Supplementary Materials and Methods

Immunohistochemistry - Double stainings. Biopsies of perilesional murine skin were double-stained for the eosinophil marker Siglec-F and the neutrophil marker Ly-6G or for C5aR1 and Ly-6G. Briefly, 6-µm cryosections were fixed with cold acetone for 10 min at -20°C. After three washes with 0.01M PBS pH 7.2, slides were blocked with Avidin/Streptavidin blocking kit (Thermo Fischer Scientific, Dreieich, Germany) in accordance with manufacturers' instructions. Afterwards, slides were blocked with 5 % (v/v) NGS for one hour and incubated with either rat anti-mouse Siglec-F antibody (BD Biosciences, San Josa, CA, USA; clone E50-2440) or with rat anti-mouse C5aR1 antibody (Bio-Rad, Munich, Germany; clone 10/92) at 4 °C overnight. Slides were washed three times with 0.01 M PBS pH 7.2, and incubated with AlexaFluor® 594 AffiniPure Goat Anti-Rat IgG (Jackson Immunoresearch, Suffolk, UK; cat. no. 112-585-003) for one hour at RT. Slides were washed and blocked with 3 % (w/v) bovine serum albumin (BSA) in 0.01 M PBS pH 7.2 for 30 min before biotinylated rat anti-mouse Ly-6G antibody (BioLegend, Koblenz, Germany; clone 1A8) was added in 3 % (w/v) BSA for an one hour-incubation at RT. After washing, slides were incubated with Streptavidin Protein, DyLight 488 (Thermo Fischer Scientific, Dreieich, Germany) for 30 min at RT. Afterwards, slides were washed again and mounted with DAPI fluoromount G (SouthernBiotech, Birmingham, AL, USA). Images were acquired on Keyence Microscope BZ-9000E series (Keyence GmbH, Neu-Isenburg, Germany) and analyzed using BZ II Analyzer (Keyence GmBH, Neu-Isenburg, Germany). Neutrophil and eosinophil infiltration in the skin was quantified by determining the percentage of the Ly-6G$^+$ area and percentage of the Siglec-F$^+$ area in the same high-power field, respectively, averaged from three 200x-magnification fields for each mouse. To quantify the extent of C5aR1/Ly6G co-expression in the skin the Hybrid cell count

function on the BZ II Analyzer software was used. Briefly, single-extraction algorithm was used to extract double positive Ly-6G^{+} (green) C5aR1^{+} (red) area in three 200x magnified images per mouse. The mean values of the positively co-stained areas were calculated. To exclude unspecific binding, matching isotype controls, i.e., rat IgG or biotinylated rat IgG were used.

Legends - Supplementary Figures

Figure S1. C5aR1 is expressed on neutrophils in the dermal infiltrate. Skin biopsies of perilesional skin obtained on day 12 in the BP-like EBA mouse model from vehicle or Coversin (2.5 mg/kg 2x daily) treated mice were stained for C5aR1 and Ly-6G$^+$ cells. (**A**) Representative pictures of Ly-6G and C5aR1 immunofluorescence stainings in 200x magnification focused on the dermis. Arrows indicate Ly-6G$^+$ cells (left panel), C5aR1$^+$ cells (middle panel), and Ly-6G$^+$/C5aR1$^+$ double-positive cells (right panel). Scale bars represent 100 µm. (**B**) Quantification of the Ly-6G$^+$/C5aR1$^+$ double-positively stained area (µm^2) per high power 200x magnification field (HPF). Results were pooled from two independent experiments and are presented as mean ± SEM with each dot representing an individual mouse (n = 10 - 12 mice/group). Data were analyzed by Mann-Whitney U test; n.s., not significant.

