

Supplemental information

**Pervasive nuclear envelope ruptures precede ECM
signaling and disease onset without activating
cGAS-STING in Lamin-cardiomyopathy mice**

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SUPPLEMENTARY FIGURE S1

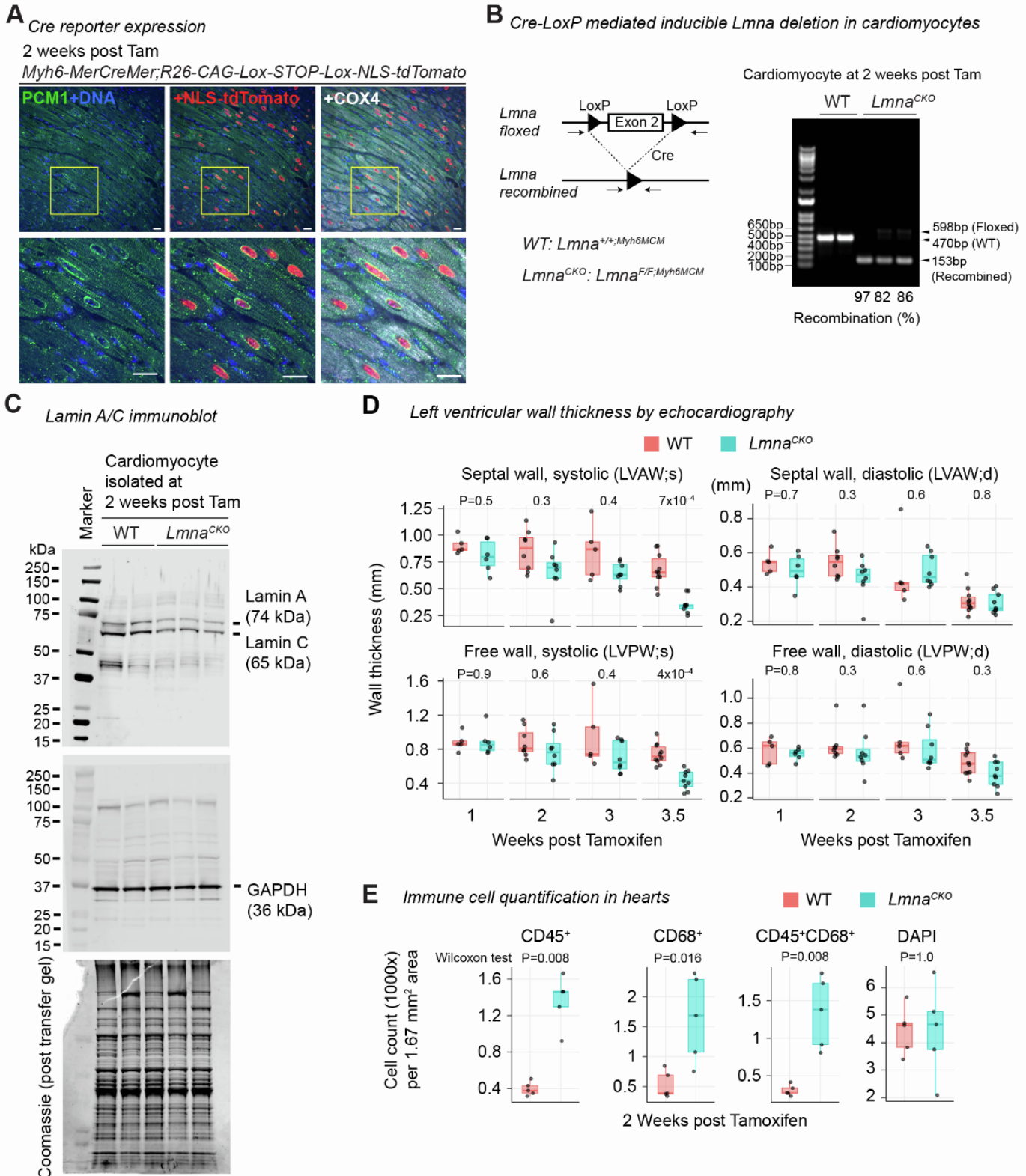


Figure S1. Characterization of adult mice with cardiomyocyte-specific *Lmna* deletion

A) Validation of cardiomyocyte-specific *Cre* activity conferred by the *Myh6-MerCreMer* transgene. The *Cre* reporter is *R26-CAG-LSL-NLS-tdTomato*. Heart sections are stained with *NLS-tdTomato*, *PCM1*, and *COX4* immunofluorescence. *PCM1* and *COX4* stain cardiomyocytes. Scale bar: 20 μ m.

