Tumor-Targeting Peptides: The Functional Screen of Glioblastoma Homing Peptides to the Target Protein FABP3 (MDGI)

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Supplementary Figures and Tables

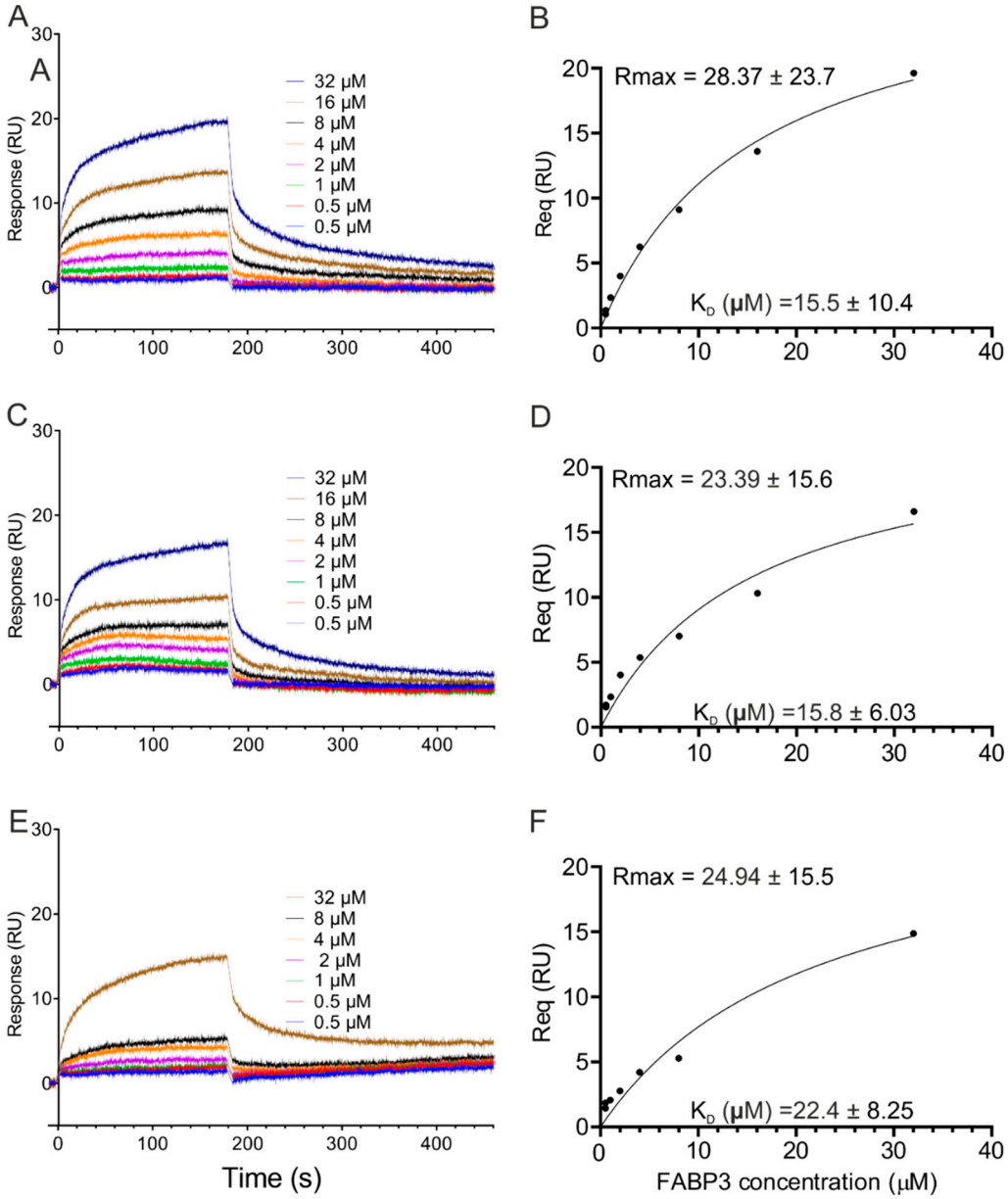

Figure S1. Measurement of the binding affinity between FABP3 and CooP peptide by using the surface plasmon resonance (SPR). (**A,B,E)** SPR sensorgrams and the steady state affinity between FABP3 and CooP peptide (**B,C,F)** from three independent experiments.

