Supporting Information for

ORIGINAL ARTICLE

The long-circulating effect of pegylated nanoparticles revisited *via* simultaneous monitoring of both the drug payloads and nanocarriers

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Received 25 August 2021; Received in revised form 19 October 2021; accepted 25 October 2021

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Table S1 Within-day precision of determination of mPEG-PCL nanoparticles (n = 3).

Organ	C (mg/mL)	**Within-day precision (%)**					
		PCL-80	$mPEG_{5k}$-9%-80	$mPEG_{5k}$-17%-80	$mPEG_{5k}$-29%-80	$mPEG_{2k}$-29%-80 nm	$mPEG_{5k}$-29%-200
Heart	0.0048	8.33	6.55	8.33	8.18	9.12	8.81
	0.078	2.92	0.51	2.69	3.90	6.37	3.32
	2.5	1.89	2.59	3.54	4.43	8.89	5.03
Liver	0.0048	8.81	5.88	7.90	4.88	6.55	6.45
	0.078	2.18	4.72	1.63	5.80	1.08	2.67
	2.5	3.39	3.45	3.90	6.74	5.81	1.86
Spleen	0.0048	7.51	8.33	9.12	7.16	6.74	4.68
	0.078	3.38	1.19	4.45	9.08	1.72	4.34
	2.5	2.02	2.41	6.94	4.35	5.87	3.50
Lung	0.0048	7.53	9.62	4.88	9.17	2.28	3.85
	0.078	3.28	2.91	3.10	3.87	3.63	2.79
	2.5	3.37	2.84	2.39	7.26	5.53	1.57
Kidney	0.0048	7.51	9.17	7.51	8.18	8.65	7.05
	0.078	8.14	2.82	8.10	4.01	1.47	1.89
	2.5	2.32	4.32	7.67	4.27	3.96	5.23
Blood	0.0048	9.35	8.92	9.96	7.90	4.17	5.09
	0.078	6.49	3.43	5.39	9.12	6.09	3.27
	2.5	2.81	4.34	5.60	4.24	6.61	4.24

Table S2 Between-day precision of determination of mPEG-PCL nanoparticles (n = 3).

Organ	C (mg/mL)	**Between-day precision (%)**					
		PCL-80	$mPEG_{5k}$-9%-80	$mPEG_{5k}$-17%-80	$mPEG_{5k}$-29%-80	$mPEG_{2k}$-29%-80	$mPEG_{5k}$-29%-200 nm
Heart	0.0048	8.81	4.88	7.90	7.51	5.68	5.88
	0.078	5.98	5.00	3.41	5.09	2.99	2.23
	2.5	2.46	1.99	1.59	6.24	7.73	6.55
Liver	0.0048	9.17	3.94	8.25	4.17	6.28	2.59
	0.078	4.36	4.72	5.30	3.17	5.01	6.51
	2.5	5.08	7.29	5.64	4.16	3.57	6.74
Spleen	0.0048	5.87	5.41	7.51	5.33	5.33	4.35
	0.078	8.25	7.19	3.24	3.22	7.14	6.68
	2.5	2.47	2.41	5.96	3.86	4.95	4.93
Lung	0.0048	4.75	9.17	4.68	6.67	6.55	4.00
	0.078	4.30	3.88	1.88	1.92	4.57	3.61
	2.5	2.20	5.97	8.92	5.82	5.80	5.05
Kidney	0.0048	7.90	5.26	7.90	5.56	8.33	4.55
	0.078	6.20	3.66	1.84	1.25	1.11	5.19
	2.5	1.23	4.86	3.99	5.33	7.41	5.99
Blood	0.0048	5.99	6.19	6.67	8.66	6.55	4.55
	0.078	2.48	1.60	0.93	5.96	6.46	9.93
	2.5	1.76	5.02	7.59	5.29	7.90	0.36

Table S3 Accuracy of determination of mPEG-PCL nanoparticles. Data are presented as mean±SD (*n*=3).

Organ	*C* (mg/mL)	**Accuracy (%)**					
		PCL-80 nm	$mPEG_{5k}$-9%-80 nm	$mPEG_{5k}$-17%-80 nm	$mPEG_{5k}$-29%-80 nm	$mPEG_{2k}$-29%-80 nm	$mPEG_{5k}$-29%-200
Heart	0.0048	95.21±7.93	93.64±6.68	98.20±6.30	98.33±6.40	96.71±3.15	92.96±7.85
	0.078	95.24±2.46	93.98±4.44	92.43±2.28	95.81±2.60	90.78±2.84	91.11±3.55
	2.5	101.04±4.29	101.42±2.78	98.46±2.73	98.69±2.56	96.59±3.11	99.33±3.01
Liver	0.0048	103.63±9.56	91.47±7.94	97.35±6.52	98.64±3.60	101.23±4.83	99.94±6.46
	0.078	101.22±6.82	93.79±3.52	94.08±2.11	90.49±1.86	94.26±1.56	90.29±4.13
	2.5	99.68±6.96	100.92±3.94	102.12±4.67	97.14±3.33	105.53±4.59	97.25±2.15
Spleen	0.0048	93.93±7.85	90.51±1.80	94.03±7.29	97.75±4.76	97.00±4.80	99.85±4.82
	0.078	90.16±3.23	90.75±2.99	93.54±2.48	90.72±3.70	90.74±4.20	92.02±1.14
	2.5	99.62±1.59	100.23±2.42	101.59±7.23	97.29±2.70	98.90±3.54	101.59±4.86
Lung	0.0048	94.65±8.47	92.97±6.30	91.33±3.14	94.29±4.90	103.53±6.29	99.39±3.14
	0.078	93.97±3.57	94.68±1.87	92.37±2.43	92.24±5.75	92.94±3.47	90.35±3.81
	2.5	99.07±5.63	98.91±1.26	101.35±5.52	94.56±7.60	98.17±2.72	97.33±2.72
Kidney	0.0048	92.00±4.76	93.81±4.80	91.86±7.24	103.99±6.49	96.17±4.79	105.81±4.74
	0.078	91.42±4.61	91.77±3.48	91.45±3.32	91.74±4.48	91.36±4.26	90.99±2.54
	2.5	99.96±2.16	97.37±2.18	102.69±1.84	99.88±3.66	99.79±5.05	100.06±3.88
Blood	0.0048	90.29±6.56	93.20±7.86	95.07±3.13	93.28±4.83	96.83±3.67	102.78±3.14
	0.078	93.59±8.53	90.74±3.96	91.32±4.06	92.81±3.70	94.53±2.72	90.32±3.20
	2.5	101.79±1.21	96.69±4.91	94.65±3.31	105.53±3.95	101.29±4.33	100.44±2.72

