Figure S1

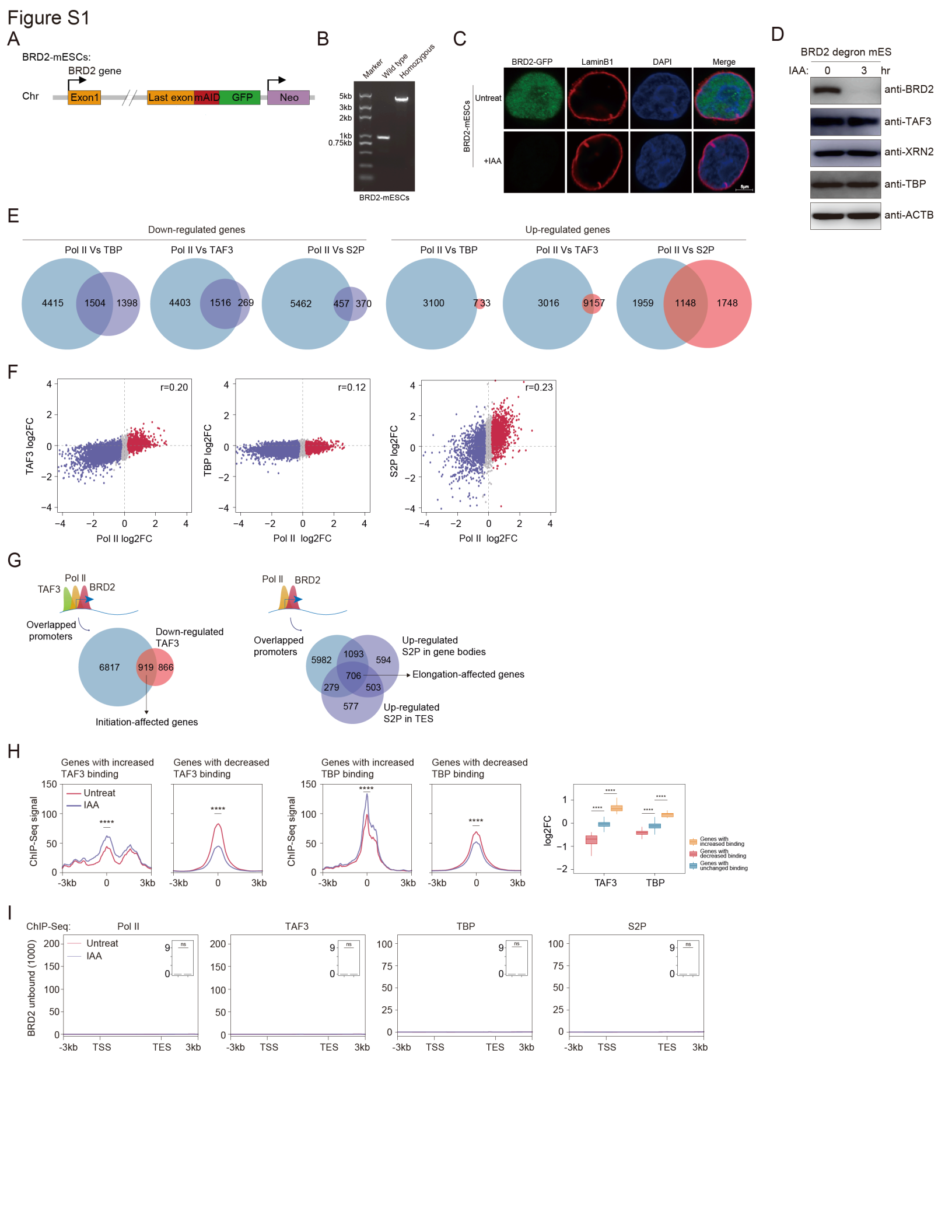
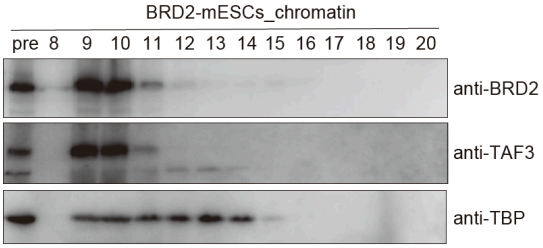
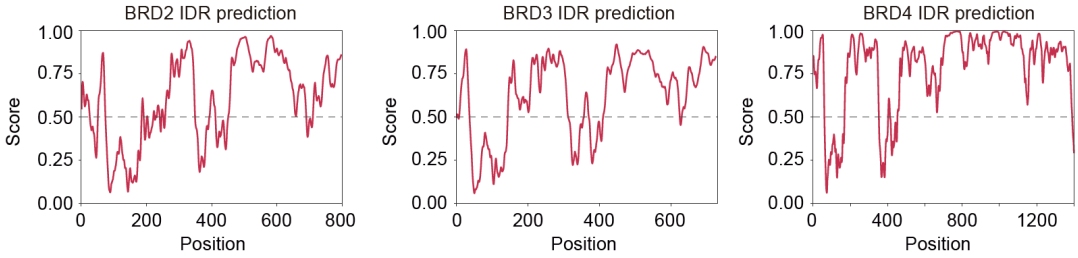

