Figure S1. Efficiency of establishment of patient-derived prostate organoids.

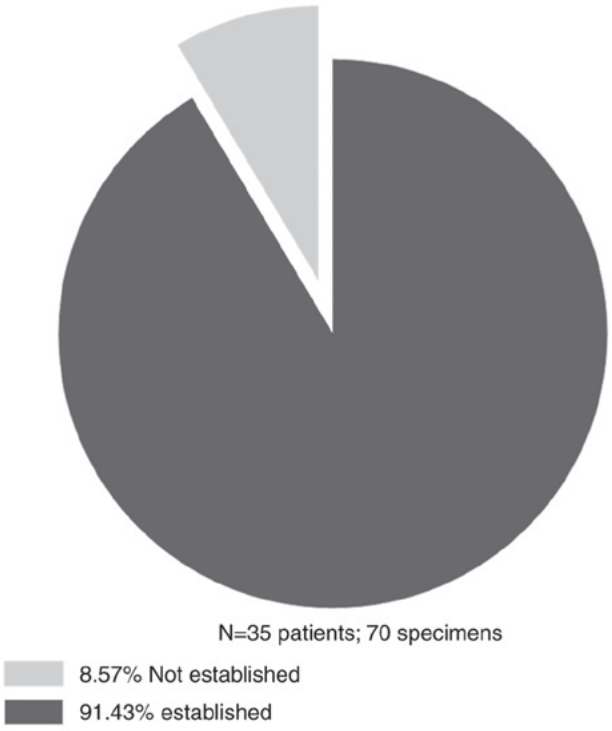

Figure S2. Characterization of patient-derived prostate tumor organoids and corresponding tissue for prostate differentiation markers. Immunohistochemistry images of organoids and corresponding tissue [patient 8; grade group 1; Gleason score, 6 (3+3)] stained with prostate differentiation markers c-MYC and ERG. Scale bar, 20 μm. Representative microscopy images were acquired using the Olympus CX41 light microscope (magnification, x20). ERG, ETS transcription factor ERG.

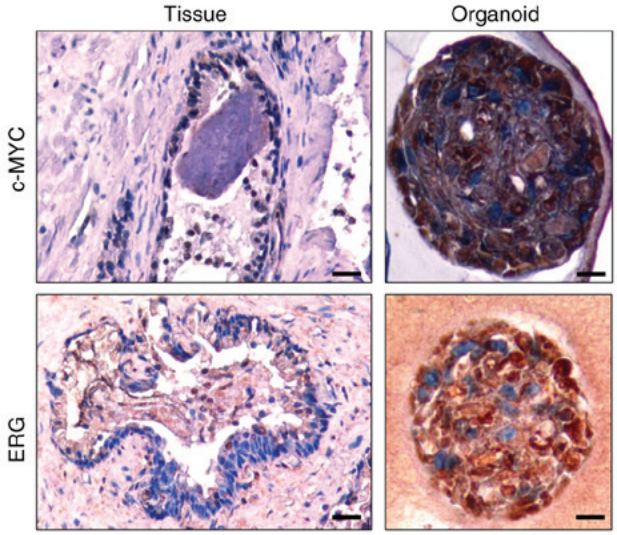

Figure S3. RNA-seq of patient-derived PCa organoids relative to corresponding tissue. RNA-Seq was performed using the Hi-Seq 2500 Illumina platform to delineate DEGs. (A) Volcano plot of DEGs. The threshold was set at P-adj<0.05. Differentially expressed transcripts (n=3,134) between PCa organoids and tissue (two biological and two technical duplicates in each group) were identified. (B) Heatmap and hierarchical cluster analysis of DEGs. Red, upregulation; blue, downregulation. Seq, sequencing; PCa, prostate cancer; DEGs, differentially expressed genes.