B) Left: Schematic for the *Lmna* *LoxP* allele and the expected recombined allele upon *Cre* expression. Arrow, PCR primer location. Right: Amplification of the wild-type, floxed, and recombined *Lmna* alleles by PCR using

primers indicated in the left panel. The recombination efficiency (%) was calculated by the intensity of the 153-bp recombined band relative to that of the 598-bp floxed band. WT n=2, *Lmna*^{CKO} n=3.

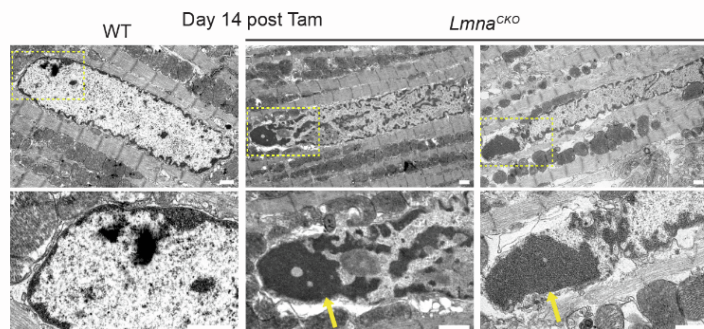
C) The whole immunoblot for **Fig. 1B**, with Coomassie staining of the original gel.

D) Echocardiography of left ventricular (LV) geometry. n=5-10 mice/genotype. P-value, Wilcoxon test.

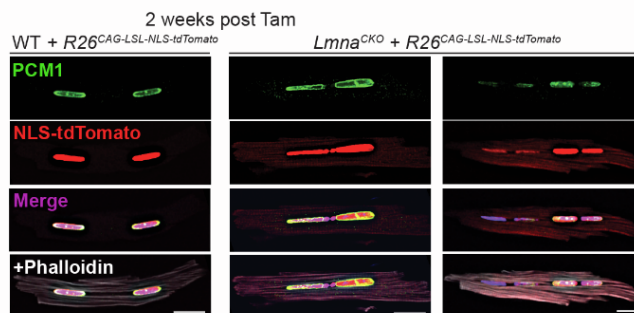
E) Quantification of CD45 and CD68-positive cells in heart sections. Representative image in **Fig. 1J**. n=5 mice/genotype. P-value, same as **D**.

