Electronic Supplementary Material (ESI) for Biomaterials Science.
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Supplemental Information

Destructive fibrotic teamwork: how both microenvironment stiffness and profibrotic Interleukin 13 impair alveolar macrophage phenotype and function

Kartik Bomb, Lina Pradhan, Qi Zhang, Bader M. Jarai, Arnab Bhattacharjee, David L. Burris, April M. Kloxin, Catherine A. Fromen**

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Table S13: DOE analysis of %PS +ve cells

Table S14: DOE analysis of normalized MFI for efferocytosis assay

Table S15: Validation of DOE model using CD80 and CD86 normalized marker expression.

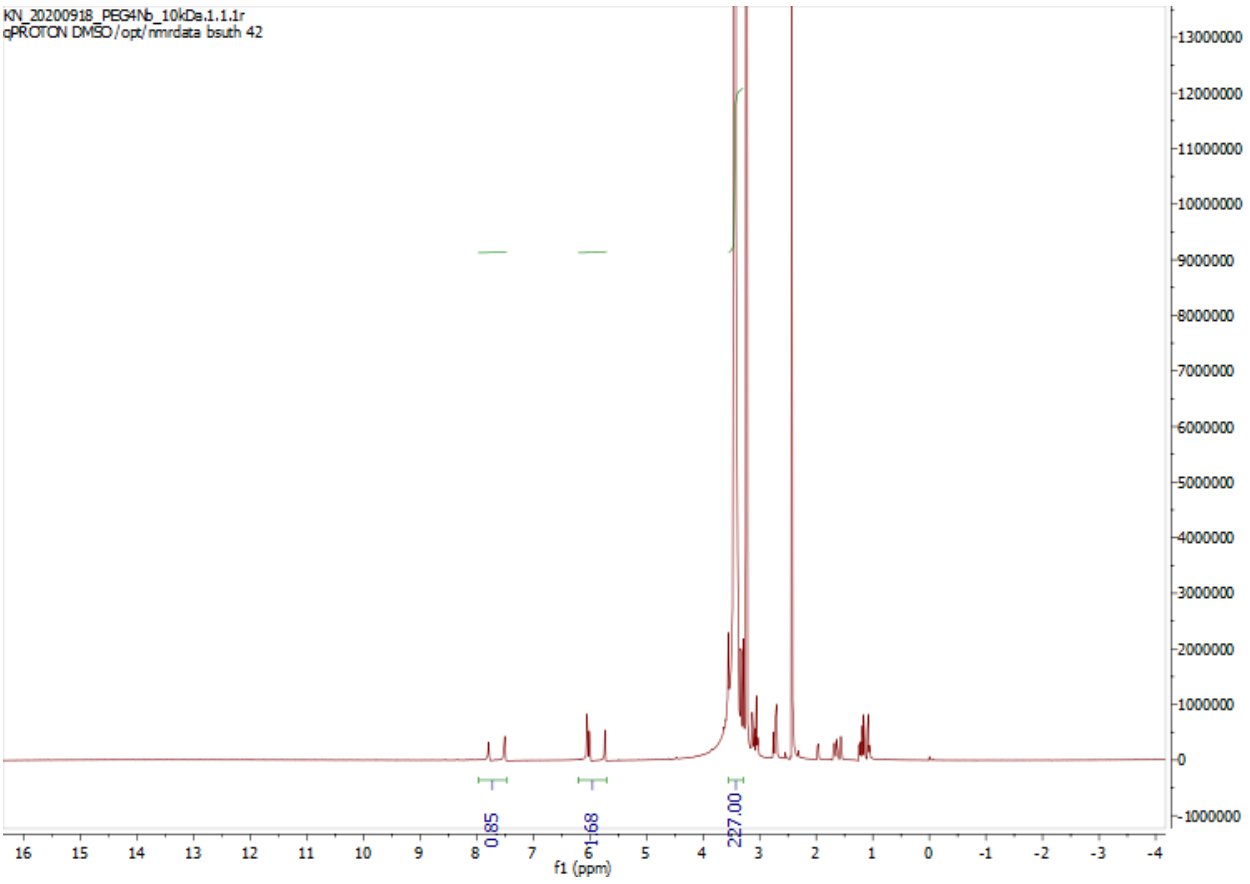

Figure S1: ^{1}H NMR spectra for PEG-4-Nb. The extent of norbornene functionalization for 4-arm PEG (10kDa) was determined by appearance of proton peaks corresponding to the norbornene double bond (2H, δ 6.20-5.86 ppm). The peaks were normalized to the PEG backbone protons (227H, δ 3.65-3.40 ppm). ~85% functionalization was achieved for the batch.

