

## Supplementary Information

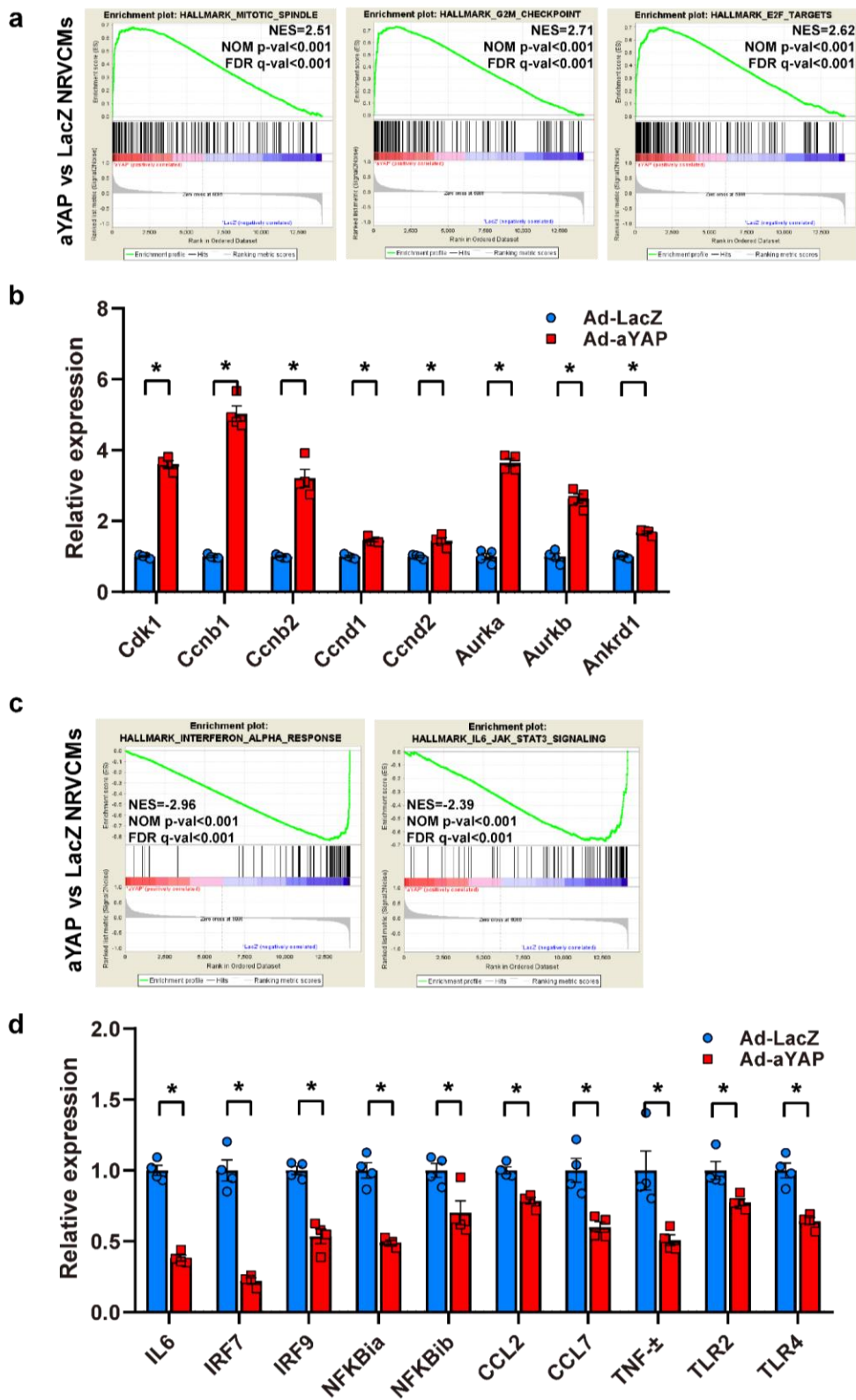
### Intercalated disc protein $Xin\beta$ is required for Hippo-YAP signaling in the heart

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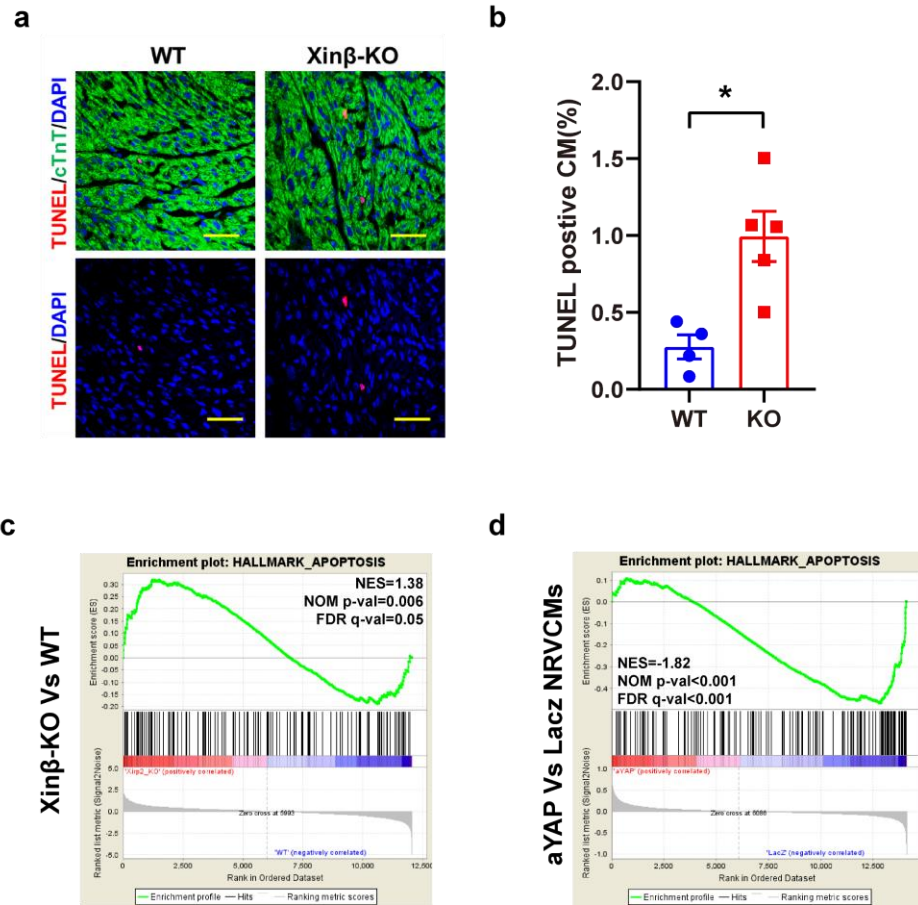
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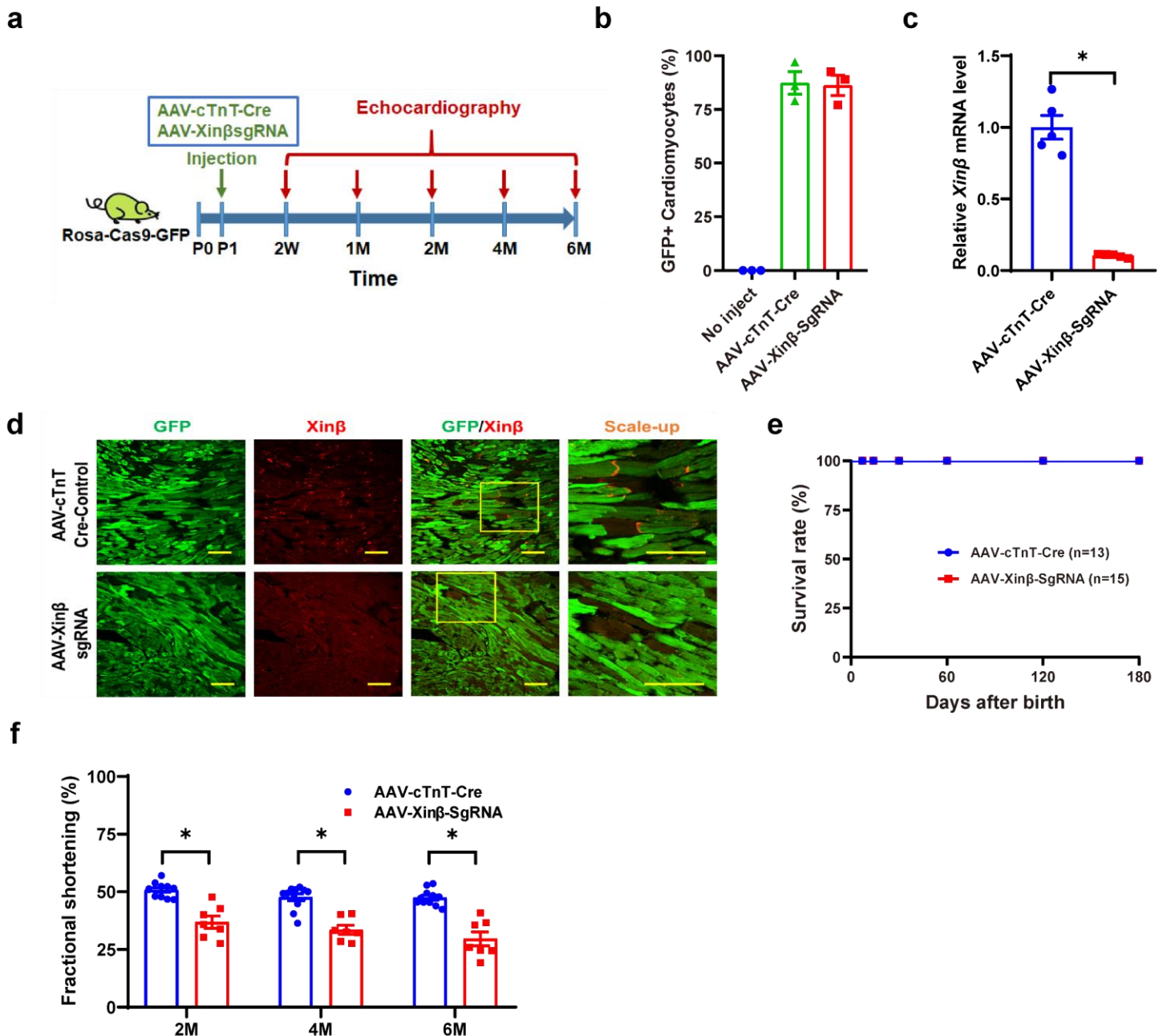
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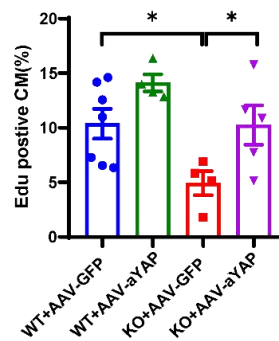
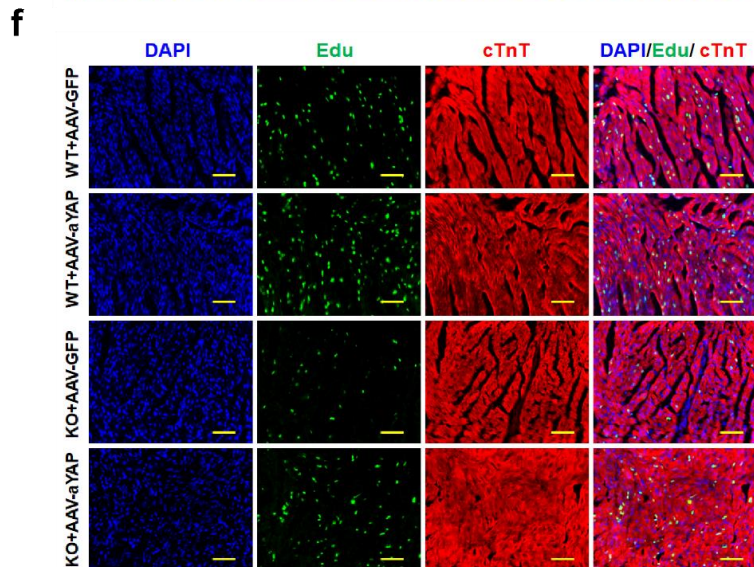
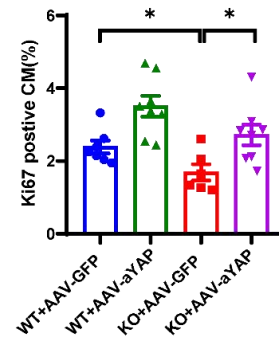
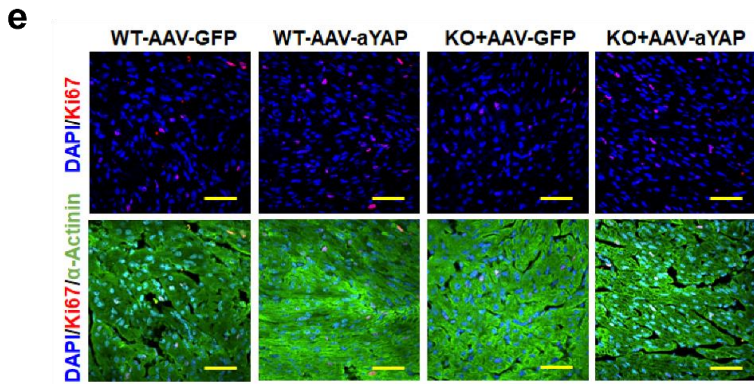
