Supplementary Materials

Methods

Blood pressure measurement: Mice were restrained on a heated holder to maintain body temperature at 37 ˚C, and anesthesia was induced with 2.5% inhaled isoflurane. Once a level plane of anesthesia was achieved, isoflurane was reduced to 1.5%, a 2-3 mm midline incision was made in the neck, and the lobes of the thymus were separated to expose the right common carotid artery. After clamping and making a small nick in the right common carotid artery, a Millar pressure transducer (model SPR-671, Houston, TX) was introduced and advanced to the ascending aorta. Systolic and diastolic blood pressure and heart rate were recorded at 1% inhaled isoflurane with the PowerLab® data acquisition system (ADInstruments, Colorado Springs, CO), and data were analyzed by using LabChart® 7 for Mac software (ADInstruments, Colorado Springs, CO). All surgical procedures were performed in accordance with protocols approved by the Animal Studies Committee of Washington University School of Medicine.

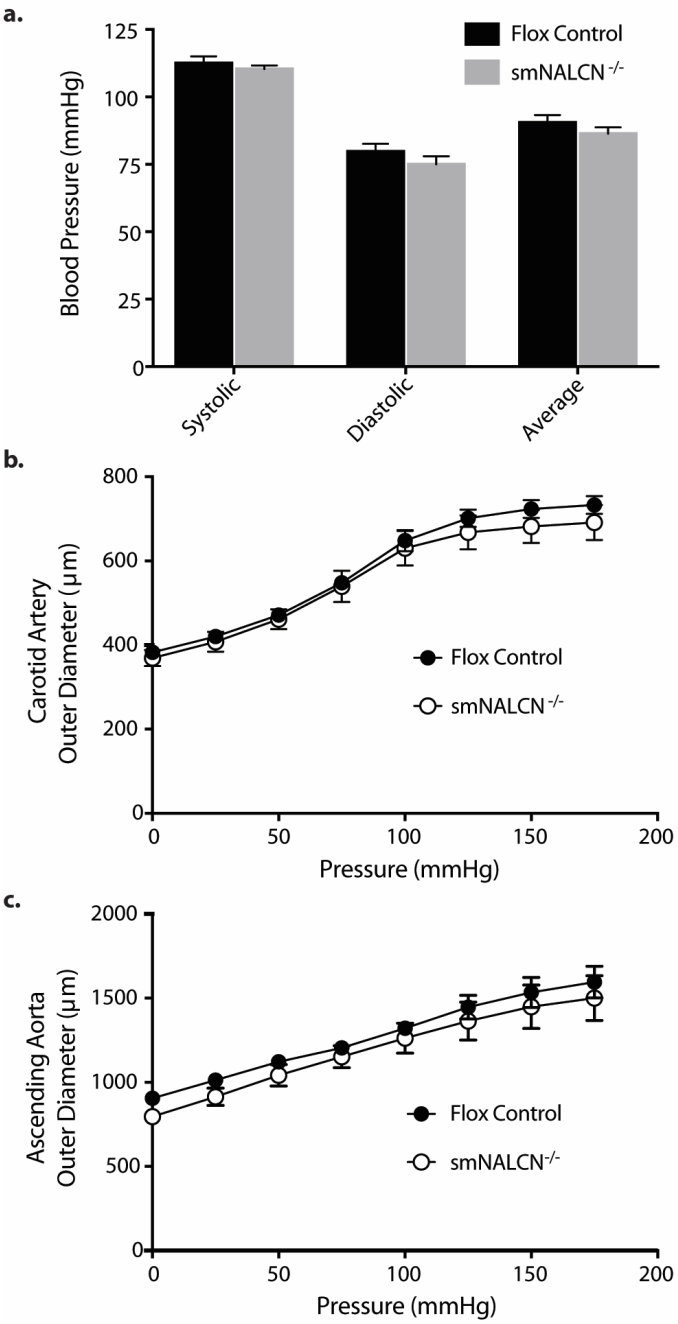
Compliance studies: Arterial pressure-diameter curve measurements were performed as previously described [36]. Briefly, after blood pressure measurement, mice were euthanized under isoflurane anesthesia, and the ascending aorta and left common carotid artery were excised and placed in physiological saline solution (PSS) composed of 130 mM NaCl, 4.7 mM KCl, 1.6 mM $CaCl_2$, 1.18 mM $MgSO_4$-$7H_2O$, 1.17 mM KH_2PO_4, 14.8 mM $NaHCO_3$, 5.5 mM dextrose, and 0.026 mM EDTA (pH 7.4). After removing surrounding fat and fascia, the vessels were mounted on a pressure arteriograph (Danish Myotechnology, Aarhus, Denmark) and maintained in PSS at 37°C. Vessels were transilluminated under a microscope connected to a charged-coupled device camera and computerized measurement system (Myoview, Danish

Myotechnology) to allow continuous recording of vessel diameters. Intravascular pressure was increased from 0 to 175 mm Hg in 25 mm Hg steps. At each step, the outer diameter of the vessel was measured and recorded. The average of three measurements at each pressure was reported.

