

Intra-epithelial non-canonical Activin A signalling safeguards prostate progenitor quiescence

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* Source data are available online for this figure.

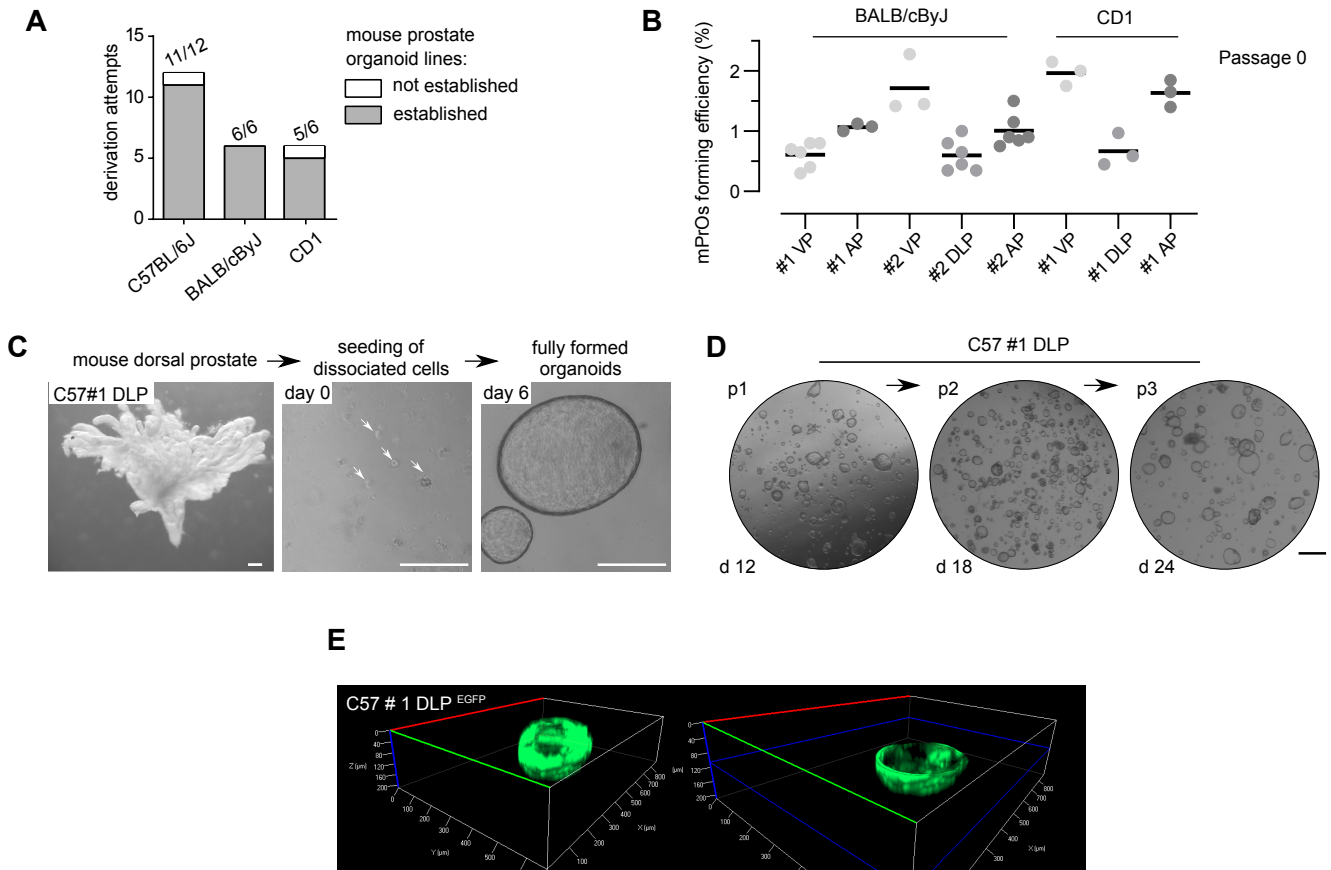


Figure S1. Establishment of a mouse prostate organoid biobank, related to Figure 1

- A Prostate organoid derivation rate from distinct mouse strains.
- B Mouse prostate organoid forming efficiency at derivation. Data points are shown with crossing line representing mean value.
- C Representative stereoscopic images at different stages of mouse prostate organoid derivation. Scale bar = 500 μ m.
- D Representative stereoscopic images of mouse prostate organoid cultures during subsequent passages. Scale bar = 1 mm.
- E Representative 3D reconstruction of a fully formed mouse prostate organoid based on live epifluorescent microscopic analysis (1 μ m section x 300 planes). On the right side, only the bottom half of the organoid is displayed.

