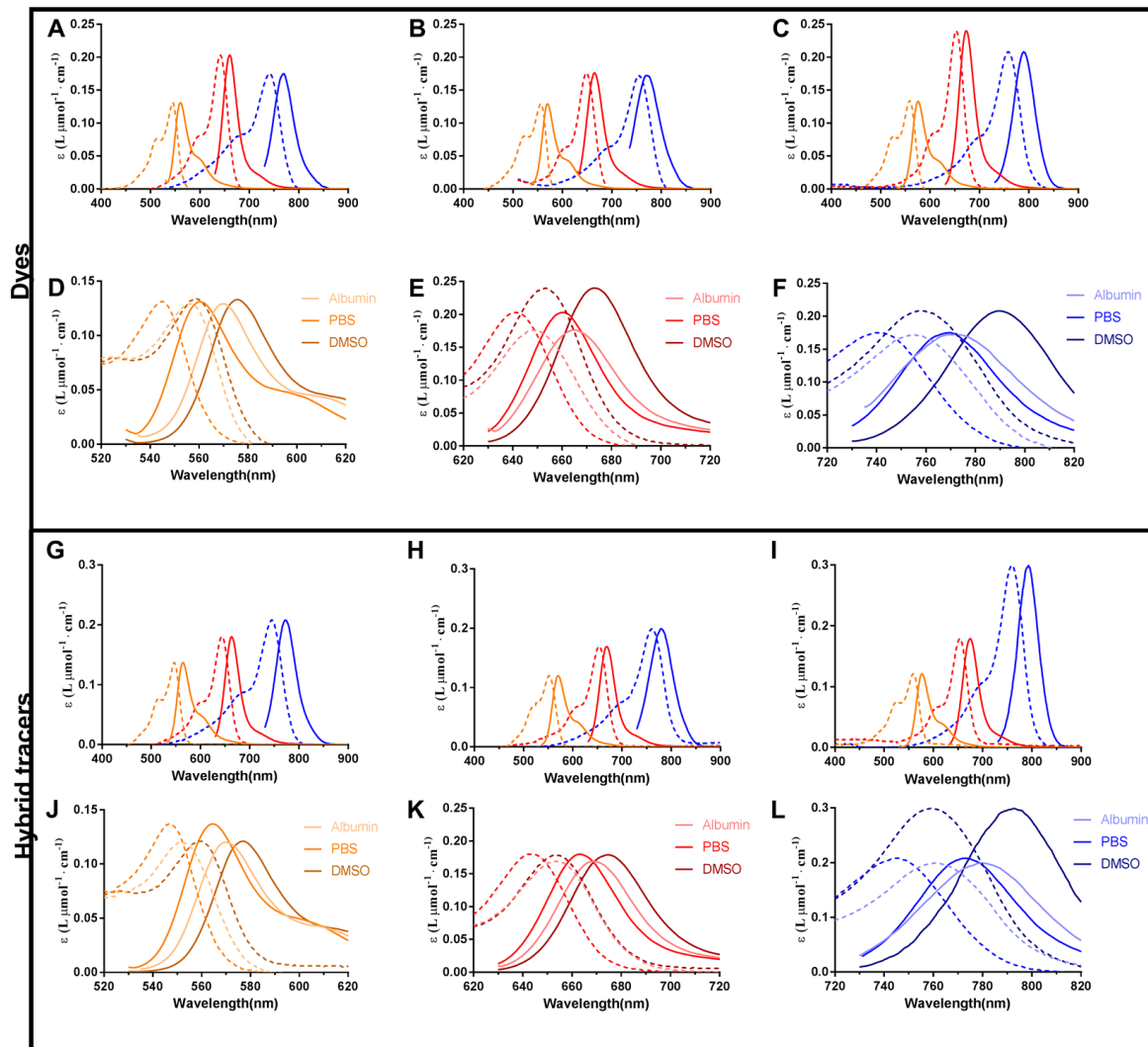


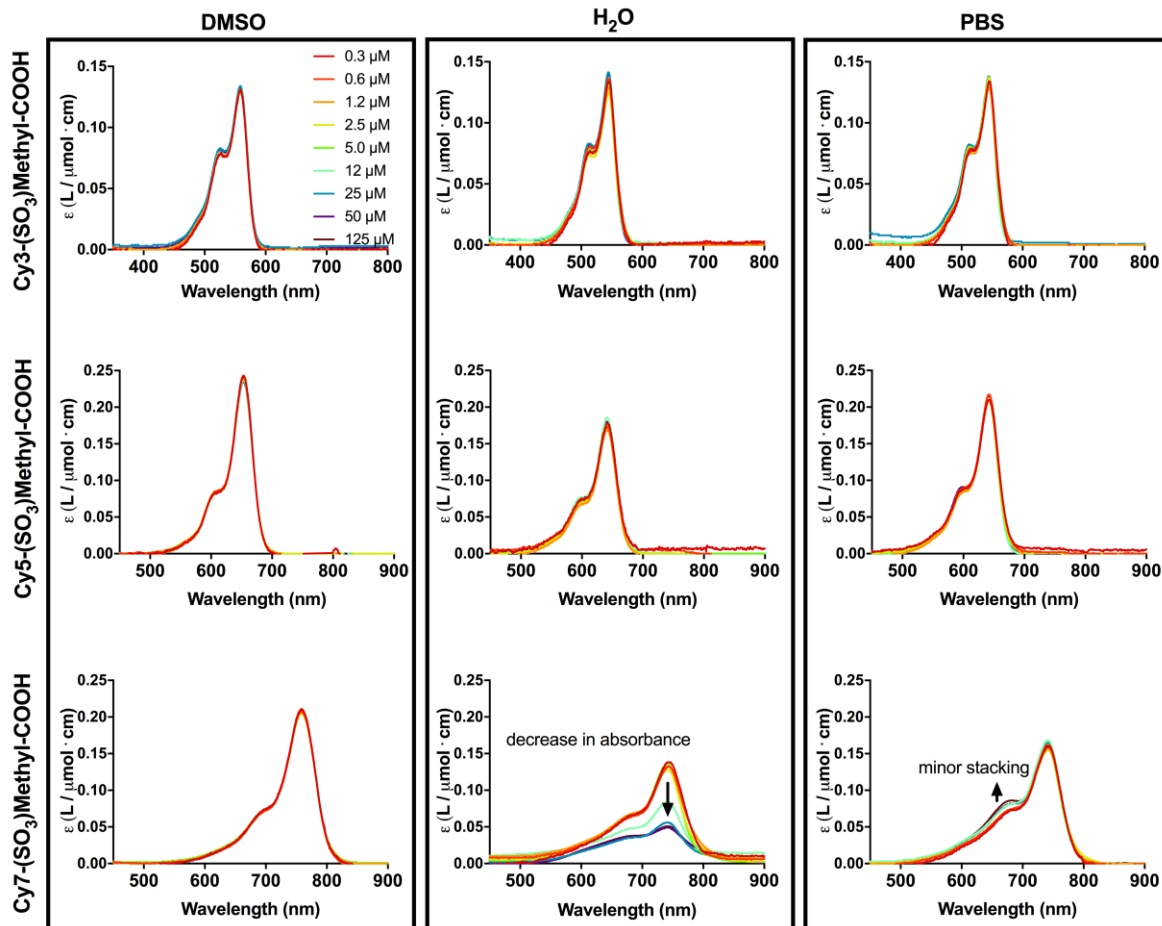
**Supplemental Table 1. (Photo)physical properties of the three unconjugated dyes.**

	<b>Cy3-(SO<sub>3</sub>)Methyl-COOH</b>	<b>Cy5-(SO<sub>3</sub>)Methyl-COOH</b>	<b>Cy7-(SO<sub>3</sub>)Methyl-COOH</b>
$\epsilon$ in PBS ( $M^{-1}\cdot cm^{-1}$ )	$1.3 \cdot 10^5$	$2.1 \cdot 10^5$	$1.6 \cdot 10^5$
$\epsilon$ in 200 mg/mL albumin in water ( $M^{-1}\cdot cm^{-1}$ )	$1.3 \cdot 10^5$	$1.8 \cdot 10^5$	$1.7 \cdot 10^5$
$\lambda_{ex}/\lambda_{em}$ in H <sub>2</sub> O and PBS (stoke shift)	545/560 (15nm)	641/660 (19nm)	741/769 (28nm)
$\lambda_{ex}/\lambda_{em}$ in 200 mg/ml albumin (stoke shift)	556/570 (14nm)	648/665 (17nm)	755/769 (14nm)
$\Phi_F$ in PBS	1 % <sup>a</sup>	13 % <sup>b</sup>	9 % <sup>c</sup>
$\Phi_F$ in 200 mg/mL albumin	9 % <sup>d</sup>	28 % <sup>d</sup>	14 % <sup>d</sup>
Relative brightness (PBS/albumin) to Cy3	1 / 1	15/ 4	8 / 2
Calculated log D <sup>e</sup>	1.50	2.02	2.55

