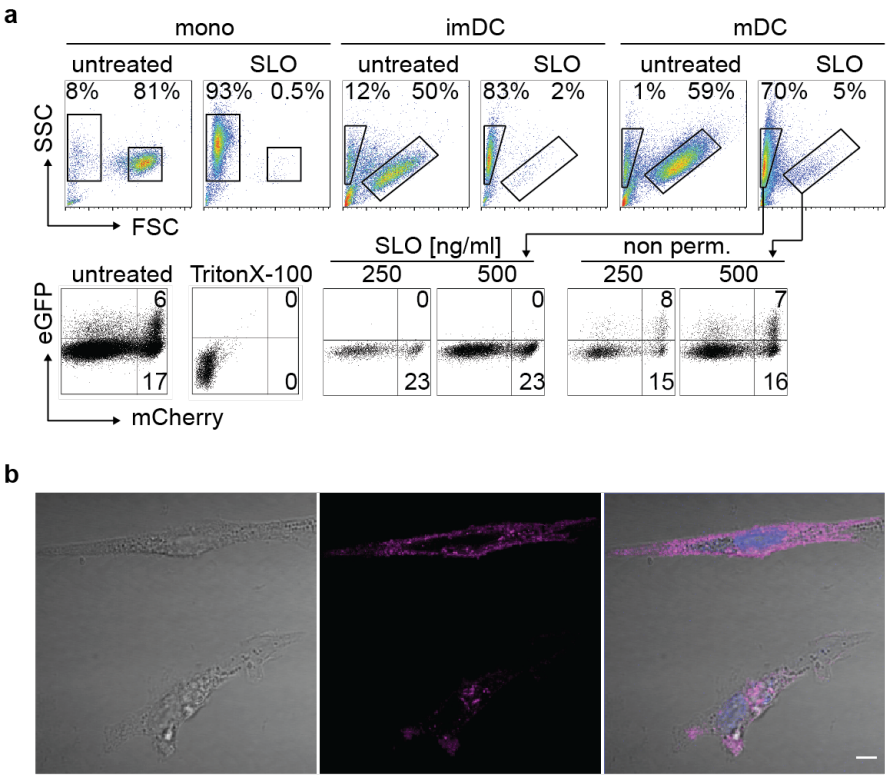
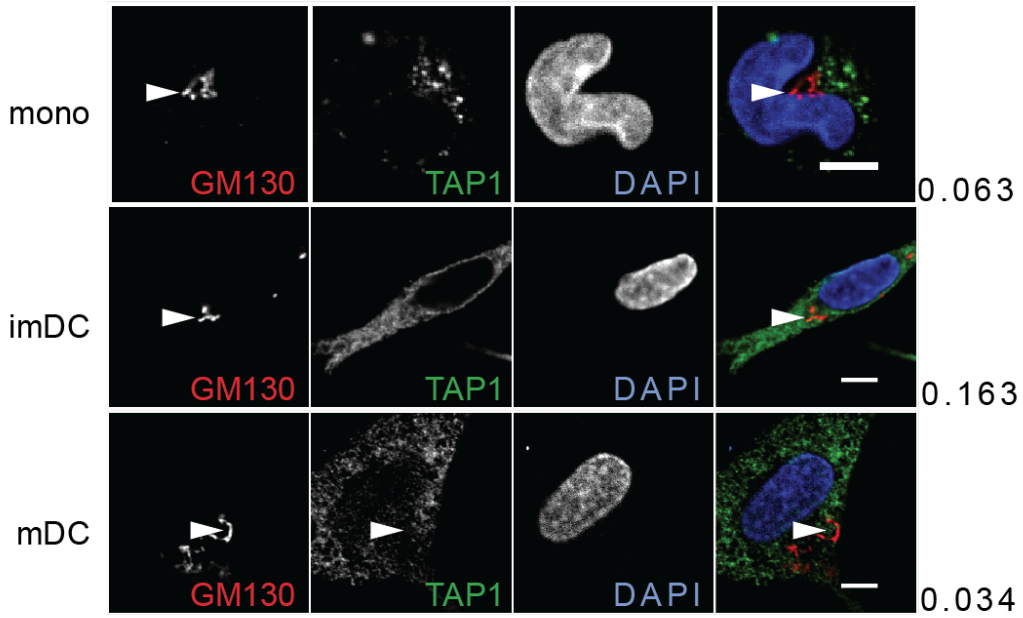
