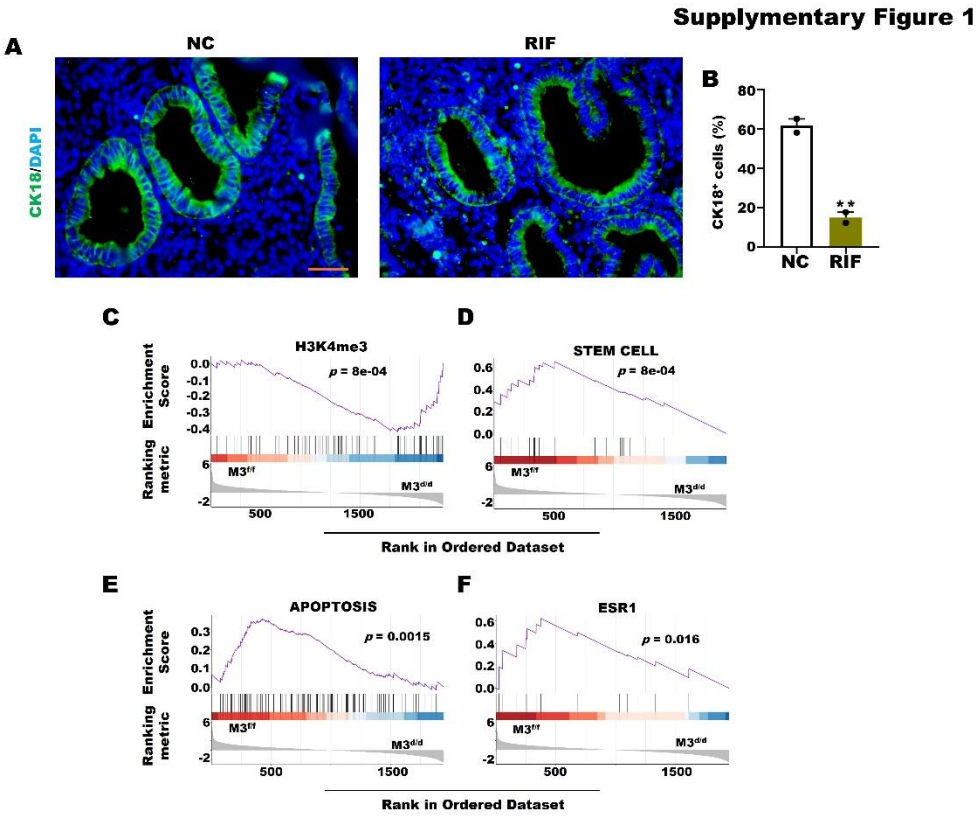
Supplementary Materials for

Mettl3/Eed/Ythdc1 regulatory axis controls endometrial receptivity and function



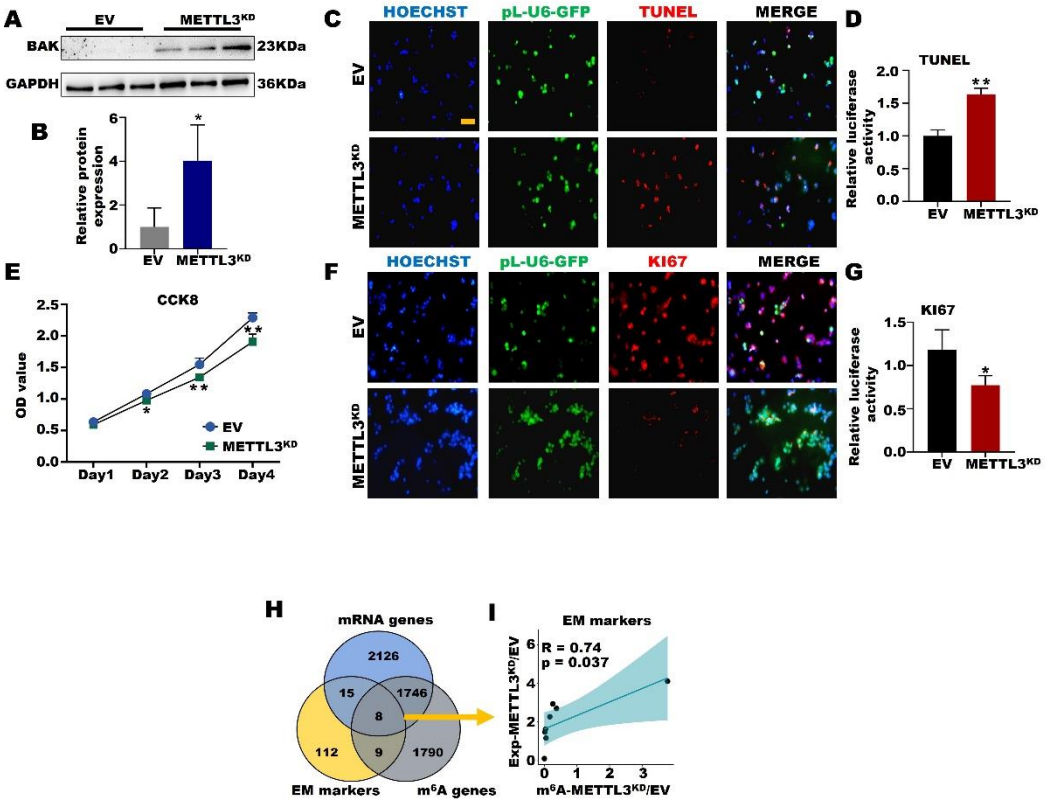
Supplementary Fig. S1 GSEA analysis for m^{6}A modificatory difference genes in RIF

