Gottlieb et al. Figure S1

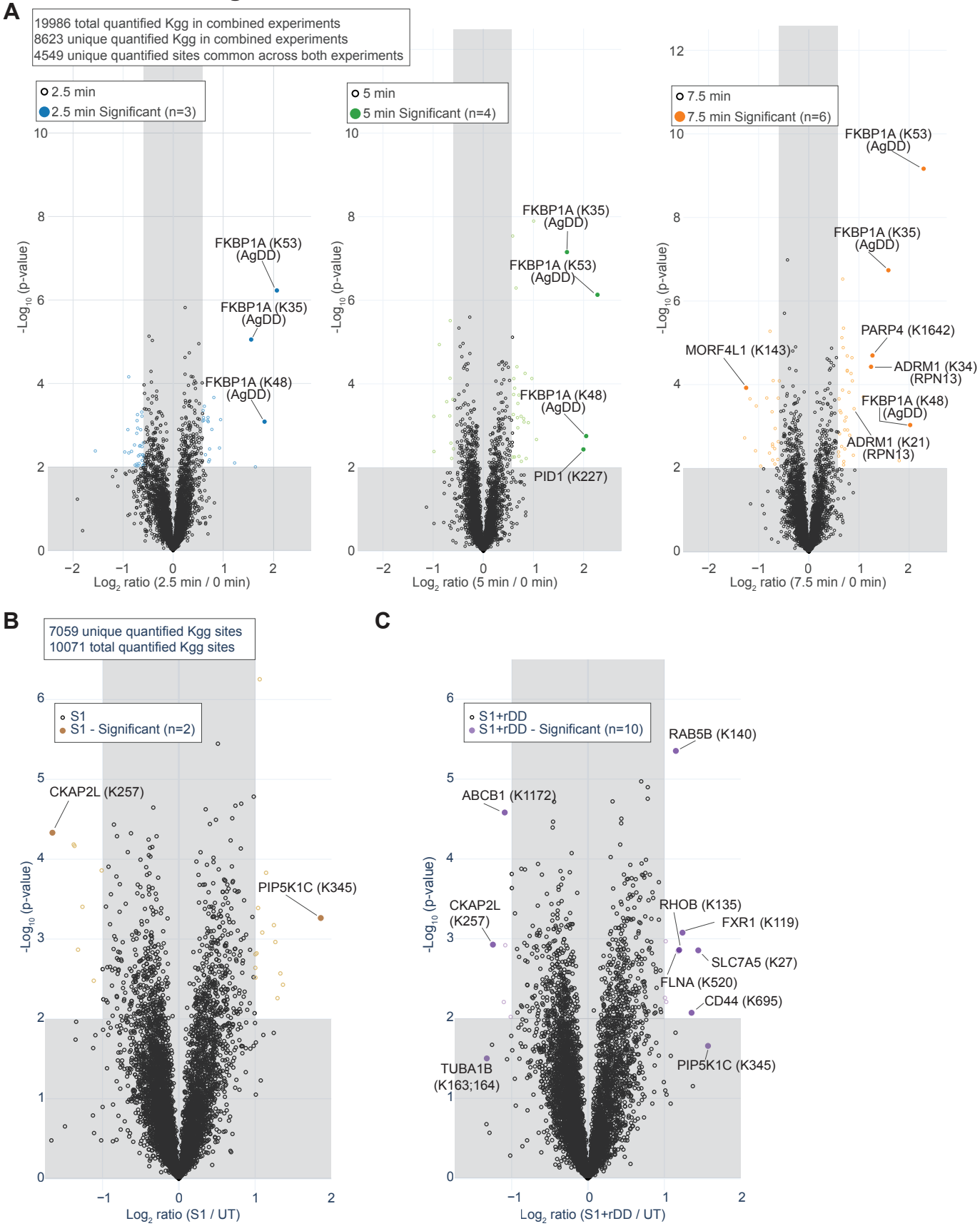

Fig. S1. S1 w/o in AgDD cells rapidly alters the ubiquitylation of PN components. (A) Volcano plots (-Log10 p-value versus log2 ratio to untreated cells) of K-GG sites at 2.5, 5 and 7.5 min following S1 w/o. Proteins with log2 <-0.585 or >0.585 ($p < 0.01$) are indicated as colored empty circles, and filled colored circles indicate statistically significant hits (Welch's t test [S0 = 0.5], corrected for multiple comparison by permutation-based false discovery rate [FDR] [1%]) (B) Volcano plot (-Log10 p-value versus log2 ratio to untreated cells) of K-GG sites in HEK293 WT cells grown in S1 vs vehicle. (C) Volcano plot (-Log10 p-value versus log2 ratio to untreated cells) of K-GG sites in HEK293 cells grown in S1 and undergoing S1 w/o (S1+rDD). (B,C) Proteins with log2 <-1 or >1 ($p < 0.01$) are indicated as colored empty circles, and filled colored circles indicate statistically significant hits (Welch's t test [S0 = 1.2], corrected for multiple comparison by permutation-based false discovery rate [FDR] [1%]).

