OMTN, Volume *32*

Supplemental information

Development of DNA aptamers for visualization of glial brain tumors and detection of circulating tumor cells

Anna S. Kichkailo, Andrey A. Narodov, Maria A. Komarova, Tatiana N. Zamay, Galina S. Zamay, Olga S. Kolovskaya, Evgeniy E. Erakhtin, Yury E. Glazyrin, Dmitry V. Veprintsev, Roman V. Moryachkov, Vladimir V. Zabluda, Irina Shchugoreva, Polina Artyushenko, Vladimir A. Mironov, Dmitry I. Morozov, Vladimir A. Khorzhevskii, Anton V. Gorbushin, Anastasia A. Koshmanova, Elena D. Nikolaeva, Igor P. Grinev, Ivan I. Voronkovskii, Daniil S. Grek, Kirill V. Belugin, Alexander A. Volzhentsev, Oleg N. Badmaev, Natalia A. Luzan, Kirill A. Lukyanenko, Georgy Peters, Ivan N. Lapin, Andrey K. Kirichenko, Petr V. Konarev, Evgeny V. Morozov, Gleb G. Mironov, Ana Gargaun, Darija Muharemagic, Sergey S. Zamay, Elena V. Kochkina, Maya A. Dymova, Tatiana E. Smolyarova, Alexey E. Sokolov, Andrey A. Modestov, Nikolay A. Tokarev, Nikolay V. Shepelevich, Anastasia V. Ozerskaya, Natalia G. Chanchikova, Alexey V. Krat, Ruslan A. Zukov, Varvara I. Bakhtina, Pavel G. Shnyakin, Pavel A. Shesternya, Valery A. Svetlichnyi, Marina M. Petrova, Ivan P. Artyukhov, Felix N. Tomilin, and Maxim V. Berezovski

Table S1. Specifications of the tissues used for the aptamer selection.

SELEX Round	Selection	Patient and tissue information			
		Gender	Age	Histologic type, grade	Tumor location
1	Positive	Female	56	Astrocytoma, Grade IV	The left temporal lobe of the brain
2	Negative	Male	54	Colon cancer metastases	The parietal lobe of the brain
		Male	59	Lung cancer metastases	The left parietal lobe of the brain
		Male	60	Meningioma	The left occipital lobe
	Positive	Male	35	Astrocytoma, Grade II	The right occipital lobe of the brain
3	Negative	Male	56	Parasagittal meningioma	The parietal lobe of the brain
	Positive	Male	23	Astrocytoma, Grade IV	The left lateral and third ventricle of the brain
4	Negative	Female	39	Melanoma metastases	The left temporal lobe of the brain
	Positive	Female	56	Astrocytoma, Grade III	The right frontal lobe, lateral ventricle of the brain
5	Negative	Female	28	Anaplastic meningioma	The left parietal lobe of the brain
	Positive	Female	45	Astrocytoma, Grade II	The right frontal lobe, lateral ventricle of the brain
6	Negative	Female	63	Kidney's cancer metastases	The temporal lobe of the brain
	Positive	Male	43	Astrocytoma, Grade III	The right frontal, parietal and temporal lobes of the brain

Table S2. Sequences of DNA aptamers binding to glioblastoma.

Family	The most abundant member	Sequence	Frequency/Number of members in a family	EC_{50}, nM	Fluorescent intensity/ % of binding
A	Gli-2	CTC CTC TGA CTG TAA CCA CGC CTG ACT ATT CCA CTG CAA CAA CTG AAC GGA CTG GAA ACA CTG GTC ATA ATC ATG GTG GCG CAT AGG TAG TCC AGA AGC C	1517/63	26	33/26
	Gli-225	AAA CAC TGG TCA TAA TCA TGG TGG C	-	35	45/20
	Cli-233	ACT ATT CCA CTG CAA CAA CTG AAC GGA CTG GAA	-	27	52/47
B	Gli-5566	CTC CTC TGA CTG TAA CCA CGG TCC GGT TCA CCT CTA GCA TTC CTG GCG TTA TTA ACG GAG CAG TCC TGT GGA GTG GGT GACG CAT AGG TAG TCC AGA AGC C	631/18	17	30/43
	Gli-55	GTC CGG TTC ACC TCT AGC ATT CCT GGC GTT ATT AAC GGA GCA GTC CTG TGG AGT GGG TGA	-	11	60/55
C	Gli-21	CTC CTC TGA CTG TAA CCA CGA TCC TGG TAA CCG ATA GCG TTC CTG AAG TGA GTA ACG TCT CAG TCC TTT GGA ATG GGT AAG CAT AGG TAG TCC AGA AGC C	111/16	103	12/26
D	Gli-78	CTC CTC TGA CTG TAA CCA CGG TGC ATG TAA AGG CGC ACA TAC CTC TTA CAT TTG CTT GCG GAG ATG CTT AAT GTA TTT ATG CAT AGG TAG TCC AGA AGC C	83/2	78	31/25
	Gli-7820	TAG AGG CGC ACA TAC CTC TT	-	108	32/16
	Gli-7819	TGC TTA ATG TAT TTA TGC A	-	N/A	12/5
E	Gli-23	CTC CTC TGA CTG TAA CCA CGT AAC CAC GCA CAT CTC CTC TGA CTG TAA CCA CGA TTC TTG AAT TCG TCC GGG GGC ATA GGT AGT CCA GAA GC C	26/2	N/A	16/43
F	Gli-41	CTC CTC TGA CTG TAA CCA CGA ACC ACG GAG TAT GAA TTT AAA TAT ATT TCT TCT GAA TGT GCA TGC GGC ATA GGT AGT CCA GAA GCC	25/2	N/A	17/42

G	Gli-42	CTC CTC TGA CTG TAA CCA CG G ACT GTA ACC ACG GTG AGG ATC TCA ATG TCC GCG CGG GGC GCA TGC GTC ATG GTG CGC GTG G CAT AGG TAG TCC AGA AGC C	24/2	N/A	15/46
Non Family	Gli-19	CTC CTC TGA CTG TAA CCA CGT GAC TAC TCC TCT GAC TGT AAC CAC GAT TCT TGA ATT CGT CCG GGG TTT GAA ATC GGG TAG CAT AGG TAG TCC AGA AGC C	14/1	N/A	17/50
	Gli-13	CTC CTC TGA CTG TAA CCA CGT ATG AAT CTG GGT ACG GGC TTG CAG TAT GTG TAT TAT GGT GTA CGC GCC ATA CGTA CGT GCA TAG GTA GTC CAG AAG CC	13/1	N/A	15/42

Table S3. Protein targets of aptamers identified by mass spectrometry.

Aptamer	Protein ID	Protein name	Number of series	Post-translational modifications	Co- localization with antibodies
Gli-233	P14136	Glial fibrillary acidic protein	3	Oxidized methionines at positions 73, 74, 342; phosphorylated serines at positions 247 and 248	+--
	Q9BQE3	Tubulin alpha-1C chain	3	Polyglycylation and polyglutamylation of the C-terminal tail of glutamic acid at various positions: glycylation (position 433); biglutamylation (445), glutamylation (447), 5-link polyglutamylation (448); triglycylation (433), 6-link polyglycylation (443), 4-link polyglycylations (447 and 448)	+++
	P02686	Myelin basic protein	2	Deamidated glutamine at positions 237 and 281	---
	P08670	Vimentin	2	No	+--
Gli-55t	P14136	Glial fibrillary acidic protein	2	Oxidized methionines at positions 73, 74	++-
	Q17RR3	Pancreatic lipase-related protein 3	2	No	---

Table S4. Properties of the aptamer binding sites on the alpha-tubulin 1C surface.

