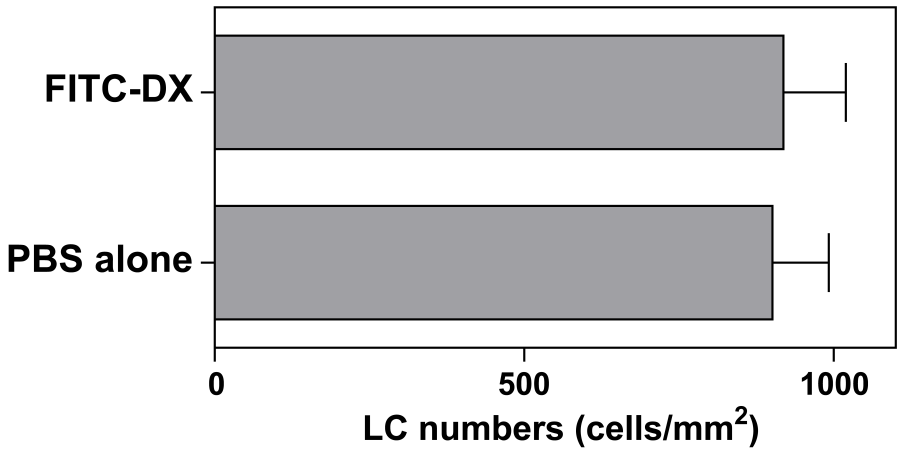
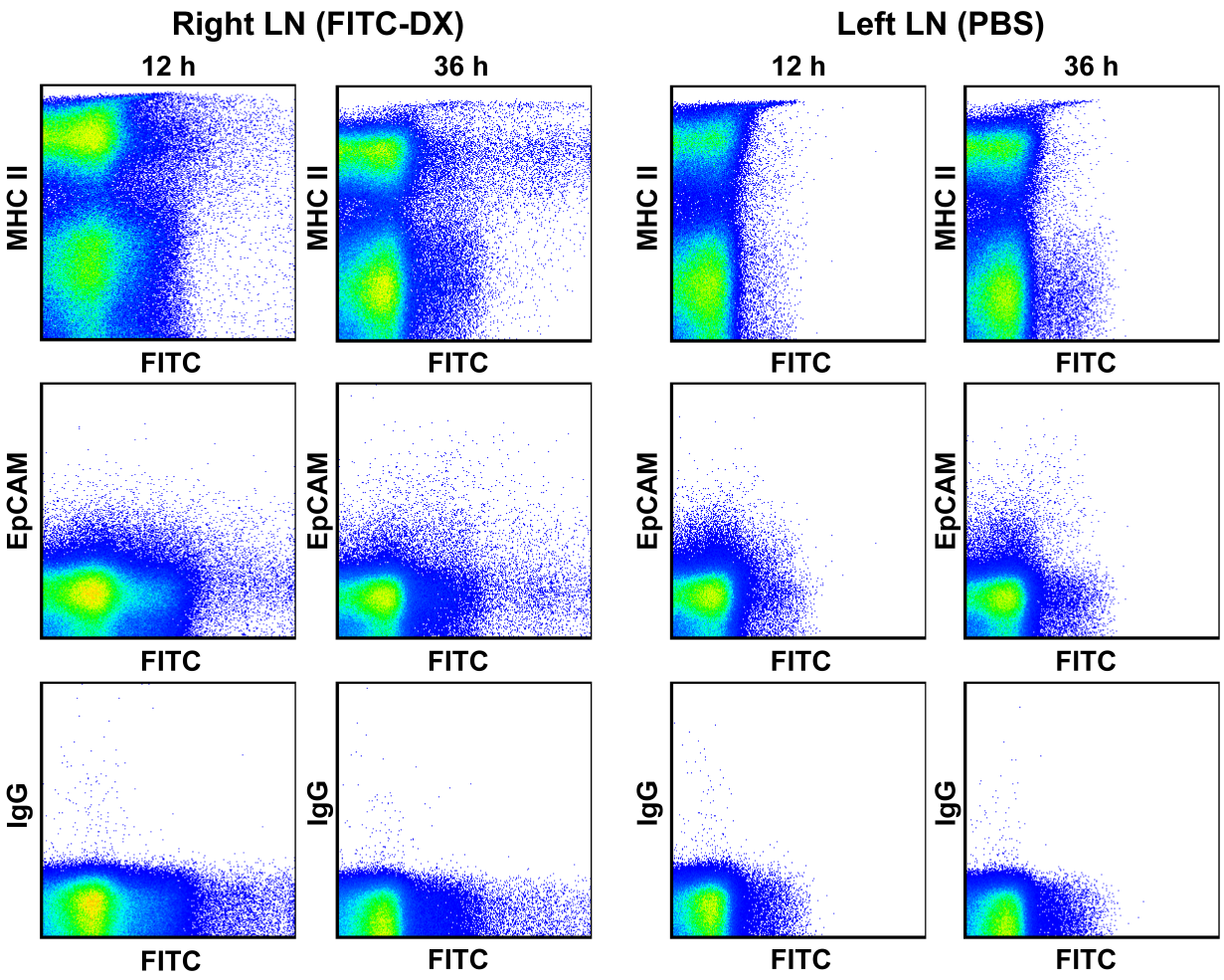
SUPPLEMENTARY MATERIAL