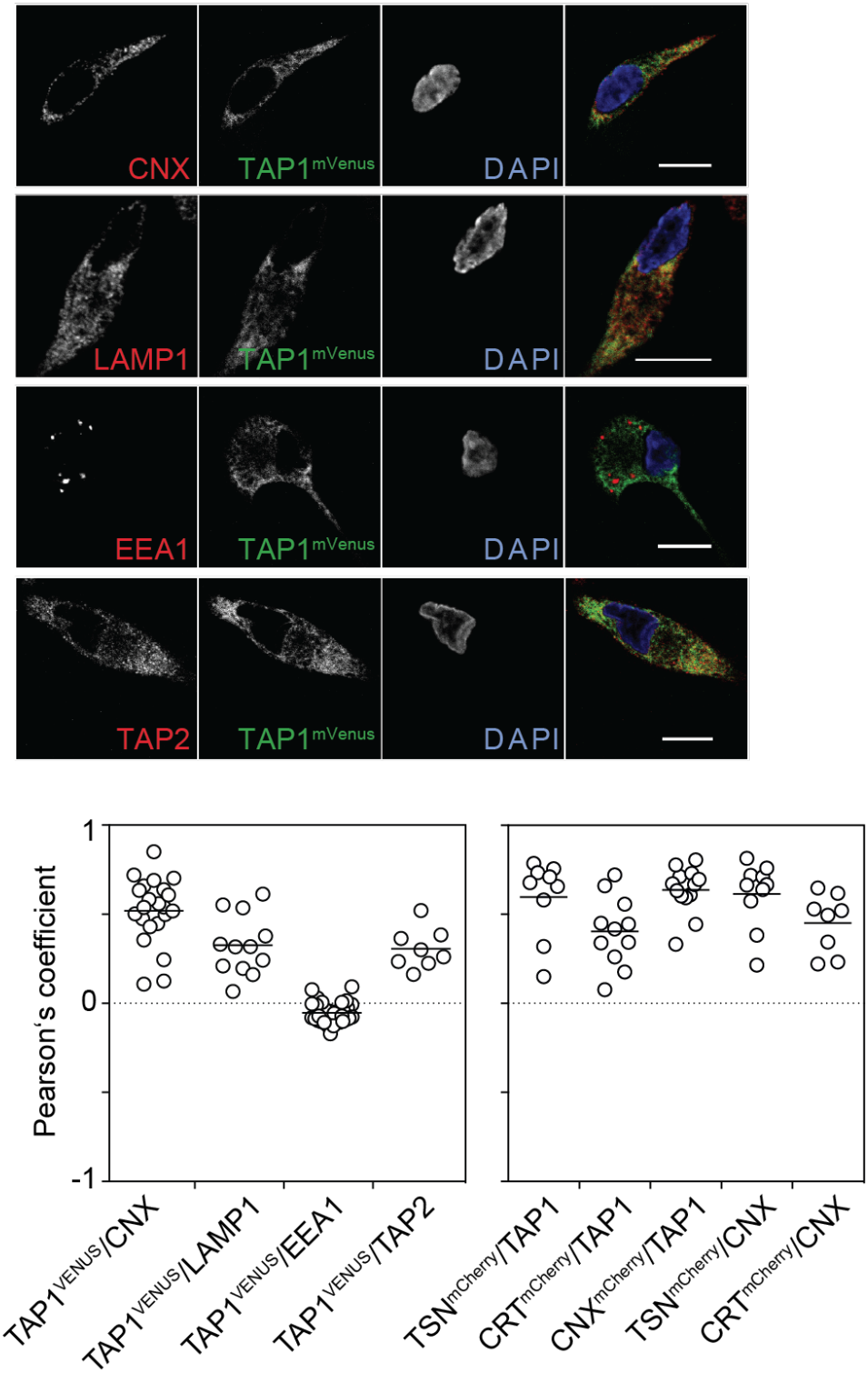
Supplementary Figures



