SUPPLEMENTAL DATA

ATPase assay based on malachite green system

ATPase assays were performed using malachite green system (19,54) to obtain kinetic parameters. The assays were performed in reaction mixture containing 50 mM Tris-HCl (pH 8.0), 20 mM $MgCl_2$, 1 mM EDTA, 0.1-3 mM ATPand 0.5 μM p97. To start the reaction, ATP was added to 50 μl of reaction mixture, the reaction was carried out at 37 °C for 15 min. Malachite green dye buffer of 400 μl containing 6 mM ammonium heptamolybdate, 120 μM malachite green, 0.06% polyvinyl alcohol, and 3.4% sodium citrate was added. Incubation at room temperature for 20 min is required for colour development, then 200 μl from each reaction was transfered to a 96-well plate for measurement of absorbance at 630 nm. Reactions containing no p97 were performed to generate a background readings of inorganic phosphate and were subtracted from the experimental results. The inorganic phosphate released was calculated based on the absorbance standard curve established by KH_2PO_4 standards. The kinetic parameters K_M and k_{cat} where calculated from the Lineweaver-Burk plot. All assays were repeated at least three times.

Circular Dichroism

Samples of wild type and mutant p97 at 0.1mg/ml in CD buffer (20mM phosphate, 150mM potassium chloride, 5mM magnesium chloride) were run on a Chirascan spectropolarimeter (Applied Photophysics) in a 0.1cm quartz cuvette.

Dynamic Light Scattering

Wild-type and mutant p97 samples at ~1mg/ml and in 250 mM KCl, 2 mM β-ME, 25 mM HEPES pH 7.5 where measured on a Zetasizer nano-series (Malvern instruments).

Mutation	k_{cat} (nmol ATP/min/µg p97)	K_M (mM)	k_{cat}/K_M (nmol ATP/min/µg p97/mM ATP)
Wild-type	0.36	0.36	1.00
A232E	0.45	0.42	1.06
R155C/N387C	0.06	0.88	0.07
R155C/N387C (DTT)	0.24	0.16	1.52

Table S1: Michaelis-Menton measurements for wild-type p97, $p97^{A232E}$ and $p97^{R159C/N387C}$. Parameters for $p97^{R159C/N387C}$ were calculated both in the presence and absence of DTT.

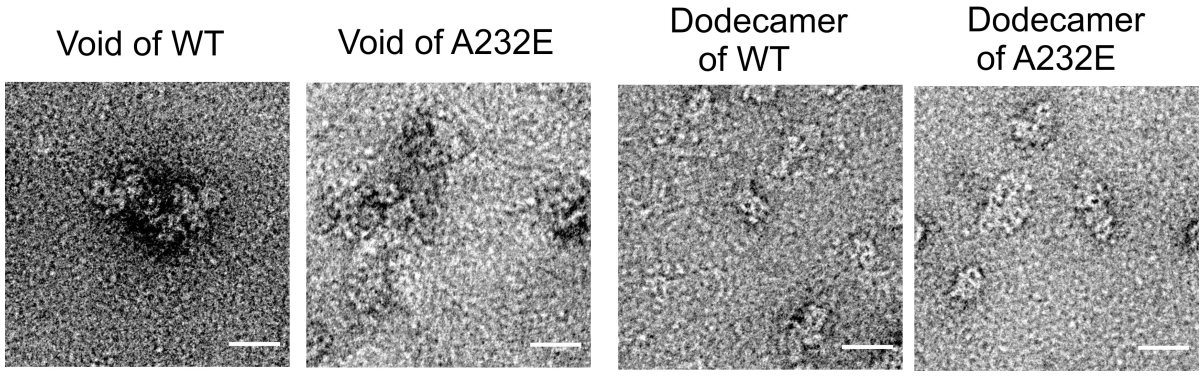

Figure S1: Negative-stain electron microscopy of wild-type p97 and $p97^{A232E}$ fractions. Micrographs of the void volume and dodecamer fractions from the gel filtration of wild-type p97 and $p97^{A232E}$ in a superose6 (GE healthcare). The scale bar represents 300Å. The void fractions show large aggregated clumps for both wild-type p97 and $p97^{A232E}$.

