SUPPLEMENTAL MATERIAL

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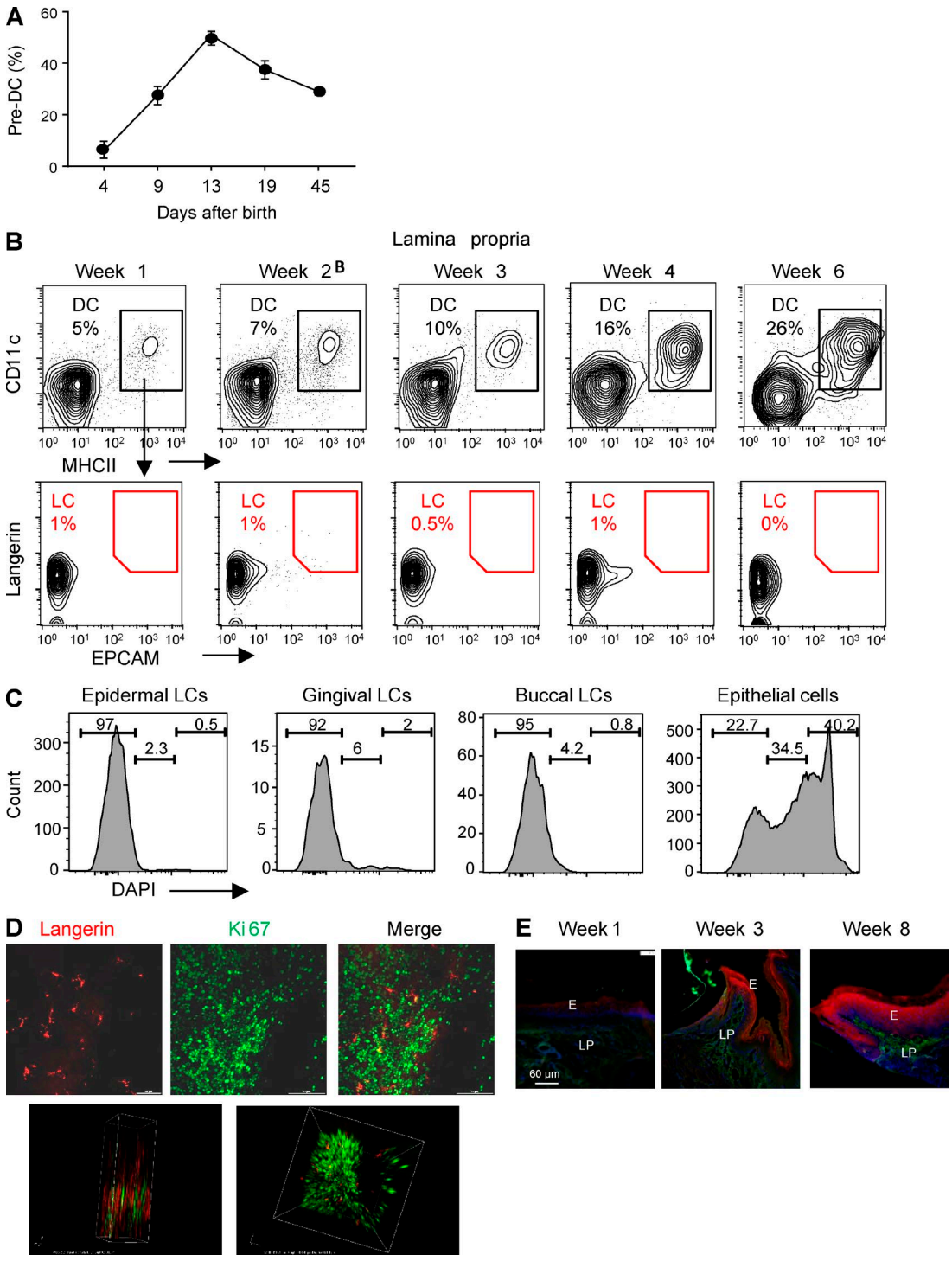

Figure S1. **Analysis of LCs in the lamina propria and steady-state proliferation of mucosal LCs. (A)** Frequencies of pre-DCs in the lamina propria on various days after birth (analysis of day 4 was performed on whole gingiva), representing the mean values ± SEM ($n = 3$). **(B)** Representative flow cytometry plots demonstrating the frequencies of MHCII$^+$CD11c$^+$ cells and LCs (CD45$^+$CD11c$^+$MHCII$^+$langerin$^+$EPCAM$^+$) in the lamina propria at various times after birth. **(C)** Flow cytometric cell cycle analysis of skin, gingival and buccal LCs as well as oral epithelial cells. Data are representative of one out of three independent experiments. **(D)** Top: Immunofluorescence analysis of gingival epithelial layers from 6-wk-old B6 mice stained for langerin (red) and Ki67. Bottom: 3D reconstruction. Confocal images are representative of one out of two independent experiments. Bars, 50 µm. **(E)** Gingival cross sections of 1-, 3-, and 8-wk-old mice were stained for TGF-β1 (red), BMP7 (green), and Hoechst (blue). Representative confocal microscopy images from one out of two independent experiments. E, epithelium; LP, lamina propria.

