

## SUPPORTING INFORMATION

### **Evaluation of a Centyrin-based Near-infrared Probe for Fluorescence-Guided Surgery of Epidermal Growth Factor Receptor Positive Tumors**

Sakkarapalayam M. Mahalingam<sup>1,2</sup>, Vadim Dudkin<sup>3</sup>, Shalom Goldberg<sup>3</sup>, Donna Klein<sup>3</sup>, Fang Yi<sup>3</sup>, Sunil Singhal<sup>4</sup>, Karyn T. O'Neil<sup>3\*</sup>, and Philip S. Low<sup>1,2\*</sup>

<sup>1</sup>Department of Chemistry and <sup>2</sup>Institute for Drug Discovery, Purdue University, West Lafayette, Indiana 47907, United States

<sup>3</sup>Janssen Research & Development, 1400 McKean Road, Springhouse PA 19477, United States

<sup>4</sup>Department of Surgery, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania 19104, United States

#### **AUTHOR INFORMATION**

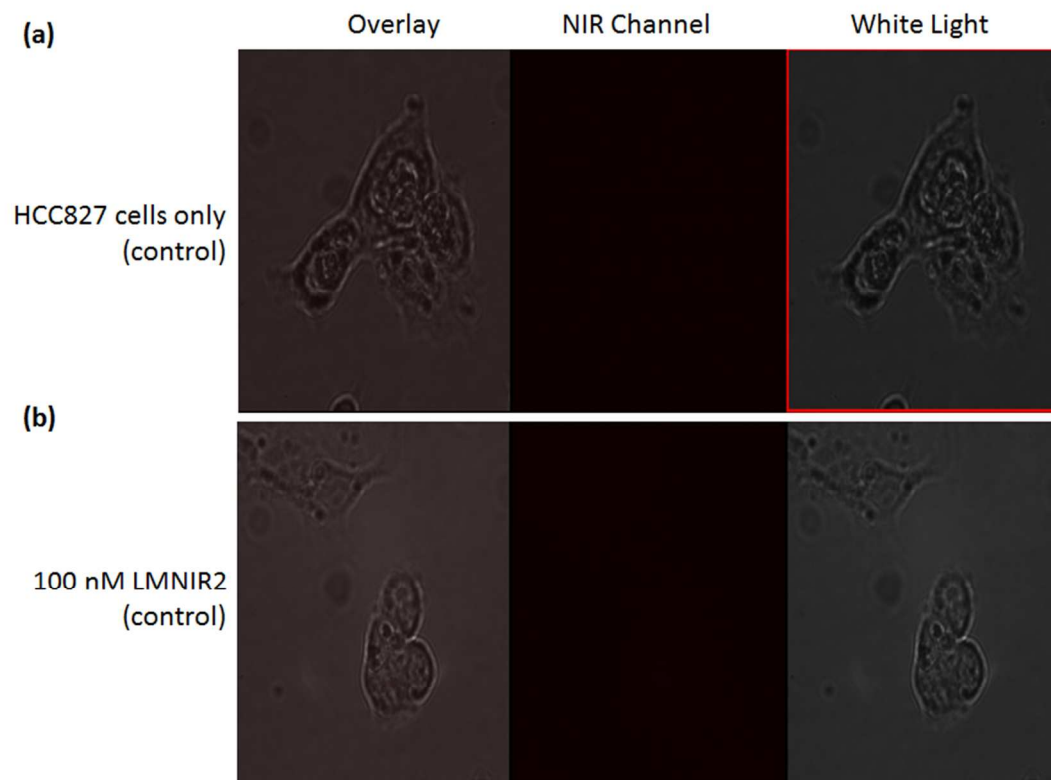
##### **Corresponding Authors**

\*E-mail: [koneil@its.jnj.com](mailto:koneil@its.jnj.com) ; E-mail: [plow@purdue.edu](mailto:plow@purdue.edu)

