SUPPLEMENTAL MATERIAL

Functional Improvement and Maturation of Rat and Human Engineered Heart Tissue by Chronic Electrical Stimulation

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$$F = \frac{3 \cdot E \cdot I \cdot \delta}{L^3}$$

E: Young‘s modulus or elastic modulus
E = 1.9 $\frac{N}{mm^2}$ = 1.9 MPa

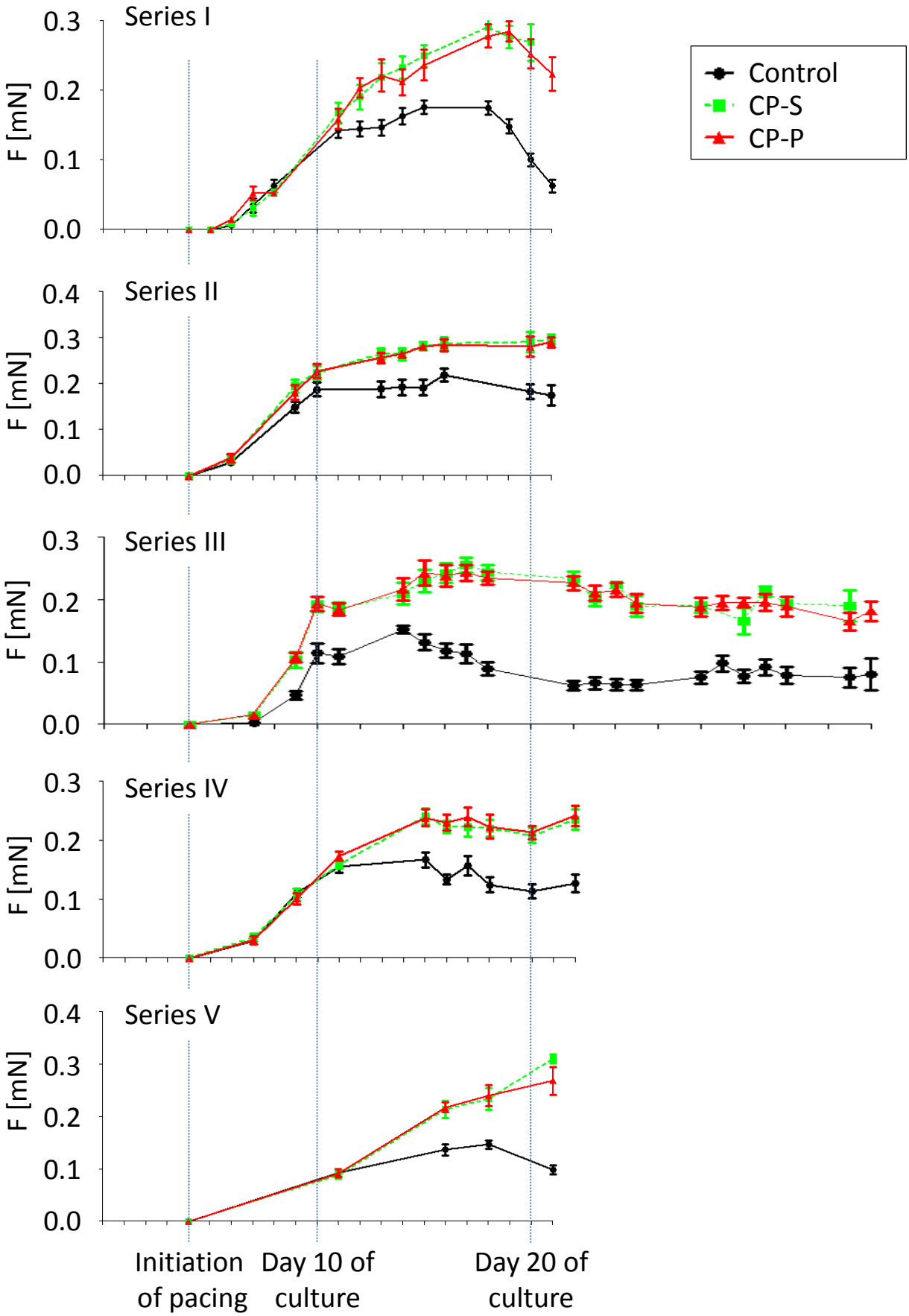
I: Second moment of area
I = 0.25 π $[R^4 - r^4]$

