The BTB-domain transcription factor ZBTB2 recruits chromatin remodelers and a histone chaperone during the exit from pluripotency

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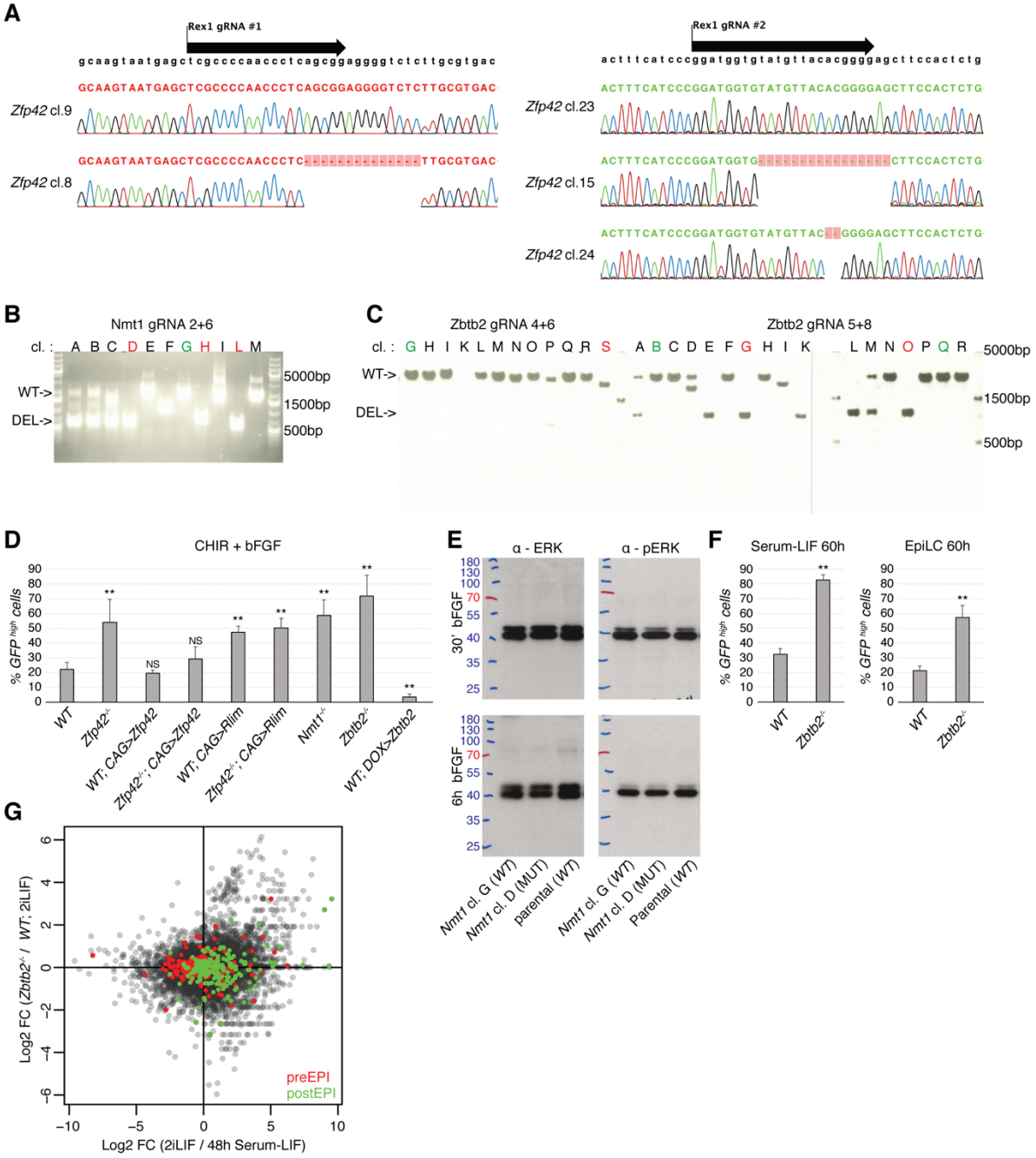
SUPPLEMENTARY INFORMATION

