P-selectin Targeting Stimulates Caveolin-1-Mediated Endothelial Transcytosis in Medulloblastoma

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Summary: Medulloblastoma (MB) is the most common malignant pediatric brain tumor with ~30% mediated by Sonic hedgehog (SHH) signaling. While SHH effector Smoothened inhibition is promising, therapeutic index is reduced by high systemic doses required for sufficient intracranial drug concentrations. Treatment-related toxicities associated with radiation therapy result in lifelong morbidity, and SHH pathway inhibition in children notably results in permanent bone defects. Here, we found that nanoparticle-mediated P-selectin targeting on tumor vasculature induces caveolin-1-dependent transcellular passage across the blood-brain barrier. A vismodegib nanocarrier that targets P-selectin exhibited striking efficacy and markedly reduced both bone-related toxicities and drug exposure to healthy brain. These findings demonstrate a potent strategy and novel mechanism for targeted intracranial pharmacodelivery that overcomes the restrictive endothelial barrier to achieve enhanced tumor-selective penetration and has therapeutic implications for other diseases within the central nervous system.

35 **Main Text:**

36 Site-directed drug delivery to brain parenchymal tissue is a desirable but elusive goal due to the distinct and highly
37 regulated blood-brain barrier (BBB). The BBB is comprised of a network of specialized endothelial cells, pericytes,
38 and astrocytes that prevent material extravasation¹. Characteristic tight junctions between adjacent brain endothelial
39 cells block paracellular transport, and the passive entry of molecules is constrained to a narrow window of size and
40 lipophilicity. These restrictive physical and functional barriers inhibit drug exposure to intracranial tissues².
41 Hydrophilic small molecule drugs are routinely excluded from the brain by tight junctions, and while many lipophilic
42 drugs are capable of entry via passive diffusion, penetration into diseased brain tissue is inefficient and typically
43 requires high drug doses often resulting in dose-limiting systemic toxicity³. As such, the integrity of the BBB can
44 considerably impact treatment efficacy. This is well evidenced in medulloblastoma wherein patients in the Sonic
45 hedgehog medulloblastoma subgroup (SHH-MB) suffer worse clinical outcomes due in part to an intact BBB limiting
46 the entry of drugs into the brain at therapeutic concentrations⁴⁻⁶. In addition, the targeted inhibition of the SHH
47 effector Smoothened (SMO) via vismodegib causes premature bone growth plate fusions in pediatric patients and
48 this is likely a result of the high doses required for therapeutic efficacy^{7,8}.

49

50 Given the many limitations for the passage of small molecules across the BBB, nanoparticles have been explored as
51 a vehicle to improve delivery into brain tissues⁹. To date, much of this work has focused on strategies that enhance
52 passive mechanisms of transport for drug loaded nanoparticles across the BBB. For instance, in diseases that result
53 in a compromised BBB, such as glioblastoma, nanostructures have been observed to extravasate through the leaky
54 vasculature to accumulate at tumor sites¹⁰. Also, researchers have implemented analogous approaches to improve
55 drug delivery past an intact BBB, by developing strategies that first disrupt this barrier^{11,12,13}. However, by permitting
56 unregulated passage across the BBB, such approaches not only abrogate the homeostatic functions of the BBB, but
57 potentially expose the brain to harmful toxins and pathogens. Alternative approaches for diseases such as SHH
58 subgroup medulloblastoma that retain BBB integrity have utilized non-targeting nano-carriers to extend systemic
59 circulation of small molecule drugs with only partial improvement of on-target toxicity profiles at high-doses¹⁴.
60 Importantly, recent work has suggested that passive entry of nanoparticles into solid tumors through gaps between
61 endothelial cells represents a minor mechanism of entry and that up to 97% of transport is through an active process
62 through endothelial cells¹⁵. However, the molecular mechanism of this active transcellular transport across
63 endothelial barriers has not yet been elucidated, and little is known of whether this transendothelial nanoparticle
64 transport occurs at the BBB.

65

66 In this study, we investigated active transcellular transport mechanisms to enhance drug delivery across an intact
67 BBB specifically to brain tumor tissue. We and others previously demonstrated targeting of fucoidan-based
68 nanoparticles to P-selectin on activated endothelial cells and found that P-selectin expression on endothelial cells
69 may be enhanced by radiotherapy (RT) to effect greater nanoparticle accumulation in tumor sites^{16,17}. Herein, we
70 found that P-selectin facilitates material transendothelial transport across an intact BBB via caveolin-1 (Cav1)

mediated transcytosis. Using a genetic mouse model of SHH-MB with an intact BBB, we found that P-selectin targeting results in active transport in tumor endothelium to enable delivery of fucoidan-based nanoparticles selectively into the tumor microenvironment, which is enhanced by RT. Fucoidan nanoparticles encapsulating the Smoothened inhibitor vismodegib (FiVis) exhibited significant effector inhibition at low drug doses, striking anti-tumor efficacy, and attenuated on-target bone-related toxicities. These findings demonstrate a novel approach for improving the therapeutic index of vismodegib for SHH-MB and present a potent adjuvant strategy for delivering drugs to treat brain tumors in combination with standard radiotherapy. Furthermore, we report an active mechanism of transendothelial transport that can be exploited to improve drug delivery across activated brain endothelial cells in conditions with an intact BBB.

Results

We first characterized the brain vasculature in a genetically engineered mouse (GEM) *Ptfla^{cre/+};Ptch1^{fl/fl}* SHH-MB model to investigate the integrity of the BBB. To assess the permeability of the BBB in mice at advanced stages of SHH-MB, symptomatic mice (14 weeks or older) were injected intravenously with TMR-dextran. We observed minimal extravasation of TMR-dextran into parenchymal brain tumor tissue (Extended Data Fig. 1). We conclude that the BBB of these mice appears to remain intact well into advanced tumor stages, and thus parallels the physiology of human patients with SHH-MB⁶.

