Supplement Methods.

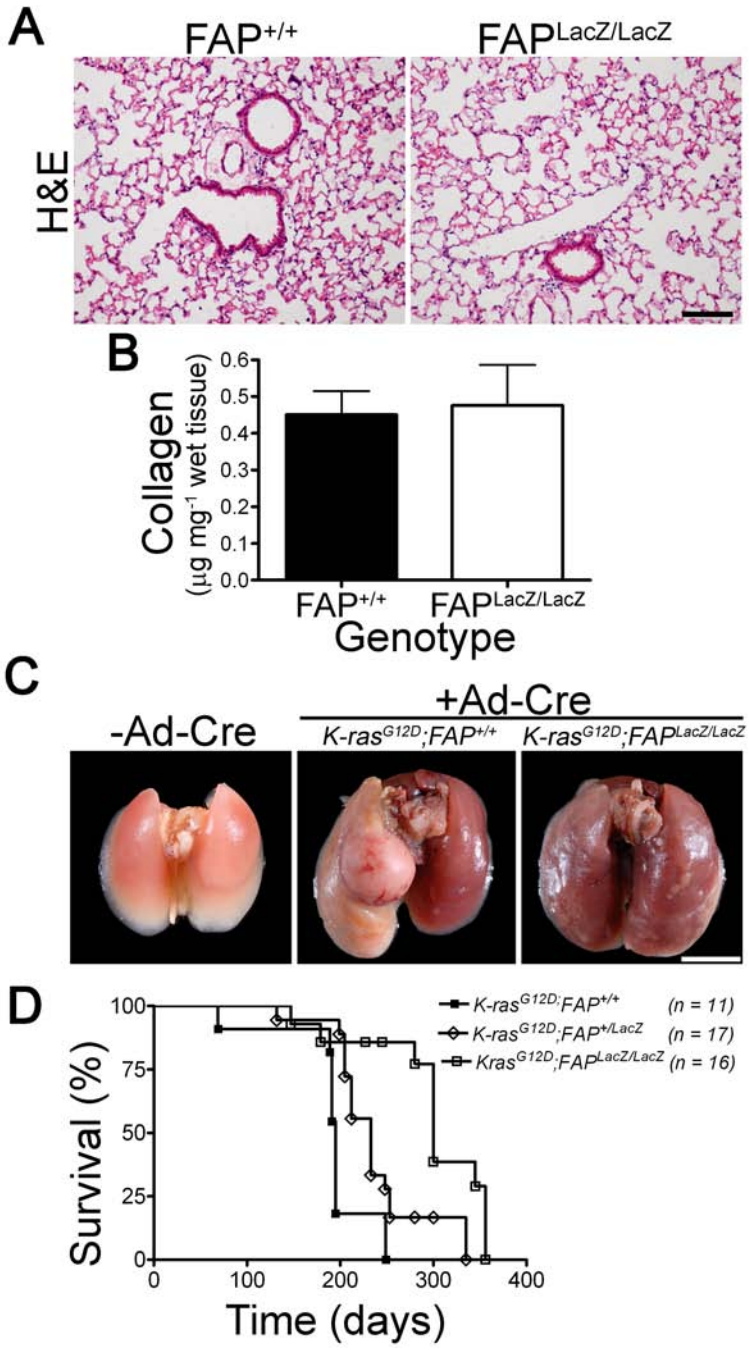
Genotype PCR analysis.

Genomic DNA was extracted from mouse tails using phenol-chloroform as described elsewhere. The *LSL-K-ras*^{G12D} allele was assessed by PCR with *Red Taq* DNA Polymerase (Sigma Chem Co.) using the primers 5'-GCATGGGTTGAGTAAGTCTGC-3' and 5'-CGCAGACTGTAGAGCAGCG-3' (ITD, Iowa). FAP primers (ITD, Iowa) were 5'-CAGCTGTGCTTTATGGGTTT (common); 5'-CCCTCGAAGGCCTACTAATC (wild type allele) and 5'-ATCTCTCGTGGGATCATTGT (neomycin cassette). The wild-type and knock-out (neomycin) amplicons migrated at 660 bp and 392 bp, respectively.

