Supplemental Material

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

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References
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).
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 dpi² 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 dpi² 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 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        |
|--------------------------------|------------------------------------------------------------|------------------------------|-----------------|
| **Animal experiments**         |                                                            |                              |                 |
| Nembutal                       | 5% sodium pentobarbital                                    | Diamondback Drugs            | Scottsdale, AZ  |
| Disposable biopsy punches      | 6-mm and 8-mm                                              | Integra Mitex, 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 μg/mL to prevent bacterial contamination of cell suspensions | ThermoFisher Scientific |
| **FACS buffer** | PBS with 1% bovine serum albumin for resuspending cells and aiding in minimizing non-specific antibody binding | Made in-house |
| **Fc Block** | Unconjugated monoclonal antibody (clone 3070) for blocking Fc receptors that can cause non-specific, 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** | 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 |

*All products were purchased from manufacturers or suppliers within the USA.*
TABLE S2 Calculation of probable macrophages from FACS analysis

| Baseline | Cell count | Total cells | Live cells | Granulocyte pool\(^{a}\) CD11b\(^{b}\) | M2 macrophages CD11b\(^{c}\)CD206\(^{c}\) | Neutrophils CD11b\(^{c}\)Ly6G\(^{c}\) | M1 macrophages CD11b\(^{c}\)CD206\(^{c}\)Ly6G\(^{c}\) |
|----------|------------|-------------|------------|-------------------------------------|-----------------------------------------|---------------------------------|---------------------------------------|
| 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                                     |
| 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                            | 7.9                                   |
| 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                                 |
| 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                                  |
| 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                                 |
| UT1      | 99         | 683100      | 99.5       | 9.57                                | 5.27                                    | 2.65                            | 1.65                                  |
| UT2      | 231        | 1438820     | 99.3       | 13.9                                | 5.79                                    | 4.35                            | 3.76                                  |
| UT3      | 179        | 1202800     | 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\(^{5}\)) 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) | 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 a | CTL | UT-D1 | UT-D3 | UT-D7 | PT-D1 | PT-D3 | PT-D7 | BT-D1 | BT-D3 | BT-D7 |
|------------------------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Sample 1               | 28.33 | 27.10 | 19.82 | 5.13  | 48.31 | 51.10 | 6.26  | 7.12  | 6.54  |
| Sample 2               | 14.36 | 34.24 | 38.63 | 35.69 | 45.88 | 10.66 | 14.44 | 43.51 | 19.79 |
| Sample 3               | 4.22  | 59.84 | 33.99 | 41.70 | 23.90 | 22.23 | 1.99  | 25.67 | 20.08 |
| Sample 4               | 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 a | CTL | UT-D1 | UT-D3 | UT-D7 | PT-D1 | PT-D3 | PT-D7 | BT-D1 | BT-D3 | BT-D7 |
|------------------------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| See note b             | 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 |
|---------------------------------------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Sample 1                               | 1.72 | -2.21 | -3.78 | 2.34  | -2.25 | -3.61 | 1.11  | -2.65 | -1.86 |
| Sample 2                               | 1.40 | -1.64 | -2.46 | 1.18  | -1.81 | -3.96 | 1.39  | -1.10 | -1.49 |
| Sample 3                               | -1.23| 1.01  | -2.48 | -1.07 | -2.43 | -2.91 | 1.99  | -2.14 | -5.14 |
| Sample 4                               | 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)

| Sample 1 | CTL | UT-D1 | UT-D3 | UT-D7 | PT-D1 | PT-D3 | PT-D7 | BT-D1 | BT-D3 | BT-D7 |
|----------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 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 |

| 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 |

#### CXCL10 (IP-10)

| Sample 1 | CTL | UT-D1 | UT-D3 | UT-D7 | PT-D1 | PT-D3 | PT-D7 | BT-D1 | BT-D3 | BT-D7 |
|----------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 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 |

| 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 |

#### GM-CSF (CSF2)

| Sample 1 | CTL | UT-D1 | UT-D3 | UT-D7 | PT-D1 | PT-D3 | PT-D7 | BT-D1 | BT-D3 | BT-D7 |
|----------|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 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 | | | | | | | | |

