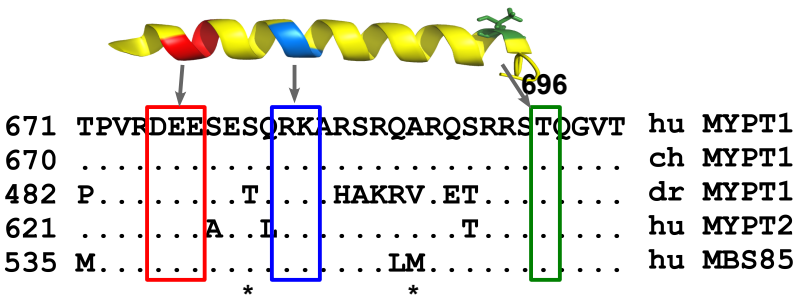
A

Helix B

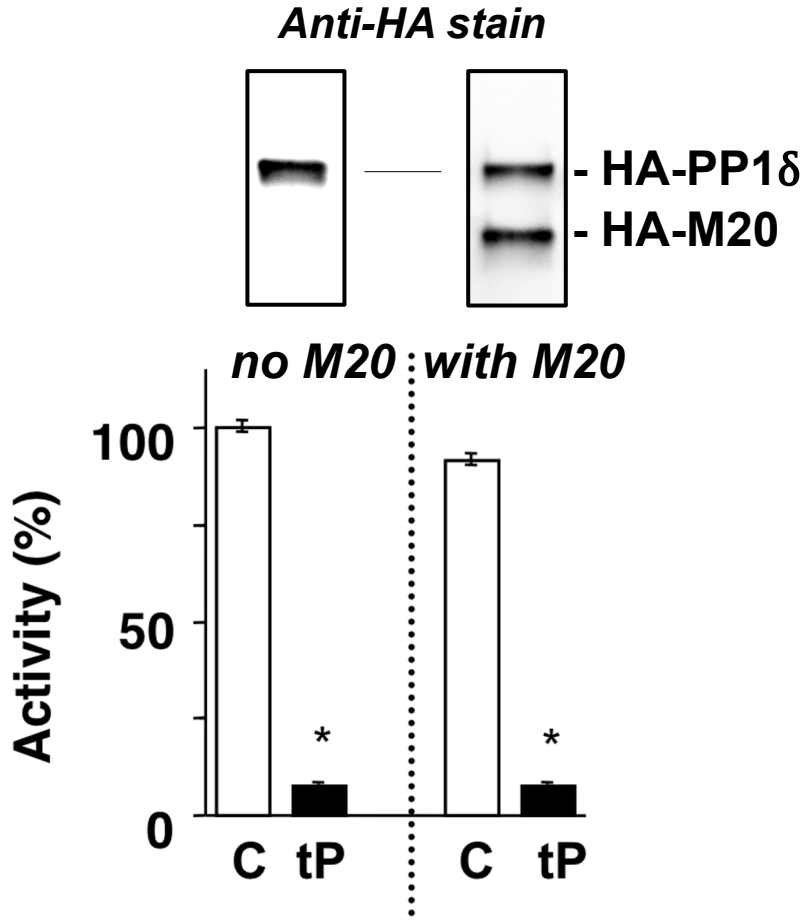
PVRDEESESQRKARSRQARQSRRSTpQGVTLTDL:(672-704)

KEGEDKSQPKSIRERRRPREKRRSTpGVSFWTQD:(829-861)

