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Fig. S1. Sequence alignment of mouse FKBP52 (mFKBP52) and the FKBP52 cDNA clone from the screen. Underlined sequences represent FKBP52 domains. The point mutation G1075A in the FKBP52 clone is marked in red.

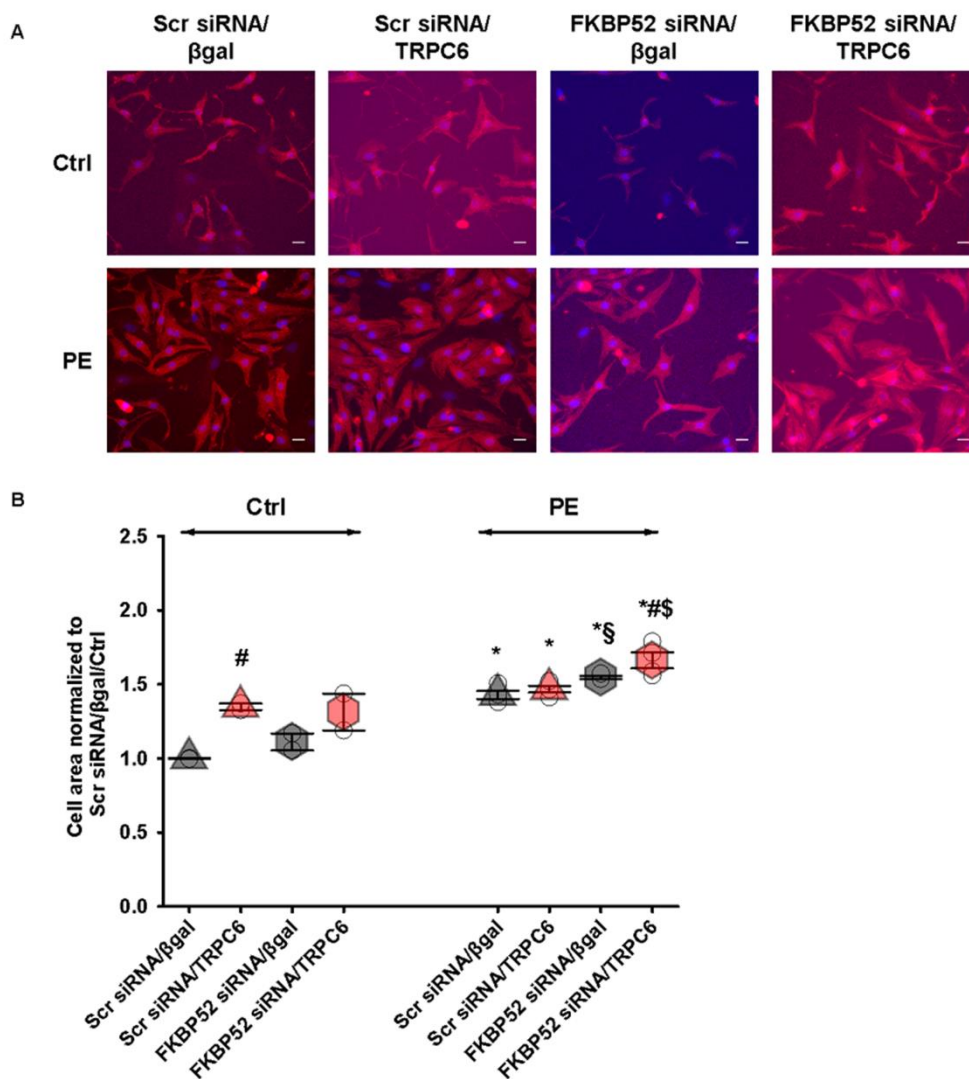


Fig. S2. Downregulation of FKBP52 results in an elevated TRPC6-induced hypertrophic response. (A) Neonatal rat cardiomyocytes (NRCs) were infected with adenoviruses encoding β gal or TRPC6 and transfected with FKBP52 (FKBP52 siRNA) or scramble siRNAs (Scr siRNA). The hypertrophic growth of NRCs was measured as an increase of the cell surface after phenylephrine (PE; 50 μ M) or control (Ctrl) stimulation for 24 h. Red, α -actinin; Blue, DAPI. Scale bar: 20 μ m. Taken with a 40 \times magnification objective. (B) Quantification of the cell area relative to Scr siRNA/ β gal/Ctrl. * P <0.05 vs. respective Ctrl stimulation, # P <0.05 vs. Scr siRNA/ β gal/Ctrl or vs. FKBP52 siRNA/ β gal/PE, respectively, \$ P <0.05 vs. Scr siRNA/ β gal/PE, $\text{\textcircled{S}}$ P <0.05 vs. FKBP52 siRNA/ β gal/PE (two-way Anova with Holm-Sidak method and unpaired t-test), $n = 2-4$, mean \pm SEM.