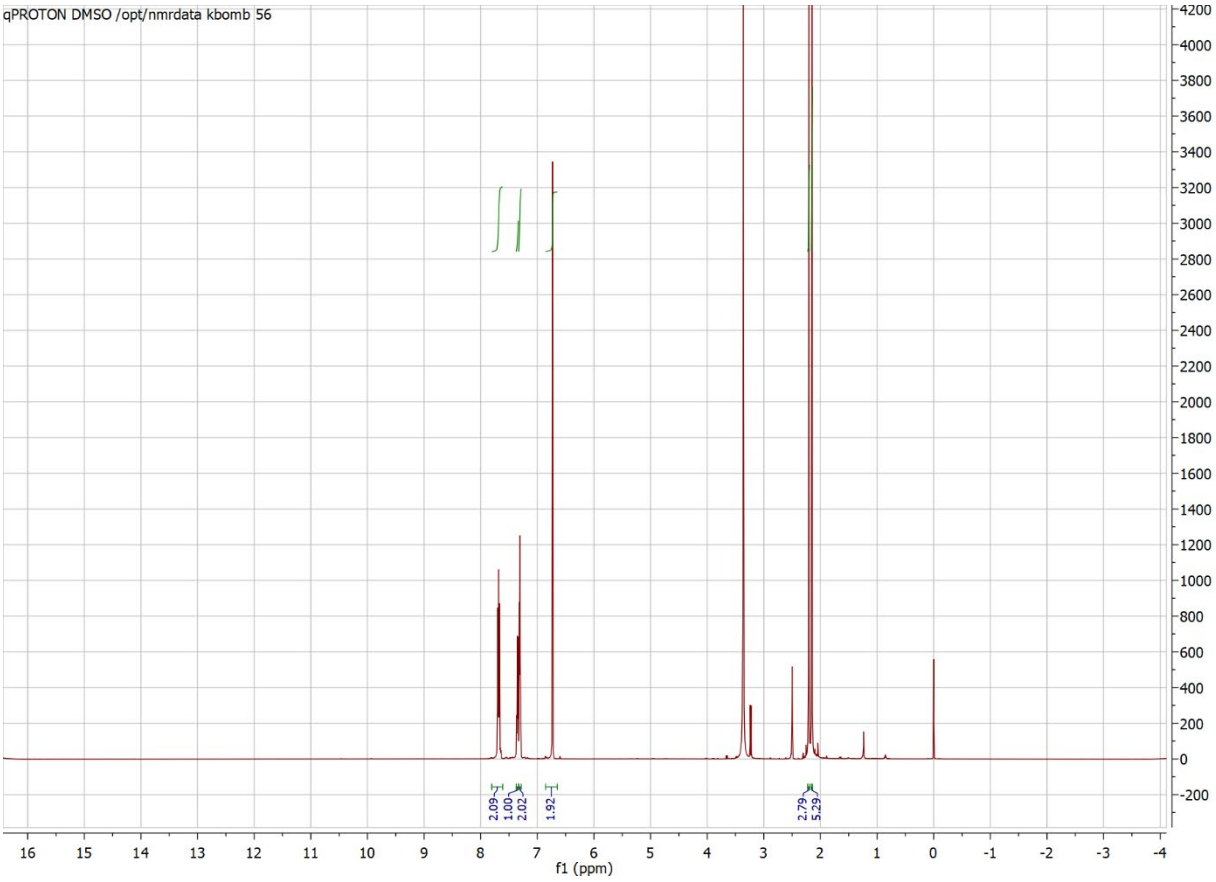

Figure S2. ^{1}H NMR spectra for LAP. The successful reaction was confirmed by appearance of the LAP protons: δ 7.59 (m, 2H), 7.44 (m, 1H), 7.36 (m, 2H), 6.78 (s, 2H), 2.12 (s, 3H), 1.90 (s, 6H).