We next examined P-selectin expression in the SHH-MB tumor microenvironment and the effects of low-dose X-ray irradiation (XRT)¹⁸. We found that P-selectin is expressed in SHH-MB tumor vasculature in the absence of radiation (Fig. 1a) and that this expression could be further enhanced following a single 2 Gy dose of XRT (Fig. 1c). Notably, the elevation of P-selectin expression following irradiation was confined to tumor regions and not apparent in adjacent, normal brain tissue (Fig. 1c). In order to assess the potential to mitigate RT-related toxicity, we sought to identify the minimal required dose of irradiation that could still achieve significant induction of endothelial P-selectin expression. We found that P-selectin expression could still be robustly induced following a single dose of 0.25 Gy XRT (Fig. 1b). P-selectin expression was observed to reach significantly elevated levels at approximately 6 hours after XRT, and these levels persisted for at least 24 hours afterwards (Fig. 1e, Extended Data Fig. 2). To confirm the clinical relevance of endothelial P-selectin expression as a potential target molecule, we examined human SHH-MB tumor tissue surgically resected from pediatric patients. IHC analysis similarly showed P-selectin expression in tumor-adjacent vasculature (Fig. 1f). To target an SHH pathway modulator to P-selectin, we synthesized nanoparticles encapsulating the Smoothened inhibitor vismodegib. We used a nanoprecipitation process, incorporating the polysaccharide fucoidan, to target P-selectin, and a near infra-red dye (IRDye783), to facilitate imaging¹⁶. The resulting fucoidan-vismodegib (FiVis) nanoparticles exhibited an average size of 80 nm +/- 10 nm and were relatively homogeneous as determined by atomic force microscopy and dynamic light scattering (DLS) (Fig. 2 a, b). The FiVis nanoparticles retained this uniform size with no observable aggregation for up to 7 days when

stored at 4°C in deionized water (Extended Data Fig. 3a). FiVis nanoparticles were also tested for their stability in serum and exhibited effective drug encapsulation for up to 12 hours as determined by quantification of free drug released into the serum mixture, which would be indicative of nanoparticle disassembly (Extended Data Fig. 3b).

We next investigated nanoparticle localization to SHH-MB tumors as a function of P-selectin expression. In non-irradiated mice, treatment with FiVis nanoparticles resulted in a slight increase in targeting to the cerebellar tumor compared to adjacent normal forebrain tissue. However, in mice that were pre-treated with irradiation (1 Gy), we observed pronounced tumor accumulation of FiVis in contrast to non-tumor-bearing WT mice where it remained in an intravascular location (Fig. 2c). We also synthesized control dextran sulfate-based vismodegib nanoparticles (DexVis) that do not exhibit affinity to P-selectin (Extended Data Fig. 4)¹⁹. These nanoparticles, exhibiting similar size and drug encapsulation characteristics to FiVis, did not demonstrate a comparable tumor localization effect, even following irradiation (Fig. 2c). In contrast, pre-treatment with 0.25 Gy XRT resulted in upwards of a 3-fold increase in FiVis nanoparticle localization at tumor sites as compared to normal adjacent forebrain tissue (Fig. 2d). In addition, localization of FiVis nanoparticles to tumors was abrogated in P-selectin knockout SHH-MB mice (SELP null SHH-MB, *Ptflacre/+*; *Ptch1fl/fl*; *Selp^{-/-}*). Quantification of the vismodegib cargo by liquid chromatography-mass spectrometry (LC-MS) found similar accumulation within cerebellar tumor regions while relatively sparing normal adjacent forebrain regions (Fig. 2e).

We proceeded to test the functional response and efficacy of FiVis nanoparticle treatment in SHH-MB mice. We used qRT-PCR to measure the expression of *Gli1*, a downstream effector of SHH pathway activation. When combined with 1 Gy XRT, FiVis treatment resulted in *Gli1* target inhibition in a dose-dependent manner (~80% inhibition with FiVis 10 mg/kg and ~90% inhibition with FiVis 20 mg/kg) (Fig. 3a). However, treatment with control DexVis nanoparticles in combination with XRT did not result in increased *Gli1* inhibition compared to XRT alone (Fig. 3b). Furthermore, the *Gli1* inhibition conferred by a combination of XRT and FiVis nanoparticle treatment was sustained even with a very low 0.25 Gy XRT dose, which alone does not affect *Gli1* levels (Fig. 3c). Of note, comparable levels of *Gli1* inhibition with free drug in SHH-MB mouse models typically requires treatment with vismodegib at doses upwards of 50 mg/kg²⁰. Using this *Gli1* inhibition data to inform nanoparticle drug dosing in a survival study, we tested the efficacy of low dose XRT and FiVis for treating mice with advanced stage SHH-MB. Treatment of mice with 10 mg/kg FiVis significantly prolonged survival on its own, however when combined with 0.25 Gy XRT, FiVis treatment at 10 mg/kg further extended survival by more than two-fold (Fig. 3d). Importantly, we did not see enhanced mouse survival following treatment with a combination of low dose 0.25 Gy XRT and free vismodegib at 10 mg/kg, suggesting that the efficacy observed with P-selectin targeted FiVis nanoparticles is likely not mediated by any potential BBB leakiness induced by this low level of ionizing radiation.

We next sought to assess the issue of on-target vismodegib-related systemic and bone toxicity that has been observed in preclinical studies in juvenile mice as well as in children with SHH-MB treated with therapeutic doses of vismodegib^{8,21,22}. In order to confer a survival benefit comparable to the FiVis nanoparticle treatment cohort, free

142 vismodegib had to be given daily at a considerably elevated 50 mg/kg dose (Extended Data Fig. 9). However, short-
143 term treatment of P10 mice with these high doses of vismodegib caused visible growth stunting (Fig. 4a). Notably,
144 these growth defects were abrogated in young mice treated with FiVis at 10 mg/kg, at which significant Gli1 inhibition
145 and survival benefit were observed in advanced stage SHH-MB mice. To assess general toxicity, mice were weighed
146 over the duration of treatment. We increased the free vismodegib dose to 100 mg/kg to better assess physiologic
147 changes at levels shown to promote significant intracranial drug levels in preclinical studies²⁰. While mice treated
148 with 100 mg/kg free vismodegib exhibited growth restriction, the weights of FiVis treated mice paralleled those of
149 vehicle-treated control mice (Fig. 4b). Closer examination of bone tissue revealed significant abnormalities in femur
150 lengths (Fig. 4c) and trabecular bone number using micro-CT (Fig. 4d) in P10 mice treated with free vismodegib
151 doses effective in SHH-MB that were not observed in mice treated with efficacious FiVis dosing.