A
BRD2-mESCs:
BRD2 gene
Chr
Exon1
Last exon
mAID
GFP
Neo
B
Marker
Wild type
Homozygous
5kb
3kb
2kb
1kb
0.75kb
BRD2-mESCs
C
BRD2-GFP
LaminB1
DAPI
Merge
BRD2-mESCs
Untreat
+IAA
5μm
D
BRD2 degron mES
IAA: 0 3 hr
anti-BRD2
anti-TAF3
anti-XRN2
anti-TBP
anti-ACTB
E
Down-regulated genes
Up-regulated genes
Pol II Vs TBP
Pol II Vs TAF3
Pol II Vs S2P
4415
1504
1398
4403
1516
269
5462
457
370
3100
733
3016
9157
1959
1148
1748
F
r=0.20
r=0.12
r=0.23
TAF3 log2FC
TBP log2FC
S2P log2FC
Pol II log2FC
G
Pol II
TAF3
BRD2
Overlapped promoters
Down-regulated TAF3
6817
919
866
Initiation-affected genes
Up-regulated S2P in gene bodies
5982
1093
594
706
279
503
577
Elongation-affected genes
Up-regulated S2P in TES
H
Genes with increased TAF3 binding
Genes with decreased TAF3 binding
Genes with increased TBP binding
Genes with decreased TBP binding
Untreat
IAA
ChIP-Seq signal
log2FC
TAF3
TBP
Genes with increased binding
Genes with decreased binding
Genes with unchanged binding
I
ChIP-Seq:
Pol II
TAF3
TBP
S2P
BRD2 unbound (1000)
Untreat
IAA
ns
-3kb
TSS
TES
3kb

