Supporting Information

Byun et al. 10.1073/pnas.1004303107

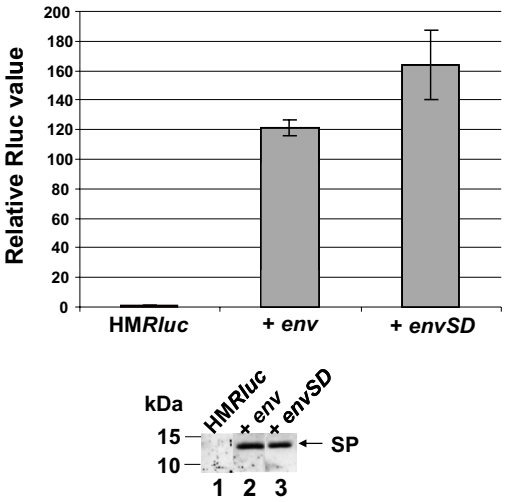

Fig. S1. Signal peptide (SP) expression does not require the splice donor site in the envelope (*env*) gene. Jurkat cells were transiently transfected with the *env* or *env* splice donor (*envSD*) mutant expression constructs in the presence of the reporter vector pHM*Rluc*. (*Upper*) Luciferase assays are expressed as in Fig. 1*D*. (*Lower*) Western blotting showed equivalent SP expression from *env* and *envSD* constructs.

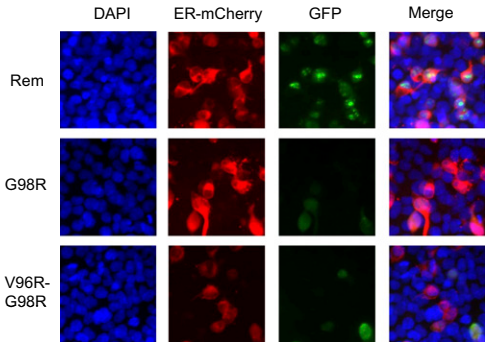

Fig. S2. Fluorescence microscopy of GFP-tagged Rem and signal peptidase cleavage-site mutants. The 293T cells were cotransfected with GFP-tagged Rem expression constructs (0.25 μg wild-type *rem*, 1 μg of G98R, and 6 μg of V96RG98R) and endoplasmic reticulum (ER)-mCherry (0.2 μg). Cells were incubated for 48 h, stained with DAPI, and analyzed by fluorescence microscopy.

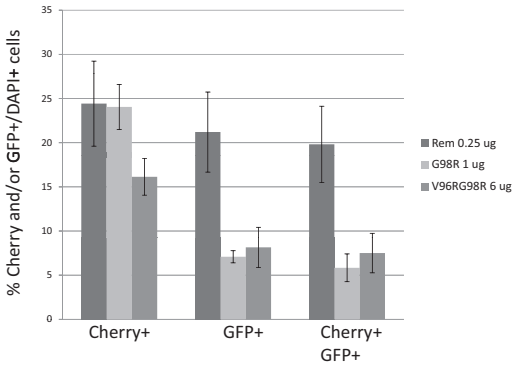

Fig. S3. Quantitation of GFP and mCherry expression in cotransfected 293T cells. Approximately 300 cells from the transfection shown in Fig. S2 were evaluated in four different fields for mCherry and GFP expression as determined by two independent observers. The mean and SDs of cell counts are shown.

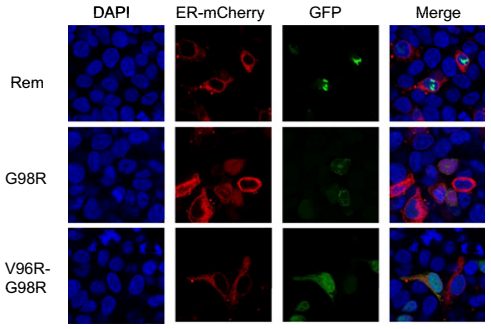

Fig. S4. Confocal microscopy of wild-type Rem and signal peptidase cleavage-site mutants in 293T cells. The transfection shown in Fig. S2 was visualized by confocal fluorescence microscopy. Because both mutants had diminished green and/or red fluorescent signals, visualization was artificially enhanced for detection.

