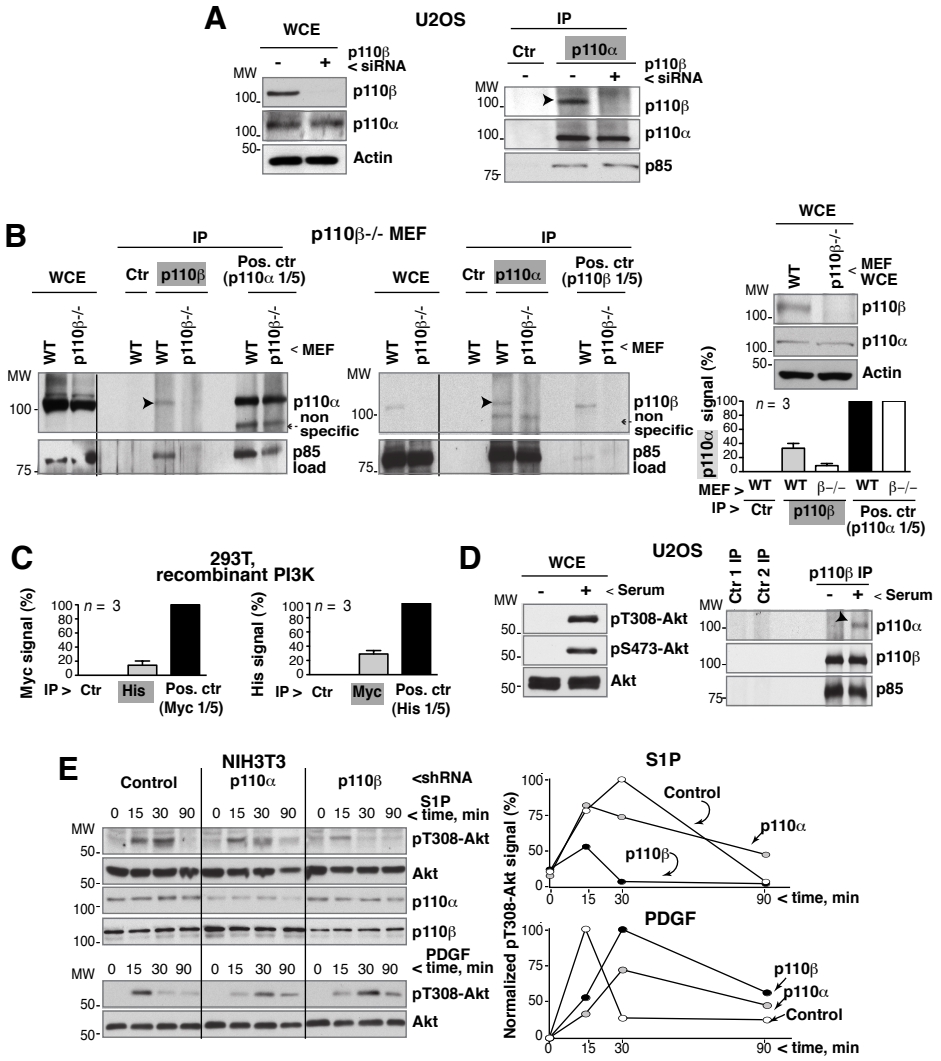

Figure S1. Specificity of p110β in complex with p110α and *vice versa*. (A) U2OS cells were transfected with control or p110β siRNA (72 h). WCE (50 μg) and indicated immunoprecipitates (from 1 mg protein extract) were analyzed in WB. The p110β signal detected in p110α precipitates was lower after p110β knockdown than in controls. Representative experiment (n = 3). (**B**) Extracts (1 mg) from exponentially growing p110β-deficient MEF, alone or reconstituted with WT p110β were immunoprecipitated with anti-p110β or -p110α Ab. A small amount (1/5) of extract (200 μg) was used to precipitate p110α (positive control); a similar control was used for p110β. Negative control included extract + protein A. WCE (50 μg) and p recipitates were analyzed in WB. The graph shows the percentage of p110α signal in p110β precipitates normalized to p85 levels and relative to the maximum (positive control p110α precipitate normalized to p85, 100%; mean ± SEM, *n* = 3). (**C**) Graphs show quantitation of the experiment in Fig. 1E. The percentage of Myc-p110α in complex with His-p110β normalized to p85 and relative to the maximum (Myc-p110α in the positive control immunoprecipitation normalized to p85, 100%; mean ± SEM, *n* = 3); quantitation