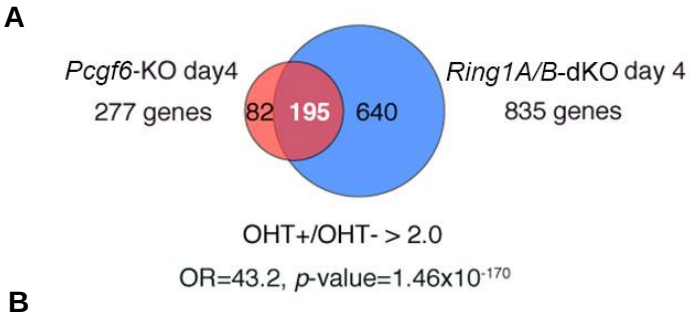
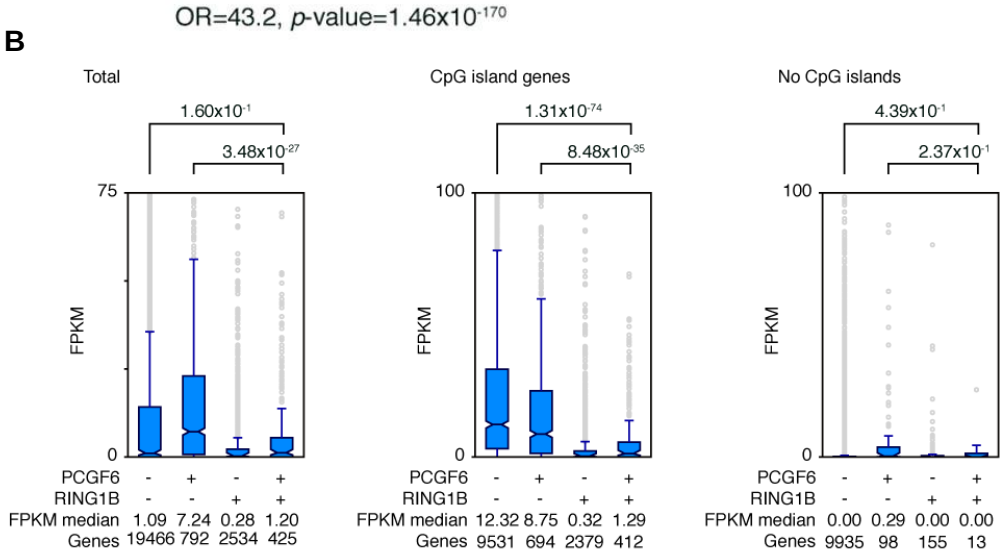
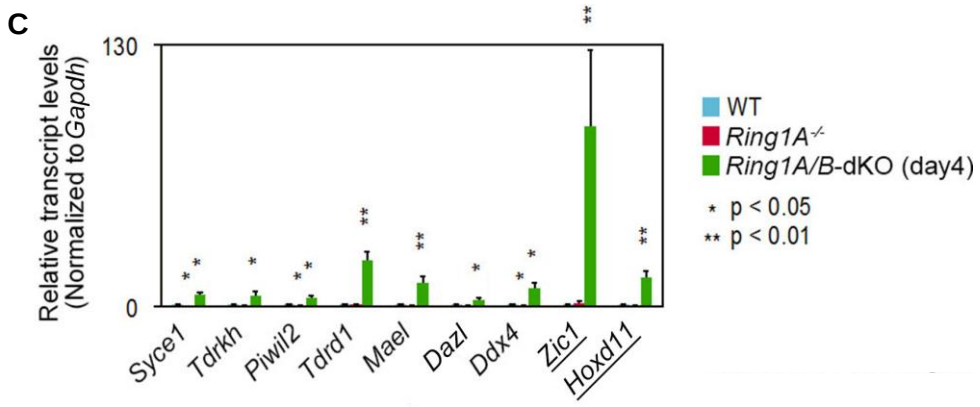
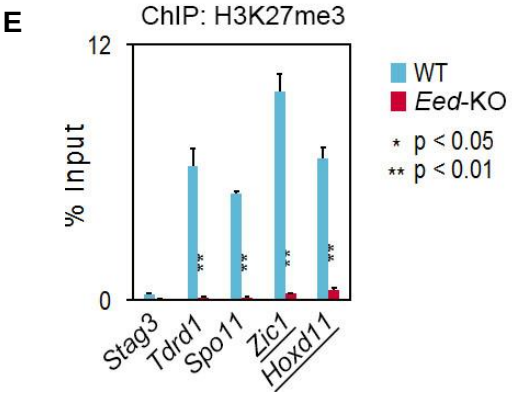
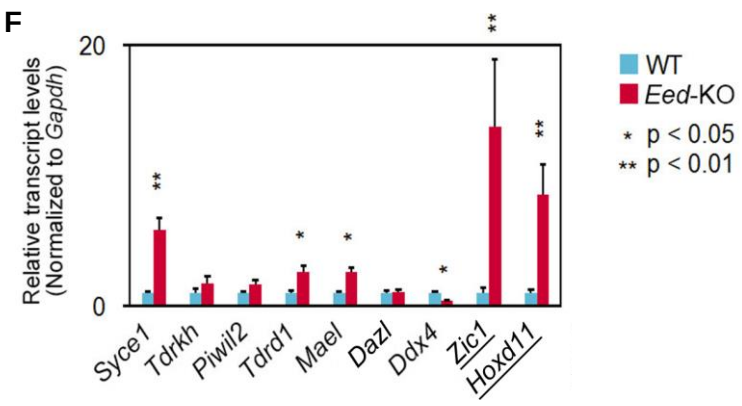
Figure 2–Figure Supplement 1