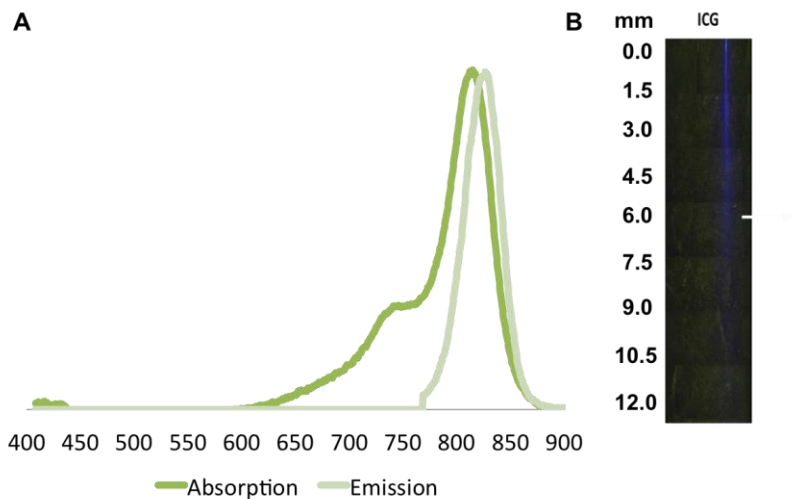
a) Relative quantum yield compared to Cy3-(SO<sub>3</sub>)COOH-(SO<sub>3</sub>)COOH in PBS ( $\Phi_F = 4\%$ ), b) Relative quantum yield compared to Cy5-(SO<sub>3</sub>)COOH-(SO<sub>3</sub>)COOH in PBS ( $\Phi_F = 27\%$ ). c) Relative quantum yield compared to Cy7-(SO<sub>3</sub>)Et-(SO<sub>3</sub>)COOH in PBS ( $\Phi_F = 13\%$ ). d) Relative increase in total fluorescence compared to the  $\Phi_F$  measured in PBS, hereby the solvent refractivity was not taken into account, e) Calculated for pH 7.4 with MarvinSketch (ChemAxon, Hungary) 15.11.9 using the consensus approach.



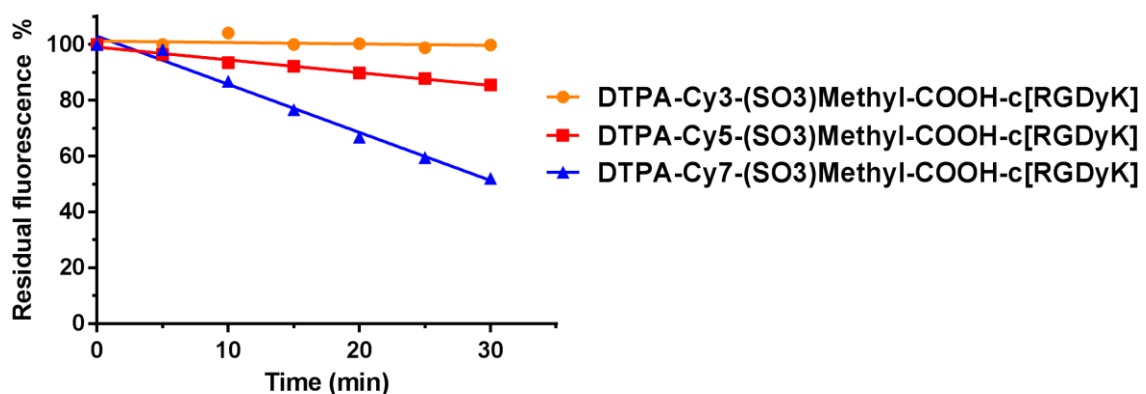
**Supplemental Fig. 1. Absorption and emission of the fluorescent dyes and hybrid tracers.** Absorption (dotted line) and emission graphs (solid line) of Cy3-(SO<sub>3</sub>)Methyl-COOH (orange), Cy5-(SO<sub>3</sub>)Methyl-COOH (red) and Cy7-(SO<sub>3</sub>)Methyl-COOH (blue) in A) PBS, B) Albumin and C) DMSO. Overview of differences per dye in Albumin, PBS and DMSO for D) Cy3-(SO<sub>3</sub>)Methyl-COOH, E) Cy5-(SO<sub>3</sub>)Methyl-COOH and E) Cy7-(SO<sub>3</sub>)Methyl-COOH. Absorption (dotted line) and emission graphs (solid line) of DTPA-Cy3-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] (orange), DTPA-Cy5-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] (red) and DTPA-Cy7-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] (blue) in A) PBS, B) Albumin and C) DMSO. Overview of differences per hybrid tracer in Albumin, PBS and DMSO for D) DTPA-Cy3-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK], E) DTPA-Cy5-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] and E) DTPA-Cy7-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK].



