

1 *Supporting Information*

2 **Tuning Pharmacokinetics to Improve Tumor Accumulation of a PSMA-**
3 **Targeted Phototheranostic Agent**

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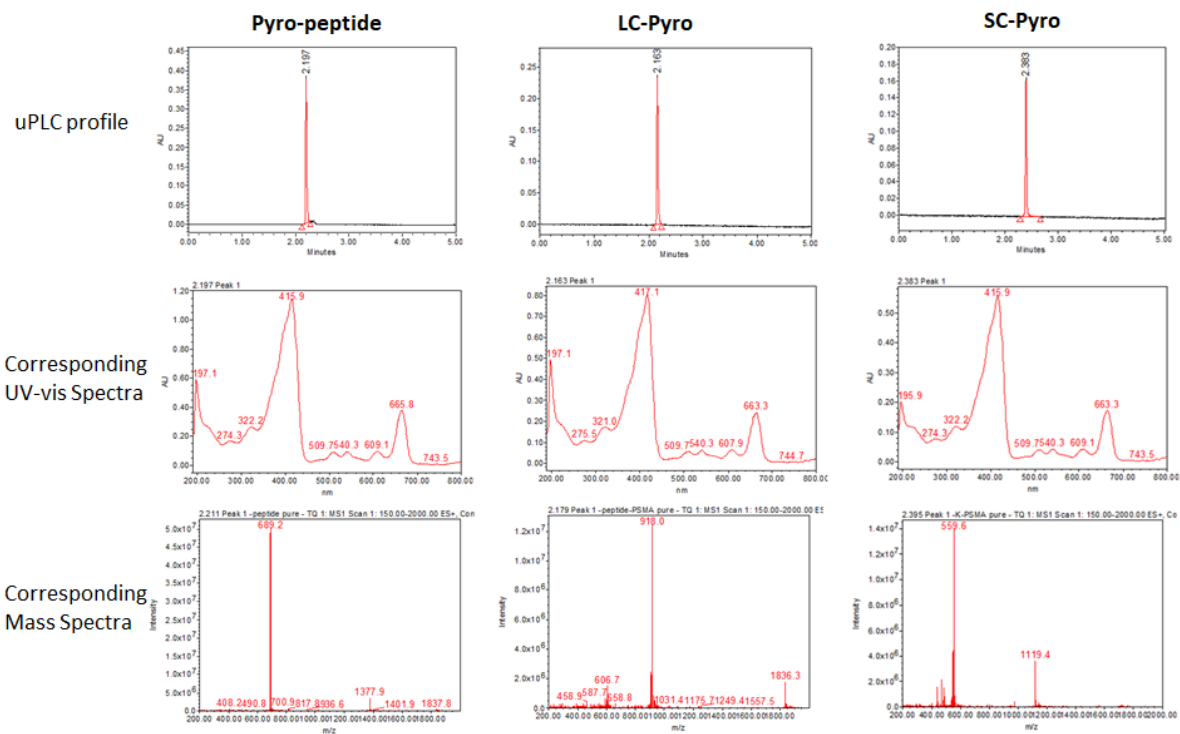
28 **Figure S6.** PET/CT image of ⁶⁴Cu-labeled LC-Pyro accumulation in mice bearing an orthotopic
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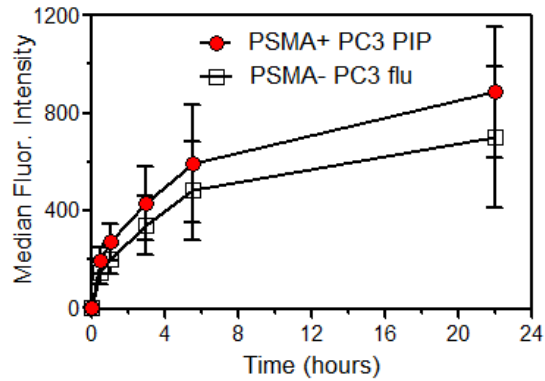
33 **Figure S8.** Immunohistochemical validation of PSMA expression in PSMA+ PC3 PIP tumors
34 post-PDT treatment.

35 **Figure S9.** Blood clearance profile of YC-9.