R: Outer radius of silicone posts (R = 0.5 mm)
r: Inner radius of silicone posts, if hollow (r = 0 mm)

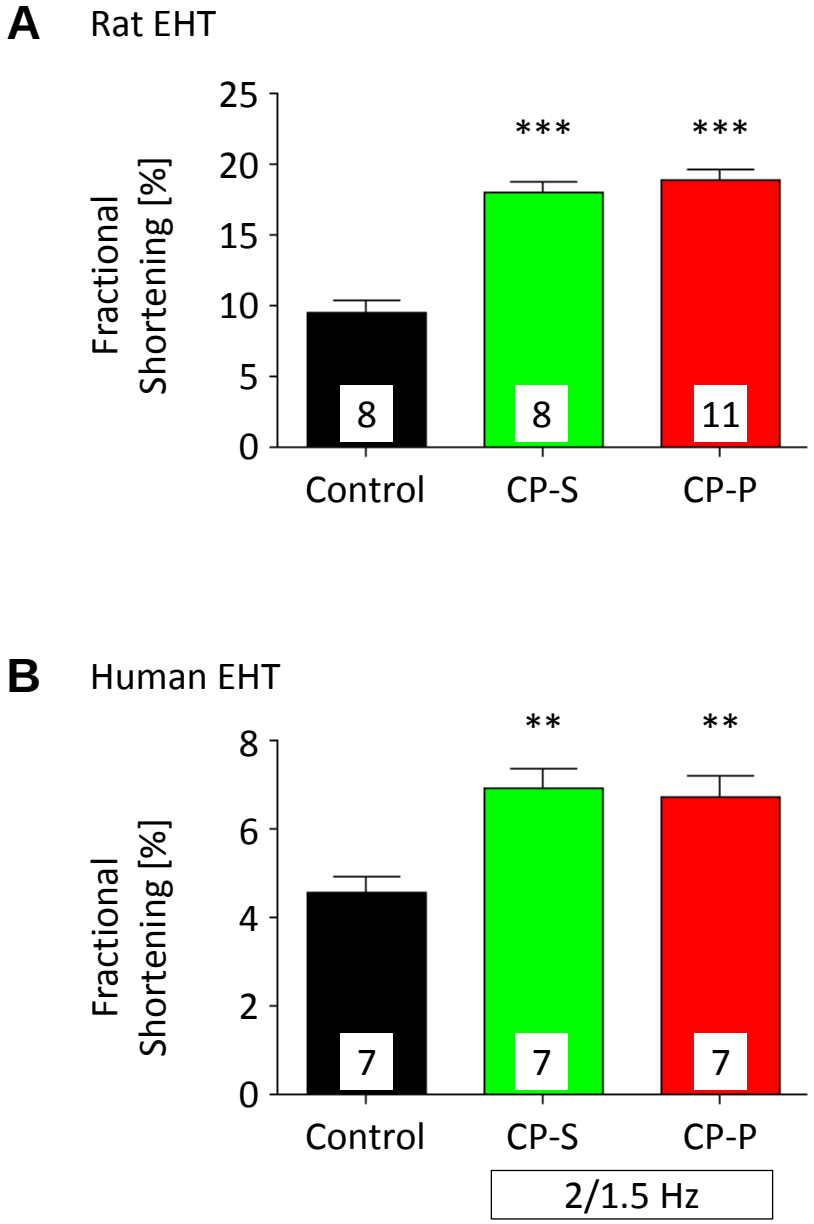
δ: Sum of deflection of both silicone posts

