

Association of Aggresomes with Survival Outcomes in Pediatric Medulloblastoma

Authors

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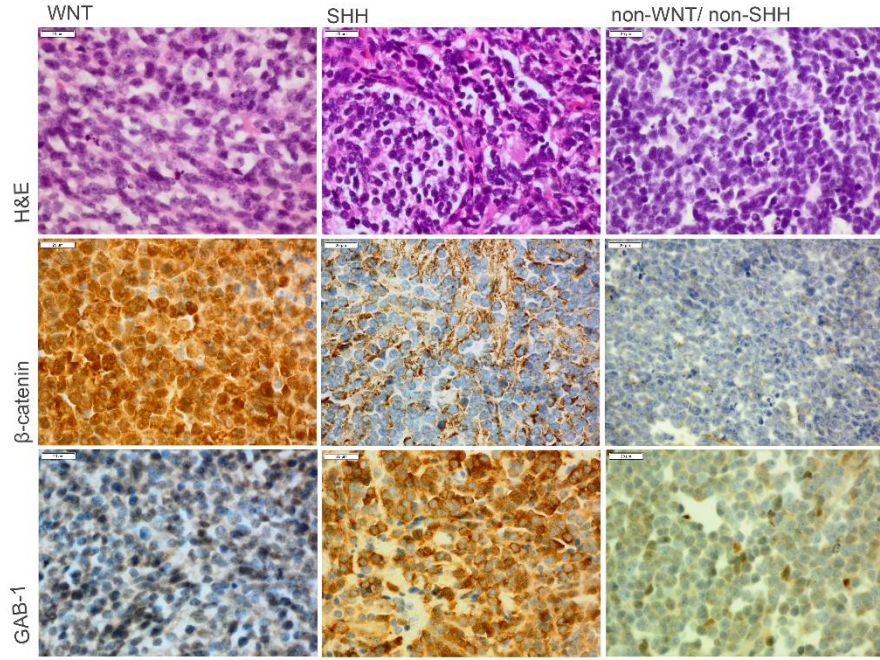
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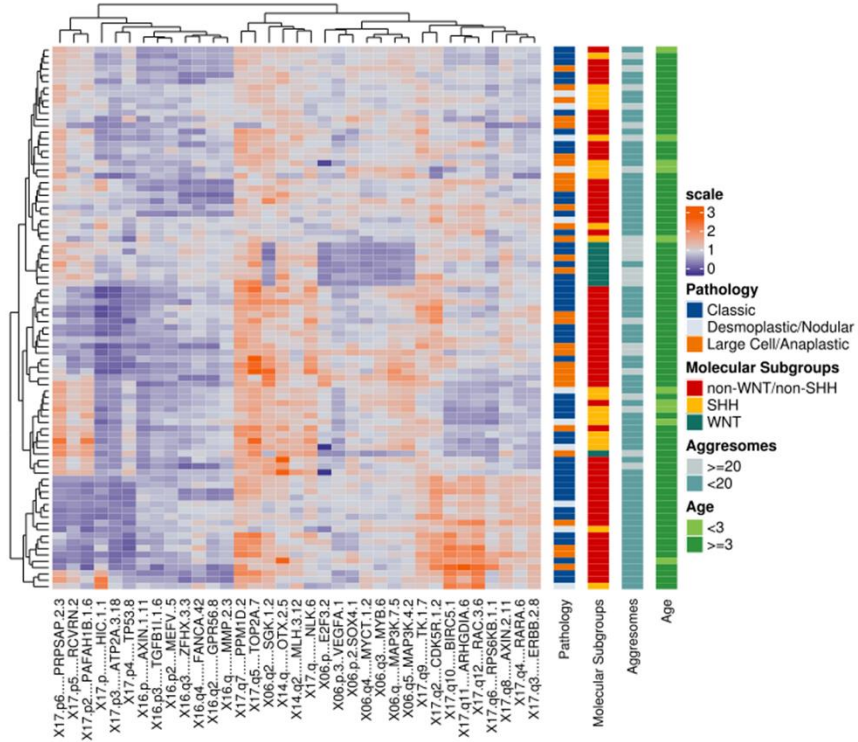
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Supplementary Figure. 1