SUPPLEMENTARY FIGURE S2

A Additional images for EM in hearts

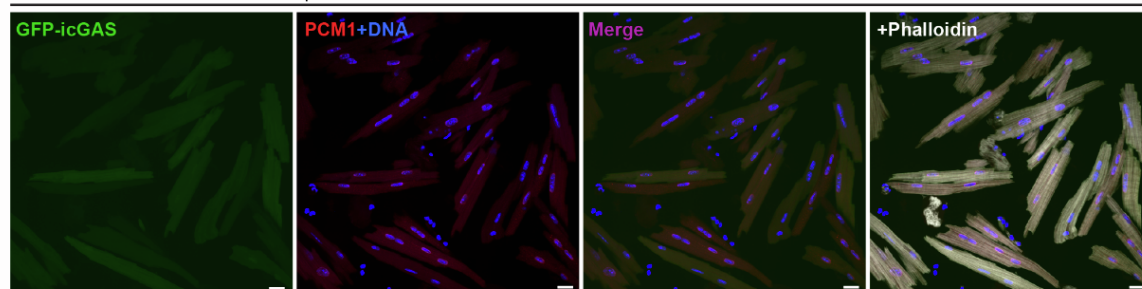


B Additional images for NLS-tdTomato signals in *Lmna*^{CKO} CMs

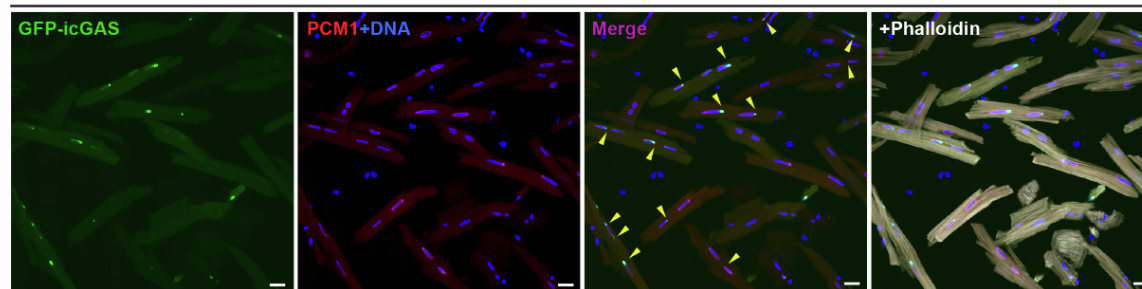


C Localization of cytoplasmic DNA sensor GFP-icGAS in isolated cardiomyocytes

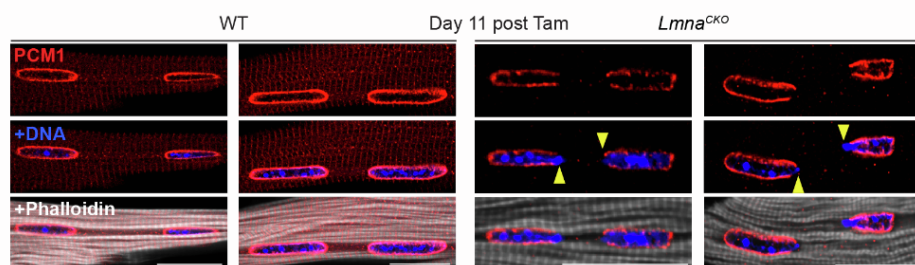
WT + AAV-GFP-icGAS 2 weeks post Tam



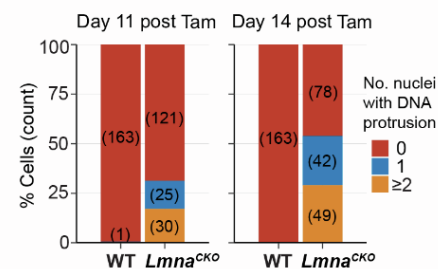
Lmna^{CKO} + AAV-GFP-icGAS 2 weeks post Tam



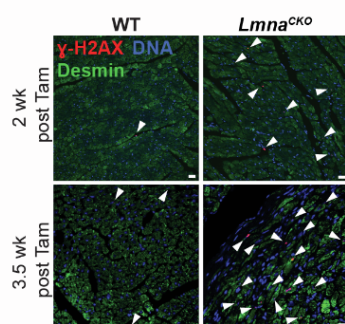
D PCM1 IF in CMs at Day 11 post Tam



E Number of nuclei with DNA protrusion from PCM1-lost nuclear tips per cell



F γ -H2AX IF in heart



G TUNEL assay in heart section

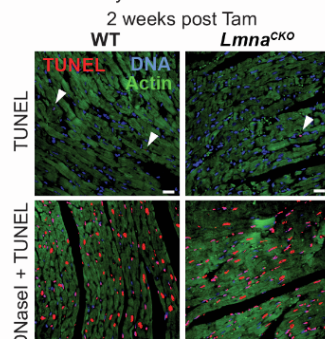


Figure S2. Characterization of nuclear envelope ruptures in *Lmna*^{CKO} cardiomyocytes

A) Top: Additional transmission electron micrographs of heart sections focusing on cardiomyocytes, related to **Fig. 2B**. Bottom: Enlarged images of the area indicated by yellow box in the upper panels. Arrow, protruded chromatin. Scale bar: 1 μ m.

