Molecular Cell, Volume *74*

Supplemental Information

TAF5L and TAF6L Maintain Self-Renewal of Embryonic Stem Cells via the MYC Regulatory Network

Davide Seruggia, Martin Oti, Pratibha Tripathi, Matthew C. Canver, Lucy LeBlanc, Dafne C. Di Giammartino, Michael J. Bullen, Christian M. Nefzger, Yu Bo Yang Sun, Rick Farouni, Jose M. Polo, Luca Pinello, Effie Apostolou, Jonghwan Kim, Stuart H. Orkin, and Partha Pratim Das

SUPPLEMENTAL INFORMATION

TAF5L and TAF6L maintain self-renewal of embryonic stem cells via the MYC regulatory network

Davide Seruggia, Martin Oti, Pratibha Tripathi, Matthew C. Canver, Lucy LeBlanc, Dafne C. Di Giammartino, Michael J. Bullen, Christian M. Nefzger, Yu Bo Yang Sun, Rick Farouni, Jose M. Polo, Luca Pinello, Effie Apostolou, Jonghwan Kim, Stuart H. Orkin and Partha Pratim Das

SUPPLEMENTAL FIGURES, TITLES AND LEGENDS

Figure S1

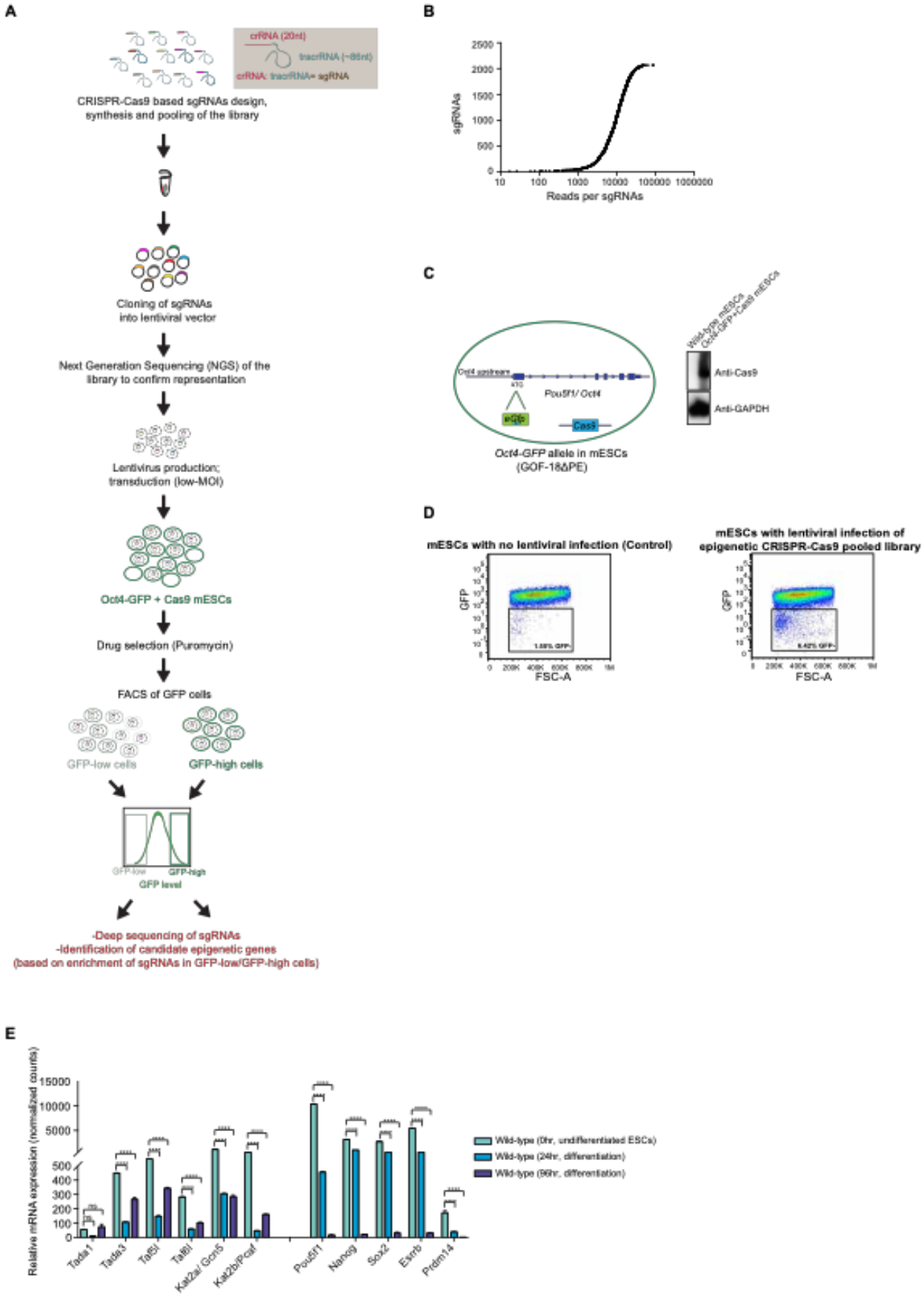

A
crRNA (20nt)
tracrRNA (~86nt)
crRNA: tracrRNA= sgRNA
CRISPR-Cas9 based sgRNAs design, synthesis and pooling of the library
Cloning of sgRNAs into lentiviral vector
Next Generation Sequencing (NGS) of the library to confirm representation
Lentivirus production; transduction (low-MOI)
Oct4-GFP + Cas9 mESCs
Drug selection (Puromycin)
FACS of GFP cells
GFP-low cells
GFP-high cells
GFP-low
GFP-high
GFP level
-Deep sequencing of sgRNAs
-Identification of candidate epigenetic genes
(based on enrichment of sgRNAs in GFP-low/GFP-high cells)
B
sgRNAs
Reads per sgRNAs
C
Oct4 upstream
ATG
Pou5f1/ Oct4
eGfp
Cas9
Oct4-GFP allele in mESCs
(GOF-18ΔPE)
Wild-type mESCs
Oct4-GFP+Cas9 mESCs
Anti-Cas9
Anti-GAPDH
D
mESCs with no lentiviral infection (Control)
mESCs with lentiviral infection of epigenetic CRISPR-Cas9 pooled library
GFP
FSC-A
E
Relative mRNA expression (normalized counts)
Tada1
Taba3
Taf5l
Taf6l
Kat2a/ Gcn5
Kat2b/Pcaf
Pou5f1
Nanog
Sox2
Esrrb
Prdm14
Wild-type (0hr, undifferentiated ESCs)
Wild-type (24hr, differentiation)
Wild-type (96hr, differentiation)

Figure S1. CRISPR-Cas9 mediated loss-of-function genetic screen identifies potential candidate epigenetic genes for the mESC state

(A) Schematic diagram represents an outline of the CRISPR-Cas9 screen.