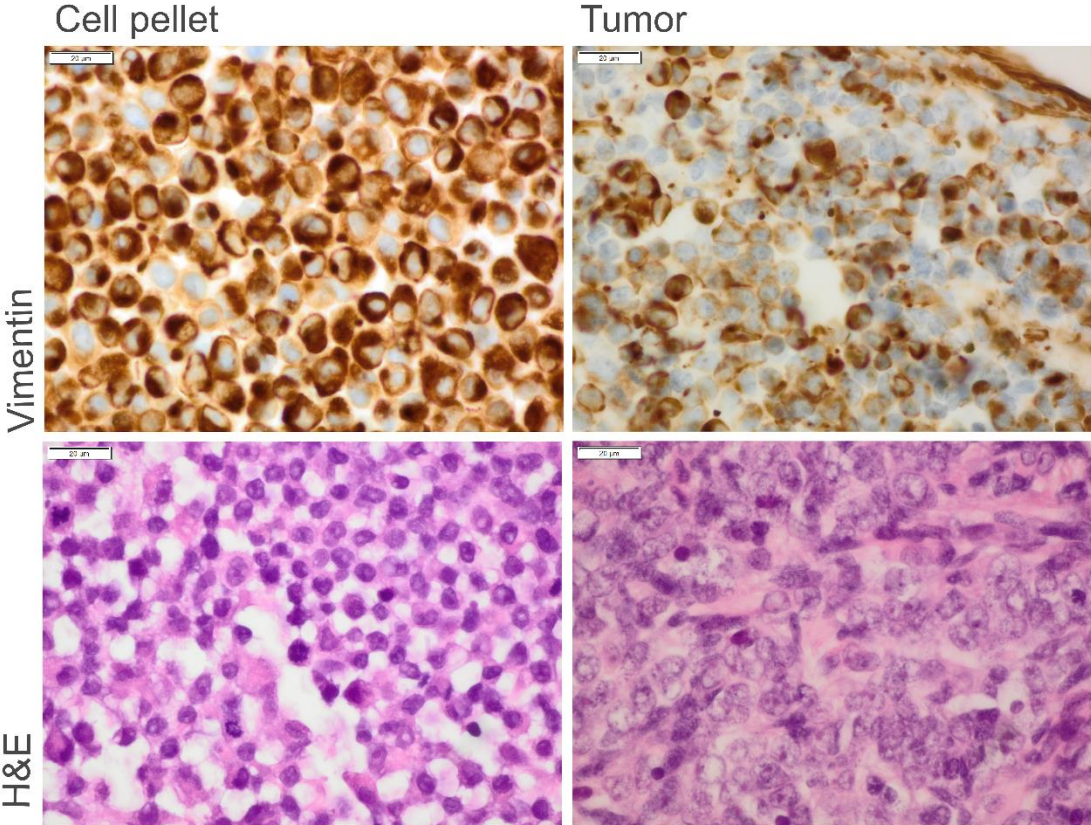
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