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Supplementary Materials for

R-loops coordinate with SOX2 in regulating reprogramming to pluripotency

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Figure S1

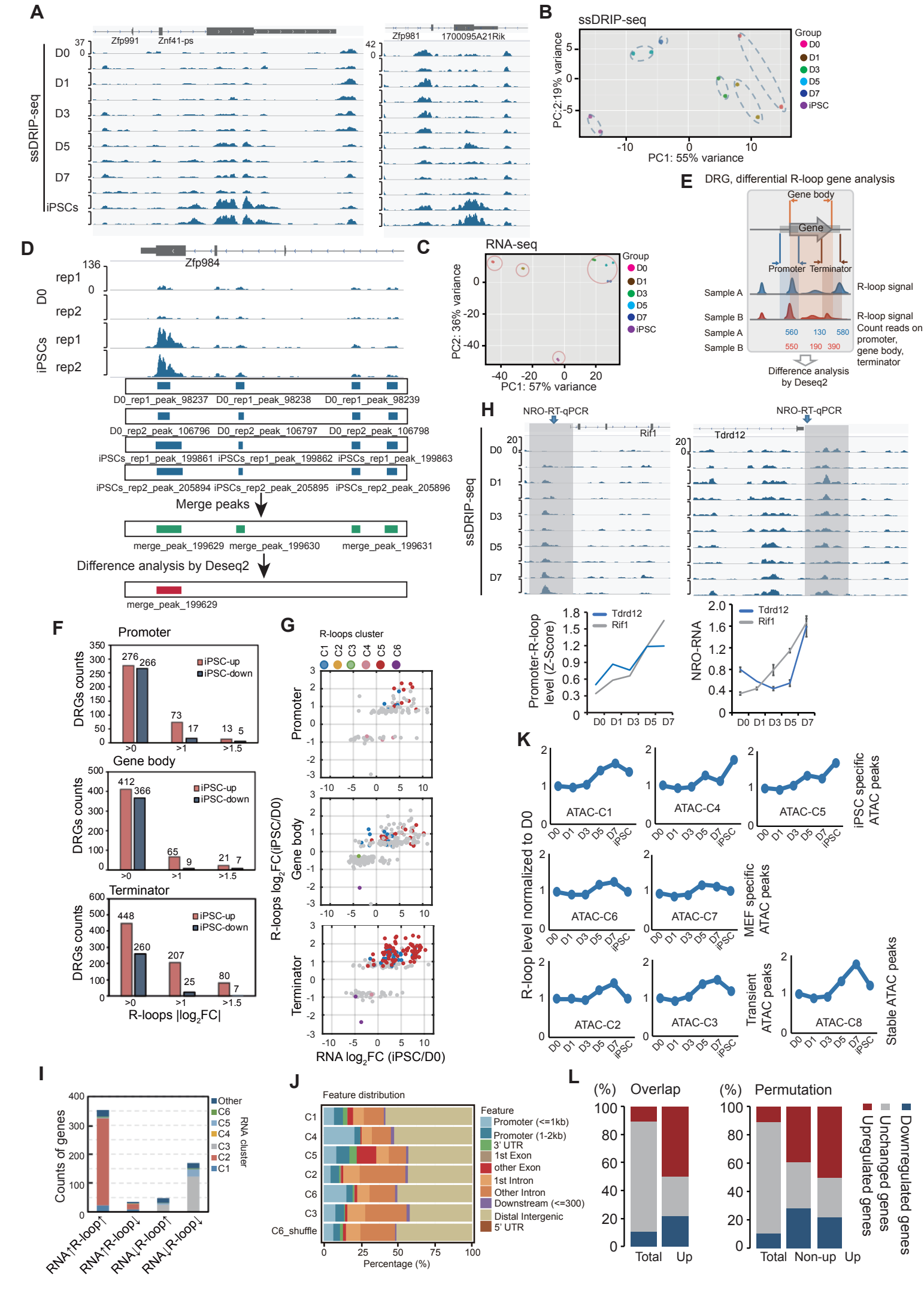
A
ssDRIP-seq
D0
D1
D3
D5
D7
iPSCs
Zfp991
Znf41-ps
Zfp981
1700095A21Rik
B
ssDRIP-seq
PC:2:19% variance
PC1: 55% variance
Group
D0
D1
D3
D5
D7
iPSC
C
RNA-seq
PC2: 36% variance
PC1: 57% variance
D
Zfp984
rep1
rep2
D0_rep1_peak_98237
D0_rep1_peak_98238
D0_rep1_peak_98239
D0_rep2_peak_106796
D0_rep2_peak_106797
D0_rep2_peak_106798
iPSCs_rep1_peak_199861
iPSCs_rep1_peak_199862
iPSCs_rep1_peak_199863
iPSCs_rep2_peak_205894
iPSCs_rep2_peak_205895
iPSCs_rep2_peak_205896
Merge peaks
merge_peak_199629
merge_peak_199630
merge_peak_199631
Difference analysis by Deseq2
merge_peak_199629
E
DRG, differential R-loop gene analysis
Gene body
Gene
Promoter
Terminator
Sample A
Sample B
R-loop signal
560
130
580
550
190
390
Count reads on promoter, gene body, terminator
Difference analysis by Deseq2
F
Promoter
Gene body
Terminator
DRGs counts
iPSC-up
iPSC-down
R-loops |log2FC|
G
R-loops cluster
C1
C2
C3
C4
C5
C6
Promoter
Gene body
Terminator
R-loops log2FC(iPSC/D0)
RNA log2FC (iPSC/D0)
H
NRO-RT-qPCR
Rif1
Tdrd12
ssDRIP-seq
Promoter-R-loop level (Z-Score)
NRO-RNA
K
ATAC-C1
ATAC-C4
ATAC-C5
ATAC-C6
ATAC-C7
ATAC-C2
ATAC-C3
ATAC-C8
R-loop level normalized to D0
iPSC specific ATAC peaks
MEF specific ATAC peaks
Transient ATAC peaks
Stable ATAC peaks
I
Counts of genes
RNA cluster
RNA↑R-loop↑
RNA↑R-loop↓
RNA↓R-loop↑
RNA↓R-loop↓
J
Feature distribution
C6_shuffle
Percentage (%)
Feature
Promoter (<=1kb)
Promoter (1-2kb)
3' UTR
1st Exon
other Exon
1st Intron
Other Intron
Downstream (<=300)
Distal Intergenic
5' UTR
L
(%) Overlap
(%) Permutation
Total
Up
Non-up
Downregulated genes
Unchanged genes
Upregulated genes

Fig. S1. Analysis of ssDRIP-seq and RNA-seq of reprogrammed cells at different stages of reprogramming. (A) The snapshots showing the distribution of ssDRIP-seq signals in two biological replicates at different stages of reprogramming. **(B)** PCA analysis of ssDRIP-seq signals of MEFs reprogrammed with OSKM at different stages of reprogramming. **(C)** PCA analysis of RNA-seq signals of MEFs reprogrammed with OSKM at different stages of reprogramming. **(D)** Schematic representation of the workflow for analyzing differential R-loop regions, see methods. **(E)** Schematic representation of the workflow for analyzing differential R-loop genes (DRGs). **(F)** Statistics of genes with increased or decreased R-loop levels ($P < 0.01$) at promoter (upper), gene body (middle), or terminator (lower) region of genes in iPSCs compared with MEFs reprogrammed with OSKM on day 0. **(G)** Scatter plots showing gene expression (x axis) and R-loops (y axis) at promoter (upper), gene body (middle), or terminator (lower) region in iPSCs compared with MEFs reprogrammed with OSKM on day 0. The concurrently changed genes were shown and colored by R-loop clusters shown in Fig. 1B. **(H)** The snapshots showing the distribution of ssDRIP-seq signals of *Tdrd12* and *Rif1* loci in two biological replicates at different stages of reprogramming (top). The changes of R-loop level in *Tdrd12* and *Rif1* loci at different stages of reprogramming (bottom left). The changes in *Tdrd12* and *Rif1* expression were measured by NRO-RT-qPCR. All qPCR data were analyzed and normalized to *Gapdh* expression (bottom right). **(I)** Statistics of concurrently changed genes from different RNA clusters, sorted by dynamic relationships between gene expression and R-loops (↑ means upregulated, ↓ means downregulated). **(J)** Distribution of differential R-loop regions from six R-loop clusters shown in Fig 1B. **(K)** R-loop level of different ATAC clusters defined by Knaupp et al. (2017). **(L)** Left, overlap of enhancers containing eRNAs and upregulated (Up) R-loops with upregulated, unchanged or downregulated expression of genes. Right, permutation test of enhancers containing eRNAs and upregulated (Up) R-loops or nonupregulated (Non-up) R-loops with upregulated, unchanged or downregulated expression of genes. The total genes were as a control.

