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SUPPORTING INFORMATION

NIR-emissive PEG-*b*-TCL Micelles for Breast Tumor Imaging and Minimally Invasive Pharmacokinetic Analysis

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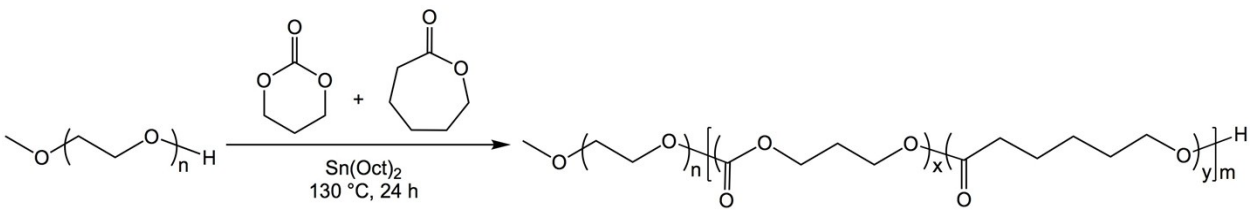


Scheme S1: Synthesis of PEG(2k)-*b*-TCL(11.2k) copolymer.

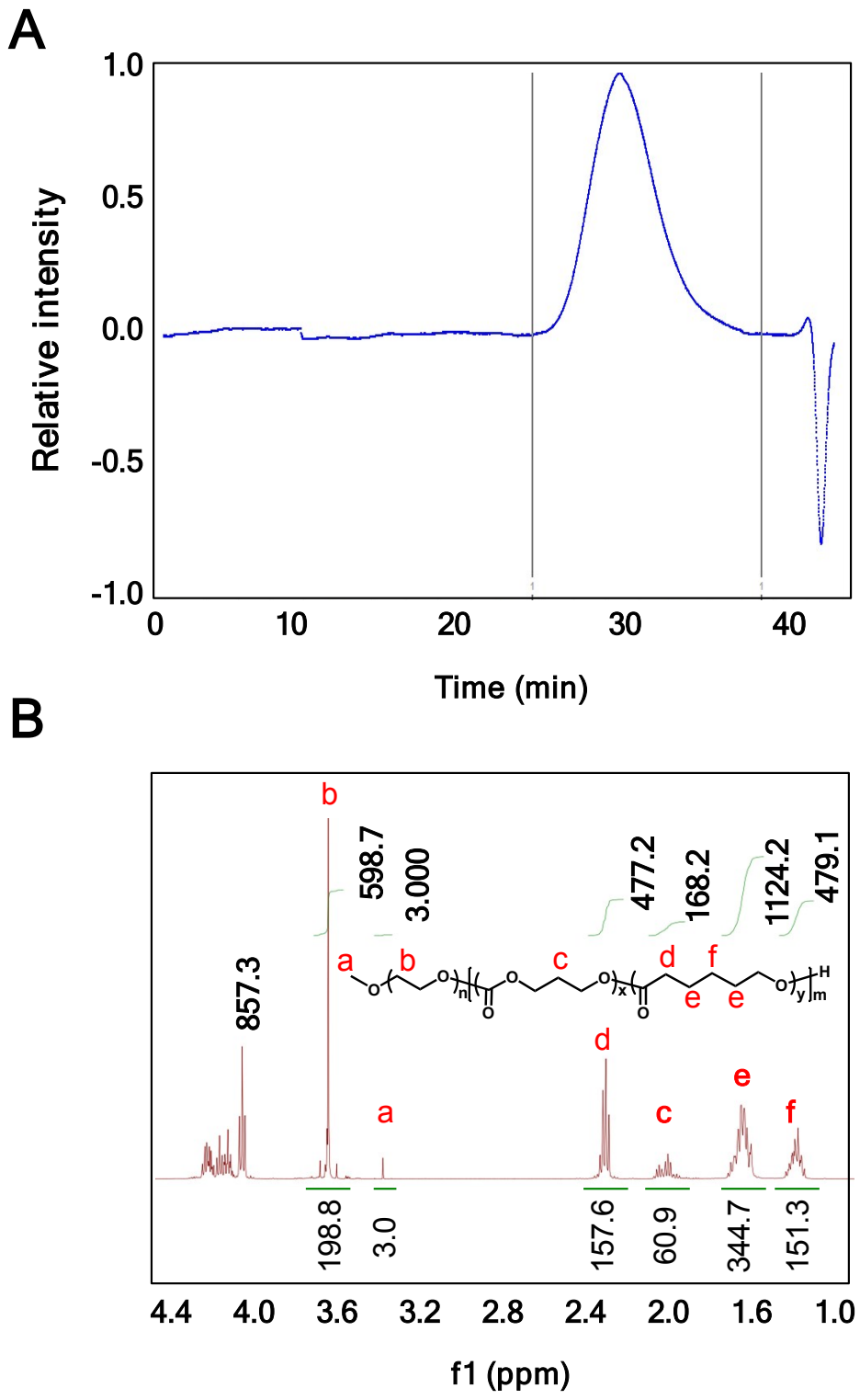


Figure S1. Characterization of PEG(2k)-*b*-TCL(11.2k) copolymer. (A) GPC-MALS and (B) ^{1}H-NMR spectrum of the copolymer in $CDCl_3$. Distinguishable ^{1}H peaks are labeled and assigned to protons in the chemical structure.

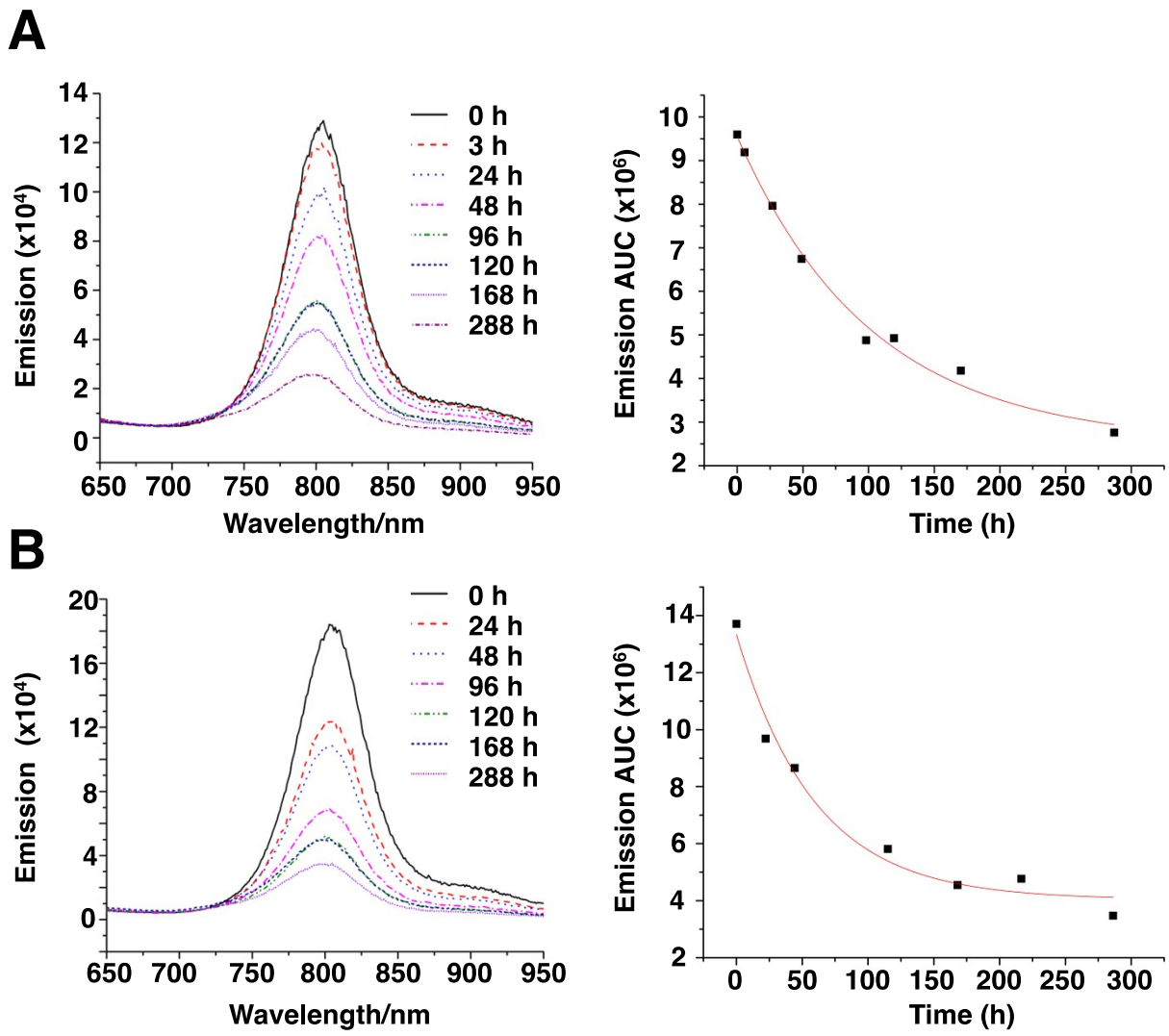
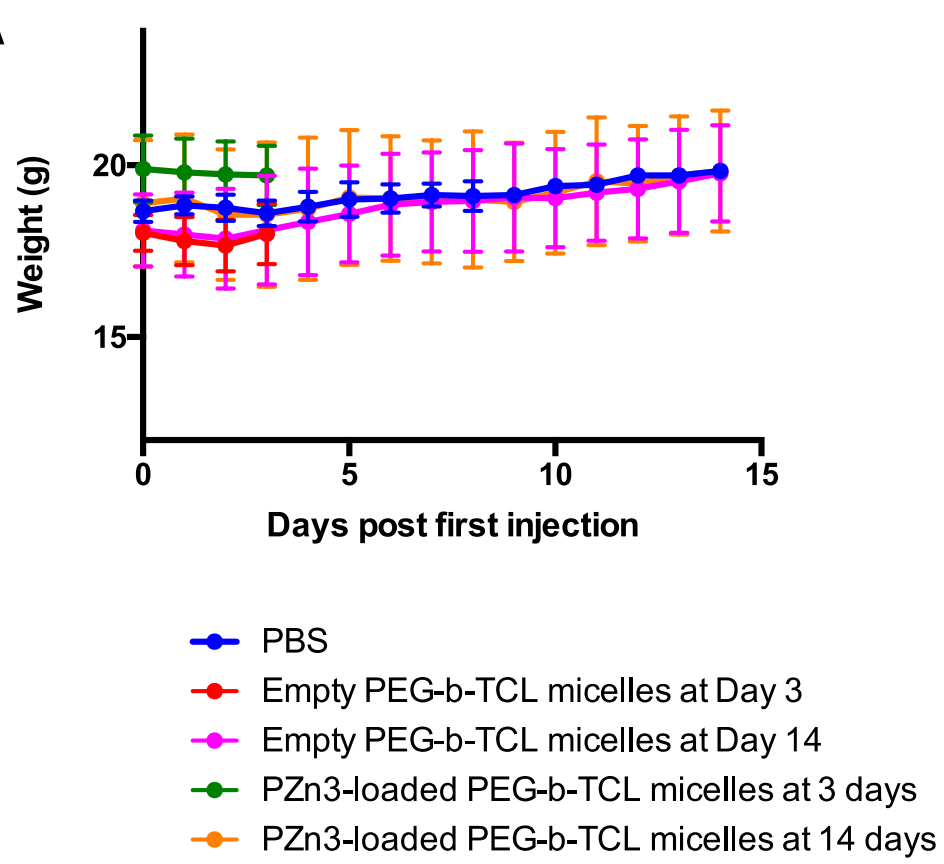