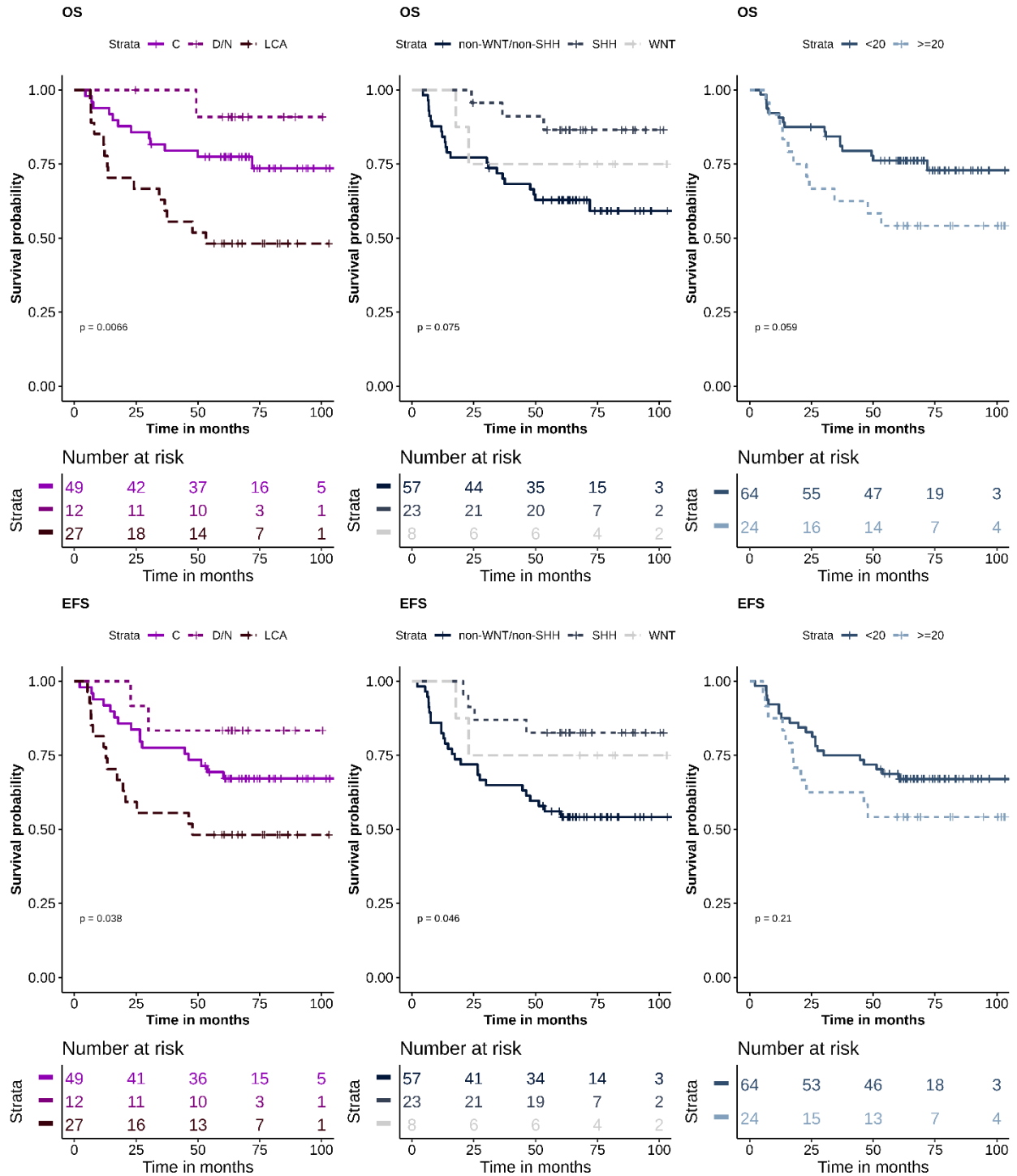
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Supplementary Figure. 2