D

Up in both *Pcgf6*-KO and *Ring1A/B*-dKO			
GO [biological process]	**N**	**X**	***P*-value**
meiotic nuclear division (GO:0007126)	125	13	4.4E-10
meiotic cell cycle (GO:0051321)	165	14	6.9E-10
meiotic cell cycle process (GO:1903046)	130	13	7.2E-10
meiosis I (GO:0007127)	80	9	1.9E-06
reproductive process (GO:0022414)	1010	23	3.1E-06
single organism reproductive process (GO:0044702)	924	22	3.5E-06
synapsis (GO:0007129)	39	7	8.3E-06
cell cycle process (GO:0022402)	766	19	3.3E-05
piRNA metabolic process (GO:0034587)	12	5	3.4E-05
homologous chromosome segregation (GO:0045143)	48	7	3.4E-05

Up in *Ring1A/B*-dKO but not in *Pcgf6*-KO			
GO [biological process]	**N**	**X**	***P*-value**
organ development (GO:0048513)	2532	99	2.6E-18
system development (GO:0048731)	3499	115	3.2E-16
multicellular organismal development (GO:0007275)	4036	123	3.8E-15
anatomical structure morphogenesis (GO:0009653)	2004	81	8.1E-15
embryonic morphogenesis (GO:0048598)	561	42	1.1E-14
skeletal system development (GO:0001501)	431	37	1.9E-14
anatomical structure development (GO:0048856)	4183	124	2.4E-14
embryo development (GO:0009790)	989	53	3.5E-13
single-organism developmental process (GO:0044767)	4666	130	3.9E-13
developmental process (GO:0032502)	4693	130	6.2E-13

Figure 2–Figure Supplement 1. Repression of target genes by PCGF6-PRC1. (A) Venn diagram depicting the overlap of more than 2-fold up-regulated genes upon deletion of *Pcgf6* (in *Pcgf6*$^{fl/fl}$*;Rosa26::CreERT2*$^{tg/+}$ ESCs; 4 days after OHT treatment) or *Ring1B* (in *Ring1A-/-;Ring1B*$^{fl/fl}$*;Rosa26::CreERT2*$^{tg/+}$ ESCs; 4 days after OHT treatment). The number of genes de-repressed in *Pcgf6*-KO and *Ring1A/B*-dKO and included in each fraction is indicated. Odds ratio (OR) and *p*-value for the overlap between the indicated 2 groups were calculated by the Student's *t*-test. **(B)** Graphic representation of expression levels for PCGF6-bound and/or RING1B-bound genes. The average, deviation and distribution of FPKM values for the respective subset of genes determined by RNA-seq analysis are shown. The box plots represent the median (horizontal line; values are indicated below the plots), interquartile range (box), range (whiskers), and outliers (circles). The number of genes included in each subset is shown at the bottom. The p-values for the difference of expression changes between the indicated 2 groups were calculated by the Student's *t*-test and are indicated above each graph. Results for total genes, genes associated with and without CpG islands are shown. **(C)** Expression levels of the indicated genes in wild-type (WT) and *Ring1A*$^{-/-}$*;Ring1B*$^{fl/fl}$*;Rosa26::CreERT2*$^{tg/+}$ ESCs before (*Ring1A-/-*) or after OHT treatment [*Ring1A/B*-dKO (day4)]. Underlined genes are canonical PRC1 targets. Expression levels were normalized to a *Gapdh* control and are depicted as fold change relative to mock (OHT-untreated) ESCs. Error bars represent standard deviation determined from at least three independent experiments. *p*-values for the expression changes upon *Ring1B* deletion were calculated by the Student's t-test. **(D)** Gene ontology (GO) analysis of genes more than 2-fold up-regulated both in *Pcgf6*-KO and *Ring1A/B*-dKO ESCs was performed using http://geneontology.org/ and is shown in the upper table. GO analysis of genes up-regulated more than 2-fold in *Ring1A/B*-dKO but not in *Pcgf6*-KO was performed as well and is shown in the lower table. The significance of the enrichment of each GO term is indicated by a *p*-value for each category of biological process. **(E)** Local levels of H3K27me3 at the indicated promoter regions in wild type (WT) and *Eed*-KO ESCs were determined by ChIP and site-specific real-time PCR. Underlined genes are canonical PRC1 targets. The relative amount of ChIPed DNA is depicted as a percentage of input DNA. Error bars represent standard deviation determined from at least three independent experiments. The *p*-values for the difference in H3K27me3 levels at the respective loci between wild type and *Eed*-KO ESCs were determined by the Student's *t*-test. **(F)** EED is dispensable for repression of genes bound by PCGF6-PRC1. Comparative expression levels of selected genes bound by PCGF6-PRC1 or canonical PRC1 (underlined) in wild type (WT) and *Eed*-KO ESCs. Expression levels were normalized to a *Gapdh* control and are depicted as fold change relative to wild-type ESCs. Error bars represent standard deviation determined from at least three independent experiments.