Supplementary Material

Supplementary Figure Legends

Figure S1 - Isogenic *Htt* allelic series ESC and NPC ChIP-seq and RNA-seq datasets are highly comparable

A) The matrices plot the pair-wise mapped read values (log2 scale) and Pearson correlation coefficients obtained in cross-correlation analyses for the wild-type (WT) and *Htt* null (dKO) and heterozygous *Htt* CAG knock-in $Hdh^{Q20/7}$, $Hdh^{Q50/7}$, $Hdh^{Q91/7}$, $Hdh^{Q111/7}$ (CAG 18/+, 48/+, 89/+ 109/+) ESC and NPC ChIP-seq datasets and the datasets previously reported by Mikkelsen et al. (Mikk) (34). In the figure labels, CAG is omitted for brevity and the different genotypes are referred as 18/+, 48/+, 89/+ 109/+. The number of mapped reads for histone H3K4me3 and histone H3K27me3 ChIP-seq data are assessed in +/- 2 kb intervals around the TSS and for histone H3K36me3 are over the entire gene body.

B) Metagene histone H3K4me3, histone H3K36me3 and H3K27me3 ChIP profiles in a region of -5/+2 Kb around the TSS are depicted as MLE: smoothed maximum likelihood enrichment estimates for groups of RefSeq genes put into 5 equal sized bins according to their RNA expression levels (calculated as RPKM: reads per million kilobase) in the RNA-seq datasets for all six members of the *Htt* isogenic ESC and NPC series, As expected, higher correlation with RNA expression is observed for genes with TSS histone H3K4me3 and H3K36me3 enrichment.

Figure S2 - Isogenic *Htt* allelic series ESC and NPC ChIP-seq datasets are highly comparable at the gene level

A-B) Venn diagrams showing the overlap of genes with TSS enriched for histone H3K4me3, histone H3K36me3 and histone H3K27me3 in the datasets from the *Htt* wild-type ESC and NPC lines used in this study and from publically available wild-type mouse ESC (51; 34) and NPC (34) datasets. The tables below report the number of genes in total, the overlapping genes and the unique genes found in each comparison. The lower degree of overlap that is observed in the NPC genes is expected for different neuronal induction methods.

C-D) Bar graphs plot the total number of TSS with the indicated assessed histone enriched marks calculated for the *Htt* wild-type (WT) and *Htt* null (dKO) ESC and NPC lines and the *Htt* CAG knock-in $Hdh^{Q20/7}$, $Hdh^{Q50/7}$, $Hdh^{Q91/7}$, $Hdh^{Q111/7}$ (CAG 18/+, 48/+, 89/+, 109/+) ESC and NPC lines. For histone H3K4me3 and histone H3K27me3, the ChIP enrichment over the INPUT sample (normalized enrichment) was evaluated in a region of +/- 2 kb around the TSS while histone H3K36me3 the normalized ChIP enrichment was calculated for the entire gene body. The histone H3K27me3 data for *Htt* wild-type and *Htt* null ESC is presented in Figure 4A.

Figure S3 - ChIP-qPCR analyses confirm differential TSS enrichment at 'bivalent' class genes in *Htt* null ESC and NPC

A, C) IGV track views of genes selected from the subsets of ' *Htt* null sensitive' and ' *Htt* null insensitive' classes from the comparisons of *Htt* wild-type (WT) and *Htt* null (dKO) ESC and NPC chromatin states summarized in Figure 4 and listed in Table S2.

B, D, E). Bar plots reporting H3K27me3 ChIP-qPCR enrichment for each gene tested is presented as average of two biologically independent ChIP preparations using histone H3K27me3 antibody (Millipore, ABE44) and for panel D a second histone H3K27me3 antibody (Millipore, 07-449). The bars represent mean +/- standard deviation of two biological and two technical replicates. * denotes p-values ≤ 0.05; ** denotes p-values ≤ 0.01. A gene desert region (Chr.6: 120,258,500-120,259,000) (GD) and the *β-actin* (*Actb*) promoter were used as negative controls for histone H3K27me3 enrichment.