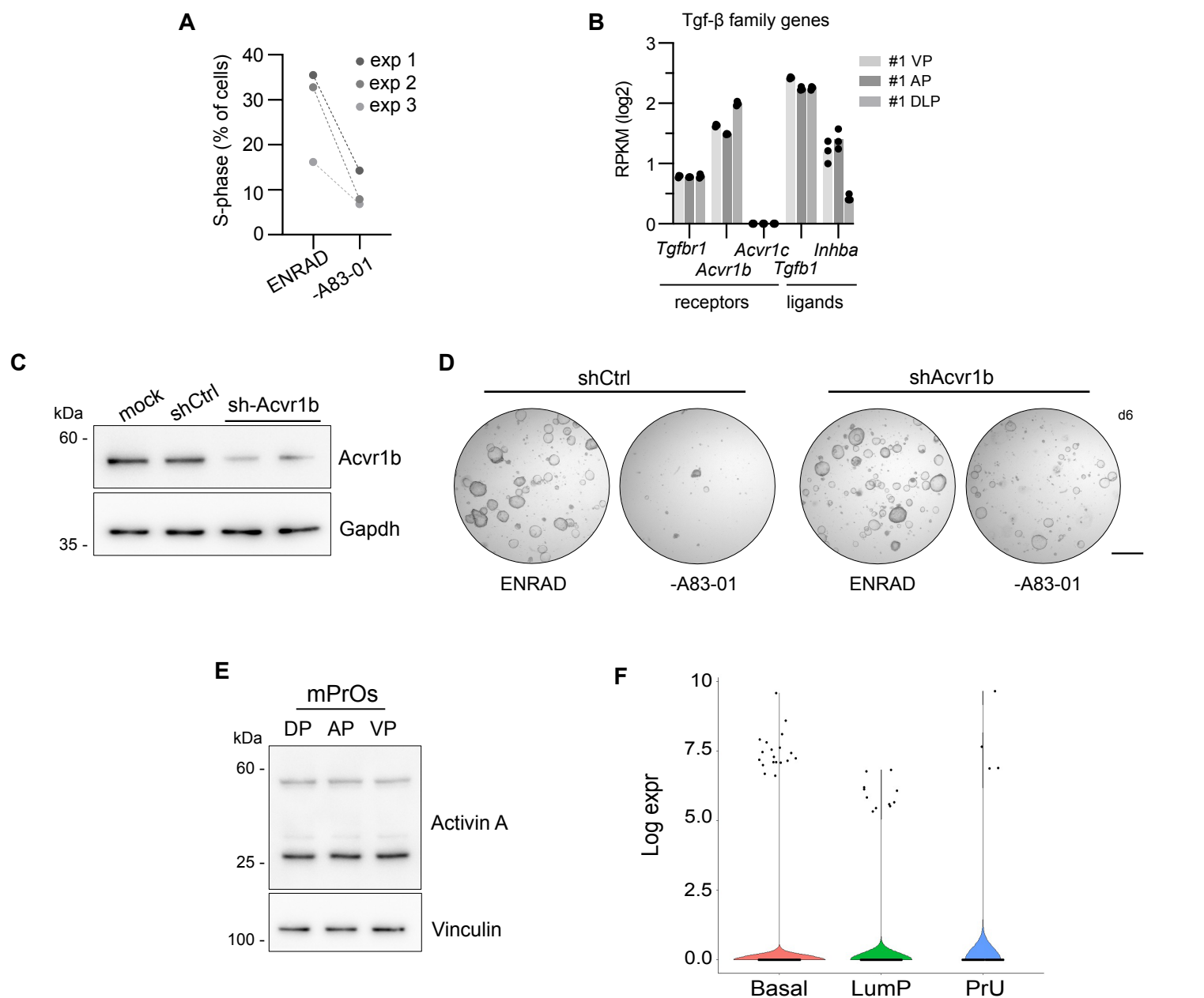


Figure S2. Inhibition of Tgf- β receptors is required for cell cycle progression in mouse prostate organoids, related to Figure 2

