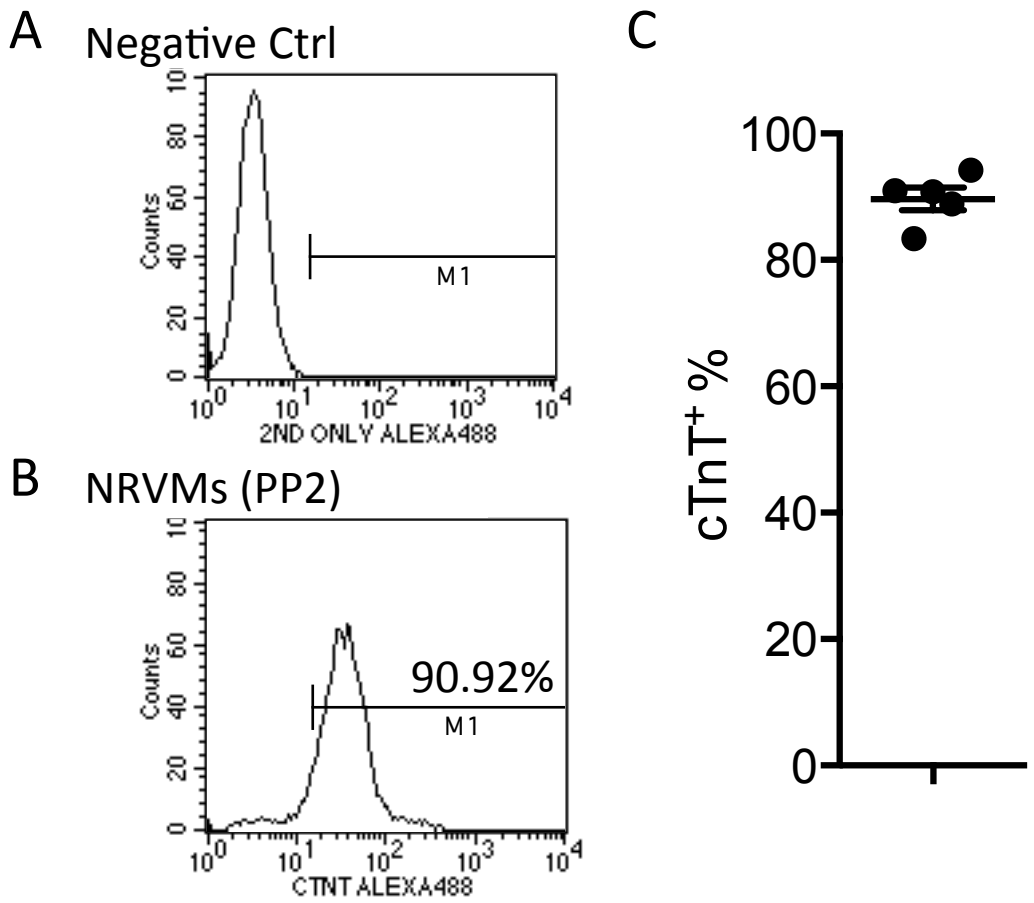
Age-dependent Functional Crosstalk Between Cardiac Fibroblasts and Cardiomyocytes in a 3D Engineered Cardiac Tissue

Supplementary Figures

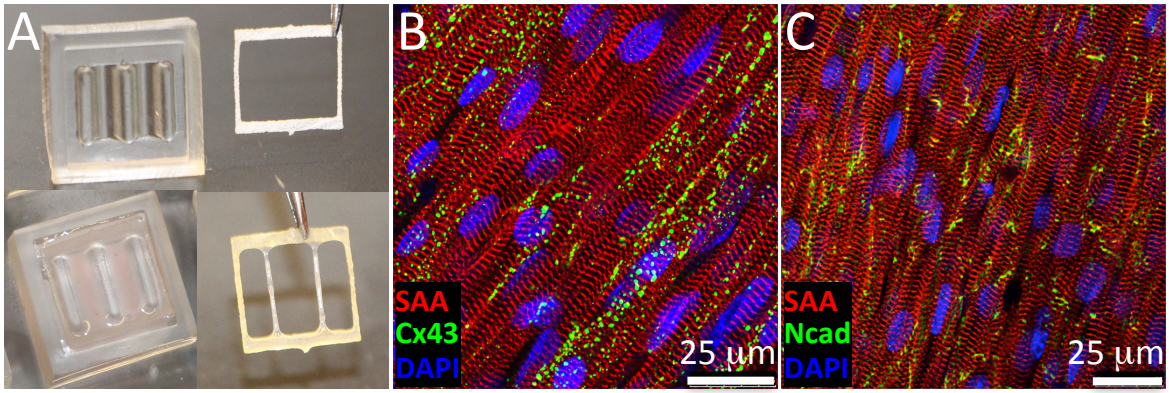
Yanzhen Li, Huda Asfour and Nenad Bursac

