**Supplemental Information**

# Genetic perturbations direct the development of distinct brain tumor types from postnatal neural stem/progenitor cells

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**Figure S1**

Representative macroscopic images (left hand side) and microscopic hematoxylin eosin stained paraffin sections (right hand side) of brain tumors, which developed upon transplantation of neural stem/progenitor cells over-expressing *HRAS* or *MYC* in combination with *Bmi1* or *Ezh2*. Three secondary tumors (first, third and fourth panel) and one primary tumor (second panel) are shown. Arrowheads point to giant cells. Scale bars: 50 µm.

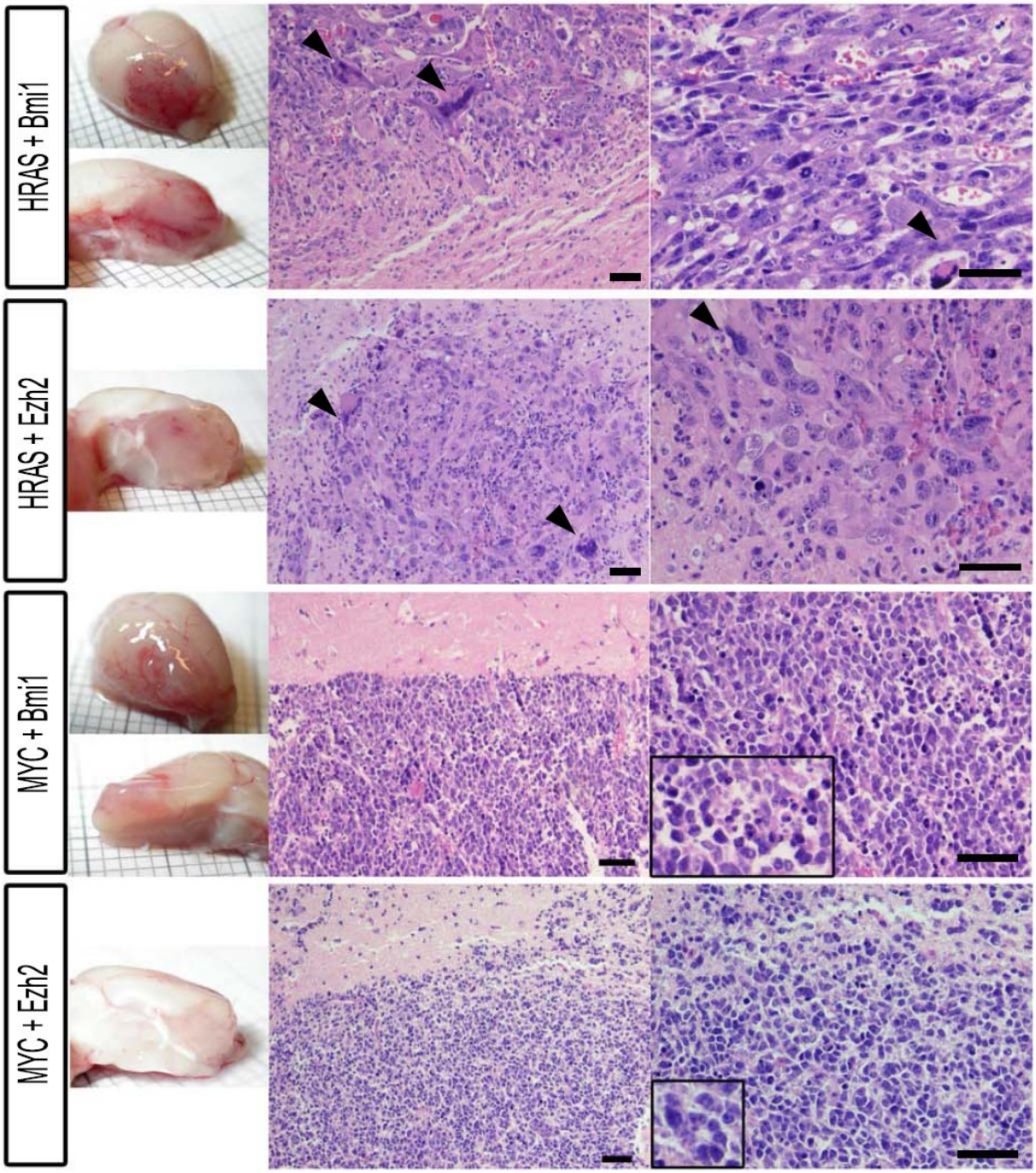
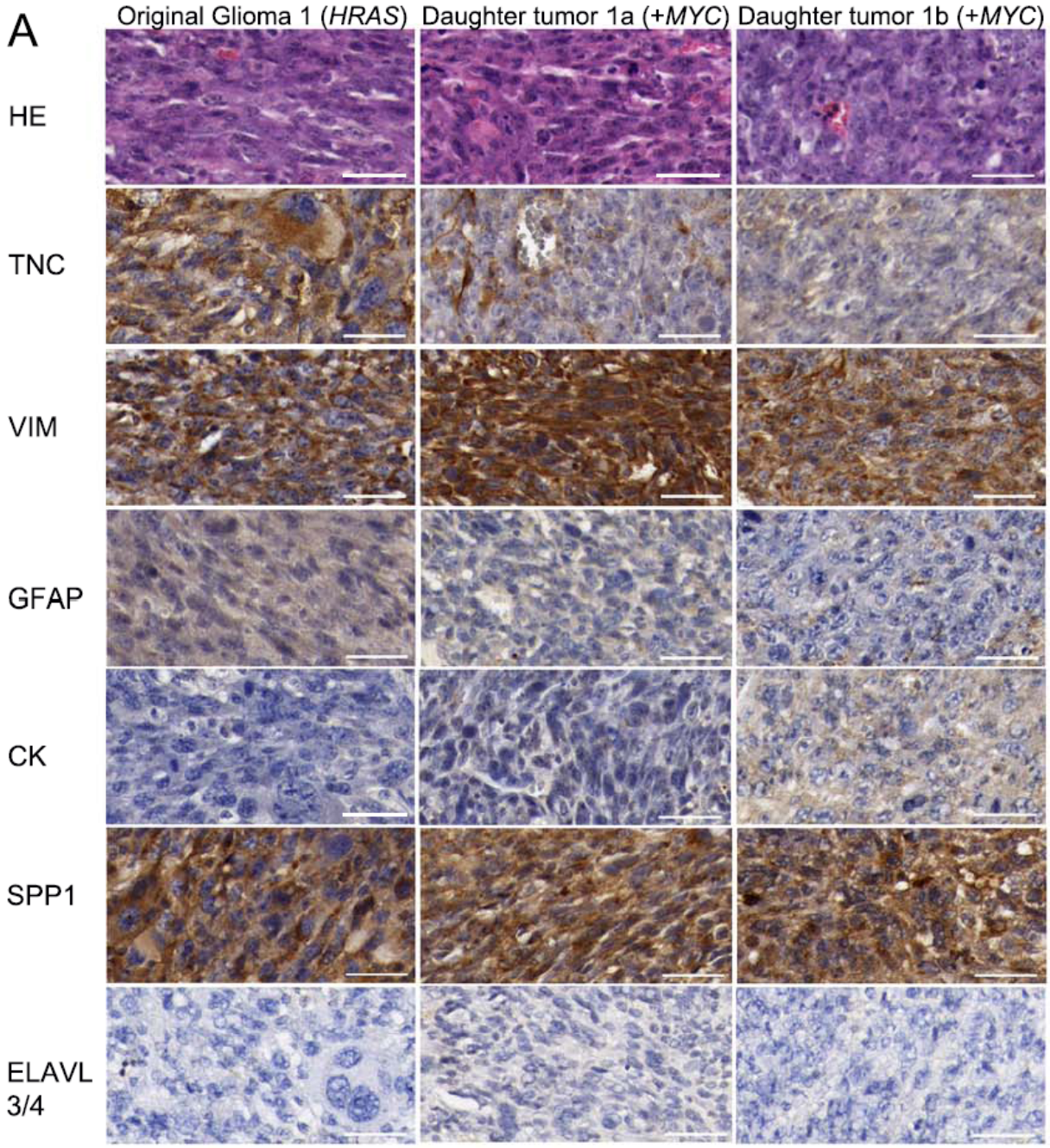
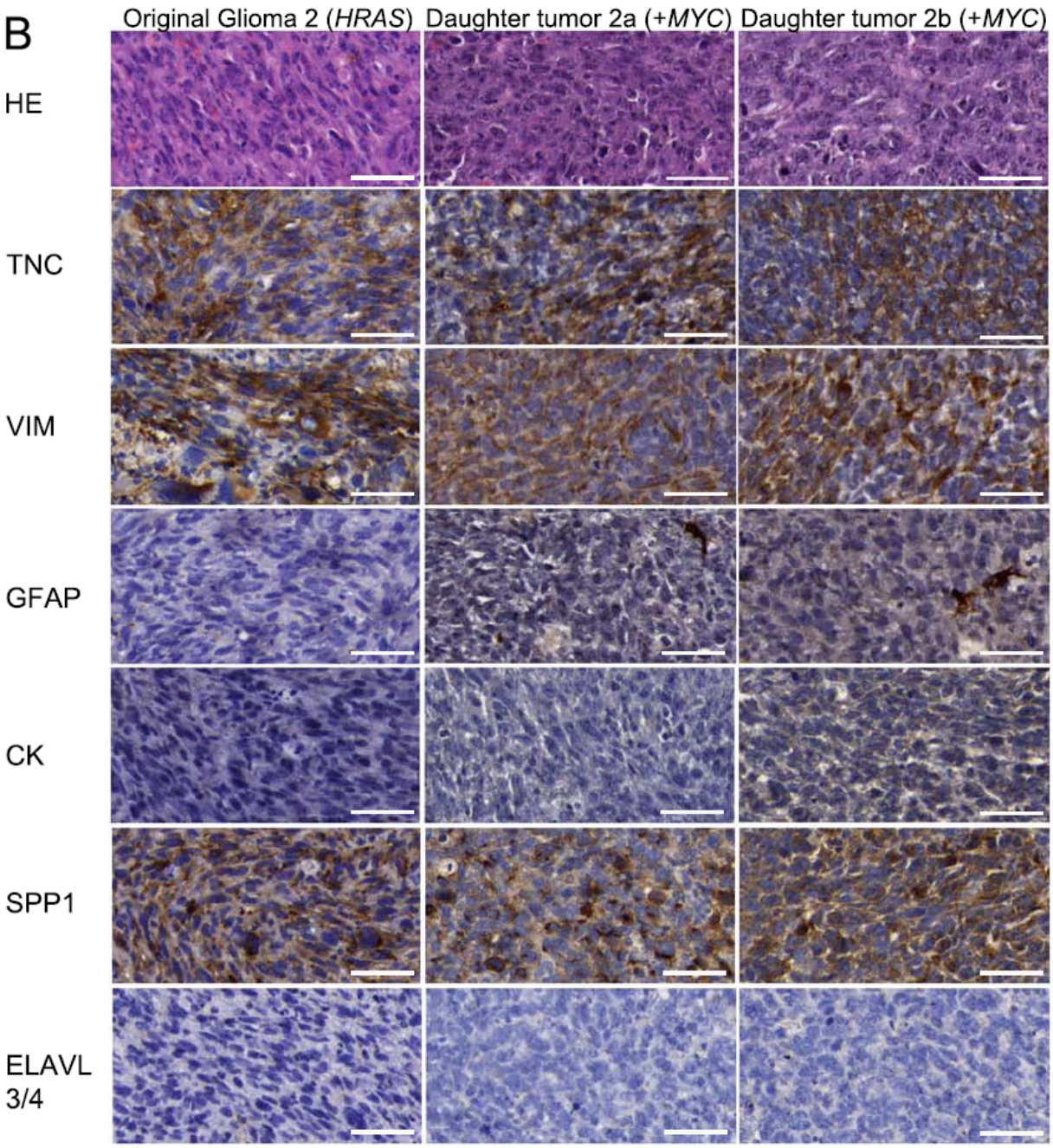