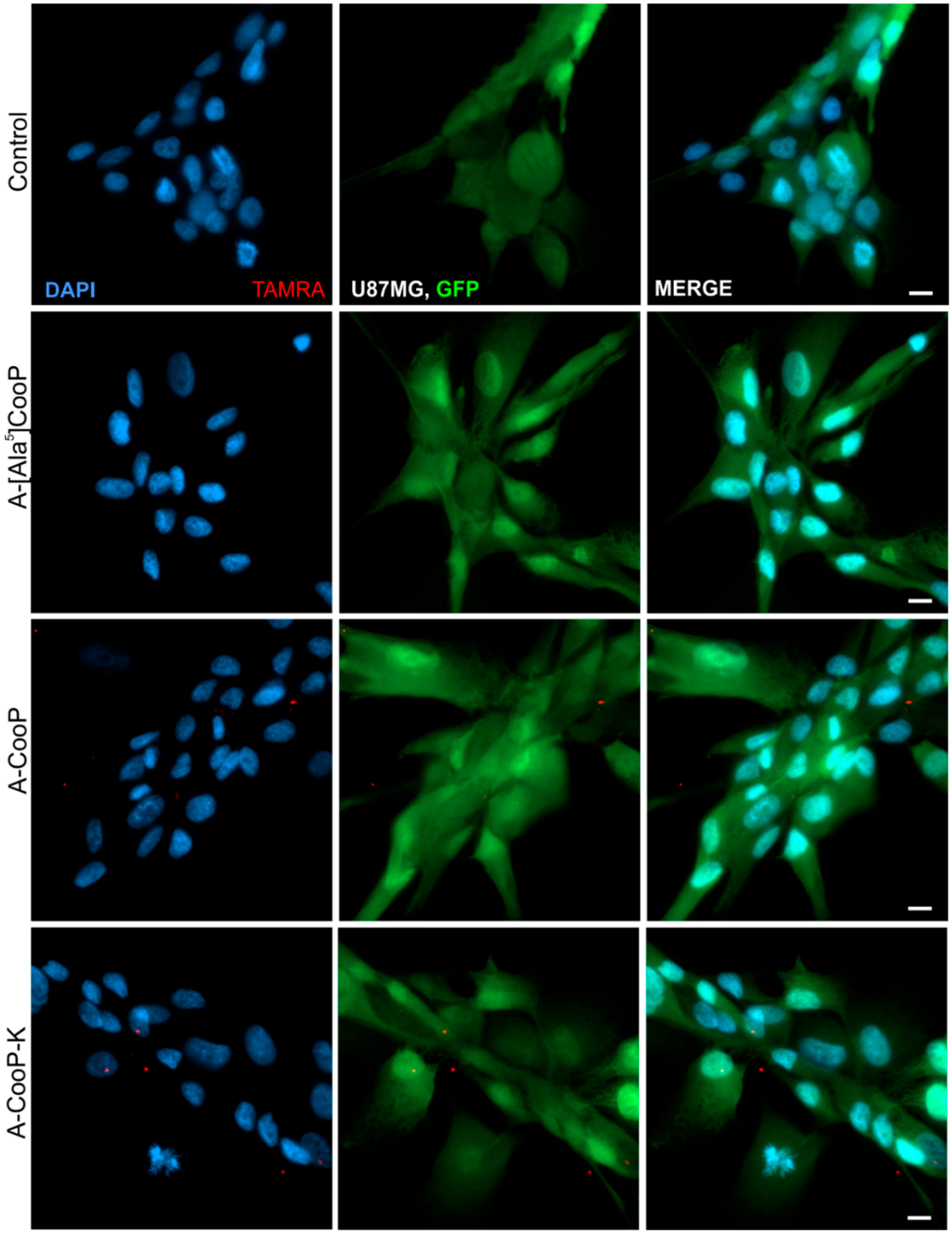

Figure S2. In vitro cellular uptake of the selected A-CooP variants by the U87MG cells. Microscopic images of the U87MG glioblastoma cells expressing GFP (green). These cells express only negligible amounts of FABP3. No significant binding or internalization of the control, A-[Ala^5]CooP, A-CooP, or A-CooP-K peptides (red) were observed.