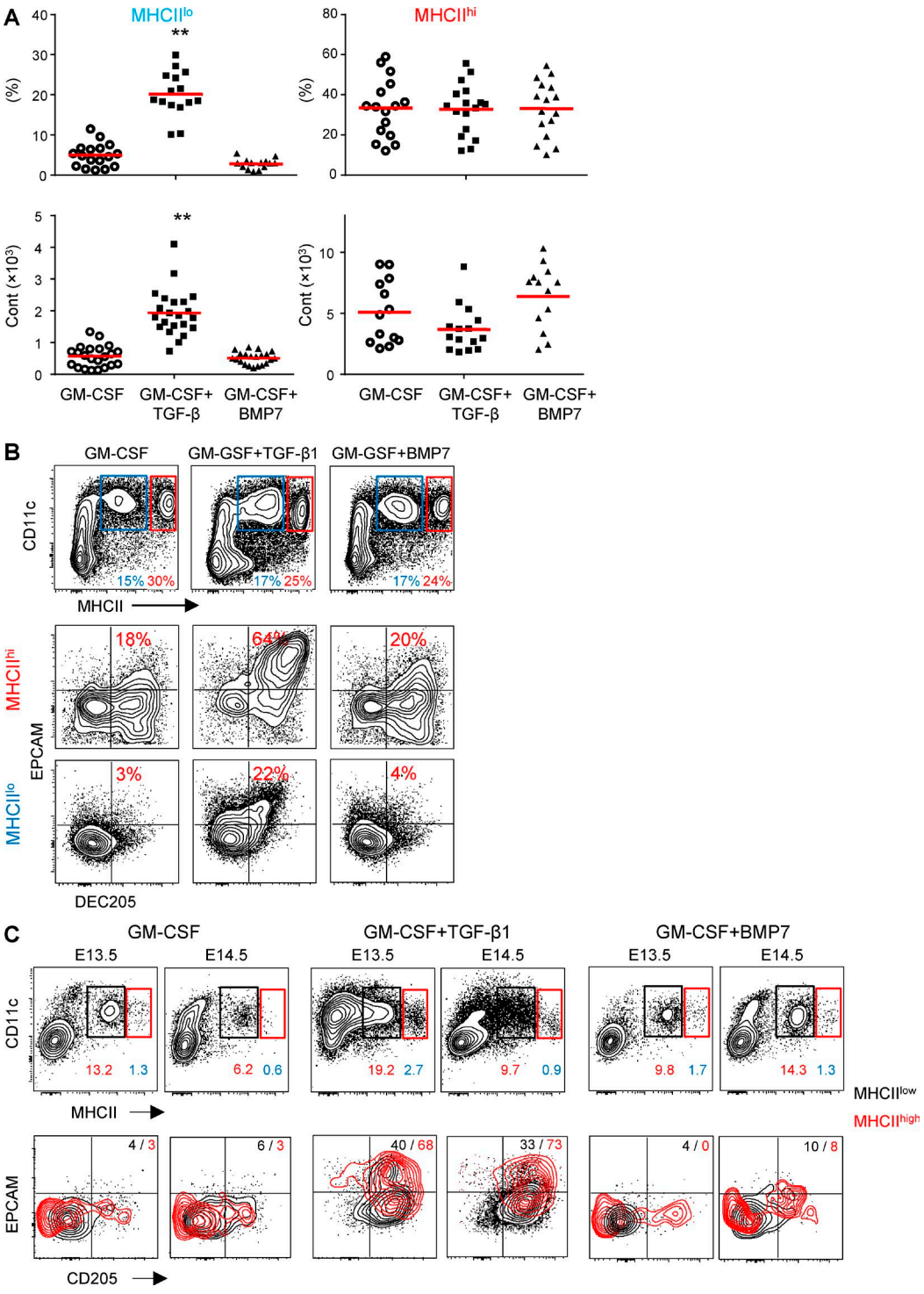

Figure S2. **Further characterization of LC-like cell differentiation from BM cells and murine fetal liver cells. (A)** Percentages and numbers of CD11c^{+}MHCII^{lo} and CD11c^{+}MHCII^{hi} populations in serum-free SM containing GM-CSF with and without TGF-β1 or BMP7. Data pooled from three independent experiments are presented. **, $P < 0.001$. **(B)** Representative FACS plots demonstrating the differentiation of LCs from BM cell in SM containing fetal calf serum and GM-SCF+TGF-β1. Representative data from one out of at least three independent experiments are shown. **(C)** Fetal liver cells were collected at E13.5 and E14.5 and cultured for 7 d in a serum-free supplemented media (FLT3L, SCF, and TNF-α) containing GM-CSF in the presence or absence of TGF-β1 or BMP7. Every 2 d, the cultures were refreshed by removing half of the media and adding same volume of fresh media containing all relevant cytokines. Representative