Figure S2

A



B



C

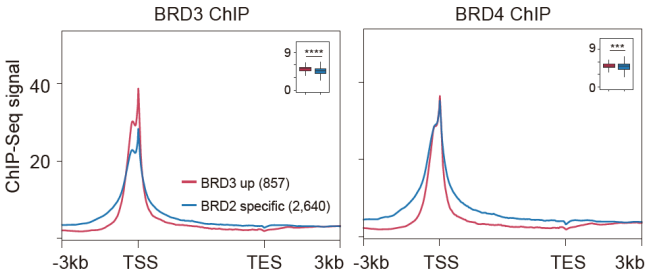
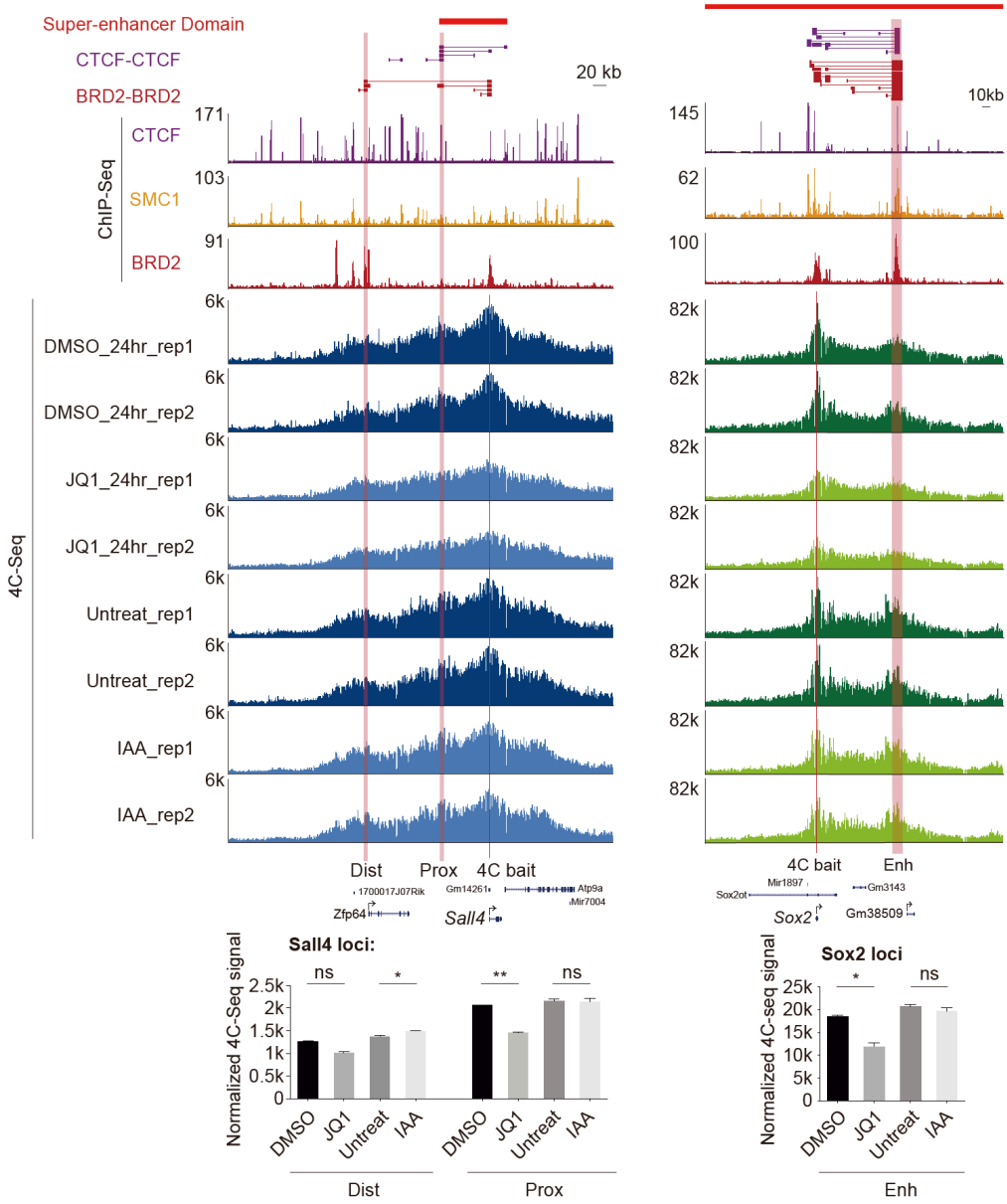


Figure S3

A


Super-enhancer Domain
CTCF-CTCF
BRD2-BRD2
20 kb
ChIP-Seq
CTCF
SMC1
BRD2
171
103
91
4C-Seq
DMSO_24hr_rep1
DMSO_24hr_rep2
JQ1_24hr_rep1
JQ1_24hr_rep2
Untreat_rep1
Untreat_rep2
IAA_rep1
IAA_rep2
6k
Dist
Prox
4C bait
1700017J07Rik
Gm14261
Atp9a
Mir7004
Zfp64
Sall4
Sall4 loci:
Normalized 4C-Seq signal
ns
*
**
ns
2.5k
2k
1.5k
1k
0.5k
0
DMSO
JQ1
Untreat
IAA
Dist
Prox
10kb
145
62
100
82k
4C bait
Enh
Mir1897
Sox2ot
Gm3143
Sox2
Gm38509
Sox2 loci
Normalized 4C-seq signal
*
ns
25k
20k
15k
10k
5k
0
DMSO
JQ1
Untreat
IAA
Enh

