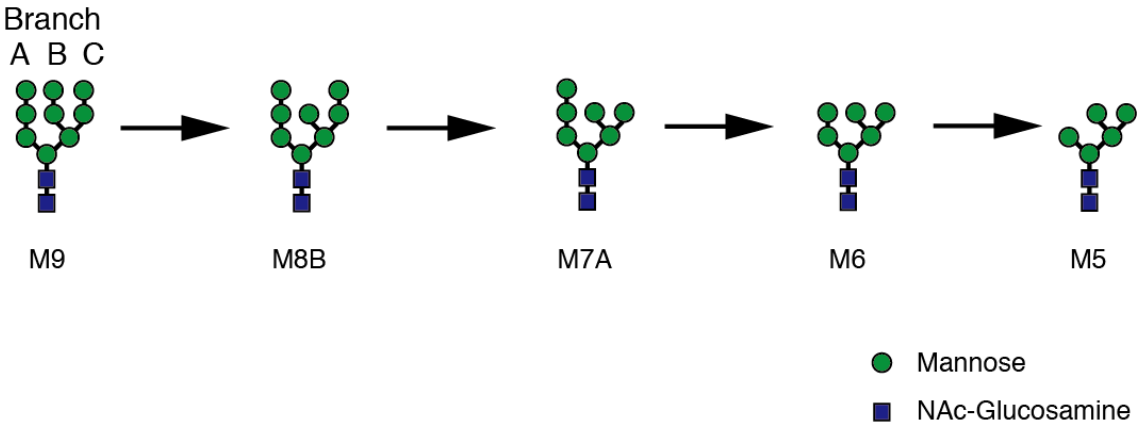
Supporting Information

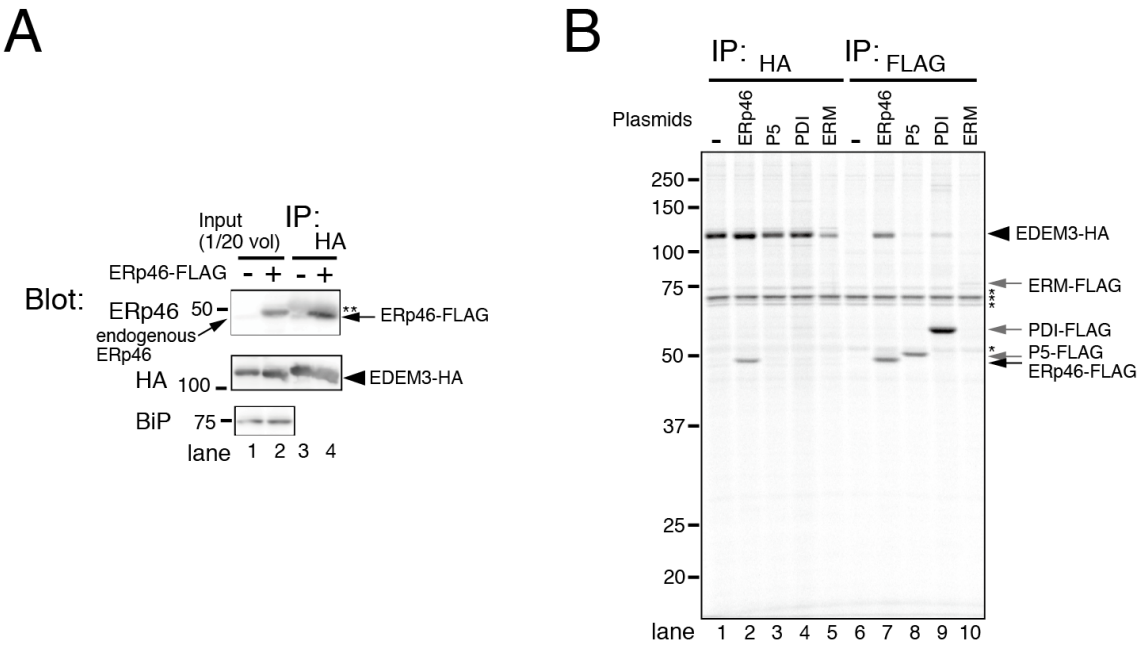
Supplemental Figures



Suppl Fig. 1

Fig. S1. N-glycan structures

N-glycan structures are presented schematically: $Man_9GlcNAc_2$ (M9), $Man_8GlcNAc_2$ isomer B (M8B), $Man_7GlcNAc_2$ isomer A (M7A), $Man_6GlcNAc_2$ (M6), and $Man_5GlcNAc_2$ (M5).

