Supplementary Information

Lipoprotein interactions with water-soluble NIR-II emitting aza-BODIPYs boost the fluorescence signal and favor selective tumor targeting

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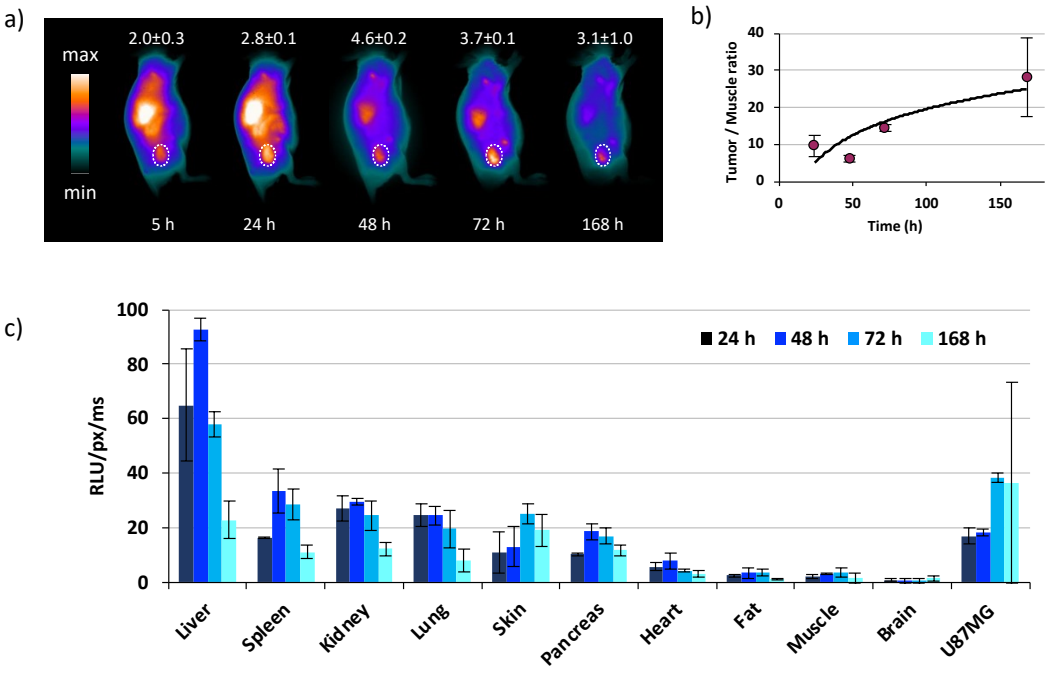


Figure S1: SWIR-WAZABY-01 NIR-II tumor-uptake capacity.
(a) NIR-II fluorescence imaging of a mouse bearing subcutaneous U-87 MG tumor, up to 7 days post SWIR-WAZABY-01 administration (n = 3 / condition). Tumor/Skin ratios are indicated on the top of the pictures, as the mean of fluorescence intensities ± standard deviation, for 3 animals. (b) Tumor/muscle ratios obtained from *ex-vivo* analyses respectively. (c) SWIR-WAZABY-01 biodistribution at different time points (n = 3 / condition). (Adapted with permission from *Kalot et al. Bioconjugate Chem 2020, copyright © 2020, American Chemical Society*)

Materials and Methods:

6 mice were implanted with U-87 MG cells orthotopically in their brain, according to the approved ethical guidelines and to the following procedure: anesthetized mice (isoflurane/air 4% for induction and 1.5% thereafter) were placed on a stereotaxic frame and 50 µL of bupivacaïne (5 mg/mL) were administered subcutaneously before scalp opening. The skull was revealed by gently removing the membranes (connective tissues) located on its external side. The point of needle injection was determined on the cranium surface (bregma 0, mediolateral 2 mm, dorso-ventral 3 mm), and a hole was drilled allowing the Hamilton syringe to be inserted (3 mm into the striatum). U-87 MG cells suspension ($1.0x10^5$ cells in 5 µL) was slowly pumped in (5 µL/min) using the needle, after which it was slowly removed (0.5 mm/30 sec). Finally, the opening was stoppered with Horsley wax and the skin was sutured. Following tumor growth, the mice were intravenously injected in their tail vein with 200 µL of 600 µM SWIR-WAZABY-01 solution diluted in PBS. *In vivo* NIR-II fluorescence imaging of the head was performed before and after SWIR-WAZABY-01 administration at 5, 24 and 48 h. Three mice were euthanized (at 24 and 48 h, respectively), and their brains were sampled for *ex-vivo* fluorescence imaging. Exposure time was optimized for each image acquisition. Acquired images were analyzed using Fiji software, and semi-quantitative fluorescence data were obtained by drawing regions of interest (ROI).

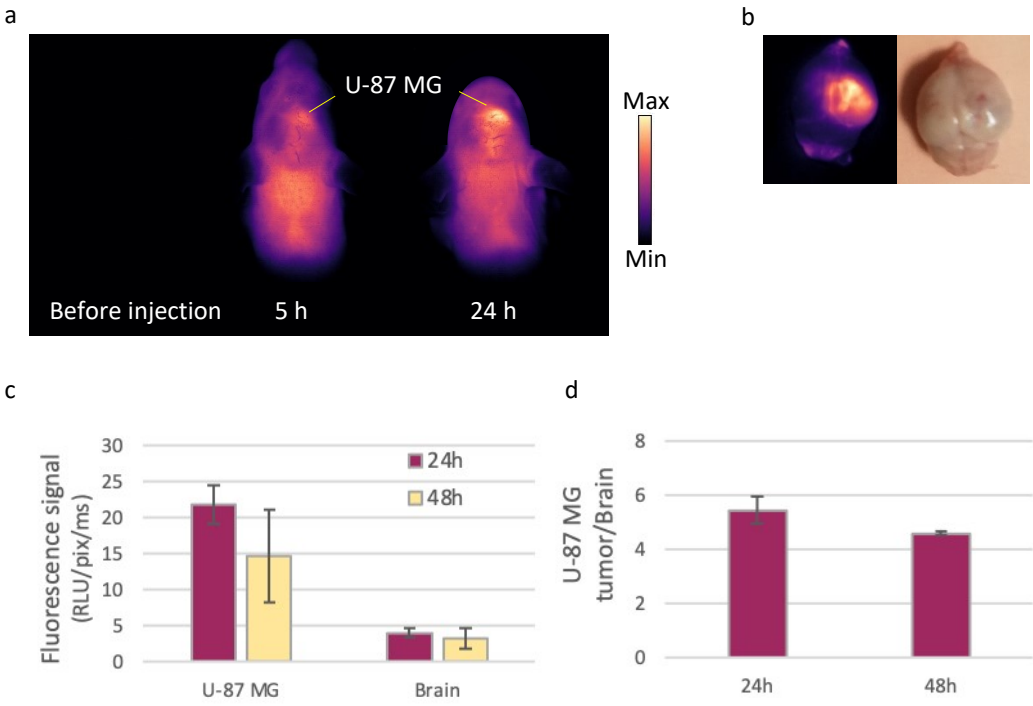


Figure S2: SWIR-WAZABY-01 accumulation in U-87 MG intracranial orthotopic tumors. (a) Example of in vivo NIR-II fluorescence imaging of mouse head, before, and 5 and 24 h post intravenous administration of SWIR-WAZABY-01 (200 µL at 600 µM). The mouse brain bears a U-87 MG tumor in the right hemisphere. (b) Ex-vivo NIR-II fluorescence image and bright field image of the excised brain of (a). (c) Quantification of U-87 MG tumor and brain fluorescence signal obtained from the NIR-II fluorescence images of dissected brains collected at 24 and 48 h. (d) U-87 MG Tumor/Brain ratios calculated from (c) data.