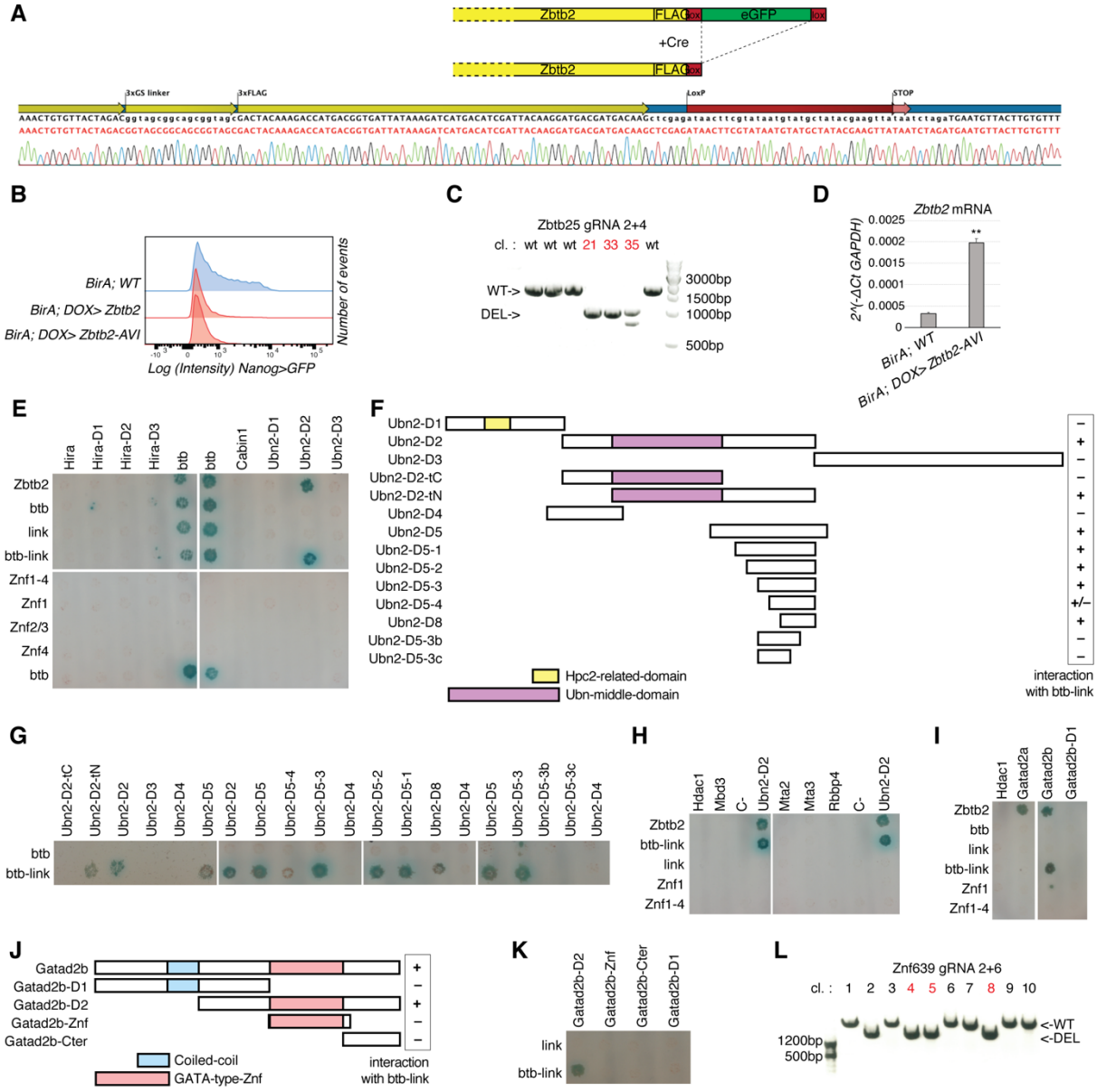
Figure S1



Supplementary Figure S1: Related to Figure 1.

