Supplemental Material

The influence of a biofilm-dispersing wound gel on the wound healing process

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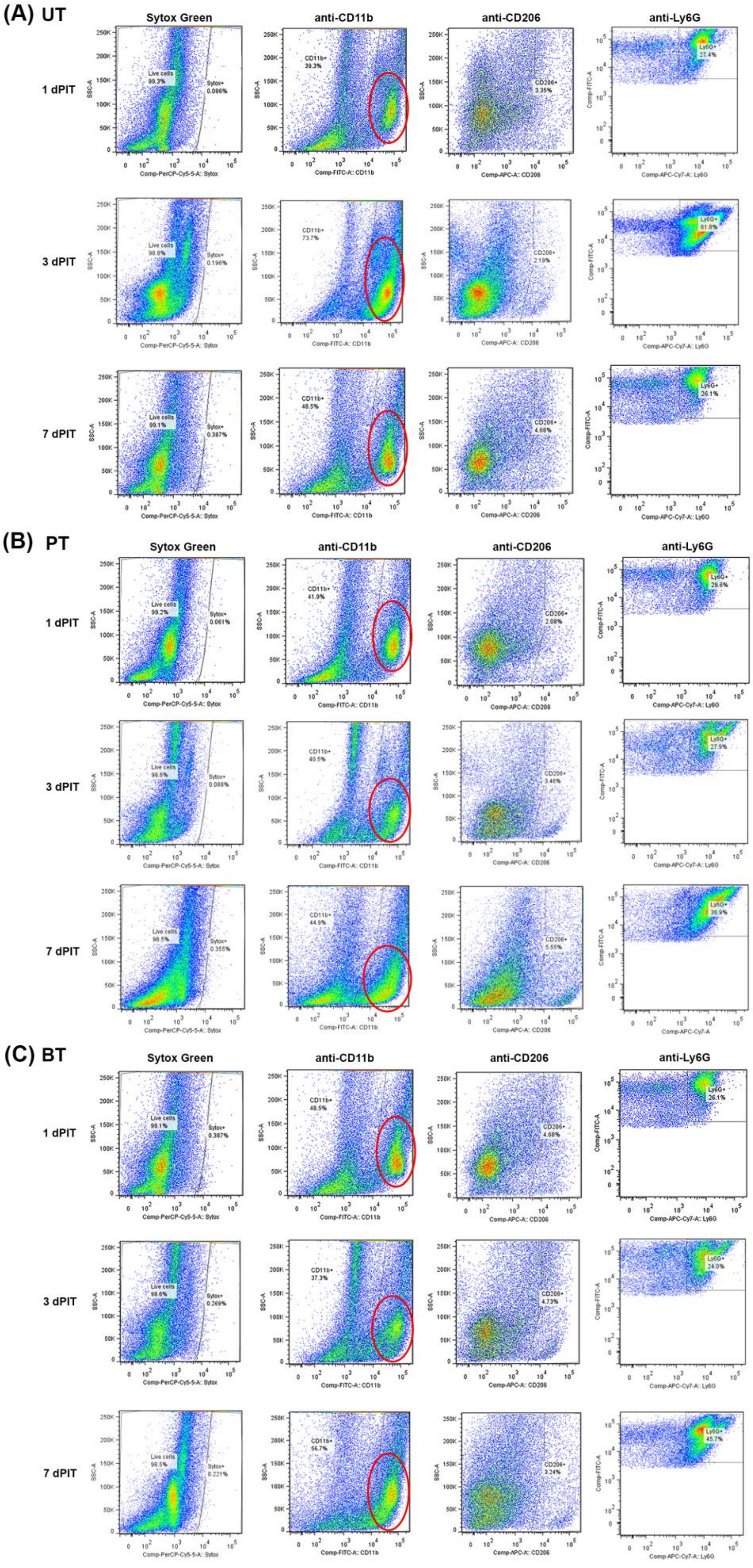
(A) UT
Sytox Green
anti-CD11b
anti-CD206
anti-Ly6G
1 dPIT
Live cells 99.3%
Sytox+ 0.086%
CD11b+ 39.3%
CD206+ 3.35%
Ly6G+ 27.4%
3 dPIT
Live cells 98.8%
Sytox+ 0.196%
CD11b+ 73.7%
CD206+ 2.19%
Ly6G+ 61.8%
7 dPIT
Live cells 99.1%
Sytox+ 0.387%
CD11b+ 48.5%
CD206+ 4.68%
Ly6G+ 26.1%
(B) PT
Sytox Green
anti-CD11b
anti-CD206
anti-Ly6G
1 dPIT
Live cells 99.2%
Sytox+ 0.061%
CD11b+ 41.9%
CD206+ 2.08%
Ly6G+ 29.6%
3 dPIT
Live cells 98.6%
Sytox+ 0.088%
CD11b+ 40.5%
CD206+ 3.46%
Ly6G+ 27.9%
7 dPIT
Live cells 98.5%
Sytox+ 0.355%
CD11b+ 44.9%
CD206+ 5.55%
Ly6G+ 36.9%
(C) BT
Sytox Green
anti-CD11b
anti-CD206
anti-Ly6G
1 dPIT
Live cells 99.1%
Sytox+ 0.387%
CD11b+ 48.5%
CD206+ 4.68%
Ly6G+ 26.1%
3 dPIT
Live cells 98.6%
Sytox+ 0.269%
CD11b+ 37.3%
CD206+ 4.73%
Ly6G+ 24.0%
7 dPIT
Live cells 98.5%
Sytox+ 0.221%
CD11b+ 56.7%
CD206+ 3.24%
Ly6G+ 45.7%
SSC-A
Comp-FITC-A
0 50K 100K 150K 200K 250K
0 10^2 10^3 10^4 10^5
Comp-PerCP-Cy5-5-A:: Sytox
Comp-FITC-A:: CD11b
Comp-APC-A:: CD206
Comp-APC-Cy7-A:: Ly6G
Comp-APC-Cy7-A

FIGURE S1 Fluorescence-activated cell sorting (FACS) analysis for immune cells. Full-thickness excision wounds were generated on the backs of adult mice (3/group) and were covered with untreated gauze (UT), gauze spread with PEG (PT), or gauze spread with BDWG (BT). Animals were euthanized at 1, 3, and 7 days post injury/treatment (dPIT) and the wound plus 2 mm of surrounding intact skin was excised and processed for FACS analysis. Scatter plots are representative of each treatment group. Scatter plots from the FACS analysis of cells from the uninjured control group are shown in Figure 3A of the main text. In the sequential gating system used, live cells were first distinguished from dead cells using SYTOX Green nucleic acid stain (Sytox Green) followed by surface staining with anti-CD11b to separate granulocytes (neutrophils, macrophages, and natural killer cells) from other live cells. The CD11b$^+$ cells were internally stained anti-CD206 to separate M2 macrophages followed by internal staining with anti-Ly6G to separate neutrophils. The CD11b+ cell population is indicated by a red oval. (A) UT mice; (B) PT mice; (C) BT mice).

