iScience, Volume *26*

Supplemental information

Pharmacological HDAC inhibition impairs pancreatic β-cell function through an epigenome-wide reprogramming

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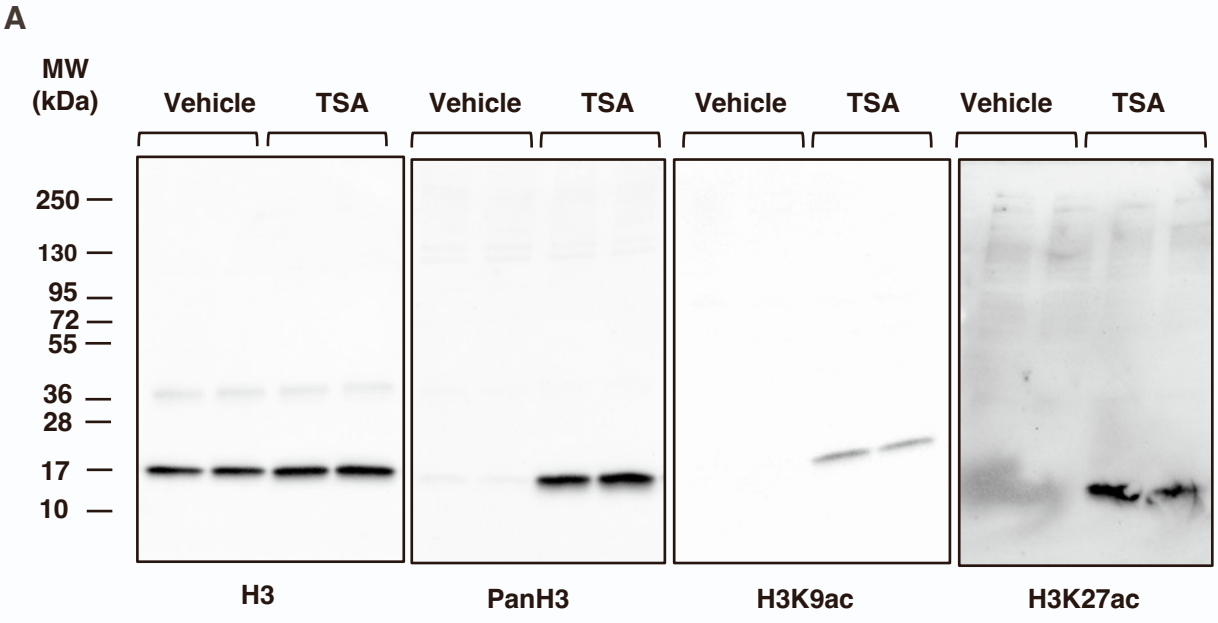
