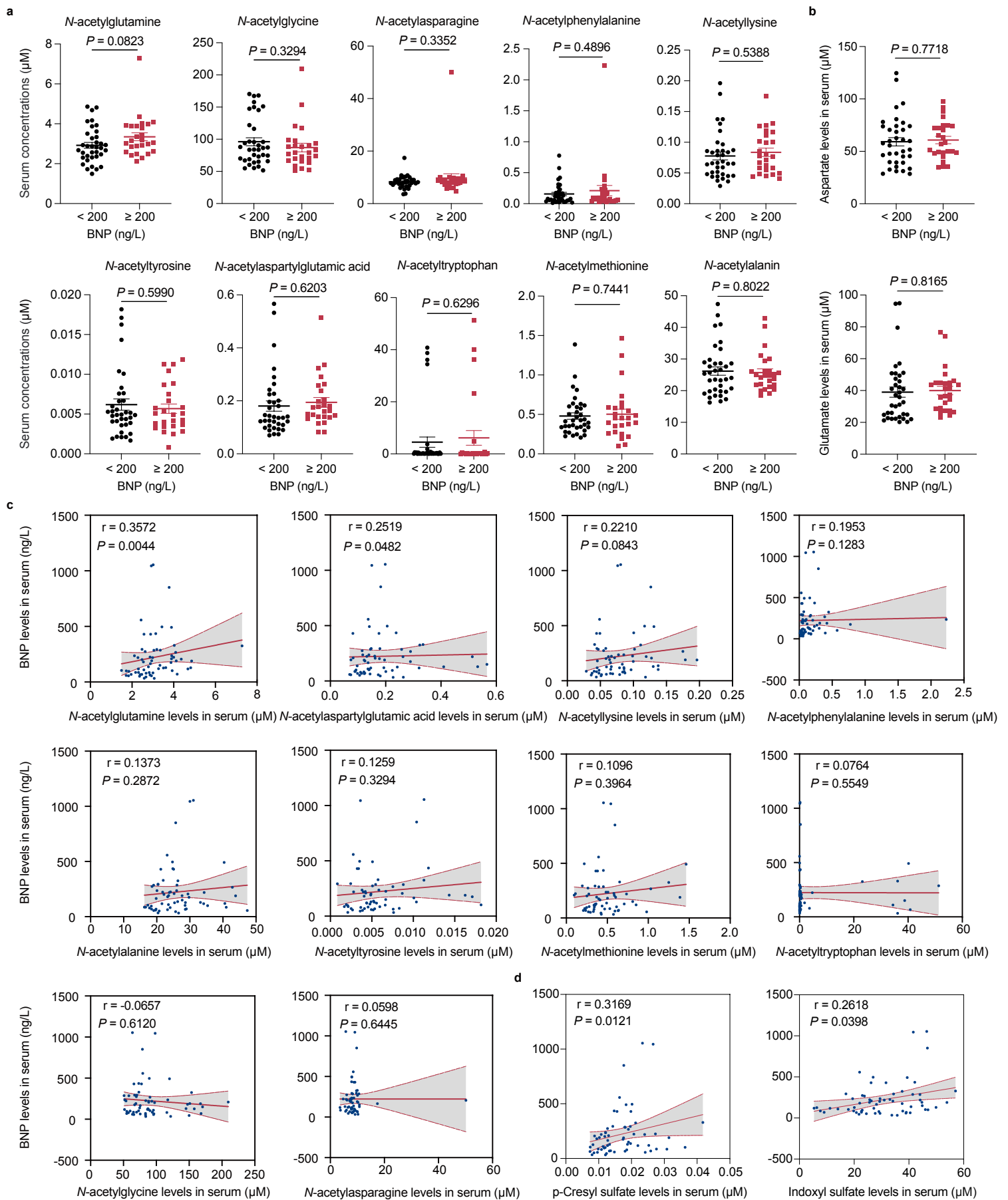


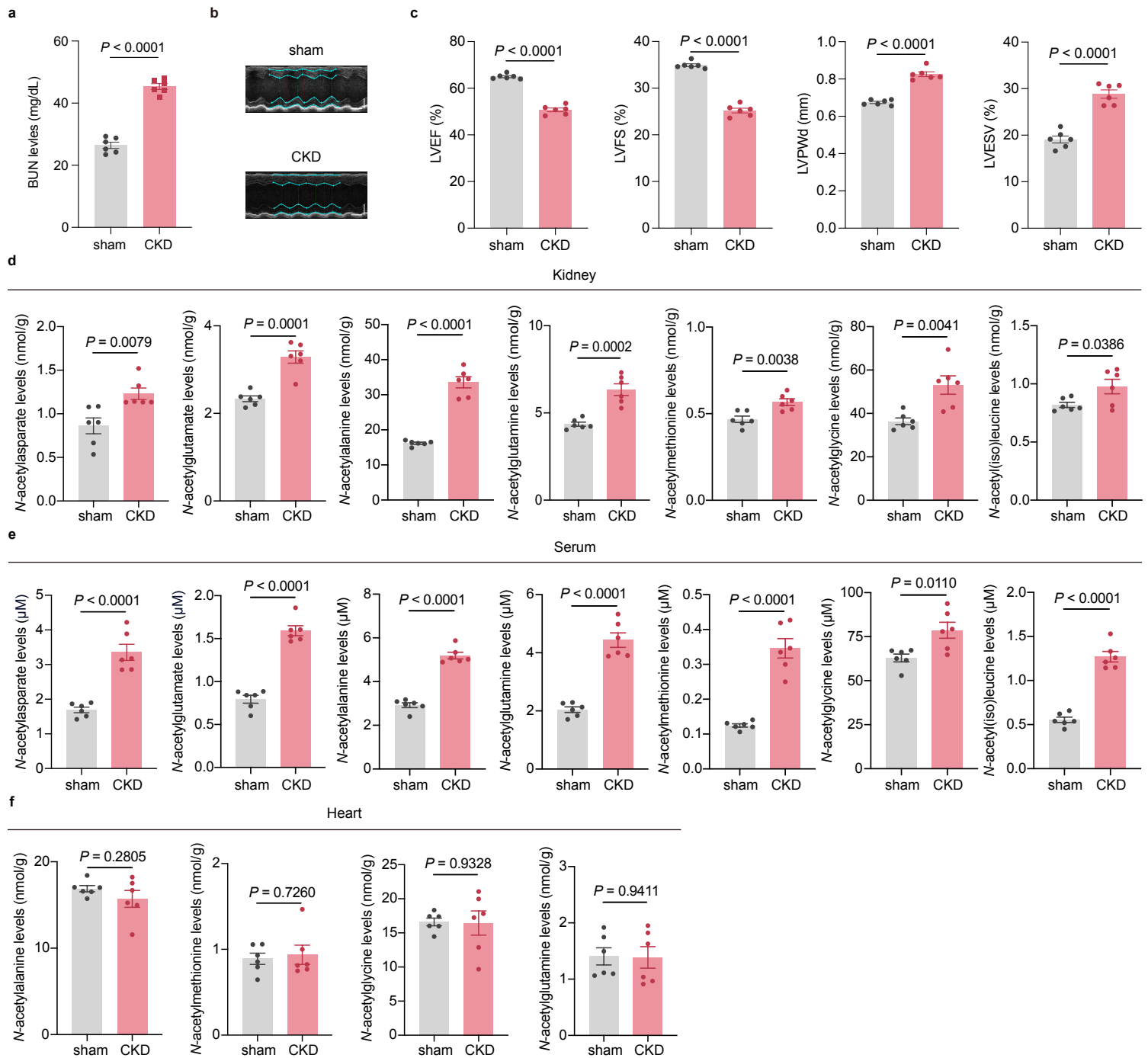


Extended Data Fig. 1. Related to Fig. 1. Elevated circulating NAA and NAG are associated with increased BNP levels in CKD patients.

a, b. Circulating *N*-acetylated amino acids (**a**), aspartate and glutamate (**b**) levels in CKD patients with BNP < 200 ng/L (*n* = 36) or ≥ 200 ng/L (*n* = 26).

c, d. Pearson correlation of *N*-acetylated amino acids (**c**), p-Cresyl sulfate and indoxyl sulfate (**d**) with BNP levels in serum; *n* = 62.

Data are presented as mean ± SEM. Statistical analysis was performed using unpaired two-tailed Student's *t*-test (**a, b**).



Extended Data Fig. 2. Related to Fig.1. CKD leads to cardiac dysfunction and elevated *N*-acetylated amino acids.