Binding site	1	2	3
Avg. # caged Na+	12.5	7.6	11.9
Avg. # h-bonds	3.9	2.7	4.8
Avg. RMSD vs lone aptamer in water	9.16	8.49	7.75

Table S5. Acute toxicity study of the aptamers in rats. The average values of body mass and weight gain.

	0.9% NaCl		The preparation of DNA-aptamers 40 µl 100 nM		The preparation of DNA-aptamers 200 µl 1000 nM	
Males	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n
Day 1, body weight, g	301.4 ± 15.4	5	301.6 ± 14.0	5	300.2 ± 20.1	5
Day 7, body weight, g	312.0 ± 17.7	5	313.0 ± 16.8	5	310.6 ± 20.0	5
Day 14, body weight, g	324.0 ± 18.1	5	329.0 ± 16.2	5	323.0 ± 20.3	5
Day 7, increase,%	3.5 ± 1.1	5	3.8 ± 1.3	5	3.5 ± 1.4	5
Day 14, increase, %	7.5 ± 1.2	5	9.1 ± 1.1	5	7.6 ± 2.0	5
Females	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n
Day 1, body weight, g	207.6 ± 11.8	5	207.2 ± 13.8	5	212.6 ± 17.5	5
Day 7, body weight, g	212.4 ± 9.9	5	213.4 ± 15.3	5	213.0 ± 10.5	5
Day 14, body weight, g	213.8 ± 6.1	5	218.0 ± 16.4	5	215.0 ± 11.8	5
Day 14, increase,%	2.4 ± 3.9	5	3.0 ± 1.9	5	0.5 ± 4.9	5
Day 14, increase,%	3.2 ± 4.1	5	5.2 ± 2.0	5	1.4 ± 5.3	5

Table S6. Acute toxicity study of the aptamers in rats. The average values of feed consumption.

	0.9% NaCl	The preparation of DNA-aptamers 40 µl 100 nM	The preparation of DNA-aptamers 200 µl 1000 nM
Males			

Day 7, g / kg / day	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n
	65.4 ± 2.7	5	64.8 ± 4.2	5	67.1 ± 3.2	5
Day 14, g / kg / day	63.9 ± 13.0	5	66.6 ± 3.4	5	68.0 ± 15.5	5
Females	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n
Day 7, g / kg / day	63.0 ± 3.2	5	62.1 ± 5.2	5	63.7 ± 4.8	5
Day 14, g / kg / day	72.3 ± 4.5	5	75.2 ± 6.0	5	76.8 ± 11.3	5

Table S7. Acute toxicity study of the aptamers in rats. The average values of motor activity.

Males	**0.9% NaCl**	**The preparation of DNA-aptamers 40 µl 100 nM**	**The preparation of DNA-aptamers 200 µl 1000 nM**
	Mean ± SD	Mean ± SD	Mean ± SD
Distance traveled, cm	(n=5)	(n=5)	(n=5)
	616 ± 154	797 ± 182	800 ± 183
Time of immobility, s	99 ± 16	85 ± 18	85 ± 10
Movement time, c	81 ± 16	95 ± 18	95 ± 10
Number of counters	14.6 ± 1.7	14.8 ± 3.1	12.8 ± 0.8
Females	Mean ± SD	Mean ± SD	Mean ± SD
	(n=5)	(n=5)	(n=5)
Distance traveled, cm	564 ± 238	663 ± 230	684 ± 108
Time of immobility, s	110 ± 24	101 ± 13	98 ± 8
Movement time, c	70 ± 24	79 ± 13	82 ± 8
Number of counters	11.2 ± 2.2	12.4 ± 1.5	12.2 ± 1.3

Table S8. Acute toxicity study of the aptamers in rats. The average values of blood pressure and heart rate.

	0.9% NaCl	**The preparation of DNA-aptamers 40 µl 100 nM**	**The preparation of DNA-aptamers 200 µl 1000 nM**
Males	Mean ± SD	Mean ± SD	Mean ± SD
	(n=5)	(n=5)	(n=5)
Heart rate, beats / min	380 ± 44	376 ± 51	422 ± 30
Blood pressure, mm, Hg,	100 ± 9	114 ± 7	114 ± 8
Females	Mean ± SD	Mean ± SD	Mean ± SD
	(n=5)	(n=5)	(n=5)
Heart rate, beats / min	403 ± 48	438 ± 37	423 ± 50
Blood pressure, mm, Hg,	112 ± 17	98 ± 18	108 ± 23

Table S9. Acute toxicity study of the aptamers in rats. The average values of clinical chemistry indices, males.

	0.9% NaCl	The preparation of DNA-aptamers 40 μl 100 nM	The preparation of DNA-aptamers 200 μl 1000 nM
	Mean ± SD	Mean ± SD	Mean ± SD
Urea, mmol / l	(n=5)	(n=5)	(n=5)
Cholesterol, mmol / l	9.3 ± 1.6	9.1 ± 1.2	9.4 ± 1.1
Triglycerides, mmol / l ALT, U / l	3.18 ± 0.29	3.13 ± 0.28	3.48 ± 0.18
AST, U / L	1.24 ± 0.17	1.33 ± 0.07	1.29 ± 0.18
General, bilirubin, μmol / l	93.4 ± 24.1	84.1 ± 12.3	91.6 ± 5.0
Creatinine, μmol / L Alkaline phosphatase, U / l Albumin, g / l	86.8 ± 15.8	89.1 ± 7.3	89.7 ± 4.4
	5.4 ± 1.4	3.7 ± 0.9	3.4 ± 1.7
Total protein, g / l	77 ± 5	72 ± 2	72 ± 3
Globulins, g / l	227 ± 44	257 ± 35	240 ± 40
	44.4 ± 1.5	43.8 ± 2.0	45.2 ± 1.7
	73.8 ± 1.8	72.1 ± 2.0	74.3 ± 2.0
	29.4 ± 1.4	28.4 ± 0.4	29.1 ± 0.5

Table S10. Acute toxicity study of the aptamers in rats. The average values of clinical chemistry indices, females.

	0.9% NaCl	The preparation of DNA-aptamers 40 μl 100 nM	The preparation of DNA-aptamers 200 μl 1000 nM
	Mean ± SD	Mean ± SD	Mean ± SD
Urea, mmol / l Cholesterol, mmol / l Triglycerides, mmol / l ALT, U / l	(n=5)	(n=5)	(n=5)
AST, U / L	7.9 ± 1.1	7.9 ± 0.4	7.8 ± 1.0
	3.53 ± 0.46	3.54 ± 0.21	3.18 ± 0.37

Bilirubin, μmol / l	1.13 ± 0.31	1.05 ± 0.11	1.02 ± 0.20
Creatinine, μmol / L	71.8 ± 17.1	80.0 ± 7.3	69.2 ± 7.9
Alkaline phosphatase, U / l	85.0 ± 7.9	91.4 ± 14.2	83.1 ± 3.2
Albumin, g / l	6.4 ± 0.9	6.0 ± 1.2	5.6 ± 1.4
Total protein, g / l	71 ± 3	67 ± 7	70 ± 4
Globulins, g / l	180 ± 45	163 ± 19	158 ± 47
	43.5 ± 1.5	42.7 ± 1.1	43.3 ± 1.1
	72.0 ± 1.8	70.8 ± 2.8	70.8 ± 1.0
	28.5 ± 1.4	28.0 ± 2.3	27.5 ± 1.2

Table S11. Acute toxicity study of the aptamers in rats. The average values of hematologic parameters and hemogram, males.

