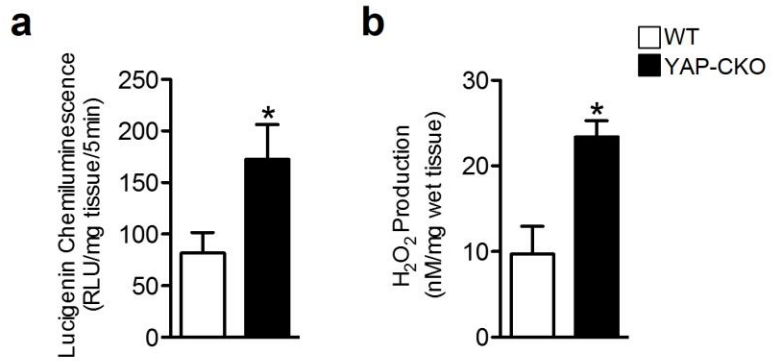
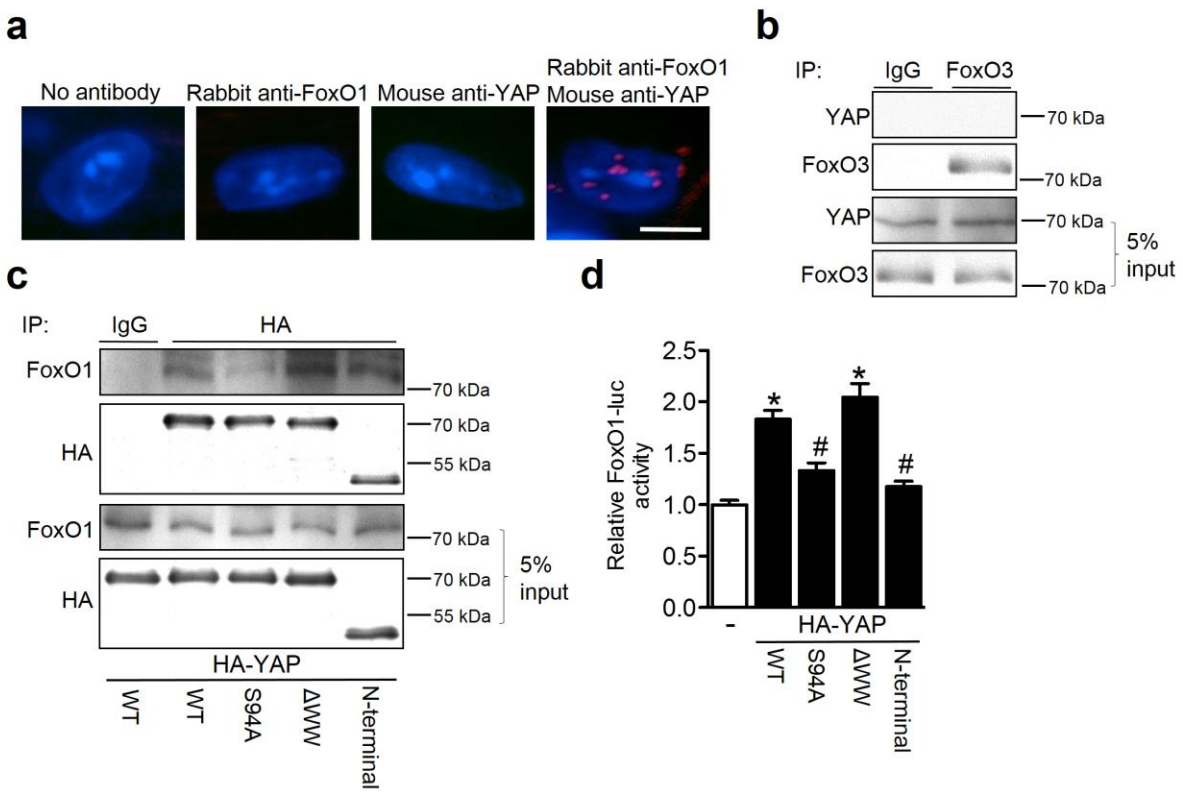