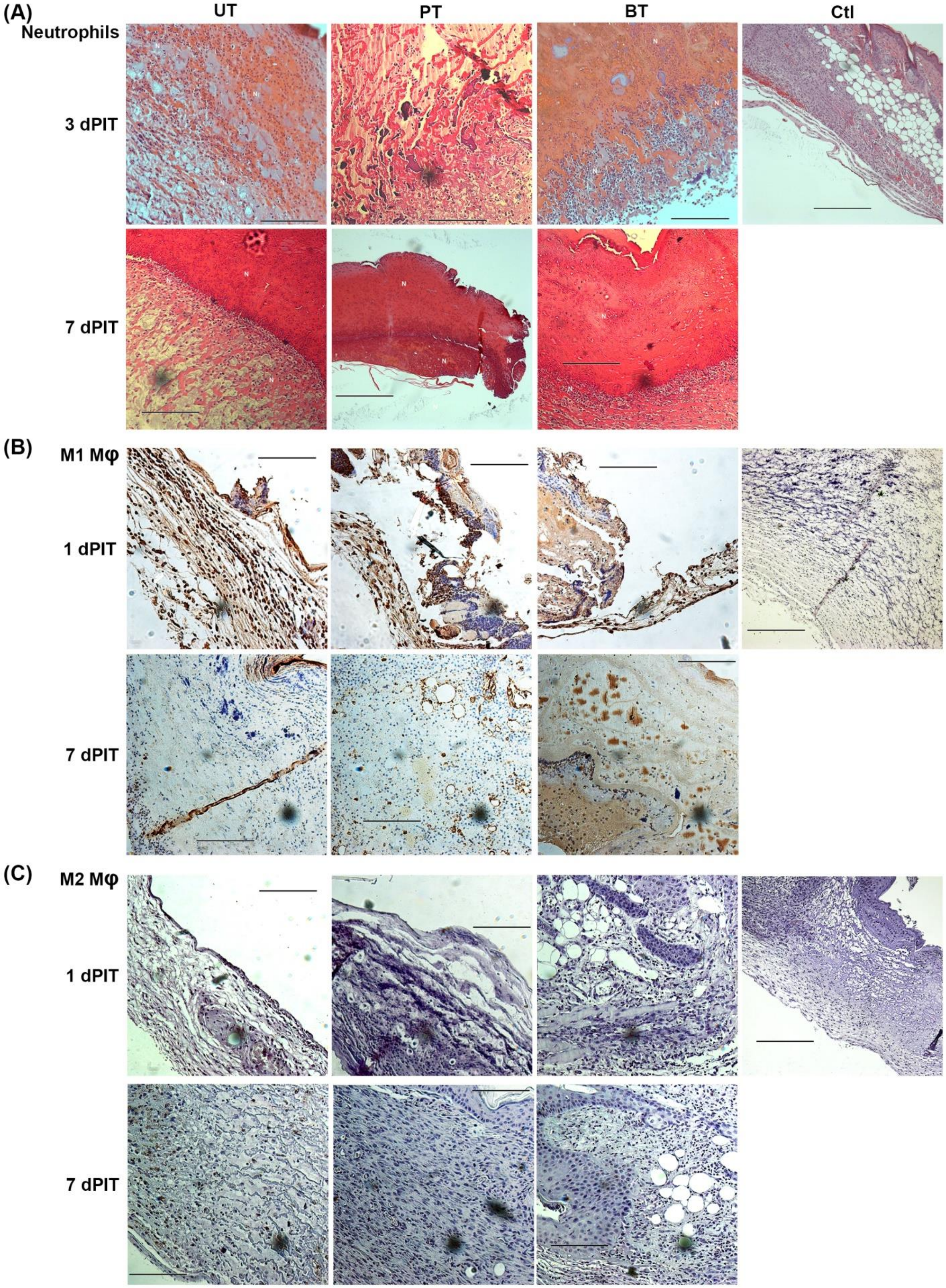
(A)
UT
PT
BT
Ctl
Neutrophils
3 dPIT
7 dPIT
N
(B)
M1 Mφ
1 dPIT
7 dPIT
(C)
M2 Mφ
1 dPIT
7 dPIT

FIGURE S2 Distribution of inflammatory cells within wound margins, beds, or scabs. Full-thickness skin wounds were generated and treated as described in Figure S1. At 1, 3, or 7 dPIT, animals were euthanized and the wound plus 2 mm of surrounding intact skin was excised, formalin fixed and submitted to the Department of Pathology, Texas Tech University Health Sciences Center for embedding and sectioning. (A) Representative photomicrographs of neutrophils that were stained with H&E at 3 and 7 dPIT. (B) Representative photomicrographs of M1 macrophages that were 3,3′-diaminobenzidine (DAB) stained using iNOS primary antibody at 1 and 7 dPIT. M1 macrophage staining was done by the Histology Research Core Facility (University of North Carolina, Chapel Hill, NC). (C) Representative photomicrographs of M2 macrophages that were DAB-stained using arginase-1 primary antibody at 1 and 7 dPIT. Photomicrographs were taken at 200×; bars, 300 dpi. Photomicrographs of neutrophils at 1 dPIT, M1 macrophages at 3 dPIT, and M2 macrophages at 3 dPIT are shown in Figure 5A, C, and E of the main text, respectively.

