Supplementary Figures

Supplementary Figure 1. Virus replication is not different in Dys^{KI} myocytes. The first cycle of virus replication was the same in Dys^{WT} and Dys^{KI} myocytes demonstrating that the virus entry and replication within a cardiac myocyte is independent of dystrophin-cleavage.

Supplementary Figure 2. Dystrophin cleavage site mutant inhibits cell membrane disruption. Cell culture data indicates that disruption of the cell membrane as assessed by Trypan-blue staining is inhibited in CVB3 infected embryonic cardiac myocytes when the protease 2A cleavage site has been mutated in Dys^{KI} myocytes.

Supplementary Figure 3. Representative images of H&E sections from both Dys^{wt} and Dys^{KI} mice 8 days post-infection, scale bar= 500μm.

Supplementary Figure 4. CVB3 administration has no deleterious effect on survival or cardiac function 8 days post-infection in mice backcrossed into C3H background. A, In C3H mice, administration of CVB3 had no effect on lifespan in Dys^{WT} nor Dys^{KI} mice up to 8 days following CVB3 infection (Kaplan-Meier survival analysis). B, Echocardiographic parameters and heart weight comparisons 8 days post-CVB3 infection. Heart weight normalized to body weight (HW/BW (mg/g)), heart weight normalized to tibia length (HW/TL (mg/ml)), and fractional shortening were not significantly different in Dys^{WT} nor Dys^{KI} mice 8 days following CVB3 infection (mean ± S.E.M, NS=not significant, each data point is represented on graphs).

Supplementary Methods

Cells

HeLa cells were a kind gift from S.A. Huber, University of Vermont (Burlington, VT), and were cultured in DMEM culture medium with 5% FBS (Invitrogen, Carlsbad, CA). Embryonic cardiac myocytes were cultured from E16.5 embryonic hearts. Each embryonic heart was separately digested with 0.25% trypsin-EDTA, at 37°C, for 20 minutes. Myocytes were isolated from digested hearts by pipetting and then culturing on 0.1% gelatin coated culture plates. Genotyping of embryonic myocytes was performed by PCR using genomic DNA isolated from the embryonic tail.

Western blot analysis

Proteins were extracted from mouse hearts and cultured embryonic cardiac myocytes. Extracted proteins were subjected to western blot analysis using an ECL detection system as described previously (19). Primary antibodies used were mouse (DYS1, Leica, Richmond, IL) and rabbit dystrophin (Santa Cruz, San Diego, CA), enterovirus VP1 (Leica).

Immunofluorescent and histological staining

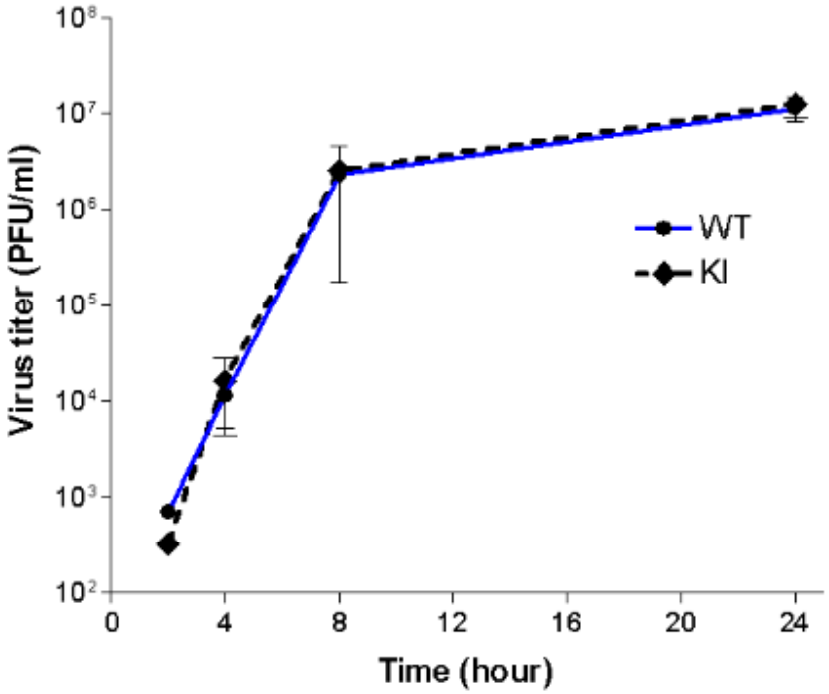
Hearts were snap-frozen by embedding in OCT Tissue Tek (Sakura Finetechnical, Torrance, CA). 10-µm sections were cut by Leica cryostat (Leica, Bannockburn, IL). Specimens were fixed with ice-cold acetone, followed by blocking and permeabilization with 2% BSA and 1% Triton X-100 in