Figure S2. Determination of the emission half-life of PZn_3 in a suspension of PEG-*b*-TCL-based micelles in plasma at 37 °C. A total of 3 experimental runs were performed to determine changes in the emission spectra over a range of λ = 650 to 950 nm (left) and to calculate the AUC of emission over time (right): (A) run 1, (B) run 2. The third experimental run is shown in Figure 2B. The half-life of emission of PZn_3-loaded PEG-*b*-TCL-based micelles was determined from the decay constant of the slope of the exponential fit and the average value is reported in the main manuscript.

A



B

Treatment group	Mice tag	BUN	CREATININE
PBS Day 14	YY 905	23	0
	YY 906	20	0.2
	YY 908	18	0
Empty PEG-b-TCL micelles at Day 3	YY 901	28	0
	YY 903	25	0
	YY 904	23	0
Empty PEG-b-TCL micelles at Day 14	YY 902	22	0
	YY 909	20	0.1
	YY 912	26	0
PZn3-loaded PEG-b-TCL micelles at 3 days	YY 911	27	0
	YY 914	31	0
	YY 915	27	0
PZn3-loaded PEG-b-TCL micelles at 14 days	YY 907	20	0
	YY 910	20	0.1
	YY 913	18	0

C

Treatment group	Mice tag	ALK Phosphatase	ALT (SGPT)	AST(SGOT)	Total Bilirubin	Direct Bilirubin	Albumin
PBS Day 14	YY 905	97	52	262	0.7	0	3.2
	YY 906	98	25	62	0.1	0	2.8
	YY 908	86	28	108	0.2	0	2.6
Empty PEG-b-TCL micelles at Day 3	YY 901	93	95	436	0.7	0	3.6
	YY 903	94	46	132	0.4	0	3.2
	YY 904	99	54	200	0.5	0.1	3.4
Empty PEG-b-TCL micelles at Day 14	YY 902	100	188	330	0.2	0	2.4
	YY 909	91	27	80	0.1	0	2.6
	YY 912	104	59	128	0.2	0.1	2.5
PZn3-loaded PEG-b-TCL micelles at 3 days	YY 911	89	40	131	0.5	0	3
	YY 914	105	47	138	0.3	0	3.3
	YY 915	102	45	117	0.4	0	3.5
PZn3-loaded PEG-b-TCL micelles at 14 days	YY 907	90	29	64	0.1	0.1	2.5
	YY 910	82	30	111	0.1	0	2.7
	YY 913	88	27	61	0.2	0.1	2.5

