SUPPLEMENTARY MATERIALS AND METHODS

Human Subjects

Blister fluids, blood and skin biopsies from EB-affected patients were obtained with the support from DEBRA Chile and DEBRA Mexico according to local rules and regulations. Clinical features of the patients are presented in Table S1. No patient-identifiable information was available to researchers and the study was not considered as human subject research. Foreskins for isolation of human ADSC were collected at Thomas Jefferson University Hospital under institutional review board-approved protocols. The use of discarded tissue for research purpose does not require patient informed consent.

Collection of blister fluid and skin biopsies

Blister fluid was collected from blisters that arose on different parts of the body (Table S1) as needle aspirates. Age of the blister (<24 hour or >98 hours) was reported by patients. Blood and blister fluid samples were collected at the same time. For several samples, the blister fluid was collected along with skin biopsy.

Isolation of human adipose-derived stem cells (ADSC)

Human ADSC were isolated according to the established protocols (Alexeev, 2014). The resultant $CD31^{-}CD45^{-}$ population defined as ADSC was grown in DMEM/F12-Glutamax media supplemented with 10% FBS and Penicillin/Streptomycin (Thermo/Fisher, Grand Island, NY).

Human peripheral blood mononucleated cells (PBMC)

Human PBMC were purchased from Stem Cell Technologies (Cambridge, MA). Cells were cultured in RPMI1640 media supplemented with 10% FBS, Penicillin/Streptomycin and 20 mM HEPES, pH 7.3 (Thermo/Fisher, Grand Island, NY).

PBMC gating strategy

Gating of different leukocyte populations was done based on the assessment of side scatter (SSC) versus forward scatter (FSC). Since FSC represents size and SSC complexity of the internal structure, differentiation of cell types in heterogeneous PBMC-derived population was done based on the correlative measurements of the FSC and SSC, according to standard distribution of the cell types.

Chemokine and cytokine antibody arrays

Human Chemokine Antibody Arrays C1 series and Human Cytokine Antibody Array C1000 series (Ray Biotech, Inc., Norcross, GA) were used in the study. Twenty μl of blister fluids (BF) or plasma were incubated with array membranes overnight at +4°C following detection protocol as devised by the manufacturer. Relative spot intensity was measured using ScanAlyse Software (Stanford, CA). Data analysis was done using RayBio® Human Chemokine and Cytokine Antibody Array Analysis Tools software (RayBiotech, Inc. Norcross, GA). Individual background values were subtracted and spot intensities were normalized according to positive control signals. Relative spot intensities are presented as means ± SDs.

ELISA assay

Assessment of most common human chemokines in BF was done using Multi-Analyte ELISArray kit (Qiagen, Valencia, CA) as advised by manufacturer. Quantitation of angiogenin was done using angiogenin-specific ELISA kit (Ray Biotech, Inc., Norcross GA) with angiogenin standards according to manufacturer protocol. Colorimetric reaction was measured using μQuant plate reader and analyzed using KC Junior software (Bio-Tech Instruments, Inc., Winooski, VT). To offset the non-specific antibody reaction, the OD value of control antigen was subtracted from that of each sample. Assays were conducted in triplicate, and these means were used for data analysis.

Fluorescence-activated cell sorting (FACS) analysis

FACS-based analysis was done using chemokine receptor-specific FITC-, Alexa488-, PerCP/Cy5.5- or PE-conjugated antibodies (BioLegend, San Diego, CA) (Table S2). Labeling of cells was done according to manufacturer recommendation. Briefly, cells were incubated with receptor-specific antibodies for 30 minutes at 4°C following washes in FACS buffer containing 0.5% FBS. Cells were fixed with 1% paraformaldehyde (PFA) in PBS (pH 7.4) and washed in FACS buffer. Cell surface expression of chemokine-receptors was assessed on the Guava EasyCyte system and analyzed using GuavaSoft 2.7 software (Millipore, Billerica, MA).