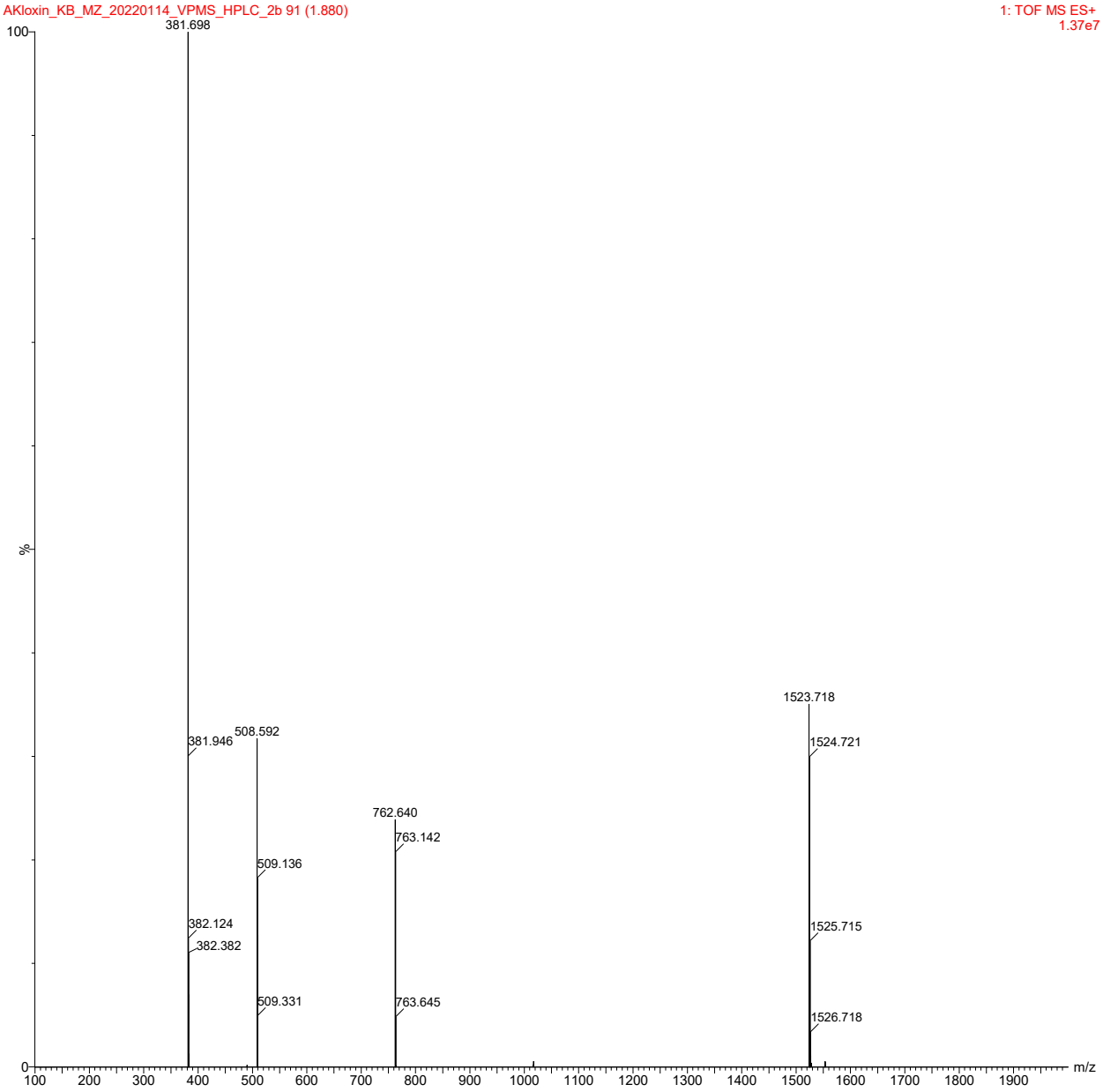

Figure S3: Mass spectrometry of dithiol linker peptide. Successful synthesis of dithiol crosslinker peptide (GCGKVPMSMRGGKGCG-amide) was confirmed by XEVO mass spectrometry. Expected molecular weight of 1524.9 g/mol. $[M + H]^+$ = 1525.9 g/mol, $[M + 2H]^+$ = 763.5 g/mol, $[M + 3H]^+$ = 509.3 g/mol, $[M + 4H]^+$ = 382.2 g/mol

