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In vivo CRISPR inactivation of Fospromotes prostate cancer progression by altering the associated AP-1 subunit Jun

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Abstract

Prostate cancer is a major global health concern with limited treatment options for advanced disease. Its heterogeneity challenges the identification of crucial driver genes implicated in disease progression. Activating protein-1 (AP-1) transcription factor is associated with cancer since the first identification of its subunits, the proto-oncogenes JUN and FOS. Whereas both *JUN* and *FOS,* have been implicated in prostate cancer, this study provides the first functional evidence that *FOS* acts as a tumor suppressor during prostate cancer progression and invasion. Data mining revealed decreased *FOS* expression in prostate cancer and a further downregulation in metastatic disease, consistent with *FOS* expression in cell lines derived from different prostate cancer stages. FOS deficiency in prostate cancer cell lines increases cell proliferation and induces oncogenic pathway alterations. Importantly, *in vivo* CRISPR/Cas9-mediated *Fos* and *Pten* double mutation in murine prostate epithelium results in increased proliferation and invasiveness compared to the abrogation of Pten alone. Interestingly, enhanced Jun expression is observed in the murine prostatic intraepithelial neoplasia lacking Fos. CRISPR/Cas9-mediated knockout of *Jun* combined with *Fos* and *Pten* deficiency diminishes the increased proliferation rate *in vivo,* but not the ability

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to form invasive disease. Overall, we demonstrate that loss of Fos promotes disease progression from clinical latent prostate cancer to advanced disease through accelerated proliferation and invasiveness, partly through Jun.

Introduction

Prostate cancer (PCa) is a paradigm of age-related cancers. It currently ranks as the second-most diagnosed cancer in men worldwide [1]. Even though most prostate cancers display an indolent phenotype, metastatic PCa is a lethal disease with limited treatment options [2,3]. PCa is also a prime example of heterogeneity and multifocality [4,5]. Therefore, the identification of genetic alterations involved in tumorigenesis and the evaluation of their phenotypic consequences *in vivo* is crucial to a better understanding of PCa progression.

The activating protein-1 (AP-1) transcription factor is implicated in a plethora of biological processes, maintaining a fine equilibrium in cell homeostasis [6,7]. The dimeric complex comprises basic region-leucine zipper (bZIP) proteins of the JUN, FOS, ATF, and MAF multigene families. In cancer, most of the AP-1 subunits are considered as either oncogenes or tumor suppressors. For instance, JUN and FOS often exhibit oncogenic properties, while JUND and JUNB show tumor-suppressive activity [8]. JUN, FOS, JUNB and JUND are implicated in PCa, together with JUN N-terminal kinase (JNK) [9–14]. However, FOS has been reported to be up- and downregulated in PCa [9,15,16] and defining its function, whether oncogenic or tumor suppressor, awaits functional assessment.

Genetically engineered mouse models (GEMM) are essential to decipher the implication of a gene products in prostate cancer [17]. Technical and methodical improvements such as CRISPR/Cas9 gene editing allows a fast validation of new driver genes in cancer research [18,19]. The present study introduces a novel PCa mouse model based on CRISPR/Cas9 technology and orthotopic viral delivery of multiplexed sgRNAs targeting *Fos, Jun,* and *Pten*. This method ensures simultaneous, multiplexed gene editing in adult mice, bypassing timely breeding schemes and allowing tumor cells to clonally expand.

We implemented CRISPR/Cas9 technology *in vivo* to analyze the role of the AP-1 subunit Fos in prostate cancer progression. By inactivating Fos and Pten simultaneously, we show that loss of Fos promotes Pten-deficient PCa progression from benign prostatic intraepithelial neoplasia (PIN) lesions to carcinoma, characterized by increased proliferation and local invasion. *In vitro* analyses revealed alterations in multiple cancer-related pathways following the loss of FOS. We further observed an upregulation of Jun/AP-1 upon Fos deletion and mice triple deficient for Pten, Fos, and Jun in the prostate epithelia displayed diminished tumor growth. Overall, our data indicate that Fos is a tumor suppressor in PCa and that alteration of Fos/AP-1 in prostate epithelium accelerates cancer progression by increasing proliferation and invasiveness, two hallmarks of cancer progression.

Methods

Human gene expression datasets

Overall survival data for FOS expression were obtained from the Human Tissue Atlas (n=483). FOS expression in non-cancer tissues vs. prostate adenocarcinoma was analysis from TCGA data by GEPIA (n= 52 normal, 497 tumorous). The following publicly available datasets were used for FOS expression in metastasis: GSE6919 (18 healthy controls, 64 primary PCa samples, 24 metastatic PCa samples)[16,20], GSE3933 (41 healthy controls, 34 primary PCa samples, 28 metastatic PCa samples, 9 metastases samples)[21]. JUN expression in non-cancer tissues vs. prostate cancer was analyzed from TCGA data by GEPIA.

Cell culture

Prostate cell lines BPH-1, PC-3 and DU145 were cultured in RPMI-1640 supplemented with 10% FBS and 1% penicillin–streptomycin and incubated at 37°C in humidified atmospheric air with 5% CO_2. Cells were repeatedly tested negative for mycoplasma contamination.

sgRNA design and cloning strategies

Human and murine single guide RNAs (sgRNA) were designed with web tools offered by either CRISPOR [22] or by Synthego. Two to three best-ranked sgRNAs based on specificity, out-of-frame and off-target scores were chosen for further validation. Human sgRNAs targeting *FOS* were cloned into the LentiCRISPRv2 plasmid (Addgene, #98290). Murine sgRNAs targeting *Pten, Fos* or *Jun* were cloned into the GFP-expressing pX458 plasmid (Addgene, #48138). Validation of guide RNA efficiency was performed in MEF cells and visually evaluated based on GFP expression rate. Following validation, murine sgRNAs were cloned into an AAV backbone plasmid with Cre expression (Addgene, #60229). WPRE and hGH poly(A) were removed and replaced by a synthetic poly(A) signal in the interest of space optimization. The plasmid carrying a sgRNA targeting only Pten was used as control (AAV9-Pten). In further cloning steps, sgRNAs targeting Fos and Jun were cloned into the plasmid (AAV9-Pten/Fos; AAV9-Pten/Fos/Jun) to achieve simultaneous gene editing. All sgRNAs were under the control of a U6 promoter. For gRNAs and primers sequence, see table S1.

