

Supplementary Information

AAV9-cBIN1 Gene Therapy Rescues Chronic Heart Failure due to Ischemic Cardiomyopathy in a Canine Model

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Supplementary Methods:

Fibrosis and Inflammation Assessment

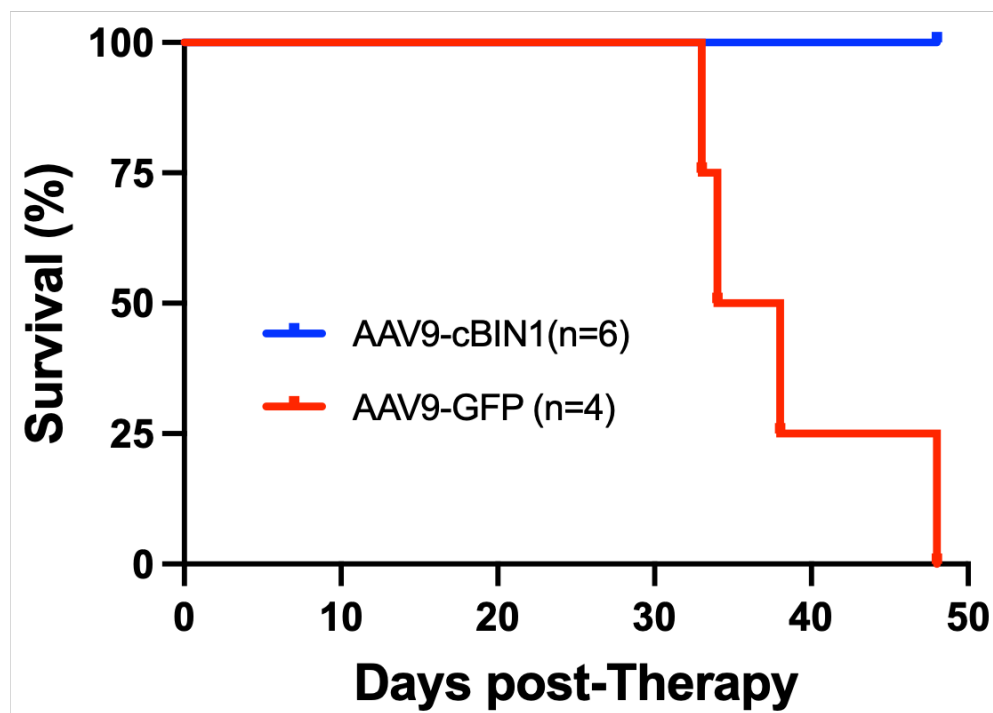
Left ventricle tissues are collected from *AAV9-cBIN1*-treated group (n=6, 3 animals, 2 samples each), *AAV9-GFP*-treated group (n=6, 3 animals, 2 samples each) and untreated HF control group (n=4, 2 animals, 2 samples each). For fibrosis and inflammation assessment, tissues are embedded in paraffin, and 10- μ m-thick sections are stained with Hematoxylin and Eosin (H&E), and Masson's Trichrome staining according to standard protocols, and mounted in permanent mounting medium. Slides are viewed using an Olympus BX51 microscope. The amount of the transmural tissue with increased fibrosis or inflammation is evaluated by the pathologist. The scale is from 0 to 3 with 0: no increased fibrosis or inflammation compared to normal hearts, 1 as mild:<25% of the tissue, 2 as moderate:>25->50% of the tissue, and 3 as severe:>50% of the tissue exhibiting increased fibrosis / inflammation. Wilcoxon matched-pairs signed rank test is used to compute the statistical comparison between *AAV9-GFP* treated group and *AAV9-cBIN1* treated group.

