Standard Treatment against paediatric BRAF-V600E Glioma Promotes Senescence and Sensitizes Tumours to BCL-xL inhibition

Supplementary Methods

BT-40 cell line culture

BT-40 cells represent a uniquely relevant human model of paediatric low-grade glioma, carrying BRAF-V600E together with CDKN2A/B loss, a combination linked to aggressive behaviour and therapeutic vulnerability to MAPK pathway inhibition. Notably, BT-40 has been extensively used to identify and validate MAPK-targeted therapies now employed clinically. Given the scarcity of robust, genetically appropriate pLGG cell lines, BT-40 provides a clinically faithful and translationally meaningful platform for this study. BT-40 cells (kind gift of Dr. P. Houghton, University of Texas) were grown in RPMI 1640 Medium, GlutaMAX™ Supplement (Gibco, catalogue no. 61870010) supplemented with 10% fetal bovine serum (Fetal Bovine Serum, qualified, Brazil, Gibco, catalogue no. 10270106, batch 2166437). Cells were routinely confirmed mycoplasma negative using the MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza, catalogue no. LT07-710) and STR profiled.

H9-derived human neural stem cell (hNSC) line culture

H9-derived human neural stem cells (hNSCs; Gibco - N7800) are a well-characterized neural stem cell line derived from the H9 (WA09) embryonic stem cell line through directed neural induction. These lines exhibit stable self-renewal and multipotency with the capacity to differentiate into major neural

lineages including neuronal-, astrocyte-, and oligodendrocyte-lineage cells under appropriate conditions. Furthermore, when stably transduced to constitutively express BRAF-V600E (H9 BRAF-V600E) cells become immortalized and growth factor independent. H9-derived hNSCs (Gibco - N7800) where grown in tumour stem media. Both wildtype H9 hNSCs and H9 BRAF-V600E cells were cultured as adherent layer (2D) on GelTrex-coated (Thermo Fisher) tissue culture-treated plates in tumour stem media (TSM) with and without growth factors, respectively. 1L media includes 475 ml Neurobasal-A (Invitrogen – 10888-022), 475 ml DMEM F-12 supplemented with HEPES (Invitrogen – 11330-032), 10 ml MEM non-essential amino acids solution (Invitrogen– 11140-050), 10 ml MEM sodium pyruvate (Invitrogen – 11360-070, 10 ml HEPES (Invitrogen– 15630-080), GlutaMAX-I (Invitrogen – 35050-061), and 10 ml pen-strep (Invitrogen – 15240-096). All H9 cells were grown in 1x B27 Supplement minus vitamin A (Invitrogen – 12587-010). H9 WT cells were grown with 20 ng/ml H-EGF (StemCell Tech – 78006), 20 ng/ml H-FGF (StemCell Tech – 78003), and 2ug/ml Heparin 0.2% (StemCell Tech – 07980). Cells were routinely confirmed mycoplasma negative using the MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza, catalogue no. LT07-710) and STR profiled.

Drugs for in vitro and in vivo testing

Dabrafenib (MedChemExpress [MCE] - HY-14660 ; Selleck – S2807), Trametinib (ApexBio - A3018; Selleck – S2673), Vinblastine (Santa Cruz Biotechnology - sc-491749; Selleck – S4505), Navitoclax (ABT-262; ApexBio - A3007), A-1155463 (MCE - HY-19725), A-1331852 (MCE - HY-19741), Obatoclax (MCE - HY-10969), Venetoclax (ABT-199; ApexBio - A8194), Piperlongumine (ApexBio - A4455), Digoxin (ApexBio - B7684).

For in vitro use, drugs were dissolved in DMSO (Santa Cruz Biotechnology - sc-202581) and stored in aliquots at −20°C. Drugs were diluted in cell culture medium and added to the cells for the durations and concentrations indicated.

For in vivo experiment, Navitoclax powder was weighed, individual aliquots were prepared. Prior to administration, vehicle (ethanol:polyethylene glycol 400:Phosal 50 PG) was added and drug was sonicated on ice into solution using Diagenode Bioruptor®, high frequency, 30 second intervals, for 15 minutes. Navitoclax was administered to mice by gavage at 50 mg per kg body weight per day (mg/kg/d) for 5 days for two cycles. Trametinib was dissolved in a DMSO 20mg/mL stock solution, individual aliquots were prepared and stored at -20°C. Prior to administration, Trametinib stock solution was diluted 1:100 in vehicle (PBS 10%w/v (2-Hydroxypropyl)-β-cyclodextrine – Sigma Aldrich C0926). Trametinib was administered to mice by I.P. injections at 1 mg/kg/d.

Generation of EGFP-LucF-BT-40 cells

For viral particles production, 293FT cell line (Invitrogen) were transfected with the plasmids pSLIEW, pMD2.G and psPAX2 (Addgene plasmids 12259 and 12260) using Lipofectamine™ 2000 Transfection Reagent (Invitrogen). For cell transduction, BT-40 cells were seeded in 24 well plates, and transduced at 70% confluence. Medium was removed by aspiration and replaced with fresh complete medium containing 5 µg/ml polybrene (Millipore). Concentrated viral supernatant was added to achieve a multiplicity of infection (MOI) of 10. After 18 hours, the medium was replaced with fresh complete medium to remove lentivirus and polybrene. Transduced cells were expanded and sorted using flow cytometry assisted cell sorting (FACS), based on EGFP fluorescence. Sorted cells were expanded and stored in liquid nitrogen.

Intracranial cell injections

A single-cell suspension (6×10^5 EGFP-LucF-BT-40) was prepared immediately before implantation in 6 weeks old NOD-SCID-gamma female mice (Charles River UK). For intracranial procedures, animals were anesthetised with isoflurane. The cranium was exposed via midline incision under aseptic conditions and 1×1mm deep hole was made through the skull to the dura. Mice were placed in a stereotactic apparatus and 60,000 cells in 2 µL were stereotactically implanted into the right striatum area using a 10µL 26G Hamilton syringe at a rate of ~ 1 µL/min. Coordinates used were 1.5mm lateral to midline, 1.5mm posterior to bregma, and 4 mm deep to cranial surface (XYZ coordinate from bregma: -1.5mm; -1.5mm; 3+1mm). A small pocket was created for the cells, which were injected 3 mm deep to the cranial surface. At the completion of infusion, the syringe needle was allowed to remain in place for a minimum of 2 minutes, then slowly manually withdrawn to minimize backflow of the injected cell suspension. The skin was closed with VetBond™ Adhesive. Mice were weighed daily for one week, and dosed for 48 hours with Metacam, at 5 mg/kg.

