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A BDNF-TrkB autocrine loop enhances senescent cell viability

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Running title: BDNF-TrkB signaling supports senescent cells

Cellular senescence is characterized by cell cycle arrest, resistance to apoptosis, and a senescence-associated secretory phenotype (SASP) whereby cells secrete pro-inflammatory and tissue-remodeling factors. Given that the SASP exacerbates age-associated pathologies, some aging interventions aim at selectively eliminating senescent cells. In this study, a drug library screen uncovered TrkB (NTRK2) inhibitors selectively capable of triggering apoptosis of senescent, but not proliferating, human fibroblasts. Senescent cells expressed high levels of TrkB, which supported senescent cell viability, and secreted the TrkB ligand BDNF. The reduced viability of senescent cells after ablating BDNF signaling suggested an autocrine function for TrkB and BDNF, including increased expression of BCL2L2 downstream of BDNF-TrkB, favoring senescent cell survival. Strikingly, treatment with TrkB inhibitors reduced the accumulation of senescent cells in aged mouse organs. We propose that the activation of TrkB by SASP factor BDNF promotes cell survival and could be exploited therapeutically to reduce the senescent-cell burden.

The cell senescence program is implemented in response to different forms of non-lethal cell damage. Senescent cells are characterized by several traits, including morphological changes, indefinite growth arrest, and metabolic reprogramming that includes altered lysosomal function with increased presence of a senescence-associated β -Galactosidase (SA- β -Gal) activity¹. However, one of their most prominent features is the senescence-associated secretory phenotype (SASP), a trait defined as the active secretion of pro-inflammatory factors and matrix-remodeling enzymes. Through these secreted proteins, senescent cells modify their environment and influence physiologic and disease processes².

On the one hand, senescence has been identified as critically beneficial for tissue repair, embryonic development, and tumor suppression; on the other, the uncontrolled accumulation of senescent cells within organs can lead to tissue dysfunction and disease³. With advancing age, senescent cells increase in tissues and organs, exacerbating a range of aging-related physiologic declines and diseases⁴. The pharmacologic removal of senescent cells using senolytics in mouse models was recently found to improve several age-associated disorders⁵. Many senolytic strategies exploit the fact that senescent cells exist in a state of potent suppression of apoptosis⁶, for instance, by inhibiting the pro-survival effect of anti-apoptotic proteins in the BCL2 family^{7,8}.

A range of roles have been identified for mitogen-activated protein kinase (MAPK) signaling pathways in senolysis⁹. To address the influence of MAPKs experimentally and systematically, we employed a chemical library including inhibitors of every step in the MAPK cascade, from the receptors down to the final effector proteins. This survey identified two drugs, both inhibitors of tropomyosin receptor kinase (Trk) activity, that effectively and specifically reduced the viability of senescent WI-38 and BJ human diploid fibroblasts. Trk receptors are best known for transducing signals from their known ligands, the neurotrophins, to modulate neuronal development and communication¹⁰. Among the Trk family, which includes TrkA, TrkB, and TrkC, we identified TrkB as being primarily responsible for maintaining senescent cell viability in this unexpected context. Further interrogation revealed that cells rendered senescent in different ways secrete the neurotrophin BDNF (brain-derived neurotrophic factor), and that in cultured fibroblasts, autocrine/paracrine activation of TrkB by BDNF sustained ERK5 activation and BCL2L2-dependent viability of senescent cells. We present evidence that inhibiting this regulatory paradigm reduces the senescent cell burden in mouse tissues, and propose that suppressing the signaling through TrkB and BDNF can be exploited to eliminate senescent cells for therapeutic benefit.

Results

MAPK-directed drug screen identifies inhibitors of Trk receptors as potential senolytic drugs

As discussed recently⁹, the role of MAPK pathways in senescence-associated apoptosis is poorly understood. To better understand how targeting MAPKs in senescence might offer therapeutic benefit, we screened a custom library (Tocris) comprising 43 compounds that inhibit different mediators of MAPK signaling cascades (**Fig. 1a**, **Supplementary Table 1**). The screen included a known senolytic, ABT-737, as a positive control, and was carried out in parallel on proliferating cells to ensure that the identified drugs selectively affected senescent cells. To strengthen our screen, we performed it on two human diploid fibroblasts lines (WI-38 and BJ) that are well-established in senescence studies. The experimental conditions were previously optimized to trigger cellular senescence in both cell types with minimal cell death. Briefly, WI-38 and BJ fibroblasts were treated with 50 or 25 μ M etoposide, respectively, refreshing the drug every three days. After ten days, when cells had reached senescence, etoposide was removed, and the different compounds from the library were tested for an additional 48 hours (**Fig. 1b**). The induction of senescence in WI-38 and BJ cells was confirmed by measuring senescence-associated beta-Galactosidase (SA- β -Gal) activity (**Extended Data Fig. 1a-d**); growth arrest in the senescent cultures was confirmed by measuring BrdU incorporation (**Extended Data Fig. 1e,f**).

Next, we assessed the effect of treatment with the drugs in the library (each at 10 μ M) for 48 h on both proliferating and senescent WI-38 or BJ cells. The impact of each drug was evaluated by direct cell counts at the end of the treatment relative to the initial cell numbers. As shown in **Fig. 1c** (further information can be found in **Supplementary Table 1**), treatment with several inhibitors caused specific decreases in the number of live senescent cells; the heat map (**Fig. 1d**) represents the percentage of senolysis caused by a certain drug (proportion of dead cells 48 h later). The drugs were grouped by the target proteins within the MAPK pathways upon which they act. We excluded from the heat map those drugs that decreased the viability of proliferating cells, such as CMPD-1 (an inhibitor of MK2 and microtubular assembly), BRD 7389 (a p90 S6K inhibitor), HI TOPK 032 (a TOPK inhibitor), and AC 710 (a PDGFR inhibitor). Strikingly, two of the drugs with the most pronounced senolytic effect were the TrkB receptor inhibitors GNF 5837 and ANA 12 (blue arrows in **Fig. 1c,d**). TrkB belongs to a family of Receptor Tyrosine Kinases (RTKs) whose ligands are neurotrophins, a group of secreted proteins crucial for engaging neuronal survival¹¹. Although GNF 5837 also inhibits TrkA and TrkC, other inhibitors included in the library selectively targeting other members of this family, such as TrkA (GW 441756) and NGFR (PD 90780), did not show any senolytic properties. Thus, in this screen, TrkB appeared to be the main Trk receptor responsible for maintaining the viability of senescent cells. Together, these data indicate that TrkB inhibitors have potential senolytic effects.

Pharmacological inhibition of TrkB selectively induces apoptotic cell death of senescent cells

We sought to explore further the ability of Trk inhibitors to selectively induce apoptosis in senescent cells. In addition to GNF 5837 and ANA 12, our analysis included PF 06273340, an additional Trk inhibitor that does not cross the blood-brain barrier (BBB)¹². Given that Trk receptor activity is essential for cognitive function¹¹, drugs incapable of crossing the BBB, such as GNF 5837 and PF 06273340¹² would have less cognitive side-effects and thus might be more attractive for therapy. ANA 12 does enter the central nervous system, and it has been proposed to be beneficial as an antidepressant¹³.

Therefore, we evaluated a range of doses of each drug (1.25 to 50 μ M) in proliferating and senescent cells (**Fig. 2a**). Analysis of viability after 48 h later revealed that the optimal senolytic doses are 10 μ M GNF 5837, 20 μ M ANA 12, and 30 μ M PF 06273340 (**Fig. 2b,c**), since they caused maximal death of senescent cells with minimal consequences on proliferating cells. These doses also significantly increased Caspase 3/7 activity, an indicator of apoptosis, in senescent cells relative to untreated senescent cells (**Extended Data Fig. 2, Fig. 2d**) as tested previously¹⁴. Importantly the same doses effectively reduced the viability of WI-38 cells that had been rendered senescent by other means –replicative senescence (RS) or ionizing radiation-induced senescence (IRIS) (**Fig. 2e; Extended Data Fig. 2b-d**). We further tested their effectiveness in reducing cell viability in other senescence models, including BJ or IMR-90 fibroblasts rendered senescent by treatment with etoposide. After confirming the induction of senescence in these models by measuring SA- β -Gal activity and BrdU incorporation (**Extended Data Fig. 2b-d**), we evaluated the effects of treatments with 10 μ M GNF 5837, 20 μ M ANA 12, or 30 μ M PF 06273340. Importantly, while these doses did not reduce the viability of proliferating cells (**Extended Data Fig. 2e**), they strongly induced death of both senescent BJ and IMR-90 cells (**Fig. 2f**). In keeping with the results seen in senescent WI-38 cells, senescent BJ and IMR-90 fibroblasts displayed elevated caspase activity in the presence of Trk inhibitors (**Fig. 2g**), supporting the notion that these cells experienced increased apoptotic death in response to these treatments.

