

Supplemental Information

**Long Non-coding RNA PEBP1P2 Suppresses
Proliferative VSMCs Phenotypic Switching
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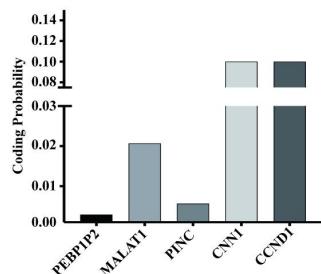
Long Non-coding RNA PEBP1P2 Suppresses Proliferative VSMCs Phenotypic Switching and Proliferation in Atherosclerosis

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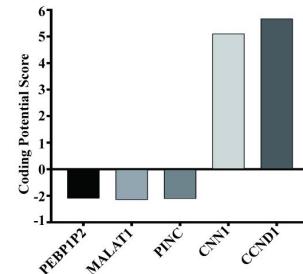
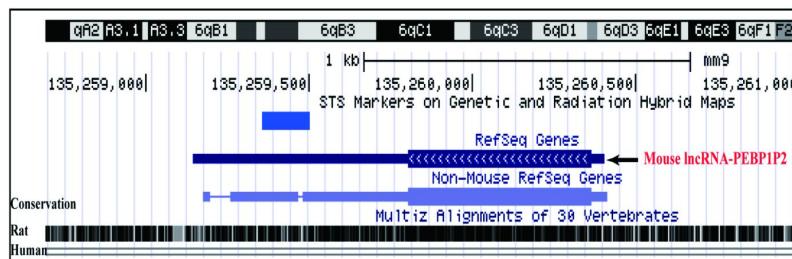
Tao Yu, Hui Xin.

A

CPAT		
ID	Coding Label	Coding Probability
PEBP1P2	no	0.0029434043899346
MALAT1	no	0.020711595574509
PINC	no	0.0056974609714006
CNN1	yes	1
CCND1	yes	1

**B**

CPC		
ID	C/NC	Coding Potential Score
PEBP1P2	noncoding	-1.09952
MALAT1	noncoding	-1.14703
PINC	noncoding	-1.10997
CNN1	coding	5.11008
CCND1	coding	5.67432

**C****D**

Score 412 bits(456)	Expect 1e-119	Identities 336/408(82%)	Gaps 0/408(0%)	Strand Plus/Plus
Query 1	GTCAACACTTAACTGGCCCTGTCCTGCTCATAAACACAGCCAGACATAGCGGTGGAGAACCTGT			60
Sbjct 1	GTCAACACTTCAAGTGCTTGTCTGCTGGTACACCAAGCCATACATAGCGATGGAGGCCAGT			60
Query 61	GCCCCTTGGGAGGGCCCGAGCCCACATAATCGGAGAGGAGTGTGTCACTGCTGATGTGATT			120
Sbjct 61	GCCCCTTGGGAGGGCCCGAGACCACCGTAATCCGAGAGGACCTTGCCGCTGCTGATGTGTT			120
Query 121	GCCCCTTCATGTTGACCACCCAGAAAATGATGCCATTCTCTGTTATTGGATCCCTTCTTGCT			180
Sbjct 121	GCCCCTTCATGTTGACCACCCAGAAAATGATGCCATTCTCTGTTATTGGATCCCTTCTTGCT			180
Query 181	GGGAGCACAGCGCTCTGTCAGGACCAAGGTCPAGACGCTCCCTGAATCAAAGACCACTCCA			240
Sbjct 181	GGGAGCACATCTGGGCTCTGTCAGGATCAAGGTCTAGAGTTCCCGGGTCAGGCCCCATCTCCA			240
Query 241	CTAAATGCTGGTGGGTCATTCTTAACCTGGGTGGGGCTCAGCACATTGCCAGCTCGTC			300
Sbjct 241	TGTAATGCTGGTGGGTCCTGTCCTTAACCTGGGTGGGGCTCAGCACATTGCCAGCTCGTC			300
Query 301	CACCAACGGCCGGGGTAGGTGACATGCCAGCAGCTCTGACCCGTCCTCCATTCTCGG			360
Sbjct 301	CACCCCTCCGGGGCTGAGGTGACCCGTCAGGTGCTGGGGCTGCTCGTCATCATCTG			360
Query 361	CAGGCTCAAGGGCCGGGACACTTGCTGAGGTCCACCGCATGGCAAG	408		
Sbjct 361	TAGGCTCAAGGGCCGGGACACTTGCTGAGGTCCACCGCATGGCAAG	408		

Sequence: (U) BLAST Results for: Nucleotide Sequences
Copy 147051

Figure S1. The characterization of PEBP1P2 in coding possibility and conservatism. (A and B)

The coding-potential assessment (CPAT, <http://lilab.research.bcm.edu/cpat/index.php>) and coding potential calculator (CPC, <http://cpc.cbi.pku.edu.cn/>) tools were used to predict the coding probability of PEBP1P2. MALAT1 and PINC served as the long non-coding RNA controls, while CNN1 and CCND1 served as the coding RNA controls. (C) Genomic location of PEBP1P2 on mouse assembly (2007) predicted by UCSC LiftOver tool. (D) The NCBI BLAST tool was used to sequence comparison between human and rat PEBP1P2.