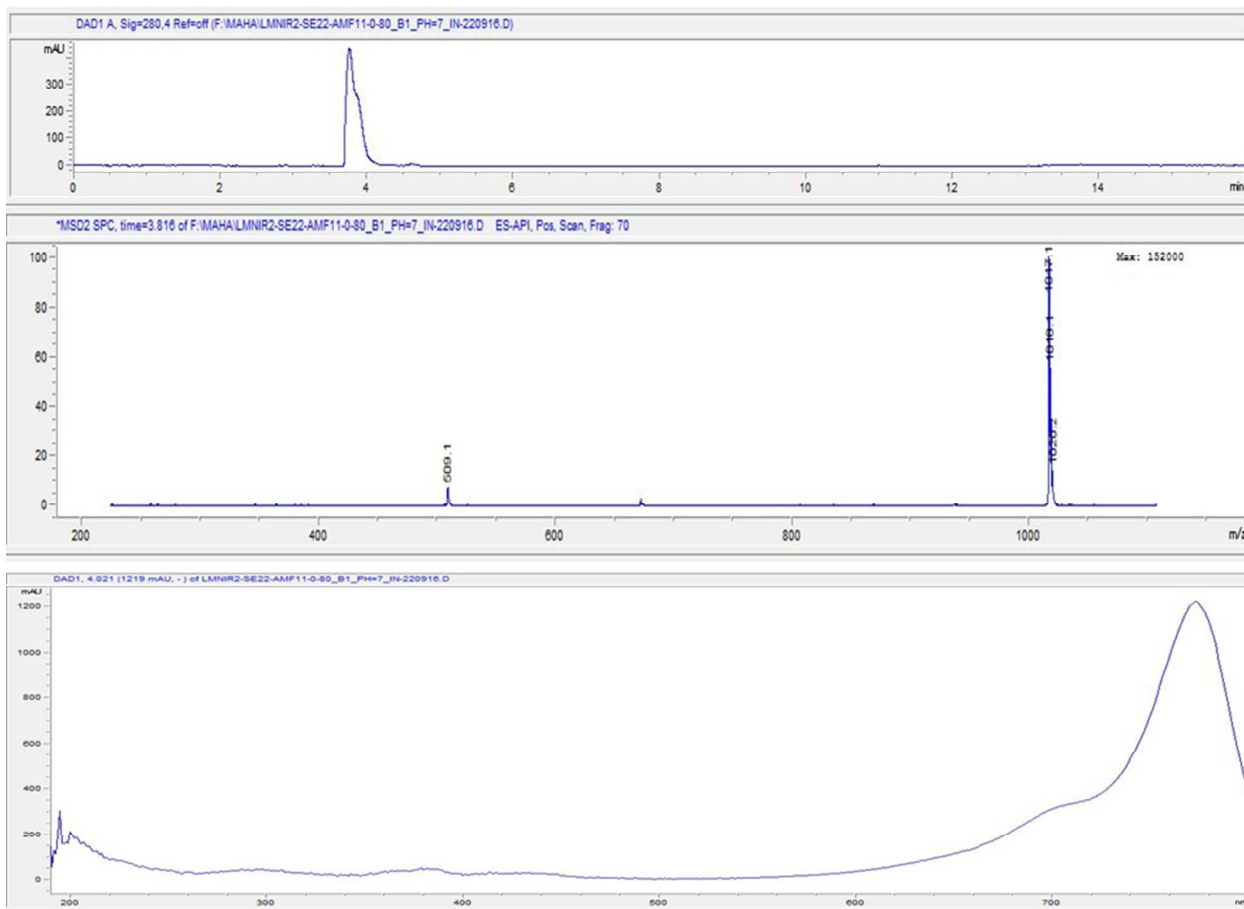
##### **ORCID**

Dr. Karyn T. O'Neil: 0000-0002-6490-5664

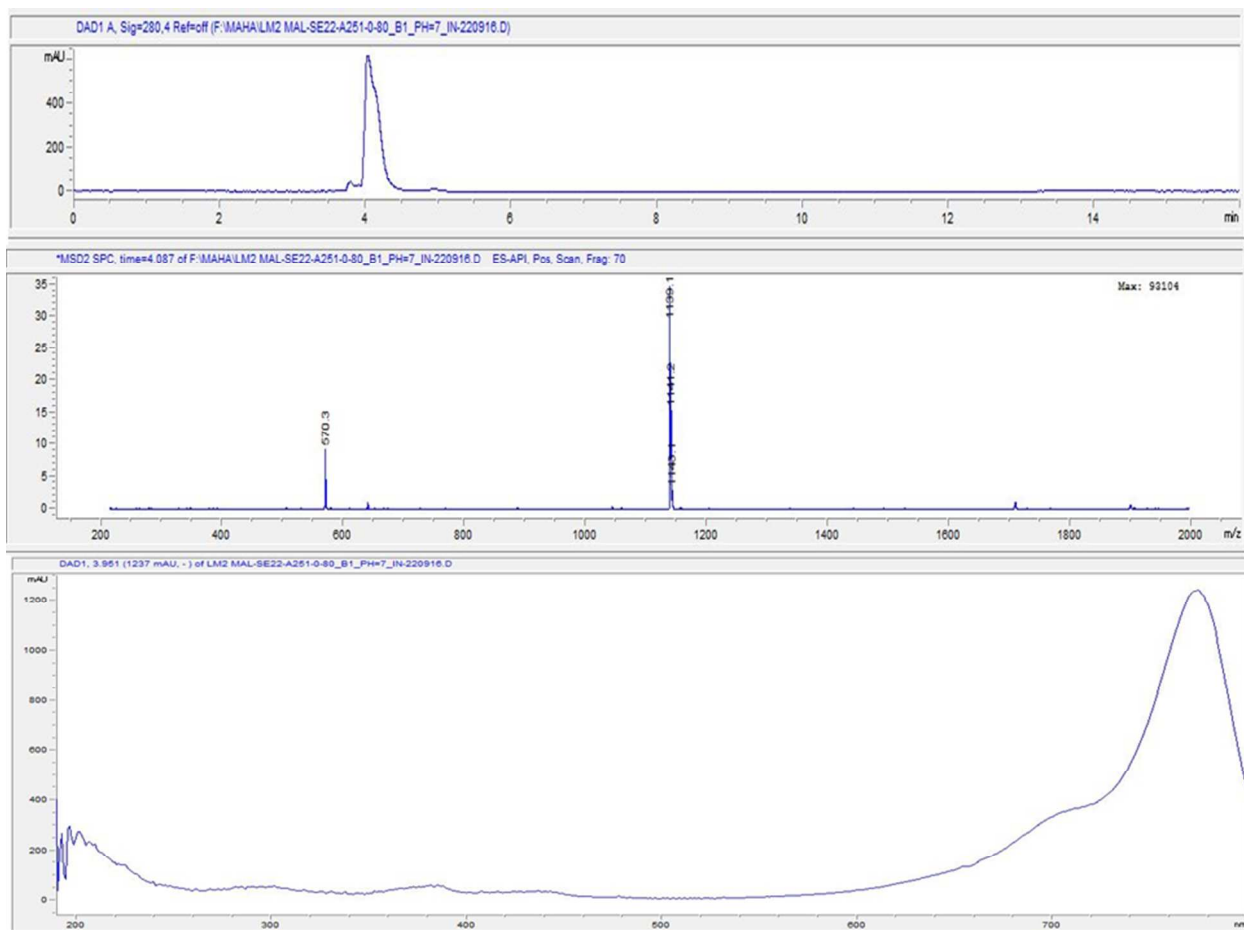
Philip S. Low: 0000-0001-9042-5528



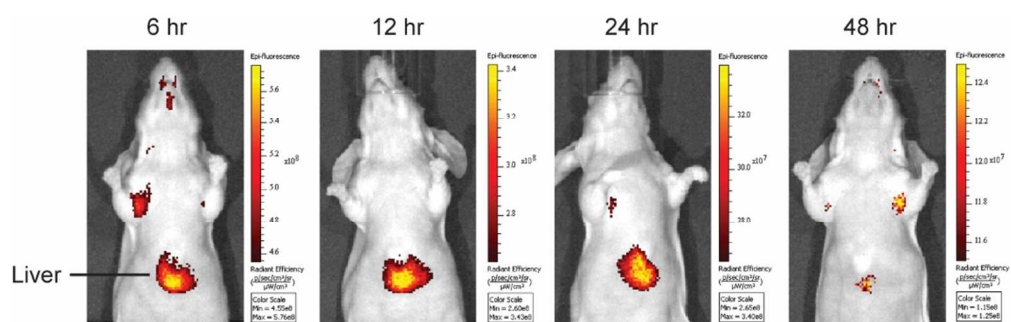
**Figure S1.