**Figure S2**

Hematoxylin eosin (HE) stained and immunohistochemistry images of original (mother) and respective daughter tumors which developed upon over-expression of *MYC* in glioma tumors (A,B) or of *HRAS* in CNS PNET tumors (C,D). Paraffin-embedded tumor sections were stained with antibodies against tenascin C (TNC), vimentin (VIM), GFAP, Cytokeratin (CK), SPP1, and ELAVL3/4. [+] Staining of a few tumor cells. V indicates blood vessel. Scale bars: 50 µm.

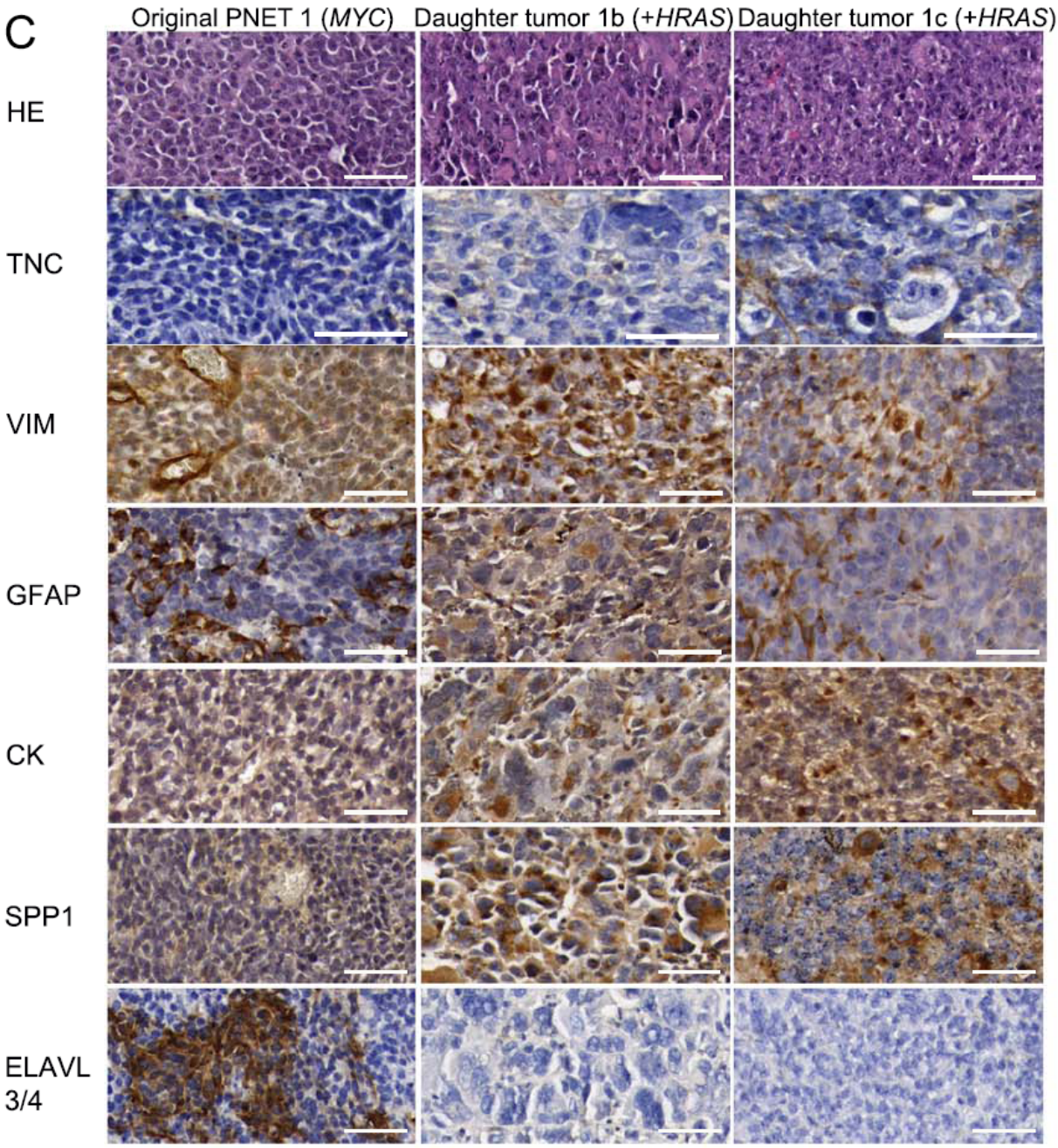



| | Original glioma 1 | Daughter tumor 1a | Daughter tumor 1b |
|---|---|---|---|
| TNC | + | - | - |
| VIM | + | + | + |
| GFAP | - | - | - |
| CK | - | - | - |
| SPP1 | + | + | + |
| ELAVL3/4 | - | - | - |



