

<b>Reagent</b>	<b>Catalog</b>	<b>Supplier</b>
Tamoxifen	#T5648	Sigma-Aldrich
Proteinase K	#P2308	Sigma-Aldrich
4% paraformaldehyde	#J19943.K2	ThermoFisher Scientific
Triton	#X100	Sigma-Aldrich
NeuroCult Basal Medium	#057001	Stem Cell Technologies
Pen/Strep	#15140122	ThermoFisher Scientific
EGF	#315-09	Peptotech
bFGF	#450-33	Peptotech
Heparin	#H3393	Sigma-Aldrich
4-hydroxytamoxifen	#H6278	Sigma-Aldrich
Neurobasal+ Medium	#A3582901	ThermoFisher Scientific
Geltrex	#A1413201	ThermoFisher Scientific
B27+	#A3582801	ThermoFisher Scientific
GlutaMAX	#35050061	ThermoFisher Scientific
FBS	#10270106	ThermoFisher Scientific
Dynabeads protein G	#10003D	ThermoFisher Scientific
RNeasy Kit	#74104	QIAGEN
SuperScript III Reverse Transcriptase	#18080-044	ThermoFisher Scientific
MyTaq Red Mix	#BIO- 25044	Bioline
PowerUp SYBR Green Mater Mix	#A25741	ThermoFisher Scientific
EcoRI	#R0101S	New England Biolabs
XhoI	#R0146S	New England Biolabs
BrdU	#B9285-1G	Sigma-Aldrich
MG132	#M7449-200ul	Sigma-Aldrich
Cycloheximide	#239765	Sigma-Aldrich
PD 98059	#HY-12028	Medchem
PLX-4032	#HY-12057	Medchem
Dapi Fluorogel II	#17985-50	Electron Microscopy Sciences
5-Bromo-4chloro-3-indolyl-beta-D-galactopyranoside	#203782	Calbiochem
DAB substrate kit	#34002	ThermoFisher Scientific
Topo Ta cloning pCR2.1	#45-0641	ThermoFisher Scientific
QIAmp DNA FFPE Tissue Kit	#56404	Qiagen
BRAF codon 600 Mutation Analysis Kit II	#BRAFX-RT64	EntroGen