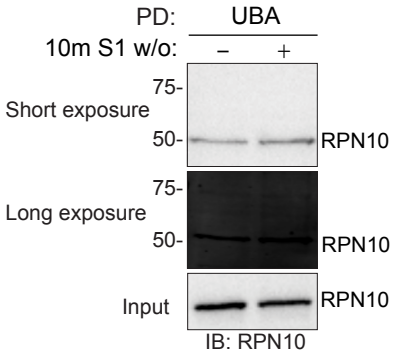

Fig S2. Ubiquitylation of RPN10 induced by S1 w/o is not detectable by immunoblotting. UBA capture of RPN10 did not reveal slower migrating bands that increased in intensity following S1 w/o.

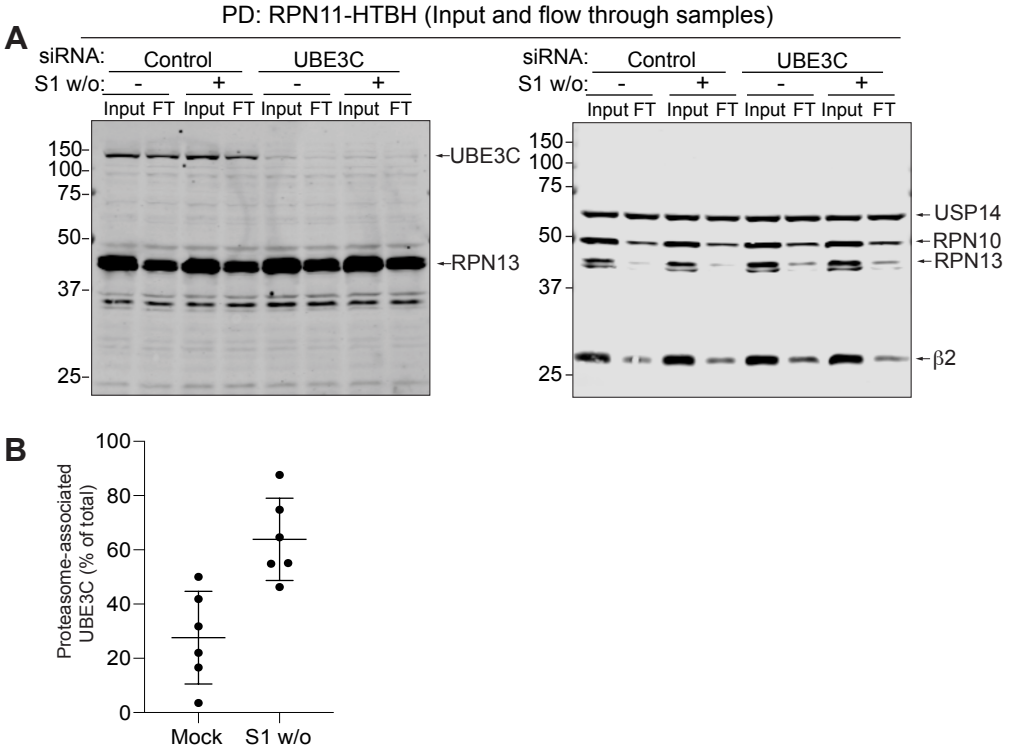

Fig S3. UBE3C-catalyzed ubiquitylation of RPN13 and AgDD at the proteasome following 10 min S1 w/o. (A) Input and flow through (FT) samples showing depletion of UBE3C and several other proteasome-associated proteins following proteasome affinity capture. (B) Increase in the fraction of total intracellular UBE3C that associates with proteasomes following S1 w/o. Error bars represent standard deviation.

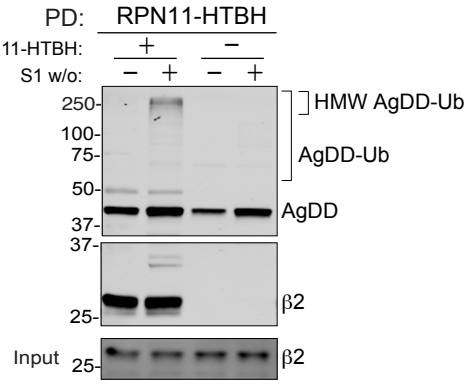

Fig S4. Ubiquitylated AgDD co-purifies with 26S proteasomes. HEK cells stably expressing AgDD alone or in combination with Rpn11-HTBH were subjected to either mock or S1 w/o for 10 min prior to harvest. Proteasomes were captured from Rpn11-HTBH expressing cells using streptavidin-coated magentic beads. Immunodetection of the 20S subunit β2 (middle panel) confirmed the capture of intact 26S proteasomes from AgDD Rpn11-HTBH cells. Immunodetection of GFP (upper panel) detected ubiquitylated AgDD in association with captured proteasomes.