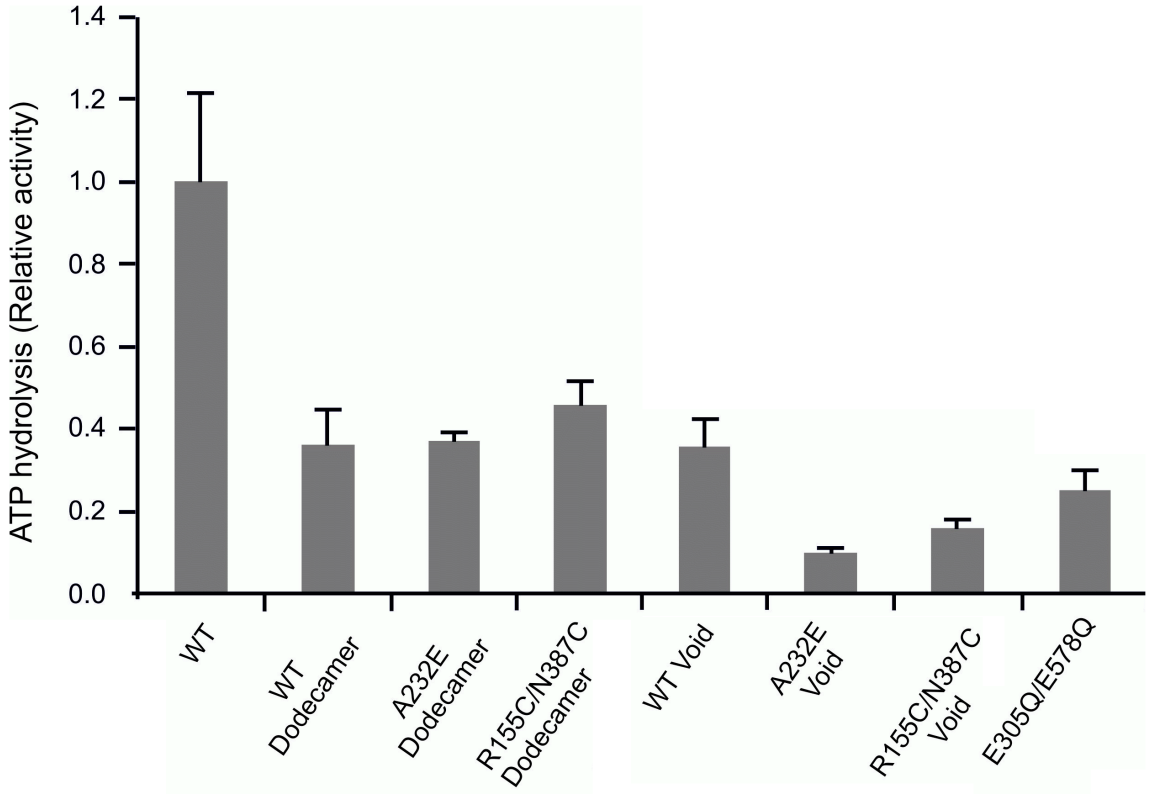

Figure S2: ATPase activities of the void volume and dodecamer fractions from size exclusion chromatography. Fractions containing wild-type p97, $p97^{A232E}$ and $p97^{R155C/N387C}$ were obtained using a superose6 column (GE healthcare). The histogram shows the rates of ATP hydrolysis averaged from three independent measurements and normalised against wild-type. Error bars indicate standard deviations.