L: Length of silicone post
L = 10 mm

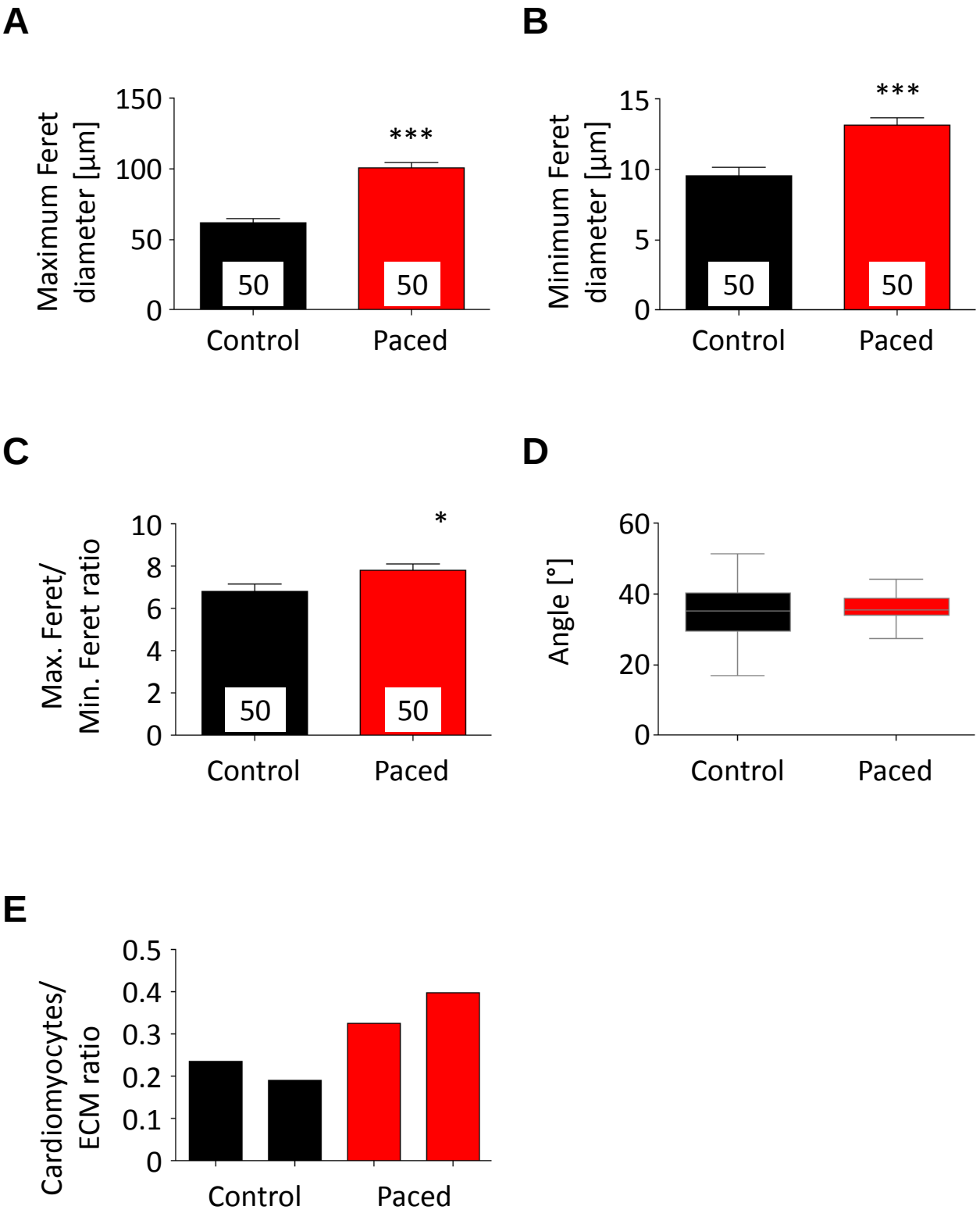
Supplemental Fig. S1 Formula for the calculation of force generated by an EHT by the deflection of the silicone posts during contractions. The calculations were validated in a previous study [11].



