Supplemental Material

MEF2B - Nox1 Signaling is Critical for Stretch-Induced Phenotypic Modulation of Vascular Smooth Muscle Cells

Rodriguez *et al.*

Supplemental Figure Legends

Supplemental Figure I. Time response of Nox1 activity in response to varied incubation times of NoxA1ds. RASMC were subjected to 24 hr of cyclic stretch and NoxA1ds (5 μM) was added at 1, 4, 12, & 24 hr before the end of cyclic stretch. SOD-inhibitable $O_2^{\cdot -}$ was measured by cytochrome *c* reduction (*n* = 7). Data are expressed as means + SEM. **P*<0.05 versus static vehicle. $^{\#}$*P*<0.05 versus cyclic stretch.

Supplemental Figure II. NoxA1ds inhibits agonist-induced $O_2^{\cdot -}$ production in RASMC. RASMC were stimulated by classical Nox agonist for 1 hr PMA (20 μM) and PDGF (5 ng/ml). SOD-inhibitable $O_2^{\cdot -}$ was measured by cytochrome c (*n* = 6). RASMC were preincubated with NoxA1ds or scrambled peptide (10 μM). Data are expressed as means + SEM. **P*<0.05 versus static control.

Supplemental Figure III. Characterization of RASMC by immunostaining. RASMC were fixed in 2% paraformaldehyde and stained with monoclonal antibodies specific for SM α-actin (A), smoothelin (B), myosin heavy chain (C), and SM22α (D). Primary antibodies were visualized by staining with Cy3-conjugated secondary antibodies (red). Nuclei were stained by Hoechst (blue) and F-actin was labeled by 488 phalloidin (green). Images were taken with an Olympus FluoView™ FV1000 confocal microscope. Unstained controls were performed omitting the primary antibody in each case.

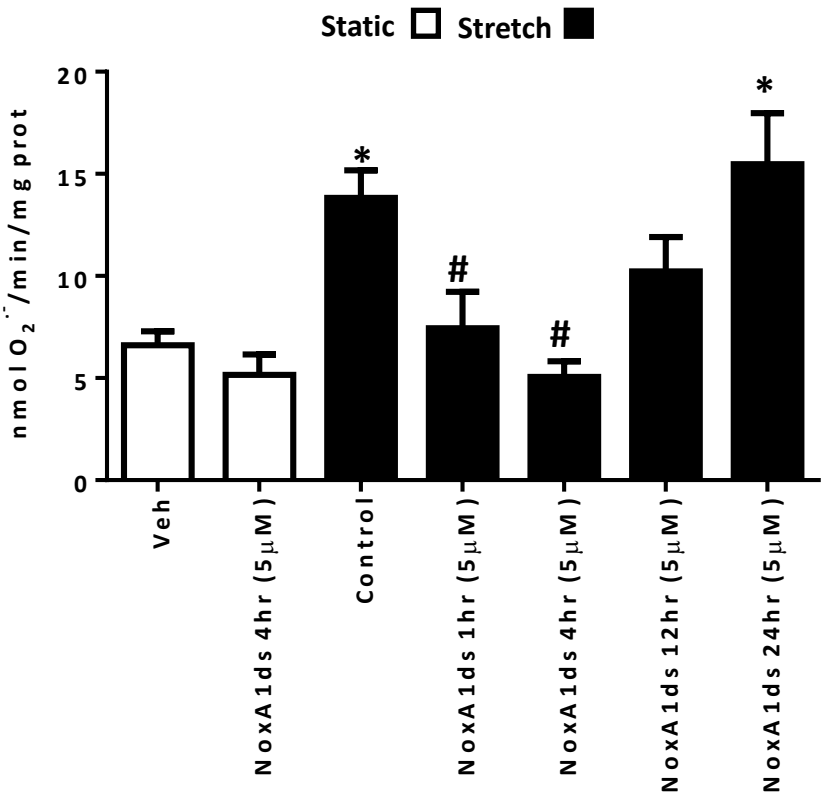
Supplemental Figure IV. Nox4 siRNA does not reverse CS-induced decreases in CNN1 (A) and increases in OPN (B) levels but augments the reduction in CNN1. RASMCs were pretreated with scrambled (Scr) siRNA or Nox4 siRNA (48 hrs) and then subjected to CS for 24 hr. CNN1 and OPN protein expression was investigated by Western blot (*n* = 3). Data are expressed as means + SEM. **P*<0.05 versus static control.