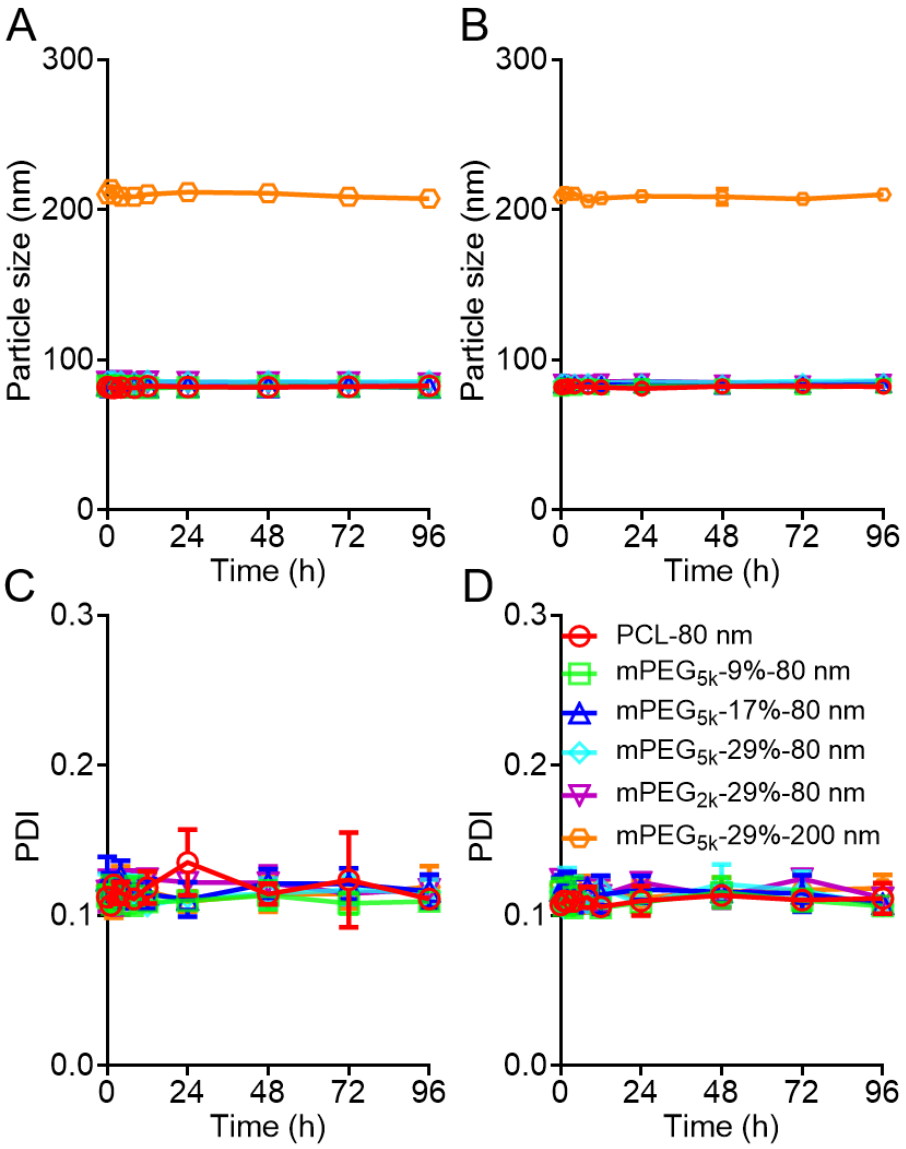

Figure S1 Physical stability of mPEG-PCL nanoparticles incubated in different media: A-B, particle size (A, purified water; B, PBS, pH=7.4); C-D, PDI (C, purified water; D, PBS, pH = 7.4). Data are presented as mean ± SD (n = 3).