Bioluminescence imaging (Firefly D-Luciferin, s.c 150mg/kg – PerkinElmer; IVIS Lumina III In Vivo Imaging System - PerkinElmer) was taken at day 12 after implantation and then every four days to monitor intracranial tumour growth. The mice were sacrificed when they exhibited weight loss >15% body weight or neurological symptoms. Brightfield and GFP of whole-mount brains were assessed with Leica M205 FA Fluorescence stereo microscope.

bulkRNA sequencing on BT-40 cells

For in vitro experiment, total RNA was extracted using RNeasy Mini kit (Qiagen). Library preparation was performed with KAPA mRNA HyperPrep kit. Sequencing was then performed on an Illumina with NextSeq2000 100cycles P2 kit. Sequencing data was then aligned with {STAR 2.7.11b}. Raw and processed data are available on GEO repository GSE294664. Secondary analysis was performed using {R 4.3.3} on RStudio (2023.06.0+421). Differential expression was performed using {DESeq2 1.38.1}, complete pipeline is available at https://gitlab.univ-nantes.fr/guihomics/plgg_tis_rnaseq. GSEA performed using the GSEA software (v4.3.2 - UC San Diego and Broad Institute). Gene sets used in this study were either obtained from public databases: gene ontology (GO), KEGG, REACTOME, molecular signatures database (Broad Institute); or published literature.

bulkRNA sequencing on BT-40 xenografts

Once tumours were established, the mice received either Vehicle or Trametinib treatment during one week. Tumour bearing brain were dissected into 1 mm3 pieces and dissociated in a solution of 0.5% w/v collagenase type 2 (Lorne Laboratories Ltd.), 0.1x Trypsin (Gibco), 50 µg/ml DNaseI (Worthington) and 2.5 µg/ml fungizone (Gibco) in Hank's Balanced Salt Solution (HBSS (Gibco) for 45 minutes at 37°C. Tissue fragments were further dissociated into single cells through mechanical trituration using a pipette and filtration through a 70 µm filter. Single cells were washed in HBSS and suspended in FACS media (PBS supplemented with 1% foetal calf serum (PAA), 25 mM HEPES and 10 µg/ml of 4',6-diamidino-2-phenylindole (DAPI, BD Pharmingen) for live/dead cell discrimination) before being analysed using a BD FACS AriaIII Flow Cytometer. GFP+ cells were collected into 4°C RLT lysis buffer and total RNA was isolated (Qiagen RNeasy Micro Kit). Total RNA was then processed for bulk RNA-sequencing analysis. Isolated RNA was processed by the SMARTer V4 low input assay kit (Clontech) to generate amplified cDNA using the strand-switching protocol. Library preparation was performed with 200 pg of amplified cDNA using the NEBNext Low Input RNA kit with 12 cycles of PCR. Sequencing was then performed on an Illumina NextSeq 2000 100cycles with 100 bp single-end reads. To remove potential contamination of host cells from the analysis, sequencing data was aligned with {STAR 2.7.11b} on both human and mouse annotated genomes (Ensembl genome 113). {XenofilteR 1.6} package (Kluin RJC, Kemper K, Kuilman T, et al. XenofilteR: computational deconvolution of mouse and human reads in tumor xenograft sequence data. *BMC Bioinformatics*. 2018;19(1):366. doi:10.1186/s12859-018-2353-5) was used to filtered out murine host reads from human graft reads. Raw and processed data are available on GEO repository GSE296000. Secondary analysis was performed using {R 4.3.3} on RStudio (2023.06.0+421). Differential expression was performed using

{DESeq2 1.38.1}, complete pipeline is available at https://gitlab.univ-nantes.fr/guihomics/plgg_tis_rnaseq. GSEA performed using the GSEA software (v4.3.2 - UC San Diego and Broad Institute). Gene sets used in this study were either obtained from public databases (gene ontology (GO), KEGG, REACTOME, molecular signatures database (Broad Institute) or published literature: senescence profiling and SASP.

Cell viability, drugs combinatorial effects and apoptosis for BT-40 experiment

Two thousand cells per well were seeded into 96-well plates and cultured for 72 h in the presence of increasing concentrations of drugs as indicated. Cell confluency was monitored by taking three brightfield pictures per well twice a week for 5 weeks. The BT-40 cells growing in large spindle-shaped clusters, confluency is represented as a ratio of measured covered cell area vs. maximum covered area. Cell viability was determined by CellTiter-Glo One Solution Assay (Promega) on a FLUOstar OPTIMA plate reader (BMG Labtech) 72h after treatment. IC50 were calculated using GraphPad Prism v9.4.0 software modelization. Drug sensitivity scores (DSS) were estimated using Breeze-pipeline.

For apoptosis assessment, 2,000 cells per well were seeded into 96-well plates and cultured for 24 h in the presence of 100nM Vinblastine, Dabrafenib or Trametinib as indicated, followed by a wash out period of 6 days. At day 7, half of the plates were fixed with PFA 4%, stain with DAPI, scanned on Zeiss Observer 7 colour fluorescence and nuclei were counted with Fiji software 1.53. On the other half, Navitoclax (0, 0.1 or 1µM) was added to the pre-treated cells for 24h. Caspase 3/7 activity was determined using 100µl Caspase-Glo® 3/7 Assay System (Promega G8090), which was added directly to the cells in 96-well plates and incubated for 1 hour before recording luminescence on a Microplate luminometer (GloMax® Navigator – Promega). For BT-40 experiments, the "no cell" blank control value has been subtracted from each, and results were normalized by DAPI-positive nuclei counted with the same treatments conditions. For H9 hNSC experiments, cell apoptosis was normalized to DMSO-treated cells.

In vitro histochemistry and immunofluorescence for BT-40 experiments

EdU incorporation staining was performed using Click-iT EdU Cell Proliferation Kit (Invitrogen) following manufacturer´s instructions. Senescence-associated (SA)-β-galactosidase staining was performed using the Senescence Cells Histochemical Staining Kit (CS0030 - Sigma-Aldrich) following manufacturers´ instructions. Immunostaining of adherent cells was performed after 10min fixation with Formalin 4% (v/v), permeabilization with 0.1% Triton X-100 and blocking with 2% BSA. Primary antibodies were

incubated for 2 hours at RT before fluorescent dye–labelled secondary antibody incubation for 45min. DAPI was used as counterstain. The list of the antibodies used is presented in **Supplementary Table S2**. Imaging was performed on Zeiss Observer 7 colour fluorescence, with Hamamatsu Flash 4.0v3 camera, using Zen Blue software v3.7. Analysis was performed with Fiji software 1.53t36.

Cell viability, SA-β-galactosidase assay and apoptosis for H9 hNSC experiments

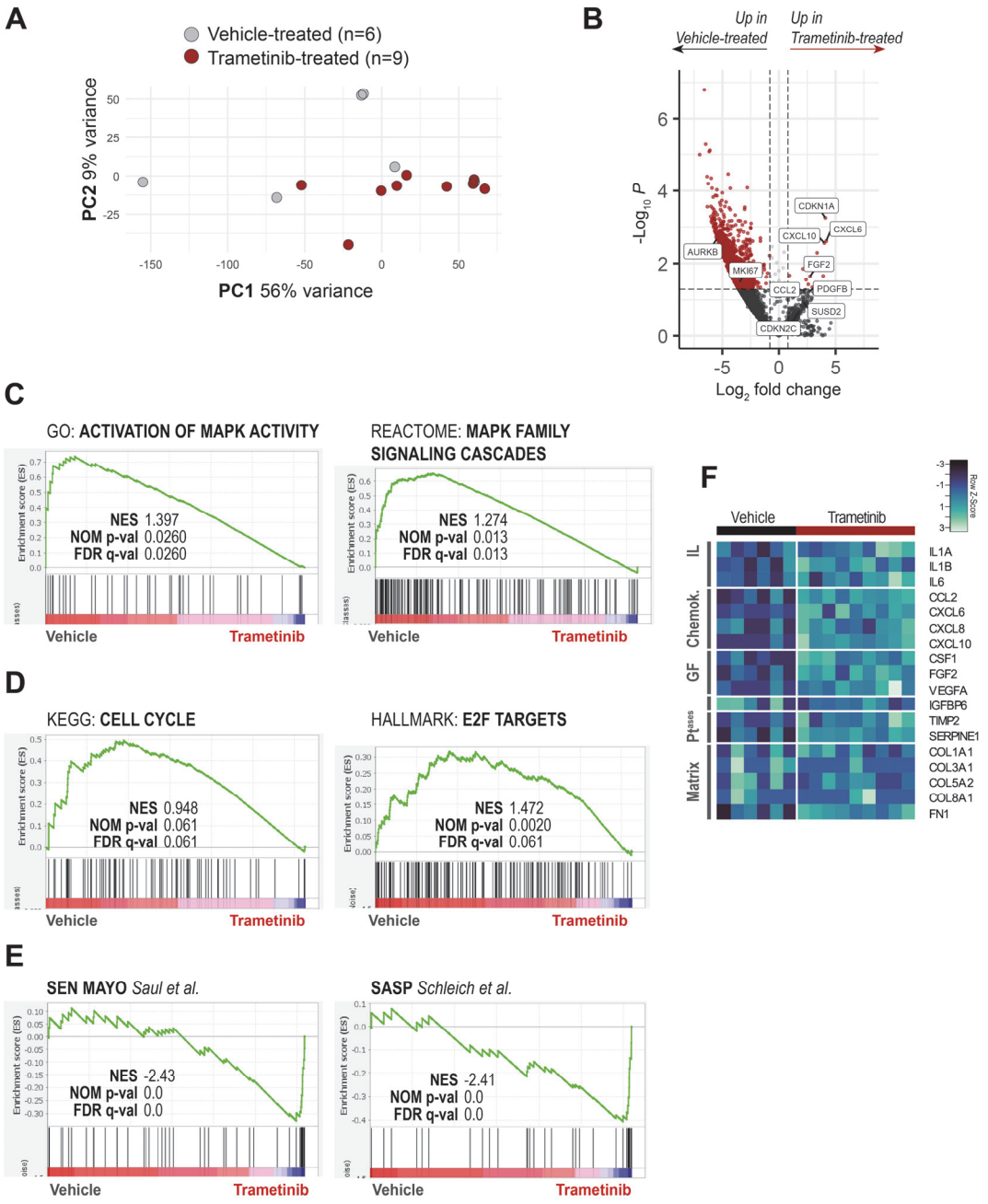
For H9 hNSC experiments, cell confluency was monitored using Incucyte (Sartorius) real-time live-cell imaging by taking the average confluency of nine brightfield pictures per well every six hours over six days.

