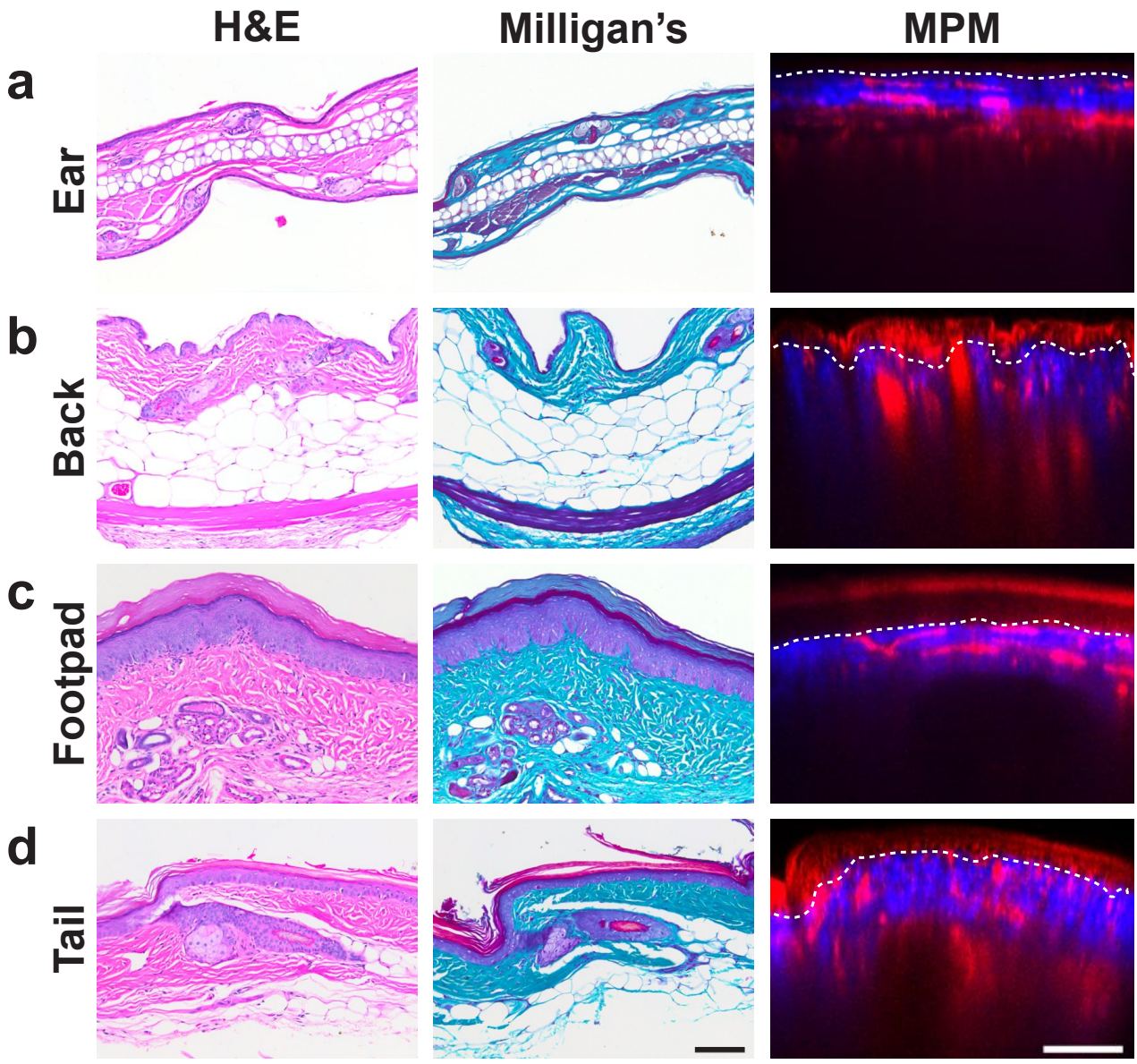
