### Supplemental information:

Supplementary Figure S1 is related to Figure 1 Supplementary Figure S2 is related to Figure 2 Supplementary Figure S3 is related to Figure 3 Supplementary Figure S4 is related to Figure 4 Supplementary Figure S5 is related to Figure 5 Supplementary Table S1 is related to Figure 4 Supplementary Table S2 is related to Figure 1 and Figure 4 Supplementary Table S3 is related to Figure 1 to Figure 5

#### Figure S1 related to Figure 1 Establishment of murine prostate cultures

A: Schematic overview of organoid establishment in 3D Matrigel culture.

**B:** Karyotyping results of a passage 52 (1-year) organoid culture (N=15). 39-41 chromosomes are considered normal, due to the assay limitations

**C:** RT-PCR analysis of prostate organoids shows that both luminal and basal markers are expressed under DHT and castrate conditions.

**D:** IHC staining of Ck5 in murine organoids under DHT and castrate conditions.

**E**: Organoids can undergo cycles of DHT addition and withdrawal. A castrate appearance is observed 5-7 days after DHT withdrawal. A folded glandular-like appearance is evident 5-7 days after DHT addition. Scale bars represent 50 microns. **F**: Brightfield pictures showing establishment of murine organoids from different lobes in the presence and absence of the TGF-β inhibitor (A83-01.) Only DLP-derived organoids can growth in the absence of the TGF-β pathway inhibitor. Scale

bars represent 50 microns.

**G**: Representative experiment of organoids formed in the absence of Noggin, R-spondin and DHT. 1000 cells were seeded in 20  $\mu$ l of Matrigel. Organoid establishment rate is diminished in the absence of Noggin, R-spondin and DHT.

**H**: Histogram showing passage number of organoids in absence of designated growth factors. EGF is essential for proliferation. TGF- $\beta$  inhibitor A83-01 is essential for growth of AP and VP derived organoids. R-spondin, Noggin and DHT are not essential for organoid survival, but influence proliferation rate.

**I**: RT-PCR of Lgr receptors in organoids. *Lgr4* is strongly expressed in organoids. *Lgr5* expression is detected. Lgr5-lacZ reporter organoids show expression of LacZ in a small subset of cells.

**J**: H&E staining of murine prostate lobes, organoids derived from their respective lobes and UGSM/recombinations. 50.000 organoid cells were recombined with UGSM from **e17.5** embryonic rats. Recombinations were harvested and analyzed 8 weeks post implantation. Organoids derived from all lobes formed glandular structures in the UGSM recombination assay. Scale bars represent 50 microns.

#### Figure S2 related to Figure 2 Basal and Luminal cells give rise to organoids

**A:** Anti-CD24 (HSA) staining on murine prostate. CD24 specifically stains luminal cells. AP is shown.

**B:** IF staining of FACS isolated CD49f-expressing basal cells and CD24-expressing luminal cells. Green: basal marker Ck5, red: luminal marker Ck8, pink: pan leukocyte markers CD11b and CD45. Scale bars represent 50 microns.

**C:** Representative brightfield images of outgrowth of single CD49f-expressing basal and CD24-expressing luminal cells. No obvious morphological differences were detected.

# Figure S3 related to Figure 3 Genetically engineered mouse prostate cancer models are recapitulated in organoids

**A:** Schematic overview of mouse strains used in experiments.

**B:** H&E staining of organoids derived from the dorsolateral lobe of wildtype, PBCre Erg and PBcre Pten/Erg mice and after transplantation in UGSM tissue recombination experiments. *In vitro* organoids and tissue from UGSM tissue recombination experiments resemble phenotypes seen *in vivo*. Scale bars represent 50 microns.

**C:** Quantitative RT-PCR analysis of *Fkbp5* expression in organoids derived from each prostate lobe from all genotypes in the presence of DHT (1 nM) and in castrate conditions (24 hours). All lobes and all genotypes are DHT responsive. Expression was normalized to *Hprt*. Results are shown as mean  $\pm$  SD.

**D**: Quantitative RT-PCR analysis of *Pten* and *Psca* expression in shPten and shScrcontrol infected organoids derived from each lobe from PBCre Rosa26 <sup>LSL-ERG</sup> mice. Upon *Pten* knockdown Ar target expression is reduced. Expression was normalized to *Hprt*. Results are shown as mean  $\pm$  SD.

**E:** Western blot analysis of PI3K pathway activation in shPten and ShScr-control infected organoids derived from each lobe from PBCre Rosa26 <sup>LSL-ERG</sup> mice. Levels of phosphorylated Akt and pS6 are increased upon *Pten* knockdown.

# Figure S4 related to Figure 4 Establishment of human prostate organoid cultures

**A:**\_Schematic overview of organoid establishment from normal human prostate tissue.

**B:** Karyotyping of passage 20 (6 months) organoids (N=15), 45-47 chromosomes were considered as normal in the conditions used in this assay

**C:** Representative experiment of relative organoid establishment in the absence of indicated growth factors

**D**: Passage number of organoids in absence of the indicated growth factors

**E**: Quantitative RT-PCR of *AR* mRNA levels in the absence of growth factors. Withdrawal of Noggin, R-spondin, FGF10 and Nicotinamide led to reduced *AR* expression. Expression was normalized to *GAPDH*. Results are shown as mean  $\pm$  SD. **F**: Quantitative RT-PCR analysis of *PSA* mRNA levels after exposure of organoids to decreasing levels of EGF (50, 5, 0.5, 0 ng/ml) for 24 hours. *PSA* mRNA levels are elevated with reduced EGF. Expression was normalized to *GAPDH*. Results are shown as mean  $\pm$  SD.

