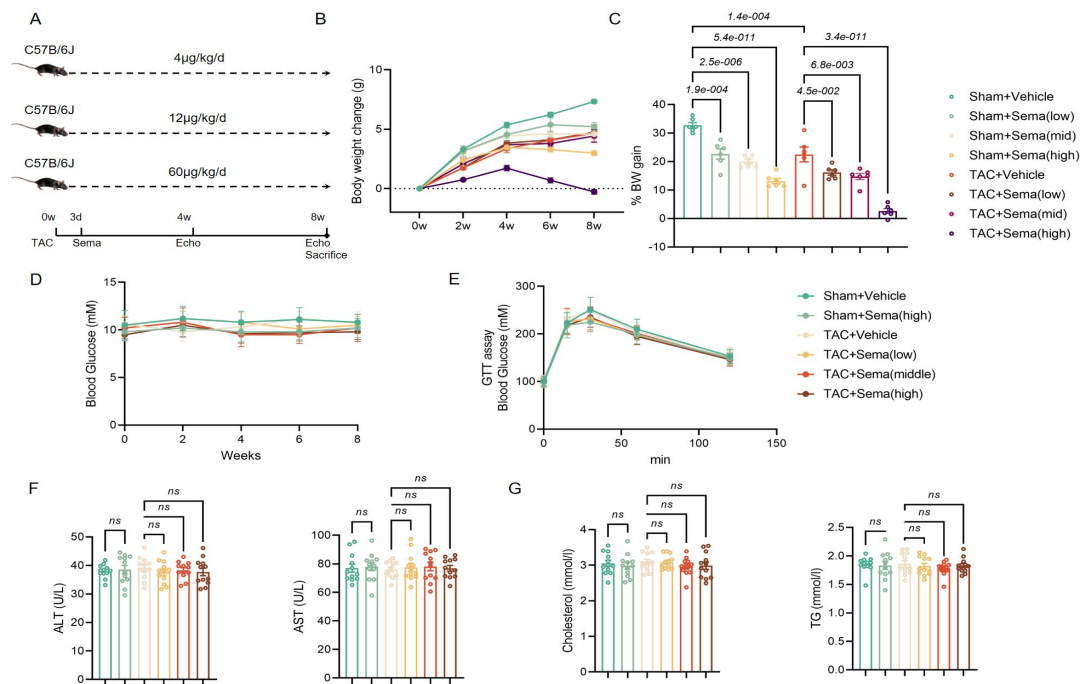
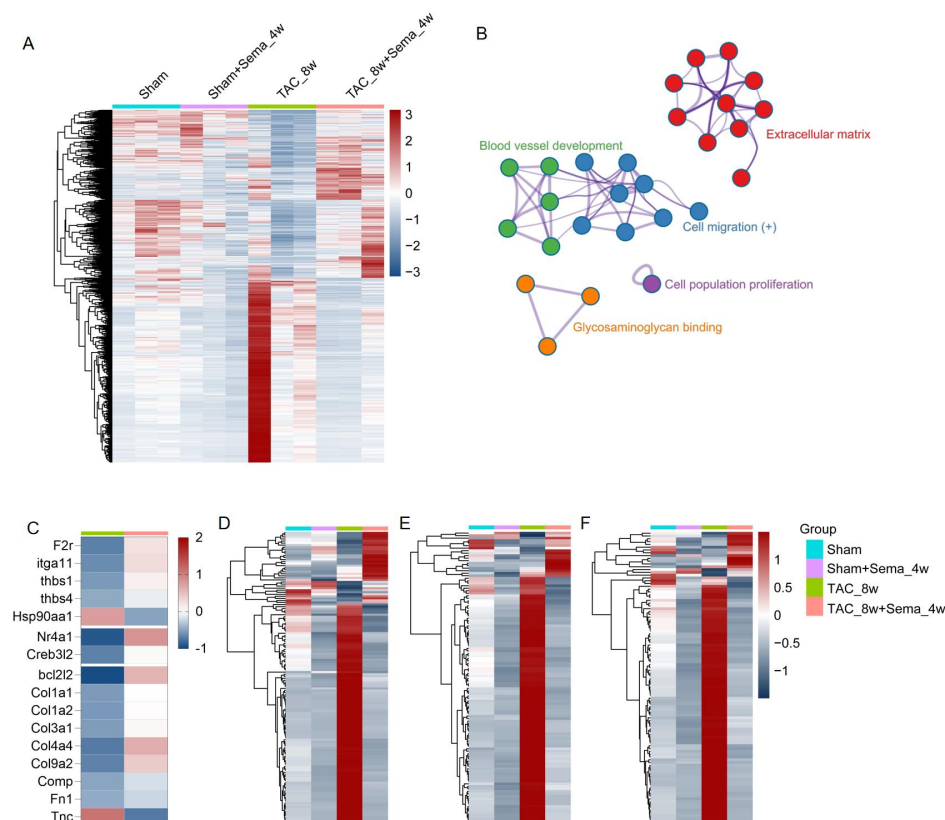


