

Supplemental Figures, videos and tables

hsa-miRNA-548v controls the viscoelastic properties of human cardiomyocytes and improves their relaxation rates

Eva Vermersch^{1,2} PhD, Salomé Neuwendel¹, Charlene Jouve¹, Andrea Ruiz Velasco¹, Céline Pereira¹, Magali Seguret¹, Marie-Elodie Cattin-Messaoudi² PhD, Sofia Lotfi², Thierry Dorval² PhD, Pascal Berson³ PhD, and Jean-Sébastien Hulot^{1,4*} MD PhD.

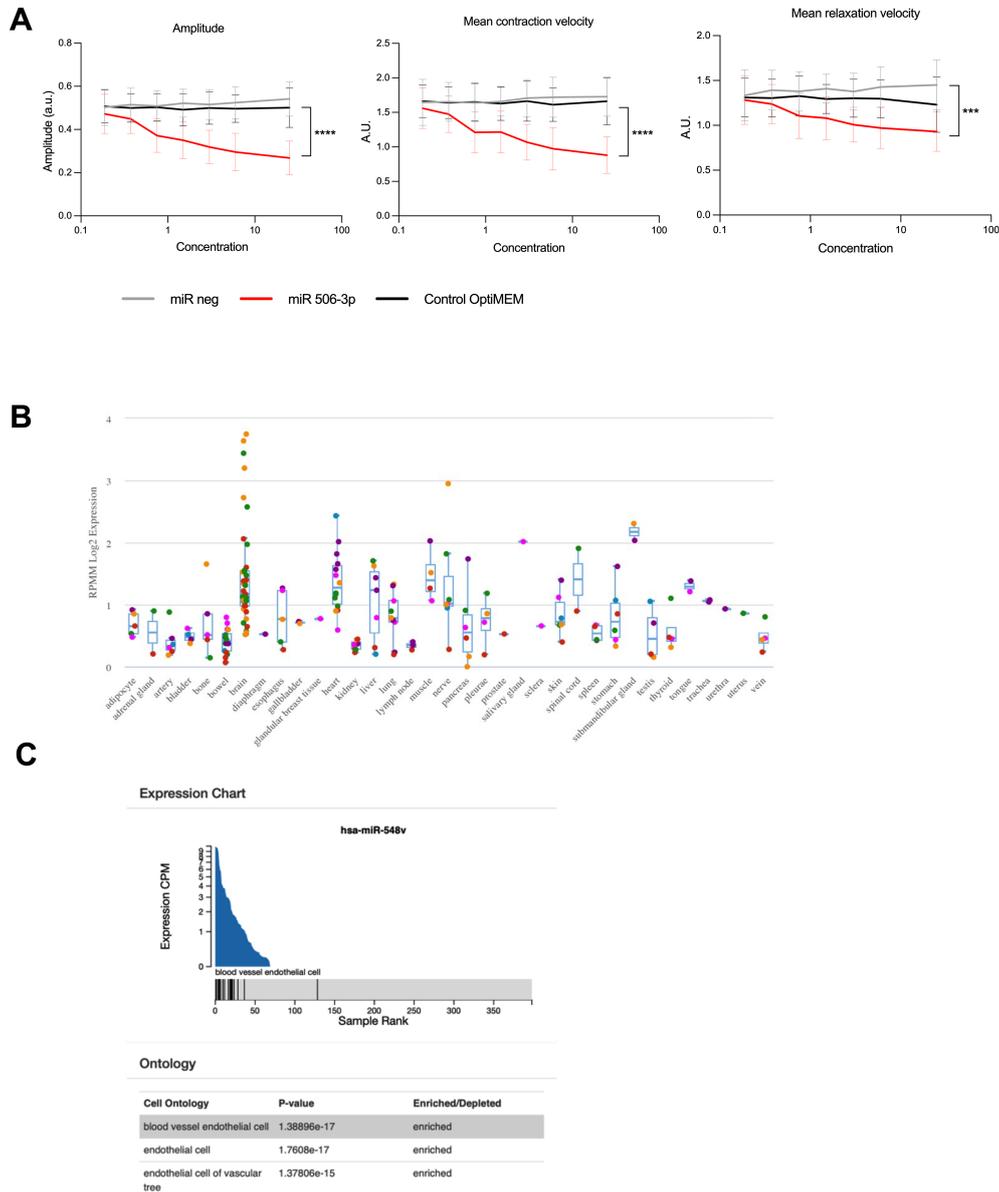


Figure Supplemental 1: HTS quality control and expression profile of hsa-miR-548V

