Wallstabe et al.:

Inflammation-Induced Tissue Damage Mimicking GvHD in Human Skin Models as Test Platform for Immunotherapeutics

Supplementary Data

S1 Supplemental material and methods

S1.1 Stimulation of human skin cells

Keratinocytes, fibroblasts or endothelial cells were stimulated with 20 ng/mL interferon gamma (IFNγ; R&D Systems, Wiesbaden, Germany) or left unstimulated as control. After 48 h, cells were detached and used for experiments.

S1.2 Mixed lymphocyte reaction (MLR)

PBMCs served as responder cells. They were either pre-stimulated with 50 ng/mL phorbol 12-myristate 13-acetate (PMA) and 2 µg/mL ionomycin (both from Sigma-Aldrich® Chemie GmbH) for 2 h, stimulated with irradiated 3rdParty-PBMCs (i3rdP, mix of PBMCs from at least five different donors) or left unstimulated. Autologous PBMCs or allogeneic human skin cells (keratinocytes, fibroblasts, endothelial cells, either IFNγ-stimulated or unstimulated) were irradiated with a total dose of 30 Gy and used as stimulator cells. Responder and stimulator cells were resuspended in CellGro® GMP DC Medium (CellGenix GmbH, Freiburg, Germany) and adjusted to a cell number of $1x10^5$ cells per well. Cells were seeded in 96-well round-bottom microtiter plates and incubated for 7 days. Proliferation of responder cells was measured via ^{3}H-thymidine incorporation. To this end, 1 µCi ^{3}H-thymidine was added to each well for the last 18 h of incubation. ^{3}H-thymidine uptake was measured by a beta-scintillation counter. All reactions were performed in triplicates and reported as mean ±SD of counts per minute (cpm).

S1.3 Flow cytometric analysis

Cells were stained for 15 min at 4°C with the following fluorchrome-conjugated antibodies: CD3 PerCP (SK7, BioLegend Inc., San Diego, CA, USA), CD25 APC (BC96, BioLegend Inc.), CD80 APC (2D10, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), CD86 APC (2331, BD Biosciences, San José, CA, USA), HLA ABC PE (G46-2.6), HLA DR PE (G46-6; both from BD Biosciences), PD L1 PE (MIH1, eBioscience, Frankfurt am Main, Germany), IgG1κ PE and IgG1κ APC (both MOPC-21, from BD Biosciences). Data were obtained using a FACSCalibur flow cytometer (BectonDickinson, Heidelberg, Germany) or CytoFlex (Beckman Coulter Inc., Brea, CA, USA) and analyzed using FlowJo 10 software (Tree Star, Ashland, OR, USA).

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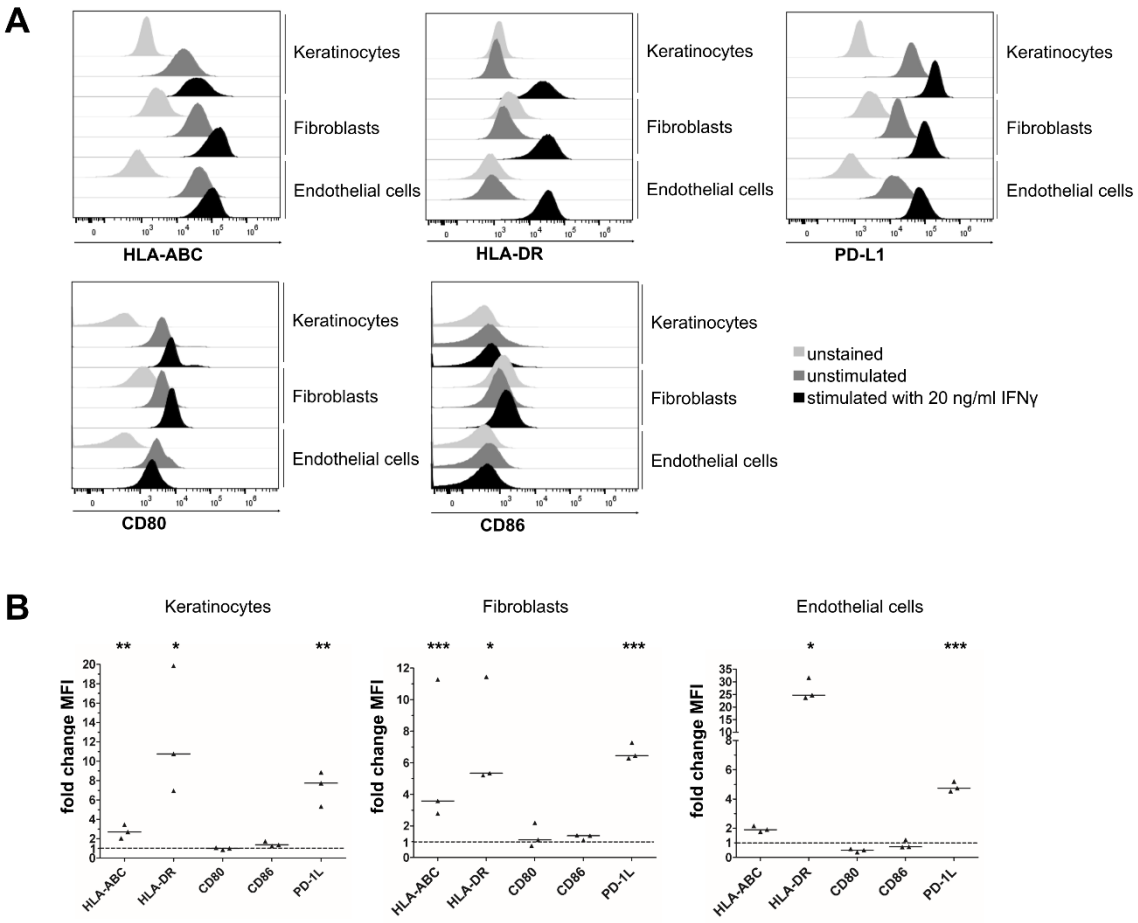


