SUPPORTING INFORMATION

Multi-Scale Optical Imaging of the Delayed Type Hypersensitivity Reaction Attenuated by Rapamycin

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Figure S1. Imaging area for data analysis of the DTH reaction. The footpad contains six skin bulges, and the area inside the six skin bulges was the 'imaging area'.



Figure S2. Distribution of GFP-expressing CX₃CR1 cells and blood vessels in the footpad at 48 h post-challenge. Water-soluble QD was *i.v.* injected into CX₃CR1-GFP mice at 48 h post-challenge. Z-stack images of the footpad were acquired. The upper and middle rows represent single-layer images, showing GFP⁺ cells (green) in the footpad skin at various vertical depths along the z-projection. The extracellular matrix in the dermis was detected by the SHG signals (blue). Blood vessels in the dermis were labeled by QD (red). The lower row represents 3D reconstruction images, showing the distribution of GFP⁺ cells in relation to the SHG and blood vessel. Scale bar = 50 µm.



Fig. S3. Data processing method of the distribution of neutrophils. A-B) The 'imaging area' (left images) inside the region of six skin bulges was draw out from the whole images detected at 4 h (A) and 48 h (B) using the large-scale scanning microscopy, and was divided into 2,200 sub-areas (right images). C) The average fluorescence intensity of sub-areas distribution percentages of neutrophils during the elicited phage of the DTH reaction.



Figure S4. H&E stain of a cross-paw section of footpad at 48 h post-challenge. H&E section reveals the infiltration of leucocytes in CX₃CR1-GFP mice compared to rapamycin-treated mice. Scale bar = $100 \mu m$.