Supplementary Materials for

First-in-human study of PET and optical dual-modality image-guided surgery in glioblastoma using ^{68}Ga-IRDye800CW-BBN

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Fig. S3. Preoperative contrast-enhancing MRI, PET imaging and residual tumor at the anterior part of tumor cavity with positive fluorescence in patient 9.

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Fig. S5. Preoperative MRI, PET imaging and intraoperative fluorescence guided surgery for a 31-year-old male recurrent GBM patient (No.12).

Fig. S6. Preoperative contrast-enhancing MRI, PET imaging and residual tumor at the splenium of corpus callosum and posterior temporal lobe with positive fluorescence in patient 14.

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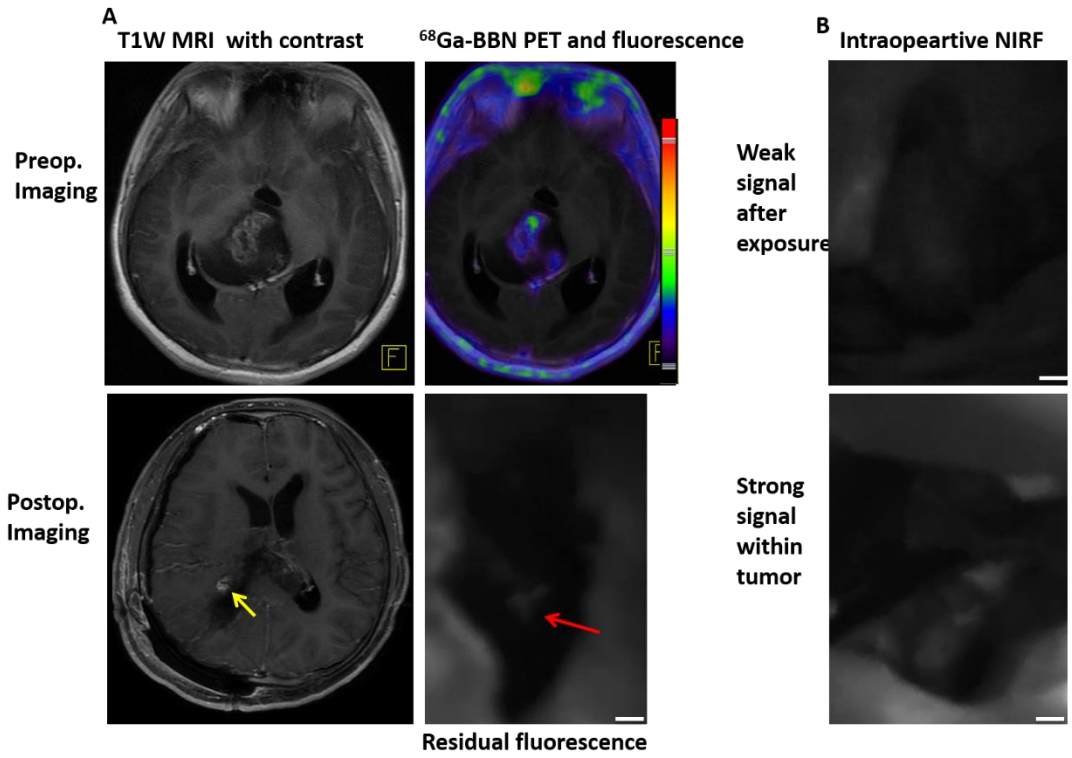

Fig. S1. Preoperative contrast-enhancing MRI, ^{68}Ga-BBN PET/MRI, intraoperative IRDye800CW-BBN NIRF signal and postoperative enhanced MRI in patient 4. This 15-year-old male was diagnosed with right thalamus gliomas. The pathology demonstrated GBM with partly oligodendroglioma, more likely secondary GBM. (A) The lower grade part of tumor did not uptake MRI contrast and PET tracer. (B) However, immediately after exposing the tumor, the weak density of fluorescence was noticed intraoperatively (upper). The fluorescence signal was much stronger within the tumor after partial resection showed the most malignant part of tumor (lower). The hematoxylin and eosin (H&E) staining respectively showed oligodendroglioma and GBM with some oligodendroglioma cells. The residual fluorescence was left around the tumor cavity because of eloquent areas (A lower right). The patient's neurological status improved postoperatively, with no newly developed deficits. Scale bar, 2mm.

