Extended Data Figure Legends

Extended Data Fig. 1 | Extended data on the implementation of senescence in WI-38 and BJ fibroblasts. **a**, Representative micrographs of SA-β-Gal staining performed in proliferating and senescent WI-38 fibroblasts. Senescence was induced by treating cells with 50 μM etoposide (Etop) for 10 days. Scale bars, 100 μm. **b**, Quantification of SA-β-Gal-positive cells in the conditions described in (**a**). **c**, Representative micrographs of SA-β-Gal staining performed in proliferating and senescent BJ fibroblasts. Senescence was induced by treating cells with 25 μM etoposide for 10 days. Scale bars, 100 μm. **d**, Quantification of SA-β-Gal-positive cells in the conditions described in (**c**). **e**, Evaluation of the proliferative ability for the conditions described in (**a**,**c**), respectively, by measuring BrdU incorporation 24 h later. Graphs (**b**,**c**,**e**,**f**) represent the mean values ±SD of n=3 experiments.

Extended Data Fig. 2 | Extended data on Trk inhibitors as senolytic compounds. **a**, Assessment of caspase 3/7 activity levels (*top*) and cell viability (*bottom*) 48 h after addition of the indicated doses of Trk inhibitors GNF 5837 (yellow), ANA 12 (green), PF 06273340 (red). **b**, Representative images of SA-β-Gal staining performed in proliferating and etoposide-induced senescent IMR-90 cells (upper images), along with proliferating and senescent (replicative or IR-induced) WI-38 fibroblasts (lower images). Scale bars represent 100 μm. **c**, Quantification of the conditions described in (**b**). **d**, Analysis of the proliferative ability of the experimental conditions described in (**b**) by BrdU incorporation assay. **e**, Cell viability observed after treatment of proliferating BJ and IMR-90 fibroblasts with the indicated Trk inhibitors. Graphs (**a**,**c-e**) represent the mean values ±SD of n=3 experiments; significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) was determined by using two-tailed Student's t-test.

Extended Data Fig. 3 | Extended data on TrkB expression throughout senescence. **a**, Representative western blot analysis of the levels of TrkB, p21, and loading control ACTB at the indicated days after treating BJ fibroblasts with 25 μM etoposide (Etop) to induce senescence. **b**, BJ cells were treated as in (**a**) and RT-qPCR analysis was performed to evaluate the levels of markers associated with different stages of senescence: early marker *TGFB1* mRNA, and late markers *p16* and *IL6* mRNAs. **c**, Representative western blot analysis of the levels of TrkB, p21, and loading control ACTB at the indicated days after treating IMR-90 fibroblasts with 50 μM etoposide to induce senescence. **d**, BJ cells were treated as in (**c**) and RT-qPCR analysis was performed to evaluate the levels of markers associated with different stages of senescence: early marker *TGFB1* mRNA, and late markers *p16* and *IL6* mRNAs. Graphs (**b**,**d**) display the mean values ±SD of n=3 experiments; significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) was determined by using two-tailed Student's t-test.

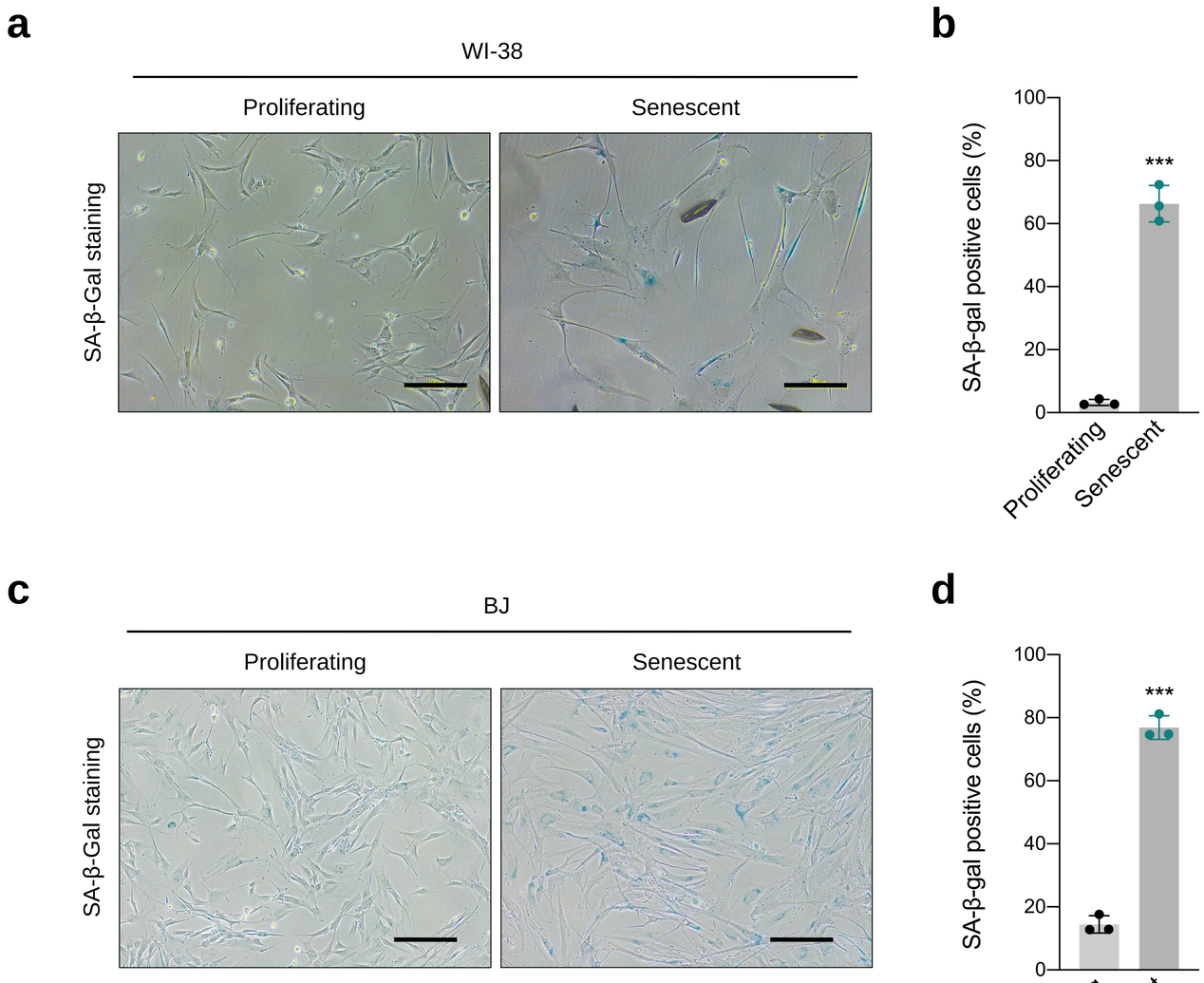
Extended Data Fig. 4 | Extended data on BDNF as a novel SASP factor. **a**, Heat maps displaying the relative levels of mRNAs encoding the indicated neurotrophins in several models of cellular senescence, as measured previously[22]. Expression values are shown by row Z-score values and represented as a graded green-black-red color scale. **b**, ELISA measurement of the levels of NGF, NT-3, and NT-4/5 in conditioned media collected for 48 h from proliferating and senescent WI-38, BJ, and IMR-90 fibroblasts. ND, not detected.

