Supplemental Methods

Cardiac differentiation protocol

hESCs were differentiated with RPMI supplemented with 1% penicillin-streptomycin (Thermo Fisher Scientific), 200 mM L-ascorbic acid (Sigma), B27 (insulin minus) supplement (Invitrogen) and the following growth factors: 9 ng/ml Activin A (R&D systems), 5 ng/ml Bone morphogenic protein-4 (BMP4) (R&D systems), 5 ng/ml bFGF (R&D systems), 1 μM CHIR99021 (Stemgent) for 3 days (with daily medium changes) to induce mesoderm formation. Cardiac fate was further specified by WNT inhibition with 5 μmol/L IWP4 (Stem cell technologies) in B27 (insulin minus) media for 3 days followed by 5 μmol/L IWP4 in B27 (insulin plus) for another 7 days. Cells were then cultured in RPMI B27 (insulin plus) media for 2 days until harvested on day 15.

Flow cytometry

For cell surface markers, staining was performed on live cells in 5% FBS in PBS blocking buffer, and for intracellular staining, cells were fixed for 10 minutes with 1% PFA in PBS and permeabilized and blocked with 0.025% Triton-X and 5% FBS in PBS. Primary antibodies were incubated for 45 minutes at 4°C then washed with the respective blocking buffer. Cells were subsequently counterstained with Alexa-fluor goat anti-mouse 488 (1:1000), Alexa-fluor goat anti-rabbit 488 (1:1000) for cell surface markers, and additionally with Hoechst33342 (1:1000) for intracellular stains for 30 minutes at 4°C. Cells were resuspended in PBS for analysis with Becton Dickinson LSR Fortessa X-20 cytometer. For cell surface markers, forward and side scatter parameters were used to separate out cell populations, with isotype controls used to evaluate nonspecific staining. For intracellular staining, Hoechst33342 and forward scatter parameters were used to separate out cell populations. CD14 and CD45 antibody staining was optimised on human cord blood monocytes (Stem Cell Technologies), and CD31 antibody staining was optimised using H9-derived endothelial cells using published methods (Orlova et al., 2014).

Quantitative RT-PCR

500 μl of Trizol was used per hCO and vortexed with a sterile stainless steel ball bearing to RNA extract from the tissue. RNA was DNase treated as per the manufacturer's instruction (Roche) before cDNA was synthesised (Thermo Fisher Scientific). SYBR Green PCR mix (Thermo Fisher Scientific) was used for RT-QPCR and StepOne software v2.3 was used to determine gene expression, using the $2^{-\Delta\Delta Ct}$ method with normalisation to no injury controls. Gene expression is presented as the fold change in expression compared to the no injury control ± SEM. Primer sequences can be found in Supplementary Table 3.

Electrical stimulation and force analysis of hCO

Pole tracking analysis was performed using a custom designed Matlab program that tracks pole movement and calculates the average force of contractions using the equations for elastic beam deflection shown below. This analysis was utilised to measure contractile force at 6-hour post cryoinjury, as opposed to the organ bath, as this method was more sensitive to minor changes in force detected at early time points. Data is represented as the average active force (μN) ±SEM.

Equation (1) General deflection of a cantilever: $F = (\frac{3EI}{L^3})\delta$

where *F*, *E*, *I*, *L*, and δ are the bending force, Young's modulus, area moment of inertia, length of pole, and resulting deflection of the post, respectively. Area moment of inertia (for a filled rectangular area with a base width of b and height h):

Equation (2): $I = \frac{bh^3}{12}$

Therefore the design equation becomes:

Equation (3): $F = \frac{3Ebh^3\,\delta}{12L^3}$

The current pole design is:
E_{PDMS} = 1500 kPa = 1.5 N/mm^2
b = 2 mm
h = 2 mm
L = 10 mm