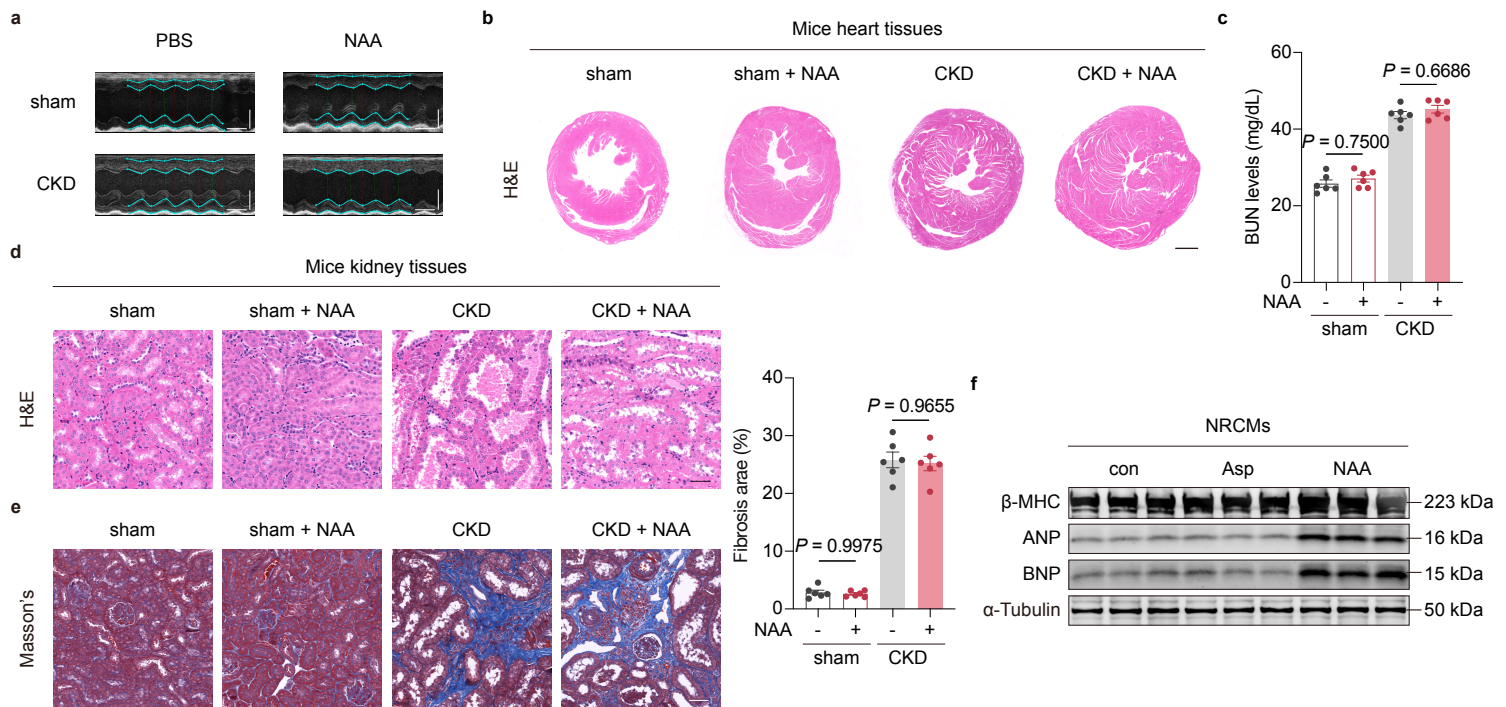
a, Elevated blood urea nitrogen (BUN) levels 8 weeks after 5/6 subtotal nephrectomy confirmed the successful establishment of CKD in mice.

b, Representative M-mode echocardiographic images of the left ventricle from sham and CKD mice. Cyan contours mark the endocardial and epicardial borders. Red dashed lines indicate end-diastole, and green dashed lines indicate end-systole. Horizontal scale bar = 0.1 s, vertical scale bar = 2 mm.

c, Echocardiographic assessment showing reduced left ventricular ejection fraction (LVEF) and fractional shortening (LVFS), and increased left ventricular end-systolic volume (LVESV) and posterior wall thickness at end-diastole (LVPWd) in CKD mice compared with sham controls.

d-f, *N*-acetylated amino acids levels in kidney (**d**), serum (**e**) and cardiac (**f**) samples from sham and CKD mice.

Data are presented as mean $\pm$ SEM, $n = 6$ mice per group. Statistical analysis was performed using unpaired two-tailed Student's *t*-test (**a**, **c**, **d-f**).



Extended Data Fig. 3. Related to Fig.1. NAA exacerbates cardiac dysfunction without altering renal injury.

a, Representative M-mode echocardiographic images of the left ventricle from sham and CKD mice with NAA treatment (200 mg per kg per day). Cyan contours mark the endocardial and epicardial borders. Red dashed lines indicate end-diastole, and green dashed lines indicate end-systole. Horizontal scale bar = 0.1 s, vertical scale bar = 2 mm.

b, Representative images of Hematoxylin and Eosin (H&E) staining of cardiac tissues from sham and CKD mice with NAA treatment. Scale bar = 1 mm.

