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Supplemental information

The related coactivator complexes SAGA and ATAC control embryonic stem cell self-renewal through acetyltransferase-independent mechanisms

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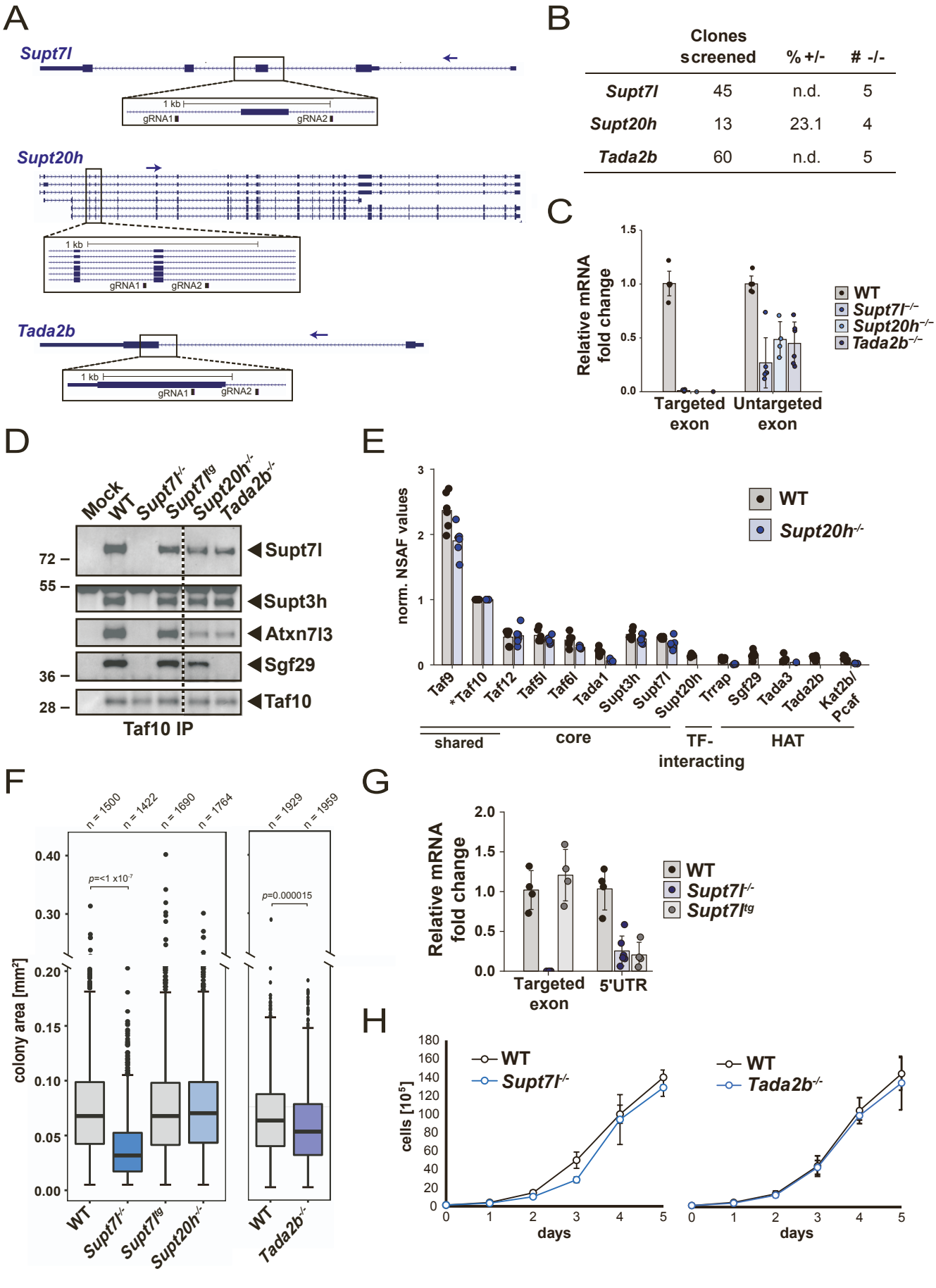
A
Supt7l
1 kb
gRNA1
gRNA2
Supt20h
1 kb
gRNA1
gRNA2
Tada2b
1 kb
gRNA1
gRNA2
B
Clones screened
% +/-
# -/-
Supt7l 45 n.d. 5
Supt20h 13 23.1 4
Tada2b 60 n.d. 5
C
Relative mRNA fold change
1.5
1.0
0.5
0.0
Targeted exon
Untargeted exon
WT
Supt7l-/-
Supt20h-/-
Tada2b-/-
D
Mock
WT
Supt7l-/-
Supt7ltg
Supt20h-/-
Tada2b-/-
72 –
55 –
36 –
28 –
Supt7l
Supt3h
Atxn7l3
Sgf29
Taf10
Taf10 IP
E
norm. NSAF values
3
2
1
0
WT
Supt20h-/-
Taf9
*Taf10
Taf12
Taf5l
Taf6l
Tada1
Supt3h
Supt7l
Supt20h
Trrap
Sgf29
Tada3
Tada2b
Kat2b/ Pcaf
shared
core
TF-interacting
HAT
F
n = 1500
n = 1422
n = 1690
n = 1764
n = 1929
n = 1959
p=<1 x10-7
p=0.000015
colony area [mm2]
0.40
0.30
0.20
0.15
0.10
0.05
0.00
WT
Supt7l-/-
Supt7ltg
Supt20h-/-
WT
Tada2b-/-
G
Relative mRNA fold change
2.0
1.5
1.0
0.5
0.0
Targeted exon
5'UTR
WT
Supt7l-/-
Supt7ltg
H
cells [10^5]
180
160
140
120
100
80
60
40
20
0
0 1 2 3 4 5
days
WT
Supt7l-/-
WT
Tada2b-/-
0 1 2 3 4 5
days