Senescence-associated (SA)-β-galactosidase staining was performed using the Senescence β-galactosidase Staining Kit (Cell Signaling - 9860) following manufacturers´ instructions.

Cell viability was determined by CellTiter-Glo One Solution Assay (Promega) on a SpectraMax M5 plate reader 72h after treatment. IC50 were calculated using GraphPad Prism v9.4.0 software modelization.

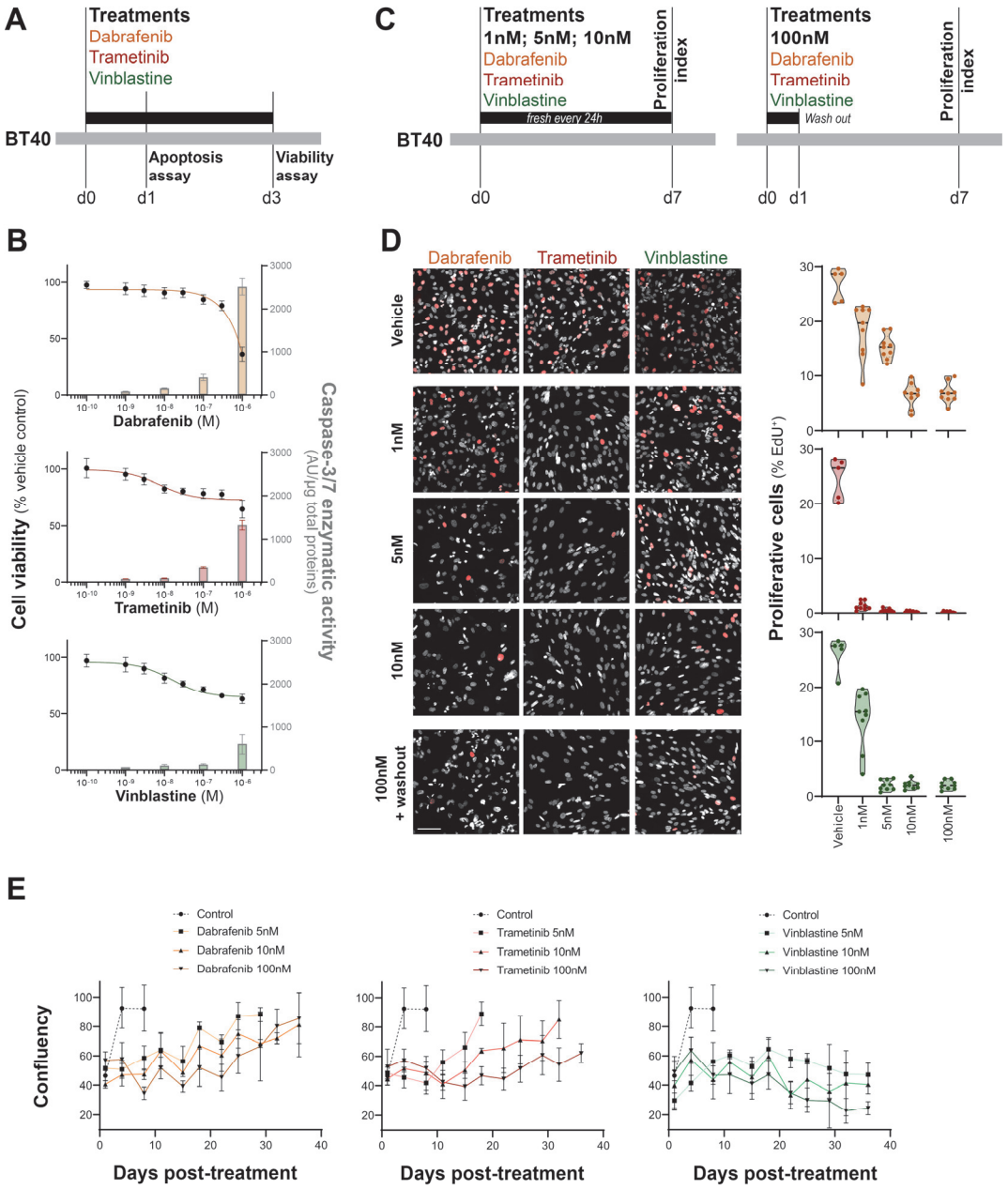
Supplementary Figures

Supplementary Figure S1



Supplementary Figure S1. Transcriptomic analysis of BT-40 orthotopic xenograft tumours treated with the MAPK inhibitor Trametinib confirms MAPK pathway inhibition and cell cycle arrest, while revealing the induction of senescence-associated features. (A) Principal component analysis plot of the 6 Vehicle-treated (grey) and the 9 Trametinib-treated (red) samples based on single-end RNAseq of flow-sorted BT-40 tumour cells. (B) Volcano plot of differentially expressed genes identified between vehicle and Trametinib-treated group. Thresholds used: p-values < 0.05 and log2 FoldChange > 1. (C-E) Geneset enrichment analysis suggest the inactivation of MAPK pathway (C), the decrease in proliferative function (D) and the enrichment for senescence and senescence-associated secretory phenotype (E) in Trametinib-treated tumour cells. (F) Heatmap highlighting the diversity of genes coding for secretory and matrix proteins associated with SASP overexpressed in the Trametinib-treated samples.

