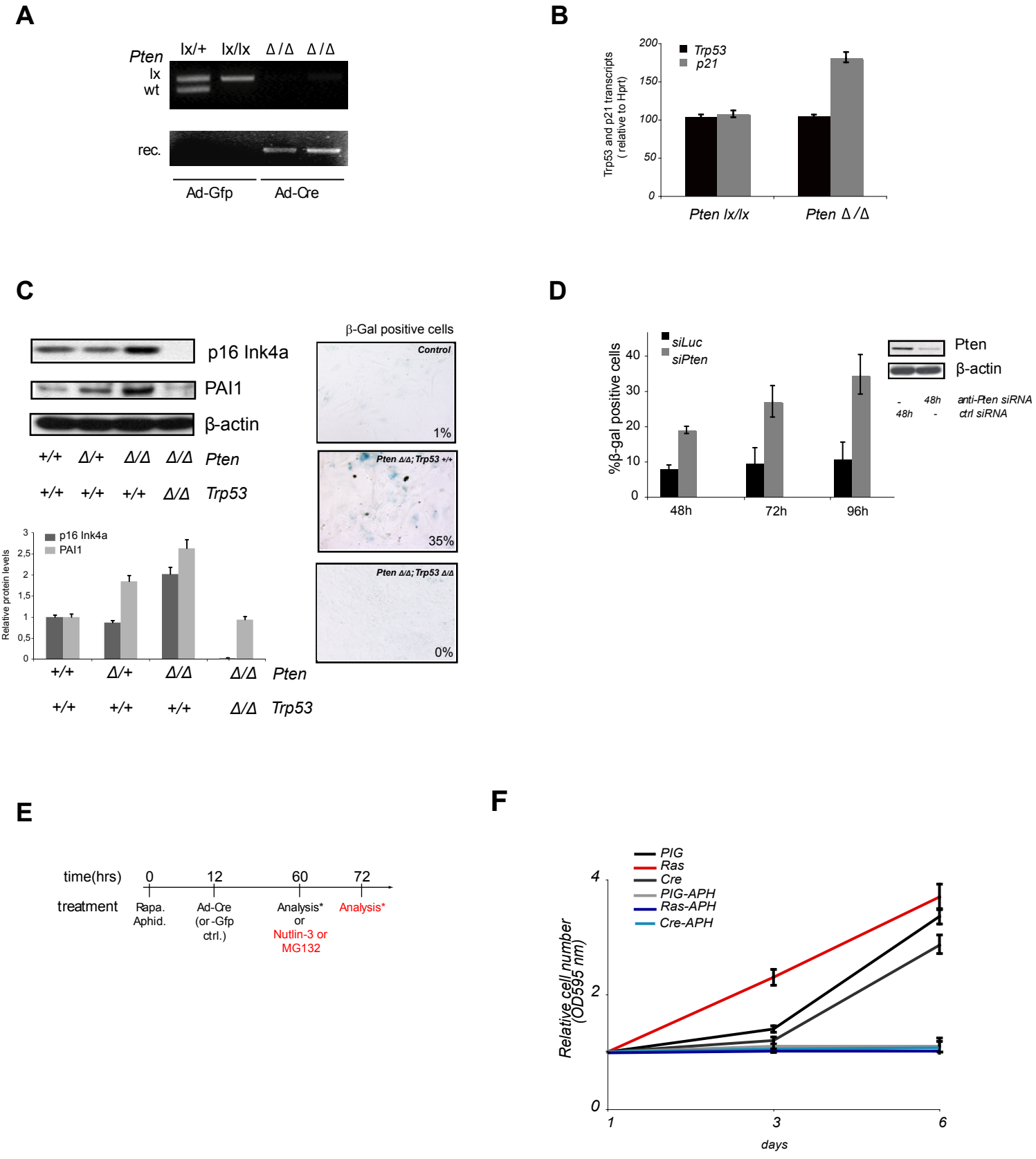
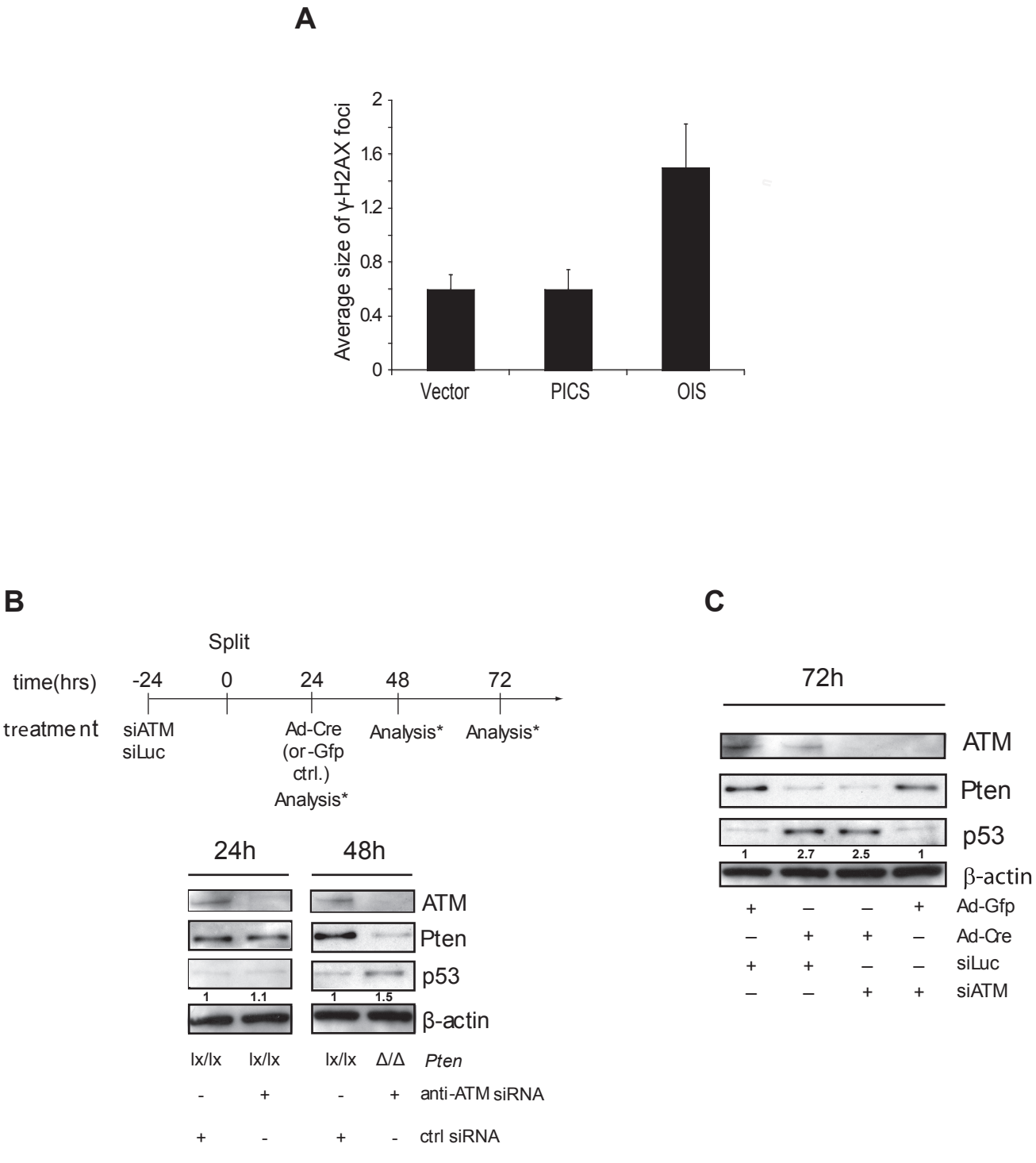