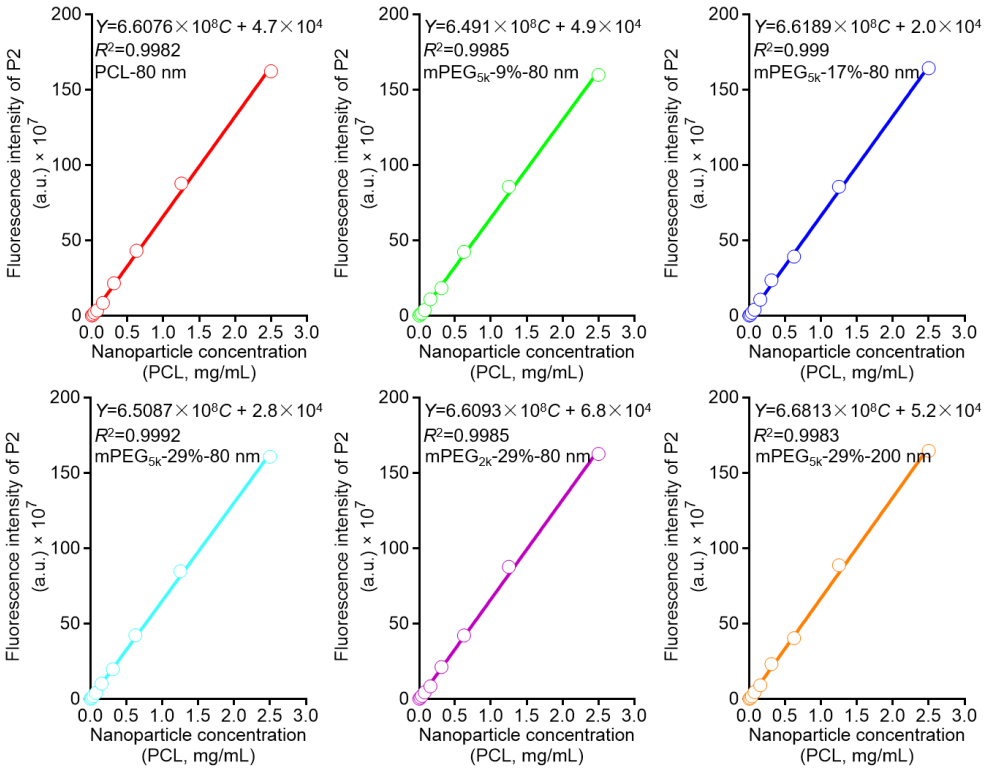

Figure S2 Calibration curves of mPEG-PCL nanoparticles in the heart obtained by linear regression of fluorescence intensity of P2 probe loaded in nanoparticle by IVIS *vs.* nanoparticle concentration. The PCL content was utilized to represent the content of nanoparticles. The nanoparticle suspension was sequentially diluted by heart homogenate.

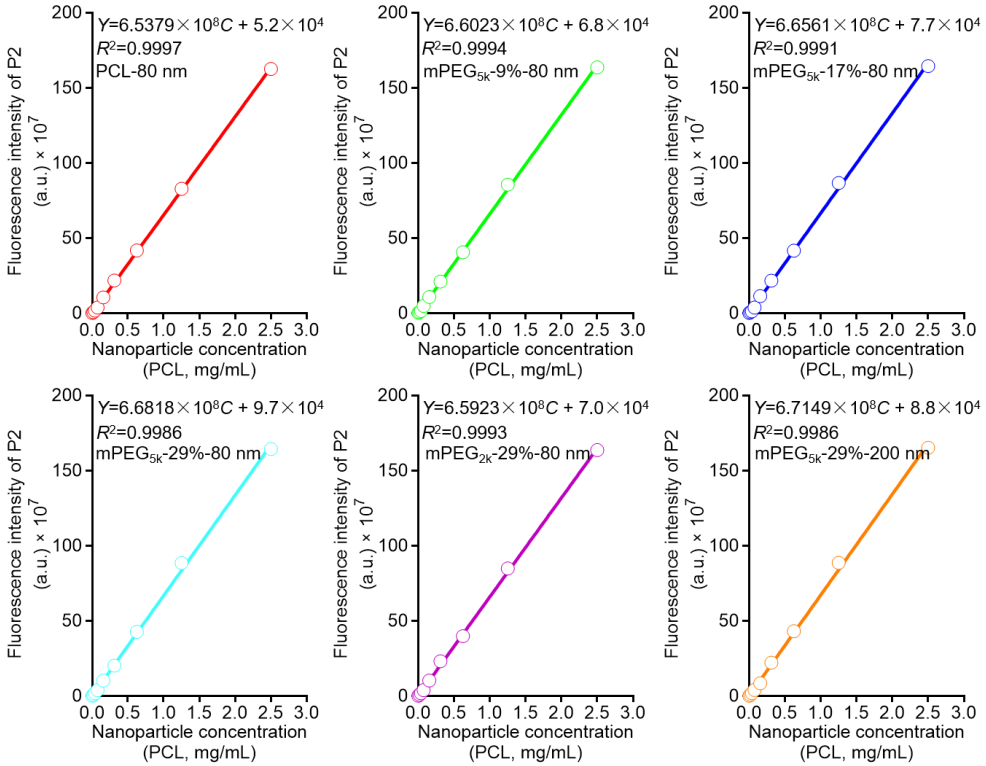

Figure S3 Calibration curves of mPEG-PCL nanoparticles in the liver obtained by linear regression of fluorescence intensity of P2 probe loaded in nanoparticle by IVIS *vs.* nanoparticle concentration. The PCL content was utilized to represent the content of nanoparticles. The nanoparticle suspension was sequentially diluted by liver homogenate.

