

## **Supplementary Information for**

### **Functional dissection of PRC1 subunits RYBP and YAF2 during neural differentiation of embryonic stem cells**

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† Contributed equally to this work.

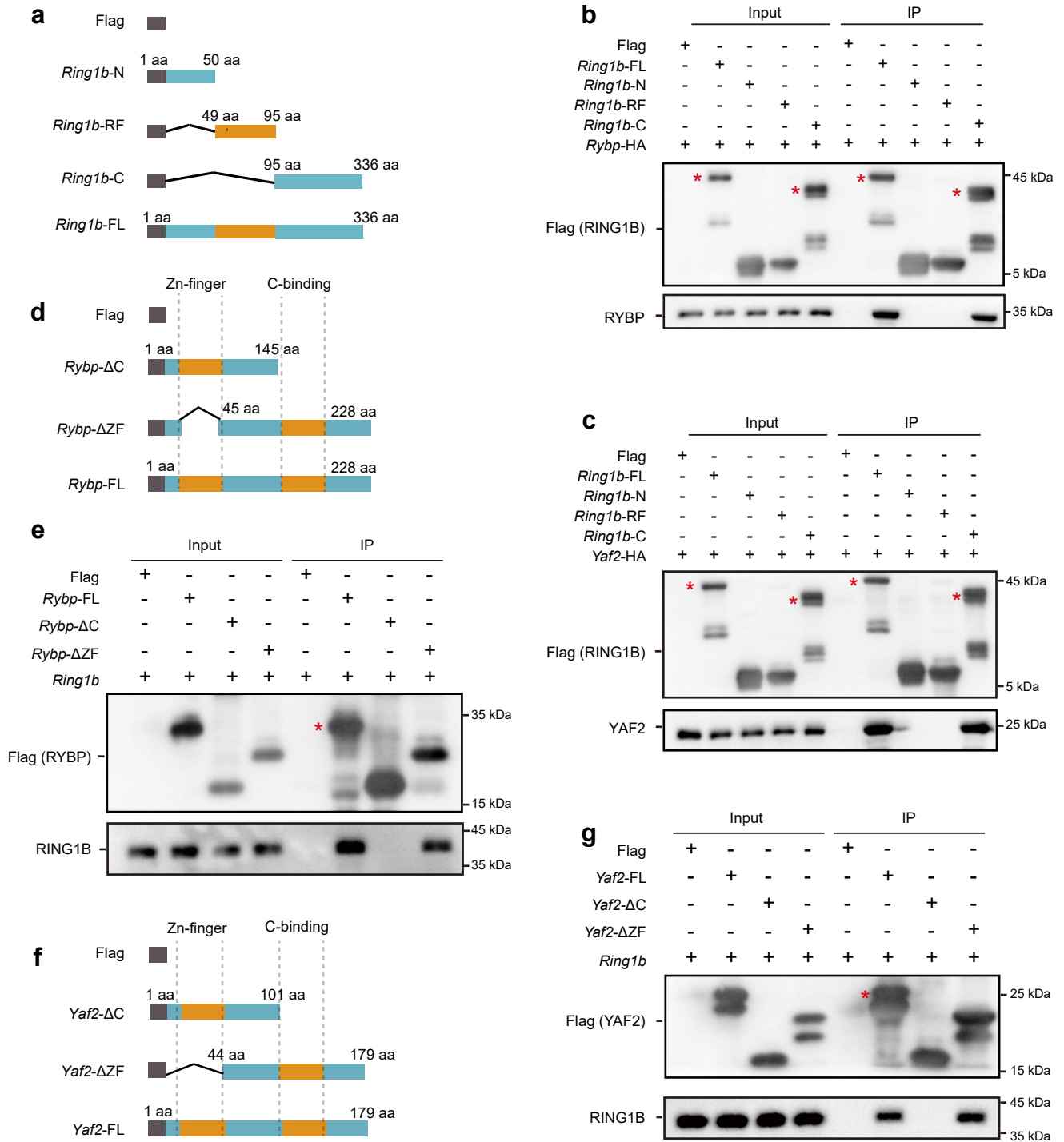
\* Corresponding author.

#### **This PDF file includes:**

Supplementary Figures 1 to 5;

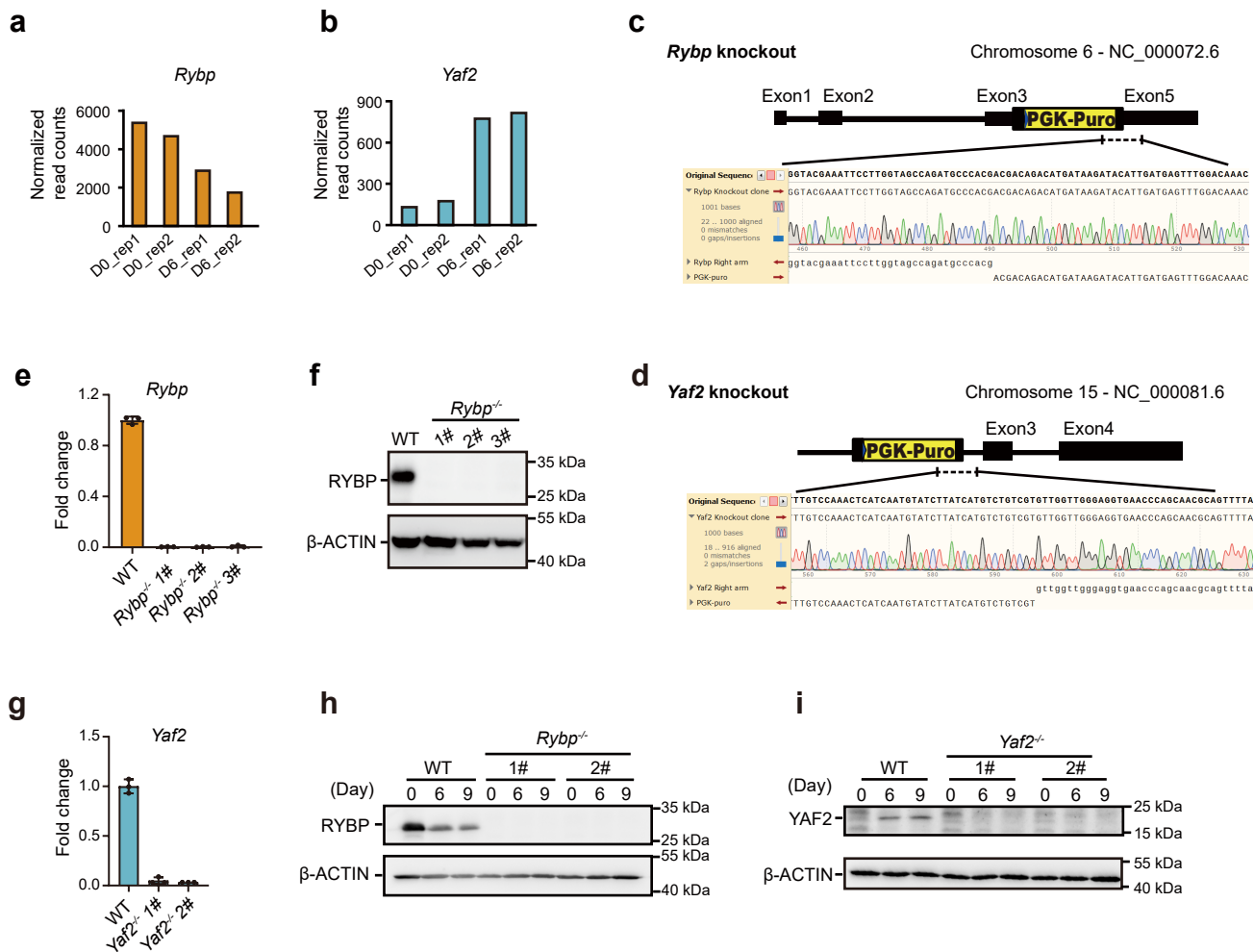
Supplementary Tables 1 to 8.

