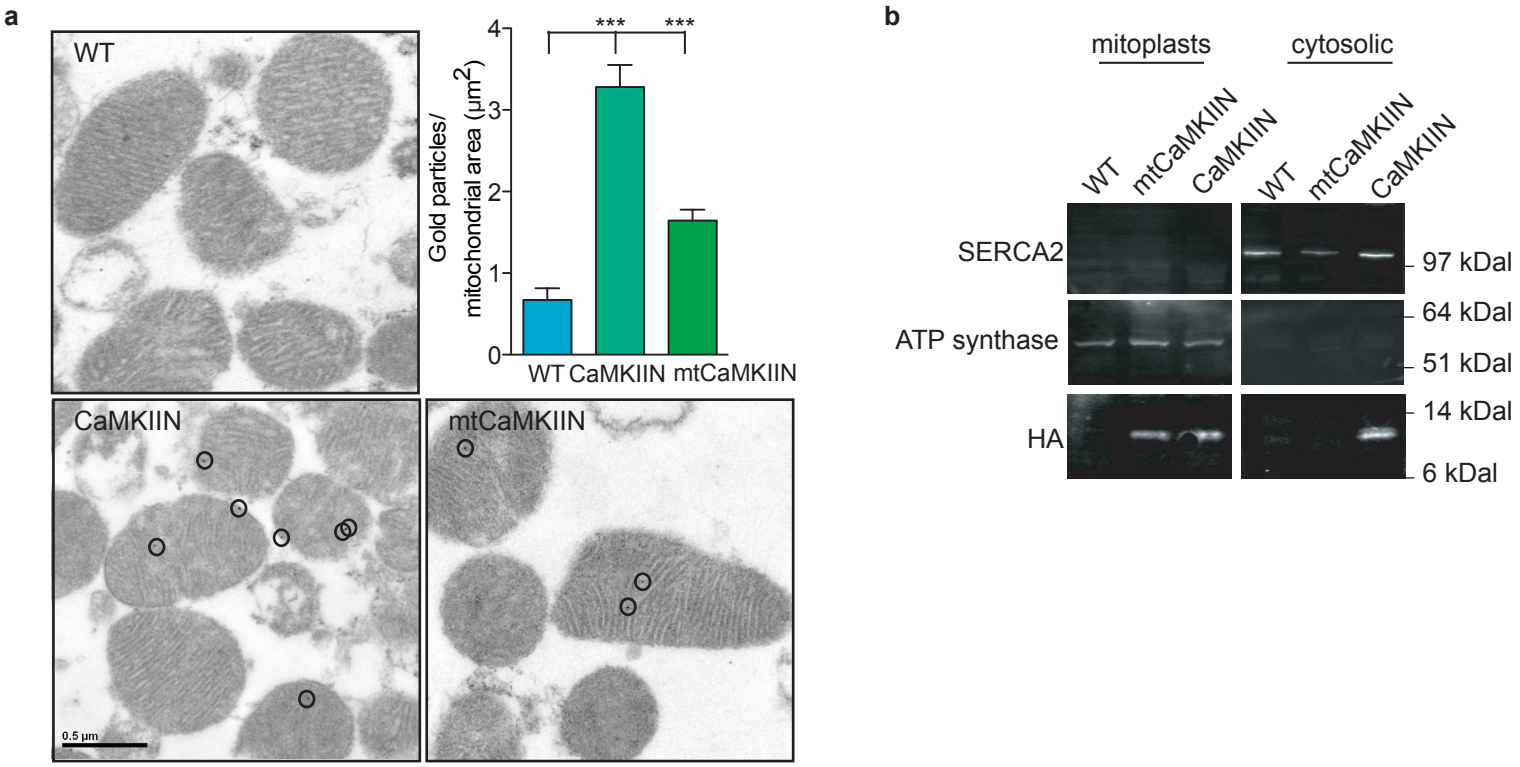
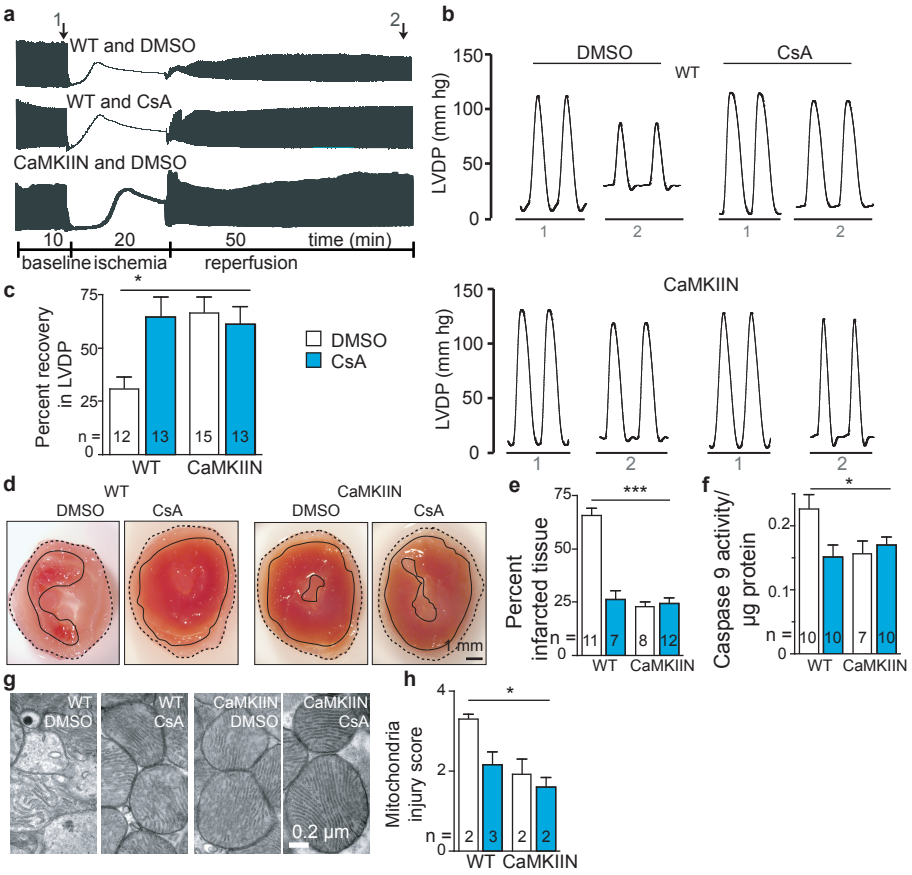
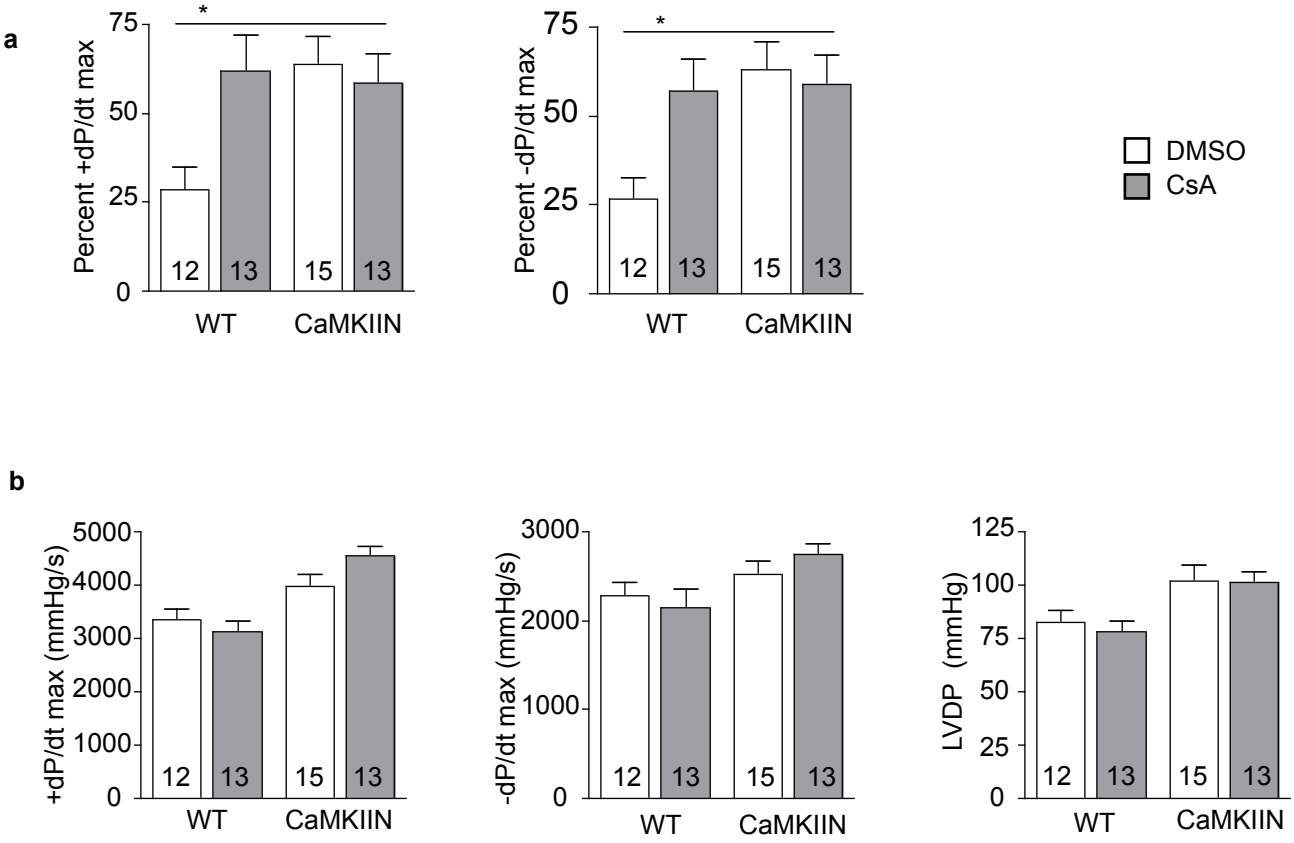


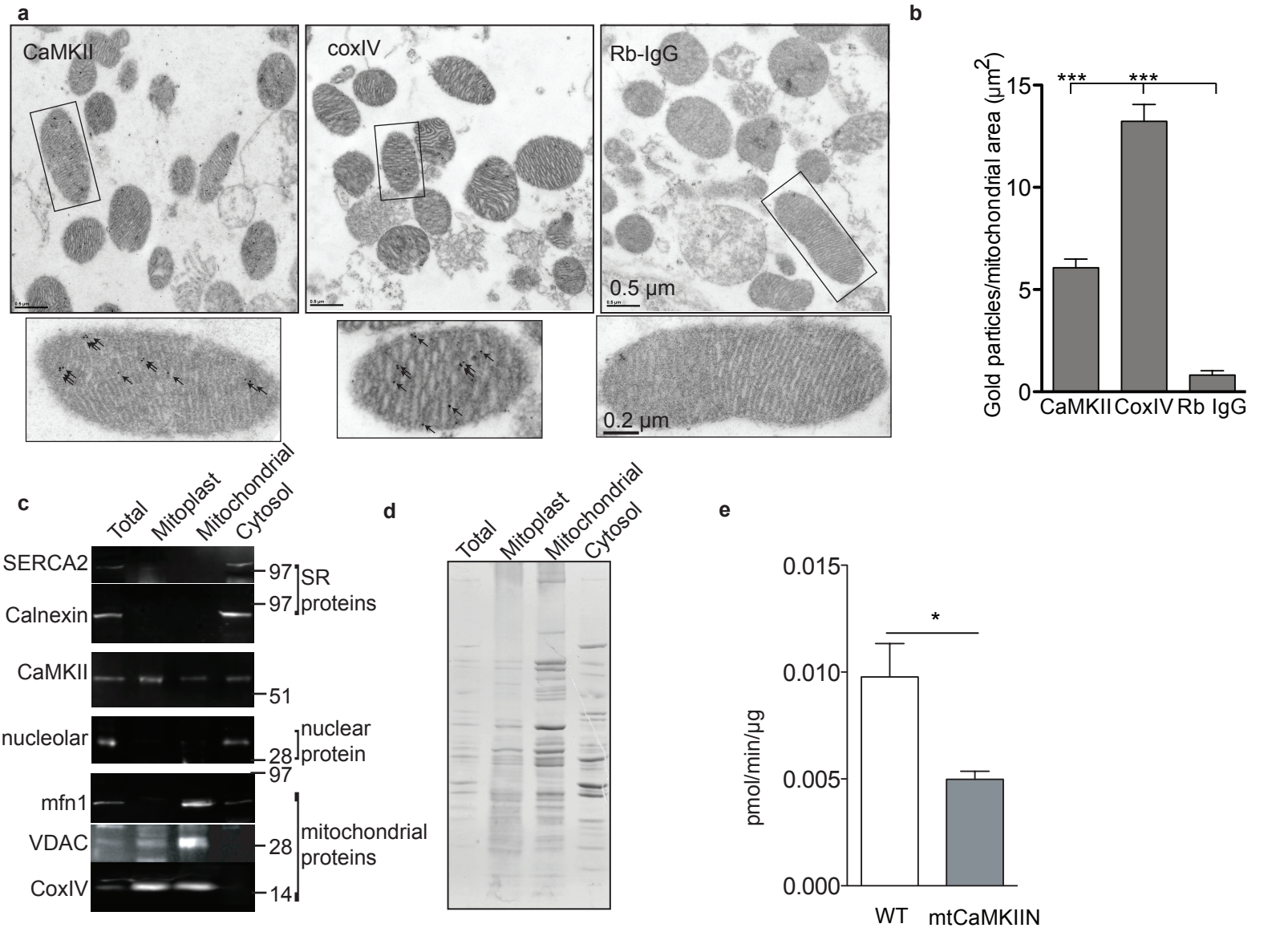
Supplemental Figure 1. CaMKIIN is expressed in mitochondria of CaMKIIN and mtCaMKIIN mouse hearts. **a**. Representative TEM images of mitochondria isolated from hearts showing HA-tagged CaMKIIN expression in CaMKIIN and mtCaMKIIN transgenic mice, but not in WT mice. Gold labeling on mitochondria is marked by black circles. Summary data (upper right) showing HA staining for n = 5 fields per condition, n = 6 hearts/genotype, (***$p < 0.0001$). Data represent mean ± s.e.m. **b**. Western analysis of mitoplast lysate from WT, mtCaMKIIN and CaMKIIN hearts. HA antibody recognizes HA-CaMKIIN in mitoplast and cytosolic lysates from CaMKIIN transgenic hearts but only in mitoplast lysates from mtCaMKIIN transgenic hearts. HA was not detected in lysates from WT hearts. SERCA2, an SR protein, is present in cytosol and absent in mitochondrial fractions. Inner mitochondrial protein, ATP synthase (5a), is present only in mitoplast fractions.



