Supporting Information

Carbonic Anhydrase IX-Targeted Near-Infrared Dye for Fluorescence Imaging of Hypoxic Tumors

Sakkarapalayam M. Mahalingam[1,2], Haiyan Chu[3], Xin Liu[1,2], Christopher P. Leamon[3] and Philip S. Low[1,2*]

[1]Department of Chemistry and [2]Institute for Drug Discovery, Purdue University, West Lafayette, Indiana 47907, United States.

[3]Endocyte Inc., 3000 Kent Avenue, West Lafayette, Indiana 47906, United States.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: plow@purdue.edu

ORCID

Philip S. Low: 0000-0001-9042-5528

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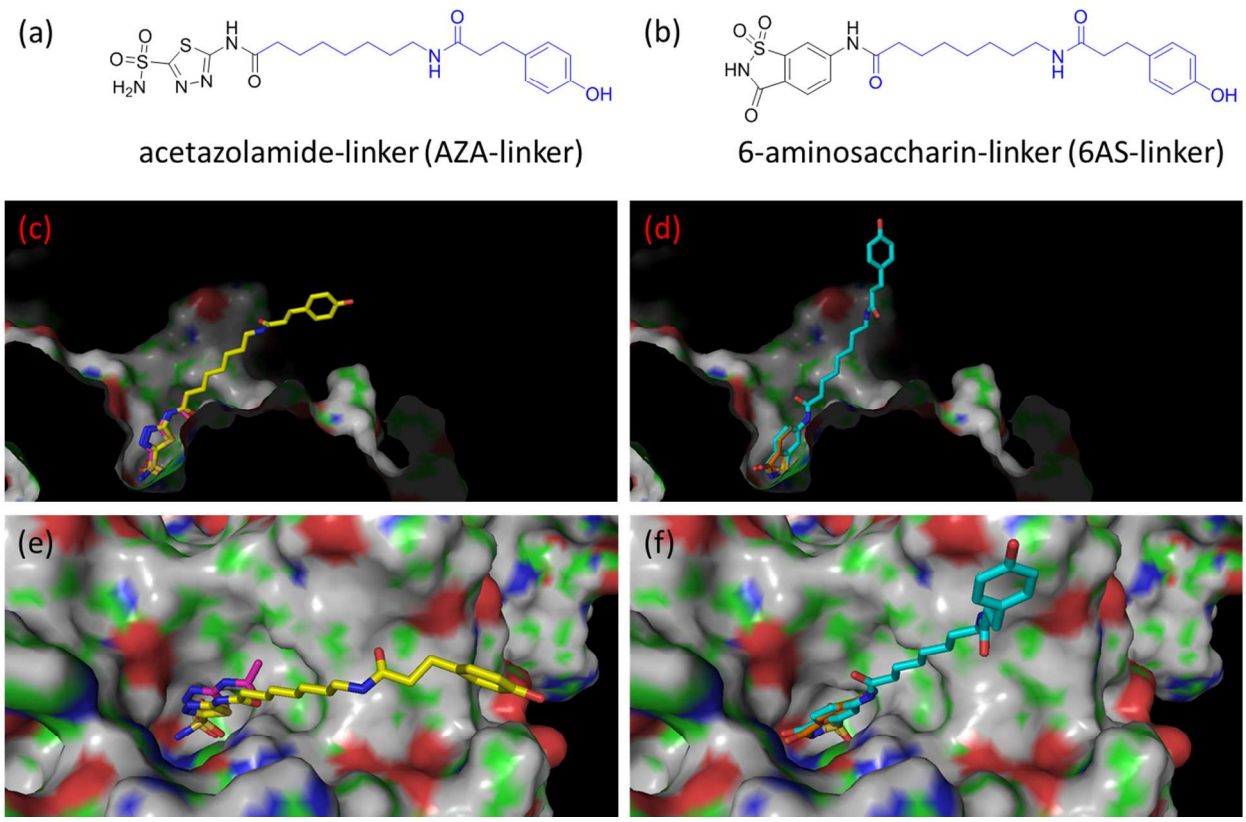
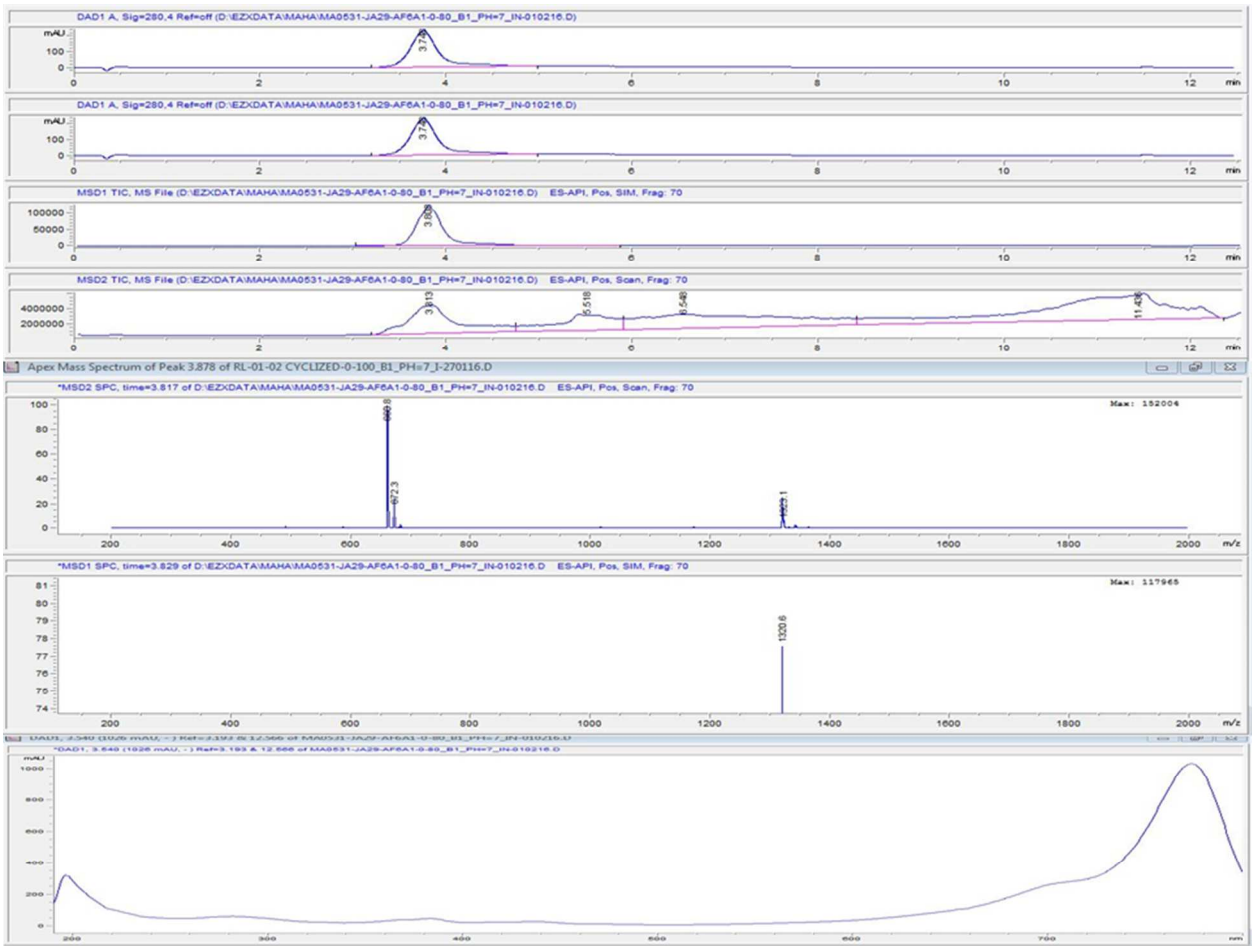
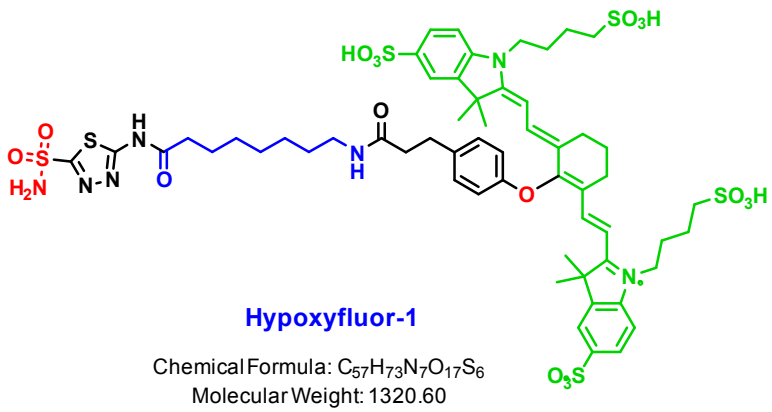