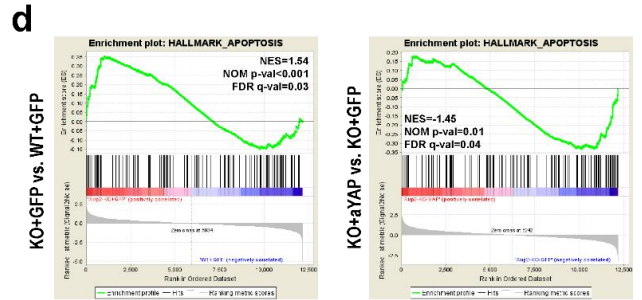
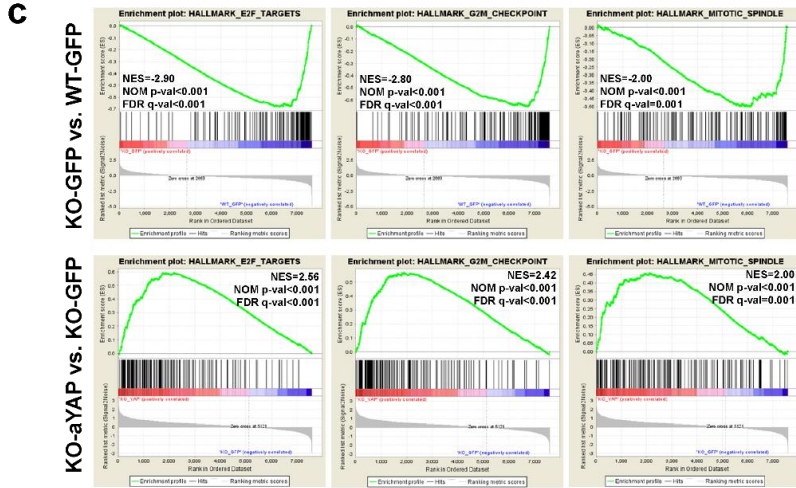
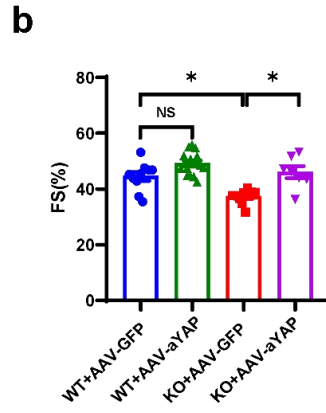
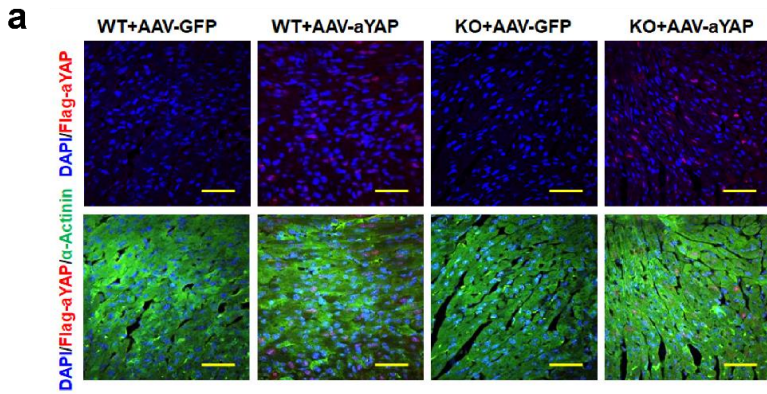
**Supplementary Figure 1. Loss of  $Xin\beta$  results in alteration of the Hippo-YAP signaling pathway in the heart. a**, Enrichment plot showing upregulation of genes related to the mitotic spindle, G2M checkpoint, and E2F targets in Ad-aYAP-treated cardiomyocytes. **b**, qRT-PCR of cell cycle related genes in Ad-aYAP cardiomyocytes. N = 4 biologically independent samples, asterisk