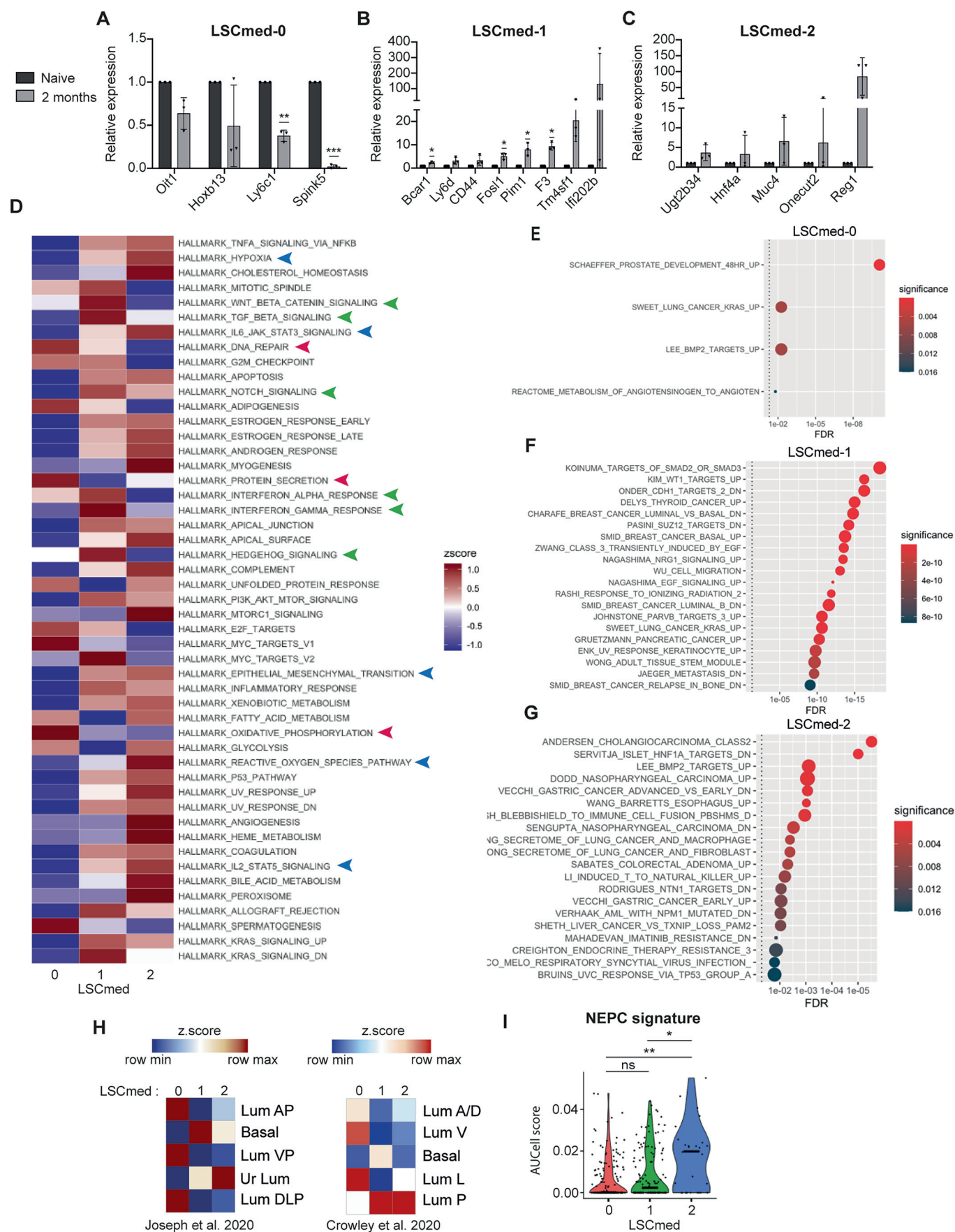


Expanded View Figures

Figure EV1. Gene set enrichment analyses of the three LSC^{med} cell subpopulations.

(A–C) The expression of selected marker genes of LSC^{med}-0 (A), LSC^{med}-1 (B), and LSC^{med}-2 (C) cells was measured by RT-qPCR in bulk LSC^{med} cells sorted from intact ($n = 3$) and 2-month-castrated ($n = 7$) *Pten^{flx/flx}* mice (biological replicates, $n = 3$ independent experiments). Data were normalized to the expression in intact mice. ns not significant, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (unpaired *t*-test with Welch's correction). Exact *p* values are reported in Appendix Table S1. Error bars represent SD.