Figure S2

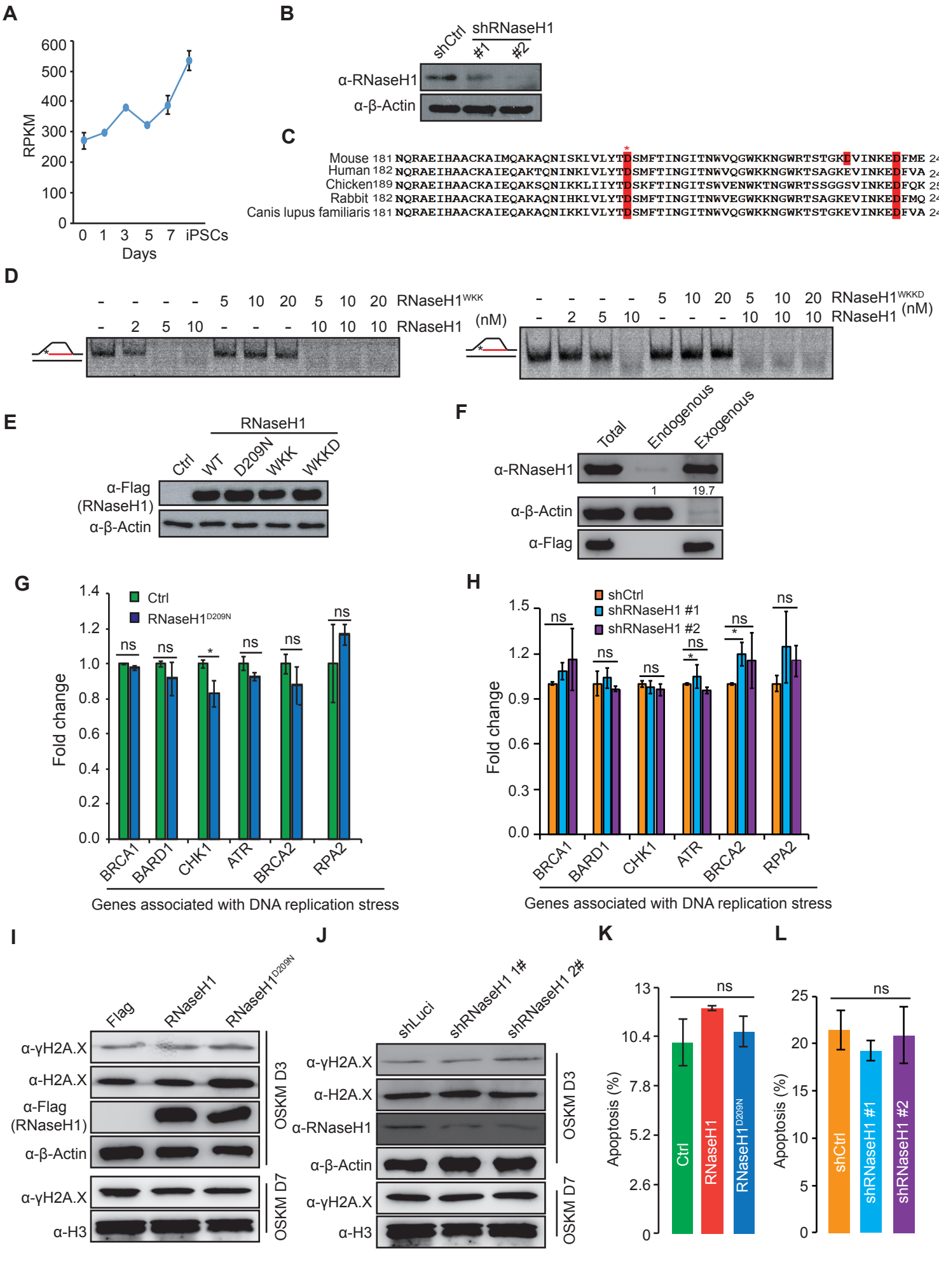
A
RPKM
600
500
400
300
200
100
0
0 1 3 5 7 iPSCs
Days
B
shCtrl
shRNaseH1
#1
#2
α-RNaseH1
α-β-Actin
C
Mouse 181 NQRAEIHAACKAIMQAKAQNISKLVLYTDSMFTINGITNWVQGWKKNGWRTSTGKDVINKEDFME 245
Human 182 NQRAEIHAACKAIEQAKTQNINKLVLYTDSMFTINGITNWVQGWKKNGWKTSAGKEVINKEDFVA 246
Chicken189 NQRAEIHAACKAIEQAKSQNIKKLIIYTDSKFTINGITSWVENWKTNGWRTSSGGSVINKEDFQK 253
Rabbit 182 NQRAEIHAACKAIEQAKAQNIHKLVLYTDSMFTINGITNWVEGWKKNGWRTSAGKEVINKEDFMQ 246
Canis lupus familiaris 181 NQRAEIHAACKAIEQAKAQNIKKLVLYTDSMFTINGITNWVQGWKKNGWKTSTGKEVINKEDFVA 245
D
RNaseH1WKK (nM)
RNaseH1 (nM)
RNaseH1WKKD (nM)
E
RNaseH1
Ctrl
WT
D209N
WKK
WKKD
α-Flag (RNaseH1)
α-β-Actin
F
Total
Endogenous
Exogenous
α-RNaseH1
1
19.7
α-β-Actin
α-Flag
G
Ctrl
RNaseH1D209N
Fold change
ns
*
BRCA1
BARD1
CHK1
ATR
BRCA2
RPA2
Genes associated with DNA replication stress
H
shCtrl
shRNaseH1 #1
shRNaseH1 #2
Fold change
Genes associated with DNA replication stress
I
Flag
RNaseH1
RNaseH1D209N
α-γH2A.X
α-H2A.X
α-Flag (RNaseH1)
α-β-Actin
OSKM D3
OSKM D7
α-H3
J
shLuci
shRNaseH1 1#
shRNaseH1 2#
α-RNaseH1
K
Apoptosis (%)
Ctrl
RNaseH1
RNaseH1D209N
L
shCtrl
shRNaseH1 #1
shRNaseH1 #2

Fig. S2. Verification of RNaseH1^{D209N} and gene expression level. (A) Expressional analysis of RNaseH1 at different stages of reprogramming (RPKM, reads per kilobase of exon model per million mapped reads of RNA-seq. Mean ±SD). **(B)** Western blot to test the knockdown efficiency of RNaseH1 in MEFs reprogrammed with OSKM, together with control plasmid (pSuper-shLuci), and two independent RNaseH1 shRNAs on day 5 in iCD1 medium. **(C)** Sequence alignment of RNaseH1 in different species. Conserved residue of catalytic domain is highlighted in red asterisk. **(D)** R-loop substrate (Bottom, 25 nM) with Cy5-labeled (*) RNA (red line) or DNA (black line) was incubated with the increasing concentrations of RNaseH1, RNaseH1^{WKK} (Left) or RNaseH1^{WKKD} (Right) in the presence of RNaseH1 (10 nM). The reaction was detected on a native polyacrylamide gel. **(E)** Western blot to test the overexpression efficiency of RNaseH1 in MEFs reprogrammed with OSKM, together with control plasmid (pMXs-Flag), pMXs-RNaseH1 and different RNaseH1 mutations on day 5 in iCD1 medium. **(F)** Western blot to test the expression of endogenous RNaseH1 and exogenous RNaseH1^{D209N}. **(G)** qRT-PCR analysis of genes associated with DNA replication stress in MEFs reprogrammed with OSKM and pMXs-Flag (Ctrl) and pMXs-RNaseH1^{D209N} on day 7. Error bars correspond to means ± SD (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ns, not significant, P ≥ 0.05, two-tailed Student's *t* test). **(H)** qRT-PCR analysis of genes associated with DNA replication stress in MEFs reprogrammed with OSKM and pSuper-shLuci (shCtrl), and two independent RNaseH1 shRNAs on day 7. Error bars correspond to means ± SD (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ns, not significant, P ≥ 0.05, two-tailed Student's *t* test). **(I)** Western blot to detect protein level of γH2A.X in MEFs reprogrammed with OSKM and pMXs-Flag, pMXs-RNaseH1, pMXs-RNaseH1^{D209N} and pSuper-shLuci (shCtrl), two independent RNaseH1 shRNAs on days 3 and 7, respectively. **(J)** Western blot to detect protein level of γH2A.X in MEFs reprogrammed with OSKM and pSuper-shLuci (shCtrl), two independent RNaseH1 shRNAs on days 3 and 7, respectively. **(K)** Cell apoptosis of MEFs reprogrammed with OSKM and pMXs-Flag, pMXs-RNaseH1, pMXs-RNaseH1^{D209N} and pSuper-shLuci (shCtrl), two independent RNaseH1 shRNAs on day 7. Error bars correspond to means ± SD (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ns, not significant, P ≥ 0.05, two-tailed Student's *t* test). **(L)** Cell apoptosis of MEFs reprogrammed with OSKM and pSuper-shLuci (shCtrl), two independent RNaseH1 shRNAs on day 7.