(B) Representation of the epigenetic CRISPR-Cas9 pooled library. The pooled library comprised of 2,335 sgRNAs was synthesized and cloned into a lentiviral vector, and deep-sequenced to ensure representation of the sgRNAs. Each dot represents an sgRNA and its corresponding read count in the pooled library. Y axis: sgRNAs; X axis: number of reads per sgRNAs.

(C) *Oct4*-GFP reporter mESC line (Yeom et al., 1996 and Szabo et al., 2002) that constitutively expresses Cas9. Cas9 expression was confirmed by Western blot.

(D) FACS analysis represents percentage (%) of GFP-ve (GFP-low) cells from *Oct4*-GFP reporter mESCs either with no lentiviral infection (control) or with lentiviral infection of epigenetic CRISPR-Cas9 pooled library at low multiplicity (MOI).

(E) mRNA expression levels of selected candidate epigenetic genes (*Taf5l, Taf6l, Tada1* and *Tada3),* ESC specific genes *(Pou5f1/Oct4, Nanog, Sox2, Esrrb, Prdm14*), and HAT complex genes (*Kat2a and Kat2b*) during differentiation – from undifferentiated ESCs to differentiated state (0 to 24 to 96 hrs).

Data are represented as mean ± SEM (n = 3); p-values were calculated using ANOVA. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; and ns (non-significant).

Related to Figure 1.

