SUPPLEMENTARY DATA

Myosin Regulatory Light Chain Diphosphorylation Slows Relaxation of Arterial Smooth Muscle

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FIGURE LEGENDS

Supplementary FIGURE S1. Effect of exogenous calmodulin and MLCK on LC_{20} phosphorylation in Triton-skinned rat caudal arterial smooth muscle. Triton-skinned rat caudal arterial smooth muscle strips were incubated for 15 min in pCa 9 solution in the absence or presence of exogenous calmodulin (CaM) (1.8 μM) and/or MLCK (10 or 50 nM). $[Ca^{2+}]$ was then increased to pCa 4.5 under otherwise identical conditions and, after steady-state force was achieved (10 and 20 min), tissues were harvested for Phos-tag SDS-PAGE and western blotting with anti-pan LC_{20}. A control experiment in which tissue was treated with microcystin at pCa 9 was included to indicate the positions of unphosphorylated (0P), mono- (1P) and diphosphorylated LC_{20} (2P) bands. Phosphorylation levels were quantified and the results are shown in Supplementary Table 1.

Supplementary FIGURE S2. Identification of thiophosphorylated forms of LC_{20} using phosphospecific antibodies. Triton-skinned rat caudal arterial smooth muscle strips were treated as shown in Fig. 8*A* and harvested at selected times for Phos-tag SDS-PAGE and western blotting with anti-pan LC_{20}, anti-pS19-LC_{20}, anti-pT18-LC_{20} and anti-pT18,pS19-LC_{20}. A control experiment (lane 1) in which tissue was treated with microcystin at pCa 9 was included to indicate the positions of unphosphorylated (0P), mono- (1P) and diphosphorylated LC_{20} (2P) bands. Tissue incubated at pCa 9 (lane 2) is also included as a control. Numbers 3 - 6 indicate tissues harvested at the times indicated in Fig. 8*A*.

Supplementary FIGURE S3. ILK does not use ATPγS as a substrate. Triton-skinned rat caudal arterial smooth muscle strips were treated as shown in ***A***. Tissues were harvested at the indicated times for Phos-tag SDS-PAGE and western blotting with anti-pan LC_{20} (***B***). Numbers 1 (pCa 9) and 2 (pCa 4.5 + ATPγS) correspond to the times indicated in Fig. 8*A*, and numbers 3 and 4 to the times indicated in panel ***A*** of this figure (i.e. tissue treated with ATPγS + microcystin at pCa 9 and following washout at pCa 9, respectively).

Supplementary FIGURE S4. Contraction of Triton-skinned rat caudal arterial smooth muscle induced by Ca^{2+} or okadaic acid in the absence of Ca^{2+}. Following control Ca^{2+}-induced contraction-relaxation cycles, Triton-skinned rat caudal arterial smooth muscle strips were treated with okadaic acid (OA; 20 μM) at pCa 9 (***A***) or with Ca^{2+} (pCa 4.5) (***B***). Relaxation followed washout of OA or removal of Ca^{2+}, respectively.

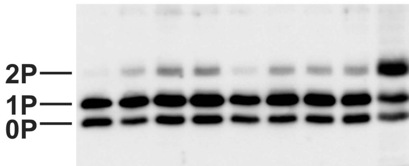
Supplementary FIGURE S5. Dephosphorylation of MYPT1 following washout of okadaic acid. Tissues were treated as in Fig. S4*A* and harvested at the indicated times as follows: under relaxed conditions at pCa 9 (pCa 9); at the peak of pCa 4.5-induced contraction (pCa 4.5); at the plateau of okadaic acid-induced contraction at pCa 9 (100% Force) and during the relaxation following washout of okadaic acid corresponding to force levels of 90, 80, 70, 60, 50 and 25% and at complete relaxation (0% Force). Tissue samples were subjected to SDS-PAGE and western blotting with anti-pT697-MYPT1 or anti-pT855-MYPT1. Blots were also probed with anti-actin (shown beneath the corresponding phosphospecific antibody blots). Identical results were obtained in 5 independent experiments.

Supplementary Table 1. Effect of exogenous calmodulin and MLCK on LC_{20} phosphorylation in Triton-skinned rat caudal arterial smooth muscle[1]

Conditions	% total LC_{20}		
	0P	1P	2P
pCa 4.5, 10 min	34.7	65.3	0
pCa 4.5, 20 min	22.6	76.0	1.4
pCa 4.5, CaM, 10 min	16.7	80.1	3.2
pCa 4.5, CaM, 20 min	14.7	81.3	4.0
pCa 4.5, CaM, MLCK (10 nM), 10 min	22.3	73.5	4.2
pCa 4.5, CaM, MLCK (10 nM), 20 min	17.8	77.7	4.5
pCa 4.5, CaM, MLCK (50 nM), 10 min	20.7	75.1	4.2
pCa 4.5, CaM, MLCK (50 nM), 20 min	24.0	70.3	5.7
MC, pCa 9	16.8	26.4	56.8

[1]These data were calculated from the western blot in Supplementary Fig. S1.

