Supplementary Information for
O-GlcNAcylation Promotes Cerebellum Development and Medulloblastoma Oncogenesis via SHH Signaling

Liping Chen\textsuperscript{a,1}, Ying Li\textsuperscript{a,1}, Zhihong Song\textsuperscript{a}, Saisai Xue\textsuperscript{b}, Fengjiao Liu\textsuperscript{a}, Xin Chang\textsuperscript{a}, Yan Wu\textsuperscript{a}, Xiaotao Duan\textsuperscript{c,2}, Haitao Wu\textsuperscript{a,b,d,e,2}

\textsuperscript{1}L.C. and Y.L. contributed equally to this work
\textsuperscript{2}To whom correspondence may be addressed. Email: wuht@bmi.ac.cn or xduan@ncba.ac.cn

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Materials and Methods

Mouse strains, genotyping and drug treatment

hGFAP-Cre (JAX: 004600), Atoh1-Cre (JAX: 011104), OGT^F/F (JAX: 004860) and R26SmoM2 (JAX: 005130) mice were maintained on a C57BL/6 background as previously described (1-4). Gli1 lacZ reporter (Gli1^lz/+) mice (JAX: 008211) (a gift from Dr. Yizheng Wang at Beijing Institute of Basic Medical Sciences) were maintained on a 129S6/SvEvTac background as previously described (5). OGT^F/F mice were crossed with hGFAP-Cre and Atoh1-Cre mice respectively to generate heterozygotes. Conditional OGT knockout (OGT^hGFAP and OGT^Atoh1 cKO) mice were obtained in the first and second generation, and OGT^F/F mice were used as controls (Ctrl). To generate Ctrl and OGT^hGFAP cKO mice that carry Gli1 lacZ reporter gene, Gli1^lz/+ mice were crossed with OGT^F/F mice to generate double heterozygotes. Heterozygotes were then crossed with hGFAP-Cre; OGT^F/F heterozygous mice. To generate OGT and SmoM2 double mutant mice (SmoM2^Atoh1; OGT^Atoh1), SmoM2 mice were crossed with OGT^F/F mice to generate heterozygotes. Heterozygotes were then crossed with Atoh1-Cre; OGT^F/F heterozygous mice. All mice were bred at the age between 8-12 weeks. For all experiments, male or female mice were selected randomly. The ages and development stages of the mice at analysis are shown in the figures and figure legends.

All brains used for phenotypic analysis were isolated from mice of a specific genotype crossed for more than five generations onto C57BL/6 background. The day of vaginal plug was considered embryonic day 0.5 (E0.5). The day of birth was considered postnatal day 0 (P0). All animals were housed in standard conditions on a 12-h light/dark cycle with ad libitum access to food and water.

All animal experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of the Beijing Institute of Basic Medical Sciences (#SYXK2019-0004). All sequences for mice genotyping are listed in the Supplementary Table S1.

For drug treatments, SmoM2^Atoh1 mice were gavaged with 12 µg/g OSMI-1 (Sigma-Aldrich, SML1621) or an equal volume of vehicle control (DMSO) daily for 16 consecutive days from P14 to P30. To induce cerebellar cell apoptosis, WT C57BL/6 mice were i.p. injected with 100 µL 20% ethanol (dissolved in 0.9% NaCl) at P7 sacrificed 2 h later for analysis (6).

Cell culture, transfection and OGT/OGA inhibitor treatment

HEK293T (ATCC, CRL-11268™) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, C11995500BT) supplemented with 10% FBS (Gibco, 10091148) in a 37 °C incubator with a humidified, 5% CO2 atmosphere. C3H/10T1/2, Clone 8 (NIH-3T3) (ATCC® CCL-162™) were cultured in Basal Medium Eagle (BME) (Gibco, 41010109) supplemented with 10% FBS (Gibco, 10091148), 2 mM L-glutamine (ATCC 30-2214) in a 37 °C incubator with a humidified, 5% CO2 atmosphere (7). Daoy cells (ATCC® HTB-186™) were cultured in Eagle's Minimum Essential Medium (EMEM) (ATCC, Catalog No. 302003) supplemented with 10% FBS (Gibco,
C11995500BT) in a 37 °C incubator with a humidified, 5% CO₂ atmosphere. NIH-3T3 and Daoy cells were purchased from ATCC.

HEK293T cells and NIH-3T3 cells were transfected using Lipofectamine 2000 (Invitrogen, 11668019) for 48 h according to the manufacturer’s protocol. Daoy cells were treated with OSMI-1 (Sigma-Aldrich, SML1621) at the final concentration of 20 μM or 40 μM; TM-G (Sigma-Aldrich, SML0244) at the final concentration of 40 μM or 80 μM; or DMSO as control. Cells were treated for 5 days for EdU and Ki67 proliferation studies, and 21 days for single-cell colony formation assay, respectively.

**Luciferase assay**

HEK293T or NIH-3T3 cells were transfected with expression plasmids for mouse Gli2 or human GLI2 and mutants, in the presence or absence of OGT, the pRL-TK luciferase reporter as internal reference, and four different luciferase reporter plasmids, pGL4.2-8GBS, pGL3.0-Bcl2 promoter, pGL3.0-Bcl2 enhancer, pGL4.2-Foxm1 (gifts from Dr. Shiwen Luo at Nanchang University), respectively, using Lipofectamine 2000 (Invitrogen, 11668019). Empty pCMV6 was used to maintain equal DNA amounts for transfection. Cells were harvested at 48 h post transfection. Luciferase activity was measured according to the manufacturer’s instructions (Promega, E1910). Data were normalized for transfection efficiency by calculating the ratio between firefly luciferase activity and Renilla luciferase activity, as previously described (8).

**Immunofluorescence staining and image processing**

Mice were anaesthetized with 1% (wt/vol) sodium pentobarbital (6 g/kg) (Sigma-Aldrich, 52944-66-8) and perfused through the left cardiac ventricle with 0.9% NaCl, followed by 4% paraformaldehyde (PFA) (Solarbio, P1110). Brains were removed and post-fixed overnight at 4 °C, cryoprotected in 15% and 30% sucrose (Sigma-Aldrich, 57-50-1) for 48 h at 4 °C, respectively, and embedded into optimal cutting temperature (O.C.T.) (SAKURA, #4583) prior to cryo-sectioning on a cryostat. Sagittal sections (20-40 μm) were taken through the midline of the cerebellum. Sections were mounted onto slides and dried at room temperature for 30 min. Tissue slides or primary spheres were rehydrated with PBS for 15 min, washed with PBST (PBS + 1% Triton X-100) for 30 min, blocked in 3% BSA in PBST (PBS + 0.3% Triton X-100) for 1 h at room temperature (RT) and incubated with primary antibodies (diluted in 3% BSA (Sigma-Aldrich, 1933) overnight at 4 °C. Primary antibodies and dilutions (diluted in 3% BSA in 0.3% PBST) were as following: anti-OGT (Abcam, ab96718, 1:200), anti-O-GlcNAc (Abcam, ab264496, 1:300), anti-NeuN (Abcam, ab104224, 1:400), anti-Calbindin (Sigma-Aldrich, C9848, 1:400), anti-BLBP (Abcam, ab32423, 1:400), anti-BrdU (Abcam, ab6326, 1:800), anti-Pax6 (MBL, PD022, 1:400), anti-Ki67 (BD Bioscience, 550609, 1:400), anti-Cleaved-Caspase-3 (Cell Signaling Technology, 9661, 1:400). After three washes in PBS at 5 min each, Alexa Fluor 568- or Alexa Fluor 488-conjugated fluorescent secondary antibodies (Biotium, 20012, 20101, 20102, 20018) (diluted in PBST + 3% BSA) were incubated at RT for 3 h (1:500). Following several washes with PBS, sections were
counterstained and mounted with fluorescent mounting medium DAPI (ZSGB-BIO, ZLI-9557). All images were acquired with an Olympus FV-1200 confocal microscope (Tokyo, Japan).

