Cell Reports, Volume *43*

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

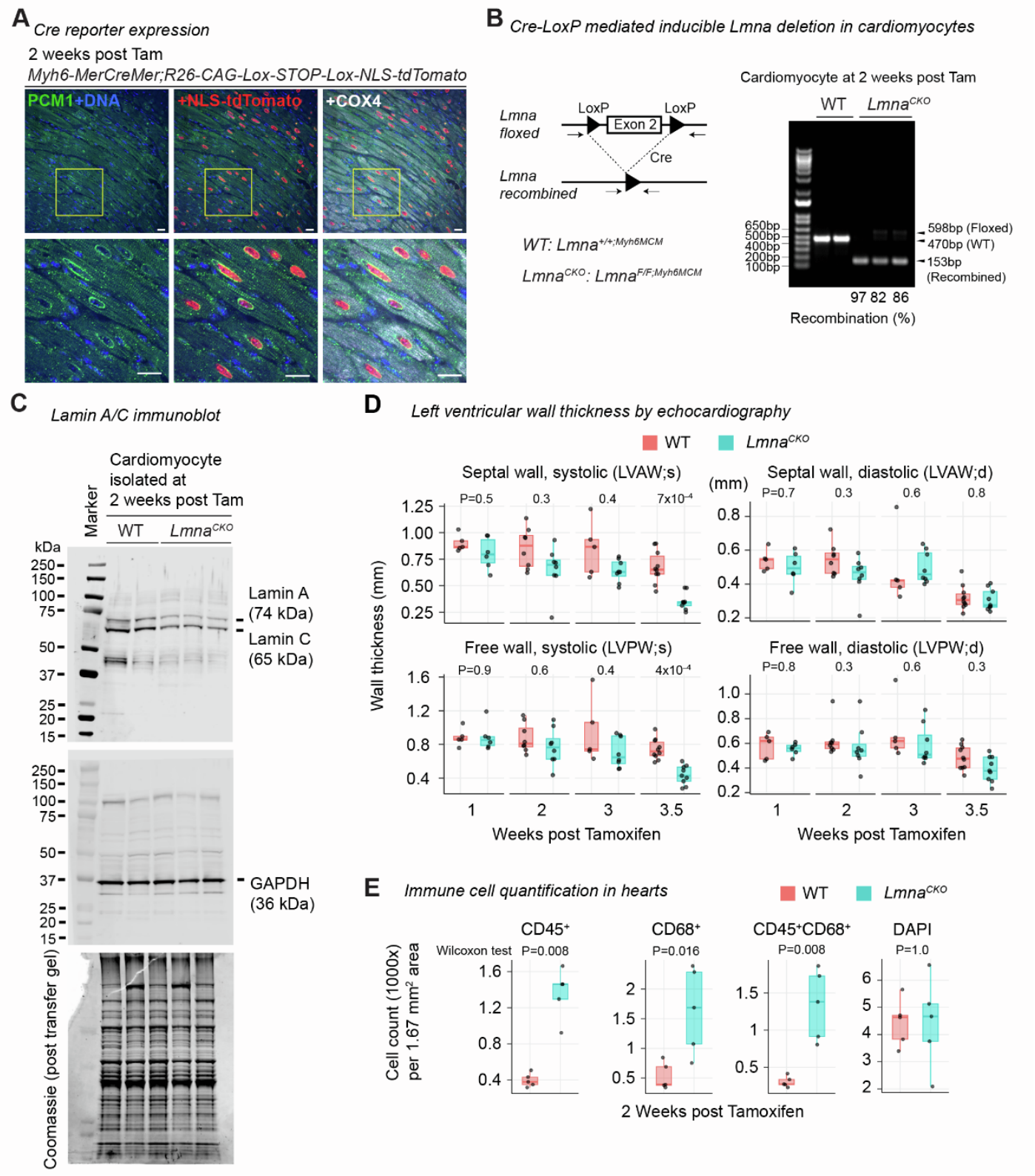

Figure S1. Characterization of adult mice with cardiomyocyte-specific *Lmna* deletion

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 µm.

