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Supplemental Information

**Delta-1 Functionalized Hydrogel Promotes
hESC-Cardiomyocyte Graft Proliferation
and Maintains Heart Function Post-Injury**

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SUPPLEMENTARY INFORMATION

Delta-1 functionalized hydrogel promotes hESC-cardiomyocyte graft proliferation and maintains heart function post-injury

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Figure S1. Related to Figure 1. Immobilized Delta-1 promotes Notch-mediated luciferase expression in 2D. (A) After culturing U2OS CSL luc/ren Notch reporter cell line on the 2D platform, Notch-driven luciferase expression (Delta) significantly increases relative to tissue culture polystyrene surfaces (Untreated) and IgG treated (Control) controls, peaking between 48 and 72 hrs. (B) Addition of fibronectin (FN) during ligand immobilization in 2D does not negatively impact Notch response, and in fact results in a slight increase in Notch signaling by CSLuc/ren luciferase analysis (Delta). (C) HESC-cardiomyocytes proliferate in response to immobilized, oriented Delta-1 (Delta) or IgG (Control) in 2D with inclusion of FN. There is a dose-dependent effect on cardiomyocyte proliferation, measured by histology as double-positive β MHC⁺/BrdU⁺ cells, with diminishing proliferation at the highest ligand density. (D) Human cardiomyocyte number increases after culture on 2D Delta-1 surfaces (Delta) compared with IgG coated surfaces (Control). The Notch inhibitor (gamma-secretase inhibitor, GSI) blocks Delta-induced proliferation and also reduces baseline proliferation. For all panels, * indicates $p < 0.05$ and error bars denote SEM.