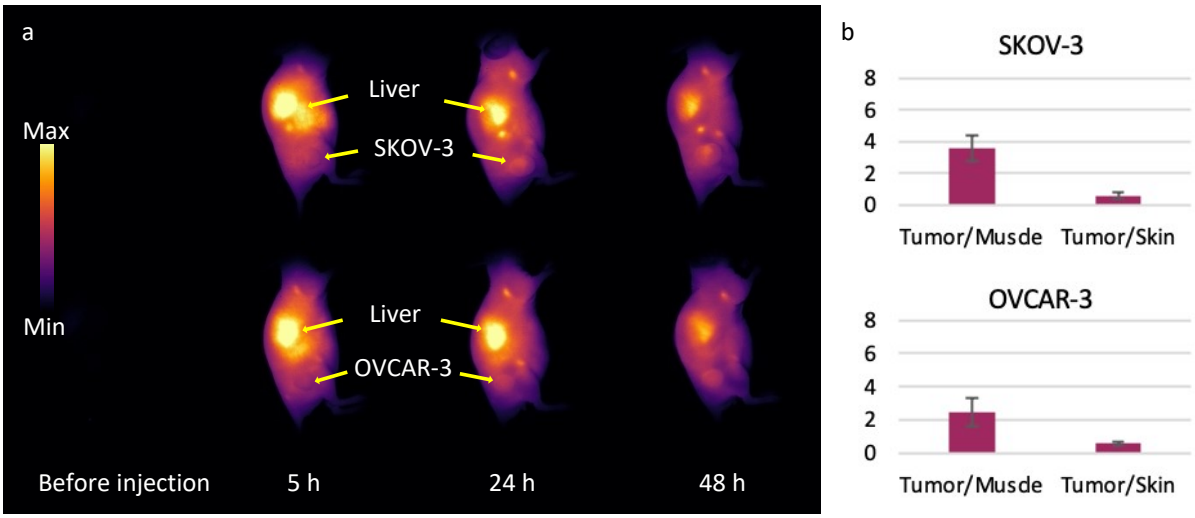
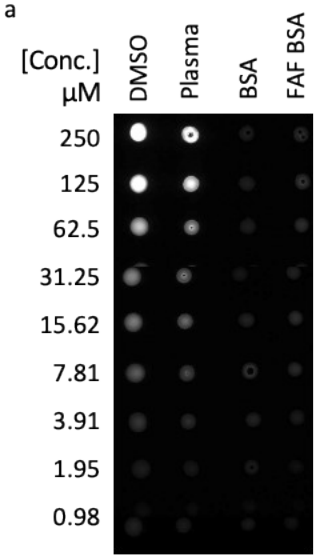
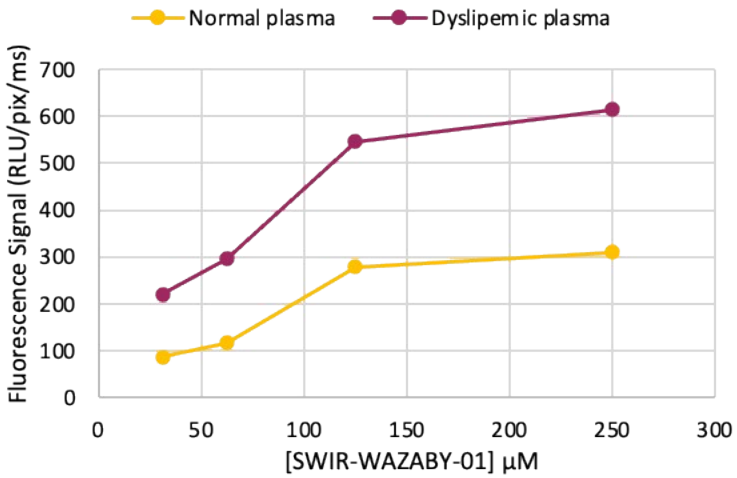