Figure S1, related to Figure 1. Generation of ESC lines inactivated for genes encoding subunits of SAGA core and HAT module. (A) Representation of the *Supt7l*, *Supt20h* and *Tada2b* loci based on UCSC genome browser views. All RefSeq transcript variants are shown. Blue arrows indicate transcription direction. Inserts highlight positions of the two gRNAs in introns flanking the first out-of-frame exon shared by all transcript variants. For *Tada2b*, the two gRNAs flank the splice acceptor site of the second and last exon. **(B)** Table showing numbers of clones screened, percentages of heterozygous (+/-) and numbers of homozygous clones (-/-). n.d., not deteremined. **(C)** RT-qPCR analyses using primers amplifying the deleted exon (left, targeted) or an untargeted exon (right). The untargeted exon primer pair revealed the induction of non-sense mediated decay (NMD). RNA polymerase III genes (*Rpph1* and *Rn7sk*) were used for normalization and results were compared to WT cells. Error bars show mean ± SD of at least 4 biological replicates, each the mean of 3 technical RT-qPCR replicates. **(D)** Western blot analysis of immunoprecipitation experiments described in Fig. 1C. **(E)** Mass spectrometry analyses of SAGA complexes purified from *Supt20h*$^{-/-}$ and WT cells. *, bait protein. **(F)** Quantification of the size of colonies analyzed by clonal assays in Fig. 1E. Colony areas were measured with ImageJ using at least two independent clones. Statistical test performed is ANOVA test. Only statistically significant ($p < 0.05$) results are indicated. **(G)** Comparison of *Supt7l* mRNA levels in *Supt7l*$^{-/-}$ and *Supt7l*^{tg} cell lines. 5'UTR primers represent the same primer pair as the untargeted primer in (C), localized within the 5' untranslated region. RNA polymerase III genes (*Rpph1* and *Rn7sk*) were used for normalization and results were compared to WT cells. Error bars show mean ± SD of at least 4 biological replicates, each the mean of 3 technical RT-qPCR replicates. **(H)** Growth curve analyses based on quantification of viable cells as assessed by trypan blue staining comparing *Supt7l*$^{-/-}$ and *Tada2b*$^{-/-}$ to WT cells in FCS+LIF+2i medium. At least two independent clones were analyzed per cell line.