B) Additional immunofluorescence images for PCM-1 with detection of native NLS-tdTomato. Scale bar: 20 μ m.

C) Immunofluorescence for PCM-1 with detection of exogenous GFP-icGAS in isolated cardiomyocytes, related to **Fig. 2H**. Phalloidin stains F-actin. Arrowhead, GFP-icGAS localization at nuclear tips. Scale bar: 20 μ m.

D) Immunofluorescence images for PCM1 in isolated cardiomyocytes at Day 11 post tamoxifen. Arrowhead, local loss of PCM1 at nuclear tips. Scale bar: 20 μ m.

E) Fraction of cardiomyocytes with DNA protrusion from PCM1-lost nuclear tips. Cardiomyocytes are stratified by the number of ruptured nuclei per cell. Only multinucleated cardiomyocytes are analyzed.

F) Immunofluorescence for γ H2AX and desmin in heart tissue sections. Desmin stains cardiomyocytes. Arrowhead, γ H2AX-stained nuclei. Scale bar: 20 μ m.

G) TUNEL assay for cell death detection in heart tissues. Arrowhead, TUNEL-positive cells. Scale bar: 20 μ m.

SUPPLEMENTARY FIGURE S3

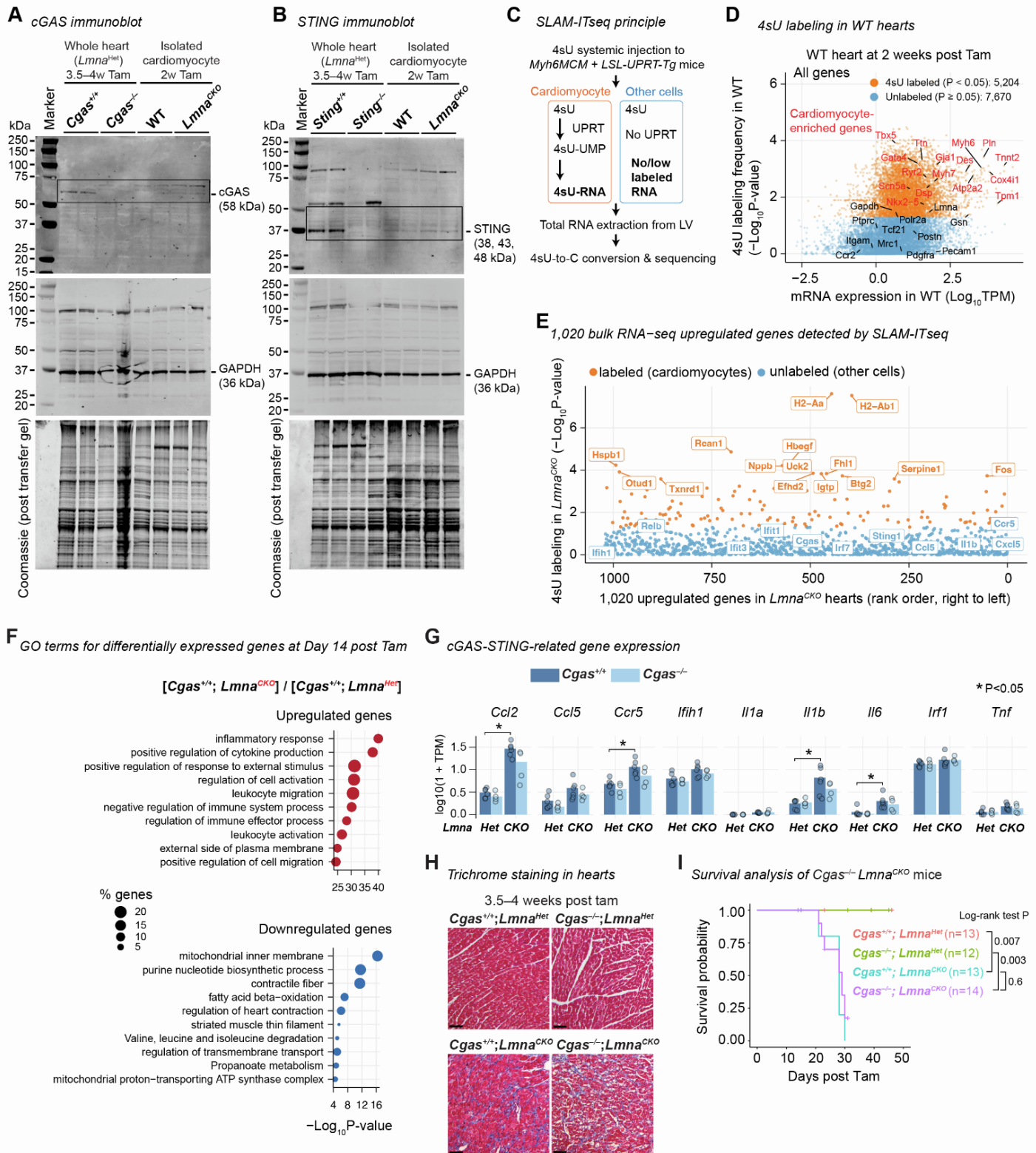


Figure S3. Analysis of the cGAS-STING pathway in *Lmna*^{CKO} hearts

A) cGAS and GAPDH immunoblots, and Coomassie staining of original gel, in hearts and isolated cardiomyocytes, related to **Fig. 3A**.

B) Same as (A), but for STING.