Therefore, Equation (4): $F = \frac{3\ x\ 1.5\ N/mm^2\ x\ 2\ mm\ x\ (2\ mm)^3}{12\ x\ (10mm)^3}\delta$

$$F = 0.006\ N/mm\ \delta$$

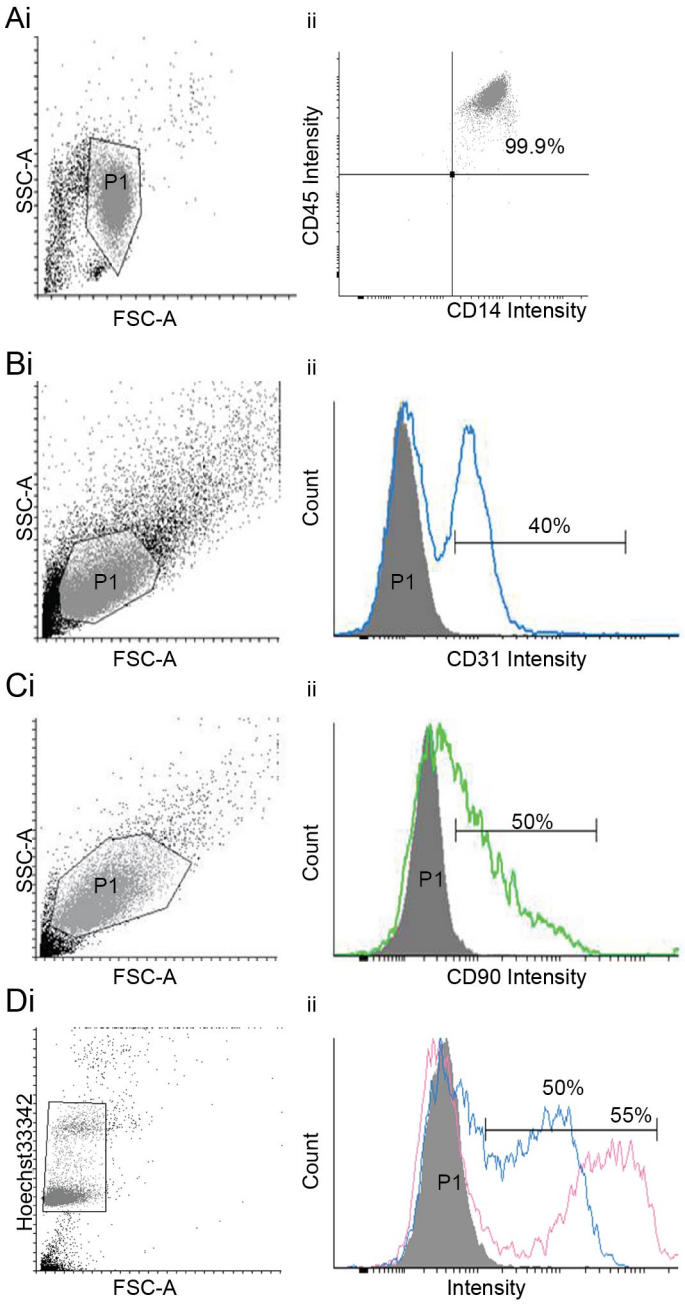
$$F = 6\ \mu N/\mu m\ \delta$$

Therefore, for every µm of pole deflection, the tissue is producing 6 µN of force.

