van Arensbergen et al. Figure S1

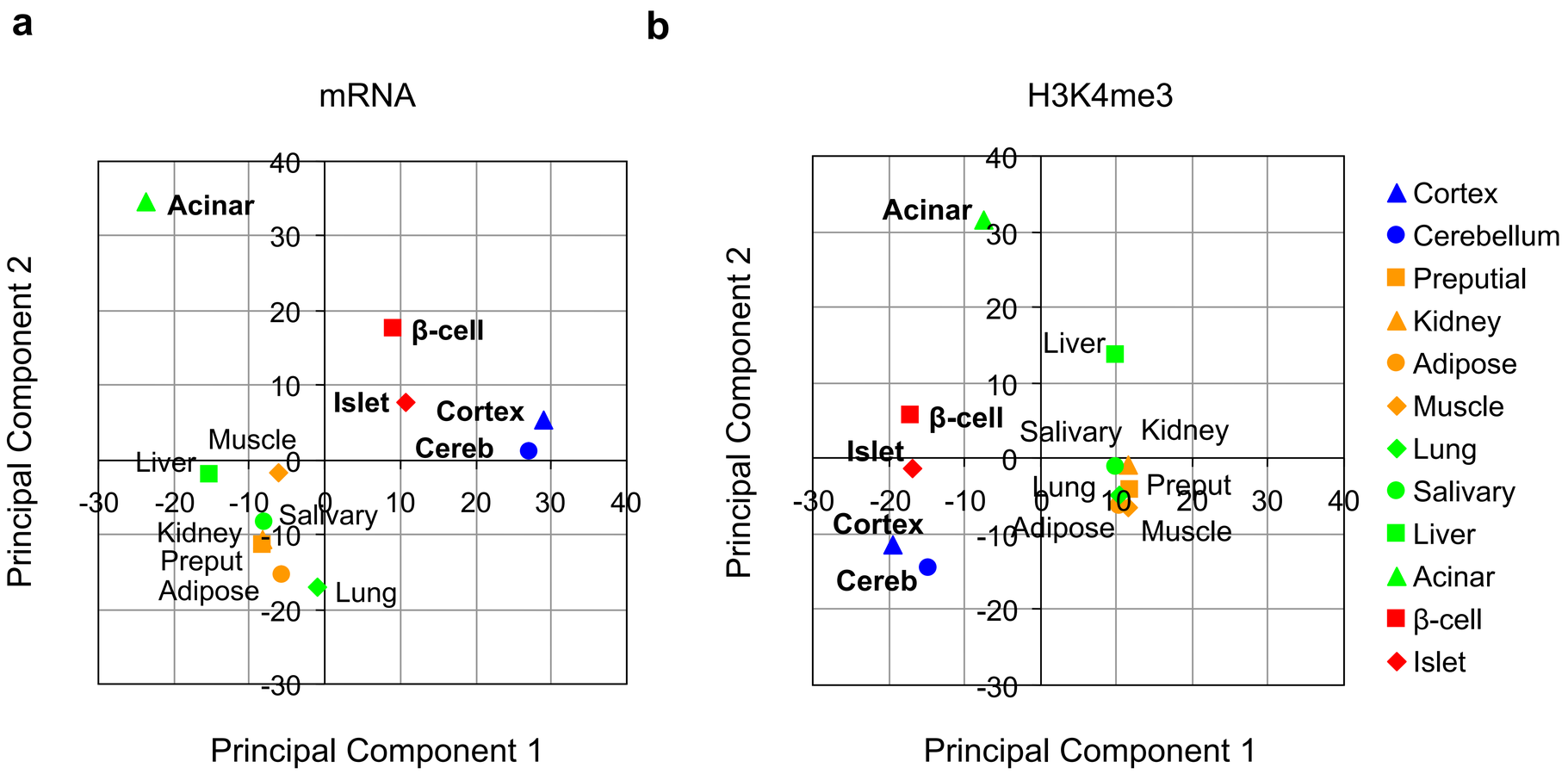



**Figure S1. β-cells and neural tissues share a gene activity program. a.** First two dimensions of the principal component analysis of mRNA expression in β-cells and 11 tissues . Together these two principal components represent 35% of the total variation of mRNA presence. Note that in both components β-cells and islets are most proximal to cortex and cerebellum. **b.** Same as in **a.** but for H3K4me3 profiles. The two components together represent 36% of the total variation.