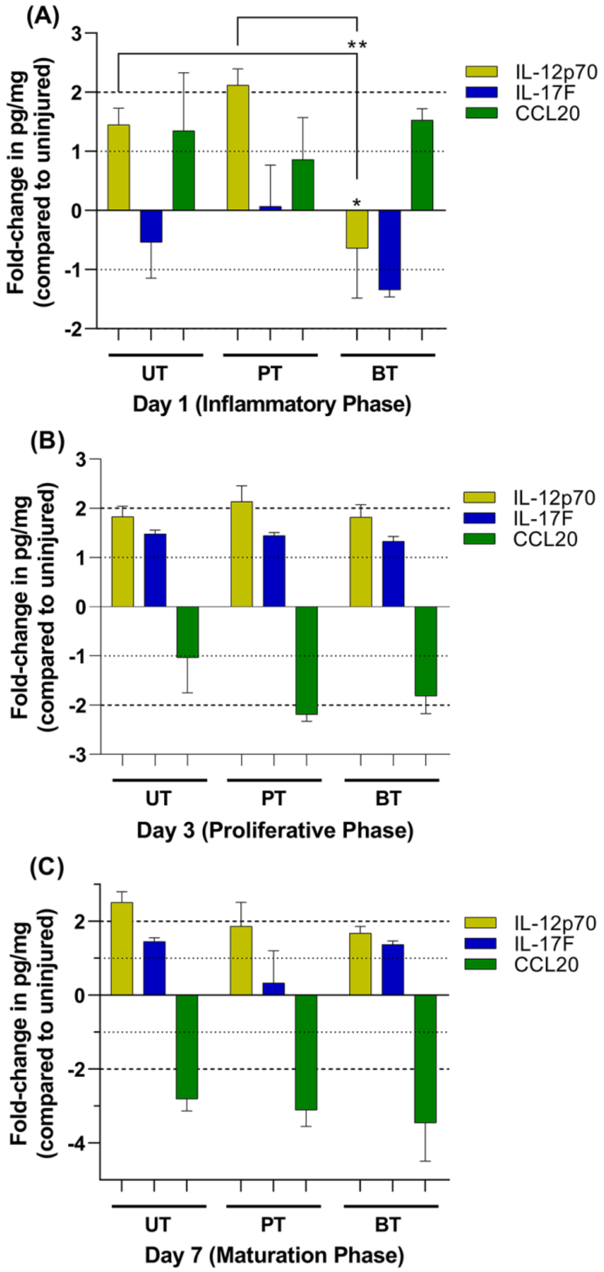

FIGURE S3 Cytokines and chemokines (C/C) with low levels of expression compared to uninjured tissue. Full-thickness 6-mm punch biopsy wounds were made on the backs of mice (4/group) and the wounds were UT, PT, or BT as described in Materials and Methods. At 1, 3, and 7 dPIT, tissue from the three biopsy wounds on a single mouse was collected using an 8-mm punch and the tissues were pooled and treated as a single sample (4 separate pools per group). An equal amount of tissue was collected from 3 uninjured mice to serve as baseline for levels of C/C within normal skin. Proteins were extracted and quantities of the C/C were measured using the U-PLEX biomarker assay with MSD DISCOVERY WORKBENCH software version 4.0. Bars represent the means of the fold-change values obtained from the 4 pools compared to the average value of the uninjured pool (Table S2). (A) Fold changes in levels of IL-12p70, IL-17F, and CCL20 at 1 dPIT; (B) Levels of the C/Cs at 3 dPIT; (C) Levels of the C/Cs at 7 dPIT. Differences in changes in expression were analyzed using one-way ANOVA with Tukey's multiple comparisons posttest; *, $P < 0.05$.

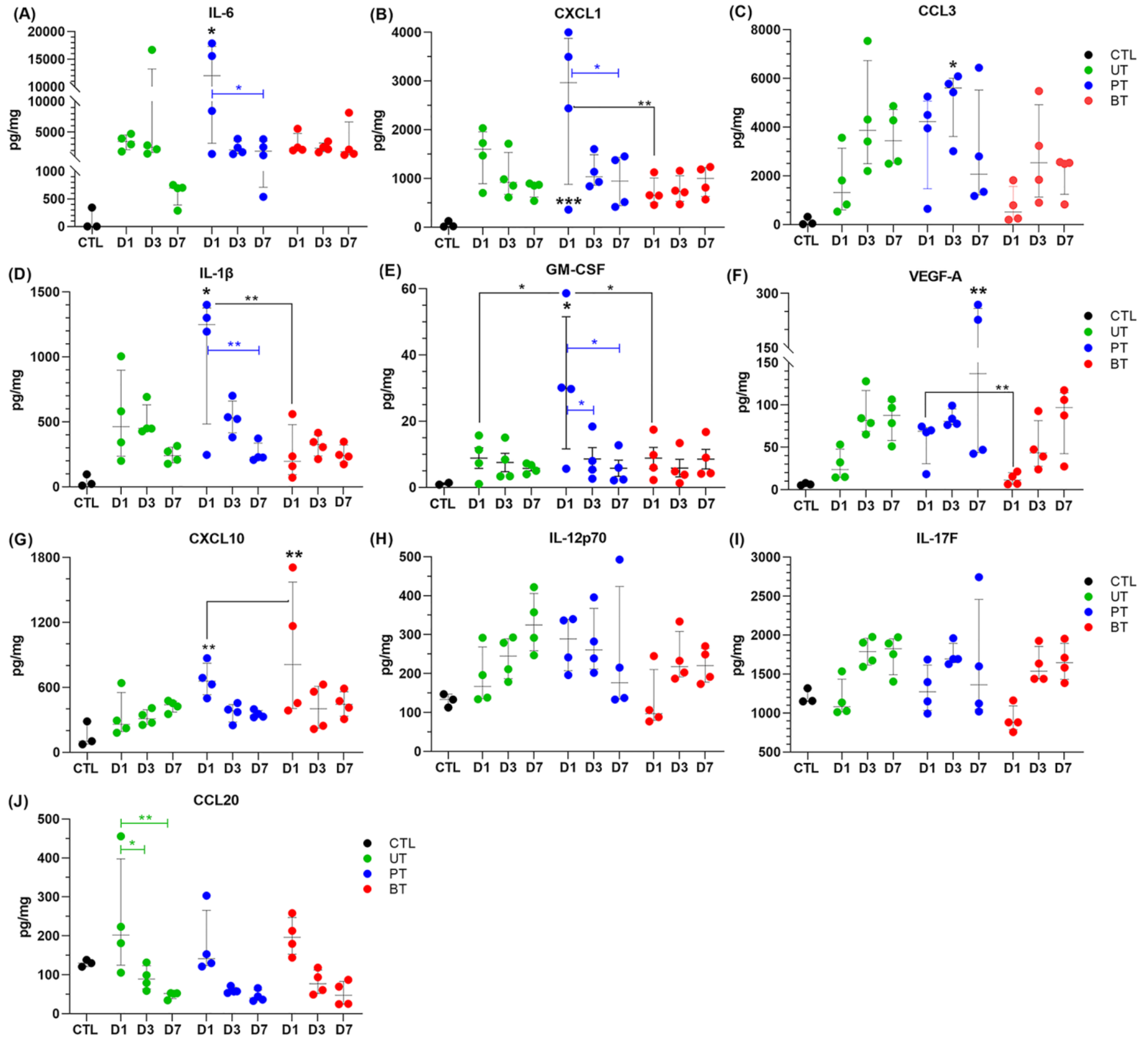

FIGURE S4 Day-to-day changes in levels of specific C/C among the different treatment groups. The preparation of the animals, collection of the samples, and determination of protein levels for each C/C are described in Figure S3. Data are reported in pg/mg and plotted for individuals (4 mice/treatment; 2-3 mice for uninjured tissue control) showing the median and quartile ranges for each C/C (A-J). One-way ANOVA with Tukey's multiple comparisons posttest was used to assess significant differences between levels in baseline control and each C/C (bolded black asterisks), between levels in one treatment and another on the same day (black brackets and nonbolded asterisks), and day to day variation within a treatment (colored brackets and asterisks); *, *P* <0.05; **, *P* <0.01; ***, *P* <0.001.