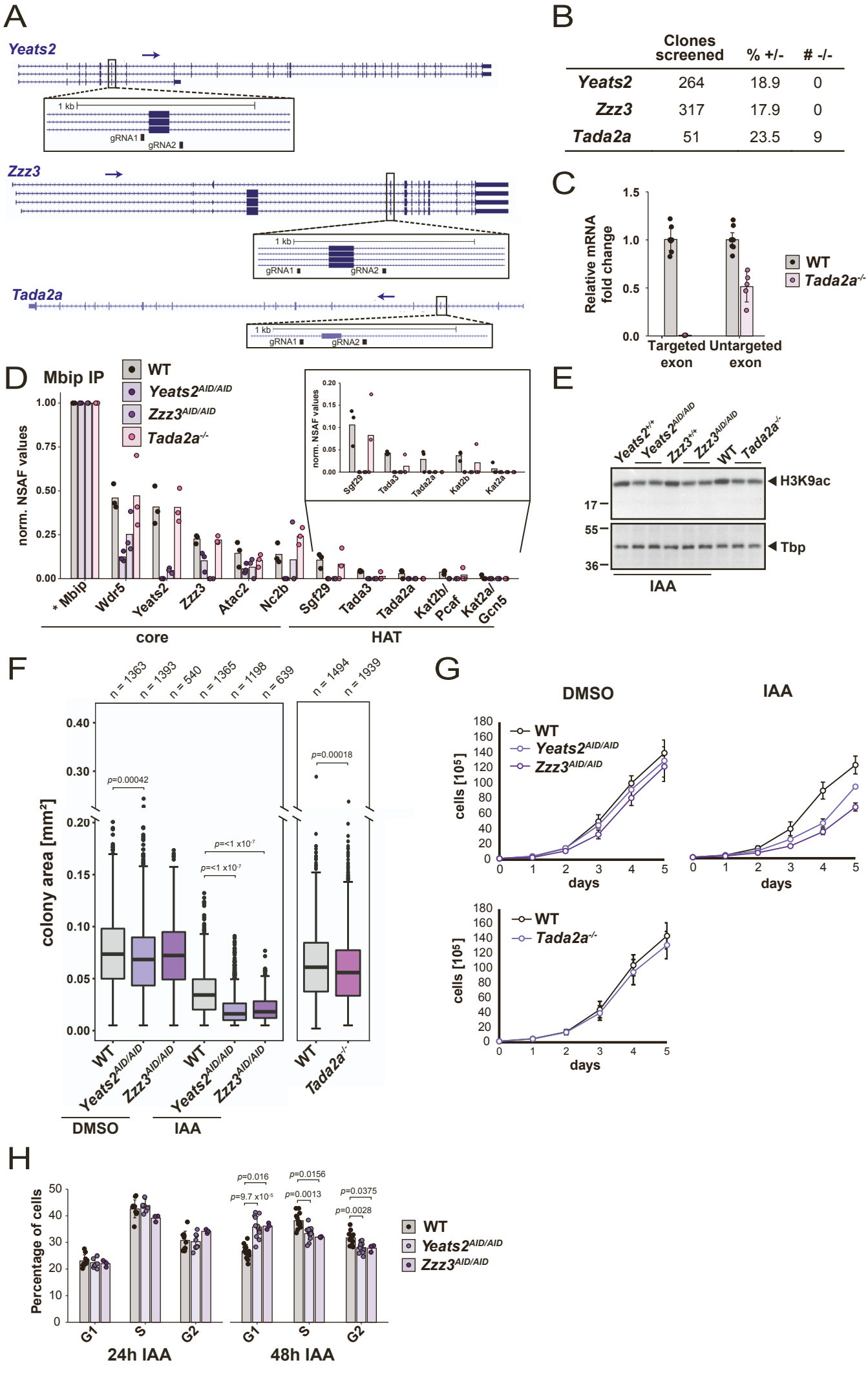
A
Yeats2
1 kb
gRNA1
gRNA2
Zzz3
Tada2a
B
Clones screened
% +/-
# -/-
Yeats2 264 18.9 0
Zzz3 317 17.9 0
Tada2a 51 23.5 9
C
Relative mRNA fold change
WT
Tada2a-/-
Targeted exon
Untargeted exon
D
Mbip IP
WT
Yeats2AID/AID
Zzz3AID/AID
Tada2a-/-
norm. NSAF values
* Mbip
Wdr5
Yeats2
Zzz3
Atac2
Nc2b
Sgf29
Tada3
Tada2a
Kat2b/ Pcaf
Kat2a/ Gcn5
core
HAT
E
Yeats2+/+
Yeats2AID/AID
Zzz3+/+
Zzz3AID/AID
WT
Tada2a-/-
H3K9ac
Tbp
IAA
F
n = 1363
n = 1393
n = 540
n = 1365
n = 1198
n = 639
n = 1494
n = 1939
colony area [mm2]
p=0.00042
p=<1 x10-7
p=0.00018
DMSO
IAA
G
DMSO
IAA
cells [105]
days
WT
Yeats2AID/AID
Zzz3AID/AID
Tada2a-/-
H
Percentage of cells
p=0.016
p=9.7 x10-5
p=0.0156
p=0.0013
p=0.0375
p=0.0028
G1
S
G2
24h IAA
48h IAA
WT
Yeats2AID/AID
Zzz3AID/AID

Figure S2, related to Figure 2. Generation of ESC lines inactivated for genes encoding subunits of ATAC core and HAT module. (A) Representation of the *Yeats2*, *Zzz3* and *Tada2a* loci based on UCSC genome browser views. All RefSeq transcript variants are shown. Blue arrows indicate transcription direction. Inserts highlight position of the two gRNAs in introns flanking the first out-of-frame exon shared by all transcript variants. **(B)** Table showing numbers of clones screened, percentages of heterozygous (+/-) and numbers of homozygous clones (-/-). **(C)** RT-qPCR analyses of $Tada2a^{-/-}$ cells using primers amplifying the deleted exon (left, targeted) or an untargeted exon (right). The untargeted exon primer pair revealed the induction of non-sense mediated decay (NMD). RNA polymerase III genes (*Rpph1* and *Rn7sk*) were used for normalization and results were compared to WT cells. Error bars show mean ± SD of at least 5 biological replicates each the mean of 3 technical RT-qPCR replicates. **(D)** Mass spectrometry analyses of Mbip immunoprecipitations (IP) of the ATAC complexes purified from $Yeats2^{AID/AID}$, $Zzz3^{AID/AID}$, $Tada2a^{-/-}$ and WT cells. *, bait protein. **(E)** Western blot analyses of H3K9ac levels examined in two independent $Yeats2^{AID/AID}$, $Zzz3^{AID/AID}$ and $Tada2a^{-/-}$ cells lines and compared to WT cells. Tbp serves as a loading control. AID cell lines were treated for 24 hours with IAA. (**F**) Quantification of the sizes of colonies analyzed by clonal assays in Fig. 2F. Colony areas were measured with ImageJ, using at least two independent clones. Statistical test performed is ANOVA test. Only statistically significant ($p < 0.05$) results are indicated. **(G)** Growth curve analyses based on quantification of viable cells as assessed by trypan blue staining from $Yeats2^{AID/AID}$, $Zzz3^{AID/AID}$ and $Tada2a^{-/-}$ cell lines compared to WT cells in the absence (DMSO) or presence of auxin (IAA) in FCS+LIF+2i medium. At least two independent clones were analyzed per cell line. **(H)** Cell cycle analyses of $Yeats2^{AID/AID}$ and $Zzz3^{AID/AID}$ cell lines were performed using propidium iodide staining after 24 hours (left panel) or 48 hours (right panel) IAA treatment and compared to WT cells. At least two independent clones were analyzed per cell line. Error bars show mean ± SD of at least 4 biological replicates. Statistical test performed is Wilcoxon rank sum test with Benjamini-Hochberg correction for multiple testing. Only statistically significant ($p < 0.05$) results are indicated.

