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Supplemental Information

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Mass Spectrometry Analysis of Ubiquitylomes

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Highly Multiplexed Quantitative Mass Spectrometry Analysis of Ubiquitylomes

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TABLES

Table S1. Related to Figure 1: Quantitative ubiquitylome results for an ubiquitin remnant IP beginning with 1 mg/sample of peptides from cells treated for 16 hours with DMSO or Bortezomib.

Table S2. Related to Figure S1: Quantitative ubiquitylome results for an ubiquitin remnant IP beginning with 10 mg/sample of peptides from cells treated for 16 hours with DMSO or Bortezomib.

Table S3. Related to Figure 2: Quantitative proteome and ubiquitylome results for an ubiquitin remnant IP beginning with 7 mg/sample of peptides from mouse brain or liver tissue.

Table S4. Related to Figure 3: Quantitative ubiquitylome results for an ubiquitin remnant IP beginning with 1 mg/sample of peptides from cells treated over a time course with Bortezomib or with DMSO for 16 hours.

Table S5. Related to Figure 4: Quantitative proteome and ubiquitylome results for an ubiquitin remnant IP beginning with 2 mg/sample of peptides from mitochondrial enriched HeLa cells undergoing mitophagy.

Table S6. Related to Figure 4: Mitochondrial ubiquitylation sites demonstrating a 1.8 fold increase in expression upon mitochondrial depolarization.

Table S7. Related to Figure 4: Mitochondrial inner membrane ubiquitylation sites demonstrating an increase in expression 6hr after mitochondrial depolarization.

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.

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

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

SUPPLEMENTAL FIGURES

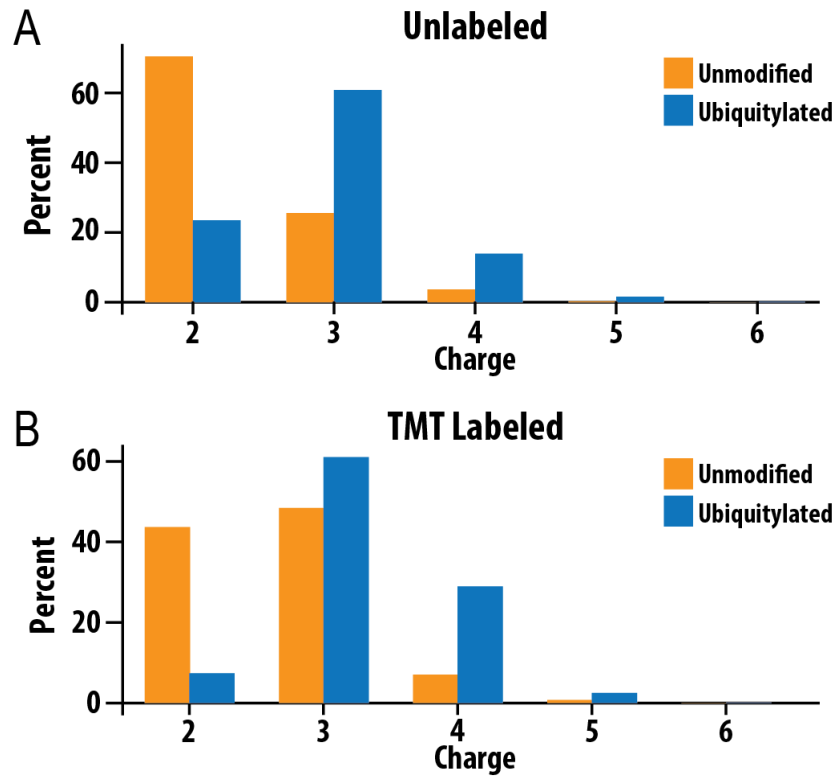


Figure S1. Related to Figure 1. Charge state distribution of identified unmodified and ubiquitylated peptides that are not labeled (A) and labeled (B) with TMT.