A-B. IF tested the positive cell number of CK18 in the RIF group. Scale bars, 50μm.

C-D. m^{6}A modificatory difference genes are enriched in H3K4me3, stem cell, apoptosis and Esr1.

Significance was calculated using t test in all panels. **p < 0.01. All error bars are ± SD. For all IF, n = 2.

Supplymentary Figure 2



Supplementary Fig. S2 Mettl3^{KD} promoted apoptosis and inhibited proliferation in endometrial cells

A-B. Western Blot tested the expression level of BAK after METTL3^{KD}.

C-D. IF tested the percentage of positive cells (TUNEL). Scale bars, 50μm.

E. ELISA method evaluated the level of CCK8.

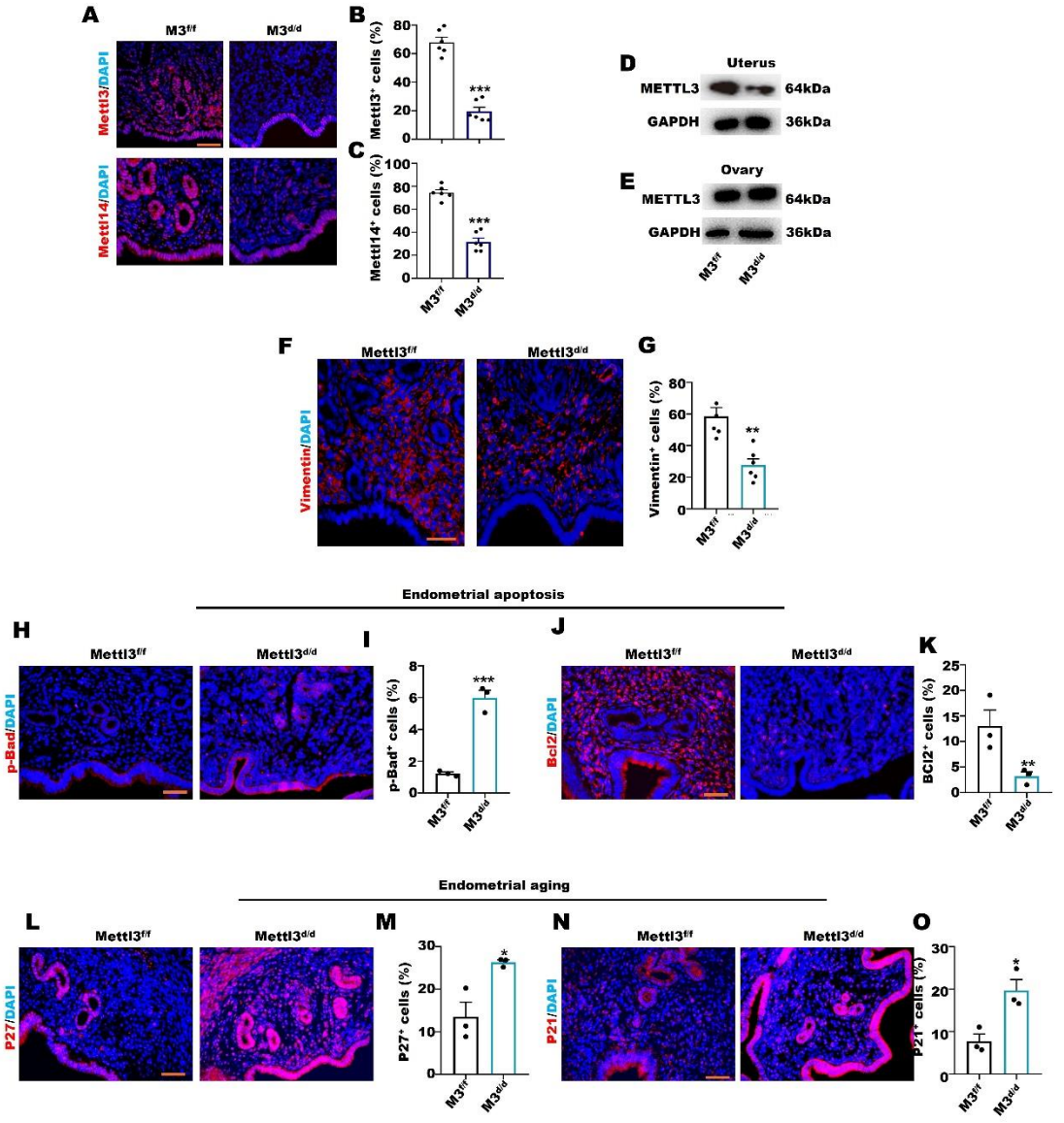
F-G. IF tested the percentage of positive cells (Ki67). Scale bars, 50μm.