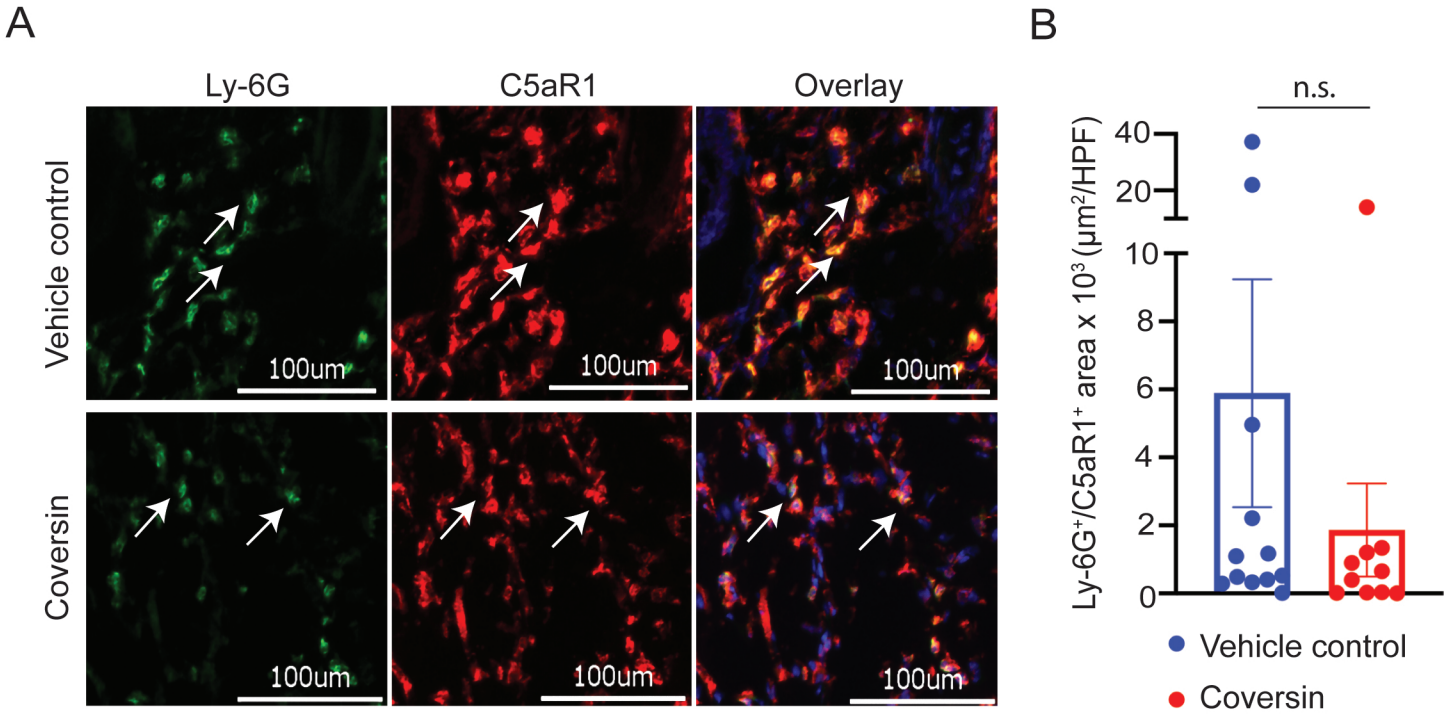
Figure S2. Effects of Coversin and PAS-L-Coversin treatment on granulocytes infiltration into the skin in the BP-like EBA model. (**A**) Representative immunofluorescence double-stainings for Ly-6G (green) and Siglec-F (red) in perilesional skin of mice harvested on day 12 of the antibody transfer BP-like EBA model. The samples stained and analyzed here are identical with those presented in figure 4 of the main manuscript. In this examination only the vehicle control, Coversin (2.5 mg/kg 2x daily), and PAS-L coversin (10 mg/kg) groups were included. Scale bars represent 100 µm. (**B**) Quantification of Ly-6G$^+$ and Siglec-F$^+$ cell infiltration determined as positive area (µm^2) per high power 200x magnification field (HPF). Results were pooled from two independent experiments and are presented as mean ± SEM with dots

representing individual mice (n = 10 - 12 mice/group). Groups were compared by Kruskal-Wallis test.

Figure S3. Effects of Coversin on granulocytes infiltration into the skin in the BP-like EBA model in the therapeutic setting. (**A**) Representative immunofluorescence double-stainings for Ly-6G (green) and Siglec-F (red) in perilesional skin of mice harvested on day 12 of the antibody transfer BP-like EBA model. The samples stained and analyzed here are identical with those presented in figure 6 of the main manuscript. In this examination only the vehicle control and the Coversin (2.5 mg/kg 2x daily) groups were included. Scale bars represent 100 µm. (**B**) Quantification of Ly-6G$^+$ and Siglec-F$^+$ cell infiltration determined as positive area (µm^2) per high power 200x magnification field. Results were pooled from two independent experiments and are presented as mean ± SEM with dots representing individual mice (n = 10 mice/group). Groups were compared by Mann-Whitney U test. *, $p < 0.05$.

