Supplementary material

Endothelial to mesenchymal transition contributes to nicotine-induced atherosclerosis

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Supplementary figures 1-6

Supplementary table 1

Figure S1

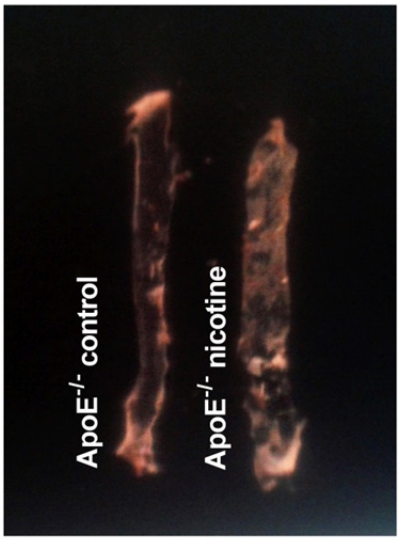

Figure S1. Oil Red O staining of aorta reveals the increase of atherosclerotic lesions induced by nicotine in ApoE$^{-/-}$ mice fed with high fat diet.

Figure S2

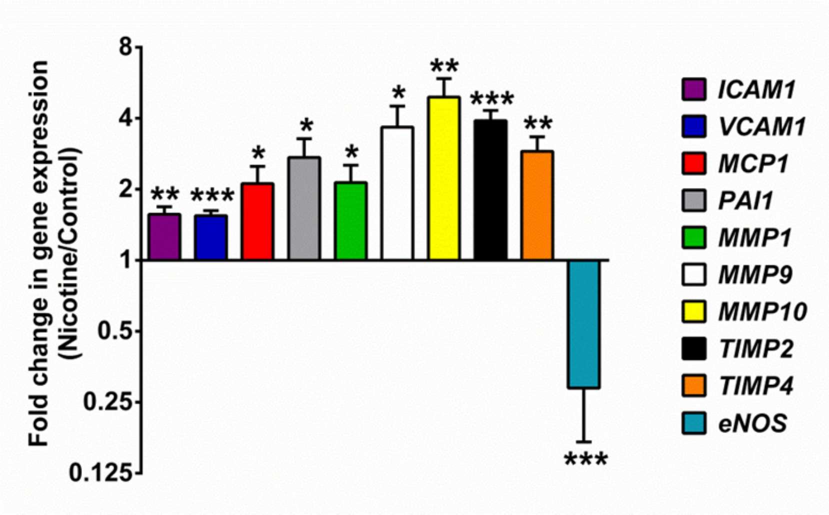

Figure S2. Nicotine increases mRNA levels of leukocyte adhesion molecules (ICAM1 and VCAM1), monocyte chemotactic protein 1 (MCP1), proinflammatory protein plasminogen activator inhibitor-1 (PAI1), matrix metalloproteinases (MMP1, 9 and 10), and TIMP metallopeptidase inhibitors (TIMP2 and 4) and decreases mRNA level of protective protein endothelial NOS (eNOS) in human aortic endothelial cells (HAECs). n = 4. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Figure S3

