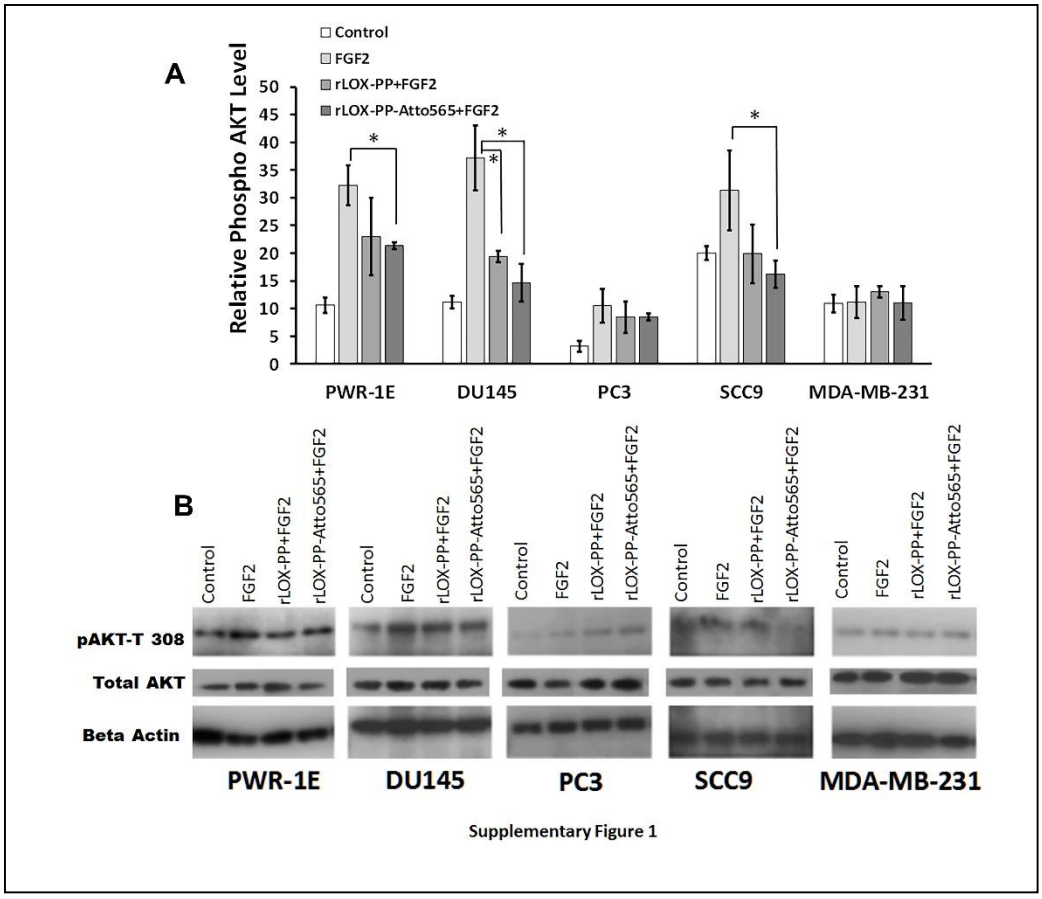

Figure S1. Control of efficacy of non-labeled rLOX-PP and rLOX-PP-Atto565 on FGF-2-stimulated AKT phosphorylation in cells. Serum starved cells for 12 hours were treated with or without 0.2 µM non-labeled rLOX-PP or 0.2 µM rLOX-PP-Atto565. After 24 h, cells were treated with or without 10 ng/ml FGF-2 for 10 minutes. Cells extracts were subjected to SDS PAGE and probed for phospho AKT, total AKT or $(His)_6$-tag antibody and band intensity against beta actin was quantified. Phospho AKT protein expression were compared in non-treated, FGF-2 treated, rLOX-PP-FGF2 or rLOX-PP-Atto565 treated cells. No significant differences were observed for the inhibition of AKT phosphorylation by rLOX-PP or rLOX-PP-Atto565. Values were expressed as fold change in normalized AKT phosphorylation relative to FGF-2 stimulated control cells lacking rLOX-PP or rLOX-PP-Atto565. Results shown are from a representative experiment performed three times with similar results. Data are means +/- SD; n = 3; *, two-tailed p-value <0.003.