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

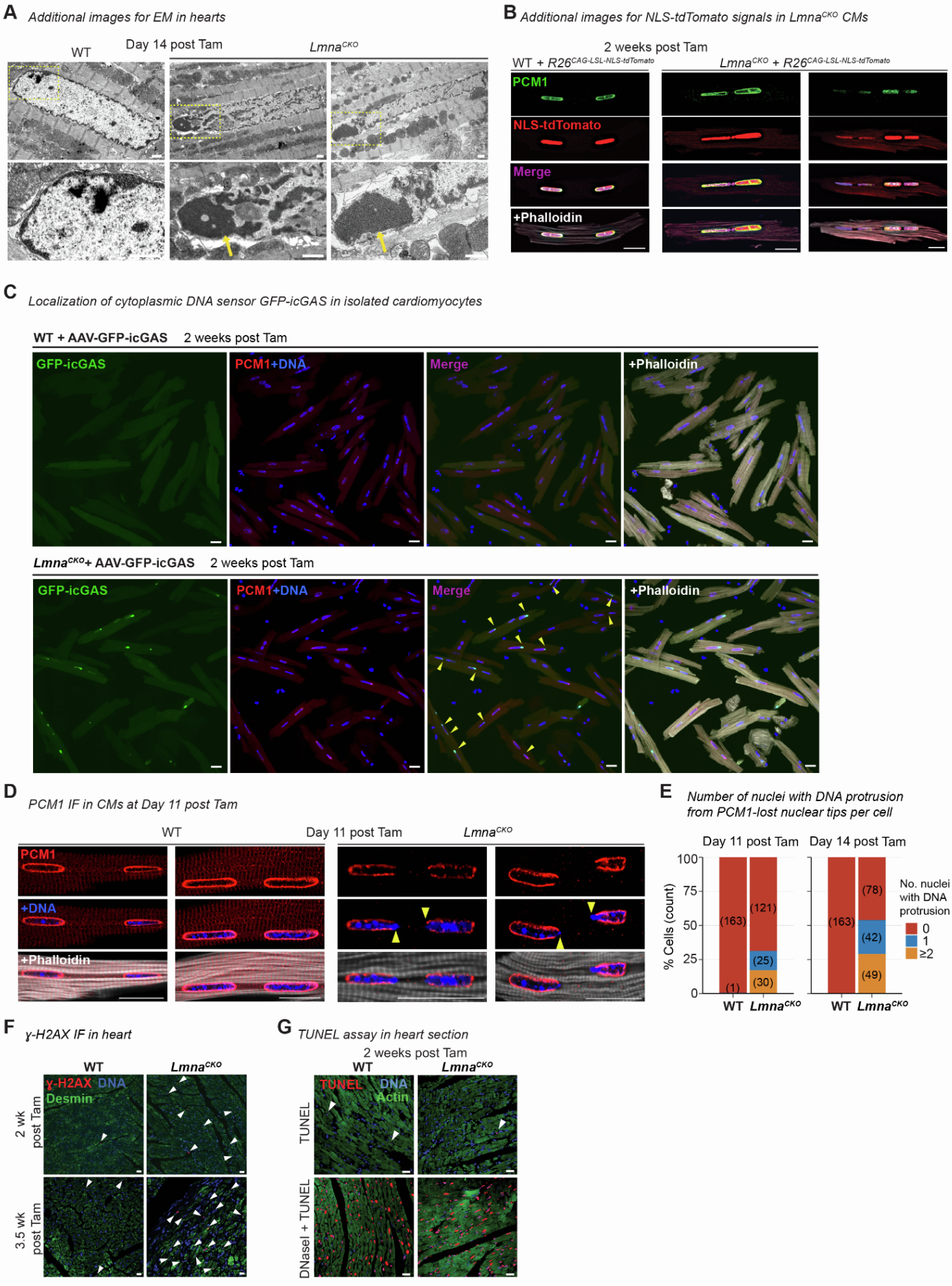

Figure S2. Characterization of nuclear envelope ruptures in *Lmna*^{CKO} cardiomyocytes

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 μm.

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

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 μm.

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

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 γH2AX and desmin in heart tissue sections. Desmin stains cardiomyocytes. Arrowhead, γH2AX-stained nuclei. Scale bar: 20 μm.

