Supporting Information

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SI Materials and Methods

Mice and Cell Culture. TRAMP (*Bl6x129*) mice (1) were backcrossed with FVB/N mice from the Jackson Laboratory for six generations. Only male littermates were used. *Hif-1α*$^{F/F}$ mice (FVB/N) were kindly provided by R. Johnson (Department of Biology, University of California, San Diego). Mice were maintained under specific pathogen-free conditions, and experimental protocols were approved by the University of California, San Diego, Animal Care Program, following National Institutes of Health guidelines. Myc-CaP cells derived from Myc (v-myc avian myelocytomatosis viral oncogene homolog)-induced prostate cancer (PC) in mice of FVB/N background (Hi-Myc) (2) were provided by C. Sawyers (Memorial Sloan Kettering Cancer Center) and were cultured under standard conditions and confirmed to be mycoplasma-free. Myc-CaP cells were s.c. injected into the flank of male FVB/N mice, as described (2). Tumor growth was measured with a caliper. Surgical procedures were described (3).

Human Specimens. Anonymous human prostate ($n = 5$), benign prostatic hyperplasia ($n = 4$), and PC ($n = 10$) paraffin-embedded blocks were provided by the Cooperative Human Tissue Network. Pathology reports were provided by the network for each sample.

Fibroblast Activation Protein Vaccination. Fibroblast activation protein (FAP) and TOPO (empty vector) vaccines were kindly provided by R.A. Reisfeld (Scripps Research Institute). The vaccines were propagated and mice were vaccinated as described (4).

Inhibitors. SB-431542 was purchased from Selleck Chemicals, and mice were i.p. injected at 10 mg/kg once a day as described (5). Pentoxifylline was purchased from Sigma-Aldrich. Sildenafil was kindly provided by Pfizer.

Cell Fractionation. Fibroblast, CD11b, CD11c, and epithelial cell fractions were isolated using the EasySep (Stem Cell Technologies) positive selection kit for CD140, CD11b, CD11c and a negative selection kit for mouse epithelial cells, respectively, according to manufacturer's instructions. Briefly, a single-cell suspension at a concentration of 1×10^8 cells/mL in PBS plus 2% (vol/vol) FBS were stained for the above-indicated PE-labeled cell markers. EasySep magnetic nanoparticles were added, and cells were collected by immunomagnetic isolation.

Histological Procedures. Mouse prostate and PC tissues were immersed in 10% (vol/vol) neutral buffered formalin before paraffin embedding and sectioning. Sections were stained and processed as described (3), using ApoTag Red TUNEL assay kit (Millipore) or antibodies for IκB kinase α (IKKα) (Imgenex), CXCL13 (R&D), mouse smooth muscle actin (SMA), SMAD2/3, FAP, IKKα (phospho T23) (Abcam), CD19 (eBioscience), B220, CD34 (BD Bioscience), CD20, CD3, and SMA (Dako). Hypoxyprobe-1 was purchased from Hypoxyprobe, Inc. Solid pimonidazole HCl was i.p. injected at 60 mg/kg 90 min before killing the mice, and its covalent adducts were stained according to the manufacturer's instructions. After immunohistochemistry or immunofluorescence, specimen sections were analyzed with a microscope (Axiovert 200; Carl Zeiss) equipped with Plan-Neofluar (10× NA 0.3, 20× NA 0.5, and 40× NA 0.95) objective lenses at room temperature. The imaging medium was air for both objective lenses used. The AxioCam 506 color camera (Carl Zeiss) and AxioVision software (Carl Zeiss) were used for image collection. Each set of stained sections was processed under identical gain and laser power setting and under identical brightness and contrast settings. The fluorochromes used were Alexa Fluor 488, 594, and DAPI. Image analysis of in situ hybridization/IHC-stained sections was performed using ImageJ software.

Analysis of RNA and Protein Expression. Total tissue RNA was prepared using RNAeasy (Qiagen). Quantitative PCR was as described (3).

Statistical Analyses. Results are expressed as means ± SEM or ± SD. Data were analyzed by Student *t*-test, using GraphPad Prism statistical program. Error bars depict SEM or SD. *P* values > 0.05 were considered insignificant, 0.01–0.05 was considered significant, 0.001–0.01 was considered very significant, and <0.001 was considered highly significant.

1. Greenberg NM, et al. (1995) Prostate cancer in a transgenic mouse. *Proc Natl Acad Sci USA* 92(8):3439–3443.
2. Watson PA, et al. (2005) Context-dependent hormone-refractory progression revealed through characterization of a novel murine prostate cancer cell line. *Cancer Res* 65(24): 11565–11571.
3. Ammirante M, Luo JL, Grivennikov S, Nedospasov S, Karin M (2010) B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature* 464(7286):302–305.
4. Loeffler M, Krüger JA, Niethammer AG, Reisfeld RA (2006) Targeting tumor-associated fibroblasts improves cancer chemotherapy by increasing intratumoral drug uptake. *J Clin Invest* 116(7):1955–1962.
5. Mikami F, et al. (2006) The transforming growth factor-beta-Smad3/4 signaling pathway acts as a positive regulator for TLR2 induction by bacteria via a dual mechanism involving functional cooperation with NF-kappaB and MAPK phosphatase 1-dependent negative cross-talk with p38 MAPK. *J Biol Chem* 281(31):22397–22408.