Figure S3

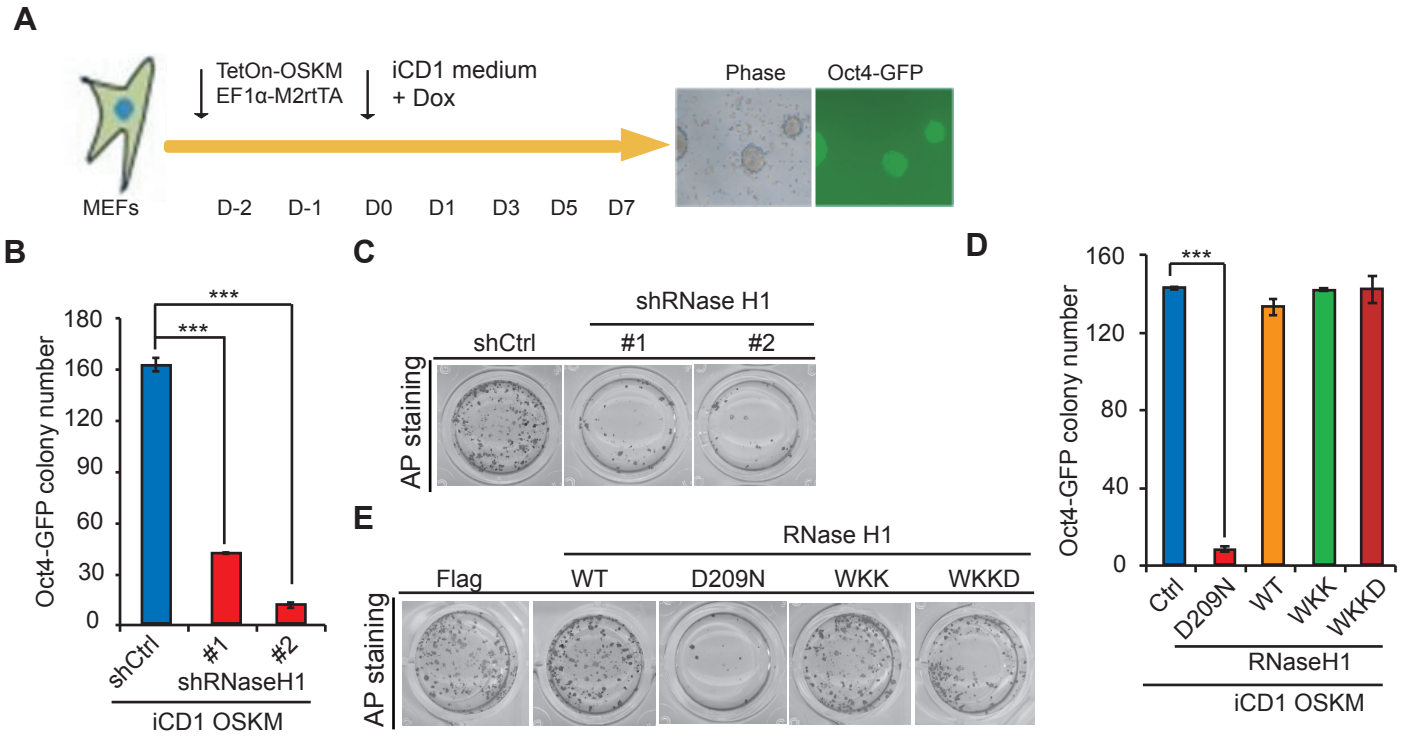
A
TetOn-OSKM
EF1α-M2rtTA
iCD1 medium
+ Dox
MEFs
D-2
D-1
D0
D1
D3
D5
D7
Phase
Oct4-GFP
B
Oct4-GFP colony number
180
160
120
90
60
30
0
***
***
shCtrl
#1
#2
shRNaseH1
iCD1 OSKM
C
shRNase H1
shCtrl
#1
#2
AP staining
D
Oct4-GFP colony number
160
120
80
40
0
***
Ctrl
D209N
WT
WKK
WKKD
RNaseH1
iCD1 OSKM
E
RNase H1
Flag
WT
D209N
WKK
WKKD
AP staining

Fig. S3. RNaseH1 activity loss inhibits reprogramming in a lentivirus-inducing system. (A) Workflow of lentivirus-inducing reprogramming system. **(B)** *Oct4*-GFP colony number of MEFs reprogrammed with lentiviral infection of OSKM and retroviral infection of pSuper-shLuci (shCtrl), plus two independent shRNAs for RNaseH1 on day 8 in iCD1 medium. Error bars correspond to means ± SD (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, two-tailed Student's *t* test). **(C)** AP staining on day 5, corresponding to Fig. S3B. **(D)** *Oct4*-GFP colony number of MEFs reprogrammed with lentiviral infection of OSKM and retroviral infection of pMXs-Flag (Ctrl), RNaseH1, and RNaseH1^{D209N} on day 8 in iCD1 medium. Error bars correspond to means ± SD (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, two-tailed Student's *t* test). **(E)** AP staining on day 5, corresponding to Fig. S3D.