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

SUPPLEMENTARY FIGURE S3

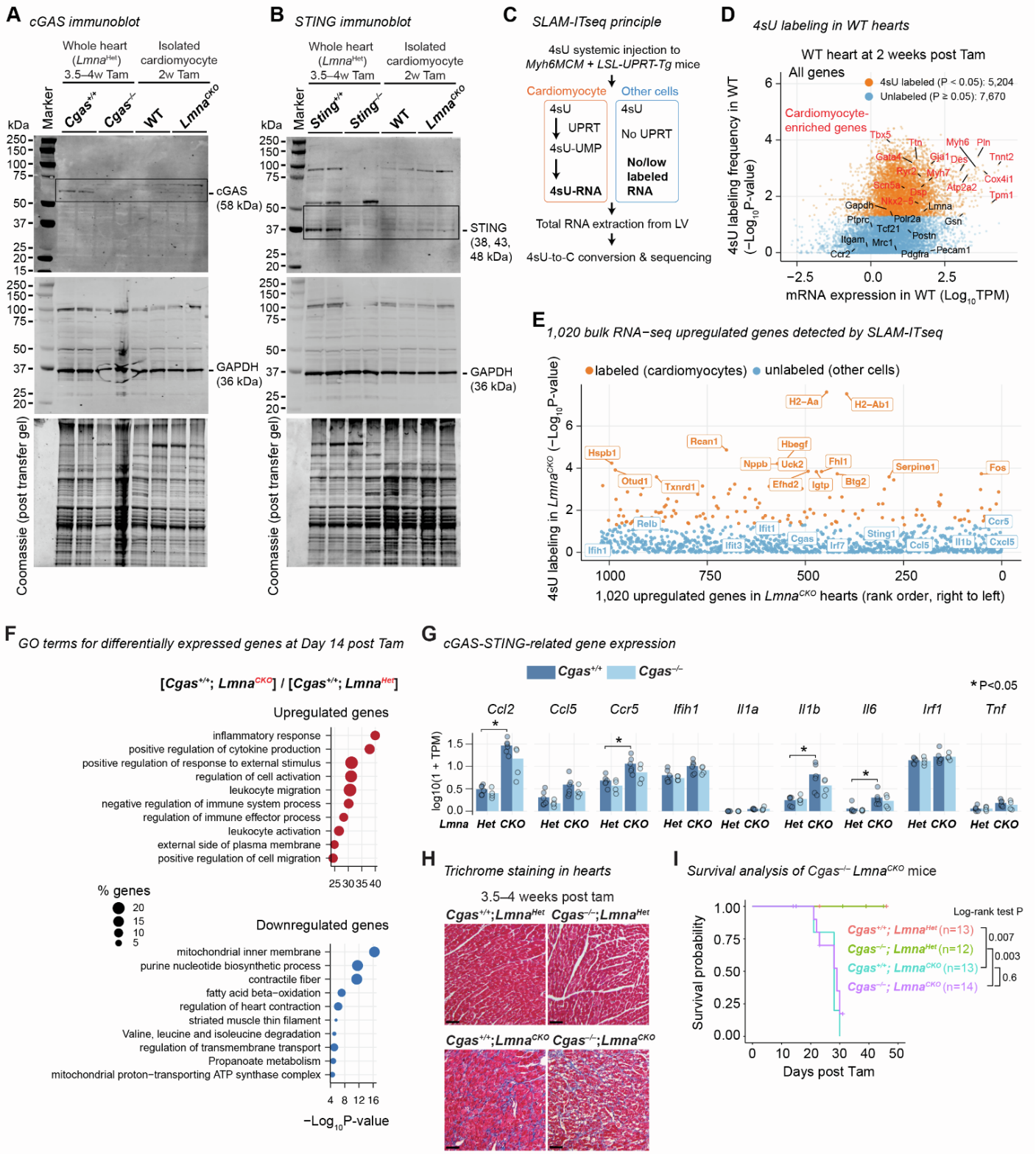

Figure S3. Analysis of the cGAS-STING pathway in *Lmna*^{CKO} hearts

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 (Log_{10} 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

