Supplemental Data

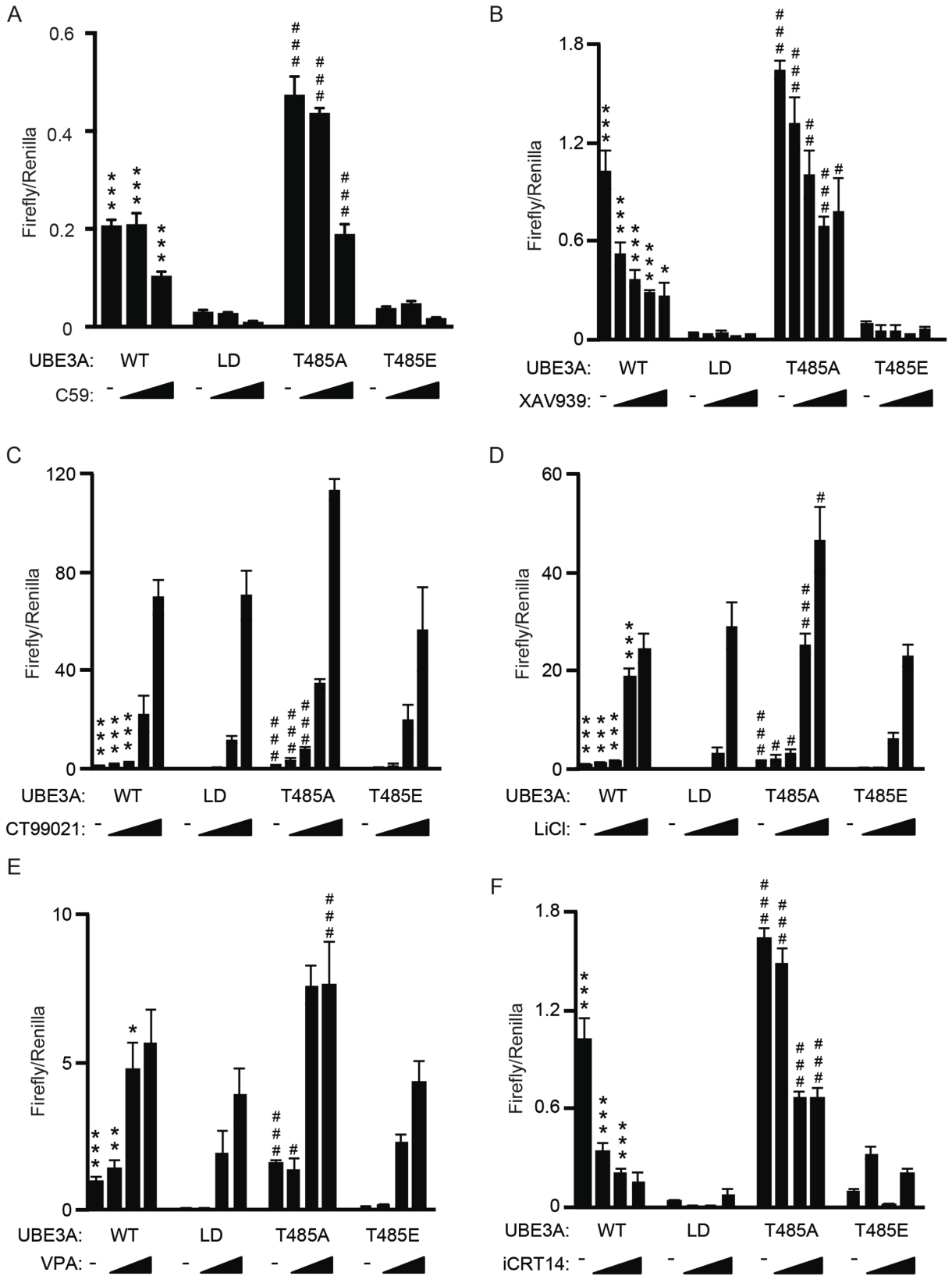

Figure S1. Epistasis experiments indicate that UBE3A acts at or downstream of the destruction complex.