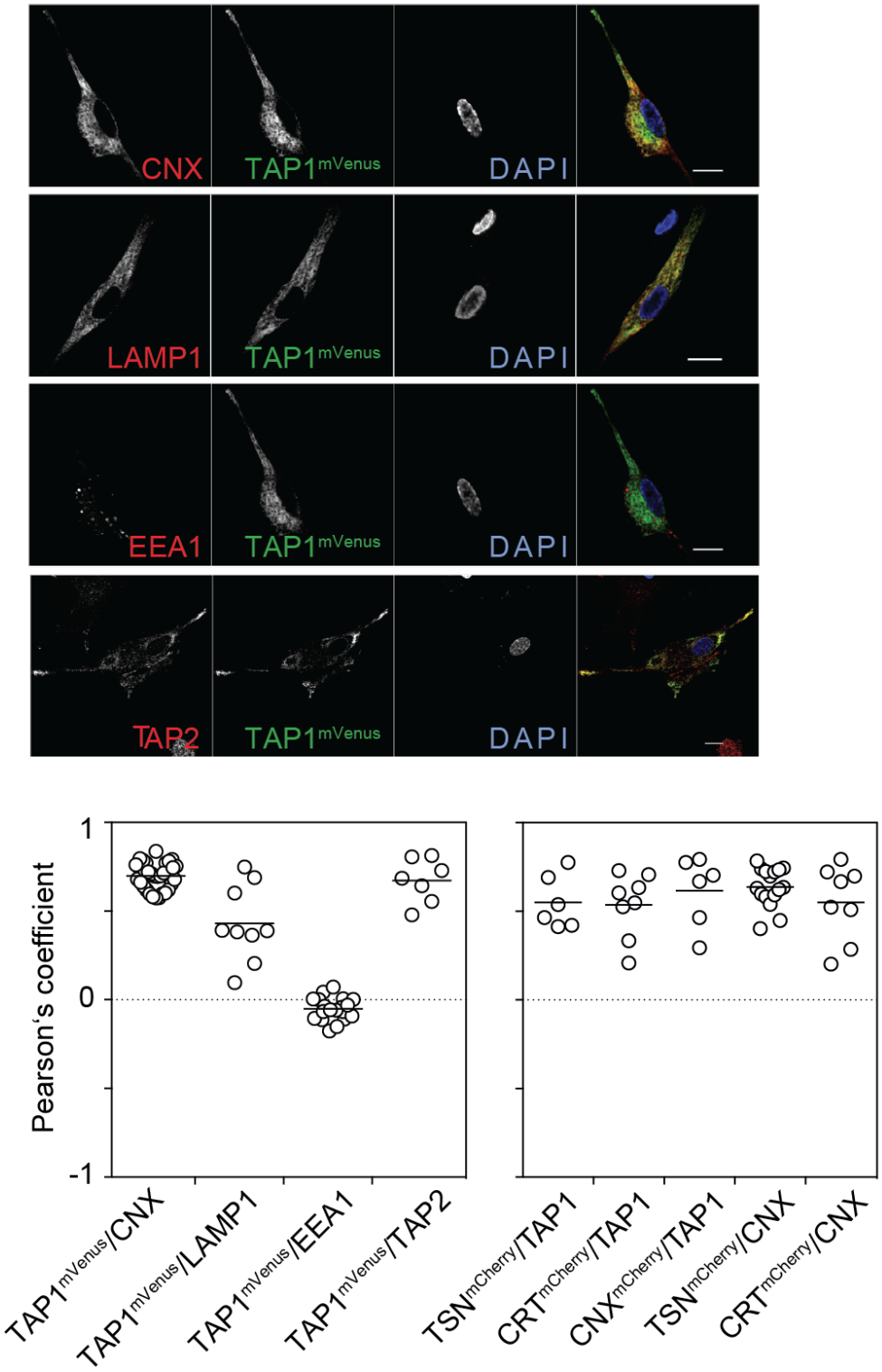
Supplementary Figure 1 Control for TAP-dependent peptide compartmentalization assay. (a) FSC/SSC characteristics upon semi-permeabilization of monocytes, imDCs and mDCs with streptolysin O (SLO) (upper panel). Cytosolic eGFP and ER-restricted mCherry expression of mDCs when left untreated, upon complete permeabilization with TritonX-100, or upon semi-permeabilization with SLO in permeabilized and non-permeabilized mDCs (lower panel). To assure permeabilization of a minimum of 70% of cells, different concentrations of SLO were applied for monocytes (250 ng/ml), imDCs (400 ng/ml), and mDCs (500 ng/ml). The degree of semi-permeabilization was assessed using mDCs expressing cytosolic eGFP (IRES-eGFP) and ER-resident mCherry (mCherry-KDEL). Upon SLO treatment no residual cytosolic eGFP fluorescence was detected, while ER-resident mCherry fluorescence was similarly abundant as in untreated cells (Supplementary Fig. 1, compare left panel and middle panels). The residual 5% of non-permeabilized mDCs (non-perm.) showed a similar eGFP and mCherry fluorescence as untreated mDCs (compare left and right in lower panels), whereas complete permeabilization by Triton X-100 treatment abolished detection of eGFP or mCherry positive cells. Thus, the SLO treatment of mDCs selectively perforated the plasma membrane while the integrity of ER membranes remained intact. (b) Montage of a bright field image and a CD83 immunolabeling (magenta) of mDCs. DAPI stain in blue indicates nucleus. Scale bars, 5 µm.