van Arensbergen et al. Figure S2

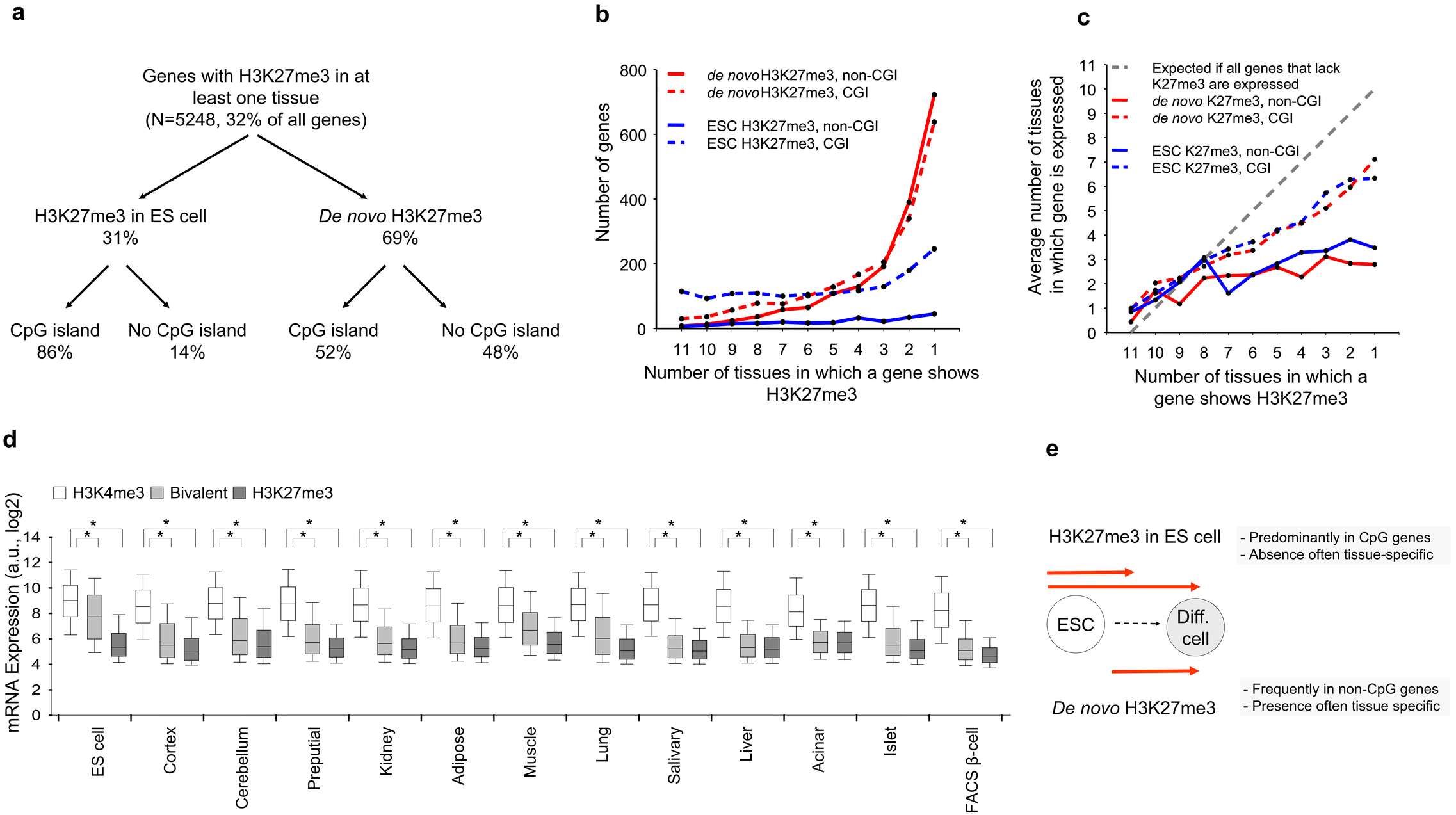



**Figure S2. H3K27me3 profiles are shaped by tissue-specific *de novo* methylation coupled with selective removal of ES cell H3K27 methylation. a.** Breakdown of H3K27me3 targets according to the presence of H3K27me3 in ES cells or only in differentiated tissues (*de novo* H3K27me3), and by the presence of CpG islands in their promoters. **b.** *De novo* H3K27me3 is highly tissue-selective in both CpG and non-CpG island genes. **c.** Genes that are targeted by H3K27me3 in only few tissues are often also inactive in tissues where they do not show H3K27me3. This is particularly true for non-CpG island genes. **d.** mRNA levels of genes marked by H3K4me3 (white), H3K27me3 (dark grey) or both (light gray). Note that while in ES cells bivalent genes are expressed at an intermediate level, in differentiated tissues expression of bivalent genes is similar or only marginally higher than genes marked by H3K27me3 only. *$P$<0.001. **e.** Summary representation of the two major patterns of tissue-specific H3K27me3 profiles.

