Supplemental Material

Detailed Materials & Methods

Antibodies and Reagents

Antibodies: - against MEF2 (#sc313, Santa Cruz Biotechnology, Santa Cruz, CA), KLF4 (#sc20691, Santa Cruz), DNMT1 (#39905, Active Motif, Carlsbad, CA), DNMT3A (#sc20703, Santa Cruz), DNMT3B (#39207, Active Motif; #MA5-16165, Thermo Scientific, Waltham, MA) TET1 (#MA5-16312, Thermo Scientific) and β-actin (#ab6276, Abcam). RG108 purchased from EMD Millipore (Billerica, Ma) and 5-azacitidine (5-aza) from Santa Cruz Biotechnology were dissolved in DMSO. DMSO influences DNA hydroxymethylation / methylation at high but not low concentration (<0.5%)[1]. The final DMSO concentration used was < 0.1%.

CpG island prediction and sequence alignment

The sequence of genes, including information on 5' UTR, transcription start site, exons and introns was obtained from *Ensembl*. Human gene sequence data were obtained from genome assembly GRCh37; Swine gene sequence was obtained from Sscrofa10.2. CpG islands were predicted by Methyl Primer Express software (Life Technologies, Grand Island, NY) using the following parameters: - CGI size 300-2000bp, C+Gs/ total bases >50%, CpGo/e> 60%.

To calculate the local alignment of sequences, the promoter sequence (3000bp upstream and 500bp downstream of TSS), mRNA (NM_004235.4, NM_001031782.2, NM_000603.4, NM_214295.1) and protein (NP_004226.3, NP_001026952.2, NP_000594.2, NP_999460.1) of human and swine KLF4 and NOS3 were compared by using EMBOSS (European Molecular Biology Open Software Suite) Water Smith-Waterman algorithm and ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

Real-time RT-PCR Analysis of pre-mature mRNA (pre-mRNA) and mRNA

Total RNA was extracted using the RNeasy kit (Qiagen, Valencia, CA). cDNA was synthesized by reverse transcribing 1 μg total RNA with 50 ng random hexamers and SuperScript III reverse transcriptase (Life Technologies). Quantitative-PCR (qPCR) was carried out in Light Cycler 480 with SYBR Green I Master (Roche, Indianapolis, IN) with 2 μl of first strand cDNA and 500 nM primers in a final volume of 20 μl in the presence and absence of reverse transcriptase to exclude contaminating genomic DNA. Reactions ran with an initial denaturation at 95 ^{o}C for 10 min. Thirty five to 45 cycles ran at: 95 ^{o}C for 10 s, 60 ^{o}C for 10 s, 72 ^{o}C for 10 s. A melting curve was run to detect the desired amplicon. Amplicon size was verified by a TapeStation 2200 (Agilent Technologies, Santa Clara, CA).

Transcriptional activity was monitored by measurement of nascent pre-mature mRNA (pre-mRNA) the PCR primers for which were designed to target at least one exon and one

intron. The primers for mature mRNA were designed to target the exons. qPCR primers are listed in Online Table I. The housekeeping gene ubiquitin B (UBB), GAPDH, or PECAM1 was used for normalization.

Methylation specific PCR and Pyrosequencing

Methylation specific PCR (MSP) and pyrosequencing was used to determine DNA methylation at specific loci. In brief, the genomic DNA was isolated by using DNeasy blood and tissue kit (Qiagen). All unmethylated cytosines in genomic DNA were converted to uracil by sodium bisulfite conversion (EpiTect kit, Qiagen), while the methylated cytosines were protected. Methylation-specific primers which target the methylated DNA (Online Table II) were designed by Methyl Primer Express software (Life Technologies). The promoter region of human KLF4 (-152/+9), human NOS3 (-369/-196), swine KLF4 (-1065/-900) and swine NOS3 (-13/+93) were tested. The methylated DNA was amplified during PCR by methylation-specific primers while the unmethylated DNA was not amplified. To normalize the input DNA, we used primers that target the UBB promoter sequence that does not contain any CpG sites. The methylation ratio was calculated as $Ect_{(region\ of\ interest)}$ / $Ect_{(UBB)}$, where E is the specific amplification efficiency and Ct is the crossing point for sequence region and UBB respectively.

After bisulfite PCR, pyrosequencing was performed on a Pyromark Q96 ID platform (Qiagen) using the PyroMark Gold Q96 Reagents (Qiagen) following manufacturer's instructions. The resulting sequences and percentage of methylated cytosine were generated and calculated by Pyro Q-CpG software (version 1.0.9). The bisulfite PCR primers targeting human KLF4 promoter were F. AGGGTTTAGAGATGGTTGGTTGAAAAT (-168/-142bp), R. biotin-ATATAAAAAAAAAACAATACCCAACACTATAC (+69/+38). The sequencing primer targeting CpG sites of human KLF4 was GGTTGGTTGAAAATTGT (-155/-139). The bisulfite PCR primers targeting swine KLF4 promoter were F. GTTTTGAGATTTTTGGTTGAAGGTATT (-830/--804), R. biotin-ACCCCACTCTAATCCCTAAATACCATT (-509/-535). The sequencing primer targeting CpG sites of swine KLF4 was TTATTTTATTAAGTGAATTTTGAGA (-775/-751).