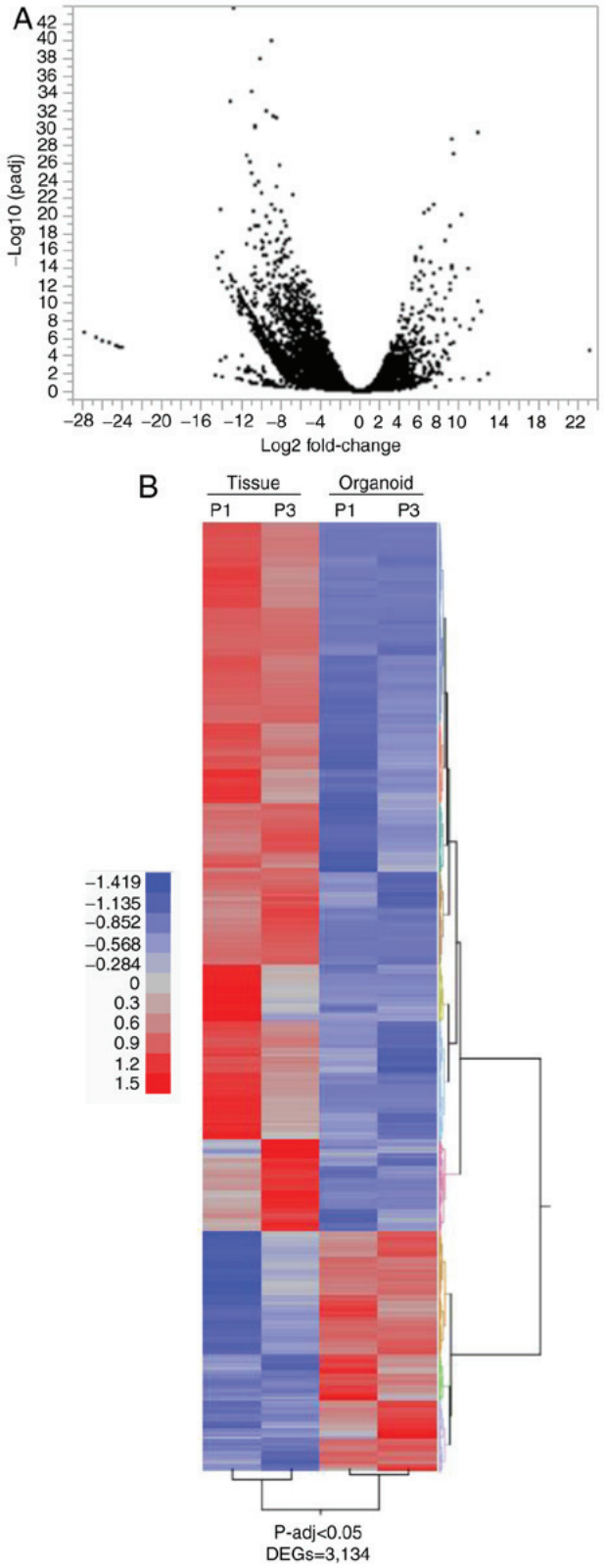

Figure S4. Pathways enriched in up- and downregulated genes in PCa organoids vs. tissue. Enrichment maps of pathways among PCa organoid samples were constructed using EnrichmentMap on Cytoscape 3.7.2 software. Each node (circle) represents a distinct pathway (red, upregulated; blue, downregulated) and edges (lines) represent the number of overlapping genes between two pathways, determined using the similarity coefficient.