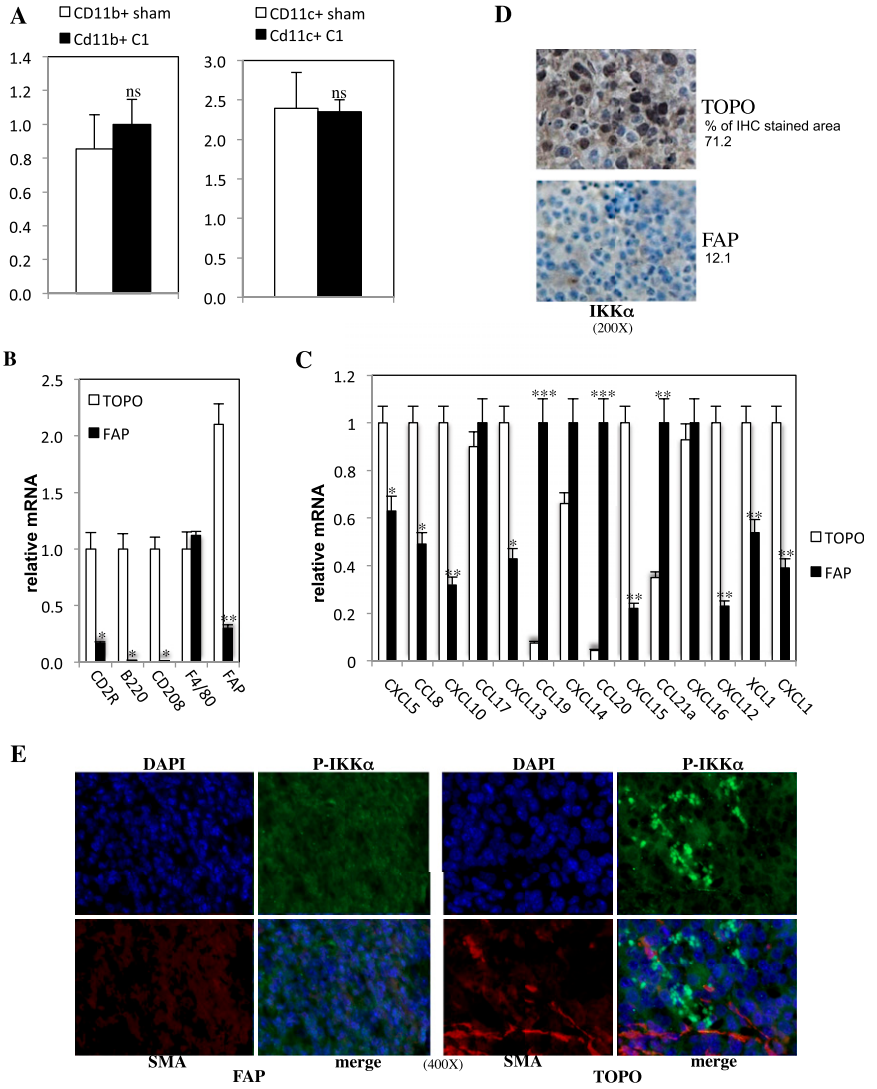

Fig. S1. (*A*) FVB/N mice (n = 10 per group) bearing Myc-CaP tumors were castrated or sham operated and killed 1 wk later. Tumors were removed and digested, and the $CD11b^+$ and $CD11c^+$ cell fractions were isolated, total RNA was extracted, and CXCL13 mRNA was quantitated by Q-PCR and normalized to cyclophilin A mRNA. Results are averages ± SD. (*B* and *C*) FVB/N mice (n = 10) were vaccinated with TOPO and FAP vaccines and Myc-CaP tumors were established as described in Fig. 1*D*. One week after castration, tumors were collected, total RNA was isolated, and expression of the indicated mRNAs was quantitated by Q-PCR, as described earlier, and normalized to cyclophilin A mRNA. Results are averages ± SD. (*D* and *E)* FVB/N mice (n = 10 per group) bearing Myc-CaP tumors were castrated or sham operated and killed 1 wk later. Tumors were analyzed for nuclear IKKα by IHC (*D*). (Magnification: 200×.) They were also analyzed for phosphorylated (P)-IKKα and SMA by IF. (Magnification: 400×.) The areas occupied by stained cells were determined as described earlier (see Fig. 1*A*).

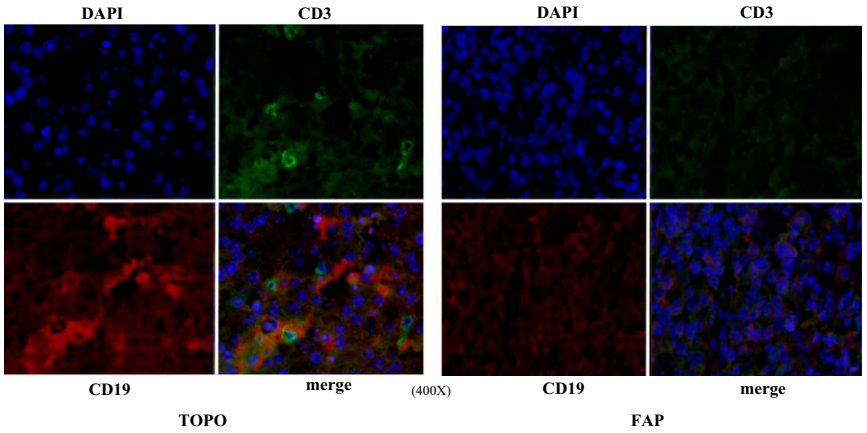

Fig. S2. Tumors from Fig. 1*D* were analyzed for the presence of $CD19^+$ and $CD3^+$ cells by IF. (Magnification: 400×.)

