SUPPLEMENTAL DATA

Sources of cell lines

HaCat (human keratinocyte cell line) was a gift from Rajiv Khanna, Brisbane, Australia. MDAMC (human breast carcinoma cell line) was a gift from Irene Joab, Paris, France. HeLa and 293 were purchased from ATCC. The EBV-transformed B lymphoblastoid cell lines MS-LCL and CM-LCL were generated by culturing PBMCs of a healthy donor with supernatant of the marmoset cell line B95.8 with RPMI-1640 + 20% FCS + 2 mM glutamine + 2 μg/ml gentamycin + 1 μg/ml cyclosporine A. The two EBV-positive Hodgkin's lymphoma cell lines RPMI6666 and L591 were purchased from ATCC and a gift from Martina Vockerodt and Dieter Kube, Göttingen, Germany, respectively. Mouse hybridomas IVA12 (anti-human MHC class II) and w6/32 (anti-human MHC class I) were purchased from ATCC.

Generation of influenza A virus matrix protein (MP1)-specific T cell clones

CD14-negative PBMCs isolated from whole blood of a lab donor (HLA-A*0201, -A*6801, -B*4402, -B*0702, -C*0501, -C*0702, -DRB1*1501, -DRB1*0401, -DRB5*01, -DRB4*01, -DQBI*0602 and –DQB1*0301) were stimulated with autologous mature DCs electroporated with in vitro transcribed Influenza A MP1-RNA, a gift from Irina Tcherepanova, Durham, NC (PBMC:DC ratio = 30:1, medium: RPMI-1640 with 5% human serum + glutamine + gentamycin). DCs were electroporated with 10 μg RNA in Opti-MEM at 300 V and 150 μF with a BioRad Gene Pulser plus Capacitance Extender (BioRad). On day 8 of PBMC/DC coculture, the stimulation was repeated and 10 U/ml IL-2 were added to enhance T cell survival. On day 21, the surviving cells were cloned by limiting dilution at 10, 1, or 0.3 cells/well and expanded in RPMI-1640 + 8% PHS + 150 U/ml rhIL-2 (Chiron) + 1μg/ml PHA-L (Sigma-Aldrich) + glutamine + gentamycin. 10^5 irradiated PBMCs/well and 10^4 irradiated LCLs/well were added as feeder cells. On day 40, expanded cells were tested in split-well IFNγ ELISPOT assays for recognition of an MP1 peptide mix (64 15-mer peptides overlapping by 10

amino acids) and the HLA-A2 immunodominant epitope $MP1_{58-66}$ (GILGFVFTL). All peptides were purchased from the Proteomics Resource Center of the Rockefeller University. MP1-specific, homogenously $CD4^+$ or $CD8^+$ clones were expanded as described above and frozen into aliquots.

Inhibitors and recombinant proteins

Chloroquine (CQ) and protease inhibitors (E64, Leupeptin and Pepstatin A) were purchased from Sigma. Recombinant human IFN-γ was purchased from ProSpec-Tany TechnoGene LTD, Israel and was used at 200 U/ml to induce MHC class II expression. Recombinant IFN-α-2b was from Schering Corporation, Kenilworth, NJ.

Generation of expression plasmids and lentiviral constructs

The cDNA of human MAP1LC3B sequence (NM_022818) was cloned into the mammalian expression vector pEGFP-C2 (Clontech). The cDNA of Influenza A/WSN/33 matrix protein 1 (MP1) was PCR-amplified from the pCAGGS/MCS-MP1 vector, a gift from Peter Palese, Mount Sinai School of Medicine, New York, with or without a stop codon at the 3' end. The PCR products then were inserted into the pEGFP-LC3 vector in place of the EGFP sequence to obtain MP1-LC3 fusion constructs. For lentiviral constructs, the EGFP-LC3, MP1-LC3 or MP1Stop-LC3 sequences were subcloned into the lentiviral vector pHR-SIN-CSGWΔNotI, a gift from Jeremy Luban, Columbia University, New York. For production of lentiviral particles, lentiviral vectors were co-transfected with the helper plasmids pCMVΔR8.91 and pMDG into 293T cells by calcium phosphate transfection. Culture supernatants containing recombinant viral particles were harvested on day 1, 2 and 3 after transfection, filtered through a 0.45 μm filter and frozen at –80ºC.