(D) Heatmap highlighting Hallmark 50 gene set enrichments of the three LSC^{med} subpopulations. Biological processes mentioned in the text are labeled with arrows color-coded according to Fig. 2A (red: LSC^{med}-0; green: LSC^{med}-1; blue: LSC^{med}-2). (E–G) Dot plots showing enrichment of gene sets belonging to the C2 chemical and genetic perturbations category (MsigDB) per LSC^{med} cluster using the hyper (2.2.0) package (hypergeometric distribution test). (H) Heatmap representation of LSC^{med}-0, LSC^{med}-1, and LSC^{med}-2 signatures (average expression, z-score) in luminal and basal cells. We interrogated murine prostate scRNAseq datasets (Data Ref: Crowley et al, 2020a; Joseph et al, 2020a) for the expression of top 50 upregulated LSC^{med} cluster 0,1,2 genes. The average LSC^{med} signature expression was determined per cell type and z-score-transformed. LSC^{med} cluster 0,1,2 z-scores were plotted for luminal (Lum), luminal progenitor (LumP), urethral luminal (Ur Lum), and basal cell types. A anterior, D dorsal, L lateral, V ventral prostate lobes. (I) NEPC signature expression intensities (AUCell score) in LSC^{med} cell subpopulations. ns not significant, * $p < 0.05$, ** $p < 0.01$ (Wilcoxon test, Holm-method adjusted). Exact *p* values: $p = 0.12$ (0 vs 1), $p = 0.0031$ (0 vs 2), and $p = 0.039$ (1 vs 2), $n = 140$ (cluster 0), $n = 130$ (cluster 1), $n = 28$ (cluster 2).



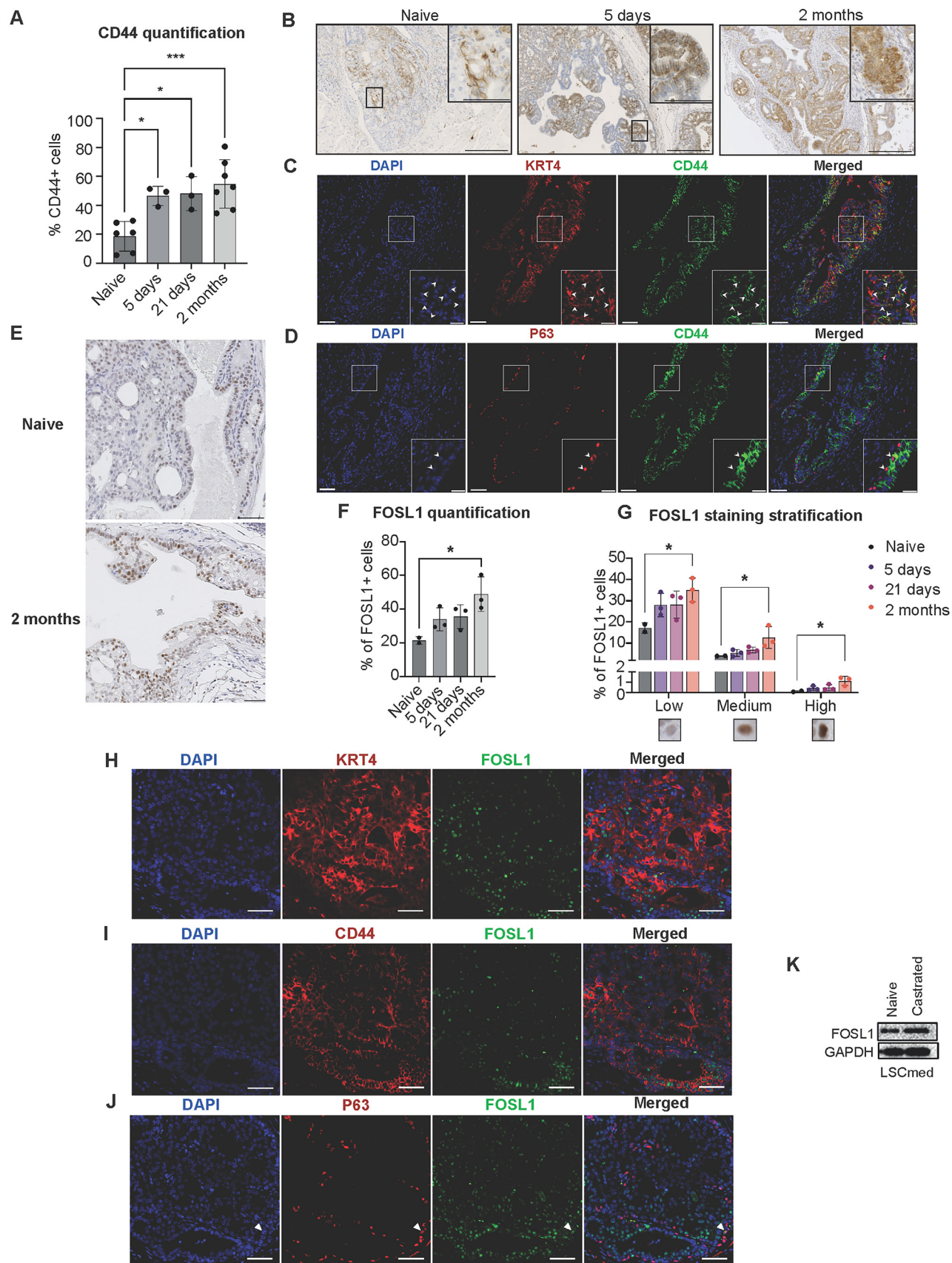


Figure EV2. Time-course analysis of CD44 and FOSL1 expression after castration in *Pten^{pc-/-}* mouse prostates.