van Arensbergen et al. Figure S3

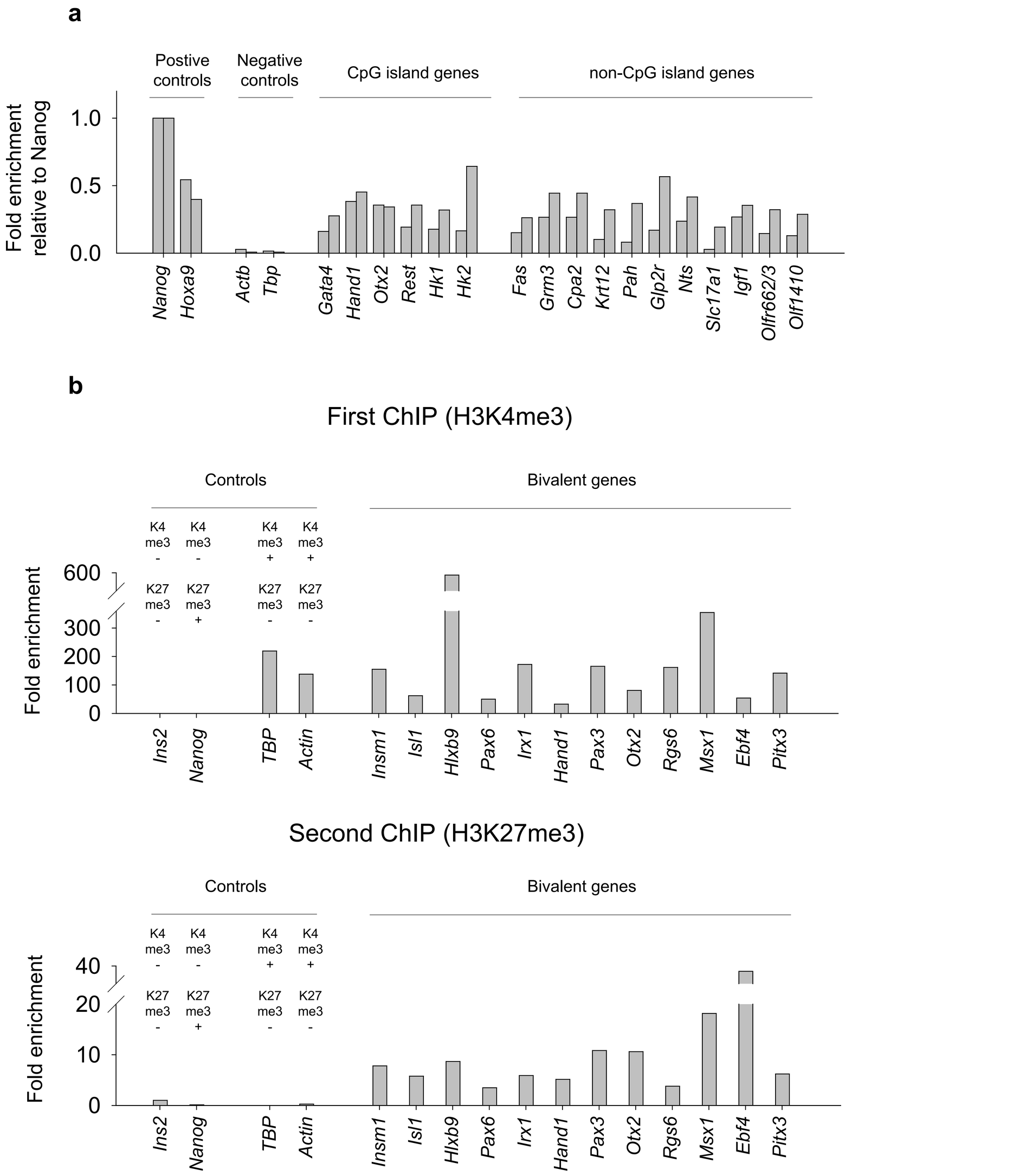
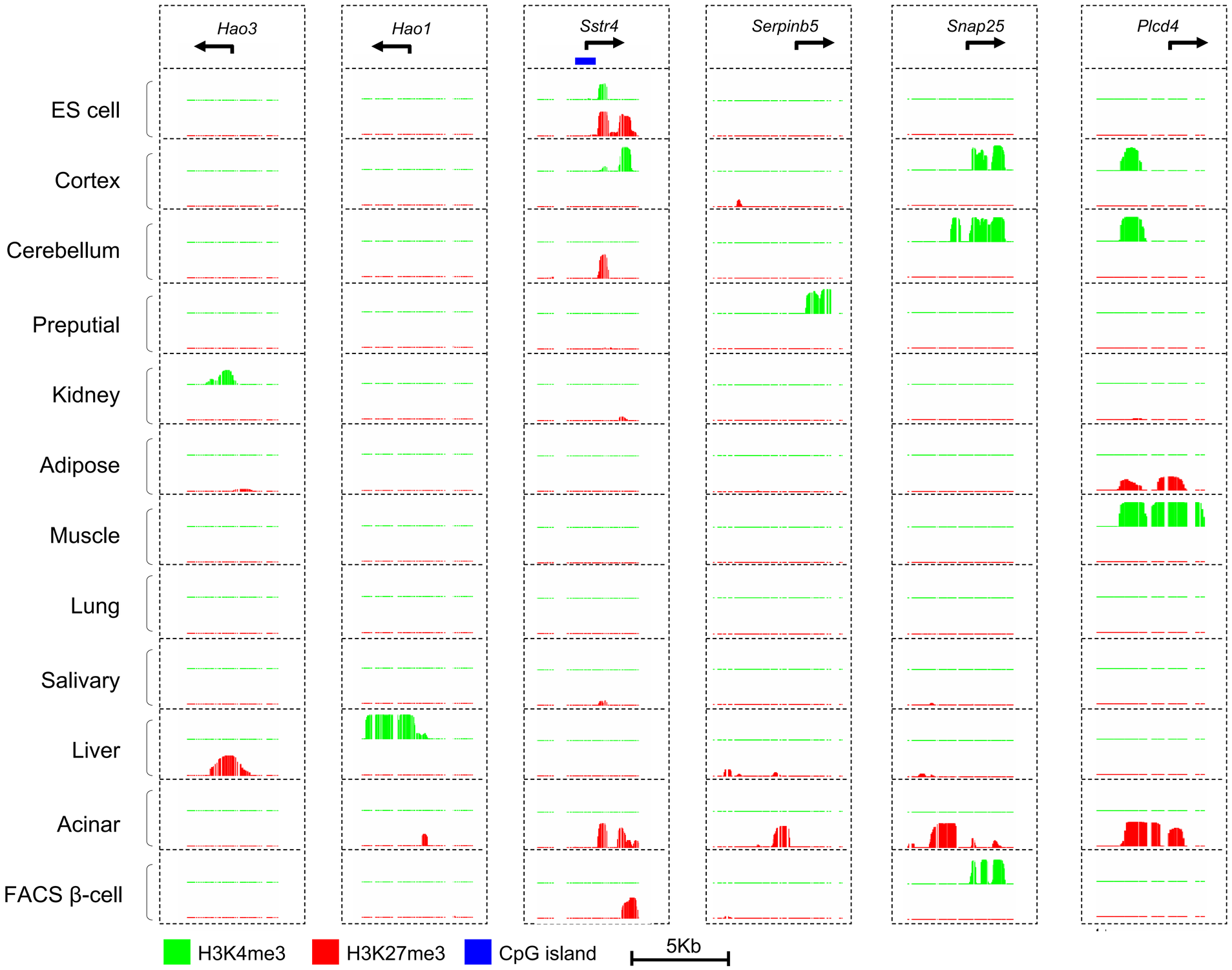



**Figure S3. Gene-specific ChIP qPCR analysis. a.** qPCR confirmations of H3K27me3 enrichment in chromatin from FACS purified β-cells. H3K27me3 targets determined by microarray hybridization were confirmed by qPCR, while housekeeping genes *Actb* and *Tbp* showed no enrichment.Targets were chosen to include genes that are selectively repressed in β-cells and non-CpG island genes. Two biological replicates are shown. ChIP/input enrichment levels were normalized to Nanog. **b.** qPCR confirmation of bivalency using sequential ChIP in acinar cell chromatin. ChIP for H3K4me3 was performed in ~5 x $10^6$ acinar cells, followed by ChIP for H3K27me3. For both ChIPs we calculated the ratio of immunoprecipitated/input DNA and expressed it as the fold-enrichment over the same ratio in the *Ins2* negative control promoter. Input DNA corresponds to genomic DNA in the first ChIP, and H3K4me3-enriched DNA in the second ChIP. The results show H3K27me3 enrichment in H3K4me3-enriched chromatin in genes found to be bivalent in array studies.