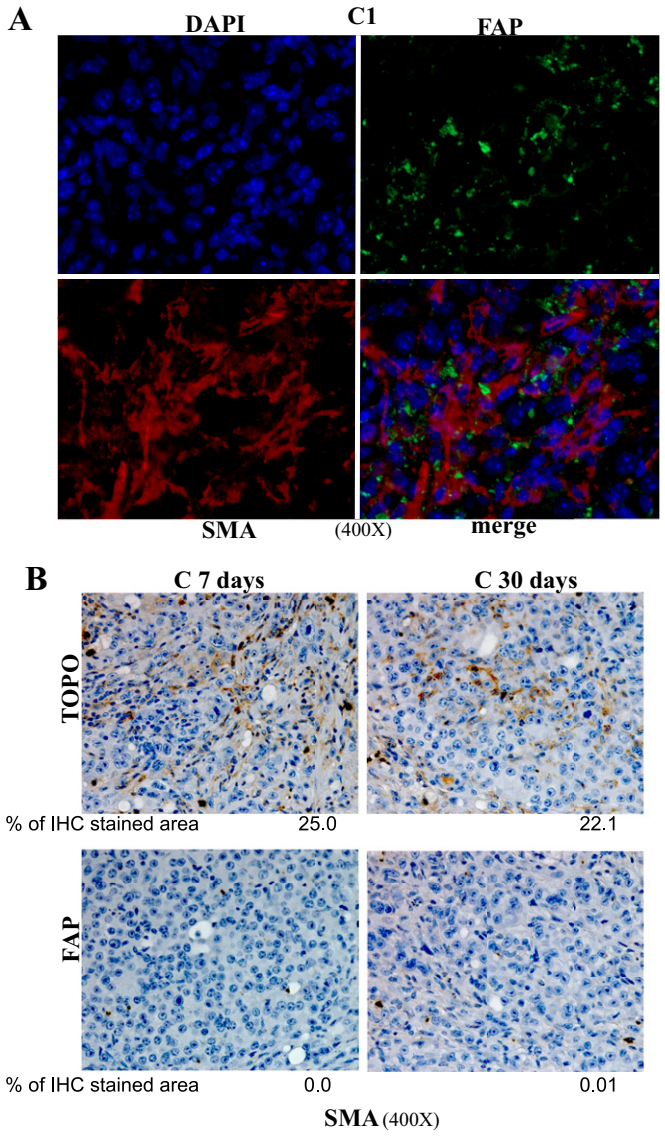

Fig. S3. (*A*) Tumors from Fig. 1*D* were analyzed for FAP and SMA expression by IF. (Magnification: 400×.) (*B*) Tumors were established as in Fig. 1*D* and were collected 1 wk and 30 d after the operation and IHC-analyzed for SMA expression. (Magnification, 400×.)