Finally, to confirm the role of apoptosis in the death caused by TrkB inhibitors, we blocked caspase activity by incubating cells with the pan-caspase inhibitor Z-VAD-FMK along with GNF 5837, ANA 12, or PF 06273340. As shown, the increased cell death and caspase 3/7 activity seen by inhibiting Trk (**Fig. 2f,g**) were reversed when cells were simultaneously treated with Z-VAD-FMK (**Figs. 2h,i**). Together, these data support the notion that inhibiting Trk enhanced pro-apoptotic signaling in senescent cells, leading to cell death.

Elevated TrkB levels in senescent cells promotes resistance to apoptosis

We then set out to further characterize the role of Trk receptors in cellular senescence. In neuronal cells, activation of Trk receptors strongly promotes cell viability through specific gene expression programs¹⁵. Although our data pointed to a prominent pro-survival role for TrkB in senescent cells, we

also analyzed the structurally similar TrkA and TrkB. We first assessed the levels of *NTRK1*, *NTRK2*, and *NTRK3* mRNAs (encoding TrkA, TrkB, and TrkC, respectively), along with the levels of *p16/CDKN2A* and *IL6* mRNAs, two markers of senescence, by reverse transcription (RT) followed by real-time quantitative (q)PCR analysis. Although we were unable to detect *NTRK3* mRNA in WI-38 fibroblasts, the levels of *NTRK1* and *NTRK2* mRNAs increased slightly, while *p16* and *IL6* mRNAs increased markedly, as expected, in senescent (S) relative to proliferating (P) cells (**Fig. 3a**). By western blot analysis, however, the levels of TrkA, TrkB, and senescence marker p21 increased robustly in the senescent populations, including WI-38, BJ, and IMR-90 cells rendered senescent by treatment with etoposide, and replicatively senescent (RS) WI-38 cells (**Fig. 3b**).

To determine the role of each Trk receptor in senescent cell viability, we reduced the levels of TrkA or TrkB by transfecting specific small interfering (si)RNAs. After silencing was achieved, we triggered senescence with etoposide and monitored cell viability. As observed in **Fig. 3c**, cells in which TrkA was silenced (siNTRK1) and control cells did not show reduced viability, while silencing TrkB (siNTRK2) significantly decreased the numbers of live cells. The efficiency of the silencing interventions was confirmed by measuring mRNA levels (**Fig. 3d**); silencing of TrkA or TrkB in proliferating cells did not affect viability (not shown). In sum, despite the rise in TrkA levels in some senescence models, the enhanced viability of senescent cells appears to be mediated by TrkB.

Next, since TrkB protein levels increased proportionally more than *NTRK2* mRNA levels in senescent cells, we evaluated the stability of TrkB protein by treating proliferating and senescent cells with cycloheximide (CHX), an inhibitor of *de novo* translation. This analysis included positive control p53 (TP53), as its stability increases in senescent cells^{16,17}. As assessed by western blot analysis (**Fig. 3e**), TrkB appeared more stable in senescent fibroblasts, as did p53, suggesting that increased TrkB protein stability contributes to its accumulation in senescent cells. A time-course analysis after triggering senescence with etoposide revealed that TrkB protein levels increased by day 2 and continued to rise until day 10 (**Fig. 3f**). Early markers of senescence, p21 protein and *TGFBI* mRNA (**Figs. 3f,g**)¹⁸⁻²⁰, as well as late markers of senescence, *p16* and *IL6* mRNAs (**Fig. 3g**), were included in the analysis. Although with different kinetics, TrkB levels also increased progressively with senescence in BJ and IMR-90 cells (**Extended Data Fig. 3**). Finally, we tested if TrkB was found on the plasma membrane of senescent cells. We enriched plasma surface proteins using a biotinylation strategy (Methods) and found that TrkB was present on the membrane of senescent cells; we included as a positive control DPP4, a protein previously reported to increase on the plasma membrane of senescent cells²¹ (**Fig. 3h**). Immunofluorescence detection of TrkB using non-permeabilizing conditions confirmed that TrkB was strongly abundant on the membrane of senescent cells, as was DPP4 (**Fig. 3i**). Together, these data indicate that TrkB protein increases during senescence in several cell systems, and contributes to ensuring senescent cell viability.

BDNF is a novel SASP member that ensures viability in senescent cells

Trk receptors are activated by a family of secreted ligands known as neurotrophins, comprising NGF, NTF3, NTF4, and BDNF¹⁵, which are essential for neuronal function. Even though BDNF is the preferred ligand of TrkB, we explored possible roles for all neurotrophins in this paradigm of cell senescence. First, by RT-qPCR analysis, we measured the levels of *NGF*, *NTF3*, *NTF4*, and *BDNF* mRNAs in WI-38, BJ, and IMR-90 fibroblasts induced to undergo senescence by etoposide treatment for ten days. Interestingly, only *BDNF* mRNA was consistently elevated in the three senescence models (**Fig. 4a**). Second, we found increased *BDNF* mRNA levels in transcriptomic datasets obtained from different models of senescence: in WI-38 cells rendered senescent by replicative exhaustion or by treatment with doxorubicin or IR; as well as in human umbilical vein endothelial cells (HUVEC) and human alveolar epithelial cells (HAEC) cells rendered senescent by treatment with IR (**Extended Data Fig. 4a**)²². Third, to test if BDNF is secreted by senescent cells, we collected media from the senescence models used in **Fig. 4a**; as shown, the levels of secreted BDNF, as analyzed by ELISA, were significantly higher in media collected from senescent cells compared to proliferating cells (**Fig. 4b**); NGF, NTF3, and NTF4 were undetectable by ELISA in either population (**Extended Data Fig. 4b**). Increased levels of BDNF and other SASP cytokines ([CXCL1 (GRO- α), IL6, HGF, or CXCL10] were also detected in media from senescent cells by using a cytokine array (**Fig. 4c**). NTF3 and NTF4 were included in this array but did not increase in the media obtained from senescent cells. Together, these data indicate that senescent cells secrete BDNF.

By immunofluorescence microscopy, we found that senescent WI-38 cells were strongly positive for BDNF (**Fig. 4d**). RT-qPCR analysis likewise revealed a rise in *BDNF* mRNA levels two days after triggering senescence with etoposide (**Fig. 4e**). Since many SASP members are regulated by NF- κ B or p53, we investigated if these two transcription factors induced BDNF production in senescent cells. Following transfection with siRNAs directed at *TP53* and *RELA* mRNAs to silence p53 and RELA, respectively, we observed that the rise in *BDNF* mRNA levels in senescent cells was dramatically suppressed by silencing p53 and modestly reduced by silencing RELA (**Fig. 4f**). The senescence-associated rise in *p21* mRNA levels, which is dependent on the transcriptional activity of p53, was similarly reduced by silencing p53, while induction of the senescence-associated induction in *IL6* mRNA, an NF- κ B-regulated mRNA, was mostly abrogated by silencing RELA. The rise in *p16* mRNA was not reversed by either silencing intervention.