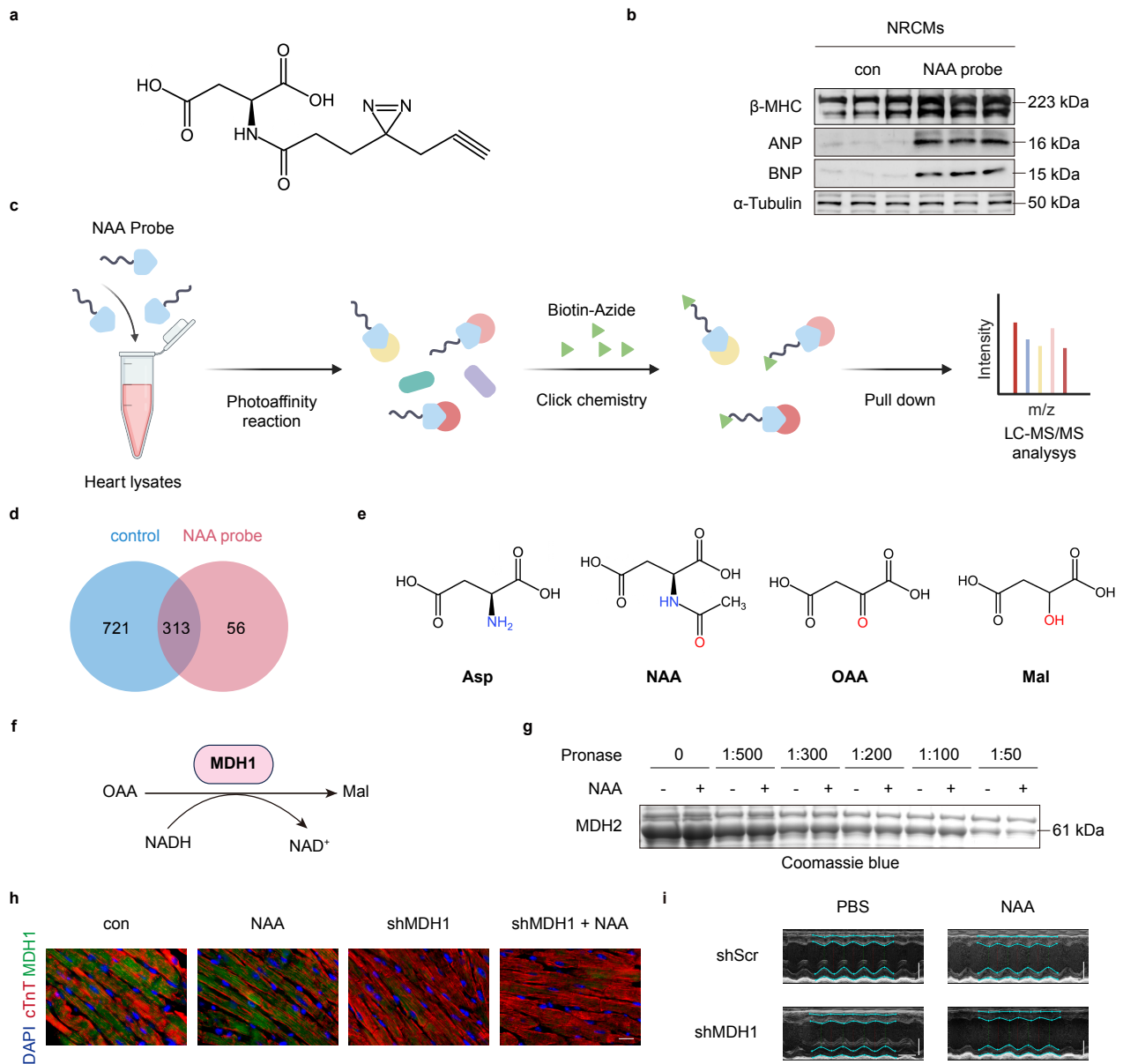
c, BUN levels showing no significant change after NAA administration.

d, Representative images of H&E staining of kidney tissues from sham and CKD mice with NAA treatment. Scale bar = 50 μm.

e, Masson's trichrome staining of kidney tissues showed that NAA treatment did not cause significant changes in collagen deposition. Scale bar = 50 μm.

f, Western blotting analysis of hypertrophic markers β-MHC, ANP, and BNP in NRCMs after 24 h treatment with Asp (0.5 mM) or NAA (0.5 mM).

Data are presented as mean ± SEM, n = 6 mice per group. Statistical analysis was performed using two-way ANOVA with Tukey's post hoc test (**c**, **e**).



Extended Data Fig. 4. Related to Fig. 2. MDH1 is the primary target mediating NAA-induced cardiac dysfunction.

a, Chemical structure of the photoaffinity-tagged NAA probe used for activity-based protein profiling (ABPP).

b, Western blotting analysis of hypertrophic markers β -MHC, ANP, and BNP in NRCMs after 24 h of treatment with 0.5 mM NAA probe.

c, Schematic of ABPP workflow: heart lysates were incubated with the NAA probe or non-tagged NAA as a control, followed by UV crosslinking, click chemistry-mediated enrichment, and mass spectrometry analysis.

d, Venn Diagram showing 56 candidate NAA-interacting proteins enriched by the NAA probe compared with control.

e, Chemical structures of aspartate (Asp), NAA, oxaloacetate (OAA), and malate (Mal).

