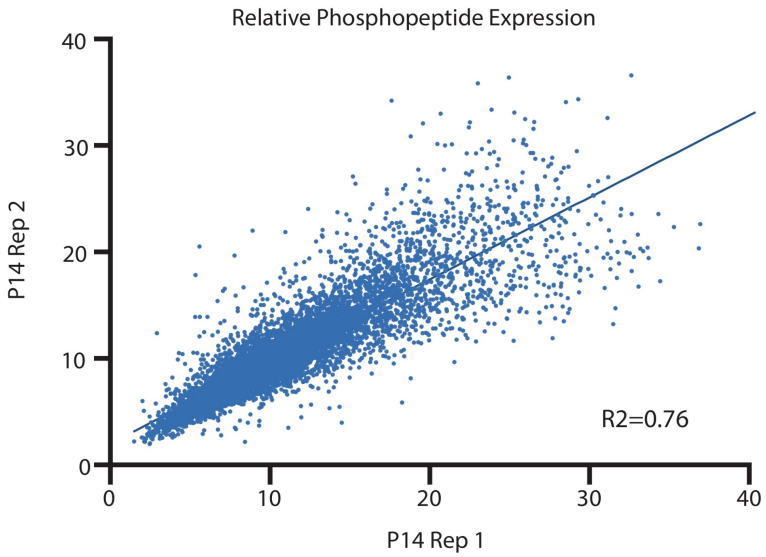

Figure S1. Relative phosphopeptide abundance between P14 biological replicates. Scatterplot and coefficient of determination of all quantified peptides and phosphopeptides shared between biological replicates (Rep1, Rep2) obtained from GNPs purified from P14 mouse cerebella, as assessed by mass spectrometry (FDR<1%). Each time point (P1, P7 and P14) was performed in biological triplicate, shown is a representative pair: total N = 3 separate cohorts of mice (each cohort = 35-40 mice).