Table S1. DNA Primer Sequences.

Primer Name	Sequence
mNALCN_F	TTTCCCCGCTGGCGCTCCTA
mNALCN_R	ACCAGCTGCCAACCACCAGC
mUNC80_F	TGTCGAAGCTTCATGTCTGG
mUNC80_R	GCTGTGGTACATTCCGAGGT
mUNC79_F	GAGCGTTCACAAAGGAGAGG
mUNC79_R	GCTAGCTTGGTTCCAGCATC
mNLF-1a_F	TTGAAAGCGTGCTGCATAAG
mNLF-1a_R	GGGCAATATGAATGGACACC
mSDHA_F	GGAACACTCCAAAAACAGACCT
mSDHA_R	CCACCACTGGGTATTGAGTAGAA
mTOP1_F	AAGATCGAGAACACCGGCATA
mTOP1_R	CTTTTCCTCCTTCGGTCTTTCC

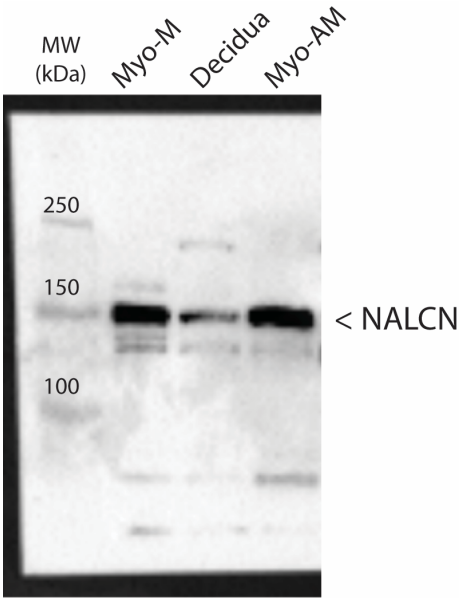
Figure S1. Cardiovascular parameters in flox control and smNALCN$^{-/-}$ males.



a) Blood pressure measurements in smNALCN-/- and flox control mice showed no difference in systolic, diastolic and mean blood pressure between the two genotypes. Pressure-diameter

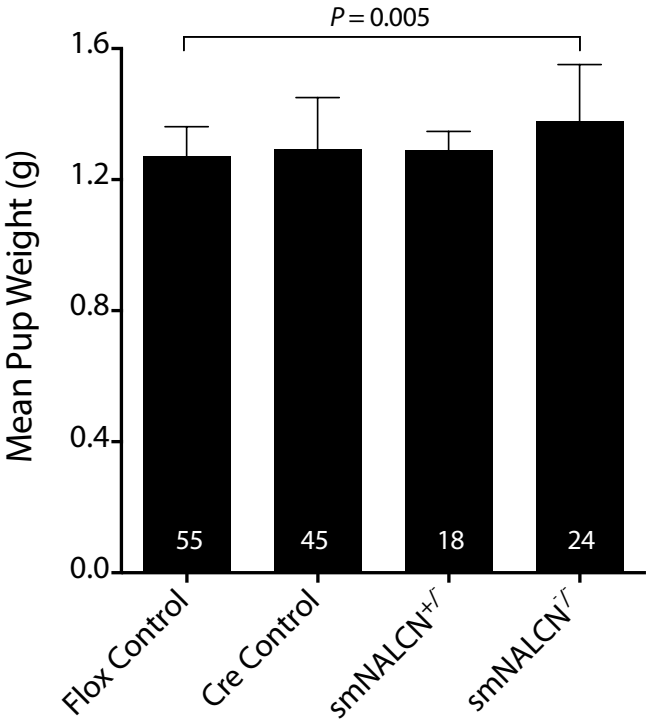
measurements of the carotid artery (b) and ascending aorta (c) showed similar vascular diameters and compliance (the slope of the pressure-diameter curve) over a range of pressures from 0 to 175 mmHg. n = 3 for smNALCN$^{-/-}$ and n= 4 for flox control mice. Shown is mean plus standard deviation for all measurements.

Figure S2. NALCN protein expression in the myometrium and decidua.



Western blot of NALCN membrane preparations from the mesometrial and anti-mesometrial myometrium (Myo-M and Myo-AM, respectively) and decidua from the same day 14 pregnant wild type dam.

Figure S3. Mean pup weight for each genotype.



Mean pup weight is represented by bars. Error bars represent the standard deviation. Pups were weighed within 24 hours of birth. Not all litters were weighed. Numbers at the bottoms of the bars indicate total number of pups from 7 flox control, 8 Cre control, 3 smNALCN$^{+/-}$, and 6 smNALCN$^{-/-}$ mice.