Cell Reports, Volume *29*

Supplemental Information

A Functional Link between Nuclear RNA Decay and Transcriptional Control Mediated by the Polycomb Repressive Complex 2

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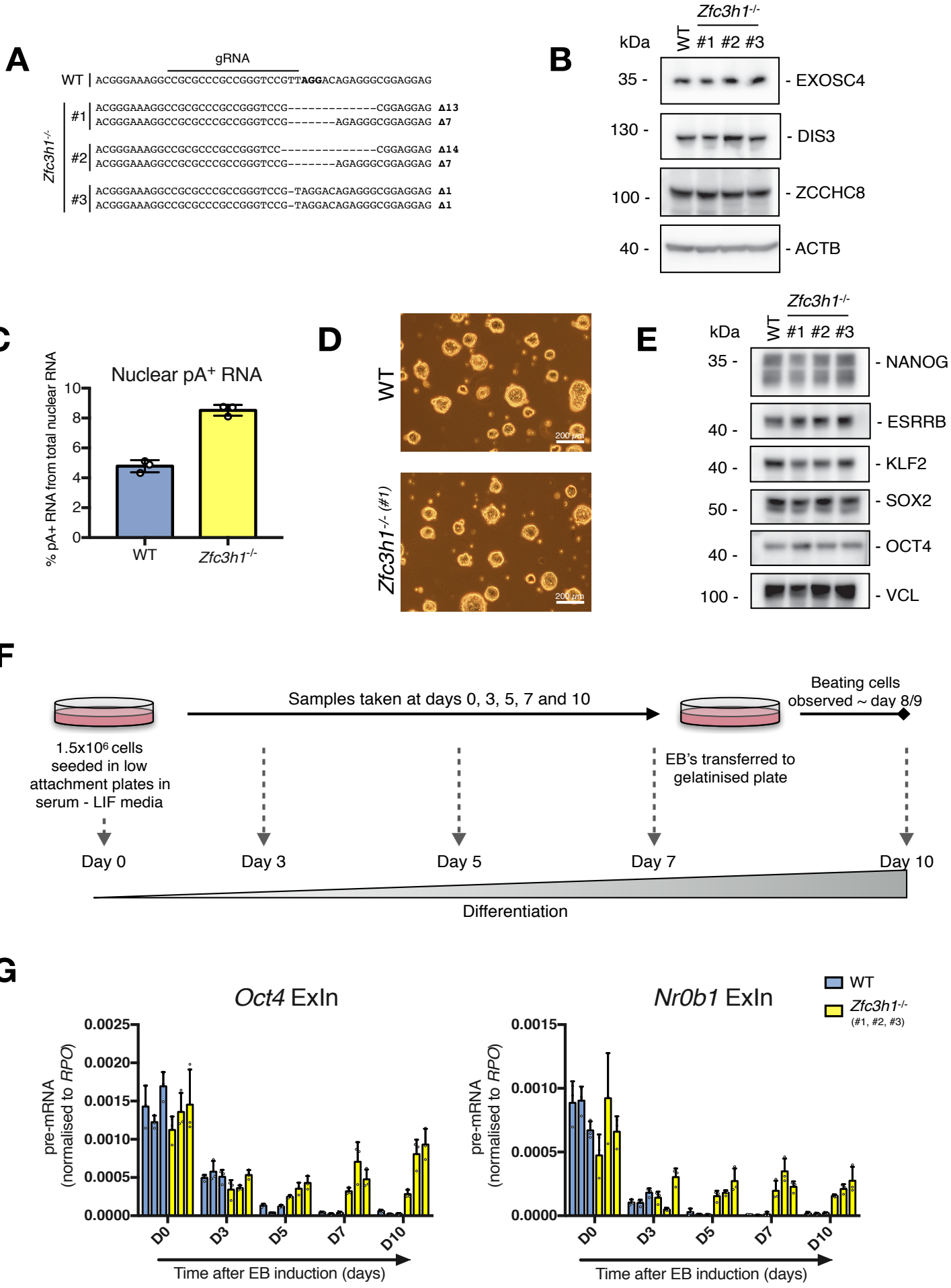
FIGURE S1, related to Figure 1

