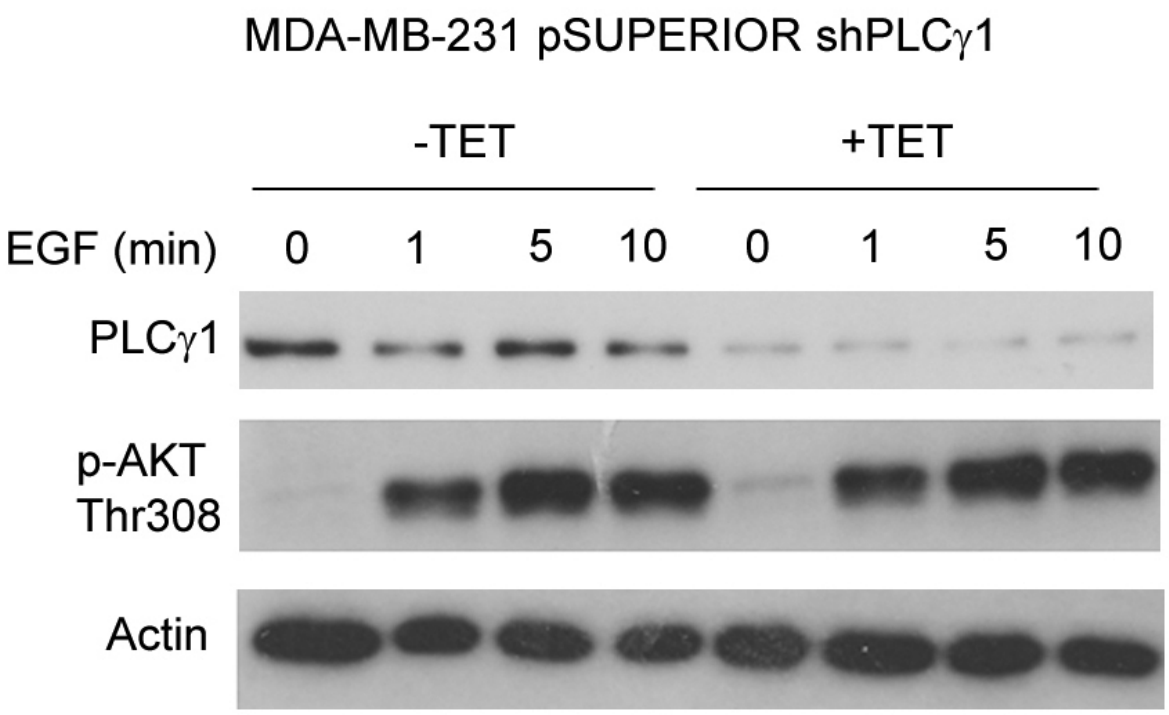

Fig. S1. PLCγ1 does not activate PDK1. Western blot analysis of lysates from serum-starved MDA-MB-231 expressing an inducible TET-Off-PLCγ1 targeting system untreated or treated with EGF for the indicated times in the presence or absence of tetracycline. Levels of PLCγ1 and the PDK1-dependent phosphorylation of Akt at its residue Thr308 were determined by western blot analysis. Equal loading was confirmed using an anti-actin antibody.