Males	**0.9% NaCl**	**The preparation of DNA-aptamers 40 µl 100 nM**	**The preparation of DNA-aptamers 200 µl 1000 nM**
	Mean ± SD (n=5)	Mean ± SD (n=5)	Mean ± SD (n=5)
Number of white blood cells (WBC), g / l	15.4 ± 1.8	17.6 ± 4.0	14.7 ± 1.2
The number of red blood cells (RBC), T / L	9.34 ± 0.17	9.13 ± 0.32	9.69 ± 0.25
The level of hemoglobin (Hb), g / l	175 ± 4	172 ± 8	179 ± 5
Hematocrit (HCT), l / l	0.534 ± 0.009	0.517 ± 0.023	0.537 ± 0.006
The mean volume of the erythrocyte (MCV), fl	57.2 ± 0.4	56.6 ± 1.3	56.0 ± 0.9
The mean hemoglobin content in the erythrocyte (MCH), pg	18.8 ± 0.5	18.9 ± 0.6	18.5 ± 0.8
The average concentration of hemoglobin in the erythrocyte (MCHC), g / l	328 ± 9	333 ± 5	329 ± 11
Latitude of distribution of red blood cells by volume - coefficient of variation (RDW),%	11.5 ± 0.3	11.6 ± 0.4	11.5 ± 0.5
The width of the distribution of red blood cells by volume - the standard deviation (RDW-SD), fl	32.4 ± 1.1	30.9 ± 1.1	31.3 ± 1.4
Number of platelets (PLT), g / l	787 ± 78	809 ± 81	729 ± 19
The average platelet count (MPV), fl	5.5 ± 0.2	5.6 ± 0.2	5.7 ± 0.2
Thrombote (PCT), sl / l	0.432 ± 0.038	0.449 ± 0.045	0.415 ± 0.015
Latitude of platelet distribution by volume - coefficient of variation (PDW),%	15.4 ± 1.1	15.8 ± 1.5	17.1 ± 1.6
Leukogram			
Staphylococcal neutrophils,%	0.2 ± 0.4	0.4 ± 0.9	0.4 ± 0.9
Staphylococcal neutrophils, g / l	0.03 ± 0.07	0.06 ± 0.14	0.05 ± 0.12
Neutrophils segmentonuclear,%	3.8 ± 1.5	4.4 ± 2.3	4.2 ± 2.5
Neutrophils segmented, G / L	0.58 ± 0.25	0.77 ± 0.41	0.61 ± 0.35
Eosinophils,%	0.6 ± 0.9	0.4 ± 0.5	0.8 ± 0.4
Eosinophils, g / l	0.08 ± 0.11	0.06 ± 0.09	0.12 ± 0.07
Basophils,%	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Basophils, g / l	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Lymphocytes,%	93.8 ± 1.6	93.8 ± 2.8	93.6 ± 2.7
Lymphocytes, g / l	14.45 ± 1.76	16.57 ± 3.95	13.77 ± 1.41
Monocytes,%	1.6 ± 0.9	1.0 ± 0.0	1.0 ± 0.0
Monocytes, g / l	0.26 ± 0.16	0.18 ± 0.04	0.15 ± 0.01

Table S12. Acute toxicity study of the aptamers in rats. The average values of hematologic parameters and hemogram, females.

Females	**0.9% NaCl**	**The preparation of DNA-aptamers 40 µl 100 nM**	**The preparation of DNA-aptamers 200 µl 1000 nM**
	Mean ± SD (n=5)	Mean ± SD (n=5)	Mean ± SD (n=5)
Number of white blood cells (WBC), g / l	11.4 ± 2.3	10.3 ± 1.6	11.2 ± 1.5
The number of red blood cells (RBC), T / L	8.55 ± 0.47	8.27 ± 0.26	8.52 ± 0.35
The level of hemoglobin (Hb), g / l	170 ± 8	164 ± 5	166 ± 5
Hematocrit (HCT), l / l	0.492 ± 0.022	0.476 ± 0.015	0.487 ± 0.022
The mean volume of the erythrocyte (MCV), fl	57.7 ± 1.3	57.5 ± 1.0	57.2 ± 0.5
The mean hemoglobin content in the erythrocyte (MCH), pg	19.9 ± 0.5	19.8 ± 0.3	19.5 ± 0.2
The average concentration of hemoglobin in the erythrocyte (MCHC), g / l	345 ± 4	344 ± 5	341 ± 6
Latitude of distribution of red blood cells by volume - coefficient of variation (RDW),%	11.6 ± 0.2	11.3 ± 0.4	11.0 ± 0.2 *
The width of the distribution of red blood cells by volume - the standard deviation (RDW-SD), fl	30.5 ± 0.2	29.9 ± 1.1	30.5 ± 0.1
Number of platelets (PLT), g / l	843 ± 96	854 ± 74	775 ± 61
The average platelet count (MPV), fl	5.2 ± 0.1	5.2 ± 0.2	5.3 ± 0.2
Thrombote (PCT), sl / l	0.441 ± 0.060	0.448 ± 0.056	0.410 ± 0.045
Latitude of platelet distribution by volume - coefficient of variation (PDW),%	13.4 ± 0.6	13.8 ± 0.8	14.0 ± 0.5
Leukogram			
Staphylococcal neutrophils,%	0.2 ± 0.4	0.2 ± 0.4	0.0 ± 0.0
Staphylococcal neutrophils, g / l	0.03 ± 0.06	0.02 ± 0.04	0.00 ± 0.00
Neutrophils segmentonuclear,%	3.4 ± 0.5	3.6 ± 2.5	2.8 ± 1.3
Neutrophils segmented, G / L	0.39 ± 0.09	0.40 ± 0.34	0.32 ± 0.16
Eosinophils,%	0.4 ± 0.5	0.6 ± 0.5	0.2 ± 0.4
Eosinophils, g / l	0.05 ± 0.07	0.06 ± 0.06	0.02 ± 0.05

Basophils,%	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Basophils, g / l	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes,%	94.6 ± 1.1	94.6 ± 2.8	96.0 ± 1.2
Lymphocytes, g / l	10.77 ± 2.16	9.72 ± 1.38	10.79 ± 1.48
Monocytes,%	1.4 ± 0.5	1.0 ± 0.0	1.0 ± 0.0
Monocytes, g / l	0.15 ± 0.03	0.10 ± 0.02 *	0.11 ± 0.02

* - P <0.05 based on the Kruskal-Wallis test for the control group

Table S13. Acute toxicity study of the aptamers in rats. The average values of hematologic parameters and myelogram, males.

