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Supplemental Information

The STAT3 Target *Mettl8* Regulates Mouse ESC Differentiation via Inhibiting the JNK Pathway

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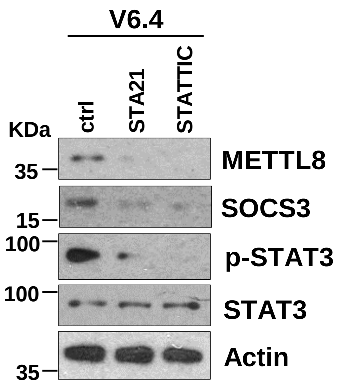
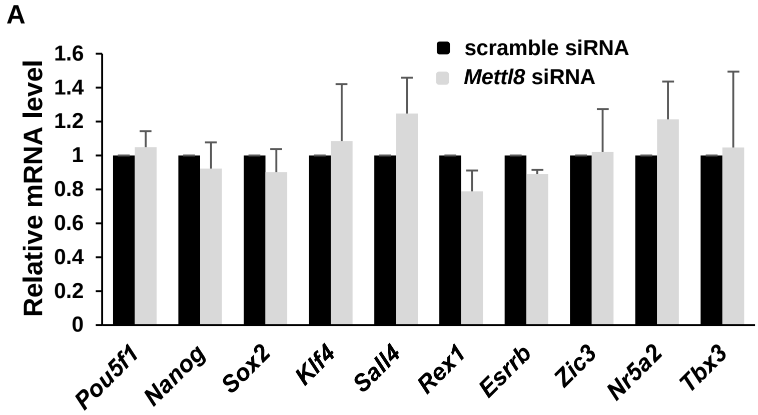


Figure S1. ***Mettl8*** **is transcriptionally regulated by STAT3. Related to Figure 1.**

V6.4 cells were treated with STA-21 and STATTIC for 6 hours and harvested. Then cell lysates were analysed by western blot.

A



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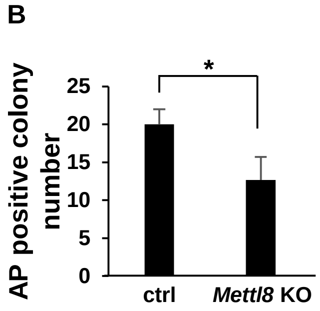

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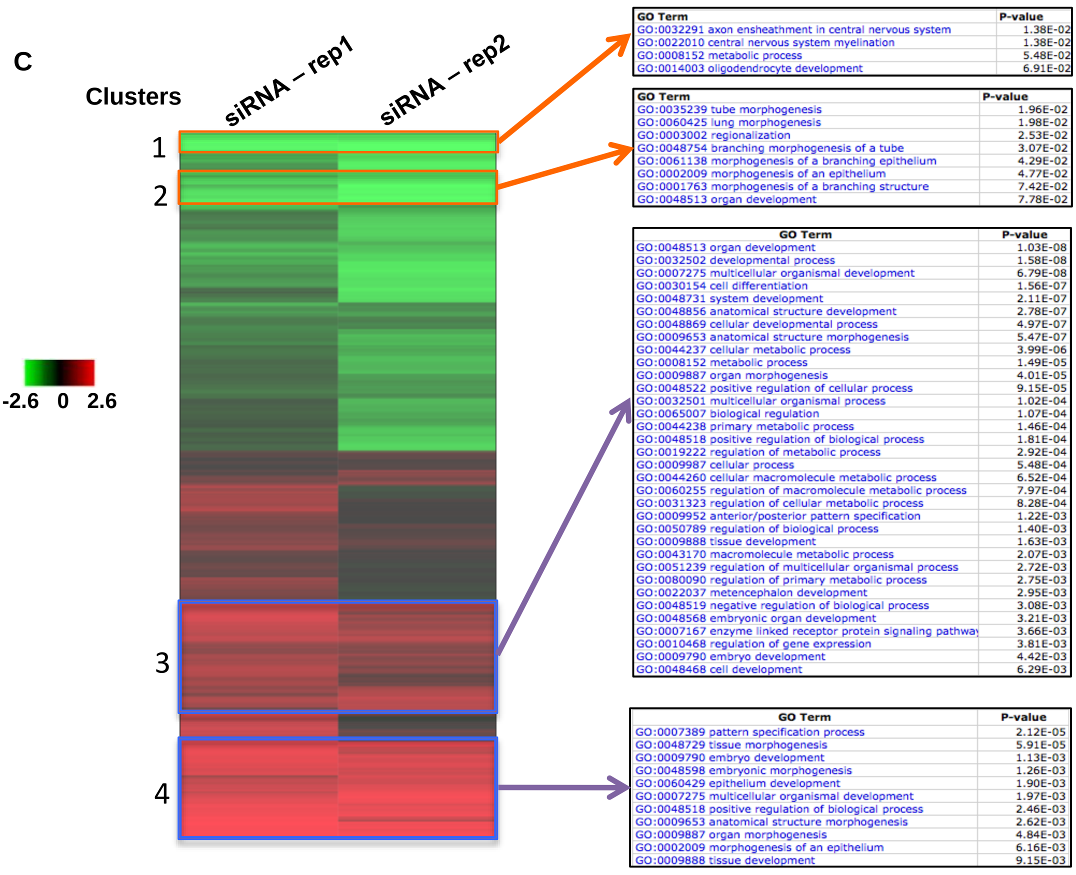

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GO:0022010 central nervous system myelination	1.38E-02
GO:0008152 metabolic process	5.48E-02
GO:0014003 oligodendrocyte development	6.91E-02

GO Term	P-value
GO:0035239 tube morphogenesis	1.96E-02
GO:0060425 lung morphogenesis	1.98E-02
GO:0003002 regionalization	2.53E-02
GO:0048754 branching morphogenesis of a tube	3.07E-02
GO:0061138 morphogenesis of a branching epithelium	4.29E-02
GO:0002009 morphogenesis of an epithelium	4.77E-02
GO:0001763 morphogenesis of a branching structure	7.42E-02
GO:0048513 organ development	7.78E-02

GO Term	P-value
GO:0048513 organ development	1.03E-08
GO:0032502 developmental process	1.58E-08
GO:0007275 multicellular organismal development	6.79E-08
GO:0030154 cell differentiation	1.56E-07
GO:0048731 system development	2.11E-07
GO:0048856 anatomical structure development	2.78E-07
GO:0048869 cellular developmental process	4.97E-07
GO:0009653 anatomical structure morphogenesis	5.47E-07
GO:0044237 cellular metabolic process	3.99E-06
GO:0008152 metabolic process	1.49E-05
GO:0009887 organ morphogenesis	4.01E-05
GO:0048522 positive regulation of cellular process	9.15E-05
GO:0032501 multicellular organismal process	1.02E-04
GO:0065007 biological regulation	1.07E-04
GO:0044238 primary metabolic process	1.46E-04
GO:0048518 positive regulation of biological process	1.81E-04
GO:0019222 regulation of metabolic process	2.92E-04
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GO:0060255 regulation of macromolecule metabolic process	7.97E-04
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GO:0050789 regulation of biological process	1.40E-03
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GO:0043170 macromolecule metabolic process	2.07E-03
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GO:0010468 regulation of gene expression	3.81E-03
GO:0009790 embryo development	4.42E-03
GO:0048468 cell development	6.29E-03