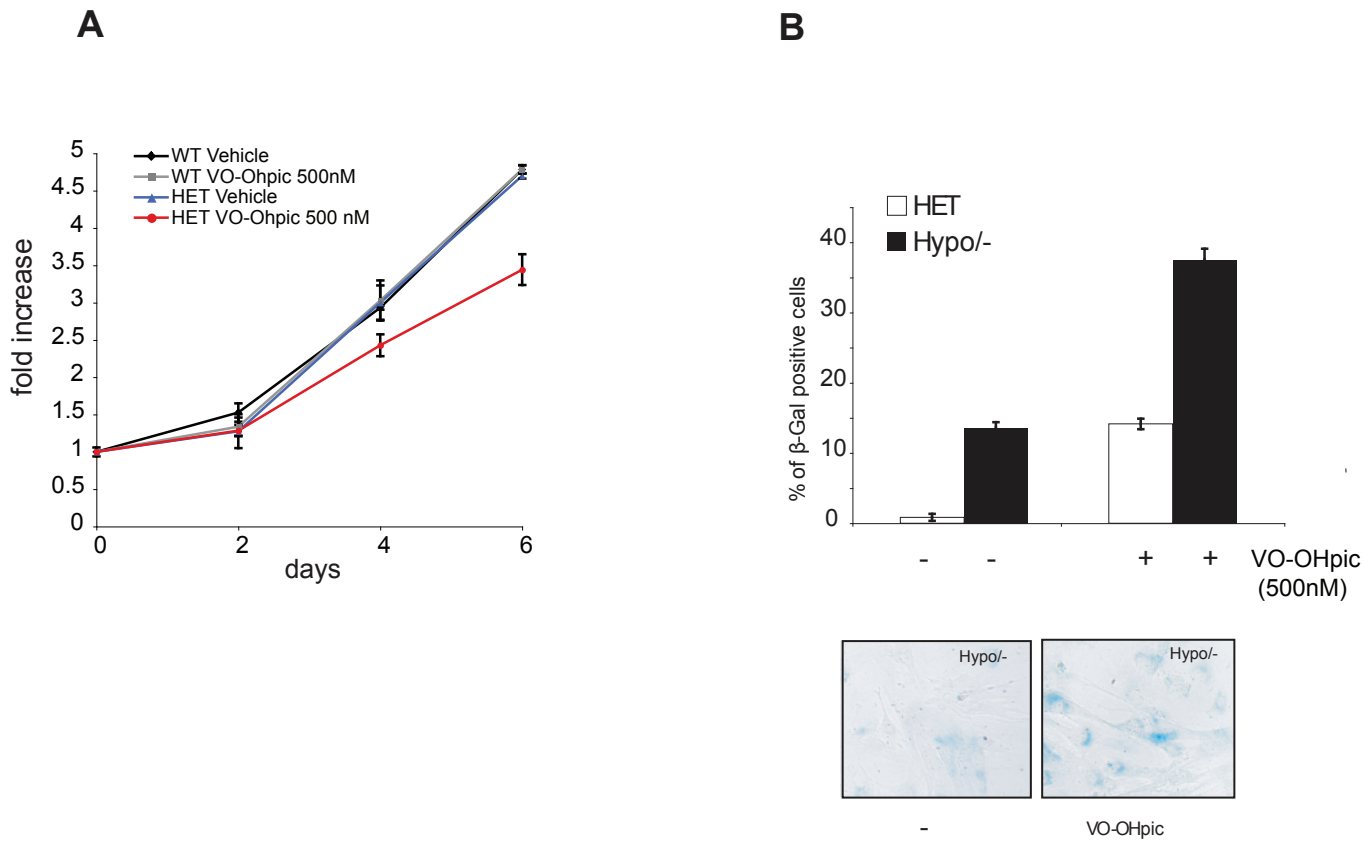
