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Supplementary Materials for

Parallel PRC2/cPRC1 and vPRC1 pathways silence lineage-specific genes and maintain self-renewal in mouse embryonic stem cells

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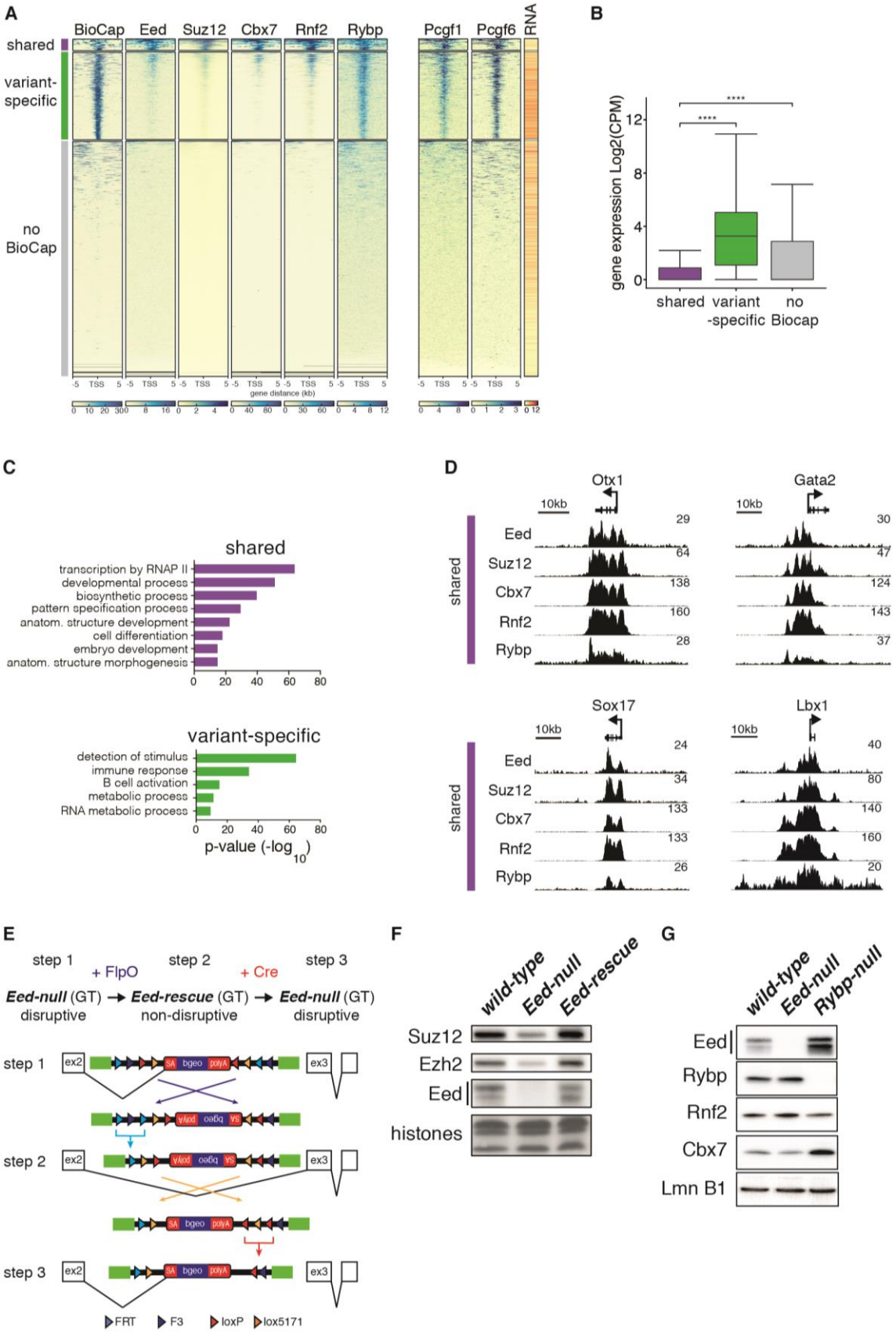

A
BioCap
Eed
Suz12
Cbx7
Rnf2
Rybp
Pcgf1
Pcgf6
RNA
shared
variant-specific
no BioCap
TSS
gene distance (kb)
B
gene expression Log2(CPM)
shared
variant -specific
no Biocap
****
C
shared
transcription by RNAP II
developmental process
biosynthetic process
pattern specification process
anatom. structure development
cell differentiation
embryo development
anatom. structure morphogenesis
variant-specific
detection of stimulus
immune response
B cell activation
metabolic process
RNA metabolic process
p-value (-log10)
D
Otx1
Gata2
Sox17
Lbx1
10kb
Eed
Suz12
Cbx7
Rnf2
Rybp
shared
E
step 1
+ FlpO
step 2
+ Cre
step 3
Eed-null (GT)
disruptive
Eed-rescue (GT)
non-disruptive
Eed-null (GT)
disruptive
ex2
ex3
SA
bgeo
polyA
FRT
F3
loxP
lox5171
F
wild-type
Eed-null
Eed-rescue
Suz12
Ezh2
Eed
histones
G
wild-type
Eed-null
Rybp-null
Eed
Rybp
Rnf2
Cbx7
Lmn B1

Fig. S1. Genomic distribution of PRC1 and PRC2 and generation of single mutant mESCs. **(A)** Heat maps of Eed, Suz12, Cbx7, Rnf2, Rybp, Pcgf1 and Pcgf6 centered around the TSS of CGI promoters (+/- 5kb) show relative enrichment in *wild-type* mESCs. TSSs are separated by k-means into "shared" (965 TSSs - violet), "variant-specific" (7,333 TSSs - green) and "noBioCap" clusters (19,712 TSSs - gray). TSSs were ranked by enrichment of canonical PRC1 (Rnf2, Cbx7), variant PRC1 (Rnf2, Rybp), PRC2 (Eed) subunits and un-methylated CGIs (BioCap). Colored scales (bottom) show dynamic range of ChIP-seq signal. **(B)** Boxplot quantification of gene expression (QuantSeq) at the three cluster from A). p-values calculated using Wilcoxon test (****: $p < 1e\text{-}04$). **(C)** Gene Ontology (GO) analysis compares enriched categories of shared (violet) and variant-specific (green) PcG target genes. **(D)** Genomic screenshots of selected "shared" Polycomb target genes encoding developmental transcription factors. **(E)** Schematic representation of gene-trap disruption and rescue of *Eed* gene expression in haploid mESC line. FlpO and Cre indicate DNA recombinases facilitating inversion at recombination sites. *Eed-null* (GT) (step 1) and *Eed-rescue* (GT) (step 2) mESCs have been used in this study. **(F)** Western blot of Eed, Suz12 and Ezh2 in wild-type, *Eed-null* and *Eed-rescue* mESCs. Ponceau staining serves as loading control. **(G)** Western blot of Eed, Rnf2, Cbx7 and Rybp in *wild-type*, *Eed-null* and *Rybp-null* mESCs. Lmn B1 serves as loading control.