Supplementary Materials and Methods

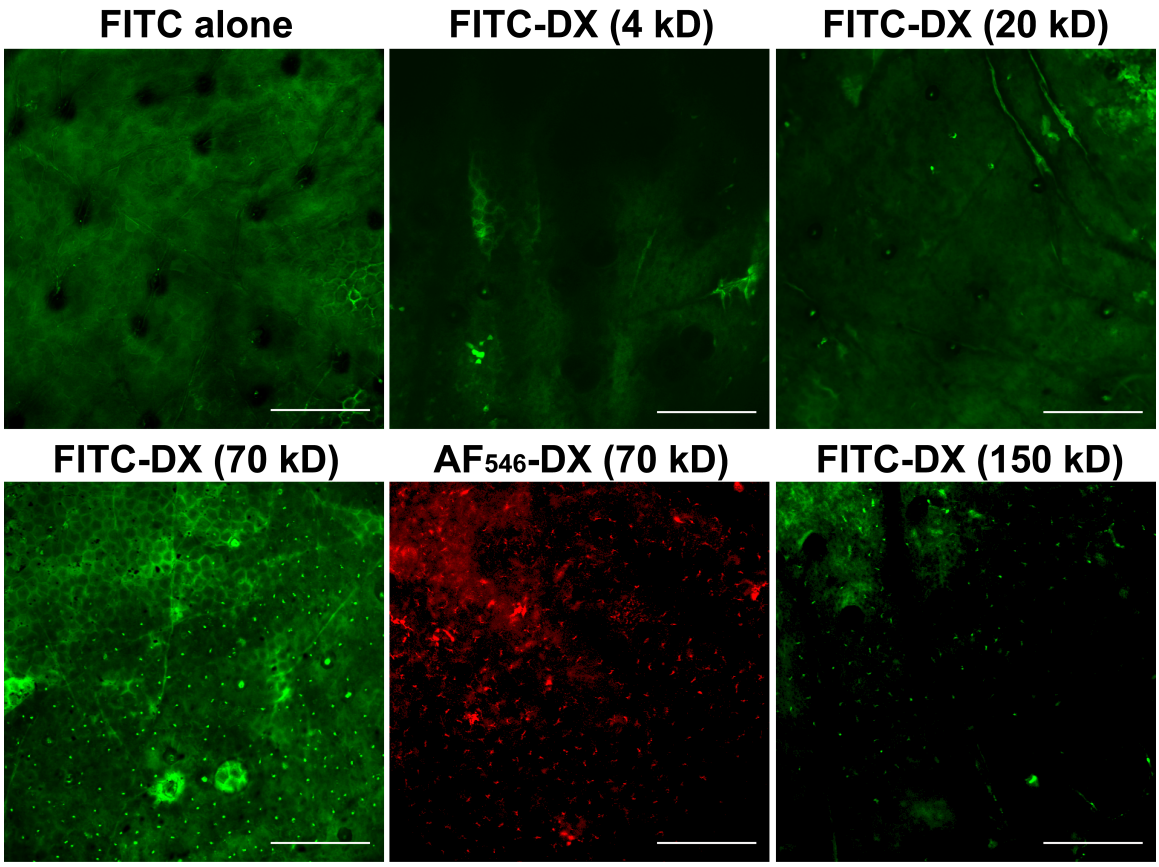
The mice were anesthetized with intraperitoneal injection of the anesthetic cocktail (ketamine, xylaxine and acepromazine) before s.c. injection of FITC-DX, FITC-mannan, or FITC-OVA probes into the ear. At different time points, the mice were anesthetized again as above and placed on an imaging stage in a supine position so that the dorsal aspect of the ear faced down on a cover slip. The ear was immersed in PBS and held between the cover slip and a microscope glass slide. During the imaging session, animals were administered oxygen through an environmental chamber and body temperature was maintained *via* a heating pad. Confocal images were acquired using a Leica TCS SP5 confocal microscope with excitation at 488 and 561 nm and detection with a 20X, 40x, or 63x Leica Plan Apo objective. Some images were recorded using an Olympus Fluoview FV1000 confocal microscope with a 20X or 40X Olympus Uplan FL N objective. The resulting 3D image stacks (sequential x-y planes separated by 1 μm) were processed using the Leica Confocal Software Package, ImageJ (NIH), and the Adobe Photoshop.



