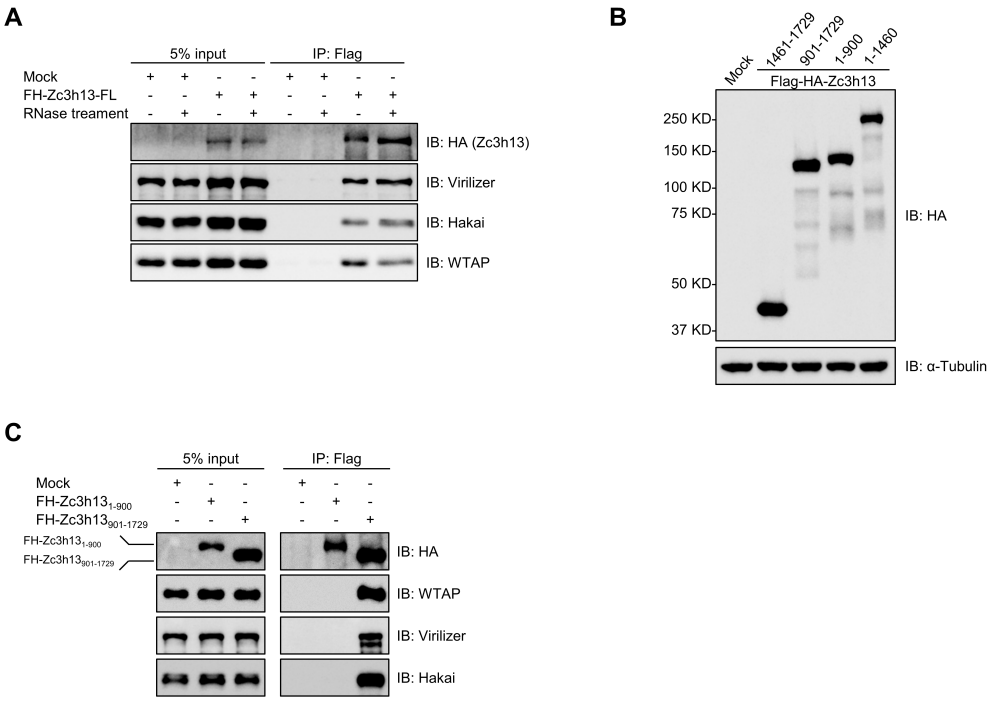
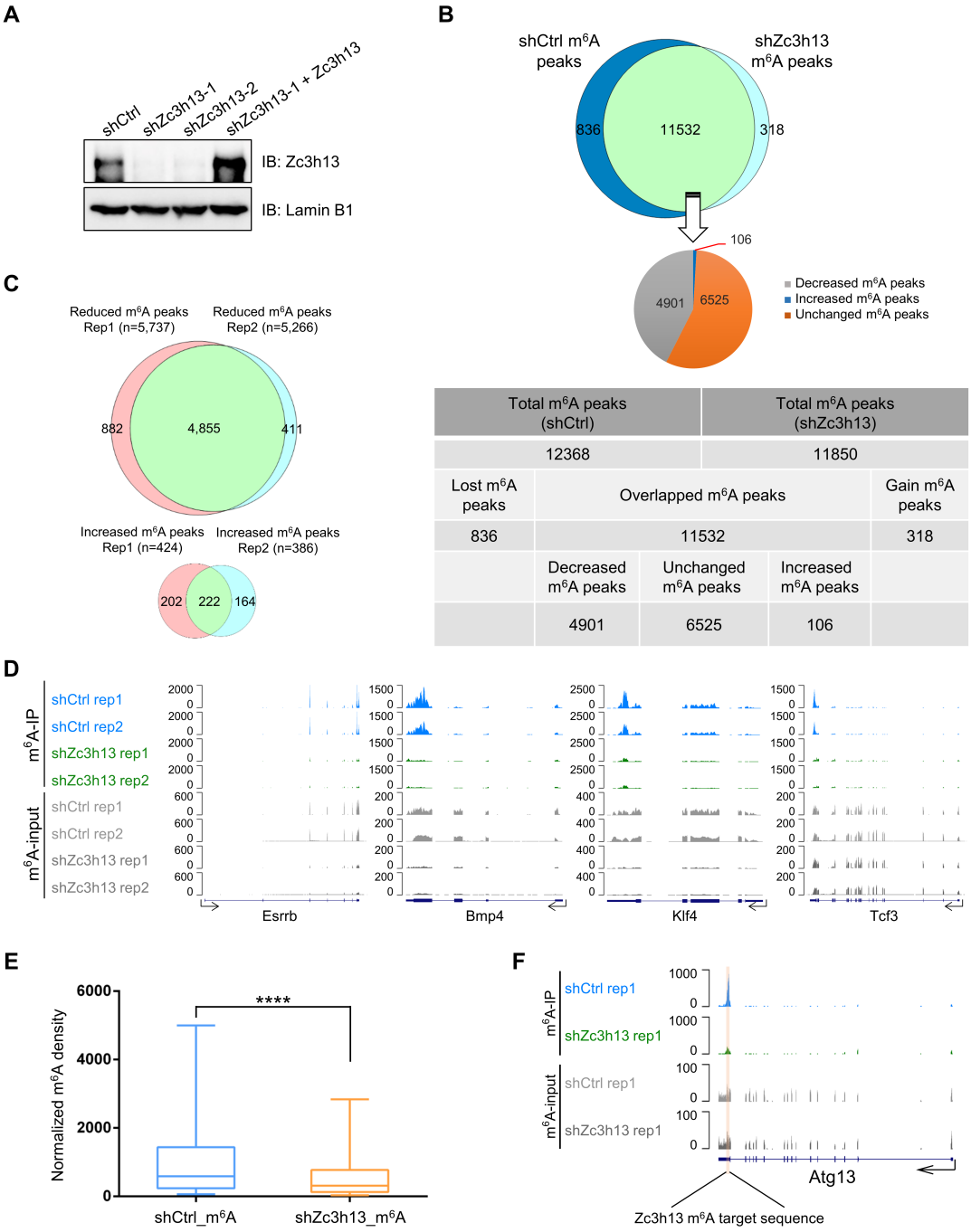


