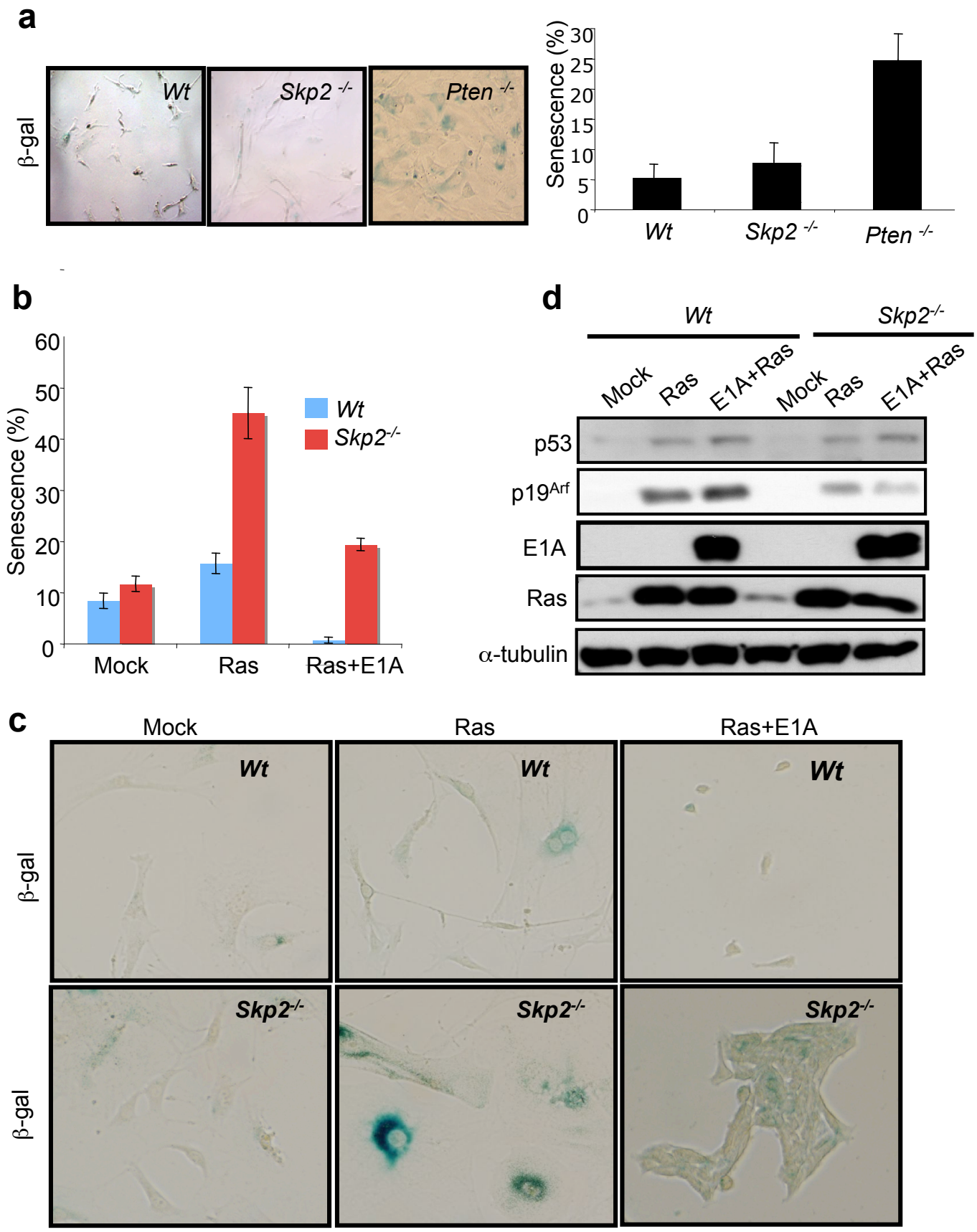

a
β-gal
Wt
Skp2 -/-
Pten -/-
Senescence (%)
30
25
20
15
10
5
0
Wt
Skp2 -/-
Pten -/-
b
Senescence (%)
60
50
40
30
20
10
0
Wt
Skp2-/-
Mock
Ras
Ras+E1A
d
Wt
Skp2-/-
Mock
Ras
E1A+Ras
Mock
Ras
E1A+Ras
p53
p19Arf
E1A
Ras
α-tubulin
c
Mock
Ras
Ras+E1A
β-gal
Wt
Wt
Wt
β-gal
Skp2-/-
Skp2-/-
Skp2-/-

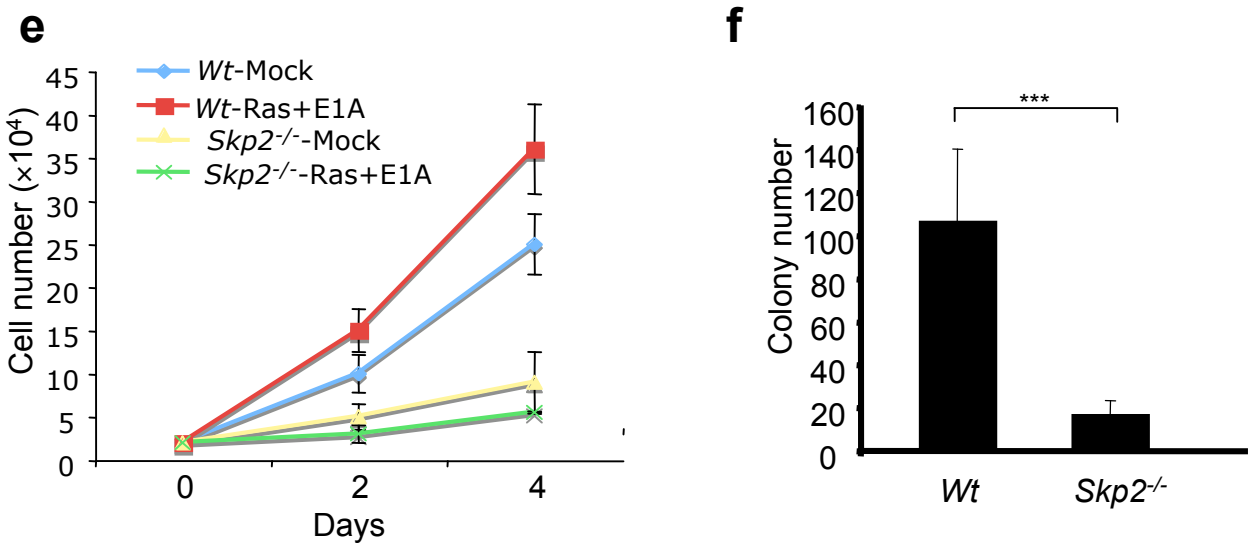


Figure S1. *Skp2* loss triggers a novel senescence response in MEFs in the context of *Pten* inactivation and *Arf* deficiency via a p19^{Arf}/p53-independent pathway. (a) Primary MEFs at passage 5 from various mouse embryos were plated for senescence assay. **(b)** Cell lysates were collected from primary MEFs of various genotypes of mouse embryos for Western blot analysis. The lysates from Wt MEFs treated with g-irradiated for 60 minutes served as a positive control for p53. **(c)** Primary *Pten*$^{+/-}$*Skp2*$^{-/-}$ MEFs infected with retroviruses expressing various control shRNA (shRNA-ctrl), p53 shRNA (shp53), or dominant negative p53 (p53-DN) were plated for senescence assay and Western blot analysis. **(d)** Primary MEFs at passage 5 from various genotypes of mouse embryos were plated for senescence assay. Results are presented as mean values ±s.d. from a representative experiment performed in triplicate. ***p<0.001 using two-tailed student's t-test, n=3.