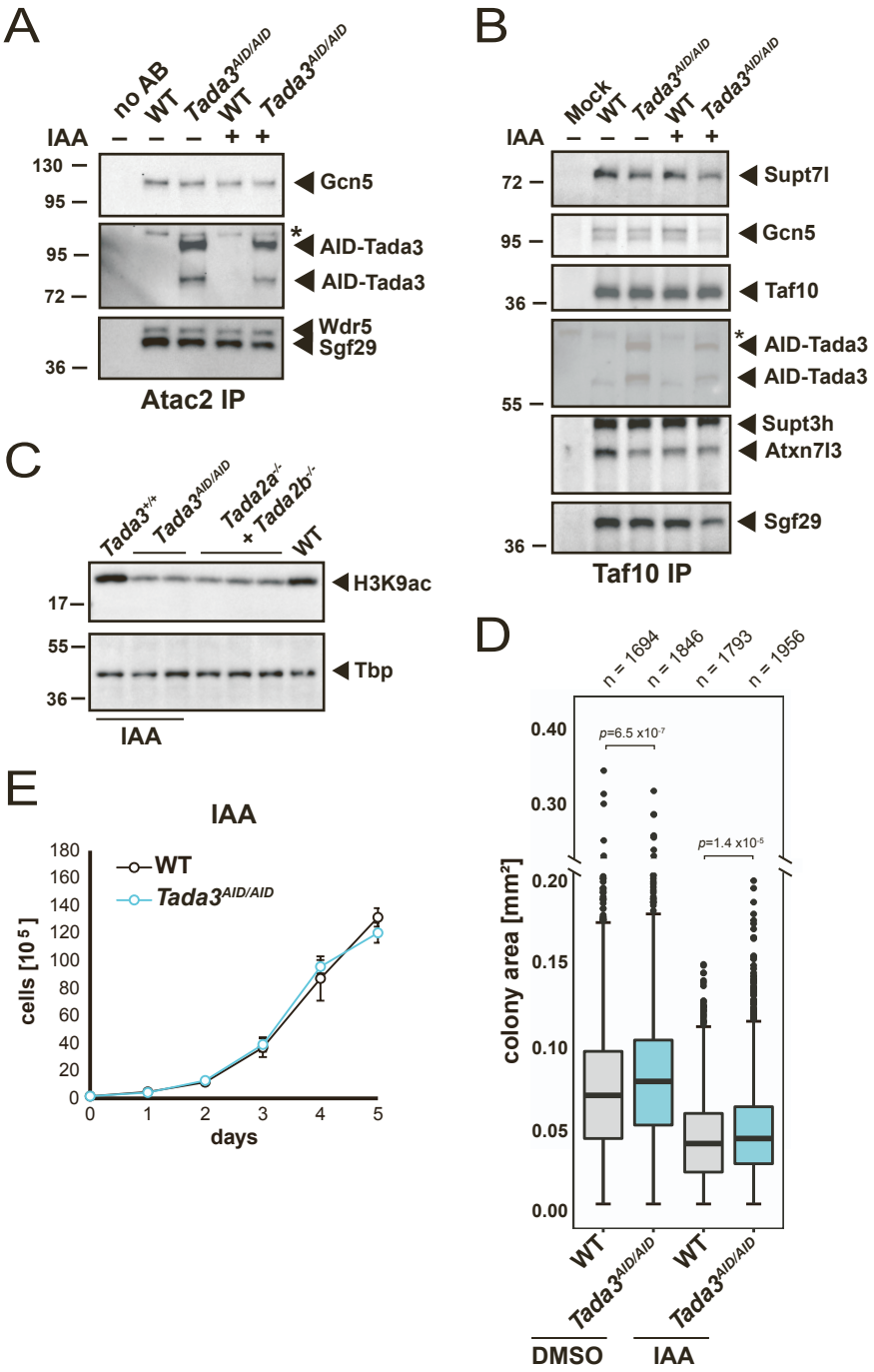
A
no AB
WT
Tada3AID/AID
WT
Tada3AID/AID
IAA – – – + +
130
95
Gcn5
95
72
*
AID-Tada3
AID-Tada3
36
Wdr5
Sgf29
Atac2 IP
B
Mock
WT
Tada3AID/AID
WT
Tada3AID/AID
IAA – – – + +
72
Supt7l
95
Gcn5
36
Taf10
55
*
AID-Tada3
AID-Tada3
Supt3h
Atxn7l3
36
Sgf29
Taf10 IP
C
Tada3+/+
Tada3AID/AID
Tada2a-/- + Tada2b-/-
WT
17
H3K9ac
55
Tbp
36
IAA
D
n = 1694
n = 1846
n = 1793
n = 1956
p=6.5 x10-7
p=1.4 x10-5
0.40
0.30
0.20
0.15
0.10
0.05
0.00
colony area [mm2]
WT
Tada3AID/AID
WT
Tada3AID/AID
DMSO
IAA
E
IAA
WT
Tada3AID/AID
cells [10 5]
180
160
140
120
100
80
60
40
20
0
0
1
2
3
4
5
days