## SUPPLEMENTAL MATERIAL

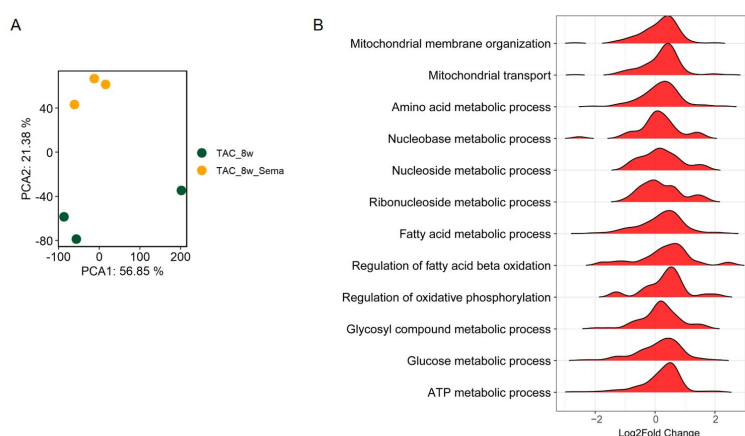
### Supplemental Figures



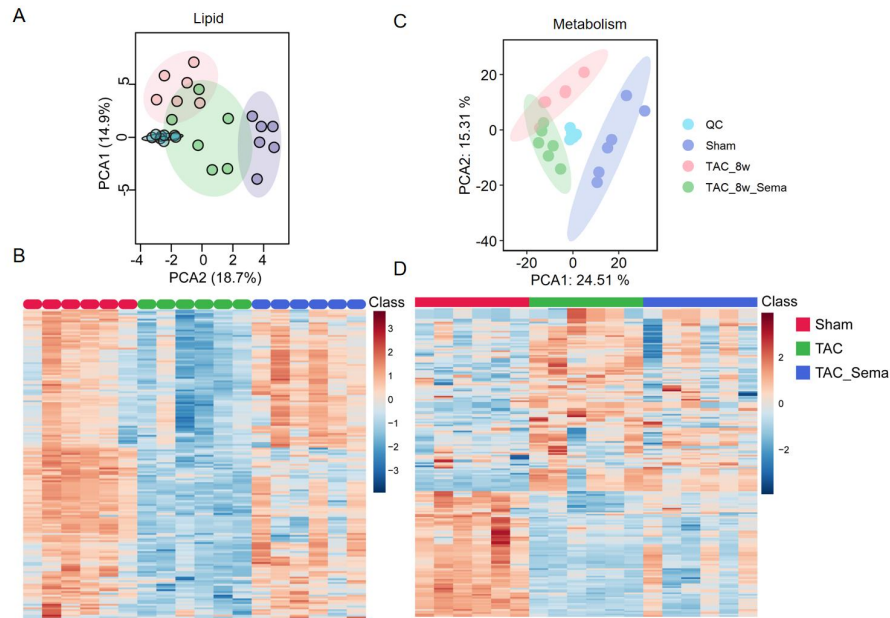
**Figure S1. Effects of different doses of Sema on body weight and glycolipids in mice that underwent TAC for eight weeks.** (A) Mouse model intervention timeline diagram. (B-C) Effect of different doses of Sema on body weight of mice after sham or TAC surgery n=6; BW gain:  $F(5, 66) = 4.917$ ,  $P = 0.0007$ . (D) Effects on fasting blood glucose in mice treated with different doses of Sema every two weeks after sham or TAC surgery (n=6). (E) The results of the GTT after eight weeks of treatment with different doses of Sema n=6. (F) Detection of the liver function indicators ALT and AST after eight weeks of treatment with different doses of Sema (n=12); ALT:  $F(5, 66) = 0.2430$ ,  $P = 0.9419$ ; AST:  $F(5, 66) = 0.09683$ ,  $P = 0.9924$ . (G) Measurement of total cholesterol and triglyceride levels after eight weeks of treatment with different doses of Sema (n=12); Cholesterol:  $F(5, 66) = 0.4508$ ,  $P = 0.8112$ ; TG:  $F(5, 66) = 0.3203$ ,  $P = 0.8991$ . All results are shown as the mean  $\pm$  SEM, and analysis using one-way ANOVA followed by Bonferroni post hoc test (C and F-G) was conducted. For the analysis in (B and D-E), repeated measures two-way ANOVA followed by Sidak post hoc test was conducted. p values are indicated. Source data are provided as a Source Data file. TAC, transverse aortic constriction; GTT, glucose tolerance test; TG, triglycerides; ALT, liver enzymes alanine aminotransferase; AST, aspartate aminotransferase.



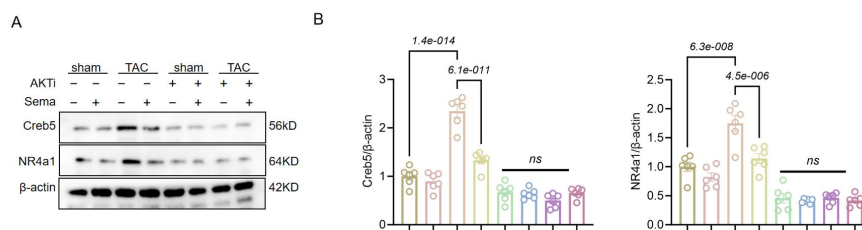
**Figure S2. Sema reverses cardiac hypertrophy, fibrosis and dysfunction in mice induced by TAC by regulating ECM deposition, cell migration, and vascular development pathways.** (A). Heat map of gene level changes in the sema reversal experiment, n=3. (B). Gene Set Enrichment Analysis (GSEA) analysis network maps show key molecular pathways involved in the cardiac remodeling process and regulated by Sema n=3. (C). Heat map showing key molecular signatures both involved in the cardiac remodeling process and regulated by Sema, n=3. (D-F). Heatmap showing changes in genes involved in the ECM deposition, cell migration, and vascularization pathways involved and regulated by Sema during the reversal experiments, n=3. All results are shown as the mean±SEM. p values are indicated.



**Figure S3. Transcriptome analysis of Sema ameliorates energy metabolism in pathological cardiac remodeling.** (A) Cardiac tissues were harvested for transcriptome analysis. PCA (principal component analysis) is shown (n=3). (B) Gene set enrichment analysis (GSEA) of mitochondrial function and energy metabolism-related pathways identified 12 significantly altered pathways (n=3). All results are shown as the mean±SEM. p values are indicated.

