Supplementary Information, Table S1. ΔcT values for expression in the data analysis, as represented in Figures 1D, E, 5E.

Upper panel: PRUNE-1 expression levels at diagnosis in our cohort of primary MB samples were correlated to metastatic stage (M-stage) of patients recorded according to the five-tiered Chang classification (M+, n= 8; M0, n=26). The normal cerebellum control samples (n = 13) were from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, USA. The analysis was performed using real-time-PCR with SYBR-green technology, and the relative expression values are shown as $2^{-\Delta Ct}$ (related to Figure 1D). Similar data also shown for MB_{Group3} (n=5) and MB_{Group4} (n=5) within our cohort dataset, as determined by real-time-PCR using Taqman probe (Applied Biosystems, ThermoScientific; Human, 58497; Hs00930871_g1) (related to Figure 1E). **Lower panel:** Time-course expression analysis of AA7.1 (100 μM) *versus* PBS treatment of MB_{Group3} D425-Med cells, for PRUNE-1, OTX2, SNAIL and PTEN. The analysis was performed using real-time-PCR with SYBR-green technology, and relative expression values are shown as $2^{-\Delta Ct}$ (related to Figure 5F).

Prune-1 Average 2^-□Ct

Normal Cerebellum	Metastatic Stage M+ (Chang)	Metastatic stage M0 (Chang)		Molecular subgroup MB Group3	Molecular subgroup MB Group4
0,017968109	0,033243768	0,03957201	0,014619849	2,874829453	1,502634025
0,012298839	0,045310444	0,018304708	0,006565009	2,120285122	0,81499226
0,020776338	0,070201483	0,04497906	0,009609183	2,256340065	1,752859196
0,015817479	0,023282933	0,014981769	0,041127263	1,068324458	1,829879814
0,014408404	0,024470329	0,014353962	0,023427584	1,428852825	0,637456357
0,015463384	0,056014777	0,169506982	0,008994276		
0,015588746	0,02669426	0,029045353	0,008678344		
0,021861051	0,054773421	0,015865455	0,011786639		
0,022665806		0,018211761	0,015949542		
0,020836826		0,020252856	0,016868025		
0,011192525		0,015369316	0,013161862		
0,019574428		0,008866973	0,012767014		
0,025000378		0,009530144	0,018291085		

Cell line	Time (Hour)	Treatment	Prune-1 Average 2^-ΔCt	OTX2 Average 2^-ΔCt	SNAIL Average 2^-ΔCt	PTEN Average 2^-ΔCt
D425-MED	0	-	0,022611483	5,946699167	0,008206207	0,08778037
	1	PBS	0,02222267	3,390477988	0,013641848	0,057594705
		100 μM AA7.1	0,009951936	3,626218642	0,012161853	0,152002014
	3	PBS	0,012854084	5,83343564	0,010104008	0,085911808
		100 μM AA7.1	0,009254939	0,598007322	0,006053971	0,781800546
	6	PBS	0,010241471	4,707353424	0,012647817	0,021091171
		100 μM AA7.1	0,000969824	0,765425767	0,008941335	0,241943297

Supplementary Information, Table S2. Mini-ontology analysis of the three independent gene signatures in MB. The analysis was performed using GO terms involved in neurogenesis or neuron differentiation (GO:0030182, GO:0048666, GO:0050767, GO:0022008) using three of four probes for PRUNE-1 (see Material and Methods). The analysis indicated *OTX2* as most likely to be statistically regulated in the high PRUNE-1 expression category in the main three gene-expression MB signatures (publically available: Kool, Gilbertson, Magic-Northcott). The other genes (*CYFIP1*, *GLI2*) were inversely correlated to PRUNE-1 expression (i.e., negative [–] scores). The *AMIGO1* gene showed minimal up-regulation, as measured by fold-change values within these expression signatures. This analysis strengthens the overall correlation between PRUNE-1 and OTX2 mRNA expression in MB.