**G**: Quantitative RT-PCR analysis of *AR* mRNA levels after exposure of organoids to decreasing levels of EGF (50, 5, 0.5, 0 ng/ml) for 24 hours. *AR* mRNA levels are

modestly elevated with reduced EGF. Expression was normalized to *GAPDH*. Results are shown as mean  $\pm$  SD.

**H:** Western blot of AR expression levels following decreasing levels of EGF (50, 5, 0.5, 0 ng/ml). pS6 levels serve as a control for induction of EGF pathway signaling. GAPDH was probed as control.

### Figure S5 related to Figure 5 Establishment of human prostate organoid cultures from luminal and basal cells

**A:** IF staining of FACS isolated CD49f-expressing basal cells and CD26-expressing luminal cells. Green: basal marker CK5, Red: luminal marker CK8, Pink: pan leukocyte markers CD11b and CD45. Scale bars represent 50 microns.

**B:** IF staining of FACS isolated CD26-expressing luminal cells. Green: luminal marker NKX3.1, red: luminal marker CK8. Quantitative RT-PCR of *NKX3.1* mRNA levels in FACS isolated CD49f-expressing basal cells and CD26-expressing luminal cells. Luminal cells express higher levers of *NKX3.1*.

**C:** IF staining of AR (pink) and EPCAM (green) in CD26-derived luminal organoids and CD49f-derived basal organoids at passage 4, cultured under standard conditions. Both cultures contain AR expressing cells.

**D**: Karyotyping of passage 12 luminal and basal organoids (4 months) showing a stable karyotype with 46 chromosomes (45-47 chromosomes were considered as normal, due to the limitations of this assay).

**E:** Representative experiment of relative organoid establishment by CD26expressing luminal cells in the absence of indicated growth factors. Organoid formation is reduced in the absence of Noggin, R-spondin and DHT.

**F:** Impact of removal of various growth factors on passaging of luminal organoids.

**G:** RT-PCR analysis of expression of Lgr receptors in human organoids and cell populations. *Lgr4* is strongly expressed in both luminal- and basal cell-derived organoids.

**H:** IHC staining for AR in luminal and basal organoids in DHT (1 nM) or castrate conditions. Nuclear AR localization is observed in the presence of DHT in both organoid types. Scale bars represent 50 microns.

**I:** Quantitative RT-PCR of *PSA* mRNA levels at decreasing EGF concentrations (50, 5, 0.5, 0 ng/ml, 24 hours) in the indicated organoid types. *PSA* mRNA levels are higher at low EGF concentrations. Expression was normalized to *GAPDH*. Results are shown as mean  $\pm$  SD.

|      | oid culture |            |        |                      |           |                      |           |                                     |
|------|-------------|------------|--------|----------------------|-----------|----------------------|-----------|-------------------------------------|
| Chro | Position    | dbSNP ID   | Change | Call p2 <sup>a</sup> | Allele    | Call p7 <sup>a</sup> | Allele    | Independent validation <sup>b</sup> |
| m    |             |            |        |                      | depth     |                      | depth     |                                     |
|      |             |            |        |                      | (ref/alt) |                      | (ref/alt) |                                     |
| 1    | 12837619    | rs19950694 | A>G    |                      | 73/3      |                      | 54/13     |                                     |
|      |             | 0          |        | 0/1                  |           | 0/1                  |           | Heterozygous in both cultures       |
| 1    | 14529983    | rs20047332 | G>A    |                      | 30/0      |                      | 21/5      |                                     |
|      | 8           | 5          |        | 0/0                  |           | 0/1                  |           | Heterozygous in both cultures       |
| 2    | 96521815    | rs11326795 | C>A    |                      | 38/1      |                      | 24/6      |                                     |
|      |             | 1          |        | 0/0                  |           | 0/1                  |           | Heterozygous in both cultures       |
| 6    | 32713788    | rs74223470 | T>G    | 0/0                  | 135/5     | 0/1                  | 80/17     | Failed                              |
| 6    | 32713793    | rs62623408 | T>G    | 0/0                  | 132/5     | 0/1                  | 85/17     | Failed                              |
| 10   | 12667812    | -          | T>A    |                      | 24/0      |                      | 16/5      |                                     |
|      | 8           |            |        | 0/0                  |           | 0/1                  |           | False positive                      |
| 16   | 88765509    | -          | T>G    | 0/0                  | 20/1      | 0/1                  | 15/8      | False positive                      |
| 9    | 10736144    | rs14657611 | T>C    |                      | 7/196     |                      | 3/161     | Homozygous alt in both              |
|      | 9           | 5          |        | 0/1                  |           | 1/1                  |           | cultures                            |
| 9    | 10736145    | rs20154043 | G>A    |                      | 9/189     |                      | 3/153     | Homozygous alt in both              |
|      | 1           | 3          |        | 0/1                  |           | 1/1                  |           | cultures                            |
| 9    | 10736145    | rs19966529 | C>G    |                      | 9/184     |                      | 3/151     | Homozygous alt in both              |
|      | 2           | 2          |        | 0/1                  |           | 1/1                  |           | cultures                            |
| 1    | 12061200    |            | CGG>C  |                      | 41/2      |                      | 29/8      | Failed                              |
|      | 2           |            |        | 0/0                  |           | 0/1                  |           |                                     |
| 10   | 12667812    | rs14091354 | GT>G   |                      | 23/0      |                      | 16/5      | False positive                      |
|      | 2           | 3          |        | 0/0                  |           | 0/1                  |           | -                                   |
| 11   | 59710498    |            | TC>T   | 0/0                  | 99/5      | 0/1                  | 84/16     | False positive                      |
| 14   | 76349013    |            | CT>C   | 0/0                  | 26/1      | 0/1                  | 17/6      | False positive                      |
| 13   | 25021144    | rs78060735 | GTCT>G | 0/1                  | 3/65      | 1/1                  | 0/72      | False positive                      |
| 16   | 88723538    | rs33956101 | T>TG   | 0/1                  | 4/24      | 1/1                  | 1/19      | False positive                      |
| Y    |             | rs20011045 | C>CT   | •                    | 4/18      |                      | 1/20      | False positive                      |
|      | 14954404    | 2          |        | 0/1                  |           | 1/1                  | •         | -                                   |