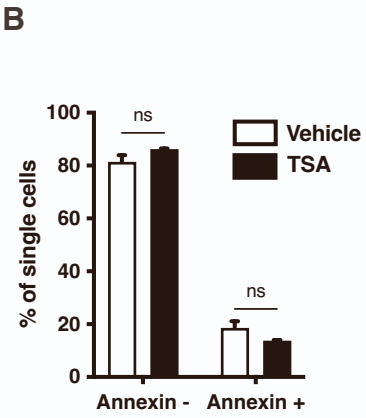
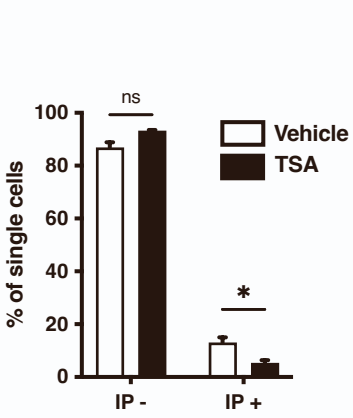
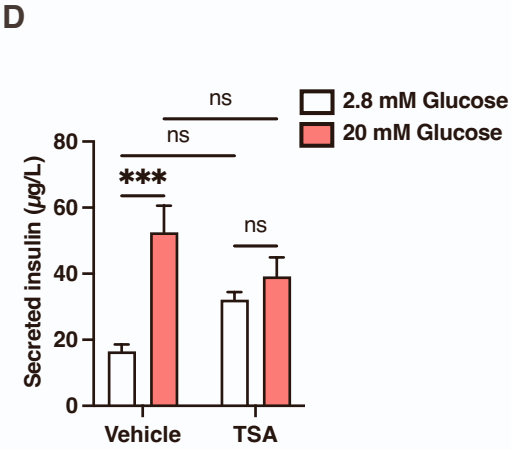
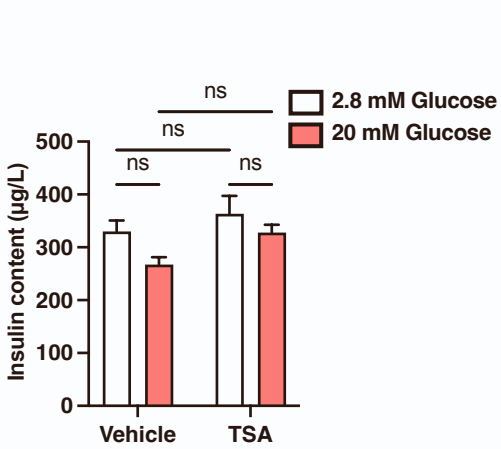
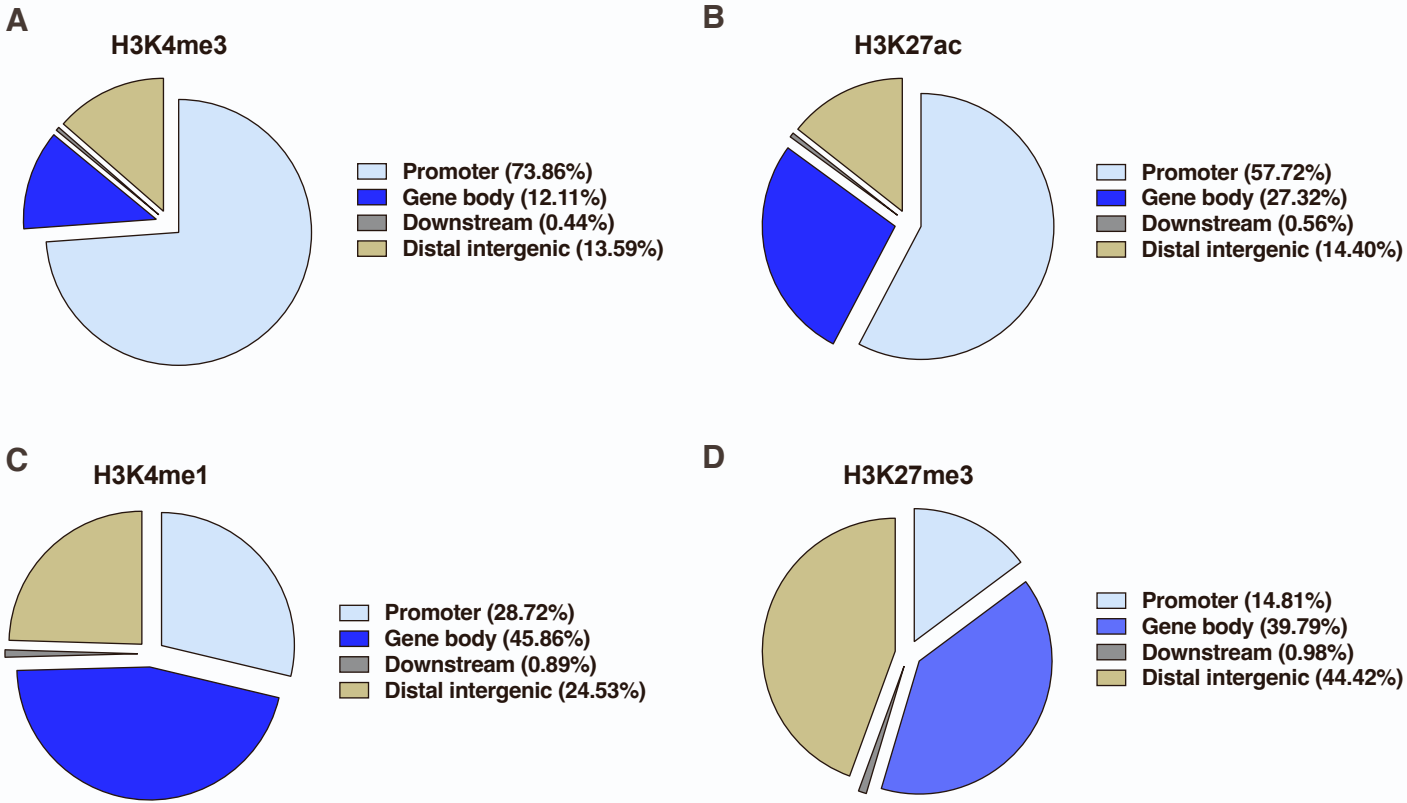