Males	**0.9% NaCl**	**The preparation of DNA-aptamers 40 µl 100 nM**	**The preparation of DNA-aptamers 200 µl 1000 nM**
	Mean ± SD (n=5)	Mean ± SD (n=5)	Mean ± SD (n=5)
Blasts (including myeloblasts)	0.4 ± 0.2	0.7 ± 0.4	0.4 ± 0.1
All granulocytes	52.5 ± 2.8	53.4 ± 3.8	50.1 ± 2.3
Neutrophilous promyelocytes	1.7 ± 0.3	1.6 ± 0.3	1.5 ± 0.4
Neutrophilic myelocytes	10.5 ± 1.6	8.6 ± 1.0	9.0 ± 0.9
Neutrophilic metamyelocytes	10.8 ± 1.4	9.2 ± 0.9	10.0 ± 0.7
Stab neutrophils	15.4 ± 1.5	17.3 ± 2.2	16.2 ± 1.1
Segmented neutrophils	9.3 ± 1.2	10.3 ± 2.2	9.1 ± 1.0
Eosinophils (of all generations)	4.3 ± 1.8	5.2 ± 2.2	3.0 ± 1.1
Basophiles (of all generations)	0.6 ± 0.5	1.3 ± 0.7	1.3 ± 0.5
All erythrocaryocytes	25.3 ± 1.9	24.0 ± 1.6	27.0 ± 1.6
Lymphocytes	19.8 ± 1.2	19.8 ± 2.3	20.7 ± 1.3
Monocytes	1.4 ± 0.3	1.1 ± 0.2	1.1 ± 0.2
Plasma cells	0.3 ± 0.1	0.7 ± 0.5	0.5 ± 0.2
Reticular cells	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
The leycoerythroblastic ratio	2.9 ± 0.3	3.1 ± 0.3	2.7 ± 0.2

Table S14. Acute toxicity study of the aptamers in rats. The average values of hematologic parameters and myelogram, females.

Females	**0.9% NaCl**	**The preparation of DNA-aptamers 40 µl 100 nM**	**The preparation of DNA-aptamers 200 µl 1000 nM**
	Mean ± SD (n=5)	Mean ± SD (n=5)	Mean ± SD (n=5)
Blasts (including myeloblasts)	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.3
All granulocytes	50.9 ± 2.4	49.9 ± 6.4	52.8 ± 3.0
Neutrophilous promyelocytes	1.8 ± 0.4	1.4 ± 0.3	1.8 ± 0.2
Neutrophilic myelocytes	7.8 ± 0.4	7.0 ± 1.7	7.5 ± 0.7
Neutrophilic metamyelocytes	10.4 ± 0.7	9.7 ± 2.4	10.5 ± 0.4
Stab neutrophils	15.0 ± 1.7	14.4 ± 1.6	16.2 ± 1.2
Segmented neutrophils	9.5 ± 1.1	9.9 ± 2.8	11.0 ± 1.1
Eosinophils (of all generations)	5.4 ± 0.8	6.2 ± 1.2	5.0 ± 1.3
Basophiles (of all generations)	1.1 ± 0.2	1.3 ± 0.7	0.9 ± 0.4
All erythrocaryocytes	25.3 ± 1.5	26.4 ± 3.9	25.0 ± 1.9
Lymphocytes	21.5 ± 1.1	21.3 ± 2.9	20.0 ± 1.3
Monocytes	1.0 ± 0.2	1.0 ± 0.3	1.0 ± 0.1
Plasma cells	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
Reticular cells	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
The leycoerythroblastic ratio	2.9 ± 0.2	2.8 ± 0.5	3.0 ± 0.3

Table S15. Acute toxicity study of the aptamers in rats. The average values of weight of organs (absolute values).

	0.9% NaCl		The preparation of DNA-aptamers 40 µl 100 nM		The preparation of DNA-aptamers 200 µl 1000 nM	
Males						
	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n
Testes, g	3.146 ± 0.123	5	3.555 ± 0.164 *	5	3.385 ± 0.173	5
Spleen, g	0.747 ± 0.055	5	0.725 ± 0.084	5	0.728 ± 0.098	5
Kidneys, g	2.223 ± 0.161	5	2.228 ± 0.126	5	2.174 ± 0.162	5
Adrenal glands, g	0.0494 ± 0.0066	5	0.0429 ± 0.0039	5	0.0440 ± 0.0053	5
Liver, g	10.85 ± 0.97	5	10.43 ± 0.36	5	10.82 ± 0.85	5
Thymus, r	0.425 ± 0.079	5	0.463 ± 0.091	5	0.486 ± 0.042	5
Heart, g	1.153 ± 0.123	5	1.152 ± 0.060	5	1.220 ± 0.086	5
Brain, g	1.678 ± 0.063	5	1.705 ± 0.031	5	1.703 ± 0.075	5
Females						
Testes, g						
Spleen, g	0.1131 ± 0.0142	5	0.1084 ± 0.0157	5	0.1094 ± 0.0129	5
Kidneys, g	0.587 ± 0.025	5	0.563 ± 0.072	5	0.541 ± 0.047	5
Adrenal glands, g	1.461 ± 0.118	5	1.462 ± 0.139	5	1.408 ± 0.132	5
Liver, g	0.0607 ± 0.0037	5	0.0566 ± 0.0031	5	0.0583 ± 0.0023	5
Thymus, r	6.87 ± 0.37	5	6.53 ± 0.94	5	6.38 ± 0.48	5
Heart, g	0.302 ± 0.024	5	0.245 ± 0.131	5	0.282 ± 0.037	5
Brain, g	0.838 ± 0.078	5	0.887 ± 0.045	5	0.867 ± 0.068	5
	1.620 ± 0.082	5	1.629 ± 0.062	5	1.600 ± 0.071	5

* - P <0.05 based on the Kruskal-Wallis test for the NaCl group.

Table S16. Acute toxicity study of the aptamers in rats. The average values of weight of organs (relative values).

	0.9% NaCl		The preparation of DNA-aptamers 40 μl 100 nM		The preparation of DNA-aptamers 200 μl 1000 nM	
Males						
	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n
Testes, g	1.021 ± 0.040	5	1.133 ± 0.037 *	5	1.096 ± 0.019	5
Spleen, g	0.242 ± 0.012	5	0.232 ± 0.030	5	0.236 ± 0.032	5
Kidneys, g	0.720 ± 0.017	5	0.710 ± 0.018	5	0.703 ± 0.032	5
Adrenal glands, g	0.0160 ± 0.0016	5	0.0136 ± 0.0007	5	0.0142 ± 0.0013	5
Liver, g	3.515 ± 0.217	5	3.327 ± 0.167	5	3.499 ± 0.096	5
Thymus, r	0.1371 ± 0.0216	5	0.1470 ± 0.0248	5	0.1573 ± 0.0106	5
Heart, g	0.373 ± 0.028	5	0.368 ± 0.031	5	0.395 ± 0.017	5
Brain, g	0.544 ± 0.015	5	0.544 ± 0.032	5	0.552 ± 0.018	5
Females						
Testes, g						
Spleen, g	0.0549 ± 0.0065	5	0.0518 ± 0.0044	5	0.0534 ± 0.0077	5
Kidneys, g	0.285 ± 0.008	5	0.270 ± 0.034	5	0.264 ± 0.029	5
Adrenal glands, g	0.710 ± 0.048	5	0.701 ± 0.041	5	0.683 ± 0.034	5
Liver, g	0.0295 ± 0.0023	5	0.0272 ± 0.0020	5	0.0283 ± 0.0010	5
Thymus, r	3.338 ± 0.197	5	3.122 ± 0.239	5	3.098 ± 0.067	5
Heart, g	0.1465 ± 0.0095	5	0.1180 ± 0.0629	5	0.1365 ± 0.0122	5
Brain, g	0.407 ± 0.032	5	0.426 ± 0.016	5	0.421 ± 0.017	5

	0.787 ± 0.029	5	0.784 ± 0.057	5	0.778 ± 0.025	5

* - P <0.05 based on the Kruskal-Wallis test for the NaCl group.