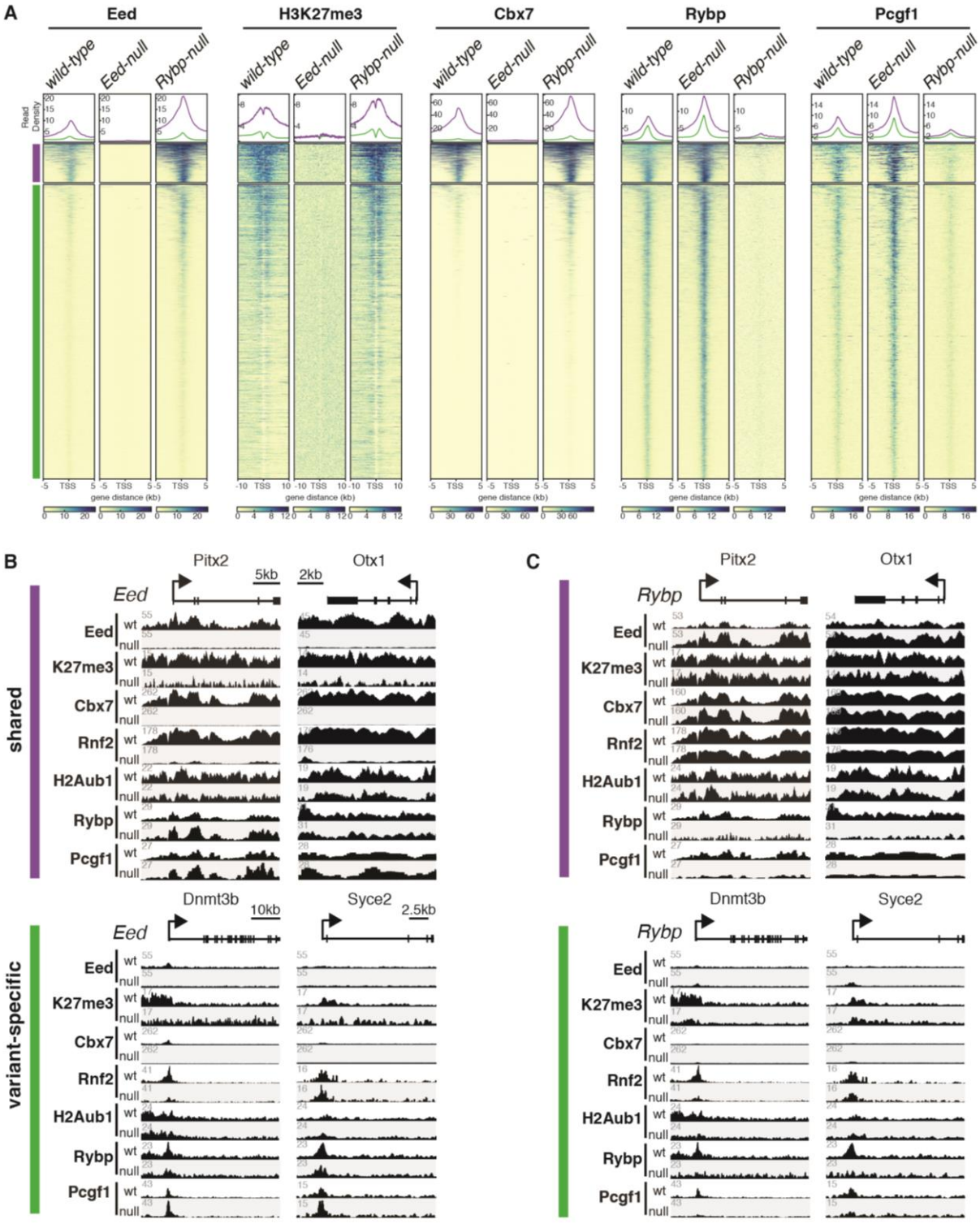

A
Eed
H3K27me3
Cbx7
Rybp
Pcgf1
wild-type
Eed-null
Rybp-null
Read Density
gene distance (kb)
B
shared
variant-specific
Pitx2
Otx1
Dnmt3b
Syce2
Eed
5kb
2kb
10kb
2.5kb
wt
null
K27me3
Cbx7
Rnf2
H2Aub1
Rybp
Pcgf1
C

Fig. S2. PcG protein occupancy and histone modifications in single mutant mESCs. (A) Meta plots and heat maps compare Eed, H3K27me3, Cbx7, Rybp and Pcgf1 enrichments centered around the TSS of CGI promoters (+/- 5kb or +/- 10kb) at "shared" (violet) and "variant-specific" (green) genes in *wild-type*, *Eed-null* and *Rybp-null* mESCs. Colored scales (bottom) show dynamic range of ChIP-seq signals. Genomic screenshots of representative "shared" and "variant-specific" Polycomb target genes display relative enrichment of PcG proteins and histone modifications (n=2) in **(B)** *wild-type* and *Eed-null* mESCs and **(C)** in *wild-type* and *Rybp-null* mESCs.

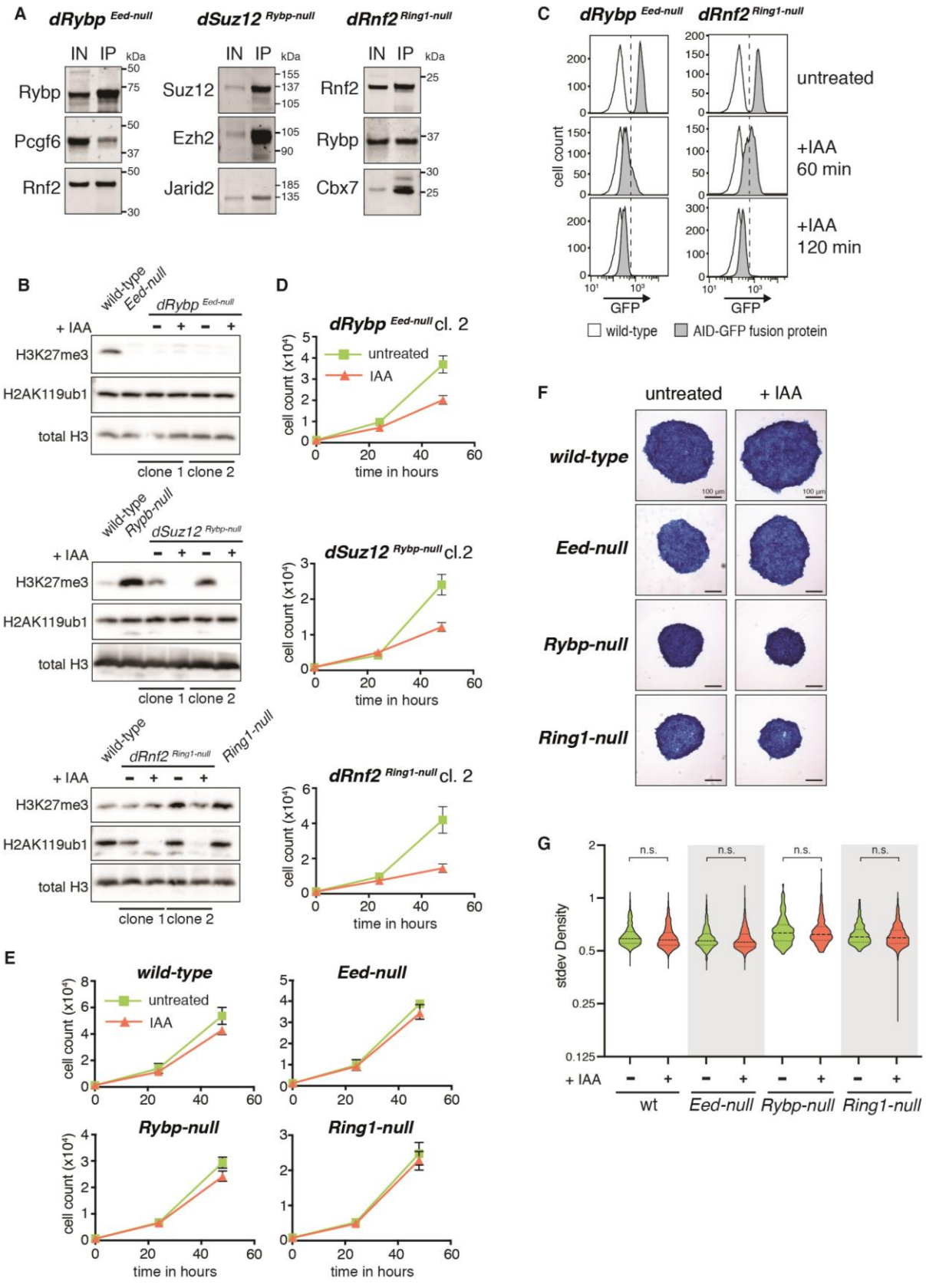

A
dRybp Eed-null
dSuz12 Rybp-null
dRnf2 Ring1-null
IN IP kDa
Rybp
Pcgf6
Rnf2
Suz12
Ezh2
Jarid2
Rnf2
Rybp
Cbx7
B
wild-type
Eed-null
dRybp Eed-null
+ IAA
H3K27me3
H2AK119ub1
total H3
clone 1 clone 2
wild-type
Rybp-null
dSuz12 Rybp-null
wild-type
dRnf2 Ring1-null
Ring1-null
C
dRybp Eed-null
dRnf2 Ring1-null
untreated
+IAA 60 min
+IAA 120 min
cell count
GFP
wild-type
AID-GFP fusion protein
D
dRybp Eed-null cl. 2
untreated
IAA
cell count (x10⁴)
time in hours
dSuz12 Rybp-null cl.2
dRnf2 Ring1-null cl. 2
E
wild-type
Eed-null
Rybp-null
Ring1-null
F
untreated
+ IAA
wild-type
Eed-null
Rybp-null
Ring1-null
G
stdev Density
n.s.
+ IAA
wt
Eed-null
Rybp-null
Ring1-null