# Supplementary Figure 1



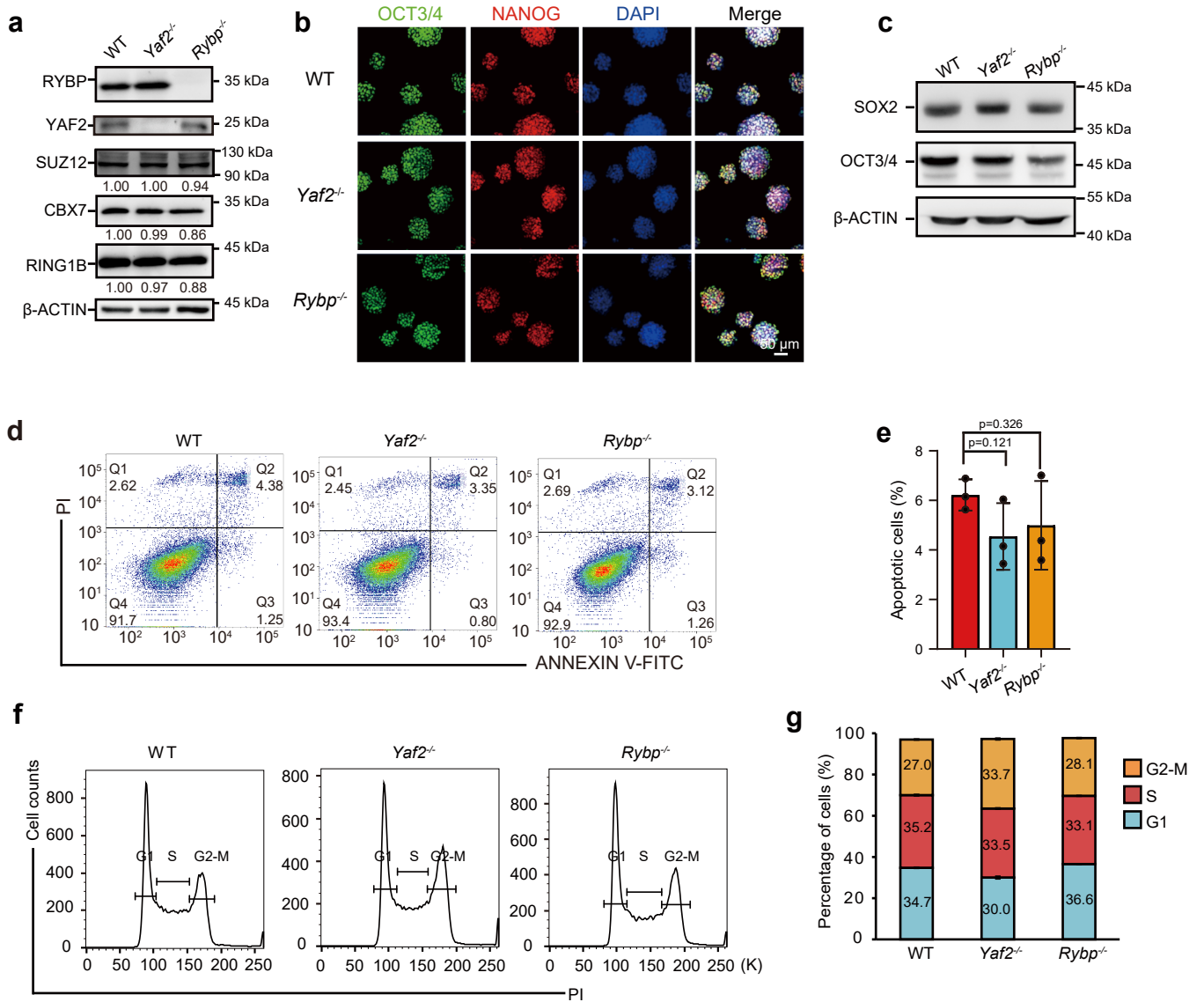
**Supplementary Figure 1. RYBP and YAF2 interact with RING1B at the C-terminus, respectively.** **a** Schematic representation of Flag-*Ring1b* and its deletions used in Flag co-IP assays. N: N-terminus; RF: ring finger region; C: C-terminus; FL: full length. **b** Flag immunoprecipitations to detect the interaction between RYBP and Flag-RING1B or Flag-tagged RING1B deletions. Asterisks indicate target proteins. **c** Flag immunoprecipitations to detect the interaction between YAF2 and Flag-RING1B or Flag-tagged RING1B deletions. Asterisks indicate target proteins. **d** Schematic representation of Flag-*Rybp* and its deletions used in Flag co-IP assays. ZF: zinc finger. **e** Detection of the interaction between RING1B and full-length RYBP or its deletions. Asterisks indicate target proteins. **f** Schematic representation of Flag-*Yaf2* and its deletions used in Flag co-IP assays. **g** Detection of the interaction between RING1B and full-length YAF2 or its deletions. Asterisks indicate target proteins. For **b**, **c**, **e** and **g**, representative immunoblots (n = 3 independent experiments) are shown. Source data are provided as a Source Data file.

