Myosin regulatory light chain, gi 15809016

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MSSKKAKTKTTKKRPQRATSNVFAMFDQSQIQEFKEAFNM 40
IDQNRDGFIDKEDLHDMLASLGKNPTDAYLDAMMNEAPGP 80
INFTMFLTMFGEKLNGTDPEDVIRNAFACFDEEATGTIQE 120
DYLRELLTTMGDRFTDEEVDELYREAPIDKKGNFNYIEFT 160
RILKHGAKDKDD 172
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(29% of the protein were covered by the identified peptides)

Myosin essential light chain, gi 17986258

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MCDFTEDQTAEFKEAFQLFDRTGDGKILYSQCGDVMRALG 40
QNPTNAEVLKVLGNPKSDEMNVKVLDFEHFLPMLQTVAKN 80
KDQGTYEDYVEGLRVFDKEGNGTVMGAEIRHVLVTLGKKM 120
TEEEVEMLVAGHEDSNGCINYEAFVRHILSG 151
```

(41% of the protein were covered by the identified peptides)

Fig. S1. Amino acid sequences of myosin subunits coimmunoprecipitated with the p75 cytosolic tail. The amino acid sequences are of human MRLC and MELC. The boxed regions denote the peptide sequences identified by mass spectrometric analysis.

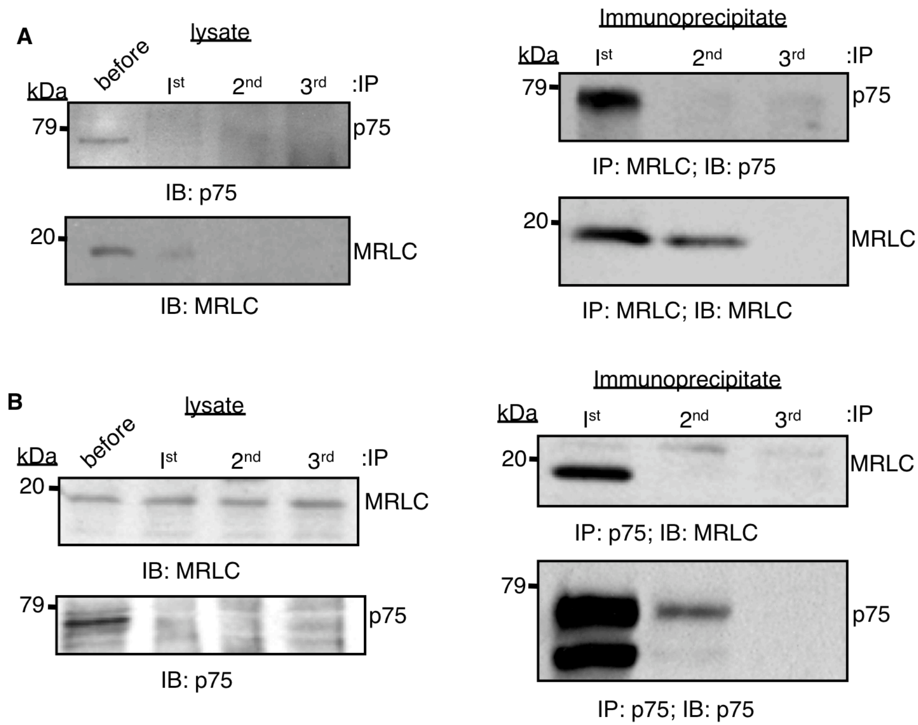

Fig. S2. Residual myosin or p75 in cell lysates after sequential immunoprecipitations. (**A**) Cell lysates analyzed by Western blotting with anti-p75 antibody after sequential immunoprecipitation of MRLC (left panel). (**B**) Cell lysates analyzed by Western blotting with anti-MRLC antibody after sequential immunoprecipitation of p75 (left panel). Right panels of (A) and (B) show coimmunoprecipitated p75 and MRLC, respectively. Blots are representative of two independent experiments.