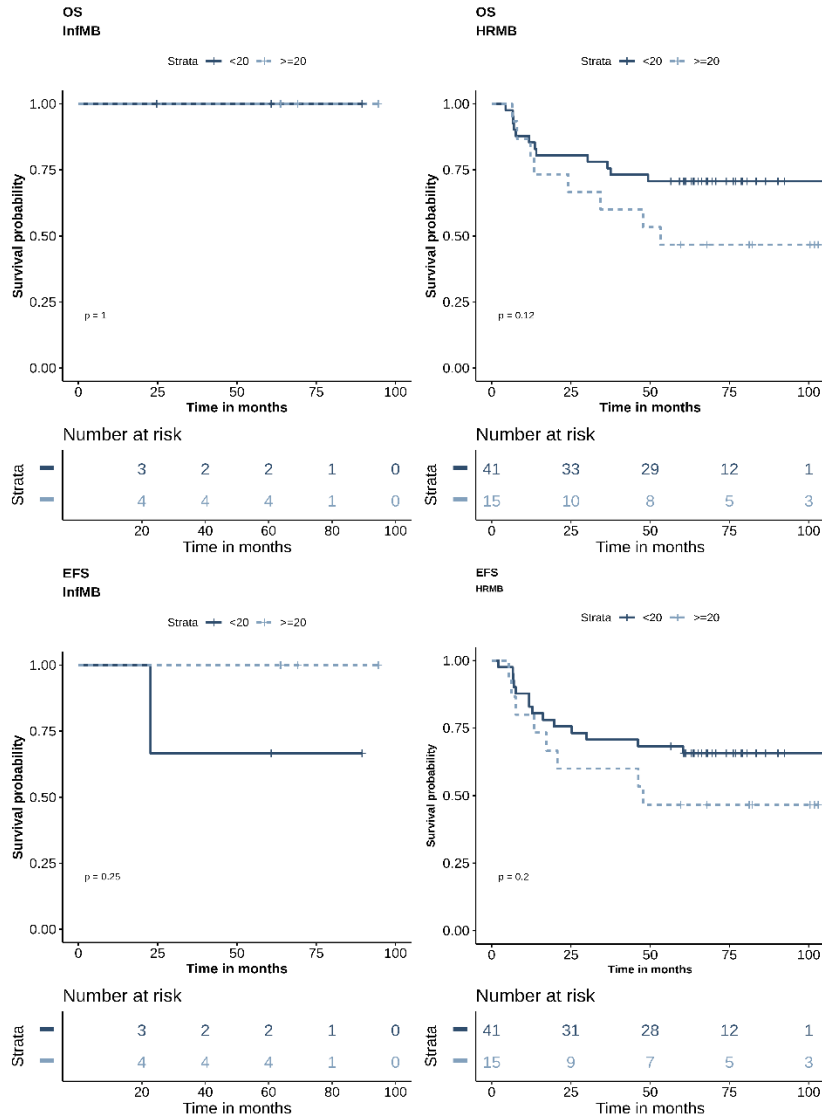


Supplementary Figure. 3

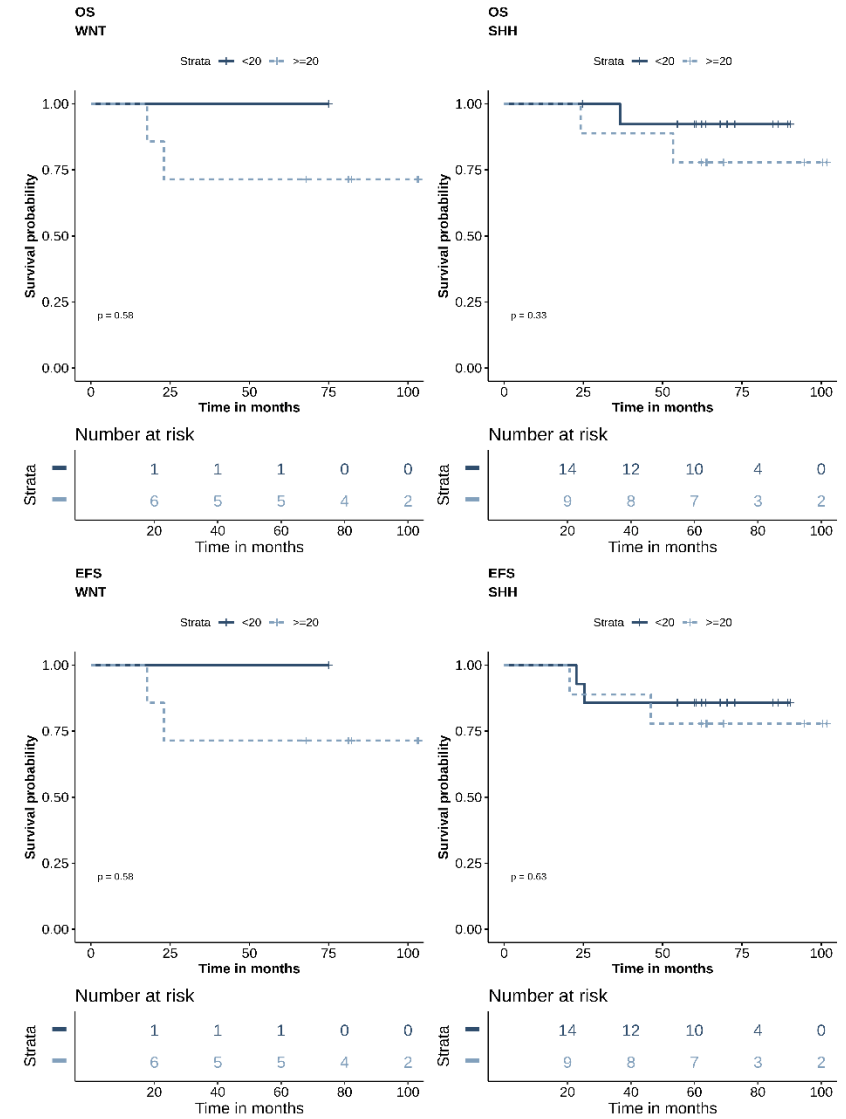


Supplementary Figure. 4

A



B



Supplementary Figure Legends

Supplementary Fig. 1. Stratification of medulloblastoma molecular subgroups. (A) Histologic evaluation was performed using H&E stained section. Nuclear β -catenin and cytoplasmic GAB-1 immunoreactivity were used as markers for WNT and SHH molecular subgroups respectively. Non-WNT/non-SHH subgroup were identified using cytoplasmic β -catenin and negative GAB-1 immunostaining; (B) Heatmap representation of MLPA DQ results. Clinicopathological and molecular annotations are provided as side bars according to the included key.

Supplementary Fig. 2. Immunohistochemical analysis of CCHE-188 cell pellet and the original tumor. Aggresomes are identified by the formation of juxta nuclear localization of vimentin in both cell line and tumor.

Supplementary Fig. 3. Survival analysis Kaplan-Meier plot of overall and event-free survival. Survival analysis separated by histological variants, molecular subgroups and percentage of aggresomes within the tumor ($\geq 20\%$ and $< 20\%$). Survival probability (y axis) and time indicated in months (x axis). *P-values* were calculated using the log-rank test.

Supplementary Fig. 4. Survival analysis Kaplan-Meier according to percentage of aggresomes within the tumor cells. (A) Patients are stratified according to clinicopathological risk criteria (InfMB and HRMB). (B) Patients are stratified using molecular subgrouping (WNT and SHH). Survival probability (y axis) and time indicated in months (x axis). *P-values* were calculated using the log-rank test.

Supplementary Table 1

ID	Gender	Pathology	β-catenin	GAB1	YAP1	M-subgroup	Vimentin	Aggresomes	M_Status	Treatment protocol
CCHE_Med_001	M	Classic	N	N	N/A	nonWnt/SHH	P	N	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_002	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_003	F	Desmoplastic/Nodular	N	90%	N/A	SHH	P	75%	M3	HRMB (CCHE 3-1-2008)