Fig. S3. Combined PcG protein depletion triggers loss of mESC self-renewal. (A) Co-immunoprecipitation of AID tagged fusion proteins from nuclear extracts of *dRybp* $^{Eed\text{-}null}$ (left), *dSuz12* $^{Rybp\text{-}null}$ (middle) and *dRnf2* $^{Ring1\text{-}null}$ (right) mESCs using FLAG antibody and analysis by western blot with indicated antibodies. **(B)** Western blots of histone modifications in *wild-type*, *Eed-null* mESCs and two independent clones of *dRybp* $^{Eed\text{-}null}$ mESCs (top), in *wild-type*, *Rybp-null* mESCs and two independent clones of *dSuz12* $^{Rybp\text{-}null}$ mESCs (middle) and in *wild-type*, *Ring1-null* mESCs and two independent clones of *dRnf2* $^{Ring1\text{-}null}$ mESCs (bottom). IAA treatment (250 µM) for 24 hours. Western blot for total histone H3 serves as loading control. **(C)** Flow cytometry histograms display expression of PcG protein fusions with AID-GFP (gray) in *dRybp* $^{Eed\text{-}null}$ (left) and *dRnf2* $^{Ring1\text{-}null}$ (right) before, after 60 mins and 120 minutes of IAA treatment. White histogram shows expression of control cells without GFP. **(D)** Proliferation assays of additional independent clones of *dRybp* $^{Eed\text{-}null}$, *dSuz12* $^{Rybp\text{-}null}$ and *dRnf2* $^{Ring1\text{-}null}$ mESCs grown in serum conditions for 72 hours without (untreated) or with 250µM IAA. Live cells were quantified by flow cytometry every 24 hours starting at 24 hours (time point 0h). Displayed are the median and standard deviation of four replicates. **(E)** Proliferation assays of *wild-type*, *Eed-null* and *Rybp-null* and *Ring1-null* mESCs grown in serum conditions for 72 hours without (untreated) or with 250µM IAA. Live cells were quantified by flow cytometry every 24 hours starting at 24 hours (time point 0h). Displayed are the median and standard deviation of four replicates. **(F)** Representative images of alkaline phosphatase staining of *wild-type*, *Eed-null* and *Rybp-null* and *Ring1-null* mESC colonies cultured in absence (untreated) or presence or 250µM IAA for five days. **(G)** Box plots display Standard Deviation of Density quantifying the degree of mESC colony heterogeneity in response to five days of IAA treatment. Statistical significance calculated using unpaired t-test (p-value <0.01)

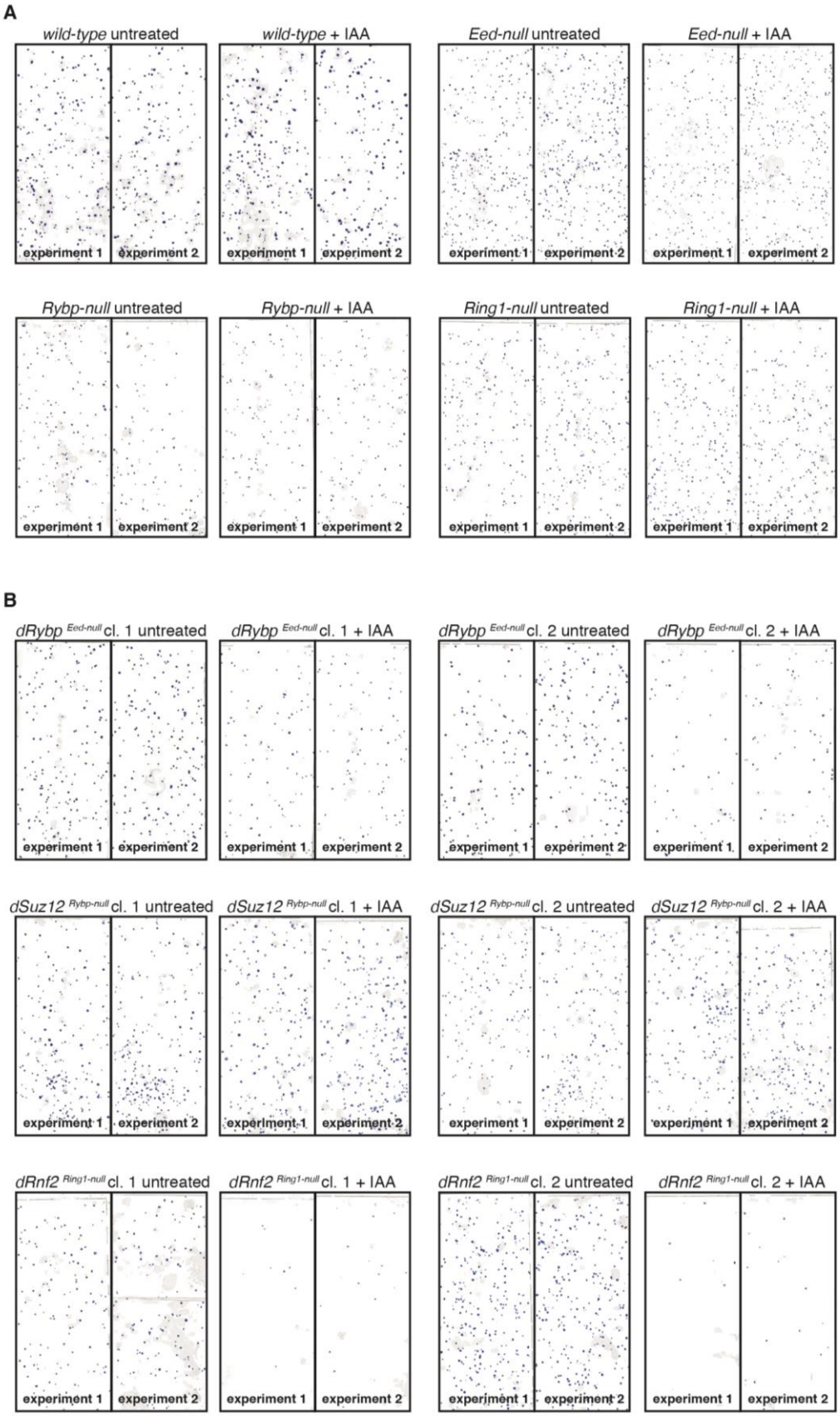

A
wild-type untreated
wild-type + IAA
Eed-null untreated
Eed-null + IAA
Rybp-null untreated
Rybp-null + IAA
Ring1-null untreated
Ring1-null + IAA
experiment 1
experiment 2
B
dRybp Eed-null cl. 1 untreated
dRybp Eed-null cl. 1 + IAA
dRybp Eed-null cl. 2 untreated
dRybp Eed-null cl. 2 + IAA
dSuz12 Rybp-null cl. 1 untreated
dSuz12 Rybp-null cl. 1 + IAA
dSuz12 Rybp-null cl. 2 untreated
dSuz12 Rybp-null cl. 2 + IAA
dRnf2 Ring1-null cl. 1 untreated
dRnf2 Ring1-null cl. 1 + IAA
dRnf2 Ring1-null cl. 2 untreated
dRnf2 Ring1-null cl. 2 + IAA

Fig. S4. AP staining of untreated and IAA-treated mESCs. (A) Slides showing AP staining from two independent experiments of *wild-type*, *Eed-null* and *Rybp-null* and *Ring1-null* mESC colonies cultured in absence (untreated) or presence or 250µM IAA for five days. **(B)** Slides showing AP staining of *dRybp* $^{Eed\text{-}null}$, *dSuz12* $^{Rybp\text{-}null}$ and *dRnf2* $^{Ring1\text{-}null}$ mESC colonies from two independently derived clones cultured in absence (untreated) or presence or 250µM IAA for five days.