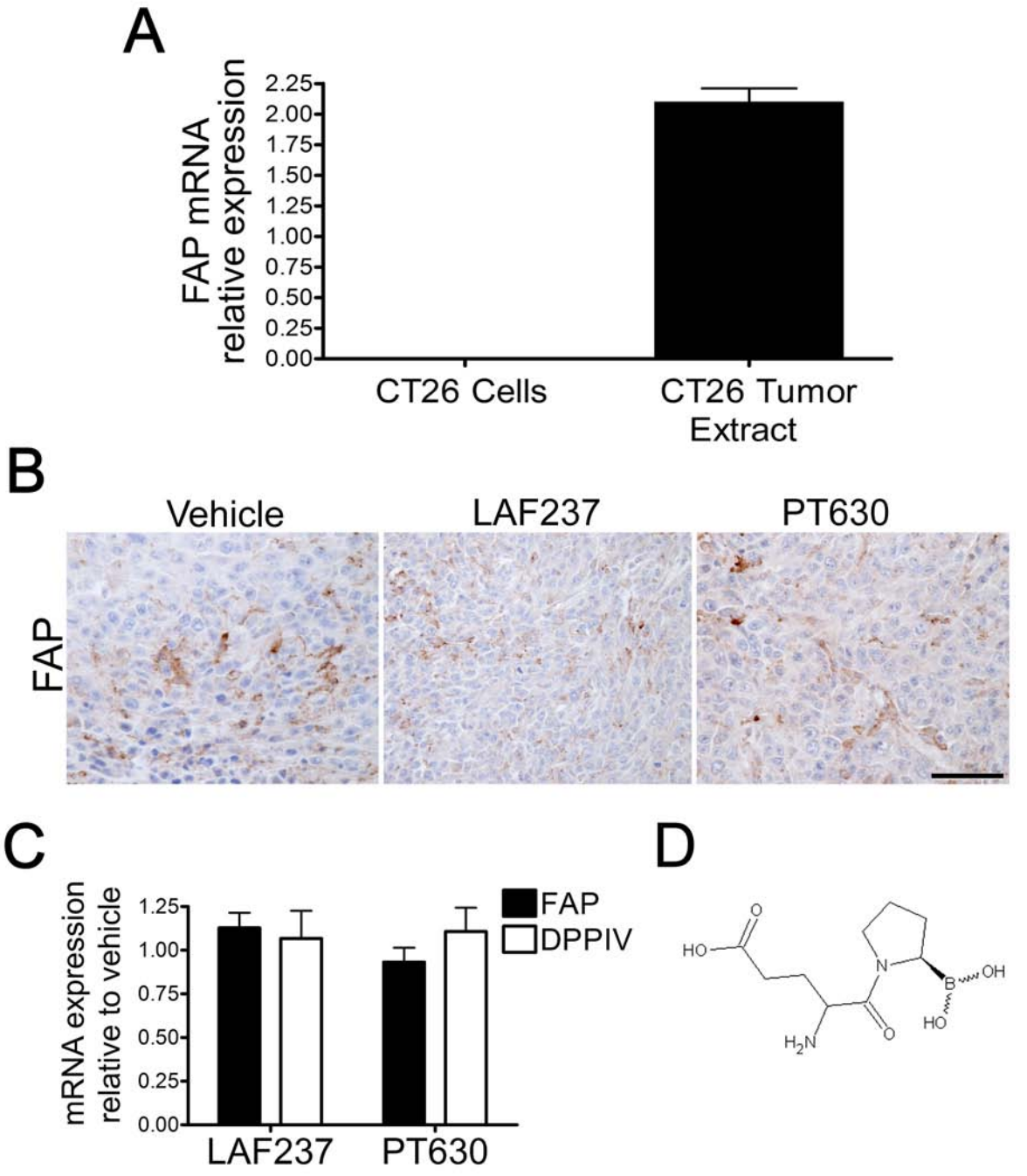
Quantitative real Time-PCR (Q-PCR) analysis.

Flank tumors (25-50 mg) were homogenized on ice in 1 ml of TRIzol (Invitrogen, Palo Alto, CA). Total RNA was extracted according to the manufacturer's protocol and then treated with Turbo DNAse (Ambion) to eliminate genomic DNA. One µg total RNA was reverse transcribed using the TaqMan reverse transcriptase kit (Applied Biosystems, Foster City, CA). Q-PCR amplification and detection was performed according to a standard dissociation protocol using the SYBR Green master mix (Sigma Chem. Co.) and a 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA). For each gene, relative expression was normalized to GADPH. Primer sequences were generated using the Primer Express software (TaqMan® Probe).

