The cardiac syndecan-4 interactome: A role for syndecan-4 in nuclear translocation of muscle LIM protein (MLP)

Sabrina Bech Mathiesen [1], Marianne Lunde[1], Jan Magnus Aronsen[1,2#], Andreas Romaine[1,3], Anita Kaupang[1], Marita Martinsen[1], Gustavo Antonio de Souza[4†], Tuula A. Nyman[4] , Ivar Sjaastad[1,3], Geir Christensen[1,3] and Cathrine Rein Carlson[1]

From the [1]Institute for Experimental Medical Research, Oslo University Hospital and University of Oslo, Oslo, Norway; [2] Bjørknes College, Oslo, Norway; [3]KG Jebsen Center for Cardiac Research, University of Oslo, Norway; [4]Department of Immunology, Institute of Clinical Medicine, University of Oslo and Rikshospitalet Oslo

Running title: *The cardiac syndecan-4 interactome reveals MLP connection*

SUPPORTING INFORMATION

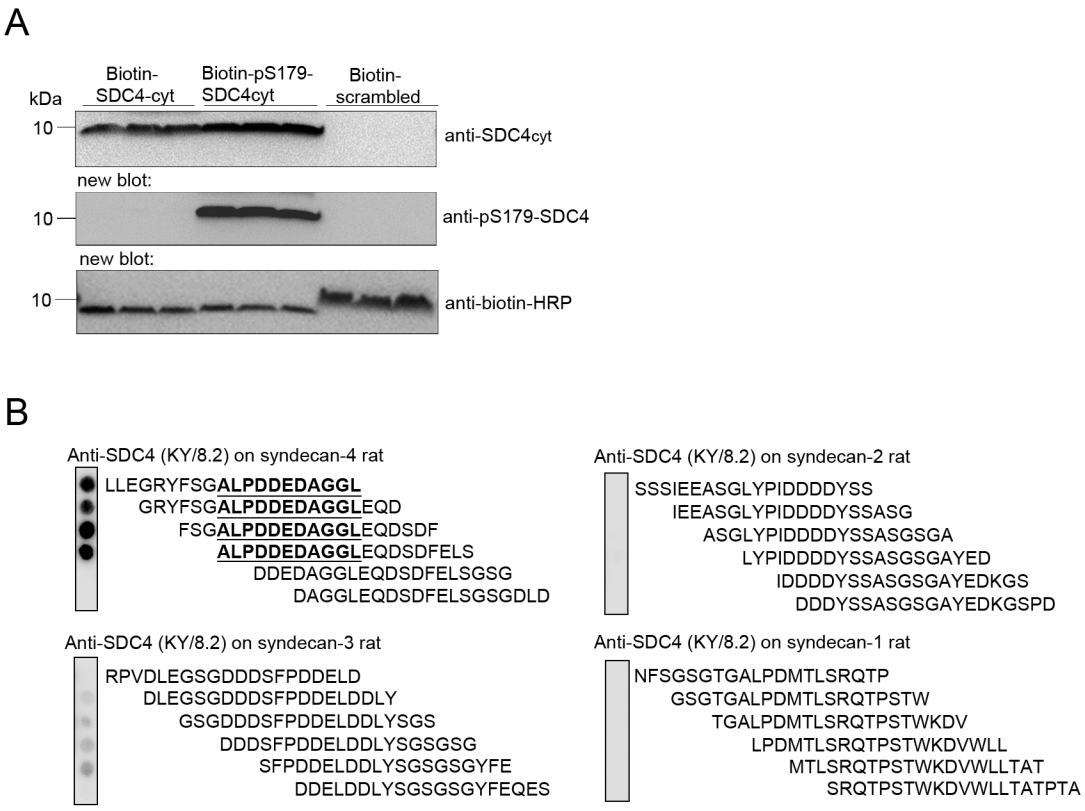

Figure S1. Validation of reagents used in the two AP-MS approaches. (A) The three biotinylated peptides used in the first AP-MS approach were validated by immunoblotting using anti-syndecan-4 (upper panel), anti-pS179-syndecan-4 (middle panel) and anti-biotin-HRP (lower panel). (B) Anti-syndecan-4 (KY/8.2) was overlayed arrays of immobilized overlapping 20-mer peptides of the syndecan-1-4 rat protein sequences. The core epitope in syndecan-4 is underlined. Syndecan-1-3 was hardly recognized.