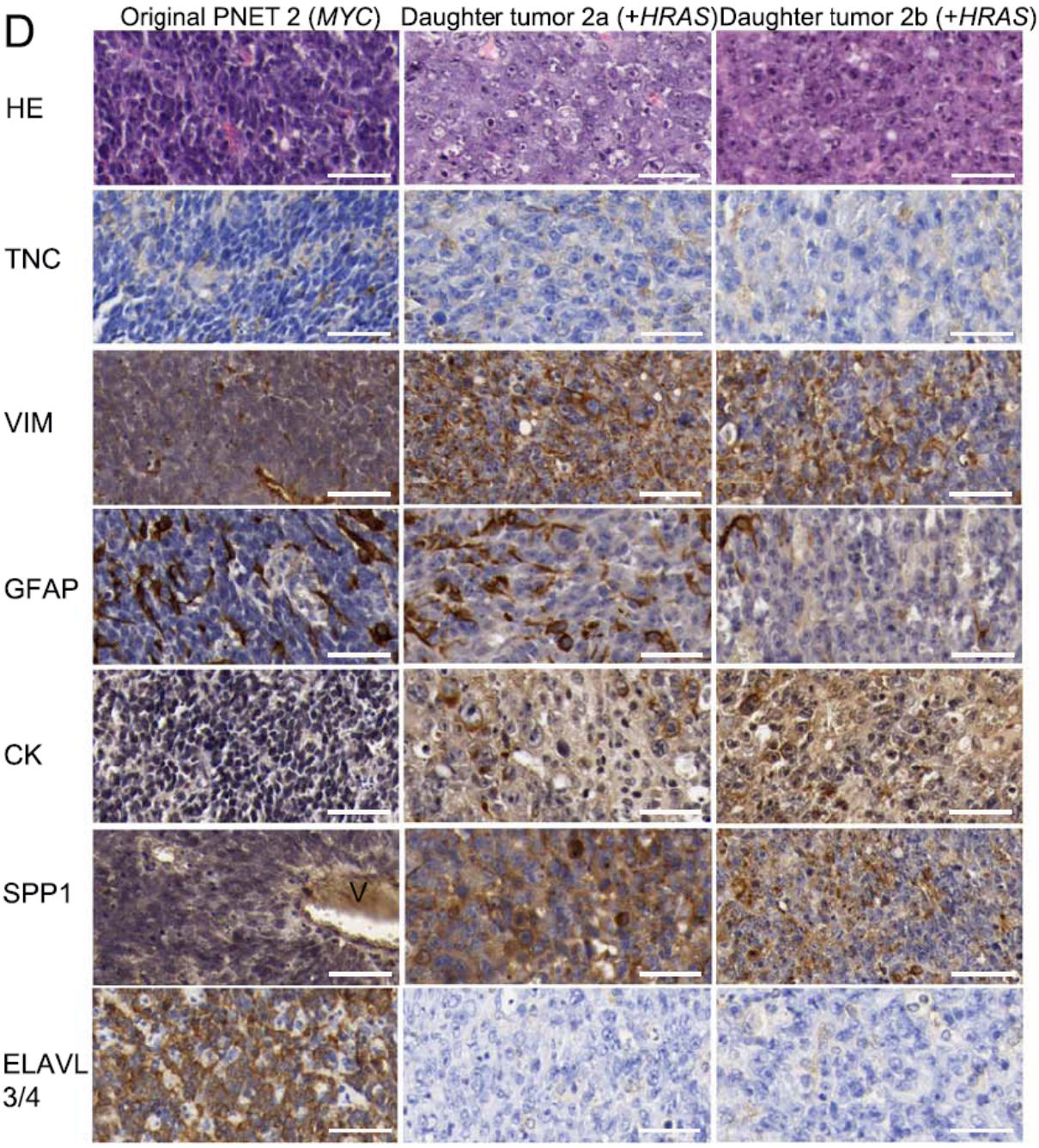

| | Original glioma 2 | Daughter tumor 2a | Daughter tumor 2b |
|---|---|---|---|
| TNC | + | + | + |
| VIM | + | + | + |
| GFAP | - | - | - |
| CK | - | - | - |
| SPP1 | + | + | + |
| ELAVL3/4 | - | - | - |

C




| | Original PNET 1 | Daughter tumor 1b | Daughter tumor 1c |
|---|---|---|---|
| TNC | - | - | - |
| VIM | - (only blood vessels +) | + | + |
| GFAP | [+] | - | [+] |
| CK | - | [+] | [+] |
| SPP1 | - | + | + |
| ELAVL3/4 | + | - | - |

D




| | Original PNET 2 | Daughter tumor 1b | Daughter tumor 1c |
|---|---|---|---|
| TNC | - | - | - |
| VIM | - (only blood vessels +) | + | + |
| GFAP | [+] | [+] | [+] |
| CK | - | [+] | [+] |
| SPP1 | - | + | + |
| ELAVL3/4 | + | - | - |

**File S1 (separate file)**

Gene sets used in this study.

**Table S1**

Overview of tumor samples used for microarray experiments. Tumors developed upon transplantation of p53-deficient postnatal neural stem/progenitor cells over-expressing different gene combinations.

| ***Initial combinatorial perturbation experiments*** | | | |
|---|---|---|---|
| **Gene combination** | **Array sample number** | **Transplantation** | **Transplanted cell number** |
| *HRAS + MYC* | 15 | Secondary | 50 |
| *HRAS + MYC* | 16 | Secondary | 50 |
| *HRAS + MYC* | 17 | Primary | 300000 |
| *HRAS + MYC* | 18 | Primary | 300000 |
| *HRAS + MYC* | 14 | Primary | 300000 |
