

**TGF- β 1-induced m6A modifications accelerate onset of nuclear cataract
in high myopia by modulating the PCP pathway**

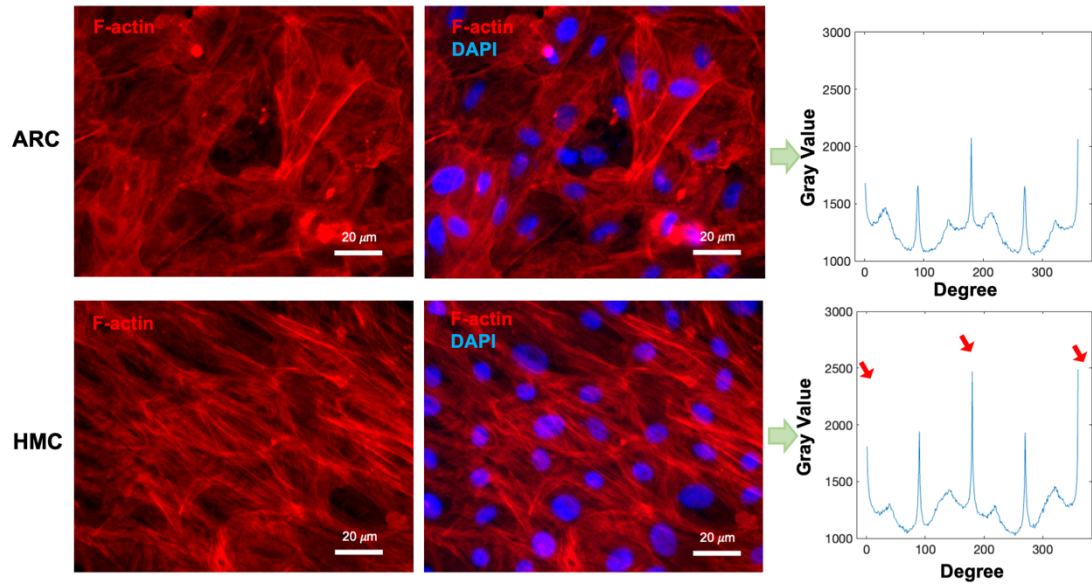
Supplementary Information

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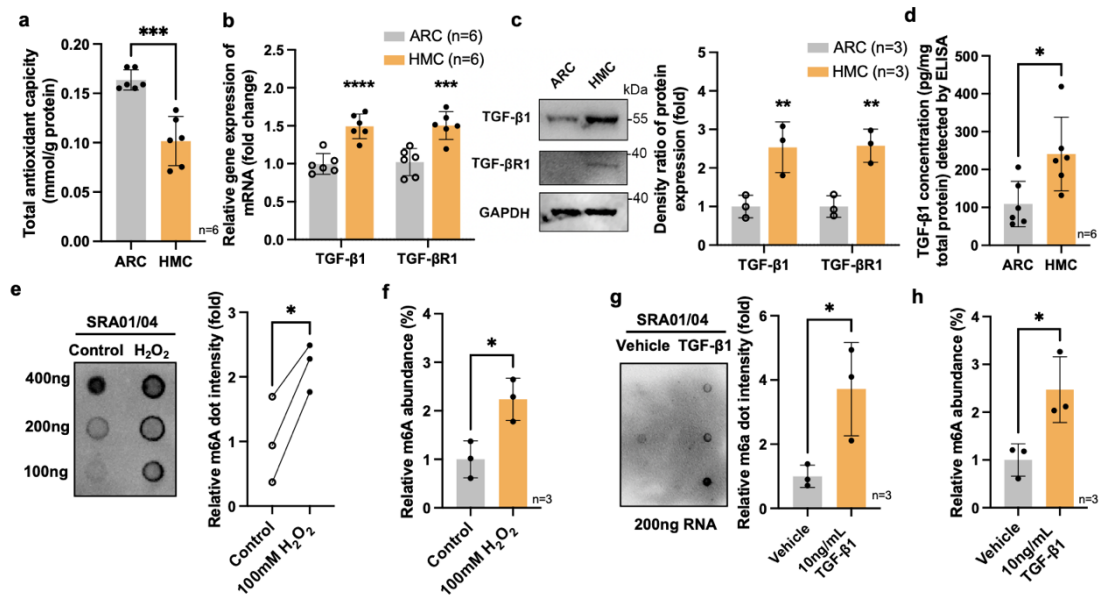
Supplementary Tab. 1 Patients' characteristics in highly myopic cataract and age-related cataract groups. Two-sided Student's t test was used to compare the continuous variables and the χ^2 test was used to compare categorical variables between HMC and ARC.