H. Venn diagram analyzed the common genes from mRNA difference genes, EM marker genes and m6A difference genes. EM = Endometrium.

I. The common genes were carried out correlation analysis with m^{6}A and mRNA levels.

Significance was calculated using t test in all panels. *p < 0.05, **p < 0.01. All error bars are ± SD. For all IF, n = 3.

Supplymentary Figure 3



Supplementary Fig. S3 Mettl3 expression level in the Mettl3^{cKO} mouse model and Mettl3^{cKO} induced endometrial function loss, apoptosis and aging increase

A-C. IF tested the percentage of positive cells with Mettl3 and Mettl14. Scale bars, 50μm.

D-E. Western Blot tested the expression level of Mettl3 in the uterus and ovary.

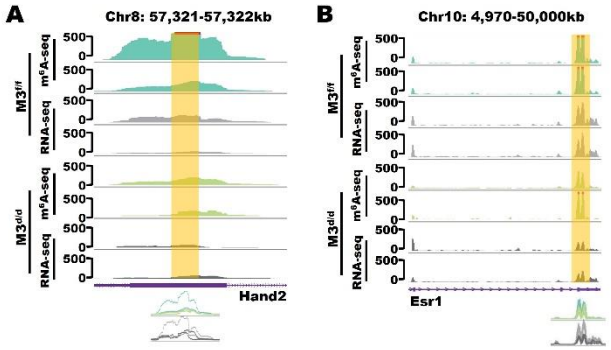
F-G. IF tested the percentage of positive cells with vimentin. Scale bars, 50μm.

H-K. IF tested the percentage of positive cells with apoptotic genes (Bcl2, p-Bad). Scale bars, 50μm.

L-O. IF tested the percentage of positive cells with aging genes (P27, P21). Scale bars, 50μm.

Significance was calculated using t test in all panels. **p < 0.01. All error bars are ± SD. For all IF, n = 3.

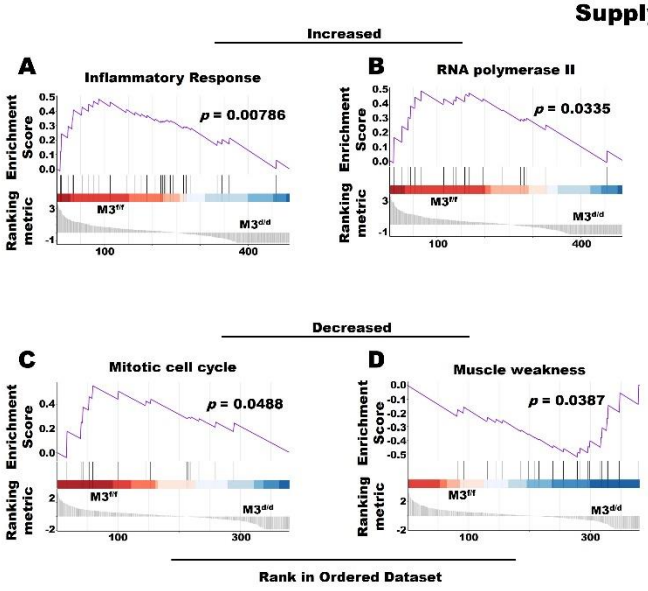
Supplymentary Figure 4



Supplementary Fig. S4 IGV demonstrated the m^{6}A and mRNA levels of endometrial receptivity genes

A-B. m^{6}A and mRNA levels of endometrial receptivity genes (Hand2 and Esr1) were compared.

