SUPPLEMENTARY DATA

circHIPK3 Sustains Ribosome Biogenesis and Protects Against Cardiac Aging by Stabilizing Ncl mRNA and Supporting its LLPS

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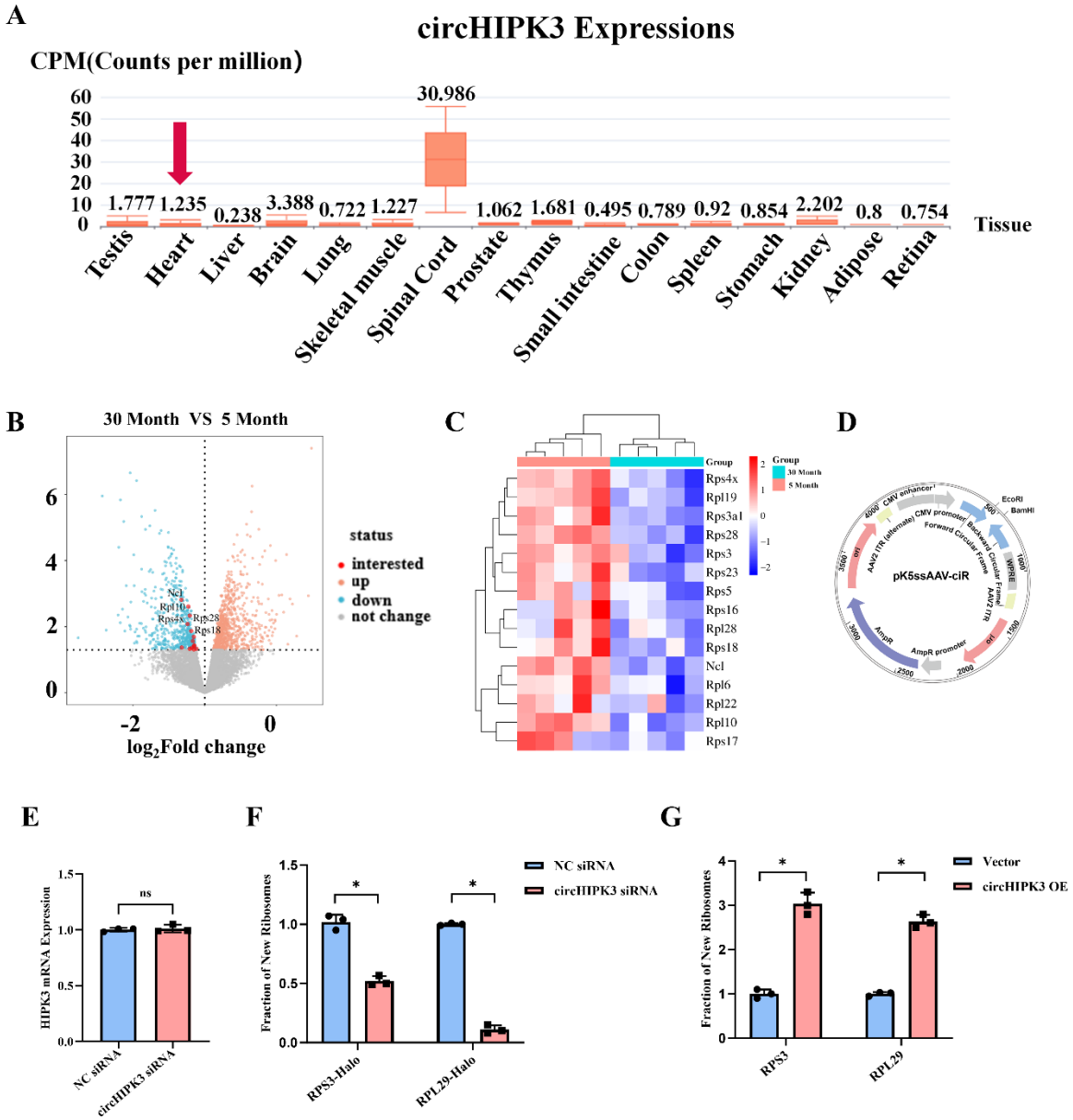
SUPPLEMENTARY DATA



Supplementary Figure 1. Tissue distribution of circHIPK3, age-related gene expression changes, and plasmid map of pK5ssAAV-ciR. (**A**) RNA-sequencing was used to determine circHIPK3 expression across multiple mouse tissues. (**B**) Volcano plot was used to visualize differential gene expression; Ncl and ribosomal protein genes. (**C**) Heatmap was used to display the expression profile of Ncl and ribosomal protein genes in 30 Month and 5 Month mouse groups. (**D**) Schematic map of the pK5ssAAV-ciR plasmid. (**E**) qRT-PCR was used to analyze the expression of linear HIPK3 mRNA after siRNA transfectionin H9C2 cells (n=3). (**F**) Quantification of Ribo-Halo after siRNA transfection (n=3). (**G**) Quantification of Ribo-Halo after transfection of overexpression plasmid (n=3). (Mann-Whitney U test). $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