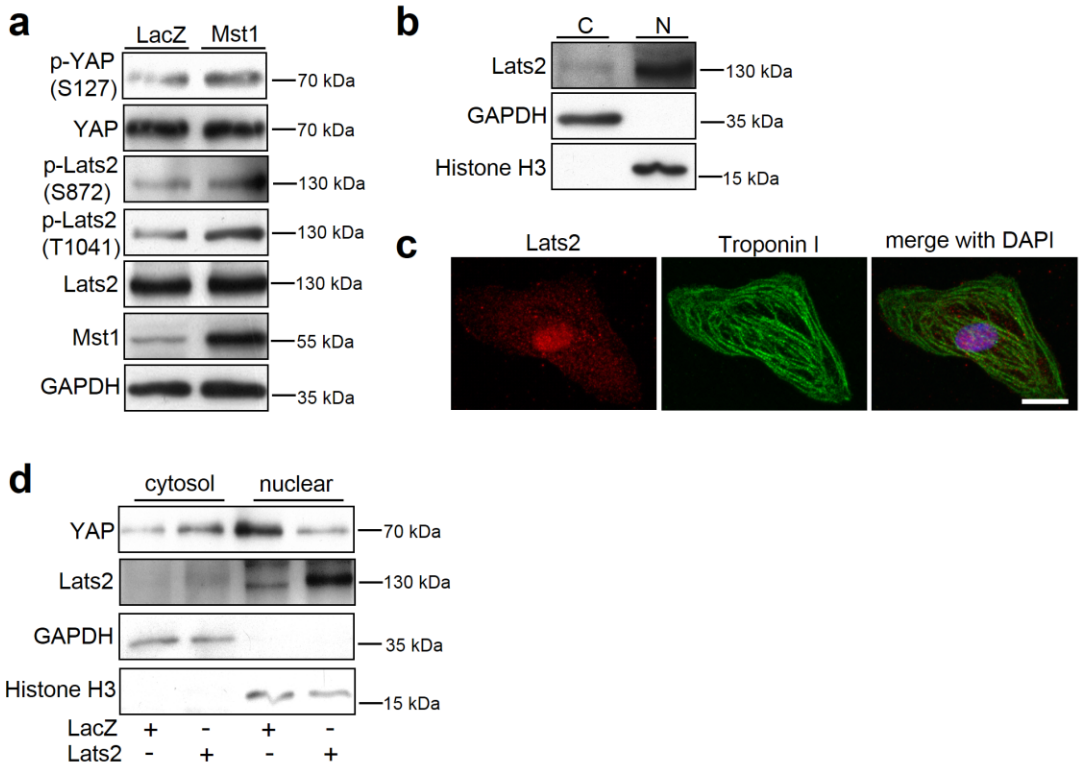
Supplementary Figure 1. **YAP KO mice show enhanced ROS accumulation in the heart.**

a, Cardiac-specific *Yap1* deletion (YAP-CKO) and control WT mouse hearts were incubated with 5 μM lucigenin. The O_2^- level was measured by lucigenin chemiluminescence (*$p<0.05$ vs. WT, n=5). **b,** YAP-CKO and control WT mouse hearts were incubated with 10 μM Amplex Red. The H_2O_2 production was measured (*$p<0.05$ vs. WT, n=4). Data shown as mean±s.e.m.. *P* values were determined using unpaired Student's t-test.



