Supplementary Figure Legends.

Supplementary Figure 1. Characterization of primary hISMCs by flow cytometry. Passage 1 primary hISMCs were used for characterization. α-smooth-muscle-actin and desmin were used as smooth muscle cell markers and CD34 was used as endothelium cell mark and measured by flow cytometry. Commercially available hISMCs were used as positive control. Figure shows the ratio of α-smooth muscle actin or CD34 positive cells to the total examined cells. (n=3)

Supplementary Figure 2. MLC phosphorylation time course and patterns in response to ECS. (A) Determination of MLC phosphorylation time course by regular SDS-PAGE. MLC(Thr18/Ser19) phosphorylation changes with different duration of ECS were compared to CCS. **(B) Determination of MLC phosphorylation changes by phos-tag SDS-PAGE.** Phosphorylated and un-phosphorylated MLC were separated by phos-tag SDS-PAGE as described in Methods. Antibody that recognizes total MLC protein was used in Western blots.

Supplementary Figure 3. Comparison of the MLC phosphorylation change in static culture, CCS and ECS groups MLC di-phosphorylation (Thr18/Ser19) (A), MLC(Ser19) phosphorylation (B) and MLC(Thr18) phosphorylation (C) were monitored by Western blotting. Representative Western blots are shown for each protein as indicated, and the ratio of the phosphorylated protein to the total protein is shown in the lower panels. (*, $p<0.05$ vs. CCS; n=5 per group in panel A; n=3 per group in panels B and C)

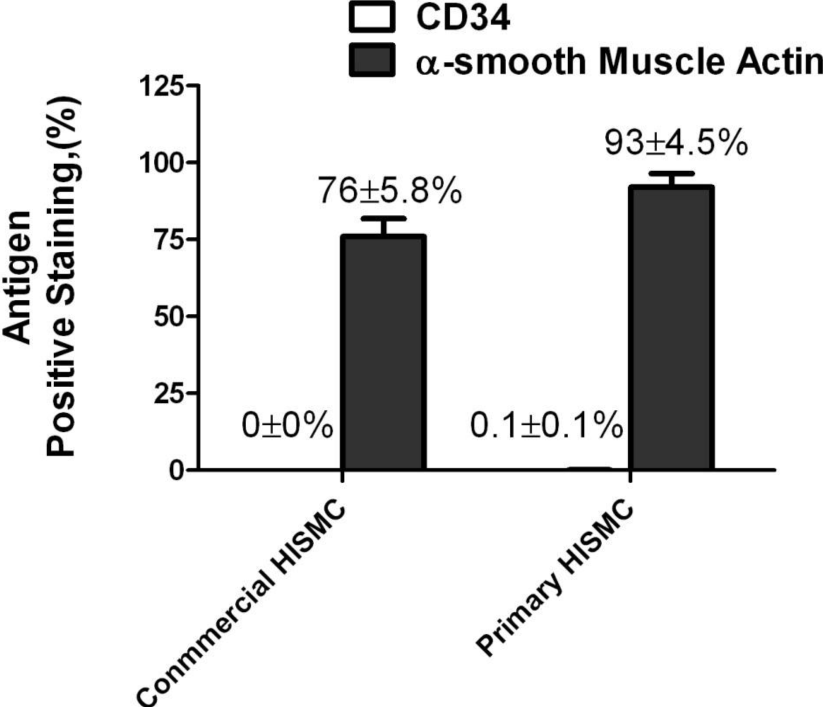
Supplementary Figure 4. Change of MLC phosphorylation with BPIPP treatment in static hISMCs. A. Change of MLC phosphorylation with BPIPP treatment in static hISMCs. HISMCs were treated with varying concentrations of BPIPP, as indicated, for 30 minutes. The ratio of phosphorylated to total MLC phosphorylation is shown. (+, $p<0.05$ vs. Veh treatment within groups; n=3 per group) **B-D. Changes in MLC phosphorylation with BPIPP treatment confirmed by immunofluorescence.** HISMCs were treated with varying concentrations of BPIPP, as indicated, for 30 minutes before immuno-staining. Phosphorylated MLC is shown in red fluorescence and nuclei are shown in blue fluorescence.

Supplementary Methods.

