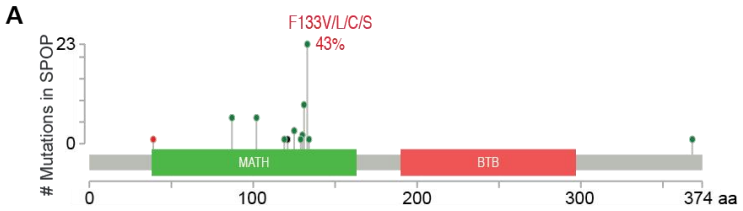
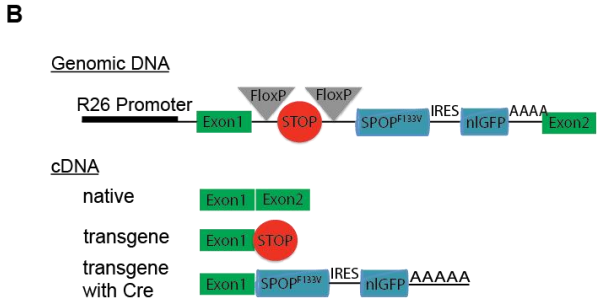
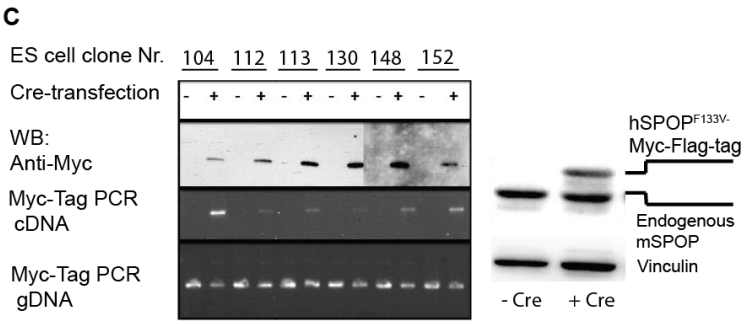
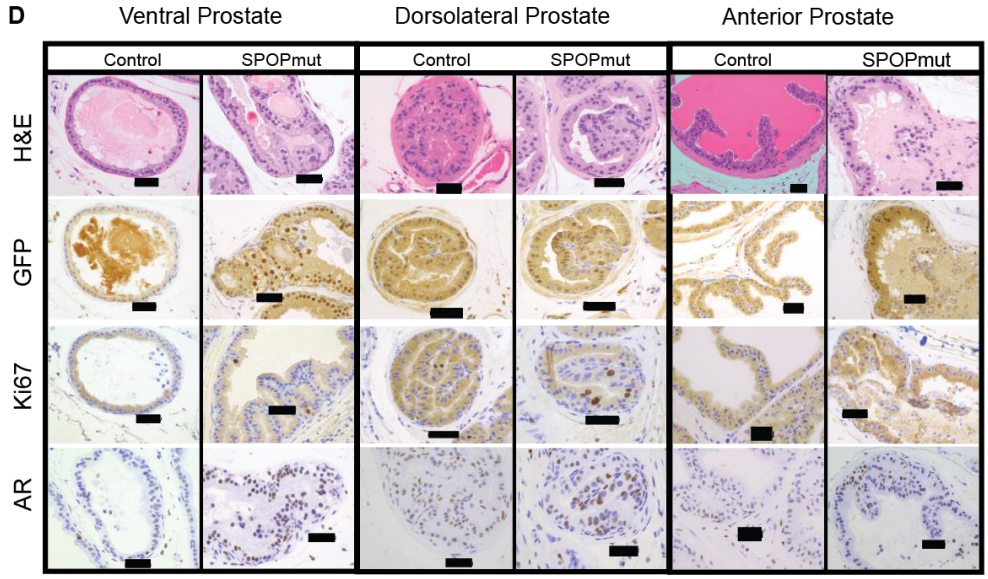
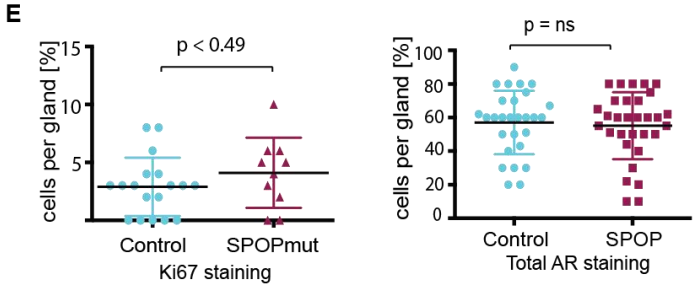

A
F133V/L/C/S
43%
Mutations in SPOP
23
0
MATH
BTB
0
100
200
300
374 aa


B
Genomic DNA
R26 Promoter
FloxP
FloxP
Exon1
STOP
SPOP^F133V
IRES
nlGFP
AAAA
Exon2
cDNA
native
Exon1
Exon2
transgene
Exon1
STOP
transgene
with Cre
Exon1
SPOP^F133V
IRES
nlGFP
AAAAA


C
ES cell clone Nr.
104
112
113
130
148
152
Cre-transfection
WB:
Anti-Myc
Myc-Tag PCR
cDNA
Myc-Tag PCR
gDNA
hSPOP^F133V-
Myc-Flag-tag
Endogenous
mSPOP
Vinculin
- Cre
+ Cre


D
Ventral Prostate
Dorsolateral Prostate
Anterior Prostate
Control
SPOPmut
H&E
GFP
Ki67
AR


E
p < 0.49
cells per gland [%]
15
10
5
Control
SPOPmut
Ki67 staining
p = ns
100
80
60
40
20
0
Control
SPOP
Total AR staining

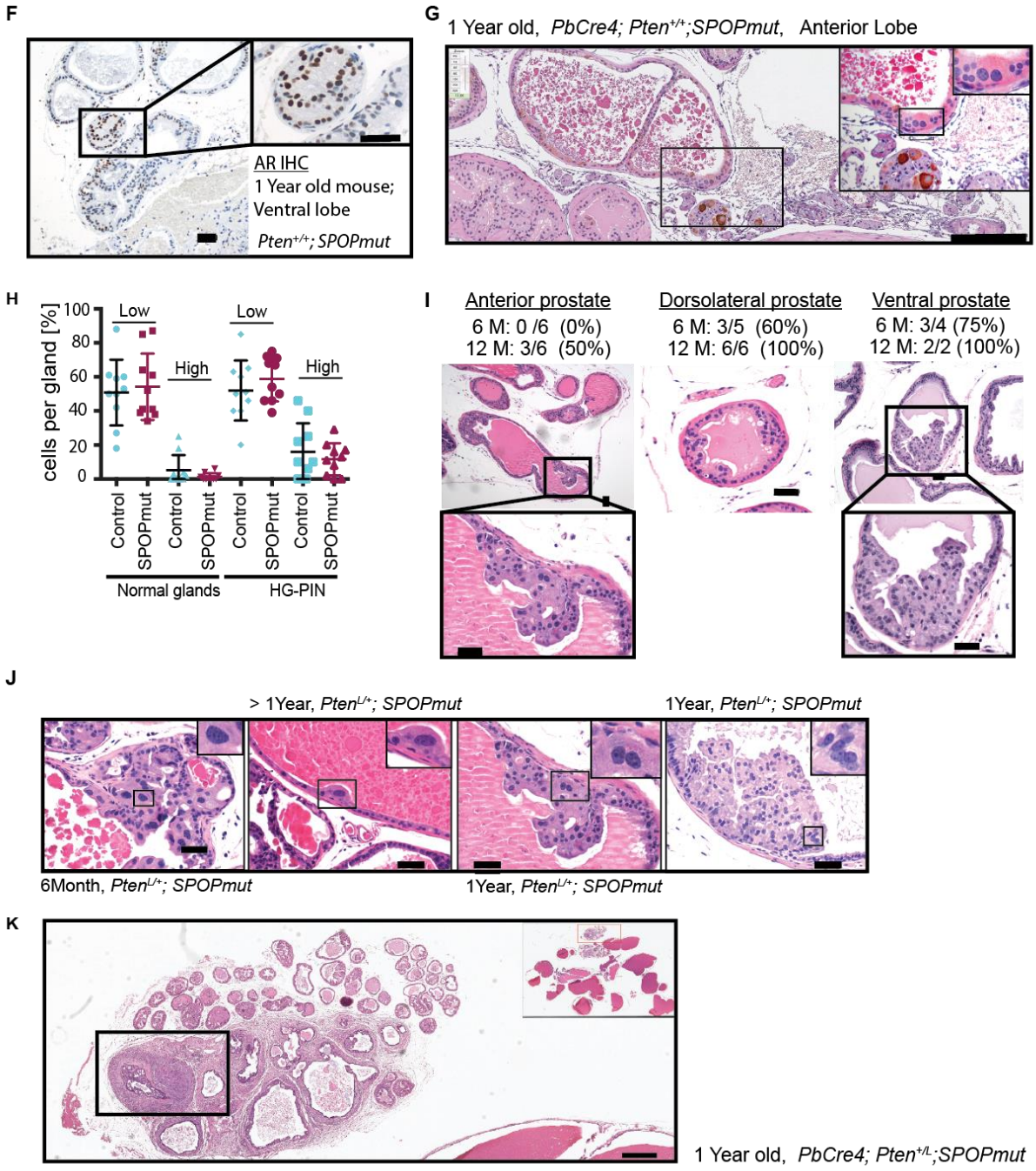


Figure S1 related to Figure 1

(A) Schematic of missense mutations in SPOP in 56 clinically localized human prostate cancers (data from TCGA[4]). 43% of all reported mutations occur at F133.

