Supporting information

EDEM1's mannosidase-like domain binds ERAD client proteins in a redox-sensitive manner and possesses catalytic activity

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Immunofluorescence microscopy – HEK293A cells were transfected with indicated EDEM1 constructs and incubated for 24 hr at 37^{o}C and 5% CO_2. Transfected cells were fixed with 3.7% paraformaldehyde in 1X phosphate-buffered saline (PBS) for 15 min at room temperature followed by permeabilization with 0.1% Triton X-100 in 1X PBS for 30 min at room temperature. Slides were rinsed in 1X PBS followed by blocking with 10% fetal bovine serum in 1X PBS for 30 min at room temperature. Cells were rinsed and stained with the indicated primary antibody solution at a 1:200 dilution factor followed by staining with the respective Alexa Fluor 488 or 594 secondary antibodies at a 1:400 dilution factor in blocking buffer. Cells were rinsed and mounted onto coverslips pretreated with VectaShield (Vector Laboratories). Images were acquired using $A1^{+}/A1R^{+}$ confocal microscope (Nikon). The microscopy data was gathered in the Light Microscopy Facility and Nikon Center of Excellence at the Institute for Applied Life Sciences, UMass Amherst with support from the Massachusetts Life Science Center.

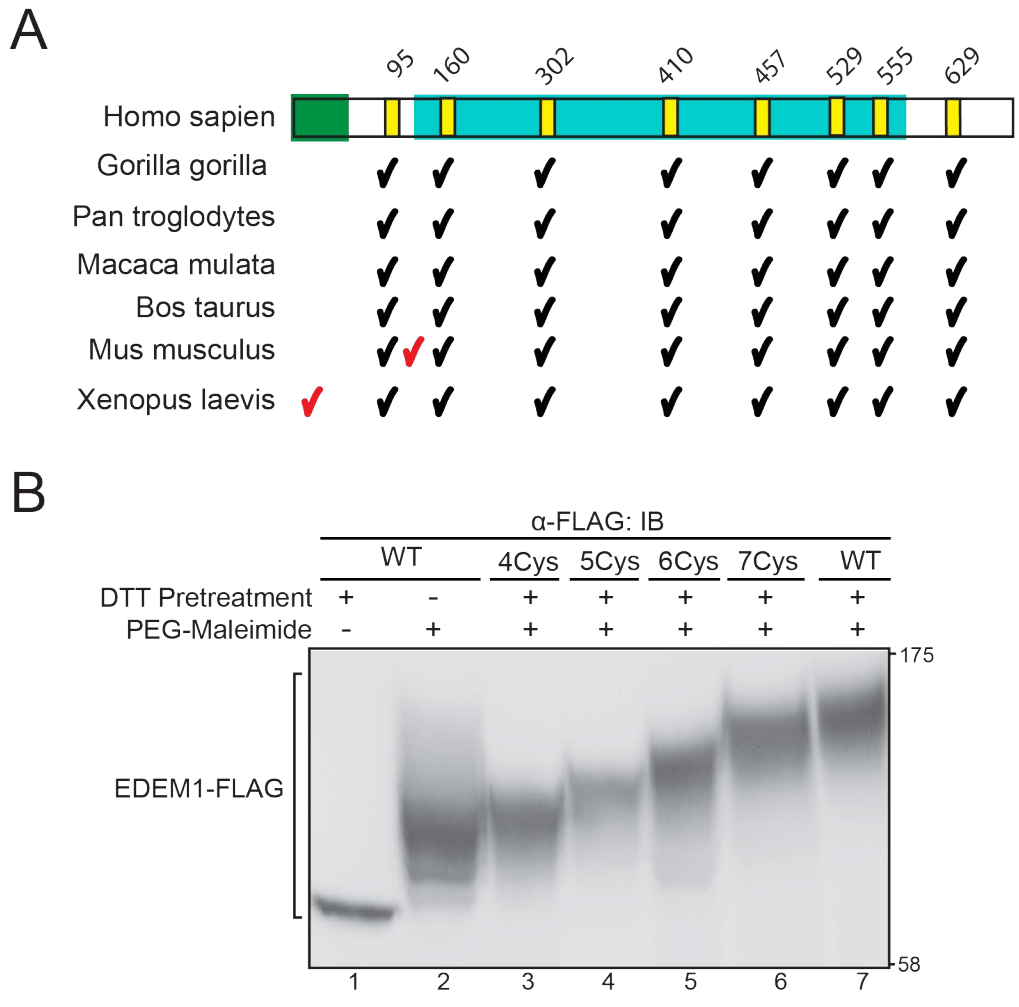