Figure S2

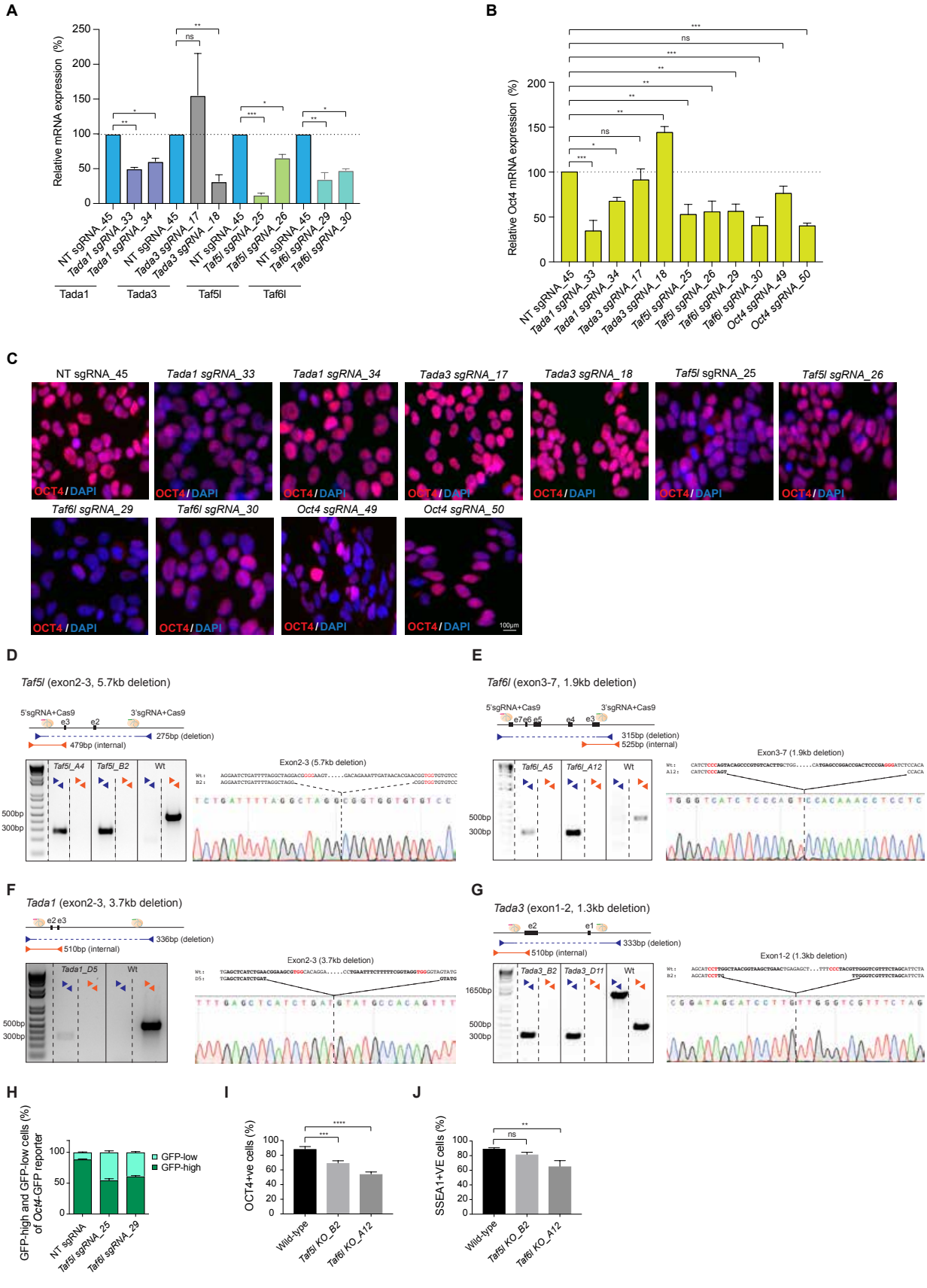

A
Relative mRNA expression (%)
NT sgRNA_45
Tada1 sgRNA_33
Tada1 sgRNA_34
NT sgRNA_45
Tada3 sgRNA_17
Tada3 sgRNA_18
NT sgRNA_45
Taf5l sgRNA_25
Taf5l sgRNA_26
NT sgRNA_45
Taf6l sgRNA_29
Taf6l sgRNA_30
Tada1
Tada3
Taf5l
Taf6l
B
Relative Oct4 mRNA expression (%)
NT sgRNA_45
Tada1 sgRNA_33
Tada1 sgRNA_34
Tada3 sgRNA_17
Tada3 sgRNA_18
Taf5l sgRNA_25
Taf5l sgRNA_26
Taf6l sgRNA_29
Taf6l sgRNA_30
Oct4 sgRNA_49
Oct4 sgRNA_50
C
NT sgRNA_45
Tada1 sgRNA_33
Tada1 sgRNA_34
Tada3 sgRNA_17
Tada3 sgRNA_18
Taf5l sgRNA_25
Taf5l sgRNA_26
Taf6l sgRNA_29
Taf6l sgRNA_30
Oct4 sgRNA_49
Oct4 sgRNA_50
OCT4 / DAPI
100μm
D
Taf5l (exon2-3, 5.7kb deletion)
5'sgRNA+Cas9
3'sgRNA+Cas9
e3
e2
275bp (deletion)
479bp (internal)
Taf5l_A4
Taf5l_B2
Wt
500bp
300bp
Exon2-3 (5.7kb deletion)
E
Taf6l (exon3-7, 1.9kb deletion)
5'sgRNA+Cas9
3'sgRNA+Cas9
e7
e6
e5
e4
e3
315bp (deletion)
525bp (internal)
Taf6l_A5
Taf6l_A12
Wt
500bp
300bp
Exon3-7 (1.9kb deletion)
F
Tada1 (exon2-3, 3.7kb deletion)
e2
e3
336bp (deletion)
510bp (internal)
Tada1_D5
Wt
500bp
300bp
Exon2-3 (3.7kb deletion)
G
Tada3 (exon1-2, 1.3kb deletion)
e2
e1
333bp (deletion)
510bp (internal)
Tada3_B2
Tada3_D11
Wt
1650bp
500bp
300bp
Exon1-2 (1.3kb deletion)
H
GFP-high and GFP-low cells (%) of Oct4-GFP reporter
GFP-low
GFP-high
NT sgRNA
Taf5l sgRNA_25
Taf6l sgRNA_29
I
OCT4+ve cells (%)
Wild-type
Taf5l KO_B2
Taf6l KO_A12
J
SSEA1+VE cells (%)
Wild-type
Taf5l KO_B2
Taf6l KO_A12

Figure S2. Validation confirms TAF5L and TAF6L are the epigenetic genes for the mESC state

(A) mRNA expression levels of Tada1, Tada3, Taf5l and Taf6l after delivery of individual sgRNAs that target coding sequences of these genes and create indels. Non-targeting sgRNA (NT sgRNA) used as control. mRNA levels were normalized to GAPDH. The two best sgRNAs (for each gene) from the screen were used.

(B) Endogenous Oct4 mRNA expression levels in the *Tada1, Tada3, Taf5l* and *Taf6l* edited cells.

(C) Immunofluorescence staining of OCT4 in the *Tada1, Tada3, Taf5l* and *Taf6l* edited cells. Scale bar is 100μm.

(D-G) Schematic diagram illustrating the strategy to generate homozygous/biallelic deletion or *KO* clones. Paired sgRNAs (5' and 3' sgRNAs) flanking critical exons of *Taf5l* (D), *Taf6l* (E), *Tada1* (F) and *Tada3* (G) were introduced to create homozygous deletions. Two PCRs (deletion PCR and internal PCR) were used for each targeted gene to identify biallelic/homozygous deletions. Sanger sequencing was used to confirm the biallelic deletions. The *KO* clones show the same deletion breakpoints in both alleles.

(H) Percentages of GFP-high and GFP-low cells of *Oct4*-GFP reporter mESCs upon delivery of non-targeting (NT) or *Taf5l* and *Taf6l* targeting sgRNAs.

(I-J) Percentages of OCT4-positive (I) and SSEA1-positive (J) cells in wild-type, *Taf5l KO* and *Taf6l* KO mESCs.

Data are represented as mean ± SEM (n = 3); p-values were calculated using ANOVA. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; and ns (non-significant).

Related to Figure 2.