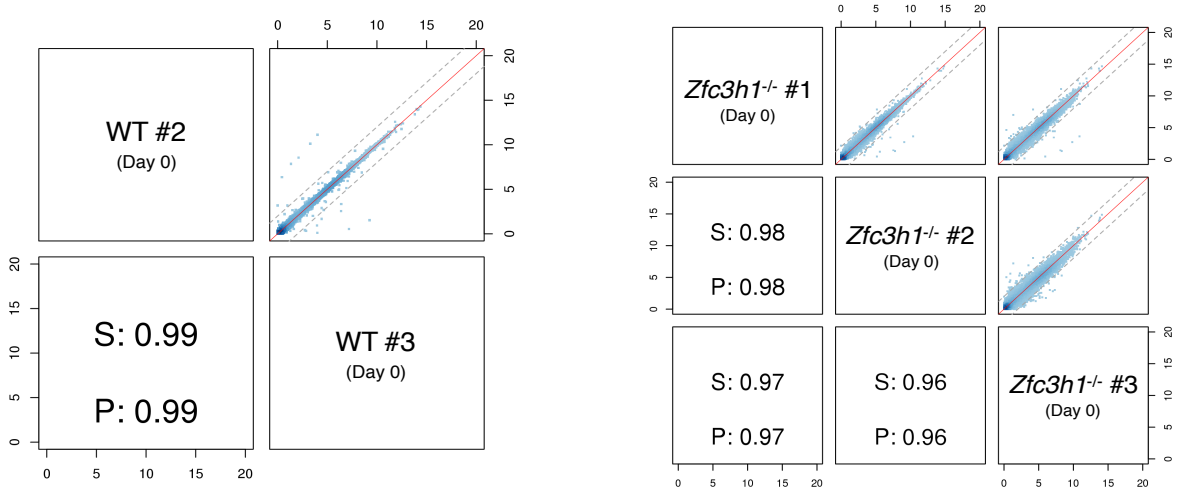


Supplemental Figure 1, related to Figure 1

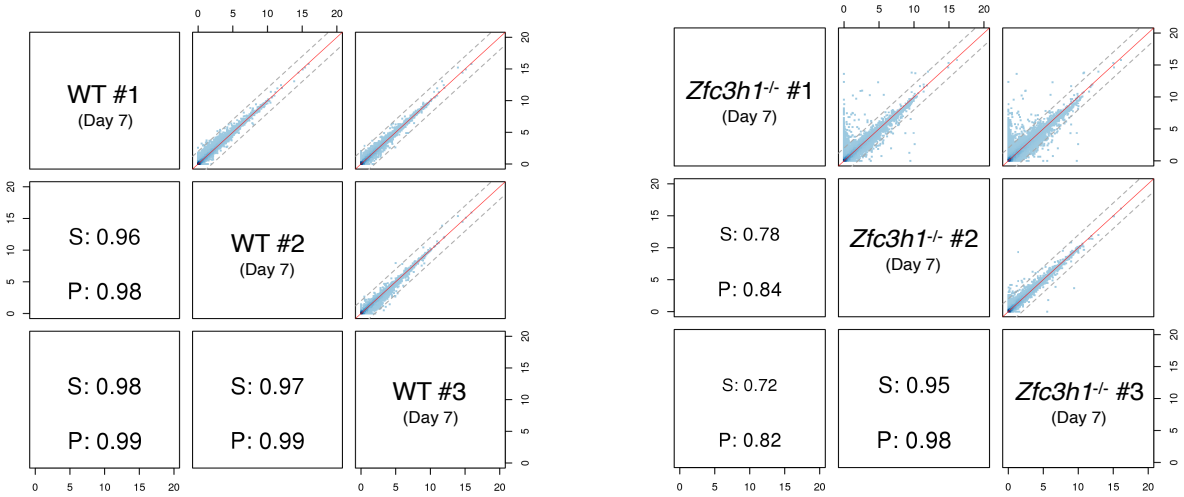
(A) Genomic validation of CRISPR/Cas9-engineered *Zfc3h1*$^{-/-}$ cell lines. PCR was carried out on genomic DNA isolated from three *Zfc3h1*$^{-/-}$ cell lines derived from single cell clones. To distinguish individual alleles, amplicons were cloned into the pCR4 vector and sequenced. The gRNA sequence is highlighted and the PAM motif is shown in bold. Base pair deletion sizes are indicated per allele (Δn). **(B)** Western blotting analysis using the same lysates as in Figure 1B. Blots were probed with the indicated antibodies and actin (ACTB) was used as a loading control. **(C)** Quantification of pA^{+} RNA purified from WT and *Zfc3h1*$^{-/-}$ nuclei. Results are shown as percentage of total nuclear RNA. Columns represent the average value of biological triplicates with error bars denoting the SD. Individual data values from technical triplicates are indicated as points. **(D)** Phase contrast microscopy images of undifferentiated (D0) WT and *Zfc3h1*$^{-/-}$ (#1) cells. Scale bars denote 200 μm. **(E)** Western blotting analysis of extracts from WT and *Zfc3h1*$^{-/-}$ cell lines to assess the expression of pluripotency markers. Blots were probed with the indicated antibodies and vinculin was used as a loading control. **(F)** Overview of the EB differentiation assay. ES cells, growing in 2i/LIF media, were transferred to serum-LIF media and seeded in low attachment plates. Samples for RNA and protein analyses were taken at the indicated days (0, 3, 5, 7, 10). At day 7, EBs were transferred to gelatinised plates and cultured for an additional 3 days. **(G)** qRT-PCR analysis of total RNA from Figure 1E but using exon-intron (ExIn) primers to amplify pre-mRNA. Results are shown relative to *Rplp0* (*RPO*) mRNA as in Figure 1E.