For immunofluorescence staining with anti-OGT (Abcam, ab96718, 1:400) and homemade anti-GLI2 S358 O-GlcNAc (1:500) in Daoy cells, cells were fixed in 4% paraformaldehyde for 10 min, washed with PBS thrice at 5 min each, punched in PBST (PBS + 0.5% Triton X-100) for 10 min, blocked in 3% BSA in PBS for 1 h at RT and incubated with primary antibodies (diluted in 3% BSA) overnight at 4 °C. Primary antibodies and dilutions (diluted in PBS + 3% BSA) were as following: anti-OGT (1:400), anti-GLI2 S358 O-GlcNAc (1:500). After three washes with PBS at 5 min each, Alexa Fluor 568-conjugated fluorescent secondary antibodies (diluted in PBS + 3% BSA) at RT for 3 h (1:500). Following several washes with PBS, cells were counterstained and mounted with fluorescent mounting medium DAPI (ZSGB-BIO, ZLI-9557). All images were acquired with an Olympus FV-1200 confocal microscope (Tokyo, Japan).

**BrdU and EdU incorporation assay**

GNP proliferation in the EGL was detected by co-labeling of bromo-2’-deoxyuridine (BrdU) (Roche, 10280879001) and 5-ethynyl-2’-deoxyuridine (EdU) (Sigma-Aldrich, 900584). Ctrl, OGT<sup>hGFAP</sup> cKO and OGT<sup>Atoh1</sup> cKO mice were firstly intraperitoneally injected with 50 mg/kg BrdU at P6. After 23 hours they were intraperitoneally injected with 10 mg/kg of 5-ethynyl-2’-deoxyuridine (EdU) (Sigma-Aldrich, 900584), and sacrificed 1 h later. BrdU/EdU co-localization staining was performed as previously reported (9-11). The ratios of EdU+/BrdU+ to BrdU+ and EdU+/BrdU- to EdU+ cells were counted to evaluate GNP proliferation. The slices were stained with EdU according to the manufacturer’s instructions (RiboBio, C00003). The sections were incubated in 2 N HCl at 37 °C for 30 min and then washed 3 times with 0.1 M borate buffer (pH 8.4). Immunofluorescent staining for BrdU was then performed as above with anti-BrdU antibody (Abcam, ab6326, 1:800).

For in vitro characterization of proliferating cells by EdU incorporation, primary GNPs and Daoy cells were incubated in medium containing 10 μg/mL EdU for 2 h. The cells were then fixed in 4% paraformaldehyde for 10 min, washed with 1% Triton X-100 PBS for 30 min, incubated in Apollo reaction solution for half an hour, then washed with 0.5% Triton X-100 PBS for 3 times. The cells were then rinsed two times with methanol, and mounted with medium containing DAPI (ZSGB-BIO, ZLI-9557).

All images were obtained with FV10-ASW 3.0 viewer software (Olympus, Japan). Given that the typical lobules were significantly disrupted in OGT<sup>hGFAP</sup> and OGT<sup>Atoh1</sup> cKO mice, to quantitatively analyze and compare the BrdU/EdU labelled proliferating GNPs within the EGL between Ctrl and OGT cKO mice, we randomly chose five different rostral regions (including lobule I to the rostral half of lobule VI) of the cerebellum vermis from five pairs of Ctrl and OGT cKO mutants for comparisons in all experiments.

**Nissl, hematoxylin and eosin (H&E), and X-gal staining**
For Nissl staining, slices were washed with PBS for 3 times, 5 min each, and immersed in 0.5% tar-violet solution (Beyotime, C0117) for 20 min. The slices were then quickly rinsed in distilled water and differentiated in 95% ethanol for 2 min. They were then sequentially dehydrated in 75% ethanol, 90% ethanol and 100% ethanol for 30 s each. Finally, the slices were sealed with neutral resin.

For H&E staining, slices were washed PBS for 3 times, 5 min each, and immersed into hematoxylin solution (Beyotime, C0105M) for 10 min. The slices were then quickly rinsed in distilled water and immersed into eosin (Beyotime, C0105M) for 3 min. They were then sequentially dehydrated in 75% ethanol, 90% ethanol and 100% ethanol for 30 s each. Finally, the slices were sealed with neutral resin.

For X-gal staining, slices were rinsed in diluent (2 mM MgCl₂, 0.01% deoxycholic acid sodium salt, 0.02% NP-40) for 10 min. The slices were then rinsed in the X-gal staining buffer ([1 mg/mL X-gal (Promega, V394A), 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide]) for 24 h at 37 °C. The slices were then washed with PBS for 3 times, 5 min each, and sealed with neutral resin.

Immunohistochemistry of human medulloblastoma tissue microarray slides

The tissue microarray slides of human medulloblastoma were purchased from Alenabio (BC17012c), Avilabio (DC-Bra01022) and Bioaitech (N035Cb01). After deparaffinization and rehydration of the sections, antigen retrieval was performed at 100 °C for 20 min in sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0). Endogenous peroxidases were neutralized with 3% peroxide for 15 min. Slices were blocked with a buffer (3% BSA in 0.3% Triton X-100) for 1 h and incubated overnight with primary antibodies. The primary antibodies used were anti-OGT (Abcam, ab96718, 1:100), anti-GAB1 (Abcam, ab59362, 1:50), and the custom rabbit-origin anti-Gli2 S355 O-GlcNAc antibody developed using O-GlcNAcylated peptide [KQSS(O-GlcNAc) ESAVC] as the antigen by ABclonal technology (Wuhan, China, 1:100). The signals were detected with the mouse and rabbit SPN kit (ZSGB-BIO, PV-9000).

RNAscope assay

Brains from OGT^hGFAP cKO and control littermates at P3 and P7 were dissected on ice and immersed into cooled isopentane. The whole brains were then snap-frozen in liquid nitrogen-chilled isopentane, embedded into O.C.T. (SAKURA, # 4583) and stored at - 80 °C. Before sectioning, the tissue blocks were equilibrated at - 20 °C for at least 1 h in a cryostat. Sagittal sections (10-15 μm) were taken through the midline of the cerebellar vermis. Sections were mounted onto slides and dried at RT for 30 min. For RNA-fluorescence in situ hybridization (FISH) assay, both Gli1 and Gli2 RNA (ACD, Cat No.311001 and 405771) was stained with the RNAscope multiplex fluorescent reagent kit v2 (ACD, Bio-technne Ltd., UK). RNAscope procedures were completed according to the manufacturer’s instruction and protocol.