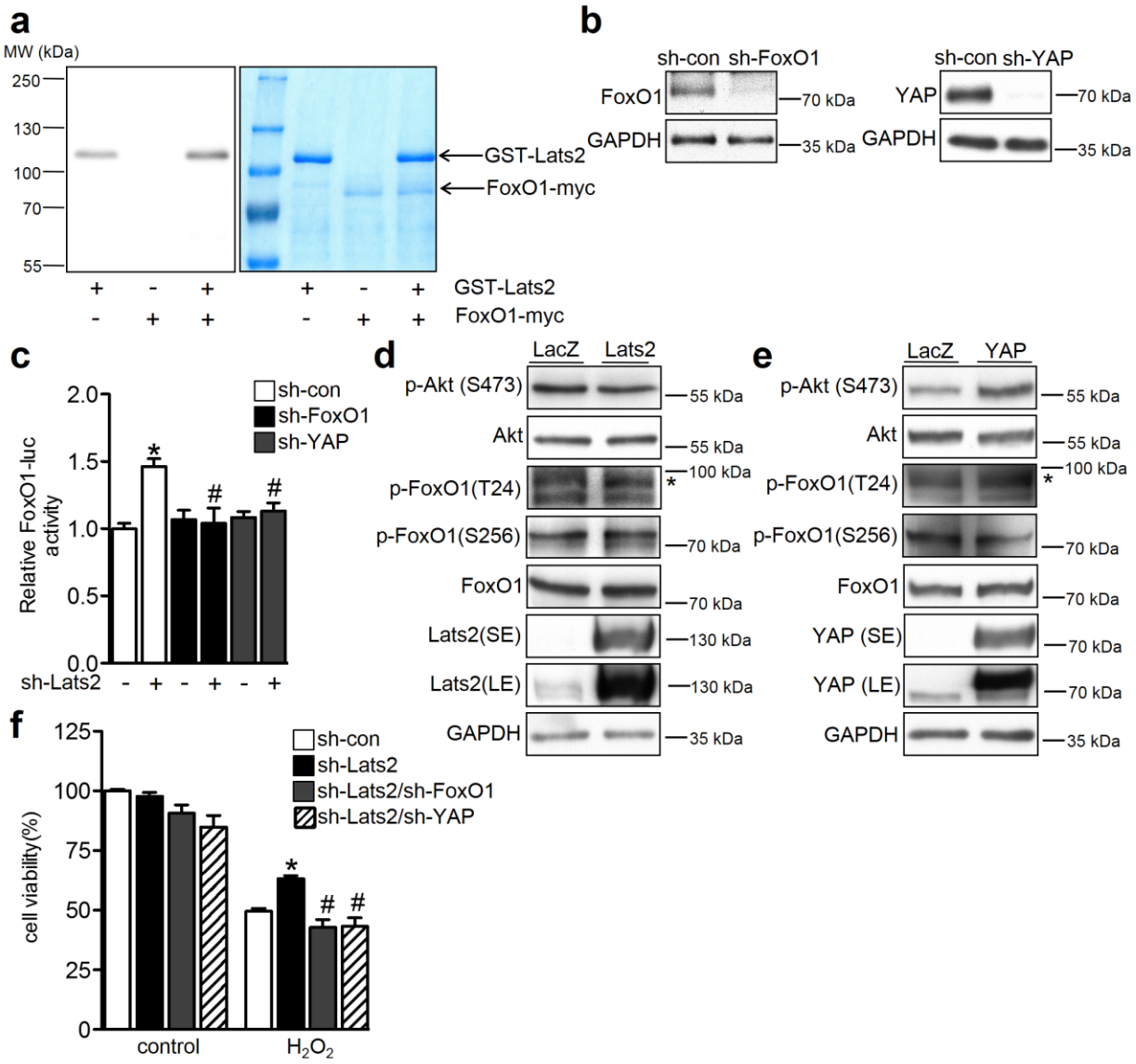
Supplementary Figure 2. Characterization of essential domains for YAP-mediated FoxO1 activation.

a, LV myocardial sections were stained with Rabbit anti-FoxO1 antibody and/or Mouse anti-YAP antibody, and *in vivo* protein–protein interaction between FoxO1 and YAP (red dots) was detected with secondary proximity probes, anti-Rabbit PLUS and anti-Mouse MINUS, using the Duolink *in situ* PLA detection kit. Scale bar, 20 μm. **b ,** Neonatal cardiomyocyte lysates were prepared and immunoprecipitated with FoxO3 antibody or control IgG. The interaction between FoxO3 and YAP was examined. YAP was not pulled down with FoxO3. Immunoblots of input lysate controls (5% of inputs) are also shown. **c,** The interaction between YAP and FoxO1 was evaluated. Cos7 cells were transfected with N-Terminal HA tagged YAP (HA-YAP) vectors containing various mutations. After 48 hours, cell lysates were prepared and immunoprecipitated with an HA antibody or control IgG, and immunoblot analyses with FoxO1 and HA antibodies were performed. Immunoblots of input lysate controls (5% of inputs) are also shown. **d**, Cardiomyocytes were transfected with 1 μg FoxO1-luc vector and the indicated expression vectors. After 48 hours, the luciferase activity was measured (*$p<0.05$ vs. empty vector, #$p<0.05$ vs. YAP/WT, n=3). Data shown as mean±s.e.m.. *P* values were determined using one-way ANOVA followed by a Newman-Keuls comparison test.



