

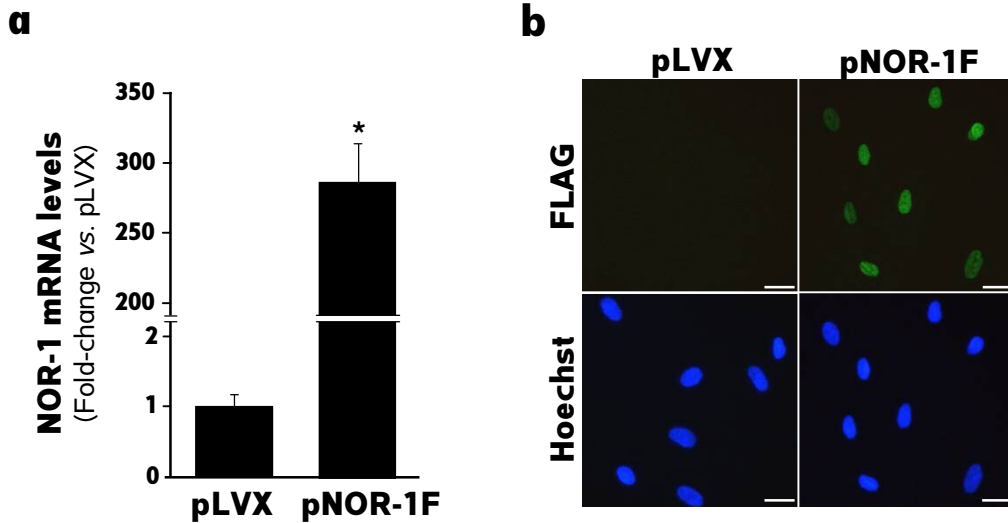
Supplementary Information

The nuclear receptor NOR-1 regulates the small muscle protein, X-linked (SMPX) and myotube differentiation