Lysate preparation and immunoblotting

Cells were lysed in ice cold lysis buffer (50 mM Tris-HCl pH 8.0, 140 mM NaCl, 1.5 mM $MgCl_2$, 0.5% NP-40 with Complete protease inhibitor cocktail from Roche) for 5 min on ice (about 10^6 cells/200 μl). Whole cells and cell debris were pelleted by low speed centrifugation (400 g, 3 min) and cleared supernatants were transferred to a new tube. Protein concentration was determined by BCA protein assay (Pierce). Samples were boiled for 5 min in the presence of 4x SDS-PAGE-loading buffer (250 mM Tris-HCl pH 6.8, 40% glycerol, 8% SDS, 0.57 M β-mercaptoethanol, 0.12% bromophenol blue). Equal amounts of protein were run on 11 or 12% SDS-PAGE gels and transferred onto a PVDF membrane (Hybond-P, Amersham Biosciences). Primary antibodies were visualized with HRP-conjugated goat anti-rabbit or anti-mouse IgG (Biorad) and the ECLplus detection system (Amersham Biosciences).

Flow cytometry

MHC class I and II surface levels on T cell targets were measured by staining cells with IVA12 or w6/32 hybridoma supernatants and AlexaFluor488-conjugated rabbit a-mouse IgG (Invitrogen-Molecular Probes). Cells were analyzed on a FACScalibur instrument (Becton-Dickinson).

Figure S1:

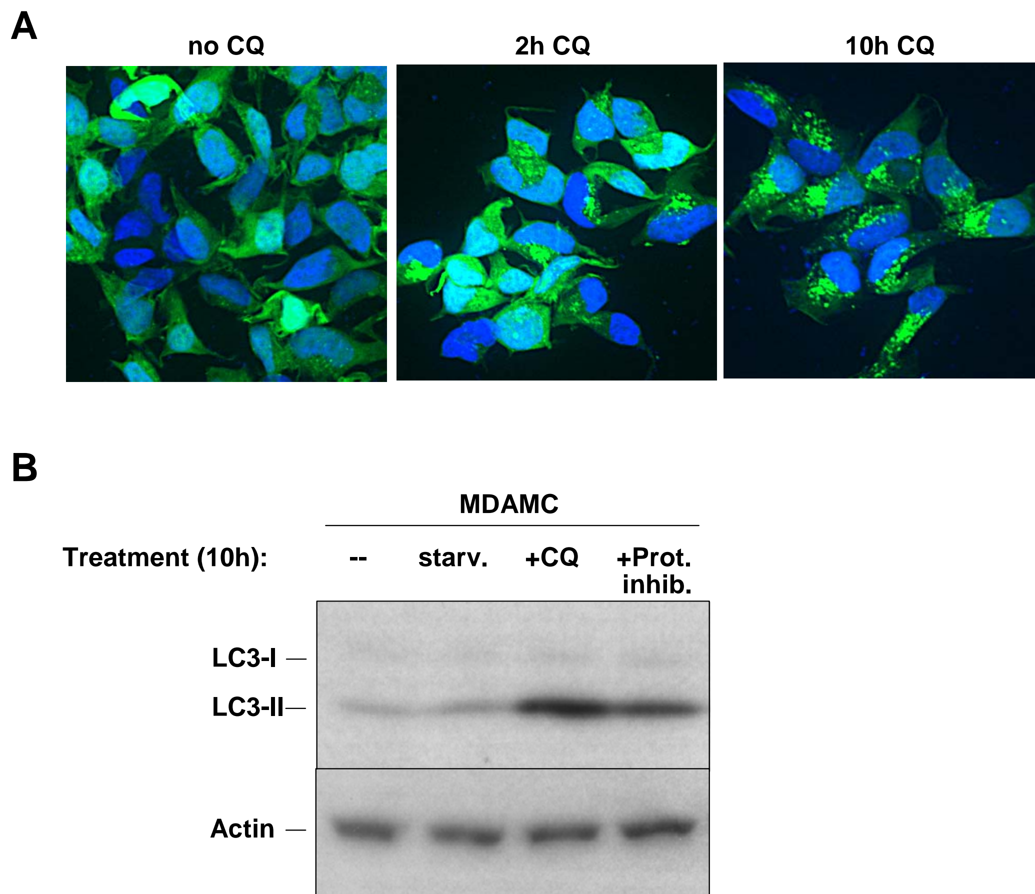


Figure S1: Chloroquine treatment induces gradual accumulation of GFP-LC3 in autolysosomes and differs substantially from nutrient starvation