- A** Quantification of cells undergoing S-phase based on flow cytometry (EdU vs. TO-PRO-3) in complete medium (ENRAD) or in the absence of A83-01 (24 hours; related to Fig 2A; n = 3 biological replicates, individual data points are shown).
- B** mRNA expression levels for selected Tgf- β family receptors and ligands. Bulk-RNAseq analysis (n = 3 biological replicates; individual data points are shown with bar graphs representing mean value).
- C** Western blot analysis for Acvr1b in control (sh-Ctrl) and Acvr1b knocked down (sh-Acvr1b) mouse prostate organoids in normal growth condition (ENRAD) and following A83-01 withdrawal (-A83-01).
- D** Representative stereoscopic images of control (sh-Ctrl) and Acvr1b knocked down (sh-Acvr1b) mouse prostate organoids in normal growth condition (ENRAD) and following A83-01 withdrawal (-A83-01). Scale bar = 1 mm.
- E** Non-reducing western blot analysis for Activin A in cell lysates of lobe-specific mouse prostate organoids.
- F** Single cell profile of *Inhba* expression in Basal, LumP and PrU epithelial cells of adult mouse prostate.

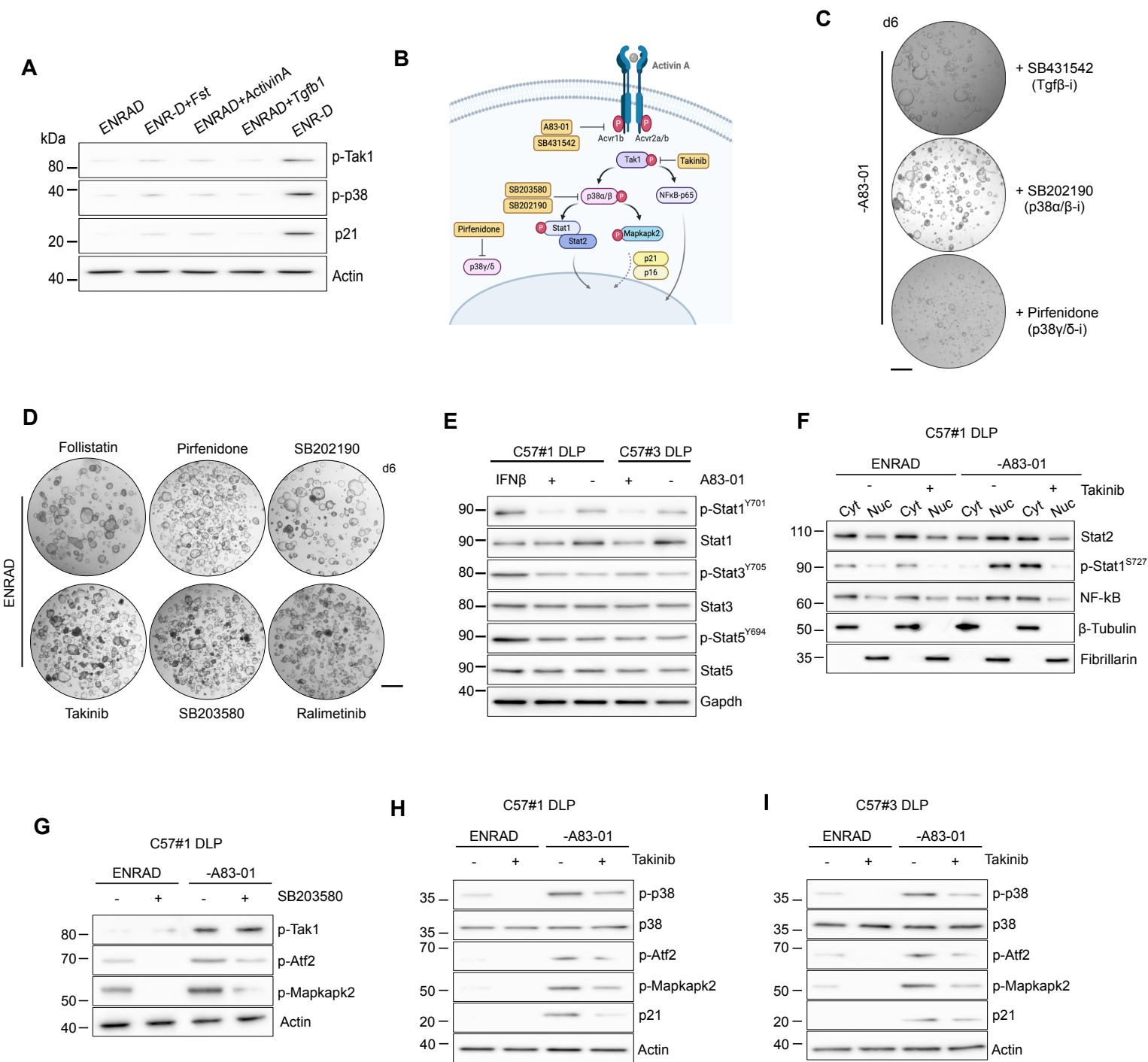


Figure S3. Tak1/p38 MAPK signalling activates immune- and stress-related pathways in prostate organoids, related to Figure 2