Figure S1



pCa:	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	9
CaM:	-	+	+	+	-	+	+	+	-
MLCK (nM):	0	0	10	50	0	0	10	50	0
MC:	-	-	-	-	-	-	-	-	+
time (min):	10	10	10	10	20	20	20	20	60

Figure S2

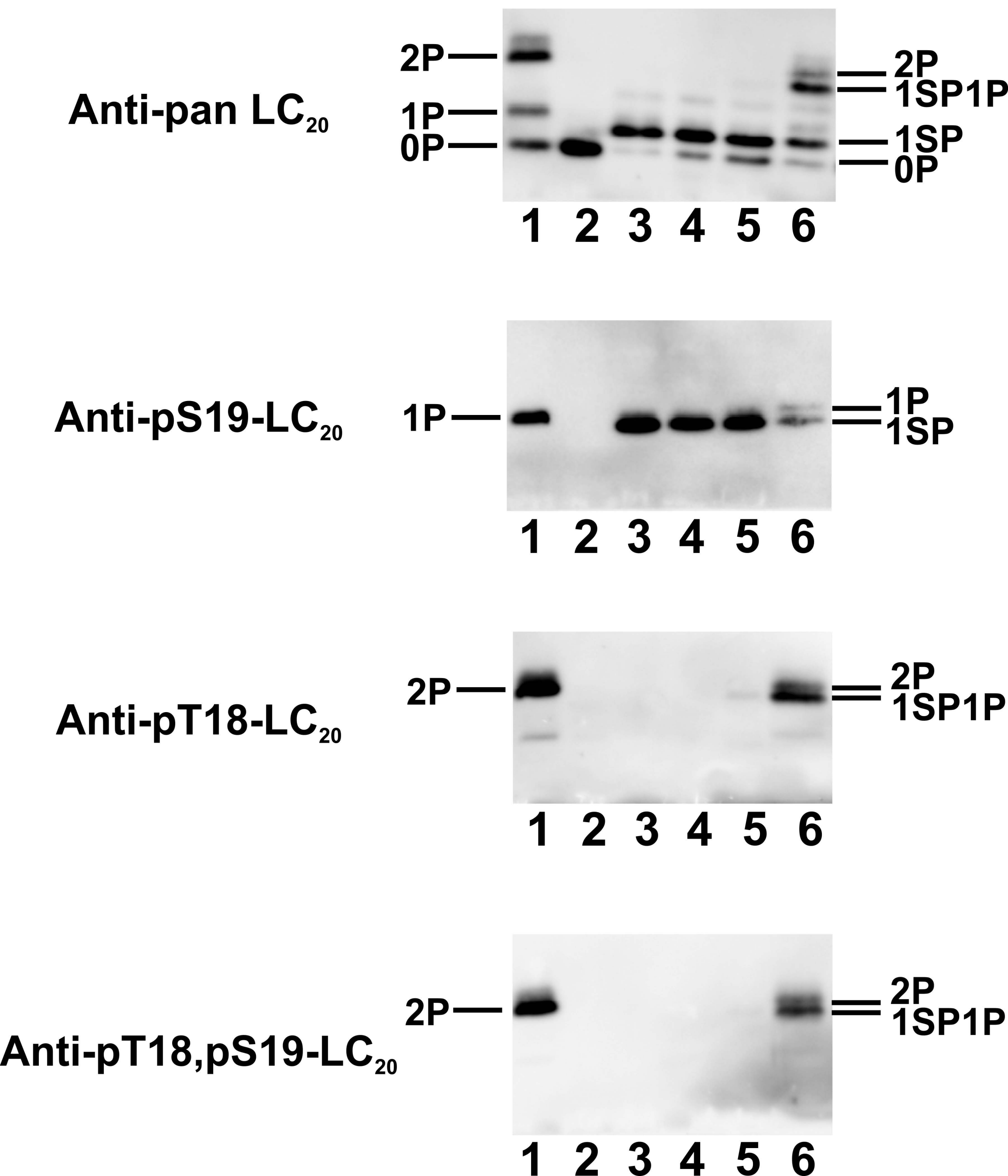
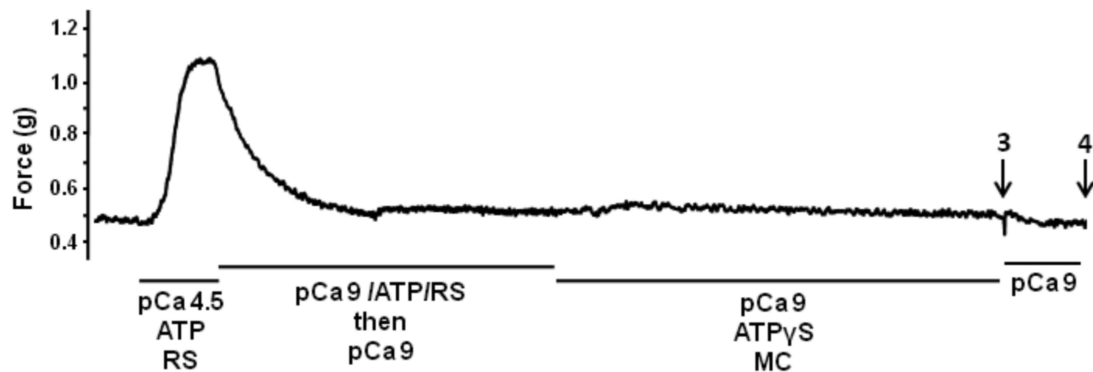