Supplemental Figure V. Cyclic Stretch causes no change in MMP2 activity. RASMC were subjected for 24 hr to stretch or static conditions. Extracellular MMP2 activity was measured by zymography (*n* = 5). RASMC were preincubated with NoxA1ds or scrambled peptide (10 µM).

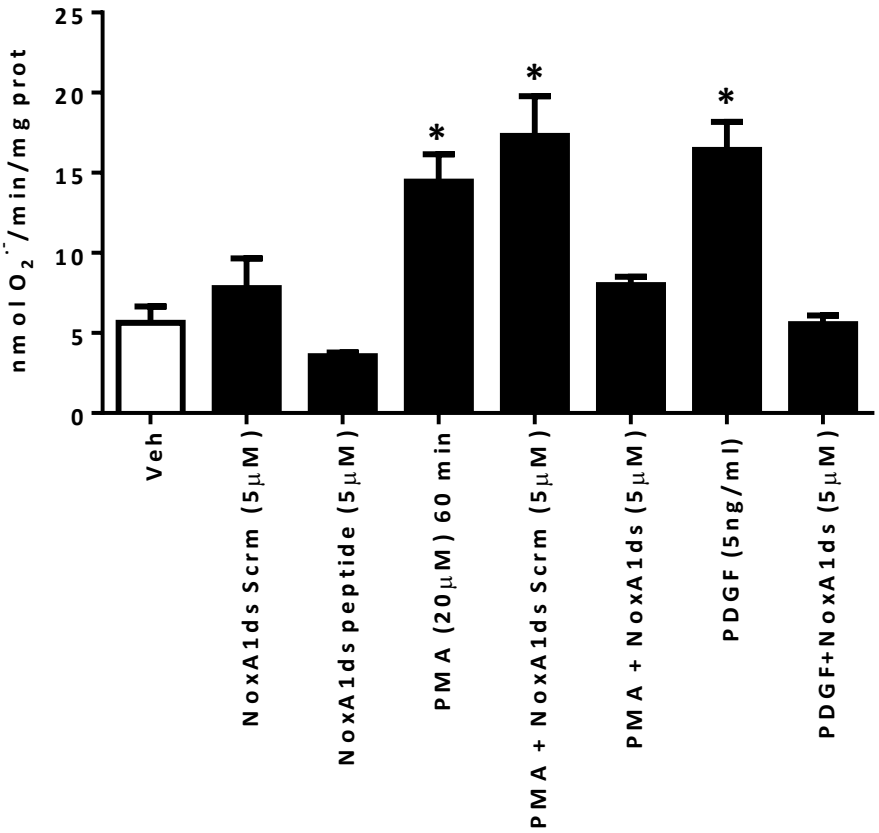
Supplemental Figure VI. MEF2B consensus sequence site is located at -438 bp upstream of the transcription initiation codon (ATG) within the Nox1 promoter region. Analyzed by ENSEMBL genome browser (www.ensembl.org) in Rattus Norvegicus (Rat): Chromosome X: 104,742,297-104,765,479 (reverse strand).