Figure S3, related to Figure 3. Generation of *Tada3*$^{AID/AID}$ cell lines. (A,B) ATAC (A) and SAGA (B) complexes were immunopurified from WT or *Tada3*$^{AID/AID}$ cells treated with DMSO or IAA for 24 hours and analyzed by western blot. **(C)** Western blot analyses of H3K9ac levels examined in two independent *Tada3*$^{AID/AID}$ cell lines and three independent *Tada2a*$^{-/-}$ + *Tada2b*$^{-/-}$ double mutant clones compared to WT cells. Tbp serves as a loading control. AID cell lines were treated for 24 hours with IAA. **(D)** Quantification of colony areas in clonal assays of *Tada3*$^{AID/AID}$ cells as shown in Fig. 3D. Colony areas were measured using ImageJ. At least two independent clones were analyzed per cell line. Statistical test performed is two-sided Welch t-test. **(E)** Growth curve analyses based on quantification of viable cells as assessed by trypan blue staining from Tada3$^{AID/AID}$ cell lines compared to WT cells in the presence of auxin (IAA) in FCS+LIF+2i medium. At least two independent clones were analyzed per cell line.

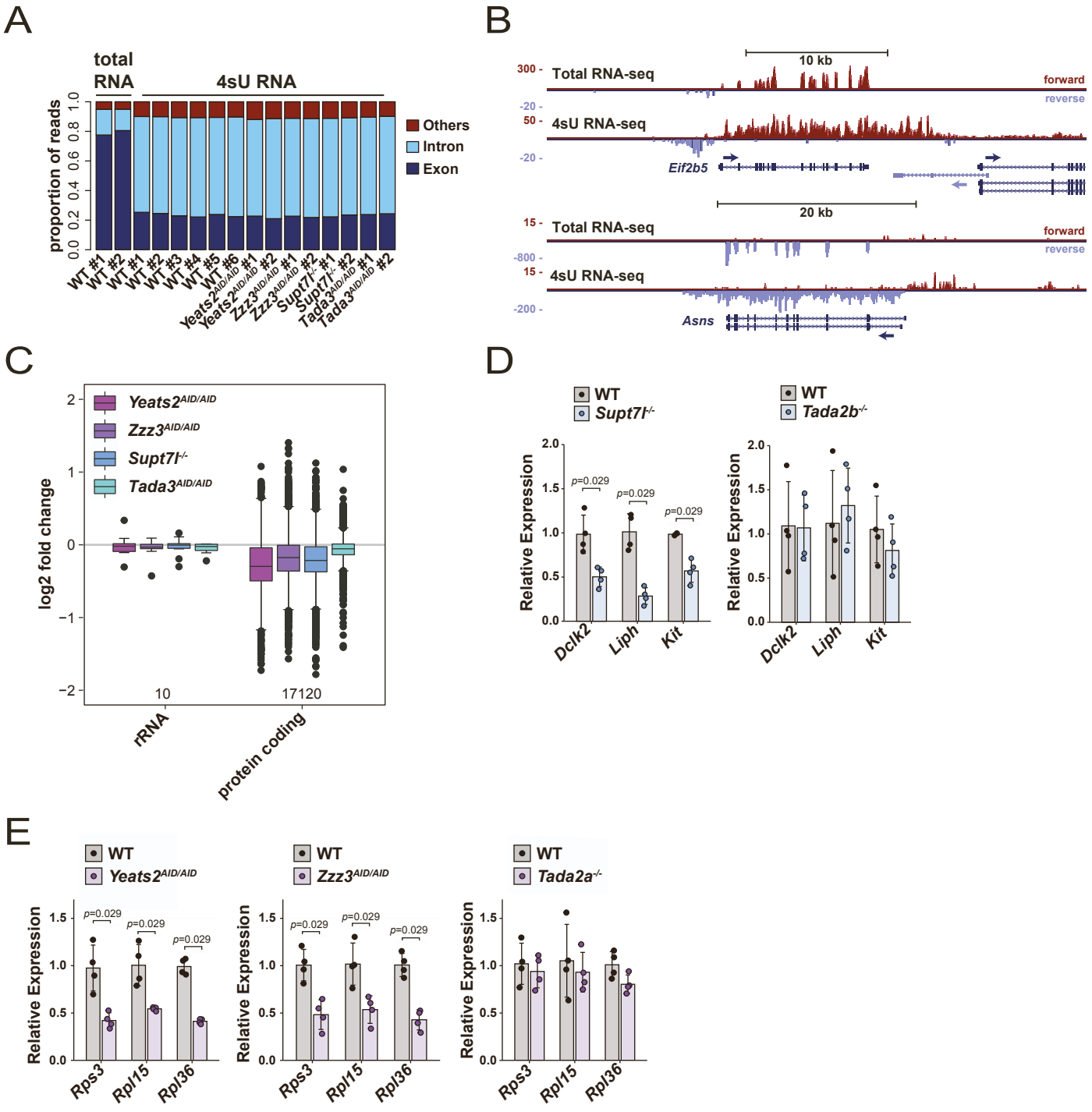
A
total RNA
4sU RNA
proportion of reads
Others
Intron
Exon
WT #1
WT #2
WT #1
WT #2
WT #3
WT #4
WT #5
WT #6
Yeats2AID/AID #1
Yeats2AID/AID #2
Zzz3AID/AID #1
Zzz3AID/AID #2
Supt7l-/- #1
Supt7l-/- #2
Tada3AID/AID #1
Tada3AID/AID #2
B
10 kb
Total RNA-seq
forward
reverse
4sU RNA-seq
Eif2b5
20 kb
Total RNA-seq
forward
reverse
4sU RNA-seq
Asns
C
Yeats2AID/AID
Zzz3AID/AID
Supt7l-/-
Tada3AID/AID
log2 fold change
10
17120
rRNA
protein coding
D
WT
Supt7l-/-
Relative Expression
p=0.029
p=0.029
p=0.029
Dclk2
Liph
Kit
WT
Tada2b-/-
Relative Expression
Dclk2
Liph
Kit
E
WT
Yeats2AID/AID
Relative Expression
p=0.029
p=0.029
p=0.029
Rps3
Rpl15
Rpl36
WT
Zzz3AID/AID
Relative Expression
p=0.029
p=0.029
p=0.029
Rps3
Rpl15
Rpl36
WT
Tada2a-/-
Relative Expression
Rps3
Rpl15
Rpl36