Plasmid construction and lentiviral packaging
Mouse Gli2 expression plasmid pCEFL3xHAMGli2 was purchased from Addgene (#37671). The cDNA of mGli2 in the pCEFL3xHAMGli2 was reorganized to incorporate the pCMV6 vector through PCR. The Gli2 mutants S273A, S355A, S361A/T362A, T791A, S844A and S355D, and human Gli2 mutants S79A, S96A, S358A, S364A/T365A, T810A, and S358D were generated with PCR using Mut Express MultiS Fast Mutagenesis Kit V2 (Vazyme, C215). The pCMV6-mGli2 and human Gli2 plasmids were amplified with point mutation primers detailed in Supplementary Table S1. The PCR product was digested with methylated DNA-specific endonuclease, DpnI, to eliminate template DNA that contained methylated nucleotides. The resulting plasmids were used to transform E. coli DH1 chemically competent cells (Transgen, CD511). After 12 h, single clones were selected and sequenced.

To generate the endogenous mGli2/hGLI2 deletion and exogenous Gli2/hGLI2 overexpression vectors, H1-shRNA sequences of mGli2 and human GLI2 were synthesized by RuiBiotech (Beijing, China) and the sequence is listed in the Supplementary Table S1. A 276 bp fragment of H1-mGli2-shRNA and a 280 bp fragment of H1-GLI2-shRNA were obtained from pUC57-mGli2/hGLI2-shRNA vector by PacI (Thermo, FD2204) digestion, respectively, and transferred to the PacI-digested FUGW vector (a gift from Dr. Zilong Qiu at Center for Excellence in Brain Science and Intelligence Technology, CAS) to construct the FUGW-mGli2/hGLI2-shRNA vector. The direction of H1-mGli2/hGLI2-shRNA fragment in FUGW-mGli2/hGLI2-shRNA was confirmed by DNA sequencing.

To construct the mGli2/hGLI2 overexpression FUGW-mGli2-shRNA plasmid, six bases were mutated in pCMV6-mGli2/hGLI2-WT plasmid to decrease the knockdown effect of shRNA, without changing the mGli2/hGLI2 amino acid sequence. mGli2 S355/hGLI2 S358 in the mutated pCMV6-mGli2/hGLI2-WT plasmid was subsequently mutated into alanine using the Mut Express MultiS Fast Mutagenesis Kit V2 (Vazyme, C215). WT Gli2/GLI2 and S355A/S358A DNA segments were amplified via PCR and purified through agarose gel electrophoresis. To achieve site-specific mutagenesis, S355A/S358A fragments were transferred to the BamHI (Thermo, FD0054) and NotI (Thermo, FD0593) digested FUGW-mGli2/hGLI2-shRNA vector using the ClonExpress Ultra One Step Cloning Kit (Vazyme, C115). The plasmids were then used to transform E. coli DH5α competent cells. Single clones were selected and sequenced. The procedures of FUGW-shRNA-hGLI2-WT and FUGW-shRNA-hGLI2-S358A construction were similar with the processes above as previously described (12).

To construct the Lenti-CRISPR-V2-OGT sgRNA-GFP plasmid, pairs of gRNAs targeting the human OGT were selected from the CRISPOR design tool (http://crispor.tefor.net/) (13). The sequences of the gRNA are listed in Supplementary Table S1. The oligos were synthesized and inserted into the Lenti-CRISPR-V2-GFP vector (a gift from Dr. Jun Yao at Tsinghua University) according to a previous report (14). The plasmid was validated by sequence analysis. For the construction of the pKH3-p300 plasmid, the full-length p300 coding sequence (CDS) was amplified via PCR from the pCMV-p300 plasmid, and the pKH3 vector was digested with NotI (Thermo, FD0593) and Xhol.
Insertion of the p300 CDS was accomplished using ClonExpress Ultra One Step Cloning Kit (Vazyme, C115). The product was used to transform *E. coli* DH5α competent cells, and single clones were selected and sequenced. For the construction of pEGFP-C1-Sufu plasmid, full-length Sufu CDS was amplified via PCR from the pCMV-Sufu plasmid. The pEGFP-C1 vector was digested with HindIII (Thermo, FD0504) and BamHI (Thermo, FD0054), which was then inserted with the amplified Sufu product using ClonExpress Ultra One Step Cloning Kit (Vazyme, C115). The plasmid was directly used to transform *E. coli* DH5α competent cells, and single clones were selected and sequenced.

The following plasmids were generously provided by other laboratories: pEGFP-C1-OGT and pEGFP-C1-OGT H498A/H558A double mutant expression plasmids (Dr. Xiaotao Duan at Beijing Institute of Pharmacology and Toxicology); p300 expression plasmid (Dr. Huiyan Li at National Center of Biomedical Analysis). pGL4.2-8GBS, pGL3.0-Bcl2 promoter, pGL3.0-Bcl2 enhancer, pGL4.2-Foxm1 luciferase reporter plasmid, pRL-TK, pKH3-3xHA-Sufu and pCMV6-hGLI2 (Dr. Shiwen Luo at Nanchang University); pFlag-HDAC1 (Dr. Huiyan Li, National Center of Biomedical Analysis, Beijing, China); pKH3-Ubiquitin (Dr. Jiyan Zhang, Beijing Institute of Basic Medical Sciences).

For lentiviral packaging, FUGW-shC, FUGW-shGli2, FUGW-shGli2+Glii2R, FUGW-shGli2+S355A, FUGW-shGli2+GLI2R, FUGW-shGli2+S358A, FUGW-shGLI2+ GLI2R, and FUGW-shGLI2+S358A plasmids were co-expressed with VSVG and psPAX2 in HEK293T cells at the ratio of 5:3:2. The medium containing virus was collected at 36 and 72 h, respectively. The medium was centrifuged at 4000 rpm and 4 °C. The resulting supernatant was filtered twice through 0.45 μm filter, and subsequently centrifuged at 80,000 g for 2 h at 4 °C. Lentivirus precipitation was resolved in DMEM/F-12 (Gibco, 11330500) medium or EMEM medium (ATCC, 302003). The titer of virus was determined by real-time quantitative PCR.

**GNP neurosphere isolation, lentivirus infection and OGT/OGA inhibitor treatment**

Primary GNPs were isolated from the cerebellum of P5 Ctrl and OGT^{TGFAP} cKO mice according to our previously published work (11). First, the cerebella from anaesthetized mice was isolated in primary medium [DMEM/F-12 containing 2% B27 (Gibco, 17504044), 20 ng/mL recombinant human epidermal growth factor (EGF) (Gibco, PHG0311L) and 20 ng/mL basic fibroblast growth factor (bFGF) (Gibco, 13256029)] on ice. After removing the meninges, the cerebellum was isolated and chopped into small pieces, which were subsequently incubated with a mixture of primary medium and 0.25% trypsin (Gibco, 25200056) (v:v /1:1) at 37 °C for 30 min. The trypsin reaction was stopped with 10% FBS and centrifuged at 1000 rpm for 5 min. The cells were then rinsed with primary medium and filtered through a 70 μm cell strainer. The filtered cells were sucked out with a pipette and gently added to the top of 10% Percoll (GE Healthcare, 17089102) separation solution along the tube wall. The solution was then centrifuged at 200 g for 20 min. The upper layer solution was gently sucked out and discarded. Next, the interlayer cell solution was gently isolated with a
Pasteur pipette and transferred into a new tube. Fresh primary medium was added into the tube. The purified GNP and granule cells were then gently dispersed with pipetting to a single cell suspension and centrifuged at 1000 rpm for 5 min. The centrifuged cells were resuspended with primary medium and seeded at a density of 1-2 x 10^6 in T12.5 cell culture flasks, and cultured at 37 °C and 5% CO₂.