Animals

Animal experiments were individually reviewed and approved by the Institutional Animal Care and Use Committee (2016-15-0201-01083). Animals were kept in pathogen-free facilities, according to institutional guidelines. ROSA26-LSL-Cas9/EGFP mice (Jax Lab, Cat No. 26175) express Cas9 and EGFP upon Cre-recombinase exposure and allow visualization of transduced cells. Details on animal procedures are described in Supplementary methods.

Histology and Immunohistochemistry

Histological sections were cut from FFPE tissue (4μm). Antigen retrieval was carried out at 100°C in citrate buffer with a pH 6 or in a Tris buffer pH 9 for 20min. Endogenous

peroxidase was blocked by incubation with 0,3% hydrogen peroxide for 5 minutes. Sections were then blocked in 2.5% BSA in TBS containing 0.1% tween20. The following primary antibodies were used: phospho-AKT (CS4060), Jun (CS9165), Ki67 (ab16667), Fos (sc-271243), GFP (CS2956) and p63 (SC-8431). Counterstaining was performed using hematoxylin. H&E stainings were performed using standard protocols.

Details of AAV production, PET/MR imaging, DNA, RNA and Protein isolation, Real-time quantitative PCR, Sanger sequencing and Indel analysis, Immunoblotting and RNA-Sequencing are described in Supplementary methods.

Statistical analyses

A Log-rank (Mantel-Cox) test was used for statistical analysis of the Kaplan-Meier survival curve. An unpaired t-test was used for analysis of two groups by using GraphPad Prism. A p-value ≤ 0.05 was considered statistical significance between the two groups.

Results

FOS is downregulated in advanced human prostate cancer

FOS/AP-1 transcription factor has been associated with prostate cancer and described either to be up- and downregulated in the tumor [9,15,20]. FOS is rarely found amplified or deleted in prostate cancer, suggesting that FOS expression is transcriptionally regulated (Fig S1). To evaluate the impact of altered FOS expression in PCa, we analyzed survival data of 483 PCa patients provided by the Human Tissue Atlas and found a positive correlation between high FOS mRNA (Fig 1A). Further analysis of different publicly available PCa data sets revealed that FOS is downregulated in primary PCa and further decreased in metastatic PCa, when compared to healthy controls or primary tumors (Fig 1B, C). Biopsies of common metastatic sites, such as lymph nodes, show a similar downregulation (Fig 1D). We next analyzed *FOS* mRNA expression in three different prostate cell lines. The benign cell line BPH1 had significantly higher expression of FOS compared to the metastatic cell lines PC3 and DU145 (Fig 1E). Overall, the human transcriptome analysis, in line with the *in vitro* data, provide evidence that FOS is implicated in PCa as a possible tumor suppressor, affecting patient overall survival.

FOS inactivation in human cancer cell lines elicits an oncological phenotype

To investigate the implication of decreased FOS expression in PCa, we generated *FOS* knock out clones in the benign human prostate cell line BPH1 by CRISPR/Cas9 gene editing. Sanger sequencing and insertion/deletion (Indel) analysis, followed by Western blot analysis (Fig 2A, B, C), validated successful KO. *FOS* KO cell clones proliferated faster than wild-type BPH1 cells (Fig 2D). RNA sequencing of BPH1 wild-type cells and *FOS* KO clones followed by gene set enrichment analysis revealed several cancer–related pathway alterations in *FOS* KO clones (Fig S2). This includes the upregulation of pathways related to androgen-regulated genes, Kras activation, JAK-STAT-signaling and epithelial-mesenchymal transition (Fig S2, S3). This analysis revealed that FOS is implicated in essential pathways in prostate epithelial cells.

We then focused our attention on specific targets with high gene expression alterations, as well as other AP-1 family members (Fig 2E, S2B). qRT-PCR confirmed the transcriptional upregulation of FGFR1, IRF1, and the AP-1 target MMP7. Downregulation could be confirmed for SPRR3 and DSP (Fig S4A). Elevated protein levels of FGFR1 and IRF1 was also observed by Western blot (Figs S4B). Taken together, these results indicate that the loss of FOS activity alters a cascade of crucial pathways in human PCa cells, modulating and promoting disease progression. The lack of FOS initiates expression changes that are well-described in other cancers and activates oncogenic signaling pathways associated with cancer aggressiveness and invasion.

CRISPR/Cas9-mediated *in vivo* gene editing of *Fos* and *Pten* in prostate epithelium

To investigate the role of *FOS* in PCa progression *in vivo*, we generated a CRISPR/Cas9-mediated *Fos* knock out (KO) in the prostate epithelium of adult ROSA26-LSL-Cas9/EGFP mice. Since the loss of *Fos* alone is not sufficient to induce prostate cancer [23], *Pten* was simultaneously targeted by a single guide RNA (sgRNA) to ensure hyper-proliferation of the prostatic tissue and PCa initiation [11,19,24,25]. Adeno-associated viral vectors harboring a Cre recombinase and sgRNAs targeting *Fos* and *Pten* were constructed (Fig 3A). Viral vectors carrying Cre recombinase and a sgRNA targeting only *Pten* served as a control, and AAV serotype 9 was chosen based on previously described superior features, such as a broad cell tropism [26].

