Supporting Information

In Vivo Optical Imaging of Acute Cell Death Using a Near-Infrared Zinc-Dipicolylamine Probe

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1. In vivo Imaging

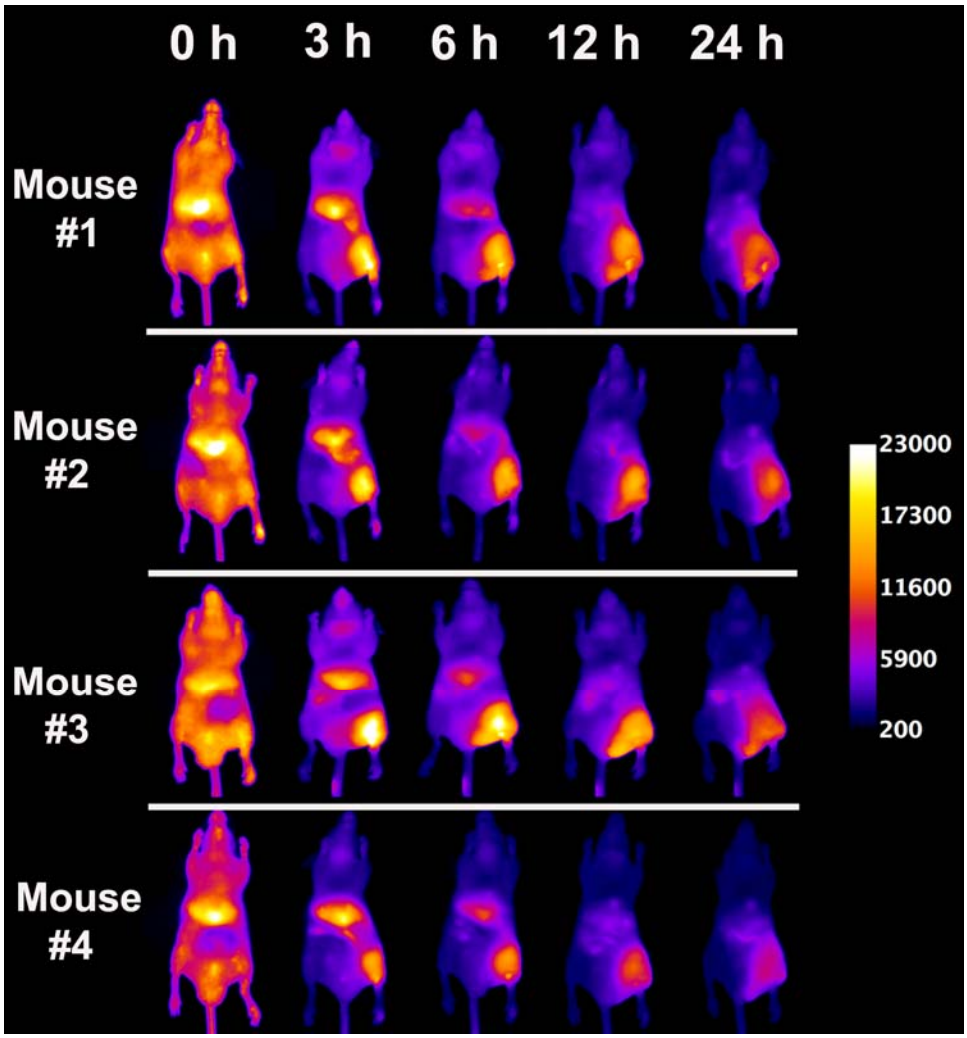

Figure S1. In vivo fluorescence montage of mice treated with ionophore (**1**) and later injected with probe **2**. Each mouse was anesthetized and injected intramuscularly with 50 μL of ionophore (0.5 mg) in the right hind leg and injected with an equal volume of saline in the left hind leg muscle. Mice were then dosed via the tail vein with probe **2** (100 μL of 1 mM) 2 h post-treatment. Mice were imaged at the indicated timepoints. Calibration bar applies to all images.

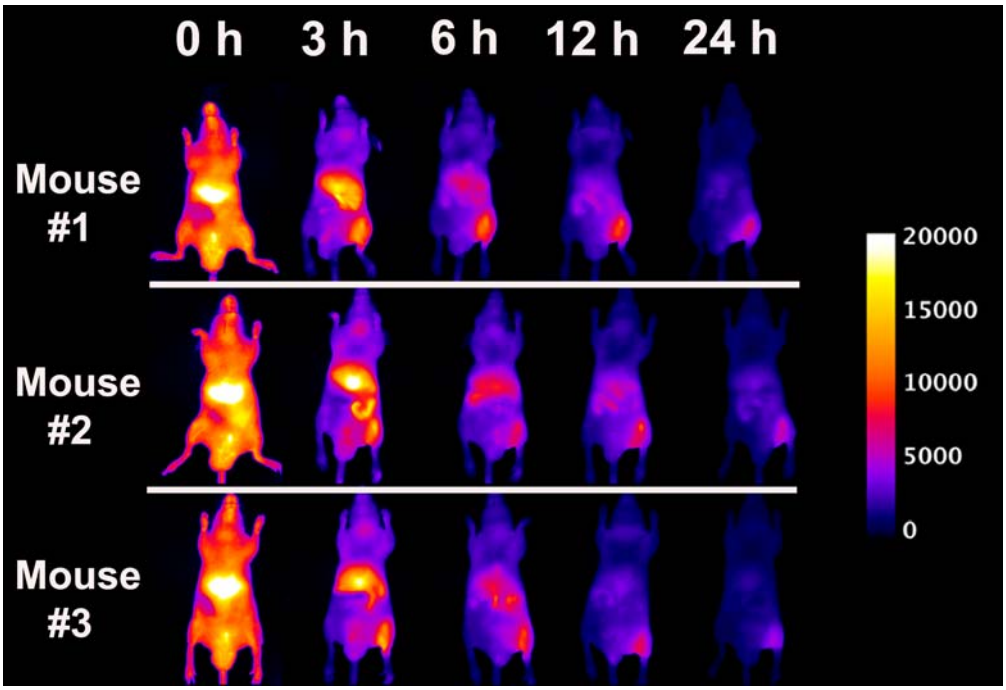

Figure S2. In vivo fluorescence montage of mice treated with ethanol and later injected with probe **2**. Each mouse was anesthetized and injected intramuscularly with 50 μL of ethanol (100 %) in the right hind leg and injected with an equal volume of saline in the left hind leg muscle. Mice were then dosed via the tail vein with probe **2** (100 μL of 1 mM) 2 h post-treatment. Mice were imaged at the indicated timepoints. Calibration bar applies to all images.

