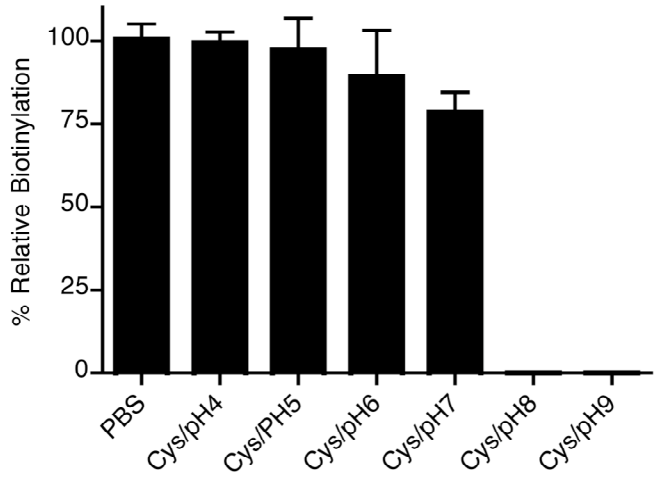
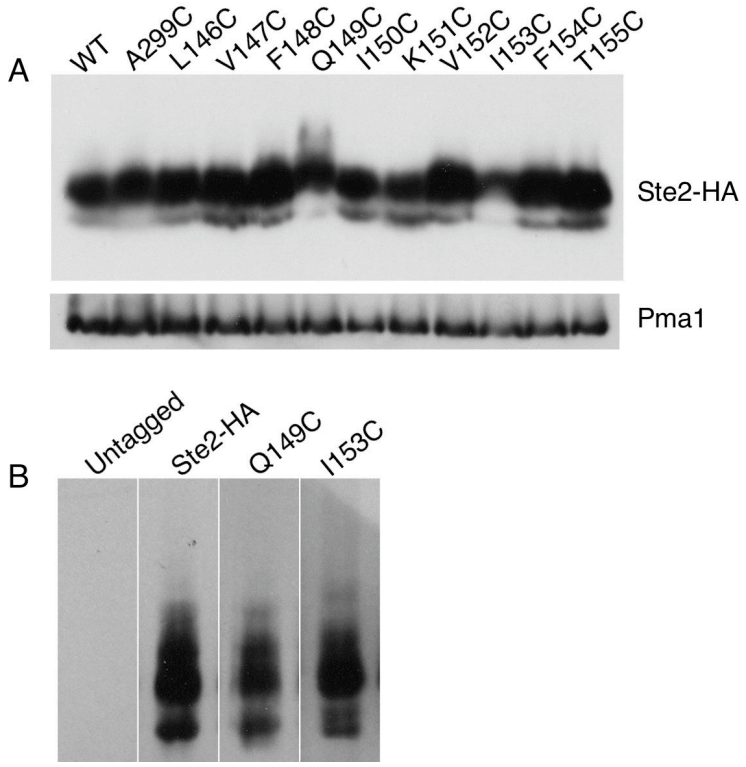


Supplementary Information Figure 1

Stability of Ste2 biotinylation in the presence of 10 mM Cys at different pH levels.

Membrane fractions containing the Ste2-A299C mutant receptors were reacted with MTSEA-biotin, washed with PBS, and then incubated either in PBS alone or in PBS with 10 mM Cys at the indicated pH for 5 min. The membrane fractions were then washed, and then the Ste2 proteins were immunoprecipitated with anti-HA monoclonal antibody immobilized beads. The degree of receptor biotinylation was quantified on Western blots probed with anti-HA to detect Ste2, and with Streptavidin to detect biotinylated Ste2. The degree of biotinylation was normalized to 100% for the level of the biotinylated A299C membrane fractions that were treated only with PBS. The results represent the average of three to six independent reactions. Bars indicate standard error.



Supplementary Information Figure 2

Western blot analysis of mutant Ste2 proteins.

(A) Western blot analysis for a representative set of the Cys-substituted Ste2 proteins. Membrane fractions were separated by gel electrophoresis, transferred to nitrocellululose, and then probe with either anti-HA to detect Ste2-HA or anti-Pma1 as a loading control.

(B) An independent analysis of the indicated membrane fractions in which the exposure times for developing the signal for the Q149C and I153C mutants on the blot were adjusted to approximate the signal for the wild-type Ste2-HA in order to compare the complexity of protein heterogeniity due to glycosylation and phosphorylation.