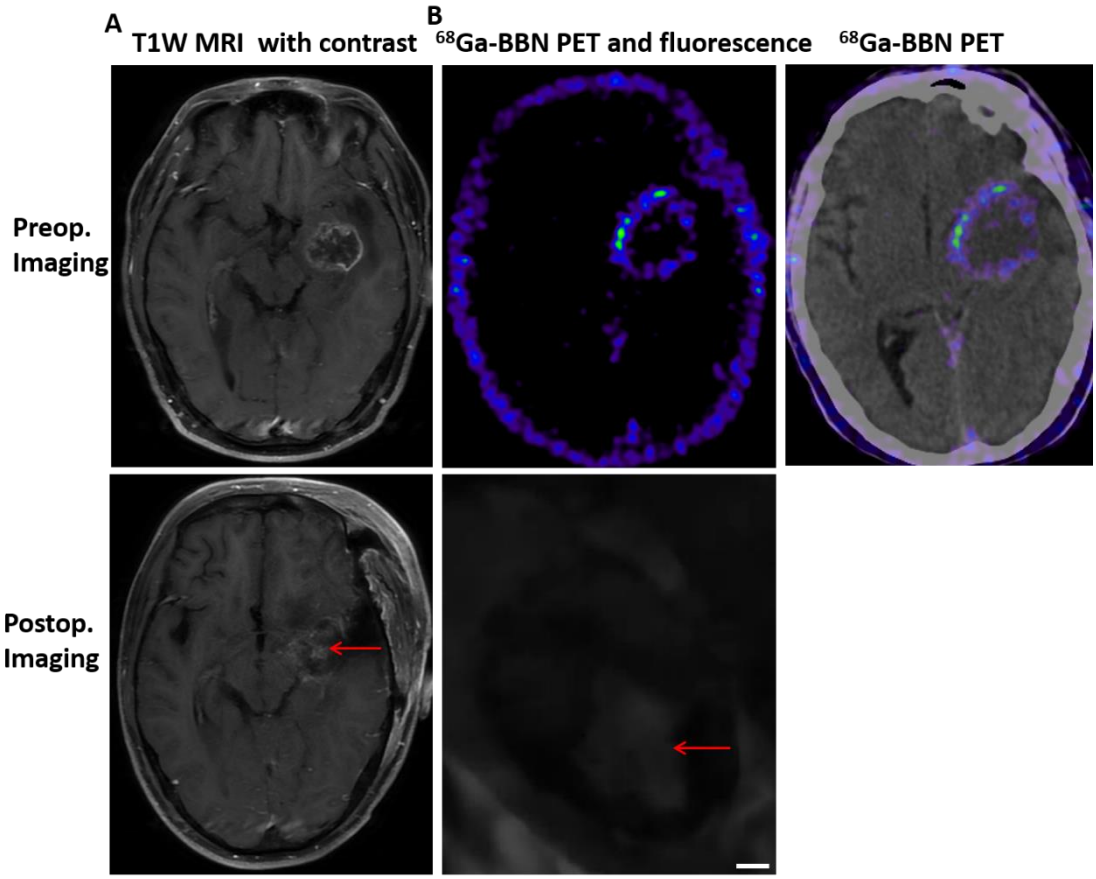

Fig.S2. Preoperative contrast-enhancing MRI, ^{68}Ga-BBN PET, postoperative enhanced MRI and intraoperative IRDye800CW-BBN NIRF imaging in patient 5. This 46-year-old male was diagnosed with left temporal and insula gliomas. The pathology demonstrated GBM with partly astrocytoma. (A) preoperative T1W MRI with contrast enhancement for a 49-year-old female patient with newly diagnosed GBM. The postoperative imaging indicated residual tumor (red arrow). (B) The preoperative ^{68}Ga-BBN PET/CT indicated larger area of BBN positive uptake compared to T1W MRI with contrast. The residual fluorescence was left in the medial part of temporal lobe, which was evident in postoperative enhancing MRI. Scale bar, 5mm.

