

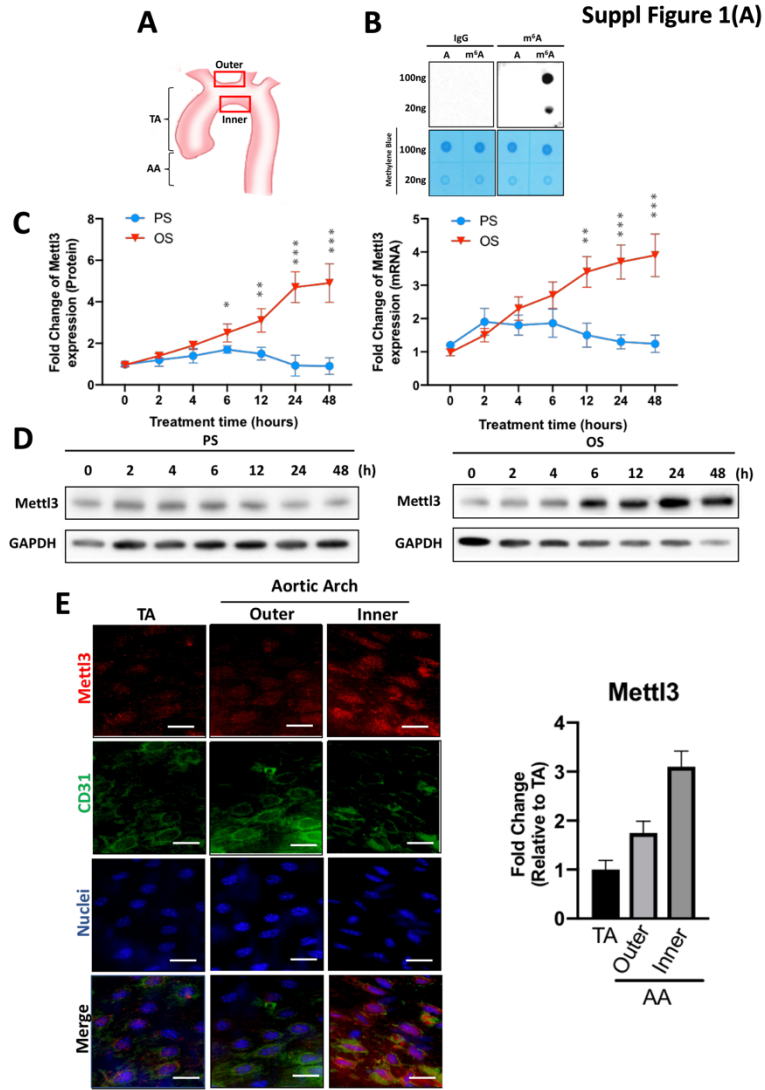
**Supplemental Table I:** The list of genes containing m<sup>6</sup>A sites that were hypomethylated by METTL3 knockdown under OS

