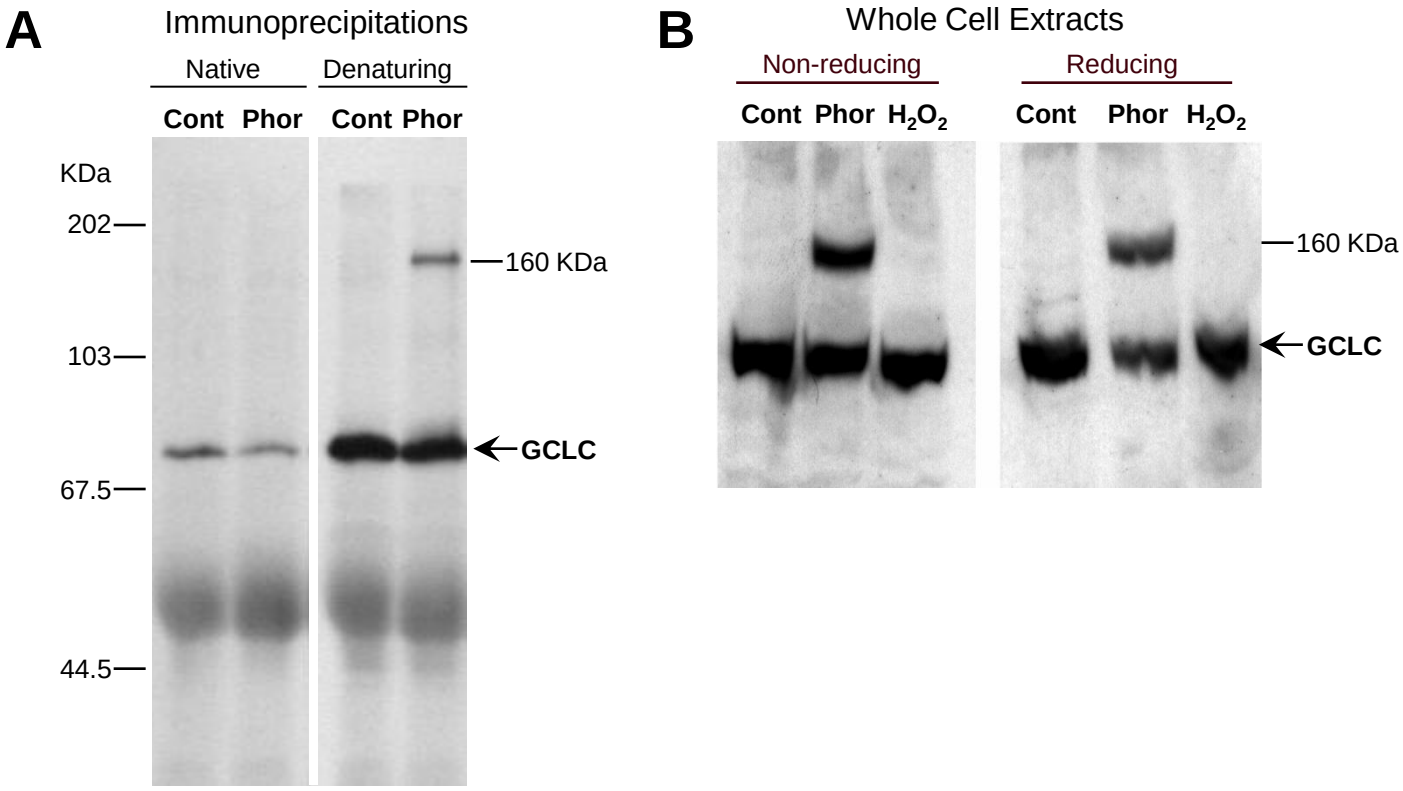
Supplemental Materials for Krejsa et al., Rapid Activation of Glutamate Cysteine Ligase

Supplemental Fig. 1. Phorone treatment induces high MW form of GCL. (A) GCLC was immunoprecipitated from identical extracts of control or phorone-treated Jurkat cells using the native or denaturing IP protocol, separated by reducing SDS-PAGE and stained for GCLC. Relative recovery was lower by native IP, and the denaturing IP strategy revealed the E^{76}-T^{91} epitope in phorone-treated cells, recovering equal amounts as from control cells. The denaturing IP protocol also recovered a ~160 KDa protein that stained for GCLC. (B) Whole cell extracts of Jurkat cells treated with phorone or H_2O_2 were resolved by non-reducing and reducing SDS-PAGE. No intermolecular disulfide was detected in GCL following oxidative stress. The high MW form in phorone-treated cells is more prominent in the whole cell extracts than in the immunoprecipitations.

Supplemental Fig. 2. Altered GCLC epitope recognition following oxidative stress. Jurkat cells treated with phorone or control cells extracts were subjected to IP using an immunoaffinity resin. (A) Serial IP of GCLC from native extracts of untreated Jurkat cells. Lanes 1-4 are sequential 2 h reactions, and the lane 5 reaction continued overnight (24 h total IP with 5 changes of resin). (B) Densitometry analysis shows the average recovery for all sequential reactions, and cumulative recovery of GCLC from the 24 h reaction. Error bars indicate standard deviations in recovered GCLC from the first three sequential reactions. (C) GCLC recovery from extracts of control and phorone-treated cells, with increasing resin volume in the IP reaction. (D) Densitometry analysis of recovered GCLC from control and treated cells.

Supplemental Figure 1



Supplemental Figure 2