAP plot showing the proper classification of islet cells. UMAP visualisation (in two dimensions [UMAP1 and UMAP2]) of 56,748 islet cells from 4 groups of normal chow (NC)-fed mice and 6 groups of high-fat diet (HFD) (8 weeks or 16 weeks)-fed mice (different colors mark distinct cell types annotated based on the expression of signature genes; each dot represents one cell). (C) Dot plot of expression of representative genes related to ion channel, insulin secretion, and ER stress in NC or HFD β cells.

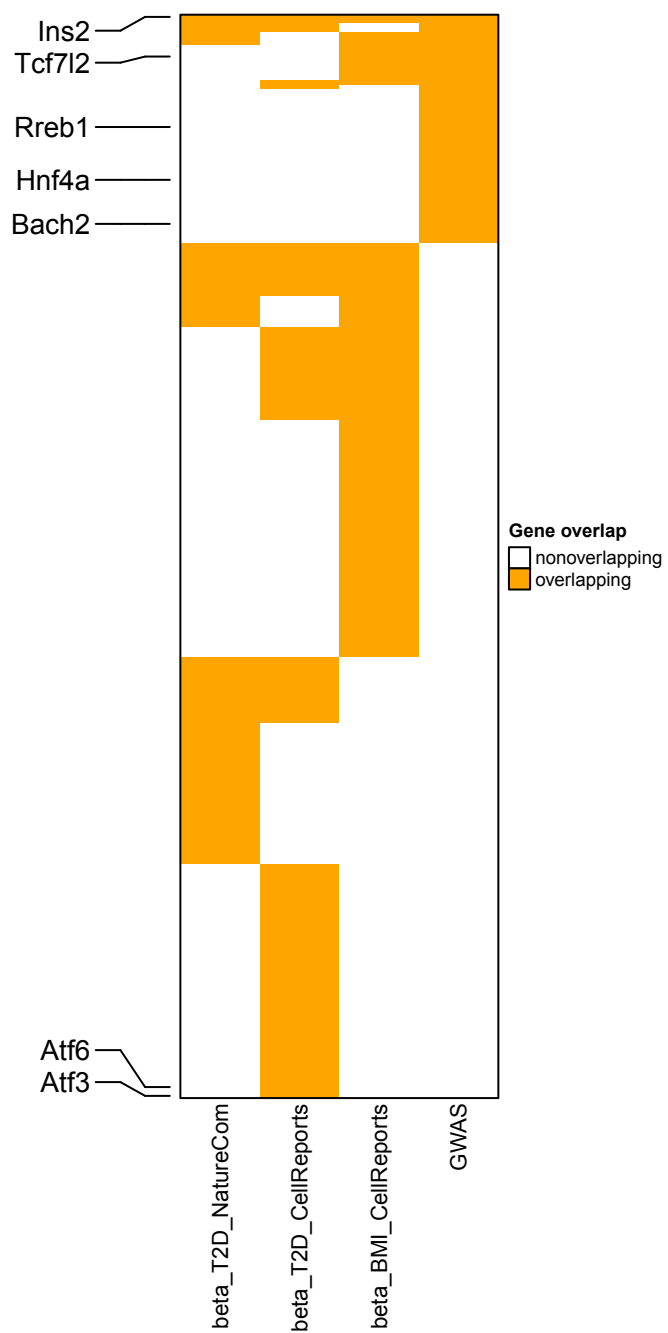


Figure S4. The overlap between top 1,000 genes in Figure 1F and T2D GWAS-associated genes and dynamic genes defined by scRNA-seq in human islets^{16,45}.

