

Supplementary material file

SET7 lysine methyltransferase mediates the up-regulation of NADPH oxidase expression, oxidative stress, and NLRP3 inflammasome priming in atherosclerosis

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Materials

Standard reagents, molecular biology enzymes and kits, cytokines, oligonucleotide primers, histology-related reagents and laboratory disposables were purchased from Sigma-Aldrich (Germany), Thermo Fisher Scientific (USA), Carl Roth (Germany), R&D Systems (USA), Roche (Switzerland), Bio-Rad (USA), Eppendorf (Germany) and TPP (Switzerland). Oxidized low density lipoprotein (oxLDL) was obtained from Thermo Fisher Scientific (USA). The SET7 pharmacological inhibitor, (*R*)-PFI-2 (hydrochloride) (8-fluoro-1,2,3,4-tetrahydro-N-[(1*R*)-2-oxo-2-(1-pyrrolidinyl)-1-[[3-(trifluoromethyl)phenyl]methyl]ethyl]-6 isoquinolinesulfonamide, monohydrochloride, C₂₃H₂₅F₄N₃O₃S • HCl, purity ≥ 95%, CAS No. 1627607-87-7), was purchased from Cayman Chemical (USA). The methyltransferase inhibitor, sinefungin ((2*S*,5*S*)-2,5-diamino-6-[(2*R*,3*S*,4*R*,5*R*)-5-(6-aminopurin-9-yl)-3,4-dihydroxyoxolan-2-yl] hexanoic acid; purity ≥ 98%, CAS No. 58944-73-3) was obtained from Abcam (UK). Primary and secondary antibodies were purchased from Santa Cruz Biotechnology (USA), Thermo Fisher Scientific (USA), R&D Systems (USA), Abcam (UK), and Diagenode (USA). The pCMV6-Entry and pCMV6-SET7 plasmids were obtained from Origene (USA).

Parameter	Values (n = 25)
Gender: Female (F)/Male (M) (N, %)	9/16 (36%/64%)
Age (years)	64.2 ± 8.3
Body-mass index (BMI) (kg/m ²)	26.1 ± 3.6
Current smoker (N, %)	16 (64%)
Former smoker (N, %)	4 (16%)
Alcohol consumption (N, %)	1 (4%)
Diabetes (N, %)	8 (32%)
Hypertension (N, %)	21 (84%)
Other cardiac diseases	
Coronary artery disease (CAD) (N, %)	9 (36%)
Peripheral artery disease (PAD) (N, %)	8 (32%)
Glucose (mg/dl)	110.8 ± 33.6
TC (mg/dl)	175.1 ± 59.4
CHOL LDL (mg/dl)	116.8 ± 50.2
CHOL HDL (mg/dl)	43.5 ± 11.3
TG (mg/dl)	120 ± 46.2
Medication	
Aspirin (N, %)	23 (92 %)
Insulin (N, %)	1 (4%)
Metformin (N, %)	7 (28%)
Sulphonylurea (N, %)	2 (8 %)
Statin (N, %)	24 (96 %)

Table S1. Clinical characteristics of the patients. TC: total cholesterol; CHOL LDL: cholesterol low-density lipoprotein fraction; CHOL HDL: cholesterol high-density lipoprotein fraction; TG: triglycerides.

