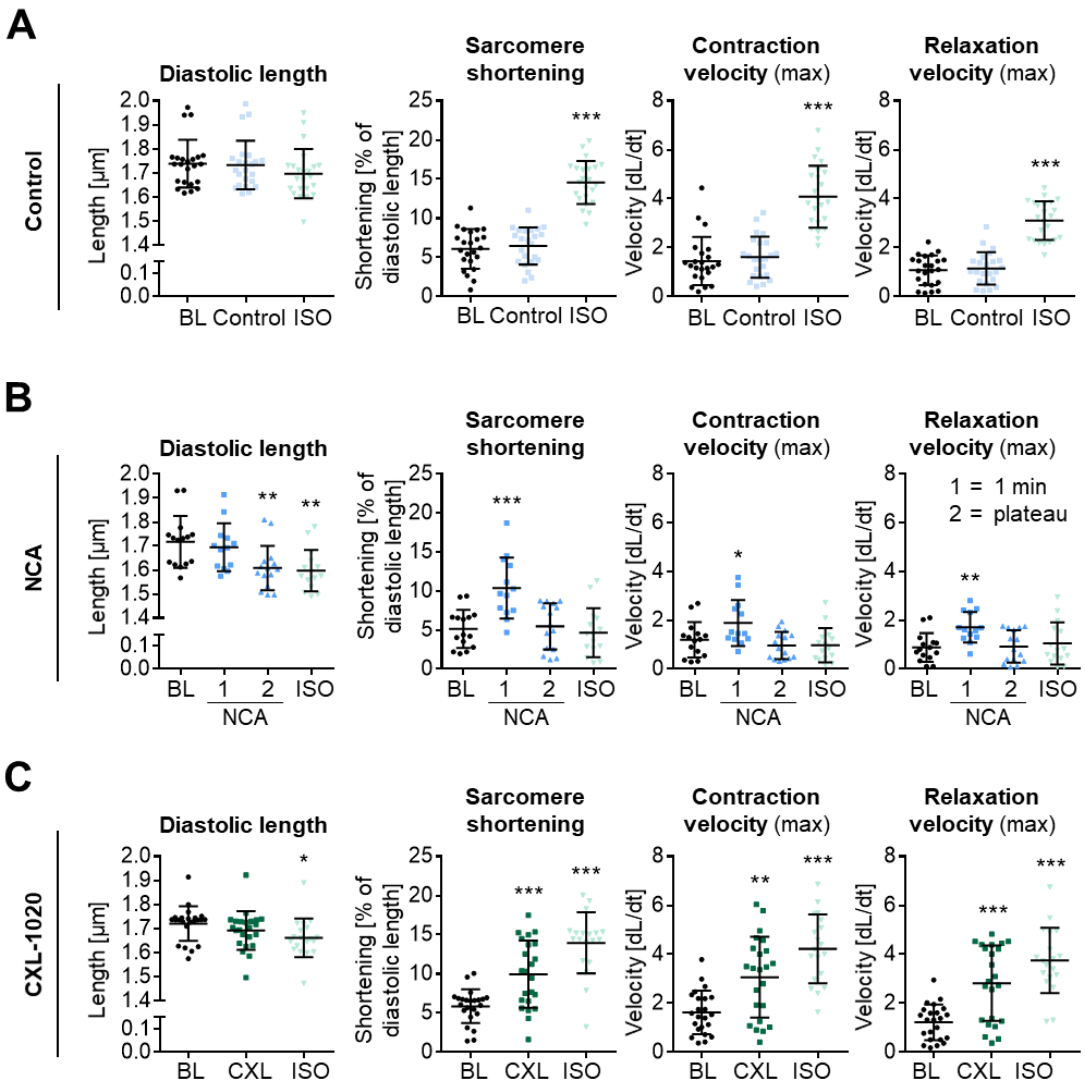
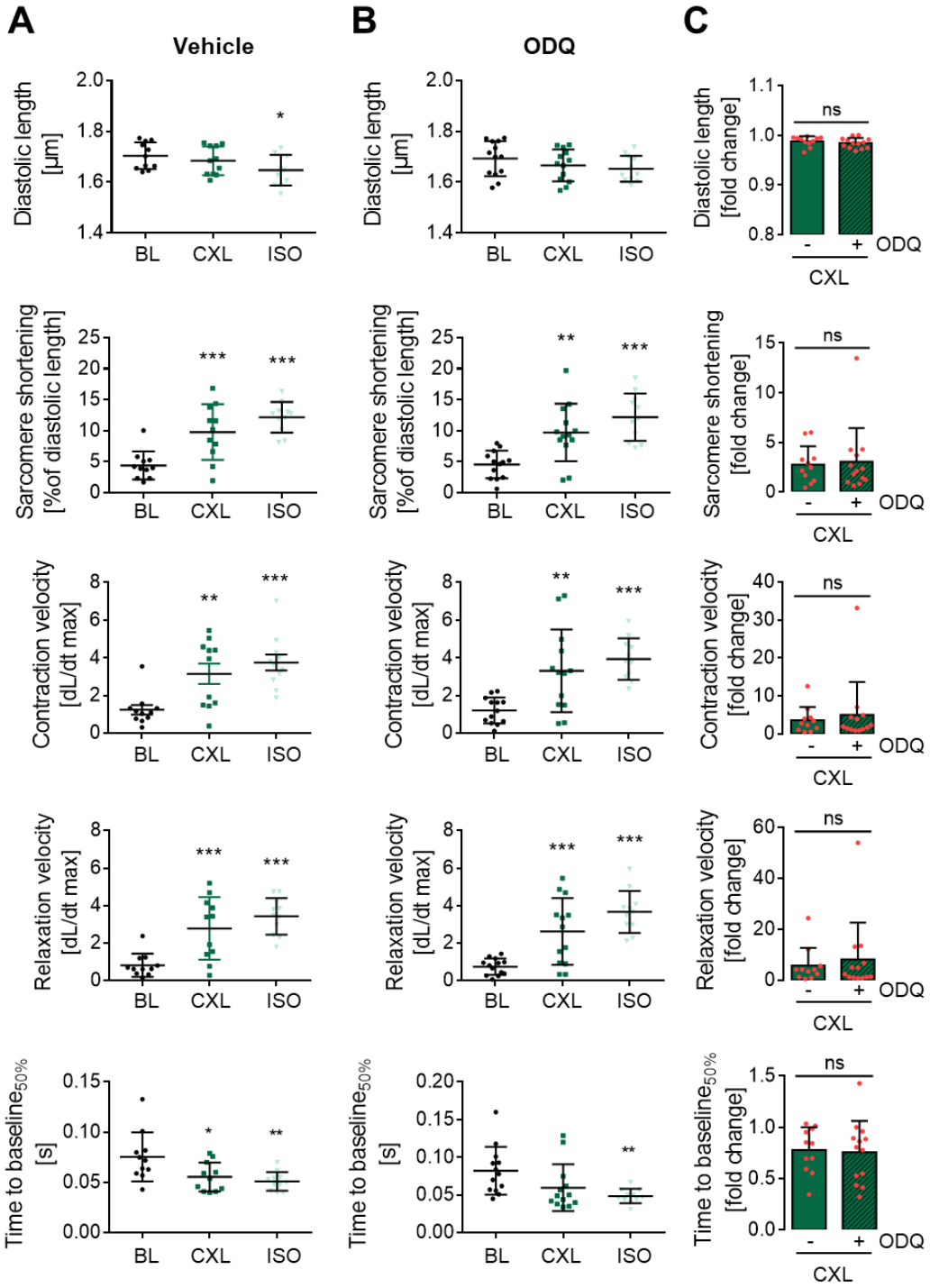
Suppl. Figure 1