Supplementary Figure 2 In monocytes, imDCs, and mDCs TAP1 does not locate to the Golgi apparatus. Immunofluorescent photos upon immunolabeling of TAP1 and the *cis* Golgi network (GM130) in monocytes, imDCs and mDCs. Pearson's value indicated on the right. Scale bar, 5 µm.

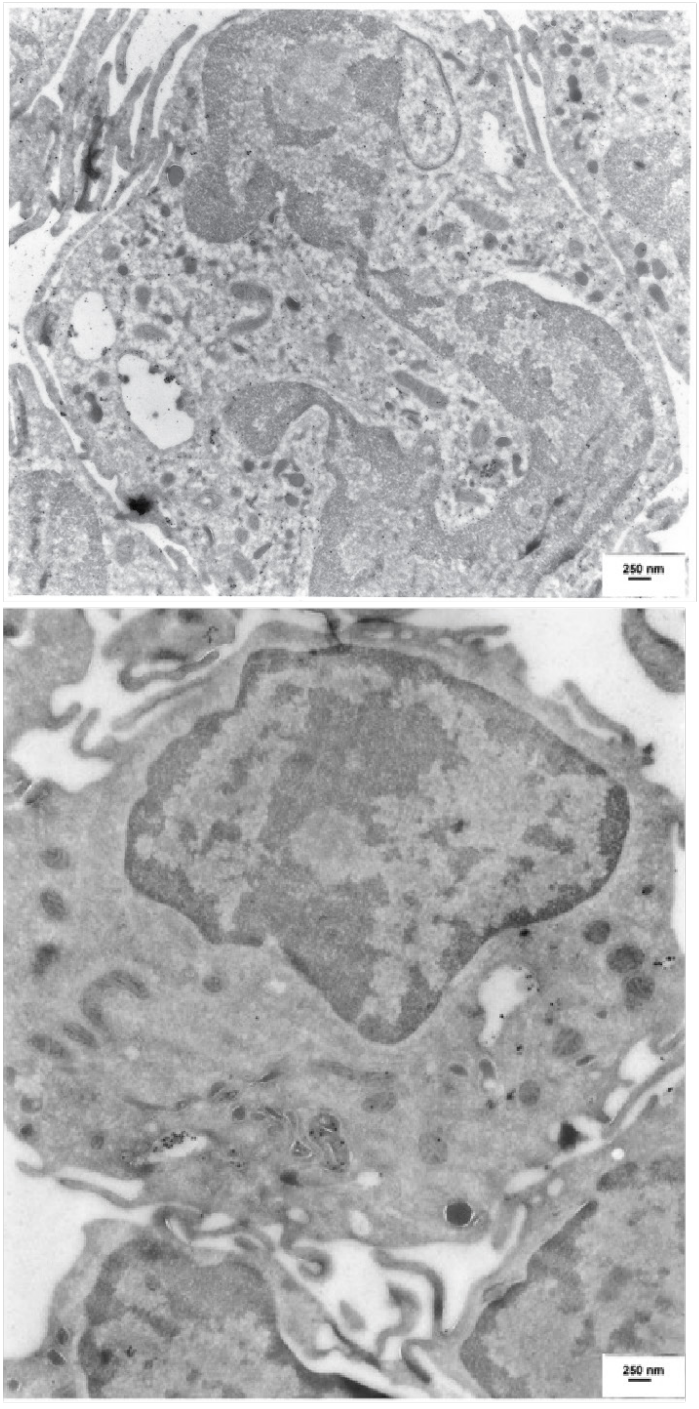


Supplementary Figure 3 In imDCs expressing TAP1^{mVenus}, TAP1 colocalizes strongly with CNX and to an intermediate degree with LAMP1 and TAP2. Immunofluorescent immunolabeling of CNX, LAMP1, EEA1, and TAP1. Scale bars, 10 µm (top panel). Pearson's coefficients were calculated using ImageJ intensity correlation analysis. White circles represent analysis of single cells obtained from imDCs of two donors (mean, left lower panel). Pearson's coefficients for the PLC components tapasin (TSN), and calreticulin (CRT) and TAP1 derived of single cells obtained from imDCs of two donors (means, right lower panel).

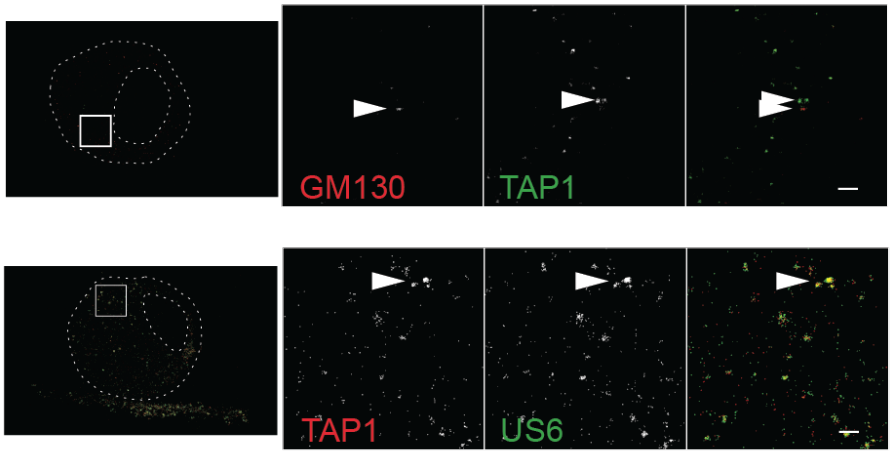


Supplementary Figure 4 In mDCs expressing TAP1^{mVenus}, TAP1 colocalizes strongly with CNX and TAP2 as well as to an intermediate degree with LAMP1. Immunofluorescent immunolabeling of CNX, LAMP1, EEA1, and TAP1. Scale bars, 10 µm (top panel). Pearson's coefficients were calculated using ImageJ Intensity correlation analysis. Statistical analysis of Pearson's coefficients of single cells obtained from mDCs of two donors (mean, left lower panel). Pearson's coefficients for the

