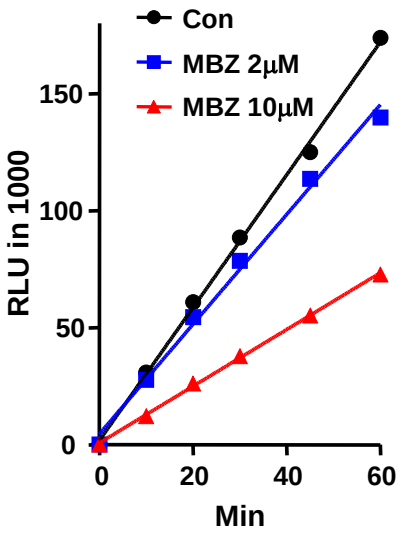
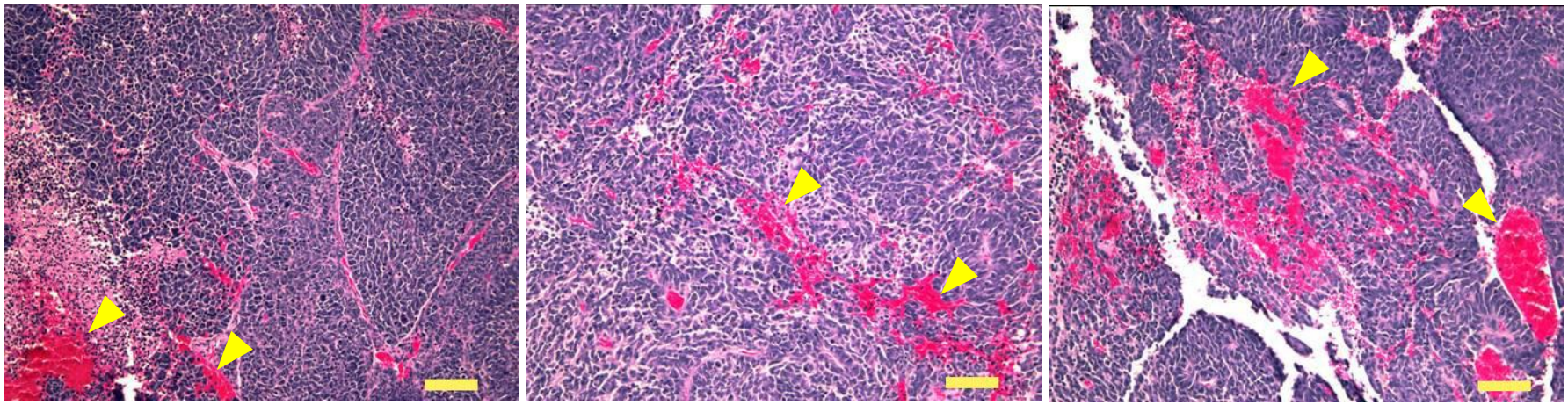
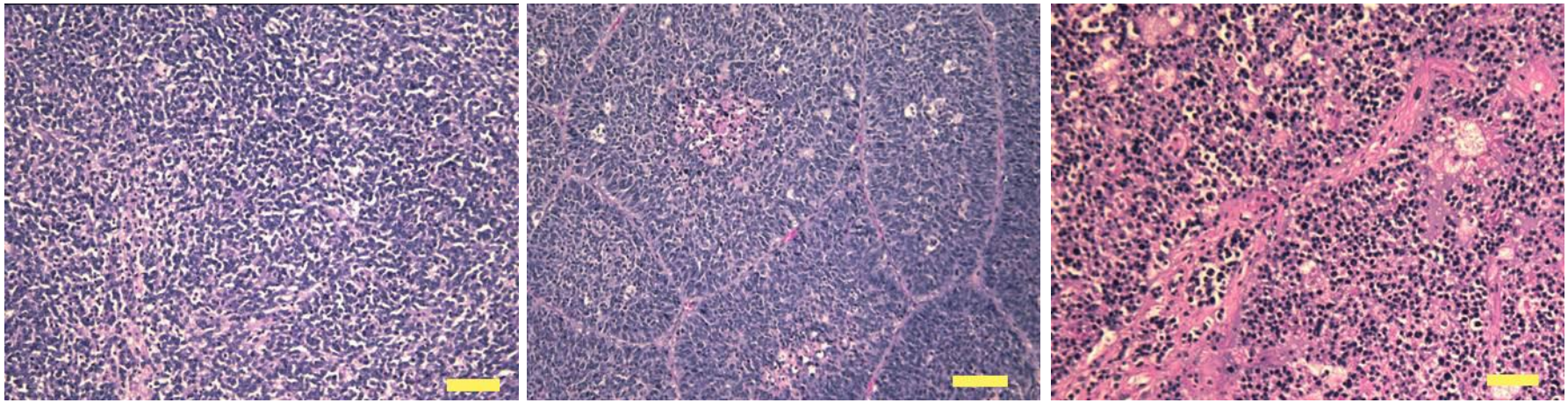