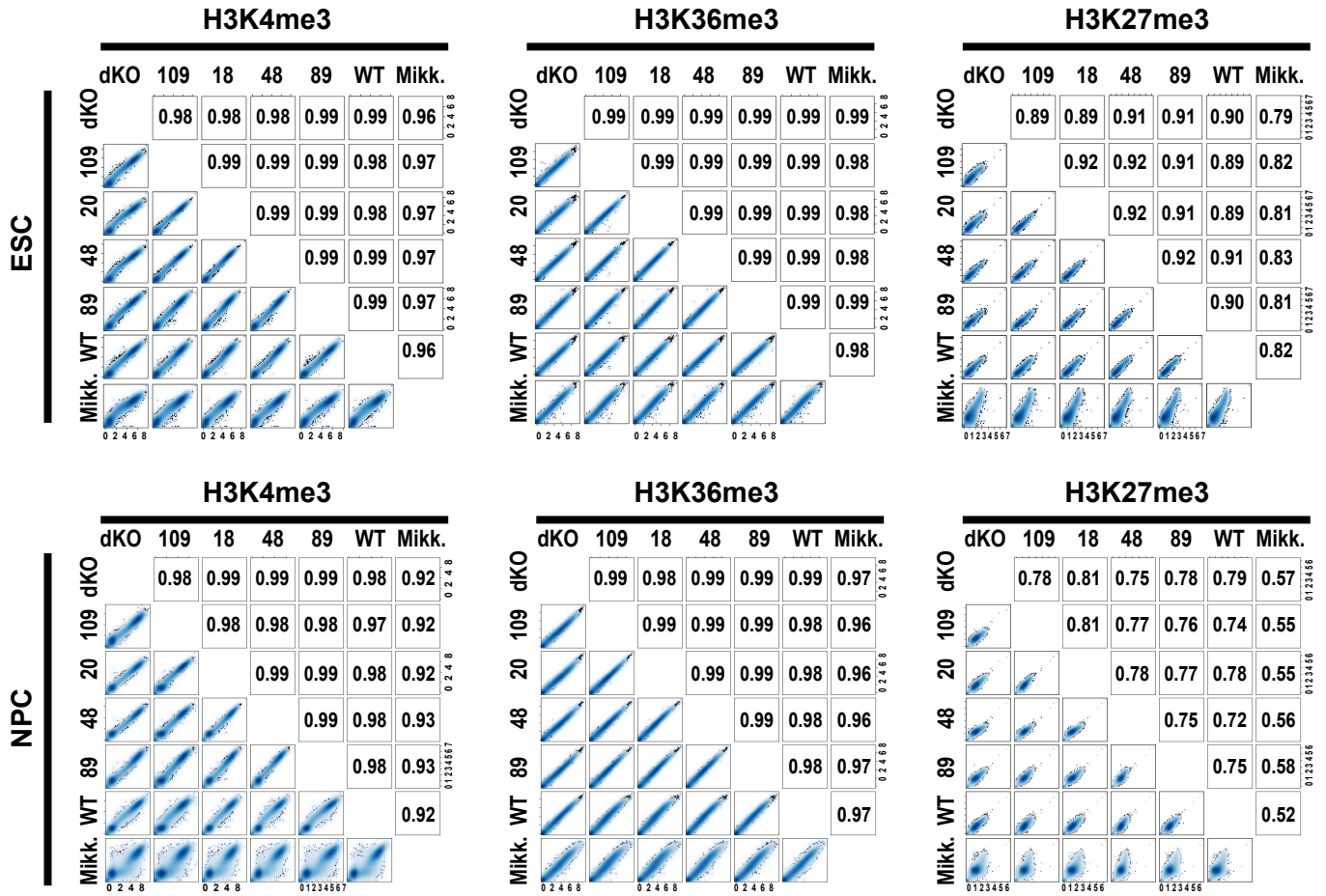
Figure S4 - ChIP-qPCR analyses confirm TSS enrichment that conforms to HD genetic criteria in the *Htt* CAG ESC and NPC lines

A, B, E, F, I) IGV track views of genes selected from the subsets with TSS that exhibit CAG-correlated increases or decreases in histone H3K27me3 or histone H3K4me3 enrichment from analyses presented in Figure 5 (listed in Table S3) are shown for the *Hdh*$^{Q20/7}$ (CAG 18/+) and *Hdh*$^{Q111/7}$ (CAG 109/+) ESC and NPC datasets.