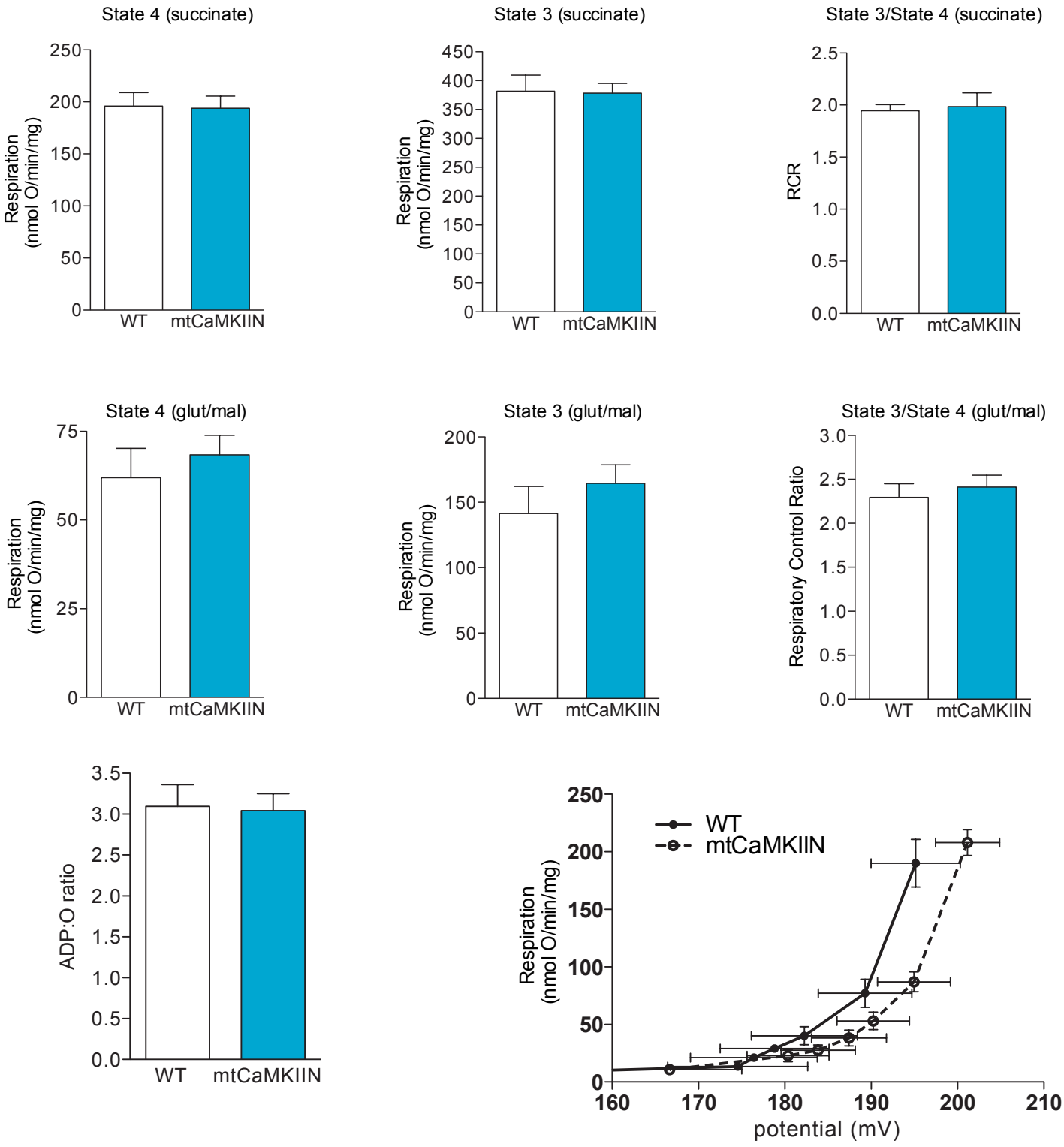
Supplemental Figure 2. Heart function is equally maintained after ischemia and reperfusion injury (I/R) by CaMKII inhibition (CaMKIIN) or cyclosporin A (CsA). **a**. Representative left ventricular developed pressure (LVDP) traces from WT hearts and CaMKIIN-expressing hearts perfused with DMSO or CsA (2 µM). The concentration of CsA in the coronary perfusate is based on blood serum levels of CsA 20 min after injection in the study by Piot et al. 2008. **b**. Expanded LVDP traces before and after I/R from time-points 1 and 2 (with arrows) in panel **a**. **c**. LVDP recovery following I/R as a percentage of the baseline value (*p = 0.029). Numerals indicate number of measurements/group. **d**. Representative triphenyl tetrazolium chloride (TTC) stained heart sections. The dark red staining represents living myocardium, the solid black outlines form boundaries demarcating viable from dead tissue and the dotted lines indicate total area at risk. **e**. Summary data from TTC stained hearts with relative area of infarct normalized to WT. Infarcted area was measured as a percentage of total at risk myocardium (indicated by the dashed lines in panel **d**, ***p < 0.0001). **f**. Caspase 9 activity from hearts treated as in panel **a** (*p = 0.034). **g**. Representative transmission electron microscopy (TEM) images from hearts treated as in panel **a**. **h**. Summary mitochondria injury scores for TEM studies by the following criteria: 0 = no detectable disruption in any mitochondria/field, 1 = cristae disrupted in one mitochondrion/field, 2 = disruption in >1 mitochondrion/field, 3 = ≥ 1 and < 50% ruptured mitochondria/field and 4 = ≥ 50% mitochondria ruptured/field (*p = 0.028, about 500 mitochondria from 10 random fields were counted/genotype and treatment). Data represent mean ± s.e.m.



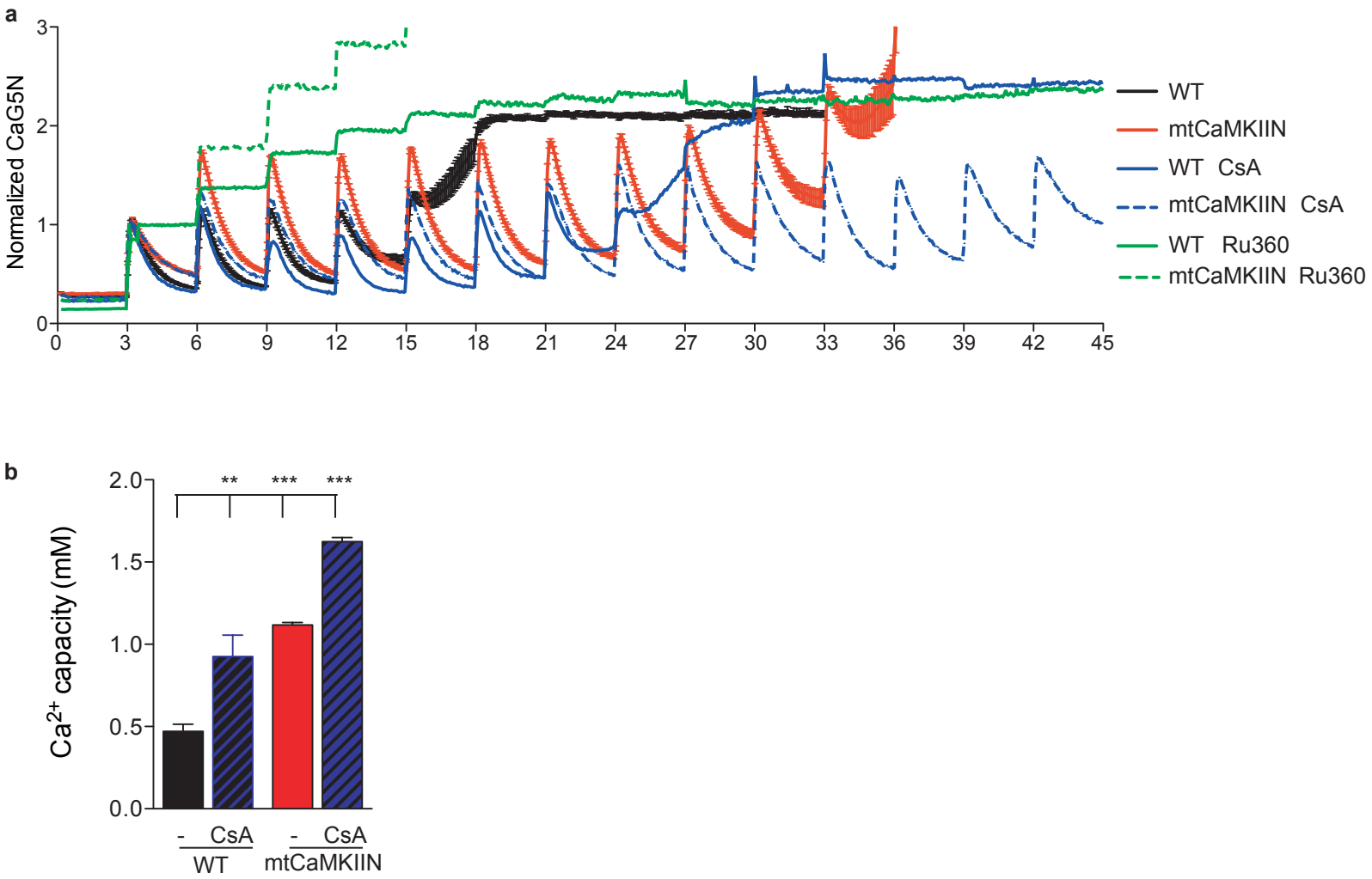
Supplemental Figure 3. Mechanical function in isolated hearts is maintained after ischemia and reperfusion by CaMKIIN expression in transgenic hearts or by CsA in WT hearts. **a**. Percent recovery of maximum contractility (+dP/dtmax) and relaxation (-dP/dtmax, from time points 1 and 2, see Supplementary Fig. 2a) in mouse hearts subjected to I/R injury. **b**. Baseline recordings for each of the parameters. *p < 0.01. Numerals in bars indicate the number of hearts studied. Data represent mean ± s.e.m.