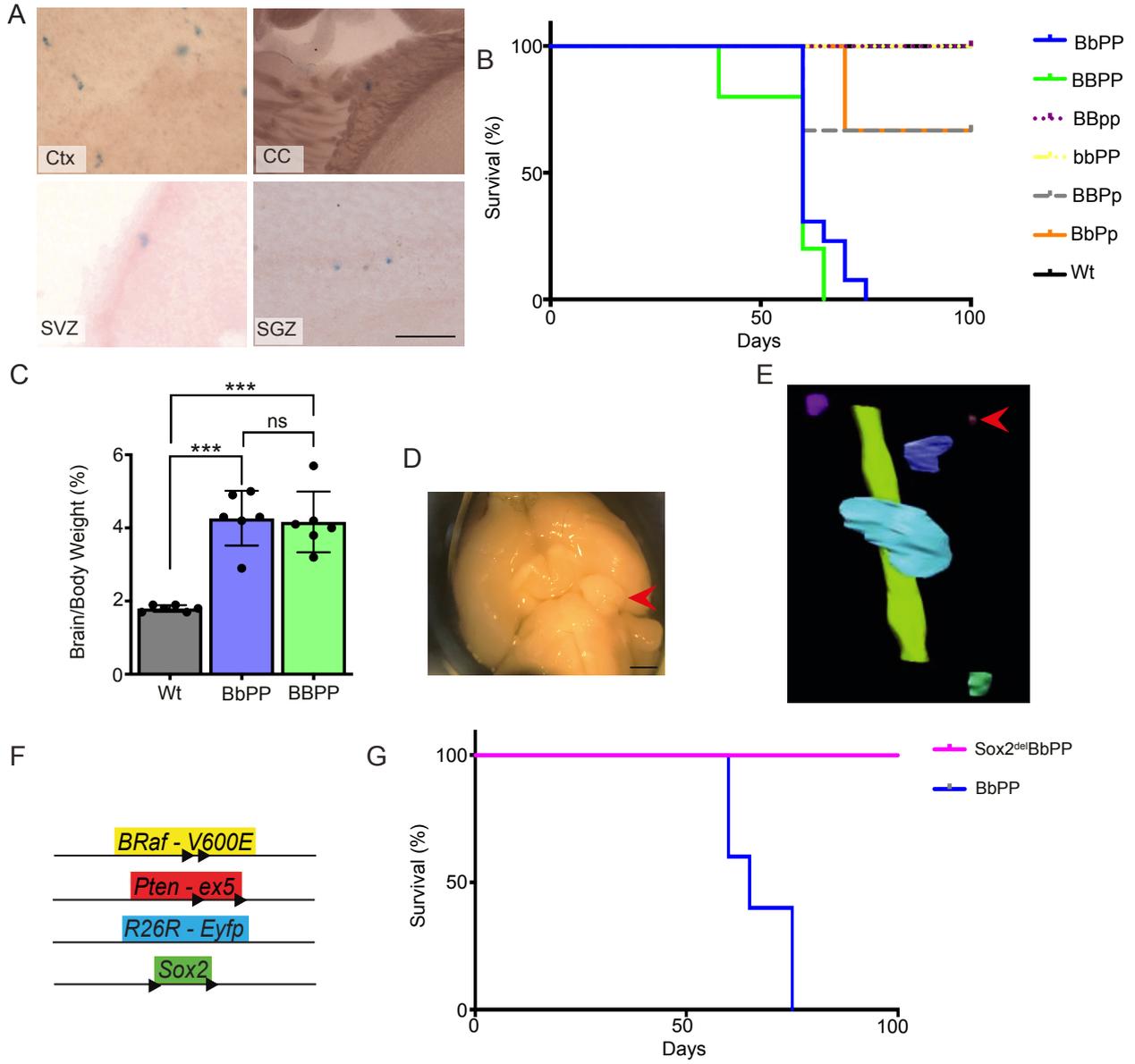
**Table 1.** Reagents list

<b>Antibody target</b>	<b>Catalog</b>	<b>Supplier</b>	<b>Concentration</b>	<b>Application</b>
Olig2	760-5050	Roche	1:1000	IHC/IF
Sox2	Sc-365823	Santa Cruz	1:200 3 µg	IHC/IF IP
Ki67	AB15580	Abcam	1:500	IHC
Gfap	MAB-12029	Immunological sciences	1:100	IHC/IF
NeuN	MAB377	Abcam	1:100	IHC
Eyfp	AB80449	Immunological Sciences	1:100	IF
Pten	Sc-7974	Santa Cruz	1:100	IF
BRAFV600E	790-5095	Roche	1:1000	IF/IHC
IDH1 R132H	MRQ-67	Roche	1:1000	IHC
S100b	GA504	DAKO	Ready to use	IHC
Sox10	Sc-365692	Santa Cruz	1:100	IHC
BrdU	Sc-32323	Santa Cruz	1:500	IF
Tubb3	T8578	Sigma-Aldrich	1:100	IHC/IF
MBP	NB600-717	Novus bio	1:100	IF
Nestin	MAB353	Millipore	1:100	IHC
Goat anti-rabbit	111-505-003	Jackson ImmunoResearch	1:200	IF
Goat anti-mouse	115-585-166	Jackson ImmunoResearch	1:200	IF
Donkey anti-rat	712-585-150	Jackson ImmunoResearch	1:200	IF
Anti-mouse/rat/rabbit	GTX83398	Gene Tex	Ready to use solution	IHC
β-Tubulin	2118	Cell Signaling Technologies	1:1000	WB
pAkt	Sc-7985	Santa Cruz	1:1000	WB
Akt	Sc-5298	Santa Cruz	1:1000	WB
pErk1,2	9101	Cell Signalling Technologies	1:1000	WB
Erk2	Sc-154	Santa Cruz	1:1000	WB
HA	Sc-7392	Santa Cruz	1:1000	WB
FLAG	F1804	Sigma-Aldrich	1:1000	WB
pT118 Sox2	PA5-143769	Invitrogen	1:1000	WB
Sox2	AB-83387	Immunological sciences	1:1000	IF/WB
Mouse-IgG	M5409	Sigma-Aldrich	3 µg	IP
Mouse anti-rabbit	Sc-2357	Santa Cruz	1:10000	WB
Goat anti-mouse	AB-2722565	Proteintech	1:10000	WB