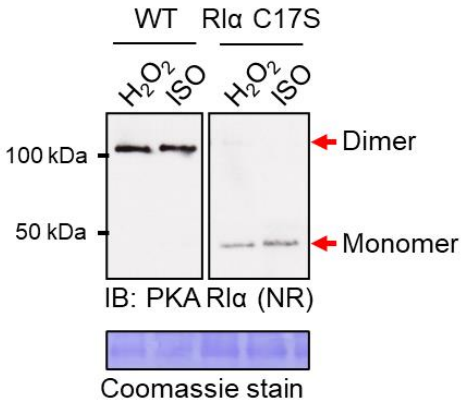
Supplementary Figure 1: A-C Contraction characteristics of single ARVMs paced at 1 Hz at basal conditions (BL), upon exposure to (**A**) vehicle (DMSO; control), (**B**) NCA (100 µmol/L) or (**C**) CXL-1020 (300 µmol/L) and in response to subsequent ISO-application (10 nmol/L). Scatter plots include NCA-values after 1 min (**1**) of exposure and at the plateau state (**2**) of the transient effect (average 4.5 min of exposure). The contractile parameters measured are the diastolic length, sarcomere shortening, as well as maximal contraction and relaxation velocities (raw data) at baseline, upon stimulation and after addition of ISO. * P<0.05, ** P<0.01, *** P<0.001 for comparison with the corresponding baseline values by one-way ANOVA with Dunnett's post-test (F and P values: a) DMSO: diastolic length F=1.14, P=0.3237; sarcomere shortening F=80.47, P<0.0001; contraction velocity (max) F=45.64, P<0.0001; relaxation velocity (max) F=165.0, P<0.0001; b) NCA: diastolic length F=5.71, P=0.0018; sarcomere shortening F=9.71, P<0.0001; contraction velocity (max) F=4.68, P=0.0056; relaxation velocity (max) F=4.18, P=0.0099; c) CXL-1020: diastolic length F=3.02, P=0.0559; sarcomere shortening F=27.23, P<0.0001; contraction velocity (max) F=19.57, P<0.0001; relaxation velocity (max) F=22.37, P<0.0001).