B

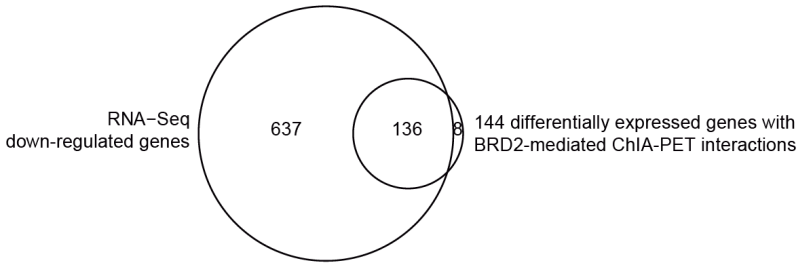

RNA−Seq
down-regulated genes
637
136
8
144 differentially expressed genes with
BRD2-mediated ChIA-PET interactions

Figure S4

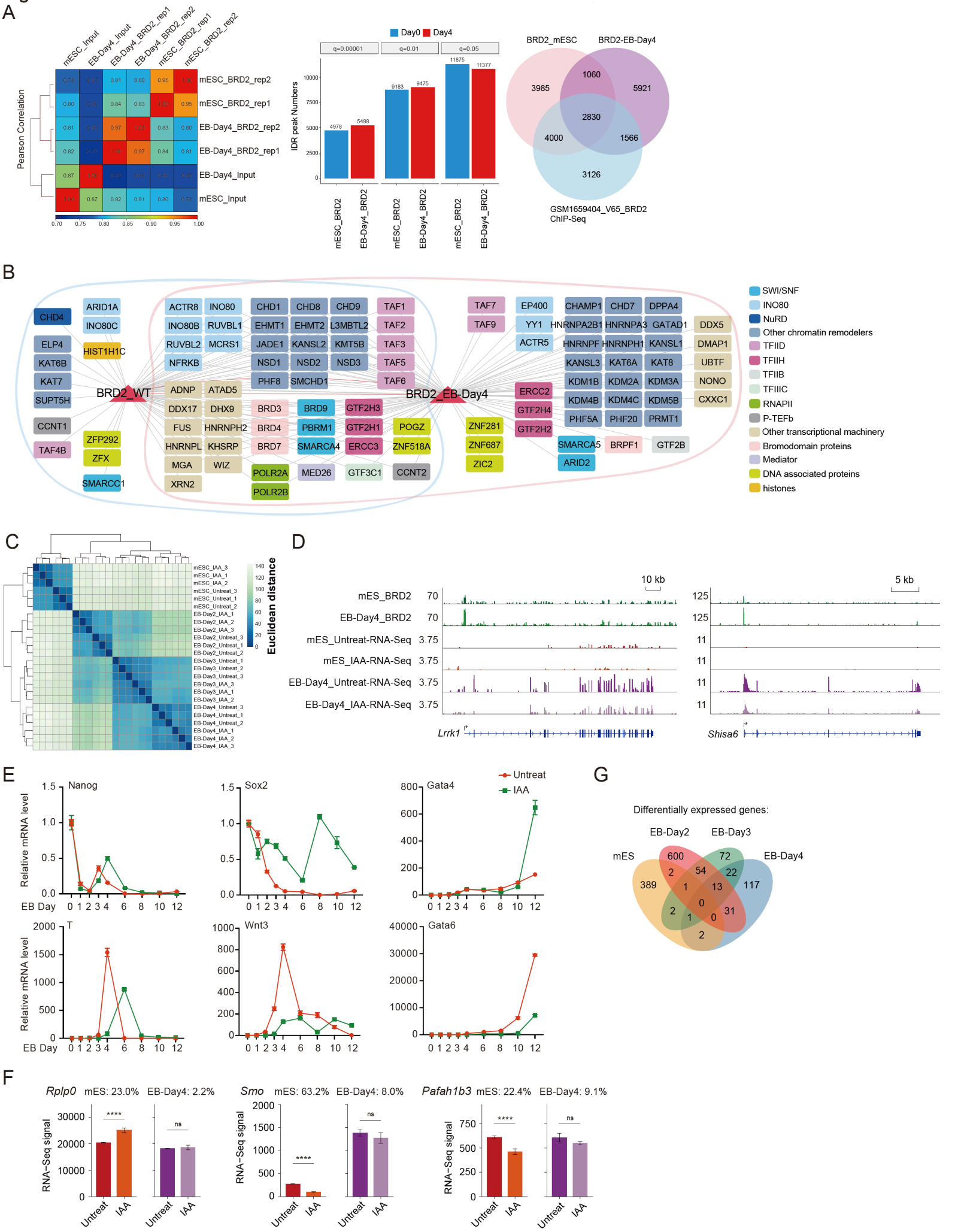
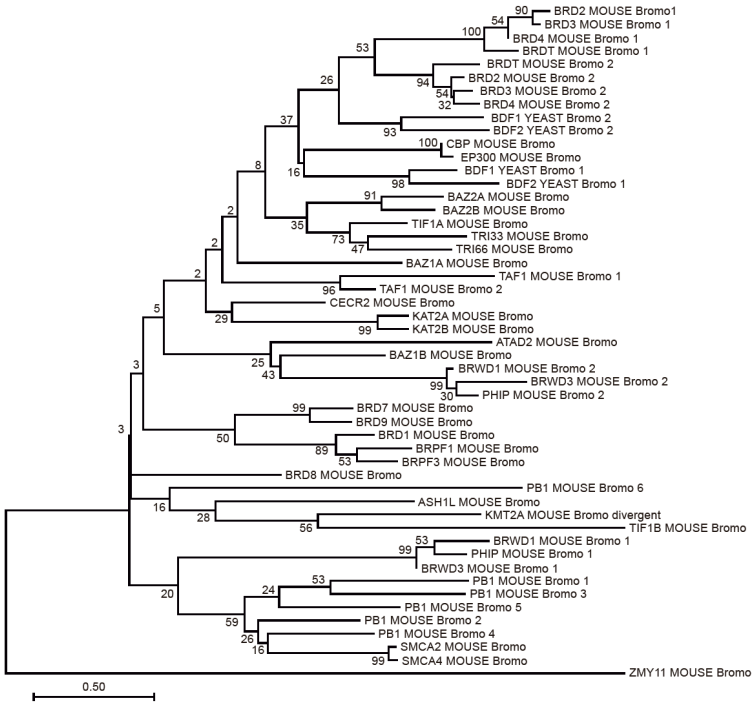