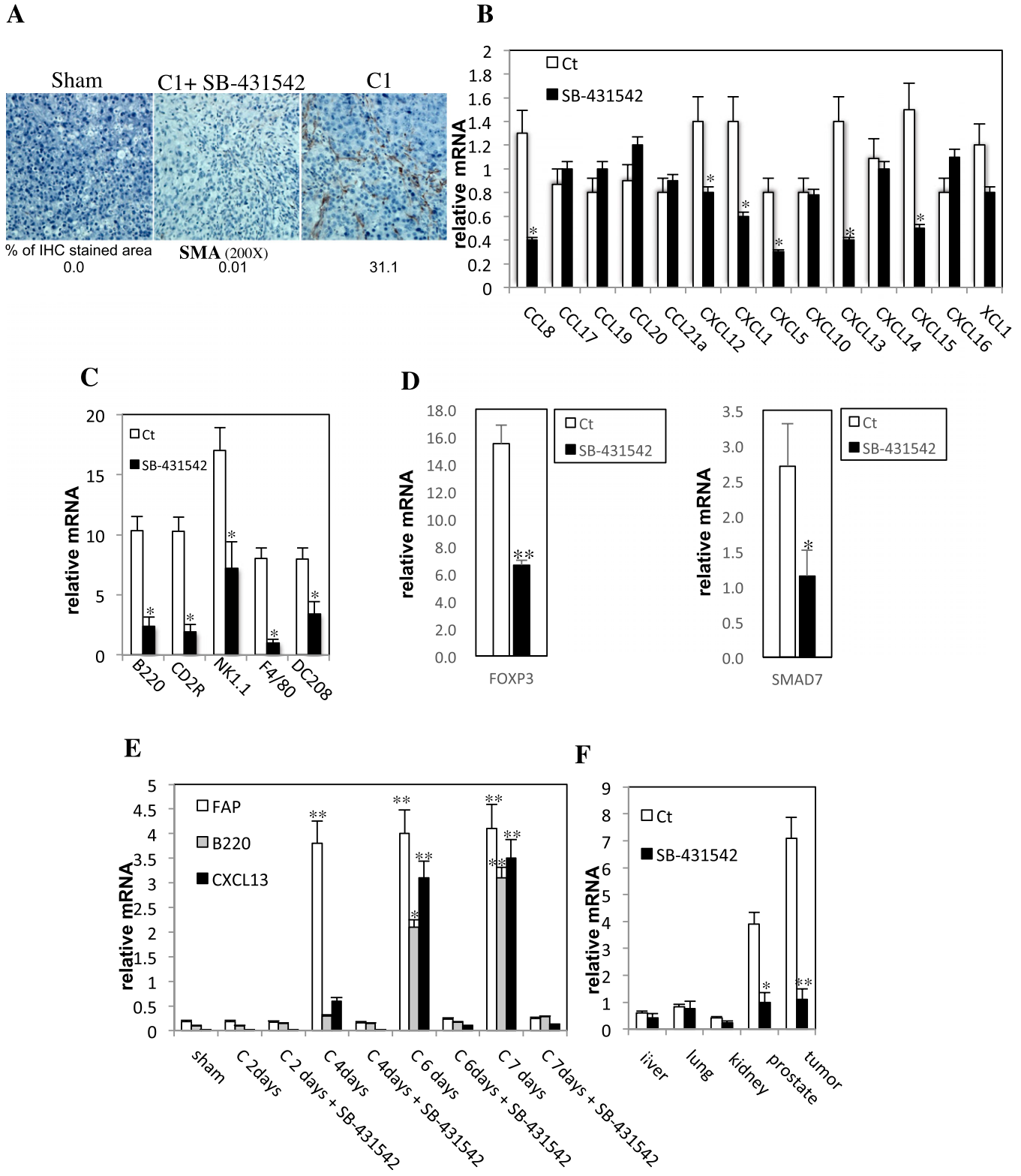

Fig. S4. FVB/N mice (n = 10) bearing Myc-CaP tumors were castrated or sham operated and i.p. injected with SB-431542 or vehicle, as described in Fig. 2*B*. (*A*) Tumors were collected 1 wk after the operation, paraffin embedded, sectioned, and IHC analyzed for SMA. (Magnification: 200×.) (*B*–*D*) Tumors were collected 1 wk after castration, RNA was extracted and the indicated mRNAs were quantitated by Q-PCR as described earlier. Results are averages ± SD. (*E*) Tumors were collected at the indicated points and the indicated mRNAs were quantitated and normalized as described earlier. Results are averages ± SD. (*F*) Tumors, liver, lungs, kidneys, and prostates were collected from Myc-Cap-inoculated FVB/N mice treated as described earlier, total RNA was isolated 1 week after castration, and SMA mRNA expression was quantitated as described earlier. Results are averages ± SD. Ct, control; FOXP3, forkhead box P3.

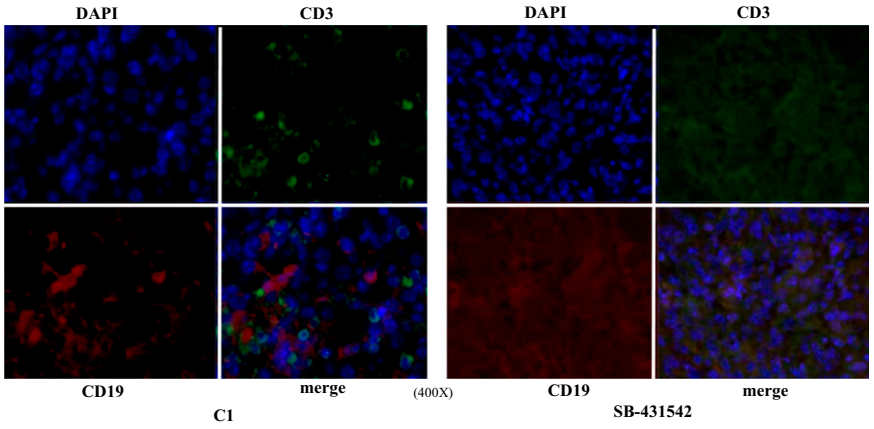

Fig. S5. Tumors from Fig. S3*A* were IF-analyzed for the presence of $CD19^+$ and $CD3^+$ cells. (Magnification: 400×.)