**Table 2.** Antibodies list

<b>Primer</b>	<b>Forward</b>	<b>Reverse</b>
Sox2-CRE	5'- AATGCTTCTGTCCGTTTGCCGG- 3'	5'- CCAGGCTAAGTGCCTTCTCTAC- 3'
BRaf	5'-TGAGTATTTTTGTGGCAACTC- 3'	5'-CTCTGCTGGGAAAGCGGC-3'
Pten	5'-CAAGCACTCTGCGAACTGAG- 3'	5'- AAGTTTTTGAAGGCAAGATGC-3'
Pten del	5'-ACTCAAGGCAGGGATGAGC-3'	5'- GCTTGATATCGAATTCCTGCAG C-3'
Sox2 del	5-CAGTCCAAGCTAGGCAGGTT-3'	5-AGGCTGAGTCGGGTCAATTA- 3'
R26R Eyfp	5'-AAAGTCGCTCTGAGTTGTTAT- 3'	5'- GGAGCGGGAGAAATGGATATG- 3' 5'GCGAAGAGTTTGTCTCAACC- 3'
Beta-gal	5'-AACAGTTGCGCAGCCTGAAT-3'	5'-TCTTCCAGATAACTGCCGTC- 3'
Sox2T118A	5'- GGAAAGCCAAGACGCTCATGAA G-3'	5'-ATGAGCGTCTTGGCTTTCCG- 3'
Gfap	5'- GAAGCTCCAAGATGAAACCAACC -3'	5'- GAGTTCTCGAACTTCCTCCTCA- 3'
Sox2	5'-ACGCCTTCATGGTATGGTCC-3'	5'- CTTCTCGGTCTCGGACAAAAGT- 3'
Actb	5'- CTGTCGAGTCGCGTCCAC-3'	5'-GCTTTGCACATGCCGGAG-3'