Figure S1. Zc3h13 C-terminal domain interacts with WTAP, Hakai and Virilizer. Related to Figure 1.

(A) Co-immunoprecipitation analysis showing Zc3h13 interacts with WTAP, Virilizer and Hakai in mESC with/without RNase treatment. Mock, mES cells transfected with empty vector; IP, immunoprecipitation; IB, immunoblotting.

(B) Western blot analysis of overexpression of the indicated truncated fragments of Zc3h13 with Flag-HA tag in mouse ESCs. α-Tubulin is used as a loading control.

(C) Interactions between Zc3h13 truncated fragments ($Zc3h13_{1\text{-}900}$ and $Zc3h13_{901\text{-}1729}$) and Virilizer, WTAP and Hakai were determined by co-immunoprecipitation. FH-Zc3h13, Flag-HA-Zc3h13; Mock, mES cells transfected with empty vector; IP, immunoprecipitation; IB, immunoblotting.



Total m^{6}A peaks (shCtrl)			Total m^{6}A peaks (shZc3h13)
12368			11850
Lost m^{6}A peaks	Overlapped m^{6}A peaks		Gain m^{6}A peaks
836	11532		318
	Decreased m^{6}A peaks	Unchanged m^{6}A peaks	Increased m^{6}A peaks
	4901	6525	106

Figure S2. MeRIP-seq data analysis of Zc3h13 kd and control mESCs. Related to Figure 2.

(A) Western blot analysis of Zc3h13 protein levels in the indicated mES cell lines. Lamin B1 is used as a loading control. shCtrl, control.

(B) Statistics of m^{6}A peaks from MeRIP-seq data of Zc3h13 kd and control mES cells. Venn diagram shows overlap of m^{6}A peaks between control and Zc3h13-depleted mESCs. Pie chart depicts the number of increased, decreased and unchanged m^{6}A peaks of above overlapping m^{6}A peaks. The table lists m^{6}A peak numbers, in consistent with Venn diagram and pie chart.

(C) The overlap of Zc3h13-dependent m^{6}A peaks between two MeRIP-seq replicates.

(D) UCSC snapshots of MeRIP-seq reads along the indicated mRNAs.

(E) Box plot of normalized m^6A reads density of all the m^6A peaks in control and Zc3h13 kd mESCs. ****$p < 0.0001$; t test.

(F) UCSC snapshots of MeRIP-seq reads of Zc3h13 target m^6A peaks on Atg13 mRNA. The region selected for minigene construction was highlighted by orange box.