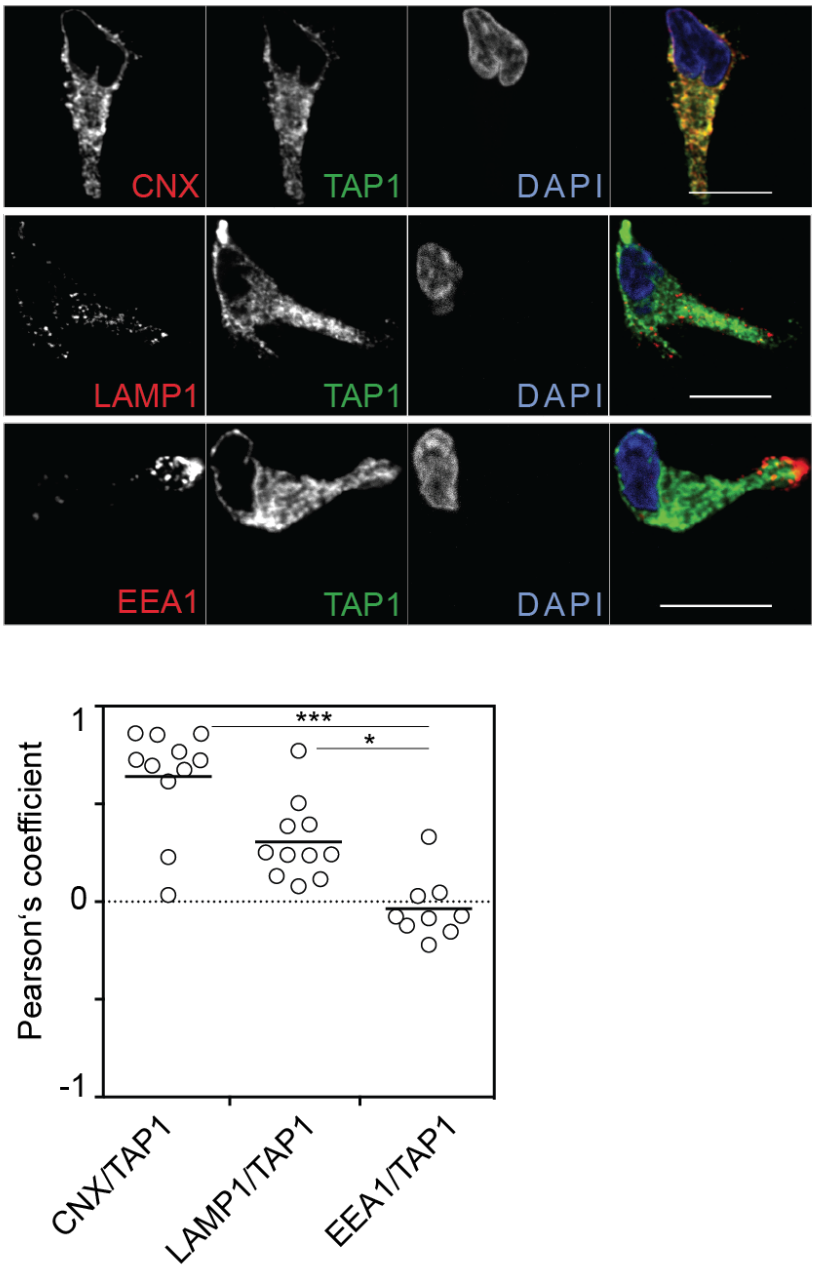
PLC components tapasin (TSN), and calreticulin (CRT) and TAP1 derived of single cells obtained from mDCs of two donors (mean, right lower panel).



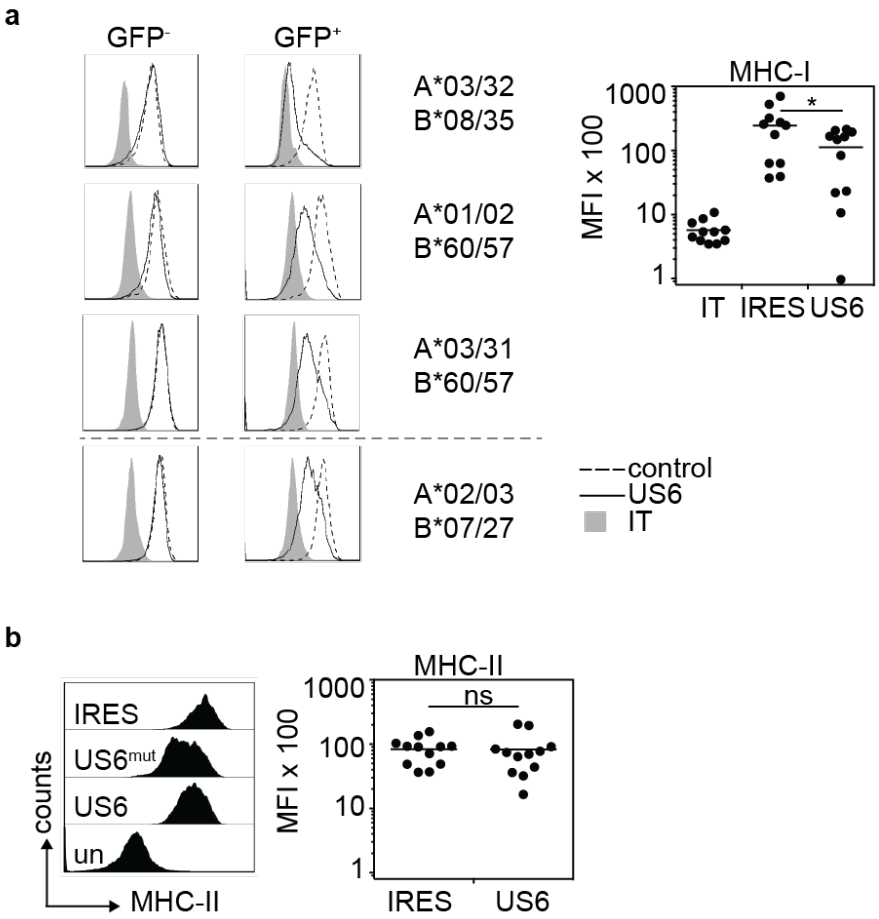
Supplementary Figure 5 High resolution microscopy illustrates that TAP is not located in the ER in monocytes, but in endosomes that are EEA1 positive. Immunoelectron microscopy of monocytes co-immunolabeling of TAP1 (15-nm gold particles) and CRT (10-nm gold particle), upper photo. EEA1 (15-nm gold particle) immunolabeling, lower photo. Scale bar, 250 nm.