For lentiviral infection, viruses containing LV-GFP, LV-Cre-GFP (Hanbio), FUGW-shC, FUGW-shGli2, FUGW-shGli2+Gli2R, FUGW-shGli2+S355A, FUGW-shGli2+GLI2R, FUGW-shGli2+S358A (Titer: 1 x 10^8 TU/mL, 10 μL) were added to the medium 4 h after seeding primary GNPs, respectively. For OGT/OGA inhibitor treatment, primary spheres were treated with OSMI-1 (10 μM or 20 μM), TM-G (40 μM or 80 μM) or DMSO as control 24 h after seeding primary GNPs. For combined lentiviral and chemical treatment, lentivirus was first added to the medium 4 h after seeing primary GNPs, 8 h later, the cells were treated with 10 μM OSMI-1, 80 μM TM-G or DMSO as control. After 6 days, cells were collected for further analysis.

To validate the specificity of anti-OGT and anti-GLI2 S358 O-GlcNAc antibodies, Daoy cells were infected with lentiviruses including lenti-CRISPR-V2, lenti-CRISPR-V2-OGT sgRNA, FUGW-shGLI2+GLI2R, and FUGW-shGLI2+S358A (Titer: 1 x 10^8 TU/mL, 10 μL), respectively, 4 h after seeding Daoy cells. Single-cell clone of lenti-CRISPR-V2 or lenti-CRISPR-V2-OGT sgRNA virus-infected Daoy cells were selected, and the knockout effect was verified by immunofluorescence staining with anti-OGT and anti-GLI2 S358 O-GlcNAc antibodies.

**Protein digestion and liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS)**

Endogenous mouse Gli2 from the cerebellum of WT mice at P7 and human GLI2 from Daoy cells were enriched by co-immunoprecipitation with anti-Gli2 and purified with Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The gel was then stained with Coomassie brilliant blue and the corresponding visualized bands were excised, de-stained with ammonium bicarbonate buffer and dehydrated in 75% acetonitrile. Following rehydration with 50 mM ammonium bicarbonate, the gel was digested with trypsin or chymotrypsin overnight. The peptides were then extracted with acetonitrile containing 0.1% formic acid and vacuum dried. MS was performed as previously described (15).

C57BL/6 mice at P15 were intraperitoneally injected with OSMI-1 (62.5 mg/kg and 125 mg/kg) or gavaged with OSMI-1 (25 mg/kg and 50 mg/kg) daily for 10 consecutive days, respectively, and with DMSO treatment as control. The mice were anaesthetized with 1% (wt/vol) sodium pentobarbital (6 g/kg) at P25. Brain tissue from anaesthetized mice were isolated in 0.9% NaCl, and the meninges and blood vessels were subsequently completely removed on ice. The tissue was washed with 0.9 % NaCl three times and homogenized with 0.9% NaCl (w/v: 1:3) on ice. For each sample, 20 μL homogenate was mixed with 20 μL acetonitrile and 100 μL acetonitrile containing 10 ng/mL propranolol (Superlco, P-055) as internal standard. The mixture was vortexed
for 1 min and centrifuged at 18,880 g for 10 min at 4 °C. 80 μL supernatant was acquired and added into the injection tube, and 2 μL sample was analyzed using an API 5000 mass spectrometer.

**Immunoprecipitation and immunoblotting**

Mouse cerebellum, primary GNP spheres, HEK293T, NIH-3T3, and Daoy cells were collected and lysed in a buffer containing 50 mM Tris (pH 7.5), 150 mM NaCl, 10 mM MgCl2, 1% Triton X-100, 10% glycerol, 1 mM EDTA, 1 mM PMSF (Beyotime, ST507), and 1% protease inhibitors (Thermo Fisher Scientific, A32965). Lysates were centrifuged at 12,000 rpm and 4 °C for 15 min. Protein samples were subjected to immunoprecipitation using primary antibodies and protein A/G magnetic agarose beads (Thermo scientific, 20423). Precipitated immunocomplexes were washed 6 times with washing buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, and 1% Triton X-100) and analyzed with immunoblot. Protein supernatants were boiled with the loading buffer for 5 min. electrophoresed in SDS-PAGE gels and transferred onto Polyvinylidene fluoride (PVDF) membranes (Millipore, IPFL00010). The membranes were blocked in PBST buffer containing 5% fat-free milk (w/v) for 1 h at RT, incubated with the corresponding antibody at 4 °C overnight, and then incubated with the secondary antibody conjugated to horseradish peroxidase for 2 h at RT. Membranes were stained with standard ECL reagents purchased from Applygen Technologies (Beijing, China) and then photographed by X-ray.

Primary antibodies used for Western blot and co-immunoprecipitation are as follows: Ptch1 (Novus, NBP1-71662, 1:1000), Ptch2 (Novus, NB200-119, 1:1000), OGT (Novus, NBP1-32791, 1:3000), Gli2 (Abcam, ab26056, 1:1000; Santa Cruz Biotechnology, sc-271786, 1:50), Gli3 (Abcam, ab123495, 1:1000), O-GlcNAc (Abcam, ab264496, 1:1000), Gli1 (Cell Signaling Technology, 2534, 1:1000), Sufu (Cell Signaling Technology, 2522, 1:2000), Acetylated-Lysine (Cell Signaling Technology, 9441, 1:1000), SMO (Santa Cruz Biotechnology, sc-166685, 1:100), Flag (Sigma-Aldrich, F1804, 1:3000), HA (Sungene Biotech, KM8004, 1:2000), Myc (Sungene Biotech, KM8003, 1:2000), GFP (Sungene Biotech, KM8009, 1:2000), β-actin (Sungene Biotech, KM9001T, 1:3000).

**RNA-Sequencing and bioinformatics analysis**

tdTomato+ cells from the cerebellum of Atoh1-Cre; Ai9 (Ctrl; Ai9) and OGT<sup>Atoh1</sup> cKO mice were collected using Fluorescence Activating Cell Sorter (FACS). Total RNA was extracted with TRIzol (Invitrogen, 15596018), following the manufacturer’s instructions. RNA quality and concentration were determined by NanoDrop spectrophotometer (Thermo Fisher Scientific). High-throughput sequencing was performed by ANOROAD using the Illumina Novaseq 6000 platform. Data analysis was performed according to Cuffdiff and R as previously described (16). Differentially expressed genes (DEGs) between the Ctrl and OGT<sup>Atoh1</sup> cKO mice were defined by 1.5-fold cutoff. GO analysis was performed with an online tool (https://www.networkanalyst.ca/faces/home.xhtml).
Multiple tests were adjusted by Benjamini-Hochberg method with `p.adjust` function in R (17, 18). GO terms with FDR < 0.05 was considered statistically significant.

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Fig. S1. High-level expression of O-GlcNAc in the cerebellum.

(A) Immunofluorescent staining with anti-OGT, anti-O-GlcNAc in the cerebellum of Ctrl mice at the indicated postnatal developmental stages. Scale bar: 1 mm.

(B) Immunofluorescent staining with anti-Pax6 and anti-O-GlcNAc reveals high level of O-GlcNAc expression in cerebellar GNPs in Ctrl mice at P7 and P14 and co-localization of Pax6 and O-GlcNAc. Scale bar: 1 mm and 200 μm.