Gene	GeneBank® Accession Number	Sequences of oligonucleotide primers
Mouse Nox1	NM_172203.2	S: 5'-CATCCAGTCTCCAAACATGACA-3' A: 5'-GCTACAGTGGCAATCACTCCAG-3'
Mouse Nox2	FJ168469.1	S: 5'-ACTCCTTGGGTCAGCACTGG-3' A: 5'-GTTCTGTCCAGTTGTCTTCG-3'
Mouse Nox4	AF276957.1	S: 5'-TGAACACAGTGAAGATTTCTTGAAC-3' A: 5'-GACACCCGTCAGACCAGGAA-3'
Mouse p22phox	NM_007806.3	S: 5'-TGGCCTGATTCTCATCACTGG-3' A: 5'-GGGACAACCTCCACAGAACTC-3'
Mouse NOS2	NM_010927.4	S: 5'-CAGAGGACCCAGAGACAAGC-3' A: 5'-TGCTGAAACATTTCTGTGC-3'
Mouse β-Actin	NM_007393.5	S: 5'-CGTGAAAAGATGACCCAGATCA-3' A: 5'-TGGTACGACCAGAGGCATACAG-3'
Human Nox1	NM_013955	S: 5'-CACAAGAAAAATCCTTGGGTCAA-3' A: 5'-GACAGCAGATTGCGACACACA-3'
Human Nox2	KU178009.1	S: 5'-TCACTTCCTCCACCAAAACC-3' A: 5'-CACCTTCTGTTGAGATCGCC-3'
Human Nox4	NM_016931	S: 5'-TGGCTGCCCATCTGGTGAATG-3' A: 5'-CAGCAGCCCTCCTGAAACATGC-3'
Human Nox5	NM_024505	S: 5'-CAGGCACCAGAAAAGAAAGCAT-3' A: 5'-ATGTTGTCTTGGACACCTTCGA-3'
Human p22phox	NM_000101.4	S: 5'-GTTTGTGTGCCTGCTGGAGT-3' A: 5'-TGGGCGGCTGCTTGATGGT-3'
Human β-Actin	NM_001101	S: 5'-CTGGCACCCAGCACAATG-3' A: 5'-GCCGATCCACACGGAGTACT-3'

Table S2. Sequences of sense (S) and antisense (A) oligonucleotide primers used in real-time PCR assays. If not indicated in table, sequences of the oligonucleotide primers used in real-time PCR assays are derived from Origene (USA).

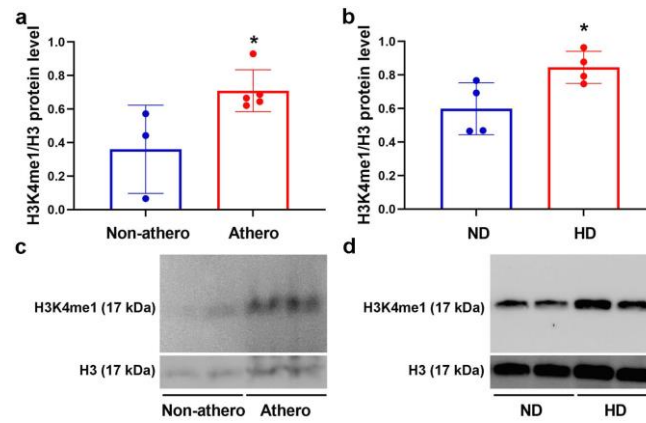


Figure S1. H3K4me1 protein level is enhanced in human carotid atherosclerotic lesions and atherosclerotic aorta of ApoE^{-/-} mice. **a-b**, Western blot analysis depicting the up-regulation of H3K4me1 protein level in atherosclerotic lesions and mouse aortic tissues, respectively. **c**, **d**, Representative blots illustrating the immunodetection of H3K4me1 and H3 proteins in human and mouse arterial tissue homogenates. n=3-5, * $p < 0.05$. p -values were considered in relation to non-athero condition. Non-athero, non-atherosclerotic STA control tissue samples. ND, normal diet; HD, high-fat, cholesterol-rich diet.

