Supplemental Data

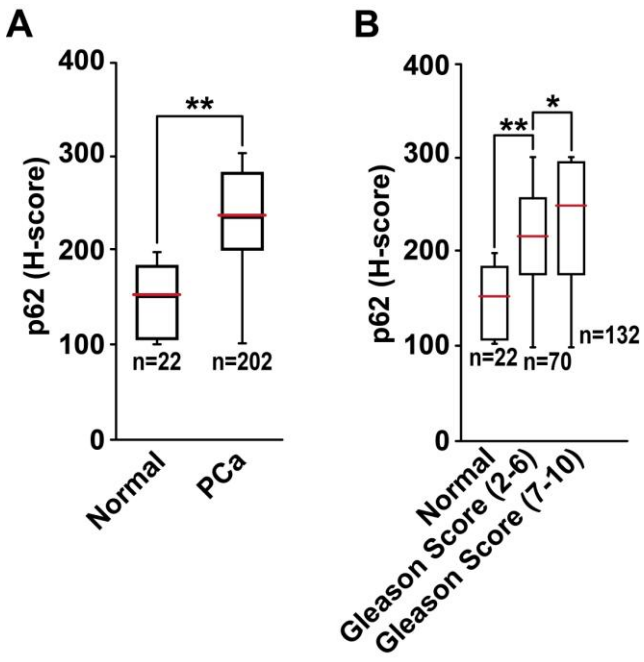
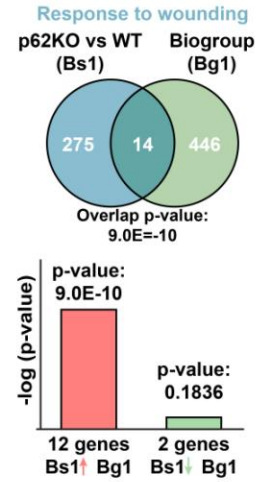


Figure S1, related to Figure 1. p62 is overexpressed in the epithelium of human prostate tumors.

(A) p62 levels are increased in the epithelium of primary PCa tumors as compared to normal samples, and (B) are upregulated upon PCa progression. Analysis of TMA of Figure 1A. The H-score, the staining intensity of p62 in epithelium x the proportion of cells with the observed intensity, was used to grade p62 expression levels in each sample. Students t test (*$p < 0.05$,**$p < 0.01$).

A

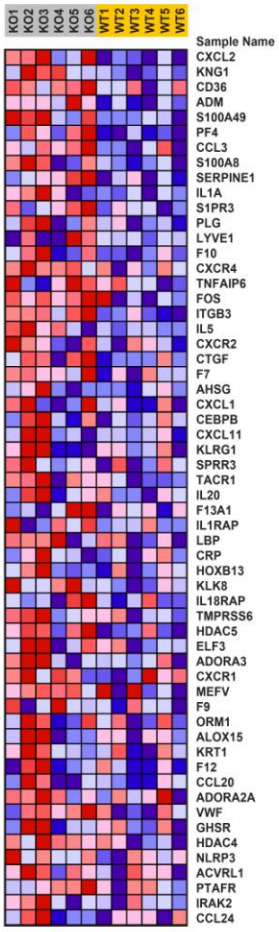


B

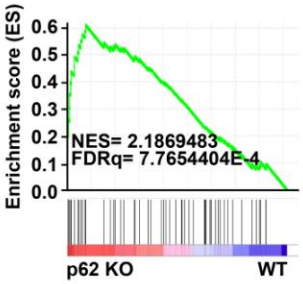
Gene sets enriched in phenotype p62 KO- Analysis with C5 database of MSigDB v. 3.1