Supplementary Figure S2

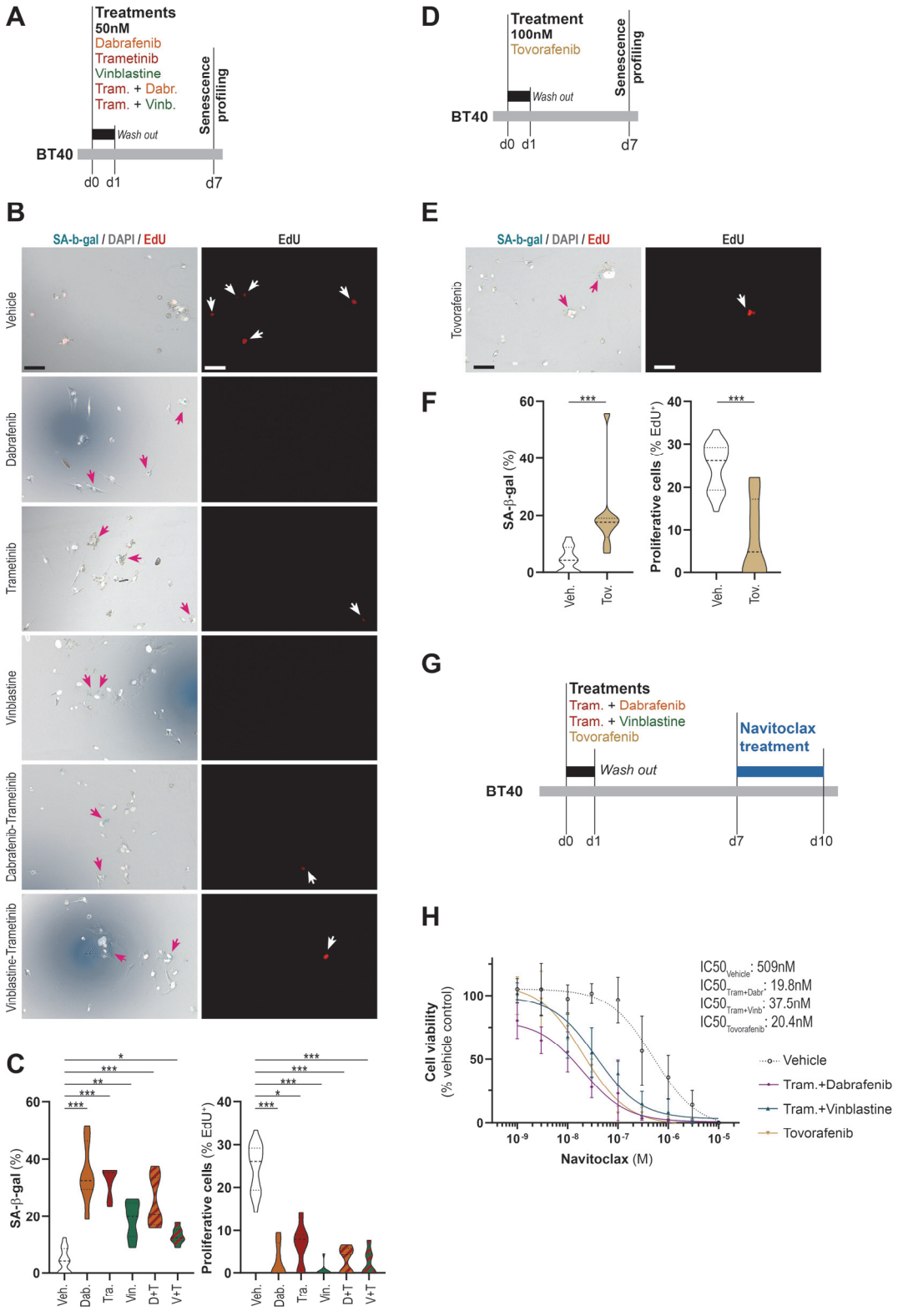


Supplementary Figure S2. In vitro treatment with MAPK inhibitors or Vinblastine induces sustained proliferation arrest in BT-40 tumour cells.

(A) A schematic of treatments and analysis kinetic for short term effect of a range of concentrations. (B) BT-40 cell viability assessed by ATP production at day 3 (line) and apoptosis assessed by caspase 3/7 activity at day 1 (bar) in the presence of increasing concentrations of Trametinib, Dabrafenib or Vinblastine. (C) A schematic of treatments and analysis kinetic for long term effect of a chronic treatment of 1, 5 or 10nM of the drugs or a 24h pulse of 100nM on BT-40 proliferation index. (D) EDU

accumulation overnight observed by Click-iT kit indicating proliferation of the treated cells at Day 7. Quantification of % of positive nuclei on the right. (E) Assessment of confluency overtime showing an overcoming of cell proliferation arrest 2 to 3 weeks post MAPK inhibitors treatment whereas vinblastine-treated cells remain non-proliferative for more than 5 weeks. Shown are mean ± SEM of three independent experiments.

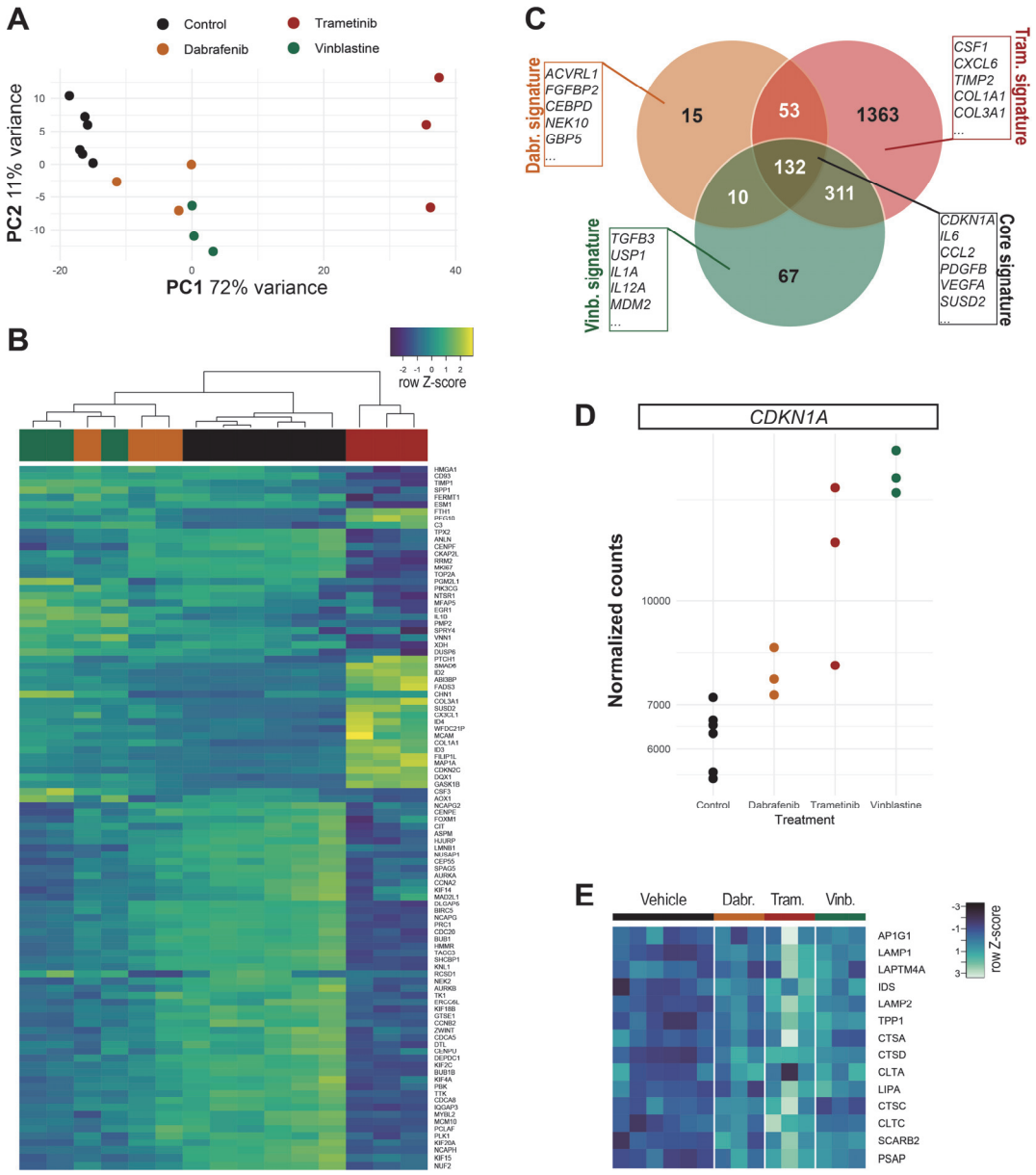
Supplementary Figure S3



Supplementary Figure S3. Dabrafenib, Trametinib, Vinblastine, Trametinib-based combinations and Tovorafenib induce sustained proliferation arrest and SA-β-gal activity in BT-40 tumour cells and sensitise treatment-induced senescent cells to Navitoclax.