Protein FC values & Average values: 

**Sample 1**: CTL 51.08, Individual FC 1.00, Average FC 29.02

**Sample 2**: CTL 154.52, Individual FC 1.00, Average FC 2.16

**Sample 3**: CTL 154.52, Individual FC 1.00, Average FC 2.07

**Sample 4**: CTL 154.52, Individual FC 1.00, Average FC 2.76
### Individual FC values^b

|        | UT-D1 | UT-D3 | UT-D7 | PT-D1 | PT-D3 | PT-D7 | BT-D1 | BT-D3 | BT-D7 |
|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| **See note** |       |       |       |       |       |       |       |       |       |
|        | 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 |
|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| **See note** |       |       |       |       |       |       |       |       |       |       |
|        | 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 |
|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| **See note** |       |       |       |       |       |       |       |       |       |       |
|        | 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<sup>b</sup> | CTL | UT-D1 | UT-D3 | UT-D7 | PT-D1 | PT-D3 | PT-D7 | BT-D1 | BT-D3 | BT-D7 |
|-------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Sample 1 | 41.27 | 19.36 | 2.52 | 12.57 | 21.68 | 22.30 | 21.78 | 19.23 | 71.28 |
| Sample 2 | 34.65 | 145.93 | 6.05 | 136.18 | 12.30 | 4.71 | 48.48 | 14.77 | 12.78 |
| Sample 3 | 26.28 | 13.06 | 6.13 | 156.26 | 15.21 | 33.57 | 18.58 | 30.45 | 18.81 |
| Sample 4 | 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

| 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<sup>b</sup> | UT-D1 | UT-D3 | UT-D7 | PT-D1 | PT-D3 | PT-D7 | BT-D1 | BT-D3 | BT-D7 |
|-----------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| See note<sup>c</sup> | 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

| 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

|       | 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

|       |       |       |       |       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|       | 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 | 2.14  | 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

|       |       |       |       |       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Sample 1 | 5.14  | 53.10 | 78.63 | 51.08 | 18.15 | 77.61 | 227.06| 15.58 | 23.75 |
| Sample 2 | 6.00  | 32.16 | 84.31 | 96.71 | 70.07 | 83.66 | 42.41 | 21.42 | 92.78 |
| Sample 3 | 7.79  | 14.97 | 127.82| 106.51| 67.60 | 76.44 | 288.77| 6.86  | 39.14 |
| 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

|       |       |       |       |       |       |       |       |       |       |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|       | 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

‡Cytokines and chemokines are listed in alphabetical order.

‡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.

‡Values under 1.00 were converted to minus numbers relative to 1.00 for ease of comparison (above and below baseline).
References
1. Batista MD, Ho EL, Kuebler PJ, Milush JM, Lanier LL, Kallas EG, et al. skewed distribution of natural killer cells in psoriasis skin lesions. Exp Dermatol. 2013 Jan;22(1):74-7. https://doi.org/10.1111/exd.12060. PubMed PMID: 23278897. Epub 2013/01/03.
2. von Bubnoff D, andres E, Hentges F, Bieber T, Michel T, Zimmer J. Natural killer cells in atopic and autoimmune diseases of the skin. J Allergy Clin Immunol. 2010 Jan;125(1):60-8. https://doi.org/10.1016/j.jaci.2009.11.020. PubMed PMID: 20109737. Epub 2010/01/30.
3. Stevens WW, Kim TS, Pujanauski LM, Hao X, Braciale TJ. Detection and quantitation of eosinophils in the murine respiratory tract by flow cytometry. J Immunol Methods. 2007 Oct 31;327(1-2):63-74. https://doi.org/10.1016/j.jim.2007.07.011. PubMed PMID: 17716680. Epub 2007/08/25.