(A) Chromatograms of *Zfp42*$^{-/-}$ (cl.8, 15, 24) and *WT* (cl.9, 23) sibling clones under the respective gRNAs used (see also Table S2).
(**B,C**) Genotyping PCR of *Nmt1* and *Zbtb2* targeted clones, showing knockout (red) and sibling control clones (green) used in this study (see also Table S2).
(D,F) Average and standard deviation (SD) of *Nanog>GFP*-high cells of biological triplicates quantified as in Fig.1B, C, D (**D**). and Fig.1F (**F**). ** indicates p-values<0.001 and NS p-values>0.1 compared to corresponding *WT* controls.
(E) Anti-ERK and anti-phospho-ERK western-blot of lysates from *Nmt1*$^{-/-}$ and *WT* clones.
(G) Scatterplot of log2FCs in gene expression of indicated contrasts. Green labels post-implantation (postEPI) and red pre-implantation epiblast (preEPI) specific genes (55).

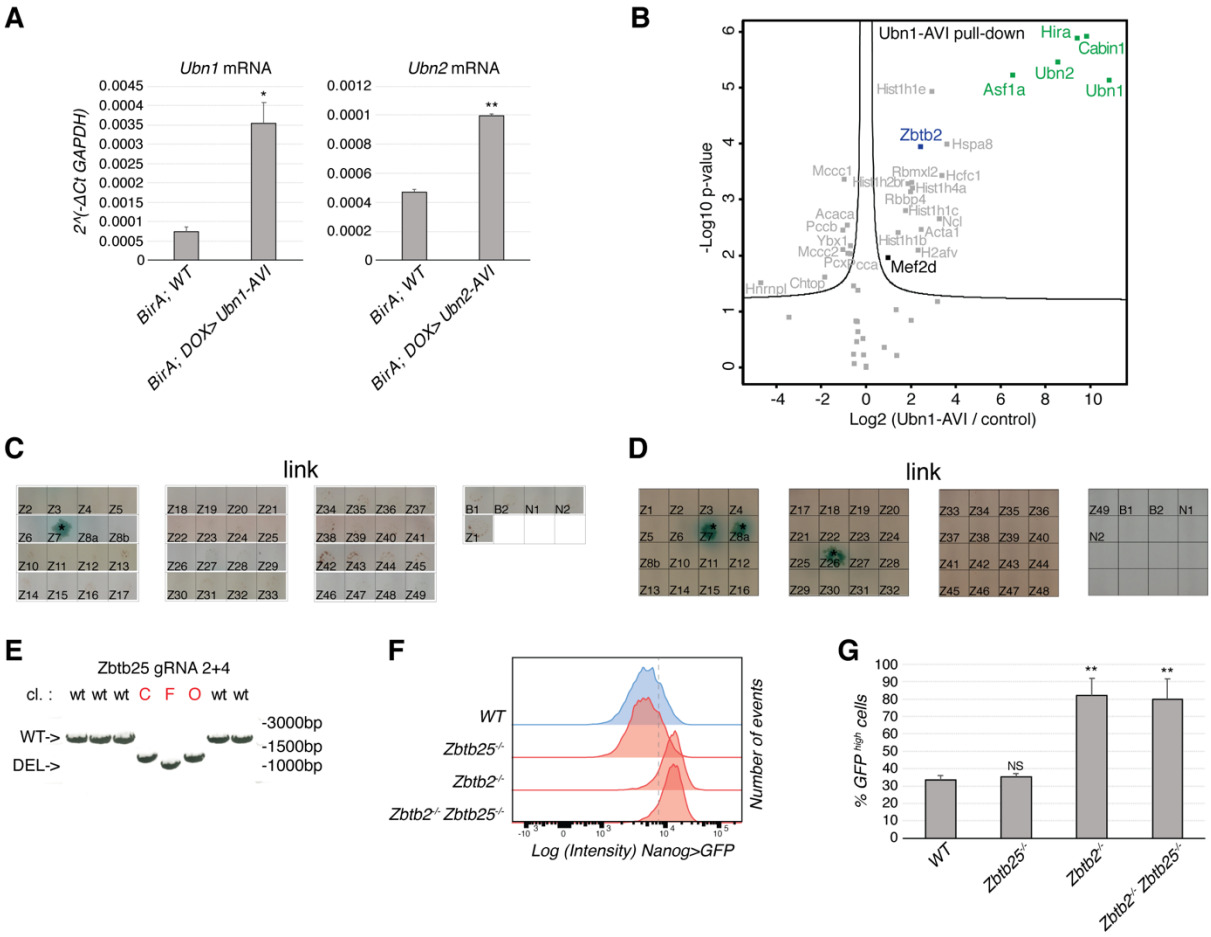
Figure S2



Supplementary Figure S2: Identification of the ZBTB2-interacting subunits and domains. Related to Figure 2.

(A) Scheme of the ZBTB2-3xFLAG knock-in strategy (see also Table S2) and chromatogram of final homozygous cell line.
(B) *Nanog>GFP* intensities after switching from 2iLIF to N2B27 +CHIR +bFGF for 3 days in the presence of DOX of indicated genotypes.