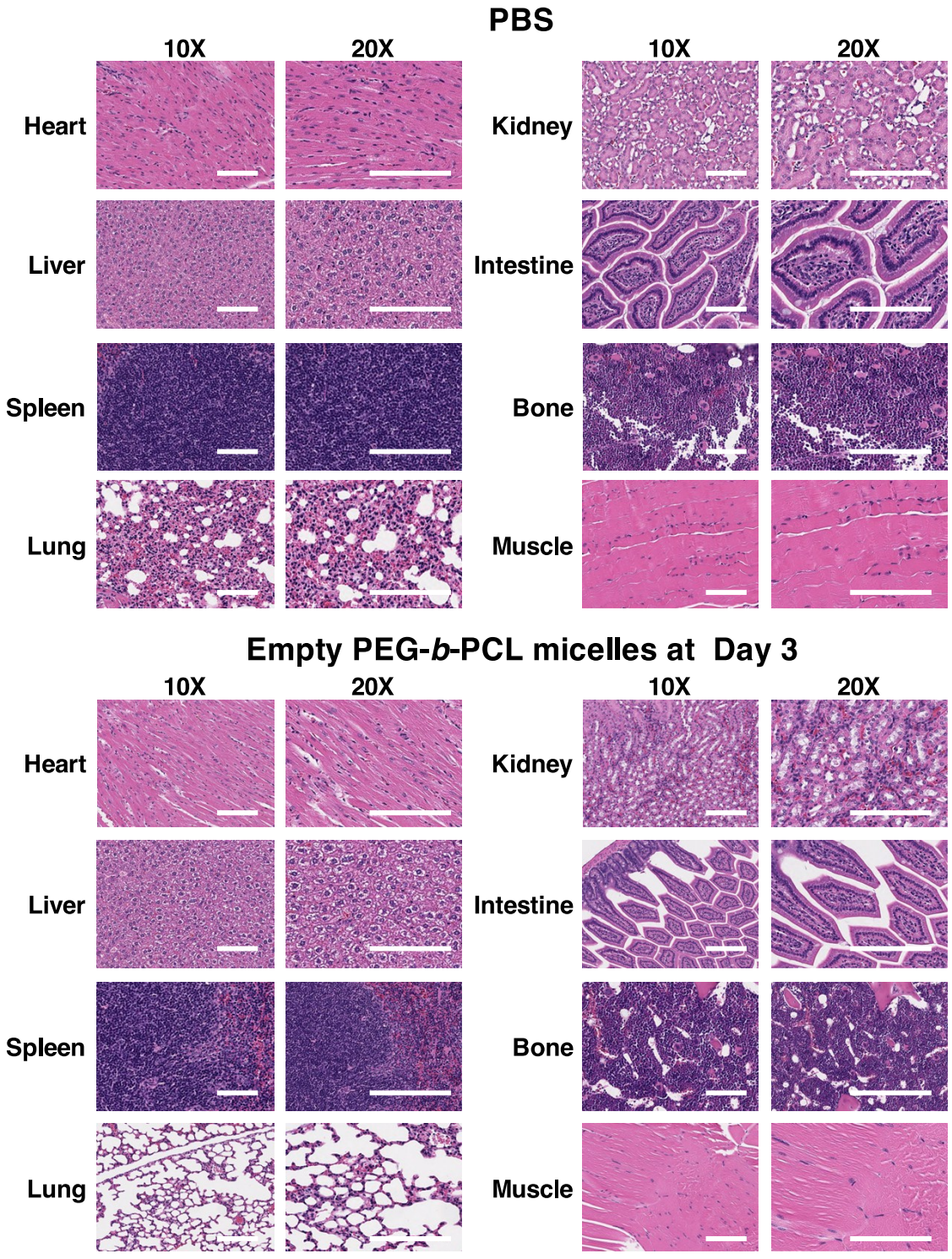
D

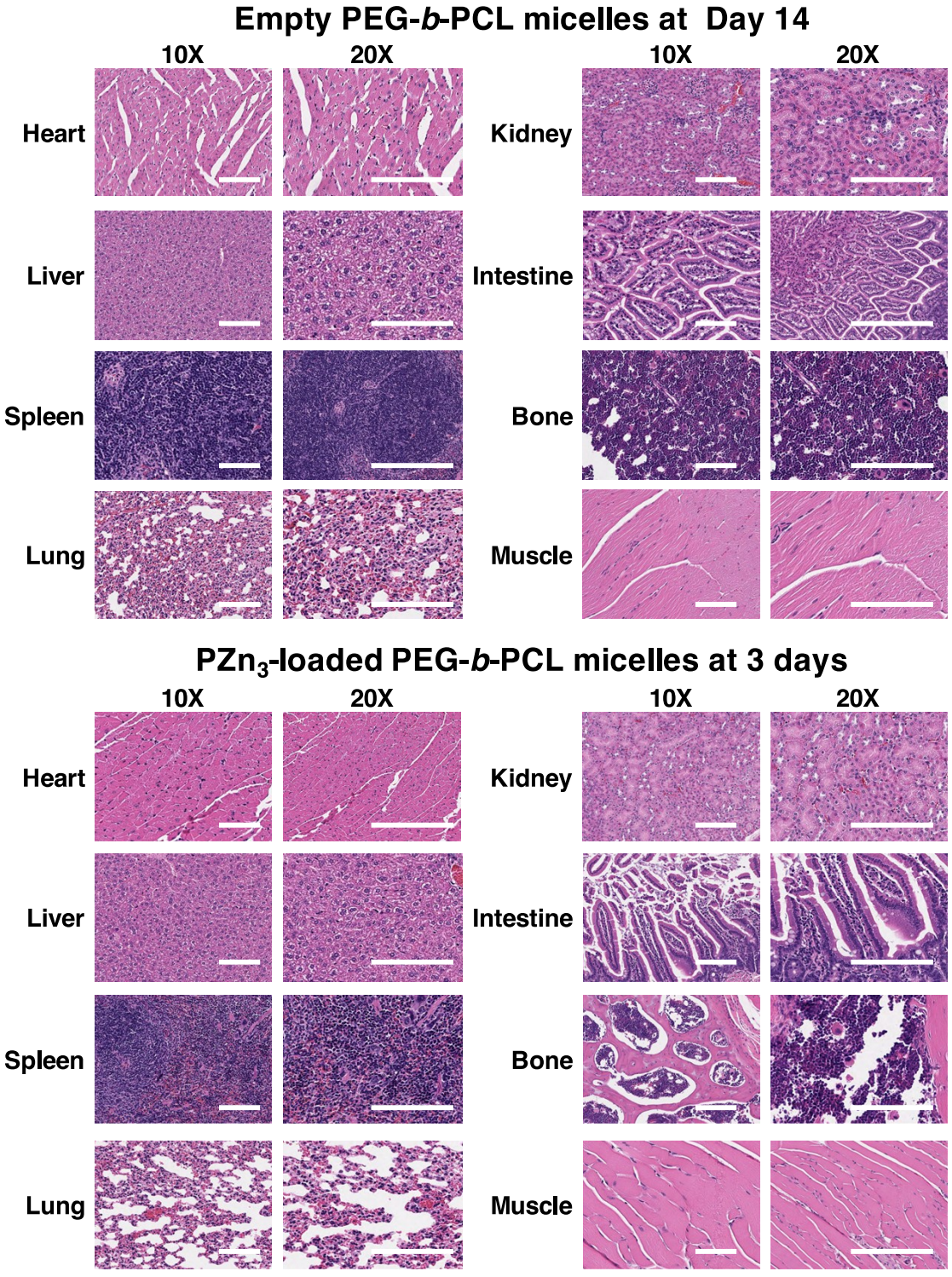
Treatment group	Mice tag	WBC (K/μL)	Hb(g/dL)	HCT(%)	PLT(K/μL)
PBS Day 14	YY 905	5	15.6	60.9	970
	YY 906	4.1	15.2	59	710
	YY 908	1.3	12.5	48.2	110
Empty PEG-b-TCL micelles at Day 3	YY 901	7	16.8	61.6	571
	YY 903	8.9	16	60.2	800
	YY 904	9.4	18.2	65.6	770
Empty PEG-b-TCL micelles at Day 14	YY 902	5.7	13.7	49.6	764
	YY 909	6.6	13.3	50.9	649
	YY 912	5.4	13.7	49.7	788
PZn3-loaded PEG-b-TCL micelles at 3 days	YY 911	7.8	15.1	59	983
	YY 914	6.5	17.3	64.1	792
	YY 915	8.7	13.5	51.1	817
PZn3-loaded PEG-b-TCL micelles at 14 days	YY 907	8.6	13.9	53.6	840
	YY 910	5.7	13.7	52.8	761
	YY 913	2.8	11.8	43.9	347