GO Term	P-value
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GO:0009790 embryo development	1.13E-03
GO:0048598 embryonic morphogenesis	1.26E-03
GO:0060429 epithelium development	1.90E-03
GO:0007275 multicellular organismal development	1.97E-03
GO:0048518 positive regulation of biological process	2.46E-03
GO:0009653 anatomical structure morphogenesis	2.62E-03
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GO:0009888 tissue development	9.15E-03

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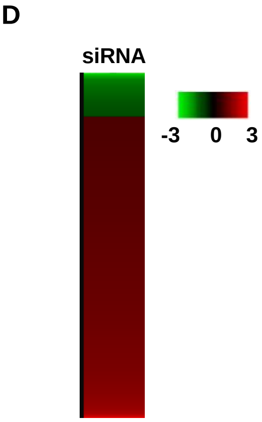

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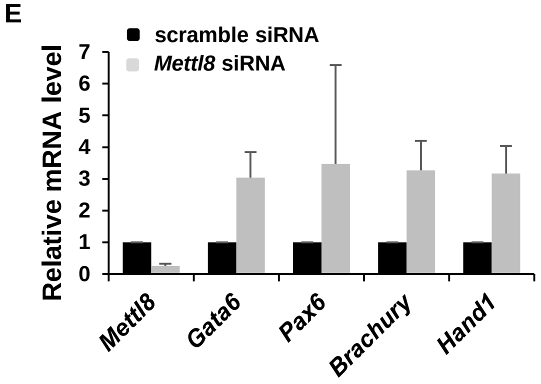


Figure S2. METTL8 affects ESCs differentiation rather than pluripotency. Related to Figure 2.

(A) Quantitative RT-PCR analysis showed no significant change in expression of the key pluripotent genes.

(B) *Mettl8* KO and control V6.4 cells were subjected to EB formation for 7 days. EBs were trypsinized and re-plated into gelatin-coated dished. After 3 days culture in ES medium, colonies were stained for AP activity and AP positive colonies were counted. Data are shown as the mean ± s.d. from 3 independent experiments.* means $P<0.05$.

(C) Microarray profiling of gene expression changes in ES cells after knocking down *Mettl8* and Log_2 transformed fold gene expression differences. siRNA-rep 1 and siRNA-rep 2 represents two independent comparisons of transcriptomes of mES cells treated with *Mettl8* siRNA compared to those treated with scramble siRNA control in two independent experiments.

(D) RNA-seq analysis of gene expression changes in ES cells after knocking down *Mettl8.* Log_2 transformed fold gene expression differences were subject to hierarchical clustering.

(E) Five upregulated developmental genes from microarray and RNA-seq results are selected for validation by realtime-PCR. The transcript levels of all genes were normalized to *Gapdh* expression level.

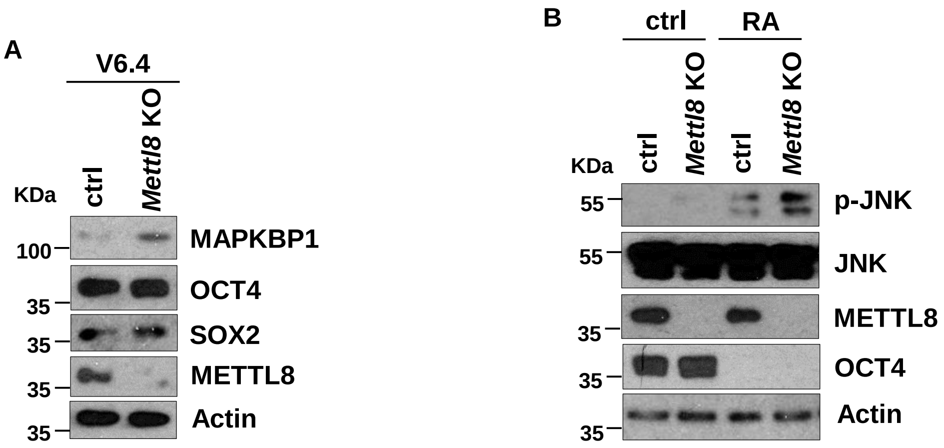
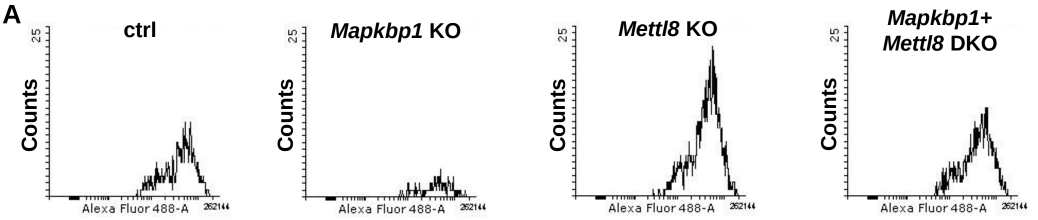


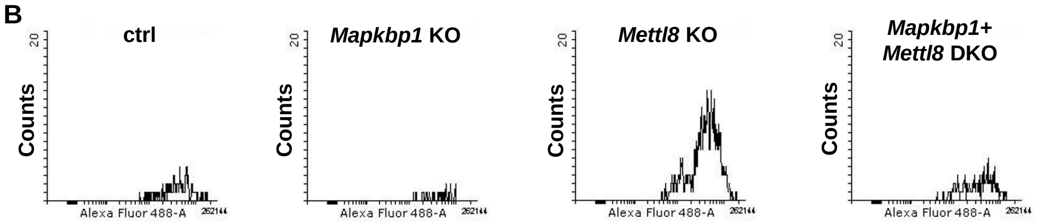
Figure S3. METTL8 inhibits JNK signaling in V6.4 mESCs. Related to Figure 6.

(A) Cell lysates of *Mettl8* KO and control V6.4 cells were analysed by western blot.

(B) *Mettl8* KO and control V6.4 cells were treated with RA for 6 days and cell lysates were analysed by western blot.