** Fluorescence microscopy images of control experiments (a) untreated HCC827 cells only, and (b) HCC827 cells incubated with 100 nM LMNIR2 followed by the usual washing as performed in Fig.1.



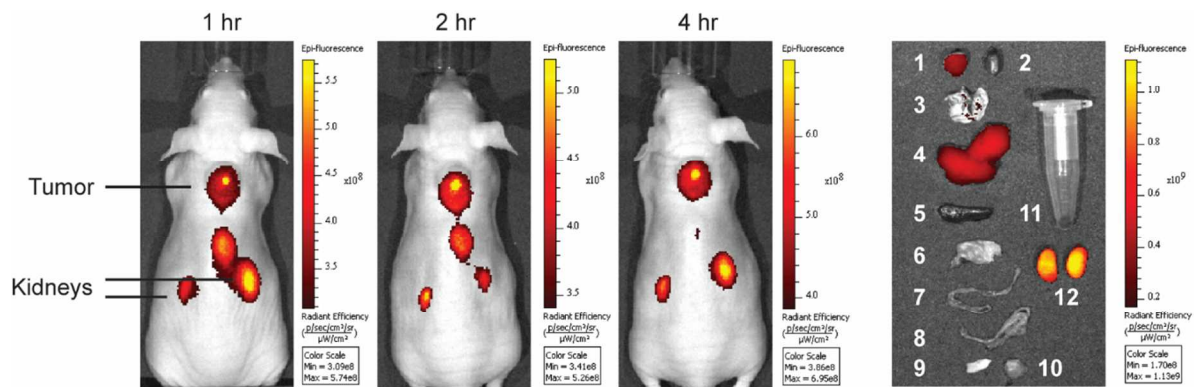
**Figure S2.** LC-MS and uv-vis characterization of LMNIR2.



