Our results indicate that SD-208 is very effective at reverting HCM CFs to a less activated state as well as inhibiting the activation seen when healthy donor CFs are cultured without serum. Additionally, SD-208 significantly reduces the concentration of sEV secreted without affecting the size or purity of the populations, indicating that sEV secretion is dependent on the activation status of the cell.

Conflict of Interest
N/A
for ambient transcripts (CellBender). Seurat pipeline was used for analysis including quality control, batch correction, dimensionality reduction, unbiased clustering and differential gene expression analysis. Scspy was used for data visualisation. Differential abundance of each cell state was examined by Milo. SCORPIUS was applied for trajectory analysis.

**Results**

This cardiotoxicity model induces decreased ejection fraction and fractional shortening with echocardiography in 6-Week mice. Data from 12 hearts (61,416 nuclei) allowed to identify 8 major cell types including cardiomyocytes (CM), endothelial and mural cells, fibroblasts, myeloid cells, B and T lymphocytes and neuronal-like cells. To analyse the changes occurring in CM, we performed unbiased clustering which defined 6 CM subpopulations. CM_Stressed is characterised by the enrichment of Nppb encoding for natriuretic peptide hormone, which is a well-known marker of cardiac stress, and Myh7, the foetal isoform of myosin heavy chain that is typically upregulated in diseased mouse hearts. CM_Intermediate also significantly enriches for Nppb, but at a lower level compared to CM_Stressed. CM_Basal expresses negligible levels of Nppb and Myh7, but shows higher expression of Angpt1, which was found to promote myocyte survival. CM_Energetic highly expresses nuclear encoded mitochondrial genes such as Cox4i1 and Atp5g1, indicating a metabolically active cell state. Amongst the two rare populations is CM_Conduction featuring HCN4, a classic pacemaker channel gene. Importantly, CM_Stressed were enriched in 6-Week group while CM_Intermediate was higher in both Dox treated groups. In contrast, CM_Basal is downregulated after Dox treatment. To determine how CM cell states relate to each other, and to test whether CM_Intermediate is a precursor of CM_Stressed, we performed trajectory analysis. By applying SCORPIUS, we uncovered a progression path from CM_Energetic, CM_Basal, CM_Intermediate to CM_Stressed, along with a list of 100 signature genes that characterise the progression. Notably, trajectory signature genes largely overlap with markers of those four states. Differential gene expression analysis confirmed that Myh7 and Nppb are upregulated in Dox-treated groups.

Abstract BS35 Figure 2

A, UMAP representation of cardiomyocytes, colour coded by 6 unbiased cell states. Each point represents a single nucleus. Marker genes are labelled next to cell state names. B-C, Milo results projected on UMAP. Each dot represents a neighbourhood consists of various numbers of nuclei. Dot size represents the number of nuclei per neighbourhood; line thickness represents the number of overlapped nuclei between neighbourhoods. Neighbourhoods were statistically tested and adjusted for multiple comparison; FDR < 0.1 was considered significant. Only significantly enriched neighbourhoods were shown. Dot colour represents log2FC against Dox-treated groups; blue, Vehicle; red, Dox-treated group (3-Day or 6-Week). D, Heatmap showing the expression of 100 trajectory signature genes. Top horizontal bars represent the progression of 4 cell states and pseudo-time, respectively. Signature genes were clustered into three modules (vertical bars on the left) corresponding to CM_Energetic (green), CM_Basal (blue) and CM_Stressed (red). E, Scatter plot and a curve showing the trajectory path of 4 cell states. Each point represents a nucleus, colour coded by cell states.
Conclusions We generated a single-nucleus dataset from Dox-treated and control mouse hearts, and identified a progression of cardiomyocytes states from basal to stressed.

Conflict of Interest NA