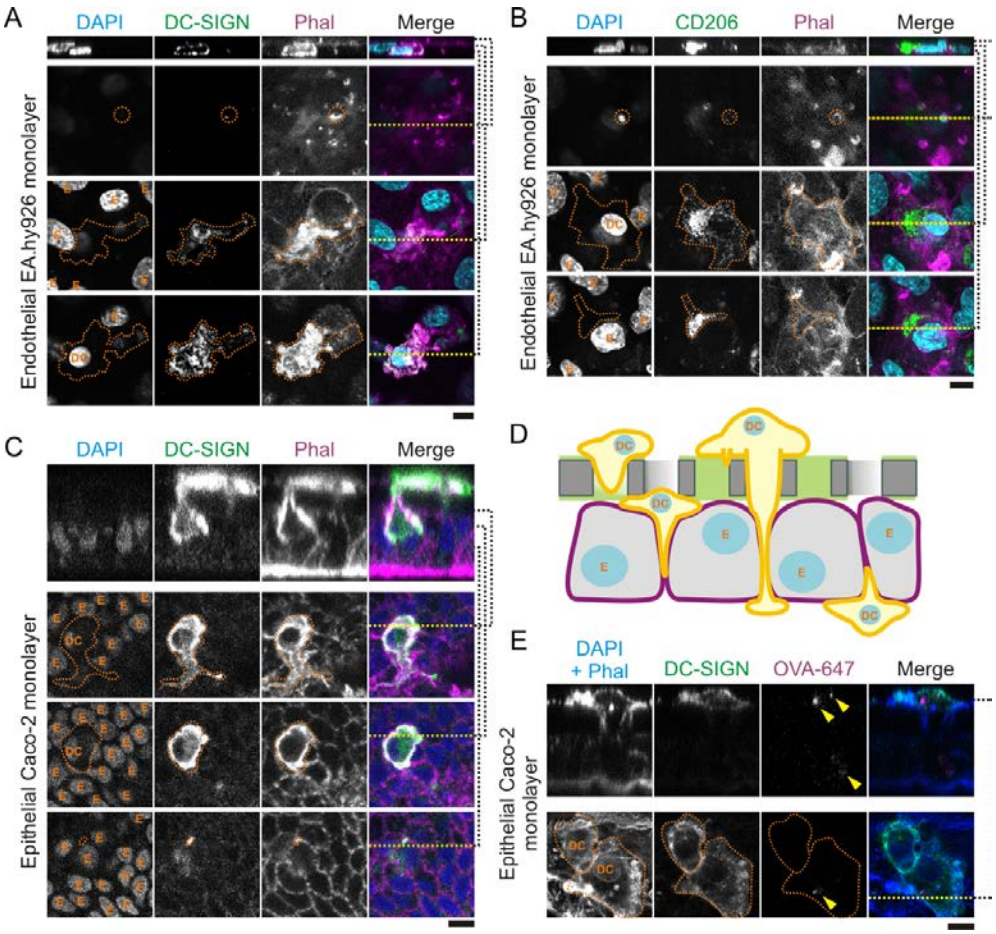
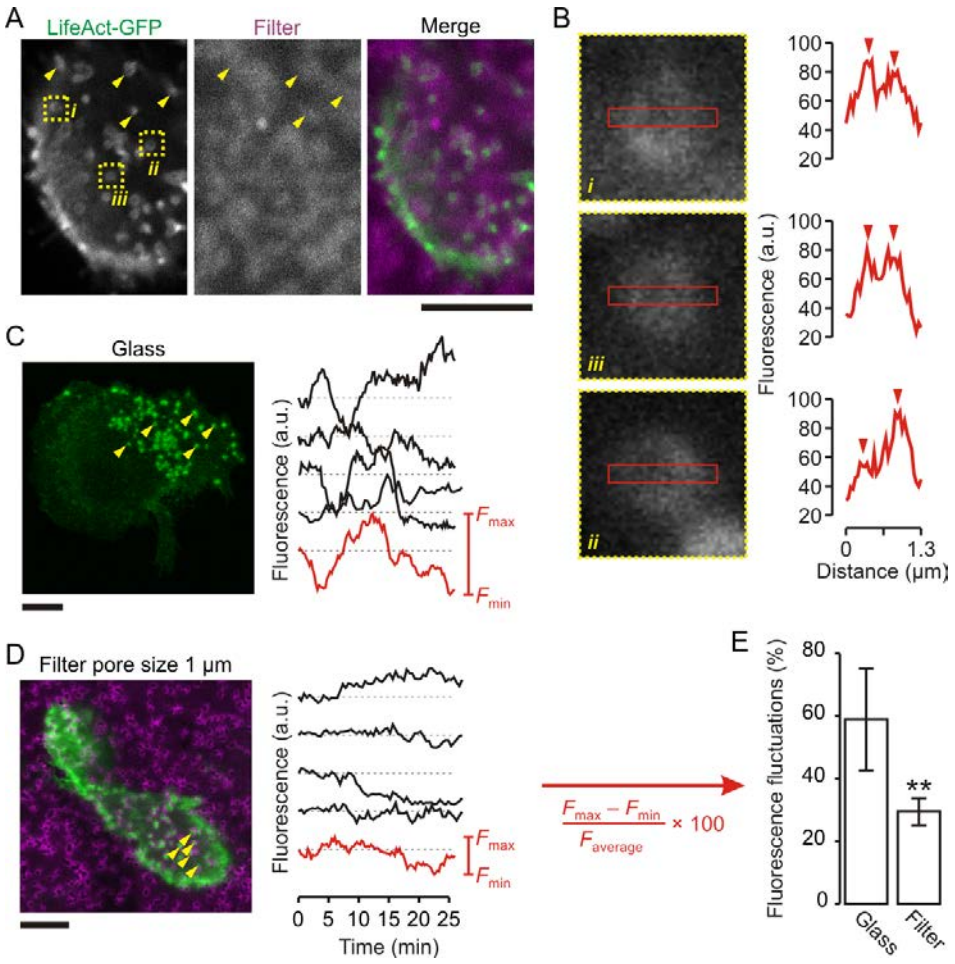