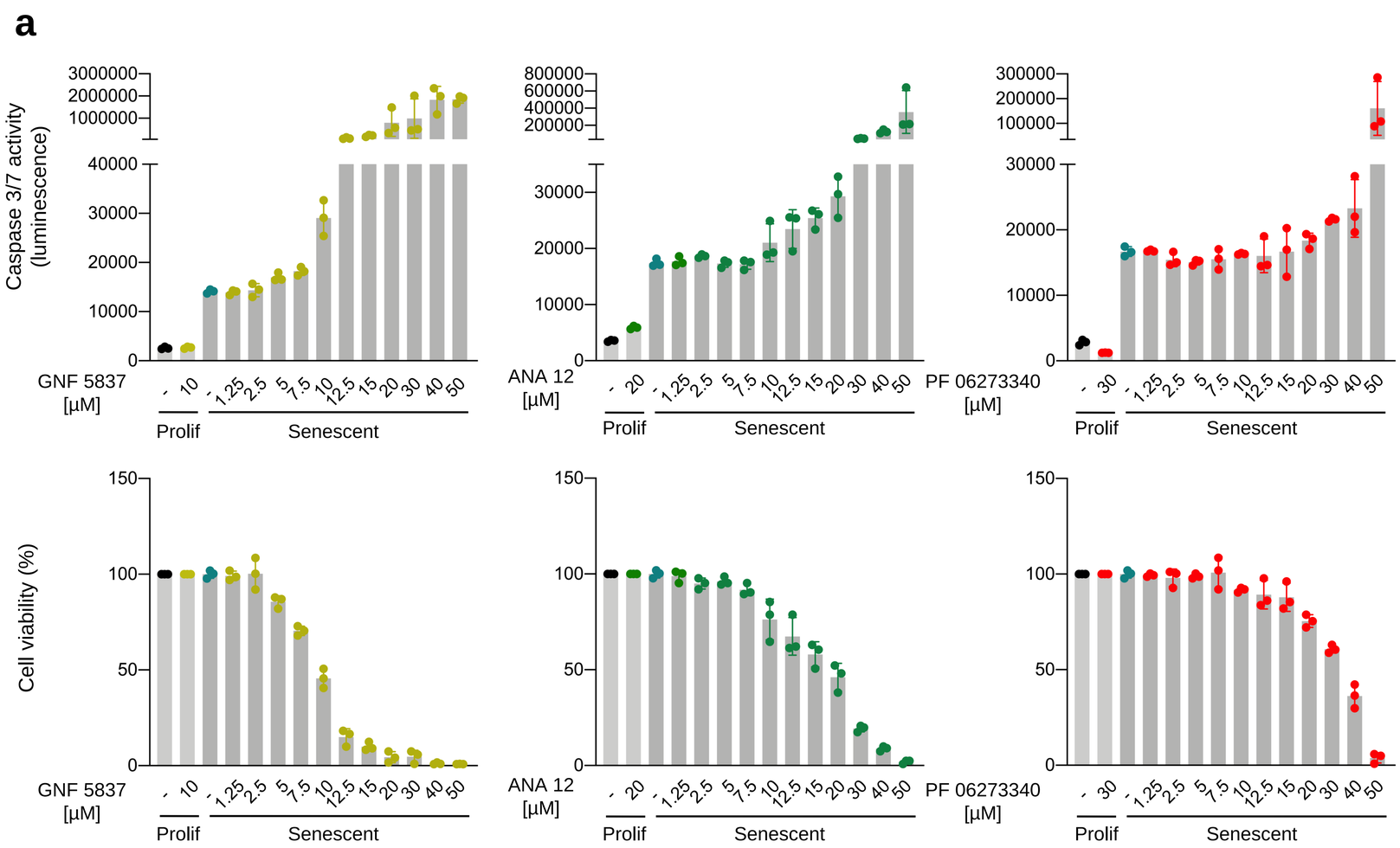
Extended Data Fig. 5 | Extended data on BDNF-TrkB-ERK5-BCL2L2 axis in senescent cells. **a**, RT-qPCR analysis of the levels of *p21*, *IL6*, *NTrk2*, and *BDNF* mRNAs at both early and late senescence [2 and 8 days after etoposide (Etop) treatment, respectively] in WI-38 fibroblasts transfected with siCtrl, siNTrk2, or siBDNF siRNAs. **b**, RT-qPCR analysis of the levels of *p21* and *IL6* mRNAs in proliferating (P) and senescent (S) WI-38 fibroblasts treated with IgG or BDNF-blocking antibodies for 48 h. **c**, Representative phase-contrast micrographs of senescent WI-38 fibroblasts that were either left untreated or treated with the ERK5 inhibitor (ERK5-in-1, 1 μM) for 48 h. **d**, The viability of proliferating WI-38 cells treated with ERK5i (ERK5-in-1, 1 μM) for 48 h. **e**, RT-qPCR analysis of the levels of *p21* and *IL6* mRNAs in senescent WI-38 cells that were either untreated or treated with ERK5i (1 μM) for 48 h. Untreated proliferating WI-38 fibroblasts were included as a control of baseline expression of each of the mRNAs. Graphs (**a**,**b**,**d**,**e**), represent the values ±SD from n=3 experiments; significance (*p < 0.05, **p < 0.01, ***p < 0.001) was determined by using two-tailed Student's t-test.