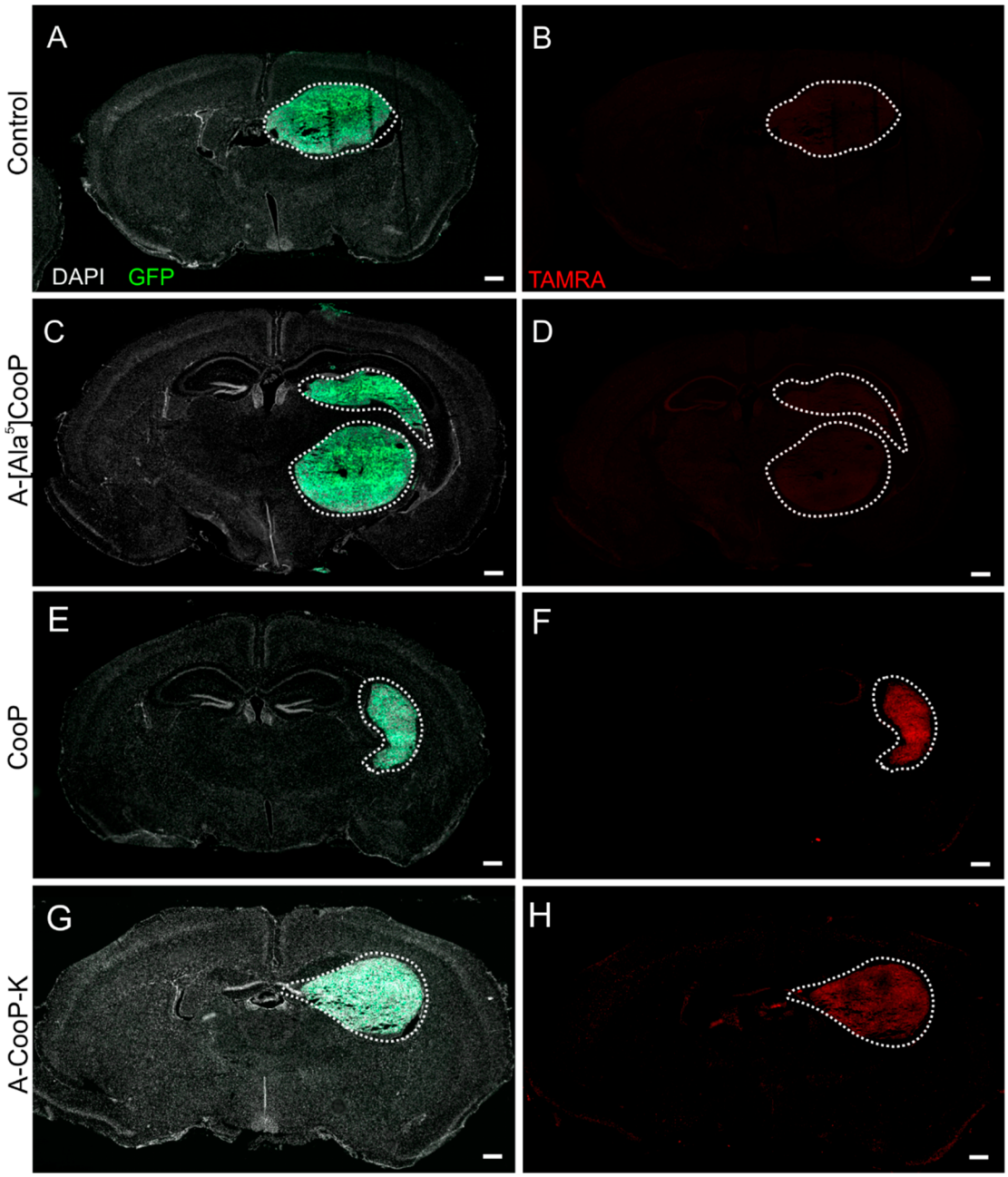

Figure S3. Homing of the A-CooP variants to the intracranial glioblastoma xenografts. The GFP-labeled patient-derived glioblastoma cells were intracranially grafted into immunocompromised mice. After 25 days fluorescently labeled peptides (100μM in 100 μl of 0.9% saline solution) were infused intravenously into tumor-bearing animals and let to circulate for 60 min. The brain tissue was collected and processed for immunofluorescence. Micrograghs show representative whole coronal section micrographs of murine brain containing the tumors (outlined with white dashed line) and the peptide-associated fluorescence in red. (**A-B) C**ontrol peptide, (**C-D)** A-[Ala^5]CooP, (**E-F)** A-CooP, and (**G-H)** A**-**CooP-K.

Table S1. MST summary table shows the calculated K_D values, response amplitude (RU), standard error of regression, signal-to-noise ratio, and the reduced X^2 values (R^2) for the different peptide variants.

Peptide variants	Target conc. (nM)	K_D (μM)	Response amplitude	Unbound	Bound	Standard error	R^2	Signal-to-noise
A-CooP	20	2.18 ± 1.15	15	842	827	2.43	0.60	7
A-[Ala^1]CooP	20	-	-	-	-	-	-	-
A-[Ala^2]CooP	20	0.09 ± 0.07	20	850	831	2.31	0.73	9
A-[Ala^3]CooP	20	0.25 ± 0.14	23	856	833	2.18	0.83	11
A-[Ala^4]CooP	20	0.11 ± 0.02	24	857	833	1.98	0.80	13
A-[Ala^5]CooP	20	-	-	-	-	-	-	-
A-[Ala^6]CooP	20	0.19 ± 0.03	14	845	831	1.34	0.73	11
A-[Ala^7]CooP	20	133 ± 75	138	839	977	6.37	0.73	23
A-[Ala^8]CooP	20	0.12 ± 0.05	24	851	828	2.34	0.84	11
KA-CooP	20	0.63 ± 0.13	15	843	828	1.72	0.76	10
A-CooP-K	20	0.07 ± 0.01	20	847	827	1.71	0.95	12
RI-A-CooP	20	24 ± 13	16	842	826	2.84	0.73	6
Negative control	20	-	-	-	-	-	-	-