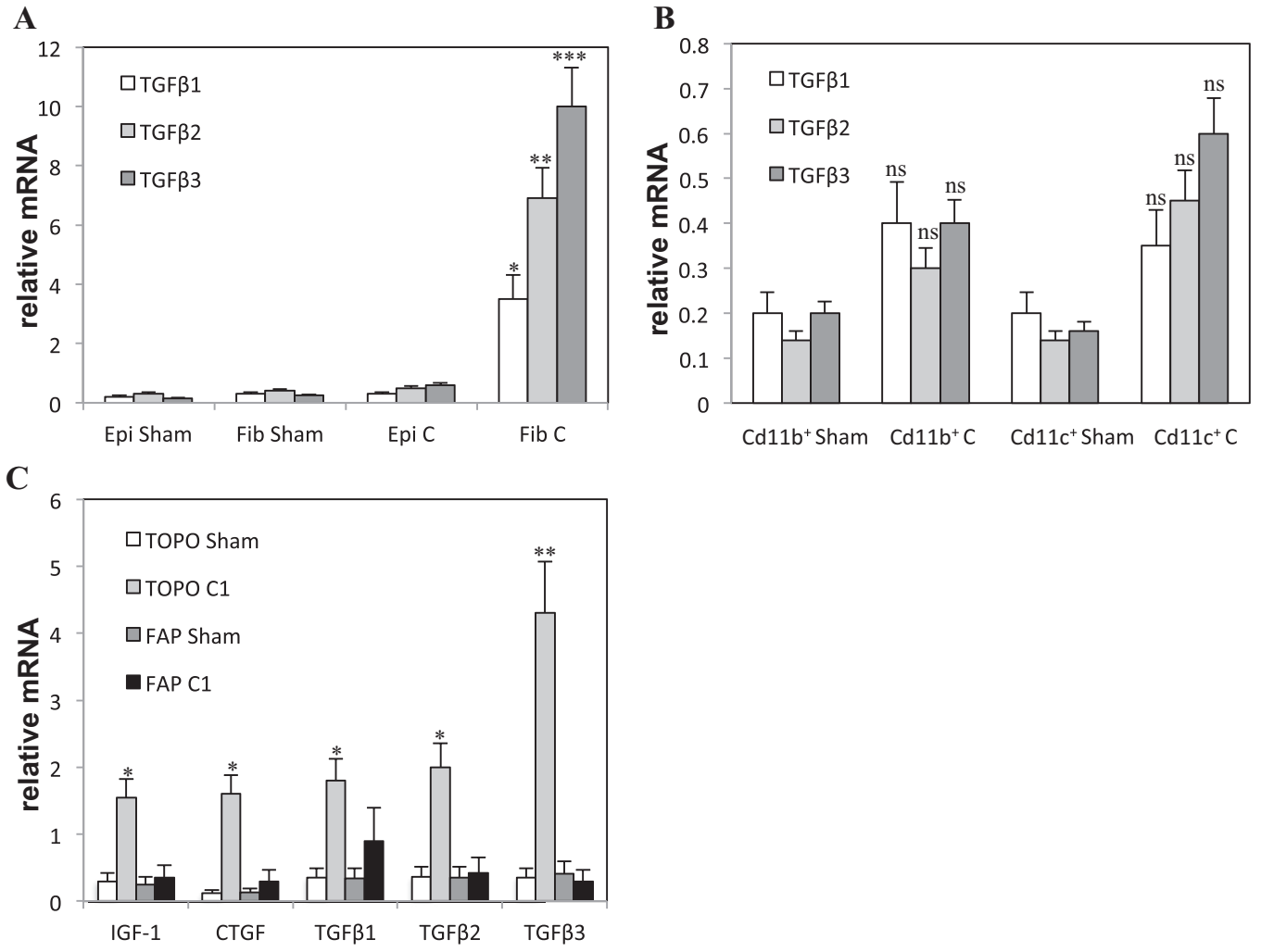

Fig. S6. (*A* and *B*) FVB/N mice ($n = 10$) bearing Myc-CaP tumors were sham operated or castrated (C), and tumors were collected 1 wk later. Fibroblasts (Fib), epithelial (Epi), $CD11b^+$, and $CD11c^+$ cells were isolated, total RNA was extracted, and TGF-β1, TGF-β2, and TGF-β3 mRNAs were quantitated by Q-PCR and normalized to cyclophilin A mRNA. Results are averages ± SD. (*C*) FVB/N mice ($n = 10$ per group) were vaccinated with TOPO or FAP vaccines, as in Fig. 1*B*, and Myc-CaP tumors were established. One week after castration, tumors were collected, total RNA was isolated, and expression of the indicated mRNAs was quantitated and normalized to that of cyclophilin A mRNA. Results are averages ± SD.