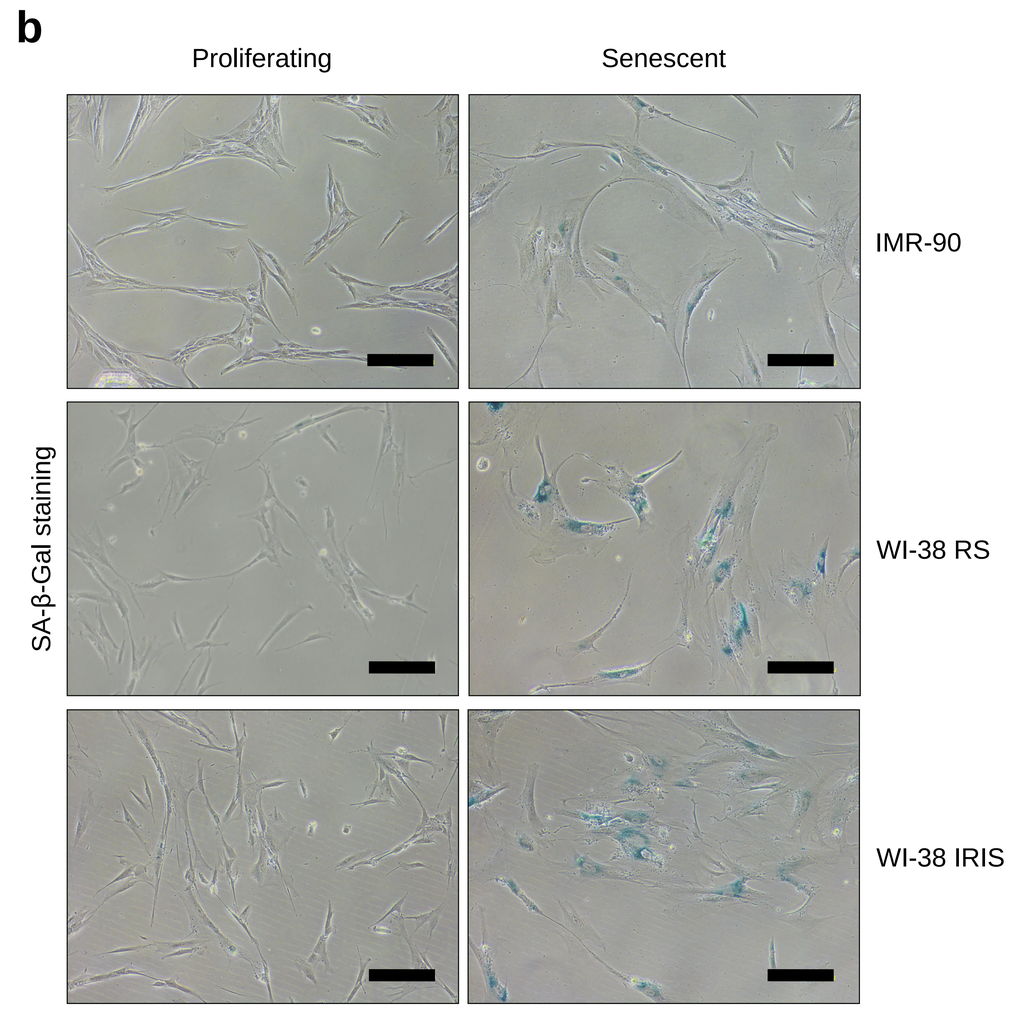
Extended Data Fig. 6 | Extended data on BDNF as a discrete marker of survival in cellular senescence. **a**, RT-qPCR analysis of the levels of *p21* and *IL6* mRNAs in WI-38 fibroblasts transfected with siCtrl or siTP53 siRNAs and then treated with 50 μM etoposide (Etop) for 48 h. Untreated siCtrl-transfected WI-38 fibroblasts were included as a control for baseline mRNA expression levels. **b**, Confirmation of p53 silencing in the experiments described in (**a**) by RT-qPCR analysis of *TP53* mRNA levels. **c**, RT-qPCR analysis of the levels of *p21* and *IL6* mRNAs in WI-38 fibroblasts treated with 50 μM etoposide for 48 h. The levels of p53 were stabilized by the addition of Nutlin-3a (N3a, 10 μM). Untreated proliferating WI-38 fibroblasts were included as a control group. **d**, Assessment of senescent cell viability in the conditions described in (**a**) and (**c**), respectively, by direct cell counting. **e**, RT-qPCR analysis of the levels of *STAT3*, *p21* and *IL6* mRNAs in WI-38 fibroblasts transfected with siCtrl or siSTAT3 siRNAs, then treated with 50 μM etoposide for 48 h. **f**, RT-qPCR analysis of the levels of STAT3 mRNA to assess the impact of siSTAT3 transfection in cells treated as described in (**e**). Viability of WI-38 cells transfected with siCtrl or siSTAT3 siRNAs, then treated for 48 h with 50 μM etoposide. Values shown in **a-f** are values ±SD; significance (*p < 0.05, **p < 0.01, ***p < 0.001) was determined by using two-tailed Student's t-test.

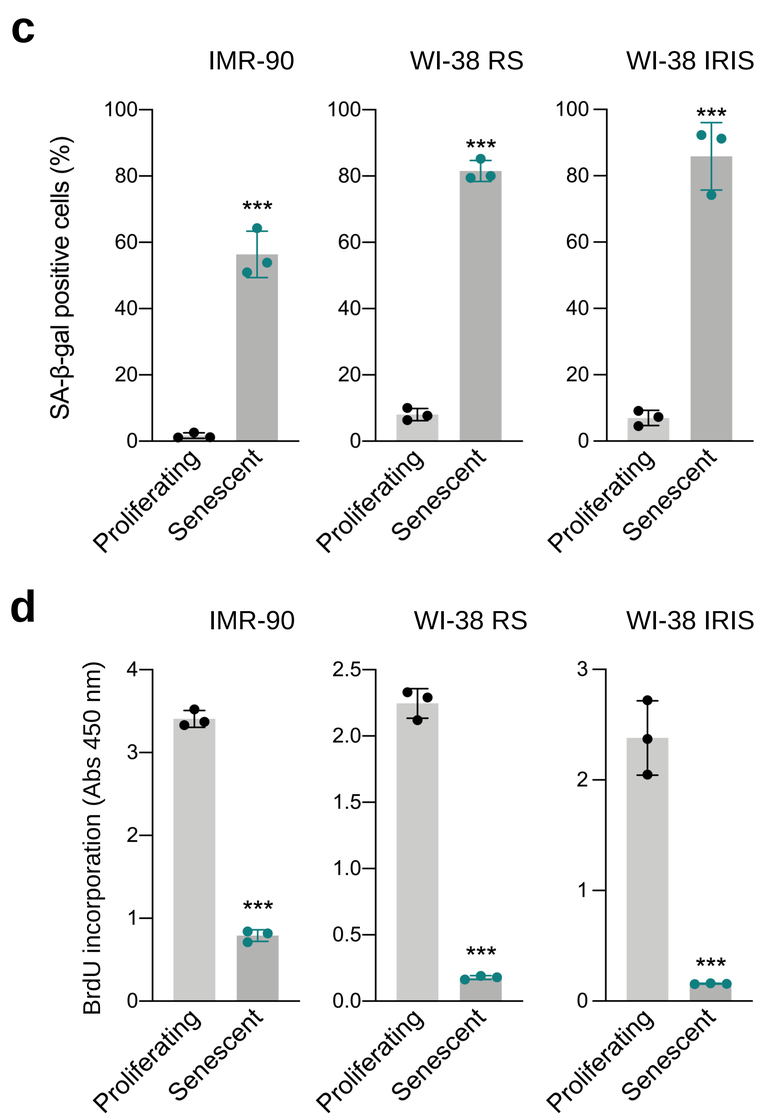
Extended Data Fig. 7 | Extended data on Trk inhibitors reducing cellular senescence markers in old mice. **a**, Serum concentrations of TIMP-1 and PAI-1 in four mouse groups shown, as analyzed by using a Bioplex instrument. **b**, Weights of the mice included in the indicated groups at the end of the experiments (young are 3 m.o., old are 24 m.o.). **c**, Representative p16 immunofluorescent micrographs from liver and lung in the indicated groups. Scale bars, 200 μm in all the images. Values shown in **a** and **b** are individual data points with the values ±SD; significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) was determined by using two-tailed Student's t-test.