Sample	*Nestin* positive cell number
ctrl	668
Mapkbp1 KO	213
Mettl8 KO	1487
Mapkbp1+*Mettl8* DKO	1055



Sample	*Gata4* positive cell number
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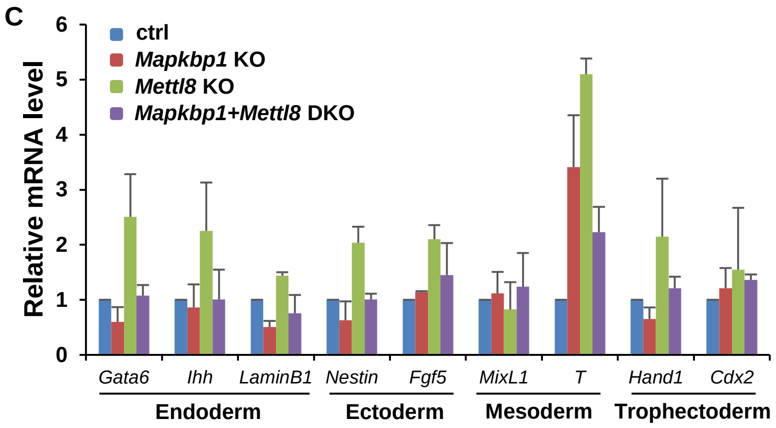
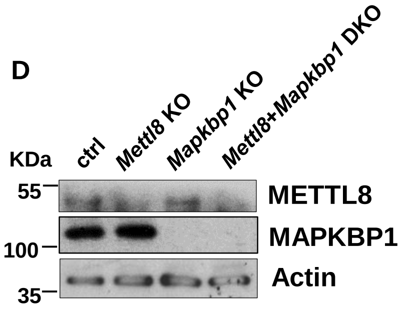
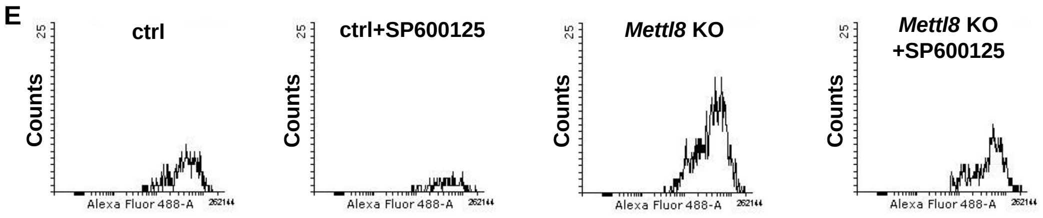
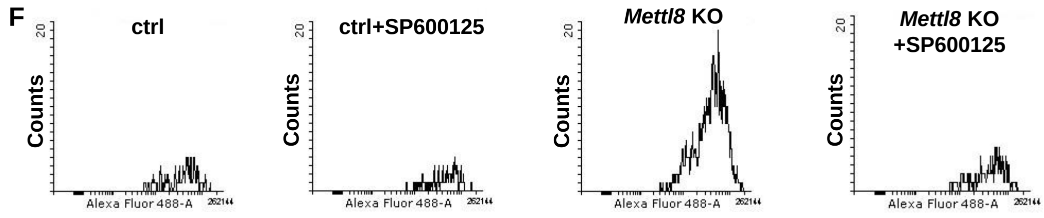
Mapkbp1 KO	72
Mettl8 KO	902
Mapkbp1+*Mettl8* DKO	288







Sample	*Nestin* positive cell number
ctrl	482
ctrl+SP600125	177
Mettl8 KO	1302
Mettl8 KO+SP600125	757



Sample	*Gata4* positive cell number
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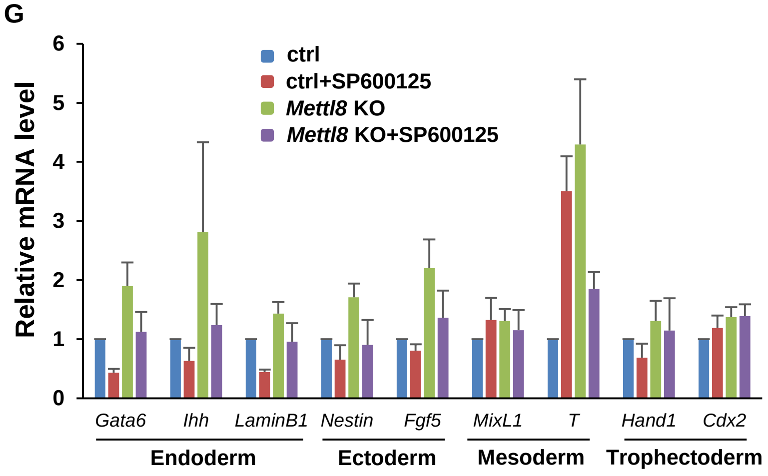
ctrl+SP600125	202
Mettl8 KO	1298
Mettl8 KO+SP600125	331



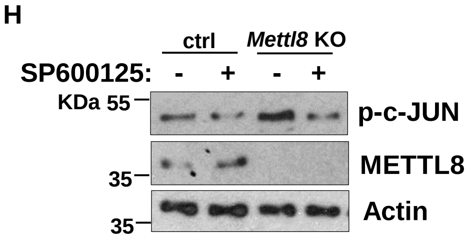


Figure S4. The METTL8-JNK pathway in ESCs differentiation. Related to Figure 7.

(A) Re-confirm the number of Nestin positive cells by FACS. *Mettl8* KO, *Mapkbp1* KO, *Mettl8* and *Mapkbp1* DKO and control E14 cells were subjected to neuron progenitor cells differentiation. Cells were stained for expression of Nestin and half of cells from one 12-well plate were analysed by FACS.

(B) Re-confirm the number of Gata4 positive cells by FACS. Mettl8 KO, *Mapkbp1* KO, *Mettl8* and *Mapkbp1* DKO and control E14 cells were subjected to cardiac differentiation. Cells were stained for expression of Gata4 and 10% cells from one 12-well plate were analysed by FACS.

(C-D) *Mettl8* KO, *Mapkbp1* KO, *Mettl8* and *Mapkbp1* DKO and control E14 cells were subjected to EB formation. Fourteen days later, (C) total RNAs were extracted and RNA levels of lineage markers were analysed by RT-PCR. Data are shown as the mean ± s.d. from 3 independent experiments. (D) Cell lysates were analysed by western blot.

(E) Re-confirm the number of Nestin positive cells by FACS. *Mettl8* KO and control E14 cells were treated with or without SP600125 and subjected to neuron progenitor cells differentiation. Cells were stained for expression of Nestin and half of cells from one 12-well plate were analysed by FACS.

(F) Re-confirm the number of Gata4 positive cells by FACS. *Mettl8* KO and control E14 cells were treated with or without SP600125 and subjected to cardiac differentiation. Cells were stained for expression of Gata4 and 10% cells from one 12-well plate were analysed by FACS.

(G-H) *Mettl8* KO and control E14 cells were treated with or without SP600125 and subjected to EB formation. Fourteen days later, (G) total RNAs were extracted and RNA levels of lineage markers were analysed by RT-PCR. Data are shown as the mean ± s.d. from 3 independent experiments. (H) Cell lysates were analysed by western blot.