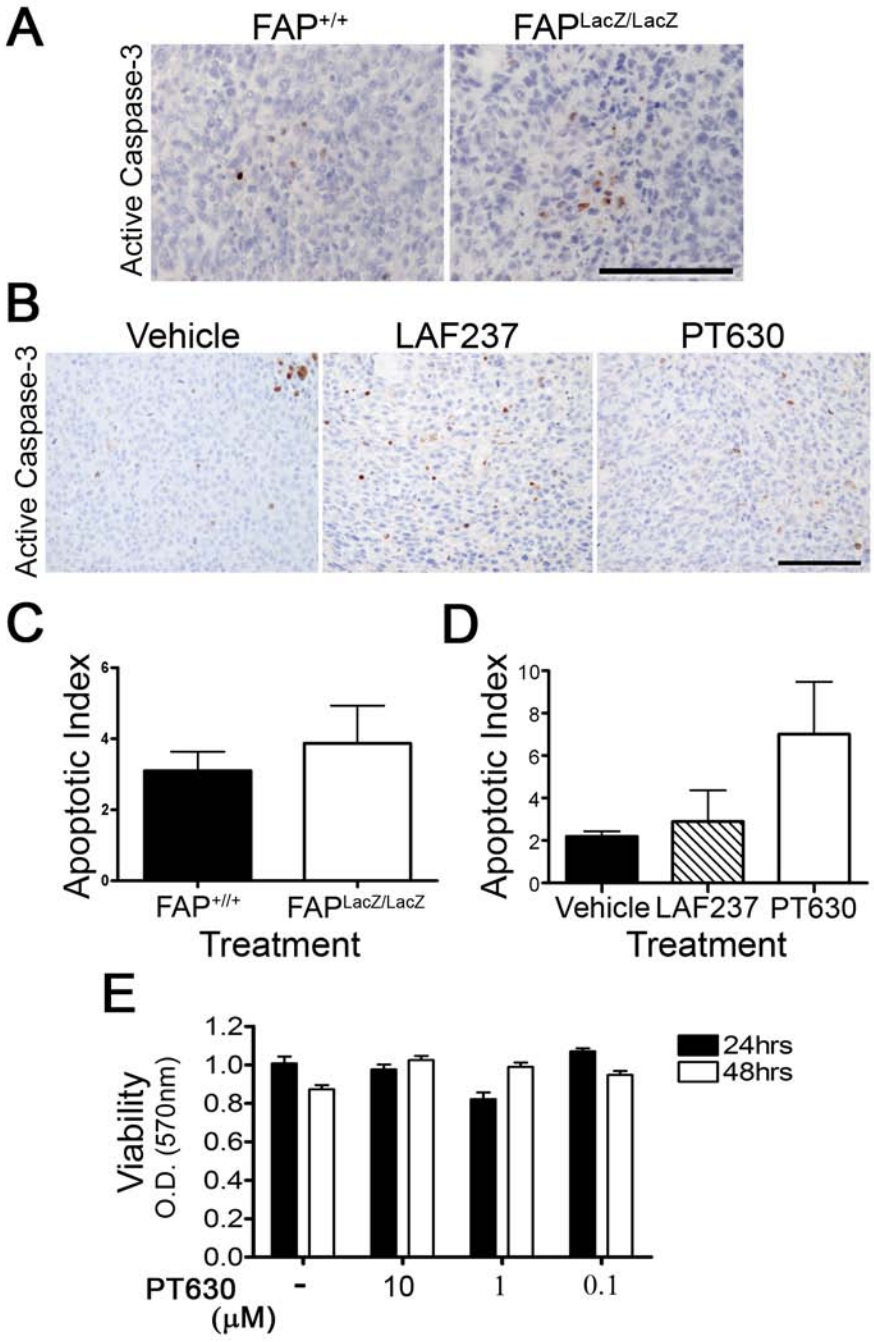
Supplement Figures.



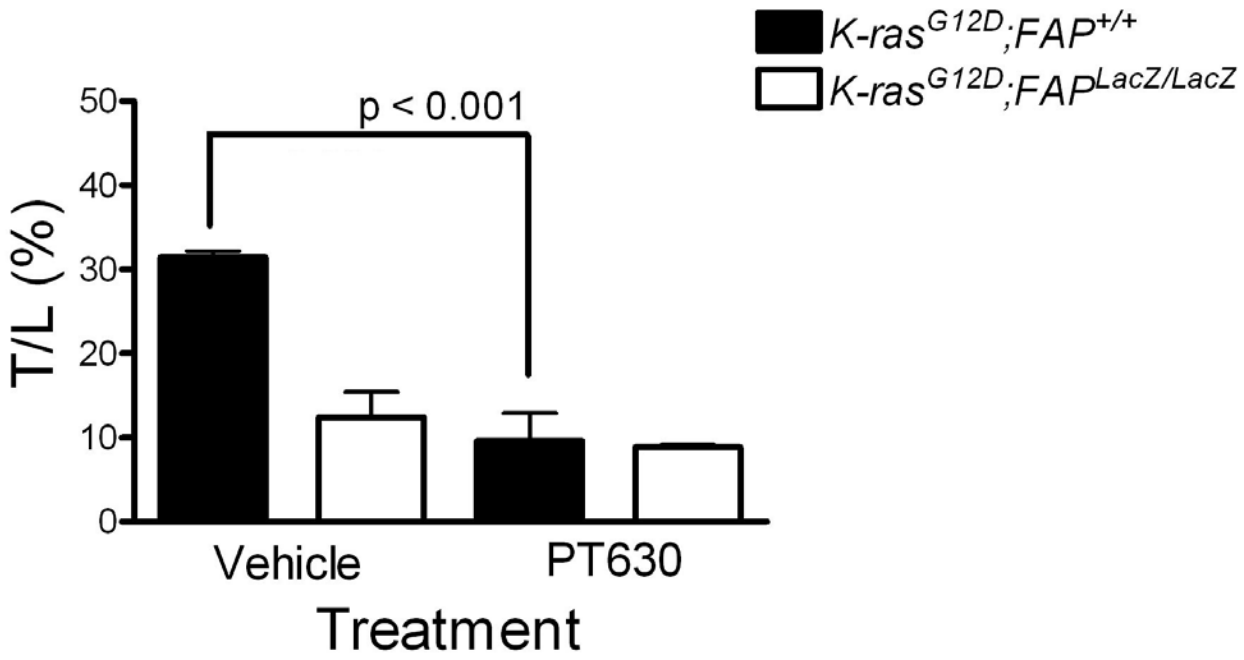
Supplement Figure 1. (A) Representative H&E micrographs of sections of lungs from FAP$^{+/+}$ and FAP$^{LacZ/LacZ}$ mice. **(B)** Collagen content of lungs from FAP$^{+/+}$ and FAP$^{LacZ/LacZ}$ mice. Data represent means $\pm$ SE of 5 lungs per genotype. **(C)** Representative macroscopic appearance of lungs from an uninfected *LSL-K-ras^{G12D};FAP$^{+/+}$* mouse (-Ad-Cre) and Ad-Cre infected (+Ad-Cre) *LSL-K-ras^{G12D};FAP$^{+/+}$* and *LSL-K-ras^{G12D}; FAP$^{LacZ/LacZ}$* mice. **(D)** Kaplan-Meier curve of Ad-Cre infected *LSL-K-ras^{G12D};FAP$^{+/+}$* (n=11), *LSL-Kras^{G12D};FAP$^{+/LacZ}$* (n=17) and *LSL-Kras^{G12D};FAP$^{LacZ/LacZ}$* (n=16).