Figure S1. Chemical structures of (a) acetazolamide-linker (AZA-linker) and (b) 6-aminosaccharin-linker (6AS-linker). Molecular modeling of side view of CA IX binding pocket with (c) AZA-linker (yellow) and (d) 6AS-linker (cyan). Molecular modeling of top view of CA IX binding pocket with (e) AZA-linker (yellow) and (f) 6AS-linker (cyan). The figures are generated using PyMOL. The unconjugated ligand AZA (magenta) and 6AS (orange) are overlaid with AZA-linker and 6AS-linker, respectively.

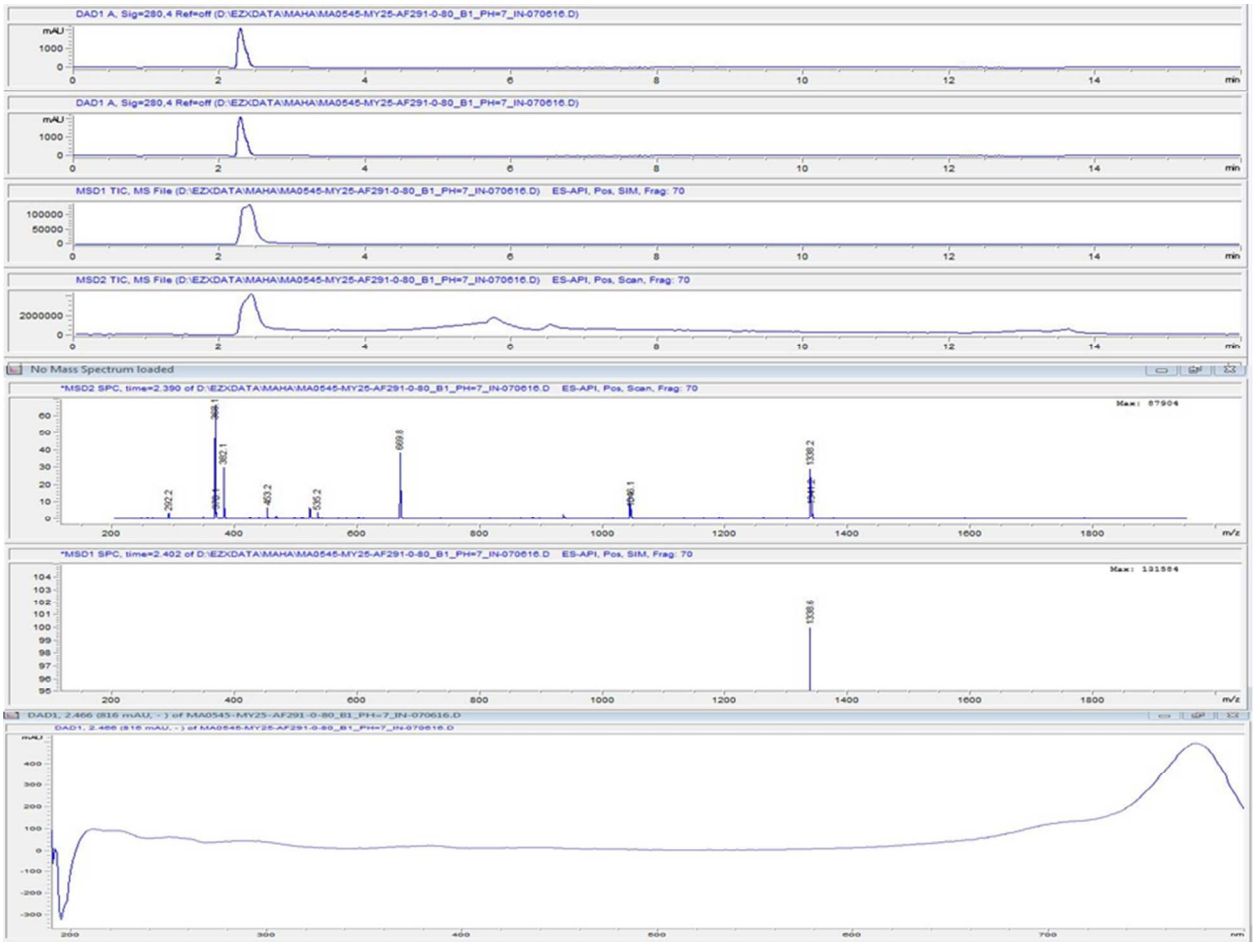


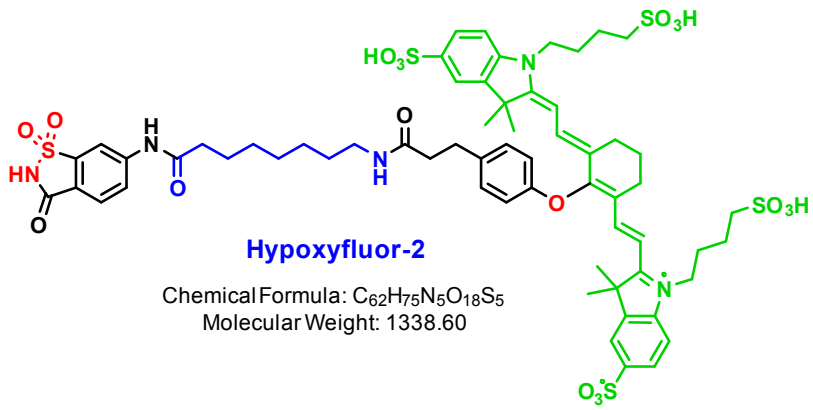


Hypoxyfluor-1

Chemical Formula: $C_{57}H_{73}N_7O_{17}S_6$
Molecular Weight: 1320.60

Figure S2. LC-MS and uv-vis characterization of Hypoxyfluor-1.





Hypoxyfluor-2

Chemical Formula: $C_{62}H_{75}N_5O_{18}S_5$
Molecular Weight: 1338.60

Figure S3. LC-MS and uv-vis characterization of Hypoxyfluor-2.