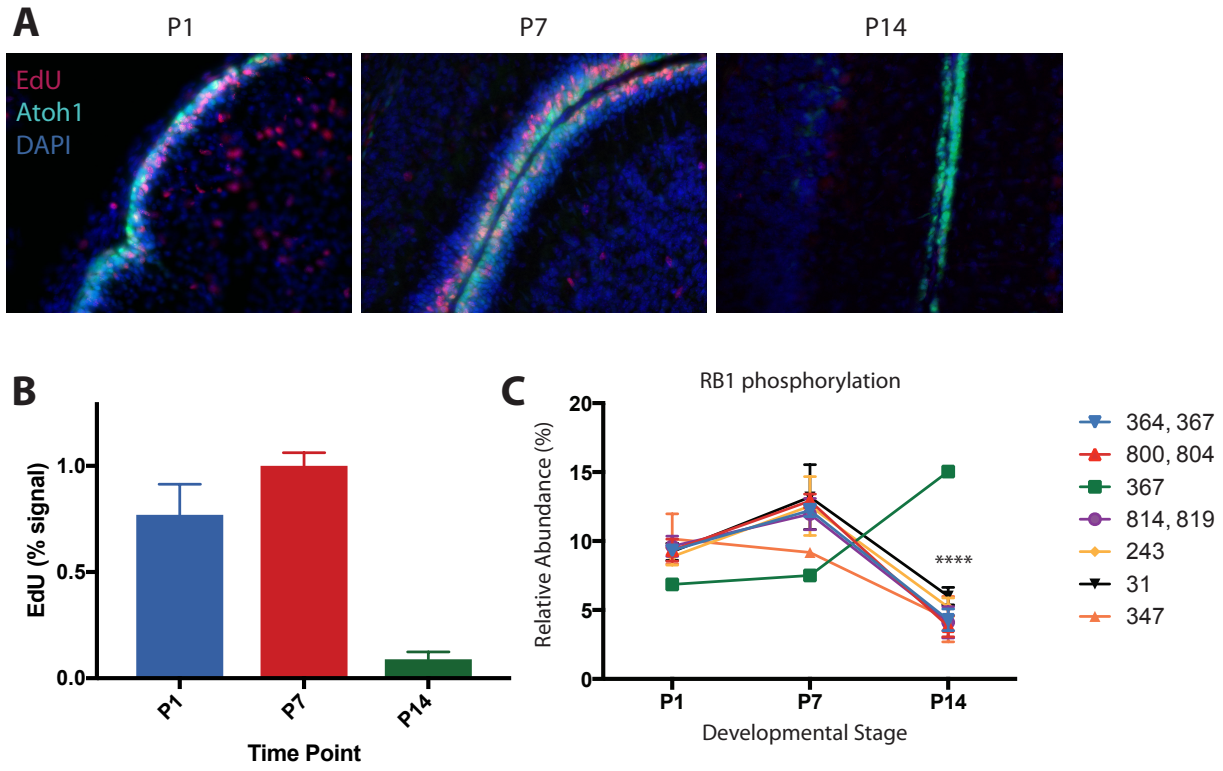

Figure S2. Relative proliferation in P1, P7 and P14 cerebellum. (A and B) Immunofluorescence assessment of EdU incorporation in P1, P7, and P14 cerebella 45 min after a pulse of EdU (A), and the relative EdU signal intensity (compared to that at P7) within Atoh1-positive regions, in P1, P7, and P14 cerebella (B). **(C)** Relative abundance of RB1 phosphorylation at the indicated 9 identified sites in the cerebella of P1, P7, and P14 wild-type mice, assessed by mass spectrometry as described in fig. 1. n=3 (separate cohorts of mice).; error bars = SD.

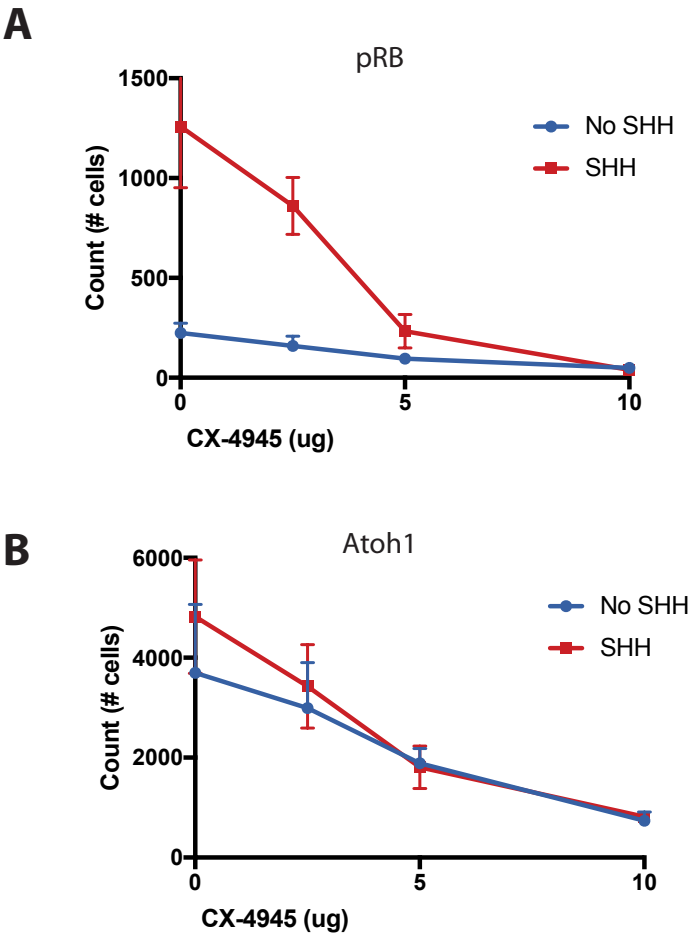

Figure S3. **GNP proliferation after CX-4945 treatment in culture. (A and B)** High-throughput single-cell immunofluorescence imaging was used to quantify XXXX the number of GNPs with detectable pRB (A) and Atoh1 (B), in GNPs cultured with (blue) or without (red) SHH at varying doses of CX-4945. n=3 (experiments); error bars = SD.