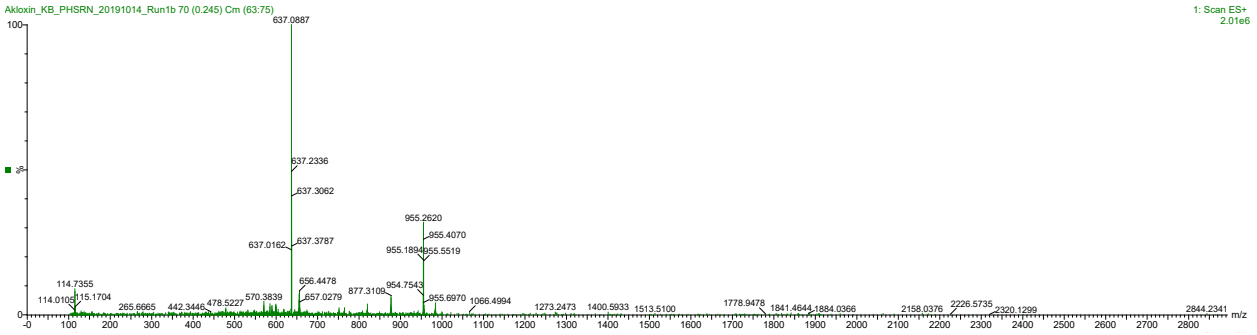

Figure S4: Mass spectrometry of integrin binding peptide. Successful synthesis of scrambled integrin binding peptide (CGGHRPSNG_{10}RGSDP-amide) was confirmed by SQD2 mass spectrometry. Expected molecular weight of 1909.9 g/mol. $[M + 2H]^+$ = 956 g/mol, $[M + 3H]^+$ = 637.7 g/mol

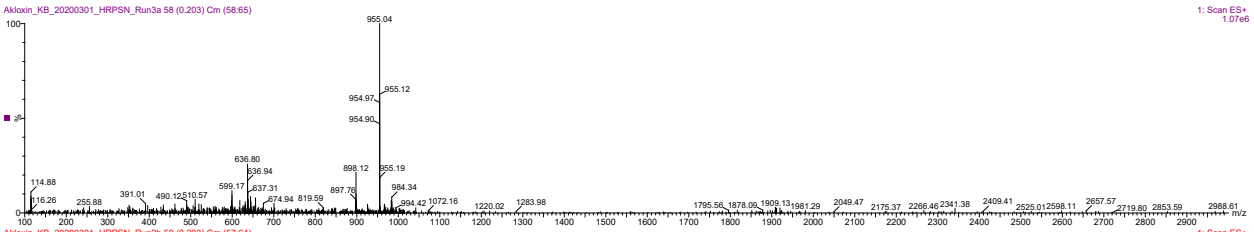

Figure S5: Mass spectrometry of scrambled integrin binding peptide. Successful synthesis of integrin binding peptide (CGGPHSRNG$_{10}$RGDSP-amide) was confirmed by SQD2 mass spectrometry. Expected molecular weight of 1909.9 g/mol. $[M + 2H]^+$ = 956 g/mol, $[M + 3H]^+$ = 637.7 g/mol

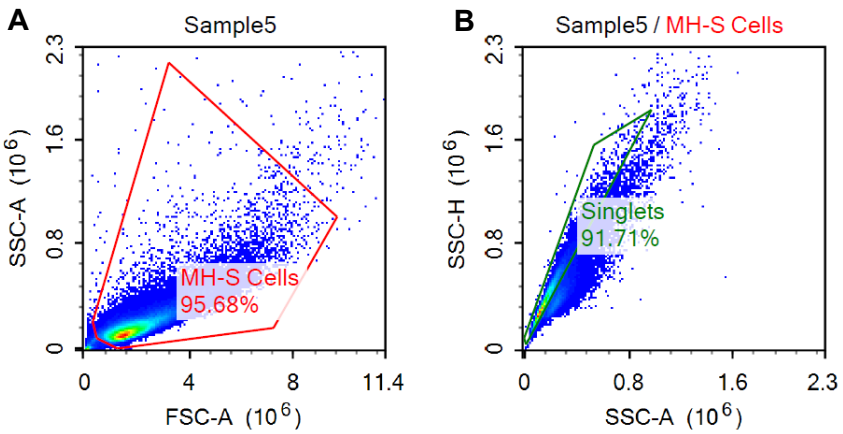

Figure S6: Representative flow cytometry gating. A) Flow cytometry (forward scatter area (FSC-A) vs side scatter area (SSC-A)) gating strategy to identify MH-S cells. B) Flow cytometry (side scatter area (SSC-A) vs side scatter height (SSC-H)) gating strategy to identify singlet MH-S cells.