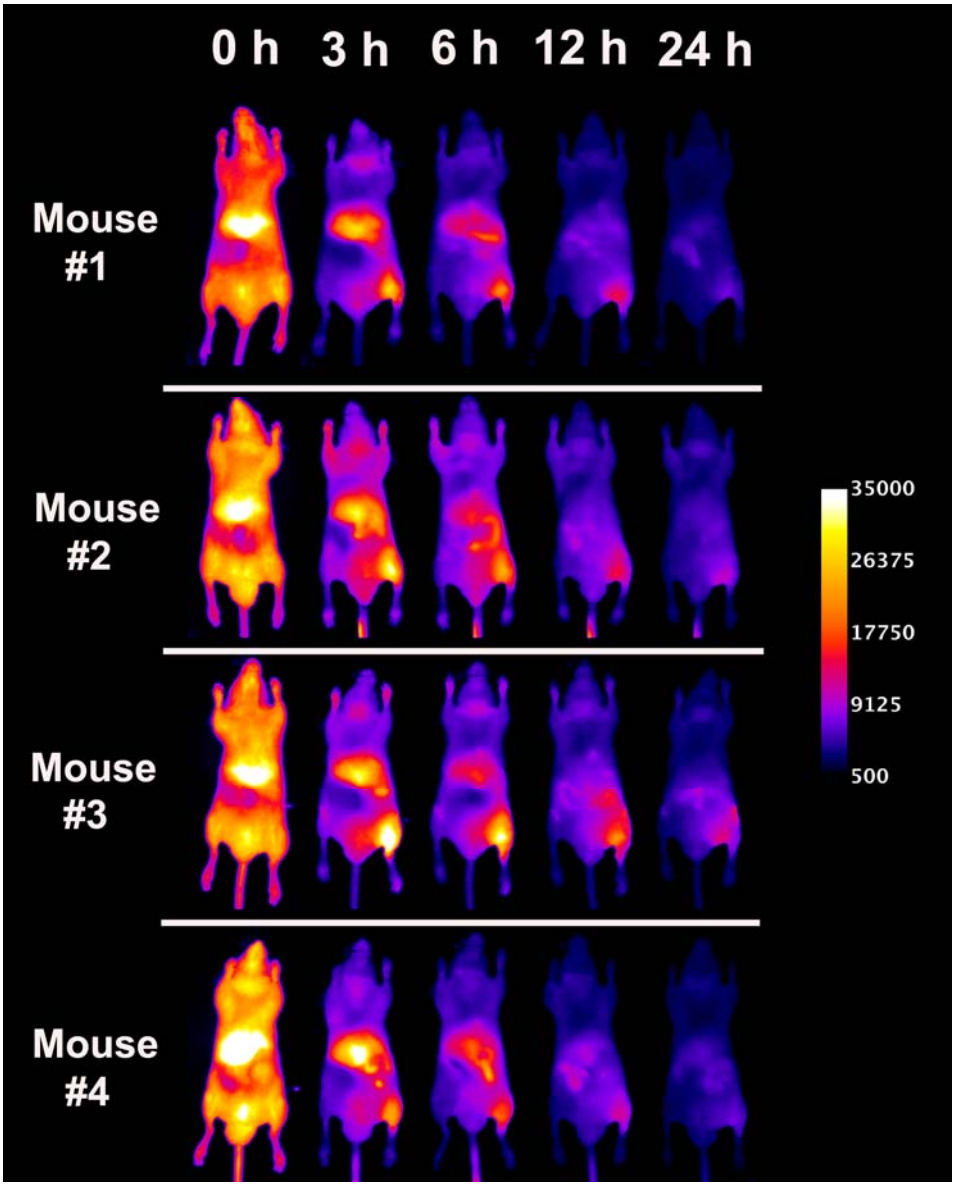

Figure S3. In vivo fluorescence montage of mice treated with ketamine and later injected with probe **2**. Each mouse was anesthetized and injected intramuscularly with 50 μL of ketamine (5.0 mg) in the right hind leg and injected with an equal volume saline in the left hind leg muscle. Mice were then dosed via the tail vein with probe **2** (100 μL of 1 mM) 2 h post-treatment. Mice were imaged at the indicated timepoints. Calibration bar applies to all images.

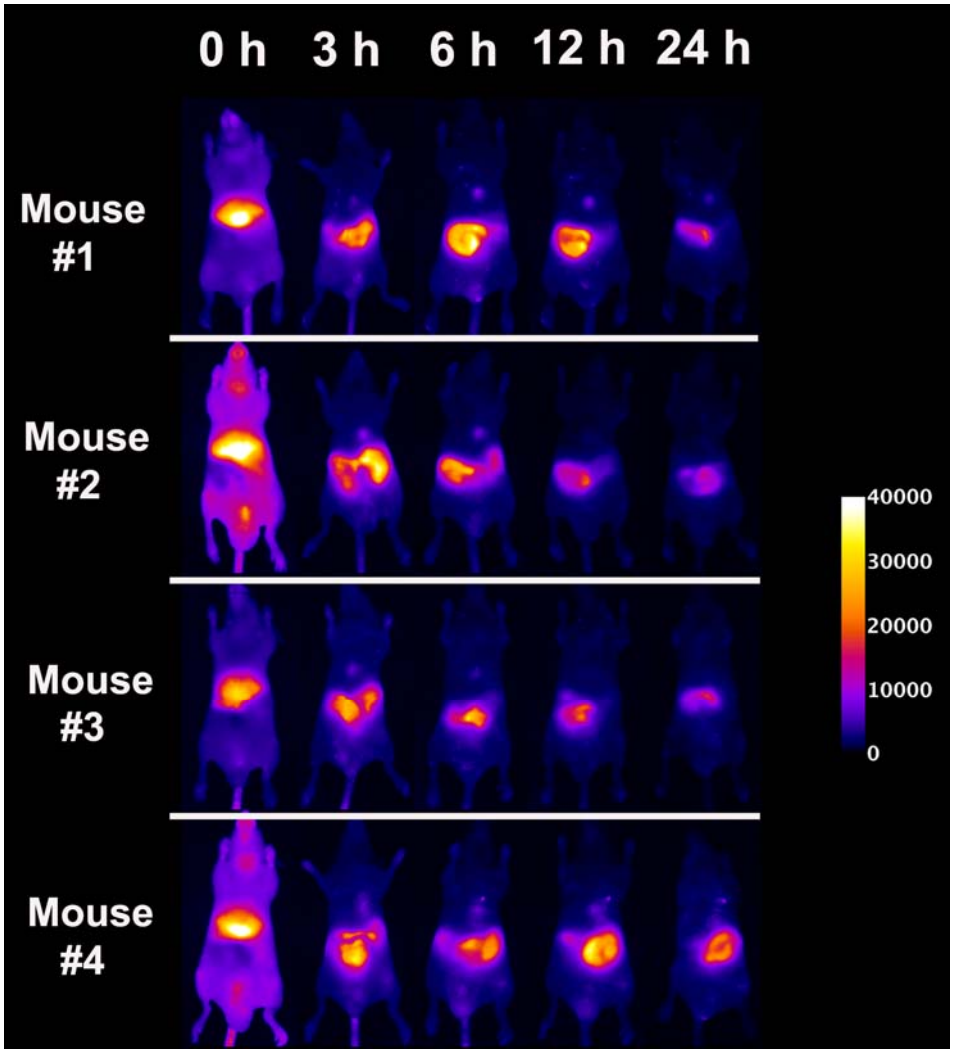

Figure S4. In vivo fluorescence montage of mice treated with ionophore (**1**) and later injected with control **3**. Each mouse was anesthetized and injected intramuscularly with 50 μL of ionophore (0.5 mg) in the right hind leg and injected with an equal volume of saline in the left hind leg muscle. Mice were then dosed via the tail vein with control **3** (100 μL of 1 mM) 2 h post-treatment. Mice were imaged at the indicated timepoints. Calibration bar applies to all images.