A Western blot analysis in mouse prostate organoids for key components of the non-canonical Tgf-β pathway in ENRAD medium or upon acute A83-01 withdrawal, with or without exogenous administration of Activin A (50 ng/mL), Tgfb1 (500 ng/mL), or Follistatin (500 ng/mL).

B Schematic view of the non-canonical Activin A signalling pathway, including inhibitors used for the experiments described in this figure.

C Representative stereoscopic images of mouse prostate organoids grown in the absence of A83-01 and in the presence of either SB431542 (Tgfβ-receptor inhibitor; 10 μM, 6 days), or SB202190 (p38α/β inhibitor; 10 μM, 6 days), or Pirfenidone (p38γ/δ inhibitor; 10 μM, 6 days). Scale bar = 1 mm.

D Representative images showing normal organoids growth in ENRAD medium conditioned with Activin A, Tak1 or p38 inhibitors. Scale bar = 1 mm.

E Western blot analysis in mouse prostate organoids for canonical IFN signalling mediators upon acute A83-01 withdrawal (IFNβ; 50 ng/mL, 6 days).

F-I Western blot analysis in mouse prostate organoids for key components of the non-canonical Tgf-β pathway and IFN signalling upon acute A83-01 withdrawal and concomitant inhibition of either Tak1 (Takinib; 5 μM, 24 hours) (F, H, I) or p38α/β (SB203580; 10 μM, 24 hours) (G).

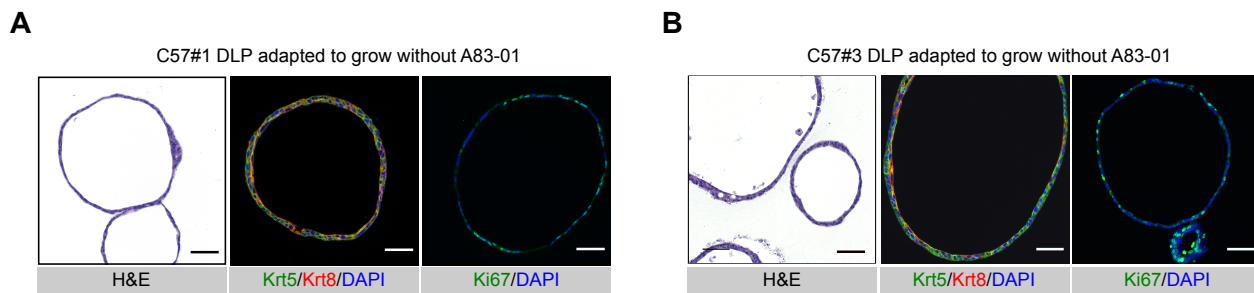


Figure S4. Mouse prostate organoids adapted to the absence of the Tgf- β receptor inhibitor A83-01 retain quasi-normal cellular identities and cytoarchitecture, related to Figure 3

A-B Representative H&E and immunofluorescence analyses of C57#1 and C57#3 DLP mouse prostate organoids upon adaptation to A83-01 withdrawal (scale bar = 50 μ m). Please note the adapted organoids retain a pattern of cytokeratins expression consistent with normal cellular identities, including basal, luminal P (lumP) and peri-urethral (PrU).