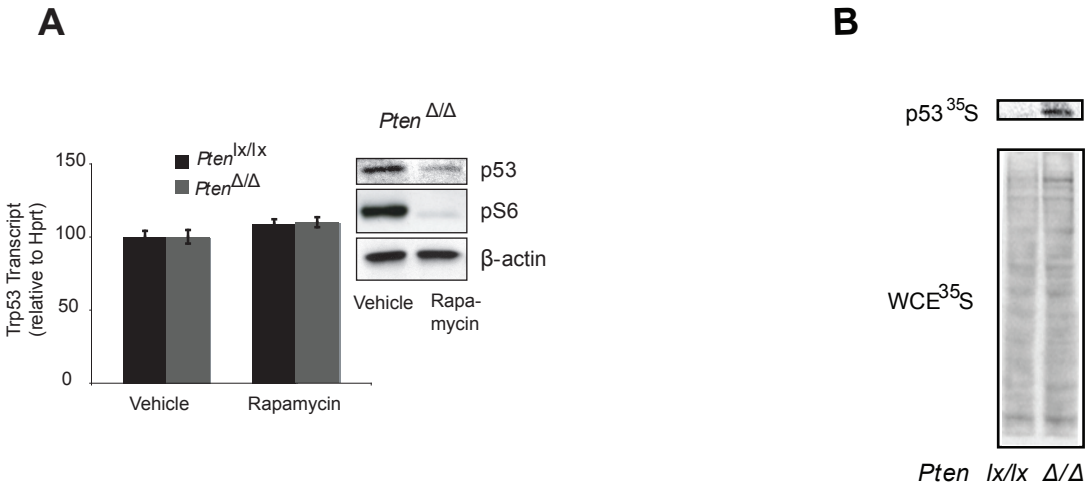
Alimonti _Supplementary Figure S1