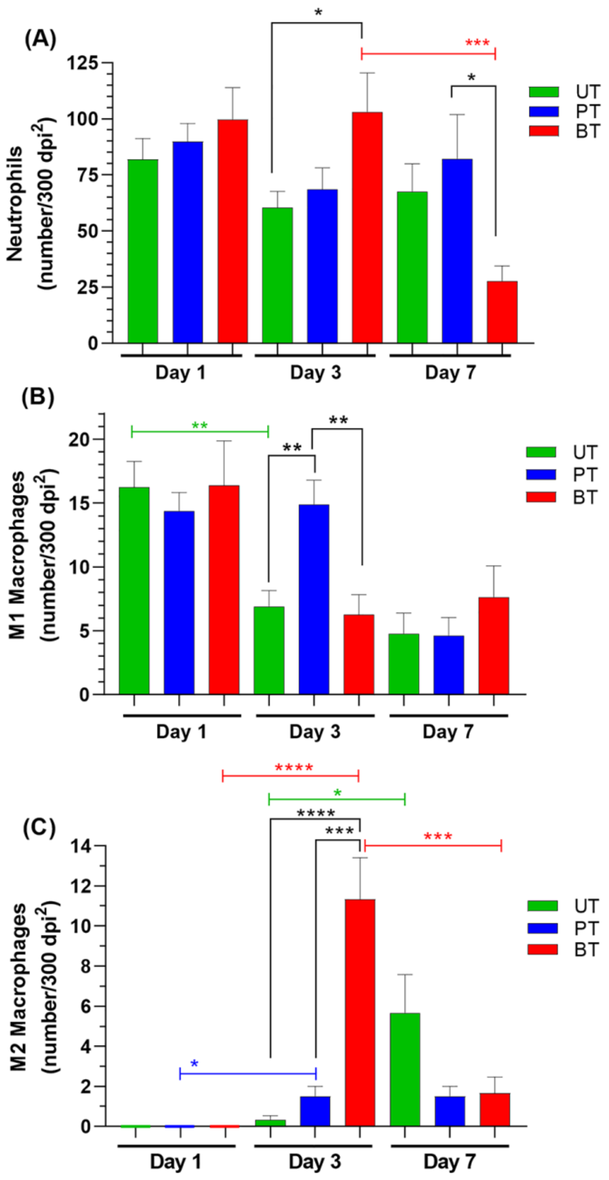

FIGURE S5 Changes in total numbers of neutrophils, M1 macrophages, and M2 macrophages from specific sites within the wounded tissue. Specific cells were counted at 200× within 300 dpi^2 grids; 3 grids were counted for wound margins, 6 for wound beds, and 9 for scabs (due to variability within the beds and scabs). Numbers from all sites were added. Values on the graphs represent the means of counts for 3 different tissue samples/treatment ± SEM. One-way ANOVA was used to detect significant differences between treatments on the same day (black brackets) and cell locations within a treatment (colored brackets); *, *P* <0.05; **, *P* <0.01; ***, *P* <0.001; ****, *P* <0.0001.

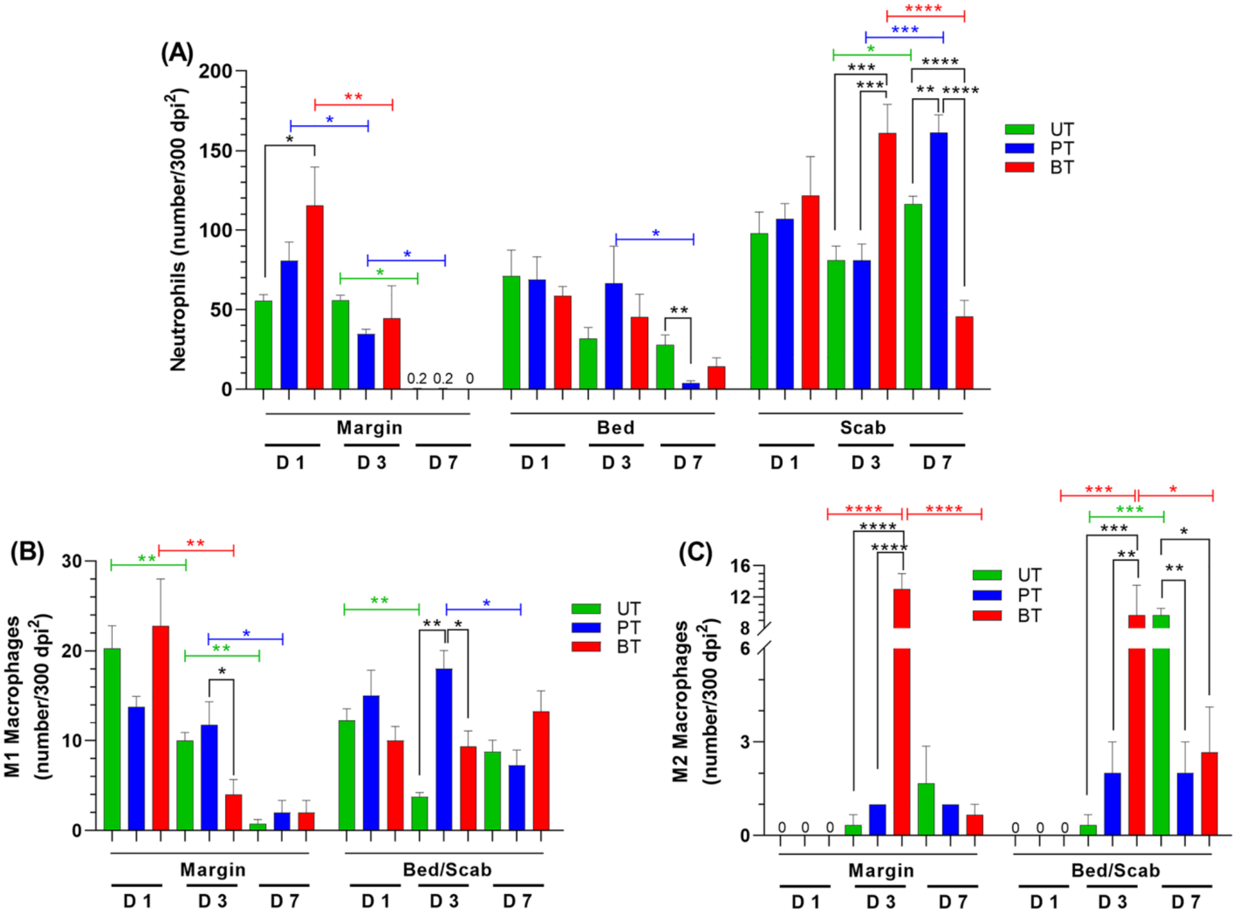

FIGURE S6 Distribution of (A) neutrophils, (B) M1 macrophages, and (C) M2 macrophages within the wound margins, beds, and scabs at 1, 3, and 7 dPIT. Specific cells were counted at 200× within 300 dpi^2 grids; 3 grids were counted for wound margins, 6 for wound beds, and 9 for scabs (due to variability within the beds and scabs). Values represent the means of cells counted at each location for 3 different tissue samples/treatment ± SEM. One-way ANOVA was used to detect significant differences between treatments on the same day (black brackets) and within a treatment on different days (colored brackets); *, *P* <0.05; **, *P* <0.01; ***, *P* <0.001; ****, *P* <0.0001.