CCHE_Med_004	F	Classic	N	15%	N	nonWnt/SHH	N	N	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_005	M	Large_Cell_Anaplastic	P	N	N/A	Wnt	P	20%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_006	F	Classic	P	N	N/A	Wnt	P	30%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_007	M	Classic	N	25%	N/A	SHH	P	85%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_008	M	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	N	N	Mx	Surgery only
CCHE_Med_009	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_010	M	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	N	N	Mx	HRMB (CCHE 3-1-2008)
CCHE_Med_011	M	Classic	P	N	N/A	Wnt	P	60%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_012	F	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	P	20%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_014	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_015	M	Classic	N	N	N/A	nonWnt/SHH	P	7%	M2	HRMB (CCHE 3-1-2008)
CCHE_Med_016	F	Classic	N	N	N/A	nonWnt/SHH	N	N	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_017	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_018	M	Large_Cell_Anaplastic	P	N	P	Wnt	P	60%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_019	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_020	F	Classic	P	N	N/A	Wnt	P	70%	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_021	F	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	N	N	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_022	F	Large_Cell_Anaplastic	N	98%	N/A	SHH	N	15%	Mx	HRMB (CCHE 3-1-2008)
CCHE_Med_023	F	Large_Cell_Anaplastic	N	90%	P	SHH	P	2%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_024	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_025	F	Classic	N	75%	N/A	SHH	P	90%	M0	InfMB (CCHE 3-1-2008)
CCHE_Med_026	M	Desmoplastic/Nodular	N	40%	N/A	SHH	N	5%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_027	M	Classic	N	N	N/A	nonWnt/SHH	N	N	Mx	HRMB (CCHE 3-1-2008)
CCHE_Med_028	M	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	N	10%	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_029	M	Desmoplastic/Nodular	N	50%	N/A	SHH	P	15%	Mx	InfMB (CCHE 3-1-2008)
CCHE_Med_030	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_031	F	Classic	P	N	N/A	Wnt	P	20%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_032	M	Classic	N	N	N/A	nonWnt/SHH	P	5%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_033	M	Large_Cell_Anaplastic	N	40%	N/A	SHH	N	10%	Mx	HRMB (CCHE 3-1-2008)