FIGURE S2, related to Figure 2

A



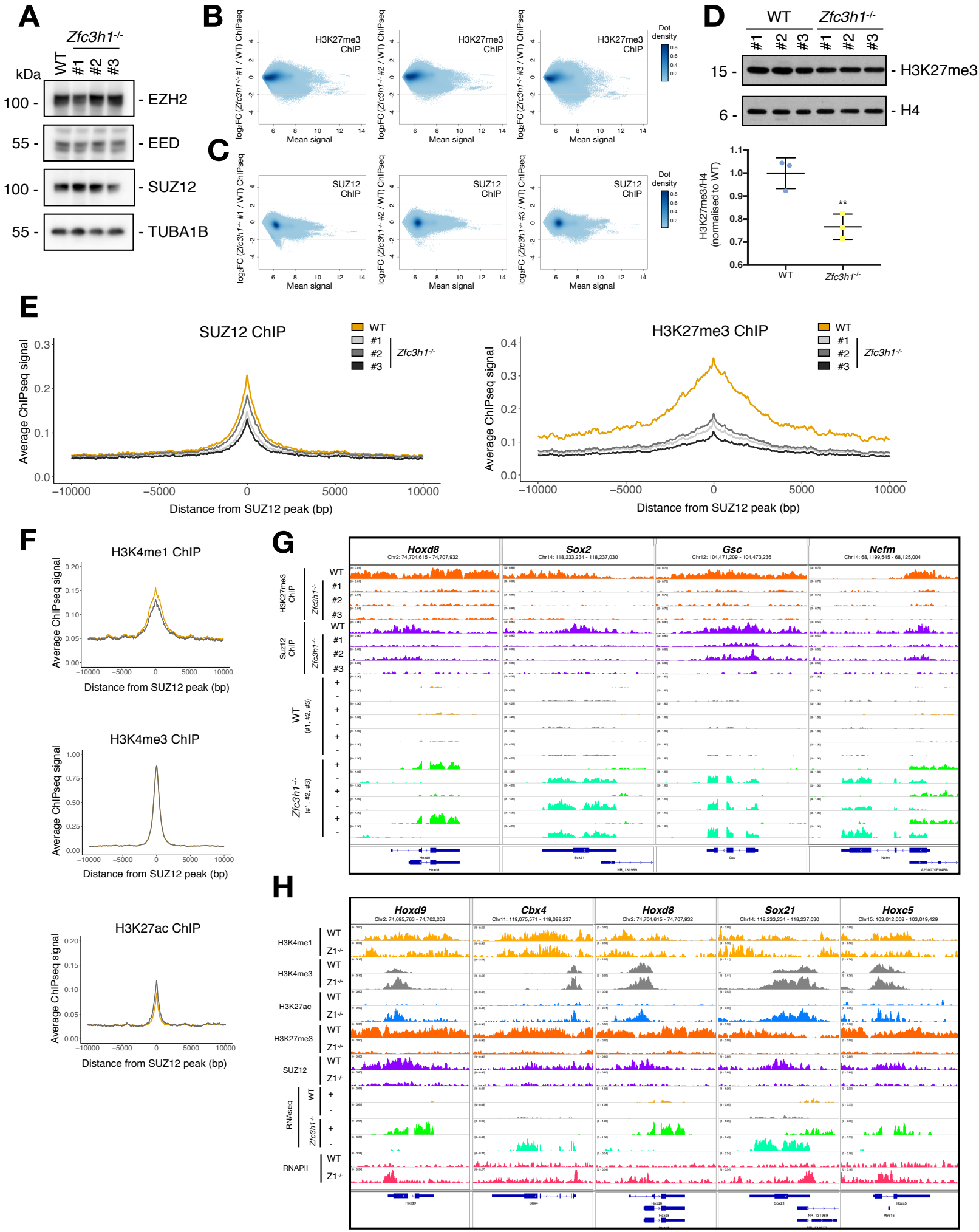
B



Supplemental Figure 2, related to Figure 2

(A) Pairwise comparisons of expression from replicate RNAseq samples from WT and *Zfc3h1*$^{-/-}$ cell lines after 7 days of EB differentiation. All genes from GENCODE were included and expression were measured as TPM. Spearman and Pearson correlation coefficients, (S) and (P) respectively, are shown for each plot. **(B)** As in (A) but with samples taken from WT and *Zfc3h1*$^{-/-}$ undifferentiated (D0) cells.