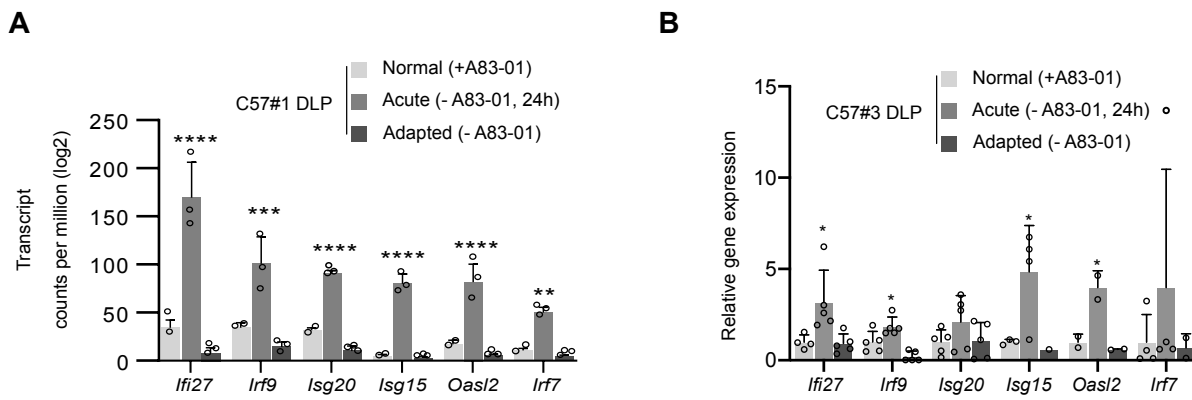


Figure S5. Non- canonical Activin A signalling regulates type-I interferon genes in mouse prostate organoids, related to Figure 3

A-B mRNA expression levels for selected type-I IFN response genes in C57#1 and C57#3 DLP mouse prostate organoids cultured in complete medium (normal), upon acute A83-01 withdrawal (acute), and adapted to grow without A83-01 (adapted). Transcription of type-I interferon genes is enhanced upon A83-01 withdrawal and reduced upon adaptation (n = 3 biological replicates, bulk-RNAseq analysis (A) or qPCR (B); individual data points are shown with bar graphs representing mean value and SD; two-way ANOVA, Tukey's test, p-value * (<0.05), ** (<0.01), *** (<0.001), **** (<0.0001).

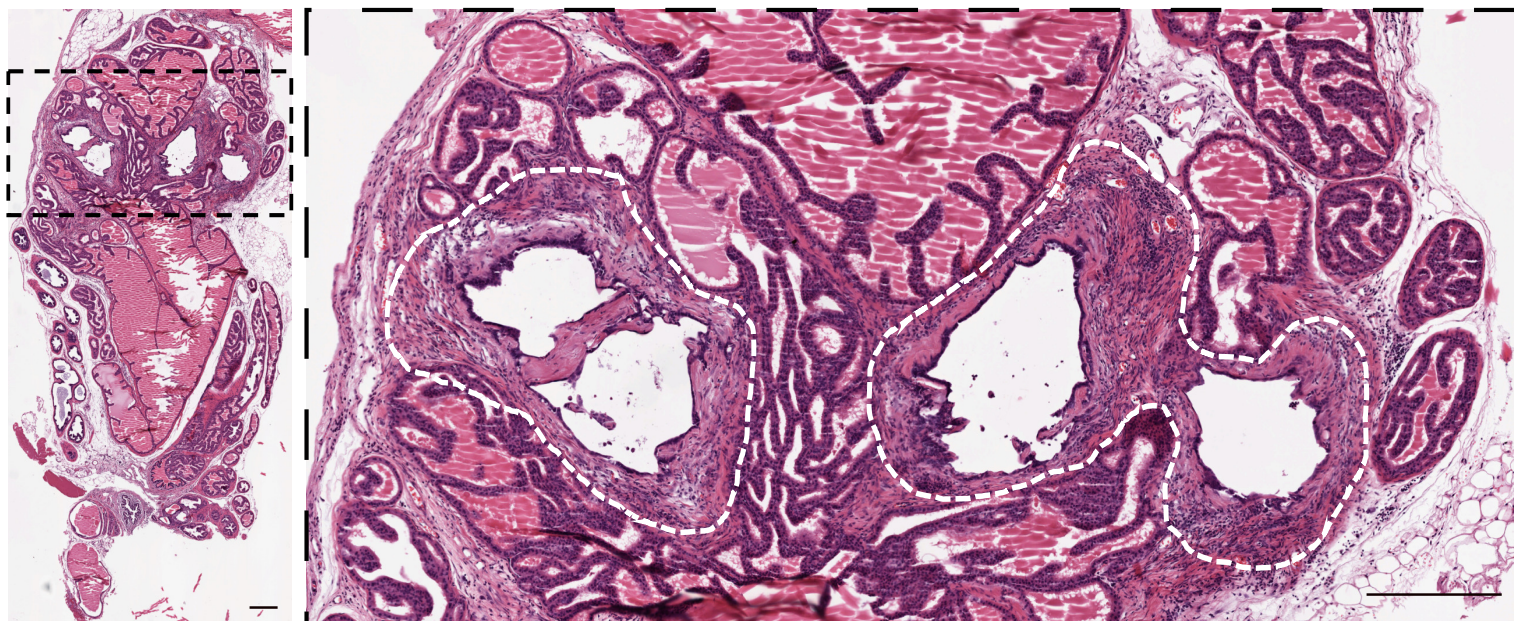


Figure S7. H&E image of the anterior lobe of adult mouse prostate transplanted with organoids adapted to grow in the absence of A83-01, related to Figure 4. Dashed white lines in the magnification delimit the reactive stroma surrounding the organoid-derived tissue. Scale bar = 0.5 mm.