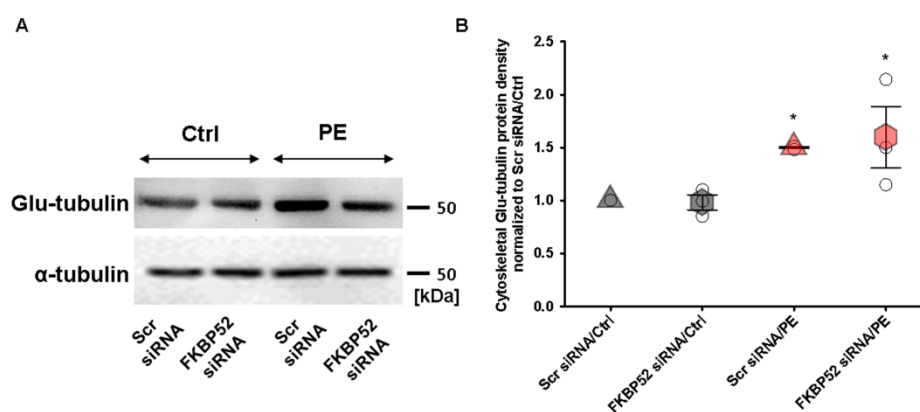


Fig S3. The stabilization of microtubules is not affected by a downregulation of FKBP52 during hypertrophic stimulation. (A) Western blot analysis of α -tubulin and Glu-tubulin expression levels in cytoskeletal extracts from neonatal rat cardiomyocytes (NRCs) after 24 h of phenylephrine (PE) or control (Ctrl) stimulation. The cells were transfected with FKBP52 or scramble siRNAs (Scr siRNA). (B) PE stimulation results in increased Glu-tubulin expression levels in FKBP52 and scr siRNA-treated NRCs. Protein expression levels were normalized to scr siRNA/Ctrl. * $P < 0.05$ vs. to scr siRNA/Ctrl or FKBP52 siRNA /Ctrl, respectively (two-way Anova with Holm-Sidak method), $n = 3$, mean \pm SEM.