We then asked if BDNF might directly affect senescent cell survival in a manner similar to how it promotes neuronal viability, through TrkB activation^{15,23}. First, we silenced BDNF by using specific siRNAs directed at *BDNF* mRNA (Ctrl siRNAs were used in control transfections), and then we triggered senescence with etoposide. As shown in **Fig. 4g**, silencing BDNF before triggering senescence significantly reduced cell viability relative to control (siCtrl) cells that reached senescence but were able to express BDNF. Importantly, BDNF silencing did not influence the survival of

proliferating cells, suggesting that it selectively promoted the survival of senescent cells; RT-qPCR analysis confirmed the silencing interventions (**Fig. 4h**). To further assess if the effect of BDNF was autocrine/paracrine, we employed blocking antibodies. Forty-eight hours after adding anti-BDNF or non-specific IgG antibodies (4 µg/ml) to the media, proliferating cells showed no change in proliferation or viability, while senescent cells showed markedly reduced viability in the presence of anti-BDNF antibodies (**Figs. 4i,j**). Moreover, caspase 3/7 activity increased by blocking BDNF with antibodies (**Fig. 4k**). In sum, our results identify BDNF as a SASP member implicated in promoting senescent cell survival and further indicate that BDNF secreted by senescent cells promotes viability in an autocrine and/or paracrine fashion.

ERK5 activation by TrkB-BDNF axis sustains senescent cell survival through BCL2L2

We then sought to investigate if the activation of TrkB by BDNF was linked to the TrkB-mediated survival of senescent cells. First, to visualize protein phosphorylation we employed phos-tag polyacrylamide gels, which capture phosphorylated proteins and reduce their migration, and compared the proportion of slow-migrating bands (phosphorylated) to those migrating at the expected size (non-phosphorylated). As shown, slow-migrating TrkB bands appeared by 2 to 3 days of etoposide treatment, increasing in intensity in subsequent days (**Fig. 5a, left**); notably, the dynamics of the rise in phosphorylation mirrored the rise in *BDNF* mRNA abundance. To test if the two processes were related, we silenced BDNF before triggering senescence and found that this intervention completely prevented the appearance of the phosphorylated TrkB band 6 days later (**Fig. 5a, right**). These data suggest that the production of BDNF is critical for inducing TrkB phosphorylation in senescent cells. Second, we tested whether both TrkB and BDNF depletion affected senescent cell viability in a similar time frame. Given that TrkB and BDNF levels rose markedly by 2 days into senescence, and that silencing either TrkB or BDNF increased cell death, we evaluated whether their depletion affected senescent cell survival at early and/or late senescence, two stages defined previously^{19,24}. We found that silencing TrkB or BDNF decreased cell survival at both early and late senescence, although the survival was more strongly reduced in late senescence (**Fig. 5b**).

To further explore the ability of BDNF and TrkB to modulate senescent cell survival, we sequenced the bulk RNA present in senescent cells in which either TrkB or BDNF were silenced, and compared it to siCtrl-transfected senescent counterparts (**Fig. 5c**). The RNA-seq data are deposited in GSE190998 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE190998>; reviewer token [crqxcgaculzcxnid](#)). GSEA analysis of RNAs differentially abundant after silencing TrkB or BDNF revealed enriched pathways of apoptosis and caspase activity (**Fig. 5d, left**). The heat map in **Fig. 5d**, comprising differentially abundant mRNAs encoding apoptosis-related proteins, revealed jointly modulated transcripts, including *BCL2L2* mRNA, which encodes a protein essential for the survival of senescent cells^{8,25}. We used RT-qPCR analysis to validate these results by measuring the levels of

BCL2L2 mRNA, along with the levels of other mRNAs encoding apoptosis-modulating proteins such as *BCL2L1* and *PUMA*^{6,8}, and included conditions in which TrkB or BDNF were silenced (**Extended Data Fig. 5a**). Notably, while the levels of p53-induced pro-apoptotic *PUMA* mRNA were not affected, the levels of *BCL2L2* mRNA, encoding the anti-apoptotic protein BCL2L2, decreased after TrkB or BDNF silencing at both early and late senescence (**Fig. 5e**). This effect appeared to be specific for *BCL2L2* mRNA, as *BCL2L1* mRNA levels were unchanged after silencing TrkB or BDNF (**Fig. 5e**), as were the levels of the p53-regulated *p21* mRNA, while *IL6* mRNA levels unexpectedly decreased after silencing TrkB or BDNF (**Extended Data Fig. 5a**). Furthermore, BDNF-blocking antibodies specifically blunted the rise in *BCL2L2* mRNA in senescent cells, while *PUMA* and *BCL2L1* mRNAs were unaffected (**Fig. 5f**). As seen in TrkB- or BDNF-silenced senescent cells (**Extended Data Fig. 5a**), *p21* mRNA levels did not change in BDNF-blocked cells while *IL6* mRNA levels decreased after treatment with BDNF-blocking antibodies (**Extended Data Fig. 5b**). These results were confirmed by western blot analysis of BCL2L2 (BCLW) expression levels; p53 levels were unchanged (**Fig. 5g**).

Next, we analyzed the pathways downstream of BDNF and TrkB signaling. Phosphorylation of ERK5 was dramatically reduced by silencing TrkB or BDNF, in both early and late senescence (**Fig. 5h**), a notable finding, given that ERK5 elevates BCL2L2 levels downstream of BDNF-TrkB for the survival of neuronal cells²⁶. We then utilized a highly selective ERK5 inhibitor (ERK5-IN-1, 1 μ M) to reduce ERK5 activity in senescent cells and found that it lowered both BCL2L2 levels and senescent-cell viability (**Figs. 5i,j** and **Extended Data Fig. 5c**), but it did not affect proliferating cells (**Extended Data Fig. 5d**). Again, in agreement with the results after silencing TrkB or BDNF (**Extended Data Fig. 5a**), inhibiting ERK5 specifically lowered the levels of *BCL2L2* and *IL6* mRNAs, but not the levels of *PUMA*, *BCL2L1*, or *p21* mRNAs (**Fig. 5i** and **Extended Data Fig. 5E**). In sum, our data suggest that activation of TrkB by BDNF specifically activates ERK5, which in turn increases BCL2L2 levels.

BDNF is a discrete marker of survival in cellular senescence

In light of our earlier findings that p53 increases BDNF production, we investigated if BDNF induction contributed to the actions of p53 in senescence. First, we treated WI-38 cells with a range of doses of etoposide that had different effects on cell proliferation and viability. Untreated cells proliferated as expected, but increasing doses of etoposide reduced proliferation to different degrees (5 to 50 μ M etoposide) or caused cell death (100 to 200 μ M etoposide) (**Fig. 6a**). *BDNF* mRNA levels increased at etoposide concentrations that reduced proliferation (≤ 50 μ M) but not at concentrations that triggered cell death (100-200 μ M) (**Fig. 6b**); by contrast, *p21* and *IL6* mRNAs accumulated with increasing DNA damage (**Fig. 6b**). Second, we modulated p53 function by silencing and chemical activation to study if p53 influenced *BDNF* mRNA levels. As shown in **Fig. 6c**, silencing p53 prevented the rise in *BDNF* mRNA elicited by 50 μ M etoposide treatment for 48 h; the same interventions also reduced *p21* mRNA but not *IL6* mRNA (**Extended Data Fig. 6a,b**). These experiments underscore a requirement for

functional p53 to induce BDNF even at early stages of senescence, despite the absence of p53 sites on the *BDNF* promoter. We then tested if increasing p53 affected BDNF levels at a senescence-inducing dose of etoposide (50 μ M) by employing Nutlin-3a (Nut3a), a p53-stabilizing compound²⁷. Notably, increasing p53 in cells treated with 50 μ M etoposide significantly decreased BDNF levels (**Fig. 6c**), resembling the reduction observed at higher levels of DNA damage; *p21* mRNA levels increased in the presence of Nut3a (**Extended Data Fig. 6c**) and Nut3a reduced cell viability (**Extended Data Fig. 6d**). These data suggest that only moderate p53 levels induce BDNF production; levels of p53 that are too low or too high prevent the rise in BDNF that promotes survival.