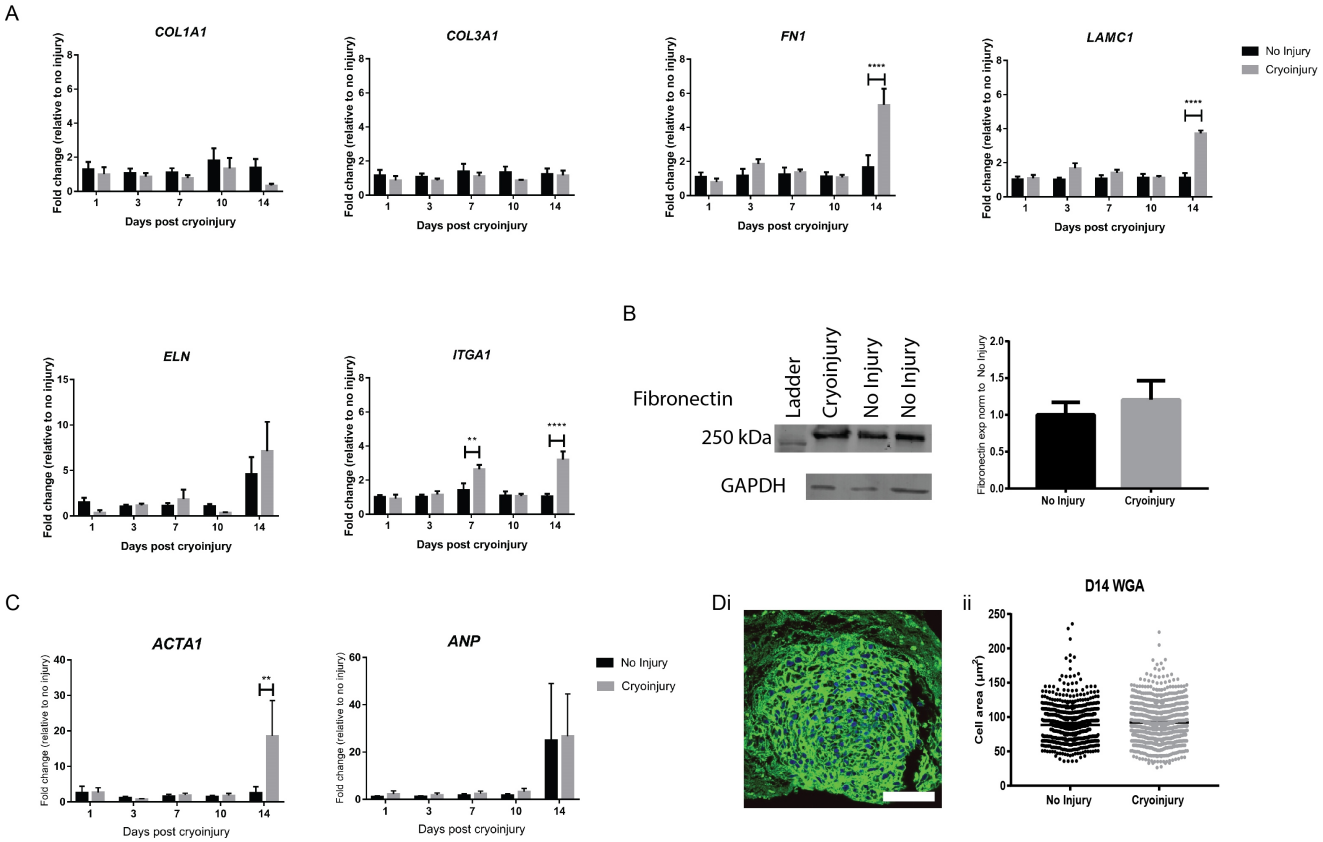
Monocyte co-culture

Human Cord Blood CD14+ Monocytes (Stem Cell Technologies) and Human Peripheral Blood CD14+ Monocytes (Stem Cell Technologies) were co-cultured in α-MEM with hCO. Following cryoinjury, hCO were placed into customised PDMS wells with monocyte suspension to maximise monocyte concentration around the hCO. 125, 000 CD14+ monocytes were co-cultured per hCO for 24 hours. hCO were removed from PDMS wells and cultured in α-MEM for a further 3 days.

