Supplementary material



Fig S1. Representative photographs of WT and PP1cβ KO animal intestines *in situ*, and **after dissection.** Irregular widths and apparent blood clots were frequently observed in KO animals.



Fig S2. Comparison of smooth muscle RLC content in bladder smooth muscles after indicated days of Tamoxifen treatment to induce knockout of PP1c β . A) A representative Coomassie-stained gel after SDS-PAGE of 2 µg of total bladder smooth muscle extract (upper), and a parallel gel immunoblotted for smooth muscle RLC (lower). Mobility of molecular weight proteins are shown as kDa. B) Scatter dot plot of RLC immunoblot signal relative to total Coomassie stained protein. Horizontal bar indicates mean \pm S.D.; dots represent individual animal samples, relative to average WT; N≥7 per group. Smooth muscle RLC content in PP1c β KO bladder extracts are not significantly different from WT.



Fig S3. Comparison of protein content in bladder smooth muscles after Triton X-100

treatment. A) Representative Coomassie-stained gel after SDS-PAGE of untreated control and Triton X-100 treated bladder smooth muscle strips. Proteins are indicated (MHC, myosin heavy chain), and mobility of molecular weight proteins are shown as kDa. B) Representative immunoblot of MYPT1 and PP1c β in parallel gels as A. C) Quantitation of MYPT1 and PP1c β as ratios of Coomassie-stained MHC and immunoblot of MYPT1, respectively. D) Ratio of RLC/MYPT1 in Triton X-100 treated bladder strips (N=6) from three distinct mouse bladders, relative to an untreated strip from the same tissue. E) Representative immunoblot of MYPT1 and RLC from 1 untreated Control and 6 distinct Triton X-100 treated bladder smooth muscle strips from 1 mouse bladder.



Fig S4. Representative blots of MYPT1 and PP1c β content in bladder smooth muscles after a-toxin vs Triton X-100 treatment. A) Representative Coomassie-stained gel after SDS-PAGE for treated bladder strips. Treatments and proteins are indicated (MHC, myosin heavy chain). Mobility of molecular weight proteins are shown as kDa. B) Representative images of Coomassie-stained myosin heavy chain (upper panels), immunoblot of MYPT1 (middle panels), and PP1c β and SM22 (lower panels) in three different bladder strips after indicated treatments. SM22, a smooth muscle-specific soluble protein is completely removed with Triton X-100 treatment, and serves as a positive control for treatment efficiency.