(B) Human *F133V-SPOP* transgene construct was integrated between Exon1 and 2 of the Rosa26 locus. FloxP-Stop-FloxP silences transgene transcription upon Cre expression. Shown are three possible cDNA transcript: native form (top), after insertion of transgene (middle) and in cells expressing Cre (bottom).

(C) Left: Screening results of 6 embryonic stem cells with and without Cre transfection shows the SPOP –Myc-Flag tagged protein and Myc-tag cDNA PCR product only after Cre transfection. Transgene construct is present in all 6 clones as shown by Myc-tag PCR product on genomic DNA. Right: Murine prostate organoids generated from *R26*^{F133V} mice show physiological levels

of human SPOP-F133V (hSPOP^{F133V}-Myc-Flag) protein in background of Cre expression. Western blot using antibody against SPOP: Top band: hSPOP^{F133V}-Myc-Flag protein, bottom band: endogenous mouse SPOP (mSPOP).

(D) Histological comparison of one year old mouse either WT (Control) or SPOP-F133V expressing (SPOPmut). Shown are H&E, and immunohistochemistry against GFP, Ki67 and AR for the ventral, dorsolateral and anterior lobes.

(E) Quantification of Ki67 (left) and AR (right) protein level in three one year old control and SPOPmut mice across all lobes. All data are means ± SEM. p values were calculated by one sample t test.

(F) Ventral prostate gland from one year old *R26*^{F133V} mouse showing high AR expression. (Scale bar: 50 μM)

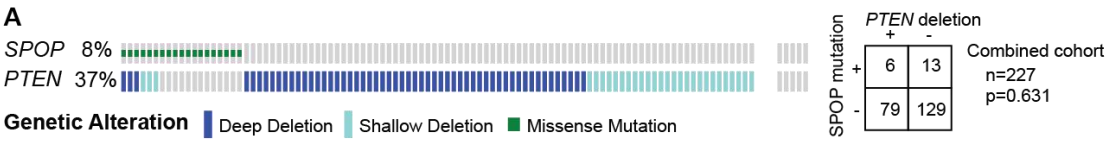
(G) Prostate gland with atypical nuclei in one year old PbCre;*R26*^{F133V} mouse. (Scale bar: 200 μM). Insets show higher magnification of same area.

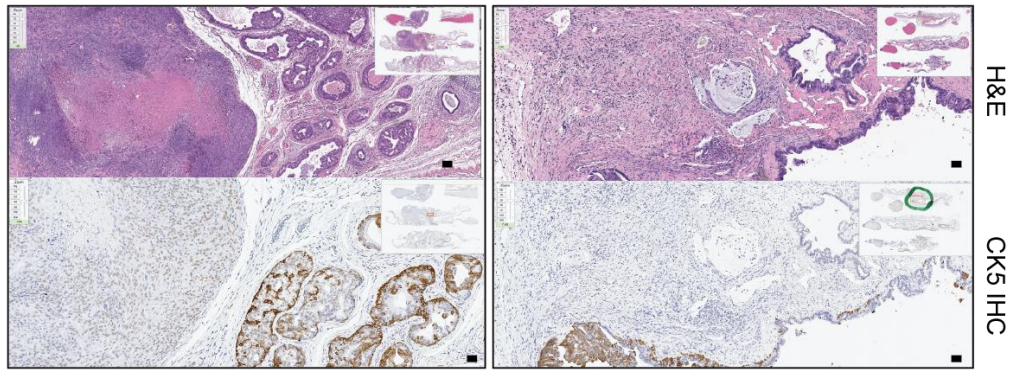
(H) Quantification of numbers of cells per gland expressing either low or high level of AR in six one year old mice. Glands were divided by histological phenotype of either normal or HG-PIN.

(I) Images showing areas of HG-PIN in *Pten*$^{L/+}$, *R26*^{F133V} mice in different lobes (Left to right, Anterior prostate, Dorsolateral prostate and Ventral prostate). On top: Summarized the prevalence of HG-PIN within each lobe at 6 and 12 month of age. (Scale bar: 50 μM). Top panel shows High magnification images and matching low magnification images of anterior prostate (left) and ventral prostate (right) bottom panel. (Scale bar: 50 μM)

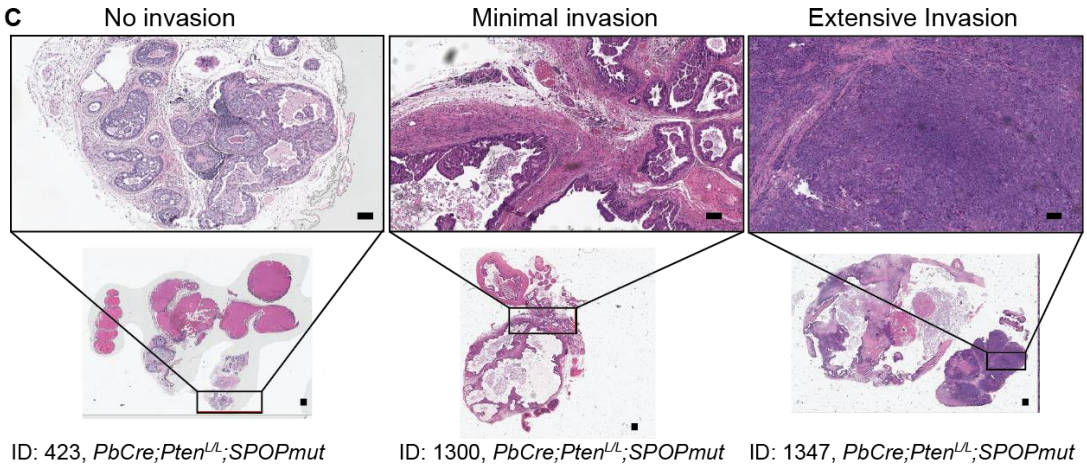
(J) Examples showing atypia in multiple *Pten*$^{L/+}$, *R26*^{F133V} mice. (Scale bar: 50 μM). Insets in upper right corner shows high magnification of a representative atypical cell. Details about age and genotype indicated below images.

(K) Area showing invasive carcinoma in *Pten*$^{L/+}$*; R26*^{F133V} mouse in a range from low to high magnification. (Scale bar: 500 μM)





ID: 272, 1 Year old, *PbCre;Pten$^{L/L}$;SPOPmut* ID: 1230, 1 Year old, *PbCre;Pten$^{L/L}$;SPOPmut*



ID: 423, *PbCre;Pten$^{L/L}$;SPOPmut* ID: 1300, *PbCre;Pten$^{L/L}$;SPOPmut* ID: 1347, *PbCre;Pten$^{L/L}$;SPOPmut*