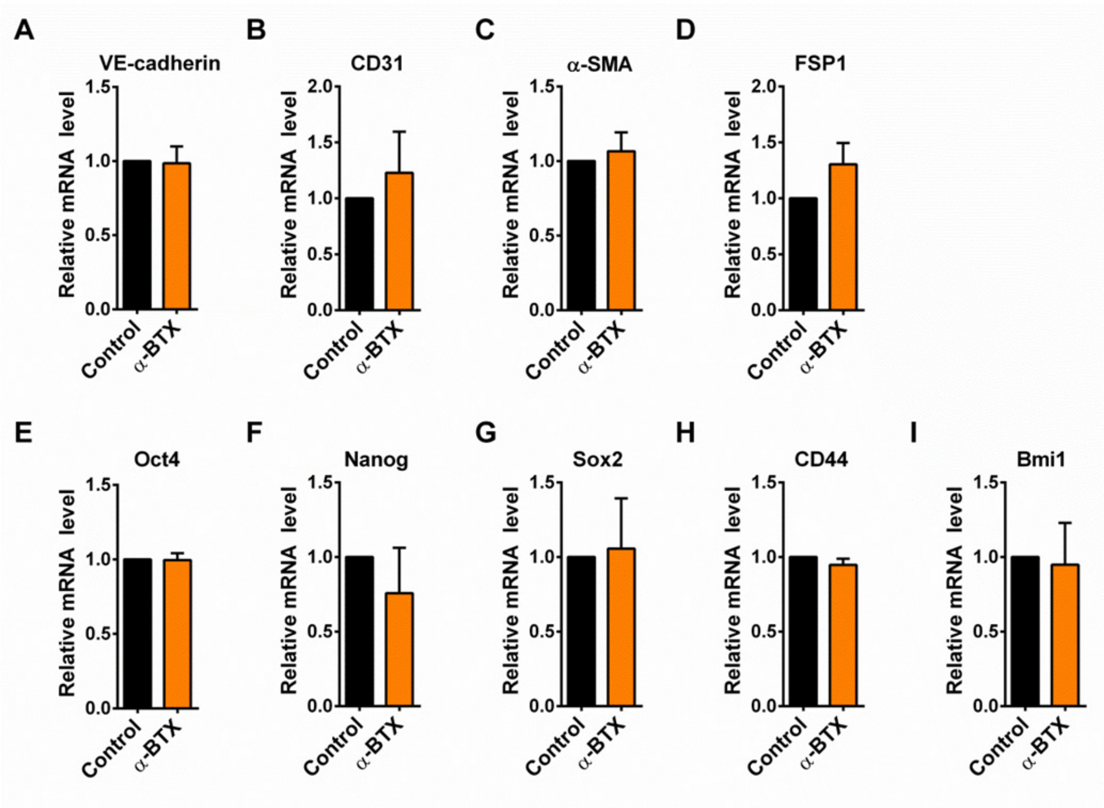

Figure S3. Blocking α7 nicotine acetylcholine receptor (α7nAChR) by α-BTX has no obvious effect on the expression of EndMT-related markers and stem cell markers in HAECs. The mRNA levels of VE-cadherin (A), CD31 (B), α-SMA (C), FSP1 (D), Oct4 (E), Nanog (F), Sox2 (G), CD44 (H) and Bmi1 (I) were determined by RT-PCR. n = 4.

Figure S4

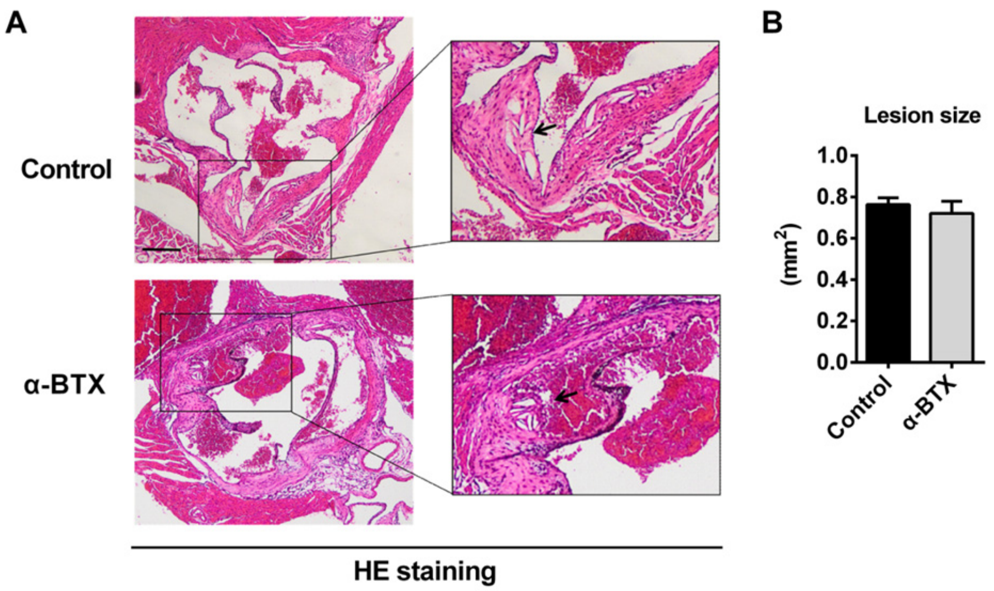

Figure S4. Blocking α7 nicotine acetylcholine receptor (α7nAChR) by α-BTX exhibited no significant changes in atherosclerotic lesions in ApoE$^{-/-}$ mice. All animals were fed with high fat diet for 8 weeks to establish atherosclerosis. Mice in α-BTX group received intraperitoneal injection of α-BTX 0.05 mg/kg once daily for 8 weeks. Mice in control group received phosphate buffered saline. (A) Hematoxylin-eosin (HE) staining of aortic root sections. Scale bar indicates 600 μm. Arrows indicate atherosclerotic plaques. (B) Quantification of the lesion area per section in the control and α-BTX groups. n = 4 mice in each group.