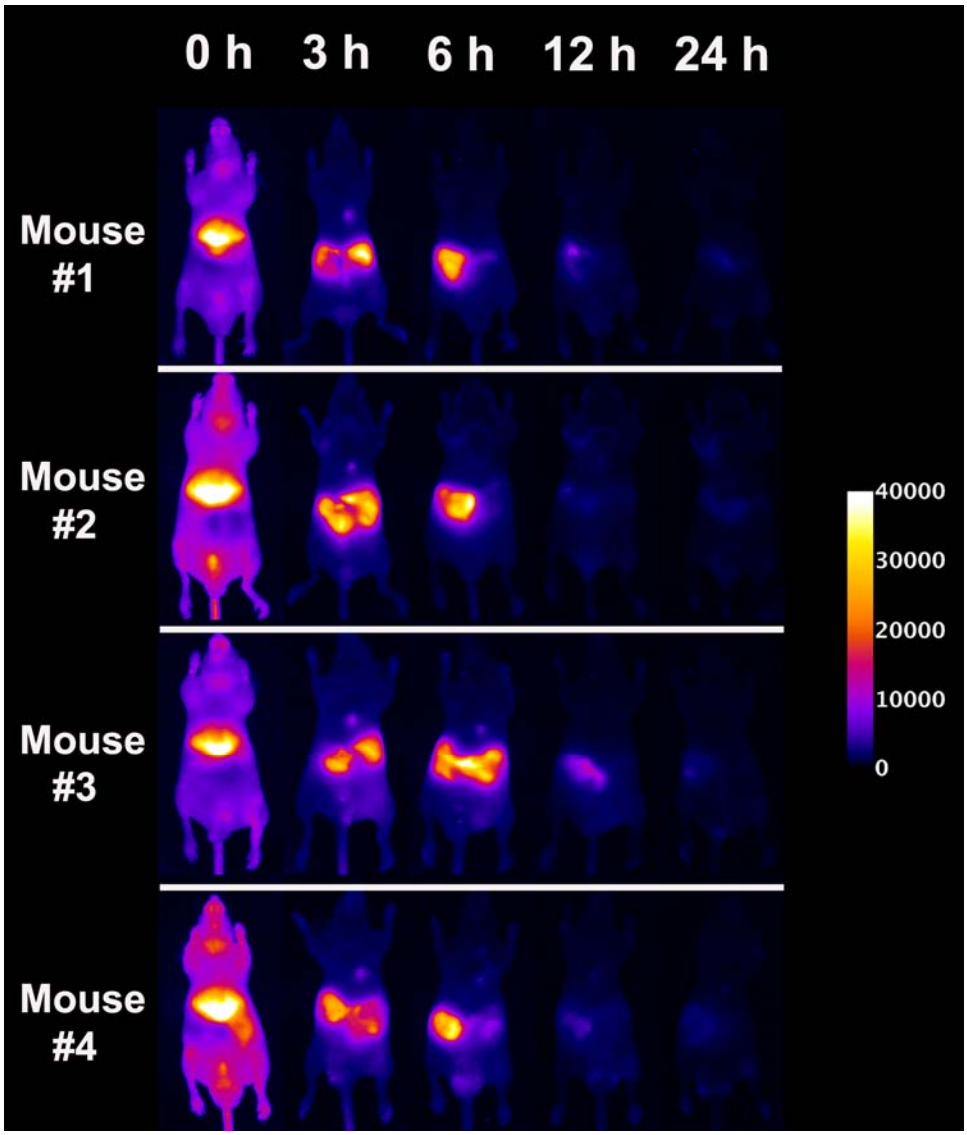

Figure S5. In vivo fluorescence montage of mice treated with ethanol and later injected with control **3**. Each mouse was anesthetized and injected intramuscularly with 50 μL of ethanol (100 %) in the right hind leg and injected with an equal volume of saline in the left hind leg muscle. Mice were then dosed via the tail vein with control **3** (100 μL of 1 mM) 2 h post-treatment. Mice were imaged at the indicated timepoints. Calibration bar applies to all images.

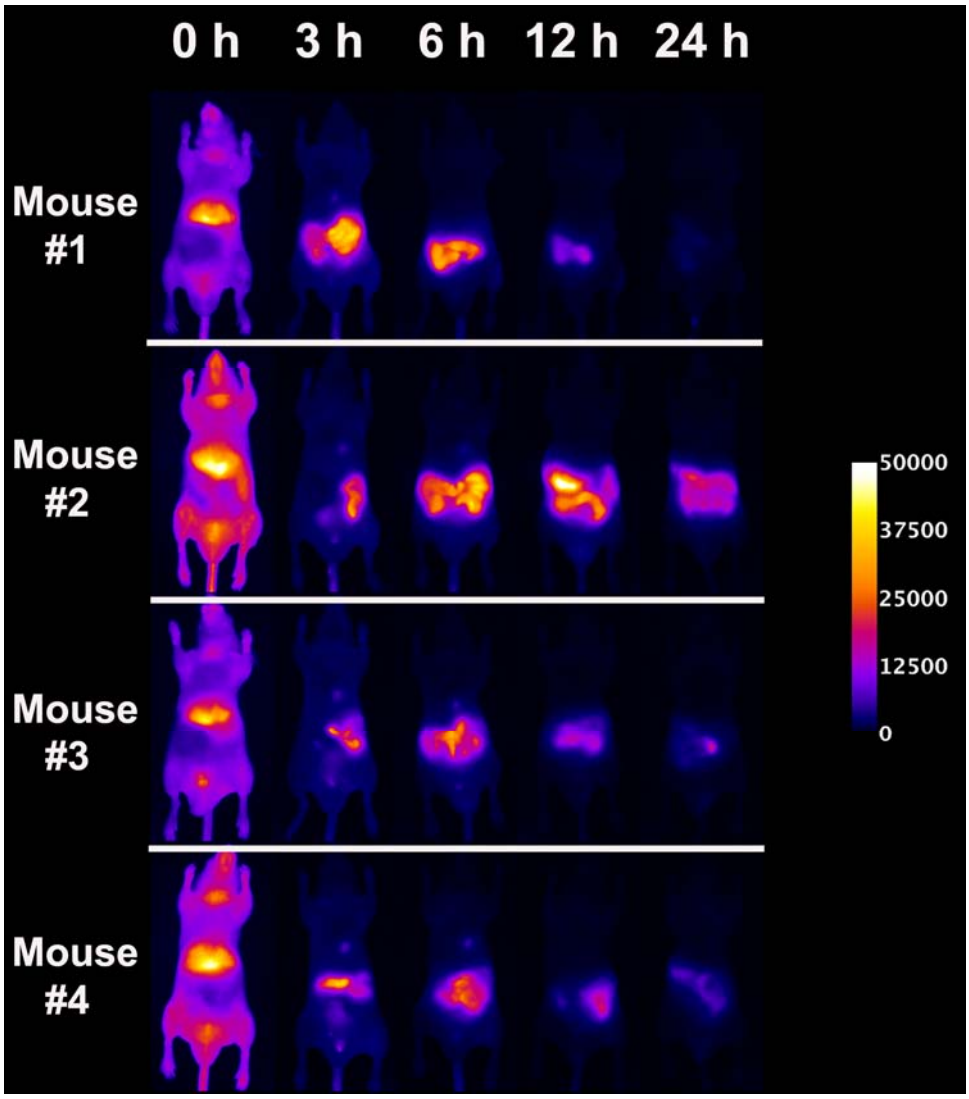

Figure S6. In vivo fluorescence montage of mice treated with ketamine and later injected with control **3**. Each mouse was anesthetized and injected intramuscularly with 50 μL of ketamine (5.0 mg) in the right hind leg and injected with an equal volume of saline in the left hind leg muscle. Mice received 100 μL of control **3** (1 mM) via tail vein injection 2 h post-treatment. Mice were imaged at the indicated timepoints. Calibration bar applies to all images.

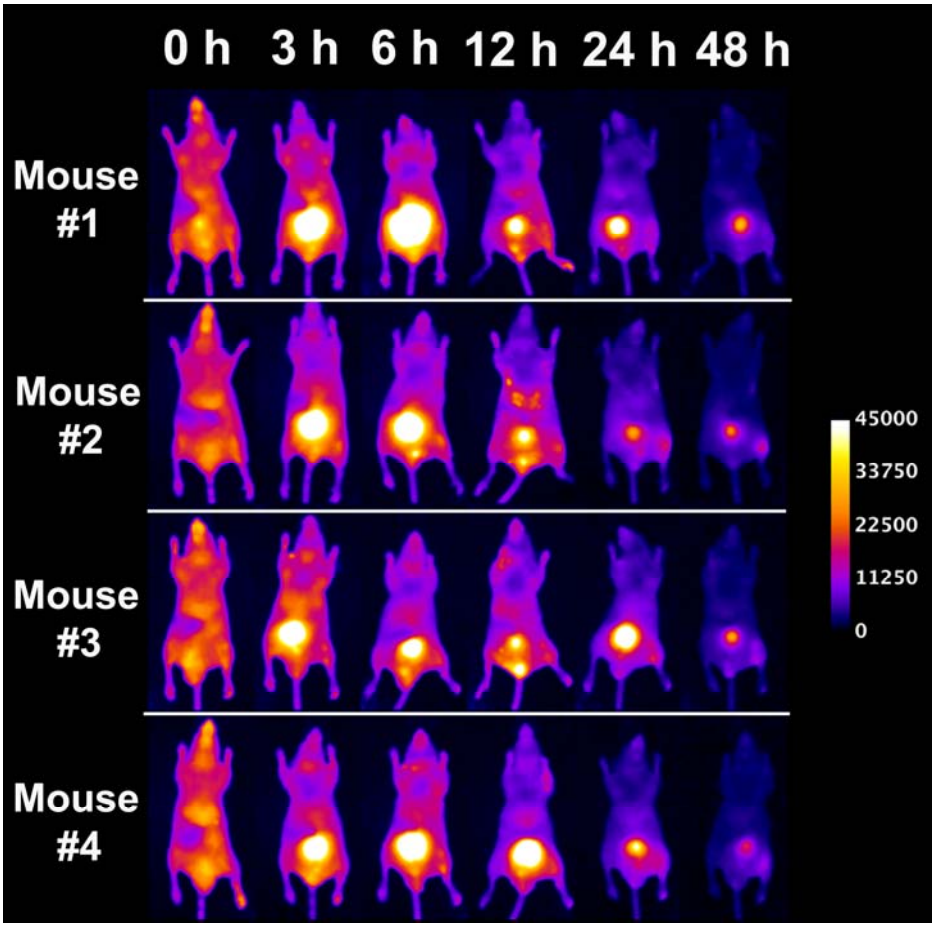

Figure S7. In vivo fluorescence montage of mice treated with ionophore (**1**) and later injected with Annexin-Vivo 750. Each mouse was anesthetized and injected intramuscularly with 50 μL of ionophore (0.5 mg) in the right hind leg and injected with an equal volume of saline in the left hind leg muscle. Mice were then dosed via the tail vein with Annexin-Vivo 750 2 h post-treatment. Mice were imaged at the indicated timepoints. Calibration bar applies to all images.