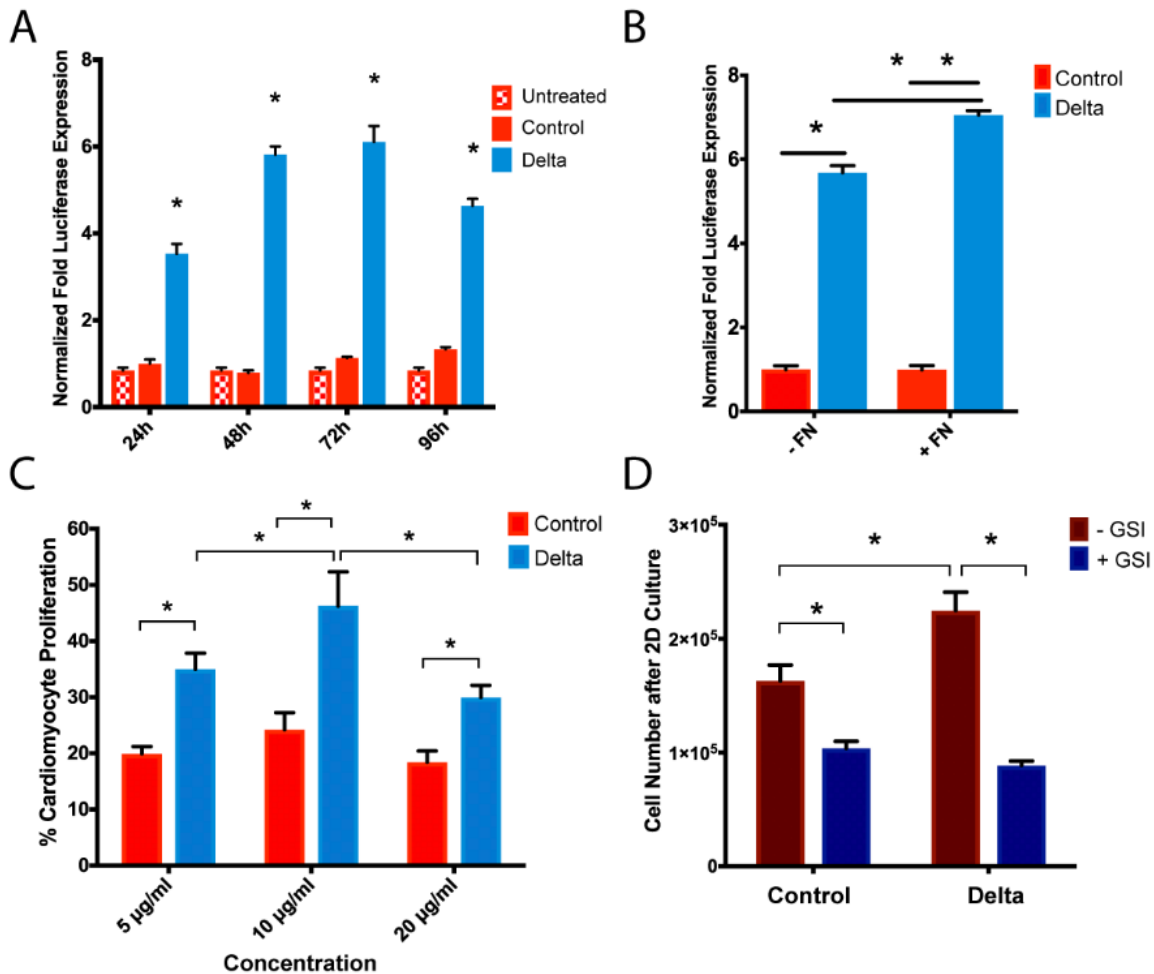


Figure S2. Related to Figure 1. Optimization of 3D gel protocol. (A) Delta-1 ligand orientation via anti-IgG binding is required to achieve detectable Notch-mediated luciferase expression in 3D gels. Samples were analyzed after 48 hrs in culture. Control uses IgG protein bound to collagen via anti-IgG (oriented) or directly to collagen (unoriented). (B) Increasing the duration of anti-IgG binding time facilitates higher Delta-1 immobilization as measured through Notch-driven luciferase expression. Samples were analyzed after 48 hrs in culture. Control uses IgG protein bound to collagen via anti-IgG. For all panels, * indicates $p < 0.05$ and error bars denote SEM.

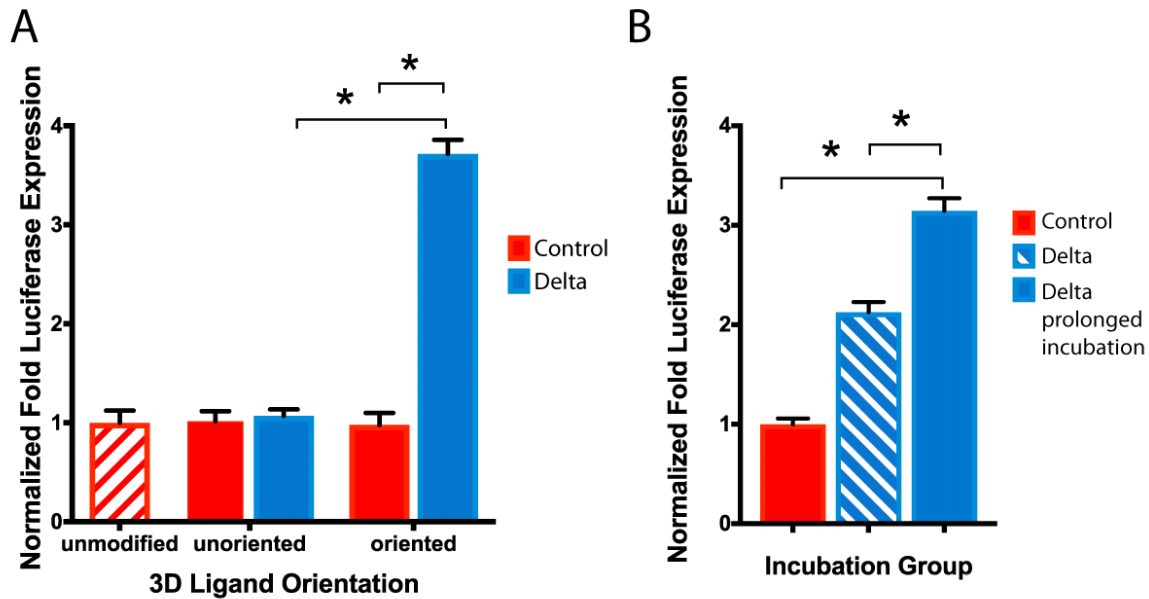


Figure S4. Related to Figures 2, 3, S2. Pilot study for reducing required cell dose. (A) Timeline for 2 week pilot study where animals received 5×10^6 hESC-CMs + IgG (Control + hESC-CMs) or Delta (Delta + hESC-CMs) gel. (B) Sum total of β MHC⁺ graft area normalized to LV area across all analyzed tissue sections. Because $n=2$, no error bars are shown. (C) β MHC⁺ graft area is normalized to fibrotic tissue area (picrosirius red area). Because $n=2$, no error bars are shown. (D) Fibrosis is identified in the myocardium by picrosirius red for collagen and healthy myocardium is counterstained with fast green. Scale bar = 5mm. (E) Transplanted human cardiomyocytes are identified with β MHC and visualized with DAB (brown) with a hematoxylin counterstain to quantify graft area. Scale bar = 100 μ m. (F) CD68 antibody, labeling monocytes and macrophages, visualized with DAB (brown) and a hematoxylin counterstain. Scale bar = 100 μ m. Quantification of macrophage content at 2 weeks in Figure 3I.