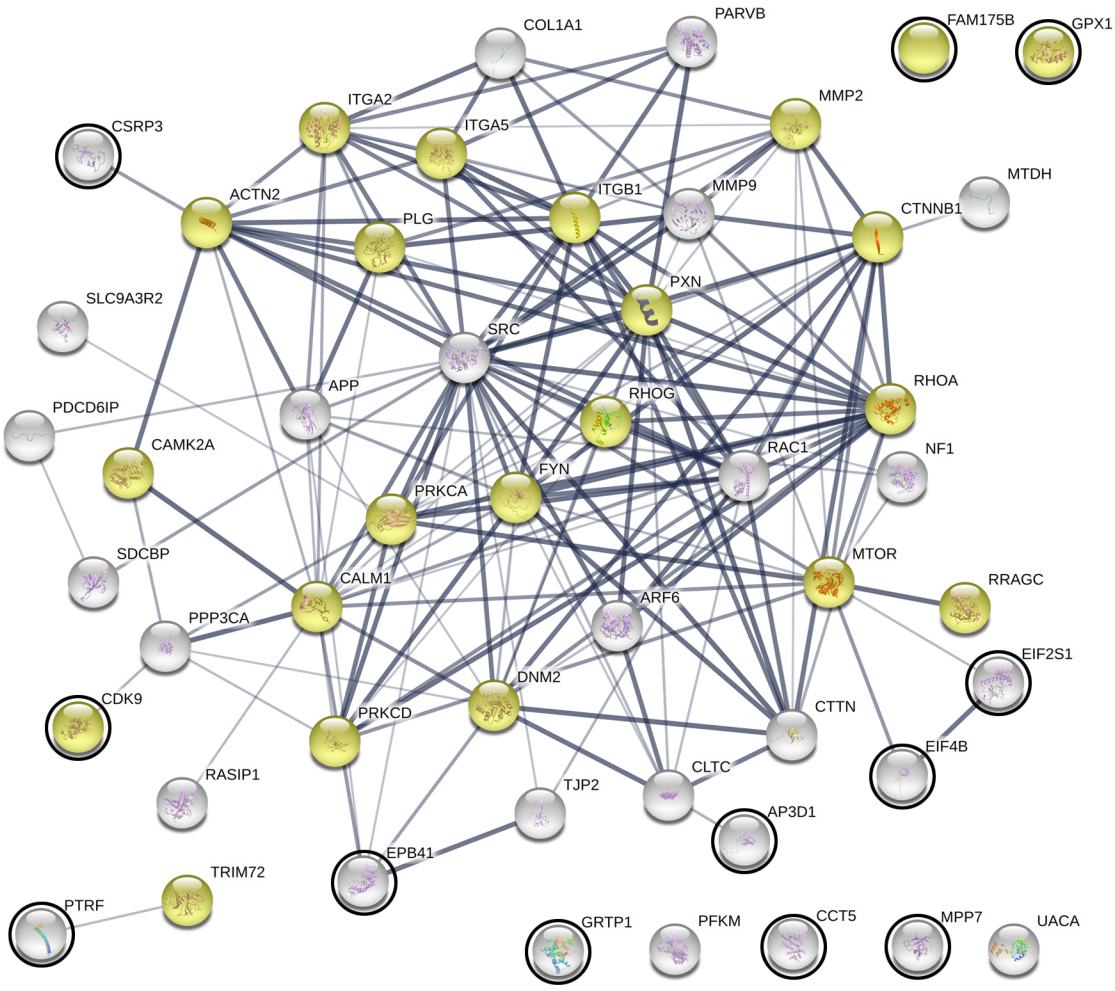

Figure S2. An illustration of the cardiac syndecan-4 network consisting of 50 proteins (STRING 10.5). Proteins involved in stress response are in yellow. Novel syndecan-4 partners identified in this study were highly connected (black circles).

