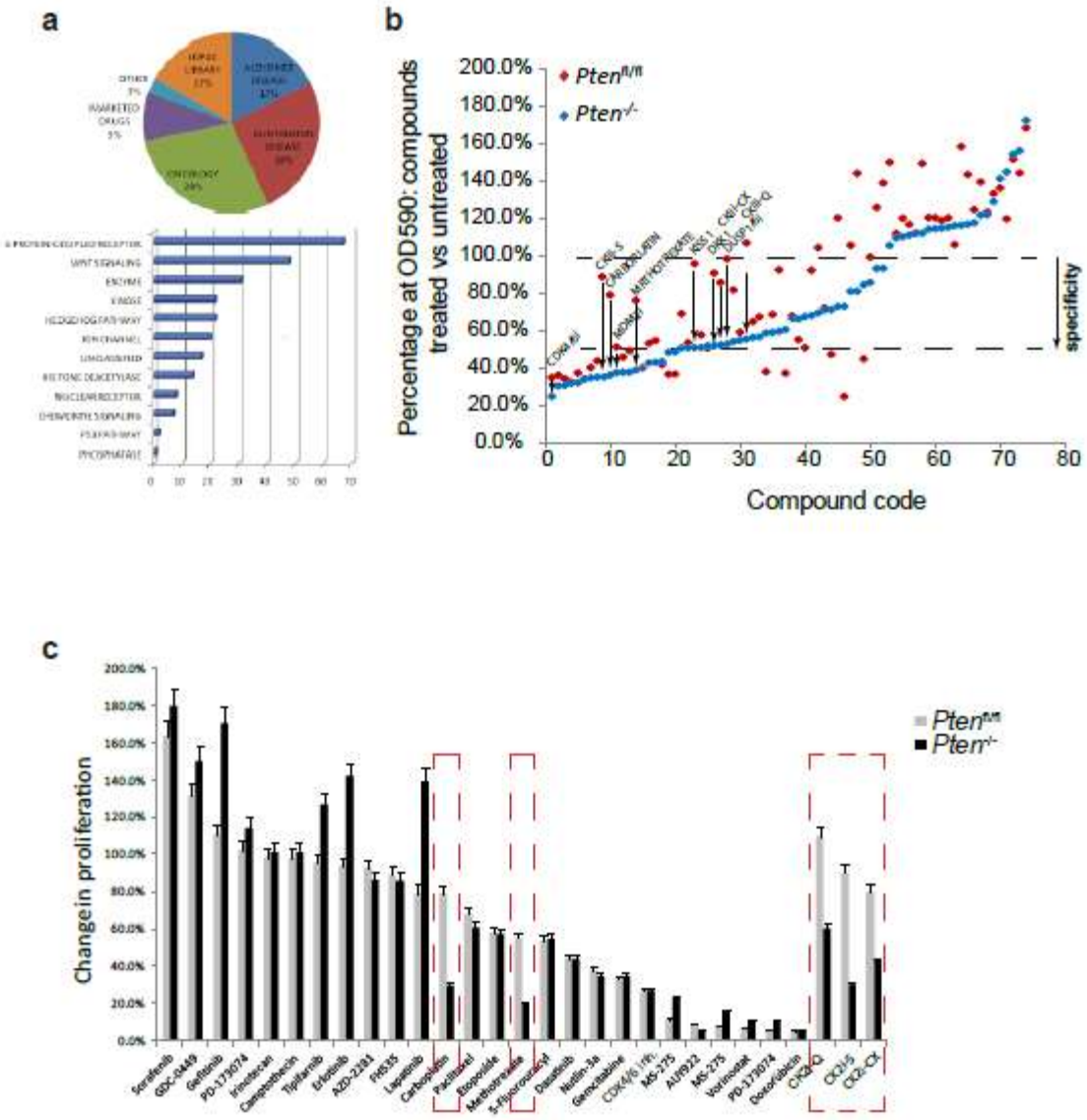
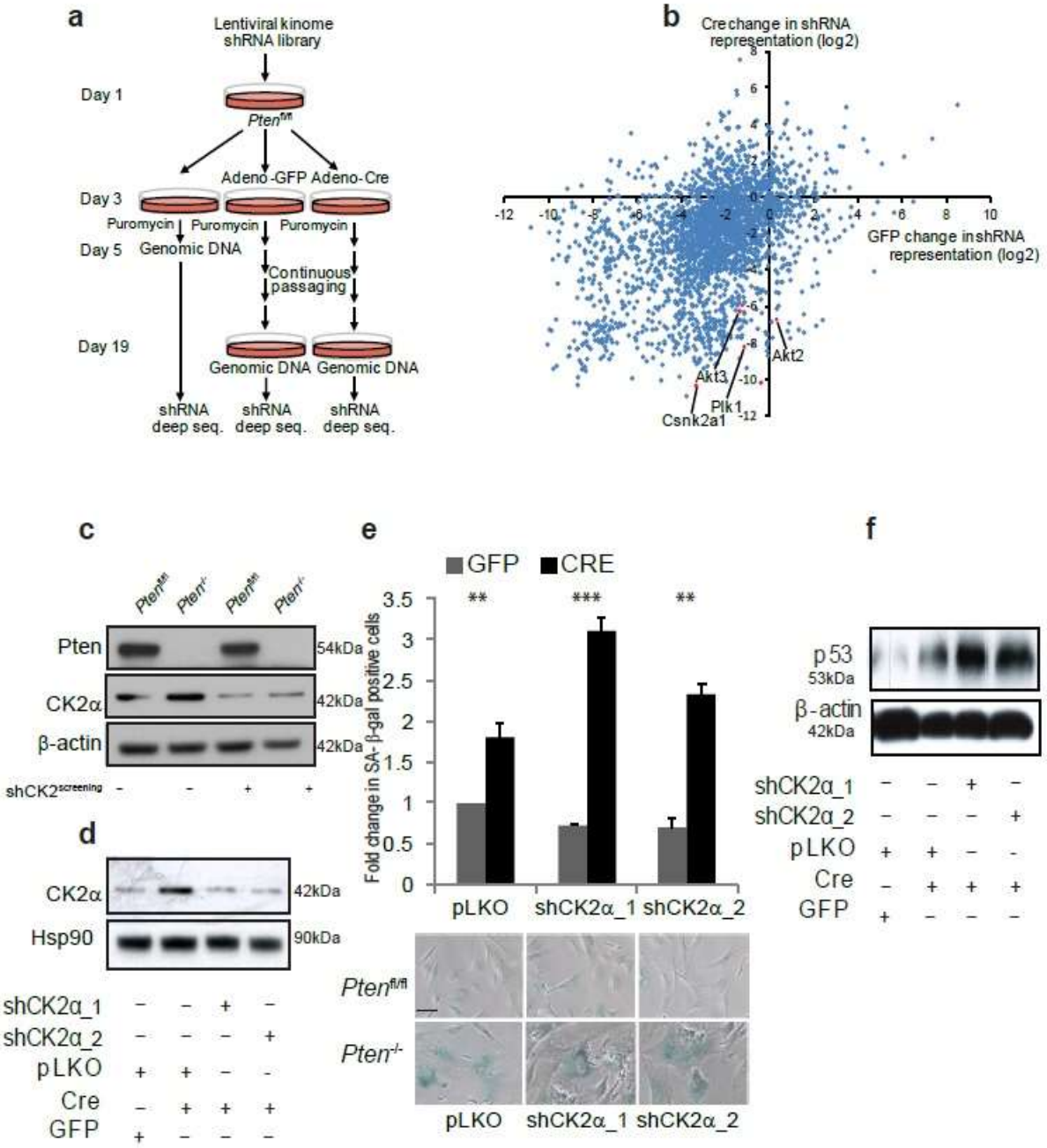