Figure S3: SWIR-WAZABY-01 tumor type-dependent accumulation. (a) *In vivo* NIR-II fluorescence imaging of mice bearing subcutaneous SKOV-3 and OVCAR-3 tumors before and 5, 24 and 48 h post intravenous administration of SWIR-WAZABY-01 (200 µL at 600 µM). Tumors and livers are indicated by yellow arrows. (b) *Ex-vivo* tumor/muscle and tumor/skin ratios calculated from excised organs at 48 h.

a



b



c

[SWIR-WAZABY-01] µM	SWIR-WAZABY-01 fluorescence signal (RLU/pix/ms)		Signal enhancement
	Normal plasma	Dyslipemic plasma	
250	309.9	614.7	1.98
125	278.1	545.2	1.96
62.5	116.7	296.2	2.54
31.25	87.0	221.1	2.54

d

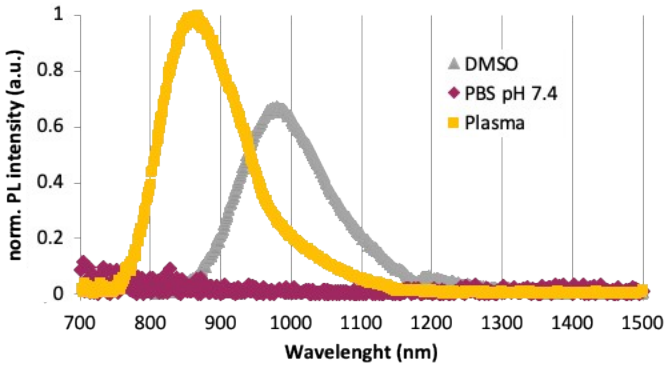


Figure S4: SWIR-WAZABY-01 solvent-dependent fluorescence. (a) Serial dilution of the SWIR-WAZABY-01 in DMSO, plasma, BSA 40 g/L, and fatty acid free (FAF)-BSA 40 g/L, showing that high fluorescence signals was observed in presence of DMSO and plasma (λ_{em} = 1,250 nm). (b) Graphical representation and (c) quantification of the SWIR-WAZABY-01 fluorescence signal following serial dilution in normal and dyslipemic plasma (from Figure 2b). (d) Normalized photoluminescence intensity of SWIR-WAZABY-01 in DMSO (grey), PBS (purple), and plasma (yellow), recorded after excitation at λ_{ex} = 808 nm.

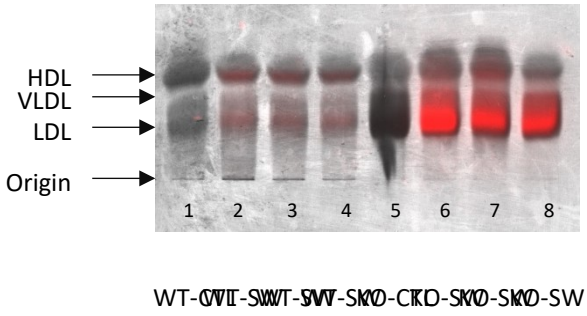


Figure S5: **Lipoprotein gel electrophoresis of mice plasmas.** Blood plasma of wild type (WT) and LDLR knockout (KO) mice was collected in control condition (CTL) or 30 min after i.v. administration of SWIR-WAZABY-01 (SW). Overlay of the SWIR-WAZABY-01 fluorescence image and black/white image of the lipoprotein electrophoresis gel. SWIR-WAZABY-01 fluorescence is designated by red color while the lipoprotein bands (LDL, VLDL, HDL) are detected in gray, thanks to Sudan Black staining. The electrophoresis indicated the absence of fluorescence for the control non-injected conditions, and a mild interaction of SW with lipoproteins of the WT mice. For KO animals, the fluorescence was strongly increased and a large part of the SW was colocalized with the LDL fraction.