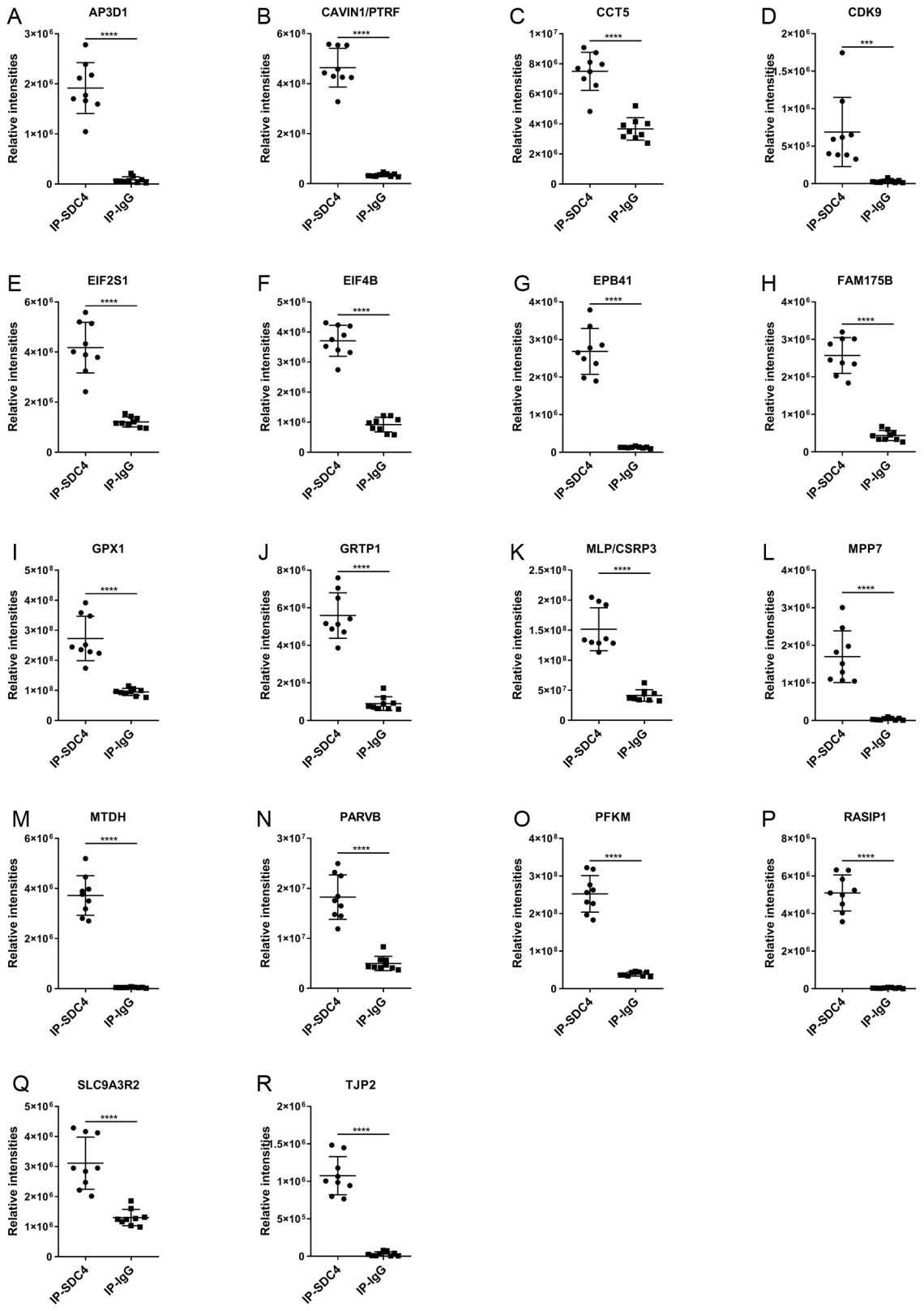

Figure S3. The relative intensities of the 21 novel partners measured by MS are given in scatter plots. (IP-SDC4 versus IP-IgG, ***, $p < 0.001$, ****, $p < 0.0001$, n=3).