Supplemental Figures

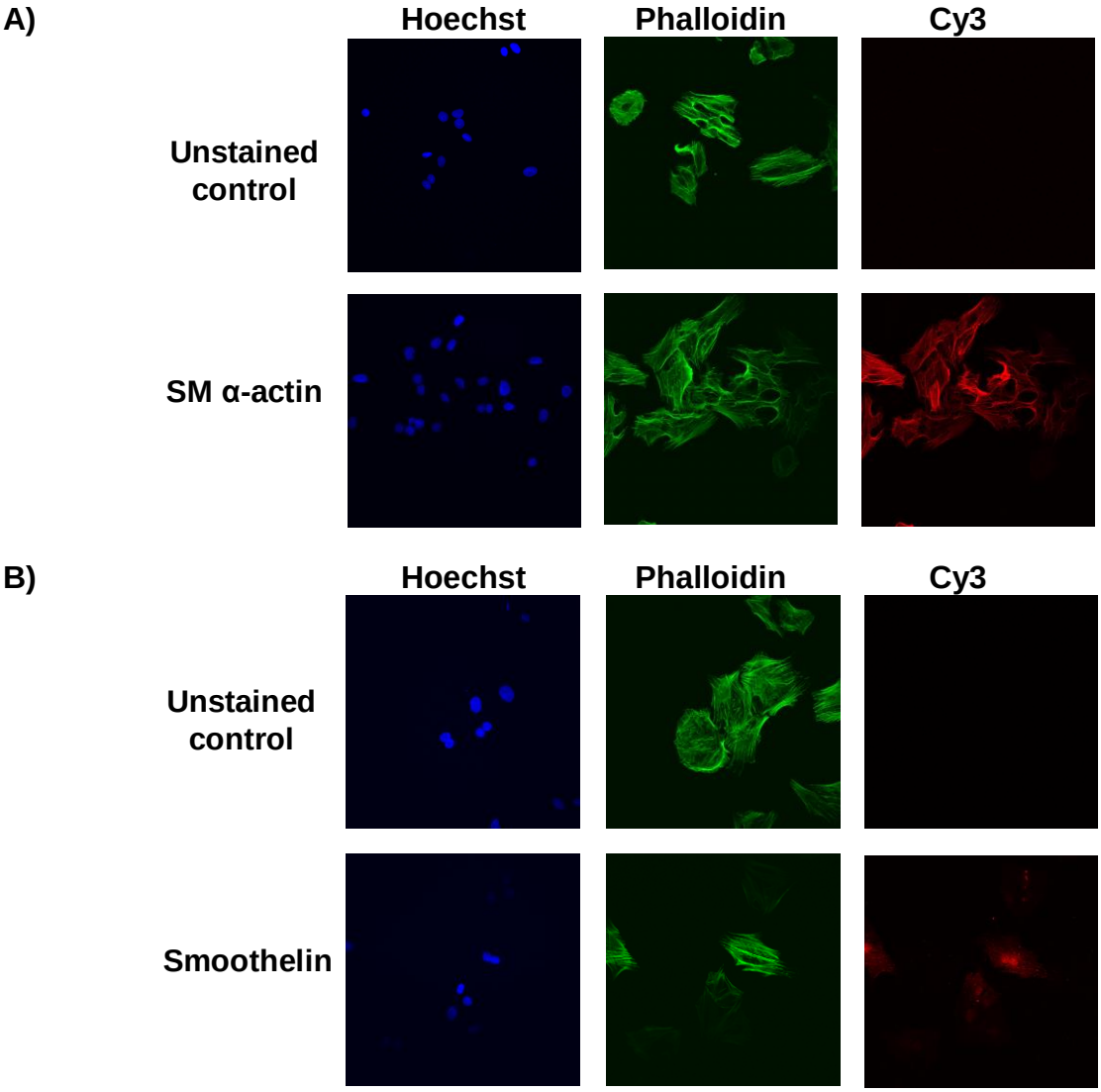
Supplemental Figure I



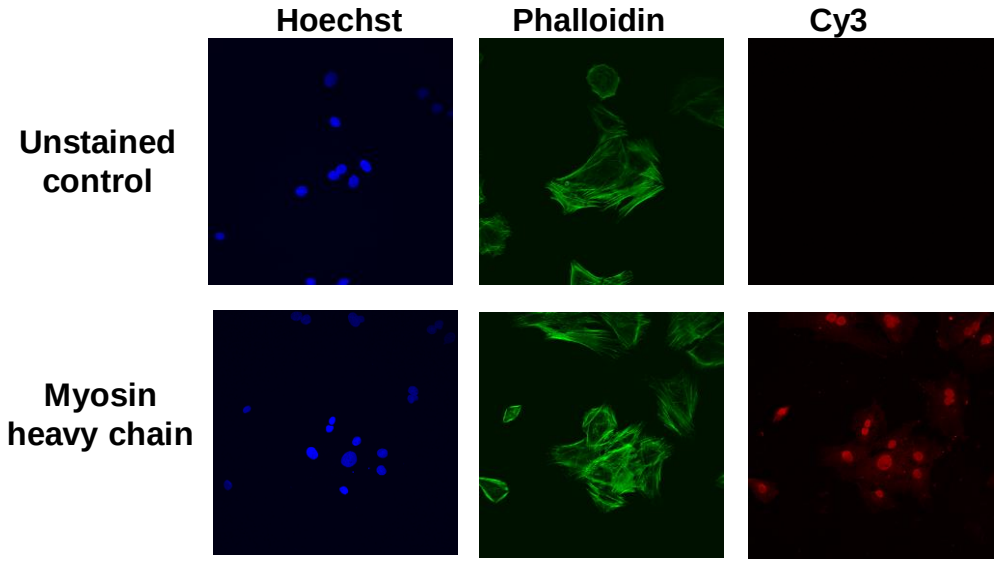
Supplemental Figure II