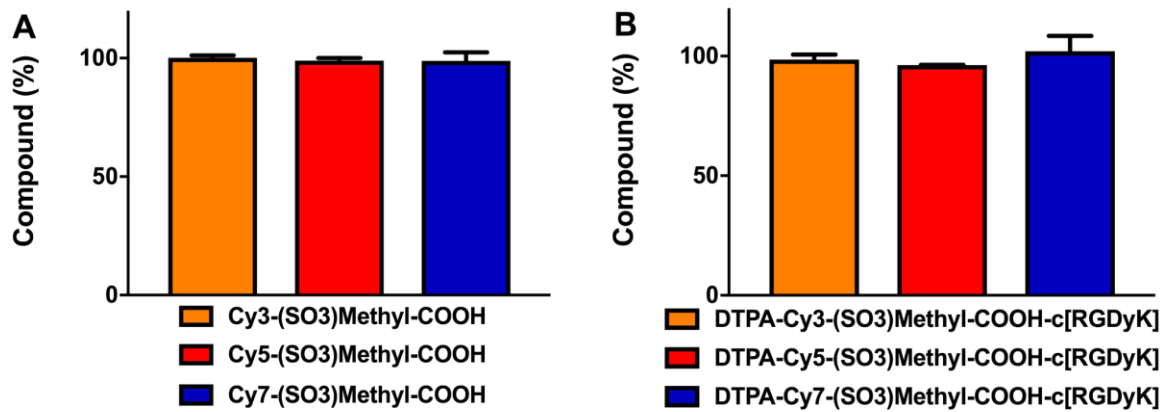
**Supplemental Fig. 2. Absorption spectrometry of the fluorescent dyes.** Concentration-normalized absorption spectra of Cy3-(SO<sub>3</sub>)Methyl-COOH (top), B) Cy5-(SO<sub>3</sub>)Methyl-COOH (middle) and C) Cy7-(SO<sub>3</sub>)Methyl-COOH (bottom) in DMSO, H<sub>2</sub>O and PBS.



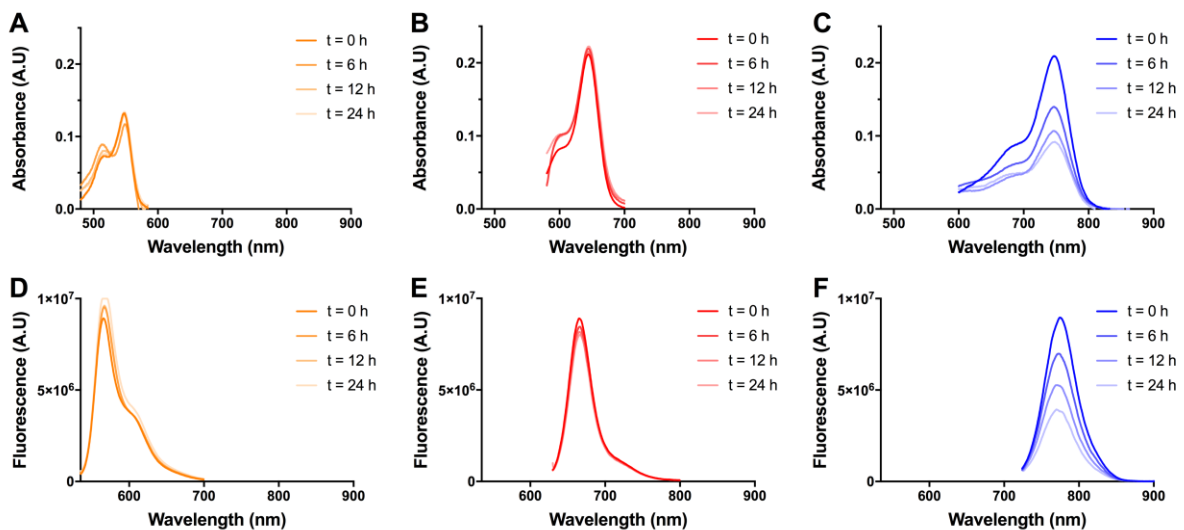
**Supplemental Fig.3. Photophysical properties and tissue penetration of Indocyanine Green (ICG).** A) Absorption and emission spectra of ICG in 200 mg/mL albumin in water. B) Tissue penetration of ICG, diluted in albumin and measured with a clinical-grade laparoscopic fluorescence camera system.