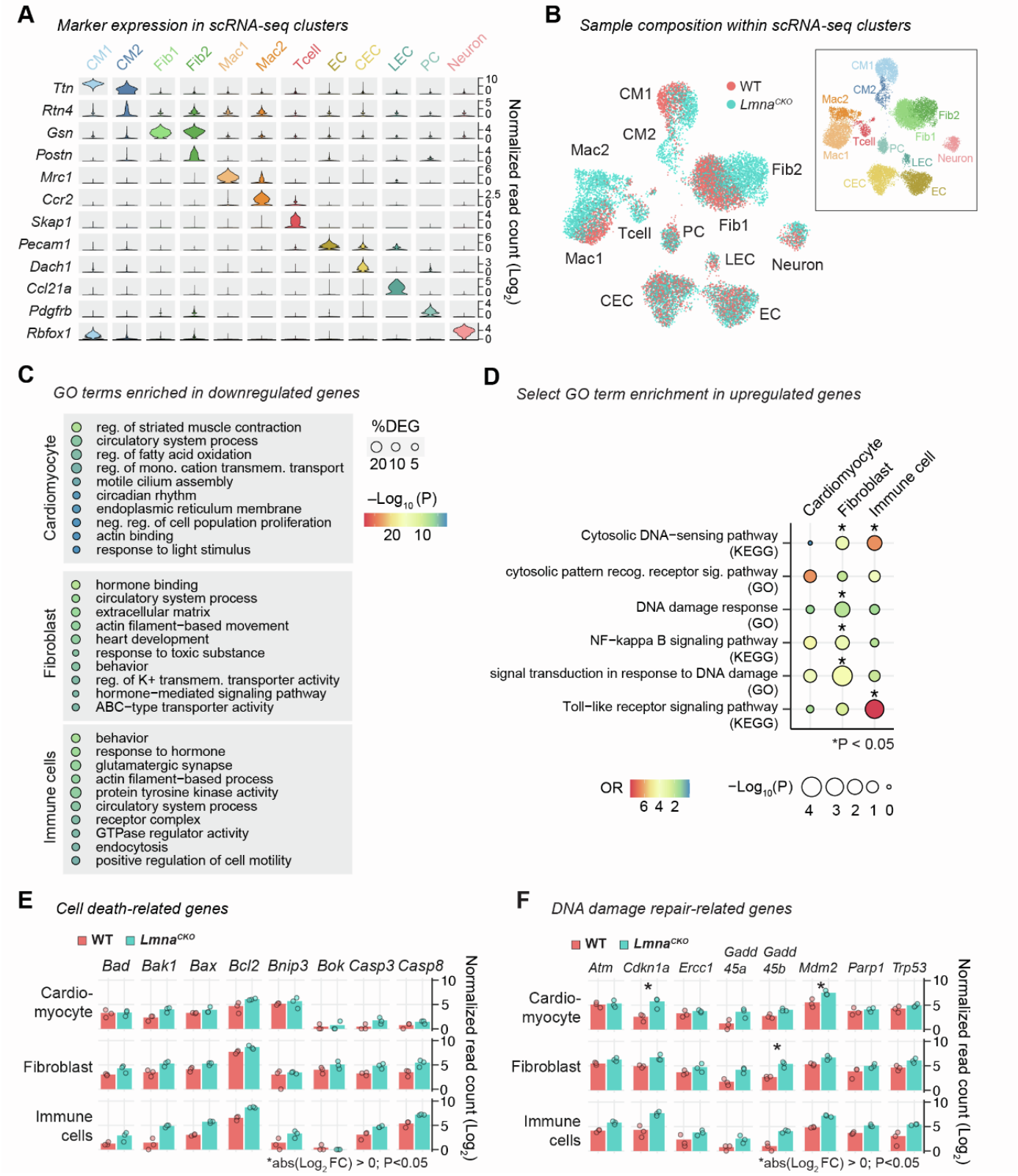

Figure S4. Single-nucleus RNA-seq analysis of *Lmna*^{CKO} hearts

A) Expression (sum of single-nucleus normalized read count across 3 mice within cell type) of maker 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.