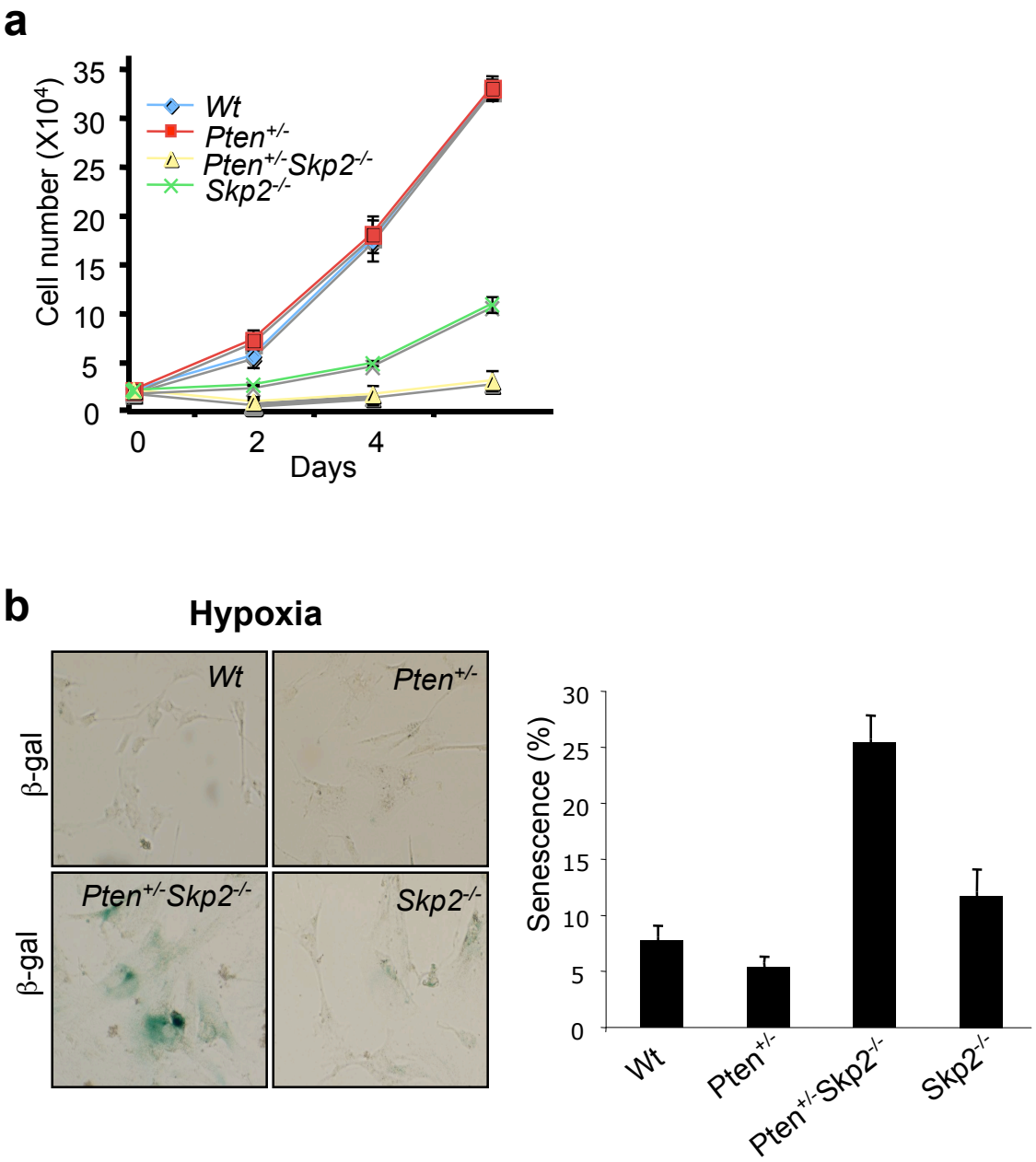


Figure S2. *Pten*$^{+/-}$*Skp2*$^{-/-}$ MEFs display growth arrest and undergo cellular senescence in conditions of hypoxia. (a) Growth curves of primary MEFs at passage 5 from various genotypes of mouse embryos. Results are mean values ±s.d. from a representative experiment performed in triplicate. **(b)** Primary MEFs at passage 4 from various mouse embryos were cultured in hypoxia condition (1% O_2) for 4 days, followed by senescence analysis. Results are presented as mean values ± s.d. from a representative experiment performed in triplicate.

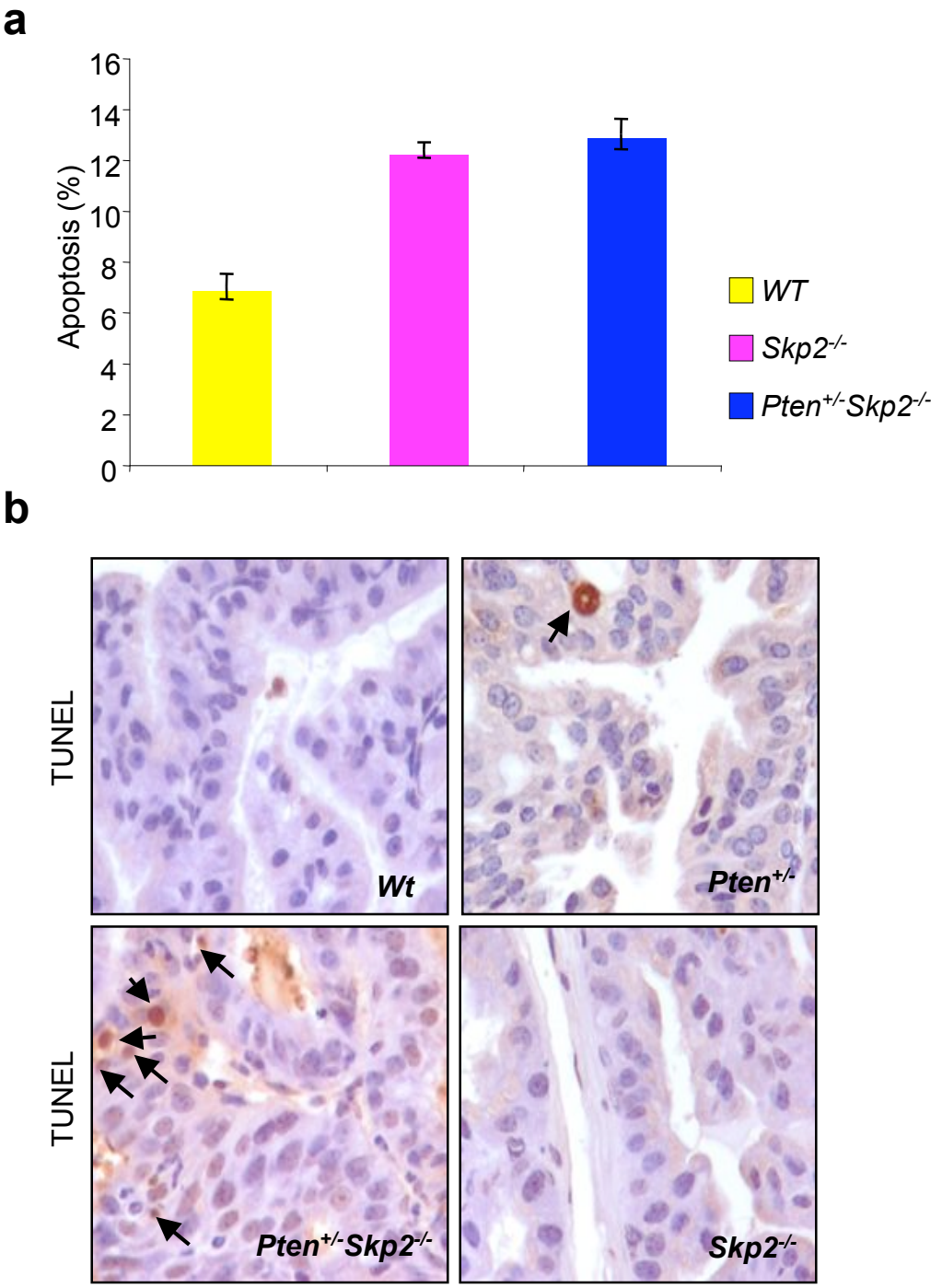


Figure S3. Apoptosis rate in MEFs and prostate samples from various genotypes. (a) Apoptosis assay in primary MEFs at passage 4 from various genotypes of mouse embryos. **(b)** TUNEL staining in prostate tissues from 3-month-old mice of various genotypes. Arrows indicate apoptotic cells.

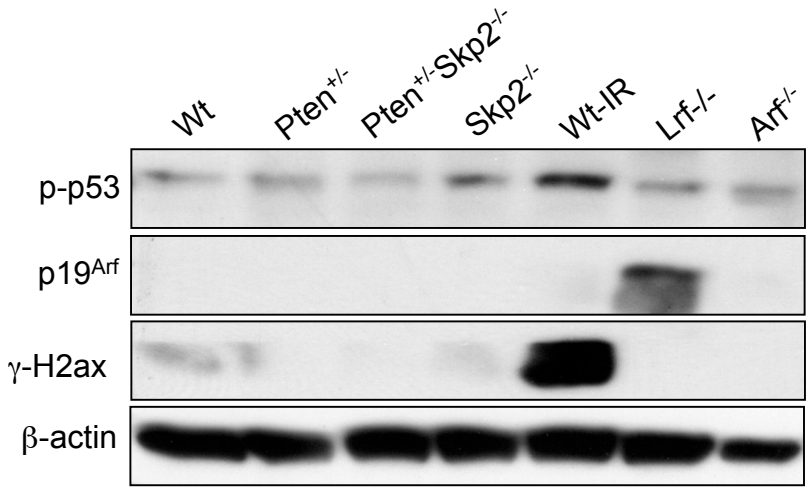


Figure S4. *Pten*$^{+/-}$*Skp2*$^{-/-}$ MEFs neither display p19^{Arf} protein induction nor evidence of DNA damage. Cell lysates were collected from primary MEFs of various genotypes of mouse embryos for Western blot analysis. The lysates from *WT* MEFs γ-irradiated for 60 minutes served as a positive control for p53. The lysates from *Lrf*$^{-/-}$ and *Arf*$^{-/-}$ served as positive and negative controls, respectively.