C) Principle of SLAM-IT-seq. Transcripts in cardiomyocytes are labeled by 4-thiouracil (4sU).

D) SLAM-IT-seq data for all genes in WT hearts at 2 weeks post tamoxifen, with the expression level on the X axis and the 4sU labeling frequency on the Y axis. Select genes known to be preferentially expressed in

cardiomyocytes (red) and those not known to be preferentially expressed in cardiomyocytes (black) are indicated. P-value, beta binomial test P-value adjusted with the Benjamini-Hochberg procedure.

E) SLAM-IT-seq data for the 1,020 upregulated genes at 2 weeks post tamoxifen, with the rank order of the fold change of upregulation on the X axis and the 4sU labeling frequency in *Lmna*^{CKO} hearts on the Y axis. Blue labeled gene: all upregulated cytokine genes. Highly labeled genes (Log₁₀ P-value > 3.5) are also indicated (orange). P-value, same as **D**.

F) The top 10 Gene Ontology terms overrepresented among differentially expressed genes in *Cgas*^{-/-}; *Lmna*^{Het} versus *Cgas*^{+/+}; *Lmna*^{Het}. P-value is computed by Metascape ⁽¹⁾.

G) Normalized RNA-seq gene expressed levels for additional cGAS-STING-related genes (bar, mean). P-value, generalized linear model by DESeq2 ⁽²⁾.

H) Masson's trichrome staining of heart sections. Scale bar: 20 μm.

I) Kaplan-Meier survival analysis. P-value, log-rank test.