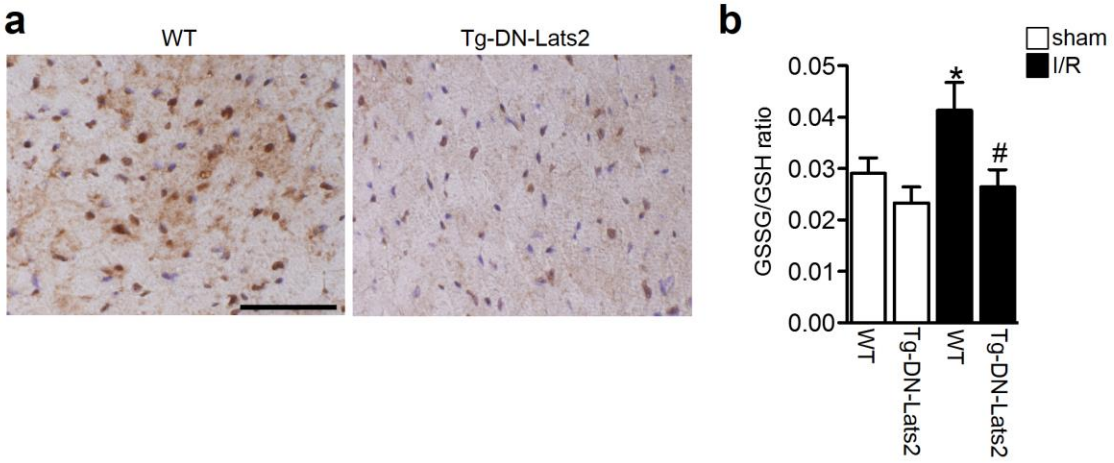
Supplementary Figure 3. Overexpression of Lats2 induces nuclear exclusion of YAP.

a, Cardiomyocytes were transduced with the indicated adenovirus for 48 hours. Lysates were used for immunoblot analysis of p-YAP (S127), YAP, p-Lats2 (T1041 and S872), Lats2, Mst1, and GAPDH. Results are representative of three individual experiments. **b-c**, Lats2 localization was examined. **b**, The cytosolic and nuclear fractions of neonatal cardiomyocytes were prepared. **c**, Myocytes were stained with Lats2 antibody (red), troponin I antibody (green), and DAPI (blue). Scale bar, 100 μm. **d**, Neonatal cardiomyocytes were transduced with the indicated adenovirus. Cytosolic and nuclear fractions were prepared in order to examine YAP localization. Results are representative of three individual experiments.



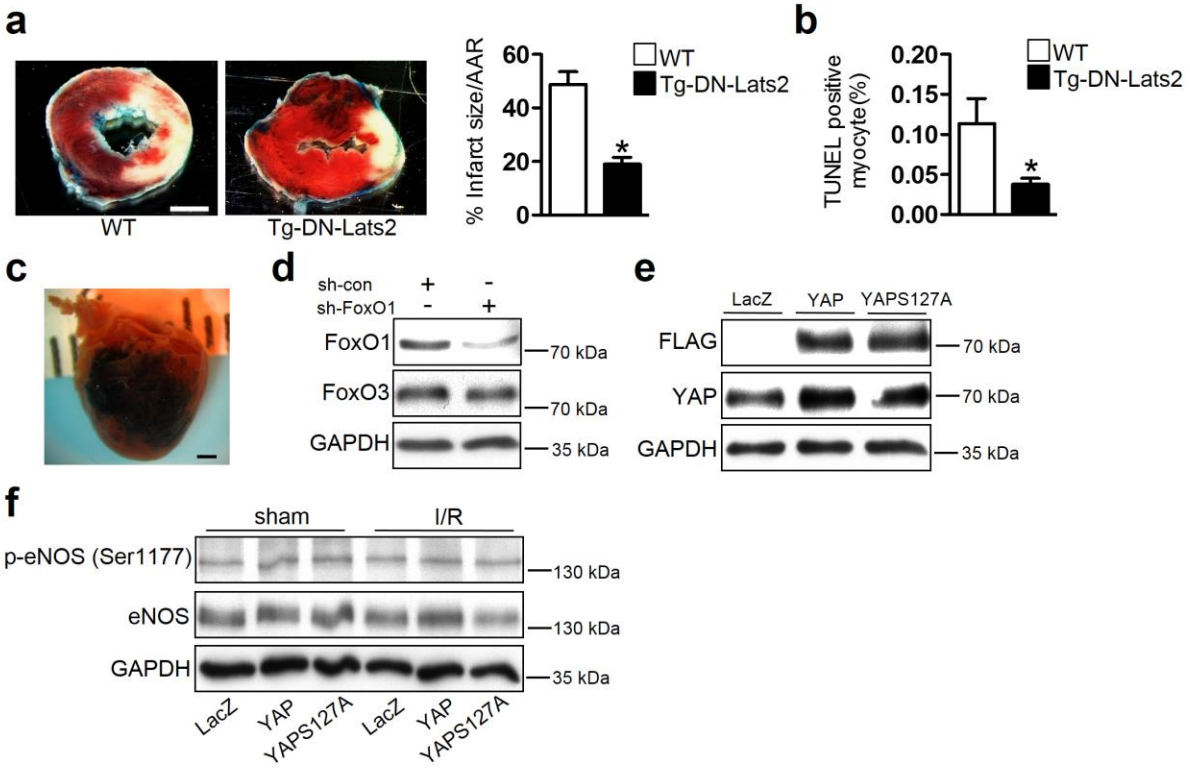
Supplementary Figure 4. Lats2-mediated FoxO1 transcriptional suppression is Akt-independent.