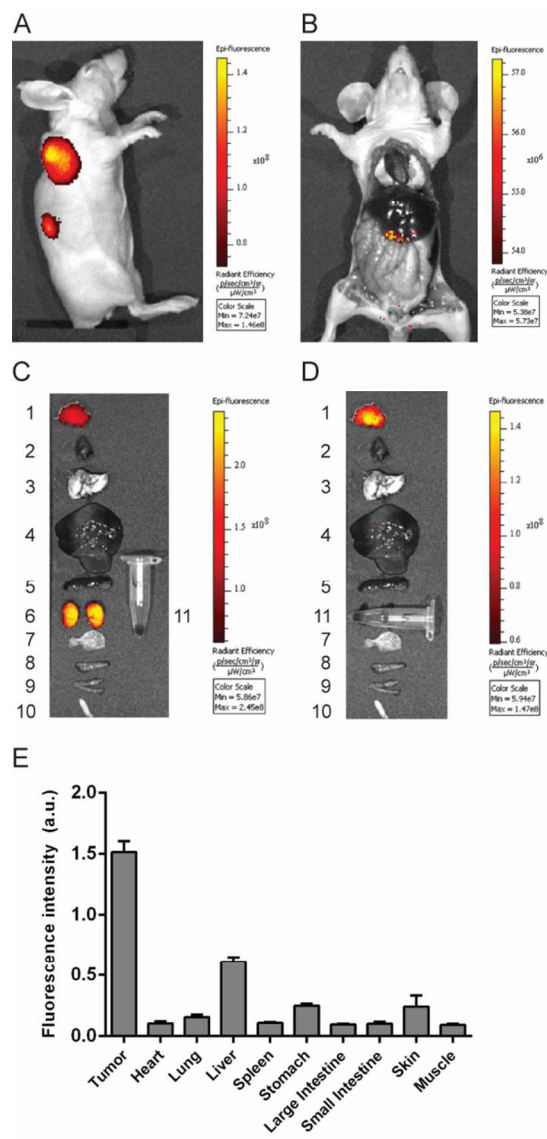
**Figure S3.** LC-MS and uv-vis characterization of LMNIR2-maleimide.



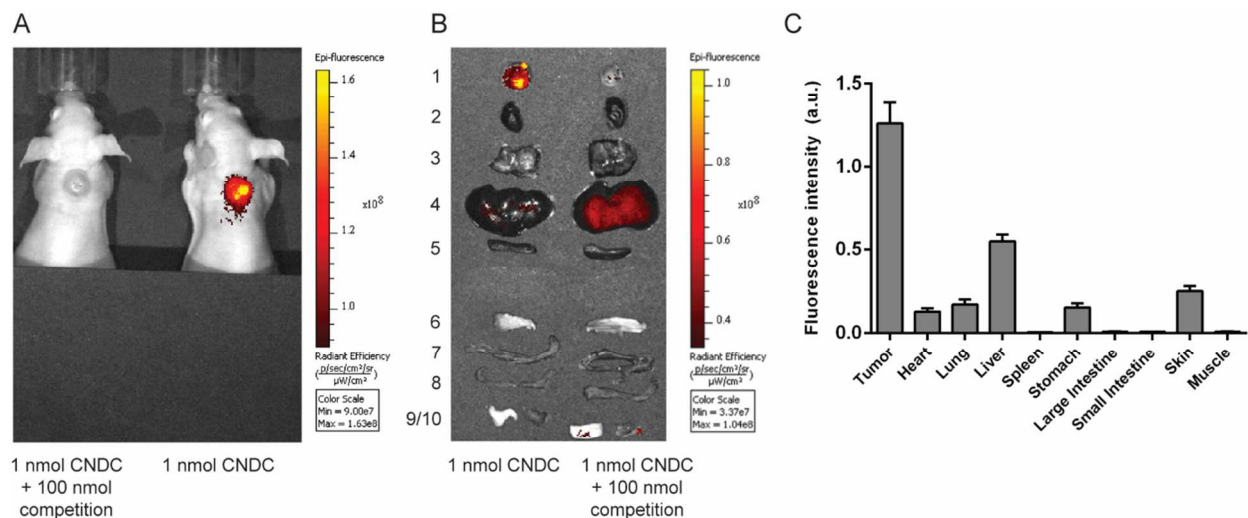
**Figure S4.** Analysis of liver clearance following tail vein injection of 10 nmol CNDC. Images were collected over a 48 h period with ventral exposure to facilitate liver imaging.