Figure S3

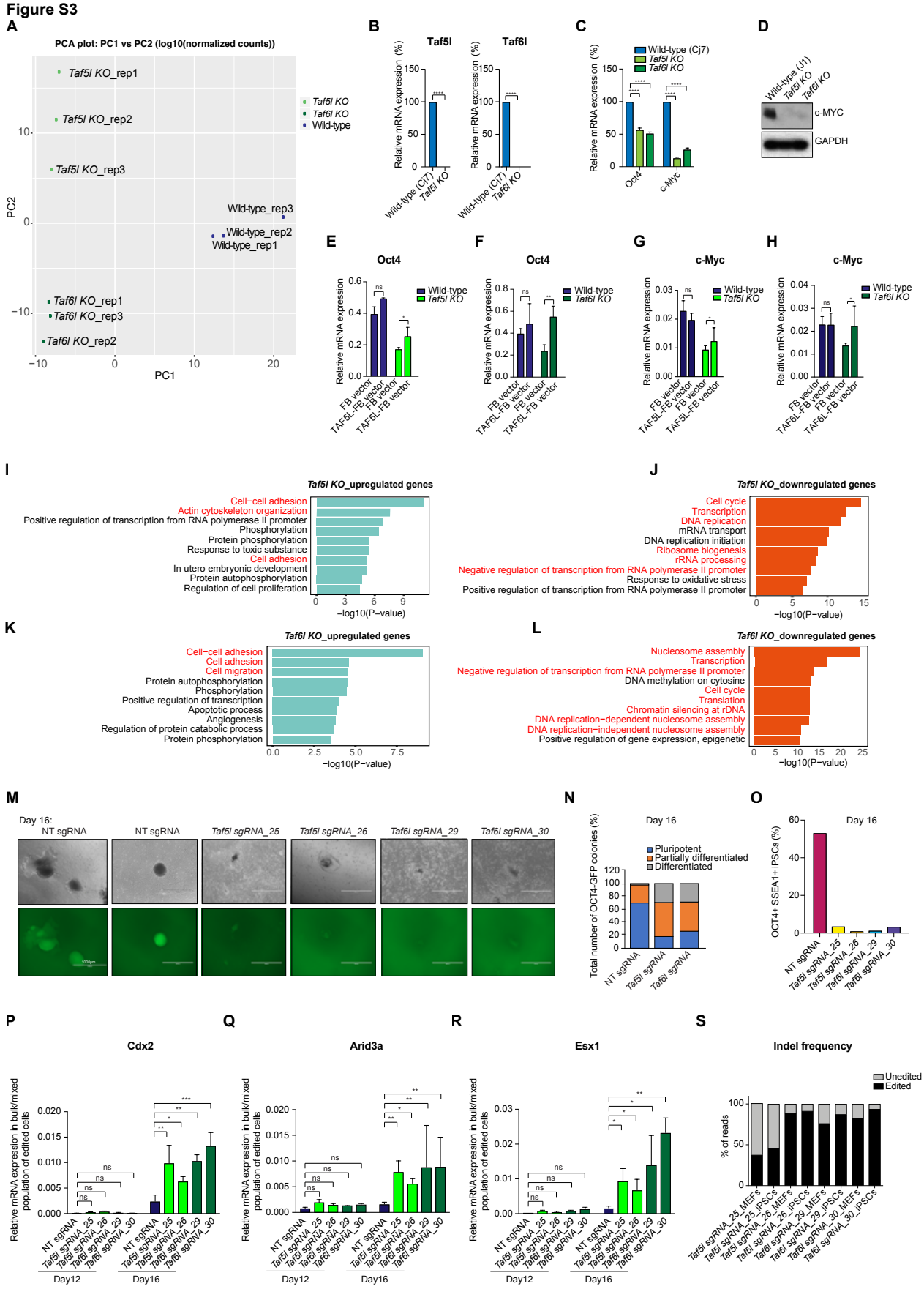

A
PCA plot: PC1 vs PC2 (log10(normalized counts))
Taf5l KO_rep1
Taf5l KO_rep2
Taf5l KO_rep3
Wild-type_rep3
Wild-type_rep2
Wild-type_rep1
Taf6l KO_rep1
Taf6l KO_rep3
Taf6l KO_rep2
Taf5l KO
Taf6l KO
Wild-type
PC1
PC2
B
Taf5l
Taf6l
Relative mRNA expression (%)
Wild-type (Cj7)
Taf5l KO
Taf6l KO
C
Wild-type (Cj7)
Taf5l KO
Taf6l KO
Relative mRNA expression (%)
Oct4
c-Myc
D
Wild-type (J1)
Taf5l KO
Taf6l KO
c-MYC
GAPDH
E
Oct4
Relative mRNA expression
Wild-type
Taf5l KO
FB vector
TAF5L-FB vector
F
Oct4
Taf6l KO
TAF6L-FB vector
G
c-Myc
H
c-Myc
I
Taf5l KO_upregulated genes
Cell−cell adhesion
Actin cytoskeleton organization
Positive regulation of transcription from RNA polymerase II promoter
Phosphorylation
Protein phosphorylation
Response to toxic substance
Cell adhesion
In utero embryonic development
Protein autophosphorylation
Regulation of cell proliferation
−log10(P−value)
J
Taf5l KO_downregulated genes
Cell cycle
Transcription
DNA replication
mRNA transport
DNA replication initiation
Ribosome biogenesis
rRNA processing
Negative regulation of transcription from RNA polymerase II promoter
Response to oxidative stress
Positive regulation of transcription from RNA polymerase II promoter
−log10(P−value)
K
Taf6l KO_upregulated genes
Cell−cell adhesion
Cell adhesion
Cell migration
Protein autophosphorylation
Phosphorylation
Positive regulation of transcription
Apoptotic process
Angiogenesis
Regulation of protein catabolic process
Protein phosphorylation
−log10(P−value)
L
Taf6l KO_downregulated genes
Nucleosome assembly
Transcription
Negative regulation of transcription from RNA polymerase II promoter
DNA methylation on cytosine
Cell cycle
Translation
Chromatin silencing at rDNA
DNA replication−dependent nucleosome assembly
DNA replication−independent nucleosome assembly
Positive regulation of gene expression, epigenetic
−log10(P−value)
M
Day 16:
NT sgRNA
NT sgRNA
Taf5l sgRNA_25
Taf5l sgRNA_26
Taf6l sgRNA_29
Taf6l sgRNA_30
N
Day 16
Pluripotent
Partially differentiated
Differentiated
Total number of OCT4-GFP colonies (%)
NT sgRNA
Taf5l sgRNA
Taf6l sgRNA
O
Day 16
OCT4+ SSEA1+ iPSCs (%)
NT sgRNA
Taf5l sgRNA_25
Taf5l sgRNA_26
Taf5l sgRNA_29
Taf6l sgRNA_30
P
Cdx2
Relative mRNA expression in bulk/mixed population of edited cells
Day12
Day16
Q
Arid3a
R
Esx1
S
Indel frequency
Unedited
Edited
% of reads
Taf5l sgRNA_25 MEFs
Taf5l sgRNA_25 iPSCs
Taf5l sgRNA_26 MEFs
Taf5l sgRNA_26 iPSCs
Taf5l sgRNA_29 MEFs
Taf5l sgRNA_29 iPSCs
Taf6l sgRNA_30 MEFs
Taf6l sgRNA_30 iPSCs

Figure S3. TAF5L and TAF6L are required for gene expression programs of mESC state; and for somatic cell reprogramming/iPSCs generation

(A) PCA plot of RNAseq data from three biological replicates of wild-type, *Taf5l KO* and *Taf6l KO.*

(B) mRNA expression levels of Taf5l and Taf6l in wild-type (CJ7) and their corresponding *KOs*.

(C) mRNA expression levels of Oct4 and c-Myc from wild-type (CJ7), *Taf5l KO* and *Taf6l KO* (from RT-qPCR).

(D) Protein levels of c-MYC in wild-type (J1) and *KOs*, using Western blot.

(E, F) Oct4 mRNA expression levels upon exogenous expression of TAF5L and TAF6L in their corresponding *KOs* and wild-type.