Supplementary Figure 1 | Compounds selection and screening of clinically approved compounds and inhibitors under pre-clinical investigation in *Pten*$^{fl/fl}$ and *Pten* $^{-/-}$ MEFs (**a**) 500 small molecule inhibitors were obtained from a larger compound library of 90,000 compounds. Criteria based on which the compounds were selected are represented in the pie graph. Classification of the small molecule inhibitors tested based on their targets. Bar graph represents the number of small molecules targeting indicated genes or pathways. (**b**) Graphical representation of various pro-senescence compounds and small molecule inhibitors identified by assessing their proliferation in both *Pten*$^{fl/fl}$ and *Pten* $^{-/-}$ MEFs. Red dots represent *Pten*$^{fl/fl}$ whereas blue dots represent *Pten* $^{-/-}$ MEFs. Values were normalized for the DMSO control and were organized according to the proliferation of *Pten* $^{-/-}$ MEFs, from the lower to higher values. (**c**) Proliferation of

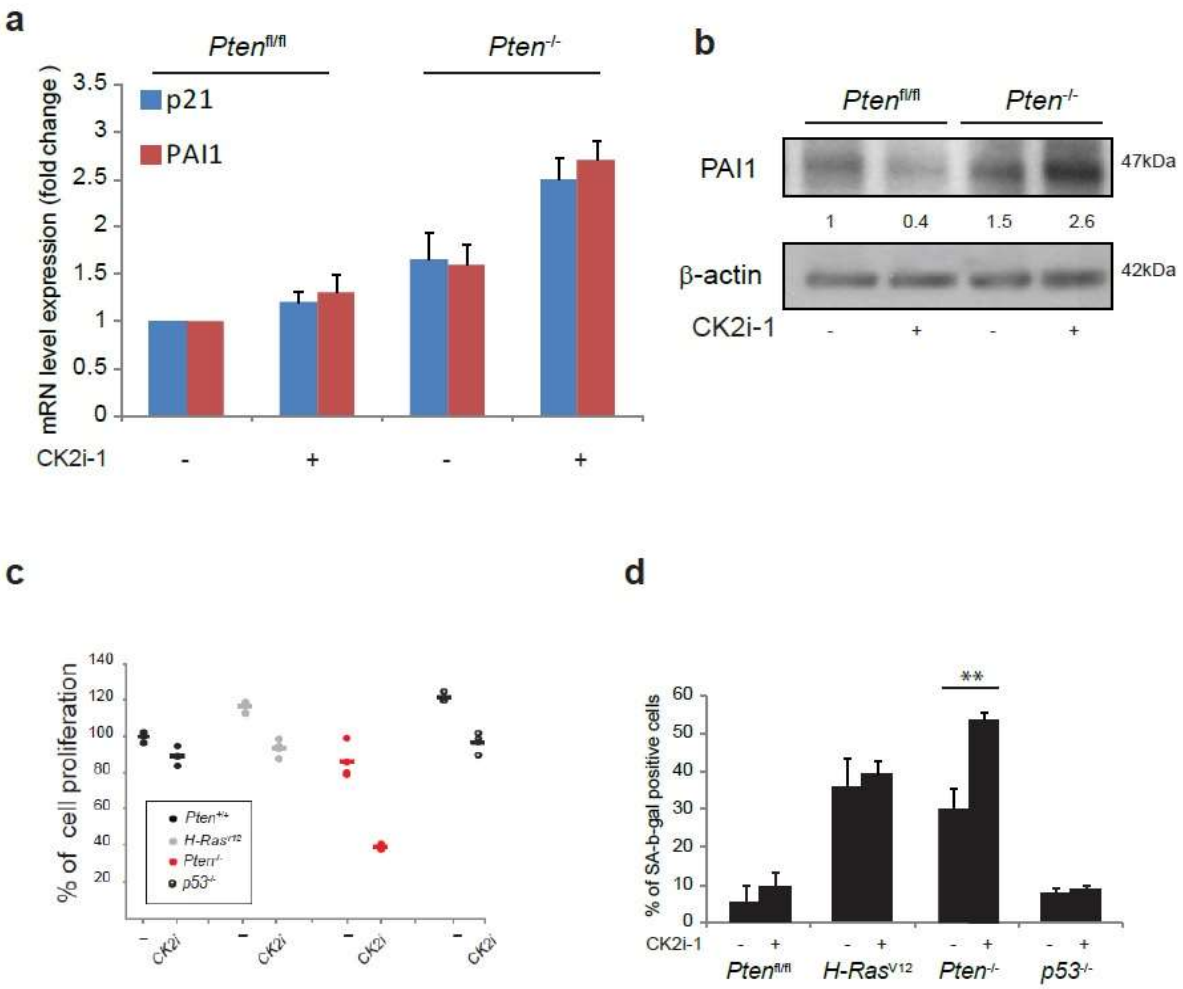
Pten$^{fl/fl}$ and *Pten* $^{-/-}$ MEFs after treatment with the indicated compounds (10 μM). They were normalized for the respective DMSO control.



