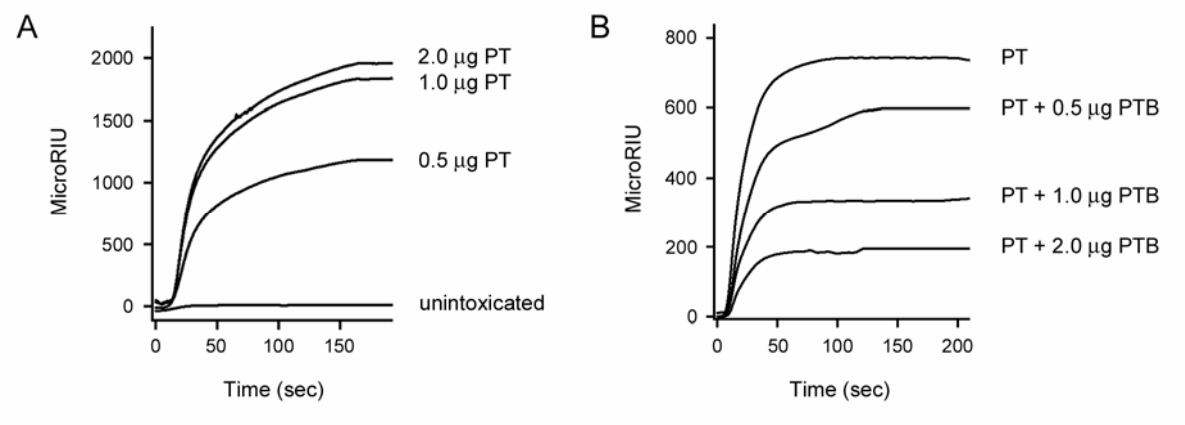

FIG S1 PT binding to the cell surface is a saturable event. (A) CHO cells were incubated for 30 min at 4°C with 2.0, 1.0, or 0.5 µg/mL of PT. Unbound toxin was removed, and cell extracts generated with a Triton X-100 lysis buffer were perfused over a SPR sensor coated with an anti-PTS1 antibody. An extract from unintoxicated cells was also used as a negative control. (B) CHO cells were incubated for 30 min at 4°C with a combination of 1 µg/mL PT and 0.5, 1.0, or 2.0 µg/mL of the PTB oligomer. Unbound toxin was removed, and cell extracts generated with a Triton X-100 lysis buffer were perfused over a SPR sensor coated with an anti-PTS1 antibody.