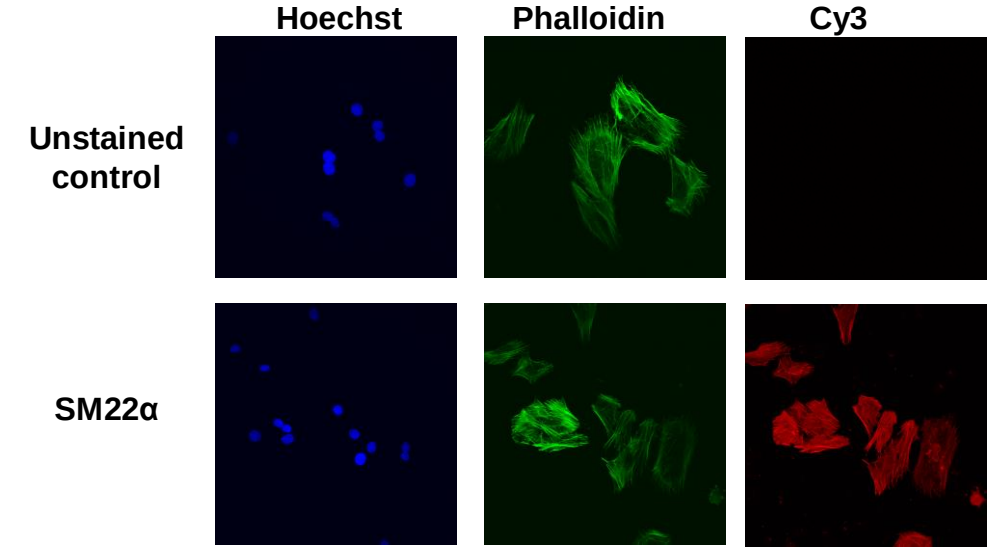
Supplemental Figure III



C)

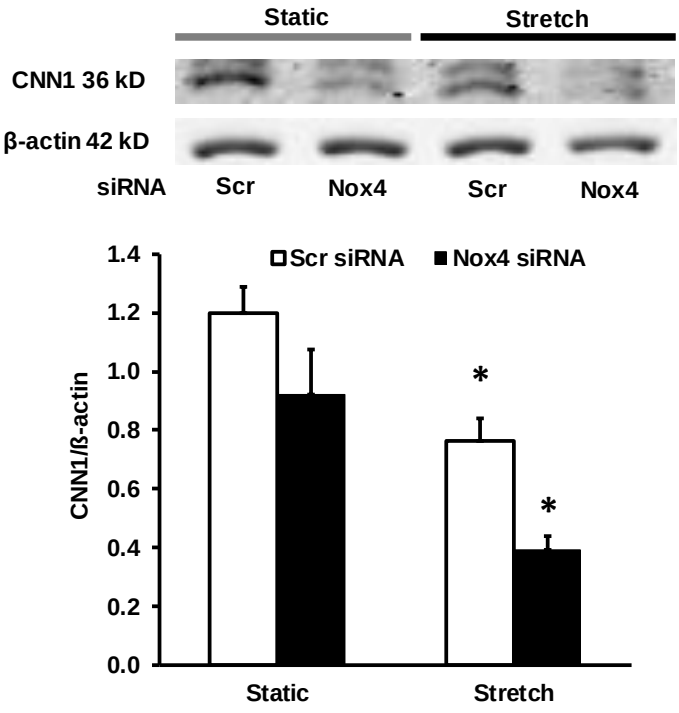

Hoechst
Phalloidin
Cy3
Unstained control
Myosin heavy chain

D)