| *HRAS + MYC* | 4 | Primary | 300000 |
| *HRAS + MYC* | 3 | Primary | 300000 |
| *MYC + Ezh2* | 1 | Primary | 300000 |
| *MYC + Ezh2* | 5 | Secondary | 50 |
| *MYC + Ezh2* | 8 | Primary | 300000 |
| *MYC + Ezh2* | 19 | Primary | 300000 |
| *MYC + Ezh2* | 20 | Secondary | 50 |
| *MYC + Bmi1* | 6 | Secondary | 5000 |
| *MYC + Bmi1* | 21 | Secondary | 5000 |
| *MYC + Bmi1* | 7 | Secondary | 5000 |
| *HRAS + Bmi1* | 22 | Primary | 300000 |
| *HRAS + Bmi1* | 9 | Primary | 300000 |
| *HRAS* | 23 | Primary | 300000 |
| *HRAS* | 2 | Secondary | 50 |
| *HRAS* | 10 | Primary | 300000 |
| *HRAS* | 24 | Secondary | 50 |
| *MYC* | 11 | Primary | 300000 |
| *MYC* | 12 | Primary | 300000 |
| *MYC* | 25 | Primary | 300000 |
| *MYC* | 13 | Secondary | 50 |
| *MYC* | 26 | Tertiary | 50 |
| ***Consecutive perturbation experiments*** | | | |
| **Gene combination** | **Array sample number** | **Transplantation** | **Transplanted cell number** |
| *HRAS (Glioma 2)* | 49 | Primary | 500000 |
| *Additional MYC (daughter 2a)* | 52 | Secondary | 5000 |
| *Additional MYC (daughter 2b)* | 53 | Secondary | 5000 |
| *MYC (PNET 1)* | 48 | Primary | 500000 |
| *Additional HRAS (daughter 1a)* | 50 | Secondary | 5000 |
| *Additional HRAS (daughter 1b)* | 51 | Secondary | 5000 |

**Table S2 (separate file)**:

Six groups of genes (A-F), which show the highest correlation to one of the three tumor types.