C, D, G, H, J) Bar graphs plot histone H3K27me3 or histone H3K4me3 ChIP enrichment for each gene tested is presented as average of two biologically independent *Hdh*$^{Q20/7}$ (CAG 18/+) and *Hdh*$^{Q111/7}$ (CAG 109/+) ESC and NPC ChIP-qPCR preparations using histone H3K27me3 antibody (Millipore, ABE44) or histone H3K4me3 antibody (Millipore, 07-473). Enrichment over INPUT was normalized to a gene desert control (Chr.6: 120,258,500-120,259,000). The bars represent the mean +/- standard deviation from two biological and two technical replicates. * denotes p-values ≤ 0.05; **) denotes p-values ≤ 0.01. Notably, the ChIP-qPCR confirmation of CAG-correlated histone H3K27me3 enrichment in *Htt* CAG expansion NPC was not technically possible due the low levels of histone H3K27me3 enrichment at this developmental stage.

Figure S1 related to Figure 3

A)



B)

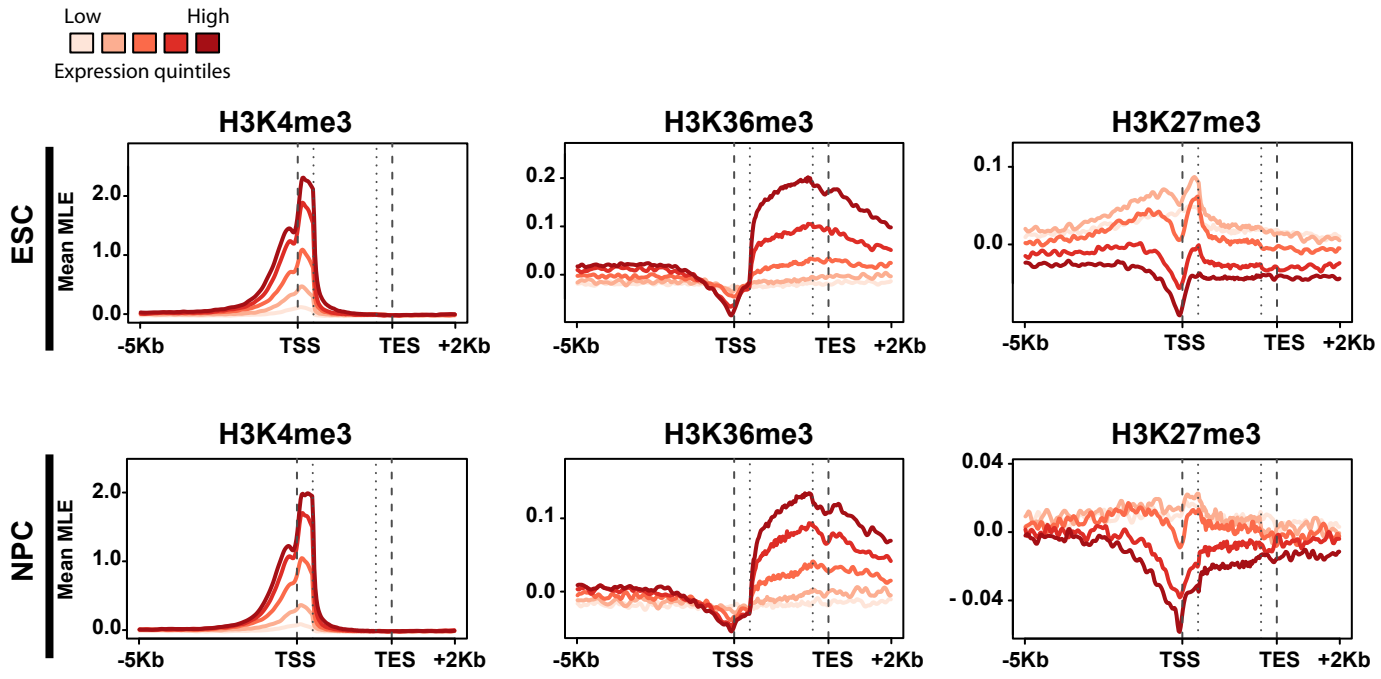
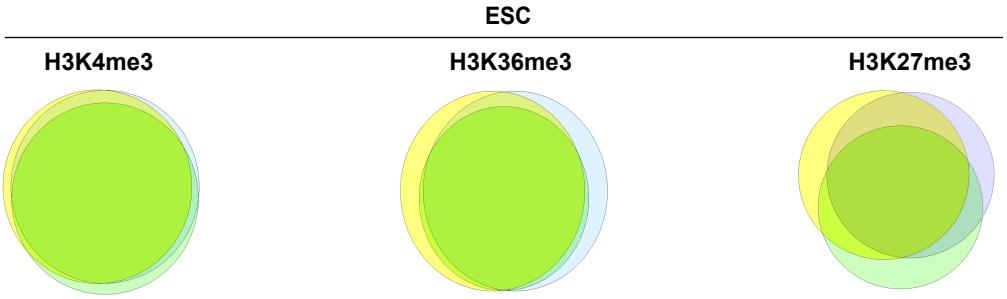