A
mESC_Input
EB-Day4_Input
EB-Day4_BRD2_rep1
EB-Day4_BRD2_rep2
mESC_BRD2_rep1
mESC_BRD2_rep2
Pearson Correlation
mESC_BRD2_rep2
mESC_BRD2_rep1
EB-Day4_BRD2_rep2
EB-Day4_BRD2_rep1
EB-Day4_Input
mESC_Input
0.70 0.75 0.80 0.85 0.90 0.95 1.00
Day0 Day4
q=0.00001
q=0.01
q=0.05
IDR peak Numbers
4978
5488
9183
9475
11875
11377
mESC_BRD2
EB-Day4_BRD2
BRD2_mESC
BRD2-EB-Day4
3985
1060
5921
2830
4000
1566
3126
GSM1659404_V65_BRD2
ChIP-Seq
B
CHD4
ARID1A
INO80C
ELP4
HIST1H1C
KAT6B
KAT7
SUPT5H
CCNT1
TAF4B
ZFP292
ZFX
SMARCC1
BRD2_WT
ACTR8
INO80
INO80B
RUVBL1
RUVBL2
MCRS1
NFRKB
ADNP
ATAD5
DDX17
DHX9
FUS
HNRNPH2
HNRNPL
KHSRP
MGA
WIZ
XRN2
CHD1
CHD8
CHD9
EHMT1
EHMT2
L3MBTL2
JADE1
KANSL2
KMT5B
NSD1
NSD2
NSD3
PHF8
SMCHD1
BRD3
BRD9
BRD4
PBRM1
BRD7
SMARCA4
POLR2A
MED26
POLR2B
GTF3C1
TAF1
TAF2
TAF3
TAF5
TAF6
GTF2H3
GTF2H1
GTF2H4
ERCC3
POGZ
ZNF518A
CCNT2
BRD2_EB-Day4
TAF7
TAF9
ZNF281
ZNF687
ZIC2
EP400
YY1
ACTR5
ERCC2
GTF2H4
GTF2H2
CHAMP1
CHD7
DPPA4
HNRNPA2B1
HNRNPA3
GATAD1
HNRNPF
HNRNPH1
KANSL1
KANSL3
KAT6A
KAT8
KDM1B
KDM2A
KDM3A
KDM4B
KDM4C
KDM5B
PHF5A
PHF20
PRMT1
SMARCA5
BRPF1
GTF2B
ARID2
DDX5
DMAP1
UBTF
NONO
CXXC1
SWI/SNF
INO80
NuRD
Other chromatin remodelers
TFIID
TFIIH
TFIIB
TFIIIC
RNAPII
P-TEFb
Other transcriptional machinery
Bromodomain proteins
Mediator
DNA associated proteins
histones
C
Euclidean distance
140
120
100
80
60
40
20
0
mESC_IAA_3
mESC_IAA_1
mESC_IAA_2
mESC_Untreat_3
mESC_Untreat_1
mESC_Untreat_2
EB-Day2_IAA_1
EB-Day2_IAA_2
EB-Day2_IAA_3
EB-Day2_Untreat_3
EB-Day2_Untreat_1
EB-Day2_Untreat_2
EB-Day3_Untreat_1
EB-Day3_Untreat_2
EB-Day3_Untreat_3
EB-Day3_IAA_3
EB-Day3_IAA_1
EB-Day3_IAA_2
EB-Day4_Untreat_3
EB-Day4_Untreat_1
EB-Day4_Untreat_2
EB-Day4_IAA_1
EB-Day4_IAA_2
EB-Day4_IAA_3
D
10 kb
5 kb
mES_BRD2
EB-Day4_BRD2
mES_Untreat-RNA-Seq
mES_IAA-RNA-Seq
EB-Day4_Untreat-RNA-Seq
EB-Day4_IAA-RNA-Seq
3.75
Lrrk1
Shisa6
E
Nanog
Sox2
Gata4
Untreat
IAA
Relative mRNA level
EB Day
T
Wnt3
Gata6
G
Differentially expressed genes:
EB-Day2
EB-Day3
mES
EB-Day4
600
72
2
54
22
389
1
13
117
0
31
2
1
0
2
F
Rplp0 mES: 23.0% EB-Day4: 2.2%
Smo mES: 63.2% EB-Day4: 8.0%
Pafah1b3 mES: 22.4% EB-Day4: 9.1%
RNA−Seq signal
****
ns
Untreat
IAA