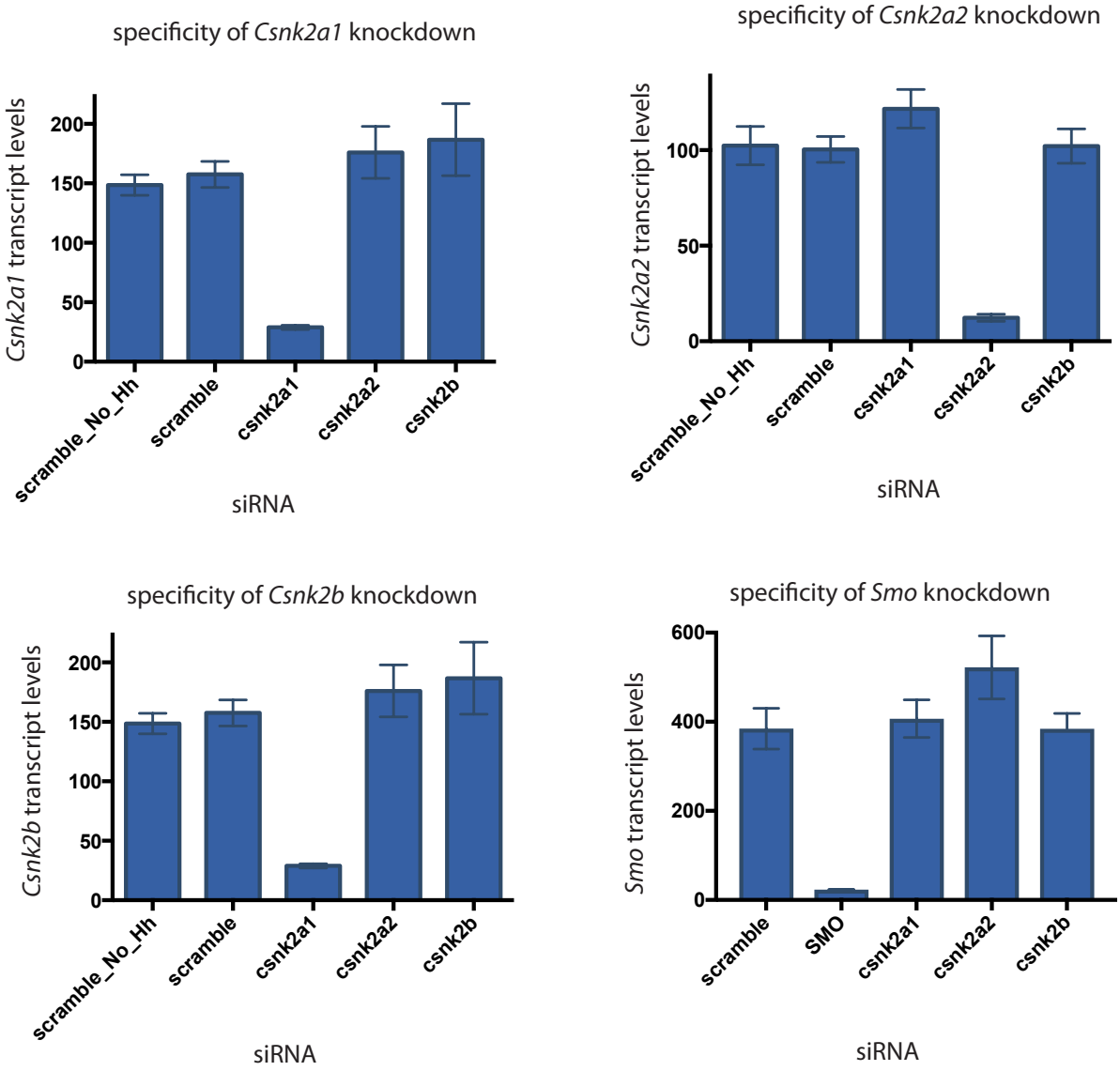

Figure S4. **Knockdown specificity of pooled siRNA constructs targeting *Csnk2a1, Csnk2a2, Csnk2b and Smo.*** *Csnk2a1*, *Csnk2a2*, *Csnk2b* and *Smo* transcript levels in NIH3T3's following 72 hours of siRNA-mediated knockdown of CSNK2A1, CSNK2A2, CSNK2B and SMO. All samples were exposed to SHH for 6 hours except scramble_No_Hh, which was not exposed to SHH. Control = NIH3T3's transfected with scramble siRNA and cultured without

SHH (scramble_No_Hh) or exposed to SHH for 6 hours (scramble). Data were obtained by qRT-PCR and normalized to GAPDH. XXXXXXXXXX. n=3 (experiments); error bars = S.D.