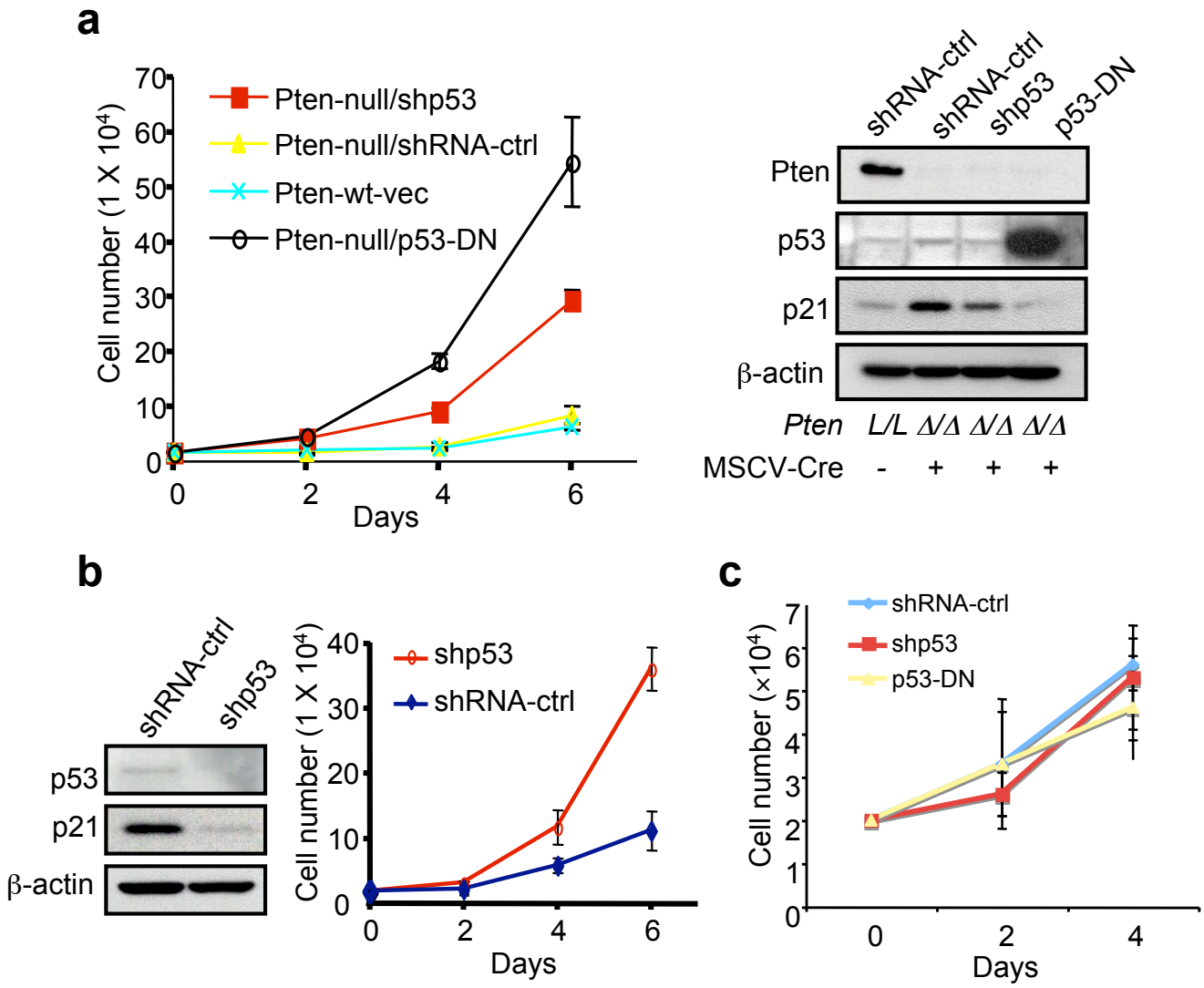


Figure S5. Effect of p53 inactivation on cell proliferation of MEFs of various genotypes. **(a)** *Pten*$^{lox/lox}$ MEFs at passage 2 were infected with retroviruses expressing control, Cre recombinase in combination of control shRNA (shRNA-ctrl), p53 shRNA (shp53), or p53 dominant negative (*p53-DN*), selected, and plated for cell proliferation (left panel) and Western blot analysis (right panel). **(b)** *Wt* MEFs were infected with retroviruses expressing control shRNA and p53 shRNA, selected, and plated for cell proliferation and Western blot analysis. **(c)** Primary *Pten*$^{+/-}$*Skp2*$^{-/-}$ MEFs were infected with retroviruses expressing shRNA-ctrl, shp53, or p53-DN as indicated, selected, and plated for cell proliferation assay. Results are presented as mean values ±s.d. from a representative experiment performed in triplicate.

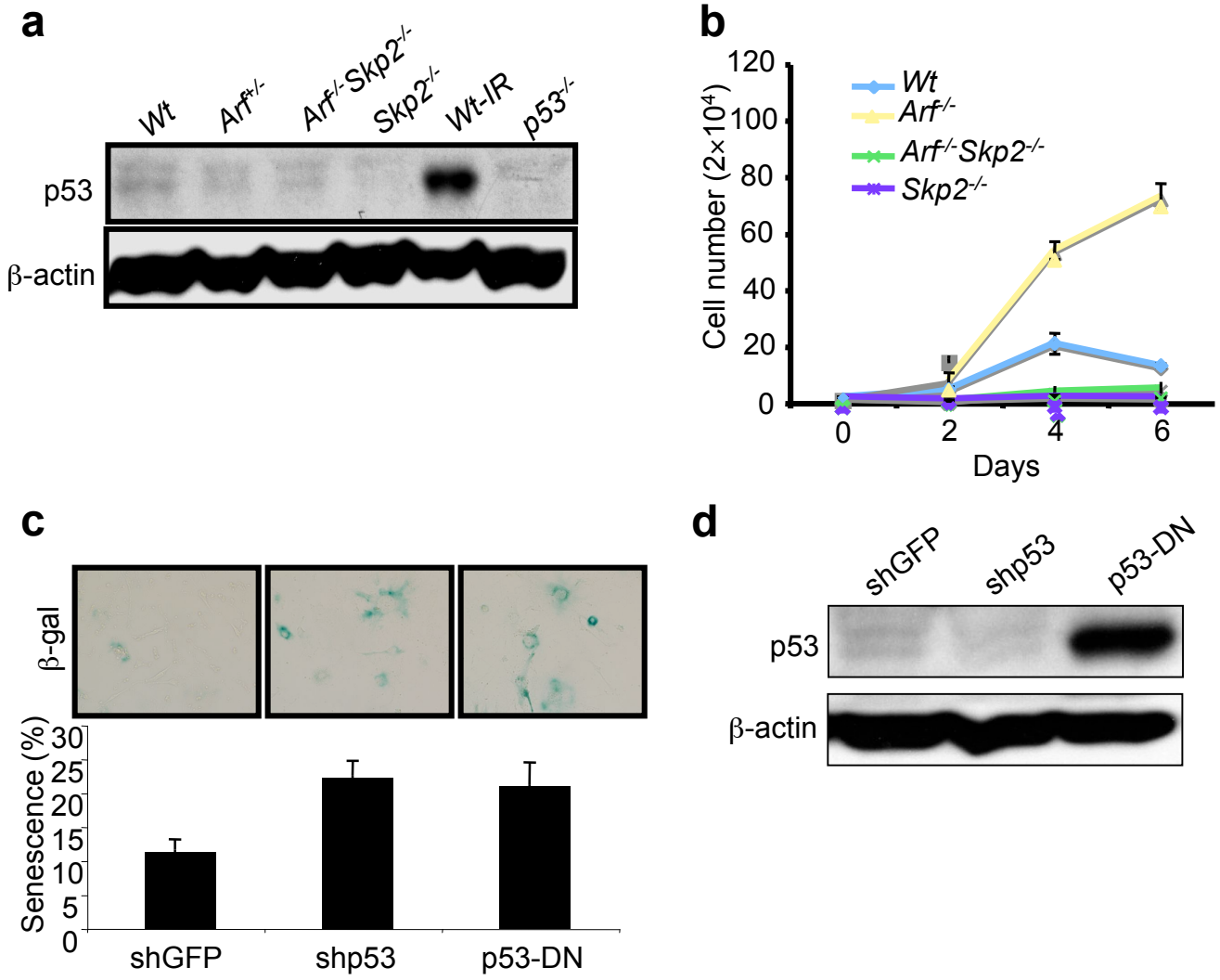


Figure S6. *Skp2* deficiency induces growth arrest and cellular senescence in the context of *Arf/p53* inactivation. (a) Cell lysates were collected from primary MEFs of various genotypes of mouse embryos for Western blot analysis. The lysates from *Wt* MEFs upon 60 minutes γ-irradiation and *p53*$^{-/-}$ MEFs served as positive and negative controls, respectively. **(b)** Growth curves of primary MEFs at passage 5 from various genotypes of mouse embryos. Results are mean values ±s.d. from a representative experiment performed in triplicate. **(c, d)** *Skp2*$^{-/-}$ MEFs at passage 2 were infected with retroviruses expressing GFP shRNA, p53 shRNA (shp53), or p53DN as indicated, selected, and plated for senescence assay **(c)** and Western blot assay **(d)**.

