Supporting Information

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SI Materials and Methods

HPC and MEF Isolation and Immortalization. For isolation of HPCs, day 10.5 embryos were dissected from timed pregnant females. HPCs, freshly isolated by trypsinization of embryo yolk sacs were used in colony formation assays or infected with a retrovirus encoding Hox-11 and neomycin-resistance in the presence of 8 μg/mL polybrene, as described (1). Hox-11 packaging cells were a kind gift from Robert Hawley (The George Washington University Medical Center, Washington, DC). Cells were not subjected to G418 selection, but the cell pools obtained were found to be neomycin-resistant and to express Hox11. Immortalized IL3-dependent HPC pools were cultured in RPMI medium containing 10% FBS and 2.5 ng/mL recombinant murine IL3 (Peprotech). Before growth factor/cytokine stimulation, HPCs were deprived of IL3 and serum for 16 h in X-VIVO-10 medium (Lonza) or for 3 h in RPMI medium supplemented with 0.1% BSA.

MEFs derived from $p110\alpha^{flox/flox}$ mice carrying a tamoxifen-inducible Cre transgene ($CreER^{T2}$) have been described (2). $CreER^{T2}$-$p110\alpha^{flox/flox}$ MEFs (or heterozygous $CreER^{T2}$-$p110\alpha^{flox/WT}$ MEFs used as controls) were immortalized by transduction with a retrovirus expressing a microRNA-based short hairpin RNA against p53 (3). Deletion of the $p110\alpha^{flox}$ allele was then induced by treating pools of immortalized MEFs with 1 μM 4-hydroxy-tamoxifen for 72 h. Effective deletion was confirmed by immunoblot analysis of p110α protein expression in cell lysates. Heterozygous (referred to as $p110\alpha^{DEL/WT}$; used as controls) or p110α-deficient (referred to as $p110\alpha^{DEL/DEL}$) MEFs were cultured in DMEM medium containing 10% serum (or 0.5% serum for serum deprivation conditions).

Colony Formation Assay. Ten thousand cells, freshly isolated from trypsinized yolk sacs, were plated in 25-mm dishes containing 2 mL methylcellulose-based medium supplemented with growth factors/cytokines (insulin, IL3, IL6, SCF, erythropoietin; Methocult GF M3434; Stem Cell Technologies). After incubation for 10–14 d in a humidified incubator at 37 °C and 5% CO_2 atmosphere, colony numbers were visually scored under a stereomicroscope.

Immunoblot Analysis. Cells lysis buffer was 50 mM Tris.HCl pH 7.4, 5 mM EDTA, 150 mM NaCl, 50 mM NaF, and 1% Triton X-100 supplemented with 2 mg/mL aprotinin, 1 mM pepstatin, 1 ng/mL leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 1 mM sodium orthovanadate. Lysates were analyzed by polyacrylamide gel electrophoresis and immunoblotting using the following antibodies: p85 (cat. no. 06-195; Millipore), p110α (cat. no. 611398; BD Transduction Laboratories), p110β, and p110δ (sc-603 and sc-7671, respectively; Santa Cruz). A mouse monoclonal antibody to p110γ was donated by Reinhard Wetzker (University of Jena, Jena, Germany). Antibodies to phospho-T308, phospho-S473, and total Akt and to phospho- and total ribosomal S6 were from Cell Signaling Technology. Antibodies to Gab2 were from Millipore (cat. no. 06-967). Immunoblot signal intensity quantitation was performed with a Bio-Rad GS-800 densitometer and QuantityOne software.

1. Yu W-M, Hawley TS, Hawley RG, Qu C-K (2002) Immortalization of yolk sac-derived precursor cells. *Blood* 100:3828–3831.
2. Graupera M, et al. (2008) Angiogenesis selectively requires the p110alpha isoform of PI3K to control endothelial cell migration. *Nature* 453:662–666.
3. Dickins RA, et al. (2005) Probing tumor phenotypes using stable and regulated synthetic microRNA precursors. *Nat Genet* 37:1289–1295.