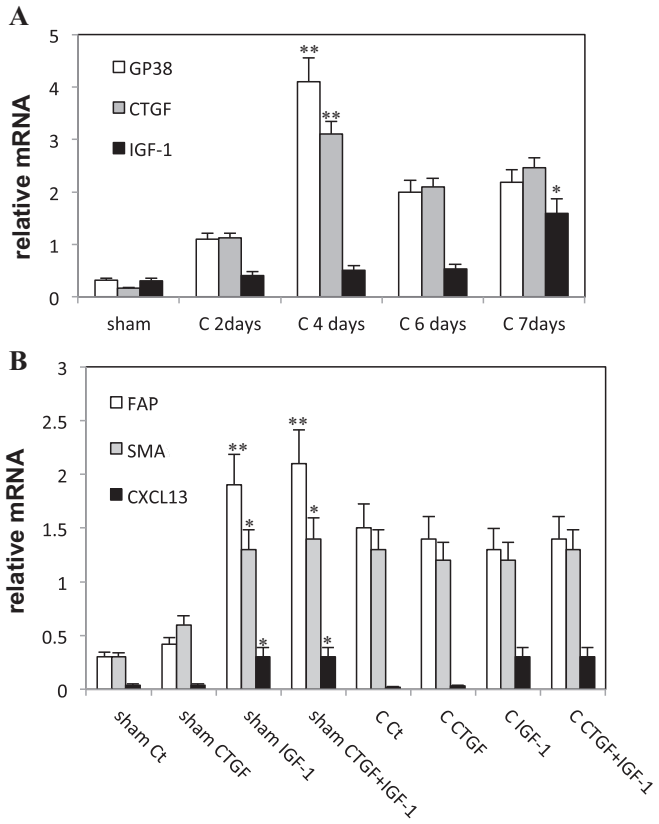

Fig. S7. (*A*) Myc-CaP tumors ($n = 10$) were established in 6-wk-old FVB/N males that were castrated or sham operated. Tumors were collected at the indicated points after operation, total RNA was isolated, and expression of the indicated mRNAs was quantitated and normalized to that of cyclophilin A mRNA. Results are averages ± SD. (*B*) Fibroblasts were purified from Myc-CaP tumors 1 wk after sham operation or castration (C) ($n = 10$ per group), plated, and stimulated for 24 h with connective tissue growth factor (CTGF; 50 ng/mL), insulin-like growth factor 1 (IGF1; 10 ng/mL), or CTGF plus IGF1. RNA was isolated, and expression of the indicated genes was analyzed as described earlier. Results are averages ± SD.

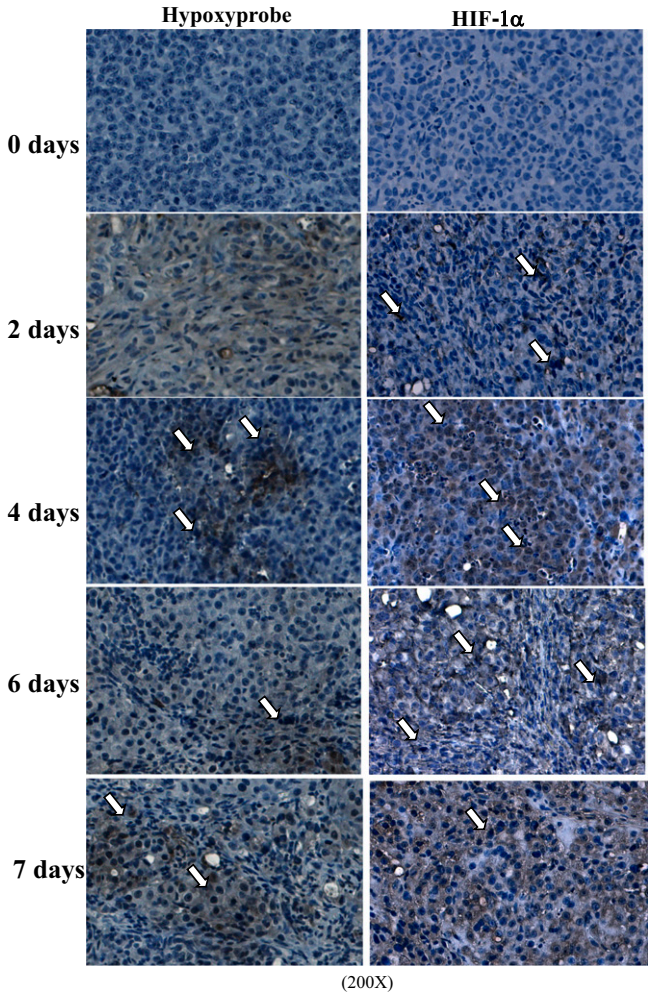

Fig. S8. Full-field image of Fig. 3*C*.

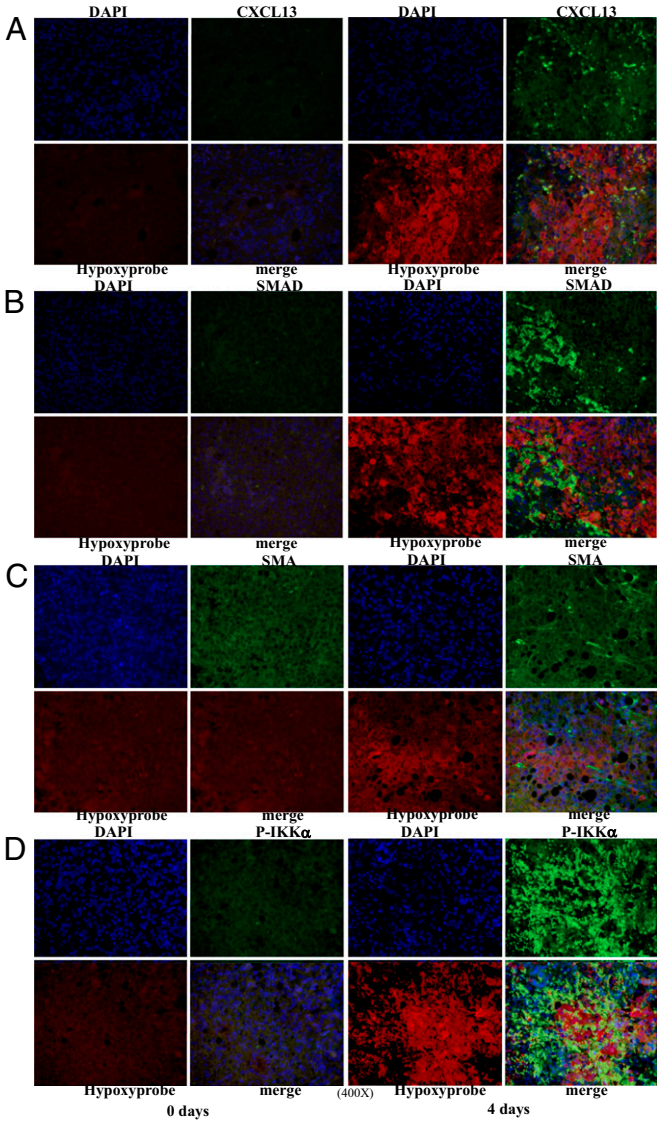

Fig. S9. (*A*–*D*) Tumors from Fig. 3*C* were stained with Hypoxyprobe and CXCL13 (*A*), SMAD (*B*), SMA (*C*), or phospho (P)-IKKα (*D*) antibodies. (Magnification: 400×.)