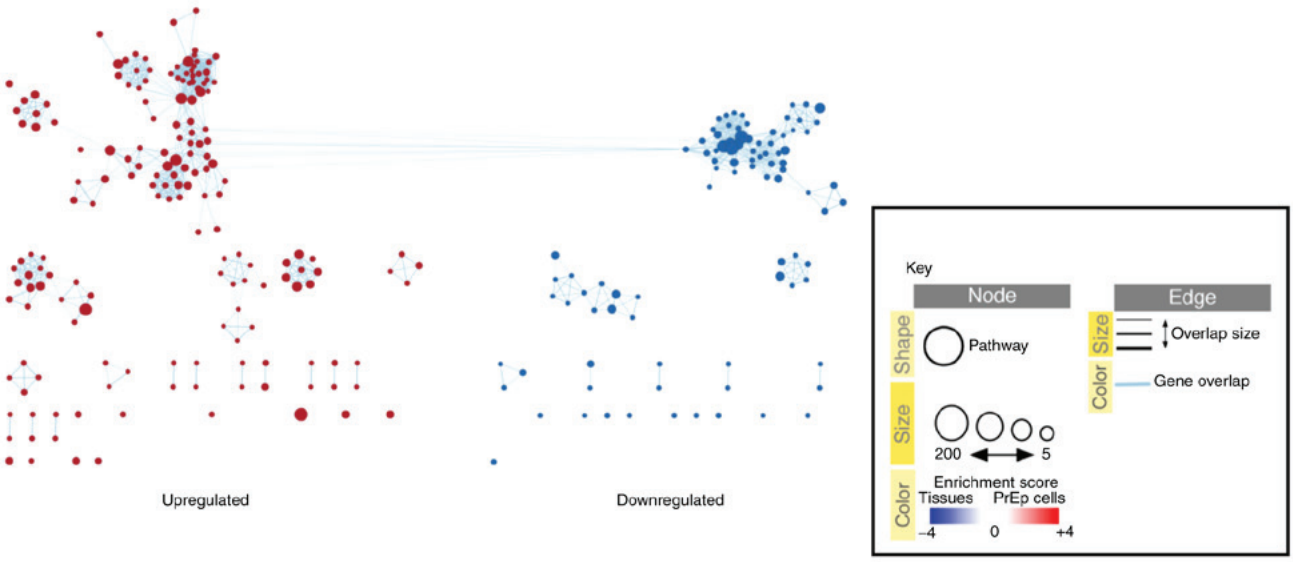

Figure S5. Gene set enrichment analysis of the top 20 signaling pathways activated in PCa organoids relative to tissue. PCa organoids exhibited enrichment for: i) Cell cycle pathways, such as 'cell_cycle_reactome', 'cell_cycle_checkpoints_reactome' and 'm_phase_reactome', among others; ii) e2f signaling, such as 'hallmark_e2f_targets'; iii) mitosis, such as 'mitotic_cell_cycle', 'mitotic_anaphase_reactome', and 'sister_chromatid_segregation' among others; and (iv) epithelial differentiation pathways, such as 'epidermis_development', 'keratinization', 'keratinocyte_differentiation', and 'skin_development' among others. PCa, prostate cancer.

Tumor organoid vs. tissue

NAME	ES	NES	NOM p-val	FDR q-val
KERATINIZATION_REACTOME	0.872	5.994	0	0
EPIDERMIS_DEVELOPMENT	0.725	5.915	0	0
CORNIFICATION	0.872	5.813	0	0
EPIDERMAL_CELL_DIFFERENTIATION	0.811	5.770	0	0
KERATINOCYTE_DIFFERENTIATION	0.827	5.754	0	0
KERATINIZATION	0.862	5.509	0	0
SKIN_DEVELOPMENT	0.710	5.309	0	0
CELL_CYCLE_MITOTIC_REACTOME	0.652	5.259	0	0
CELL_CYCLE_REACTOME	0.640	5.013	0	0
HALLMARK_G2M_CHECKPOINT	0.720	5.002	0	0
HALLMARK_E2F_TARGETS	0.750	4.904	0	0
MITOTIC_CELL_CYCLE	0.527	4.742	0	0
MITOTIC_CELL_CYCLE_PROCESS	0.520	4.700	0	0
FORMATION_OF_THE_CORNIFIED_ENVELOPE_REACTOME	0.871	4.626	0	0
CELL_CYCLE_CHECKPOINTS_REACTOME	0.729	4.594	0	0
M_PHASE_REACTOME	0.629	4.361	0	0
MITOTIC_METAPHASE_AND_ANAPHASE_REACTOME	0.715	4.122	0	0
SISTER_CHROMATID_SEGREGATION	0.768	4.108	0	0
CELL_CYCLE_CHECKPOINT	0.703	4.088	0	0
MITOTIC_ANAPHASE_REACTOME	0.715	4.060	0	0