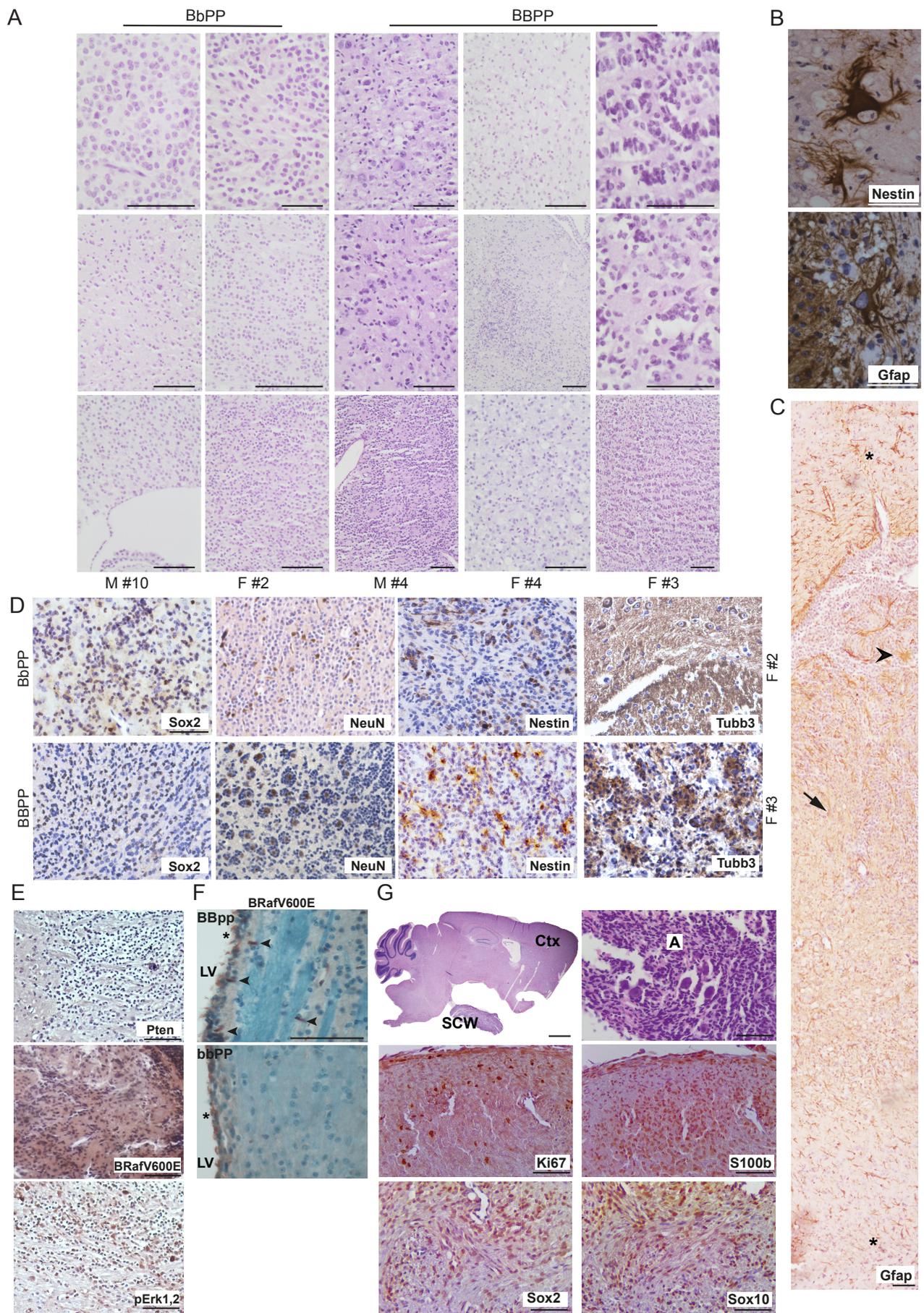
**Table 3.** Primers list.