PBS, and incubated with primary antibodies as follows: mouse dystrophin (DYS1, Leica) and rabbit dystrophin (Santa Cruz, CA), enterovirus VP-1 (Leica), CVB3-K1 (a gift from A. Henke, Friedrich Schiller University, Jena, Germany) (1), and α-sarcomeric actinin (Sigma). Target proteins were visualized with secondary antibodies conjugated with fluorophores (Alexa 488 and 594, 1:250; Invitrogen) and Hoechst nuclear stain. Fluorescein-conjugated wheat germ agglutinin (Vector Laboratories, Burlingame, CA) was used to demarcate individual myocytes for quantitation purposes. Hematoxylin and eosin (H & E) and von Kossa staining procedures were completed by the UCSD Moores Cancer Center Histology and Immunochemistry Core. EBD, H & E, and von Kossa stained tissue sections were visualized using the Nanozoomer Digital Pathology System at the UCSD Neurosciences Microscopy Core (NS047101). Image J was used to quantitate captured images.

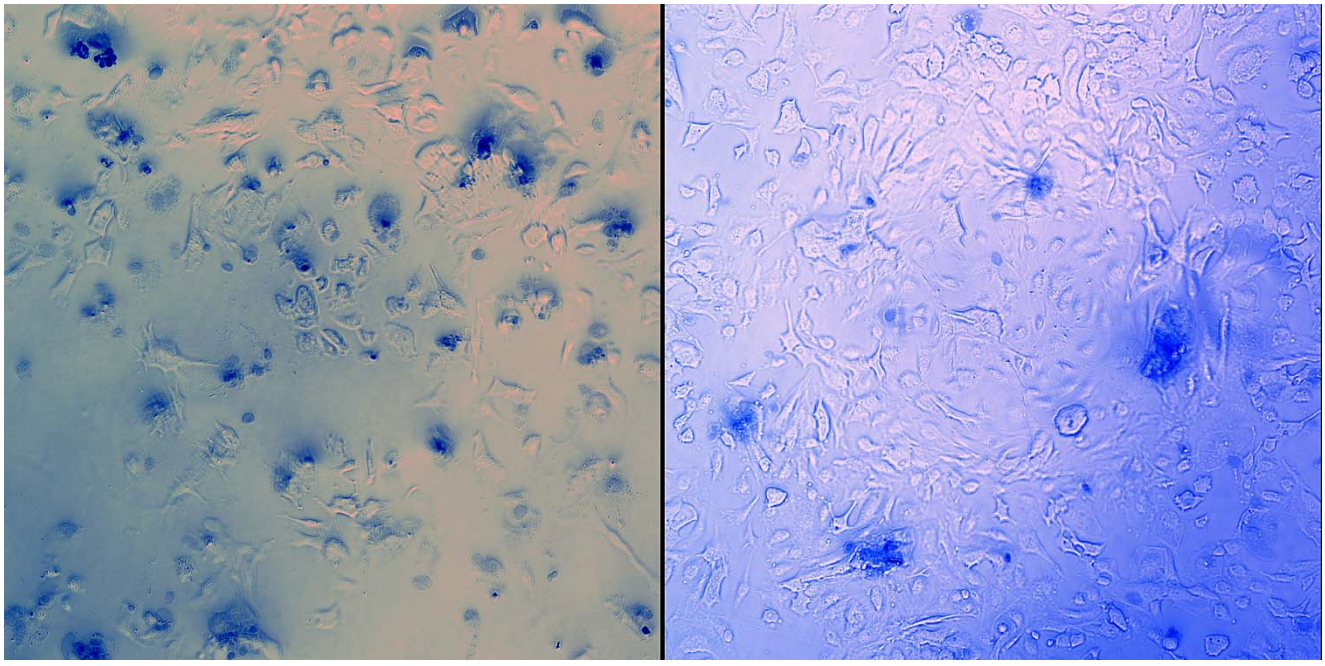
Mouse echocardiography

M-mode echocardiograms were performed on tamoxifen treated mice and virus infected Dys^{WT} and Dys^{KI} as described previously (19).