Figure S4

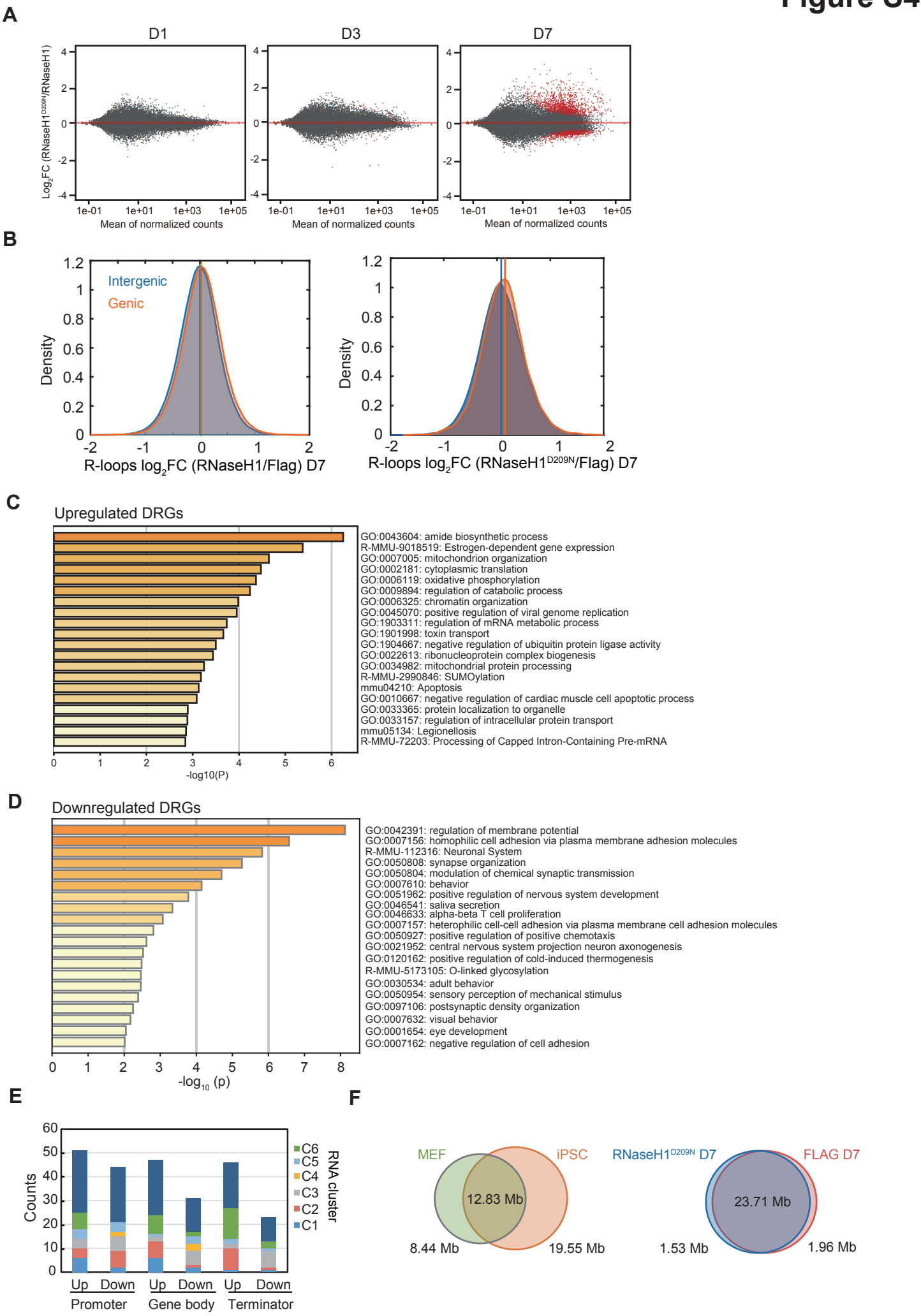
A
D1
D3
D7
Log2FC (RNaseH1D209N/RNaseH1)
Mean of normalized counts
B
Intergenic
Genic
Density
R-loops log2FC (RNaseH1/Flag) D7
R-loops log2FC (RNaseH1D209N/Flag) D7
C
Upregulated DRGs
GO:0043604: amide biosynthetic process
R-MMU-9018519: Estrogen-dependent gene expression
GO:0007005: mitochondrion organization
GO:0002181: cytoplasmic translation
GO:0006119: oxidative phosphorylation
GO:0009894: regulation of catabolic process
GO:0006325: chromatin organization
GO:0045070: positive regulation of viral genome replication
GO:1903311: regulation of mRNA metabolic process
GO:1901998: toxin transport
GO:1904667: negative regulation of ubiquitin protein ligase activity
GO:0022613: ribonucleoprotein complex biogenesis
GO:0034982: mitochondrial protein processing
R-MMU-2990846: SUMOylation
mmu04210: Apoptosis
GO:0010667: negative regulation of cardiac muscle cell apoptotic process
GO:0033365: protein localization to organelle
GO:0033157: regulation of intracellular protein transport
mmu05134: Legionellosis
R-MMU-72203: Processing of Capped Intron-Containing Pre-mRNA
-log10(P)
D
Downregulated DRGs
GO:0042391: regulation of membrane potential
GO:0007156: homophilic cell adhesion via plasma membrane adhesion molecules
R-MMU-112316: Neuronal System
GO:0050808: synapse organization
GO:0050804: modulation of chemical synaptic transmission
GO:0007610: behavior
GO:0051962: positive regulation of nervous system development
GO:0046541: saliva secretion
GO:0046633: alpha-beta T cell proliferation
GO:0007157: heterophilic cell-cell adhesion via plasma membrane cell adhesion molecules
GO:0050927: positive regulation of positive chemotaxis
GO:0021952: central nervous system projection neuron axonogenesis
GO:0120162: positive regulation of cold-induced thermogenesis
R-MMU-5173105: O-linked glycosylation
GO:0030534: adult behavior
GO:0050954: sensory perception of mechanical stimulus
GO:0097106: postsynaptic density organization
GO:0007632: visual behavior
GO:0001654: eye development
GO:0007162: negative regulation of cell adhesion
-log10 (p)
E
Counts
RNA cluster
C6
C5
C4
C3
C2
C1
Up Down Up Down Up Down
Promoter
Gene body
Terminator
F
MEF
iPSC
12.83 Mb
8.44 Mb
19.55 Mb
RNaseH1D209N D7
FLAG D7
23.71 Mb
1.53 Mb
1.96 Mb

Fig. S4. Analysis of ssDRIP-seq and RNA-seq of MEFs reprogrammed with OSKM and RNaseH1^{D209N} at different stages of reprogramming. (**A)** MA plots showing the DEGs (red dot) of RNaseH1^{D209N}/RNaseH1 at different time points during reprogramming. **(B)** Kernel density of log_2 fold change (RNaseH1/Flag, D7, Left) and log_2 fold change (RNaseH1^{D209N}/Flag, D7, Right) of intergenic (blue) or genic (containing promoter, terminator, and terminator, orange) R-loop levels on total R-loop regions, presented by histograms. Mean of intergenic (blue) or genic (orange) R-loop log_2 fold change was shown as vertical line. (**C)** GO categories of the upregulated DRGs by RNaseH1^{D209N} compared with the control. **(D)** GO categories of the downregulated DRGs by RNaseH1^{D209N} compared with the control. **(E)** Statistics of Prom-/Gb-/Term- concurrently changed genes shown in Fig. 3D with up- or downregulated RNA levels, sorted by RNA clusters shown in Fig. 1D. **(F)** Intersections of ATAC peaks. Left, MEFs and iPSCs. Right, RNaseH1^{D209N} and Flag on day 7. The size of the genomic regions covered by peaks was shown.