Gene-expression signatures	Kool			Gilbertson			MAGIC-Northcott		
	p-value (High vs. Low)	Bonferroni (p-value (High vs. Low)	Fold-Change (High vs. Low)	p-value (High vs. Low)	Bonferroni (p-value) (High vs. Low)	Fold-Change (High vs. Low)	p-value (High vs. Low)	Bonferroni (p value (High vs. Low)	Fold-Change (High vs. Low)
NEURON_DIFFERENTIATION; GO-0030182									
OTX2	**0.0116917**	**1**	**2.92372**	**0.0107397**	**1**	**2.35364**	**4.48176E-07**	**0.0113357**	**2.13026**
AMIGO1	0.000203993	1	1.5837	0.0382765	1	1.27153	3.65589E-05	0.924684	1.19368
CYFIP1	8.99872E-05	1	-1.71509	0.10151	1	-1.20427	2.94717E-07	0.00745428	-1.28958
GLI2	0.00365488	1	-2.12231	0.00730009	1	-1.9128	5.32173E-07	0.0134603	-1.92626
NEURON_DEVELOPMENT									
GO-0048666									
OTX2	**0.0116917**	**1**	**2.92372**	**0.0107397**	**1**	**2.35364**	**4.48176E-07**	**0.0113357**	**2.13026**
AMIGO1	0.000203993	1	1.5837	0.0382765	1	1.27153	3.65589E-05	0.924684	1.19368
CYFIP1	8.99872E-05	1	-1.71509	0.10151	1	-1.20427	2.94717E-07	0.00745428	-1.28958
GLI2	0.00365488	1	-2.12231	0.00730009	1	-1.9128	5.32173E-07	0.0134603	-1.92626
REGULATION_OF_NEUROGENESIS; GO-0050767									
AMIGO1	0.000203993	1	1.5837	0.0382765	1	1.27153	3.65589E-05	0.924684	1.19368
NEUROGENESIS									
GO-0022008									
OTX2	**0.0116917**	**1**	**2.92372**	**0.0107397**	**1**	**2.35364**	**4.48176E-07**	**0.0113357**	**2.13026**
CYFIP1	8.99872E-05	1	-1.71509	0.10151	1	-1.20427	2.94717E-07	0.00745428	-1.28958
GLI2	0.00365488	1	-2.12231	0.00730009	1	-1.9128	5.32173E-07	0.0134603	-1.92626

Supplementary Table S3. Gene expression correlation analyses between MYC family members (i.e., c-MYC, N-MYC) and PRUNE-1, TGFB1 and OTX2.

Gene expression correlation analysis for c-MYC and N-MYC relating to PRUNE-1, TGF-β1 (TGFB1) and OTX2 transcripts in MB samples from the Cavalli dataset (n=763 tumour samples).

Variable 1	Variable 2	Correlation coefficient (r)	Significance(p)	Correlation strength
c-MYC	PRUNE-1	0.173	1.5e-06	Positive (weak)
	TGFB1	0.420	5.8e-34	Positive (moderate)
	OTX2	0.255	8.0e-13	Positive (weak)
N-MYC	PRUNE-1	-0.439	3.3e-37	Negative (moderate)
	TGFB1	-0.258	4.6e-13	Negative (weak)
	OTX2	-0.556	3.1e-63	Negative (moderate)

Supplementary Information, Table S4: Bioluminescence analysis from *in-vivo* mouse trials.

Upper panel (*in-vivo* trial with AdV-CPP): *In-vivo* bioluminescence analysis from cerebellum as the region of interest in nude mice (n = 6) implanted with MB_{Group3} cells (1×10^5) stably expressing the firefly luciferase gene (D425-Luc cells), 24 h after infection *in vitro* with multiplicity of infection 100 of adenoviral particles type 5 carrying CPP (AdV-CPP), and empty viral particles (AdV-Mock) as control. Tumorigenesis was followed *in vivo* for up to 28 days after tumour implantation (related to Figure 4D). **Lower panel (*in-vivo* trial with AA7.1):** As for upper panel, initially with mice (n = 12) implanted with D425-Luc MB_{Group3} cells (1×10^5). Fourteen days after primary tumours were established (from implantation; day 0), the mice were grouped based on bioluminescence values, and treated *in vivo* with AA7.1 (60 mg/kg every 2 days), or with PBS as vehicle control, for 14 further days (related to Figure 5B).