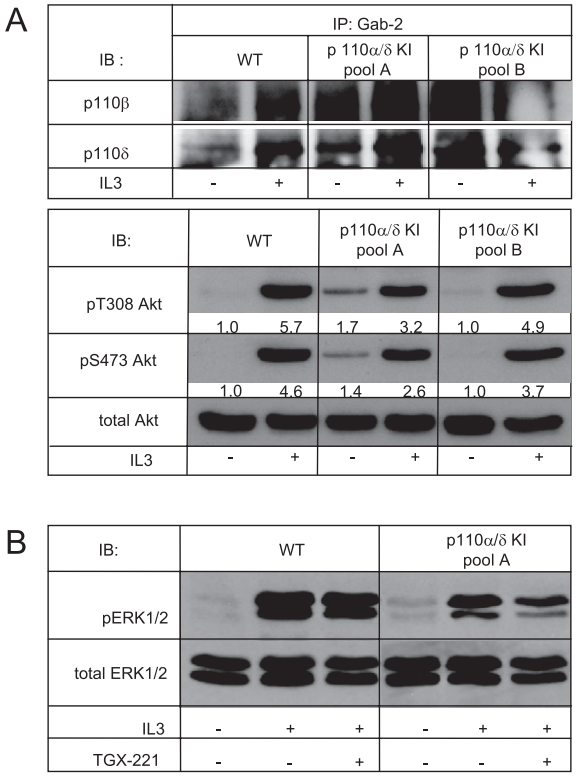

Fig. S1. Association of p110β with Gab2 and phosphorylation of Akt and ERK in p110α/δ KI HPCs. (*A*) HPCs were deprived of serum and IL3 for 3 h and stimulated with 20 ng/mL IL3 for 5 min at 37 °C, followed by cell lysis. Immunoprecipitates (made with Gab2 antibody on 1.5 mg of total protein) were immunoblotted with the indicated anti-p110 antibodies (*Upper*) or 50 μg of total protein was immunoblotted using the indicated antibodies to Akt (*Lower*). Relative (fold over vehicle-treated WT cells) signal intensities, indicated under each blot, were quantified by densitometry. (*B*) WT and p110α/δ KI HPCs cultured in the presence or absence of 500 nM TGX-221 for 24 h were stimulated with 20 ng/mL IL3 for 5 min at 37 °C, followed by lysis, immunoblot, and analysis of ERK1/2 phosphorylation using the indicated antibodies.

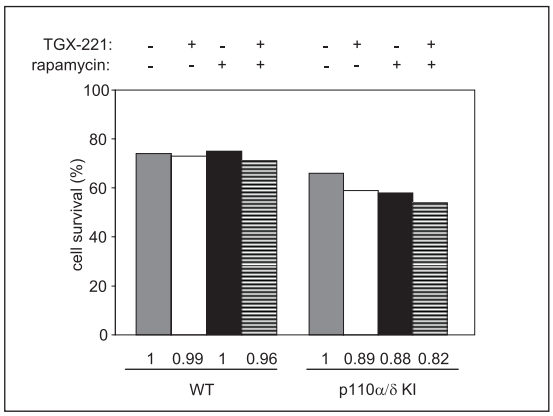

Fig. S2. Rapamycin does not induce apoptosis in either WT HPCs or HPCs lacking class IA PI3K activity. The fraction of HPCs surviving 24-h treatment with 1 μM TGX-221 alone, or in combination with 20 nM rapamycin, was determined by FACS analysis of multicaspase activation.