Figure S1. **EDEM1 Cys are conserved across species and posseses approximately four reactive thiols at steady state** (**A**) Cartoon representation of amino acid sequence alignments (Clustal Omega) of EDEM1 from various species (Accession #: NP_055489.1, XP_004033576.1, JAA36785.1, NP_001252679.1, AAI51306.1, NP_619618.1, and NP_001091440.1) depicting conserved and non-conserved Cys residues, black and red check marks, respectively. (**B**) FLAG-tagged EDEM1 WT, EDEM1 C95/302/555/629S (4Cys), EDEM1 C95/302/629S (5Cys), EDEM1 C95/302S (6Cys), and EDEM1 C95S (7Cys) were transfected in HEK293T cells. 5 mM DTT was added for 1 h where inidcated (+). Cells were lysed in sample buffer containing 5 mM peg-maleimide (+ PEG-Maleimide) or 20 mM NEM (- PEG-Maleimide). Proteins were resolved on 8% SDS-PAGE and immunoblotted with FLAG antibody. Gel represents data acquired from three independent experiments.

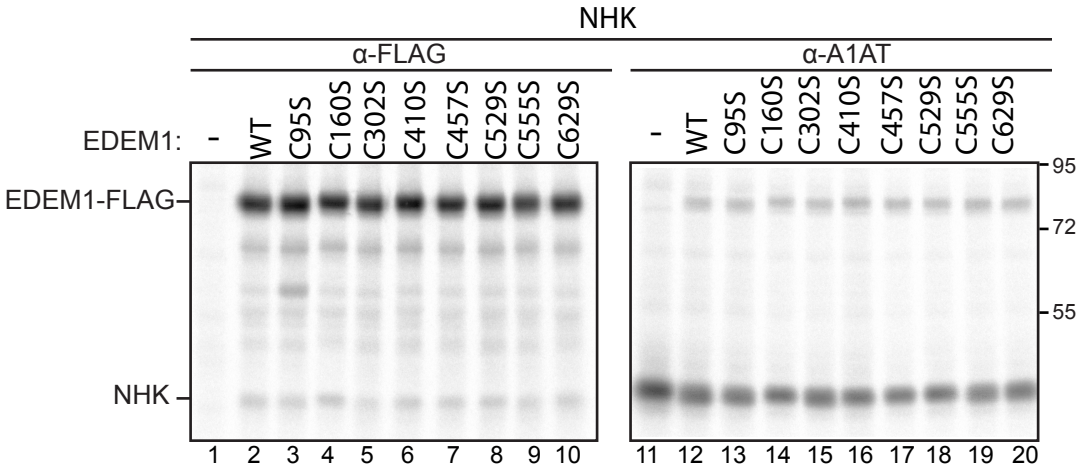

Figure S2. **EDEM1 possessing individual Cys/Ser mutations binds NHK under harsh detergent conditions** FLAG-tagged EDEM1 WT, C95S, C160S, C302S, C410S, C457S, C529S, C555S, C629S and empty plasmid (-) were co-expressed in HEK293T cells with NHK. The proteins were radiolabeled with [^{35}S]-Cys/Met for 30 min and chased for 1 hr. Cells were lysed in MNT buffer containing Triton X-100. Half of the cell lysate was subjected to anti-A1AT immunoprecipitation while the other half to anti-FLAG immunoprecipitation, and washed in buffer containing 0.1% SDS. Proteins were resolved on 9% reducing SDS-PAGE. Gels are representative of three independent experiments.