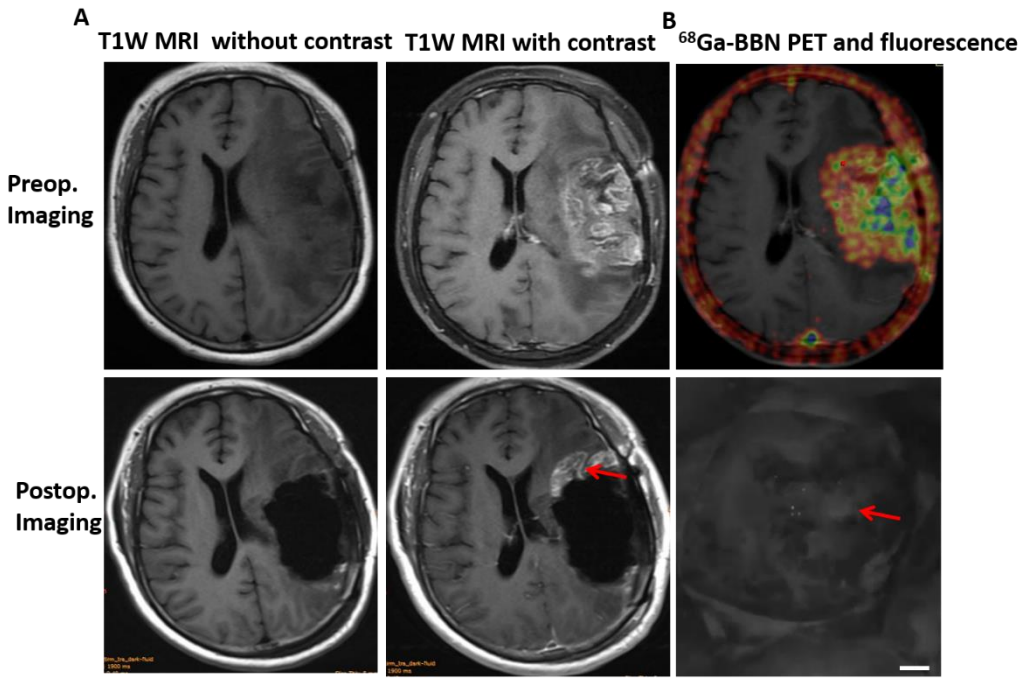

Fig. S3. Preoperative contrast-enhancing MRI, PET imaging and residual tumor at the anterior part of tumor cavity with positive fluorescence in patient 9. (A) preoperative T1W MRI without and with contrast enhancement for a 49-year-old female patient with recurrent GBM in the left frontal, parietal and insula lobes. The postoperative T1W MRI with contrast indicated residual tumor (red arrow). (B)The preoperative 68Ga-BBN indicated larger area of BBN positive uptake compared to T1W MRI with contrast. Because of eloquent position, the residual part of tumor at the anterior part of tumor cavity was also fluorescent positive. Scale bar, 2mm.