Fig. S1: Expression of surface molecules relevant for APCs on primary skin cells isolated from adult skin samples
Keratinocytes, fibroblasts or endothelial cells were stimulated with 20 ng/mL IFNγ or left unstimulated as a control for 48 h. 24 and 72 h of incubation were also tested initially; no significant differences were observed (data not shown). (A) Histograms show representative examples of flow cytometric analysis of surface expression of HLA ABC, HLA DR, CD80, CD86 and PD-1L on human primary skin cells stimulated with or without IFNγ. (B) Scatter plots summarize the results of three different donors and the median of the three experiments. Relative fold change was determined as ratio comparing IFNγ-stimulated cells to unstimulated controls. No change of surface expression is depicted as dashed line (fold change level of 1.0) (*n* = *3*). MFI, mean fluorescent intensity.

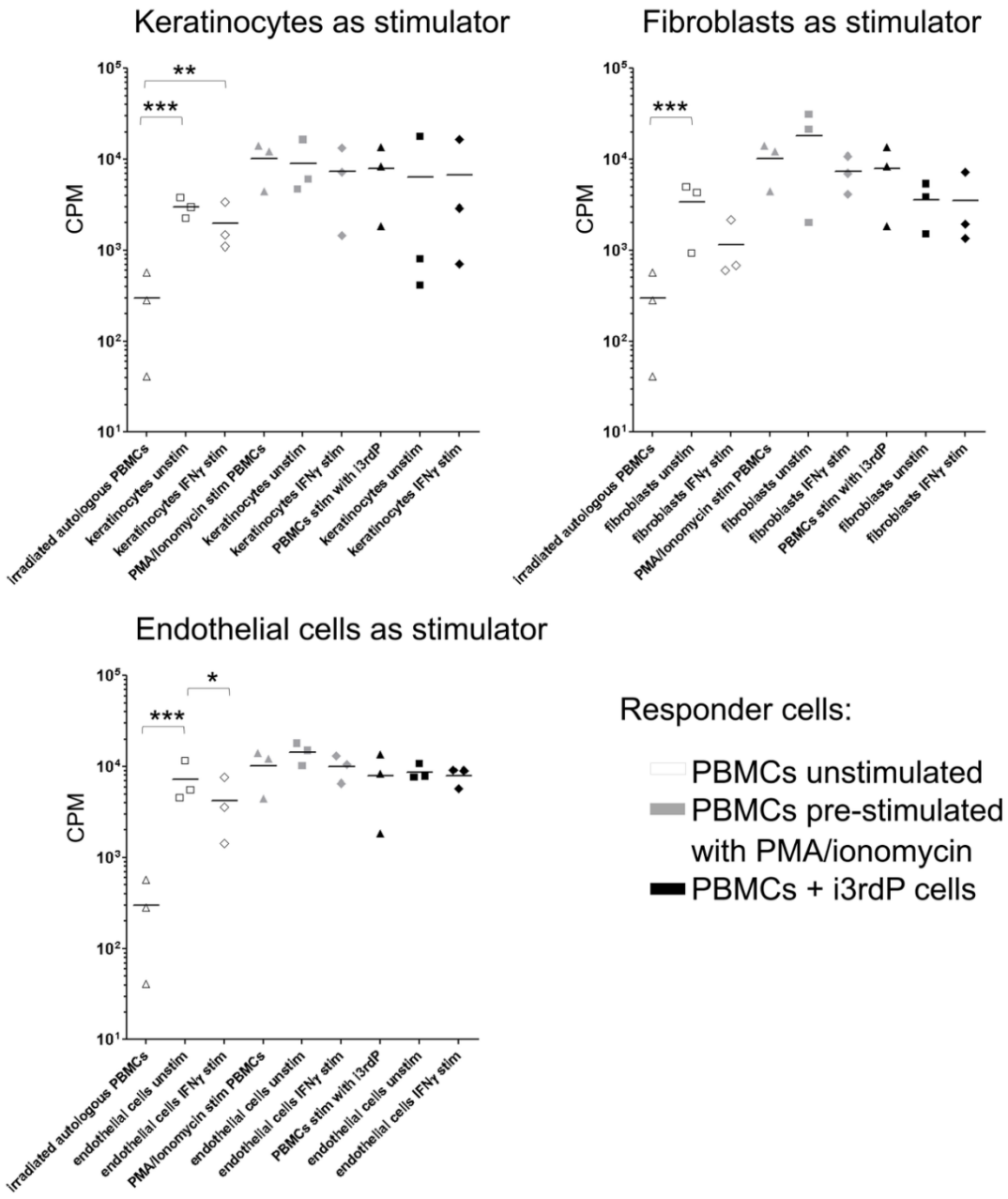


Fig. S2: Functional analysis of primary skin cells isolated from adult skin samples in MLRs
Keratinocytes, fibroblasts or endothelial cells were stimulated with 20 ng/mL IFNγ or left unstimulated as control for 48 h. The proliferation rate of PBMCs in an MLR was measured after 7 days using the ^{3}H-thymidine incorporation assay. PBMCs as responder cells were left unstimulated (white symbols), pre-stimulated with PMA/ionomycin (grey symbols) or mixed with i3rdP cells (black symbols). The proliferative response to irradiated allogeneic human primary skin cells as stimulator cells (unstimulated or stimulated with IFNγ) was tested for each of these settings ($n = 3$). * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0001$; cpm, counts per minute