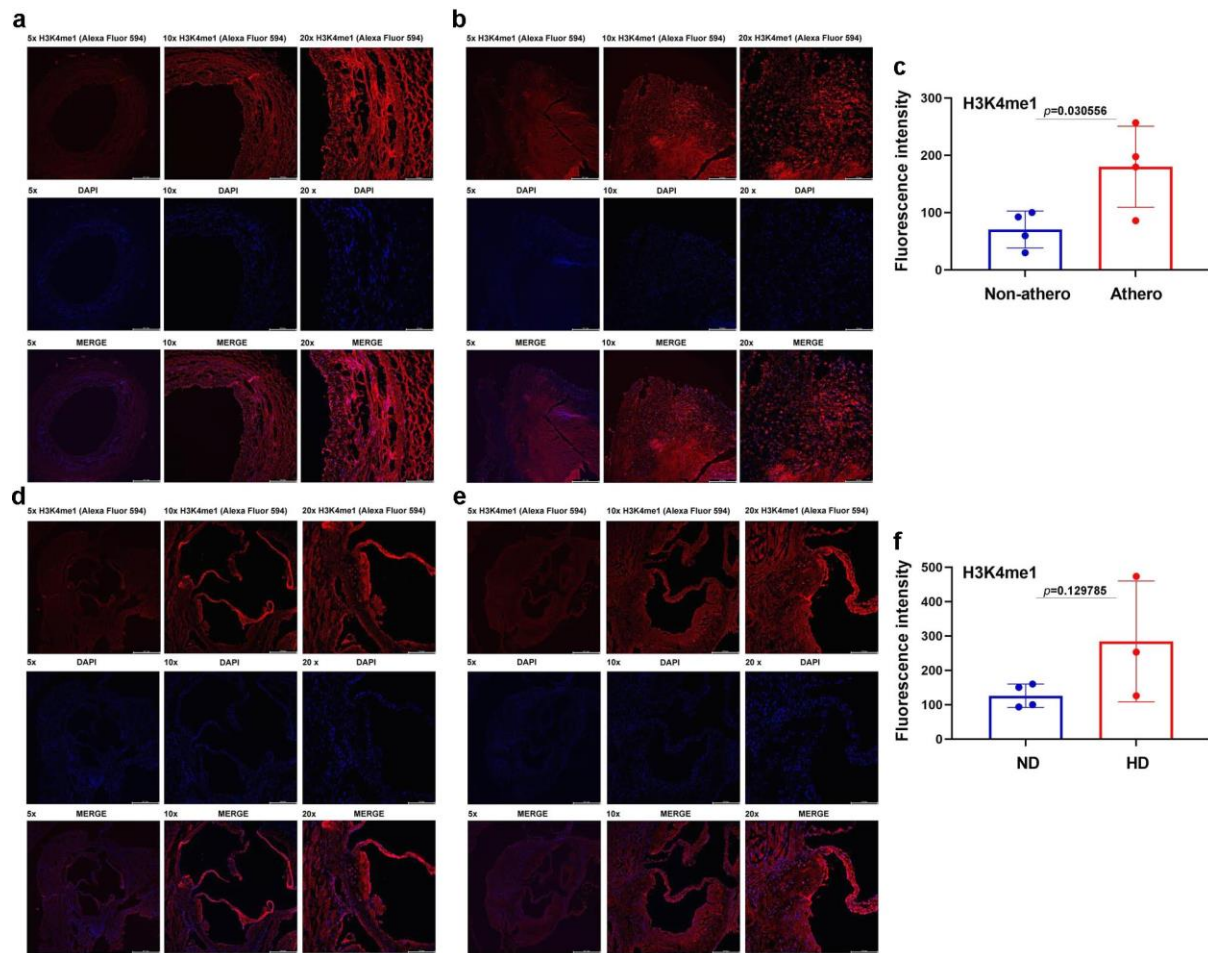


Figure S2. IF microscopy examination showing the expression pattern of H3K4me1 in vascular cells within **(a, b)** non-atherosclerotic arterial wall (left panel) and in the area of infiltrated immune cells in human atherosclerotic lesions (right panel), and **(d, e)** aortic root-derived atherosclerotic lesions obtained from ApoE^{-/-} (ND) (left panel) or ApoE^{-/-} (HD) mice (right panel). **(c, f)** Quantification of H3K4me1 probe fluorescence intensity in human and mouse tissue specimens, respectively. n=3-4.