# Supplementary Figure 2



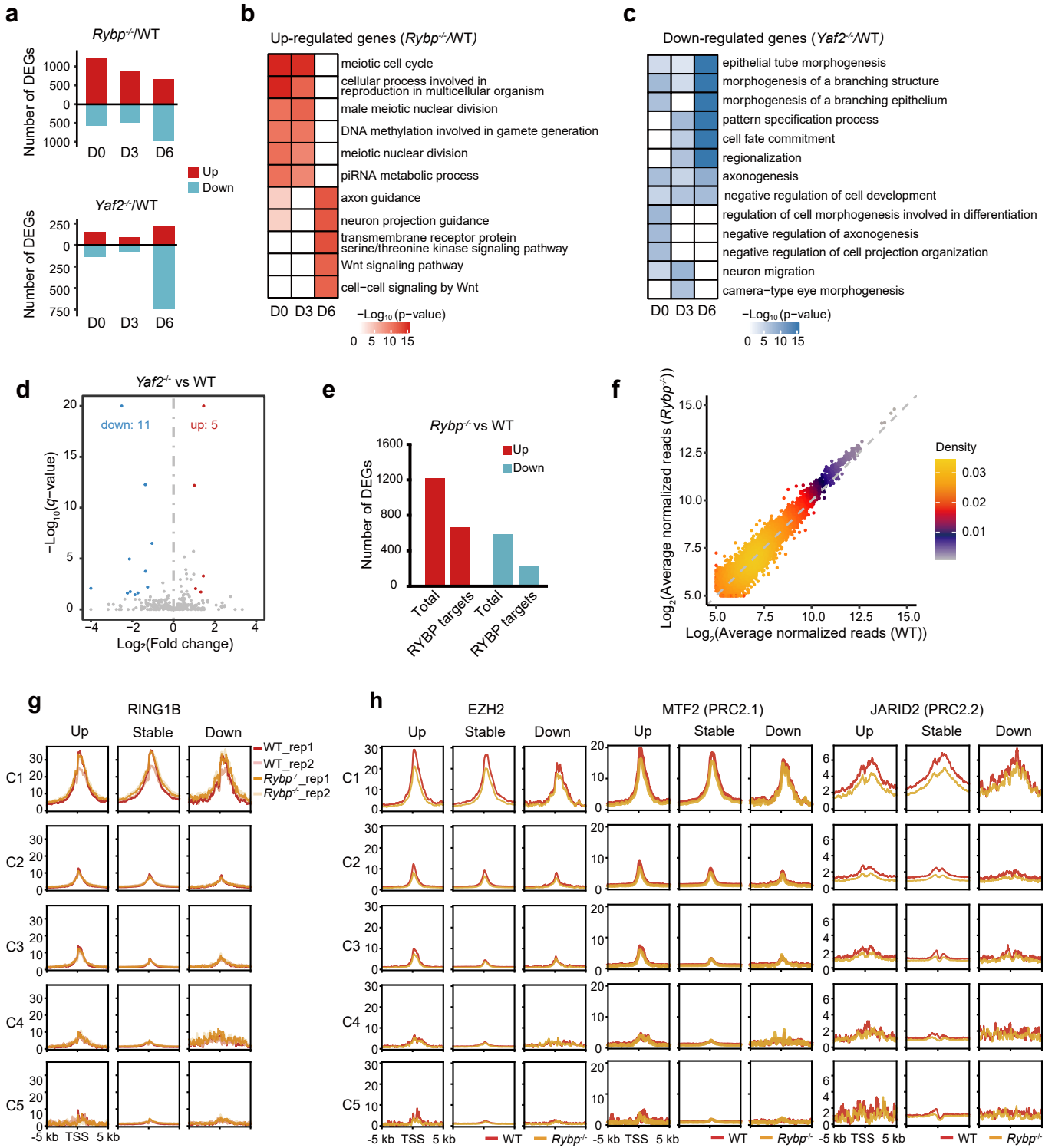
**Supplementary Figure 2. Expression of *Rybp* and *Yaf2* during mESC neural differentiation and generation of *Rybp*<sup>-/-</sup> or *Yaf2*<sup>-/-</sup> mESCs.** **a, b** Bar plots showing normalized read counts for *Rybp* (**a**) and *Yaf2* (**b**) expression during mESC neural differentiation from our previous RNA-seq data<sup>4</sup>. **c, d** Upper: Schematic overview depicting the targeting strategy for *Rybp* and *Yaf2* loci using CRISPR/Cas9. PGK: human phosphoglycerol kinase promoter; puro: puromycin resistance gene. Bottom: The insert for either *Rybp* knockout or *Yaf2* knockout was confirmed by Sanger sequencing. **e** RT-qPCR analysis of *Rybp* expression in both wild-type and *Rybp*<sup>-/-</sup> mESCs. The results are shown relative to wild-type (n=3 independent experiments). **f** Western blot analysis of RYBP expression in both wild-type and *Rybp*<sup>-/-</sup> mESCs. **g** RT-qPCR analysis of *Yaf2* expression in both wild-type and *Yaf2*<sup>-/-</sup> mESCs. The results are shown relative to wild-type (n=3 independent experiments). **h** Western blot analysis of RYBP expression in both wild-type and *Rybp*<sup>-/-</sup> mESCs and neural-differentiated cells. **i** Western blot analysis of YAF2 expression in both wild-type and *Yaf2*<sup>-/-</sup> mESCs and neural-differentiated cells. For **e** and **g**, data are represented as the mean values  $\pm$  s.d.s. Source data are provided as a Source Data file.