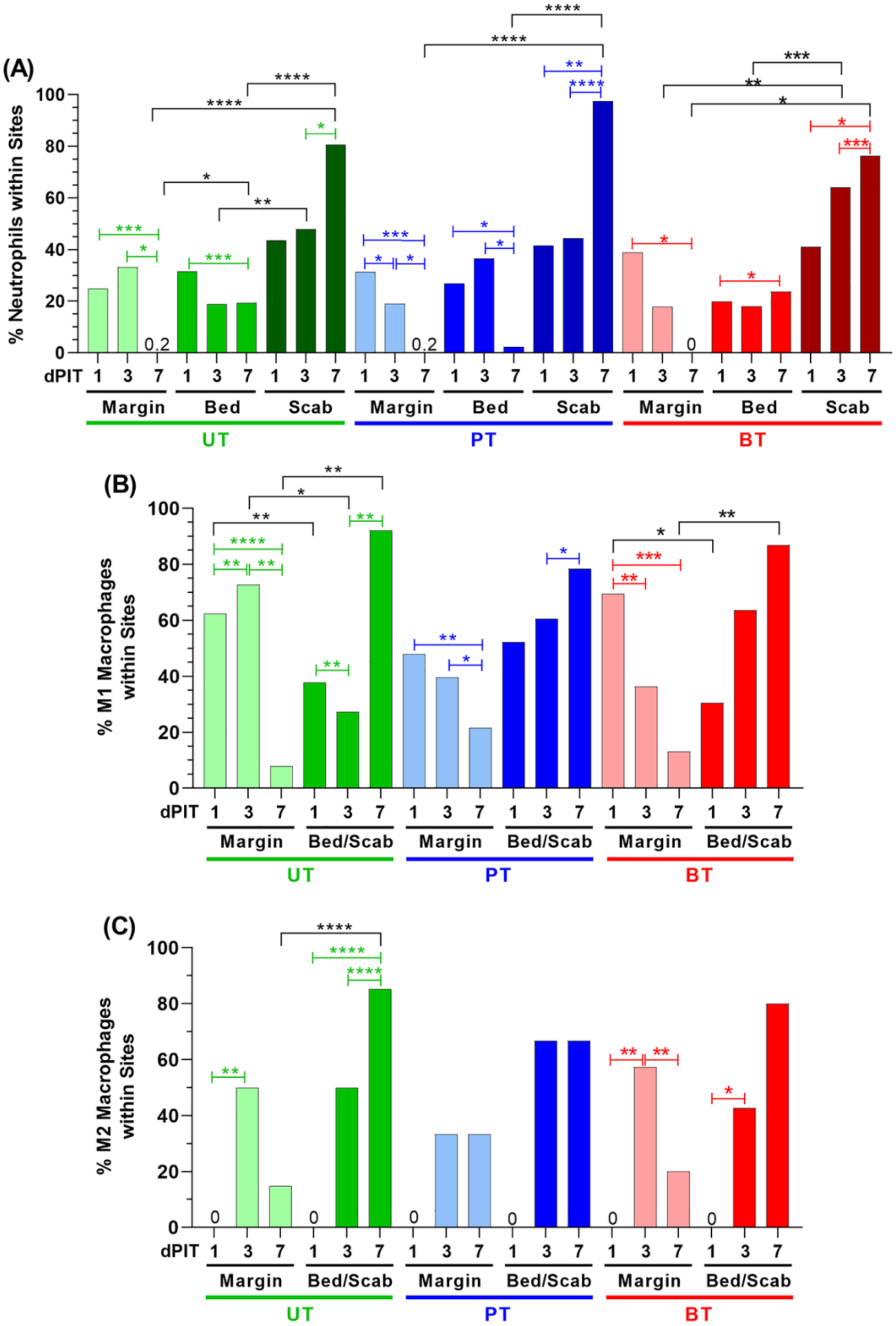

FIGURE S7 Percent changes in inflammatory cells within margins, beds, and scabs of the wounds from day to day by treatment. Data are graphed as percentages for visualization of the day-to-day shifts of neutrophils (A), M1 macrophages (B), and M2 macrophages (C) within the wound margins, beds, and scabs for each treatment. Values for beds and scabs were combined for the macrophages due to lower numbers. Percentages were calculated from the numbers of specific cells counted at 200× within 300 dpi^2 grids; 3 grids were counted for wound margins, 6 for wound beds, and 9 for scabs (due to variability within the beds and scabs). Bars represent the percentages counted at each location for 3 different tissue samples/treatment. One-way ANOVA run on the counted cells was used to detect significant shifts in cell populations from one site to another (black brackets and asterisks) and changes in percentages from day to day within the same treatment (colored brackets and asterisks); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

TABLE S1 Detailed descriptions and sources for antibodies, products, and reagents used in this study.

Product	**Description**	**Manufacturer/Source**	**Location**[a]
Animal experiments			
Nembutal	5% sodium pentobarbital	Diamondback Drugs	Scottsdale, AZ
Disposable biopsy punches	6-mm and 8-mm	Integra Miltex, Integra LifeSciences	Princeton, NJ
OPSITE	Transparent, moisture permeable, adhesive dressing	Smith and Nephew	Andover, MA
General histology			
10% formalin	Tissue preservative	MilliporeSigma	St. Louis, MO
H&E stain		ThermoFisher Scientific	Waltham, MA
Permount	Mounting medium for coverslipping tissue sections	ThermoFisher Scientific	
Immunohistochemistry			
Pan macrophage primary antibody	Rabbit anti-human, anti-mouse polyclonal anti-F4/80 antibody for staining all macrophages; unconjugated	Invitrogen	Carlsbad, CA
Anti-iNOS primary antibody	Rabbit, anti-human, anti-mouse polyclonal antibody for staining M1 macrophages; unconjugated	Invitrogen	
Normal goat serum	Used at 20% to block non-specific antibody binding in tissue	abcam	Cambridge, MA
Anti-arginase 1 (ARG1) primary antibody	Rabbit anti-mouse ARG1/liver arginase polyclonal antibody for staining M2 macrophages; unconjugated	Novus Biologicals	Littleton, CO
Anti-IgG secondary antibody	Biotinylated goat anti-rabbit IgG; recognizes both heavy and light chains of antibodies	Vector Laboratories	Burlingame, CA
Avidin-biotinylated enzyme complex	Enzymatic detection of biotinylated molecules via horseradish peroxidase; VECTASTAIN ABC-HRP Kit	Vector Laboratories	
DAB staining	3,3′-diaminobenzidine substrate for horseradish peroxidase colorimetric detection	Two-component DAB kit; Biogenex	Fremont, CA
Hematoxylin Gill 2	Formulated for counterstaining DAB-stained tissue sections	Richard Allan Scientific	San Diego, CA
Fluorescence-activated cell sorting analysis			
RPMI 1640 medium	Cell culture medium for preparation cells from excised wound tissues; contains glutathione, biotin, vitamin B12, and para-aminobenzoic acid	ThermoFisher Scientific	
Liberase TL	Collegenase I and collagenase II with a low concentration of thermolysin; to dissociate cells within tissue; thermolysin low	MilliporeSigma	St. Louis, MO
DNase I	Bovine pancreatic deoxyribonuclease I (grade II) for isolation of cells from tissue samples	MilliporeSigma	