(A) 293 cells stably transfected with a GFP-LC3 reporter construct were left untreated (no CQ) or were treated with 50 µM chloroquine (CQ) for 2 or 10 hours. Cells were fixed, stained with DAPI and analyzed by fluorescence microscopy. Inhibition of lysosomal acidification with CQ leads to a gradual accumulation of GFP-LC3-labeled autophagosomes over time. Representative fields from one experiment out of two are shown.

(B) MDAMC cells were left untreated (--), cultured in Hanks Balanced Salt Solution (starv.), treated with 50 µM chloroquine (+CQ) or with the protease inhibitors E64 (28 µM), Leupeptin (40 µM) and Pepstatin A (15 µM) (+Prot. inhib.) for 10 hours. Whole cell lysates were run on a 12% SDS-PAGE

gel and LC3-I and II were visualized by anti-LC3 Western blotting. Actin blot demonstrates equal protein loading. While nutrient starvation induces LC3-II only weakly, inhibition of lysosomal proteases by treatment with CQ or the protease inhibitors E64, Leupeptin and Pepstatin A leads to a strong accumulation of LC3-II. One of two experiments is shown.

Figure S2:

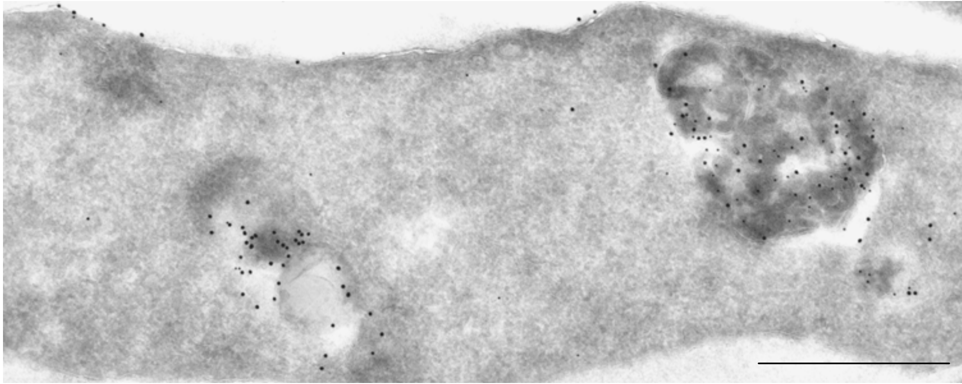
293T HaCat MDAMC
IFN: -- α γ -- α γ -- α γ
*
*
LC3-I
LC3-II

Figure S2: Recombinant IFNs do not affect macroautophagy in human epithelial cells

Human epithelial cell lines (293T, HaCat and MDAMC) were treated for 24 h with 1000 U/ml recombinant human IFN-α or IFN-γ or were left untreated (--). Whole cell lysates were prepared and equal amounts of protein were run on a 12% SDS-PAGE gel. LC3-I and –II were visualized by anti-LC3 Western blotting. The high molecular weight bands marked with an asterisk (*) are proteins that cross-react with the LC3 antiserum and demonstrate equal protein loading. LC3-II levels and hence macroautophagy are not affected by the IFN treatment.