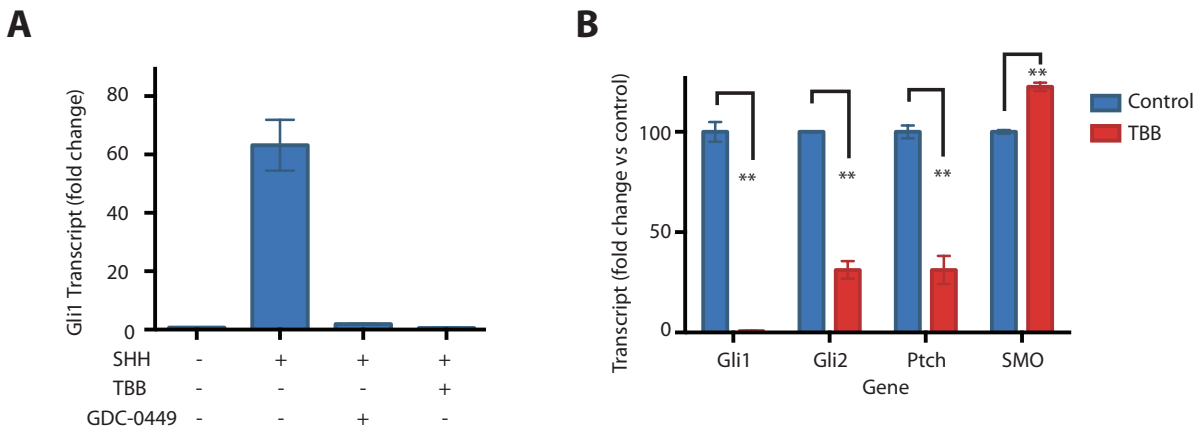

Figure S5. ***Gli1*** **and Hh-associated transcript expression in NIH3T3 cells after CK2 inhibition. (A)** Change in *Gli1* transcript in NIH-3T3 cells after 6 hours of exposure to SHH with or without CK2 inhibitor TBB (50 μM) or SMO inhibitor GDC-0449 (100 nM). **(B)** Change in transcript abundance of Gli2 target genes *Gli1*, *Gli2*, *Ptch* and non-Gli2 target gene *Smo* after exposure to SHH with or without CK2 inhibitor TBB (50 μM). Data were obtained by qRT-PCR and normalized to GAPDH. n=3 (experiments).; error bars = SD.