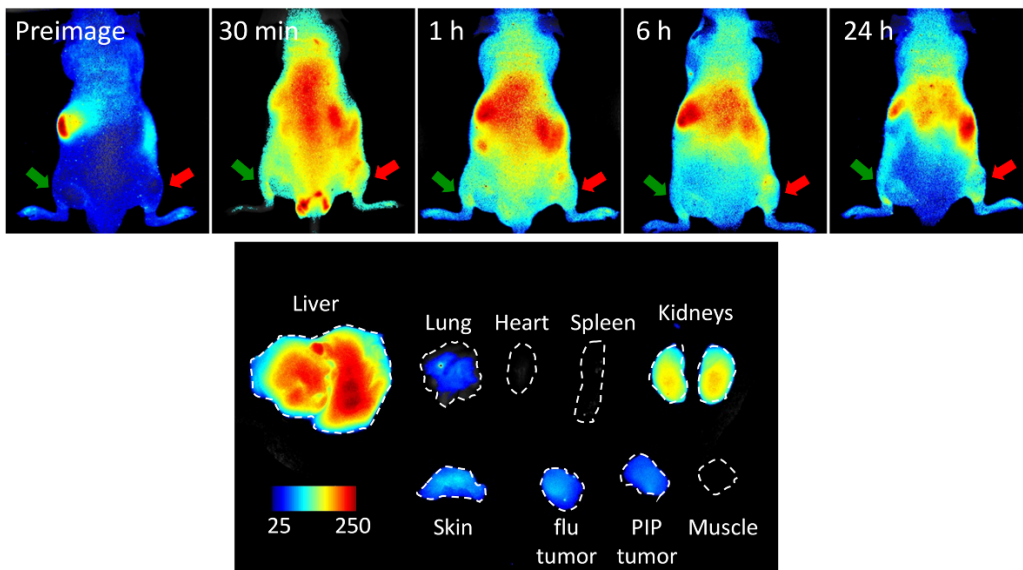
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37 **Figure S1.** Chemical characterization of Pyro-peptide, LC-Pyro and SC-Pyro; uPLC retention
 38 profile (Top); Corresponding UV-vis absorbance spectra (Middle) and mass spectra (Bottom).



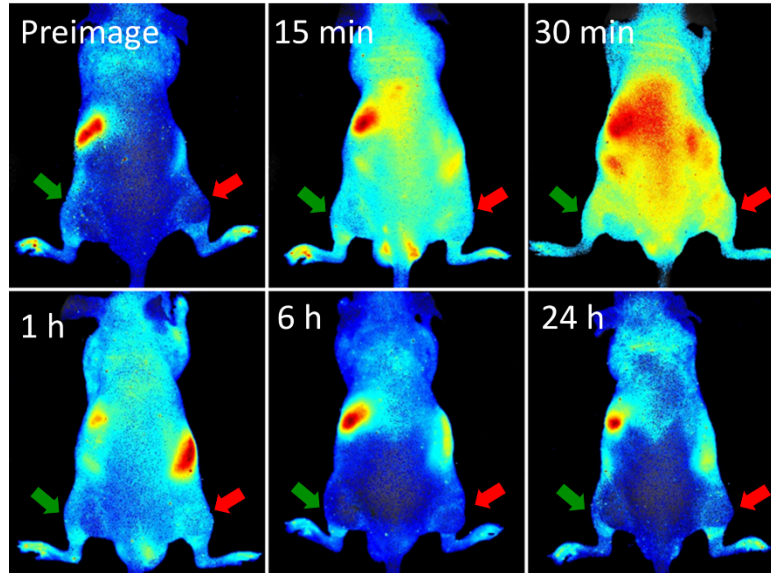
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40 **Figure S2.** Flow cytometry time-dependent LC-Pyro uptake in PSMA+ PC3 PIP and PSMA-
 41 PC3 flu cell lines. Each point represents the median fluorescence intensity \pm the SD of three
 42 independent measurements.