**Table S3 (separate file)**:

ER stress relation of Group A genes (higher expressed in AT/RT-like cells as compared to glioma and PNET cells).

**Table S4 (separate file)**:

ER stress relation of genes up-regulated in mouse embryonic fibroblasts upon deletion of the AT/RT tumor suppressor gene *Snf5* and also up-regulated in human AT/RT (SNF5KO_GS_UP genes, Isakoff et al., 2005)

**Table S5**:

Gene set enrichment analysis to identify molecular signatures of pathways that correlate best to the gene expression profile of the three tumor types (vs = versus). Results with a family wise error rate (FWER) below 0.01 are shown. NES = absolute normalized enrichment score.

| | Gene Set (GS) | GS SIZE | NES | FWER p-value |
|---|---|---|---|---|
| **PNET** | | | | |
| vs AT/RT-like | - | - | - | - |
| vs Glioma | HSA03010_RIBOSOME | 64 | 2.32 | 0.000 |
| | MRNA_PROCESSING_REACTOME | 98 | 2.00 | 0.008 |
| **Glioma** | | | | |
| vs AT/RT-like | GPCRDB_OTHER | 47 | 2.06 | 0.000 |
| vs PNET | HSA04510_FOCAL_ADHESION | 186 | 2.22 | 0.000 |
| | HSA04512_ECM_RECEPTOR_INTERACTION | 80 | 2.19 | 0.000 |
| | INTEGRIN_MEDIATED_CELL_ADHESION_KEGG | 89 | 2.07 | 0.000 |
| | PROSTAGLANDIN_SYNTHESIS_REGULATION | 27 | 2.06 | 0.000 |
| | HSA01430_CELL_COMMUNICATION | 119 | 2.00 | 0.000 |
| | BLOOD_CLOTTING_CASCADE | 18 | 1.98 | 0.000 |
| | HSA05222_SMALL_CELL_LUNG_CANCER | 83 | 1.97 | 0.000 |
| | HSA04060_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION | 212 | 1.95 | 0.001 |
| | CCMX_INTEGRINCELLADH | 172 | 1.94 | 0.003 |
| | BREAST_CANCER_ESTROGEN_SIGNALING | 83 | 1.92 | 0.005 |
| | HSA05120_EPITHELIAL_CELL_SIGNALING_IN_HELICOBACTER_PYLORI_INFECTION | 65 | 1.91 | 0.005 |
| | HSA04640_HEMATOPOIETIC_CELL_LINEAGE | 64 | 1.90 | 0.005 |
| | HSA04620_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY | 95 | 1.90 | 0.005 |
| **AT/RT-like** | | | | |
| vs Glioma | HSA00970_AMINOACYL_TRNA_BIOSYNTHESIS | 36 | 2.18 | 0.000 |
| | DNA_REPLICATION_REACTOME | 41 | 2.11 | 0.001 |
| vs PNET | BREAST_CANCER_ESTROGEN_SIGNALING | 83 | 2.08 | 0.000 |
| | HSA04640_HEMATOPOIETIC_CELL_LINEAGE | 64 | 2.04 | 0.000 |
| | HSA01430_CELL_COMMUNICATION | 119 | 2.02 | 0.000 |
| | HSA04060_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION | 212 | 2.00 | 0.002 |
| | PROSTAGLANDIN_SYNTHESIS_REGULATION | 27 | 1.95 | 0.003 |
| | HSA04620_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY | 95 | 1.93 | 0.005 |
| | BLOOD_CLOTTING_CASCADE | 18 | 1.92 | 0.006 |