van Arensbergen et al. Figure S4

**a**




**b**

| | *Hao3* | *Hao1* | *Sstr4* | *Serpinb5* | *Snap25* | *Plcd4* |
|---|---|---|---|---|---|---|
| ES cell | 11 | 10 | 41 | 19 | 13 | 27 |
| Cortex | 10 | 11 | 457 | 15 | 9644 | 153 |
| Cerebellum | 10 | 11 | 36 | 16 | 10637 | 231 |
| Preputial | 11 | 14 | 37 | 1064 | 15 | 30 |
| Kidney | 3089 | 13 | 43 | 23 | 13 | 44 |
| Adipose | 11 | 10 | 64 | 19 | 11 | 31 |
| Muscle | 12 | 11 | 52 | 23 | 11 | 1383 |
| Lung | 12 | 11 | 63 | 16 | 12 | 30 |
| Salivary | 11 | 14 | 47 | 62 | 13 | 29 |
| Liver | 17 | 2914 | 50 | 24 | 13 | 39 |
| Acinar | 18 | 16 | 65 | 23 | 14 | 40 |
| FACS β-cell | 10 | 9 | 28 | 16 | 3961 | 48 |

**Figure S4. Examples of genes with context-selective repression.** H3K27me3-mediated repression often occurs in only a subset of tissues in which a gene is inactive. Profiles of H3K4me3 and H3K27me3 (**a.**) and expression levels (**b.**) are shown for selected genes.