(**A**) HEK293T cells were transfected with the BAR reporter, TK-*Renilla*, and empty vector or the indicated UBE3A plasmid. Cells were treated with DMSO, or increasing concentrations (0, 0.1 μM, 1 μM) of the porcupine inhibitor C59 (inhibits WNT secretion) before firefly luciferase quantitation. Values are shown as the mean ± standard error of firefly/*Renilla* ratios. N=3 for each condition. Note that a reduction in BAR activity is seen across all conditions at the 1 μM dose. ***p<0.0005; WT UBE3A compared to UBE3A LD; $^{###}$p<0.0005, UBE3A^{T485A} compared to UBE3A^{T485E}, two-sample t-test (two-tailed).

(**B**) Cells were treated with DMSO, or increasing concentrations (100 nM, 1 μM, 5 μM, or 10 μM) of the Tankyrase inhibitor XAV939 before firefly luciferase quantitation. Values are shown as the mean ± standard error of firefly/*Renilla* ratios. N=4 for each condition. ***p<0.0005, *p<0.05, WT UBE3A compared to UBE3A LD; $^{###}$p<0.0005, $^{##}$p<0.005, $^{#}$p<0.05, UBE3A^{T485A} compared to UBE3A^{T485E}, two-sample t-test (two-tailed).

(**C**) Cells were treated with DMSO, or increasing concentrations (0.625 μM, 1.25 μM, 2.5 μM or 5 μM) of the GSK3β inhibitor CT99021 before firefly luciferase quantitation. Values are shown as the mean ± standard error of firefly/*Renilla* ratios. N=4 for each condition. **p<0.005, UBE3A^{T485A} compared to UBE3A^{T485E}. ***p<0.0005, WT UBE3A compared to UBE3A LD; $^{###}$p<0.0005, UBE3A^{T485A} compared to UBE3A^{T485E}, two-sample t-test (two-tailed).

(**D**) Cells were treated with DMSO, or increasing concentrations (0.2 mM, 2 mM, 10 mM or 20 mM) of the non-specific GSK3β inhibitor LiCl before firefly luciferase quantitation. Values are shown as the mean ± standard error of firefly/*Renilla* ratios. N=4 for each condition. ***p<0.0005, WT UBE3A compared to UBE3A LD; $^{###}$p<0.0005, $^{#}$p<0.05, UBE3A^{T485A} compared to UBE3A^{T485E}, two-sample t-test (two-tailed).

(**E**) Cells were treated with DMSO, or increasing concentrations (0.2 mM, 2 mM, or 10 mM) of the β-catenin-dependent transcription activator valproic acid (VPA) before firefly luciferase quantitation. Values are shown as the mean ± standard error of firefly/*Renilla* ratios. N=4 for each condition. ***p<0.0005, **p<0.005, *p<0.05, WT UBE3A compared to UBE3A LD; $^{###}$p<0.0005, $^{#}$p<0.05, UBE3A^{T485A} compared to UBE3A^{T485E}, two-sample t-test (two-tailed).

(**F**) Cells were treated with DMSO, or increasing concentrations (10 nM, 100 nM, 500 nM) of the β-catenin-dependent transcription inhibitor iCRT14 before firefly luciferase quantitation. Values are shown as the mean ± standard error of firefly/*Renilla* ratios. N=4 for each condition. ***p<0.0005, WT UBE3A compared to UBE3A LD; $^{###}$p<0.0005, UBE3A^{T485A} compared to UBE3A^{T485E}, two-sample t-test (two-tailed).