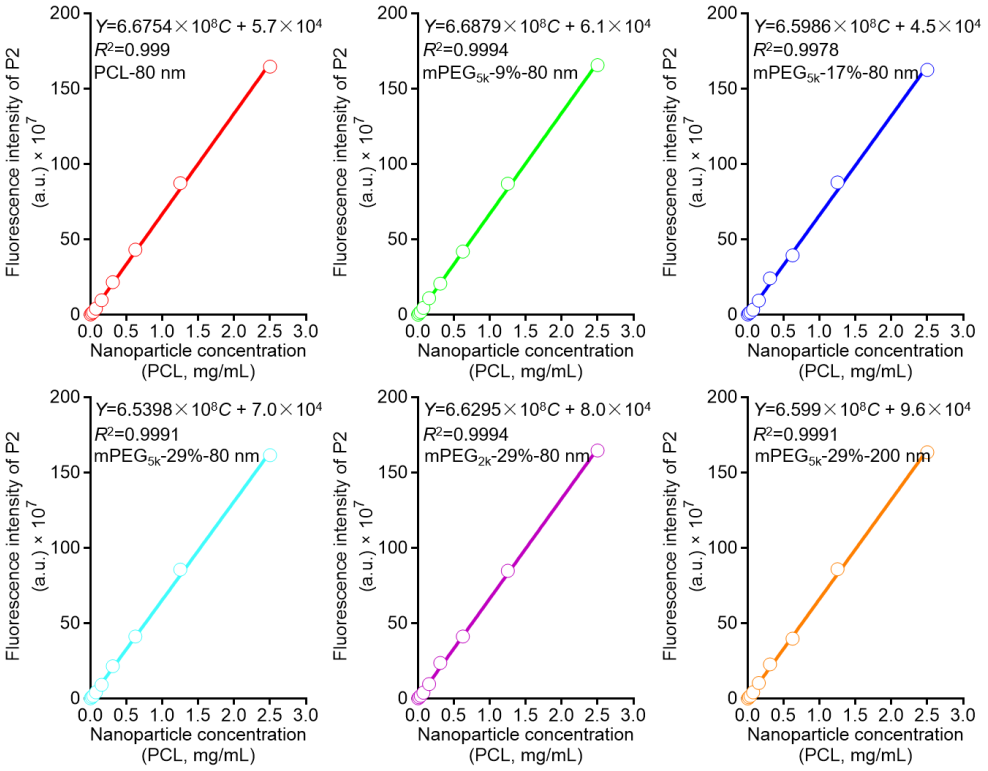

Figure S4 Calibration curves of mPEG-PCL nanoparticles in the spleen obtained by linear regression of fluorescence intensity of P2 probe loaded in nanoparticle by IVIS *vs.* nanoparticle concentration. The PCL content was utilized to represent the content of nanoparticles. The nanoparticle suspension was sequentially diluted by spleen homogenate.

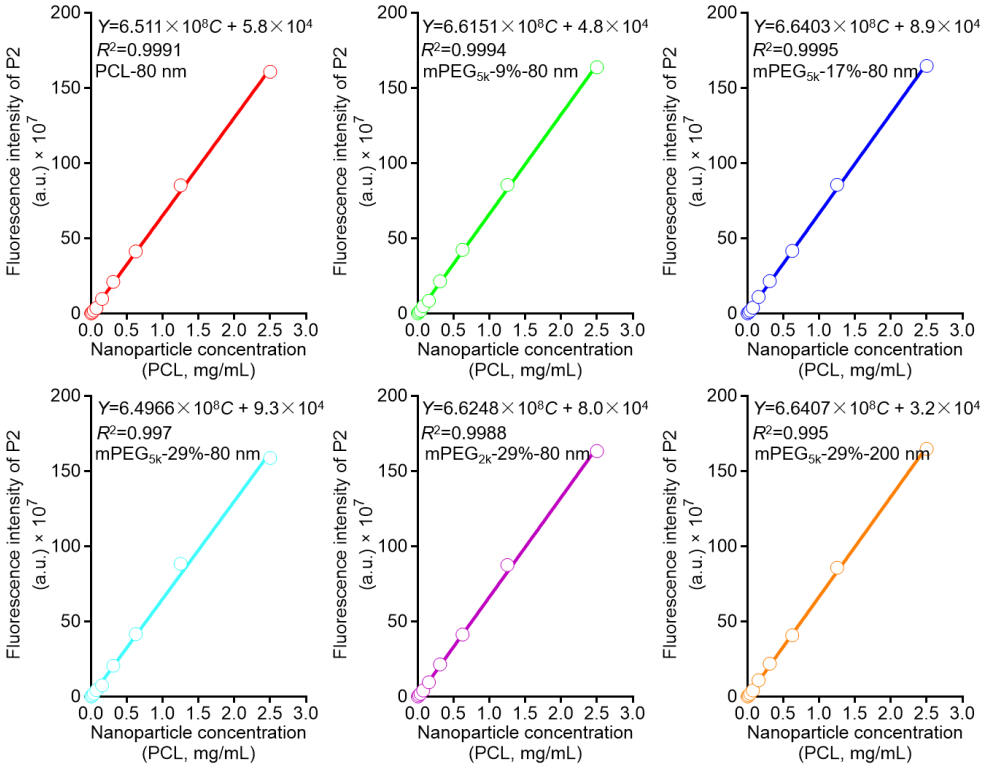

Figure S5 Calibration curves of mPEG-PCL nanoparticles in the lungs obtained by linear regression of fluorescence intensity of P2 probe loaded in nanoparticle by IVIS *vs.* nanoparticle concentration. The PCL content was utilized to represent the content of nanoparticles. The nanoparticle suspension was sequentially diluted by lung homogenate.