Histological and immunofluorescence analyses

EB skin samples were transported in Michele's Transport Media (American MasterTech Scientific, Inc., Lodi, CA). Samples were embedded in Optimal Cutting Temperature (OCT) compound (Sakura Finetek USA, Inc., Torrance, CA), frozen, and cryosectioned. Seven micron sections were fixed in 4% PFA in PBS and used for indirect immunofluorescence analysis. A list of the primary antibodies is provided in Table S2. After blocking with 1% BSA in PBS for 1 h, sections were incubated with primary antibodies overnight at +4°C. Immunocomplexes were detected with AlexaFluor^{488}- and/or AlexaFluor^{594}-labeled secondary antibodies (Thermo/Fisher, Grand Island, NY). Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) (Sigma, St. Louis, MO). Slides were covered with Fluorosafe reagent (Millipore, Billerica, MA) and analyzed by fluorescent microscopy.

***In vitro* migration in Transwell chambers**

PBMC ($3x10^6$ cells) or ADSC ($1x10^6$ cells) in 250 μl of culture media were loaded into the top chamber of the 8-μm pore size Transwell. Control or BF-containing culture media was placed into a lower chamber. At indicated time points, cells were aspirated and removed from the top chamber. Cells that migrated to the undersides of the porous membrane and to the lower chamber were collected and used for analysis.

Migration in collagenous matrix

For the assessment of ADSC migration in collagenous matrix, cultures were initiated by preparing an acellular collagenous layer (100 μl) on top of the porous membrane of the 12-well Transwell chamber by mixing 10x Eagle's MEM, L-glutamine, dialyzed FBS,

NaHCO3, and rat tail type I collagen. After polymerization, a cellular layer was created by mixing the same components with $5x10^4$ Vybrant DiO-labeled ADSC per well (200 μl). After polymerization, a separating acellular layer (600 μl) was created on top. The migration unit was completed by the formation of the acellular layer with or without BF (100 μl) (BF diluted at 1:10 ratio). When completed, migration units were placed into a Transwell plate and culture media was added to the lower chamber and on the top of the migration unit. Plates were incubated for 4 days under standard tissue culture condition. On the second and third days, BF (1:10 ratio) was added on top of the migration units. After 4 days, migration units were fixed in 10% buffered formalin for 4 h at room temperature and frozen in OCT compound. Ten μm cryosections were used for direct and indirect immunofluorescence analysis.

Plasmid DNA construction and delivery using Amaxa nucleofection

cDNA encoding human CXCR1 and CXCR2 chemokine receptors were generated from total RNA isolated from human PBMC using SurepScript III reverse transcriptase (Thermo/Fisher, Grand Island, NY), following amplification of the cDNA using PfuUltra II fusion HS DNA polymerase (Agilent technologies, Santa Clara, CA) with gene-specific primers. The resultant cDNA was ligated into pEF6-TOPO mammalian expression vector (Thermo/Fisher, Grand Island, NY) under the control of the human elongation factor 1 promoter. Integrity of the plasmids and expression of the proteins were assessed by direct DNA sequencing. Expression of CXCR1 and CXCR2 from the resultant plasmids was verified by RT-PCR and Western blot analyses. Amaxa nucleofection reaction was used for plasmid DNA delivery into ADSC. The Amaxa Nucleofector II device with Kit V was used (Lonza, Basel, Switzerland). ADSC ($1x10^6$ cells per reaction) were mixed with nucleofection solution and DNA per manufacturer protocol. The T-027 program was used

for nucleofection. After the reaction, cells were incubated in standard tissue culture conditions, analyzed for protein expression and used in experiments.

Table S1. Source of blister fluid samples collected from patients affected with different types of epidermolysis bullosa.