Cell strainer	40 µm sterile cell strainer for isolating primary cells to obtain a uniform single-cell suspension	Falcon; Corning Life Sciences	Corning, NY
Fetal bovine serum	For preparation of cell suspensions	R&D Systems	Minneapolis, MN
Penicillin-Streptomycin solution	Penicillin at 10,000 U/mL and streptomycin at 10,000 ug/mL to prevent bacterial contamination of cell suspensions	ThermoFisher Scientific	
FACS buffer	PBS with 1% bovine serum albumin for resuspending cells and aiding in miniminzing non-specific antibody binding	Made in-house	
Fc Block	Unconjugated monoclonal antibody (clone 3070) for blocking Rc receptors that can cause nonspecific, false-positive antibody staining of cells	BD Biosciences	San Jose, CA
SYTOX Green nucleic acid stain	Green-fluorescent nuclear and chromosome counterstain that is impermeable to live cells; indicator of dead cells within a population	Invitrogen	
Anti-CD11b	Rat anti-mouse, unlabeled, monoclonal antibody (clone M1/70) for staining granulocytes (neutrophils, monocytes, macrophages, eosinophils, and natural killer cells)	BD Biosciences	
Anti-Ly6G	Rat anti-mouse, allophycocyanin (APC)-Cy7 conjugated, monoclonal antibody (clone 1A8) for staining neutrophils	BD Biosciences	
Cytofix/Cytoperm	Fixation (4.2% formaldehyde [w/w]) and permeabilization buffer	BD Biosciences	
Anti-iNOS	Rabbit anti-mouse phycoerythrin (PE) conjugate monoclonal antibody (clone D6B6S) for staining M1 macrophages	Cell Signaling Technology	Beverly, MA
Anti-CD206	Rat anti-mouse conjugated monoclonal antibody (clone MR6F3) for staining M2 macrophages	Invitrogen, eBioscience	
Cytokine/chemokine analysis			
Protein lysis buffer	Buffer containing 150 mM NaCl; 20 mM Tris, pH 7.5; 1 mM EDTA; 1 mM EGTA, 1% Triton X-100	Meso Scale Discovery	Rockville, MD
Halt protease and phosphatase inhibitor cocktail	Contains inhibitors targeting aminopeptidases, cysteine and serine proteases, serine/threonine and protein tyrosine phosphatases	ThermoFisher Scientific	

[a]All products were purchased from manufacturers or suppliers within the USA.

TABLE S2 Calculation of probable macrophages from FACS analysis

			Percentage of cells in each category				
Baseline Control	**Cell count**	**Total cells**	**Live cells**	**Granulocyte pool[a] CD11b$^+$**	**M2 macrophages CD11b$^+$CD206$^+$**	**Neutrophils CD11b$^+$Ly6G$^+$**	**M1 macrophages[b] CD11b$^+$CD206$^-$Ly6G$^-$**
Uninjured	30	247800	99.5	9.32	4.72	1.75	2.85
Uninjured	31	218860	98.4	6.46	4.25	2.21	0
Uninjured	31	218860	98.4	6.46	4.26	2.31	0
1 dPIT							
UT1	64	2336000	99.3	39.3	3.35	20	15.95
UT2	105	699300	98.8	44.4	2.01	29.9	12.49
UT3	134	913880	99.1	38	2.68	27.4	7.92
PT1	223	1623440	99.2	48.2	2.01	27.3	18.89
PT2	148	976800	99.2	41.9	2.08	29.6	10.22
PT3	216	1563840	99.4	48.7	2.49	30.5	15.71
BT1	90	734400	99	49.6	3.55	32.5	13.55
BT2	76	2143200	99.1	48.5	4.68	26.1	17.72
BT3	197	1438100	98.6	38.8	4.63	14.6	19.57
3 dPIT							
UT1	90	572400	98.9	73.7	2.19	59.6	11.91
UT2	94	659880	99.1	79.2	0.952	69.9	8.348
UT3	183	1354200	99.1	71.6	1.9	61.8	7.9
PT1	183	1171200	98.3	38.6	2.02	23.6	12.98
PT2	61	2092300	98.6	40.5	3.46	27.9	9.14
PT3	184	1361600	98.8	74	0.958	62.4	10.642
BT1	104	684320	98.6	37.3	4.73	24	8.57
BT2	143	1012440	98.8	37	7.33	21.3	8.37
BT3	162	1130760	98.8	70.6	1.87	58.8	9.93
7 dPIT							
UT1	124	892800	98	40	5.72	31.8	2.48
UT2	134	1098800	98.2	44	4.17	35.2	4.63
UT3	160	1184000	98.9	52.1	3.39	44.3	4.41
PT1	34	263840	95.8	67.6	1.3	61.2	5.1
PT2	118	849600	98.7	35	7.19	24.9	2.91
PT3	36	256320	98.5	44.9	5.55	36.9	2.45
BT1	135	1044900	97.8	59.3	2.06	45.7	11.54
BT2	107	749000	98.5	56.7	3.24	44.2	9.26
BT3	111	843600	98.7	53.3	5.52	31.8	15.98
10 dPIT							
UT1	99	683100	99.5	9.57	5.27	2.65	1.65
UT2	231	1436820	99.3	13.9	5.79	4.35	3.76
UT3	179	1202880	99.5	10.6	5.58	3.9	1.12
PT1	232	1498720	99.5	12	4.82	4.95	2.23
PT2	258	1578960	99.5	15.3	5.51	6.11	3.68
PT3	133	938980	98.9	19.9	4.05	13.1	2.75
BT1	64	409600	99.2	28.8	6.2	18.7	3.9
BT2	157	963980	99.5	11	4.57	4.21	2.22
BT3	180	120600	99.5	14.7	5.83	5.9	2.97

[a] The granulocyte pool consists of neutrophils, macrophages (M1 and M2), eosinophils, and natural killer cells.