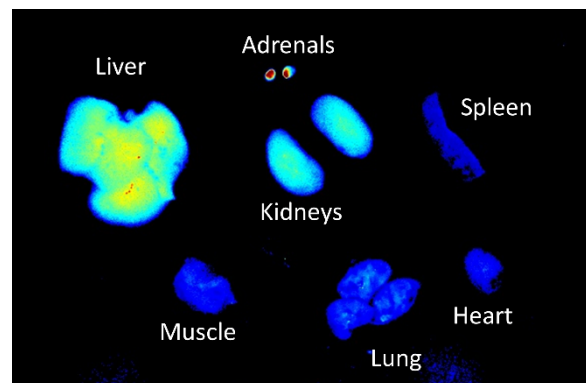
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44 **Figure S3.** Representative fluorescence images of a mouse bearing dual PSMA+ PC3 PIP (red
 45 arrow) and PSMA- PC3 flu (green arrow) tumors that were intravenously injected with an LC-
 46 Pyro derivative unconjugated to the PSMA small molecule affinity ligand (20 nmol). Mice were
 47 imaged at 0, 30 min, 1 h, 6 h and 24 h post-intravenous injection and major organs were excised
 48 and imaged *ex vivo*; n = 3.



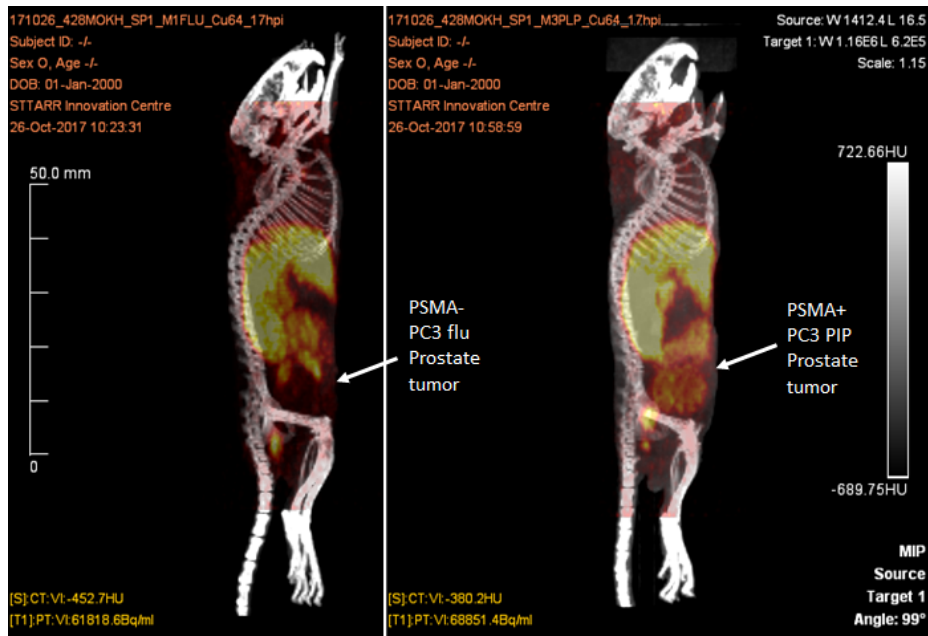
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50 **Figure S4.** Representative fluorescence images of a mouse bearing dual PSMA+ PC3 PIP (red
 51 arrow) and PSMA- PC3 flu (green arrow) tumors that were intravenously injected with SC-Pyro
 52 (20 nmol) at 0, 15 min, 30 min, 1 h, 6 h and 24 h post-injection; n = 3.



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54 **Figure S5.** Fluorescence *ex vivo* biodistribution of major clearance organs in a mouse injected
 55 with LC-FITC 24 h post-injection.