***In vivo* trial with AdV-CPP** **Total Flux (P/S) values from the Region Of Interest**						
	AdV-MOCK			**AdV-CPP**		
Mice	**1**	**2**	**3**	**4**	**5**	**6**
T0	6,04E+07	4,66E+07	3,86E+07	5,64E+07	1,75E+07	8,89E+07
T14	8.96E+07	2.10E+07	5.01E+07	2.92E+07	2,85E+07	1.28E+07
T21	5.31E+08	6.03E+08	6.85E+08	6,43E+08	6,50E+08	6.27E+08
T28	9.31E+09	1.71E+10	1.07E+10	7.75E+09	5.72E+09	5.66E+09

***In vivo* trial with AA7.1** **Total Flux (P/S) values from the Region Of Interest (ROI)**						
	Vehicle (PBS)					
Mice	**1**	**2**	**3**	**4**	**5**	**6**
T0	2,79E+04	2,06E+04	1,05E+05	2,10E+04	1,49E+05	2,59E+05
T14	4,13E+05	2,16E+05	1,80E+05	6,46E+04	6,65E+04	4,90E+05
T21	3,81E+06	3,08E+06	3,35E+06	3,22E+06	6,38E+06	1,08E+06
T28	3,54E+07	1,65E+07	2,36E+07	3,40E+07	3,40E+07	2,91E+07
	AA7.1 (60mg/Kg/every two days)					
Mice	**7**	**8**	**9**	**10**	**11**	**12**
T0	4,77E+04	2,41E+05	7,69E+04	1,77E+04	2,40E+05	8,93E+04
T14	1,94E+05	1,49E+06	2,34E+04	5,13E+04	8,42E+04	4,94E+04
T21	1,22E+06	1,58E+06	6,38E+05	3,96E+05	2,41E+06	2,72E+06
T28	1,42E+07	3,70E+06	1,94E+06	5,73E+06	3,76E+06	4,93E+06

Supplementary Information, Table S5. Haematological and biochemical analyses of AA7.1 toxicity *in vivo*.

Haematological and biochemical markers of hepatic and renal functions from Balb/c mice treated *in vivo* with increasing doses of AA7.1 (15 to 60 mg/kg) administered once daily for 1 week (toxicity study), and from nude xenograft mice implanted with D425-Luc and treated *in vivo* with AA7.1 (60 mg/kg every 2 days) (pre-clinical trial). Mice treated with PBS were used as vehicle controls. Data are expressed as means from three different mice. No significant differences were detected between mice treated with AA7.1 and PBS. WBC= White Blood Cells; LYM= lymphocytes; MONO= monocytes; GRAN= granulocytes; HGB= Hemoglobin; HCT= hematocrit; RBC= Red Blood Cell; MVC= Mean Corpuscular Volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration; PLT= Platelets; GPT= glutamic-pyruvic transaminase; GOT= glutamic oxaloacetic transaminase; CRE= creatinine; BUN= Blood Urea Nitrogen.

	Reference values		**Toxycity *in vivo* study (Balc/C mice) 5 days treatment**				***In vivo* pre-clinical trial (nude mice) 14 days treatment**	
			CTR	**AA7.1 (mg/Kg/day)**			**CTR**	**AA7.1 (mg/Kg)**
	Unit	**Values**	**PBS**	**15**	**30**	**60**	**PBS**	**60**
WBC	**10^9/l**	**6.3 - 10.1**	1.6	3.1	3.65	3.4	1.9	3.2
LYM	**10^9/l**	**3.4 - 7.0**	1.25	2.75	3.2	2.75	0.8	1.6
MONO	**10^9/l**	**0.1 - 0.5**	0.15	0.15	0.2	0.2	0.2	0.3
GRAN	**10^9/l**	**1.5 – 3.2**	0.9	0.25	0.35	0.45	0.9	1.3
HGB	**g/dl**	**11.5 – 15.1**	10.8	13.4	12.4	13.8	12.7	13.3
HCT	**%**	**36.6 – 47.4**	32.15	39.5	36.75	41.85	33.2	36.2
RBC	**10^{-2}/l**	**5.2 – 6.8**	6.065	8.14	7.41	8.37	7.48	7.95
MCV	**fl**	**64.6 – 76.2**	53.05	48.5	49.7	50	44.4	45.5
MCH	**pg**	**21.1 – 24.5**	17.85	16.5	16.75	16.55	16.9	16.8
MCHC	**g/dl**	**29.5 – 33.9**	33.8	34	33.75	33.05	38.2	36.9
PLT	**10^9/l**	**250 - 610**	493	489.5	443	591.5	300	300
GPT	**U/l**	**up to 60**	46	34.5	32	27	35	61
GOT	**U/l**	**54 - 269**	77	68	76.5	84.5	79	82
CRE	**mg /dl**	**0.3 - 1**	0.35	0.3	0.4	0.3	0.4	0.4
BUN	**mg/dl**	**17-28**	31.45	30.4	25.55	31.45	30.6	31.2