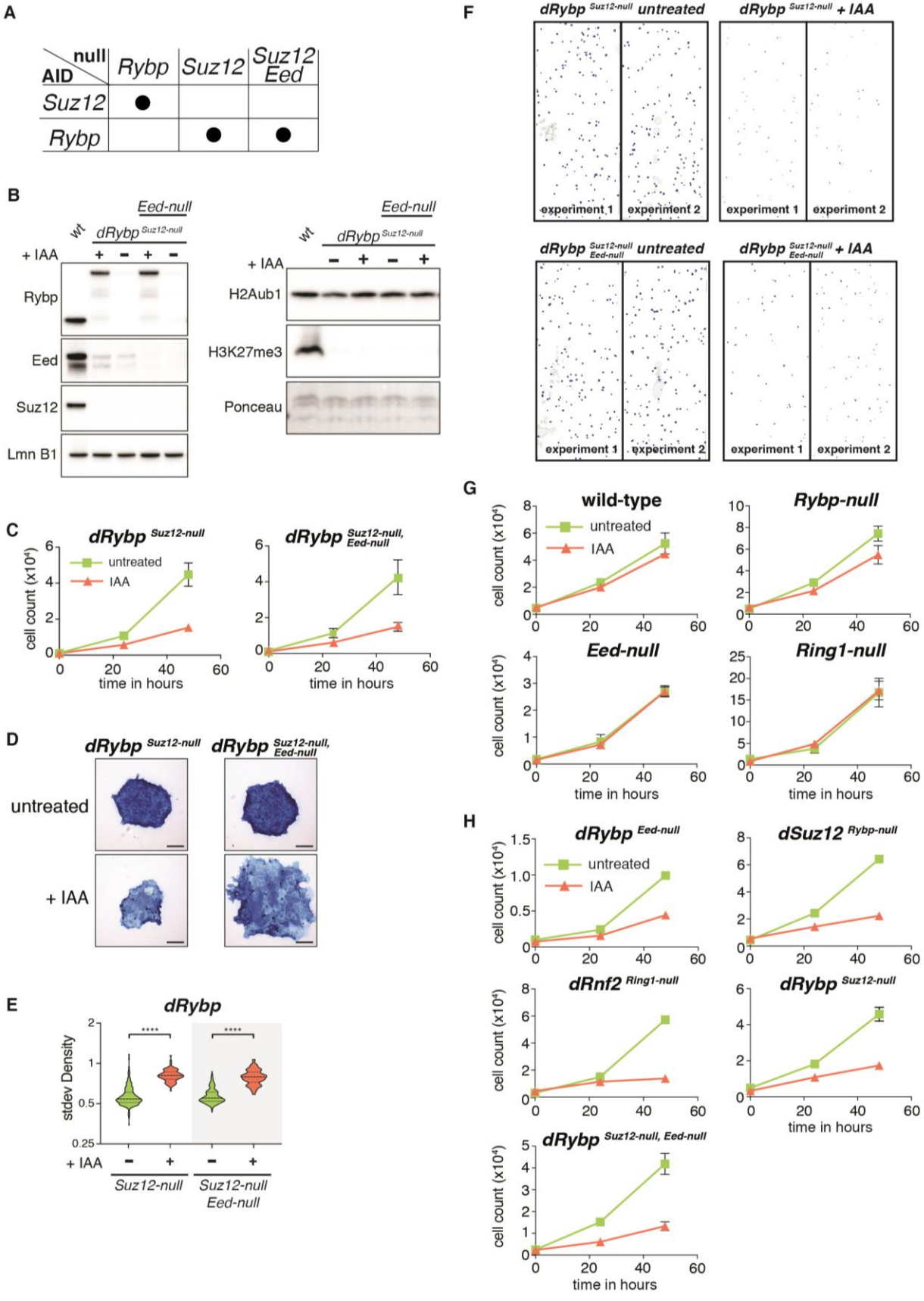
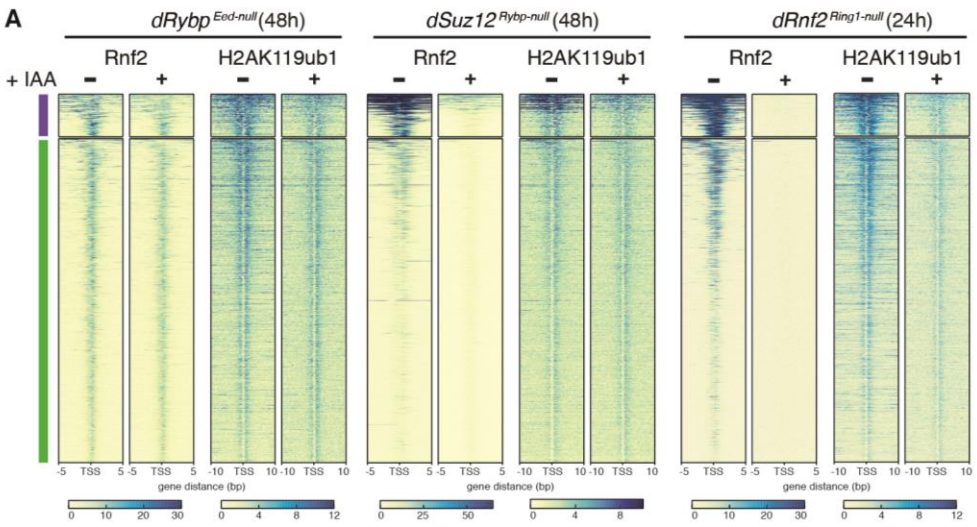
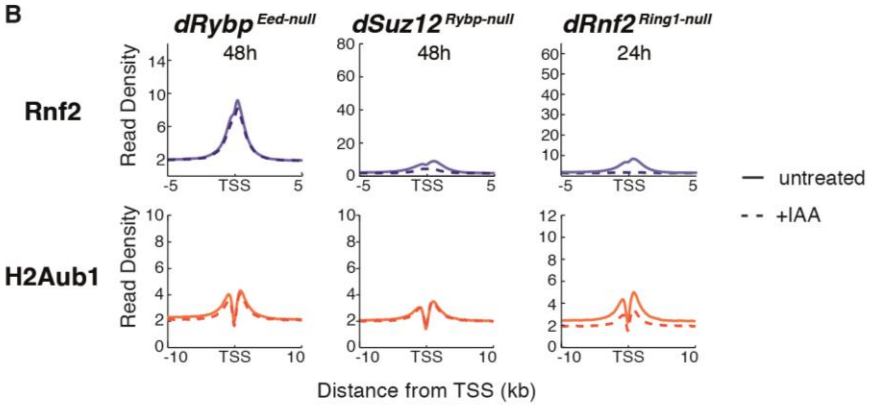

A
null
AID
Rybp
Suz12
Suz12 Eed
Suz12
Rybp
B
Eed-null
wt
dRybp Suz12-null
+ IAA
Rybp
Eed
Suz12
Lmn B1
H2Aub1
H3K27me3
Ponceau
C
dRybp Suz12-null
dRybp Suz12-null, Eed-null
untreated
IAA
cell count (x10⁴)
time in hours
D
dRybp Suz12-null
dRybp Suz12-null, Eed-null
untreated
+ IAA
E
dRybp
stdev Density
****
+ IAA
Suz12-null
Suz12-null Eed-null
F
dRybp Suz12-null untreated
dRybp Suz12-null + IAA
dRybp Suz12-null Eed-null untreated
dRybp Suz12-null Eed-null + IAA
experiment 1
experiment 2
G
wild-type
Rybp-null
Eed-null
Ring1-null
untreated
IAA
cell count (x10⁴)
time in hours
H
dRybp Eed-null
dSuz12 Rybp-null
dRnf2 Ring1-null
dRybp Suz12-null
dRybp Suz12-null, Eed-null

Fig. S5. Consequences of Rybp degradation in Suz12-null and Suz12/Eed-null mESCs. (A) Table summarizes engineered degron mESCs with inducible degradation of either Rybp or Suz12. **(B)** Western blot of PcG proteins and histone modifications in *wild-type*, *dRybp* $^{Suz12\text{-}null}$ and *dRybp* $^{Suz12\text{-}null,\ Eed\text{-}null}$ mESCs (left). IAA treatment (250 µM) for 24 hours leads to degradation of AID-fusion protein. Lamin B1 (Lmn B1) and Ponceau staining serve as loading controls. **(C)** Proliferation assays of *dRybp* $^{Suz12\text{-}null}$ and *dRybp* $^{Suz12\text{-}null,\ Eed\text{-}null}$ mESCs grown in serum conditions for 72 hours without (untreated) or with 250µM IAA. Live cells were quantified by flow cytometry every 24 hours. Displayed is the median and standard deviation of four replicate measurements starting at 24 hours IAA (time point 0h). **(D)** Representative images of Alkaline phosphatase staining of *dRybp* $^{Suz12\text{-}null}$ and *dRybp* $^{Suz12\text{-}null,\ Eed\text{-}null}$ mESC colonies cultured in absence (untreated) or presence or 250µM IAA for five days. **(E)** Box plots display Standard Deviation of Density quantifying the degree of mESC colony heterogeneity in response to five days of IAA treatment. Statistical significance calculated using unpaired t-test (p-value <0.01). **(F)** Slides showing AP staining from two independent experiments of *dRybp* $^{Suz12\text{-}null}$ and *dRybp* $^{Suz12\text{-}null,\ Eed\text{-}null}$ mESC colonies cultured in absence (untreated) or presence or 250µM IAA for five days. **(G)** Proliferation assays of *wild-type*, *Eed-null*, *Rybp-null* and *Ring1-null* mESCs grown in 2i conditions for 72 hours without (untreated) or with 250µM IAA. Live cells were quantified by flow cytometry every 24 hours. Displayed is the median and standard deviation of four replicates. **(H)** Proliferation assays of *dRybp* $^{Eed\text{-}null}$, *dRnf2* $^{Ring1\text{-}null}$, *dSuz12* $^{Rybp\text{-}null}$ and *dRybp* $^{Suz12\text{-}null}$ mESCs grown in 2i conditions for 72 hours without (untreated) or with 250µM IAA. Live cells were quantified by flow cytometry every 24 hours starting at 24 hours (time point 0h). Displayed is the median and standard deviation of four replicates.