Several mechanisms can explain how p53 could both induce and repress BDNF production depending on the extent of DNA damage. STAT3 was recently reported to promote BDNF expression in the lung upon injury with acid²⁸ and we found that phosphorylation of STAT3 at Tyrosine 705 decreased in IMR-90 fibroblasts committed to apoptosis but remained phosphorylated in senescence-committed cells (Anerillas et al., submitted). Therefore, we silenced STAT3 by transfection with siRNA (**Extended Data Fig. 6e**) and evaluated BDNF induction by 50 μ M etoposide treatment 48 h later. As shown in **Fig. 6d**, the rise in *BDNF* mRNA triggered by senescence-inducing etoposide (50 μ M) was prevented by STAT3 silencing; by contrast, *p21* mRNA levels were not affected and the slight rise in *IL6* mRNA was suppressed (**Extended Data Fig. 6e**). Despite the observation that silencing STAT3 lowered BDNF levels, silencing STAT3 did not reduce cell viability, suggesting that STAT3 might be part of multiple factors governing the survival-apoptosis balance in early senescence (**Extended Data Fig. 6f**). Interestingly, both p53 silencing and p53 activation decreased STAT3 phosphorylation (**Fig. 6e**), mirroring the effects seen for BDNF. Over the doses tested, p53 levels increased linearly along with DNA damage, but STAT3 phosphorylation at Tyr705 peaked at 25 μ M, when p53 levels were moderately elevated. Finally, we evaluated whether both p-STAT3 and BDNF were simultaneously activated at a single-cell level during senescence by double immunofluorescence staining of senescent WI-38 cells with antibodies recognizing p-STAT3(Tyr705) and BDNF. Interestingly most BDNF-positive cells were p-STAT3(Tyr705)-positive, but virtually every p-STAT3(Tyr705)-positive cell was BDNF-positive (**Fig. 6g,h**). Together, these experiments indicate that p53 can both induce or repress BDNF depending on the level of DNA damage and that p-STAT3(Tyr705) critically mediates BDNF induction in senescence. In turn, BDNF secretion by senescent cells can trigger signaling through TrkB $\rightarrow$ ERK5 $\rightarrow$ BCL2L2 to ensure survival in an autocrine manner (**Fig. 6i**).

Delivery of TrkB inhibitors reduces senescent cell burden in old mice

Finally, we tested the relevance of the BDNF-TrkB paradigm on senescent cell viability *in vivo* using C57BL/6J mice. We chose naturally aged mice as a well-established and relevant *in vivo* cellular senescence model²⁹. The appearance of senescent cells in older tissues such as lung and liver has been

documented by different methods, such as assessment of SA- β -Gal activity or immunostaining for senescence markers like p16³⁰⁻³². Initially, we studied the extent to which p16- and BDNF-positive cells colocalized in tissues in 24 month-old (m.o.) mice. Interestingly, ~one-half of the p16-positive cells in lung and liver were also BDNF-positive (**Fig. 7a**), suggesting that the two proteins were co-expressed in aging.

We then tested the ability of Trk inhibitors to reduce cell senescence markers in old mice. We administered the Trk inhibitors identified in **Fig. 1** by monthly injections into 21 m.o. mice until they were 24 m.o. (**Fig. 7b** and extended information in Methods). We assayed the same inhibitors we had tested in culture (GNF 5837, ANA 12, and PF 06273340), but halted treatments with ANA 12 due to toxicity; in fact, ANA 12 was a poor candidate for therapy due to its ability to penetrate the BBB¹³. Compared with control mice [vehicle-treated 24 m.o. and untreated young (3 m.o.) mice], GNF 5837 and PF 06273340 had promising effects on senescence markers and traits. The increases in levels of *p16* and *p21* mRNAs observed in kidney, lung, and liver of old mice were significantly mitigated by each drug (**Fig. 7c**); the same trends were seen for *Il6* and *Il1b* mRNAs, but they did not reach significance in all organs (**Fig. 7c**). These observations indicate that these drugs, which do not cross the BBB, were able to decrease the levels of prominent senescence markers such as *p16* or *p21* mRNAs in several tissues in aged mice.

We then studied senescence-related serum markers that were recently found to increase with age³³. One such marker, GDF15, increased markedly in old mice; this increase was significantly mitigated by treatment with PF 06273340, while GNF 5837 showed the same trend without reaching significance (**Fig. 7d**). Other serum markers (TIMP-1 and PAI-1) were not elevated with age (**Extended Data Fig. 7a**) and thus they could not be used to test the effects of these drugs. In addition, the drugs did not affect body weight (**Extended Data Fig. 7b**). Immunofluorescence analysis revealed that both GNF 5837 and PF 06273340 treatments reduced the percentage of p16-positive cells in all three organs tested (**Fig. 7e** and **Extended Data Fig. 7c**). As was the case for GDF15 serum levels, PF 06273340 appeared to be more effective than GNF 5837 in reducing p16 levels.

Finally, we assessed the canonical SA- β -Gal marker in three organs. Remarkably, both drug treatments reduced the age-associated increases observed both in the intensity and the number of SA- β -Gal-positive cells in kidney, lung, and liver (**Figs. 7g,h**). Together, we propose that the reduction in senescence markers is evidence that treatments with GNF 5837 and PF 06273340 are promising strategies to reduce the senescent cells that accumulate with aging. Although GNF 5837 and PF 06273340 do not cross the BBB and thus do not eliminate senescent cells in the central nervous system, this may be an advantageous trait, as it avoids interference with BDNF-TrkB signaling in the brain.

Discussion

Strategies to remove detrimental senescent cells from tissues and organs are gaining interest in the clinic, particularly for the treatment of chronic age-related diseases. Given that senescent cells bring about a proinflammatory state through the SASP, they have been found to exacerbate many aging-associated pathologies, including cardiovascular disease, neurodegeneration, cancer, and sarcopenia^{4,34}. Therefore, extensive efforts are underway to develop pharmacological approaches to eliminate them with minimum side effects^{5,35}. With increasing recognition that MAPK signaling cascades are implicated in senescence⁹, we systematically surveyed the effectiveness of MAPK inhibitors in achieving senolysis. The discovery that Trk inhibitors selectively reduced senescent cell viability (**Fig. 1**) led us to propose a novel role for TrkB in supporting the survival of senescent cells. Although a role for TrkB in senescence was not previously described, TrkB is a well-established pro-survival receptor in neurons^{10,11,15} and thus we set out to characterize the function of TrkB in senescent cell survival.

Interestingly, the levels of TrkB ligand BDNF, a neurotrophin, were found specifically elevated in several senescence models (**Extended Data Fig. 4**), and moreover, interfering with BDNF function by silencing or blocking antibodies caused a specific reduction in the viability of senescent cells (**Figs. 4, 5**). Our results indicated that BDNF was required for the pro-survival influence of TrkB, akin to the enhanced survival of neuronal cells elicited by BDNF upon TrkB activation³⁶; as in neurons, this regulation appeared to be mediated in senescent cells in an autocrine/paracrine manner. The active secretion of molecules by senescent cells through the SASP is a key feature of the response to damage. SASP factors mount a repair program that includes the promotion of inflammation, recruitment of immune cells, and remodeling of the tissue architecture. The discovery that the SASP factor BDNF promotes senescent cell survival by activating TrkB uncovers an important new dimension of the SASP in strengthening the anti-apoptotic attribute of senescent cells.

The finding that a neuronal survival paradigm is utilized by senescent cells to support viability was interesting, but it was not entirely unexpected, as activation of TrkB by BDNF (and more generally Trk receptors by neurotrophins) has been found to occur outside of the nervous system, particularly in developmental processes associated with the kidney, heart, muscle, and immune system³⁷. In addition, the BDNF-TrkB axis has been implicated in tissue repair following lung injury, periodontal wounding, and bone repair^{28,38,39}, even though senescence was not studied in such paradigms. On the other hand, aberrant activation of the BDNF-TrkB axis has been described in age-related pathologies such as lung fibrosis, sarcopenia, cancer, and kidney disease⁴⁰⁻⁴⁴. Interestingly, all these processes, from the developmental responses to the age-associated declines and diseases, have been linked to cellular senescence⁴⁵⁻⁵⁰. Thus, it will be important to determine if BDNF contributes to these paradigms through its actions on TrkB-expressing senescent cells.