(C) Immunofluorescent staining with anti-NeuN, anti-Calbindin, anti-BLBP and anti-O-GlcNAc reveals O-GlcNAc expression in cerebellar GCs, Purkinje cells (PC) and Bergman glial (BG) cells in Ctrl mice at P14 and co-localization of NeuN, Calbindin, BLBP with O-GlcNAc, respectively. Scale bar: 50 μm.

a, anterior; d, dorsal; p, posterior; v, ventral; IGL, internal granular layer; PCL, Purkinje layer; ML, molecular layer; EGL, external granular layer. Related to Fig. 1.
Fig. S2. Low-level O-GlcNAcylation and OGT expression in the OGT<sup>hGFAP</sup> cKO cerebellum.

(A) Immunofluorescent staining with anti-O-GlcNAc and anti-Pax6 in the cerebellum of Ctrl and OGT<sup>hGFAP</sup> cKO mice at P7. Scale bar: 50 μm.

(B-F) Representative immunoblot (B) and quantification of OGT in the cerebellum of Ctrl and OGT<sup>hGFAP</sup> cKO at P3 (C) or P7 (E), and O-GlcNAc in the cerebellum of Ctrl and OGT<sup>hGFAP</sup> cKO at P3 (D) or P7 (F) (n = 3), respectively. Unpaired t-test. All data represent mean ± SEM. ***P < 0.001. IGL, internal granular layer; PCL, Purkinje layer; EGL, external granular layer. Related to Fig. 1.
**Fig. S3.** Disrupted histoarchitecture of cerebellar vermis in both OGTGFAP and OGTAbh1 cKO mice. (A) Immunofluorescent staining with anti-Pax6, anti-NeuN, anti-Calbindin, and anti-BLBP in Ctrl, OGTGFAP, and OGTAbh1 cKO mutant cerebellum at P14. Scale bar: 100 μm. (B) Number of NeuN+ ectopic granule neurons outside the IGL at P14 in Ctrl and OGTGFAP cKO mutant mice (n = 5). Unpaired t-test. (C) Number of Calbindin+ ectopic PCs outside the PCL in Ctrl and OGTGFAP cKO mice at P14 (n = 5). Unpaired t-test. (D) Number of BLBP+ ectopic BGs outside the PCL in Ctrl and OGTGFAP cKO mice at P14 (n = 5). Unpaired t-test. (E) Number of NeuN+ ectopic granule neurons outside the IGL in Ctrl and OGTAbh1 cKO mice at P14 (n = 5). Unpaired t-test. (F) Number of calbindin+ ectopic PCs outside the PCL in Ctrl and OGTAbh1 cKO mice at P14 (n = 5). Unpaired t-test. (G) Number of BLBP+ ectopic BGs outside the PCL in Ctrl and OGTAbh1 cKO mice at P14 (n = 5). Unpaired t-test. All data represent mean ± SEM. ****P < 0.0001. IGL, internal granular layer; PCL, Purkinje layer; ML, molecular layer. Related to Fig. 1.
Fig. S4. Ablation of OGT in NSCs or GNPs disrupts the proliferative ability of GNPs but not apoptosis.

(A) Immunofluorescent staining showing GNP proliferation with anti-Ki67 and anti-Pax6 in Ctrl, \( OG^{T^{\text{hGFAP}} \text{cKO}} \) and \( OG^{T^{\text{Atoh1}}} \text{cKO} \) mutant mice at P7. Boxed areas are shown at higher magnification to illustrate Ki67+ cells in the Pax6+ rostral and caudal EGL areas. Scale bar: 1 mm and 100 \( \mu \)m.

(B) Quantification of the ratio of Ki67+/Pax6+ cells in the cerebellar EGL of Ctrl and \( OG^{T^{\text{hGFAP}}} \text{cKO} \) mice at P7 (n = 5). Unpaired t-test.

(C) Quantification of the ratio of Ki67+/Pax6+ cells in the rostral area of the cerebellar EGL of Ctrl and \( OG^{T^{\text{Atoh1}}} \text{cKO} \) mice at P7 (n = 5). Unpaired t-test.

(D and E) Immunofluorescent staining of apoptotic GNPs by labeling cells with Cleaved-Caspase-3 and NeuN in Ctrl, \( OG^{T^{\text{hGFAP}}} \text{cKO}, \ OG^{T^{\text{Atoh1}}} \text{cKO}, \) and the positive control mice intraperitoneally injected with 20% ethanol at P7, respectively. Scale bar: 1 mm.

(F and G) Quantification of Cleaved-Caspase-3+ cells in the cerebellum of Ctrl, \( OG^{T^{\text{hGFAP}}} \text{cKO}, \ OG^{T^{\text{Atoh1}}} \text{cKO}, \) and ethanol injection mice at P7, respectively (n = 5). One-way ANOVA and Dunnett’s multiple comparisons test.

All data represent mean ± SEM. ****P < 0.0001, n.s., no significance. EGL, external granular layer; ML, molecular layer; PCL, Purkinje layer; IGL, internal granular layer. Related to Fig. 1.
Fig. S5. Ablation of OGT decreases the expression of Gli transcripts in the EGL.

(A and B) The expression of Gli1 transcripts in Ctrl and OGT<sup>hGFAP</sup> cKO mutant cerebellum by RNAscope <i>in situ</i> hybridization at P3 (A) and P7 (B), respectively. Boxed areas are shown at higher magnification to illustrate Gli1 mRNA expression in the EGL. Scale bar: 1 mm and 50 μm, respectively.

(C and D) Quantification of the ratio of Gli1 mRNA level in the EGL of Ctrl and OGT<sup>hGFAP</sup> cKO mice at P3 (C) and P7 (D), respectively (n = 5). Unpaired <i>t</i>-test.

(E and F) The expression of Gli2 transcripts in Ctrl and OGT<sup>hGFAP</sup> cKO mutant cerebellum by RNAscope <i>in situ</i> hybridization at P3 (E) and P7 (F), respectively. Boxed areas are shown at higher magnification to illustrate Gli2 mRNA expression in the EGL. Scale bar: 1 mm and 50 μm, respectively.

(G and H) Quantification of the ratio of Gli2 mRNA level in the EGL of Ctrl and OGT<sup>hGFAP</sup> cKO mice at P3 (G) and P7 (H), respectively (n = 5). Unpaired <i>t</i>-test.

All data represent mean ± SEM. ***P < 0.001, EGL, external granular layer. Related to Fig. 2.
Fig. S6. OGT regulates Shh pathway activation via its enzyme activity

(A) Schematic diagram of the experimental procedure for B-E.

(B-E) Representative immunoblot (B) and quantification of Gli1 (C), Gli2 (D) and OGT (E) in LV-GFP and LV-Cre-GFP treated GNPs (n = 3). Unpaired t-test.

(F-J) Representative immunoblots (F) and quantification of Gli1 (G), Gli2 (H), OGT (I) and O-GlcNAc (J) expression in GNPs treated with OSMI-1, TM-G, or DMSO as control (n = 3). One-way ANOVA and Dunnett’s multiple comparisons test.

(K) Schematic diagram of the experimental procedure for L.

