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Supplemental Information

RNA Aptamers Targeting Integrin $\alpha5\beta1$ as Probes for Cyto- and Histofluorescence in Glioblastoma

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SUPPLEMENTAL DATA

Supplemental figures

Figure S1.

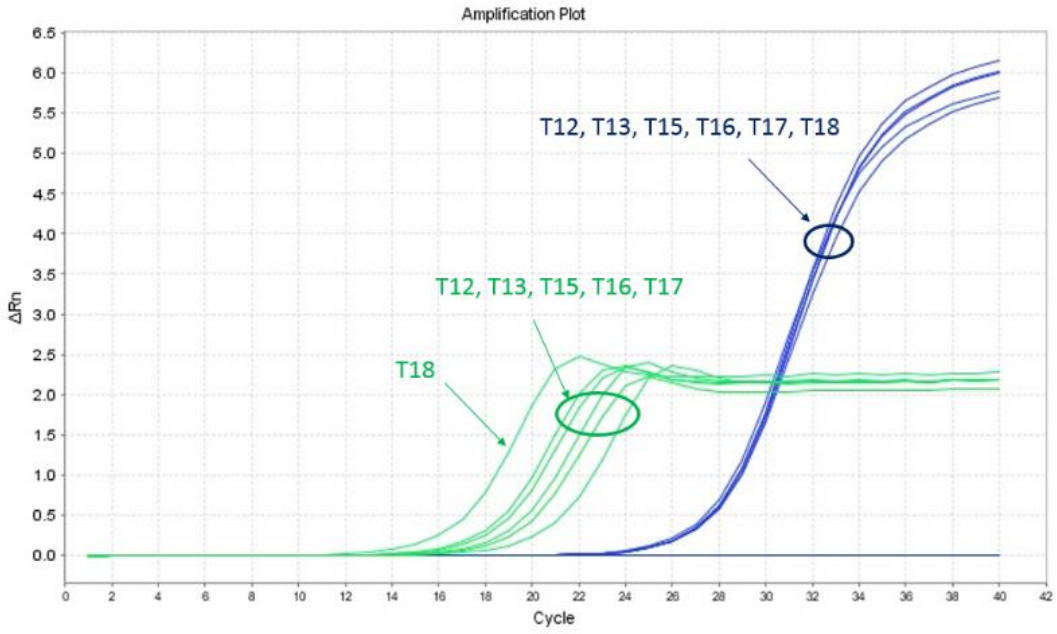


Figure S2.