Hydroxymethylation detection

Hydroxymethylation was quantified by restriction enzyme based qPCR (EpiMark 5-hmC and 5-mC Analysis Kit, New England Biolabs, Ipswich, MA). In brief, 5-hydroxymethylcytosine (5-hmC) in genomic DNA was first glucosylated by T4 β-glucosyltransferase (T4-BGT) to glucosylated 5-hydroxymethylcytosine (5-ghmC). The genomic DNA was then digested by restriction endonuclease (HpaII and MspI). HpaII cleaves only unmodified CCGG site; any modification (5-mC, 5-hmC or 5-ghmC) blocks the cleavage of CCGG by HpaII. MspI cleaves unmodified cytosine, 5-mC and 5-hmC, but not 5-ghmC. If the CpG site contains 5-hmC, PCR

product will be detected after glucosylation and MspI digestion, but not in the non-glucosylated control reaction. If the CpG site contains 5-mC and/or 5-hmC, PCR product will be detected after HpaII digestion, The glucosylated and digested DNA was quantified by qPCR with primers flanking only one CCGG site (Online Table III). Data were normalized to uncut DNA.

Western blot

Total cell lysates were prepared in a buffer containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and a protease inhibitor mixture (PMSF, aprotinin, and sodium orthovanadate). Cytoplasmic and nuclear extract were prepared by using NE-PER Nuclear and Cytoplasmic Extraction kit (Thermo Scientific) as described previously[18]. 15-20 μg of protein was separated by 4-12% SDS/PAGE gradient gel and analyzed by Western blot using the designated antibodies.

Chromatin immunoprecipitation (ChIP)-PCR Assay

ChIP-PCR assays were performed in accordance with protocols from Abcam. Following flow exposure, cells were fixed with 0.75% formaldehyde for 10 min at room temperature. Glycine was added to a final concentration of 125 mM for 5 min to neutralize formaldehyde. Cells collected by scraping were sonicated to an average fragment size 200-700bp using a Covaris E200 sonicator (Woburn, MA). Immunoprecipitation analysis was carried out using anti-DNMT3A, anti-TET1 or anti-MEF2 antibody. Immunoprecipitated DNA fragments were used as templates for qPCR. The ChIP-PCR primers targeting KLF4 promoter were listed in Online Table IV.

The transcription factor binding sites were predicted by Transcription Element Search System (TESS, www.cbil.upenn.edu/cgi-bin/tess). The primers flanking MEF2, DNMT3A and TET1 binding sites in the KLF4 promoter (-161/-25bp) were F.CGAGATGGCTGGTTGAAAACTG, R.AGGCACGAATGGGGAGTTATG. The primers targeting NOS3 (-167/-15bp) were F. CGTGGAGCTGAGGCTTTAGA, R.AGCCCTGGCCTTTTCCTTAG. The primers targeting ACTA2 were F.CAGGCCAAGGATGTGACTTATAG, R.CATGAACCCAGCCAAATCC. Because ACTA2 promoter was heavily methylated and comparable in endothelia in different hemodynamic environments (Online Figure VIII), the ChIP-qPCR data were normalized to ACTA2.

Gel shift assay

Single-stranded oligonucleotides containing MEF2 binding sites (5'FAM-CCCTTTCTCGCTATTTAAAGTATCAAAACCATAACTCCCCATTCGTGCCT) were 5' labeled with FAM (Fluorescein amidite), PAGE purified and used as probe. A 10 μg nuclear protein extract from HAEC were incubated with 500 fmol oligonucleotide probe, 1 μg sonicated salmon

sperm DNA and 10X loading buffer dye (Affymetrix, Santa Clara, CA) for 30 min at room temperature. 15 µl protein and DNA mixture were separated in a 6% DNA retardation gel (Life Technologies). The fluorescent signal was captured by a Fujifilm LAS-3000 Imager.

0.4 µg anti-MEF2 antibody (C-21, Santa Cruz) was pre-incubated with nuclear protein for 10 min in the competition study. Wild type oligonucleotides containing MEF2 binding sites CCCTTTCTCGCTATTTAAAGTATCAAAACCATAACTCCCCATTCGTGCCT were used as specific competitor. In mutant oligonucleotides CCCTTTCTTACTATTTAAAGTATCAAAACCATAACTCCCCATTTATGCCT, two CG dinucleotides were changed to TA. Five, 20, and 50-fold molar excess of competitor and mutant oligonucleotides were used in competition binding.

Methylation of promoter constructs and luciferase reporter assay

Human KLF4 promoter sequence (-69/+1250), which contains MEF2 binding site (-64/-55), was inserted upstream of the luciferase reporter gene in pEZX-PG04 vector (GeneCopoeia, Rockville, MD). The CpG sites in the construct were *in vitro* methylated by methylase M.SssI (NEB). Methylated and mock-methylated constructs were purified by DNA clean kit (Zymo Research, Irvine, CA). The methylation status and concentration were verified by using methylation-sensitive HpaII enzyme digestion, followed by TapeStation analysis. HAEC were plated and grown on 24-well plates 48 hr prior to transfection. Cells in 24-well plate at 60-80% confluence were transfected with 100 ng plasmid by Lipofectamine 2000 (Invitrogen). Luciferase activities were measured by using luminescence assay Kit (GeneCopoeia) 72 hr after transfection. The luminescent signal was measured by an EnVision 2103 multilabel plate reader (PerkinElmer).