Appendix Table S1. Small molecules used in this study

Small molecules	Source	Identifier
A83-01 (f.c. 200 nM)	Tocris	2393
R-Spondin 1 c.m. (f.c. 10% v/v)	R&D	3710-001-01
Noggin (f.c. 100 ng/mL)	PeproTech	120-10C
DHT (f.c. 10 nM)	Merk	10300
Egf (f.c. 50 ng/mL)	PeproTech	315-09
Y-27632 (f.c. 10 μ M)	Calbiochem	146986-50-7
SB203580 (f.c. 10 μ M)	Tocris	1202
SB202190 (f.c. 10 μ M)	PreproTech	1523072
SB431542 (f.c. 10 μ M)	Sigma Aldrich	S4317
SD-208 (f.c. 100 nM, 1 μ M)	Selleckchem	S7624
Ralimetinib* (f.c. 1 μ M)	Selleckchem	S1494
Pirfenidone (f.c. 10 μ M)	Cayman	13986
Takinib (f.c. 5 μ M)	Sigma Aldrich	SML2216
IFN β (f.c. 50 ng/mL)	SinoBiol	50708-MCCH5
Activin A (f.c. 50 ng/mL)	R&D Systems	338-AC
Tgfb1 (f.c. 500 ng/mL)	SinoBiol	50698-M08H
Follistatin (f.c. 500 ng/mL)	PeproTech	120-13
MAB3381 (f.c. 1 μ g/mL)	R&D	MAB3381

Appendix Table S2. Antibodies used in this study

Antibody	WB	IF	Source	Identifier
β-Actin	1:2000		Sigma-Aldrich	A2228; RRID:AB_476697
β-Tubulin	1:4000		SCBT	sc-5274; RRID:AB_476697
Activin A	1:1000		R&D	MAB3381; RRID:AB_2125864
Akt	1:2000		CST	9272; RRID:AB_329827
Akt pS473	1:1000		CST	4060; RRID:AB_2315049
Akt pT308	1:1000		CST	9275; RRID:AB_329828
Ar	1:500	1:500	SCBT	sc-816; RRID:AB_1563391
Atf2 pT71	1:1000		CST	9221; RRID:AB_2561045
Atm	1:1000		CST	2873; RRID:AB_2062659
Atm pS1981	1:1000		CST	5883; RRID:AB_10835213
c-Myc	1:1000		Abcam	ab32072; RRID:AB_731658
Chk1	1:1000		CST	2360; RRID:AB_2080320
Chk1 pS35	1:1000		CST	2348; RRID:AB_331212
Cyclin D1	1:1000		Abcam	ab134175; RRID:AB_2750906
Cyclin E1	1:1000		CST	20808; RRID:AB_2783554
Ki67		1:200	eBioscience	14-5698-82; RRID:AB_10854564
Egfr		1:200	Abcam	ab52894; RRID:AB_869579
Erk	1:1000		CST	9102; RRID:AB_330744
Erk pT/Y204	1:1000		CST	4370; RRID:AB_2315112
Fibrillarin	1:500		Abcam	ab4566; RRID:AB_304523
Gapdh	1:4000		Thermo Fisher	MA515738; RRID:AB_10977387
Krt5	1:500	1:500	BioLegend	905901; RRID:AB_2565054
Krt8/18	1:1000	1:1000	Abcam	ab53280; RRID:AB_869901
Krt8		1:200	Merk	MABT329; RRID:AB_2891089
Krt7		1:250	Abcam	ab68459; RRID:AB_1139824
Mapkapk2 pT334	1:1000		CST	3041; RRID:AB_330726
p16	1:500		Abcam	ab211542; RRID:AB_2891084
p21	1:500		Abcam	ab109520; RRID:AB_10860537
Ppp1r1b		1:400	Invitrogen	MA5-14968; RRID:AB_10981784
Aevr1b		1:500	R&D Systems	AF1477; RRID:AB_2242240
p38	1:1000		CST	9212; RRID:AB_330713
p38 pT180/Y182	1:500		CST	9215; RRID:AB_331762
Smad2 pS465/7	1:1000		CST	8828; RRID:AB_2631089
Smad2/3	1:1000		CST	8685; RRID:AB_10889933
Smad4	1:1000		SCBT	sc-7966; RRID:AB_627905
Stat1	1:1000		BD Biosciences	610186; RRID:AB_397585
Stat1 pS727	1:1000		CST	9177; RRID:AB_2197983
Stat1 pY701	1:1000		CST	9171; RRID:AB_331591
Stat2	1:1000		CST	4597; RRID:AB_2198305
Stat3	1:1000		CST	9132; RRID:AB_331588
Stat3 pY705	1:1000		CST	9131; RRID:AB_331586
Stat5	1:1000		CST	9358; RRID:AB_659905
Stat5 pY694	1:1000		CST	9359; RRID:AB_823649
Tak1	1:1000		CST	5206; RRID:AB_10694079
Trp53	1:500		Abcam	ab26; RRID:AB_303198
Cd24a		1:800	eBioscience	17-0242-82; RRID:AB_10870773
Sca-1		1:800	BioLegend	122514; RRID:AB_756199
Parp	1:1000		CST	9542S; RRID:AB_2160739
Cleaved-Caspase 3	1:1000		CST	9661S; RRID:AB_2341188

