Electronic Supplementary Material (ESI) for Nanoscale.

Multi-component bioresponsive nanoparticles for synchronous delivery of Docetaxel and TUBB3 siRNA to lung cancer cells

Claudia Conte[a,b]*, Patrícia Monteiro[a], Pratik Gurnani[a], Snow Stolnik[a], Francesca Ungaro[b], Fabiana Quaglia[b], Philip Clarke[c], Anna Grabowska[c], Maria Kavallaris[d,e] and Cameron Alexander[a]*

[a]Division of Molecular Therapeutics and Formulation, School of Pharmacy, University of Nottingham, NG7 2RD, UK; [b]Drug Delivery Laboratory, Department of Pharmacy, University of Napoli Federico II, 80131 Napoli, Italy; [c]The BioDiscovery Institute and Faculty of Medicine & Health Sciences, University of Nottingham, NG7 2RD, UK; [d]Tumour Biology and Targeting Program, Children's Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, Sydney, NSW 2052 , Australia; [e]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology and Australian Centre for NanoMedicine, University of New South Wales, Sydney, NSW 2052, Australia; [f]School of Women's and Children's Health, Faculty of Medicine, UNSW Sydney, NSW, Australia 2052.

* Corresponding authors

claudia.conte@unina.it (C. Conte), cameron.alexander@nottingham.ac.uk (C. Alexander)

Table S1. Composition and properties of NPs loaded with Luc-siRNA.

Code	siRNA (% w/w)	Yield (%)	Mean D_H (nm)	P.I.	ζ (mV)	siRNA Actual loading[a] (Entr. Eff %[b])
Luc-siRNA/PLGA-PEG	0.1	65	175	0.115	-7.4	0.1 (95)
Luc-siRNA/PLGA-SS-PEG	0.1	71	152	0.184	-9.3	0.1 (94)

[a] Actual loading is expressed as the amount (mg) of drug encapsulated *per* 100 mg of NPs.

Table S2. Composition and properties of NPs loaded with Cy™3-siRNA.

Code	siRNA (% w/w)	Mean D_H (nm)	P.I.	ζ (mV)	siRNA Actual loading[a]
Cy™3-siRNA/ PLGA-PEG	0.1	161	0.125	-6.4	0.1
Cy™3-siRNA/ PLGA-SS-PEG	0.1	148	0.117	-8.2	0.1

[a] Actual loading is expressed as the amount (mg) of drug encapsulated *per* 100 mg of NPs.

Table S3. IC_{50} values (μg/mL) of free DTX and DTX- NPs with or without TUBB3-siRNA in A549 and H1299 lung cancer cells after 72 h of treatment.

	A549			**H1299**		
	IC_{50} (μg/mL±SD)	P.F.[a]	P.F.[b]	IC_{50} (μg/mL±SD)	P.F.[a]	P.F.[b]
Free DTX	102.6±2.0	-	-	0.4±0.1	-	-
DTX/PLGA-PEG	132.8±1.4	<1	-	3.4±0.1	<1	-
TUBB3-DTX/PLGA-PEG	98.7±1.7	<1	1.3	2.9±0.1	1.2	<1
DTX/PLGA-SS-PEG	79.8±1.9	1.3	-	8.3±0.2	<1	-
TUBB3-DTX/PLGA-SS-PEG	6.5±2.0	16	12.3	0.4±0.04	<1	16

[a] Potentiating factor values of NPs versus free DTX
[b] Potentiating factor values of DTX/*TUBB3*-NPs versus the corresponding DTX-NPs

Table S4. Composition and properties of NPs loaded with a scrambled siRNA.