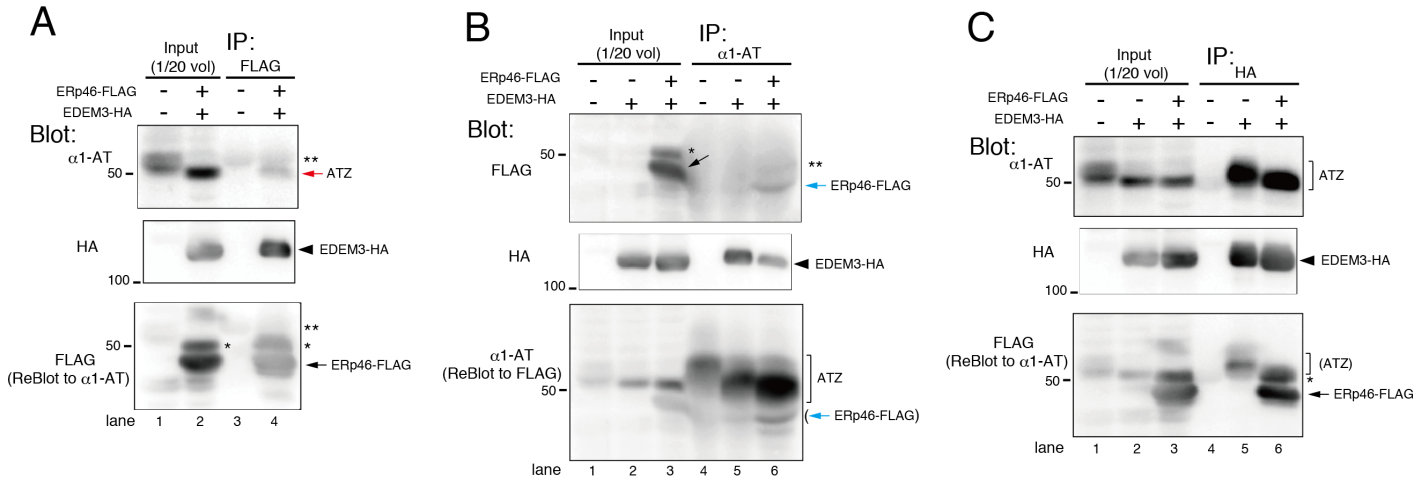


Suppl Fig. 2

Fig. S2. Association of EDEM3 with ERp46

(A) EDEM3-HA associates with endogenous ERp46. HEK293 cells were transfected with EDEM3-HA and ERp46-FLAG, and immunoprecipitated using anti-HA antibody. Endogenous ERp46 co-immunoprecipitated with EDEM3-HA.