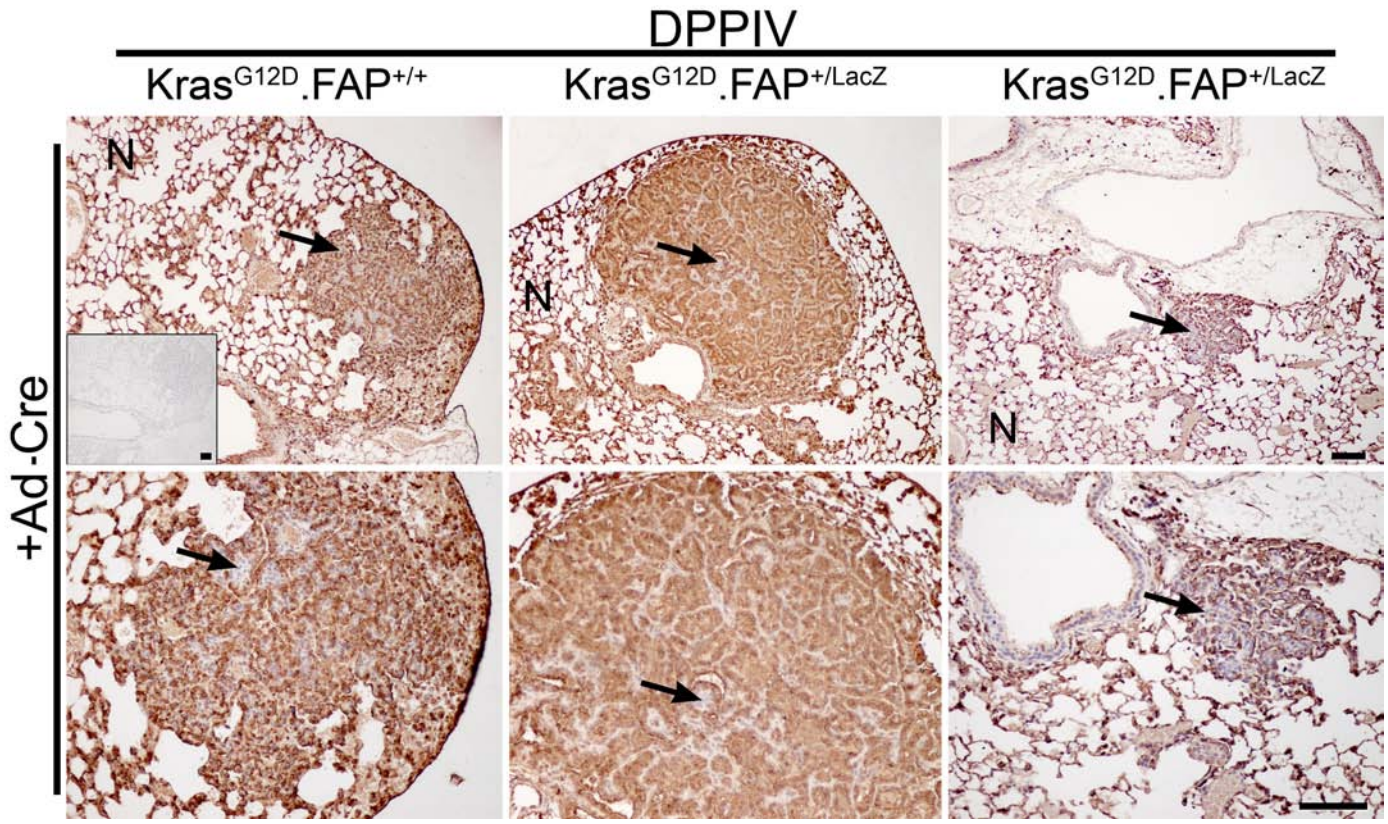
Supplement Figure 2. Anti-tumor activity of PT630 is not due to inhibition of induced FAP expression in tumors in vivo. FAP is not expressed in cultured CT26 cells at the RNA **(A)** or protein level (data not shown) but is induced in vivo in CT26 tumors from wild-type mice **(B,C)**. Neither PT630 nor LAF237 had any effect on FAP protein expression by IHC **(B)** nor FAP or DPPIV mRNA levels in CT26 tumors. Magnification in (B), 60x objective; bar=50 µm. **(A,C)** Values are expressed as means ± SE for 5 tumors from two independent experiments. **(D)** PT630 chemical structure.