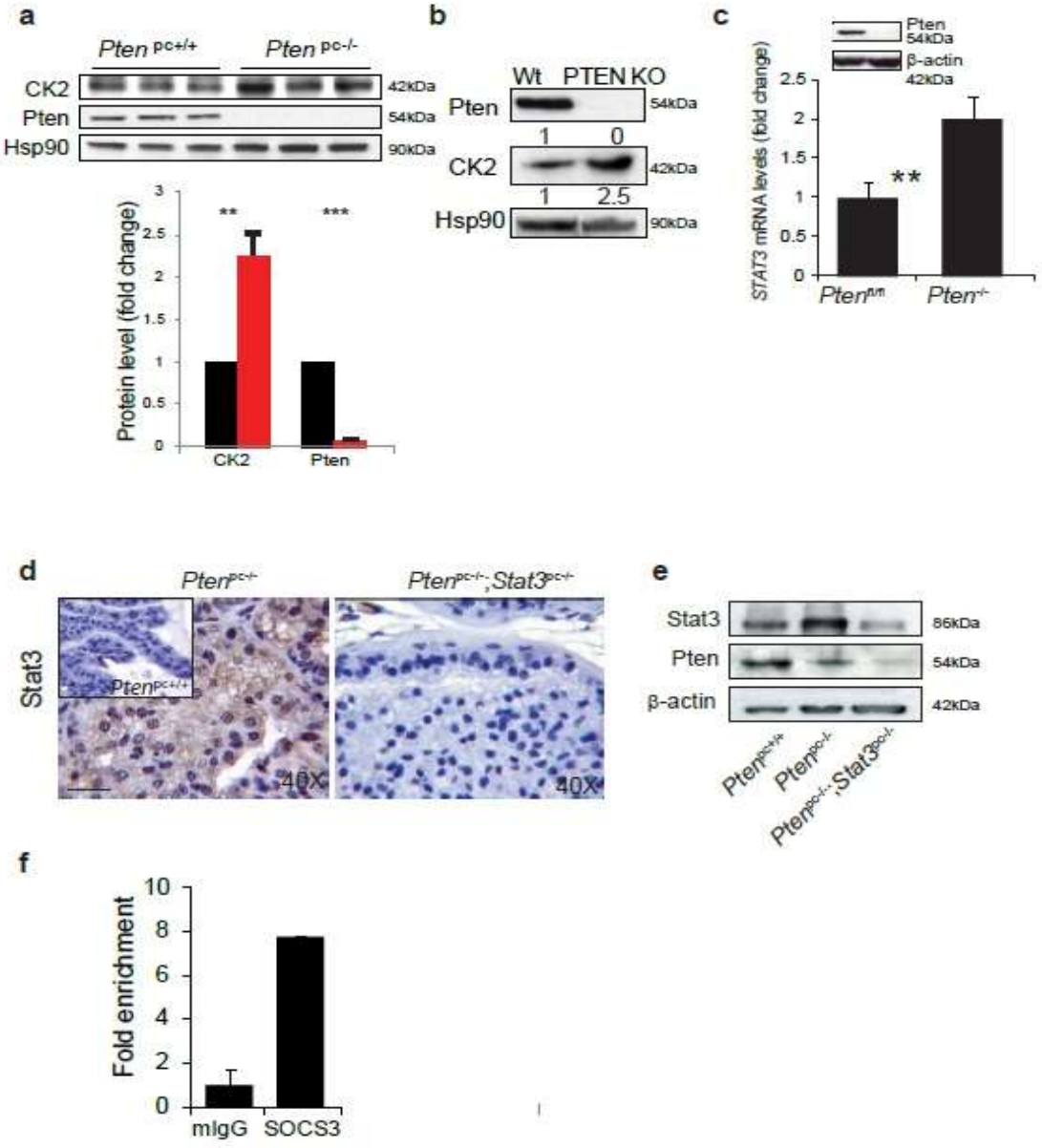
Supplementary Figure 2 | ShRNA screening

(**a**) Schematic overview of the experimental procedure for population-based screening of the shRNA kinome library. (**b**) Distribution of the changes in the representation of each shRNA at day 19 compared to day 5 in Adeno-GFP infected cells (x-axis) or Adeno-Cre infected cells (y-axis) (right panel). Data are plotted as log2 (shRNA representation late/shRNA representation early). Red dots highlight shRNAs that target selected genes. (**c,d**) Western blot analysis for CK2α in MEFs infected with an empty vector and either with the shRNAs against CK2α used in the screening. (c) or two shCK2 α (shCK2α_1 and shCK2α_2) used for the target validation (d). (**e**) Quantification (upper panel) and images (lower panel) of SA-β-gal positive cells in *Pten*$^{fl/fl}$ (GFP) and *Pten* $^{-/-}$ (Cre)

MEFs infected. Scale bar 10μM (**f**) Western blot analysis for p53 in the cells infected as described above in (d).



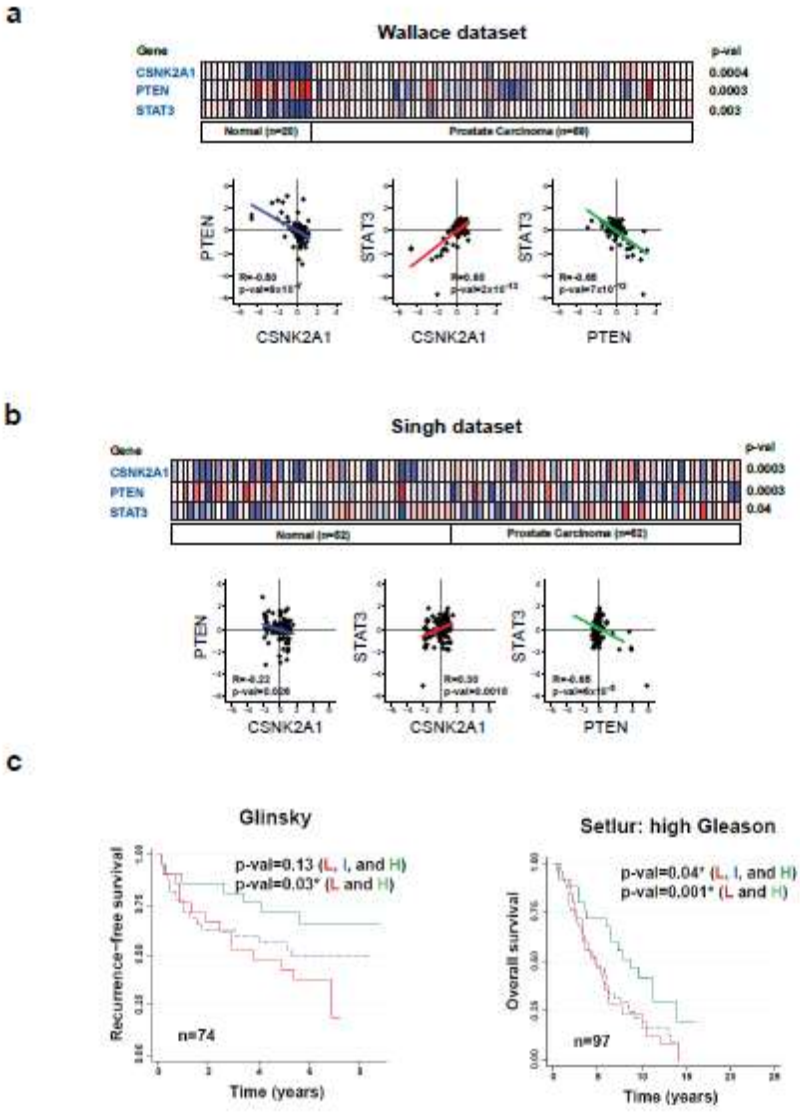
Supplementary Figure 3 | Senescence markers and specificity of CK2i for the *Pten*-/- genetic background (**a**) p21 and PAI1 mRNA in *Pten*$^{fl/fl}$ and *Pten* $^{-/-}$ MEFs treated with CK2IIi-Q. (**b**) WB for PAI1 in *Pten*$^{fl/fl}$ and *Pten* $^{-/-}$ MEFs treated with CK2IIi-Q. (**c**) Selective growth arrest induced by CK2i in *Pten*-/- cells when compared to other genetic backgrounds. (**d**) Enhanced and selective SA-β-gal staining induced by CK2i in *Pten*-/- cells when compared to other genetic backgrounds. Data are represented as mean ± s.d. n ≥ 3. p value indicates the statistical significance as measured by student t-test. (*p<0.05,**p<0.01, ***p<0.001).