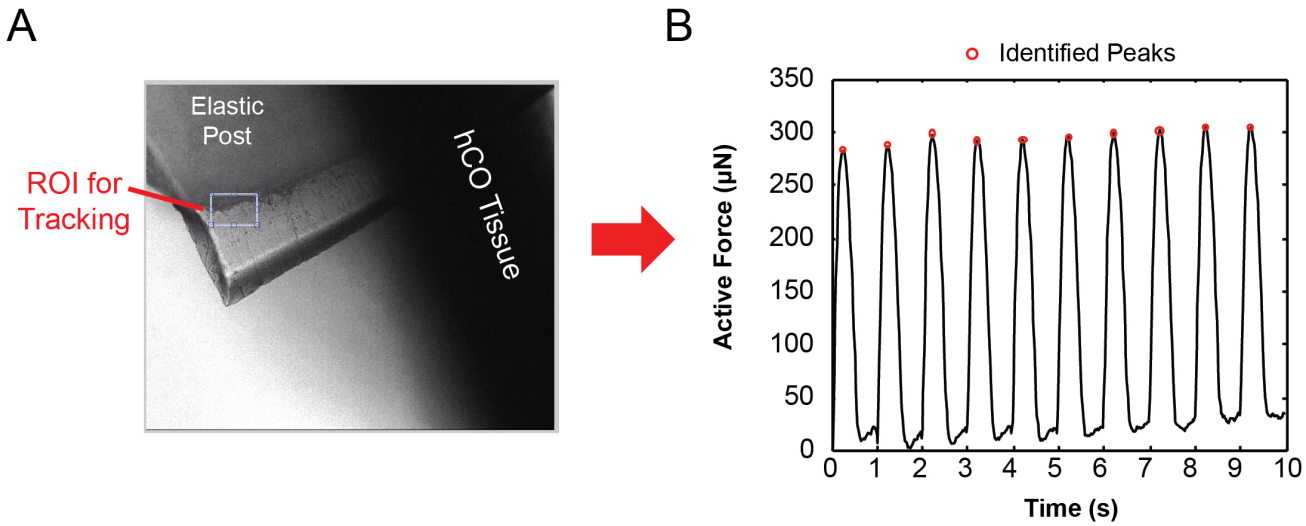
Supplemental Figures



Supplementary Figure 1: Flow cytometry for positive controls. Ai) Gating strategy for positive control for CD45 and CD14 antibodies using live human monocytes **ii** Isotype control and CD45 and CD14 quantification. **Bi)** Positive control for CD31 using live cells from endothelial differentiation **ii** Isotype control and CD31 quantification. **Ci)** Gating strategy for CD90 antibody using cells from cardiac differentiation **ii** Isotype control and CD90 quantification. **Di)** Gating strategy for CD90 antibodies harvested cardiac differentiation **ii** Isotype control and α-actinin (blue) and cardiac troponin T (pink) quantification.