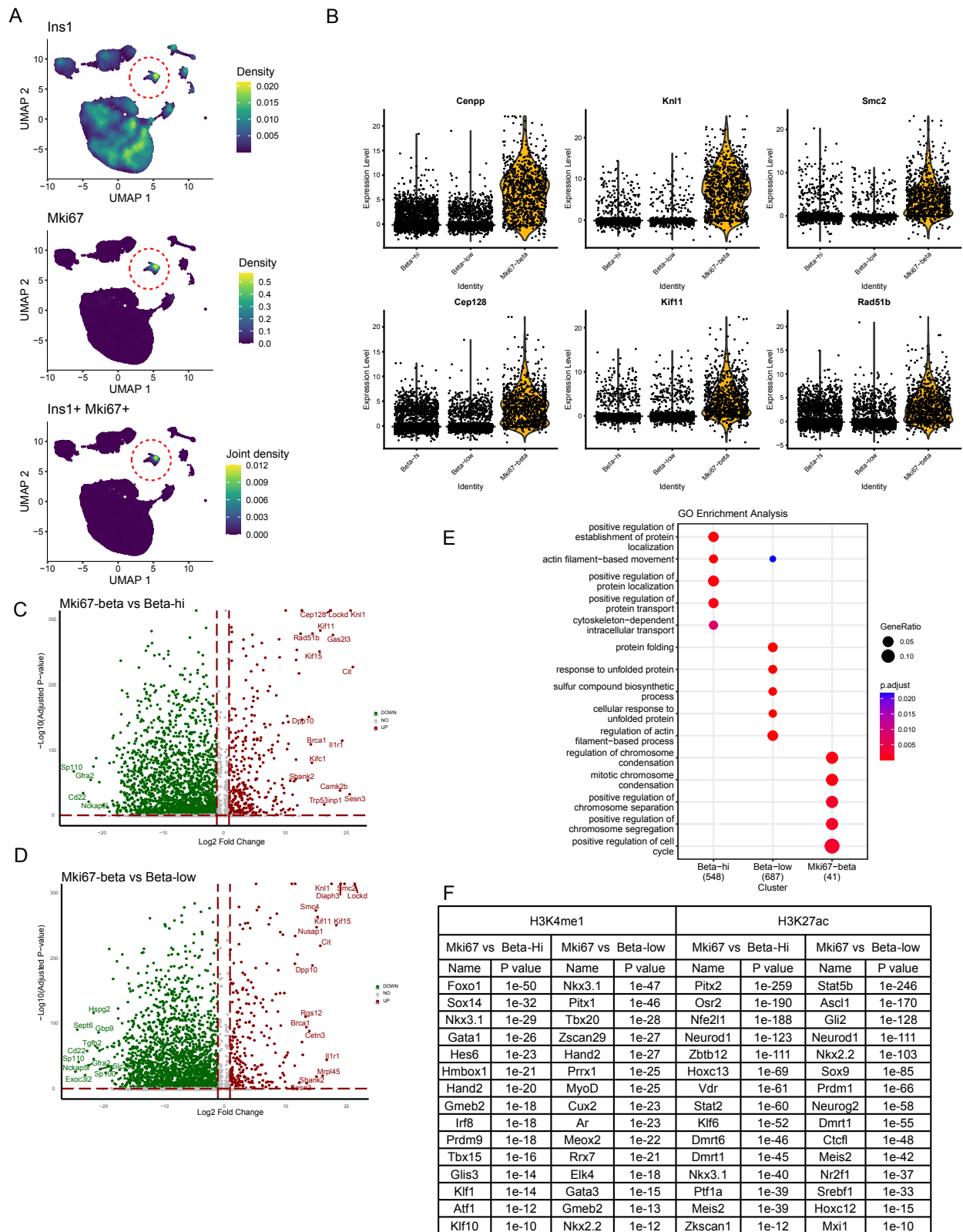


Figure S5. The Mki67 β cell population features high expression of genes related to the cell cycle.

(A) Visualization of *Mki67*, *Ins1*, and joint expression of *Mki67* and *Ins1* in UMAP space using the kernel density estimation method in the R package Nebulosa. (B) Violin plots show the expression of genes related to cell cycle (*Cennp*, *Kn11*, *Smc2*, *Cep128*, *Kif11* and *Rad51b*) in Beta-hi, Beta-low and Mki67-beta populations. (C-D). Volcano plot of differentially expressed genes in Mki67-beta vs. Beta-hi (C), or Mki67-beta vs. Beta-low (D). Differentially expressed genes were selected by adjusted p-value < 0.01 & $|\log_2(\text{fold change})| > 1$. The x-axis shows the fold change in gene expression between Mki67-beta and Beta-hi, and the y-axis shows adjusted p value. Dots represent individual genes. Gray dots represent genes that are not differentially expressed. Red and green dots represent genes that are significantly upregulated or downregulated in Mki67+ beta cells, respectively. (E) Summary of statistically enriched GO biological processes in beta-hi, beta-low or Mki67-beta populations based on Genomatix Pathways System (GePS) functional enrichment analysis. The dot size reflects the number of genes enriched in the Gene Ontology (GO) term and the color reflects the corresponding statistical significance (P-value). (F) Table of selected enriched TF motifs when comparing H3K4me1 or H3K27ac enhancers in Mki67+ β cells to those in beta-hi or beta-low cells.

