Supplementary materials for

# Src Homology 2 domains enhance tyrosine phosphorylation in vivo by protecting binding sites in their target proteins from dephosphorylation

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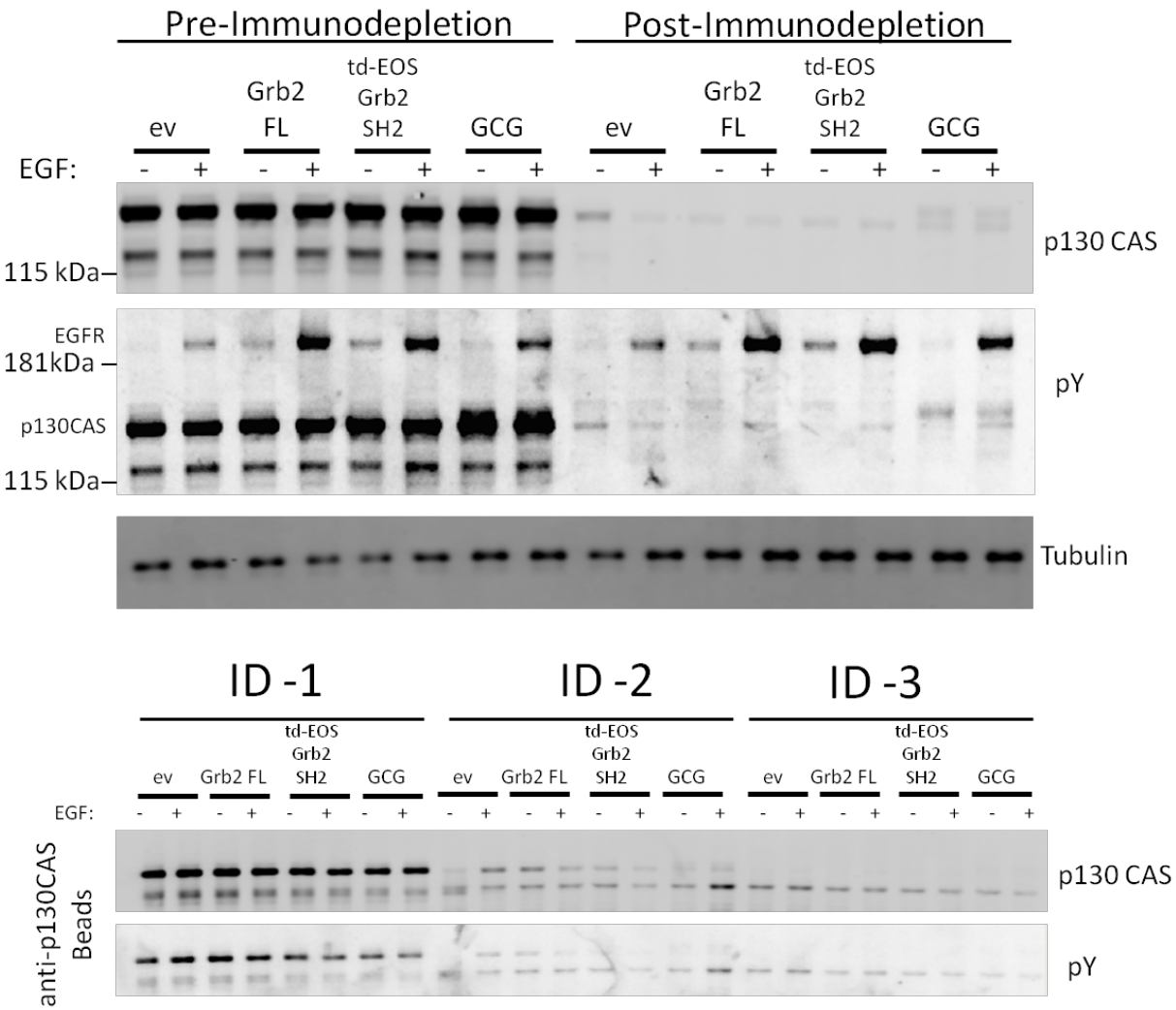
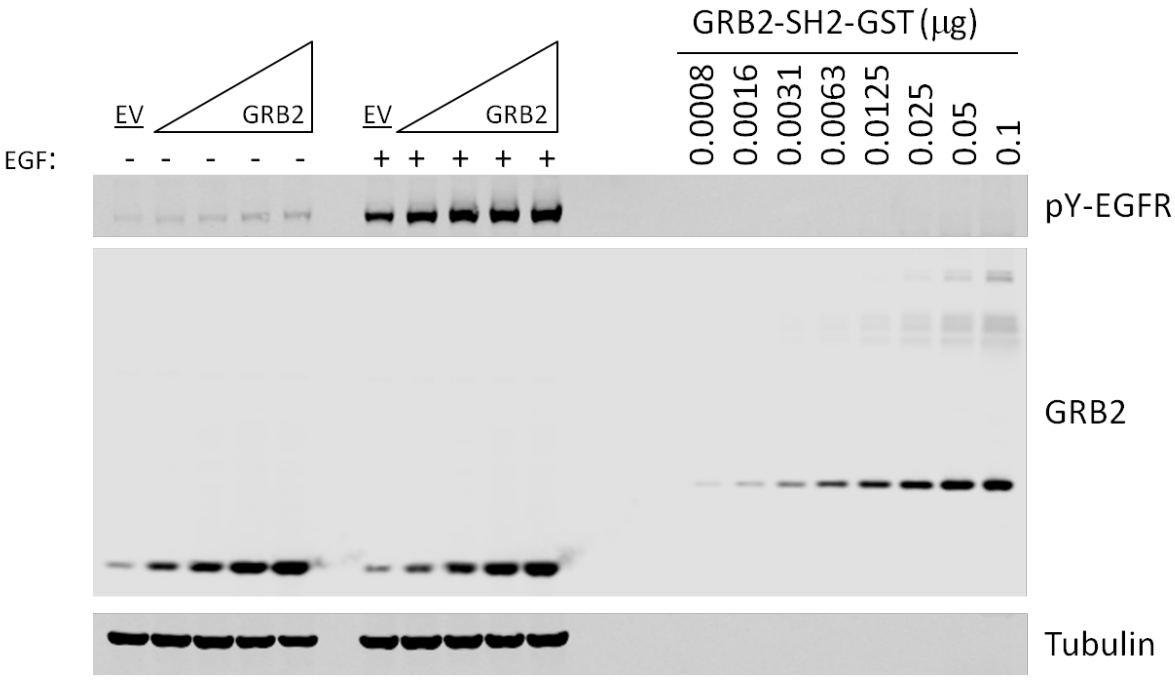
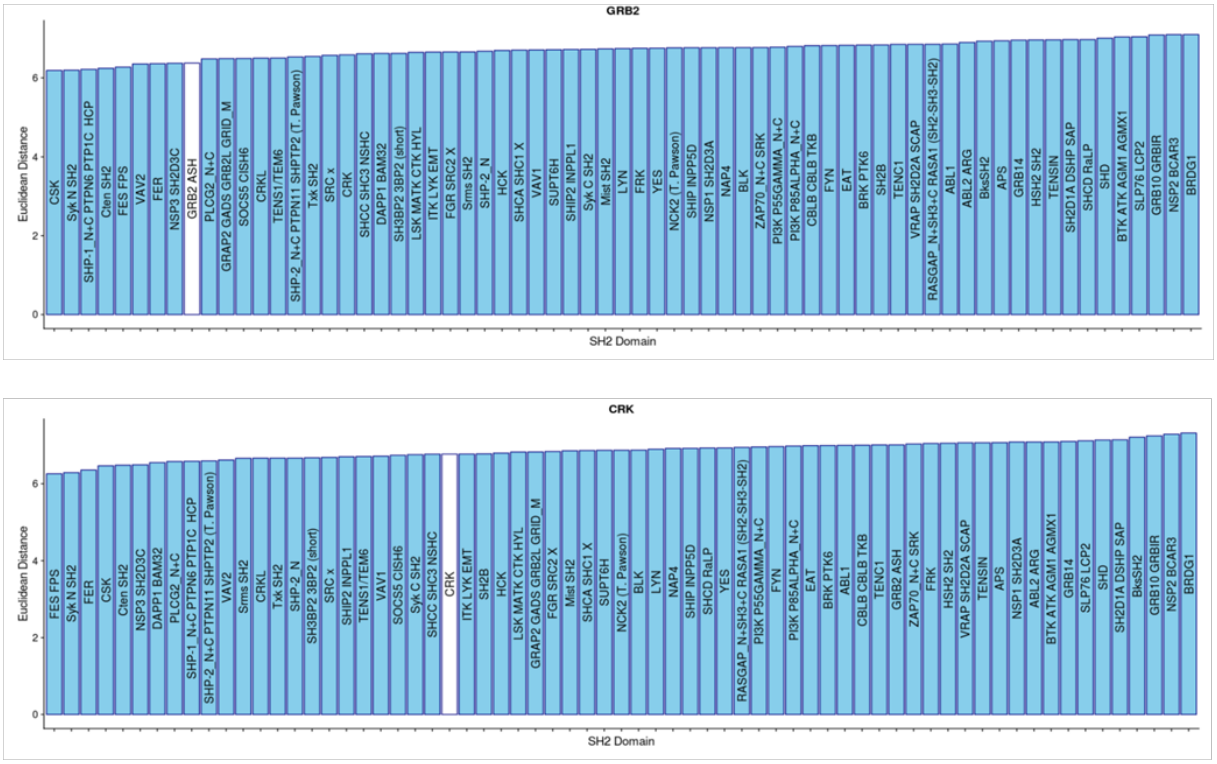
Provided as a separate spreadsheet file:



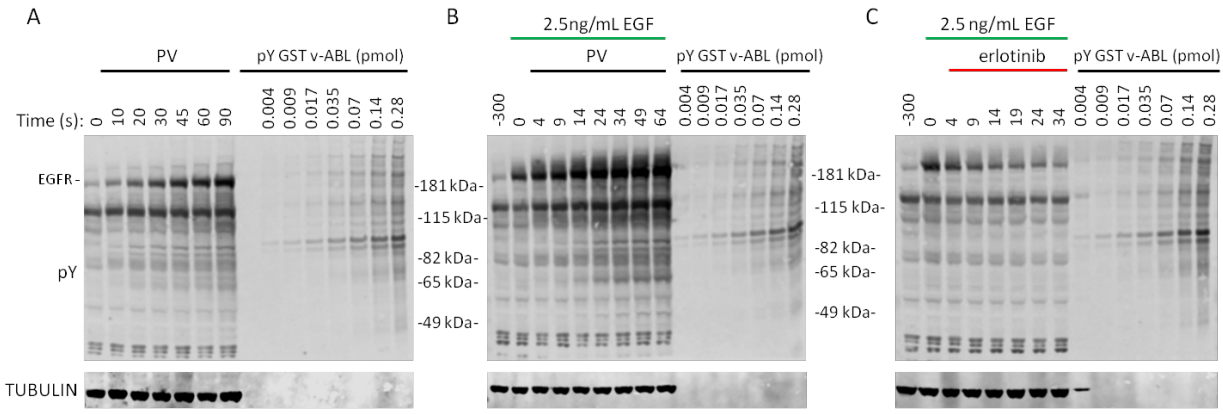

**Supplemental Figure 1:** Identification of p130CAS as the protein whose phosphorylation is enhanced by expression of GCG. Lysates expressing SH2 domain constructs as in Figure 1 were immunodepleted with antibody to p130CAS bound to agarose beads. Immunodepetion resulted in near complete loss of the phosphotyrosine band that is enhanced by GCG expression. The amount of bead-bound p130CAS and phosphorylated p130CAS associated with the antibody bound beads after each successive immunoprecipitation in shown in the bottom panel. Antibodies used for immunoblotting are indicated on right. ID = immunodepletion.




**Supplemental Figure 2:** Quantification of GRB2 expression in COS1 cells. Western blot of lysates of COS1 cells transfected with increasing amount of full length GRB2 with and without EGF simulation (2.5 ng/ml). The amount of GRB2 was quantified by comparison with serially diluted recombinant GRB2-SH2-GST run on the same gel (right). Filters were probed with anti-pY, anti-GRB2, or anti-tubulin as indicated to right.




**Supplemental Figure 3:** Comparison of Euclidian distances: A) Bar graph depicting the Euclidian distance between a PWM created from sites whose phosphorylation is enhanced by GRB2 SH2 expression and PWMs for all 67 SH2s assessed by phosphopeptide binding by Tinti et al. The location of the PWM for GRB2 SH2 peptide binding data is shown in white. Smaller distances mean greater similarity. B) Bar graph depicting the Euclidian distance between a PWM for sites whose phosphorylation was enhanced by GCG expression and PWMs for all 67 SH2s assessed by Tinti et al. The location of the PWM for CRK SH2 peptide binding data is shown in white.




**Supplemental Figure 4:** Immunoblots for absolute quantification of EGFR phosphorylation and dephosphorylation rates in COS1 Cells. A) Western blot of lysates from COS1 cells treated with pervanadate (PV) for the indicated times. B) Western blot of lysates from COS1 cells treated with 2.5 ng/ml EGF for 5 min (time = 0 s) followed by treatment with pervanadate for the times indicated. C) Western blot of lysates from COS1 cells treated with 2.5 ng/ml EGF for 5 min and then treated with erlotinib for the times indicated. For all panels, phosphorylated GST-ABL standard was run on the right at the amounts listed.

**Supplemental Table 1:** iTRAQ MS data and SH2-enhanced phosphosites. *MS Raw Data* tab lists all phosphopeptides identified by MS. The protein name, accession number, peptide sequence, relative abundance, and standard deviation of all peptides identified are shown. Peptides phosphorylated at serine and/or threonine are listed, but were not included in our analysis. Mean abundance values are such that the sum for each peptide equals eight. *GCG ENHANCED* tab lists the proteins, sequence and fold change in abundance for peptides that displayed increased abundance in GCG-transfected cells (over empty vector-transfected) for both starved and EGF treatment conditions. *GRB2 ENHANCED* tab lists the protein, sequence and fold change in abundance for peptides enhanced in tdEOS-GRB2 SH2 and full length GRB2-transfected cells (over empty vector-transfected) for both starved and EGF treatment conditions. Data from GCG *ENHANCED* and *GRB2 ENHANCED* were used to create the LOGOs in Figure 5B and C.