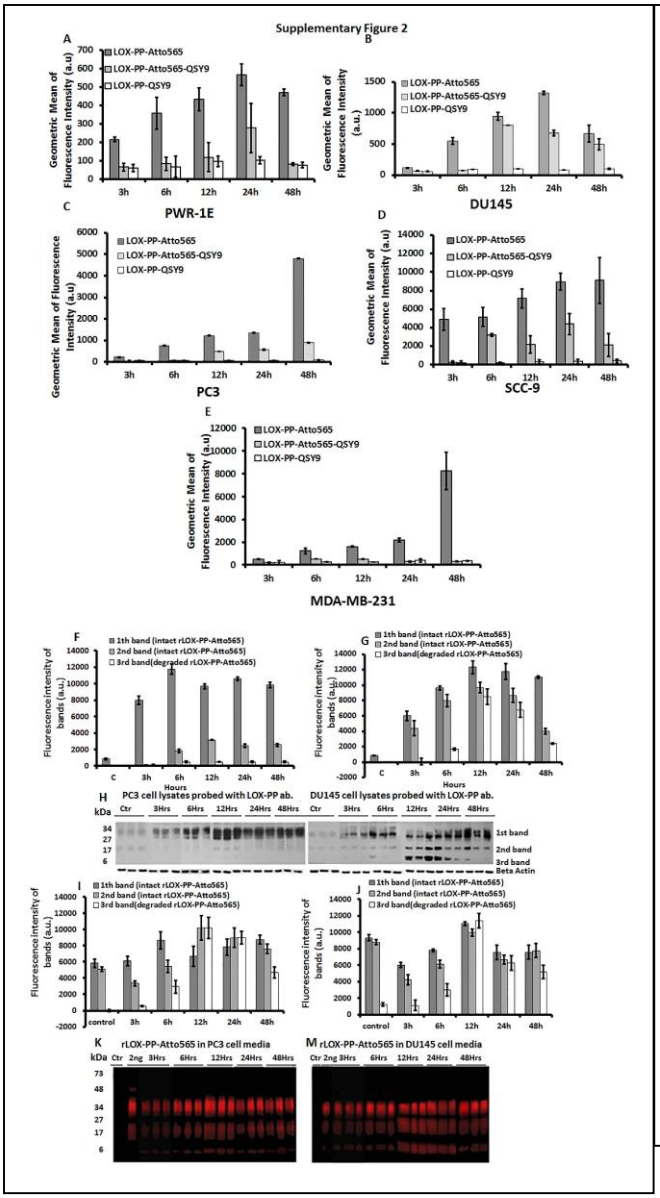

Figure S2. Degradation of LOX-PP as a function of time. PWR-1E (**A**), DU145 (**B**), PC3(**C**), SCC9 (**D**) and MDA-MB-231(**E**) cells were cultured with either rLOX-PP-Atto565 (dark gray), double labeled rLOX-PP-Atto565-QSY9 (light grey) or with quencher labeled rLOX-PP-QSY9 only (white) for different time intervals at 37º C under standard cell culture conditions. Cell-associated fluorescence was determined by flow cytometry as a function of time for all cell lines. Data show time-dependent uptake of LOX-PP-Atto565, with no evidence for intracellular degradation until time points longer than 3 hours in all cell lines. For confirmation of the flow cytometry degradation assay (**A-E**), cell extracts and media samples were subjected to SDS PAGE. Cell extract samples were respectively probed with LOX-PP antibody and band intensity against beta actin was quantified (**F-H**). Evidence for intracellular degradation of rLOX-PP-Atto565 occurred at time points later that 3 hours, as expected. Media samples from PC3 and DU145 cells visualized with a Molecular Imager Pharos-FX (Bio-Rad) (**I-M**) similarly showed little evidence of degradation until after 3 hours. This experiment was performed at least three times with the same outcomes.