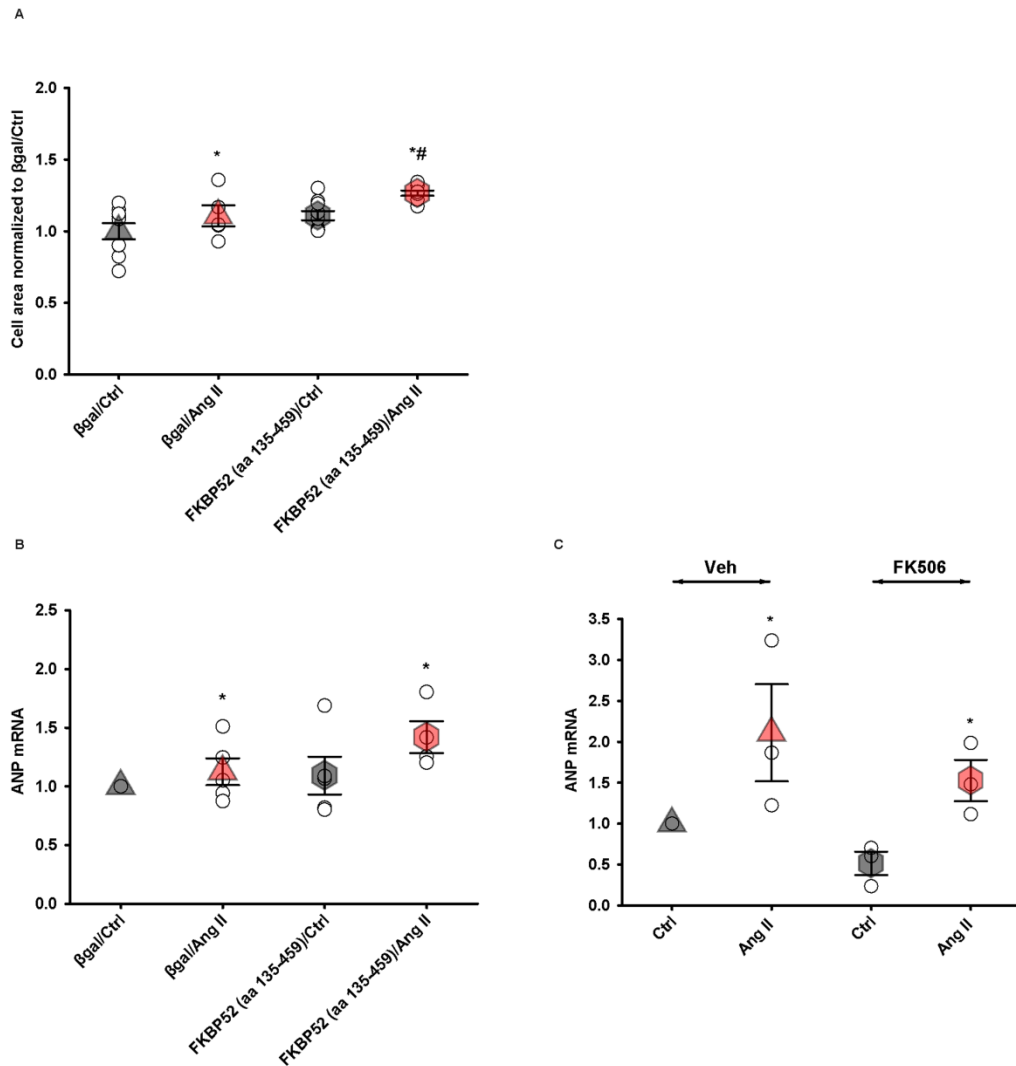


Fig S4. FKBP52 (aa 135-459) affects an angiotensin II-stimulated hypertrophic response differently than FK506. (A) Quantification of the cell area after 24 h of angiotensin II (Ang II; 100 nM) or control (Ctrl) stimulation. NRCs were infected with FKBP52 (aa 135-459) or β gal. Data are shown relative to β gal-infected controls. * P <0.05 vs. β gal/ctrl, # P <0.05 vs. β gal/Ang II (two-way Anova with Holm-Sidak method), $n = 5-9$, mean \pm SEM. (B) mRNA levels of atrial natriuretic peptide (ANP) were determined after 24 h of Ang II or control (Ctrl) stimulation *via* qPCR from NRCs expressing β gal or FKBP52 (aa 135-459). * P <0.05 vs. β gal or FKBP52 (aa 135-459)/ctrl (two-way Anova with Holm-Sidak method), $n = 4-5$, mean \pm SEM. (C) mRNA levels from NRCs treated with FK506 (2 μ M) or vehicle (Veh). * P <0.05 vs. Ctrl (two-way Anova with Holm-Sidak method), $n = 3$, mean \pm SEM. * P <0.05.

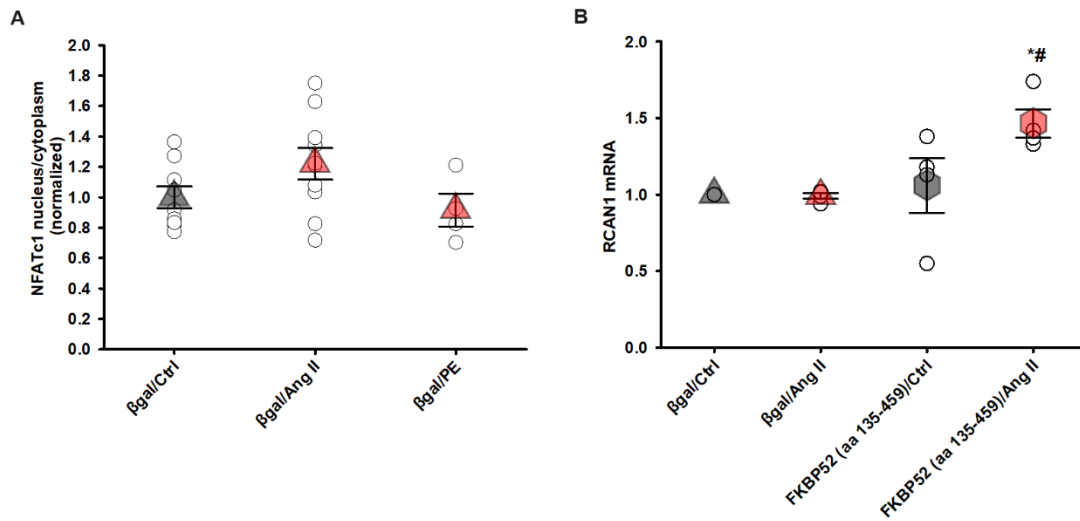


Fig. S5. NFATc1 and RCAN1 activation in response to agonist stimulation in neonatal rat cardiomyocytes. (A) Average quantified values of NFATc1-GFP localized to the nucleus in response to angiotensin II (Ang II; 100 nM), phenylephrine (PE; 50 μ M) or control (Ctrl) stimulation. Neonatal rat cardiomyocytes (NRCs) were infected with β gal. Data are shown relative to β gal/Ctrl (One-way Anova with Holm-Sidak method), $n = 4-10$, mean \pm SEM. (B) Regulator of calcineurin 1 (RCAN1) expression levels as parameter of the activation of calcineurin in NRCs. mRNA levels of RCAN1 were measured after 24 h of Ang II or control (Ctrl) stimulation *via* qPCR. NRCs were infected with β gal or FKBP52 (aa 135-459). Average quantified values relative to β gal/Ctrl. * $P < 0.05$ vs. FKBP52 (aa 135-459)/Ctrl, # $P < 0.05$ vs. β gal/ Ang II (two-way Anova with Holm-Sidak method), $n = 4$, mean \pm SEM.