Figure S3:

A



B

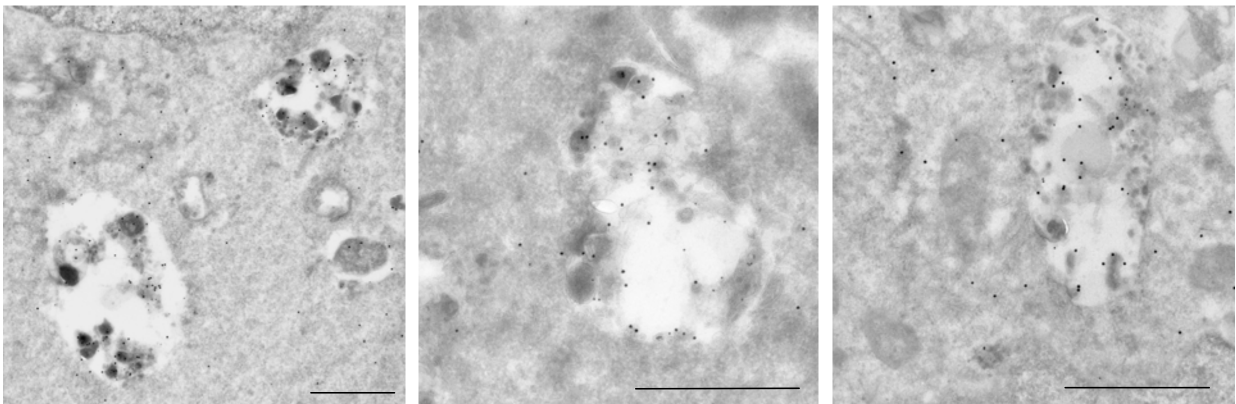


Figure S3: Immuno-electron microscopy of MHC class II/GFP-LC3 labeled cryosections

(A) Ultrathin cryosections of PFA-fixed MDAMC-GFP-LC3 cells were double-labeled with anti-HLA-DR antiserum/15 nm gold particles and anti-GFP antiserum/10 nm gold particles and analyzed by electron microscopy. MHC class II labeling can be seen both on GFP-LC3-positive electron-dense multivesicular compartments and on the plasma membrane. One representative field from one experiment out of three is shown. Scale bar: 1 μm.

(B) MDAMC-GFP-LC3 cells were treated with 50 μM CQ for 10h and ultrathin crysections were double-labeled for MHC class II (10 nm gold) and GFP (15 nm gold) and analyzed by electron-microscopy. Double-labeled multivesicular compartments frequently appear expanded and swollen,

with a diameter of >1 μm and some empty space. Three representative fields from one experiment out of three are shown. Scale bar: 1 μm.

Figure S4:

