Supplementary Materials and Methods:

Generation, Validation and Characterization of LMD-PSMA cells.
LMD cells were isolated from lung metastasis derived MDA-MB-231 xenograft in a female NOD-SCID mouse (provided by S. Nimmagadda, JHU). LMD cells were repeatedly transduced with LV-PSMA, a VSV-G pseudotyped lentivirus encoding PSMA and packaged in 293T cells (provided by L. Huang and E. Jaffee, JHU) to generate LMD-PSMA cells.

Western Blot.
Cells were washed with 1x PBS and resuspended in RIPA lysis buffer (Sigma, St. Louis, MO. Cat# R0278-50ML) supplemented with protease inhibitor cocktail (Roche, Indianapolis, IN. Cat# 11836153001). Cell lysate was incubated on ice (30 minutes), centrifuged (10 minutes) at 4°C, and Total protein concentration measured using the Micro BCA Protein Assay Kit (Thermo Scientific, Rockford, IL. Cat# 23225) according to manufacturer's protocol. Equal amounts of proteins were separated by SDS–PAGE and transferred to a PVDF membrane. After blocking with 5% nonfat dry milk in PBS (1 hour) at room temperature (RT), the blot was incubated with primary antibody overnight at 4°C. The membrane was probed with anti-mouse HRP conjugated secondary antibody (1 hour), and developed using ECL plus (GE Healthcare, Pittsburgh, PA. Cat# RPN2133) detection reagent according to manufacturer's protocol.

ELISA/Reverse Westerns.
PSMA (positive)/(negative) cells were plated on 96 well plates and grown to 90% confluency, washed with 1xPBS and fixed with 4% Paraformaldehyde (30 minutes) at RT. After fixation, cells were washed with 1x PBS and blocked with 1% BSA in PBS (1 hour) at room temperature, incubated with primary antibody at 37^{o}C (1 hour), and probed with secondary antibody conjugated with HRP. ELISA substrate was added and absorbance measured (OD).

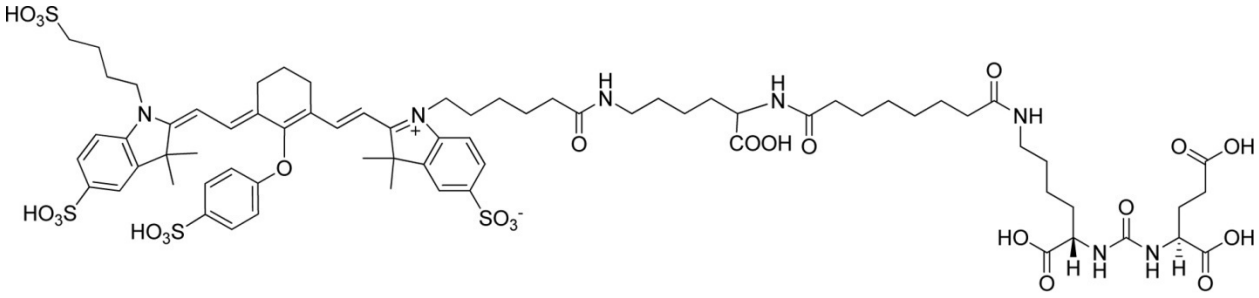
In vitro Cell Growth.
Cells were plated in 6 well plates in complete media and cell viability was quantified by cell count using the Countess (Invitrogen, NY. Cat# C10227). Cells were either re-plated to analyze if confluency may play a role on cell growth or re-seeded at a lower density to ensure cells were kept in log phase. No difference was observed in growth when cells were kept in log phase or grown to confluence.

Colony Formation Assay.
1000 cells were resuspended in 0.35% agarose solution in complete media and plated in a 6 well plate containing a base layer of 0.5% agarose. Plates were incubated at 37°C and 5% carbon dioxide (CO_2) and fresh media was added twice/week. After 24 days, plates were scored for colonies.

Wound Healing Assay.
Cells were plated in a 6 well plate and grown to 95% confluency, washed in PBS and a scratch was formed using a sterile 1000uL pipette tip. Fresh media was added and phase contrast images were captured 21h, 26h, 31h, and 43h post scratch along five sections of the scratch. Distance between the scratch was measured at three locations for each image.

YC-27 Synthesis.
The YC-27 compound was synthesized as described in the Chen, Y., *et al.* A low molecular weight PSMA-based fluorescent imaging agent for cancer, 2009 Biochemical and biophysical research communications paper. This compound is now commercially available as IRDye® 800CW YC-27 through LI-COR (Lincoln, NE. Cat# 926-27000), which is pending a toxicological study for an Investigational New Drug (IND) approval from the FDA. The absorbance maximum of the compound is 774 nm, and the emission maximum is 792 nm, which is unchanged in biological media.