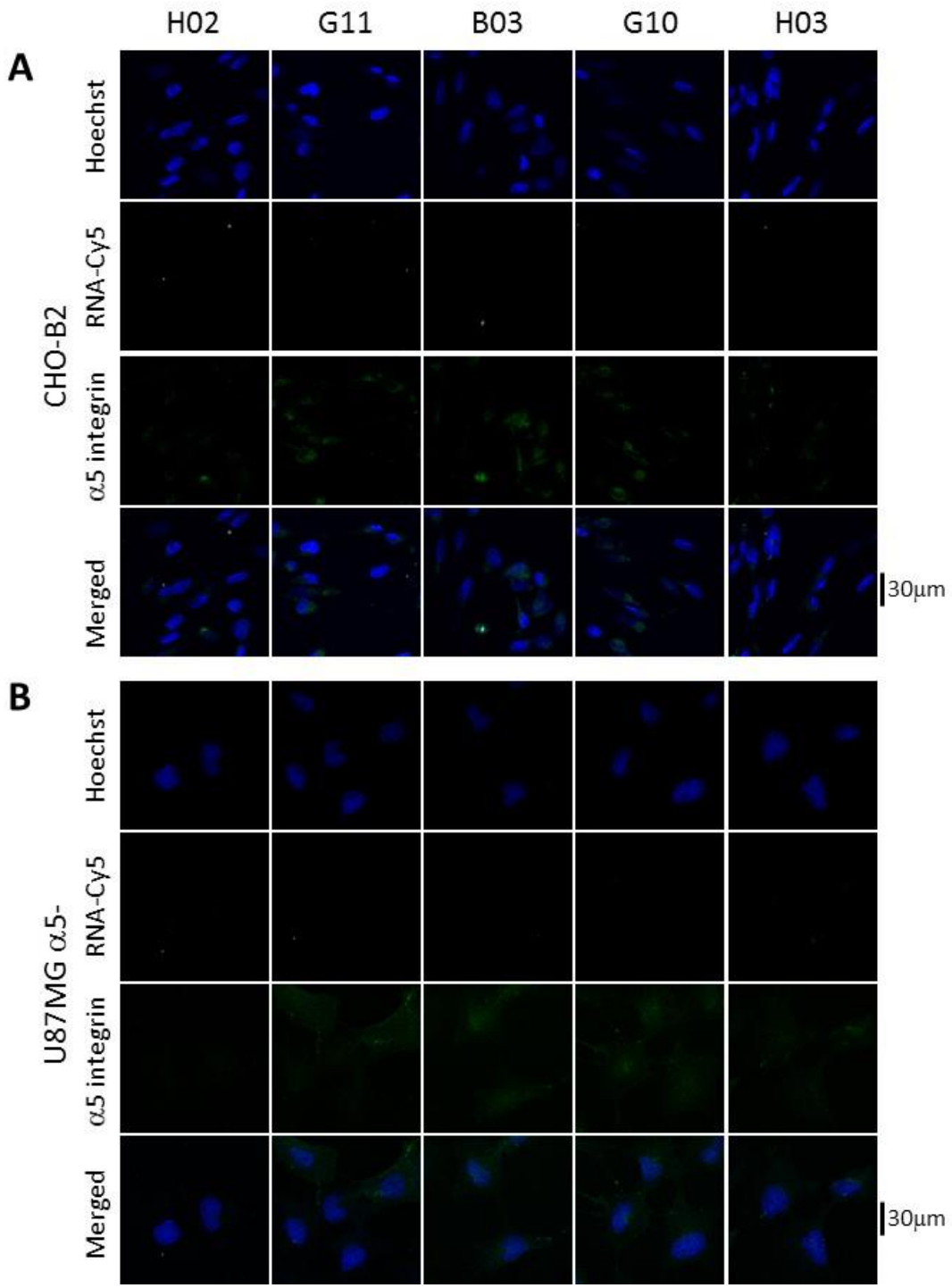

H02
G11
B03
G10
H03
A
CHO-B2
Hoechst
RNA-Cy5
α5 integrin
Merged
30μm
B
U87MG α5-
Hoechst
RNA-Cy5
α5 integrin
Merged
30μm

Figure S3.