Table S1 related to Figure 4 Whole exome sequencing analysis of early and late passage prostate organoid cultures

DNA isolated from prostate organoid cultures passage 2 (p2) and passage 7 (p7) were whole exome sequenced and differences in genetic variation between the cultures were determined.

<sup>a</sup>Genotype calls by GATK: 0/0, homozygous reference call; 0/1, heterozygous call; 1/1, homozygous alternative call. <sup>b</sup>Candidate somatic variation was independently validated by capillary sequencing. Candidate variation were considered false positive (not *de novo*) if the mutation was not validated (not present) or present in both cultures. All the variation that could be validated were false positives.

#### **Extended experimental procedures**

#### Isolation & culture of prostate epithelial cells

Murine prostates were isolated from wildtype C57Bl/6, Rosa<sup>LSL-ERG</sup>, Pten<sup>loxP/loxP</sup>, Pten<sup>loxP/loxP</sup>, Rosa<sup>LSL-ERG</sup> or Lgr5-LacZ mice. All lines were crossbred to C57bl/6 background. Murine prostates were divided into three lobe pairs, the Anterior Prostate (AP), Dorsolateral Prostate (DLP) and Ventral Prostate (Marker et al., 2003). Subsequently each lobe was placed in 5 mg ml<sup>-1</sup> collagenase type II (Gibco) in ADMEM/F12 and digested for 1 to 2 hours at 37°C. Glandular structures were washed with ADMEM/F12 and centrifuged at 100\*G. Subsequently structures were digested in 5 ml TrypLE (Gibco) with the addition Y-27632 10 µM (Sigma) for 15 min at 37°C. Trypsinized cells were washed and seeded in growth factor reduced Matrigel (Corning). Murine prostate epithelial cells were cultured in ADMEM/F12 supplemented with B27 (Life technologies), 10 mM Hepes, Glutamax (Life technologies) and Penicillin/Streptavidin and contained following growth factors: EGF 5-50 ng/ml (Peprotech), R-spondin1 conditioned medium or 500 ng/ml

recombinant R-spondin1 (Peprotech), Noggin conditioned medium or 100 ng/ml recombinant Noggin (Peprotech) and the TGF-\u00b3/Alk inhibitor A83-01 (Tocris). Dihydrotestosterone (Sigma) was added at 0.1-1 nM final concentration. Murine prostate organoids were passaged either via trituration with a glass Pasteur pipet or trypsinization with TrypLE for 5 min at 37 °C. Passage was performed every week with either a 1:3 (castrate conditions) or a 1:5 (+DHT conditions) ratio. Human prostate samples were obtained from patients undergoing radical prostatectomy according to guidelines from the UMC Utrecht. Prostate samples were palpitated by a pathologist to identify regions of normal tissue before sectioning, then tissue status of the sample was confirmed by histology. Prostate pieces were minced and digested in 5 ml of 5 mg ml<sup>-1</sup> collagenase type II (Gibco) in complete prostate medium (described below) and digested for 14-16 hours at 37°C gently shaking. Subsequently structures were digested in 5 ml TrypLE (Gibco) with addition of Y-27632 10 μM (Sigma) for 15 min at 37 °C. Trypsinized cells were washed and seeded in growth factor reduced Matrigel (Corning). Human prostate organoids were cultured in ADMEM/F12 containing growth factors as above, with the addition of 10 ng/ml FGF10 (Peprotech), 5 ng/ml FGF2 (Peprotech), 1 µM Prostaglandin E<sub>2</sub> (Tocris), SB202190 (10 µM) (Sigma-Aldrich), 10 mM Nicotinamide (Sigma-Aldrich) and Dihydrotestosterone (Sigma-Aldrich) was added at 0.1-1 nM final concentration. Human prostate organoids were passaged either via trituration with a glass Pasteur pipet or trypsinization with TrypLE for 5 min at 37 °C. Passage was performed with a 1:2 ratio every 7 to 14 days. A detailed summary of medium composition and growth factor function can be found in supplementary table 2.

#### **UGSM essay**

UGSM recombination essay was performed as described previously (Xin et al., 2003). In short 50.000 organoid cells from passage 8 were recombined with 250.000 UGSM cells and placed under the kidney capsule of nude mice. Recombinations were harvested 8 weeks post implantation. All mouse work was performed in accordance with national guidelines and regulations of the Hubrecht institute or Memorial Sloan Kettering Cancer Center.