Figure S5

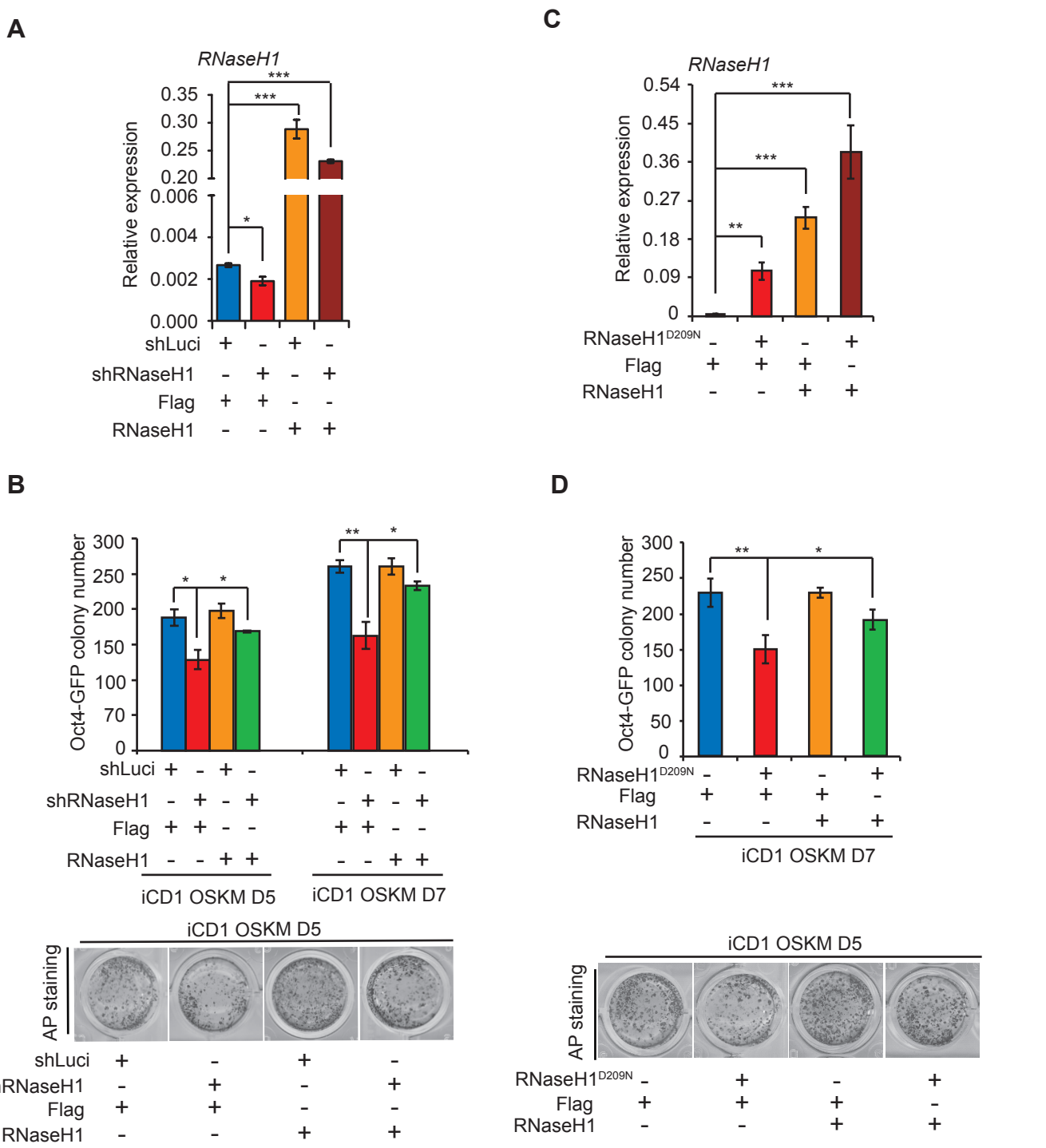
A
RNaseH1
Relative expression
0.35
0.30
0.25
0.20
0.006
0.004
0.002
0.000
***
***
*
shLuci + - + -
shRNaseH1 - + - +
Flag + + - -
RNaseH1 - - + +
C
RNaseH1
Relative expression
0.54
0.45
0.36
0.27
0.18
0.09
0
***
***
**
RNaseH1D209N - + - +
Flag + + + -
RNaseH1 - - + +
B
Oct4-GFP colony number
300
250
200
150
100
70
0
*
*
**
*
shLuci + - + - + - + -
shRNaseH1 - + - + - + - +
Flag + + - - + + - -
RNaseH1 - - + + - - + +
iCD1 OSKM D5
iCD1 OSKM D7
iCD1 OSKM D5
AP staining
shLuci + - + -
shRNaseH1 - + - +
Flag + + - -
RNaseH1 - - + +
D
Oct4-GFP colony number
300
250
200
150
100
50
0
**
*
RNaseH1D209N - + - +
Flag + + + -
RNaseH1 - - + +
iCD1 OSKM D7
iCD1 OSKM D5
AP staining
RNaseH1D209N - + - +
Flag + + + -
RNaseH1 - - + +

Fig. S5. Overexpression of RNaseH1 rescues the negative effect of inhibition of RNaseH1 loss on reprogramming. (A) qRT-PCR to test the expression of *RNaseH1* in MEFs reprogrammed with OSKM and retroviral infection of pMXs-Flag, RNaseH1, and control shLuci, RNaseH1 shRNA, on day 5 in iCD1 medium. Error bars correspond to means ± SD (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, two-tailed Student's *t* test). **(B)** Top: *Oct4*-GFP colony number of MEFs reprogrammed with OSKM and retroviral infection of pMXs-Flag, RNaseH1, and control shLuci, RNaseH1 shRNA, on days 5 and 7, respectively, in iCD1 medium. The retrovirus was added at the same time. Bottom: AP staining on day 5. Error bars correspond to means ± SD (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, two-tailed Student's *t* test). **(C)** qRT-PCR to test the expression of *RNaseH1* in MEFs reprogrammed with OSKM and retroviral infection of pMXs-Flag, RNaseH1, and RNaseH1^{D209N} on day 5 in iCD1 medium. Error bars correspond to means ± SD (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, two-tailed Student's *t* test). **(D)** Top: *Oct4*-GFP colony number of MEFs reprogrammed OSKM and retroviral infection of pMXs-Flag, RNaseH1, and RNaseH1^{D209N} on day 7 in iCD1 medium. The retrovirus was added at the same time. Bottom: AP staining on day 5. Error bars correspond to means ± SD (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, two-tailed Student's *t* test).

Figure S6

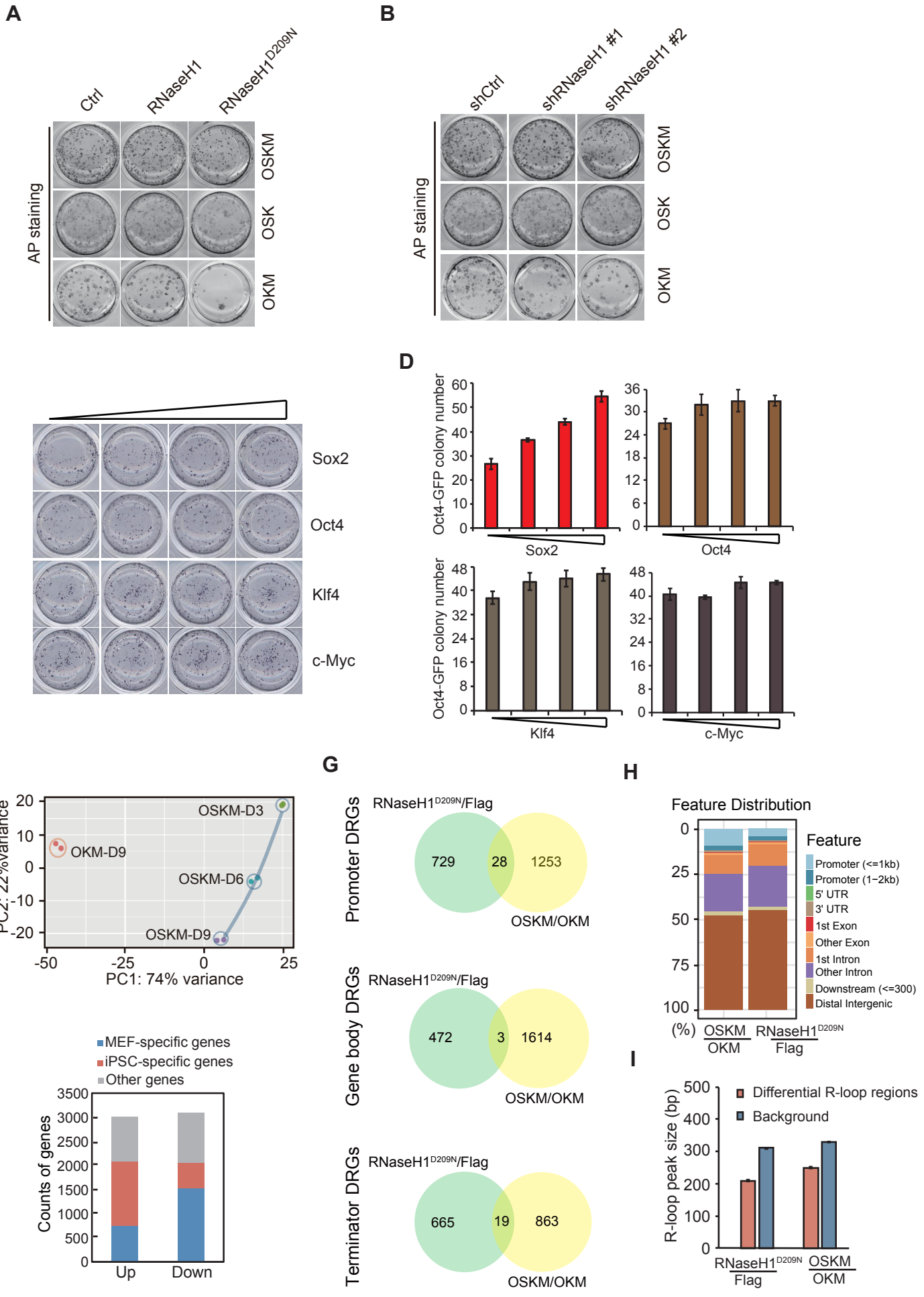
A
Ctrl
RNaseH1
RNaseH1D209N
AP staining
OSKM
OSK
OKM
B
shCtrl
shRNaseH1 #1
shRNaseH1 #2
AP staining
OSKM
OSK
OKM
C
Sox2
Oct4
Klf4
c-Myc
D
Oct4-GFP colony number
Sox2
Oct4
Klf4
c-Myc
E
PC2: 22%variance
PC1: 74% variance
OSKM-D3
OKM-D9
OSKM-D6
OSKM-D9
F
MEF-specific genes
iPSC-specific genes
Other genes
Counts of genes
Up
Down
G
Promoter DRGs
Gene body DRGs
Terminator DRGs
RNaseH1D209N/Flag
729
28
1253
OSKM/OKM
RNaseH1D209N/Flag
472
3
1614
OSKM/OKM
RNaseH1D209N/Flag
665
19
863
OSKM/OKM
H
Feature Distribution
Feature
Promoter (<=1kb)
Promoter (1−2kb)
5' UTR
3' UTR
1st Exon
Other Exon
1st Intron
Other Intron
Downstream (<=300)
Distal Intergenic
(%)
OSKM
OKM
RNaseH1D209N
Flag
I
Differential R-loop regions
Background
R-loop peak size (bp)
RNaseH1D209N
Flag
OSKM
OKM