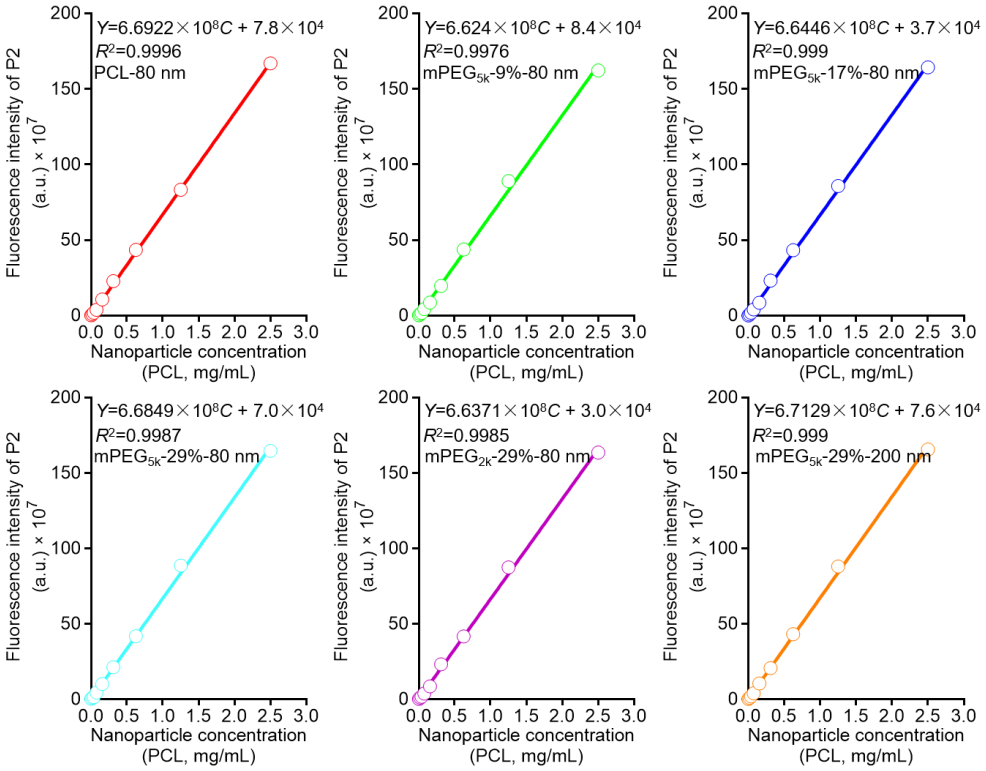

Figure S6 Calibration curves of mPEG-PCL nanoparticles in the kidneys obtained by linear regression of fluorescence intensity of P2 probe loaded in nanoparticle by IVIS *vs.* nanoparticle concentration. The PCL content was utilized to represent the content of nanoparticles. The nanoparticle suspension was sequentially diluted by kidney homogenate.

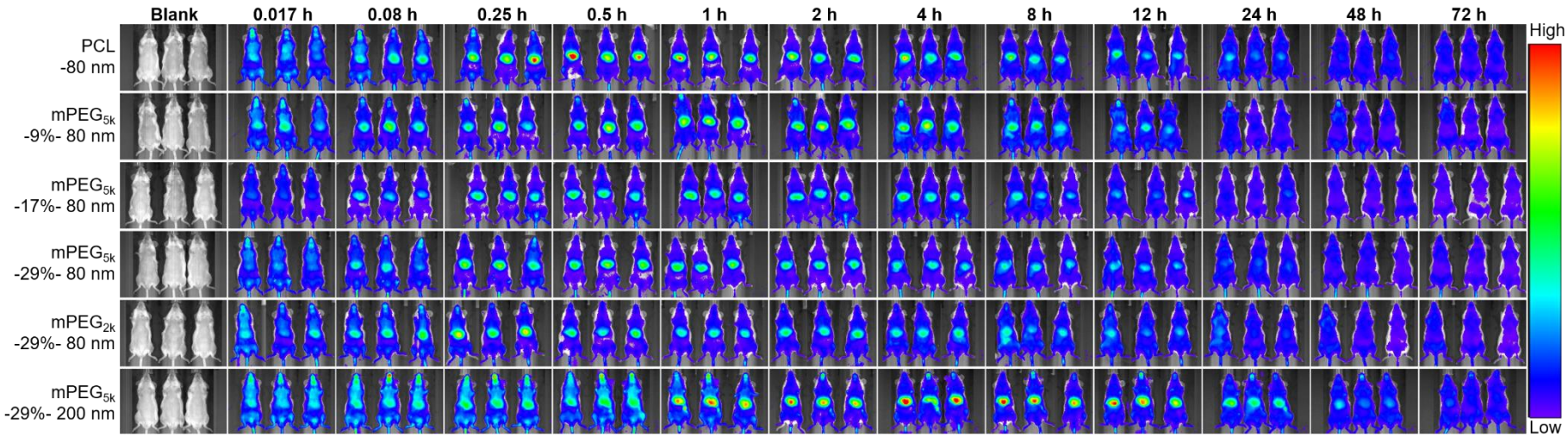

Figure S7 Live imaging of the distribution of P2-loaded mPEG-PCL nanoparticles after intravenous administration.

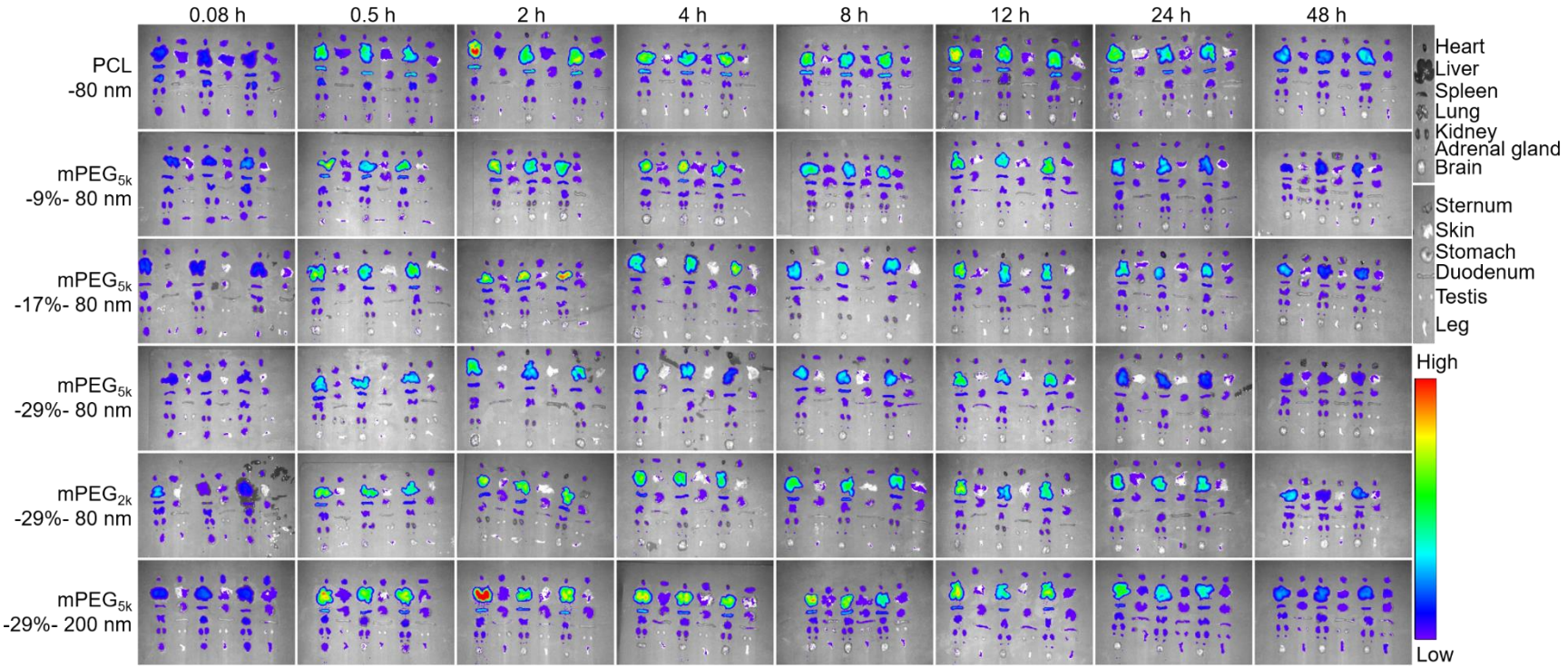

Figure S8 Live imaging of *ex vivo* organs and tissues dissected after intravenous administration of P2-loaded mPEG-PCL nanoparticles.