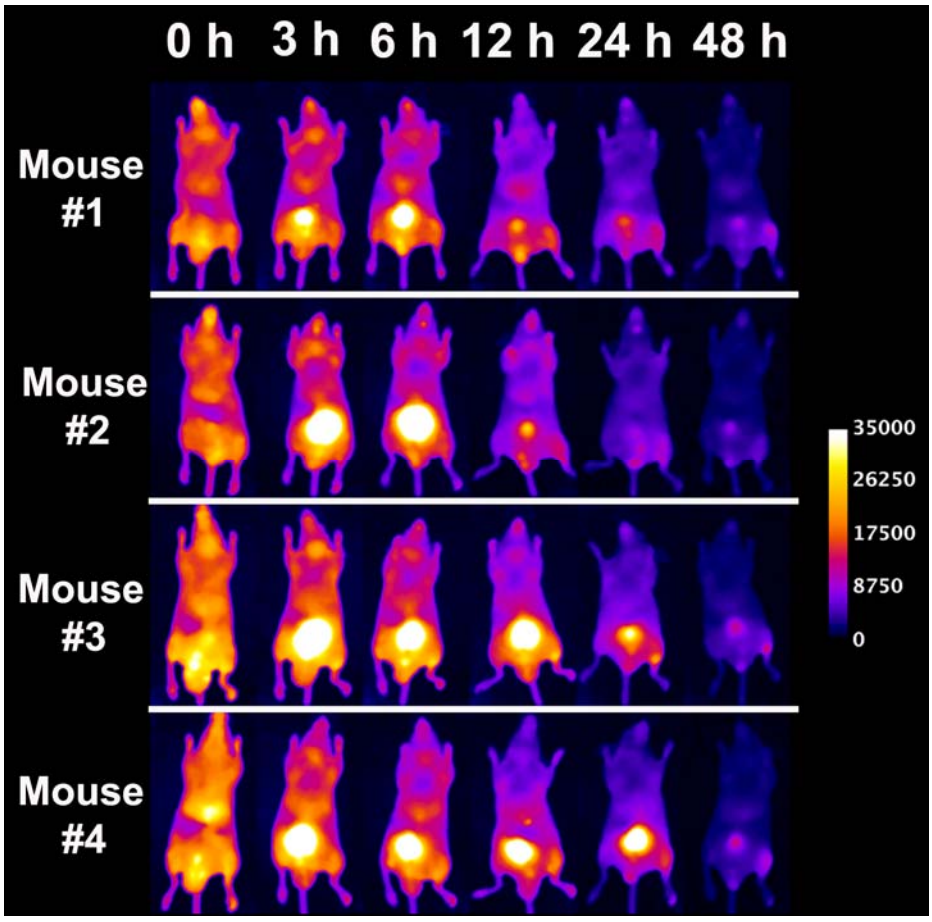

Figure S8. In vivo fluorescence montage of mice treated with ethanol and later injected with Annexin–Vivo 750. Each mouse was anesthetized and injected intramuscularly with 50 μL of ethanol (100 %) in the right hind leg and injected with an equal volume of saline in the left hind leg muscle. Mice were then dosed via the tail vein with Annexin Vivo-750 2 h post-treatment. Mice were imaged at the indicated timepoints. Calibration bar applies to all images.

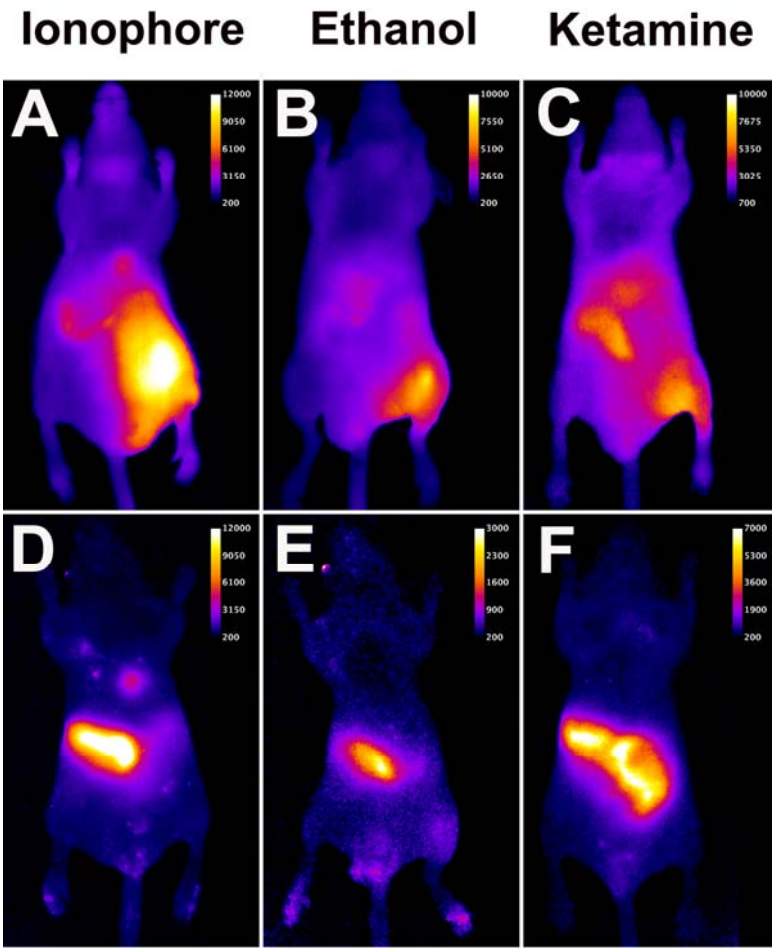

Figure S9. Representative near-infrared fluorescence images of treated mice 24 h post-injection of either probe **2** (A-C) or control **3** (D-F). Each mouse was injected intramuscularly with the indicated cytotoxin in the right hind leg and injected with saline in the left hind leg muscle. Mice were then injected via the tail vein with either probe **2** or control **3** (100 μL of 1 mM) 2 h post-treatment.

2. In vivo image analysis

Target to non-target (T/NT) and signal to noise (S/N) ratios were used to determine the targeting ability of probe **2**, control **3**, and Annexin-Vivo 750 for acute tissue damage in living mice. The target to non-targets ratios were computed by the following formula:

$$T/NT = \frac{\text{(mean pixel intensity of the treated leg)}}{\text{(mean pixel intensity of the contralateral leg)}}$$

T/NT ratios were calculated by drawing identical regions of interests (ROI) around the treated leg (Target, T) and the same anatomical location on the contralateral leg of each animal (Non-Target, NT). The mean pixel intensities for the target and non-target regions were measured and recorded for each animal.

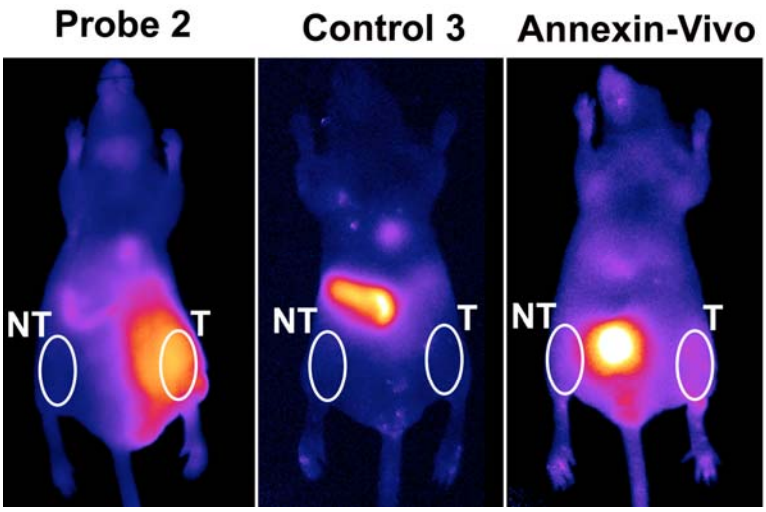

Figure S10. Representative target (T) and non-target (NT) regions of interest (ROI) for in vivo fluorescence images. Identical ROIs were used in Figures S1-S8.

Table S1. In vivo target to non-target ratios (T/NT) for probe **2**, control **3**, and Annexin-Vivo 750. T/NT ± standard error of the mean.