(C) *Zbtb25* genotyping PCR of *Zbtb2*$^{-/-}$; *Zbtb25*$^{-/-}$ clones; red labels clones used in this study.
(D) qPCR of *Zbtb2* relative to *GAPDH* in *WT* and ZBTB2-AVI overexpressing cells. Average and SD of technical triplicates, ** indicates p-value<0.001.
(E,G,H,I,K) Colony growth on QDOXA plates of strains expressing indicated proteins. Bait constructs are vertical and prey constructs are horizontal.
(F,J) Diagram of *Ubn2* (**F**) and *Gatad2b* (**J**) constructs used for Y2H analysis; + and – indicates positive and negative interactions as in (**G**,**I** and **K**).
(L) Genotyping PCR of *Znf639*$^{-/-}$ clones; red labels clones used in this study.

Figure S3



Supplementary Figure S3: Lack of genetic interaction between ZBTB2 and ZBTB25. Related to Figure 3.

(A) qPCR of *Ubn1* and *Ubn2* relative to *GAPDH* in *WT* and UBN1-AVI- or UBN2-AVI-overexpressing cells. Average and SD of technical triplicates, * indicates p-value<0.01 and ** p-value<0.001.

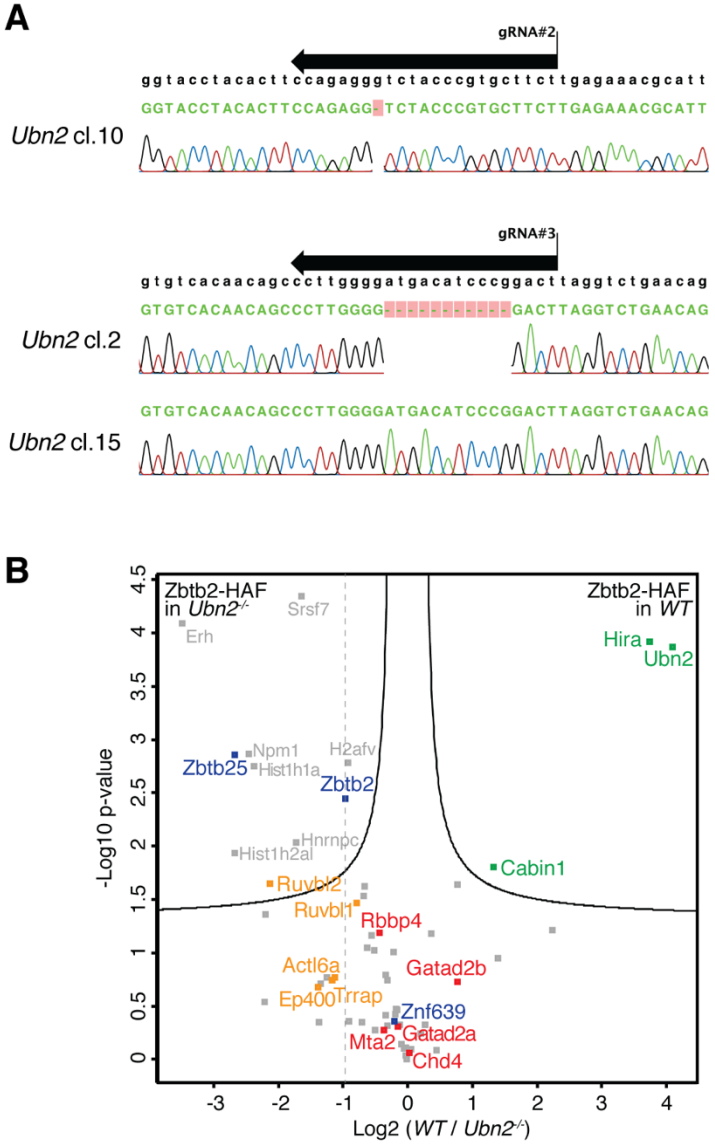
(B) Volcano plot of protein enrichments in AP-MS of mESCs overexpressing UBN1-AVI compared to control BirA-only-expressing mESCs. ZBTB2 is indicated in blue and HiRA subunits in green.