Suppl. Figure 2



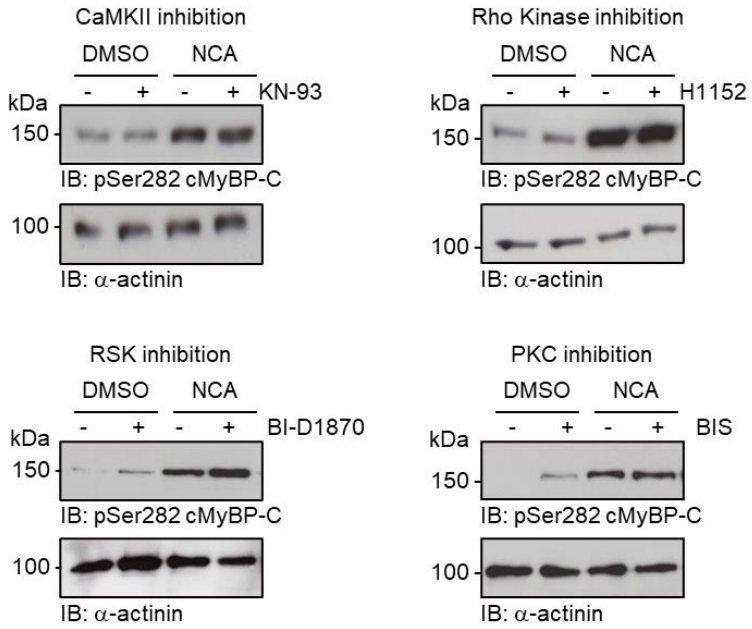
Supplementary Figure 2: Impact of ODQ-pretreatment on CXL-1020-mediated effect on ARVM contractility. Contraction characteristics of single ARVMs paced at 1 Hz were recorded after exposure to **A** vehicle (DMSO) under basal conditions (BL), upon exposure to CXL-1020 (300 µmol/L) and in response to subsequent ISO-application (10 nmol/L) or **B** after pretreatment with ODQ (0.3 µmol/L, 10 min) under basal conditions (BL), upon exposure to CXL-1020 (300 µmol/L) and in response to subsequent ISO-application (10 nmol/L). * P<0.05, ** P<0.01, *** P<0.001 for comparison with the corresponding baseline values by one-way ANOVA with Dunnett's post-test (F and P values: a) DMSO pretreatment: diastolic length F=2.86, P=0.0729; sarcomere shortening F=16.54, P<0.0001; contraction velocity (max) F=9.66, P=0.0006; relaxation velocity (max) F=14.81, P<0.0001; time to baseline$_{50\%}$ F=6.18, P=0.0058 b) ODQ pretreatment: diastolic length F=1.30, P=0.2850; sarcomere shortening F=13.60, P<0.0001; contraction velocity (max) F=11.29, P=0.0002; relaxation velocity (max) F=17.28, P<0.0001; time to baseline$_{50\%}$ F=4.91, P=0.0134).**C** Scatter plots compare effects of vehicle (DMSO) or ODQ pretreatment on subsequent CXL-1020 exposure. ns: non-significant.

