

Figure 1–Figure Supplement 1

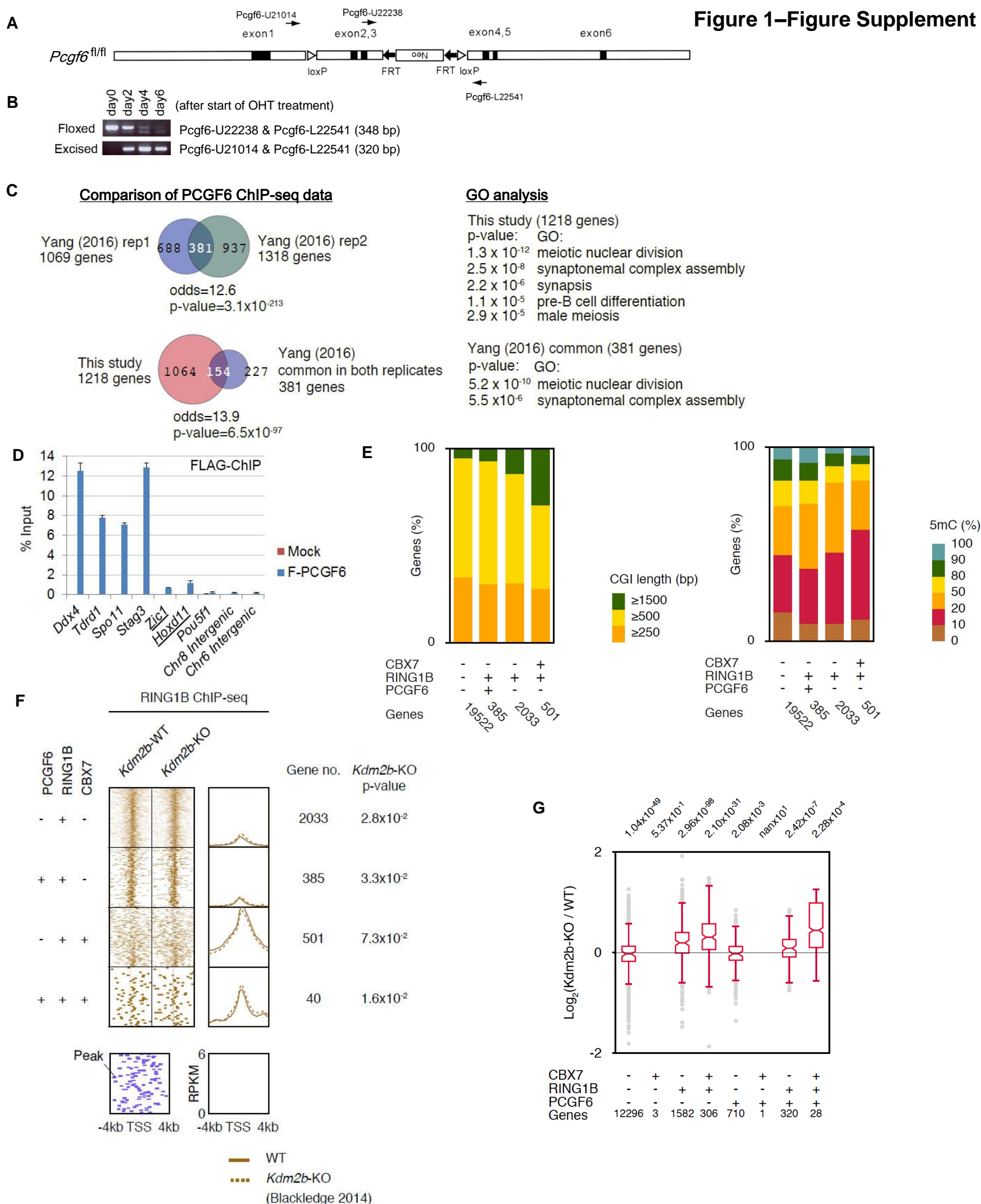


Figure 1–Figure Supplement 1. Generation of a *Pcgf6* conditional allele and properties of CpG islands at PCGF6-PRC1 target genes. (A) Schematic representation of the construct for conditional targeting of the *Pcgf6* locus. The targeting construct contains an FRT (closed arrows)-flanked neomycin resistance gene (neo), and the second and the third exons (closed rectangles) of the mouse *Pcgf6* gene are flanked by two loxP sites (open triangles). **(B)** Genomic PCR using the indicated primers demonstrating the kinetics of the excision of the loxP-flanked region in *Pcgf6*^{fl/fl}; *Rosa26::CreERT2*^{tg/+} ESCs after OHT treatment. **(C)** Comparison of the PCGF6 ChIP-seq data in this study with those reported in a previous paper (Yang et al., 2016). **(D)** ChIP-qPCR data showing a strong binding of FLAG-tagged PCGF6 to representative PCGF6 targets (*Ddx4*, *Tdrd1*, *Spo11* and *Stag3*) determined by ChIP-seq analysis shown in Figure 1 and a marginal binding to known canonical PRC1 targets (underlined). **(E)** Comparison of CpG island (CGI) length (left) and 5-methylcytosine (5mC) levels (right) of CBX7-, RING1B-, and/or PCGF6-bound genes. The number of genes included in each subset is shown at the bottom. **(F)** A heat map view of RING1B distribution in ± 4kb genomic regions around TSS in wild type (WT), and *Kdm2b*-KO ESCs. RING1B-bound genes are further subclassified by binding of PCGF6 and CBX7 (left). Average distribution and p-value for its change in *Kdm2b*-KO are also shown (right). **(G)** Gene expression changes in *Kdm2b*-KO ESCs. Box plot representation of gene expression changes induced by deletion of *Kdm2b* for genes bound by CBX7, PCGF6 and/or RING1B. The box plots represent the median (horizontal line), 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 determined by the Student's *t*-test and are indicated above each graph.