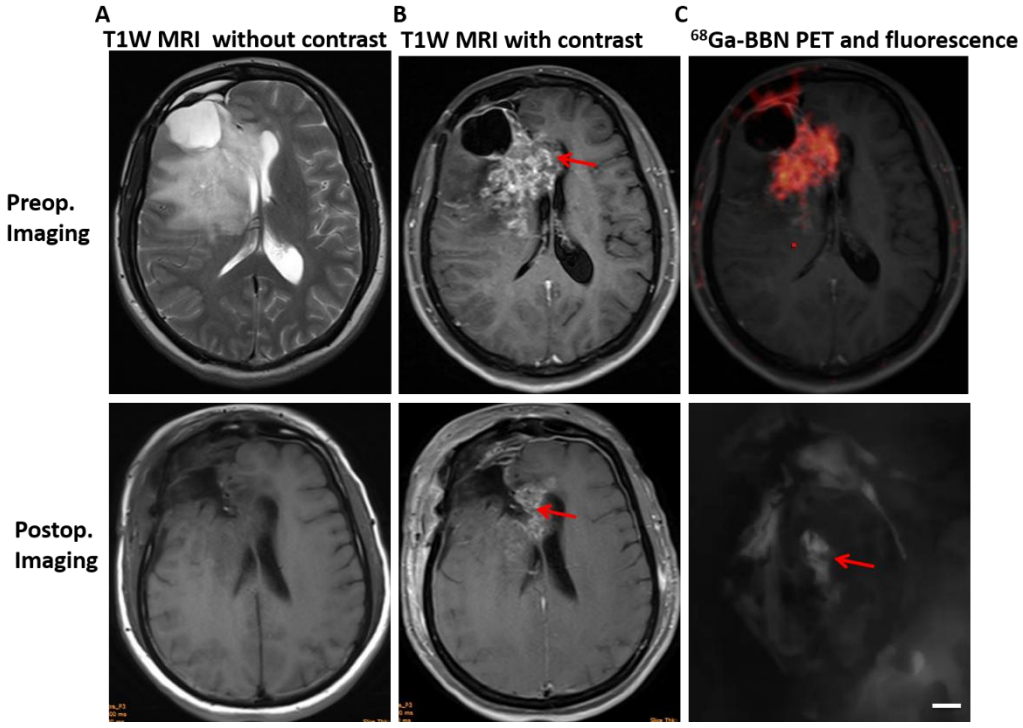

Fig. S4. Preoperative contrast-enhancing MRI, PET imaging and residual tumor at the genu of corpus callosum with positive fluorescence in patient 11. (A, B) preoperative T1W MRI without and with contrast enhancement for a 39-year-old female patient with recurrent GBM in the right frontal and corpus callosum. The postoperative T1W MRI with contrast indicated residual tumor at the genu of corpus callosum and anterior horn of lateral ventricle (red arrow). (C) The preoperative ^{68}Ga-BBN also indicated similar area of BBN positive uptake compared to T1W MRI with contrast. Because of eloquent position, the residual tumor part was significantly fluorescent positive. Scale bar, 5mm.

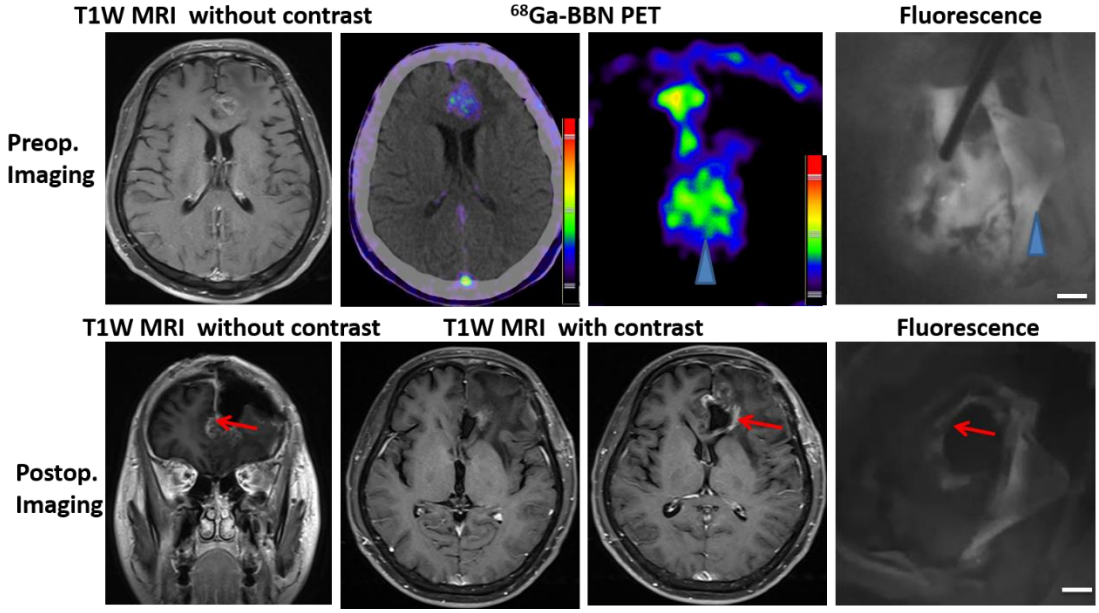

Fig. S5. Preoperative MRI, PET imaging and intraoperative fluorescence guided surgery for a 31-year-old male recurrent GBM patient (No.12). Preoperative T1W MRI with contrast enhancement and ^{68}Ga-BBN PET showed tumor at the left frontal lobe partly involving genu of corpus callosum and medial part of right frontal lobe. The area of positive BBN uptake was larger than that of MRI contrast enhancement. Immediately after dura opening, the fluorescent positive tumor was shown with obvious boundary and dura (blue trigone). The postoperative T1W MRI with contrast indicated residual tumor at the eloquent areas including the genu of corpus callosum and medial right frontal lobe (red arrow), and with the similar shape of residual fluorescent signal around the tumor cavity. Scale bar, 2mm.

