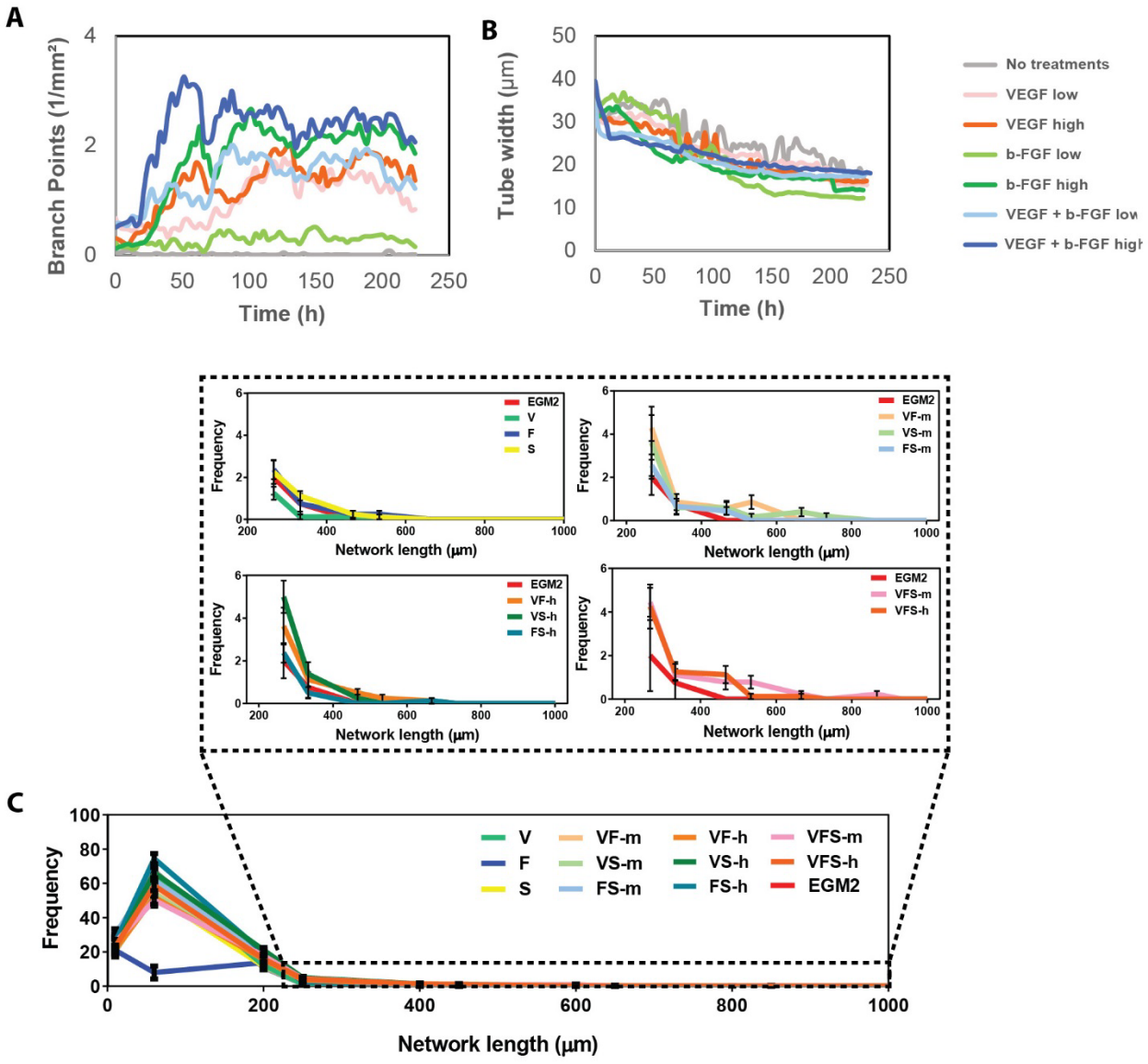
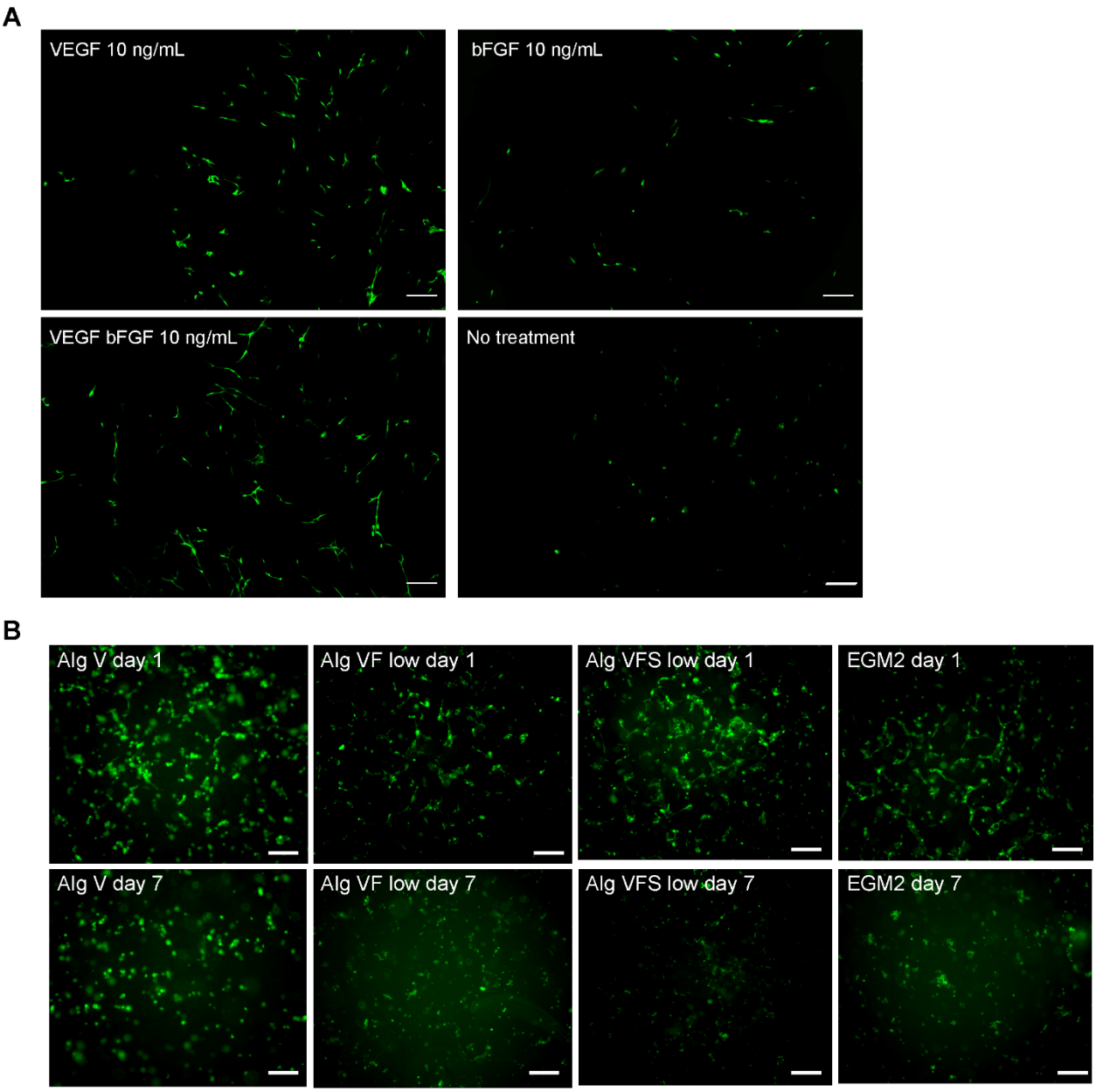
Supplemental Material

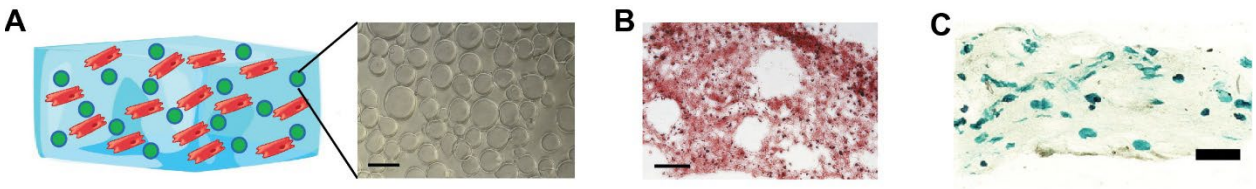
"Engineered human myocardium with local release of angiogenic proteins improves vascularization and cardiac function in injured rat hearts" by Munarin, F., Kant, R.J., Rupert, C.E., Khoo, A. and Coulombe, K.L.K.



Supplemental Figure S1. 2D network formation assay. Quantification of A) number of branch points and B) tube width from network masks. C) Histogram representing the length dispersion of HUVECs network-like structures formed on the 2D assay. The graphs reported in the dotted box show an enlarged tail of the length histogram (note y-axis values) to better highlight differences among double and triple combinations of growth factors.