Figure S5

A



B

Bromodomain 1:

```
human   BRD2      74 RVTNQLQYLHKVVMKALWKHQFAWPFRQPVDAVKLGLPDYHKIIKQPMDMGTIKRRLENNYYWAASECMQDFNTMFTNCYIYNKPTDDIVLMAQTLEKIFLQKVASMPQEEQ 185
human   BRD3      34 RKTNQLQYMQNVVVKTLWKHQFAWPFYQPVDAIKLNLPDYHKIIKNPMDMGTIKKRLENNYYWSASECMQDFNTMFTNCYIYNKPTDDIVLMAQALEKIFLQKVAQMPQEEV 145
human   BRD4      58 RQTNQLQYLLRVVLKTLWKHQFAWPFQQPVDAVKLNLPDYYKIIKTPMDMGTIKKRLENNYYWNAQECIQDFNTMFTNCYIYNKPGDDIVLMAEALEKLFLQKINELPTEET 169
human   BRDT      27 RLTNQLQYLQKVVLKDLWKHSFSWPFQRPVDAVKLQLPDYYTIIKNPMDLNTIKKRLENKYYAKASECIEDFNTMFSNCYLYNKPGDDIVLMAQALEKLFMQKLSQMPQEEQ 138
mouse   Brd2      73 RVTNQLQYLHKVVMKALWKHQFAWPFRQPVDAVKLGLPDYHKIIKQPMDMGTIKRRLENNYYWAASECMQDFNTMFTNCYIYNKPTDDIVLMAQTLEKIFLQKVASMPQEEQ 184
Chicken Brd2      27 RVTNQLQYLHKVVMKALWKHQFAWPFRQPVDAVKLGLPDYHKIIKQPMDMGTIKRRLENNYYWGAAECMQDFNTMFTNCYIYNKPTDDIVLMAQTLEKIFLQKVAQMPPEEQ 138
Xenopus Brd2      74 RSTNQLQYLHKAVVKSLWKHQFSWPFRQPVDAVKLGLPDYHKIIKQPMDMGTVKKRLENNYYWSALECMQDFNTMFTNCYIYNKPTDDIVLMAQSLEKMFLQKVAQMPQEEQ 185
Drosophila Fs(1)h 34 RNTNQLQYLIKTVMKVIWKHHFSWPFQQPVDAKKLNLPDYHKIIKQPMDMGTIKKRLENNYYWSAKETIQDFNTMFNNCYVYNKPGEDVVVMAQTLEKVFLQKIESMPKEEL 145
```