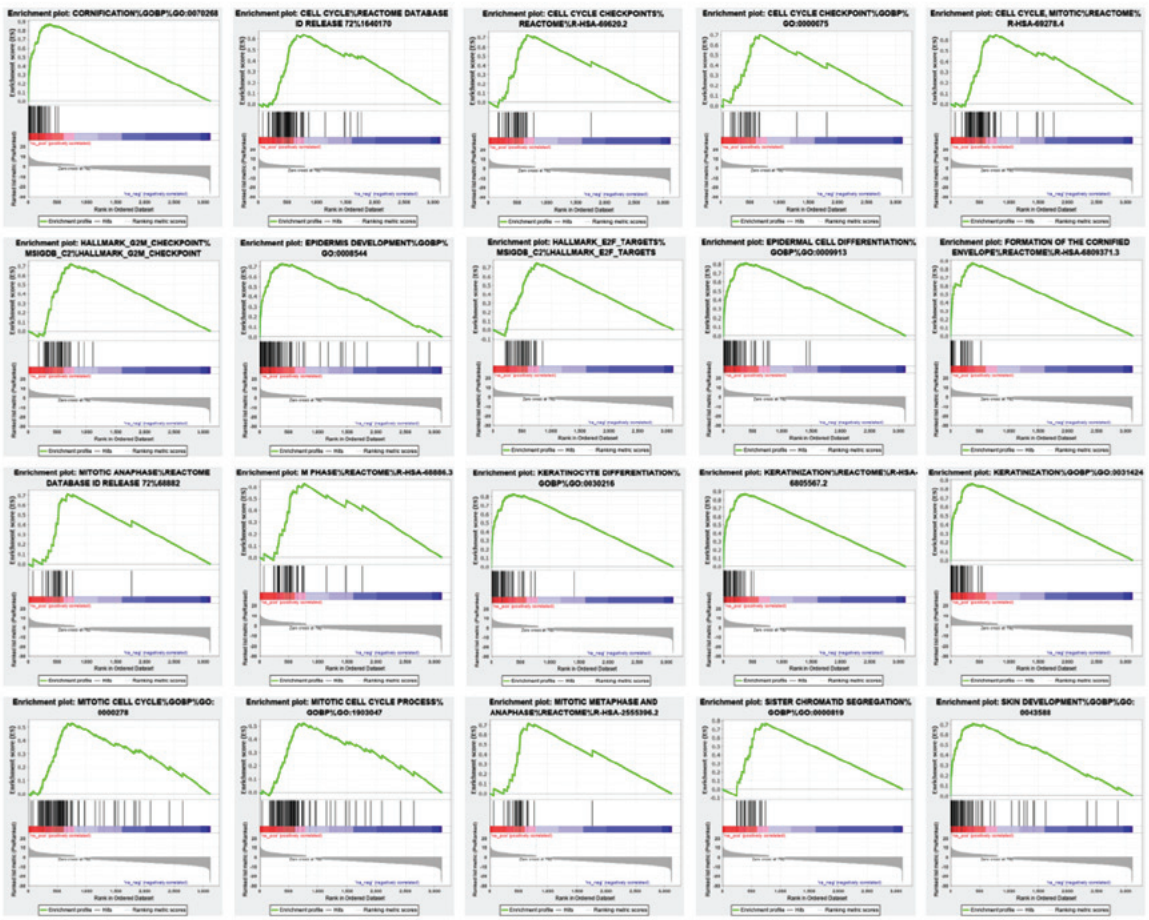

Figure S6. Effect of withdrawal of factors from human prostate growth medium on organoid growth. (A) OFC [treatment F(10,11)=134.3, P<0.0001]. (B) Quantification of the average diameter of G1 PCa organoids [treatment F(10,529)=113.4, P<0.0001]. (C) OFC [treatment F(10,11)=210.0, P<0.0001]. (D) Quantification of the average diameter of G1 PCa organoids. One-way ANOVA followed by Bonferroni multiple comparisons: Treatment F(10,529)=110.9, P<0.0001. (E) Quantification of OFC of G1 PCa organoids from three random PCa patients with similar clinical manifestations [grade group 2; Gleason score, 7 (3+4)] [treatment F(10,22)=3.793, P=0.0044]. Bonferroni post hoc analysis was performed to determine simple factor effects. (F) Quantification of the average diameter of G1 PCa organoids from three patients. One-way ANOVA followed by Bonferroni multiple comparisons: Treatment F(10,22)=3.79, P=0.0044). Data are presented as the mean ± SEM of three independent experiments. *P<0.05; **P<0.01; ***P<0.001 vs. CTRL. OFC, organoid formation count; G, generation; PCa, prostate cancer; EGF, epidermal growth factor; DHT, dihydrotestosterone; FGF, fibroblast growth factor; NOG, noggin; RSPO, R-spondin; PGE2, prostaglandin E2; SB, SB202190; A83, A83-01; NAC, N-acetylcysteine.