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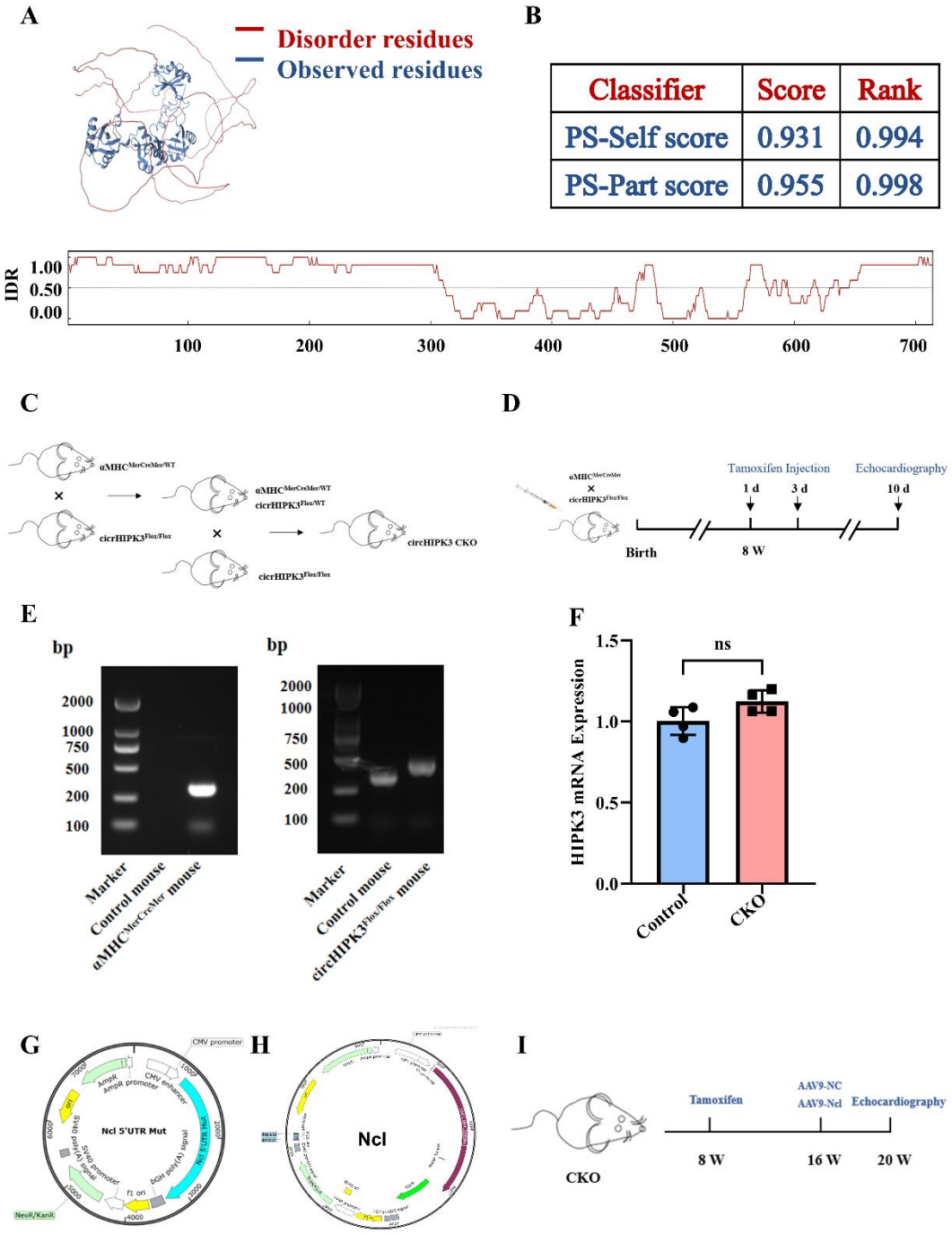

Supplementary Figure 2. Prediction of Ncl IDR and the generation cardiomyocyte-specific tamoxifen-induced circHIPK3 knockout mice. (**A, B**) Prediction of Ncl IDR, with sequences scoring>0.5 indicating IDR regions. (**C-E**) Generation cardiomyocyte-specific tamoxifen-induced circHIPK3 knockout mice. (**F**) qRT-PCR was used to analyze the expression of linear HIPK3 mRNA in heart tissues (n=4). (**G**) Schematic map of the Ncl 5' UTR Mut plasmid. (H) Ncl overexpression in mice via tail vein injection. (Mann-Whitney U test). $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