(G, H) c-Myc mRNA expression levels upon exogenous expression of TAF5L and TAF6L in their corresponding *KOs* and wild-type.

(I-L) Gene ontology (GO) term analysis (biological process) of up and downregulated genes from *Taf5l* and *Taf6l KOs*.

(M) Morphology and GFP fluorescence of transgene-independent iPSC colonies (at day 16 after reprogramming) that were generated upon perturbation of *Taf5l* and *Taf6l* using sgRNAs. Non-targeting sgRNA used as a control. Scale bar is 1000 μm.

(N) Percentages of pluripotent iPSCs, partially differentiated and differentiated cells after reprogramming (at day 16) upon perturbation of *Taf5l* and *Taf6l* using sgRNAs. Non-targeting sgRNA used as a control.

(O) FACS analysis of OCT4+ and SSEA1+ iPSCs at day 16.

(P-R) mRNA expression levels of Cdx2, Arid3a and Esx1 in bulk/mixed populations of edited cells after reprogramming at day 12 and day 16, upon perturbation of *Taf5l* and *Taf6l* using sgRNAs. Non-targeting sgRNA used as control.

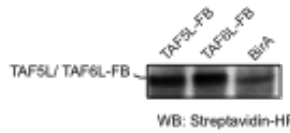
(S) Percentages of indels (insertions and deletions) of the targeted regions of *Taf5l* and *Taf6l* genes were quantified using targeted amplicon sequencing. Indel frequencies of *Taf5l* and *Taf6l* genes were measured before (from mCherry+ reprogrammable MEFs, as MEFs were transduced with viral particles of sgRNAs carrying mCherry and Cas9) and after (bulk/mixed populations of edited cells) reprogramming at day 16. Percentages indicate the edited reads over the total number of reads.

Data are represented as mean ± SEM (n = 3); p-values were calculated using ANOVA. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; and ns (non-significant).

Related to Figure 3.

Figure S4

A



B

TAF5L interacting partners identified by MS (150mM salt)

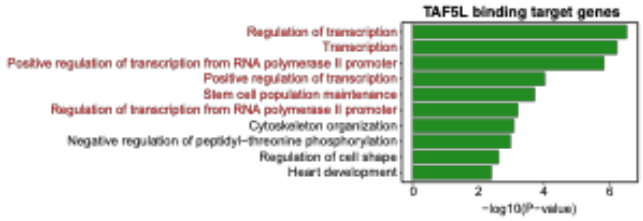
Interacting partners		TAF5L-FB (no. of peptides)	BirA (no. of petides)
Trrap	HAT	30	0
Taf6l	HAT	16	0
Taf5l	HAT	16	0
Taf9	HAT	14	0
Supt20h	HAT	11	0
Taf9b	HAT	10	0
Tada1	HAT	9	0
Tada3	HAT	8	0
Supt7l	HAT	8	0
Flna		6	0
Hnrnph1		4	0
L1td1		3	0
Supt3	HAT	3	0
Sf3b3		3	0
Sgf29		3	0
Kat2b	HAT	3	0
Atxn7l3		3	0
Taf10	HAT	3	0
Cpne8		3	0

C

TAF6L interacting partners identified by MS (150mM salt)

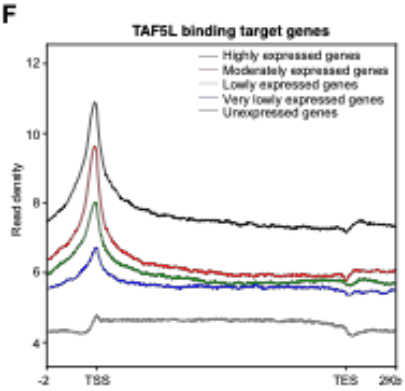
Interacting partners		TAF6L-FB (no. of peptides)	BirA (no. of petides)
Trrap	HAT	77	0
Taf6l	HAT	47	0
Taf5l	HAT	39	0
Supt7l	HAT	22	0
Supt20h	HAT	20	0
Taf9	HAT	17	0
Tada3	HAT	16	0
Taf9b	HAT	14	0
Sf3b3		12	0
Kat2b	HAT	11	0
Tdh		11	0
Supt3	HAT	11	0
Tada1	HAT	9	0
Taf10	HAT	9	0
Hadha		7	0
Atp5b		6	0
Kat2a	HAT	6	0
Tada2b	HAT	6	0
Atxn7l2		5	0
Hsp90ab1		5	0
Lonp1		4	0
Sgf29		4	0
Supt20	HAT	5	0
Trap1		3	0
Pdha1		3	0
Atad3		3	0
Pdhb		3	0
Hnrnph1		3	0
Ywhaz		3	0

D

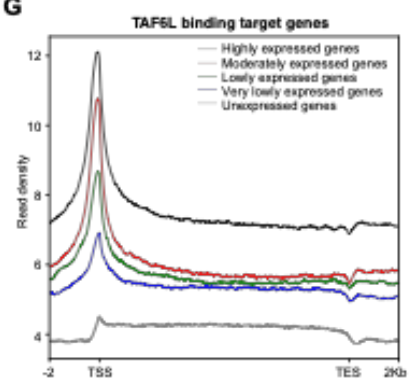


E

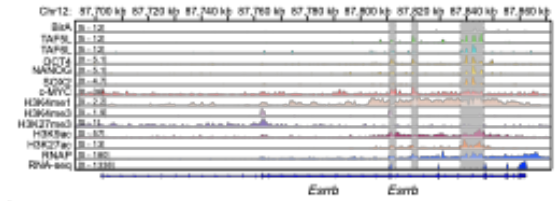
F



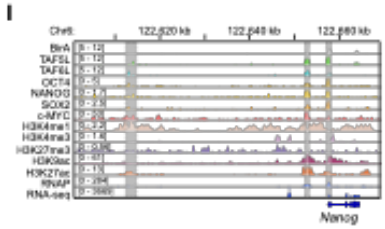
G



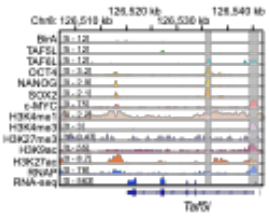
H



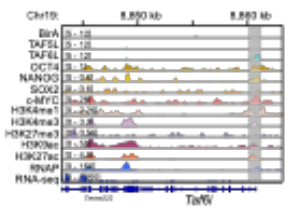
I



J



K



L

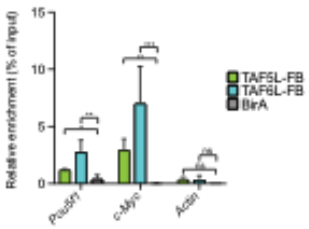


Figure S4. TAF5L and TAF6L belong to the MYC and CORE regulatory modules but mainly regulate the MYC module activity

(A) Generation of flag-biotin (FB)-tagged TAF5L and TAF6L mESC lines. The biotinylated forms of TAF5L and TAF6L were detected using streptavidin.