Supplementary Tab. 2 Reagents and resources used in this study.

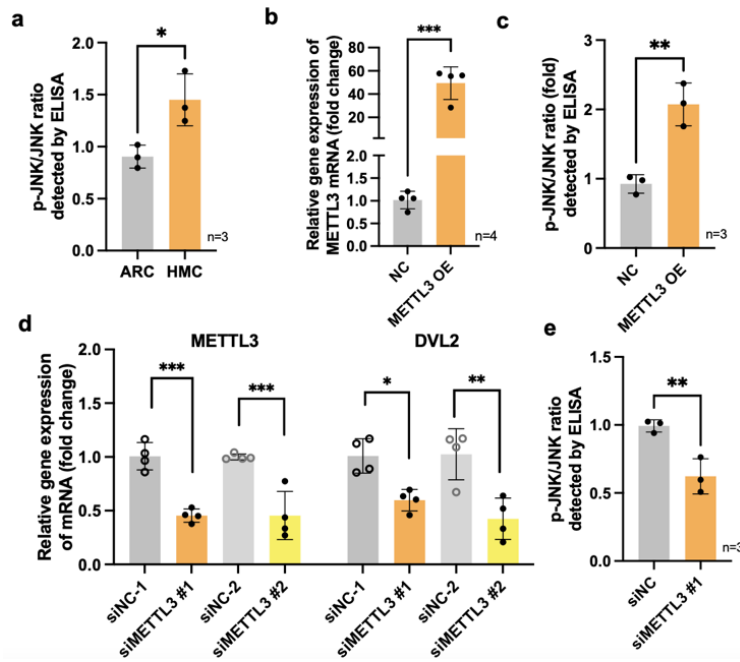
Supplementary data file (see [xlsx](#)): List of differentially methylated RNA sites identified by MeRIP-seq, including both upregulated and downregulated sites in HMC versus ARC.



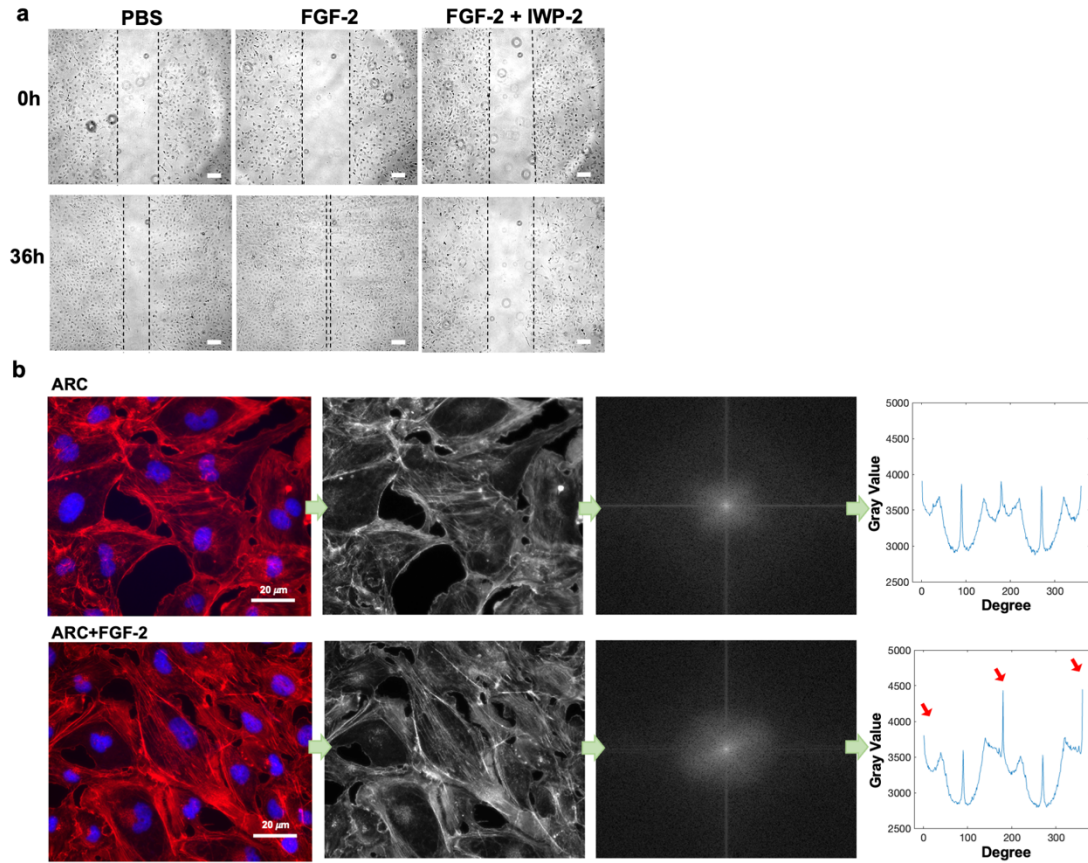
Supplementary Fig. 1 The immunofluorescent staining of F-actin in primary culture of human lens epithelial cells from age-related cataract (ARC) and highly myopic cataract (HMC) patients. Scale bar: 50 μm . The 2D Fast Fourier Transform (2D FFT) was conducted to obtain quantitative data of cell alignment. Red arrows show the intensity peaks in 0°, 180°, and 360°. Scale bar: 20 μm .