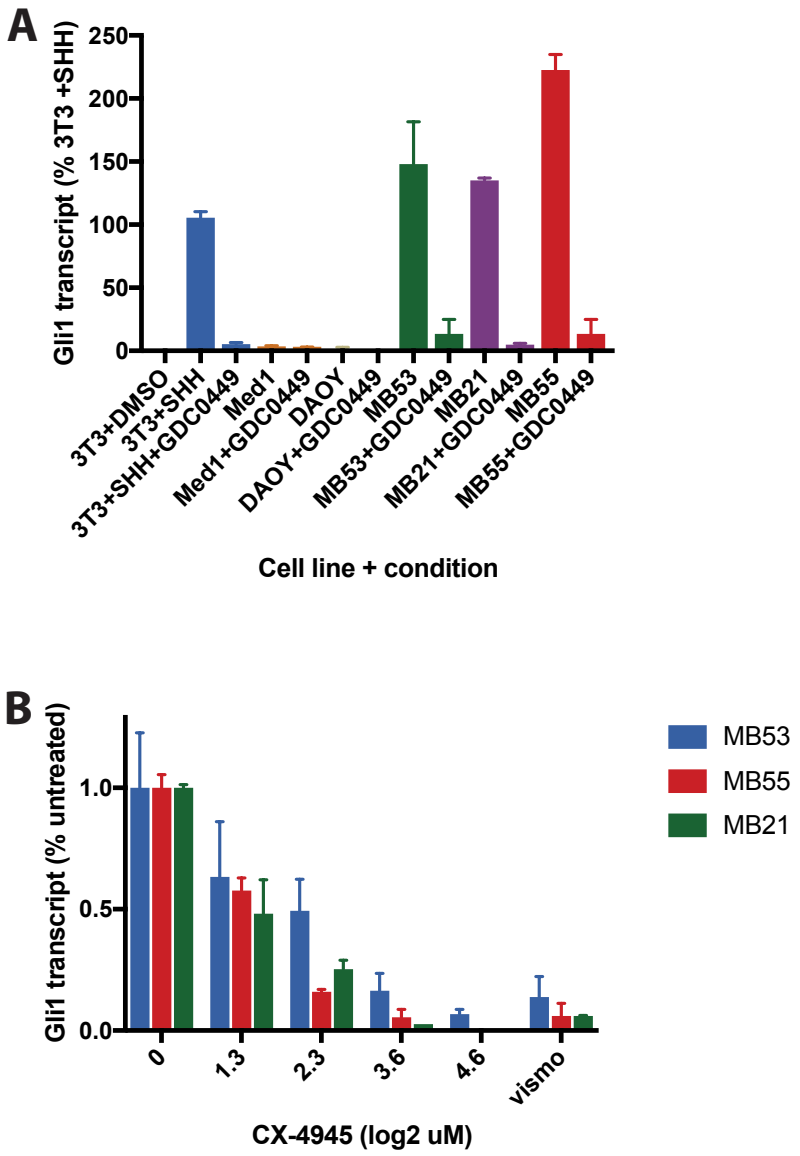

Figure S6. ***Gli1*** **transcript expression in cultured SHH MB cell lines. (A)** *Gli1* transcript expression in Med1, DAOY, MB53, MB21 and MB55 MB cell lines treated with DMSO or GDC-0449 (100nM) for 6 hours, relative to each in NIH3T3 cells cultured in SHH for 24 hours. **(B)** *Gli1* transcript abundance (relative to untreated cells) in MB53, MB55 and MB21 cells treated with CK2 inhibitor CX-4945 (10uM) or vismodegib (100nM GDC-0449; vismo) for 6 hours. Data were obtained by qRT-PCR XXXX and normalized to GAPD XXXX. n=3 (experiments).; error bars = SD.