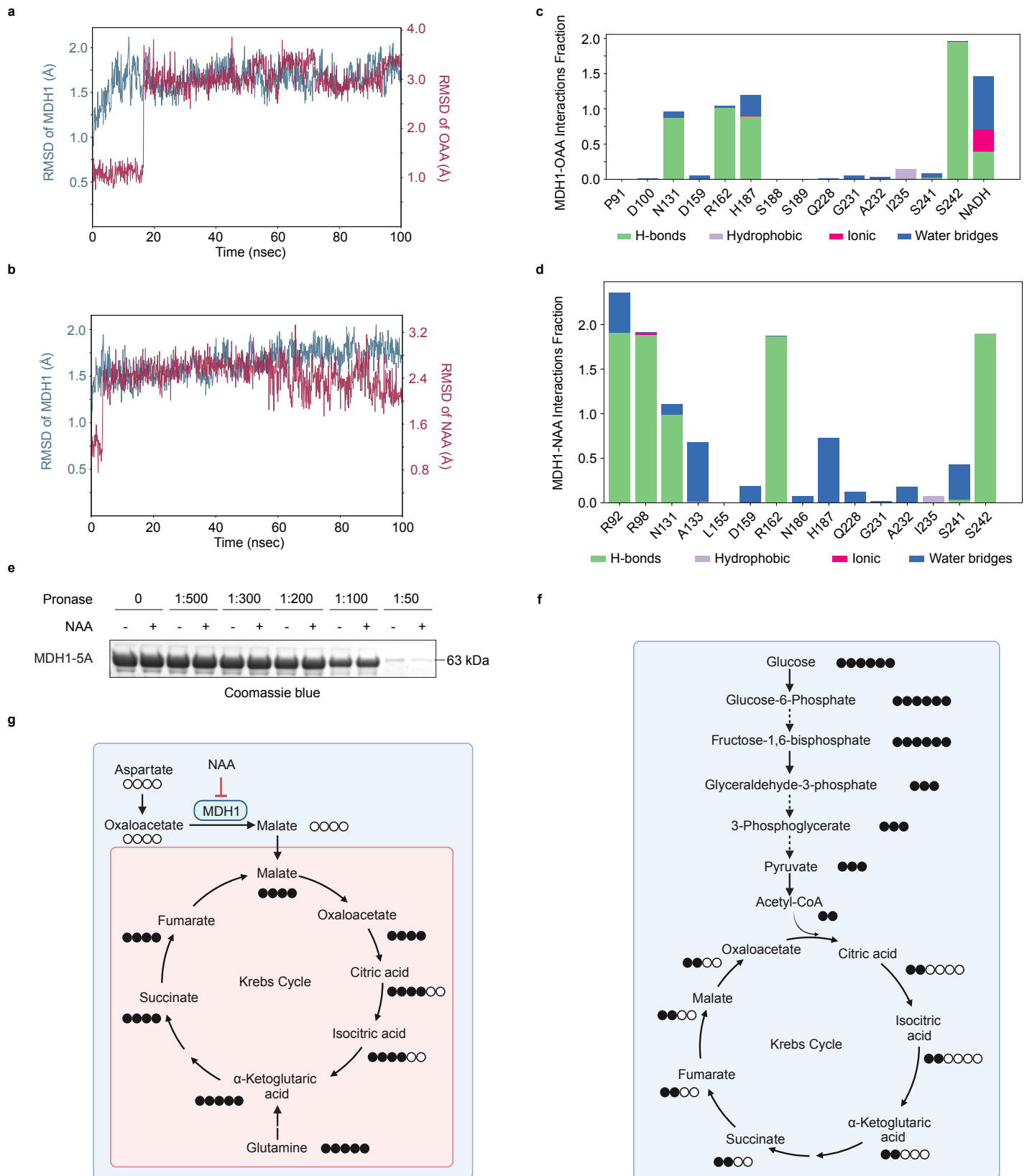
f, Schematic illustrating MDH1-catalyzed conversion of OAA to Mal.

g, DARTS assay showing that NAA did not alter the stability of purified recombinant MDH2 against Pronase digestion.

h, Representative immunofluorescence images of cardiac tissue confirming cardiomyocyte-specific MDH1 knockdown. Scale bar = 20 μ m; n = 6 mice per group.

i, Representative M-mode echocardiographic images of the left ventricle from con, NAA, shMDH1, and shMDH1 + NAA. Cyan contours mark the endocardial and epicardial borders. Red dashed lines indicate end-diastole, and green dashed lines indicate end-systole. Horizontal scale bar = 0.1 s, vertical scale bar = 2 mm; n = 6 mice per group.

Schematic in **c** was created using BioRender (<https://BioRender.com>).



Extended Data Fig. 5. Related to Fig. 2 and 3. NAA binds MDH1 and modulates glycolysis and TCA cycle activity.

a, b. Molecular dynamics simulation showing Root Mean Square Deviation (RMSD) trajectories of MDH1-OAA (**a**) and MDH1-NAA (**b**) complexes.