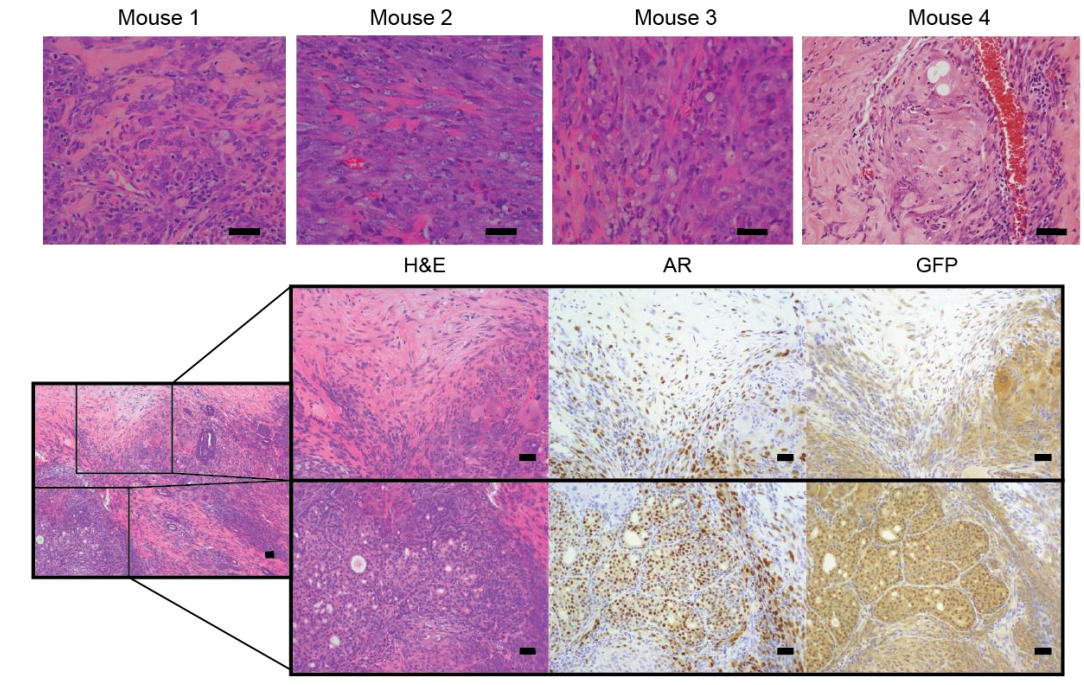


Figure S2 related to Figure 2

(A) Analysis of *SPOP* mutations and *PTEN* deletions in 227 advanced, metastatic human prostate cancers. There is no evidence of mutual exclusivity: PTEN deletions occur in 32% of *SPOP* mutant samples and 38% of *SPOP* wt samples ($p = 0.831$).

(B) Scanned H&E slides of one year old $Pten^{L/L}$; $R26^{F133V}$ mice (top right) showing the whole extracted prostate. Representative areas with H&E staining as well as CK5 IHC as conformation for invasion with higher magnification. (Scale bar: 50μM)

(C) Photomicrographs of prostates with either no invasion (top), minimal invasion (middle) and extensive invasion (bottom). Right panel: Scanned H&E slide of extracted prostate. Left panel: representative high magnification of area of HG-PIN, minimal invasion and extensive invasion. (Scale bar: 50μM)

(D) Top panel: Mice expressing SPOP-F133V in a $PbCre;Pten^{L/L}$ background develop invasive carcinoma with transition into sarcomatoid differentiation. Shown are four representative regions of poorly differentiated carcinoma in individual mice.

Bottom panel: Invasive Carcinoma (bottom panel) as well as invasive carcinoma transitioning into sarcomatoid differentiation (top panel) remain AR and GFP positivity. Shown is the H&E as well as AR and GFP staining of one year old $PbCre;Pten^{L/L};R26^{F133V}$ mouse. (Scale bar: 50μM)

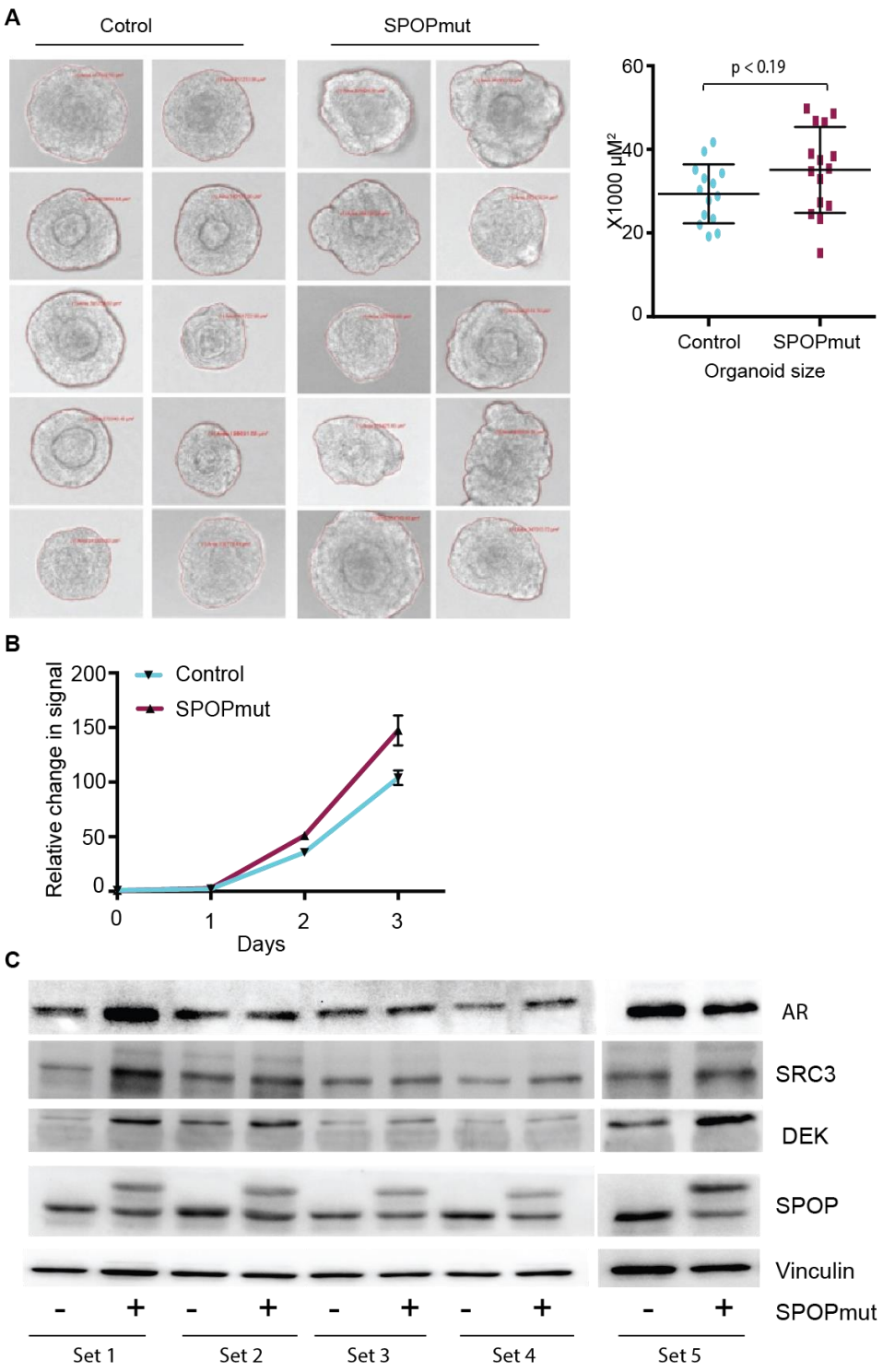
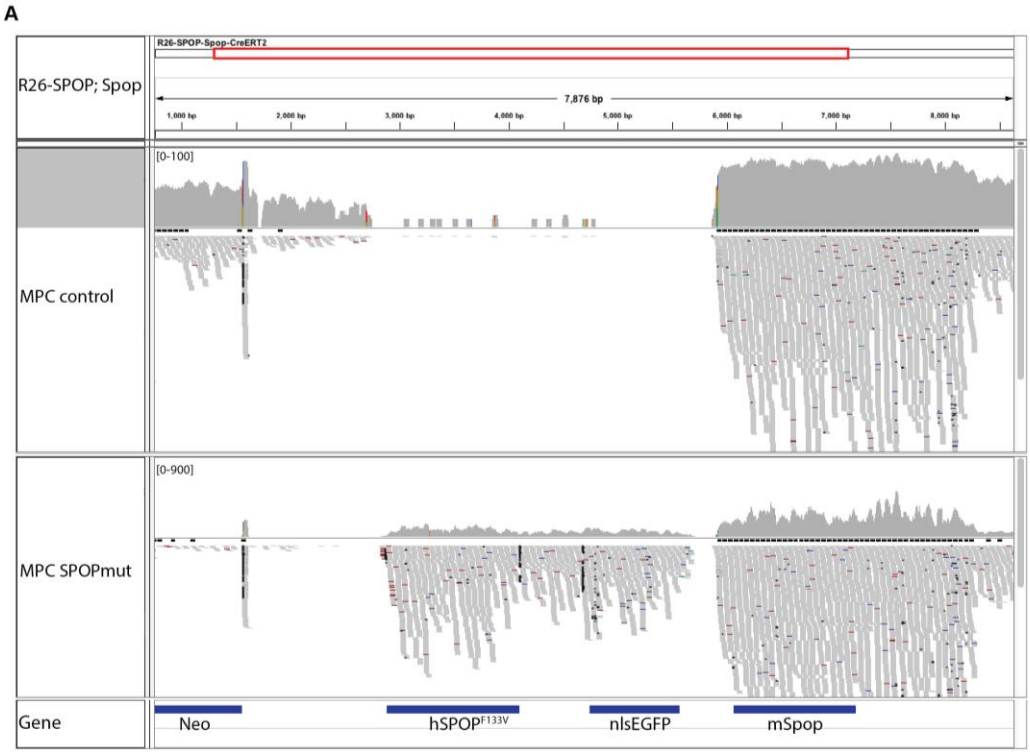