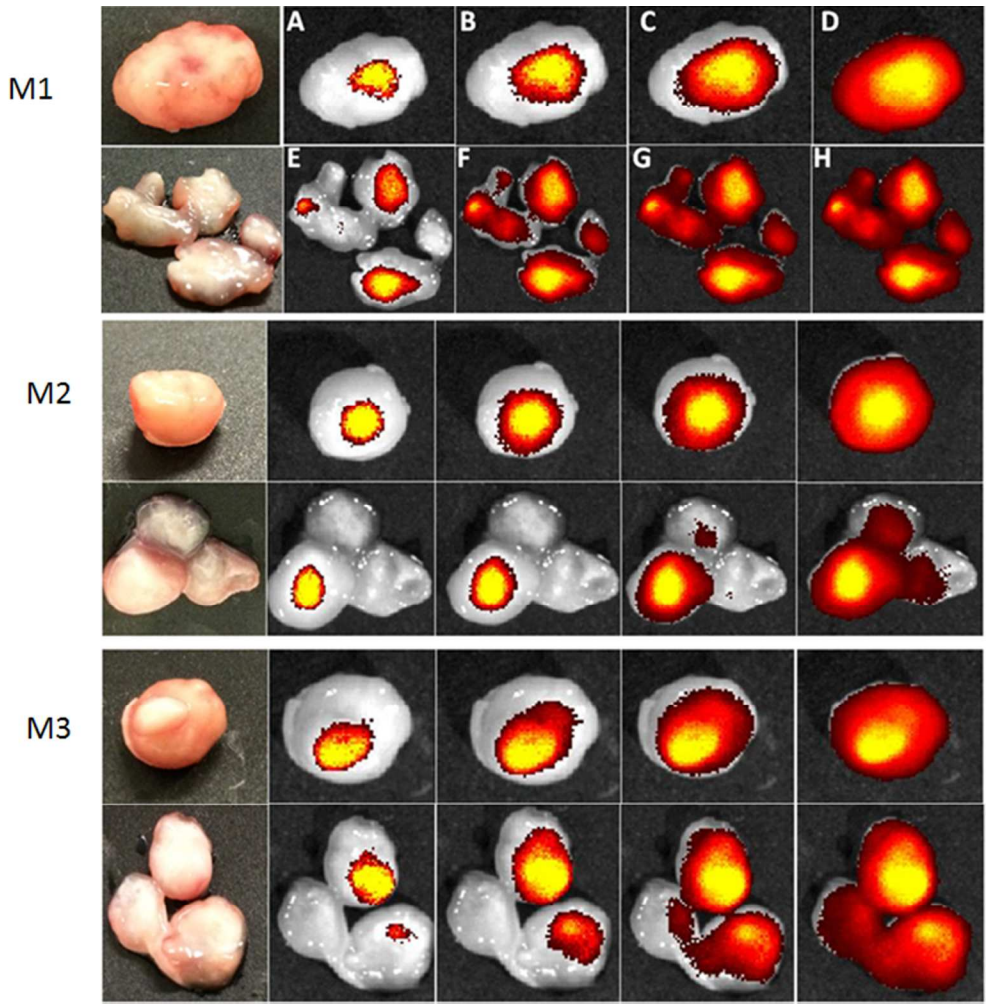


Figure S4. *Ex vivo* optical imaging of solid tumors in HypoxyFluor-1 treated tumor bearing mice. Mice bearing HT-29 tumors (CA IX-positive) were intravenously injected with 10 nmol HypoxyFluor-1 and euthanized and dissected 4 h post-injection. Fluorescence intensities of whole tumor (panels A-D) and tumor slices (E-H) were determined using a Lumina 2 Fluorescence Imager.

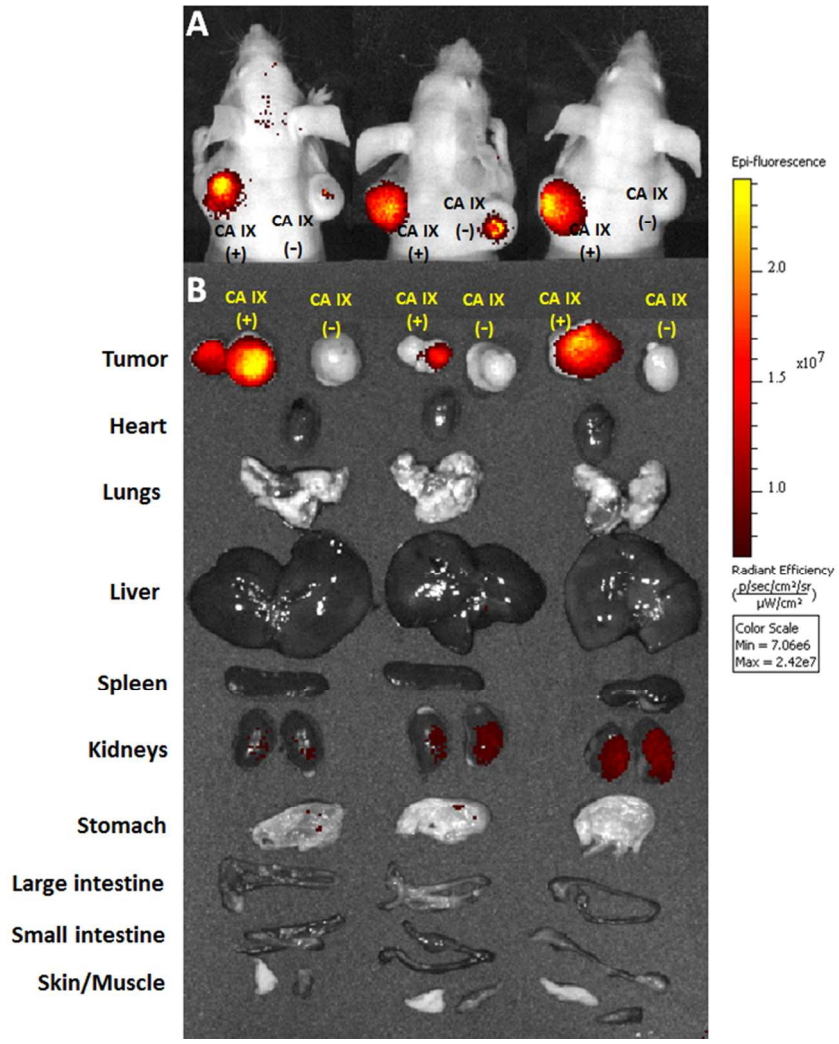


Figure S5. *In vivo* optical imaging of 10 nmol HypoxyFluor-2 treated mice. Mice bearing CA IX-positive HT-29 tumor on left and CA IX-negative MDA-MB-231 tumor on right shoulder were injected via tail vein with 10 nmol HypoxyFluor-2 and fluorescence images were acquired 4 h post-injection by i.v.