(**C,D**) Colony growth on QDOXA plates of control matings for experiments presented in Fig.3B,C,F **(C)** and in Fig.3D **(D)** using ZBTB2's link region as control bait construct.

(E) *Zbtb25* genotyping PCR of *Zbtb25*$^{-/-}$ clones; red labels clones used in this study.

(F,G) *Nanog>GFP* intensities after switching from 2iLIF to Serum-LIF after 60h of indicated genotypes (**F**). Dashed line indicates the threshold for quantification of GFP-high cells as in **(G)**. **(G)** average and SD of biological triplicates; ** indicates p-values<0.001 and NS p-values>0.1 compared to the *WT* control.

Figure S4

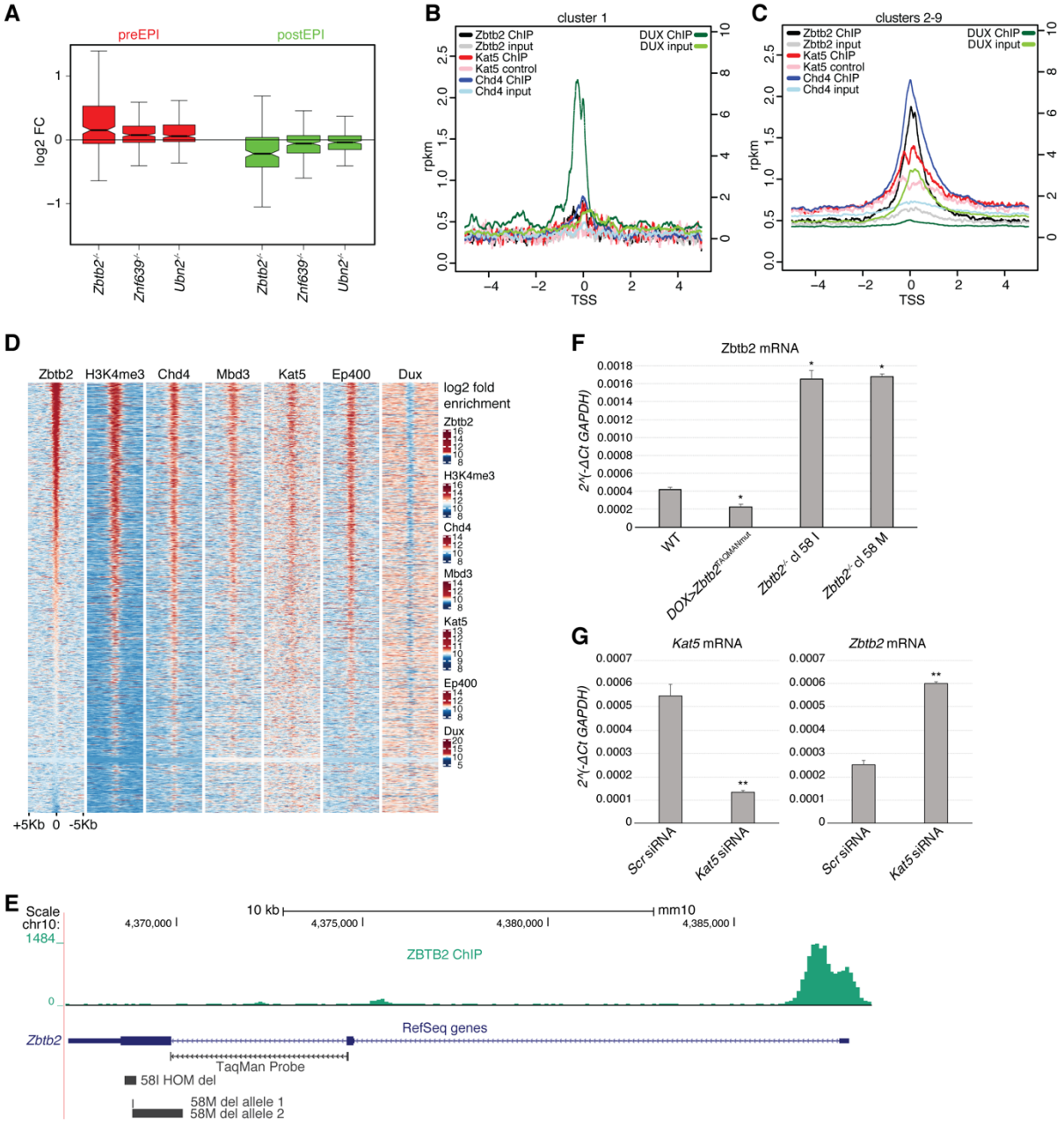


Supplementary Figure S4: The Ep400 interaction is not mediated by the HiRA complex. Related to Figure 4.

(A) Chromatograms of *Ubn2*$^{-/-}$ (cl.10,2) and *WT* (cl.15) sibling clones under the respective gRNAs used (see also Table S2).

(B) Volcano plot of protein enrichments in AP-MS of overexpressed ZBTB2-HAF in *WT* compared to *Ubn2*$^{-/-}$ mESCs. ZBTB2 and partner TFs are indicated in blue. The dashed line marks enrichment of the ZBTB2-HAF bait protein which is similar to that of NuRD subunits in red and Ep400 subunits in orange, while interaction with HiRA subunits in green is comparatively reduced in *Ubn2*$^{-/-}$ mESCs.

Figure S5



Supplementary Figure S5: ZBTB2 binds and represses its own promoter. Related to Figure 5.