Chemical structure of the YC-27 compound.



Primers used in amplifying mPSMA.
Intron/exon spanning primers that bind near the PSMA active site and amplify a 329 bp product.

Primer Name	Primer Sequence
PSMA F	5' CCTAACAAAAGAGCTGCAAAGCCCA 3'
PSMA R	5' CAAATACCATCGCTCCTCGAACCTG 3'

Serial dilution of PSMA positive cells to determine limit of detection.
PIP, Flu, LMD-PSMA, LMD and LNCaP cells were harvested and washed in KRB buffer (KRB buffer pH 7.4, Sigma-Aldrich, St. Louis, MO. Cat# K4002-10X1L). 5E6 cells in 1ml were incubated in 1 nM YC-27 (17) in KRB buffer at 37°C for 30 min with rotation. Cells were washed 3x with KRB. Two fold dilutions of cells starting at 400,000 cells/well were plated in a flat bottom 96 well plate in duplicate. The plate was imaged with the Classic Odyssey® scanner (Li-Cor, Lincoln, NE) and the Pearl® Impulse imager (Li-COR, Lincoln, NE) at 85 and 170 micron resolution respectively.

Supplementary Table:

		Animals with PSM detected after surgery	
		LumiNIR™ Guided	**White light Guided**
Immediate post surgery	Imaged by PEARL®	0/8 (0%)	4/10 (40%)
	Palpable tumor	0/8 (0%)	0/10 (0%)
7 Days post surgery	Imaged by PEARL®	0/8 (0%)	4/10 (40%)
	Palpable tumor	0/8 (0%)	1/10 (10%)
14 Days post surgery	Imaged by PEARL®	0/8 (0%)	4/10 (40%)
	Palpable tumor	0/8 (0%)	2/10 (20%)
21 Days post surgery	Imaged by PEARL®	0/8 (0%)	4/10 (40%)
	Palpable tumor	0/8 (0%)	4/10 (40%)
30 Days post surgery	Imaged by PEARL®	0/8 (0%)	4/10 (40%)
	Palpable tumor	0/8 (0%)	4/10 (40%)

Supplementary Table S1. Residual tumor post surgery as determined by imaging and palpation.
Aathymic Nu/Nu mice harboring PSMA-positive xenografts were administered 19.1 μg/kg (HED of 100 μg for a 70 Kg male) YC-27 via tail vein injection. In a subset of the animals (8) the tumors were resected with the guidance of LumiNIR™ system. In the rest of the animals (10) the tumors were resected under white light. All animals were imaged with the LumiNIR™ and the PEARL® immediate post surgery. The animals were also imaged 7, 14, 21 & 30 days post surgery, each time19.1 μg/kg YC-27 was administered via tail vein injection 6 hrs prior to imaging. The animals were also examined for palpable tumors.

Author contributions:
B.P.N. and J.B.E. were primarily responsible for writing the manuscript, M.C. and W.H.C. helped to edit it. R.R. and M.G.P. oversaw the overall preparation. B.P.N., J.B.E., M.C., W.H.C., R.M., and R.R. helped in conducting the experiments. B.P.N. contributed to figures 2, 3, 4, 5, 6, S2, S7, S8, S9, S10, S11, S12, Supplementary Table 1, and supplementary movies 1, 2, 3. J.B.E. contributed to figures 1, 2, S2, S7, and Supplementary movie 1. M.C. contributed to figures 1, 2, 3, 4, 6, S2, S3, S4, S5, S6, S7, S9, S10, and Supplementary movies 2, 3. W.H.C. contributed to figures 1, 2, 3, 4, 5, 6, S1, S2, S5, S6, S7, S8, S9, S10, S11, S12, Supplementary Table 1, and Supplementary movies 1, 2, 3. A.M. contributed to Supplementary figure 3B, he also optimized the method of generating paired PSMA +/- tumors which were used in figures 3, S8. R.M. and R.R. contributed to figure 2 and Supplementary movies 2, 3. W.H.C. and M.C. analyzed, documented and generated figures for all the experiments with the help of B.P.N. W.H.C. and M.C. developed the light source "LumiNIR", which was named by B.P.N. Y.C. and R.C.M. developed and synthesized the compound, YC-27, in M.G.P.'s lab. S.E.L. contributed towards the experimental design and data analysis of the Supplementary figures 3, 4, and 5 which were conducted in his lab. All other experiments were conducted in R.R.'s lab. B.P.N., J.B.P., M.C., W.H.C., and R.R. helped in the experimental designs of those.

Competing interests:
Under a licensing agreement between LI-COR and the Johns Hopkins University, Drs. Pomper, Chen, and Mease are entitled to a share of royalty received by the University on sales of products described in this article. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies. All other authors declare that they have no competing interests.