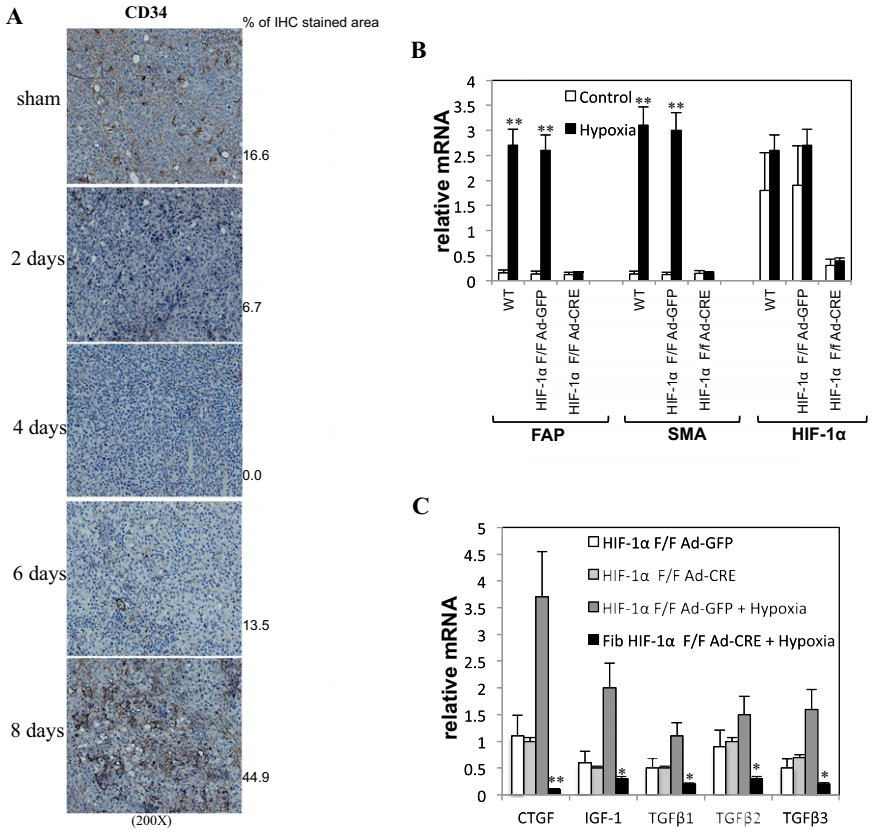

Fig. S10. (*A*) FVB/N mice bearing Myc-CaP tumors were castrated or sham operated, and tumors were collected at the indicated points after castration and stained for CD34 by IHC. (Magnification: 200×.) (*B*) Tumor-associated fibroblasts were purified from *Hif-1α*$^{F/F}$ mice bearing Myc-CaP tumors, plated, and infected with adenovirus-Cre (Ad-Cre) or adenovirus-GFP (Ad-GFP) and incubated in either a normal cell incubator or an hypoxic chamber (1% O_2) for 24 h. Total RNA was isolated, and expression of the indicated mRNAs was quantitated and normalized to that of cyclophilin A. Results are averages ± SD (n = 6). (*C*) Tumor-associated fibroblasts were isolated from *Hif-1α*$^{F/F}$ mice bearing Myc-CaP tumors, plated, infected with Ad-Cre or Ad-GFP, and cultured under normoxic or hypoxic conditions for 24 h. Expression of the indicated mRNAs was quantitated as described earlier. Results are averages ± SD (n = 6).