(A) Schematic of the treatment and analysis timeline for assessing the long-term effects of a 24 h pulse treatment with the indicated drugs (50 nM) on senescence-associated readouts in BT-40 cells. (B) At day 7, BT-40 cells show increased SA-β-gal staining and reduced EdU incorporation following treatment with all drugs and combinations, whereas vehicle-treated cells did not. (C) Quantification of the SA-β-Gal and EdU positive cells. Kruskal-Wallis test, Dunn's post-test; *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$. (D) Schematic of the Tovorafenib treatment and analysis timeline to assess the long-term effects of a 24 h pulse treatment with 100 nM Tovorafenib on senescence-associated readouts in BT-40 cells. (E) At day 7, BT-40 show increased SA-β-gal staining and reduced EdU incorporation following Tovorafenib treatment. (F) Quantification of SA-β-Gal and EdU-positive cells. Mann-Whitney test; ***: $p<0.001$. (G) Schematic of the experimental design. Cells were treated with the indicated drugs for 24 h, followed by a 6-day washout period in the absence of drug. At day 7, vehicle-treated proliferating cells and treatment-induced senescent BT-40 cells were treated with navitoclax for 3 days. (H) Cell viability, assessed by ATP production, of treatment-induced senescent BT-40 cells (Tovorafenib, gold; Trametinib + Dabrafenib, pink; Trametinib + Vinblastine, green) or proliferating BT-40 cells (dotted black) following 72 h treatment with Navitoclax. IC50 values were calculated from cell viability measurements.

Supplementary Figure S4

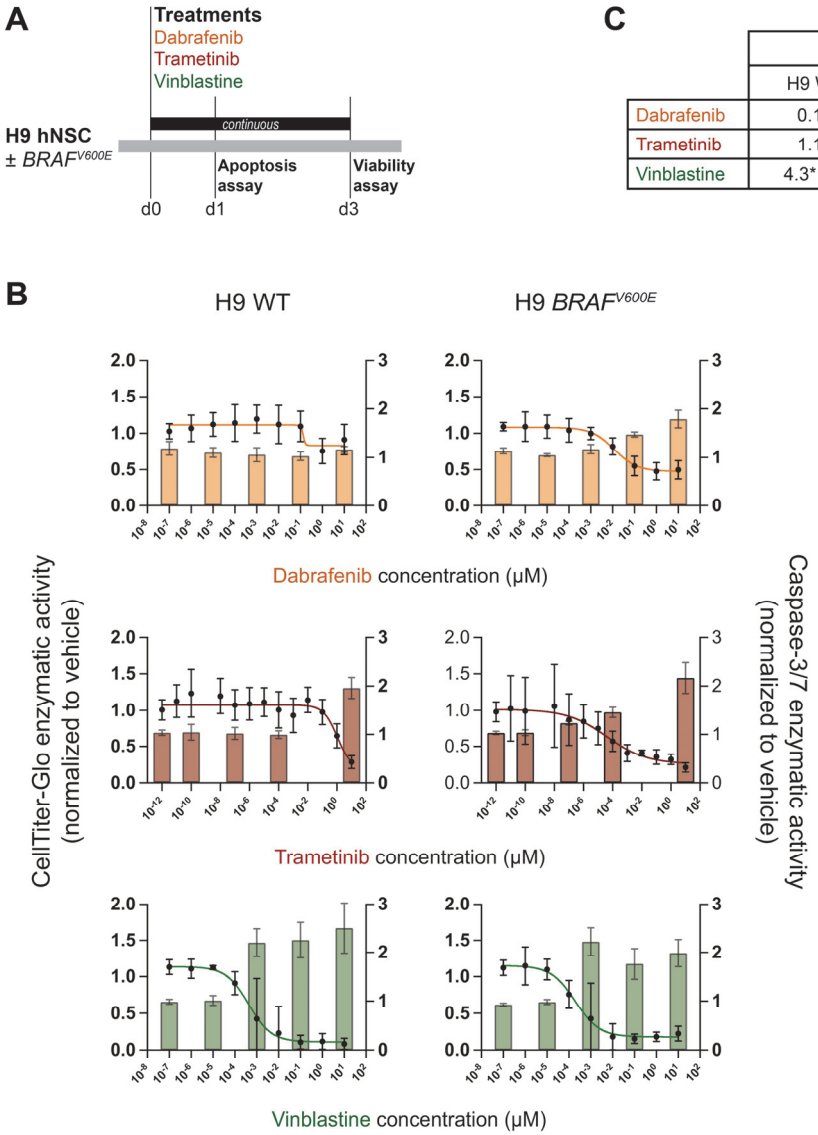


Supplementary Figure S4. Transcriptomic analysis by RNAseq of Treatment-induced senescent BT-40 cells reveals both shared and drug-specific gene expression signatures.

(A) Principal component analysis plot of the 6 proliferative controls (black), the 3 Trametinib-induced senescent (red), the 3 Dabrafenib-induced senescent (orange) and the 3 Vinblastine-induced senescent (green) samples based on RNAseq of BT-40 tumour cells. (B) Heatmap of the 500 most variable genes enabling segregation between the different conditions. (C) Venn-diagram of the number of differentially expressed genes identified between proliferative and treatment-induce senescent state, classified according to inducing treatment, revealing core and treatment-specific gene signatures. (D)