RANK	MSigDB	Size	ES	NES	NOM p-val	FDR q-val	FWER p-val
1	GENERATION_OF_A_SIGNAL_INVOLVED_IN_CELL_CELL_SIGNALING	26	0.61	1.85	0.001	0.222	0.247
2	G_PROTEIN_SIGNALING_COUPLED_TO_CYCLIC_NUCLEOTIDE_SECOND_MESSENGER	95	0.46	1.82	0	0.166	0.344
3	CYCLIC_NUCLEOTIDE_MEDIATED_SIGNALING	97	0.46	1.78	0	0.181	0.499
4	HUMORAL_IMMUNE_RESPONSE	29	0.56	1.75	0.006	0.182	0.599
5	HORMONE_SECRETION	16	0.64	1.74	0.006	0.172	0.664
6	RESPONSE_TO_WOUNDING	171	0.41	1.73	0	0.15	0.685
7	DETECTION_OF_ABIOTIC_STIMULUS	19	0.61	1.73	0.007	0.137	0.708
8	CELL_CELL_SIGNALING	364	0.38	1.72	0	0.132	0.746
9	RESPONSE_TO_EXTERNAL_STIMULUS	274	0.38	1.71	0	0.125	0.771
10	NEGATIVE_REGULATION_OF_MULTICELLULAR_ORGANISMAL_PROCESS	27	0.55	1.69	0.008	0.14	0.838
11	SECOND_MESSENGER_MEDIATED_SIGNALING	141	0.41	1.69	0	0.137	0.861
12	NEUROLOGICAL_SYSTEM_PROCESS	343	0.37	1.68	0	0.135	0.876
13	INFLAMMATORY_RESPONSE	113	0.41	1.65	0.001	0.172	0.944
14	DETECTION_OF_STIMULUS	41	0.49	1.65	0.006	0.16	0.945
15	ECTODERM_DEVELOPMENT	69	0.44	1.65	0.004	0.15	0.945
16	POTASSIUM_ION_TRANSPORT	56	0.46	1.64	0.004	0.147	0.953
17	REGULATION_OF_MULTICELLULAR_ORGANISMAL_PROCESS	129	0.4	1.64	0.001	0.146	0.959
18	DEFENSE_RESPONSE	209	0.38	1.64	0.001	0.139	0.96
19	G_PROTEIN_SIGNALING_COUPLED_TO_CAMP_NUCLEOTIDE_SECOND_MESSENGER	60	0.45	1.63	0.009	0.136	0.964
20	SYNAPTIC_TRANSMISSION	162	0.38	1.62	0.001	0.141	0.975

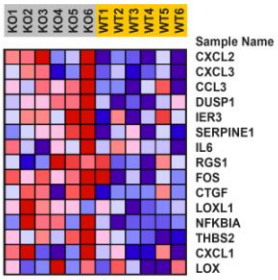
C



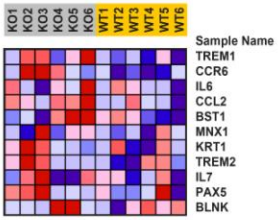
D



E



F



G

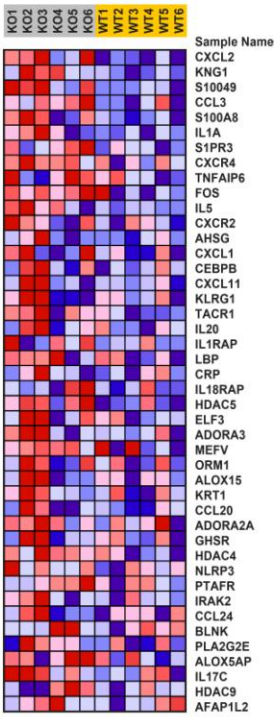


Figure S2, related to Figure 2. p62 deficiency in the stroma promotes a CAF and an inflammatory phenotype.

(A) Genes differentially expressed between orthotopic tumors from WT and p62 KO mice were subjected to NextBio analysis to identify biosets that contain similar genes. Venn diagrams show the number of common and unique genes in both sets. (B and C) Differentially expressed genes in orthotopic tumors from p62 KO mice analyzed by GSEA against C5 biological processes of the MSigDB database. (B) The top-ranked twenty gene sets enriched in p62 KO tumors are shown. (C) Leading edge genes of the “response to wounding” gene set. (D and E) Differentially expressed genes in orthotopic tumors from p62 KO mice analyzed by GSEA against C2 curated gene sets of the MSigDB database. (D) GSEA plot of the selected “stromal stimulation” gene set. (E) Leading edge genes of gene set of D. (F and G) Leading edge genes of selected gene sets enriched in p62 KO tumors from analysis shown in (B).