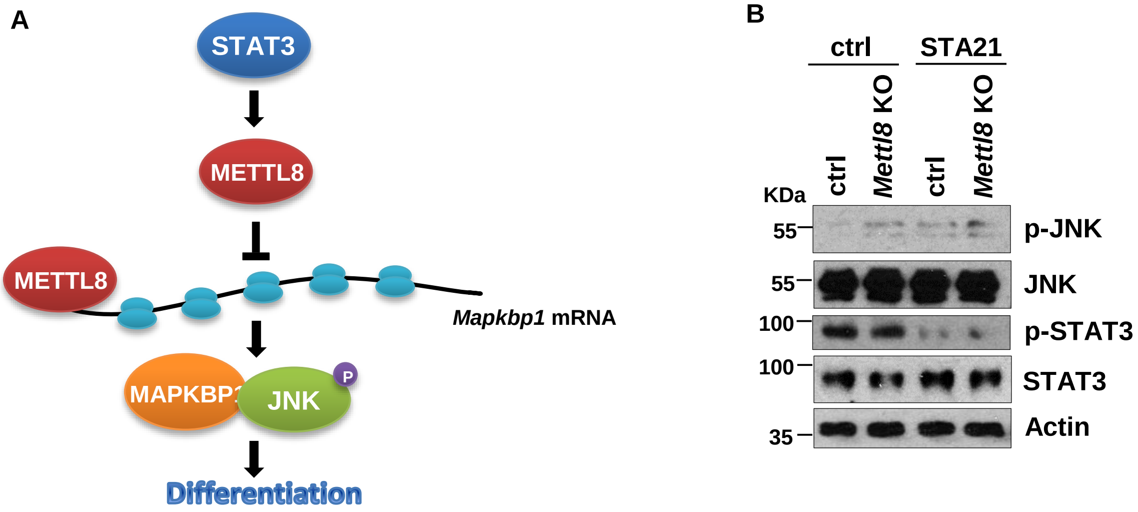
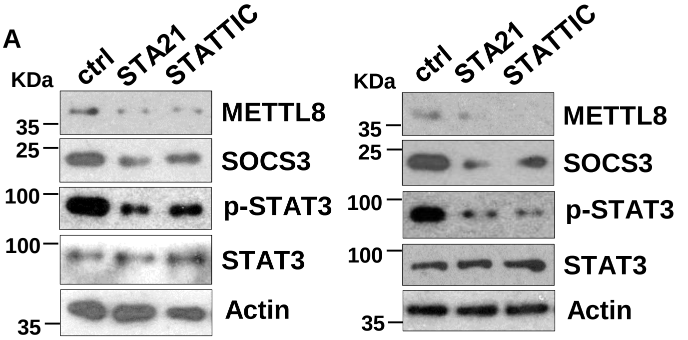


Figure S5. The STAT3-METTL8-JNK pathway in mouse ESCs

(A) A proposed model illustrating the role of the STAT3-METTL8-MAPKBP1-JNK pathway in mouse ESCs.

(B) Control and *Mettl8* KO E14 cells were treated RA for 4 days. Then cells were treated with or without STA21 for 12 hours and cell lysates were analysed by western blot.

A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25
100
100
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SOCS3
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STAT3
Actin
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25
100
100
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STAT3
Actin

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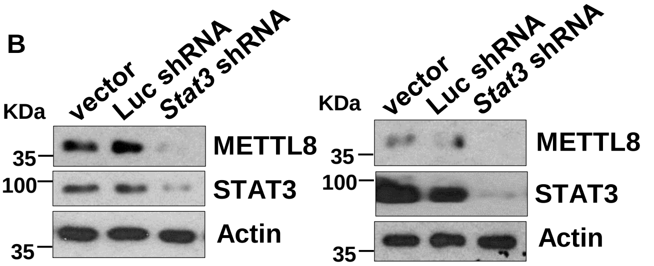
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KDa
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100
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STAT3
Actin
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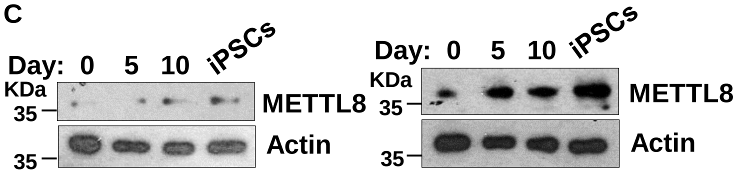
METTL8
STAT3
Actin

C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35
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METTL8
Actin
Day: 0 5 10 iPSCs
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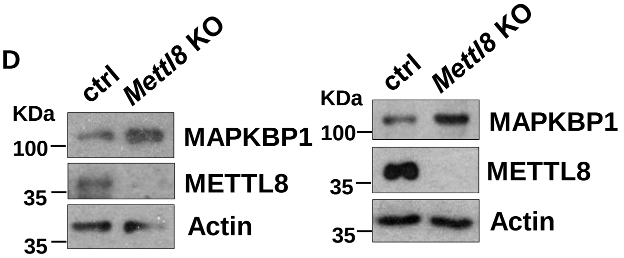
35
35
METTL8
Actin

D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100
35
35
MAPKBP1
METTL8
Actin
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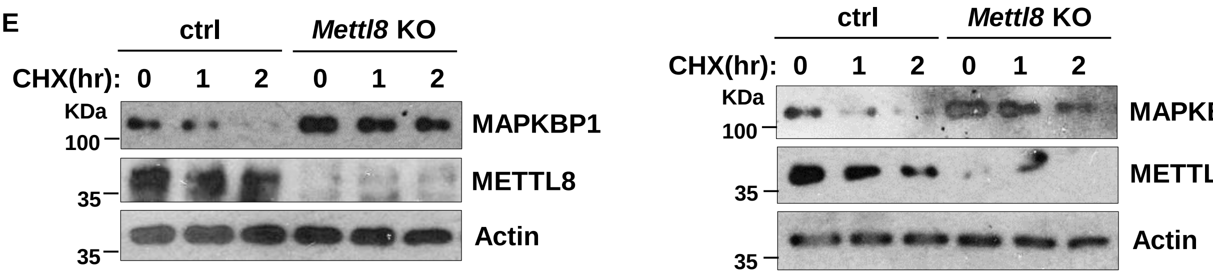
KDa
100
35
35
MAPKBP1
METTL8
Actin