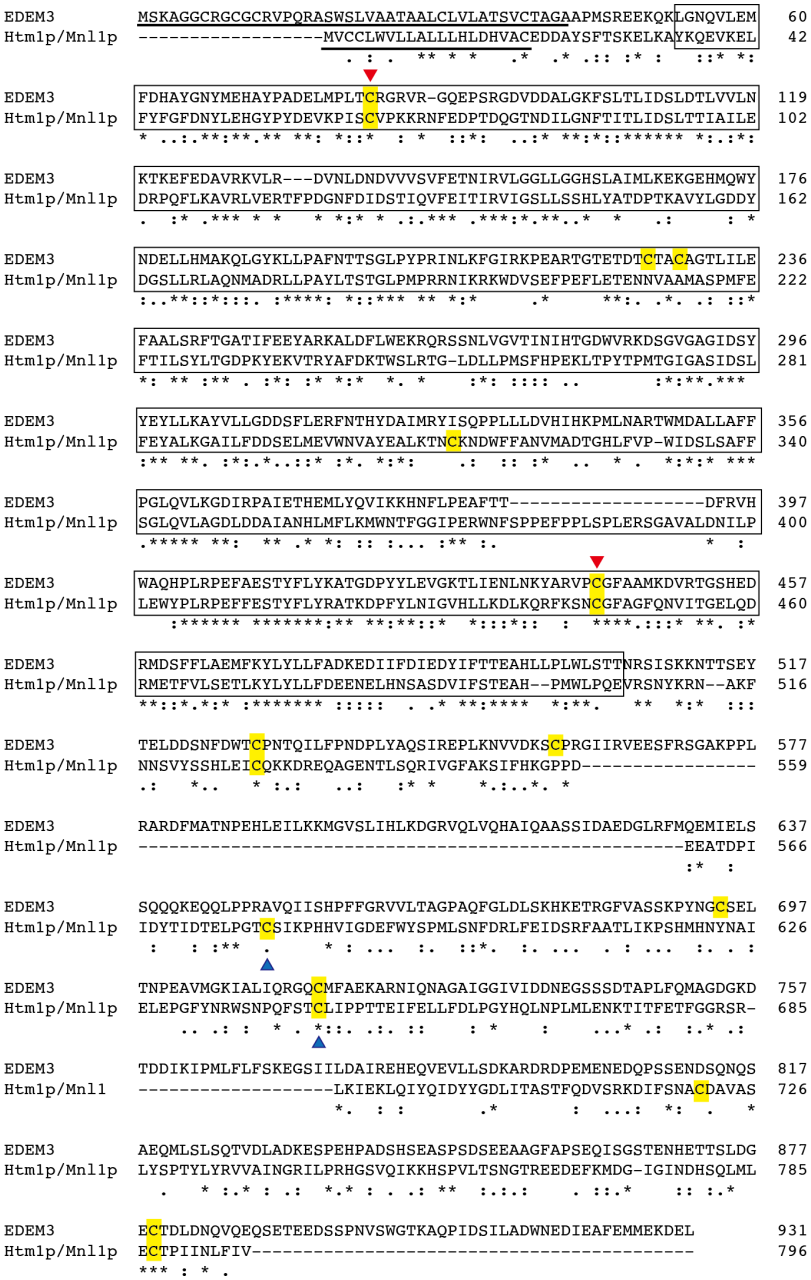
(B) Co-immunoprecipitation of ERp46 with EDEM3. HEK293 cells were transfected with EDEM3-HA and FLAG-tagged oxidoreductases (ERp46, P5, and PDI) or ERM, and metabolically labeled for 1 h. Cell lysates were immunoprecipitated with anti-HA or anti-FLAG antibody.