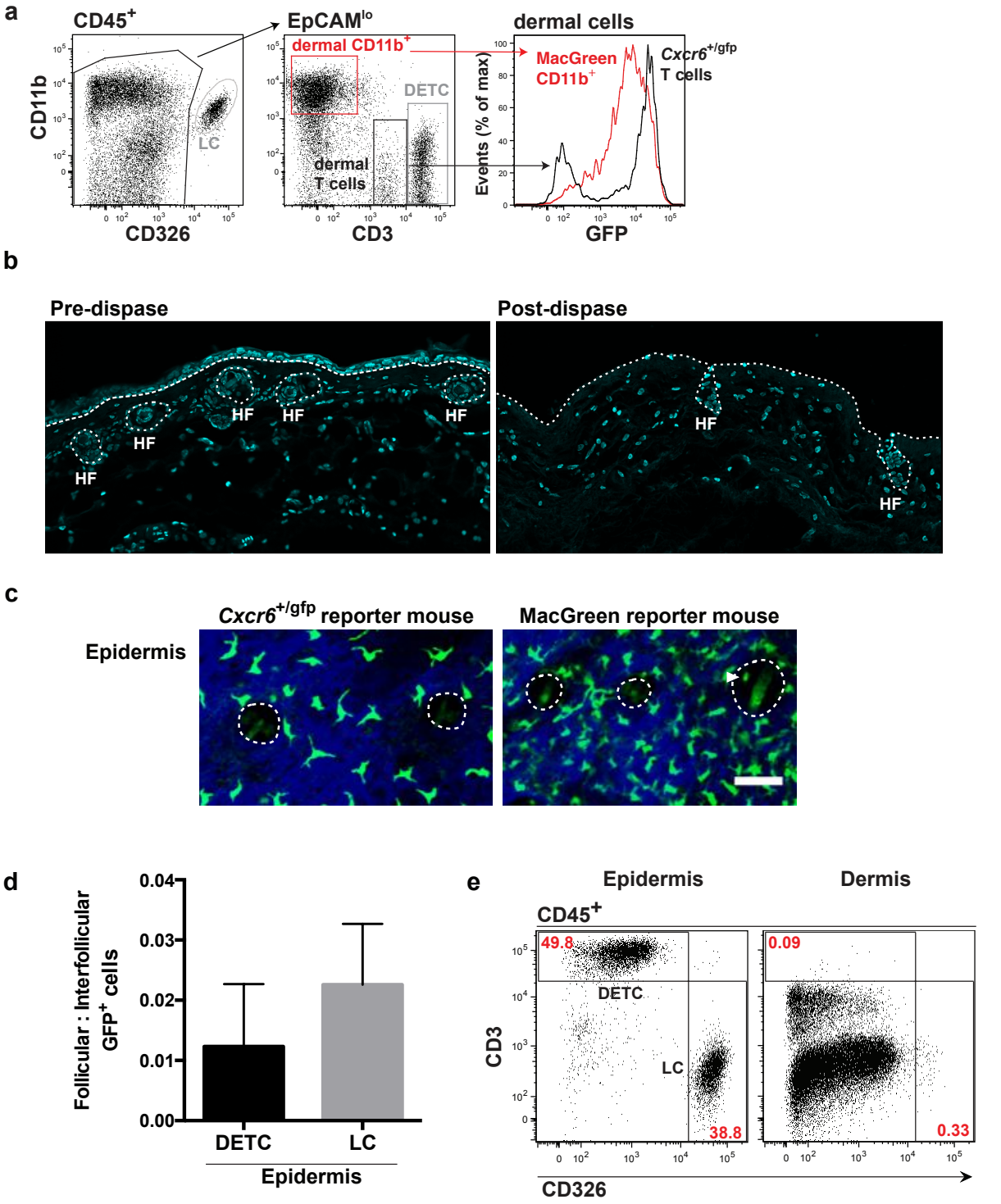
Supplementary Figure 1