Supplementary Figure 4 | CK2 protein levels in *Pten*$^{pc+/+}$ and *Pten*$^{pc-/-}$ and efficient depletion of Pten and Stat3 proteins in *Pten*$^{pc-/-}$;*Stat3*$^{pc-/-}$ prostate.

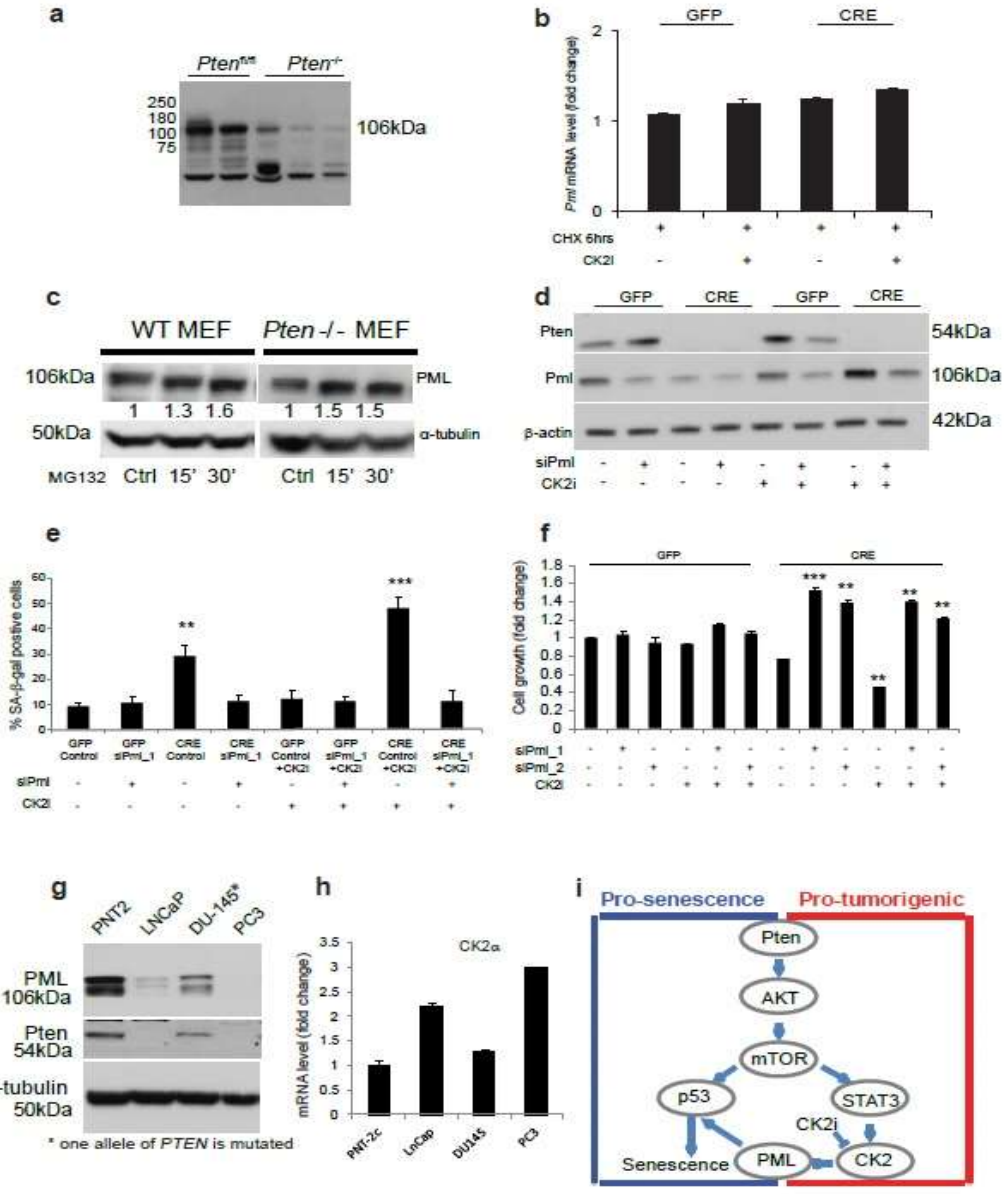
(**a**) Western blot for CK2 and Pten in *Pten*$^{pc+/+}$ normal prostate and *Pten*$^{pc-/-}$ prostate tumors (left panel). Quantification of the western blot (right panel). (**b**) WB shows the status of both PTEN and CK2 in the human HCT116 cell lines. (**c**) *Stat3* mRNA levels in Pten in *Pten*$^{fl/fl}$ and *Pten*$^{-/-}$ MEFs. Inset, western blot showing the status of Pten in those MEFs. (**d**) IHC showing Stat3 staining in *Pten*$^{pc-/-}$ and *Pten*$^{pc-/-}$;*Stat3*$^{pc-/-}$ prostate tumors. Inset, Stat3 staining in *Pten*$^{pc+/+}$ normal prostate. Scale bar: 50μM. (**e**) Western blot showing the efficient depletion of both Pten and Stat3 in *Pten*$^{pc-/-}$;*Stat3*$^{pc-/-}$ prostate tumors. (**f**) A Chip assay was performed with chromatin from MEFs using

antibody against Total Stat3. The immunoprecipitated DNA was amplified by qPCR, using primers specific for Socs3. Data are represented as mean ± s.d. n ≥ 3. p value indicates the statistical significance as measured by student t-test. (*p<0.05,**p<0.01, ***p<0.001)



Supplementary Figure 5 | Correlation between PTEN/CK2/STAT3 in human prostate cancer (**a,b**) Heat map analysis for *PTEN*, *CK2* and *STAT3* mRNA expression levels in the Wallace (a) and Singh (b) prostate cancer datasets. Expression of these genes is significantly deregulated in prostate cancer samples when compared with normal prostate samples. *PTEN* expression is downregulated whereas *CK2α* and STAT3 are over expressed. Corresponding p-values for each gene is shown (T-test analysis). Total number of normal and tumor samples analyzed is included in the panels. For both datasets, scatter plot graphs shown inverse correlation between *PTEN* and *CK2* or *PTEN* and *STAT3* mRNAs, and direct correlations between *CK2* and *STAT3* (lower panels in a and b). Pearson correlation (R), significance p-values, and regression lines are shown in each paired comparison. (**c**) Kaplan-Meier curves for disease-free (biochemical recurrence) (Glinsky dataset) and overall survival (Setlur dataset) in patients with tumors expressing different levels of *PTEN* and *CK2*. Patients were stratified into low (L), intermediate (I) and high (H) group levels depending on the values of *PTEN* and *CK2* mRNA levels (see also Methods). Patients having low *PTEN* expression and high *CK2* expression are associated with a worst disease-free and overall survival when compared with the other groups. Number of patients in each cohort is indicated in the panels. P-

values were calculated using log-rank test. Asterisks indicate significant differences between patient groups.