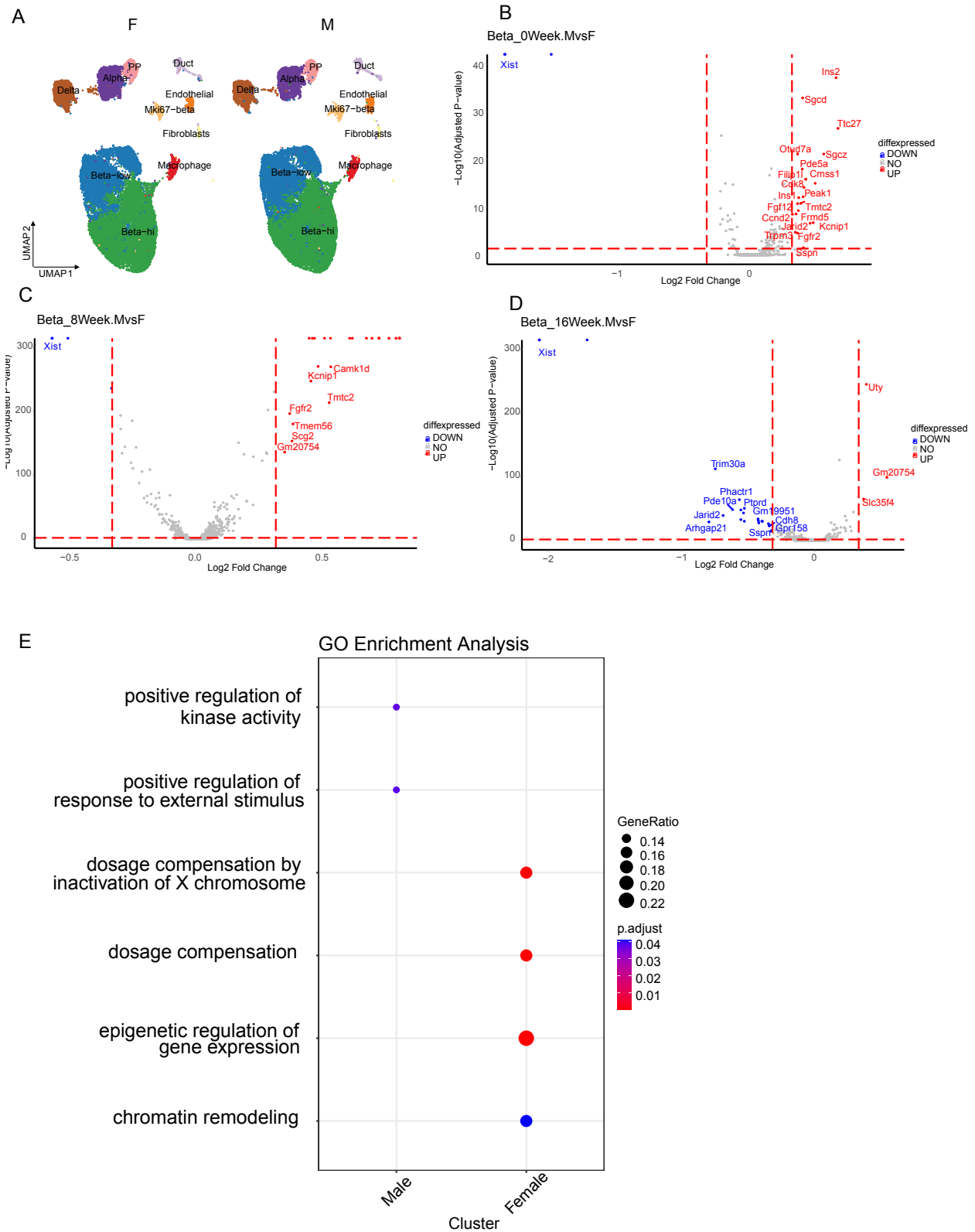


Figure S6. β cell transcriptome analysis reveals sexually dimorphic genes in mice.

(A). UMAP visualizations of populations defined by scRNA in islets from male (left) or female (right) mice. (B-D) Differential gene expression in male or female β cells in islets under 0, 8, or 16-week HFD. In the volcano plots, the male-biased and female-biased genes are labeled by blue and red dots, respectively. Differentially expressed genes were defined by adjusted p-value < 0.05 & $|\log_2(\text{fold change})| > 0.1$. (E). Functional enrichment analysis for male- or female-biased genes.

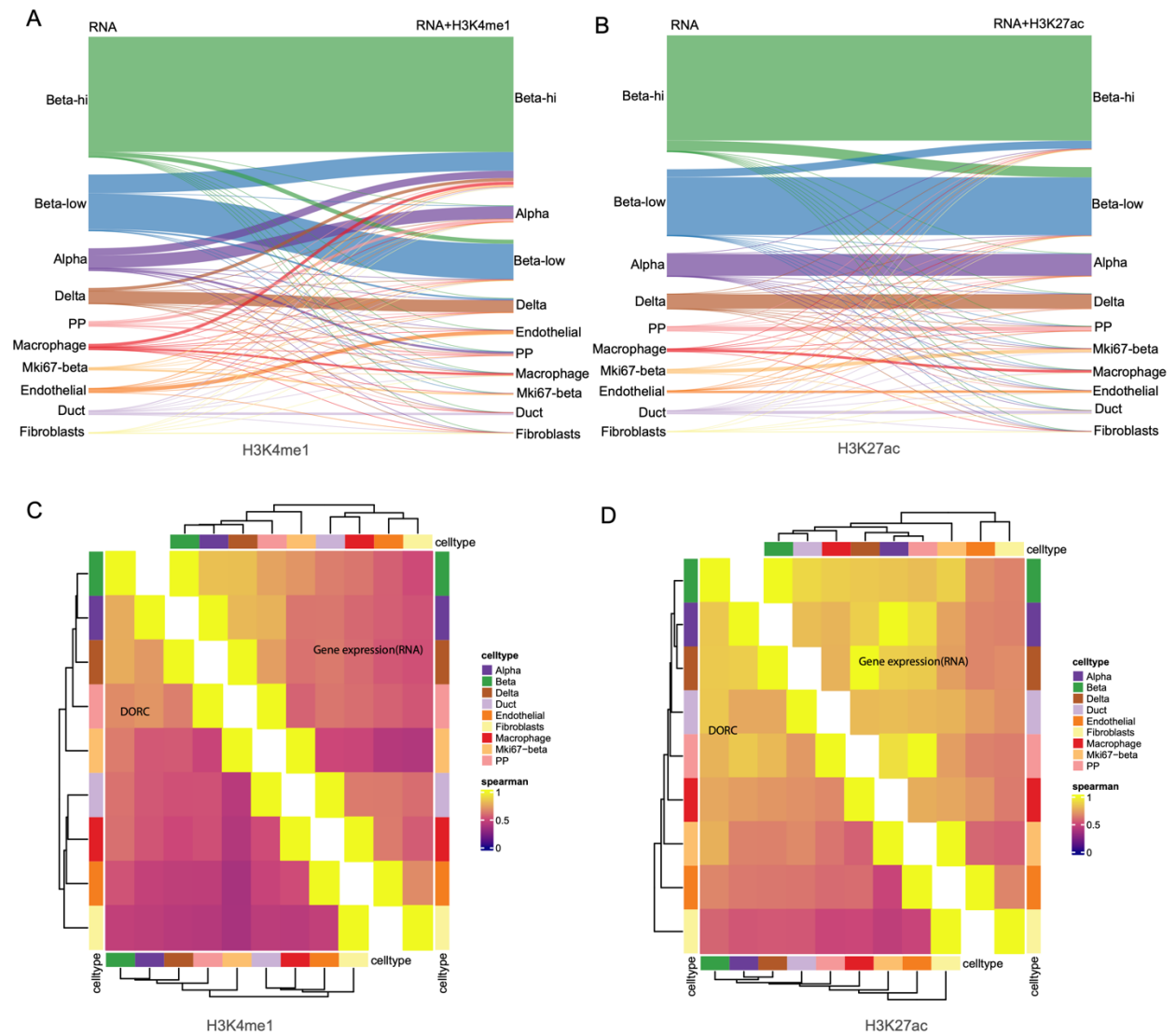
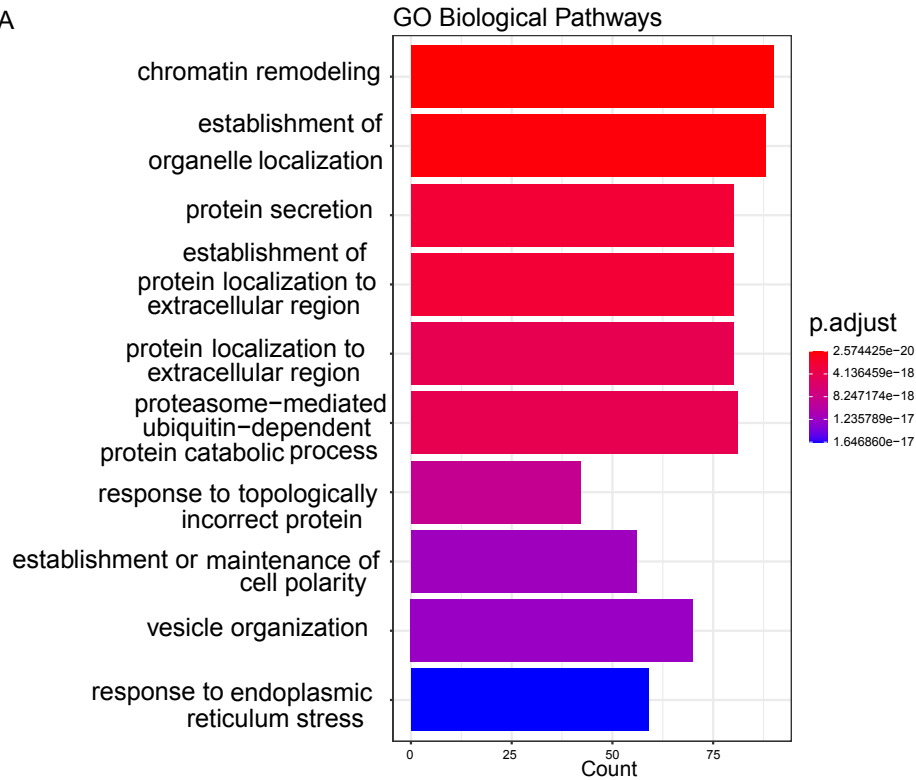


