Supplemental Figure Legends.

Figure S1. Showing pregnancy increases MYPT1 mRNA levels in wildtype and smtnl1-/- mice, although deletion of SMTNL1 alone has no effect. RT PCR results of MYPT1 mRNA levels in uterine smooth muscle from aged matched pregnant and non pregnant WT and smtnl-/- mice. Results shown are mean values of 3 experiments ±SEM.

Figure S2. Interaction of MYPT1 and SMTNL1 by overlay (FarWestern) analysis. Flag-SMTNL1 and GST-MYPT1 were incubated with 10 nM Flag-SMTNL1 (left), with 10 nM GST-MYPT1 (middle) and were probed with anti-Flag and anti-GST antibodies, respectively. Control proteins (1μg of PKA and AMPK) were used to show specificity as well as control overlay experiments conducted with with anti-His and anti-GST antibodies (right).

Figure S3. Pregnancy and SMTNL1 deletion promote increased myosin phosphatase activity and dephosphorylation of endogenous myosin LC20 phosphorylation on Ser19 *in vivo*. A. Effect of pregnancy and SMTNL1 deletion on the phosphorylation level of $LC20^{Ser19}$ in smooth muscle lysate by Western blots using phospho-LC20 Ser19 antibody (upper Western-blot panel). Anti-LC20 antobody is used for loading control (lower Western-blot panel). Relative changes in the phosphorylation were determined by densitometric analysis of the blots (top panel). Data represent mean ±SEM for n=3 independent experiments. B. Pregnancy and SMTNl1 deletion increase endogenous myosin phosphatase activity in thoracic aorta (n=3, ± SEM).

Figure. S1

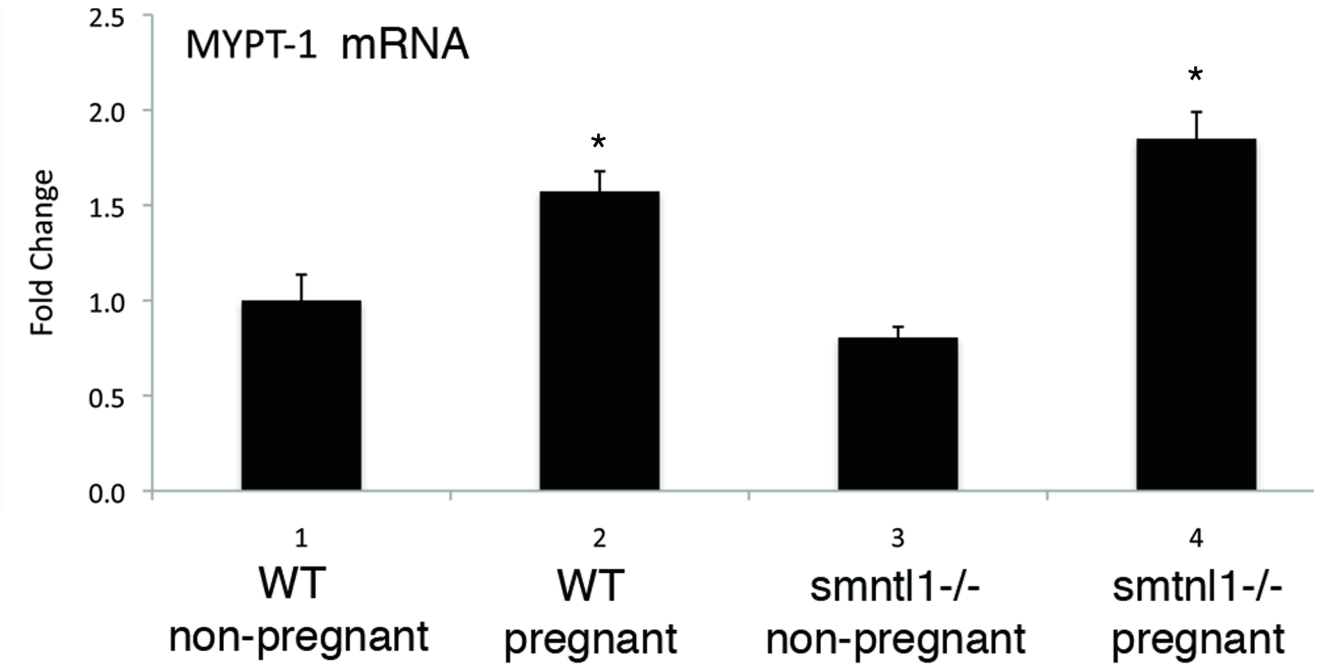

MYPT-1 mRNA
Fold Change
2.5
2.0
1.5
1.0
0.5
0.0
*
*
1
2
3
4
WT
non-pregnant
WT
pregnant
smntl1-/-
non-pregnant
smtnl1-/-
pregnant