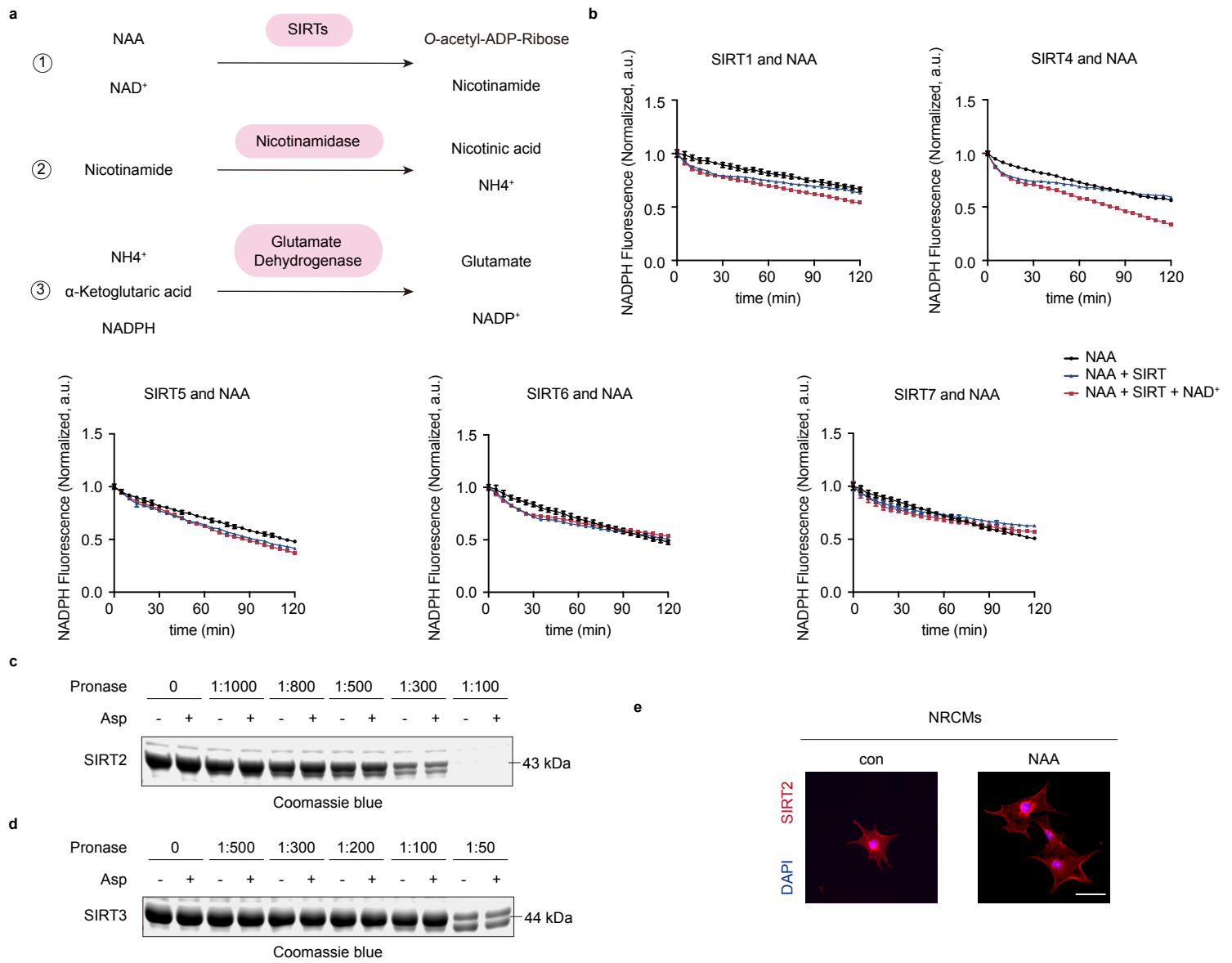
c, d. Interactions fraction analysis from molecular dynamics simulations of MDH1-OAA (**c**) and MDH1-NAA (**d**) complex.

e. DARTS assay showing that mutation of critical residues (R92A/R98A/N131A/R162A/S242A; MDH1-5A) abolished NAA-MDH1 interaction.

f. Schematic of $^{13}\text{C}_6$ -glucose tracing experiments used to assess the impact of 24 h of treatment with 0.5 mM NAA on glycolysis and TCA cycle activity.

g. Schematic of $^{13}\text{C}_6$ -glutamine tracing experiments used to assess the impact of 24 h of treatment with 0.5 mM NAA on TCA cycle activity.

Schematic in **f, g** were created using BioRender (<https://BioRender.com>).