#### FACS

Trypsinized cells were filtered using a 42-micron mesh to ensure a single cell suspension. Cells were blocked for 10 min with 2% FCS in ADMEM/F12. Human cells were stained using CD49f-alexa 647 conjugated antibody (1:200, GoH3, BD Biosciences) and CD26-FITC conjugated antibody (1:200, M-A261, eBioscience) in 0.05% FCS in ADMEM/F12 for 2 hours on ice. Murine cells were stained using a Cd49f-PE conjugated antibody (1:200, GoH3, BD Biosciences) and CD24-alexa 647 conjugated antibody (1:200, 30-F1, eBioscience) in 0.05% FCS in ADMEM/F12 for 2 hours on ice. Cells were sorted using MoFlo (Dako).

#### Karyotyping

Karyotyping was performed as described previously (Sato et al., 2009). In short, organoids were treated with Colcemid (Gibco) for 16 hours. Cells were trypsinized, washed and fixed using MeOH: acetic acid 1:1. Cells were washed 3x with MeOH: acetic acid 1:1. Cells were plated from height on glass slides and metaphase spreads were visualized using DAPI. Slides were mounted with vectashield and analyzed on a DM6000 Leica microscope. Images were processed using Leica LAS software.

#### Genetic analysis

From a single prostate organoid culture DNA was isolated from an early (p2) and a late (p7) passage. DNA sequence libraries were generated from 1 µg of DNA using standard protocols (Illumina). The sequence libraries were subjected to whole exome enrichment using the SureSelectXT Human All Exon V5 capture design according to manufacturer's protocol (Agilent) and subsequently sequenced with paired-end (2 x 100 bp) runs using Illumina HiSeq 2500 sequencers to a average depth of 100 x base coverage in the capture design. Sequence reads were mapped against human reference genome GRCh37 using Burrows-Wheeler Aligner (BWA) 0.7.5a (Li and Durbin, 2009) with settings 'bwa mem -c 100 -M' resulting in samplespecific BAM files. Genome Analysis Toolkit (GATK) (DePristo et al., 2011)Haplotypecaller was used to call SNPs and indels on all samples simultaneously. Variants are flagged as PASS only if they do not meet the following criteria: OD < 2.0, MO < 40.0, FS > 60.0, HaplotypeScore > 13.0, MORankSum < -12.5, ReadPosRankSum < -8.0, snpclusters >=3 in 35bp. For indels: QD < 2.0, FS >200.0, ReadPosRankSum < -20.0. We only considered positions that were covered at least 20 x in both p2 and p7 cultures. To obtain canditate variants which are specifically induced by long-term culturing, we filtered for base substitutions and indels that were uniquely called in p7 culture with a minimal alternative allele frequency of 0.15 while allowing a maximal alternative allele frequency of 0.05 in the p7 culture. Furthermore, to account for possible loss-of-heterozygocity of variant positions during long-term cultering, we filtered for positions that were called heterozygous in the p2 culture and homozygous mutant in the p7 culture. This filtering resulting in the identification of 17 candidate variant positions (Table S1). To validate these candidate variants we PCR amplified the loci harboring the variants (primers are available upon request), followed by cappillary sequencing (Table S1). The data for the whole exome sequencing were deposited to the EMBL European Nucleotide Archive, accession number: ERP006541.

#### Immunohistochemistry and Immunofluorescence

Organoids were fixed using 4% Paraformaldehyde at 4°C overnight. Fixed organoids were washed and transferred to paraffin according to standard techniques. Immunohistochemistry was performed as described previously (Sato et al., 2009). In short, antigen retrieval was performed by boiling samples for 20 minutes with either sodium citrate or EDTA buffers. Blocking was performed using 1% BSA in PBS0 for 30 minutes at room temperature. Following antibodies were used for

staining on murine and human prostate organoids AR (1:1000, N-20, Santa Cruz) CK5 (1:2000, AF-138, Covance), CK8 (1:50, C-51, Santa Cruz), PSA (1:1000,Dako), p63 (1:800, 4A4, Millipore). Stainings were visualized using bright vision (Dako). LacZ staining of organoids was done as described previously (Sato et al., 2009).

For immunofluorescent stainings organoids were washed using ADMEM/F12 to remove matrigel. Subsequently organoids were fixed using 4% PFA in PBS for 1 hour on ice, followed by permeabilization/blocking with 1% BSA, 1% Triton X-100 in PBS0 for 30 min on ice. Stainings were performed in 0.05% BSA 0.1% Triton X-100 in PBS0 overnight at 4°C. Organoids were stained using the following antibodies AR (1:200, N-20, Santa Cruz) Ck5 (1:500, AF-138, Covance), Ck8 (1:50, C-51, Santa Cruz), NKX3.1 (Rabbit, 1:100, Kind gift of Michael Shen). Subsequently organoids were washed with PBS0, 3x 10 minutes and subsequently stained with alexa-fluor (-488, -568, -647) conjugated antibodies in 0.05% BSA 0.1% triton X-100 in PBS0 for 1 hour in the dark at room temperature. DNA was stained using DAPI. Images were made using a Leica Sp5 inverted microscope. Images were processed using Leica LAS software.

#### RNA isolation, cDNA preparation, qPCR

RNA was isolated from organoids using Qiagen RNeasy kits according to manufacturers protocol. 100-1000 ng RNA was reverse-transcribed to cDNA using GoScript reverse transcriptase (Promega) according to manufacturers protocol. Quantitative RT-PCR was performed using IQ<sup>™</sup> SYBR green mix (Bio-Rad) according to manufacturers protocol. QPCR was performed using primers from supplementary table 3.