Transfection of DNMT3A-specific shRNA

Ten µg DNMT3A-specific shRNA expression plasmids (Origene, Rockville, MD) were transfected into 60-80% confluent HAEC in 100mm culture dishes by using Lipofectamine 2000. Scramble shRNA plasmids were used as control. After 2 days of transfection, the mRNA of DNMT1, 3A and 3B were examined by RT-PCR. After successful knock-down of DNMT3A, the confluent HAEC on glass slide were subjected to flow for 2 days.

Reference

1. Thaler R, Spitzer S, Karlic H, Klaushofer K, Varga F. DMSO is a strong inducer of DNA hydroxymethylation in pre-osteoblastic MC3T3-E1 cells. *Epigenetics.* 2012; 7:635-651.

Online Tables

Online Table I. Primers for qPCR analysis

Online Table I A. Primers for qPCR analysis of human mRNA and pre-mRNA

	Human Genes	Primers	Amplicon Size (bp)
1	KLF4, pre-mRNA	F. TCCCATCTTTCTCCACGTTCG	131
		R. ACCACACCCACGAAAACCC	
2	NOS3, pre-mRNA	F.CCAGCGCCAGAACACAGGTAAG	119
		R.GCCCTCCCGACTCAGCTACAA	
3	KLF4, mRNA	F. TCTCCAATTCGCTGACCCATC	136
		R. ATCGGATAGGTGAAGCTGCAG	
4	NOS3, mRNA	F.CTCATGGGCACGGTGATG	152
		R. ACCACGTCATACTCATCCATACAC	
5	DNMT1, mRNA	F. AGCACAAACTGACCTGCTTC	172
		R. ATGGGGCCAAGATTTTTGCC	
6	DNMT3A, mRNA	F. TCTTCGTTGGAGGAATGTGC	153
		R. AAAAGCACCTGCAGCAGTTG	
7	DNMT3B, mRNA	F. AAACCCAACAACACGCAACC	162
		R. ATTTGTCTTGAGGCGCTTGG	
8	DNMT3L, mRNA	F. TCCCCTTGAGATGTTCGAAACC	104
		R. AAAAGCCCAAACTCGTCAGC	
9	TET1, mRNA	F. CAACAGTAAGCCTTCGTCACTG	137
		R. CGGATGGCATCAGCGAATAAG	
10	TET2, mRNA	F. AACAAGGCAGTGCTAATGCC	133
		R. TGGTTTTCTGCACCGCAATG	
11	TET3, mRNA	F. TCAACCGTGAGATGAGTCGTG	150
		R. AGTTGCAGTTGGGTGGTTTC	
12	TDG, mRNA	F. ACAACTGATGGCTGAAGCTC	109
		R. TTTTGGAGCCTCTTGCACTG	
13	THBD, mRNA	F.ACAGGTGCCAGATGTTTTGC,	136
		R. TTTTCGCACTCGTCGATGTC	
14	MCP-1, mRNA	F.CCAGCAGCAAGTGTCCCAAAG	115
		R. TGCTTGTCCAGGTGGTCCATG	
15	GADD45B, mRNA	F. GAGTCGGCCAAGTTGATGAATG	136
		R. TGTCGTTGTCACAGCAGAAG	
16	MBD4, mRNA	F. GCCAAAGACTCAGAACACAACG	154
		R. CTGGTTGGTGAGCAGTTGTTG	
17	SMUG1, mRNA	F. TTGGCCAAGCAGATGACAAC	129
		R. AGTGTCAAAACTGCCATGGC	

18	UBB, mRNA	F. GAGGTGGAGCCCAGTGACA R. ATGTTGTAGTCAGAAAGAGTGCGG	137

Online Table I B. Primers for qPCR analysis of porcine mRNA and pre-mRNA

	Swine Genes	Primers	Amplicon Size (bp)
1	KLF4, pre-mRNA	F. CACGTCTAGGCGGTGTAGG R. TTTGCTTCATGTGGGAGAGC	127
2	KLF4, mRNA	F. ACGCTGGCACTCTTAGTTCC R. AGGACAAGAGATACCGTCAGTG	129
3	NOS3, pre-mRNA	F. AGGCCAGGGACTTCATCAAC R. ACCATTTCTAGGTCTCGACAGG	116
4	NOS3, mRNA	F. AGGCCAGGGACTTCATCAAC R. ACACCAGCTCATTCTCTCGAAG	138
5	GAPDH, mRNA	F.CCTGTGACTTCAACAGTGACAC R. CCCTGTTGCTGTAGCCAAATTC	123
6	PECAM1, mRNA	F.CCTCGCCCATTTCCTACCAACTTT R.CAGACTCCACCTCCTCGCTCAG	237
7	UBB, mRNA	F. GGGAAGGTGGGAAAGAGGTAG R. AGCATTGAAATTCCCGTTGGG	105

Online Table II. Primers for MSP analysis.

Online Table II A. Primers for MSP analysis of human gene promoter.

