

## Supplemental Information

### PCGF6 Regulates Stem Cell Pluripotency as a Transcription Activator via Super-Enhancer Dependent Chromatin Interactions

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#### Supplemental Figures

**Figure S1. PCGF6 maintains mESC identity. Related to Figure 1.** (A-E) Heatmaps of the marker gene expression for pluripotency (A), Trophectoderm (B), Mesoderm (C), Ectoderm (D), and Endoderm (E) by using RNA-Seq data from shEV and sh*Pcgf6* knockdown ESCs. The scale represents fold changes.

**Figure S2. PCGF6 regulates SE associated genes. Related to Figure 3.** (A) Overlapping of the targeted regions of PCGF6/OCT4, PCGF6/SOX2 and PCGF6/NANOG. (B) Overlapping of the SE associated genes with downregulated or upregulated genes after *Pcgf6* knockdown, respectively. (C) Relative expression of SE associated genes were significantly downregulated or upregulated after *Pcgf6* knockdown. The scale represents  $\log_2$  (fold change).

**Figure S3. PCGF6 coordinates OCT4 in regulating SEs. Related to Figure 4.** (A) Percentage of OCT4 binding to PCGF6/H3K4me3 or PCGF6/H3K27me3 target genes, respectively. (B) Percentage of MED1 binding to PCGF6/OCT4 shared regions. (C) Depletion of OCT4 by dox treatment decreased the enrichment of PCGF6 at the SEs of *Oct4*, *Sox2*, *Nanog*, *Klf4*, *Pim1*, and *Agtrap*. Data are presented as mean  $\pm$  SD from three independent replicates. \*\*p < 0.01 compared with control cells. \*p < 0.05 compared with control cells.

**Figure S4. PCGF6 and OCT4 participate in 3D chromatin. Related to Figure 5.** Percentage of promoter-promoter or promoter-enhancer interactions regulated by PCGF6.

**Figure S5. PCGF6 and OCT4 regulate proliferation genes. Related to**

**Figure 6.** (A) Colony formation assay for ESCs after *Pcgf6* or *Oct4* knockdown. Individual colonies were stained for AP activity. (B) Cell cycle analysis for ESCs after *Pcgf6* or *Oct4* knockdown. \*\*p < 0.01 compared with control cells. \*p < 0.05 compared with control cells. (C) Overexpression efficiency of *Ccnd3* and *Polr3gl* in mESCs was validated by Real-Time Quantitative PCR (qPCR). Data are presented as mean  $\pm$  SD from three independent replicates. \*\*p < 0.01 compared with shEV+EV cells. \*p < 0.05 compared with shEV+EV cells. (D) Cell proliferation was evaluated by cell counting kit 8 cell viability assay. Data are presented as means  $\pm$  SD from three independent replicate experiments. The experiments were performed in triplicate. (E) Expression changes of pluripotency genes in response to knockdown of *Pcgf6* were rescued by introduction of *Ccnd3* or *Polr3gl*. Data are depicted as mean  $\pm$  SD from three independent replicates. \*\*p < 0.01 compared with shEV+EV cells. \*p < 0.05 compared with shEV+EV cells. ##p < 0.01 compared with sh*Pcgf6*+EV cells. #p < 0.05 compared with sh*Pcgf6*+EV cells.

### **Supplemental Tables**

Table S1. Primers used in this study for RT-qPCR and CHIP-qPCR.