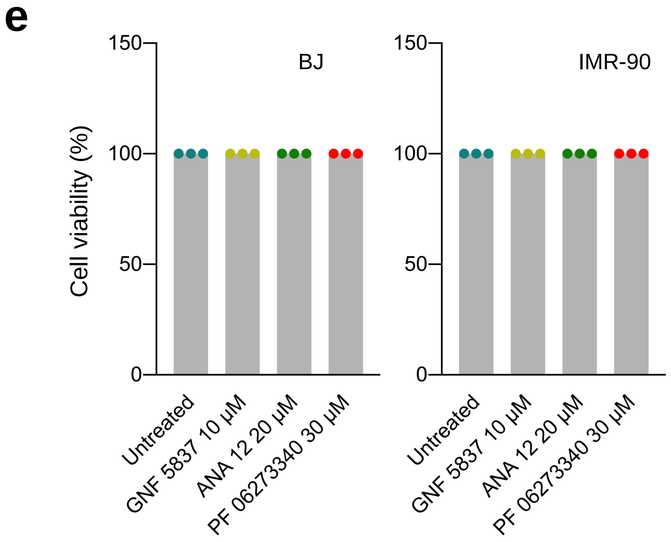
Anerillas et al., Extended Data Fig. 1

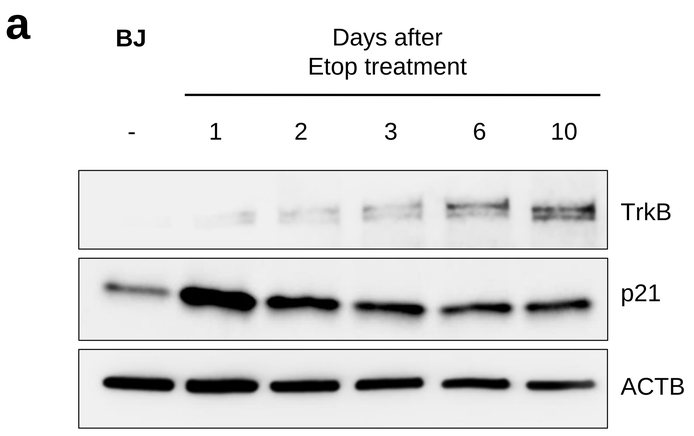


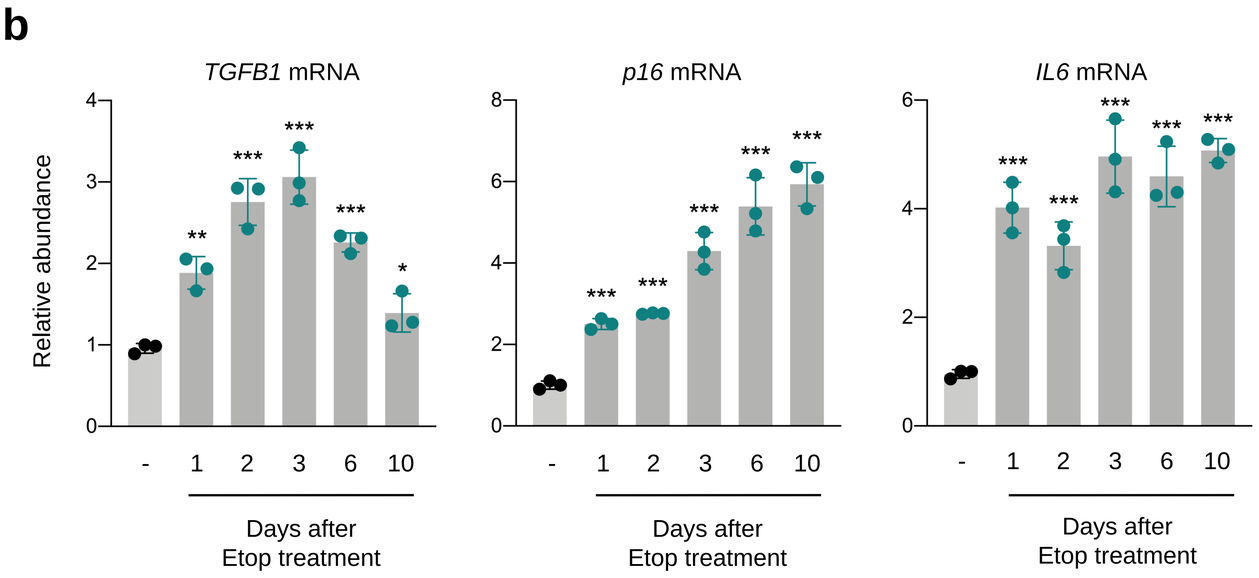

a
Caspase 3/7 activity (luminescence)
GNF 5837 [μM]
ANA 12 [μM]
PF 06273340 [μM]
Prolif
Senescent
Cell viability (%)