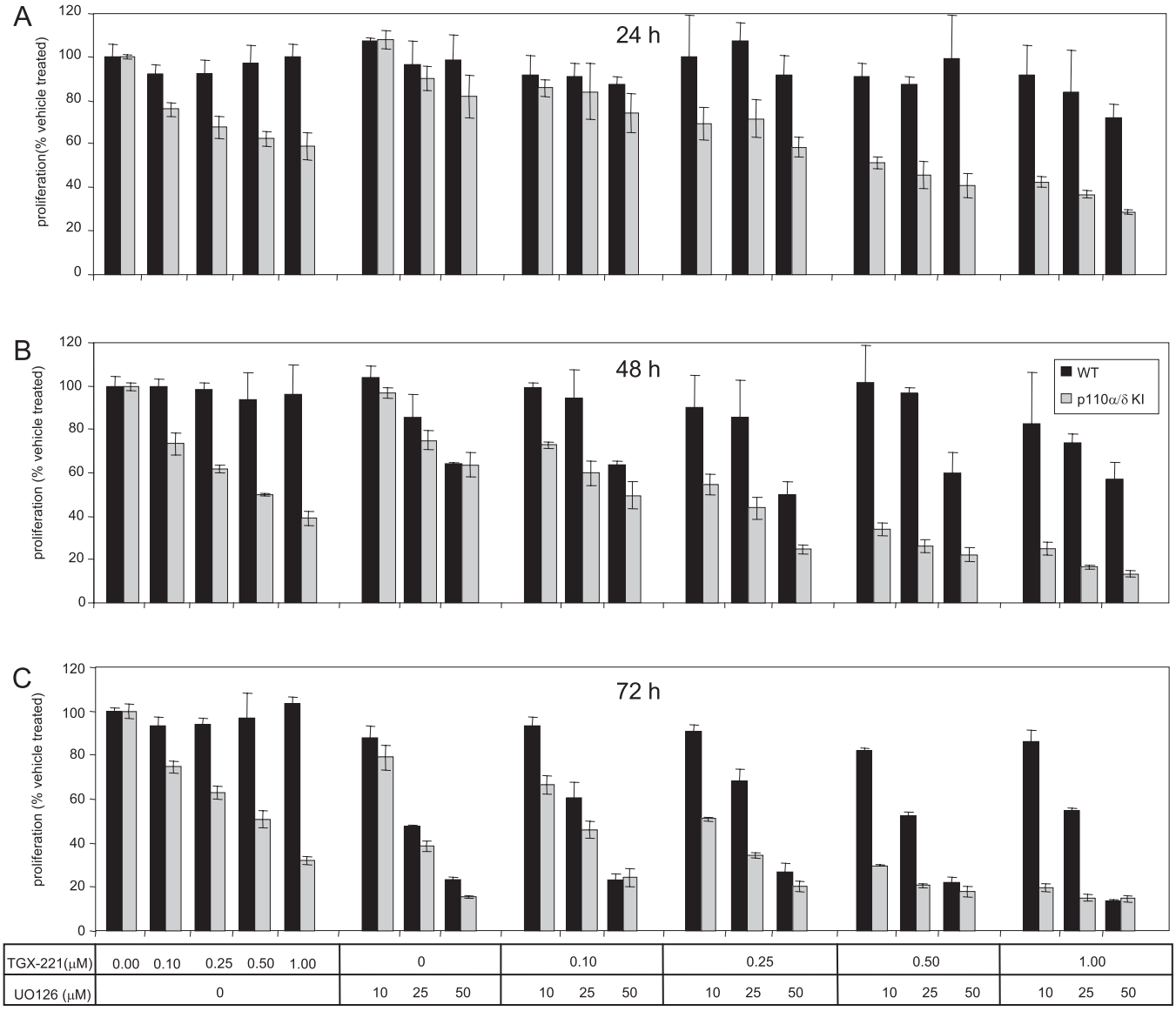

Fig. S3. Time- and dose-dependent effect of TGX-221 combined with the MEK inhibitor UO126 on HPC proliferation, assessed by MTS assay after 24 h (*A*), 48 h (*B*), and 72 h (*C*). A representative experiment performed in triplicate is shown (values = mean ± SD).

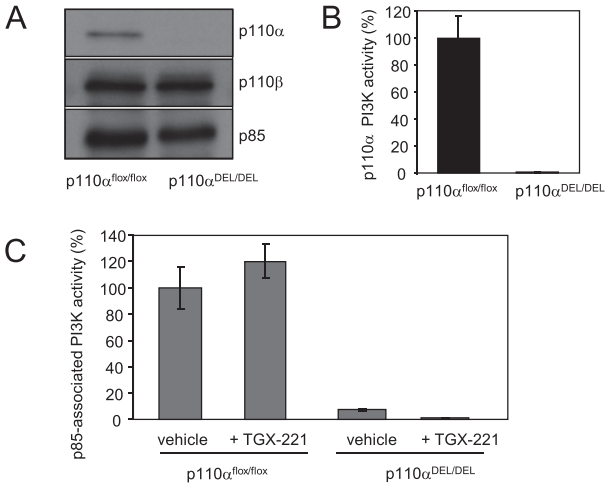

Fig. S4. Efficiency of conditional deletion of p110α and its effect on p85-associated PI3K activity. Deletion efficiency of the floxed p110α allele following treatment of MEFs with 1 μM 4-hydroxy-tamoxifen for 72 h was assessed by immunoblot analysis with the indicated antibodies (*A*) and PI3K activity assay in p110α immunoprecipitates (*B*). (*C*) The impact of p110α deletion on total cellular class IA PI3K activity was assessed by PI3K assay on p85 immunoprecipates (in the presence or absence of 100 nM TGX-221) made from cell lysates of the designated genotypes Representative experiments are shown (values = mean ± SD). $p110\alpha^{flox/flox}$ designates MEFs treated with vehicle; $p110\alpha^{DEL/DEL}$ designates $p110\alpha^{flox/flox}$ MEFs treated with 4-hydroxy-tamoxifen.