	Human Genes	Methylation Primers	Genomic DNA Sequence
1	KLF4, (-1520/-1328)	F.GGTGGGGTTGAATTTTTC R.TAAATTAACTCGACCCTCCC	GGTGGGGTTGAACTCCTCGAGGTCATAGGAAGGAGGACCT CGGTTTCCCTGCCAAAACACTCTCATCCCCCAGCTGCAAC CCTGGCAGCCCTTGGCCAGAGCCAAGCTAGAGGATGGAA GTCACACTCAAGGGCGTCCGACTCTGGATCTTAGAGGGAT TCCTGGCGTTGGCAGGGAGGGCCGAGTCAATTCA
2	KLF4, (-1339/-1141)	F.CGAGTTAATTTATTCGGGGC R.TCGAACACCGAACAAAAAAT	CGAGTCAATTCATTCGGGGCGAGATGCATTCCAAGAATGC TTCTGTGGTCGGCGAGGAGTGGAAAAATCAGGTATCACAA ATATTCTGAGGAAACCTAATAGGTACCAGAATCGCAGGGTT CAAACAGGTGACAACGACAAAGGACATTGGCAACTAGAAC TCAAAAAGGGGTGAGCGCACTCTCTGCCCGGTGCCCGA
3	KLF4, (-808/-648)	F.TTTTCGGTGGGTAAGGAC, R.AACAACTCGTTCCTTCGACT	TTCCCGGTGGGCAAGGACGCCTGCGGCGGGCTCGGTGTG CACTTTTTGAGAGCTAGGATCCCACCGCATCGCTCCACTTC CTGGCACATGGTCACTTCCCAGGGAAGTCCCTTTAGGGGG GCCTGACCTACCCGGAGCCTGAGCCGAAGGAACGAGTTGT C
4	KLF4, (-152/+9)	F. TGGTTGAAAATTGTTTTCGC, R. CAAAAAAATATTCCGCCGA	TGGTTGAAAACTGTCTCCGCGCCTCACAGTCCCTCTTCTCT CCCTTATGCCTCTGAGTAAATCTTGAGCCCTTTCACTCCCT TTCTCGCTATTTAAAGTATCAAAACCATAACTCCCCATTCGT GCCTCTTTCCGCCTGTTCCCGGCGGAACACTCCTCTG
5	NOS3, (-369/-196)	F.GAGGTCGAAGGTTTTTTC R.ACGCCTAAATTCCGCTAT	GAGGCCGAAGGCCCTTCCGTCTGGTGCCACATCACAGAAG GACCTTTATGACCCCCTGGTGGCTCTACCCTGCCACTCCC CAATGCCCCAGCCCCCATGCTGCAGCCCCAGGGCTCTGCT GGACACCTGGGCTCCCACTTATCAGCCTCAGTCCTCACAG CGGAACCCAGGCGT
6	THBD, (-802/-681)	F. CGAAATTGGTTAGGTTGATTC R. AAATCGCGAAAACACTATCA	CGAAACTGGCCAGGCTGACTCGCTCCCACGCGCCCGCCC CTACCCGGCGCCGCAGCAATTCACCTGCCACCGCCTCTGA GCCGGGTCCGGACTTCGGCGCCCTGACAGTGTCCCCGCG ACTT
7	UBB, (+538/+718)	F.ATAGTGGGTTTTGTTGATTTGA R.CCTTTCTCACACTAAAATTCCA	ACAGTGGGCCTTGTTGACCTGAGGGGGGCGAGGGCGGTT GGCGCGCGCGCGCGTTGACGGAAACTAACGGACGCCTAA CCGATCGGCGATTCTGTCGAGTTTACTTCGCGGGGAAGGC GGAAAAGAGGTAGTTTGTGTGGTTTCTGGAAGCCTTTACTT

			TGGAATCCCAGTGTGAGAAAGG

Online Table II B. Primers for MSP analysis of porcine gene promoter.

	Swine Genes	Methylation Primers	Genomic DNA Sequence
1	KLF4, (-1065/-900)	F.GGTGGGTAGTGATAGTCGTC, R.ACGAAATAAAAATCAACCGA	GGTGGGCAGTGACAGCCGCCGCGGGCTCCGTGTGACCTT ATCAGGAACCGGGGTTCCAATGCGTCGCTCCACTTTCTGG CATGGGGCCACTTTCCAGCCAAGTCCCTCTCGGTGGGCTT GACCGCTCGAGACAAGGGAGCGAGTTGCCGGTTGACCTTT ACCTCGC
2	NOS3, (-13/+93)	F.AGCGGTCGAAGGTGATAC R.ATTCTTCACGCGAAAAAACT	AGCGGCCGAAGGTGACACGCTTCTCTCTCATGACCCCAGC CCGGCTCCCAACAGCCCCCCGCTCACCCGGCCCCCAGAG GGGCCCAAGTTCCCTCGCGTGAAGAAC
3	UBB, (-2358/-2255)	F. GTTGTTGTGTTAGGAGGAATTT R. TCACAAAAAAAAACTATACCCC	GCTGCTGTGTTAGGAGGAACTTATTAAATCTGTTAACAGTG ACCACAGCAGCAACGTGAACAGAGACGCTGATTGGAACTG GGGGGCATAGTTTCTCTTTGTGA

Online Table III. Primers for restriction enzyme qPCR analysis.