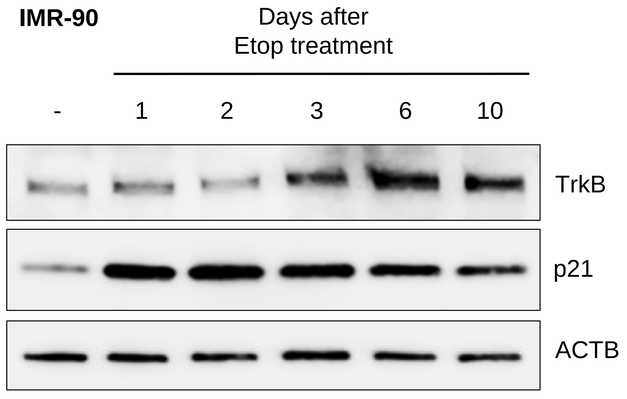

b
Proliferating
Senescent
SA-β-Gal staining
IMR-90
WI-38 RS
WI-38 IRIS

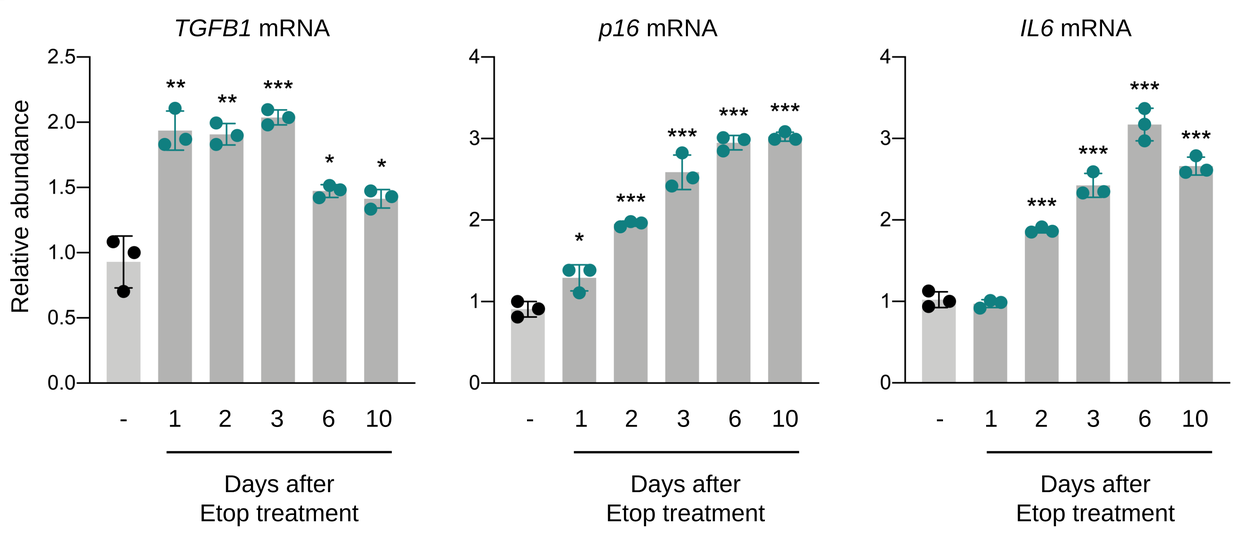

c
IMR-90
WI-38 RS
WI-38 IRIS
SA-β-gal positive cells (%)
Proliferating
Senescent
d
BrdU incorporation (Abs 450 nm)


e
BJ
IMR-90
Cell viability (%)
Untreated
GNF 5837 10 μM
ANA 12 20 μM
PF 06273340 30 μM