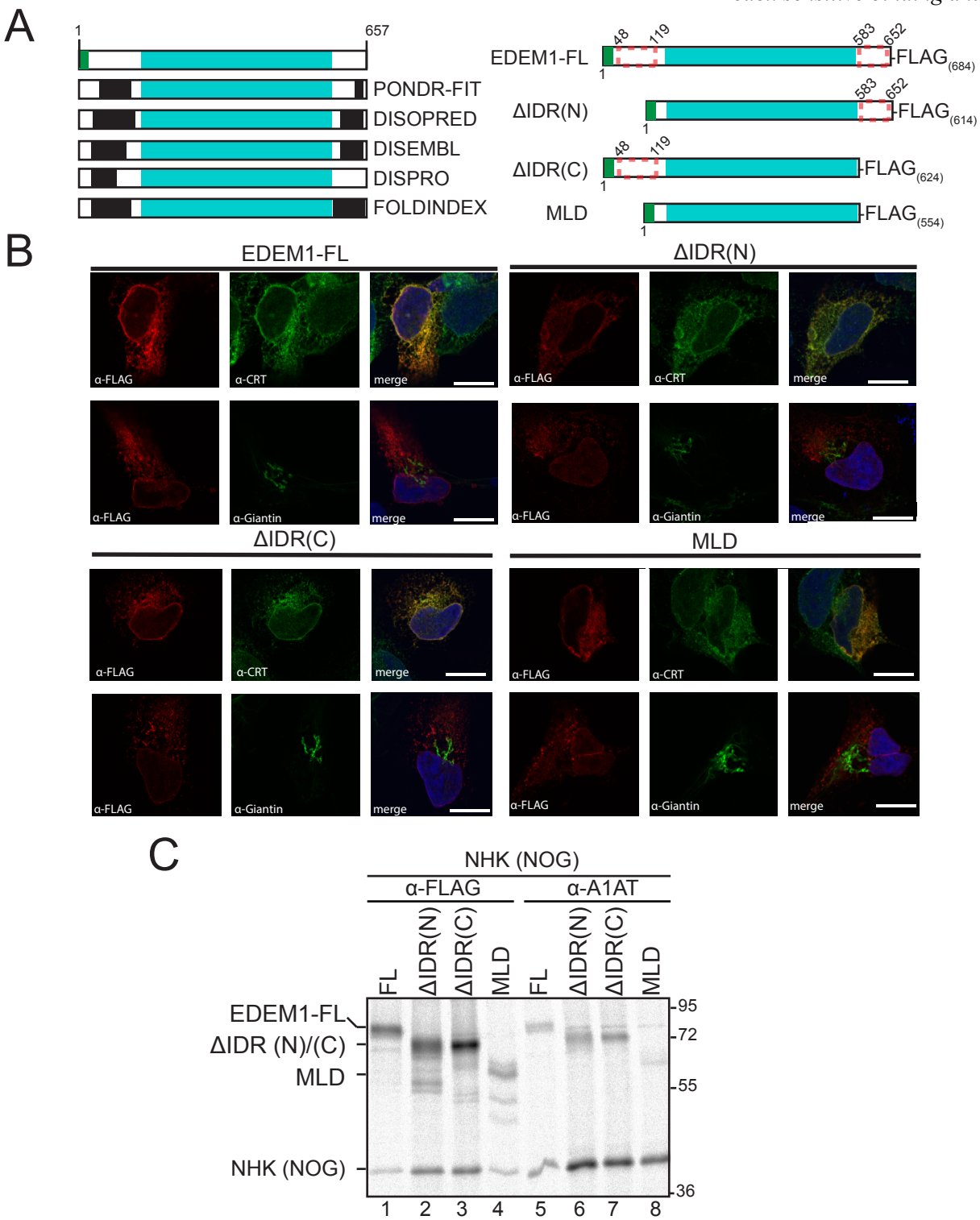

Figure S3. **EDEM1 lacking either IDR is targeted to the ER and binds non-glycosylated substrates**
(**A**) (Left) Cartoon representation of human EDEM1 depicting signal sequence (green), MLD (cyan), and predicted disordered regions (black). (Right) FLAG-tagged EDEM1 constructs lacking either IDRs (ΔIDR N and ΔIDR C) or both (MLD). (**B**) Images of exogenously expressed FLAG-tagged EDEM1, EDEM1 ΔIDR (N), EDEM1 ΔIDR (C), and MLD obtained by confocal microscopy. Fixed cells were stained with FLAG, CRT (ER), or Giantin (Golgi) antisera. Nuclei were visualized by DAPI staining (Blue).Scale bars correspond to 10 μm. (**C**) FLAG-tagged EDEM1 WT, ΔIDR (N), ΔIDR (C), and MLD were co-expressed in HEK293T cells with NHK NOG. The proteins were radiolabeled with [^{35}S]-Cys/Met for 30 min and chased for 1 hr. Cells were lysed in MNT buffer. Half of the cell lysate was subjected to anti-FLAG immunoprecipitation while the other half to anti-A1AT immunoprecipitation. Proteins were resolved on 9% reducing SDS-PAGE. Gel is representative of three independent experiments.