Supplementary Figure S1

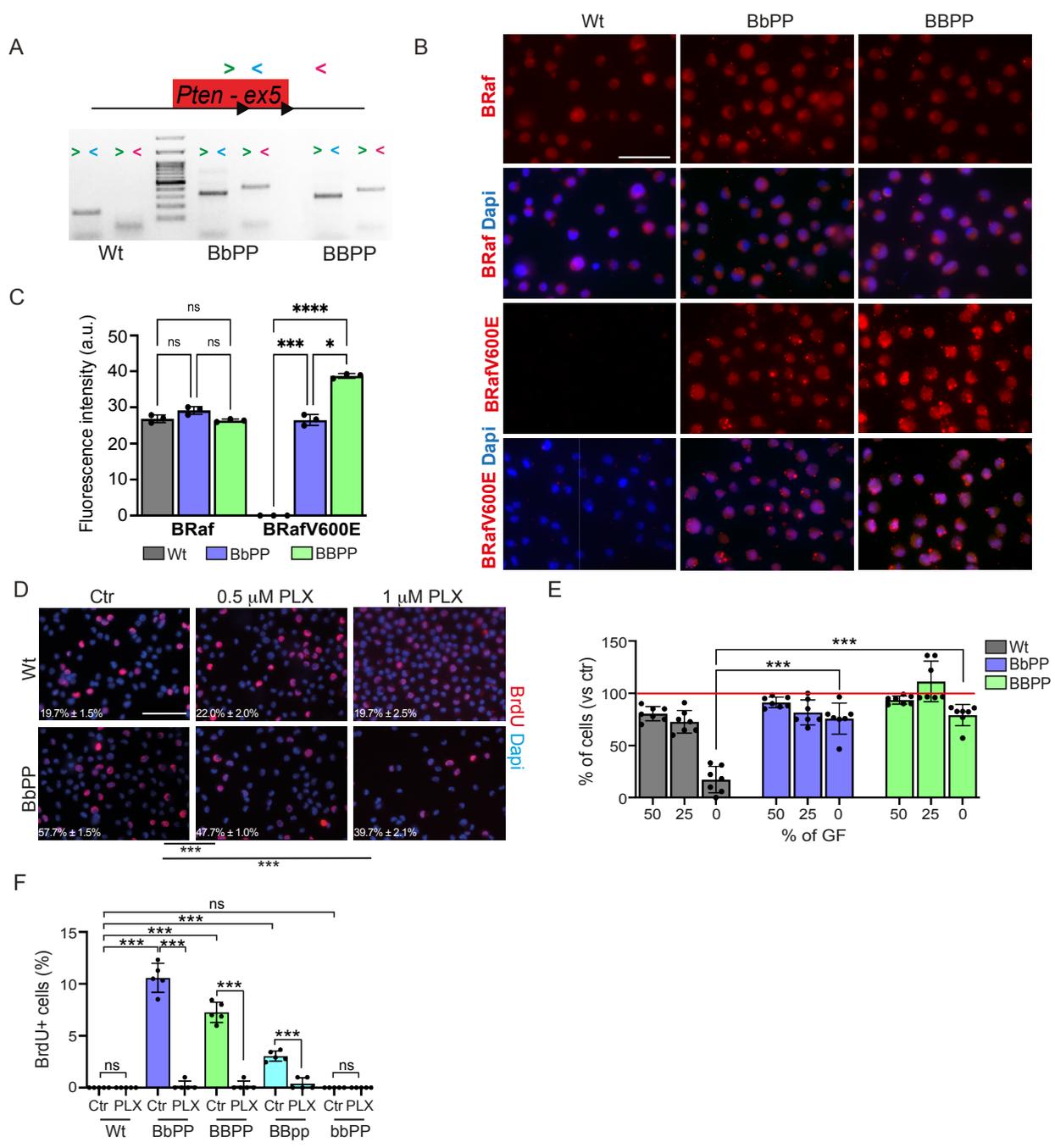
## Supplementary Figure 1

**(A)**  $\beta$ -gal staining of adult brain sections 10 days post tamoxifen administration.  $\beta$ -gal positive cells were found in the cerebral cortex (Ctx), corpus callosum (CC) sub-ventricular zone (SVZ), and sub-granular zone (SGZ). Scale bar: 50  $\mu$ m. **(B)** Kaplan-Meier survival curve for BbPP (n=13), BBPP (n=12), BBpp (n=3), bbPP (n=7), BBPp (n=8), BbPp (n=8), and Wt (n=12) mice, aged until symptomatic. **(C)** Bar chart showing the ratio of brain/body weight of Wt, BbPP, and BBPP mice (n=6 for each genotype), \*\*\*p < 0.001, ns=non-significant (Student's *t*-test, two-tailed). **(D)** Stereomicroscopic inspection of a BbPP brain, showing a mass on the ventral surface of the brainstem associated to CN V. Scale bar=100  $\mu$ m. **(E)** Ultra-high-frequency ultrasound system (UHFUS) micro-imaging, showing DRG masses in a BbPP mouse. The light green color represents the spinal cord. All the other colors represent different dimensions of the tumor masses. Violet (0.637 mm<sup>3</sup>), purple (1.208 mm<sup>3</sup>), light blue (9.391 mm<sup>3</sup>), green (0.871 mm<sup>3</sup>). Arrowhead points to the orange mass (0.162 mm<sup>3</sup>), representing a laterovertebral affected sympathetic ganglion. Unaffected ganglia are not detectable by this imaging system. To perform the ultrasonography animals were anesthetized with isoflurane and inspected utilizing a micro-imaging UHFUS, the Vevo3100 Imaging System (VisualSonics) equipped with an ultra-high-frequency transducer (MX155D, 40MHz, axial resolution 40 $\mu$ m) combined with the software provided by the manufacturer (VevoStrain Analysis software package, Version 1.5.0). **(F)** Schematic representation of the *Sox2*<sup>loxP/loxP</sup>, *Pten*<sup>loxP/loxP</sup>, *BRafV600E*, *R26R-Eyfp* transgene construct. **(G)** Kaplan-Meier survival curve of *Sox2*<sup>del</sup>*BbPP* mice vs *Sox2*<sup>w</sup>*BbPP* (n=5 for each genotype). 0 corresponds to tamoxifen administration in 1-month-old mice.