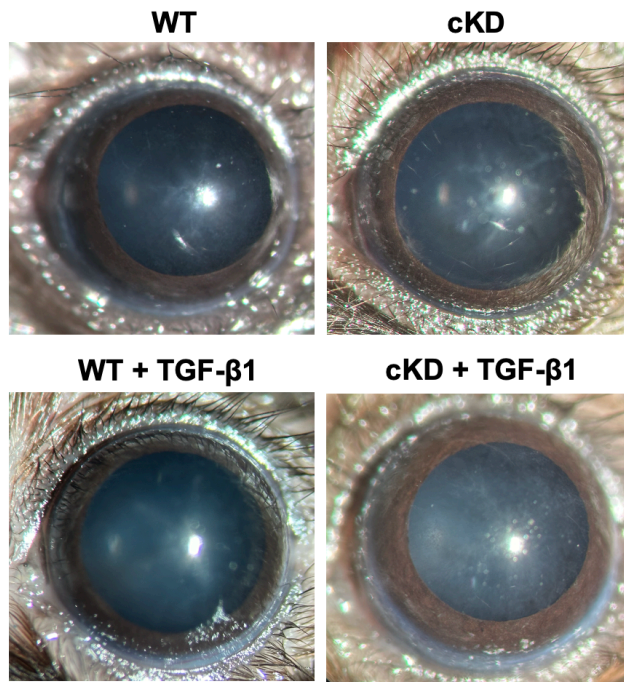
Supplementary Fig. 2 **a**. Total antioxidant capacity in lens epithelia of age-related cataract (ARC) and highly myopic cataract (HMC) patients ($n = 6$, $p = 0.0002$). **b**. TGF-β1 and TGF-βR1 gene expressions detected by qRT-PCR in lens epithelia of ARC and HMC patients ($n = 6$, TGF-β1 $p < 0.0001$, TGF-βR1 $p = 0.0001$). **c**. Total TGF-β1 and TGF-βR1 protein expressions detected by Western Blot in lens epithelia of ARC and HMC patients ($n = 3$, TGF-β1 $p = 0.0055$, TGF-βR1 $p = 0.0047$). **d**. Total TGF-β1 level of in lens epithelia of ARC and HMC patients ($n = 6$, $p = 0.0179$). **e, f**. The dot blot assay (**e**) and colorimetric quantification (**f**) of m6A abundance in the total RNAs extracted from SRA 01/04 cells treated with 100 μM H₂O₂ ($n = 3$, **e**: $p = 0.0255$; **f**: $p = 0.0201$). **g, h**. The dot blot assay (**g**) and colorimetric quantification (**h**) of m6A abundance in the total RNAs extracted from SRA 01/04 cells treated with 10 ng/mL TGF-β1 ($n = 3$, **g**: $p = 0.0345$; **h**: $p = 0.0290$). The band or dot density in Western blot or dot blot was normalized to loading control as a ratio for statistical analysis. Data present are mean ± SD. Level of significance was detected using two-sided unpaired Student's t test (**a, d, f-h**), paired Student's t test (**e**), and two-way ANOVA with Šidák's multiple comparisons test (**b, c**). n =biological replicates, **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$.