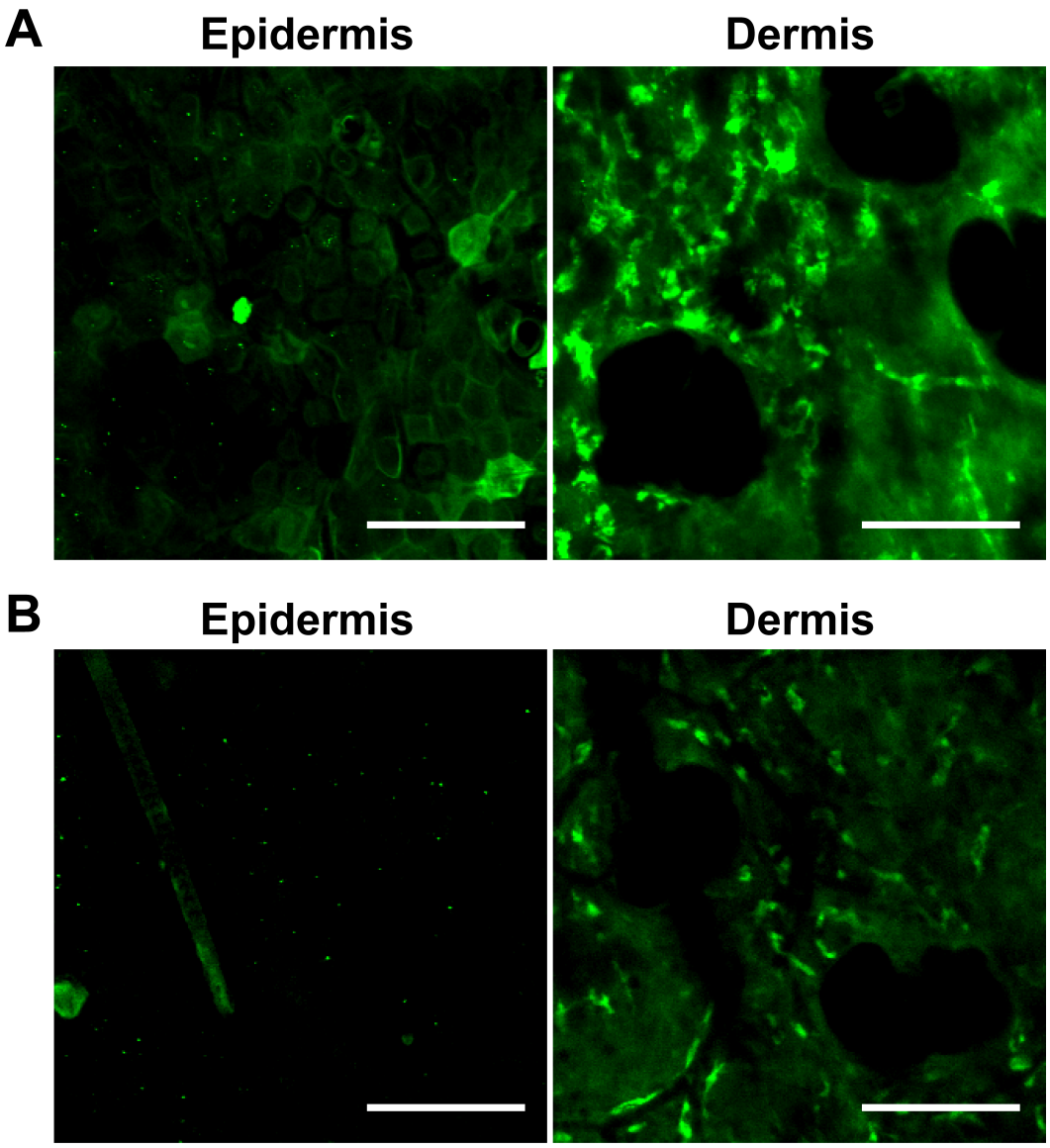
Supplementary Figure S1: Surface LC densities after local injection of FITC-DX probes. FITC-DX (70 kDa) or PBS alone was s.c. injected into the right or left ear of the same BALB/c mice (n = 5). At 24 hours after the injection, epidermal sheets were prepared from the ear skin to count the number of LCs after staining with AF_{546}-conjugated anti-MHC II mAb. Data shown are the means ± SD of LC numbers.



