Supplementary Figure Legends

Figure S1. Mouse SHH medulloblastomas do not show amplification of the *miR-17~92* cluster. *miR-17~92* amplification in mouse medulloblastoma (MB) samples by FISH using BAC clones RP23-132K20 for *miR-17-92* (green) and RP23-35N6 for band A3 on mouse chromosome 14 (red) assessed. None of the ten medulloblastoma samples exhibited *miR-17~92* amplification. Representative images of *miR-17~92* FISH are shown in two medulloblastomas (**A** and **B**).

Figure S2. 8-mer LNA-antimiRs inhibit miR-17 and miR-19a. QRT-PCR analysis of relative levels of miR-17 (**A**) and miR-19a (**B**) from RNAs extracted from whole cerebellum of a 1-month-old mouse (1 mo Cb), medulloblastoma cells (MB cells) left untreated (Unt), or treated for 48 hours *in vitro* with scrambled LNA control (Scr), antimiR-17 (17) or antimiR-19 (19). The relative miR-17 and miR-19a levels were quantified from three independently-derived tumors.

Figure S3. *In vitro* treatment with 8-mer LNA-antimiRs of MB cells from independently derived spontaneous MBs in [*Ptch1+/-;Cdkn2c-/-*] mice inhibits cell proliferation. (**A**) Two-to-five spontaneous MBs from [*Ptch1+/-;Cdkn2c-/-*] mice were purified and $2X10^6$ MB cells were plated per well in a 12-well culture plate then treated with varying concentrations of tiny LNAs (2 μM, 5 μM, 8 μM and 15 μM) or left untreated for 48 hours. (**B**) Using the same experimental design, MB cells from an additional 8-10 MBs were cultured and treated with a fixed concentration of 8 μM tiny LNAs (scrambled, antimiR-17, antimiR-19 or antimiR-17 and antimiR-19 [antimiR-combo]) or left

untreated. In both (**A**) and (**B**), the number of live MB cells were counted from each well and expressed as a percentage of the number of cells per well in the untreated condition (equals 100%). (**C-D**) $2X10^6$ cultured tumor cells from at least three MBs were treated with tiny LNAs for 48 hours and pulsed with 10 μM BrdU for 2 hours prior to harvest or harvested for assessment of apoptosis. (**C**) To assess BrdU incorporation, cells were harvested, fixed, stained with an antibody to BrdU and 7-Aminoactinomycin D (a DNA intercalating dye) prior to cell cycle analysis using FACS. (**D**) Harvested cells were stained with Annexin V and analyzed using FACS. In both (**C**) and (**D**), FACS analysis was completed on 10,000 events (1 event=1 cell). (**E**) $2X10^6$ cultured tumor cells from at least three MBs were cultured on matrigel-coated 4-chamber slides and fixed after treatment with tiny LNAs for 48 hours. The number of Ki-67 immunoreactive and DAPI-counterstained cells from each treatment condition (untreated, scrambled, antimiR-17 and antimiR-19) were counted and expressed as a percentage of DAPI-counterstained cells. At least 400 cells were counted per condition. Error bars indicate SD. *, **, *** Indicate P≤0.05, P≤0.01 and P≤0.001 versus untreated cells; #, ##, ### indicate P≤0.05, P≤0.01 and P≤0.001 versus scrambled. Black bars=untreated, grey bars=scrambled LNA, red bars=antimiR-17 and blue bars=antimiR-19.

Figure S4. *MiR-17~92* does not target *Smad5* or *Smad4*. Oligonucleotides containing putative miR-17 and miR-19 binding sites from the *Smad5* 3'-UTR and *Smad4* 3'-UTR sequences were designed and cloned into the pmirGLO vector according to manufacturer's instructions. The day before transfection, SAOS-2 cells, maintained in DMEM supplemented with 10% FBS, 4 mM glutamine and 100 units each of penicillin and streptomycin (GIBCO) at 37°C and 8% CO, were seeded in 24-well plates (3.0 x

10^4 cells per well). The next day, 10 ng of pmirGLO reporter plasmid was co-transfected with 0-200 ng of MSCV-miR-17~92-IRES-GFP using Fugene HD (Promega; 3.5:1 DNA) according to manufacturer's instruction. pCMV6 (a gift from Mark E. Hatley, SJCRH) was used to maintain the total amount of DNA per transfection. The cells were lysed 48 hours later, and dual-luciferase reporter assay (Promega, E1910) performed on Synergy 2 Biotek microplate reader according to manufacturer instructions. Bar graphs of Smad5 3'-UTR (**A**) and *Smad4* 3'-UTR (**B**) reporter assays in the SAOS-2 osteosarcoma cell line.

Figure S5. MiR levels of transplantable and non-transplantable [*Ptch1+/-;Cdkn2c-/-*] and [*Ptch1+/-;Trp53-/-*] flank tumors. (**A**) RNAs were extracted from [*Ptch1+/-;Cdkn2c-/-*] MBs that were either transplantable (n=6) or non-transplantable (n=3), and then subjected to qRT-PCR analysis of relative miR-17 (P=0.03) and miR-19a (P=0.04) levels. * Indicates P≤0.05 compared to transplantable tumors. (**B**) Relative miR-17 and miR-19a levels from RNA extracted from the whole cerebellum of 1-month-old mice (1 mo Cb; n=3) and from three [*Ptch1+/-;Trp53-/-*] flank MBs (tumors A-C). Error bars indicate SD.

Figure S6. FAM-labeled tiny LNAs are present in tissues 14 days after MB cell pre-treatment and transplantation. MB cells were purified from a medulloblastoma that spontaneously arose in a *[Ptch1+/-;Trp53-/-]* mouse. MB cells were either left untreated or pre-treated for 30 minutes with FAM-labeled tiny LNAs (scrambled, antimiR-17 or antimiR-19). 2 x 10^6 cells treated or untreated were immediately allografted into the

flanks of recipient mice, three mice per treatment condition. Mice were sacrificed 14 days after transplant, at which point, flank tumors were palpable. Tumors were sectioned, stained for the FAM-label using an anti-FAM-conjugated AlexaFluor 594 and the presence of tiny LNAs was visualized by confocal microscopy (20X objective, zoom 6). Representative images of flank tumors that arose from MB cells either left untreated or treated with scrambled LNA, antimiR-17 or antimiR-19. Note the presence of FAM (magenta) within tumor cells; nuclei are stained with DAPI (blue). Scale bar = 10 μm.