in the reciprocal assay was similar. (**D**) U2OS cells were serum-starved (19 h) and activated by serum addition (1 h). Extracts (1 mg) were immunoprecipitated with anti-p110β Ab. Negative controls were protein A + Ab (Ctr 1) and protein A + cell extract (Ctr 2). Extracts (50 μg) and precipitates were analyzed in WB. (**E**) NIH3T3 cells transfected with control, p110α or p110β shRNA (72 h) were incubated in serum-free medium (2 h), activated by addition of S1P (10 mM) or PDGF (50 nM) for the times indicated, and extracts analyzed in WB. The image shows a 2-min exposure for PDGF and a 20-min exposure for S1P. Graphs show the percentage of pAkt signal normalized to Akt levels and relative to the maximum for each stimulus (100%; mean ± SEM, *n*=3).

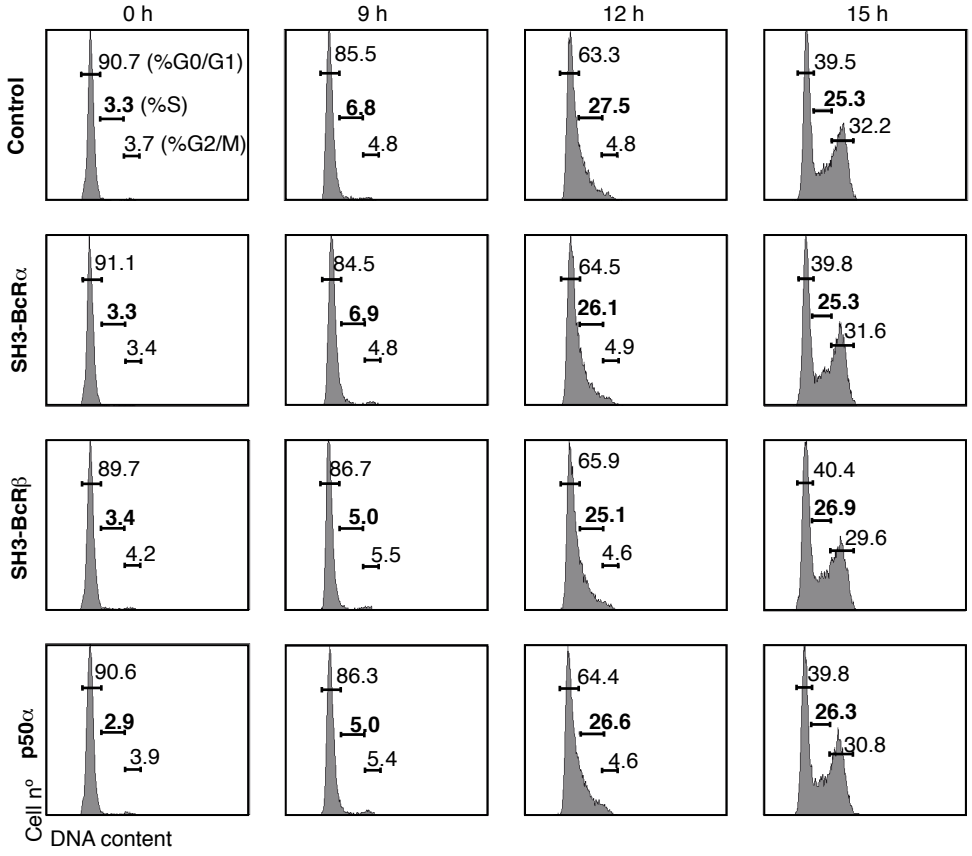

Figure S2. Cell cycle entry is not essentially affected by SH3-BcRα, SH3-BcRβ, or p50α expression. NIH3T3 cells were transfected with cDNA encoding SH3-BcRα, SH3-BcRβ or p50α (48 h) and incubated in serum-free medium (19 h), then induced to enter cell cycle by serum addition (10%). The figure shows representative cell cycle profiles analyzed by measuring DNA content in flow cytometry at various times after serum addition. Numbers indicate the percentage of cells in each phase.