# Supplementary Figure 3



**Supplementary Figure 3. Effects of knocking out either *Rybp* or *Yaf2* on apoptosis and cell cycle.** **a** Western blot analysis of RYBP, YAF2, SUZ12, CBX7 and RING1B expression in wild-type, *Rybp*<sup>-/-</sup> and *Yaf2*<sup>-/-</sup> mESCs. **b** Immunofluorescence of OCT3/4 (green) and NANOG (red) in wild-type, *Rybp*<sup>-/-</sup> and *Yaf2*<sup>-/-</sup> mESCs. DNA was stained with DAPI (blue). Scale bar, 50 μm. **c** Western blot analysis of OCT3/4 and SOX2 expression in wild-type, *Rybp*<sup>-/-</sup> and *Yaf2*<sup>-/-</sup> mESCs. **d** FACS analysis of the percentage of apoptotic cells in wild-type, *Rybp*<sup>-/-</sup> and *Yaf2*<sup>-/-</sup> mESCs. Annexin V and PI were used to discriminate necrotic cells (Q1), late apoptotic cells (Q2), early apoptotic cells (Q3) and intact cells (Q4). **e** Statistical analysis of the total apoptotic cells, including early apoptotic cells and late apoptotic cells, in panel **(d)** (n=3 independent experiments). **f** FACS analysis of the cell cycle in wild-type, *Rybp*<sup>-/-</sup> and *Yaf2*<sup>-/-</sup> mESCs. **g** Statistical analysis of the cell cycle distribution in panel **(f)** (n=3 independent experiments). For **a-c**, representative immunoblots (n = 3 independent experiments) are shown. For **e**, data are represented as the mean values ± s.d.s with the indicated significance from two-sided *t* test. Source data are provided as a Source Data file.