(A, B) The expression of CD44, chosen as a selected marker of LSC^{med}-1 cells was assessed by immunohistochemistry in intact *Pten^{pc-/-}* mice ($n = 6$) and 5 days ($n = 3$), 21 days ($n = 3$), and 2 months ($n = 7$) post-castration. Both membrane and diffuse cytoplasmic staining was observed as previously reported in PCa (Omara-Opyene et al, 2004). (A) Quantification of CD44-positive cells was performed by QuPath. $*p < 0.05$, $***p < 0.001$ (one-way ANOVA). Exact p values: $p = 0.00231$ (5 days), $p = 0.00169$ (21 days), $p = 0.0005$ (2 months). (B) Representative images are shown for the indicated time points. Scale bars = 200 μm and 50 μm in insets. (C, D) Immunofluorescence analysis of CD44, KRT4 and p63 showing (arrowheads) frequent co-immunostaining of CD44 and KRT4 in the luminal epithelium (C) and occasional co-immunostaining of CD44 and p63 in the basal layer (D). Representative images from castrated mice are shown. Scale bars = 50 and 10 μm in insets. (E-G) The expression of FOSL1 was assessed by immunohistochemistry in intact *Pten^{pc-/-}* mice ($n = 2$) and 5 days ($n = 3$), 21 days ($n = 3$), and 2 months ($n = 3$) post-castration. (E) Representative images from intact and 2-month castrated mice are shown. Scale bars = 50 μm . (F) Quantification of nuclear FOSL1-positive cells was performed by QuPath. $*p < 0.05$ (One-way ANOVA). Exact p values: $p = 0.2383$ (5 days), $p = 0.1748$ (21 days), $p = 0.0132$ (2 months). (G) For each timepoint, FOSL1-positive cells were stratified by QuPath according to low, medium and high nuclear staining intensity, as illustrated. $*p < 0.05$ (one-way ANOVA); exact p values are reported in Appendix Table S1. (H-J) Immunofluorescence analysis of FOSL1, KRT4, CD44, and p63 showing frequent co-immunostaining of FOSL1 and KRT4 (H) or CD44 (I) in the luminal epithelium, and occasional co-immunostaining of FOSL1 and p63 in the basal layer (arrowhead) (J). Representative images from castrated mice are shown. Scale bars = 50 μm . (K) Western blot showing the upregulation of FOSL1 expression in sorted LSC^{med} cells after castration (FOSL1/GAPDH ratio increased by 46%). Each lane represents two pooled animals.