Appendix Table S3. Primers used in this study

Oligonucleotides	Source
<i>Ifi27 fw</i> :ggcttcattgtctccaaga	This paper
<i>Ifi27 rv</i> :accttcagtgctccaagtgc	This paper
<i>Isg20 fw</i> :cttctgaaaggcaagctggt	This paper
<i>Isg20 rv</i> :tcttgtagcaggcgtta	This paper
<i>Irf9 fw</i> :ctcttggtcagcgccttg	This paper
<i>Irf9 rv</i> :tccagaaatgtagggtgc	This paper
<i>Isg15 fw</i> :gagctagagcctgcagcaat	This paper
<i>Isg15 rv</i> :taagaccgtcctggagcact	This paper
<i>Oasl2 fw</i> :ctccggttggtgaagttctg	This paper
<i>Oasl2 rv</i> :gtcgcggtagttacagga	This paper
<i>Irf7 fw</i> :gaagaccaactccgctgtg	This paper
<i>Irf7 rv</i> :agcattgctgaggctcactt	This paper
<i>Trp63 fw</i> :agaacggcgatggtacgaag	This paper
<i>Trp63 rv</i> :tctcacgacctctcactggt	This paper
<i>Ar fw</i> :gccaggagtggtgtgtgccg	This paper
<i>Ar rv</i> :aagttgcggaagccaggcaagg	This paper
<i>Tmprss2 fw</i> :gccgcctccggagatttaag	This paper
<i>Tmprss2 rv</i> :aggtgacctgagttcaatgc	This paper
<i>Pscs fw</i> :gctcactgcaaccatgaaga	This paper
<i>Pscs rv</i> :gctaagtaggtggccagcag	This paper
<i>Nkx3.1 fw</i> :cggaccctaggagggactc	This paper
<i>Nkx3.1 rv</i> :cacctgagtgtagagaaggc	This paper
<i>Mme fw</i> :ctccaacttctcccaatccc	This paper
<i>Mme rv</i> :tcgagcagctgatttatgcagt	This paper
<i>Pbsn fw</i> :tgcacagtatgaaggagcat	This paper
<i>Pbsn rv</i> :tccgtgtccatgatacgctg	This paper
<i>Fkbp5 fw</i> :agccgggaagcctaagtttg	This paper
<i>Fkbp5 rv</i> :acttgctccctgaagtaca	This paper
<i>Tgm4 fw</i> :ctagagtcttgacagggtgc	This paper
<i>Tgm4 rv</i> :tggtactcggatgtgtggtg	This paper
<i>Sbp fw</i> :ccagattgtaccacaaaccctc	This paper
<i>Sbp rv</i> :ccgtctccagaaggaagtc	This paper
<i>Sdha fw</i> :tggtgagaacaagaagcatca	This paper
<i>Sdha rv</i> :cgctacaaccacagcatca	This paper
<i>Tbp fw</i> :cggtgcgctattttctccgc	This paper
<i>Tbp rv</i> :gtggggaggccaagccctga	This paper
<i>Actb fw</i> :atccagctcctagcacgcacg	This paper
<i>Actb rv</i> :gtccccgcttgccgcagtg	This paper
<i>Gapdh fw</i> :gagagtgttctcgtcccg	This paper
<i>Gapdh rv</i> :actgtgccgttgaattgcc	This paper