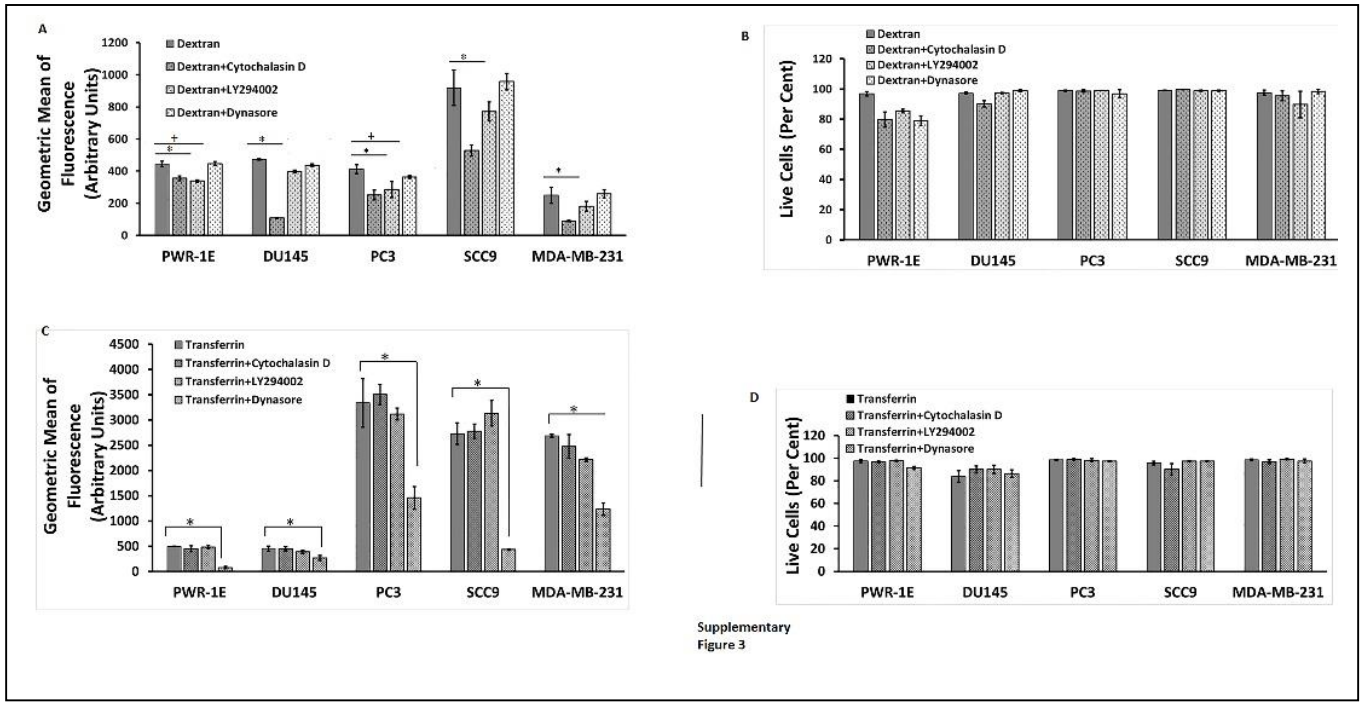

Figure S3. Uptake of 10 μM 10 kDa dextran-Bodipy-fl (after 3 hours) and 10 μM Transferrin-FITC (after 45 minutes) in presence or absence of Cytochalasin D, LY294002 or Dynasore. (**A**) 10 kDa dextran-Bodipy-fl uptake was quantified by flow cytometry in the absence (solid dark gray bar) and in the presence of 1.5 μM cytochalasin D (dashed dark gray bar), 200 μM LY294002 (dashed light gray bar) or 100 μM dynasore (dashed white bar). Data are means +/- SD; n = 3; *, two-tailed p-value<0.03 and n = 3; +, two-tailed p-value<0.02. (**B**) Aliquots of 10 kDa dextran-Bodipy-fl treated cells analyzed in panel A were applied to LIVE/DEAD® Fixable Near-IR stain assay to show the percentage of live cells in each cell lines; in the absence (solid dark gray bar) and in the presence of cytochalasin D (dashed dark gray bar), LY294002 (dashed light gray bar) or dynasore (dashed white bar). (**C**) Transferrin-FITC uptake was quantified by flow cytometry in the absence (solid dark gray bar) and in the presence of 1.5 μM cytochalasin D (dashed dark gray bar), 200 μM LY294002 (dashed light gray bar) or 100 μM dynasore (dashed white bar). Data are means +/- SD; n = 3; *, two-tailed p-value<0.009. (**D**) Aliquots of Transferrin-FITC treated cells analyzed in panel A were applied to LIVE/DEAD® Fixable Near-IR stain assay to show the percentage of live cells in each cell lines; in the absence (solid dark gray bar) and in the presence of cytochalasin D (dashed dark gray bar), LY294002 (dashed light gray bar) or dynasore (dashed white bar). Data are means +/- SD, n = 3.