	0 h	3 h	6 h	12 h	24 h	48 h
Ionophore						
2	1.13 ± 0.04	4.90 ± 0.22	5.75 ± 0.28	6.20 ± 0.43	5.97 ± 0.42	NP
3	1.09 ± 0.03	1.35 ± 0.11	1.22 ± 0.06	1.48 ± 0.03	1.04 ± 0.01	NP
Annexin-Vivo	1.15 ± 0.06	1.16 ± 0.07	1.31 ± 0.10	1.56 ± 0.20	1.80 ± 0.17	2.48 ± 0.25
Ethanol						
2	1.14 ± 0.10	3.15 ± 0.13	3.23 ± 0.45	3.74 ± 0.20	4.15 ± 0.25	NP
3	1.15 ± 0.06	1.67 ± 0.24	1.32 ± 0.13	1.29 ± 0.11	1.25 ± 0.09	NP
Annexin-Vivo	1.09 ± 0.04	1.10 ± 0.04	1.22 ± 0.08	1.53 ± 0.04	1.96 ± 0.06	2.43 ± 0.21
Ketamine						
2	1.09 ± 0.06	3.69 ± 0.17	3.77 ± 0.22	3.37 ± 0.23	2.83 ± 0.09	NP
3	1.11 ± 0.04	1.79 ± 0.14	1.26 ± 0.10	1.35 ± 0.09	1.13 ± 0.04	NP
Annexin-Vivo	NP	NP	NP	NP	NP	NP

3. Ex vivo imaging

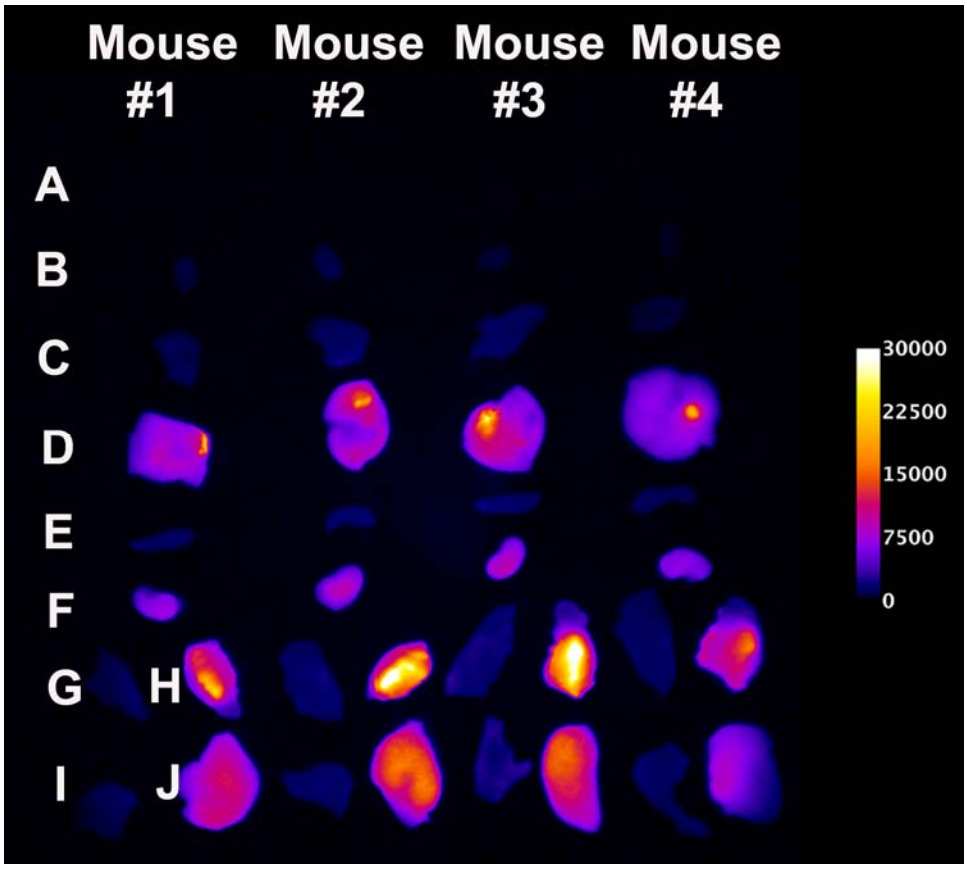

Figure S11. Ex vivo fluorescence imaging of probe **2** in tissues from ionophore (**1**) treated mice. The ex vivo image was acquired 24 h post-injection of **2**. Tissues are in the following order: blood (A), heart (B), lungs (C), liver (D), spleen (E), kidney (F), control muscle (G), ionophore treated muscle (H), skin from control leg (I), and skin from ionophore treated leg (J). The fluorescence intensity bar applies to all tissues.

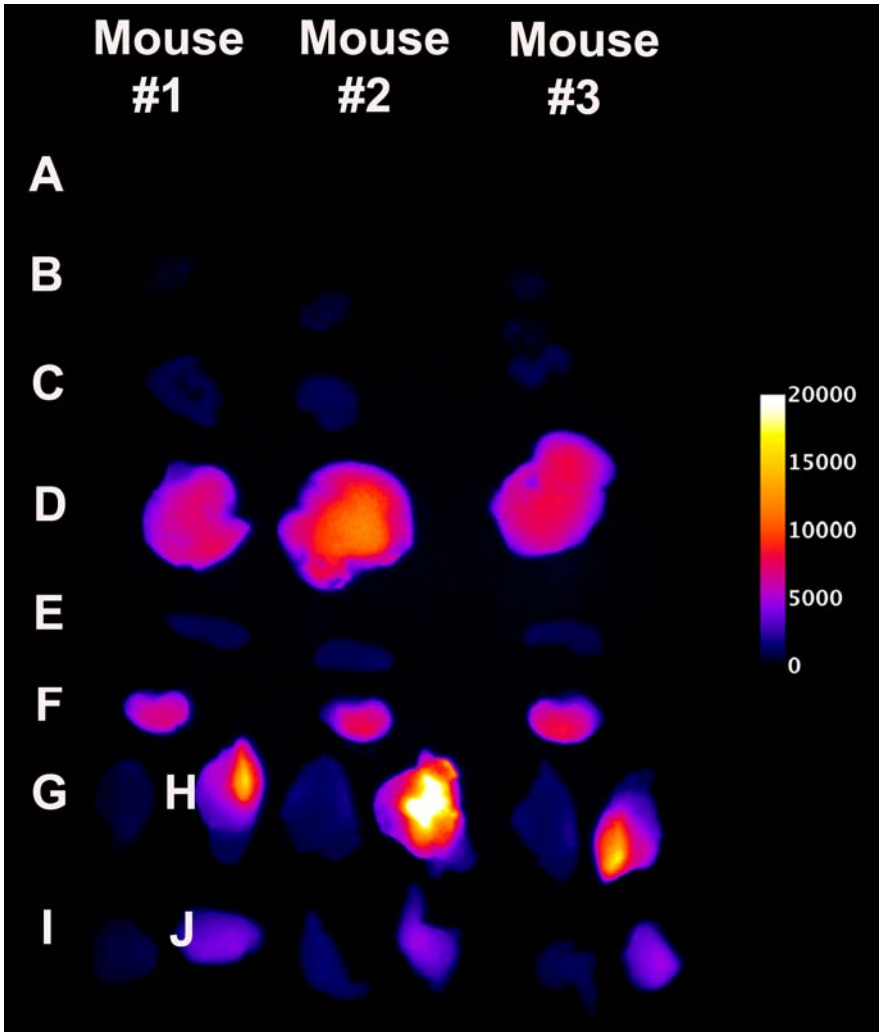

Figure S12. Ex vivo fluorescence imaging of probe **2** in tissues from ethanol treated mice. The ex vivo image was acquired 24 h post-injection of **2**. Tissues are in the following order: blood (A), heart (B), lungs (C), liver (D), spleen (E), kidney (F), control muscle (G), ethanol treated muscle (H), skin from control leg (I), and skin from ethanol treated leg (J). The fluorescence intensity bar applies to all tissues.