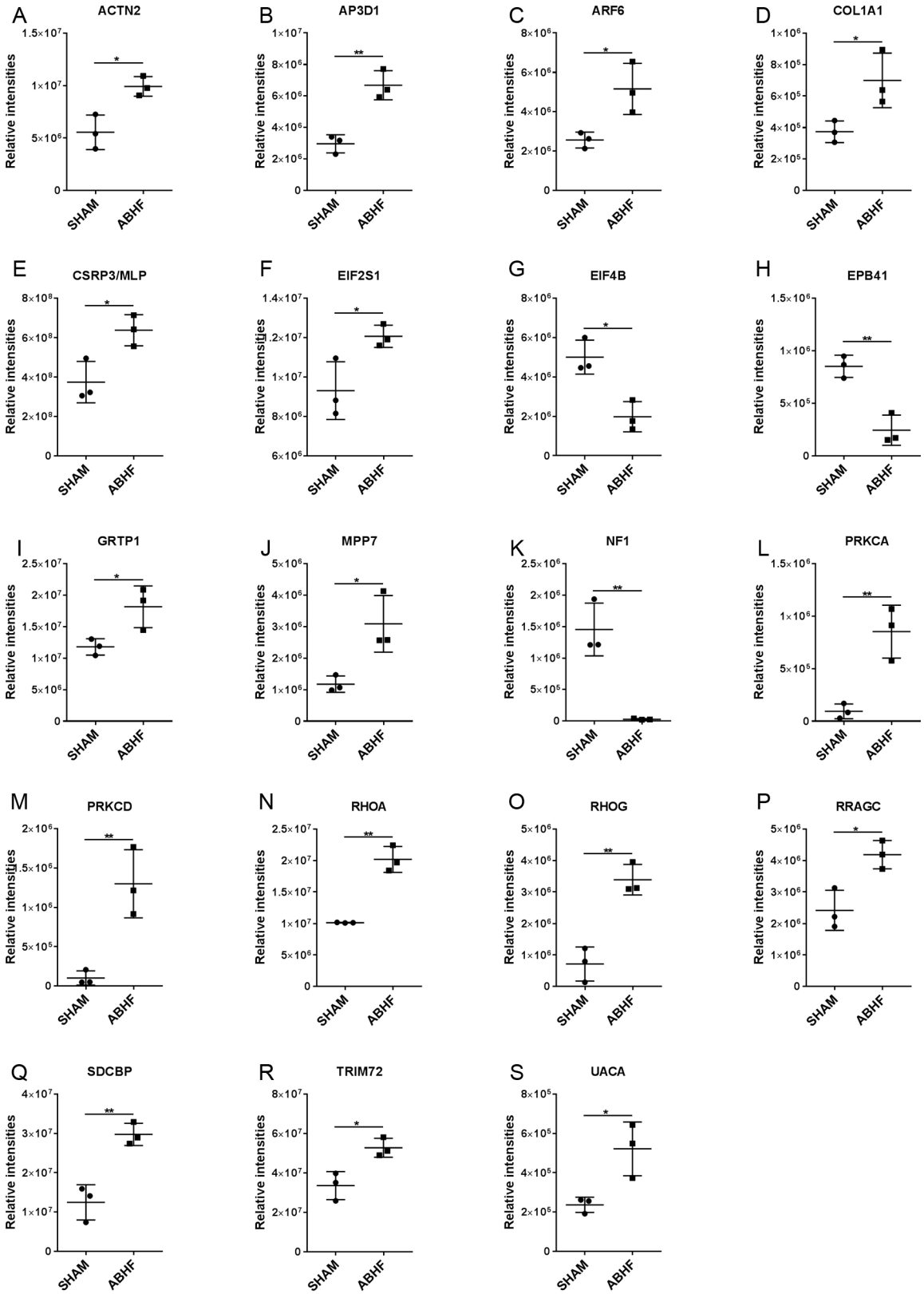

Figure S4. The relative intensities of the 19 partners with an altered interaction with syndecan-4 after aortic banding (ABHF) are given in scatter plots. SHAM animals were used as control. (*, $p < 0.05$; **, $p < 0.01$, n=3).