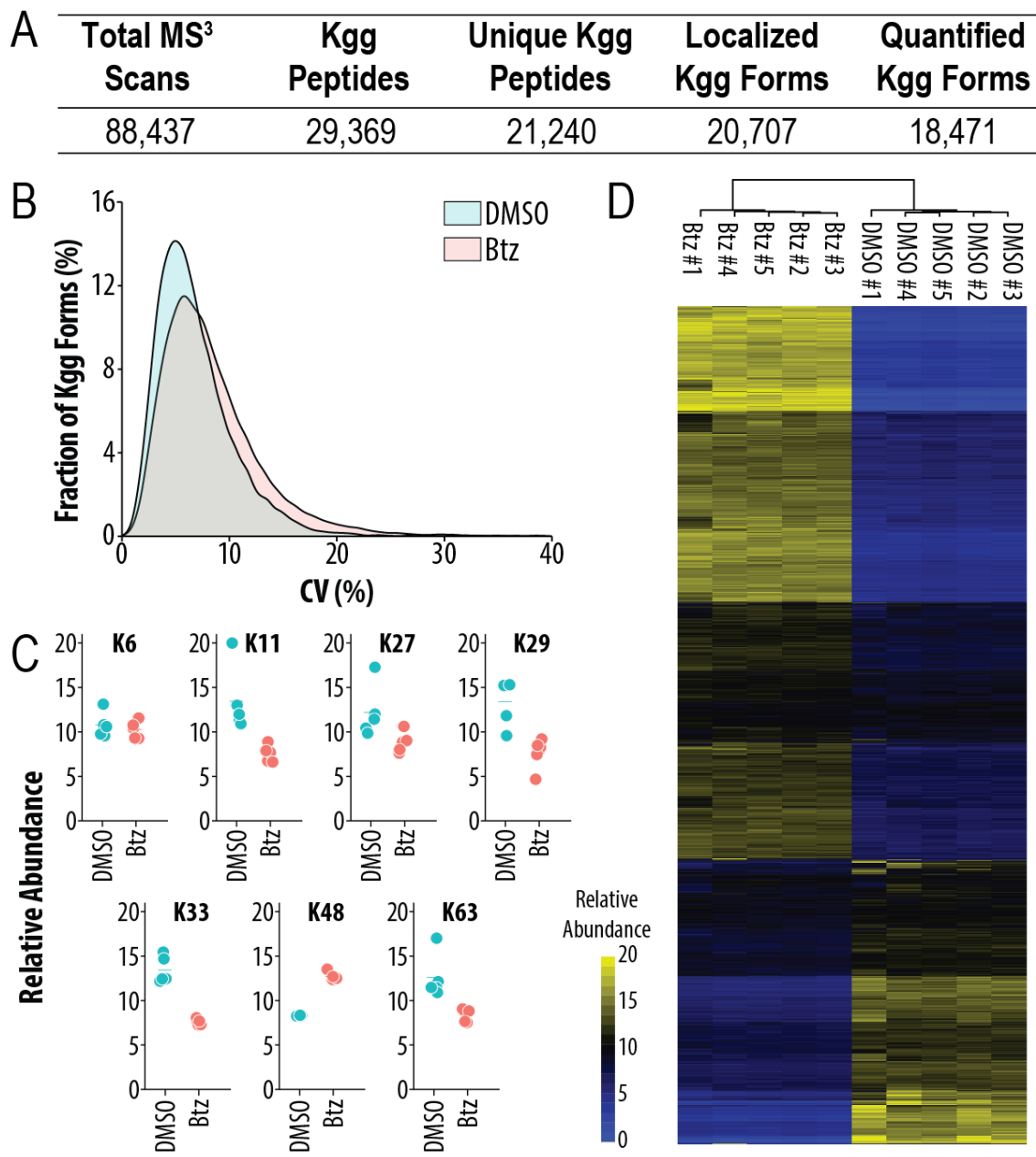


Figure S2. Related to Figure 1. Ubiquitylome analysis beginning with 10 mg of peptide input per sample.

- (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.