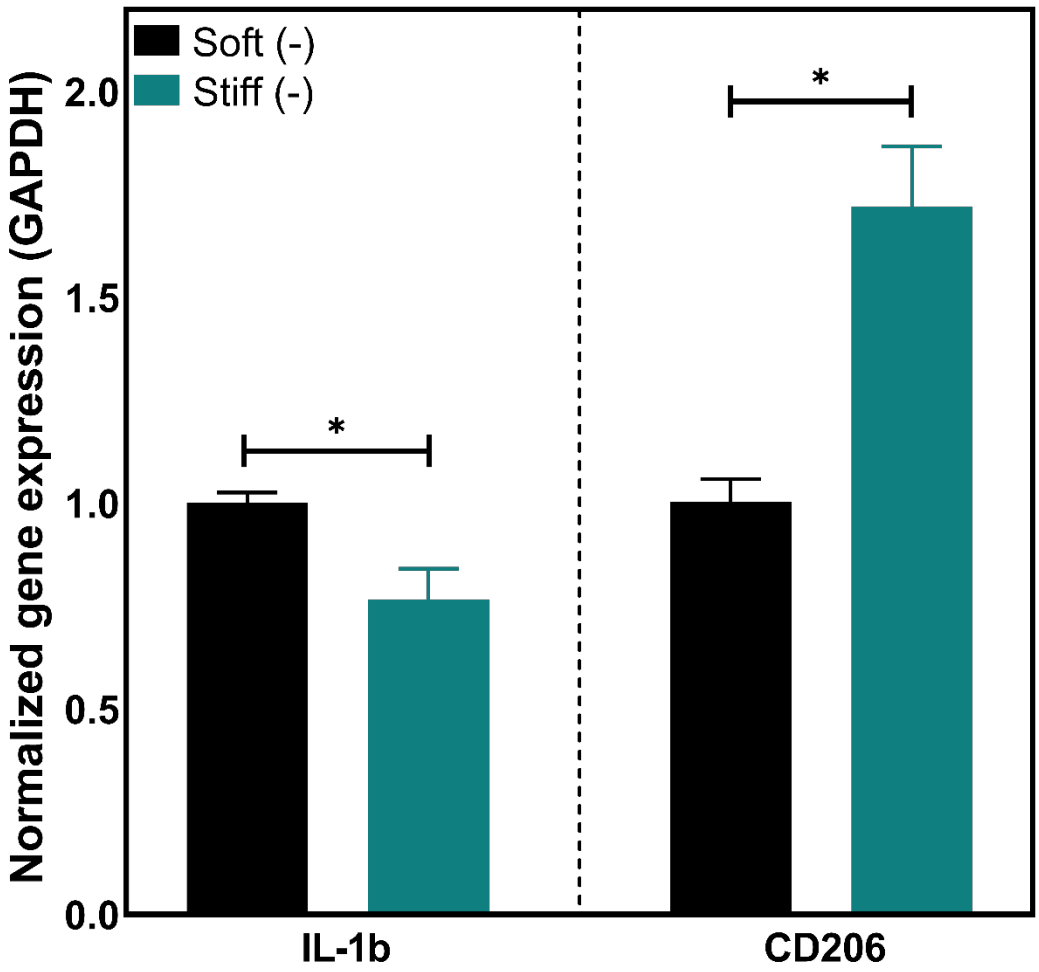

Figure S7: Gene expression analysis for Soft (-) and Stiff (-) hydrogels. Downregulation of M1 marker (IL-1β) and upregulation of M2 marker (CD206) was also confirmed on gene level using RT-qPCR analysis.