In senescent cells, the activation of TrkB by BDNF increased progressively as senescence advanced (**Figs. 4, 5**). The fact that this signaling paradigm is active in cells facing sublethal levels of DNA damage, but not in cells exposed to higher levels of damage that do not trigger senescence and instead result in cell death (**Fig. 6**), supports the existence of a well-orchestrated molecular program to efficiently induce senescence within a specific range of injury. In this regard, in cells facing damage that might result in either senescence or death, BDNF appears to be a better predictor of a path to senescence than other, more established, senescence markers such as p21 or IL6, which are also strongly induced by acute DNA damage but are not always good indicators of senescence⁵¹⁻⁵³. To test this possibility, a comprehensive analysis of BDNF production in a wide range of senescence models is warranted. Furthermore, given that depleting BDNF levels in cells undergoing senescence drastically reduces viability (**Fig. 4, 5**), BDNF appears to function not only as a robust marker of cell survival, but also as a key effector of the anti-apoptotic program of senescence. In sum, the activation of BDNF-TrkB in senescent cells supports the notion that senescence is a developmental program set in motion by cell damage that shares previously unrecognized molecular similarities with other cell responses such as neuronal survival.

Interestingly, the discovery that the activation of TrkB by BDNF enhanced ERK5 signaling to increase the levels of BCL2L2 had additional parallels with the neuronal responses, as a similar, BCL2L2-dependent program is activated in neurons to persist within the nervous system²⁶. Together with the fact that *BCL2L2* mRNA was identified as a core senescence-associated protein elevated in multiple cellular senescence models²⁵, and the fact that the senolytic drug ABT-737, an inhibitor of BCL2L2, potently sensitizes senescent cells to apoptotic death^{7,8}, a prominent role for BCL2L2 in senescent cell survival is increasingly apparent. In further support for this notion, we recently found that BCL2L2, but not BCL2L1, contributed to sustaining cell viability in early senescence in a model of senescence that relied on the activation of the oncoprotein SRC (Anerillas et al., submitted).

A major caveat that must be overcome before the BDNF-TrkB paradigm can be exploited therapeutically is the fact that BDNF-TrkB activity is required to maintain a fully functional nervous system⁵⁴. Since Trk inhibition is used to treat chronic pain⁵⁵, various compounds have been developed that prevent adverse side effects within the central nervous system. Two out of the three drugs tested in this work, GNF 5837 and PF 06273340, are peripherally restricted Trk inhibitors, i.e., they cannot cross the blood-brain barrier^{12,56}, and thus could be exploited for removing senescent cells through BDNF-TrkB inhibition without impacting neuronal function. These compounds reduced senescence-associated markers in tissues such as kidney, lung, and liver in a mouse model of senescence triggered by old age (**Fig. 7**). Further exploration of the impact of GNF 5837 and PF 06273340 in mice is warranted, particularly in other senescence models, in order to determine if their beneficial actions are similar to those of other senolytics^{4,34}. Eventually, it will be essential to study if these or other TrkB inhibitors are viable strategies to reduce the burden of senescent cells in human conditions of dysfunction and disease.

In summary, TrkB inhibitors reduce senescent cells in aged mouse organs. In sum, we have identified a role for BDNF in promoting cell survival through the activation of TrkB, and propose that this signaling paradigm might be exploited therapeutically to lower senescent cells in older organs.

Methods

Cell culture and treatment. Human IMR-90 (ATCC), WI-38 (Coriell Institute), and BJ fibroblasts (ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco), Penn/Strep (Gibco), Sodium Pyruvate (Gibco), and non-essential amino acids (Gibco) in a 5% CO₂ incubator. Cells were maintained at low population doubling levels (PDL), between PDL15 and PDL25. Etoposide was added to the media as indicated every three days; control conditions included equivalent concentrations of vehicle (DMSO). The remaining drugs utilized were refreshed every 48 hours. The drugs used are listed (**Supplementary Table 1**).

Cells were transfected with RNAiMAX (Invitrogen) following the manufacturer's instructions. Briefly, cells at 50% confluency were transfected with ON-TARGETplus SMARTPool (Dharmacon) non-targeting siCtrl (Catalog ID: D-001810-10-05), siNTrk1 (Catalog ID: L-003159-00-0005), siNTrk2 (Catalog ID: L-003160-00-0005), siTP53 (Catalog ID: L-003329-00-0005), siRELA (Catalog ID: L-003533-00-0005), siBDNF (Catalog ID: L-017626-00-0005), or siSTAT3 (Catalog ID: L-003544-00-0005) siRNAs at a final concentration of 25 nM. Twenty-four hours later, additional treatments were initiated as indicated, and viability was assessed by direct cell counting and represented as the percentage of remaining cells compared to the number of cells present at the beginning of the experiment. All cell counts were performed manually by using ImageJ and performed in at least three independent replicates. From each replicate, three fields were randomly selected and counted.

RT-qPCR analysis. Samples (tissues or cells) were lysed in either Tri-Reagent (Invitrogen) or RLT buffer (Qiagen), and the lysate was processed with the QIAcube (Qiagen) to purify total RNA, which was then reverse-transcribed (RT) to create cDNA using Maxima reverse transcriptase (Thermo Fisher Scientific) and random hexamers. Real-time, quantitative (q)PCR analysis was then performed using SYBR Green mix (Kapa Biosystems), and the relative expression was determined by the $2^{-\Delta\Delta C_t}$ method and normalized to *ACTB* mRNA levels.

The primers used for human transcripts, each forward (F) and reverse (R) were:

GTTACGGTCGGAGGCCG and GTGAGAGTGGCGGGGTC for *p16/CDKN2A* mRNA;
 AGTCAGTTCCTTGTGGAGCC and CATGGGTTCTGACGGACAT for *p21/CDKN1A* mRNA;
 AGTGAGGAACAAGCCAGAGC and GTCAGGGGTGGTTATTGCAT for *IL6* mRNA;
 CTTCCAGCCGAGGTCCTT and CCCTGGACACCAACTATTGC for *TGFB1* mRNA;
 GATGGTGGCCTACCTGGAGA and AGAGCTGTGAACTCCGCCCA for *BCL2L2* mRNA;
 GGCTTGACATCATTGGCTGAC and CATTGGGCCGAACCTTTCTGGT for *BDNF* mRNA;
 GCAAGGCTGATAACGCTGAGGA and CCTGGGCATCAGCGGTCAATG for *NTF4* mRNA;
 CAAGCAGATGGTGGACGTTAAGG and TCGCAGCAGTTCGGTGTCCATT for *NTF3* mRNA;

ACCCGCAACATTACTGTGGACC and GACCTCGAAGTCCAGATCCTGA for *NGF* mRNA;
 CCGACACTGTGGTCATTGGCAT and CAGTTCTCGCTTCAGCACGATG for *NTRK3* mRNA;
 TCGTGGCATTTCGAGATTGG and TCGTCAGTTTGTTCGGGTAAA for *NTRK2* mRNA;
 CACTAACAGCACATCTGGAGACC and TGAGCACAAGGAGCAGCGTAGA for *NTRK1* mRNA;
 CATGTACGTTGCTATCCAGGC and CTCCTTAATGTCACGCACGAT for *ACTB* mRNA;
 GACCTCAACGCACAGTACGAG and AGGAGTCCCATGATGAGATTGT for *PUMA* mRNA;
 GAGCTGGTGGTTGACTTTCTC and TCCATCTCCGATTCAGTCCCT for *BCL2L1* mRNA;
 CCTCAGCATCTTATCCGAGTGG and TGGATGGTGGTACAGTCAGAGC for *TP53* mRNA;
 ATGTGGAGATCATTGAGCAGC and CCTGGTCCTGTGTAGCCATT for *RELA* mRNA; and
 CAGCAGCTTGACACACGGTA and AAACACCAAAGTGGCATGTGA for *STAT3* mRNA.