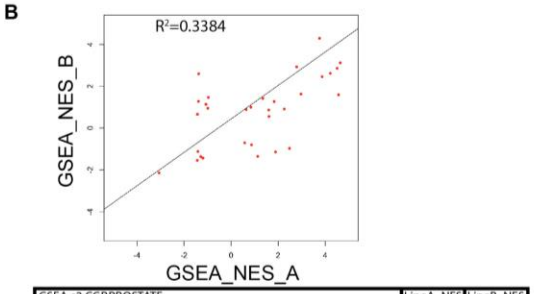
Figure S3 related to Figure 3

(A) Shown are 10 organoids grown from a single cells either expressing SPOP-F133V (SPOPmut) or not (Control). Size was measured two weeks post seeding. p values were calculated by one sample t test.

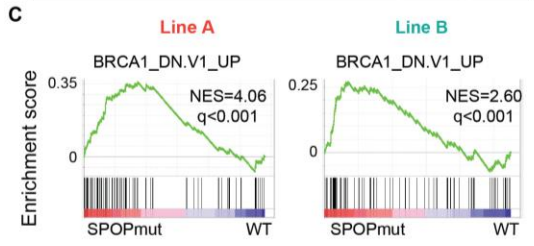
(B) Cell proliferation assay for PbCre-Neg(Control) and PbCre;Pten$^{+/+}$,SPOPmut cell lines. All data are means ± SEM

(C) Western blot for AR, SRC3, DEK and SPOP of 5 independently generated organoid lines with (+) and without (-) CreERT2 activation





GSEA.c2.CGPPROSTATE	LineA_NES	LineB_NES
LIU_PROSTATE_CANCER_DN	4.64	3.12
SCHAEFFER_PROSTATE_DEVELOPMENT_48HR_UP	4.57	1.59
ACEVEDO_FGFR1_TARGETS_IN_PROSTATE_CANCER_MODEL_DN	4.51	2.86
SCHAEFFER_PROSTATE_DEVELOPMENT_48HR_DN	4.22	2.62
KONDO_PROSTATE_CANCER_WITH_H3K27ME3	3.87	2.46
WALLACE_PROSTATE_CANCER_RACE_UP	3.76	4.29
SCHAEFFER_PROSTATE_DEVELOPMENT_12HR_UP	2.97	1.63
LIU_VAV3_PROSTATE_CARCINOGENESIS_UP	2.79	2.93
SCHAEFFER_PROSTATE_DEVELOPMENT_6HR_UP	2.48	-0.97
TOMLINS_PROSTATE_CANCER_DN	2.26	0.91
YEGNASUBRAMANIAN_PROSTATE_CANCER	1.89	-1.14
ACEVEDO_FGFR1_TARGETS_IN_PROSTATE_CANCER_MODEL_UP	1.83	1.27
LI_PROSTATE_CANCER_EPIGENETIC	1.61	0.56
KONDO_PROSTATE_CANCER_HCP_WITH_H3K27ME3	1.6	0.86
WANG_PROSTATE_CANCER_ANDROGEN_INDEPENDENT	1.35	1.42
SCHAEFFER_PROSTATE_DEVELOPMENT_12HR_DN	1.13	-1.35
SETLUR_PROSTATE_CANCER_TMPRSS2_ERG_FUSION_UP	0.87	-0.8
SCHAEFFER_SOX9_TARGETS_IN_PROSTATE_DEVELOPMENT_UP	0.84	1
SCHAEFFER_SOX9_TARGETS_IN_PROSTATE_DEVELOPMENT_DN	0.64	0.89
WALLACE_PROSTATE_CANCER_RACE_DN	0.58	-0.7
OUYANG_PROSTATE_CANCER_PROGRESSION_UP	-0.97	1.47
WANG_HCP_PROSTATE_CANCER	-0.99	0.95
LIU_PROSTATE_CANCER_UP	-1.07	1.14
OUYANG_PROSTATE_CANCER_MARKERS	-1.2	-1.43
OUYANG_PROSTATE_CANCER_PROGRESSION_DN	-1.29	-1.36
YAMASHITA_METHYLATED_IN_PROSTATE_CANCER	-1.38	2.6
SCHAEFFER_PROSTATE_DEVELOPMENT_AND_CANCER_BOX4_DN	-1.39	1.28
WALLACE_PROSTATE_CANCER_UP	-1.41	-1.12
HWANG_PROSTATE_CANCER_MARKERS	-1.43	0.66
TOMLINS_PROSTATE_CANCER_UP	-1.44	-1.54
SCHAEFFER_PROSTATE_DEVELOPMENT_6HR_DN	-3.06	-2.14



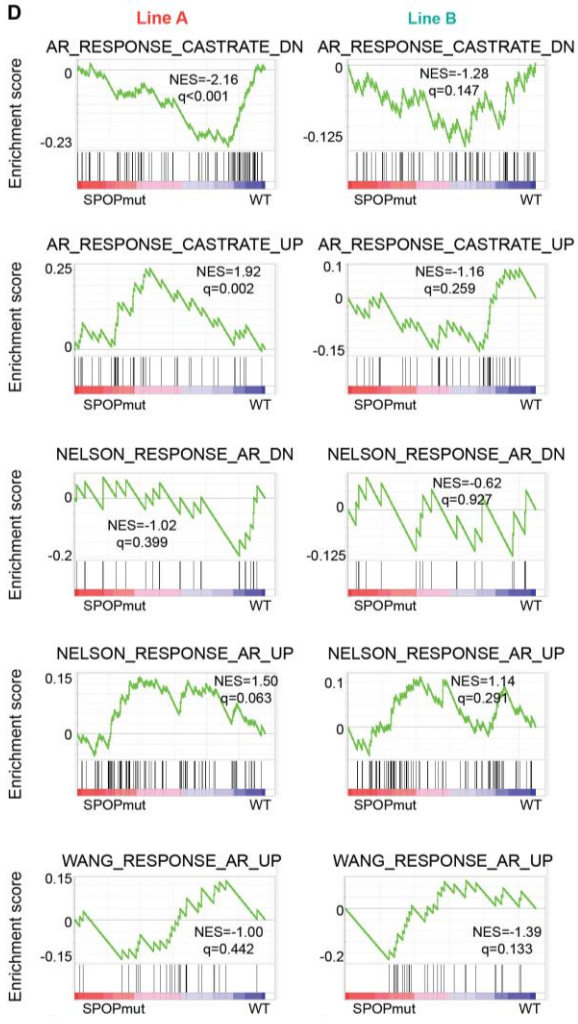


Figure S4 related to Figure 4

(A) IGV snapshot of Neo-cassette, hSPOP^{F133V}, and mSpop before (MPC control) and after (MPC SPOPmut) 4OH-Tamoxifen treatment, CreERT2 activation and GFP selection.

(B) Multiple independently derived organoids expressing SPOP-F133V show strong concordance across prostate gene sets (top panel). Concordance of GSEA normalized enrichment score (NES) from two independent mouse SPOP-F133V cell lines, based on prostate cancer related gene signatures downloaded from MSigDB shown in the bottom panel.

(C) GeneSetEnrichentAnalysis (GSEA) of the SPOP mutant profiling in mouse prostate organoids against BRCA1_DN.V1_UP.

(D) Shown is Gene Set Enrichment Analysis (GSEA) of SPOP mutant profiling in mouse prostate organoids compared to a mouse prostate specific AR-dependent gene sets as well as human AR-dependent gene sets. Mouse gene set defined by changes resulting from mouse castration, (AR_Response_Castrate_UP; AR_Response_Castrate_Down) Gene sets downloaded from MSigDB, (Neslon_Response_AR_DW; Neslon_Response_AR_UP; Wang_Response_AR_UP,).