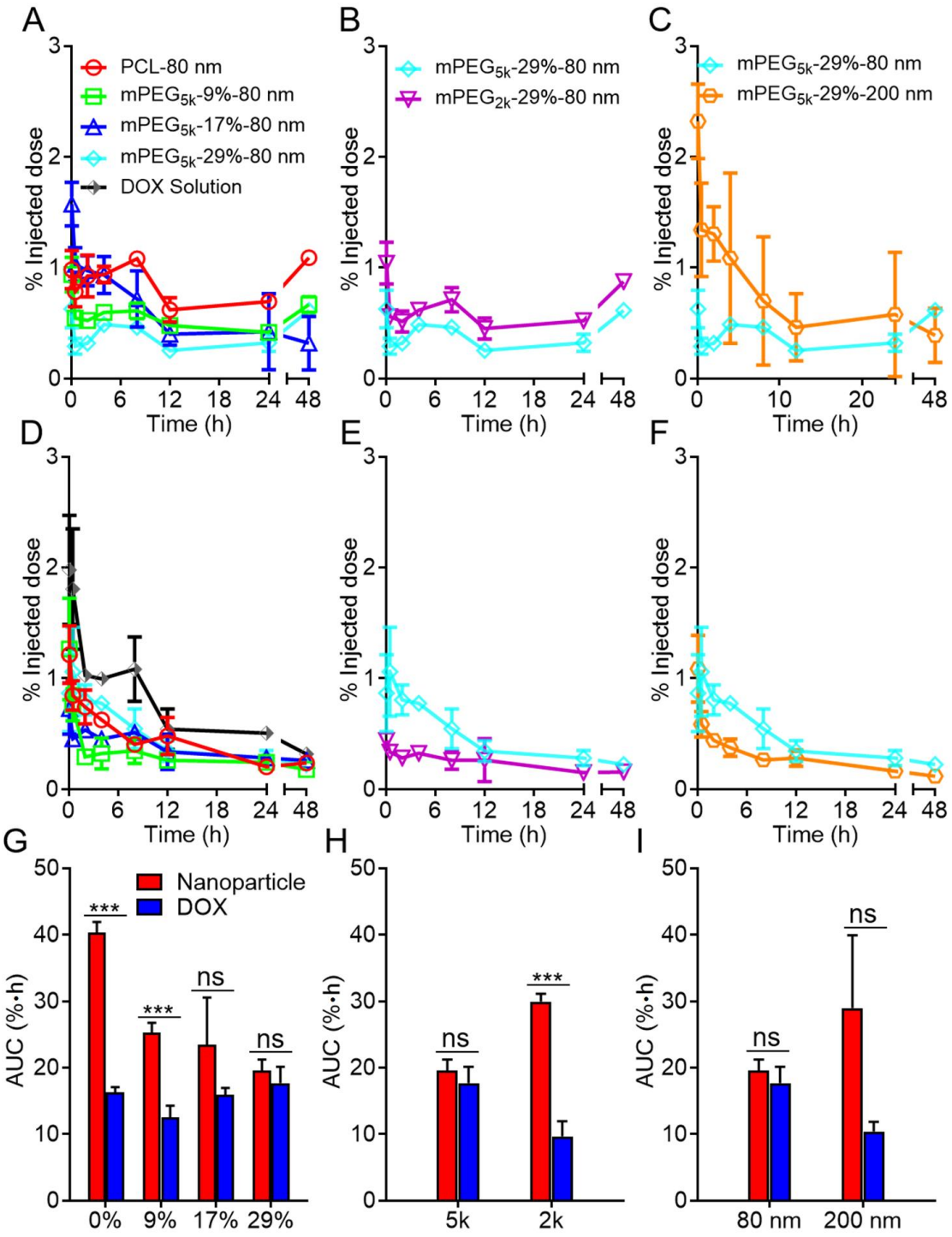

Figure S9 Distribution content of nanoparticle (A-C) and DOX (D-F) encapsulated in mPEG-PCL nanoparticle in the heart as highlighted by comparing PEGylation density (A, D), PEG chain length (B, E) and particle size (C, F), respectively, while bar plot G, H & I present corresponding AUC values. Data are presented as mean ± SD (n = 3). ***P < 0.001, ns, not significant.

