Supplementary Materials

Transforming Growth Factor β1 Modulates Sex Differences in Cardiac Myofibroblast Activation on Hydrogel Biomaterials

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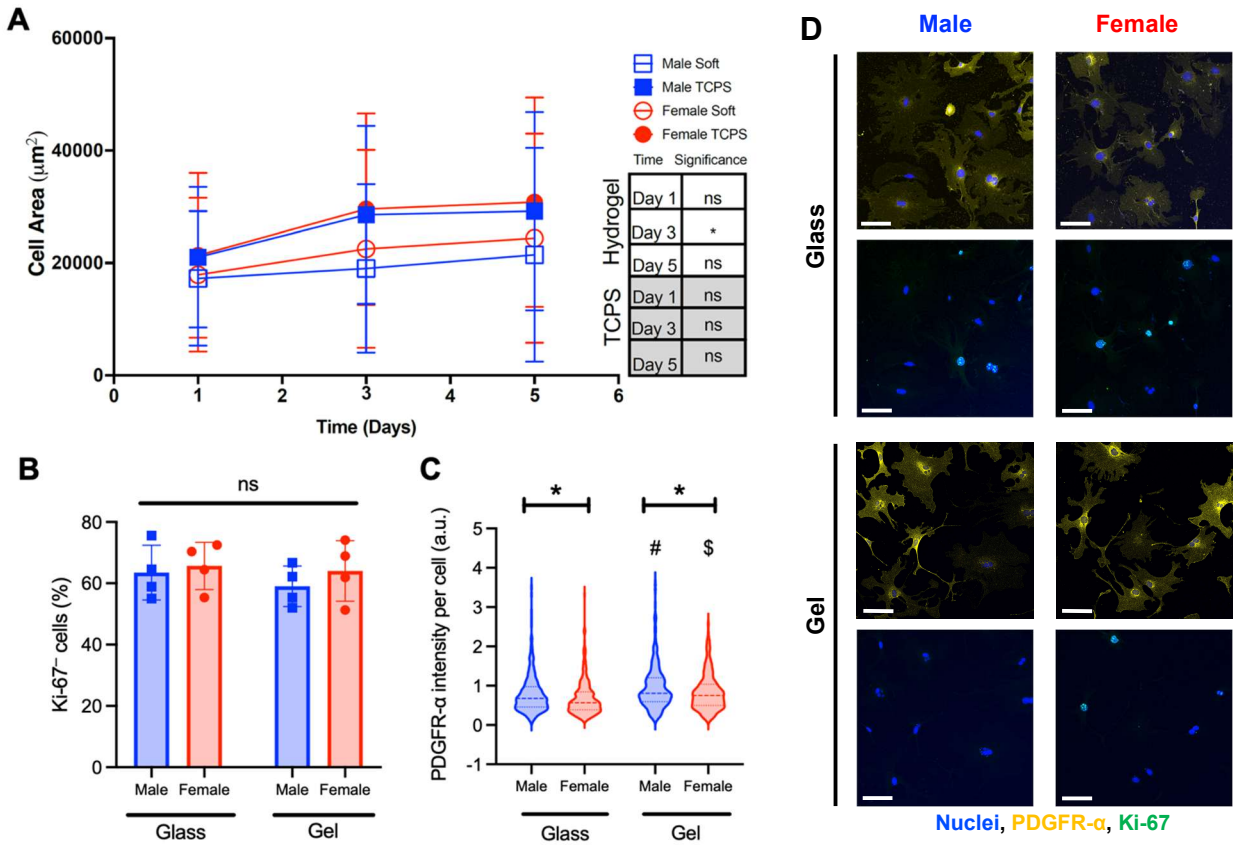
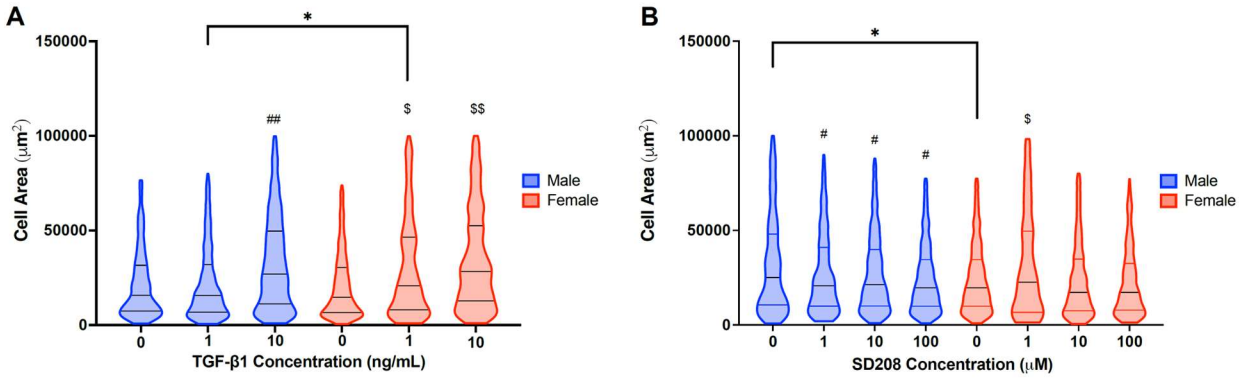
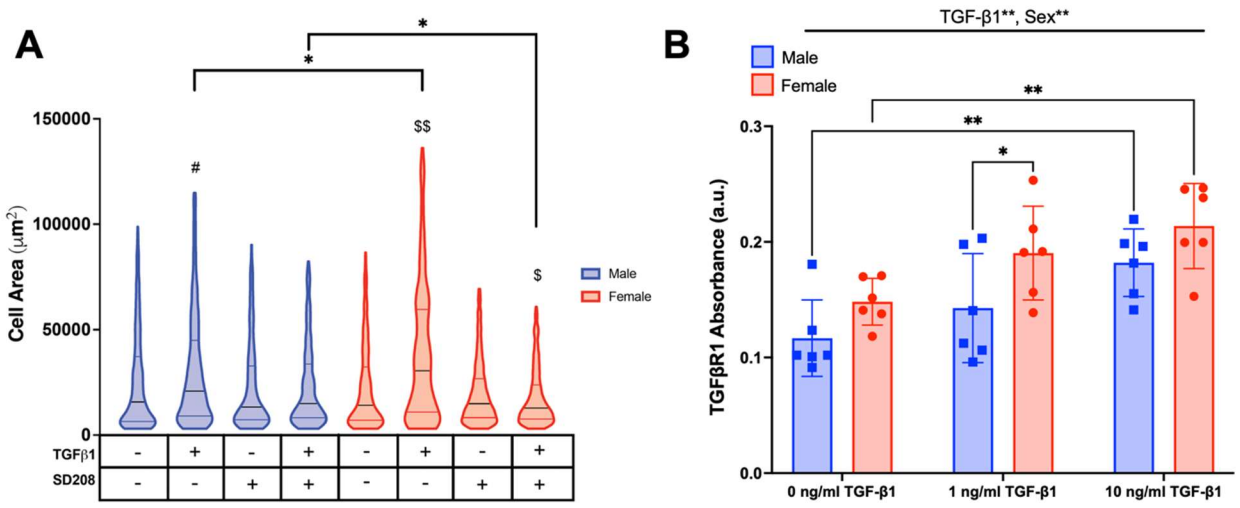


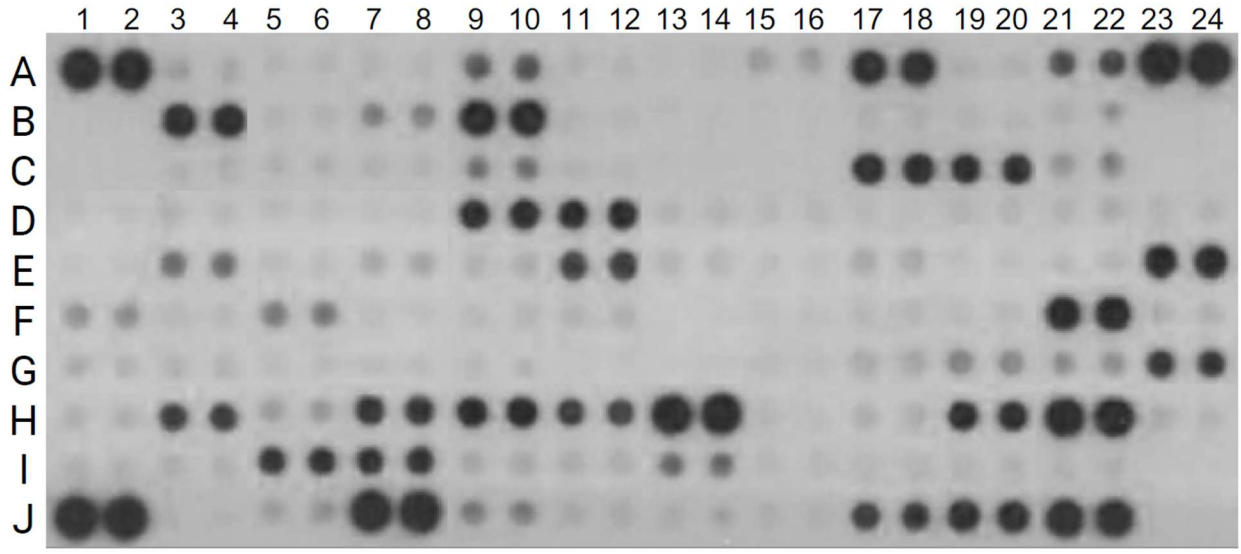
Supplementary Figure 1: Phenotype of cardiac fibroblasts cultured on hydrogels or TCPS. (**A**) Quantification of cell area in male and female CFs cultured on hydrogels and TCPS at various time points. N=3 hydrogels, mean ± S.D. shown. Significance was determined using two-way nonparametric ANOVA (P <0.05) and a Cohen's d test (*d > 0.2), denoting effect size between sex in the table provided. (**B**) Quantification of Ki-67$^-$ cells (%) for male and female cardiac fibroblasts cultured on glass and hydrogels. N=4 gels, n>196 cells per gel, mean ± S.D. shown. Significance was determined using two-way ANOVA with Tukey's post-hoc test. (**C**) Quantification of PDGFR-α mean intensity per cell for male and female cardiac fibroblasts cultured on glass and hydrogels. N=4 hydrogels, n>196 cells per gel. Statistical significance was determined using two-way ANOVA (P<0.05) with Tukey's post-hoc test and effect size determined using Cohen's d test (*d > 0.2) denoting effect size between sex. Hashtag indicates effect size in male group relative to glass control ($^{\#}$d > 0.2); dollar sign indicates effect size in female group relative to glass control ($^{\$}$d > 0.2). (**D**) Representative immunofluorescence images of PDGFR-α (yellow) and Ki-67 (green) in male and female cardiac fibroblasts cultured on glass and hydrogels. Scale is 100 μm.