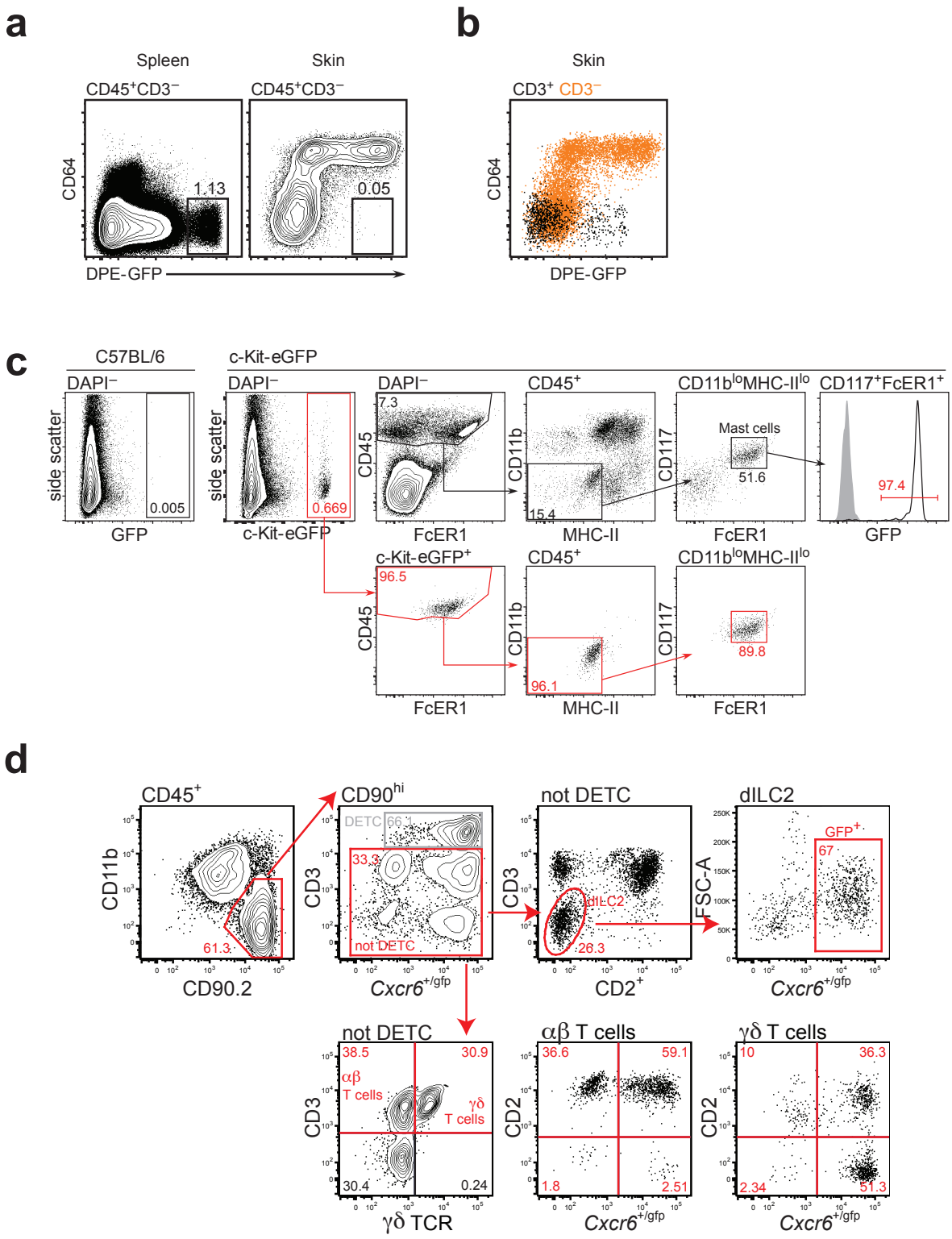
Supplementary Figure 1. Multiphoton imaging recapitulates site-specific structural differences in murine skin. Vertical sections of skin from the (a) ear pinnae (b) dorsal back (c) footpad and (d) tail of albino C57BL/6-*Tyr*$^{c\text{-}2J}$/J mice were stained with hematoxylin and eosin (H&E, *left column*) or Milligan's trichrome stain (*middle column*). Far *right column* represents a cross-sectional reconstruction from 3-dimensional *ex vivo* multiphoton microscopy (MPM) of tissue from mT/mG mice, showing cell-membrane fluorescence (red) and SHG signals (blue). Dotted line indicates dermo-epidermal junction. Scale bar 100 μm. Representative images from n = 2 mice shown for histology and MPM studies.