## Supplementary Figure 2

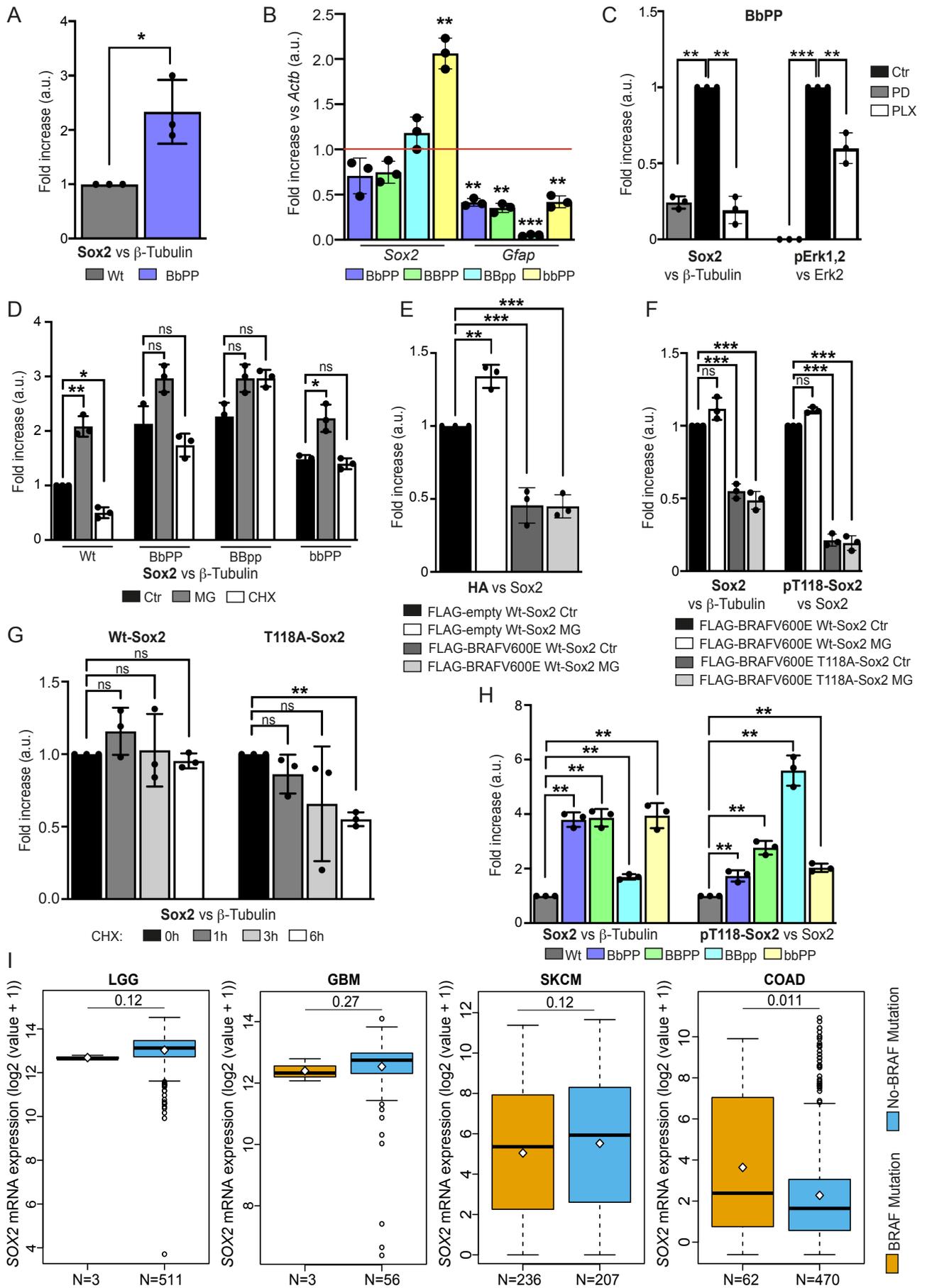
**(A)** H&E-stained sections of brain tumors in BbPP (M#10 adult male sample and F#2 adult female sample) and BBPP (M#4 adult male sample and F#4 and F#3 adult female samples) mice. Scale bar: 50 $\mu$ m. **(B)** Higher magnification images of Nestin- and GFAP-positive reactive astrocytes. Scale bar: 50 $\mu$ m. **(C)** IHC analyses on BbPP tumor section probed for Gfap. Asterisks indicate astrocytes with typical stellate morphology, while arrowheads mark reactive astrocytes. Gfap-positive cells (arrows) are present within the tumor mass but are absent in oligodendroglioma-like areas. Scale bar: 50 $\mu$ m. **(D)** IHC analysis on BbPP (F#2) and BBPP (F#3) affected brains probed for Sox2, NeuN, Nestin, and Tubb3. Scale bar: 50 $\mu$ m. **(E)** IHC analysis on BbPP affected brain probed for Pten, BRafV600E, and pERK1,2. Scale bar: 50 $\mu$ m. **(F)** IHC analysis on BBpp (upper panel) and bbPP (lower panel) brain section probed for BRafV600E. Arrowheads indicate BRafV600E-positive cells in BBpp samples, which are not observed in bbPP sections. Lateral ventricle (LV). The asterisk indicates nonspecific staining. Scale bar: 50 $\mu$ m. **(G)** H&E of brainstem-associated mass of CN V from BBPP mice (left upper panel; schwannoma (SCW), cortex (Ctx)). Scale bar: 2 mm. The right panel represents 40x magnification. IHC (remaining panels) for Ki67, S100b, Sox2, and Sox10. The letter A denotes Antoni A areas. Scale bar: 50 $\mu$ m.