The primers used for mouse transcripts, each forward (F) and reverse (R) were:

CTGCAAGAGACTTCCATCCAG and AGTGGTATAGACAGGTCTGTTGG for *Il6* mRNA;
 GAAATGCCACCTTTTGACAGTG and TGGATGCTCTCATCAGGACAG for *Il1b* mRNA;
 CCCAACGCCCCGAAGT and GCAGAAGAGCTGCTACGTGAA for *p16/Cdkn2a* mRNA;
 TTCTTTGCAGCTCCTTCGTT and ATGGAGGGGAATACAGCCC for *Actb* mRNA;
 and TTGCCAGCAGAATAAAAGGTG and TTTGCTCCTGTGCGGAAC for *p21/CDKN1a* mRNA.

Caspase 3/7 activity. Apoptosis-related Caspase 3/7 activity was assayed by using the Caspase-Glo 3/7 Assay System (Promega). Briefly, Caspase-Glo® 3/7 solution was added directly to each well. The plate was then shaken vigorously for 30 sec and incubated at 25 °C in the dark for 30 to 180 min. Luminescence was then measured using a GloMax plate reader (Promega).

Immunofluorescence analysis in cultured cells. Cells were fixed in 4% PFA (paraformaldehyde) for 10 min and then permeabilized by incubating cells with 0.2 % TX-100 for 5 min at 25 °C, unless stated otherwise. After blocking with 10% goat serum for 1 h, primary antibodies were added in the same 10% goat serum buffer overnight at 4°C (phosphorylated STAT3 (Tyr705), 1:50, Cell Signaling 9145; BDNF, 1:40, Santa Cruz Biotechnology sc-65514, TrkB, 1:40, Santa Cruz Biotechnology sc-377218; DPP4, 1:100, Cell Signaling 67138).

Fluorescent signals were detected by adding fluorescent secondary antibodies (Invitrogen, Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 568; and Goat anti-Rabbit IgG (H+L), Superclonal Recombinant Secondary Antibody, Alexa Fluor 488) in 10% goat serum buffer (1:1000) for 1 h at 25 °C. Finally, DAPI was used to counterstain the nuclei; images were taken using a fluorescence microscope (BZ-X Analyzer, Keyence) and analyzed with ImageJ.

Western blot analysis. Protein extracts were obtained by lysing cells with a denaturing buffer containing 2% sodium dodecyl sulfate (SDS) (Sigma-Aldrich) in 50 mM HEPES. After boiling and

sonication, whole-cell protein extracts were separated by size through polyacrylamide gels and transferred to nitrocellulose membranes (Bio-Rad). Membranes were blocked with 5% non-fat dry milk and immunoblotted. Specific primary antibodies were used that recognized phosphorylated p38 MAPK (T180/Y182) (Biolegend, Ref. 903501), phosphorylated SAPK/JNK (T183/Y185) (81E11) (Cell Signaling, Ref. 4668S), phosphorylated ERK1/2 (T202/Y204) (Biolegend, 675502), phosphorylated AKT (Ser473) (Cell Signaling, 4060S), phosphorylated STAT3 (Y705) (D3A7) XP® (Cell Signaling, 9145S), p53 (DO-1) (Cell Signaling, 18032S), ACTB (β -Actin C4) (Santa Cruz Biotechnology, sc-47778), and BCL2L2/BCL-w (Cell Signaling, 2724S), phosphorylated PKC α / β II (Thr638/641) (Cell Signaling, 9375S), phosphorylated ERK5 (Thr218/Tyr220) (Cell Signaling, 3375), p21 (Santa Cruz Biotechnology, sc-53870), TrkA (Cell Signaling, 2510S), and TrkB (Santa Cruz Biotechnology, sc-136990). After incubation with the required secondary antibodies conjugated with horseradish peroxidase (HRP; Jackson Immunoresearch), the chemiluminescent signals were detected by using the Chemidoc system (Biorad).

Isolation of cell surface proteins. Cell surface proteins were isolated by using the Pierce™ Cell Surface Biotinylation and Isolation Kit (ThermoFisher Scientific, Catalog No. A44390). Briefly, cells were washed twice with PBS and cell surface proteins were labeled for 10 min at 25 °C with a solution of EZ-Link Sulfo-NHS-SS-Biotin, a membrane-impermeable biotinylation reagent, followed by several washes in TBS. Cells were then lysed with the detergent provided in the kit and the same amount of protein extract between different samples was used for the subsequent pulldown. The biotinylated proteins were captured by biotin-affinity purification with a NeutrAvidin™ Agarose Resin (Product No. 29200, ThermoFisher Scientific), followed by several washes with the Wash Buffer. Dithiothreitol (DTT) (10 mM) was used in the elution buffer to reduce the disulfide bonds in the biotin label and release the bound proteins.

Mice. All mouse work, including the import, housing, experimental procedures, and euthanasia, were approved by the Animal Care and Use Committee (ACUC) of National Institute on Aging (NIA). C57BL/6J Mice were provided standard chow *ad libitum* and maintained under a 12:12 h light/dark cycle. For the delivery of Trk inhibitors, 21-month-old mice were injected intraperitoneally for three months and euthanized at 24 months of age. The treatments were performed at the beginning of each month for 5 consecutive days, at the indicated doses (GNF 5837 at 20 mg/kg, PF 06273340 25 mg/kg).

Bioplex analysis of mouse serum. Mouse serum was collected, allowed to clot for 2 h at 25 °C, and centrifuged for 20 min at 2000 $\times$ g. Serum was removed and frozen at -80 °C. For the multiplex assay, serum was thawed and centrifuged at 16,000 $\times$ g for 4 min. A custom murine Luminex Assay kit was designed by R&D Biosystems to include the following analytes: GDF-15, PAI-1, and TIMP-1. Serum

was diluted 1:2 using Calibrator Diluent RD6-52 provided in the kit. Standards (provided with the kit), blanks and serum were incubated with the microparticle cocktail for 2 h at 25 °C, followed by incubation with a biotin-antibody cocktail for 1 h. A final incubation of 30 min with Streptavidin-PE was performed with shaking at 25 °C before running the plate on the Biorad Bioplex-200 Instrument. Each incubation was followed by three sets of washes with wash buffer (provided in the kit). Instrument settings were adjusted to the following: 50 µl sample volume, Bio-Plex MagPlex Beads (Magnetic), Double Discriminator Gates set at 8000 and 23,000, low RP1 target value for the CAL2 setting, 50 count/region. The results were analyzed with the Bio-Plex Manager software.

BrdU incorporation. Cells were incubated with (4 µg/ml) 5-Bromo-2'-deoxyuridine (BrdU) diluted in DMEM media with 10% FBS for 24 h. BrdU incorporation was measured following the manufacturer's protocol (Cell Signaling Technology). Briefly, cells were fixed and denatured prior to the addition of BrdU mouse mAb, and BrdU was detected using the GloMax plate reader (Promega).

Detection of SA-β-Gal. Senescence-associated β-Galactosidase (SA-β-Gal) activity was assessed following the manufacturer's instructions (Cell Signaling Technology). For the *in vivo* samples, dried slides were pre-washed with PBS plus 1 mM Magnesium Chloride before the addition of staining solution. All the slides were stained at once for 16 h at 37 °C. The resulting images were taken by a fluorescence microscope (BZ-X Analyzer, Keyence) and analyzed with ImageJ.

Tissue processing. To perform RT-qPCR analysis in tissue samples, organ pieces were flash-frozen in liquid nitrogen and preserved at -80 °C until required. To extract RNA, tissue samples were introduced in Tri-Reagent (Invitrogen) and disrupted using a tissue homogenizer (Bertin Instruments). RNA extraction was then carried out as indicated by the manufacturer's instructions.