Supplementary Figure 1.



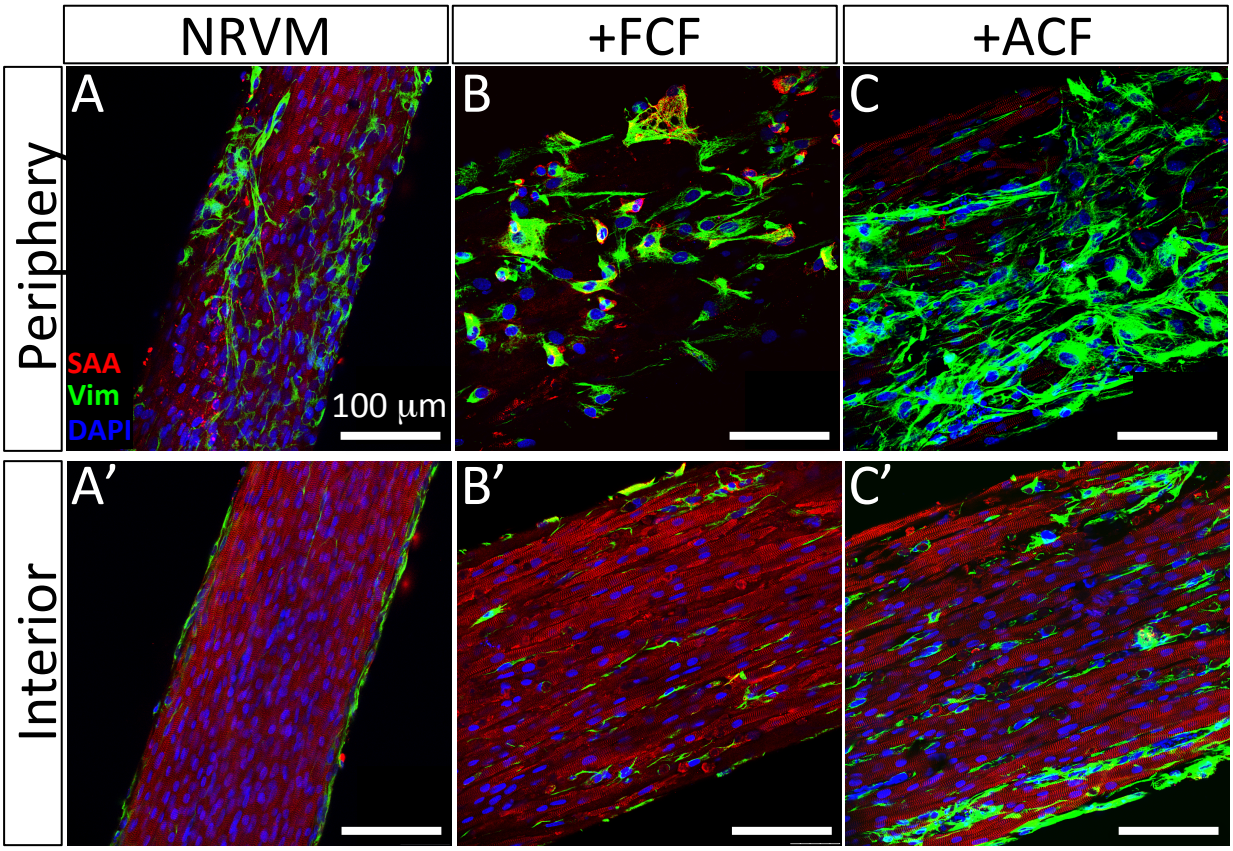
Supplementary Figure 1. FACS analysis of cTnT+ cells in freshly isolated NRVMs. (A) Representative negative control sample of NRVMs with only secondary antibody staining. (**B**) Representative PP2 (preplate 2) NRVMs stained with cTnT primary antibody and Alexa 488 secondary antibody. 90.92% of cells are cTnT+. **(C)** Percentage of cTnT+ cells in the NRVM population from N=5 independent cell isolations.