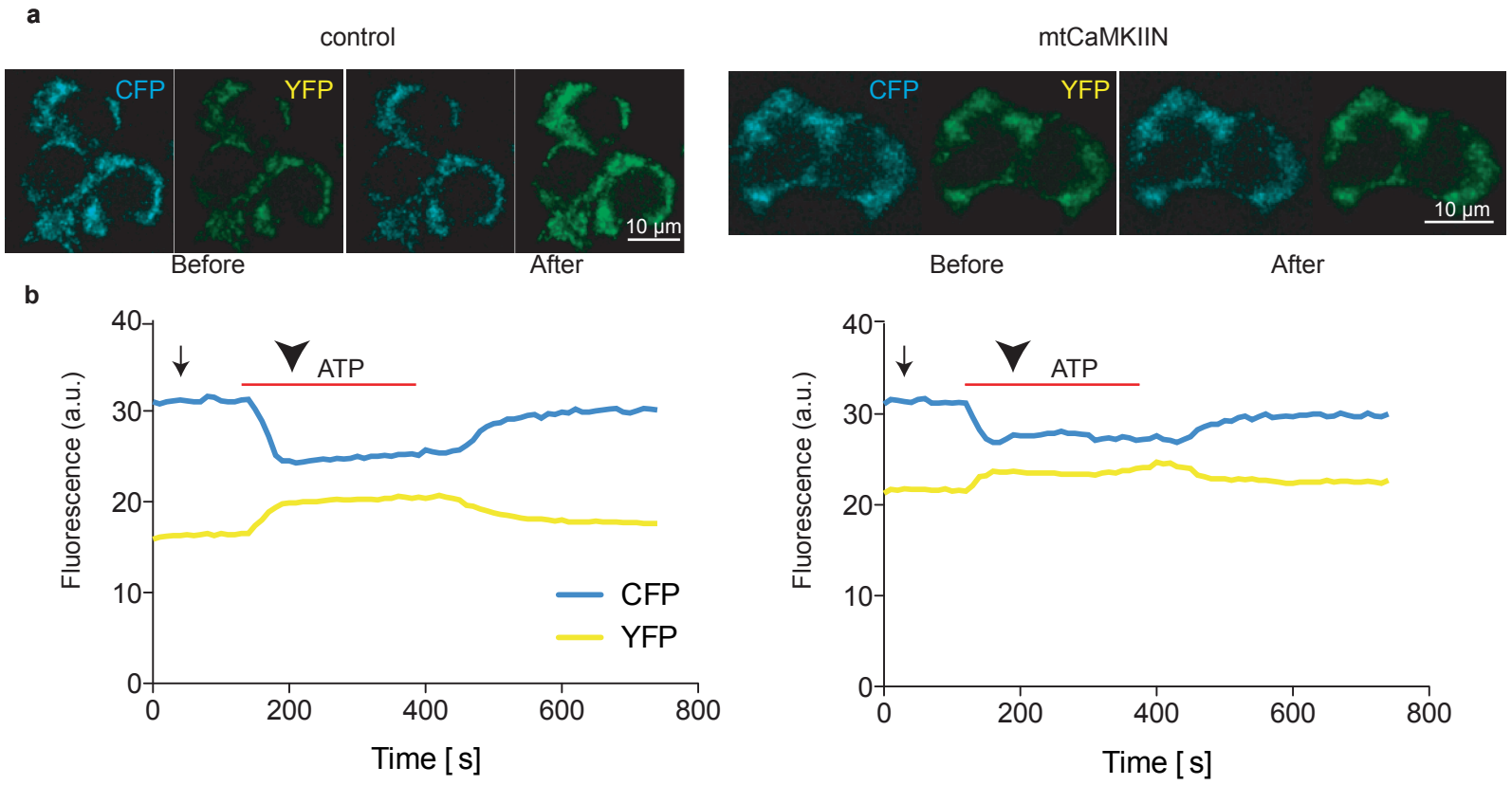
Supplemental Figure 4. CaMKII associates with mitochondria. **a**. Representative immuno-EM images of isolated mitochondria with antibodies against CaMKII or CoxIV (arrows). **b**. Summary data of gold labeling for CaMKII, using CoxIV and rabbit IgG primary antibodies as positive and negative controls, respectively. n = 5 fields per antibody. **c**. Immunodetection of CaMKII in mitochondria. Western blot shows sarcoplasmic reticulum (SR) proteins in total and cytosol cell fractions, nuclear proteins in total and cytosol cell fractions and outer mitochondrial proteins (mfn1 and VDAC)predominantly in whole mitochondrial fraction while inner membrane space and inner membrane protein (CoxIV) are in both mitochondrial fractions. **d**. Coomassie stain of gel used in **c**. **e**. CaMKII activity assay measuring Ca^{2+} and calmodulin-dependent activity in isolated mitochondria (n = 4 measurements/ genotype, *p = 0.024). Data represent mean ± s.e.m.