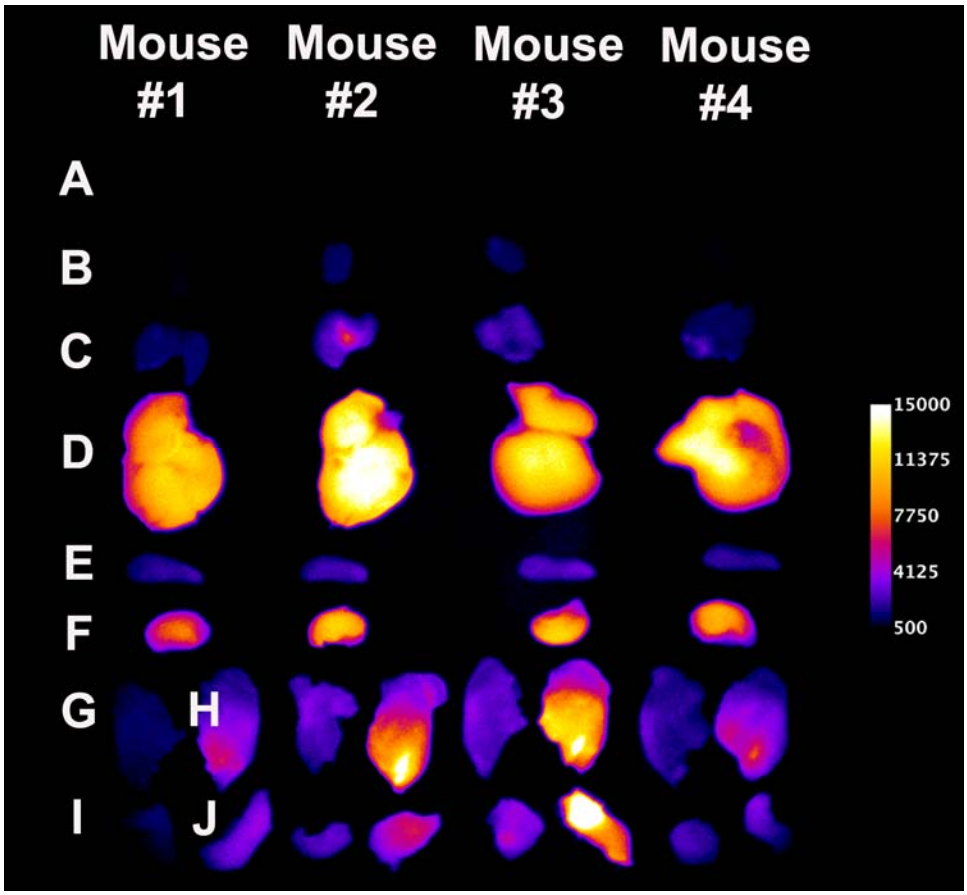

Figure S13. Ex vivo fluorescence imaging of probe **2** in tissues from ketamine treated mice. The excised tissues were imaged 24 h post-injection of probe **2**. Tissues are in the following order: blood (A), heart (B), lungs (C), liver (D), spleen (E), kidney (F), control muscle (G), ketamine treated muscle (H), skin from control leg (I), and skin from ketamine treated leg (J). The fluorescence intensity bar applies to all tissues.

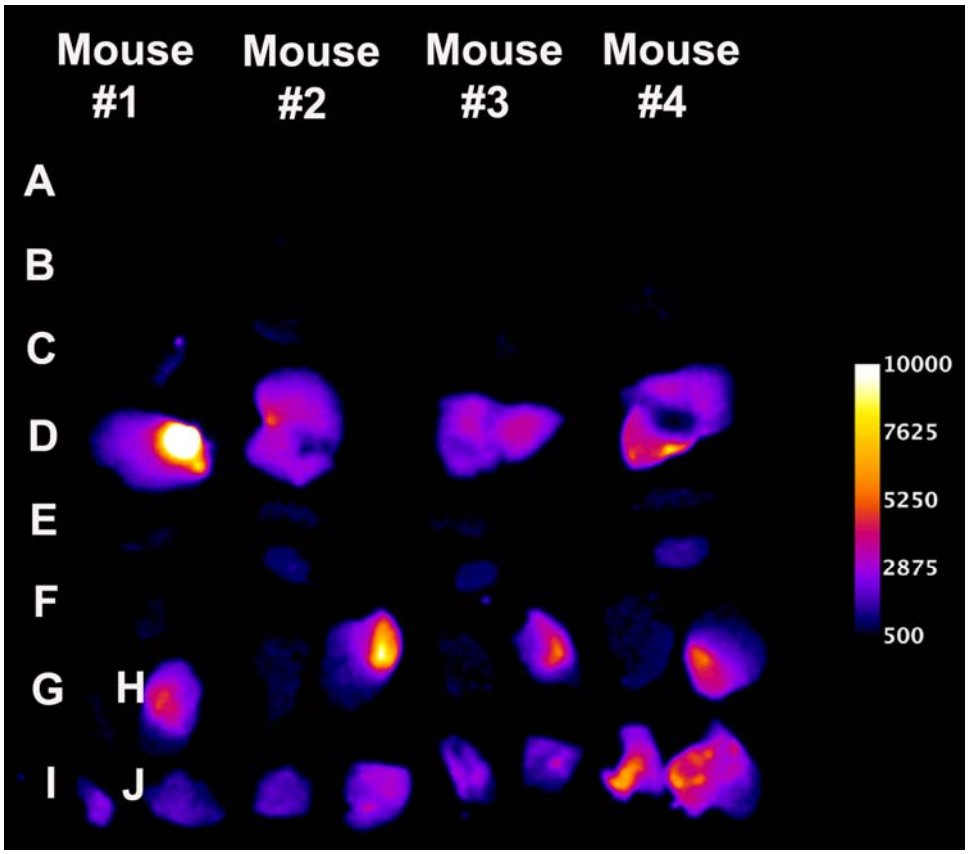

Figure S14. Ex vivo fluorescence imaging of control **3** in tissues from ionophore (**1**) treated mice. The ex vivo image was acquired 24 h post-injection of control **3**. Tissues are in the following order: blood (A), heart (B), lungs (C), liver (D), spleen (E), kidney (F), control muscle (G), ionophore treated muscle (H), skin from control leg (I), and skin from ionophore treated leg (J). The fluorescence intensity bar applies to all tissues.

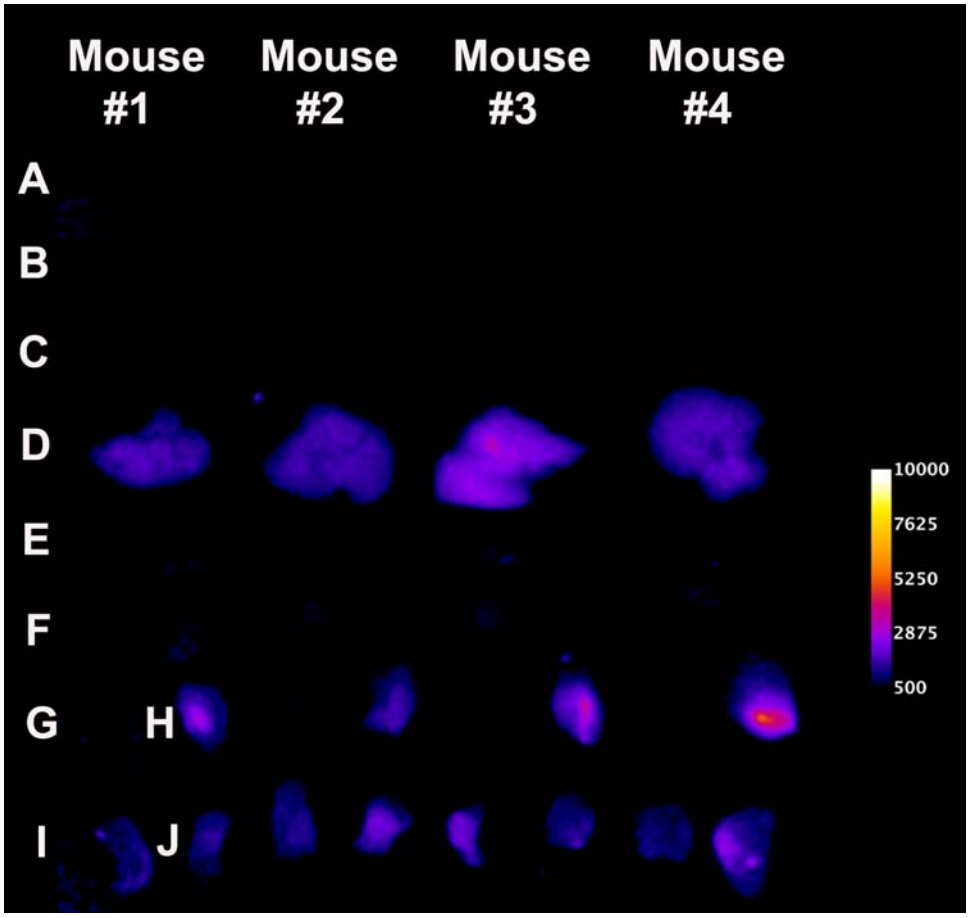

Figure S15. Ex vivo fluorescence imaging of control **3** in tissues from ethanol treated mice. The excised tissues were imaged 24 h post-injection of control **3**. Tissues are in the following order: blood (A), heart (B), lungs (C), liver (D), spleen (E), kidney (F), control muscle (G), ethanol treated muscle (H), skin from control leg (I), and skin from ethanol treated leg (J). The fluorescence intensity bar applies to all tissues.