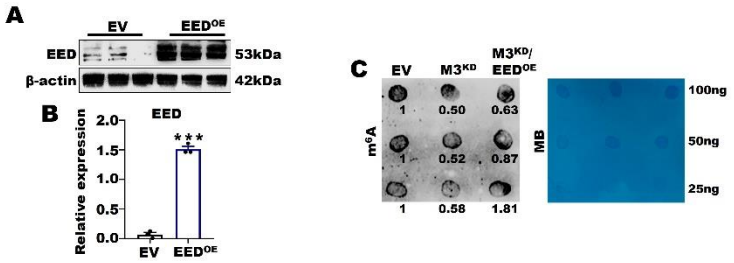
Supplymentary Figure 5



Supplementary Fig. S5 GSEA analysis for m^{6}A modificatory difference genes in Mettl3$^{d/d}$

A-D. The increased m^{6}A modificatory difference genes enriched in the inflammatory response and RNA polymerase II. The decreased m^{6}A modificatory difference genes are enriched in the mitotic cell cycle and muscle weakness.

Supplymentary Figure 6

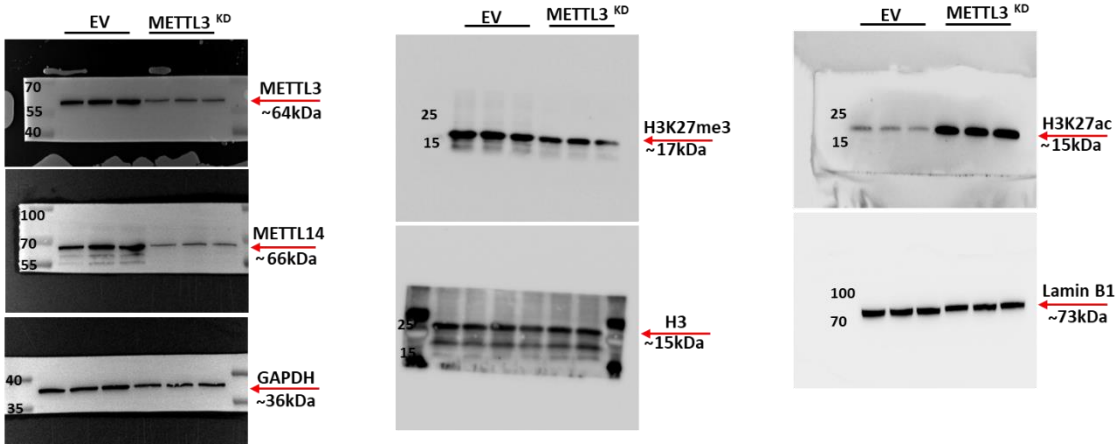


Supplementary Fig. S6 EED overexpression rescued the m^{6}A level in METTL3^{KD}

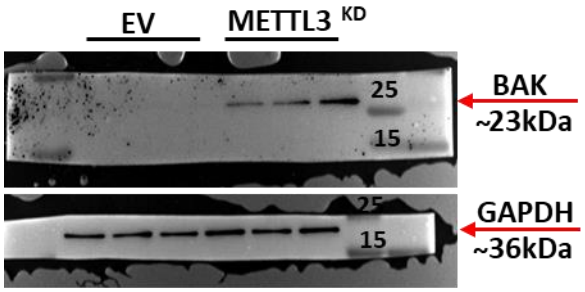
A-B. Western Blot tested the expression level of EED in the EED^{OE} group.

C. Dot Blot detected the m^{6}A level in the EV, METTL3^{KD} and METTL3^{KD}/EED^{OE} groups.

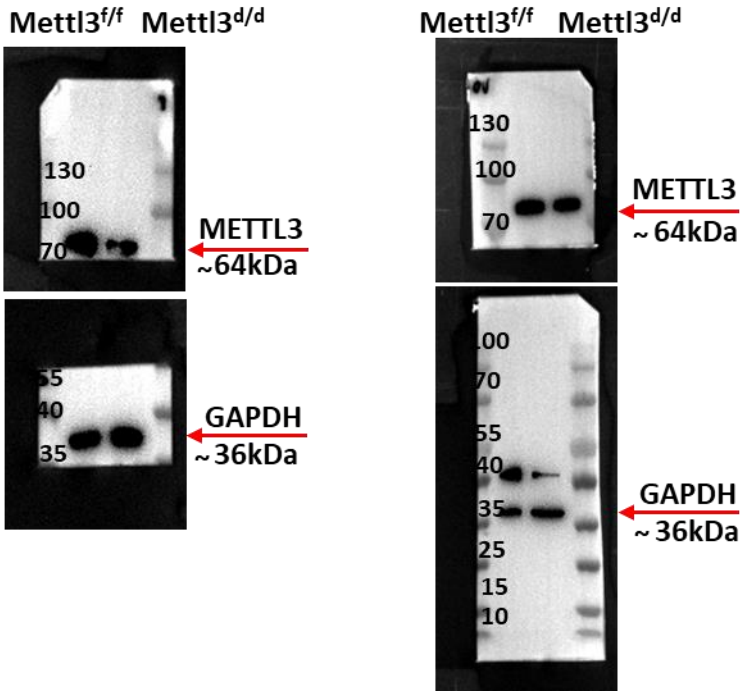
Significance was calculated using t test in all panels. ***p < 0.001. All error bars are ± SD.