152

153 We next investigated the mechanism of material transport across the BBB. We observed from immunohistological
154 analysis of brains obtained from the above SHH-MB treatment studies that Cav1 expression was selectively
155 upregulated in endothelial cells within SHH-MB tumor regions and not within normal brain regions when SHH-MB
156 mice were treated with FiVis (Fig. 5, a, b) but not in SHH-MB mice treated with low dose XRT alone (Fig. 5c). This
157 finding suggests that P-selectin engagement with fucoidan-based nanoparticles on endothelial cells might stimulate
158 a caveolae-mediated transendothelial transport mechanism. To further investigate transcytosis mechanisms, we
159 evaluated the uptake of FiVis nanoparticles in bEnd.3 murine brain endothelial cells. We found that 0.25 Gy XRT
160 significantly enhanced P-selectin expression on bEnd.3 cells *in vitro* similar to our *in vivo* SHH-MB GEM model
161 studies (Extended Data Fig. 6a). Notably, this XRT dose also resulted in enhanced uptake of FiVis nanoparticles into
162 bEnd.3 cells (Extended Data Fig. 6b). We next interrogated endocytosis pathways that may potentially mediate this
163 nanoparticle uptake. For pharmacological inhibition of either caveolin-dependent endocytosis or clathrin-dependent
164 endocytosis, we treated bEnd.3 cells with methyl- β -cyclodextrin (CD) or chlorpromazine (CPZ), respectively²³.
165 While treatment with CPZ did not affect nanoparticle uptake in bEnd.3 cells, groups treated with CD showed
166 significantly reduced FiVis uptake in a dose-dependent manner (Fig. 5d, Extended Data Fig. 6b). This result
167 suggested FiVis entry into murine brain endothelial cells was mediated by caveolin-dependent endocytosis. To
168 further investigate caveolin-mediated transcytosis, we assessed nanoparticle uptake using caveolin-1 knockout
169 (Cav1KO) bEnd.3 cells. We observed that FiVis uptake into Cav1KO cells was marginal, as compared to wildtype
170 (WT) cells (Fig. 5e). The WT bEnd.3 cells demonstrated elevated levels of nanoparticle uptake with increasing doses
171 of FiVis. We next sought to determine whether Cav1 also contributes to the transcytosis of FiVis nanoparticles across
172 brain endothelial cells. Cav1KO and WT bEnd.3 cells were cultured on porous transwell inserts to directly assess
173 transcellular transport. Confluent monolayers of the cells were evaluated for integrity by transendothelial electrical
174 resistance (TEER) and paracellular permeability (Extended Data Fig. 7c,d). FiVis nanoparticles introduced to the
175 upper chamber of the insert were quantified by fluorescence in the bottom chamber over the course of a 4-hour
176 incubation. The passage of FiVis across the cells and into the bottom chamber was significantly restricted in Cav1
177 KO compared to WT cells (Fig. 5f). Of note, FiVis nanoparticles were observed to have remained intact following

178 passage across bEnd.3 cells into the bottom chamber and found to be equivalent in size to those used at the onset of
179 the assay, as measured by dynamic light scattering (Fig. 5g), consistent with a transcytosis mechanism of transport.
180
181 To investigate the role of Cav1 in material blood-brain barrier transport *in vivo*, we bred the Cav1 null allele onto
182 our SHH-MB GEM model to generate homozygous Cav1 null SHH-MB mice (*Ptfla*^{cre/+}; *Ptchl*^{fl/fl}; *Cav1*^{-/-}). These
183 Cav1 null SHH-MB tumor mice showed similar latency and penetrance as Cav1 wild-type SHH-MB mice with
184 resultant tumors having similar histological features and levels of P-selectin expression (Extended Data Fig. 8 a,b).
185 We administered FiVis to these Cav1 KO SHH-MB mice following low dose XRT and observed that nanoparticles
186 localized to tumor vasculature presumably due to intact endothelial P-selectin targeting but did not appear to
187 extravasate into the brain tumor parenchyma (Fig. 5h). Furthermore, the SHH pathway target inhibition was
188 considerably diminished in Cav1 null SHH-MB mice, as evident by the abrogation of Gli1 target inhibition following
189 treatment with FiVis nanoparticles, as compared to that in Cav1 WT SHH-MB mice (Fig. 5i). These results support
190 a role for Cav1 in this active nanoparticle transport process across the BBB. A proposed transport model for fucoidan-
191 based nanoparticle drug delivery across the blood-brain barrier is shown in Fig. 5j.
192

193 Discussion

194 The highly specialized endothelial cells that comprise the BBB act as a physiological barrier that regulates the entry
195 of molecules into the central nervous system. While this is favorable for maintaining homeostasis under normal
196 conditions, it is an impediment to effective drug delivery for the treatment of diseases that manifest in parenchymal
197 brain tissue. We report that one way to overcome this pervasive challenge is by stimulating the active transport
198 machinery of brain endothelial cells. Here we show that fucoidan-based nanoparticle targeting of P-selectin on tumor
199 vasculature facilitates caveolin-1 dependent transendothelial transport and enhances drug delivery across an intact
200 blood-brain barrier. Using a combination of nanoengineering and genetic approaches, we provide evidence for
201 fucoidan-based nanoparticle targeting to P-selectin expressing brain endothelial cells *in vitro* and to P-selectin
202 expressing SHH-MB endothelium *in vivo*. In addition, using both pharmacological and genetic approaches *in vitro*
203 and *in vivo*, we show that the cellular entry and transendothelial BBB transport of fucoidan nanoparticles to SHH-
204 MB tumor tissue is caveolin-1 dependent and less likely through passive passage through interendothelial spaces as
205 suggested in recent studies¹⁵. This starkly contrasts previous efforts that have aimed to improve passive delivery
206 of drugs into brain tissue through disruption of tight junctions in the BBB that consequently might impact the
207 structural integrity of the neurovascular unit.
208

209 Our study presents novel mechanistic insights for nanoengineering a specific receptor-ligand interaction to facilitate
210 targeted and controlled delivery of nanoparticle-encapsulated therapeutic agents across the blood-brain barrier.
211 Previous work by others supports a role for P-selectin mediated engagement and caveolin-1 interaction. Notably,
212 activated endothelial cells have been shown to have significant colocalization of P-selectin and caveolin-1, a primary

213 constituent of caveolae²⁴. Our data supports the notion that the tendency for P-selectin to partition into caveolae may
214 effectively prime P-selectin bound FiVis nanoparticles for uptake by caveolae-mediated endocytosis and subsequent
215 transcytosis across the BBB. Although beyond the scope of this current study, it is currently not known whether
216 engagement of fucoidan nanoparticles with P-selectin activates a signaling pathway to mediate caveolin-1 dependent
217 cellular entry of nanoparticles or if it is mediated through more direct mechanical forces. Along these lines, previous
218 work has shown that PSGL-1 binding to P-selectin on endothelial cells has shown involvement of intracellular PI3K
219 and Src kinases within leukocytes, however little is known about involved signaling pathways within endothelial
220 cells following P-selectin engagement²⁵. Furthermore, recent work has also shown that CD44 and/or spectrin
221 cytoskeletal networks on endothelial cells can restrict selectin mobility to maintain apical density and clustering thus
222 allowing for leukocyte rolling through PSGL-1²⁴. Interestingly, activated endothelial cells following tumor necrosis
223 factor-alpha stimulation also resulted in apical distribution and restricted mobility of caveolae with subsequent
224 colocalization of caveolae with P-selectin. Future studies will be important to distinguish whether fucoidan
225 nanoparticle engagement with P-selectin on activated endothelial cells results in involvement of either endogenous
226 signaling pathways or alterations in CD44 and/or spectrin cytoskeletal networks for subsequent cellular entry.