a, FoxO1-myc was incubated with GST-Lats2 and the phosphorylation status of FoxO1 and Lats2 was examined by autoradiography. **b**, Representative immunoblots are shown examining the knockdown efficiency of ad-sh-FoxO1 and ad-sh-YAP. Cardiomyocytes were transduced with the indicated adenovirus for 72 hours. Lysates were used for immunoblot analysis of FoxO1, YAP, and GAPDH. **c**, Cardiomyocytes were transfected with 1 μg FoxO1-luc vector. After 6 hours, myocytes were transduced with the indicated virus for 72 hours and luciferase activity was measured (*p<0.05 vs. sh-con/sh-Lats2(-), #p<0.05 vs. sh-con/sh-Lats2(+), n=4). **d-e**, Cardiomyocytes were transduced with the indicated adenovirus for 48 hours. Lysates were used for immunoblot analysis of p-Akt (S473), Akt, p-FoxO1 (T24 and S256), FoxO1, Lats2, YAP, and GAPDH. *The p-FoxO1 (T24) antibody also cross-reacted with p-FoxO3 (T32). Results are representative of three individual experiments (SE, short exposure; LE, long exposure). **f**, Cardiomyocytes transduced with the indicated adenovirus were treated with or without H_2O_2 and cell viability was examined (*p<0.05 vs. sh-con/H_2O_2, #p<0.05 vs. sh-Lats2/H_2O_2, n=4). Data shown as mean±s.e.m.. *P* values were determined using one-way ANOVA followed by a Newman-Keuls comparison test.



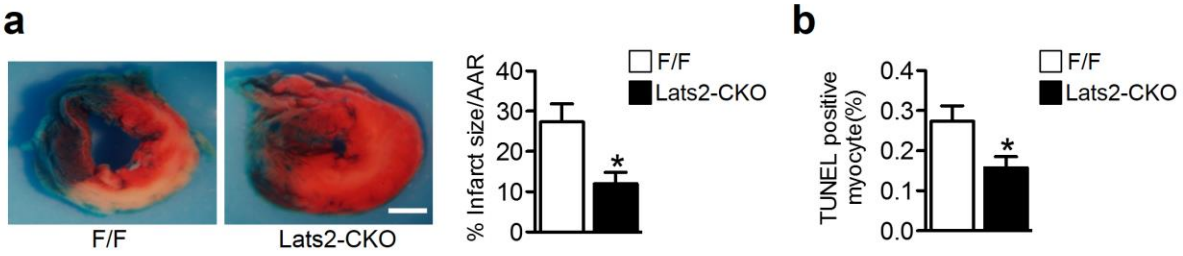
Supplementary Figure 5. Lats2 activation enhances ROS accumulation in the heart in response to I/R.

a-b, Tg-DN-Lats2 and control WT mice were subjected to ischemia for 45 minutes followed by 24 hours of reperfusion. **a**, Representative immunohistochemistry staining for 8-OHdG in LV myocardial sections from the ischemic area in Tg-DN-Lats2 and WT mice. Scale bar, 100 μm. **b**, The tissue GSSG/GSH ratio was determined in Tg-DN-Lats2 and WT mice (*$p<0.05$ vs. WT/sham, #$p<0.05$ vs. WT/I/R, n=8). Data shown as mean±s.e.m.. *P* values were determined using one-way ANOVA followed by a Newman-Keuls comparison test.