Table S1, related to Figure 4: (Provided as an Excel file)
Significantly differentially expressed genes (FDR<=0.2) between SPOPmut and control in MPC Line A

Table S2, related to Figure 4: (Provided as an Excel file)
Gene Set Enrichment Analysis (GSEA) of SPOP mutant profile in MPC Line A (FDR<=0.05)

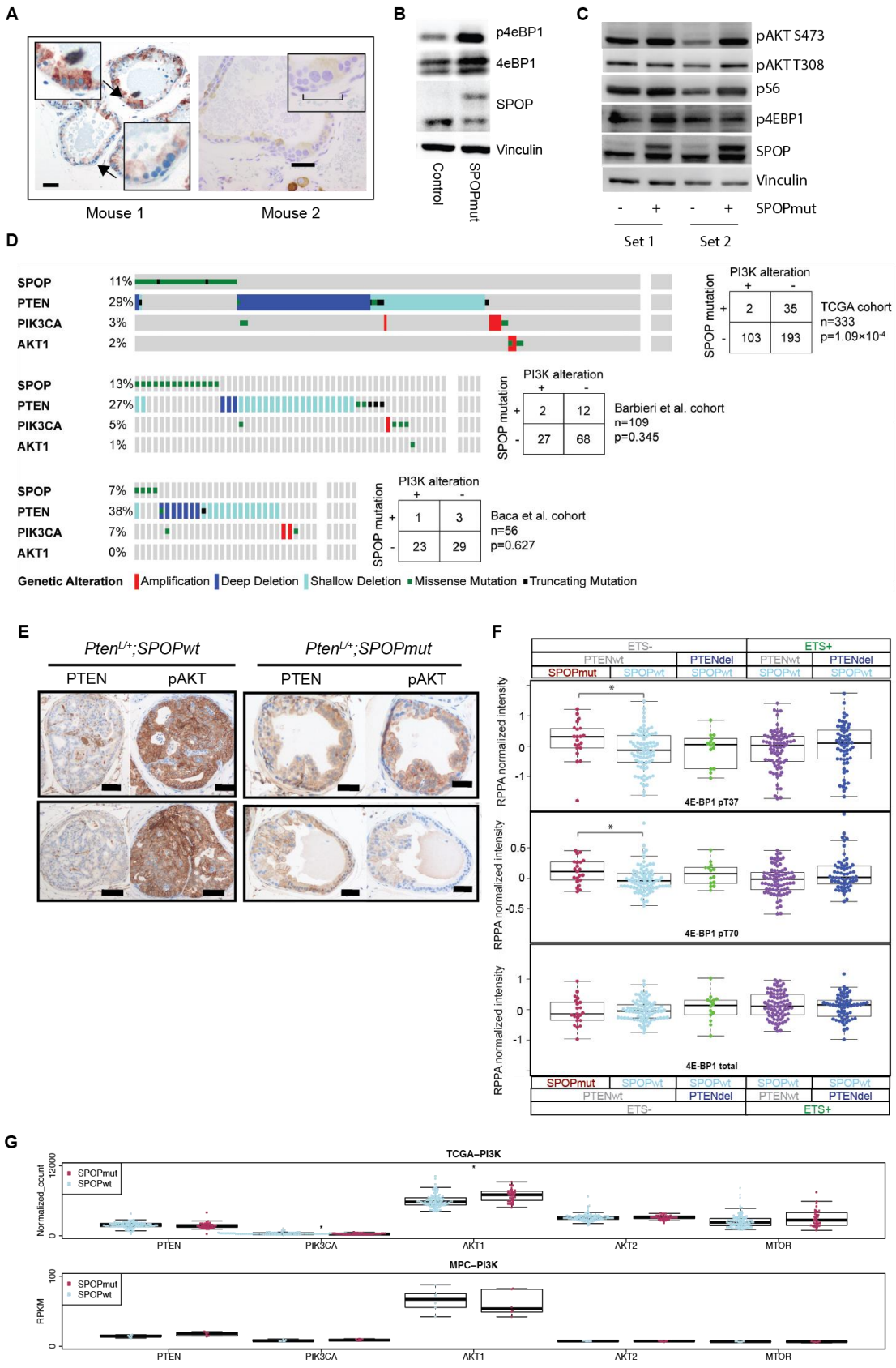

A
Mouse 1
Mouse 2
B
p4eBP1
4eBP1
SPOP
Vinculin
Control
SPOPmut
C
pAKT S473
pAKT T308
pS6
p4EBP1
SPOP
Vinculin
SPOPmut
Set 1
Set 2
D
SPOP 11%
PTEN 29%
PIK3CA 3%
AKT1 2%
PI3K alteration
SPOP mutation
2 35 103 193
TCGA cohort
n=333
p=1.09×10-4
SPOP 13%
PTEN 27%
PIK3CA 5%
AKT1 1%
2 12 27 68
Barbieri et al. cohort
n=109
p=0.345
SPOP 7%
PTEN 38%
PIK3CA 7%
AKT1 0%
1 3 23 29
Baca et al. cohort
n=56
p=0.627
Genetic Alteration
Amplification
Deep Deletion
Shallow Deletion
Missense Mutation
Truncating Mutation
E
PtenL/+;SPOPwt
PtenL/+;SPOPmut
PTEN
pAKT
F
ETS-
ETS+
PTENwt
PTENdel
SPOPmut
SPOPwt
RPPA normalized intensity
4E-BP1 pT37
4E-BP1 pT70
4E-BP1 total
G
TCGA–PI3K
MPC–PI3K
Normalized_count
RPKM
PTEN
PIK3CA
AKT1
AKT2
MTOR

Figure S5 related to Figure 5

(A) Majority of atypical looking cells in *PbCre; Pten*$^{+/+}$*;R26*^{F133V} mice show strong pS6 staining. Shown are representative regions in two individual one year old mice. Inset shows high power images of atypical nuclei.

(B) Western blotting of *Pten*$^{+/+}$ (Control) and *Pten*$^{+/+}$*,R26*^{F133V} (SPOPmut) cell lysates for peEBP1 and Vinculin.

(C) Western blot showing protein levels of PI3K/MTOR pathway components for two independently generated Control and PbCre-SPOPmut cell lines

(D) Shown are molecular features (SPOP and PI3K pathway alterations) of three publicly available data sets (TCGA[4], Barbieri et al.[1] and Baca et al.[6] cohorts). Data sets were downloaded from cBioPortal. Exclusivities between SPOP mutant and PI3K pathway alteration were tested by Fisher's exact test for each data set

(E) PTEN and pAKT IHC staining in *PbCre, Pten*$^{L/+}$ and *PbCre, Pten*$^{L/+}$*, R26*^{F133V} mice

(F) Box plot of protein intensity for phospho-T37, phospho-T70 and total 4eBP1 based on reverse-phase protein array data from 250 TCGA human prostate cancer samples. Y-axis represents the normalized protein intensity, and x-axis represents molecular subtypes based on SPOP mutant, PTEN deletion and ETS fusion. The normalized intensity differences were tested by Wilcoxon signed-rank test between subtypes.

(G) Gene expression (RNA-seq) of selected components of the PI3K pathway in SPOP mutant and wt settings in 135 human prostate cancer samples (top panel, TCGA) and mouse prostate organoids (bottom). Expression levels were compared using Wilcoxon signed-rank test. * indicates p-value < 0.05.

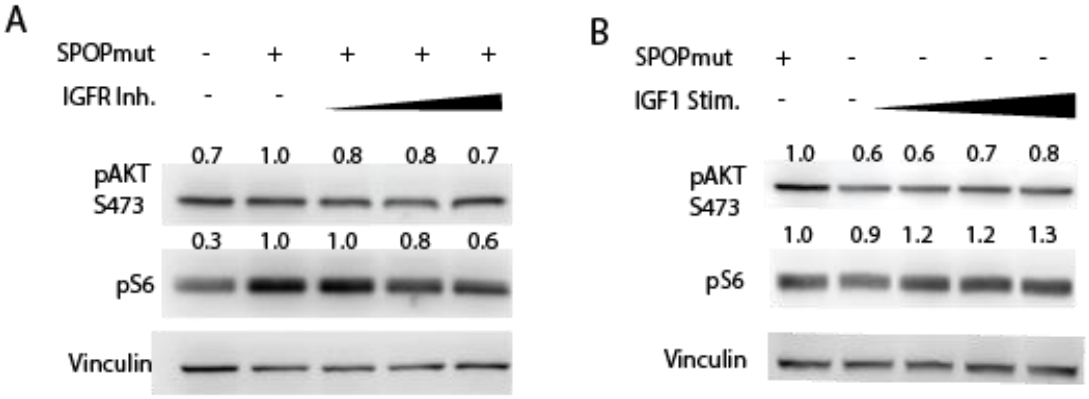


Figure S6 related to Figure 6

(A) Mouse prostate cells, with Pten$^{L/L}$ background, without (-) or with SPOP-F133V (+) expression. Shown are pAKT , pS6 and Vinculin protein expression with increasing IGF1 receptor inhibition (0, 0.1, 10, 50μM). Cells were treated with inhibitor over night. **(B)** Mouse prostate cells, with Pten$^{L/L}$ background, without (-) or with SPOP-F133V (+) expression. Shown are pAKT , pS6 and Vinculin protein expression with increasing IGF1 protein stimulation (0, 5, 10, 50ng)