(B, C) Interacting partners of TAF5L and TAF6L are displayed. These interacting partners were identified through pull down of the biotinylated version of TAF5L and TAF6L using streptavidin beads, followed by mass spectrometry.

(D, E) Gene ontology (GO) term analysis (biological processes) of TAF5L and TAF6L binding target genes.

(F, G) Distribution of TAF5L and TAF6L binding target genes around the five different metagenes– ranked by their expression levels in mESCs.

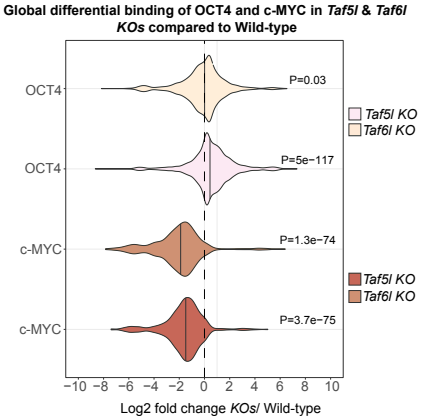
(H-K) Genomic tracks of ChIP intensities of several factors, including TAF5L and TAF6L, and histone marks binding at *Esrrb, Nanog, Taf5l* and *Taf6l* gene loci. RNAseq tracks represent expression of these genes.

(L) ChIP-qPCR analyses of TAF5L and TAF6L binding at *Oct4, c-Myc* and *Actin* gene loci. Relative enrichment is shown as % of input (all the factors multiplied with 10^6).

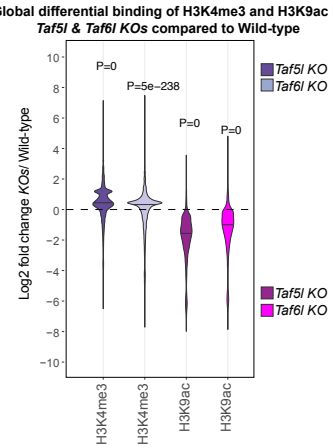
Related to Figure 4.

Figure S5

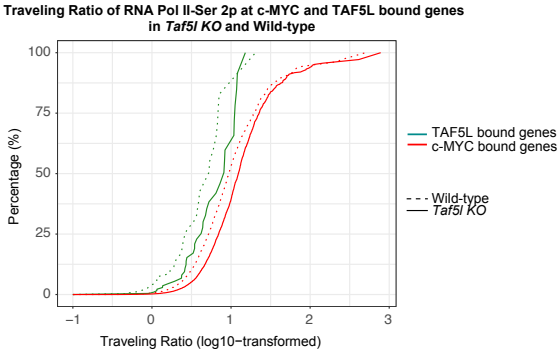
A



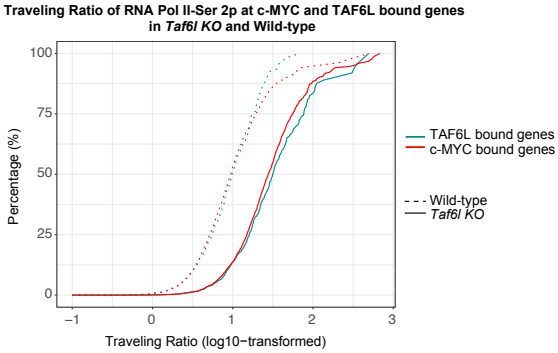
B



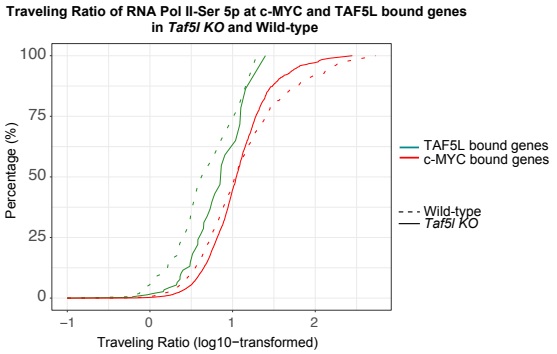
C



D



E



F

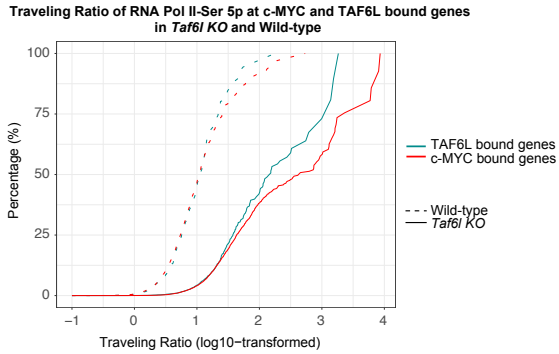


Figure S5. Predominantly TAF5L/TAF6L modulate H3K9ac deposition and c-MYC recruitment at the TAF5L/TAF6L target genes to activate their gene expression through RNA Pol II pause release

(A) Global differential binding of OCT4 and c-MYC in *Taf5l* and *Taf6l KOs* compared to wild-type.

(B) Global differential binding of H3K9ac and H3K4me3 in *Taf5l* and *Taf6l KOs* compared to wild-type.

(C) The traveling ratio (TR) of RNA Pol II-Ser 2p at c-MYC and TAF5L bound genes in *Taf5l KO* and wild-type.

(D) The traveling ratio (TR) of RNA Pol II-Ser 2p at c-MYC and TAF6L bound genes in *Taf6l KO* and wild-type.