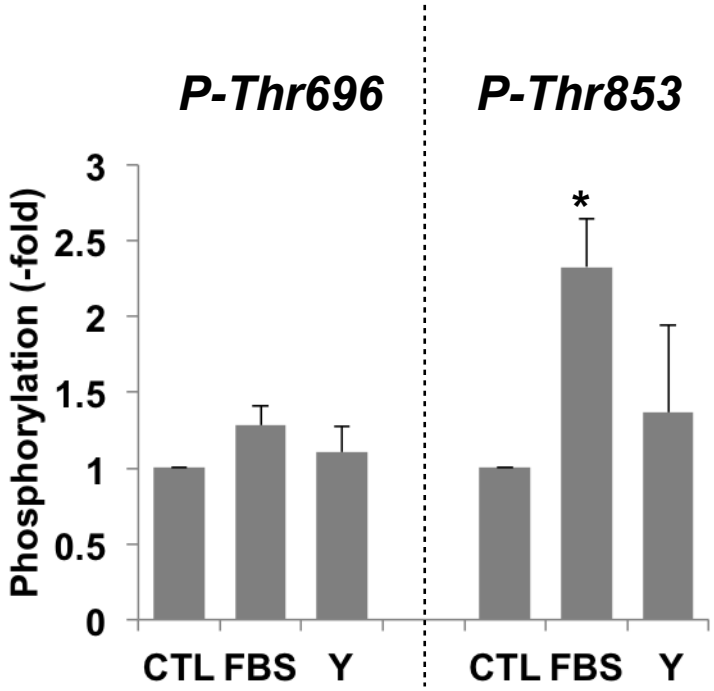
B



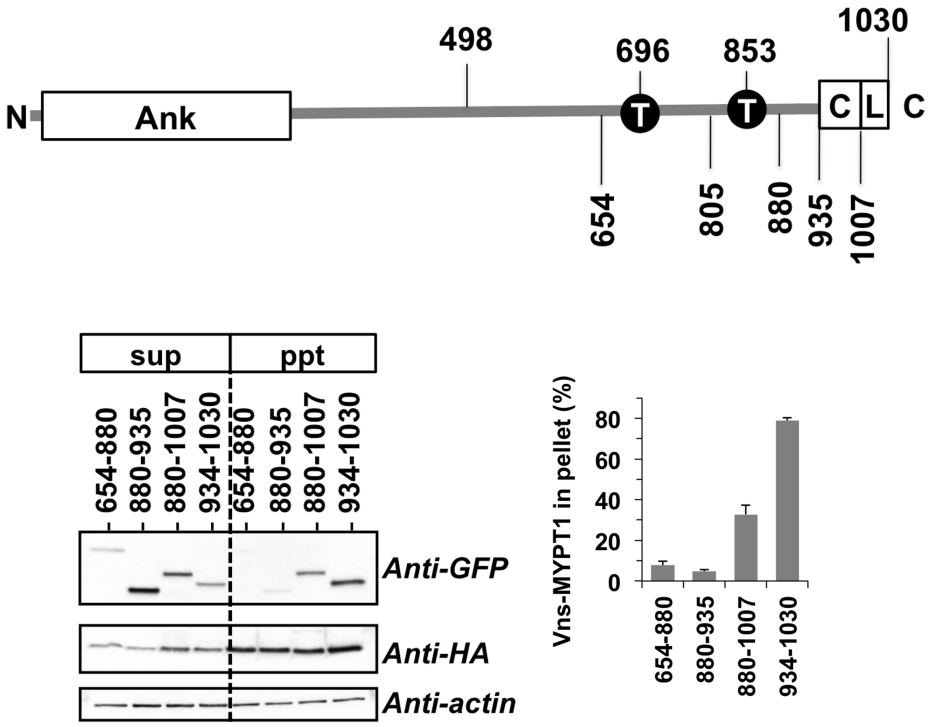
Supplemental figure S1: Sequence alignment and 3D structure of human MYPT1 phosphorylation sites. (A) Thr696 vs Thr853. Box indicates the residues consisting of alpha-helix. Red indicates Pro residues. (B) Similarlity around MYPT1 Thr696. The 3D structure of the autoinhibitory domain was shown. The conserved DEE, RK regions and Thr696 were indicated in red, blue and green, respectively.



Supplemental figure S2: Effects of M20 subunit of the MLCP complex. Human M20 cDNA fragment was synthesized (GenScript) and inserted into pHA3 vector (gifted by Dr. Ian Macara, University of Virginia). COS1 cells were transiently transfected overnight with vectors for fvm-MYPT1 (1μg) and HA-PP1δ (1μg) in the absence (left) or presence (right) of HA-M20 vector (1μg). The MLCP complex was extracted and purified as described in Experimental Procedures section. Aliquots (1.5μg for the dimer or 0.7μg for the trimer) were subjected to immunoblotting using anti-HA antibody. The MLCP assay was performed with control (C) and the MLCP complex thiophosphorylated using ROCK (tP). Mean value ± SEM was obtained from triplicate assay. * indicates $p<0.05$ vs. control (C). The specific activity of the untreated dimeric MLCP was set as 100%.



Supplemental figure S3: Phosphorylation of MYPT1 in leiomyosarcoma cells. Assay was performed as described in Fig. 5A. The density ratio of phospho-MYPT1 / actin was obtained in each sample. The value of control (CTL) was set as 1. Mean value ± SEM was obtained from three independent experiments. * indicates $p<0.05$ (n=3, student's t-test against CTL).



Supplemental figure S4: (Top) Schematic illustration of human MYPT1 structure. (Bottom) Solubility of Vns-MYPT1 fragments. COS1 cells expressing Vns-MYPT1 fragments indicated were lysed with the buffer (10mM HEPES (pH7.4) including 0.2M NaCl, 5mM $Mg(OAc)_2$, 2mM EGTA and 0.1% Triton X-100), and subjected to subcellular fractionation using airfuge (30min at 80,000rpm). Supernatant and pellet resuspended with the equilent volume of the buffer were subjected immunoblotting using antibodies indicated. Staining intensity were measured by densitometry. % precipitation was defined as density(pellet) / {density(pellet) + density(sup)} x100. Mean values ± SEM was obtained from three independent assays.