SUPPLEMENTAL MATERIAL

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Figure S1. **Decision workflow for artifacts removal.** (A) Red channel in 2D. (B) Green channel in 2D. (C) Red channel in 3D. (D) Distribution of red signal versus green-to-red signal motivating the choice of the threshold value 0.25 for the green-to-red signal to distinguish between ordinary MC (<0.25) and GFP⁺RFP⁺ MCs or artifacts (>0.25). Selection of other thresholds was done analogously based on distributions of corresponding parameters in all objects. Related to Fig. 1.
Figure S2. **Dynamic analysis of DC-to-MC interactions over time.** (A) Computing of overlap change between MCs and DCs in time-lapse series. (B) Computing characteristics of contacts between MCs and DCs. Related to Fig. 5.
Figure S3. Analysis of CD45.2 and H-2b MHCII expression by SJL/J\textsuperscript{B6} BM chimera DCs. (A) The chimerism of SJL/J\textsuperscript{B6} mice was determined and quantified according to the expression of CD45.1 and CD45.2 on blood leukocytes 2 wk after BM transplantation. (B–D) CD45.1, CD45.2, and MHCII haplotype H2\textsuperscript{b} expression in ear skin CD11c\textsuperscript{+}DCs of untreated SJL/J mice (B) and C57BL/6 mice (C) and in ear skin of SJL/J\textsuperscript{B6} BM chimera mice (D) 24 h after DNP8 administration was analyzed using flow cytometry. Related to Fig. 8.
Figure S4. **Sorting of skin DCs and MCs from SJL/J86 BM mice.** (A) Donor BM replaced ear skin DCs (CD11c+, MHCII H2b+, CD45.2+) were sorted from SJL/J86 BM chimera 24 h after DNFB. The sorting control proved 100% expression of CD11c and H2b, thereby excluding contamination with other cell subsets. Control DCs were sorted from SJL/J (H2s) mice 24 h after DNFB as cell subset expressing CD11c and CD45.1, but no expression of MHCII H2b and CD45.2 (not depicted). The sorting control proved 100% expression of CD11c and CD45.1, thereby excluding contamination with other cell subsets. Related to Fig. 9.

**Video 1.** **DC migrational arrest upon DNFB administration.** (A–D) Longitudinal and side-matched time-lapse series of DCs/Ms mouse ear skin after DNFB (A), and 1 h (B), 6 h (C), and 7.5 h (D) after DNFB administration. MCs are represented in red and DCs in green. Blood vessels are depicted by a vascular tracer (blue). Skin inflammation is associated with a pronounced increase in plasma (and vascular dye) leakage (B–D). DC/MC-independent yellow structures correspond to autofluorescence (including hairs, corneocytes, melanocytes, and structures in blood flow). Time-lapse series is depicted as maximum intensity projection; time of observation, 29 min, each. Bar, 30 µm. Refers to Fig. 2.

**Video 2.** **Time-lapse series in DCs/Ms mouse ear skin before DNFB treatment.** MCs are represented in orange, DCs in green; areas of DC-to-MC colocalization appear as yellow contact zones; DC/MC-independent yellow structures correspond to autofluorescence (including hairs, corneocytes, melanocytes, and structures in blood flow). Time-lapse series is depicted as a 3D-rendered z-stack; time of observation, 30 min. Bar, 20 µm. Refers to Fig. 3 B.
Video 3. **Time-lapse series in DC<sup> GFP</sup>/MC<sup> GFP</sup> mouse ear skin 12 h after DNFB treatment.** MCs are represented in orange, DCs in green; areas of DC-to-MC colocalization appear as yellow contact zones; DC/MC-independent yellow structures correspond to autofluorescence (including hairs, corneocytes, melanocytes, and structures in blood flow). Time-lapse series is depicted as a 3D-rendered z-stack; time of observation, 60 min. Bar, 20 µm. Refers to Fig. 3 B.

Video 4. **Time-lapse series in DC<sup>GFP</sup>/MC<sup>GFP</sup> mouse ear skin 24 h after DNFB treatment.** MCs are represented in orange, DCs in green; areas of DC-to-MC colocalization appear as yellow contact zones; DC/MC-independent yellow structures correspond to autofluorescence (including hairs, corneocytes, melanocytes, and structures in blood flow). Time-lapse series is depicted as a 3D-rendered z-stack; time of observation, 60 min. Bar, 20 µm. Refers to Fig. 3 B.