#### Lentiviral infections

Lentiviral infections were performed as described previously (Koo et al., 2012) using pLKO.1-puro-UbC-TagFP635 from Sigma targeting *Pten* (CGACTTAGACTTGACCTATAT) or control Scramble (CCTAAGGTTAAGTCGCCCTCG). In short, 100.000 single cells were infected with an MOI 1\*10^3. Infection was done during centrifugation for 1 hour at 600\*G RT. Cells were subsequently placed at 37 °C. 5% CO<sub>2</sub> for 3 hours to recover. Cells were plated in Matrigel and 24 hours post seeding 1  $\mu$ g/ml puromycin was applied for 2 days to ensure only infected cells remained.

#### Western blot

Samples were lysed using RIPA buffer (50 mM Tris-HCL pH 8.0, 150 mM NaCL, 0.1% SDS, 0,5% Na-Deoxycholate, 1% NP-40) containing Complete<sup>TM</sup> protease and phosphatase inhibitors (Roche). Protein content was quantified using standard Bradford assay. 10  $\mu$ g of protein was loaded on gradient polyacrylamide gels (4-

12%) and subsequently transferred to a PVDF membrane. Membranes were probed with antibodies directed against AR (1:000, N-20, Santa Cruz), GAPDH (1:2000 Abcam) AKT (1:1000 Cell Signaling #9272), phosphorylated-AKT (Ser473) (1:1000 Cell Signaling #4060) and Phosphorylated-S6 (Ser235/Ser236) (1:1000, Cell Signaling #2211). Following washes membranes were incubated with secondary HRP conjugated antibodies (goat anti-Rabbit HRP 1:2000, anti goat HRP 1:2000 and Rabbit anti-mouse 1:2000 all GE healthcare). ECL (G&E healthcare) Signal was detected using LAS 5000 (GE healthcare)

#### Supplementary Table S2 related to Figure 1 & Figure 4:

Basic medium was as in Sato *et al.* (Sato et al., 2009); ADMEM/F12 medium supplemented with B27 (life technologies), 10 mM Hepes, Glutamax (life technologies) and penicillin/streptavidin (Sato et al., 2009).

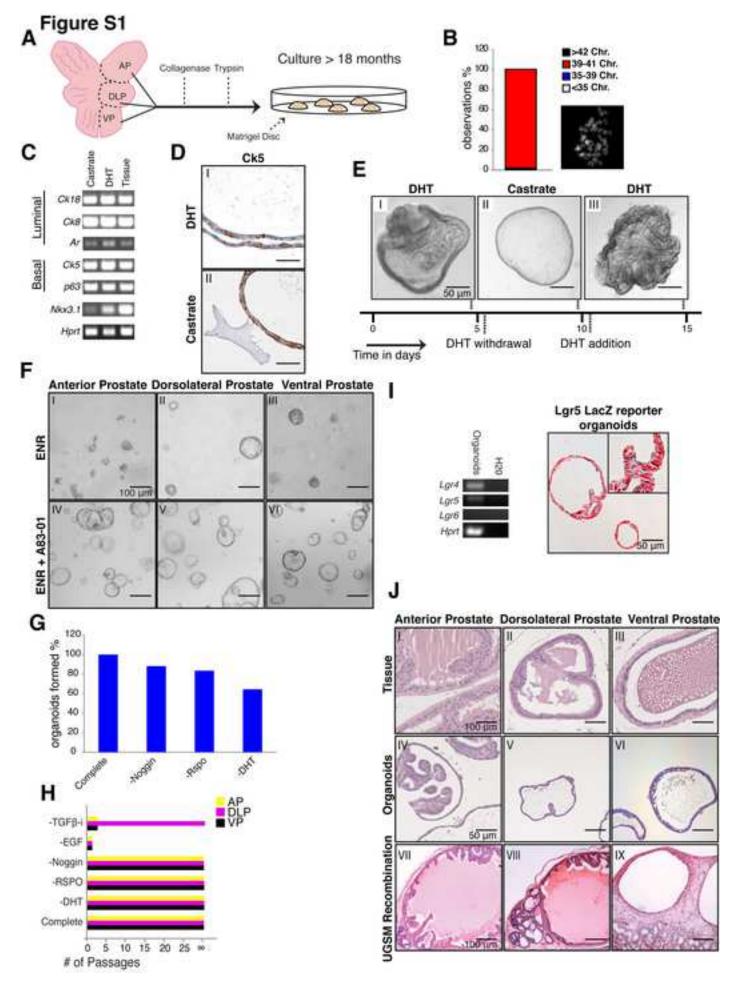
| FACTOR           | MOUSE<br>ORGANOID<br>CULTURE              | HUMAN<br>ORGANOID<br>CULTURE              | Source   | FUNCTION  |
|------------------|---|---|--|---|
| EGF              | 50 ng/ml                                  | 5 ng/ml                                   | Peprotech  | Mitogen, essential for all organoid growth  |
| EGF              | 50 lig/lill                               | 5 lig/ lill                               | Peprotech  | Enhances organoid   |
|                  |   |   |  | growth and  |
|                  | 10% conditioned                           | 10% conditioned                           | Peprotech or   | establishment. Induces  |
|                  | medium or 100                             | medium or 100                             | Hubrecht Institute for                                     | expression of Androgen  |
| NOGGIN           | ng/ml                                     | ng/ml                                     | condition medium   | Receptor.   |
| R-SPONDIN        | 10% conditioned<br>medium or 500<br>ng/ml | 10% conditioned<br>medium or 500<br>ng/ml | Peprotech or<br>Hubrecht Institute for<br>condition medium | Mitogen enhances<br>organoid growth and<br>establishment. Induces<br>expression of Androgen<br>Receptor |
| A83-01           | 200 nM                                    | 500 nM                                    | Tocris   | Enhances organoid<br>establishment. Essential<br>for continued organoid<br>growth                       |
|                  |   |   |  | Enhances Androgen   |
| FGF10            |   | 10 ng/ml                                  | Peprotech  | Receptor expression.  |
| FGF2             |   | 5 ng/ml                                   | Peprotech  | Mitogen enhances<br>organoid growth and<br>establishment.   |
| Prostaglandin E2 |   | 1 μΜ                                      | Tocris   | Mitogen enhances<br>organoid establishment.<br>Essential for continued<br>organoid growth.              |
| Nigotinomido     |   | 10 mM                                     | Sigma Aldrich  | Enhances organoid<br>growth and<br>establishment. Induces<br>expression of Androgen                     |
| Nicotinamide     |   | 10 mM                                     | Sigma-Aldrich  | Receptor.<br>Inhibits stress-induced  |
|                  |   |   |  | keratinization of basal cell  |
| SB202190         |   | 10 µM                                     | Sigma-Aldrich  | derived organoids.  |
| DHT              | 1 nM                                      | 1 nM                                      | Sigma-Aldrich  | Enhances organoid<br>establishment and<br>growth.   |
| Y-27632          | 10 µM                                     | 10 μM                                     | Sigma-Aldrich  | Enhances organoid   |