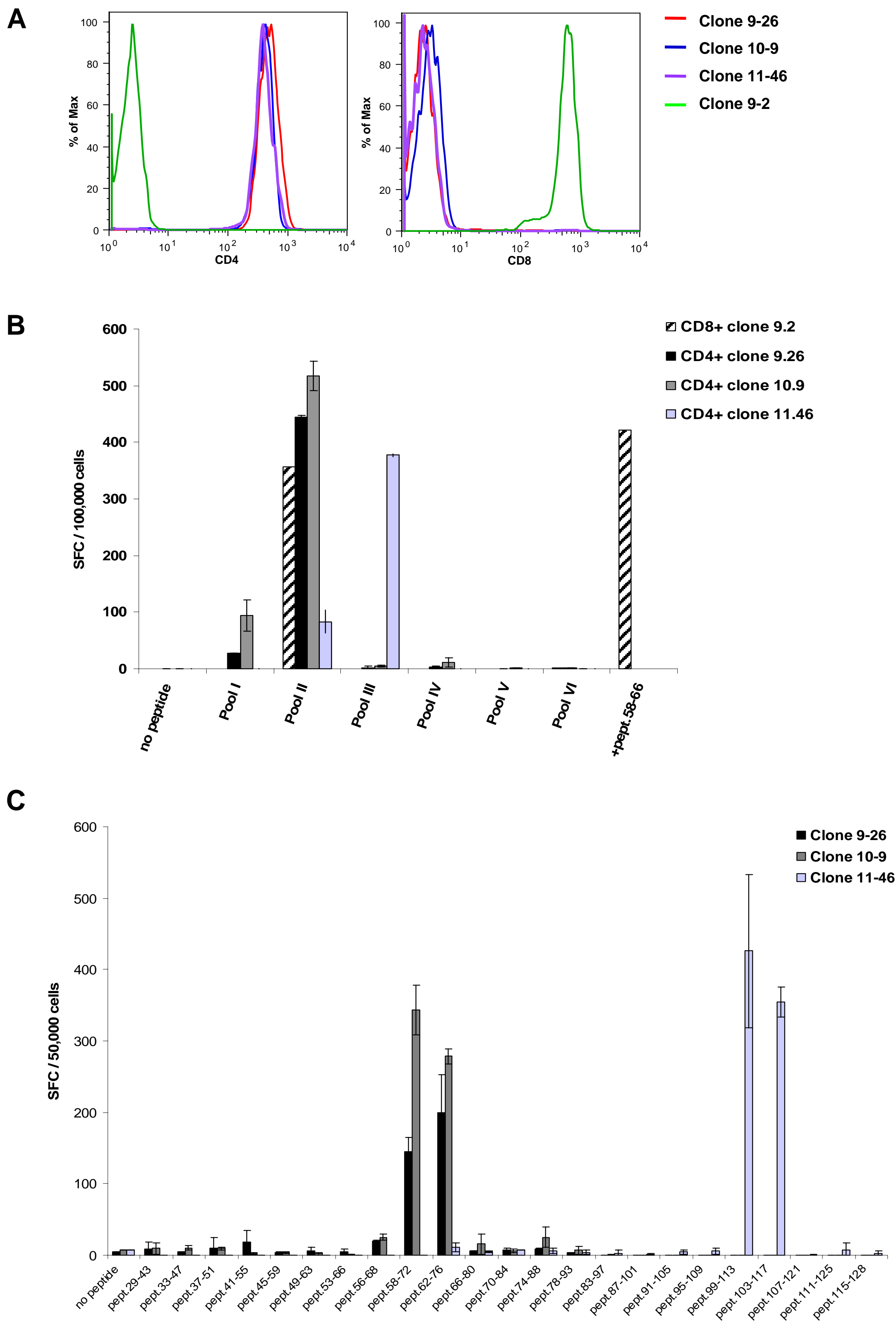


Figure S4: Characterization of Influenza MP1-specific $CD4^+$ and $CD8^+$ T cell clones

(A) CD4 and CD8 expression of T cell clones was analyzed by flow cytometry. Clones 9.26, 10.9 and 11.46 were homogenously $CD4^+CD8^-$ and clone 9.2 was homogenously $CD8^+CD4^-$.

(B) Recognition of MP1 peptides by $CD4^+$ T cell clones in IFN-γ ELISPOT assays. An MP1 peptide library (64 15-mer peptides overlapping by 10 amino acids) was divided in 6 subpools covering MP1 amino acid positions 1-51 (pool I), 41-88 (pool II), 78-128 (pool III), 118-163 (pool IV), 152-203 (pool V) and 193-252 (pool VI). Clones 9.2, 9.26 and 10.9 responded specifically to pool II and clone 11.46 to pool III. In addition, the $CD8^+$ T cell clone 9.2, but not the $CD4^+$ T cell clones, recognized the HLA-A2 restricted MP1 epitope 58-66. Error bars indicate standard deviations.

(C) MP1-specific $CD4^+$ T cell clones were tested for recognition of individual peptides covering MP1 amino acid sequence 29-128, including all peptides of MP1 pools II and III. Clones 9.26 and 10.9 specifically recognized peptide epitope $MP1_{62-72}$ and clone 11.46 was specific for epitope $MP1_{103-113}$. Error bars indicate standard deviations.

Figure S5:

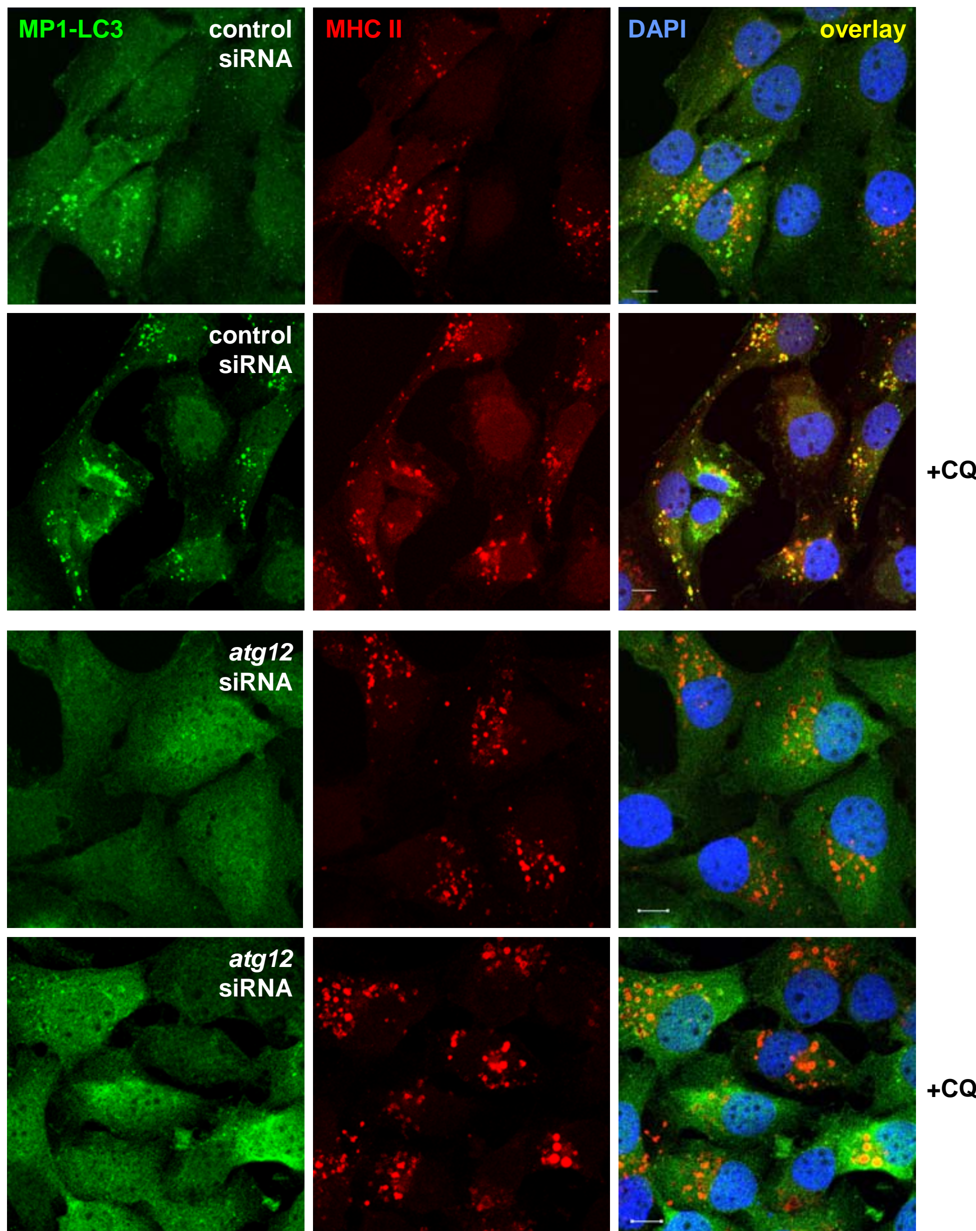


Figure S5: Macroautophagy is required for delivery of MP1-LC3 to MHC class II loading compartments

MDAMC cells stably expressing MP1-LC3 were transfected with control siRNA (specific for *firefly luciferase*) or siRNA specific for *atg12*. After 36 h, cells were treated with 200 U/ml IFNγ to upregulate MHC class II expression and were cultured for another 36 h. To prevent degradation of MP1-LC3 by lysosomal proteases, cells were treated with 50 μM chloroquine (CQ) during the last 6

hours of the culture, where indicated (+CQ). Cells were fixed, stained with MP1- and MHC class II-specific antibodies and DAPI and analyzed by confocal microscopy. Scale bar: 10 µm. Representative fields from one experiment out of two are shown. In control siRNA-treated cells, a substantial fraction of MP1-LC3-containing vesicles can be observed to colocalize with MHC class II compartments, whereas this colocalization is completely abrogated after *atg12* knockdown.