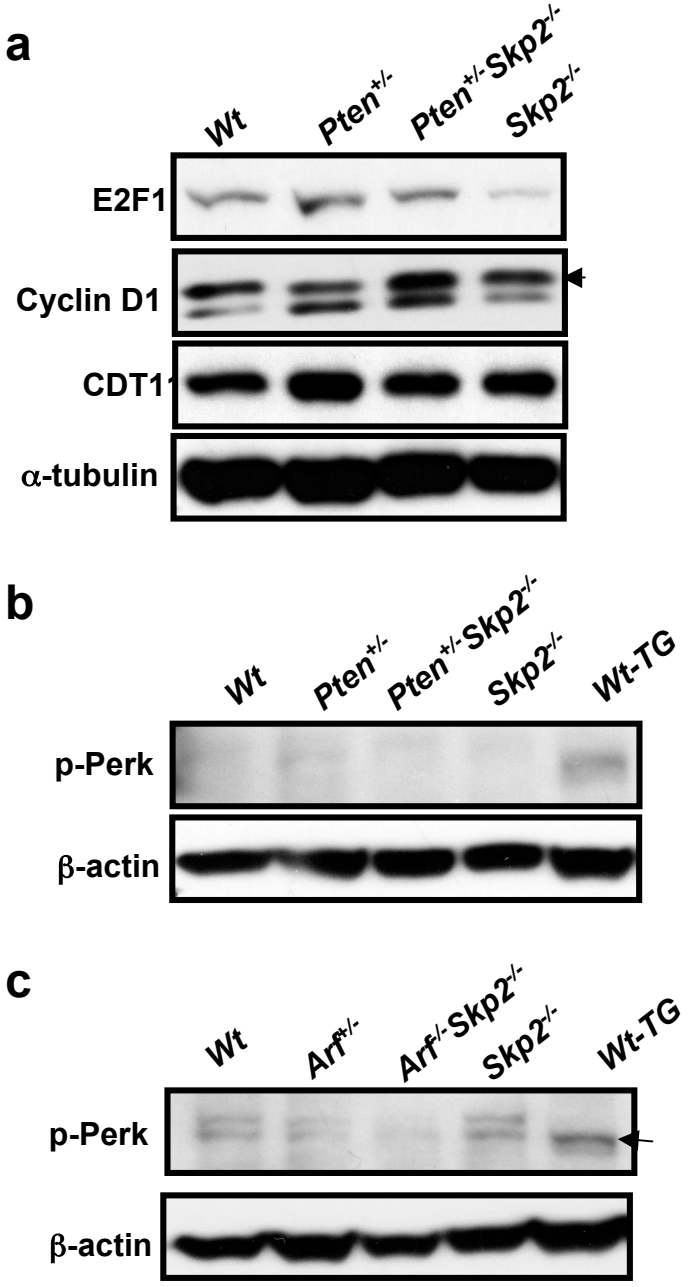


Figure S7. p-Perk levels are not induced in *Pten*$^{+/-}$*Skp2*$^{-/-}$ and *Arf*$^{-/-}$*Skp2*$^{-/-}$ MEFs. (a) Cell lysates were collected from primary MEFs of various genotypes of mouse embryos for Western blot analysis. **(b, c)** Cell lysates were collected from primary MEFs of various genotypes of mouse embryos for Western blot analysis. The lysates from *Wt* MEFs treated with 10 μM thapsigargin (TG) for 20 min served as a positive control.

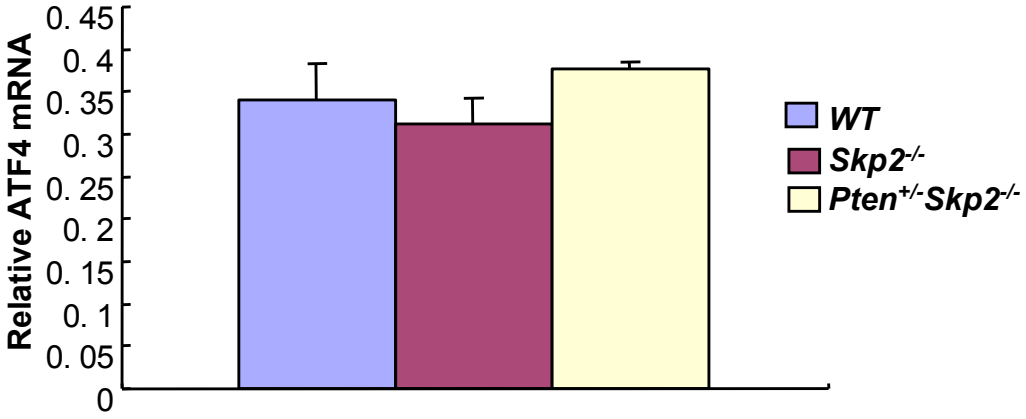


Figure S8. ATF4 mRNA level is not upregulated in *Pten*$^{+/-}$*Skp2*$^{-/-}$ MEFs. Primary MEFs of various genotypes of mouse embryos were harvested and ATF4 mRNA levels were analyzed by real-time PCR.

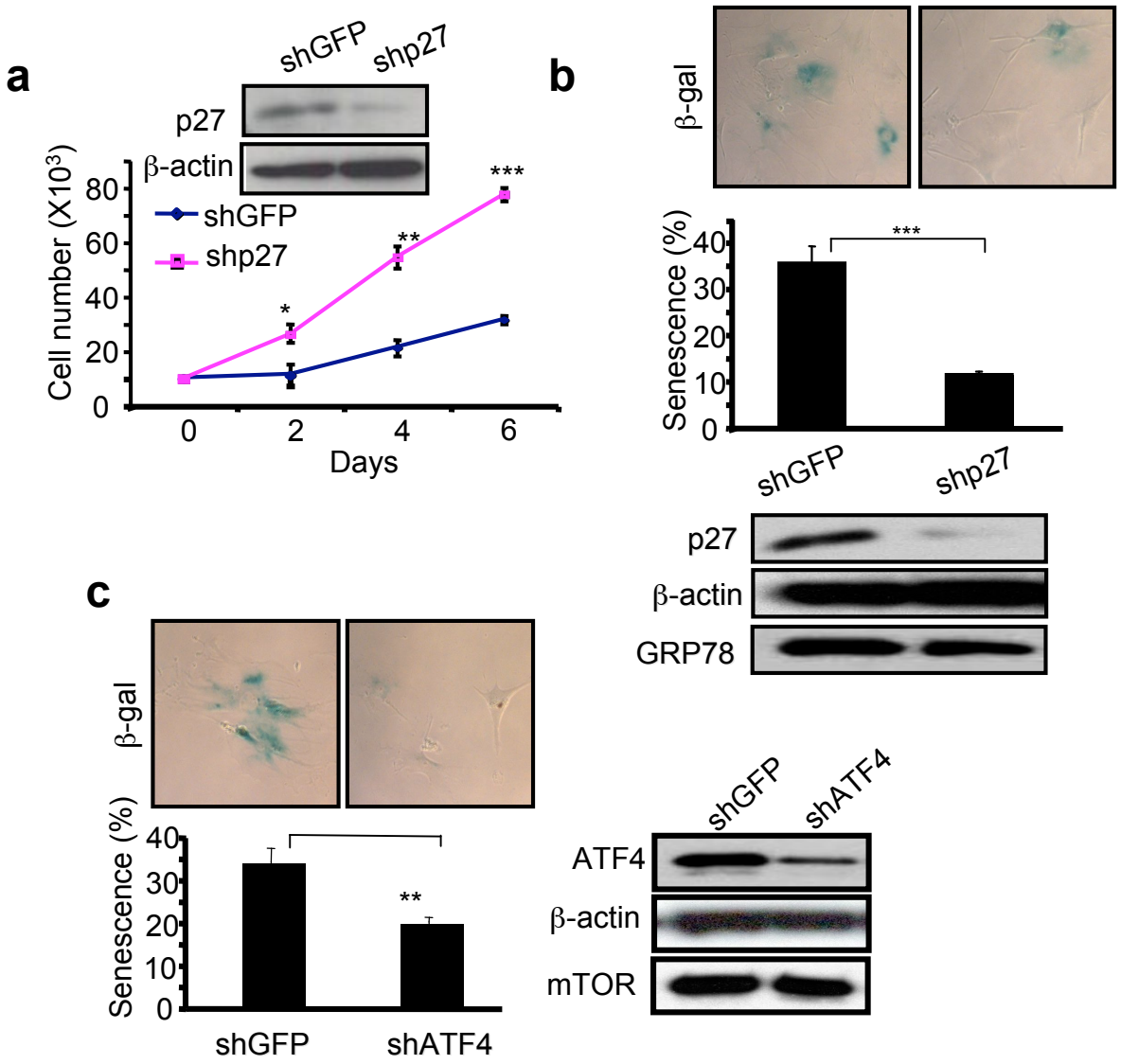


Figure S9. p27 and ATF4 upregulation contributes to cellular senescence in *Pten*$^{+/-}$*Skp2*$^{-/-}$ MEFs. (a, b) *Pten*$^{+/-}$*Skp2*$^{-/-}$ MEFs at passage 2 were infected with retroviruses expressing GFP shRNA or p27 shRNA as indicated, selected, and plated for cell proliferation **(a)** and senescence assays **(b)**. The results are mean values ± s.d. from a representative experiment performed in triplicate. **(c)** *Pten*$^{+/-}$*Skp2*$^{-/-}$ MEFs at passage 2 were infected with retroviruses expressing GFP shRNA or ATF4 shRNA as indicated, selected, and plated for western blot analysis and senescence assays. The results are mean values ± s.d. from a representative experiment performed in triplicate. *p<0.05, **p<0.01, ***p<0.001 using two-tailed student's t-test, n=3.