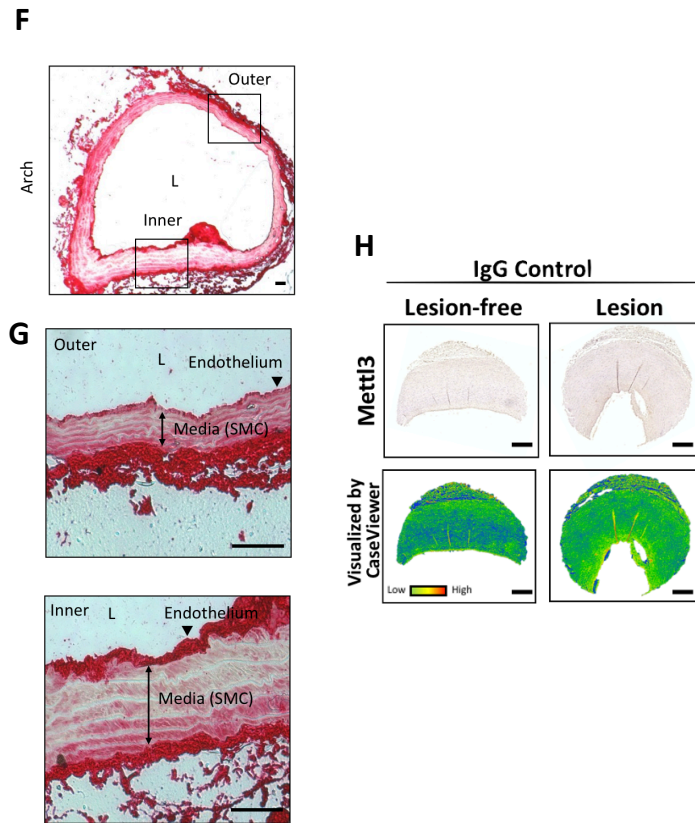
OS_siCtrl						OS_siMETTL3									
Chr	Start	End	-log10(P-value)	Log2 Fold Change	Strand	Gene	Chr	Start	End	-log10(P-value)	Log2 Fold Change	Strand	Gene	Feature	Fold
chr8	141,194,251	141,194,264	22.661	5.583	+	DENND3	chr8	141,194,270	141,194,353	31.600	3.451	+	DENND3	3' UTR	0.228
chr12	1,490,575	1,490,608	46.544	5.103	+	ERC1	chr12	1,490,575	1,490,602	15.809	3.034	+	ERC1	3' UTR	0.238
chr8	91,960,419	91,960,437	37.744	5.712	-	RUNX1T1	chr8	91,960,431	91,960,473	11.099	3.968	-	RUNX1T1	CDS	0.299
chr7	150,573,229	150,573,238	27.127	4.665	+	GIMAP4	chr7	150,573,244	150,573,289	5.540	3.017	+	GIMAP4	3' UTR	0.319
chr9	131,260,675	131,260,760	30.905	4.711	-	FAM78A	chr9	131,260,556	131,260,616	5.262	3.111	-	FAM78A	3' UTR	0.330
chr11	35,619,499	35,619,508	26.062	4.862	+	FJX1	chr11	35,619,350	35,619,384	31.883	3.321	+	FJX1	CDS	0.344
chr8	27,672,631	27,672,668	31.254	5.151	+	SCARA3	chr8	27,672,678	27,672,700	10.747	3.633	+	SCARA3	3' UTR	0.349
chr20	50,891,296	50,891,302	57.548	4.631	-	ADNP	chr20	50,891,259	50,891,274	22.040	3.128	-	ADNP	3' UTR	0.353
chr3	42,536,959	42,537,017	32.324	5.794	+	VIPR1	chr3	42,537,258	42,537,316	11.809	4.367	+	VIPR1	3' UTR	0.372
chr7	44,883,034	44,883,039	67.183	6.007	+	PURB	chr7	44,883,003	44,883,040	85.366	4.674	-	PURB	3' UTR	0.397
chr19	52,612,852	52,612,875	17.752	4.427	-	ZNF83	chr19	52,612,881	52,612,947	7.246	3.139	-	ZNF83	3' UTR	0.410
chr12	12,330,321	12,330,342	78.319	4.666	-	MANSC1	chr12	12,330,482	12,330,550	28.095	3.397	-	MANSC1	CDS	0.415
chr8	17,645,678	17,645,682	55.911	4.966	-	MTUS1	chr8	17,645,683	17,645,700	28.793	3.727	-	MTUS1	3' UTR	0.424
chr17	5,501,517	5,501,523	12.538	4.941	-	NLRP1	chr17	5,501,475	5,501,527	8.537	3.708	-	NLRP1	3' UTR	0.426