Orthotopic AAV injections into the anterior lobe allow the transduction of only a subset of prostate epithelial cells [19]. The viral particles can circulate within the organ and also transduce cells in the dorsolateral and ventral prostate lobes (Fig S5A). AAV particles expressing only Cre or YFP protein were not sufficient to induce prostate cancer (Fig S5B). In contrast, virus expressing Cre and sgRNAs targeting either *Pten* or *Pten* and *Fos* induces hyperplastic lesions. Edited lesions can be visualized by GFP expression following the stop cassette deletion in the ROSA-LSL-Cas9/EGFP allele (Fig 3B) [18]. Three months post-injection, isolated prostates of $Pten^{\Delta P}$ and $Fos^{\Delta P}/Pten^{\Delta P}$ mice demonstrated a strong GFP signal, prostate enlargement and an increase in tissue density when compared to age-matched, untreated controls (Fig 3B). Nine months post-injection, macroscopic tumors were observed in both groups (Fig 3B). Disease progression was followed by PET/MR imaging using an ^{18}[F]-fluorodeoxyglucose (FDG) tracer to visualize increased glucose uptake (Fig S6). Quantification of the prostate tumor volume at the timepoint of termination showed further enlargement when Fos and Pten were depleted, compared to only Pten-deficiency (Fig 3C). To confirm CRISPR-mediated KO mutations, gene editing was investigated by Sanger sequencing. Prostate biopsies obtained from $Pten^{\Delta P}$ and $Fos^{\Delta P}/Pten^{\Delta P}$ mice had efficient *in vivo* gene editing with knockout efficiencies up to 80% (Fig 3D, S7) and demonstrated a comparable insertion or deletion (Indel) distribution for the target genes (Fig S7D). Indel formation in *Fos* and *Pten* in $Fos^{\Delta P}/Pten^{\Delta P}$ mice occurred with similar frequencies, confirming the simultaneous KO of both genes (Fig 3E). Screening of five tumors from each group indicated the absence of off-target effects of CRISPR mediated mutations (Fig S7E). Thus, we successfully induced CRISPR/Cas9-mediated gene knockouts of either *Pten* or *Pten* in combination with *Fos* in the prostate epithelium. Notably,

combined knockout of *Pten* and *Fos* resulted in significantly larger tumors, when compared to $Pten^{\Delta P}$ mice, demonstrating a tumor suppressor function of *Fos* during PCa progression.

Loss of Fos in the murine prostate epithelium is associated with invasive cancer

To further characterize the enlarged prostate tumors isolated from *Fos*-deficient mice, histological examination was performed. Tissue sections of murine prostates obtained at 9 months post-injection showed mouse prostatic intraepithelial neoplasia (mPIN) in the anterior (AP) and dorsal-lateral prostate (DLP) in both $Pten^{\Delta P}$ and $Fos^{\Delta P}/Pten^{\Delta P}$ mice (Fig 4A). Epithelial cells exhibited atypical proliferation with the characteristic cribriform growth pattern of murine PCa and foci of nuclear atypia and altered stroma were observed for both genotypes (Fig S8). Increased phospho-AKT activity was also seen in abnormal areas, indicating that Pten has been inactivated and clonal expansion of transformed cells has been induced (Fig 4A). Immunohistochemistry for Fos visualized positive cells in Pten-deficient mice, co-localized with increased phospho-AKT. Fos staining was absent in $Fos^{\Delta P}/Pten^{\Delta P}$ validating the loss of Fos protein expression after *in vivo* CRISPR gene editing (Fig 4A). Staining for Ki67 showed an enhanced proliferation rate in $Fos^{\Delta P}/Pten^{\Delta P}$ mice compared to $Pten^{\Delta P}$ mice after 3 and 9 months (Fig 4B), consistent with an increased tumor volume (Fig 3C). Strikingly, while $Pten^{\Delta P}$ mice showed hyperproliferation directed into the lumen and an intact gland architecture, some $Fos^{\Delta P}/Pten^{\Delta P}$ mice displayed locally invasive cancer with epithelial cells invading the nearby stroma. Invasive epithelial cells in $Fos^{\Delta P}/Pten^{\Delta P}$ mice were positive for phospho-AKT and negative for Fos (Fig 4C, S9). Invasive foci demonstrated a loss of the basal cell marker p63 (Fig 4D) and invading cells were GFP-positive as a result of Cre expression upon viral delivery (Fig 4D). These findings confirmed that the invasive cells were initially target by CRISPR/Cas9. In total, 7 out of 30 mice showed progression to invasive carcinoma in DLP when both *Fos* and *Pten* were knocked out in prostate epithelium 9 months post-injection, while no invasive events were observed in mice with single *Pten* knockout (Fig 4E). In conclusion, the loss of the AP-1 transcription factor subunit Fos increases tumor size of Pten-deficient lesions and drives PCa progression towards invasive disease.

Increased Jun expression with loss of Fos in murine and human prostate cancer

Changes in AP-1 gene expression were observed when FOS was depleted in human BPH1 cells, including upregulation of JUN. To address if Jun levels were changed *in vivo* in murine prostate we performed histological analysis. Increased Jun staining was observed in transformed lesions compared to healthy prostate tissue, where it was restricted to a few basal cells, and this increase was further pronounced in Fos-deficient samples (Fig 5A). Furthermore, an similar increase in phospho-ERK was observed (Fig 5A). High Jun and p-ERK were also observed when FOS was inactivated by CRISPR in the human metastatic prostate cell line DU145 (Fig 5B, C). The changes in these pro-oncogenic pathways are consistent with accelerated cell proliferation (Fig 5D). JUN expression analysis in a cohort of non-cancer prostate samples and tumor biopsies revealed increased expression in prostate cancer (Fig 5E). Taken together, these results indicate that JUN upregulation is a conserved event in mouse and human prostate cancer, in particular when FOS is lost.

Increased Jun levels drive proliferation in mice with compound knockout of *Pten* and *Fos*

To evaluate the role of Jun in the progression towards aggressive disease, we generated an *in vivo* model with a simultaneous knockout of *Jun*, *Fos* and *Pten* (hereafter $Jun^{\Delta P}/Fos^{\Delta P}/Pten^{\Delta P}$). Histological analysis of tissue sections from triple knockout mice showed loss of Jun in edited foci with remaining Jun positive cells in unedited tissue areas (Fig 6A). Successful *in vivo* gene editing was further validated by Sanger sequencing, followed by Indel analysis (Fig 6B). Of note, the *Jun* KO efficiency appeared to be lower than for *Fos* and *Pten*. This could indicate negative selection for cells with a knockout of both AP-1 transcription factors. Consistently, CRISPR KO efficiency was higher in prostate samples from 3 months post-injection than 9 months in this setting (Fig 6B). $Jun^{\Delta P}/Fos^{\Delta P}/Pten^{\Delta P}$ mice displayed decreased proliferation, illustrated by the reduction of Ki67 positive cells (Fig 6C) and a tendency towards reduced tumor size compared to $Fos^{\Delta P}/Pten^{\Delta P}$ mice (Fig 6D). However, triple deficient prostate samples had a similar incidence of micro-invasive events, as observed in $Fos^{\Delta P}/Pten^{\Delta P}$ mice (Fig 6E). These data demonstrate that enhanced Jun expression plays a role in the altered proliferation of the prostate tumor with compound KO of *Fos* and *Pten* but that the loss of Fos drives prostate cancer invasion independently of Jun (Fig 7).