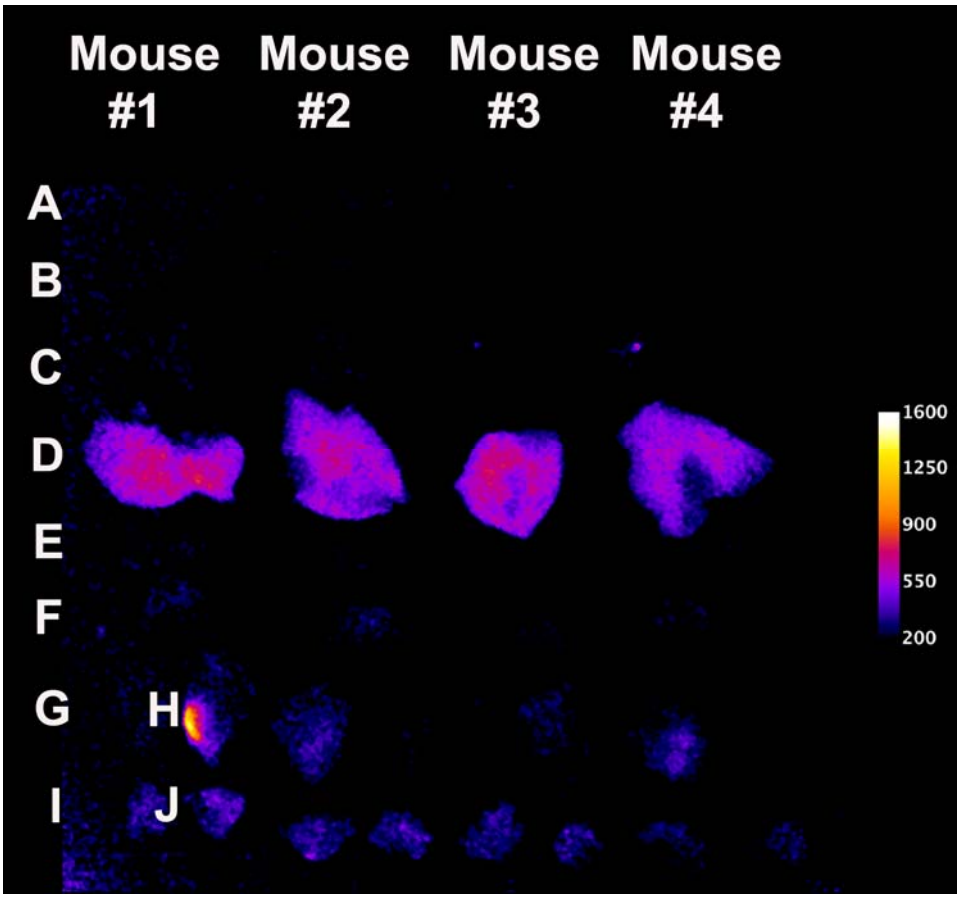

Figure S16. Ex vivo fluorescence imaging of control **3** in tissues from ketamine treated mice. The ex vivo image was acquired 24 h post-injection of control **3**. Tissues are in the following order: blood (A), heart (B), lungs (C), liver (D), spleen (E), kidney (F), control muscle (G), ketamine treated muscle (H), skin from control leg (I), and skin from ketamine treated leg (J). The fluorescence intensity bar applies to all tissues.

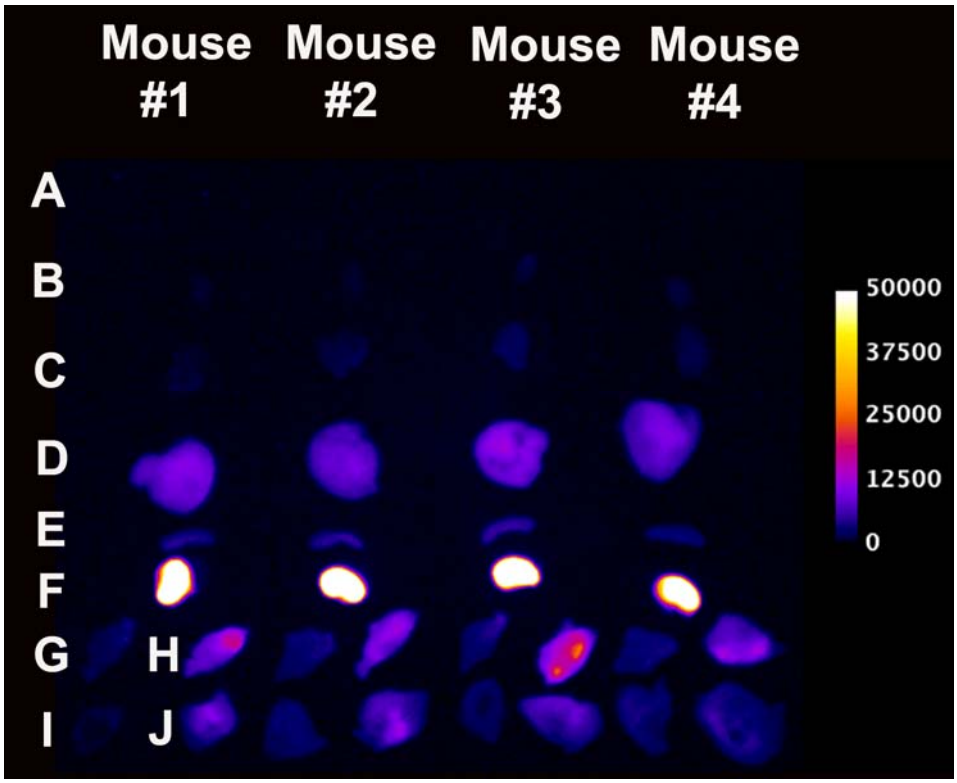

Figure S17. Ex vivo fluorescence imaging of Annexin-Vivo 750 in tissues from ionophore (**1**) treated mice. The ex vivo image was acquired 24 h post-injection of probe. Tissues are in the following order: blood (A), heart (B), lungs (C), liver (D), spleen (E), kidney (F), control muscle (G), ionophore treated muscle (H), skin from control leg (I), and skin from ionophore treated leg (J). The fluorescence intensity bar applies to all tissues.

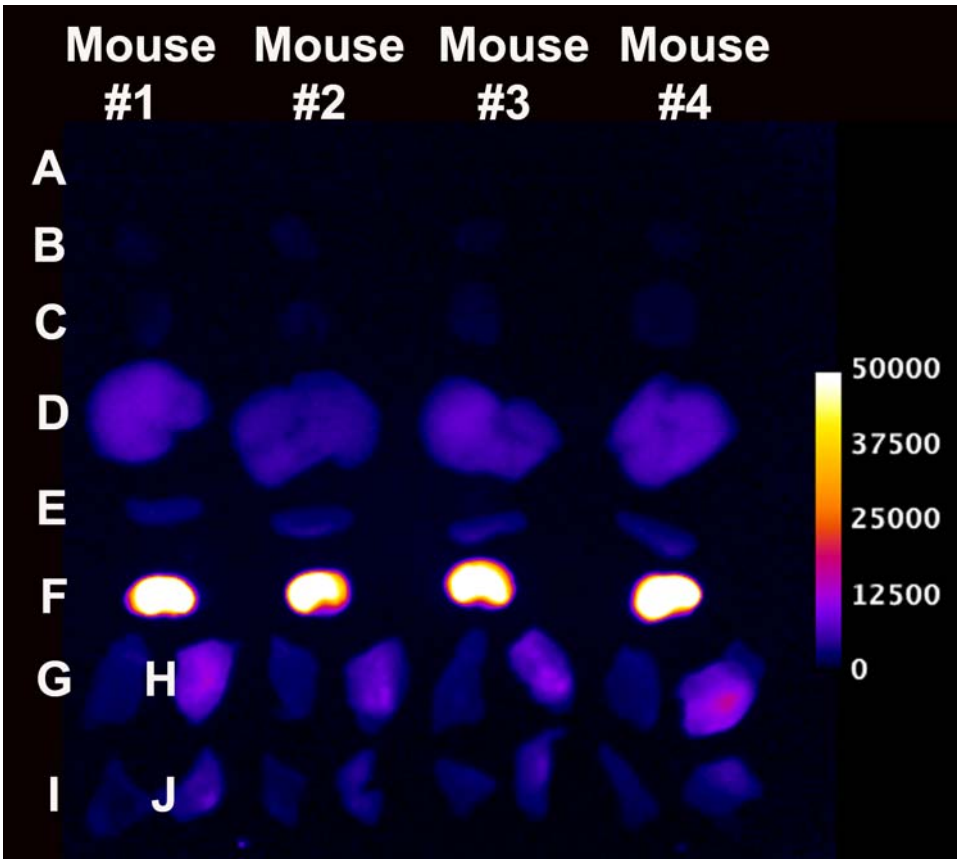

Figure S18. Ex vivo fluorescence imaging of Annexin-Vivo 750 in tissues from ethanol treated mice. The ex vivo image was acquired 24 h post-injection of the probe. Tissues are in the following order: blood (A), heart (B), lungs (C), liver (D), spleen (E), kidney (F), control muscle (G), ethanol treated muscle (H), skin from control leg (I), and skin from ethanol treated leg (J). The fluorescence intensity bar applies to all tissues.