Figure S7. Cell identities defined by multi-omic datasets recapitulate RNA-based definition.

(A-B). Sankey plots showing the cell identities defined by Paired-Tag RNA profiles (left) or joint modalities of RNA-H3K4me1(A), or RNA-H3K27ac(B) (right). (C-D) The pair-wise correlation (Spearman correlation coefficient) of cell clusters defined by RNA (top right) or H3K4me1/H3K27ac (bottom left). The correlations of H3K4me1 or H3K27ac were calculated based on the cluster-average DORC scores of each locus. The RNA correlation was calculated based on the cluster-average expression of each individual gene.

A



B

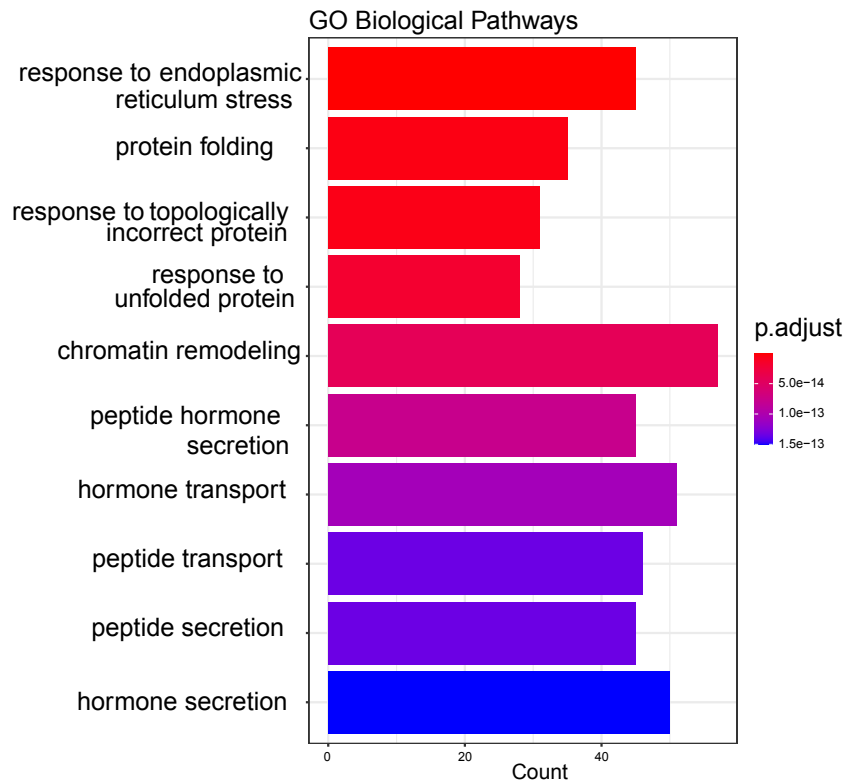


Figure S8. Gene ontology for genes in Figure 2K (A) and 2L(B) showing enrichment in distinct enriched categories related to ER-stress, inflammation, and beta cell function.