[b] Natural killer cells are found in very low numbers in normal skin tissue.[1,2] No eosinophils (which express low levels of CD11b in noninflamed settings[3]) were seen in any of the H&E-stained tissues. Therefore, the residual CD11b$^+$ cells are most likely M1 macrophages.

TABLE S3 Changes in cytokine/chemokine expression in response to different treatments

Protein expression in pg/mg of protein

CCL3 (MIP-1α)[a]	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
Sample 1	318.65	3566.61	3412.78	2495.57	645.59	6082.60	6434.30	788.64	896.17	823.77
Sample 2	41.50	1807.81	4311.31	4863.82	4494.15	5777.54	1341.90	1817.80	5479.07	2491.43
Sample 3	17.60	530.99	7534.22	4279.29	5250.85	3009.00	2799.02	251.00	3232.47	2527.87
Sample 4		820.54	2195.56	2599.47	3952.21	5431.19	1171.88	194.32	1838.89	2565.47
CTL Average	125.92									

Individual FC values[b]	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
		28.33	27.10	19.82	5.13	48.31	51.10	6.26	7.12	6.54
		14.36	34.24	38.63	35.69	45.88	10.66	14.44	43.51	19.79
		4.22	59.84	33.99	41.70	23.90	22.23	1.99	25.67	20.08
		6.52	17.44	20.64	31.39	43.13	9.31	1.54	14.60	20.37
Average FC	1.00	13.35	34.65	28.27	28.48	40.31	23.32	6.06	22.73	16.69

Protein expression in pg/mg of protein

CCL20 (MIP-3α)	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
Sample 1	138.16	223.03	58.63	34.31	302.95	57.48	35.88	144.02	48.89	69.56
Sample 2	120.49	180.83	78.87	52.71	152.61	71.57	32.71	179.55	118.15	87.06
Sample 3	129.97	105.42	131.29	52.19	121.20	53.20	44.53	258.19	60.59	25.20
Sample 4		455.69	98.84	51.23	129.82	57.02	65.76	212.70	93.47	24.26
CTL Average	129.54									

Individual FC values[b]		UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
See note[c]		1.72	0.45	0.26	2.34	0.44	0.28	1.11	0.38	0.54
		1.40	0.61	0.41	1.18	0.55	0.25	1.39	0.91	0.67
		0.81	1.01	0.40	0.94	0.41	0.34	1.99	0.47	0.19
		3.52	0.76	0.40	1.00	0.44	0.51	1.64	0.72	0.19

Conversion to FC above/below baseline	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
		1.72	-2.21	-3.78	2.34	-2.25	-3.61	1.11	-2.65	-1.86
		1.40	-1.64	-2.46	1.18	-1.81	-3.96	1.39	-1.10	-1.49
		-1.23	1.01	-2.48	-1.07	-2.43	-2.91	1.99	-2.14	-5.14
		3.52	-1.31	-2.53	1.00	-2.27	-1.97	1.64	-1.39	-5.34
Average FC	1.00	1.35	-1.04	-2.81	0.86	-2.19	-3.11	1.53	-1.82	-3.46

Protein expression in pg/mg of protein

CXCL1 (KC/GRO)	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
Sample 1	123.38	2032.84	852.55	539.89	358.74	1133.93	1450.77	647.72	467.22	1235.88
Sample 2	19.37	1726.46	1712.96	869.93	3493.98	928.08	414.82	1125.89	1155.56	812.77
Sample 3	10.50	1469.09	981.33	895.17	3997.87	836.94	1374.32	454.20	749.46	1183.93
Sample 4		700.36	612.88	852.15	2436.81	1600.11	516.33	653.70	716.47	568.63
CTL Average	51.08									

Individual FC values[b]	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
		39.80	16.69	10.57	7.02	22.20	28.40	12.68	9.15	24.19
		33.80	33.53	17.03	68.40	18.17	8.12	22.04	22.62	15.91
		28.76	19.21	17.52	78.26	16.38	26.90	8.89	14.67	23.18
		13.71	12.00	16.68	47.70	31.32	10.11	12.80	14.03	11.13
Average FC	1.00	29.02	20.36	15.45	50.35	22.02	18.38	14.10	15.12	18.60

Protein expression in pg/mg of protein

CXCL10 (IP-10)	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
Sample 1	286.33	639.90	345.49	476.63	498.95	370.67	323.25	1165.87	214.50	472.54
Sample 2	103.40	293.81	408.62	454.44	687.05	455.78	355.11	1708.19	625.68	307.12
Sample 3	73.85	180.01	252.25	353.35	627.57	249.54	397.63	455.15	560.92	412.69
Sample 4		223.35	273.28	423.54	868.57	395.54	329.13	386.42	245.48	588.14
CTL Average	154.52									

Individual FC values[b]	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
		4.14	2.24	3.08	3.23	2.40	2.09	7.54	1.39	3.06
		1.90	2.64	2.94	4.45	2.95	2.30	11.05	4.05	1.99
		1.16	1.63	2.29	4.06	1.61	2.57	2.95	3.63	2.67
		1.45	1.77	2.74	5.62	2.56	2.13	2.50	1.59	3.81
Average FC	1.00	2.16	2.07	2.76	4.34	2.38	2.27	6.01	2.66	2.88

Protein expression in pg/mg of protein

GM-CSF (CSF2)	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
Sample 1	1.38	15.66	8.37	3.99	5.61	5.40	12.71	5.96	1.28	8.97
Sample 2	0.82	11.32	3.33	5.01	58.64	7.98	2.15	17.41	13.39	16.71
Sample 3	--	1.02	15.01	6.70	29.73	2.61	5.92	9.78	3.80	4.27
Sample 4		7.33	3.35	7.24	30.14	18.36	2.37	2.24	4.81	4.14
CTL Average	1.10									

Individual FC values[b] See note[c]	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
	14.27	3.03	64.71	5.11	4.92	11.58	15.87	1.16	8.18
	10.32	3.03	4.57	53.44	7.27	1.96	15.87	12.21	15.23
	0.93	13.69	6.11	27.10	2.38	5.40	8.92	3.46	3.90
	6.68	3.06	6.60	27.47	16.73	2.16	2.05	4.39	3.77

Conversion to FC above/below baseline	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
		14.27	3.03	64.71	5.11	4.92	11.58	15.87	1.16	8.18
		10.32	3.03	4.57	53.44	7.27	1.96	15.87	12.21	15.23
		-1.08	13.69	6.11	27.10	2.38	5.40	8.92	3.46	3.90
		6.68	3.06	6.60	27.47	16.73	2.16	2.05	4.39	3.77
Average FC	1.00	7.55	5.70	20.50	28.28	7.82	5.27	10.67	5.31	7.77