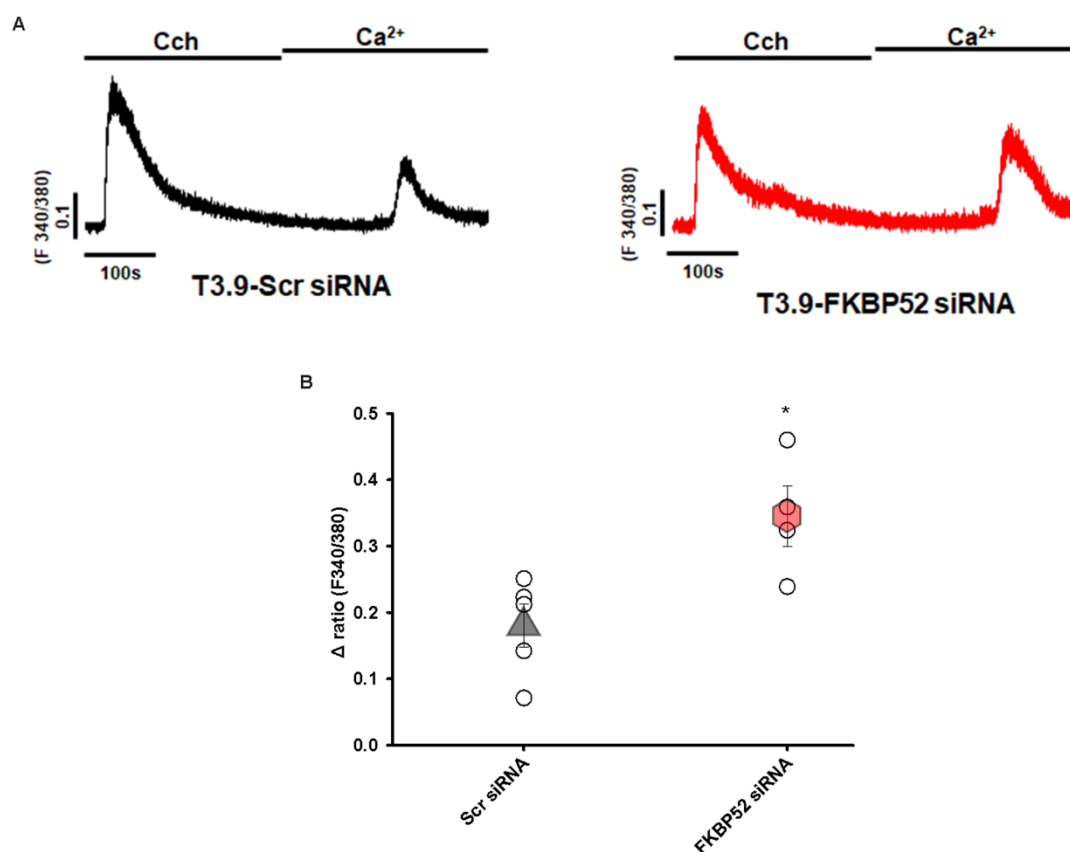


Fig. S6. TRPC3-dependent Ca²⁺ signals in HEK 293 cells are dependent on FKBP52. (A) Representative Ca²⁺ recordings of HEK 293 cells stably expressing TRPC3 (T3.9). The cells were treated with scramble (Scr siRNA) or FKBP52 siRNAs. A TRPC3-related Ca²⁺ influx was measured according to a classical Ca²⁺ re-addition protocol. T3.9 cells were first stimulated with the G_q protein-coupled receptor agonist carbachol (Cch, 300 μM) in nominally Ca²⁺-free conditions and subsequently perfused with an external solution containing CaCl₂. This manoeuvre resulted in a Cch-dependent Ca²⁺ release from internal stores followed by a Ca²⁺ influx from the extracellular space. The TRPC3-mediated Ca²⁺ influx was significantly enhanced in cells treated with siRNAs against FKBP52. (B) Mean Δ ratio values were calculated by subtracting the peak value after Ca²⁺ re-addition from the baseline value *P<0.05 vs. Scr siRNA (unpaired t-test), *n* = 4-5, mean ± SEM. The mean Δ ratio of the Ca²⁺ release (peak-baseline) was not different between the groups. P= 0.34.