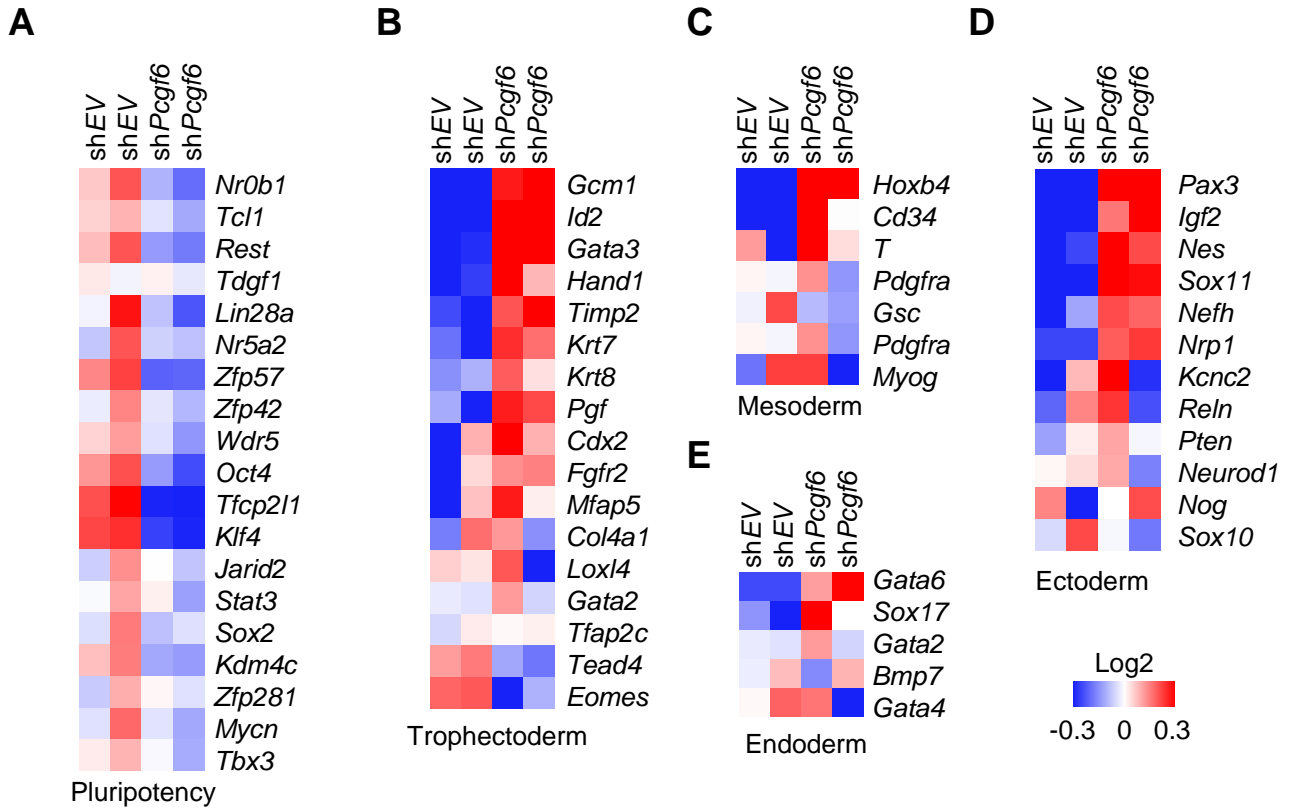
Table S2. Published GEO datasets used in this study.

Table S3. RNA-Seq data on genes that are differentially expressed after 5 days of *Pcgf6* KD.

Table S4. List of PCGF6 target genes used in this study.

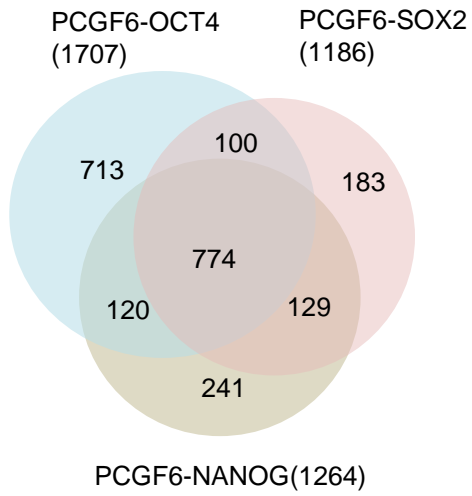
Table S5. List of OCT4 target genes used in this study