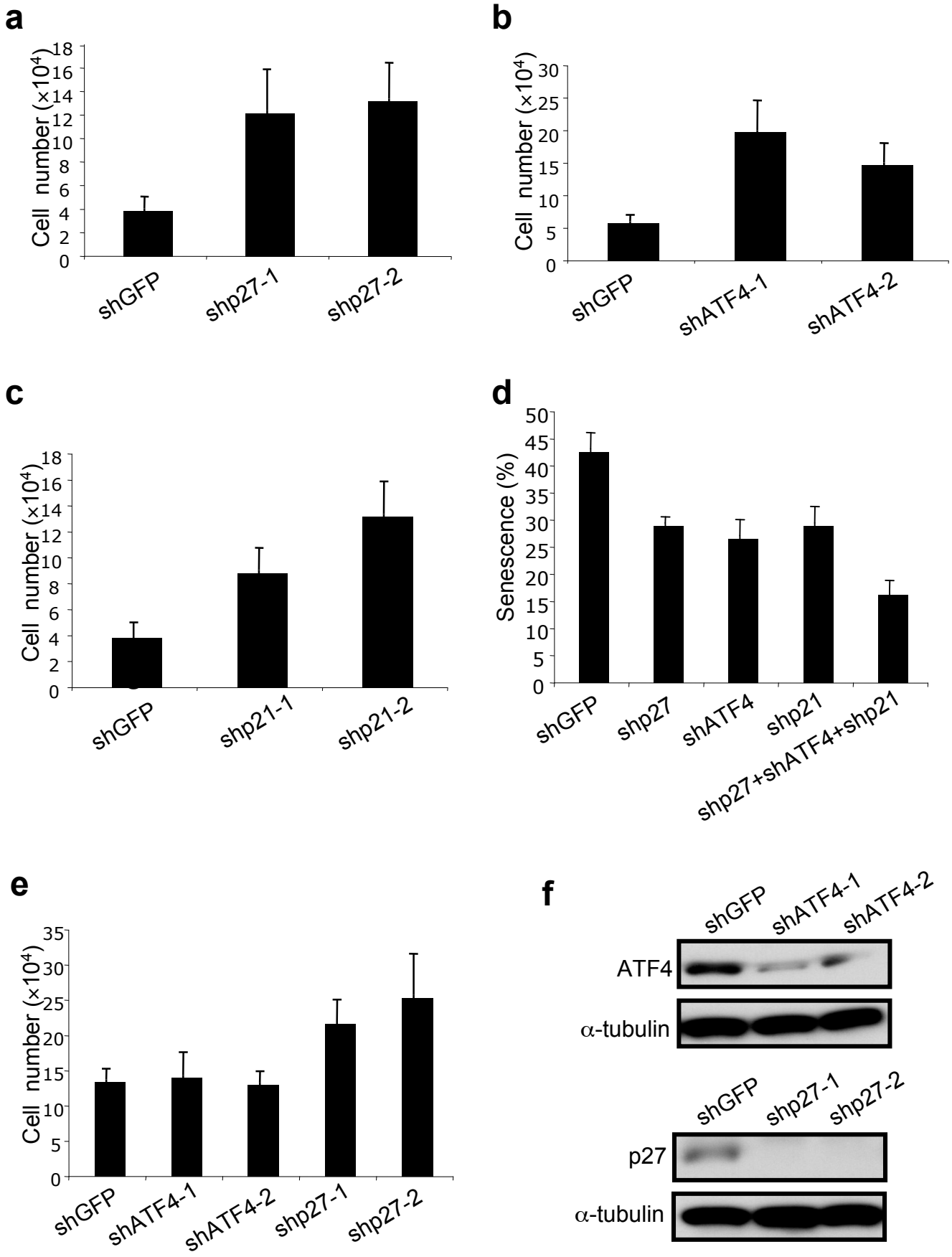
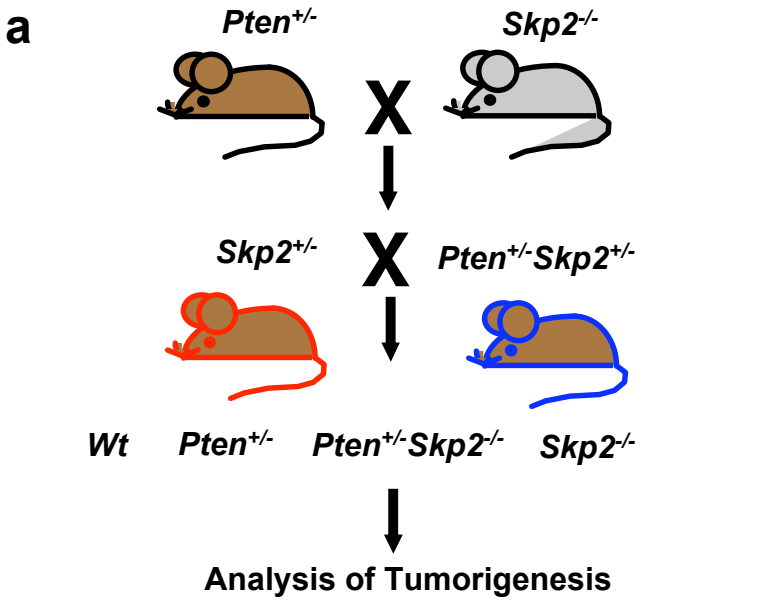
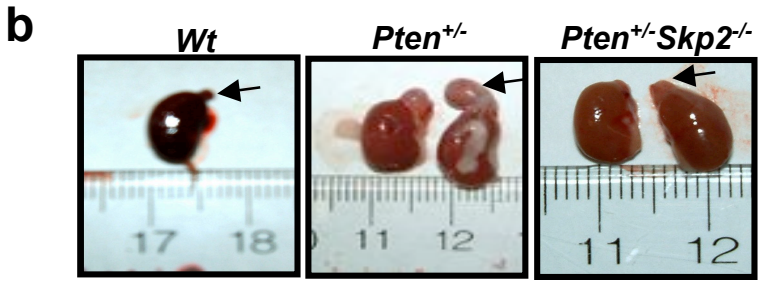
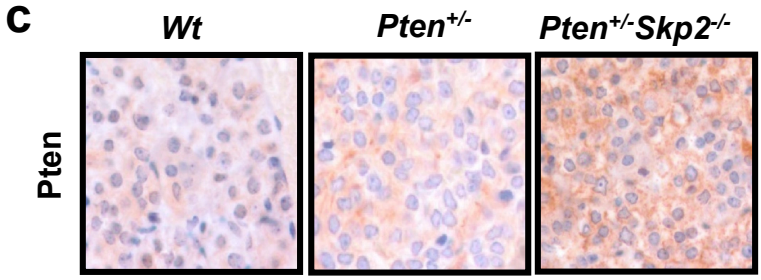

a
Cell number (×10^4)
0
2
4
6
8
10
12
14
16
18
shGFP
shp27-1
shp27-2
b
Cell number (×10^4)
0
5
10
15
20
25
30
shGFP
shATF4-1
shATF4-2
c
Cell number (×10^4)
0
2
4
6
8
10
12
14
16
18
shGFP
shp21-1
shp21-2
d
Senescence (%)
0
5
10
15
20
25
30
35
40
45
50
shGFP
shp27
shATF4
shp21
shp27+shATF4+shp21
e
Cell number (×10^4)
0
5
10
15
20
25
30
35
shGFP
shATF4-1
shATF4-2
shp27-1
shp27-2
f
shGFP
shATF4-1
shATF4-2
ATF4
α-tubulin
shGFP
shp27-1
shp27-2
p27
α-tubulin

Figure S10. Silencing of p27, ATF4 and p21 reverses cell growth arrest and cellular senescence in *Skp2*$^{-/-}$ MEFs. (a-c) *Pten*$^{+/-}$*Skp2*$^{-/-}$ MEFs at passage 2 were infected with lentiviruses expressing GFP shRNA **(a-c)**, p27 shRNA **(a)**, ATF4 shRNA **(b)** and p21 shRNA **(c)** as indicated, selected, and plated for cell proliferation assay. 10,000 cells were plated in a 12-well plate and 4 days later the cell number was counted. Results are mean values ±s.d. from a representative experiment performed in triplicate. **(d)** *Pten*$^{+/-}$*Skp2*$^{-/-}$ MEFs at passage 2 were infected with lentiviruses expressing individual p27 shRNA, ATF4 shRNA, p21 shRNA, or in combination as indicated, selected, and plated for senescence assay and Western blot analysis. **(e, f)** *Skp2*$^{-/-}$ MEFs at passage 2 were infected with lentiviruses expressing GFP, p27, or ATF4 shRNAs as indicated, selected, and plated for cell proliferation assays by direct cell counting **(e)** and Western blot analysis **(f)**.