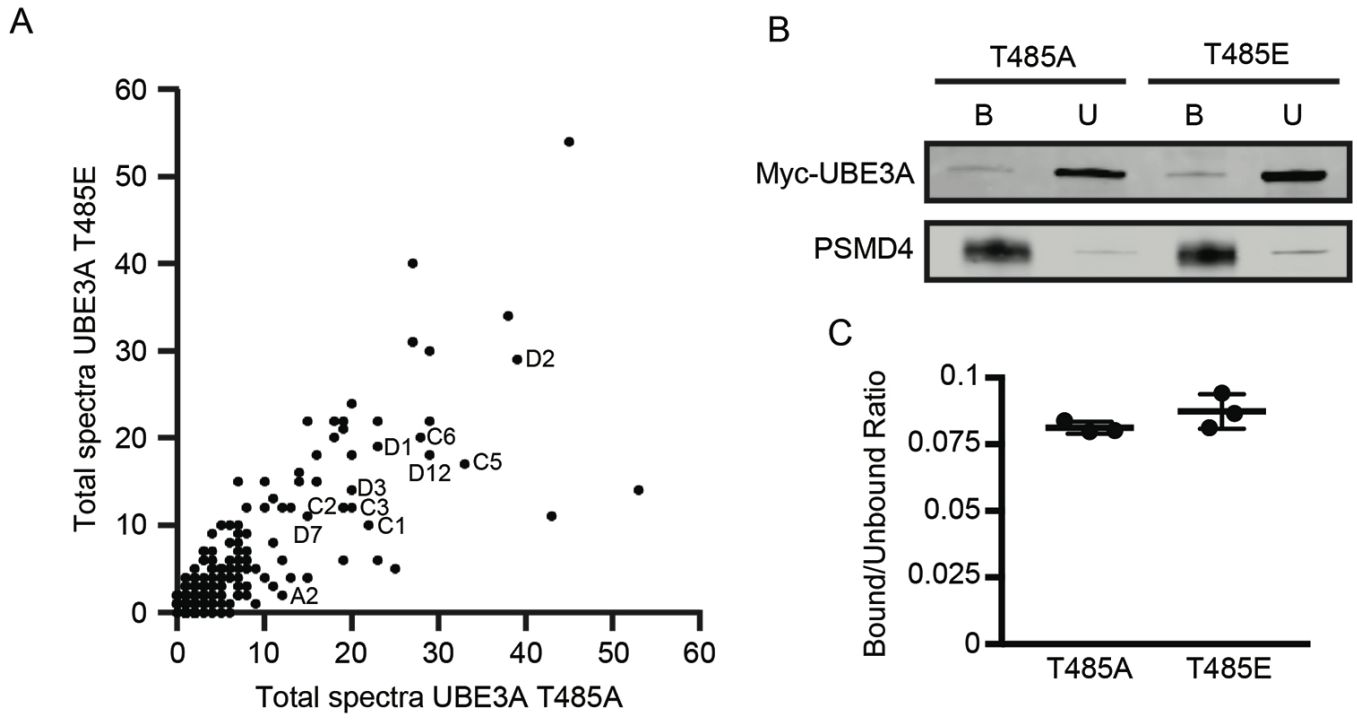

Figure S2. UBE3A phosphorylation does not affect its association with interacting proteins or the proteasome.

(**A**) Scatter plot showing spectral counts of UBE3A interacting proteins identified by mass spectrometry analysis. Each dot represents a protein identified to interact with the UBE3A^{T485A} and UBE3A^{T485E} mutant by mass spectrometry analysis. Several of the interacting proteasome subunits are indicated (see Table S3 for full list).

(**B** and **C**) Representative western blot and quantification (**C**) of isolated proteasomes from HEK293T cells expressing Myc-tagged UBE3A^{T485A} or UBE3A^{T485E} mutants. UBE3A abundance was analyzed in the proteasome bound (B) and unbound (U) fractions. Values are shown as the mean ± standard deviation of the ratio of bound/unbound UBE3A. N=3, two-sample t-test (two-tailed).