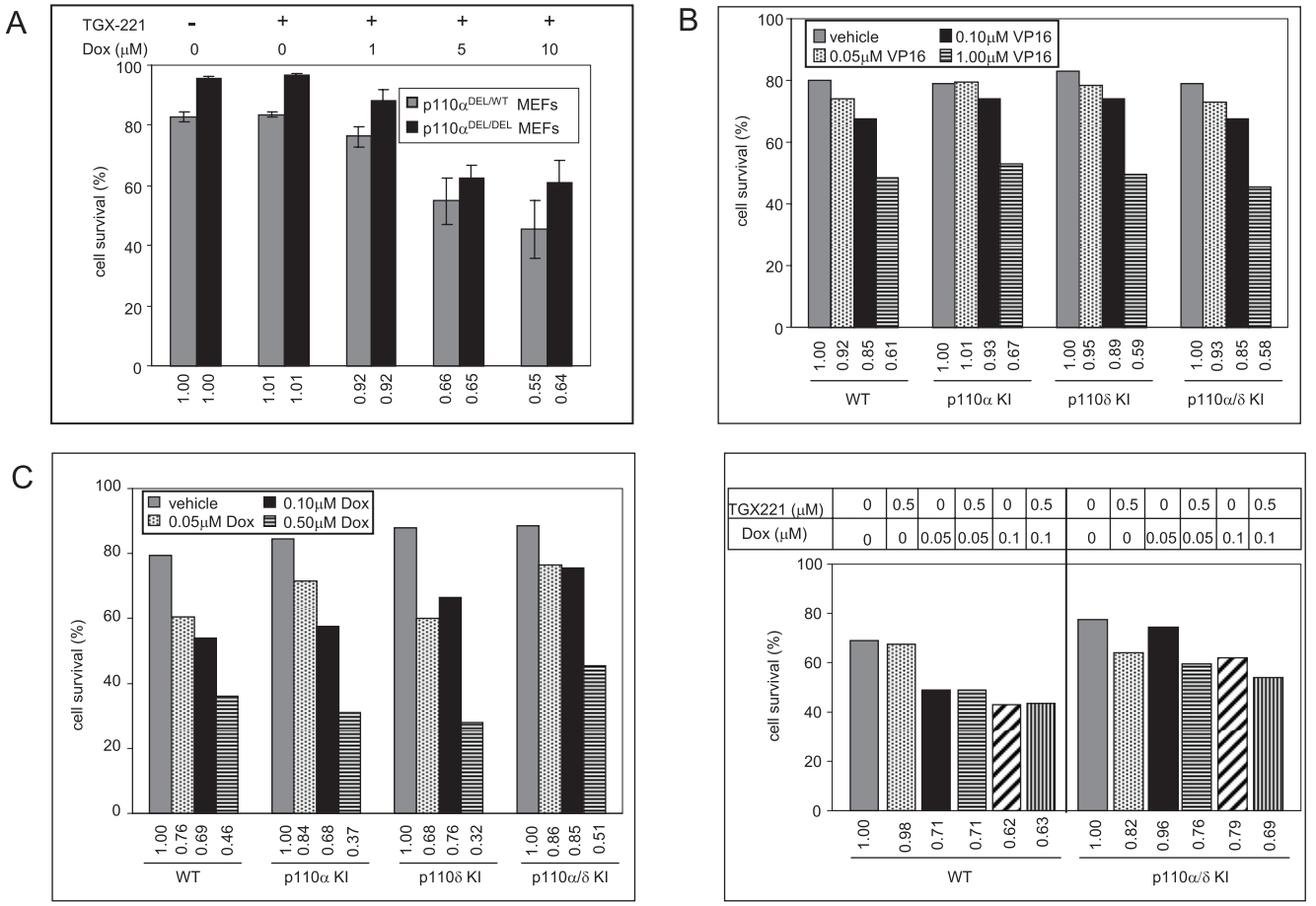

Fig. S5. Inactivation of class IA PI3K does not sensitize MEFs or HPCs to apoptosis induced by genotoxic drugs. The fraction of MEFs (*A*) or HPCs (*B* and *C*) surviving 24-h treatment with the indicated concentrations of doxorubicin (Dox) (*A*), etoposide (VP-16) (*B*), or doxorubicin in the absence (*Left*) or presence (*Right*) of the indicated concentrations of TGX-221 (*C*) was determined following detection of apoptotic cells by FACS analysis of multicaspase activation. Relative change in survival from vehicle-treated cells of each genotype is indicated under each bar graph. Pooled data from three independent experiments are shown in *A*. Representative experiments are shown in *B* and *C*.