a
Pten+/-
Skp2-/-
X
Skp2+/-
X
Pten+/-Skp2+/-
Wt
Pten+/-
Pten+/-Skp2-/-
Skp2-/-
Analysis of Tumorigenesis


b
Wt
Pten+/-
Pten+/-Skp2-/-
17
18
11
12
11
12


c
Wt
Pten+/-
Pten+/-Skp2-/-
Pten

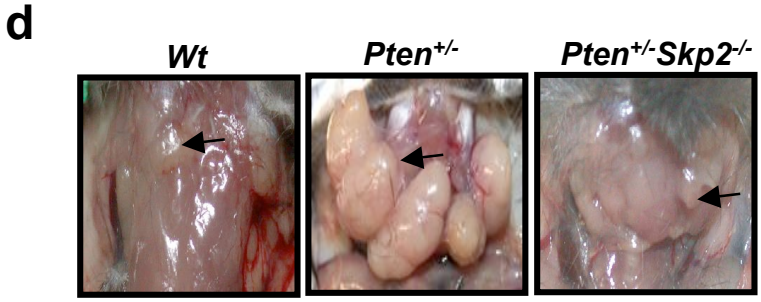

d
Wt
Pten+/-
Pten+/-Skp2-/-

Figure S11. *Skp2* deficiency abrogates adrenal tumour formation and lymphoadenopathy in *Pten* heterozygous mutants. **(a)** Scheme of *Pten*$^{+/-}$ and *Skp2*$^{-/-}$ mice breeding strategy for analysis of tumourigenesis. **(b)** Post-mortem analysis of adrenal tissues from 12-month-old *Wt*, *Pten*$^{+/-}$, and *Pten*$^{+/-}$*Skp2*$^{-/-}$ mice. **(c)** Adrenal tissues from 12-month-old mice of various genotypes were obtained for Pten IHC analysis. **(d)** Post-mortem analysis of sub-mandibular lymph nodes obtained from 5-month-old female mice of various genotypes.

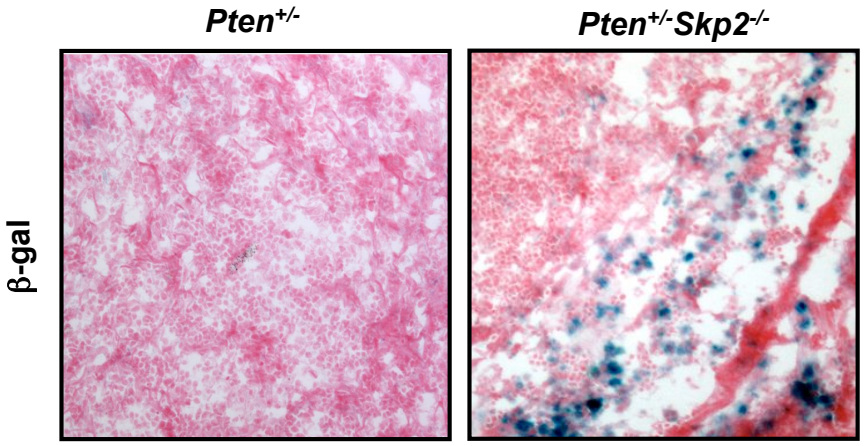


Figure S12. Cellular senescence occurs in lymphoid tissues from *Pten*$^{+/-}$*Skp2*$^{-/-}$ mice *in vivo*. Senescence analysis of lymphoid tissue from 5-month-old *Pten*$^{+/-}$ and *Pten*$^{+/-}$*Skp2*$^{-/-}$ mice, as determined by β-gal staining.

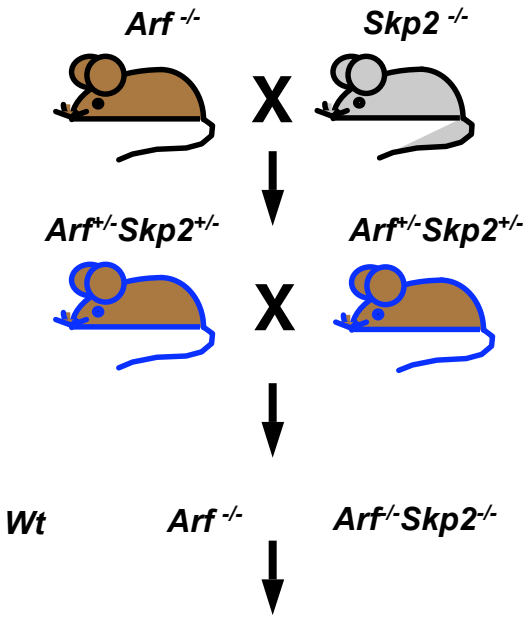
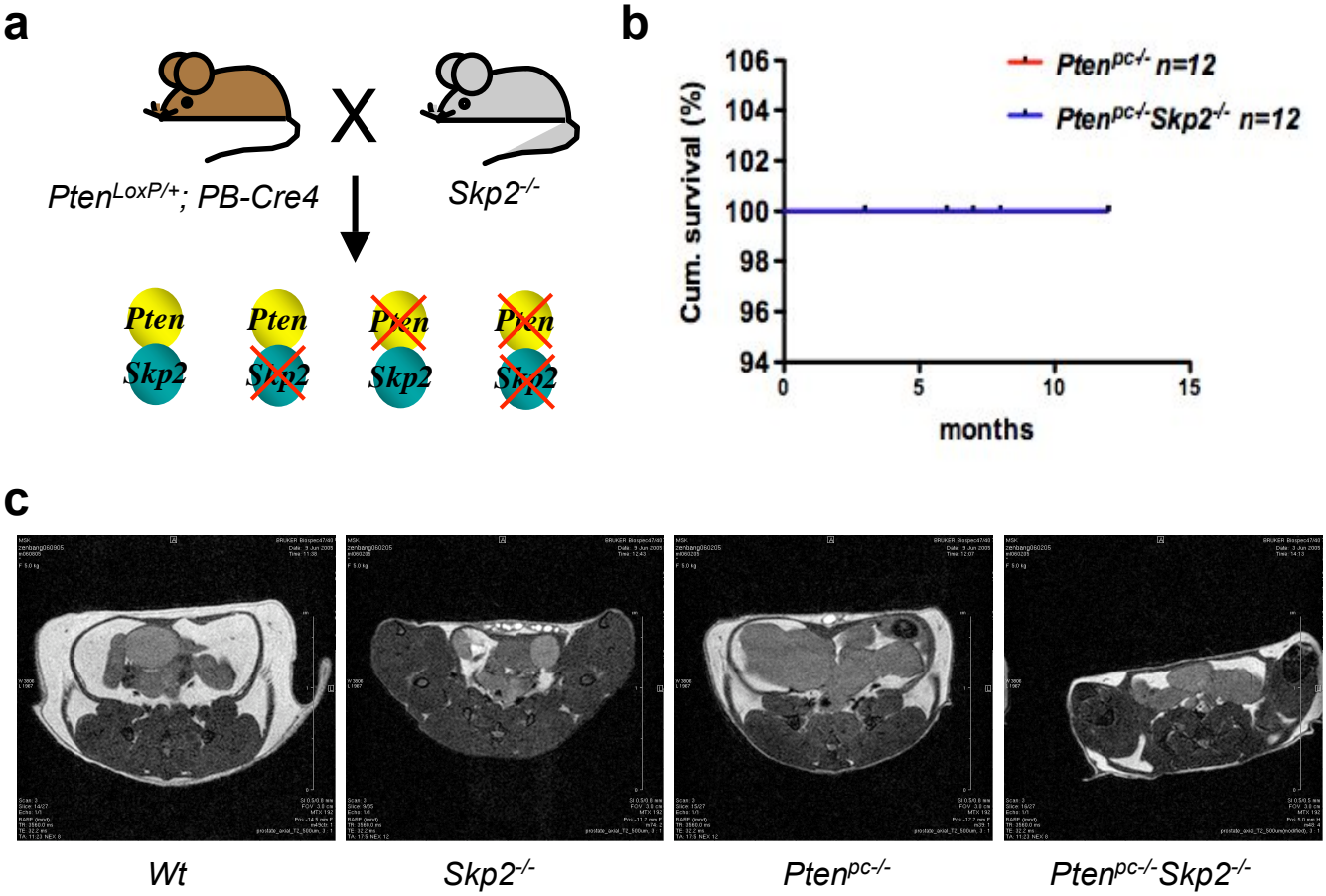


Figure S13. Scheme of *Arf*$^{-/-}$ and *Skp2*$^{-/-}$ mice breeding strategy for analysis of tumorigenesis.


a
Pten^LoxP/+; PB-Cre4
X
Skp2^-/-
Pten
Skp2
b
Pten^pc-/- n=12
Pten^pc-/-Skp2^-/- n=12
Cum. survival (%)
106
104
102
100
98
96
94
0
5
10
15
months
c
Wt
Skp2^-/-
Pten^pc-/-
Pten^pc-/-Skp2^-/-
d
Wt
Pten^pc-/-
Skp2^-/-
Pten^pc-/-Skp2^-/-
e
Pten^-/-
Pten^pc-/-Skp2^-/-
(N=4)
Invasive cancer (%)
80
70
60
50
40
30
20
10
0
*
**
**
AP
VP
DLP
6 months of age