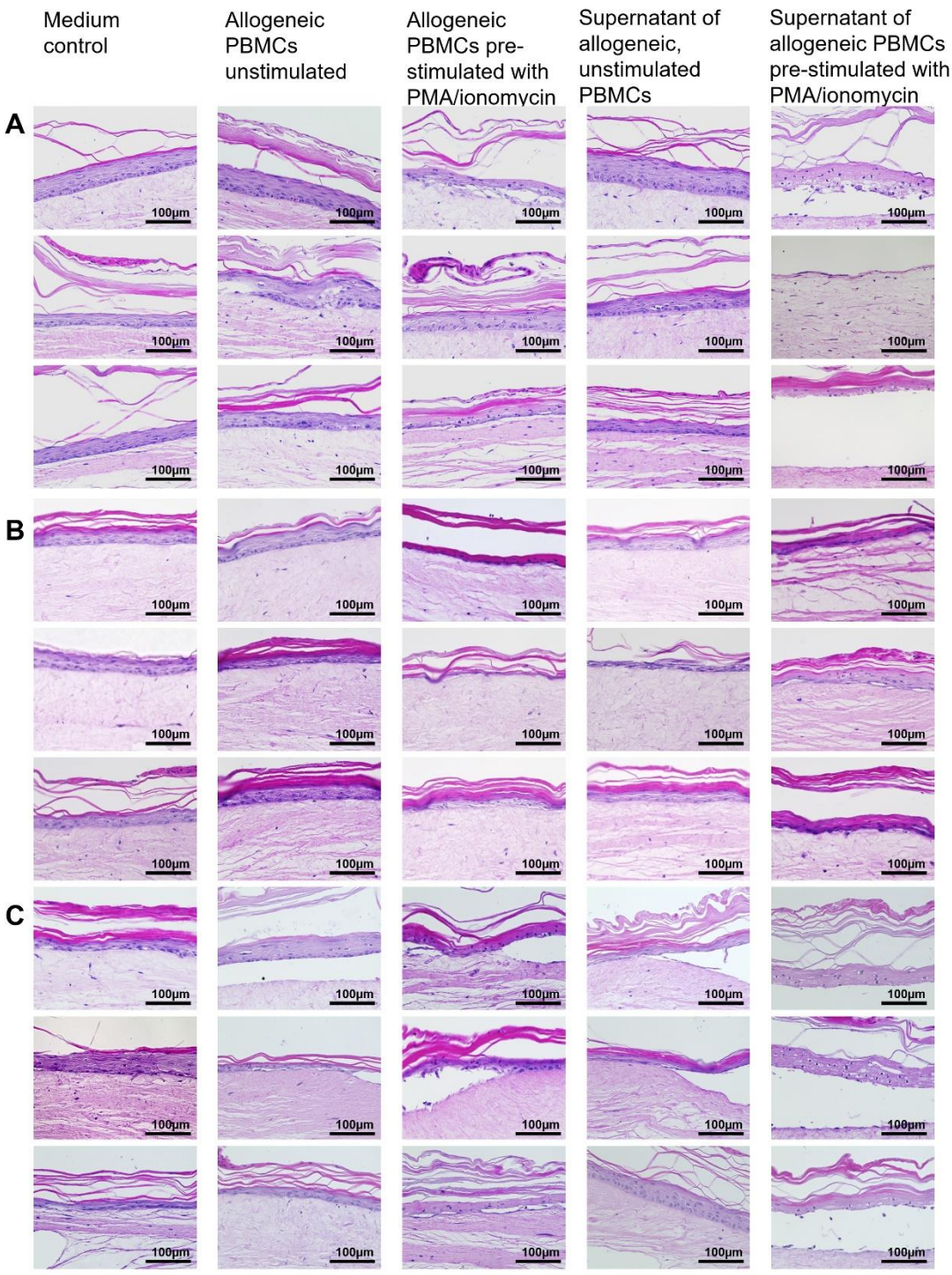