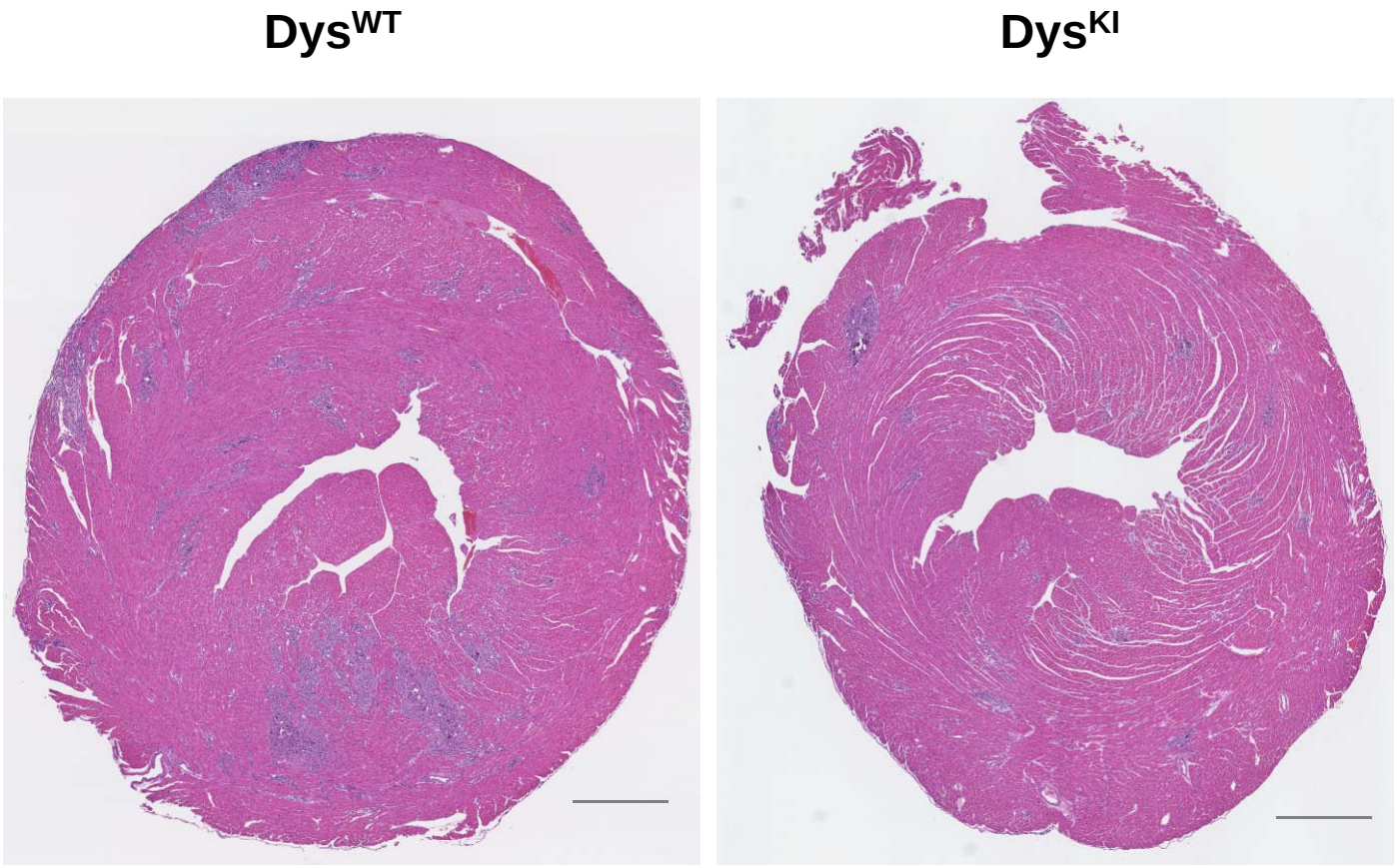
Supplementary Figure 1



Supplementary Figure 2

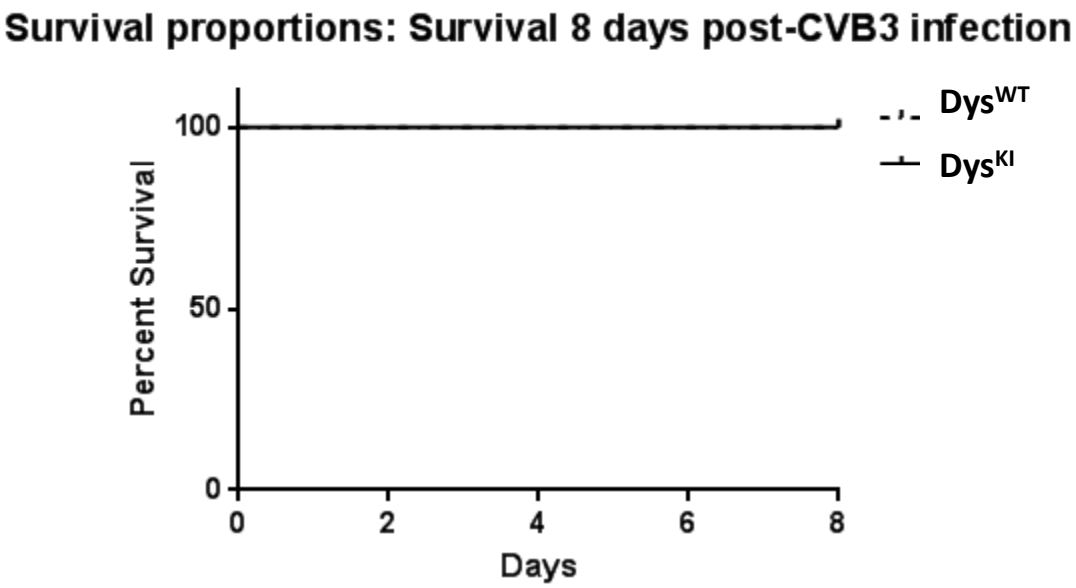


Supplementary Figure 3



Supplementary Figure 4

A



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