## Supplemental experimental procedures

### Animal procedures

In the pilot study, female and male 4-8 weeks old wild-type C57Bl/6 mice were used for transplantations, for the main study female 4-8 weeks old wild-type C57Bl/6 mice received intracranial cell transplants. Mice were anesthetized by intraperitoneal injection of ketamine/xylazine solution. Cell concentrations were determined with CASY cell counter (CASY-TT; Innovatis; Reutlingen, Germany) or in case of lower cell numbers by a Hemacytometer. In case of 1000 or more transplanted cells, cells were harvested by centrifugation and resuspended in 3 µL PBS. In case of lower transplanted cell numbers, cells were injected in a 2-5 µl suspension in neurosphere medium. Injections were performed with 10µL gastight Hamilton syringes (blunt end 26G needles; Hamilton Bonaduz AG, Switzerland) into the right frontal brain lobe (2 mm lateral/1 mm anterior to the bregma, and 3 mm depth).
Transplanted mice of the pilot study were monitored for 6 months, mice of the main study for 8 months. Animals which showed symptoms such as ataxia, reduced movements or seizures were sacrificed and brains were isolated. Macroscopic brain images were taken with a Nikon Coolpix 4500 camera. A part of the tumor tissue was removed to isolate tumor cells by FACS. The remaining brain tissue was used for paraffin and cryosections. All animal procedures were performed with consent from the ethical committee at Lund University.

### Viral vectors and transduction

Human *c-Myc* was kindly provided by Rogier Versteeg, human $V^{12}$ *Ha-Ras* cDNA was obtained by PCR using genomic DNA from RasB8 mice (kindly provided by Abhijit Guha; Ding et al.). To generate mouse *Bmi1*, *Ezh2*, and *FoxM1* cDNA, RNA was isolated from neurosphere cultures that were established from postnatal wt mouse LVW tissue, reverse-transcribed and used as a template for PCR with gene-specific primers. $6x10^6$ cells of the ecotropic retroviral packaging cell line EcoPack2-293 (Clontech) were plated in 12 ml DMEM medium per T75 CellBind tissue culture flasks (Corning). The DMEM medium contained 1g/L glucose (Lonza), 10% FBS, 1 mM sodium pyruvate (both from Biochrom), 4 mM Glutamine, 100 units/mL Penicillin/Streptomycin (Invitrogen), and 2.5µg/mL Amphotericin B (PAA). 18-24h later, the medium was changed to transfection medium (DMEM medium as above with 25 µM chloroquine), and cells were transfected by the calcium phosphate method (as described in Mangassarian et al. 1999). After 16h, the medium was removed, cells were briefly treated with 15% glycerol in HeBS (25 mM HEPES, 140 mM NaCl, 0.75 mM $Na_2HPO_4$, all from Sigma), and grown in neurosphere medium for 48h. Cell supernatant was harvested, filtered by passing through a 0.45 µm PVDF membrane filter (Millipore) to remove remaining EcoPack2-293 cells and debris, and concentrated by centrifugation (2h, 20000x g). Viral pellets were resuspended in a final volume of 1 mL neurosphere medium containing 4 µg/mL polybrene (Sigma Aldrich) and applied to $3x10^6$ dissociated neurosphere cells per gene combination in 15 mL Falcon tubes for 4h at 37°C and 5% $CO_2$. The neurosphere cells were then harvested by centrifugation, resuspended in 15 mL fresh neurosphere medium without polybrene, seeded into T75 flasks with UltraLowAttachment surface (Corning), kept at 37°C and 5% $CO_2$, and FACS sorted after approximately 7 days.

### RT-PCR

For cDNA synthesis, 2 µg of total RNA were incubated with 5 µl 10 mM dNTPs (Fermentas, Burlington, Canada) and 2.5 µL random primers (500ng/µL) in a 35 µl reaction volume at 70°C for 5 min followed by a short incubation on ice and reverse transcription at 37°C for 60 min upon addition of 200 U M-MLV reverse transcriptase (Promega, Madison, WI), 0.5 U RNAsin (Promega), and 10 µl 5x M-MLV reaction buffer (Promega) to a final reaction volume

of 50 µL. 2 µL of this solution were used as template in a 50 µL polymerase chain reaction (PCR) with 0.4 µM of the forward and reverse primers.
Reaction conditions for the amplification of oncogene sequences were: 35 cycles (94°C for 45 sec, 55°C for 45 sec, 72°C for 45 sec). The following forward primers were used:
mEzh2_end_for (ATGGTGACCACAGGATAGGC)
mFoxM1_end_for (TTTCAGCCAACCGTTCTCTC)
Ha-Ras_end_for (GGATGCCTTCTACACGTTGG)
H_cMyc_end_for (AAAGGCCCCCAAGGTAGTTA), and
Bmi1_for (AGGTGTTCCCTCCACCTCTT).
CMMP 3' end rev (CGGATCCCCCTGATCCTC) was used as a reverse primer.
For the XBP1 PCR, primers hXBP1_Fw (GGAGTTAAGACAGCGCTTGG) and hXBP1_Rv (ACTGGGTCCAAGTTGTCCAG) were used. Reaction conditions were: 30 cycles (94°C for 45 sec, 61°C for 45 sec, 72°C for 90 sec).