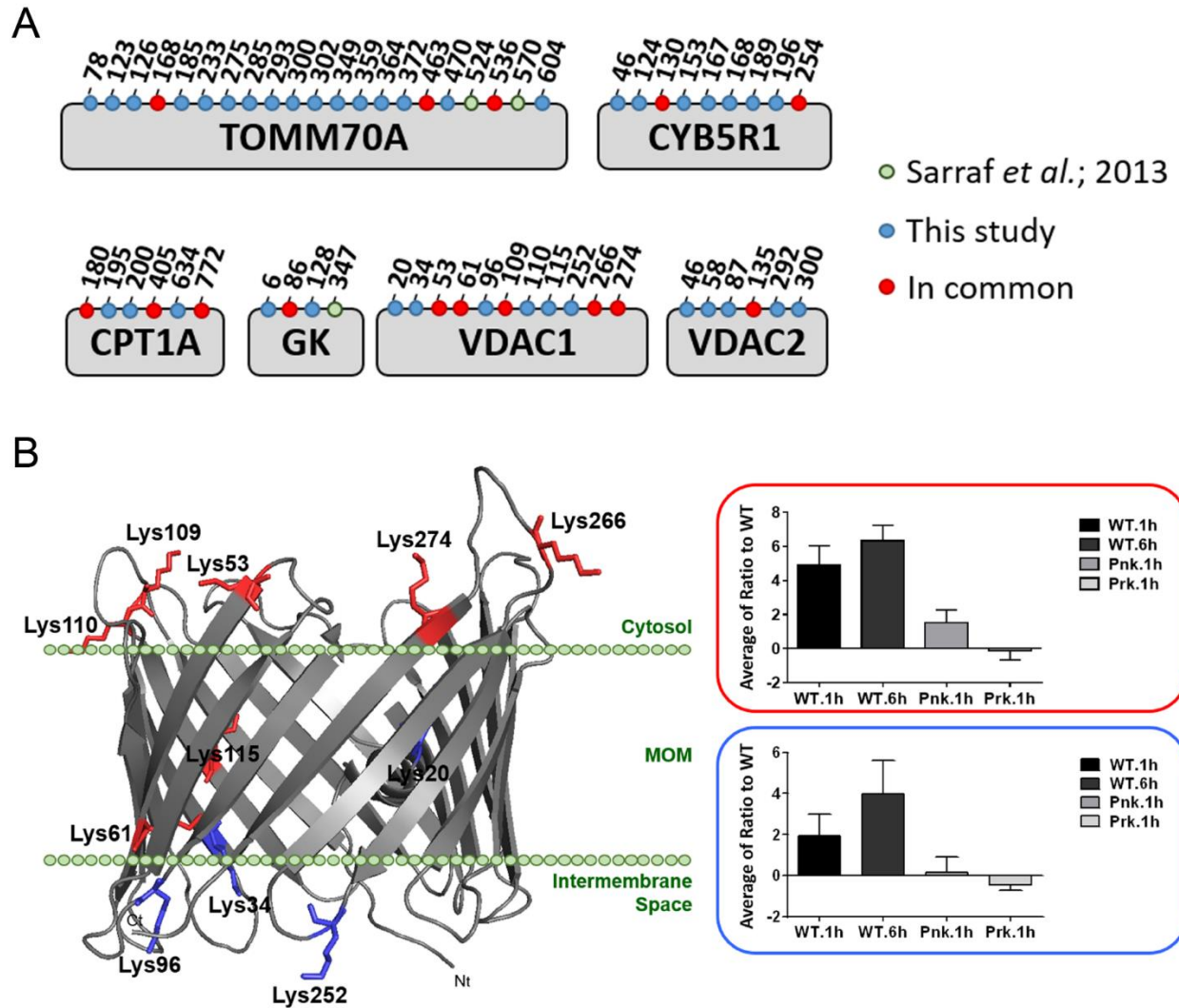


Figure S3. Related to Figure 4. Ubiquitylome analysis of mitochondrial outer membrane PARKIN-dependent substrates.

(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).