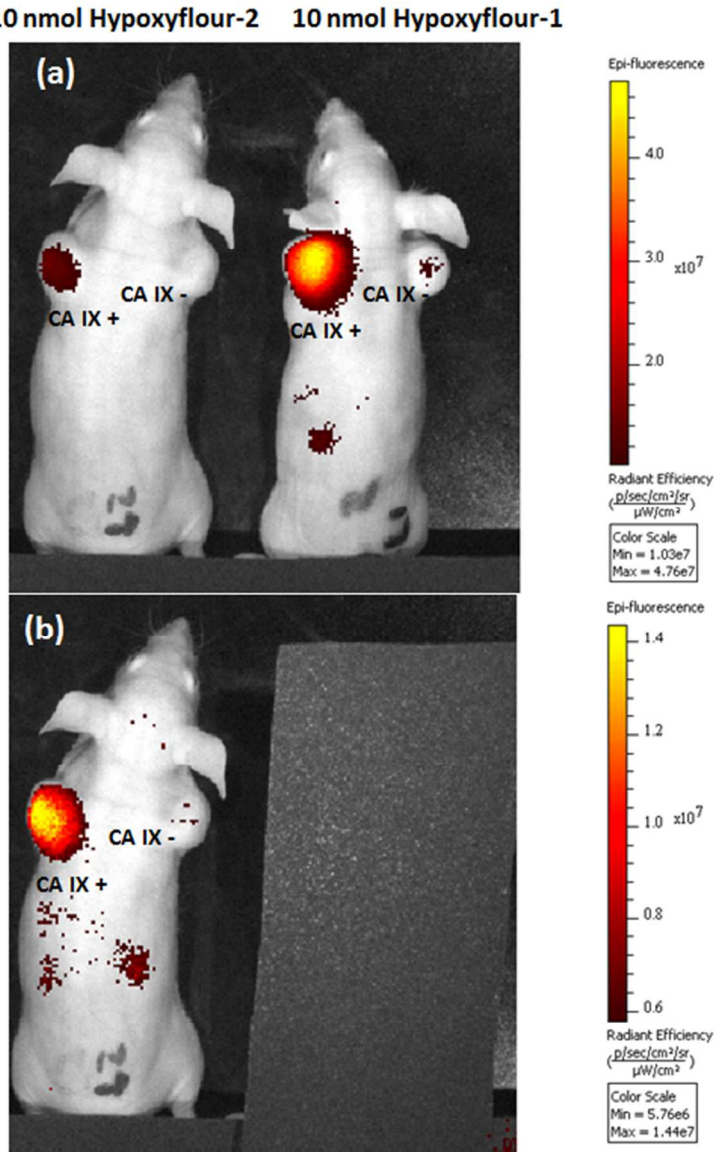


Figure S6. Representative images of HypoxyFluor-1 and HypoxyFluor-2 treated HT-29 tumor bearing mice. Mice bearing HT-29 tumors were injected via tail vein with 10 nmol HypoxyFluor-1 and HypoxyFluor-2 and fluorescence images were acquired 4 h post-injection by i.v.

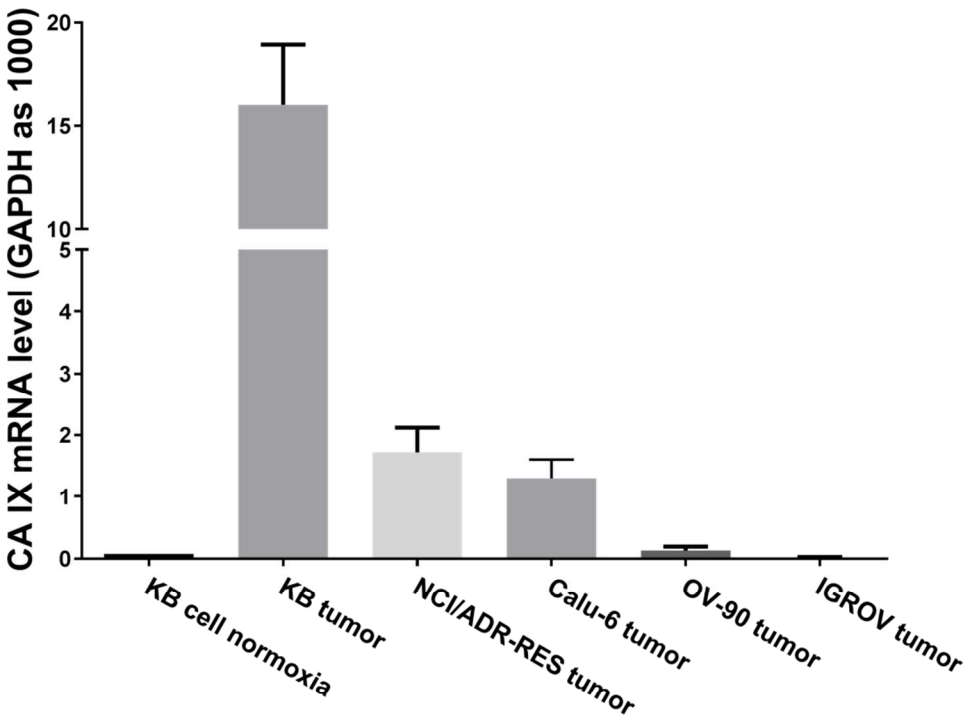


Figure S7. CA IX gene expression in human cancer cell lines and xenograft tumors. KB, Calu-6, IGROV, OV90, NCI/ADR-RES cancer cells were implanted subcutaneously in NOD-SCID mice. When tumor size reached 300-500 mm^3, the whole tumors were collected and CA IX expression in the tumors was analyzed as described in the Materials and Methods section.