Figure S5

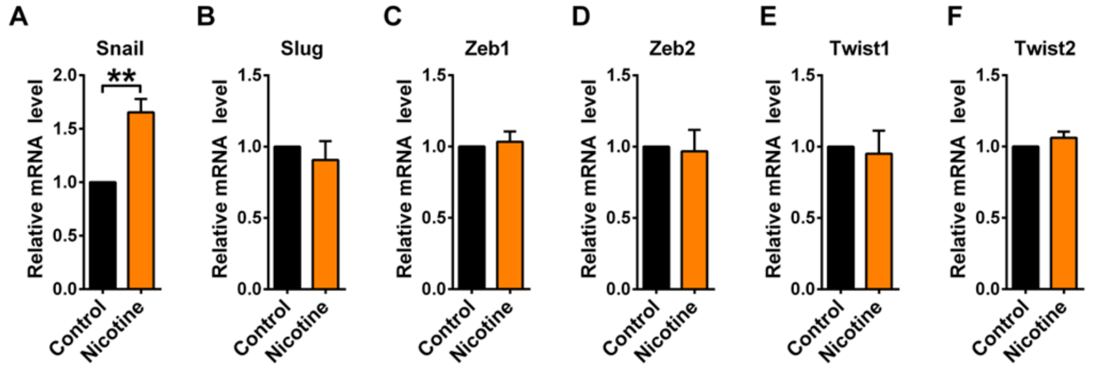

Figure S5. Transcription factors Snail was upregulated in HAECs after treatment with nicotine (500 nM). The mRNA levels of Snail (A), Slug (B), Zeb1 (C), Zeb2 (D), Twist1 (E) and Twist2 (F) were determined by RT-PCR. n = 3-4. ***P* < 0.01.

Figure S6

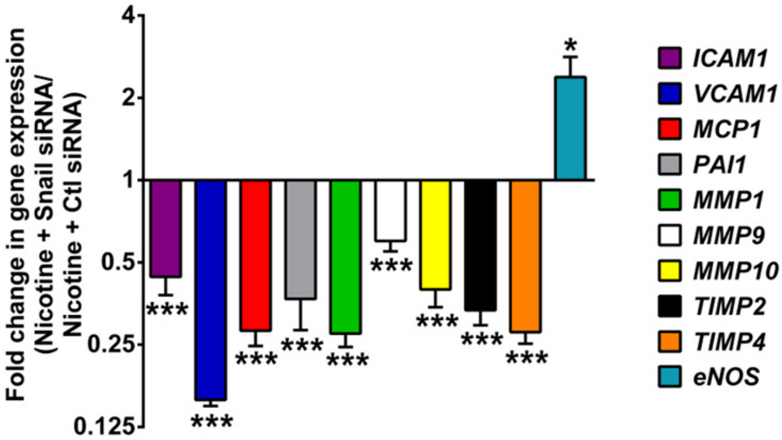

Figure S6. Snail knockdown decreases mRNA levels of ICAM1, VCAM1, MCP1, PAI1, MMP1, MMP9, MMP10, TIMP2, and TIMP4 and increases mRNA level of eNOS in nicotine-treated human aortic endothelial cells (HAECs). n = 3-4. *$P < 0.05$, ***$P < 0.001$.

Table S1. Primers used for qRT-PCR.