FACS plots demonstrate the percentages of CD11c^{+}MHCII^{lo} and CD11c^{+}MHCII^{hi} population as well as their capacity to further differentiate into LC-like cells (CD205^{+}EPCAM^{+}). Representative data from one out of three independent experiments are shown.

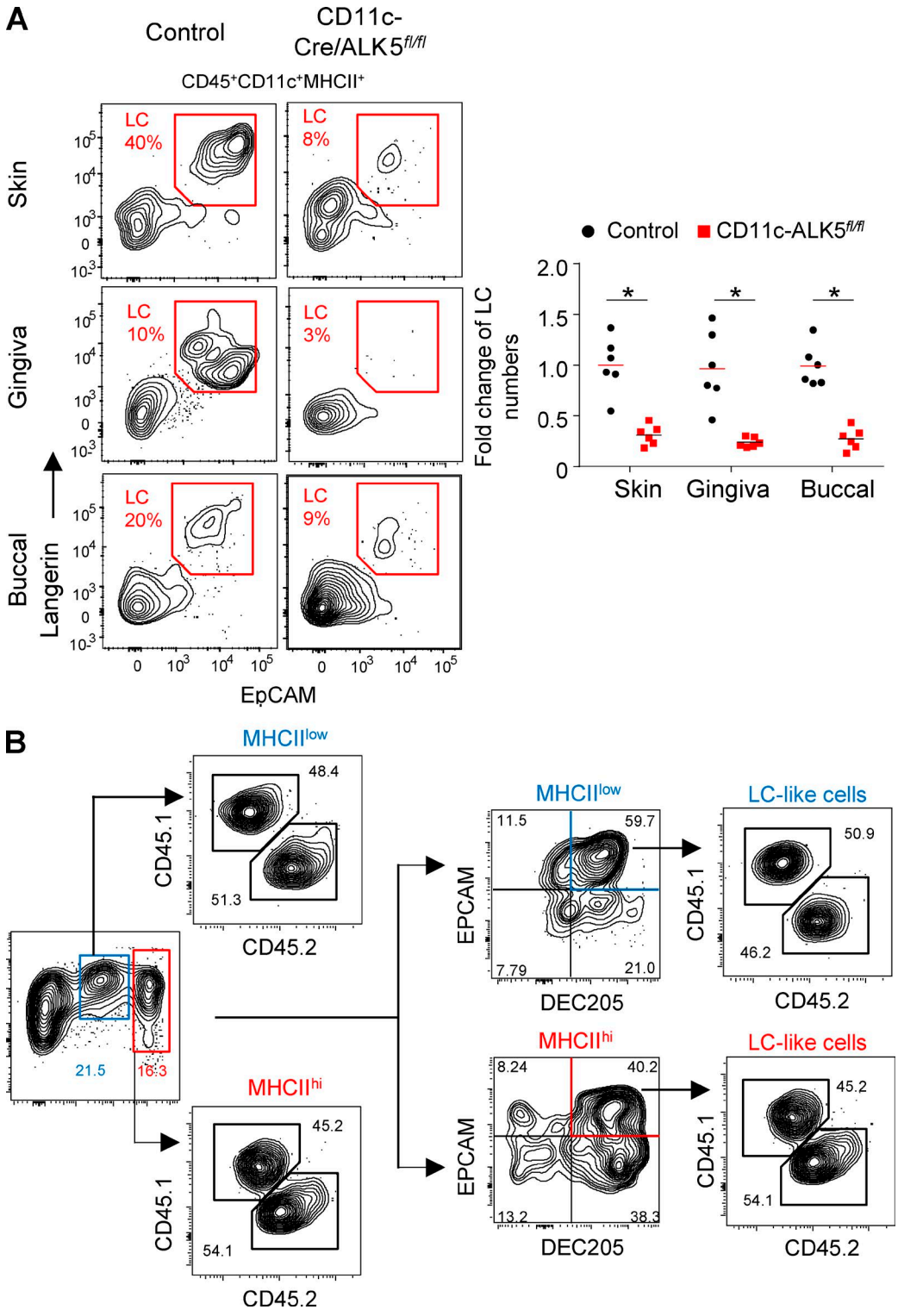

Figure S3. **ALK5 is essential for LC differentiation. (A)** Whole skin, gingival, and buccal tissue was collected from of CD11c-cre/ALK5$^{fl/fl}$ and littermate controls for tissue flow cytometry analysis. Representative FACS plots demonstrate the frequencies of live LCs (PI$^-$CD45$^+$CD11c$^+$MHCII$^+$langerin$^+$EPCAM$^+$) in each tissue. Graph presents the fold change in LC numbers; fold change was calculated based on absolute LC numbers and was normalized to control group. Data were pooled from two independent experiments and represent the mean (n = 6). **(B)** BM cells of mixed chimeric mice (CD11c-cre/ALK5$^{fl/fl}$ [CD45.1]/WT [CD45.1]) in a ratio of 3:1 were cultured for 5 d in a serum-free SM containing GM-CSF and TGF-β1. Representative FACS plots demonstrate the percentages of CD45.2$^+$ versus CD45.1$^+$ cells among MHCII^{lo} and MHCII^{hi} population as well as in EPCAM$^+$DEC205$^+$ LC-like cells. *, P < 0.05.

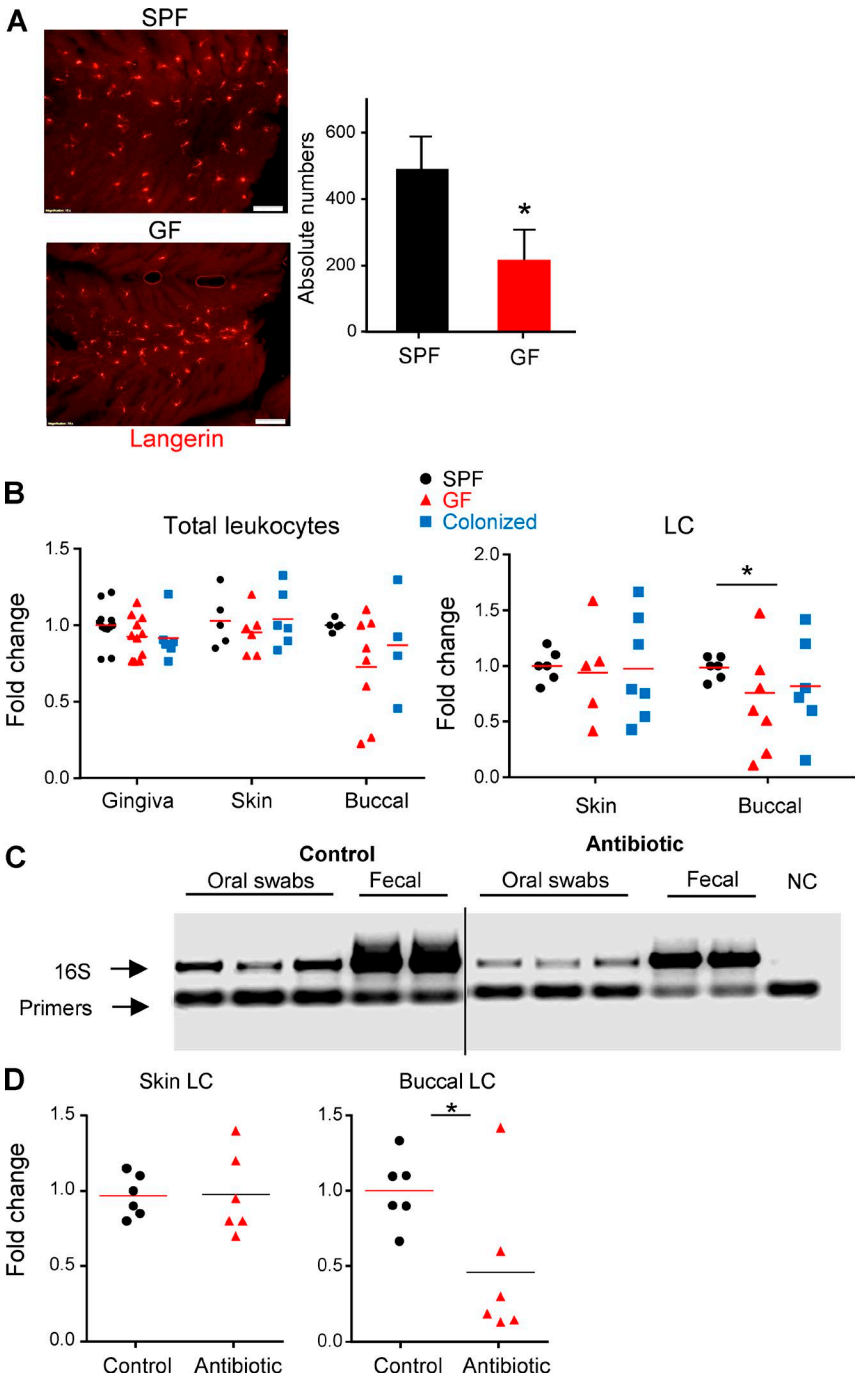

Figure S4. **Microbiota regulates the levels of oral mucosa, but not skin, LCs. (A)** Oral epithelial layers from adult Swiss Webster SPF and GF mice were stained for Langerin (red) and analyzed by confocal microscopy. Quantification of LCs was done by counting langerin$^+$ cells in 10 fields of each sample. Representative immunofluorescence image and graph from one out of three independent experiments. Each experiment included at least three separately analyzed mice. **(B)** Gingival, buccal, and skin tissue was harvested from SPF, GF, and GF mice cocaged with SPF mice for 2 mo and analyzed by flow cytometry to identify live leukocytes (CD45$^+$) and LCs (CD45$^+$CD11c$^+$MHCII$^+$langerin$^+$EPCAM$^+$). Graph presents the fold change in the numbers of the indicated cells in SPF, GF, and cohoused mice (n = 6–9). Fold change was calculated based on absolute cell numbers and was normalized to SPF mice. Data pooled from two or three independent experiments are shown as the mean values. **(C and D)** Oral swabs and fecal samples were obtain from SPF B6 mice treated with a broad-spectrum antibiotic cocktail for 2 mo. cDNA was extracted from the samples and analyzed by PCR for the expression of ribosomal 16S gene representing the microbial load. Representative data from one out of two independent experiments are shown. (D) Graph demonstrates the effect of antibiotic treatment on buccal and skin LCs. Data present the fold change in absolute LC numbers and were normalized to control mice (n = 6). Data pooled from two independent experiments are shown as the mean values. *, $P < 0.05$.