227
228 Although we demonstrate efficacy with fucoidan-encapsulated vismodegib as proof of principle for this P-selectin
229 nanotargeting strategy in a SHH-MB GEM model, there are several other potential clinical applications for this
230 approach. Many hydrophobic drugs including standard chemotherapeutics (such as vincristine, doxorubicin,
231 paclitaxel, etc.) and molecularly targeted small molecules (including trametinib, tazemetostat, etc.) have been
232 successfully incorporated into fucoidan-based nanoparticles^{16,17}. Along these lines, we have recently reported a
233 computational algorithm (Q-SNAP) that predicts the feasibility of encapsulating drugs into fucoidan-based
234 nanoparticles for tailored applications²⁶. Further clinical applicability of this P-selectin targeting approach likely
235 extends beyond medulloblastoma to other intracranial tumors including glioma^{10,17}. Combination therapies to address
236 tumor heterogeneity and treatment resistance mechanisms have often resulted in additive dose-limiting toxicities.
237 The increased therapeutic indices observed with our P-selectin nanotargeting approach may allow tumor cell
238 autonomous and non-autonomous combination drug treatment strategies to facilitate tolerability in patients. In
239 addition, several central nervous system disorders including multiple sclerosis^{27,28}, ischemic stroke²⁹, and focal
240 epilepsy³⁰ have been shown to upregulate restricted endothelial P-selectin expression at sites of disease exacerbation
241 where leukocyte trafficking plays a role in disease pathogenesis. These settings may provide additional opportunities
242 for the targeted delivery of therapeutic agents specifically to sites of intracranial disease to enhance efficacy while
243 minimizing neurotoxicity and systemic toxicities. In conclusion, we anticipate that the continued investigation and
244 development of methods that harness and improve the active uptake of nanoparticles across the blood-brain barrier
245 and potentially other endothelial barriers will be instrumental for improving the efficacy of nanomedicines.

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Fig. 1: Low dose irradiation induces P-selectin expression on tumor vasculature in medulloblastoma. Immunofluorescence of P-selectin (green) and vasculature (CD31, red) in SHH-MB brain tumor tissues: (a) in non-irradiated mice (b) 4 hours after 0.25 Gy XRT and (c) 4 hours after 2 Gy XRT. (d) Immunofluorescence of adjacent non-tumor tissue taken from the midbrain region of whole-body irradiated tumor-bearing mice. (e) Time-course of P-selectin expression in SHH-MB tumor tissue following 2 Gy XRT quantified from Western blot analysis. $*P < 0.05$ (unpaired t test). Data are means $\pm$ SEM. (f) Representative human SHH-MB tumor tissue immunostained (red) for P-selectin expression. Hematoxylin counterstain (blue) indicates SHH-MB tumor tissue.

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Fig. 2: P-selectin-targeted nanoparticles preferentially target SHH-MB tumors following low-dose irradiation. (a) Atomic force micrograph of FiVis nanoparticles. (b) Histogram of FiVis nanoparticle diameter measured by dynamic light scattering, showing an average diameter of 80 ± 10 nm. (c) Representative near-infrared images (dorsal view) of brains from WT and SHH-MB mice administered with FiVis nanoparticles. $n=3$ mice per group in each of two independent experiments. (d) Near-IR fluorescence intensities (top) of FiVis nanoparticles in tumor regions (cerebellum) and non-tumor regions (forebrain) of WT, SHH-MB, and P-selectin (SELP) null SHH-MB mice. Bottom: Representative dorsal images of brains demarcated by cerebellum and forebrain (yellow) for quantification. $n=3$ mice per group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (unpaired t test); ns, not significant. Data are means $\pm$ SEM. (e) LC-MS/MS quantification of vismodegib in cerebellar and forebrain tissue of mice administered with 0.25 Gy XRT and 10 mg/kg FiVis. $n=5$ mice; * $P < 0.05$ (paired t test). Data are means $\pm$ SEM.

Fig. 3: FiVis nanoparticles synergize with low dose irradiation to enhance Gli1 target inhibition and survival in SHH-MB. Quantitative real-time PCR analysis of Gli1 target inhibition in advanced stage SHH-MB tumors following indicated treatments. (a) qRT-PCR analysis of Gli1 expression comparing free vismodegib to FiVis at indicated doses either alone or in combination with ionizing radiation. (b) qRT-PCR analysis of Gli1 expression comparing treatment of P-selectin targeting FiVis to control nontargeting DexVis at indicated doses in combination with ionizing radiation. (c) qRT-PCR analysis of Gli1 expression following FiVis treatment at indicated dose with very low dose 0.25 Gy ionizing radiation. Data in (a-c) are means $\pm$ SEM. $n = 3$ mice per group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ (unpaired t test); ns, not significant. (d) Kaplan-Meier survival analysis of advanced stage SHH-MB mice treated with either fractionated 0.25Gy XRT, low dose FiVis, free vismodegib, and combinations thereof at indicated doses. Survival of untreated SHH-MB mice is shown in black. XRT doses of 0.25 Gy alone or in combination with respective drugs were given every other day x 8 doses. * $P = 0.0198$ (Mantel-Cox) for XRT + FiVis (orange) compared to XRT + vismodegib (pink). $P = \text{ns}$ (Mantel-Cox) when XRT + vismodegib (p) is compared to XRT alone (green).

Fig. 4: FiVis nanoparticles abrogate vismodegib treatment-related bone toxicity in juvenile mice. (a) Body length of mice at 6 weeks age following two day treatment at P10 with vismodegib (Free Vis), FiVis, or DMSO vehicle at indicated doses. Representative image; $n=3$ mice per group. (b) Mouse body weight during 6 weeks following two day treatment of P10 mice with vismodegib (100 mg/kg), or FiVis nanoparticles (10 mg/kg), or DMSO. (c) Femur length at 6 weeks following two day treatment of P10 mice with vismodegib or FiVis nanoparticle treatments. $n=3$ mice per group; $*P < 0.05$ (paired t test). Data are means $\pm$ SEM. (d) Representative micro-computed tomography (μ CT) 3D reconstruction images of trabecular bone in the distal femoral metaphysis taken from mice treated with DMSO control, FiVis nanoparticles (20 mg/kg), or free vismodegib (100 mg/kg). BV/TV= trabecular bone volume/total volume, Tb.N = trabecular number, Tb.Th = trabecular thickness.

Fig. 4: Passage of FiVis nanoparticles across the BBB is mediated by Caveolin-1.

