Supporting Information

Schmit et al. 10.1073/pnas.1323004111

SI Materials and Methods

Surgical Procedures. Replication-deficient adenovirus expressing Cre recombinase (Ad-Cre) under the control of CMV promoter was purchased from the University of Iowa. The intrabursal injection was done as described previously (1). The left ovary was Ad-Cre–injected and the right ovary was mock injected as a control. Mice were monitored closely following the injection for tumor formation or any signs of illness and were killed after predefined periods of time or when the animals became moribund. All animal procedures were approved by the Institutional Animal Care and Use Committees of Dana-Farber Cancer Institute.

Mouse Tissue Histopathology. All mice were examined at necropsy for gross organ abnormalities. The ovaries were collected, fixed in 10% formalin, and sent to the Dana-Farber/Harvard Cancer Center (DF/HCC) Histopathology Core Facility for H&E staining and histopathological evaluation.

Immunohistochemical Analysis. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded sections according to standard protocols. Briefly, sections were deparaffinized, the antigens retrieved by boiling in citrate buffer, and incubated over night with the following antibodies: p-Akt (Invitrogen), p-S6 ribosomal protein (Cell Signaling), PTEN (Cell Signaling), Cytokeratin 8 (Troma I, University of Iowa), and Ki67 (Vector Laboratories). Biotinylated secondary antibody, avidin-biotin complex solution, and 3,3′-diaminobenzidine were from Vector Laboratories; sections were counterstained with Meyer's Hematoxylin.

Growth Curves. Two thousand ovarian surface epithelial (OSE) cells were seeded per well into 12-well dishes in full growth medium. For serum-starved conditions, the medium was replaced after 6–18 h to medium without FBS and growth factors. After the indicated times, the dishes were fixed, stained with Crystal Violet for 30 min, and dried overnight. The stain was released and measured in a plate reader at an OD of 590.

Allograft Transplantation Studies. OSE cell lines were grown in vitro to about 80% confluency before being trypsinized and counted. Totals of 2×10^6 cells (PK, PKA, and PKB) or 5×10^6 cells (K, KA, KB, P, PA, and PB) were mixed 1:1 with matrigel and injected s.c. into NCrNu recipient mice. Mice were monitored closely for tumor formation or any signs of illness and were killed when the animals became moribund or their tumors reached 2 cm. For in vivo drug studies, BYL719 (provided by Novartis Pharmaceuticals) was formulated in 0.5% methylcellulose and administered at 45 mg/kg orally (p.o.) once daily, KIN193 (purchased from MedChemexpress) was formulated in 7.5% NMP and 40% PEG-400 in H_2O and administered at 20 mg/kg i.p. once daily. Tumor sizes were calculated using the formula (length × width^2)/2. After the mice were killed, the tissue at the injection site was dissected, formalin fixed, and submitted to DF/HCC Histopathology Core Facility for H&E staining and histopathological evaluation.

Western Blot. Whole ovaries and tumors were homogenized and lyzed in RIPA buffer. OSE cells were lyzed in RIPA buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% NP-40, protease inhibitors). Lysates were analyzed by Western blotting with the following antibodies: p110α, p110β, p-Akt, Akt, p-S6, PTEN, p53 (Cell Signaling), α-tubulin, vinculin, pan-cytokeratin (Sigma), pan-rat sarcoma (Calbiochem), estrogen receptor, and progesterone receptor (Santa Cruz).

Genotyping of OSE Cells. Cells were lysed in 150 mM NaCl, 20 mM Tris H 7.5, 5 mM EDTA, 0.5% SDS, and 0.25 mg/mL Proteinase K for 30 min at 37 °C. Genomic DNA was extracted with phenol/chloroform and precipitated with 0.3 M sodium acetate and isopropanol. PCR was performed with the following primers:

p110α: CTGTGTAGCCTAGTTTAGAGCAACCATCTA and CCTCTCTGAACAGTTCATGTTTGATGGTGA

p110β: CTCAAACTAGTGACTAGAAGCTGTGAC and GATCGAGGCCATTAGAGAAGACCGT

Pten: ACTCAAGGCAGGGATGAGC, AATCTAGGGCCTCTTGTGCC and GCTTGATATCGAATTCCTGCAGC

Kras: CCATGGCTTGAGTAAGTCTGC and CGCAGACTGTAGAGCAGCG.

1. Rodon J, Dienstmann R, Serra V, Tabernero J (2013) Development of PI3K inhibitors: Lessons learned from early clinical trials. *Nat Rev Clin Oncol* 10(3):143–153.

