SUPPLEMENTARY FIGURE LEGENDS

Figure S1: SHP-1 inhibits tyrosine phosphorylation of GIV after stimulation with EGF. COS7 cells transfected with vector alone (lanes 1 and 3), GIV-FLAG alone (lanes 2 and 4), or GIV-FLAG and HA-SHP-1 WT (lane 5) were serum starved (-) and subsequently stimulated with EGF (50 nM, +) for 10 min. Equal aliquots of lysates (bottom) were incubated with anti-FLAG mAb and protein G agarose beads. Immune complexes (top) were analyzed by two-color immunoblotting (IB) for GIV and pTyr using the Li-COR Odyssey Infrared Western Blot Imaging System. Single channel images for GIV and pTyr are displayed in grayscale which show that immunoprecipitated GIV was phosphorylated on tyrosine(s) exclusively after EGFR stimulation (compare lanes 2 and 4). Yellow pixels in the overlay of GIV (red) and pTyr (green) images (Merge panels) confirm that GIV was phosphorylated on tyrosine(s) after EGF treatment (lane 4). This EGF-dependent tyrosine phosphorylation of GIV was undetectable in cells co-transfected with HA-SHP-1 (lane 5). Expression of GIV and SHP-1 in all lysates was analyzed by immunoblotting (IB) for FLAG, GIV, SHP-1 and tubulin (bottom).

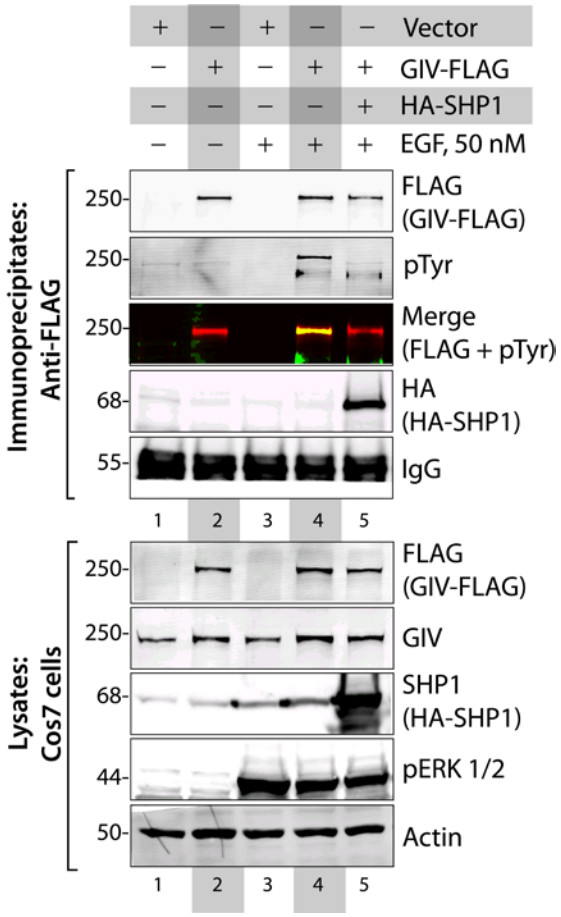

Figure S1

Figure S2: GIV is tyrosine phosphorylated after stimulation of LPA receptor. COS7 cells transfected with vector alone (lane 1), or co-transfected with Src-HA and GIV-FLAG (lanes 2, 3) were serum starved (-) and subsequently stimulated with 10 µM LPA (+) for 20 min. Equal aliquots of lysates (bottom) were incubated with anti-FLAG mAb and protein G agarose beads. Immune complexes (top) were analyzed by two-color immunoblotting (IB) for GIV and pTyr using the Li-COR Odyssey Infrared Western Blot Imaging System. Grayscale image for GIV (top panel) shows that GIV-FLAG is immunoprecipitated in lanes 2 and 3, but not in vector control (lane 1). pTyr panel shows that tyrosine phosphorylation occurred upon ligand stimulation (lane 3). Yellow pixels of the overlaid GIV-FLAG (red) and pTyr (green) images (Merge panel) confirm that the immunoprecipitated GIV-WT is phosphorylated on tyrosine(s) exclusively after LPA treatment (compare lanes 2 and 3). Adequate stimulation of cells by LPA, as determined by activation of ERK1/2, and expression of GIV-FLAG and Src-HA in lysates were analyzed by immunoblotting (IB) for FLAG, GIV, SHP-1, HA (Src-HA), phospho-ERK1/2 and tubulin (bottom).

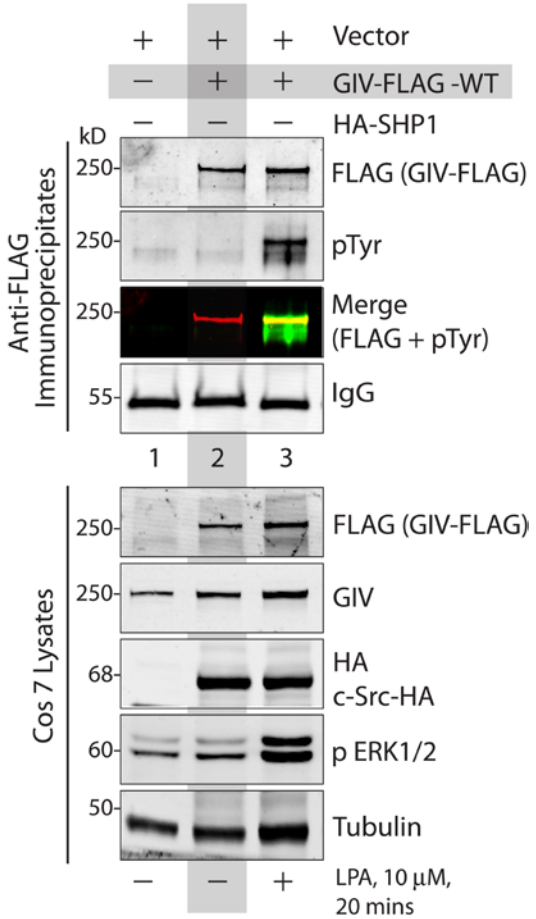

Figure S2

Figure S3: GIV, but not Gαi3, interacts with SHP-1 in HEK cells. Equal aliquots of HEK lysates (bottom) were incubated with either rabbit preimmune (lane 1) or anti-SHP-1 (lane 2) IgGs, and protein A agarose beads. Lysates and immune complexes (top) were analyzed for GIV, SHP-1, and Gαi3 by immunoblotting (IB). SHP-1 was immunoprecipitated efficiently and specifically by anti-SHP-1 (lane 2), but not control (lane 1) IgG. GIV, but not Gαi3 was detected in SHP-1-bound protein complexes (lane 2).

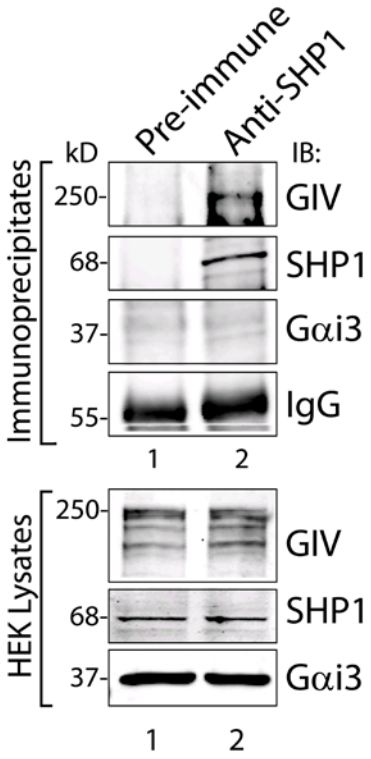

Figure S3

Figure S4: Wild-type (WT), but not the catalytically inactive C453S (CS) mutant of SHP-1 inhibits tyrosine phosphorylation of GIV and the formation of phospho-GIV-p85α(PI3K) complexes after EGF stimulation. COS7 cells were transfected with vector alone (lane 1), GIV-FLAG alone (lanes 2, 3), GIV-FLAG and HA-SHP-1 WT (lane 4), or GIV-FLAG and HA-SHP-1 CS mutant (lane 5). Cells were serum starved (-) and subsequently stimulated with 50 nM EGF (+) for 10 min. Equal aliquots of lysates (bottom) were incubated with anti-FLAG mAb and protein G agarose beads. Immune complexes (top) were analyzed by two-color immunoblotting (IB) for GIV and pTyr as well as p85α using the LI-COR Odyssey Infrared Western Blot Imaging System. Grayscale image for GIV (top panel) shows that GIV-FLAG is immunoprecipitated in lanes 2-5, but not in vector control (lane 1). pTyr panel shows that tyrosine phosphorylation occurred upon ligand stimulation (lane 3), undetectable in cells transfected with HA-SHP-1 WT (lane 4), and restored in cells expressing HA-SHP-1 CS (lane 5). Yellow pixels in the overlay of GIV-FLAG (red) and pTyr (green) images (Merge panel) confirm that tyrosine-phosphorylation of GIV is inhibited in the presence of catalytically active SHP-1 (lane 4), but not the catalytically inactive SHP-1 CS (lane 5). Equal aliquots of lysates (bottom) were analyzed for FLAG, GIV, p85α , SHP-1, and tubulin by immunoblotting (IB).

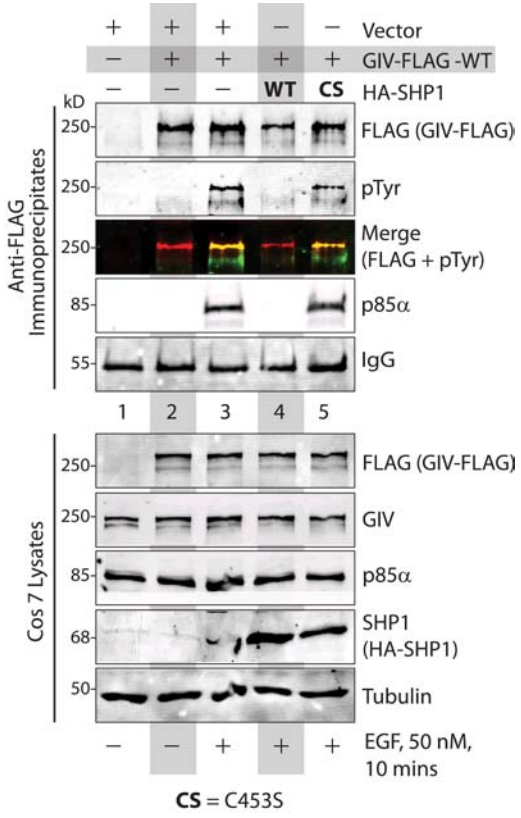

Figure S4