Code	siRNA (% w/w)	Mean D_H (nm)	P.I.	ζ (mV)	siRNA Actual loading[a] (Entr. Eff %[b])
scrambled-siRNA/PLGA-PEG	0.1	176±4	0.12	-1.6±2.3	0.1 (95)
scrambled-siRNA/PLGA-SS-PEG	0.1	187±3	0.15	-1.9±4.4	0.1 (94)

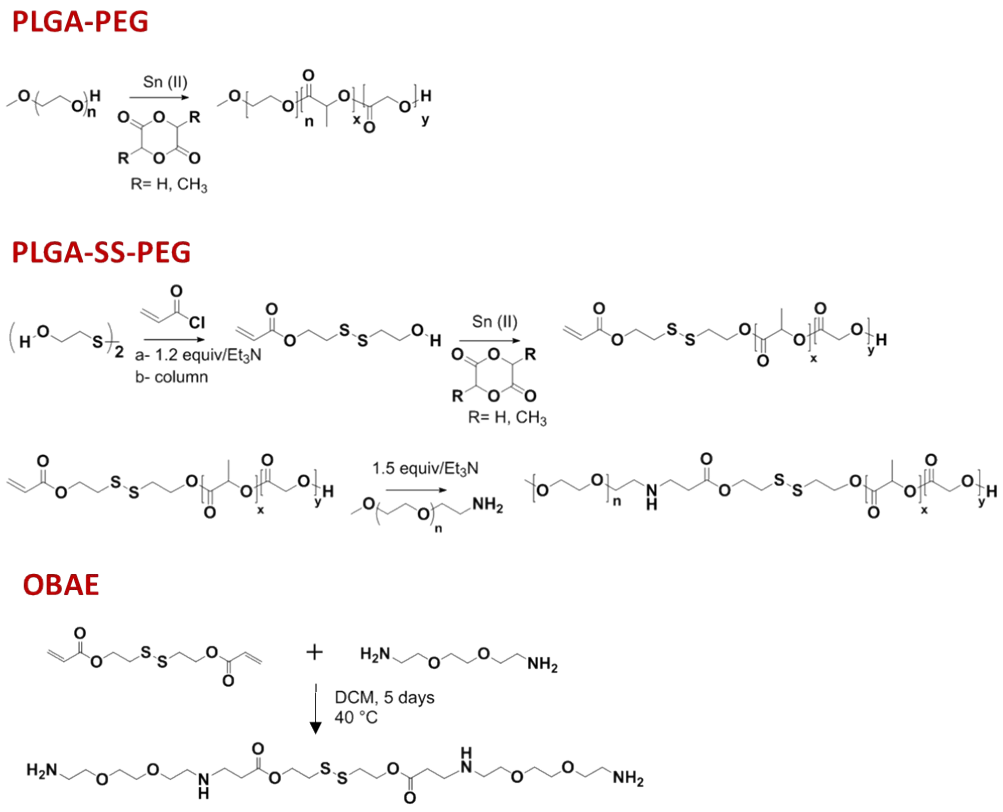


Figure S1. Schematic illustration of synthesis of the materials

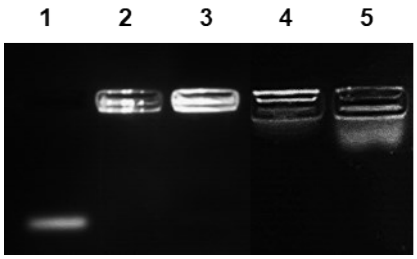


Figure S2. Gel retardation assay of siRNA-loaded NPs. Lane 1: naked TUBB3-siRNA; lane 2-3: TUBB3-DTX/PLGA-PEG NPs and TUBB3-DTX/PLGA-SS-PEG NPs; lane 4-5: TUBB3-DTX/PLGA-PEG NPs and TUBB3-DTX/PLGA-SS-PEG NPs after incubation in GSH 10 mM for 30 minutes.