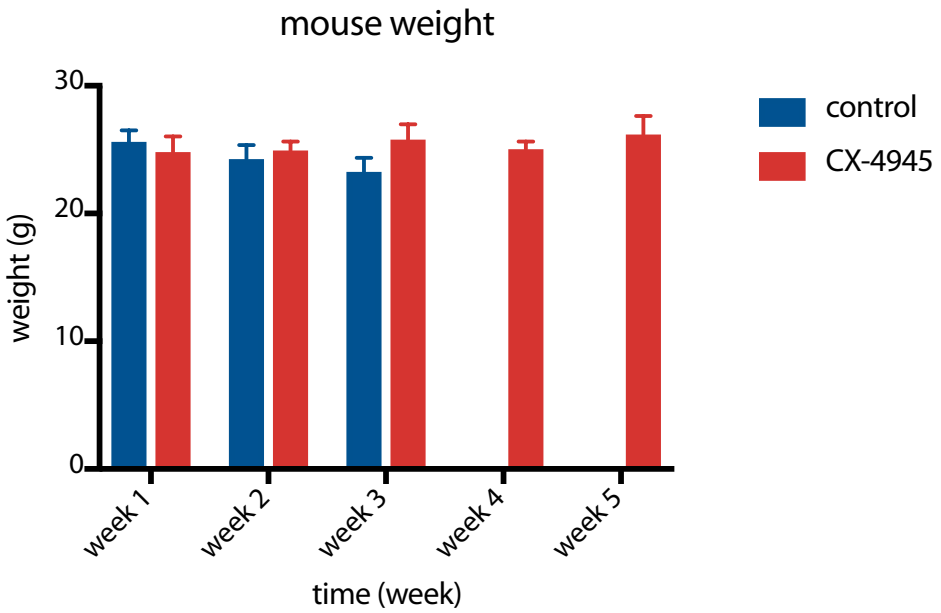

Figure S7. **Weight of control or CX-4945-treated mice with cerebellar SHH MB.** Average weight of mice with *Ptch*$^{+/-}$*;Tpr53*$^{-/-}$*;*SmoD477G cerebellar MB allografts, treated with either the CK2 inhibitor CX-4945 (37.5mg/kg, p.o., twice daily) or vehicle control (DMSO, .p.o, twice daily). Treatment was initiated 72 hours after tumor cells were injected. Week = week following tumor cell injection.XXXXXXXXXX. n=7 mice; error bars = SD.

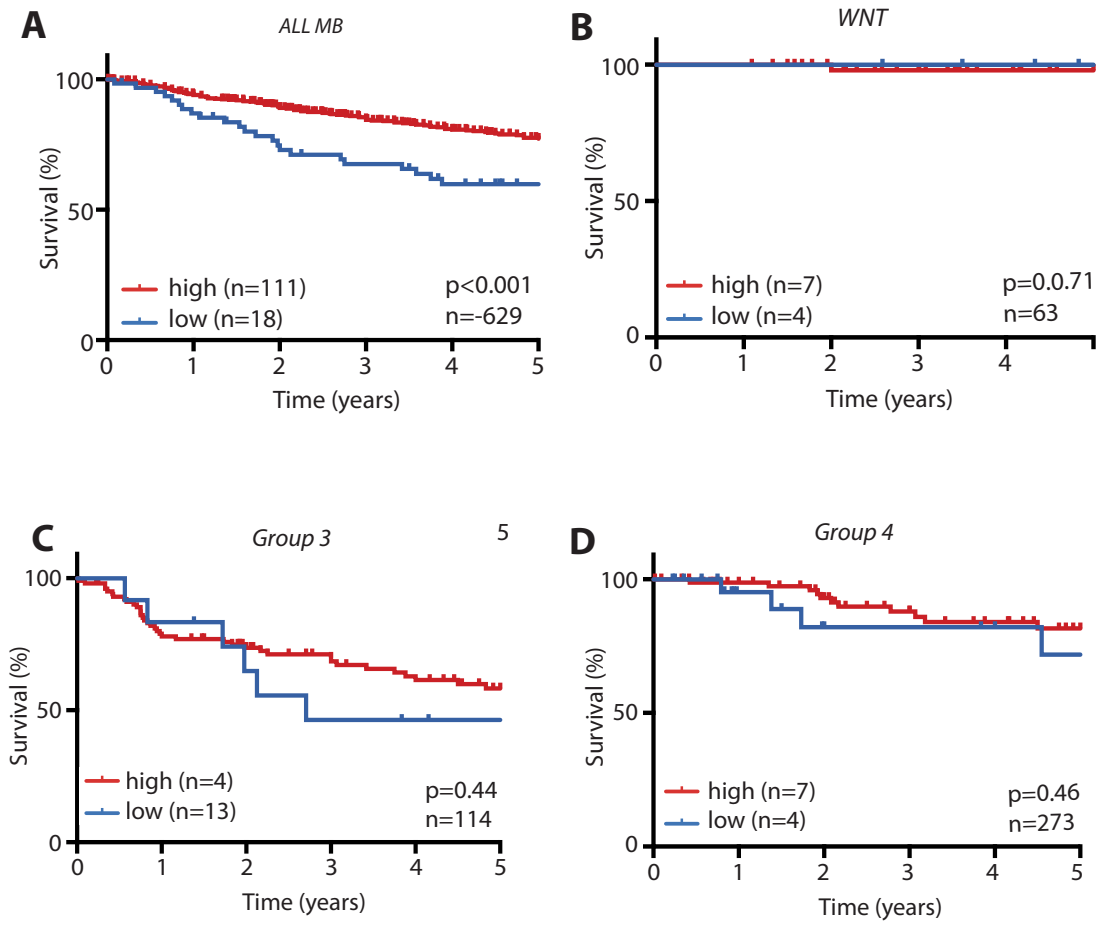

Figure S8. ***CSNK2A1*** **expression and 5-year survival of patients with SHH MB. (A to D)** Kaplan-Meier analysis of *CSNK2A1* expression (encoding CK2) and 5-year survival in all patients with MB (A; n=129), patients with WNT-subtype MB (B; n=11), patients with Group 3-subtype MB (C; n=17) and patients with Group 4-subtype MB (D; n=11).