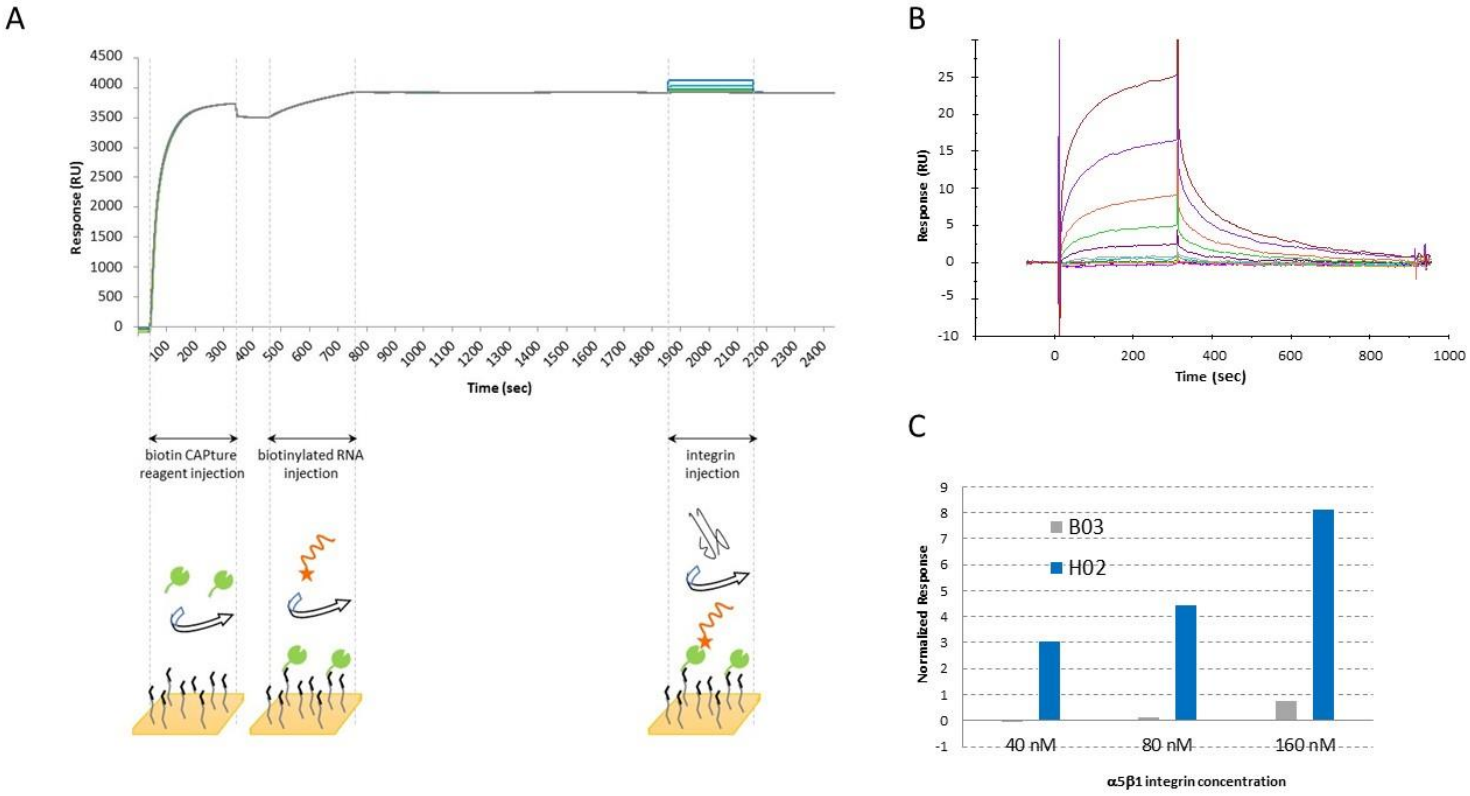

A
Response (RU)
Time (sec)
biotin CAPture reagent injection
biotinylated RNA injection
integrin injection
B
Response (RU)
Time (sec)
C
Normalized Response
B03
H02
40 nM
80 nM
160 nM
α5β1 integrin concentration

Figure S4.

