Expression analysis using Affymetrix gene chips

Total RNA was isolated using QIAshredder and RNeasy Mini Kit spin columns (Qiagen, Hilden, Germany). Traces of genomic DNA were removed by on-column DNase digestion with RNase-free DNase set (Qiagen). All further steps were performed according to the manufacturer’s instructions (Affymetrix, High Wycombe, UK) and as described previously²².

Flow cytometry analyses

Intracellular staining of DCs was performed with the Cytofix/Cytoperm kit (BD) according to the manufacturer’s instructions. Staining of activated CD11/CD18 was performed as follows: DCs were harvested at the indicated time-points, washed and resuspended in PBS with 10 mM HEPES, 2% BSA (Roche, Basel, Switzerland) and 10 µg/mL of mAB24, CBRM1/5 or isotype controls, respectively. Cells were incubated for 10 min at 37°C, washed and fixed with 4% PFA (Carl Roth GmbH, Karlsruhe, Germany).

Western blot analyses

Cells were lysed in buffer containing 50 mM tris-HCl pH 7.2, 137 mM NaCl, 2 mM EDTA, 10% glycerol (all from Carl Roth GmbH, Karlsruhe, Germany), 1% NP-40 (Boehringer Mannheim, Mannheim, Germany), 1% octyl β-D-glucopyranoside, 2 mM PMSF, 20 mM NaF, and 2 mM NaVO₃ (all from Sigma). Subsequent to SDS-PAGE, transfer to PVDF
membranes (Millipore, Billerica, USA) and incubation with the appropriate primary and HRP-linked secondary antibodies, detection was performed with ECL Plus Western blotting detection system (GE Healthcare, Freiburg, Germany).

**siRNA transfections**

RNAi against the target sequence of human CYTIP was performed using 10 µg ON-TARGET plus SMARTpool siRNA (Dharmacon, Lafayette, USA). As control, 10 µg of a non-targeting, fluorescein-labeled Allstars Negative Control siRNA (Qiagen) was used. siRNAs were delivered into immature DCs by electroporation and DCs were subsequently matured as described previously\(^2^3\).

**Transwell migration assays**

Migration assays and quantification of migrated cells were performed as described previously\(^2^2\). Differing from the above mentioned protocol, the transwell inserts (Costar, London, UK) were coated over night with 20 µg/mL fibronectin (Sigma).

**T-cell-adhesion assay**

96-well microtest plates (BD Labware Europe, Meylan Cedex, France) were coated with 0.01% poly-L-Lysine. 5x10\(^5\) DCs were added to the wells and allowed to adhere for 1 hour at 37°C. Freshly isolated bulk human T-cells were stained with the Vybrant CFDA-SE Cell Tracer Kit (Invitrogen) according to the manufacturer’s instructions. Wells were washed and 2x10\(^5\) T-cells were added to each well and incubated for 45 min at 37°C. Unbound cells were removed by washing. Quantification of bound T-cells was performed using a Victor\(^2^\) multilabel counter (PerkinElmer, Jugesheim, Germany) with excitation
and emission wavelengths of 485 nm and 535 nm, respectively. Normalization was performed by setting the adhesion to mock-infected DCs as 1.

**Figure S1. Mature dendritic cells are efficiently infected with herpes simplex virus type 1 (TIF, 37 KB)**

Flow cytometric analysis demonstrates that monocyte-derived DCs are efficiently infected with HSV-1 EGFP at a MOI of 1. DCs were analyzed for expression of EGFP at the indicated time-points postinfection (pi), gated on viable cells. Dashed line indicates mock-infected DCs; solid line, HSV-1-infected DCs. Shown is one representative experiment out of three.
Figure S2. Individual tracks of mock-infected and HSV-1-infected DCs in collagen gels (TIF, 674 KB)