A
dRybp Eed-null (48h)
dSuz12 Rybp-null (48h)
dRnf2 Ring1-null (24h)
Rnf2
H2AK119ub1
+ IAA
gene distance (bp)


B
dRybp Eed-null
dSuz12 Rybp-null
dRnf2 Ring1-null
48h
24h
Rnf2
H2Aub1
Read Density
TSS
untreated
+IAA
Distance from TSS (kb)

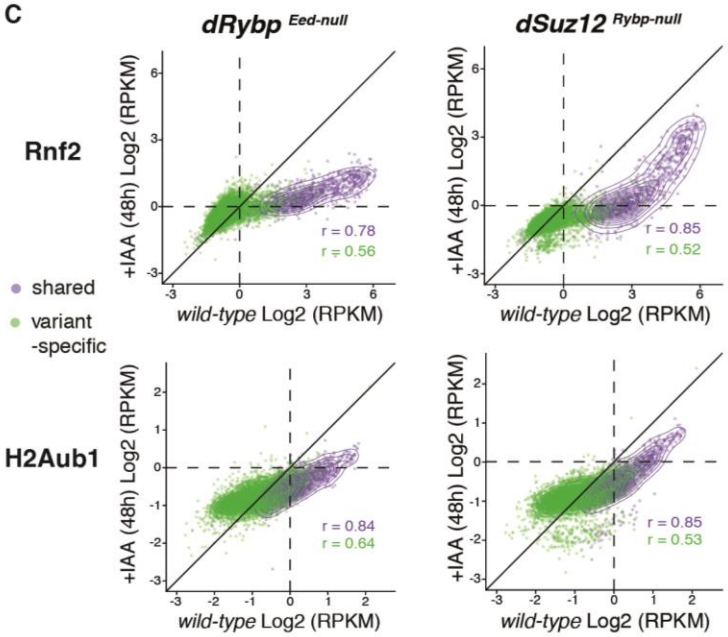

C
dRybp Eed-null
dSuz12 Rybp-null
Rnf2
H2Aub1
shared
variant -specific
+IAA (48h) Log2 (RPKM)
wild-type Log2 (RPKM)
r = 0.78
r = 0.56
r = 0.85
r = 0.52
r = 0.84
r = 0.64
r = 0.85
r = 0.53

Fig. S6. Rnf2 occupancy and H2AK119ub1 in IAA-treated degron mESCs. (A) Heat maps of ChIP-seq of Rnf2 and H2AK119ub1 around CGI TSSs (+/- 5kb or +/- 10kb) at "shared" (violet) and "variant-specific" (green) genes in *dRybp* $^{Eed\text{-}null}$, *dSuz12* $^{Rybp\text{-}null}$ and *dRnf2* $^{Ring1\text{-}null}$ mESCs before and after IAA treatment as indicated. Colored scales (bottom) show dynamic range of ChIP-seq. **(B)** Meta plots of Rnf2 and H2AK119ub1 (H2Aub1) ChIP-seq at "variant-specific" genes in *dRybp* $^{Eed\text{-}null}$, *dSuz12* $^{Rybp\text{-}null}$ and *dRnf2* $^{Ring1\text{-}null}$ mESCs before and after IAA treatment as indicated in Fig. 3C). **(C)** Density scatter plot of Rnf2 (top, +/- 5kb around TSS) and in H2AK119ub1 (H2Aub1) (bottom, +/- 10kb around TSS) signals in wild-type and IAA-treated *dRybp* $^{Eed\text{-}null}$ (left) and *dSuz12* $^{Rybp\text{-}null}$ (right) mESCs. r - Pearson's correlation coefficient, "shared" TSSs (violet), "variant-specific" TSSs (green).

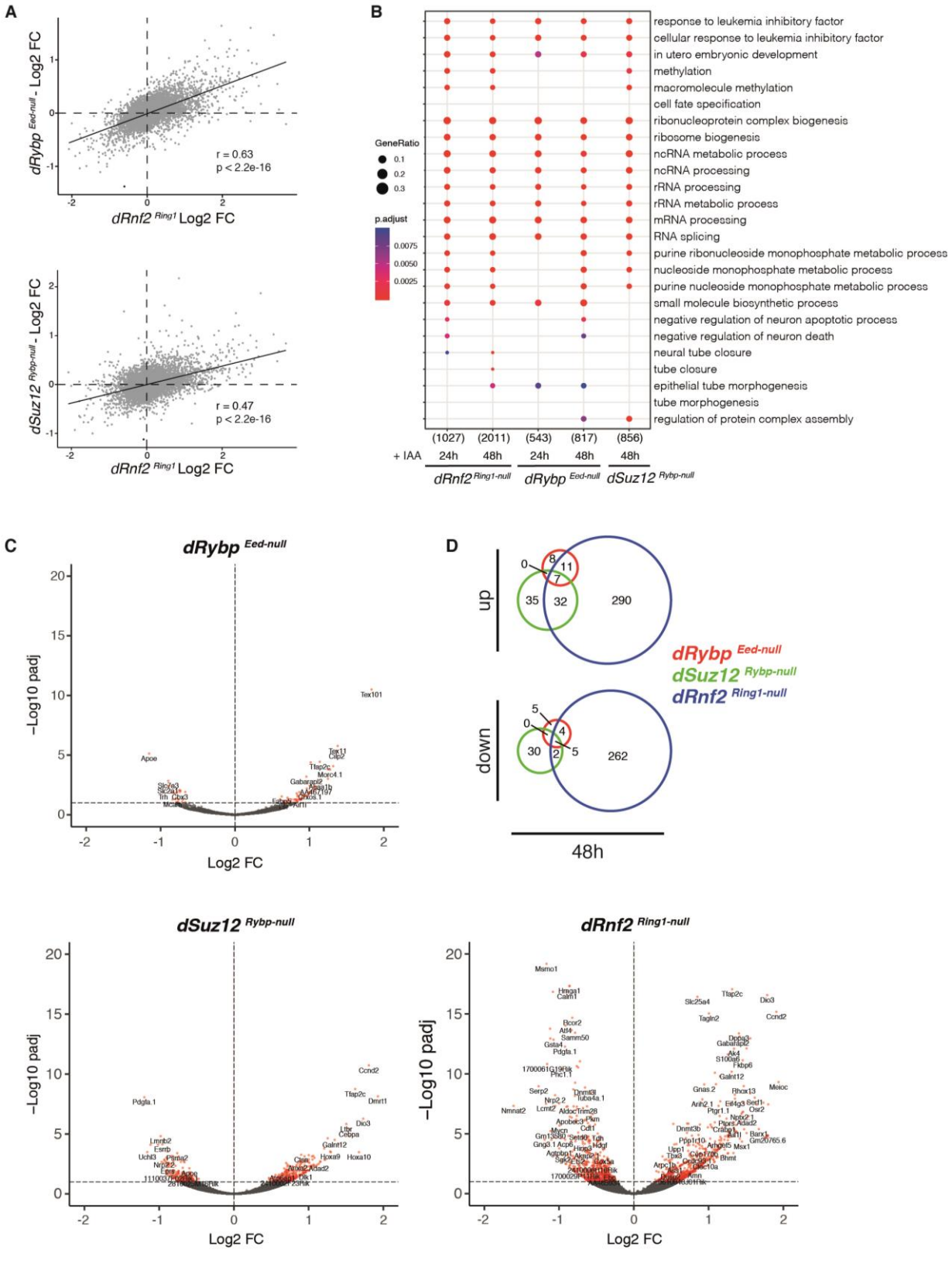

A
dRybp Eed-null - Log2 FC
dRnf2 Ring1 Log2 FC
r = 0.63
p < 2.2e-16
dSuz12 Rybp-null - Log2 FC
dRnf2 Ring1 Log2 FC
r = 0.47
p < 2.2e-16
B
response to leukemia inhibitory factor
cellular response to leukemia inhibitory factor
in utero embryonic development
methylation
macromolecule methylation
cell fate specification
ribonucleoprotein complex biogenesis
ribosome biogenesis
ncRNA metabolic process
ncRNA processing
rRNA processing
rRNA metabolic process
mRNA processing
RNA splicing
purine ribonucleoside monophosphate metabolic process
nucleoside monophosphate metabolic process
purine nucleoside monophosphate metabolic process
small molecule biosynthetic process
negative regulation of neuron apoptotic process
negative regulation of neuron death
neural tube closure
tube closure
epithelial tube morphogenesis
tube morphogenesis
regulation of protein complex assembly
GeneRatio
0.1
0.2
0.3
p.adjust
0.0075
0.0050
0.0025
(1027) (2011) (543) (817) (856)
+ IAA 24h 48h 24h 48h 48h
dRnf2 Ring1-null
dRybp Eed-null
dSuz12 Rybp-null
C
dRybp Eed-null
−Log10 padj
Log2 FC
dSuz12 Rybp-null
dRnf2 Ring1-null
D
up
down
48h
dRybp Eed-null
dSuz12 Rybp-null
dRnf2 Ring1-null

Fig. S7. Impact of combined PcG protein depletion on gene regulation. (A) Scatter plot compares gene expression changes between *dRybp* $^{Eed\text{-}null}$ and *dRnf2* $^{Ring1\text{-}null}$ (top), and *dSuz12* $^{Rybp\text{-}null}$ and *dRnf2* $^{Ring1\text{-}null}$ mESCs following 48h of IAA treatment. r and p denote Pearson correlation coefficient and P-value. **(B)** Analysis of Gene Ontology categories of downregulated genes in response to IAA treatment of *dRybp* $^{Eed\text{-}null}$, *dSuz12* $^{Rybp\text{-}null}$ and *dRnf2* $^{Ring1\text{-}null}$ mESCs at 24h and 48h. The color indicates the significance and the size represents the fraction of genes in each category. **(C)** Volcano plots show gene expression changes of IAA-treated relative to untreated *dRybp* $^{Eed\text{-}null}$, *dSuz12* $^{Rybp\text{-}null}$ and *dRnf2* $^{Ring1\text{-}null}$ mESCs cultured in 2i conditions at 48h. Differential gene expression (red, padj < 0.1) was calculated by averaging two independent clones per genotype, each sequenced in triplicates. Displayed are annotations of differentially expressed genes. **(D)** Venn diagrams show overlap of differentially expressed genes in *dRybp* $^{Eed\text{-}null}$ (red circles), *dSuz12* $^{Rybp\text{-}null}$ (green) and *dRnf2* $^{Ring1\text{-}null}$ (blue) mESCs cultured in 2i conditions at 48h of IAA treatment.