|  |  | establishment. Not   |
|--|--|----------------------|
|  |  | essential for growth |

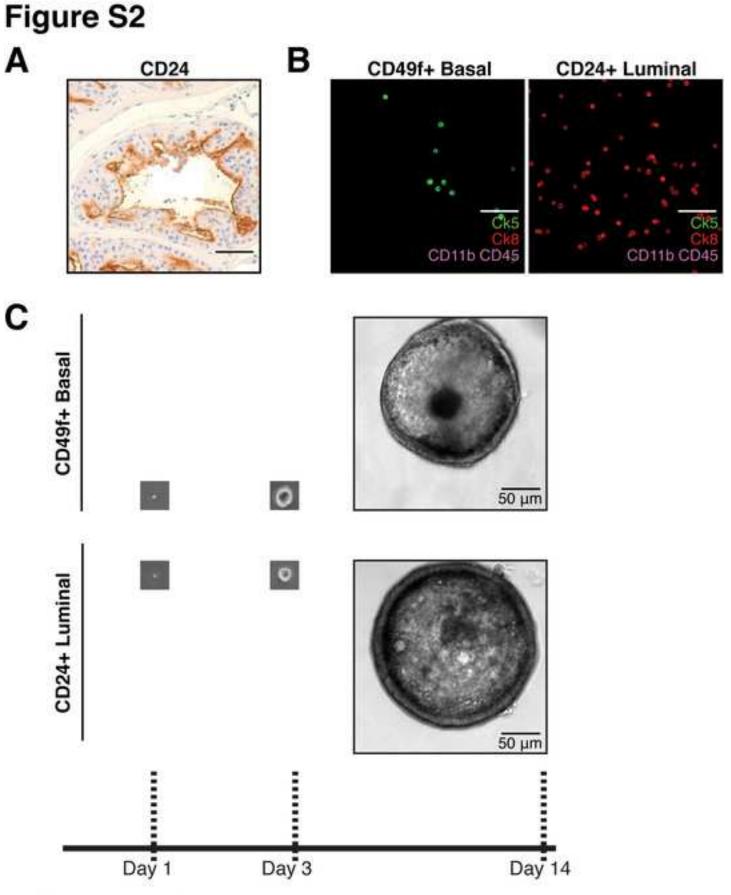
### Supplementary Table 3 related to Figure 1, Figure 2, Figure 3, Figure 4 and Figure 5:

| Gene     | Forward/Reverse | 5'-3' Primer sequence      | Species    |  |
|----------|-----------------|----------------------------|------------|--|
| Ck8      | FW              | AGCTCGCTCTCGAACCTCCGT      | M.Musculus |  |
|          | REV             | GCTCCAGGAAGCGCACCTTGTC     | M.Musculus |  |
| Ck18     | FW              | ACCTGAGGGCTCAGATCTTTGCGA   | M.Musculus |  |
|          | REV             | AGGGTGCGTCTCAGCTCCGT       | M.Musculus |  |
| Ck5      | FW              | GTGGGGGCAGCCGCTCTTTC       | M.Musculus |  |
|          | REV             | TCCAGGAACCGCACCTTGTCG      | M.Musculus |  |
| p63      | FW              | AGCAGAAAAGAGGAGAGAGCAGC    | M.Musculus |  |
|          | REV             | GGTTCGTGTACTGTGGCTCAC      | M.Musculus |  |
| Probasin | FW              | TGACGGGCCTTGGCAAACAA       | M.Musculus |  |
|          | REV             | TGCTTGGACAGTTGTCCGTGTCC    | M.Musculus |  |
| Nkx3.1   | FW              | ACGCAGTCCAAGCGGCTCAC       | M.Musculus |  |
|          | REV             | TGTGCATCAGACTCGGGTTCAGTC   | M.Musculus |  |
| Ar       | FW              | GCCAGGAGTGGTGTGTGCCG       | M.Musculus |  |
|          | REV             | AAGTTGCGGAAGCCAGGCAAGG     | M.Musculus |  |
| Psca     | FW              | GCTCACTGCAACCATGAAGA       | M.Musculus |  |
|          | REV             | GCTAAGTAGGTGGCCAGCAG       | M.Musculus |  |
|          |                 | Sequence proprietary to SA |            |  |
| Fkbp5    | FW              | bioscienses                | M.Musculus |  |
|          |                 | Sequence proprietary to SA |            |  |
|          | REV             | bioscienses                | M.Musculus |  |
| Lgr4     | FW              | GCTGCGGACTCTGGACTTAT       | M.Musculus |  |
|          | REV             | CCCATTCAGGCCTTCCGTAG       | M.Musculus |  |
| Lgr5     | FW              | CTGAGACAGGTTCCGGAGGA       | M.Musculus |  |
|          | REV             | GAGATGCAGAACCACGAGGC       | M.Musculus |  |
| Lgr6     | FW              | GCTGCACAATCTGGAGACAC       | M.Musculus |  |
|          | REV             | GGCACCATTCAAAGATAGCGT      | M.Musculus |  |
| Hprt     | FW              | AAGCTTGCTGGTGAAAAGGA       | M.Musculus |  |
|          | REV             | TTGCGCTCATCTTAGGCTTT       | M.Musculus |  |
| СК8      | FW              | ACCTGCAGGAAGGGATCTCCG      | H.Sapiens  |  |
|          | REV             | AGGCCACCGCGAAAGTTGCT       | H.Sapiens  |  |
| CK18     | FW              | TGCCAGCTCTGGGTTGACCG       | H.Sapiens  |  |
|          | REV             | TGTGGTGCTCTCCTCAATCTGCTG   | H.Sapiens  |  |
| CK5      | FW              | CGAGGAATGCAGACTCAGTGGAGA   | H.Sapiens  |  |
|          | REV             | GCTTCCACTGCTACCTCCGGC      | H.Sapiens  |  |
| p63      | FW              | CCTGGAGCCAGAAGAAAGGACAGC   | H.Sapiens  |  |
|          | REV             | CAGGTTCGTGTACTGTGGCTCACT   | H.Sapiens  |  |
| PSA      | FW              | TGGGGACCACCTGCTACGCC       | H.Sapiens  |  |
|          | REV             | TCGGTGATCAGAATGACCCACGAG   | H.Sapiens  |  |
| AR       | FW              | GGCGGGCCAGGAAAGCGACT       | H.Sapiens  |  |
|          | REV             | TCCCTGGCAGTCTCCAAACGCAT    | H.Sapiens  |  |
| NKX3.1   | FW              | CGTCCAAGCCGCTCACGTCC       | H.Sapiens  |  |
|          | REV             | CCCAAGTGCCTTTCTGGCTCGG     | H.Sapiens  |  |
| B-MSP    | FW              | TCTCCTGGGCAGCGTTGTGA       | H.Sapiens  |  |
|          | REV             | ACCCACAGGTGTAGAAACAAGGGT   | H.Sapiens  |  |
| LGR4     | FW              | GTGCTTTGCAGTCTTTGCGT       | H.Sapiens  |  |
|          | REV             | CCTGTAGGGTGGGCAGATTG       | H.Sapiens  |  |
| LGR5     | FW              | TCAATCCCTGCGTCTGGATG       | H.Sapiens  |  |
|          | REV             | TGTGGAGCCCATCAAAGCAT       | H.Sapiens  |  |
| LGR6     | FW              | GACCCCCTGACGGCTTA          | H.Sapiens  |  |
|          | REV             | ATTGTTCTGCAGCATCAGGATTTTC  | H.Sapiens  |  |
| GAPDH    | FW              | GGTATCGTGGAAGGACTCATG      | H.Sapiens  |  |
|          | REV             | ATGCCAGTGAGCTTCCCGTTC      | H.Sapiens  |  |

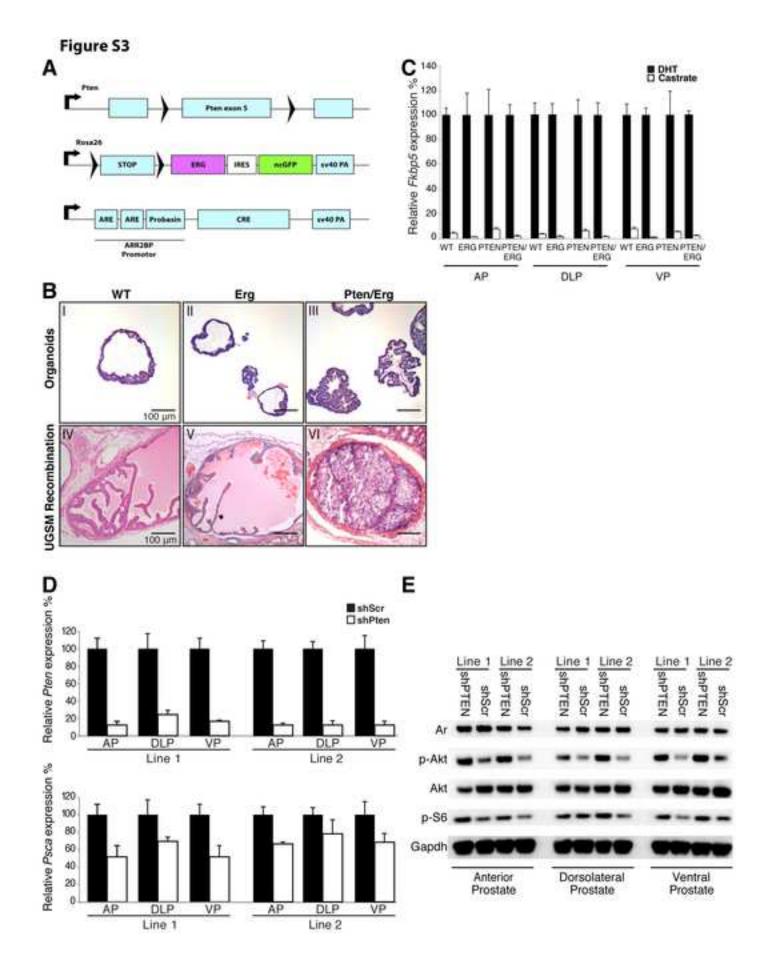
Supplemental Figure 1 Click here to download high resolution image