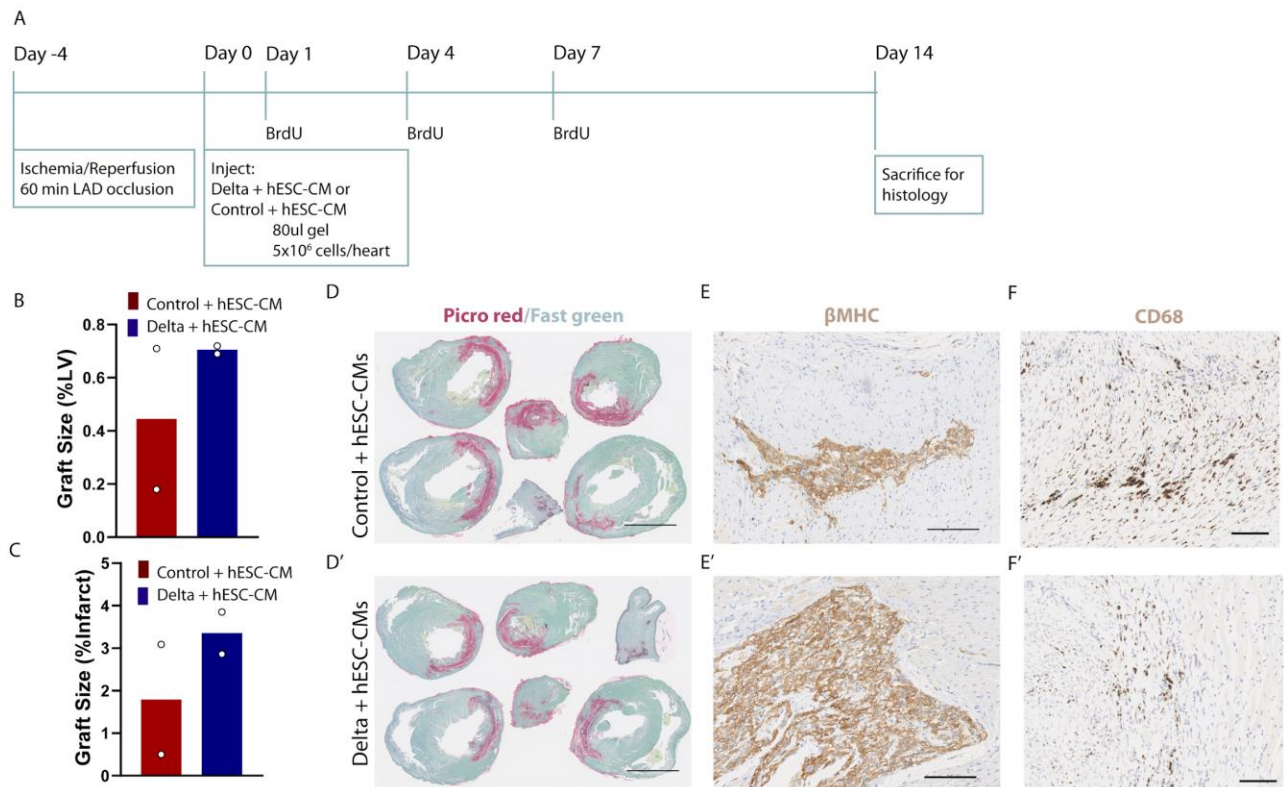


Figure S5. Related to Figures 2, 3. Gel and cell characterization prior to implantation. (A) Implanted gels bound with Delta-1 (Delta) produced a Notch-mediated increase in luciferase expression. Data is shown as fold increase of firefly vs. renilla luciferase, and normalized to IgG controls (Control). *indicates $p < 0.05$, and error bars represent SEM. (B) Implanted hESC-cardiomyocytes were greater than 70% cTnT+ by flow cytometry. The cell population Forward Scatter (FSC-A) and Side Scatter (SSC-A) plots are shown on the left. Cardiac troponin T (cTnT) is detected by PE, where data is shown as a histogram of PE expression. The isotype control (red) is used as a negative gating control for the stained population (blue).