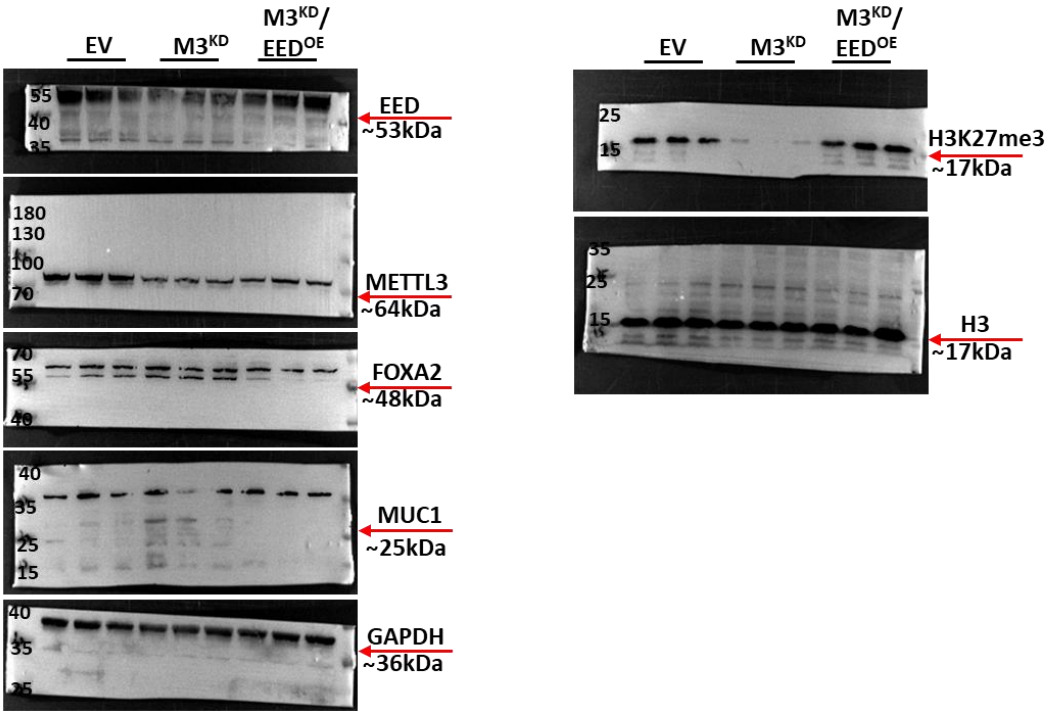
Supplementary Fig. S7 Uncropped western blot images corresponding to Fig. 2