# Supplement figure 1

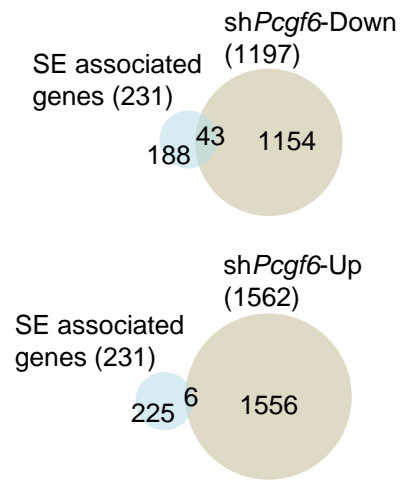


# Supplement figure 2

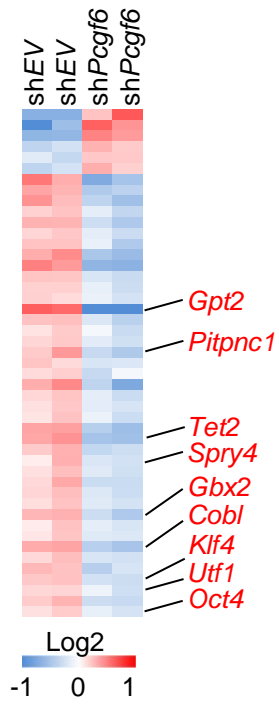
**A**



**B**

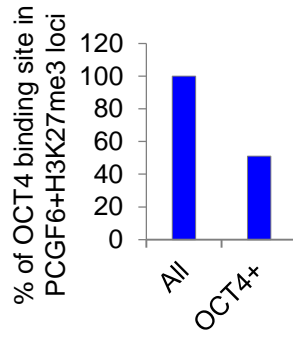
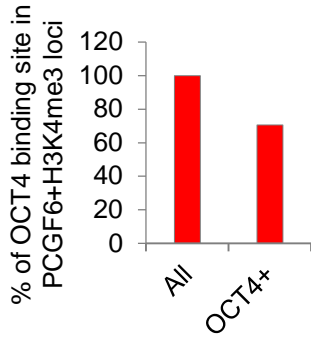


**C**

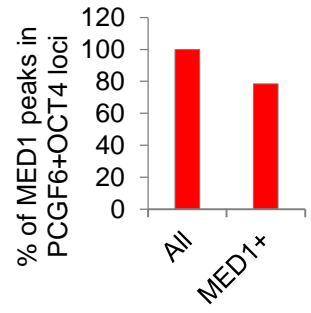


### Supplement figure 3

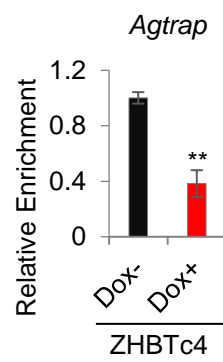
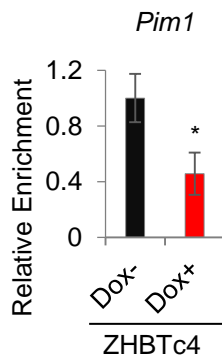
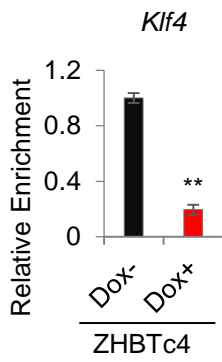
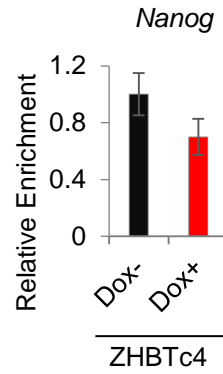
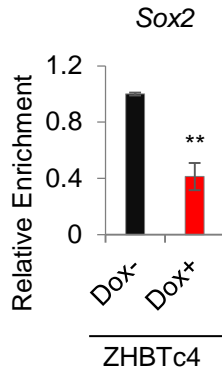
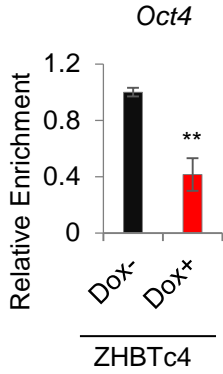
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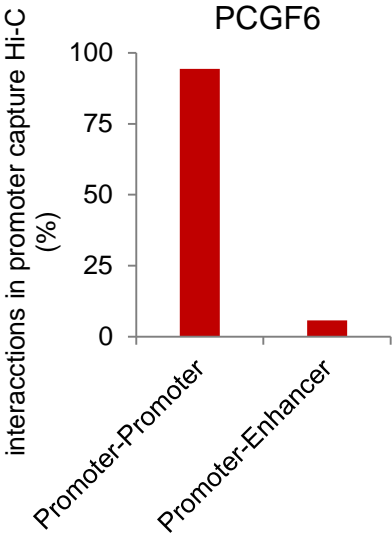
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**C**