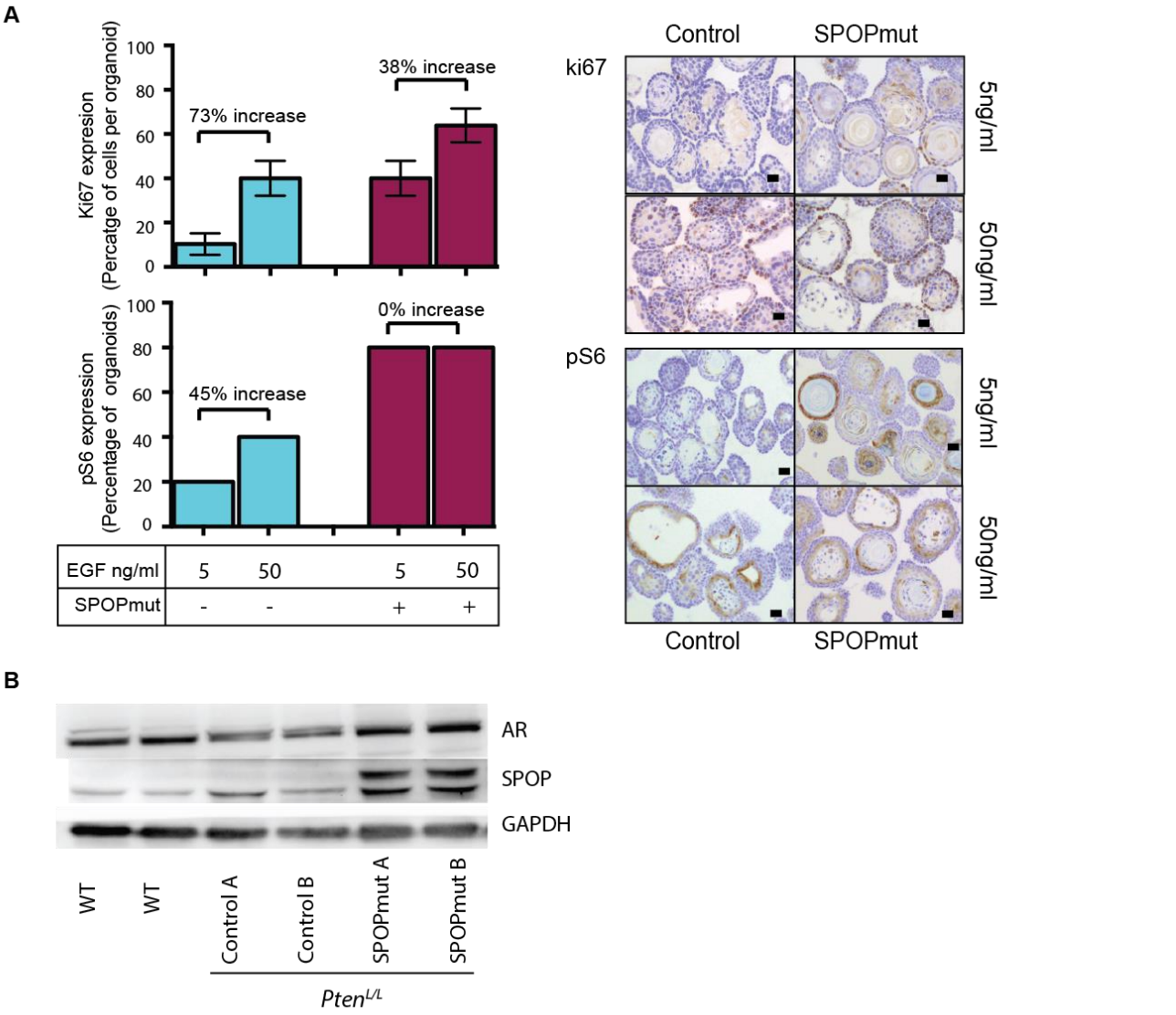

A
Ki67 expresion (Percatge of cells per organoid)
73% increase
38% increase
pS6 expression (Percentage of organoids)
45% increase
0% increase
EGF ng/ml 5 50 5 50
SPOPmut - - + +
Control
SPOPmut
ki67
pS6
5ng/ml
50ng/ml
B
AR
SPOP
GAPDH
WT
WT
Control A
Control B
SPOPmut A
SPOPmut B
Pten L/L
C
p = 3.65×10-5
TCGA_AR_score
SPOPmut
SPOPwt
D
p = 0.05
p = 0.04
p = 9.93×10-4
SPOPmut
SPOPwt
SPOPwt
SPOPwt
SPOPwt
PTENwt
PTENdel
PTENwt
PTENdel
ETS-
ETS+

Figure S7 related to Figure 7

(A) Control organoids as well as SPOP-F33V expressing organoids (SPOPmut) were cultured in 5ng/ml or 50ng/ml EGF for 7 days. Cell pellets were fixed and stained for Ki67 as well as pS6 protein and quantified. All data are means ± SEM.

(B) Western blotting of AR, SPOP and GAPDH of mouse prostate tissue from one yeard old wild type mice (WT), *PbCre,Pten*$^{L/L}$ mice (Control) and *PbCre,Pten*$^{L/L}$, *R26*^{F133V} mice (SPOPmut).

(C-D) *SPOP* mutation and relation to AR transcriptional score across disease subtypes. AR score determined as previously described[1] .**(C)** shows box plot of AR transcriptional score for molecular subtypes based on *SPOP* mutation vs. wild-type alone. (D) shows box plot of AR transcriptional score for molecular subtypes based on *SPOP* mutant status (SPOPmut and SPOPwt), *PTEN* deletion (PTENdel and PTENwt) and *ETS* fusion (ETS+ and ETS-). The AR transcriptional score differences were tested by Wilcoxon signed-rank test between subtypes.