Appendix Table S4. Deposited RNA sequencing data produced in this study

RNA sequencing data	Source
C57BL/6J #1 AP	This paper
C57BL/6J #1 DLP	This paper
C57BL/6J #1 VP	This paper
C57BL/6J #2 AP	This paper
C57BL/6J #2 DLP	This paper
C57BL/6J #2 VP	This paper
C57BL/6J #3 AP	This paper
C57BL/6J #3 DLP	This paper
C57BL/6J #3 VP	This paper
C57BL/6J #1 DLP Activin A Adapt.	This paper
C57BL/6J #1 DLP shSmad4	This paper
C57BL/6J #3 DLP Activin A Adapt.	This paper
C57BL/6J #3 DLP shSmad4	This paper
BALB/cByJ #1 AP	This paper
BALB/cByJ #1 DLP	This paper
BALB/cByJ #1 VP	This paper
BALB/cByJ #2 AP	This paper
BALB/cByJ #2 DLP	This paper
BALB/cByJ #2 VP	This paper
CD1 #1 AP	This paper
CD1 #1 DLP	This paper
CD1 #1 VP	This paper
CD1 #2 AP	This paper
CD1 #2 DLP	This paper
CD1 #2 VP	This paper

Appendix Table S5. Chemicals and recombinant proteins used in this study

Reagent	Source	Identifier
Matrigel	Corning	356231
BME-2	AMSBIO	3533
Dispase II (f.c. 1-10 mg/mL)	Life Tech	17105041
TrypLE	Life Tech	12605010
Collagenase II (f.c. 1 mg/mL)	Life Tech	17101015
Polybrene (f.c. 4 µg/mL)	Sigma Aldrich	H9268
Propidium iodide (f.c. 1 µM)	Life Tech	P3566
RNAse A (f.c. 0.5 µg/mL)	Life Tech	12091021
TO-PRO-3	Life Tech	T3695
Collagen type-I	Corning	354249
ATRA	Merck	R2625
Paraformaldehyde (f.c. 4% v/v)	Sigma Aldrich	P6148
Citrate-based buffer	Vector Lab	H3300
Hoechst 33342	Abcam	ab145597
FluorSave	Merck	345789
Vectastain Elite ABC peroxidase kit	Vector Lab	PK-6100
DPX mounting medium	Sigma	06522
Gill haematoxylin	Merck	GH5232
Eosin Y	Merck	HT110132
RNeasy Plus Micro kit	Qiagen	74034
iScript kit	Biorad	1708891
Phusion Universal qPCR kit	Life Tech	F566L
qPCRBIO SyGreen Mix	PCRBiosystems	PB20.14-05
TruSeq mRNA library prep kit	Illumina	RS-122-2101
Halt Protease inhibitor	Life Tech	87786
Phosphatase Inhibitor Mix II	Serva	3905501
NE-PER kit	Life Tech	78833
BCA Protein Assay kit	Thermo Fisher Sci.	23225
PVDF membrane	Merck	GE10600023
ECL LiteAblot plus kit A+B	Euroclone	GEHRPN2235
Click-iT protein enrichment kit	Thermo Fisher Sci.	C10416
Click-iT Plus EDU kit	Thermo Fisher Sci.	C10632
Quantikine ELISA kit	R&D Systems	DAC00B
Nocodazole (f.c. 15 µM)	Sigma Aldrich	SML1665
ProLong Antifade	Invitrogen	P36934
21XMouse Multicolor FISH kit	MetaSystems	D-0425-060-DI
DAPI	Abcam	6843.2
XMP X-probe	MetaSystems	D-1420-050-OR
XMP Y-probe	MetaSystems	D-1421-050-FI
DAPI Antifade	MetaSystems	D-0902-500-DA
Cy3 PNA probe	DAKO Cytomation	K5326

Appendix Table S6. Software and algorithms used in this study

Software and algorithms	Source
FlowJo v.10	FlowJo
ZEN	Zeiss
ImageJ v.2.0.0-rc-69/1.52i	Rueden, et al., 2017
Bio-Rad CFX Manager (v.3.1)	Bio-Rad
GraphPad PRISM (v.6.01)	Graphpad
EdgeR-3.20.9	Robinson et al., 2010
STAR-v2.6.0	Dobin et al., 2013
GSEA (v4.0.3)	UCSD/Broad Institute
DESeq2	Love et al., 2014
Alliance LD2	UVITEC
Perseus	Max-Planck Inst. of Bioch.
ISIS	MetaSystems