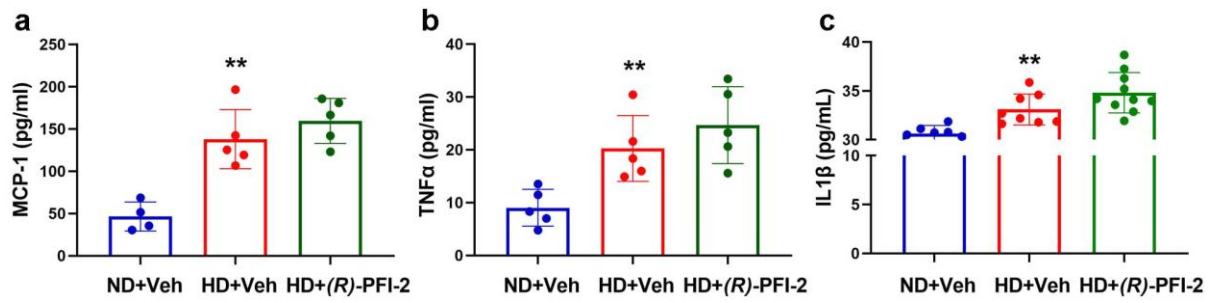


Figure S3. SET7 inhibition does not significantly affect plasma levels of **(a)** MCP-1, **(b)** TNFα, and **(c)** IL1β in hypercholesterolemic ApoE^{-/-} mice. n=4-10, ** $p < 0.01$. p -values were considered in relation to ND + Veh condition.

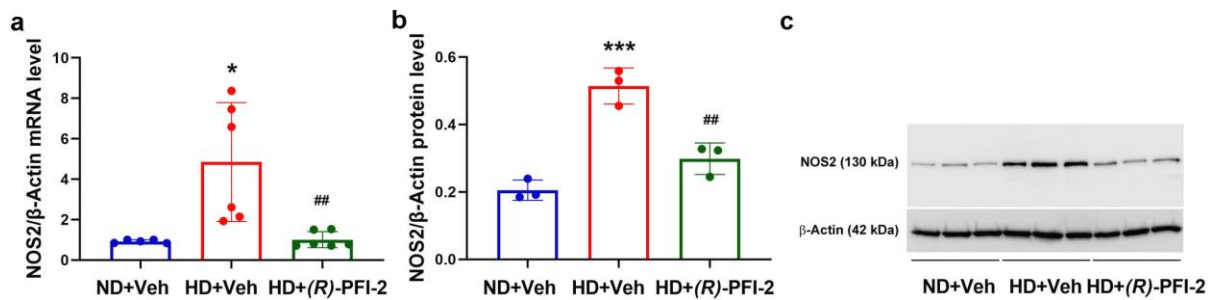


Figure S4. SET7 blockade prevents the up-regulation of NOS2 mRNA and protein levels in the atherosclerotic aorta of ApoE^{-/-} mice. **a**, Gene expression analysis of SET7 mRNA level. **b**, Western blot-associated densitometric quantification of SET7 relative protein level. **c**, Representative immunoblot showing SET7 protein detection. n=3-6, * $p < 0.05$, *** $p < 0.001$. p -values were considered in relation to ND + Veh condition. ## $p < 0.01$. p -values were considered in relation to HD + Veh condition.