Supplementary Figure 2



Supplementary Figure 2. Identification of epidermal and dermal leukocyte subsets by flow cytometry and assessment of epidermal contamination in dermal preparations arising from hair follicles. (a) Dot plots of CD45$^+$ leukocytes within the ear skin of wild-type C57BL/6 mice with identification of CD326$^+$CD11b$^+$ Langerhans cells (LC, grey gates) and CD3^{hi} dendritic epidermal T cells (DETC, grey gates) as well as CD326^{lo}CD11b$^+$ dermal cells (red gate) and CD3^{int} dermal T cells (black gate). Histogram comparing GFP intensity of CD11b$^+$ cells in MacGreen mice (in red) and dermal T cells in *Cxcr6*$^{+/gfp}$ mice (in black). (b) Representative confocal microscopy images of C57BL/6 mouse ear sections before and after dispase treatment. DAPI$^+$ nuclei shown in blue. Dotted lines indicate the dermo-epidermal junction and hair follicle (HF) shafts. (c) Representative z-projections of GFP$^+$ DETC (*left*) and LC (*right*) in the epidermis of *Cxcr6*$^{+/gfp}$ and MacGreen mice respectively, as detected by multiphoton microscopy. Underneath the epidermis, the ECM in the superficial dermis was detected by SHG signals (blue). Dotted line indicates hair follicles. Arrowhead indicates a hair follicle-associated LC. (d) Ratio of HF-resident (follicular) to interfollicular distribution of GFP$^+$ DETC and LC in the epidermis of *Cxcr6*$^{+/gfp}$ and MacGreen mice respectively. (e) Representative dot plots of CD45$^+$ leukocytes within the epidermis (*left*) and dermis (*right*) following dispase separation. Gates indicate CD3^{hi} DETC or CD326^{hi} Langerhans cells. Scale bar 50 μm. Data are representative of (a) 3 independent experiments with > 2 mice per strain each time, (b) 2 mice, (c-d) 2 mice per strain, (e) > 20 independent experiments with > 2 mice per strain.

Supplementary Figure 3



Supplementary Figure 3. Flow cytometric identification of indicated leukocyte populations in C57BL/6, DPE-GFP, cKit-eGFP and *Cxcr6*$^{+/gfp}$ mice. (a) Dot plots of leukocytes isolated from spleen (left) and skin (right) of DPE-GFP mice. Cells were pre-gated to exclude CD3^{+} T cells. Gates identify GFP^{hi}CD64^{-} plasmacytoid dendritic cells. (b) GFP vs. CD64 expression by CD3^{+} T cells (black) and CD3^{-} cells (orange) isolated from the skin of DPE-GFP mice. (c) Dot plots of C57BL/6 and cKit-eGFP mouse skin. Gating strategy identifies the fraction of mast cells that express GFP (top panels) and what proportion of GFP^{hi} cells in cKit-eGFP mice are mast cells (bottom panels). (d) Dot plots of CD45^{+} leukocytes within the ear skin of old (> 12 months) *Cxcr6*$^{+/gfp}$ mice showing the identification of CD90^{hi} dendritic epidermal T cells (DETC, grey gates), CD3^{-}CD2^{-} dermal group 2 innate lymphoid cells (dILC2, red gate), as well as CD3^{int} dermal αβ T cells and γδ T cells (red gates). Gate frequencies including GFP expression are also shown. Data are representative of 3 independent experiments with > 2 mice per group.