van Arensbergen et al. Figure S5

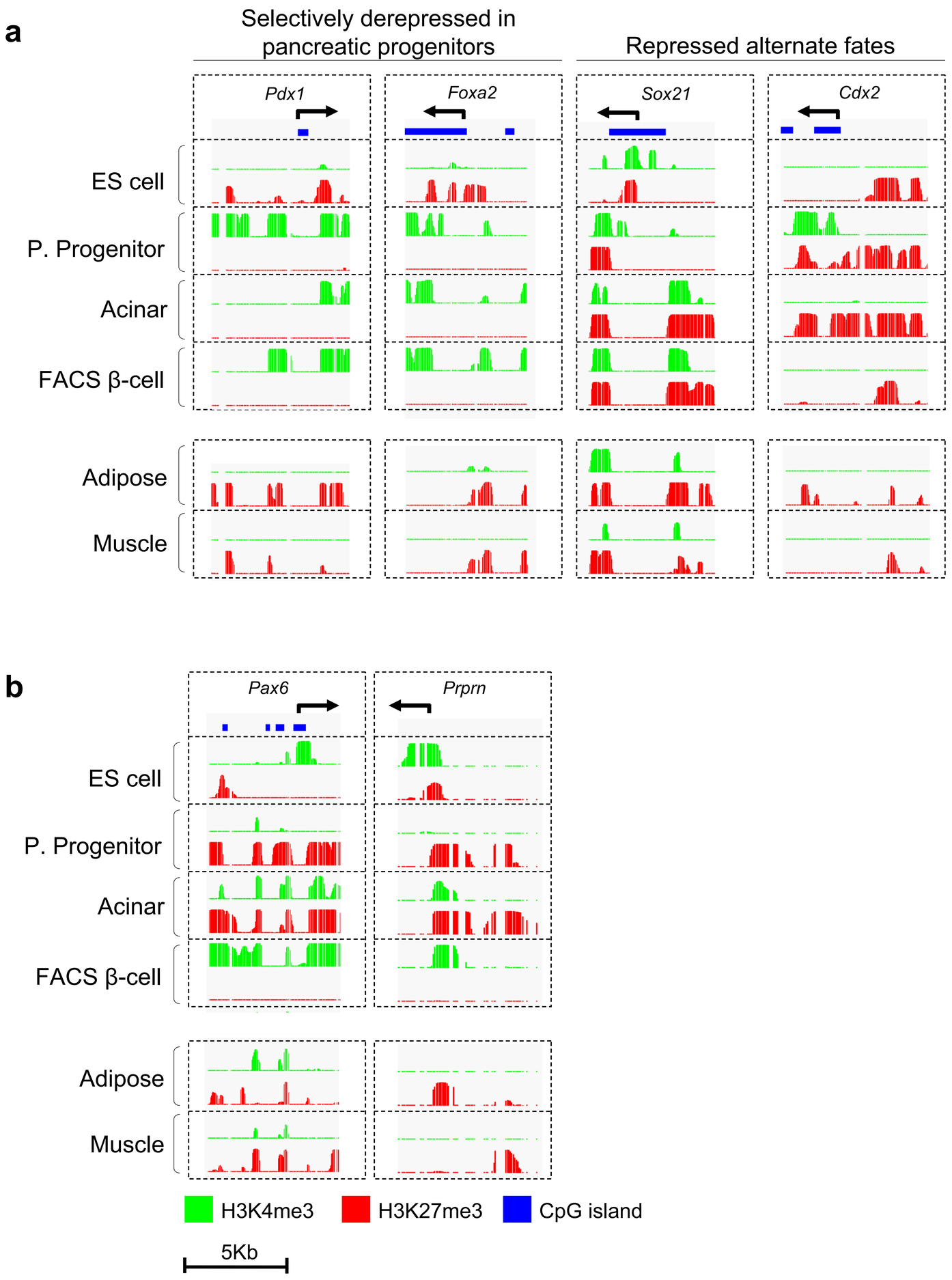
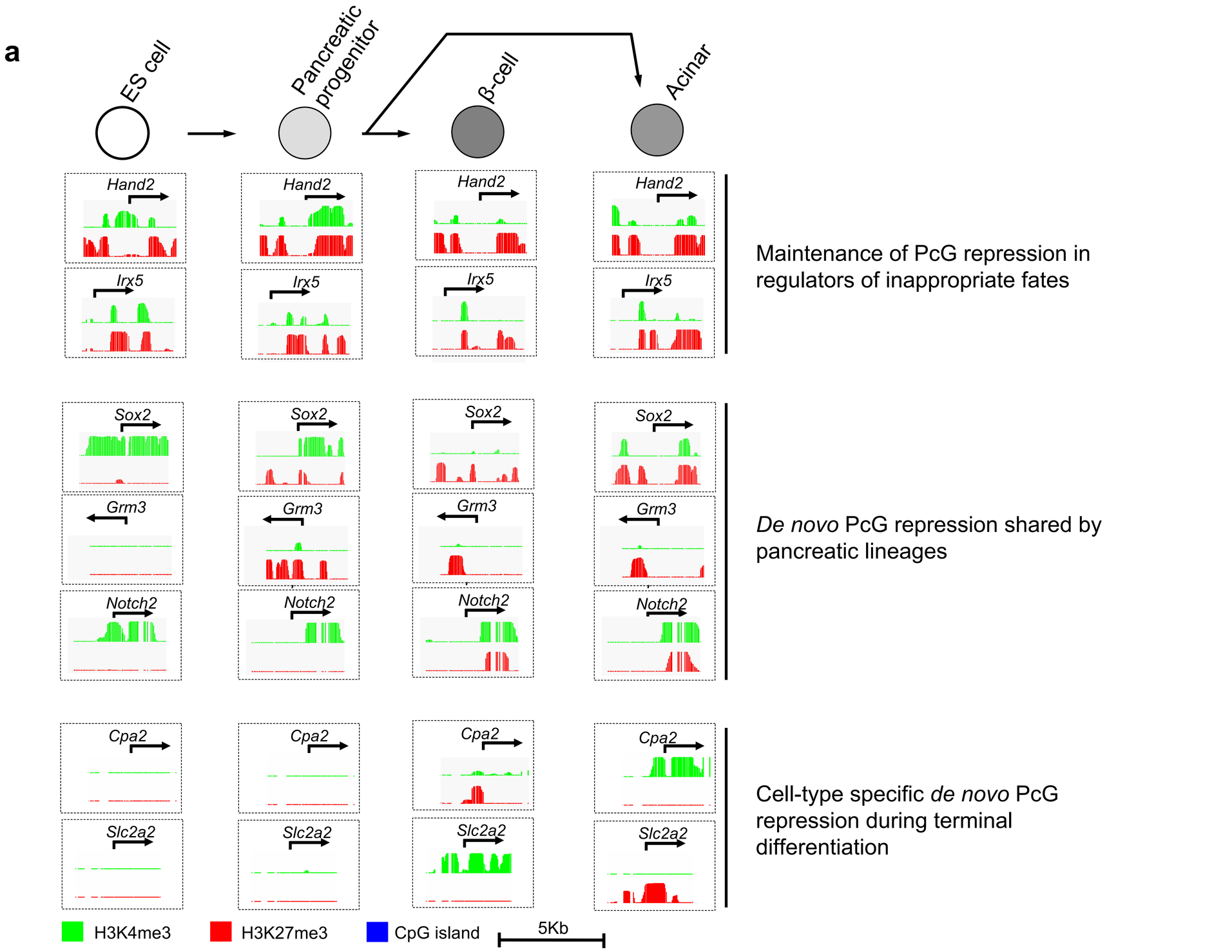
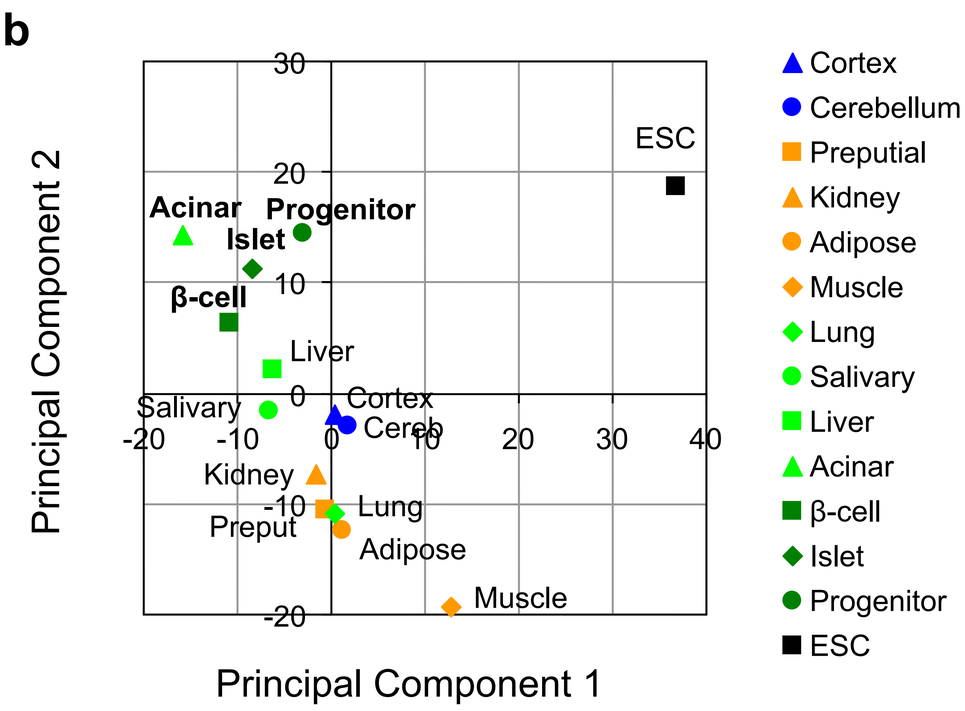



**Figure S5. Repression patterns in pancreatic progenitors. a.** Pancreatic progenitors exhibit as expected a selective absence of H3K27me3 repression in known stage-specific regulators (*Pdx1, Foxa2*), and are enriched in H3K27me3 in genes associated to alternate endoderm fates (*Sox21, Cdx2*). **b.** In *Pax6* and the β-cell autoantigen *Ptprn* a bivalent state is observed in ES cells, whereas only H3K27me3 is present in pancreatic progenitors prior to their activation in differentiated β-cells.