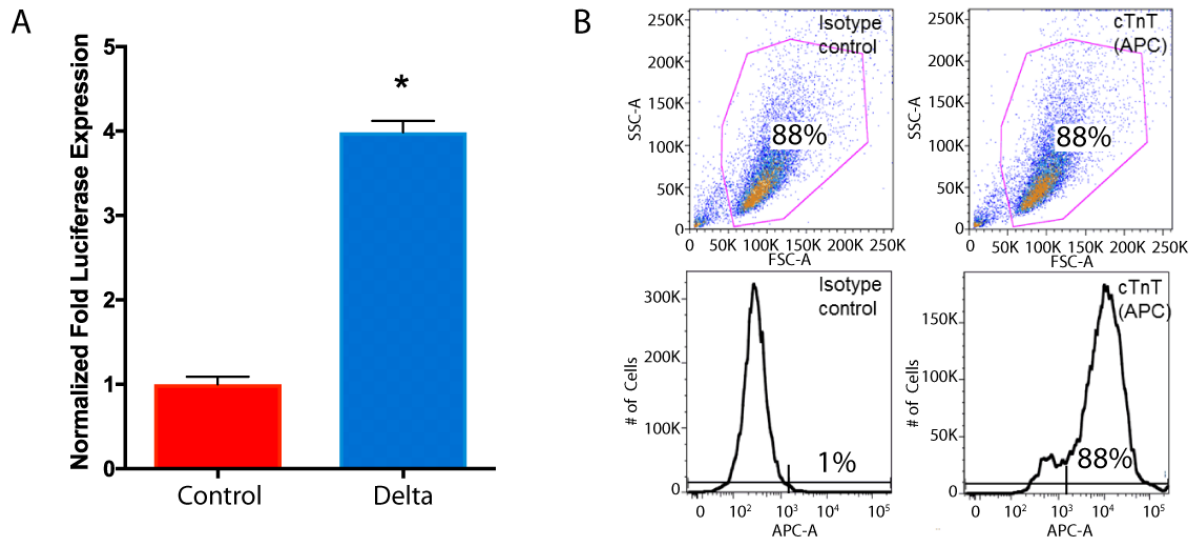


Figure S6. Related to Figure 5. Left ventricular internal dimensions do not vary among groups by echocardiography. (A) Individual traces of left ventricular internal dimensions (LVID) are shown at baseline, one, and three months for each group at diastole (LVIDd) with group averages summarized in (C). (B) Left ventricular internal dimensions are shown at systole (LVIDs) for individual hearts with group averages shown in (D). For all panels, ns = $p > 0.05$, * $p < 0.05$, ** $p < 0.005$, *** $p < 0.0005$, **** $p < 0.0001$ and error bars represent SEM. P-values were calculated by 2-way ANOVA followed by Tukey's multiple comparisons test.