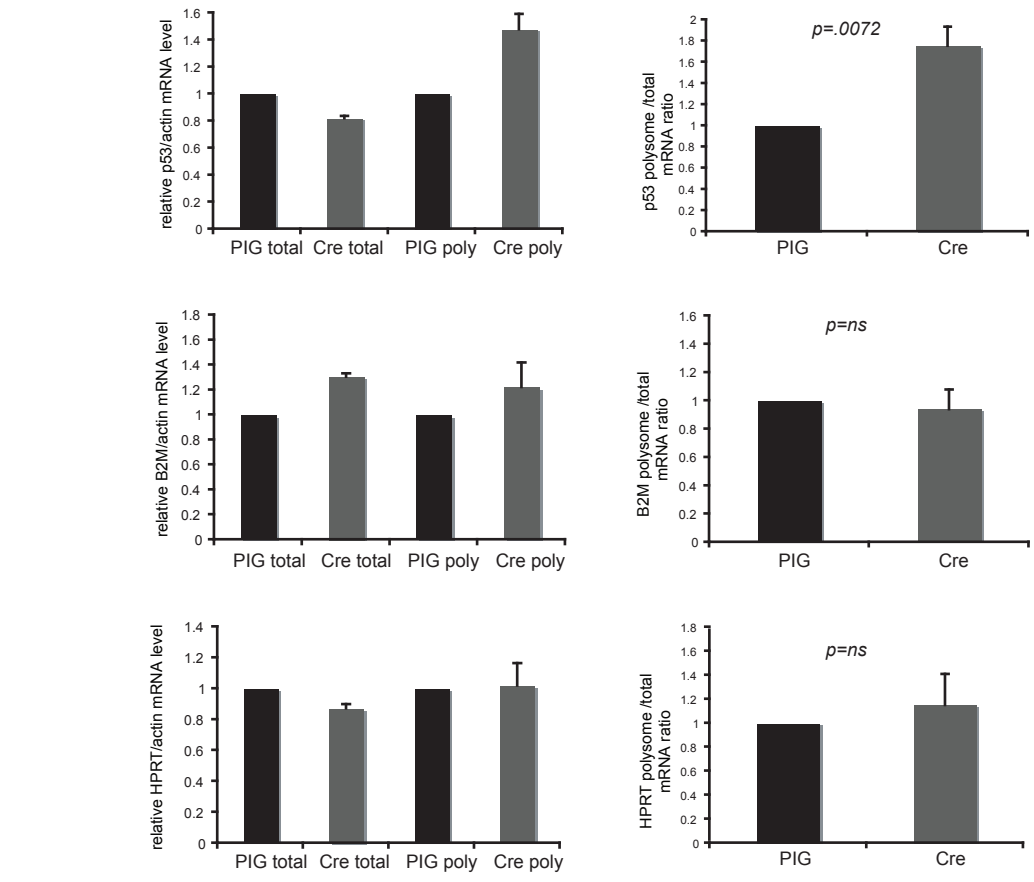



A
Average size of γ-H2AX foci
2
1.6
1.2
0.8
0.4
0
Vector
PICS
OIS
B
Split
time(hrs)
-24
0
24
48
72
treatment
siATM
siLuc
Ad-Cre
(or-Gfp
ctrl.)
Analysis*
Analysis*
Analysis*
24h
48h
ATM
Pten
p53
1
1.1
1
1.5
β-actin
lx/lx
lx/lx
lx/lx
Δ/Δ
Pten
-
+
-
+
anti-ATM siRNA
+
-
+
-
ctrl siRNA
C
72h
ATM
Pten
p53
1
2.7
2.5
1
β-actin
+
–
–
+
Ad-Gfp
–
+
+
–
Ad-Cre
+
+
–
–
siLuc
–
–
+
+
siATM