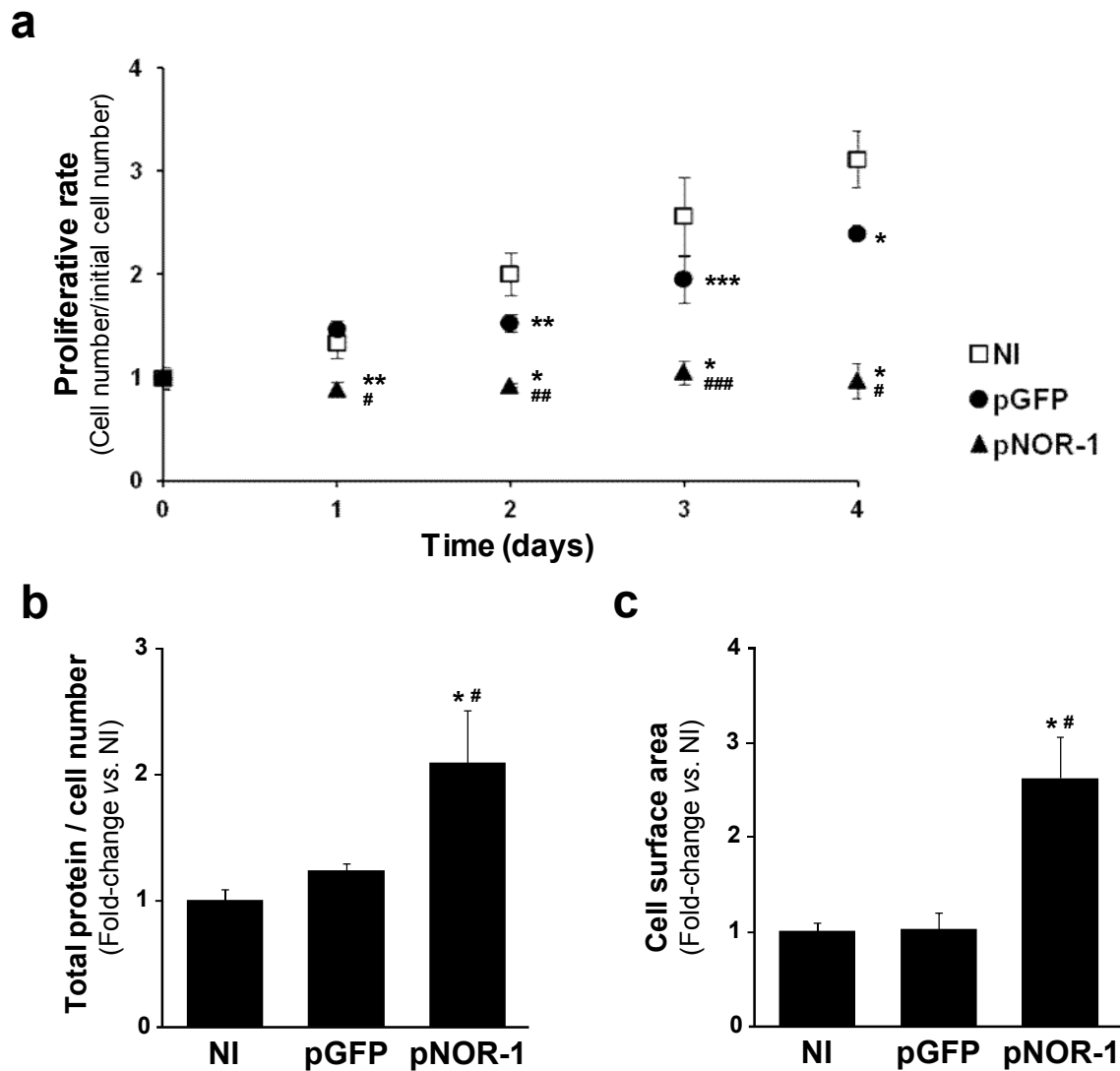
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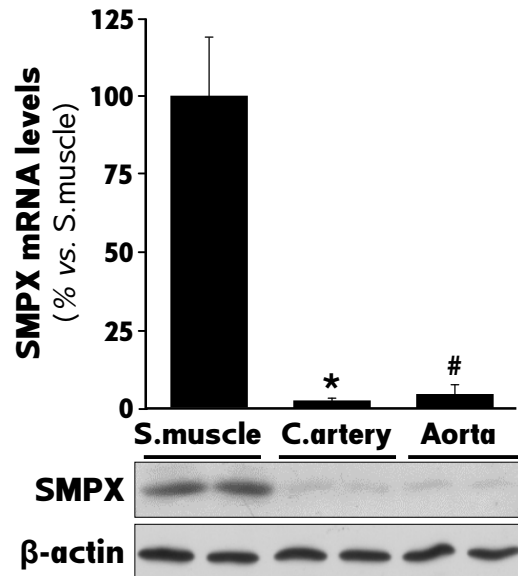
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Supplementary Fig. S1. Lentiviral over-expression of NOR-1 in human VSMC. (a) Human VSMC were transduced with pLVX or pLVX/NOR-1-FLAG (pNOR-1F) lentivirus, and NOR-1 over-expression was verified by real-time PCR (n = 6). *P < 0.0001 vs. cells transduced with pLVX. (b) Immunocytochemical analysis for NOR-1 using an antibody against the FLAG sequence (FLAG), from cells transduced as indicated in (a). Bottom panels: fluorescent labelling of nuclei using Hoechst. Bars: 25 μ m.



Supplementary Fig. S2. Lentiviral NOR-1 over-expression reduces VSMC proliferative rate and promotes VSMC hypertrophy. (a) VSMC were transduced with a lentiviral vector to over-express NOR-1 (pLVX/NOR-1; pNOR-1), or a control lentiviral vector expressing EGFP (pLVX/EGFP; pGFP), and the proliferative rate of VSMC cultures was determined by daily cell counting. Non-infected cells (NI) were used as a control. (b and c) Relative cellular protein content (total protein content/ cell number) (b) and relative cell surface area (c) of VSMC treated as indicated in (a). *, **, ***, $P < 0.0001$, $P = 0.002$, $P = 0.0077$, respectively vs. NI. #, ##, ###, $P < 0.0001$, $P = 0.0003$, $P = 0.0006$, respectively vs. pGFP.