Tables 17-20 can be found in Supplemental Videos and Spreadsheets

Table S17. Acute toxicity study of the aptamers in rats. The individual blood biochemistry data.

Table S18. Acute toxicity study of the aptamers in rats. The individual hematology data.

Table S19. Acute toxicity study of the aptamers in rats. The individual hematology data.

Table S20. Acute toxicity study of the aptamers in rats. The individual data on the mass of organs.

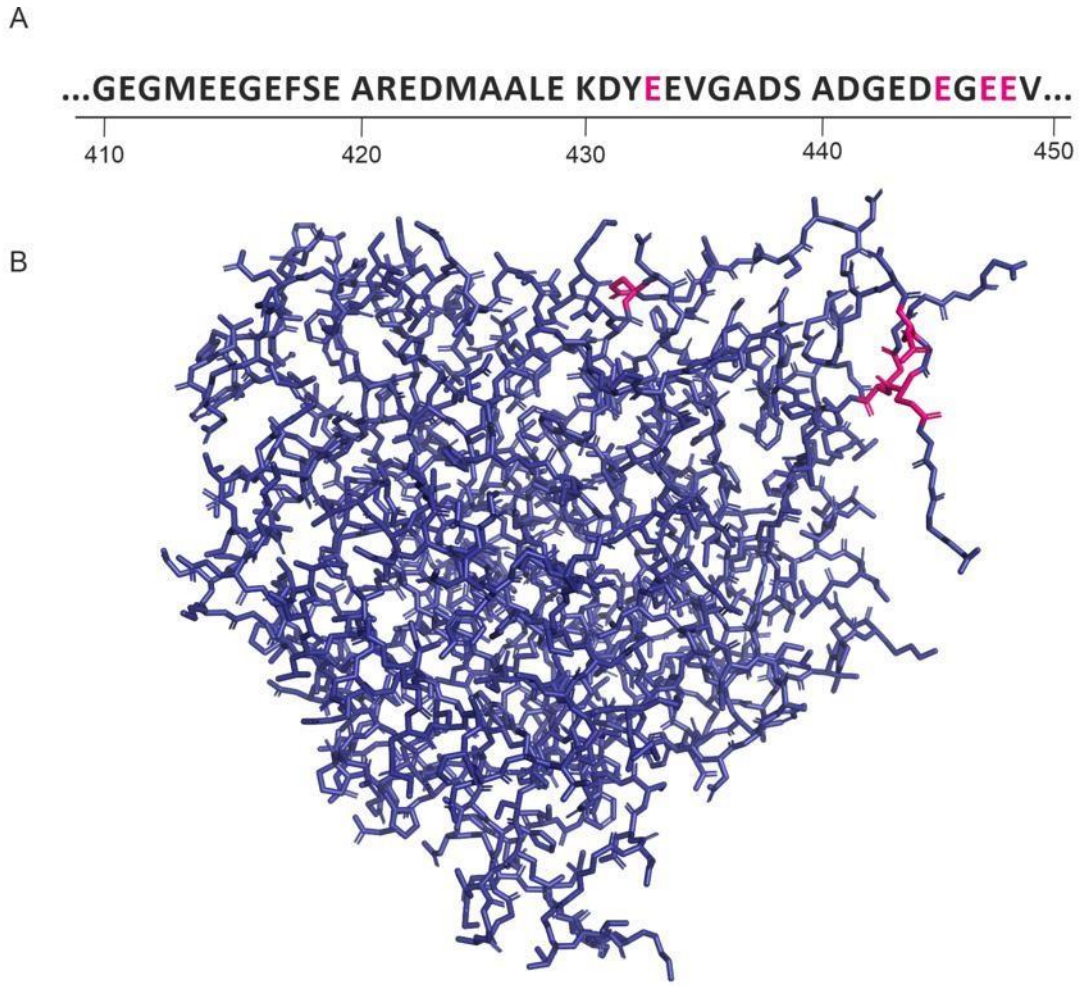

Figure S1. Post-translational modifications of tubulin alpha-1C (TUBA1C)

(A) Localization of PTMs in the protein sequence: monoglycylation of residue #433, and polyglutamylation of residues #445 (2x), #447 (1x), and #448 (5x). (B) A tertiary structural molecular model of TUBA1C.

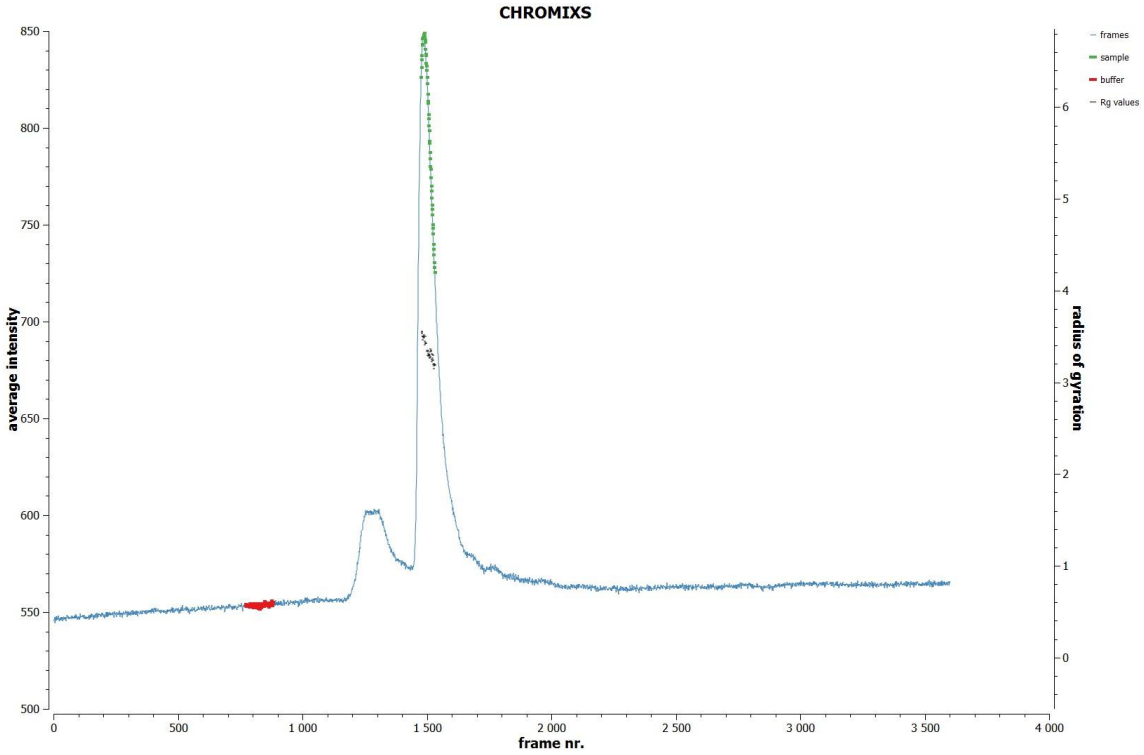

Figure S2. SEC-SAXS plot representing the chromatogram for the Gli-55 aptamer

Each of the 3600 points is an individual SAXS measurement. The highest peak shows the maximum concentration of the sample flowing through the HPLC column. Values of the gyration radius (black dots), estimated from the sample SAXS data highlighted by green dots, show the decreasing slope. It means that the peak contains a contribution of different components of the solution.