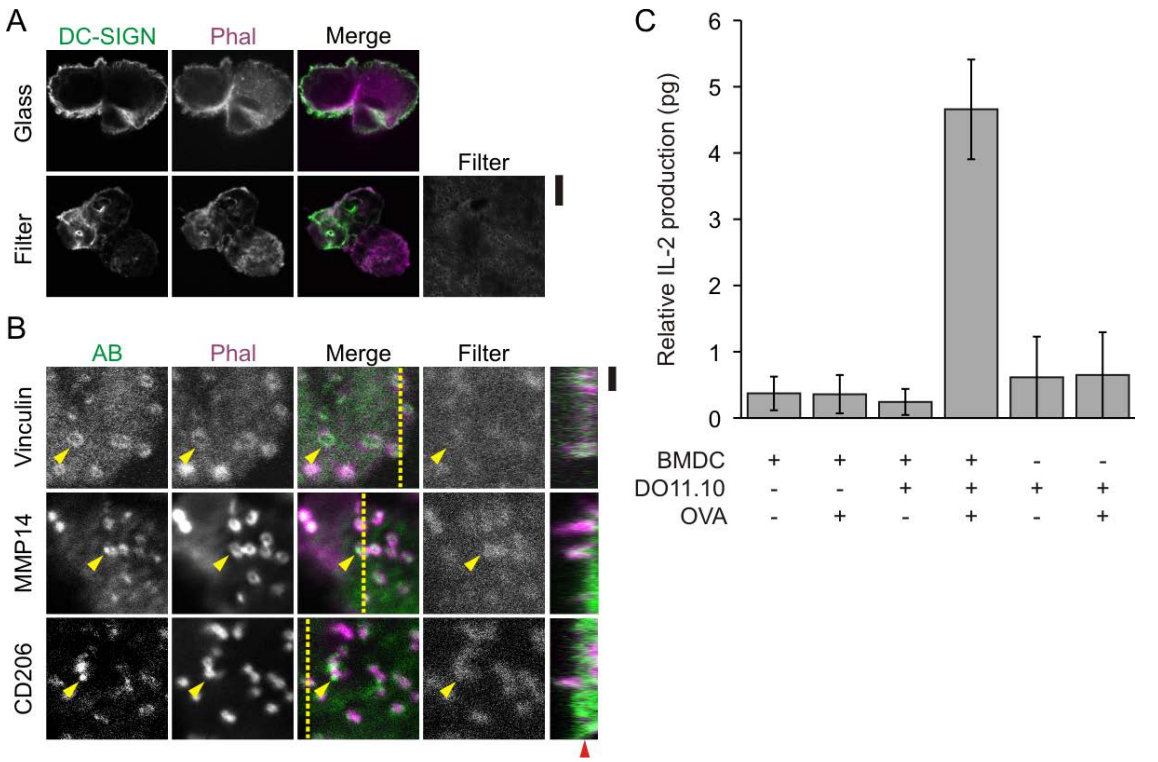