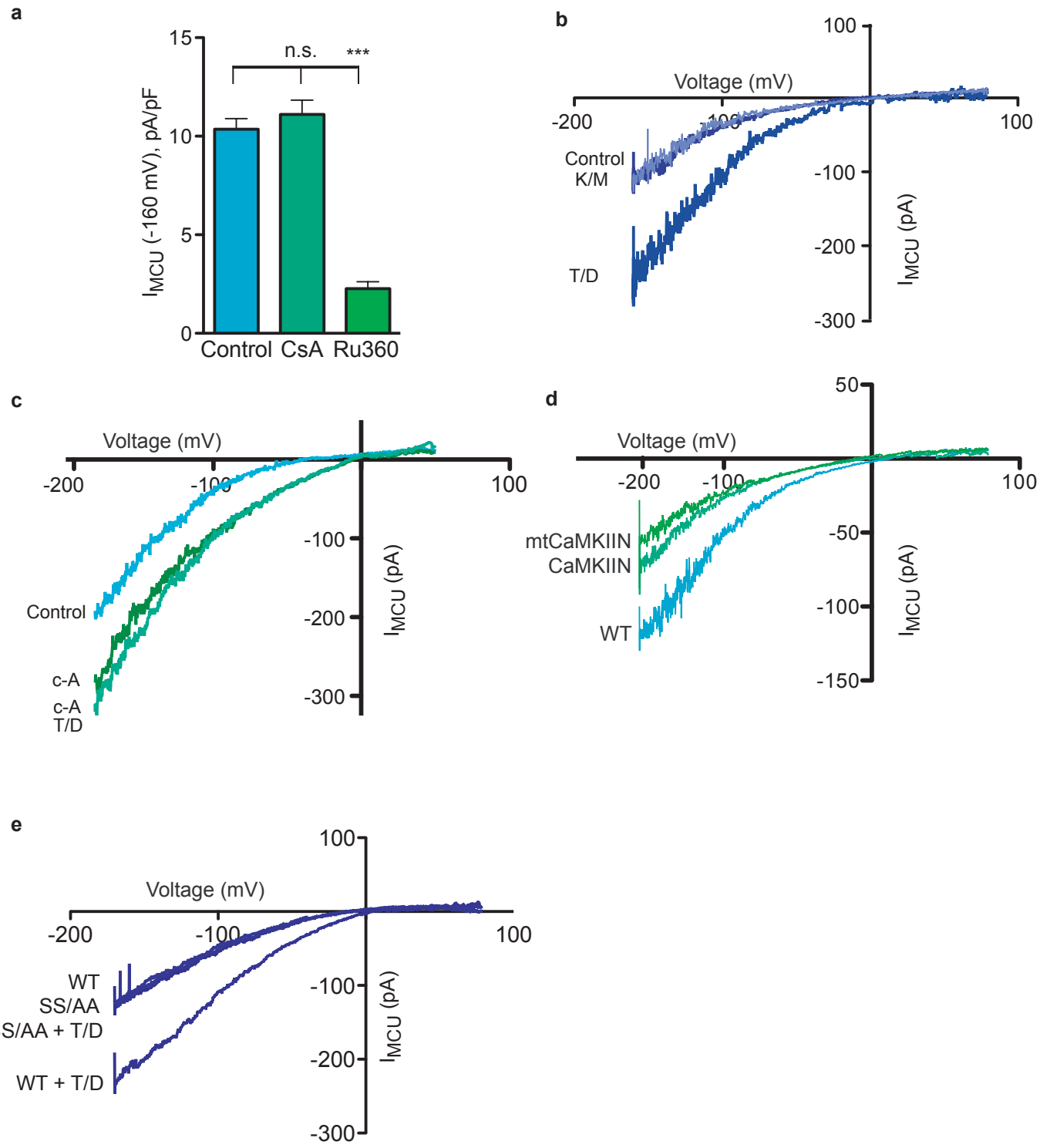
Supplemental Figure 5. Mitochondrial respiration and membrane potential are not different between WT and mtCaMKIIN mitochondria. Both membrane potential and respiration rate showed no significant differences under state 3 or 4 conditions or in coupling of inner mitochondrial membrane potential and respiration in mtCaMKIIN compared with control mice using succinate or glutamate and malate (glut/mal) as mitochondrial substrates. The ADP:O and the relationship between respiration and membrane potential were determined in succinate. Mitochondrial samples were prepared from WT and mtCaMKIIN hearts (n = 5 or 7 samples/group for mitochondria respiring in glut/mal or succinate, respectively) from 2-month old mice. Data represent mean ± s.e.m.