E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KDa
100
35
35
MAPKBP1
METTL8
Actin
ctrl
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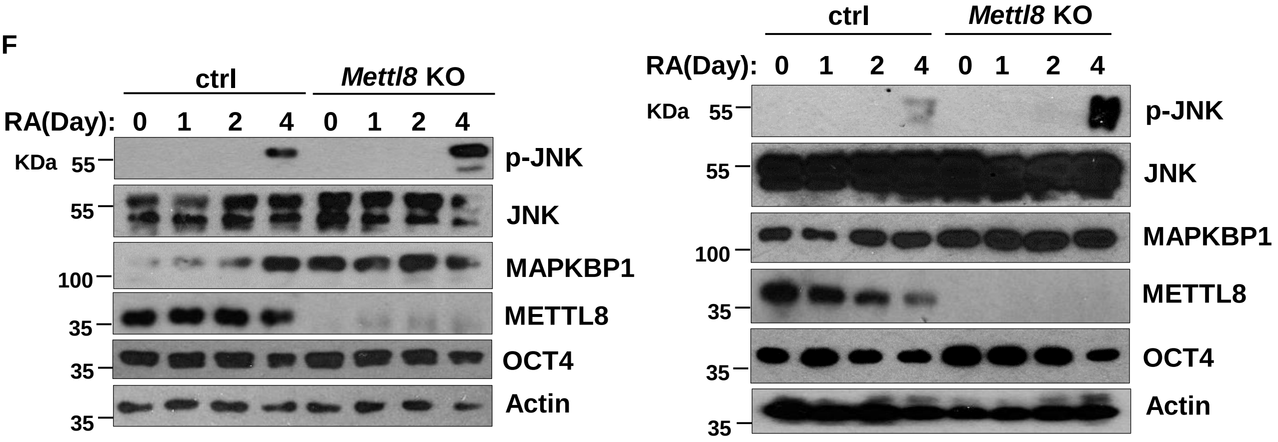
CHX(hr): 0 1 2 0 1 2
KDa
100
35
35
MAPKBP1
METTL8
Actin

F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55
100
35
35
35
p-JNK
JNK
MAPKBP1
METTL8
OCT4
Actin
ctrl
Mettl8 KO
RA(Day): 0 1 2 4 0 1 2 4
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100
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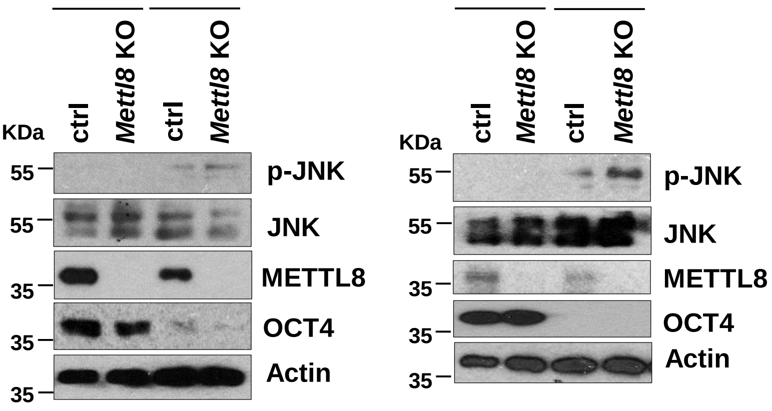
G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Actin
ctrl
EB
ctrl
Mettl8 KO
ctrl
Mettl8 KO
KDa
55
55
35
35
35
p-JNK
JNK
METTL8
OCT4
Actin

Figure S6. Replicates of western blot quantifications

(A) Replicates of Fig.1C

(B) Replicates of Fig.1E

(C) Replicates of Fig.3B

(D) Replicates of Fig.5C

(E) Replicates of Fig.5D

(F) Replicates of Fig.6B

(G) Replicates of Fig.6C

Fig1C

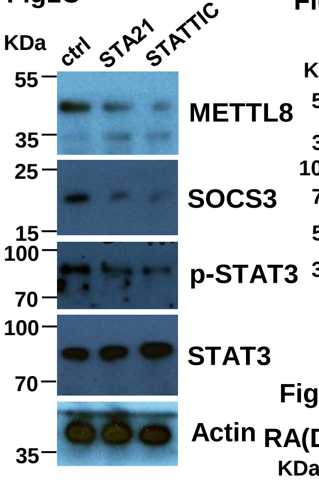


Fig1E

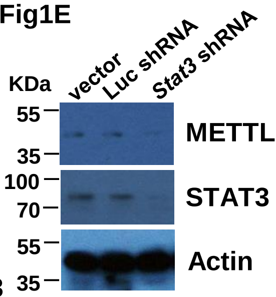
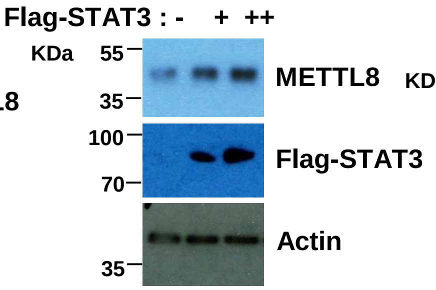


Fig1G



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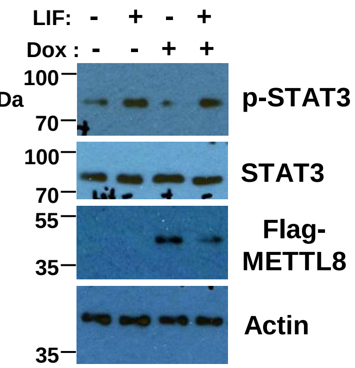


Fig2D

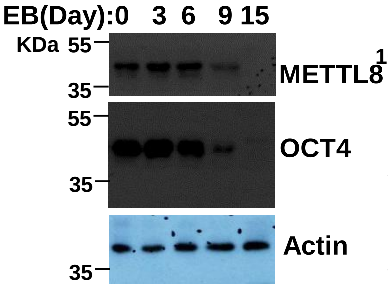


Fig2B

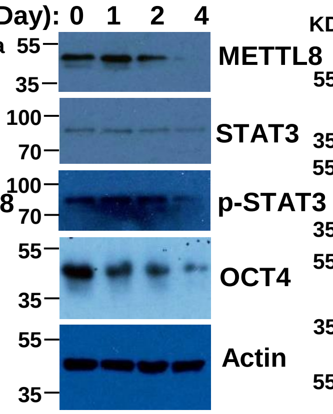


Fig2G

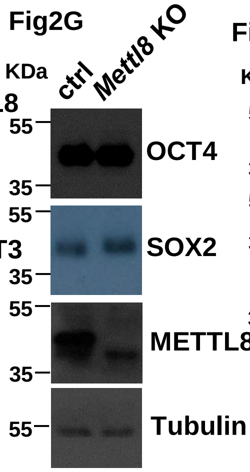


Fig2H

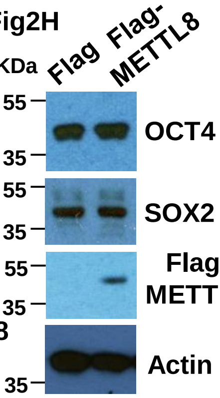
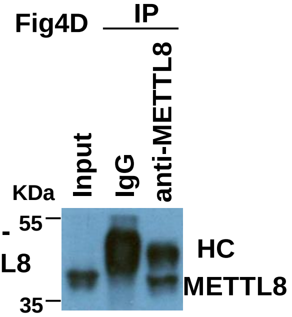