A HTS Quality control: impact of different concentrations of miR negative control (grey) compared to the addition of transfection media only (black), or miR-506-3p (red) on amplitude, mean contraction velocity and mean relaxation velocity. N=3 screens. *** $p < 0.001$, **** $p < 0.0001$ for miR-506-3p as compared to controls, ANOVA with post-hoc comparisons. **B** MiRNATissueAtlas2 results for hsa-miR-548v. **C** Fantom5 results for hsa-miR-548v showing an exclusive expression in endothelial cells

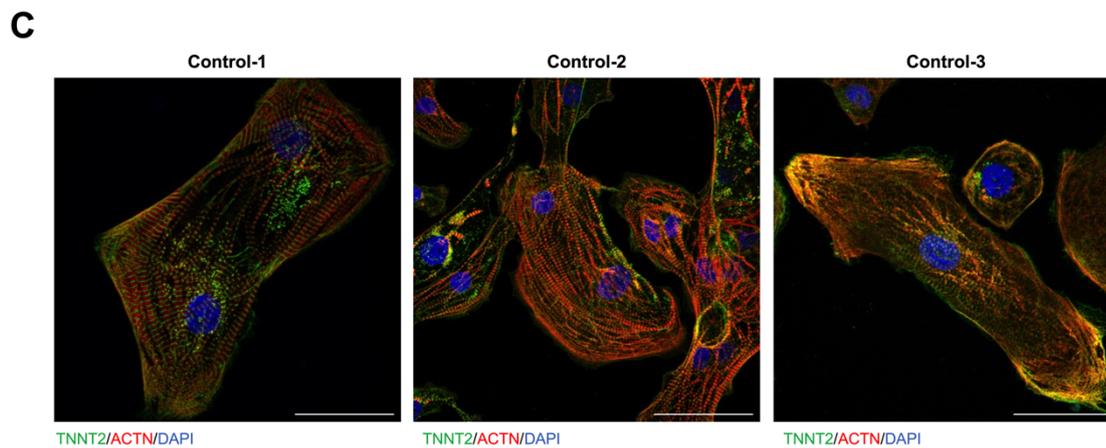
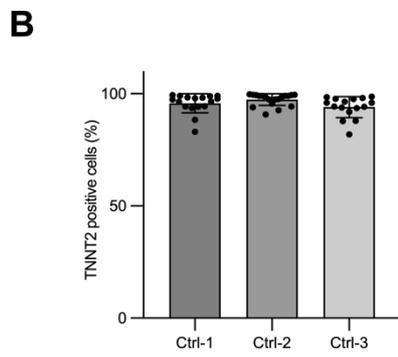
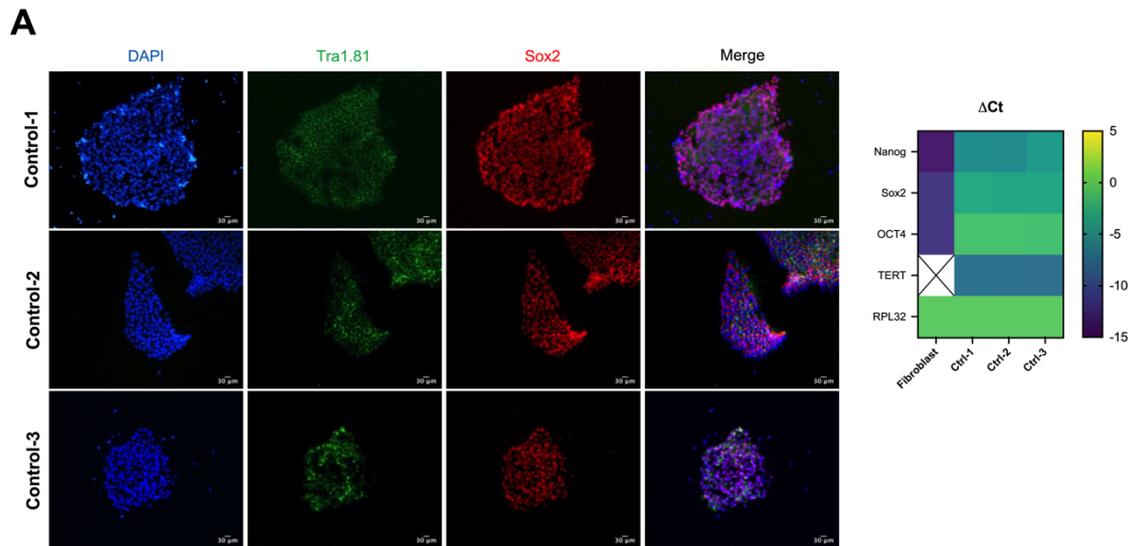