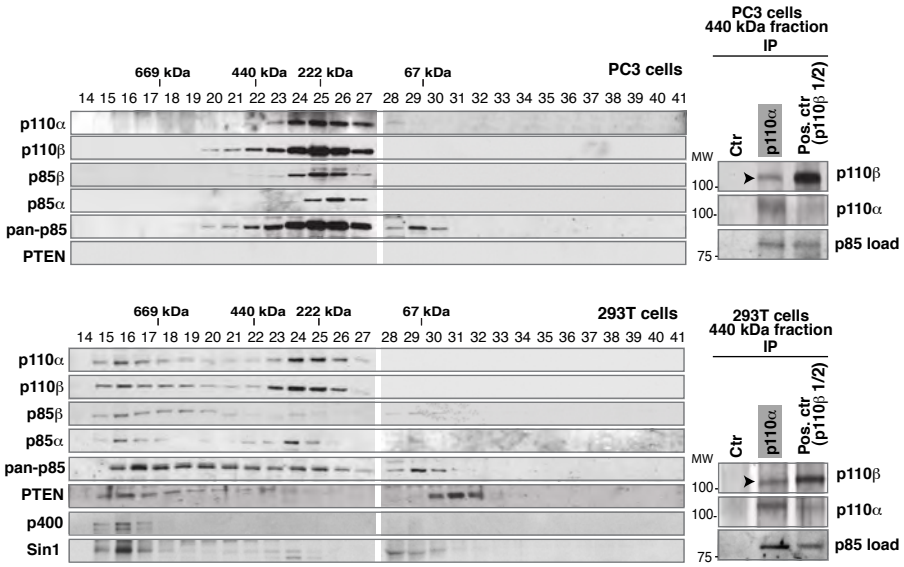

Figure S3. PTEN-deficient cells contain p110α/p110β complexes. Extracts of 293T or PC3 cells were separated by gel filtration and fractions tested in WB with indicated Ab; goat anti-mouse PTEN was used for PTEN WB. p110α/p110β complexes in the ~440 kDa fraction were analyzed by immunoprecipitation (500 µg) with anti-p110α Ab and in WB with anti-p110β Ab. An immunoprecipitation of p110β (250 µg) was used as positive control. Representative experiment; n = 3 with similar results. Arrowheads indicate p110β isoform in complex with p110α.