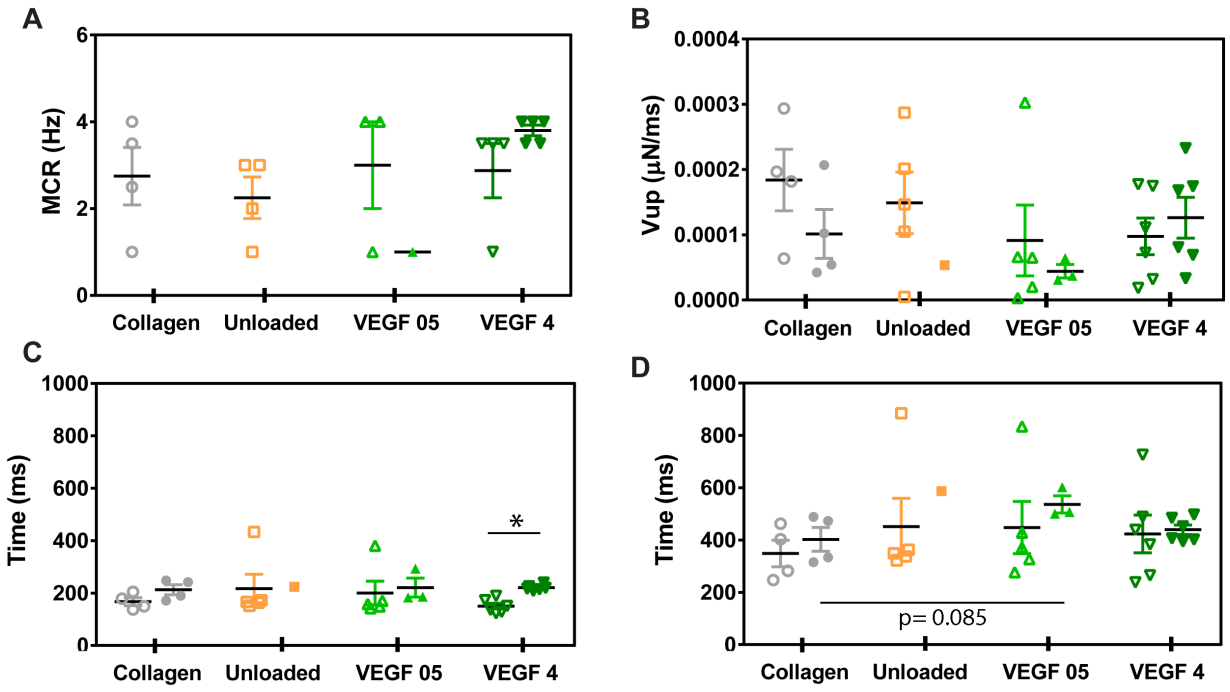


Supplemental Figure S2. Representative images of HUVEC networks in the 2D and 3D network formation assay. A) HUVECs transfected with CytoLight Green and cultured for 6 days onto normal human dermal fibroblasts in the 2D network formation assay. B) HUVECs stained with green fluorescent cell tracker and immobilized in collagen/alginate microspheres hydrogel show rapid formation of capillary-like networks at 1 day of culture and decline at 7 days. Scale bars= 200 μm.

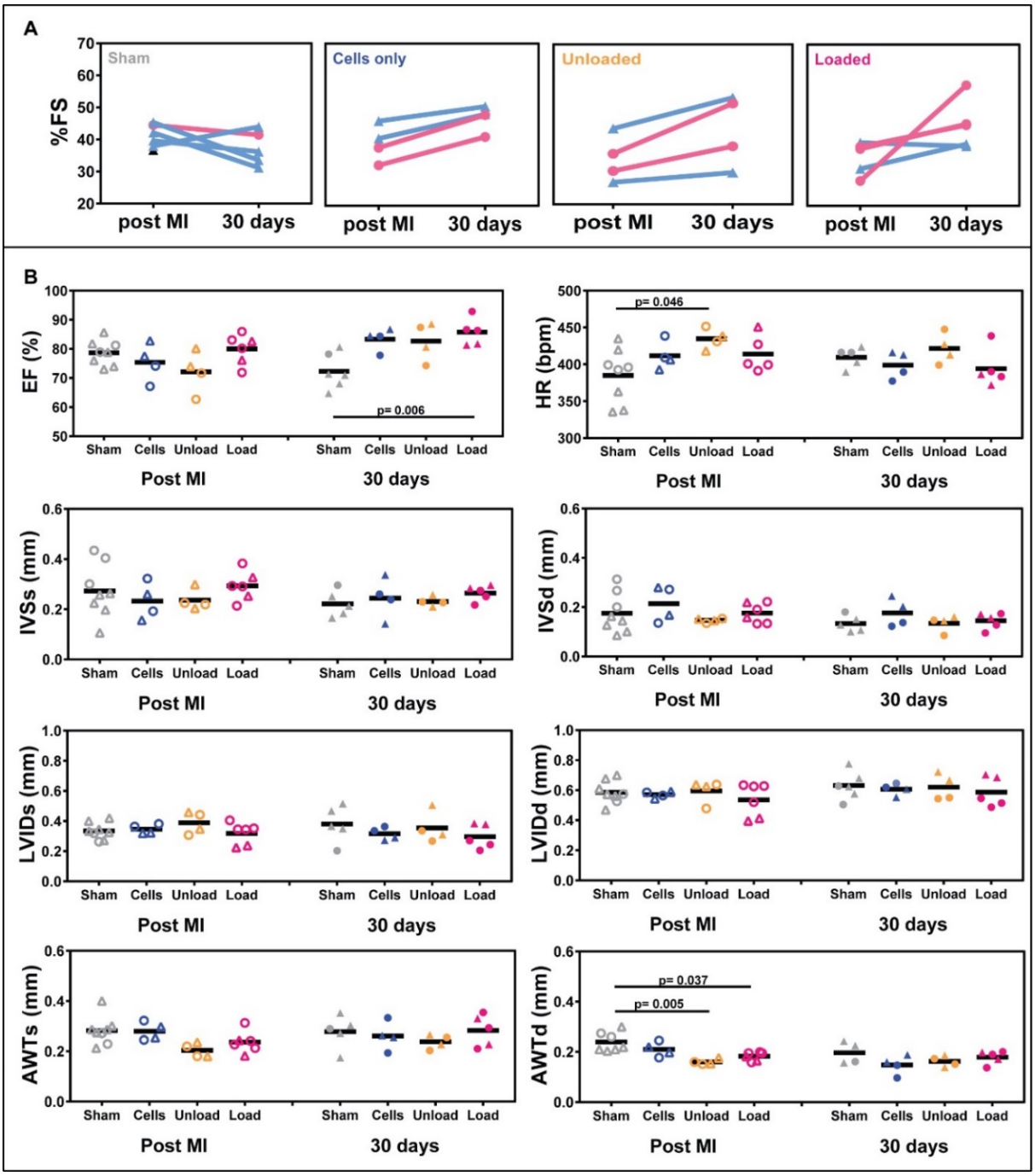


Supplemental Figure S3. Construct development. A) Scheme of the collagen – alginate microspheres scaffold, containing immobilized hiPSC-derived cardiomyocytes for cardiac function