Supplementary Figure 6 | Pml in MEFs and cancer cell lines

(**a)** Entire scan for the western blot showing the different isoforms of Pml in 2 *Pten*$^{pc+/+}$ normal prostate and 3 *Pten*$^{pc-/-}$ prostate tumors. For our analysis we have quantified the bands around 106 KDa. (**b)** *Pm1* mRNA level in *Pten*$^{fl/fl}$ and *Pten*$^{-/-}$ MEFs treated with cyclohexamide (CHX) for 6hrs in presence or absence of Quinalizarin (CK2i). (**c)** Western blot showing the protein level of Pml in *Pten*$^{fl/fl}$ and *Pten*$^{-/-}$ MEFs treated with proteasome inhibitor MG132. Ctrl= control untreated cells. (d) Western blot analysis for ml, Pten and β-actin in MEFs with an additional siPml in the absence or presence of CK2i. (e) SA-β-gal quantification of d. (**f**) Proliferation assay in *Pten*$^{fl/fl}$ and *Pten*$^{-/-}$ MEFs transfected with two different siPml. (g) PML and PTEN protein levels in different prostate cancer cells lines. (h) CK2α mRNA levels in different prostate cancer cell lines. (i) Proposed model. Loss of *Pten* initiates tumorigenesis but at the same time, promotes a concomitant senescence response opposing tumor progression. However, *Pten* loss also favors the up-regulation of CK2 (promoting event) that in turn promotes evasion of cellular senescence by impacting on the

level of Pml. Inhibition of CK2 stabilize the level of Pml potentiating senescence in tumor ad advance stages. Data are represented as mean ± s.d. n ≥ 3. p value indicates the statistical significance as measured by student t-test was used throughout. (*p<0.05,**p<0.01, ***p<0.001).

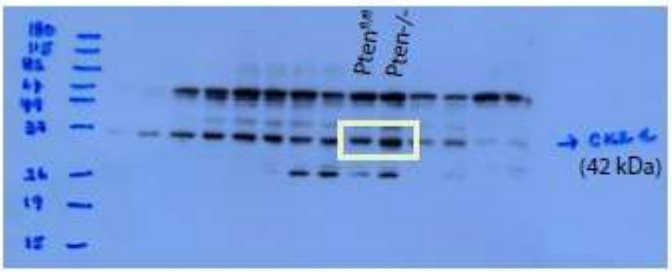


Fig. 2a

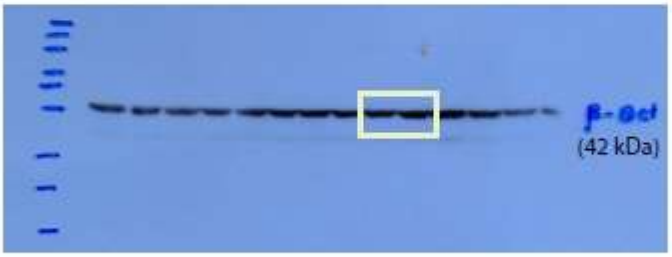


Fig. 2a

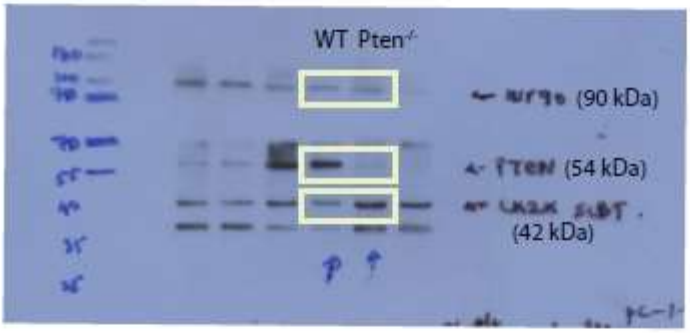


Fig. 2b

Fig. 2c

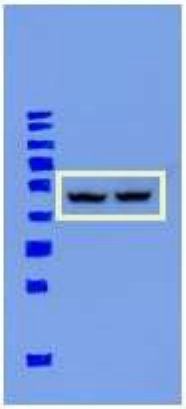


Fig. 2c

b-actin (42 kDa)

Supplementary Figure 7: Selected images of the western blots.

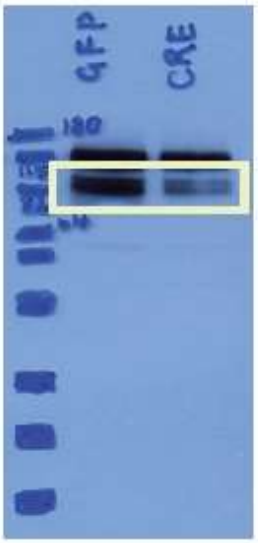
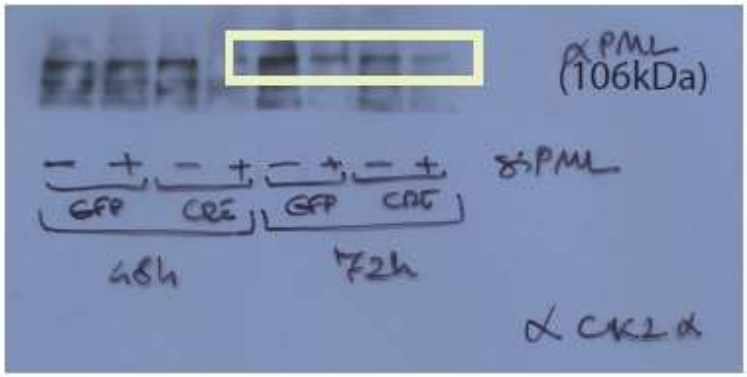


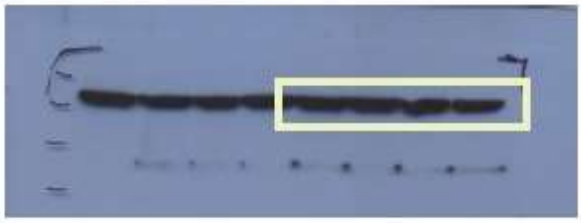
Fig. 3a
Pml
(106kDa)

Fig. 3a
(90kDa)

Fig. 3f

$Pten^{fl/fl}$ $Pten^{-/-}$





b-actin
(42 kDa)