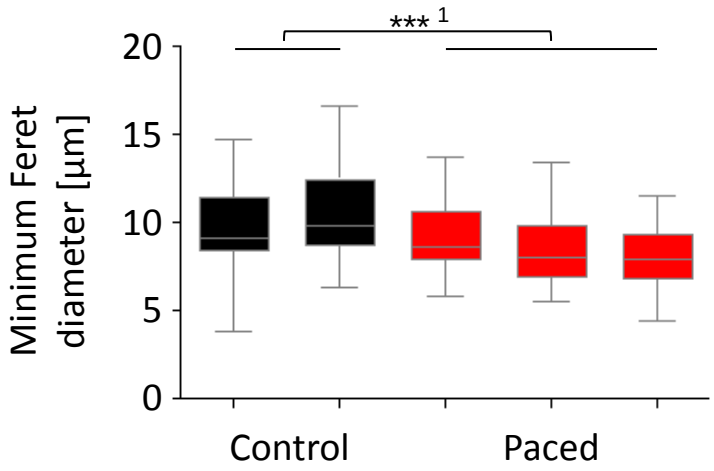
Supplemental Fig. S2 Impact of chronic pacing with 0.5 Hz on contractile parameters of 5 series of rEHTs. CP-S stands for chronic pacing group in which the stimulator was switched off transiently for measurement (i.e. spontaneous beating, depicted in green). CP-P are the same EHTs but with stimulator switched on (i.e. paced, depicted in red).



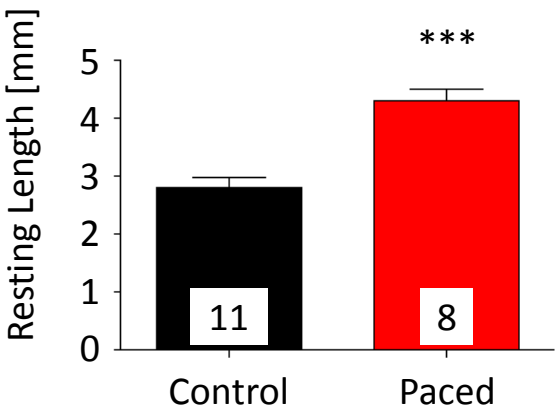
Supplemental Fig. S3 Fractional shortening of representative series of A) rat EHTs after 16 days of pacing and B) human EHTs after 10 days of high frequency pacing. CP-S stands for chronic pacing group in which the stimulator was switched off transiently for measurement (i.e. spontaneous beating, depicted in green). CP-P are the same EHTs but with stimulator switched on (i.e. paced, depicted in red).



Supplemental Fig. S4 Quantitative analyses of control and paced rEHTs. A) Maximum Feret diameters, B) minimum Feret diameters, C) ratios of maximum to minimum Feret diameters and D) angle of maximum Feret diameter of cardiomyocytes to the horizontal line. 25 cardiomyocytes per EHT (two per group) were analyzed for A-D. E) Ratio of section area covered by cardiomyocytes to ECM area.



Supplemental Fig. S5 Minimum Feret diameters of cardiomyocytes of two control and three paced hEHTs. 25 cardiomyocytes were analyzed per hEHT. [1]The p-value for all minimum Feret diameters of cardiomyocytes of control hEHTs tested against all minimum Feret diameters of paced hEHTs was < 0.001.



Supplemental Fig. S6 Resting length of rEHTs as measure of passive stiffness. Length was determined on day 21 of culture (after 17 days of chronic pacing). Control rEHTs had a mean resting length of 2.8 mm and continuously paced rEHTs of 4.3 mm (+ 53%).