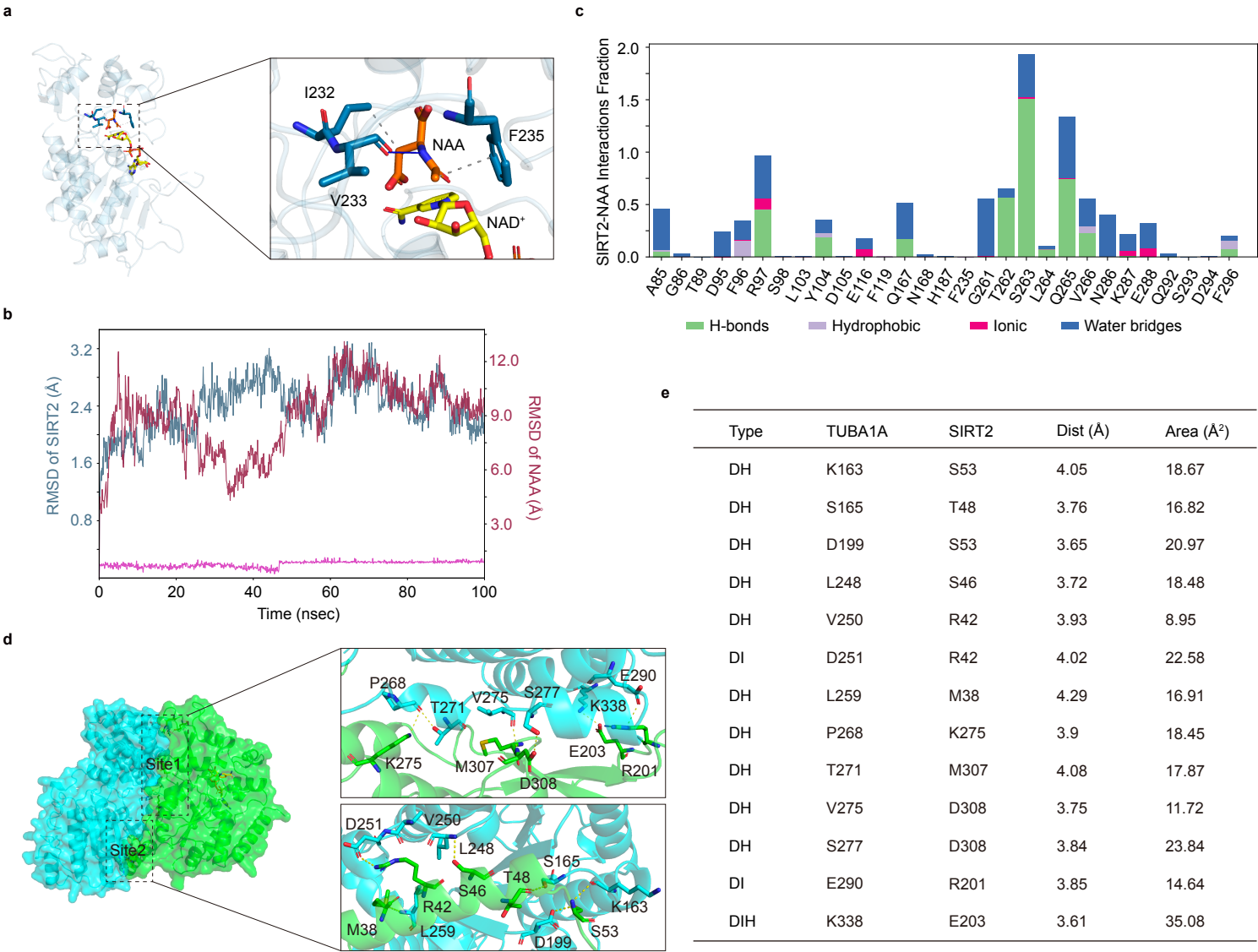
Extended Data Fig. 6. Related to Fig. 4. NAA was identified as a previously unrecognized substrate of SIRT2.

a, Schematic of the enzyme-coupled assay used to monitor NAA hydrolysis by recombinant SIRTs. NADPH consumption was measured as a surrogate readout for enzymatic activity.

b, Enzyme-coupled assays showing minimal or undetectable NAA hydrolase activity of recombinant SIRT1, SIRT4, SIRT5, SIRT6, and SIRT7 in the presence of NAD⁺. Data are presented as mean ± SEM; n = 3 independent experiments.

c, d, DARTS assays showing that aspartate, the hydrolysis product of NAA, did not increase protease resistance of SIRT2 (**c**) or SIRT3 (**d**).

e, Immunofluorescence images showing nuclear-cytoplasmic distribution of SIRT2 in NRCMs after 24 h treatment with 0.5 mM NAA. Scale bar = 50 μm; n = 3 independent experiments.



Extended Data Fig. 7. Related to Fig. 4. Structural and dynamic analyses of NAA binding to SIRT2.