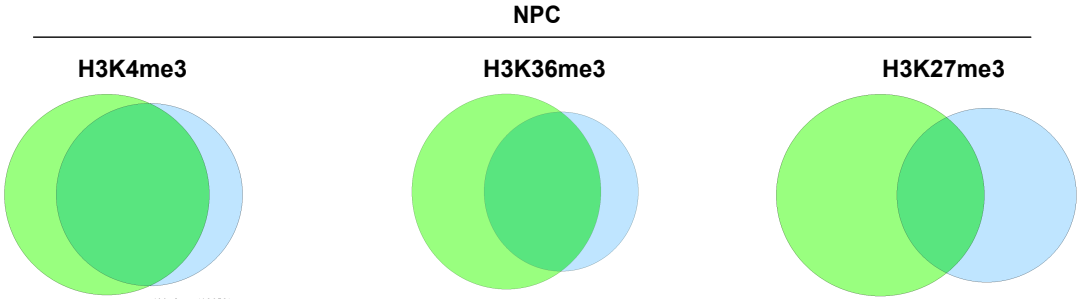
Figure S2 related to Figure 3 and 4

A)



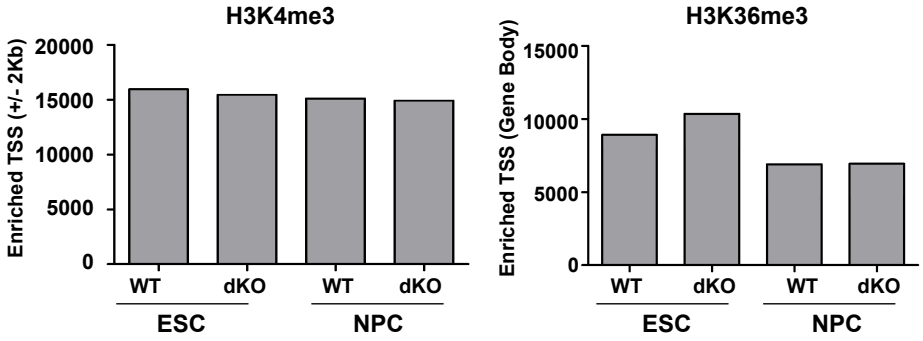
	Goren	This study	Mikkelsen	Overlap	Goren Only	This study Only	Mikkelsen Only
H3K4me3	16767	16656	16407	15890	269	381	104
H3K36me3	11736	12707	9960	9333	767	1751	136
H3K27me3	6161	5784	5936	3983	991	1101	746

B)



	This study	Mikkelsen	Overlap	This study Only	Mikkelsen Only
H3K4me3	15623	12952	12736	2887	216
H3K36me3	12303	8194	7427	4876	767
H3K27me3	2385	1773	698	1687	1075

C)



D)