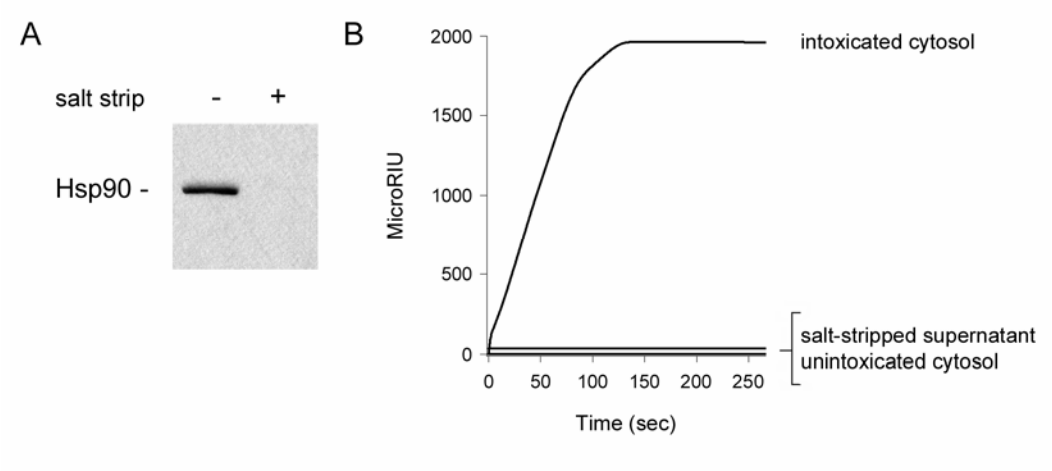

FIG S2 Translocated PTS1 is not membrane-associated. CHO cells pulse-loaded at 4°C with 1 µg/mL of PT were chased for 3 h at 37°C in toxin-free medium. Membrane pellet and cytosolic supernatant fractions from digitonin-permeabilized cells were collected at the end of the chase. The membrane pellet containing intact organelles was bathed three times in 1 mL of PBS containing 0.8 M NaCl for 20 min at 4°C. Proteins in the salt-stripped supernatant were collected after a 5 min, 13,800 x g spin re-pelleted the membranes. (A) Western blot analysis demonstrated the peripheral association of Hsp90 with the membrane fraction was disrupted by salt treatment. (B) The salt-stripped supernatant was perfused over an SPR sensor coated with an anti-PTS1 antibody. The cytosolic fractions from intoxicated and unintoxicated cells were also perfused over the sensor as positive and negative controls, respectively. Control experiments using purified PTS1 in 0.8 M NaCl ensured the high salt concentration did not interfere with PTS1 binding to the antibody-coated sensor.