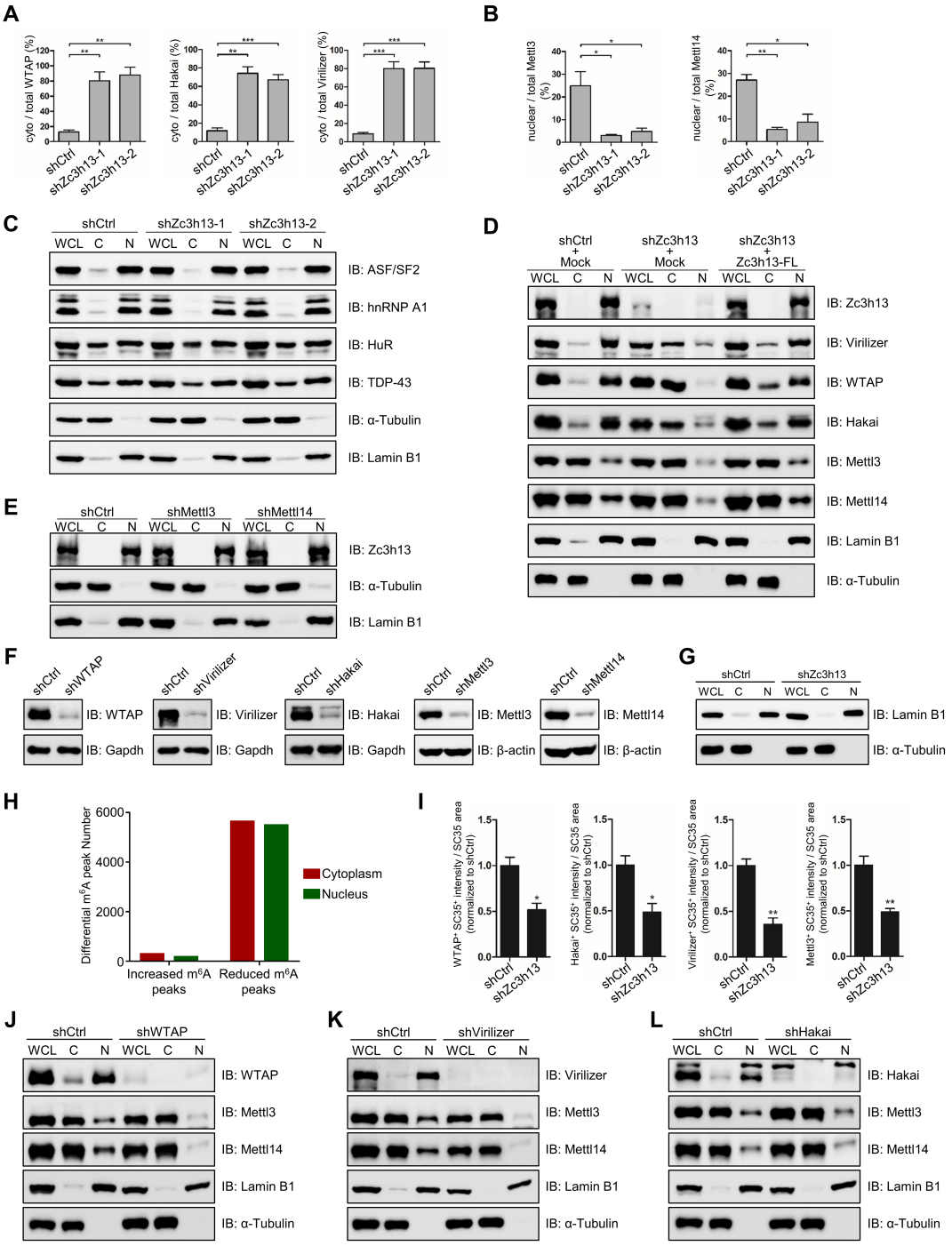


Figure S3. Inhibition Zc3h13 complex component regulates the cellular localizations of WTAP, Virilizer, Hakai, Mettl3 or Mettl14 in mESCs. Related to Figure 3.

(A) Western blot quantification of cytoplasmic WTAP, Virilizer and Hakai in Zc3h13 kd and control mESCs using Image Lab software. For each protein, the ratio of intensity of cytoplasmic fraction relative to whole cell lysate was displayed. Data are represented as mean ± SEM from three biological replicates. **p < 0.01; ***p < 0.001; t test.

(B) Western blot quantification of nuclear Mettl3 and Mettl14 in Zc3h13 kd and control mESCs using Image Lab software. For each protein, the ratio of intensity of nuclear fraction relative to whole cell

lysate was displayed. Data are represented as mean ± SEM from three biological replicates. *p < 0.05; **p < 0.01; t test.