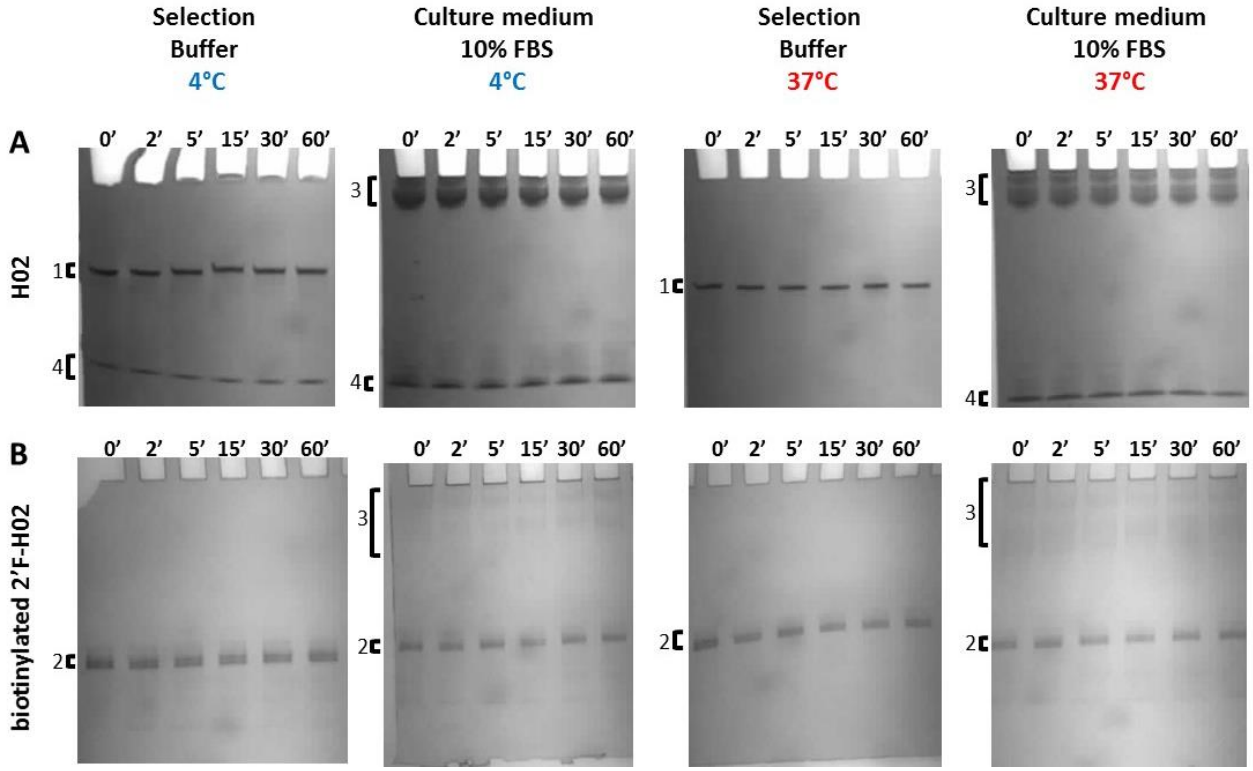

Selection
Buffer
4°C
Culture medium
10% FBS
4°C
Selection
Buffer
37°C
Culture medium
10% FBS
37°C
A
H02
B
biotinylated 2'F-H02
0' 2' 5' 15' 30' 60'
1
2
3
4

Figure S5.

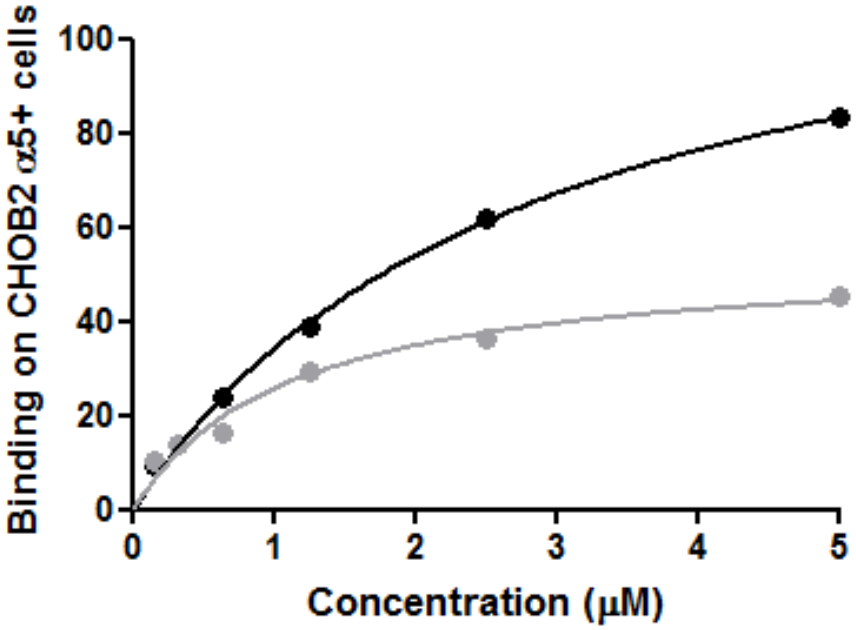
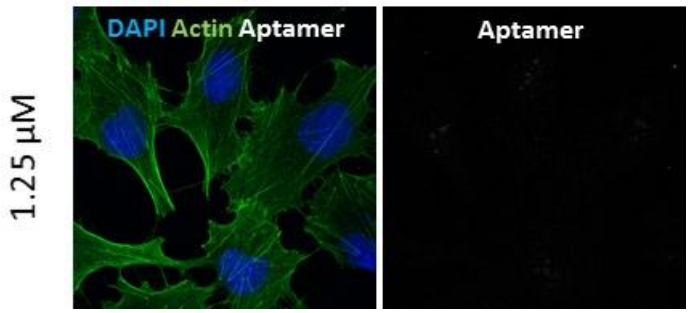