Supplementary Table 1

Langerhans cells (MacGreen mouse)	**Mean (%)**
percentage of GFP^+ cells that are Langerhans cells	100
percentage of Langerhans cells that are GFP^+	100
Citation: Puttur *et al.* 2010	

Dendritic epidermal T cells (*Cxcr6*$^{+/gfp}$ mouse)	**Mean (%)**
percentage of GFP^+ cells that are dendritic epidermal T cells	97.34
percentage of dendritic epidermal T cells that are GFP^+	99.67
Citation: Sumaria *et al.* 2011	

Mast cells (c-Kit-eGFP mouse)	**Mean (%)**
percentage of GFP^+ cells that are mast cells	100
percentage of mast cells that are GFP^+	100
Citation: Berrozpe *et al.* 2006	

Dermal dendritic cells (CD11c-YFP mouse)	**Mean (%)**
percentage of YFP^+ cells that are dermal dendritic cells	100
percentage of dermal dendritic cells that are YFP^+	64
Citation: Ng *et al.* 2008	

Dermal T cells (*Cxcr6*$^{+/gfp}$ mouse)	**Mean (%)**
percentage of GFP^+ cells that are dermal T cells	71.91
percentage of dermal T cells that are GFP^+	79.96

Individual T cell subsets including dILC2 cells (*Cxcr6*$^{+/gfp}$ mouse)	**< 3 mo. (%)**	**> 12 mo. (%)**
percentage of GFP^+ cells that are dermal αβ T cells	23.23	25.38
percentage of GFP^+ cells that are dermal γδ T cells	52.83	60.73
percentage of GFP^+ cells that are dILC2 cells	19.60	11.56
percentage of dermal αβ T cells that are GFP^+	50.06	58.44
percentage of dermal γδ T cells that are GFP^+	86.86	86.08
percentage of dILC2 cells that are GFP^+	65.55	61.97
Citation: Sumaria *et al.* 2011; Roediger *et al.* 2013		

Macrophages (DPE-GFP mouse)	**Mean (%)**
percentage of $CD3^-GFP^{hi}$ cells that are macrophages	100.00
percentage of macrophages that are GFP^+	41.83
Citation: Abtin *et al.* 2014	

Supplementary Table 1. Fluorescent protein expression in reporter mice used in the Immune Atlas. Data tables detailing the mean percentage of fluorescence-positive cells that represented each of the indicated immune subpopulations and the percentage of each of indicated immune subpopulations that expressed GFP or YFP within the relevant transgenic reporter mice.

Supplementary Table 2

Mast cells	Ear	Back	Footpad	Tail
Mean (per mm^2)	695.8	273.5	223.2	237
Std. Deviation	25.27	38.71	157.3	90.53
Std. Error of Mean	14.59	22.35	90.84	52.27

DDC	Ear	Back	Footpad	Tail
Mean (per mm^2)	692.4	724.6	593.2	485.9
Std. Deviation	269.4	262.8	309.6	91.44
Std. Error of Mean	155.5	151.8	178.7	52.79

T cells	Ear	Back	Footpad	Tail
Mean (per mm^2)	217.7	554.6	156.8	120.6
Std. Deviation	48.42	204.6	11.98	23.9
Std. Error of Mean	27.96	118.1	6.916	13.8

Macrophages	Ear	Back	Footpad	Tail
Mean (per mm^2)	1842	891.9	1933	797.4
Std. Deviation	247.3	212.4	27.37	245.5
Std. Error of Mean	142.8	122.6	15.8	141.7

Supplementary Table 2. Table of dermal leukocyte densities expressed per mm^2. Data tables are mean ± S.D. and ± S.E.M. of mast cells, dermal dendritic cells (DDC), dermal T cells and macrophage densities expressed as per mm^2 according to site.