CCHE_Med_034	F	Desmoplastic/Nodular	N	N	P	SHH	N	1%	M0	InfMB (CCHE 3-1-2008)
CCHE_Med_035	M	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	P	2%	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_036	M	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	P	5%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_038	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_039	M	Classic	N	N	N/A	nonWnt/SHH	P	7%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_040	M	Classic	N	N	N/A	nonWnt/SHH	P	25%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_041	F	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	P	15%	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_042	F	Desmoplastic/Nodular	N	98%	N/A	SHH	N	N	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_045	F	Classic	N	N	N/A	nonWnt/SHH	N	N	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_046	F	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	N	N	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_047	M	Classic	N	N	N/A	nonWnt/SHH	P	5%	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_048	M	Classic	N	2%	N	nonWnt/SHH	N	2%	M2	HRMB (CCHE 3-1-2008)
CCHE_Med_049	M	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	N	1%	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_050	M	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	N	2%	M3	HRMB (CCHE 3-1-2008)
CCHE_Med_051	M	Classic	N	90%	N/A	SHH	N	10%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_052	F	Classic	N	N	N/A	nonWnt/SHH	N	1%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_053	M	Classic	P	N	N/A	Wnt	P	10%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_054	M	Classic	N	N	N/A	nonWnt/SHH	N	1%	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_055	M	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	P	20%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_056	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M3	Surgery only
CCHE_Med_057	F	Classic	N	25%	N/A	SHH	P	N	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_058	F	Large_Cell_Anaplastic	N	80%	N/A	SHH	P	80%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_059	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_060	F	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	N	2%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_061	M	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	N	N	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_062	F	Large_Cell_Anaplastic	N	85%	N/A	SHH	P	80%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_063	F	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	P	20%	M3	HRMB (CCHE 3-1-2008)
CCHE_Med_065	F	Desmoplastic/Nodular	N	25%	N/A	SHH	N	N	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_066	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M3	HRMB (CCHE 3-1-2008)
CCHE_Med_068	F	Classic	N	N	N/A	nonWnt/SHH	N	N	M2	HRMB (CCHE 3-1-2008)
CCHE_Med_069	F	Classic	N	N	N/A	nonWnt/SHH	N	5%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_070	F	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	P	70%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_071	M	Classic	N	N	N/A	nonWnt/SHH	N	5%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_072	M	Desmoplastic/Nodular	N	N	FWN	nonWnt/SHH	P	1%	M3	HRMB (CCHE 3-1-2008)
CCHE_Med_073	M	Classic	N	N	N/A	nonWnt/SHH	P	3%	M3	HRMB (CCHE 3-1-2008)