Figure S3

A



B

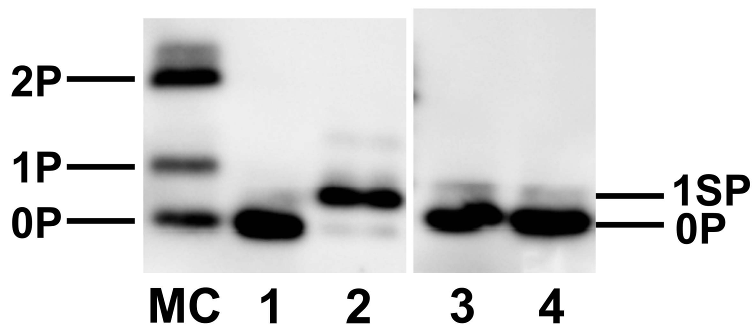
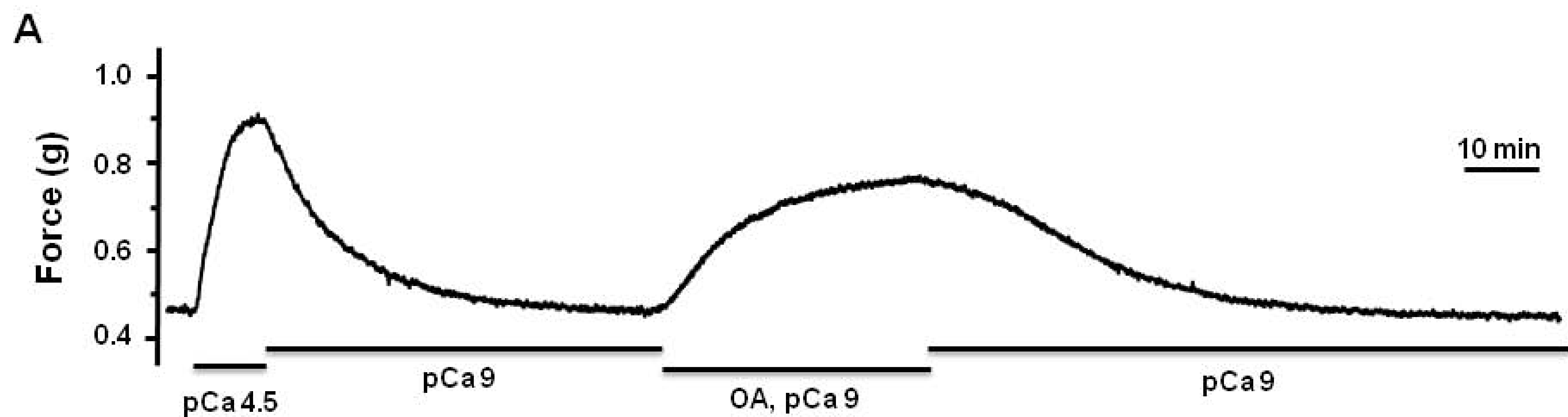


Figure S4



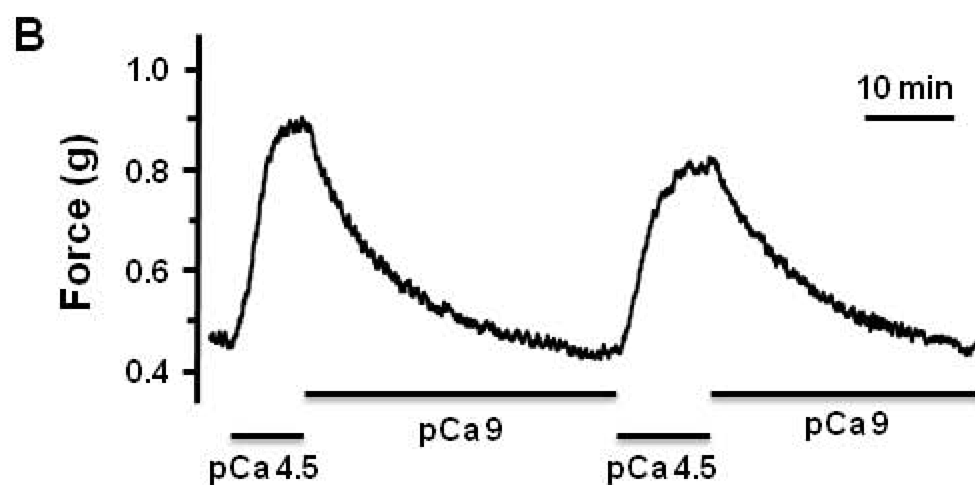


Figure S5

pT697

actin

pT855

actin

pCa 9 | pCa 4.5 | 100 | 90 | 80 | 70 | 60 | 50 | 25 | 0

% Force