Figure Supplemental 2: Quality control of hiPS control cell lines:

A Control-1, Control-2 and Control-3 pluripotency evaluation by immunofluorescence (left) and qPCR (right). **B** Quality control of TroponinT2 positive cells by flow cytometry for Control-1 (n=17), Control-2 (n=19) and Control-3 (n=16). **C** Representative images of control cardiomyocytes stained for TroponinT2 (Red), Actinin (Green) and DAPI (Blue). Scale bar = 30 μ m.

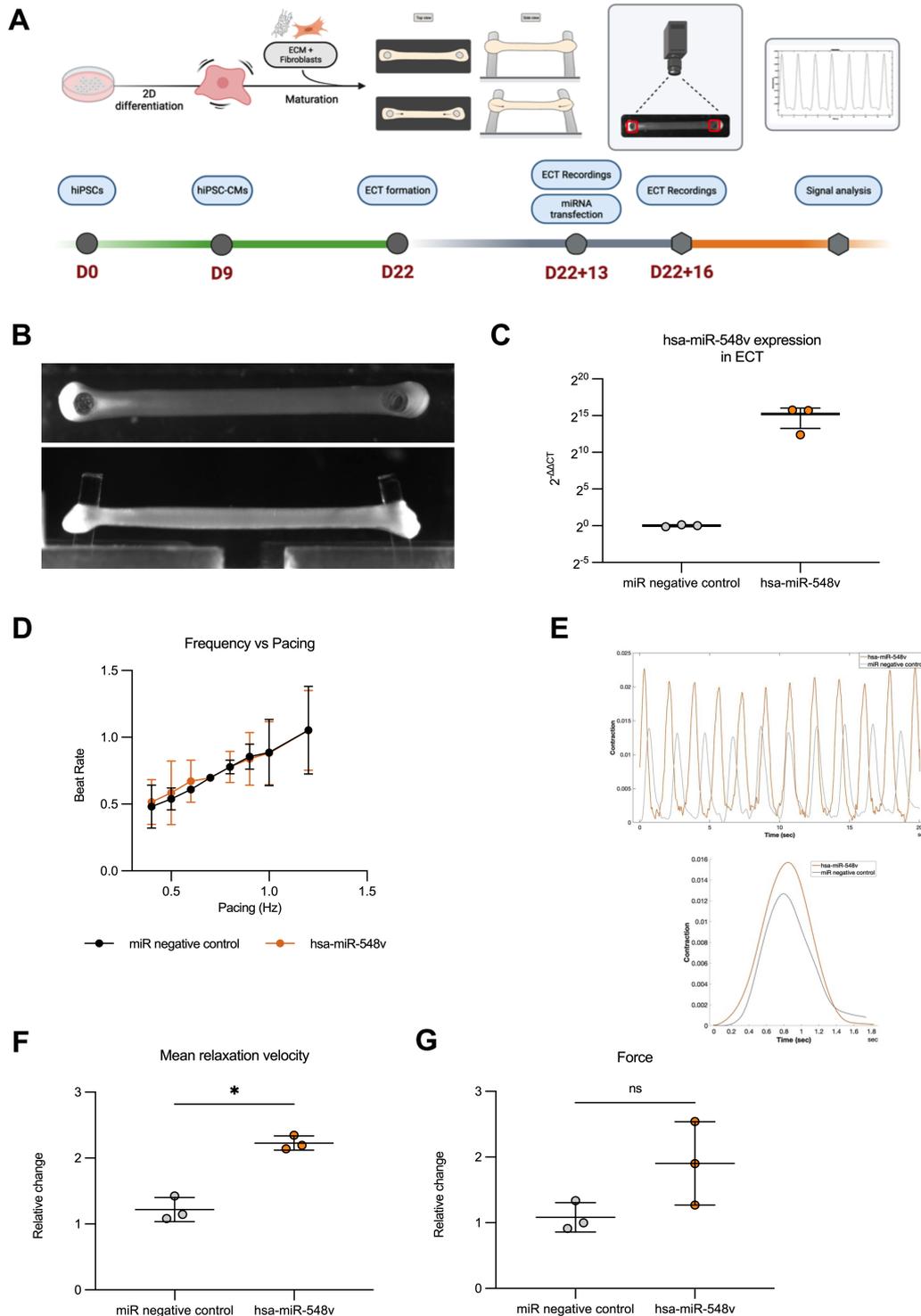
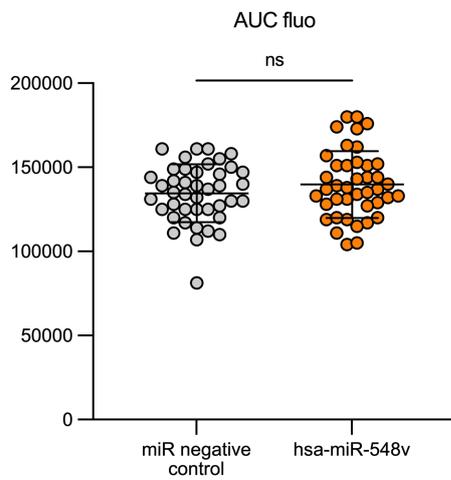
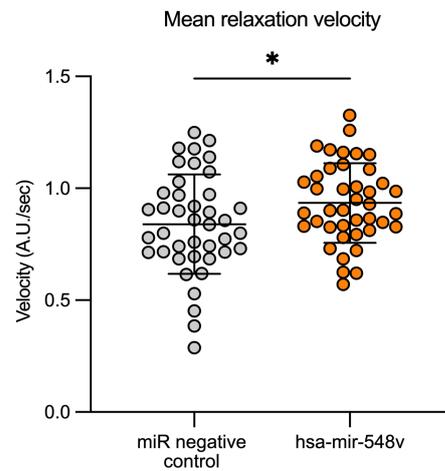


Figure Supplemental 3: hsa-miR-548v effect in hiPSC-CM-based engineered cardiac tissues (hECT)

A Experimental workflow. The green arrow represents the experiments until hECT formation. The grey arrow corresponds to baseline and 3 days post transfection hECTs' video recordings. The orange arrow corresponds to the post-processing of videos to acquire and analyze motion parameters. **B** Representative top and side view of hECT. **C** Evaluation of hsa-miR-548v expression levels 3 days after transfection (n=3 per groups). **D** Average beat rate according to pacing. (n=5 per group). **E** Representative recordings of beat-to-beat motion from hECTs transfected with hsa-miR-548v (orange) or miR negative control (grey). Averaged contraction/relaxation cycle recorded from ECTs transfected with hsa-miR-548v (orange) or miR negative control (grey). **F** Relative mean relaxation velocities in hECT 3 days after transfection with hsa-miR-548v (n=3) or miR negative control (n=3). (*, p < 0.05). **G** Relative developed force in hECT 3 days post transfection with hsa-miR-548v (n=3) or miR negative control (n=3).

