JEM

Supplemental material

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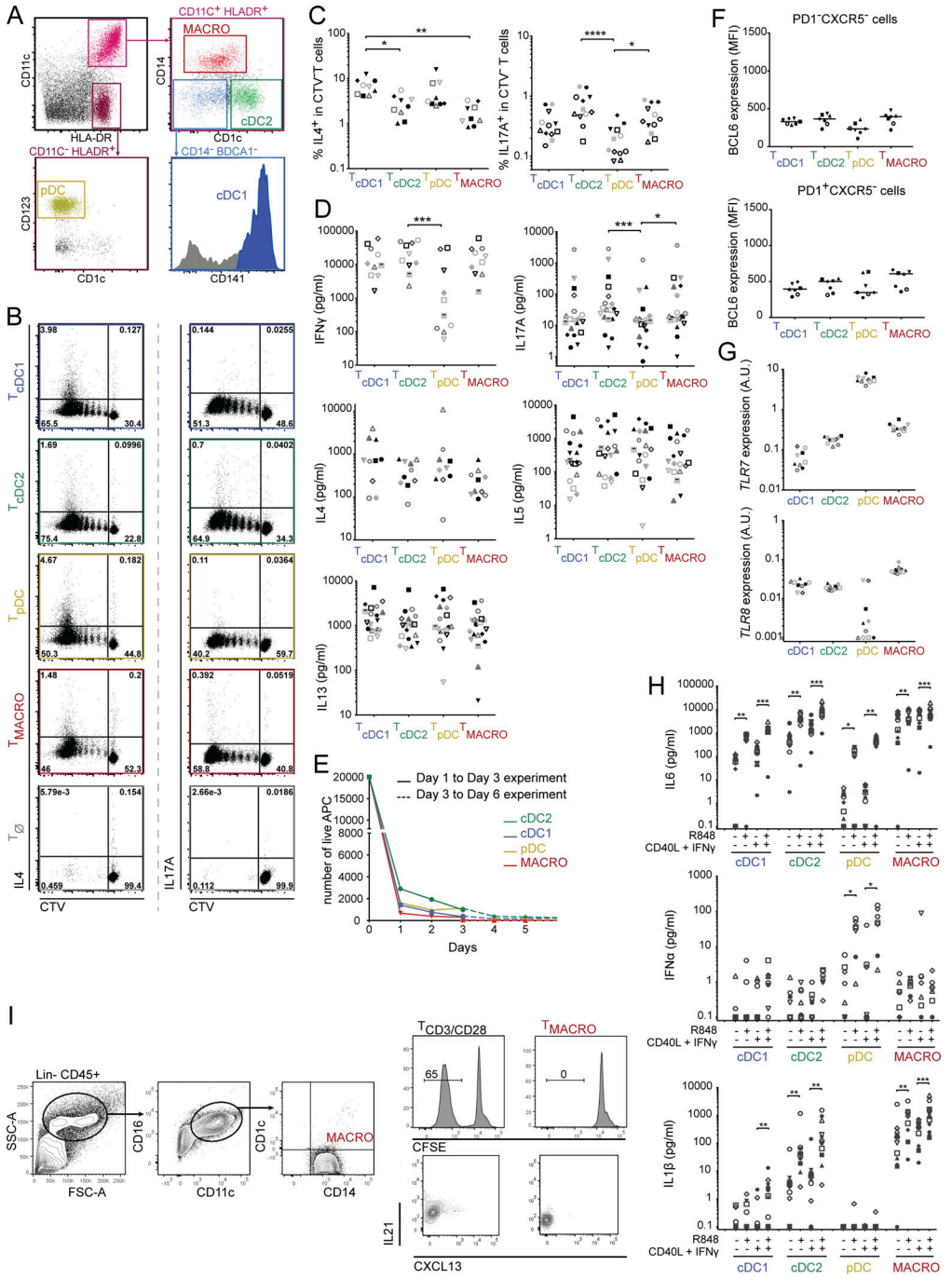

Figure S1. **Th cell polarization by DC subsets and macrophages. (A)** Gating strategy for cell sorting of tonsil APCs. pDCs were CD11c⁻HLADR⁺CD1c⁻CD123⁺. Macrophages and cDCs were positive for CD11c and HLADR. cDC1s were CD14⁻CD1c⁻CD141⁺. cDC2s were CD14⁻CD1c⁺. Macrophages (MACRO) were CD14⁺CD1c⁻. **(B–E)** Purified human tonsil DC subsets and CD14⁺ macrophages were co-cultured with allogeneic naive CD4⁺ T cells. T cells polarized with cDC1s, cDC2s, pDCs, or macrophages are termed T_{cDC1}, T_{cDC2}, T_{pDC}, and T_{MACRO}, respectively. **(B and C)** Cytokine production was analyzed by intracellular staining after restimulation with PMA and ionomycin in the presence of brefeldin A. **(B)** Representative staining, gated on live CD4⁺ T cells. $T_{Ø}$ corresponds to T cells cultured without APCs. **(C)** Percentage of divided cells (CTV⁻) producing IL-4 (n = 9) or IL-17A (n = 12). **(D)** Cytokine secretion was analyzed