Supplementary Information

Materials and Methods

Protein expression and purification

Purification of rabbit skeletal and bovine cardiac actin:

Rabbit skeletal muscle and bovine c-actin were prepared from dried acetone powder obtained from fresh rabbit psoas muscle or bovine hearts (obtained from the local slaughterhouse) as described [6]. G-actin was stored in G-buffer (5 mM HEPES-OH, pH 7.4, 0.1 mM CaCl2, 0.5 mM NaN3, and 0.2 mM ATP, pH 7.4) at 4°C or flash frozen in liquid nitrogen after addition of 5% succhrose at stored at -80°C.

Preparation of subfragment 1 from skeletal muscle and bovine cardiac myosin:

The myosin containing fractions from the acetone powder preparation were mixed with glycerol (1:1) and stored at -20°C until use. For preparation of myosin subfragment 1 (myosin-S1) the respective stored myosin was dialysed against low salt buffer to induce myosin precipitation. After centrifugation at 12,000 rpm for one hour the myosin containing precipitate was digested with chymotrypsin [10] for 8 to 10 min. After protease inactivation by addition of 1 mM PMSF the digest was dialysed against 50 mM imidazole, pH 7.0, over night at 4°C. After centrifugation at 12,000 rpm for one hour the supernatant was loaded on a DEAE-column equilibrated 50 mM imidazole-buffer, pH 7.0, and eluted with a salt gradient from 0-150 mM NaCl following the described procedure. The fractions containing pure myosin-S1 identified by SDS-Page were pooled and concentrated by Viva-Spin (Millipore, Witten, Germany). The myosin-S1 concentration was determined photometrically (ε = 11.3 cm-1 mg/ml-1).

Expression and purification of recombinant gelsolin deletion mutant G4-6:

Escherichia coli strain Rosetta (DE3) pLysS (Novagen) was transformed by pCOLDII-G4-6 plasmid and grown in Luria-Bertani medium containing 75 µg/ml ampicillin and 25 µg/ml chloramphenicol to the optical density of 0.6 measured at 600 nm. Protein expression was induced by incubation on ice for 30 min and shaking for 23 h at 20 °C in the presence of 1 mM isopropylthio-D-galactoside (IPTG). Harvested cells from 1 l culture were lysed by sonication (10 x 10 sec) in a binding buffer (10 mM Tris-HCl, pH 8.0, 50 mM KCl, 5 mM $CaCl_2$, 7 mM ß-mercaptoethanol, 1 mM PMSF, 0.01 mg/ml leupeptin, 1 mM ATP) and gently shaken for 45 min at 4°C. After centrifugation at 12,000 x g for 30 min, the sterile filtered supernatant was applied to a Ni^{2+}-affinity column by use of FPLC (AKTA Purifier, GE Amersham). After washing with 6 column volumes low salt buffer (10 mM Tris-HCl, pH 8.0, 50 mM KCl, 5 mM $CaCl_2$) and high salt buffer (10 mM Tris-HCl, pH 8.0, 350 mM KCl, 5 mM $CaCl_2$) gelsolin G4-6 was eluted by an imidazole-gradient (0-1 M, pH 8.0) and the combined G4-6 fractions were dialyzed against 50 mM Tris-HCl (pH 8.0), 50 mM KCl, 1 mM $CaCl_2$ and 1 mM DTT. The protein was concentrated with VIVASPIN 20 concentrator (Sartorius Stedim Biotech, Aubagne, France), supplemented with 3 % sucrose, flash frozen in liquid N2 and stored at -80°C.

Expression and Purification of Recombinant Actin:

The human cardiac muscle wild type α-actin and the mutants were expressed in the *baculovirus*/*Sf21*-system [2]. The preparation of recombinant *baculoviruses* was performed according to the manufacturer´s recommendation (Invitrogen) by transforming the pFastBac Dual vectors into DH10Bac *E.coli* cells, bacmid isolation and transfection into *Sf21* insect cells using Cellfectin II (Invitrogen). Infected *Sf21*-cells were harvested after 3 days and stored at -80 °C.

Purification of tag-free wild-type cardiac α-actin and both mutants was performed by affinity chromatography using the gelsolin deletion mutant G4-6 as described [7, 8], see Figure S1. The cells from 2-5 l culture were lysed by sonication and pipetting in 150 ml of KCl-free lysis-buffer (10 mM Tris-HCl, pH 8.0, 5 mM $CaCl_2$, 20 mM imidazole, 4 % Triton-X, 1 mg/ml Tween-20, 5 mM DTT, and a protease inhibitor cocktail). After addition of purified gelsolin G4-6 (10 mg/ 4 x 107 cells) the

solution was gently stirred at 4°C overnight. After centrifugation at 230,000g for 30 min (OptimaTM LE-80K Ultracentrifuge, BECKMAN COULTER) the supernatant was sterile filtered and applied to a Ni2+-affinity column by use of a FPLC (AKTA Purifier, GE Amersham). The resin was washed with Tris-HCl buffer (10 mM, pH 8.0) including 1 mM $CaCl_2$. Actin was eluted with EDTA-Buffer (10 mM Tris-HCl, pH 8.0, 1.5 mM EDTA) and dialysed against Tris-HCl-buffer (10 mM, pH 8.0) including 0.2 mM $MgCl_2$ and 1 mM DTT. G4-6 was recovered by elution with 1 M imidazole and dialysed against Tris-HCl-Buffer (10 mM, pH 8.0) including 50 mM KCl, 1 mM $CaCl_2$ and 1 mM DTT. The proteins were concentrated with VIVASPIN 20 concentrators supplemented with 3% sucrose, flash frozen in liquid N2 and stored at -80 °C. The different purification steps and the purity of the finally obtained c-actin samples was checked by 12% SDS-Page (as shown in Figure 2 of the main text for wt c-α-ctin and the p.E361G mutant). Western blot analysis of the α-cardiac actins was performed with a monoclonal anti-cardiac actin antibody provided by Progen Biotechnik (Heidelberg, Germany). The nomenclature and numeration of human cardiac α-actin wild-type (wt), and of the mutants p.A295S, p.R312K/H and p.E361G were in accordance to that of the Universal Protein Resource database.

Purification of mammalian cardiac tropomyosin:

Cardiac α-tropomyosin was chromatographically purified from acetone dried powder prepared from pig heart according to [3] and then labelled with pyrene-maleimide under denaturing conditions according to [5].

Expression and purification of human cardiac troponin subunits and troponin reconstitution:

For protein expression, the plasmids encoding wildtype human cTnC and the N-terminal fragment C0C2 of cMyBP-C were transformed into *E.coli* T7 Express lysY cells while the plasmids encoding wild type hcTnI and hcTnT were transformed into *E. coli* BL21 (DE3). The isolation of hcTnC was performed following the method described [1]. Human cTnI was purified according to [9] and hcTnT according to [4]. For the reconstitution of wild type hcTn troponin complex, the individual

References

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