Supplemental Data

Table S1 Synthesized wild-type and mutant GSTP1 peptides for EGFR-dependent phosphorylation. Putative phospho-acceptor tyrosine residues and their mutations are bolded and underlined.

GSTP1 Peptide	Sequence	Region Covered Amino Acids
GSTP1-Y7	TVV**Y**FPVRGR	4-13
GSTP1-Y3/Y7	PP**Y**TVV**Y**FPVRGR	1-13
GSTP1-Y63	GDLTL**Y**QSNT	58-67
GSTP1-Y118	EAGKDD**Y**VKALPGQL	112-126
GSTP1-Y198	FLASPE**Y**VNLPINGN	192-206
GSTP1-Y3D/Y7	PP**D**TVV**Y**FPVRGR	4-13
GSTP1-Y3/Y7D	PP**Y**TVV**D**FPVRGR	1-13
GSTP1-Y3D/Y7D	PP**D**TVV**D**FPVRGR	1-13
GSTP1-Y63D	GDLTL**D**QSNT	58-67
GSTP1-Y118D	EAGKDD**D**VKALPGQL	112-126
GSTP1-Y198D	FLASPE**D**VNLPINGN	192-206

Table S2 Sequence of primers used in PCR cloning for mutagenesis. Each primer contains underline sequences for Gateway cloning.

Primer	Sequence 5'→3'
GSTP1-WT-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGCCGCCCTACACCGTG
GSTP1-Y3F-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGCCGCCC**TTC**ACCGTGGTCTATTT
GSTP1-Y7F-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGCCGCCCTACACCGTGGTC**TTT**TT
GSTP1-Y3F/Y7F-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATGCCGCCC**TTC**ACCGTGGTC**TTT**TT
GSTP1-R	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGTTTCCCGTTGCCATTG
GSTP1-Y198F-R1	GTTGCCATTGATGGGGAGGTTCAC**GAA**CTCAGGGGAGGCC
GSTP1-Y198F-R2	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTGTTTCCCGTTGCCATTGATGGG

Materials and Methods

Analysis of phosphorylation sites in EGFR phosphorylated GSTP1 by mass spectrometry. EGFR-phosphorylated GSTP1, prepared as described earlier, was TCA-precipitated, resolubilized in a solution of 7M urea, 2M thiourea, 2% CHAPS, 10 mM DTT, 2% ampholytes pH 3-10 and 0.01% bromophenol blue, applied to immobilized pH gradient strips (pH 4-7) and subjected to isoelectric focusing. The strips were reduced and alkylated and second dimension gel electrophoresis performed on a 4-12% acrylamide gel gradient. After SYPRO Ruby fluorescent (Invitrogen, Carlsbad, CA) staining, protein spots were robotically excised, reduced with DTT, alkylated with iodoacetamide, and proteolytically digested with trypsin. Liquid chromatography-Tandem MS (LC-MS/MS) of the tryptic digests was performed by Proteomic Research Service, Inc. (Ann Arbor, MI). Details of the protocols used are available at www.prsproteomics.com. The MS/MS data were analyzed

using the MASCOT MS/MS Ions Search (www.matrixscience.com). *DeNovo* sequence analysis was performed using the Scaffold Software (Proteome Software Inc., Portland, OR), followed by manual validation.

Molecular dynamics simulations. These studies focused on Tyr7, primarily, because it is a critical residue required for GSTP1 active site function and we identified it as a principal residue phosphorylated by EGFR. For the simulation, we imported x-ray crystallographic data from the Brookhaven Protein DataBank and using the Insight II modeling program (Accelerys Software, San Diego), created the 3D structure of the GSH-bound GSTP1 monomer with and without the hydroxyl group of Tyr7 phosphorylated. The modeled structures were soaked in a cubic box filled with water molecules and subjected to energy minimization and long-duration molecular dynamics simulation, using the NAMD program 2.5 running on a 5 node Scyld Beowulf linux cluster. The coordinate and parameter files for input were generated using the `psfgen' utility in the CHARMM PARAM 22 topology file, and the all atom CHARMM PARAM 22 force field was used to describe the potential energy. Newton's equations of motion were integrated using a multiple time step velocity Verlet algorithm. Van der Waals interactions were switched smoothly from 8.0 Å to 10.0 Å, with the atom pair distance list maintained up to 11.5 Å. Long-range electrostatics were treated by the particle mesh Ewald (PME) algorithm using a 68 x 81 x 68 grid and a β-spline interpolation of the 4th order. Van der Waals interactions were calculated every one time step and full electrostatics calculation every two time steps. The SHAKE algorithm was used to restrain all hydrogen-heavy atom bond lengths to a tolerance of 10^{-5} Å. Water molecules were described by a TIP3 potential, and periodic boundary conditions were applied to the system. The dielectric constant was set to 1.0. After minimization for 10,000 steps, the system was 'heated' to 300K in 25K/250 steps increments and maintained at 300K by velocity scaling for a further 10 ps. Only water molecules moved freely. A harmonic constraint (force constant of 20.0 Kcal/mole/Å^2) was applied to the minimized complex and simulated for 50 ps to prevent instabilities resulting from energy conservation due to initial bad contacts. An NPT ensemble simulation was used for the rest of the dynamics run and the pressure maintained at 1.01325 bar using the Langevin pistin. After another 10 ps of simulation, the force constant was reduced to 1.0 Kcal/mole/Å^2 and the process continued for another 10 ps. A time step of 1.0 fs was maintained during all simulations and the entire system moved freely for 8.2 ns at 2.0 fs time steps. The results of analyses of energies and structure frames of the simulated system were extracted using the VMD software and illustrations were produced with both the VMD and SYBYL software.