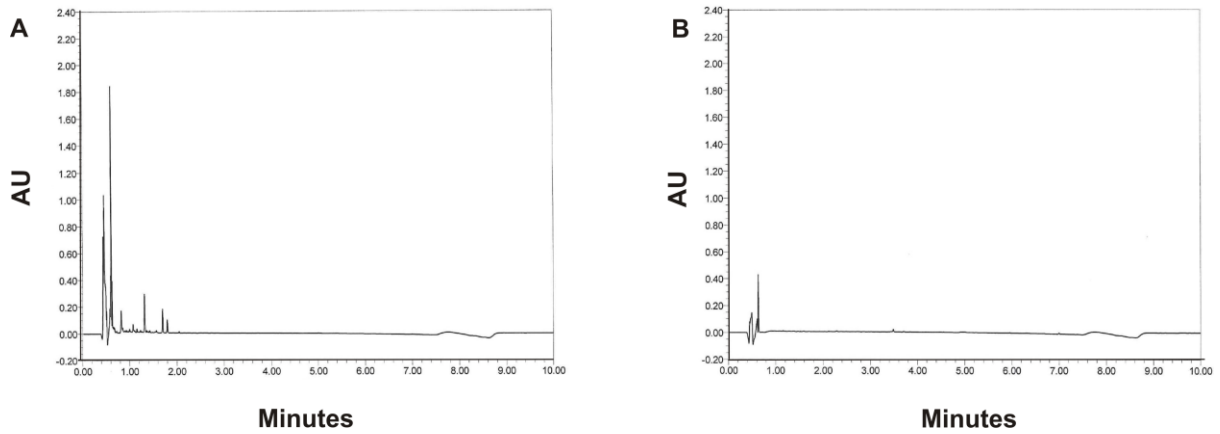
**Supplemental Fig. 4. Photo stability of the hybrid tracers.** The decrease in fluorescence of DTPA-Cy3-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] (orange), DTPA-Cy5-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] (red) and DTPA-Cy7-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] (blue) observed during fluorescent exposure of the tracers using a clinical grade fluorescence laparoscope.



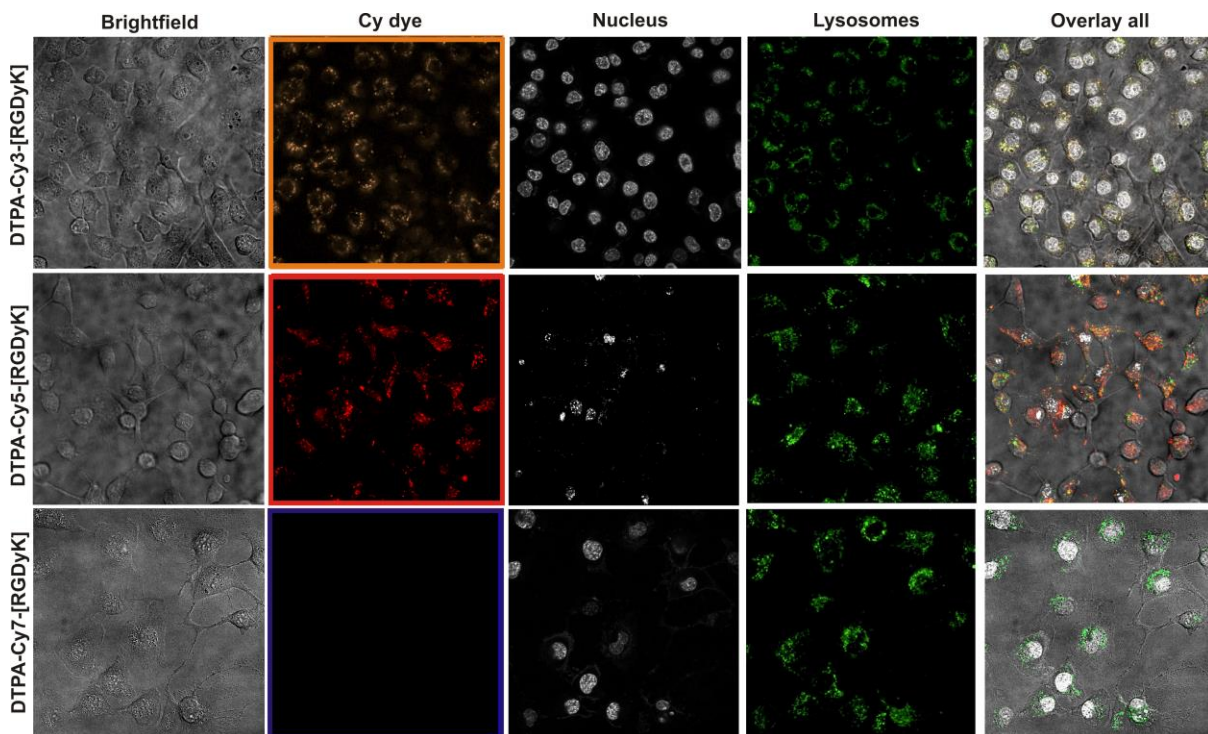
**Supplemental Fig. 5. Stability of the dyes and hybrid tracers towards glutathione.** Stability of the dyes (A) and hybrid compounds (B) in 0.5 mM glutathione in HEPES (pH 7.4) at 37 °C measured by HPLC at t = 6 hours. With Cy3-(SO<sub>3</sub>)Methyl-COOH and DTPA-Cy3-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] in orange, Cy5-(SO<sub>3</sub>)Methyl-COOH and DTPA-Cy5-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] in red, and Cy7-(SO<sub>3</sub>)Methyl-COOH and DTPA-Cy7-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] in blue.