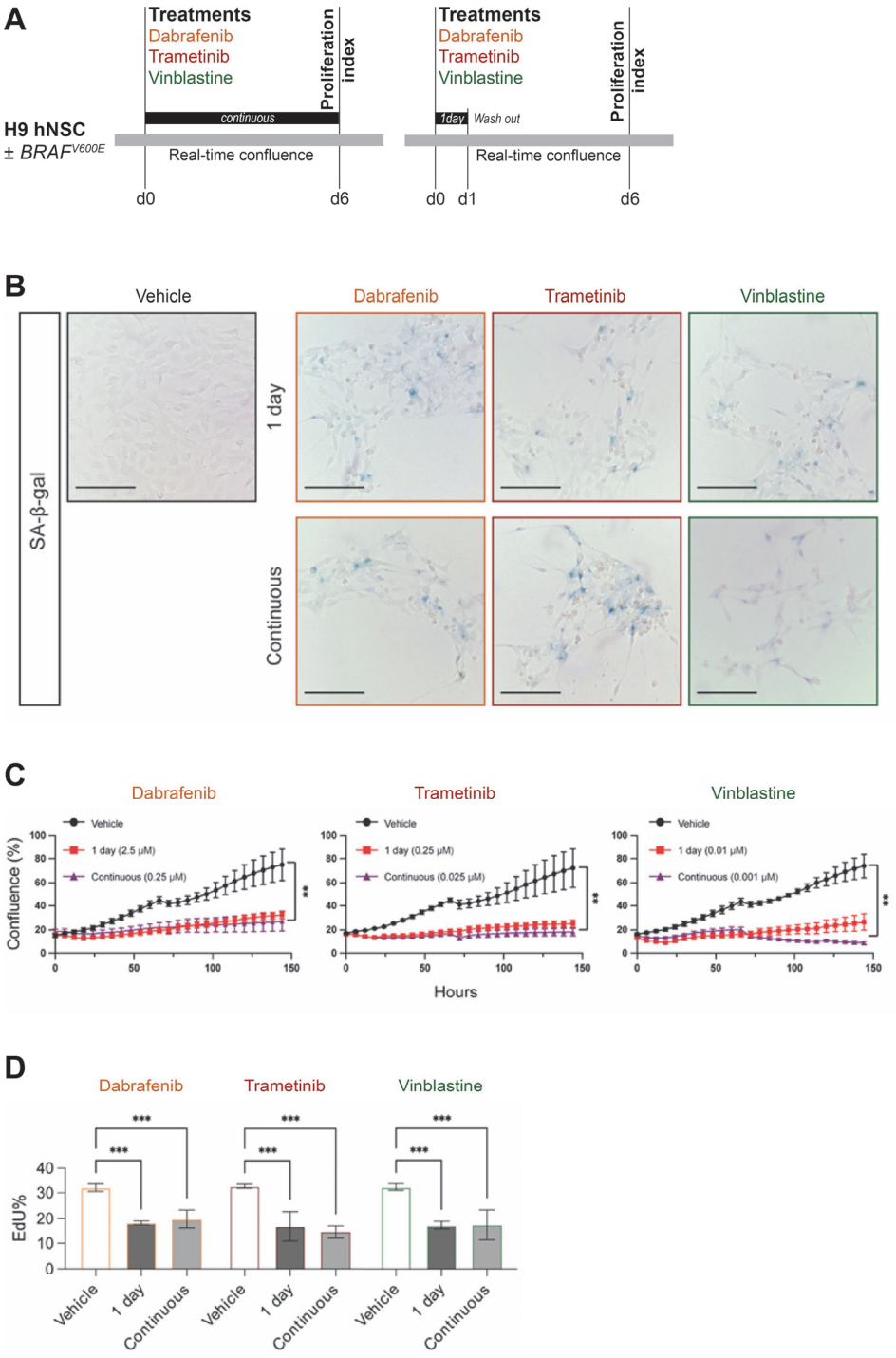
Difference in *CDKN1A* ($p21^{CIP1}$ coding gene) normalized counts across the proliferative and treatment-induced senescence states. (E) Heatmap highlighting the diversity of genes coding for proteins associated with lysosomal compartment overexpressed in the treatment-induced senescence samples.

Supplementary Figure S5



Supplementary Figure S5. H9-derived human neural stem cells expressing BRAF-V600E are selectively sensitive to MAPK pathway inhibition. (A) Schematic of the short-term treatment and analysis timeline used to assess dose-dependent drug effects. (B) Wild-type H9-derived human neural stem cells (H9 WT) and H9 hNSCs expressing BRAF-V600E (H9 BRAF-V600E) were treated with increasing concentrations of Dabrafenib, Trametinib, or Vinblastine. Cell viability was assessed by ATP content at day 3, and apoptosis was measured by caspase-3/7 activity at day 1. Data represent mean ±SD from at least three independent experiments. (C) IC50 values were calculated from cell viability measurements.

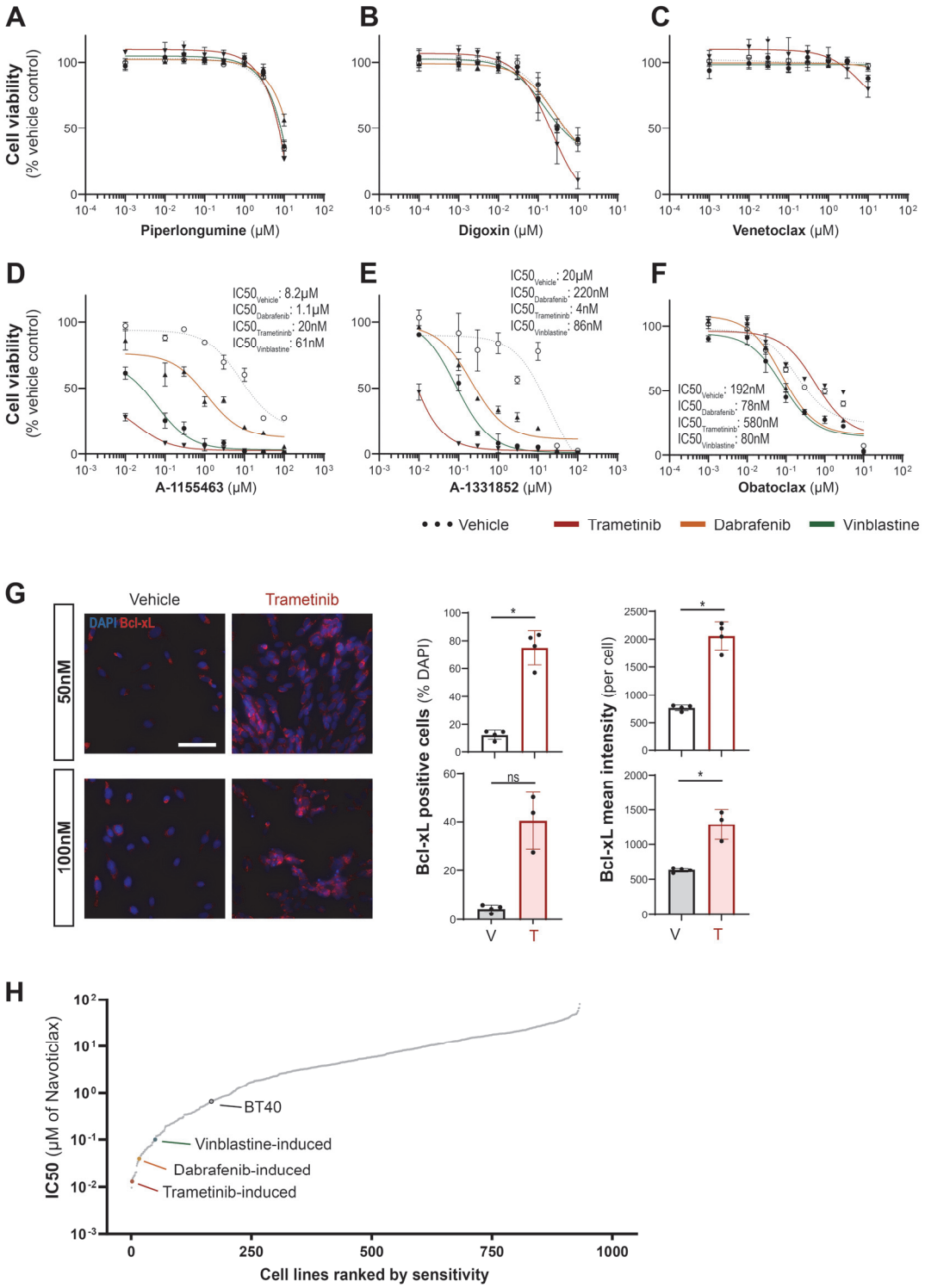
Supplementary Figure S6



Supplementary Figure S6. Dabrafenib, trametinib, and vinblastine induce growth arrest and cellular senescence in H9-derived human neural stem cells expressing BRAFV600E. (A) Schematic of the six-day continuous and one-day treatment timelines used to assess senescence induction and growth arrest. Drug concentrations were as follows: Dabrafenib (continuous, 0.25 µM; one-day, 2.5 µM), Trametinib (continuous, 0.025 µM; one-day, 0.25 µM), and Vinblastine (continuous, 0.001 µM; one-day, 0.01 µM). (B) At day 6, both continuous and one-day treated H9-derived human neural stem cells

expressing BRAF-V600E (H9 BRAF-V600E) exhibited SA-β-gal staining following treatment with all drugs, whereas vehicle-treated cells did not. (C) Cellular confluence of H9 BRAF-V600E cells treated as shown in (A) was measured by Incucyte real-time live-cell imaging every 6 hours from nine locations per well. Both continuous and one-day treatments with all drugs resulted in significant growth arrest. Data represent mean ± SD from four independent experiments. Statistical significance was determined by comparing area under the curve (AUC) values using an unpaired Student's *t*-test (**$p < 0.01$). (D) EdU incorporation was assessed by flow cytometry following a 2-hour pulse. Statistical significance was determined by one-way ANOVA (***$p < 0.001$).