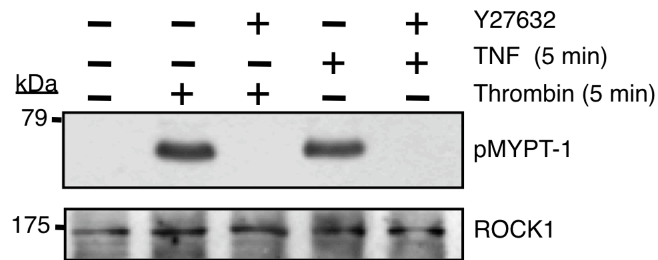

Fig. S3. Specificity of the antibody used to immunoprecipitate ROCK1. Endothelial cells were stimulated with TNF (2 ng/ml) or thrombin (5 U/ml). ROCK1 was immunoprecipitated with an isoform-specific antibody, and immunoprecipitates were resuspended in 60 ml of the assay buffer. 10 ml of the resuspended immunoprecipitates were used to perform an activity assay in the presence or absence of 20 μM Y27632. Pure MYPT-1 (~80 kD) was used as the substrate. Phosphorylation of MYPT-1 was determined by Western blotting analysis. Data are representative of three independent experiments.

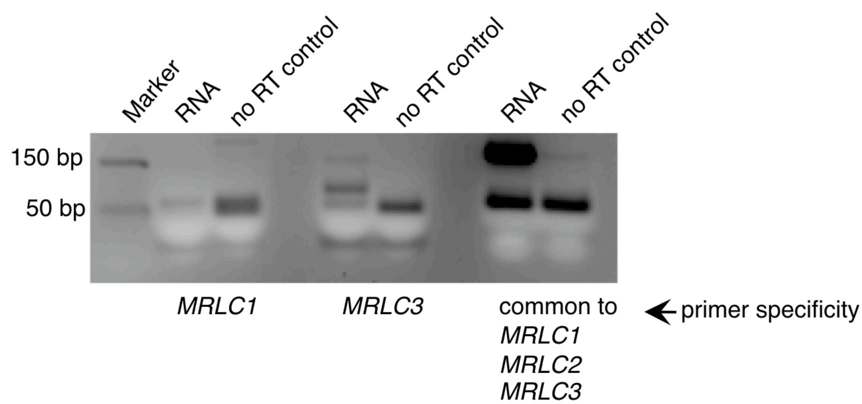

Fig. S4. MRLC isoforms in endothelial cells. *MRLC2* and *MRLC3*, but not *MRLC1*, are expressed in endothelial cells. Expression of the indicated *MRLC* isoforms in endothelial cells was determined by reverse-transcribing mRNA followed by PCR analysis with *MRLC1*-specific or *MRLC3*-specific primers or with a primer pair capable of amplifying all three isoforms. RT: Reverse Transcriptase. Data are representative of three independent experiments. *MRLC1*-specific primers: Forward: 5'-CACAAATGCAAGCTCACCAAG-3'; Reverse: 5' -AGACCCTGTCTACAGGTGC-3' (amplicon length = 143 bp). *MRLC3*-specific primers: Forward: 5'-CAGTAACAGGACCCAGAGGAC-3'; Reverse: 5'-CGTCTCACCCAGACTGAAGTG-3' (amplicon length = 153 bp). Primer set common to all three isoforms: Forward: 5'-CCTCACCATGTTTGGGGAGAAGCTGAACG-3'; Reverse: 5'-CATGTCGAGTAGGTGAAGGAGTAGAC-3' (amplicon length = 171 bp).