E

Treatment group	NE (K/μL)	LY (K/μL)	MO (K/μL)	EO (K/μL)
PBS Day 14	2.3	2.3	0.4	0
	1.5	2.3	0.3	0
	0.6	0.7	0.1	0
Empty PEG-b-TCL micelles at Day 3	3.1	3.3	0.4	2.4
	4.2	3.9	0.7	0.1
	4	4.4	0.5	0.3
Empty PEG-b-TCL micelles at Day 14	3.1	2.2	0.2	0.1
	2.5	2.5	0.4	0.1
	2.4	2.6	0.4	0
PZn3-loaded PEG-b-TCL micelles at 3 days	3	4.3	0.4	0.1
	2.7	3	0.8	0
	3.6	4.5	0.4	0.2
PZn3-loaded PEG-b-TCL micelles at 14 days	4	3.9	0.5	0.2
	3.1	2.2	0.2	0.1
	1.1	1.5	0.2	0.1

Figure S3. Assessment of gross, serologic, and hematologic toxicities of BALB/c mice at 3 or 14 days after a single dose administration of PBS, empty (i.e. unloaded), or PZn_3-loaded PEG-*b*-TCL micelles. The accompanying subfigures depict: A) the daily weights, B) the serology panel for biomarkers of renal function, C) the serology panel for biomarkers of hepatic function, D) the complete blood count (CBC) and E) the white blood cell differential counts; terminal blood draws were performed by cardiac puncture and major organs were harvested for H&E analysis (see Figure S4). White blood cell (WBC), hemoglobin (Hb), hematocrit (Hct), platelets (Plt), neutrophils (Ne), lymphocytes (Ly), monocytes (Mo), eosinophils (Eo), and Basophils (Ba).


PBS
10X
20X
10X
20X
Heart
Kidney
Liver
Intestine
Spleen
Bone
Lung
Muscle
Empty PEG-b-PCL micelles at Day 3
10X
20X
10X
20X
Heart
Kidney
Liver
Intestine
Spleen
Bone
Lung
Muscle


Empty PEG-b-PCL micelles at Day 14
10X
20X
10X
20X
Heart
Kidney
Liver
Intestine
Spleen
Bone
Lung
Muscle
PZn3-loaded PEG-b-PCL micelles at 3 days
10X
20X
10X
20X
Heart
Kidney
Liver
Intestine
Spleen
Bone
Lung
Muscle