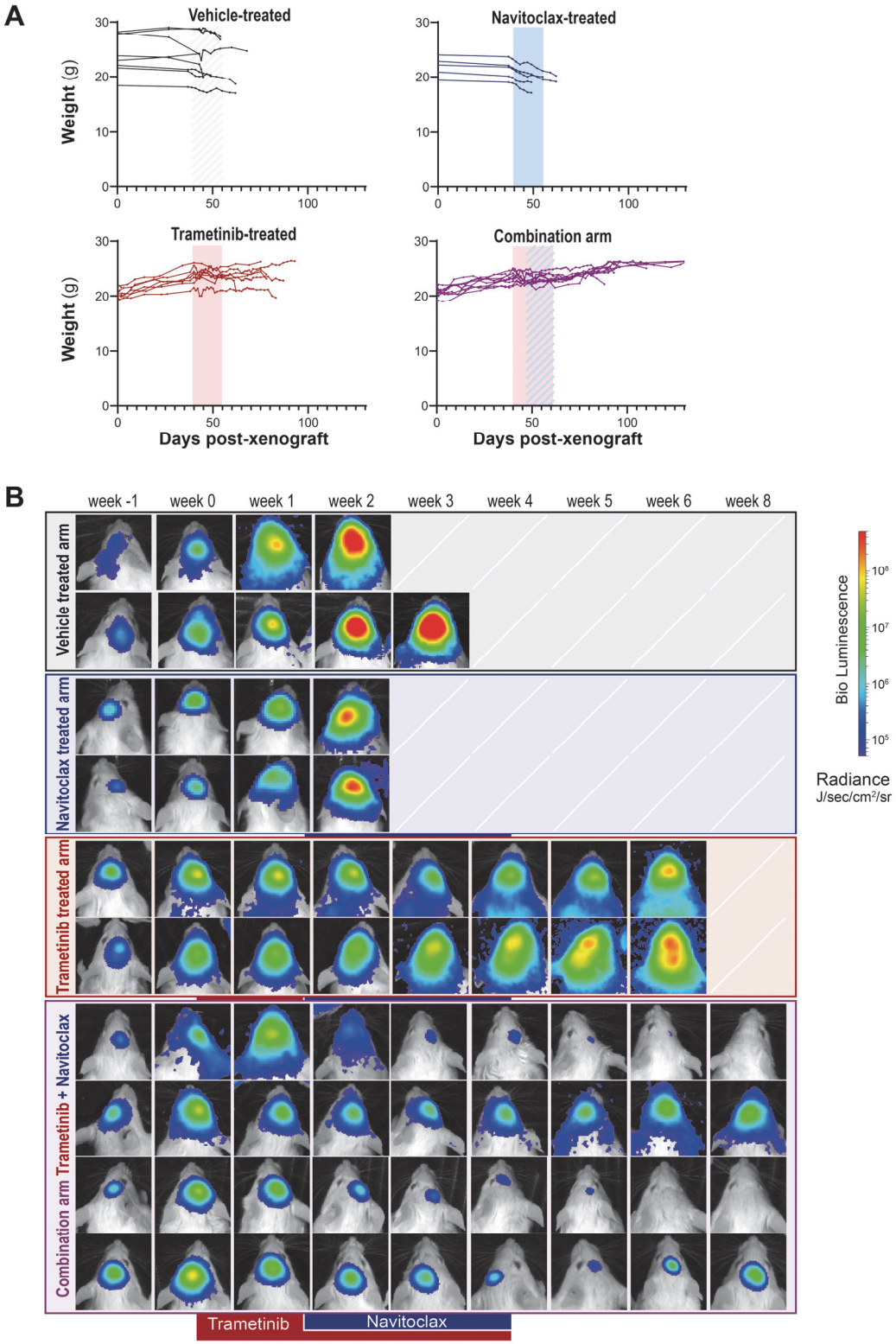
Supplementary Figure S7



Supplementary Figure S7. Treatment-induced senescent BT-40 cells are selectively sensitive to the Bcl-xL inhibitors A-1331852 and A-1155463. Assessment of viability by ATP-production of treatment-induced senescent (Dabrafenib: orange, Trametinib: red, Vinblastine: green) or proliferating (dotted black) BT-40 cells treated for 72 hours with known senolytics (Piperlongumine (A), Digoxin (B)) or BH3-mimetics Venetoclax (C), A-1155463 (D), A-1331852 (E) and Obatoclax (F) at indicated concentrations.