Protein expression in pg/mg of protein

IL-1β	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
Sample 1	95.64	1004.35	427.48	314.94	246.16	535.88	371.29	233.80	213.11	346.99
Sample 2	21.87	581.22	691.51	271.50	1300.53	522.32	226.20	559.79	415.43	232.51
Sample 3	8.92	199.59	449.89	207.77	1400.74	380.13	206.80	157.85	306.16	245.59
Sample 4		341.68	448.29	176.51	1194.96	700.02	228.31	70.91	344.01	172.54
CTL Average	42.14									

Individual FC values[b]	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
		23.83	10.14	7.47	5.84	12.72	8.81	5.55	5.06	8.23
		13.79	16.41	6.44	30.86	12.39	5.37	13.28	9.86	5.52
		4.74	10.68	4.93	33.24	9.02	4.91	3.75	7.26	5.83
		8.11	10.64	4.19	28.35	16.61	5.42	1.68	8.16	4.09
Average FC	1.00	12.62	11.97	5.76	24.57	12.68	6.13	6.06	7.59	5.92

Protein expression in pg/mg of protein

IL-6	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
Sample 1	343.04	4719.51	2213.46	287.85	1437.73	2478.93	2549.89	2490.62	2199.13	8150.42
Sample 2	0.00	3962.30	16687.21	691.78	15571.69	1406.29	538.17	5543.90	1688.90	1461.60
Sample 3	0.00	3004.69	1493.45	700.63	17868.43	1739.01	3838.75	2124.25	3481.71	2150.91
Sample 4		1846.12	2847.05	750.43	8460.80	3902.90	1232.40	2010.96	2553.71	1278.65
CTL Average	114.35									

Individual FC values[b]	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
		41.27	19.36	2.52	12.57	21.68	22.30	21.78	19.23	71.28
		34.65	145.93	6.05	136.18	12.30	4.71	48.48	14.77	12.78
		26.28	13.06	6.13	156.26	15.21	33.57	18.58	30.45	18.81
		16.14	24.90	6.56	73.99	34.13	10.78	17.59	22.33	11.18
Average FC	1.00	29.59	50.81	5.31	94.75	20.83	17.84	26.61	21.70	28.51

Protein expression in pg/mg of protein

IL-12p70	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
Sample 1	147.07	292.01	279.04	246.98	196.01	395.67	492.81	105.96	186.94	191.49
Sample 2	133.18	196.01	178.54	357.46	336.67	201.99	137.30	244.85	202.73	173.14
Sample 3	112.91	138.12	292.69	421.87	339.94	239.16	215.29	88.02	232.71	249.10
Sample 4		134.00	210.88	292.01	241.29	282.47	133.18	76.81	333.39	270.08
CTL Average	131.05									

Individual FC values[b]		UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
See note[c]		2.23	2.13	1.88	1.50	3.02	3.76	0.81	1.43	1.46
		1.50	1.36	2.73	2.57	1.54	1.05	1.87	1.55	1.32
		1.05	2.23	3.22	2.59	1.82	1.64	0.67	1.78	1.90
		1.02	1.61	2.23	1.84	2.16	1.02	0.59	2.54	2.06

Conversion to FC above/below baseline	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
		2.23	2.13	1.88	1.50	3.02	3.76	-1.24	1.43	1.46
		1.50	1.36	2.73	2.57	1.54	1.05	1.87	1.55	1.32
		1.05	2.23	3.22	2.59	1.82	1.64	-1.49	1.78	1.90
		1.02	1.61	2.23	1.84	2.16	1.02	-1.71	2.54	2.06
Average FC	1.00	1.45	1.83	2.51	2.12	2.14	1.87	-0.64	1.82	1.69

Protein expression in pg/mg of protein

IL-17F	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
Sample 1	1154.29	1534.04	1976.51	1755.74	1147.93	1959.74	2742.44	879.32	1438.42	1383.96
Sample 2	1316.70	1025.56	1593.09	1892.33	1396.11	1628.26	1122.44	1160.63	1926.11	1954.15
Sample 3	1147.93	1012.48	1903.61	1970.93	1686.49	1692.29	1598.96	879.32	1634.11	1709.65
Sample 4		1135.20	1674.89	1402.17	992.78	1692.29	1019.02	755.21	1438.42	1581.32
CTL Average	1206.31									

Individual FC values[b]		UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
See note‡		1.27	1.64	1.46	0.95	1.62	2.27	0.73	1.19	1.15
		0.85	1.32	1.57	1.16	1.35	0.93	0.96	1.60	1.62
		0.84	1.58	1.63	1.40	1.40	1.33	0.73	1.35	1.42
		0.94	1.39	1.16	0.82	1.40	0.84	0.63	1.19	1.31

Conversion to FC above/below baseline	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
		1.27	1.64	1.46	-1.05	1.62	2.27	-1.37	1.19	1.15
		-1.18	1.32	1.57	1.16	1.35	-1.07	-1.04	1.60	1.62
		-1.19	1.58	1.63	1.40	1.40	1.33	-1.37	1.35	1.42
		-1.06	1.39	1.16	-1.22	1.40	-1.18	-1.60	1.19	1.31
Average FC	1.00	-0.54	1.48	1.46	0.07	1.45	0.34	-1.35	1.33	1.37

Protein expression in pg/mg of protein

VEGF-A	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
Sample 1	5.14	53.10	78.63	51.08	18.15	77.61	227.06	15.58	23.75	27.35
Sample 2	6.00	32.16	84.31	96.71	70.07	83.66	42.41	21.42	92.78	117.25
Sample 3	7.79	14.97	127.82	106.51	67.60	76.44	268.77	6.86	39.14	105.97
Sample 4		14.45	65.25	78.56	74.43	99.09	46.98	6.34	47.40	87.58
CTL Average	6.31									

Individual FC values[b]	CTL	UT-D1	UT-D3	UT-D7	PT-D1	PT-D3	PT-D7	BT-D1	BT-D3	BT-D7
		8.42	12.46	8.10	2.88	12.30	35.98	2.47	3.76	4.33
		5.10	13.36	15.33	11.10	13.26	6.72	3.39	14.70	18.58
		2.37	20.26	16.88	10.71	12.11	42.60	1.09	6.20	16.79
		2.29	10.34	12.45	11.80	15.70	7.45	1.01	7.51	13.88
Average FC	1.00	4.54	14.11	13.19	9.12	13.34	23.19	1.99	8.05	13.40

CTL, tissue collected from uninjured mice; UT, tissue from untreated mice; PT, tissue from PEG-treated mice; BT, tissue from BDWG-treated mice; FC, fold-change

[a] Cytokines and chemokines are listed in alphabetical order.

[b] Fold change values were calculated by dividing the individual value in pg/mg by the average of the control values in pg/mg; that is, the baseline for comparison of all cytokines and chemokines is 1.

[c] Values under 1.00 were converted to minus numbers relative to 1.00 for ease of comparison (above and below baseline).

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