Figure. S2

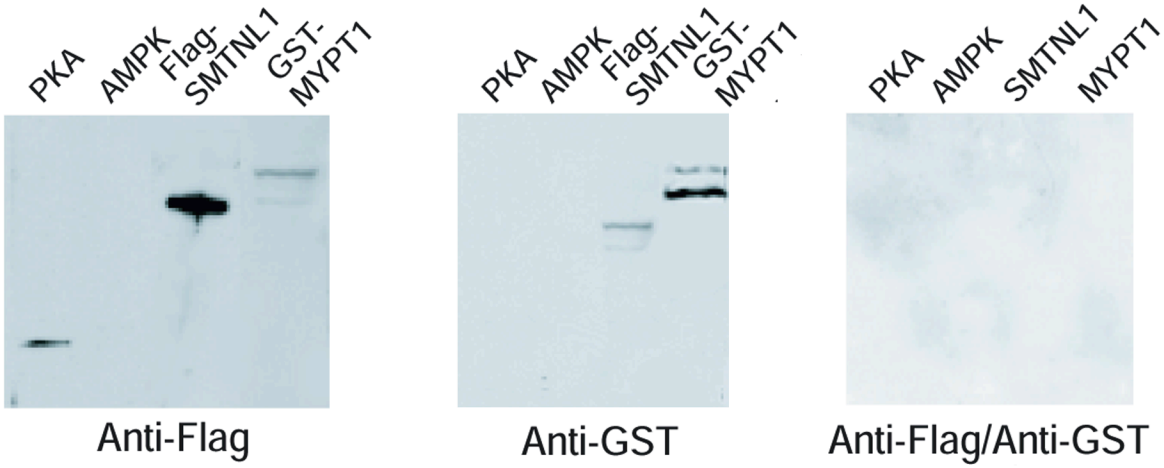
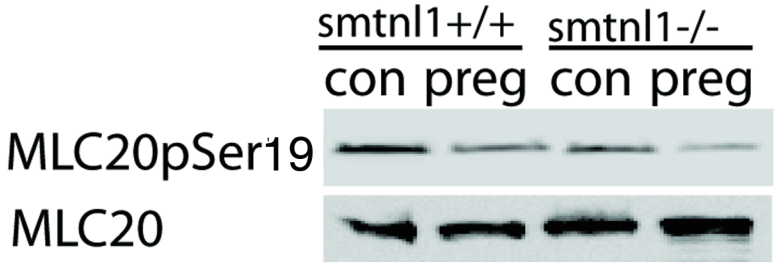
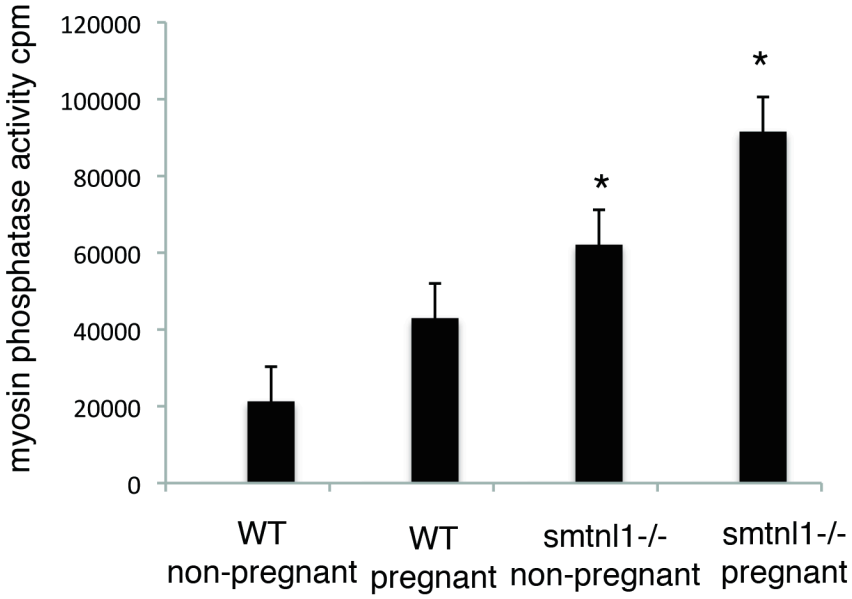

PKA
AMPK
Flag-SMTNL1
GST-MYPT1
Anti-Flag
PKA
AMPK
Flag-SMTNL1
GST-MYPT1
Anti-GST
PKA
AMPK
SMTNL1
MYPT1
Anti-Flag/Anti-GST

Figure. S3

A.


smtnl1+/+
smtnl1-/-
con preg con preg
MLC20pSer19
MLC20

B.


myosin phosphatase activity cpm
120000
100000
80000
60000
40000
20000
0
*
*
WT non-pregnant
WT pregnant
smtnl1-/- non-pregnant
smtnl1-/- pregnant