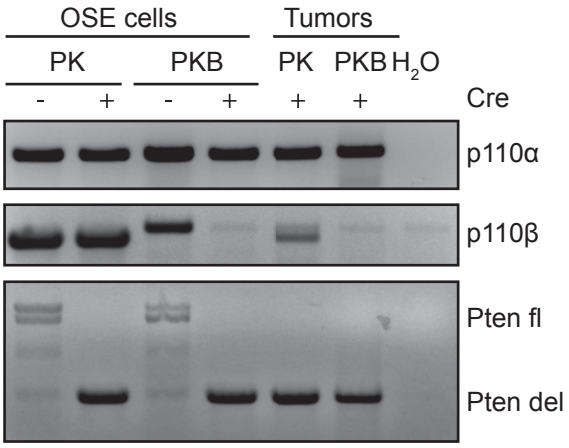

Fig. S1. Genotyping shows loss of p110β in PKB tumor tissue. OSE cells or tumor tissues with the indicated genotypes were lyzed and purified and used as template for genotyping PCR. PK, expression of oncogenic *Kras* and deletion of *Pten*; PKB, additional depletion of *p110β*; fl, floxed allele; del, deleted allele.

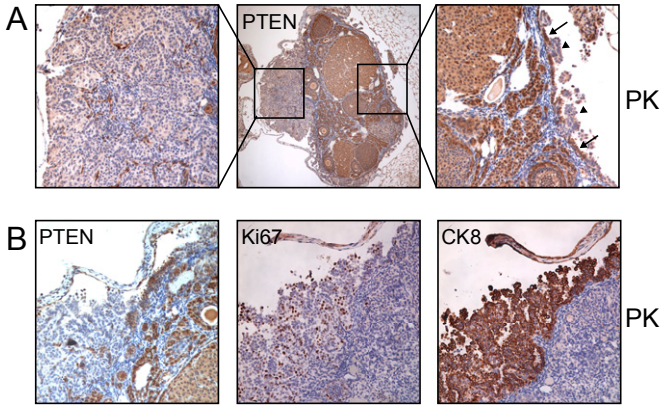

Fig. S2. Immunohistochemical analysis of ovaries after Ad-Cre injection. (*A*) Immunohistochemical staining of PTEN in ovary sections from mice with expression of oncogenic *Kras* and deletion of *Pten* (PK) at 4 wk after Cre injection. (*Center*) Section with tumor tissue on the left side and seemingly normal epithelium on the right side. Further magnification into the tumor region shows loss of PTEN in this region (*Left*). Further magnification into the epithelium shows regions with a single layer of epithelial cells that stain positive for PTEN (*Right*, arrows). In some places, the epithelium is proliferating into multiple layers and has lost PTEN expression (*Right*, arrowheads) (*B*) Immunohistochemical staining of PTEN, Ki67, and Cytokeratin 8 (CK8) in ovary sections from mice with expression of oncogenic *Kras* and deletion of *Pten* (PK) at 4 wk after Cre injection.

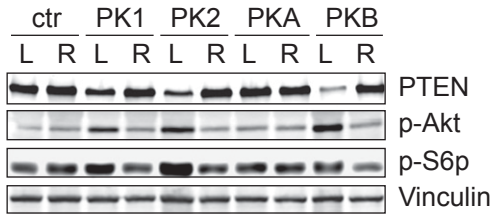

Fig. S3. Western blot analysis of whole ovary preparations. Mice with the indicated genotypes were administered Ad-Cre recombinase (left ovary, L) into the bursa of the ovary or mock injected as a control (right ovary, R). At 12 wk after injection, ovaries were isolated from killed animals, homogenized, and subjected to signaling analyses via Western blot with the indicated antibodies. PK, expression of oncogenic *Kras* and deletion of *Pten*; PKA, additional deletion of *p110α*; PKB, additional deletion of *p110β*.

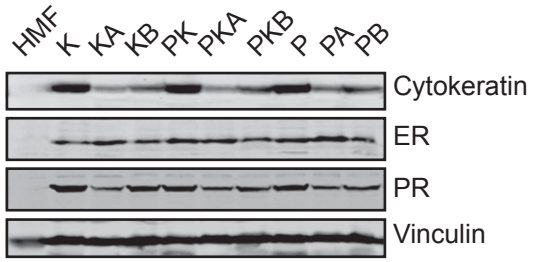

Fig. S4. Characterization of OSE cells. Western blot analysis of ovarian surface epithelial (OSE) cells with expression of oncogenic *Kras* (K), deletion of *Pten* (P), additional deletion of *p110α* (PKA, KA, and PA), and additional deletion of *p110β* (PKB, KB, and PB). All OSE lines express cytokeratin, estrogen receptor (ER), and progesterone receptor (PR). HMF, human mammary fibroblasts.

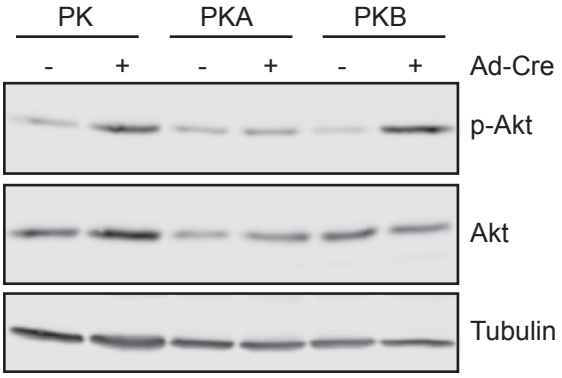

Fig. S5. Loss of p110α in OSE-PK prevents p-Akt increase. OSE cells with the indicated genotypes before Cre treatment or after one round of Ad-Cre treatment were serum starved overnight and analyzed in Western blot with the indicated antibodies. Expression of oncogenic *Kras* and deletion of *Pten* (PK), additional deletion of *p110α* (PKA), and additional deletion of *p110β* (PKB).