CCHE_Med_074	M	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	N	N	M1	Surgery only
CCHE_Med_075	F	Desmoplastic/Nodular	N	40%	N/A	SHH	P	60%	M1	InfMB (CCHE 3-1-2008)
CCHE_Med_077	F	Classic	N	90%	N/A	SHH	P	80%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_078	M	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	P	90%	M3	HRMB (CCHE 3-1-2008)
CCHE_Med_079	F	Classic	N	N	N/A	nonWnt/SHH	N	N	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_080	M	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	P	40%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_081	F	Classic	N	7%	P	SHH	P	40%	M1	InfMB (CCHE 3-1-2008)
CCHE_Med_082	F	Desmoplastic/Nodular	N	45%	N/A	SHH	P	35%	Mx	InfMB (CCHE 3-1-2008)
CCHE_Med_083	F	Desmoplastic/Nodular	N	98%	N/A	SHH	N	N	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_084	F	Classic	N	N	N/A	nonWnt/SHH	P	N	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_085	F	Classic	N	N	N/A	nonWnt/SHH	N	1%	M0	InfMB (CCHE 3-1-2008)
CCHE_Med_086	M	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	P	20%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_087	M	Desmoplastic/Nodular	N	N	FWN	nonWnt/SHH	N	8%	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_088	F	Classic	N	N	N/A	nonWnt/SHH	N	N	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_089	F	Classic	N	N	N/A	nonWnt/SHH	P	5%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_090	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M3	HRMB (CCHE 3-1-2008)
CCHE_Med_091	M	Large_Cell_Anaplastic	P	N	N/A	Wnt	P	20%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_092	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M3	HRMB (CCHE 3-1-2008)
CCHE_Med_093	F	Classic	N	N	N/A	nonWnt/SHH	P	10%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_094	F	Large_Cell_Anaplastic	N	90%	N/A	SHH	P	10%	M0	HRMB (CCHE 3-1-2008)
CCHE_Med_095	F	Large_Cell_Anaplastic	N	N	N/A	nonWnt/SHH	P	10%	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_096	F	Large_Cell_Anaplastic	N	N	N	nonWnt/SHH	P	55%	M1	HRMB (CCHE 3-1-2008)
CCHE_Med_097	F	Classic	N	N	N/A	nonWnt/SHH	N	N	M3	HRMB (CCHE 3-1-2008)
CCHE_Med_098	M	Classic	N	N	N/A	nonWnt/SHH	N	N	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_099	M	Desmoplastic/Nodular	N	60%	N/A	SHH	N	2%	M0	SRMB (CCHE 3-1-2008)
CCHE_Med_100	M	Classic	N	85%	N/A	SHH	P	5%	M0	SRMB (CCHE 3-1-2008)

FWN= Focal weak nuclear

Supplementary Table 2

	OS		EFS	
	C-Index	AUC	C-Index	AUC
5 % Aggresomes	0.5	0.51	0.53	0.51
10 % Aggresomes	0.53	0.54	0.52	0.54
20 % Aggresomes	0.58	0.59	0.55	0.59
30 % Aggresomes	0.5	0.5	0.52	0.52
60 % Aggresomes	0.52	0.53	0.5	0.51
MS	0.62	0.62	0.62	0.62
WNT	0.58	0.51	0.58	0.51
SHH	0.65	0.65	0.56	0.57
non-WNT/non-SHH	0.62	0.64	0.60	0.62
≥ 20% Aggresomes + MS	0.76	0.79	0.77	0.79
< 20 Aggresomes + MS	0.60	0.62	0.59	0.60

Supplementary Table 3

Antibody	Manufacturer (catalog no.)	Application	Dilution	Localization	Target
β -catenin	Dako (IS702)	IHC	1:100	Nuclear	WNT subgroup
GAB1	GeneTex (GTX111253)	IHC	1:100	Cytoplasmic \pm membranous	SHH subgroup
YAP1	Santa Cruz Biotechnology (G-6; sc-376830)	IHC	1:100	Cytoplasmic	Confirm WNT, SHH subgroup
Vimentin	Ventana (790-2917)	IHC	1:100	Cytoplasmic	Aggresomes
Vimentin	Abcam (ab8978)	IF	1:50	Cytoplasmic	Aggresomes
Secondary goat anti-rabbit	Cell Signaling Technology (Alexa Fluor 488)	IF	1:500		
HDAC6	Cell Signaling Technology (7558)	IF	1:200	Cytoplasmic	HDAC6
Secondary goat anti-mouse	Cell Signaling Technology (AlexaFluor 555)	IF	1:500		

HDAC6 = histone deacetylase 6, IHC= Immunohistochemistry, IF= Immunofluorescence