Video 5. **DC/MC single-pair time-lapse series in DC<sup>GFP</sup>/MC<sup>GFP</sup> mouse ear skin before DNFB treatment.** MC is represented in orange, DC in green; areas of DC-to-MC colocalization appear as yellow contact zones; DC/MC-independent yellow structures correspond to autofluorescence (including hairs, corneocytes, melanocytes, and structures in blood flow). Time-lapse series is depicted as a 3D-rendered z-stack; time of observation, 30 min. Bar, 5 µm. Refers to Fig. 4 A.

Video 6. **Close-up time-lapse series in DC<sup>GFP</sup>/MC<sup>GFP</sup> mouse ear skin before DNFB treatment.** MCs are represented in orange, DCs in green; areas of DC-to-MC colocalization appear as yellow contact zones; DC/MC-independent yellow structures correspond to autofluorescence (including hairs, corneocytes, melanocytes, and structures in blood flow). Time-lapse series is depicted as a 3D-rendered z-stack; time of observation, 30 min. Bar, 10 µm. Refers to Fig. 4 A.

Video 7. **DC/MC single-pair time-lapse series in DC<sup>GFP</sup>/MC<sup>GFP</sup> mouse ear skin 8 h after DNFB treatment.** MC is represented in orange, DC in green; areas of DC-to-MC colocalization appear as yellow contact zones; DC/MC-independent yellow structures correspond to autofluorescence (including hairs, corneocytes, melanocytes, and structures in blood flow). Time-lapse series is depicted as a 3D rendered z-stack; time of observation, 30 min. Bar, 5 µm. Refers to Fig. 4 B.

Video 8. **Close-up time-lapse series of DC<sup>GFP</sup>/MC<sup>GFP</sup> mouse ear skin 8 h after DNFB treatment.** MCs are represented in orange, DCs in green; areas of DC-to-MC colocalization appear as yellow contact zones; DC/MC-independent yellow structures correspond to autofluorescence (including hairs, corneocytes, melanocytes, and structures in blood flow). Time-lapse series is depicted as a 3D-rendered z-stack; time of observation, 34 min. Bar, 10 µm. Refers to Fig. 4 B.

Video 9. **DC/MC single-pair time-lapse series in DC<sup>GFP</sup>/MC<sup>GFP</sup> mouse ear skin 14 h after DNFB treatment.** MC is represented in orange, DC in green; areas of DC-to-MC colocalization appear as yellow contact zones; DC/MC-independent yellow structures correspond to autofluorescence (including hairs, corneocytes, melanocytes, and structures in blood flow). Time-lapse series is depicted as a 3D-rendered z-stack; time of observation, 60 min. Bar, 5 µm. Refers to Fig. 4 C.
Video 10. Close-up time-lapse series of DC<sup>GFP</sup>/MC<sup>RFP</sup> mouse ear skin 14 h after DNFB treatment. MCs are represented in orange, DCs in green; areas of DC-to-MC colocalization appear as yellow contact zones; DC/MC-independent yellow structures correspond to autofluorescence (including hairs, corneocytes, melanocytes, and structures in blood flow). Time-lapse series is depicted as a 3D-rendered z-stack; time of observation, 60 min. Bar, 8 µm. Refers to Fig. 4 C.

Video 11. DC/MC single-pair time-lapse series in DC<sup>GFP</sup>/MC<sup>RFP</sup> mouse ear skin 24 h after DNFB treatment. MC is represented in orange, DC in green; areas of DC-to-MC colocalization appear as yellow contact zones; DC/MC-independent yellow structures correspond to autofluorescence (including hairs, corneocytes, melanocytes, and structures in blood flow). Time-lapse series is depicted as a 3D-rendered z-stack; time of observation, 60 min. Bar, 7 µm. Refers to Fig. 4 D.

Video 12. Close-up time-lapse series of DC<sup>GFP</sup>/MC<sup>RFP</sup> mouse ear skin 24 h after DNFB treatment. MCs are represented in orange, DCs in green; areas of DC-to-MC colocalization appear as yellow contact zones; DC/MC-independent yellow structures correspond to autofluorescence (including hairs, corneocytes, melanocytes, and structures in blood flow). Time-lapse series is depicted as a 3D-rendered z-stack; time of observation, 30 min. Bar, 10 µm. Refers to Fig. 4 D.

Video 13. Single z-plane time-lapse series of a GFP<sup>+</sup>RFP<sup>+</sup>MC in contact with DCs in DC<sup>GFP</sup>/MC<sup>RFP</sup> mouse ear skin 24 h after DNFB treatment, showing the intercellular transfer of GFP<sup>+</sup> vesicles. MC is represented in red, DCs in green. Time of observation, 22 min. Bar, 5 µm. Refers to Fig. 5 D.