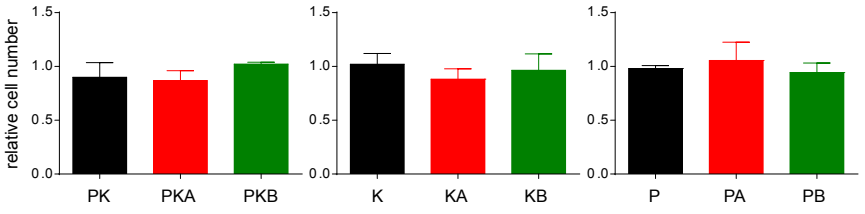

Fig. S6. Immortalized OSE cells have similar proliferation rates in full-growth medium. OSE cells with the indicated genotypes were seeded at the same densities, grown for 6 d in full-growth medium containing FBS and growth factors, and stained with Crystal Violet. Shown are averages and SDs from two independent experiments performed in triplicates. Expression of oncogenic *Kras* (K), deletion of *Pten* (P), additional deletion of *p110α* (PKA, KA, and PA), and additional deletion of *p110β* (PKB, KB, and PB).

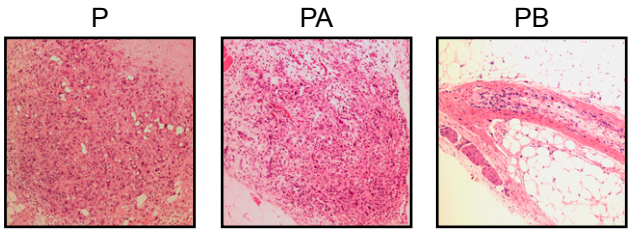

Fig. S7. OSE-P cells are dependent on the p110β isoform when injected into the mammary fat pad. OSE cells with the indicated genotypes were injected into the mammary fat pad. Shown are H&E stainings for one typical example each. Deletion of *Pten* (P), additional deletion of *p110α* (PA), and additional deletion of *p110β* (PB).

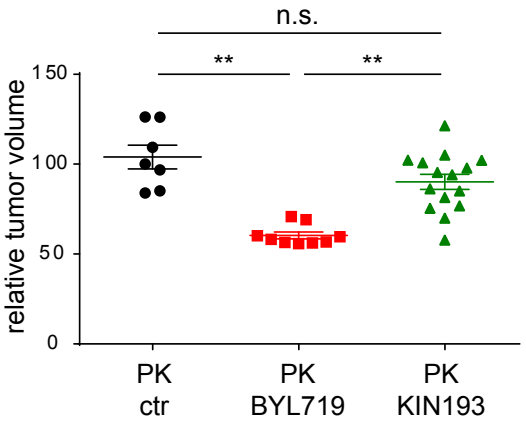

Fig. S8. Pharmacological inhibition of p110α inhibits growth of established PK tumors. Ovarian surface epithelial cells from mice with expression of oncogenic *Kras* and deletion of *Pten* (PK) were injected s.c. into the flank of NCrNu recipient mice. Treatment with pharmacological inhibitors (BYL719, 45 mg/kg once daily p.o.; KIN193, 20 mg/kg once daily i.p.) was started one week after injection when the tumors had established. After 3 wk of treatment, mice were killed and tumor sizes were measured with a caliper. Shown are single tumor sizes with mean values and SEMs. $^{**}P < 0.0001$, n.s., not significant.

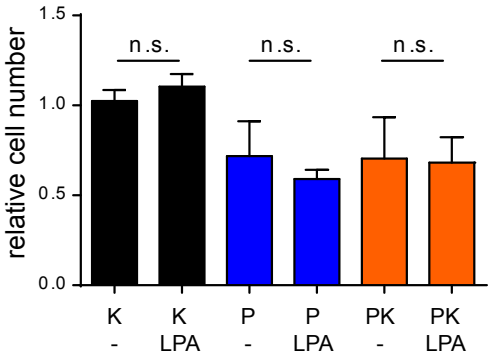

Fig. S9. Lysophosphatidic acid (LPA) does not increase OSE cell proliferation in medium without FBS or growth factors. OSE cells with the indicated genotypes were seeded at the same densities, grown for 10 d in medium without FBS or growth factors but containing 1 μM LPA, and stained with Crystal Violet. Shown are averages and SDs from two independent experiments performed in triplicates. Expression of oncogenic *Kras* (K), deletion of *Pten* (P), and combination of both lesions (PK). n.s., not significant.