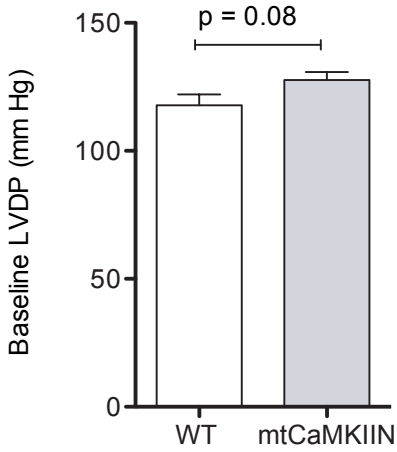
Supplemental Figure 6. Mitochondiral calcium retention and capacity in permeabilized cardiomyocytes. **a**. CaG5N fluorescence traces, normalized to the first peak, show mitochondrial Ca^{2+} uptake kinetics in saponin-permeabilized and blebbistatin- (100 μM) and thapsigargin- (5 μM) treated cardiomyocytes isolated from WT or mtCaMKIIN transgenic hearts. Each Ca^{2+} transient was detected with CaG5N in response to an addition of 100 μM Ca^{2+} (at times indicated). n = 4 experiments for WT and mtCaMKIIN. Representative traces shown for cardiomyocytes from WT and mtCaMKIIN hearts additionally treated with CsA (5 μM) or Ru360 (0.1 μM). **b**. Total extra-mitochondrial Ca^{2+} cleared by mitochondria for WT and mtCaMKIIN cardiomyocytes, with and without CsA, n = 7 (WT), 4 (WT with CsA), 6 (mtCaMKIIN) and 4 (mtCaMKIIN with CsA), **p < 0.001 ***p<0.0001. Data represent mean ± s.e.m.



Supplemental Figure 7. Mitochondrial-targeted cameleon monitored Ca^{2+} uptake in HeLa cells. **a**. Targeting of cameleon 4mtD3cpv and mtCaMKIIN or control vector (myc-pcDNA) to mitochondria after 24 h of transfection in HeLa cells. The CFP fluorescence and YFP FRET signals are differentially responsive to addition of ATP. **b**. Representative tracings of basal FRET (YFP, F535) and the related CFP (F480) fluorescence in cells transfected with 4mtD3cpv along with mtCaMKIIN or control. As indicated, cells were stimulated with 100 μM ATP. Individual fluorescent responses of 4mtD3cpv that corresponded to the experiments shown in **a**. Summary data shown in Fig. 1f are taken from relative fluorescent intensities at arrow and arrowhead.



Supplemental Figure 8. CaMKII increases I_{MCU}. **a**. I_{MCU} is sensitive to Ru360 (10 nM) but not CsA (5 μM). n = 7 (control), 6 (CsA) and 5 (Ru360), ***p < 0.0001. Data represent mean ± s.e.m. **b**. Representative traces showing I_{MCU} is increased by a constitutively activated CaMKII mutant (T/D), with 0.2 mM Ca^{2+} and ATP, but not with a kinase inactive CaMKII (K/M), CaM and ATP. **c**. Representative traces showing I_{MCU} is increased by calyculin A (c-A) and no additional increase in I_{MCU} occurred with addition of activated CaMKII. **d**. Representative traces showing reduced I_{MCU} recorded with 0.2 mM Ca^{2+} in mitoplasts from CaMKIIN and mtCaMKIIN compared to WT hearts. **e**. Constitutively active CaMKII, T/D increases I_{MCU} on mitoplasts isolated from HEK cells overexpressing WT MCU, but not mutated (serines 57 and 92 to alanines, SS/AA) MCU.



Supplemental Figure 9. Left ventricular developed pressure (LVDP) recorded from WT and mtCaMKIIN Langendorff-perfused hearts. n = 14 hearts/genotype, data represent mean ± s.e.m.