(C) Western blot analysis of cytoplasmic and nuclear fractions of hnRNP A1, ASF/SF2, HuR and TDP-43 in Zc3h13 knockdown and control mESCs.

(D) Western blot analysis of cytoplasmic and nuclear fractions of WTAP, Virilizer, Hakai, Mettl3 and Mettl14 in Zc3h13 kd, rescue and control mESCs.

(E) Western blot analysis of cytoplasmic and nuclear fractions of Zc3h13 in Mettl3 or Mettl14 knockdown and control mESCs.

(F) Western blot analysis of WTAP, Virilizer, Hakai, Mettl3 and Mettl14 knockdown efficiency in the indicated kd mES cell lines. Gapdh or β-actin is used as a loading control. shCtrl, control.

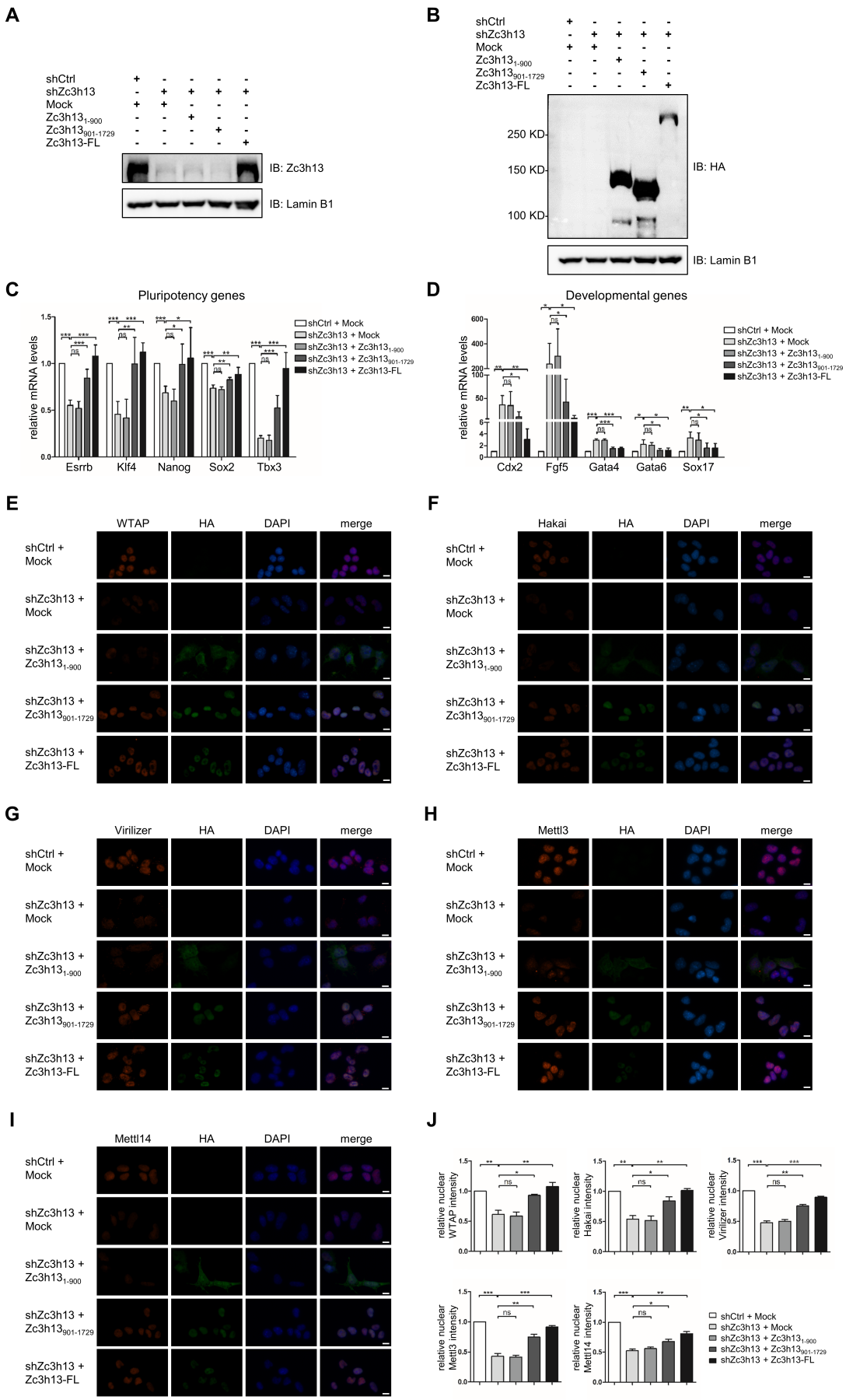
(G) Western blot analysis of cytoplasmic and nuclear fractions of Zc3h13 knockdown and control mESCs used for m^6A LC-MS/MS and MeRIP-seq.