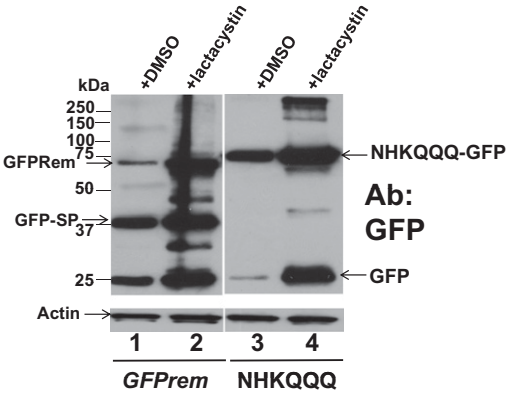

Fig. S5. Lactacystin increases the amount of Rem relative to SP. (*Upper*) Expression vectors for *GFPrem* or an α1-antitrypsin mutant (NHKQQQ) tagged with GFP were transfected into HC11 cells. Cells were treated with DMSO alone (lanes 1 and 3) or lactacystin in DMSO (lanes 2 and 4). Extracts were analyzed by Western blotting with GFP-specific antibody. Some free GFP was generated with both constructs. (*Lower*) Extracts also were analyzed with actin-specific antibody.

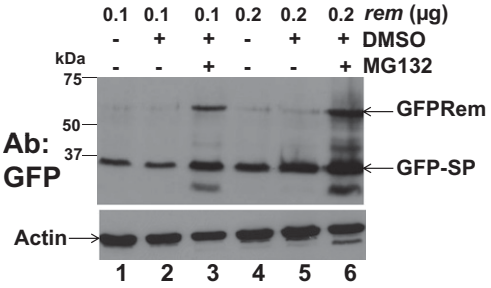

Fig. S6. MG132 increases the amount of Rem relative to SP. *GFPrem* (0.1 or 0.2 μg) was transfected into HC11 cells before treatment with DMSO alone or with MG132 in DMSO. Extracts were prepared and analyzed by Western blotting with GFP-specific antibody (*Upper*). Incubation of Western blots with actin-specific antibody confirmed equal protein loading (*Lower*). Quantitation of the results revealed that the ratio of Rem to SP changed from 0.41 in the absence MG132 to 0.75 in the presence of MG132 (compare lanes 5 and 6).

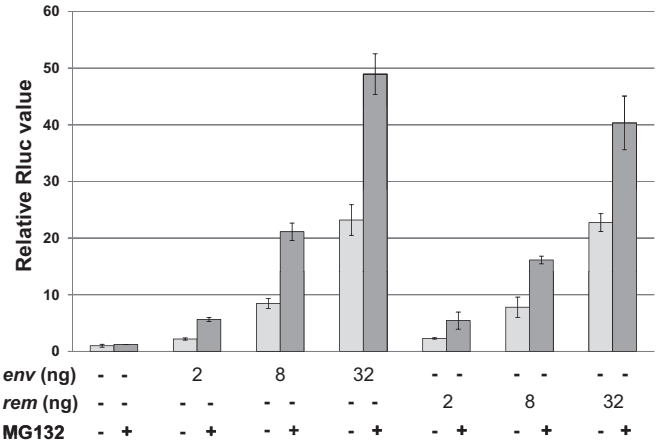

Fig. S7. Reporter activity is increased in the presence of MG132. HC11 cells were transfected with the indicated amounts of *env* or *rem* before treatment with DMSO alone or with MG132 in DMSO. Extracts were assayed for luciferase (Rluc); results are reported as described in Fig. 1*D*.

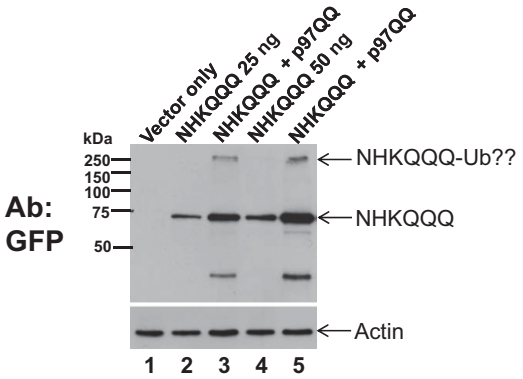

Fig. S8. The α1-antitrypsin mutant NHKQQQ accumulates in the presence of DN p97. The 293T cells were transfected with 25 or 50 ng of plasmid DNA encoding NHKQQQ in the absence of a dominant-negative p97 (lanes 2 and 4) or in the presence of 5 μg of DNA encoding the dominant-negative p97 (lanes 3 and 5). The high-molecular-mass bands in lanes 3 and 5 may represent polyubiquitinated protein. Whole-cell lysates were prepared, and 15 μg of protein extract was used for blotting with antibodies specific for GFP (*Upper*) or actin (*Lower*). Lane 1 shows results of cells transfected with pcDNA3 only. All transfections received the same amount of total DNA.