Mature DCs were either mock-infected (left panel) or HSV-1-infected (right panel). Subsequent to the infection procedure, cells were used for 3D migration assays in pure collagen gels (upper panel) and gels containing 20 µg/mL fibronectin (lower panel). HSV-1 strongly inhibits migration of DCs in both conditions. Chemotaxis towards a CCL19 gradient was monitored by bright-field time-lapse videomicroscopy beginning 90 min postinfection. Cells were imaged at a frame rate of 2 min over 180 frames. Shown are individual tracks of 30 randomly selected cells for each condition of one representative experiment out of three.
Figure S3. Efficient silencing of CYTIP in mature DCs (TIF, 82 KB)

Flow cytometric analysis of CD83, CD86, HLA-DR, CCR7 and CYTIP of untreated DCs (upper panel), cells electroporated with 10 µg of a non-targeting control siRNA (middle panel) and DCs electroporated with 10 µg CYTIP siRNA (lower panel) shows that maturation of DCs is not affected by RNAi of CYTIP. CYTIP was efficiently silenced by RNAi 48 h after electroporation. The empty profiles in the histograms indicate stainings with isotype controls. Shown is one representative experiment out of three.
Figure S4. RNAi of CYTIP in DCs enhances their adhesion to fibronectin (TIF, 69 KB)

Mature DCs (mock) and DCs electroporated with 10 µg of a non-targeting siRNA (control siRNA) or 10 µg CYTIP targeting siRNA (CYTIP siRNA) were allowed to bind to fibronectin. RNAi of CYTIP significantly increases the adhesion to fibronectin. Error bars indicate ± SD. ***$P<.001$, ns indicates not significant. The experiment was repeated 3 times independently and each single adhesion experiment was performed in quadruplicate.
Figure S5. Silencing of CYTIP in mature DCs impairs their migration (TIF, 679 KB)

Immature DCs were electroporated with 10 µg of a non-targeting control siRNA (left panel) or with 10 µg siRNA targeting CYTIP (right panel). Four hours later, maturation cocktail was added and DCs were matured for 48 hours. Subsequently, mature DCs were used in 3D migration assays towards CCL19 in pure collagen gels (upper panel) or in gels containing 20 µg/mL fibronectin (lower panel). Silencing of CYTIP strongly impairs chemotaxis of DCs in collagen gels containing fibronectin. Migration was monitored by bright-field time-lapse videomicroscopy. Cells were imaged at a frame rate of 2 min. For each condition, 30 individual tracks of randomly selected cells of one representative experiment out of three are visualized.
Figure S6. Migration timecourse of mock-infected and HSV-1-infected DCs (TIF, 218 KB)

3D migration assays of mock- or HSV-1 infected DCs towards a CCL19 gradient up to 6 hours postinfection in (A) fibronectin coated gels or (B) gels that contain ICAM-1-Fc coated beads show that HSV-1-infection impairs DC chemotaxis. Where indicated, LFA-1 blocking antibody was added in a concentration of 20 µg/mL. Blockade of LFA-1 partially restores migration. Cells were imaged at a frame rate of 2 min imaged by bright-field time-lapse videomicroscopy. The average speed was calculated as the step length /min (including non-moving periods) of 30 randomly selected cells and displayed in µm/min. One representative experiment out of three is shown.
Figure S7. T-cell detachment from HSV-1-infected DCs is impaired (TIF, 70 KB)

T-cells adhere stronger to infected DCs as shown by the T-cell adhesion assay. Mock-infected and HSV-1-infected DCs were applied to poly-L-lysine coated 96-well micro-test plates 4 h postinfection. Subsequently, CFSE-labeled bulk T-cells were allowed to adhere to the immobilized DCs for 45 min. After washing, adherent cells were quantified by measuring CFSE-fluorescence in a Victor² multilabel counter. Normalization was performed by setting the adhesion to mock-infected DCs to 1. Error bars indicate ± SD. *P<.05. The experiment was repeated 3 times independently and each single experiment was performed in quadruplicate.