Figure S4, related to Figure 4. Quantification of newly synthesized mRNAs from mutant ESC cell lines. (A) Proportion of reads found on genomic elements for total RNA-seq and 4sU RNA-seq experiments shown in Fig. 4. Besides reads aligning against exons and introns, reads matching exon-intron junctions, exon-intergenic junctions and intergenic regions are represented as 'others'. **(B)** Genome browser views comparing total RNA-seq and 4sU RNA-seq results at two loci (*Eif2b5*, top panel and *Asns*, bottom panel). Blue arrows indicate transcription direction. Forward and reverse strands are shown. **(C)** Boxplot representation of log2 fold changes for rRNA and protein coding genes of 4sU-seq experiments of the different mutant cell lines. A threshold of 1 read was used. **(D, E)** Quantification of purified 4sU-labelled, newly synthesized mRNAs by RT-qPCR in *Supt7l*$^{-/-}$ and *Tada2b*$^{-/-}$ cell lines (D) and in *Yeats2*$^{AID/AID}$, *Zzz3*$^{AID/AID}$ and *Tada2a*$^{-/-}$ cell lines (E). Genes identified as significantly downregulated by 4sU RNA-seq in *Supt7l*$^{-/-}$ or *Yeats2*$^{AID/AID}$ and *Zzz3*$^{AID/AID}$ were selected for analyses. mRNA levels were normalized to the expression of at least two genes from the spike-in mRNA and results were compared relative to those in WT cells. Error bars show mean ± SD of four biological replicates each the mean of 3 technical RT-qPCR replicates. Statistical test performed is two-sided Wilcoxon-Mann-Whitney test. Only statistically significant ($p < 0.05$) results are indicated.