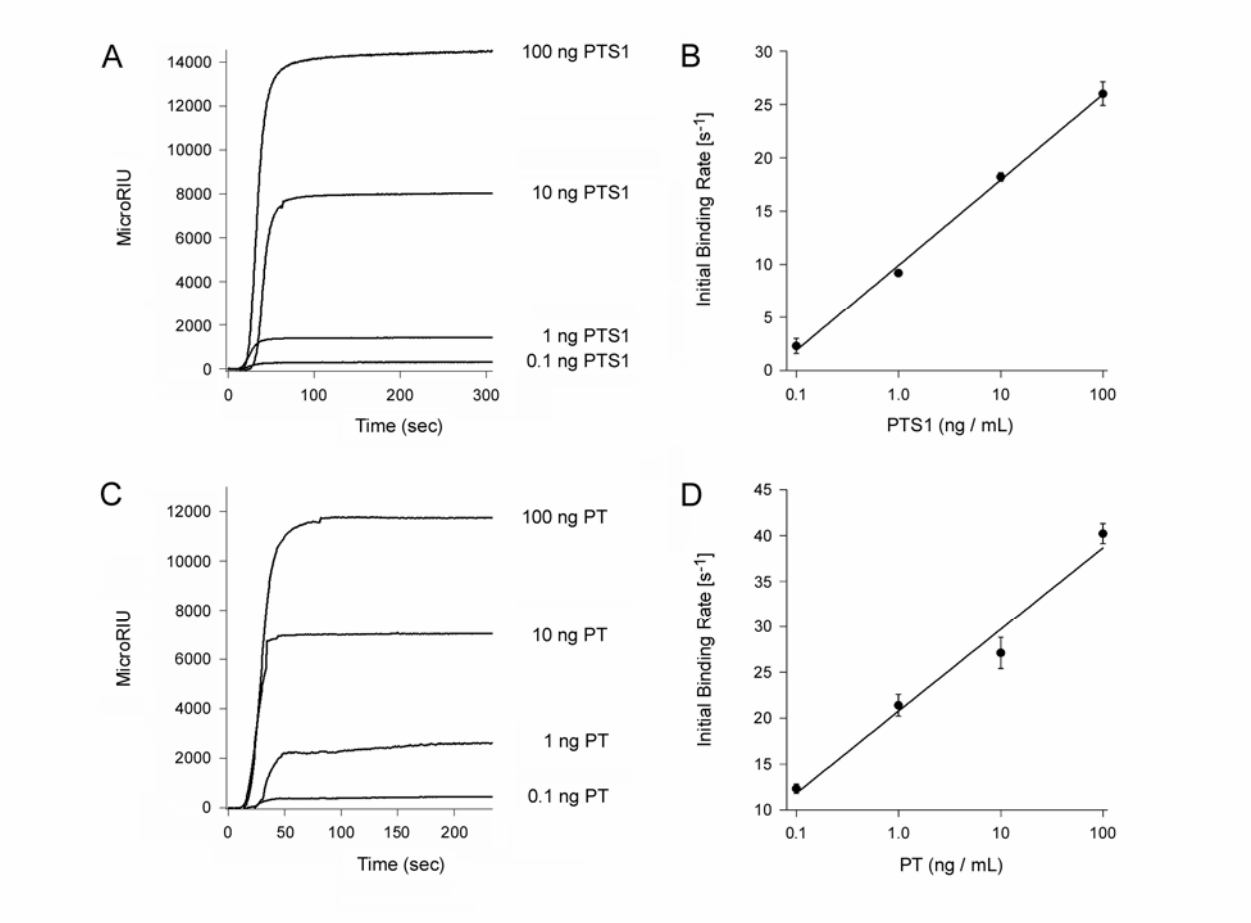

FIG S3 Standard curves for calculating unknown quantities of PTS1 and PT. (A) Known quantities of PTS1 were perfused over an SPR sensor coated with an anti-PTS1 antibody. Ligand was removed from the perfusion buffer after 200 sec. (B) Initial binding rates for the PTS1 standards were plotted as a function of protein concentration. Data from three separate experiments are presented as averages ± standard deviations. (C) Known quantities of PT were perfused over an SPR sensor coated with an anti-PTS4 antibody. Ligand was removed from the perfusion buffer after 200 sec. (D) Initial binding rates for the PT standards were plotted as a function of protein concentration. Data from three separate experiments are presented as averages ± standard deviations.