A**B****Figure Supplemental 4: Calcium imaging in hiPSC-CMs monolayers**

A Area under the curve – calcium transient analysis in fluo4 loaded hiPSC-CMs transfected with miR negative control (n=42) or hsa-miR-548v (n=41).

B Mean relaxation velocity extracted from bright field records of hiPSC-CMs transfected with miR negative control (n=41) or hsa-miR-548v (n=41). *, $P < 0.05$, T test.

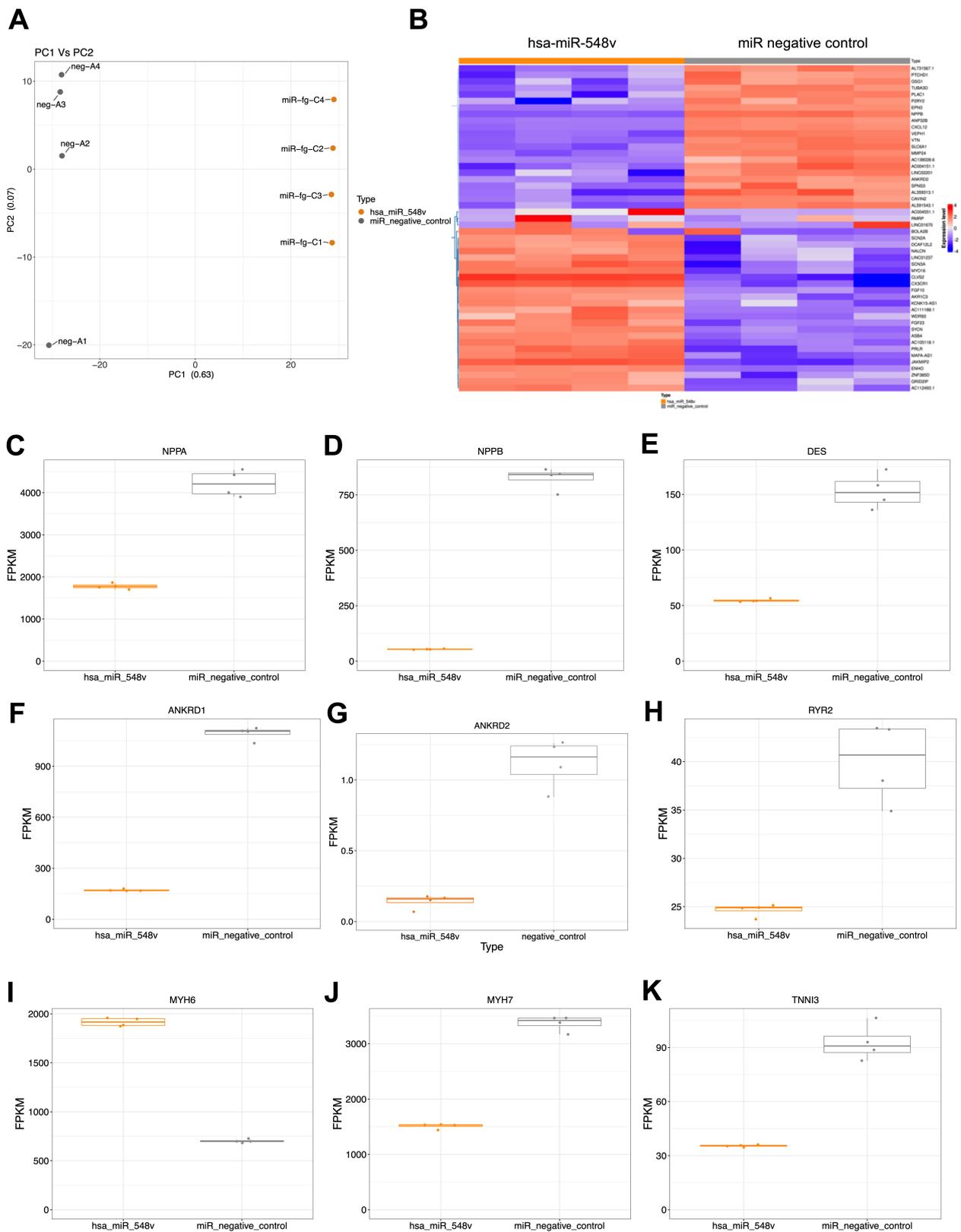


Figure Supplemental 5: Analysis of RNA-sequencing between control and hsa-miR-548v transfected hiPSC-CMs. A Principal component analysis based on