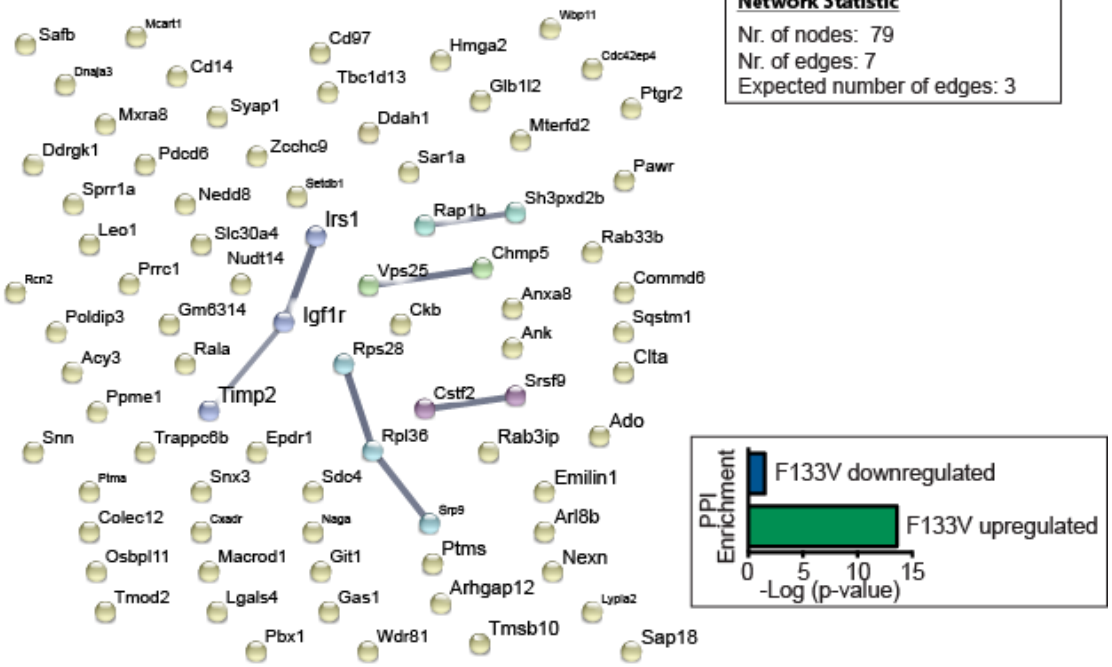
A

De novo SPOP binding site analysis

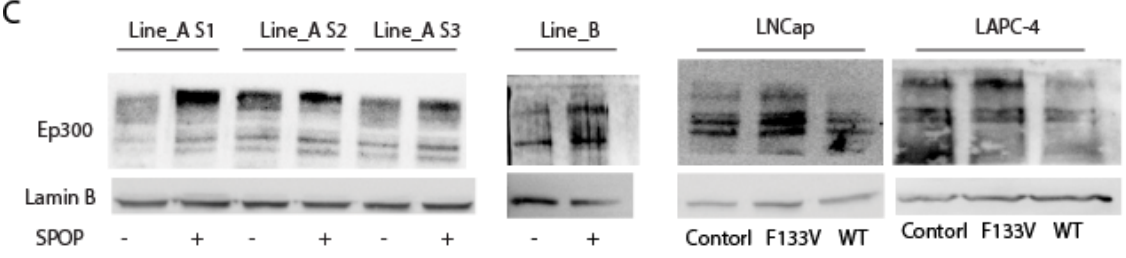
	Fitness	Hits (seqs)	Pattern
1	**16.6802**	**40 (24)**	**S-S-S-x(2)-S**
2	**16.6802**	**26 (23)**	**L-x(2)-L-L-x-S**
3	16.1802	24 (21)	S-x(0,1)-A-S-x(2)-S
4	16.1802	28 (21)	S-L-x(2)-L-x(0,1)-L
5	16.1802	33 (23)	S-x(0,1)-L-x(2)-L-L
6	16.1802	27 (21)	S-x(2)-S-S-x(0,1)-L
7	16.1802	32 (21)	S-x(1,2)-S-S-L
8	16.1802	26 (20)	S-S-x(0,1)-S-x-E
9	16.1802	37 (21)	S-x(0,1)-S-S-L
10	16.1802	31 (23)	S-x(0,1)-S-S-x-E
11	16.1802	45 (21)	S-x(2)-S-S-x(0,1)-S
12	16.1802	45 (21)	S-x(2)-S-x(0,1)-S-S
13	16.1802	51 (21)	S-x(1,2)-S-S-S
14	16.1802	33 (21)	L-S-x(1,2)-S-S
15	16.1802	33 (21)	L-x(0,1)-S-x(2)-S-S

B

Protein-Protein interaction in down regulated proteins



C



D

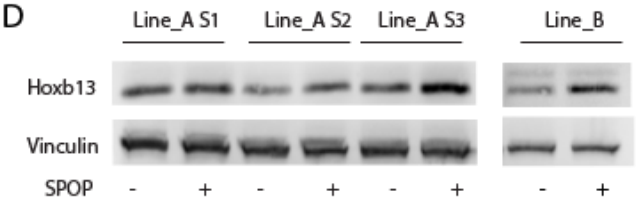


Figure S8 related to Figure 8

(A) Top 15 de novo motifs. De novo motif analysis was performed on the top 100 SPOP-F133V upregulated proteins using PRATT (http://www.ebi.ac.uk).

(B) Network analysis of proteins downregulated by SPOP-F133V expression. STRING database (string-db.org). Network nodes represent proteins with decreased expression, edges represent protein-protein associations, including physical and functional interactions. Top inset: network statistics for presented network. Bottom inset: Protein-protein interaction (PPI) enrichment score for proteins with significantly decreased expression (blue) and significantly increased expression (green), plotted as -Log10(p-value).

(C) Ep300 **p**rotein expression level shown by western blot in two independent mouse cell lines (Line_A and Line_B) without (-) or with SPOP-F133V expression (+), in LNCap and LAPC-4 cell transiently transfected with either empty (Control), SPOP-F133V (F133V) or SPOP-WT (WT) plasmids.

(D) Hoxb13 **p**rotein expression level shown by western blot in two independently generated mouse cell lines (Line_A and Line_B) without (-) or with SPOP-F133V expression (+).

Table S3, related to Figure 8: (Provided as an Excel file)
Unbiased proteome-wide profiling of control and SPOP mutant mouse prostate cells using label-free MS/MS

Table S4, related to Figure 8: (Provided as an Excel file)
Ingenuity pathway analysis of altered proteins

Table S5, related to Figure 8: (Provided as an Excel file)
Significantly altered proteins containing SBC

Table S6, related to Figure 8: (Provided as an Excel file)
List of proteins, of top 100 upregulated proteins, containing de novo sequence S-S-S-x(2)-S and sequence L-x(2)-L-L-x-S

Supplemental Experimental Procedure

Gene Targeting and mouse breeding

Genotyping has been carried out with a customized TagMan assay targeting the h*SPOP*-Flag-Myc sequence as well as PCR targeting the wild-type Rosa26 region.

Protein analysis

All IHC staining was done on a BOND-III, Automated IHC stainer, Leica. Immnofluorescence staining was carried out manually. For detailed protocol see supplemental experimental procedures. For protein extraction, cell pellets were collected in cold PBS and RIPA buffer was used for extraction. Fresh tissue was homogenized with the Dounce homogenizer. Buffer condition as described in manual by Active Motif, Universal Megnatic Co-IP Kit.

Antibodies to the following were used for IHC, western blotting and Immunofluorescence staining: rabbit AR N-20 (Santa Cruz, sc-816, 1:1000 for western blotting), rabbit AR (Abcam, ab108341, 1: 100 for IHC), rabbit GFP (Invitrogen, A11122,1:300 for IHC), rabbit Ki67 (Vector laboratories, K451, 1:200 for IHC), rabbit 4eBP1 (Cell signaling, #9466, 1:1000 for western blotting), rabbit p4eBP1 Thr70 (Cell signaling, #9455, 1:1000 for western blotting), rabbit S6 (Cell signaling, #2217, 1:1000 for western blotting), rabbit pS6 Ser235/236(Cell signaling, #2211, 1:400 for IHC, 1:1000 for western blotting), rabbit AKT (Cell signaling, #4691, 1:1000 for western blotting), rabbit pAKT Ser473 (Cell signaling, #4060, 1:25 for IHC, 1:1000 for western blotting), rabbit pAKT Thr308 (Cell signaling, #13038, 1:1000 for western blotting), rabbit Vinculin (Abcam, ab129002, 1:10000 for western blotting), chicken GAPDH (EMD Millipore, AB2302, 1:500 for western blotting) , rabbit PTEN (Cell Signaling, #9559, 1:1000 for western blotting), rabbit DEK (Proteintech, 16448-1-AP, 1:1000 for western blotting), rabbit SRC3 (Cell Signaling, #2126, 1:1000 for western blotting), chicken Cytokeratin 5 (Biolegend, #905901, 1:500 for Immunofluorescence staining, 1:2000 for IHC) rabbit Cytokeratin 8+18 (Abcam, ab53280, 1:200 for Immunofluorescence staining), rabbit SPOP (costume made by epidemics), rabbit SRC3 (Sell signaling, #2126, 1:50 for IHC), rabbit Ep300 (Abcam, #ab3164, 1:200), rabbit Hoxb13 (Santa Cruz, #sc-66923, 1:1000).

RNA extraction, cDNA synthesis, qPCR analysis

RNA extraction of cell pellets as well as frozen cores from fresh frozen tissue was done as described by manufacture using the automated Maxwell®16 system (Promega, AS1280 and AS1270). cDNA synthesis was transcribed by using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, #4374966). Quantitative PCR was done on the Roche Light Cycler 480. Sequence of used primer :

Primer name	Seuqence (5´-3´)
R26-WT-5F (1028F)	TGGGCCTGGGAGAATCCCTT
R26-TA-WT-3F (1236R)	TCCCGACAAAACCGAAAATC
SPOP-Flag-MycTag	TagMan assay designed by Life technologies
mus_NKX3.1_F2	ATGCTTAGGGTAGCGGAGC
mus_NKX3.1_R2	TGCGGATTGCCTGAGTGTC
mus_FKBP5_F1	TGAGGGCACCAGTAACAATGG
mus_FHBP5_R1	CAACATCCCTTTGTAGTGGACAT
mus_PSCA_F1	GCTCACTGCAACCATGAAGA
mus_PSCA_R1	GCTAAGTAGGTGGCCAGCAG
mus_Rpl38_F1	AGGATGCCAAGTCTGTCAAGA
mus_Rpl38_R1	TCCTTGTCTGTGATAACCAGGG
mus_SRC3_F1	AGTGGACTAGGCGAAAGCTCT
mus_SRC3 _R1	GTTGTCGATGTCGCTGAGATTT
mus_IGF1_F1	CACATCATGTCGTCTTCACACC
mus_IGF1_R1	GGAAGCAACACTCATCCACAATG
h_Rpl27_F1	CATGGGCAAGAAGAAGATCG
h_Rpl27_R1	TCCAAGGGGATATCCACAGA

FFPE slide preparation for immunofluorescence staining.

