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Supplemental Information

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TABLES

FIGURES

SUPPLEMENTAL FIGURES

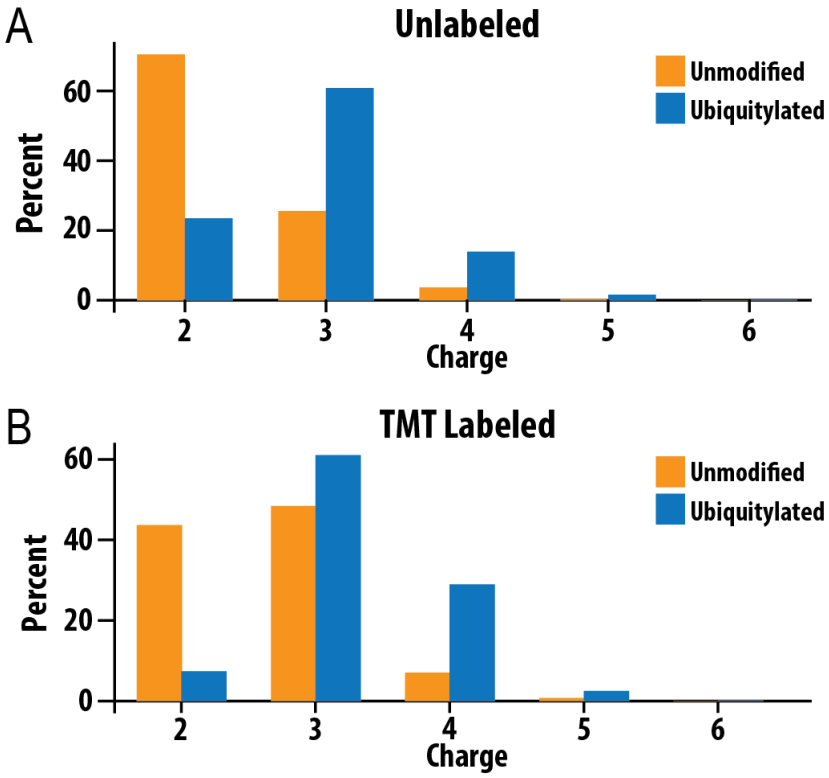

Figure S1. Related to Figure 1. Charge state distribution of identified unmodified and ubiquitylated peptides that are not labeled (A) and labeled (B) with TMT.

A

Total MS³ Scans	Kgg Peptides	Unique Kgg Peptides	Localized Kgg Forms	Quantified Kgg Forms
88,437	29,369	21,240	20,707	18,471