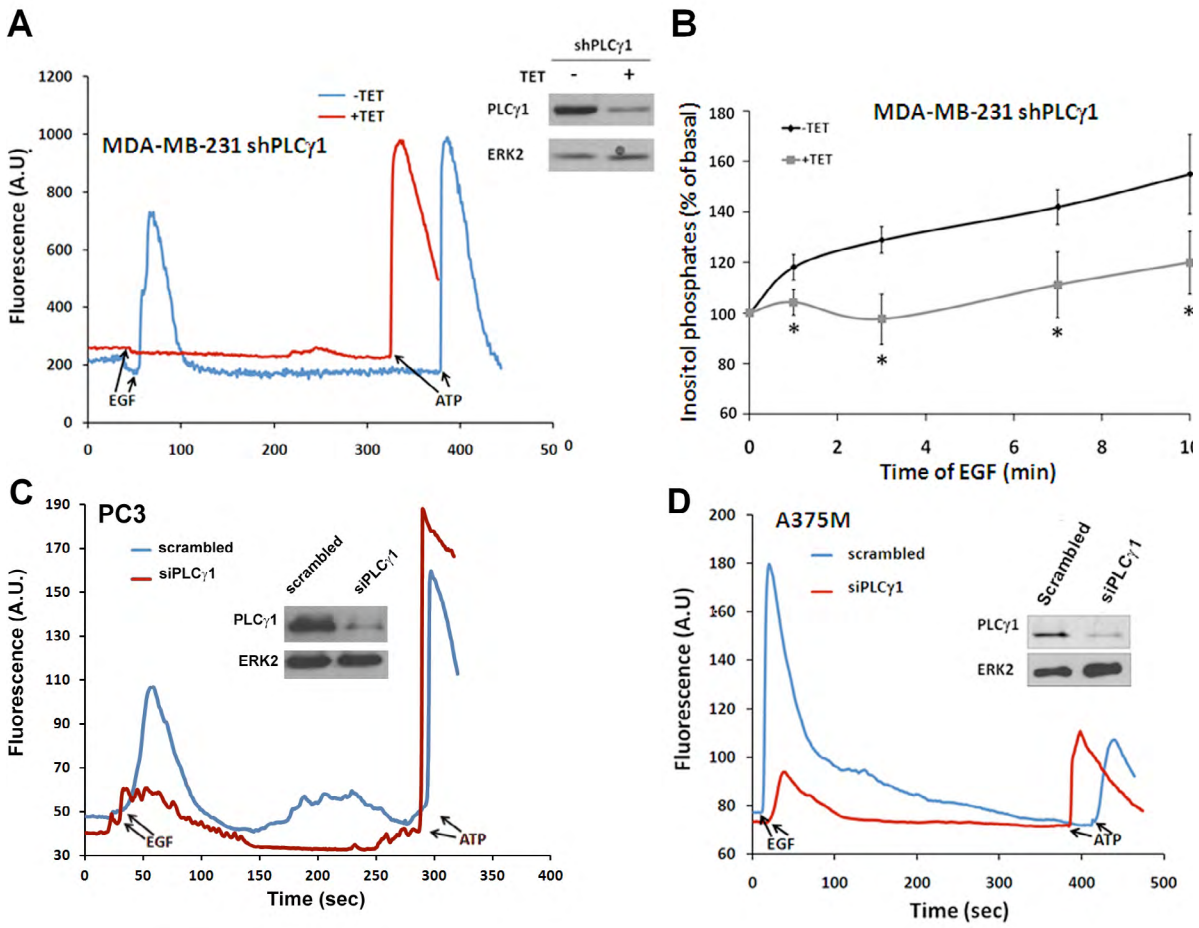

Fig. S2. PLCγ1 and PDK1 regulate the EGF-induced increase of intracellular calcium and inositol phosphates accumulation. (A) Intracellular calcium mobilization upon EGF stimulation in MDA-MB-231 expressing the inducible TET-Off-PLCγ1 targeting system. Data are from one experiment representative of three independent experiments. Levels of PLCγ1 upon treatment with tetracycline are shown. **(B)** Inositol phosphates accumulation induced by EGF stimulation in MDA-MB-231 expressing the inducible TET-Off-PLCγ1 targeting system. Data are means ± s.e.m. from four independent experiments. **P*<0.05. **(C)** Intracellular calcium mobilization measured upon EGF stimulation of PC3 cells. Cells were transiently transfected with siRNA targeting PLCγ1 or PDK1 or with scrambled siRNA as control and intracellular calcium measurements were performed in the three sets of cells. For clarity, results from cells transfected with PLCγ1-targeting siRNA are presented here whereas results from cells transfected with PDK1-targeting siRNA are presented in a separated graph in Fig. 2A. Results from cells transfected with scrambled siRNA which were used as control for both PDK1 and PLCγ1 siRNAs-expressing cells are presented in both graphs. Western blot shows protein level of PLCγ1 in transfected cells. Data are from one experiment representative of four independent experiments. **(D)** Intracellular calcium mobilization measured upon EGF stimulation of A375M cells. Cells were transiently transfected with siRNAs targeting PLCγ1 or PDK1 or scrambled siRNA as control. Data are from one experiment representative of three independent experiments. Western blot shows protein level of PLCγ1 in transfected cells. For clarity, results from cells transfected with PLCγ1-targeting siRNAs are presented here whereas results from cells transfected with PDK1-targeting are presented in Fig.2C.