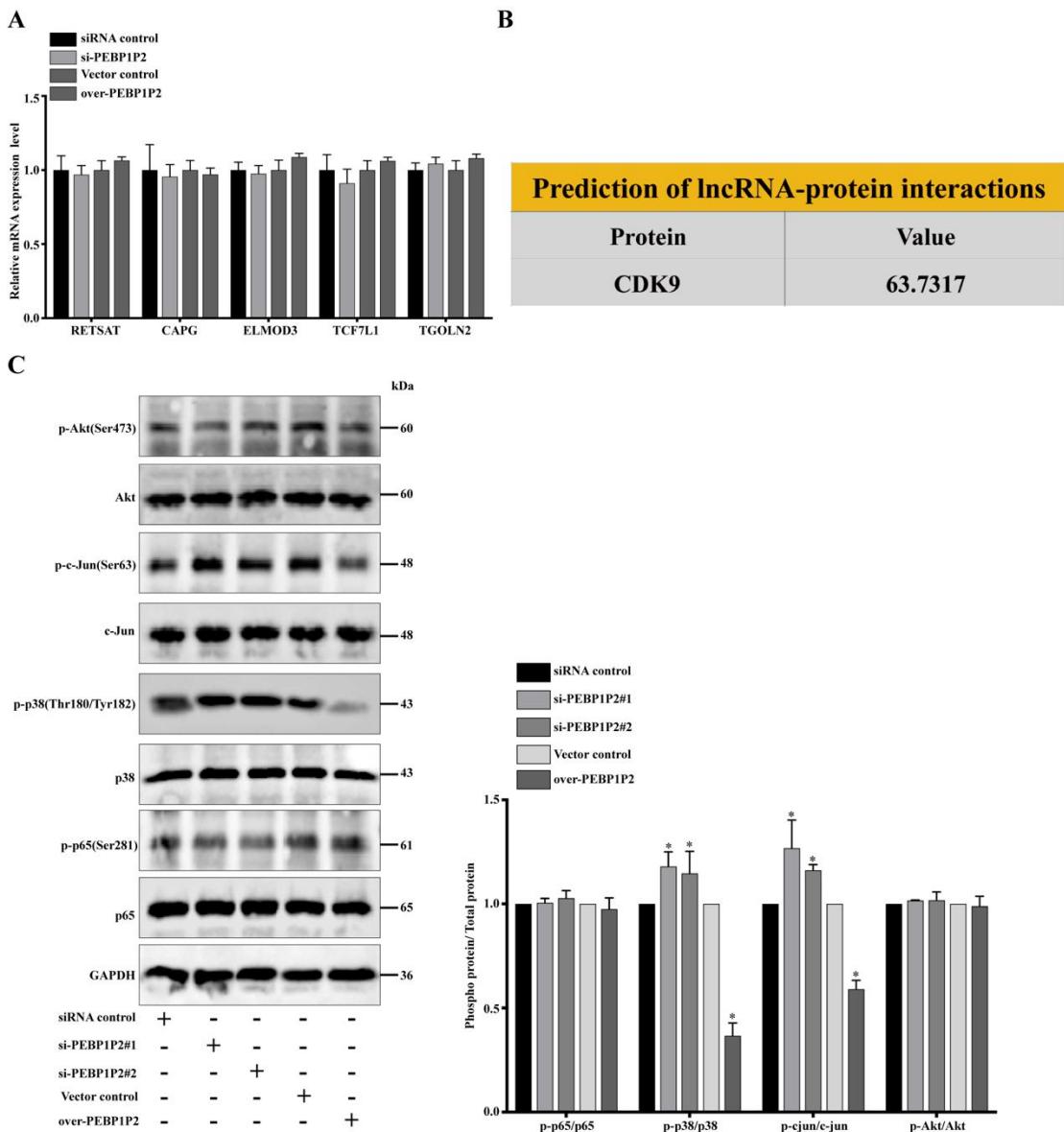


Figure S2. PEBP1P2 may work in trans. (A) The mRNA expression level of PEBP1P2 neighbor genes after PEBP1P2 knockdown or overexpression in VSMCs were measured by qRT-PCR. (B) The prediction of lncRNA-protein interactions tool (<http://bioinfo.bjmu.edu.cn/lncpro/#>) was used to predict the potential PEBP1P2/ CDK9 interaction. (C) Detection of PEBP1P2 knockdown or overexpression on the protein expression of NF- κ B p65, p-NF- κ B p65, p38MAPK, p-p38MAPK, c-Jun, p-c-Jun, Akt, and p-Akt by western blot. Data are presented as mean \pm SD. n=3. *P < 0.05 vs Ctl.