Deparafinization: 10-20minutes baking in oven for 60°C. Xylene treatment, 2 times for 10minutes each. Rehydration (2 minutes each), 100% ethanol, 95% ethanol, 70% ethanol, 50% ethanol, 100% dH2O. Antigenretrival: immerse slides into boiling citrate buffer, pH6.0 (30-40 min). Wait until buffer is cooled down followed by two washed is PBS (5min). Permeabilization: Incubate section in 0.5% PBST for 15 min followed by two washes in PBS (5min each). Serum blocking: 60 minutes incubation in 10% goat serum, 2% BSA in PBS. Primary antibody: Overnight incubation of section in Antibody dilution in 5% goat serum,1%BSA PBS solution, followed by one wash in 0.1% PBST and two wash steps in PBS (5 minutes each). Secondary antidoby: 60 minutes incubation with fluorescent conjugated secondary antibodies (Alexa fluor 488 and 555, Life technologies, A-11008 and Abcam, ab150170, (1:500 in 5% goat serum, 1%BSA PBS solution)) at room temperature for 1 hour. Three wash steps in PBS (5minute each), mounted with fluorescent mounting medium with DAPI (ProLong Gold antifade reagent with DAPI, Lifetechnologies, P36935), and cover sections with coverslip.

RNA-seq library preparation, sequencing and analysis.

Organoids (5 pairs from model A and one pair from model B of SPOP mutant and controls) were prepared for RNA sequencing using TruSeq RNA Library Preparation Kit v2. Each sample was sequenced with the HiSeq 2500 to generate 2×51-bp paired-end reads.

Reads (FASTQ files) were mapped to the mouse reference genome sequence (mm10/GRCm38 http://hgdownload.cse.ucsc.edu/downloads.html - mouse) using STAR v2.4.0j(Dobin et al., 2013), and the resulting BAM files were subsequently converted into mapped-read format (MRF) using RSEQtools(Habegger et al., 2011). Quantification of gene expression was performed via RSEQtools using GENCODE as reference gene–annotation set (http://www.gencodegenes.org/mouse_releases/9.html). Expression levels (RPKM) were estimated by counting all nucleotides mapped to each gene and were normalized by the total number of mapped nucleotides (per million) and the gene

length (per kb). ComBat(Johnson et al., 2007) was used to remove the batch effect for the downstream gene expression analysis.
Differential expression analysis were performed using the Mann-Whitney Wilcoxon test after transforming the RPKMs via $\log_2$(RPKM + 1) from mouse samples or RSEMs via $\log_2$(RSEM+1) from human samples. Multiple-hypothesis testing was considered by using Benjamini-Hochberg (BH; FDR) correction. The RNA-seq and reverse-phase protein array data of human prostate cancer samples were downloaded from TCGA (https://tcga-data.nci.nih.gov/tcga/), and genomic subclass information shown as described (Cancer Genome Atlas Research, 2015). Gene ontology analysis of SPOP regulated gene sets from hierarchical clustering and heatmap was performed using DAVID (Huang da et al., 2009). The statistical significance of the overlap between two groups of genes was tested using online method (http://nemates.org/MA/progs/overlap_stats.html).
GSEA (Subramanian et al., 2005) was performed using JAVA program (http://www.broadinstitute.org/gsea) and run in pre-ranked mode to identify enriched signatures. For the SPOP mutant profile in mouse prostates, genes were ranked from the most overexpression to the most underexpression. We used the gene sets in the Molecular Signature Database (MSigDB) (Subramanian et al., 2005) and added AR_RESPONSE_CASTRATE_DN and AR_RESPONSE_CASTRATE_UP defined by genes downregulated and upregulated in response to castration in wild-type mice (Carver et al., 2011). The GSEA plot, normalized enrichment score and q-values were derived from GSEA output, and sum of normalized enrichment score was calculated for each MSigDB oncogenic signature.

SRC3 knock down

Transient knockdown of *Src3* was done by transiently transfecting mouse organoid cells with siRNA targeting Src3 (SMARTpool, Dharmacon, #L-047722-02-0005). Matching controls were transfected with same concentrations of scrambled siRNA (ON-TARGETplus Non-targeting Pool, Dharmacon, #D-001810-10-20). Cells were incubated with siRNA (50,100 or 200nM) and DharmaFECT 1 (Dharmacon, #T-2001-02) for 48h before media change. Samples for RNA analysis was collected after 48h and samples for protein analysis after 72h.

IGF1R inhibition and IGF1 stimulation

Mouse prostate cells were treated over night with 0, 0.1, 10, 50 µM IGF1-receptor specific inhibitor (OSI-906 (Linsitinib), Selleckchem. For IGF1 stimulation, mouse prostate cells were treated over night with 0, 5, 10, 50 ng IGF1 protein (Abcam, # ab9861).

Mass spectrometry

Samples were treated with SDS-PAGE loading buffer supplied with 10 mM DTT for 5 min at 85ºC. The proteins were alkylated by the addition of iodoacetamide to the final concentration of 15 mM. Protein samples were subjected to SDS-PAGE and the whole lanes were cut out and digested with trypsin in-gel for 2 hours. The acquisition cycle consisted of a survey MS scan in the normal mode followed by twelve data-dependent MS/MS scans acquired in the rapid mode. Dynamic exclusion was used with the following parameters: exclusion size 500, repeat count 1, repeat duration 10 s, exclusion

time 45 s. Target value was set at 10^4 for tandem MS scan. The precursor isolation window was set at 2 *m/z*. The complete analysis comprised three independent biological replicates. Protein samples were extracted, dried and resuspended in 0.1% formic acid with 5% acetonitrile prior to loading onto a trap EASY-column (Thermo Scientific) coupled to an in-house made a nano HPLC column (20 cm x 75 um) packed with LUNA C18 media. Analysis was performed on Velos Pro mass spectrometer (Thermo Scientific) operated in data-dependent mode using 120-min gradients in EASY-nLC system (Proxeon) with 95% water, 5% acetonitrile (ACN), 0.1% formic acid (FA) (solvent A), and 95% ACN, 5% water, 0.1% FA (solvent B) at a flow rate of 220 nl/min.

MS data analysis

The resulting spectrum files were transformed into MGF format by MSConvert software and interrogated by MASCOT 2.4 search engine using mouse UniProt database concatenated with reverse sequences for estimation of false discovery rate (FDR) and with a list of common contaminants. The search parameters were as follows: full tryptic search, 2 allowed missed cleavages, peptide charges +2 and +3 only, MS tolerance 1 Da, MS/MS tolerance 0.5 Da. Permanent post-translational modifications was: cysteine carbamidomethylation. Variable post-translational modifications were: protein N-terminal acetylation, Met oxidation and N-terminal Glutamine to pyro-Glutamate conversion. The remaining analysis was performed as in (Poliakov et al., Mol Cell Proteomics. 2011 Jun;10(6)). To summarize, the minimal ion score threshold was chosen such that a peptide false discovery rate (FDR) below 1% was achieved. The peptide FDR was calculated as: 2 × (decoy_hits)/(target + decoy hits).

Spectral counts for all detected proteins were assembled using an in-house written Python script. The adjustment of spectral counts was done by the same script as in (Poliakov et al., Mol Cell Proteomics. 2011 Jun;10(6)).

Statistical analysis was performed by in-house written script called GLEE (Global Logarithm Error Estimation), which is a Python emulation of PLGEM software (Pavelka et al., BMC Bioinformatics. 2004 Dec 17;5:203) with modifications. Briefly, the main differences between GLEE and PLGEM are: a) GLEE uses cubic fit instead of linear in PLGEM and b) GLEE uses no binning and the fit is performed on the all data points and c) GLEE does not accept NSAF data (normalized spectral abundance factor). GLEE standalone Python application can be obtained here: https://github.com/lponnala/glee-py-gui. A web-based application can be found here: https://lponnala.shinyapps.io/glee/. Only proteins having p-value < 0.01 were considered for further analysis.

ScanProsite (http://prosite.expasy.org/scanprosite) was used to search the known SPOP Binding Consensus motif in significantly upregulated and downregulated proteins.

PRATT (http://www.ebi.ac.uk) was used for De novo motif analysis in top 100 SPOP-F133V upregulated proteins.

Supplemental References

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