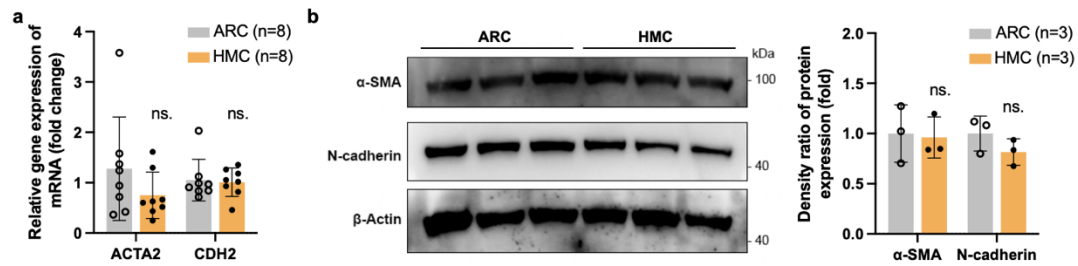
Supplementary Fig. 3 a. p-JNK/total-JNK concentration ratio detected by ELISA in lens epithelia of ARC and HMC patients ($n = 3$, $p = 0.0257$). **b.** METTL3 gene expression detected by qRT-PCR in SRA 01/04 cells transfected with METTL3 overexpression (OE) plasmid ($n = 4$, $p = 0.0005$). **c.** p-JNK/total-JNK concentration ratio detected by ELISA in SRA 01/04 cells transfected with METTL3 OE plasmid ($n = 3$, $p = 0.0281$). **d.** METTL3 and DVL2 gene expressions detected by qRT-PCR in SRA 01/04 cells transfected with two different METTL3 siRNAs (siMETTL3 #1 and #2) ($n = 4$, METTL3: siMETTL3 #1 $p = 0.0006$, siMETTL3 #2 $p = 0.0006$; DVL2: siMETTL3 #1 $p = 0.0139$, siMETTL3 #2 $p = 0.0003$). **e.** p-JNK/total-JNK concentration ratio detected by ELISA in SRA 01/04 cells transfected with siMETTL3 #1 ($n = 3$, $p = 0.0094$). Data present are mean $\pm$ SD. Level of significance was detected using two-sided two-way ANOVA with Šídák's multiple comparisons test (**d**) or Student's *t* test (**a-c**, **e**). n = biological replicates, *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$.



Supplementary Fig. 4. a. Representative images of cell migration test in human lens epithelial cell line SRA 01/04 treated with 200 ng/mL fibroblast growth factor 2 (FGF-2) alone or together with 20 μ M WNT production inhibitor IWP-2 by wound healing test. PBS was used as vehicle control. Scale bar: 50 μ m. **b.** The 2D Fast Fourier Transform (2D FFT) analysis of F-actin staining in primary culture of human lens epithelial cells from age-related cataract (ARC) patients and ARC treated with FGF-2. Red arrows show the intensity peaks in 0°, 180°, and 360°. Scale bar: 20 μ m.



Supplementary Fig. 5 Slit-lamp images of mouse lens in Prox1-CreERTwt/wt;Mettl3flox/flox mice (WT), Prox1-CreERTct2/wt;Mettl3flox/flox mice (cKD), WT and cKD mice after intravitreal injection of active TGF- β 1.