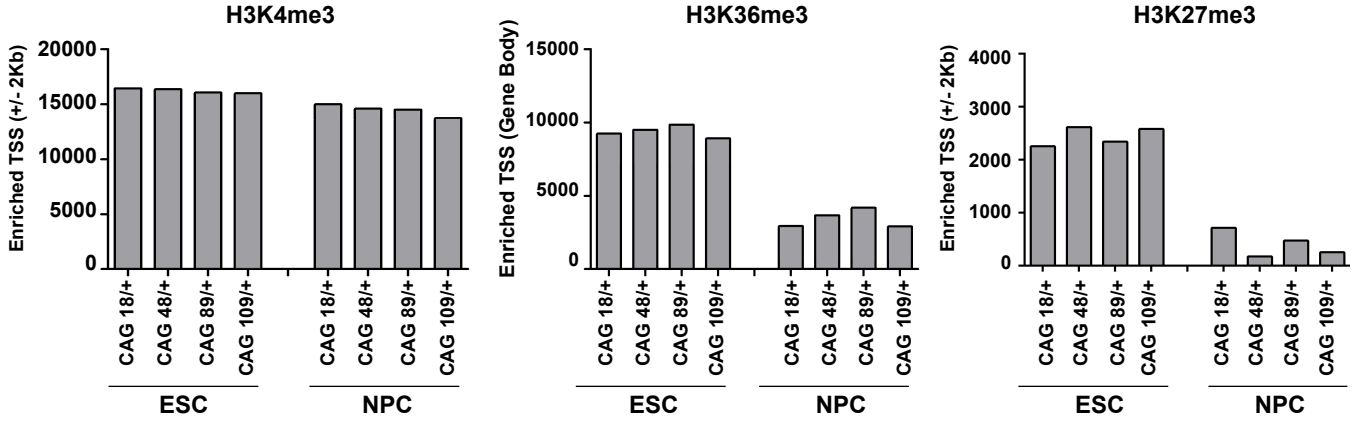


Figure S3 related to Figure 4

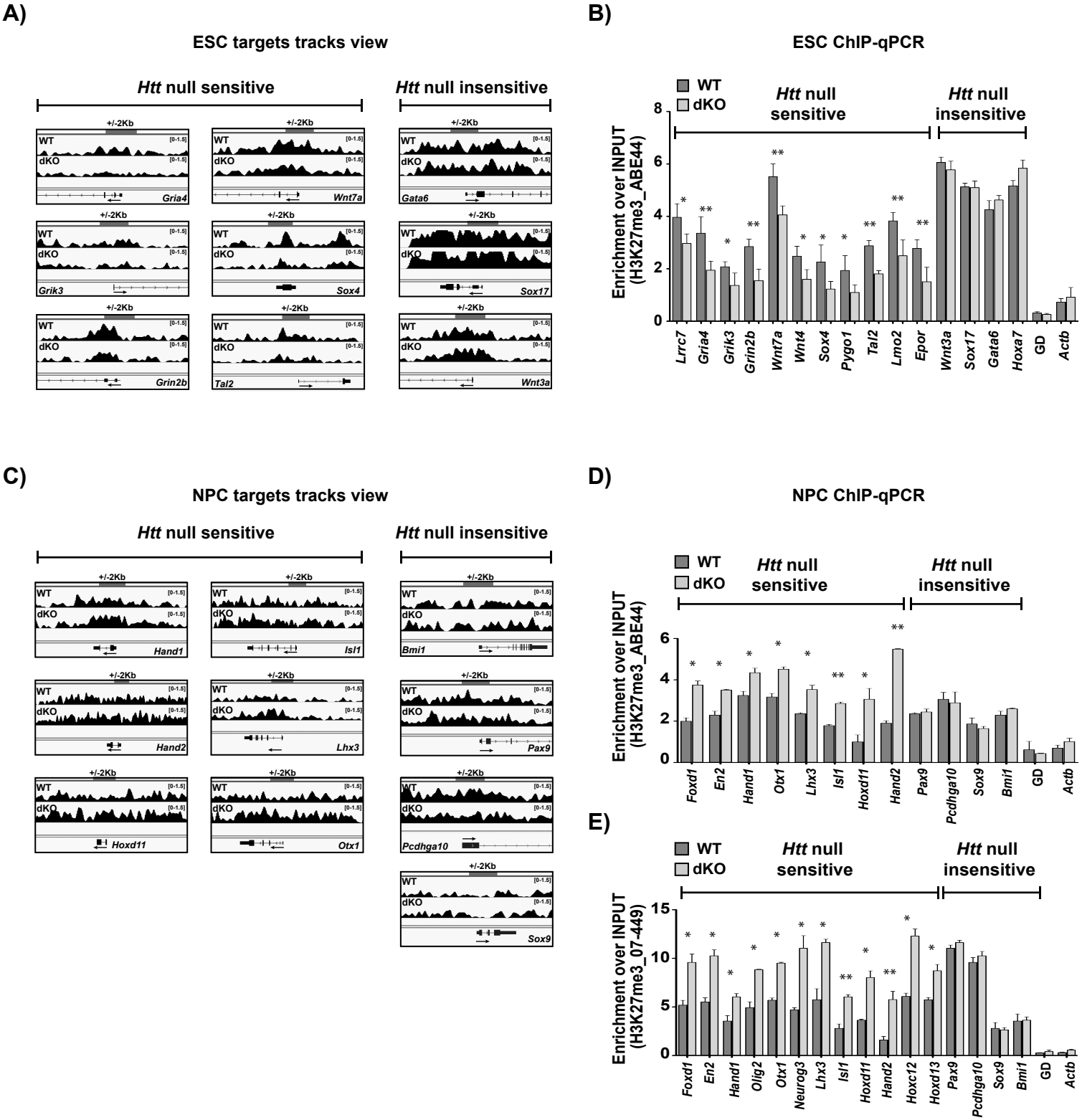


Figure S4 related to Figure 5