Binding on CHOB2 α5+ cells
100
80
60
40
20
0
0
1
2
3
4
5
Concentration (μM)

Figure S6.



Supplemental legends

Figure S1. Amplification plot showing the evolution of RNA pools from rounds 12, 13, 15, 16, 17 and 18 (named T12, T13, T15, T16, T17 and T18, in green on the figure). Curves in blue display the amplification of the α5 gene used as an internal control.

Figure S2. A & B. Monitoring of the binding of five Cy5-labeled aptamers H02, G11, B03, G10 and H03 at 5 µM, at 4°C, using confocal microscopy, on **A.** CHO-B2 and **B.** U87MG α5- cell lines. Nuclei, counterstained with Hoechst are represented in royal blue. Aptamers, coupled to Cy5, are represented in white. α5 labeling is similar to Figure 2. Merged images are shown.

Figure S3. SPR experiments. **A.** Eight different successive cycles show the reproducibility of biotinylated aptamer injections. One cycle corresponds to biotin CAPture reagent injection, biotinylated aptamer injection and integrin injections. On the sensorgrams, colors correspond to the five integrin concentrations described in the legend of Figure 4B. **B.** Positive controls: Integrin α5β1 (at concentrations ranging from 0.12 to 130 nM) was injected for 300 sec at a flow rate of 30 µl/min on a CM5 sensor chip immobilized with 760 RU of fibronectin. **C.** SPR binding responses for the interaction between integrin α5β1 injected at three concentrations (40, 80 and 160 nM, x-axis) and biotinylated

aptamers B03 (grey) and H02 (blue). The report point has been taken 5 sec before the end of integrin injection. Responses were normalized according to the level of biotinylated aptamers captured on the CAP sensor chip.

Figure S4. Stability of RNA aptamers **A.** H02 and **B.** biotinylated H02-2'F. Aptamers were incubated at 5 μM on U87MG α5+ cells for 0, 2, 5, 15, 30 and 60 min in four different conditions outlined above figures: selection buffer and culture medium supplemented with 10% FBS, at 4°C and 37 °C. One single band at the middle of the gel corresponds to the full size H02 aptamer (label n.1) and biotinylated H02-2'F aptamer (label n.2). In culture medium supplemented with 10% FBS, bands at the top of the acryl/urea gels correspond to molecules present in the culture medium and FBS (label n.3). When present, bands at the bottom of gels correspond to the migration of bromophenol present in the loading buffer (label n.4, dye front). Gels for biotinylated aptamer H02-2'F migrated for a longer time than gels for aptamer H02.

Figure S5. Binding profiles of Cy5-aptamers H02 (black) and G11 (grey) at concentrations of 0.15, 0.3, 0.6, 1.25, 2.5 and 5 μM incubated on ice with a constant amount of CHO-B2 α5+ cells and analyzed by flow cytometry.

Figure S6. Monitoring of the binding of the Cy5-labeled aptamer H02 at 1.25 μM at 4°C using confocal microscopy on U87MG α5+ cells. Nuclei, counterstained with Hoechst are represented in royal blue. Aptamers, coupled to Cy5, are represented in white. Actin labeling is represented in green. Merged images and aptamer labeling are represented to the left and to the right, respectively.

Supplemental tables

Table S1. Selection conditions in SELEX process.