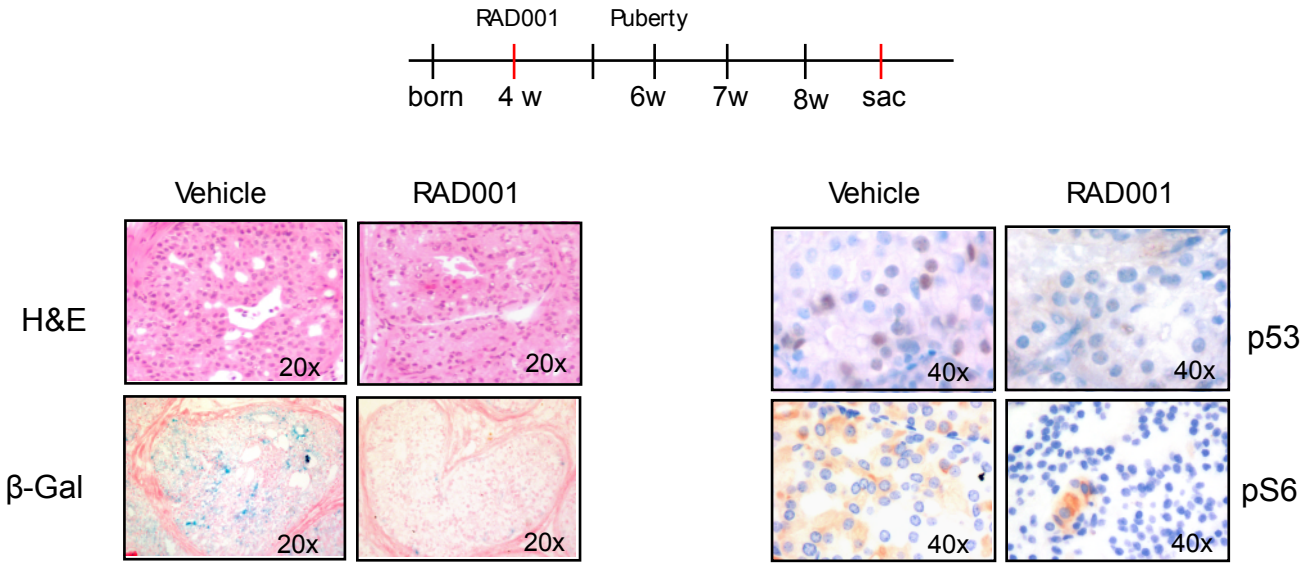
Alimonti_Supplementary Figure S3



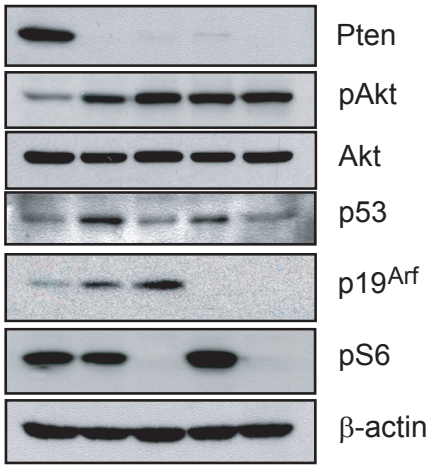

A
Pten Δ/Δ
Pten lx/lx
Pten Δ/Δ
Trp53 Transcript (relative to Hprt)
150
100
50
0
Vehicle
Rapamycin
p53
pS6
β-actin
Vehicle
Rapa-mycin
B
p53 35S
WCE35S
Pten lx/lx Δ/Δ


C
relative p53/actin mRNA level
PIG total
Cre total
PIG poly
Cre poly
p53 polysome /total mRNA ratio
p=.0072
PIG
Cre
relative B2M/actin mRNA level
B2M polysome /total mRNA ratio
p=ns
relative HPRT/actin mRNA level
HPRT polysome /total mRNA ratio
p=ns