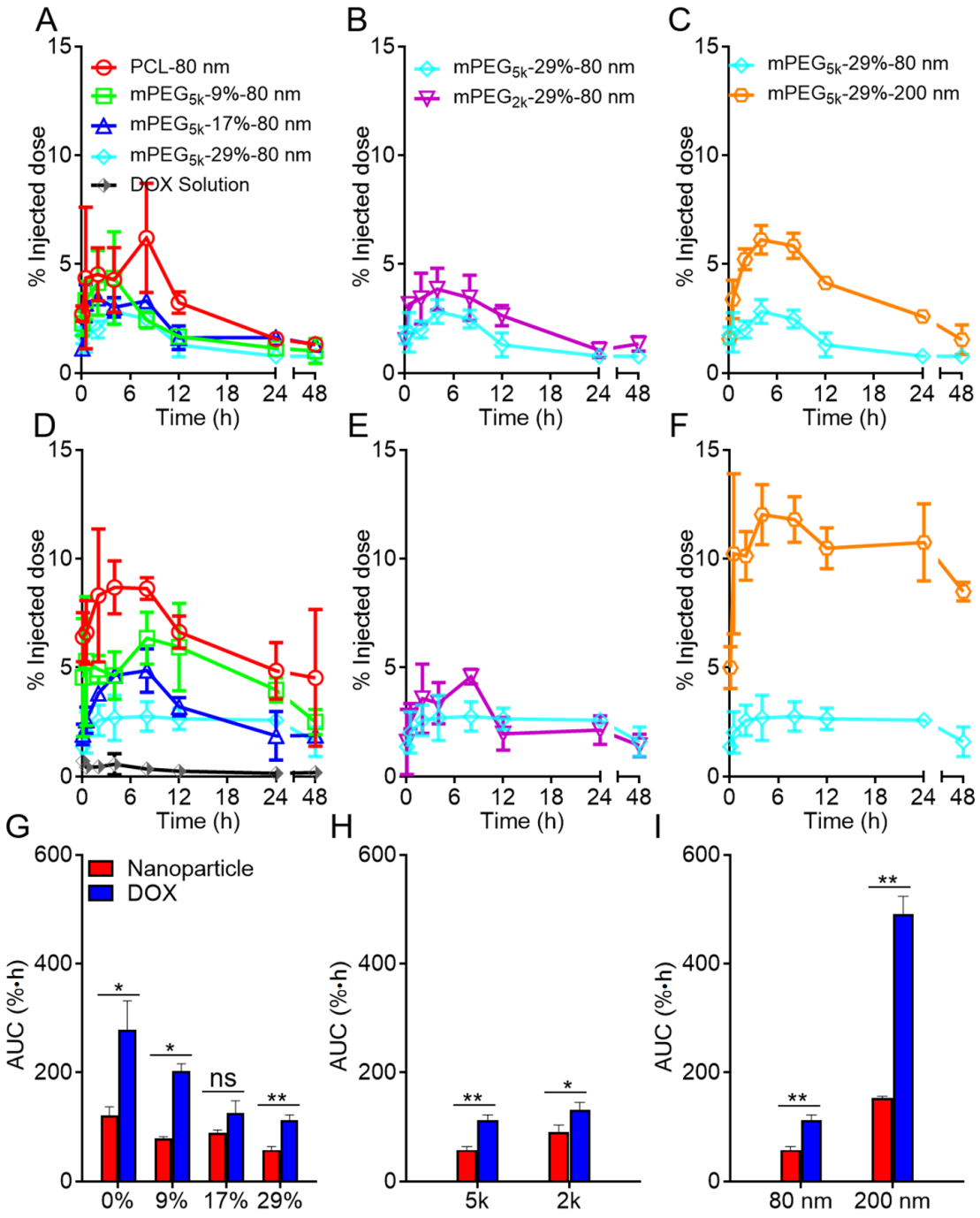

Figure S10 Distribution content of nanoparticle (A-C) and DOX (D-F) encapsulated in mPEG-PCL nanoparticle in the spleen as highlighted by comparing PEGylation density (A, D), PEG chain length (B, E) and particle size (C, F), respectively, while bar plot G, H & I present corresponding AUC values. Data are presented as mean ± SD ($n = 3$). *$P < 0.05$. **$P < 0.01$. ns, not significant.