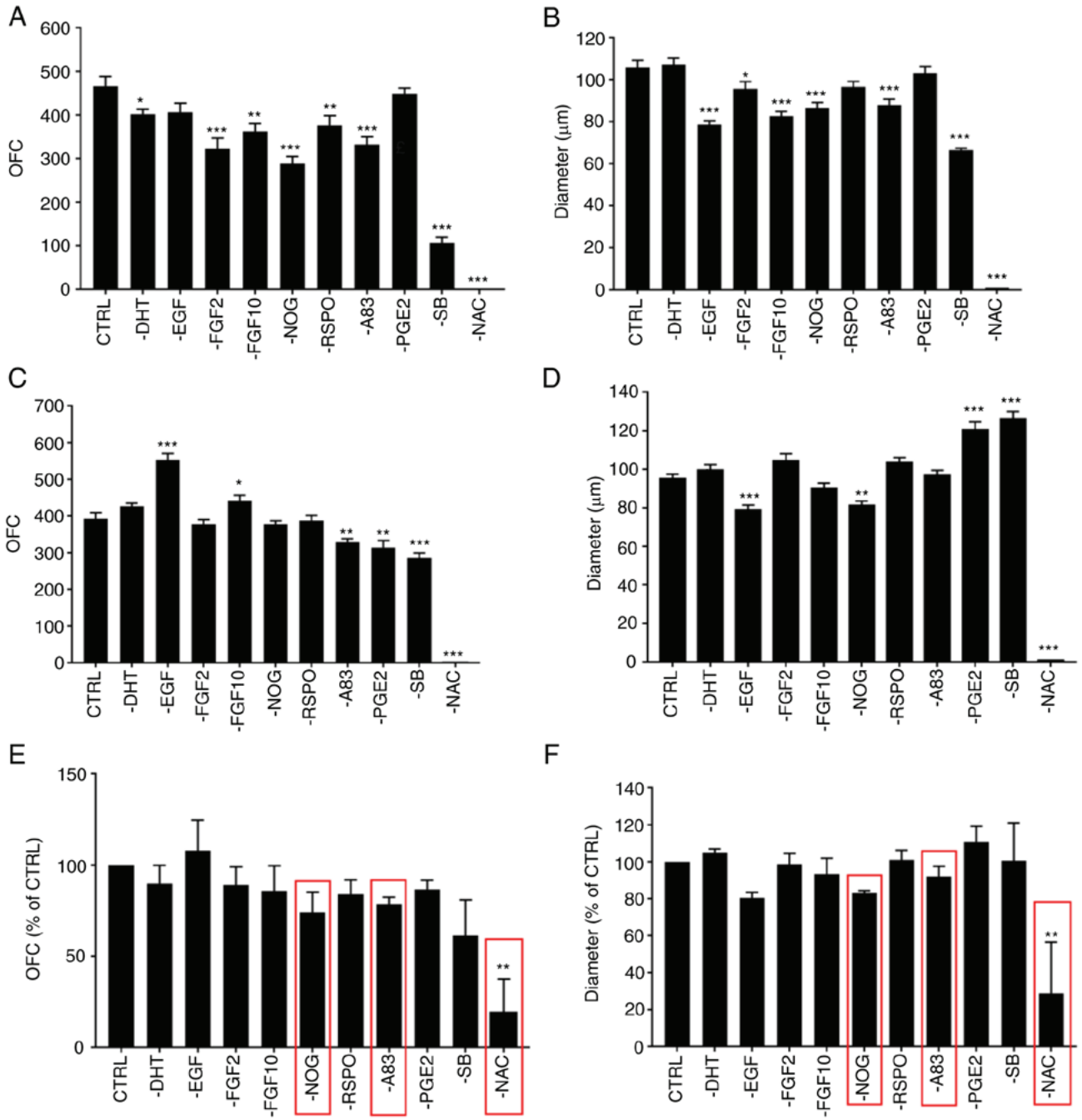

Table SI. Clinical characteristics of patients used for RNA sequencing.

Patient no.	Age, years	PSA, ng/ml	Gleason score	ISUP grade group	TNM staging	Prostate weight, g
1	66	4.62	6 (3+3)	1	T2b, N0, M0	35
2	55	8.30	7 (3+4)	2	-[a]	45

ISUP, International Society of Urological Pathology; PSA, prostate-specific antigen. [a]TNM staging of patient 2 was missing from the patient medical record.