chr13	109,756,093	109,756,163	31.861	6.246	-	IRS2	chr13	109,755,594	109,755,638	24.286	5.018	-	IRS2	3' UTR	0.427
chr1	64,692,106	64,692,127	14.129	4.820	+	CACHD1	chr1	64,691,951	64,691,977	7.521	3.597	+	CACHD1	3' UTR	0.428
chr3	48,158,817	48,158,827	52.498	6.053	-	CDC25A	chr3	48,158,828	48,158,837	30.830	4.832	-	CDC25A	3' UTR	0.429
chr6	138,424,226	138,424,240	400.000	5.264	-	NHSL1	chr6	138,424,165	138,424,173	52.901	4.062	-	NHSL1	CDS	0.435
chr2	236,165,632	236,165,632	13.168	4.655	-	GBX2	chr2	236,165,564	236,165,613	15.962	3.473	-	GBX2	3' UTR	0.441
chr19	16,893,950	16,893,985	13.578	4.355	-	CPAMD8	chr19	16,894,292	16,894,334	4.289	3.266	-	CPAMD8	CDS	0.470
chr9	128,107,706	128,107,792	47.431	4.580	+	SLC25A25	chr9	128,107,476	128,107,534	15.254	3.545	+	SLC25A25	3' UTR	0.488
chr21	37,512,439	37,512,446	56.790	5.045	+	DYRK1A	chr21	37,512,447	37,512,509	400.000	4.069	+	DYRK1A	CDS	0.509
chr10	100,548,243	100,548,321	49.926	4.169	+	HIF1AN	chr10	100,548,202	100,548,322	33.677	3.211	+	HIF1AN	3' UTR	0.515
chr6	12,120,476	12,120,527	20.548	3.934	+	HIVEP1	chr6	12,121,101	12,121,114	8.892	3.003	+	HIVEP1	CDS	0.525
chr4	20,619,406	20,619,413	18.667	4.103	+	SLIT2	chr4	20,619,196	20,619,274	18.912	3.177	+	SLIT2	3' UTR	0.526
chr19	40,033,505	40,033,610	11.023	4.494	-	ZNF780B	chr19	40,033,502	40,033,619	9.793	3.594	-	ZNF780B	3' UTR	0.536
chr12	50,986,944	50,986,954	14.508	4.233	-	SLC11A2	chr12	50,986,954	50,987,003	10.784	3.361	-	SLC11A2	3' UTR	0.546
chr16	20,735,093	20,735,102	49.917	5.425	-	THUMPD1	chr16	20,735,103	20,735,121	35.148	4.553	-	THUMPD1	3' UTR	0.547
chr15	90,229,379	90,229,429	12.290	4.643	+	SEMA4B	chr15	90,229,378	90,229,391	14.470	3.848	+	SEMA4B	3' UTR	0.576