the 1000 most variant genes. **B** Top 50 differentially expressed genes in hsa-miR-548v vs miR negative control. **C-K** level of expression of **C** *NNPA*, **D** *NNPB*, **E** *DES*, **F** *ANKRD1*, **G** *ANKRD2*, **H** *RYR2*, **I** *MYH6*, **J** *MYH7*, **K** *TNNI3*

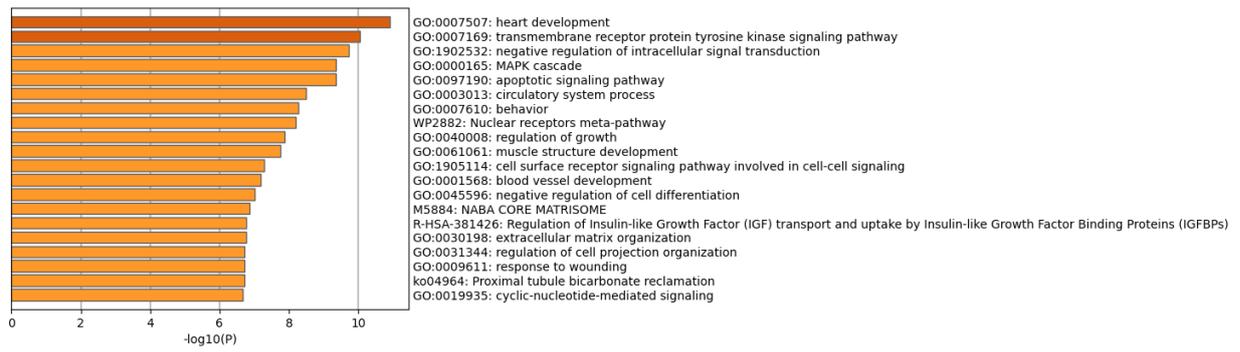
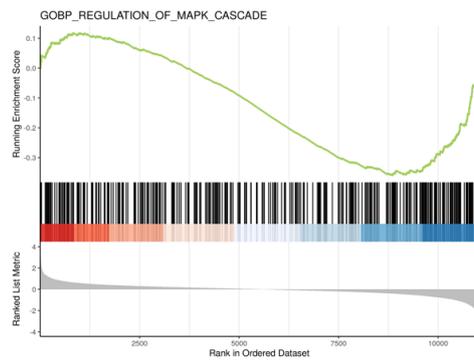
A**B**

Figure Supplemental 6: Pathway analysis of RNA-sequencing between control and hsa-miR-548v transfected hiPSC-CMs.

A GSEA enriched pathways.

B Down-regulation of the MAPK cascade in response to hsa-miR-548v transfer.