P<0.05. **c**, Enrichment plot showing downregulation of genes related to the interferon- $\alpha$  response and IL6-JAK-STAT3 signaling in Ad-aYAP-treated cardiomyocytes. **d**, qRT-PCR of innate immune response genes in Ad-aYAP-treated cardiomyocytes. N = 4 biologically independent samples, asterisk P<0.05. Exact P-values can be found in the Source Data.



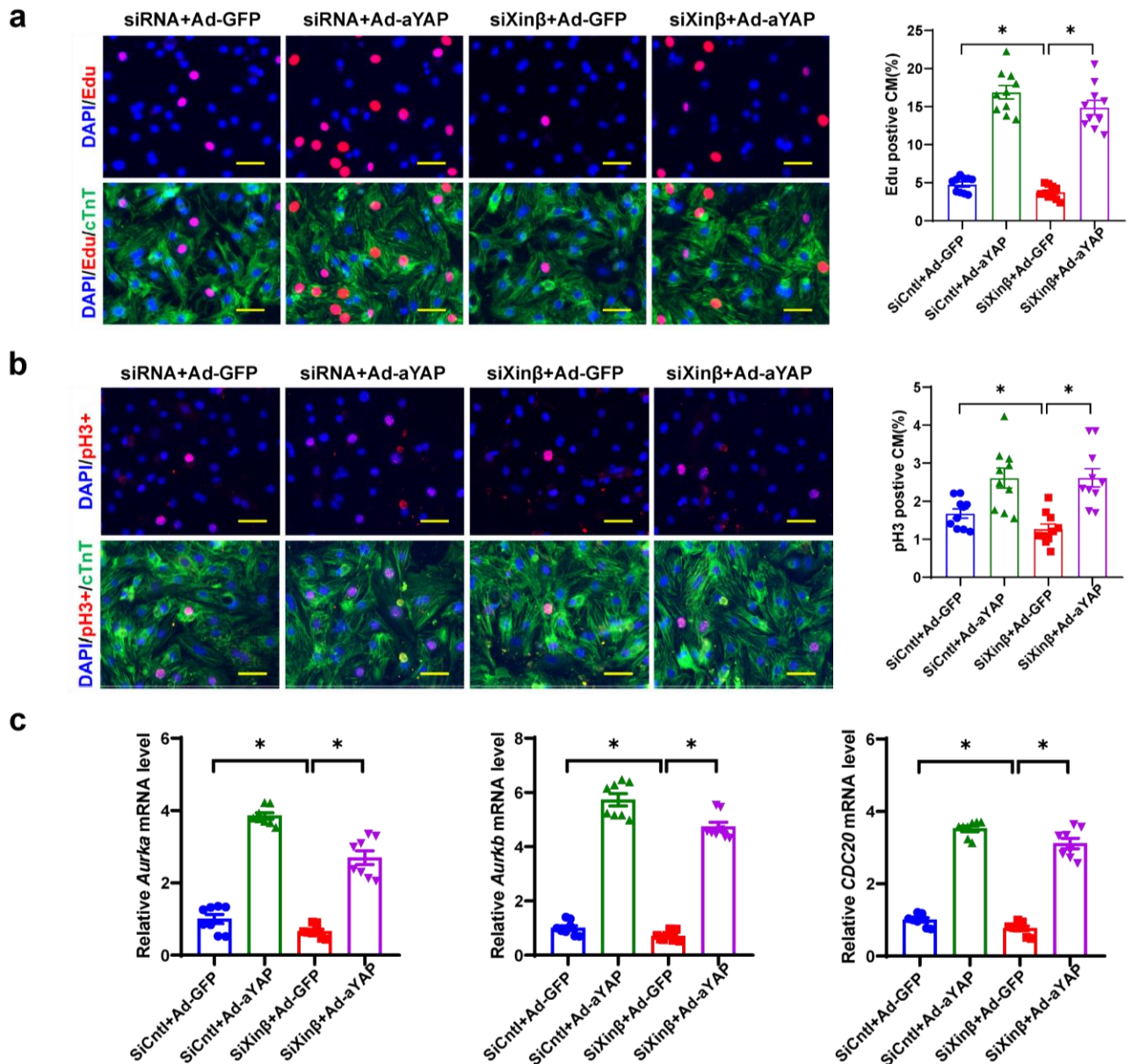
**Supplementary Figure 2. Increased apoptosis in cardiomyocytes of *Xinβ*<sup>KO</sup> hearts.