Figure S1

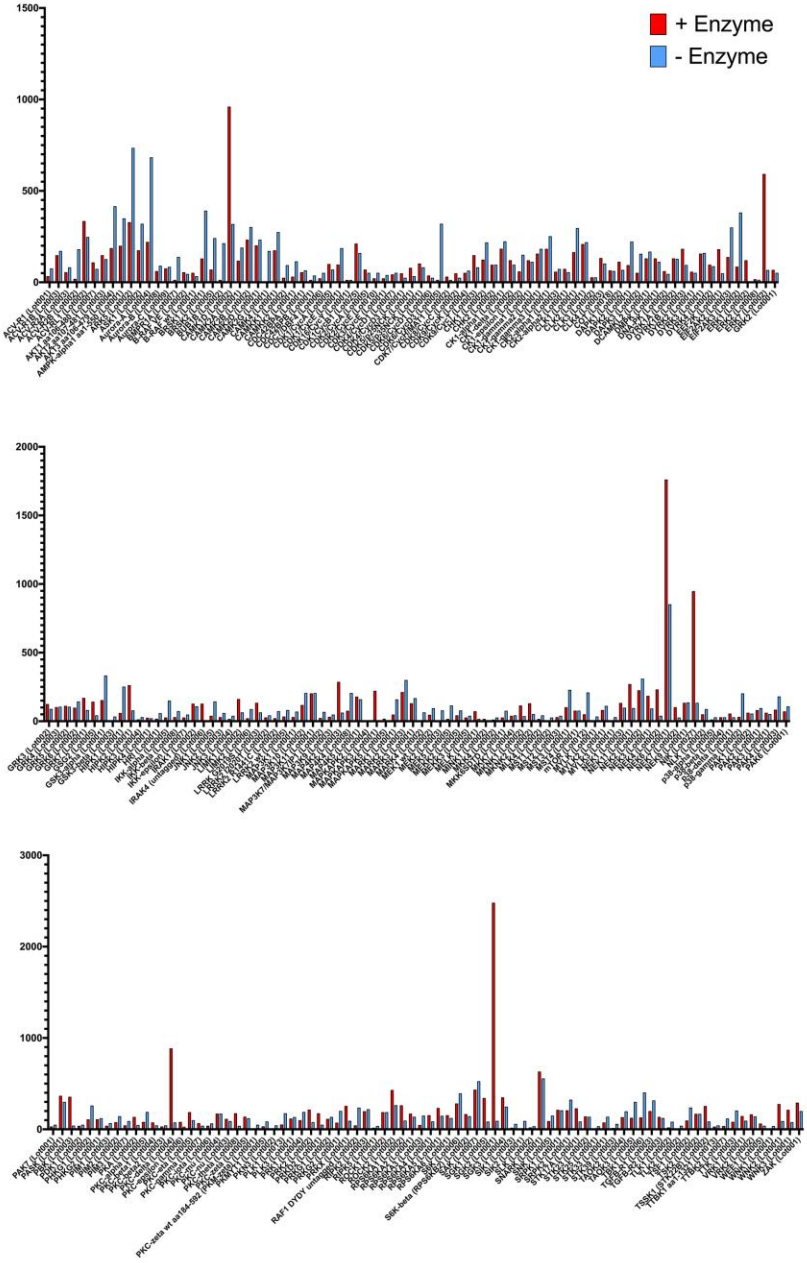