(A) Boxplot of log2 fold differential expression after 48h in Serum-LIF of pre-implantation (preEPI, red) and post-implantation (postEPI, green) epiblast specific genes (55) in indicated genotypes compared to *WT* cells.

(B,C) ZBTB2 (53), KAT5 (60) and CHD4 (25) (left scale), and DUX (17) (right scale) ChIP-seq rpkm centered on TSSs of cluster 1 (**B**) and cluster 2-9 genes (**C**), according to Fig. 5D.

(D) Heatmap of ZBTB2 (53), H3K4me3 (79), CHD4, MBD3 (25), KAT5, EP400 (60) and DUX (17) log2 fold ChIP-seq enrichment over respective controls at accessible (ATACseq, not shown) TSSs.

(E) Diagram of the *Zbtb2* locus, showing the ZBTB2 peak at the TSS, and the deletions in the *Zbtb2*$^{-/-}$ clones 58I and 58M that give rise to transcripts that are detectable by the qPCR probe (TaqMan Probe) against endogenous *Zbtb2*.

(F) qPCR of endogenous *Zbtb2* relative to *GAPDH* in *WT* cells, *WT* cells over-expressing a *Zbtb2* construct that cannot be detected by the qPCR probe (TAQMANmut) and the *Zbtb2*$^{-/-}$ clones depicted in Fig. S5E. Average and SD of technical triplicates; * indicates p-values<0.01 compared to *WT*.
(**G**) qPCR of *Kat5* and *Zbtb2* relative to *GAPDH* upon *Kat5* or control siRNA transfection. Average and SD of technical triplicates; ** indicates p-value<0.001 compared to control siRNA.