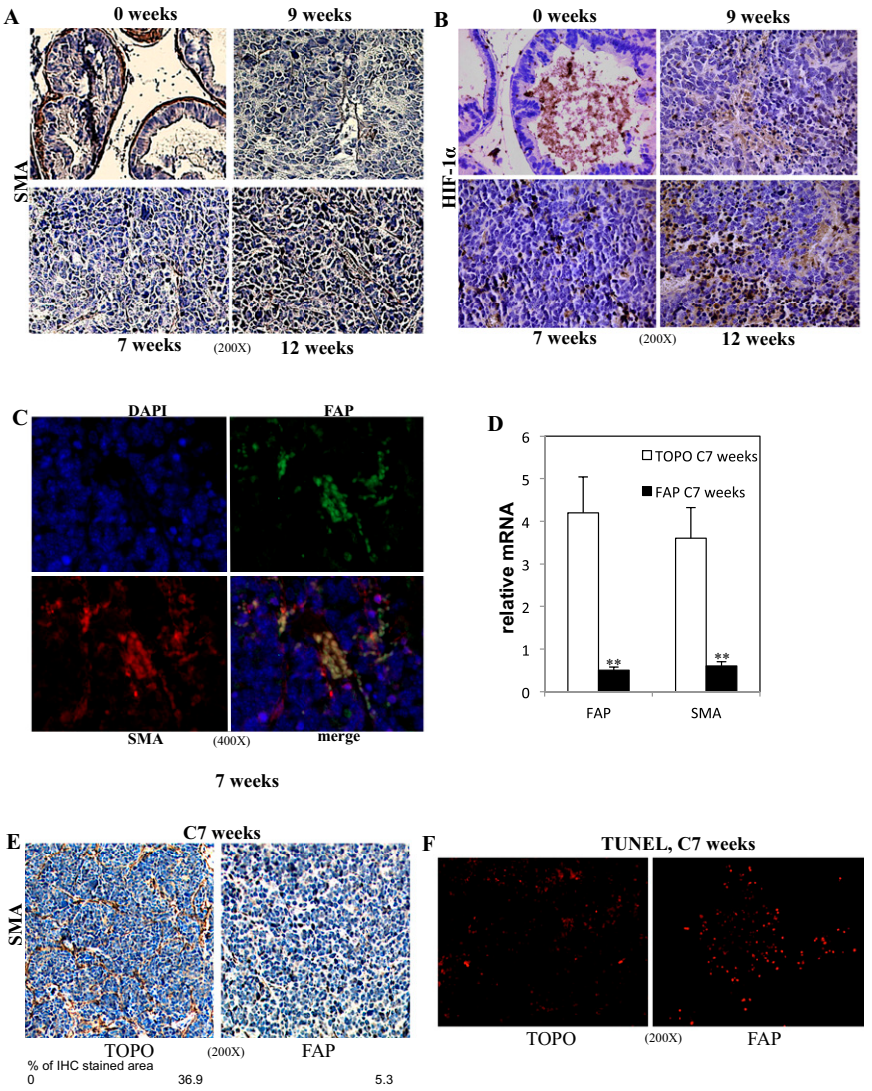

Fig. S11. (*A*–*C*) Twelve-week-old TRAMP mice ($n = 10$) were castrated or sham operated, and their tumors were collected at the indicated times after castration. Tumors were fixed, paraffin embedded, sectioned, and analyzed for SMA (*A*) and HIF-1α (*B*) by IHC (magnification: 200×) and for FAP and SMA by IF (magnification: 400×). (*D*) TRAMP mice ($n = 10$ per group) were vaccinated with TOPO or FAP vaccines, as described in Fig. 5*E*, and sham operated or castrated when 12 wk old, and tumors were collected 7 wk later and analyzed for FAP and SMA mRNA expression by Q-PCR. Results are averages ± SD. (*E*) Tumors were established, collected, and processed as in Fig. 5*E* and were analyzed for SMA expression by IHC 7 wk after castration. (Magnification: 200×.) The areas occupied by SMA^+ cells were determined as described earlier. (*F*) The same tumors were also analyzed by TUNEL staining (Magnification: 200×.)

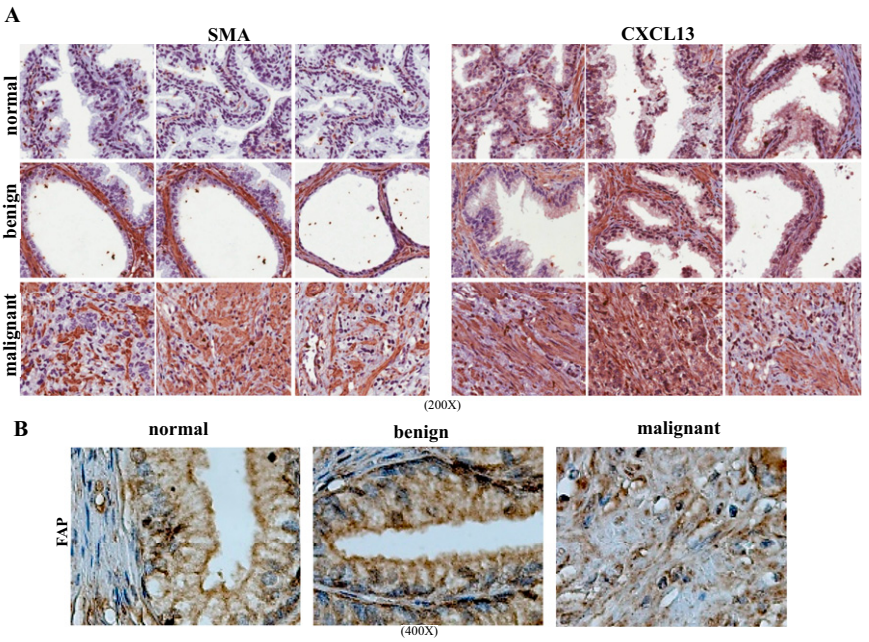

Fig. S12. (*A*) An additional 3 cases for each normal, benign, and malignant human prostate sample, as described in Fig. 6*A*, were analyzed for SMA and CXCL13 expression by IHC. (Magnification: 200×.) (*B*) Tumors from Fig. 6*A* were analyzed by IHC for FAP expression. (Magnification: 400×.)