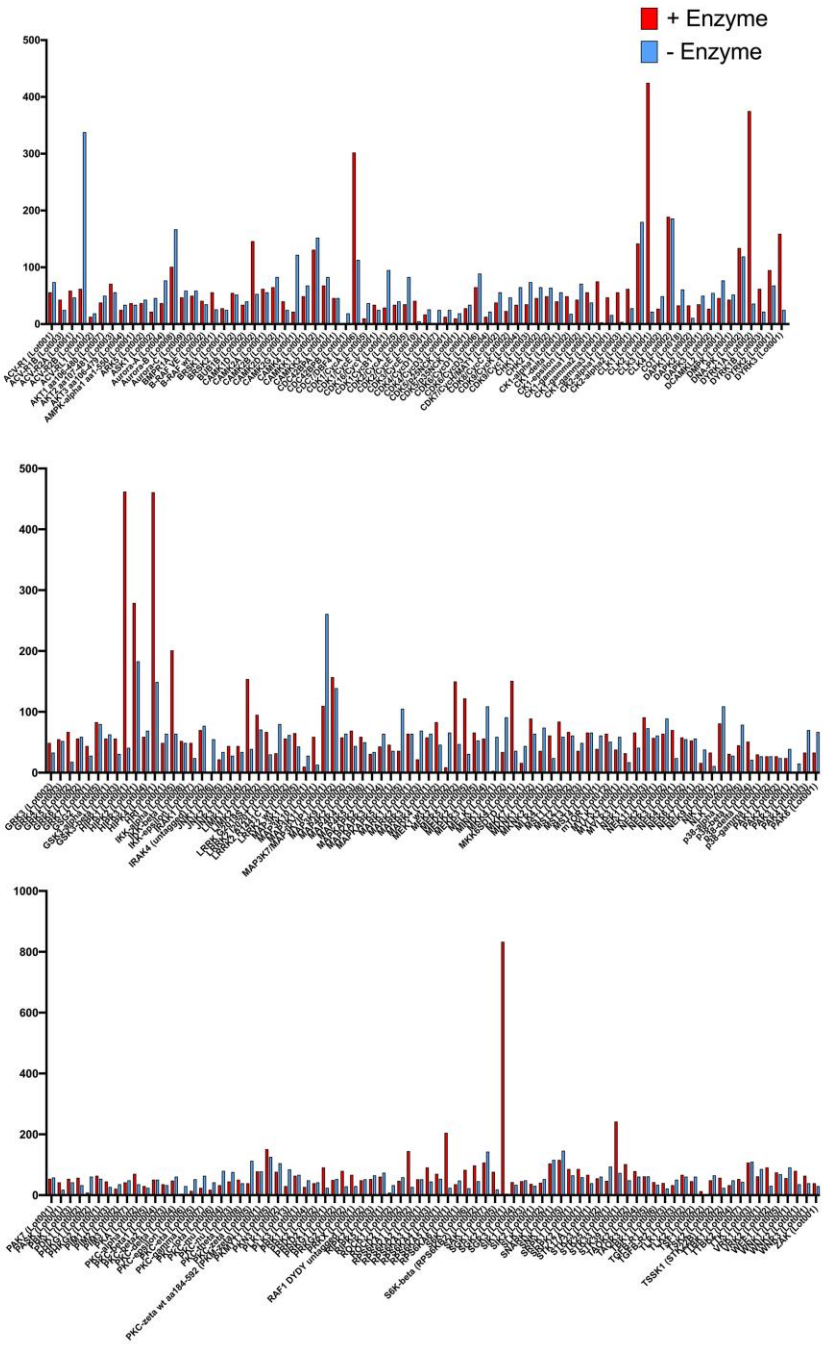