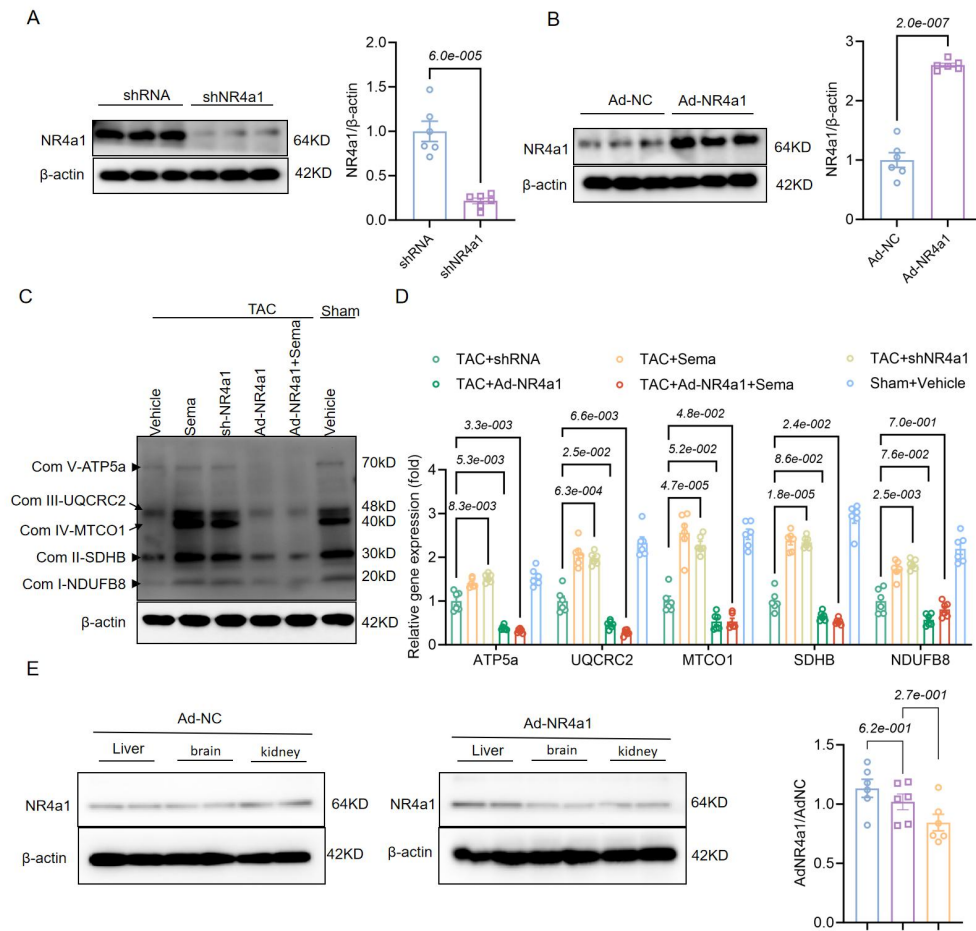


**Figure S4. Effects of 8 weeks of Sema treatment on lipid and energy metabolism in pathological cardiac remodeling.** (A-B) Untargeted metabolomics analysis of lipidomics and metabolomics-related total heatmap and PCA map (n=6). (C-G) Metabolome-based analysis of the energy metabolism-related total heatmap and PCA map (n=6). All results are shown as the mean±SEM. p values are indicated. PCA, principal component analysis.