(H) The numbers of differential m^6A peaks of cytoplasmic or nuclear fractions in Zc3h13 kd versus control mESCs.

(I) Quantification of fluorescence intensity of indicated proteins at nuclear speckles (SC35-staining region) in Zc3h13 kd and control mES cells using Image-Pro Plus software. Data are represented as mean ± SEM from three biological replicates. *p < 0.05; **p < 0.01; t test.

(J-L) Western blot analysis of cytoplasmic and nuclear fractions of Mettl3 and Mettl14 in WTAP kd (J), Virilizer kd (K), or Hakai kd (L) mESCs and control cells.

For western blot analysis of fractionation assays (C-E, G, J-L), Lamin B1 and α-Tubulin were used as nuclear and cytoplasmic markers, respectively. WCL, whole cell lysate; C, cytoplasmic fraction; N, nuclear fraction. shCtrl, control.


A
shCtrl
shZc3h13
Mock
Zc3h13 1-900
Zc3h13 901-1729
Zc3h13-FL
IB: Zc3h13
IB: Lamin B1
B
shCtrl
shZc3h13
Mock
Zc3h13 1-900
Zc3h13 901-1729
Zc3h13-FL
250 KD
150 KD
100 KD
IB: HA
IB: Lamin B1
C
Pluripotency genes
relative mRNA levels
Esrrb
Klf4
Nanog
Sox2
Tbx3
shCtrl + Mock
shZc3h13 + Mock
shZc3h13 + Zc3h13 1-900
shZc3h13 + Zc3h13 901-1729
shZc3h13 + Zc3h13-FL
D
Developmental genes
relative mRNA levels
Cdx2
Fgf5
Gata4
Gata6
Sox17
shCtrl + Mock
shZc3h13 + Mock
shZc3h13 + Zc3h13 1-900
shZc3h13 + Zc3h13 901-1729
shZc3h13 + Zc3h13-FL
E
WTAP
HA
DAPI
merge
shCtrl + Mock
shZc3h13 + Mock
shZc3h13 + Zc3h13 1-900
shZc3h13 + Zc3h13 901-1729
shZc3h13 + Zc3h13-FL
F
Hakai
HA
DAPI
merge
shCtrl + Mock
shZc3h13 + Mock
shZc3h13 + Zc3h13 1-900
shZc3h13 + Zc3h13 901-1729
shZc3h13 + Zc3h13-FL
G
Virilizer
HA
DAPI
merge
shCtrl + Mock
shZc3h13 + Mock
shZc3h13 + Zc3h13 1-900
shZc3h13 + Zc3h13 901-1729
shZc3h13 + Zc3h13-FL
H
Mettl3
HA
DAPI
merge
shCtrl + Mock
shZc3h13 + Mock
shZc3h13 + Zc3h13 1-900
shZc3h13 + Zc3h13 901-1729
shZc3h13 + Zc3h13-FL
I
Mettl14
HA
DAPI
merge
shCtrl + Mock
shZc3h13 + Mock
shZc3h13 + Zc3h13 1-900
shZc3h13 + Zc3h13 901-1729
shZc3h13 + Zc3h13-FL
J
relative nuclear WTAP intensity
relative nuclear Hakai intensity
relative nuclear Virilizer intensity
relative nuclear Mettl3 intensity
relative nuclear Mettl14 intensity
shCtrl + Mock
shZc3h13 + Mock
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shZc3h13 + Zc3h13-FL