Gene	Species	Primer Sequence （5'→3'）
CD31	Mouse	F ACGCTGGTGCTCTATGCAAG R TCAGTTGCTGCCCATTCATCA
VE-cadherin	Mouse	F TCAACGCATCTGTGCCAGAGAT R CACGATTTGGTACAAGACAGTG
α-SMA	Mouse	F CCACCGCAAATGCTTCTAAGT R GGCAGGAATGATTTGGAAAGG
smMHC	Mouse	F AAGCTGCGGCTAGAGGTCA R CCCTCCCTTTGATGGCTGAG
VE-cadherin	Human	F CAGCCCAAAGTGTGTGAGAA R TGTGATGTTGGCCGTGTTAT
CD31	Human	F GAGTCCAGCCGCATATCC R TGACACAATCGTATCTTCCTTC
α-SMA	Human	F TGACAATGGCTCTGGGCTCTGTAA R TTCGTCACCCACGTAGCTGTCTTT
FSP1	Human	F GTCCACCTTCCACAAGTAC R TGTCCAAGTTGCTCATCAG
Oct4	Human	F GCAAAGCAGAAACCCTCGTGC R ACCACACTCGGACCACATCCT
Nanog	Human	F CAAAGGCAAACAACCCACTT R TCTGCTGGAGGCTGAGGTAT
Sox2	Human	F ATGGGTTCGGTGGTCAAGT R GCTCTGGTAGTGCTGGGACA
CD44	Human	F AAGGTGGAGCAAACACAACC R ACTGCAATGCAAACTGCAAG
Bmi1	Human	F TCCACAAAGCACACACATCA R CTTTCATTGTCTTTTCCGCC
α1 nAchR	Human	F GCTCTGTCGTGGCCATCAA R CCGGAAAGCGACCAGCCAGA
α2 nAchR	Human	F GTGGAGGAGGAGGACAGA R CTTCTGCATGTGGGGTGATA
α3 nAchR	Human	F CAGAGTCCAAAGGCTGCAAG R AGAGAGGGACAGCACAGCAT
α4 nAchR	Human	F CTCACCGTCCTTCTGTGTC R CTGGCTTTCTCAGCTTCCAG
α5 nAchR	Human	F CTTCACACGCTTCCCAAACT R CTTCAACAACCTCACGGACA
α6 nAchR	Human	F TCCATCGTGGTGACTGTGT R AGGCCACCTCATCAGCAG
α7 nAchR	Human	F GTACGCTGGTTTCCCTTTGA R CCACTAGGTCCCATTCTC
α9 nAchR	Human	F GAAAGCAGCCAGGAACAAAG R GCACTTGGCGATGTACTCAA
α10 nAchR	Human	F ACACAAGTGCCCTGAGACCT R TCCCATCGTAGGTAGGCATC
β1 nAchR	Human	F CTACGACAGCTCGGAGGTCA R GCAGGTTGAGAACCACGACA

β2 nAchR	Human	F R	GGCATGTACGAGGTGTCCTT CACCTCACTCTTCAGCACCA
β3 nAchR	Human	F R	AACAGTTCCGTTTGATTTCACGAT CCCTGATGACCAAGGTCATC
β4 nAchR	Human	F R	TCCCTGGTCCTTTTCTTCCT TGCAGCTTGATGGAGATGAG
γ nAchR	Human	F R	CGCCTGCTCTATCTCAGTCA GGAGACATTGAGCACAACCA
δ nAchR	Human	F R	CAGATCTCCTACTCCTGCAA CCACTGATGTCTTCTCACCA
ε nAchR	Human	F R	TCAAGGTCACCCTGACGAAT GTCGATGTCGATCTTGTTGA
ICAM1	Human	F R	CTTTCATTGTCTTTTCCGCC ATGCCCAGACATCTGTGTCC
VCAM1	Human	F R	GGGAAGATGGTCGTGATCCTT TCTGGGGTGGTCTCGATTTTA
MCP1	Human	F R	CAGCCAGATGCAATCAATGCC TGGAATCCTGAACCCACTTCT
PAI-1	Human	F R	ACCGCAACGTGGTTTTCTCA TTGAATCCCATAGCTGCTTGAAT
MMP1	Human	F R	AAAATTACACGCCAGATTTGCC GGTGTGACATTACTCCAGAGTTG
MMP9	Human	F R	TGTACCGCTATGGTTACACTCG GGCAGGGACAGTTGCTTCT
MMP10	Human	F R	TGCTCTGCCTATCCTCTGAGT TCACATCCTTTTCGAGGTTGTAG
TIMP2	Human	F R	AAGCGGTCAGTGAGAAGGAAG GGGGCCGTGTAGATAAACTCTAT
TIMP4	Human	F R	CCACTCGGCACTTGTGATTC CATCCTTGACTTTCTCAAACCCT
eNOS	Human	F R	TGATGGCGAAGCGAGTGAAG ACTCATCCATACACAGGACCC
Snail	Human	F R	GCCTTCAACTGCAAATACTGC CTTCTTGACATCTGAGTGGGTC
Slug	Human	F R	CGAACTGGACACACATACAGTG CTGAGGATCTCTGGTTGTGGT
Zeb1	Human	F R	GATGATGAATGCGAGTCAGATGC ACAGCAGTGTCTTGTTGTTGT
Zeb2	Human	F R	CAAGAGGCGCAAACAAGCC GGTTGGCAATACCGTCATCC
Twist1	Human	F R	TCGGACAAGCTGAGCAAGATT GCAGCTTGCCATCTTGGAGT
Twist2	Human	F R	GGCGCAAGTGGAATTGGGATG CCGGGTCTTCTGTCCGATGT
GADPH	Mouse Human	F R	AAGAAGGTGGTGAAGCAGGC TCCACCACCCAGTTGCTGTA