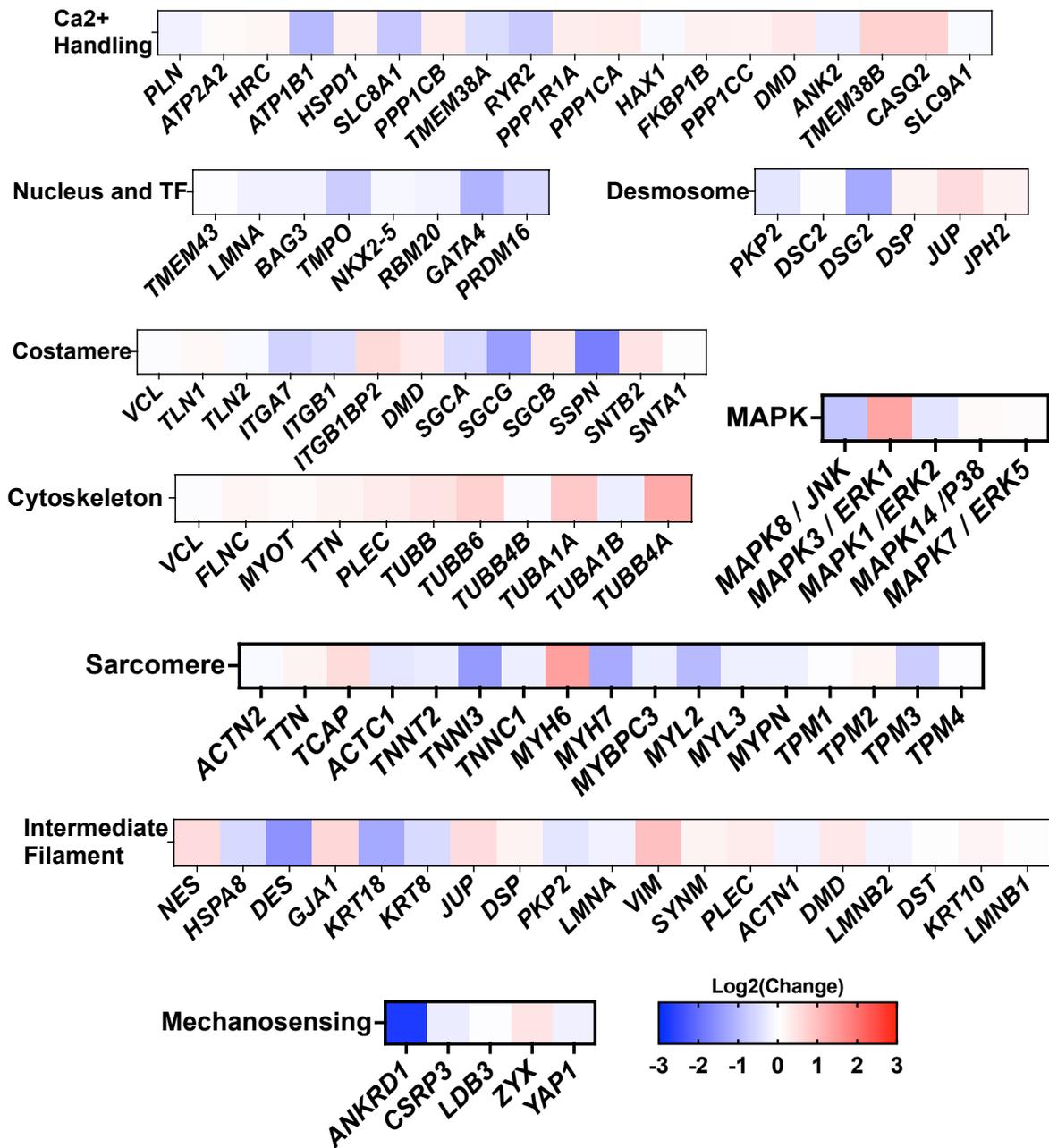


Figure Supplemental 7: Differential analysis of key genes of cardiomyocytes architecture

Supplemental Video V1: Bright field (BF) recording during HTS

Supplemental Video V2: Vector flow analysis of BF analysis during HTS

Supplemental Video V3: Beating ECT generated as linear macrostructure imaged by high-speed microscopy

Supplemental Video V4-V6: Beating ring-shaped ECT generated with the three control hiPS cell lines.

Supplemental Video V7: Beating ring-shaped ECT generated with the BRAF T599R mutated hiPSC-CMs

Supplemental Table 1: Overexpressed genes in hsa-miR-548v transfected cells compared to miR negative control transfected cells

Supplemental Table 2: Underexpressed genes in hsa-miR-548v transfected cells compared to miR negative control transfected cells