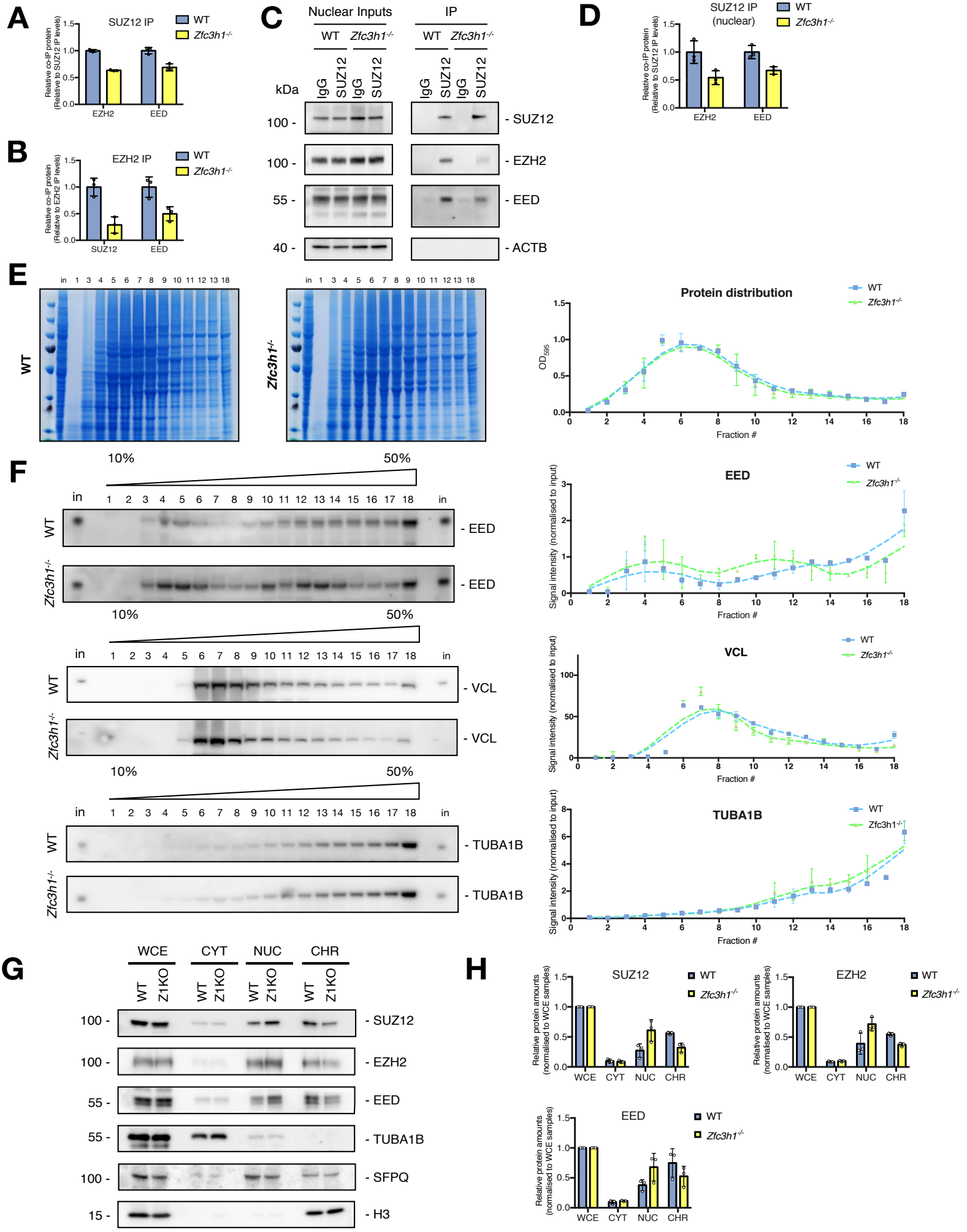
FIGURE S3, related to Figure 3



Supplemental Figure 3, related to Figure 3

(A) Western blotting analysis of PRC2 components using samples prepared as in Figure 1B. Blots were probed with the indicated antibodies and Tubulin (TUBA1B) was used as a loading control. **(B-C)** MA plots of H3K27me3 (B) and SUZ12 (C) ChIPseq profiles in *Zfc3h1*$^{-/-}$ vs. WT cell lines as in Figure 3A, but showing all 3 replicate experiments. **(D)** Top panel: Western blotting analysis of global H3K27me3 levels from three biological replicates of WT and *Zfc3h1*$^{-/-}$ ES cells. Blots were probed with H3K27me3-specific antibody and H4 was used as a loading control. Bottom panel: Quantification of western results from top panel. H3K27me3 values were normalised to H4. Individual points indicate biological replicates. **(E)** Mean SUZ12 and H3K27me3 ChIPseq signals as in Figure 3C-D, but showing all 3 replicate experiments **(F)** Average H3K4me1, H3K4me3 and H3K27ac ChIPseq signals from WT and *Zfc3h1*$^{-/-}$ cell lines in regions centred on SUZ12 ChIP peaks as in Figure 3C-D. **(G)** Genome browser views of 4 PRC2 target genes as in Figure 3G, but showing all biological replicate experiments. **(H)** Genome browser views of 5 PRC2 target genes. Displayed tracks include H3K4me1, H3K4me3, H3K27ac, H3K27me3, SUZ12 and RNAPII ChIPseq data as well as RNAseq data from WT and *Zfc3h1*$^{-/-}$ (#1) cell lines. RNAseq tracks on both strands are shown (+ and – respectively). Gene models are based on RefSeq. Genome coordinates (mm10) are indicated for each panel.