For histological analysis, tissue biopsies were immediately fixed in 4% PFA in PBS at 4 °C overnight. The following day, tissues were cryoprotected in 30% sucrose solution in PBS at 4 °C overnight, and subsequently included in OCT embedding compound, and stored at -80 °C until needed. For tissue cutting, OCT blocks were processed using a cryostat at -20 °C to obtain 10-µm slices that were mounted onto Superfrost™ Plus Microscope Slides (Fisher Scientific) and dried out overnight at 25 °C. Before incubating with antibodies, antigen retrieval was performed with a sodium citrate-based buffer (Abcam) following the manufacturer's instructions. Tissue slides were then permeabilized with 0.2% TX-100 in PBS for 10 min, blocked with goat serum blocking solution (Biolegend) for 1 h, and incubated overnight with the antibodies. The antibodies used for immunofluorescence recognized BDNF [EPR1292] (ab108319) (1:100 dilution in blocking solution) and CDKN2A/p16INK4a [2D9A12] (ab54210) (1:250 dilution in blocking solution). Fluorescent secondary antibodies

(Invitrogen) were added in 10% goat serum for 1 h at 25 °C and slides were mounted with ProLong Diamond Antipode Mountant with DAPI (Life Technologies) and coverslips.

RNA-seq analysis. RNA was extracted with RLT buffer (Qiagen) and purified using the QIAcube system (Qiagen) using RNeasy plus. The quality and quantity of RNA were assessed using the Agilent RNA 6000 nano kit on the Agilent Bioanalyzer. High-quality RNA (125 ng) was used for sequencing the library prep using Illumina TruSeq Stranded mRNA Library prep kit following the manufacturer's protocol (Illumina, San Diego, CA). The quality and quantity of the libraries were checked using the Agilent DNA 1000 Screen Tape on the Agilent Tapestation. Paired-end sequencing was performed for 103 cycles with an Illumina NovaSeq sequencer. BCL files were de-multiplexed and converted to FASTQ files using bcl2fastq program (v2.20.0.422). FASTQ files were trimmed for adapter sequences using Cutadapt version v1.18 and aligned to human genome hg19 Ensembl v82 using STAR software v2.4.0j. featureCounts (v1.6.4) software was used to generate gene counts. The Bioconductor package DESeq2 version 1.30.0⁵⁷ in R (version 4.0.3) was used to compare counts levels; statistical testing was carried out with Wald test. Transcripts were considered differentially regulated if absolute log2 fold change was > 1 and the Benjamini–Hochberg adjusted p-value was < 0.05. Functional analysis of the differentially expressed genes was performed by using normalized counts in the GSEA platform⁵⁸. RNA-seq data are deposited in GSE190998 (reviewer token [crqygaculzcxnid](#); <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE190998>).

Statistical Analysis. Data are presented as the means ± standard deviation (S.D.) of at least n=3 independent experiments. Individual data points are displayed in all the bar plots. Significance was established using two-tailed Student's t test (*, p < 0.05, **, p < 0.01, ***, p < 0.001). All analyses were performed with Prism 9.

Figure Legends

Fig. 1 | Screening of a library of drugs directed at MAPK signaling proteins unveils Trk inhibitors as potential senolytic compounds. **a**, Overview of the experimental strategy of the survey. Briefly, senescent and proliferating WI-38 and BJ fibroblasts were treated for 48 h with a collection of MAPK inhibitors, whereupon cell viability was assessed. **b**, Schematic representation of the protocol used to reach full senescence using etoposide (Etop, 25 and 50 μ M for BJ and WI-38 cells, respectively) for 10 days, refreshing etoposide twice, before treating the cell cultures with each of the drugs in the library for an additional 48 h. **c**, Bar plot displaying the % change of proliferating and senescent WI-38 fibroblasts (top graph) and BJ fibroblasts (bottom graph) after treatment with the drug library for 48 h (day 12) relative to the time before drug addition (day 10). Red line, percentages of cells present at each condition right before starting the treatments at day 10. **d**, Heat map representing the percentage of senolysis (percentage of cell death) after 48 hours when comparing day 12 to day 10 in senescent cells. Drugs were grouped by the target proteins inhibited within the MAPK superfamily. Graphs in **c** represent the mean values $\pm$ SD of n=3 experiments. See also **Extended Data Fig. 1**.

Fig. 2 | Pharmacological inhibition of TrkB selectively induces apoptotic death of senescent cells. **a**, Cell viability curves obtained after testing a range of concentrations of Trk inhibitor drugs (GNF 5837, ANA 12, PF 06273340) for 48 h in either senescent or proliferating WI-38 fibroblasts. **b**, Representative micrographs of select doses for each drug tested in (**a**). Black bar, 100 μ m. **c,d**, Cell viability (**c**) and caspase 3/7 (**d**) activity were assessed 48 h after treatment as indicated in (**b**). **e**, Cell viability assessment in WI-38 cells that had reached replicative senescence (RS) and IR-induced senescence (IRIS), and then were treated for 48 h with the same doses of Trk inhibitors utilized in (**b**). **f,g**, Cell viability and caspase 3/7 activity were assessed in BJ and IMR-90 fibroblasts that had been rendered senescent by exposure to etoposide (Etop, 25 and 50 μ M for BJ and WI-38 cells, respectively) for 10 days, and then treated for 48 h with the same doses of Trk inhibitors utilized in (**b**). **h,i**, Assessments of cell viability (**h**) and caspase 3/7 (**i**) activity measured in cells rendered senescent by etoposide for 10 days, then treated for 48 h with different Trk inhibitors, either alone or in combination with the pan-caspase inhibitor Z-VAD-FMK. Graphs in **a**, **c-i**, represent the mean values $\pm$ 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. See also **Extended Data Fig. 2**.

Fig. 3 | Increased TrkB levels in senescent cells promotes resistance to apoptosis. **a**, RT-qPCR analysis of the levels of *NTRK1*, *NTRK2*, and *NTRK3* mRNAs (encoding Trk receptors) and senescence markers *p16* mRNA and *IL6* mRNA in WI-38 cells rendered senescent by treatment with etoposide (Etop, 50 μ M) for 10 days. Data were normalized to the levels of *GAPDH* mRNA. **b**, Representative

western blot analysis of the levels of TrkA, TrkB, senescence marker p21, and loading control ACTB, in WI-38, BJ, and IMR-90 fibroblasts that were proliferating (P) or were rendered senescent (S) by etoposide treatment for 10 days. WI-38 cells rendered senescent by replicative exhaustion (RS) were included in this analysis. **c**, Cells were transfected with control siRNA (siCtrl) or with siRNAs directed at TrkA and TrkB (siNTrk1 and siNTrk2, respectively), and the percentage of live cells were counted at the indicated times (0-10 days) after treatment with a dose of etoposide (50 μ M) that triggered senescence. **d**, RT-qPCR analysis of *NTRK1* and *NTRK2* mRNA levels after ten days in the presence of with 50 μ M etoposide, to assess the efficacy of the siRNA interventions. Data were normalized to the levels of *GAPDH* mRNA. **e**, Representative western blot analysis of the levels of TrkB, labile protein p53, and loading control ACTB in proliferating and senescent cells at the times shown after incubating the cells with CHX (50 μ g/ml). **f**, Representative western blot analysis of the levels of TrkB, senescence marker p21, and loading control ACTB. **g**, RT-qPCR analysis of the levels of *TGFB1* mRNA (an early senescence marker), *p16* mRNA (a late senescence marker), and *IL6* mRNA (a late senescence marker). Data were normalized to the levels of *GAPDH* mRNA. **h**, Pulldown of cell-surface proteins from WI-38 cells that were either proliferating (P) or rendered senescent (S) by etoposide treatment (50 μ M, 10 days). The presence of TrkB in the cell-surface pulldown and DPP4, a known senescent cell surface marker, was assessed by western blot analysis. Ponceau staining of the transferred samples served to assess differences in sample loading and transfer. **i**, Immunofluorescence micrographs to identify cells positive for TrkB (red), DPP4 (green), and merged signals (orange) in proliferating and senescent cells (etoposide 50 μ M, 10 d) in non-permeabilizing conditions. White scale bar, 100 μ m. Graphs (**a,c,d,g**) display the mean values $\pm$ 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. See also **Extended Data Fig. 3**.