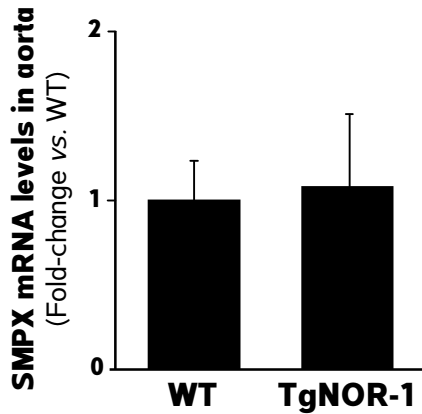
Supplementary Fig. S3. SMPX is expressed in human arteries. (a) SMPX mRNA levels determined by real-time PCR in human skeletal muscle (S. muscle; n = 4), and human vessels: coronary arteries (C. artery; n = 7) and aorta (n = 6). Data are expressed as mean \pm s.e.m. *P = 0.0001 and #P = 0.0003 vs. S. muscle. (b) Representative Western blot showing SMPX protein levels in these tissues. Levels of β -actin were used as a loading control in Western blot experiments.

a

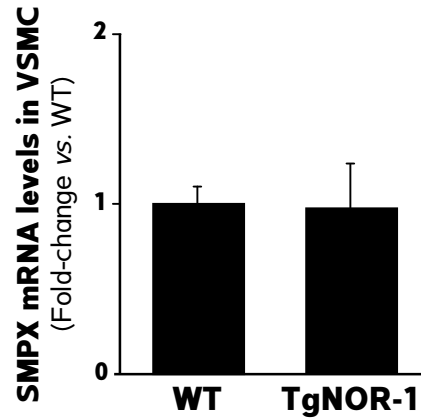
NBRE c.s. TGACCTTT
| | | | | | | |

| | |
|-------------------|--|
| Human | AAGTAAGATACCATAGCCACCTCCCGGCT TGACATTT GAGC-CTGCTTTCCGCACGGTCAAGCCT |
| Orangutan | AAGTAAGATACCATAGCCACCTCCCGGCT TGACATTT GAGC-gcttTccgCaCggtcaggccttT |
| Chimpanzee | AAGTAAGATACCATAGCCACCTCCCGGCT TGACATTT GAGC-gcttTccgCaCggtcaagccttT |
| Gorilla | AAGTAAGATACCATAGCCACCTCCCGGCT TGACATTT GAGC-gcttTccgCaCggtcaagccttT |
| Cow | A-GTAAGATtCCAgAGCCACCTtCCGGCT TGACATTT GAGCcCTGCTTTCaGCACGGTCAAGCCT |
| Pig | A-GTAAGATtCCgTAGCCACCTtCCGGCT TGACATTT GAGCcCTGCTTTCaGCACGGTCAAGCCT |
| Mouse | A-GaAAGATAaCgTAGCCACCTCaGGCT TGA tATcTGAGCcCTGCTTTCaCACGGTCAAGCCT |
| Rat | A-GTAAGATAaCgTAGCCACCTCaGCT TGA tATcTGAGCcCTGCTTTCaCAtGGTCAAGCCT |