Supplementary Figure 2.



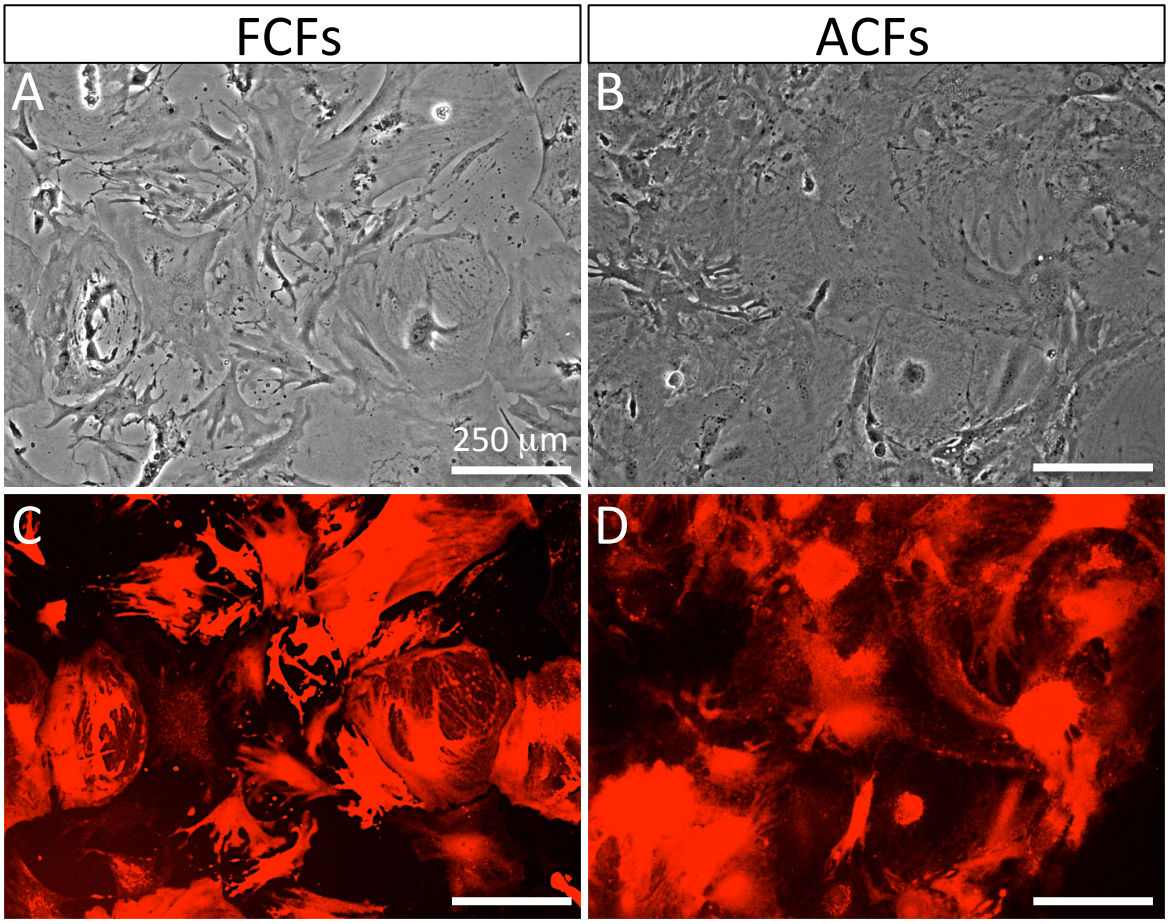
Supplementary Figure 2. Fabrication and structure of 3D engineered cardiac tissue bundles. (A) Cells were encapsulated in fibrin/matrigel matrix and cast into a PDMS mold (upper left) containing a nylon frame (upper right). Frames with cardiac bundles (bottom left) were removed from PDMS molds the day after assembly and cultured free-floating for 2 weeks (bottom right). **(B-C)** Representative images of control NRVM bundles stained for sarcomeric α-actinin (SAA, red), Connexin (Cx43, green) or N-cadherin (Ncad, green) and DNA (DAPI, blue).