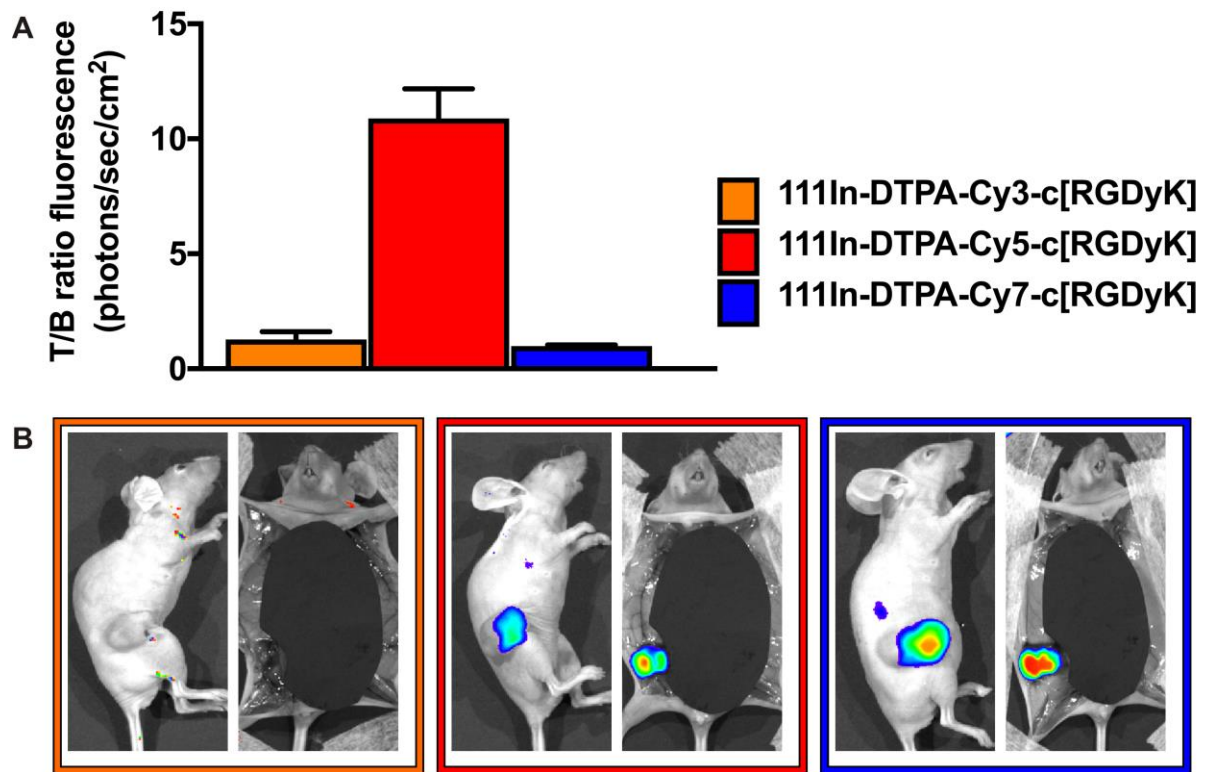
**Supplemental Fig. 6. Serum stability of hybrid constructs.** Assessment of stability using absorbance of (1 μM) of A) DTPA-Cy3-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] (orange), B) DTPA-Cy5-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] (red) and C) DTPA-Cy7-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] (blue) in bovine serum at 37 °C over time. B) Assessment of stability using fluorescence over time of D) DTPA-Cy3-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK], E) DTPA-Cy5-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] and F) DTPA-Cy7-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK].