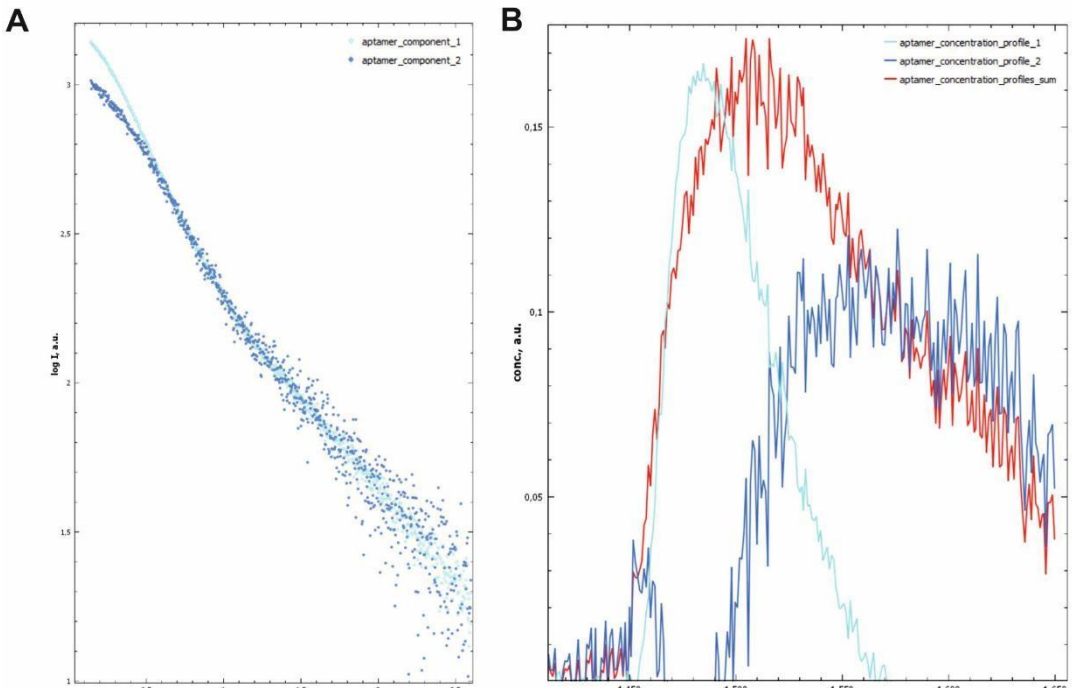

Figure S3. EFAMIX decomposition of SEC-SAXS data from the Gli-55 monomer-dimer mixture

(A) SAXS profiles decomposed from the SEC-SAXS data. (B) Concentration profiles for two components and their sum.

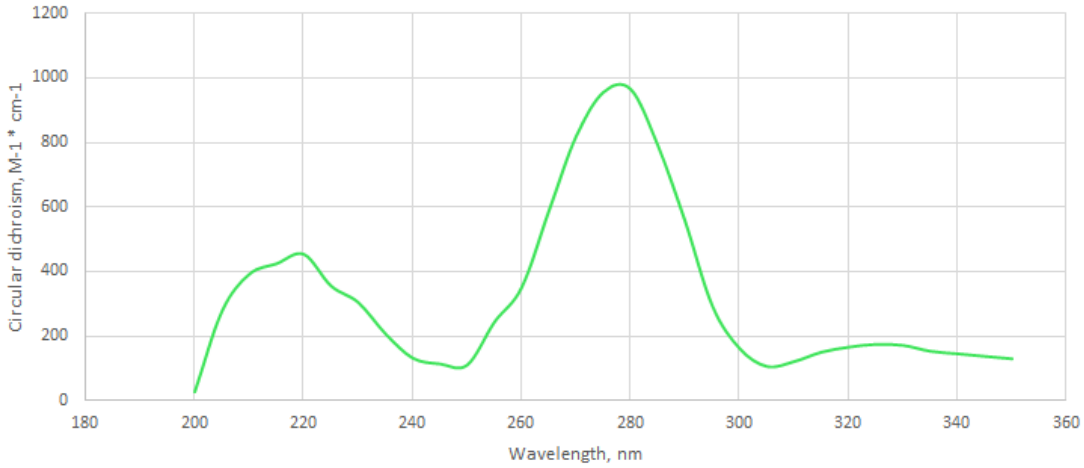

Figure S4. The circular dichroism (CD) spectroscopy of the aptamer Gli-55

Peaks at 220 and 280 nm wavelengths indicate DNA duplex formation.

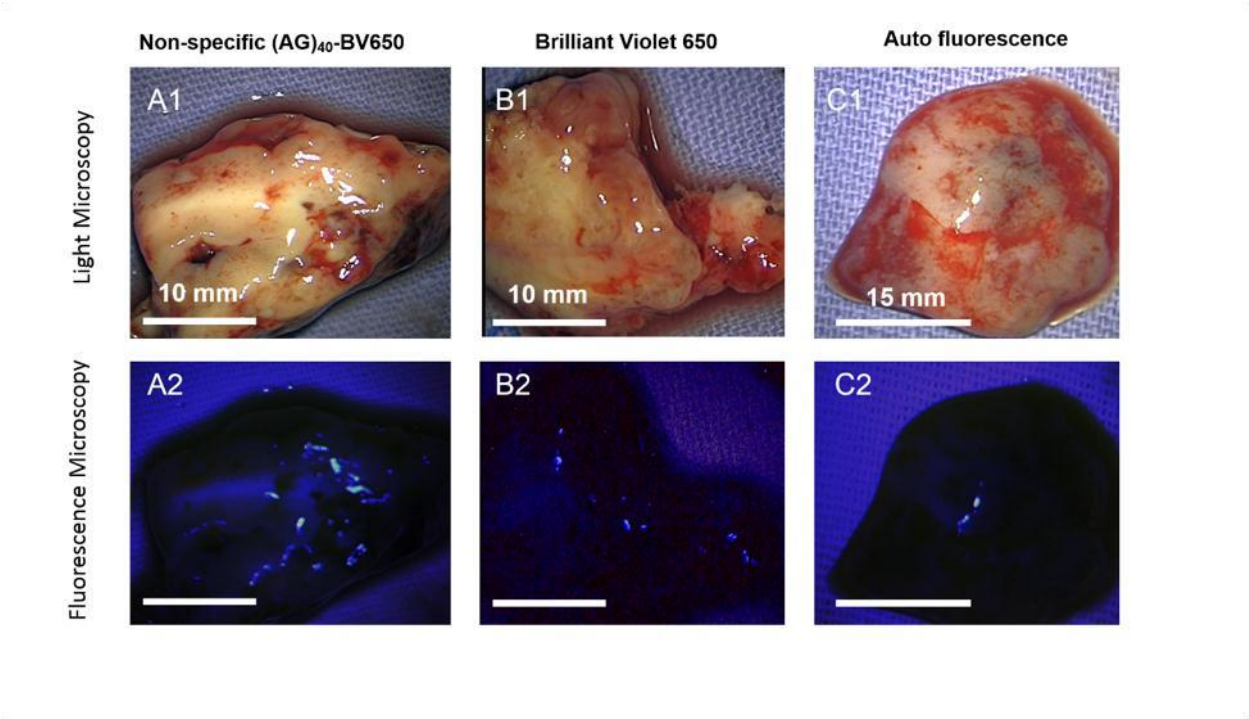

Figure S5. Astrocytoma, Grade IV staining controls

(A) Non-specific oligonucleotide $(AG)_{40}$ conjugated with Brilliant Violet 650. (B) Free dye Brilliant Violet 650. (C) Autofluorescence. Magnification 8×.