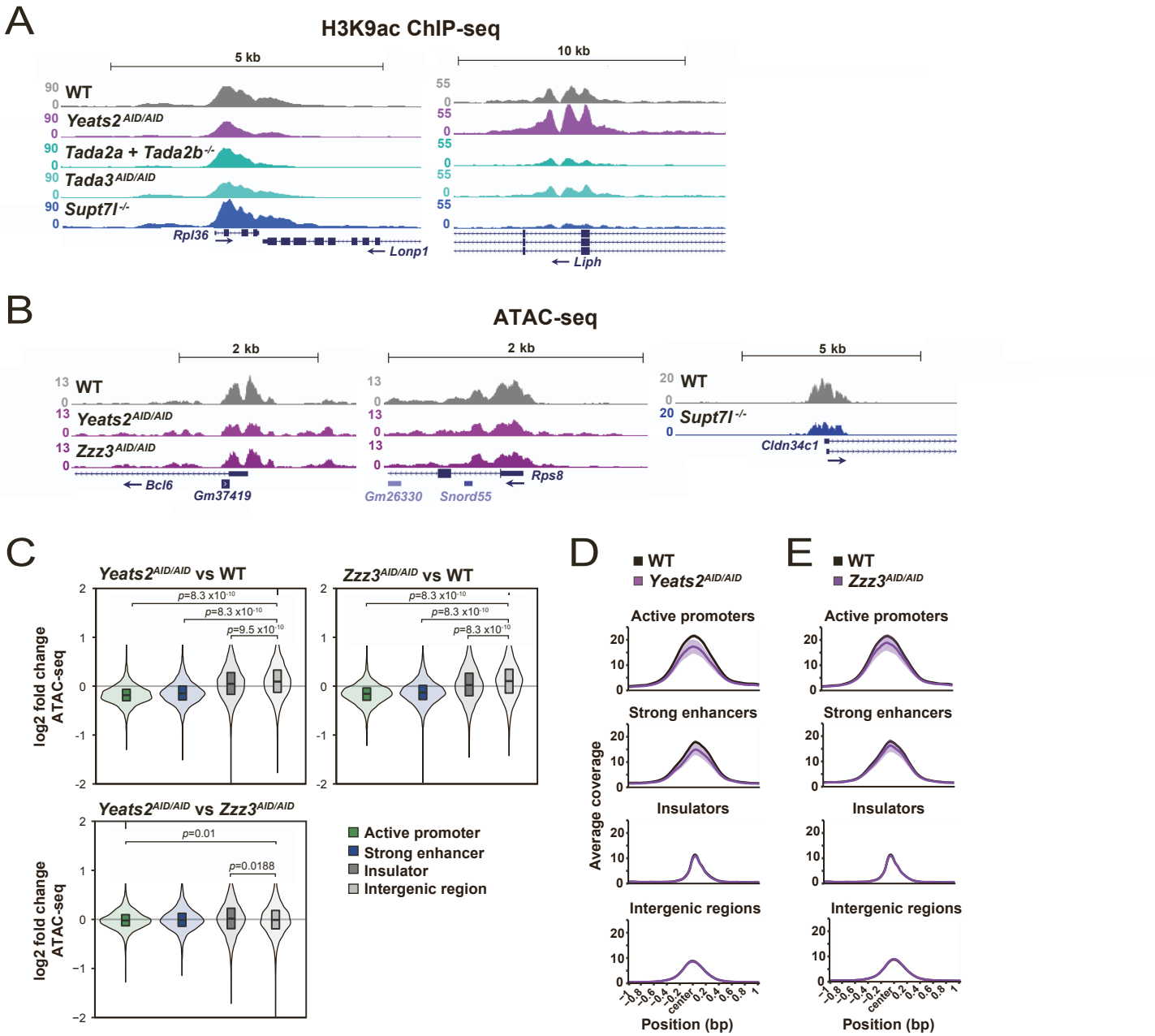
A
H3K9ac ChIP-seq
5 kb
10 kb
WT
Yeats2 AID/AID
Tada2a + Tada2b -/-
Tada3 AID/AID
Supt7l -/-
Rpl36
Lonp1
Liph
B
ATAC-seq
2 kb
2 kb
5 kb
WT
Yeats2 AID/AID
Zzz3 AID/AID
Supt7l -/-
Bcl6
Gm37419
Rps8
Gm26330
Snord55
Cldn34c1
C
Yeats2 AID/AID vs WT
Zzz3 AID/AID vs WT
Yeats2 AID/AID vs Zzz3 AID/AID
log2 fold change ATAC-seq
p=8.3 x10-10
p=9.5 x10-10
p=0.01
p=0.0188
Active promoter
Strong enhancer
Insulator
Intergenic region
D
WT
Yeats2 AID/AID
Active promoters
Strong enhancers
Insulators
Intergenic regions
Average coverage
Position (bp)
E
WT
Zzz3 AID/AID
Active promoters
Strong enhancers
Insulators
Intergenic regions
Position (bp)

Figure S5, related to Figure 5. Additional analyses of ATAC-seq and ChIP-seq datasets. (A) UCSC genome browser views of H3K9ac ChIP-seq signal in WT, *Yeats2*$^{AID/AID}$, *Tada2a*$^{-/-}$ + *Tada2a*$^{-/-}$, *Tada3*$^{AID/AID}$ and *Supt7l*$^{-/-}$ cell lines. Cells were treated for 24 hours with IAA. Blue arrows indicate transcription direction. **(B)** UCSC genome browser views of ATAC-seq signal at promoters in WT, *Yeats2*$^{AID/AID}$, *Zzz3*$^{AID/AID}$ and *Supt7l*$^{-/-}$ cell lines. Cells were treated for 24 hours with IAA. Blue arrows indicate transcription direction. **(C)** Violin plots showing log2 fold change of ATAC-seq peaks for selected chromHMM chromatin states comparing *Yeats2*$^{AID/AID}$, *Zzz3*$^{AID/AID}$ cells to WT cells or comparing *Yeats2*$^{AID/AID}$ with *Zzz3*$^{AID/AID}$ cell lines. Statistical test performed is ANOVA test. Only statistically significant ($p < 0.05$) results are indicated. **(D,E)** Metagene plots of chromatin accessibility centered on ATAC-seq peaks of the indicated regions comparing WT cells (black lines) to *Yeats2*$^{AID/AID}$ (purple lines) (D) or to *Zzz3*$^{AID/AID}$ cells (purple lines) (E).

Supplemental Table 1, related to STAR methods: CRISPR-Cas9 gRNA table. The following table shows the gRNA sequences used to generate the cell lines presented in this study including the type of modification, the targeted gene, the targeted region of the gene, the sequence of the gRNA, the PAM sequence and the strand of the gRNA. KO, knockout; AID, auxin-inducible degron; E, exon.