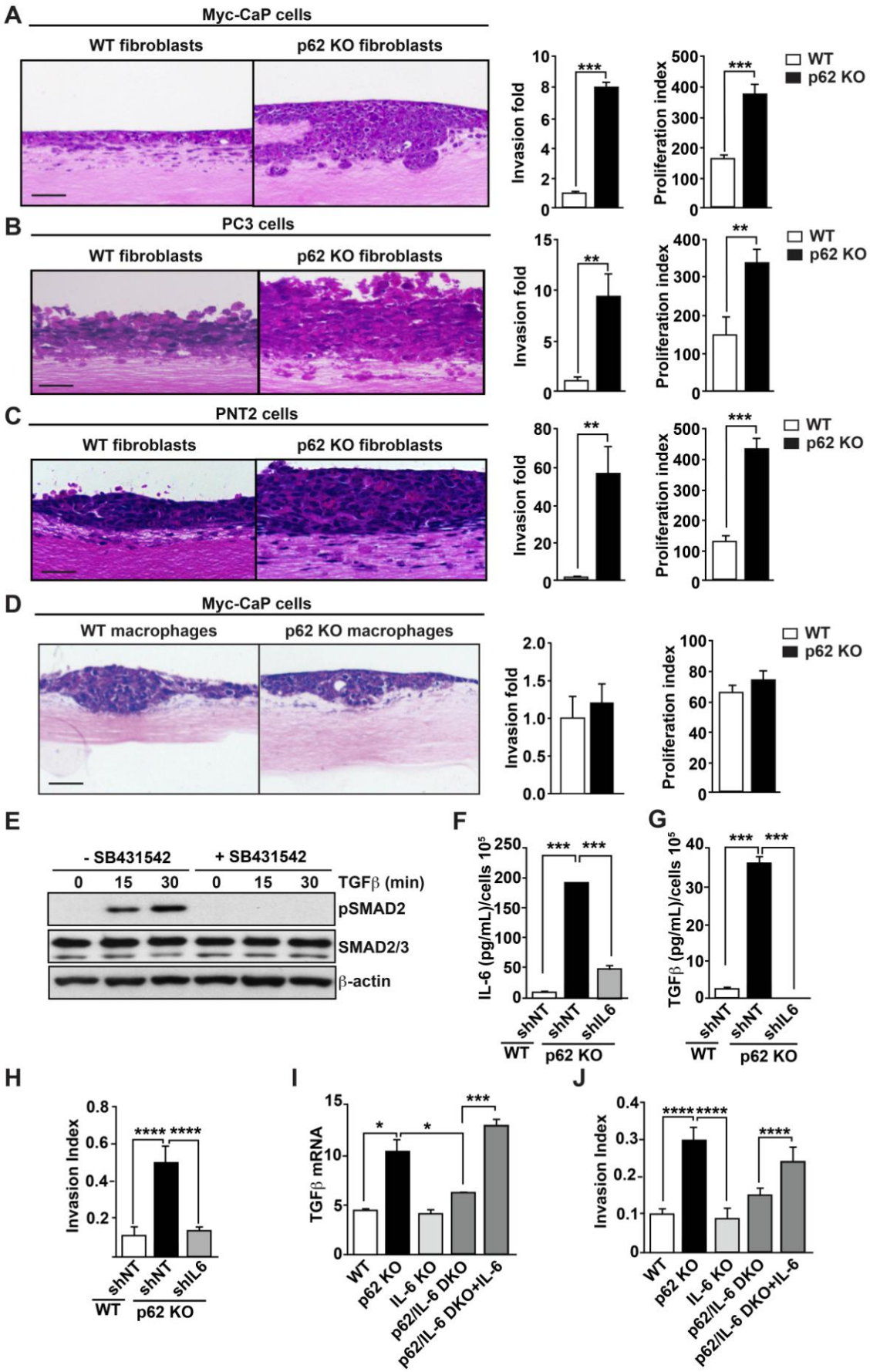

A
Myc-CaP cells
WT fibroblasts
p62 KO fibroblasts
Invasion fold
Proliferation index
WT
p62 KO
B
PC3 cells
WT fibroblasts
p62 KO fibroblasts
Invasion fold
Proliferation index
WT
p62 KO
C
PNT2 cells
WT fibroblasts
p62 KO fibroblasts
Invasion fold
Proliferation index
WT
p62 KO
D
Myc-CaP cells
WT macrophages
p62 KO macrophages
Invasion fold
Proliferation index
WT
p62 KO
E
- SB431542
+ SB431542
0
15
30
0
15
30
TGFβ (min)
pSMAD2
SMAD2/3
β-actin
F
IL-6 (pg/mL)/cells 10^5
shNT
shNT
shIL6
WT
p62 KO
G
TGFβ (pg/mL)/cells 10^5
shNT
shNT
shIL6
WT
p62 KO
H
Invasion Index
shNT
shNT
shIL6
WT
p62 KO
I
TGFβ mRNA
WT
p62 KO
IL-6 KO
p62/IL-6 DKO
p62/IL-6 DKO+IL-6
J
Invasion Index
WT
p62 KO
IL-6 KO
p62/IL-6 DKO
p62/IL-6 DKO+IL-6

Figure S3, related to Figure 3. Loss of p62 in the prostate stroma promotes tumorigenesis in the prostate epithelium.

(A-C) H&E-stained organotypic cultures of different type of PCa cells: Myc-CaP (A), PC3 (B) and PNT2 (C) combined with fibroblasts from WT and p62 KO mice. Right panels show quantification of PCa cell invasion and proliferation of the organotypic experiments. (D) H&E-stained organotypic cultures of Myc-CaP cells with macrophages from WT and p62 KO mice. Right panels show quantification of PCa cell invasion and proliferation of the organotypic experiments. (E) The TGFβ □□□□□□□□□ SB431542 effectively blocks TGFβsignaling as determined by immunoblot with pSMAD2. (F-H) Selective knockdown of IL-6 in p62 KO fibroblasts reverted increased IL-6 (F) and TGFβ Ģ production as determined by ELISA, as well as PCa invasion as determined by modified Boyden chamber assay (H). (I) Addition of IL-6 to p62/IL-6 DKO fibroblasts restored TGFβlevels. IL-6 was added for 48 hr and TGFβ □□□□levels were determined by RT-PCR. (J) PCa invasion determined by modified Boyden chamber assay in fibroblasts from the different genotypes. IL-6 was exogenously added to p62/IL-6 DKO fibroblasts. Data are means ± SEM (n = 4). *p < 0.05, **p < 0.01. ***p < 0.001, ****p < 0.0001. Scale bars, 100 μm.