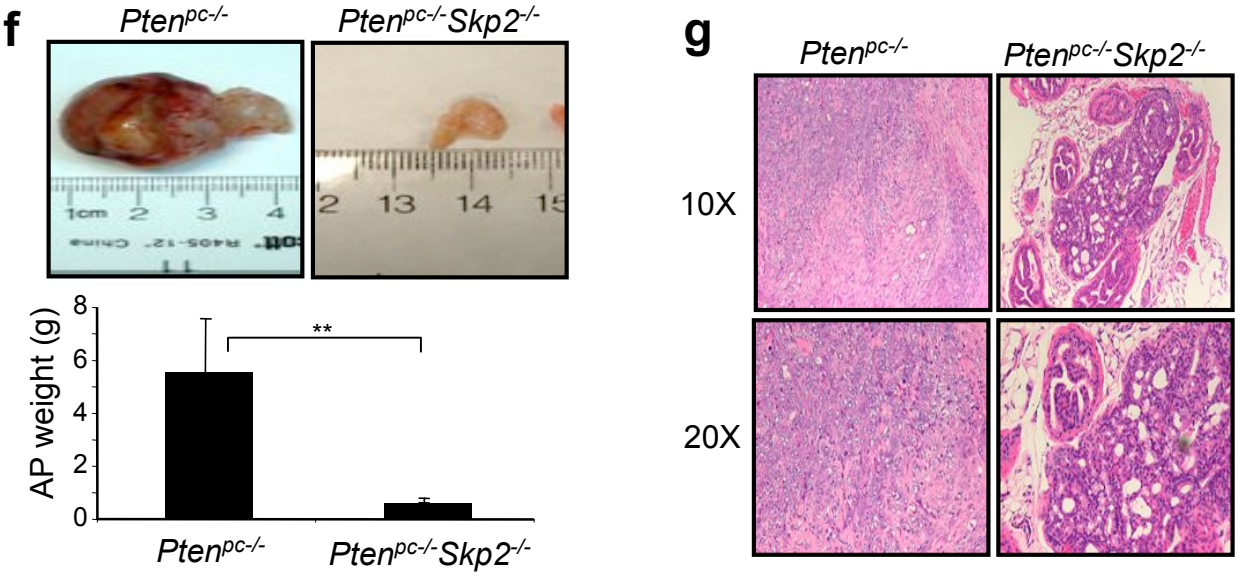


Figure S14. *Skp2* deficiency restricts prostate cancer development upon complete *Pten* inactivation in the prostate (a) Scheme of *Pten*$^{LoxP/LoxP}$*; Pb-Cre4* and *Skp2*$^{-/-}$ mice breeding strategy for analysis of prostate tumorigenesis. **(b)** Kaplan-Meier plot of cumulative survival of *Pten*$^{pc-/-}$ and *Pten*$^{pc-/-}$*Skp2*$^{-/-}$ mutants. **(c)** MRI comparison of AP tumours from 24-week-old *Skp2*$^{-/-}$, *Pten*$^{pc-/-}$, and *Pten*$^{pc-/-}$ *Skp2*$^{-/-}$ mutants, and *Wt* controls. **(d)** Histopathological analysis of AP in various genotypes of mice at 24 weeks of age reveals normal glands in *Wt* and *Skp2*$^{-/-}$ mice but PIN lesions in *Pten*$^{pc-/-}$*Skp2*$^{-/-}$ mice and invasion (arrow) in *Pten*$^{pc-/-}$ mice. **(e)** Quantification of percentages of invasive prostate cancer in each lobe of *Pten*$^{pc-/-}$ and *Pten*$^{pc-/-}$*Skp2*$^{-/-}$ mice at 24 weeks of age. AP: anterior prostate lobe; VP: ventral prostate lobe; DLP: dorsal lateral prostate lobe. In each lobe, individual glands displaying invasive features were scored versus the total glands. Results are mean values ±s.d. *p<0.05, **p<0.01 using two-tailed student's t-test, n=4. **(f)** Post mortem analysis of AP from 15-month-old *Pten*$^{pc-/-}$ and *Pten*$^{pc-/-}$*Skp2*$^{-/-}$ mice was performed. Actual lobe sizes and weights were presented. Results are mean values ± s.d. **p<0.01 using two-tailed student's t-test, n=3. **(g)** Histopathological analysis of AP from 15-month-old *Pten*$^{pc-/-}$ and *Pten*$^{pc-/-}$*Skp2*$^{-/-}$ mice. *Pten*$^{pc-/-}$ mice developed invasive prostate cancer, which was profoundly inhibited in *Pten*$^{pc-/-}$ *Skp2*$^{-/-}$ mice.

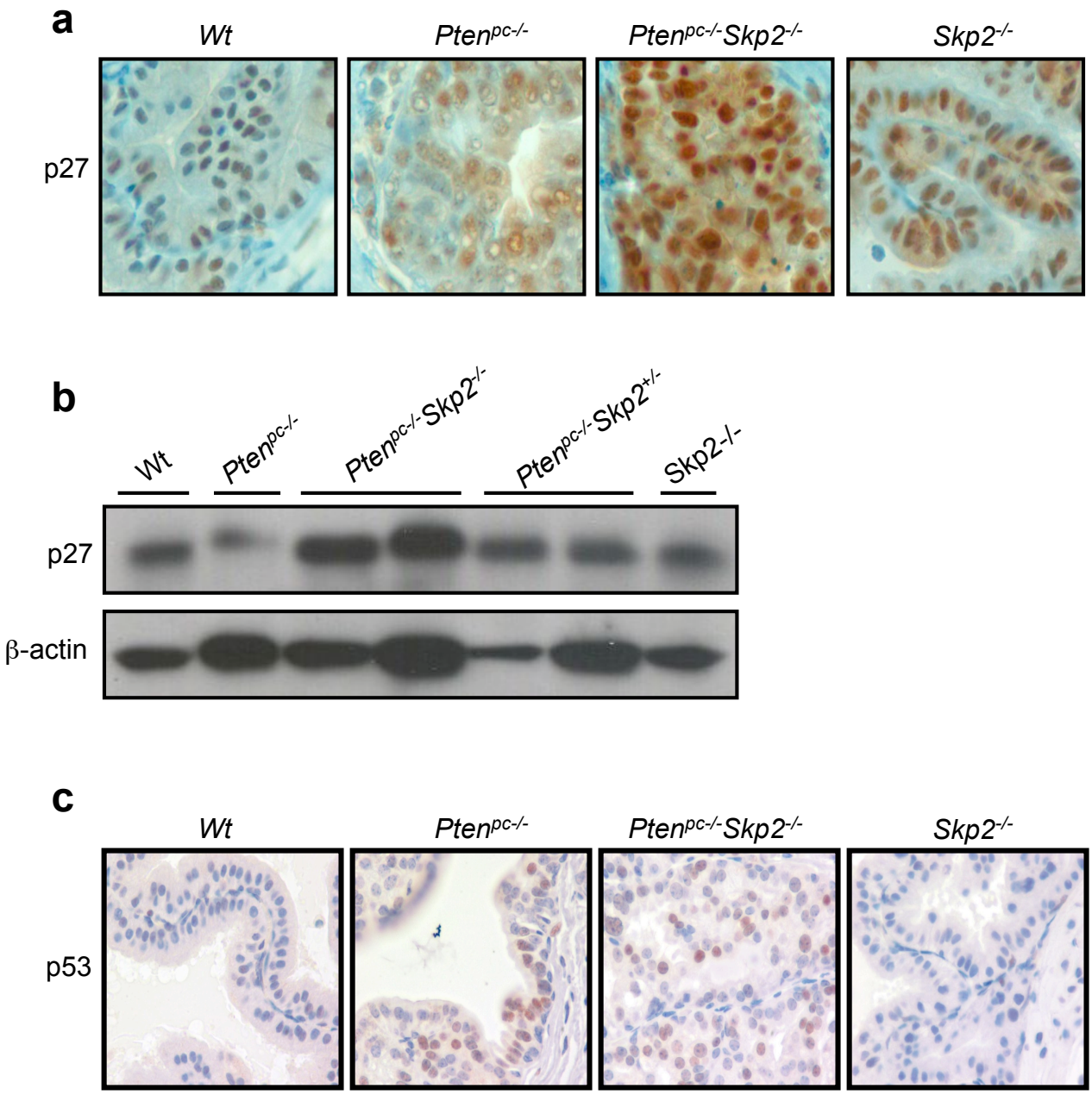


Figure S15. *Skp2* deficiency cooperates with *Pten* inactivation to induce p27 expression, but not p53 expression, in the prostate. (**a**, **b**) AP from 6-month-old mice of indicated genotypes was obtained for p27 IHC (**a**) and Western blot analysis (**b**). **(c)** AP from 6-month-old mice of indicated genotypes was obtained for p53 IHC.

