Supplementary Materials and Methods:

Western Blot Antibodies

MyoD primary antibodies used were rabbit C-20, Santa Cruz Biotechnology, 1:200, mouse, BD Biosciences, 1:150 and rabbit 6975b (developed in Dr. Stephen Tapscott’s lab), β-actin loading control (Abcam, 1:5000) used with appropriate HRP-conjugated secondary antibodies (Jackson Immunoresearch, 1:8000) for chemiluminescent detection.

qRT-PCR primer sequences

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<tr>
<th>Gene Name</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<td>Ppia</td>
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Supplementary Figure legends

Supplementary Figure 1:

Comparative analysis of MyoD +/-; SmoA2, MyoD +/-; SmoA2 and MyoD-/-; SmoA2 mice

A, Photograph shows MyoD +/-; SmoA2 and MyoD +/-; SmoA2 littermates at P18. MyoD +/-; SmoA2 and MyoD +/-; SmoA2 (not in image) littermates are indistinguishable at this stage when clinical signs of tumorigenesis are absent. MyoD +/-; SmoA2 mice obtained in sub-mendelian ratios, appeared smaller with compromised health and the majority (6 out of total 8 obtained) did not survive beyond approximately 3 weeks of postnatal life. B, Hematoxylin-Eosin stained representative horizontal sections obtained from cerebella of P19 MyoD +/-;
SmoA2, MyoD +/-; SmoA2 and MyoD -/-; SmoA2 littermates without any clinical signs of tumors, show no major differences in histopathology. Scale bar: 100μm.

Supplementary Figure 2
Mouse medulloblastomas with one allele of MyoD trend towards higher Ki67 index with reduction in total number of MyoD+ cells as well as cellular expression
A, Quantitative image analysis of Ki67 immunofluorescence data on stage-matched MyoD +/-; SmoA2 and MyoD +/-; SmoA2 tumors show a trend towards increased proliferation in the latter group (n=5, p=0.06; one-tailed Student t-test). B, Immunofluorescence for MyoD in Ki67+ tumors in MyoD +/-; SmoA2 and MyoD +/-; SmoA2 mice show reduced number of MyoD+ positive cells in the latter group (n=5 per group, p<0.05). Scale bar: 100um. C, Cumulative distribution functions plotted as normalized MyoD maximum intensity on the x-axis and the proportion of cells at each intensity level on the y-axis, show that the MyoD +/-; SmoA2 tumors appear to have both reduced number of MyoD+ cells (B) as well as reduced expression of MyoD at a cellular level (p<0.05, K-S test) compared to MyoD +/-; SmoA2 tumors. The dotted line indicates the intensity threshold that was used to qualify cells as MyoD positive or MyoD negative.

Supplementary Figure 3
MyoD is expressed in proliferating tumor cells in SmoA1, SmoA2 and Ptch conditional knock out medulloblastoma mouse models
MyoD (green) is localized exclusively in Ki67+ (red) tumor cells (arrows) as determined by immunofluorescence analysis in representative tumors from three models of Shh-driven
medulloblastoma – SmoA1, SmoA2 and \textit{Ptc}^{F/F} Math1-Cre conditional knockout (\textit{Ptc} cko).

DAPI was used as the nuclear counterstain. Scale Bar: 100\,\mu m.

**Supplementary Figure 4**

MyoD expression is higher in the tumor periphery compared to the inner core

Immunofluorescence analysis shows a higher number of MyoD+ tumor cells towards the periphery of the SmoA2 tumors (n=4, \( p < 0.05 \)), a Shh driven medulloblastoma which originates from the hyperproliferative EGL. The abundance of MyoD+ cells decrease towards the inner core of the tumor. Scale Bar: 100\,\mu m

**Supplementary Figure 5: Heterozygous loss of MyoD does not influence its canonical targets in the myogenic differentiation program**

Compared to adult WT cerebellum, known targets of MyoD, \textit{Myog} and \textit{Myf5} are increased in both MyoD+/+; SmoA2 and MyoD+/-; SmoA2 tumors with no significant inter-group difference. \textit{Cdh15}, \textit{Desmin} remain unchanged. \textit{Id3} levels are increased approximately 2-fold in MyoD+/-; SmoA2 compared to MyoD+/+; SmoA2 tumors (*\( p = 0.006 \) as determined by two-tailed Student t-test). \textit{Ppia} was used for data normalization. All data are mean \textpm\,s.e.m

**Supplementary Figure 6**

\textbf{MYOD is expressed across different molecular subgroups of medulloblastoma}

Log\textsubscript{2} fold change in expression of \textit{MyoD} in adult and fetal cerebella, the four subgroups of medulloblastoma in a non-overlapping validation series. Data are presented as box-whisker
plots, showing the central location and distribution of the fold change in expression for each subgroup.