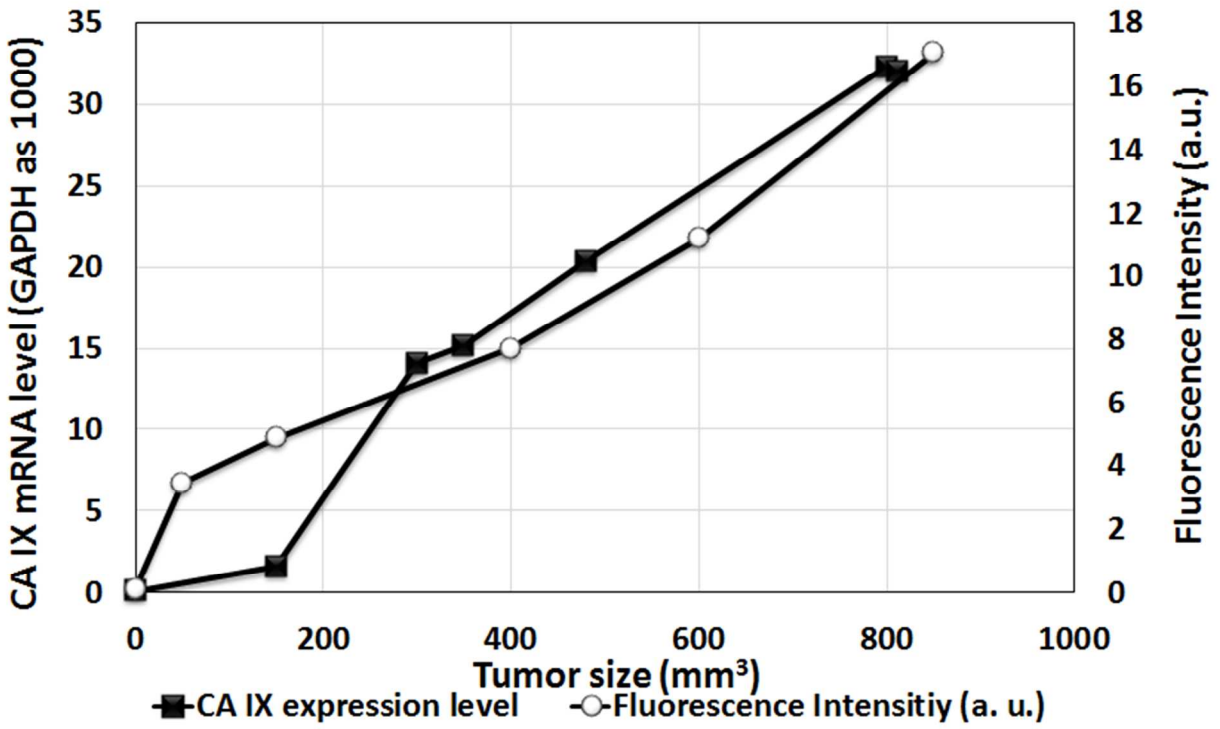


Figure S8. Effect of KB tumor size on both CA IX gene expression and HypoxyFluor-1 uptake 4h following intravenous injection of 10 nmol HypoxyFluor-1. KB cells were implanted subcutaneously in NOD-SCID mice and allowed to grow to the indicated sizes prior to resection and analysis for CA IX mRNA levels (solid squares) or HypoxyFluor-1 fluorescence (open circles).