chr1	183,926,300	183,926,337	30.423	3.909	+	RGL1	chr1	183,926,325	183,926,338	12.829	3.115	+	RGL1	3' UTR	0.577
chr1	64,692,127	64,692,203	17.537	4.684	+	CACHD1	chr1	64,691,977	64,692,027	10.374	3.973	+	CACHD1	3' UTR	0.611
chr1	25,567,560	25,567,571	49.834	5.119	+	LDLRAP1	chr1	25,567,582	25,567,662	47.335	4.416	+	LDLRAP1	3' UTR	0.614
chr9	107,485,495	107,485,551	22.813	4.174	-	KLF4	chr9	107,485,546	107,485,578	25.326	3.483	-	KLF4	3' UTR	0.619
chr8	41,932,095	41,932,128	21.374	4.449	-	KAT6A	chr8	41,931,910	41,931,981	13.533	3.800	-	KAT6A	3' UTR	0.638
chr8	13,085,663	13,085,671	59.849	4.667	-	DLC1	chr8	13,085,676	13,085,682	400.000	4.075	-	DLC1	3' UTR	0.664
chr6	107,068,577	107,068,644	6.504	4.256	-	BEND3	chr6	107,068,444	107,068,507	8.048	3.731	-	BEND3	3' UTR	0.695
chr9	4,860,420	4,860,445	6.742	3.565	+	RCL1	chr9	4,860,419	4,860,471	14.365	3.053	+	RCL1	3' UTR	0.701
chr16	25,239,375	25,239,406	7.507	4.259	-	ZKSCAN2	chr16	25,239,326	25,239,415	12.385	3.811	-	ZKSCAN2	3' UTR	0.733
chr7	129,212,462	129,212,517	22.898	4.937	+	SMO	chr7	129,212,460	129,212,518	11.643	4.514	+	SMO	3' UTR	0.746
chr8	23,117,106	23,117,112	20.718	4.162	+	TNFRSF10C	chr8	23,117,104	23,117,147	26.657	3.823	+	TNFRSF10C	3' UTR	0.791
chr19	2,477,946	2,477,959	25.701	3.579	+	GADD45B	chr19	2,477,945	2,477,957	400.000	3.259	+	GADD45B	3' UTR	0.801
chr6	137,878,690	137,878,768	7.258	4.194	+	TNFAIP3	chr6	137,878,705	137,878,773	13.204	3.877	+	TNFAIP3	CDS	0.803
chr7	55,206,343	55,206,372	31.308	5.142	+	EGFR	chr7	55,206,372	55,206,440	41.353	4.944	+	EGFR	3' UTR	0.872
chr2	239,052,438	239,052,520	7.334	4.390	-	HDAC4	chr2	239,052,525	239,052,609	9.673	4.215	-	HDAC4	3' UTR	0.886
chr11	6,391,889	6,391,894	40.787	3.423	+	SMPD1	chr11	6,391,903	6,391,965	47.744	3.382	+	SMPD1	CDS	0.972