Supplementary Figure 1



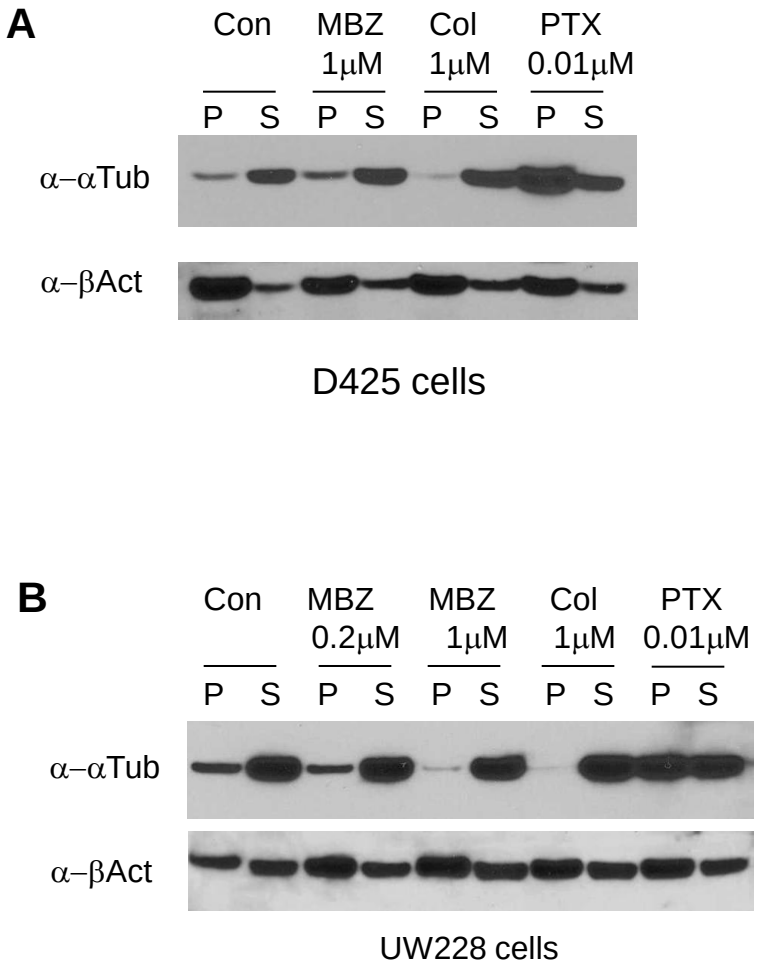
Control



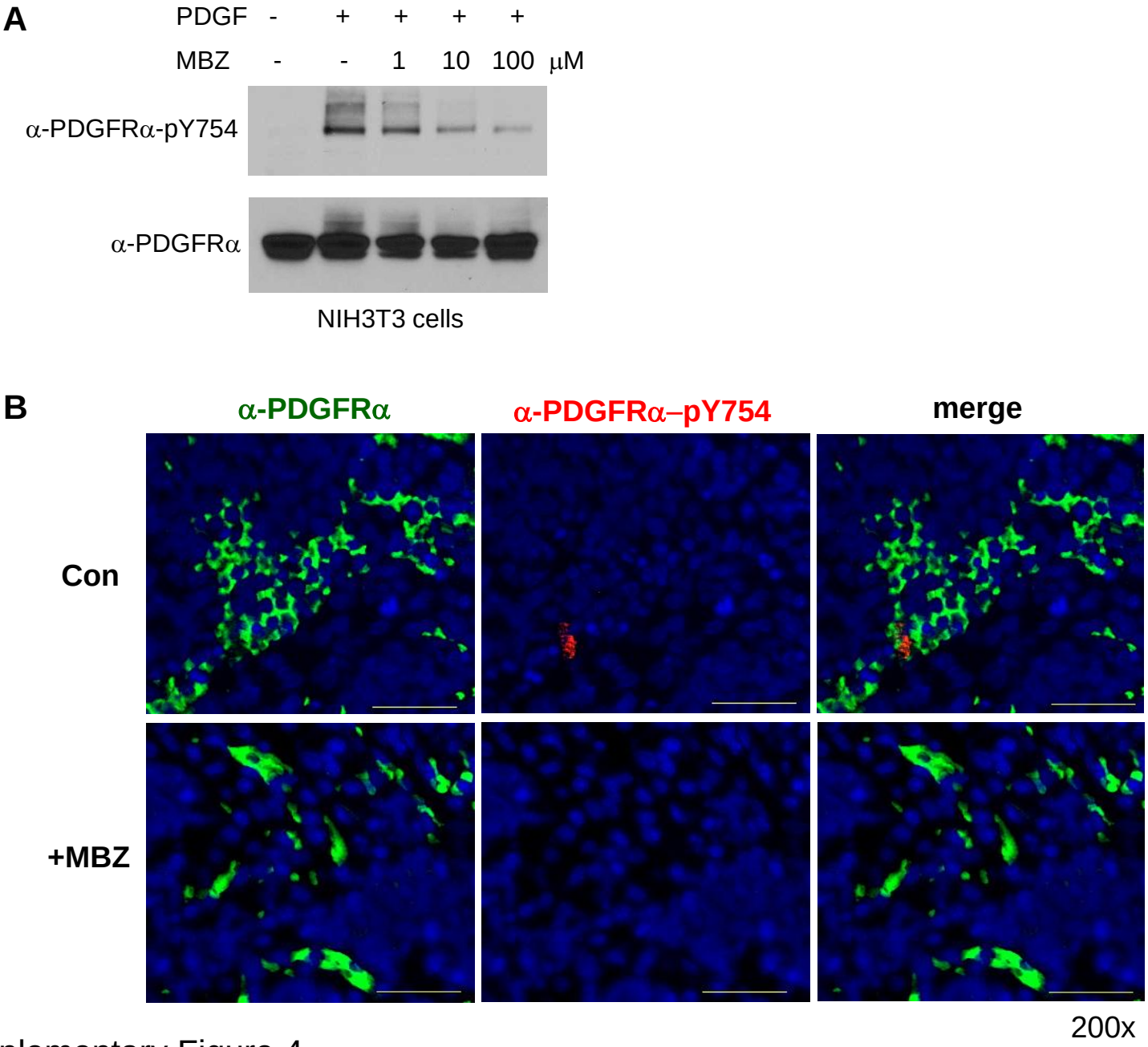
MBZ

H&E 100x

Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

MB cell lines	D283	D341	D425	D458	D487	DAOY	MHH1	UW228
IC50 in μM	0.13	0.15	0.22	0.39	0.18	0.13	1.0	0.50

Supplementary Table 1

Supplementary Figure 1. Effect of MBZ on the time course of VEGFR2 kinase assay.

The VEGFR2 kinase assay used 50 μM ATP and 1 μg poly (Glu_4,Tyr_1) peptide as substrates with 20 ng purified VEGFR2 (aa789-1356) and MBZ at indicated concentrations. ADP converted from ATP was measured by a luminescence reader as relative light unit (RLU).

Supplementary Figure 2. MBZ-treated PTCH Mutant medulloblastoma showed fewer intra-tumoral hemorrhages.