**Supplemental Fig. 7.** UPLC chromatogram of DTPA-Cy7-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK]. A) PBS sample and B) filtrate after one time washing with water of the serum sample.



**Supplemental Fig. 8. Fluorescence confocal imaging.** Fluorescence confocal images of GE $\beta$ 3 cells after incubation with DTPA-Cy3-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] (DTPA-Cy3-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK]; in orange), DTPA-Cy5-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] (DTPA-Cy5-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK]; in red) or DTPA-Cy7-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] (DTPA-Cy7-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK]; in blue). Counterstainings were used to highlight the nuclei (white) and lysosomes (green). The overlay shows the localization of staining in the cells.



**Supplemental Fig. 9. In vivo fluorescence-based tumor-to-background ratios.** A) Analysis of the tumor-to-background (T/B) ratio of <sup>111</sup>In-DTPA-Cy3-c[RGDyK] (orange), <sup>111</sup>In-DTPA-Cy5-c[RGDyK] (red) and <sup>111</sup>In-DTPA-Cy7-c[RGDyK] (blue) based on fluorescence intensity signals (photons/sec/cm<sup>2</sup>) with B) corresponding fluorescence images obtained using an IVIS Spectrum.

**Supplemental Table 2. Relative scoring of DTPA-Cy3-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] to previously reported hybrid c[RGDyK] analogues studied under identical conditions.**

Cy7 versus c[RGDyK] Dyes FINAL	Blood	Brain	Lungs	Heart	Liver	Kidneys	Spleen	Stomach	Intestines	Tumor	Muscle (paw)	Fat (neck)	Mammary gland	Tumor:muscle	Tumor:Fat (neck)
DTPA-Ac-c[RGDyK]	0.427	0.341	0.469	0.422	0.844	0.382	0.569	0.411	0.636	0.418	0.338	0.533	0.355	1.148	0.798
DTPA-c[RGDyK]	0.461	0.435	0.620	0.639	0.874	0.401	0.624	0.711	0.789	0.496	0.508	0.669	0.429	0.937	0.721
DTPA-Cy5-Sulfonate-COOH-c[RGDyK]	0.929	0.901	1.430	1.344	1.166	1.455	1.244	1.075	1.253	1.025	1.004	1.073	0.701	0.948	0.912
DTPA-Cy5-Methyl-COOH-c[RGDyK]	1.182	0.892	2.010	2.182	3.402	1.416	1.754	1.448	1.979	1.195	1.180	1.803	0.997	0.950	0.642
DTPA-Cy5-QAmine-COOH-c[RGDyK]	0.499	0.502	1.041	0.933	4.344	0.906	0.989	0.940	0.847	0.755	0.657	0.799	0.559	1.069	0.895
DTPA-Cy5-(SO <sub>3</sub> )Sulfonate-COOH-c[RGDyK]	0.470	0.333	0.716	0.665	0.521	1.364	0.609	0.634	0.530	0.604	0.430	0.555	0.337	1.372	1.082
DTPA-Cy5-(SO <sub>3</sub> )Methyl-COOH-c[RGDyK]	0.520	0.246	0.515	0.406	0.385	0.513	0.329	0.559	0.462	0.640	0.321	0.346	0.202	1.912	1.890
DTPA-Cy5-(SO <sub>3</sub> )QAmine-COOH-c[RGDyK]	0.483	0.291	0.582	0.599	0.674	0.754	0.603	0.554	0.577	0.596	0.413	0.580	0.319	1.377	0.977
DTPA-Cy5-(SO <sub>3</sub> )Sulfonate-(SO <sub>3</sub> )COOH-c[RGDyK]	0.323	0.330	0.765	0.737	1.009	1.726	0.697	0.626	0.749	0.543	0.418	0.487	0.353	1.259	1.075
DTPA-Cy5-(SO <sub>3</sub> )Methyl-(SO <sub>3</sub> )COOH-c[RGDyK]	0.425	0.353	0.754	0.724	1.194	0.941	0.711	0.719	0.785	0.568	0.599	0.598	0.391	0.940	0.997
DTPA-Cy5-(SO <sub>3</sub> )QAmine-(SO <sub>3</sub> )COOH-c[RGDyK]	0.323	2.567	0.517	1.223	1.670	0.844	0.541	0.615	0.909	0.312	0.405	0.427	0.669	0.983	0.813
DTPA-Cy3-(SO <sub>3</sub> )Methyl-COOH-c[RGDyK]	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
DTPA-Cy7-(SO <sub>3</sub> )Methyl-COOH-c[RGDyK]	1.883	1.153	1.860	2.208	3.097	2.766	2.068	1.710	1.907	1.881	2.194	1.872	2.910	0.921	1.008

