

Table S1. Antibodies or DAPI and the conditions used for multidimensional fluorescence staining

Stain Order	Target	Antibody Clone (host species) or lot# /Company	Dilution	Opal dye (Dilution)	CITY/Nation
1	Collagen III	ab23445 (Mouse)/Abcam	1/200	520 (1:100)	Cambridge/UK
2	CA9	ab107257 (Mouse)/Abcam	1/400	570 (1:100)	Cambridge/UK
3	MMP1	ab52631 (Rabbit)/Abcam	1/200	620 (1:100)	Cambridge/UK
4	CD3E	ab52959 (Rabbit)/Abcam	1/400	690 (1:100)	Cambridge/UK
7	DAPI	C0060/Solarbio	1/500		Beijing/China

Table S2. Procedures for multidimensional fluorescence staining

Antigen	Collagen III	CA9	MMP1	CD3E	DAPI
Antigen retrieval	AR6	AR6	AR9	AR6	
	20 min	20 min	20 min	20 min	
Wash	TBST	TBST	TBST	TBST	
	10 min	10 min	10 min	10 min	
Blocking	10 min	10 min	10 min	10 min	
	@RT	@RT	@RT	@RT	
Inc. with Primary antibody	Over night	Over night	Over night	Over night	10 min
	@4°C	@4°C	@4°C	@4°C	@RT
Wash	TBST	TBST	TBST	TBST	TBST
	10 min	10 min	10 min	10 min	10 min
Inc. with Opal HRP Polymer Ms+Rb	10 min	10 min	10 min	10 min	
	@RT	@RT	@RT	@RT	
Wash	TBST	TBST	TBST	TBST	Mounting
	10 min	10 min	10 min	10 min	

@RT: At room temperature; @4°C: At 4°C

Table S3. Results of univariate Cox regression

	HR	P value
Age	0.996	0.563
Gender		
Female	Reference	
Male	0.972	0.811
Tumor Location		0.001
Pons	Reference	
Midbrain	0.381	0.001
Medulla	0.401	0.012
DIPG		
N	Reference	
Y	2.882	<0.001
WHO Grade		
4	Reference	
3	0.344	0.006
2	0.076	<0.001
1	<0.001	0.971
Driver Mutation		<0.001
Wildtype	Reference	
H3F3A	10.733	<0.001
HIST1H3B	120.301	<0.001
IDH1	1.939	0.205
Radiotherapy		
Not received	Reference	
Received	0.967	0.892
Chemotherapy		
Not received	Reference	
Received	0.624	0.058