Fig. 3f

$Pten^{fl/fl}$ $Pten^{-/-}$

CK2i + + + +

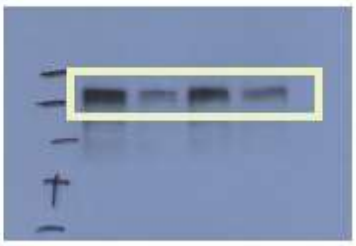
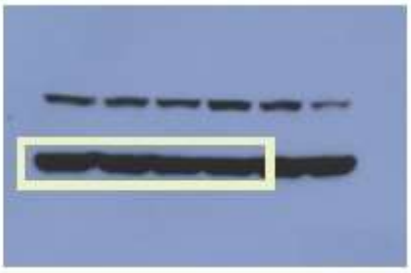
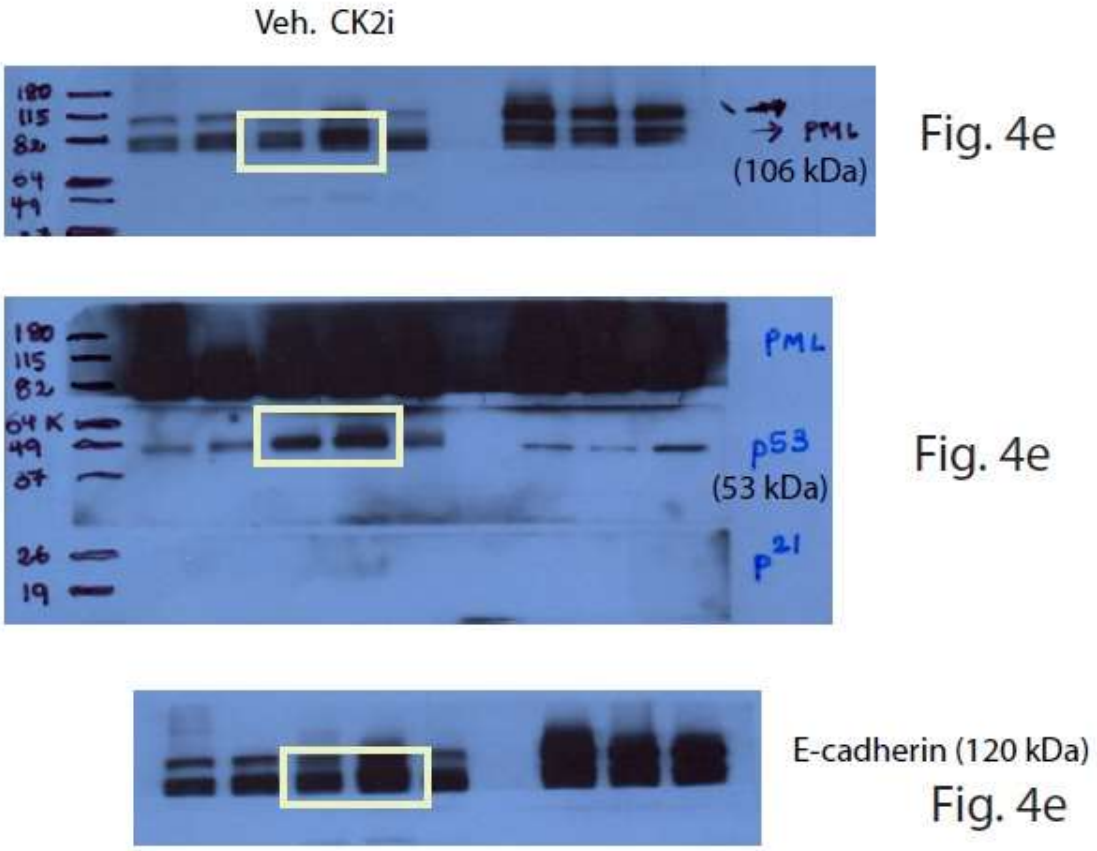


Fig. 3f
Pml
(106kDa)



b-actin
(42 kDa)

Supplementary Figure 7 (continued)



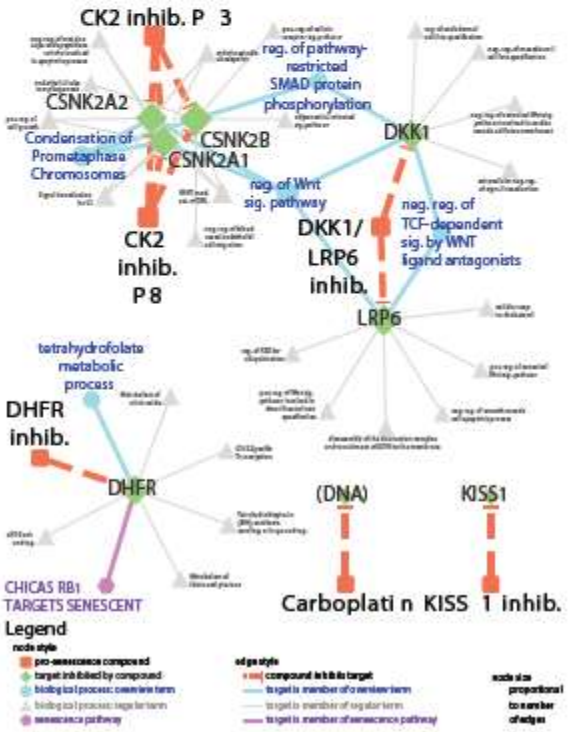
Supplementary Figure 7 (continued)

a

Selective inhibitors	n	Selectivity % of inhibition compared to untreated control (IC 50)	%SA-β-Gal increase compared to untreated control (IC 50)
CK2α	3	wt 5±2%; -/- 53±5% wt 5±2%; -/- 58±7% wt 7±3%; -/- 51±3%	wt 3±1%; -/- 45±3% wt 6±1%; -/- 56±4% wt 4±2%; -/- 48±8%
KISS1	1	wt 6±3%; -/- 51±4 %	wt 2±1%; -/- 27±3%
DKK1	1	wt 9±3%; -/- 50±4%	wt 10±2%; -/- 62±5%
DIHYDROFOLATE REDUCTASE	1	wt 20±5%; -/-60±5%	wt 5±2%; -/- 20±3%
Carboplatin	1	wt19±4%; -/- 60±4%	wt 12±2%; -/- 55±5%

Positive hits identified in the screening

b



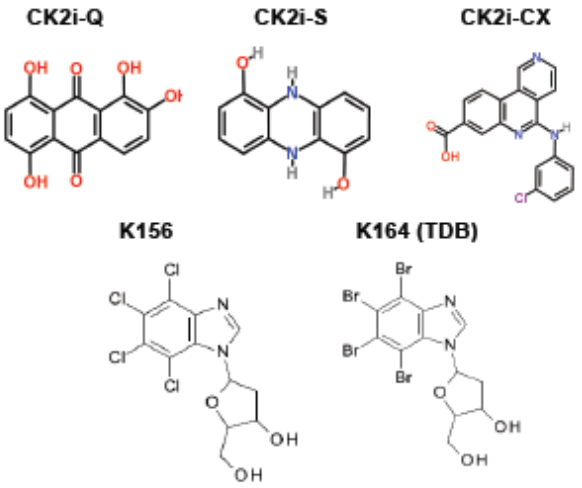
Supplementary Table 1 Positive hits and Systematic mapping of each pro-senescence compound's targets and each target's membership in biological processes in general and senescence pathways in particular. (**a**) Positive hits identified in the screening (**b**) Pro-senescence compounds were submitted to Drug Bank to complete the list of targets they inhibit. Functional annotation for each target was achieved by interrogating all experimentally verified biological processes comprised in the Gene Ontology (GO) and the Reactome database. From every group of terms, the most interconnected term was selected as its overview term and highlighted in blue. In addition, every target was linked to any senescence pathway comprised in any of the major bioinformatics resources and displayed in purple. Including biological processes that are not experimentally verified would have annotated KISS1 with the GO-terms positive regulation of growth hormone secretion and positive regulation of luteinizing hormone secretion. Abbreviations: activation (act.), inhibitor (inhib.), mediated (med.), negative (neg.), positive (pos.), regulation (reg.), response (resp.) and signaling (sig.).