(L) Relative luciferase activity in NIH-3T3 cells co-transfected with luciferase reporter plasmids Bcl2 promoter and Renilla luciferase reporter plasmid pRL-TK for 72 h, and subsequently treated with
DMSO, SAG (0.1 μM) for 48 h, OSMI-1 (7.5 μM or 15 μM) or TM-G (40 μM or 80 μM) for 24 h (n = 3). One-way ANOVA and Dunnett’s multiple comparisons test. All data represent mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. n.s., no significance. Related to Fig. 2.
**Fig. S7.** Protein-protein interaction between OGT and Shh signaling pathway molecules (A-L) Representative blots of co-immunoprecipitation of OGT and Shh signaling pathway molecules Ptc1 (A and B), Ptc2 (C and D), Smo (E and F), Suf (G and H), Gli3 (I and J) and Gli1 (K and L), in cerebellar tissue from WT mice at P7. Related to Fig. 3.
Fig. S8. Potential O-GlcNAc sites in mouse Gli2 and human GLI2 in vivo

(A-D) Potential mouse Gli2 O-GlcNAc site S273 (A), S361 and T362 (B), T791 (C), S844 (D) in vivo. Gli2 was purified from mouse cerebellum and analyzed by MS.

(E-H) Potential human GLI2 O-GlcNAc site S79 (E), S96 (F), S364 and T365 (G), T810 (H) in vivo. GLI2 was purified from Daoy cells and analyzed by MS.

Related to Fig. 3.
Fig. S9. O-GlcNAc of Gli2 S355 promotes its transcriptional activity
(A) Schematic diagram of the experimental procedure for B-I.
(B-E) Relative luciferase activity in HEK293T cells co-transfected for 48 h with pCMV6 control, WT Gli2 or Gli2 mutants S273A, S355A, S361A/T362A, T791A, S844A, together with luciferase reporter plasmids 8GBS (B), Bcl2 promoter (C), Bcl2 enhancer (D), or Foxm1 (E) and Renilla luciferase reporter plasmid pRL-TK (n = 12). One-way ANOVA and Dunnett's multiple comparisons test.
(F-I) Relative luciferase activity in HEK293T cells co-transfected for 48 h with pCMV6 control, WT Gli2 or Gli2 mutant S355D, together with luciferase reporter plasmids 8GBS (F), Bcl2 promoter (G), Bcl2 enhancer (H) or Foxm1 (I) and Renilla luciferase reporter plasmid pRL-TK (n = 21). One-way ANOVA and Dunnett's multiple comparisons test.

All data represent mean ± SEM. ***P < 0.001. Related to Fig. 3.
Fig. S10. O-GlcNAc of human GLI2 occurs at S358

(A) Potential human GLI2 O-GlcNAc site S358 in vivo. GLI2 was purified from Daoy cells and analyzed by MS.

(B and C) Representative immunoblot (B) and quantification (C) of O-GlcNAc levels of immunoprecipitated human GLI2 WT and mutants S79A, S96A, S358A, S364/T365A, T810A. HEK293T cells were transfected for 48 h with Myc-tagged WT or the indicated mutant Gli2 construct (n = 3). One-way ANOVA and Dunnett’s multiple comparisons test.
(D-G) Relative luciferase activity in HEK293T cells co-transfected for 48h with pCMV6 control, GLI2 WT or GLI2 mutants together with luciferase reporter plasmids 8GBS (D), Bcl2 promoter (E), Bcl2 enhancer (F), or Foxm1 (G) and Renilla luciferase reporter plasmid pRL-TK (n = 9). One-way ANOVA and Dunnett's multiple comparisons test.

(H-K) Relative luciferase activity in HEK293T cells co-transfected for 48 h with pCMV6 control, WT human GLI2 or GLI2 mutant S355D, together with luciferase reporter plasmids 8GBS (H), Bcl2 promoter (I), Bcl2 enhancer (J) or Foxm1 (K) and Renilla luciferase reporter plasmid pRL-TK (n = 30). One-way ANOVA and Dunnett’s multiple comparisons test.

All data represent mean ± SEM. ***P < 0.001, ****P < 0.0001. Related to Fig. 3.
Fig. S11. O-GlcNAc of GLI2 S358 mediates the proliferation of GNPs

(A) Schematic diagram of the Lentivirus-Gli2 shRNA-GLI2-Myc-GFP plasmid with endogenous mouse Gli2 knockdown and overexpression of exogenous shRNA-resistant human Gli2 or GLI2 S358A mutant. Four constructs were utilized: shRNA control (shC), Gli2 shRNA knockdown (shGli2), GLI2 shRNA knockdown and overexpression of the shRNA-resistant human GLI2 (shGli2 + GLI2R) or shRNA-resistant human GLI2 S358A mutant (shGli2 + S358A).

(B) Schematic diagram of experimental timeline and procedure for C-J.

(C-E) Representative immunoblot (C) and quantification of the Gli2 (D) or Myc (E) protein level in GNPs 7 days after infection with shC, shGli2, shGli2 + GLI2R or shGli2 + S358A lentivirus, respectively (n = 3). One-way ANOVA and Dunnett’s multiple comparisons test.

(F) Immunofluorescent staining with anti-EdU, anti-Ki67, anti-Pax6 antibodies reveals the proliferation of GFP⁺ or Pax6⁺ GNPs in neurospheres at DIV 6. Scale bar: 100 μm.

(G) The diameter distribution of GNP neurospheres at DIV6 (n = 3). Two-way ANOVA and Tukey’s multiple comparisons test.

(H-J) Quantification of the ratio of EdU⁺GFP⁺ cells to GFP⁺ cells (H), Ki67⁺GFP⁺ cells to GFP⁺ cells (I) and Ki67⁺Pax6⁺ cells to Pax6⁺ cells (J) in GNP neurospheres at DIV 6 (n = 6). One-way ANOVA and Dunnett’s multiple comparisons test.

All data represent mean ± SEM. **P < 0.01, ***P < 0.001, ****P < 0.0001. n.s., no significance. Related to Fig. 4.
Fig. S12. O-GlcNAc of Gli2 S355 does not alter Gli2 ubiquitination nor the binding between Gli2 with HDAC1 or Sufu.

(A) Representative immunoblot of ubiquitinated Gli2 immunoprecipitated from HEK293T cells transfected with pCMV6 control, Myc-tagged Gli2, GFP-OGT and HA-Ub.
(B) Representative immunoblot of ubiquitinated Gli2 immunoprecipitated from HEK293T cells transfected with pCMV6 control, Myc-tagged Gli2 or Myc-tagged Gli2 S355A, and HA-Ub.
(C) Representative immunoblot of HDAC1 immunoprecipitated from HEK293T cells transfected with pCMV6 control or Myc-tagged Gli2, Flag-HDAC1 and GFP-tagged OGT.
(D) Representative immunoblot of HDAC1 immunoprecipitated from HEK293T cells transfected with pCMV6 control or Myc-tagged Gli2 and Flag-HDAC1. The transfected cells were treated with OSMI-1 (10 μM), TM-G (80 μM) or DMSO for 36 h, respectively.
(E) Representative immunoblot of HDAC1 immunoprecipitated from HEK293T cells transfected with pCMV6 control, Myc-tagged Gli2 or Myc-tagged Gli2 S355A, and Flag-HDAC1.
(F) Representative immunoblot of Sufu immunoprecipitated from HEK293T cells transfected with pCMV6 control, Myc-tagged Gli2 or Myc-tagged Gli2 S355A and HA-Sufu.

Related to Fig. 5.
Fig. S13. O-GlcNAc regulates the activation of SHH pathway and the proliferation of human SHH-subtype medulloblastoma in vitro.

(A) OD value (450nm) of Daoy cells stained with CCK8 for 2 h. The cells were treated with OSMI-1 (20 μM, 40 μM), TM-G (40 μM, 80 μM) or DMSO as control for the designated number of days (n = 5). Two-way ANOVA and Tukey’s multiple comparisons test.