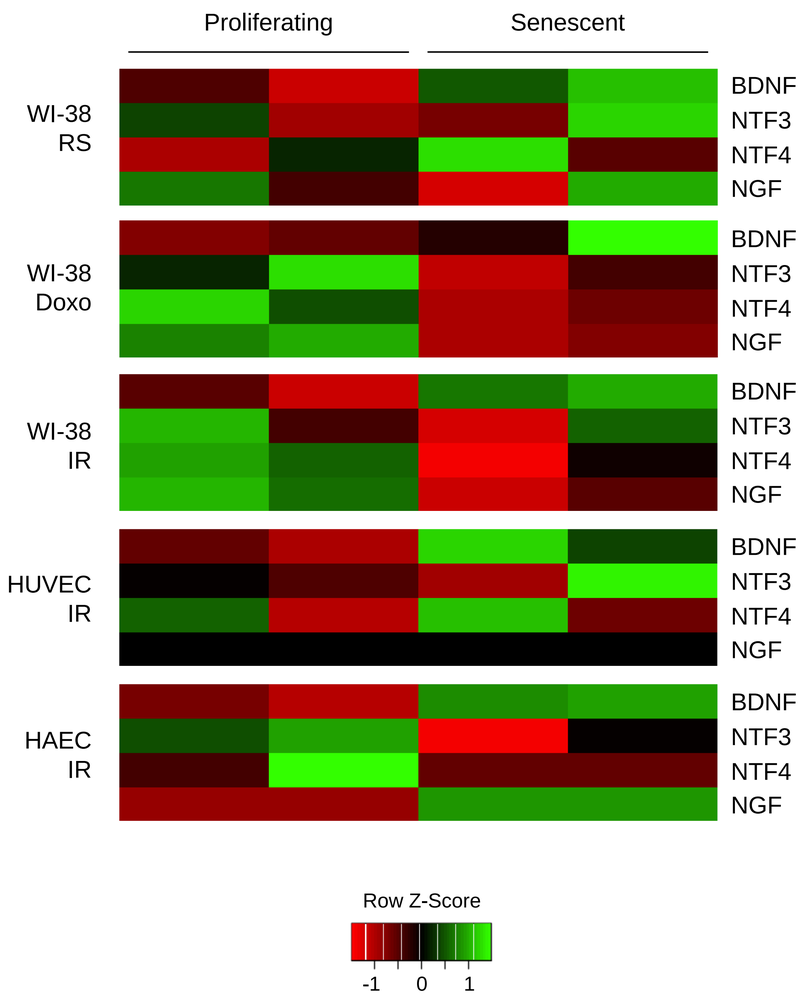

a
BJ
Days after
Etop treatment
-
1
2
3
6
10
TrkB
p21
ACTB


b
TGFB1 mRNA
p16 mRNA
IL6 mRNA
Relative abundance
Days after
Etop treatment

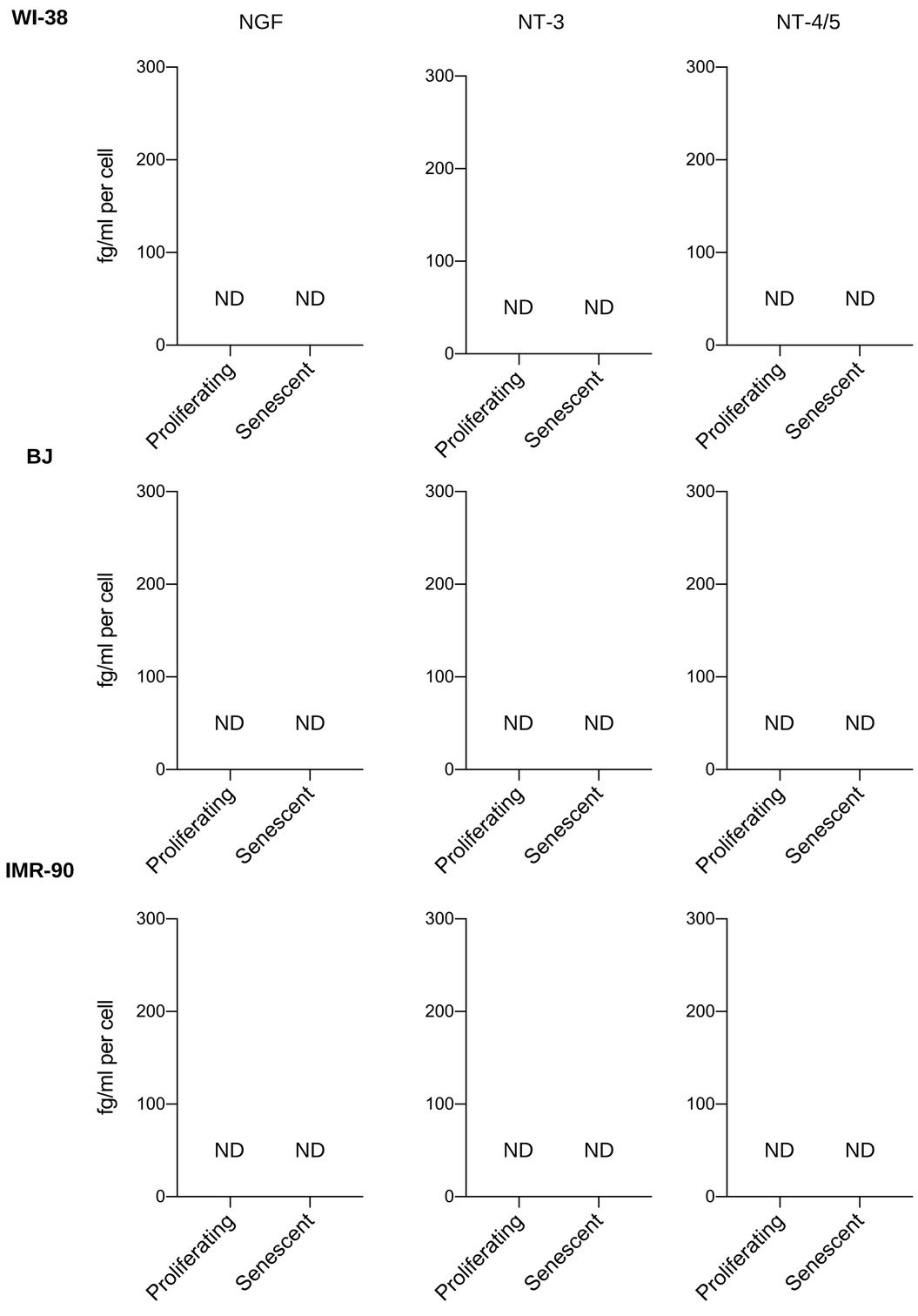

c
IMR-90
Days after
Etop treatment
-
1
2
3
6
10
TrkB
p21
ACTB


d
TGFB1 mRNA
p16 mRNA
IL6 mRNA
Relative abundance
Days after
Etop treatment

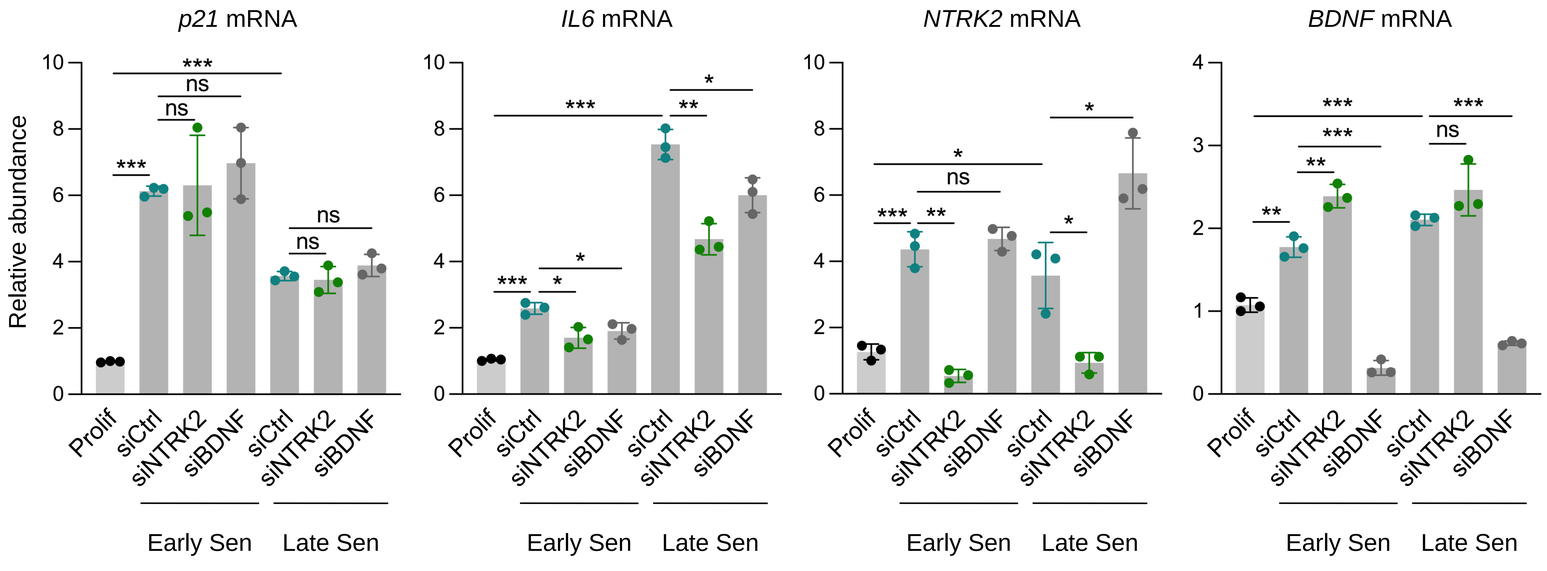
a


Proliferating
Senescent
WI-38 RS
WI-38 Doxo
WI-38 IR
HUVEC IR
HAEC IR
BDNF
NTF3
NTF4
NGF
Row Z-Score
-1
0
1