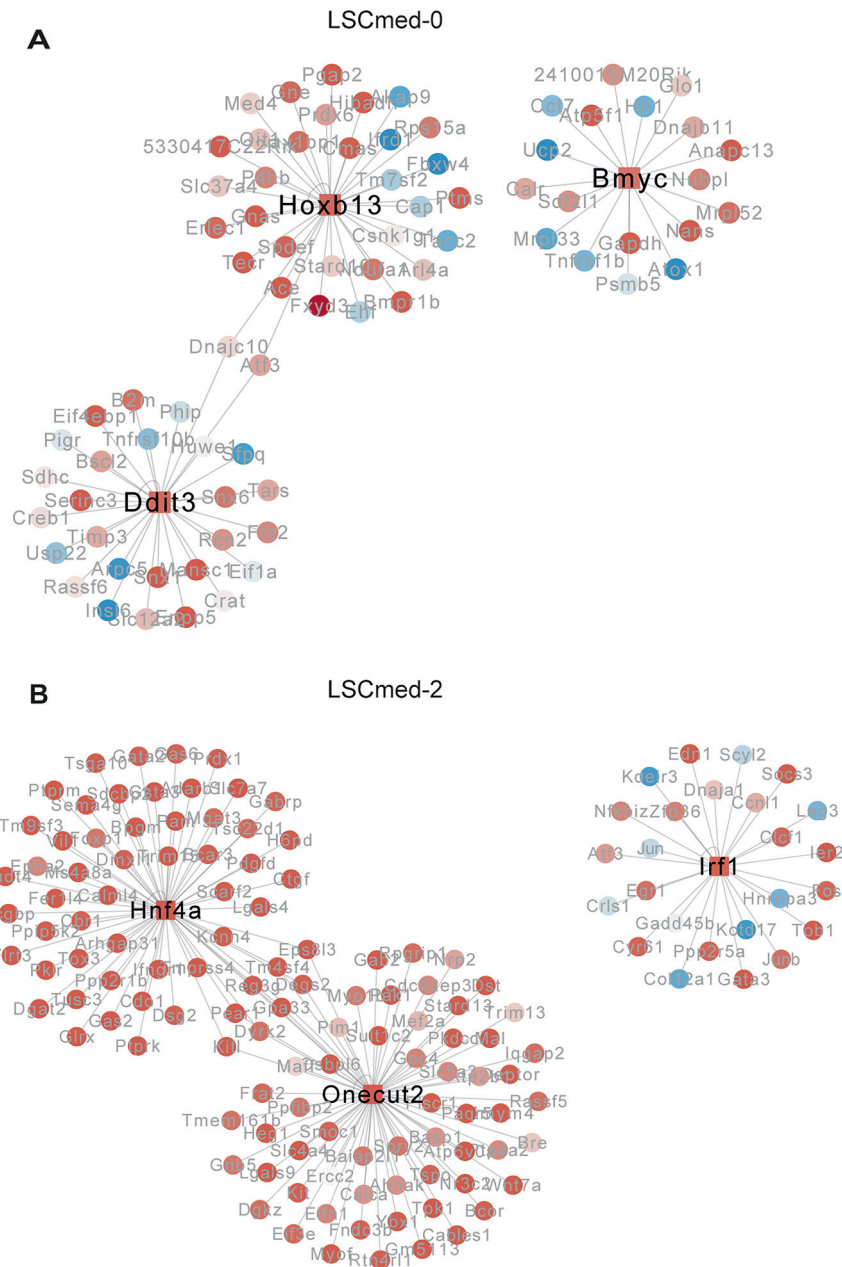
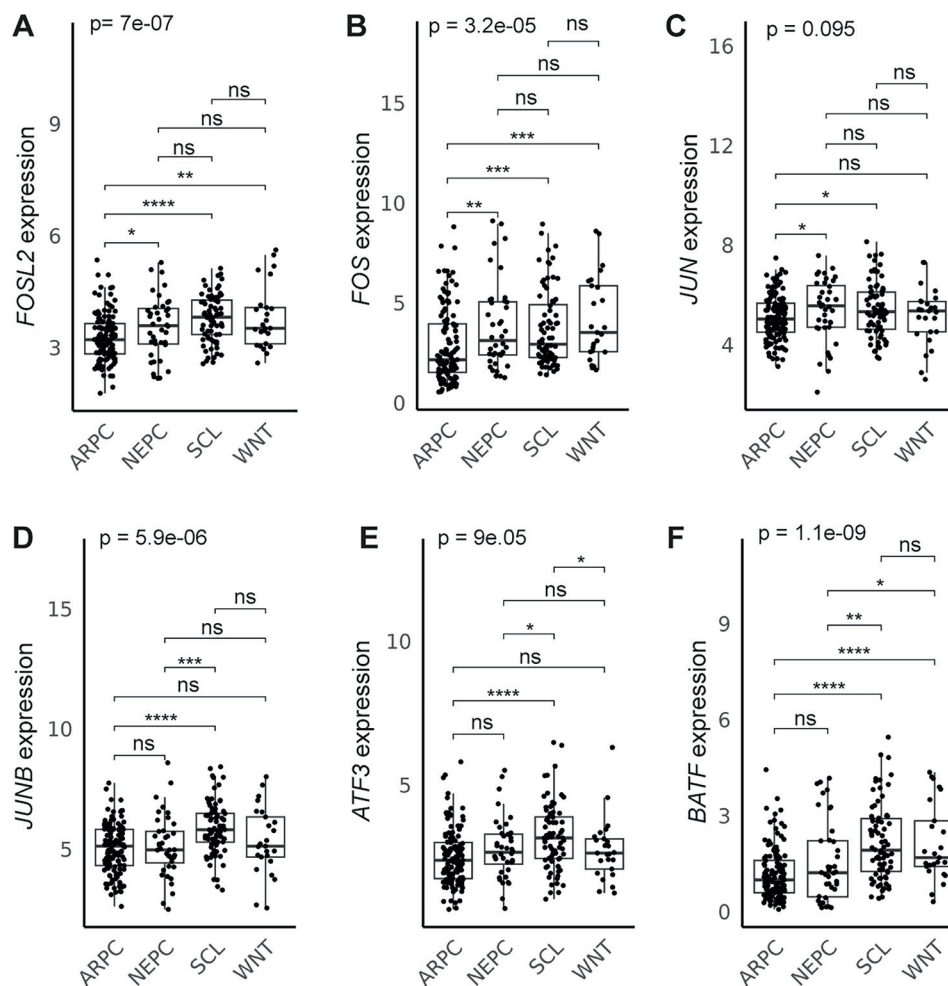
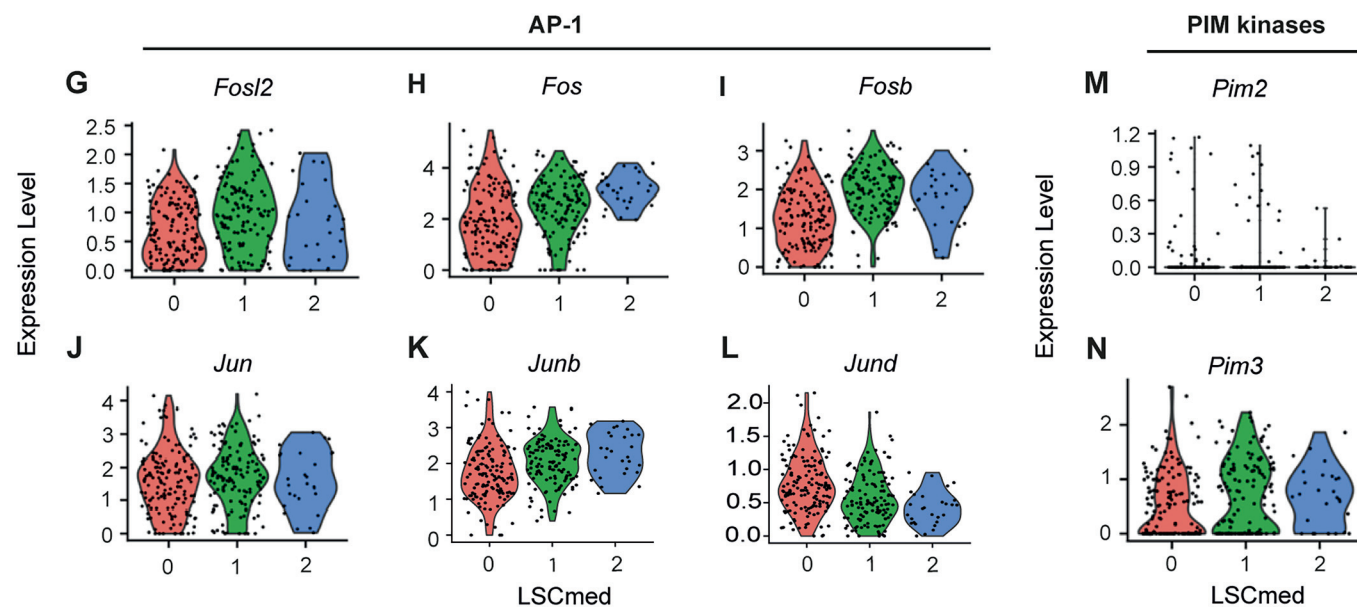