Suppl. Figure 3



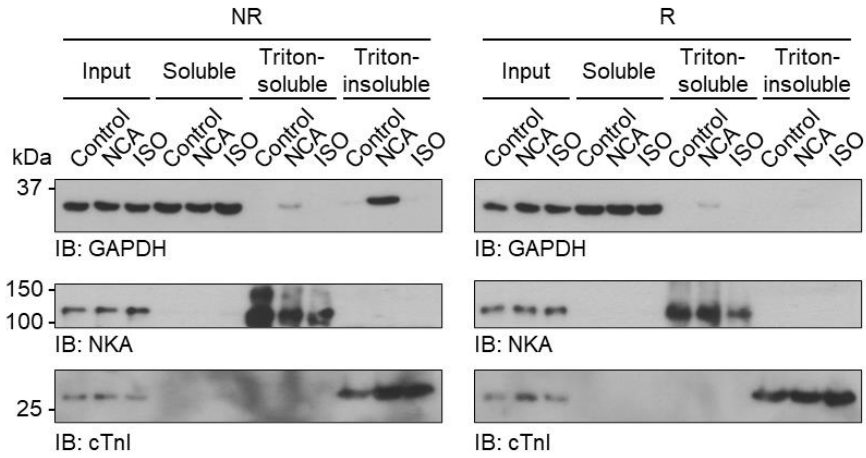
Supplementary Figure 3: Validation of the anti-PKA-RIα antibody to detect the oxidation state of PKA-RIα. Homogenates of adult mouse ventricular myocytes (AMVM) isolated from wildtype (WT) or Cys17Ser PKA-RIα knock-in mice were exposed to hydrogen peroxide (H_2O_2; 100 µmol/L, 10 min) or ISO (10 nmol/L, 10 min) and western immunoblot analysis performed under non-reducing (NR) conditions using a PKA-RI specific antibody. Equal protein loading was demonstrated by Coomassie staining. The blot is representative of the results obtained from four independent AMVM preparations.