Figure S4. C5aR1 and BLT1 are abundantly expressed in perilesional skin of BP patients. This supplementary figure shows the immunofluorescences pictures of the inlays of Fig. 8A/B/C of the main manuscript threefold digitally enlarged. (**A**) Expression of C5aR1 and (**B**) of BLT1 in the skin of healthy controls and in perilesional skin of BP patients. Arrows indicate C5aR1$^+$ and BLT1$^+$ cells, respectively. (**C**) Immunofluorescence stainings for CD68, MPO, and MBP in perilesional skin of BP patients and their co-expression with BLT1. Arrows indicate respective positive cells. Dashed lines indicate the dermal-epidermal junction. Scale bars represent 50 µm.

Figure S1


A
Ly-6G
C5aR1
Overlay
Vehicle control
Coversin
100um
B
n.s.
Ly-6G+/C5aR1+ area x 10³ (μm²/HPF)
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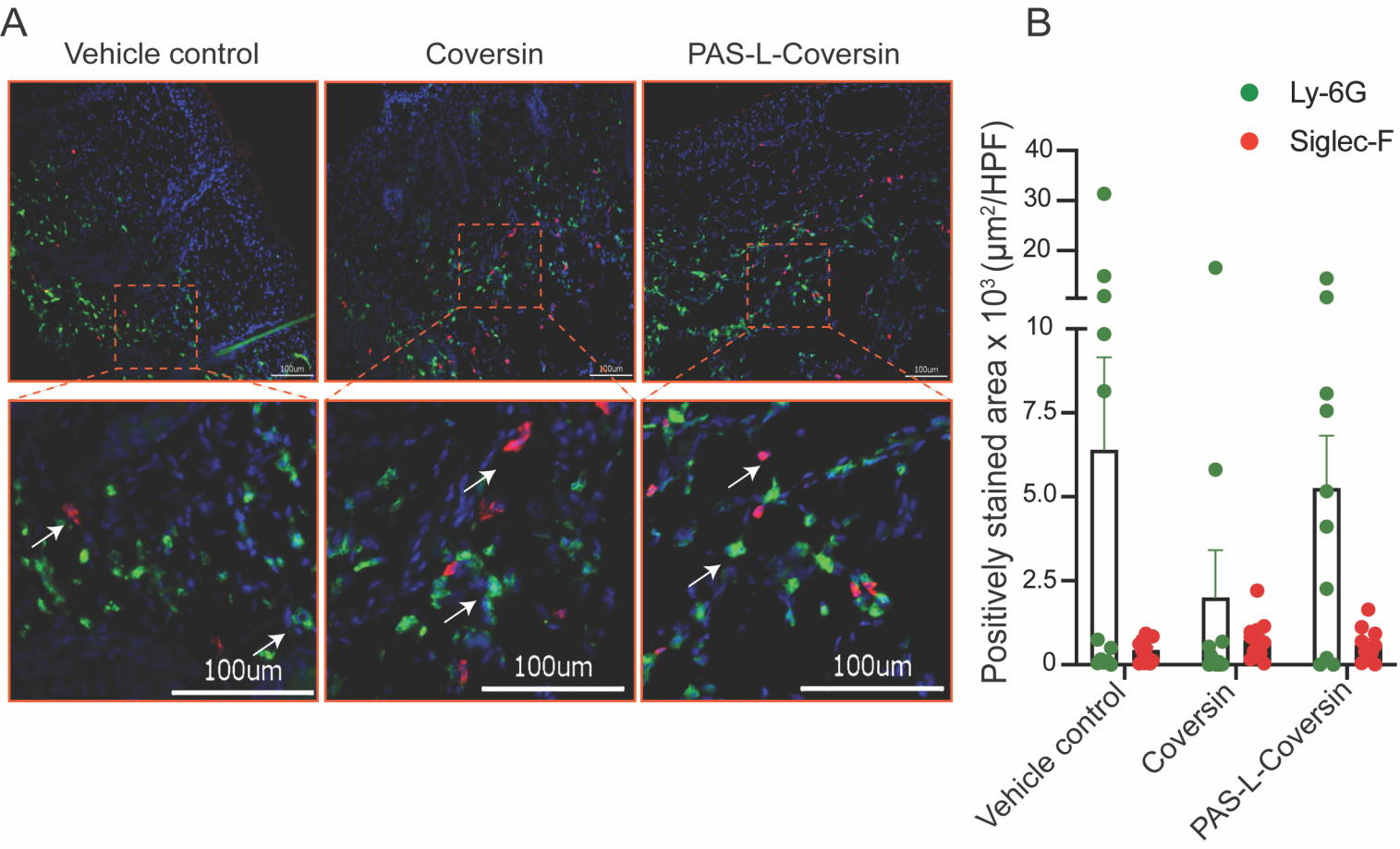
20
10
8
6
4
2
0
Vehicle control
Coversin