Fig. 4 | BDNF is a novel SASP member that ensures viability in senescent cells. **a**, Heat map display of the relative abundance of mRNAs encoding the different neurotrophins in both proliferating and senescent WI-38, BJ, or IMR-90 fibroblasts (50, 25, and 50 μ M, respectively, 10 days), as assessed by RT-qPCR analysis. **b**, ELISA measurement of BDNF levels in conditioned media collected for 48 h in the same experimental groups shown in (**a**). **c**, Cytokine array analysis of the abundance of several SASP factors, including neurotrophins BDNF, NTF3, and NTF4, in proliferating and senescent (Etop, 50 μ M, 10 days) WI-38 cells. **d**, Immunofluorescent micrographs of BDNF signals in WI-38 cells processed as in (**c**). Scale bar, 100 μ m. **e**, RT-qPCR analysis of *BDNF* mRNA levels at the times shown as WI-38 cells progress towards senescence. Data were normalized to *GAPDH* mRNA levels. **f**, RT-qPCR analysis of the levels of *BDNF*, *p21*, *IL6*, and *p16* mRNAs (as well as the levels of *TP53* and *RELA* mRNAs, to monitor the silencing interventions) in senescent cells transfected with either control siRNA (siCtrl) or siRNAs that specifically lowered the abundance of p53 or RELA. Proliferating cells

transfected with siCtrl were utilized as a control for the baseline expression of each of the mRNAs analyzed. **g**, Percentage of WI-38 fibroblasts relative to the starting cell numbers after transfecting them with siCtrl or siBDNF and then inducing senescence (50 μ M Etop). Cell numbers were measured by direct cell counting at each of time point. **h**, RT-qPCR analysis of *BDNF* mRNA levels in the cells studied in (**g**) at day 10. **i**, Number of remaining cells after 48 h of treatment with either IgG or BDNF-blocking antibodies (4 μ g/ml each) in P and S WI-38 fibroblasts treated as in (**g**), represented as the percentage of live cells relative to initial cell counts. **j**, Representative micrograph of the experiments plotted in (**i**), only for S cells. Scale bar, 100 μ m. **k**, Caspase 3/7 activity analysis performed in senescent WI-38 fibroblasts treated for 48 h with either IgG or BDNF-blocking antibodies as described in (**i**). Graphs (**b,e-i,k**) represent the mean values $\pm$ 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. See also **Extended Data Fig. 4**.

Fig. 5 | ERK5 activation by TrkB-BDNF axis sustains senescent cell survival through BCL2L2. **a**, Western blot analysis performed with PhosTag gels to assess TrkB phosphorylation across the senescence time course (*left*), as well as in cells transfected to express normal (siCtrl) or reduced (siBDNF) levels of BDNF (*right*). ACTB was used as a loading control. **b**, WI-38 fibroblasts were transfected with siCtrl, siNTrk2, and siBDNF and treated with 50 μ M etoposide (Etop) the next day to induce senescence. Cell viability was then measured by direct cell counting at early senescence (2 days after adding etoposide) and late senescence (8 days after adding etoposide). **c**, Schematic of the experimental procedure followed for RNA-seq analysis to evaluate transcriptomic differences among WI-38 fibroblasts transfected with siCtrl, siNTrk2, and siBDNF. **d**, Transcriptomic analysis of WI-38 cells in which NTrk2 or BDNF were silenced. Shown are GSEA gene set associations with the stated conditions (*left*), and a heat map depicting genes related to apoptosis that changed significantly in senescent cells after silencing TrkB as well as in senescent cells after silencing BDNF (*right*). **e**, RT-qPCR analysis of the levels of *PUMA*, *BCL2L2*, and *BCL2L1* mRNAs in early or late senescent WI-38 cells transfected with siCtrl, siNTrk2 or siBDNF, as defined in (**b**). **f**, RT-qPCR analysis of the levels of *PUMA*, *BCL2L2*, and *BCL2L1* mRNAs in proliferating and senescent cells treated with either IgG or BDNF-blocking (4 μ g/ml each) antibodies for 48 h. **g**, Representative western blot analysis of the levels of BCLW (BCL2L2), p53, and loading control ACTB in cells treated as described in (**e,f**). **h**, Representative western blot analysis of known effector proteins downstream of BDNF-TrkB in other cellular systems, including p-AKT (S473), p-ERK1/2 (T202/Y204), p-p38 (T180/Y182), p-JNK (T183/Y185), p-STAT3 (Y705), p-PKC α/β II (T638/641), and p-ERK5 (T218/Y220). Proteins were assessed in cells that were processed as explained in (**e,f**). **i**, RT-qPCR analysis of the levels of *BCL2L2*, *PUMA*, and *BCL2L1* mRNAs in senescent WI-38 cells (50 μ M etoposide, 10 days) that were either left untreated or treated with an ERK5 inhibitor (ERK5i, ERK5-IN-1) for 48 h at 1 μ M. **j**, WI-38

fibroblasts were treated with the ERK5i as described in (i), and cell viability was measured by direct cell counting of the remaining viable cells. Graphs (b,e,f,i,j), represent the values $\pm$ 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. See also **Extended Data Fig. 5**.

Fig. 6 | BDNF is a marker of surviving senescent cells. a,b, WI-38 fibroblasts were treated with the doses of etoposide (Etop) shown; 48 h later, cell viability was assessed by direct cell counts and represented as percentages referred to initial counts (the red dashed line) (a), and RT-qPCR analysis was used to measure the levels of *BDNF*, *p21*, and *IL6* mRNAs (normalized to *GAPDH* mRNA) (b). c, Assessment of *BDNF* mRNA levels in WI-38 cells treated with etoposide 50 μ M for 48 h after silencing p53 (siTP53; siCtrl in control transfections) or inducing p53 levels (by treatment with Nutlin-3a, N3A, 10 μ M). Controls included siCtrl-transfected and untreated WI-38 cells, respectively. d, RT-qPCR analysis of *BDNF* mRNA levels in WI-38 cells transfected with either siCtrl or siSTAT3 siRNAs and treated for 48 h with 50 μ M etoposide. e, Representative western blot analysis of p-STAT3 (Y705) and p53 protein levels in WI-38 cells treated as explained in (c). f, Representative western blot analysis of p-STAT3 (Y705) and p53 protein levels in the experimental groups shown in (a). g,h, Representative immunofluorescence analysis of colocalized signals for p-STAT3 (Y705) (green) and BDNF (red). DAPI was used to stain nuclei. Co-staining for p-STAT3 and BDNF appears as an orange signal (orange arrows). Red arrows point to BDNF-only positive cells. White scale bar, 100 μ m. Signals from the experiment in (g) were quantified (h). Fractions in color represent the percentage $\pm$ SD of the resulting staining for each group described: unstained, BDNF+, p-STAT3/BDNF+, and p-STAT3+. i, Schematic depicting the proposed model described in this study. Values shown in a-d are the means $\pm$ SD; significance (*p < 0.05, **p < 0.01, ***p < 0.001) was determined by using two-tailed Student's t-test. See also **Extended Data Fig. 6**.

Fig. 7 | Treatment with TrkB inhibitors reduces senescent cells in organs from old mice. a, Immunofluorescence staining of BDNF (green) and p16 (red) in lung and liver from 24-month old (m.o.) C57BL/6J mice. b, Schedule of administration of TrkB inhibitors GNF 5837 and PF 06273340 to normally aging mice. c, RT-qPCR analysis of the abundance of *p16*, *p21*, *Il6*, and *Il1b* mRNAs in kidney, lung and liver in the different mouse cohorts studied. The levels of these mRNAs were normalized to *Actb* mRNA levels. d, GDF15 concentration in sera from the four mouse cohorts, as measured with a Bioplex instrument. e,f, Percentages of p16-positive cells in kidney, lung, and liver of the four mouse cohorts (e); representative images of p16-positive cells found in kidney in the different groups tested (f). g,h, Representative images (g) and quantification (h) of SA- β -Gal activity assessment performed in the same four mouse cohorts described in (c). Scale bars, 200 μ m. Values shown in c-e,h

are individual data points with the values $\pm$ SD; significance (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) was determined by using two-tailed Student's t-test. See also **Extended Data Fig. 7**.

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a **b** **c** **d** **e** **f** **g** **h**

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