Supplementary Figure 3.



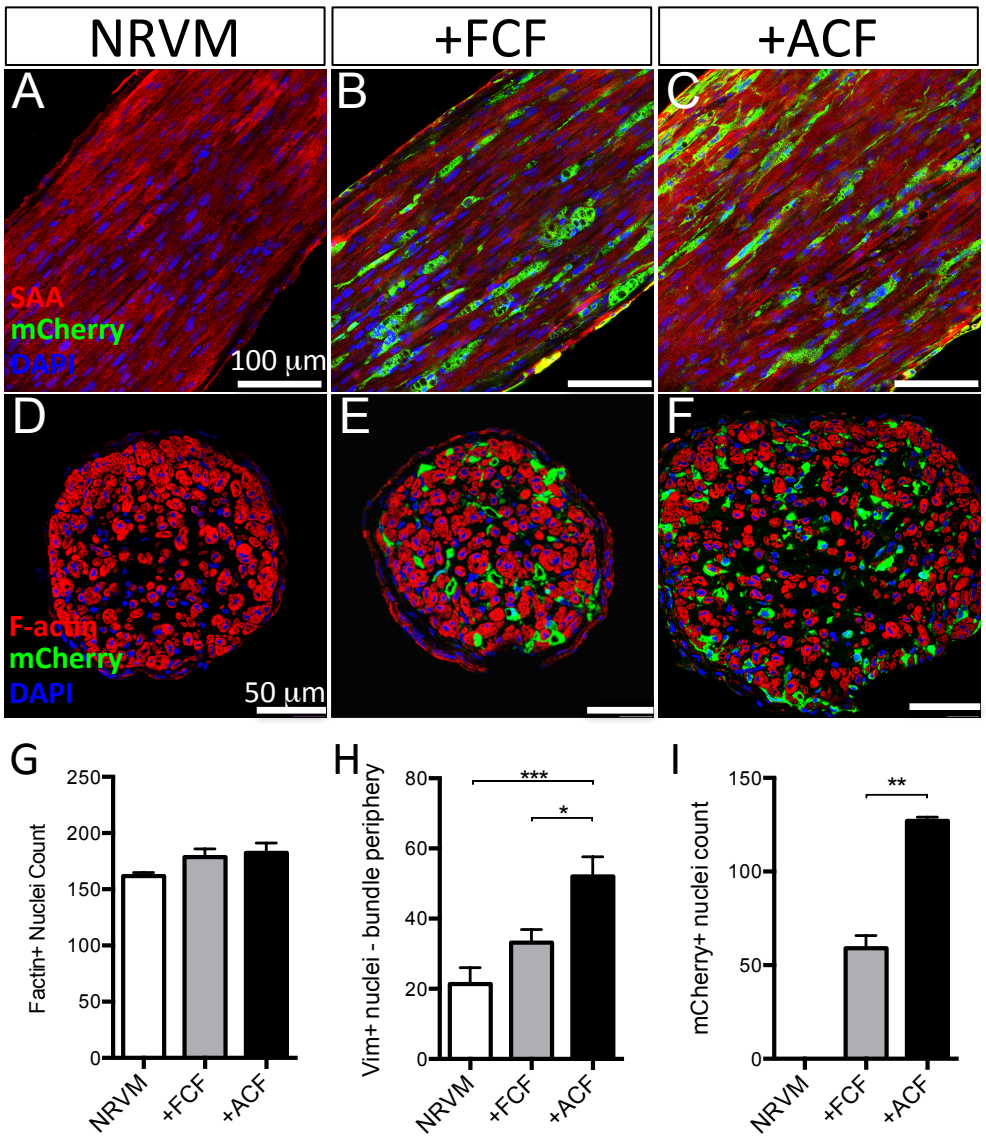
Supplementary Figure 3. Distribution of cardiac fibroblasts in engineered tissue bundles. (A-C and A'-C') Representative images of NRVM, NRVM + 30% fetal cardiac fibroblasts (+FCF) and NRVM + 30% adult cardiac fibroblasts (+ACF) bundles, stained for sarcomeric α-actinin (SAA, red), Vimentin (Vim, green) and DNA (DAPI, blue). Panels A-C show outer surface (periphery) of the bundles. Panels A'-C' show interior of the bundles.