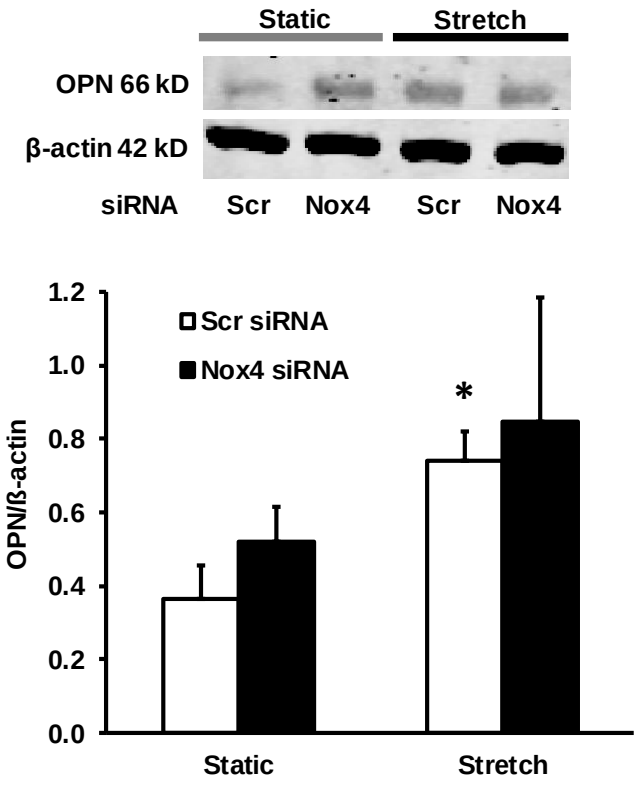

Hoechst
Phalloidin
Cy3
Unstained control
SM22α

Supplemental Figure IV

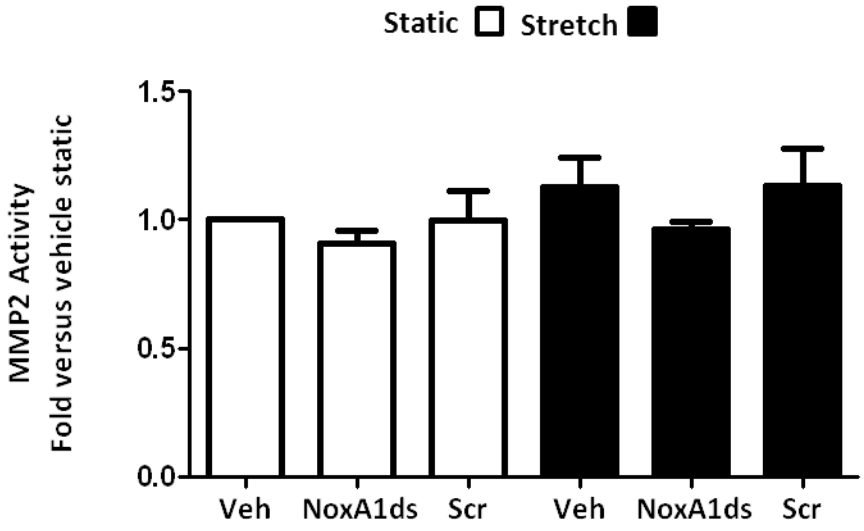
A)



B)


Static
Stretch
OPN 66 kD
β-actin 42 kD
siRNA
Scr
Nox4
Scr
Nox4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
OPN/ß-actin
Scr siRNA
Nox4 siRNA
*
Static
Stretch

Supplemental Figure V



Supplemental Figure VI