**Immunohistochemistry**

Paraffin sections were rehydrated, boiled in citrate buffer (10 mM Sodium citrate, 0.05% Tween-20; pH6.0) for antigen retrieval, incubated in 0.35% $H_2O_2$, and blocked with 5% donkey serum/PBS. Primary antibodies were applied in PBS for 16h at 4°C (PBS only served as negative control); Biotin-conjugated secondary antibodies (1:500; Jackson Immunoresearch, West Grove, PA) were applied for 90 mins at room temperature. The R.T.U vectastain kit and DAB peroxidase substrate were used according to the manufacturer's instructions (VectorLabs, Burlingame, CA). Finally, the tissue sections were counterstained with Haematoxylin, dehydrated, and embedded in Entellan. The following primary antibodies were used: anti-BAF47 (SMARCB1/SNF5; 1/100; BD Biosciences, San Diego, CA), anti-pan-Cytokeratin (1:100; DAKO, Glostrup, Denmark), anti-glial fibrillary acidic protein (GFAP; 1:500, DAKO), anti-human neuronal protein HuC/HuD (ELAVL3/4; 1:400; Molecular Probes/Invitrogen, Carlsbad, CA), anti-Tenascin (1:400; kind gift of A. Faissner; Ruhr-University, Bochum, Germany), anti-Osteopontin/SPP1 (1:400; R&D Systems, Minneapolis, MN), anti-Vimentin (1:400; Progen Biotechnik, Heidelberg, Germany). Images of paraffin sections were taken with an Olympus BX50 camera or ScanScope scanner. Images of 10 µm cryosections were taken with an Axiovert 200M microscope using AxioVision 4.5 software (Zeiss).

**Western blot analysis**

Cells were harvested at 50-70% confluency, pelleted by centrifugation, and dissolved in RIPA buffer (10 mM Tris/HCl (pH 7.5), 1 mM EDTA, 1% Triton X-100, 0.1% SDS, 0.1% sodium deoxycholate, 100 mM NaCl) supplemented with protease inhibitors (2mM PMSF and 1x Complete Mini, Roche). Supernatants were collected, protein concentrations were determined using the BCA assay (Pierce/Thermo Scientific), and proteins were separated in a 10% SDS-polyacrylamide gel. Western blotting followed standard protocols using nitrocellulose membranes (Amersham/GE Healthcare). Signal intensities were analyzed with ImageJ (http://rsb.info.nih.gov/ij/).

**Culture of human cell lines**

The CHLA-02-ATRT cell line was obtained from ATCC and kept in neurosphere medium (see experimental procedures). The human malignant rhabdoid tumor cell line LM (provided by Rupert Handgretinger, Tuebingen, Germany) was kept according to Versteege et al. (Nature 1998). DAOY medulloblastoma cells (provided by Michael Grotzer, Zurich, Switzerland) were kept in EMEM (Lonza) supplemented with 10% FBS (Biochrom), 2 mM glutamine, 100 units/mL Penicillin/Streptomycin (Invitrogen), and 2.5 µg/mL Amphotericin B (PAA). SW1783 and Hs683 glioma cell lines (provided by Matthias Simon, Bonn, Germany) and were kept in DAOY medium with 1 mM sodium pyruvate, MEM non essential amino

acids, and MEM vitamin solution (all from Invitrogen). MCF7-SNF5-KD#73 cells (Xu et al., 2010) were cultured in DMEM (4.5g/L glucose, Invitrogen), 10% FBS (Tet System Approved, Clontech), 2 mM glutamine, and 100 units/mL Penicillin/Streptomycin (Invitrogen). For the knockdown of SMARCB1, tetracycline (1 µg/mL) was added on day 1 and 3, and cells harvested at day 4. At day 4, cells were treated with DTT (5 mM) or Thapsigargin (5 µM). All cells were kept in T75 tissue culture flasks (Techno Plastic Products, Switzerland).

**Apoptosis assay**

500,000 MCF7-SNF5-KD#73 cells (Xu et al., 2010) were plated into T75 tissue culture flasks (Techno Plastic Products, Switzerland). For knockdown experiments, tetracycline (1 µg/mL) was added on day 1 and day 3. In case of Bortezomib-treated cells, the drug (final concentration 10 nM) was added for 12 hours on day 4 (at 70-85% confluency). Cells were carefully washed with PBS and dissociated by trypsinization. Aliquots of single cell suspensions containing $1x10^5$ cells in 100 µL PBS were stained with AnnexinV-APC and 7AAD according to the manufacturer's instructions (BD Biosciences) for 15 min at RT. Subsequently, 400 µL of AnnexinV binding buffer was added (10 mM Hepes, 140 mM NaCl and 2.5 mM $CaCl_2$), cell suspensions were placed on ice and immediately analyzed with a FACSCalibur flow cytometer (BD Biosciences). The data analysis was performed using the FlowJo software (Tree Star). For statistical analyses, an unpaired t-test was conducted using Prism 5.0 (GraphPad, LaJolla, CA, USA).