Supplementary Figure 4

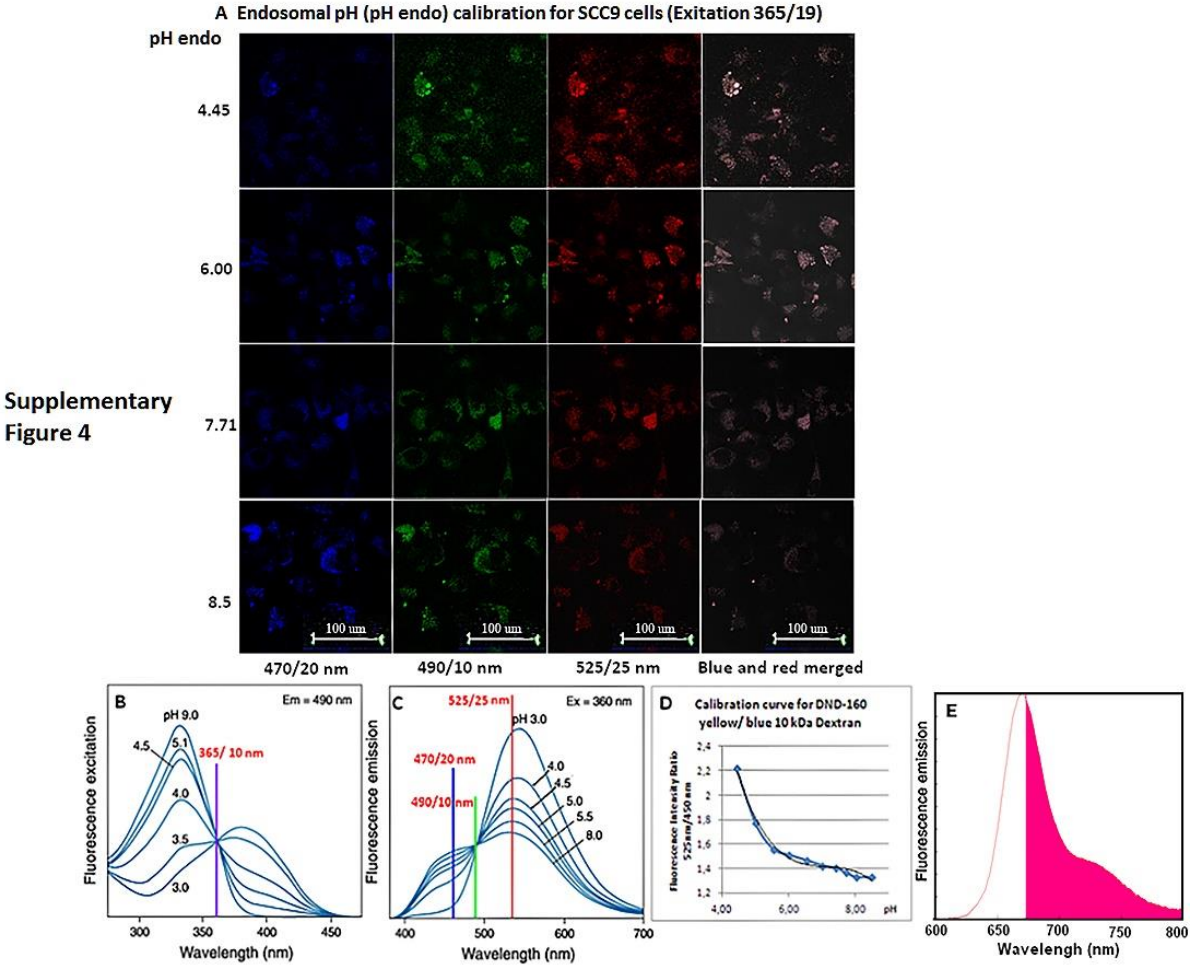

Figure S4. Endosomal pH (pH endo) calibration curve of Lysosensor Yellow/Blue dextran for SCC9 cells. In A, artificially colored images show the emission intensity of Lysosensor Yellow/Blue dextran at 470 nm/20 nm (blue), 490 nm/10 nm (green) and 525 nm/25 nm (red) in SCC9 cells at selected pHs (4.45, 6.00, 7.71 and 8.50). Emission isobestic points (green) were used to control intensity level of each sample. Blue and red images were merged to show the emission intensity shift (images were collected EC-Plan Neofluar 40x/1.30 oil DIC M27 objective). In B and C, figures show an excitation spectrum shift when Yellow/Blue dextran is emitted at 490 nm and the emission spectrum shift when it is excited at 360 nm. The panels B and C also show excitation (365 nm/10 nm-purple) and emission points (470 nm/20 nm-blue, 490 nm/10 nm-green and 525/25 nm-red) used in the experiments. Panel D shows the calibration curve generated based on the 525/470 nm emission ratio of Lysosensor Yellow/Blue dextran against pH in SCC9 cells. In E, the Atto647N emission spectrum is shown. Artificially magenta-colored emission integral of rLOX-PP-Atto647N was collected to avoid overlap between Yellow/Blue dextran, and the rLOX-PP-Atto647N emission spectrum in rLOX-PP-Atto647N treated samples.