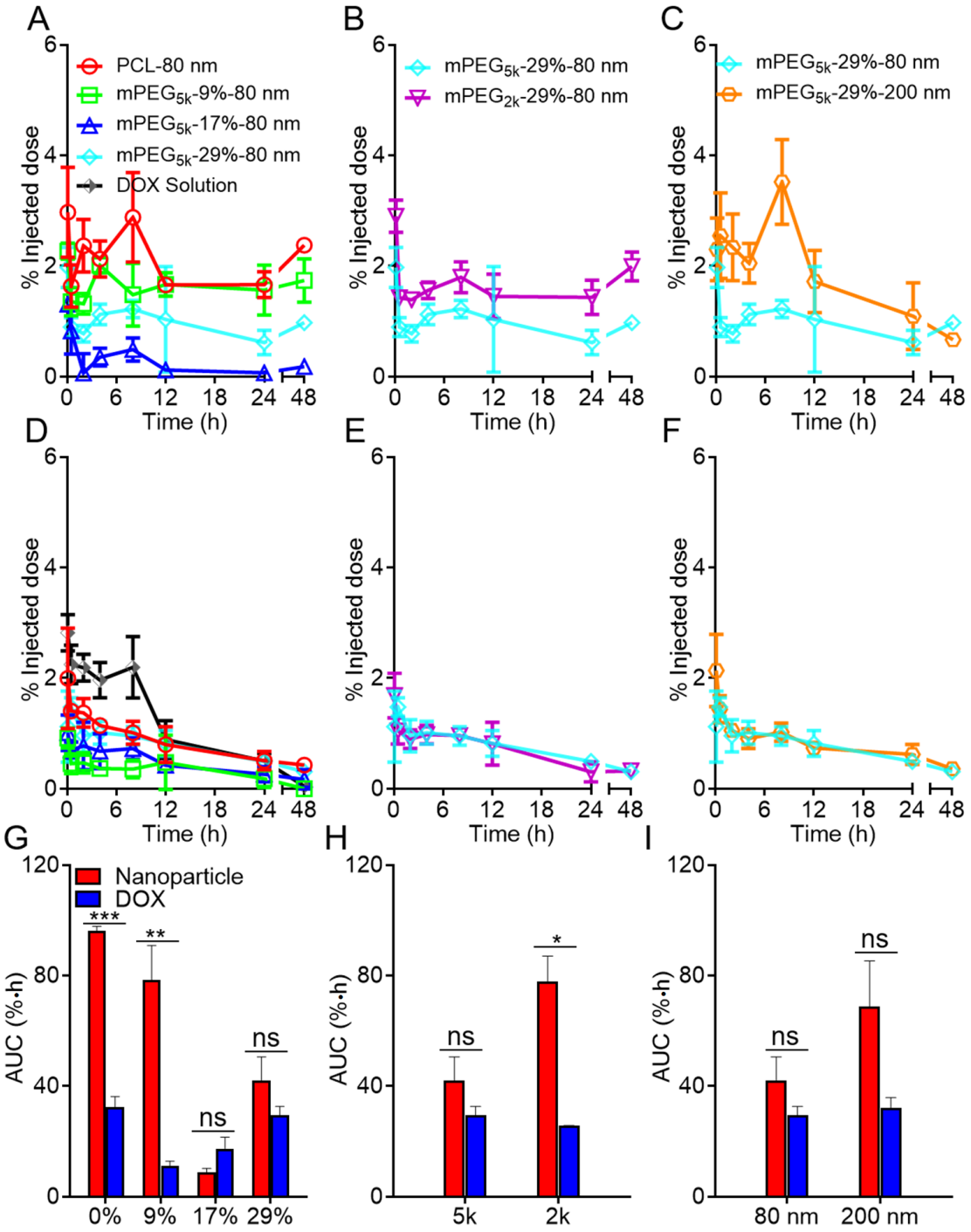

Figure S11 Distribution content of nanoparticle (A-C) and DOX (D-F) encapsulated in mPEG-PCL nanoparticle in the lungs as highlighted by comparing PEGylation density (A, D), PEG chain length (B, E) and particle size (C, F), respectively, while bar plot G, H & I present corresponding AUC values. Data are presented as mean ± SD ($n = 3$). *$P < 0.05$, **$P < 0.01$. ***$P < 0.001$. ns, not significant.

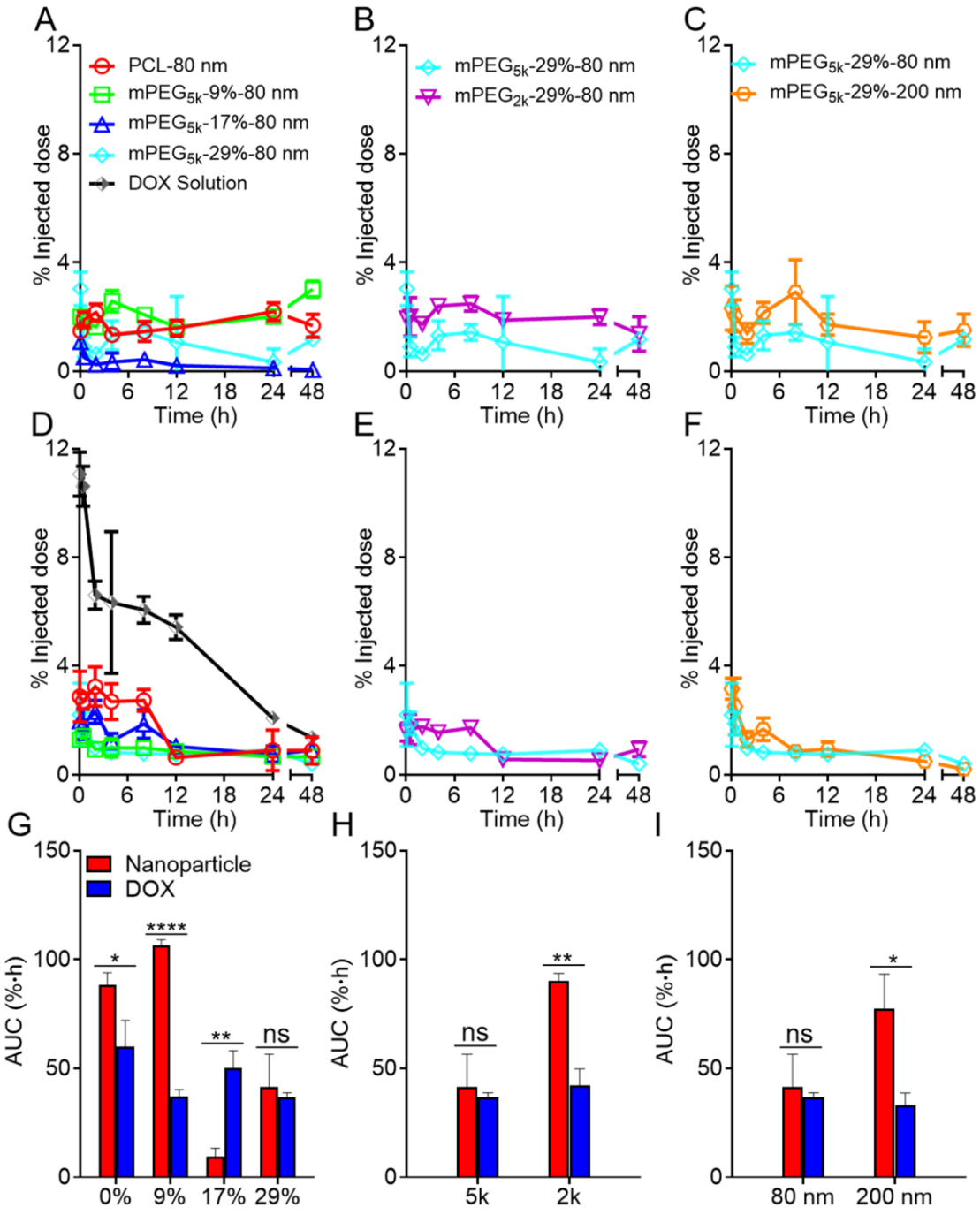

Figure S12 Distribution content of nanoparticle (A-C) and DOX (D-F) encapsulated in mPEG-PCL nanoparticle in the kidneys as highlighted by comparing PEGylation density (A, D), PEG chain length (B, E) and particle size (C, F), respectively, while bar plot G, H & I present corresponding AUC values. Data are presented as mean ± SD ($n = 3$). *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$. ****$P < 0.0001$. ns, not significant.