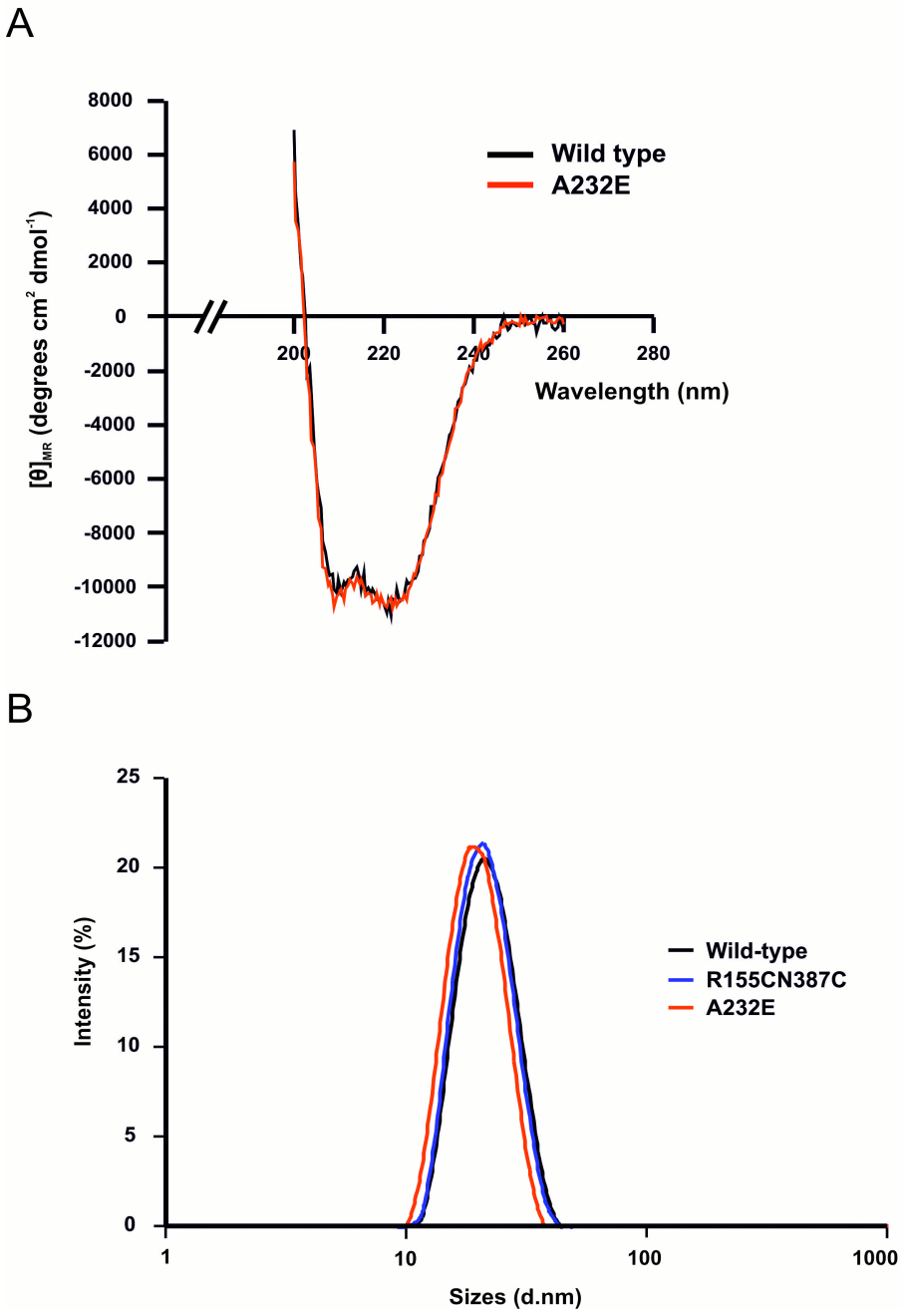

Figure S3: Circular dichroism and dynamic light scattering of wild-type and p97 mutants. A. Spectra for wild-type p97 and $p97^{A232E}$ between wavelengths 200 and 260nm are plotted against mean residue ellipticity (degrees cm^2 $dmol^{-1}$). B. Dynamic light scattering for wild-type p97, $p97^{A232E}$ and $p97^{R155C/N387C}$. Representative average particle sizes in solution of wild type p97, $p97^{R155C/N387C}$ and $p97^{A232E}$ show similar distributions.

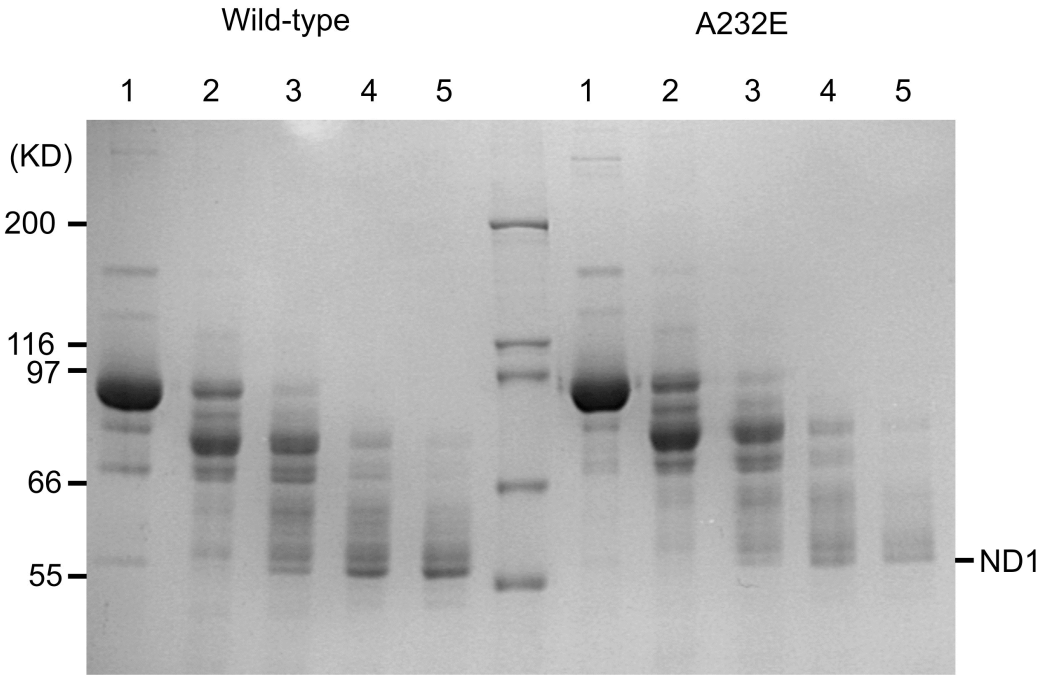

Figure S4: Limited proteolysis for wild-type p97 and $p97^{A232E}$. Reactions were analyzed by SDS-PAGE with lanes 1 to 5 showing p97 samples incubated with trypsin for 0, 10, 20, 30, and 40 min respectively. The band corresponding to the N-D1 p97 fragment (labeled), as confirmed by N-terminal peptide sequencing, is more stable in wild-type p97 than $p97^{A232E}$.