Type	Target	Region	Sequence	PAM	Strand
KO	*Supt20h*	5'E4	TCGCTTGCACTCACTCGT	AGG	+
		3'E4	GTAGAGCAGTCCAGTCGG	AGG	-
	Supt7l	5'E3	ACCAGTACGTATTCAGAG	TGG	+
		3'E3	ACCATCTCCCTCGCCCCG	AGG	+
	Tada2b	5'E2	CCTACATAGATGTACCTGAG	CGG	-
		E2	TTATGAGATAGAGTATGACC	AGG	+
	Tada2a	5'E3	GCTACAGGTAGTCTTCCCTG	CGG	-
		3'E3	CTGCTGTGTAGTAGACAGAG	TGG	-
	Yeats2	5'E6	TCACTGAAACAGTATTCAGT	AGG	-
		3'E6	CCGTTACTGCATATTCACAG	TGG	+
	Zzz3	5'E5	GACTAGGTACTTCGTAACTC	AGG	+
		3'E5	AGATATCACTGCATTACATG	GGG	-
AID	*Yeats2*	E1	TGTTCGCTTGATTCCAGACA	TGG	-
	Tada3	E1	GGAGGCCGGCCTAATCATGC	AGG	-
		E1	ACAAACCTGCATGATTAGGC	CGG	+
	Zzz3	E1	GTGTTACAAGATCAACAGTG	GGG	+
	Zzz3	Last E	TCCCAGCCAACAGATGACAT	GGG	+
HA-tagged	*Tada2a*	Last E	AGATAGACGTGAACAAAACC	CGG	-
		Last E	AAGGAATGTGAACAGTCAGA	GGG	-

Supplemental Table 2, related to STAR methods: Table of primers used for RT-qPCR analysis. This table contains the primers used for RT-qPCR analyses show in this study including the gene name and forward and reverse primer sequences. KO, knockout.

Target	Forward Primer [5` => 3`]	Reverse Primer [5` => 3`]	Information
KO validation			
Supt20h	TACATCGTGGAAAGTGCTCAG	ACCTCGGGTTCTTTTTCACAT	targeted exon
	AAGACAAACTTTTGCTTGAGAGC	CCGGTTATAGAGCAGCCTATTG	untargeted exon
Supt7l	ATTGTGGCGACTGCTTGATAG	ACCCAGAGAGTGACTTTTACCG	targeted exon
	GCAGTTCCCACATAAGAAGCA	AGCCGCGTATACCACTCCT	untargeted exon/5'UTR
Tada2b	TACATGCTAACGTAGTGCTCCATC	AGTTCGGCTTCGGCAACT	targeted exon
	CTGTTCAGTCACACCAGCTACT	CACCAGAAGATGCTGAGCAATG	untargeted exon
Tada2a	CTCTGCAGGGCGGCTTATC	CAAGGGAGATCAAGCAGCCATC	targeted exon
	TCCAGCTGGGATCAAGAACAG	TCACTCGAGGATTTGAGTACAAGA	untargeted exon
Pluripotency genes			
Pou5f1	CTAGCATTGAGAACCGTGTGAG	GATTGGCGATGTGAGTGATCT	Oct4
Sox2	GCGGAGTGGAAACTTTTGT	CGGGAAGCGTGTACTTATCCTT	
Tfcp2l1	ACTACAACCAGCACAACTCTGG	CCCATTCTCAGGAGATAGCTG	
Nanog	CTCCAGCAGATGCAAGAACTC	CTTGCACTTCATCCTTTGGTTT	
Esrrb	GAGGACTCCGCCATCAAAT	TAGTGGTAGCCAGAGGCAATGT	
Klf4	GTGGGTTAGCGAGTTGGAAA	GTGCAGCTTGCAGCAGTAAC	
House-keeping genes			
Rpph1	GGGGGAGAGTAGTCTGAATTGG	CGGAGCTTGGAACAGACTCA	
Rn7sk	TTCCCCGAATAGAGGAGGAC	TGGACCTTGAGAGCTTGTTTG	
4sU RNA-seq validation			
Dclk2	TGCTTTCTTTCACCTCACTCTTC	ACGTTAATCCGAACTGGTCTGT	
Liph	TTGGTCACATTTAAGCTCCCCA	CTCCGTGAGACTGATGCTCTAC	
Kit	ATCCATCCATCCAGCACAATCA	GTCCATCTGACAAAGTCGGGAT	
Rps3	ATCAGACCATCCACAAACTTCA	GCTTCGGTTCATTATGGAGAGT	
Rpl15	CCAGTCTTCGAGCTTTATCAGG	GACGTGATGCGCTTTCTTCT	
Rpl36	CACCAAACACACCAAGTTCG	CGCTTGTCCTTGGACACTTT	
dβTub56d	GCAGTTCACCGCTATGTTCA	CACCAGATCGTTCATGTTGC	D. melanogaster spike
dRpl12	AAGGGAACCTGCAAGGAAGT	CCCTCGTTCAGTTCGTCAATA	D. melanogaster spike
dGapdh	ACCGAACTCGTTGTCGTACC	ACCGACTTCTTCAGCGACAC	D. melanogaster spike
yAct1	ACTACCGCCGAACGTGAAAT	GGGAGGAAGATTGAGCAGCA	(Tourigny et al. 2018)
RPG time course auxin treatment			
Rps24 ns	AGTAATTGCTGAAAGGCCAGAG	CTGGTCCGGATGGTTACTGT	
Rpl29 ns	GGGCCTGTTATATGCTACCTCA	TTCTTCAGGCCTTTCTTGTTGT	
Rps5 ns	TATCTGAGGCTGGTCCCACTAC	CTTGGCATACTTCTCCTTCACA	