by CBA after restimulation with anti-CD3/CD28 beads. Each symbol represents an individual donor (n = 10 for IFN-γ and IL-4, n = 19 for IL-5, IL-13, and IL-17A). **(E)** Number of live APCs at different days after the start of the co-culture as evaluated by flow cytometry. Cultures were analyzed either from day 1 to day 3, or from day 3 to day 6 (n = 3 in each series). Mean is shown. **(F)** MFI of Bcl6 staining in PD-1⁻CXCR5⁻ cells and PD-1⁺CXCR5⁻ cells at day 4 (n = 7). **(G)** Relative expression of *TLR7* and *TLR8* mRNA compared with housekeeping genes in purified tonsil DC subsets and macrophages. **(H)** Purified DCs and macrophages were cultured for 24 h with or without R848 (TLR7/8 ligand), CD40L, and IFN-γ. Cytokine secretion was analyzed by CBA (n = 12). Each symbol represents an individual donor. A.U., arbitrary units. **(I)** Lung macrophages were isolated from nondiseased parts of the lungs of cancer patients and co-cultured with allogeneic naive CD4⁺ T cells for 6 d. Gating strategy for cell sorting is shown. Proliferation profiles and intracellular stainings for IL-21 and CXCL13 of naive CD4⁺ T cells cultured with anti-CD3/CD28 beads (positive control) or lung macrophages are shown. Representative results of four different donors. SSC-A, side scatter; FSC-A, forward scatter. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001; Wilcoxon test.

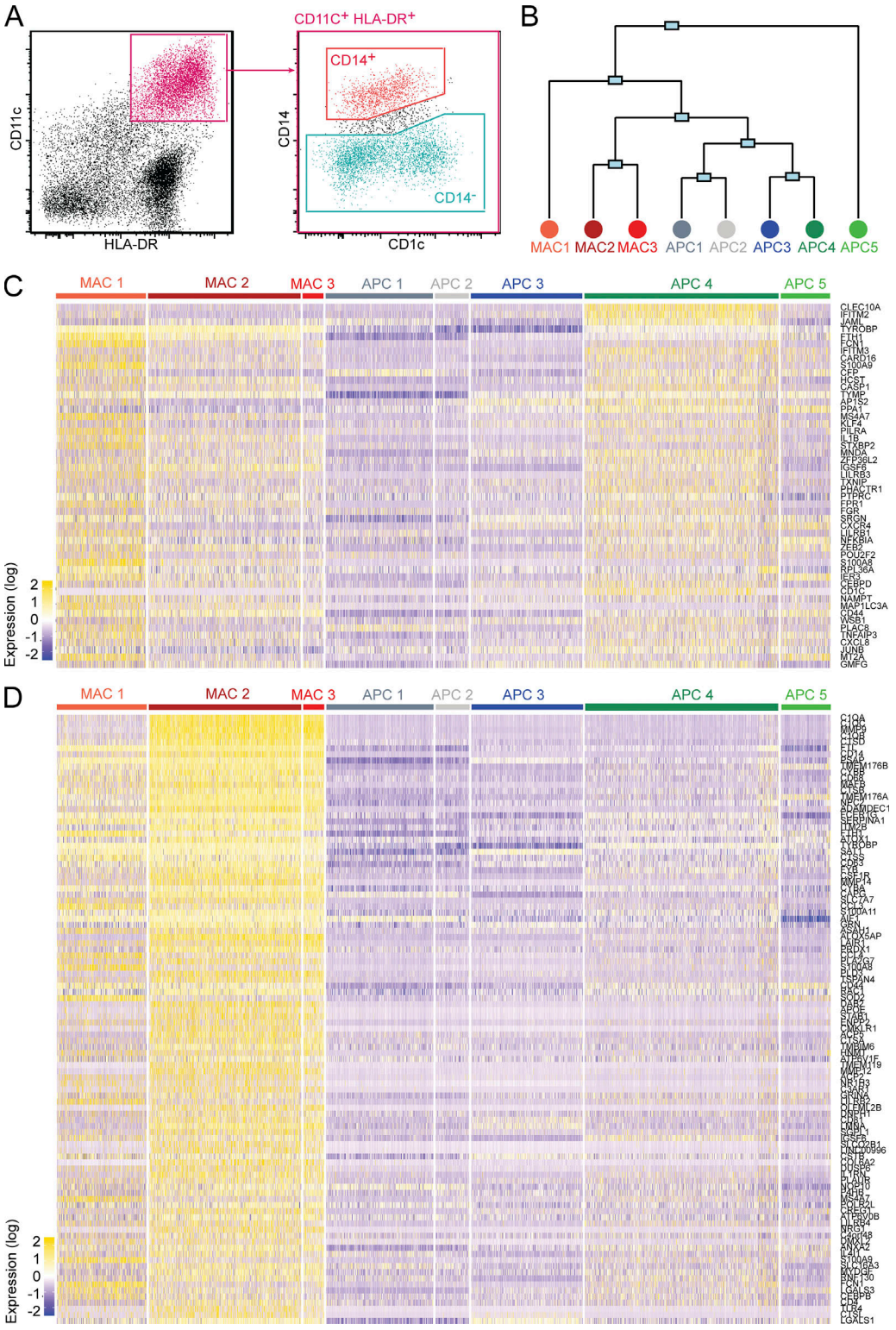

Figure S2. **Tonsil CD14⁺ cells do not contain a population of DCs. (A)** Purified tonsil HLA-DR⁺CD11c⁺CD14⁻ cells and HLA-DR⁺CD11c⁺CD14⁺ cells were analyzed by single-cell RNA-seq. Gating strategy for cell sorting. **(B)** Hierarchical clustering tree of meta-cells. Colors correspond to clusters from Fig. 3. **(C)** Heatmap of scaled expression (log values of UMI) for the calculated top 50 "putative conserved genes" between cluster MAC1 and cluster APC4 compared with all other clusters. **(D)** Heatmap of scaled expression (log values of UMI) for the calculated top 100 "putative conserved genes" between cluster MAC1 and cluster MAC2 compared with all other clusters.