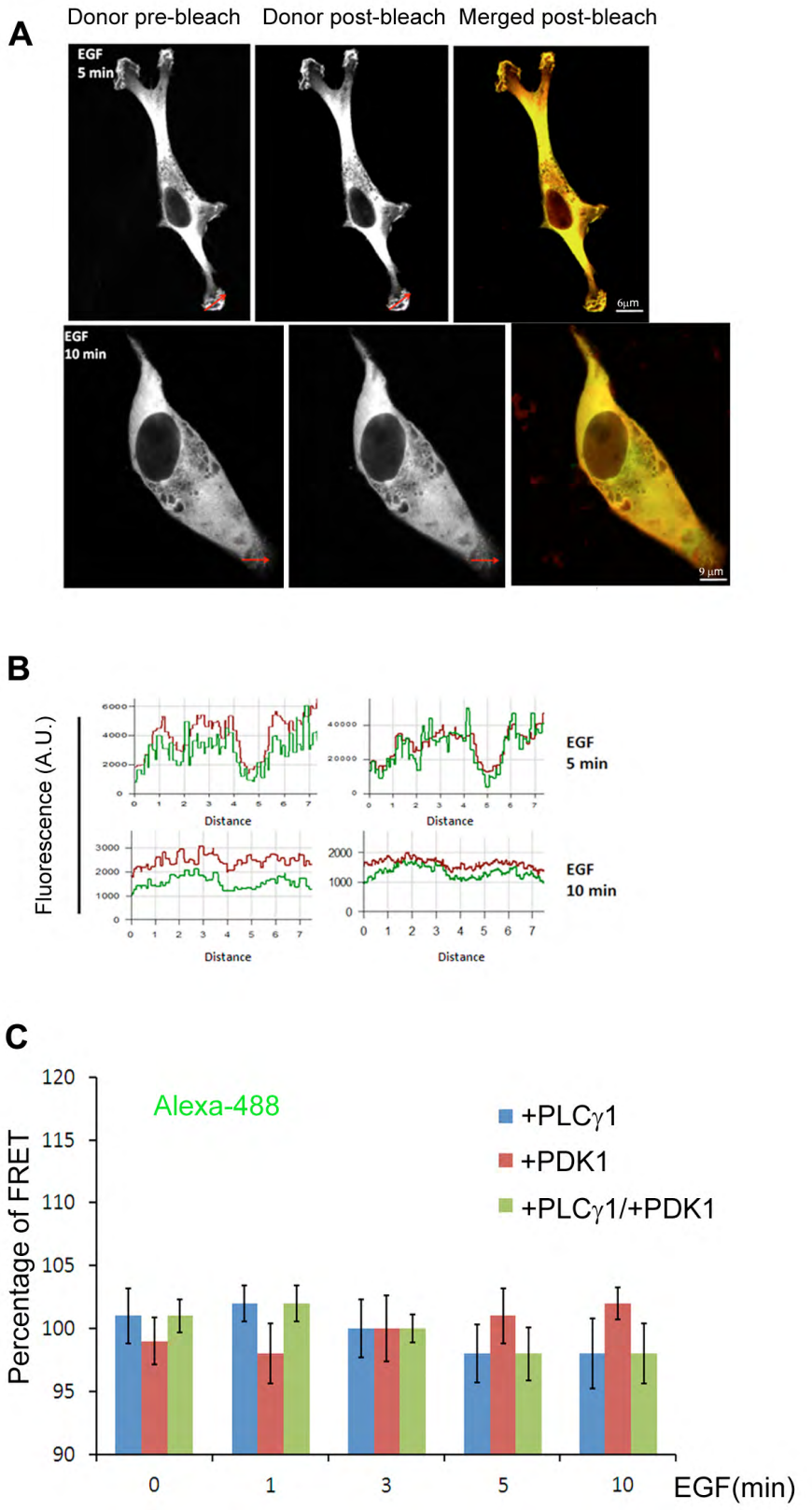

Fig. S3. FRET analysis of PLCγ1 and PDK1 association. (A,B) Confocal microscopy images of FRET analysis in serum-starved MDA-MB-231 cells co-expressing PLCγ1 (green) and PDK1 (red) untreated or stimulated with EGF for 5 and 10 minutes. The green and red lines in the charts show the fluorescence of donor and acceptor respectively, before and after acceptor photobleaching measured along the indicated red arrow in cells shown in the panel above. **(C)** Quantification of FRET measured by acceptor photobleaching in serum-starved MDA-MB-231 overexpressing PLCγ1 alone (+PLCγ1) or PDK1 alone (+PDK1) or co-expressing PLCγ1 and PDK1 (+PLCγ1/+PDK1), untreated or treated with EGF for the indicated time points, single-stained with Alexa-488-conjugated secondary antibody. Graph is the means ± s.e.m. from two independent experiments examining a total of 30 cells per time point.