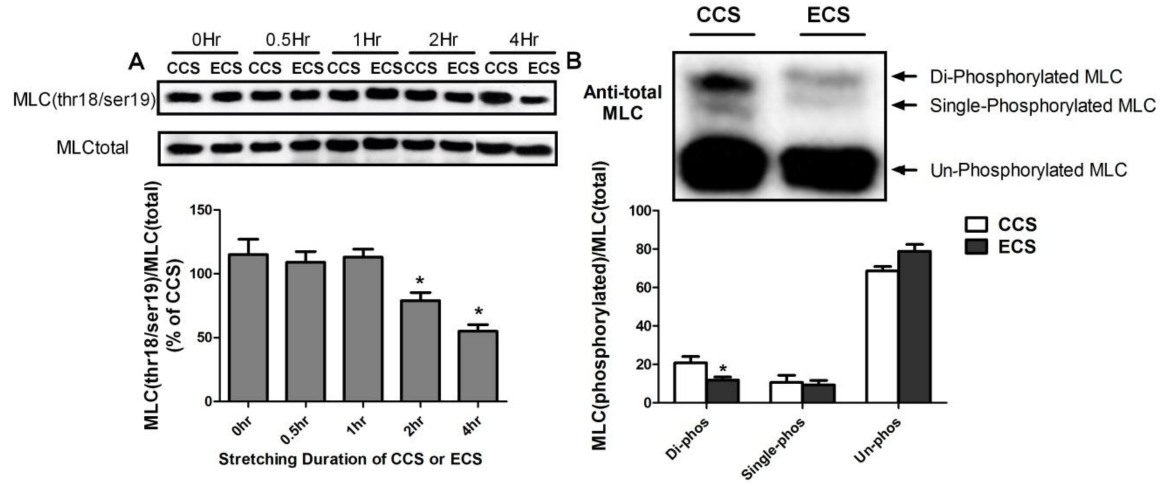
Immunofluorescence

HISMCs were grown on poly-D-lysine-coated cover slips. After 30 minutes BPIPP or vehicle treatment, cells were fixed with 4% paraformaldehyde (25°C, 10 minutes). After removing paraformaldehyde and permeabilizing hISMCs with 0.2% Triton X-100 (5 minutes, 25°C), cells were blocked with 10% goat serum. HISMCs were washed and incubated with MLC antibody overnight at 4°C. Goat anti-rabbit Alexa Fluor®594 was used as secondary antibody (Invitrogen, Grand Island, NY). Nuclei were stained with Dapi (Invitrogen, Grand Island, NY). HISMCs were imaged using Zeiss Axio Observer Z1 microscope (Zeiss, Jena, Germany).

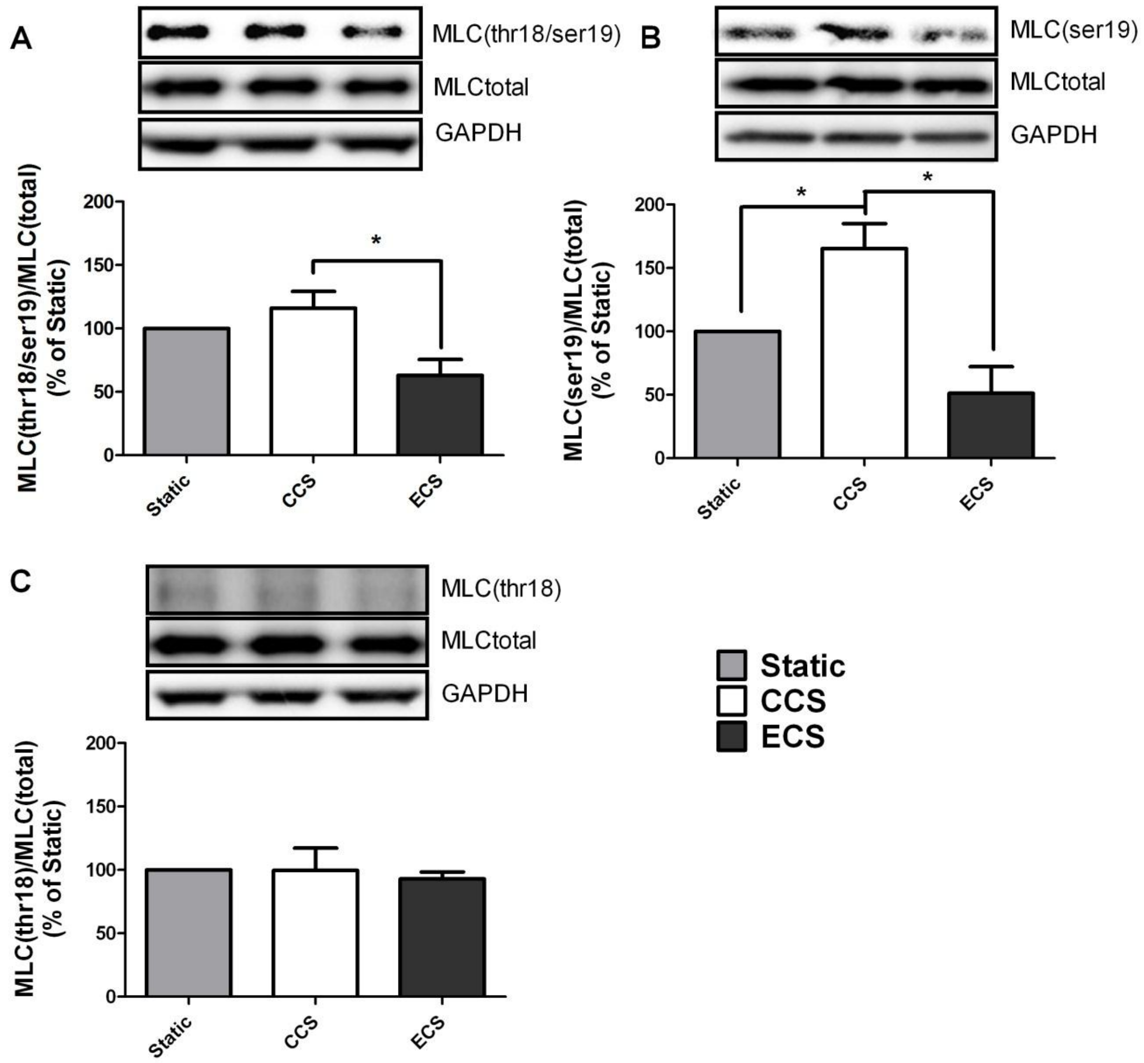
Sup. Fig. 1



Sup. Fig. 2



Sup. Fig. 3



Sup. Fig. 4