**Supplemental Table II: Primer information**

Gene Name	Gene #	Primer name	Sequence	Tm	Product size	
<b>Mettl3</b>	NM_019852.4	hMettl3QF	TGGGGGTATGAACGGGTAGA	60	149	RT-PCR
		hMettl3QR	TGGTTGAAGCCTTGGGGATT			
<b>Ythdf1</b>	NM_017798	hYthdf1QF	GACTTTGAGCCCTACCTTACTG	55	131	RT-PCR
		hYthdf1QR	GACCACGGAGCCTCATTG			
<b>Ythdf2</b>	NM_016258	hYthdf2QF	TCTGGAAAAGGCTAAGCAGG	57	145	RT-PCR
		hYthdf2QR	CTTTTATTTCCCACGACCTTGAC	57		
<b>Ythdf3</b>	NM_152758	hYthdf3QF	TAGGTGTTGTACCTGTCACT	55	122	RT-PCR
		hYthdf3QR	ACGTCCATTCTCAGATTCC	55		
<b>KLF4</b>	NM_004235.6	hKlf4QF	CCGCTCCATTACCAAGAGCT	60	76	RT-PCR
		hKlf4QR	ATCGTCTCCCTCTTTGGC			
<b>NLRP1</b>	NM_033004.4	hNLRP1_F	G TTCAGGGATGCTGGAAATA	55	207	RT-PCR
		hNLRP1_R	CTGGAGGGATCAGAGTAGTT	55		
<b>NLRP1</b>	NM_033004.4	hNLRP1_pos1F	TCTTCCAGTGCTTG CAGGC	60	200	meRIP-qPCR
		hNLRP1_pos1R	GATGTTCCAGGTGGTGAGG	60		
		hNLRP1_pos2F	CTGAAGGAGACCCATCCTCAC	57	139	meRIP-qPCR
		hNLRP1_pos2R	ACCCAAGAAGGGTCAGCCA	57		
		hNLRP1_pos3F	AGCATCTCAGGGAATGTCCA	60	160	meRIP-qPCR
		hNLRP1_pos3R	TGACACAGCCAGAGGCAATG	60		
<b>KLF4</b>	NM_004235.6	hKlf4_pos1F	AAGAAGAAGGATCTCGGCCA	60	155	meRIP-qPCR
		hKlf4_pos1R	CATGTCAGACTCGCCAGGTG	60		
		hKlf4_pos2F	ACCTTACCAGTGTGACTGGG	60	131	meRIP-qPCR
		hKlf4_pos2R	ACCTGGAAAATGCTCGGTCTG	60		
		hKlf4_pos3F	TGGTCAAGTCCCAACTGAGTC	60	181	meRIP-qPCR
		hKlf4_pos3R	TCCACAACCTTCCAGTCACCC	60		
<b>M13</b>		M13_F	TGTAAAACGACGGCCAGT	55		RIP
		M13_R	CAGGAAACAGCTATGAC	55		
<b>Mettl3/APPA</b>		Mettl3/APPA-F	gcagttgtagtgctgccccacccgoggatattccatggaa	55		site-directed mutagenesis
		Mettl3/APPA-R	ttocatgtaatatccgoggtggggcagccatcacaaactgc	55		