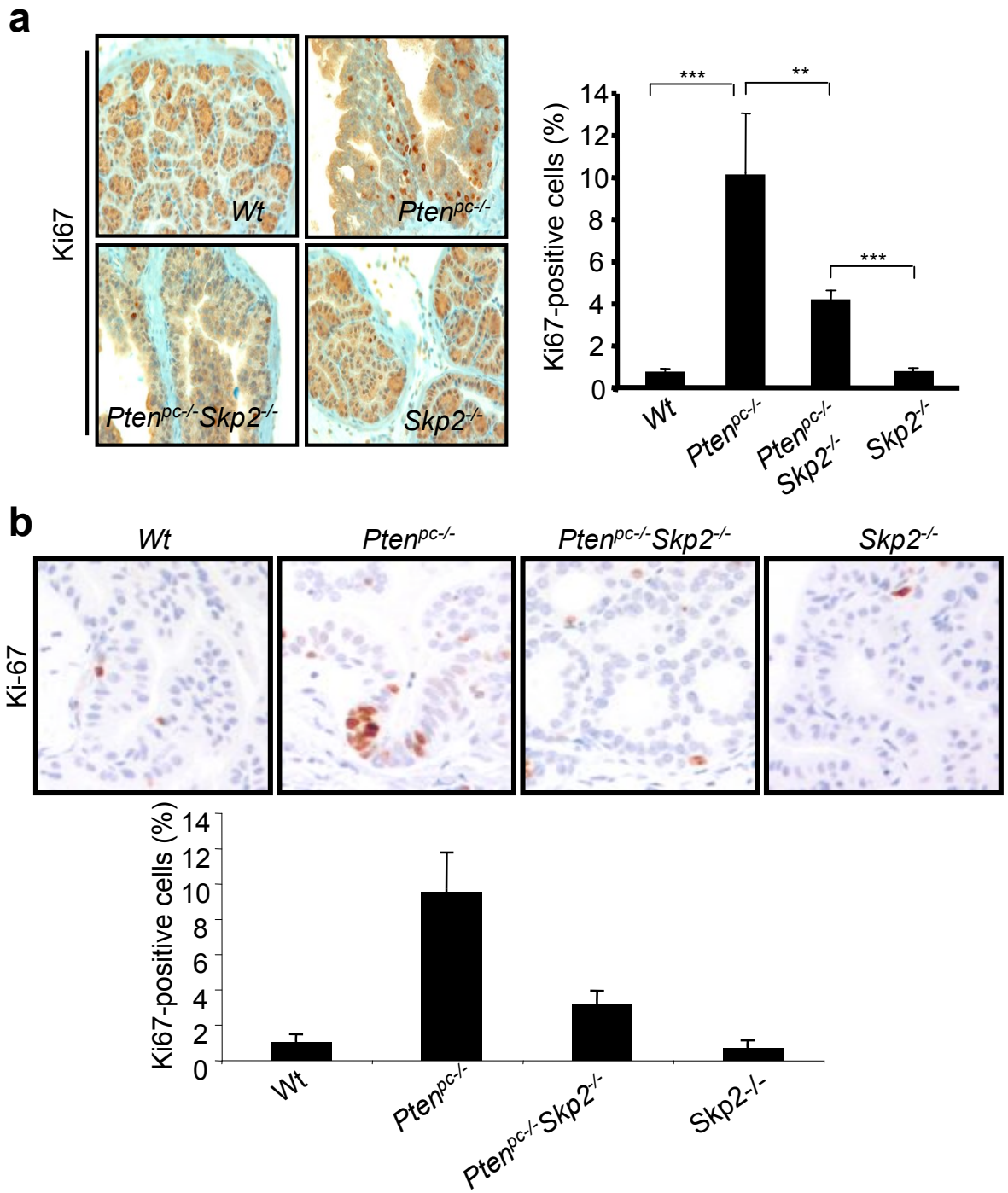


Figure S16. *Skp2* deficiency restricts cell proliferation. (a) Quantification of Ki-67 staining of AP from 3-month-old mice. Representative sections from three mice were counted for each genotype. Results are mean values ±s.d. **p<0.01, ***p<0.001 using two-tailed student's t-test, n=3. **(b)** Quantification of Ki-67 staining of AP from 6-month-old mice. Representative sections from three mice were counted for each genotype. Results are mean values ± s.d.

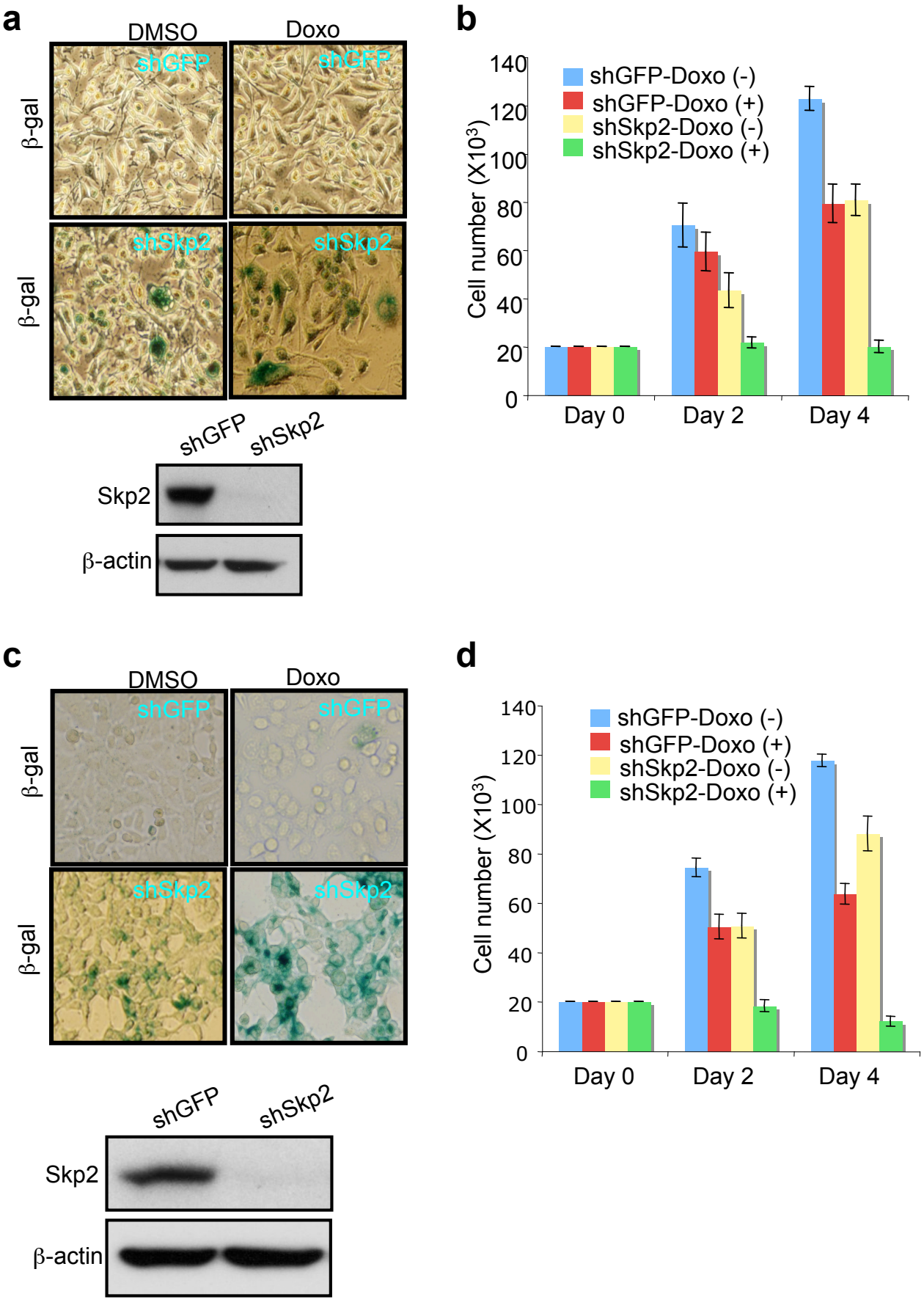

a
DMSO
Doxo
shGFP
shGFP
β-gal
shSkp2
shSkp2
β-gal
shGFP
shSkp2
Skp2
β-actin
b
shGFP-Doxo (-)
shGFP-Doxo (+)
shSkp2-Doxo (-)
shSkp2-Doxo (+)
Cell number (X10³)
140
120
100
80
60
40
20
0
Day 0
Day 2
Day 4
c
DMSO
Doxo
shGFP
shGFP
β-gal
shSkp2
shSkp2
β-gal
shGFP
shSkp2
Skp2
β-actin
d
shGFP-Doxo (-)
shGFP-Doxo (+)
shSkp2-Doxo (-)
shSkp2-Doxo (+)
Cell number (X10³)
140
120
100
80
60
40
20
0
Day 0
Day 2
Day 4

Figure S17. *Skp2* knockdown induces cellular senescence and cooperates with DNA damage agent to trigger cellular senescence in human prostate cancer cells. (a, c) PC3 cells **(a)** and DU145 **(c)** infected with lentiviruses expressing Skp2 shRNA were treated with vehicle or 30 nM Doxorubicin (Doxo) for 4 days and harvested for senescence assay. **(b, d)** PC3 cells **(b)** and DU145 **(d)** infected with lentiviruses expressing Skp2 shRNA were treated with vehicle or 30 nM Doxorubicin (Doxo) for various time points and harvested for cell proliferation. The results are mean values ± s.d. from a representative experiment performed in triplicate.