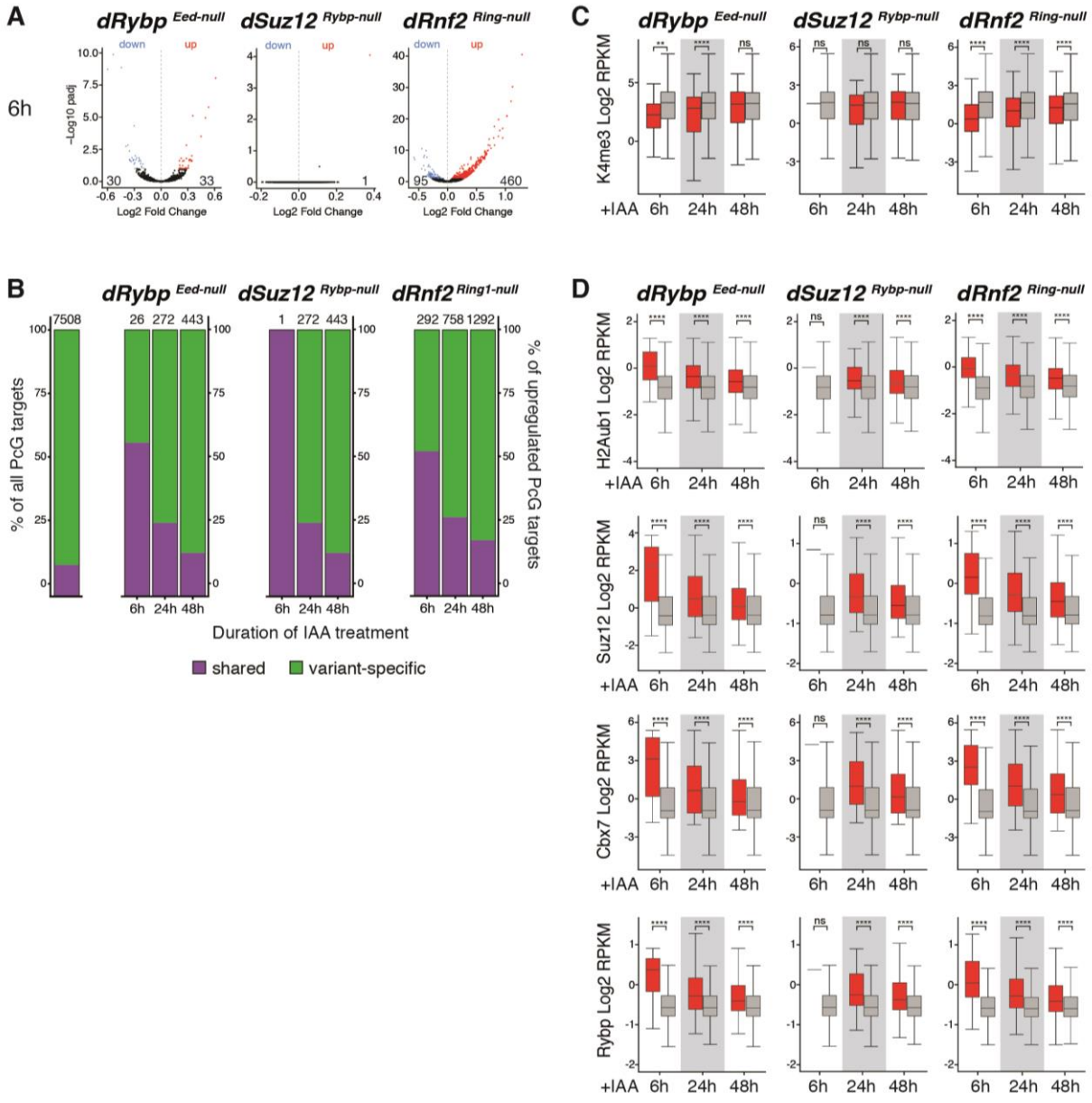

A
dRybp Eed-null
dSuz12 Rybp-null
dRnf2 Ring-null
6h
down
up
−Log10 padj
Log2 Fold Change
30
33
1
95
460
B
dRybp Eed-null
dSuz12 Rybp-null
dRnf2 Ring1-null
% of all PcG targets
% of upregulated PcG targets
7508
26 272 443
1 272 443
292 758 1292
6h 24h 48h
Duration of IAA treatment
shared
variant-specific
C
dRybp Eed-null
dSuz12 Rybp-null
dRnf2 Ring-null
K4me3 Log2 RPKM
+IAA 6h 24h 48h
D
H2Aub1 Log2 RPKM
Suz12 Log2 RPKM
Cbx7 Log2 RPKM
Rybp Log2 RPKM
ns

Fig. S8. Genes with large Polycomb domains are most vulnerable to PRC1 loss. (A) Volcano plots show gene expression changes of IAA-treated relative to untreated *dRybp* $^{Eed\text{-}null}$, *dSuz12* $^{Rybp\text{-}null}$ and *dRnf2* $^{Ring1\text{-}null}$ mESCs at 6h. down (blue) – number of repressed genes, up (red) – number of upregulated genes. Differential gene expression (padj < 0.1) was calculated by averaging two independent clones per genotype, each sequenced in triplicates. **(B)** Bar graphs show the fractions of "shared" and "variant-specific" genes among all Polycomb target genes (left) and the fraction of differentially expressed "shared" and "variant-specific" genes (padj < 0.1) at 6h, 24h and 48h of IAA-treated *dRybp* $^{Eed\text{-}null}$, *dSuz12* $^{Rybp\text{-}null}$ and *dRnf2* $^{Ring1\text{-}null}$ mESCs. **(C)** Box plots display *wild-type* levels of H3K4me3 (+/- 10kb around TSS) of genes upregulated (red) or unchanged (grey) at 6h, 24h and 48h of IAA treatment of *dRybp* $^{Eed\text{-}null}$, *dSuz12* $^{Rybp\text{-}null}$ and *dRnf2* $^{Ring1\text{-}null}$ mESCs. Significance (p-value) calculated using Wilcoxon test (**: $p < 0.01$; ****: $p < 0.001$; ****: $p < 1e\text{-}04$). **(D)** Box plots display *wild-type* levels of H2AK119ub1 (H2Aub1) (+/- 10kb around TSS), Suz12, Cbx7 and Rybp (all +/- 5kb around TSS) of genes upregulated (red) or unchanged (grey) at 6h, 24h and 48h of IAA treatment of *dRybp* $^{Eed\text{-}null}$, *dSuz12* $^{Rybp\text{-}null}$ and *dRnf2* $^{Ring1\text{-}null}$ mESCs. Significance (p-value) calculated using Wilcoxon test (**: $p < 0.01$; ****: $p < 0.001$; ****: $p < 1e\text{-}04$).