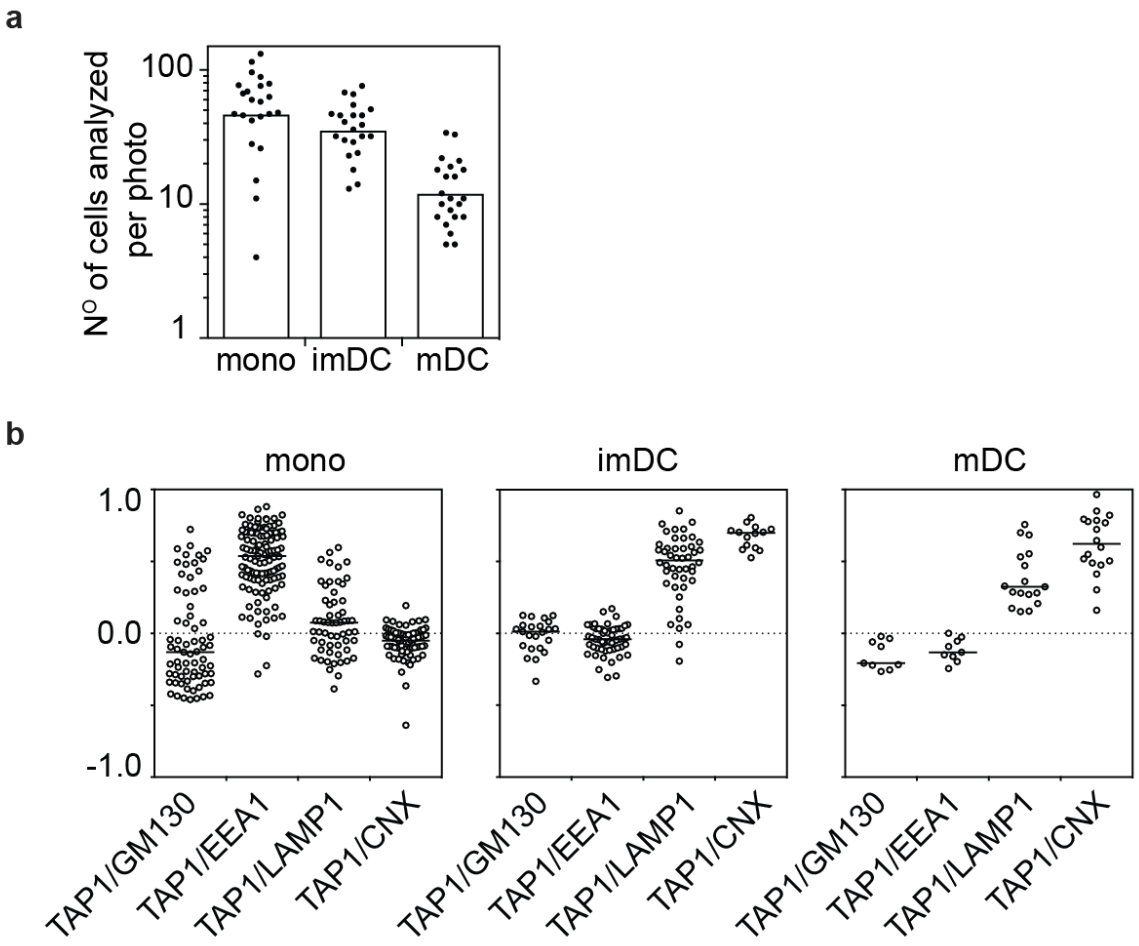
Supplementary Figure 6 STORM controls. STORM of mDCs stained for TAP1 and GM130, upper panel, and immunolabeling for TAP1 and US6, lower panel.



Supplementary Figure 7 In BDCA3$^+$ DCs TAP1 shows strong colocalization with CNX, intermediate colocalization with LAMP1 and no colocalization with EEA1. 5 x 10^5 BDCA3$^+$ MACS-enriched DC were seeded in Lab-Tek chambers and stained for indicated marker-proteins. Immunofluorescent immunolabeling of CNX, LAMP1, EEA1, and TAP1. Scale bars, 10 µm. Pearson's coefficients were calculated using ImageJ Intensity correlation analysis. Statistical analysis of single cells obtained from BDCA3$^+$ cells of one donor (means). ***: $p \leq 0.001$; *: $p \leq 0.05$; Kruskal-Wallis test with Dunn's correction for multiple comparison.