(full figure legend on pg. 14)

Fig. 4: Passage of FiVis nanoparticles across the BBB is mediated by Caveolin-1. Immunofluorescence of Cav1 (white) and CD31 (green) in advanced SHH-MB mouse tumors following treatment with (a) 0.25 XRT + FiVis, (b) FiVis only, or (c) XRT only. (d) Uptake of FiVis nanoparticles into bEnd.3 cells following pre-treatment with pharmacological inhibitors of endocytosis pathways as measured by flow cytometry. CPZ = Chlorpromazine (inhibitor of clathrin-mediated endocytosis); M β CD = methyl- β -cyclodextrin (inhibitor of caveolae-dependent endocytosis). Uptake was compared to a group of cells administered nanoparticles but no inhibitor. Data are means $\pm$ SEM. *** P < 0.001, **** P < 0.001 (one-way ANOVA); ns, not significant. (e) Uptake of FiVis nanoparticles at indicated doses in Cav1 wildtype (WT) bEnd.3 cells compared to homozygous Cav1 knockout (KO) bEnd.3 cells. Data are means $\pm$ SEM *** P < 0.001, **** P < 0.001 (one-way ANOVA); ns, not significant. (f) Quantification of FiVis transcytosis across a monolayer of wildtype or Cav1 KO bEnd.3 cells at indicated incubation times using a transwell assay. Data are means $\pm$ SEM. * P < 0.05 (one-way ANOVA); ns, not significant. (g) Dynamic light scattering assessment of FiVis nanoparticle size in apical and basolateral chambers of a transwell assay across a bEnd.3 cell monolayer. Data are means $\pm$ SEM. ns, not significant (unpaired t test) (h) Near-IR imaging of FiVis nanoparticles at 6 hours following XRT and nanoparticle administration at indicated doses in advanced stage Cav1 wildtype SHH-MB or Cav1 null SHH-MB tumors. Representative images, n =3 mice per group (i) Quantitative real-time PCR analysis of Gli1 target inhibition in advanced stage Cav1 wildtype SHH-MB and homozygous Cav1 null SHH-MB tumors following indicated XRT and FiVis treatments. qRT-PCR analysis for Cav1 is shown to indicate Cav1 status in wildtype and Cav1 knockout SHH-MB tumors. Data are means $\pm$ SEM. n = 3 mice per group, ** P < 0.01, *** P < 0.001 (unpaired t test); ns, not significant. (j) Schematic of proposed mechanism for FiVis nanoparticle passage across the BBB.

Methods

Preparation of Vismodegib Nanoparticles

Fucoidan-encapsulated vismodegib nanoparticles (FiVis) were prepared by a nanoprecipitation method adopted from previous work by our group^{16, 17}. In a microcentrifuge tube, the aqueous phase was prepared by combining the following solutions: 400 μ L of fucoidan (15 mg/mL), 50 μ L of IR-783 (2 mg/mL), 50 μ L of IR-820 (2 mg/mL), and 100 μ L of 0.01mM sodium bicarbonate. While gently vortexing this mixture, 50 μ L vismodegib (20mg/ml, dissolved in DMSO) were added dropwise. Upon complete addition of the organic phase, vortexing was ceased and the resultant semi-opaque emulsion was centrifuged for 15 minutes at 30,000g. Pellets were resuspended in 200 μ L of deionized water and further diluted as necessary, based on the vismodegib concentration of the mixture. Working stocks of FiVis used for in vivo and in vitro experiments were prepared at a concentration 2.5 mg of vismodegib per mL of nanoparticle mixture.

Nanoparticle Characterization

The concentration of vismodegib in the nanoparticle suspension was quantified using HPLC. Prior to analysis, nanoparticles were diluted 1:10 in DI water. An aliquot of this dilution was then mixed with acetonitrile at a ratio of 1:4 in order to extract vismodegib from the nanoparticles. Samples were then analyzed on an Agilent 1260 Infinity II HPLC system with an InfinityLab Poroshell 120 EC-C18, 4.6 x 75 mm, 2.7 μ m, analytical LC column. The mobile phase was comprised of acetonitrile and/or DI water, each containing 0.1% TFA. Chromatographic separation was achieved by gradient elution with acetonitrile (0% to 95%) at a flow rate of 1 mL/min. A single peak corresponding to vismodegib was characteristically observed with absorbance at 260 nm and a retention time of 3.04 minutes. The size and zeta potential of the nanoparticles were determined using dynamic and electrophoretic light scattering measurements acquired with a Malvern Zetasizer Nano ZS.

Flow Cytometry

Murine brain endothelial (bEnd.3) cells were plated in a 12-well plate at a density of 150,000 cells/well in 1mL of media (DMEM, 10% FBS, 1% P/S). Once confluent, cells in treatment groups receiving ionizing radiation were exposed to 0.25 Gy XRT. After 1 hour, cells were collected, transferred to microcentrifuge tubes, and fixed on ice using 2% paraformaldehyde. Fixed cells were washed twice with PBS and then resuspended in 100 μ L FACS buffer (PBS with 2%FBS). Cells were stained with 2 μ L of anti-P-selectin antibody (Biolegend, Cat #148310) and incubated at room temperature for 30 minutes. Cells were then washed twice with PBS, resuspended in 300 μ L of FACS buffer, and transferred to FACS tubes for analysis. Data was collected on a BD LSR II flow cytometer and analyzed using FCS Express Software. To quantify the effects of endocytosis inhibitors on nanoparticle uptake, bEnd.3 cells were plated in a 12-well plate at a density of 150,000 cells/well in 1mL of media. Upon reaching confluency, cells were treated with media containing chlorpromazine (5, 6.25, 7.5, 8.75 μ g/mL), methyl- β -cyclodextrin

(5.0, 6.25, 7.50, 8.75 mM), or regular media. After 8 hours, cells were washed with PBS and treated with nanoparticles (1:100 dilution of FiVis in complete DMEM media). Cells were incubated with nanoparticles for 30 minutes at 37°C. Afterwards, cells were washed twice with PBS and resuspended in freshly prepared FACS buffer containing propidium iodide as a viability stain. Data was collected on a BD LSR II flow cytometer, using the APC-Cy7 channel (excitation with 633 nm red laser, detection with 780/60 nm bandpass filter) to detect fluorescent signal from the IR-dyes within the nanoparticles. As previously, data was analyzed using FCS Express Software.

Fluorescence Microscopy

Similar to the nanoparticle uptake experiments using flow cytometry as a readout, bEnd.3 cells were plated in a 12-well plate at a density of 150,000 cells/well in 1mL of media. As above, once the cells were confluent, they were treated with media containing chlorpromazine (5.0, 6.25, 7.50, 8.75 µg/mL), methyl-β-cyclodextrin (5.0, 6.25, 7.50, 8.75 mM), or regular media. After 8 hours, the cells were washed with PBS and treated with nanoparticles (1:100 dilution of FiVis in complete DMEM media). The cells were then incubated with nanoparticles for 30 minutes at 37°C. After incubation with nanoparticles, cells were washed with HBSS and then stained with an HBSS solution containing both a membrane dye (CellMask Green, diluted 1:1000) and a nuclear dye (Hoescht 33342, diluted 1:10000). Following a 15-minute incubation at 37°C, cells were washed twice more with HBSS. Images were taken using an Olympus IX51 fluorescence microscope equipped with XM10IR Olympus camera and an X-Cite Xenon lamp. ImageJ software was used to process the data to create overlays of images taken from different channels.