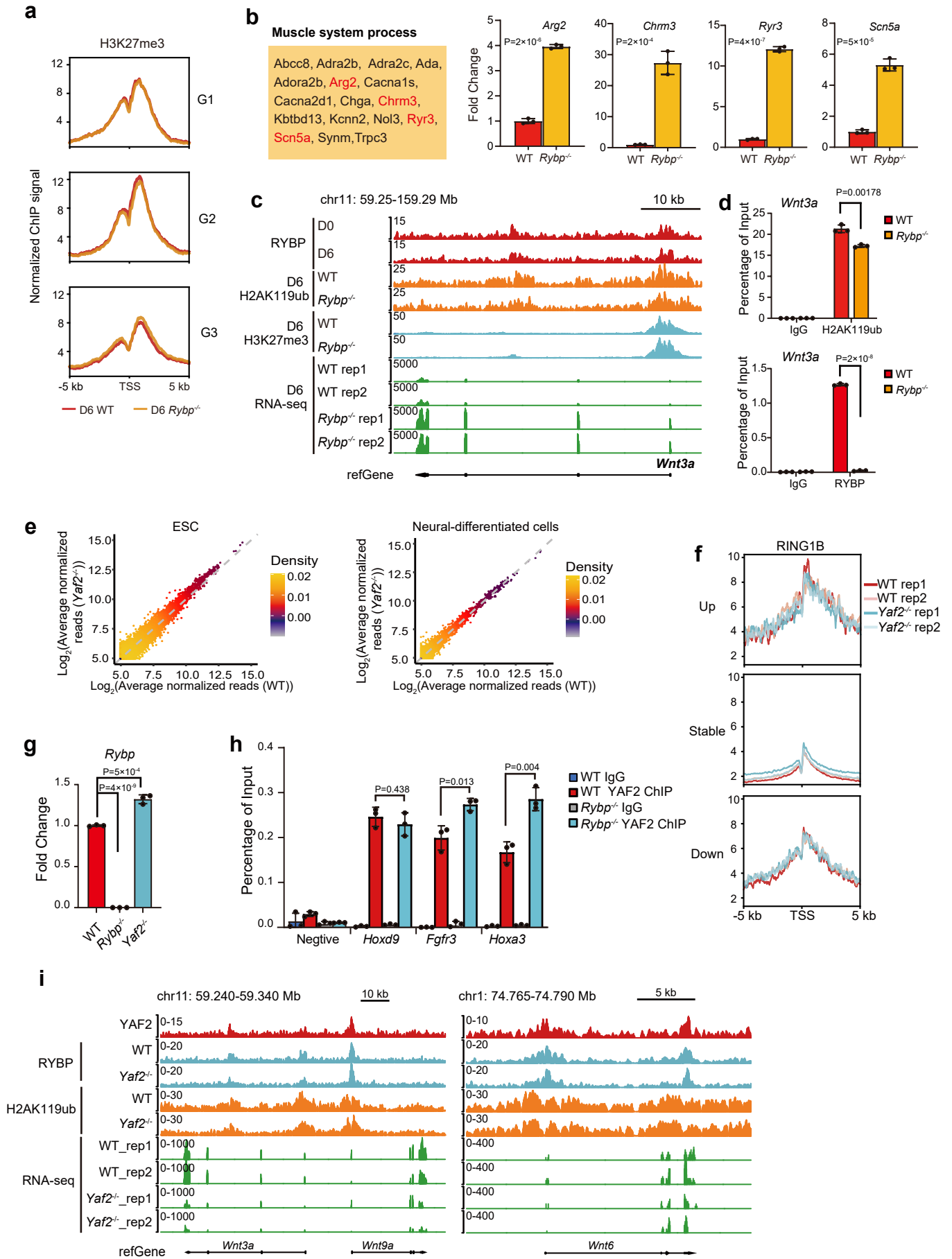
# Supplementary Figure 4





**Supplementary Figure 4. Effect of loss of either *Rybp* or *Yaf2* on gene expression and PRC2 binding.** **a** Bar plots showing the number of differentially expressed genes between *Rybp*<sup>-/-</sup> mESCs and wild-type mESCs (upper) or between *Yaf2*<sup>-/-</sup> mESCs and wild-type mESCs (bottom) during neural differentiation. **b** GO analysis of the biological functions of upregulated target genes in *Rybp*<sup>-/-</sup> mESCs relative to wild-type mESCs during neural differentiation. The results are expressed as -Log<sub>10</sub>(p value). **c** GO analysis of the biological functions of downregulated target genes in *Yaf2*<sup>-/-</sup> mESCs relative to wild-type mESCs during neural differentiation. The results are expressed as -Log<sub>10</sub>(p value). **d** Scatter plot showing changes in YAF2 target genes between wild-type and *Yaf2*<sup>-/-</sup> mESCs. **e** Bar plots showing the number of DEGs among RYBP target genes and total DEGs after *Rybp* knockout in mESCs. **f** Scatter plot showing the comparison of normalized RING1B ChIP signals in wild-type and *Rybp*<sup>-/-</sup> mESCs. **g** Average enrichment of RING1B around TSS regions for different RYBP target genes between wild-type and *Rybp*<sup>-/-</sup> mESCs from Fig. 2d. **h** Average enrichment of EZH2, MTF2 and JARID2 around TSS regions for different RYBP target genes between wild-type (red line) and *Rybp*<sup>-/-</sup> (orange line) mESCs from Fig. 2d. Source data are provided as a Source Data file.

# Supplementary Figure 5



**Supplementary Figure 5. Analysis of the derepressed RYBP target genes after loss of either *Rybp* or *Yaf2*.** **a** Average enrichment of H3K27me3 in the promoters of different classes of genes between wild-type and *Rybp*<sup>-/-</sup> neural-differentiated cells on day 6 from Fig. 3c. **b** RT-qPCR analysis of the expression of muscle-related genes in wild-type and *Rybp*<sup>-/-</sup> neural differentiated cells on day 3 (n=3 independent experiments). **c** Representative genomic tracks showing the normalized signal for RYBP, H2AK119ub, H3K27me3 and RNA-seq data at the *Wnt3a* gene locus in neural-differentiated cells on day 6. **d** CHIP-qPCR showing relative enrichment of H2AK119ub and RYBP in the promoter region of *Wnt3a* in both wild-type and *Rybp*<sup>-/-</sup> neural-differentiated cells on day 6. IgG was used as the negative control. (n=3 independent experiments) **e** Scatter plots showing the comparison of normalized RING1B ChIP signals between wild-type and *Yaf2*<sup>-/-</sup> mESCs (left) and between wild-type and *Yaf2*<sup>-/-</sup> neural differentiated cells on day 6 (right). **f** Average normalized RING1B ChIP signal in the promoters of upregulated, stable and downregulated YAF2 target genes. **g** RT-qPCR analysis of the expression of *Rybp* in wild-type, *Rybp*<sup>-/-</sup> and *Yaf2*<sup>-/-</sup> neural differentiated cells on day 6 (n=3 independent experiments). **h** CHIP-qPCR showing the relative enrichment of YAF2 in the promoter regions of *Hoxd9*, *Fgfr3* and *Hoxa3* in both wild-type and *Rybp*<sup>-/-</sup> mESCs (n=3 independent experiments). **i** Genomic tracks showing YAF2, RYBP, H2AK119ub and RNA-seq at the *Wnt3a*, *Wnt9a* and *Wnt6* gene loci in wild-type and *Yaf2*<sup>-/-</sup> neural differentiated cells on day 6. For **b**, **d**, **g** and **h**, data are represented as the mean values ± s.d.s with the indicated significance from two-sided *t* test. Source data are provided as a Source Data file.

**Supplementary Table 1. Oligonucleotide information for PCR in this study.**

Name	Sequence (5'-3')
pSin- <i>Ring1b</i> (FL-HA)-EcoR I -F	acaaggatgacgacgataagATGTCTCAGGCTGTGCAGAC
pSin- <i>Ring1b</i> (FL-HA)-EcoR I -R	cgggccgctttctcgaggccTCAAGCGTAATCTGGAACATCGTATGGGTATTTGTGCTCCTTGGTGG
pSin- <i>Ring1b</i> (FL no Flag)-F	gtcgtgaggaattggccaccATGTCTCAGGCTGTGCAGAC
pSin- <i>Ring1b</i> (FL no Flag)-R	ggggccgctttctcgaggccTCATTTGTGCTCCTTGGTGG
pSin-Flag- <i>Ring1b</i> (N)-F	cgacgataaggaattcATGTCTCAGGCTGTGC
pSin-Flag- <i>Ring1b</i> (N)-R	tatggctagcgaattcTCACATTAATCACTGTGTAGACTTC
pSin-Flag- <i>Ring1b</i> (RF)-F	cgacgataaggaattcTTAATGTGCCCAATTT
pSin-Flag- <i>Ring1b</i> (RF)-R	tatggctagcgaattcTCAAACCAGTTTTTCCGACAGG
pSin-Flag- <i>Ring1b</i> (C)-F	cgacgataaggaattcGTTTCTAAAAGATCACTAAGGCC
pSin-Flag- <i>Ring1b</i> (C)-R	tatggctagcgaattcTCATTTGTGCTCCTTGGTGGG
pSin- <i>Rybp</i> (FL)-EcoR I -F	atgacgacgataaggaattcATGACCATGGGCGACAAGAA
pSin- <i>Rybp</i> (FL)-EcoR I -R	ctcatatggctagcgaattcTCAGAAAGATTTCATTCA
pSin- <i>Rybp</i> ( $\Delta$ ZF)-EcoR I -F	atgacgacgataaggaattcGATGTGCGGAAAGGCACCTC
pSin- <i>Rybp</i> ( $\Delta$ C)-EcoR I -R	ctcatatggctagcgaattcCACCTTGAGGTGTGGTTTGT
pSin- <i>Rybp</i> (FL-HA)-EcoR I -F	gtcgtgaggaattggccaccATGACCATGGGCGACAAGAA
pSin- <i>Rybp</i> (FL-HA)-EcoR I -R	ggggccgctttctcgaggccTCAAGCGTAATCTGGAACATCGTATGGGTAgaaagattcatcattcactgctg
pSin- <i>Yaf2</i> (FL)-EcoR I -F	atgacgacgataaggaattcATGGGCGACAAGAAGAGCCC
pSin- <i>Yaf2</i> (FL)-EcoR I -R	ctcatatggctagcgaattcTTAGTGAGACTCTCCGTTGAGGG
pSin- <i>Yaf2</i> ( $\Delta$ ZF)-EcoR I -F	atgacgacgataaggaattcGTGCGGAAGGGCACCTCCAC
pSin- <i>Yaf2</i> ( $\Delta$ C)-EcoR I -R	ctcatatggctagcgaattcTTAGGTTTTCTGTGACAGTTCT
pSin- <i>Yaf2</i> (FL-HA)-EcoR I -F	gtcgtgaggaattggccaccATGGGCGACAAGAAGAGCCC
pSin- <i>Yaf2</i> (FL-HA)-EcoR I -R	ggggccgctttctcgaggccTCAAGCGTAATCTGGAACATCGTATGGGTAgtgagactctccgttgagg
pET28a- <i>Yaf2</i> -mCherry-F	tgggtcgggatccgaattcATGGGCGACAAGAAGAGCCC
pET28a- <i>Yaf2</i> -mCherry-R	cggatctcattgtttaaacgaTTACTTGTACAGCTCGTCCATGCCG
pGEX4T-GST- <i>Ring1b</i> -F	atctggtccgctggatccATGTCTCAGGCTGTGCAGAC
pGEX4T-GST- <i>Ring1b</i> -R	ggcagatcgtcagtcagtcTCATTTGTGCTCCTTGGTGGGTG