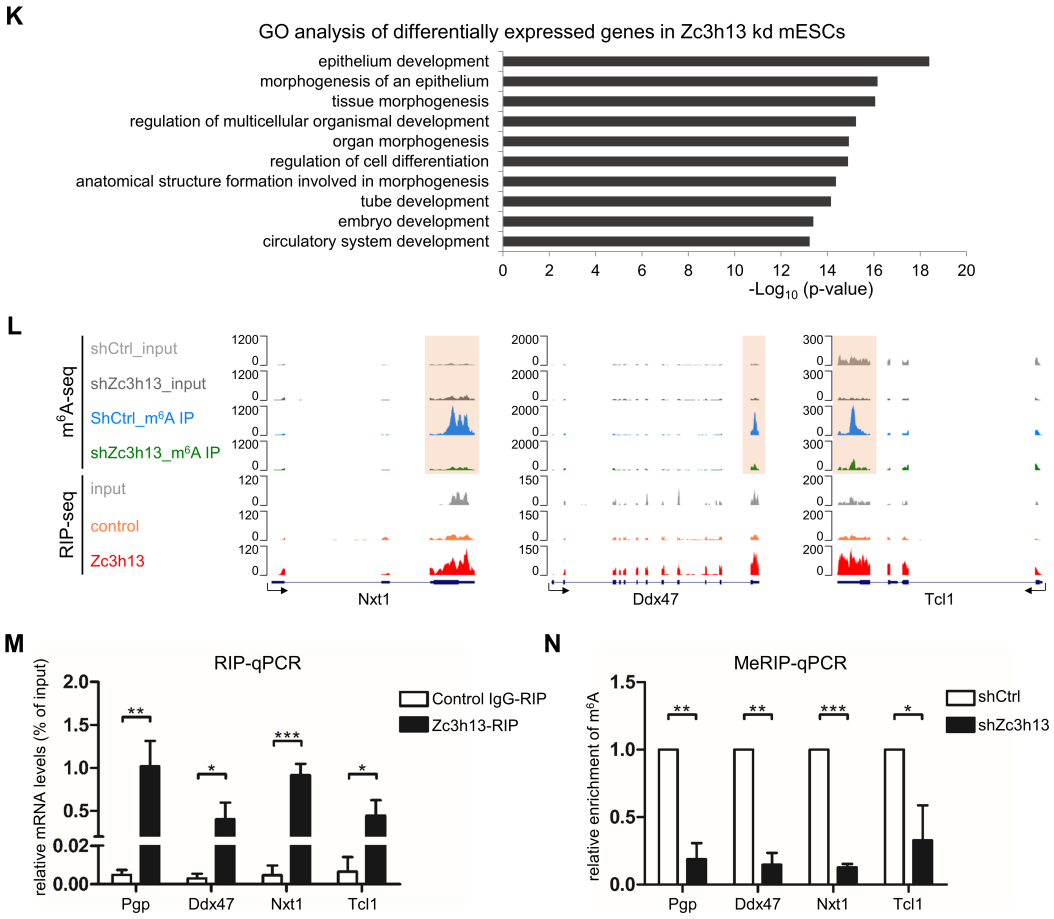

Figure S4. C-terminal domain of Zc3h13 (aa 901-1729) significantly rescues the dampened self-renewal capacity and nuclear localization of complex components in Zc3h13 knockdown mESCs. Related to Figure 4.

(A) Western blot analysis of Zc3h13 knockdown efficiency in the indicated mES cell lines. Lamin B1 is used as a loading control. ShCtrl, control; Mock, mES cells transfected with empty vector; FL, full length.

(B) Western blot analysis of overexpression of the indicated Zc3h13 truncations with Flag-HA tag in the indicated mESCs. Lamin B1 is used as a loading control.

(C and D) RT-qPCR analysis of pluripotency genes (C) and differentiation genes (D) in the indicated mES cell lines. Data are represented as mean ± SD from five biological replicates. *p < 0.05; **p < 0.01; ***p<0.001; ns, no significance; t test.

(E-I) Immunofluorescence analysis of WTAP (E), Hakai (F), Virilizer (G), Mettl3 (H), Mettl14 (I) in the indicated rescue mES cell lines. Flag-HA-Zc3h13-full length (FL), Flag-HA-Zc3h13$_{1\text{-}900}$, or Flag-HA-Zc3h13$_{901\text{-}1729}$ were overexpressed in Zc3h13 kd mESCs, detected with WTAP (red), Hakai (red), Virilizer (red), Mettl3 (red), Mettl14 (red), HA (green, Zc3h13 constructs) antibodies and DAPI (blue, cell nuclei). Mock, mES cells transfected with empty vector. Scale bar, 10 μm.

(J) Quantification of relative nuclear immunofluoresence intensity of indicated proteins in the indicated mES cell lines using Image-Pro Plus software. Data are represented as mean ± SEM from three biological replicates. *p < 0.05; **p < 0.01; ***p<0.001; ns, no significance; t test.