regeneration and alginate microspheres for the release of proangoiogenic growth factors. The enlarged image represents a bright field image of alginate microspheres, showing reproducible morphology and narrow size dispersion. Scale bar= 100 μm. B) Picrosirius red staining of a collagen-alginate microspheres scaffold. Microspheres are dissolved when sectioning the paraffin blocks (5 μm of thickness), leaving void spaces in the slice. Scale bar= 50 μm C) Thick frozen section (50 μm) of a collagen – alginate microspheres scaffold cultured for 7 days and stained with alcian blue indicate homogeneous dispersion of the microspheres in the scaffold. Scale bar = 200 μm.

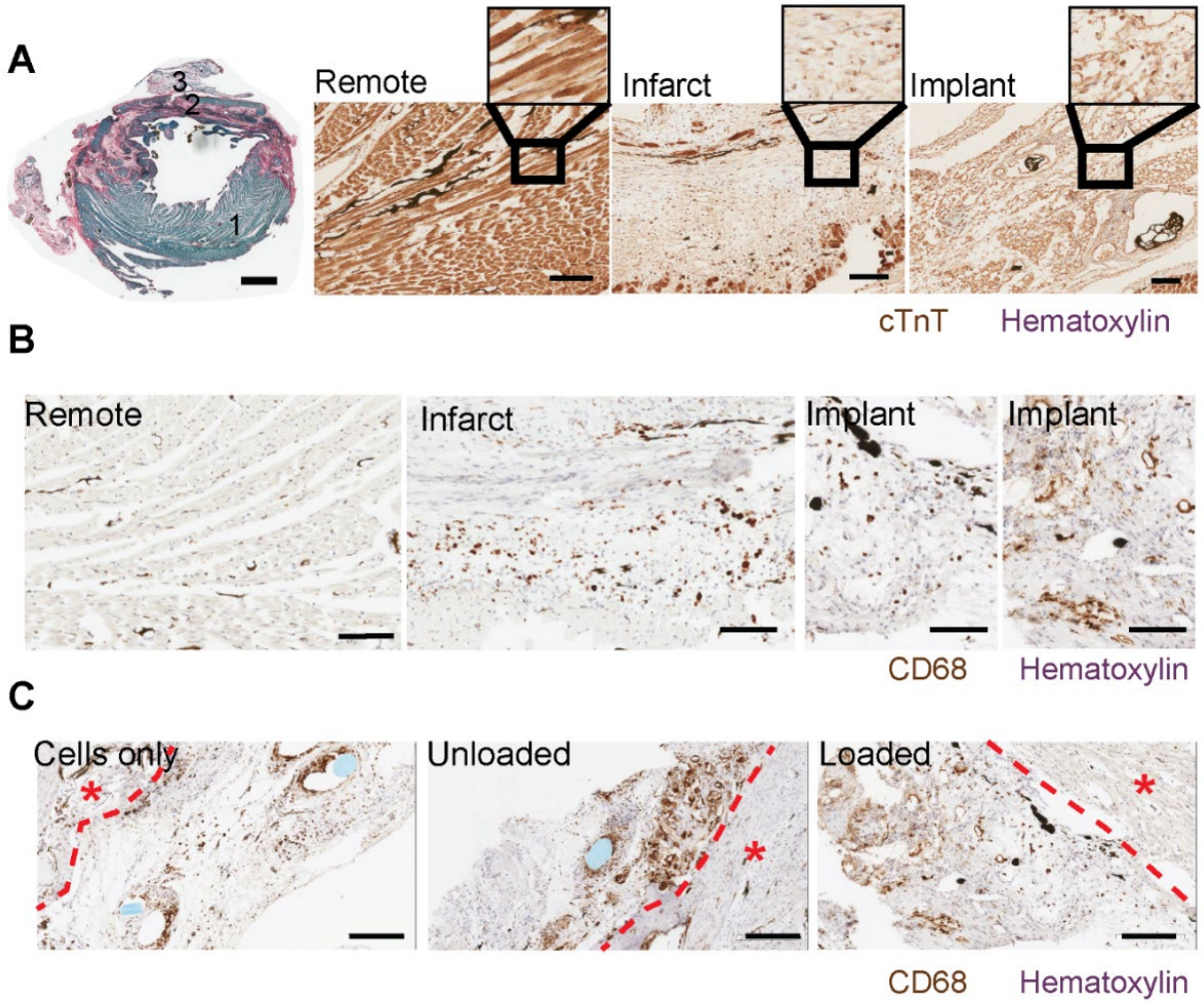