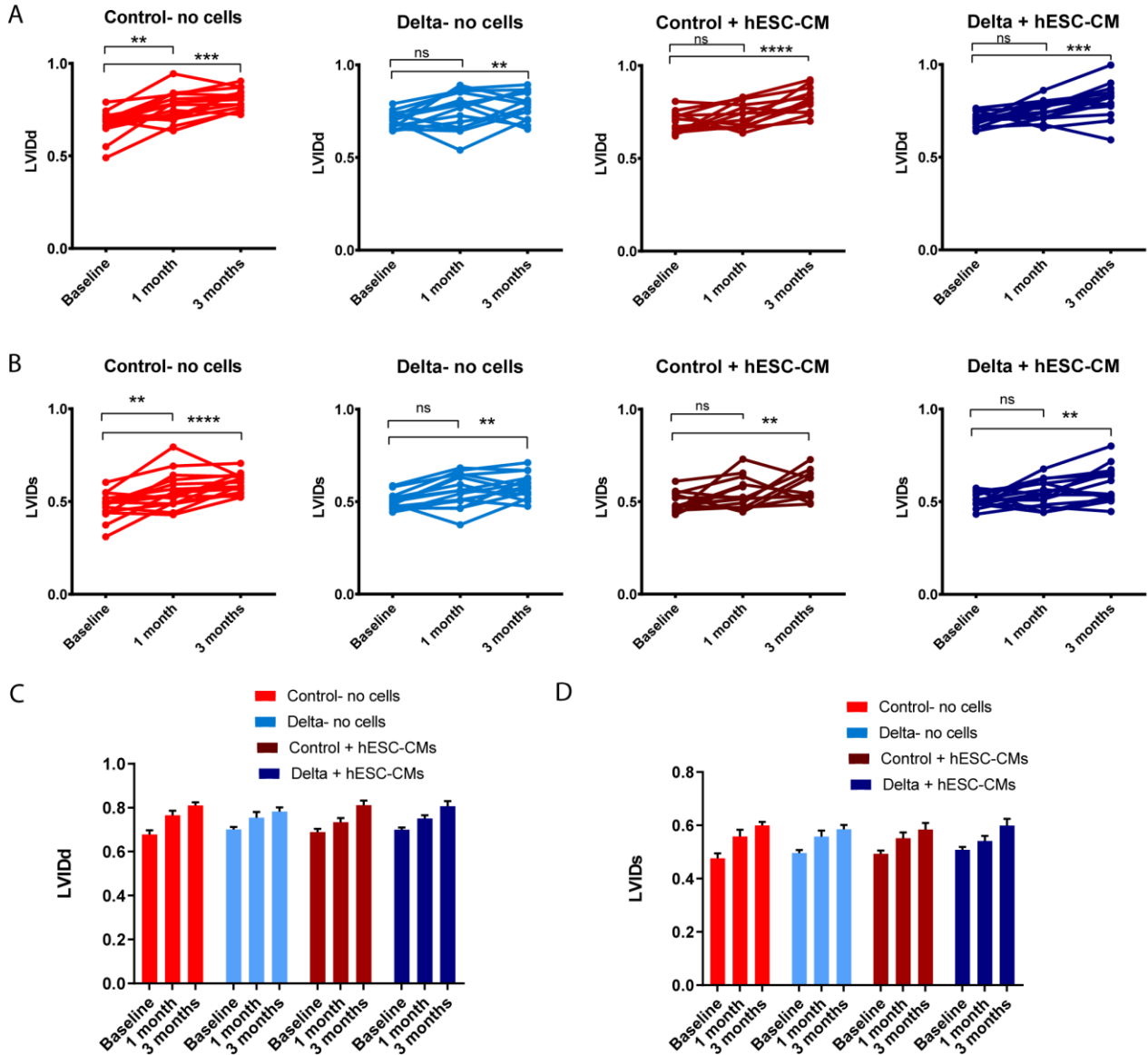
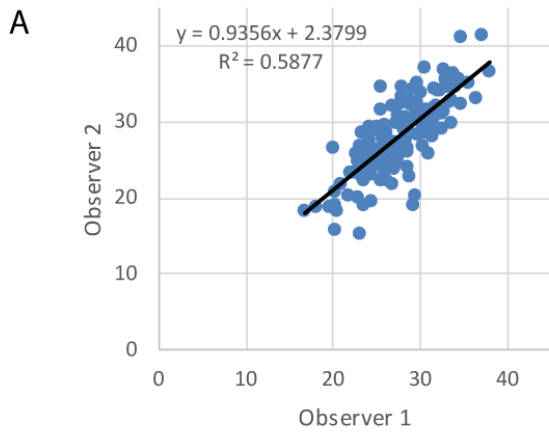
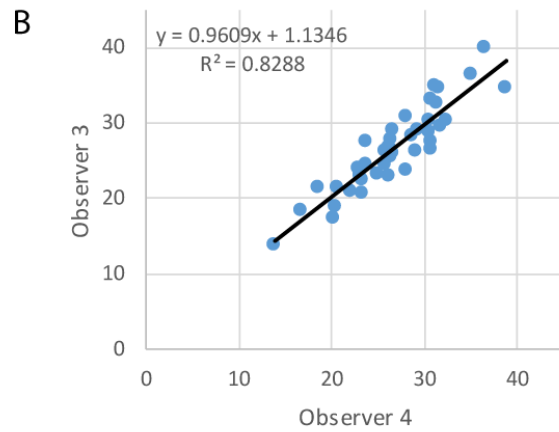


Figure S7. Related to Figure 5. Echocardiography interobserver variability. (A) Two independent, blinded scientists performed analysis of echocardiography with the correlation of measurements shown here for all time points (n=135). The best fit line equation and R² value are shown on the graph with Pearson correlation coefficient shown below. Surgery and echo analysis for 14 animals from the Control- no cell and Delta- no cell groups was performed at a later time than the initial experiment and on a different ultrasound. The correlation of observer measurements is shown separately for these animals at all time points (n=42) in (B).



Pearson correlation coefficient 0.767



Pearson correlation coefficient 0.910