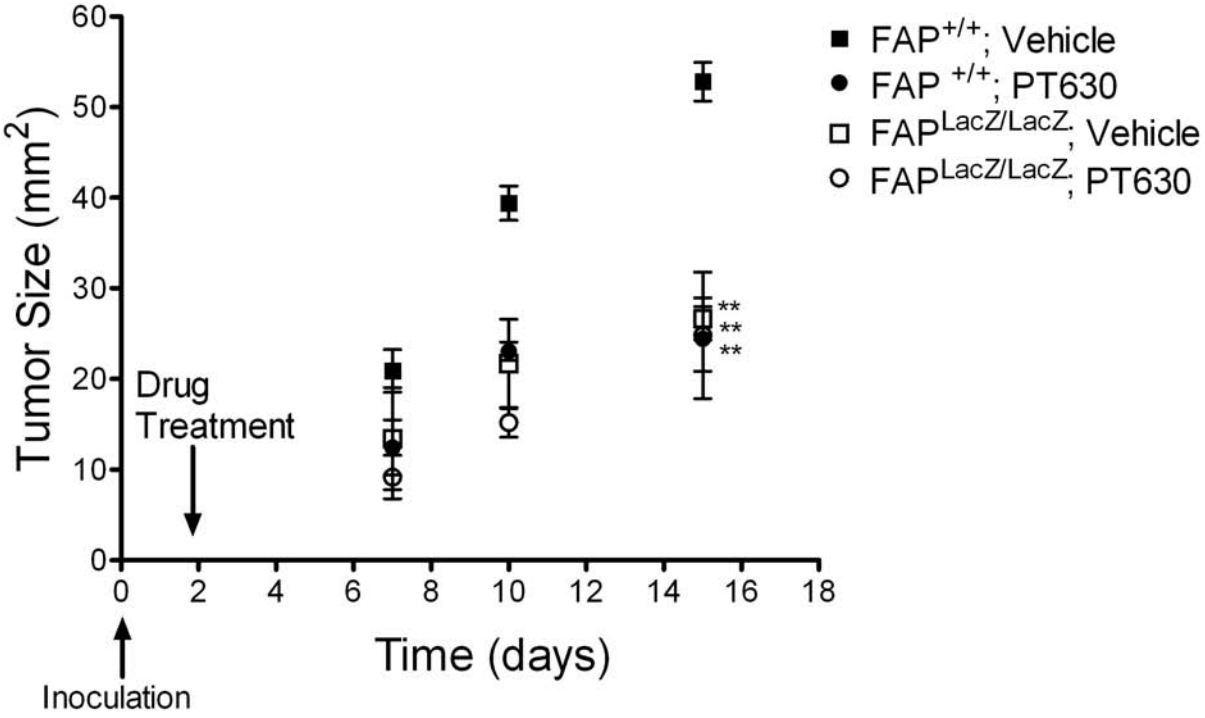
Supplement Figure 3. PT630 does not directly affect tumor cell apoptosis or tumor cell proliferation. Representative active caspase-3 immunostaining in CT26 tumors from **(A)** $FAP^{+/+}$ versus $FAP^{LacZ/LacZ}$ mice and **(B)** drug treated $FAP^{+/+}$ mice. Magnification, 60x objective; bar=100 μm. Values are expressed as means ± SE for ten animals from two independent experiments. Quantification of **(A)** and **(B)** are shown in **(C)** and **(D)**, respectively. **(E)** Cultured CT26 cells viability was assessed after treatment with 0.1-10 μM PT630 for 24 or 48hrs *in vitro*. Values are expressed as means ± SE of triplicates from 3 independent experiments.