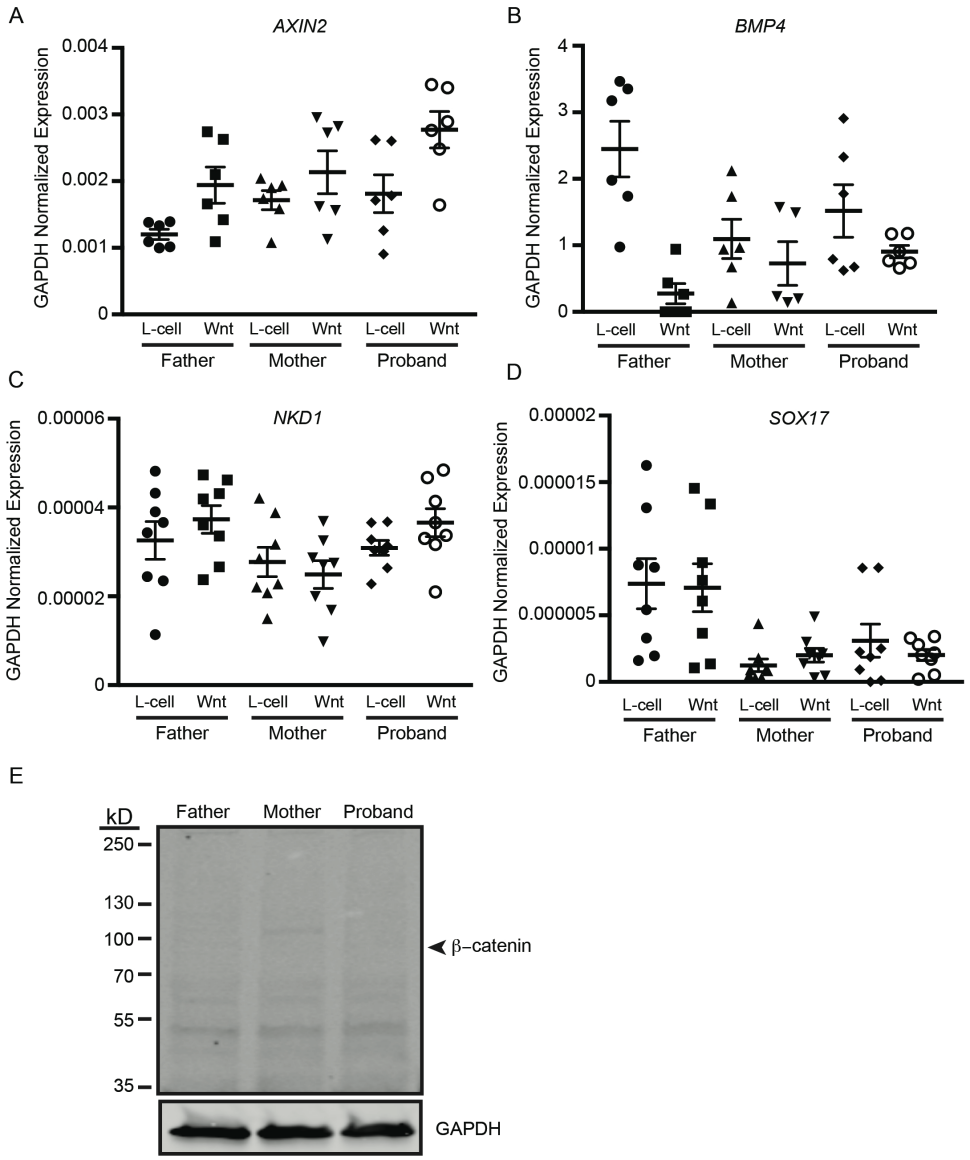

Figure S3. Immortalized lymphocytes are not responsive to WNT stimulation.

(**A** – **D**). Lymphocytes were grown in L-cell CM and WNT3A CM and the induction of WNT-responsive genes *AXIN2* (**A**), *BMP4* (**B**), *NKD1* (**C**), and *SOX17* (**D**), were analyzed by qPCR. Values are shown as the mean ± standard deviation of transcript levels normalized to GAPDH. N=6 for each condition. No statistically significant gene expression was detected for any target gene in the absence or presence of WNT, two-sample t-test (two-tailed).

(**E**) Representative western blot showing the absence of detectable β-catenin in immortalized lymphocyte cell lines from the father, mother, and autism proband (Simon's Simplex Collection; Family ID: 13873).