Discussion

In this study, we analyzed the function of the AP-1 transcription factor FOS in PCa initiation and the progression to invasive disease. By combining data obtained from patient transcriptome records, *in vitro* analyses and a novel CRISPR/Cas9-driven mouse model, we demonstrate that *Fos*-deficiency in the prostate epithelium drives invasive prostate carcinoma. Cancer progression in the absence of Fos was partly driven by the upregulation of Jun (Fig 7). We thus show a close interaction between different AP-1 transcription factors in cancer progression.

In our CRISPR/Cas9 mouse model, tumorigenesis is initiated by clonal expansion of single, edited cells that face natural selection pressure. Based on this model, we could show that the additional deletion of *Fos* in *Pten*-deficient epithelial cells led to increased proliferation and local invasion when compared to *Pten* KO tumors. Tumorigenesis occurs alongside a natural selection process of favorable and unfavorable mutations in our mouse model. We did not find any tumors without loss of Pten and elevated p-Akt levels. This indicates that neither the loss of Fos alone, nor Fos and Jun double-deficiency is sufficient to drive tumorigenesis. This is in agreement with previous observations, where loss of AP-1 subunits in the prostate epithelium failed to initiate prostate cancer. Additional mutations, such as the loss of Pten, have to be present [11,23]. The slightly lower CRISPR-KO efficiency rate observed in $Fos^{\Delta P}/Pten^{\Delta P}$ compared to $Pten^{\Delta P}$ mice could indicate less efficient gene editing when multiple mutations are generated by CRISPR compared to targeting a single gene. In $Jun^{\Delta P}/Fos^{\Delta P}/Pten^{\Delta P}$ mice, we observed similar Indel frequency as in *Fos* and *Pten*, but the Indel frequency was diminished for *Jun*, especially in samples obtained 9 months post-injection. Though, samples that were isolated 3 months post-injection demonstrate higher efficiency rates. Therefore, we speculate that a positive selection for tumors with unscathed Jun activity occurs during tumor progression. This further suggests an oncogenic function of

Jun in prostate cancer progression, which is in agreement with an increased proliferation index. Ultimately, these results show that CRISPR/Cas9-induced tumors reflect a degree of natural selection during tumor evolution.

Numerous oncogenic signaling pathways intersect with AP-1 transcription factor complexes, and their target genes are implicated in proliferation, invasion and metastasis [8,27]. FOS is primarily associated with a pro-oncogenic activity, overexpression in tumor cells and poor overall survival [15,28]. Here, we demonstrate a tumor suppressor function of FOS in PCa with decreased expression in primary cancer, which is more prevalent in metastatic disease. Consequently, we show that FOS expression is significantly diminished in metastatic PCa cell lines compared to the benign hyperplasia cell line BPH1. This downregulation in advanced prostate disease has also been reported for other AP-1 subunits, such as JUNB [11] and the c-Jun N-terminal kinase (JNK) [13]. Loss of JunB in the prostate epithelium, in combination with Pten inactivation, drives benign prostate lesions to invasive prostate cancer [11]. Here we have shown that loss of Fos also facilitates an invasive prostate cancer, and JunB and Fos may form dimers in prostate epithelial cells to maintain a non-invasive phenotype. Interestingly, only once before Fos was functionally shown to be a tumor suppressor and this was in murine rhabdomyosarcoma when combined with mutated *Trp53* [29]. We observed here a tumor suppressor function of *Fos* in combination with loss of Pten, another strong tumor suppressor gene. These results indicate that while primarily acting as an oncogene, Fos can switch to a context-specific, “cooperative” tumor suppressor. Whether this cooperativity extends to other tumor suppressor genes in other cancer types needs further exploration.

Appropriate activation of AP-1 transcription factor complexes depends on a finely-tuned balance between every single member. Even though some of the dimeric subunits might share similar functions [8,30], the loss of one factor affects expression of other subunits. The altered expression of one subunit will not only influence the expression of other AP-1 members but also their downstream target genes, resulting in a complex phenotype. Among other deregulations, we observed that loss of FOS increased JUN expression. JUN upregulation is implicated in various human cancers [8,9] and reciprocal regulation of Jun and Fos has been observed in the context of liver regeneration (EF Wagner, unpublished) and liver cancer [31]. Several JUN target genes are involved in cell proliferation and survival. JUN, for instance, downregulates the tumor suppressors p53 and p21 and activated Cyclin D1[32]. Intriguingly, while the loss of Jun in combination with Pten inactivation had no effect on the progression of the benign lesion [11], additional inactivation of Fos led to decreased cell proliferation and a smaller size of triple deficient prostate tumors, when compared to only loss of Fos and Pten. This indicates that the oncogenic function of Jun in PCa operates in aggressive Fos-deficient tumors rather than in benign Fos proficient lesions. Interestingly, the invasive properties of Fos deficient PCa were not affected by inactivation of Jun, indicating that this function of Fos is independent of Jun. The mechanistic basis of the enhanced invasiveness is at the moment unclear but could involve alterations in the tumor stroma and/or immune infiltrates, as it was observed in invasive JunB-deficient prostate tumors [11], or yet to be identified epithelial cell-autonomous changes. Utilizing our CRISPR/Cas9-driven PCa model in mice with stroma/immune-cell specific manipulations and/or *in vivo* screens will certainly provide therapeutically-relevant insights.