Figure EV3. Transcription factor networks in LSC^{med}-0 and LSC^{med}-2 cells.
The transcription factor networks of the 3 most pertinent regulons of LSC^{med}-0 (A) and LSC^{med}-2 cells (B) are represented. See Fig. 4C showing the same analysis for LSC^{med}-1 cells.

Human PCa subtypes

LSC^{med} cell subpopulations

◀ **Figure EV4. Expression of AP-1 family members and Pim kinases in human PCa subtypes and LSC^{med} cell subpopulations.**

(A–F). Expression of *FOSL2* (A), *FOS* (B), *JUN* (C), *JUNB* (D), *ATF3* (E), and *BATF* (F) in SU2C patient cohort (Data Ref: Abida et al, 2019a), according to the tumor molecular subtype ($n = 125$ ARPC, $n = 40$ NEPC, $n = 76$ SCL, $n = 25$ WNT). Boxplots display median (line), 25th–75th percentiles (box), whiskers at $1.5 \times \text{IQR}$, and individual outliers. ns not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ (Kruskal–Wallis with Holm-corrected Wilcoxon post hoc tests). See Appendix Table S1 for exact p values. (G–N). Expression of *Fosl2* (G), *Fos* (H), *Fosb* (I), *Jun* (J), *Junb* (K), and *Jund* (L), and of PIM kinases *Pim2* (M) and *Pim3* (N), in the three of LSC^{med} cell subpopulations.