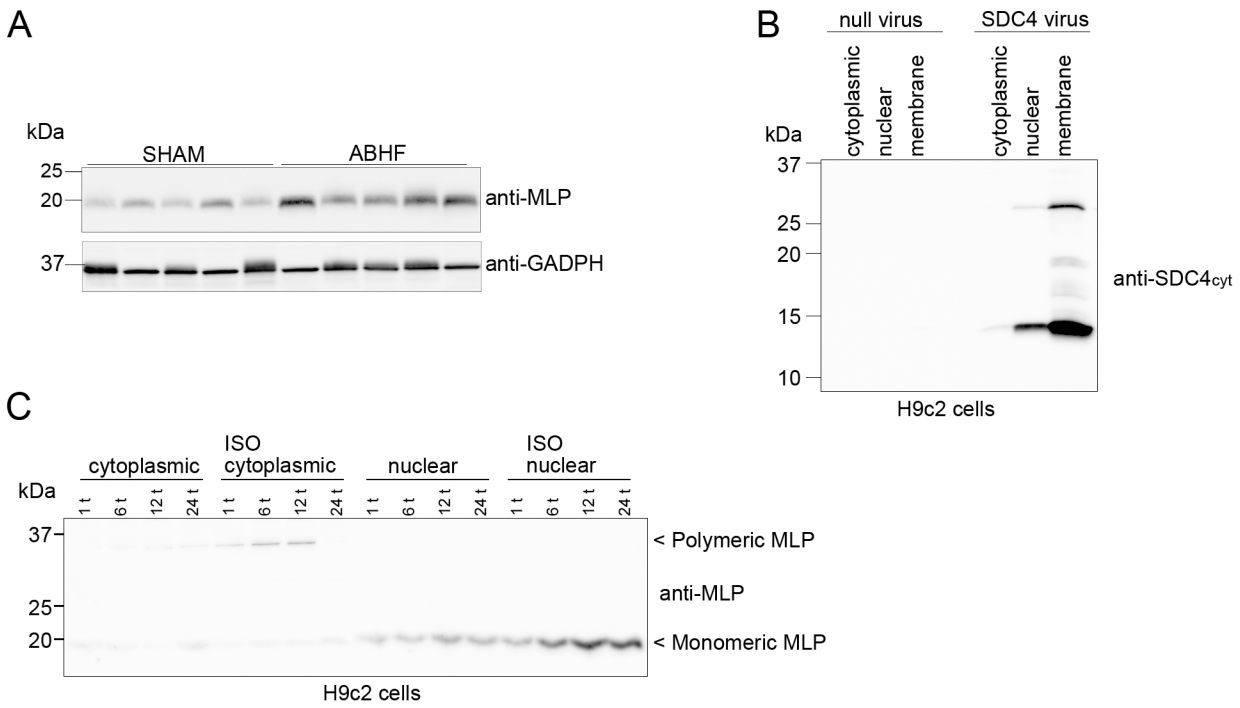

Figure S5. Protein levels of MLP, overexpression of syndecan-4 with syndecan-4 virus and the time point of MLP nuclear translocation after ISO stimulation. (A) Immunoblot of the SHAM and ABHF lysates used to determine MLP levels in figure 6C. (B) Lysates from H9c2 cells transduced with either null or syndecan-4 (SDC4) virus and subsequently fractionated into cytoplasmic, nuclear and membrane fractions. 25 µg protein was loaded per well. (C) H9c2 cells were stimulated with ISO for the given time points and fractionated into cytoplasmic and nuclear fractions. Non-stimulated cells were used as control. In line with previous studies (14), only monomeric MLP was found in the nucleus.

14. Boateng, S. Y., Belin, R. J., Geenen, D. L., Margulies, K. B., Martin, J. L., Hoshijima, M., de Tombe, P. P., and Russell, B. (2007) Cardiac dysfunction and heart failure are associated with abnormalities in the subcellular distribution and amounts of oligomeric muscle LIM protein. American journal of physiology. Heart and circulatory physiology 292, H259-269