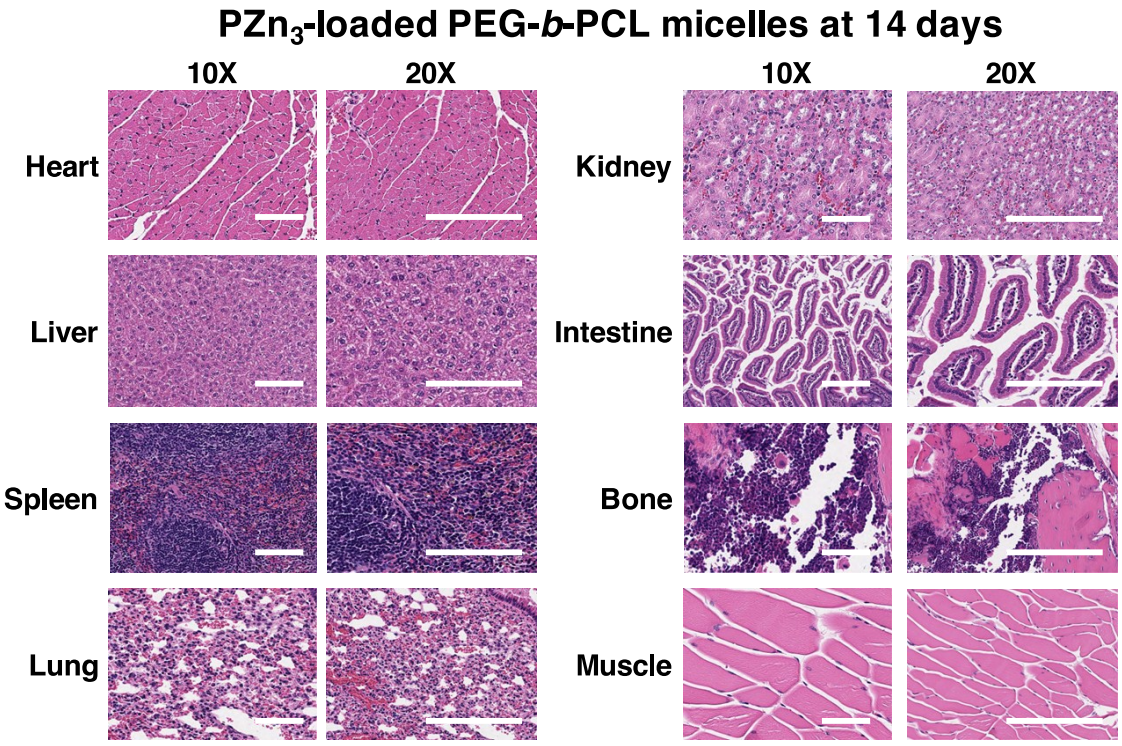


Figure S4. H&E of organs harvested from BALB/c mice at at 3 or 14 days after a single dose administration of PBS, empty (i.e. unloaded), or PZn_3-loaded PEG-*b*-TCL micelles. BALB/c mice at 4-6 weeks of age were randomly grouped (n=3 mice per group per time point). Each mouse was treated with a single dose of the following by IV administration: PBS, empty (i.e. unloaded) or PZn3-loaded PEG-b-TCL micelles. The mice were monitored and weighed daily; and, they were sacrificed at 3 days or at 14 days after administration. Scale bar: 200 μm.

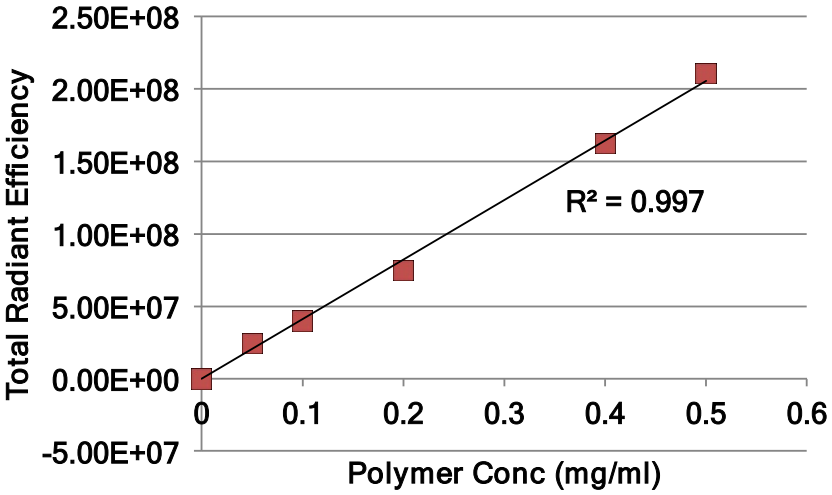


Figure S5. Quantification of the concentration of PEG-*b*-TCL-based micelles based on the emission of PZn_3 loaded in their cavities. Standard curves depicting the linear correlation between the fluorescence intensity of PZn_3 and the concentration of PZn_3-loaded PEG-*b*-TCL-based micelles in plasma. The standard curve was generated using known concentrations of micelles that were added to mouse plasma, collected into heparinized capillary tubes, and imaged with the IVIS Kinetic® Imaging instrument (λ_{ex}= 745 nm, λ_{em}= 810-875 nm).