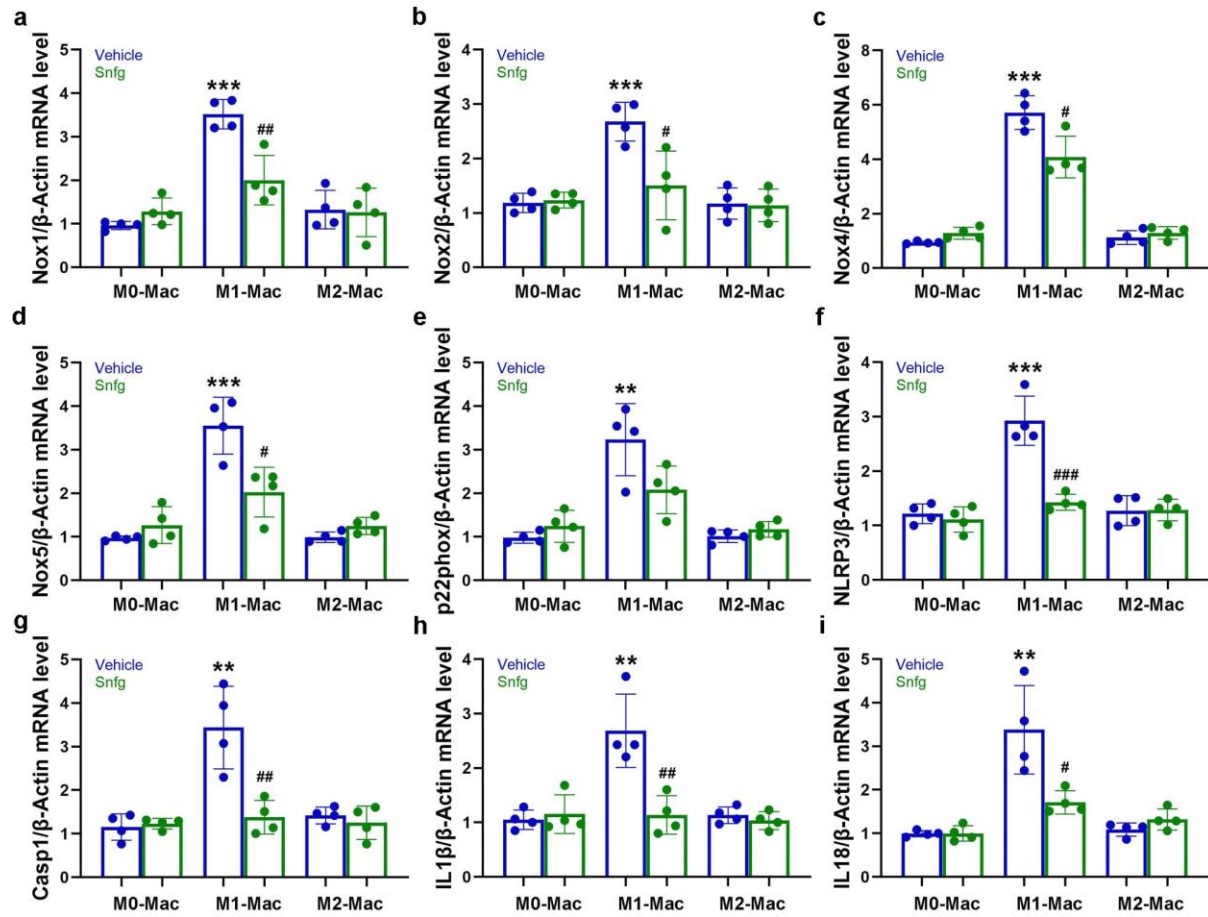


Figure S5. Sinefungin (Snfg), a potent SET7 inhibitor mitigates the mRNA expression of Nox catalytic subunits and NLRP3 inflammasome in cultured human M1-Mac. **a-i**, Gene expression profiling of Nox1, Nox2, Nox4, Nox5, p22phox, NLRP3, Casp1, IL1 β , and IL18. $n=4$, ** $p<0.01$, *** $p<0.001$. p -values were considered in relation to vehicle-exposed M0-Mac. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$. p -values were considered in relation to vehicle-treated M1-Mac.