Category	P-value	No. of affected genes	Gene names (upregulated, downregulated).
Top network			
Immune cell trafficking	‡	35	AMPD3, BIRC3, BMP2, CCL4, CCL3L1/CCL3L3, CD14, CISH, CXCL1, Cxcl12, CYP7B1, EMP1, ENPP2, ENPP3, EREG, IER3, MGST1, MSLN, NOV, PENK, PTGES, RCN3, RNF149, SERPINA3, SERPINE2, Slpi (includes others), SMPDL3A, STMN1, TGFB3, THBS2, TLR2, TNF, TNFAIP2, TNFRSF11B, TNIP1, VCAM1
Top associated disease and disorder			
Inflammatory response	$p<10^{-10}$	24	CALCRL, CCL11, CCL13, CCL3L1/CCL3L3, CCL4, CD14, CX3CR1, CXCL1, CXCL2, CXCL3, CXCL6, EDN1, GREM1, IL1A, IL1B, IL1RN, IL4R, IL6, JAK2, OLR1, SDC1, TLR2, TNF, TREM1
Top cellular function			
Cellular movement	$p<10^{-10}$	45	CALCRL, CCL11, CCL13, CCL2, CCL20, CCL3L1/CCL3L3, CCL4, Ccl6, CD44, CDH13, CTGF, CX3CR1, CXCL1, Cxcl12, CXCL2, CXCL3, CYR61, DPP4, EDN1, ENPP2, ERBB3, FGF7, FLT1, FYN, GREM1, GUCY1A3, GUCY1B3, HAS1, ICAM1, IL1B, IL1RN, IQUB, ITGB2, JAK2, NFKBIA, Nrg1, NTRK3, PDPN, PREX1, RAMP2, RAMP3, SEMA3F, STMN1, TNF, TNFRSF11B
Top canonical pathways			
1) Granulocyte adhesion and diapedesis"	$p<10^{-12}$	32	IL1A, ICAM1, CXCL1, CCL20, SDC4, CXCL3, CCL13, CCL2, Ccl6, MMP19, TNFRSF11B, CSF3R, VCAM1, CXCL11, SDC1, MMP28, PF4, CCL11, CXCL6, ITGB2, IL18, ITGAM, CCL4, CCL7, IL1RN, JAM3, Cxcl12, CCL3L1/CCL3L3, IL1B, CXCL2, TNF, MMP9
2) Agranulocyte adhesion and diapedesis"	$p<10^{-11}$	31	IL1A, MYH6, ICAM1, CXCL1, CCL20, SDC4, CXCL3, CCL13, CCL2, Ccl6, ACTA1, MMP19, VCAM1, CXCL11, MMP28, PF4, CCL11, CXCL6, MYL7, ITGB2, IL18, CCL4, CCL7, IL1RN, JAM3, Cxcl12, CCL3L1/CCL3L3, IL1B, CXCL2, TNF, MMP9
Top upstream regulator			
TNF-α	$p<10^{-31}$	53	ADORA2A, AMPD3, BIRC3, BMP2, CCL13, CCL3L1/CCL3L3, CCL4, CD14, CD44, CISH, CXCL1, Cxcl12, CXCL2, CXCL3, CXCL6, CYP7B1, EMP1, ENPP2, ENPP3, EREG, FST, ICAM1, IER3, IL1A, IL1B, IL6, MGST1, MMP9, MSLN, NFKBIA, NOS2, NOV, PENK, PLSCR1, PTGES, PTPRN, RCN3, RNF149, SERPINA3, SERPINE2, Slpi (includes others), SMPDL3A, SOCS3, STMN1, TAGLN, TGFB3, THBS2, TLR2, TNF, TNFAIP2, TNFRSF11B, TNIP1, VCAM1

Supplemental Table S1 IPA Pathway analysis (Interactive pathway analysis of complex 'omics data, Release 2013-05, Ingenuity Systems). Differentially expressed was defined as > 1.5 fold upregulation or < 0.66 fold downregulation and an FDR-corrected p-value < 0.05. ‡ IPS does not calculate p-values for networks.