Fig4D



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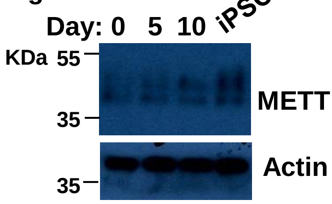

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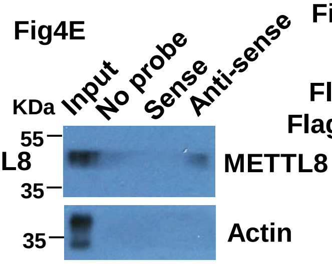


Fig4C

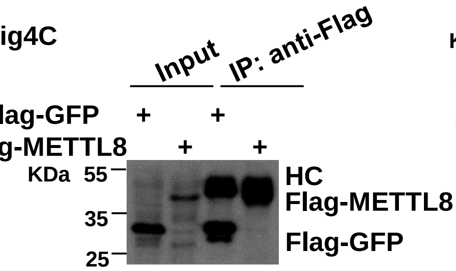


Fig4B

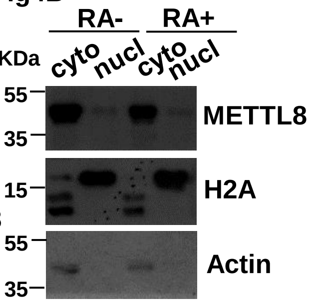


Fig5C

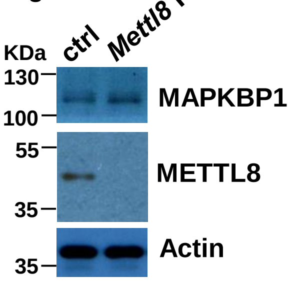


Fig5D

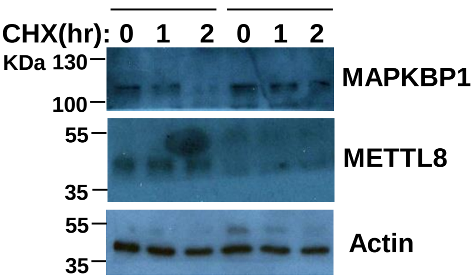


Fig6B

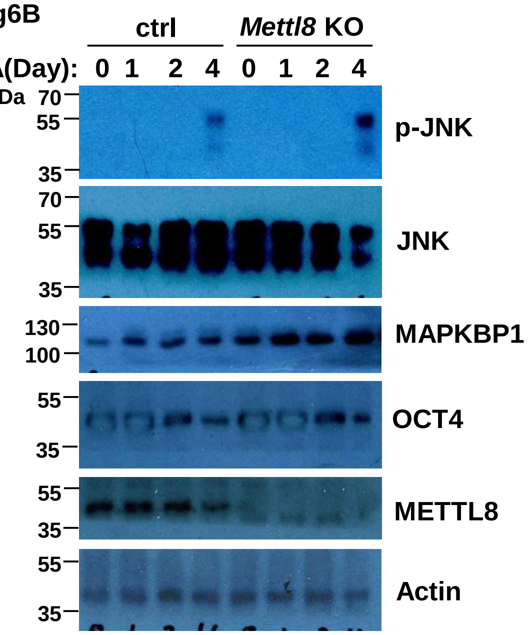


Fig6C

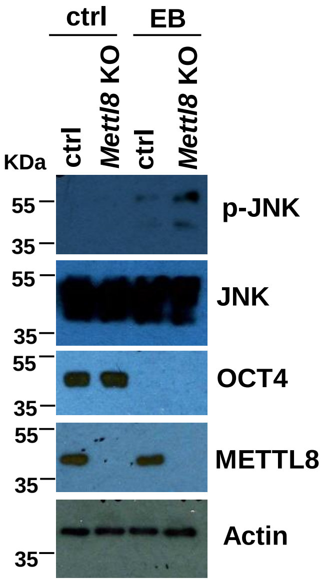


Fig6D

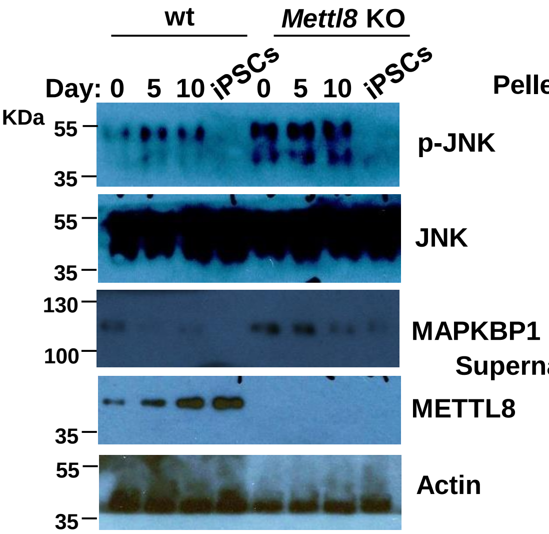
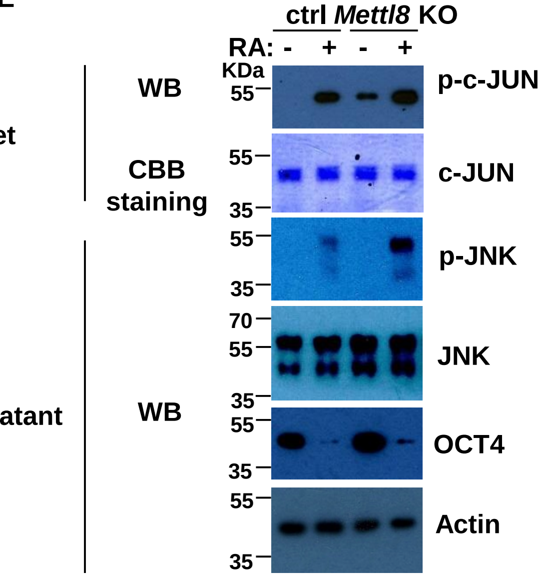
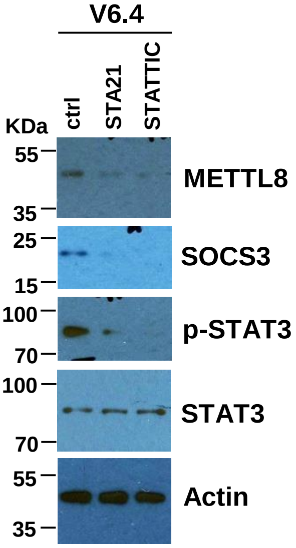