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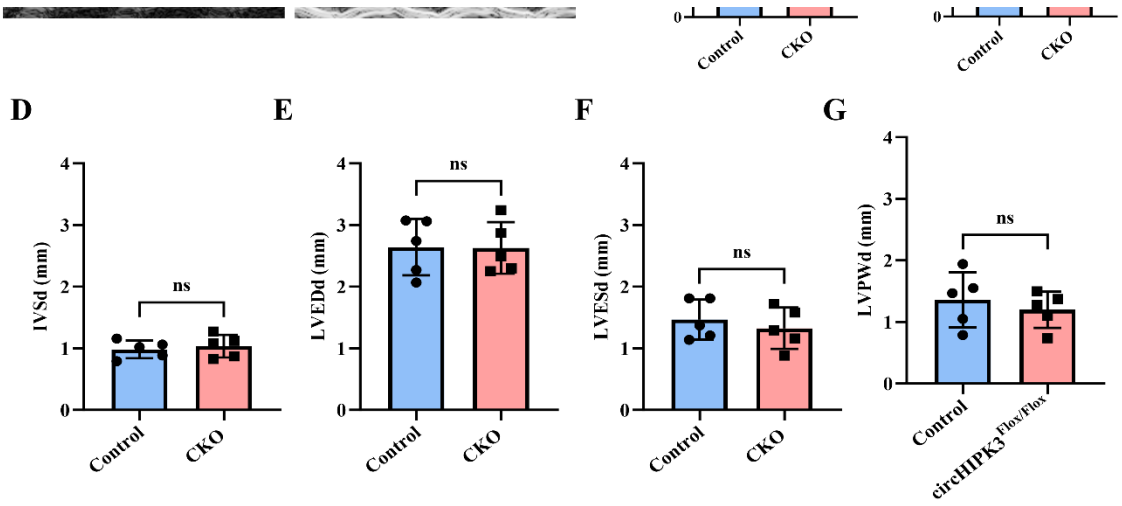

Supplementary Figure 3. Baseline cardiac morphology and function prior to tamoxifen induction. (**A**) Echocardiography was used to evaluate Control and CKO mice cardiac function (n=5). (**B**) Ejection fraction (EF) (n=5). (**C**) Fractional shortening (FS) (n=5). (**D**) Interventricular septal thickness at diastole (IVSd) (n=5). (**E**) Left ventricular end-diastolic diameter (LVEDd) (n=5). (**F**) Left ventricular end-systolic diameter (LVESd) (n=5). (**G**) Left ventricular posterior wall thickness at diastole (LVPWd) (n=5). (Mann-Whitney U test). $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

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Supplementary Table 1 Primer used for qPCR

mRNA	Sequence（5'—3'）
circHIPK3（Mouse）	F: GGATCGGCCAGTCATGTATC
	R: ACCGCTTGGCTCTACTTTGA
circHIPK3（Rat）	F: GGATCGGCCAGTCATGTATC
	R: ACCGCTTGGCTCTACTTTGA
Gapdh（Mouse）	F: AAATGGTGAAGGTCGGTGTG
	R: TGAAGGGGTCGTTGATGG
Gapdh（Rat）	F: CAACGGGAAACCCATCACCAT
	R: AGATGATGACCCTTTTGGCCCC
p21（Mouse）	F: CCTGGTGATGTCCGACCTG
	R: CCATGAGCGCATCGCAATC
p21（Rat）	F: GGGATGCATCTATCTTGTGATATGT
	R: AGACGACGGCATACTTTGCT
36B4（Mouse）	F: ACTGGTCTAGGACCCGAGAAG
	R: TCAATGGTGCCTCTGGAGATT
Ncl（Mouse）	F: CAGGGAACAGTTTGGTGGGT
	R: GCTGAGTGCCTTCAGCTACA
Ncl（Rat）	F: TTCATTACCCGCCGATCCAG
	R: TGGACTCTCCGTGGGTTTTG
47S pre-rRNA（Mouse）	F: GCTTGTTTCTCCCGATTGC
	R: CGCGAACAACTGAGAAAAGT
47S pre-rRNA（Rat）	F: GTTCCGCTCACACCTCAGAT
	R: CAAGTGCGTTCGAAGTGTCG
28S rRNA（Mouse）	F: AGCCGACTTAGAACTGGTGC
	R: GGCAGAAATCACATCGCGTC
28S rRNA（Rat）	F: AGCCGACTTAGAACTGGTGC
	R: GGCAGAAATCACATCGCGTC
18S rRNA（Mouse）	F: GGCCGTTCTTAGTTGGTGGA
	R: CCCGGACATCTAAGGGCATC
18S rRNA（Rat）	F: GGCCGTTCTTAGTTGGTGGA
	R: CCCGGACATCTAAGGGCATC
5.8S rRNA（Mouse）	F: CTTAGCGGTGGATCACTCGG
	R: GCAAGTGCGTTCGAAGTGTC
5.8S rRNA（Rat）	F: CTTAGCGGTGGATCACTCGG
	R: GCAAGTGCGTTCGAAGTGTC

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Supplementary Table 2 siRNA sequence

siRNA	Sequence
si-circHIPK3	GTGCAAGAATAGTAACTGA

Supplementary Table 3 The sequence of RNA pull down probe

Probe Number	Sequence（5'-3'）
1	/5bio/-ATACCTGTAGTAGCGAGATT
2	/5bio/-CCATACCTGTAGTAGCGAGA
3	/5bio/-AGGCCATACCTGTAGTAGCG
4	/5bio/-TGAGGCCATACCTGTAGTAG
5	/5bio/-TGTGAGGCCATACCTGTAGT

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