Fig. S6. Sox2 induces reprogramming by maintaining high level of R-loops. (A) AP staining corresponding to Fig 4A. **(B)** AP staining corresponding to Fig. 4B. **(C)** AP staining of MEFs reprogrammed with OSKM and RNaseH1^{D209N} plus TetON-inducible Sox2, Oct4, Klf4 or c-Myc in iCD1 medium with 20, 50, 100, 500 ng/mL doxycycline. **(D)** *Oct4*-GFP colony number of MEFs reprogrammed with OSKM and RNaseH1^{D209N} plus TetON-inducible Sox2, Oct4, Klf4 or c-Myc in iCD1 medium with 20, 50, 100, 500 ng/mL doxycycline. **(E)** PCA analysis of RNA-seq signals in OSKM- and OKM-reprogrammed MEFs at the indicated time points during reprogramming. **(F)** Statistics of genes with expression changes under both conditions (OSKM/OKM, D9, shown in Fig 4H) with up- or downregulated RNA levels, denoted as MEF-specific genes (dark blue), iPSC-specific genes (tangerine), and other genes (gray). **(G)** Venn diagram showing the overlap of differential R-loop regions in OSKM/OKM D9 (yellow), RNaseH1^{D209N}/Flag D7 (green) from promoter (top), gene body (middle), or terminator (bottom) region. Counts of mutual or unique differential R-loop regions were shown. **(H)** Distribution of differential R-loop regions of RNaseH1^{D209N}/Flag, and OSKM/OKM. **(I)** Bar chart showing the peak size of differential R-loop regions of RNaseH1^{D209N}/Flag, and OSKM/OKM. The background peaks were used as a control.

Figure S7

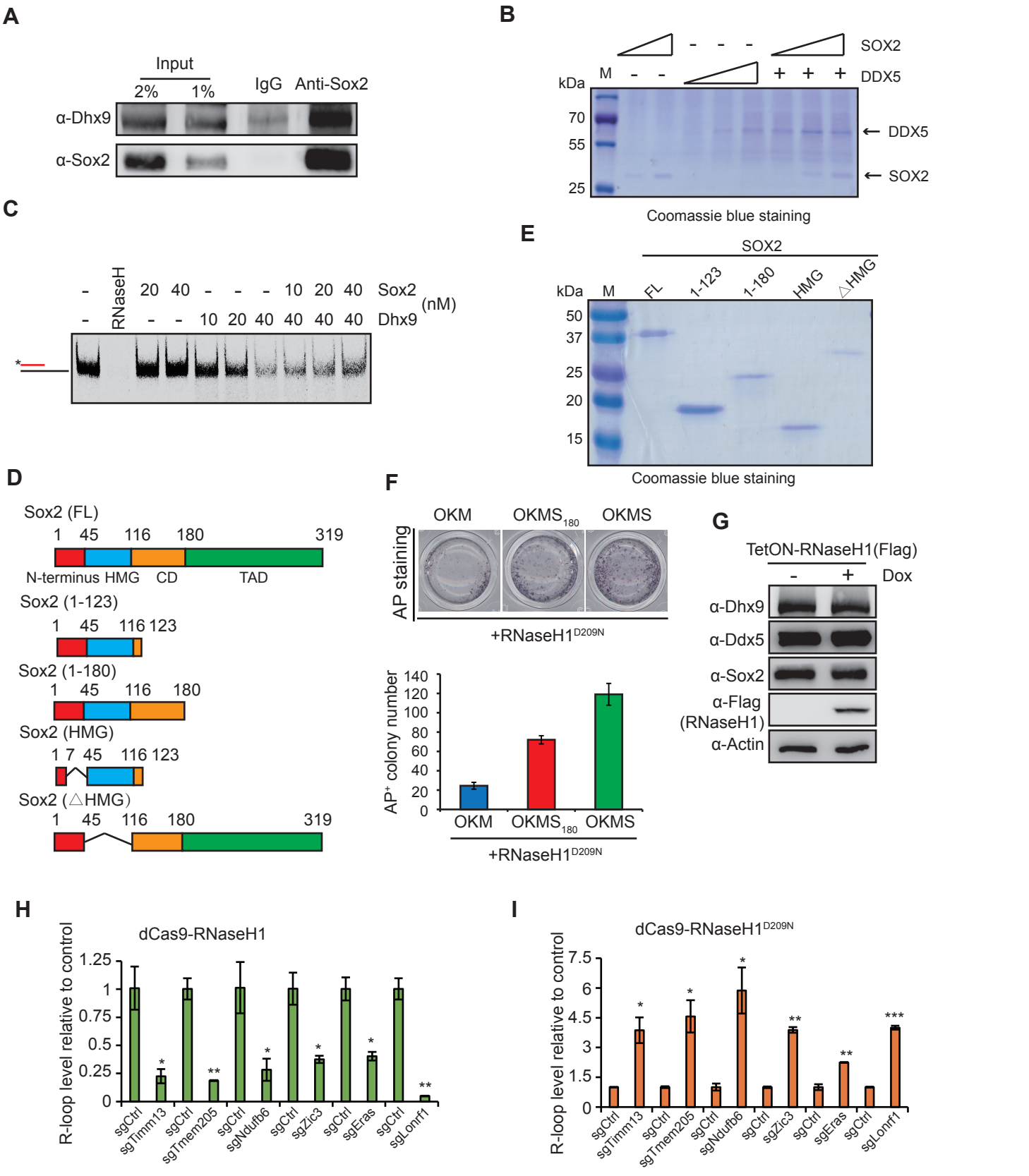
A
Input
2%
1%
IgG
Anti-Sox2
α-Dhx9
α-Sox2
B
SOX2
DDX5
kDa
M
70
55
25
DDX5
SOX2
Coomassie blue staining
C
RNaseH
Sox2
Dhx9
(nM)
E
SOX2
kDa
M
FL
1-123
1-180
HMG
△HMG
50
37
25
20
15
Coomassie blue staining
D
Sox2 (FL)
1 45 116 180 319
N-terminus HMG CD TAD
Sox2 (1-123)
1 45 116 123
Sox2 (1-180)
1 45 116 180
Sox2 (HMG)
1 7 45 116 123
Sox2 (△HMG)
1 45 116 180 319
F
OKM
OKMS180
OKMS
AP staining
+RNaseH1D209N
AP+ colony number
140
120
100
80
60
40
20
0
OKM
OKMS180
OKMS
+RNaseH1D209N
G
TetON-RNaseH1(Flag)
Dox
α-Dhx9
α-Ddx5
α-Sox2
α-Flag
(RNaseH1)
α-Actin
H
dCas9-RNaseH1
R-loop level relative to control
1.25
1
0.75
0.5
0.25
0
sgCtrl
sgTimm13
sgCtrl
sgTmem205
sgCtrl
sgNdufb6
sgCtrl
sgZic3
sgCtrl
sgEras
sgCtrl
sgLonrf1
I
dCas9-RNaseH1D209N
R-loop level relative to control
7.5
6
4.5
3
1.5
0
sgCtrl
sgTimm13
sgCtrl
sgTmem205
sgCtrl
sgNdufb6
sgCtrl
sgZic3
sgCtrl
sgEras
sgCtrl
sgLonrf1