Figure 2S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Vehicle control
Coversin
PAS-L-Coversin
100um
100um
100um
B
Ly-6G
Siglec-F
Positively stained area x 10³ (µm²/HPF)
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30
20
10
7.5
5.0
2.5
0
Vehicle control
Coversin
PAS-L-Coversin

Figure S3

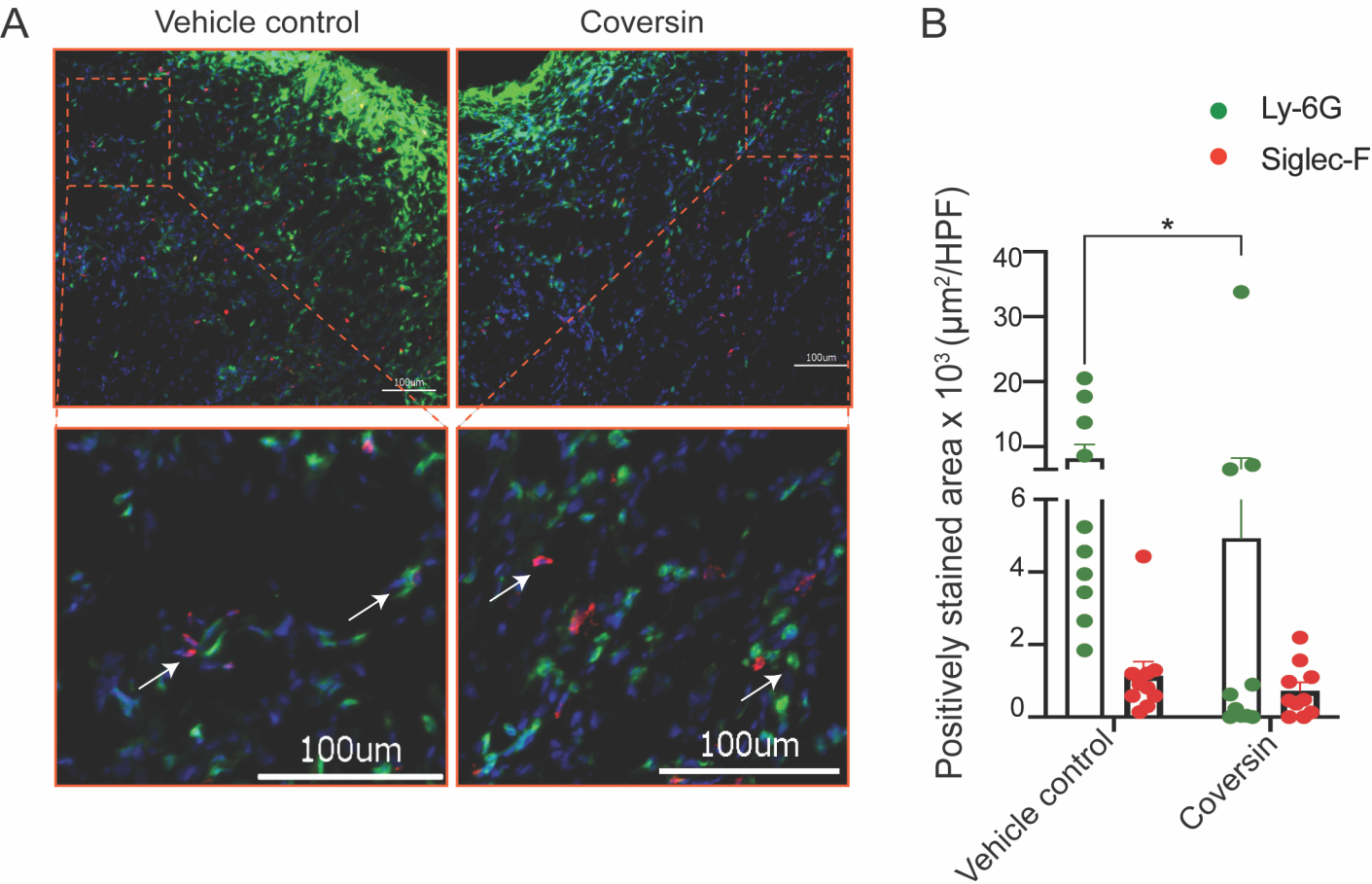