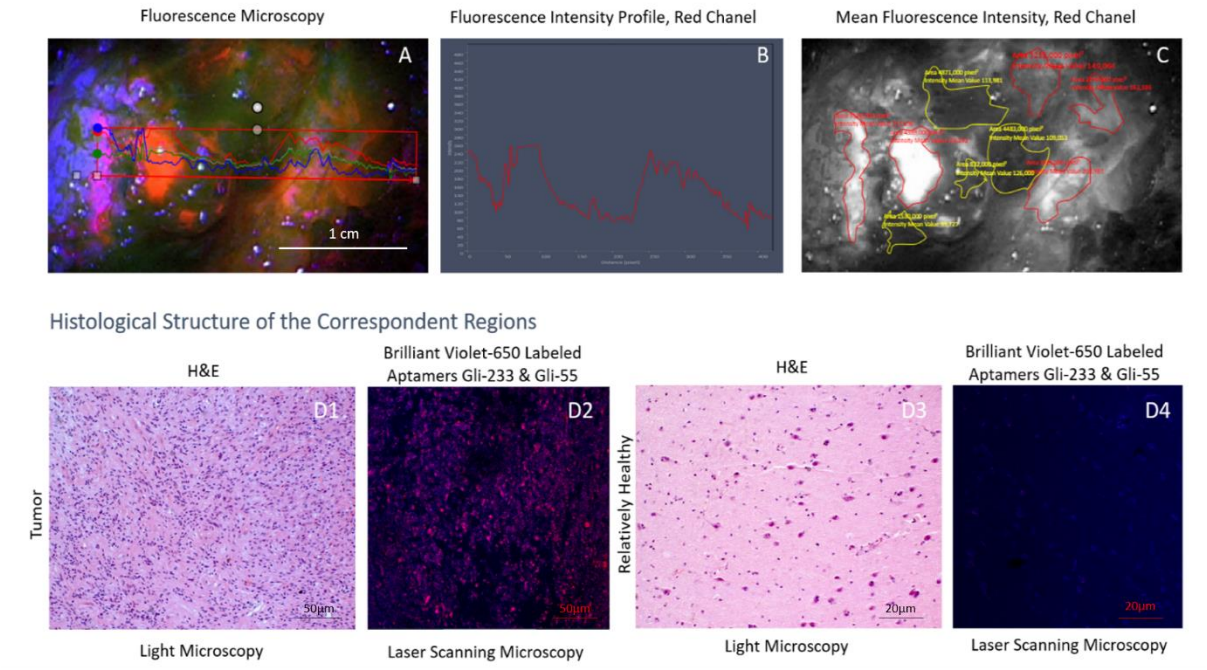

Figure S6. Tumor to background ratio (TBR) estimation

(A) Fluorescence microscopy of astrocytoma tissues *ex vivo*. (B) Fluorescence intensity profile of red channel. (C) The mean fluorescence intensity (MFI) for different zones of tumor and background. (D) Histological structure (1, 3) and laser scanning microscopy (2, 4) of the resected tissues defined as the tumor (1, 2) and relatively healthy brain tissue (the biopsy from the background area) (3, 4**)**. True tumor detection is defined by a TBR>1, when tumor MFI is greater than background MFI. The average TBR calculated from MFI is 1.898 for margin zones TBR=1.4, for the tumor zones TBR=4.5.

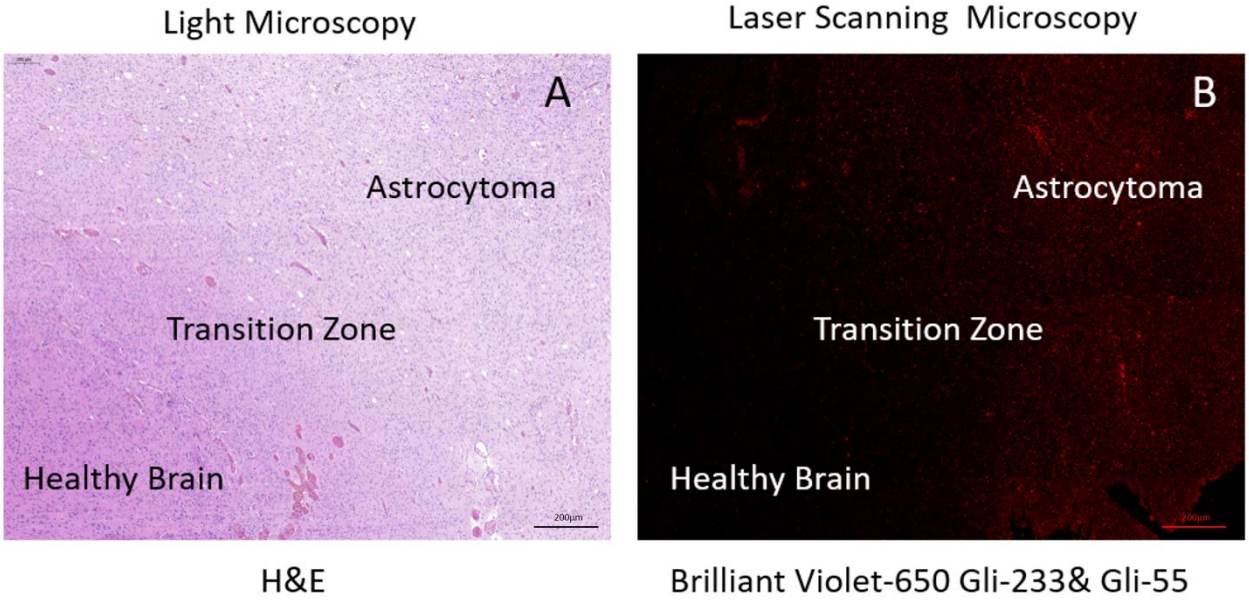

Figure S7. Brilliant violet-650 Gli-233 & Gli-55 reveal glioma boundaries in the transition zone of the tumor

(A) Histological structure of the resected tissues from a transition zone of astrocytoma tissues. (B) Laser scanning microscopy of the resected tissues from a transition zone of astrocytoma tissues.