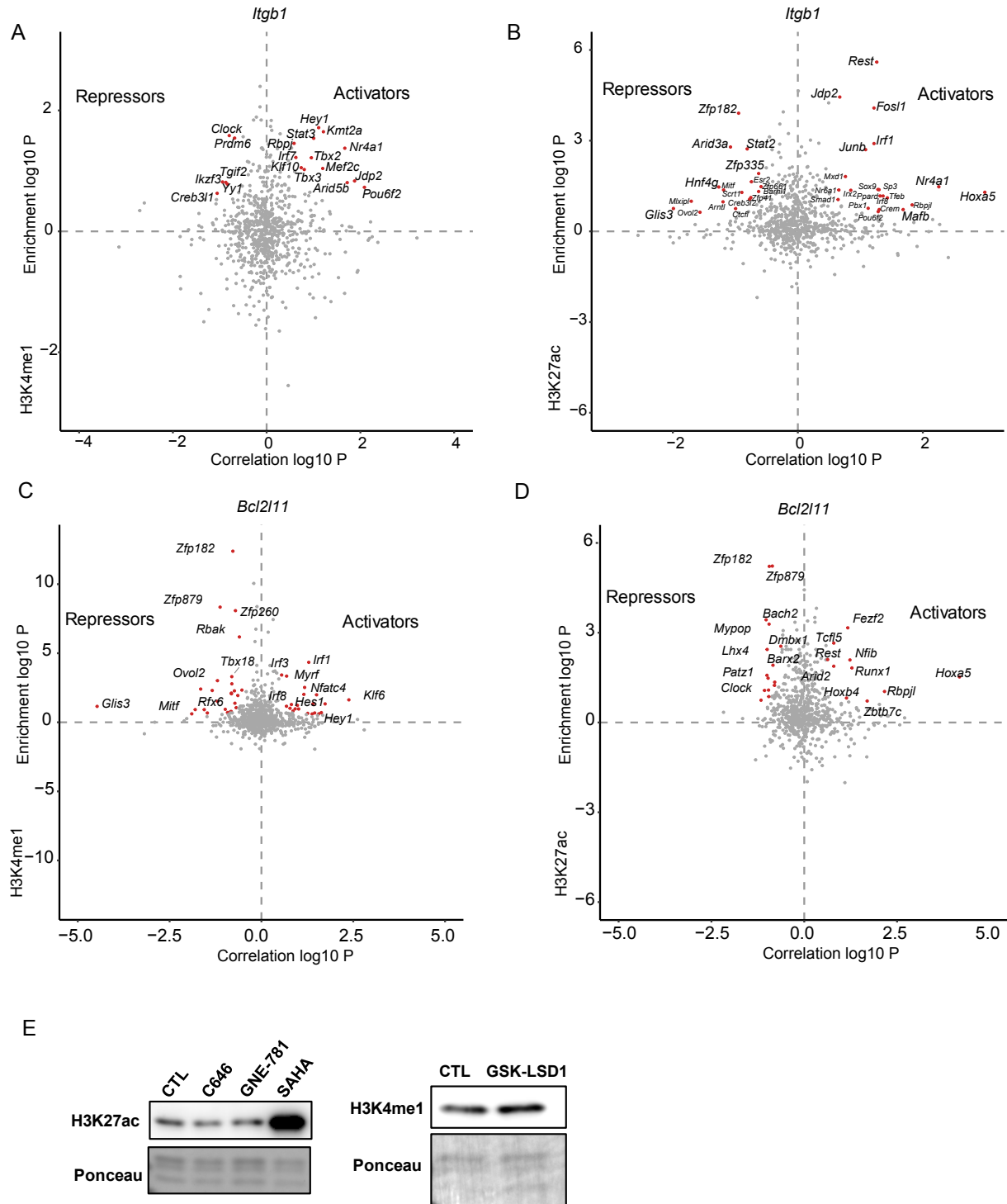


Figure S9. Using gene regulatory network (GRN) to identify TF modulators for individual genes.

(A-B) TF activators or repressors for *Itgb1* based on H3K4me1 (A) or H3K27ac (B). (C-D) TF activators or repressors for *Bcl2l11* based on H3K4me1 (C) or H3K27ac (D). Red dots are

significant TFs defined by $\text{abs}(\text{regulation score}) \geq 0.5$ ($-\log_{10}$ scale), with all other TFs shown in gray. **(E)** Western blotting of H3K27ac and H3K4me in MIN6 cells treated with SAHA (5 μM), GNE-781 (1 μM), C646 (5 μM), GSK-LSD1 (5 μM) for 24 h.

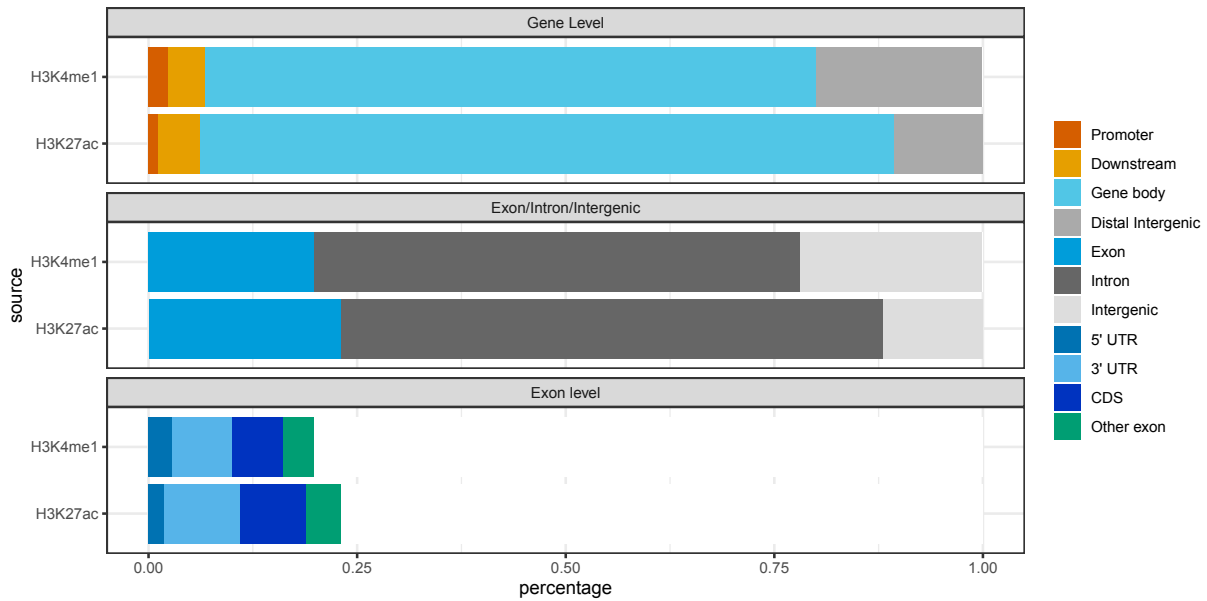


Figure S10. Stacked bar plot showing the distribution of H3K4me1 and H3K27ac peaks in different genomic regions. Promoters are defined as genomic regions encompassing 200 bp upstream and *downstream* of the TSS. Intragenic regions contain 5' UTR, exons, introns, and 3' UTR. Downstream means 2000 bp downstream of the transcription end site. Distal intergenic regions are genomic regions except downstream and promoter regions.

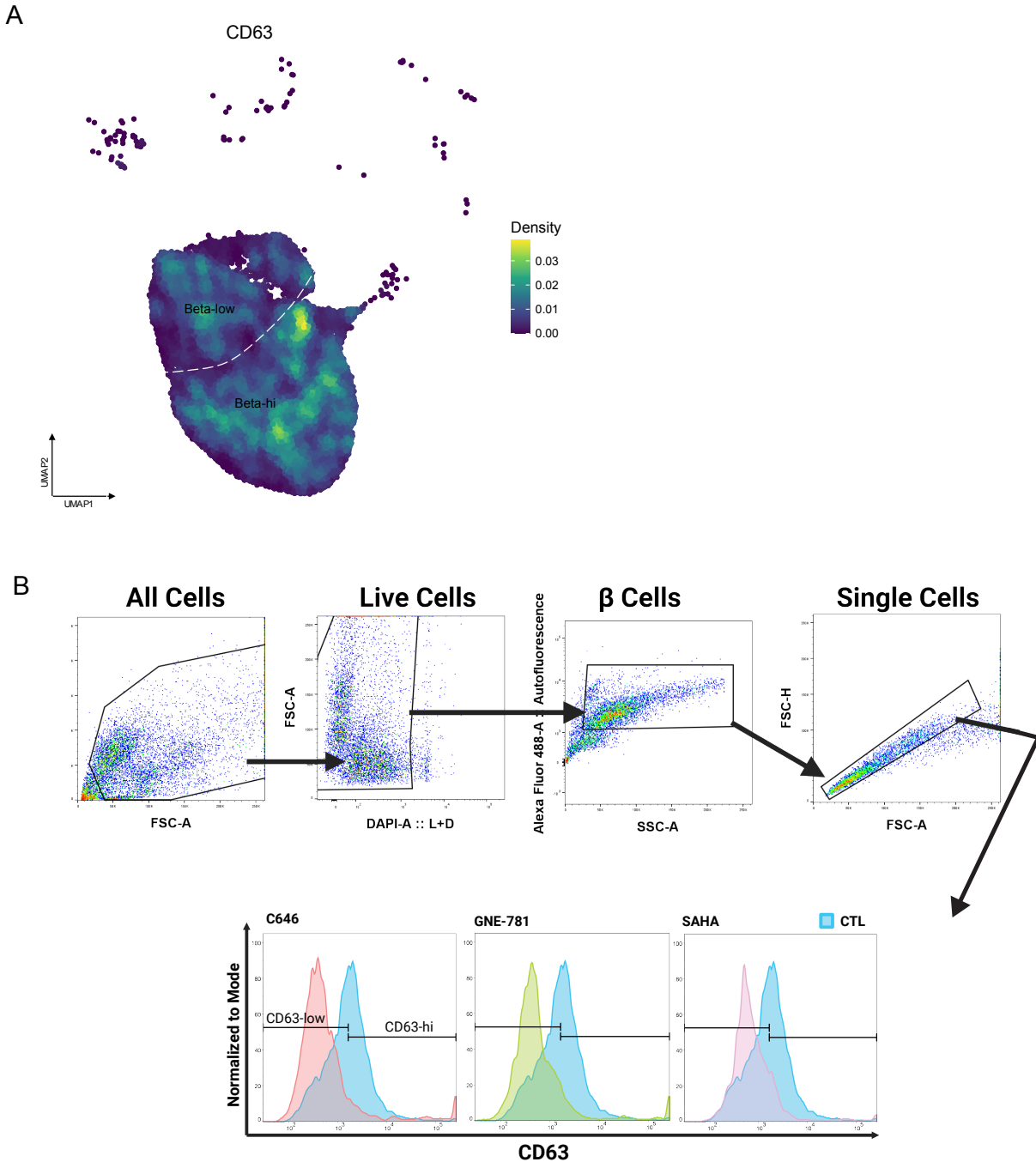


Figure S11. H3K27ac level influences the heterogeneity of β cells.

(A) UMAP showing restricted expression patterns of CD63 in β cell population. (B) Scatterplots showing the sequential gating protocol to isolate CD63-hi and CD63-low β cells from mice islets treated with SAHA, GNE-781, or C646 by FACS. Source numerical data are available in source data.

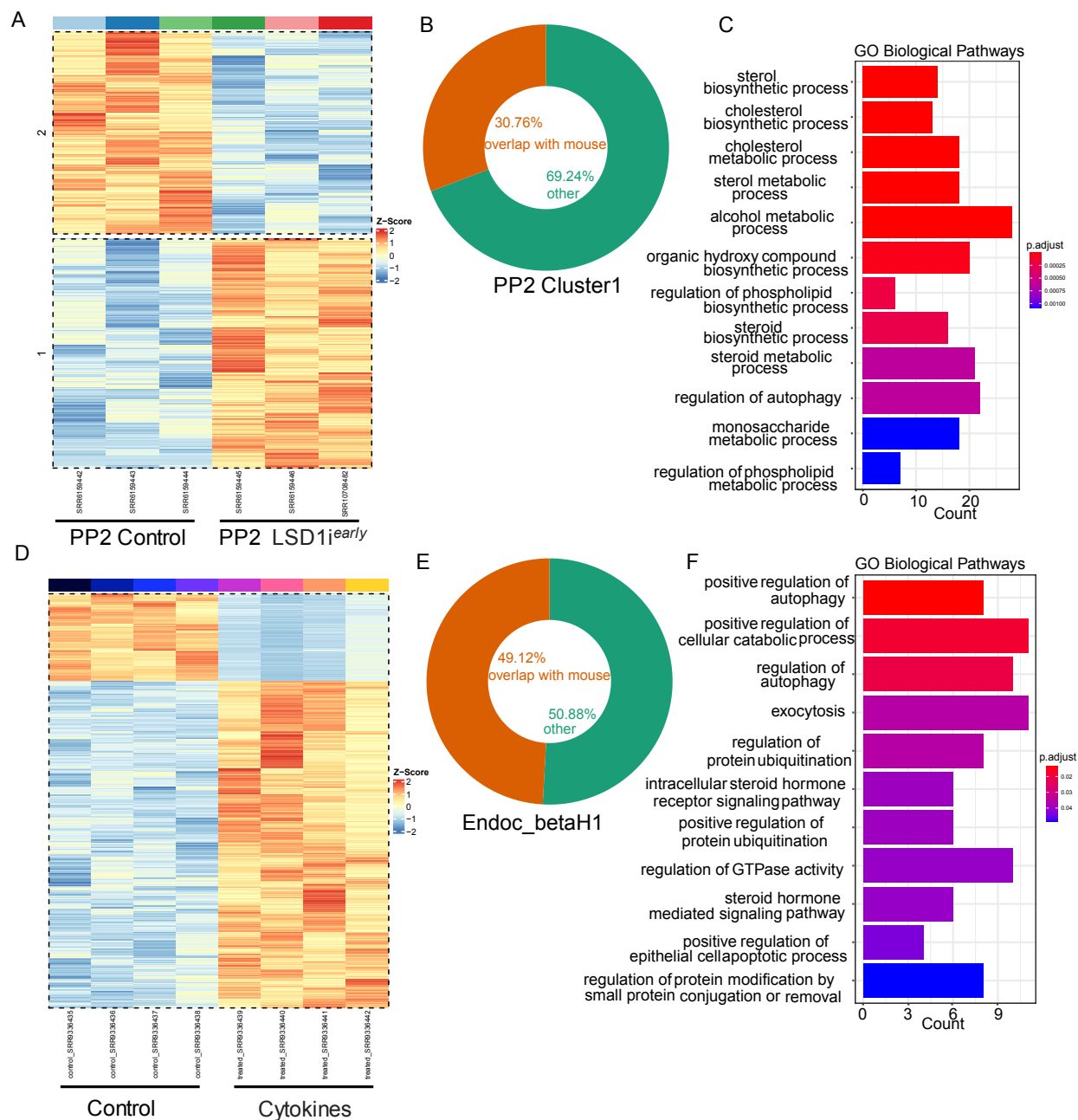


Figure S12. The H3K4me1/H3K27ac-RNA linkage is conserved in human beta cells.

(A). Heatmap showing significantly altered gene expression in PP2 cells (late pancreatic progenitor cells) after LSD1 inhibition (LSD1^{early}) in Vinckier et al.⁶⁵. (B). Pie chart showing the percentage of LSD1^{early} induced genes (clusters1) with a mouse homolog that has significant H3K4me1-RNA association. (C). Gene ontology for genes in (B). (D). Heatmap showing differential H3K27ac peaks in EndoC-βH1 cells treated with cytokine mix (data from Ramos-Rodriguez, M et. al.⁶⁶). (E). Pie chart showing the percentage of (D)-associated genes with a

mouse homolog that has significant H3K27ac-RNA association. **(F)** Gene ontology for human-mouse overlapping genes in **(E)**.

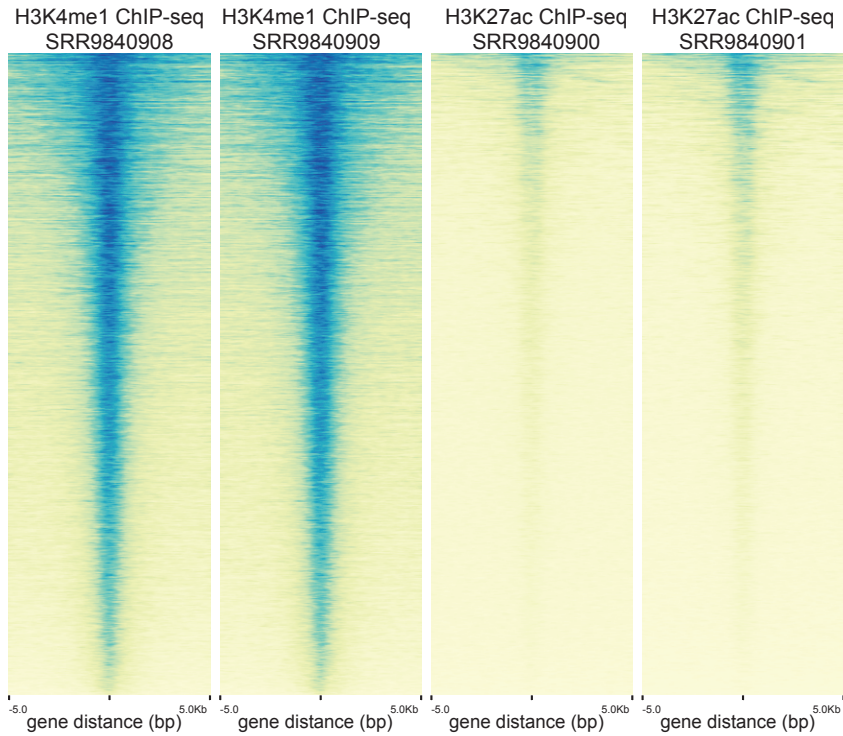


Figure S13. Primed enhancers defined by Paired-tag are consistent with published bulk ChIP-seq.

Heatmap showing previously published bulk H3K4me1 and bulk H3K27ac ChIP-seq at 14,116 primed enhancer regions defined in this study. The rows were sorted by the descending signals of H3K4me1.

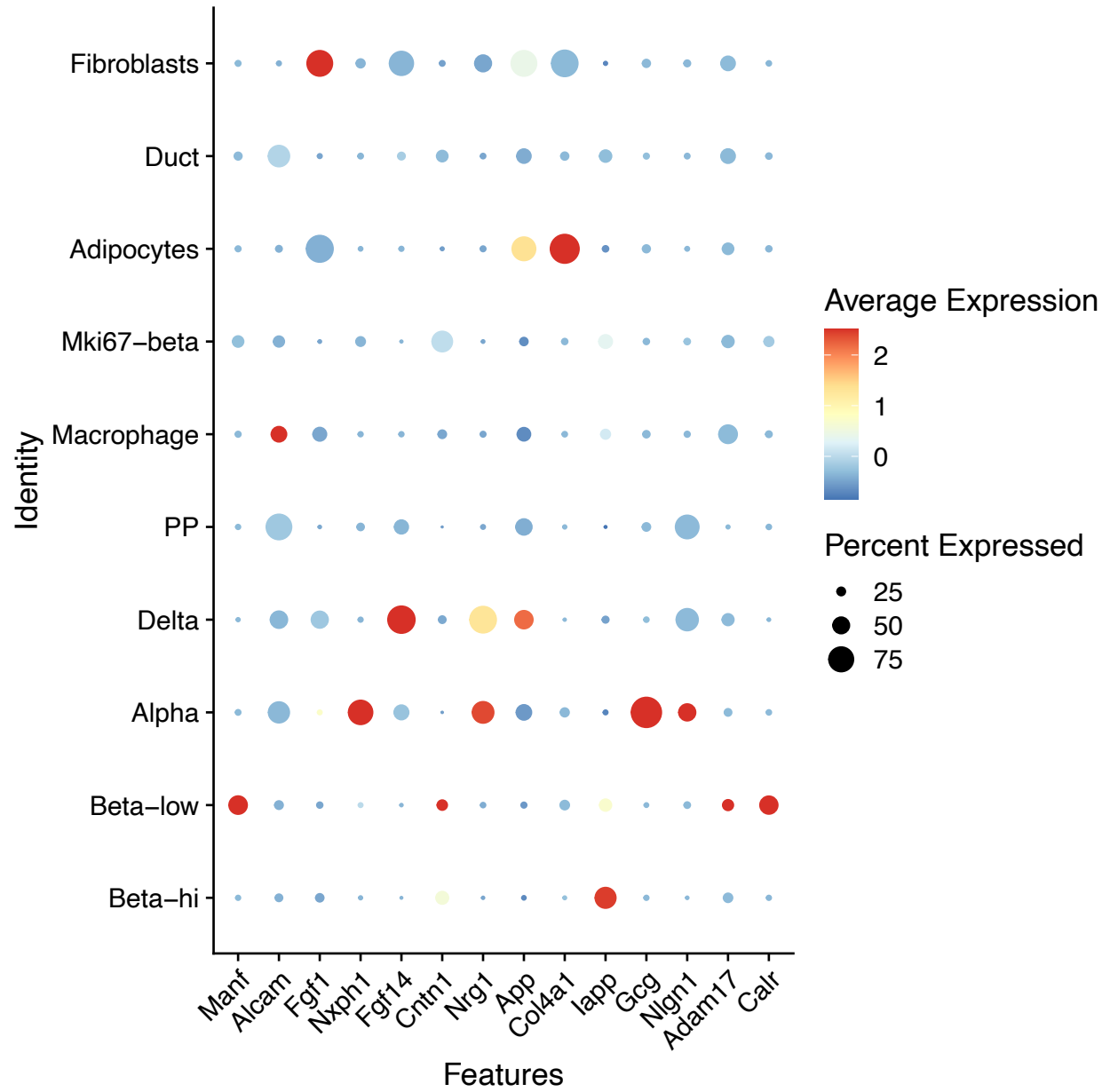


Figure S14. Expression of ligands (Figure 6A) in different cell populations.

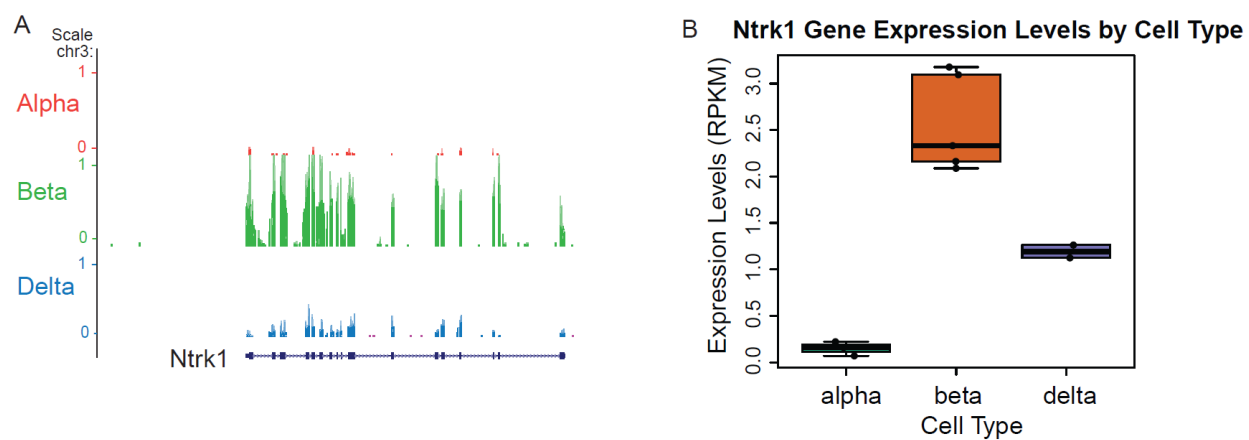


Figure S15. Expression of Ntrk1 (TrkA) in α , β , and δ cells.

(A) Genome browser plot showing the expression of Ntrk1 in purified mouse α , β , and δ cells from DiGruccio MR et. al.⁸⁵. **(B)** Box plot of Ntrk1 quantification in (A). n=3 for alpha, n=5 for beta, n=2 for delta.