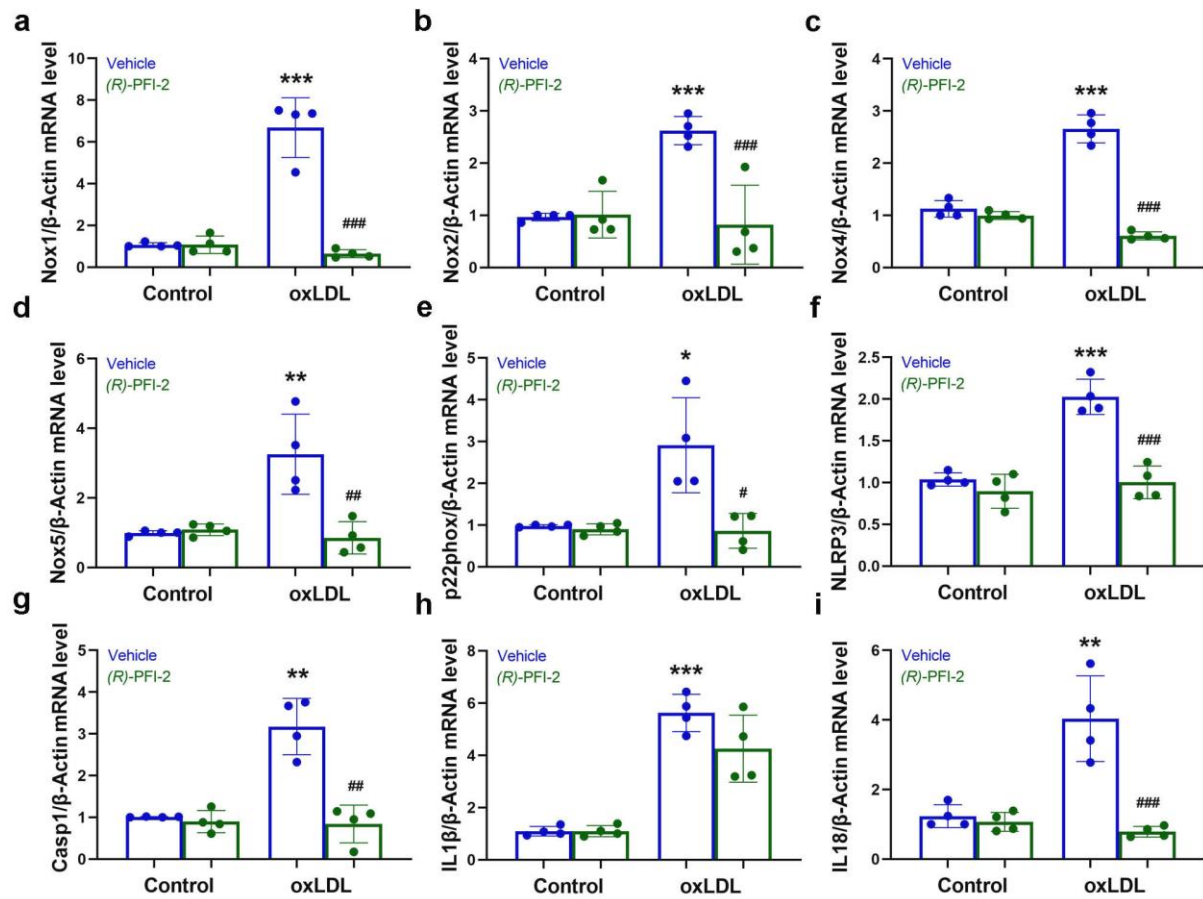


Figure S6. Pharmacological blockade of SET7 enzymatic activity suppresses the oxLDL-induced mRNA expression of Nox catalytic subunits and NLRP3 inflammasome in human Mac. Gene expression profiling of (a) Nox1, (b) Nox2, (c) Nox4, (d) Nox5, (e) p22phox, (f) NLRP3, (g) Casp1, (h) IL1 β , and (i) IL18 in Mac. $n=4$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. p -values were considered in relation to vehicle-exposed M0-Mac. # $p<0.05$, ## $p<0.01$, ### $p<0.001$. p -values were considered in relation to oxLDL-exposed Mac.