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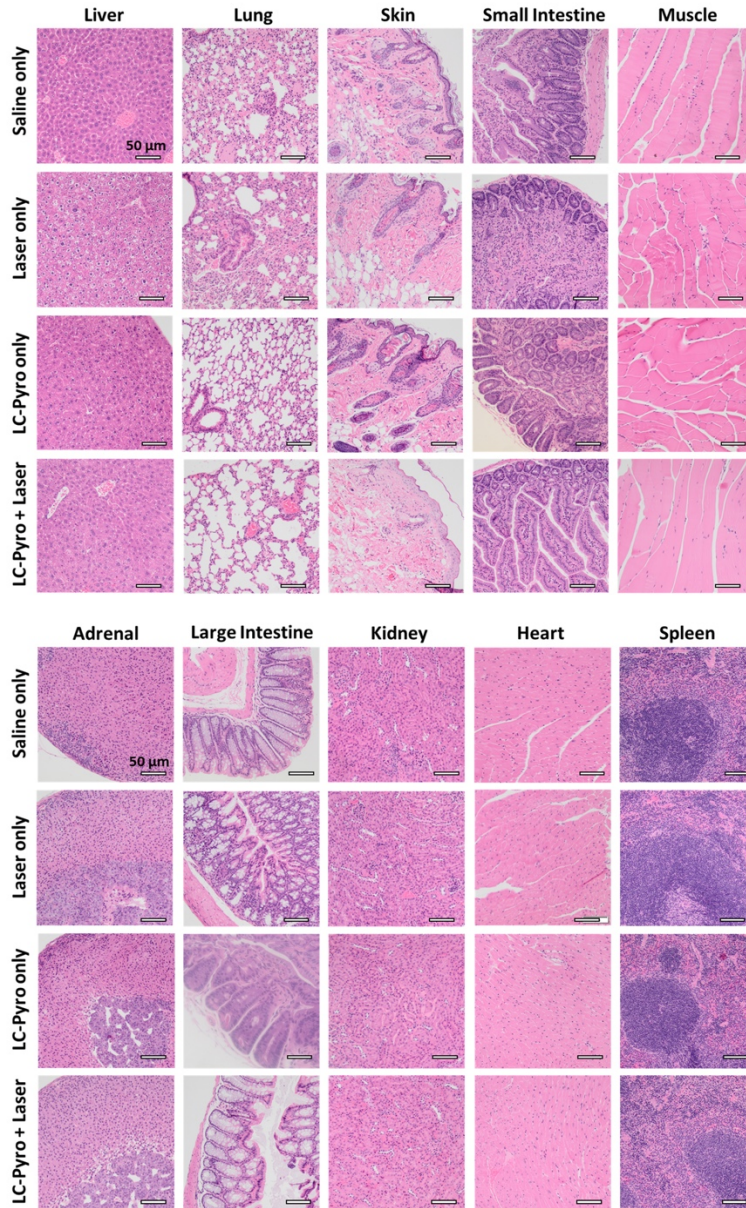
57 **Figure S6.** Lateral view PET/CT images of mice bearing either a PSMA- PC3 flu (left) or

58 PSMA+ PC3 PIP (right) orthotopic prostate tumor 17 h post-injection of ^{64}Cu -LC-Pyro.

Organ	PSMA- PC3 flu	PSMA+ PC3 PIP
Liver	18.11 ± 4.43	16.61 ± 3.39
Kidney	21.52 ± 1.27	22.68 ± 3.38
Lung	3.81 ± 1.98	3.47 ± 0.76
Heart	2.00 ± 0.83	1.99 ± 0.45
Spleen	6.85 ± 2.93	8.68 ± 2.10
Adrenals	9.55 ± 0.65	7.36 ± 2.38
Blood	1.74 ± 0.03	2.35 ± 0.92
Bladder	2.60 ± 1.00	5.45 ± 2.51
Urine	1.92 ± 0.71	2.00 ± 0.92
Brain	0.16 ± 0.02	0.18 ± 0.03
Muscle	1.13 ± 0.98	0.98 ± 0.77
Stomach	3.25 ± 0.63	2.85 ± 0.22
Skin	3.81 ± 2.18	2.38 ± 0.69
Fat	2.32 ± 1.88	1.49 ± 1.12
S. Intestine	4.33 ± 2.08	4.70 ± 1.39
L. Intestine	4.44 ± 1.69	4.84 ± 1.96
Tumor	2.30 ± 0.09	9.74 ± 2.26
Prostate	3.38 ± 1.22	4.14 ± 0.53
Sem. Ves.	1.09 ± 0.53	1.16 ± 0.47
Testes	1.59 ± 0.57	1.66 ± 0.26
Feces	14.35 ± 5.79	21.74 ± 8.96