a

	Experiment 1		Experiment 2	
shRNA identifier	GFP log2(T1/T0)	CRE log2(T1/T0)	GFP log2(T1/T0)	CRE log2(T1/T0)
NM_010407.3-435e1c1_Hck	-1.8	-7.6	-2.0	-10.1
NM_008746.4-113e1c1_Ntrk3	-0.5	-7.9	-3.4	-8.8
NM_007788.2-1069e1c1_Camk2a1	-2.0	-6.7	-3.3	-10.4
NM_008011.1-1007e1c1_Fgfr4	-0.4	-6.5	-1.0	-9.4
NM_009288.1-1620e1c1_Stk10	-0.6	-7.2	-2.7	-8.3
NM_177395.2-1559e1c1_Map3k9	-1.3	-7.8	-2.1	-7.8
NM_007783.2-905e1c1_Cak	-2.2	-7.4	0.9	-7.8
NM_053067.1-254e1c1_Epgn	-0.7	-6.2	-2.7	-8.6
NM_148945.1-783e1c1_Rps6ka3	-1.6	-4.6	-0.4	-10.2
NM_010570.2-486e1c1_Irs1	-0.3	-6.4	-2.9	-8.2
NM_172584.1-105e1c1_Epk1	0.0	-5.1	-2.8	-9.3
NM_023813.2-1254e1c1_Camk2d	0.5	-6.8	-3.2	-7.6
NM_016693.2-2782e1c1_Map3k5	-2.2	-6.5	-1.8	-7.7
NM_134250.1-136e1c1_Havcr2	0.1	-6.6	-2.1	-7.7
NM_145962.1-912e1c1_Pank3	-0.7	-5.5	-0.1	-8.7
NM_009873.1-866e1c1_Cdk6	-0.8	-5.1	-2.5	-9.2
NM_010547.1-732e1c1_Ikbkg	-1.3	-7.3	-2.0	-6.9
NM_007560.3-1503e1c1_Bmpr1b	-1.5	-6.5	-1.5	-7.7
XM_285897.2-868e1c1_Kar2	-2.6	-6.2	-1.3	-7.7
NM_007710.1-178e1c1_Ckm	-0.6	-5.8	-3.0	-8.0
NM_022014.1-841e1c1_Rs3k	-1.1	-6.0	0.1	-7.7
XM_194822.4-4852e1c1_Taf1	-1.6	-6.3	-2.3	-7.4
NM_008828.1-425e1c1_Pgk1	-0.6	-6.8	-1.6	-6.8
NM_019730.1-411e1c1_Nme3	-2.6	-6.5	2.1	-7.1
NM_010570.2-3168e1c1_Irs1	-1.2	-8.7	-2.2	-4.9
NM_013726.1-152e1c1_Dbf4	-1.5	-7.6	-1.7	-6.0
NM_175294.2-228e1c1_Nucks1	-2.8	-6.1	0.8	-7.4
NM_009535.1-1923e1c1_Yes1	0.1	-4.3	-3.3	-9.2
NM_016695.1-789e1c1_Mpp2	-1.1	-5.9	-1.9	-7.6
NM_008924.1-1372e1c1_Prkar2a	-0.7	-6.1	-0.5	-7.2
NM_021479.1-809e1c1_Tesk3	-2.2	-6.4	-1.1	-6.9
NM_144549.2-1060e1c1_Titb1	0.0	-6.1	-2.9	-7.2
NM_031190.1-453e1c1_Pgk2	-1.0	-7.0	-2.2	-6.2
NM_027165.1-463e1c1_Cdk3	-1.3	-5.9	1.3	-7.3
NM_007928.1-113e1c1_Mark2	-0.5	-4.5	-2.5	-8.6
NM_010292.3-1296e1c1_Gck	-2.0	-6.4	-1.3	-6.8
XM_487772.1-2308e1c1_Tnni3k	-2.2	-7.9	-1.5	-5.2
NM_013871.2-539e1c1_Mapk12	1.7	-5.6	-2.8	-7.5
XM_147046.2-1111e1c1_Atr	-1.9	-6.8	-1.2	-6.3
NM_001001983.1-6236e1c1_Pikfyve	-0.4	-4.6	-0.1	-8.4
NM_016681.2-1008e1c1_Chek2	-1.5	-7.6	-1.4	-5.5
NM_016686.3-466e1c1_Cib2	-2.4	-7.8	-0.2	-5.2
NM_011282.1-1365e1c1_Rpe1	-1.8	-7.5	-1.4	-5.5
NM_008925.1-1694e1c1_Prkcah	-1.6	-8.3	0.3	-4.7
NM_029634.1-866e1c1_Ihpk2	-0.7	-6.1	-2.3	-6.8
NM_021461.2-435e1c1_Mknk1	-1.2	-7.7	0.3	-5.2
NM_007398.2-124fe1c1_Acvr2a	1.2	-4.5	-2.0	-8.4
NM_011494.2-931e1c1_Stk16	-0.2	-7.1	-3.2	-5.7
NM_007434.2-280e1c1_Akt2	-1.7	-6.1	0.3	-6.7
NM_009516.2-892e1c1_Wee1	1.3	-5.1	-2.8	-7.7
NM_011841.1-162fe1c1_Mapk7	-2.2	-6.0	-0.8	-6.8
NM_007908.2-1207e1c1_Eef2k	-0.6	-5.9	-2.7	-6.9
NM_011795.2-865e1c1_Akt3	-2.2	-6.4	-1.3	-6.3
NM_013724.1-4234e1c1_Nrk	-1.0	-5.2	1.6	-7.4
NM_007483.2-1060e1c1_Speg	-1.7	-6.2	-1.2	-6.4
NM_019374.1-769e1c1_Pftk1	-1.3	-7.0	-1.7	-5.5
NM_178907.1-1287e1c1_Mapkapk3	-1.0	-8.6	-2.7	-3.9
NM_008810.1-658e1c1_Pgk6	-2.4	-5.7	-1.2	-6.8
NM_011121.2-1464e1c1_Ptk1	-2.0	-4.2	-1.1	-8.2
NM_009184.1-657e1c1_Ptk6	1.0	-9.2	-3.4	-3.2
XM_194716.2-1230e1c1_Lats1	-1.4	-5.3	-2.4	-7.1
NM_011851.1-865e1c1_Gsk1	-0.5	-6.9	-1.2	-5.5
NM_016695.2-477e1c1_Ak2	-1.0	-6.3	-2.1	-6.1
XM_485948.1-4424e1c1_Lmtk1	-1.4	-5.4	0.1	-6.9
NM_198703.1-4167e1c1_Wnk1	-0.9	-4.4	-3.0	-7.7
NM_028105.2-1360e1c1_Adck1	-2.2	-7.9	-1.2	-4.3
NM_019703.2-1378e1c1_Pfkp	-1.5	-5.7	1.6	-6.4
NM_023580.2-1293e1c1_Epha1	-2.2	-6.7	-1.4	-5.4
NM_029428.1-788e1c1_Brsk2	-1.8	-7.0	-0.3	-5.1
XM_111790.4-439e1c1_Pak6	-2.2	-5.3	-1.4	-6.8
NM_008194.1-277e1c1_Gyk	-1.8	-4.5	-1.2	-7.6
NM_007397.1-1398e1c1_Acvr2b	0.0	-5.0	-2.2	-7.0
NM_181591.2-1564e1c1_Txndc3	-0.5	-5.3	-3.3	-6.7
NM_008193.2-213e1c1_Guk1	-1.7	-4.4	-2.1	-7.6
NM_010387.1-2968e1c1_Magi1	-1.1	-6.9	-0.5	-5.1