** **a**, TUNEL assays in P7.5 *Xinβ*<sup>KO</sup> and control hearts. DAPI marks nuclei, cTnT labels cardiomyocytes. Scale bars = 100  $\mu$ m. **b**, Quantification of TUNEL staining data. N = 5 biologically independent samples and 4 biologically independent samples, respectively, asterisk P<0.05. **c**, Enrichment plot showing upregulation of genes related to apoptosis in P7.5 *Xinβ*<sup>KO</sup> hearts. **d**, Enrichment plot showing downregulation of genes related to apoptosis in Ad-aYAP-treated cardiomyocytes. Exact P-values can be found in the Source Data.



**Supplementary Figure 3. AAV9-CAS strategy to generate a *Xin $\beta$*  mutation in adult mice.** **a**, Schematic diagram of the mutation strategy. **b**, Quantification of GFP positive cardiomyocytes from AAV-*cTnT-Cre* or AAV-*Xin $\beta$ <sup>sgRNA</sup>* injected mice. N = 3 biologically independent samples, asterisk P<0.05. **c**, qRT-PCR quantification of *Xin $\beta$*  expression in the hearts of AAV-*cTnT-Cre* or AAV-*Xin $\beta$ <sup>sgRNA</sup>* injected mice. N = 5 biologically independent samples, asterisk P<0.05. **d**, Immunohistochemical staining to detect *Xin $\beta$*  expression in adult hearts from AAV-*cTnT-Cre* or AAV-*Xin $\beta$ <sup>sgRNA</sup>* injected mice. Scale bars = 20  $\mu$ m. **e**, Survival curve of AAV-*cTnT-Cre* or AAV-*Xin $\beta$ <sup>sgRNA</sup>* injected mice. N = 13 or 15 biologically independent samples, respectively. **f**, Cardiac fractional shortening (FS%) in AAV-*cTnT-Cre* or AAV-*Xin $\beta$ <sup>sgRNA</sup>* injected mice at 2, 4, and 6 months (M) of age. N = 11 and 7 biologically independent samples, respectively, asterisk P<0.05. Exact P-values can be found in the Source Data.