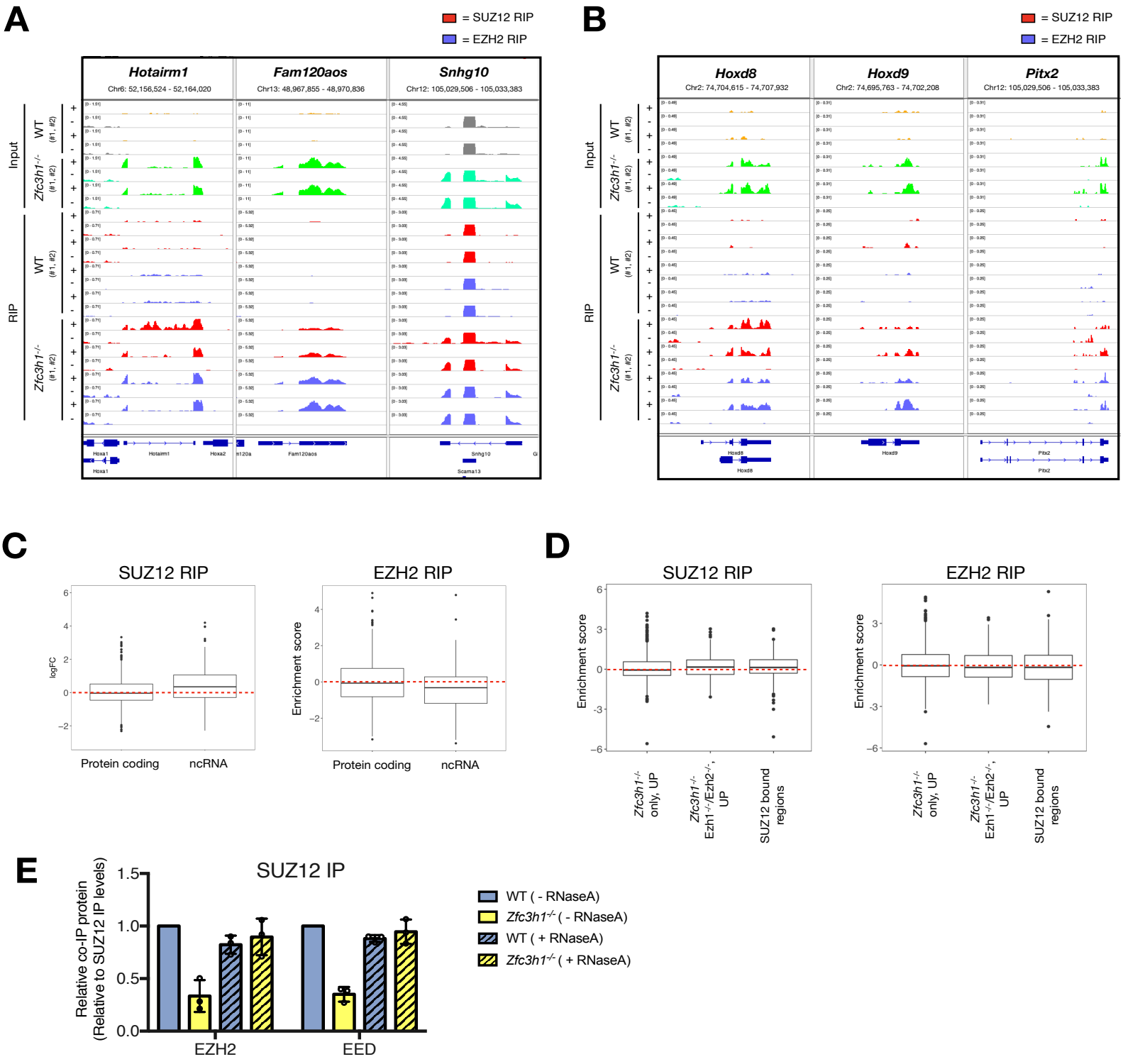
FIGURE S4, related to Figure 4



Supplemental Figure 4, related to Figure 4

(A) Quantification of western blotting analysis from Figure 4A, and including 2 additional replicates (data not shown). EZH2 and EED values from IP samples were normalised to SUZ12 values from IP samples. Individual points indicate replicates. **(B)** As in (A) but for results from Figure 4B, and including 2 additional replicates (data not shown). SUZ12 and EED values from IP samples were normalised to EZH2 values from IP samples. **(C)** Western blotting analysis of SUZ12 IPs as in Figure 4A, but using lysates prepared from isolated nuclei. Actin (ACTB) was used as a loading control for nuclear inputs. **(D)** As in (A) but for results from Figure S4C. EZH2 and EED values from nuclear IP samples were normalised to SUZ12 values from nuclear IP samples. **(E)** Total protein content in glycerol gradient sedimentation assays from Figure 4C and 4D. Left panel: Input and 2% of indicated fractions were separated on SDS-PAGE gels and stained with Coomassie Blue 250G. Right panel: Protein concentration from each fraction was measured by Bradford assay. Axes show the OD_{595} reading (Y) in each fraction (X) for WT (blue) and *Zfc3h1*$^{-/-}$ (green) samples. Values are the average of two replicates and error bars show the SD. A smoothed curve was fit to show the distribution of protein throughout the gradient. **(F)** Left panels: As in Figure 4C but showing the sedimentation of EED, Vinculin (VCL) and Tubulin (TUBA1B) in glycerol gradients from WT and *Zfc3h1*$^{-/-}$ cell extracts. Right panels: Quantification of western blots as in Figure 4C. **(G)** Western blotting analysis of equivalent lysates isolated from whole cell extract (WCE), cytoplasmic (CYT), nucleoplasmic (NUC) and chromatin-associated (CHR) fractions of WT and *Zfc3h1*$^{-/-}$ cells. Tubulin (TUBA1B), SFPQ and H3 were used as cytoplasmic, nucleoplasmic and chromatin-associated protein markers, respectively. **(H)** Quantification of western blots from (G). Columns show the average values from biological triplicates and error bars denote the SD. Individual points indicate replicates.