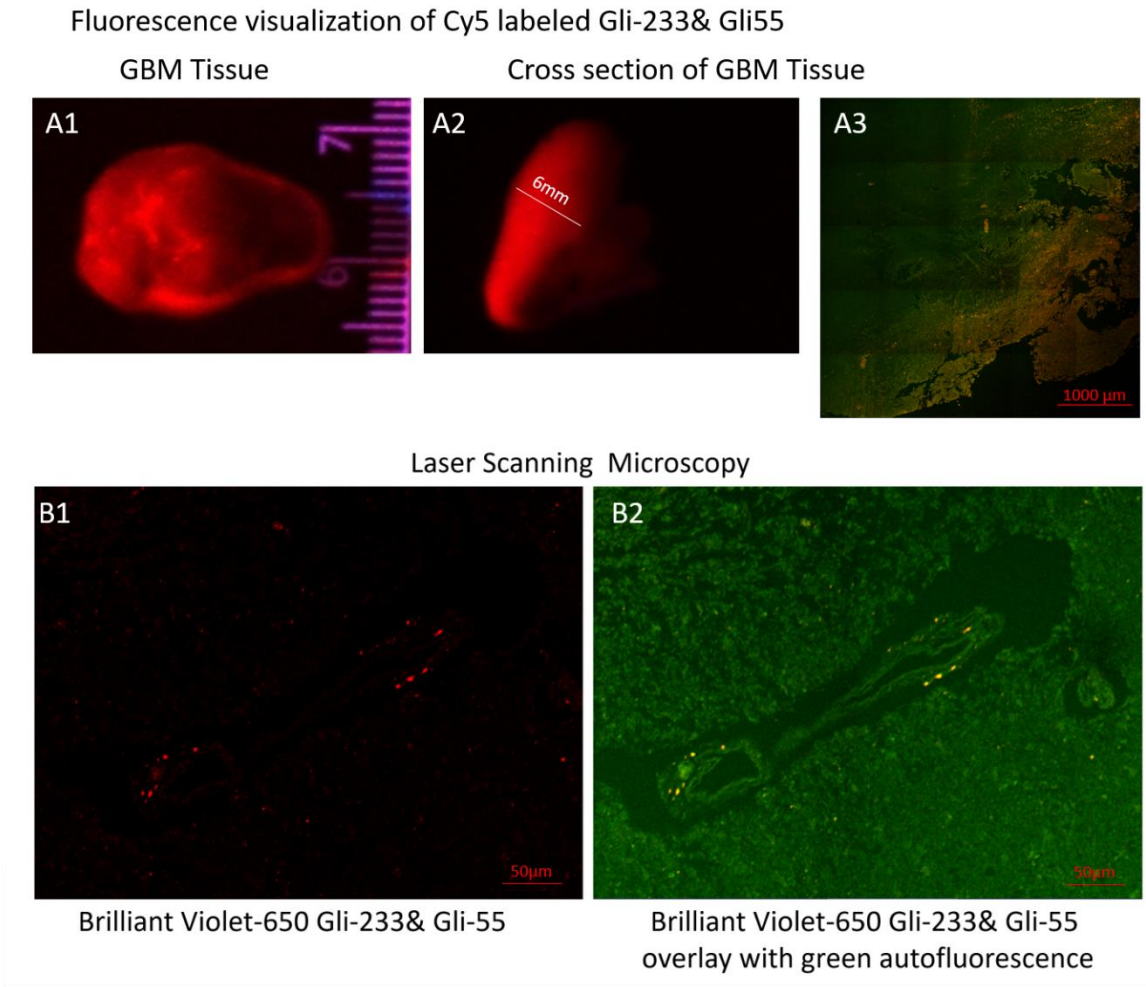

Figure S8. Biodistribution of Brilliant violet-650 Gli-233 & Gli-55 in the astrocytoma, Grade III tissues

(A) Fluorescent aptamers penetrates 6 mm deeply into the tumor. (B) Fluorescent aptamers do not stain the vasculature.

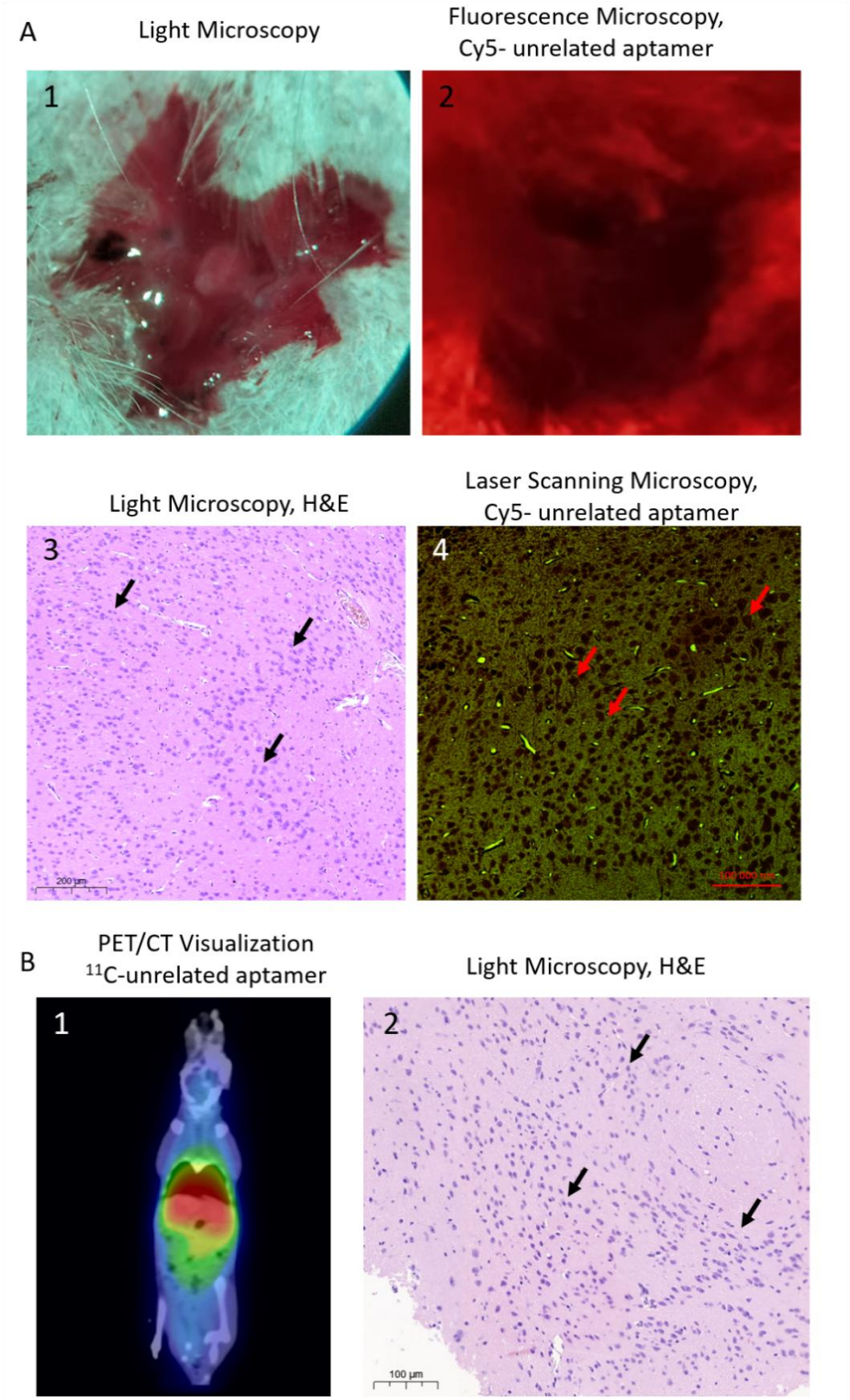

Figure S9. Controls with the unrelated aptamer for *in vivo* visualization of xenotransplantated human glioma in mice. (A) Fluorescence-assisted *in situ* visualization of the tumor in mice brain stained with the Cy5 labeled aptamer Gli-233 during the surgical intervention *in vivo* (2) supported with the light microscopy (1) of the correspondent area, laser scanning (Cy5 labeled aptamer – red channel; autofluorescence – green channel) (4) and light (3) microscopy of same tissues stained with hematoxylin and eosin. (B) PET/CT visualization (1), and histological analyses the tumor in the brain (2). Black and red arrows out astrocytoma cells. Magnification 4×, 10×, 40×.