Supplementary Fig. 6 a. ACTA2 and CDH2 gene expressions detected by qRT-PCR in lens epithelia of ARC and HMC patients ($n = 8$, ACTA2 $p = 0.1873$, CDH2 $p = 0.9886$). **b.** α -SMA and N-cadherin protein expressions detected by Western Blot in lens epithelia of ARC and HMC patients ($n = 3$, α -SMA $p = 0.9686$, N-cadherin $p = 0.5190$). The band density in Western blot was normalized to loading control as a ratio for statistical analysis. Data present are mean $\pm$ SD. Level of significance was detected using two-sided two-way ANOVA with Šídák's multiple comparisons test (**a-b**). n = biological replicates, ns. = not significant.

Supplementary Table 1. Patients' characteristics in highly myopic cataract and age-related cataract groups. Two-sided Student's t test was used to compare the continuous variables and the χ^2 test was used to compare categorical variables between HMC and ARC.

A. For qRT-PCR, Western Blot, Immunostaining, and Primary cell culture analysis

	HMC	ARC	P value
Age-matched 45-55 years old group			
Number	117	117	
Age	50.85 $\pm$ 3.23	51.79 $\pm$ 3.78	0.1176
Max	55	55	
Min	45	45	
Sex (Female%)	65/117	68/117	0.7919
AL	28.74 $\pm$ 2.13	23.35 $\pm$ 0.69	<0.0001
Max	35.51	24.72	
Min	26.06	22.01	
Laterality (OD%)	51.28% (60/117)	52.14% (61/117)	>0.9999
Ethnicity	Asian	Asian	
Age-matched 56-65 years old group			
Number	240	240	
Age	60.43 $\pm$ 3.14	60.67 $\pm$ 2.78	0.0961
Max	65	65	
Min	56	56	
Sex (Female%)	137/240	138/240	>0.9999
AL	28.78 $\pm$ 2.62	23.41 $\pm$ 0.61	<0.0001
Max	34.88	24.48	
Min	22.53	22.07	
Laterality (OD%)	50.00% (120/240)	50.83% (122/240)	0.9273
Ethnicity	Asian	Asian	

HMC = highly myopic cataract, ARC = age-related cataract, AL = axial length.

Data present are mean $\pm$ SD.

B. Donor lens samples for SEM.

	Control	ARC	HMC	P value
Age	50.80 ± 2.77	53.40 ± 2.07	51.00 ± 4.42	0.0532
Max	55	55	55	
Min	45	50	45	
Sex	60% (6/10)	40% (4/10)	40% (4/10)	0.5853
(Female%)				
AL	22.94 ± 0.50	23.34 ± 0.62	28.84 ± 1.25	<0.0001
Max	23.8	24.4	30.9	
Min	22.2	22.5	27.0	
Ethnicity	Asian	Asian	Asian	
LOCSIII	N1	C3.3N3.5	N3.5	
(averaged)				

HMC = highly myopic cataract, ARC = age-related cataract, AL = axial length, LOCSIII = Lens Opacity Classification System III.

Data present are mean ± SD.

C. Sample list for MERIP-seq

Year	of	Date of surgery	Sample	Name_m	Sex	Age	Ethnicity	laterarity	Axial	LOCSIII	DM	HBP	Mix of
surgery			value	asked					length				sample
2020		10.26	OK	lym	male	64	Asian	OD	24.48	C4N2	no	no	ARC-1
2020		10.26	OK	cxh	male	64	Asian	OD	23.77	C3N3	no	no	ARC-1
2020		10.12	OK	csl	female	60	Asian	OS	22.19	C2N3	no	no	ARC-1
2020		10.26	OK	jhh	female	60	Asian	OS	22.88	C3N3	no	no	ARC-2
2020		10.19	OK	laq	female	64	Asian	OD	24.29	C4N3	no	no	ARC-2
2020		10.19	OK	yqh	male	63	Asian	OS	23.03	C3N3	no	no	ARC-2
2020		10.12	OK	yhm	female	56	Asian	OS	23.66	C3N3	no	no	ARC-3
2020		10.12	OK	ymj	female	60	Asian	OS	24.03	C3N3	no	no	ARC-3
2020		10.19	OK	csc	female	57	Asian	OS	23.76	C3N3	no	no	ARC-3
2020		10.12	OK	yxm	male	65	Asian	OD	27.2	N4	no	no	HMC-1
2020		10.14	OK	glm	male	64	Asian	OS	30.67	N3	no	no	HMC-1
2020		10.26	OK	yjg	male	51	Asian	OD	35.41	N3	no	no	HMC-1
2020		11.2	OK	zzh	male	57	Asian	OD	30.56	N3	no	no	HMC-2
2020		11.2	OK	gf	male	60	Asian	OS	33.16	N3	no	no	HMC-2
2020		11.2	OK	thj	male	65	Asian	OD	31.44	N3	no	no	HMC-2
2020		10.26	OK	wh	female	47	Asian	OD	27.34	N3,5	no	no	HMC-3
2020		10.26	OK	shl	male	61	Asian	OS	30.37	N4	no	no	HMC-3
2020		10.19	OK	wcz	male	58	Asian	OS	36.18	N4	no	no	HMC-3