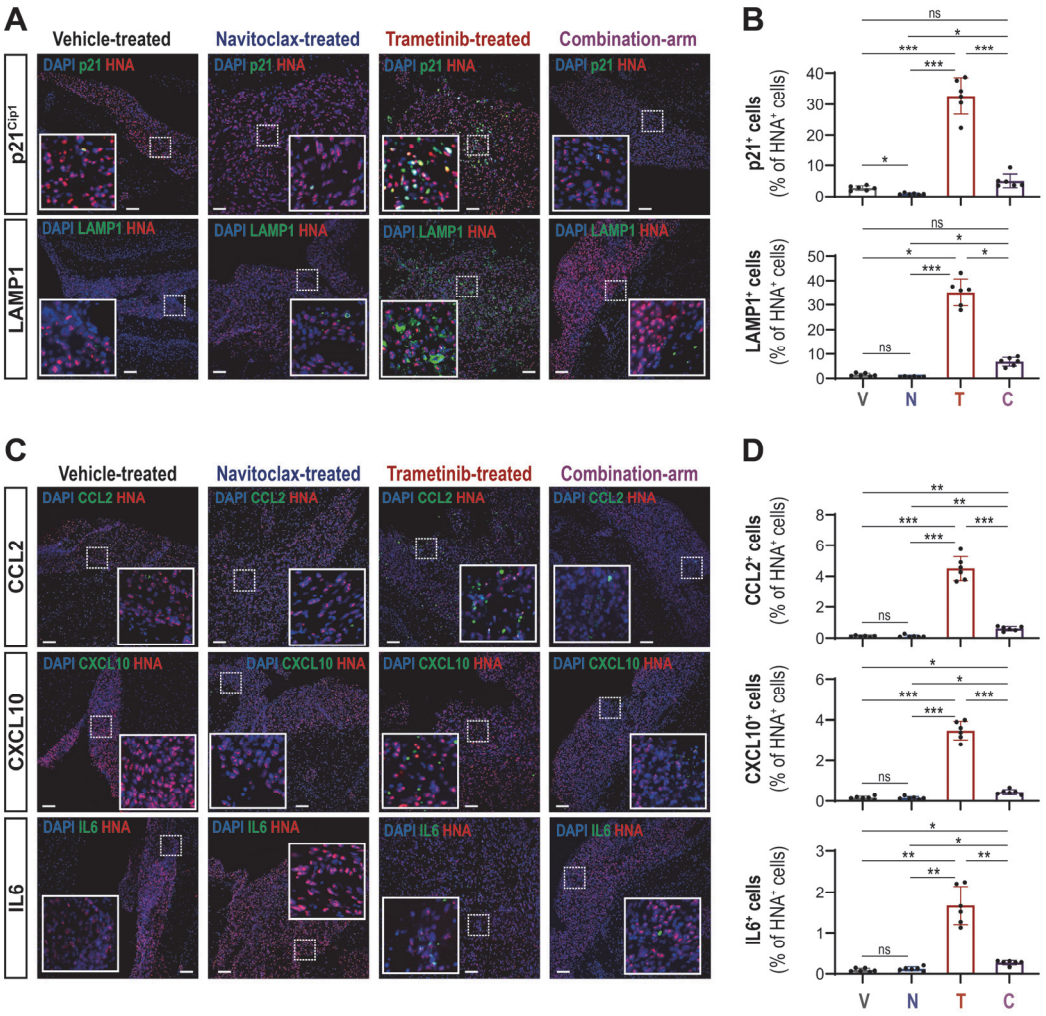
Shown are mean ± SEM of three independent experiments. (G) Immunofluorescence staining of Bcl-xL (red) at day 4 in vehicle-treated and Trametinib-treated BT-40 cultured cells at indicated concentration. DAPI in blue. Scale bars: 100 µm. Quantification of positive cells percentage and mean fluorescence intensity per cell on the right. Mann-Whitney test; *: $p<0.05$. (H) 928 cell lines screened by the Genomics of Drug Sensitivity in Cancer (GDSC) cell lines database ranked by Navitoclax IC50. Parental BT-40, not included in GDSC database, and treatment-induced senescent BT-40 cells have been inserted into their corresponding positions in the ranking according to experimentally calculated IC50: 2^{nd} for trametinib-induced senescence, 17^{th} for dabrafenib-induced senescence, and 50^{th} for vinblastine-induced senescence.

Supplementary Figure S8



Supplementary Figure S8. Individual follow-up of mice bearing orthotopic BT-40 xenograft tumours treated with Trametinib, Navitoclax, or sequential Trametinib followed by combination therapy. (A) Weight of each animal per group. Human endpoint is fixed when weight drop by >10% ; (B) BLI representation across the time, before, during and after treatments for representative animals in each group.

Supplementary Figure S9



Supplementary Figure S9. Combination therapy with Trametinib and Navitoclax reduces senescence and SASP marker expression in BT-40 tumour cells in vivo.

(A) Double immunofluorescence against Human Nuclear Antigen (HNA –red) and the senescence markers p21 or LAMP1 (green). Scale bars: 200µm. (B) Quantification of the proportion of HNA positive cells expressing p21 and LAMP1 analysed in (A). Note the overall reduction of senescent marker expression in the combination compared with the Trametinib-only arms. (C) Double immunofluorescence against Human Nuclear Antigen (HNA –red) and the SASP markers CCL2, CXCL10 or IL6 (green). Scale bars: 200µm. (D) Quantification of the proportion of HNA positive cells expressing the markers used in (C). Note the overall reduction of SASP marker expression in the combination arm compared to Trametinib-only treated arm. Data show mean ± SEM of n = 3 BT-40 tumours per marker. Kruskal-Wallis test, Dunn's post-test; *: p<0.05, **: p<0.01, ***: p<0.001

Supplementary Table S1. BH3 mimetics used in this study.

Compound	Inhibitory profile (K_i cell free assays)				Clinical trial status	Example clinical trial	Achievable non-toxic C_{max} (nM)
	Bcl-2	Bcl-xL	Bcl-w	Mcl-1			
Venetoclax	<0.01	48	245	>440	MHRA approved	NCT04401748	2533.4 ± 1612
Navitoclax	<1	<0.05	<1	550 (+/-40)	Phase III	NCT02591095	6607 ± 3262
Obatoclax	0.2	1-7	1-7	1-7	Phase I	NCT00600964	7-38
A-1331852	6	<0.01	4	142	Preclinical	n.a	n.a.
A-1155463	74	<0.01	8	>444	Preclinical	n.a	n.a

Supplementary Table S2. Primary and secondary antibodies used in this study.

Application	Target	Host	Clonality	Clone	Supplier	Catalogue No	Dilution	Antigen Retrieval Method	Conjugate
Primary FFPE	Human nuclei antigen	Mouse	Monoclonal	3E1.3	Sigma-Aldrich	MAB4383	1:100	Tris- EDTA pH9.0	
Primary FFPE	Ki67	Rabbit	Monoclonal	SP6	Abcam	ab16667	1: 500	Tris-EDTA pH9.0	
Primary In vitro	p21	Rabbit	Polyclonal	M-19	Cell Signaling Technology	#5487	1:400	Tris- EDTA pH9.0	
Primary FFPE	p21	Rabbit	Polyclonal	N/A	eBioscience	14-6715-81	1:100	Tris-EDTA pH9.0	
Primary FFPE	p21	Mouse	Monoclonal	SXM30	BD Pharmingen	6431	1:100	Tris-EDTA pH9.0	
Primary FFPE	Lyzozomal β-Galactosidase (GLB1)	Rabbit	Polyclonal	N/A	Proteintech	15518-1AP	1:100	Tris-EDTA pH9.0	
Primary FFPE	LAMP-1	Rabbit	Polyclonal	N/A	Abcam	ab42170	1:200	Tris-EDTA pH9.0	
Primary In vitro	CCL2	Rabbit	Monoclonal	N/A	R&D	MAB679R	1:1000	N/A	
Primary FFPE	CCL2	Rabbit	Polyclonal	N/A	Invitrogen™	15365431	1:100	Tris-EDTA pH9.0	
Primary In vitro	IL1B	Rabbit	Monoclonal	N/A	R&D	MAB201	1:200	Tris- EDTA pH9.0	
Primary FFPE	CXCL10	Rabbit	Polyclonal	N/A	Proteintech	10937-1-AP-20UL	1:100	Tris-EDTA pH9.0	
Primary FFPE	IL6	Goat	Polyclonal	N/A	R&D systems	AF-406-N	1:100	Tris-EDTA pH9.0	
Primary FFPE	IBA1	Rat	Monoclonal	EPR16589	Abcam	ab283346	1:100	Tris-EDTA pH9.0	
Primary FFPE	CD68	Rabbit	Polyclonal	N/A	Aviva Systems Bio	OABB00472	1: 500	Tris-EDTA pH9.0	
Primary FFPE	Lamin B1	Rabbit	Polyclonal	N/A	Abcam	ab16048	1: 500	Tris-EDTA pH9.0	
Primary FFPE	Cleaved Caspase	Rabbit	Polyclonal	Asp175	Cell Signaling	#9661	1:1000	Tris- EDTA pH9.0	
Primary In vitro	Bcl-xL	Rabbit	Monoclonal	E18	Abcam	ab32370	1:500	N/A	
Secondary	Mouse Ig	Goat	Polyclonal	Thermo	A11001		1:250		Alexa Fluor® 488
Secondary	Mouse Ig	Goat	Polyclonal	Thermo	A11004		1:250		Alexa Fluor® 568
Secondary	Rabbit Ig	Goat	Polyclonal	Thermo	A11008		1:250		Alexa Fluor® 488
Secondary	Rabbit Ig	Goat	Polyclonal	Thermo	A11036		1:250		Alexa Fluor® 568