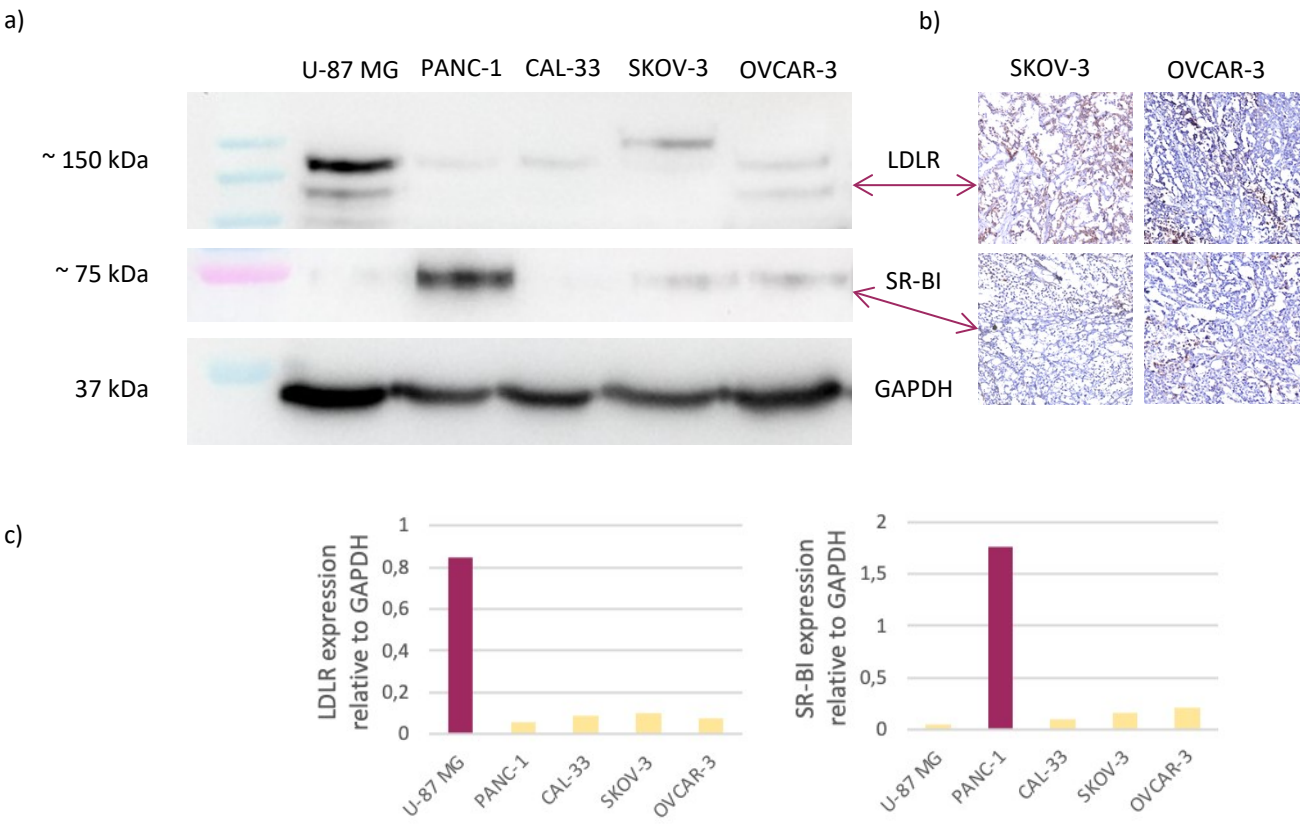


Figure S6: Lipoprotein receptors expression profile in tumor cells (a) and resected tumors (b). (a) Lipoprotein receptors (LDLR, SR-BI) expression in U-87 MG, PANC-1, CAL-33 cells, SKOV-3 and OVCAR-3 cells assessed by western blotting of whole cell extracts. GAPDH served as a loading control. (b) Histological sections of SKOV-3 and OVCAR-3 and their immunolabelling for LDLR and SB-BI. (c) Quantification of LDLR and SR-BI expression relative to GAPDH from (a) data.