Supplemental Movie S1. Cardiac constructs containing $2x10^6$ hiPSC-derived cardiomyocytes and 50mg/mL alginate microspheres loaded with 4 μg VEGF beat with uniform syncytium at 7 days of culture.



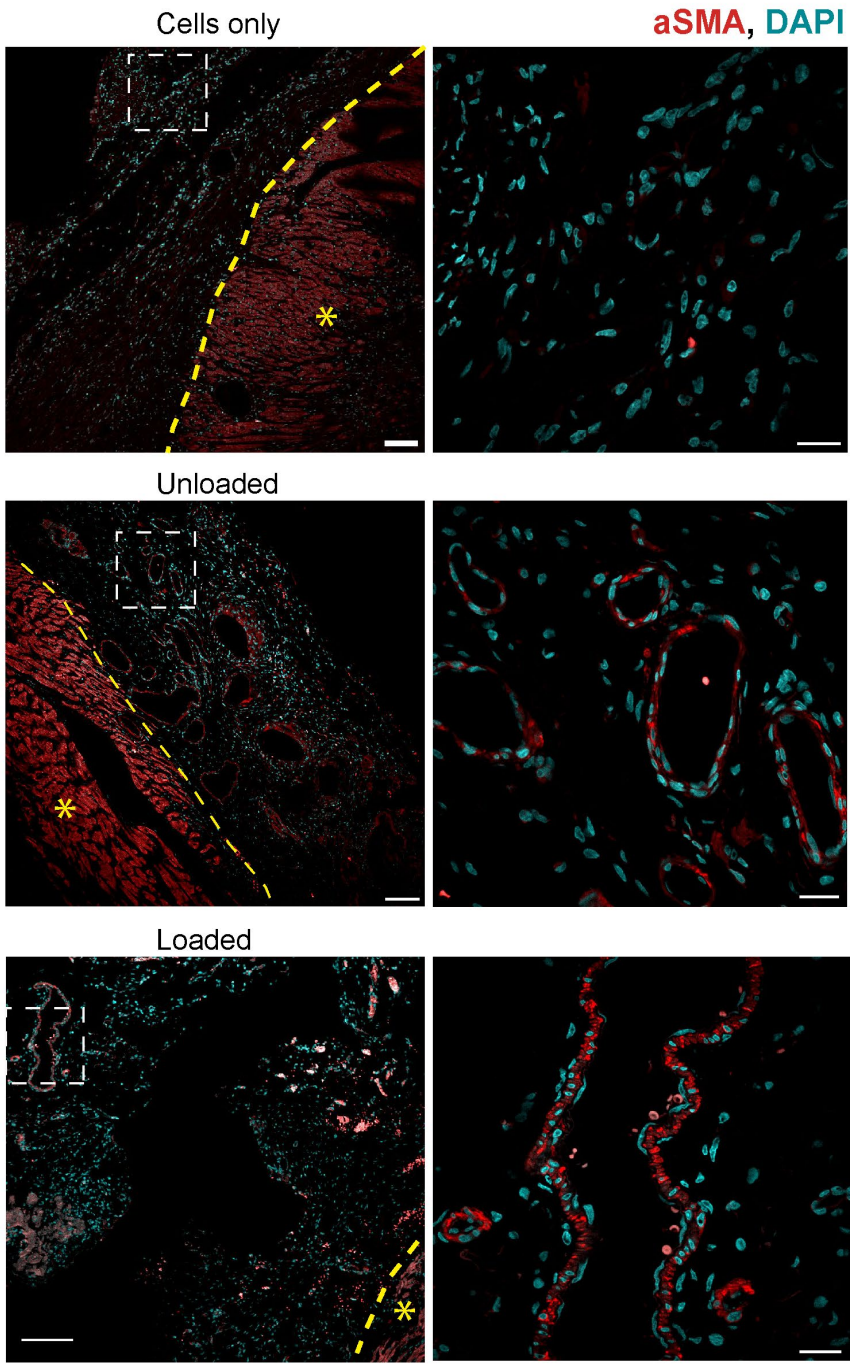
Supplemental Figure S4. Contraction and relaxation kinetics of hiPSC-derived cardiomyocyte tissues with alginate microspheres. A) The fastest pacing frequency that engineered tissues can follow during force-frequency protocols is denoted as the maximum capture rate (MCR). The B) upstroke velocity, C) time to 50% relaxation (T50), and D) time to 90% relaxation (T90) of contractions for engineered tissues measured at 15% stretch.



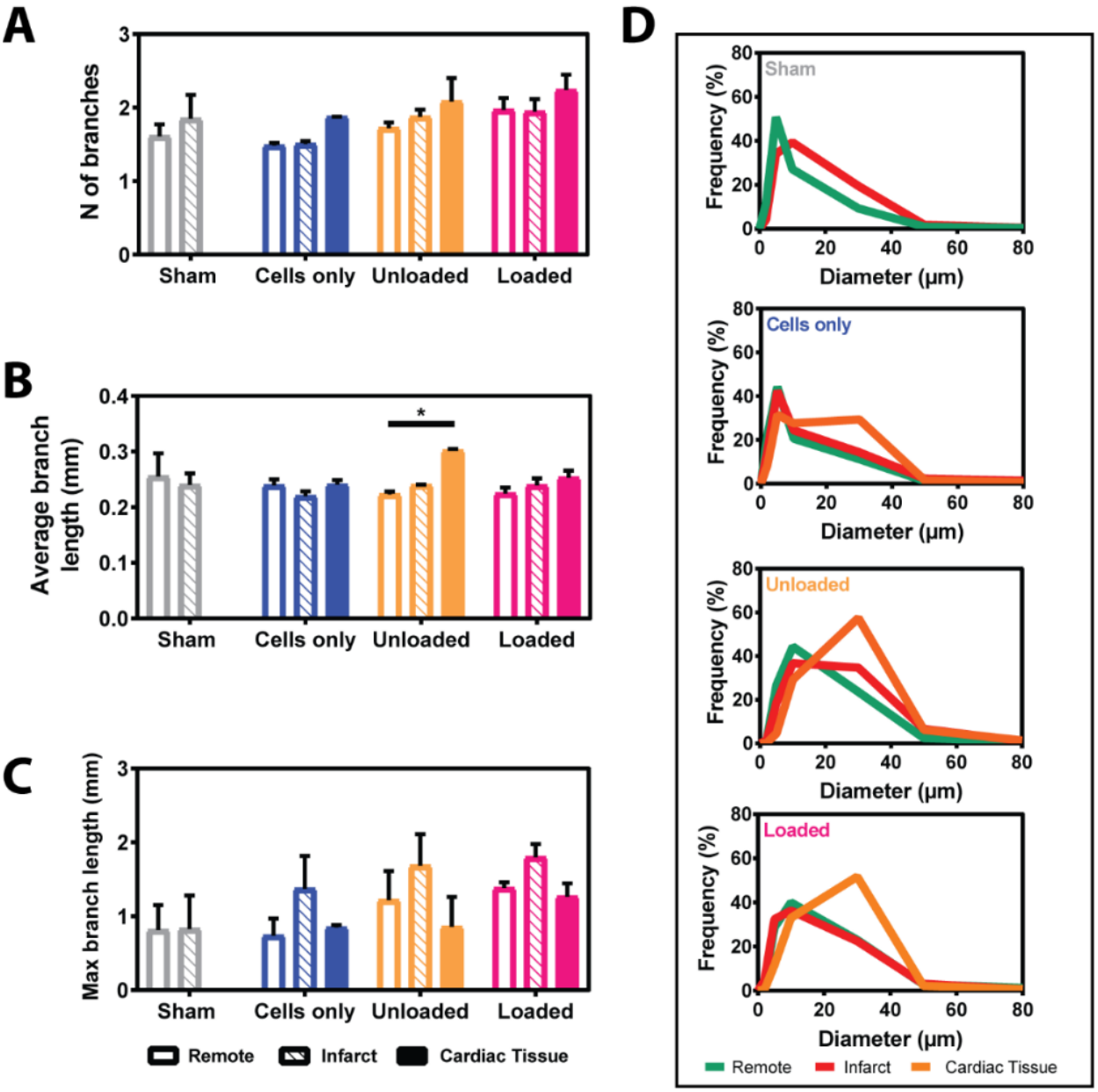
