SUPPORTING INFORMATION

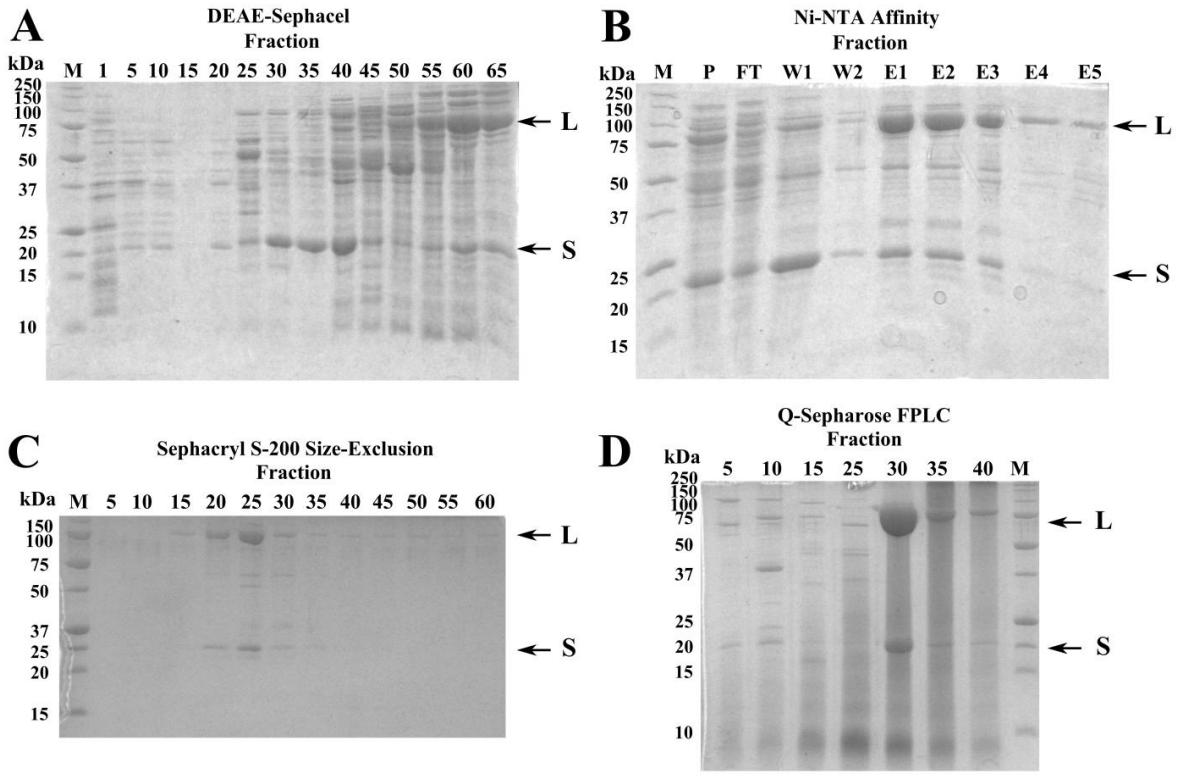

Figure S1: SDS-PAGE analysis of the purification of calpain-2-IS1. A) SDS-PAGE of eluted proteins from DEAE-Sephacel anion-exchange chromatography in 25 mM Tris-HCl (pH 7.5), 5 mM EDTA and 10 mM β-mercaptoethanol buffer. Protein was eluted using a linear salt gradient from 0-0.75 M NaCl. B) SDS-PAGE of pooled DEAE-Sephacel fractions (P), flow-through (FT), wash (W1/2) and elution fractions (E1-5) from Ni-NTA affinity chromatography. His-tagged protein was eluted with 250 mM imidazole. C) SDS-PAGE of fractions collected from Sephacryl S-200 size-exclusion chromatography. Proteins were eluted with buffer containing 25 mM Tris-HCl (pH 7.5), 5 mM EDTA, 2 % v/v glycerol and 10 mM β-mercaptoethanol buffer. D) SDS-PAGE of purified recombinant calpain-2-IS1 eluted from Q-Sepharose anion-exchange chromatography. Calpain-2-IS1 was eluted using a salt gradient of 0-1 M NaCl. Calpain-2-IS1 catalytic (L) subunit and 28 kDa small regulatory subunit (S) bands are indicated by black arrows.

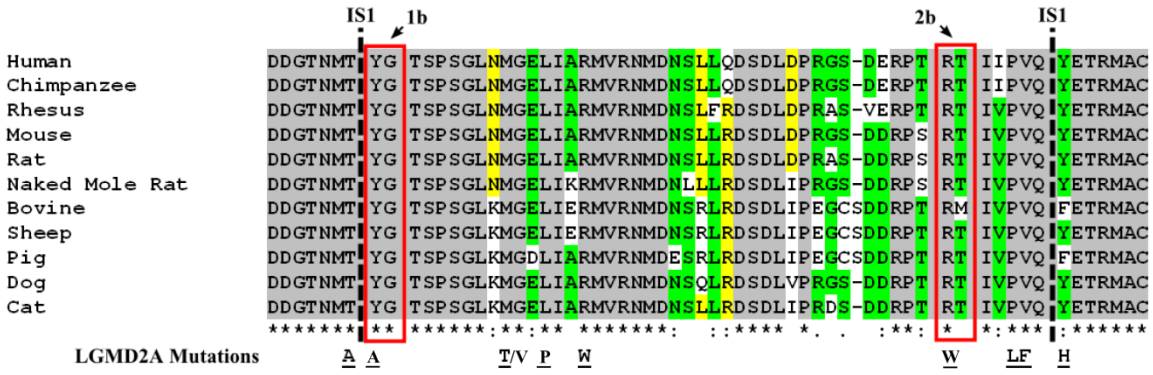

Figure S2: Sequence alignment of mammalian calpain-3 IS1 and LGMD2A causing mutations. The scissile bonds for the intramolecular autolytic cleavage (1b) and intermolecular autolytic cleavage (2b) of IS1 are highlighted by red rectangles. The degree of sequence conservation with human IS1 is shown, where grey indicates fully conserved residues, green indicates highly conserved residues and more weakly conserved residues are shown in yellow and white. LGMD2A mutations in humans are listed below the alignment.