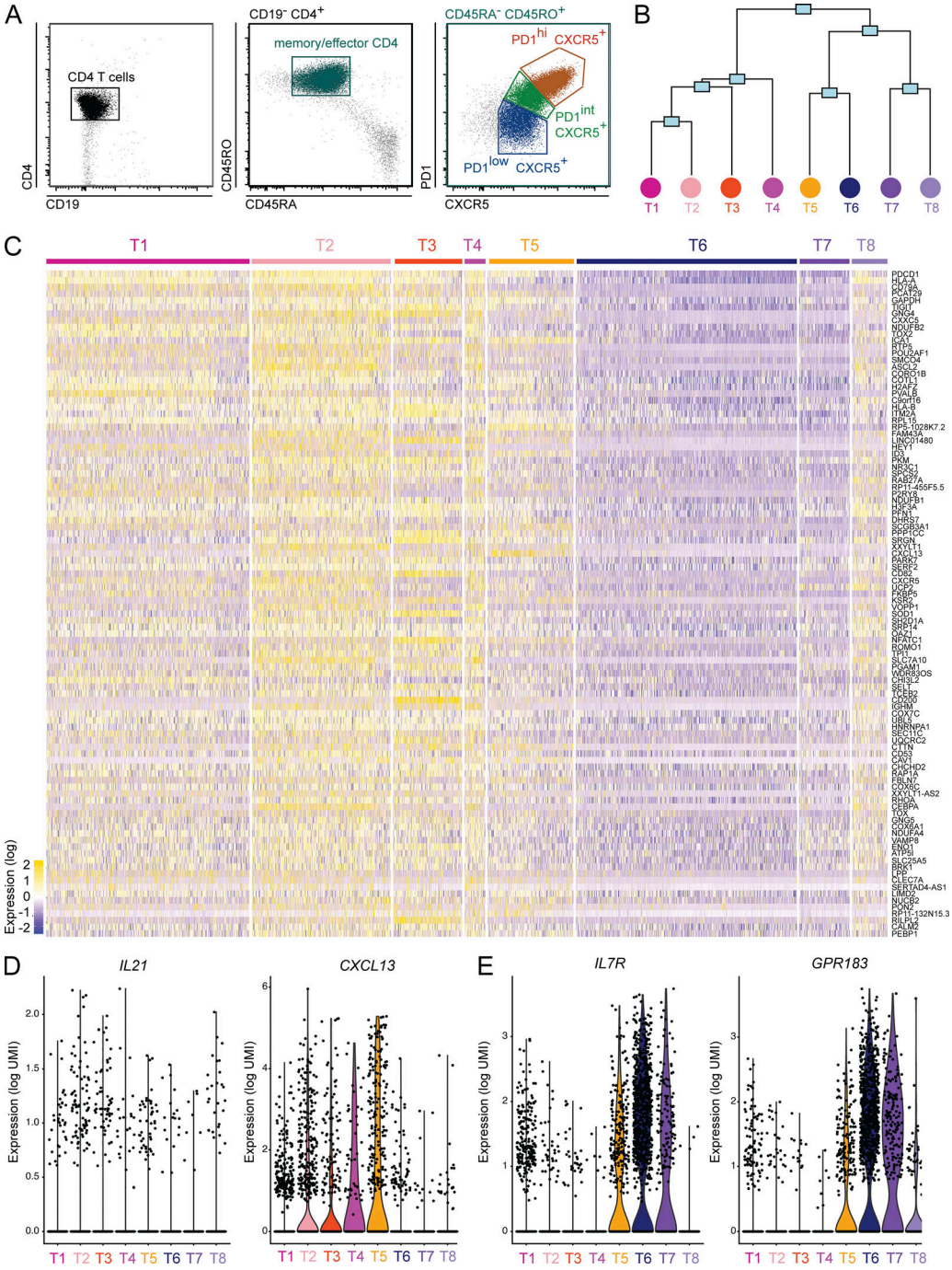

Figure S3. **Tfh cells comprise two distinct effector states. (A)** Tonsil CD4⁺CD45RA⁻CD45RO⁺ cells were purified as CXCR5⁺PD-1^{low}, CXCR5⁺PD-1^{int}, and CXCR5⁺PD-1^{high} cells and were analyzed by single-cell RNA-seq. Gating strategy for cell sorting. **(B)** Hierarchical clustering tree of metacells. Colors correspond to clusters from Fig. 4. **(C)** Heatmap of scaled expression (log values of UMI) for the calculated top 100 "putative conserved genes" between clusters T1–T5 compared with all other clusters. **(D and E)** Scaled expression (log values of UMI) in individual cells for *IL21* and *CXCL13* (D) or *IL7R* and *GPR183* (E).