Supplemental Figure S5. Heart function assessed by echocardiography. A) Comparison of % fractional shortening for each animals at 3 days post MI and 30 days after implantation surgery. Each line represent the %FS of a single rat, and the black dot (sham group) indicates a rat that died 21 days after receiving the implant. B) Dimensional parameters (ejection fraction EF, heart rate HR, interventricular septum thickness IVS, left ventricular internal diameter LVID and anterior wall thickness AWT at end diastole and end systole) measured by echocardiography to assess cardiac output 3 days after the infarct and 30 days after implantation surgery.



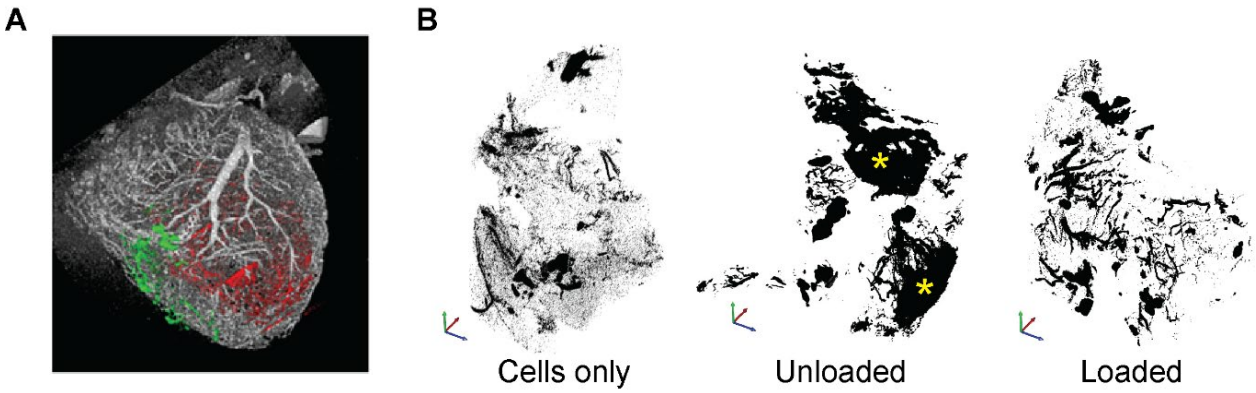
Supplemental Figure S6. Histological assessment of test groups at 1 month. A) Picrosirius red-fast green staining on whole cross-sections of a heart implanted with collagen-alginate microspheres cardiac constructs releasing VEGF, bFGF and SHH, harvested 30 days after implantation. Numbers represent remote (1), infarct (2) and implant (3) regions (scale bars= 1mm). cTnT (middle and right panels) stains cardiomyocytes in the regions of interest at 1 month. Scale bars = 100 μm. B) CD68 is used to identify macrophages in the remote, infarct and implant areas (in a protein-loaded construct). Scale bars = 100 μm. C) Macrophage infiltration in the cells-only, unloaded and loaded implants at 1 month show macrophage recruitment around alginate microspheres. Host tissue is identified with red asterisk and dashed lines. Scale bars = 200 μm.