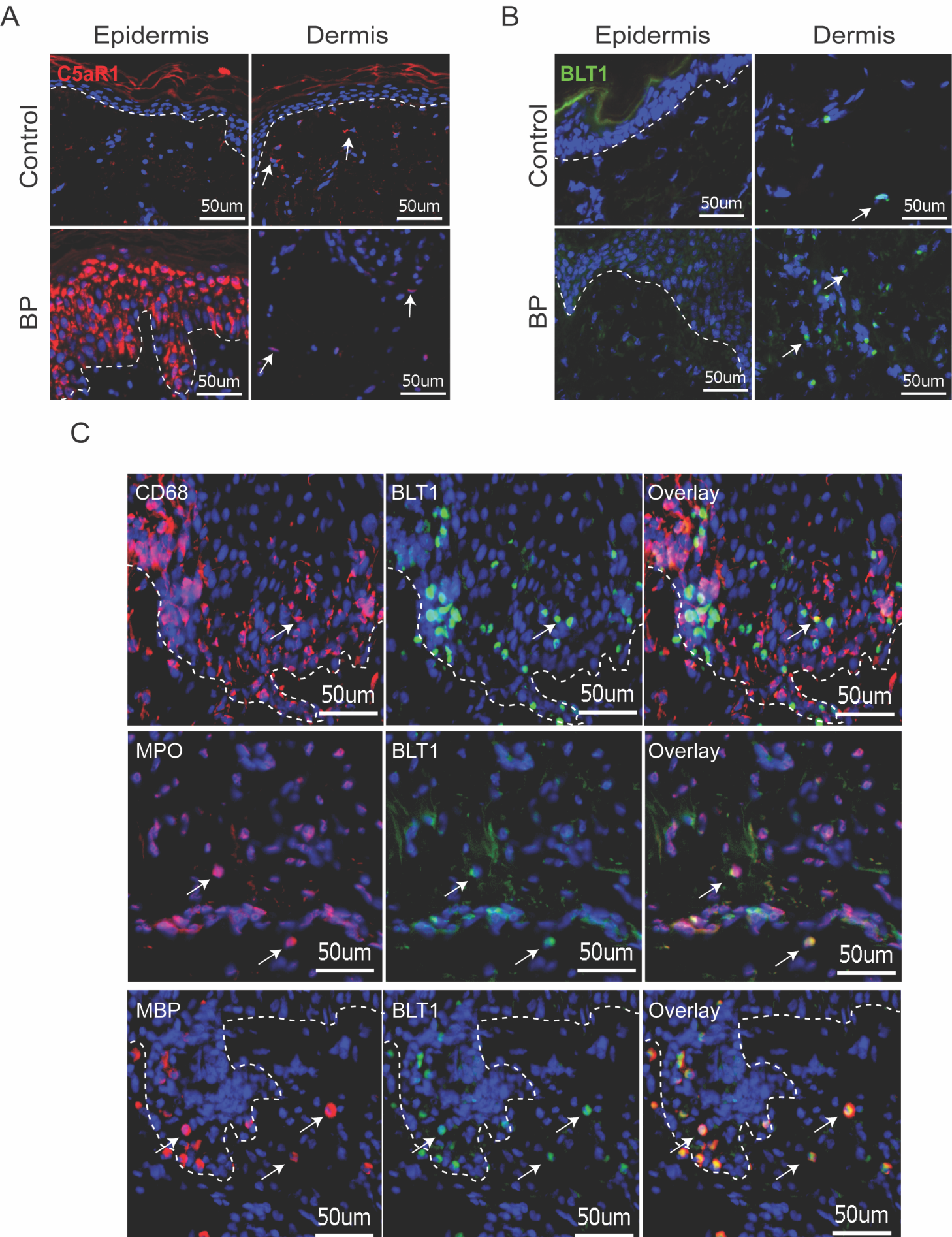

A
Vehicle control
Coversin
100um
100um
100um
100um
B
Ly-6G
Siglec-F
Positively stained area x 10³ (μm²/HPF)
40
30
20
10
6
4
2
0
*
Vehicle control
Coversin

Figure S4


A
Epidermis
Dermis
C5aR1
Control
BP
50um
B
Epidermis
Dermis
BLT1
Control
BP
50um
C
CD68
BLT1
Overlay
MPO
BLT1
Overlay
MBP
BLT1
Overlay
50um