Supplementary Figure 4.



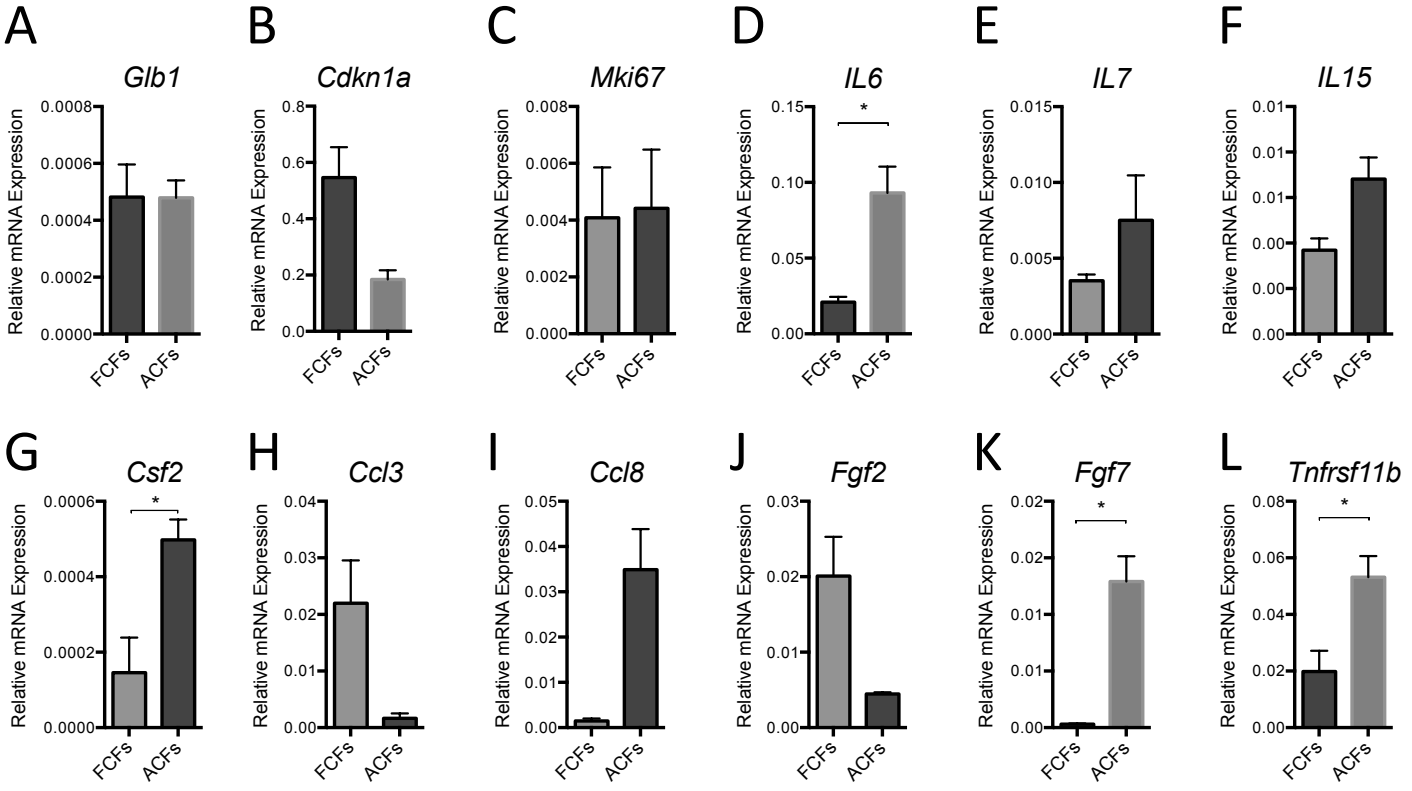
Supplementary Figure 4. Labeling fetal and adult cardiac fibroblasts with mCherry fluorescence. (A-B) Representative bright field images of FCFs and ACFs at passage 3. (C-D) mCherry expression in FCFs and ACFs after lentiviral transduction (with pRRL-CAG-mCherry-Puro) and puromycin selection.

Supplementary Figure 5.