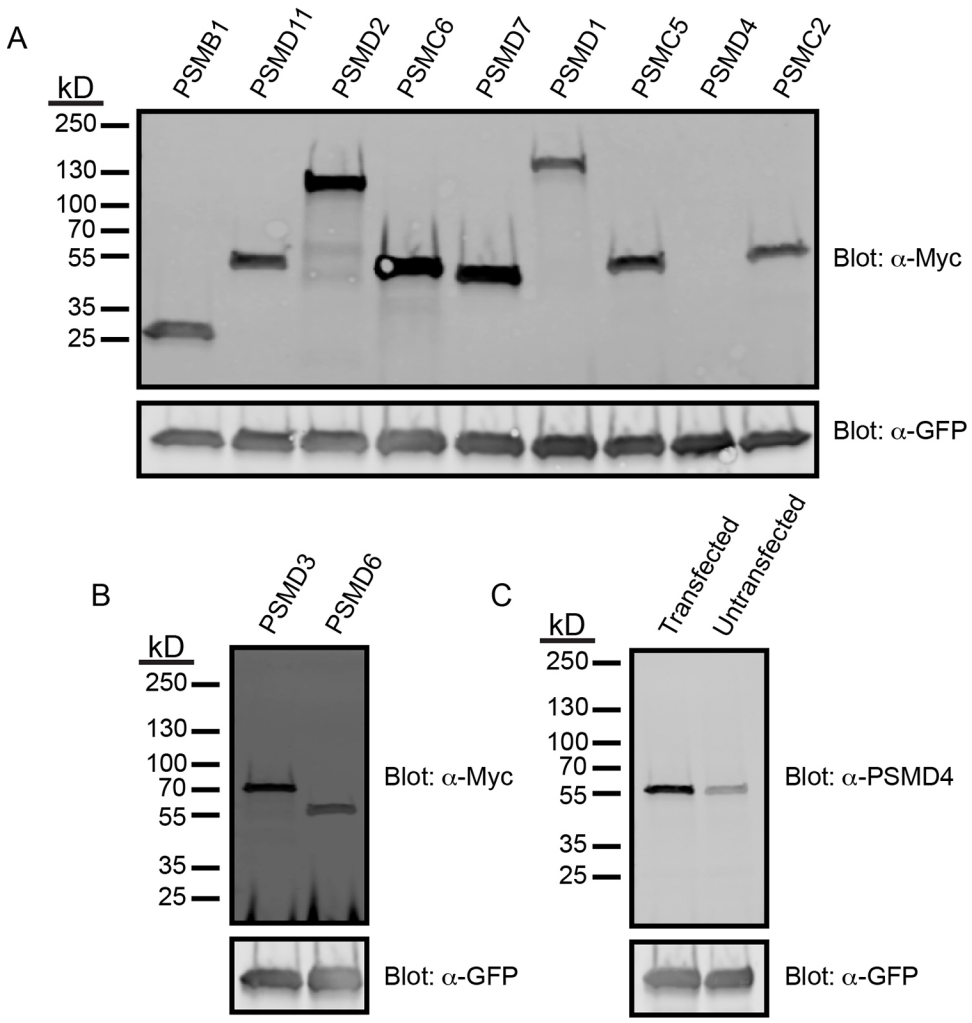

Figure S4. All plasmids used in rescue experiments overexpress proteasome subunit protein in HEK293T cells.

(**A**) HEK293T cells were co-transfected with plasmids encoding GFP and the indicated proteasome subunit. Western blots were performed with anti-Myc antibody to detect the epitope tag on each subunit, and with anti-GFP antibody to confirm transfection. Note that PSMD4 is untagged, and not reactive with anti-Myc antibody. See (**C**).

(**B**) Western blot performed with anti-Myc and anti-GFP antibodies to confirm PSMD3 and PSMD6 overexpression.

(**C**) Protein lysates from HEK293T cells transfected with GFP alone, or co-transfected with GFP and PSMD4 plasmids were analyzed by western blot with anti-PSMD4 and anti-GFP antibodies.

Supplemental Tables

Table S1. High confidence UBE3A interacting proteins identified by mass spectrometry.

We identified high confidence interacting proteins from (24) based on the following criteria: 1) proteins must have a normalized weighted D (NWD) score greater than or equal to 1 in replicate experiments, 2) the interaction must be specific to UBE3A and not NEDD4 (negative control).

Table S2. Identification of negative regulators of WNT signaling by genome-wide siRNA screening in HEK293T cells.

Genes that, when knocked down, enhanced WNT reporter gene expression by at least 50% ($p < 0.05$) from (39) are shown.

Table S3. UBE3A T485A and UBE3A T485E interacting proteins identified by mass spectrometry in HEK293T cells.

Table S4. Putative UBE3A substrates identified by SILAC and ubiquitin remnant immunoaffinity profiling in HEK293T cells.

The proteins listed exhibited at least a 20% enrichment in ubiquitination in cells expressing UBE3A T485A as compared to cells expressing UBE3A T485E. Core proteasome subunits are highlighted.

Table S5. Rescue of UBE3A-dependent WNT activation by proteasomal subunits.

Table S6. BAR reporter responses in HEK293T cells upon knockdown of proteasome subunits.