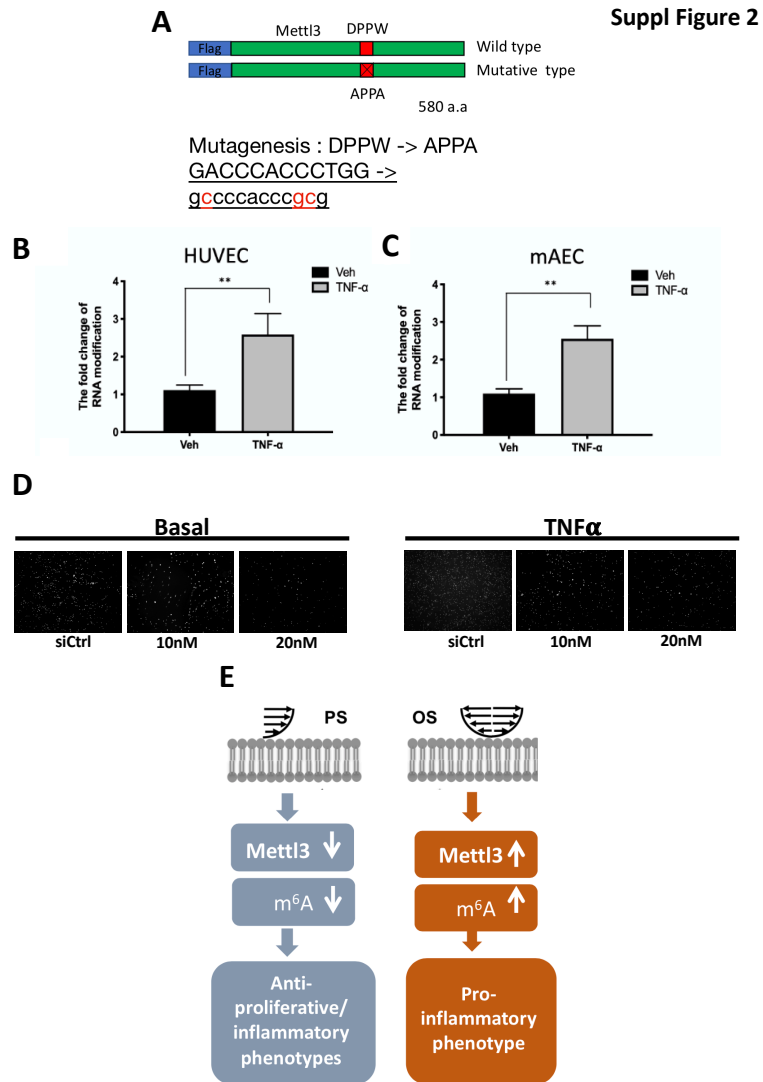
Supplemental Figure 1





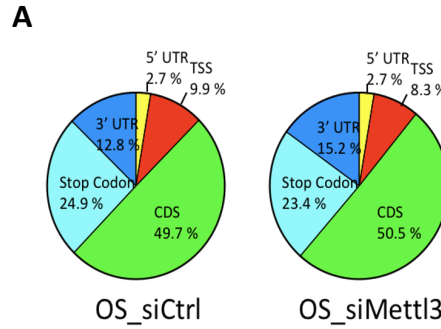
**Supplemental Figure 1: Comparison of METTL3 expression in the endothelium of the inner AA and the outer AA from wild-type control mice.** (A) Schemes showing the regions of thoracic aorta (TA) and aortic arch (AA). (B) Specificity validation of m6A dot blot. (C) Shear pattern- and time-dependent increase of METTL3 mRNA and (D) protein expression over a 48-hour time course. (E) Left: En face immunofluorescence staining showing the expression of METTL3 in arterial endothelium of TA and outer and inner parts of AA. Right: quantification showing mean fluorescence intensity values relative to TA with SD error bars. (F-G) Immunohistochemistry of METTL3 protein expression in the endothelium of the outer AA (Panel F, Upper) and the inner AA (Panel F, Lower) from control untreated C57BL/6 mice. (H) Specificity validation of METTL3 immunofluorescence.

Supplemental Figure 2



**Supplemental Figure 2: METTL3 as a mediator of the pro-atherogenic process in ECs in response to disturbed flow.** (A) Schemes showing the sequence for wild-type METTL3 and its catalytic mutant form, METTL3 APPA. (B~C) LCMS analysis showing the m6A modification of mRNA with or without TNF- $\alpha$  stimulation in both HUVECs (Panel B) and HAECs (Panel C). (D) Representative images of monocyte adhesion on TNF- $\alpha$ -stimulated HUVECs with or without Mettl3 knockdown. (E) Scheme showing METTL3 as a mediator of the pro-atherogenic inflammatory effects in ECs subjected to disturbed flow.