Suppl Fig. 3

Fig. S3. Association of ATZ with EDEM3 and ERp46

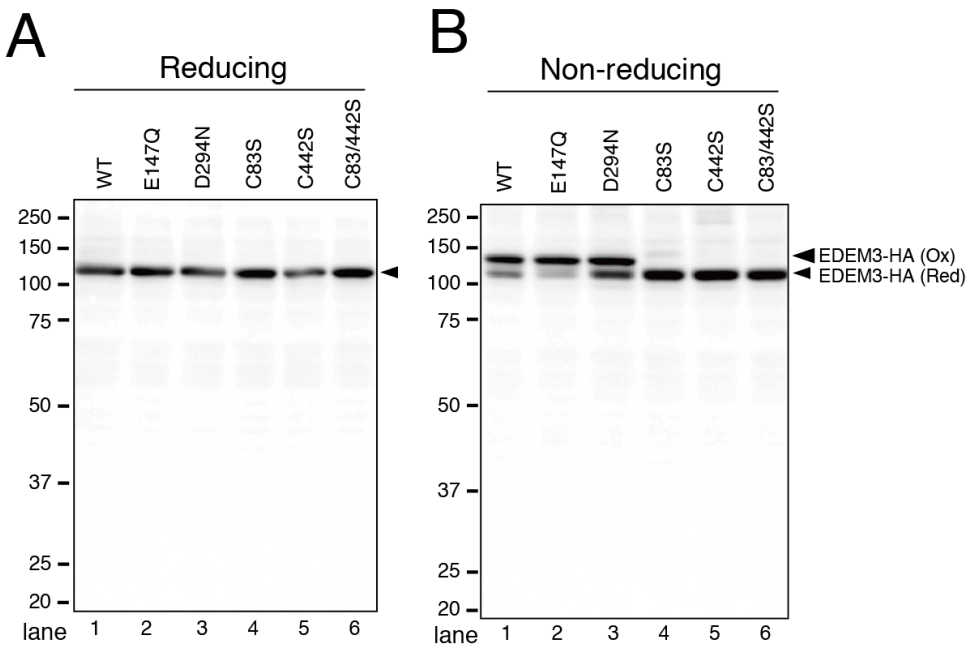
HEK293 cells were transfected with ATZ, EDEM3-HA and ERp46, and immunoprecipitated with anti-FLAG antibody (A), anti-α1-AT antibody (B), or anti-HA antibody (C). ATZ co-immunoprecipitated with ERp46-FLAG was indicated by a red arrow in (A), and ERp46 co-immunoprecipitated with ATZ was shown by a blue arrow in (B). ATZ co-immunoprecipitated with EDEM3-HA was shown by a bracket in (C), and EDEM3-HA co-immunoprecipitated with ATZ was indicated by an arrowhead in (B). Asterisks indicate a signal sometimes detected by the FLAG antibody in cells transfected with ERp46-FLAG, and ** indicate immunoglobulin heavy chains used for the precipitation of each proteins.



Suppl Fig. 4

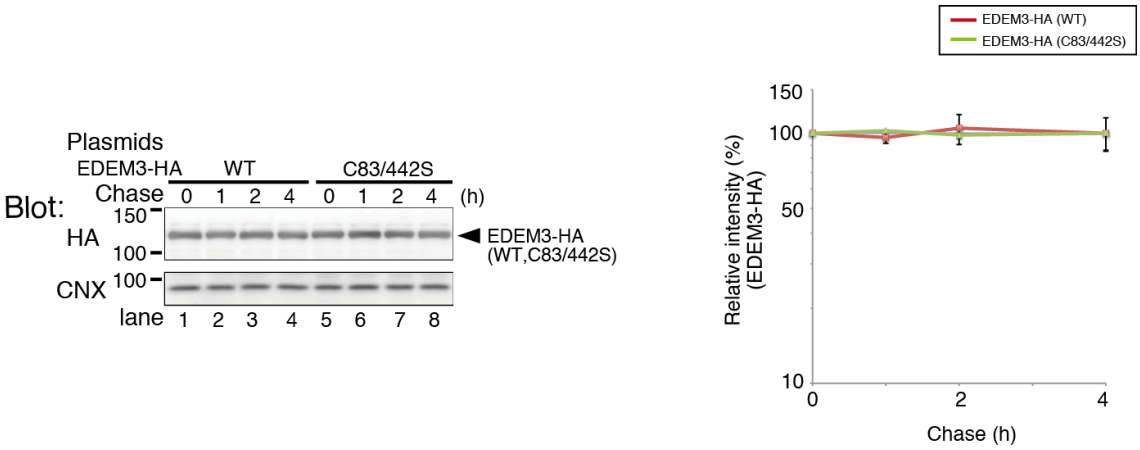
Fig. S4. Amino acid sequence alignment of EDEM3 (*Mus musculus*) and Htm1p/Mnl1p (*S. cerevisiae*).