Supplementary Figure 8 TAP inhibition down-regulates MHC I on the surface of mDCs (a) Histogram of MHC I surface expression of moDCs transduced with a control vector (dashed line), expressing US6 (solid line), and isotype immunolabeling (grey filled) for four out of eleven donors that were haplotyped. Upper three haplotypes are TAP dependent and below dashed line is a TAP independent haplotype. Of note, even the TAP independent haplotype showed a reduction of MHC I expression. Statistics of MHC I surface expression of US6-expressing mDCs, regardless of haplotype. *: $p \leq 0.05$. Kruskal-Wallis test with Dunn's correction for multiple comparison (circles represent donors, means shown as black line). (b) Histogram of MHC II expression from a TAP dependent haplotype (left panel). IRES, vector control; $US6^{mut}$, inactive US6 mutant; US6, active US6; un, unstained. Statistics for MHC II expression. Kruskal-Wallis test with Dunn's correction for multiple comparison (Circles represent donors, means shown as black line).



Supplementary Figure 9 Exemplary immunofluorescence data set of one representative donor. (a) Distribution of the cells per photo analyzed for each cell type. Each dot represents a photo. (b) Pearson's values calculated for each cell identified on at least three photos from the same donor for the indicated immunolabeling pairs (Circles represent cells, means shown as black line).

Supplementary Materials and Methods

Antibodies us in flow cytometry and microscopy

Antibodies used for FACS analysis: anti-CD14 PacificBlue, clone: MΦP9.1, Becton Dickinson; anti-CD40 FITC, clone: 5C3, Biolegend; anti-CD83 APC, clone: HB15e, Becton Dickinson; anti-CD86 PE, clone: IT2.2, BioLegend; anti-HLA-ABC PE-Cy5, clone: W6/32, eBioscience; anti-HLA-ABC Streptavidin, clone: W6/32, eBioscience; anti-HLA-DR APC-Cy7, clone: L234, BioLegend; anti-CD45R0 PacificBlue, clone: UCHL1, Biolegend; anti-CD69 PE-Cy7, clone: FN50, BioLegend. anti CD8 APC, clone: RPAT4, BD Bioscience; anti-TNF-α PE-Cy7, clone: MAb11, BD Bioscience; IL-2 PacificBlue, clone: MQ1-17H12, BioLegend.

The following antibodies were used for immunolabeling: mouse monoclonal anti-EEA1, abcam; mouse monoclonal anti-TAP1 (148.3) hybridoma[1]; mouse monoclonal anti-TAP2 (435.3) hybridoma[1]; rabbit monoclonal anti-calnexin, abcam; rabbit monoclonal αanti-LAMP-1, EPITOMICS; rabbit polyclonal anti-calnexin, abcam; rabbit polyclonal anti-calreticulin, Sigma; rabbit polyclonal anti-EEA1, abcam; rabbit polyclonal anti-GM130, EPITOMICS; rabbit polyclonal anti-OSTC, abcam. As secondary antibody reagents the AlexaFluor-coupled antibodies from Invitrogen were used (donkey anti-goat AlexaFluor568; donkey anti-goat AlexaFluor647; donkey anti-mouse AlexaFluor568; donkey anti-rabbit AlexaFluor568; goat anti-mouse AlexaFluor488; goat anti-mouse AlexaFluor647; goat anti-rabbit AlexaFluor647). We analyzed for monocytes an average of 58 ± 16, for imDC 39 ± 8, and for mDC 14 ± 4 cells per photo **(Supplementary Fig. 9a)**. To compensate the different cell/photo ratios for monocytes and mDCs, for monocytes 3 photos and for DCs 5 photos were analyzed for each immunolabeling condition **(Supplementary Fig. 9b)**. The Pearson's coefficients were calculated for every immunolabeling combination of every cell subset of each donor and means were generated and statistically analyzed **(Fig. 4 and Supplementary Fig. 2,3,4,6)**.

STORM *microscopy*

The protocol for N-STORM sample preparation (http://nic.ucsf.edu/dokuwiki/lib/exe/fetch.php?media=nikon_storm_sample_preparation.pdf) was used. Briefly, affinity purified donkey anti-mouse IgG (H+L) (Jackson Immuno Research Europe) antibodies were labeled with activator-reporter pairs of AlexaFluor405-AlexaFluor647 fluorophores and affinity purified donkey anti-rabbit IgG (H+L) (Jackson Immuno Research Europe) antibodies were marked with activator-reporter pairs of Cy3-AlexaFluor647 in the molar ratio: activator dye : antibody : reporter dye = 2-3 : 1 : 0.6-1. After immunolabeling cells were kept in buffer at 4 °C until image recording. For superresolution imaging a Nikon N-Storm Ti eclipse inverted microscope equipped with four activation/imaging lasers of 405 nm (100 mW, Coherent Cube Diode Laser), 488 nm (65 mW), 561nm (100 mW, Coherent Sapphire) and 647 nm (150 mW), an EMCCD camera

(Ixon DV 897DCS-BV, Andor) and an autofocus system (Nikon Perfect Focus) was used. Lasers were attenuated/shuttered by an AOTF and coupled into a single mode optical fiber. The fiber output was collimated and focused to the back focal plane of an oil immersion objective (Nikon TIRF 100x Plan Apo 1.49 NA) on the microscope. Before starting the 2-channel image acquisition with a pixel resolution of 512 x 512 in TIRF mode the reporter fluorophores (AlexaFluor647) were switched to the dark-state by illuminating the sample with light of the imaging/deactivation laser (647 nm). Then data capturing of the Alexa405-Alexa647 dye pair was performed by imaging cycles comprised of one frame of 405 nm activation laser illumination followed by three frames of 647 nm imaging/deactivation laser illumination. Subsequently, the photo switchable dye pair Cy3-Alexa647 was activated by one frame of 561 nm laser illumination followed by another series of three frames of 647 nm laser illumination. Data acquisition and image analysis was done by using NIS Elements Microscope Imaging Software (Nikon), which used only the first out of the three imaging frames for image reconstruction and provided algorithms for crosstalk reduction between channels as well as drift correction by autocorrelation. To register enough events for reconstructing a reliable 2-color super resolution image data recording took on average 15 to 20 min at a rate of 20 frames per second. During N-Storm imaging cells were incubated in an imaging buffer consisting of 50 mM Tris/HCl pH 8.0, 0.5 mg/ml glucose oxidase from *Aspergillus niger*, 40 µg/ml catalase from bovine liver, 10% D(+) glucose, and 100 mM mercaptoethylamine (MEA). The imaging buffer was always freshly prepared from 3 stock solutions consisting of (1) 10 mg/ml glucose oxidase, 2 mg/ml catalase, 50 mM Tris/HCl pH 8.0, 2.5 ml glycerol; (2) 50 mM Tris/HCl pH 8.0, 10% glucose; (3) 50 mM Tris/HCl pH 8.0, 1 M MEA,. To obtain 1 mL of imaging buffer 850 µL of (2) were mixed with 100 µL of (3) and 50 µL of (1).

Electron microscopy

50 nm cryosections were cut at -120 ºC using diamond knives in a cryoultramicrotome (Leica) and transferred with a mixture of sucrose and methylcellulose onto formvar-coated copper grids. The grids were placed on 2% gelatine. Ultrathin frozen sections were incubated at room temperature with mouse monoclonal anti-TAP1 (148.3) hybridoma[1]; rabbit polyclonal anti-EEA1, abcam, followed by a bridging rabbit anti-mouse and incubation with protein A-conjugated colloidal 10 nm gold (EM Lab) as first marker. In double labeling experiments the monoclonal was used first with 15-nm gold particles and after in between fixation with 1% glutaraldehyde for 10 min the incubation with the rabbit antibodies, against calreticulin (ABR), labeled with 10-nm gold particles was done. After immuno labeling, the sections were embedded in a mixture of methylcellulose and uranyl acetate and examined with a Philips CM10 electron microscope (FEI company).

Analysis of TAP-dependent peptide compartmentalization

TAP-dependent peptide transport was analyzed on the basis of translocated and glycosylated fluorescent peptides. The human histone H3-derived reporter epitope NST-F (RRYQNSTC$^{(F)}$L), fluorescein labeled, was used for this assay. Per sample 2 x 10^5 cells were transferred into a 1.5 ml reaction tube. Cells were washed twice with DPBS (350 *xg*; 10 min). Supernatant was discarded and the pellet was resuspended with 50 µl of a cell type specific concentration of streptolysin O (SLO). Monocytes were treated with 250 ng/ml, imDCs with 400 ng/ml and mDCs with 500 ng/ml. After a 15 min incubation at 4 °C, cells were centrifuged (1 min, 400 *x*g) and the pellet was resuspended either in transport buffer (10 nM peptide, 10 mM ATP in 10 mM $MgCl_2$ in DPBS) or ADP buffer (10 nM peptide, 10 mM ADP in 10 mM $MgCl_2$ in DPBS). Samples were incubated at 37 °C and 350 rpm for 15 min. Transport was blocked by the addition of 150 µl stop-buffer (20 mM EDTA in 10 mM $MgCl_2$ in DPBS). Subsequently cells were immediately analyzed in the flow cytometer.

Visualization of immunofluorescence

The program ImageJ (v1.46) was used to produce overlays and image montages as presented in the results section. For the analysis of transduced imDCs and mDCs ImageJ's intensity correlation analysis plug-in was used. The cells were identified and marked as regions of interest (ROI) and correlation coefficients were calculated for the signals from selected channels (blue, green, and red) in the respective ROI.

Statistical analysis of immunofluorescence

In detail, the program analyzed each pixel and determined the grey scale value assigned to each pixel. After background subtraction, these pixels were clustered to determine cell size and shape using a density-clustering algorithm. After cells were defined for the selected channels (blue, green, and red) a correlation coefficient for these cells was calculated. For our purpose, for every individual cell the Pearson's coefficient of TAP1 with one of the subcellular organelle markers was calculated.

1 Meyer, T. H., van Endert, P. M., Uebel, S., Ehring, B. & Tampe, R. Functional expression and purification of the ABC transporter complex associated with antigen processing (TAP) in insect cells. *FEBS Lett* **351**, 443-447 (1994).