Fig6E



FigS1A



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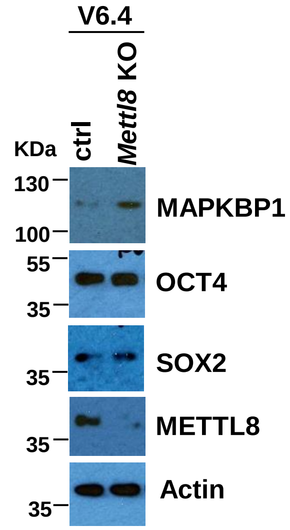
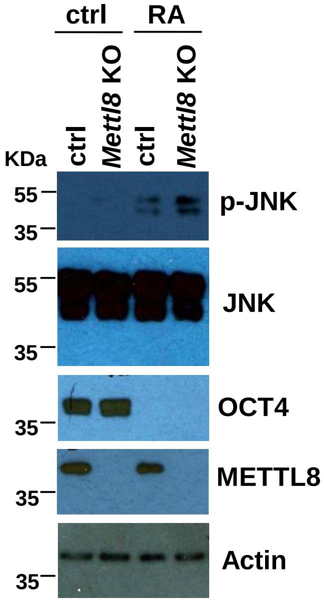
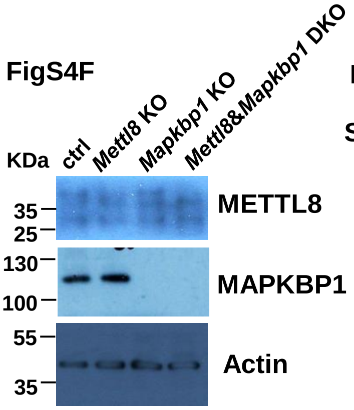


FigS3B



FigS4F



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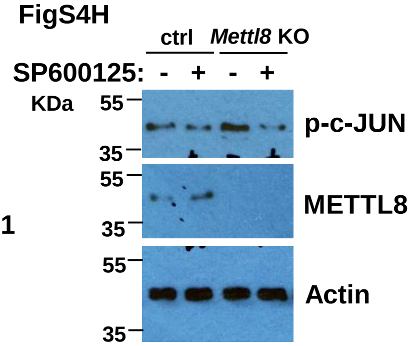

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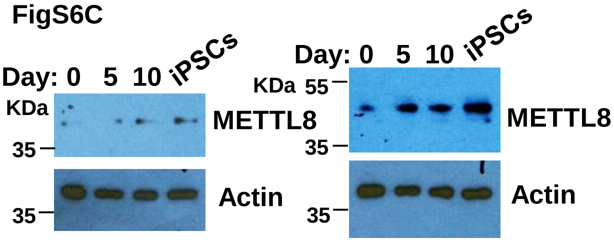

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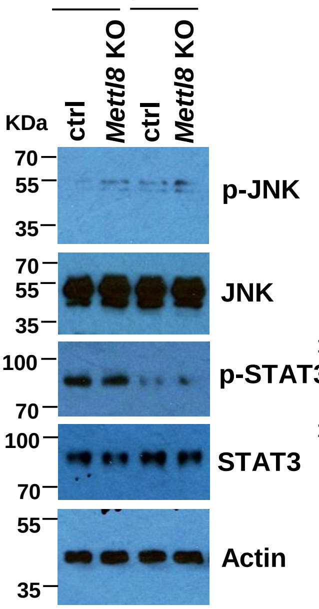

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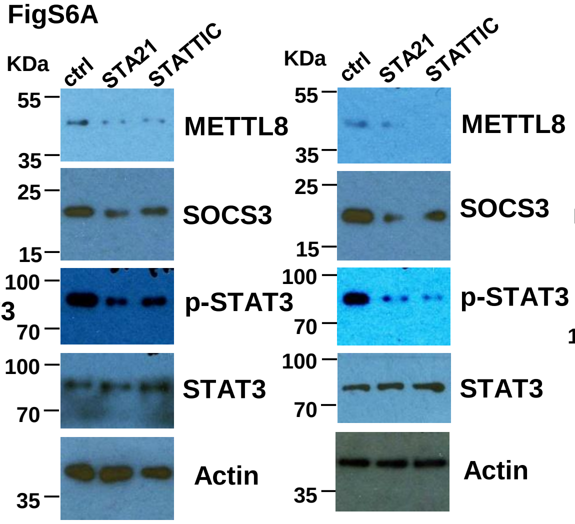

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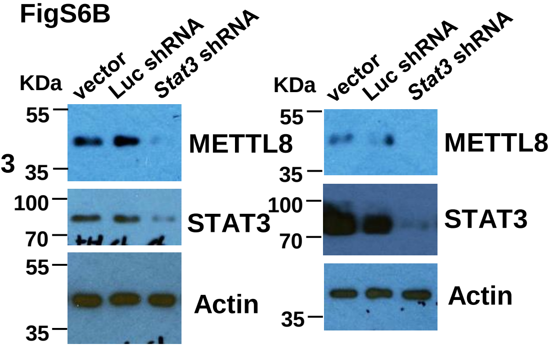

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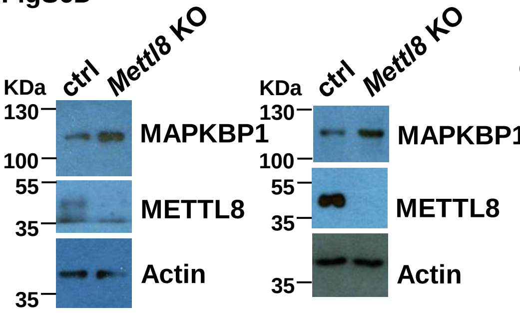

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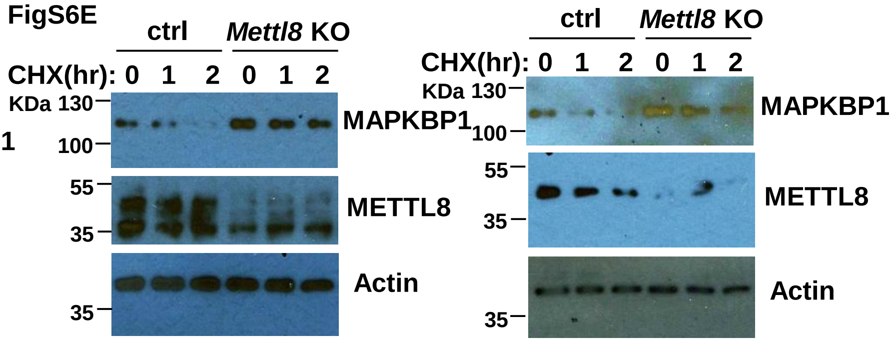