4. Ex vivo image analysis

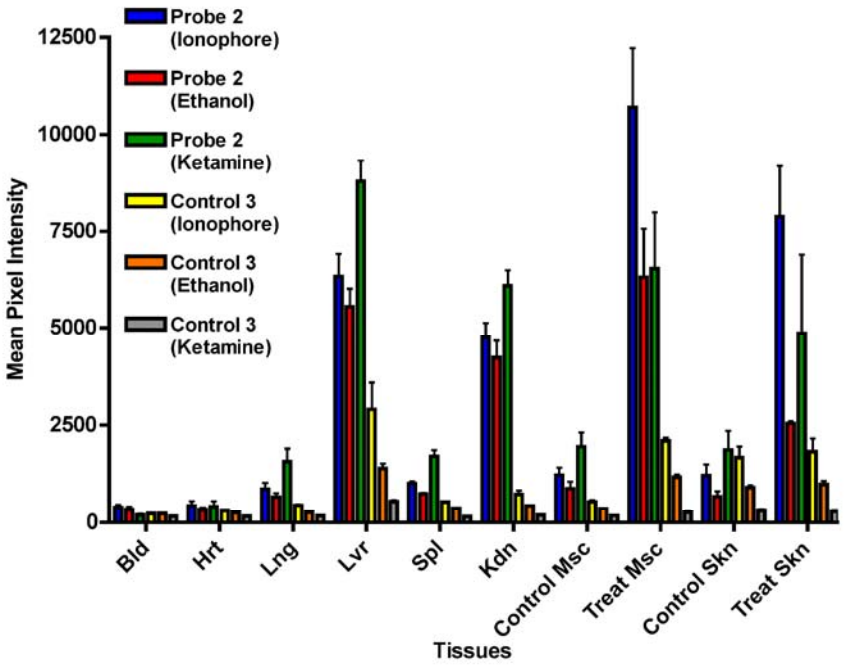

Figure S19. Bar graph showing ex vivo tissue distribution of probe **2** and control **3** in treated mice. Error bars represent the standard error of the mean. N = 4 except n = 3 for probe **2** (Ethanol). $P < 0.001$ for ionophore treated muscle with probe **2** vs. the ionophore treated muscle with control **3**. $P < 0.007$, $P < 0.04$, $P < 0.02$ for treated muscle vs control muscle with probe **2** (Ionophore), probe **2** (Ethanol), and probe **2** (Ketamine), respectively. $P < 0.005$ for ethanol and ketamine treated muscle with probe **2** vs. ethanol and ketamine treated muscle with control **3**.

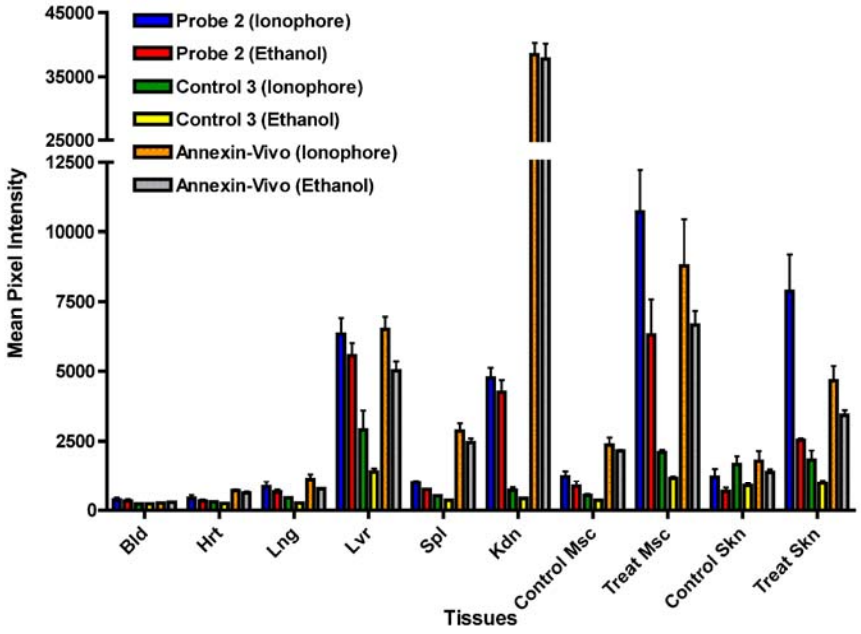

Figure S20. Bar graph showing ex vivo tissue distribution of probe **2**, control **3**, and Annexin-Vivo 750 in treated mice. Error bars represent the standard error of the mean. N = 4 except n = 3 for probe **2** (Ethanol).

5. Histology

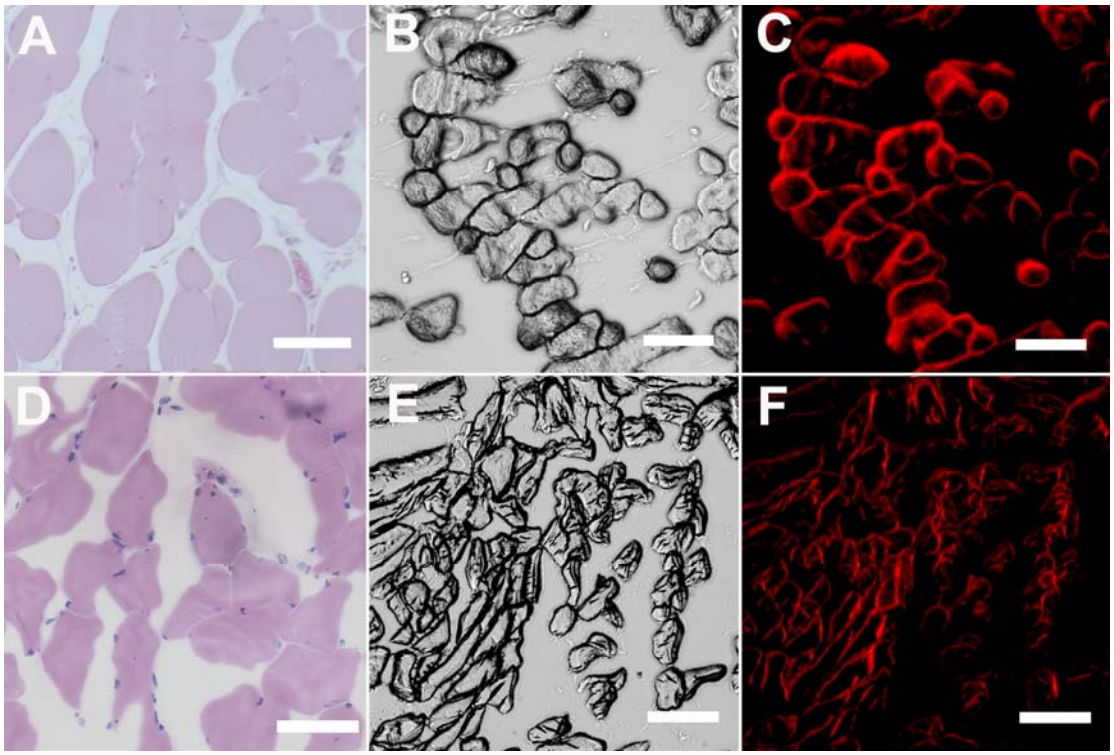

Figure S21. Histological micrographs of probe **2** accumulation in tissue sections from damaged leg muscles treated with either ethanol (A-C) or ketamine (D-F). Micrographs were stained with hematoxylin and eosin (A, D) or left unstained. Unstained micrographs were imaged in the brightfield (B, E) and NIR (C, F) filter sets. Scale bar = 200 μm for panels A and D. Scale bar = 50 μm for panels B, C, E, F.