(E) The traveling ratio (TR) of RNA Pol II-Ser 5p at c-MYC and TAF5L bound genes in *Taf5l KO* and wild-type.

(F) The traveling ratio (TR) of RNA Pol II-Ser 5p at c-MYC and TAF6L bound genes in *Taf6l KO* and wild-type.

Related to Figure 5.

Figure S6

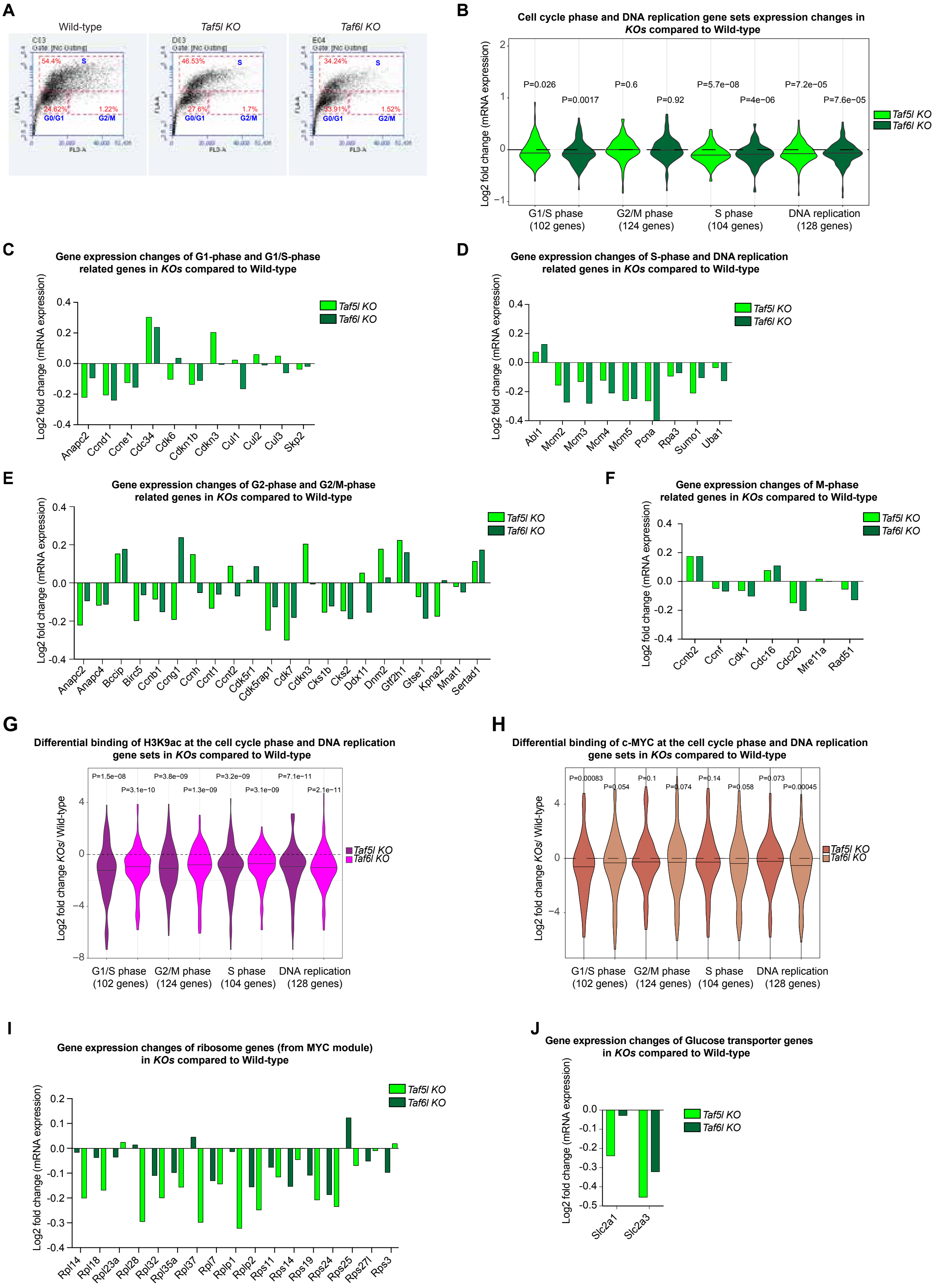

A
Wild-type
Taf5l KO
Taf6l KO
54.4%
S
24.62%
1.22%
G0/G1
G2/M
46.53%
27.6%
1.7%
34.24%
33.91%
1.52%
FL3-A
B
Cell cycle phase and DNA replication gene sets expression changes in KOs compared to Wild-type
Log2 fold change (mRNA expression)
P=0.026
P=0.0017
P=0.6
P=0.92
P=5.7e−08
P=4e−06
P=7.2e−05
P=7.6e−05
G1/S phase (102 genes)
G2/M phase (124 genes)
S phase (104 genes)
DNA replication (128 genes)
Taf5l KO
Taf6l KO
C
Gene expression changes of G1-phase and G1/S-phase related genes in KOs compared to Wild-type
Log2 fold change (mRNA expression)
Anapc2
Ccnd1
Ccne1
Cdc34
Cdk6
Cdkn1b
Cdkn3
Cul1
Cul2
Cul3
Skp2
Taf5l KO
Taf6l KO
D
Gene expression changes of S-phase and DNA replication related genes in KOs compared to Wild-type
Log2 fold change (mRNA expression)
Abl1
Mcm2
Mcm3
Mcm4
Mcm5
Pcna
Rpa3
Sumo1
Uba1
Taf5l KO
Taf6l KO
E
Gene expression changes of G2-phase and G2/M-phase related genes in KOs compared to Wild-type
Log2 fold change (mRNA expression)
Anapc2
Anapc4
Bccip
Birc5
Ccnb1
Ccng1
Ccnh
Ccnt1
Ccnt2
Cdk5r1
Cdk5rap1
Cdk7
Cdkn3
Cks1b
Cks2
Ddx11
Dnm2
Gtf2h1
Gtse1
Kpna2
Mnat1
Sertad1
Taf5l KO
Taf6l KO
F
Gene expression changes of M-phase related genes in KOs compared to Wild-type
Log2 fold change (mRNA expression)
Ccnb2
Ccnf
Cdk1
Cdc16
Cdc20
Mre11a
Rad51
Taf5l KO
Taf6l KO
G
Differential binding of H3K9ac at the cell cycle phase and DNA replication gene sets in KOs compared to Wild-type
Log2 fold change KOs/ Wild-type
P=1.5e−08
P=3.1e−10
P=3.8e−09
P=1.3e−09
P=3.2e−09
P=3.1e−09
P=7.1e−11
P=2.1e−11
G1/S phase (102 genes)
G2/M phase (124 genes)
S phase (104 genes)
DNA replication (128 genes)
Taf5l KO
Taf6l KO
H
Differential binding of c-MYC at the cell cycle phase and DNA replication gene sets in KOs compared to Wild-type
Log2 fold change KOs/ Wild-type
P=0.00083
P=0.054
P=0.1
P=0.074
P=0.14
P=0.058
P=0.073
P=0.00045
G1/S phase (102 genes)
G2/M phase (124 genes)
S phase (104 genes)
DNA replication (128 genes)
Taf5l KO
Taf6l KO
I
Gene expression changes of ribosome genes (from MYC module) in KOs compared to Wild-type
Log2 fold change (mRNA expression)
Rpl14
Rpl18
Rpl23a
Rpl28
Rpl32
Rpl35a
Rpl37
Rpl7
Rplp1
Rplp2
Rps11
Rps14
Rps19
Rps24
Rps25
Rps27l
Rps3
Taf5l KO
Taf6l KO
J
Gene expression changes of Glucose transporter genes in KOs compared to Wild-type
Log2 fold change (mRNA expression)
Slc2a1
Slc2a3
Taf5l KO
Taf6l KO