(K) GO biological process enrichment analysis of the 577 differentially expressed genes in Zc3h13

knockdown versus control mES cells.

(L) UCSC snapshots of MeRIP-seq and Zc3h13 RIP-seq reads along indicated mRNAs. Transcription directions are showed by arrows. UCSC ranges are shown at the left side of each track.

(M) RIP-qPCR analysis of Zc3h13 binding level of the indicated mRNAs. Data are represented as mean ± SD from three biological replicates. *$p < 0.05$; **$p < 0.01$; ***$p<0.001$; t test.

(N) MeRIP-qPCR analysis of m^6A level in the indicated mRNAs in control and Zc3h13 kd mESCs. shCtrl, control. Data are represented as mean ± SD from three biological replicates. *$p < 0.05$; **$p<0.01$; ***$p < 0.001$; t test.

Table S2. Oligonucleotides used in this study. Related to STAR Methods.

Gene Name	Primers (5'-3')	
Zc3h13 cloning primers		
ppB-Zc3h13	Forward	ATAACCGGTATGTCAAAAATTAGGAGAAAGGTC
	Reverse	ACGCGTCGACCTAGGACACACACAGCTCCTG
ppB-Zc3h13(1-900)	Forward	ATAACCGGTATGTCAAAAATTAGGAGAAAGGT
	Reverse	ACGCGTCGACTTATTTCTTGGACTTCCTCTCCTC
ppB-Zc3h13(901-1729)	Forward	ACGGACCGGTCGCTATAGAAATGAAGGGAGTC
	Reverse	ACGCGTCGACCTAGGACACACACAGCTCCTG
ppB-Zc3h13(1-1460)	Forward	ATAACCGGTATGTCAAAAATTAGGAGAAAGGTC
	Reverse	ACGCGTCGACCTACTCATGTTCACTCTCTAATTT
ppB-Zc3h13(1461-1729)	Forward	CGCACCGGTAGAGATCTGGAAGGCAGCTC
	Reverse	ACGCGTCGACCTAGGACACACACAGCTCCTG
shRNA primers		
shZc3h13-1	Forward	CCGGGACACGTGCTTTGGTTCATTTCTCGAGAAATGA ACCAAAGCACGTGTCTTTTTG
	Reverse	AATTCAAAAAGACACGTGCTTTGGTTCATTTCTCGAG AAATGAACCAAAGCACGTGTC
shZc3h13-2	Forward	CCGGCATGAGGATAGTCAGGTATTTCTCGAGAAATAC CTGACTATCCTCATGTTTTTG
	Reverse	AATTCAAAAACATGAGGATAGTCAGGTATTTCTCGAG AAATACCTGACTATCCTCATG
shWTAP	Forward	CCGGGGAAAGTACACAGATCTTAATCTCGAGATTAAG ATCTGTGTACTTTCCTTTTTG
	Reverse	AATTCAAAAAGGAAAGTACACAGATCTTAATCTCGAG ATTAAGATCTGTGTACTTTCC
shHakai	Forward	CCGGCCATTACAATCCTAACTCTTTCTCGAGAAAGAG TTAGGATTGTAATGGTTTTTG
	Reverse	AATTCAAAAACCATTACAATCCTAACTCTTTCTCGAGA AAGAGTTAGGATTGTAATGG
shVirilizer	Forward	CCGGGCGTGTTCCTTCAGCACTTGTCTCGAGACAAGT GCTGAAGGAACACGCTTTTTG
	Reverse	AATTCAAAAAGCGTGTTCCTTCAGCACTTGTCTCGAG ACAAGTGCTGAAGGAACACGC
shMettl3	Forward	CCGGCGTCAGTATCTTGGGCAAATTCTCGAGAATTTG CCCAAGATACTGACGTTTTTG
	Reverse	AATTCAAAAACGTCAGTATCTTGGGCAAATTCTCGAG AATTTGCCCAAGATACTGACG
shMett14	Forward	CCGGGCTGGACCTGGGATGATATTACTCGAGTAATATC ATCCCAGGTCCAGCTTTTTG
	Reverse	AATTCAAAAAGCTGGACCTGGGATGATATTACTCGAG TAATATCATCCCAGGTCCAGC