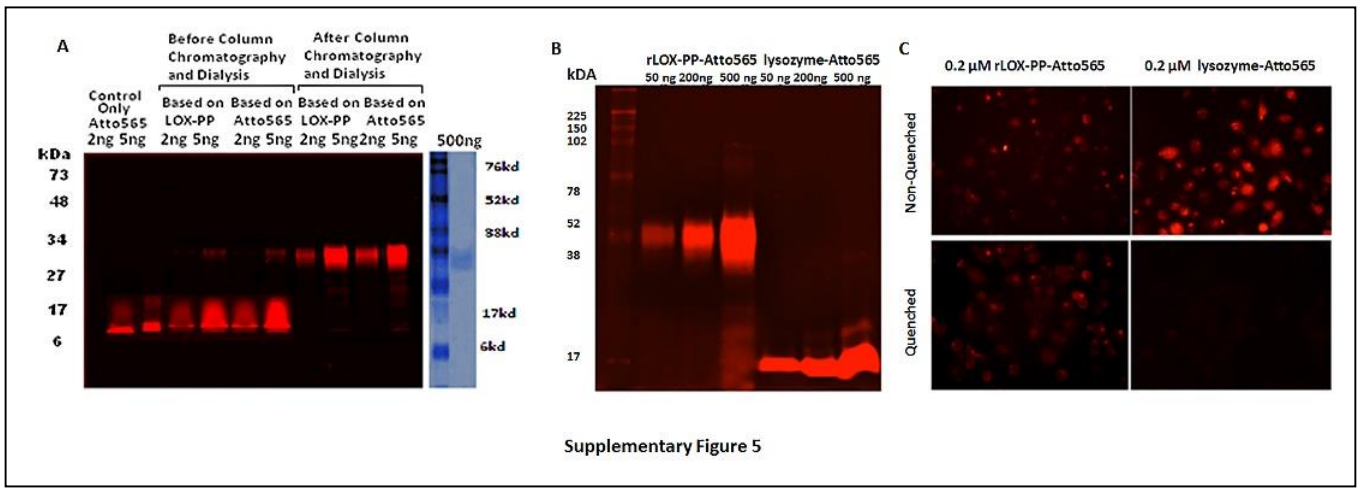

Figure S5. SDS PAGE of rLOX-PP-Atto565 and the evaluation of its uptake in comparison with that of lysozyme-Atto565 uptake in PC3 cells. (**A**) Atto565 labeled rLOX-PP (rLOX-PP-Atto565) purification was monitored by SDS PAGE and visualized by fluorescence and by Coomassie Blue staining. Before and after G-25 gel chromatography and dialysis equivalent amounts of dye and compared to the mobility of free dye. Data indicate that rLOX-PP-Atto565 is a pure protein and does not contain any free dye. Labeled and purified rLOX-PP-Atto565 and lysozyme-Atto565 were subjected to SDS-PAGE and imaged with Molecular Imager Pharos-FX (Bio-Rad) to confirm removal of free dye (**B**). PC3 cells were treated with 0.2 µM rLOX-PP-Atto565 or 0.2 µM lysozyme-Atto565 for 6 hours. Before quenching with 16 µM Nε-(Carboxymethyl)lysine-BSA-QSY9 (CML-BSA-QSY9) quencher, 0.2 µM lysozyme-Atto565 (**C-top**) appeared to emit more fluorescence than 0.2 µM rLOX-PP-Atto565 (**C, top panels**). After quenching, rLOX-PP-Atto565 fluorescence remained (**C, bottom left**), but lysozyme-Atto565 fluorescence was eliminated (**C, bottom right**).