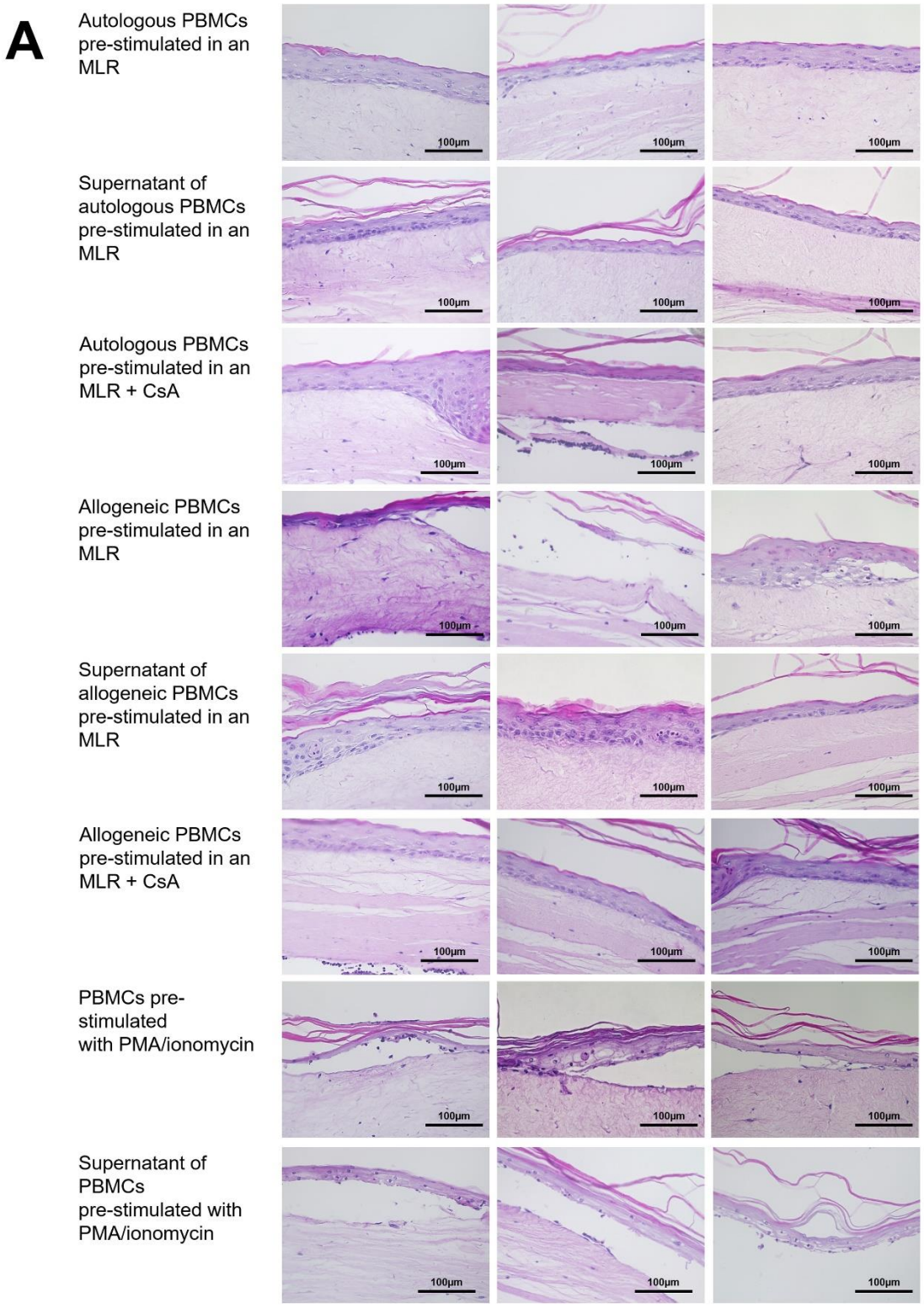