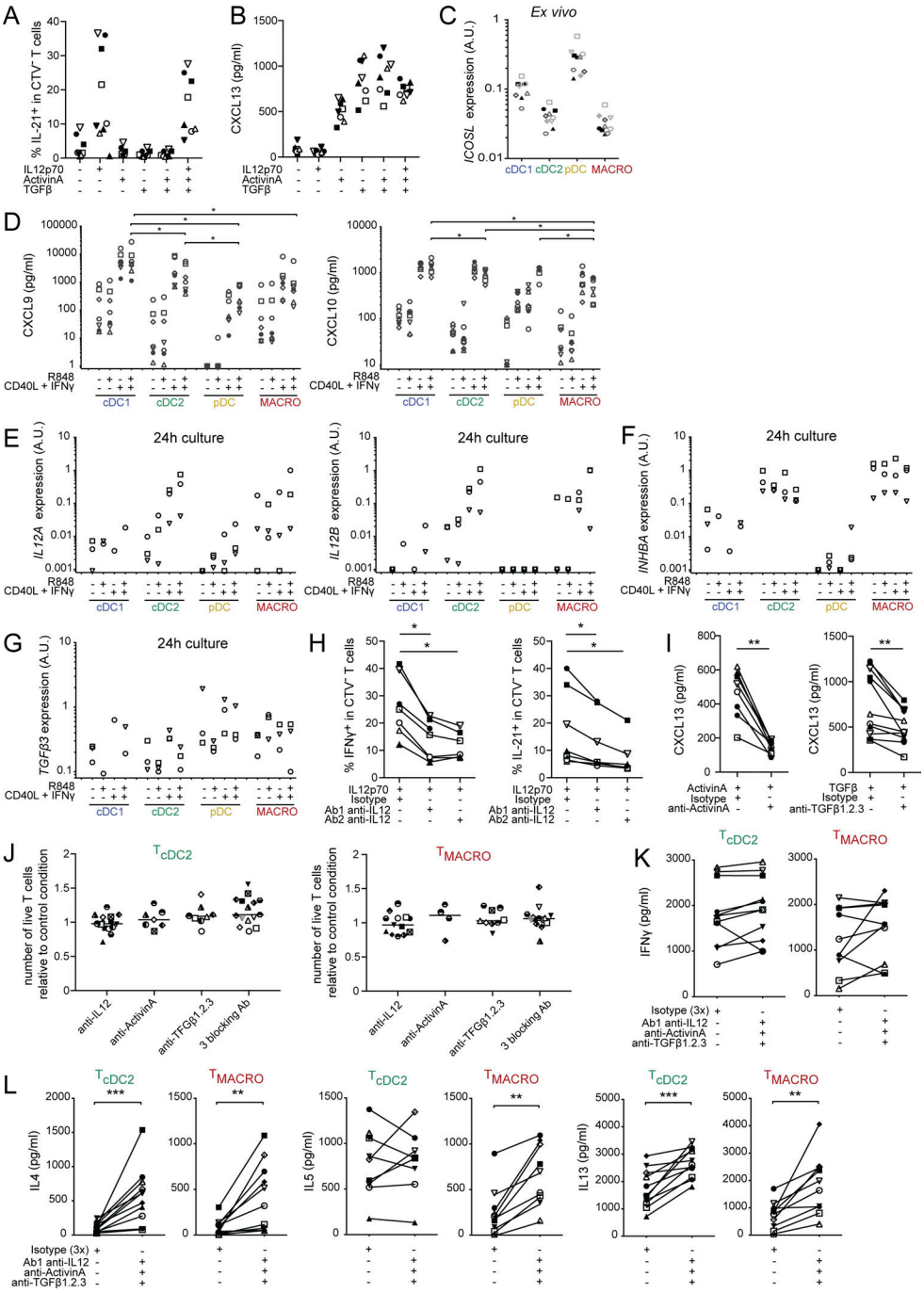

Figure S4. **Analysis of IL-12, activin A, and TGFβ in Th cell polarization. (A and B)** Naive CD4+ T cells were cultured in the presence of recombinant IL12p70, activin A, and/or TGFβ1. Each symbol represents an individual donor. **(A)** IL-21 production was analyzed by intracellular staining after restimulation with PMA and ionomycin in presence of brefeldin A (n = 8). **(B)** CXCL13 secretion was analyzed by ELISA after restimulation with anti-CD3/CD28 beads (n = 8). **(C)** *ICOSL* mRNA expression in purified human tonsil DC subsets and macrophages was measured directly after cell sorting (n = 10). Each symbol represents an individual donor. **(D–G)** Purified DCs and macrophages were cultured for 24 h with or without R848, CD40L, and IFN-γ. Chemokine secretion was analyzed by CBA (D) and mRNA expression by RT-PCR (E–G). Each symbol represents an individual donor. n = 12 (D); n = 3, except for cDC1 (n = 1–3; E–G). **(H)** Naive CD4+ T cells were cultured in presence of recombinant IL12p70 and blocking antibodies against IL-12 (two different clones termed Ab1 and Ab2) or isotype control. IL-21 and IFN-γ production was analyzed by intracellular staining after restimulation with PMA and ionomycin in presence of brefeldin A (n = 7). **(I)** Naive CD4+ T cells were cultured in presence of