**Supplementary Table 2. sgRNA sequences used in this study.**

<b>Name</b>	<b>sequence (5'-3')</b>
pX330- <i>Yaf2</i> -deletion-F	caccgTATCGCTTGTAGGATCGTAA
pX330- <i>Yaf2</i> -deletion-R	aaacTTACGATCCTACAAGCGATAc
pX330- <i>Rybp</i> -deletion-F	caccgTGTATGCTGTTAGCTTCACT
pX330- <i>Rybp</i> -deletion-R	aaacAGTGAAGCTAACAGCATACAc

**supplementary Table 3. Antibody information and catalog number in this study.**

REAGENT or RESOURCE	SOURCE	IDENTIFIER	Dilution
Rabbit polyclonal anti-RING1B	Abcam	Cat# ab101273, RRID:AB_10711495	1:1000 for WB
Rabbit polyclonal anti-RING1B	CST	Cat#5694, RRID:AB_10705604	1:100 for ChIP
Rabbit polyclonal anti-RYBP	Millipore	Cat# AB3637, RRID:AB_11213333	1:1000 for WB, 1:100 for ChIP
Rabbit polyclonal anti-YAF2 (for WB)	Bethyl Laboratories	Cat# A303-654A, RRID:AB_11204594	1:1000 for WB
Rabbit polyclonal anti-YAF2 (for ChIP)	Our lab	NA	1:100 for ChIP
Mouse monoclonal anti-FLAG	Sigma-Aldrich	Cat# F1804, RRID:AB_262044	1:1000 for WB
Rabbit polyclonal anti-HA tag	Abcam	Cat# ab9110, RRID:AB_307019	1:1000 for WB
Mouse monoclonal anti- $\beta$ -ACTIN	Sigma-Aldrich	Cat# A2228, RRID:AB_476697	1:4000 for WB
Rabbit polyclonal anti-TUBB3	Abcam	Cat# ab18207, RRID:AB_444319	1:1000 for WB
Rabbit polyclonal anti-PAX6	Biolegend	Cat# 901301, RRID:AB_2565003	1:1000 for WB, 1:100 for flow cytometry stain
Mouse monoclonal anti-NESTIN	Cell Signaling Technology	Cat# 33475, RRID:AB_2799037	1:1000 for WB, 1:100 for flow cytometry stain
Mouse monoclonal anti- $\beta$ -Tubulin	RayBiotech	Cat# RM2003, RRID:AB_2773015	1:1000 for WB
Rabbit polyclonal anti-Non-phospho (Active) $\beta$ -Catenin (Ser33/37/Thr41)	Cell Signaling Technology	Cat# 8814, RRID:AB_11127203	1:1000 for WB
Rabbit monoclonal anti-H2AK119ub	Cell Signaling Technology	Cat# 8240, RRID:AB_10891618	1:100 for ChIP
Rabbit monoclonal anti-H3K27me3	Cell Signaling Technology	Cat# 9733, RRID:AB_2616029	1:100 for ChIP
Rabbit polyclonal anti-H3K27ac	Active Motif	Cat# 39133, RRID:AB_2561016	1:100 for ChIP
Mouse monoclonal anti-OCT3/4	Santa Cruz Biotechnology	Cat# sc-5279, RRID:AB_628051	1:1000 for WB, 1:100 for IF
Rabbit polyclonal anti-NANOG	Novus	Cat# NB100-58842, RRID:AB_2150116	1:100 for IF
Rabbit polyclonal anti-SUZ12	Active Motif	Cat# 39357, RRID:AB_2614929	1:1000 for WB
Rabbit polyclonal anti-CBX7	Abcam	Cat# ab21873, RRID:AB_726005	1:1000 for WB
Rabbit monoclonal anti-SOX2	Cell Signaling Technology	Cat# 23064, RRID:AB_2714146	1:1000 for WB
Rabbit monoclonal anti-EZH2	Cell Signaling Technology	Cat# 5246, RRID:AB_10694683	1:100 for ChIP
Rabbit polyclonal anti-MTF2	Proteintech	Cat# 16208-1-AP, RRID:AB_2147370	1:100 for ChIP
Rabbit monoclonal anti-JARID2	Cell Signaling Technology	Cat# 13594, RRID:AB_2798269	1:100 for ChIP
Goat Anti-Mouse IgG (H+L) HRP secondary antibody	KangChen Bio-tech	Cat# KC-MM-035	1:5000 for WB
Goat Anti-Rabbit IgG (H+L) HRP secondary antibody	KangChen Bio-tech	Cat# KC-RB-035	1:5000 for WB
Goat anti-mouse IgG Alexa 594 secondary antibody	Invitrogen	Cat# A11032, RRID:AB_2534091	1:500 for flow cytometry stain
Goat anti-rabbit IgG Alexa 647 secondary antibody	Invitrogen	Cat# A21245, RRID:AB_141775	1:500 for flow cytometry stain