	Human Genes	Primers	Amplicon Size (bp)
1	KLF4, (CCGG-9)	F. TGAGCCCTTTCACTCCCTTTC R. AACTGGGAGCCTCAAGAGAAG	120
2	KLF4, (CCGG-804)	F. AGATCGCCGTTTCCTATTCCC R. AGTGACCATGTGCCAGGAAG	117
3	NOS3, (CCGG-137)	F.CGTGGAGCTGAGGCTTTAGA R.AGCCCTGGCCTTTTCCTTAG	156
4	NOS3, (CCGG-194)	F.TTATCAGCCTCAGTCCTCACAG R.GCTCTAAAGCCTCAGCTCCAC	86
5	NOS3, (CCGG-745)	F.TCAGCCCTAGTCTCTCTGCTG R. TCAAGTGGGGGACACAAAAG	115

Online Table IV. Primers for ChIP-PCR analysis of human KLF4.

	Human Genes	Primers	Amplicon Size (bp)
1	KLF4, (-2594/-2484)	F. TCTATGTGTCCAGACCCCATAC R.AGGGGATTAATGTGGAGCCATG	111
2	KLF4, (-1471/-1352)	F. TGCCAAAACACTCTCATCCC R.AACGCCAGGAATCCCTCTAAG	120
3	KLF4, (-778/-643)	F. CTCGGTGTGCACTTTTTGAGAG R.CAAAGGACAACTCGTTCCTTCG	139
4	KLF4, (+215/+285)	F. TGGATGAGTCACGCGGATAATC R. CTGCTAGCATACGCGCTTG	71

Online Figures I - VIII
Figures Legends

Online Figure I. DF regulation of KLF4 and NOS3 promoter methylation and hydroxymethylation.

Confluent HAEC were subjected to UF or DF for 2 days. Methylation and hydroxymethylation of promoter was determined by restriction enzyme qPCR targeting (**A**) KLF4 (CCGG-804) and (**B**) NOS3 (CCGG -745 and -194). ①input, ②5hmC, ③5mC and 5hmC, ④input, ⑤background, ⑥5mC and 5hmC. Data are normalized to ①uncut DNA and expressed as mean ± SEM. *Different from UF, $P < 0.05$, n= 4.

Online Figure II. MEF2 chromatin enrichment in KLF4.

The chromatin of HAEC were cross-linked and sonicated. MEF2 chromatin loading was mapped in KLF4 promoter by ChIP-qPCR using 4 pairs of primer. Data are normalized to input DNA and are expressed as mean ± SEM fold of UF.

Online Figure III. Induction of MEF2 binding to KLF4 promoter sequence by hemodynamic forces.

Confluent HAEC were subjected to UF or DF for 2 days. 10 μg of nuclear protein was incubated with FAM labeled oligonucleotides with sequence of the MEF2 binding site from the KLF4 promoter (-74/-25) for 30 min. The oligonucleotides are not methylated. Anti-MEF2 antibody was pre-incubated with nuclear protein before adding the oligonucleotides. The oligonucleotides and proteins were separate in 6% DNA retardation gel. The fluorescent signal was captured by a Fujifilm LAS-3000 Imager. Representative image is shown. The arrow head indicates a shifted band.

Online Figure IV. Methylation of KLF4 promoter reporter construct.

The human KLF4 promoter sequence (-69/+1250), which contains MEF2 binding sequence, was inserted upstream of the luciferase reporter gene in pEZX-PG04 vector. The construct were *in vitro* methylated by methylase M.SssI**.** Methylated and unmethylated construct were examined by methylation-sensitive restriction enzyme HpaII. The digested DNA was analyzed in TapeStation 2200. Representative image were shown.

Online Figure V. Effects of knock-down DNMT3A.

DNMT3A-specific shRNA expression plasmids were transfected into 60-80% confluent HAEC by Lipofectamine 2000. Scrambled shRNA plasmids were used as control. After 2 days of transfection, the mRNA of DNMT1, 3A and 3B were examined by RT-PCR. Data are normalized

to UBB and expressed as mean ± SEM fold of scrambled control. *Different from scramble shRNA control $P < 0.05$. n= 4.

Online Figure VI. Effects of RG108 and 5-azacytidine on KLF4 promoter methylation.