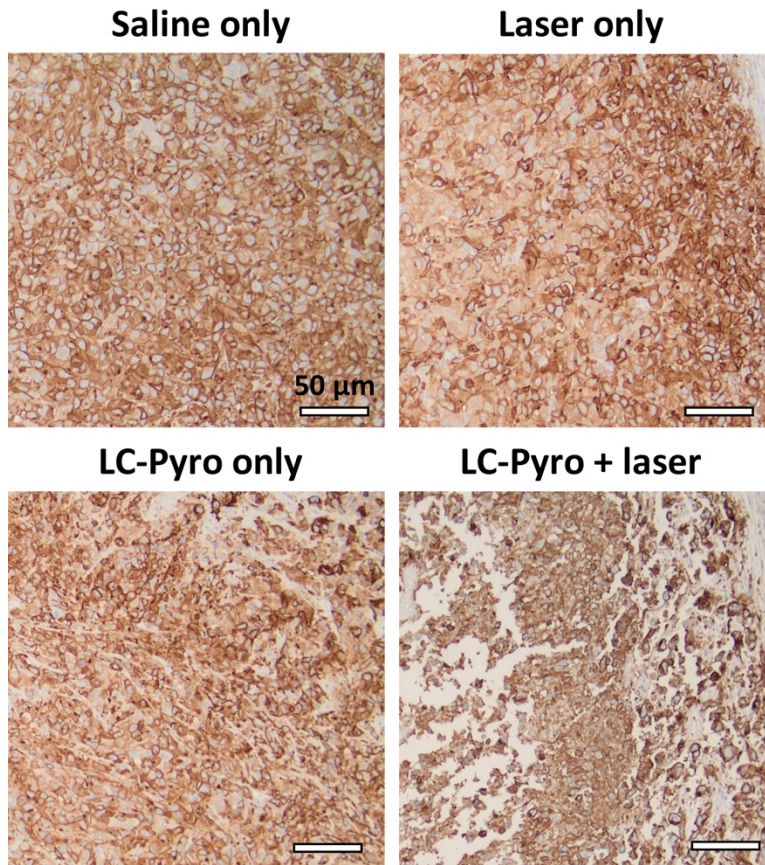
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60 **Table S1.** Corresponding ⁶⁴Cu-LC-Pyro biodistribution from **Figure 5** of main organs and
61 PSMA- PC3 flu and PSMA+ PC3 PIP tumors quantified via gamma counting (n = 4 for PSMA+
62 PC3 PIP; n = 3 for PSMA- PC3 flu, ***P* < 0.01; ****P* < 0.001). Data is represented as mean ±
63 SD.



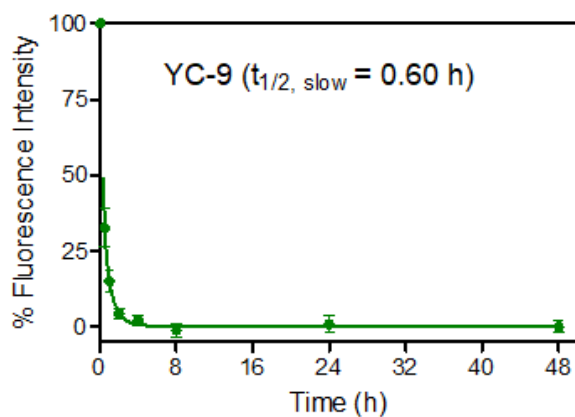
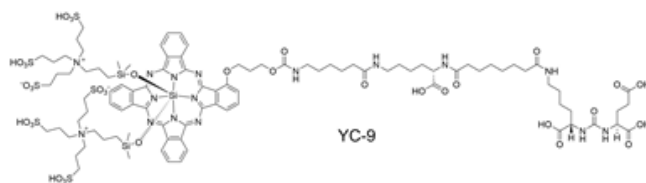
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65 **Figure S7.** H&E evaluation of organ toxicity 24 h post-treatment from each cohort; 1) Saline
 66 only; 2) Laser only; 3) LC-Pyro only and 4) LC-Pyro + Laser. Organs include the liver, lung,
 67 skin, small intestine, muscle, adrenal, large intestine, kidney, heart and spleen. Histological
 68 slices reveal no adverse side effects on healthy tissues after treatment. Scale = 50 μm.



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70 **Figure S8.** Representative PSMA staining sections of PSMA+ PC3 PIP tumors post-PDT
71 treatment from each cohort; 1) Saline only; 2) Laser only; 3) LC-Pyro only and 4) LC-Pyro +
72 Laser. Scale = 50 μm. PDT did not significantly alter expression of PSMA.



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74 **Figure S9.** Chemical structure of reported compound YC-9¹ and its plasma blood clearance
 75 profile in BALB/c mice (n = 5). The profile fits into a two-compartment model with a slow half-
 76 life of 0.60 h.

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78 **References:**

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