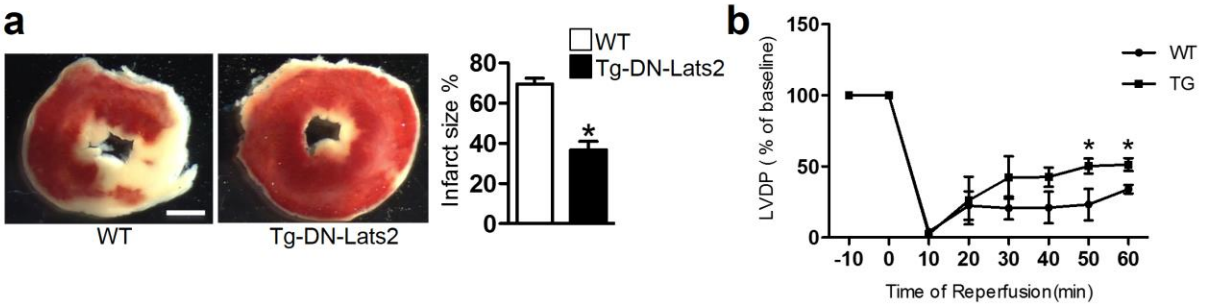
Supplementary Figure 6. Tg-DN-Lats2 mice reduce I/R injury.

a-b, Tg-DN-Lats2 and control WT mice were subjected to ischemia for 45 minutes followed by 24 hours of reperfusion. **a**, Gross appearance of LV tissue sections after Alcian blue and triphenyltetrazolium chloride staining in Tg-DN-Lats2 and WT mice. The myocardial infarct area/area at risk (% infarct size/AAR) was determined as described (*$p<0.05$ vs. WT, n=5). Scale bar, 1 mm. **b**, LV myocardial sections were subjected to TUNEL staining. The number of TUNEL-positive myocytes was expressed as a percentage of total nuclei detected by DAPI staining (*$p<0.05$ vs. WT, n=5). **c**, Purified LacZ control adenovirus (1×10^9 opu) was injected into the mouse heart. A representative image of the heart with X-gal staining three days after Ad-LacZ injection. Scale bar, 1 mm. **d**, Purified adenovirus (1×10^9 opu) was administered by direct injection to the LV free wall (two sites, 25 μl/site). Five days after injection, heart homogenates were used to examine the efficiency of FoxO1 knockdown. A representative immunoblot indicating that ad-sh-FoxO1 specifically decreased FoxO1 expression but not FoxO3 expression. **e-f**, Purified adenovirus (1×10^9 opu) was administered by direct injection to the LV free wall (two sites, 25 μl/site). **e**, Three days after injection, heart homogenates were used to examine the efficiency of transduction of ad-YAP and ad-YAP (S127A) containing N-terminal FLAG tag. FLAG was also used to examine exogenous gene expression. **f**, Three days after injection, mice were subjected to ischemia for 30 minutes followed by 24 hours of reperfusion. Sham and ischemic area samples were used for immunoblot analysis of p-eNOS (Ser1177), eNOS, and GAPDH. Data shown as mean±s.e.m.. *P* values were determined using unpaired Student's t-test.