**Supplementary Table 4. Primer sequences used for RT-qPCR in this study.**

<b>Name</b>	<b>Foward Primer (5'-3')</b>	<b>Reverse Primer (5'-3')</b>
<i>Gapdh</i>	AACTTTGGCATTGTGGAAGGGCTCA	TTGGCAGCACCCAGTGGATGCAGGGA
<i>Arg2</i>	TCCTCCACGGGCAAATTCC	GCTGGACCATATTCCACTCCTA
<i>Chrm3</i>	CCTCGCCTTTGTTTCCCAAC	TTGAGGAGAAATTCCCAGAGGT
<i>Ryr3</i>	CTGAGCTGGTCCACTTTGTA	GAGGTCACCTAATCCCCTTCA
<i>Scn5a</i>	ATGGCAAACCTCCTGTTACCTC	CCACGGGCTTGTTTTTCAGC
<i>Pax6</i>	GCAGATGCAAAAGTCCAGGTG	CAGGTTGCGAAGAACTCTGTTT
<i>Tubb3</i>	ACTTGGAACCTGGAACCATGG	GGCCTGAATAGGTGTCCAAAGG
<i>Map2</i>	GCCAGCCTCAGAACAAACAG	AAGGCTTGGGAGGGAAGAAC
<i>Nestin</i>	CCCTGAAGTCGAGGAGCTG	CTGCTGCACCTCTAAGCGA
<i>Sox1</i>	GCACACAGCGTTTTCTCGG	ACATCCGACTCCTCTTCCC
<i>Yaf2</i>	CCCTGGGGAAAAAGTATCGCT	CCTGACCCTTTCGGGACATC
<i>Rybp</i>	CAACGTCACCGTCATTATCACAG	CATCATTCACTGCTGACATGTCG
<i>Wnt3a</i>	AATTTGGAGGAATGGTCTCTCGG	CAGCAGGTCTTCACTTCACAG
<i>Wnt6</i>	GCAAGACTGGGGGTTTCGAG	CCTGACAACCACACTGTAGGAG
<i>Wnt9a</i>	GGCCCAAGCACACTACAAG	AGAAGAGATGGCGTAGAGGAAA

**Supplementary Table 5. Primer sequences used for ChIP-qPCR in this study.**

<b>Name</b>	<b>Sequence (5'-3')</b>
<i>Wnt3a</i> -F	GGTGACCTTAAGTCTGCGCT
<i>Wnt3a</i> -R	TCTCAGGCCCTAAGTCCTCC
<i>Wnt6</i> -F	AGAGAGAGTCAAACGGCTGC
<i>Wnt6</i> -R	TACTGCCAATGCTGAAGGGG
<i>Hoxa3</i> -F	TGCCCTAACAGTTTGGCGTC
<i>Hoxa3</i> -R	GCAGCCTGGTCCTAACGTG
<i>Hoxd9</i> -F	AAAAGGACTGAGTGCTCCGC
<i>Hoxd9</i> -R	GGAACGAGTTGCACGGGAAT
<i>Fgfr3</i> -F	TTTAGCGCCTGCTAGGACTG
<i>Fgfr3</i> -R	GACACCCGACGCAAGAAGTA
Negative ctrl-F	CTTCTCACGGCCCCAATCAT
Negative ctrl-R	CATCCCTGTAGCGACCCATC



**Supplementary Table 6. Mapping statistic and peak calling information for ChIP-seq data.**

IP	Treatment	Cells	Total reads	Mappin g ratio	Unique reads	Unique reads (subsample)	Control reads	Number of peaks
RYBP	Wild-type	mESCs	46610491	97.73	26420512	—	27473567	10191
YAF2	Wild-type	mESCs	47948159	97.92	25667850	—	21668926	504
RYBP	Wild-type	neural differentiated cells on day 6	46890923	97.74	29032555	—	20930723	6991
YAF2	Wild-type	neural differentiated cells on day 6	39512031	97.62	25455192	—	20930723	6733
RYBP	<i>Yaf2</i> <sup>-/-</sup>	neural differentiated cells on day 6	40420126	97.23	25249341	—	22208382	9742
YAF2	<i>Rybp</i> <sup>-/-</sup>	neural differentiated cells on day 6	38118491	97.8	24101282	—	24090025	9020
H2AK119ub	Wild-type	mESCs	49895173	41.44	14179265	14179265	24836635	23152
H2AK119ub	<i>Rybp</i> <sup>-/-</sup>	mESCs	29276653	83.19	16660210	16660210	27473567	6510
H2AK119ub	<i>Yaf2</i> <sup>-/-</sup>	mESCs	51420275	80.33	29682587	14984156	21668926	17164
H2AK119ub	Wild-type	neural differentiated cells on day 6	34442358	88.99	24625869	14985097	20930723	34029
H2AK119ub	<i>Rybp</i> <sup>-/-</sup>	neural differentiated cells on day 6	29067210	86.07	20078291	14984753	24090025	39849
H2AK119ub	<i>Yaf2</i> <sup>-/-</sup>	neural differentiated cells on day 6	33948990	85.42	23531078	14983559	22208382	41678
H3K27me3	Wild-type	mESCs	36791122	92.76	23701592	—	24836635	68776
H3K27me3	<i>Rybp</i> <sup>-/-</sup>	mESCs	32308684	94.08	20847570	—	27473567	70771
H3K27me3	<i>Yaf2</i> <sup>-/-</sup>	mESCs	30648343	93.45	19804866	—	21668926	65625
H3K27me3	Wild-type	neural differentiated cells on day 6	29054469	84.57	17516424	—	20930723	17106
H3K27me3	<i>Rybp</i> <sup>-/-</sup>	neural differentiated cells on day 6	40306905	87.4	24413112	—	24090025	18139
H3K27me3	<i>Yaf2</i> <sup>-/-</sup>	neural differentiated cells on day 6	29746850	85.35	17781997	—	22208382	16140
EZH2	Wild-type	mESCs	37154383	90.31	17685477	—	24836635	10403
EZH2	<i>Rybp</i> <sup>-/-</sup>	mESCs	51726087	91.12	22917974	—	27473567	6261
EZH2	<i>Yaf2</i> <sup>-/-</sup>	mESCs	44744908	90.45	21079457	—	21668926	8293
MTF2	Wild-type	mESCs	35083775	90.07	17421943	—	24836635	5258
MTF2	<i>Rybp</i> <sup>-/-</sup>	mESCs	55362595	89.79	24695369	—	27473567	6386
MTF2	<i>Yaf2</i> <sup>-/-</sup>	mESCs	53795477	89.69	24616676	—	21668926	6264
JARID2	Wild-type	mESCs	30333656	80.08	13623305	—	24836635	2833
JARID2	<i>Rybp</i> <sup>-/-</sup>	mESCs	35353350	65.33	11911870	—	27473567	2615
JARID2	<i>Yaf2</i> <sup>-/-</sup>	mESCs	36593547	89.36	20491309	—	21668926	4995
H3K27ac	Wild-type	mESCs	40832081	94.3	21452788	—	24836635	33998
H3K27ac	<i>Rybp</i> <sup>-/-</sup>	mESCs	42250404	93.78	21132180	—	27473567	31015
RING1B_rep1	Wild-type	mESCs	38203769	86.2	17190604	10000000	24836635	12282
RING1B_rep2	Wild-type	mESCs	31620588	81.3	12939006	10000000	24836635	10104
RING1B_rep1	<i>Rybp</i> <sup>-/-</sup>	mESCs	47161455	85.9	20465203	10000000	27473567	12349
RING1B_rep2	<i>Rybp</i> <sup>-/-</sup>	mESCs	27849080	80.7	11402658	10000000	27473567	7900
RING1B_rep1	<i>Yaf2</i> <sup>-/-</sup>	mESCs	38946166	87	18129307	10000000	21668926	11181
RING1B_rep2	<i>Yaf2</i> <sup>-/-</sup>	mESCs	29074955	80.3	11395642	10000000	21668926	6735
RING1B_rep1	Wild-type	neural differentiated cells on day 6	42970425	86.6	19518458	10000000	20930723	6453
RING1B_rep2	Wild-type	neural differentiated cells on day 6	27827564	81.2	11452011	10000000	20930723	3945
RING1B_rep1	<i>Rybp</i> <sup>-/-</sup>	neural differentiated cells on day 6	38115125	85.2	17092121	10000000	24090025	7793
RING1B_rep2	<i>Rybp</i> <sup>-/-</sup>	neural differentiated cells on day 6	30223096	79.9	11591762	10000000	24090025	4619
RING1B_rep1	<i>Yaf2</i> <sup>-/-</sup>	neural differentiated cells on day 6	38730079	86	17578510	10000000	22208382	3760
RING1B_rep2	<i>Yaf2</i> <sup>-/-</sup>	neural differentiated cells on day 6	30831114	79.6	11860033	10000000	22208382	3477