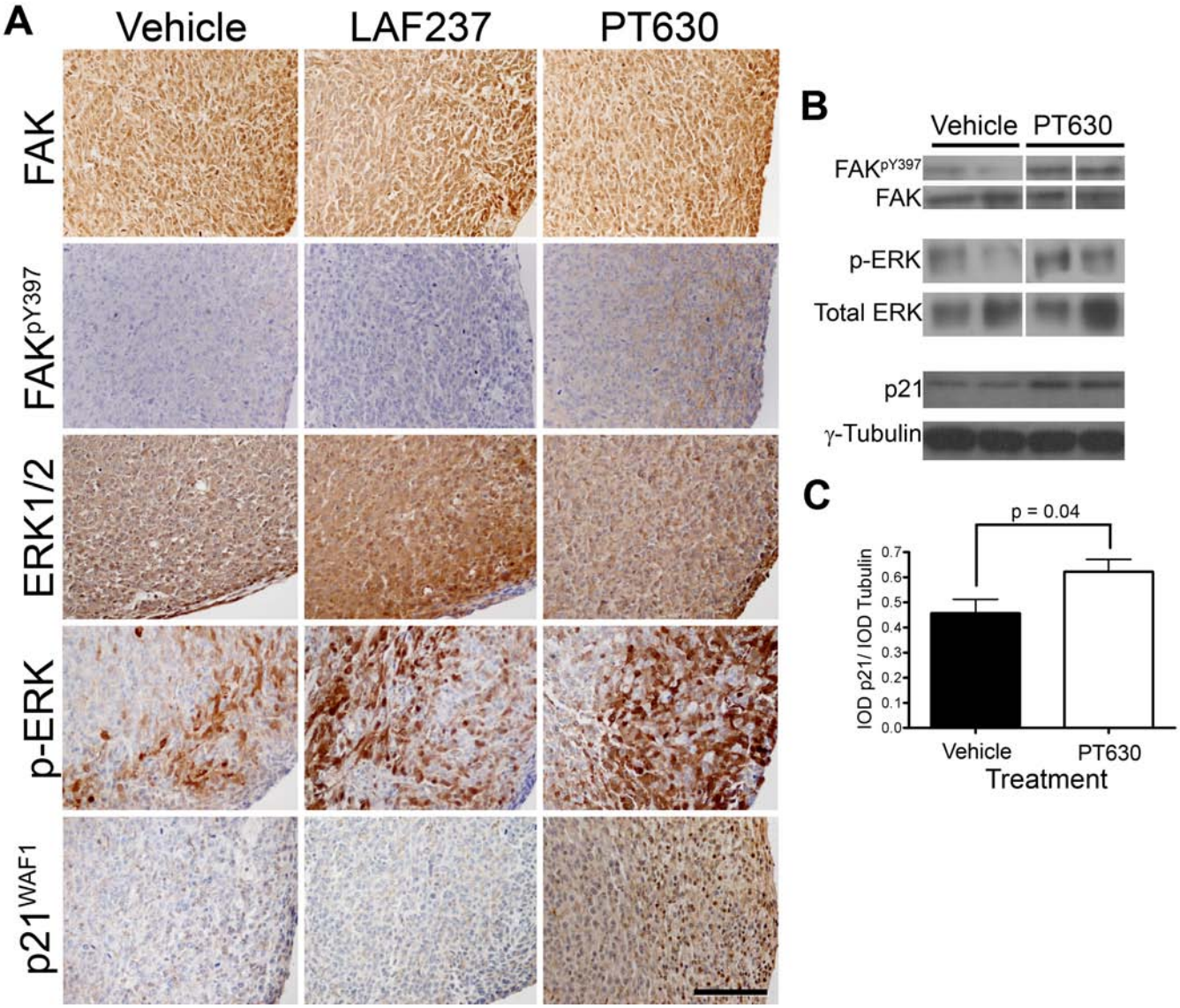
Supplement Figure 4. PT630 reduced formation of K-ras^{G12D}-driven lung tumors in *LSL-K-ras^{G12D};FAP$^{+/+}$* but not in FAP-deficient mice. The ratio of tumor-to-lung area (T/L) was quantified in *LSL-K-ras^{G12D};FAP$^{+/+}$* and *LSL-K-ras^{G12D};FAP$^{LacZ/LacZ}$* mice treated with vehicle control or PT630 for 4 weeks starting 4 weeks post-Ad-Cre infection (n=3-5 animals per group).