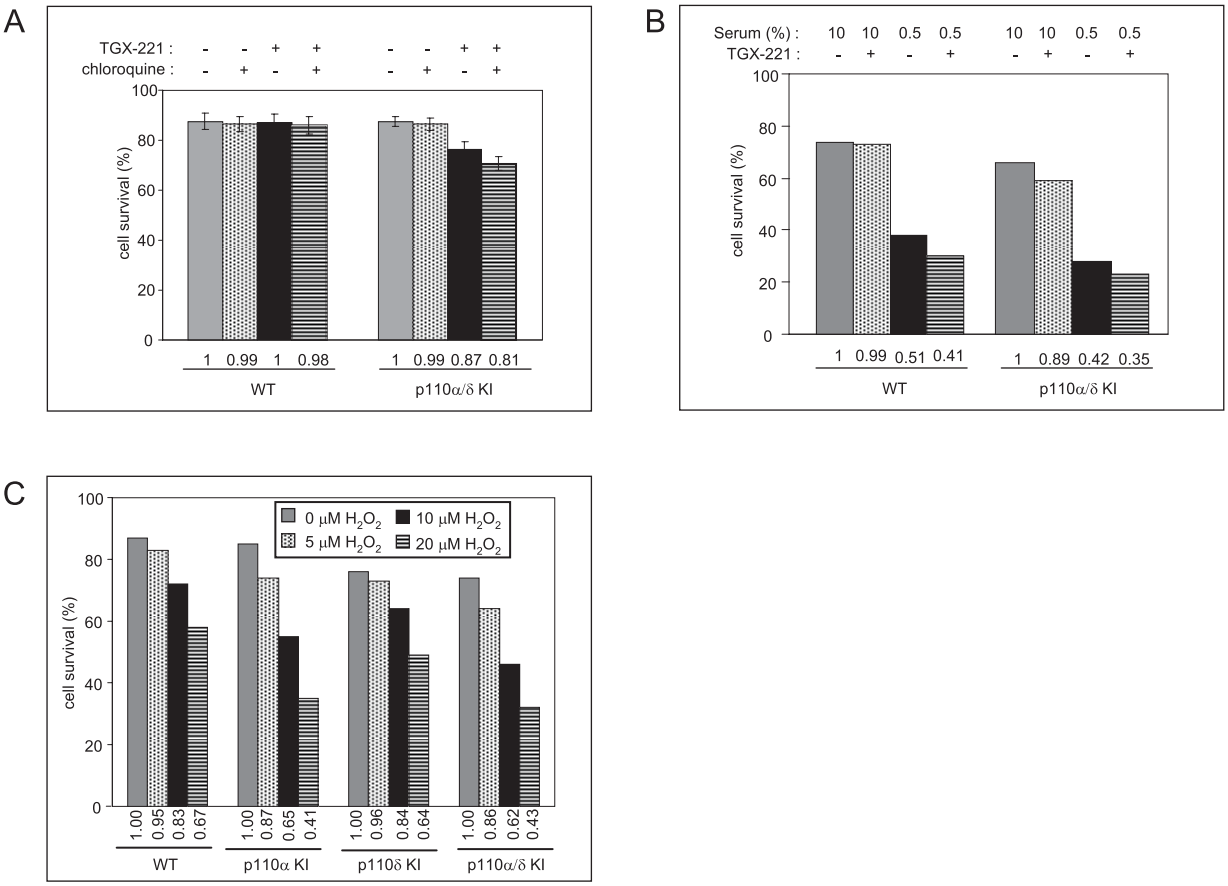

Fig. S6. Inactivation of class IA PI3K does not sensitize HPCs to apoptosis induced by autophagy inhibition, serum deprivation, or oxidative stress. (*A*) The fraction of HPCs surviving 24-h treatment with 1 μM TGX-221 alone, or in combination with 10 μM chloroquine. (*B*) Fraction of HPCs surviving 24-h culture in media supplemented with 0.5% serum in the presence of 1 μM TGX-221. (*C*) Fraction of HPCs surviving 24-h treatment with the indicated concentrations of H_2O_2. Survival was determined following detection of apoptotic cells by FACS analysis of multicaspase activation. Relative change in survival from vehicle-treated cells of each genotype is indicated under each bar graph. Representative experiments are shown, except for *A* (pooled data from two independent experiments performed in triplicate).