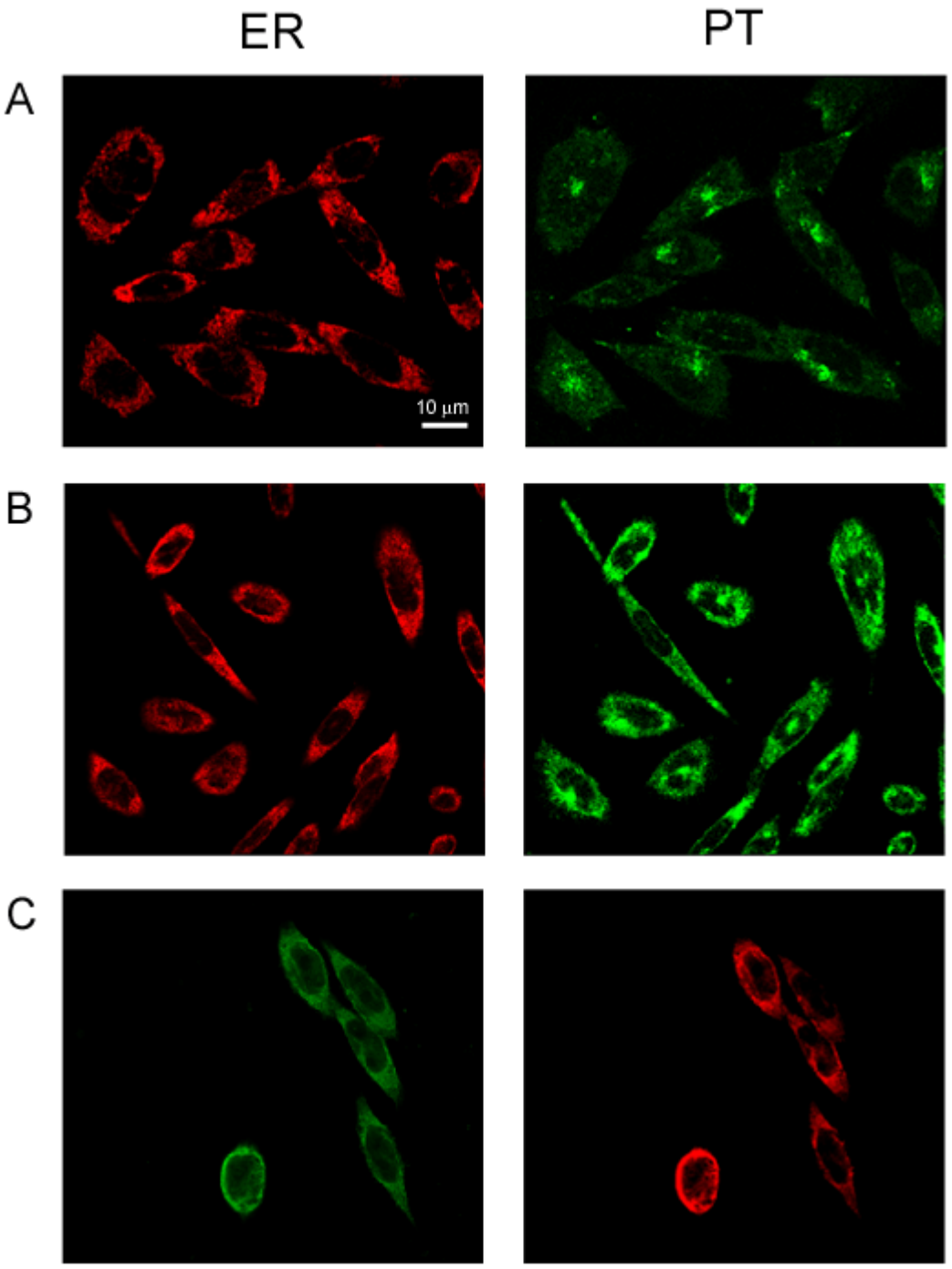

FIG S4 PT reaches the ER of PBA- or glycerol-treated cells. CHO cells pulse-loaded at 4°C with 1 µg/mL of PT were chased for 3 h at 37°C in toxin-free medium with (A) no additions, (B) 100 µM PBA, or (C) 10% glycerol. Confocal microscopy then compared the location of resident ER markers (left panels) to the distribution of PT (right panels) using antibodies against (A, B) PDI and PTS1 or (C) BiP and PTS4. The punctate, perinuclear distribution of PT in untreated cells partially shifted to a web-like pattern that co-localized with PDI or BiP in cells treated with PBA or glycerol.

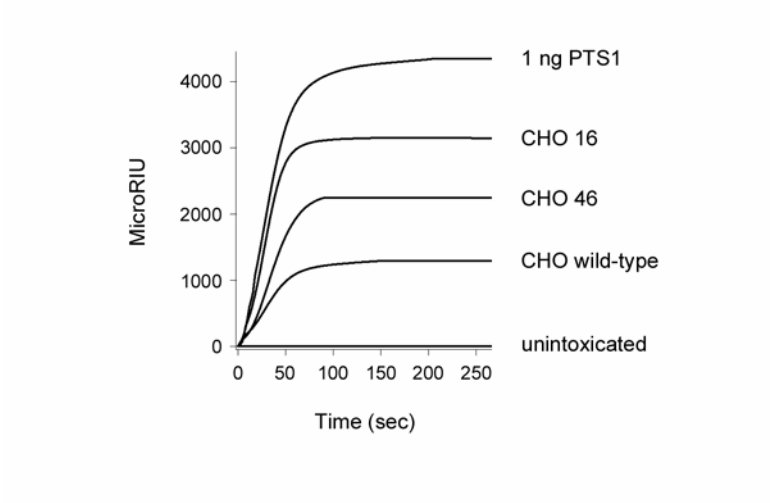

FIG S5 CHO mutants 16 and 46 secrete more PTS1 than the parental cell line. Wild-type CHO cells, mutant 16, and mutant 46 were pulse-loaded at 4°C with 1 μg/mL of PT and chased for 3 h at 37°C in toxin-free medium. The extracellular medium was collected at the end of the chase and perfused over an SPR sensor coated with an anti-PTS1 antibody. To serve as a reference point, a known quantity of purified PTS1 (1 ng/mL) was also perfused over the sensor. Before perfusion, media samples were normalized to the total protein content (as determined by Bradford assay) from each corresponding cell lysate.

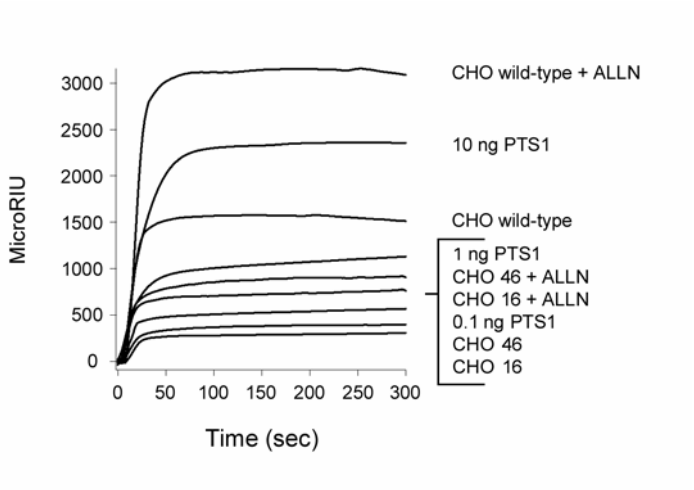

FIG S6 Proteasome inhibition results in elevated levels of cytosolic PTS1. Wild-type CHO cells, CHO mutant 16, and CHO mutant 46 were incubated with PT at 4°C for 30 min. Unbound toxin was removed, and the cells were chased for 3 h at 37°C in toxin-free medium lacking or containing 100 μM of the proteasome inhibitor ALLN. Cytosolic fractions from digitonin-permeabilized cells and known quantities of PTS1 standards were then perfused over an SPR slide coated with an anti-PTS1 antibody. Ligand was removed from the perfusion buffer after 200 sec.