(B) Percentage of single cell colony formation from Daoy cells treated with OSMI-1 (20 μM, 40 μM), TM-G (40 μM, 80 μM) or DMSO as control at day 21 (n = 3). One-way ANOVA and Dunnett’s multiple comparisons test.

(C) Immunofluorescent staining of EdU and Ki67. Cells were treated with OSMI-1 (20 μM, 40 μM), TM-G (40 μM, 80 μM) or DMSO as control for 5 days. Scale bar: 100 μm. Boxed area, scale bar: 20 μm.

(D and E) Quantitative analysis of the percentage of dividing EdU+ (D) and Ki67+ (E) cells (n = 5). One-way ANOVA and Dunnett’s multiple comparisons test.

(F-I) Representative immunoblot (F) and quantification of the expression of GLI1 (G), GLI2 (H), and GLI3 (I) in Daoy cells treated with OSMI-1, TM-G or DMSO for 6 days (n = 3). One-way ANOVA and Dunnett’s multiple comparisons test.

All data represent mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. n.s., no significance. Related to Fig. 6.
**Fig. S14.** Detection of OGT inhibitor OSMI-1 in brain lysates by LC-MS/MS

(A and B) MS analysis of OSMI-1 in the mouse brain following oral gavage administration of either OSMI-1 or DMSO as a negative control (A). The same brain lysates with propranolol and OSMI-1 were used as positive controls (B). (a) peak reflecting propranolol (10 ng/mL), which is used as internal standard, (b) peak reflecting OSMI-1.

(C and D) MS analysis (C) and quantification (D) of OSMI-1 in brain lysates from mouse intraperitoneally injected with OSMI-1 (62.5 mg/kg and 125 mg/kg) (n = 6). (a) peak reflecting propranolol (10 ng/mL) as internal standard, (b) peak reflecting OSMI-1, showing its presence in the brain after crossing the blood-brain barrier. One-way ANOVA and Dunnett’s multiple comparisons test.

(E and F) MS analysis (E) and quantification (F) of OSMI-1 in brain lysates from mouse gavaged with OSMI-1 (25 mg/kg and 50 mg/kg) (n = 6). (a) peak reflecting propranolol (10 ng/mL) as internal standard, (b) peak reflecting OSMI-1, showing its presence in the brain after crossing the blood-brain barrier. One-way ANOVA and Dunnett’s multiple comparisons test.

All data represent mean ± SEM. **P < 0.01. Related to Fig. 6.
Fig. S15. OGT inhibitor OSMI-1 suppresses the development of Shh-subtype medulloblastoma
(A) Schematic diagram of the experimental procedure for B-I.
(B) Kaplan-Meier curves of SmoM2<sup>Atoh1</sup> Mut mice treated with DMSO (brown), or OSMI-1 (purple) (n = 10). Log-rank test.
(C) Brain micrographs and H&E light micrographs of midsagittal sections through cerebellar vermis at P30. Scale bar: 5 mm, 2 mm.
(D) Cerebella weight of SmoM2<sup>Atoh1</sup> + DMSO mice and SmoM2<sup>Atoh1</sup> + OSMI-1 mice at P30 (n = 10). Unpaired t-test.
(E-G) Representative immunoblots (E) and quantification of O-GlcNAc of Gli2 S355 (F) and AcGli2 (G) immunoprecipitated from SmoM2<sup>Atoh1</sup> cerebellum with DMSO or OSMI-1 at P30 (n = 3). Unpaired t-test.
(H and I) Immunofluorescent staining (H) and quantification (I) of the ratio of Ki67<sup>+</sup> in Pax6<sup>+</sup> GNPs of Shh-subtype medulloblastoma at P30 (n = 5). Scale bar: 50 μm. Unpaired t-test.
All data represent mean ± SEM. **P < 0.01, ***P < 0.001, ****P < 0.0001. Related to Fig. 6.
Fig. S16. Immunofluorescent staining of Daoy cells using anti-OGT and anti-GLI2 S358 O-GlcNAc antibodies in vitro

(A) Schematic diagram of the Lenti-CRISPR-V2-OGT-sgRNA-GFP vector with human OGT specific sgRNA (OGT sgRNA), and the Lenti-CRISPR-V2-GFP as control (V2-vector).

(B) Schematic diagram of experimental timeline and procedure for C-F.

(C and D) Representative immunoblot (C) and quantification (D) of the OGT protein level in single cell clonal amplified Daoy cells infected with control (V2-vector) or OGT sgRNA lentivirus, respectively (n = 4). Unpaired t-test.

(E and F) Immunofluorescent staining (E) and quantification (F) of OGT in indicated lentivirus infected Daoy cells (n = 5). Scale bar: 50 μm. Unpaired t-test.

(G) Schematic diagram of the Lenti-FUGW-GLI2 shRNA-GLI2-Myc-GFP vector with endogenous human GLI2 knockdown and overexpression of exogenous shRNA-resistant human GLI2 (shGLI2+GLI2R) or GLI2 S358A mutant (shGLI2+S358A).

(H) Schematic diagram of experimental timeline and procedure for I-L.

(I and J) Representative immunoblot (I) and quantification (J) of the GLI2 S358 O-GlcNAc protein level in Daoy cells infected with shGLI2 + GLI2R or shGLI2 + S358A lentivirus, respectively (n = 4). Unpaired t-test.

(K and L) Immunofluorescent staining (K) and quantification (L) of GLI2 S358 O-GlcNAc in indicated lentivirus infected Daoy cells (n = 5). Scale bar: 50 μm. Unpaired t-test.

All data represent mean ± SEM. ****P < 0.0001. Related to Fig. 6.
Fig. S17. Linear regression reveals a positive correlation between OGT, GLI2 S358 O-GlcNAc and GAB1* expression.

(A) Representative immunohistochemistry images showing non-Shh (left panel) and Shh subgroup (right panel) clinical MB samples stained with indicated antibodies. Scale bar: 20 μm.