SUPPLEMENTARY FIGURE S4

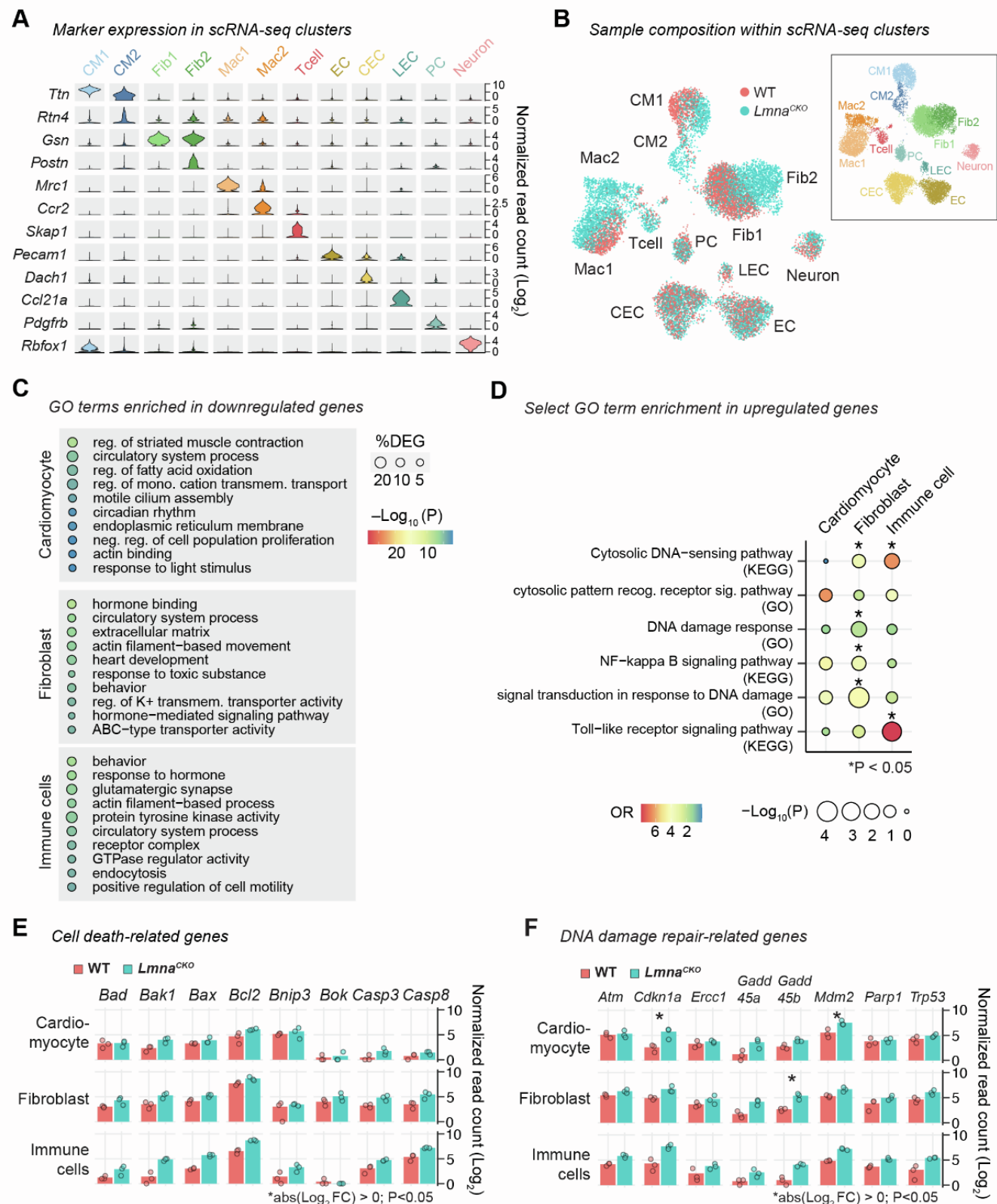


Figure S4. Single-nucleus RNA-seq analysis of *Lmna*^{CKO} hearts
A) Expression (sum of single-nucleus normalized read count across 3 mice within cell type) of marker genes used to classify single nuclei to the cell types indicated along the Y axis, related to **Fig. 4A**.
B) snRNA-seq UMAP plot for 14,111 nuclei, colored by sample genotype. Inset, UMAP colored by cell type for reference.

- C)** GO terms enriched among downregulated genes within indicated cell type. P-value is computed by Metascape ⁽¹⁾.
- D)** Enrichment for select GO terms relevant to cytosolic DNA sensing among upregulated genes. P-value, FDR-adjusted P-value in Fisher's exact test.
- E)** Expression (mean of single-nucleus normalized read count within cell type) of cell death-related genes. Dot, mean within individual mice. Bar, mean across three mice. P-value, generalized linear model by DESeq2 ⁽²⁾.
- F)** Same as (C), but for DNA damage repair-related genes.

REFERENCES

1. Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A.H., Tanaseichuk, O., Benner, C., and Chanda, S.K. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat. Commun.* *10*, 1523.
2. Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* *15*, 550.