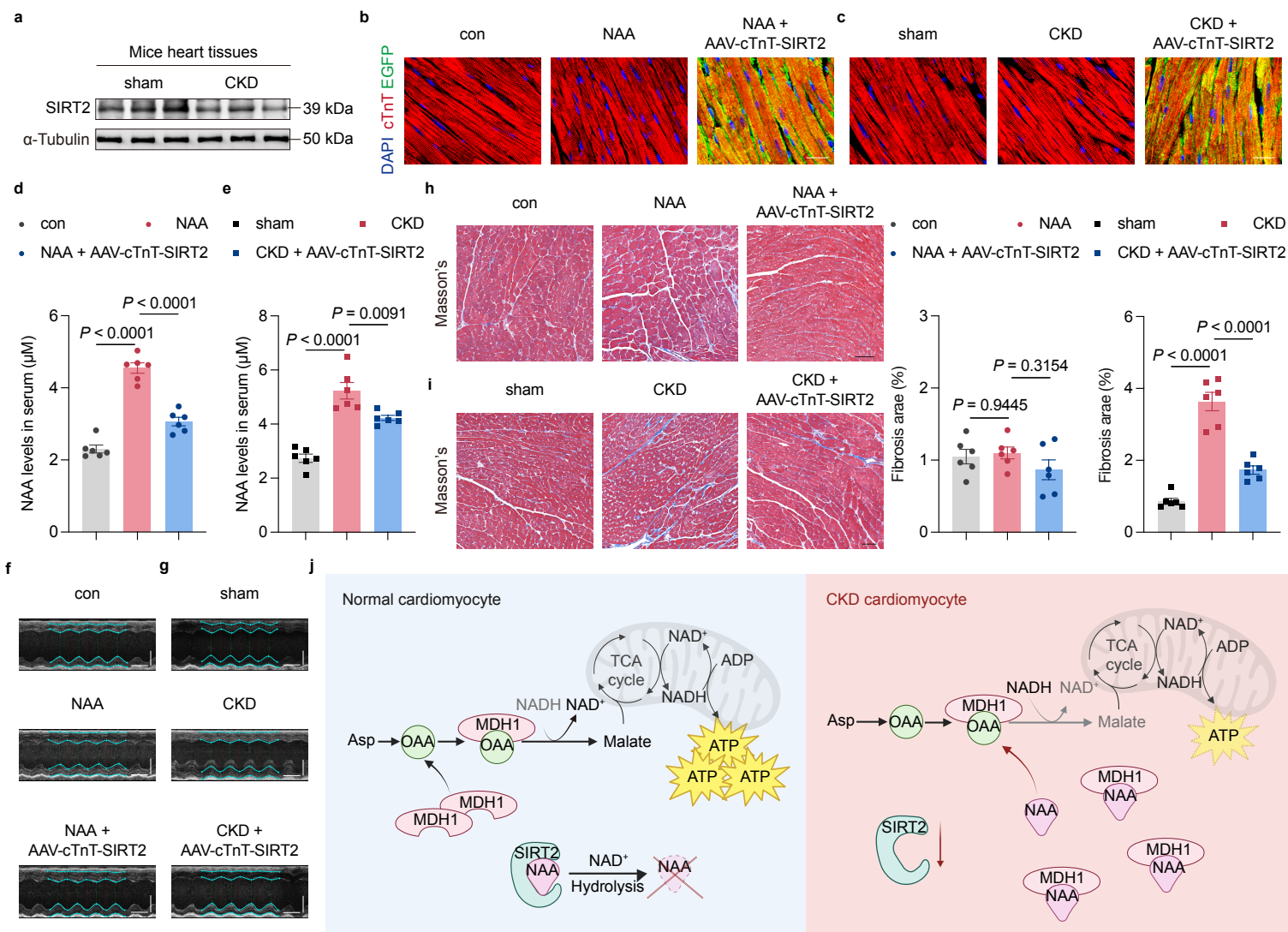
a, Molecular docking of NAA in the active site of SIRT2, showing interactions with residues Ile232, Val233, and Phe235 in the L4 loop.

b, Molecular dynamics simulation showing RMSD trajectories of SIRT2-NAA complexes.

c, Molecular dynamics simulation showing interactions fraction between NAA and key SIRT2 residues.

d, Schematic representation of the protein-protein interaction between SIRT2 and its canonical substrate TUBA1A, showing binding across two spatially separated sites. SIRT2 is shown in green, and TUBA1A is shown in blue.

e, Detailed amino acid-level interactions between SIRT2 and TUBA1A, including direct hydrogen bonds (DH), direct interactions (DI), and direct hydrogen bond interactions (DIH).



Extended Data Fig. 8. Related to Fig. 5. SIRT2 overexpression rescues NAA-induced cardiac dysfunction.

a, Western blotting analysis showing decreased levels of SIRT2 in hearts from CKD mice.

b, c, Immunofluorescence staining of cardiac tissue confirming cardiomyocyte-specific overexpression of EGFP-tagged SIRT2 in NAA-treated (**b**) or CKD mice (**c**). Scale bar = 25 μm; n = 6 mice per group.

d, e, Serum NAA levels in NAA-treated (**d**) and CKD (**e**) mice with or without SIRT2 overexpression; n = 6 mice per group.

f, g, Representative M-mode echocardiographic images of the left ventricle from NAA-treated (**f**) and CKD (**g**) mice with or without SIRT2 overexpression. Cyan contours mark the endocardial and epicardial borders. Red dashed lines indicate end-diastole, and green dashed lines indicate end-systole. Horizontal scale bar = 0.1 s, vertical scale bar = 2 mm; n = 6 mice per group.

h, i, Masson's trichrome staining of heart tissue from NAA-treated (**h**) and CKD (**i**) mice, showing that SIRT2 overexpression attenuates interstitial fibrosis. Scale bar = 50 μm; n = 6 mice per group.

j, Schematic model illustrating the role of NAA and SIRT2 in CKD-associated cardiac dysfunction.

Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA with Tukey's post hoc test (**d, e, h, i**). Schematic in **j** was created using BioRender (<https://BioRender.com>).