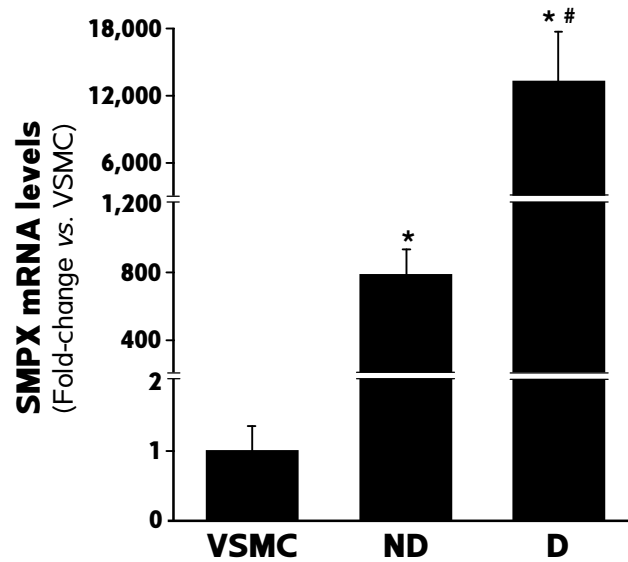
b



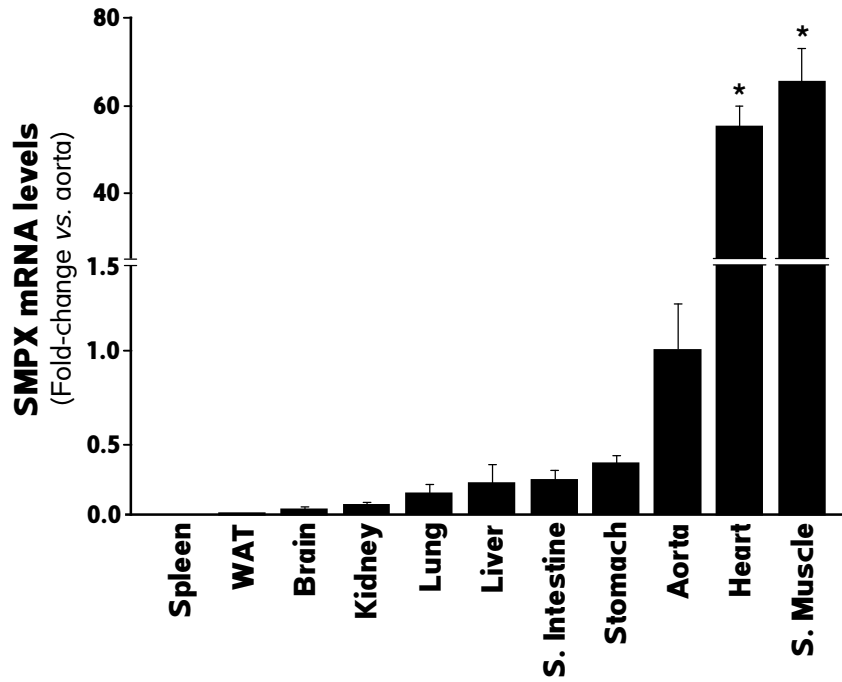
c



Supplementary Fig. S4. SMPX does not seem to be regulated by NOR-1 in mouse. (a) Alignment of the proximal region corresponding to the SMPX promoter from different species including human and mouse. Non-conserved positions are indicated in lower case. The NBRE consensus sequence (NBRE c.s.) is shown at the top. (b and c) SMPX was not regulated by NOR-1 transgenesis in mice. SMPX mRNA levels were analyzed by real-time PCR in mouse aorta from wild-type (WT; n = 7) and transgenic mice that specifically over-express human NOR-1 in VSMC (TgNOR-1; n = 9) (b), and in cultures of VSMC from these animals (n = 6) (c). Data are expressed as mean \pm s.e.m.



Supplementary Fig. S5. SMPX is highly expressed in HSMM. mRNA levels of SMPX (analysed by real-time PCR) in human VSMC, and in HSMM non-differentiated (ND) and differentiated (D) to myotubes (after the exposition to 2% horse serum during five days) (n = 6). *P < 0.0001 vs. VSMC; #P < 0.0001 vs. ND.



Supplementary Fig. S6. SMPX expression in mouse tissues. Expression levels of SMPX analysed by real-time PCR in a set of mouse tissues. WAT: white adipose tissue; S. intestine: small intestine; S. muscle: skeletal muscle. Data are expressed as mean \pm s.e.m. (n = 7). *P < 0.0001 vs. aorta.