Supplementary Table 2. Reagents and resources used in this study.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-TGF- β 1	Abcam	ab170874
Anti-TGF beta Receptor I	Abcam	ab235578
Anti-METTL3	Abcam	ab195352
Anti-DVL2	Santa Cruz Biotechnology	sc-8620
Anti-WNT11	Santa Cruz Biotechnology	sc-365032
Anti-IGF2BP3	Abcam	ab177477
Anti- β -Catenin non-phospho (active) S37/T41	Abcam	ab246504
Anti-phosphor-JNK	Santa Cruz Biotechnology	sc-293136
Anti-GAPDH	Abcam	ab8245
Anti- β -Actin	Sigma-Aldrich	A5441
Peroxidase AffiniPure Goat Anti-Mouse IgG (H+L)	Jackson ImmunoResearch	115-035-003
Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L)	Jackson ImmunoResearch	111-035-003
Alexa Fluor® 488 AffiniPure Donkey Anti-Rabbit IgG (H+L)	Jackson ImmunoResearch	711-545-152
Alexa Fluor® 488 AffiniPure Goat Anti-Mouse IgG (H+L)	Jackson ImmunoResearch	115-545-003
TRITC Phalloidin	Yeesen	40734ES75
FITC conjugate WGA	Sigma-Aldrich	L4895
Anti-m6A antibody	Abcam	ab208577
Biological samples		
Lens from healthy donors	Eye bank, Eye and ENT Hospital of Fudan University	N/A
Lens epithelia and lens epithelial cells from cataract surgery patients	Eye and ENT Hospital of Fudan University	N/A
Chemicals, peptides, and recombinant proteins		
Animal-Free Recombinant Human TGF- β 1 (CHO derived), active	Pepro Tech	AF-100-21C
Recombinant Mouse TGF-beta 1 Protein, active	R&D Systems	7666-MB-005
Glutaraldehyde	Sigma-Aldrich	Cat# G5882
Osmium tetroxide	Ted Pella	Cat#18451
TRIzol reagent	Invitrogen	15596018
Immobilon ECL Ultra Western HRP Substrate	Sigma-Aldrich	WBULS0100
Actinomycin D	Cell Signaling Technology	#15021
RIPA lysis buffer	Beyotime	P0013B
Nitrocellulose membrane	Beyotime	FFN02
Corn oil	Sigma-Aldrich	CAS#10540-29-1

Tamoxifen	Sigma-Aldrich	T5648-5G
Lipofectamine 3000	ThermoFisher	L3000015
4-12% precast mini polyacrylamide gels	GenScript	SurePAGE™
0.5% tropicamide and 0.5% phenylephrine	Santen Pharmaceutical Co., Ltd	N/A
ProDots Recombinant Human FGF basic/FGF2 (146 aa) Protein	R&D Systems	PRD233-10
Wnt Antagonist II, IWP-2	Merck	681671-10MGCN
Critical commercial assays		
Human/mouse/rat TGF-β1 ELISA kit, total	Multi Sciences	EK981-96
PathScan Phospho-SAPK/JNK (Thr183/Tyr185) Sandwich ELISA Kit	Cell Signaling Technology	#7325
PathScan Total SAPK/JNK Sandwich ELISA Kit	Cell Signaling Technology	#7330
RNeasy Micro Kit	Qiagen	74004
EpiQuik m6A RNA Methylation Quantification Kit (Colorimetric)	EpiGentek	P-9005-96
GenSeq™ m6A RNA IP kit	GenSeq Inc.	GS-ET-001
Magna RIP RNA-Binding Protein Immunoprecipitation Kit	Millipore	17–700
Cell Counting Kit-8 (CCK-8)	DOJINDO	CK04
HiFiScript gDNA removal cDNA Sythesis Kit	ComWin Biotech	CW2582M
Bicinchoninic Acid Assay	Beyotime	P0012
Deposited data		
M ⁶ A-sequencing sequencing data	NCBI Gene expression omnibus (GEO)	GSE244037
Experimental models: Cell lines		
SRA 01/04	OEbiotech	N/A
Experimental models: Organisms/strains		
C57BL/6J	Jackson Laboratory	Cat# 000664
C57BL/6J, <i>Mettl3</i> flox/flox	GemPharmatech	Strain NO.T006659
C57BL/6J, Tg(Prox1-Cre-ERT2)	GemPharmatech	Strain NO.T010829
Oligonucleotides		
Human DVL2 for MERIP-qPCR Forward: GCAGCTACATGGCCCAAATC Reverse: CCATTGGGCCCTTCTAACC	This paper	In this study
Human WNT11 for MERIP-qPCR Forward: GGTCCCTTGAGCAGAGTCCTC Reverse: TGGCTACAACCCCTACACAG	This paper	In this study
Human TGFBR1 for RT-qPCR Forward: CTCTTCAAAAAGTGGGTCTGTG Reverse: CATCAACATGAGTGAGATGCAG	This paper	In this study

Human METTL3 for RT-qPCR Forward: GGAATCACCTCCGACACTC Reverse: AAGCTGCACTTCAGACGAAT	This paper	In this study
Human WTAP for RT-qPCR Forward: CTGACAAACGGACCAAGTAATG Reverse: AAAGTCATCTTCGGTTGTGTTG	This paper	In this study
Human METTL14 for RT-qPCR Forward: ACCAAAATCGCCTCCTCCCAAATC Reverse: AGCCACCTCTTTCTCCTCGGAAG	This paper	In this study
Human ALKBH5 for RT-qPCR Forward: CCCGAGGGCTTCGTCAACA Reverse: CGACACCCGAATAGGCTTGA	This paper	In this study
Human FTO for RT-qPCR Forward: TGGGTTCATCCTACAACGG Reverse: CCTCTTCAGGGCCTTCAC	This paper	In this study
Human IGF2BP1 for RT-qPCR Forward: GATGAAGGCCATCGAACTTTC Reverse: GGGGTGGAATATTCGGATTTG	This paper	In this study
Human IGF2BP2 for RT-qPCR Forward: GATGAACAAGCTTTACATCGGG Reverse: GATTTTCCCATGCAATTCCACT	This paper	In this study
Human IGF2BP3 for RT-qPCR Forward: GAGGCGCTTTCAGGTAAAATAG Reverse: AATGAGGCGGGATATTCGTAT	This paper	In this study
Human WNT11 for RT-qPCR Forward: AGCTCACCTGACTTCTGCAT Reverse: CACATAAGGTCGCAGCTGTC	This paper	In this study
Human DVL2 for RT-qPCR Forward: TTGGCTCCATCATGAAGGGT Reverse: TCACCTGCAAAAGCATGTCC	This paper	In this study
Mouse DVL2 for RT-qPCR Forward: AACTTTGAGAACATGAGCAACG Reverse: ATAGATGAGGAGCCAGGGTAAG	This paper	In this study
Mouse METTL3 for RT-qPCR Forward: CGCTGCCTCCGATGTTGATCTG Reverse: TCTCCTGACTGACCTTCTTGCTCTG	This paper	In this study
Human GAPDH Endogenous Reference Genes Primers	Sangon Biotech	B662104-0001
Mouse GAPDH Endogenous Reference Genes Primers	Sangon Biotech	B662304-0001
Software and algorithms		
ImageJ	National Institutes of	1.53c

	Health	
MATLAB	Mathworks	version R2021b
SPSS	IBM	22.0
Prism 9	GraphPad	Version 9.1.1