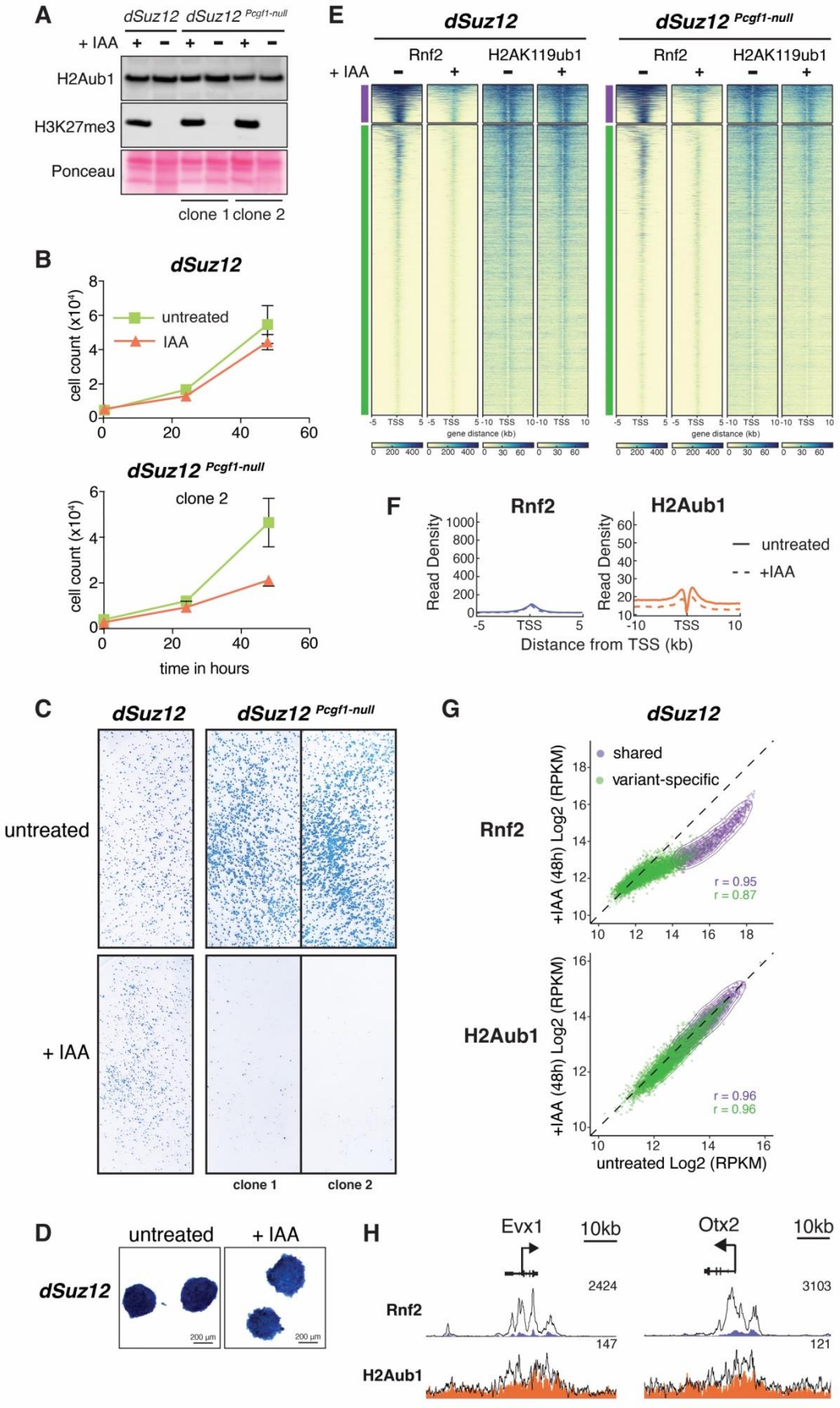

A
dSuz12
dSuz12 Pcgf1-null
+ IAA
H2Aub1
H3K27me3
Ponceau
clone 1
clone 2
B
dSuz12
untreated
IAA
cell count (x10⁴)
dSuz12 Pcgf1-null
clone 2
time in hours
C
dSuz12
dSuz12 Pcgf1-null
untreated
+ IAA
clone 1
clone 2
D
untreated
+ IAA
dSuz12
200 µm
E
dSuz12
dSuz12 Pcgf1-null
Rnf2
H2AK119ub1
+ IAA
gene distance (kb)
F
Rnf2
H2Aub1
Read Density
untreated
+IAA
Distance from TSS (kb)
G
dSuz12
shared
variant-specific
Rnf2
H2Aub1
+IAA (48h) Log2 (RPKM)
untreated Log2 (RPKM)
r = 0.95
r = 0.87
r = 0.96
r = 0.96
H
Evx1
Otx2
10kb
Rnf2
H2Aub1
2424
147
3103
121

Fig. S9. Consequences of combined depletion of Pcgf1 and Suz12. (A) Proliferation assays of *dSuz12* mESCs grown in serum conditions for 72 hours without (untreated) or with 250µM IAA. Live cells were quantified by flow cytometry every 24 hours. Displayed is the median and standard deviation of four replicate measurements starting at 24 hours IAA (time point 0h). **(B)** Proliferation assays of *dSuz12* mESCs (top) and a second mESC clone of *dSuz12* $^{Pcgf1\text{-}null}$ (bottom) grown in serum conditions for 72h without (untreated) or with 250µM IAA. Live cells were quantified by flow cytometry every 24h. Displayed is the median and standard deviation of four replicate measurements starting at 24h IAA (time point 0h). **(C)** Slides show AP staining of *dSuz12* mESC colonies and two independent clones of *dSuz12* $^{Pcgf1\text{-}null}$ mESC colonies cultured in absence (untreated) or presence or 250µM IAA for five days. **(D)** Representative images of alkaline phosphatase staining of *dSuz12* mESC colonies cultured in absence (untreated) or presence or 250µM IAA for five days. **(E)** Heat maps of ChIP-seq of Rnf2 and H2AK119ub1 around CGI TSSs (+/- 5kb or +/- 10kb) at "shared" (violet) and "variant-specific" (green) genes in *dSuz12* and *dSuz12* $^{Pcgf1\text{-}null}$ mESCs before and after 48 hours of IAA treatment. Colored scales (bottom) show dynamic range of ChIP-seq. **(F)** Meta plots of Rnf2 and H2AK119ub1 (H2Aub1) ChIP-seq at "variant-specific" genes in *dSuz12* $^{Pcgf1\text{-}null}$ mESCs before and after 48 hours of IAA treatment corresponding to Fig. 6E). **(G)** Density scatter plot of Rnf2 (top, +/- 5kb around TSS) and in H2AK119ub1 (H2Aub1) (bottom, +/- 10kb around TSS) signals in untreated and IAA-treated *dSuz12* mESCs. r - Pearson's correlation coefficient, "shared" TSSs (violet), "variant-specific" TSSs (green). **(G)** Genomic screenshots of Rnf2 (violet) and H2AK119ub1 (H2Aub1 – orange) enrichments at two representative "shared" Polycomb target genes in untreated and IAA-treated *dSuz12* $^{Pcgf1\text{-}null}$ mESCs. Superimposed are levels in wild-type mESCs (black line).

Table. S1. Antibodies used for western blot

Name	Cat #	Vendor	Host	Dilution
Rnf2	5694S	Cell Signaling	rabbit	1:1000
Cbx7	07-981	Merck Millipore	rabbit	1:1000
Rybp	41787S	Cell Signaling	rabbit	1:1000
Eed	61203	Active Motif	mouse	1:500
LaminB1	ab16048	Abcam	rabbit	1:5000
Ring1	2820S	Cell Signaling	rabbit	1:1000
Suz12	3737S	Cell Signaling	rabbit	1:1000
Pcgf1	NBP1-82767	Novus Biologicals	rabbit	1:750
Goat anti-Rabbit IgG HRP	656120	Invitrogen	goat	1:10000
anti-Mouse IgG HRP	W4021	Promega	goat	1:2500