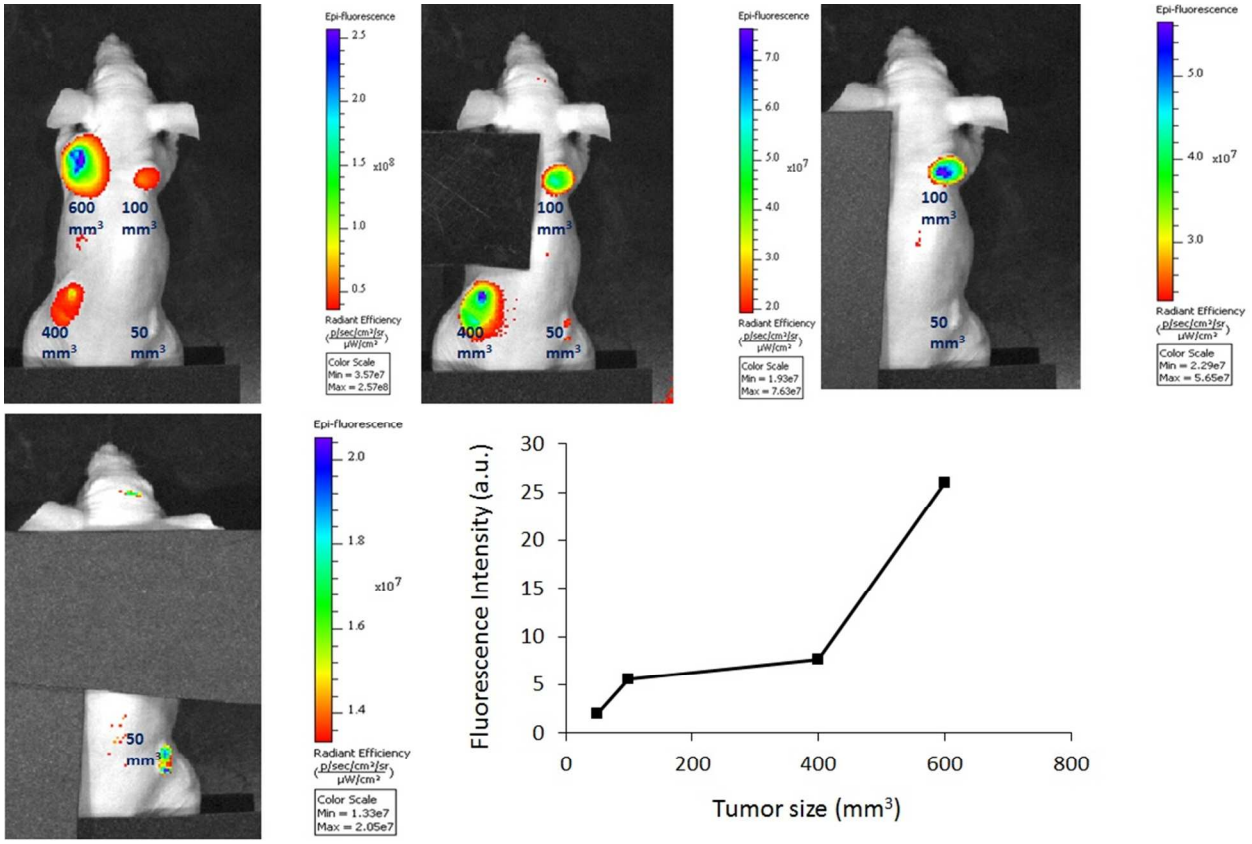


Figure S9. *In vivo* optical imaging of 10 nmol HypoxyFluor-1 treated mice. Mouse bearing different sizes of CA IX-positive KB tumors was injected via tail vein with 10 nmol HypoxyFluor-1 and fluorescence images were acquired 4 h post-injection by i.v.

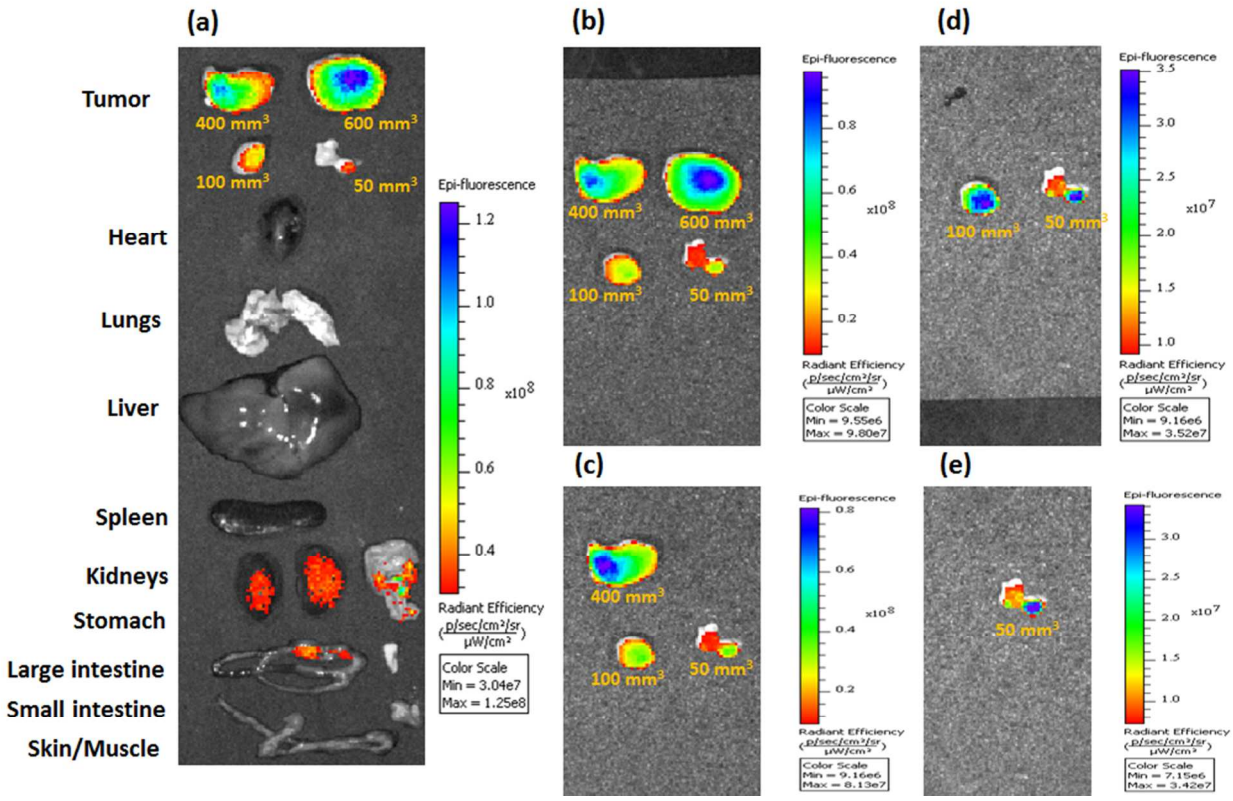


Figure S10. Accumulation of HypoxyFluor-1 in different sizes of KB tumors, internal tissues and organs of mice from Figure S7. Tissues and organs were excised after whole animal imaging and the fluorescence images of the isolated tissues were acquired.