Favorable significant differences ( $p \leq 0.05$ ) in uptake were colored green, unfavorable significant changes were noted in red. Insignificant differences ( $p > 0.05$ ) from <sup>111</sup>In-DTPA-Cy5-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] were left uncolored.

**Supplemental Table 3. Relative scoring of DTPA-Cy7-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] to previously reported hybrid c[RGDyK] analogues studied under identical conditions .**

Cy7 versus c[RGDyK] Dyes FINAL	Blood	Brain	Lungs	Heart	Liver	Kidneys	Spleen	Stomach	Intestines	Tumor	Muscle (paw)	Fat (neck)	Mammary gland	Tumor:muscle	Tumor:Fat (neck)
DTPA-Ac-c[RGDyK]	0.227	0.296	0.252	0.191	0.273	0.138	0.275	0.240	0.333	0.222	0.154	0.285	0.122	1.247	0.792
DTPA-c[RGDyK]	0.245	0.377	0.333	0.290	0.282	0.145	0.302	0.416	0.414	0.264	0.231	0.357	0.148	1.017	0.715
DTPA-Cy5-Sulfonate-COOH-c[RGDyK]	0.493	0.781	0.768	0.609	0.377	0.526	0.601	0.629	0.657	0.545	0.457	0.573	0.241	1.029	0.904
DTPA-Cy5-Methyl-COOH-c[RGDyK]	0.628	0.774	1.081	0.988	2.713	0.512	0.848	0.847	1.037	0.635	0.538	0.963	0.343	1.032	0.636
DTPA-Cy5-QAmine-COOH-c[RGDyK]	0.265	0.435	0.560	0.423	1.403	0.327	0.478	0.550	0.444	0.402	0.300	0.427	0.192	1.161	0.888
DTPA-Cy5-(SO <sub>3</sub> )Sulfonate-COOH-c[RGDyK]	0.250	0.288	0.385	0.301	0.168	0.493	0.294	0.371	0.278	0.321	0.196	0.296	0.116	1.489	1.074
DTPA-Cy5-(SO <sub>3</sub> )Methyl-COOH-c[RGDyK]	0.276	0.214	0.277	0.184	0.124	0.186	0.159	0.327	0.242	0.340	0.146	0.185	0.069	2.075	1.875
DTPA-Cy5-(SO <sub>3</sub> )QAmine-COOH-c[RGDyK]	0.256	0.253	0.313	0.271	0.218	0.273	0.292	0.324	0.303	0.317	0.188	0.310	0.110	1.495	0.969
DTPA-Cy5-(SO <sub>3</sub> )Sulfonate-(SO <sub>3</sub> )COOH-c[RGDyK]	0.171	0.286	0.411	0.334	0.326	0.624	0.337	0.366	0.393	0.288	0.190	0.260	0.121	1.366	1.067
DTPA-Cy5-(SO <sub>3</sub> )Methyl-(SO <sub>3</sub> )COOH-c[RGDyK]	0.225	0.306	0.405	0.328	0.385	0.340	0.344	0.420	0.412	0.302	0.273	0.319	0.134	1.020	0.989
DTPA-Cy5-(SO <sub>3</sub> )QAmine-(SO <sub>3</sub> )COOH-c[RGDyK]	0.171	2.225	0.278	0.554	0.539	0.233	0.262	0.360	0.477	0.166	0.184	0.228	0.230	1.068	0.807
DTPA-Cy3-(SO <sub>3</sub> )Methyl-COOH-c[RGDyK]	0.531	0.867	0.538	0.453	0.323	0.362	0.484	0.585	0.524	0.532	0.456	0.534	0.344	1.086	0.992
DTPA-Cy7-(SO <sub>3</sub> )Methyl-COOH-c[RGDyK]	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Favorable significant differences ( $p \leq 0.05$ ) in uptake were colored green, unfavorable significant changes were noted in red. Insignificant differences ( $p > 0.05$ ) from <sup>111</sup>In-DTPA-Cy5-(SO<sub>3</sub>)Methyl-COOH-c[RGDyK] were left uncolored.