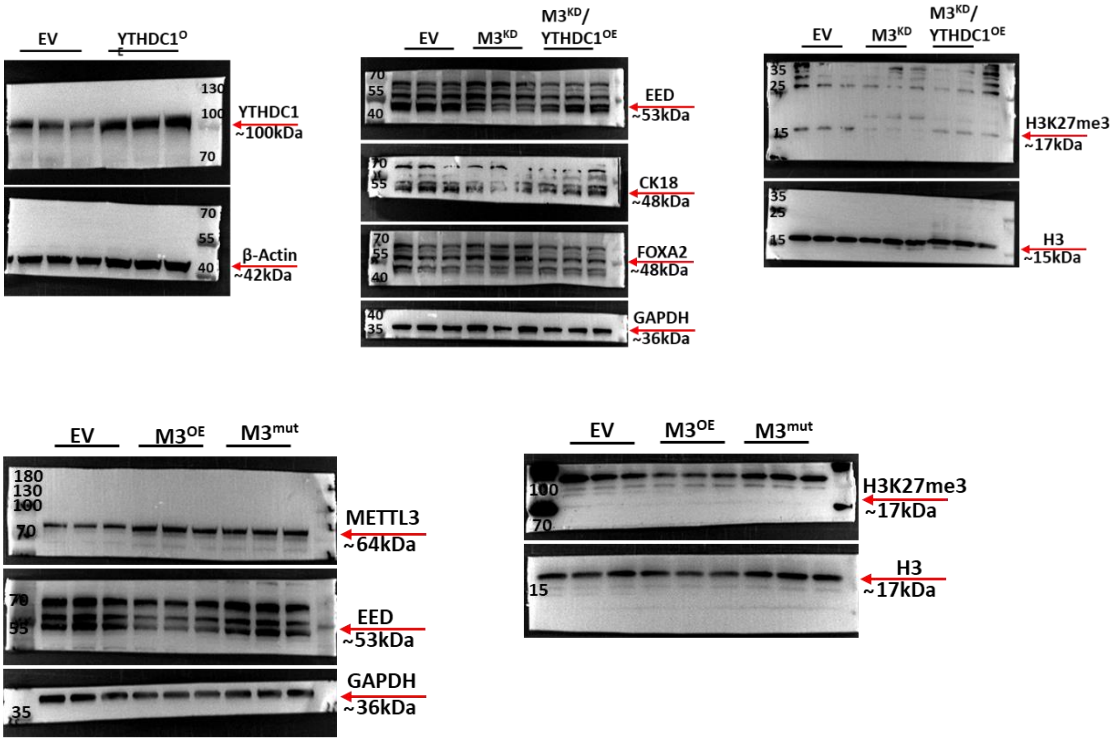
Supplementary Fig. S8 Uncropped western blot images corresponding to Supplementary Fig. 2



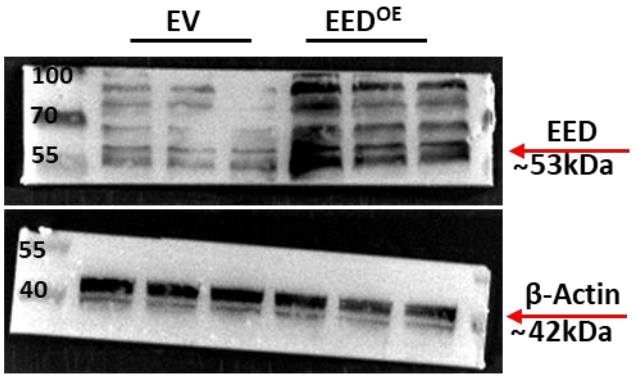
Supplementary Fig. S9 Uncropped western blot images corresponding to Supplementary Fig. 3



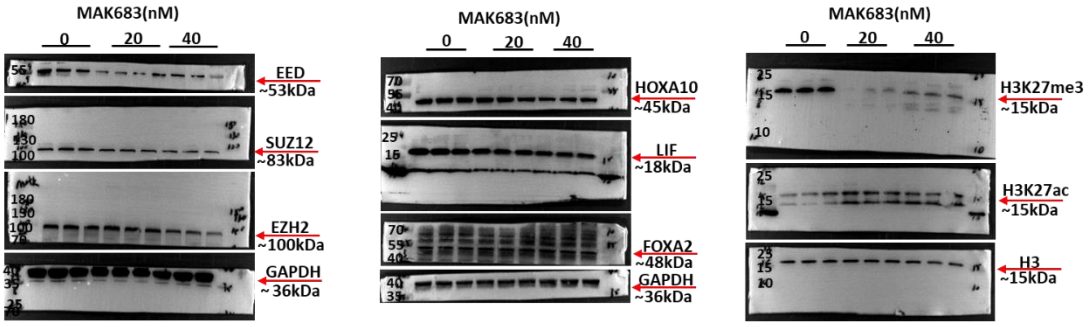
Supplementary Fig. S10 Uncropped western blot images corresponding to Fig. 6.



Supplementary Fig. S11 Uncropped western blot images corresponding to Fig. 7.



Supplementary Fig. S12 Uncropped western blot images corresponding to Supplementary Fig. 6.



Supplementary Fig. S13 Uncropped western blot images corresponding to Fig. 8.