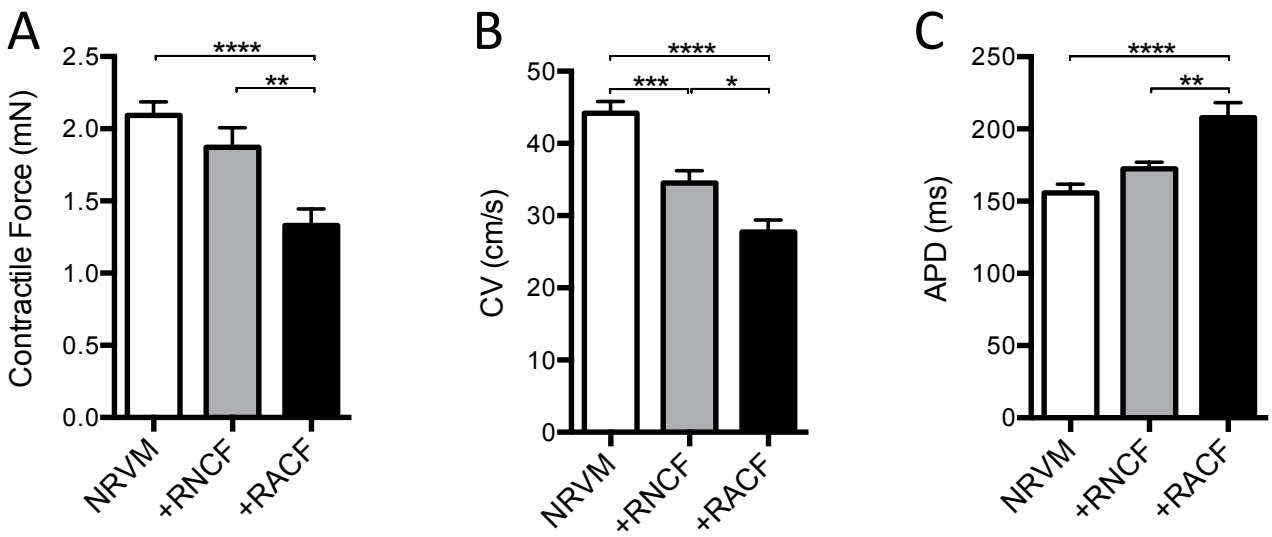
Supplementary Figure 5. Cellular localization and composition in cardiac bundles. (**A-C**) Representative whole bundle stainings for sarcomeric α-actinin (SAA, red), mCherry (DsRed antibody staining, green) and DNA (DAPI, blue) in NRVM, +FCF, and +ACF bundles. (**D-F**) Representative cross-sectional stainings for filamentous actin (Factin, red), mCherry (green), and DNA (DAPI, blue) in NRVM, +FCF and +ACF. **(G-H)** Average nuclei counts in Factin^{+} areas (**G**) and Vimentin^{+} areas at the periphery (**H**) of bundle cross-sections (n=6 bundles per group). **(I)** mCherry+ nuclei per bundle cross-section (n=3 bundles per group). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

Supplementary Figure 6.



Supplementary Figure 6. Differential expression of genes related to senescence in FCFs and ACFs. (A) Quantitative PCR (qPCR) analysis of *Glb1* **(A)**, *Cdkn1a* **(B)**, *Mki67* **(C)**, *IL6* **(D)**, *IL7* **(E)**, *IL15* **(F)**, *Csf2* **(G)**, *Ccl3* **(H)**, *Ccl8* **(I)**, *Fgf2* **(J)** , *Fgf7* **(K)** and *Tnfrsf11b* **(L)**. The relative mRNA expression was normalized to house keeping gene *B2M* and shown as $2^{-\Delta CT}$ (N=3 independent experiments). *P < 0.05.

Supplementary Figure 7.



Supplementary Figure 7. Effects of rat neonatal (rNCFs) and adult (rACFs) cardiac fibroblasts on cardiac bundle function. (A) Maximum contractile forces recorded during 1 Hz pacing at optimal tissue length. **(B)** Conduction velocity (CV) at 2 Hz stimulation rate. **(C)** Action potential duration (APD) measured from activation time to 80% repolarization from bundles paced at 2 Hz (N=3 independent experiments, n=13-15 bundles per group). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.