Fig. S7. Sox2 is involved in regulation of R-loop. (A) Co-IP analysis of interaction between Sox2 and Dhx9. **(B)** Coomassie blue staining of purified recombinant mouse Sox2 and Ddx5, corresponding to Fig. 5F. M: marker. **(C)** The RNA/DNA + ssDNA substrate (25 nM) was incubated with increasing concentrations of deletions of Sox2 in the presence of Dhx9. The reaction was detected on a native polyacrylamide gel. **(D)** Schematic diagrams of deletions of Sox2 expression constructs. **(E)** Coomassie blue staining showing different deletions of recombinant mouse Sox2. (**F**) AP staining of MEFs reprogrammed with OKM, $OKMS_{180}$ or OSKM plus $RNaseH1^{D209N}$ in iCD1 medium on day 4 (top). Quantified number of AP positive colonies (botom). **(G)** Western blot to detect protein level of Dhx9, Ddx5 and Sox2 in TetON-RNaseH1 mESCs before and after adding 2 μg/mL doxcycline. **(H)** DRIP-qPCR to monitor R-loop level at the selected sites modulated by dCas9-RNaseH1 during reprogramming. **(I)** DRIP-qPCR to monitor R-loop level at the selected sites modulated by dCas9-$RNaseH1^{D209N}$.

Table S1. Sequences of shRNA and sgRNA Oligos.

shRNA

pSuper-shRNaseH1#1	GATCCCCGCCTTTGTCAGGAGCTCTTCATTCAAGAGATGAAGAGCTCCTGACAAAGGCTTTTTA
	AGCTTAAAAAGCCTTTGTCAGGAGCTCTTCATCTCTTGAATGAAGAGCTCCTGACAAAGGCGGG
pSuper-shRNaseH1#2	GATCCCCGAGAACAAGTACAGGGAAATTCAAGAGATTTCCCTGTACTTGTTCTCTTTTTA
	AGCTTAAAAAGAGAACAAGTACAGGGAAATCTCTTGAATTTCCCTGTACTTGTTCTCGGG

sgRNA

Timm13	F	CACCGTATTGTACGCATGCGCAGCG
	R	AAACCGCTGCGCATGCGTACAATAC
Ndufb6	F	CACCGAGGATAGACTAGTATGAGTC
	R	AAACGACTCATACTAGTCTATCCTC
Tmem205	F	CACCGGGCATAATCCCCGTTCCACG
	R	AAACCGTGGAACGGGGATTATGCCC
Zic3	F	CACCGGAGACCCCCTAGGCGCTACC
	R	AAACGGTAGCGCCTAGGGGGTCTCC
Eras	F	CACCGAGACTGGGGGTTTGATCGCT
	R	AAACAGCGATCAAACCCCCAGTCTC
Lonrf1	F	CACCGCCTCCACATACTCCGAAAAG
	R	AAACCTTTTCGGAGTATGTGGAGGC

Table S2. Primers Used for qPCR.

Application	Species	Gene	Forward primer(5'-3')	Reverse primer(5'-3')
RT-qPCR	Mouse	*RNaseH1*	CCAGGCCACCCCTTAAATGT	CCAGCCCTGAACCCAGTTAG
RT-qPCR	Mouse	*Gapdh*	AACTTTGGCATTGTGGAAGGGCTCA	TTGGCAGCACCAGTGGATGCAGGGA
RT-qPCR	Mouse	*Brca1*	ATGAGCTGGAGAGGATGCTG	CTGGGCAGTTGCTGTCTTCT
RT-qPCR	Mouse	*Brca2*	TCTGCCACTGTGAAAAATGC	TCAAGCTGGGCTGAAGATT
RT-qPCR	Mouse	*Bard1*	TGGTATGCCAGCCAGGAAAA	CTTCATGCAACGGTGTCCA
RT-qPCR	Mouse	*Chk1*	TGCATTTGGATTCCTGTGGC	AGTTGAACTTCTCCATAGGCAC
RT-qPCR	Mouse	*Atr*	TGCAACAAATCGGTCGATGG	GTCATGATTCGCATGGGCAC
RT-qPCR	Mouse	*Rpa2*	CAGCTTGGTGGAGTCTGCTT	TAGTCACAATCTGCGGTGGC
DRIP-qPCR	Mouse	*Sox2*	AACCAGAAGAACAGCCCGGA	CTCCTGGGCCATCTTACGC
DRIP-qPCR	Mouse	*Esrrb*	AGGAGCCATTCCATCCCATT	ATCCCAACCCTCCCTCTACA
DRIP-qPCR	Mouse	*Tfcp2l1*	GTCAGTGTTCAGAGCGAGGA	AGTTGCTCAGGGCGAGATTG
DRIP-qPCR	Mouse	*Nr5a2*	AAGGGAAGTGACAGCCCAAG	ACCCAGTAGGCAAAACGCAT
DRIP-qPCR	Mouse	*Lin28a*	GCCTACACCTTACTGGGCAC	AGTTGACCGGTAGTTGGGTG
DRIP-qPCR	Mouse	*Lrrc2*	CCCTTGCCTGAGAGCAGATT	TGTTTGCTTGCTTGTTCGCT
DRIP-qPCR	Mouse	*Slc24a3*	CCTCTTCTCTGACTATTGGTGCAT	TCGAATGTAGCCCCAGTCCT
DRIP-qPCR	Mouse	*Lvrn*	CGTCCTCAGTGTCCATCCATC	GGATGAGCAGAAGTAGGGCT
DRIP-qPCR	Mouse	*Pcdh9*	TCACAACATCATGCCATTCATC	GAGGCCTACCTAACCCTTCAC
NRO-RT-qPCR	Mouse	*Tdrd12*	ACAGAGGATGCAAGCTGGTC	ATGGTGCAACCTGTATGGGG
NRO-RT-qPCR	Mouse	*Rif1*	TGAGAGGCACGTGAAACGAT	AAAATACCGCCCTTCAGCCT
NRO-RT-qPCR	Mouse	*Gapdh*	CCCTTGATATGGTGCAACCTG	CACAAACATGGGGGCATCG

Table S3. Lists of S9.6 co-IP interactome.

Gene Name	Ensembl ID
Rpl18	ENSMUSG00000059070
Mta2	ENSMUSG00000071646
Ighv1-56	ENSMUSG00000094862
Hnrnpl	ENSMUSG00000015165
Atp5o	ENSMUSG00000022956
Prpf8	ENSMUSG00000020850
Snip1	ENSMUSG00000050213
Ilf3	ENSMUSG00000032178
Rps6	ENSMUSG00000028495
Cct3	ENSMUSG00000001416
Elavl1	ENSMUSG00000040028
Tardbp	ENSMUSG00000041459
Rps17	ENSMUSG00000061787
Smchd1	ENSMUSG00000024054
Rpl23	ENSMUSG00000071415
Abce1	ENSMUSG00000058355
Chd4	ENSMUSG00000063870
Rpl6	ENSMUSG00000029614
Mcm3	ENSMUSG00000041859
Rps4x	ENSMUSG00000031320
Utp20	ENSMUSG00000004356
Kifc1	ENSMUSG00000079553
Rfc2	ENSMUSG00000023104
Atp5f1b	ENSMUSG00000025393
L1td1	ENSMUSG00000087166
Bop1	ENSMUSG00000022557
Snrnp70	ENSMUSG00000063511
Rps3	ENSMUSG00000030744
Snrnp200	ENSMUSG00000003660
Fxr1	ENSMUSG00000027680
Rpl3	ENSMUSG00000060036
Gatad2b	ENSMUSG00000042390
Brd4	ENSMUSG00000024002
Rbmxl1	ENSMUSG00000037070
Ncl	ENSMUSG00000026234
Tuba1b	ENSMUSG00000023004
Hmga1	ENSMUSG00000046711
Ddx21	ENSMUSG00000020075
Rps20	ENSMUSG00000028234
Atad3	ENSMUSG00000029036
Vdac1	ENSMUSG00000020402
Hspa1l	ENSMUSG00000007033
Srp72	ENSMUSG00000036323
Nono	ENSMUSG00000031311
Utp15	ENSMUSG00000041747
Hnrnpr	ENSMUSG00000066037
Wdr75	ENSMUSG00000025995
Rrs1	ENSMUSG00000061024
Rpl13a	ENSMUSG00000074129
Dnajc2	ENSMUSG00000029014
Smc3	ENSMUSG00000024974
Supt16	ENSMUSG00000035726