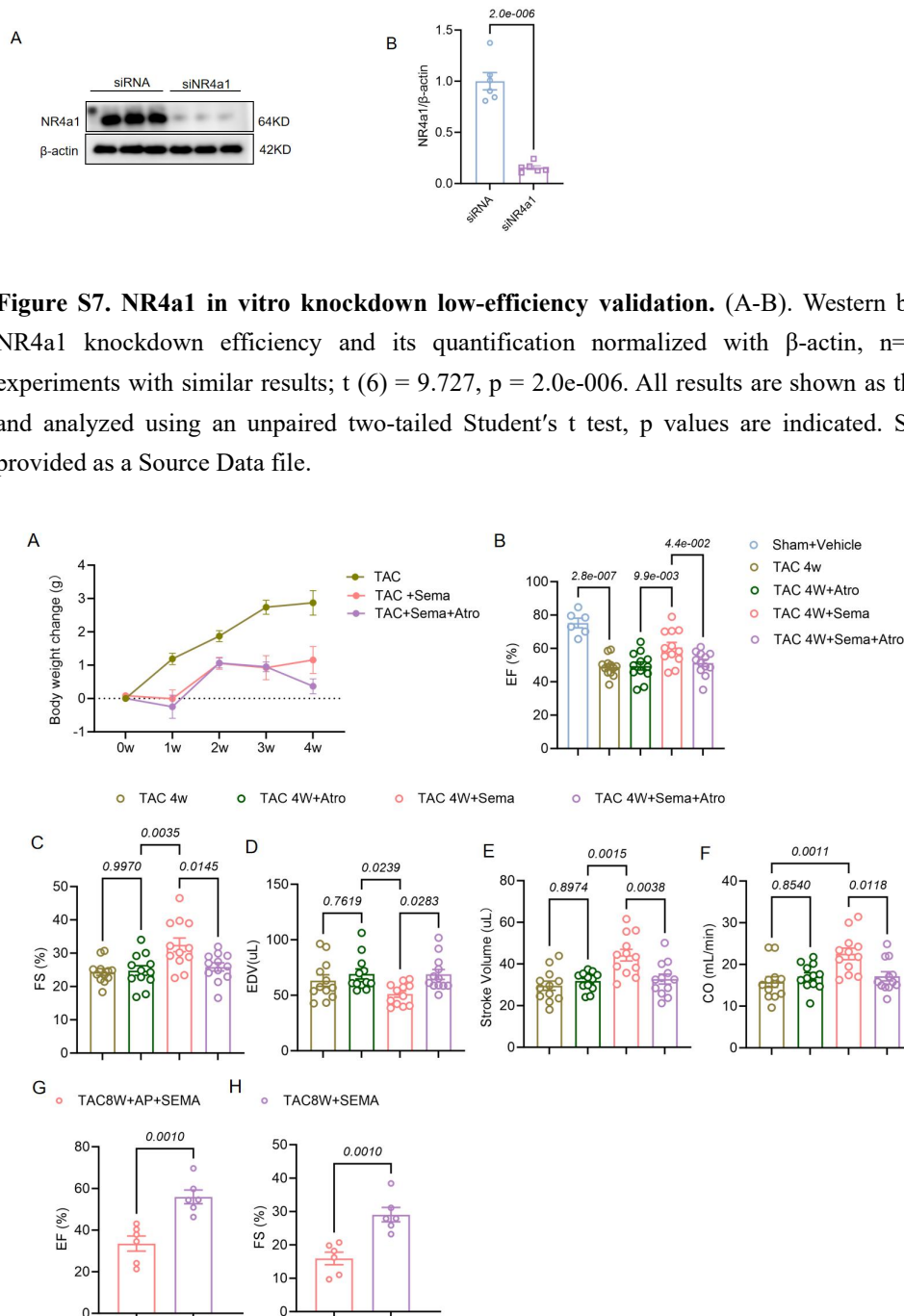


**Figure S5. The therapeutic effects of Sema and elevated Creb5/NR4a1 due to myocardial hypertrophy can be counteracted after the use of AKT inhibitors.** (A) Protein blot levels of Creb5 and NR4a1 under stimulation with AKT inhibitors in the Sema or vehicle groups (n=6 independent experiments with similar results). (B) Quantitative protein map of Creb5 and NR4a1, normalized to β-actin (n=6); Creb5/β-actin: F (7, 40) = 62.37, P = 1.62e-019; NR4a1/β-actin: F (7, 40) = 39.19, P = 5.96e-016. All results are shown as the mean±SEM, and analysis using one-way ANOVA followed by Bonferroni post hoc test was conducted. p values are indicated. Source data are provided as a Source

Data file.