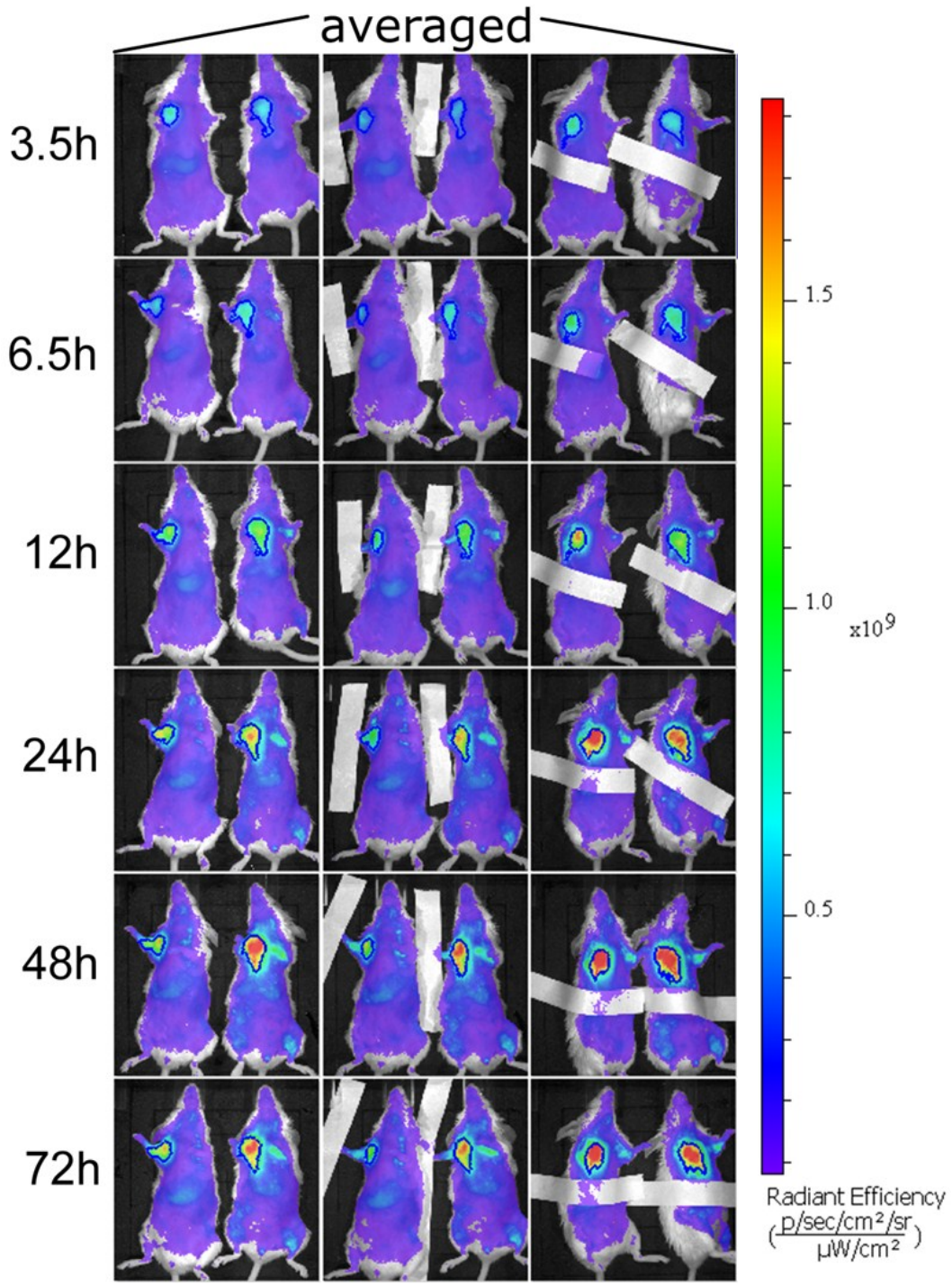


Figure S6. ***In vivo*** **tumor accumulation of PZn_3-loaded PEG-*b*-TCL-based micelles**. Whole-animal optical imaging was performed to quantify PZn_3 fluorescence emission signals from micelles at various time points after systemic administration via IV tail-vein injection. Representative images demonstrate the 3 positions that were used for each mouse and at each time point to determine the average tumor radiant efficiencies. Mice were treated with 110 nm-diameter PZn_3-loaded PEG-*b*-TCL-based micelles and were imaged using the IVIS® Kinetic Imaging instrument (λ_{ex}= 745 nm, λ_{em}= 810-875 nm).