Animals

All mice in this study were maintained under protocols approved by the Institutional Animal Care and Use Committee at Weill Cornell Medicine and Memorial Sloan Kettering Cancer Center. SHH-MB mice (*Ptfla*^{cre/+}; *Ptch1*^{fl/fl}) were generated by intercrossing *Ptfla*^{cre/+} mice³¹ with *Ptch1*^{fl/fl} mice³² and maintained on a C57BL/6 background. *Cav1* null (JAX Stock No:007083) and *Selp* null mice (JAX Stock No: 008432) were bred with SHH-MB mice to generate *Ptfla*^{cre/+}; *Ptch1*^{fl/fl}; *Cav1*^{-/-} and *Ptfla*^{cre/+}; *Ptch1*^{fl/fl}; *Selp*^{-/-} SHH-MB mice, respectively^{33,34}. The genotype of each mouse was confirmed by PCR genotyping of tail biopsy using primers for *Ptch1*, *Cre*, *Cav1*, and *Selp* (see Table S3 for primer sequences). Both sexes were used for all studies. Animals were housed on a 12-hour light/dark cycle and were given access to food and water ad libitum. The number of tumors and/or mice analyzed are provided in the Results section and/or in the figure legends.

Assessment of Mouse SHH-MB Blood-Brain Barrier Integrity

A bolus of 100 µL of a 10 mg/mL 70kDa Dextran-TMR solution (Life Technologies) was injected via the tail vein into tumor-bearing SHH-MB mice at advanced symptomatic stages, as previously described⁶. Brains were then removed two hours later without perfusion, fixed overnight in 4% paraformaldehyde, embedded in OCT, and then

sections prepared at a thickness of 12µm. Immunofluorescent staining on tissue sections was performed using antibodies against CD31 and P-selectin with appropriate secondary antibodies, counterstained with DAPI to visualize nuclei, and then coverslipped using Fluoro-Gel mounting medium as described below. Detection of TMR-Dextran in context of P-selectin and CD31 immunostaining was imaged using a fluorescent microscope (Zeiss Axioobserver) and TIFF images post-processed using Adobe Photoshop CS6.

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***In Vivo* Treatment Studies**

SHH-MB mice at advanced symptomatic stages (domed head, weight loss, ataxia) were treated with whole body ionizing radiation (XRT) using a RS2000 small animal irradiator (Rad Source) at the indicated doses (0 Gy, 0.25 Gy, 1 Gy, or 2 Gy) alone, with free vismodegib alone, with nanoparticle-encapsulated vismodegib (FiVis or DexVis) alone, or in combinations as indicated in the figure legends. When used in combination, drugs were administered via intraperitoneal injection two hours following XRT administration and assayed at the indicated timepoints for western blot and qRT-PCR studies. Treatment for survival studies were given on alternating days for a total of 8 doses starting on day 0, with all treatments in all experimental groups ending on day 16.

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Immunohistochemistry

Immunohistochemical staining of murine SHH MB tissues was performed at the Molecular Cytology Core Facility of Memorial Sloan Kettering Cancer Center. Brain tissues were harvested from SHH-MB mice and fixed in 4% PFA overnight. Fixed tissues were embedded in OCT and frozen sections were prepared at a thickness of 12µm. Heat antigen retrieval (95 °C for 20 minutes) was performed with citric acid buffer (pH=6), and sections were blocked for 30 minutes with 10% normal rabbit serum in PBS. Sections were incubated with primary antibodies (CD31, P-selectin, Cav1) overnight at 4 °C and secondary antibodies for 1 hour at room temperature (see Table S1 for antibody details). Slides were counterstained with Hoechst 33258 dye (Invitrogen) and coverslipped with Fluoro-Gel mounting medium (Electron Microscopy Sciences). Immunohistochemical detection of human SHH-MB tissue was performed at the Weill Cornell Medicine Center for Translational Pathology. De-identified human SHH-MB tissue was molecularly characterized using genome-wide methylation classification approaches as previously described³⁵. Tumor tissue was formalin fixed, paraffin embedded, and prepared as 5 µm tissue sections. Immunophenotyping was performed on a Leica Bond III system using the modified protocol J. Sections were pre-treated using heat-mediated antigen retrieval with sodium citrate buffer (pH=6, epitope retrieval solution 1) for 30 minutes. Sections were then incubated with P-selectin antibody (Lifespan Biosciences; LS-B3656) for 60 minutes at room temperature and detected using an alkaline phosphatase conjugated compact polymer system. Fast Red was used as the chromagen. The section was then counterstained with hematoxylin and mounted with micromount. All images were taken with a bright-field and fluorescence microscope (Zeiss Axio Observer) or digital Panoramic Slide Scanner (3D Histech, Budapest Hungary). TIFF images (with no compression) were post-processed using Adobe Photoshop CS6.

502 **Immunoblotting and Quantification**

503 SHH-MB tissue was dissected from tumor bearing mice following 2 Gy irradiation, homogenized through tissue
504 sonication in tissue lysis buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM EDTA, 0.5% NP-40, 100 mM NaF, 2 mM
505 Na₃VO₄, 10 mM Na₄P₂O₇) supplemented with protease inhibitor (Sigma P8849). Protein concentration was
506 determined using the Pierce BCA protein assay kit (ThermoFisher), and proteins were separated by NuPAGE Novex
507 10% Bis-Tris precast gels (ThermoFisher) and then electrophoretically transferred to PVDF membranes. Membranes
508 were incubated with Odyssey Blocking Buffer (LI-COR Biosciences) and then incubated with primary antibodies
509 for P-selectin, p53, and GAPDH and the appropriate secondary antibodies and analyzed using near-infrared imaging
510 using the LI-COR Odyssey CLx Imaging System. The antibodies are described in Table S2.

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512 **qRT-PCR**

513 Total mRNA was isolated from dissected mouse SHH-MB brain regions using TRI Reagent (Molecular Research
514 Center, Inc). Reverse transcription was performed with the iScript cDNA synthesis kit (Bio-Rad), and qpCR was
515 performed using SsoAdvanced Universal SYBR(R) Green Supermix (Bio-Rad) according to the manufacturer's
516 instructions. Fold-changes in expression were calculated using the $\Delta\Delta$ CT method. The *Gapdh* gene was used to
517 normalize the results. The primer sequences used are described in Table S3.