**Figure S6. Effects on mitochondrial function after NR4a1 knockdown or overexpression.** (A) Western blot analysis of NR4a1 knockdown efficiency and its quantification normalized to  $\beta$ -actin (n=6 independent experiments with similar results);  $t(6) = 6.614$ ,  $p = 6.0e-005$ . (B) Western blot analysis of NR4a1 overexpression efficiency and its quantification with  $\beta$ -actin as a standard (n=6 independent experiments with similar results);  $t(6) = 12.49$ ,  $p = 2.0e-007$ . (C-D) Western blot analysis of NR4a1 overexpression or knockdown of mitochondrial respiratory chain proteins in mice, normalized to  $\beta$ -actin (n=6 independent experiments with similar results);  $F(20, 120) = 10.53$ ,  $P = 4.1e-018$ ,  $F(2.387, 71.62) = 59.11$ ,  $P = 2.4e-017$ ,  $F(5, 30) = 207.6$ ,  $P = 2.5e-022$ ,  $F(30, 120) = 2.802$ ,  $P = 4.0e-005$ . (E) Western blot of protein expression in mouse liver, kidney, brain after NR4a1 overexpression (n=6 independent experiments with similar results);  $F(2, 15) = 4.237$ ,  $P = 3.5e-002$ . All results are shown as the mean $\pm$ SEM, and analyzed (A, B) using an unpaired two-tailed Student's t test. For the analysis in (D), repeated measures two-way ANOVA followed by Sidak post hoc test was conducted, p values are indicated. Source data are provided as a Source Data file.



**Figure S8. Cardioprotection induced by Sema is mediated by a muscarinic mechanism.** (A) Effect of Atro on body weight of mice after TAC surgery n=12. (B-E) Cardiac function of mice after TAC 4W (EF, FS, EDV and Stroke Volume) n=12; EF:  $F(4, 49) = 15.02$ ,  $P = 4.4e-008$ ; FS:  $F(3, 44) = 6.564$ ,  $P = 0.0009$ ; EDV:  $F(3, 44) = 3.837$ ,  $P = 0.0159$ ; Stroke Volume:  $F(3, 44) = 8.680$ ,  $P = 0.0001$ . (F) The Cardiac output of mice n=12;  $F(3, 44) = 6.471$ ,  $P = 0.0010$ . (G-H) Cardiac function of mice after TAC 8W (EF and FS) n=6; EF:  $t(6) = 4.565$ ,  $p = 0.0010$ ; FS:  $t(6) = 4.599$ ,  $p = 0.0010$ . All results are shown as the mean  $\pm$  SEM, and analysis using one-way ANOVA followed by Bonferroni post hoc test (A-F) was conducted. For the analysis in (G and H), using an unpaired two-tailed Student's t test. p values are indicated. Source data are provided as a Source Data file. CO, Cardiac output; EF, ejection fractions;

FS, fractional shortening.