FIGURE S5, related to Figure 4



Supplemental Figure 5, related to Figure 4

(A) Genome browser views of 3 PAXT target genes (*Hotairm1, Fam120aos* and *Snhg10*). Displayed tracks include RNAseq data from input samples along with SUZ12 RIPseq and EZH2 RIPseq data from WT and *Zfc3h1*$^{-/-}$ cell lines. SUZ12 and EZH2 RIPseq tracks were coloured red and blue respectively. RNAseq tracks on both strands are shown (+ and – respectively) and include biological duplicates (#1 and #2). Gene models are based on RefSeq. Genome coordinates (mm10) are indicated for each panel. **(B)** As in (A) but displaying 3 PRC2 target genes (*Hoxd8, Hoxd9* and *Pitx2*). **(C)** Box plot distribution showing relative enrichment of RNAs in SUZ12 and EZH2 RIPseq samples from WT and *Zfc3h1*$^{-/-}$ cells as indicated. Transcripts shown are protein coding and ncRNAs, that were upregulated in *Zfc3h1*$^{-/-}$ RNAseq data ($log_2FC > 0.5$, $FDR < 0.05$). Enrichment scores were defined as log_2FC ((*Zfc3h1*$^{-/-}$ IP – *Zfc3h1*$^{-/-}$ input) – (WT IP – WT input)). **(C)** Box plot distributions as in (E) but for transcripts upregulated ($log_2FC > 0.5$, $FDR < 0.05$) in *Zfc3h1*$^{-/-}$ ES cells alone, both *Zfc3h1*$^{-/-}$ and *Ezh1*$^{-/-}$*Ezh2*$^{-/-}$ ES cells or transcripts from loci overlapping with SUZ12 peaks that show a log2FC < 0 in *Zfc3h1*$^{-/-}$ ChIPseq data. **(D)** Quantification of western blots from Figure 4F, and including two additional replicates (data not shown). Values were plotted as in Figure S4A.

SUPPLEMENTAL TABLES

Table S1, related to STAR methods

sgRNA primers

Name	Sequence
Zfc3h1_CRISPR_2_F	CACCGGCCGCGCCCGCCGGGTCCGTT
Zfc3h1_CRISPR_2_R	AAACAACGGACCCGGCGGGCGCGGCC

Table S2, related to STAR methods

qRT-PCR primers

Name	Forward	Reverse
Oct4	CAGCAGATCACTCACATCGCCA	GCCTCATACTCTTCTCGTTGGG
Sox2	AACGGCAGCTACAGCATGATGC	CGAGCTGGTCATGGAGTTGTAC
Klf2	CACCTAAAGGCGCATCTGCGTA	GTGACCTGTGTGCTTTCGGTAG
Esrrb	GACATTGCCTCTGGCTACCACT	ACTTGCGCCTCCGTTTGGTGAT
Gata4	GCCTCTATCACAAGATGAACGGC	TACAGGCTCACCCTCGGCATTA
Tbxt	ATCCACCCAGACTCGCCCAATT	CTCTCACGATGTGAATCCGAGG
Nkx2-5	CTCCGATCCATCCCACTTTA	AGTGTGGAATCCGTCGAAAG
Oct4 ExIn	GGGCTGTTGCCAAGTCAAATAC	CCAGGGTCTCCGATTTGCATAT
Nr0b1 ExIn	GGACCGTGCTCTTTAACCCAG	CAAGTGGGCTCCAAAACCCC
Hoxd8 ExIn	GGCGAGGACCCAGACCAC	GCAGATTTCTCCCTTTCTCCCC
Hoxd9 ExIn	CTCGCTCCCTTGTCTGCAAAC	CTCGCTCCCTTGTCTGCAAAC
Snhg10	GCCTTCCATGCCTCACCG	GAATCAGAGGATCCTGCAAG
Snhg20	CCGAAGGCGATCGTGGGAT	CAACACAGAAGCAGCCAGTG
Snhg13 (Dancr)	GAAACCCGTGACTGAATGGC	GGAAACTCACATGGCCCTC
Hotairm1	GCACTGGGACCAAAGGGAG	GCACTGGGACCAAAGGGAG
Rplp0 (RPO)	TCCATTGTGGGAGCAGAC	CAGCAGTTTCTCCCAGAGC
GAPDH	TTGATGGCAACAATCTCCAC	CGTCCCGTAGACAAAATGGT