Supplemental Table 3: List of oligonucleotides used for CRISPR-Cas9 experiments

Figure 5E full unedited gels

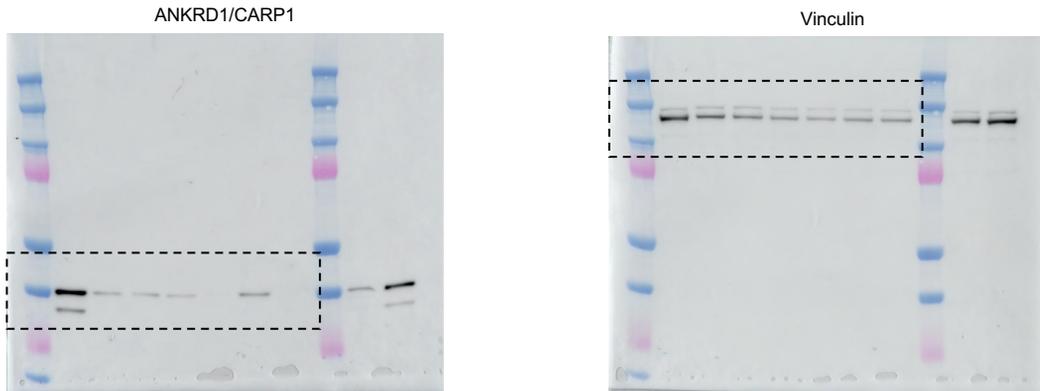


Figure 6C full unedited gels

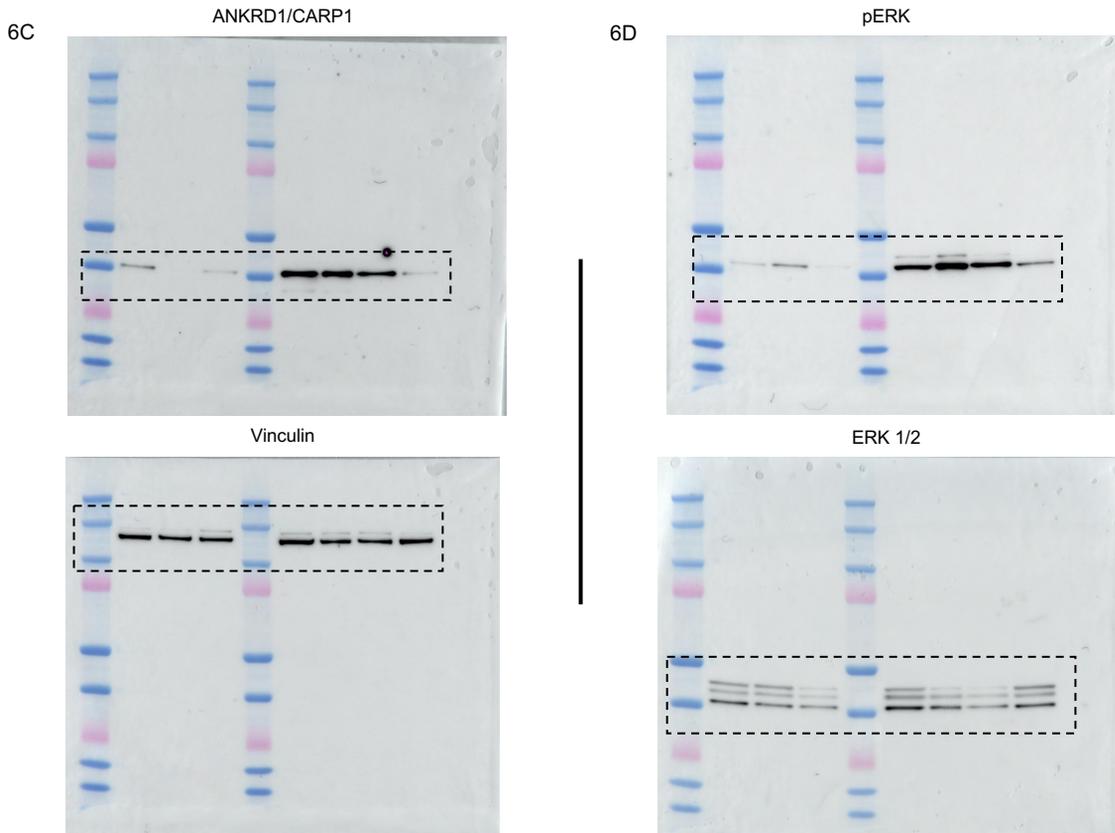


Figure 6D full unedited gels