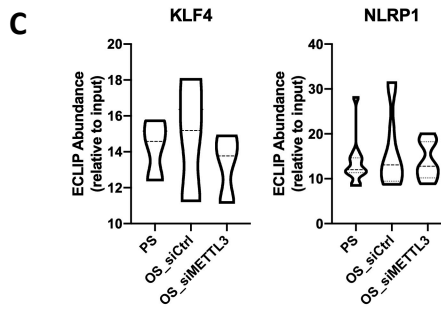
Supplemental Figure 3



Suppl Figure 3

**B**

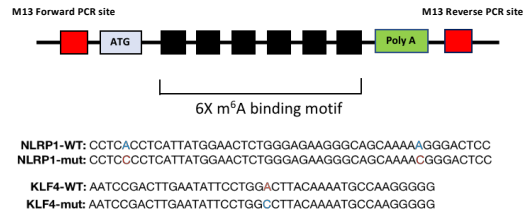
ID	Location	Family	Fold-enrich	ID	Location	Family	Fold-enrich
CD40	Plasma Membrane	transmembrane receptor	0.205	SLC7A2	Plasma Membrane	transporter	2.750
SLC11A2	Plasma Membrane	transporter	0.170	RIIP7	Nucleus	transcription	2.095
SCN1B	Plasma Membrane	ion channel	0.162	ENKAF	Extracellular Space	cytokine	2.044
PLATE	Plasma Membrane	other	0.161	LAMP5	Plasma Membrane	other	2.001
TMPT1	Extracellular Space	cytokine	0.142	DCBLD2	Plasma Membrane	other	4.087
CST3	Plasma Membrane	transmembrane receptor	0.142	MD1	Nucleus	transcription	3.881
LIR3A	Plasma Membrane	transmembrane receptor	0.140	PANX2	Nucleus	enzyme	0.981
CD57	Plasma Membrane	other	0.140	SLC22A10	Plasma Membrane	transporter	0.492
TNFRSF5	Extracellular Space	cytokine	0.141	COL3A1	Extracellular Space	other	0.498
CCDC14	Plasma Membrane	G-protein coupled receptor	0.094	CTTR	Nucleus	transcription	0.492
TNFRSF1B	Plasma Membrane	transmembrane receptor	0.094	STC17	Nucleus	transcription	0.517
PTM1	Plasma Membrane	transmembrane receptor	0.090	LRP1	Nucleus	transporter	0.819
PC4B	Cytosol	enzyme	0.093	PANX4	Nucleus	enzyme	1.527
SIMAD	Plasma Membrane	transmembrane receptor	0.090	LALP	Cytosol	transcription	1.214
RNF138	Cytosol	enzyme	0.090	TYLDP	Nucleus	other	1.527
SOXPT1	Plasma Membrane	transmembrane receptor	0.027	BAZ1B	Nucleus	transcription	0.98
AC10A	Plasma Membrane	kinase	1.315	PARC	Nucleus	enzyme	1.581
PSEN1	Plasma Membrane	peptidase	1.325	PANCC	Nucleus	other	1.593
HLA	Extracellular Space	transcription regulator	1.470	PARC2	Nucleus	other	1.707
HES1F	Extracellular Space	growth factor	1.477	PARL2	Nucleus	other	1.706
HEST1	Plasma Membrane	ion channel	1.038	SMAD3	Nucleus	transcription	2.048
PVR	Plasma Membrane	other	1.940	IFCC2	Nucleus	transcription	2.113
INDC5	Cytosol	enzyme	1.060	IFCC3	Nucleus	transcription	2.113
EDN1	Extracellular Space	cytokine	1.814	LOX	Extracellular Space	enzyme	2.048
SLC14A1	Plasma Membrane	transporter	1.623	PANX5	Nucleus	other	2.060
IKK2	Plasma Membrane	kinase	1.038	TGFR1	Plasma Membrane	kinase	2.038
MEI	Plasma Membrane	transporter	1.623	CTSD1	Nucleus	transcription	1.716
MIT	Plasma Membrane	kinase	1.767	PANCA	Nucleus	other	2.848
NRX1A	Cytosol	transcription regulator	1.775	BP1A1	Nucleus	transcription	3.551
NFY1	Cytosol	transcription regulator	1.828	METL3	Nucleus	enzyme	3.201
VP1	Extracellular Space	other	2.071	NLRP1	Cytosol	other	0.453
EDS	Plasma Membrane	G-protein coupled receptor	2.102	SMYD3	Cytosol	enzyme	0.452
MYC	Nucleus	transcription regulator	2.208	PCSKD	Cytosol	transcription	0.452
CEP350	Extracellular Space	other	2.202	CEP1	Cytosol	enzyme	0.522
IL1A	Extracellular Space	cytokine	2.247	APP	Plasma Membrane	other	0.578
SELE	Plasma Membrane	transmembrane receptor	2.247	PANX1	Plasma Membrane	transporter	1.689
LTR	Plasma Membrane	transmembrane receptor	2.385	KLF4	Nucleus	transcription	2.980
AOL	Plasma Membrane	kinase	2.485	TDRP	Cytosol	other	2.470
ABCA1	Plasma Membrane	transporter	2.676				



**Supplemental Figure 3: Involvement of METTL3-hypermethylated genes in atherosclerosis.** (A) The pie chart depicts the relative frequency of peaks in OS-treated ECs with or without *Mettl3* knockdown. The pie chart depicts the relative frequency of peaks that map to each feature type, with a peak log2 fold enrichment  $\geq 3$  and p-value  $\leq 0.001$ . (B) The list of genes affected by METTL3-knockdown under OS. (C) The eCLIP abundance of NLRP1 and KLF4 among PS-treated cells, OS-treated cells with either siCtrl or siMETTL3.

## Supplemental Figure 4

## Suppl Figure 4



**Supplemental Figure 4:** The plasmid design of NLRP1 and KLF4 RNA fragments with either wild-type (WT) or mutated (mut) m6A sites.