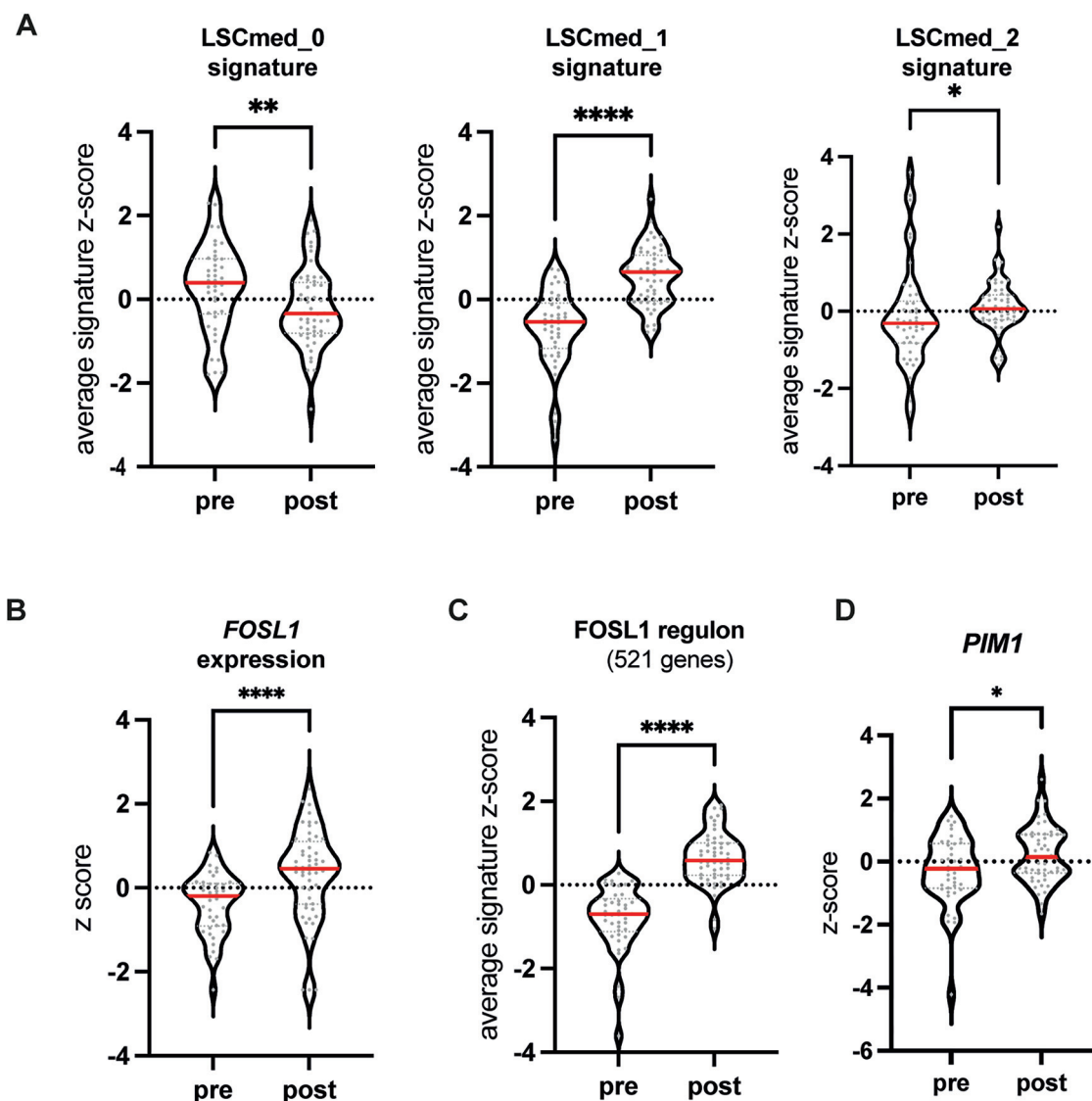


Figure EV5. Induction of *FOSL1*, *FOSL1* regulon, *PIM1* and LSC^{med} cluster 1 and 2 expression features upon enzalutamide treatment in human prostate cancer (DARANA study).

In the DARANA (Dynamics of Androgen Receptor Genomics and Transcriptomics After Neoadjuvant Androgen Ablation) study (ClinicalTrials.gov [NCT03297385](https://clinicaltrials.gov/ct2/show/study/NCT03297385)), high-risk prostate cancer patients were treated with neoadjuvant enzalutamide for 3 months before prostatectomy, with tumor biopsies taken before and after treatment. RNA sequencing data of naive ("pre", $n = 42$) and neoadjuvantly-treated ("post", $n = 52$) samples (Data Ref: Linder et al, 2022b) was interrogated for LSC^{med} cluster signatures (A), *FOSL1* (B), *FOSL1* regulon (C), and *PIM1* (D) expression. Enzalutamide treatment induces *FOSL1*, *FOSL1*-regulon and LSC^{med}-1 and LSC^{med}-2 gene expression and decreases LSC^{med}-0 gene expression. * $p < 0.05$; ** $p < 0.01$, **** $p < 0.0001$ (Wilcoxon test). Exact p values: $p = 0.0092$ (LSC^{med}-0), $p = 1.02 \times 10^{-40}$ (LSC^{med}-1), $p = 0.0146$ (LSC^{med}-2), $p = 8.82 \times 10^{-18}$ (*FOSL1*), $p = 5.46 \times 10^{-58}$ (*FOSL1* regulon), $p = 0.0378$ (*PIM1*)