Rounds	Yeast tRNA (mg/ml)	Nb of washes (2-5 min/wash)	Counter-selection incubation time	Positive-selection incubation time
		cell-SELEX		
1	/	1	/	30 min
2	/	1	/	30 min
3	/	4	/	20 min
4	/	4	15 min	20 min
5	/	4	20 min	15 min
6	/	7	25 min	10 min
7	/	7	/	20 min
		protein-SELEX		
8	/	3	15 min[a]	20 min
9	/	3	15 min[b]	20 min
10	/	6	20 min[b]	20 min
		cell-SELEX		
11	/	9	/	25 min
12	/	6	13 min	30 min
13	/	8	3 min x 6 wells[c]	30 min
14	1	8	3 min x 6 wells[c]	30 min
15	1	8	3 min x 6 wells[c]	25 min
16	1	8	3 min x 6 wells[c]	17 min
17	1	10	3 min x 6 wells[c]	15 min
18	1	10	25 min	10 min

[a]Incubation on protein A-sepharose

[b]Incubation on protein A-sepharose, followed by incubation on Cetuximab

[c]The RNA pool was incubated for 3 minutes on 6 successive adherent cells containing wells

Table S2. Sequences of all primers, library, and aptamers described in this study. Fixed sequences of library and aptamers are written in dark red.

Name	Sequence
Primer P3'	5'-GTGTGACCGACCGTGGTGC-3'
Primer P5'	5'-TAATACGACTCACTATAGGTTACCAGCCTTCACTGC-3'
RNA library	5'-GGUUACCAGCCUUCACUGC-N_{30}-GCACCACGGUCGGUCACAC-3'
Aptamer H02	5'- GGUUACCAGCCUUCACUGCGGACGGACAGAGAGUGCAACCUGCCGUGCCGCACCACGGUCGGUCACAC-3'
Aptamer G11	5'- GGUUACCAGCCUUCACUGCGGAGUACGCACACUUGGUGUUAGCGUCCCCGCACCACGGUCGGUCACAC-3'
Aptamer H03	5'- GGUUACCAGCCUUCACUGCCACAGCGGCAUUGAAAGCUGAUGACAGGCCGCACCACGGUCGGUCACAC-3'
Aptamer B03	5'-GGUUACCAGCCUUCACUGCGCCCAGUCACUGCACGAAUCAGUGAUGGCCGCACCACGGUCGGUCACAC-3'
Aptamer G10	5'- GGUUACCAGCCUUCACUGCCGAUGCAGGAUGGCCAACGUGUACCUGCCGCACCACGGUCGGUCACAC-3'

Supplemental Methods

Tracking the enrichment of RNA pools

50 pmoles of RNA pools from rounds 12, 13, 15, 16, 17 and 18 (named T12, T13, T15, T16, T17 and T18) were added to confluent α5- cells (cultivated in 24 well plates) for 15 min at 37°C. Supernatants were recovered and added to confluent α5+ cells for 15 min at 37°C. After washes, RNAs were extracted as described in the M&M section of the manuscript. RNA molecules were transcribed into cDNA using the iScript™ cDNA Synthesis kit (Bio-Rad) and then real time qPCR was performed using the Fast SYBR Green Master Mix and the StepOne Plus Real time PCR system (Applied Biosystem). Primers sequences used for PCR were 5'-GGTTACCAGCCTTCACTGC-3' and 5'-GTGTGACCGACCGTGGTG-3' for aptamer amplification and 5'-GGGCAGCAGGACAGGGTTAC-3' and 5'-GCCTTGCCAGAAATAGCTTCC-3' for α5 amplification. Data were analyzed with the StepOne Software v2.3.

Stability assay

RNA aptamers at 5 μM in selection buffer or in culture medium (EMEM) supplemented with 10% FBS were incubated on adherent U87MG α5+ cells at 4°C and 37°C for 0, 2, 5, 15, 30 min and 1 h. Samples were loaded on a denaturing (7 M urea) 12% polyacrylamide gel and revealed using a Stains all solution (Sigma-Aldrich).