Eif4a1	ENSMUSG00000059796
Msh6	ENSMUSG00000005370
Top2a	ENSMUSG00000020914
Krt75	ENSMUSG00000022986
Rpl32	ENSMUSG00000057841
Uqcrc2	ENSMUSG00000030884
Utp6	ENSMUSG00000035575
Imp3	ENSMUSG00000032288
Rpl17	ENSMUSG00000062328
Gm8994	ENSMUSG00000094973
Dhx15	ENSMUSG00000029169
Ftsj3	ENSMUSG00000020706
Rpl7	ENSMUSG00000043716
Nvl	ENSMUSG00000026516
Srrm2	ENSMUSG00000039218
Ptbp1	ENSMUSG00000006498
Eef1g	ENSMUSG00000071644
Utp18	ENSMUSG00000054079
Hnrnpul2	ENSMUSG00000071659
Mrpl11	ENSMUSG00000024902
Tra2a	ENSMUSG00000029817
Rpl36	ENSMUSG00000057863
Rps16	ENSMUSG00000037563
Acsl3	ENSMUSG00000032883
Atp5c1	ENSMUSG00000025781
Nop2	ENSMUSG00000038279
Vdac2	ENSMUSG00000021771
Hist2h4	ENSMUSG00000091405
Srsf7	ENSMUSG00000024097
Kif2c	ENSMUSG00000028678
Alyref	ENSMUSG00000025134
Glyr1	ENSMUSG00000022536
Hnrnpk	ENSMUSG00000021546
Kpna2	ENSMUSG00000018362
Utp3	ENSMUSG00000070697
Nat10	ENSMUSG00000027185
Rps27l	ENSMUSG00000036781
Pak1ip1	ENSMUSG00000038683
p16	ENSMUSG00000030483
Krt19	ENSMUSG00000020911
Eif6	ENSMUSG00000027613
Atp5f1a	ENSMUSG00000025428
Raly	ENSMUSG00000027593
Rpl10	ENSMUSG00000008682
Gatad2a	ENSMUSG00000036180
Sgpl1	ENSMUSG00000020097
Pin4	ENSMUSG00000079480
Mcm2	ENSMUSG00000002870
Ncapg	ENSMUSG00000015880
Dnmt1	ENSMUSG00000004099
Rbmxl2	ENSMUSG00000073894
Smc2	ENSMUSG00000028312
Igkc	ENSMUSG00000076609
Isg20l2	ENSMUSG00000048039
Phb2	ENSMUSG00000004264
Noc2l	ENSMUSG00000095567

Hnrnpm	ENSMUSG00000059208
Ddx42	ENSMUSG00000020705
Srsf5	ENSMUSG00000021134
Eef2	ENSMUSG00000034994
Ddx27	ENSMUSG00000017999
Rpsa	ENSMUSG00000032518
Hnrnpa1	ENSMUSG00000046434
Anln	ENSMUSG00000036777
Fcf1	ENSMUSG00000021243
Apc2	ENSMUSG00000020135
Poldip3	ENSMUSG00000041815
Rfc3	ENSMUSG00000033970
Gtpbp4	ENSMUSG00000021149
Vars	ENSMUSG00000007029
Eif2a	ENSMUSG00000027810
H2afy	ENSMUSG00000015937
Mocs3	ENSMUSG00000074576
GAPDH	ENSMUSG00000057666
Phf5a	ENSMUSG00000061360
Sf3a2	ENSMUSG00000020211
Rpl27a	ENSMUSG00000046364
Smpd4	ENSMUSG00000005899
Ddx5	ENSMUSG00000020719
Hnrnpc	ENSMUSG00000060373
Hspa8	ENSMUSG00000015656
Rpl21	ENSMUSG00000041453
Rps26	ENSMUSG00000025362
Ctcf	ENSMUSG00000005698
Rpl27	ENSMUSG00000063316
Rpl15	ENSMUSG00000012405
Rplp0	ENSMUSG00000067274
Wdr12	ENSMUSG00000026019
Snrpb	ENSMUSG00000027404
Hist1h2aa	ENSMUSG00000060081
Rpl7a	ENSMUSG00000062647
Mcm5	ENSMUSG00000005410
Dcaf13	ENSMUSG00000022300
Slc25a3	ENSMUSG00000061904
Krr1	ENSMUSG00000063334
Sfxn1	ENSMUSG00000021474
Rpl34	ENSMUSG00000062006
Trim28	ENSMUSG00000005566
Snrpd3	ENSMUSG00000020180
Vim	ENSMUSG00000026728
Msh2	ENSMUSG00000024151
Adnp	ENSMUSG00000051149
Aifm1	ENSMUSG00000036932
Rpf2	ENSMUSG00000038510
Slc25a4	ENSMUSG00000031633
Rrp12	ENSMUSG00000035049
Prpf40a	ENSMUSG00000061136
Hells	ENSMUSG00000025001
Srrt	ENSMUSG00000037364
Dhx8	ENSMUSG00000034931
Slc16a1	ENSMUSG00000032902
Hnrnph1	ENSMUSG00000007850

Ruvbl1	ENSMUSG00000030079
Ssrp1	ENSMUSG00000027067
Nifk	ENSMUSG00000026377
Ass1	ENSMUSG00000076441
mKIAA0034	ENSMUSG00000047126
Bms1	ENSMUSG00000030138
Ran	ENSMUSG00000029430
Rpl30	ENSMUSG00000058600
Tufm	ENSMUSG00000073838
Mki67	ENSMUSG00000031004
Ddx18	ENSMUSG00000001674
Ddx3x	ENSMUSG00000000787
Rpl22	ENSMUSG00000028936
Ezh1	ENSMUSG00000006920
Hnrnpf	ENSMUSG00000042079
Wdr36	ENSMUSG00000038299
Spout1	ENSMUSG00000039660
Prpf19	ENSMUSG00000024735
Wdr46	ENSMUSG00000024312
Rpl10a	ENSMUSG00000037805
Gm10036	ENSMUSG00000058064
Rps8	ENSMUSG00000047675
Hist1h2bq	ENSMUSG00000069307
Eif3d	ENSMUSG00000016554
Phb	ENSMUSG00000038845
Rpl4	ENSMUSG00000032399
Fyttd1	ENSMUSG00000022800
Rpl18a	ENSMUSG00000045128
Tbl3	ENSMUSG00000040688
Mcm6	ENSMUSG00000026355
Rpl8	ENSMUSG00000003970
Hnrnpa2b1	ENSMUSG00000004980
Ybx1	ENSMUSG00000028639
Tmpo	ENSMUSG00000019961
Rack1	ENSMUSG00000020372
Hdac2	ENSMUSG00000019777
Rps23	ENSMUSG00000049517
Actc1	ENSMUSG00000068614
Rps27a	ENSMUSG00000020460
Ddx51	ENSMUSG00000029504
Abcf1	ENSMUSG00000038762
Orc1	ENSMUSG00000028587
Trip12	ENSMUSG00000026219
Lbr	ENSMUSG00000004880
Actb	ENSMUSG00000029580
Lmnb1	ENSMUSG00000024590
Pdcd11	ENSMUSG00000025047
Smarca4	ENSMUSG00000032187
Uhrf1	ENSMUSG00000001228
Slc25a5	ENSMUSG00000016319
Trap1	ENSMUSG00000005981
Utp14a	ENSMUSG00000063785
Pcbp1	ENSMUSG00000051695
Cpsf7	ENSMUSG00000034820
Mcm4	ENSMUSG00000022673
Rrp15	ENSMUSG00000001305

Rpl19	ENSMUSG00000017404
Zc3h18	ENSMUSG00000017478
Baz2a	ENSMUSG00000040054
Rfc1	ENSMUSG00000029191
Srp68	ENSMUSG00000020780
Imp4	ENSMUSG00000026127
Tdh	ENSMUSG00000021953
Dhx9	ENSMUSG00000042699
Smarca5	ENSMUSG00000031715