Survival Analysis

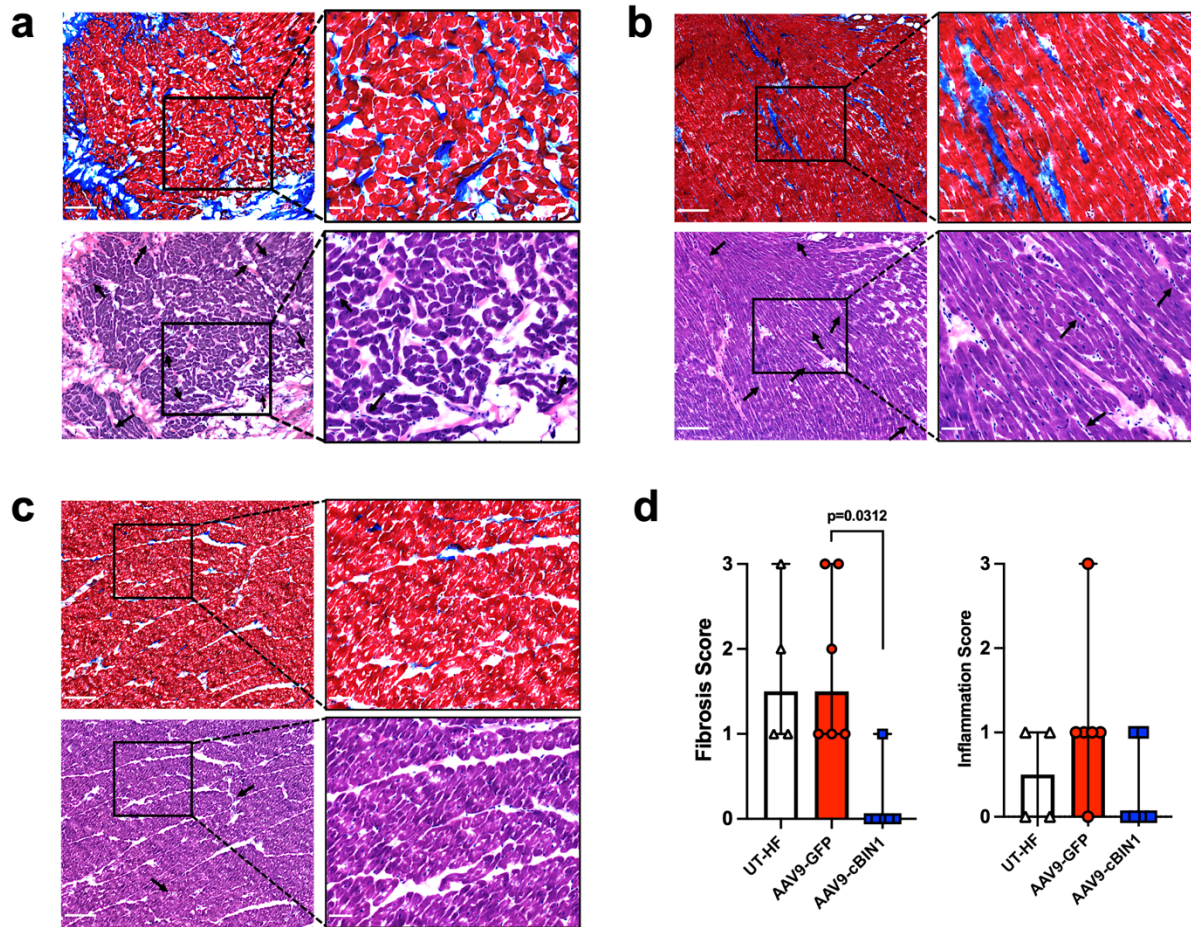
The Kaplan-Meier curve and log-rank (Mantel-Cox) test is finally used to compare the survival curves between *AAV9-GFP*-treated group and *AAV9-cBIN1*-treated group as shown in Supplementary Figure 1. All analyses are conducted in GraphPad PRISM 9.0. A p-value of < 0.05 is considered statistically significant.

By day 48 post-treatment, the *AAV9-cBIN1* treated group is significantly improved survival compared to the *AAV9-GFP* treated group ($p = 0.001$), as shown in Supplementary Figure 1. The median survival time for the *AAV9-GFP* treated group was 36 days. In contrast, the animals that received *AAV9-cBIN1* treatment were still alive at the end of the study. The hazard ratio (Mantel-Haenszel) for *AAV9-GFP*-treated group relative to *AAV9-cBIN1*-treated group was 41.3 (95% CI: 4.44 to 385), indicating a significantly higher risk of mortality in the *AAV9-GF*- treated group.

Supplementary Figure 1. Kaplan-Meier survival curves.



Supplementary Figure 2. Fibrosis and inflammation score assessment.



Top row – a, b, and c: Masson's trichrome staining. **Bottom row – a, b, and c:** H&E staining. **a** No-treatment. **b** Control treatment - AAV9-GFP. **c** Gene therapy - AAV9-*cBIN1*. Scale bars: 200 μ m - **left panel – a, b, and c**, and 50 μ m - **right panel – a, b, and c**. The top row demonstrates the presence of interstitial fibrosis (blue). The bottom row reflects the presence of inflammatory cell infiltration (highlighted with a black arrow) in the myocardial interstitium. **d** Degree of fibrosis (**left panel**) and inflammation (**right panel**) as median values of transmural sections on a semiquantitative scale. The median rank difference is significant for fibrosis ($p=0.0312$) and is not significant for inflammation ($p=0.3125$). UT-HF refers as untreated heart failure canine model – heart

obtained at time 0 and did not receive any post-HF treatment. Data presented in **d** are shown as 'median' and error bars are shown as 'range'.

Source Data File:

The numerical data used for Supplementary Figures 1-2 are included as source data file in Supplementary Data 1.