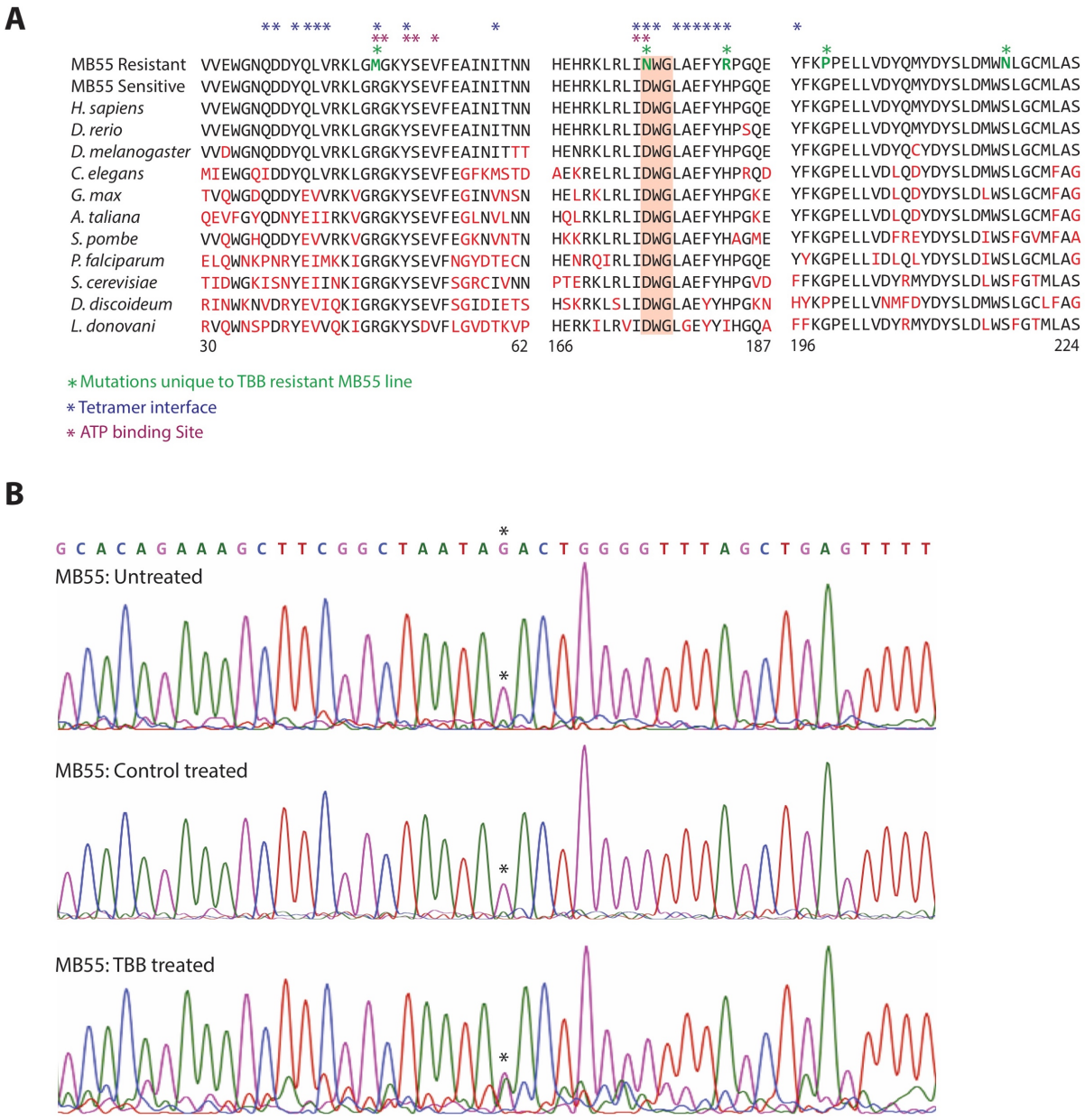

Figure S9. Sequence conservation of CSNK2A1 mutations in TBB-resistant murine SHH MB cells. (A) Peptide sequence conservation of the protein encoded by *CSNK2A1* across species. The 5 mutations found in the TBB-resistant MB55 medulloblastoma cell line are R47M, D175N, G199P and S217N. Green asterisks indicate mutated residues in TBB-resistant MB55 medulloblastoma cell line compared to the original, TBB-sensitive MB55 medulloblastoma cell line. Blue asterisks indicate residues which interact with other CK2 subunits (Csnk2a2 and Csnk2b). Purple asterisks indicate ATP-binding domain. Pink box: DWG domain. (B) Electropherogram of the DNA sequence in untreated, control (DMSO)-treated and TBB-treated

MB55 cells. Asterisk in each indicates the position of the DNA mutation resulting in Asp^{175}-to-Asn^{175} amino acid change.

Table S1: Phosphoproteome of GNPs isolated at P1, P7 and P14. Complete phosphoproteome of GNPs isolated from mouse cerebellum dissected at P1, P7 and P14. Every time point was performed in biological triplicate (each replicate = 15-40 cerebellum). A single murine SHH MB sample, obtained from a Ptch+/- mouse, is included for comparison.

Table S2: Phosphopeptides of enriched kinase motifs. Phosphopeptides enriched in 6 protein kinase CK2 consensus motifs that were prominent among 1,522 protein sequences that had phosphorylation changes in mouse GNPs between P1, P7 and P14.

siRNA sequences	
Csnk2a1-1	Sense: cau uga auu aga ucc acg udTdT Antisense: acg ugg auc uaa uuc aau gdTdT
Csnk2a1-2	Sense: aac auu gaa uua gau cca cgu dT dT Antisense: pac gug gau cua auu caa ugu u dT dT