Supplementary Figure S3

### Supplementary Figure 3

**(A)** PCR analysis for *Pten* deletion on Wt, BbPP and BBPP NSPCs DNA extracts. > (green) represents common forward primer, < (light blue or red) represents reverse primers mapping to two different regions of *Pten* locus. **(B)** Representative IF experiments on Wt, BbPP, or BBPP aNSPCs for BRaf and BRafV600E. Scale bar: 50 $\mu$ m. N=3. **(C)** Quantification of BRaf and BRafV600E fluorescence intensity in Wt, BbPP, and BBPP aNSPCs \* $p < 0.05$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ , ns=non-significant. (two-way ANOVA) N=3 **(D)** Representative BrdU IF experiments on mock (DMSO) or PLX (0,5  $\mu$ M or 1  $\mu$ M) treated Wt or BbPP cells. BrdU incorporation in BbPP cells was significantly decreased after PLX treatment, \*\*\*\* $p < 0.0001$  (Student's *t*-test, two-tailed). Scale bar: 50 $\mu$ m. N=3. **(E)** Bar chart representing the aNSPCs numbers from Wt, BbPP, or BBPP mice growing in serial dilutions of GFs. \* $p < 0.05$ , \*\*\*\* $p < 0.0001$  (Student's *t*-test, two-tailed). N=3. **(F)** Percentage of BrdU incorporating cells from Wt, BbPP, BBPP, BBpp, or bbPP mice under differentiation conditions after mock or PLX treatment, versus control (Ctr). \*\*\* $p < 0.001$ , ns=non-significant (Student's *t*-test, two-tailed). N=3. Quantification of BrdU-positive cells was obtained from 5 consecutive tissue slices at 40x magnification. The percentage of cells was evaluated as the ratio of positively stained cells to total nuclei.



Supplementary Figure S4

#### Supplementary Figure 4

**(A)** Bar graph showing the densitometric quantification of Sox2 protein levels normalized to  $\beta$ -Tubulin, based on WB analysis on cell extracts from Wt or BbPP NSPCs. \* $p < 0.05$  (Student's *t*-test, two-tailed) N=3. **(B)** Bar graphs showing RT-PCR analysis of Sox2 and Gfap gene expression in BbPP, BBPP, BBpp, or bbPP samples. Expression levels are normalized to Actb and are presented relative to Wt (which is set to 1) \*\* $p < 0.01$ , \*\*\* $p < 0.001$  (Student's *t*-test, two-tailed). N=3. **(C)** Bar graph showing the densitometric quantification of Sox2 protein levels normalized to  $\beta$ -Tubulin and pERK1,2 protein levels normalized to ERK2, based on WB analysis on cell extract from BbPP NSPCs following PD or PLX treatment, versus control (Ctr). \*\* $p < 0.01$ , \*\*\* $p < 0.001$  (Student's *t*-test, two-tailed). N=3. **(D)** Bar graph showing the densitometric quantification of Sox2 protein levels normalized to  $\beta$ -Tubulin based on WB analysis on cell extracts from Wt, BbPP, BBpp, or bbPP following MG or CHX treatment, versus control (Ctr). \* $p < 0.05$  \*\* $p < 0.01$ , ns= non-significant (Student's *t*-test, two-tailed). N=3. **(E)** Bar graph showing the densitometric quantification of HA levels normalized to Sox2 based on WB analysis on IP complexes from HEK293T transfected with FLAG-empty Wt-Sox2 or FLAG-BRafV600E Wt-Sox2 treated with MG132 for 5h. \*\* $p < 0.01$ , \*\*\* $p < 0.001$  (Student's *t*-test, two-tailed). N=3. **(F)** Bar graph showing the densitometric quantification of Sox2 levels normalized to  $\beta$ -Tubulin and pT118-Sox2 normalized to Sox2, based on WB analysis on cell extracts from HEK293T transfected with FLAG-BRafV600E Wt- or FLAG-BRafV600E T118A-Sox2 and treated with MG132 for 5h. \*\*\* $p < 0.001$ , ns= non-significant (Student's *t*-test, two-tailed). N=3 **(G)** Bar graph showing the densitometric quantification of Sox2 protein levels normalized to  $\beta$ -Tubulin, based on WB analysis on cell extracts from HEK293T transfected with FLAG-BRafV600E Wt- or FLAG-BRafV600E T118A-Sox2 following 1, 3, 6h of CHX treatment. \*\* $p < 0.01$ , ns= non-significant (Student's *t*-test, two-tailed). N=3. **(H)** Bar graph showing the densitometric quantification of Sox2 protein levels normalized to  $\beta$ -Tubulin and pT118-Sox2 protein levels normalized to Sox2, based on WB analysis on cell extracts from Wt, BbPP, BBPP, BBpp, or bbPP NSPCs. \*\* $p < 0.01$  (Student's *t*-test, two-tailed). N=3. **(I)** Boxplots showing the SOX2 mRNA expression levels in BRAF-mutated and non-mutated tumors obtained by analyzing the publicly available TCGA PanCancer Atlas dataset on the cBioPortal database (Wilcoxon test with Benjamini-Hochberg adjustment). Low-grade glioma (LGG), Glioblastoma (GBM), Skin cutaneous melanoma (SKCM), Colorectal adenocarcinoma (COAD).