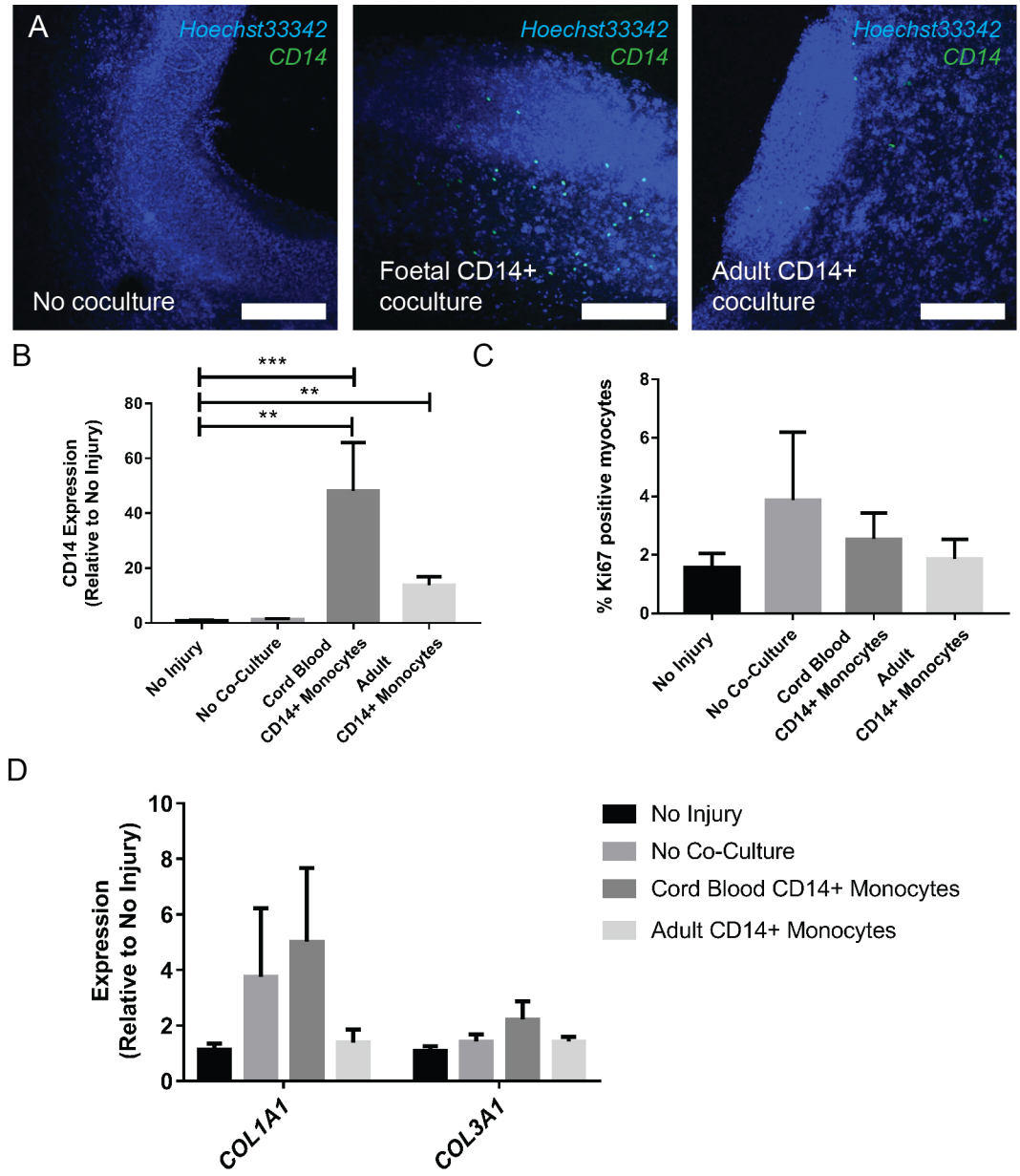


Supplementary Figure 2: Cryoinjury of human cardiac organoid (hCO) does not induce fibrosis or pathological hypertrophy. A Gene expression of extracellular matrix and integrin genes *COL1A1, COL3A1, ELN, FN1, ITGA1, LAMC1,* at 1, 3, 7, 10 and 14 days post cryoinjury (n=3-6, 2 experiments). ** $p < 0.01$, **** $p < 0.0001$ using two-way ANOVA with Sidak's multiple comparison post-test. **B** GAPDH (37kD) and Fibronectin (252kD) protein expression detected by Western Blot with quantification of Fibronectin expression normalised to no injured HES3 hCO (n=6, 2 experiments). **C** Gene expression of hypertrophy associated genes (*ANP* and *ACTA1)* in response to cryoinjury at 1, 3, 7, 10 and 14 days post cryoinjury (n=7, 2 experiments). ** $p < 0.01$ using two-way ANOVA with Sidak's multiple comparison post-test. **Di** High magnification immunofluorescent image of a 6 μm section of HES3 NKX2-5$^{eGFP/w}$ hCO stained with Wheat Germ Agglutinin (WGA) and Hoechst33342 at 14 days post injury. Scale bar = 50μm. **ii** Quantification of cardiomyocyte cell size at 14 days post cryoinjury (n =5, 3 sections per hCO, over 3 experiments). Pooled data are presented as a scatter plot, not significantly different using Student's t-test ($p < 0.05$).



Supplementary Figure 3: Tracking of elastic post for active force measurements.

A Still frame of elastic post, hCO and region of interest (ROI) for tracking. **B** Representative active force trace for analysis from a video.