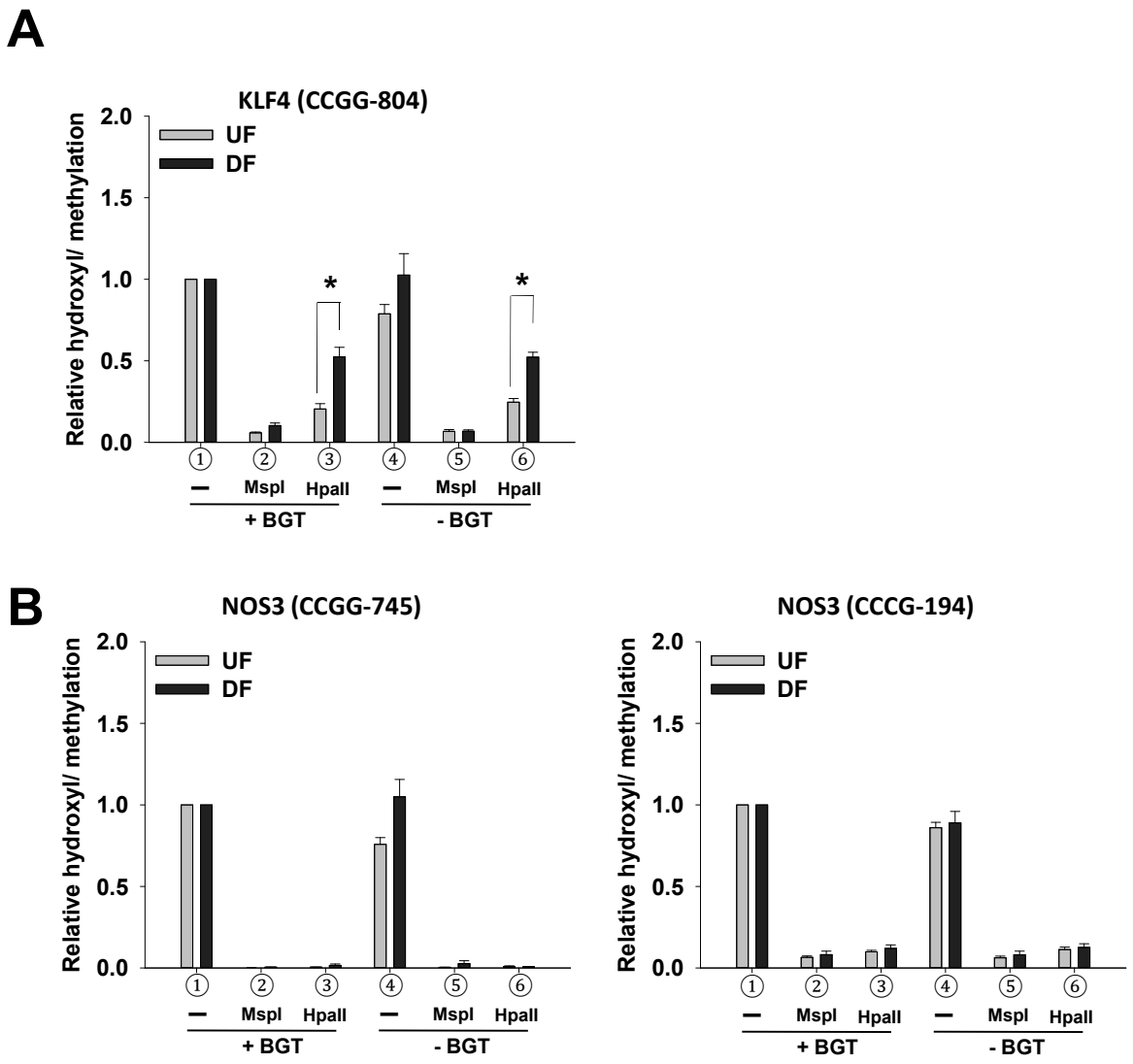
(**A**) Confluent HAEC under static condition (no flow) were treated with vehicle (DMSO), 4-100 μM RG108 or 0.2-5 μM 5-azacytidine (5-Aza) for 2 days. KLF4 promoter methylation (1339/-1141 and -152/+9) were analyzed by MSP. Data are normalized to UBB promoter without CpG sites and expressed as mean ± SEM fold of control. (**B**) Confluent HAEC were subjected to flow (UF and DF) with RG108 (20 μM) or vehicle (DMSO) for 2 days. KLF4 promoter methylation (-1520/-1328, 1339/-1141 and -808/-648) were analyzed by MSP. Data are normalized to UBB promoter and expressed as mean ± SEM fold of UF. *Different from UF. #Different from vehicle control. $^{*,\#}P < 0.05$. n= 4.

Online Figure VII. Effects of RG108 on NOS3 and THBD promoter methylation.

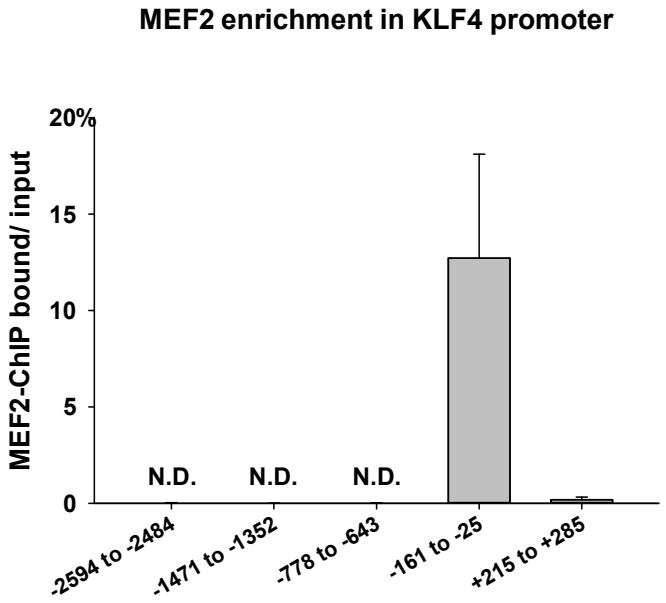
Confluent HAEC were subjected to UF and DF with RG108 (20 μM), or vehicle (DMSO) for 2 days. NOS3 promoter with low CpG density (CpGo/e <0.4) and THBD promoter with high CpG density (CpGo/e >0.6) were analyzed by MSP. Data are normalized to UBB promoter and expressed as mean ± SEM fold of UF. n= 4.