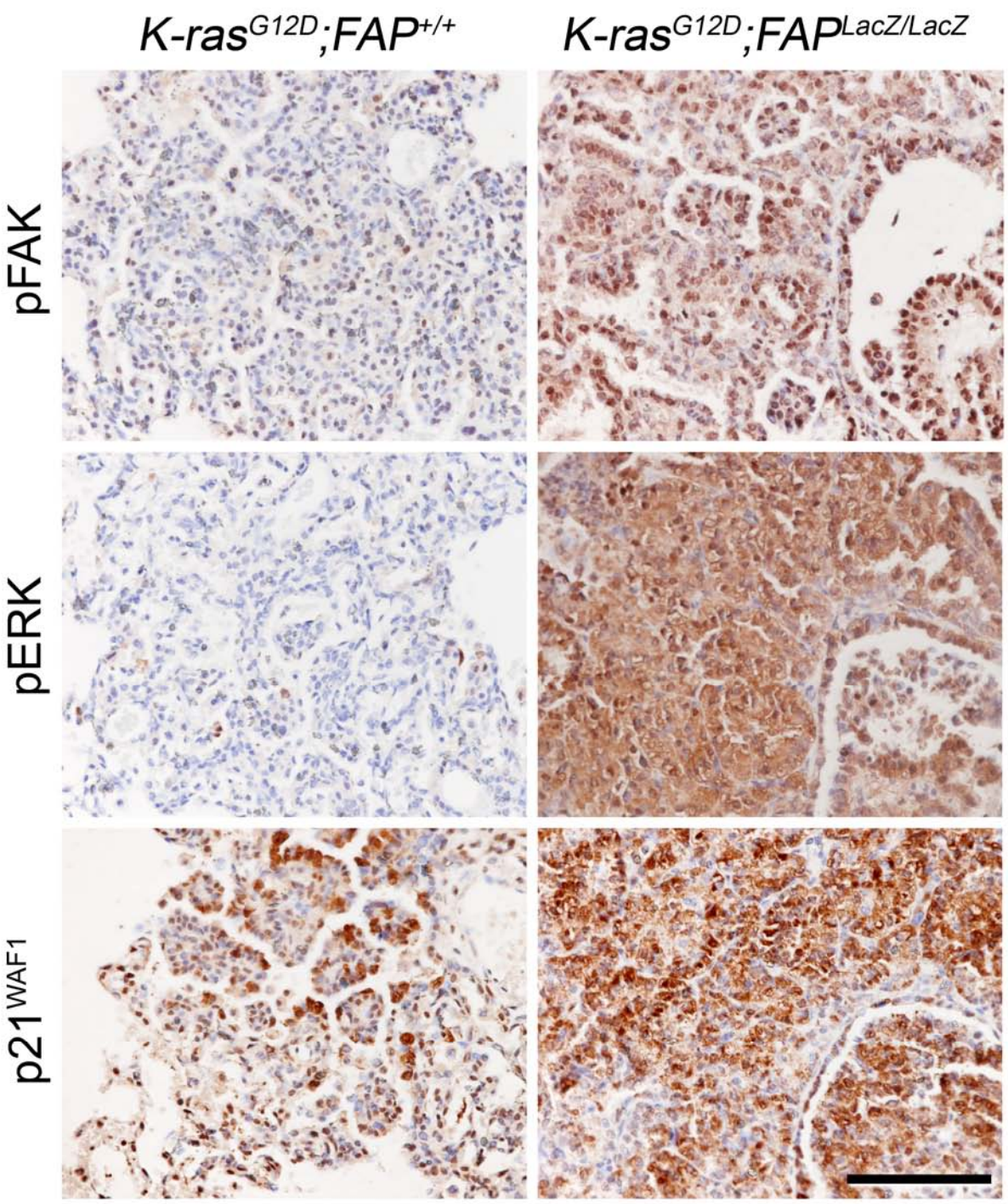
Supplement Figure 5. DPPIV stains non-tumor cells in tumor nodules and normal (N) uninvolved areas of lung but not tumor cells (arrows) in lungs from Ad-Cre infected *LSL-K-ras^{G12D};FAP$^{+/+}$*, *LSL-K-ras^{G12D};FAP$^{+/LacZ}$* and *LSL-K-ras^{G12D};FAP$^{LacZ/LacZ}$* mice. Top panel, magnification, 10x objective; bottom panel, magnification, 20x objective. Inset depicts a representative section stained with isotype matched control antibody; bar=100 µm.