Supplementary Figure 4. hCO co-culture with monocytes does not induce fibrosis or alter cardiomyocyte proliferation. A High magnification image of cryoinjured hCO stained with CD14 and Hoechst33342 following no co-culture, co-culture with cord blood (fetal) CD14+ monocytes or adult peripheral blood CD14+ monocytes. Scale bars = 50 μm. **B** qPCR analysis of CD14 (monocyte) expression in the culture conditions compared to no injury and no co-culture (n=7-10, over 3 experiments) **p<0.01, ***p<0.001 using ANOVA with Dunn's multiple comparison post-test. **C** Quantification of Ki-67 positive cardiomyocytes at 3 days post cryoinjury (n=5-7, over 2 experiments). **D** qPCR analysis of extracellular matrix genes *COL1A1and, COL3A1* at 3 days post cryoinjury compared to no injury no co-culture (n=8-10, over 3 experiments).

Supplemental Tables

Supplementary Table 1: hCO composition

Component per hCO	Volume
7.3mg/ml acid solubilized collagen 1	36µl
10x DMEM	4.3µl
0.1mol/L NaOH	4µl
Cell number	5.5 x 10^5
Cell suspension	4.6 x 10^6cells/ml α-mem

Supplementary Table 2: Basal medium composition (no-phenol)

Component	Final Concentration
Sodium Chloride (NaCl)	117mmol/L
Potassium Chloride (KCl)	5mmol/L
Sodium Bicarbonate (NaHCO3)	18mmol/L
Monosodium Phosphate (NaH2PO4)	1mmol/L
Calcium Chloride (CaCl2)	1.21mmol/L
D-glucose	5.6mmol/L

Supplementary Table 3: Primer sequence of genes of interest analysed with qPCR (all at 250 nM).

Gene	Sequence
h18S Forward	5'-GCTGAGAAGACGGTCGAACT-3'
h18S Reverse	5'-CGCAGGTTCACCTACGGAAA-3'
hCOL1A1 Forward	5'-GTGCTAAAGGTGCCAATGGT-3'
hCOL1A1 Reverse	5'-ACCAGGTTCACCGCTGTTAC-3'
hCOL3A1 Forward	5'-CCAGGAGCTAACGGTCTCAG-3'
hCOL3A1 Reverse	5'-CAGGGTTTCCATCTCTTCCA-3'
hITGA1 Forward	5'- CTGCTGCTGGCTCCTCAC-3'
hITGA1 Reverse	5'- CCAAACATGTCTTCCACCG-3'
hFN1 Forward	5'- CCATAAAGGGCAACCAAGAG-3'
hFN1 Reverse	5'- ACCTCGGTGTTGTAAGGTGG-3'
hLAMC1 Forward	5'-CAGTACCCCAGCTCCATCAA-3'
hLAMC Reverse	5'-GTAAATGGCAAAGCTCTCCG-3'
hELN Forward	5'-CAGTTGGTACCCAAGCACCT-3'
hELN Reverse	5'-AGGTGGCTATTCCCAGTGTG-3'
hANP Forward	5'- TCTGCCCTCCTAAAAAGCAA-3'
hANP Reverse	5'- TGTCCTCCCTGGCTGTTATC-3'
hACTA1 Forward	5'- ACCCAGATCATGTTTGAGACC-3'
hACTA1 Reverse	5'- TCATAAATGGGCACGTTGTG-3'
hMYH6 Forward	5'- CTCCTCCTACGCAACTGCCG-3'
hMYH6 Reverse	5'- CGACACCGTCTGGAAGGATGA-3'
hMYH7 Forward	5'- GACCAGATGAATGAGCACCG-3'
hMYH7 Reverse	5-' GGTGAGGTCGTTGACAGAACG-3'
hTTN TOTAL Forward	5'- GTAAAAAGAGCTGCCCCAGTGA-3'
hTTN TOTAL Reverse	5'- GCTAGGTGGCCCAGTGCTACT-3'
hTTN N2B Forward	5'- CCAATGAGTATGGCAGTGTCA-3'
hTTN N2B Reverse	5'- TACGTTCCGGAAGTAATTTGC-3'

Supplementary References

Orlova, V. V., Van Den Hil, F. E., Petrus-Reurer, S., Drabsch, Y., Ten Dijke, P. & Mummery, C. L. 2014. Generation, expansion and functional analysis of endothelial cells and pericytes derived from human pluripotent stem cells. *Nat Protoc,* **9,** 1514-31.