Murine Langerin+ dermal dendritic cells prime CD8+ T cells while Langerhans cells induce cross-tolerance.

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Figure S1
Figure S1: Langerin targeting combined with imiquimod adjuvant fails to induce cytotoxic responses.

(A) In vivo killing of OVA-loaded target cells. C57BL/6 mice were immunized i.d. into both ears with 0.5µg ISO/OVA isotype control, Langerin/OVA or DEC/OVA in addition to topical imiquimod application (+imiq). Seven days later, CFSE-labeled OVA-loaded target cells and CTO-labeled unloaded control cells were transferred i.v. Specific lysis of OVA-loaded target cells was measured in the blood one day later, and in both blood and ear-draining lymph nodes after two days. Data of individually analysed mice are pooled from two independent experiments (ISO/OVA+imiq: 6 mice; Langerin/OVA+imiq: 4 mice; DEC/OVA+imiq: 6 mice) and compared using one-way ANOVA (p<0.0001) followed by Tukey’s test (n.s.: non significant, p>0.05). (B-C) Tumor protection assay. C57BL/6 mice were immunized i.d. into both ears with PBS or 0.5µg ISO/OVA isotype control, Langerin/OVA or DEC/OVA in addition to topical imiquimod application (+imiq). One month after immunization, $10^5$ B16 melanoma cells expressing OVA were implanted subcutaneously. Tumor growth (B) and survival of the recipient mice (C) were monitored three times a week. Data are pooled from two independent experiments (PBS+imiq: 9 mice; ISO/OVA+imiq: 10 mice; Langerin/OVA+imiq: 8 mice; DEC/OVA+imiq: 9 mice). Tumor growth was compared using Student’s unpaired t-test (day 16, p=0.0030; day 18, p=0.0026). Survival curves were compared using a Mantel-Cox test.
Figure S2
Figure S2: Distribution of targeting mAb is not changed by the addition of adjuvants

0.5µg of PE-coupled full-length anti-Langerin L31 antibody or rat IgG2a isotype control were injected intradermally into each ear of C57BL/6 mice, left untreated (n.t.) or with adjuvants imiquimod (imiq) or poly(I:C)+anti-CD40 (pIC/40). Two days later, mice were sacrificed and auricular lymph nodes collected, digested and analysed by flow cytometry. Left column: Gating strategy for total CD11c+ (Blue line) or targeted CD11c+ (Red line). Targeted cells appear as red dots in the following columns. Percentages show the proportion of targeted cells in a given quadrant or gate. Results are representative of two independent experiments.
Figure S3
Figure S3: Differential expression of immunoregulatory receptors and IL-10 by DCs

Both ears of C57BL/6 mice were treated with imiquimod (imiq), poly(I:C) and anti-CD40 (pIC/40), or left untreated (nt). Two or four days later, a cell suspension was obtained from four auricular lymph nodes of two similarly treated mice and analysed by flow cytometry. Measurements, each comprising cells from two mice, are pooled from two independent experiments (A) Representative surface stainings and (B) percentages of cells expressing CD273/PD-L1, CD274/PD-L2, CD275/ICOSL in CD11c+ Langerin+ CD103\textsuperscript{neg} LCs and CD11c+ Langerin+ CD103+ dDCs. None of the differences reached statistical significance (nt: n=7; imiq - day 2: n=3; imiq - day 4: n=4; pIC/40 - day 2: n=3; pIC/40 - day 4: n=4; One-way ANOVA: LCs, p=0.9809; Langerin+ dDCs, p=0.9802). (C) Surface staining for OX40-ligand and intracellular expression of IL-10 in CD11c+ Langerin-/+ DCs after a 3h incubation with Brefeldin A (nt: n=4; imiq - day 2: n=2; imiq - day 4: n=2; pIC/40 - day 2: n=2; pIC/40 - day 4: n=2).
Figure S4: Selective Langerin expression in DC subsets following irradiation and bone marrow transfer.

C57BL/6 or Langerin−/− mice were lethally irradiated with a total dose of 10 Gy. Immediately after irradiation, 5x10^6 bone marrow cells from Langerin−/− or C57BL/6 were transferred intravenously, respectively. 8-12 weeks later, ear skin and skin-draining lymph nodes were obtained from chimeras. (A) Principle of irradiation/reconstitution experiments and their consequences on skin dendritic cells. (B) Epidermal sheets were stained with anti-Langerin Ab, followed with chicken anti-rat Ig/Alexa 594, and counterstained with anti-MHCII/FITC. Scale bars represent 100µm. (C) Langerin expression by CD45+ CD11c+ cells from digested epidermis. (D) CD11c+ Langerin+ lymph node DCs were stained for CD103 and EpCAM. Data are representative of eight reconstitution experiments.
Figure S5
**Figure S5: Langerin/OVA plus poly(I:C) and anti-CD40 yields tolerance only in the absence of dermal DC targeting.**

Both ears were immunized with Langerin/OVA or isotype control ISO/OVA and treated with poly(I:C) and anti-CD40 (pIC/40). One week later, mice were injected i.p. with anti-DEC-205/OVA and poly(I:C) (DEC/OVA+pIC). Two weeks after the primary immunisation, CFSE-labeled OVA-loaded target cells and CTO-labeled unloaded control cells were transferred i.v. Specific lysis of OVA-loaded target cells was measured in the blood one day later. Data are pooled from two independent experiments and compared using Student’s unpaired t-tests. **(A)** C57BL/6 mice (ISO+pIC/40→aDEC+pIC: 3 mice; L31+pIC/40→aDEC+pIC: 3 mice); **(B)** Chimeric mice: data from individually analysed mice are pooled from three independent experiments and compared using Student’s unpaired t-test (wt→wt: [ISO+pIC/40→aDEC+pIC]: 7 mice, [L31+pIC/40→aDEC+pIC]: 8 mice; LKO→wt: [ISO+pIC/40→aDEC+pIC]: 8 mice, [L31+pIC/40→aDEC+pIC]: 8 mice; wt→LKO [ISO+pIC/40→aDEC+pIC]: 5 mice; [L31+pIC/40→aDEC+pIC]: 5 mice).