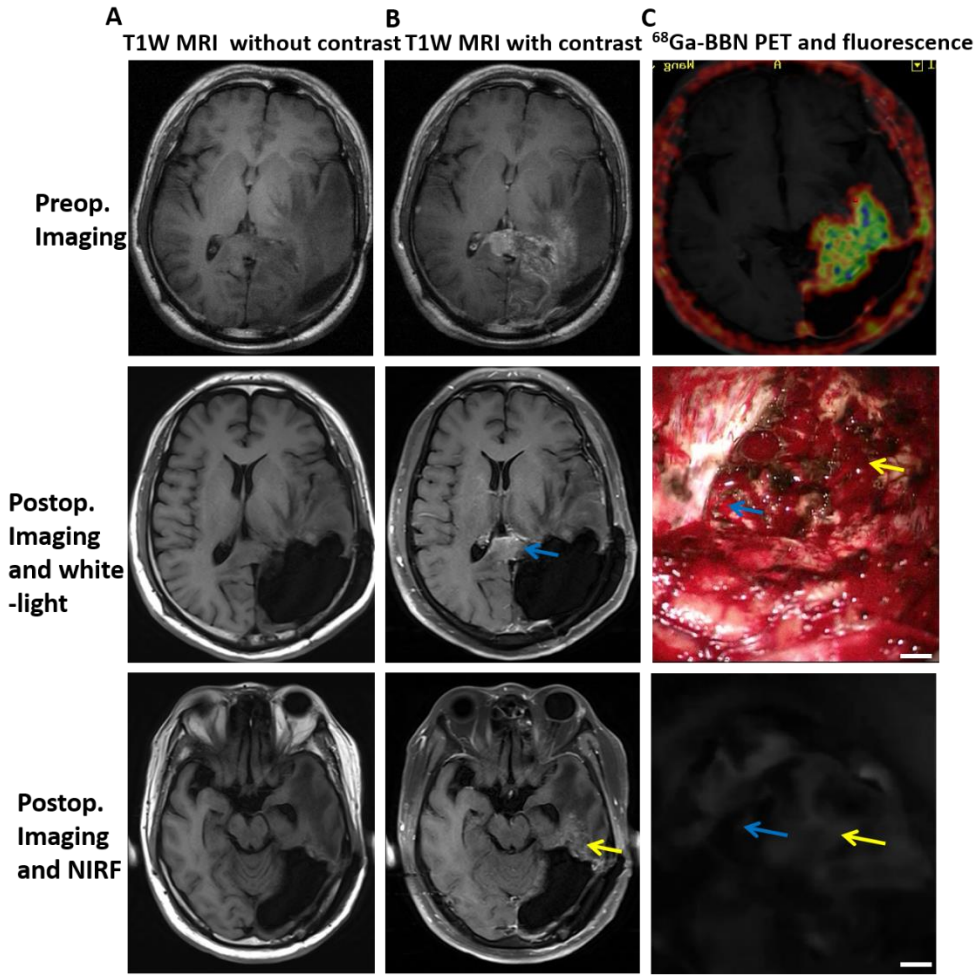

Fig.S6. Preoperative contrast-enhancing MRI, PET imaging and residual tumor at the splenium of corpus callosum and posterior temporal lobe with positive fluorescence in patient 14. (A, B) preoperative T1W MRI without and with contrast enhancement for a 42-year-old male patient with recurrent GBM in the left temporal and occipital lobe, partly involving corpus callosum. The postoperative imaging indicated residual tumor at the splenium of corpus callosum (blue arrow) and posterior temporal lobe (yellow arrow), which were language functional areas. (C) The preoperative ^{68}Ga-BBN also indicated larger area of BBN positive uptake compared to MRI. The residual tumor parts were significantly fluorescent positive, but not obvious in the cavity because of previous postoperative chemotherapy and radiotherapy. Scale bar, 2mm.