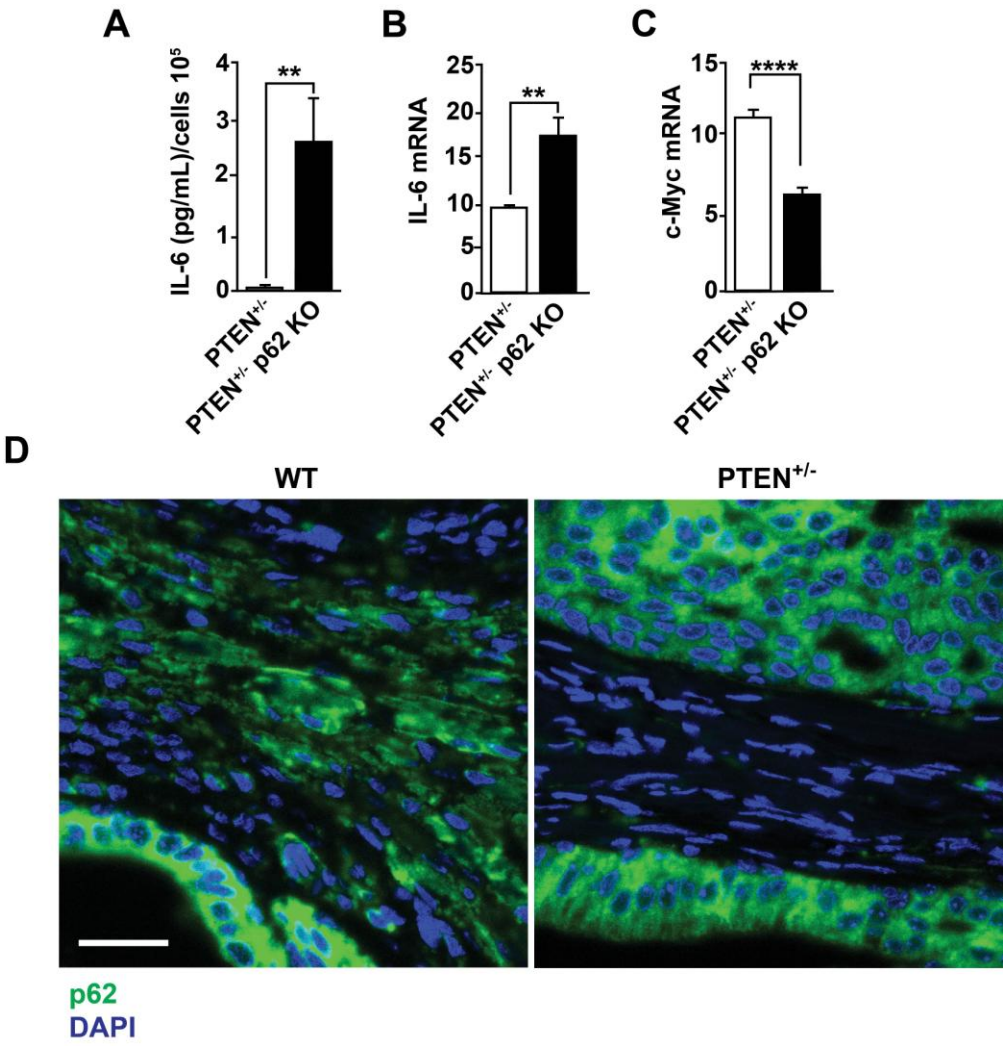