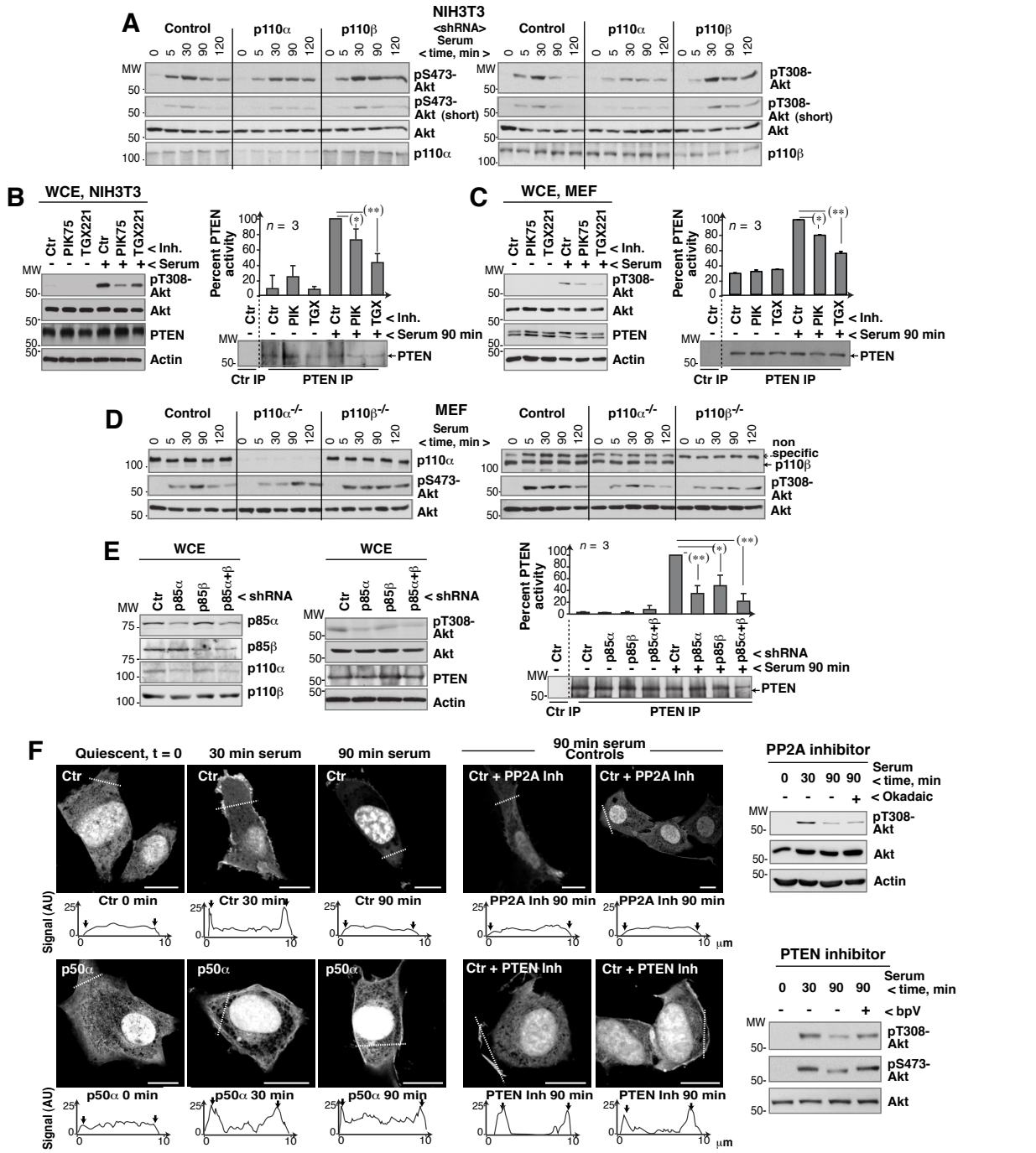

Figure S4. p50α expression disrupts PTEN-regulated membrane PI(3,4,5)P3 levels. (A) NIH3T3 cells were transfected with shRNA (72 h), serum-starved (19 h) and serum-stimulated for various times. Silencing efficiency and pAkt levels were tested in WB. (**B**) Quiescent NIH3T3 cells were serum-stimulated (30 or 90 min). Before stimulation, some cells were incubated (30 min) with PIK75 (0.08 μM) or TGX221 (30 μM) and then with serum. The PIK75 and TGX221 effect on pAkt levels was analyzed at 30 min post-activation. To determine PTEN activity, 90 min-activated cell extracts (250 μg) were immunoprecipitated with anti-PTEN Ab and tested in a phosphatase assay using PI(3,4,5)P3 as substrate; PTEN levels were analyzed in WB. The graph shows PTEN activity normalized to PTEN levels and relative to the maximum (90 min in controls; mean ± SEM, n = 3). (**C**) WT MEF were treated as for NIH3T3 cells in (B), and pAkt levels and PTEN activity in MEF extracts were analyzed as in (B). (**D**) Control, p110α or p110β-deficent MEF were examined as in (A). (**E**) NIH3T3 cells were transfected with shRNA (72 h). WB was used to confirm silencing efficiency in extracts from cells in exponential growth. Cells were serum-starved (19 h) and then serum-stimulated (90 min); PTEN activity was analyzed as in (B). (**F**) NIH3T3 cells were cotransfected with GFP-Btk-PH and control or p50α cDNA (48 h). Cells were incubated in serum-free medium (2 h) and then serum-stimulated (30 or 90 min). After 30 min serum treatment, some control cells were incubated with PTEN inhibitor (bpV, 100 nM) or okadaic acid (100 nM), and collected at 90 min. Images show representative cells examined by confocal microscopy (central z-sections) to visualize PI(3,4,5)P3 localization. WB analysis shows PTEN inhibitor activity as an increase in pAkt levels at 90 min. Quantitation along the dashed lines as in Fig. 7F. Student's t test, * P <0.05; ** P <0.01.