A Putative miR-23b promoter

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Pro23bmt

Online Figure I. A. Illustration of the luciferase construct containing miR-23b putative promoter region. **B.** The reporter constructs with either the wild type (Pro23b) and mutated (Pro23bmt) KLF2 binding motif were co-transfected with Adv-Ctrl or Adv-KLF2. 72 h post-transfection, firefly luciferase activity in cell lysate was measured and normalized on renilla luciferase (n=4, *P<0.05 vs. Adv-Ctrl/Pro23b; #P<0.05 vs. Adv-KLF2/Pro23b).

Pro23b





CCNH 3'UTR



Online Figure III. The seed region of miR-23b (red) and its target sequence at the CCNH 3'-UTR (blue) are conservative over mammalian species.



Online Figure IV. A. CCNH knockdown decreases EC proliferation. ECs were transfected with the plasmid expressing CCNH shRNA or pcDNA3 and pulse-labeled with BrdU during the last 4 h of transfection. 72 h post-transfection, the fraction of BrdU-positive cells was analyzed with flow cytometry (n=4, *P<0.05 vs. pcDNA3). **B.** CCNH overexpression attenuates the PS-reduction of EC proliferation. ECs were transfected with the plasmid expressing HA-CCNH or pcDNA3 for 48 h then subjected to 24-h PS or ST. After pulse-labeling cells with BrdU, the fraction of BrdU-positive cells was analyzed with flow cytometry (n=4, *P<0.05 vs. pcDNA3/ST; #P<0.05 vs. pcDNA3/PS).



P-CTD (Ser5)/DAPI

Online Figure V. The time course study of the PS-regulation of CTD phosphorylation of Pol II. ECs were subjected to PS flow for indicated times or under ST. The Ser5 phosphorylation of CTD under PS and ST was examined with immunofluorescence staining. Results are representative of three experiments with similar results. Scale bar, 20 um.



Online Figure VI. The effects of CCNH perturbation on the CTD phosphorylation of Pol II. **A.** ECs were transfected with the plasmid expressing CCNH shRNA or pcDNA3. 72 h post-transfection, the levels of CTD phosphorylation (Ser5) and CCNH expression were determined by Western blot. **B.** ECs were transfected with the plasmid expressing HA-CCNH or pcDNA3 for 48 h then subjected to 24-h PS or ST. The levels of CTD phosphorylation (Ser5) and CCNH expression were determined by Western blot. The bar graphs are the densitometry analyses of Western blot (n=3, *P<0.05 vs. pcDNA3/ST or pcDNA3 only; #P<0.05 vs. pcDNA3/PS; §P<0.05 vs. HA-CCNH/ST).



Online Figure VII. Fluorescence in situ hybridization (FISH) staining of miR-23b in ECs under PS and OS using the DIG-labeled miR-23b detection probe. Scale bar, 20 um.



Online Figure VIII. Flow disturbance in partial ligated rat carotid arteries. Left: the schematic presentation of the **A.** sham surgery (sham) or **B.** partial carotid ligation (PL) in Sprague-Dawley rat. Right: ultrasonography measurements of flow velocities in left carotid artery (LCA) one-week post-operation. Results are representative of three experiments with similar results. White arrows indicate the blood flow with reversal oscillation. ICA: internal carotid artery; ECA: external carotid artery; OA: occipital artery; STA: superior thyroid artery.



Online Figure IX. Immunofluorescence staining of vWF (left, red) and CD45 (right, red) in frozen sections of LCAs from sham, PL, PM-Ctrl/PL and PM23b/PL. EL: elastic lamina (green), DAPI: nuclear staining (blue). Results are representative images of immunofluorescence staining on serial section samples from four animals with similar results. Scale bar, 100 um.



Online Figure X. The role of KLF2 in regulating EC proliferation. **A.** ECs were transfected with siKLF2 or siCtrl for 48 h and then subjected to 24-h PS or ST. After pulse-labeling cells with BrdU, the fraction of BrdU-positive cells was analyzed with flow cytometry (n=4, *P<0.05 vs. siCtrl/ST; #P<0.05 vs. siCtrl/PS). **B.** The efficiency of siKLF2 knockdown on KLF2 mRNA level was examined with qRT-PCR (n=4, *P<0.05 vs. siCtrl). **C.** ECs were transduced with adenovirus expressing KLF2 (Adv-KLF2) or GFP (Adv-Ctrl) for 72 h. During the last 4 h of incubation, the fraction of BrdU-positive cells was analyzed with flow cytometry (n=4, *P<0.05 vs. Adv-Ctrl).