**Supplementary Table 7. Normalization information for calibrated ChIP-seq data.**

IP	Treatment	Cells	Reads mapped to mm10	Reads mapped to dm6	dm6/mm10	Scale factor
H2AK119ub	Wild-type	mESCs	14160715	14682	0.00103681	1
H2AK119ub	<i>Rybp</i> <sup>-/-</sup>	mESCs	16645528	18550	0.00111441	0.930365718
H2AK119ub_subsample	<i>Yaf2</i> <sup>-/-</sup>	mESCs	14984157	15843	0.00105732	0.980606867
H2AK119ub_subsample	Wild-type	neural differentiated cells on day 6	14985097	14903	0.00099452	1.042523599
H2AK119ub_subsample	<i>Rybp</i> <sup>-/-</sup>	neural differentiated cells on day 6	14984753	15247	0.0010175	1.018978982
H2AK119ub_subsample	<i>Yaf2</i> <sup>-/-</sup>	neural differentiated cells on day 6	14983559	16441	0.00109727	0.944902048
H3K27me3	Wild-type	mESCs	23701592	24848	0.00104837	1
H3K27me3	<i>Rybp</i> <sup>-/-</sup>	mESCs	20847570	20270	0.0009723	1.078240431
H3K27me3	<i>Yaf2</i> <sup>-/-</sup>	mESCs	19804866	22152	0.00111851	0.937287631
H3K27me3	Wild-type	neural differentiated cells on day 6	17516424	88591	0.0050576	0.207285902
H3K27me3	<i>Rybp</i> <sup>-/-</sup>	neural differentiated cells on day 6	24413112	129641	0.0053103	0.197421611
H3K27me3	<i>Yaf2</i> <sup>-/-</sup>	neural differentiated cells on day 6	17781997	85738	0.00482162	0.21743082

IP	Treatment	Cells	Reads mapped to mm10	Reads mapped to hg38	hg38/mm10	Scale factor
EZH2	Wild-type	mESCs	35370954	2355352	0.06659	1
EZH2	<i>Rybp</i> <sup>-/-</sup>	mESCs	45835948	4101678	0.08948605	0.744138375
EZH2	<i>Yaf2</i> <sup>-/-</sup>	mESCs	42158914	3321154	0.07877703	0.845297234
MTF2	Wild-type	mESCs	34843886	2519624	0.0723118	1
MTF2	<i>Rybp</i> <sup>-/-</sup>	mESCs	49390738	4611920	0.09337621	0.713136716
MTF2	<i>Yaf2</i> <sup>-/-</sup>	mESCs	49233352	5723928	0.11626119	0.572762123
JARID2	Wild-type	mESCs	27246610	1295582	0.04755021	1
JARID2	<i>Rybp</i> <sup>-/-</sup>	mESCs	23823740	1580470	0.06634013	0.716763884
JARID2	<i>Yaf2</i> <sup>-/-</sup>	mESCs	40982618	2384156	0.05817481	0.817367677

**Supplementary Table 8. Mapping statistic and peak calling information for ATAC-seq data.**

Sample	Treatment	Cells	Total reads	Mapping ratio	Unique reads	Number of peaks
Replicate 1	Wild-type	mESCs	30790196	97.59	17090337	49139
Replicate 2	Wild-type	mESCs	30527311	97.52	17533725	46610
Replicate 1	<i>Rybp</i> <sup>-/-</sup>	mESCs	36677783	97.46	19305413	61943
Replicate 2	<i>Rybp</i> <sup>-/-</sup>	mESCs	33560589	97.29	19305055	55007