Figure S1. In vitro screening of S/T kinases identifies a subset of kinases that phosphorylate S36 of PTOV1. An N-terminally biotinylated PTOV1 peptide encompassing S36 was incubated in a streptavidin-coated FlashPlate and subject to a radiometric kinase assay with 245 individual S/T protein kinases. To account for signal from kinase autophosphorylation, each assay (+ peptide) was normalized to a control with the kinase but no peptide.

Figure S2



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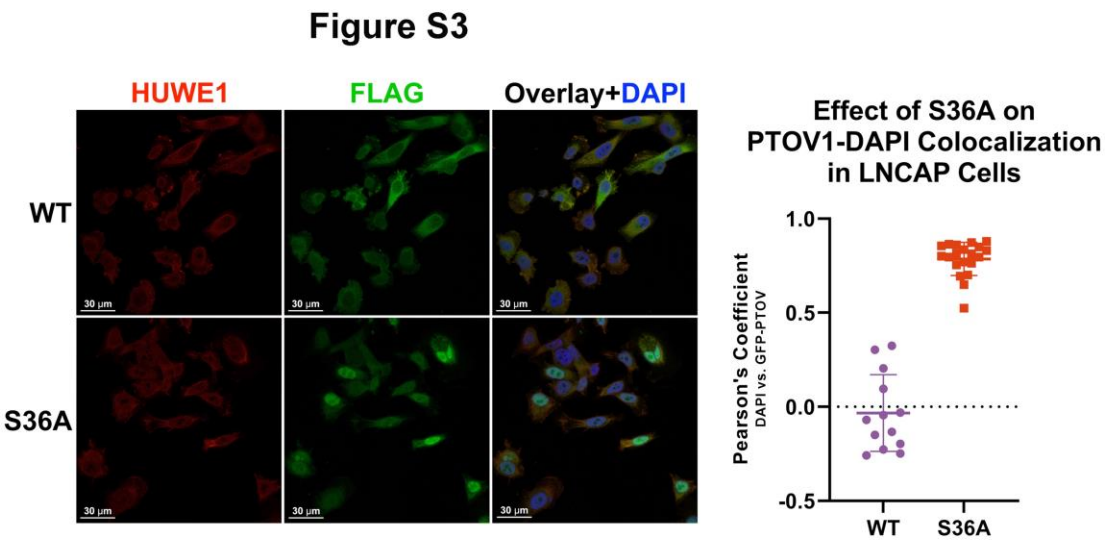

Figure S3. Loss of phosphorylation at S36 causes PTOV1 translocation to the nucleus in LNCaP cells. LNCaP cells stably expressing FLAG-PTOV1 WT or S36A were imaged and analyzed as in Figure 3C.