Online Figure XI. The role of CAK in Pol II and CDK phosphorylation. The ECs were transfected with the active form of CAK complex or vehicle control. 24 h post-transfection, the phosphorylation levels of the CTD of Pol II, CDK2, and CDK4 were determined with Western blot. Results are representative of three experiments with similar results.



Online Figure XII. The role of KLF2 in regulating CAK pathway. ECs were transduced with adenovirus expressing KLF2 (Adv-KLF2) or GFP (Adv-Ctrl) for 72 h. the levels of CTD phosphorylation, Pol II, CCNH, CDK7 and KLF2 were determined with Western blot. Results are representative of three experiments with similar results.



Online Figure XIII. The efficiency of AM23b and PM23b transfection in ECs. **A.** ECs were transfected with Cy3-labeled PM-Ctrl at 0, 25 or 50 nM. 48 h post-transfection, the transfection efficiency was examined with a fluorescent microscope. The images were representative images of triplicate experiments with similar results. Scale bar, 100 um. **B-C.** ECs were transfected with **B.** AM23b or AM-Ctrl and **C.** PM23b or PM-Ctrl. 48 h post-transfection, miR-23b levels were determined with qRT-PCR (n=4, *P<0.05 vs. AM-Ctrl or PM-Ctrl).



Online Figure XIV. A. The mRNA levels of Von Willebrand factor (vWF), α -smooth muscle actin (SMA), and internal control gene (GAPDH) in intima RNA extracted from PLor sham-operated LCAs were determined with RT-PCR following by gel electrophoresis. **B.** The mRNA levels of vWF and GAPDH in intima RNA extracted from PM-Ctrl/PL or PM23b/PL LCAs were determined with RT-PCR following by gel electrophoresis. The expression of the EC marker, vWF, indicate the RNA samples are mainly from endothelium, not the underneath smooth muscle cells (lack of SMA expression).



Online Figure XV. Negative control images of FISH staining in **A.** EC culture and **B.** frozen section. Results are representative images of three biological repeats. Scale bar, 20 um.

Online Table I. Primer Sequences

Gene	Forward 5'-3'	Reverse 5'-3'
CCNA	GTCACCACATACTATGGACATG	AAGTTTTCCTCTCAGCACTGAC
CCND	AGACCTGCGCGCCCTCGGTG	GTAGTAGGACAGGAAGTTGTTC
CCNE	GTCCTGGCTGAATGTATACATGC	CCCTATTTTGTTCAGACAACATG
CCNH [#]	GCATTGACGGATGCTTACCT	TGACATCGCTCCAACTTCTG
CDK1	CAGGATGTGCTTATGCAGGA	GGCCAAAATCAGCCAGTTTA
CDK2	CATTCCTCTTCCCCTCATCA	CAGGGACTCCAAAAGCTCTG
CDK4	TTCTGGTGACAAGTGGTGGA	CTGGTCGGCTTCAGAGTTTC
CDK7	GGCCGGACATGTGTAGTCTT	CATTTTCAGTGCCTGTGTGG
p27	TGCAACCGACGATTCTTCTACTC	CAAGCAGTGATGTATCTGAT
KLF2	AGACCTACACCAAGAGTTCGCATC	CATGTGCCGTTTCATGTGCAGC
GAPDH	ATGACATCAAGAAGGTGGTG	CATACCAGGAAATGAGCTTG
CDK1-TSS	AAGAAGAACGGAGCGAACA	GCTAGAGCGCGAAAGAAAGA
CDK2-TSS	GAAACTTGGACCCAAAGCAG	CCAGGCCTTTCTATTGGTCA
CDK4-TSS	TCAGCCTTCAGACCGGTAGT	ATTGCTCTGAGGAGCAGAGG
Ki67*	CATGGGGATTCTGAGGCTAA	GGATCACTGCTTGCTCTTCC
KLF2*	GAGTCAACGGATTTGGTCGT	TTGATTTTGGAGGGATCTCG
SM α-actin*	ACTCTGGAGATGGCGTGACT	GTCCAGAGCGACATAGCACA
vWF*	TGCTGCCCAGAGTATGAGTG	ACACAGCTGCAAGTGCATTC

*Primers used for amplifying rat cDNA sequences; *Primer used for amplifying for both human and rat cDNA sequences.