α-Mannosidase domain is boxed, and signal sequences are underlined. Cys residues in the mature proteins are highlighted in yellow. Red triangles indicate the two Cys residues in the α-mannosidase domain that form disulfide bonds. Blue triangles indicate the Cys residues that make mixed disulfide bridges with Pdi1p in yeast. * indicates identical amino acids, and : and . indicate similar amino acids.



Suppl Fig. 5

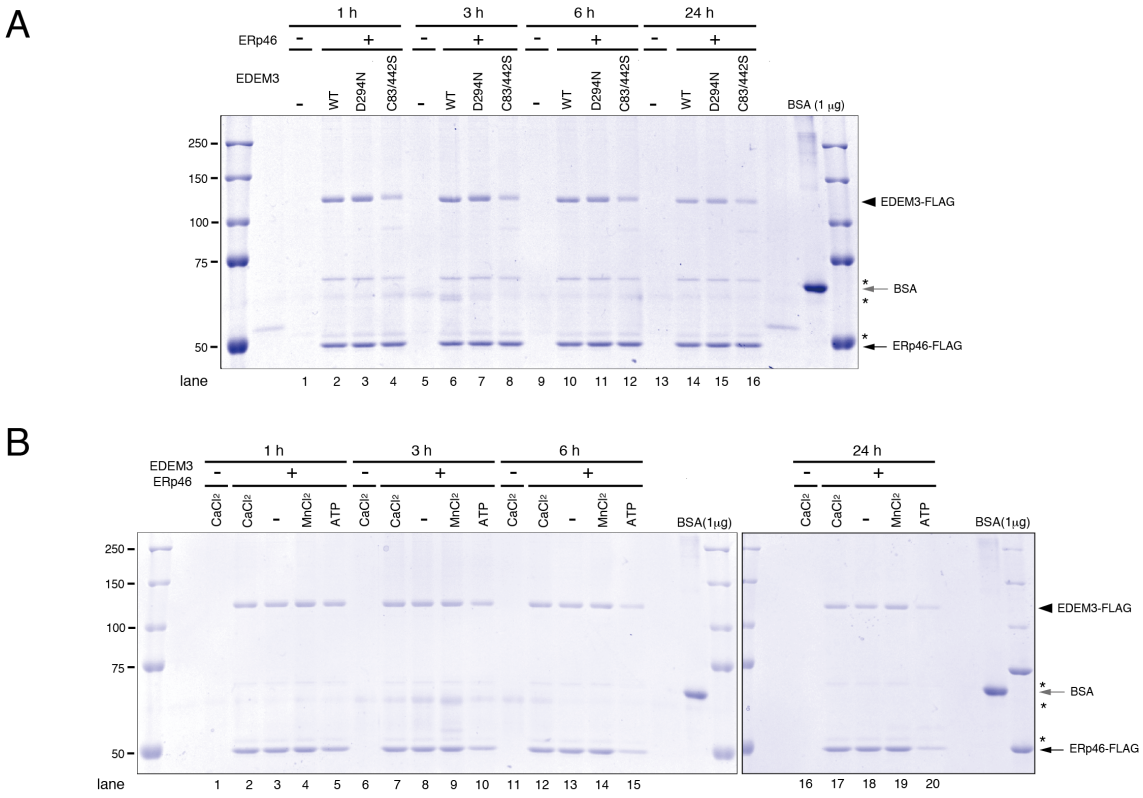
Fig. S5. EDEM3 mutants separated under reducing and non-reducing conditions.
EDEM3-HA WT and the indicated mutants were separated by SDS-PAGE under reducing (A) and non-reducing conditions (B).



Suppl Fig. 6

Fig. S6. Stability of WT EDEM3 and its Cys mutant.