Table S1. General Characteristics of the CHD Patients and Healthy People.

Clinical parameters	Control (n=29)	CHD (n=27)	P
Age, year	24.21 ± 2.04	69.67 ± 8.85*	< 0.001
Male, %	15 (51.7)	18 (66.7)	0.26
BMI, kg/m ²	21.41 ± 2.42	23.15 ± 2.93*	0.019
SBP, mmHg	118 ± 8.96	142.15 ± 23.82*	< 0.001
DBP, mmHg	62.97 ± 3.28	85.26 ± 15.72*	< 0.001
CHO, mmol/L	3.72 ± 0.94	4.60 ± 1.28*	0.005
HDL, mmol/L	2.20 ± 0.34	1.20 ± 0.35*	< 0.001
LDL, mmol/L	0.92 ± 0.49	2.84 ± 1.00*	< 0.001
TG, mmol/L	1.20 ± 1.00	1.71 ± 0.91	0.050
Hypertension, %	0 (0)	16 (59.3) [#]	< 0.001
Smoking, %	8 (27.6)	15 (55.6) [#]	0.035

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CHO, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride. P value is calculated with t-test (*) or Mann-Whitney test (#) to compare continuous variables (presented as mean ± SD) or categorical variables (presented as no. (%)), respectively. *p < 0.05 vs Ctl, #p < 0.05 vs Ctl.

Table S2. siRNA sequences.

siRNA Names	Sequences (5'→3')
siRNA Control sense	UUCUCCGAACGUGUCACGUTT
siRNA Control antisense	ACGUGACACGUUCGGAGAATT
si-PEBP1P2 #1 sense	GCUCAUAAACCAGCCAGACTT
si-PEBP1P2 #1 antisense	GUCUGGCUGGUUUUAUGAGCTT
si-PEBP1P2 #2 sense	CCAUUUCUCGCAGGCUCAATT
si-PEBP1P2 #2 antisense	UUGAGCCUGCGAGAAAUGGTT
si-CDK9 #1 sense	GGGAGAUCAAGAUCCUUCATT
si-CDK9 #1 antisense	UGAAGGAUCUUGAUCUCCCTT
si-CDK9 #2 sense	GCUGCAAGGGUAGUUAUAUATT
si-CDK9 #2 antisense	UAUAUACUACCUUGCAGCTT

Table S3. Primer Sequences for qRT-PCR

Primer Names	Sequences (5'→3')
Hu-GAPDH Forward	GTCTCCTCTGACTTCAACAGCG
Hu-GAPDH Reverse	ACCACCCCTGTTGCTGTAGCAA
Ra-Gapdh Forward	GCCCATCACCATCTCCAGGAG
Ra-Gapdh Reverse	GAAGGGGCGGAGATGATGAC
Hu/Ra-PEBP1P2 Forward	ACTTTAGTGGCCTGTCCTGCTCA
Hu/Ra-PEBP1P2 Reverse	TGACGCCAACCCAGGTTAAGAATA
Hu-CCDN1 Forward	TCTACACCGACAACCTCCATCCG
Hu-CCDN1 Reverse	TCTGGCATTTGGAGAGGAAGTG
Hu- α -SMA Forward	GTGTTGCCCTGAAGAGCAT
Hu- α -SMA Reverse	GCTGGGACATTGAAAGTCTCA
Hu-CNN1 Forward	CCAACGACCTGTTGAGAACACC
Hu-CNN1 Reverse	ATTTCGCTCCTGCTTCTCTGC
Hu-SMHC Forward	CGCCAAGAGACTCGTCTGG
Hu-SMHC Reverse	TCTTCCCCAACCGTGACCTTC
Hu-CDK9 Forward	CCATTACAGCCTTGCAGGAGAT
Hu-CDK9 Reverse	CAGCAAGGTATGCTCGCAGAA
Hu-RETSAT Forward	GAAGAGGCTGCGAACACATCC
Hu-RETSAT Reverse	CCTCAAACCACTCGTAGGCAGT
Hu-CAPG Forward	CAGGTGGAGATTGTCACTGATGG
Hu-CAPG Reverse	CTGGGCATTGCCTGTCAGCT
Hu-ELMOD3 Forward	CGGCTCCAAGTTGACTGTGCC
Hu-ELMOD3 Reverse	GAGTCATCACCAAGGTAGAGCA
Hu-TCF7L1 Forward	TCGTCCTGGTCAACGAGT
Hu-TCF7L1 Reverse	ACTTCGGCGAAATAGTCCCG
Hu-TGOLN2 Forward	GGAGAGCAGCCACTTCTTGCA
Hu-TGOLN2 Reverse	CCAAACGTTGGTAGTCAGTGGC