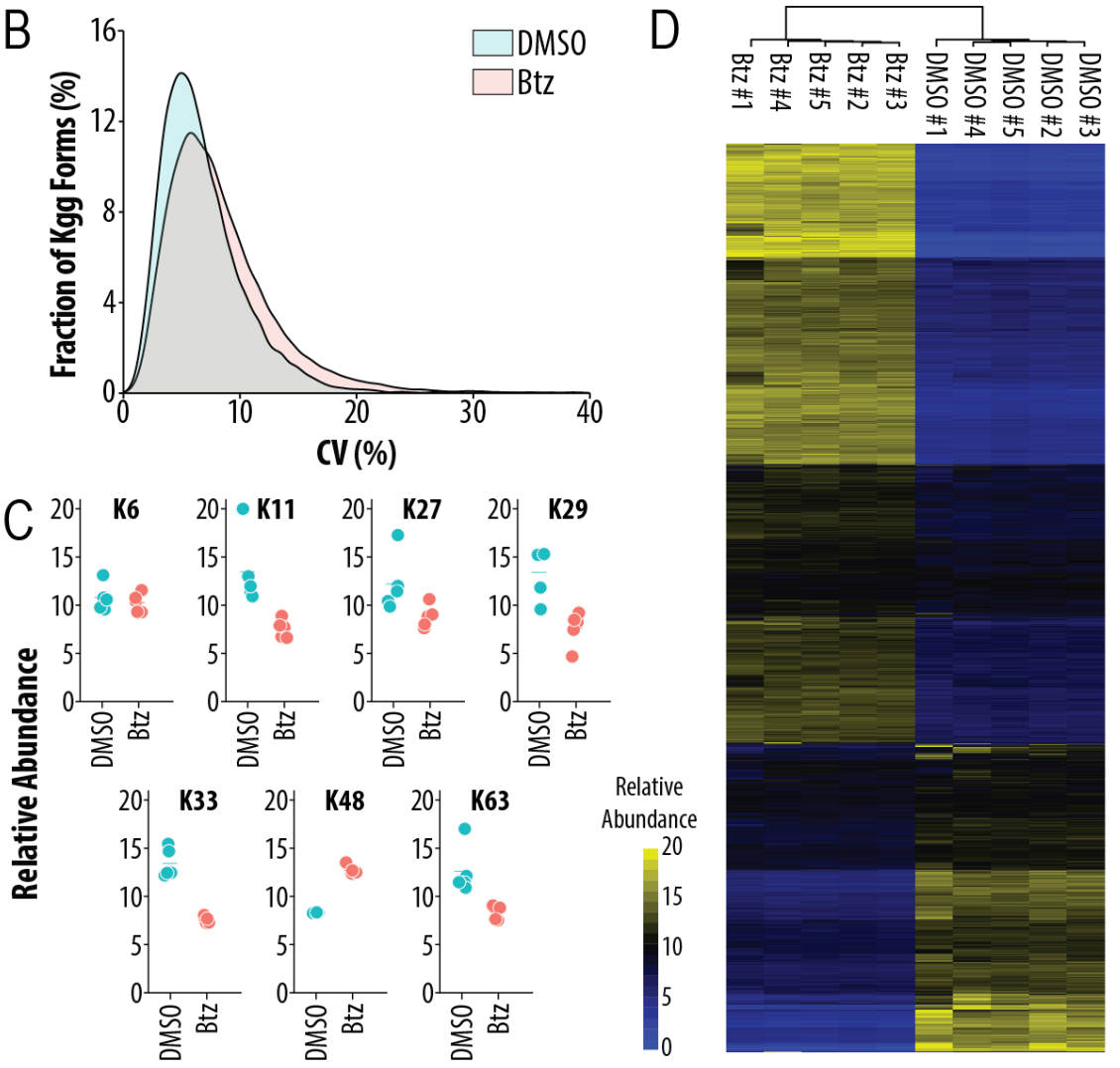

Figure S2. Related to Figure 1. Ubiquitylome analysis beginning with 10 mg of peptide input per sample.

(A) Summary statistics of experiment.

(B) Coefficient of variation (N = 5) for either DMSO or bortezomib treated cells.

(C) Quantitative values for ubiquitylated lysine residues on ubiquitin.

(D) Global changes in ubiquitylation upon bortezomib treatment.

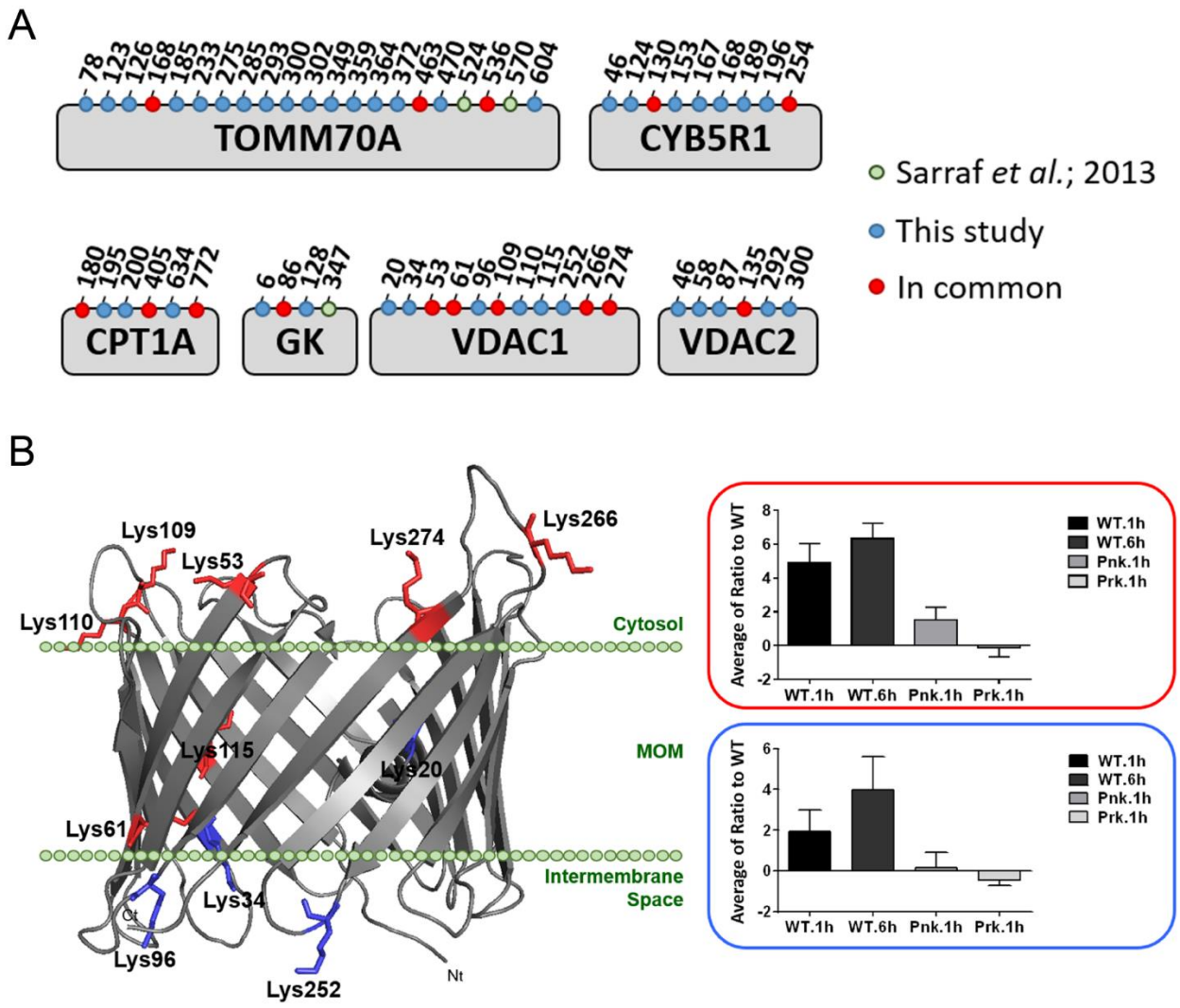

Figure S3. Related to Figure 4. Ubiquitylome analysis of mitochondrial outer membrane PARKIN-dependent substrates.

(A). A deeper ubiquitin coverage is achieved by isobaric labeling-based analysis (this study; in blue) when compared to conventional metabolic labeling techniques (Sarraf et al., 2013; in green).

(B) VDAC1 residues that are freely exposed to the cytosolic face get ubiquitylated faster (upon 1h treatment, highlighted in red) than residues facing the mitochondrial intermembrane space (upon 6h treatment, highlighted in blue). VDAC1 structure was rendered using Pymol software (PDB: 2jk4).