Fig. S3: Inflammation-induced damage in collagen skin models (juvenile foreskin skin cells, three donors)
Collagen skin models were generated using primary keratinocytes and fibroblasts isolated from human juvenile foreskins. For each experiment, every skin model was built from only one individual tissue donor. Allogeneic PBMCs were used from another donor. Culture medium, allogeneic unstimulated PBMCs or allogeneic pre-stimulated PBMCs (unspecific stimulation with PMA/ionomycin) were injected into the matrix of collagen skin models and co-cultured for 48 h. Three experiments were performed in triplicates. (A-C) HE-stained histological cross sections of collagen skin models are presented. Representative sections from skin models derived from three skin donors are shown (donor A, donor B, donor C). Scale bars represent 100 µm.


A
Autologous PBMCs pre-stimulated in an MLR
Supernatant of autologous PBMCs pre-stimulated in an MLR
Autologous PBMCs pre-stimulated in an MLR + CsA
Allogeneic PBMCs pre-stimulated in an MLR
Supernatant of allogeneic PBMCs pre-stimulated in an MLR
Allogeneic PBMCs pre-stimulated in an MLR + CsA
PBMCs pre-stimulated with PMA/ionomycin
Supernatant of PBMCs pre-stimulated with PMA/ionomycin
100µm