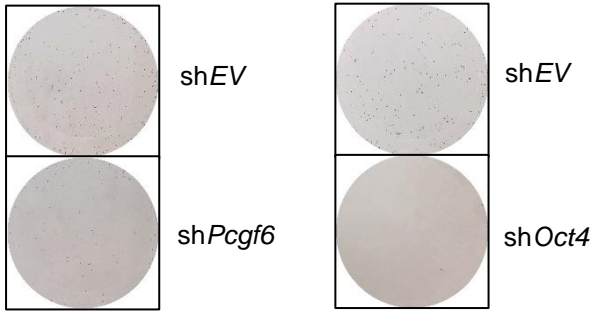


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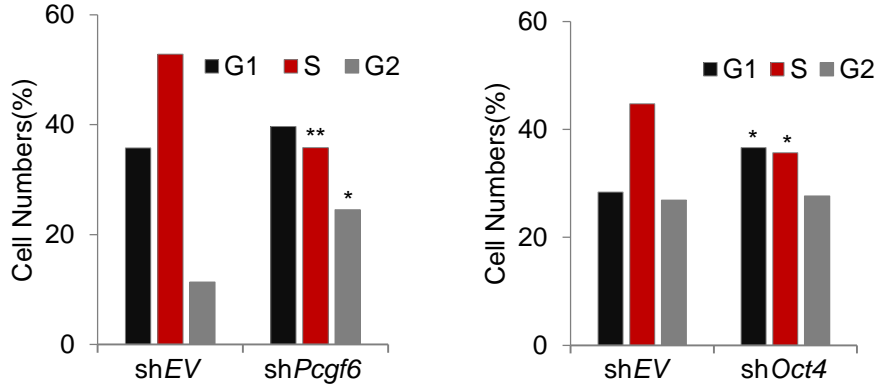


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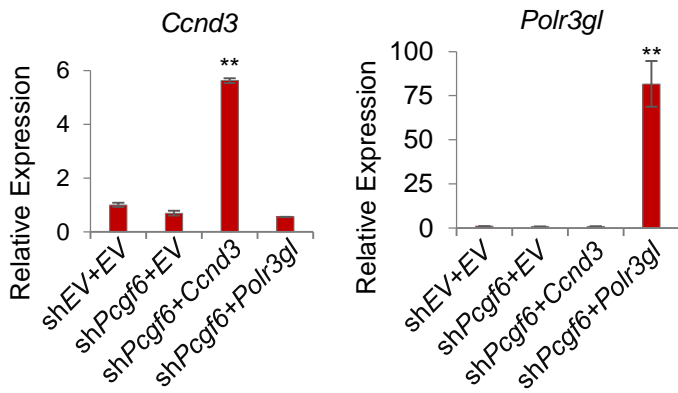
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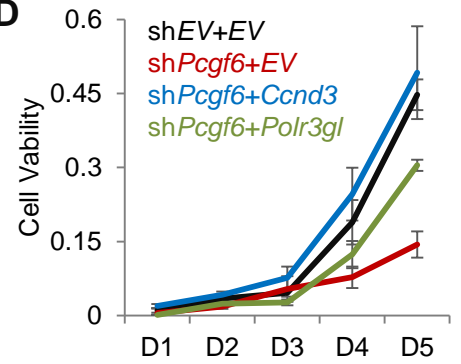
**B**



**C**



**D**



**E**