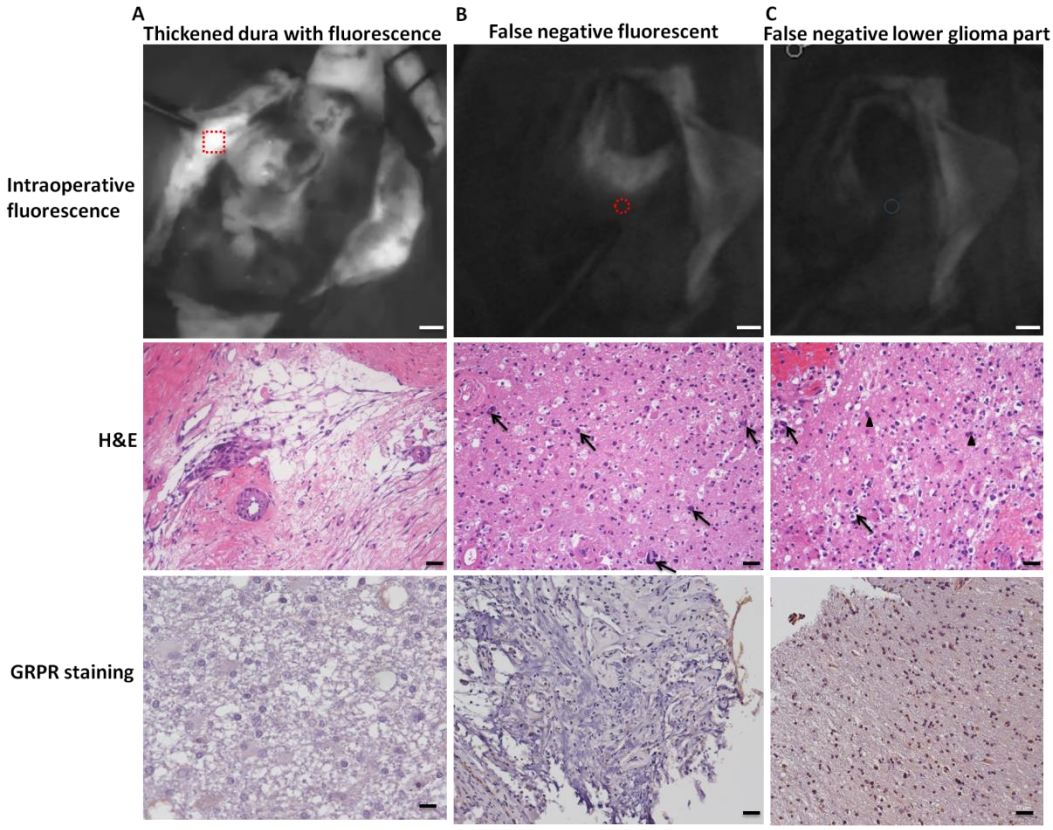

Fig. S7. False positive and negative florescence specimens confirmed by pathology diagnosis. **(A)** In the Patient 7 with right frontal lobe recurrent GBM, the dura was obviously thickened and fluorescent positive but the specimen (red dashed square) stained with hematoxylin and eosin (H&E) showed no tumor cells and no GRPR positive cells. Scale bar, 2mm for row 1; 50um for row 2 and 20um for row 3. **(B)** In the Patient 9 with recurrent secondary GBM, the tissue from fluorescent negative cortical area (red dashed cycle) showed scattered glioblastoma cells and GRPR positive cells. Scale bar, 2mm for row 1; 50um for row 2 and 3. **(C)** In the tumor cavity of Patient 9 with recurrent secondary GBM, the fluorescent negative area (blue cycle) showed lower grade part of tumor but however scattered GRPR positive cells, including oligodendroglioma (black arrow) and astrocytoma (black triangle). Scale bar, same with (B).

Table S1. Patients' discomfort and safety issue (liver and renal toxicity and pyrogenic effect) of IRDye800CW-BBN tracer after intraoperative utilization.

