*Single-cell RNA sequencing*

*Virus propagation and infection*

The Nancy strain of CVB3 was procured from the American Type Culture Collection (ATCC, Manassas, VA, USA), and the virus was titrated in Vero cells (ATCC)**. The adherent Vero cells were grown to 80 to 90% confluence in 75cm2 flasks in EMEM/10% fetal bovine serum (FBS) and were later infected with CVB3 with multiplicity of infection 1 in EMEM containing no FBS. After incubation at 37⁰ C for 1 hour with gentle intermittent rocking, maintenance medium (EMEM/2% FBS) was added. Based on the cytopathic effect of virus during the next 1 to 2 days, supernatants containing virus were harvested. After determining 50%** tissue culture infective dose (TCID50) values based on the Reed-Muench method, the virus stocks were aliquoted and preserved at -**80⁰ C. To infect mice, virus stock** diluted in 1x PBS to contain 10,000 TCID50 in 100 µl was administered intraperitoneally (i.p.). Animals were monitored closely, cages were changed once in 2 days, and body weights were taken daily until termination. In addition, an alternative food and fluid source, trans gel diet (ClearH2O, Portland, ME, USA), was placed on the cage floor as needed.

*Heart single cell preparation*

Single cell suspensions from mouse hearts were prepared as previously described (Pinto 2013, 2016). Briefly, male CVB3-infected mice on day 21 post-infection or their age-matched healthy control mice were euthanized using 2% CO2 in an asphyxiation chamber as per the IACUC guidelines. The hearts were perfused using Perfusion Buffer (1 × DPBS with 0.8 mM CaCl2, 5 ml/min for 5 minutes) until the liver was completely blanched and appeared pale yellow/brown in color. Next, hearts were isolated, and their atria and valves were removed, and the whole heart was minced to ~0.5-1 mm cubes using surgical scissors. Minced heart tissue was digested in 3 mL of Digestion Buffer (2 mg/mL Collagenase IV [Worthington Biochemical], 1.2 units/mL Dispase II [Sigma-Aldrich], in Perfusion Buffer) for ~45 minutes at 37° C using a rotating holder, with tissue suspension triturated once in every 15 minutes with 1000 μl wide-bore micropipette tips. The cell suspension was filtered through a 70 μm nylon filter mesh to remove any residual undigested tissue pieces. The filtrate was then diluted in ~15 mL Perfusion Buffer, and the cells were pelleted at ~200 × G for 20 minutes with no centrifuge brakes engaged. Cell supernatant was then aspirated and the pellet was re-suspended in ~15 mL of 1 × HBSS (Sigma-Aldrich) + 0.8 mM CaCl2. The cells were pelleted again as described above. In order to remove the unwanted debris, a debris removal kit was used as per the manufacturer’s guidelines (Milteny biotec..). The final cell pellet free of debris was resuspended in 1000 uL of 2% FBS in RPMI medium for downstream cell sorting using flow cytometry.

*Flow cytometry and sorting etc.*

*Sample processing and sequencing*

Two technical replicates, with n=5 to 6 mice per treatment group were used for heart sample processing. Approximately 16,000 cells were loaded into a single channel of the 10X Genomics chromium system with a target recovery of ~10,000 cells using the chromium v2 and v3 single cell reagent kit. cDNA was synthesized after the cell capture and lysis and amplified for 11 cycles

as per the manufacturer’s protocol (10X Genomics, Pleasanton, CA). Amplified cDNA was used to construct 3’ expression libraries and the libraries were pooled and run on an Illumina HiSeq 4000. Each lane consisted of 150 base-pair; paired-end reads. FASTQ files were aligned to the murine genome (mm10) using the CellRanger v3.0.2 pipeline as described by the manufacturer. Across aligned cells the mean number of reads per cell was 39,923, with an average of 95.3% of reads mapped to the mm10 genome.

*Single-cell data processing and analysis*

Initial processing of cells isolated form the heart in myocarditis run 1 (n=2,617), myocarditis run 2 (n=10,618), control run1 (n=1,528), and control run 2 (n=8,201) was performed using the Seurat R package (v3.0.2) (cite [here](https://www.nature.com/articles/nbt.4096) and [here](https://www.cell.com/cell/fulltext/S0092-8674(19)30559-8)). Samples were normalized using the SCTtransform approach (cite [here](https://genomebiology.biomedcentral.com/articles/10.1186/s13059-019-1874-1)) with default settings. The transformed data was then used into a single data set using canonical correlational analysis and mutual nearest neighbors (MNN) as described by Stuart et alia (cite [here](https://www.cell.com/cell/fulltext/S0092-8674(19)30559-8)). Dimensional reduction to form the uniform manifold approximation and project (UMAP) utilized the top 30 calculated dimensions and a resolution of 0.6. The schex R package (v1.1.5) was used to visualize mRNA expression of lineage-specific or highly differential markers by converting the UMAP manifold into hexbin quantifications of the proportion of single-cells with the indicated gene expressed. Default binning was set at 80, unless otherwise indicated in the figure legend.

Cell type identification utilized the SingleR (v1.0.1) R package (cite [here](https://www.nature.com/articles/s41590-018-0276-y)) with correlations of the single-cell expression values with transcriptional profiles from pure cell populations in the Immune Genome Project (Immgen) (cite [here](https://www.nature.com/articles/ni1008-1091)). In addition to correlations, canonical markers for cell lineages were utilized and are available in Supplemental Table X. Differential gene expression utilized the Wilcoxon rank sum test on count-level mRNA data. For differential gene expression across clusters or subclusters, *FindAllMarkers* function in the Seurat package using the log-fold change threshold > 0.25, minimum group percentage = 10%, and the pseudocount = 0.1. Differential comparisons between condition utilized the *FindMarkers* function in Seurat, without filtering and a pseudocount = 0.1. Multiple hypothesis correction was reported using the Bonferroni method. Cell cycle regression was performed in Seurat using the *CellCycleScoring* function and genes derived from Nestorowa et alia (cite [here](https://ashpublications.org/blood/article/128/8/e20/35749/A-single-cell-resolution-map-of-mouse)). Genes were isolated by calling *cc.genes.updated.2019* in R and then converting into murine nomenclature.Gene set enrichment analysis was performed using the escape R package (v0.99.0). Differential enrichment analysis was performed using the *getSignificance* function in escape that is based on the limma R package linear fit model. Cell trajectory The code for all analysis is available at <https://github.com/ncborcherding/Lasrado_Myocarditis> (this link is private until publication).

*Statistical Analysis*

Statistical Analyses were performed in R (v3.6.3). Two-sample significance testing utilized Welch’s T test, with significance testing for more than three samples utilizing one-way analysis of variance (ANOVA) with Tukey honest significance determination for correcting multiple comparisons. Two-proportion Z-tests was performed using the total number of cells in each condition as the number of trials and without a prior for proportion.