Figure S4

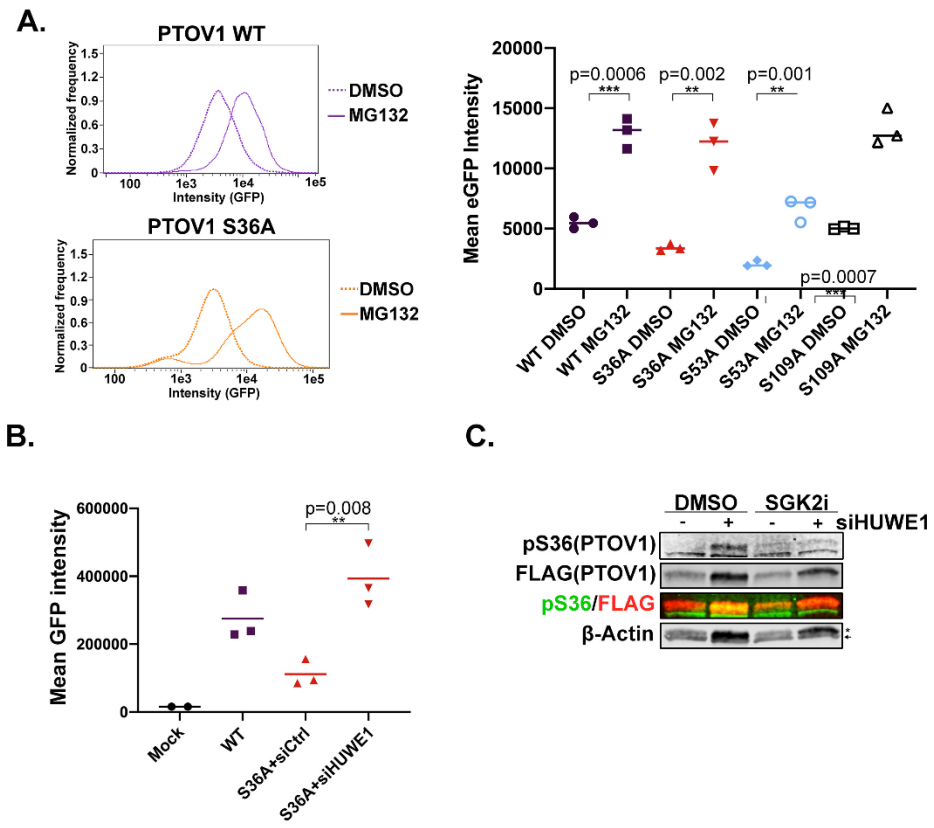

Figure S4. PTOV1 is degraded via the proteasome in a HUWE1-dependent manner. A) PC3 cells stably expressing GPF-tagged PTOV1 WT and mutants were treated with DMSO or MG132 as in Figure 4B and analyzed for GFP levels by imaging flow cytometry. Left panels show GFP intensity for the indicated PTOV1 constructs and right panel shows average GFP signal from three biological replicates. Error bars represent SEM and p-values were calculated using a Student's t-test from three biological replicates. B) PC3 cells stably expressing GFP-PTOV1 WT or S36A were transfected with siRNA as in Figure 5B, followed by analysis of GFP levels by imaging flow cytometry. Quantification was done as in panel A graph for the indicated number of biological replicates. C) PC3 cells stably expressing FLAG-PTOV1 WT were transfected with siHUWE1 then treated with SGK2 inhibitor or vehicle as in Figure 2C, followed by immunoblotting with the indicated antibodies. The arrow indicates β-Actin, while the asterisk indicates FLAG(PTOV1).