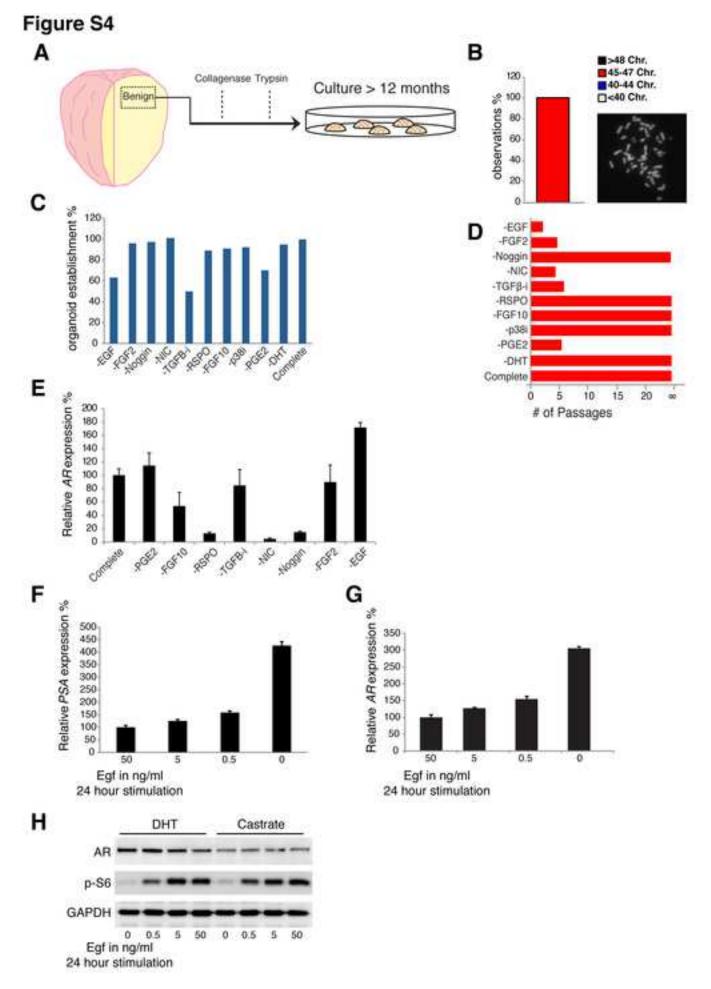
Supplemental Figure 2 Click here to download high resolution image



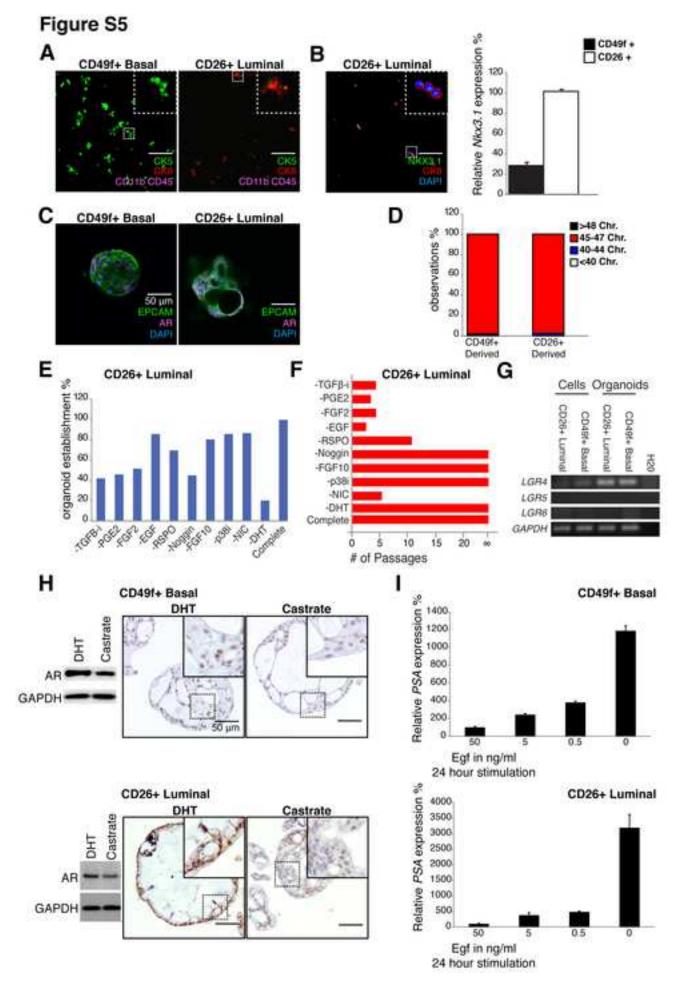
Time post establishment



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Supplemental Figure 5 Click here to download high resolution image



Conflict of Interest form