Table S1. The primers used for genotyping were studied

Gene	Forward sequence (5′-3′)	Reverse sequence (5′-3′)
Mettl3-P1P2	GTTTGCACAAGGAGTATTT	TCATCTGGGGAAGGAGGAGTG
Mettl3-P3P4	GTCCCGGAACTCTCACAC	GGCTCAGTCCTCCTCACA
PgrCre-WT	GCGACTCTACACCCACTTCCTC	GCCACTGTCCTCTTCCACCTC
PgrCre	CCTCCTCCCTCTGCCCCTA	CCACATTCTCCTTTCTGATTCTCC

Table S2. Designations, sequences of shRNA and plasmid amplicons

Name	Sequence from 5'-3'
shNC (H)	GGTTCTCCGAACGTGTCACGT
shMETTL3 (H)	GCCAAGGAACAATCCATTGTT
Empty vector(H)	pCDNA3.1(Beijing Tsingke Biotech)
METTL3 OE (H)	GenBank Accession No.NP_062826.2
METTL3 MUT(H)	TGGCTGACCCACCCTGGGATAT
EED OE(H)	GenBank Accession No.NP_001294936.1
YTHDC1 OE (H)	GenBank Accession No.NP_001026902.1

Table S3 Key antibodies resources

REAGENTor RESOURCE	SOURCE	IDENTIFIER	Antibodies used
Rabbit anti-m6A	Cell Signaling Technology	Cat# 56593S; RRID: AB_2799515	Dot blot: 1:3000
Rabbit anti-m6A	Millipore	Cat# ABE572-I; RRID: AB_2892214	RIP 0.5 μg
Mouse anti-m6A	Millipore	Cat# ABE1006	RIP 5 μg
Rabbit anti-METTL3	Abcam	Cat# ab195352;	WB 1:1000,

		RRID: AB_2721254	IF 1:500, IP 4 μg
Rabbit anti-METTL3	ABclonal	Cat# A8370; RRID: AB_2770344	IB 1:1000
Rabbit anti-METTL14	Novus	Cat# NBP1-81329; RRID: AB_11021780	IF 1:500
Rabbit anti-EED	ProteinTech	Cat# 16818-1-AP; RRID: AB_2262065	WB 1:1000, IF 1:500, IP 4 μg
Rabbit anti-EED	ABclonal	Cat# A5371; RRID: AB_2766181	IB 1:1000
Rabbit anti-EZH2	ProteinTech	Cat# 21800-1-AP; RRID: AB_10858790	WB 1:1000, IF 1:500, IP 4μg
Rabbit anti-SUZ12	ABclonal	Cat# A7786; RRID: AB_2863561	WB 1:1000, IF 1:500, IP 4 μg
Rabbit anti-HAND2	ABclonal	Cat# A7044; RRID: AB_2767599	WB 1:1000, IF 1:500
Rabbit anti-MUC1	ABclonal	Cat# A19081; RRID: AB_2862573	WB 1:1000, IF 1:500
Rabbit anti-LIF	ProteinTech	Cat#26757-1-AP; RRID: AB_2880624	WB 1:1000, IF 1:500
Rabbit anti-HOXA10	ProteinTech	Cat# 26497-1-AP; RRID: AB_2880533	WB 1:1000, IF 1:500
Rabbit anti-FOXA2	ProteinTech	Cat# 22474-1-AP; RRID: AB_2879110	WB 1:1000, IF 1:500
Mouse anti-CK18	Abcam	Cat# ab668; RRID: AB_305647	WB 1:1000, IF 1:500
Rabbit anti-YTHDC1	ProteinTech	Cat# 14392-1-AP; RRID: AB_2878052	WB 1:1000, IP 4 μg IB 1:1000
Rabbit anti-H3K27me3	ActiveMotif	Cat# 39155; RRID: AB_2561020	WB 1:1000, IF 1:1000, 0.5 μg per CUT&Tag
Rabbit anti-H3K27ac	Abcam	Cat# ab177178; RRID: AB_2828007	WB 1:1000, IF 1:1000, 0.5 μg per CUT&Tag
Rabbit anti-H3	ProteinTech	Cat# 17168-1-AP; RRID: AB_2716755	WB 1:10000
Rabbit anti-Lamin B1	ProteinTech	Cat# 12987-1-AP; RRID: AB_2136290	WB 1:10000
Rabbit anti-GAPDH	ProteinTech	Cat# 10494-1-AP; RRID: AB_2263076	WB 1:10000
Mouse anti-GAPDH	ProteinTech	Cat# 60004-1-Ig; RRID: AB_2107436	WB 1:10000
Mouse anti-β-Actin	ProteinTech	Cat# 66009-1-Ig; RRID: AB_2687938	WB 1:1000
Rabbit anti-Ki67	Abcam	Cat# ab15580;	IF 1:1000

