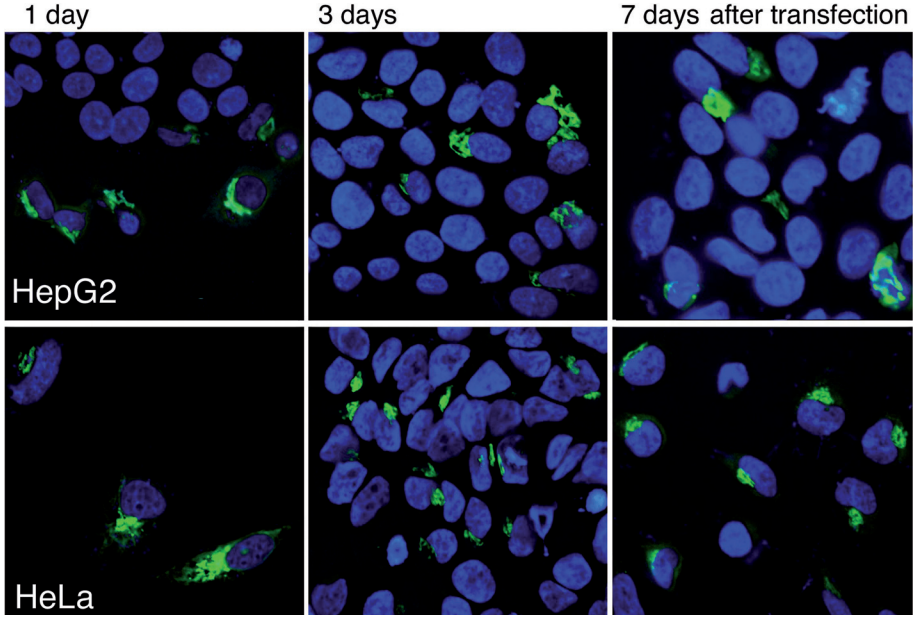
Cell. Mol. Life Sci.
DOI 10.1007/s00018-009-9038-1