Bromodomain 2:

```
human   BRD2      345 KLSEQLKHCNGILKELLSKKHAAYAWPFYKPVDASALGLHDYHDIIKHPMDLSTVKRKMENRDYRDAQEFAADVRLMFSNCYKYNPPDHDVVAMARKLQDVFEFRYAKMPDEP 457
human   BRD3      307 KLSEHLRYCDSILREMLSKKHAAYAWPFYKPVDAEALELHDYHDIIKHPMDLSTVKRKMDGREYPDAQGFAADVRLMFSNCYKYNPPDHEVVAMARKLQDVFEMRFAKMPDEP 419
human   BRD4      349 KVSEQLKCCSGILKEMFAKKHAAYAWPFYKPVDVEALGLHDYCDIIKHPMDMSTIKSKLEAREYRDAQEFGADVRLMFSNCYKYNPPDHEVVAMARKLQDVFEMRFAKMPDEP 461
human   BRDT      268 KVTEQLRHCSEILKEMLAKKHFSYAWPFYNPVDVNALGLHNYYDVVKNPMDLGTIKEKMDNQEYKDAYKFAADVRLMFMNCYKYNPPDHEVVTMARMLQDVFETHFSKIPIEP 380
mouse   Brd2      344 KLSEQLKHCNGILKELLSKKHAAYAWPFYKPVDASALGLHDYHDIIKHPMDLSTVKRKMENRDYRDAQEFAADVRLMFSNCYKYNPPDHDVVAMARKLQDVFEFRYAKMPDEP 456
Chicken Brd2      292 KLSEQLKYCNGILKELLSKKHAAYAWPFYKPVDASALGLHDYHEIIKHPMDLSTIKRKMENRDYHDAQEFAADVRLMFSNCYKYNPPDHDVVAMARKLQDVFEFSYAKMPDEP 404
Xenopus Brd2      339 KLSEQLKYCNGILKELLSKKHAAYAWPFYKPVDVSALGLHDYYDIIKHPMDMSTIKKKMDSREFKDAQEFAAAIRLMFSNCYKYNPPDHDVVAMARKLQDVFEFSYAKMPDEP 451
Drosophila Fs(1)h 476 KLSDALKSCNEILKELFSKKHSGYAWPFYKPVDAEMLGLHDYHDIIKKPMDLGTVKRKMDNREYKSAPEFAADVRLIFTNCYKYNPPDHDVVAMGRKLQDVFEMRYANIPDEP 588
```

Dimerization domain:

```
human   BRD2      511 DSEEERAHRLAELQEQLRAVHEQLAALSQGPISKPKRKREKKEKKKK 557
human   BRD3      454 DSEEERATRLAELQEQLKAVHEQLAALSQAPVNKPKKKKEKKEKEKK 500
human   BRD4      502 DSEEERAQRLAELQEQLKAVHEQLAALSQPQQNKPKKKEKDKKEKKK 548
human   BRDT      414 DSEDERVKRLAKLQEQLKAVHQQLQVLSQVPFRKLNKKKEKSKKEKK 460
mouse   Brd2      509 DSEEERAHRLAELQEQLRAVHEQLAALSQGPISKPKRKREKKEKKKK 555
Chicken Brd2      460 DSEEERANRLAELQEQLRAVHEQLAALSQGPVSKPKKKREKKKKKKS 506
Xenopus Brd2      491 DSEEERANRLAELQEQLRAVHEQLAALSQGPISKPKKKREKKKKKK 537
Drosophila Fs(1)h 648 NSDEERSARLKMLESKLLGLQEEIRKLSEEASAKKKAKKLKEKKKS 694
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