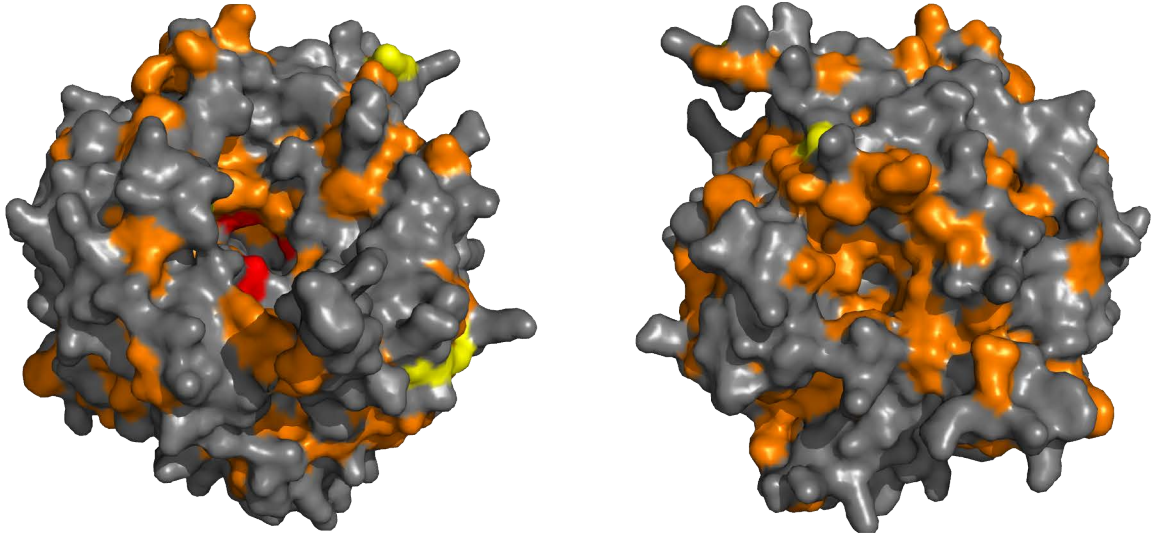

Figure S4. Structural model of the EDEM1 MLD exhibits surface-exposed hydrophobic patches
(Left) Front view of the MLD surface model (Phyre 2.0) (1) depicting three putative catalytic residues (red), Cys residues (yellow), and hydrophobic residues (orange), and Cys residue (yellow). (Right) Back view.

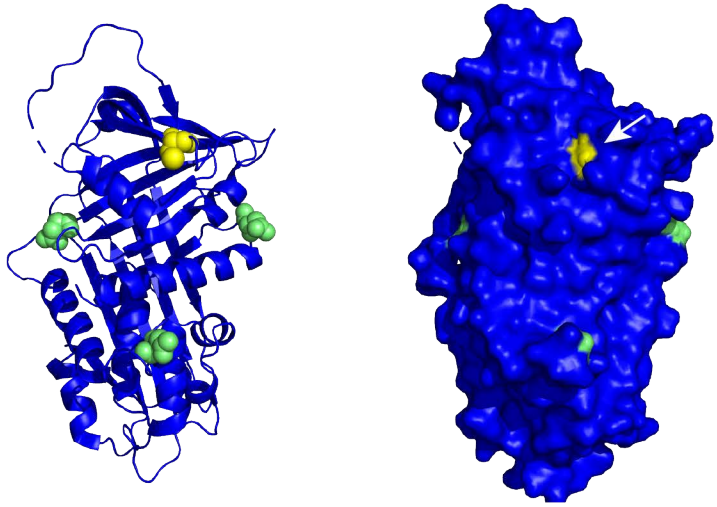

Figure S5. A1AT Cys256 is partially burried
(Left) Ribbon representation of A1AT (PDB: 3DRM) depicting three glycosylation sites (green) and Cys256 (yellow). (Right) A1AT surface model showing partially burried Cys256 (arrow).

REFERENCES

1. Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., and Sternberg, M. J. E. (2015) The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols*. **10**, 845