Online Figure VIII. ACTA2 promoter methylation.

The endothelia were isolated from a UF region of swine descending thoracic aorta and DF region of swine aorta arch. Swine ACTA2 promoter methylation was examined by bisulfite Sanger sequencing with the primer (F. GTGGAATGTAGTGGAAGAGATT, R. AAAACTAATTCCACAATCCCAC) on an ABI 3730 DNA Analyzer (Invitrogen). Data were analyzed by Sequencher version 5.1 sequence analysis software (Gene Codes Corporation, Ann Arbor, MI). n=4.



Online Figure I



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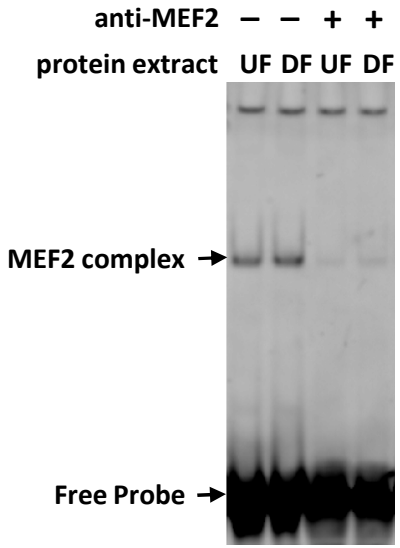

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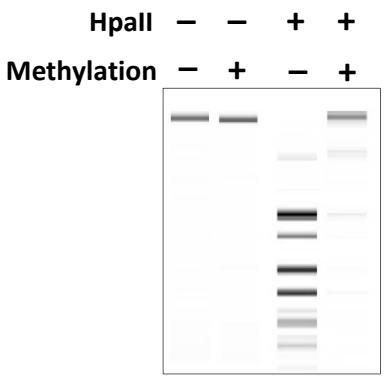

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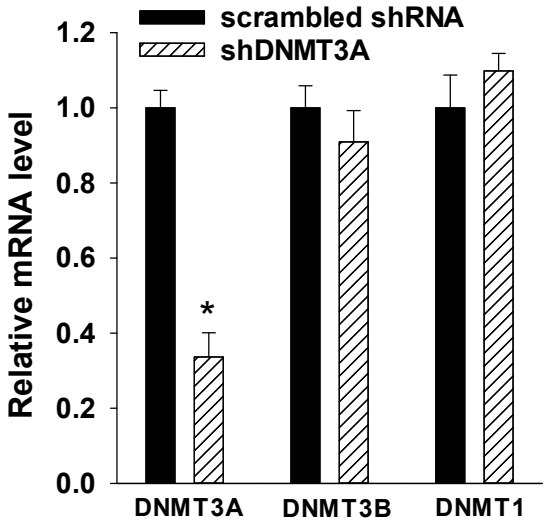

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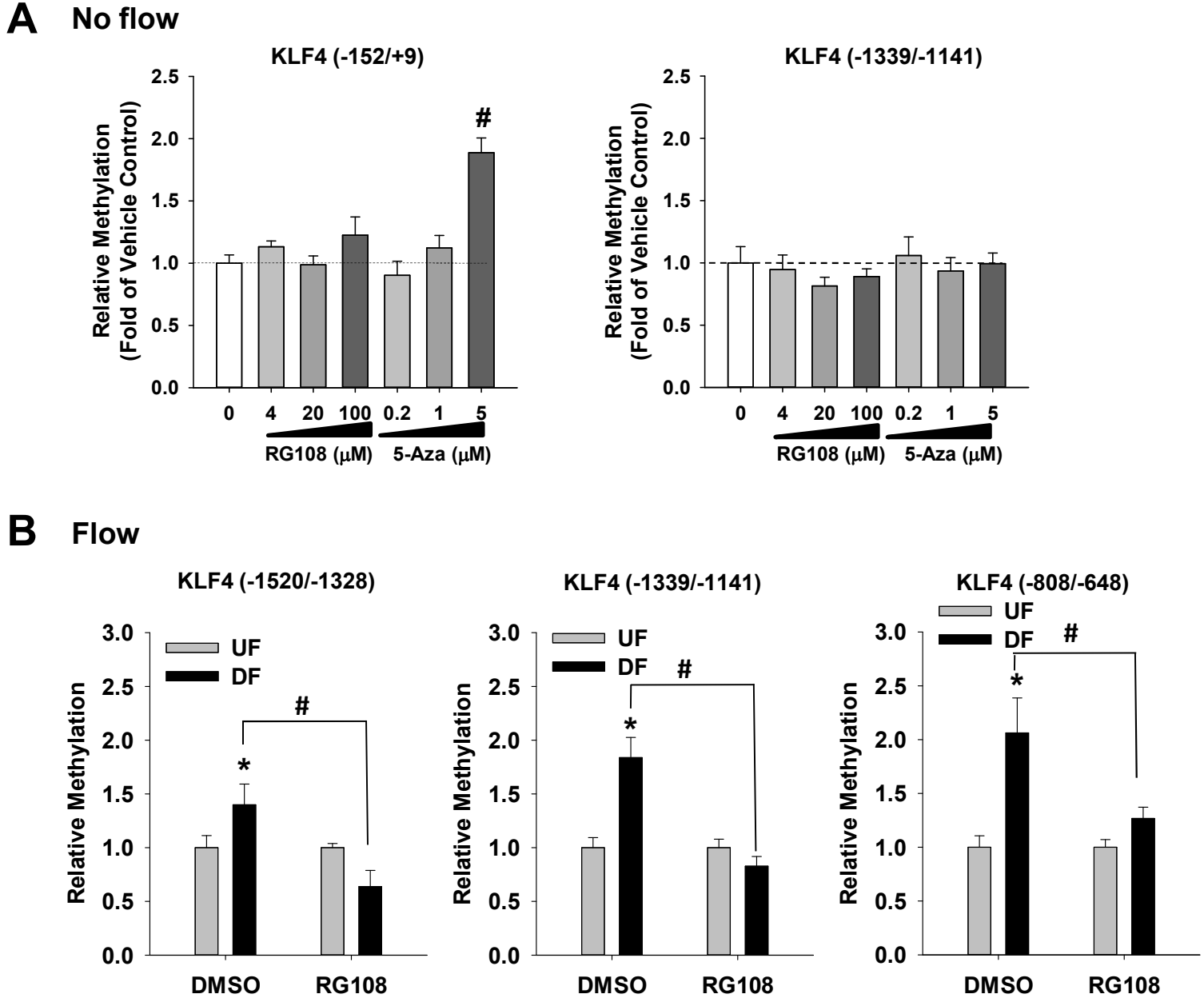