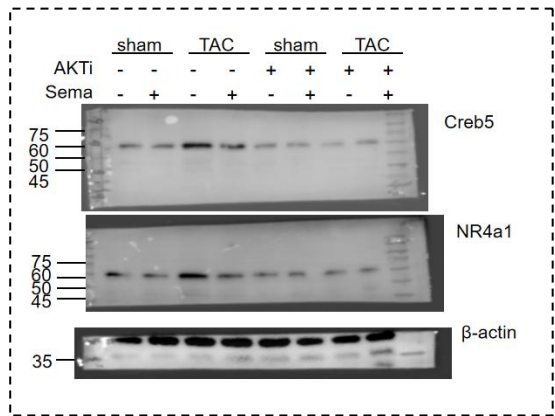
Supplemental Tables

Table S1

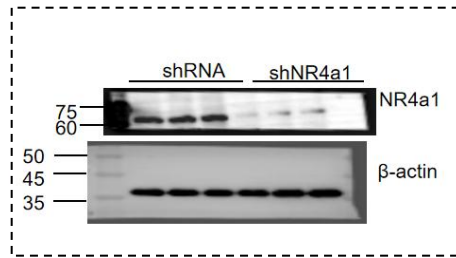
| Transcriptomic profiling and metabolism analysis |                                                                                                   |
|--------------------------------------------------|---------------------------------------------------------------------------------------------------|
| REAGENT                                          | Uniform Resource Identifier                                                                       |
| GSEA                                             | <a href="http://www.gsea-msigdb.org/gsea">http://www.gsea-msigdb.org/gsea</a>                     |
| Metascope                                        | <a href="http://www.metascope.org/gp/index.html">http://www.metascope.org/gp/index.html</a>       |
| Cytoscape                                        | <a href="http://cytoscape.org">http://cytoscape.org</a>                                           |
| R                                                | <a href="http://www.r-project.org">http://www.r-project.org</a>                                   |
| MetaboAnalyst                                    | <a href="http://www.metaboanalyst.ca">http://www.metaboanalyst.ca</a>                             |
| MetDNA                                           | <a href="http://www.metdna.zhulab.cn">http://www.metdna.zhulab.cn</a>                             |
| OSI                                              | <a href="http://www.nxhh.net/qkwenda/1460782.html">http://www.nxhh.net/qkwenda/1460782.html</a>   |
| Inter Self-built libraries                       | /                                                                                                 |
| MS-DIAL                                          | <a href="http://prime.psc.riken.j.msdiol/main.html">http://prime.psc.riken.j.msdiol/main.html</a> |

Western blot images

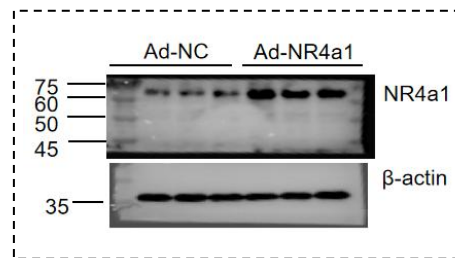
Figure S5A



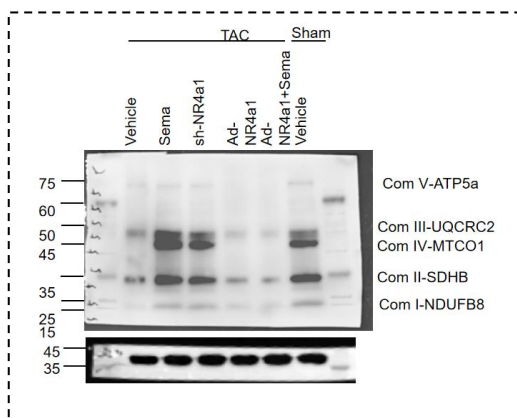
**Figure S6A**



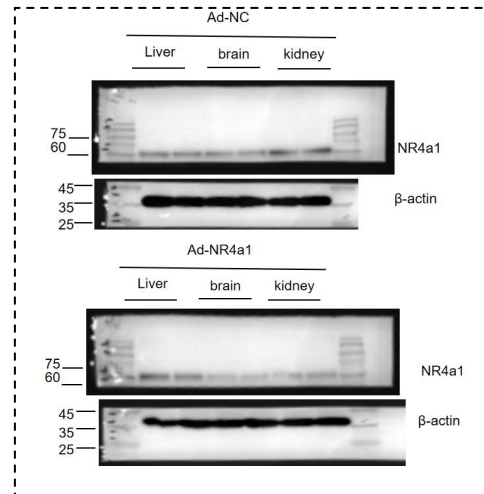
**Figure S6B**



**Figure S6C**



**Figure S6E**



**FigureS7A**