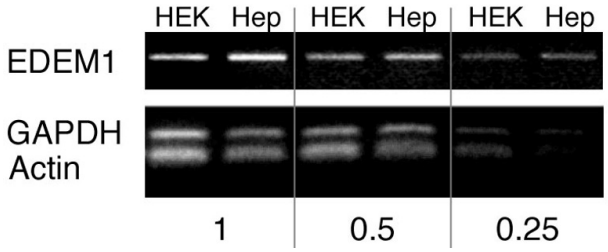
Cellular and Molecular Life Sciences

Electronic supplementary material

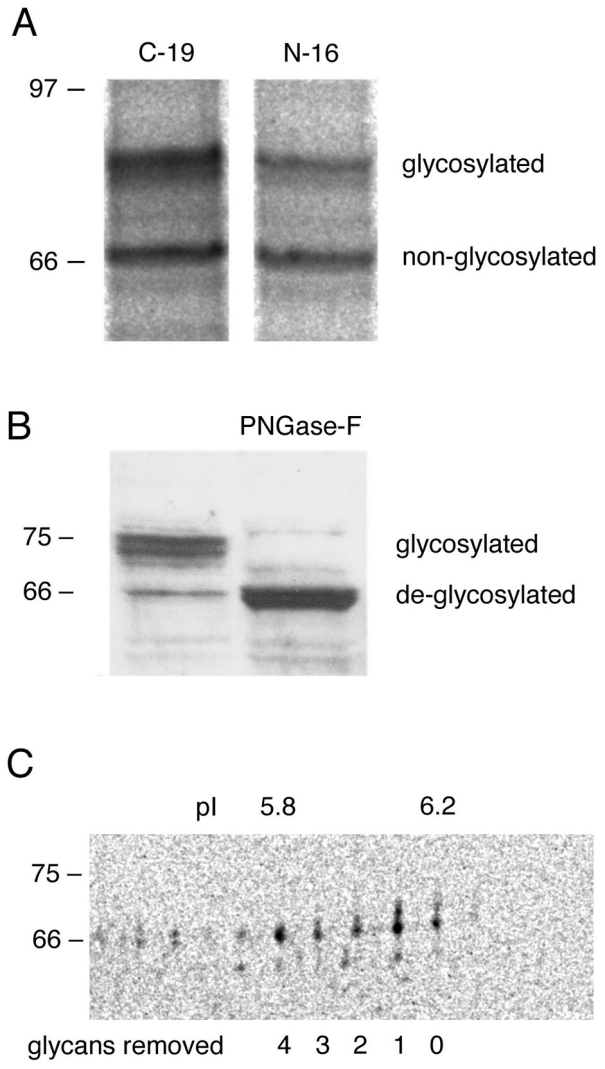
Supplemental Figures



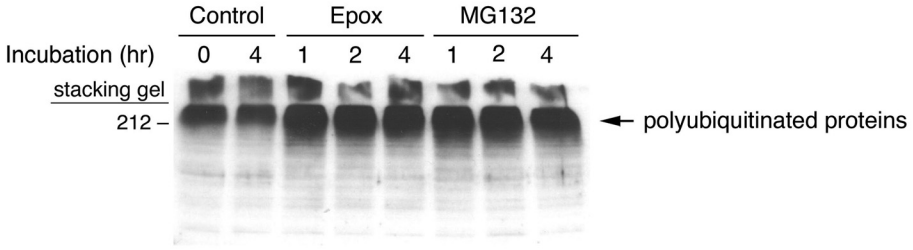
Supplemental Figure 1. Control experiments for EDEM1 overexpression. Cells were transfected with p3xFLAG-CMV control vector/ pEGFP-C1-rat endomannosidase-CMV vector mixture (ratio of 10:1). Cells expressing only the Golgi-located EGFP serving as a reporter protein show no signs of cytotoxicity up to 7 days after transfection.



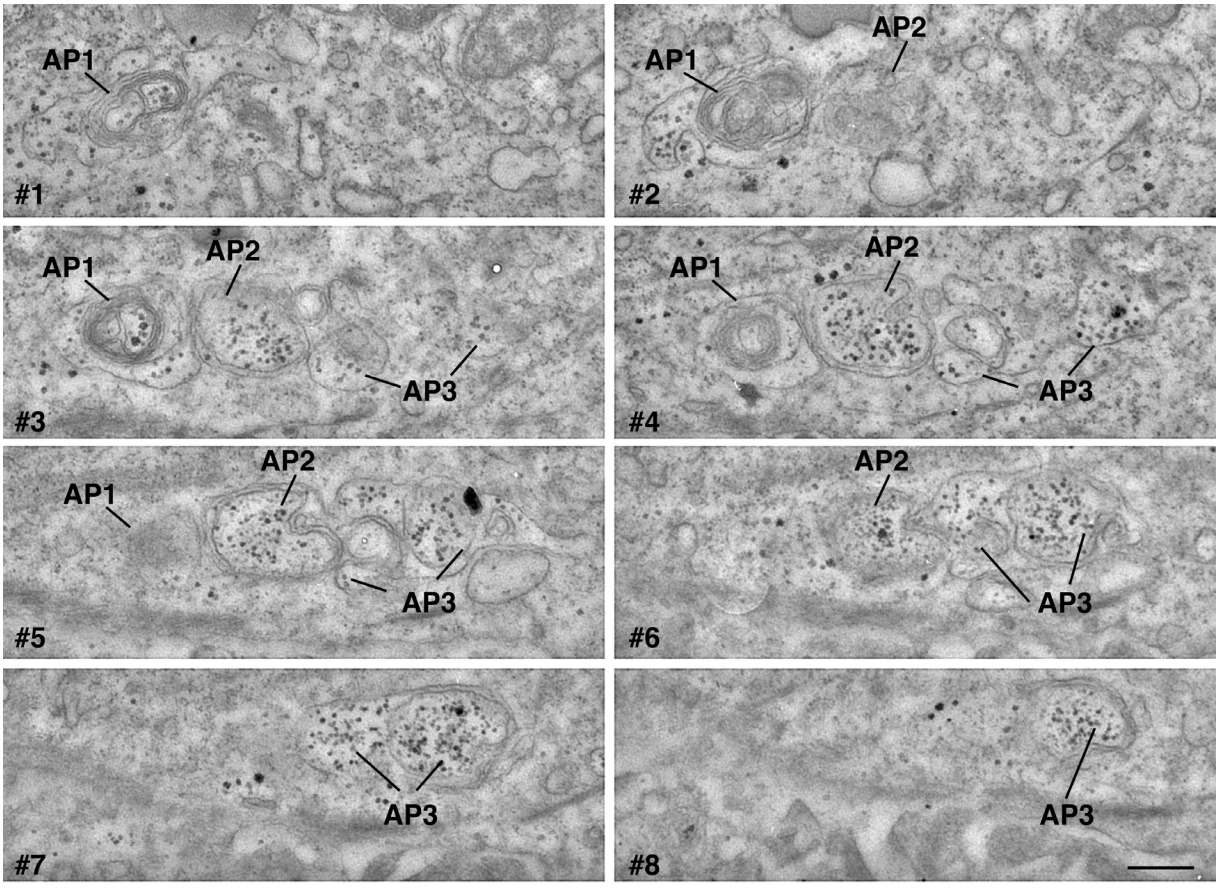
Supplemental Figure 2. RT-PCR analysis of mRNA for EDEM1 in HEK293 (HEK) and HepG2 (Hep) cells. By serial dilution of cDNA, HEK293 cells compared to HepG2 cells contain about four times less EDEM1 mRNA.