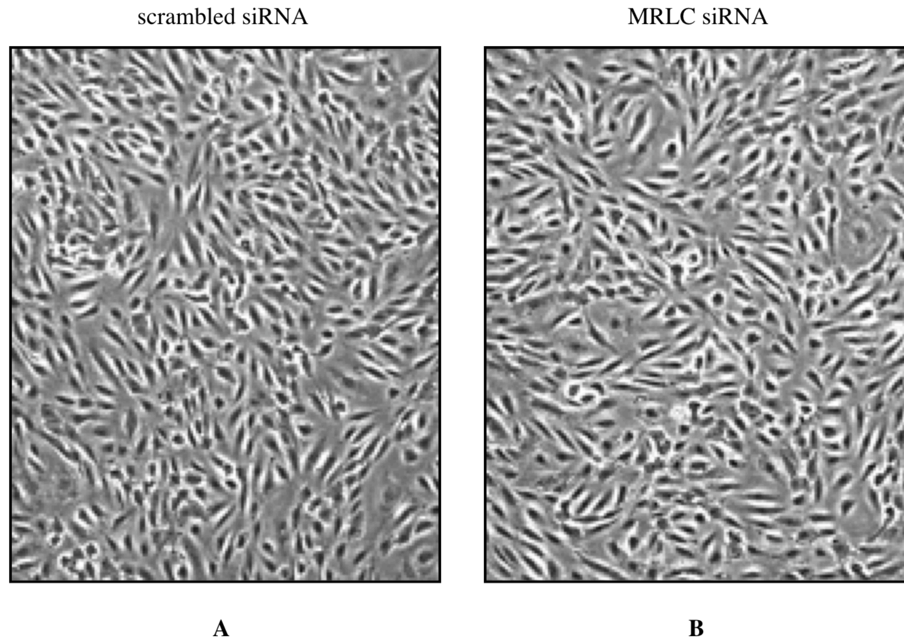

Fig. S5. L Light microscopic imaging of endothelial cells deficient in MRLCs. (**A** and **B**) MRLC-depleted endothelial cells appear normal. Light microscopy image (40×) of endothelial cells transfected with (A) scrambled siRNA or with (B) a combination of *MRLC2*- and *MRLC3*-specific siRNAs. Images were taken 36 hours after transfection. Images are representative images of three independent experiments.

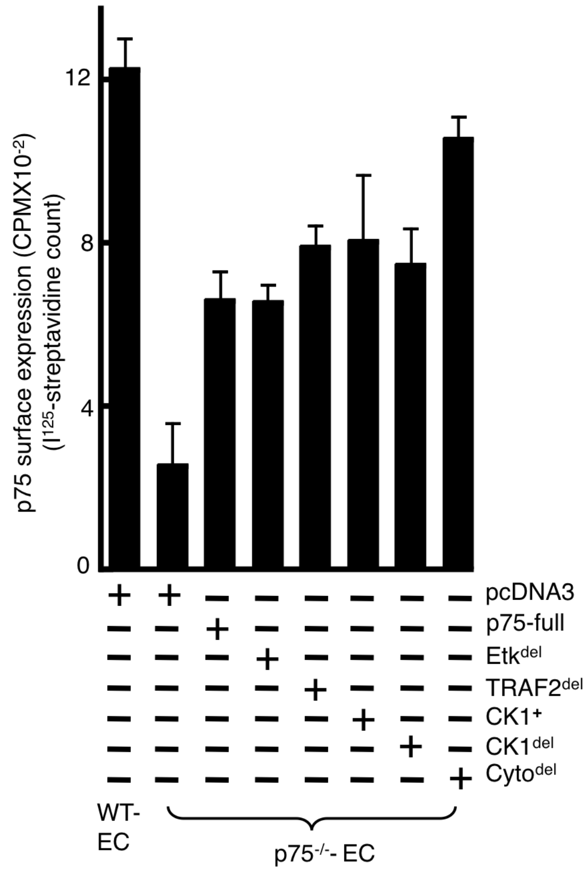

Fig. S6. Abundance of reconstituted p75 and its deletion mutants. Cell-surface abundances of full-length p75 protein and of its deletion mutants were measured with a biotin-conjugated antibody targeting the extracellular domain of p75 and followed by ^{125}I-streptavidin. Experiments were performed in triplicate with independent endothelial cell isolates, and data are means ± SD.

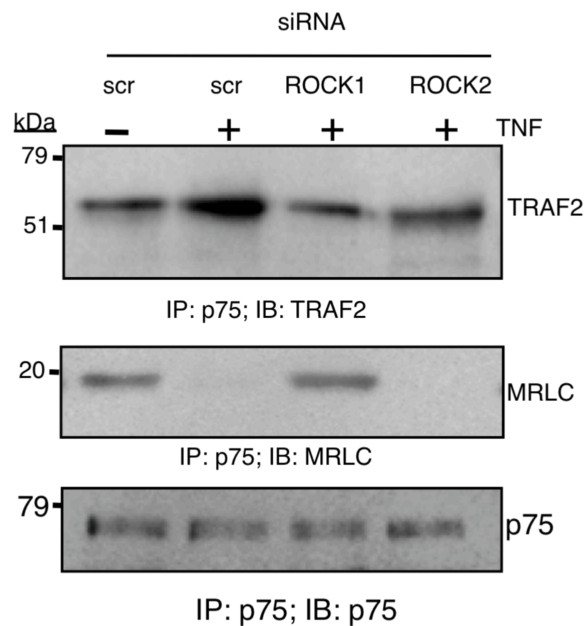

Fig. S7. The binding of TRAF2 to endogenous p75. Cells transfected with the indicated siRNAs were left untreated or were treated with TNF (2 ng/ml) for 5 min before being subjected to immunoprecipitation with antibody against endogenous p75. The immunoprecipitates were subjected Western blotting analysis for coimmunoprecipitated TRAF2 or MRLC with specific antibodies. Data are representative of two independent experiments.