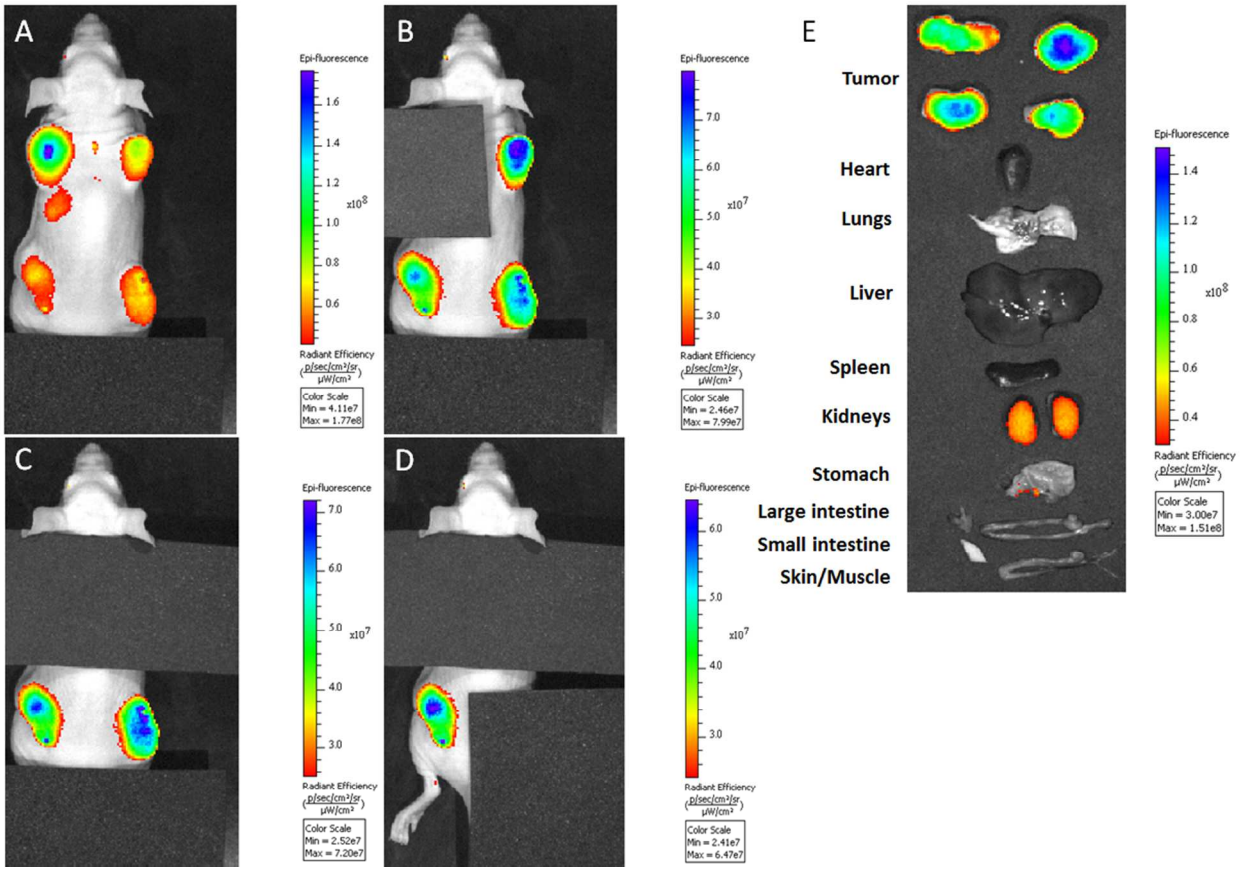


Figure S11. *In vivo* optical imaging of 10 nmol HypoxyFluor-1 treated mice. Mouse bearing different sizes of CA IX-positive KB tumors was injected via tail vein with 10 nmol HypoxyFluor-1 and fluorescence images were acquired 4 h post-injection by i.v. Tissues and organs were excised after whole animal imaging and the fluorescence images of the isolated tissues were acquired.

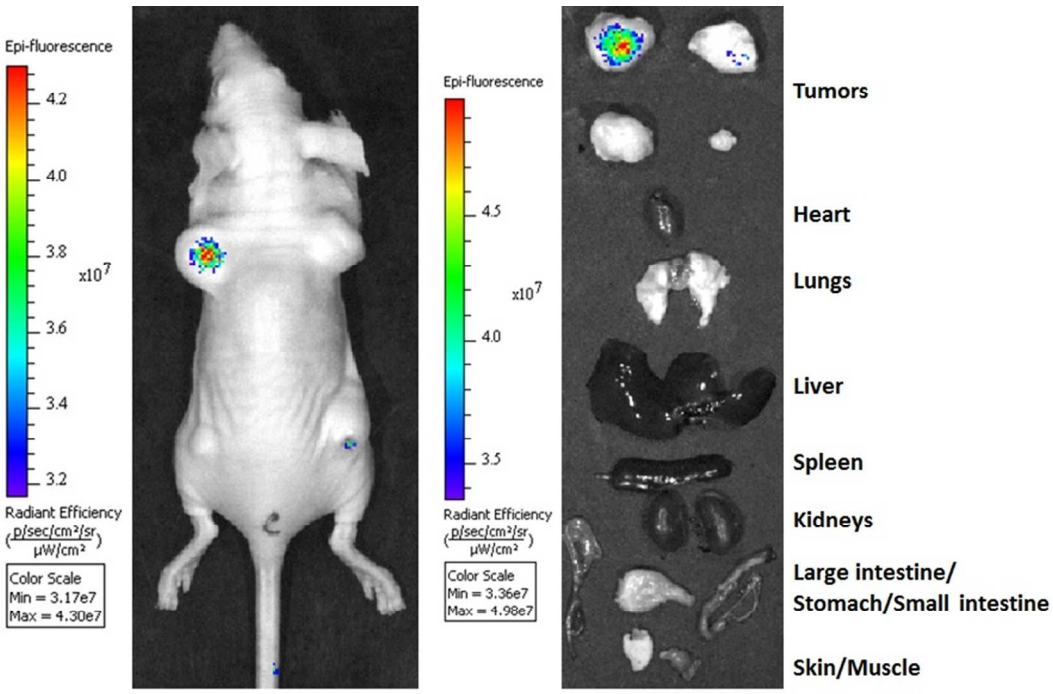


Figure S12. *In vivo* optical imaging of 10 nmol HypoxyFluor-1 treated mice. Mice bearing CA IX-positive KB tumors were injected via tail vein with 10 nmol HypoxyFluor-1 in combination with 100-fold excess CA IX inhibitor and fluorescence images were acquired 4 h post-injection by i.v.