In conclusion, we have identified FOS as a tumor suppressor in prostate cancer. *FOS* expression is negatively correlated with disease progression and by applying CRISPR technology we show that loss of *FOS* alters multiple pathways, including AP-1 itself and initiates invasiveness and proliferation, partly by JUN. Therefore, expression of AP-1 subunits including FOS should be considered as prognostic marks for PCa progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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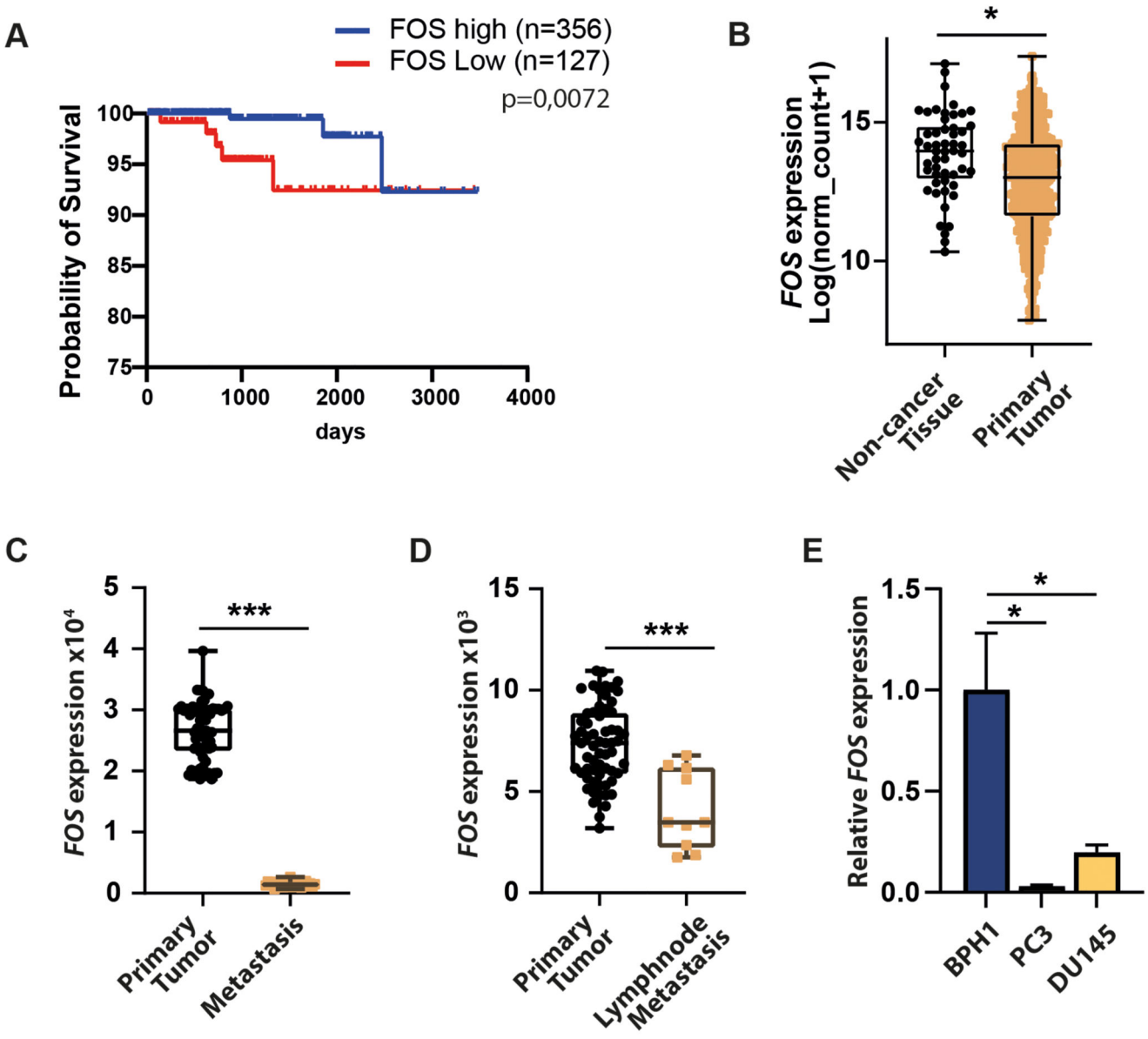

Figure 1. *FOS* expression is downregulated in primary and metastatic prostate cancer

A) Overall survival for prostate cancer patients with either high or low mRNA expression of *FOS* (n=483; p=0,0072). B) *FOS* gene expression in normal human prostate and cancer tissues (n= 52 normal, 497 tumor, p=0,003). Analysis of *FOS* expression in primary and metastatic prostate tissues from (C) a study by Chandran UR et al., (n= 64 primary cancer, 24 metastatic cancer, p<0,0001) and (D) a study by Lapointe J et al., (n= 62 primary cancer, 10 lymph node metastases, p=<0,0001). (E) Relative FOS expression in three PCa cell lines (* p<0,05; *** p<0,001).