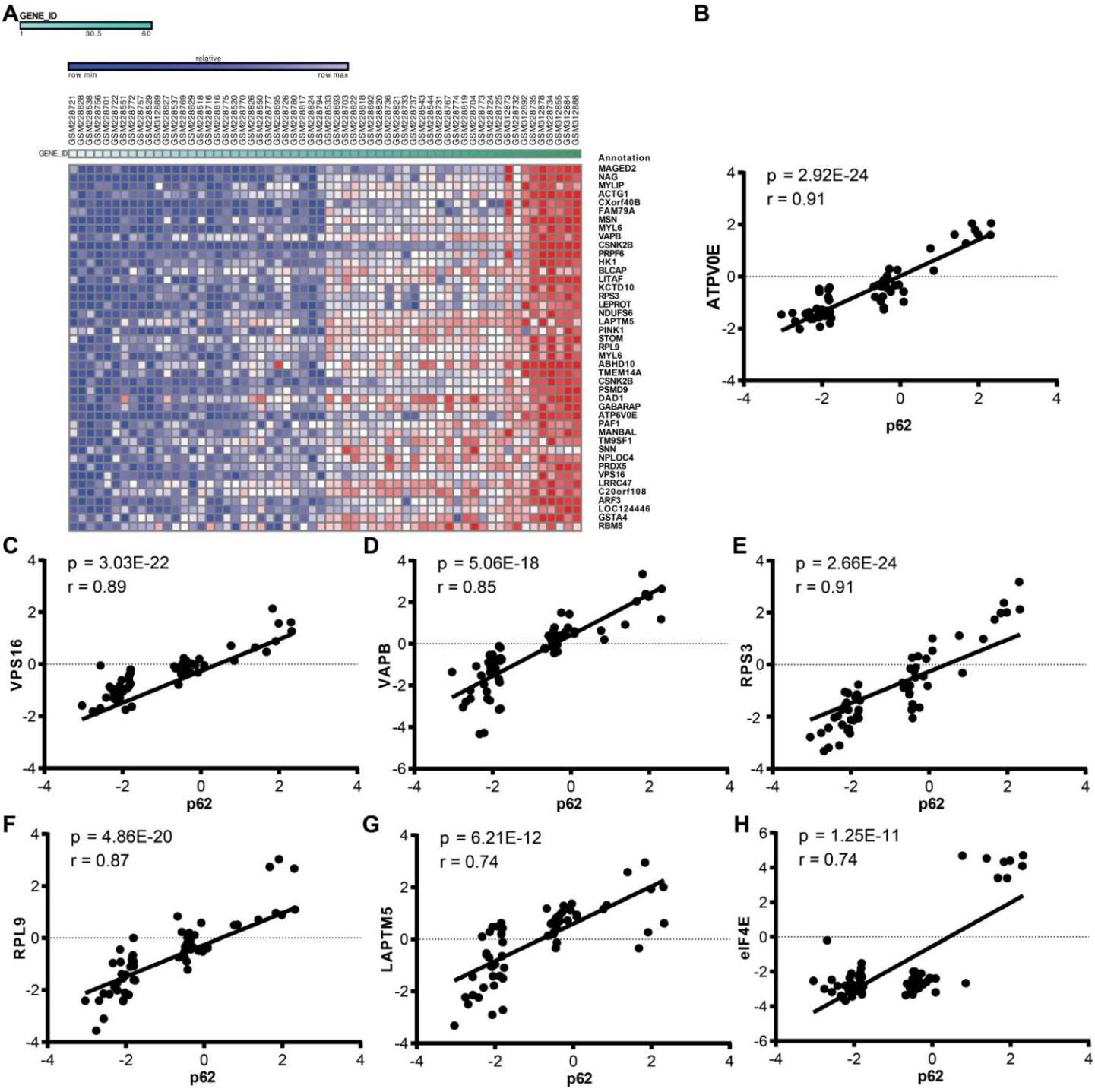