Figure S6. TAF5L/TAF6L maintain self-renewal of mESCs through MYC regulatory module/network

(A) FACS plots of cell cycle profiling from wild-type, *Taf5l KO* and *Taf6l KO*.

(B) Gene expression changes of different phases of the cell cycle and DNA replication gene sets in *Taf5l* and *Taf6l KOs* compared to wild-type.

(C-F) Gene expression changes of several individual genes related to different phases of the cell cycle and DNA replication in *Taf5l* and *Taf6l KOs* compared to wild-type.

(G, H) Differential binding of H3K9ac (G) and c-MYC (H) at the cell cycle phase and DNA replication gene sets in *Taf5l* and *Taf6l KOs* compared to wild-type.

(I) Gene expression changes of ribosome genes of MYC module in *Taf5l* and *Taf6l KOs* compared to wild-type.

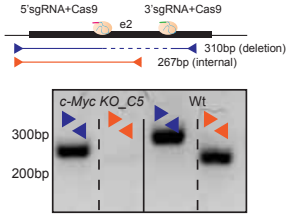
(J) mRNA expression levels of glucose transporter genes in *Taf5l* and *Taf6l KOs* compared to wild-type.

Related to Figure 6.

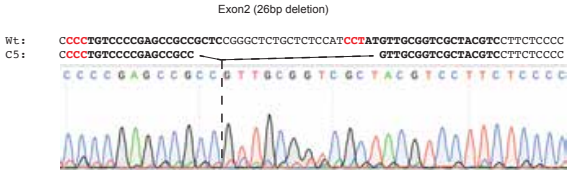
Figure S7

A

c-Myc (exon2; 26bp deletion– out-of-frame)



B

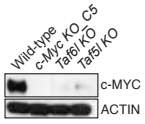


C

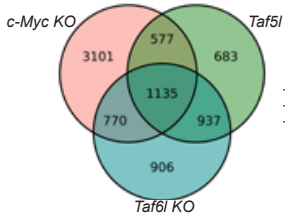
```
Wt:    MPLNVNFTNRNYDLDYDSVQPYFICDEEENFYHQQQQSELQPPA
PSEDIWKKFELLPTPPLSPSRRSGLCSPSYVAVATSFSPREDDDGGGGNFS
TADQLEMMTELLGGDMVNQSFICDPDDETFIKNIIIQDCMWSGFSAAAKLV
SEKLASYQAARKDSTSLSPARGHSVCSTSSLYLQDLTAAASECIDPSVVFP
YPLNDSSSPKSCTSSDSTAFSPSSDSLLSSESSPRASPEPLVLHEETPPTT
SSDSEEEQEDEEEIDVVSVEKRQTPAKRSESGSSPSRGHSKPPHSPLVLKR
CHVSTHQHNYAAPPSTRKDYPAAKRAKLDSGRVLKQISNNRKCSSPRSSDT
EENDKRRTHNVLERQRRNELKRSFFALRDQIPELENNEKAPKVVILKKATA
YILSIQADEHKLTSEKDLLRKRREQLKHKLEQLRNSGA*

c-Myc KO_C5:MPLNVNFTNRNYDLDYDSVQPYFICDEEENFYHQQQQSE
LQPPAPSEDIWKKFELLPTPPLSPSRRCGRYVLLPKGRR*
```

D



E



Taf5l-DE vs c-Myc-DE: p < 1e-323
Taf6l-DE vs c-Myc-DE: p < 1e-323
Taf6l-DE vs Taf5l-DE: p < 1e-323

Figure S7. TAF5L/TAF6L and c-MYC work together, as well as independently for gene regulation

(A) Strategy to generate homozygous deletion or *KO* clone of *c-Myc* in mESCs. Paired sgRNAs (5' and 3' sgRNAs) were used to target exon 2 of *c-Myc* that create homozygous deletion/*KO* of *c-Myc*. Two PCRs (deletion PCR and internal PCR) were used to identify biallelic/homozygous deletion.

(B) Sanger sequencing showing a biallelic 26bp deletion at the *c-Myc* coding sequence.

(C) The protein sequence of wild-type c-MYC, and the predicted protein sequence upon 26bp deletion of *c-Myc* gene (*c-Myc KO– clone C5*) are shown. The 26bp deletion created a frameshift mutation that resulted in a premature stop codon**.**

(D) c-MYC protein levels detected by Western blot in wild-type, *c-Myc KO*, *Taf5l KO* and *Taf6l KO* mESCs.

(E) Venn diagram illustrates overlapping and non-overlapping differentially expressed genes in *c-Myc, Taf5l* and *Taf6l KOs*.

Related to Figure 6.