Figure S5

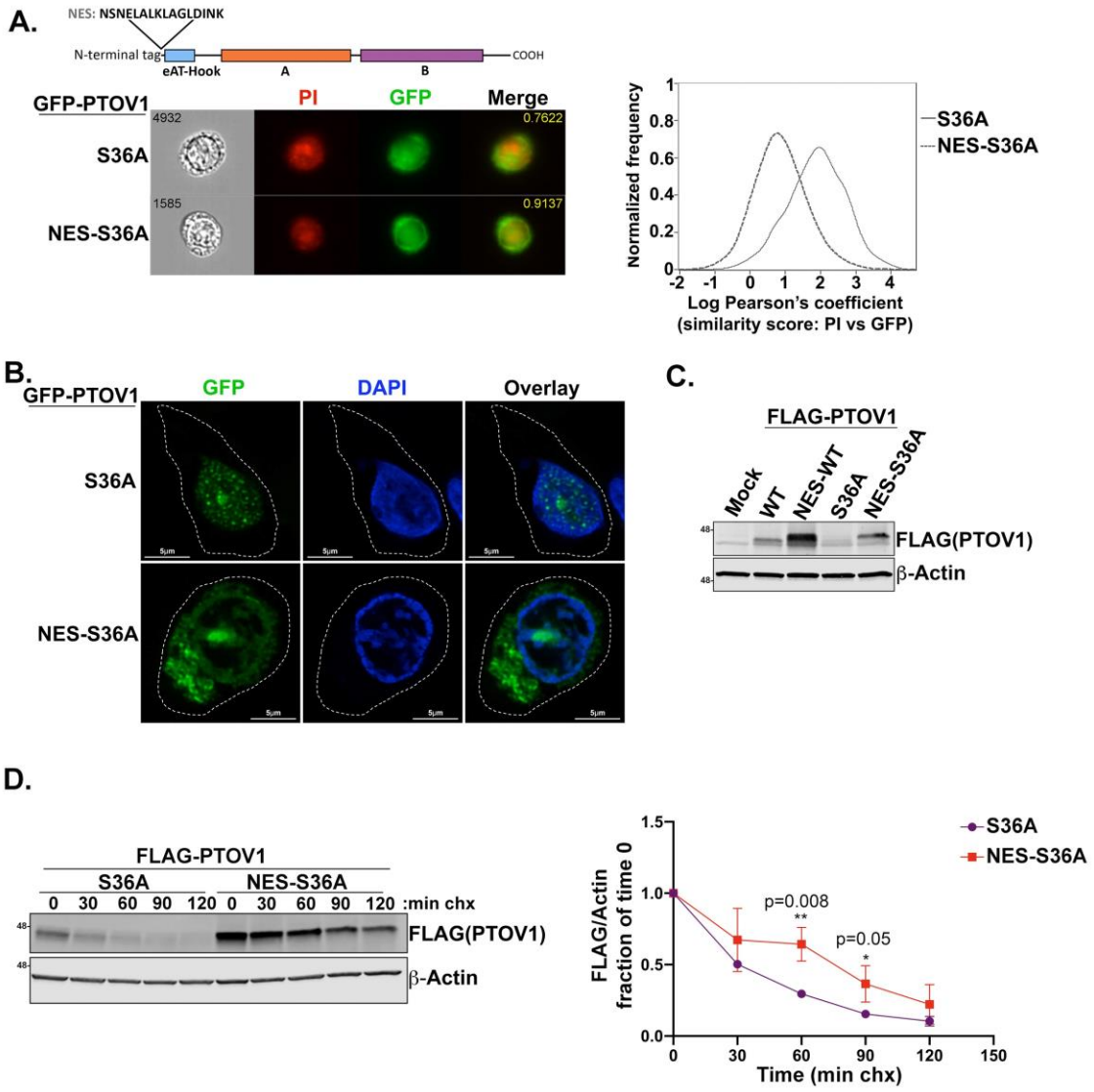

Figure S5. PTOV1 nuclear localization is required for degradation. A) Upper schematic shows the insertion site of the NES at the N-terminus of PTOV1. Lower plots show representative images from an imaging flow cytometry analysis of GFP-PTOV1 S36A and NES-GFP-PTOV1 S36A in HEK-293T cells. Lower right panel shows quantification from imaging flow cytometry analysis of PTOV1 localization to the nucleus as in Figure 3B. B) HEK-293T cells transfected with GFP-PTOV1 S36A and NES-GFP-PTOV1 S36A were visualized by confocal imaging as a corollary to imaging flow cytometry analysis in panel 6A. C) HEK-293T were transfected with indicated PTOV1 constructs and immunoblotted for FLAG(PTOV1) and β-Actin. D) HEK-293T cells overexpressing FLAG-PTOV1 S36A and NES-FLAG-PTOV1 S36A were treated and quantified as in Figure 4A. Quantification is from three biological replicates. Error bars represent SD; p-values were calculated using a two-tailed Student's t-test.

Figure S6

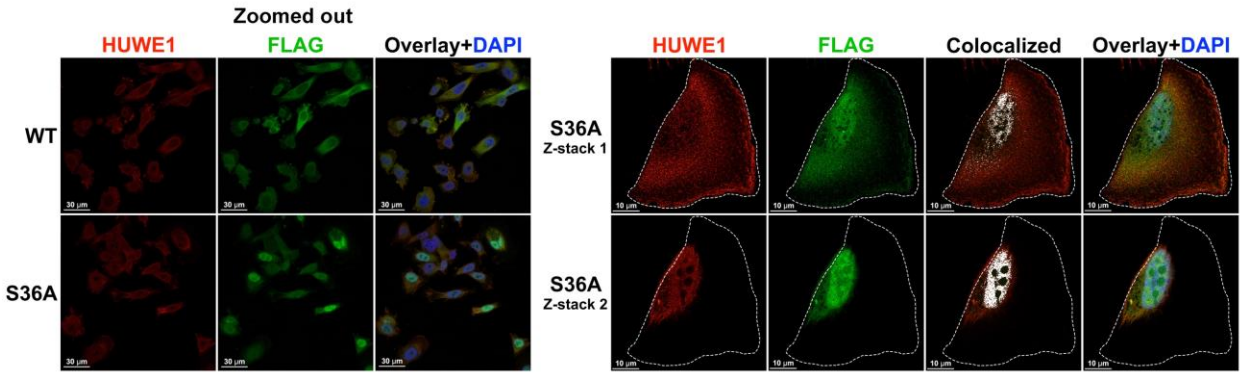

Figure S6. Endogenous HUWE1 overlaps with PTOV1 S36A in the nucleus. PC3 cells stably expressing FLAG-PTOV1 WT or S36A were immunostained for FLAG and endogenous HUWE1, treated with DAPI, then analyzed by confocal imaging. Panels on left show zoomed out images, while panels on right show detail through two layers of the Z-stack to better visualize nuclear HUWE1.