Mice bearing PTCH1+/-, p53-/- allograft medulloblastoma were treated with MBZ. As the tumor growth reached the terminal stage where the mice showed enlarged skull and morbidity, brain was taken from the control and MBZ-treated mice. Brain sections of three mice in each group were stained by H&E and presented. Yellow arrow heads indicate the intra-tumoral bleeds (red color). All scale bars are 30 μm.

Supplementary Figure 3. MBZ's effects on D425 and UW228 medulloblastoma cells.

(A) D425 cells were incubated with MBZ, colchicine (Col) or paclitaxol (PTX) at indicated concentrations for 24 h. Following the lysing of cells with hypotonic buffer, the lysates were separated by centrifugation. The pellets (P) containing polymerized tubulin were re-suspended in lysis buffer and loaded with the supernatant (S) containing depolymerized tubulin on SDS-PAGE for anti-α-tubulin (αTub) Western blot.

(B) Similar to A, UW228 cells were treated with MBZ, colchicine (Col) or paclitaxol (PTX) at indicated concentrations for 24 h and the polymerized and depolymerized tubulin were analyzed by anti-α-tubulin (αTub) Western blot. Equal amount of protein was analyzed by Western blotting.

Supplementary Figure 4. Analyzing the MBZ's inhibition on PDGFRα

(A) MBZ inhibited the autophosphorylation of PDGFRα in NIH3T3 cells. NIH3T3 cells were starved for 5 h in 0.5% FBS and treated with MBZ 30 min prior to the stimulation of 50 ng/ml PDGF-AA that lasted 5 min. Western blot of anti-PDGFRα-pY754 antibody indicated the autophosphorylation of tyrosine 754 and the levels of PDGFRα were reflected by the anti- PDGFRα western blot.

(B) Anti- PDGFRα (left panels, green) and anti-PDGFRα-pY754 (middle panels, red) staining of PTCH+/-, p53-/- medulloblastoma allografts untreated or treated with MBZ. The nuclei were visualized by DAPI staining (blue). Autophosphorylation of PDGFRα-Y754 appeared to be rare among the PDGFRα-expressing cells. All pictures were taken with the same setting as in Figure 6B and C. All scale bars are 30 μm.

Supplementary Table 1. IC50 of medulloblastoma cell lines with MBZ

IC50s of a panel of medulloblastoma cell lines were determined by incubating the cells with MBZ at a range of concentrations for 3 days.

Antibodies

The following antibodies were used: rabbit anti-PDGFRα antibody (#3164, Cell Signaling Technology), rabbit anti-PDGFRα-pY754 antibody (AB5460, Abcam), mouse anti-PDGFRα antibody (AB96569, Abcam), mouse anti-α-tubulin (#CP06, Calbiochem) and anti-actin-HRP (Santa Cruz, C-11).

Tubulin Polymerization Assay

The tubulin polymerization assay was performed as described previously.[7] Cells were lysed by re-suspension in hypertonic buffer (2mM EGTA, 1mM MgCl2, 0.5% NP40, 2mM phenylmethylsulfonyl fluoride, 20 mM Tis-HCl pH 6.8 and protease inhibitors) and vortexed briefly. Subsequently, the samples were centrifuged at 13,000 g for 10 min at room temperature (RT). The supernatant (depolymerized tubulin) was transferred to a new tube and the pellet (polymerized tubulin) was re-suspended in hypertonic buffer. Equal amount of protein was analyzed by Western blotting.

Cells Growth Assay

Medulloblastoma cell lines were used in the study were obtained from the Duke University Brain Tumor Center or indicated in the references: D283Med (D283), D341Med (D341), D425Med (D425), D458Med (D458), D487Med (D487), DAOY, MHH1 and UW228.[12] The viable cells were measured with Cell Counting Kit-8 (Dojindo Laboratories, Japan) containing WST-8 tetrazolium salt at 450 nm on a PerkinElmer VICTOR3 plate reader. IC50s were determined by incubating

cells at a range of concentrations for 72 h and calculated by GraphPad Prism 5.0 using the log(inhibitor) vs. response function and non-linear fit.