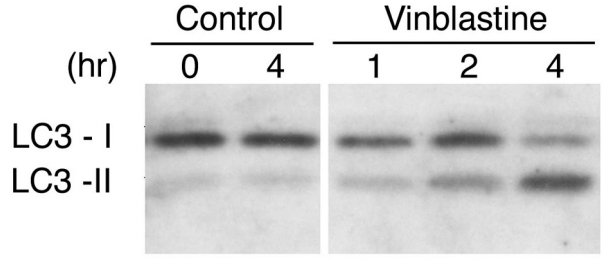
Supplemental Figure 3. The used EDEM1 anti-peptide antibodies react with glycosylated and non-glycosylated EDEM1 in Western blots. (*A*) Affinity-purified antibody C-19 raised against a C-terminal peptide of human EDEM1 (left) and affinity-purified antibody N-16 raised against a peptide distal of the signal peptide of human EDEM1 (right) recognize glycosylated and non-glycosylated EDEM1 forms. (*B*) Immunoprecipitated ^{35}S-labeled EDEM1 (left) was subjected to PNGase F (right) and resolved by SDS-PAGE. Affinity-purified anti-C19 EDEM1 antibody reacts with differentially glycosylated and non-glycosylated EDEM1 (left) and recognizes a single band following PNGase F treatment (right). (*C*) Immunoprecipitated ^{35}S-labeled EDEM1 was subjected to PNGase F and resolved by 2-D gel electrophoresis in IPG strips (pH 5.3–6.5). Spots at positions 0–4 correspond to exposed asparagine content produced by deglycosylation of differentially glycosylated EDEM1.



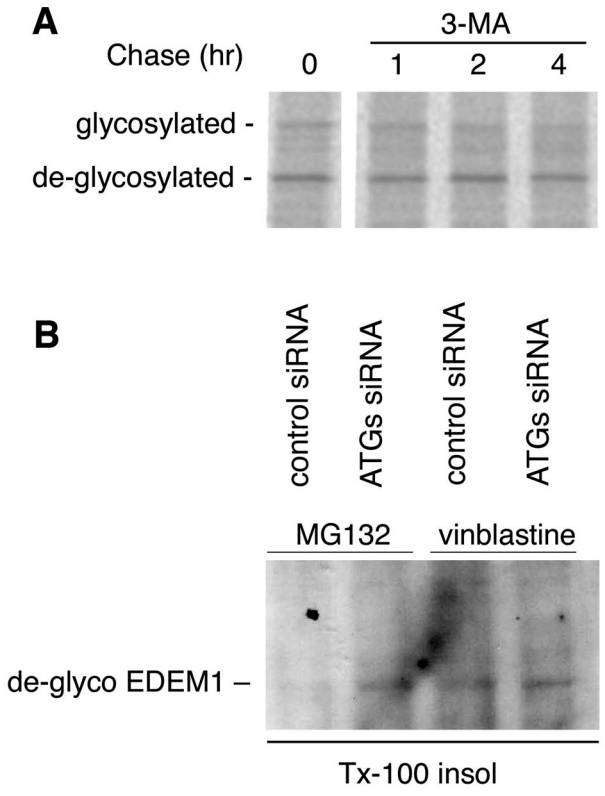
Supplemental Figure 4. Proteasome inhibitor treatment results in accumulation of polyubiquitinated proteins. Western blot with anti-ubiquitin antibodies of an aliquot of cell lysate from the same experiment shown in Figure 2 demonstrates presence of polyubiquitinated proteins and indicates efficiency of proteasome inhibitor treatment.



Supplemental Figure 5. EDEM1is present in autophagosomes. Immunoperoxidase preembedding labeling for EDEM1 in vinblastine-treated (30 min) HepG2 cells. Eight consecutive serial ultrathin sections demonstrate EDEM1 in a group of typical autophagosomes. Bar, 400 nm.



Supplemental Figure 6. Vinblastine treatment of HepG2 cells prevents autophagosome-lysosome fusion. In pulse-chase experiments, vinblastine treatment resulted in a time-dependent increase of the autophagosome membrane-anchored LC3A-II form.



Supplemental Figure 7. Inhibition of autophagosome formation by 3-methyladenine or ATG siRNA knockdown. (*A*) In pulse-chase experiments, 3-methyladenine blocks clearance of EDEM1. (*B*) In Western blots, ATG siRNA knockdown combined with MG 132 (left) or vinblastine (right) treatment does not additionally induce the formation of Triton X-100 insoluble EDEM1. MG 132 treatment in control siRNA knockdown HepG2 cells does not provoke aggregation of EDEM1 in contrast to vinblastine.