(B-D) Linear regression analysis shows positive linear relationship between OGT and GAB1* Shh subgroup MB (B), GLI2 S358 O-GlcNAc and GAB1* Shh subgroup MB (C), GLI2 S358 O-GlcNAc and OGT (D), respectively. The intensity of immunohistochemistry staining signal for each antibody was quantitatively analyzed and compared for each MB sample (n = 18). Pearson product-moment correlation coefficient analysis. All data represent mean ± SEM. Related to Fig. 6.
Fig. S18. Working model illustrating that OGT-mediated O-GlcNAcylation of Gli2 impairs its association with p300 and promotes Gli-mediated Shh signaling activation, which in turn affects GNP neurogenesis, cerebellar development and the growth of Shh-subtype medulloblastoma. Related to Figs. 1-6.
| Primers Used for Genotyping | Sequence |
|----------------------------|----------|
| Primer for genotyping: hGFAP-Cre-F | GCCTGCATTACCGGTCAATGCAACGA |
| Primer for genotyping: hGFAP-Cre-R | GTGGAGATGAGGTGCGGCAACACCATT |
| Primer for genotyping: Atoh1-Cre-F | CCGGCAATGTTTACAGAAC |
| Primer for genotyping: Atoh1-Cre-R | AGTGGTTAGCTGGCCTAAGT |
| Primer for genotyping: OGT loxP-F | CATCCTCCAGCCCCAACAATG |
| Primer for genotyping: OGT loxP-R | GACGAAGAAGGGAGGAGAC |
| Primer for genotyping: SmoM2-1 | AAAGTCGCTCTGAGTTGTTCT |
| Primer for genotyping: SmoM2-2 | GCGAAGAGTTTGCTCTTCAACC |
| Primer for genotyping: SmoM2-3 | GGAGCGGGAGAATGGAATG |
| Primer for genotyping: Gli1 lacZ reporter-F | GGGATCTGTGCTCGAAACTG |
| Primer for genotyping: Gli1 lacZ reporter-R | AGGTGAGACGACTGCAACTG |
| Primer for genotyping: A9-F | GCCATTAAGCAGCCTATCC |
| Primer for genotyping: A9-R | CTGTTTCTGTACGGCTAGG |
| Primer for pCMV6-mGli2-WT-F | TATAGGGCGCCGGGGAATTGTGGAAGA |
| Primer for pCMV6-mGli2-WT-R | AGATGAGTTCTCGAGCGCGCCGG |
| Primer for Gli2 mutants: Gli2 S273A-F | ATGGACATCTGGCTGCTGGTCCTCA |
| Primer for Gli2 mutants: Gli2 S273A-R | AGCAGCCAGATGTCTAGAGCCACT |
| Primer for Gli2 mutants: Gli2 S355A-F | AACCAGAACAAGAAGCGCCAG |
| Primer for Gli2 mutants: Gli2 S355A-R | GCGGGAGCTGTCTGCTGCT |
| Primer for Gli2 mutants: Gli2 S361A/T362A-F | TGTGAGCGGCCCGGTCAATCCTG |
| Primer for Gli2 mutants: Gli2 S361A/T362A-R | TTCACGCGCCCGCTCAGCTGG |
| Primer for Gli2 mutants: Gli2 T791A-F | AAGAGACAGCTCGAGAGGAC |
| Primer for Gli2 mutants: Gli2 T791A-R | TGGCGGAGCTGTCTCTTCTGAG |
| Primer for Gli2 mutants: Gli2 S844A-F | TCTCCAGATCGCCCTGCTGGAAC |
| Primer for Gli2 mutants: Gli2 S844A-R | AGCGGAGCTCGAGAGGAGGTCATA |
| Primer for Gli2 mutants: Gli2 S355D-F | AAGAGAGCGACGTCGAGTGCAG |
| Primer for Gli2 mutants: Gli2 S355D-R | TGACTGCTGTCTTCTGCTTGG |
| Primer for GLI2 mutants: GLI2 S79A-F | GGTACCATTACGACGGCCTCAGTGGTCC |
| Primer for GLI2 mutants: GLI2 S79A-R | CATGAGGCTCGTAATGGTACCTTCCTTT |
| Primer for GLI2 mutants: Gli2 S96A-F | CAGCGGGAGCGGTCTCGAGCAT |

Table S1. Primers used for genotyping and plasmid constructions
| Primer used for GLI2 mutants: Gli2 S96A-R | CGATGACAGGGCTGCCGCTGAGGGCA GGGGGCC |
|-----------------------------------------|-------------------------------------|
| Primer used for GLI2 mutants: GLI2 S358A-F | ACAAGCAGAGCGCTGAGTCGGCCGTC ACAGCA |
| Primer used for GLI2 mutants: GLI2 S358A-R | GCGCTCTGCTTGTTCTGGTTGCTCACTAGT |
| Primer used for GLI2 mutants: GLI2 S364A/T365A -F | CAGTGAGTCGGCCGTCAGCGCCGC CGTGTTCT |
| Primer used for GLI2 mutants: GLI2 S364A/T365A -R | CGGCGCTGACGGCCGACTCAGTGCAGTC |
| Primer used for GLI2 mutants: GLI2 T810A-F | GGAGCGCCGCGACAGCTCCGCCAGCA CTCTTTAG |
| Primer used for GLI2 mutants: GLI2 T810A-R | CGGAGCTGTCGCGGCGCTCCTGCAGTA TGGGCG |
| Primer used for GLI2 mutants: GLI2 S358D-F | ACAAGCAGAGCGATGAGTCGGCCGTC AGCAGCA |
| Primer used for GLI2 mutants: GLI2 S358D-R | GACTCATCGCTCTGCTTGTTCTGGGT |
| Sequence of H1-Gli2 shRNA targeting Gli2 mRNA | TTAATTAAAAAACGCCCAGCAGAATCT CTGCTCTTAAAGCTCTTTAGCCTC |
| Primer for sequencing H1-Gli2 shRNA | CTCGCTGCGCCCTCGTCTGA |
| Primer used for Gli2 synonymous mutations on the shRNA targeting site: mGli2-shRNA-F | GCTCATGACACCTCAGGAGGAGGG CATCTTTACTTC |
| Primer used for Gli2 synonymous mutations on the shRNA targeting site: mGli2-shRNA-R | GAGCTGCTTAA |
| Sequence of H1-GLI2 shRNA targeting GLI2 mRNA | TTAATTAAAAAACGCTCTACTACG GCGAGCTTCTTAAATCTGGCCGTAG |
| Primer for sequencing H1-GLI2 shRNA | CTCGCTGCGCCCTCGTCTGA |
| Primer used for GLI2 synonymous mutations on the shRNA targeting site: GLI2-shRNA-F | GTATTATTATGGGCAAATCCACATGTAC GAAAGGATG |
| Primer used for GLI2 synonymous mutations on the shRNA targeting site: GLI2-shRNA-R | TTGCCCATAATAATACAGCATGCTGGA TCCGGGGC |
| Primer Name                                      | Sequence                      |
|-------------------------------------------------|-------------------------------|
| OGT-sgRNA-F                                     | CACCGCGCCATTTCAAAGACCGTACT    |
| OGT-sgRNA-R                                     | AAACAGTACGGTCTTGAATGGCGC      |
| Primer for sequencing FUGW-shRNA-mGli2-F        | AGGTCGACTCTAGGAGATCCATGGAGA   |
|                                                 | TTTCTGCCCAGCC                  |
| Primer for sequencing FUGW-shRNA-mGli2-R        | GACCGGTACCCGGAGATCCATGGAGA    |
|                                                 | TACTGTCGTCATCTTGGTAAATCC      |
| Primer for sequencing FUGW-shRNA-hGli2-F        | AGGTCGACTCTAGGAGATCCATGGAGA   |
|                                                 | GTTCTGCCCAGCC                  |
| Primer for sequencing FUGW-shRNA-hGli2-R        | GACCGGTACCCGGAGATCCATGGAGA    |
|                                                 | TACTGTCGTCATCTTGGTAAATCC      |
| Primer used for pEGFP-C1-Sufu-F                 | TCAGATCTCGAGCTCAAGCTTCCATGG   |
|                                                 | CGGAGCTCGCCCTGAGC              |
| Primer used for pEGFP-C1-Sufu-R                 | TTATCTAGATCCGGTGGATCCATGGAT   |
|                                                 | TACCCCTGAGACCTGAC              |
| Primer used for pKH3-p300-F                      | AAGGATCTGGAAACCCCTGAGACCATTGG|
|                                                 | CCAGAGAATGTTGGTGAGAAC         |
| Primer used for pKH3-p300-R                      | GCTGGGTGTGCGCGGCTAGTTGATGG    |
|                                                 | CTAAGTGTACTCTGTGAGAGG         |