EB type[1]	Age (years)	Gender[2]	Location of blister	Country of Origin
RDEB				
RDEB, generalized	2	M	Neck	Mexico
RDEB, generalized	2	M	Forehead	Mexico
RDEB	2	F	Left thigh, Left ankle, Right foot	Chile
RDEB, generalized	5	F	Left palm, Left leg	Mexico
RDEB, generalized severe	6	F	Left palm, Left thigh, Right leg	Mexico
RDEB, generalized	7	M	Left palm, Right leg	Mexico
RDEB, generalized	11	F	Left palm, Right upper arm, Right leg	Mexico
RDEB, generalized severe	11	F	Left abdomen, Left thigh, Right upper arm, Left lumbar, Right thigh	Chile
RDEB, generalized	12	F	Right hand, Left palm, Forehead	Mexico
RDEB	12	F	Left palm, Left thigh, Left ankle	Chile
RDEB, generalized	14	F	Left hand, Back	Mexico
RDEB, generalized	15	F	Left palm, left thigh, Right arm	Mexico
RDEB, generalized severe	15	M	Right hand, Left palm, Right ankle, Right heel, Left leg	Chile
RDEB, generalized severe	17	M	Right back shoulder, Right forearm, Left knee	Chile
RDEB, generalized	19	F	Left hand, Right forearm, Left knee, Upper back	Mexico
RDEB, generalized	21	F	Left hand, Left leg	Mexico
RDEB, generalized	28	M	Left palm, Left leg	Mexico
RDEB, generalized	30	M	Left palm, Right leg	Mexico
RDEB, generalized severe	30	F	Left wrist, Left palm, Left elbow	Chile
RDEB, generalized	33	F	Left palm, Neck, Left leg	Mexico
RDEB, generalized	44	F	Right hand, Right leg	Mexico
RDEB, generalized	45	F	Left palm, Right arm	Mexico
RDEB, generalized	47	F	Left forearm, Left palm, Left upper arm	Mexico
EBS				
EBS	2	F	Left palm, Right leg	Mexico

EBS	4	F	Left leg, Right hand	Mexico
EBS	5	F	Left hand, Right leg	Mexico
EBS	15	M	Left palm, Right Foot	Mexico
EBS	52	F	Right inguinal, Right abdomen	Chile
JEB				
JEB, generalized intermediate	3 mo	F	Pelvis, Right lumbar waist, Right lumbar hip	Chile
JEB, generalized intermediate	4	M	Right leg	Chile
JEB, generalized severe	8	M	Left leg, Right thigh	Chile
DDEB-pruriginosa				
DDEB, pruriginosa	30	F	Arm	Mexico
DDEB, pruriginosa	34	F	Left leg	Mexico

[1]EB type included recessive dystrophic epidermolysis bullosa (RDEB), epidermolysis bullosa simplex (EBS), junctional epidermolysis bullosa (JBS), dominant dystrophic epidermolysis bullosa (DDEB).

[2]Gender: M-male; F-female.

Table S2. List of commercial antibodies used in the study.					
Antigen	**Manufacturer**	**Catalog number**	**Application[1]**	**Fluorochrome**	**Dilution**
CXCR1	BioLegend	320608	FACS	PE	1:20
CXCR2	BioLegend	320712	FACS	488	1:20
CCR2	BioLegend	357205	FACS	PE	1:20
CCR3	BioLegend	310719	FACS	FIT-C	1:20
CCR4	BioLegend	359411	FACS	PE	1:20
CCR5	BioLegend	313710	FACS	488	1:20
CD45	BD Pharmingen	555480	IF frozen	unconjugated	1:200
CXCR2	Bioss	bs-1629R	IF frozen	unconjugated	1:100
CXCR1	Abcam	ab14936	IF frozen	unconjugated	1:50
CD45RO	Dako	M0742	IF frozen	unconjugated	1:200
CD45RA	eBioscience	14-0458-82	IF frozen	unconjugated	1:200
CD11b	ThermoFisher	MA1-91659	IF frozen	unconjugated	1:100
CD16b	BD Pharmingen	555404	IF frozen	unconjugated	1:50

The commercial antibodies used in this study are listed together with their source and the method of usage.
[1]Antibodies were used for fluorescence activated cell sorting (FACS) and indirect immunofluorescence (IF) staining on frozen sections.

Figure S1.

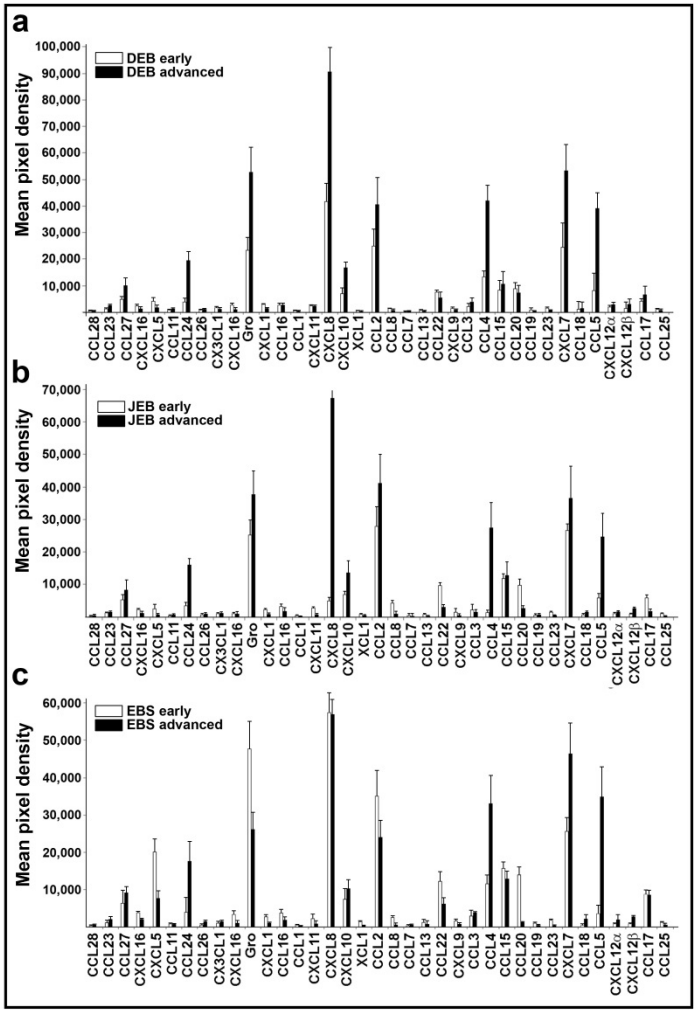

Figure S1. Array analysis of chemokines in early and advanced blisters. Chemokines were assessed by antibody array in blister fluids from early and advanced blister (as indicated). Data is presented as a mean pixel density ± SD for each EB type.

Figure S2

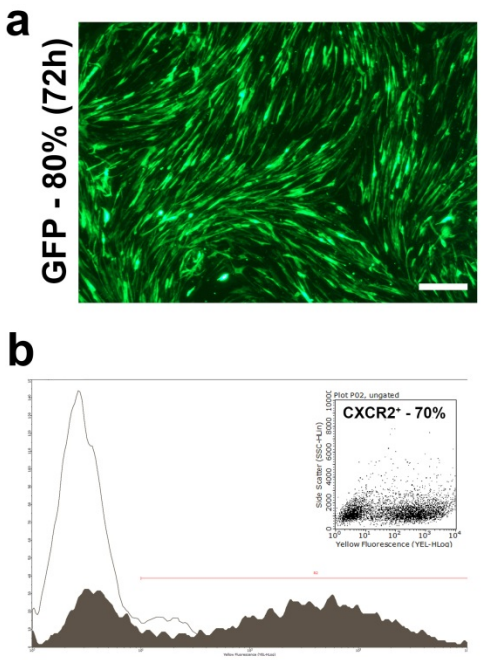

Figure S2. Amaxa nucleofection and CXCR2 expression in primary ADSC. (a) Representative micrograph depicting uniform expression of the green fluorescent protein (green) in human primary ADSC nucleofected with pMaxGFP plasmids (Lonza, Basel, Switzerland) detected as direct fluorescence 72 h after nucleofection. Scale bar - 100 μm. Percentage of GFP-positive cells is shown to the right of the image. (b) Representative profiles of control (open) and CXCR2-transduced (shaded) ADSC assessed by FACS 72 h after nucleofection of ADSC with pEF1-hCXCR2 plasmid. Percentage of $CXCR2^{+}$ cells is shown in the density plot.

Figure S3

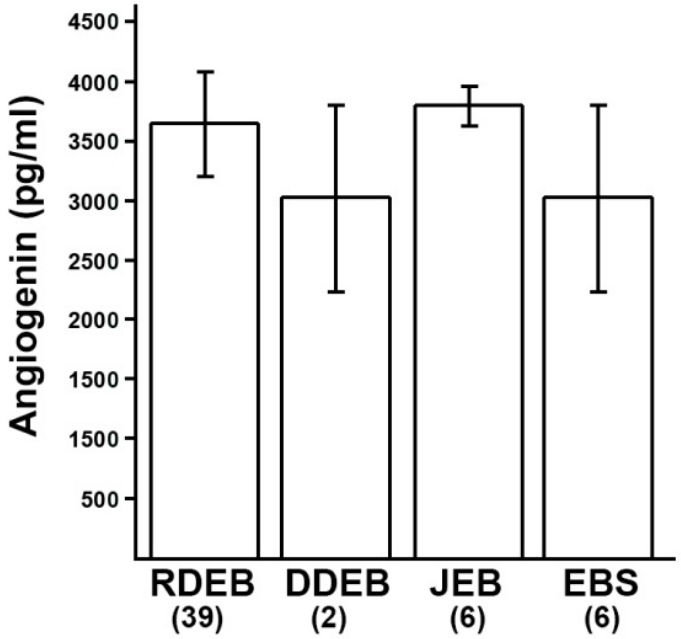

Figure S3. Quantitation of Angiogenin in blister fluids by ELISA assay. Angiogenin was quantitated by ELISA (Ray Biotech) against angiogenin standard. Blister fluids were diluted 10 times and used for the analysis. Data is presented as mean angiogenin concentration (pg/ml) ± SD. EB types and total number of samples used for the analysis indicated below the columns.

Figure S5a

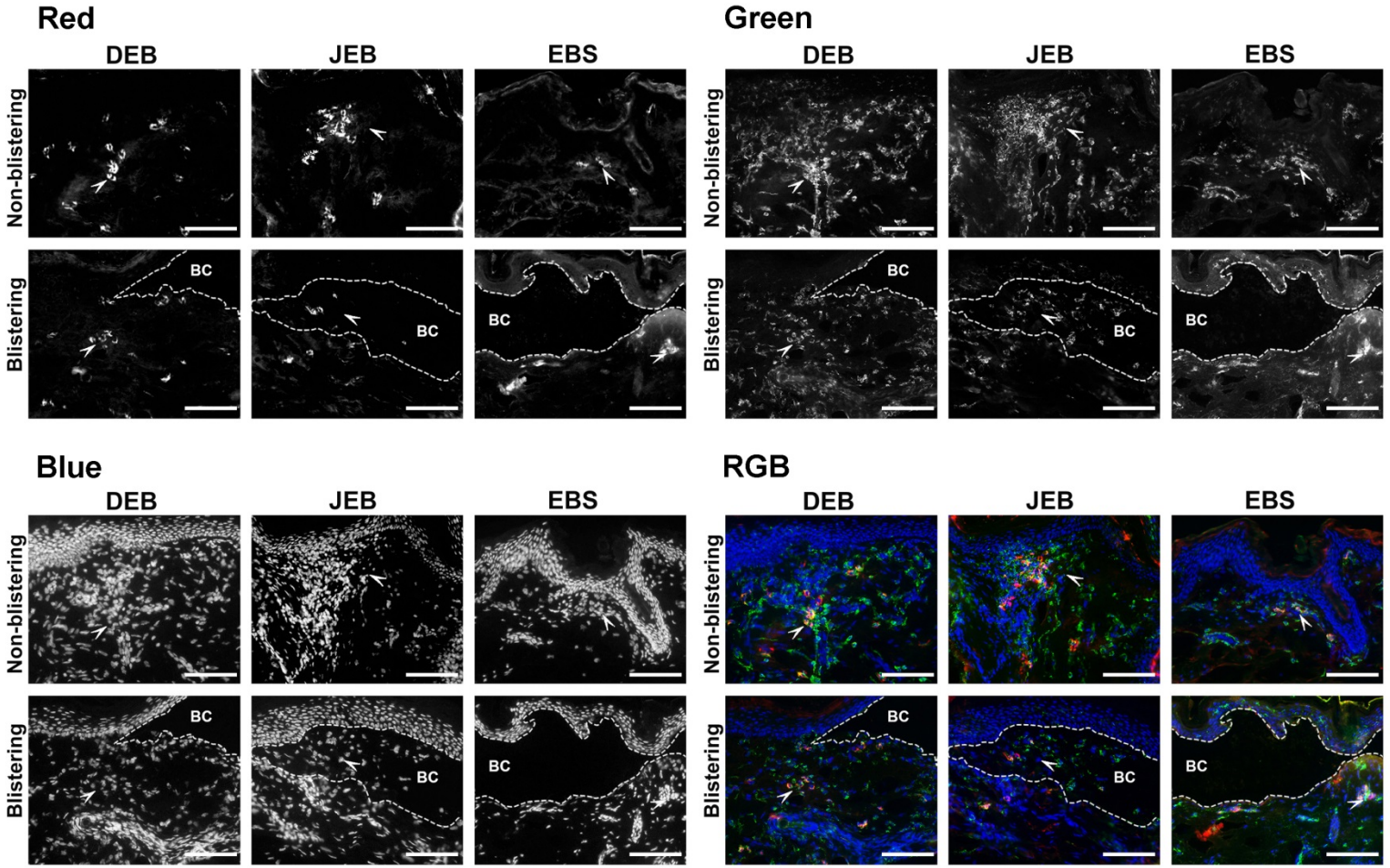

Figure S5a. Infiltration of the DEB-, JEB- and EBS-affected blistering and non-blistering skin with $CD45^+$ leukocytes. Detected antigens are shown in individual channels: CXCR2 - Red and CD45 -Green. Blue - DAPI nuclear staining. RGB images represent merged red, green and blue channels. BC - blister cavity. White arrowheads point to representative groups of $CXCR2^+$ $CD45^+$ leukocytes. Scale bar - 100 μm.