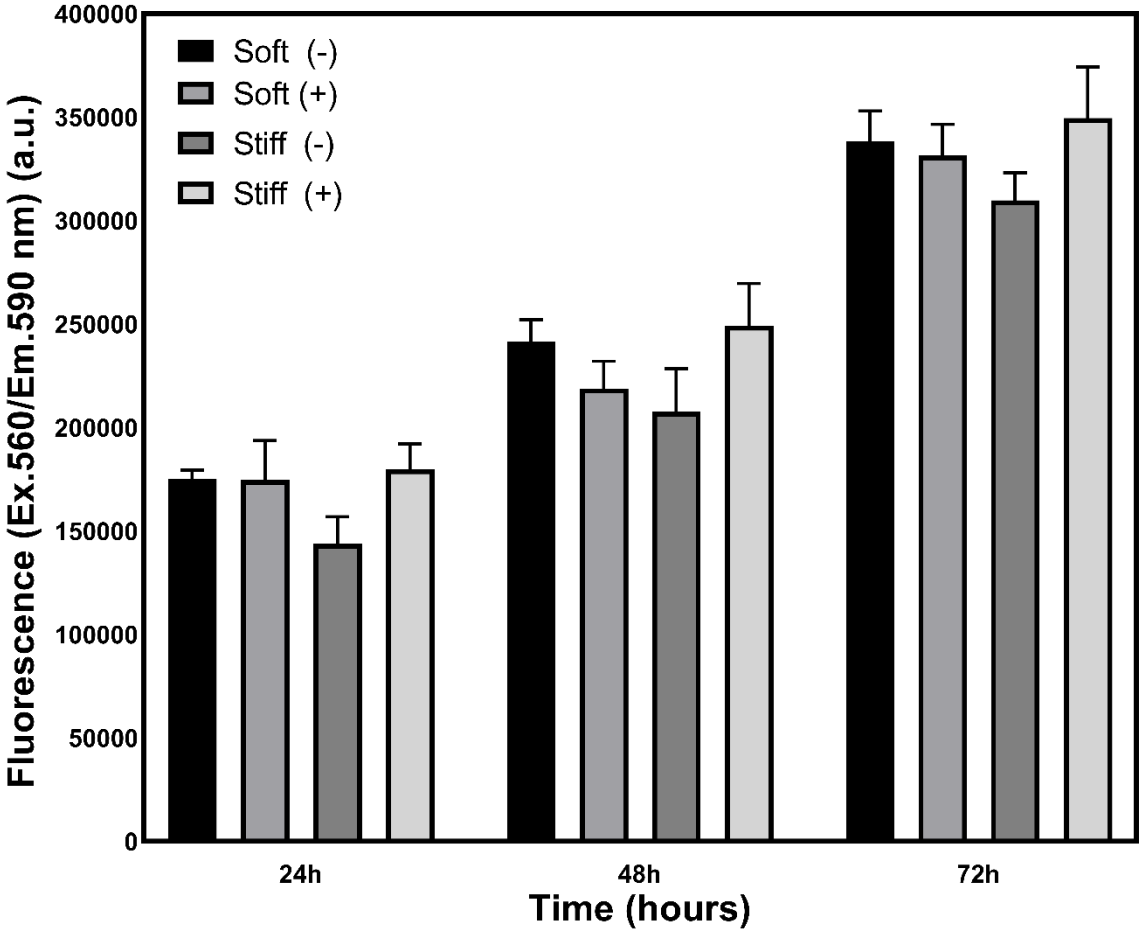

Figure S8: Metabolic activity of MH-S cells on hydrogels over time. Cell metabolic activity was assessed for cells seeded on hydrogels after 24, 48, and 72 hours in culture by an alamarBlue assay. Fluorescence was measured on a plate reader (Ex. 560, Em/ 590).

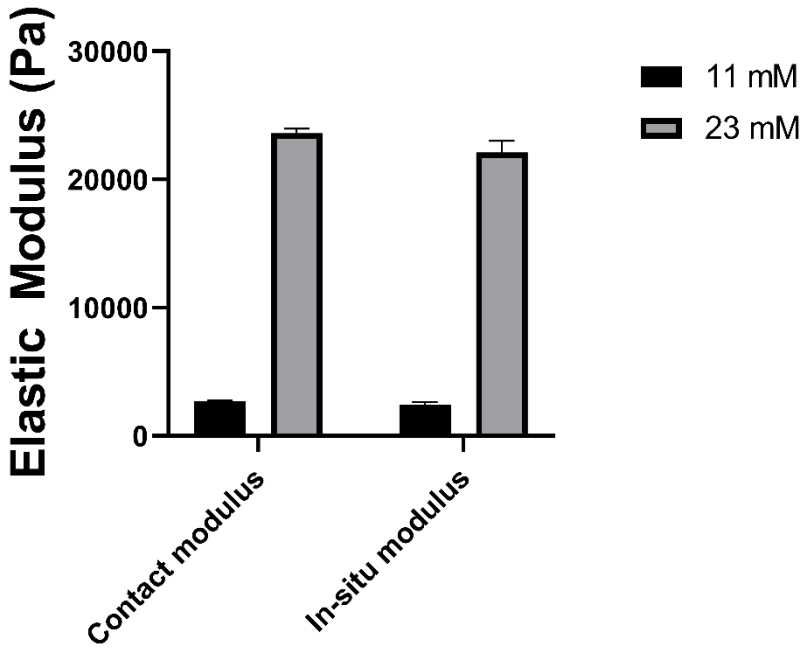

Figure S9: Comparison between the contact modulus and the *in situ* modulus of the hydrogels. Elastic modulus of the hydrogels for soft (11 mM) and stiff (23 mM) was measured using microindentation and in situ rheology.

	Forward primer	Reverse primer
GAPDH	CCTCGTCCCGTAGACAAAATG	TCTCCACTTTGCCACTGCAA
CD206	CTCTGTTCAGCTATTGGACGC	CGGAATTTCTGGGATTCAGCTTC
IL-1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT

Table S1. RT-PCR primers used to measure gene expression. Forward and reverse primers used to measure the relative gene expression of CD206 and IL-1β normalized to housekeeping gene (GAPDH)

	Soft (-)	Soft (+)	Stiff (-)	Stiff (+)
TGF-β (pg/mL)	1442.3 ± 182.3	2137.6 ± 170.7	1400.1 ± 165.6	1957.8 ± 191.6
CCL2 (pg/mL)	7432.2 ± 294.4	9556 ± 126.5	7505.7 ± 932	8558 ± 538.4
TNF-α (pg/mL)	12.6 ± 2	10.9 ± 1.9	11.1 ± 2.9	8.5 ± 1.9
IL-1β (pg/mL)	1.8 ± 0.8	1.6 ± 0.3	1.4 ± 0.7	0.84 ± 0.52

Table S2: ELISA analysis of secreted cytokines. Cytokine analysis for soluble factors secreted by MH-S cells was performed using ELISA. Cells were cultured on soft or stiff hydrogels and polarized with or without IL13 for 24 hours. At least 3 technical replicates were analyzed for each condition.

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		181.32	1.95	92.84	0.000	
Stiffness	42.06	21.03	1.95	10.77	0.000	1.00
IL13	19.31	9.65	1.95	4.94	0.001	1.00
Stiffness*IL13	5.89	2.94	1.95	1.51	0.170	1.00

Regression Equation in Uncoded Units

Cell area = 181.32 + 21.03 Stiffness + 9.65 IL13 + 2.94 Stiffness*IL13

Table S3: DOE analysis of cell area: Two-way ANOVA was performed to identify the significance of each term in promoting increased cell spread.

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		1.7662	0.0464	38.07	0.000	
Stiffness	0.3203	0.1601	0.0464	3.45	0.005	1.03
IL13	1.1160	0.5580	0.0464	12.03	0.000	1.02
Stiffness*IL13	-0.0962	-0.0481	0.0464	-1.04	0.320	1.02

Regression Equation in Uncoded Units

CD206 = 1.7662 + 0.1601 Stiffness + 0.5580 IL13 - 0.0481 Stiffness*IL13

Table S4: DOE analysis of normalized CD206 marker expression: Two-way ANOVA was performed to identify the significance of each term in promoting increased normalized CD206 MFI.

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		0.76460	0.00716	106.77	0.000	
Stiffness	-0.13866	-0.06933	0.00716	-9.68	0.000	1.00
IL13	-0.25020	-0.12510	0.00716	-17.47	0.000	1.00
Stiffness*IL13	0.08195	0.04097	0.00716	5.72	0.000	1.00

Regression Equation in Uncoded Units

CD86 = 0.76460 - 0.06933 Stiffness - 0.12510 IL13 + 0.04097 Stiffness*IL13

Table S5: DOE analysis of normalized CD86 marker expression: Two-way ANOVA was performed to identify the significance of each term in promoting decreased normalized CD86 MFI.

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		0.8584	0.0155	55.46	0.000	
Stiffness	-0.2556	-0.1278	0.0155	-8.25	0.000	1.00
IL13	0.0537	0.0268	0.0155	1.73	0.121	1.00
Stiffness*IL13	0.0812	0.0406	0.0155	2.62	0.030	1.00

Regression Equation in Uncoded Units

CD80 = 0.8584 - 0.1278 Stiffness + 0.0268 IL13 + 0.0406 Stiffness*IL13

Table S6: DOE analysis of normalized CD80 marker expression: Two-way ANOVA was performed to identify the significance of each term in promoting decreased normalized CD80 MFI.

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		1.2025	0.0308	39.01	0.000	
Stiffness	-0.0770	-0.0385	0.0308	-1.25	0.236	1.00
IL13	0.4343	0.2172	0.0308	7.05	0.000	1.00
Stiffness*IL13	-0.0477	-0.0238	0.0308	-0.77	0.454	1.00

Regression Equation in Uncoded Units

TGF-β = 1.2025 - 0.0385 Stiffness + 0.2172 IL13 - 0.0238 Stiffness*IL13

Table S7: DOE analysis of normalized TGF-β secretion: Two-way ANOVA was performed to identify the significance of each term in normalized TGF-β secretion.

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		1.1118	0.0205	54.26	0.000	
Stiffness	-0.0622	-0.0311	0.0205	-1.52	0.157	1.01
IL13	0.2137	0.1068	0.0205	5.21	0.000	1.01
Stiffness*IL13	-0.0721	-0.0360	0.0205	-1.76	0.106	1.01

Regression Equation in Uncoded Units

CCL2 = 1.1118 - 0.0311 Stiffness + 0.1068 IL13 - 0.0360 Stiffness*IL13

Table S8: DOE analysis of normalized CCL2 secretion: Two-way ANOVA was performed to identify the significance of each term in normalized CCL2 secretion.

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		0.8416	0.0433	19.45	0.000	
Stiffness	-0.1625	-0.0812	0.0433	-1.88	0.085	1.00
IL13	-0.1776	-0.0888	0.0433	-2.05	0.063	1.00
Stiffness*IL13	-0.0232	-0.0116	0.0433	-0.27	0.793	1.00

Regression Equation in Uncoded Units

TNF-α = 0.8416 - 0.0812 Stiffness - 0.0888 IL13 - 0.0116 Stiffness*IL13

Table S9: DOE analysis of normalized TNF-α secretion: Two-way ANOVA was performed to identify the significance of each term in normalized TNF-α secretion.

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		0.7754	0.0898	8.63	0.000	
Stiffness	-0.3155	-0.1578	0.0898	-1.76	0.107	1.01
IL13	-0.2248	-0.1124	0.0898	-1.25	0.237	1.01
Stiffness*IL13	-0.0912	-0.0456	0.0898	-0.51	0.622	1.01

Regression Equation in Uncoded Units

IL-1β = 0.7754 - 0.1578 Stiffness - 0.1124 IL13 - 0.0456 Stiffness*IL13

Table S10: DOE analysis of normalized IL-1β secretion: Two-way ANOVA was performed to identify the significance of each term in normalized TNF-α secretion

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		43.830	0.540	81.09	0.000	
Stiffness	-5.325	-2.663	0.540	-4.93	0.001	1.02
IL13	-3.483	-1.741	0.540	-3.22	0.009	1.00
Stiffness*IL13	0.502	0.251	0.540	0.46	0.652	1.02

Regression Equation in Uncoded Units

%PC +ve cells = 43.830 - 2.663 Stiffness - 1.741 IL13 + 0.251 Stiffness*IL13

Table S11: DOE analysis of %PC +ve cells. Two-way ANOVA was performed to identify the significance of each term in regulating uptake of phosphatidylcholine (PC) coated particles

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		0.8582	0.0144	59.65	0.000	
Stiffness	-0.1649	-0.0825	0.0144	-5.73	0.000	1.02
IL13	-0.1170	-0.0585	0.0144	-4.06	0.002	1.00
Stiffness*IL13	0.0018	0.0009	0.0144	0.06	0.952	1.02

Regression Equation in Uncoded Units

Normalized MFI = 0.8582 - 0.0825 Stiffness - 0.0585 IL13 + 0.0009 Stiffness*IL13

Table S12: DOE analysis of normalized MFI for phagocytosis assay. Two-way ANOVA was performed to identify the significance of each term in modulating normalized MFI for phagocytosis assay.

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		56.188	0.715	78.53	0.000	
Stiffness	-7.558	-3.779	0.715	-5.28	0.001	1.00
IL13	-3.428	-1.714	0.715	-2.40	0.043	1.00
Stiffness*IL13	2.472	1.236	0.715	1.73	0.122	1.00

Regression Equation in Uncoded Units

%PS +ve cells = 56.188 - 3.779 Stiffness - 1.714 IL13 + 1.236 Stiffness*IL13

Table S13: DOE analysis of %PS +ve cells. Two-way ANOVA was performed to identify the significance of each term in regulating uptake of phosphatidylserine (PS) coated particles

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		0.7858	0.0134	58.51	0.000	
Stiffness	-0.2304	-0.1152	0.0134	-8.58	0.000	1.00
IL13	-0.1115	-0.0558	0.0134	-4.15	0.003	1.00
Stiffness*IL13	0.0865	0.0433	0.0134	3.22	0.012	1.00

Regression Equation in Uncoded Units

Normalized MFI = 0.7858 - 0.1152 Stiffness - 0.0558 IL13 + 0.0433 Stiffness*IL13

Table S14: DOE analysis of normalized MFI for efferocytosis assay. Two-way ANOVA was performed to identify the significance of each term in modulating normalized MFI for efferocytosis assay.

Predicted CD80 normalized MFI	Measured CD80 normalized MFI	Predicted CD86 normalized MFI	Measured CD86 normalized MFI
0.83	0.84 ± 0.07	0.89	0.88 ± 0.03

Table S15: Validation of DOE model using CD80 and CD86 normalized marker expression. DOE model for CD80 and CD86 marker expression was validated on MH-S cells cultured on hydrogels (E ~ 10-12 kPa) without IL13.