Patient No.	Transient nausea (Y/N)	glutamic-pyruvic transaminase (ALT, U/L)	glutamic-oxaloacetic transaminase (AST, U/L)	Creatinine (umol/L)	Fever (Temperature higher than 38.5℃, Y/N)	Which postoperative day fever occurred	Fever differential diagnosis
1	Y	91.4	13.4	21.1	N	/	/
2	Y	88.5	12	21	N	/	/
3	Y	96.2	22	19	N	/	/
4	Y	89	15	12	N	/	/
5	Y	87.9	5.56	19	N	/	/
6	Y	28	21	35.2	N	/	
7	Y	30	17	67.1	Y	5	Aseptic meningitis
8	N	21	20	76.4	N	/	
9	Y	93.7	17.4	17.5	Y	3	Aseptic meningitis
10	Y	92.7	15.1	28	Y	5	Postoperative central nervous system infection（PCNSI）
11	Y	93.5	47.7	18.2	Y	4	Aseptic meningitis
12	N	88.5	25	16.3	N	/	/
13	N	90.4	25.8	19.5	Y	7	PCNSI
14	Y	91.7	19	25	N	/	/

Table S2. The specimen location, fluorescence intensity and histological pathology in this cohort of glioma patients.

Patient No.	**Sample** position	**NIRF signal**	**Pathology**	**WHO grading**
1	The tumor core	Strong	GBM	IV
	Tissue along the margin of ex vivo tissue	Negative	Brain tissue	/
	Tissue along the cavity	Strong	GBM	IV
2	Tumor center	Strong	GBM	IV
	Tissue along the cavity	Strong	GBM	IV
3	The tumor core	Strong	GBM	IV
	Tissue 1 along the cavity	Negative	Swollen brain tissue, gliosis with lymphocytes invasion	/
	Tissue 2 along the cavity	Negative	Swollen brain tissue, gliosis with lymphocytes invasion	/
4	The tumor 1	Strong	GBM	IV
	Tissue 1 along the cavity	Strong	GBM	IV
	Tissue 2 along the cavity	Strong	GBM	IV
5	The tumor core 1	strong	GBM	IV
	The tumor core 2	strong	GBM	IV
	The lower part of tumor	Faint	Astrocytoma	III
	Tissue along the cavity	Negative	Brain tissue	/
6	The tumor 1 in the cortical	positive	GBM	IV
	The tissue adjacent to tumor1	negative	Brain tissue	/
	The tumor core 1	positive	GBM	IV
	The tumor core 2	positive	GBM	IV
	Tissue 1 along the cavity	negative	Brain tissue	/
	Tissue 2 along the cavity	negative	Brain tissue	/
	Residual tumor along the cavity	positive	GBM	IV
7	The tumor core	positive	GBM	IV
	Tissue along the cavity	negative	Brain tissue	/
8	The tumor core 1	positive	GBM	IV
	The tumor core 2	positive	GBM	IV
	Residual tumor 1 along the cavity	positive	GBM	IV
	Residual tumor 2 along the cavity	positive	GBM	IV
9	The tumor core	Strong	GBM, including oligodendroglioma	IV
	Tissue along the cavity	Strong	Anaplastic oligoastrocytoma, partly GBM	III-IV
10	The dura mater	Strong	Fibrous tissue	/
	Tissue along the cavity	Strong	GBM	IV

11	The tumor core	Strong	GBM	IV
	Tissue along the cavity	Strong	Brain tissue, partly small foci of tumor	/
12	Tissue along the cavity	Negative	Atypical reactive gliosis, partly small foci of tumor	/
	Tissue along the cavity	Strong	Cyst wall fibrous tissue, with macrophages, partly GBM cells	/
	Tissue along the cavity	Strong	GBM，partly oligodendroglioma	IV
	Tissue along the cavity	Negative	oligoastrocytoma	II
13	The tumor margin	Strong	GBM, with oligodendroglioma	IV
	The tumor core	Strong	GBM, with oligodendroglioma	IV
14	The tumor core 1	Strong	GBM	IV
	The tumor core 2	Strong	GBM	IV
	Tissue along the cavity	Negative	Brain tissue	/

GBM, Glioblastoma Multiform.

Table S3. Performance measures of fluorescence in determining the specimen pathology.

		Pathology			
		Tumors	Normal tissue	The mixtures of normal tissue and tumor	Total
NIRF signal	Positive	29	0	2	31
	Negative	1	9	1	11
	Total	30	9	3	42