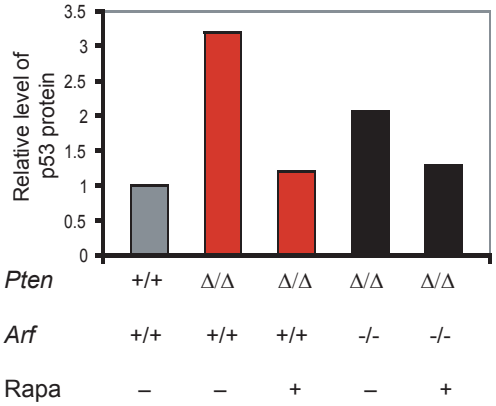
Alimonti_Supplementary Figure S5



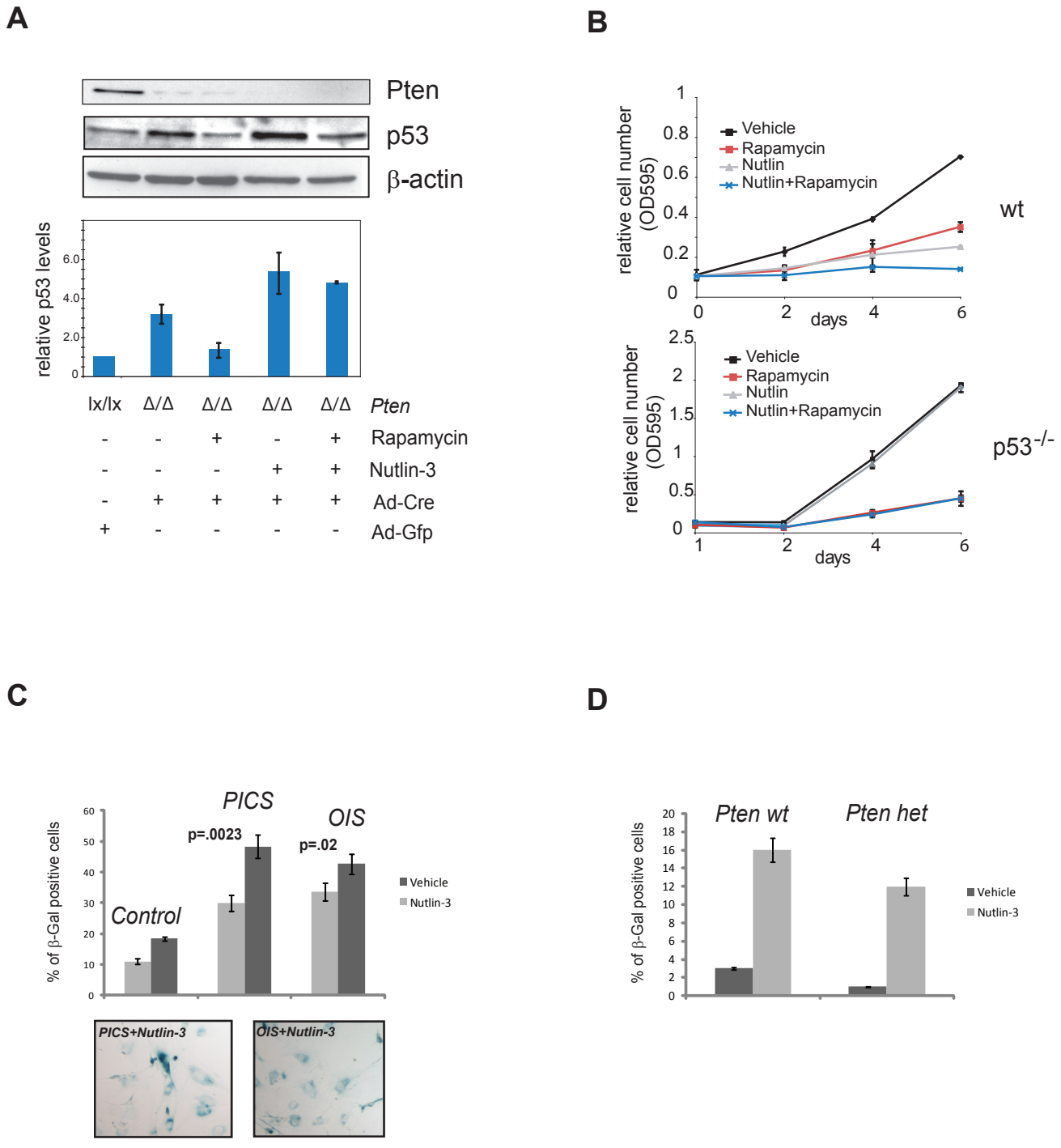
A


Pten
pAkt
Akt
p53
p19Arf
pS6
β-actin
Pten +/+ Δ/Δ Δ/Δ Δ/Δ Δ/Δ
Arf +/+ +/+ +/+ -/- -/-
Rapa – – + – +
(20 nM, 24hrs)