van Arensbergen et al. Figure S6



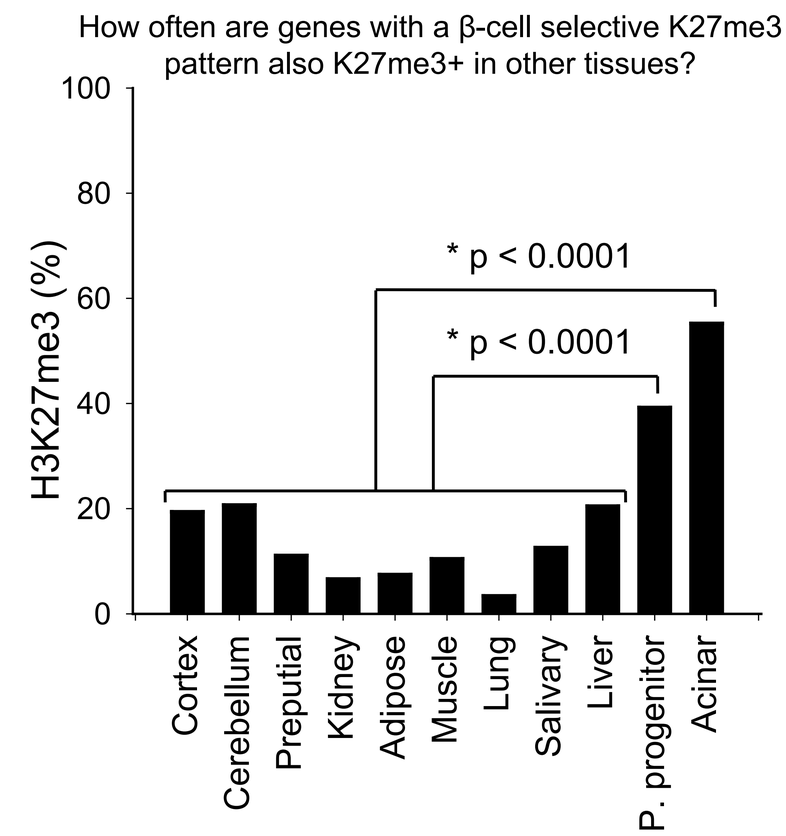




**Figure S6. H3K27me3 repression in β-cells is acquired in a stage-specific manner and is shared with other endoderm tissues. a.** Stage-specific requirements for PcG repression during β-cell development. **b.** β-cell H3K27me3 enrichment profiles resemble those of other endoderm derived tissues, including pancreatic progenitors and acinar cells. We show the first two dimensions of the principal component analysis which together represent 32% of the total variation. **b.** Genes that are H3K27me3+ in β-cells and no more than 3 other tissues are more often H3K27me3+ in pancreatic progenitors and acinar cells than in other cell-types.