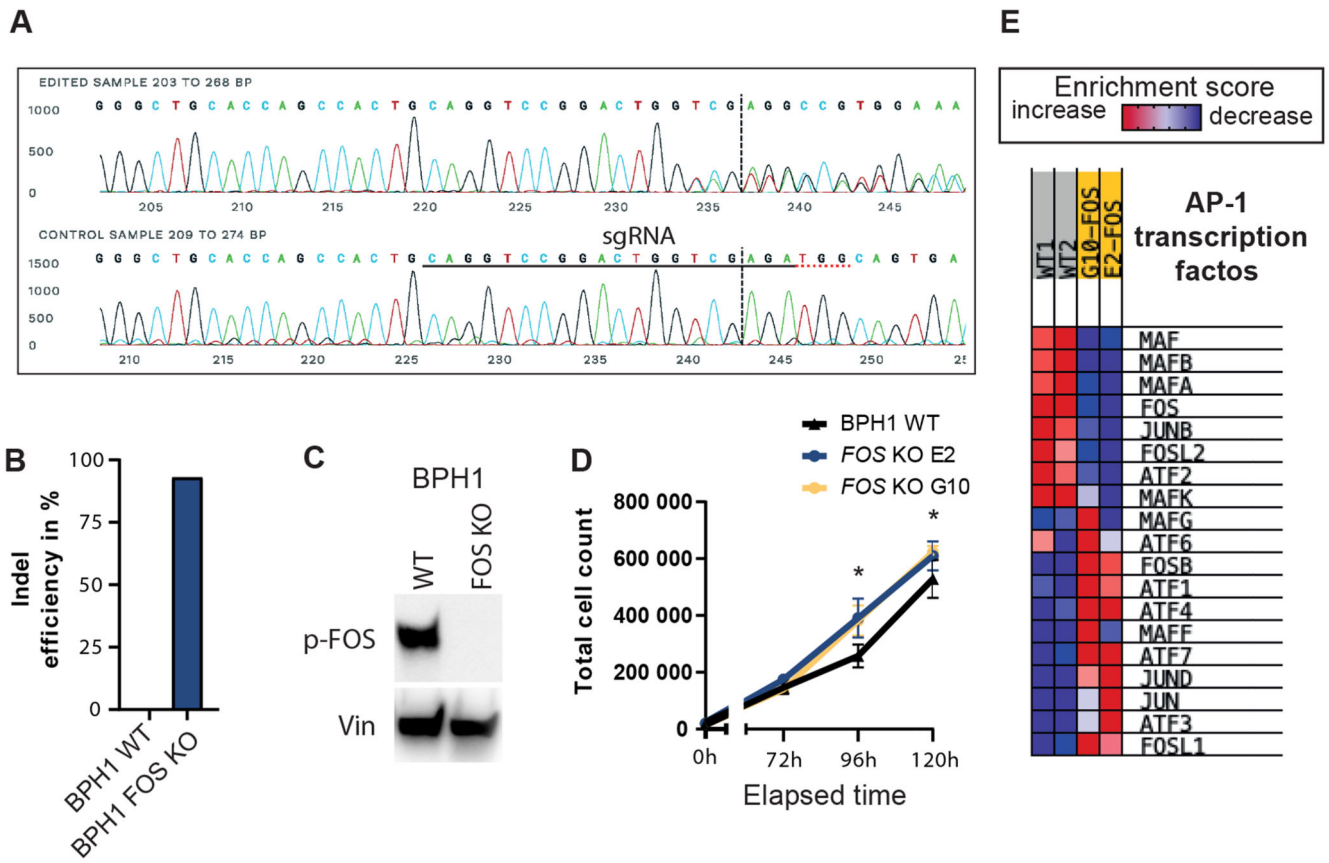

Figure 2. *FOS* depletion in benign human prostate cells alters oncogenic pathways.

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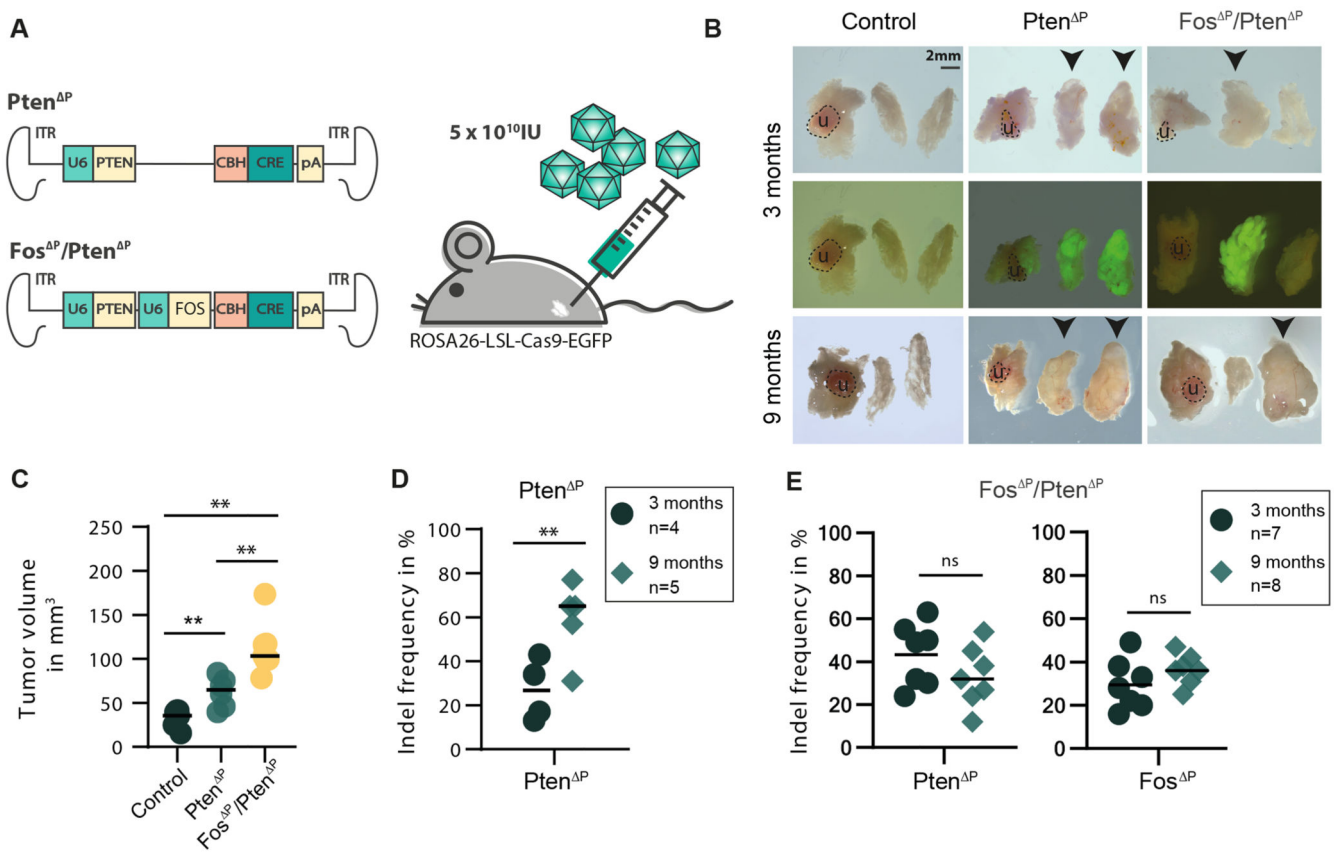

Figure 3. Combined knockout of Fos and Pten in murine prostate leads to tumor formation.