MeRIP-qPCR primers		
Ndc1	Forward	ACTGTGTTCATTACCCTGCTG
	Reverse	CAAGCACCACGACAAAGGAA
Tbx3	Forward	AAATAAACCACGGGCCTTCC
	Reverse	TTTAGTGCTCCCTCCCAGAC
Naa30	Forward	GGAACTGACAGCAAGGAACG
	Reverse	AACAGCTGCATGGGAATCAC
Pex11b	Forward	CTGGCTTTGAAGTTTCGGCT
	Reverse	TGAGCACATCCAGCAAGAGA
Pgp	Forward	TGCGTGTCCCAGGAGTATG
	Reverse	GTCTTCAGGCTACAGGTGGA
Ddx47	Forward	GAAAGACTTCTGCTCGCCTG
	Reverse	CAGGTGAGGAATGCAAGCTG
Nxt1	Forward	CAACAAACAGCGGGACTTCA
	Reverse	TGCTATCTTCCACACCGTGT
Tcl1	Forward	GGTTTGCATATCCCTGGCTG
	Reverse	CTTTGATTGCAGAGCGGTGT
RIP-qPCR primers		
Pgp	Forward	TGCGTGTCCCAGGAGTATG
	Reverse	GTCTTCAGGCTACAGGTGGA
Ddx47	Forward	GAAAGACTTCTGCTCGCCTG
	Reverse	CAGGTGAGGAATGCAAGCTG
Nxt1	Forward	CAACAAACAGCGGGACTTCA
	Reverse	TGCTATCTTCCACACCGTGT
Tcl1	Forward	GGTTTGCATATCCCTGGCTG
	Reverse	CTTTGATTGCAGAGCGGTGT
RT-qPCR primers		
Esrrb	Forward	TTTCTGGAACCCATGGAGAG
	Reverse	AGCCAGCACCTCCTTCTACA
Klf4	Forward	CCAGCAAGTCAGCTTGTGAA
	Reverse	GGGCATGTTCAAGTTGGATT
Nanog	Forward	CAGGTGTTTGAGGGTAGCTC
	Reverse	CGGTTCATCATGGTACAGTC
Oct4	Forward	GGCGTTCTCTTTGGAAAGGT
	Reverse	CTTCGGGCACTTCAGAAACA
Sox2	Forward	AAGGGTTCTTGCTGGGTTTT
	Reverse	AGACCACGAAAACGGTCTTG
Tbx3	Forward	AGGAGCGTGTCTGTCAGGTT
	Reverse	GCCATTACCTCCCCAATTTT
Cdx2	Forward	GAAACCTGTGCGAGTGGATG
	Reverse	CAGCCAGCTCACTTTTCCTC
Fgf5	Forward	CCGGATGGCAAAGTCAATGG
	Reverse	ACTCTCGGCCTGTCTTTTCA

Foxa2	Forward	ATGGGCCCAGTCACGAACAAA
	Reverse	ACACAGACAGGTGAGACTGCT
Gata4	Forward	CCTGGAAGACACCCCAATCT
	Reverse	TTAATGAGGGGCCGGTTGAT
Gata6	Forward	TTCTACACAAGCGACCACCT
	Reverse	CACCAAGAATCCTGTCGCAC
Sox17	Forward	CACAACGCAGAGCTAAGCAA
	Reverse	CGCTTCTCTGCCAAGGTC
Eomes	Forward	CAGGCGCATGTTTCCTTTCT
	Reverse	ATCTCCTGCCTCATCCAGTG
T	Forward	ACCCAGCTCTAAGGAACCAC
	Reverse	GCTGGCGTTATGACTCACAG
Gapdh	Forward	CTGCGACTTCAACAGCAACT
	Reverse	GAGTTGGGATAGGGCCTCTC
Zc3h13 target m^{6}A sequence for minigene reporters		
Atg13 fragment inserted into minigene reporter (wt)		CACCCTTTGTGAAGCAGCTGAGGACAGTCCTTGCTGA GGCTCTGTTACAGCCAGCTTCCAGCTCGACGGCCCGA GTCCTCGCCAAGTCCGGCACTGGGC
Atg13 fragment inserted into minigene reporter (Mutant)		CTCCCTTTGTGTTGCTGCTGTGGTCTGTCCTTGCTGTG GCTCTGTTTCTGCCTGCTTCCTGCTCGTCGGCCCGTGT CCTCGCCTTGTCCGGCTCTGGGC
MeRIP-qPCR primers for minigene reporters		
Atg13	Forward	AAGCTTCGATTAGTGAACGGA
	Reverse	AAGATCTCTGTCTCGAGCCG