van Arensbergen et al. Figure S7

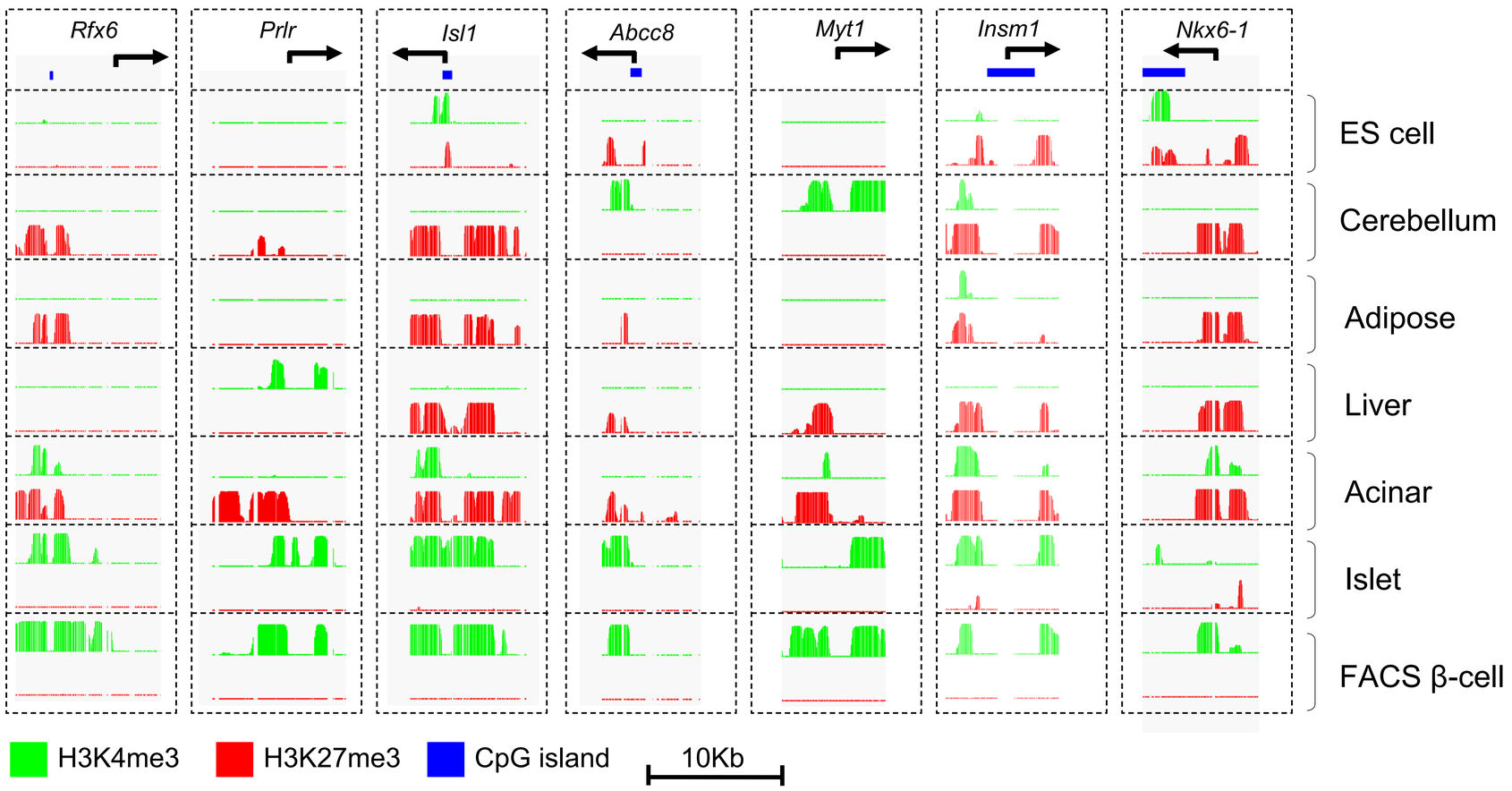
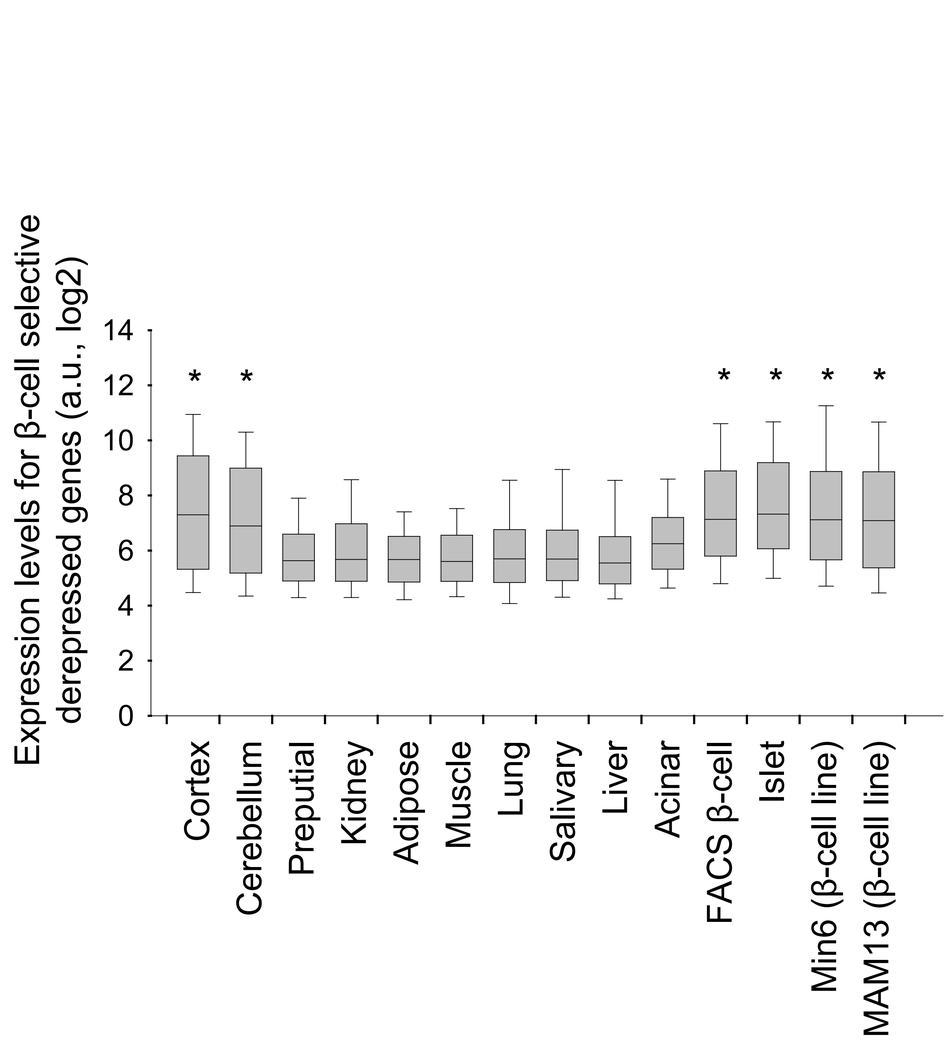



**Figure S7. H3K27me3 from acinar contamination does not contribute to the β-cell histone methylation profile.** Histone methylation in known β-cell genes that show H3K27me3 in acinar, demonstrating negligible effects of contamination in purified β-cells.