Supplementary Figure S2: Detection of FITC$^+$ cells in skin draining LN. FITC-DX (70 kDa) or PBS alone was s.c. injected into the right or left ear of the same BALB/c mice. At 12 or 36 hours after the injection, right and left cervical LNs were examined for the presence of FITC$^+$ cells after staining with mAb against MHC II or EpCAM or with isotype-matched control IgG.



Supplementary Figure S3: Uptake of FITC-DX probes of different molecular sizes. BALB/c mice received s.c. injection of FITC probes conjugated with DX with the indicated molecular sizes or 70 kDa AF_{546}-DX probes. At 36 hours after the injection, the mice were anesthetized and place under a confocal microscope to record fluorescence images. Scale bar: 200 μm.



Supplementary Figure S4: Uptake of FITC-OVA and FITC-mannan probes. BALB/c mice received s.c. injection of FITC-OVA probes (**a**) or FITC-mannan (**b**) into ear skin. At 48 hours after the injection, the mice were placed under a confocal microscope to record FITC fluorescence signals in the ear skin. Data shown are compiled x-y plane fluorescence images in the epidermal and dermal compartments. Scale bar: 100 μm.

Supplementary Movie Legends

Supplementary Movie S1: 3D distribution of FITC-DX probes immediately after injection. BALB/c mice received s.c. injection of 70 kD FITC-DX probes into ear skin. Immediately after the injection, the mice were placed under a confocal microscope to detect FITC-associated fluorescence signals in the ear skin. Data shown are sequential x-y plane fluorescence images at the indicated z-axis depths (μm) from the skin surface.

Supplementary Movie S2: 3D distribution of FITC-DX probes 12 hours after injection. BALB/c mice received s.c. injection of 70 kD FITC-DX probes into ear skin. At 12 hours after the injection, the mice were placed under a confocal microscope to detect FITC-associated fluorescence signals in the ear skin. Data shown are sequential x-y plane fluorescence images at the indicated z-axis depths (μm) from the skin surface.

Supplementary Movie S3: 3D distribution of FITC-DX probes 24 hours after injection. BALB/c mice received s.c. injection of 70 kD FITC-DX probes into ear skin. At 24 hours after the injection, the mice were placed under a confocal microscope to detect FITC-associated fluorescence signals in the ear skin. Data shown are sequential x-y plane fluorescence images at the indicated z-axis depths (μm) from the skin surface.

Supplementary Movie S4: 3D distribution of FITC-DX probes 48 hours after injection. BALB/c mice received s.c. injection of 70 kD FITC-DX probes into ear skin. At 48 hours after the injection, the mice were placed under a confocal microscope to detect FITC-associated fluorescence signals in the ear skin. Data shown are sequential x-y plane fluorescence images at the indicated z-axis depths (μm) from the skin surface. Note that linear and patchy areas showing high FITC signals represent an experimental noise caused by uneven placement of the ear under the confocal microscopy. In these areas, high fluorescence signals on the skin surface remained detectable even in deeper z-axis levels.

Supplementary Movie S5: Fate of locally injected FITC-OVA probes. BALB/c mice received s.c. injection of FITC-OVA probes into ear skin. At 48 hours after the injection, the mice were placed under a confocal microscope to detect FITC-associated fluorescence signals in the ear skin. Data shown are sequential x-y plane fluorescence images at the indicated z-axis depths (μm) from the skin surface.

Supplementary Movie S6: Fate of locally injected FITC-mannan probes. BALB/c mice received s.c. injection of FITC-mannan probes into ear skin. At 48 hours after the injection, the mice were placed under a confocal microscope to detect FITC-associated fluorescence signals in the ear skin. Data shown are sequential x-y plane fluorescence images at the indicated z-axis depths (μm) from the skin surface.

Supplementary Movie S7: Spatial relationship between internalized DX probes and MHC class II molecules. Anesthetized I-Aβ-EGFP knock-in mice received s.c. injection of 70 kD AF_{546}-DX probes into the ear skin. At 24 hours, the mice were examined under a confocal microscope. Images in the epidermal component (i.e., 0-19 μm z-axis depth range from the skin surface) are rotated to illustrate the spatial relationship between the internalized DX probes and MHC class II molecules. Scale bar: 200 μm.

Supplementary Movie S8: Spatial relationship between internalized DX probes and Langerin molecules. Anesthetized Langerin-EGFP-DTR knock-in mice received s.c. injection of 70 kD AF_{546}-DX probes into the ear skin. At 24 hours, the mice were examined under a confocal microscope. Images in the epidermal component (i.e., 0-19 μm z-axis depth range from the skin surface) are rotated to illustrate the spatial relationship between the internalized DX probes and Langerin molecules. Scale bar: 200 μm.