Suppl. Figure 4



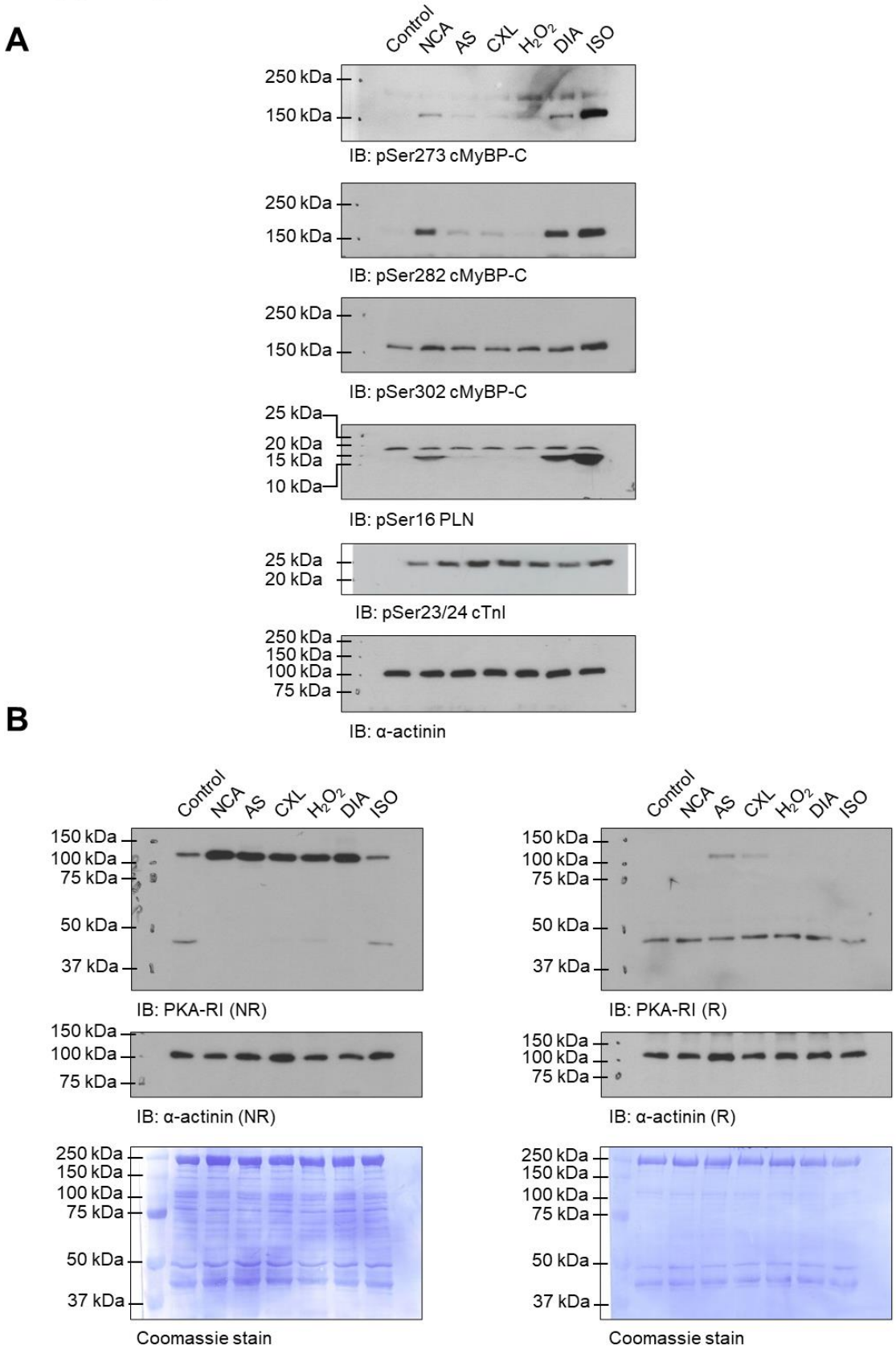
Supplementary Figure 4: Contribution of kinases to oxidant-mediated phosphorylation cMyBP-C at Ser282. ARVMS were pretreated with vehicle (DMSO) or KN-93 (20 µmol/L; 30 min) to inhibit Ca^{2+}-calmodulin-dependent kinase, BI-D1879 (10 µmol/L; 30 min) to inhibit p90 ribosomal S6 kinase, H1152 (300 nmol/L; 4 hours) to inhibit Rho-kinase or Bisindolylmaleimide (BIS; 2 µmol/L; 15 min) to inhibit protein kinase C prior to exposure to vehicle (DMSO) or NCA (100 µmol/L; 30 min). Immunoblot analysis was performed for pSer282 cMyBP-C. Equal protein loading was confirmed by blotting for α-actinin.

Suppl. Figure 5



Supplementary Figure 5: Quality control of ARVM fractionation. ARVMs were exposed to (control), NCA (100 µmol/L, 30 min) or ISO (10 nmol/L, 10 min). Cells were harvested under non-reducing (NR) or reducing (R) conditions and separated into soluble, Triton-soluble and Triton-insoluble fraction. Fractionation success was analyzed by immunoblotting (IB) using antibodies against the following marker proteins: GAPDH (soluble fraction), NKA (Triton-soluble fraction) and cTnI (Triton-insoluble fraction containing the myofilaments).

Suppl. Figure 6



Supplementary Figure 6: A Overall view of western immunoblots from Figure 2B and **B** Figure 3B of the manuscript including relevant molecular weight markers.a