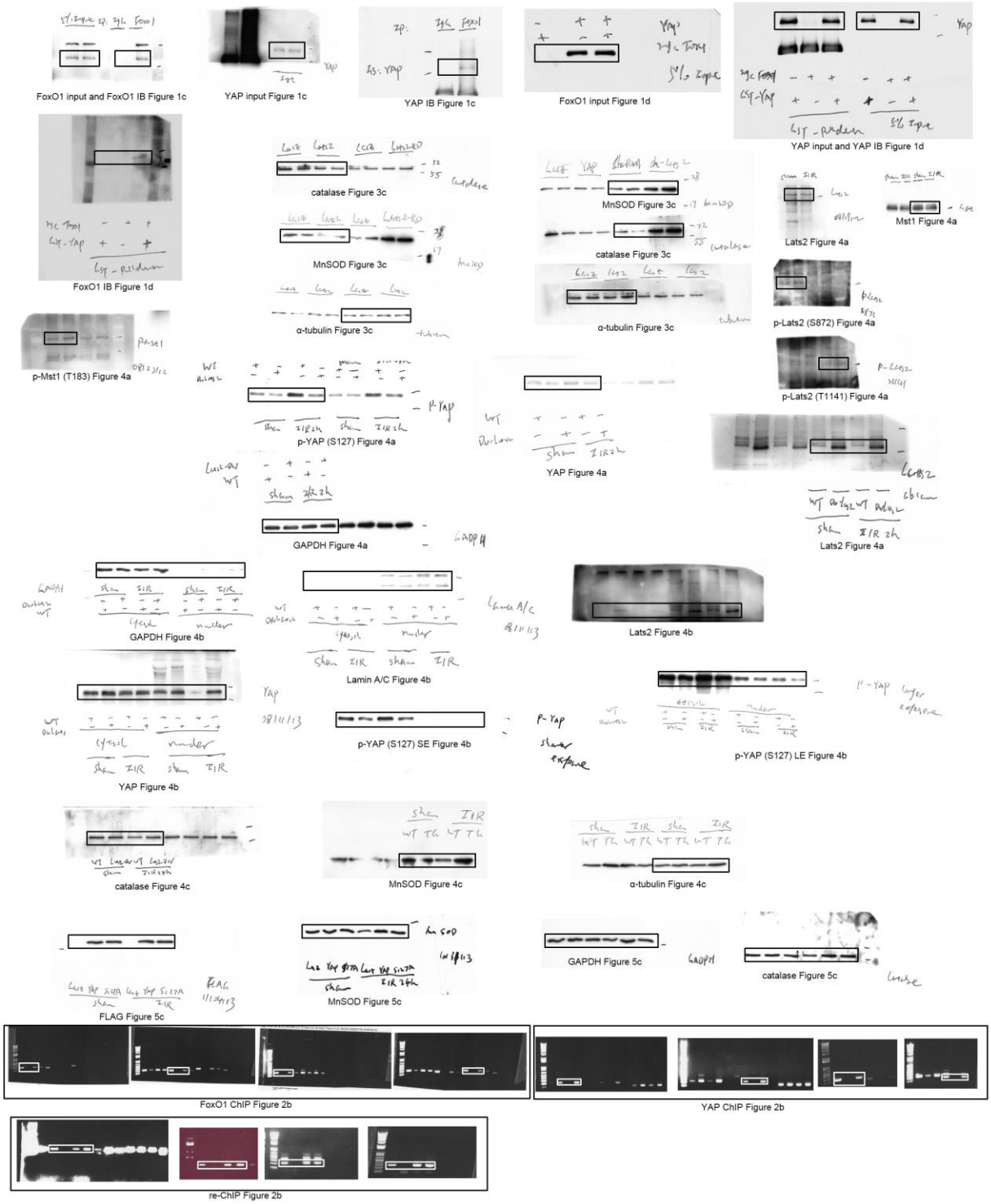
Supplementary Figure 7. Lats2 CKO mice reduce I/R injury.

a, Lats2-CKO and control floxed (F/F) mice were subjected to ischemia for 30 minutes, followed by 24 hours of reperfusion. Gross appearance of LV tissue sections after Alcian blue and triphenyltetrazolium chloride staining in Lats2-CKO and F/F mice. The myocardial infarct area/area at risk (% infarct size/AAR) was determined as described (*p<0.05 vs. F/F, n=3). Scale bar, 1 mm. **b**, Lats2-CKO and control floxed (F/F) mice were subjected to ischemia for 30 minutes, followed by 24 hours of reperfusion. The number of TUNEL-positive myocytes was expressed as a percentage of total nuclei detected by DAPI staining (*p<0.05 vs. WT, n=4-5). Data shown as mean±s.e.m.. *P* values were determined using unpaired Student's t-test.



Supplementary Figure 8. Tg-DN-Lats2 mice exhibit better cardiac function after I/R.

a-b, The hearts of Tg-DN-Lats2 or WT mice were subjected to 30 minutes of global ischemia and 60 minutes of reperfusion. **a**, Gross appearance of LV tissue after triphenyltetrazolium chloride staining. The infarct area/total LV was measured (*p<0.05 vs. WT, n=5). Scale bar, 1 mm. **b**, LV developed pressure (LVDP) was measured. The baseline level is designated as 100% (*p<0.05 vs. WT, n=5). Data shown as mean±s.e.m.. *P* values were determined using unpaired Student's t-test.



Supplementary Figure 9. Full blots and gels for main text figures.