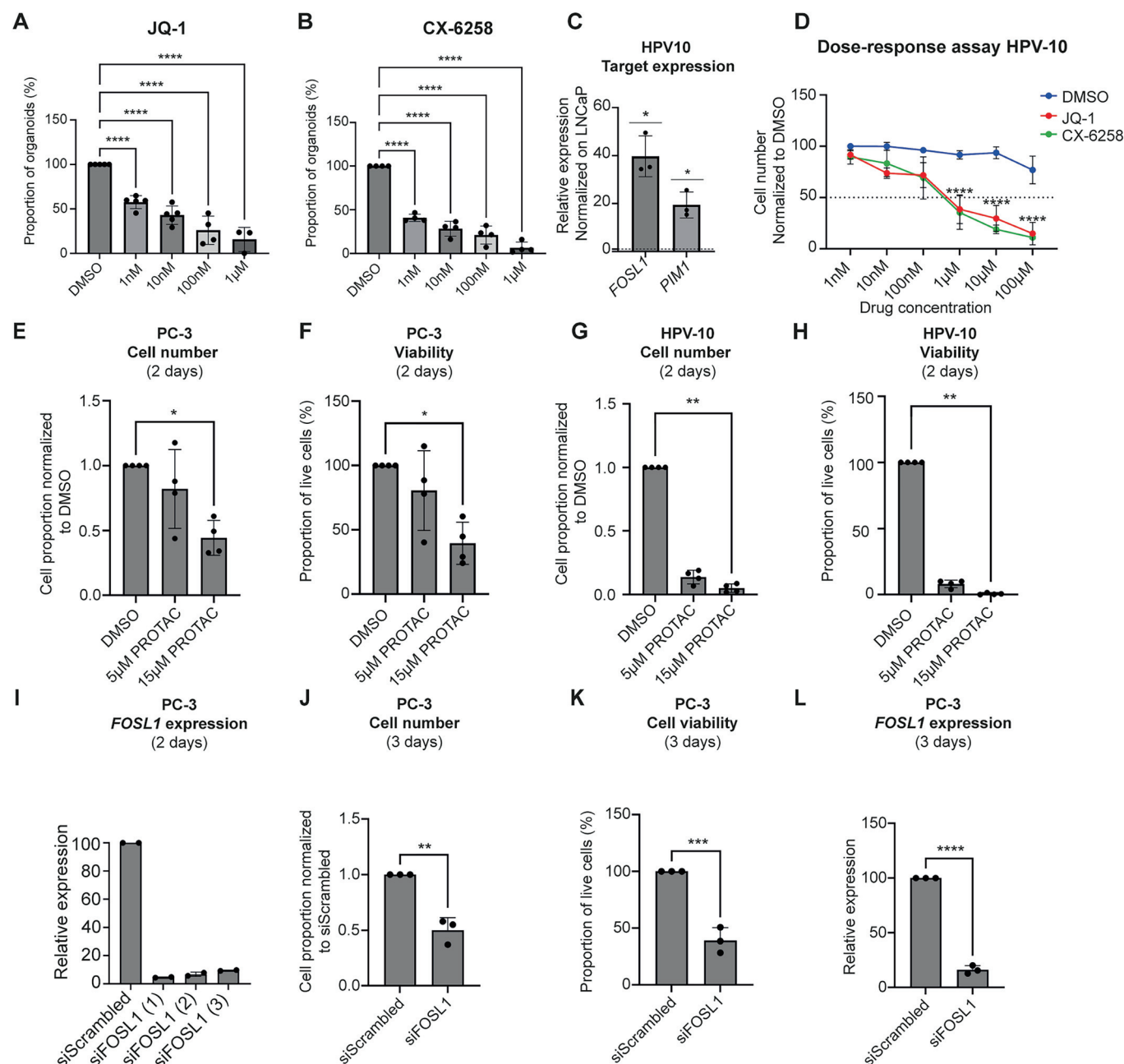
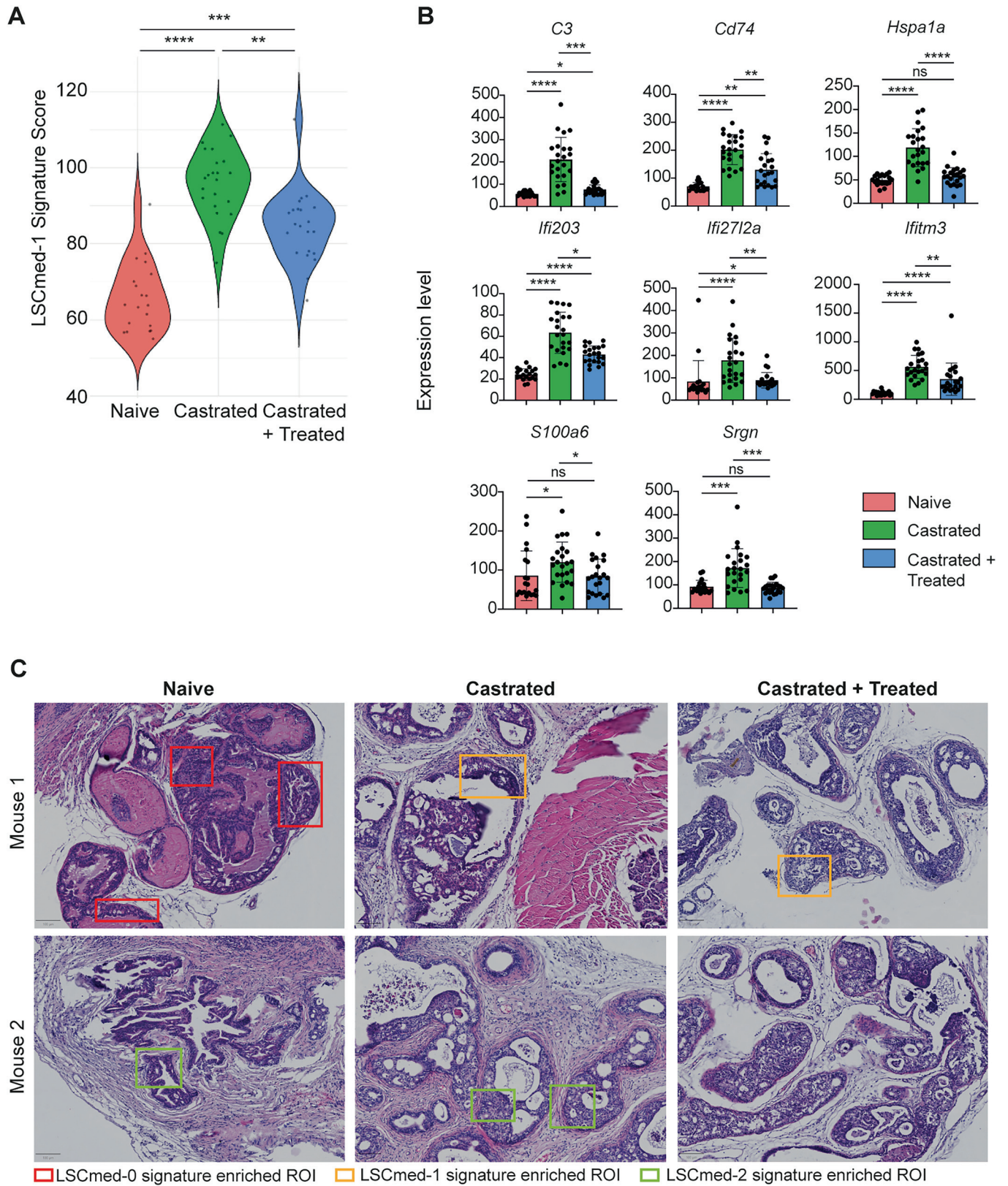