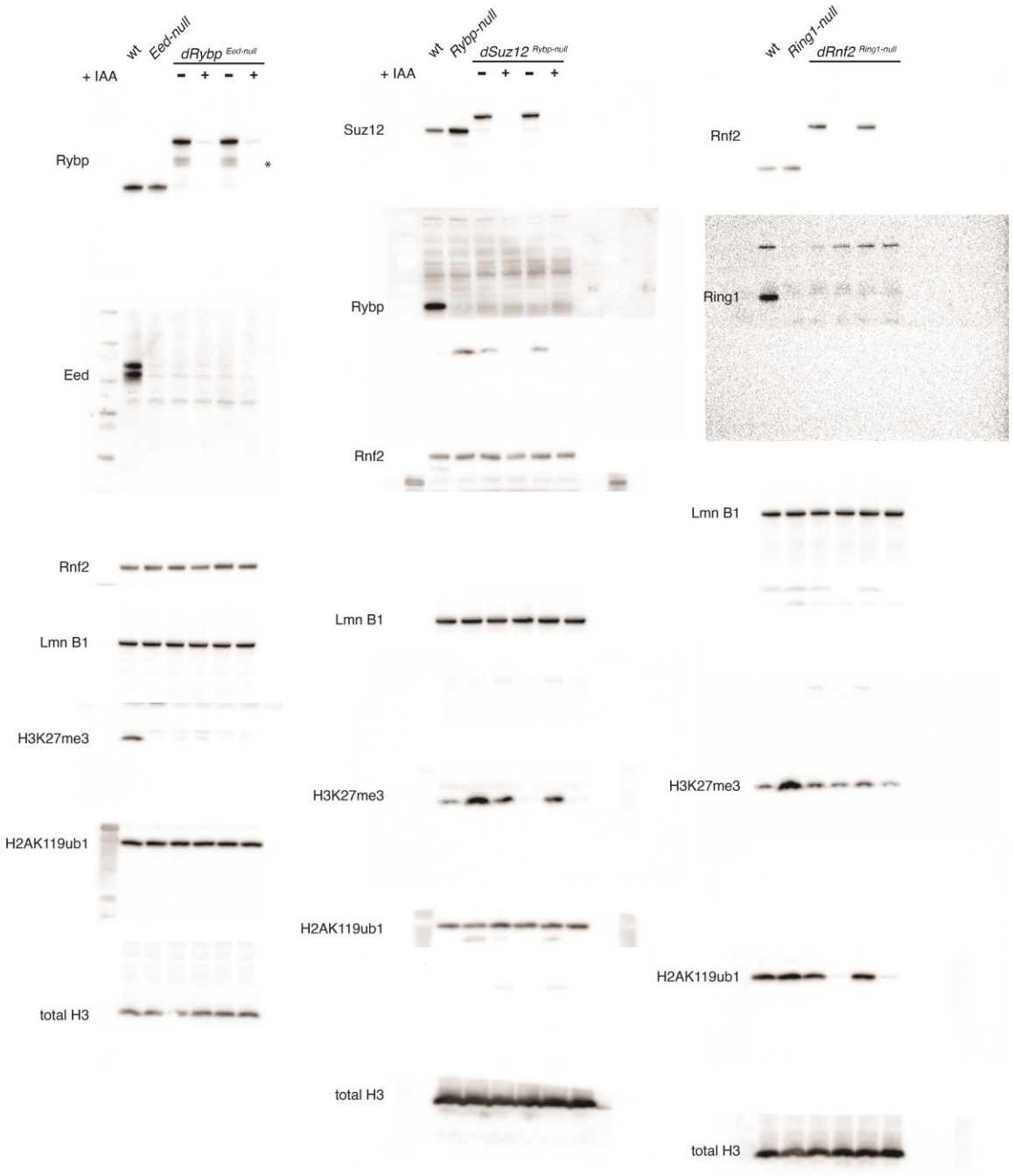
Table. S2. DNA constructs and sgRNAs

Construct	sgRNA sequence (5' - 3')
CP045_Rnf2_homology_arms_GFP_AID	
CP047_Rybp_homology_arms_AID_GFP	
CP048_Suz12_homology_arms_AID_GFP	
CP049_sgRNA_Rnf2	GCACAGCCTGAGACATTTCT
CP051_sgRNA_Rybp	GAATCTTTCTGAGATTGCACA
CP052_sgRNA_Suz12	GCAGTGTCTGTTCAAAACATG
CP053_RRL-SFFV-OsTir1-3xMyc-T2A-Puro	
jz031_Suz12-null	CCAATAAGACAAGTCCCTAC
jz030_Suz12-null	CTGTTTAGAGTAACTCGTCC
jz037_Ring1-null	TCTTTTGTTGTTGCAGGCCC
jz036_Ring1-null	ATCGTCACCGCCCTGCGGAG
SG005_sgRNA_Pcgf1	TGAGGTCCGCCATCTCCGAA
SG006_sgRNA_Pcgf1	CCTTCTCTGTTCCTAGAACA
HFM027-ZFHD1-FLAG-Pcgf3-P2A-mCherry	

Table. S3. mESC lines

ID	Genotype
AN3-12	wild-type (parental haploid mESC line)
515D10-E6	*Eed-null* mESCs (*Eed* gene-trap)
515D10-A12	*dRybp* $^{Eed\text{-}null}$ clone 1
515D10-C3	*dRybp* $^{Eed\text{-}null}$ clone 2
515D10-Suz12D8-RybpB4	*dRybp* $^{Suz12\text{-}null,\ Eed\text{-}null}$
515D10-Suz12D8-RybpB4-EedrescA4	*dRybp* $^{Suz12\text{-}null}$
AN3E9-A1	*Rybp-null* mESCs (*Rybp* CRISPR KO)
AN3E9-A8	*dSuz12* $^{Rybp\text{-}null}$ clone 1
AN3E9-B11	*dSuz12* $^{Rybp\text{-}null}$ clone 2
AN3-Suz12D8	*dSuz12* clone 1
AN3-Suz12G7	*dSuz12* clone 2
AN3-Suz12D8-P1A2	*dSuz12* $^{Pcgf1\text{-}null}$ clone 1
AN3-Suz12D8-P1A3	*dSuz12* $^{Pcgf1\text{-}null}$ clone 2
D10B3C10-G9	*Ring1-null* mESCs (*Ring1* CRISPR KO)
D10B3C10-B10	*dRnf2* $^{Ring1\text{-}null}$ clone 1
D10B3C10-C2	*dRnf2* $^{Ring1\text{-}null}$ clone 2

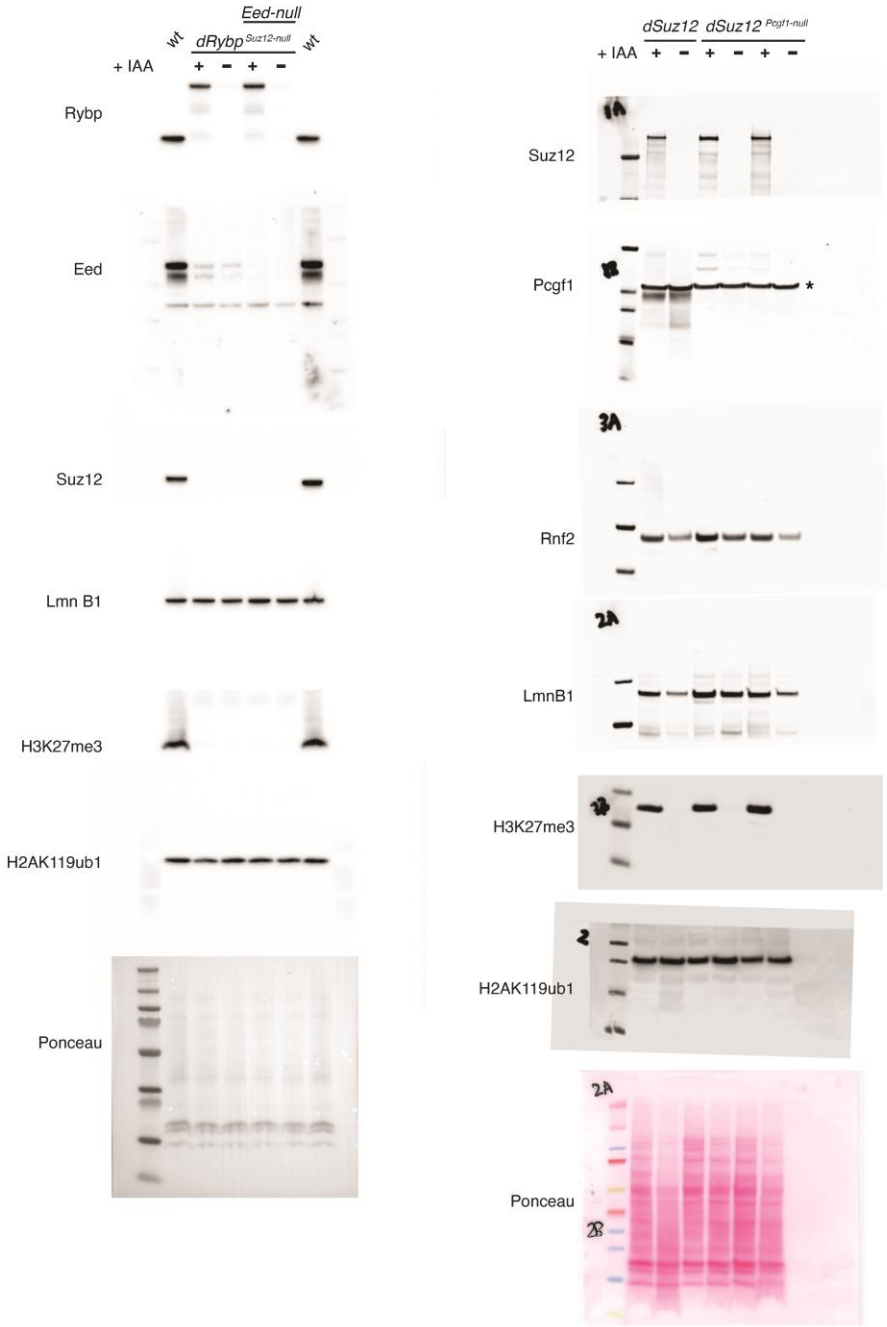
Fig. 2A and Fig. S3B



Uncropped western blots of proteins from Fig. 2A and fig. S3B. Western blots of PcG protein expression and histone modifications in wild-type, Eed-null mESCs and two independent clones

of dRybp Eed-null mESCs (left), in wild-type, Rybp-null mESCs and two independent clones of dSuz12 Rybp-null mESCs (center) and in wild-type, Ring1-null mESCs and two independent clones of dRnf2 Ring1-null mESCs (right). IAA treatment (250 μM) for 24 hours. Lmn B1 and total histone H3 serve as loading controls.

Fig. S5B, Fig. Fig. 6A and Fig. S9A



Uncropped western blots of proteins from figs. S5B, S6A, and S9A. Western blots of PcG protein expression and histone modifications in wild-type, dRybp Suz12-null mESCs and dRybp

Suz12-null, Eed-null mESCs (left) and in dSuz12 mESCs and two independent clones of dSuz12 Pcgf1-null mESCs (right). IAA treatment (250 μM) for 24 hours. Lmn B1 and Ponceau membrane staining serve as loading controls.