Supplementary Figure 2: Cell area for cardiac fibroblasts treated with TGFβ-1 or SD208. Quantification of cell area in male and female CFs cultured on hydrogels and TCPS when treated with either (**A**) 0, 1, and 10 ng/mL TGFβ-1 or (**B**) with 0, 1, 10, and 100 μM of SD208. N=3 hydrogels, n>432 cells, mean ± S.D. shown. Significance was determined using two-way nonparametric ANOVA (P <0.05) and a Cohen's d test (*d > 0.2, **d > 0.5, ***d > 0.8, and ****d > 1.4) denoting effect size between sex; asterisk indicates effect size in male group ($^{\#}$d > 0.2, $^{\#\#}$d > 0.5, $^{\#\#\#}$d > 0.8, and $^{\#\#\#\#}$d > 1.4) denoting effect size relative to male control; dollar sign indicates effect size in female group ($^{\$}$d > 0.2, $^{\$\$}$d > 0.5, $^{\$\$\$}$d > 0.8, and $^{\$\$\$\$}$d > 1.4) denoting effect size relative to female control.



Supplementary Figure 3: Cell area for cardiac fibroblasts treated with combined media. (**A**) Quantification of cell area for male and female cardiac fibroblasts cultured on hydrogels TGF-β1 and SD208. N=3 hydrogels, mean ± S.D. shown. Significance was determined using two-way nonparametric ANOVA ($P<0.05$) and a Cohen's d test (*d > 0.2, **d > 0.5, ***d > 0.8, and ****d > 1.4) denoting effect size between sex; asterisk indicates effect size in male group ($^{\#}$d > 0.2, $^{\#\#}$d > 0.5, $^{\#\#\#}$d > 0.8, and $^{\#\#\#\#}$d > 1.4) denoting effect size relative to male control; dollar sign indicates effect size in female group ($^{\$}$d > 0.2, $^{\$\$}$d > 0.5, $^{\$\$\$}$d > 0.8, and $^{\$\$\$\$}$d > 1.4) denoting effect size relative to female control. (**B**) Quantification of TGFβR1 protein expression in male and female cardiac fibroblasts were treated with 0, 1, or 10 ng/mL TGF-β1 via in-cell ELISA. n=6 per group, mean ± S.D. shown. Significance was determined using two-way ANOVA ($P<0.05$) with Tukey's post-hoc test. Main effects of TGF-β1 treatment ($P=0.0011$) and sex ($P=0.0092$) were significant.



Supplementary Figure 4: Sample Cytokine Array Blot. Row/column IDs for each cytokine spot is provided in Supplementary Table 2.