**Supplementary Figure 4. Heart-specific YAP<sup>S127A</sup> overexpression rescues cardiac gene expression in *Xinβ*<sup>KO</sup> mice.** **a**, Immunohistochemical staining to detect expression of Flag-tagged YAP in the hearts of P7.5 *Xinβ*<sup>KO</sup> and control mice injected with AAV-*aYAP* (YAP<sup>S127A</sup>) or control AAV-*GFP*. DAPI marks nuclei,  $\alpha$ -actinin labels cardiomyocytes. Scale bars = 40  $\mu$ m. **b**, Fractional shortening (FS%) of P7.5 *Xinβ*<sup>KO</sup> and control mice injected with AAV-*YAP* or control AAV-*GFP*. N = 7 biologically independent samples (*Xinβ*<sup>KO</sup>) and 15 or 10 biologically independent samples (control), respectively, asterisk P<0.05. **c**, Enrichment plot showing restored expression of genes related to cell proliferation in P7.5 *Xinβ*<sup>KO</sup> mice after injection of AAV-*aYAP*. **d**, Enrichment plot showing inhibition of the expression of genes related to apoptosis in P7.5 *Xinβ*<sup>KO</sup> mice after injection of AAV-*aYAP*. **e**, Ki67 staining of hearts from P7.5 *Xinβ*<sup>KO</sup> and control mice injected with AAV-*aYAP* or control AAV-*GFP*. DAPI marks nuclei,  $\alpha$ -actinin labels cardiomyocytes. Quantification is shown in the right panel. Scale bars = 40  $\mu$ m, N = 6 biologically independent samples (*Xinβ*<sup>KO</sup> AAV-*GFP*) or 7 biologically independent samples (*Xinβ*<sup>KO</sup> AAV-*aYAP*) and 7 biologically independent samples (control AAV-*aYAP* and AAV-*GFP*), respectively, asterisk P<0.05. **f**, EdU incorporation in hearts from P7.5 *Xinβ*<sup>KO</sup> and control mice injected with AAV-*aYAP* or control AAV-*GFP*. DAPI marks nuclei, cTnT labels cardiomyocytes. Quantification is shown in the right panel. Scale bars = 40  $\mu$ m. N = 5 biologically independent samples (*Xinβ*<sup>KO</sup> AAV-*aYAP*), 4 (*Xinβ*<sup>KO</sup> AAV-*GFP*), 4 biologically independent samples (control AAV-*aYAP*), and 7 biologically independent samples (control AAV-*GFP*), asterisk P<0.05. Exact P-values can be found in the Source Data.



**Supplementary Figure 5. Regulation of cardiomyocyte proliferation by *Xinβ* and YAP.** **a**, Primary neonatal (P1) rat ventricular cardiomyocytes (NRVMs) were transfected with siRNA against *Xinβ* (*siXinβ*) or control siRNA. Cells were transduced with adenovirus expressing either an active YAP (Ad-aYAP, which contains the S127A mutation [*YAP<sup>S127A</sup>]*) or Ad-GFP. Cells were incubated with EdU (10  $\mu$ M). After one day, cultures were fixed and stained with antibodies for EdU. Cardiac troponin T (cTnT) marks cardiomyocytes. DAPI stains nuclei. Scale bars = 70  $\mu$ m. Quantification of percentages of EdU<sup>+</sup> cardiomyocytes is presented in the right panel. N = 10 biologically independent samples, asterisk P<0.05. **b**, Primary neonatal (P1) rat cardiomyocytes were transfected with siRNA against *Xinβ* (*siXinβ*) or control siRNA. Cells were transduced with an active YAP (Ad-aYAP) or control (Ad-GFP). After one day, cultures were fixed and stained with antibodies for phospho-histone H3 (pH3). Cardiac troponin T (cTnT) marks cardiomyocytes. DAPI stains nuclei. Scale bars = 70  $\mu$ m. Quantification of percentages of pH3<sup>+</sup> cardiomyocytes is presented in the right panel. N = 10 biologically independent samples, asterisk P < 0.05. **c**, Quantitative RT-PCR (qRT-PCR) analyses of the expression of cell proliferation marker genes in NRVMs cultured in the above conditions. N = 8 biologically independent samples, asterisk P<0.05. Exact P-values can be found in the Source Data.