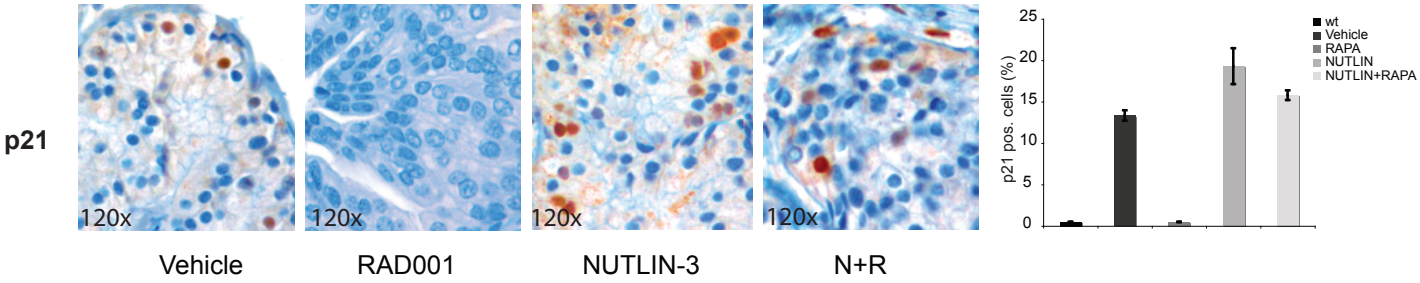
B


Relative level of p53 protein
3.5
3
2.5
2
1.5
1
0.5
0
Pten +/+ Δ/Δ Δ/Δ Δ/Δ Δ/Δ
Arf +/+ +/+ +/+ -/- -/-
Rapa – – + – +

Alimonti_Supplementary Figure S7



Alimonti _Supplementary Figure S8



Supplementary Figure Legends

Supplementary Figure S1 | Inactivation of Pten by Cre-mediated recombination or siRNA and experimental timeline. **(A)** Efficiency of Adenoviral-Cre mediated recombination of the *Pten*$^{lx/lx}$ allele as scored by PCR amplification. Note that while the *Pten* locus is quantitatively recombined, residual Pten protein may remain due to either mRNA or protein stability. **(B)** *Trp53* and p21 transcript levels in control (*Pten lx/lx)* and *Pten null (Pten Δ/Δ)* MEFs as measured by real time PCR. Error bars are S.D. **(C)** Upper left panel: Western analysis for the senescence markers p16^{ink4a} and PAI-1 for the indicated genotypes. Lower left panel: Quantification of p16^{ink4a} and PAI-1 protein levels for the indicated genotypes. Rigth panels: β-Galactosidase staining in the *Control, Pten null (Pten Δ/Δ; Trp53 +/+)* and *Pten null;Trp53 null (Pten Δ/Δ; Trp53Δ/Δ)MEFs.* Numbers indicate the percentage of β-Gal positive cells. **(D)** Quantification of senescence associated β-Galactosidase staining in *Pten*$^{+/-}$ MEFs in response to treatment with siRNA against *Pten* for times indicated. Error bars are S.D. The insert shows western analysis for Pten in the same cells at 48 hours. **(E)** Experimental timeline of PICS induction through infection with Adenoviral-Cre (Ad-Cre) and its combination with additional drug treatments (in red). Asterisks denote analysis through either western blotting or β-Galactosidase staining for senescence. **(F)** Growth curve analysis of *Pten*$^{lx/lx}$ primary MEF cells infected with a retroviral control vector, H-Ras^{V12}, or Cre and subsequently treated with aphidicolin (+APH) after infection. Error bars are S.D.

Supplementary Figure S2 | Molecular and genetic characterization of PICS. **(A)** Quantification of the average size of γ-H2AX foci in PICS and OIS. Error bars are S.D. **(B)** Experimental timeline of PICS induction through Ad-Cre infection in combination with knock-down of ATM by siRNA. Asterisks denote analysis through either western blotting or senescence associated β-Galactosidase staining. Lower panel: Western analysis showing p53 levels in response to knock-down of ATM by siRNA after 24 hours (i.e. at the time of adenoviral infection) and 48 hours (i.e. 24 hours after adenoviral infection) as outlined in the timeline. Numbers indicate densitometrically determined protein levels for p53 relative to β-actin. **(C)** Western analysis for p53-levels in primary *Pten*$^{lx/lx}$ MEFs in the absence of ATM (siATM) (i.e. final time point of

experimental timeline in (**B)**). Numbers indicate densitometrically determined protein levels for p53 relative to β-actin.

Supplementary Figure S3 | Pharmacological inactivation of PTEN drives senescence in absence of hyper-proliferation of the wild type cells. **(A)** Growth curve of *Pten*^{wt} (WT) and *Pten*$^{+/-}$ (HET) MEFs, treated with the indicated concentration of the PTEN inhibitor VO-OHpic. Error bars are S.D. **(B)** Quantification of Senescence associated β-Galactosidase staining (upper panel) and its staining (lower panel) for *Pten*$^{+/-}$ (HET) and *Pten*$^{hy/-}$ (Hypo/-) MEFs (which express Pten at ~30 % of wild type levels(13)) treated in the presence or absence of the PTEN inhibitor VO-OHpic.