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519 **Nanoparticle Fluorescence Imaging and Quantification**

520 Nanoparticle localization in brains of SHH-MB mice were analyzed ex vivo using a LI-COR Odyssey CLx Imaging
521 System. Brains were scanned at a depth of 1 mm from the dorsal surface at 42 μ m resolution in the 800 nm channel
522 to detect the fluorescence emission from the IRDye783 incorporated into the FiVis nanoparticles. Images were
523 quantified using Image Studio Software, Version 5.2.5 (LI-COR Biosciences). In brief, forebrain and cerebellar areas
524 for quantification were demarcated and the relative signal per demarcated area was defined as the sum of the pixel
525 density per demarcated area. The relative signal per demarcated area was normalized to pixel density signal from
526 forebrain regions of untreated mice. For consistency, total cerebellar regions were demarcated, which included SHH
527 MB tumor regions, and compared to demarcated forebrain regions.

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529 **Vismodegib Bone Toxicity Studies**

530 Juvenile C57BL/6 wild-type mice (age P10) were administered either FiVis (10 mg/kg or 20 mg/kg) or free
531 vismodegib (100 mg/kg) twice daily for a total of 4 doses and compared to vehicle control mice at 6 weeks age.
532 Skeletal effects of each treatment were assessed by measurement of femur lengths using calipers. micro-CT (μ CT)
533 analysis was conducted using a Scanco Medical μ CT 35 system at the Citigroup Biomedical Imaging Core as
534 previously described described³⁶. Briefly, an isotropic voxel size of 7 μ m was used to image the distal femur. Scans
535 were conducted in 70% ethanol and used an X-ray potential of 55 kVp, an X-ray intensity of 0.145 mA, and an

integration time of 600 ms. μ CT analysis was performed by an investigator blinded to the treatment of the animals under analysis. All endpoint μ CT analysis was carried out on 6-week-old mice.

Nanoparticle Transwell Assay

Mouse brain endothelial cells (bEnd.3) were seeded on the upper surface of the membrane in polyester transwell inserts (0.4 μ M pore size, 1×10^8 per cm^2 pore density, 8.4 mm diameter) at a density of 1×10^5 cells per well. Media was changed every other day and cells were cultured for 5-7 days, until a confluent monolayer formed. Before initiating the transport studies, transendothelial electrical resistance (TEER) across cell layers was measured until they reached a TEER of $30 \Omega \times \text{cm}^2$. Once bEnd.3 cells monolayers reached this threshold TEER, endothelial paracellular barrier function was evaluated by measuring the permeability of cells to 70-kDa tetramethylrhodamine-dextran (TMR-Dextran). The concentration of TMR-Dextran was determined by measuring fluorescence (excitation at 555 nm and emission at 580 nm) using a TECAN plate reader. After confirming restriction to paracellular transport, transport studies with FiVis nanoparticles were carried out by adding 200 μ L of FiVis nanoparticles (20 μ g/ml) to the upper chamber of the insert. At various timepoints thereafter, the entire basal well volume was removed and assayed for nanoparticle concentration by measurement of fluorescence (excitation at 790 nm and emission at 815 nm) and for nanoparticle size using dynamic light scattering.

Mass Spectrometry for Quantification of Vismodegib in Brain Tissue

Analysis of vismodegib concentrations in brain tissue were performed by Integrated Analytical Solutions (IAS). As with efficacy studies, mice were irradiated with 0.25 Gy XRT. Two hours later, mice were injected intraperitoneally with nanoparticles containing 10 mg/kg vismodegib. After 4 hours, mice were sacrificed, and brain tissue was sectioned into two regions: forebrain (normal tissue) and cerebellum (tumor tissue). The tissue of each region was weighed and snap- frozen prior to LC-MS analysis by IAS. A standard curve that was generated by adding known amounts of vismodegib to homogenized brain tissue from non-treated mice. The concentrations of vismodegib in forebrain and cerebellar tumor tissue samples were then calculated to determine mass of vismodegib (ng) per gram of tissue.

Statistical Analyses

All data are shown as the mean $\pm$ s.d. or mean $\pm$ s.e.m., unless otherwise indicated. For comparison between two groups, unpaired, two-tailed Student's *t* tests were used. Analysis of variance (ANOVA) followed by a post hoc test for multiple comparisons (Dunnnett's) was used for comparison of groups of 3 or more. For Kaplan-Meier survival analysis, log-rank (Mantel-Cox) test was used. GraphPad Prism Version 9.1.0 software was used for statistical analysis. *P* < 0.05 was considered statistically significant and additional indicators of statistical significance are provided accordingly in the text or in figure legends. Statistical analysis was performed on measurements taken from distinct samples.

571 **Extended Data**

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575 **Extended Data Fig. 1: Maintenance of BBB integrity in SHH-MB GEM model after 0.25 Gy XRT.**

576 (a-d) Immunofluorescence staining of representative advanced stage *Ptfla^{cre};Ptch1^{fl/fl}* SHH-MB tumor two hours
577 after intravenous administration of 70 kDa tetramethylrhodamine (TMR)- dextran. *n*=3 mice. CD31 is used to
578 visualize tumor endothelium and DAPI to visualize nuclei and indicate SHH-MB tumor tissue. P-selectin and TMR-
579 dextran are indicated as shown.

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583 **Extended Data Fig. 2: Timecourse of P-selectin expression in SHH-MB following ionizing radiation.**
584 Western blot analysis of representative advanced stage *Ptfla^{cre};Ptch1^{fl/fl}* SHH-MB tumors following 2 Gy irradiation.
585 P-selectin protein expression was assessed at 2, 4, 6, and 24 hour time points (n=2 mice at each time point) and
586 compared to untreated SHH-MB controls. P53 immunoblotting is shown as a positive control for response to ionizing
587 radiation. GAPDH immunoblotting is shown as a loading control.
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591 **Extended Data Fig. 3: Size and drug encapsulation stability of FiVis nanoparticles.** (a) Size of FiVis
592 nanoparticles measured by DLS over extended periods of time in storage at 4°C. Data are means ± SEM. ns, not
593 significant (unpaired t test). (b) Drug release profile of FiVis nanoparticles incubated at 37°C (25% adult bovine
594 serum in PBS). Evaluated by HPLC quantification of vismodegib released into solution over time. (c) Size, PDI, and
595 drug encapsulation characteristics of resuspended FiVis nanoparticles after lyophilization.

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599 **Extended Data Fig. 4: Characterization of dextran sulfate-based nanoparticles.** (a) Dynamic light scattering
600 characterization of DexVis nanoparticles. (b) HPLC quantification of the concentration of vismodegib per batch of
601 DexVis and FiVis nanoparticle formulation. (c) Calculation of encapsulation efficiency for FiVis and DexVis
602 nanoparticles. Data are means $\pm$ SEM. ns, not significant (unpaired t test). (d) Drug release profile of FiVis and
603 DexVis nanoparticles incubated at 37°C (25% adult bovine serum in PBS). Evaluated by HPLC quantification of
604 vismodegib released into solution over time.