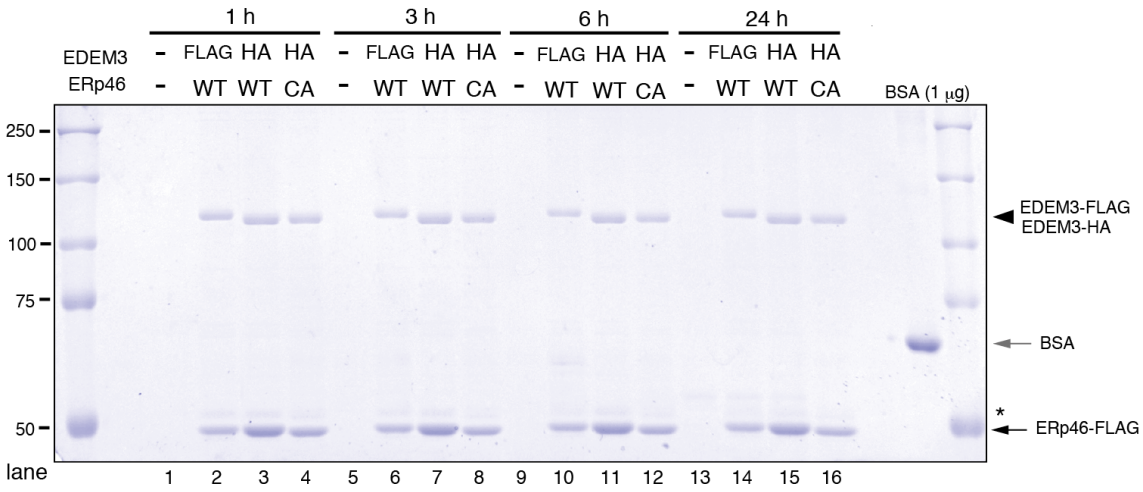
HEK293 cells expressing WT EDEM3-HA or C83/442S mutant were treated with 100 μM of cycloheximide, and chased for the indicated periods. EDEM3-HA remained in the cells were analyzed by western blotting and quantified (right). Error bars show S.D. of three independent experiments. The differences were statistically not significant (two-tailed Student's *t*-test).



Suppl Fig. 7

Fig. S7. CBB staining of the recombinant EDEM3 and ERp46 proteins used for *in vitro* analysis.

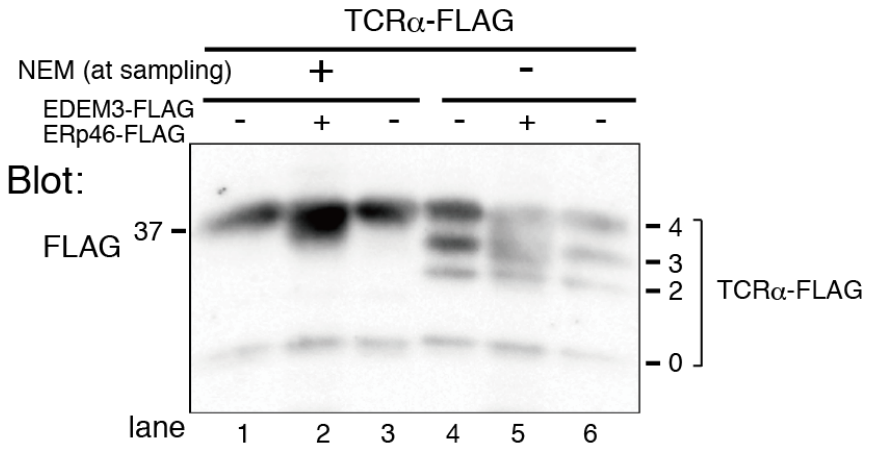
The upper parts of the gel used for the western blot analyses in Fig. 5D (A) and F (B) were stained with CBB.



Suppl Fig. 8

Fig. S8. CBB staining of the recombinant EDEM3 and ERp46 proteins used for *in vitro* analysis.

The upper part of the gel used for the western blot analysis in Fig. 6F was stained with CBB.



Suppl Fig. 9

Fig. S9. Mannose trimming from TCRα *in vitro*.

TCRα-FLAG was purified from HEK293 cell lysate in the presence or absence of NEM, and then incubated with co-purified EDEM3 and ERp46 for 24 h. The numerals on the right side indicate the number of N-glycans on TCRα-FLAG.