Supplementary Figure S4 | mTOR mediated p53 translation is essential for senescence upon *Pten*-loss. **(A)** *Trp53* transcript levels in *Pten*$^{lx/lx}$ MEFs infected with Ad-Cre (*Pten*$^{\Delta/\Delta}$) and treated with or without Rapamycin as measured by real time PCR (left panel). Error bars are S.D. Western blot analysis (right panel) on the same cells shows decreased level of p53 protein and S6 phosphorylation levels after treatment with or without Rapamycin. **(B)** Effect of *Pten* loss on p53 translation as measured by [S^{35}]-methionine incorporation. p53 protein was immunoprecipitated from *Pten*$^{lx/lx}$ (Ad-GFP infected) and *Pten*$^{\Delta/\Delta}$ (Ad-Cre infected) MEFs that had been labelled for 30 min with [S^{35}]-methionine. Analysis of whole cell-extracts (WCE) shows equal amounts of [S^{35}]-methionine incorporated into the *Pten*$^{lx/lx}$ and *Pten*$^{\Delta/\Delta}$ MEFs. **(C)** Real time analysis of total p53, β-2Microglobulin (B2M), HPRT mRNA levels and the respective mRNA loaded on polysomes (left panel) in *Pten*$^{\Delta/\Delta}$ MEFs (Ad-Cre infected) and control *Pten*$^{lx/lx}$ MEFs (Ad-GFP infected). Right panels show the ratio between the level of mRNA loaded on the polysome and the respective total mRNA. Note that each value was normalized to total β-actin expression and β-actin loaded on the polysome. The polysome profile of total RNA was analyzed by sucrose gradient centrifugation. B2M and HPRT were used as internal controls. Error bars are S.D. *p* indicates the statistical significance as measured by Student's t-test; *ns*: not statistically significant.

Supplementary Figure S5 | Effect of mTOR inhibition on β-Galactosidase staining and p53 *in vivo*. Senescence associated β-Galactosidase staining and immunohistochemical staining for p53 and phospho-S6 (pS6) in prostates from 8-week old *Pten*$^{pc-/-}$ mice treated with vehicle or RAD001 (10 mg/kg/day) as outlined in the timeline (upper panel). Note that treatment was started before puberty (i.e. before activation of the probasin-Cre).

Supplementary Figure S6 | Effect of *p19*^{Arf} inactivation in PICS. **(A)** Western blot analysis of *Pten*$^{-/-}$;p19$^{Arf+/+}$ and *Pten*$^{-/-}$;p19$^{Arf-/-}$ MEF cells after treatment with rapamycin for 24 hours. **(B)** Quantification of p53 protein levels in *Pten*$^{-/-}$ p19$^{Arf+/+}$ and *Pten*$^{-/-}$ p19$^{Arf-/-}$ MEF cells treated as in (**A**).

Supplementary Figure S7 | Modulation of PICS through Nutlin-3 and Rapamycin *in vitro*. **(A)** Western analysis for p53 and its quantification (upper and lower panel respectively) from *Pten*$^{lx/lx}$ MEFs undergoing PICS (Ad-Cre infected) and treated with rapamycin and/or Nutlin-3 according to the experimental scheme shown in Supplemental Figure S1E. Error bars are S.D. from three independent experiments. **(B)** Growth curves of primary wt (upper panel) and p53-/- (lower panel) MEFs that have been treated with rapamycin and/or Nutlin-3 as indicated. Error bars are S.D. **(C)** Quantification of senescence associated β-Galactosidase staining (day 4 after selection) in Control (*Pten*$^{lx/lx}$ MEFs infected with Gfp), *PICS* (*Pten*$^{lx/lx}$ MEFs infected with Cre) and OIS (*Pten*$^{lx/lx}$ MEFs infected with Ras^{V12}) in response to Nutlin-3 treatment added 3 days after selection. Lower panels: β-Galactosidase staining in PICS and OIS after treatment with Nutlin-3. Error bars are S.D. **(D)** Quantification of senescence associated β-Galactosidase staining in *Pten*^{wt} and *Pten*$^{+/-}$ MEFs after treatment with Nutlin-3. Error bars are S.D.

Supplementary Figure S8 | p21 staining *in vivo* after RAD001, Nutlin-3 and combined treatment. Immunohistochemical staining for p21and its quantification (right panel) in prostate tumors from mice in the indicated treatment groups.