

Figure 2–Figure Supplement 1

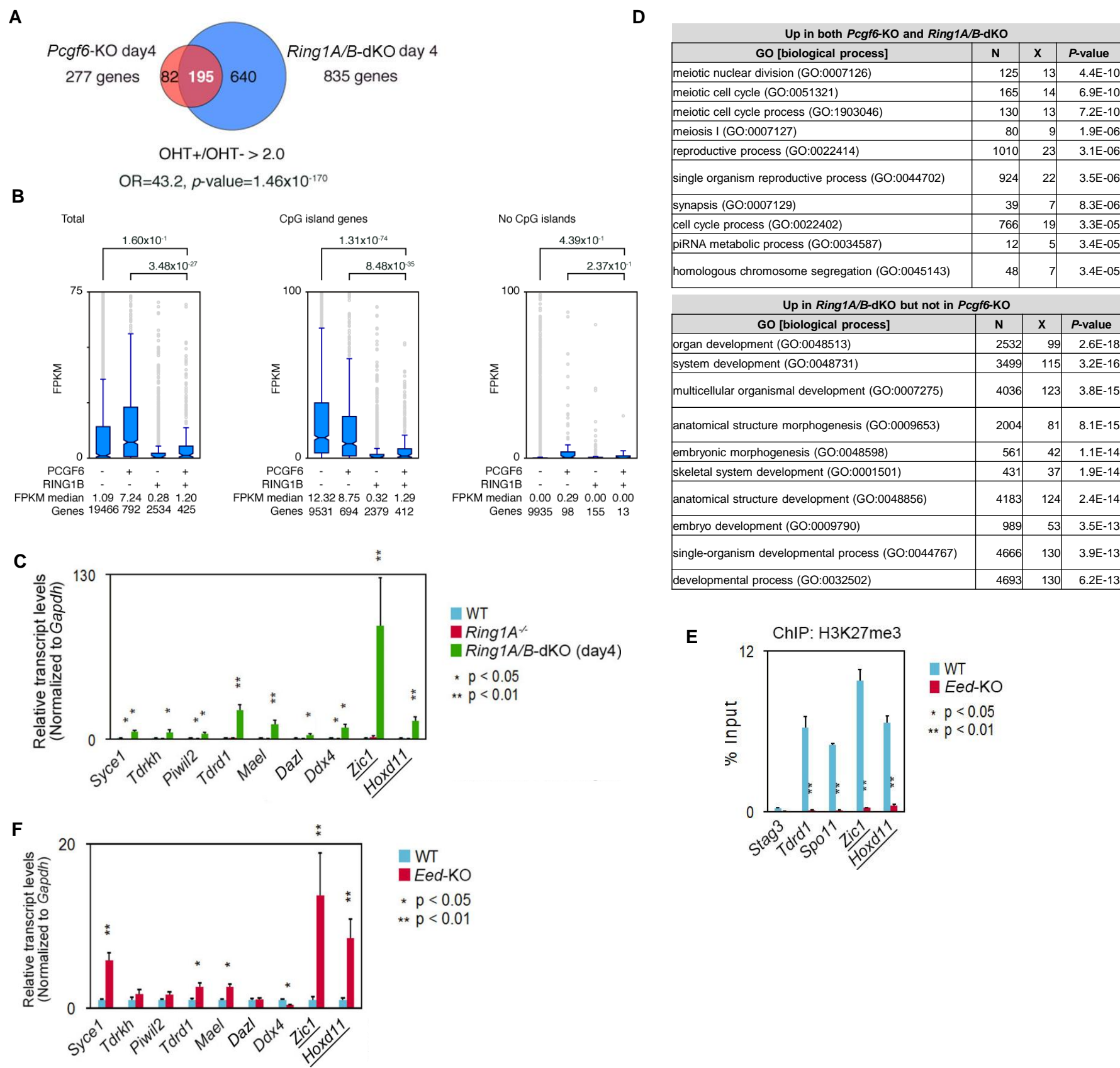


Figure 2–Figure Supplement 1. Repression of target genes by PCGF6-PRC1. (A) Venn diagram depicting the overlap of more than 2-fold up-regulated genes upon deletion of *Pcgf6* (in *Pcgf6*^{fl/fl}; *Rosa26::CreERT2*^{tg/+} ESCs; 4 days after OHT treatment) or *Ring1B* (in *Ring1A*^{-/-}; *Ring1B*^{fl/fl}; *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.