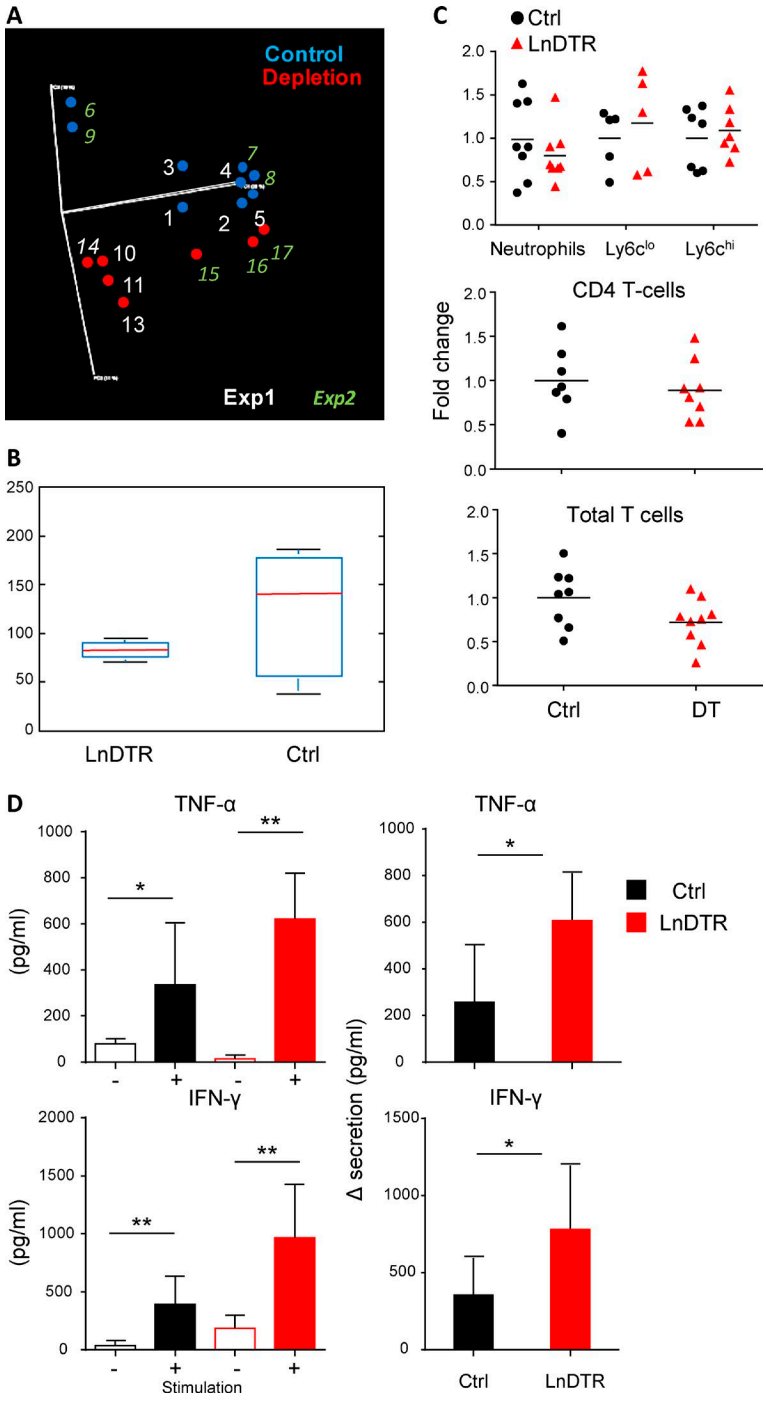

Figure S5. **Prolonged depletion of langerin-expressing cells alters oral microbiota and augments secretion of proinflammatory cytokines by splenocytes. (A)** Principal coordinates analysis of weighted UniFrac distances based on 16S rRNA of LnDTR mice versus littermate control. Data pooled from two independent experiments are shown, and numbers assign each mouse to its respective experiment ($P < 0.05$). **(B)** Alpha diversity plot presenting taxa richness in samples of both groups of mice and represent the mean ± SEM ($n = 7–8$). **(C)** Langerin-DTR (LnDTR) and littermate (Ctrl) were injected with DT on a weekly basis for 4 mo. Gingival tissues were processed and analyzed by flow cytometry to quantify neutrophils ($CD45^+LY6G^+Ly6C^-CD11b^+$), $Ly6C^{low}$ monocyte ($CD45^+Ly6C^{low}CD11b^+$), $Ly6C^{high}$ monocyte ($CD45^+Ly6C^{high}CD11b^+$), CD4 T cells ($CD45^+CD3^+CD4^+$), and total T cells ($CD45^+CD3^+$). Graphs present the fold change in absolute cell numbers and were normalized to Ctrl mice ($n = 9$). Representative graph of one out of three independent experiments is shown (mean). **(D)** Mouse splenocytes were harvested from both groups and restimulated with 5 ng/ml PMA and 500 ng/ml ionomycin. Concentrations of TNF-α and IFN-γ cytokines in the supernatants were quantified by ELISA; graphs present total secretion levels as well as the net secretion (Δ; $n = 5$). Data from one out of two independent experiments are shown as the mean values ± SEM. *, $P < 0.05$; **, $P < 0.001$.