Supplementary Figure 1. Transmigration of dendritic cells through monolayers of endothelial and epithelial cells. (A) Confocal images of human dendritic cells cultured in inserts with gelatin impregnated polycarbonate filters with pore sizes of 5 µm. A monolayer of the endothelial cell line EA.hy926 (6 – 10 µm thick) was present on the opposite side of the filter. Dendritic cells were immunostained for DC-SIGN (green), actin was visualized by phalloidin-Alexa fluor 633 (magenta) and the nucleus by DAPI (cyan). The dotted outline indicates the position of a dendritic cell based on the DC-SIGN signal. Nuclei from endothelial cells are marked 'E'. Nuclei from dendritic cells are marked 'DC'. Several confocal sections underneath of the filter surface are shown (marked by the dashed yellow lines). (B) Same as A, but now for CD206 (green) instead of DC-SIGN. (C) Same as panel A, but now with a monolayer of the epithelial cell line Caco-2 (28 – 32 µm thick). (D) Schematic diagram of dendritic cell migration through the filter insert and through the endothelial or epithelial monolayer. (E) Uptake of Alexa fluor 647-labeled ovalbumin (OVA-647; magenta) through a monolayer of Caco-2 cells. Cells were immunostained for DC-SIGN (green); nuclei and actin were stained with DAPI and phalloidin-CruzFluor405 (Phal; Santa Cruz Biotechnology; both blue). Yellow arrow heads mark OVA-647 positive intracellular compartments. Scale bars, 10 µm.