Supplementary Table 1: RT-qPCR Primer Sequences

Gene Name	Primer Sequence	Direction
B2M	GGTCTTTCTGGTGCTTGTCT	Forward
	ACGTAGCAGTTCAGTATGTTCG	Reverse
COL1A1	TGGTGAAGCAGGCAAGC	Forward
	GAAACCTCTCTCGCCTCTTG	Reverse
FN	GAGCTATCCATTTCACCTTCAGA	Forward
	TTGTTCGTAGACACTGGAGAC	Reverse
ACTA2	TCAGCGTTCAGCCTCC	Forward
	CCAGAGCCATTGTCGC	Reverse

Supplementary Table 2: Cytokine Array Protein Targets

Spot(s)	Protein Name	Spot(s)	Protein Name	Spot(s)	Protein Name
A1, A2	Reference Spots	D13, D14	DKK-1	G17, G18	IL-27 p28
A3, A4	Adiponectin/Acrp30	D15, D16	DPPIV/CD26	G19, G20	IL-28A/B
A5, A6	Amphiregulin	D17, D18	EGF	G21, G22	IL-33
A7, A8	Angiopoietin-1	D19, D20	Endoglin/CD105	G23, G24	LDL R
A9, A10	Angiopoietin-2	D21, D22	Endostatin	H1, H2	Leptin
A11, A12	Angiopoietin-like 3	D23, D24	Fetuin A/AHSG	H3, H4	LIF
A13, A14	BAFF/BLyS/TNFSF13B	E1, E2	FGF acidic	H5, H6	Lipocalin-2/NGAL
A15, A16	C1q R1/CD93	E3, E4	FGF-21	H7, H8	LIX
A17, A18	CCL2/JE/MCP-1	E5, E6	Flt-3 Ligand	H9, H10	M-CSF
A19, A20	CCL3/CCL4/MIP-1α/β	E7, E8	Gas 6	H11, H12	MMP-2
A21, A22	CCL5/RANTES	E9, E10	G-CSF	H13, H14	MMP-3
A23, A24	Reference Spots	E11, E12	GDF-15	H15, H16	MMP-9
B3, B4	CCL6/C10	E13, E14	GM-CSF	H17, H18	Myeloperoxidase
B5, B6	CCL11/Eotaxin	E15, E16	HGF	H19, H20	Osteopontin (OPN)
B7, B8	CCL12/MCP-5	E17, E18	ICAM-1/CD54	H21, H22	Osteoprotegerin/TNFRSF11B
B9, B10	CCL17/TARC	E19, E20	IFN-γ	H23, H24	PD-ECGF/Thymidine phosphorylase
B11, B12	CCL19/MIP-3β	E21, E22	IGFBP-1	I1, I2	PDGF-BB
B13, B14	CCL20/MIP-3α	E23, E24	IGFBP-2	I3, I4	Pentraxin 2/SAP
B15, B16	CCL21/6Ckine	F1, F2	IGFBP-3	I5, I6	Pentraxin 3/TSG-14
B17, B18	CCL22/MDC	F3, F4	IGFBP-5	I7, I8	Periostin/OSF-2
B19, B20	CD14	F5, F6	IGFBP-6	I9, I10	Pref-1/DLK-1/FA1
B21, B22	CD40/TNFRSF5	F7, F8	IL-1α/IL-1F1	I11, I12	Proliferin
C3, C4	CD160	F9, F10	IL-1β/IL-1F2	I13, I14	Proprotein Convertase 9/PCSK9
C5, C6	Chemerin	F11, F12	IL-1ra/IL-1F3	I15, I16	RAGE
C7, C8	Chitinase 3-like 1	F13, F14	IL-2	I17, I18	RBP4

C9, C10	Coagulation Factor III/Tissue Factor	F15, F16	IL-3	I19, I20	Reg3G
C11, C12	Complement Component C5/C5a	F17, F18	IL-4	I21, I22	Resistin
C13, C14	Complement Factor D	F19, F20	IL-5	J1, J2	Reference Spots
C15, C16	C-Reactive Protein/CRP	F21, F22	IL-6	J3, J4	E-Selectin/CD62E
C17, C18	CX3CL1/Fractalkine	F23, F24	IL-7	J5, J6	P-Selectin/CD62P
C19, C20	CXCL1/KC	G1, G2	IL-10	J7, J8	Serpin E1/PAI-1
C21, C22	CXCL2/MIP-2	G3, G4	IL-11	J9, J10	Serpin F1/PEDF
D1, D2	CXCL9/MIG	G5, G6	IL-12 p40	J11, J12	Thrombopoietin
D3, D4	CXCL10/IP-10	G7, G8	IL-13	J13, J14	TIM-1/KIM-1/HAVCR
D5, D6	CXCL11/I-TAC	G9, G10	IL-15	J15, J16	TNF-α
D7, D8	CXCL13/BLC/BCA-1	G11, G12	IL-17A	J17, J18	VCAM-1/CD106
D9, D10	CXCL16	G13, G14	IL-22	J19, J20	VEGF
D11, D12	Cystatin C	G15, G16	IL-23	J21, J22	WISP-1/CCN4