Csnk2a2-1	Sense: aag aag aua aaa cga gag gdTdT Antisense: dTdT uuc uuc uau uuu gcu cuc c
Csnk2a2-2	Sense: aaa aga aga uaa aac gag agg dT dT Antisense: pcc ucu cgu uuu auc uuc uuu u dT dT
Csnk2b-1	Sense: aag uac cag caa gga gac u dTdT Antisense: agu cuc cuu gcu ggu acu u dTdT
Csnk2b-2	Sense: aaa agu acc agc aag gag acu dT dT Antisense: pag ucu ccu ugc ugg uac uuu u dT dT

Primer sequences	
Csnk2a1-1	Forward: CCCCGAGAGTACTGGGATTA Reverse: TTCAAAAACCAAGGCAGGG
Csnk2a1-2	Forward: TGTTCGAAAATTAGGTAGGGGCA Reverse: CTGGCCAGCATACAACCCAA

Table S3: Primer and siRNA sequences. Sequences for the CK2 siRNAs and *Csnk2a1* primers used in this study. siRNA sequences were kindly provided by Dr. David Seldin, (Boston University School of Medicine).

Brain, left cerebellum, resection

Integrated diagnosis:	medulloblastoma, SHH-activated,TP53 wild-type, no amplification of MYC or MYCN PTCH1 not deleted
Histological classification:	medulloblastoma, anaplastic variant WHO grade IV
Molecular information:	SHH molecular subgroup (IHC) p53 wild-type pattern (IHC) no significant gain or amplification of MYCN no significant gain or amplification of MYC no deletion of PTCH1

Comments:
This medulloblastoma is in the anaplastic variant by histology and the SHH molecular subgroup by immunophenotype. Immunoreactivity for p53 is present in the minority of tumor cells. Significant gain or amplification of MYC or MYCN is not appreciated by FISH. Similarly, PTCH1 deletion is not appreciated.

Table S4: Pathological report for the primary human MB sample (ST01). Pathology information on the primary human MB sample obtained from surgery, grown in mice, and cultured ex vivo for various assays in this study (Fig. 5).

Movie S1:

Movie S2: