EXPERIMENTAL PROCEDURES:

Indirect Immunofluorescence—MCF-7 derived cells were grown as subconfluent monolayer cultures on glass coverslips overnight. Alexa Fluor-conjugated human transferrin, cholera toxin subunit B, goat-anti-mouse and goat-anti-rabbit antibody were purchased from Molecular Probes (Eugene, OR). Cells were treated with inhibitors for 1 hr or transfected with siRNA constructs as described above and then cooled on ice for 15 min, prior to incubation with Alexa-labeled ligand on ice for 1 h. Cells were then washed three times with cold PBS and incubated with pre-warmed RPMI 1640 for 0 or 15 min. Cells were washed with PBS, fixed with 4% paraformaldehyde/PBS, and mounted using VECTASHIELD mounting media (Vector Laboratories, Burlingame, CA). Cells were imaged using a Nikon Eclipse E800 fluorescent microscope with Metamorph 5.0 imaging software, (Downington, PA), or a Zeiss Axiovert 200 motorized fluorescence microscope with deconvolution (Axiovision 3.1) and cooled CCD camera (Zeiss Axiocam HRm).

FIGURE LEGENDS:

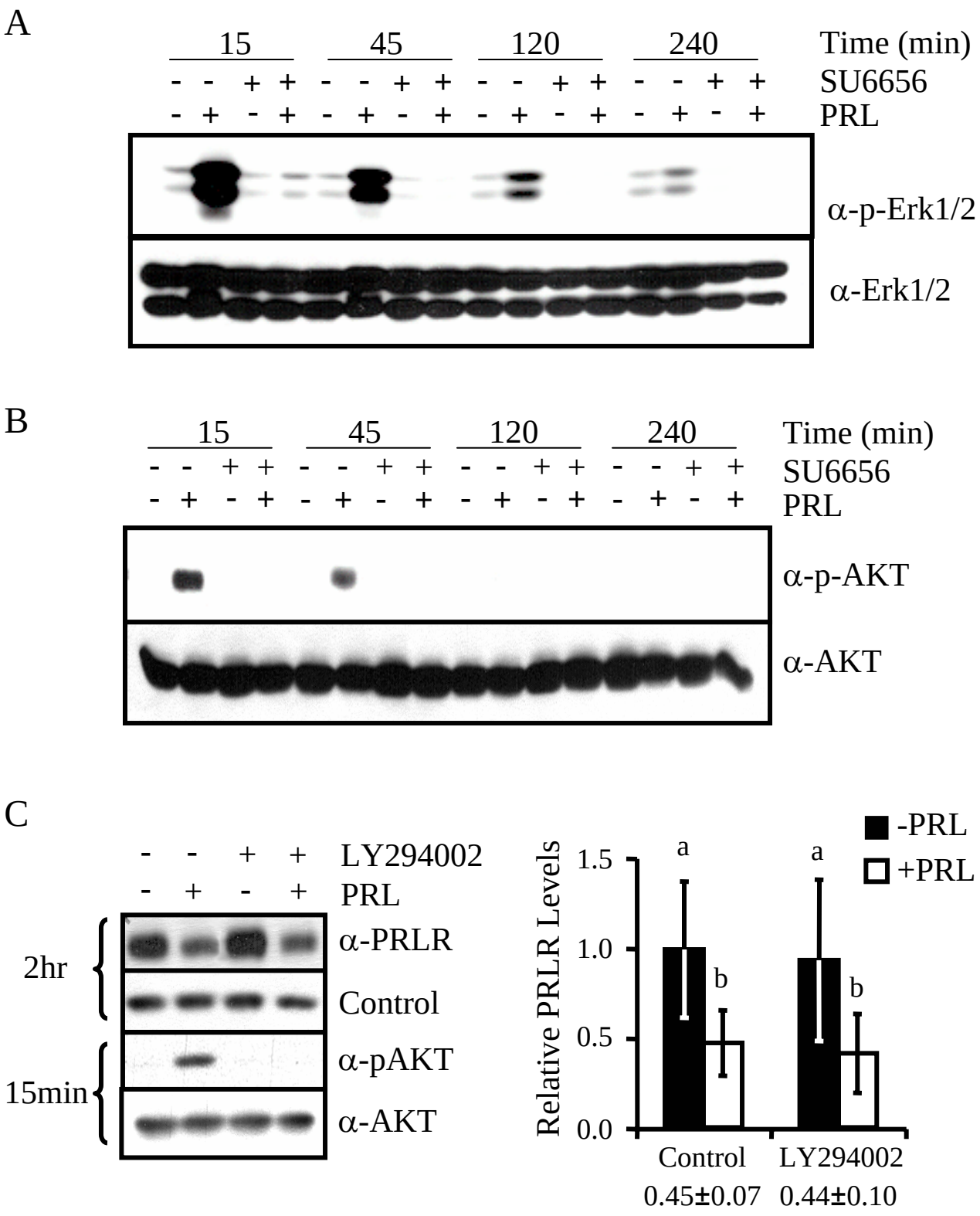
Supplemental Fig. 1. SFKs signals downstream to ERK1/2 and PI3K

A, B, serum-starved MCF-7 cells were pretreated with vehicle or 10mM SU6656 for 1 hr at 37°C, then treated +/- 4nM PRL for 15, 45, 120, or 240min. Cellular proteins were subjected to Western analysis as indicated. ***C,*** serum-starved MCF-7 cells were pretreated vehicle or 20μM LY294002 for 1 hr at 37°C, then treated +/- 4nM PRL for an additional 2 hr at 37°C. Cellular proteins were subjected to Western analysis as indicated. lPRLR levels were quantitated from at least three independent experiments, and normalized to vehicle-treated controls (mean +/- S.D.). Treatments were compared using one-way ANOVA with Student-Newman-Keuls post-test. Different letters indicate significant differences among treatments ($P \leq 0.05$). Numbers below charts indicate percent ligand-induced down-regulation compared to untreated samples for each inhibitor.

Supplemental Fig. 2. Inhibition of src family kinases blocks cholera toxin B but not transferrin internalization.

Serum-starved MCF-7 cells were pretreated with vehicle, 10μM PP1, 10μM SU6656, 25μM AG490, 10μM U0126, or 20μM LY294002 for 1 hr followed by labeling with Alexa-fluro-labeled ligands, cholera toxin B (CTB) or transferrin (TFN), for 1hr on ice. Internalization was initiated by warming of the cells to 37°C for 0 or 15min. Fluorescent ligands were visualized as described in *Experimental Procedures*.

Supplemental Fig. 1



Supplemental Fig. 2