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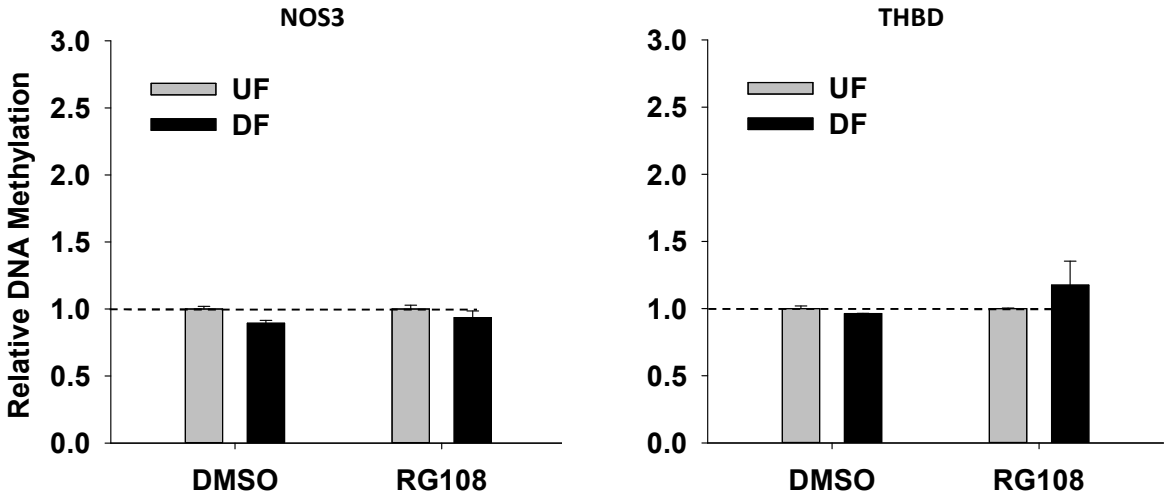
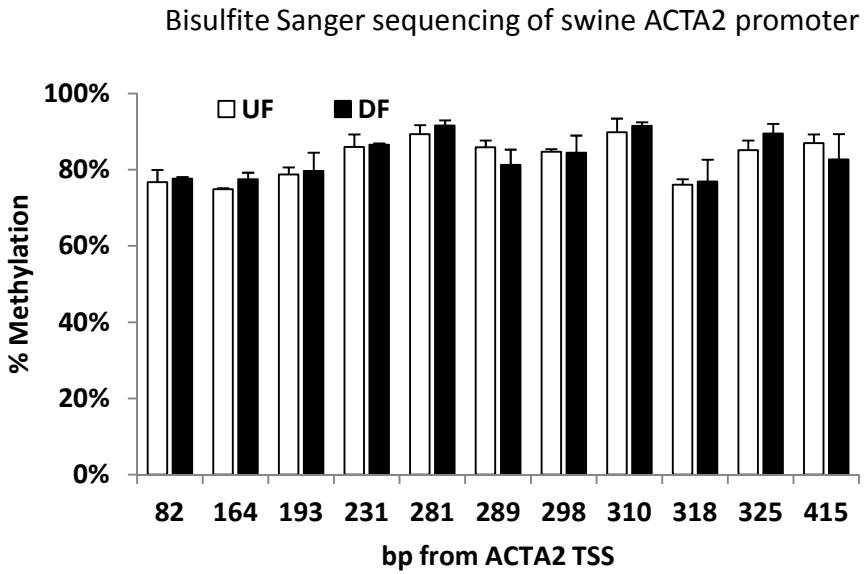


Online Figure VII



Online Figure VIII