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Extended Data Fig. 5: Modulation of caveolin-1 expression in bEnd.3 cells. (a) Western blot for Cav1 expression following CRISPR-Cas9-mediated knockout of caveolin-1 in bEnd.3 cells. (b) Cav1 expression in bEnd.3 cells measured by qPCR. WT bEnd.3 cells were treated with FiVis nanoparticles to induce Cav1 expression above baseline. Data are means $\pm$ SEM, $*P < 0.05$ (unpaired t test). ns, not significant.

Extended Data Fig. 6: FiVis uptake into bEnd.3 cells following low dose XRT and pharmacological inhibition of endocytosis pathways. (a) Murine brain endothelial (bEnd.3) cells were assayed by flow cytometry for P-selectin expression following low dose irradiation. The percentage of live cells expressing P-selectin is shown for non-irradiated (0 Gy) and irradiated (0.25 Gy) cells. Data are means $\pm$ SEM, $*P < 0.05$ (unpaired t test). (b) Fluorescence images of bEnd.3 cells incubated with FiVis nanoparticles. Non-irradiated control cells (left) were cultured using standard media. Irradiated groups received 0.25 Gy XRT and were cultured using standard media or media containing endocytosis inhibitors. CPZ = Chlorpromazine (inhibitor of clathrin-mediated endocytosis); CD= methyl- β -cyclodextrin (inhibitor of caveolae dependent endocytosis). Color Legend: Red, IR-783/820 dye in FiVis nanoparticles; Green, CellMask Green plasma membrane stain; Blue, DAPI nuclear stain.

Extended Data Fig. 7: Characterization of transwell assay for assessment of FiVis passage across murine brain endothelial cells. Quantification of FiVis passage across transwell membrane inserts. Vismodegib in the (a) top chamber or (b) bottom chamber was quantified by HPLC. (c) Trans-endothelial electrical resistance (TEER) measurements of wildtype (WT) and caveolin-1 KO (Cav1 KO) bEnd.3 cells prior to incubation with FiVis nanoparticles. Data are means $\pm$ SEM. ns, not significant (unpaired t test). (d) Fluorescence quantification of 70kDa TMR-Dextran passage into bottom chamber for measurement of paracellular transport across monolayers of WT or Cav1 KO bEnd.3 cells. Data are means $\pm$ SEM. ns, not significant (unpaired t test).

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642 **Extended Data Fig. 8: P-selectin expression in *Cav1*^{-/-} SHH-MB mice.** Immunofluorescent staining of P-
643 selectin in (a) wild-type Cav1 and (b) homozygous Cav1 null SHH MB tumor tissue. CD31 is used to visualize
644 tumor endothelium and DAPI (blue) to visualize nuclei.

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647 **Extended Data Fig. 9: Survival of SHH-MB mice treated with free vismodegib and FiVis nanoparticles.**
648 Kaplan-Meier survival analysis of advanced stage SHH-MB mice treated with either 10 mg/kg free vismodegib (red),
649 50 mg/kg free vismodegib (green), or low dose ionizing radiation combined with FiVis nanoparticle (purple) at
650 indicated doses. Survival of untreated advanced stage SHH-MB mice is shown in blue. $P=ns$ (Mantel-Cox) for
651 comparison of groups treated with 50 mg/kg vismodegib (green) and 0.25 Gy XRT + FiVis 10 mg/kg (purple).
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653 **Table S1: Antibodies used for Immunohistochemistry (IHC)**

Primary Antibodies	Company	Dilution
rabbit anti-P-selectin (LS-B3578/57409)	LSBio	1:500
rat anti-CD31 (550274)	BD Biosciences	1:500
rat anti-CD34 (553731)	BD Biosciences	1:500
mouse anti-human CD62 (P-selectin)	LSBio	1:50
Secondary Antibodies	Company	Dilution
Donkey anti-rat (Secondary Antibody, Alexa Fluor 488)	Invitrogen	1:1000
Donkey anti-rabbit (Secondary Antibody, Alexa Fluor 647)	Invitrogen	1:1000

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656 **Table S2: Antibodies used for Western Blot (WB)**

Primary Antibodies	Company	Dilution
rabbit anti-P-selectin (LS-B3578/57409)	LSBio	1:1000
rabbit anti-P53 (CM5)	Leica	1:2000
rabbit anti-GAPDH (D16H11)	Cell Signaling	1:2000
Secondary Antibody	Company	Dilution
IRDye® 800RD Goat anti-Rabbit IgM	LI-COR	1:10000

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658 **Table S3: Sequence information for RT-qPCR**

Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')
mGli1	CTTGTGGTGGAGTCATTGGA	GAGGTTGGGATGAAGAAGCA
mCav-1	GCGACCCCAAGCATCTCA	ATGCCGTCGAAACTGTGTGT
mGAPDH	CGTCCCGTAGACAAAATGGT	TCAATGAAGGGGTCGTTGAT
mP-selectin	CTATACCTGCTCCTGCTACCCAGGC	TTCACTCCACTGACCAGAGCCAGTG

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660 **Table S4: Sequence Information for CRISPR/Cas9 mediated knockout of *CAV1***

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Target	ID	Sequence 1 - Forward* (5'→3')	Sequence 2 - Reverse* (5'→3')
Cav-1	sg1	CAAGCATCTCAACGACGACG	CGTCGTCGTTGAGATGCTTG
	sg2	ATGTGATTGCAGAACCAGAA	TTCTGGTTCTGCAATCACAT
GFP	sgGFP	CACCGGGGCGAGGAGCTGTTCACCG	AAACCGGTGAACAGCTCCTCGCCCC
*Sequences ordered as oligonucleotide duplexes from IDT (Integrated DNA Technologies).			

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Authors contributions: D.T. H.K., D.A.H and G.P.R. conceived the project, designed experiments, and wrote the manuscript. H.K. and Y.S. optimized FiVis and DexVis nanoparticle synthesis. H.K. and D.T. synthesized and characterized nanoparticles for in vivo studies. J.V. and J.S. performed in vivo experiments. J.V. performed qRT-PCR, western blot analysis, near-infrared nanoparticle imaging. J.G. performed immunohistochemistry on tissue samples. D.T. performed bEnd.3 cell pharmacology studies and flow cytometry experiments. T.I. and D.T. characterized and performed transwell assays and nanoparticle stability studies. D.R.B. and M.S. molecularly characterized human medulloblastoma samples. M.B.G performed and analyzed μ CT and in vivo bone toxicity experiments. D.A.H. and G.P.R. supervised the research.

Competing interests: D.A.H. is a cofounder and officer with equity interest of Goldilocks Therapeutics Inc., LipidSense Inc., and Nirova Biosense Inc., as well as a member of the scientific advisory board of Concarlo Holdings LLC and Nanorobotics Inc.

Data and materials availability: All referenced data is available in the manuscript or the supplementary materials. Additional data that support the findings of this study can be made available from the corresponding authors upon reasonable request.