A
MW (kDa)
Vehicle
TSA
Vehicle
TSA
Vehicle
TSA
Vehicle
TSA
250
130
95
72
55
36
28
17
10
H3
PanH3
H3K9ac
H3K27ac

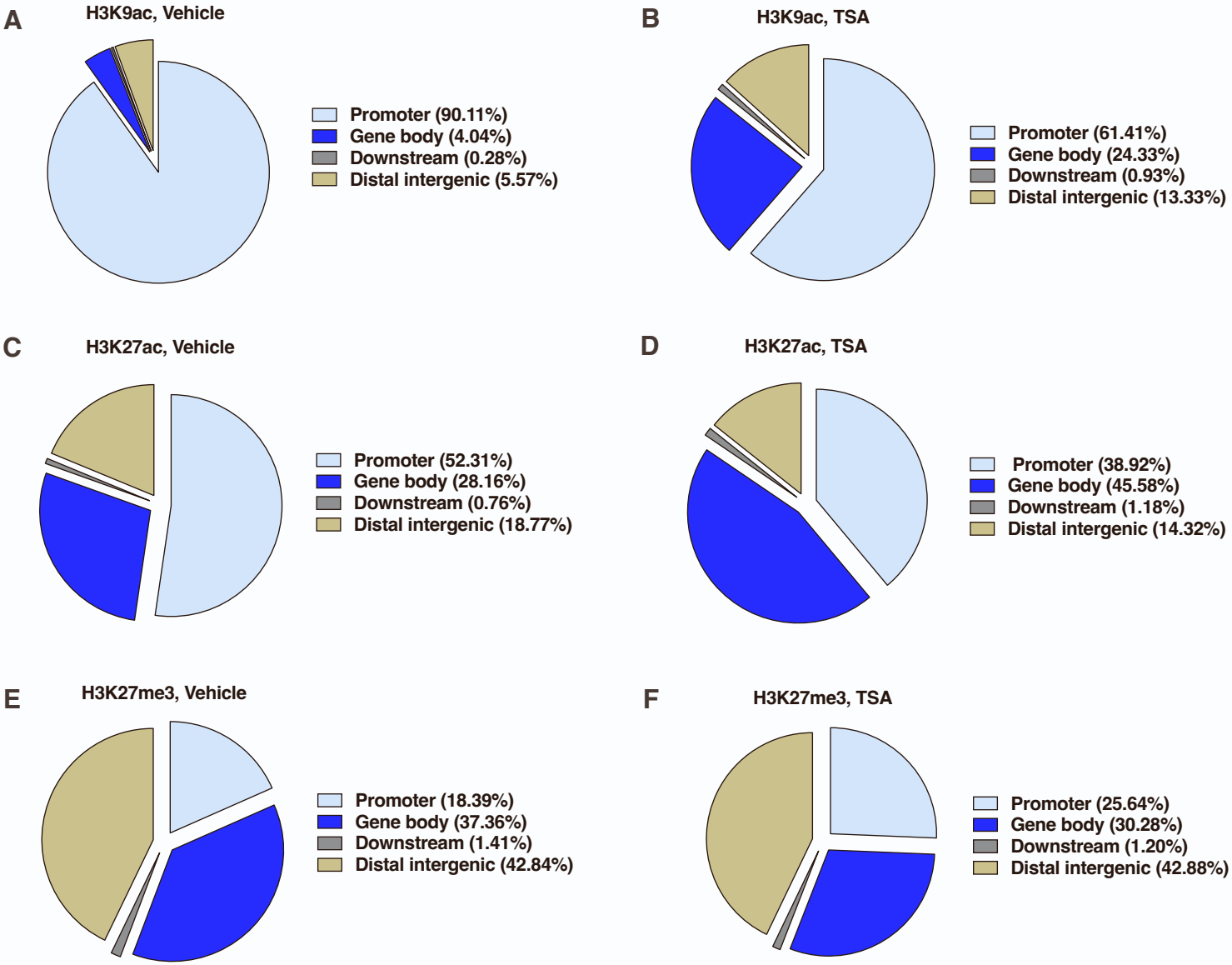

B
% of single cells
100
80
60
40
20
0
ns
ns
Vehicle
TSA
Annexin -
Annexin +

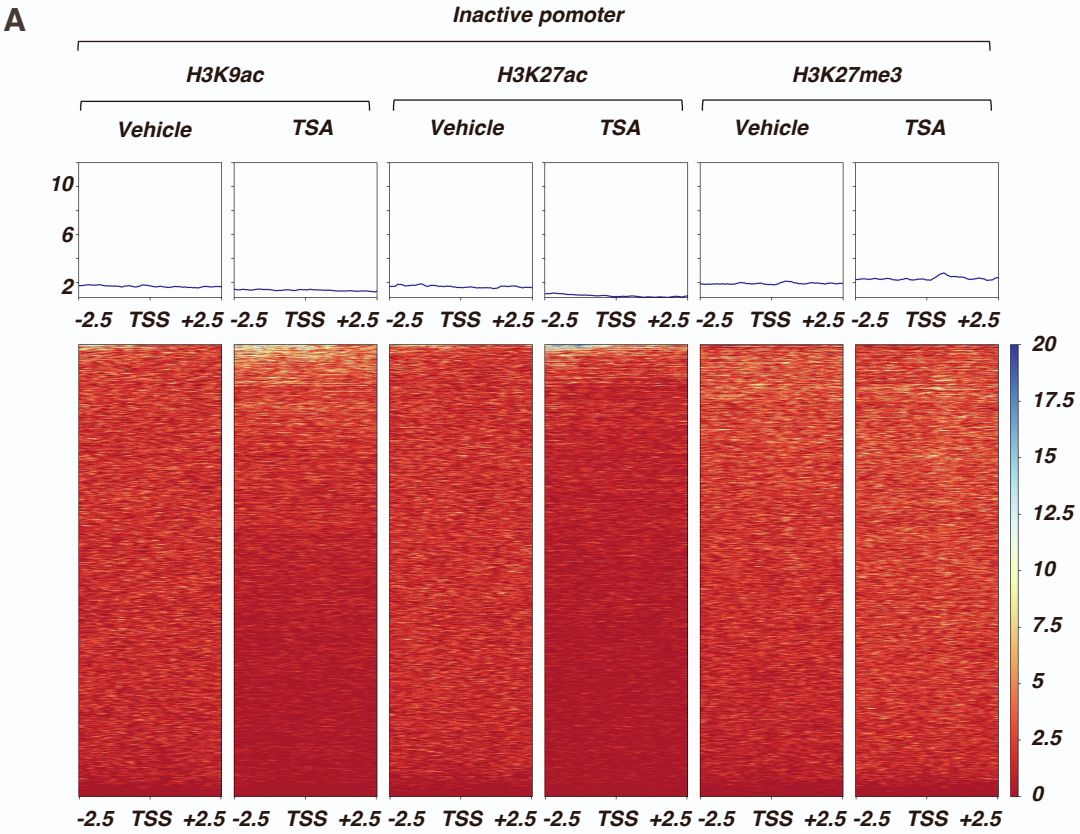

C
% of single cells
100
80
60
40
20
0
ns
*
Vehicle
TSA
IP -
IP +

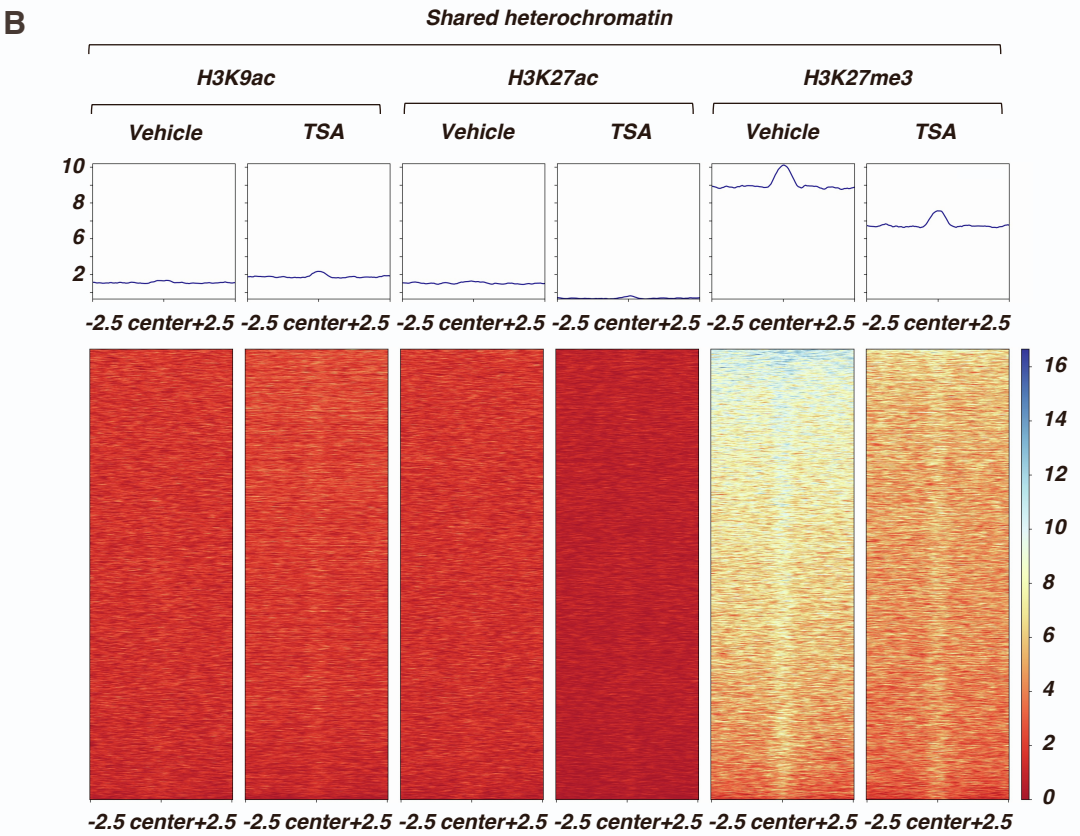

D
Secreted insulin (µg/L)
80
60
40
20
0
ns
ns
***
ns
2.8 mM Glucose
20 mM Glucose
Vehicle
TSA

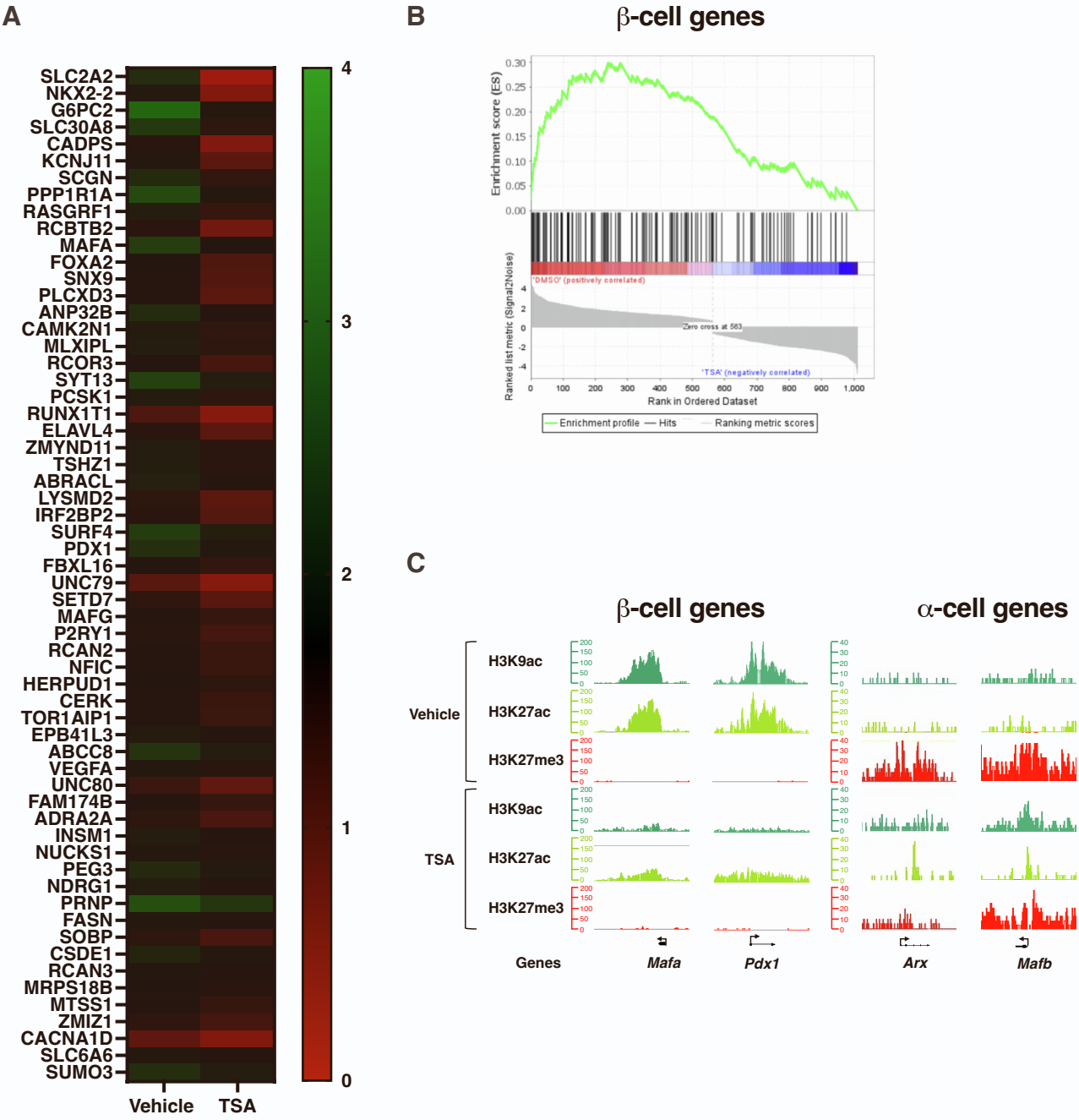

E
Insulin content (µg/L)
500
400
300
200
100
0
ns
ns
ns
ns
2.8 mM Glucose
20 mM Glucose
Vehicle
TSA

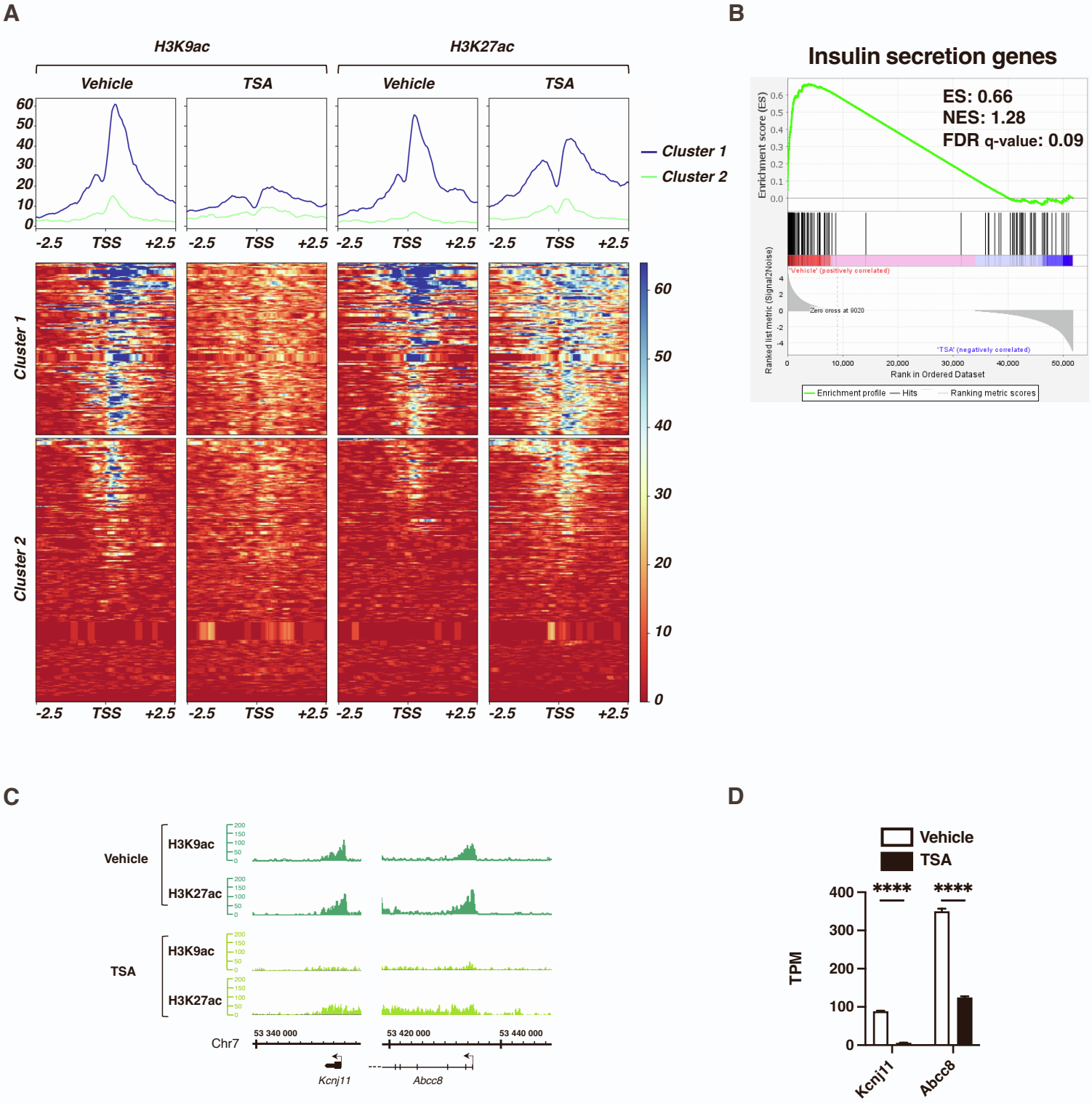

A
H3K4me3
Promoter (73.86%)
Gene body (12.11%)
Downstream (0.44%)
Distal intergenic (13.59%)
B
H3K27ac
Promoter (57.72%)
Gene body (27.32%)
Downstream (0.56%)
Distal intergenic (14.40%)
C
H3K4me1
Promoter (28.72%)
Gene body (45.86%)
Downstream (0.89%)
Distal intergenic (24.53%)
D
H3K27me3
Promoter (14.81%)
Gene body (39.79%)
Downstream (0.98%)
Distal intergenic (44.42%)


A
H3K9ac, Vehicle
Promoter (90.11%)
Gene body (4.04%)
Downstream (0.28%)
Distal intergenic (5.57%)
B
H3K9ac, TSA
Promoter (61.41%)
Gene body (24.33%)
Downstream (0.93%)
Distal intergenic (13.33%)
C
H3K27ac, Vehicle
Promoter (52.31%)
Gene body (28.16%)
Downstream (0.76%)
Distal intergenic (18.77%)
D
H3K27ac, TSA
Promoter (38.92%)
Gene body (45.58%)
Downstream (1.18%)
Distal intergenic (14.32%)
E
H3K27me3, Vehicle
Promoter (18.39%)
Gene body (37.36%)
Downstream (1.41%)
Distal intergenic (42.84%)
F
H3K27me3, TSA
Promoter (25.64%)
Gene body (30.28%)
Downstream (1.20%)
Distal intergenic (42.88%)


A
Inactive pomoter
H3K9ac
H3K27ac
H3K27me3
Vehicle
TSA
Vehicle
TSA
Vehicle
TSA
10
6
2
-2.5 TSS +2.5 -2.5 TSS +2.5 -2.5 TSS +2.5 -2.5 TSS +2.5 -2.5 TSS +2.5 -2.5 TSS +2.5
20
17.5
15
12.5
10
7.5
5
2.5
0
-2.5 TSS +2.5 -2.5 TSS +2.5 -2.5 TSS +2.5 -2.5 TSS +2.5 -2.5 TSS +2.5 -2.5 TSS +2.5


B
Shared heterochromatin
H3K9ac
H3K27ac
H3K27me3
Vehicle
TSA
Vehicle
TSA
Vehicle
TSA
10
8
6
2
-2.5 center+2.5 -2.5 center+2.5 -2.5 center+2.5 -2.5 center+2.5 -2.5 center+2.5 -2.5 center+2.5
16
14
12
10
8
6
4
2
0
-2.5 center+2.5 -2.5 center+2.5 -2.5 center+2.5 -2.5 center+2.5 -2.5 center+2.5 -2.5 center+2.5


A
SLC2A2
NKX2-2
G6PC2
SLC30A8
CADPS
KCNJ11
SCGN
PPP1R1A
RASGRF1
RCBTB2
MAFA
FOXA2
SNX9
PLCXD3
ANP32B
CAMK2N1
MLXIPL
RCOR3
SYT13
PCSK1
RUNX1T1
ELAVL4
ZMYND11
TSHZ1
ABRACL
LYSMD2
IRF2BP2
SURF4
PDX1
FBXL16
UNC79
SETD7
MAFG
P2RY1
RCAN2
NFIC
HERPUD1
CERK
TOR1AIP1
EPB41L3
ABCC8
VEGFA
UNC80
FAM174B
ADRA2A
INSM1
NUCKS1
PEG3
NDRG1
PRNP
FASN
SOBP
CSDE1
RCAN3
MRPS18B
MTSS1
ZMIZ1
CACNA1D
SLC6A6
SUMO3
Vehicle
TSA
4
3
2
1
0
B
β-cell genes
Enrichment score (ES)
'DMSO' (positively correlated)
Zero cross at 563
'TSA' (negatively correlated)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores
C
β-cell genes
α-cell genes
Vehicle
TSA
H3K9ac
H3K27ac
H3K27me3
Genes
Mafa
Pdx1
Arx
Mafb


A
H3K9ac
H3K27ac
Vehicle
TSA
Vehicle
TSA
Cluster 1
Cluster 2
-2.5
TSS
+2.5
Cluster 1
Cluster 2
B
Insulin secretion genes
ES: 0.66
NES: 1.28
FDR q-value: 0.09
Enrichment score (ES)
Ranked list metric (Signal2Noise)
'Vehicle' (positively correlated)
Zero cross at 9020
'TSA' (negatively correlated)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores
C
Vehicle
H3K9ac
H3K27ac
TSA
H3K9ac
H3K27ac
Chr7
53 340 000
53 420 000
53 440 000
Kcnj11
Abcc8
D
Vehicle
TSA
TPM
****
****
Kcnj11
Abcc8

Supplementary Figure S1. Effect of TSA treatment on histone acetylation, apoptosis, proliferation and insulin levels in Min6 cells, Related to Figure 1. (A) Levels of histone H3, global acetylation (PanH3ac) and acetylation of lysine 9 (H3K9ac) and lysine 27 (H3K27ac) were monitored by western blot analysis in vehicle- and TSA- treated Min 6 cells (0.5 µM, 16h, n=2). **(B-C)** Annexin V (B) and propidium iodide (C) labelling of TSA-treated Min6 cells (0.5 µM, 16h, n=3). Vehicle (DMSO 0.1%, n=3) was used as negative control. Annexin - : annexin V negative cells, Annexin + : annexin V positive cells. IP - : propidium iodide negative cells, IP + : propidium iodide positive cells. Results in A and B are displayed as % of single cells recorded by FACS +/- SEM. * $p<0.05$, ns: not significant. **(D-E)** Glucose-stimulated insulin secretion of TSA-treated cells (0.5 µM, 16h, n=7). Vehicle (DMSO 0.1%) was used as a control. Results are presented mean +/- SEM of secreted insulin (D) and insulin content (E) in response to 2.8 mM and 20 mM glucose. * $p<0.05$, *** $p<0.001$ (Two-way Anova). ns=not significant.

Supplementary Figure S2. Genomic distribution of histone marks in Min6 cells, Related to Figure 2. (A-D) Individual genomic distribution of H3K4me3, H3K4me1, H3K27ac and H3K27me3 in Min6 cells. The percentage of peaks for each histone mark within 4 distinct genomic segments is displayed in a pie chart (promoter (TSS +/- 2.5 kb), gene body, downstream of genes and distal intergenic regions).

Supplementary Figure S3. Genomic distribution of histone marks in Min6 cells upon TSA treatment, Related to Figure 5. (A-F) Individual genomic distribution of H3K9ac (A-B), H3K27ac (C-D) and H3K27me3 (E-F) in vehicle- (A, C and E) and TSA-treated (B, D and F) Min6 cells. The percentage of peaks for each histone mark within 4 distinct genomic segments is displayed in a pie chart (promoter (TSS +/- 2.5 kb), gene body, downstream of genes and distal intergenic regions.

Supplementary Figure S4. Genomic distribution of histone marks in inactive promoters and shared heterochromatin in Min6 cells upon TSA treatment, Related to Figure 5. (A) H3K9ac, H3K27ac and H3K27me3 signal in conserved inactive promoters in vehicle- and TSA-treated Min6 cells. Heatmap and mean signal centered on TSS +/- 2.5kb are displayed. **(B)** H3K9ac, H3K27ac and H3K27me3 signal in conserved heterochromatin in vehicle- and TSA-treated Min6 cells. Heatmap and mean signal centered on TSS +/- 2.5kb are displayed.

Supplementary Figure S5. Differential gene expression and histone acetylation upon TSA treatment in Min6 cells, Related to Figure 6. (A) Heatmap representing gene expression levels of β-cell genes in vehicle and TSA-treated Min6 cells. **(B)** Enrichment plot from Gene Set Enrichment Analysis (GSEA). GSEA was conducted with 60 genes set enriched in β cells. **(C)** Examples of functional genomic regions of β-cell (*Pdx1, Mafa)* and α-cell genes (*Arx, Mafb*) in vehicle and TSA-treated Min6 cells showing H3K9ac, H3K27ac and H3K27me3 marks. Figures are adapted from the IGB genome browser screenshots.

Supplementary Figure S6. Differential gene expression and histone acetylation profiles of genes involved in insulin secretion upon TSA treatment in Min6 cells, Related to Figure 6. (A) Heatmap displaying H3K9ac and H3K27ac level in response to TSA treatment in Min6 cells within TSS-centered promoters of insulin secretion-associated genes (n=583 transcripts). K-means clustering (2 clusters) was applied to discriminate TSA-remodeled promoters (cluster 1; 118 genes, Supplementary Table S2) from unaffected ones (cluster 2, 168 genes, Supplementary Table S3). **(B)** Enrichment plot from Gene Set Enrichment Analysis (GSEA). GSEA was conducted with 118 genes belonging to cluster 1. **(C)** Examples of remodeled promoters (*Abcc8* and *Kcnj11)* in vehicle and TSA-treated Min6 cells showing H3K9ac and H3K27ac marks. Figures are adapted from the IGB genome browser screenshots. **(D)** Expression levels (TPM) of Abcc8 and Kcnj11 in vehicle- and TSA-treated Min6 cells. **** $p<0.005$ (Two-way Anova)).