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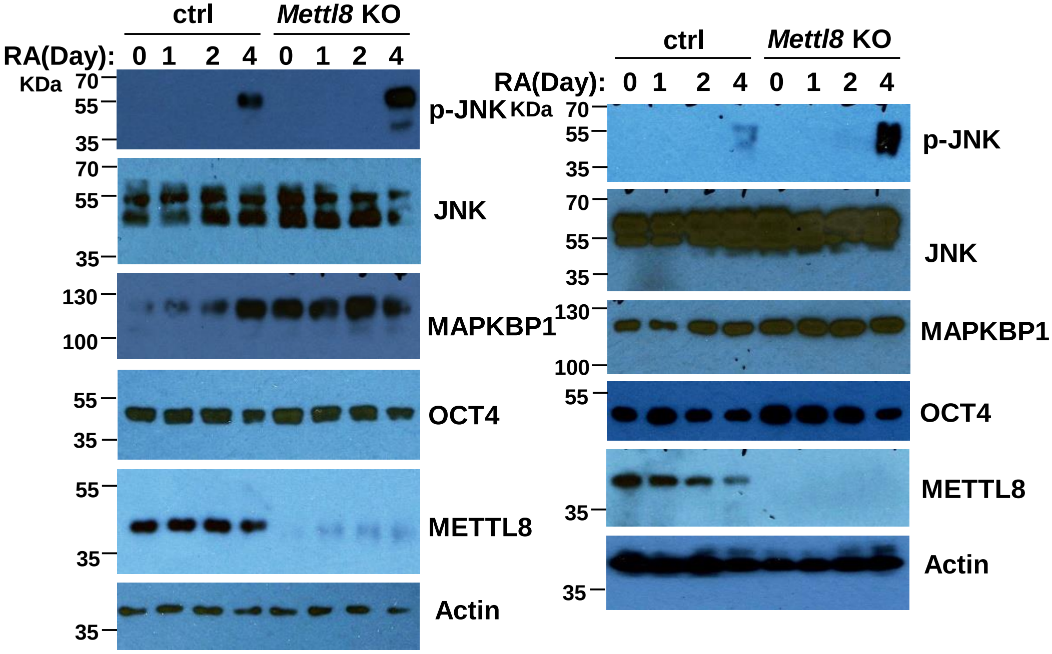

FigS6G

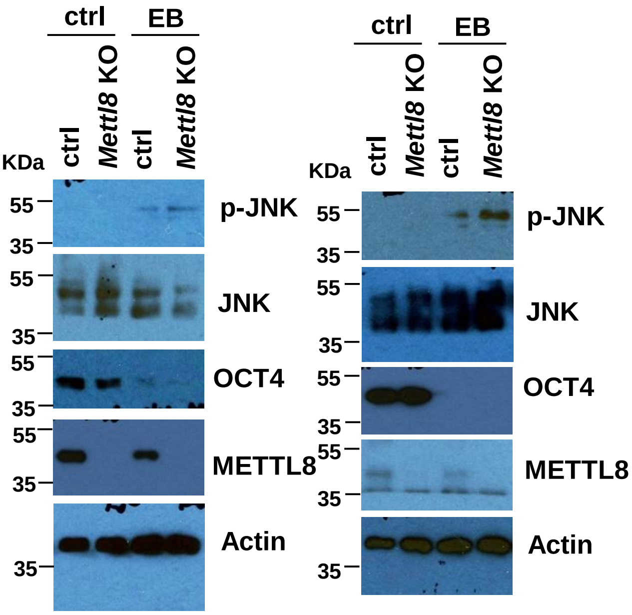

Figure S7. Uncropped images of Western blot

Table S4. List of primers sequences

Primers for qRT-PCR	
Gene	**Sequence (5’-3’)**
Actin	Fw: CTGTCCCTGTATGCCTCTG
	Rev: ATGTCACGCACGATTTCC
Oct4	Fw: TCTTTCCACCAGGCCCCCGGCTC
	Rev: TGCGGGCGGACATGGGGAGATCC
Sox2	Fw: TAGAGCTAGACTCCGGGCGATGA
	Rev: TTGCCTTAAACAAGACCACGAAA
Nanog	Fw: CTCATCAATGCCTGCAGTTTTTCA
	Rev: CTCCTCAGGGCCCTTGTCAGC
Rex1	Fw: ACGAGGTGAGTTTTCCGAAC
	Rev: CCTCTGTCTTCTCTTGCTTC
Esg1	Fw: ATATCCCGCCGTGGGTGAAAGTTC
	Rev:ACTCAGCCATGGACTGGAGCATCC
Fbx15	Fw: GTTGGAATCTGCTTCTACAG
	Rev: CTTCACCAAGATTTCCGATG
Cdx2	Fw: CGAGCCCTTGAGTCCTGTGA
	Rev: AACCCCAGGGACAGAACCA
Hand1	Fw: GCCAAGGATGCACAAGCA
	Rev: GGGCTGCTGAGGCAACTC
Gata6	Fw: CTTGCGGGCTCTATATGAAACTCCAT
	Rev: TAGAAGAAGAGGAAGTAGGAGTCATAGGGACA
Ihh	Fw: ACGTGCATTGCTCTGTCAAGT
	Rev: CTGGAAAGCTCTCAGCCGGTT
Laminin B1	Fw: CCCCAATCTCTGTGAACCATG
	Rev: GCAATTTGCACCGACACTGA
Fgf5	Fw: TGCGTCCGCGATCCA
	Rev: TCAGGGCCACGTACCACTCT
Nestin	Fw: TGAGGGTCAGGTGGTTCTG
	Rev: AGAGCAGGGAGGGACATTC
Pax6	Fw: GCATGCAGAACAGTCACAGCGGA
	Rev: ACTCCCGTTTATACTGGGCTATTT
T	Fw: ATCACCAGCCACTGCTTTC
	Rev: CCATTACATCTTTGTGGTCGTTTC
Mixl1	Fw: ACTTTCCAGCTCTTTCAAGAGCC
	Rev: ATTGTGTACTCCCCAACTTTCCC
Mapkbp1	FW: TCCTGACCGTCCTACTCTGA
	REV: TTTCCTAGGCTCACCAAGGG
Mettl8	FW: CCACCCAGGAAGAGTCTCAG
	REV: GGTTACACCCATGGTCAGGA
Primers for CRISPR	
Name	**Sequence (5’-3’)**
Mapkbp1 1	FW: caccgCGTTGACAATCATGTCATGC
	REV: aaacGCATGACATGATTGTCAACGc
Mapkbp1 2	FW: caccgCTTGCTTGTGACCCCCGATC
	REV: aaacGATCGGGGGTCACAAGCAAGc
Mettl8 1	FW: caccgGAAGTTGCATTTGCCGACTG
	REV: aaacCAGTCGGCAAATGCAACTTCc
Mettl8 2	FW: caccgTTGCATTTGCCGACTGAGGC
	REV: aaacGCCTCAGTCGGCAAATGCAAc

Biotin labelled probes for Mapkbp1 mRNA pull down	
Name	**Sequence (5’-3’)**
Probe1	Sense: TTCTGTATCACGTCGTCGGG
	Antisense: CCCGACGTGATACAGAA
Probe2	Sense: TGCAAGAGAGCCCTAGTGTT
	Antisense: AACACTAGGGCTCTCTTGCA