b

CK2 inhibitors	*Pten*$^{fl/fl}$	*Pten*$^{-/-}$
	IC50	
CK2i-Q	6.4 µM	2.9 µM
CK2i-S	15.4 µM	9.2 µM
CK2i-CX	21.7 µM	11.3 µM
K156	41.1 µM	28.5 µM
K164	4.4 µM	2.3 µM



Supplementary Table 2 ShRNA screening and CK2 inhibitors. (**a**) List of hits from two independent experiments. Numbers represent log2 values of changes in the representation of each shRNA (end time point T1/early time point T0). (**b**) Table representing the IC50 in *Pten*$^{fl/fl}$ and *Pten* $^{-/-}$ MEFs of a series of five CK2 inhibitors under pre-clinical and clinical development **(**Upper panel).Chemical structure of the five CK2 inhibitors tested in the experiment above (Lower panel). CK2i-Q is referred to Quinalizarin, CK2i-S is referred to phenazine deravative and CK2i-CX is referred to CX-4945.

	PTEN mutation status	PTEN protein level	Reference
MCF-7	wt	high	Lao H Saal *et al*, Nat. Gentetics 2007
22rv1	wt	high	Fraser M et al, Clin Cancer Res. 2012
MDA-MB 175 VII	wt	high	Lao H Saal *et al*, Nat. Gentetics 2007
HCC 1500	wt	high	Cesar G Sanchez *et al*, Breast Cancer Research 2011
A375	wt	high	Hensin Tsao et al, JID 2004
LnCaP	Loss	Low	Alimonti A *et al*, JCI 2010
U87MG	mutated	Low	Shenghua Wen *et al*, PNAS 2001
MDA-Pca-2b	wt	Low	Alimonti A et al, JCI 2010
ZR-75-1	323T>G (L108R)	Low	Lao H Saal *et al*, Nat. Gentetics 2007

Supplementary Table 3 *PTEN* status in different human cell lines.

a

Variable	HR[1]	95% CI[2]	p-val
PTEN/CSNK2A1 ratio	0.64	0.40 to 1.01	0.05*
Age	1.03	0.99 to 1.07	0.09
Gleason	1.88	1.28 to 2.75	0.001*

[1] HR: Hazard ratio.
[2] CI: confidence interval.
* Significant p-values

b

Variable	HR[1]	95% CI[2]	p-val
PTEN/CSNK2A1 ratio	0.15	0.04 to 0.53	0.003*
Age	1.06	0.99 to 1.13	0.09
Gleason	1.79	1.22 to 2.62	0.003*
$\log_{10}$(prePSA)	2.57	0.69 to 9.60	0.2
SMS[3]	2.01	0.84 to 4.84	0.1

[1] HR: Hazard ratio.
[2] CI: confidence interval.
[3] SMS: surgical margins status
* Significant p-values

Supplementary Table 4 Multivariate Cox. Multivariate Cox regression including the *PTEN/CSNK2A1* ratio and prostate cancer clinical variables in the Glinsky dataset (a) and Setlur data set (b).

hCK2A1P-Mut-1_Fwd: 5'-GAATAGAAAGTGGGGCTGCAACCCTAATTTAAAACGAGGGGT-3'
hCK2A1P-Mut-1_Rev: 5'-TTAAATTAGGGTTGCAGCCCCACTTTCTATTCAAACTTGGAG -3'

hCK2A1P-Mut-2_Fwd: 5'-AAGAGACTTCAGCGCACAGCATGCTTGGCTCTACA-3'
hCK2A1P-Mut-2_Rev: 5'-TGTAGAGCCAAGCATGCTGTGCGCTGAAGTCTCTT-3'

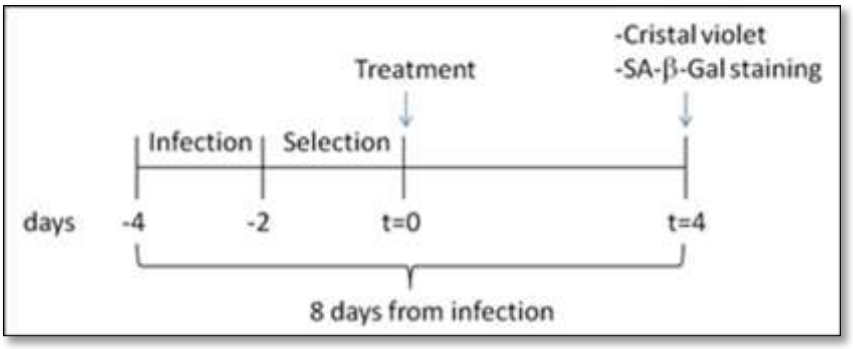
hCK2A1P-Mut-3_Fwd: 5'- CGCATGGTTCTTGGCAAGCAAGGGGGGCCAGCTGGGTGAAGT -3'
hCK2A1P-Mut-3_Rev: 5'-GCTGGCCCCCCTTGCTTGCCAAGAACCATGCGCGACCTGCAG -3'

hCK2A1P-Mut-4_Fwd: 5'- AGCTGGGTGAAGGGAGGGCAACCTGGGTACCGCCATCTTAAC -3'
hCK2A1P-Mut-4_Rev: 5'-CGGTACCCAGGTTGCCCTCCCTTCACCCAGCTGGCCCCCCTT -3'

hCK2A1P-Mut-5_Fwd: 5'-TGGGTCAAACCAACTGGGCACCTCATGGGAGGTTCGTGTT-3'
hCK2A1P-Mut-5_Rev: 5'-AACACGAACCTCCCATGAGGTGCCCAGTTGGTTTGACCCA-3'

Supplementary Table 5 Primers sequence. Primers used to mutate STAT3 binding site on CK2a promoter.

Supplementary Methods



Timeline of the infections

In our screening we used retroviral infection to inactivate *Pten* in MEFs at early passage (passage 3). *Pten*$^{lx/lx}$ MEFs were infected with either Retro-GFP or Retro-Cre for 2 days (t=-2) and then selected with puromycin for additional 2 days (t=0). MEFs were then splited and treated with either DMSO or different compounds. Four days after treatment both proliferation and SA-β-Gal staining were assessed (8 days after infection. By using this method senescence and proliferation are assessed 8 days after infection