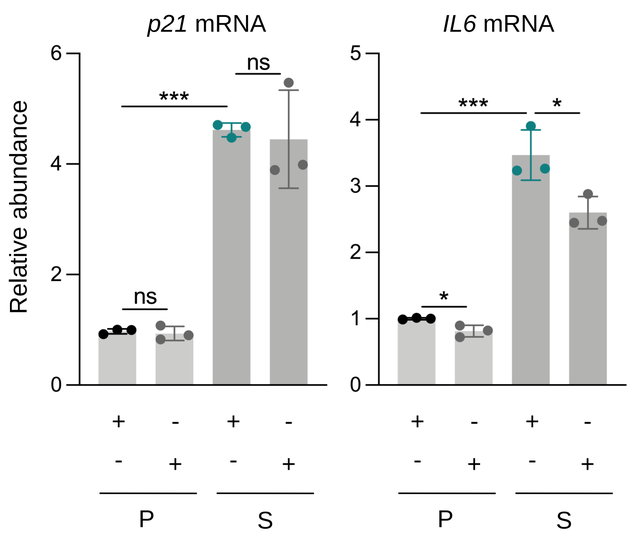
b


WI-38
BJ
IMR-90
NGF
NT-3
NT-4/5
fg/ml per cell
300
200
100
0
ND
Proliferating
Senescent

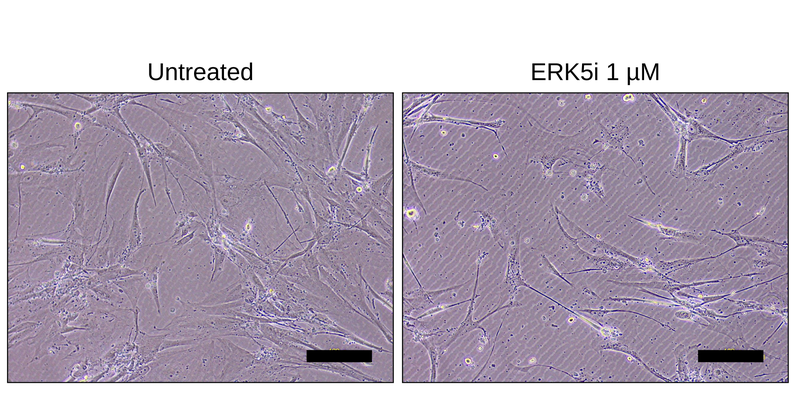
a


p21 mRNA
IL6 mRNA
NTRK2 mRNA
BDNF mRNA
Relative abundance
Prolif
siCtrl
siNTRK2
siBDNF
Early Sen
Late Sen