		RRID: AB_443209	
Rabbit anti-Bcl2	Abcam	Cat# ab182858; RRID: AB_2715467	IF 1:500
Rabbit anti-Phospho-Bad	Cell Signaling Technology	Cat# 5284; RRID: AB_560884	IF 1:200
Rabbit anti-Bim	Cell Signaling Technology	Cat# 2933; RRID: AB_1030947	IF 1:200
Rabbit anti-Bak	Cell Signaling Technology	Cat# 12105; RRID: AB_2716685	WB 1:1000
Rabbit anti-p21	ProteinTech	Cat# 10355-1-AP; RRID: AB_2077682	IF 1:500
Rabbit anti-p27	ProteinTech	Cat# 25614-1-AP; RRID: AB_2880161	IF 1:500
Rabbit anti-Caspase3	ProteinTech	Cat# 19677-1-AP; RRID: AB_10733244	IF 1:500
Alexa Fluor 488 anti-Mouse	ThermoFisher	Cat# A21202; RRID: AB_141607	IF 1:500
Alexa Fluor 555 anti-Rabbit	ThermoFisher	Cat# A31572; RRID: AB_162543	IF 1:500
HRP*Goat Anti RabbitIgG(H+L)	ProteinTech	Cat# RGAR001; RRID: AB_3073505	WB 1:10000
HRP* Goat Anti Mouse IgG(H+L)	ProteinTech	Cat# RGAM001; RRID: AB_3068333	WB 1:10000
IPKine™ HRP, Mouse Anti-Rabbit IgG LCS	Abbkine	Cat# A25022; RRID: AB_2893334	WB 1:10000
Normal Rabbit IgG Control	R&D Systems	Cat# AB-105-C; RRID: AB_354266	IP 4 μg

Table S4 Key reagent

REAGENTor RESOURCE	SOURCE	IDENTIFIER
DMEM Media	Gibco	Cat# C11995500CP
Fetal bovine serum	Gibco	Cat# 10099141C
Penicillin streptomycin -	Gibco	Cat# 15140122
Puromycin	Sangon Biotech	Cat# A610593
poly-HEMA	Sigma	Cat# 041M0024U
Triton X-100	Sigma	Cat# 93443; CAS# 9036-19-5
MAK683	MedChemExpress	Cat# HY-103663
DAPI	Beyotime	Cat# C1005
Lipofectamine™ 2000	Invitrogen	Cat# 11668019
Skim milk	BD	Cat# 232100
DTT	Invitrogen	Cat# 772590

RNase inhibitor	TaKaRa	Cat# 2313B
Maxima H Minus	Thermo Scientific	Cat# EP0752
Methylene blue	Aladdin	Cat# M196499
Hybond N+ membranes	GE Healthcare	Cat# RPN303C
PVDF membrane	Millipore	Cat# IPVH00010
Protease inhibitor	Roche	Cat# 11873580001
CCK8	Beyotime	Cat# C0039
In Situ Cell Death Detection Kit	Roche	Cat# 12156792910
Hoechst 33258	Beyotime	Cat# C1017
RNeasy MinElute Cleanup Kit	QIAGEN	Cat#74204
SMARTer Stranded Total RNA-Seq Kit v3 Pico Input Mammalian	TaKaRa	Cat# 634488
Protein A Dynabeads	Thermo Scientific	Cat# 10002D
Protein G Dynabeads	Thermo Scientific	Cat# 10004D
Protein G-Agarose	Roche	Cat# 11243233001
Qubit RNA HS Assay	Thermo Scientific	Cat# Q32852
Equalbit 1x dsDNA HS Assay Kit EQ121	Vazyme	Cat# EQ121-02
NP-40 Surfact-Amps	Thermo Scientific	Cat# 28324
Na-deoxycholate	Sigma	Cat# D6750
CUT&Tag Kit	Vazyme	Cat# TD903-01
ATAC-Seq Library Prep Kit	Vazyme	Cat# TD711-02
β-mercaptoethanol	Sigma	Cat# 63689
RNAiso Plus	Takara	Cat# 9109
Turbo DNAase	Invitrogen	Cat# BL698A

List of abbreviations

Abbreviation	Full title
m^6A	N6-methyladenosine
RIF	Recurrent implantation failure
H3K27me3	Histone H3 methylation at lysine 27
PCR2	Polycomb Repressive Complex 2
EED	Embryonic Ectoderm Development
EZH1/2	Enhancer of Zeste 1 and 2
SUZ12	Suppressor of Zeste 12
MB	Methylene blue
mRNA	Messenger RNA
GD 4	Gestation day 4

IF	Immunofluorescence
PMSF	Phenylmethylsulfonyl fluoride
RIP	RNA Immunoprecipitation
Co-IP	Co-immunoprecipitation
MeRIP-seq	m^6A RNA immunoprecipitation sequencing
SD	Standard Deviation
ESCs	Embryonic stem cells
TEs	Transposable elements
FTO	Fat mass and obesity-associated protein
ERVs	Endogenous retroviruses