Figure EV6. Targeting FOSL1 and PIM1 in Club-like cells in vitro.

(A, B) Dose-response of JQ-1 (A) and CX-6258 (B) on the number of organoids formed by sorted *Pten^{pc-/-} LSC^{med}* cells. Data are normalized to the DMSO condition (biological replicates, $n = 5$ (A) and $n = 4$ (B) independent experiments). **** $p < 0.0001$ versus DMSO (ANOVA analysis and Dunnett's post hoc test). Exact p values are reported in Appendix Table S1. (C) Expression of *FOSL1* and *PIM1* in human HPV-10 cells determined by RT-qPCR. The results are normalized to the values obtained in LNCaP cells, represented by the horizontal dotted line (biological replicates, $n = 3$ independent experiments). * $p < 0.05$ versus LNCaP cells (unpaired t -test with Welch's correction), $p = 0.0157$ (*FOSL1*), $p = 0.0278$ (*PIM1*). (D) Dose-response of JQ-1 and CX-6258 on the number of adherent HPV-10 cells. Data were normalized to the DMSO condition (each dot is the average of three biological replicates). **** $p < 0.0001$ versus DMSO (ordinary two-way ANOVA with Šidák multiple comparisons test). Exact p values are reported in Appendix Table S1. (E–H) Human PC-3 (E, F) and HPV-10 (G, H) cells were treated with T5224-PROTAC ('PROTAC') at 0, 8, and 24 h. The cells were collected at 48 h. The cell number (E, G) and cell viability (adherent + in suspension) (F, H) were determined by trypan blue staining (biological replicates, $n = 4$ independent experiments). The data were normalized to DMSO (control condition). * $p < 0.05$, ** $p < 0.01$ (ANOVA analysis and Dunn's post hoc test). (I) Human PC-3 cells were treated with siRNA (siScrambled or three different *FOSL1* siRNA, as indicated) for 6 h and the cells were collected at 48 h. The expression of *FOSL1* was measured by RT-qPCR (biological replicates, $n = 2$ independent experiments). The data were normalized to siScrambled (control condition). The three siRNA *FOSL1* showed similar efficacy. **** $p < 0.0001$ (ANOVA analysis and Dunnett's post hoc test). Exact p values are reported in Appendix Table S1. (J–L) Human PC-3 cells were treated with siScrambled or siFOSL1(1) for 6 h and the cells were collected at 72 h. Cell number (J) and cell viability (adherent + in suspension) (K) were determined by trypan blue staining. The expression of *FOSL1* (L) was measured by RT-qPCR. The data are normalized to siScrambled (biological replicates, $n = 3$ independent experiments). ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (two-tailed t -test). Exact p values are reported in Appendix Table S1. All error bars in this figure represent SD.



◀ **Figure EV7. Digital spatial profiling of EpCAM⁺ cells in prostates from *Pten*^{pc-/-} mice non-castrated or castrated and treated with vehicle or JQ-1 and CX-6258.**

(A) Violin plot of the LSC^{med}-1 signature score in selected ROIs of non-castrated (naïve), castrated vehicle-, or castrated JQ-1 and CX-6258-treated *Pten*^{pc-/-} mice. $n = 10\text{--}13$ ROI per mouse, two mice per condition. ns: not significant. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ (nonparametric Kruskal-Wallis statistic tests and nonparametric Wilcoxon post hoc test). Exact p values are reported in Appendix Table S1. (B) Transcript levels of the eight most downregulated genes (p value < 0.05 and a log2 fold change estimate of < 0.5) of the LSC^{med}-1 and LSC^{med}-2 cell signatures after the treatment in castrated mice. ns not significant, * $p < 0.05$, ** $p < 0.001$, *** $p < 0.001$, **** $p < 0.0001$ (nonparametric Kruskal-Wallis and Dunn post hoc test). Exact p values are reported in Appendix Table S1. $n = 20$ (naïve), $n = 23$ (castrated), $n = 22$ (castrated + treated). (C) Representative H&E staining of prostates from naïve, castrated vehicle-, and castrated JQ-1 and CX-6258-treated *Pten*^{pc-/-} mice. The colored squares represent LSC^{med}-0, LSC^{med}-1, and LSC^{med}-2-enriched ROIs (color code indicated at the bottom of the image). Scale bar: 100 μm . $n = 2$ mice per condition.