recombinant activin A or TGFβ1 and blocking antibodies against activin A or TGFβ1,2,3, or isotype control. CXCL13 secretion was analyzed by ELISA after restimulation with anti-CD3/CD28 beads (n = 8 for activin A and n = 11 for TGFβ). **(J–L)** Purified cDC2s or macrophages were co-cultured with naive CD4+ T cells in the presence of blocking antibodies against IL-12, activin A, and TGFβ1,2,3, or the same total concentration of isotype control. T cells polarized with cDC2 or CD14+ macrophages are termed T_{cDC2} and T_{MACRO}, respectively. **(J)** Number of live cells in cultures with blocking antibodies relative to that in cultures with isotype control antibodies. Each symbol represents an individual donor (n = 4–15). Median is shown. **(K and L)** Cytokine secretion was analyzed by CBA after restimulation with anti-CD3/CD28 beads. Each symbol represents an individual donor (n = 11). A.U., arbitrary units. *, P < 0.05; **, P < 0.01; ***, P < 0.001; Wilcoxon test.

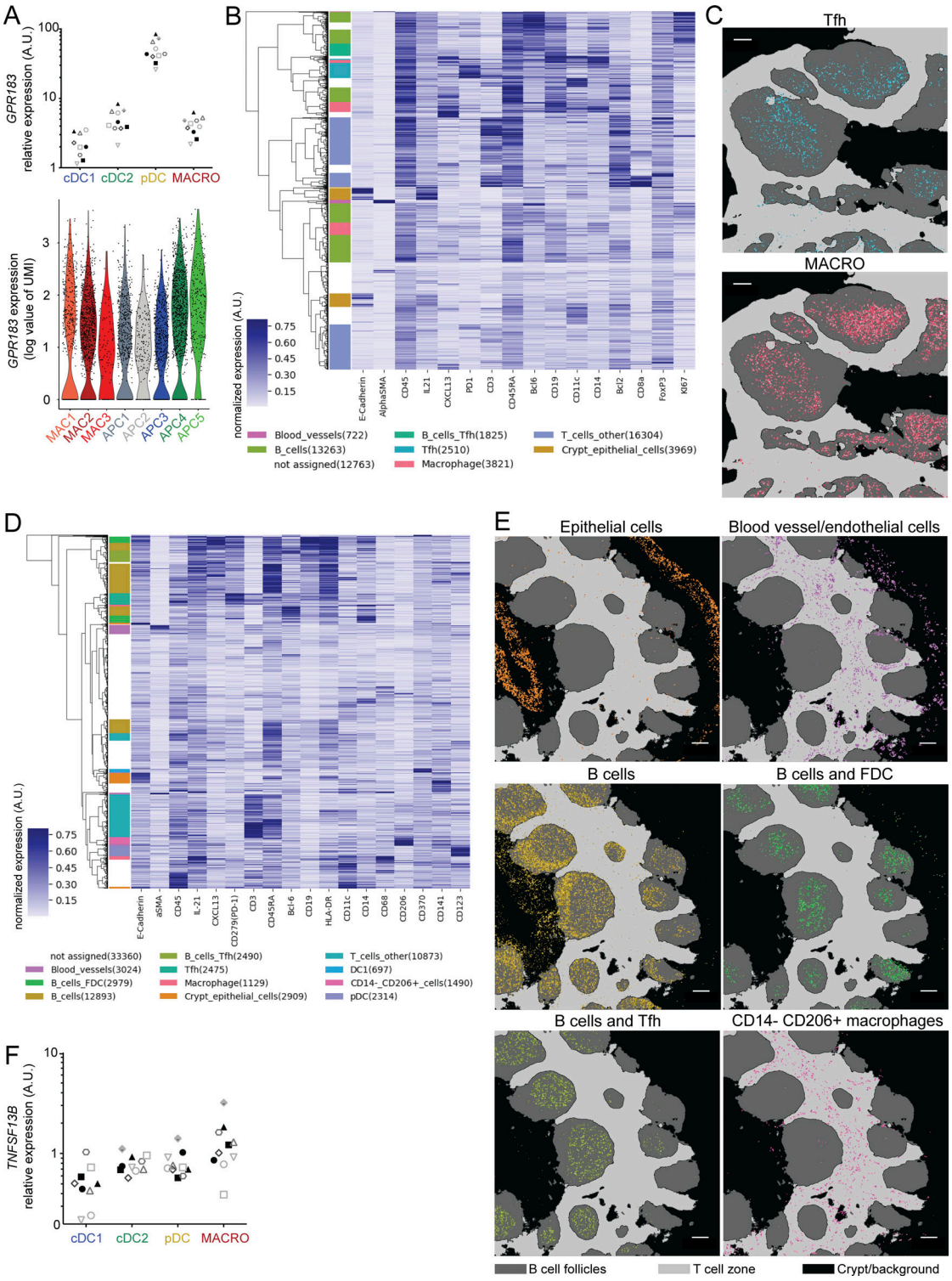

Figure S5. **Analysis of in situ localization in tonsils. (A)** *GPR183* mRNA expression (encoding EBI2) in purified human tonsil DC subsets and macrophages was measured directly after cell sorting (n = 10). Each symbol represents an individual donor. Scaled expression for *GPR183* (log values of UMI) in individual cells from the APC single-cell RNA-seq dataset. **(B–E)** Tonsil sections were analyzed by imaging mass cytometry. **(B and D)** Heatmap showing normalized expression for individual markers and unsupervised clustering of individual cells (B: first experiment; D: second series, representative of two different donors). Clusters with the same cellular identity were grouped for visualization. Each color indicates a cluster. Number of cells in each cluster is shown. **(C)** Topographic representation of cells identified as Tfh cells and CD14$^+$ macrophages (from the first experiment). Bars, 200 µm. **(E)** Topographic representation of cells identified as crypt epithelial cells, blood vessels and endothelial cells, B cells, cluster with B cells and FDC, cluster with B cells and Tfh cells, CD14$^-$CD206$^+$ macrophages (from the second series, representative of two different donors). Bars, 200 µm. **(F)** *TNFSF13B* mRNA expression (encoding B-cell activating factor) in purified human tonsil DC subsets and macrophages was measured directly after cell sorting (n = 10). Each symbol represents an individual donor. A.U., arbitrary units.

Tables S1, S2, and S3 are provided online as separate Excel files. Table S1 shows top genes per cluster, related to Fig. 3. Table S2 shows gene signatures used in Fig. 3. Table S3 shows top genes per cluster, related to Fig. 4.