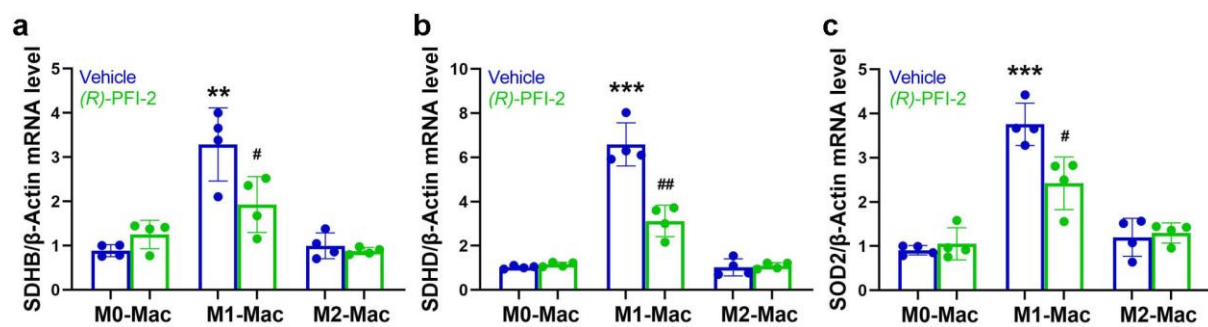


Figure S7. Pharmacological inhibition of SET7 reduces the mRNA expression levels of **(a)** SDHB, **(b)** SDHD, and **(c)** SOD2 in cultured mouse M1-Mac. $n=4$, ** $p<0.01$, *** $p<0.001$. p -values were considered in relation to vehicle-exposed M0-Mac. # $p < 0.05$, ## $p < 0.01$. p -values were considered in relation to vehicle-treated M1-Mac.

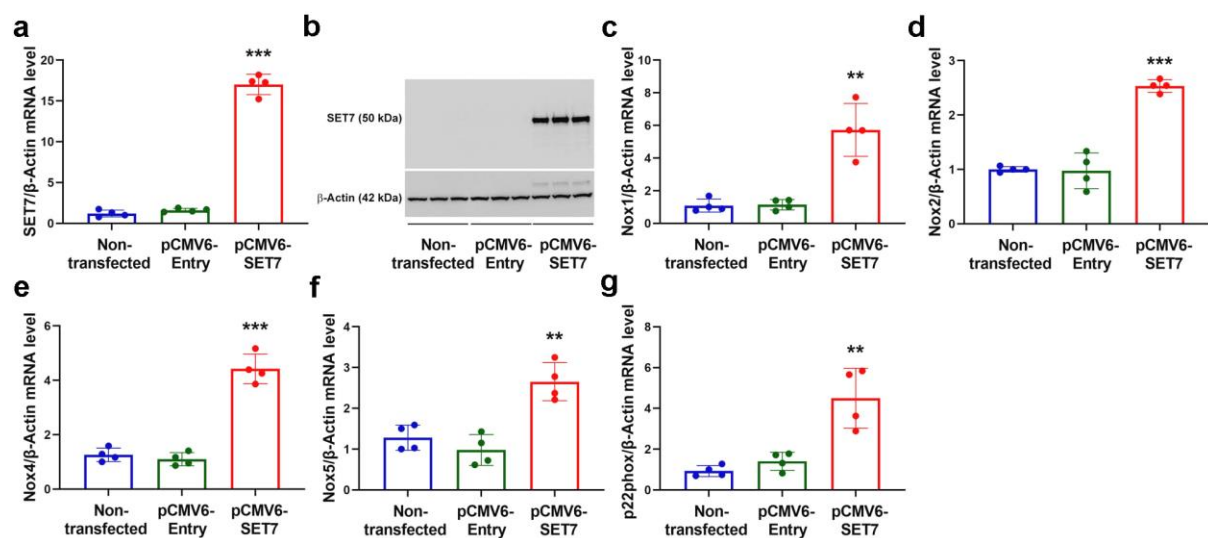


Figure S8. Overexpression of SET7 promotes the up-regulation of Nox catalytic subunit mRNA levels in HEK293 cells. **a**, Validation of SET7 mRNA overexpression in pCMV6-SET7-transfected cells. **b**, Representative immunoblot depicting the augmented SET7 protein level in pCMV6-SET7-transfected cells. **c-g**, Gene expression analysis indicating transcriptional activation of Nox1, Nox2, Nox4, Nox5, and p22phox subunits in HEK293 cells overexpressing SET7. $n=4$, ** $p<0.01$, *** $p<0.001$. p -values were considered in relation to pCMV-6 Entry condition.