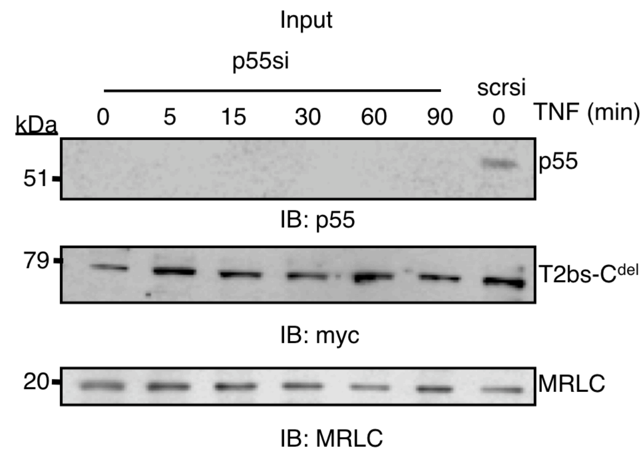

Fig. S8. Western blotting analysis of the input sample used in measuring the kinetics of myosin and TRAF2 binding. Western blotting analysis of the input sample that was used to immunoprecipitate T2bs-C^{del} in Fig. 7F. 5% of the total lysate was analyzed with specific antibodies for p55, T2bs-C^{del}, and MRLC. Data are representative of three independent experiments.

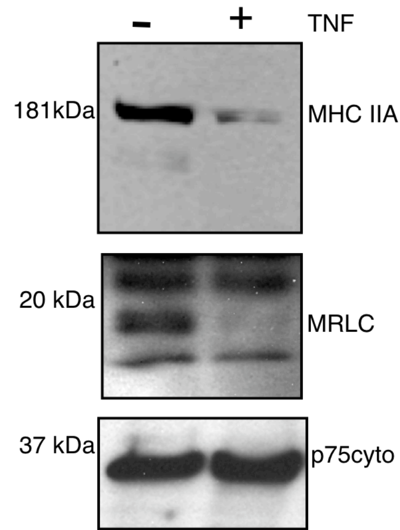

Fig. S9. Coimmunoprecipitation of MHC IIA with p75. Cells were left untreated or were treated with TNF (2 ng/ml) for 5 min before being subjected to immunoprecipitation of the p75 cytosolic tail (p75cyto). Samples were analyzed by Western blotting coimmunoprecipitated MHC IIA or MRLC with specific antibodies. Data are representative of three independent experiments.

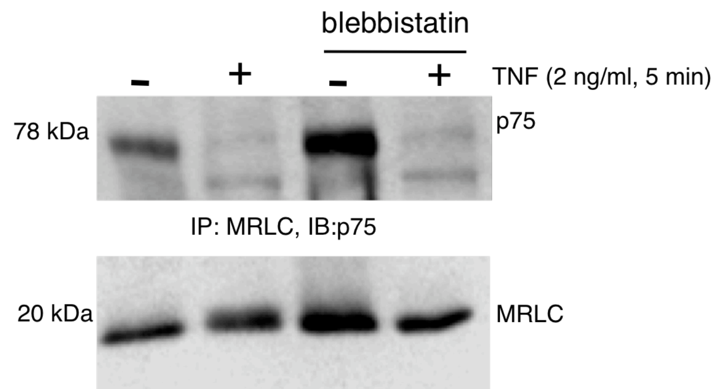

Fig. S10. Blebbistatin does not block the interaction between p75 and myosin. Endothelial cells that were left untreated or were treated with 30 mM blebbistatin were subjected to immunoprecipitation with an antibody against myosin and the samples were analyzed by Western blotting for coimmunoprecipitated p75. Data are representative of two independent experiments.