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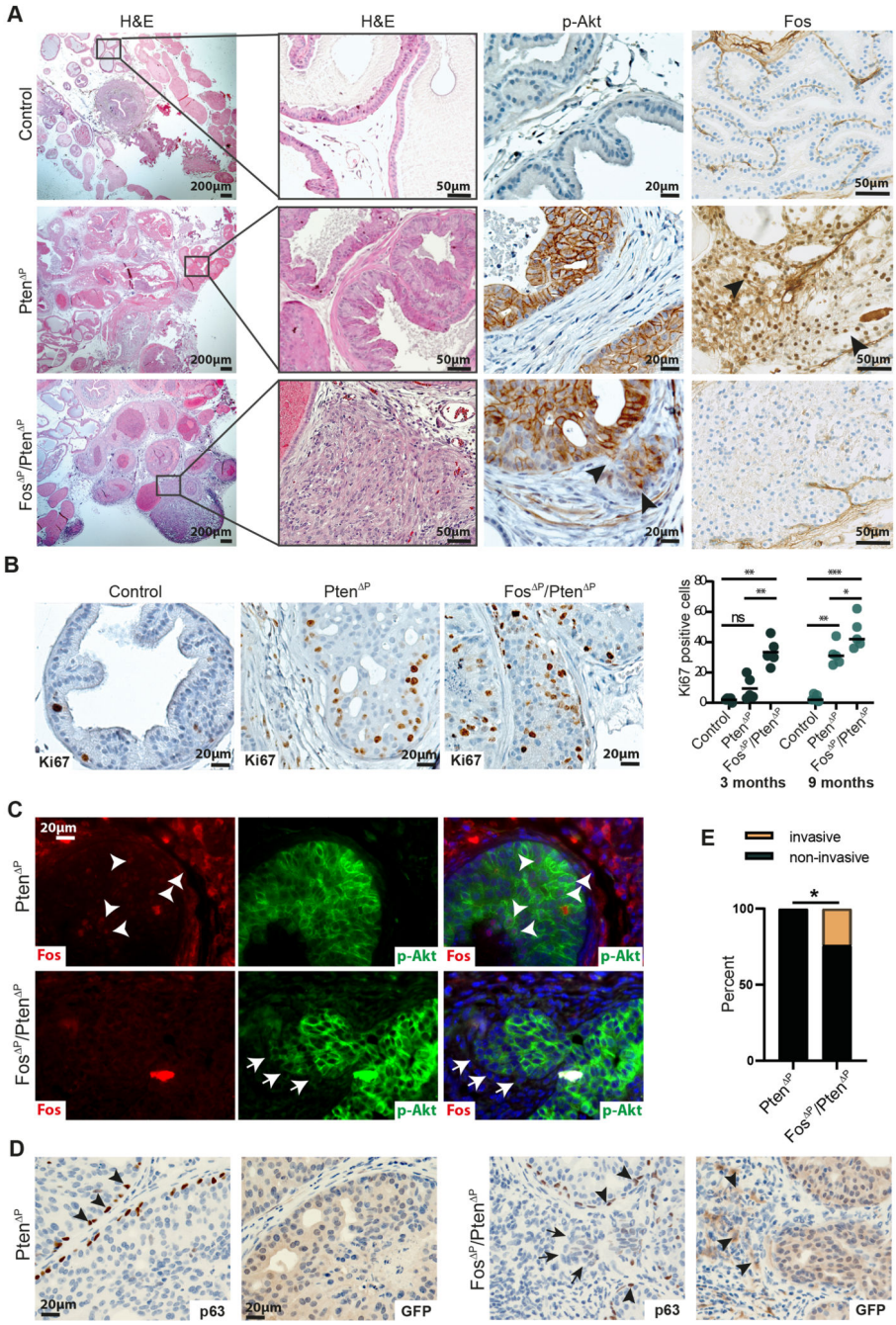

Figure 4. Loss of *Fos* in combination with *Pten* drives invasive prostate cancer *in vivo*.

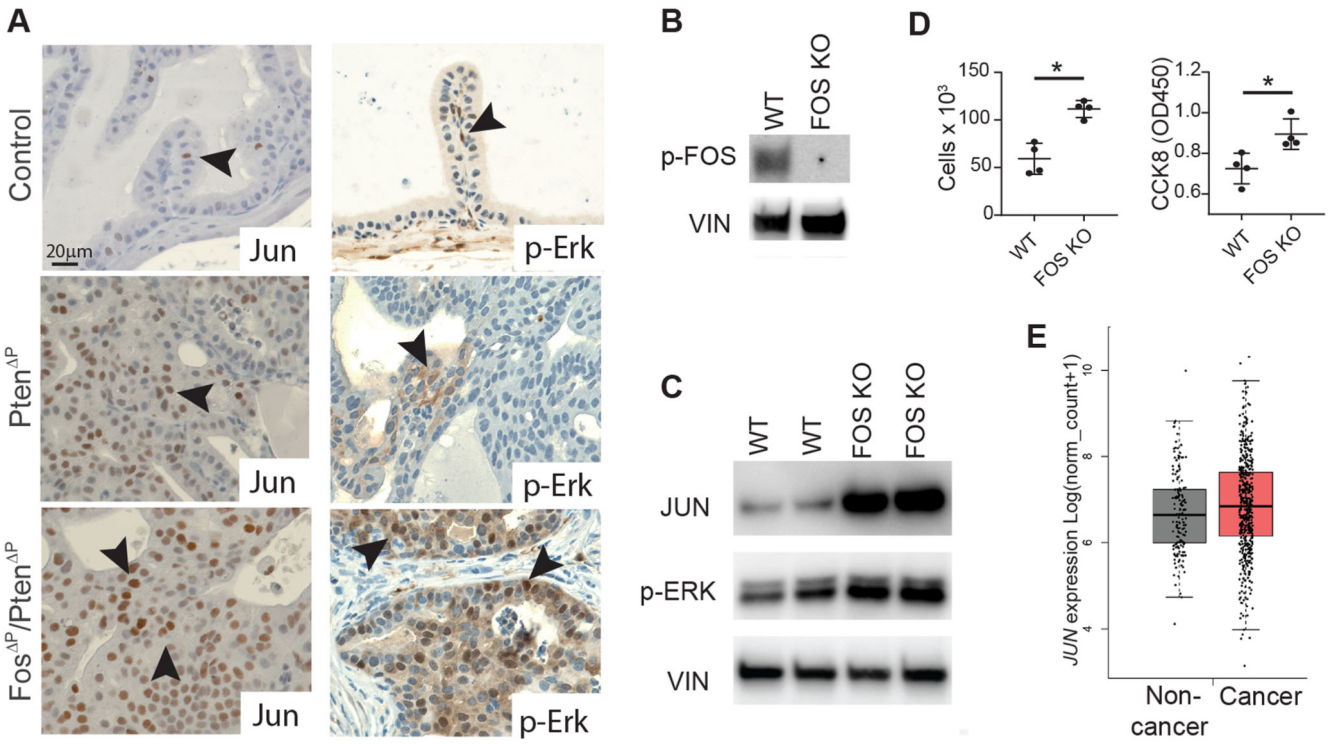

Figure 5. Jun is upregulated in prostate cancer.