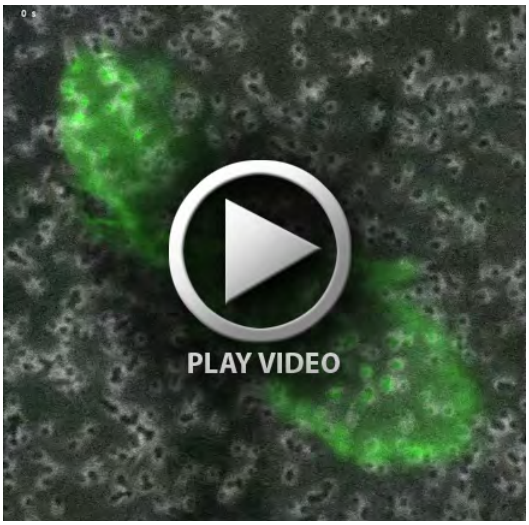


Supplementary Figure 2. Dynamics of podosome-like structures. (A) Live cell confocal imaging of human dendritic cells transfected with the actin reporter LifeAct-GFP (green) and cultured on filter with 1 µm pore size impregnated with gelatin-Alexa fluor 633 (Filter; magenta). The filter was gently pressed towards the cover slip to achieve a higher imaging resolution and to allow visualization of the LifeAct-GFP distribution in rings (compare with figure 3B). Yellow arrow heads mark randomly selected actin cores. (B) Magnifications of actin rings from panel A (marked *i–iii* in panel A) and fluorescence intensity distributions through the cross-sections marked by the red boxes (*y*-averaging). Orange arrow heads mark intensity peaks at the edges of the actin rings. (C–D) Live cell imaging of dendritic cells expressing LifeAct-GFP (green) and cultured on glass (C) and filter with 1 µm pore size impregnated with gelatin-Alexa fluor 633 (magenta) (D). Shown are the fluorescence intensities over time of LifeAct-GFP at randomly selected actin-rich cores (yellow arrow heads). Original data is in Supplementary Movies 1 and 2. (E) Quantifications of the fluctuations of LifeAct-GFP fluorescence from panels C–D. The change was calculated as the difference of the maximum and the minimum fluorescence intensities ($F_{max} - F_{min}$; see panels C–D) divided over the average fluorescence intensity $F_{average}$ (**, $P = 0.003$; 2-tailed unpaired t-test; 50 podosomes from multiple cells of 3 independent preparations). Scale bars, 10 µm.



Supplementary Figure 3. CHO cells, mouse bone marrow derived dendritic cells and T-cell activation. (A) Confocal images of CHO cells heterogeneously expressing recombinant DC-SIGN and cultured on glass or filters with 1 µm pore size and impregnated with Alexa fluor 633-labeled gelatin (Filter; grey). Actin was labeled with phalloidin-Alexa fluor 546 (Phal; magenta) and DC-SIGN was visualized by immunostaining (green). (B) Confocal images of mouse bone marrow derived dendritic cells (BMDCs) cultured on filters with 1 µm pore size and impregnated with Alexa fluor 633-labeled gelatin (Filter; grey). Actin was labeled with phalloidin-Alexa fluor 546 (magenta) and vinculin, MMP-14 and CD206 were visualized by immunostaining (AB; green). The yellow lines indicate the positions of the orthogonal views. The yellow arrows heads indicate randomly chosen actin cores. The red arrow head indicates the approximate filter surfaces. (C) IL-2 production by mouse hybridoma DO11.10 T-cells induced by BMDCs that were loaded with OVA-antigen through filters and measured after 6 days of T-cell activation. Due to (i) the great sensitivity of T-cells, (ii) the limited amount of dendritic cells we could culture on the filters (~10^3 cells) and (iii) the inability to completely wash OVA from the gelatin coated filters (due to non-specific binding), we cannot exclude the possibility that antigen was directly taken up from the medium by the BMDCs during the prolonged T-cell incubation step or that antigen leaked out of the BMDCs or was regurgitated to be presented to the T-cells in *trans* rather than in *cis*. Experiments were independently repeated at least 3 times. Data from a representative experiment is shown and error bars indicate the spread of data for at least 2 technical repeats. Scale bars, A: 10 µm; B: 2 µm.

Movie 1.



Movie 2.

Movie 3.

Supplementary Movie 1. Dynamics of podosome-like structures on glass substrate. Live cell confocal imaging of human dendritic cells transfected with the actin reporter LifeAct-GFP (green) and cultured on glass. Original data for supplementary figure 2C. The real time of each frame is printed on the top left.

Supplementary Movie 2. Dynamics of podosome-like structures on filter substrate. Live cell confocal imaging of human dendritic cells transfected with the actin reporter LifeAct-GFP (green) and cultured on filter with 1 µm pore size impregnated with Alexa fluor 633-labeled gelatin (magenta). Original data for supplementary figure 2D. The real time of each frame is printed on the top left.

Supplementary Movie 3. Uptake of OVA-647 antigen by dendritic cells through the filter. Live cell confocal imaging of human dendritic cells transfected with the actin reporter LifeAct-GFP (green) and cultured for 1 hr on filter with 1 µm pore size impregnated with gelatin. Prior to imaging (i.e. a few seconds before t = 0), a droplet containing 5 µg ml^{-1} of OVA-647 (magenta) was applied to the opposite side of the filter. Original data for figure 7F. The real time of each frame is printed on the top left.