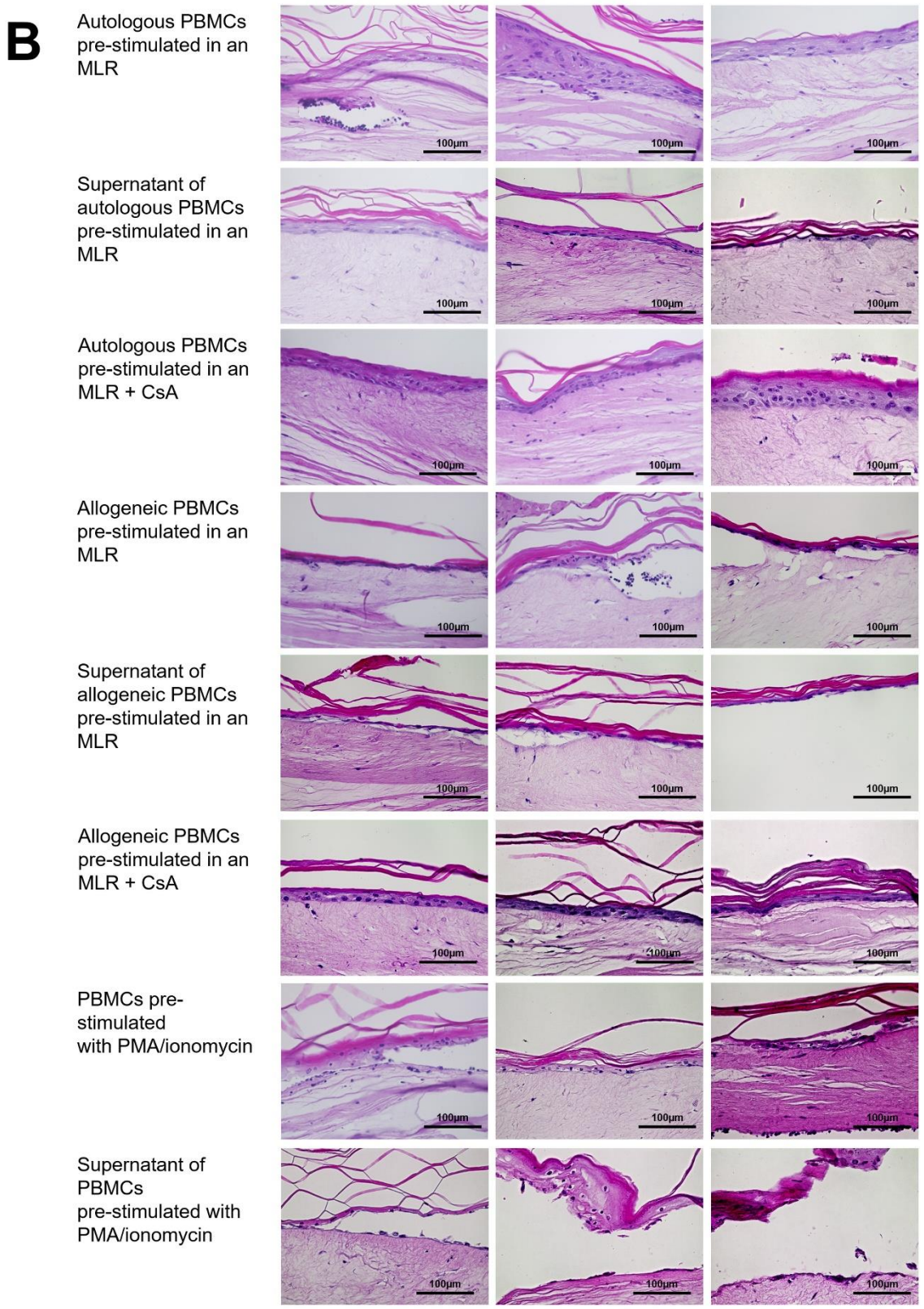

B
Autologous PBMCs pre-stimulated in an MLR
Supernatant of autologous PBMCs pre-stimulated in an MLR
Autologous PBMCs pre-stimulated in an MLR + CsA
Allogeneic PBMCs pre-stimulated in an MLR
Supernatant of allogeneic PBMCs pre-stimulated in an MLR
Allogeneic PBMCs pre-stimulated in an MLR + CsA
PBMCs pre-stimulated with PMA/ionomycin
Supernatant of PBMCs pre-stimulated with PMA/ionomycin
100µm

Fig. S4: Inflammation-induced damage in collagen skin models (adult skin cells from punch biopsies, two donors)
Collagen skin models were built-up from adult skin cells isolated from 4 mm punch biopsies. For each experiment, every skin model was built from only one individual tissue donor. PBMCs from the same donor (autologous) or another donor (allogeneic) were used. Culture medium (data not shown), autologous, allogeneic (specific stimulation) or PMA/ionomycin pre-stimulated PBMCs (unspecific stimulation) were injected into the matrix of collagen skin models. Additionally, medium supernatants were collected from unstimulated or pre-stimulated PBMCs, injected into collagen skin models and used as culture medium. Furthermore autologous and allogeneic PBMCs pre-stimulated in an MLR cultured with 200 ng/ml CsA were injected into the matrix. Collagen skin models were pre-treated with CsA one day before injection. Each condition was co-cultured for 48 hours. Two experiments were performed in triplicates (n = 2). (A-B) HE-stained histological cross sections of collagen skin models are presented. Representative sections from skin models derived from two skin donors are shown (donor A, donor B). Scale bars indicate 100 µm.