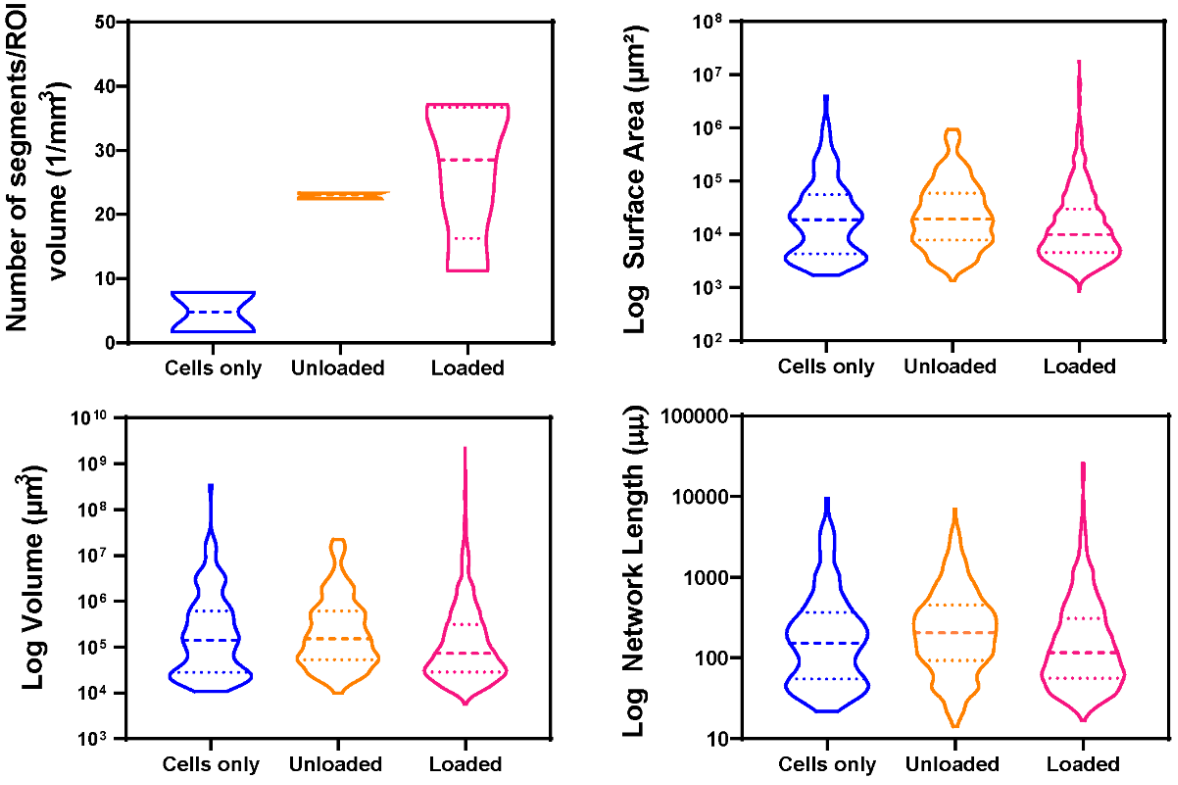
Supplemental Figure S7. Presence of αSMA-positive cells surrounding lumens is confirmed in implants containing unloaded and loaded alginate microspheres. Histological assessment with alpha smooth muscle actin (αSMA) antibody shows αSMA-positive structures with diameters appropriate for arterioles (20 – 100 μm) in the implants containing unloaded or loaded alginate microspheres. Images on the left panels shows the heart (yellow asterisk) and the implant (demarcated with the yellow dashed line) at lower magnification, scale bars = 100 μm. The white dashed squares indicate the regions represented at higher magnification on the right (scale bars = 20 μm).



Supplemental Figure S8. Vascular tree quantification. Quantification of number of branches (A), average length (B) and maximum length (C) of branches from skeletonization algorithms applied to the 3D reconstruction of the heart samples. D) Histograms showing the dimensions of the vasculature measured in remote, infarct and cardiac tissues regions.



Supplemental Figure S9. **3D reconstruction of vasculature in the infarcted heart.** A) Perfused blood vessels are detected in the infarct (red) and implanted cardiac tissue (green). B) representative 3D reconstructions of capillaries penetrating in the cell-only, unloaded and loaded implants. Large sinuses (indicated in the unloaded group with yellow stars) were excluded from the quantification.



Supplemental Figure S10. **Quantification of angiogenesis and revascularization in the cardiac tissues.** Additional angiogenic metrics measured with the automated analysis of 3D-reconstructed vessels in the engineered constructs.