van Arensbergen et al. Figure S8



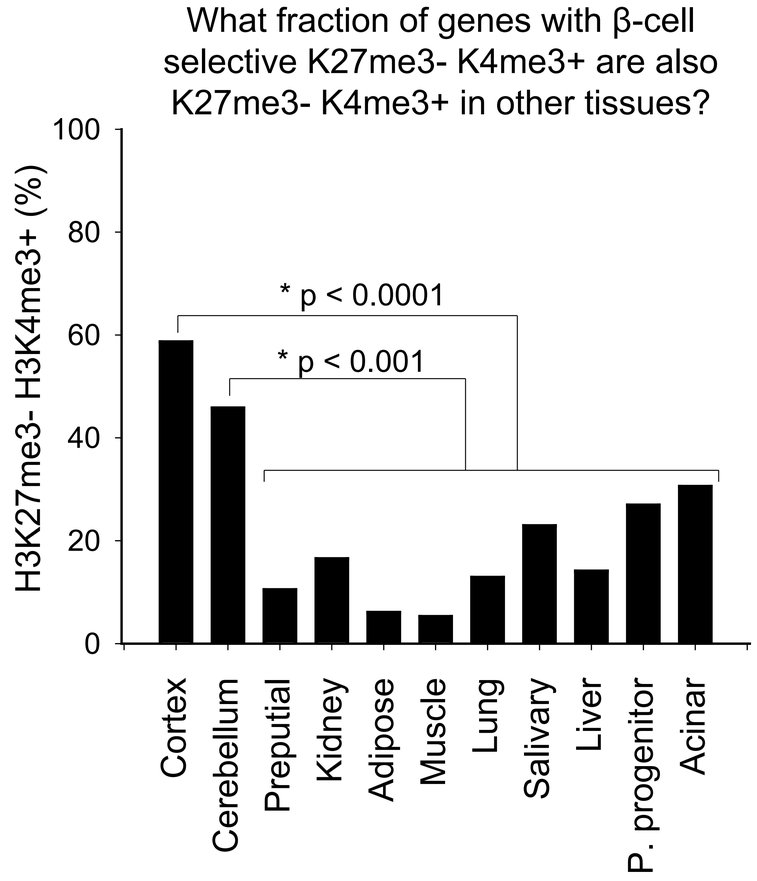




**Figure S8. β-cells and neural tissues share a selective absence of H3K27me3 repression. a.** The set of 249 genes with selective absence of PcG repression in β-cells displays enriched mRNA expression in β-cells, islets, β-cell lines and neural tissues (*$P$<0.001 relative to 8 other tissues). **b.** Genes with selective absence of PcG in β-cells also show absence of H3K27me3 and presence of H3K4me3 in nearly 60% of cases in cortex and 46% in cerebellum, in contrast to on average 16% in other tissues. P values correspond to comparisons between brain and cerebellum vs. each other tissue. Genes with selective absence of PcG in β-cells are defined as those that are H3K27me3- H3K4me3+ in β-cells but are H3K27me3+ in >50% of tissues.

van Arensbergen et al. Figure S9

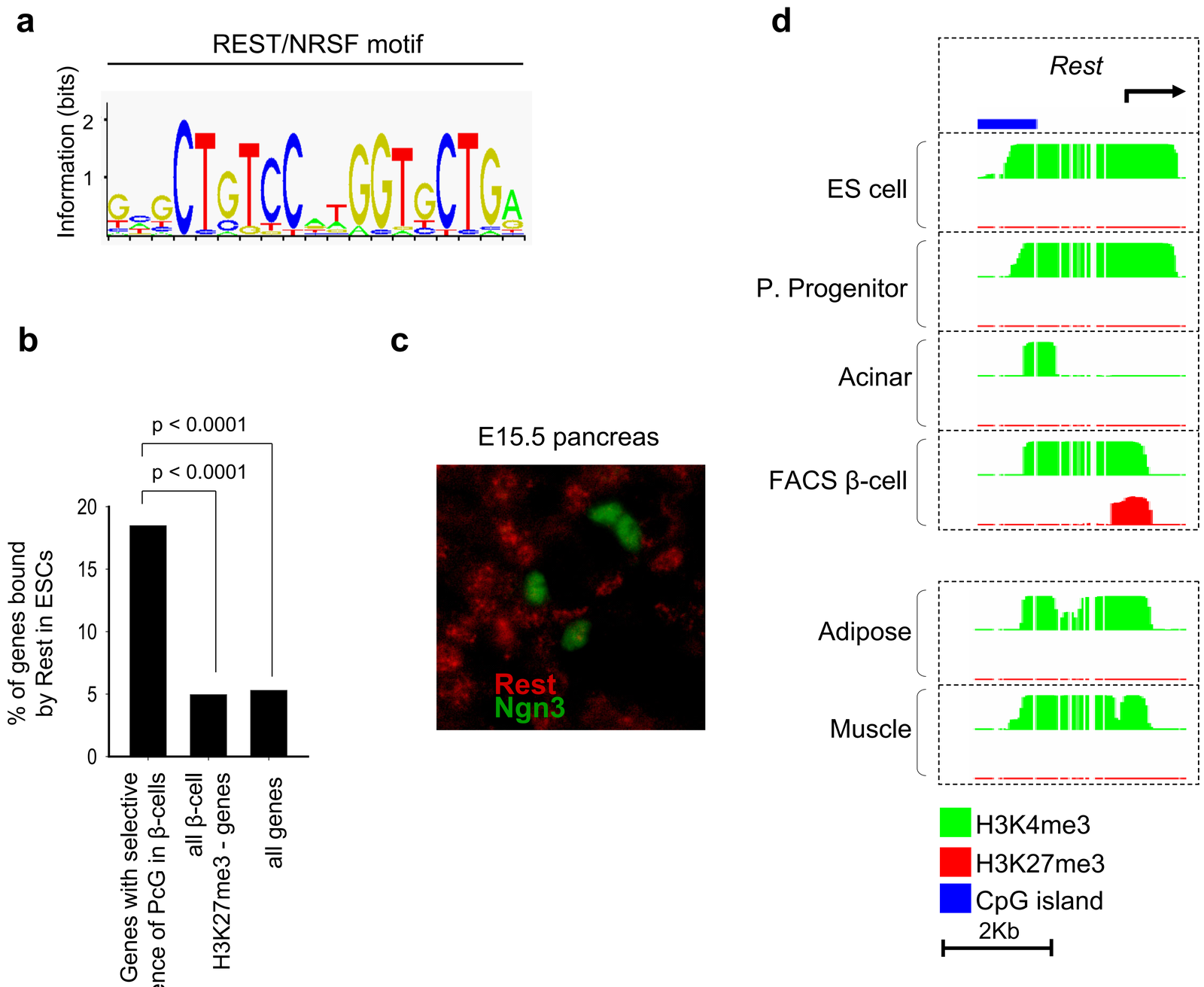



**Figure S9. The selective derepression of a neural program in β-cells is associated with the inactivation of *Rest*. a.** A scan for evolutionary conserved sequence motifs overprepresented in +/- 10 kb of the set of 249 genes with β-cell selective absence of PcG in β-cells resulted in a single most significantly enriched sequence (Fisher $P=7.8 \times 10^{-13}$) matching the known REST motif. **b.** REST binding is highly enriched among genes with selective absence of PcG in β-cells. ChIP was performed using an antibody recognizing REST in ES cells, and the frequency of REST binding among the 249 genes with selective absence of PcG in β-cells was compared to all genes negative for H3K27me3 in β cells, or to all genes. **c.** Immuofluorescence of E15.5 mouse embryos revealed REST (red) expression in pancreatic epithelial cells but only rarely in $Ngn3^{+}$ endocrine progenitors (green), suggesting that *Rest* expression is extinguished around the onset of pancreatic endocrine differentiation. **d.** The *Rest* gene is enriched in the active H3K4me3 histone modification in multipotent progenitors, acinar cells, and other non-neuronal cells, but is enriched in H3K27me3 in pancreatic β-cells.