Figure S5c

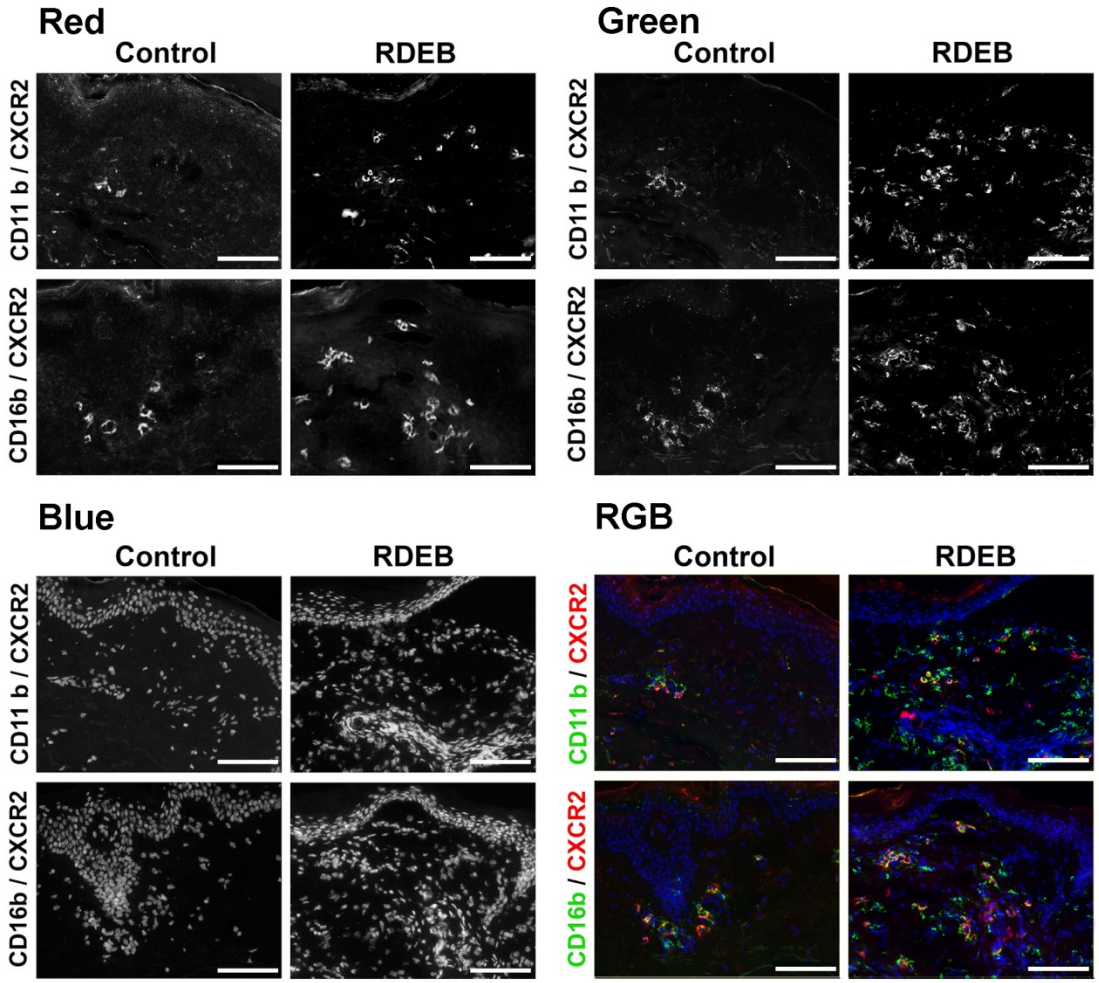

Figure S5c. Characterization of skin-infiltrating CXCR2^{+} cells in control and EB-affected skin. Detected antigens are shown in individual channels: CXCR2 - Red and CD11b or CD16b - Green. Blue - DAPI nuclear staining. RGB images represent merged red, green and blue channels. Scale bar - 100 μm.

Figure S6c

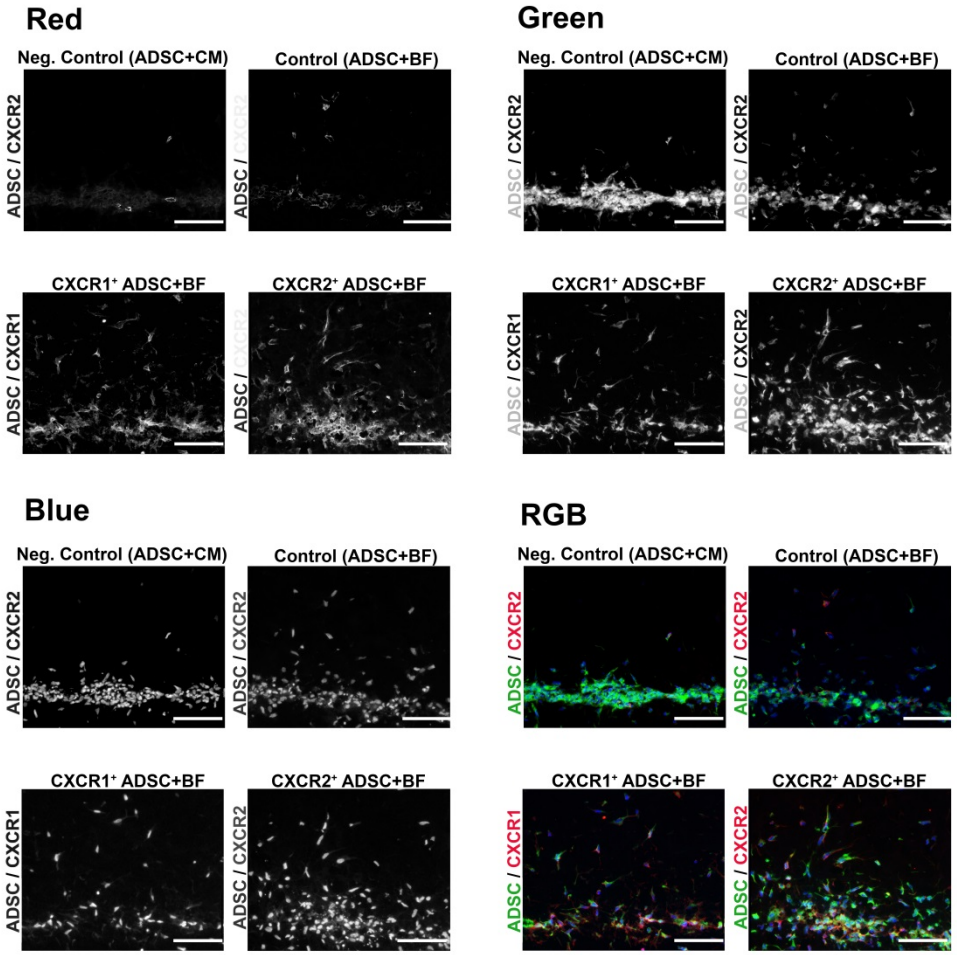

Figure S6c. Analysis of blister fluid-induced ADSC migration in collagenous matrix. Detected antigens are shown above the panels in individual channels: $CXCR1^+$ or $CXCR2^+$ - Red and vibrant DiO-labeled ADSC - Green. Blue - DAPI nuclear staining. RGB images represent merged red, green and blue channels. Scale bar - 100 μm.