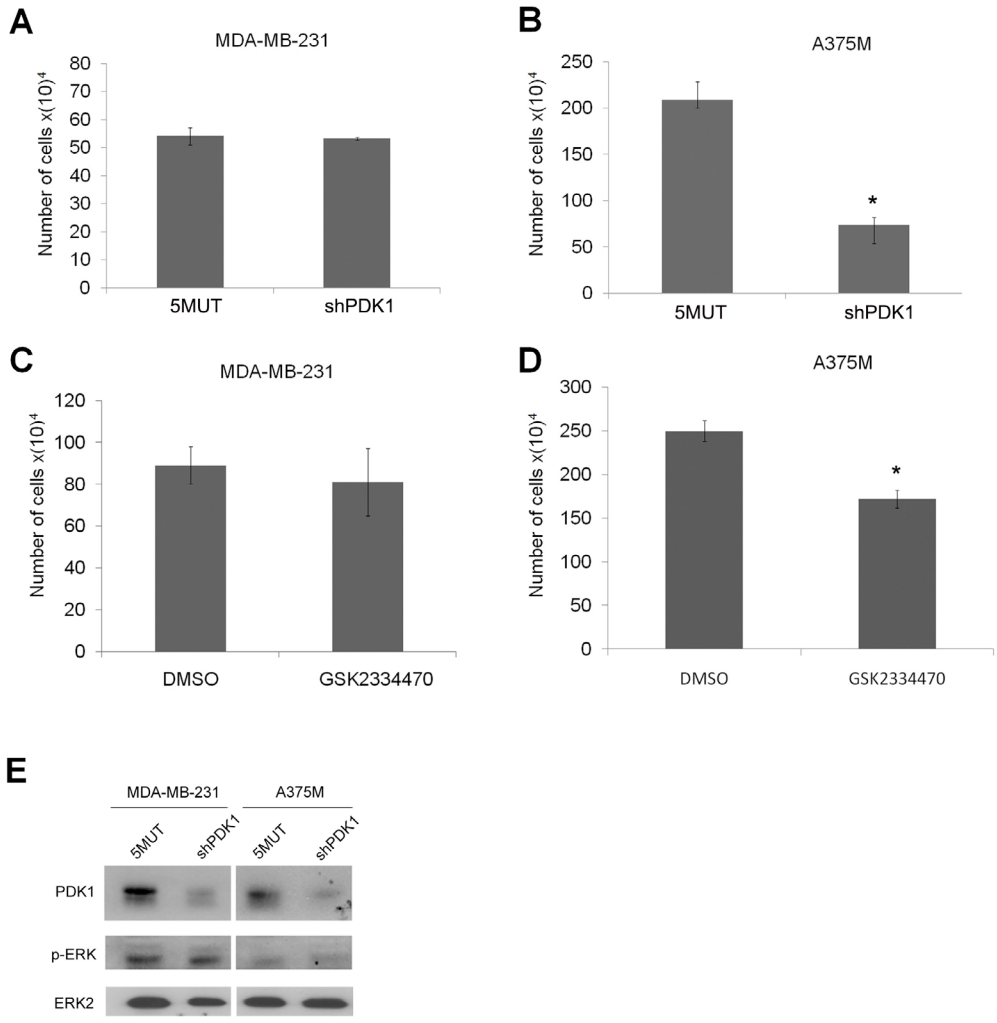

Fig. S4. Effect of PDK1 on proliferation of MDA-MB-231 and A375M. (A,B) Cell proliferation of MDA-MB-231 and A375M stably transfected with shRNA targeting PDK1 or the respective 5MUT control sequence was assessed by manual cell counting after 72 hours from plating in normal growing conditions. **(C,D)** Cell proliferation of MDA-MB-231 and A375M was assessed by manual cell counting after 72 hours of treatment with 1 μM GSK2334470 in normal growing conditions. Data in the right panel are means ± s.e.m. from three independent experiments. **P*<0.05. **(E)** Western blot analysis of lysates of MDA-MB-231 and A375M stably transfected with shRNA targeting PDK1 or a 5MUT non targeting sequence, cultured in normal growing media showing phosphorylation level of ERK. Total ERK levels were assessed after membrane stripping.