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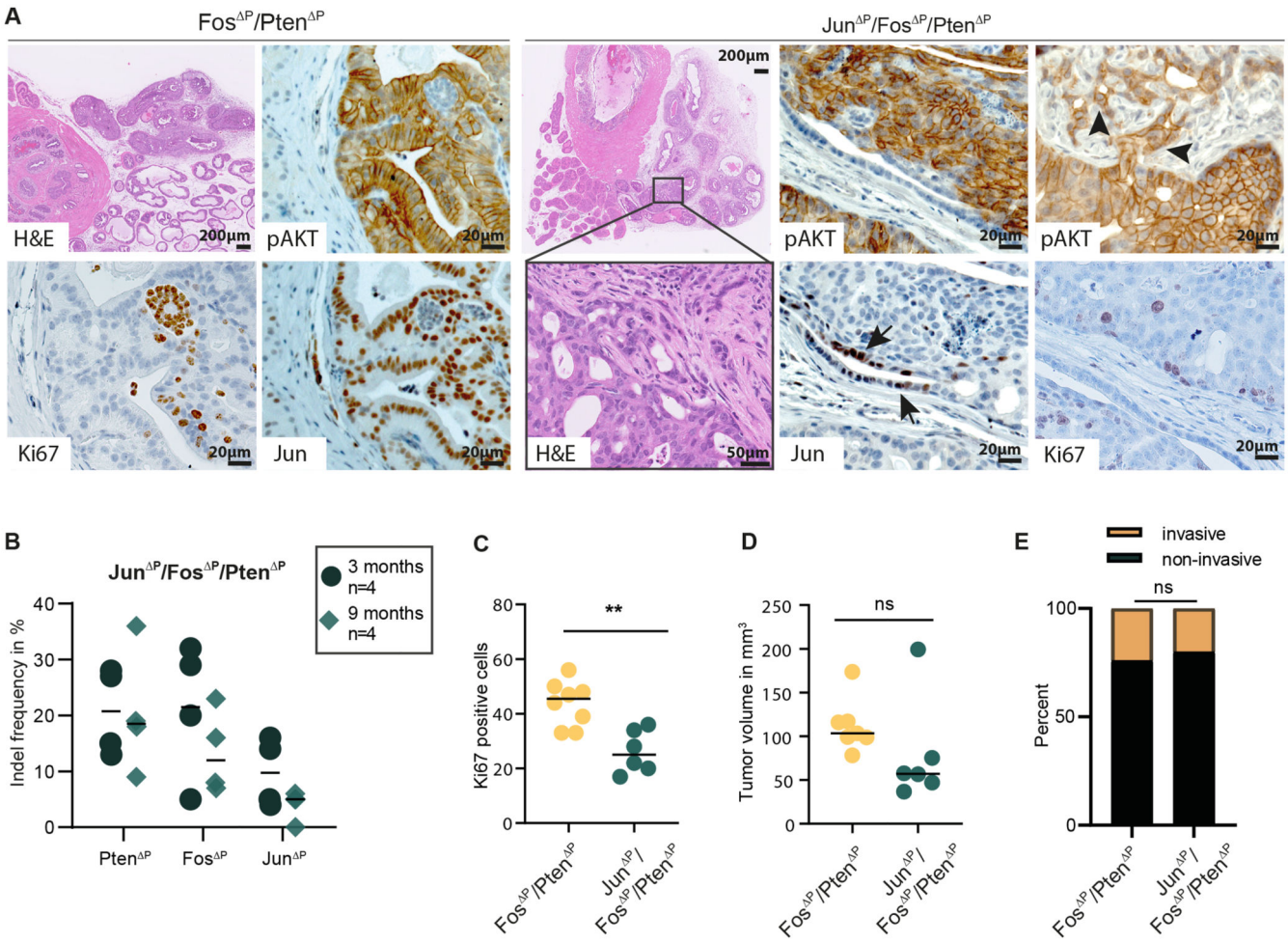

Figure 6. Jun drives proliferation in Fos-deficient prostatic tissues.

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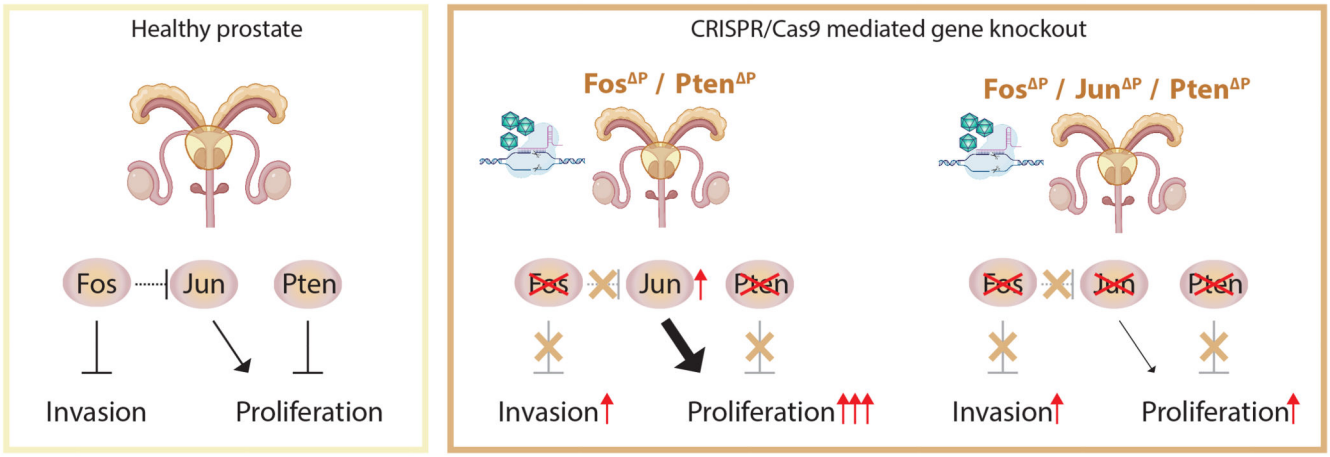

Figure 7. Model of Fos/Jun implication in prostate cancer.

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