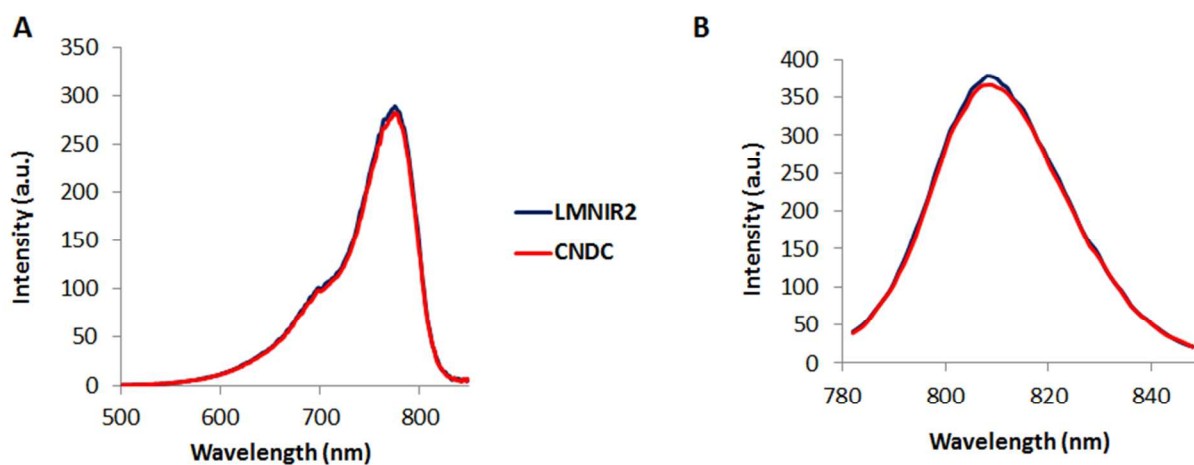
**Figure S5.** Representative images of HCC827 tumor bearing mice treated with 5 nmol CNDC. Mice were injected via tail vein with 5 nmol CNDC and fluorescence images were acquired over a 4 h period. Organs and tissues were dissected after whole animal imaging at 4 h and are labeled as follows: 1) tumor, 2) heart, 3) lungs, 4) liver, 5) spleen, 6) stomach, 7) small intestine, 8) large intestine, 9) skin, 10) muscle, 11) blood, 12) kidneys.



**Figure S6.** Imaging HCC827 tumor-bearing mice 24 h after a tail vein injection of 5 nmol of CNDC. Whole animal imaging to reveal **(A)** tumor and kidney uptake, and **(B)** liver uptake. Evaluation of internal CNDC accumulation in **(C)** all organs, **(D)** all organs excluding kidneys, and **(E)** quantification of the tissue fluorescence in **D** (data are mean  $\pm$  SD of  $n=3$ ). Dissected organs/tissues are labeled as follows: 1) tumor, 2) heart, 3) lungs, 4) liver, 5) spleen, 6) kidneys, 7) stomach, 8) small intestines, 9) large intestines, 10) skin, 11) blood.



**Figure S7. (A)** Representative images of HCC827 tumor-bearing mice treated with 1 nmol CNDC with or without competition. Mice were injected via tail vein with 1 nmol CNDC in the presence or absence of a 100-fold excess of 83v2Cys to block all vacant receptor binding sites. Fluorescence images were acquired 4 h post-injection. **(B)** Organs and tissues (excluding kidneys) were dissected after whole animal imaging at 4 h and are labeled as follows: 1) tumor, 2) heart, 3) lungs, 4) liver, 5) spleen, 6) stomach, 7) small intestine, 8) large intestine, 9) skin, 10) muscle. **(C)** Quantification of mean fluorescence intensity  $\pm$  SD for 3 mice.



**Figure S8. (A)** Excitation (B) emission spectra of a  $1\mu\text{M}$  solution of LMNIR2 (blue) and CNDC (red) in PBS.