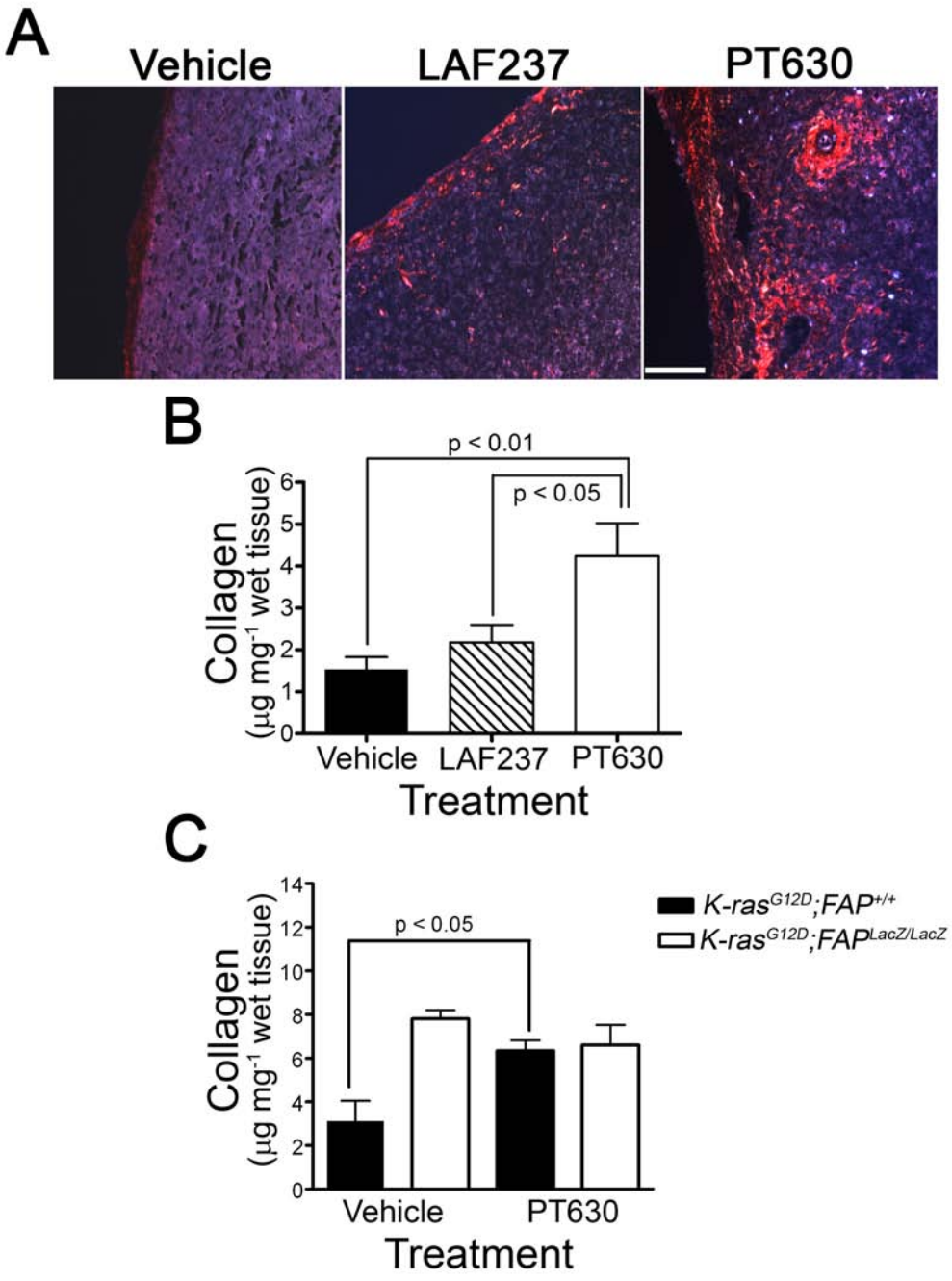
Supplement Figure 6. PT630 inhibits CT26 tumor growth in $FAP^{+/+}$ but not $FAP^{LacZ/LacZ}$ mice. CT26 tumor cells were injected s.c. in $FAP^{+/+}$ and $FAP^{LacZ/LacZ}$ Balb/c mice. Mice were randomized and treated with PT630 or vehicle control. Tumor size was measured using calipers (n=10-12 animals total per genotype, 5-6 per group in each of 2 independent experiments). **P< 0.001.



Supplement Figure 7. Inhibition of FAP enzymatic activity increases $p21^{WAF1}$ via ECM-mediated signaling through FAK, and ERK. Immunohistochemical **(A)** and immunoblot analysis **(B)** of total FAK and ERK1/2, phospho-FAK^{Y397} and phospho-ERK. **(A)** Representative photomicrographs of paraffin-embedded sections from CT26 treated tumors (n=10 tumors/group from two independent experiments). Magnification, 40x objective; bar=100 µm. **(B)** Total FAK and ERK1/2 were immunoprecipitated from total CT26 tumors extracts from treated animals followed by immunoblotting with the indicated antibodies (n=8 tumors). White vertical lines between lanes indicate that samples were run in non-consecutive lanes but on the same gel. **(C)** Quantification of $p21^{WAF1}$ by densitometry. Data represent means $\pm$ SE of 10 tumors per group.



Supplement Figure 8. Deletion of FAP increases p21^{WAF1} in LSL-K-ras^{G12D}-driven lung tumors via ECM-mediated signaling. Representative micrographs of immunohistochemical analysis of phospho-FAK, phospho-ERK and p21^{WAF1}. Magnification, 40x objective; bar=100 µm.



Supplement Figure 9. PT630 increases collagen disorganization and content. **(A)** Sections of CT26 treated tumors stained with Picro-Sirius red and visualized under polarized light. Magnification 40x objective; bar=80 µm. **(B)** Collagen content of CT26 tumors (50-60 mm2) from vehicle control, LAF237 and PT630-treated mice. Data represent means ± SE of 20 tumors per group. **(C)** Collagen content of lungs from drug treated Ad-Cre infected *LSL-K-ras^{G12D};FAP$^{+/+}$* and *LSL-K-ras^{G12D}; FAP$^{LacZ/LacZ}$* mice. Data represent means ± SE of 3-9 lungs per group. Collagen was quantified by measuring hydroxyproline.