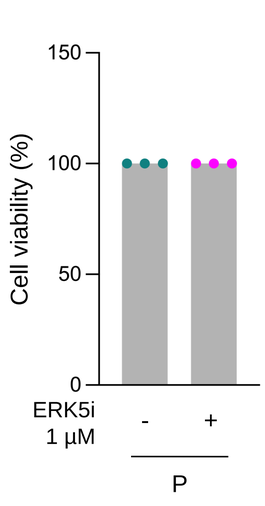
b


p21 mRNA
IL6 mRNA
Relative abundance
P
S

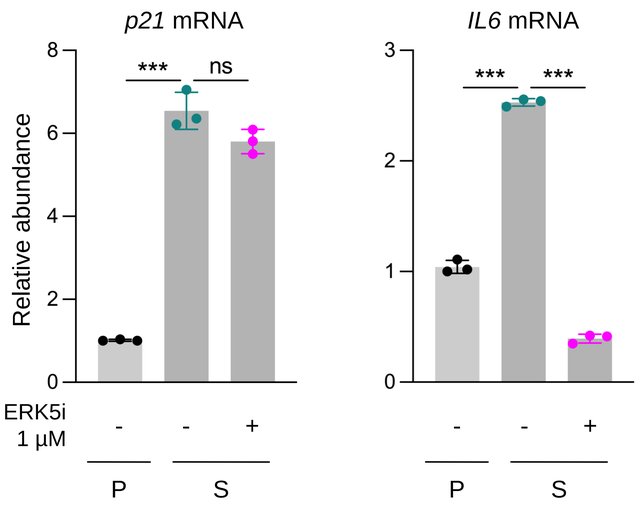
c

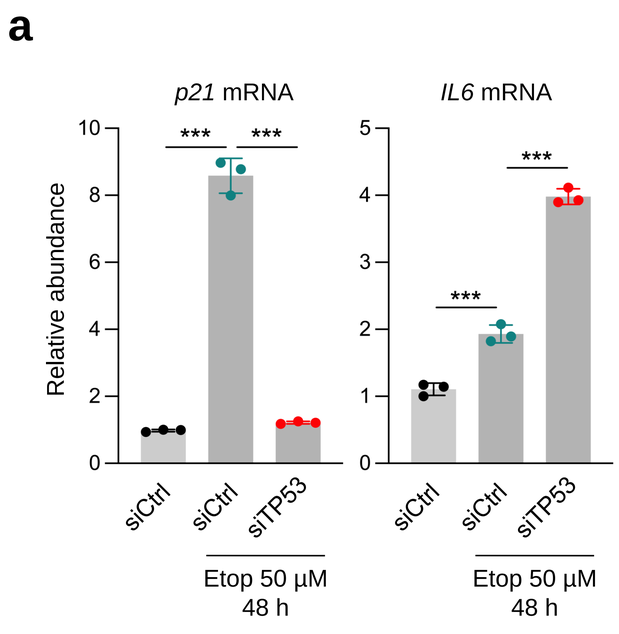

Untreated
ERK5i 1 µM

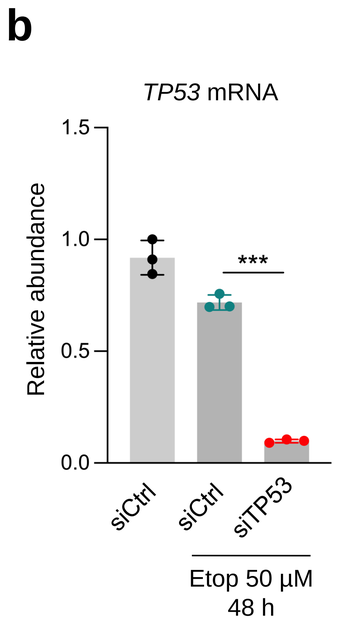
d

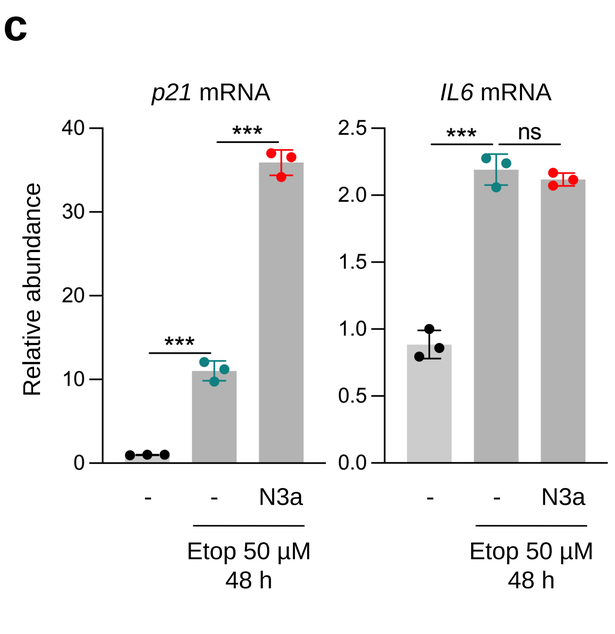

Cell viability (%)
ERK5i
1 µM
P

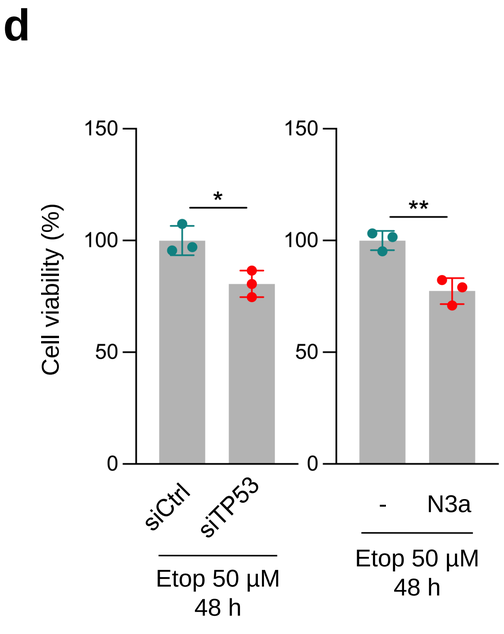
e

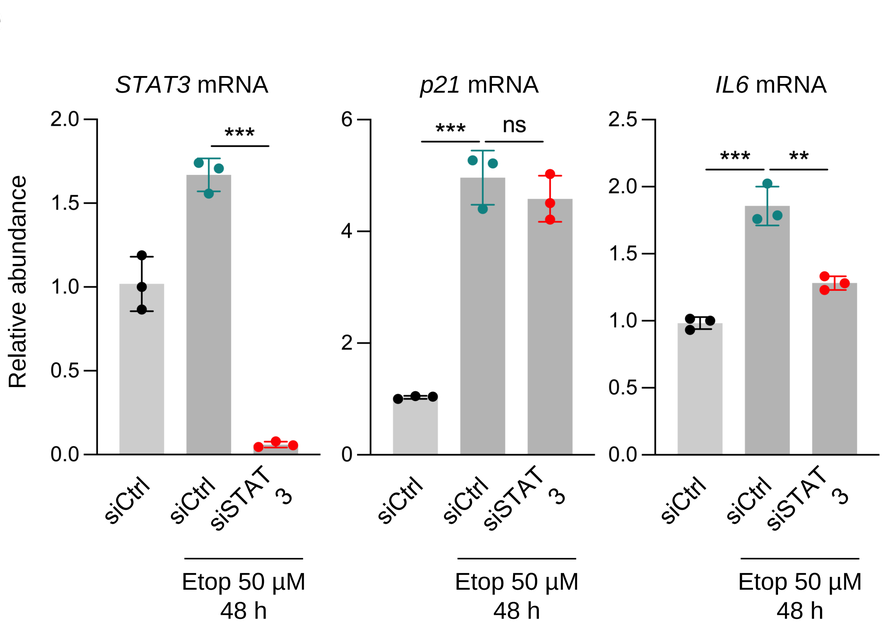

p21 mRNA
IL6 mRNA
Relative abundance
ERK5i
1 µM
P
S

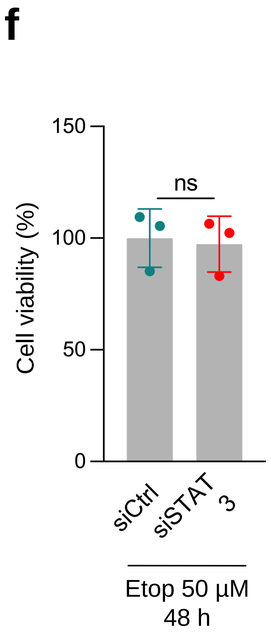

a
p21 mRNA
IL6 mRNA
Relative abundance
***
***
***
***
siCtrl
siCtrl
siTP53
siCtrl
siCtrl
siTP53
Etop 50 µM
48 h
Etop 50 µM
48 h


b
TP53 mRNA
Relative abundance
***
siCtrl
siCtrl
siTP53
Etop 50 µM
48 h


c
p21 mRNA
IL6 mRNA
Relative abundance
***
***
***
ns
-
-
N3a
-
-
N3a
Etop 50 µM
48 h
Etop 50 µM
48 h

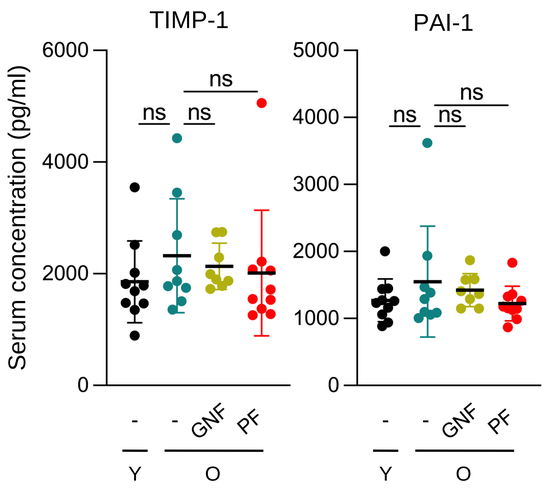

d
Cell viability (%)
*
**
siCtrl
siTP53
-
N3a
Etop 50 µM
48 h
Etop 50 µM
48 h


e
STAT3 mRNA
p21 mRNA
IL6 mRNA
Relative abundance
***
***
ns
***
**
siCtrl
siCtrl
siSTAT3
siCtrl
siCtrl
siSTAT3
siCtrl
siCtrl
siSTAT3
Etop 50 µM
48 h
Etop 50 µM
48 h
Etop 50 µM
48 h

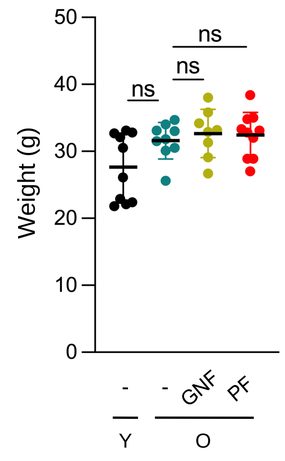

f
Cell viability (%)
ns
siCtrl
siSTAT3
Etop 50 µM
48 h

Anerillas et al., Extended Data Fig. S7

a



b



c