Figure S4, related to Figure 6. p62 role in prostate stroma of PTEN$^{+/-}$ mice.

(A-C) IL-6 ELISA (A) and RT-PCR of IL-6 (B) and c-Myc (C) in prostate stromal cells from PTEN$^{+/-}$ and PTEN$^{+/-}$/p62 KO mice (n = 6). (D) p62 levels are downregulated in the prostate stroma of PTEN$^{+/-}$ mice. Immunostaining of p62 (green) and DAPI (blue) were performed in prostate sections of WT and PTEN$^{+/-}$ mice of 9 months of age (n = 4). **p < 0.01, ****p < 0.0001. Scale bar, 25 µm. Results are presented as means ± SEM.


A
GENE_ID
1
30.5
60
relative
row min
row max
GENE_ID
Annotation
MAGED2
NAG
MYLIP
ACTG1
CXorf40B
FAM79A
MSN
MYL6
VAPB
CSNK2B
PRPF6
HK1
BLCAP
LITAF
KCTD10
RPS3
LEPROT
NDUFS6
LAPTM5
PINK1
STOM
RPL9
MYL6
ABHD10
TMEM14A
CSNK2B
PSMD9
DAD1
GABARAP
ATP6V0E
PAF1
MANBAL
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PRDX5
VPS16
LRRC47
C20orf108
ARF3
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GSTA4
RBM5
B
p = 2.92E-24
r = 0.91
ATPV0E
p62
C
p = 3.03E-22
r = 0.89
VPS16
p62
D
p = 5.06E-18
r = 0.85
VAPB
p62
E
p = 2.66E-24
r = 0.91
RPS3
p62
F
p = 4.86E-20
r = 0.87
RPL9
p62
G
p = 6.21E-12
r = 0.74
LAPTM5
p62
H
p = 1.25E-11
r = 0.74
eIF4E
p62

Figure S5, Related to Figure 7. p62 signature in human stroma.

(A) Heat map of the top 25 genes differentially expressed (FDR > 0.0002 and fold > 1.5) in the top 25% versus the bottom 25% of human stroma samples ranked by p62 expression levels. Expression data were extracted from the GSE9014 dataset. Blue and red in the heatmap indicate genes that are underexpressed or overexpressed, respectively. (B-H) A positive significant correlation of gene expression between p62 and ATPV0E (B), VPS16 (C), VAPB (D), RSP3 (E), RPL9 (F), LAPTM5 (G), and eIF4E (H) is shown for human stromal samples from the GSE9014 dataset.