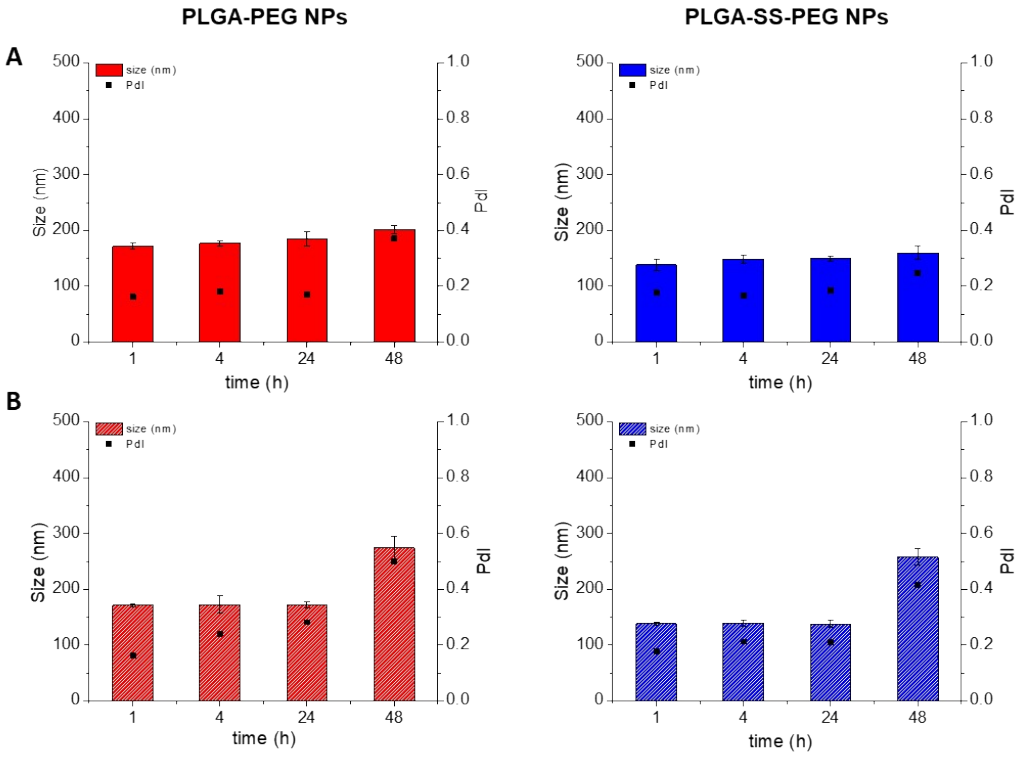


Figure S3. Stability of NPs in PBS pH 7.4 (A) or PBS pH 7.4 with Fetal Bovine Serum 10% (B). Results are expressed as mean ± SD of three experiments.

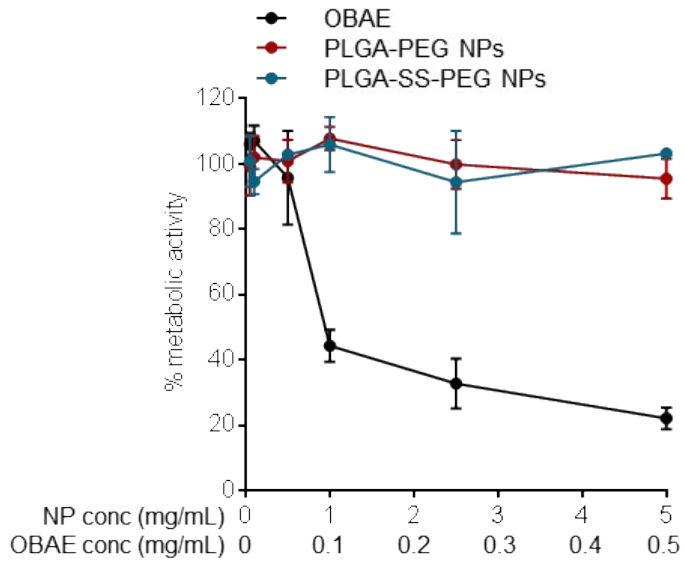


Figure S4. Cytotoxicity of unloaded NPs against H1299 cancer cells compared to free OBAE (24 h of incubation). Concentration of free OBAE is in the same range entrapped into NPs (0.005-0.5 mg/mL).

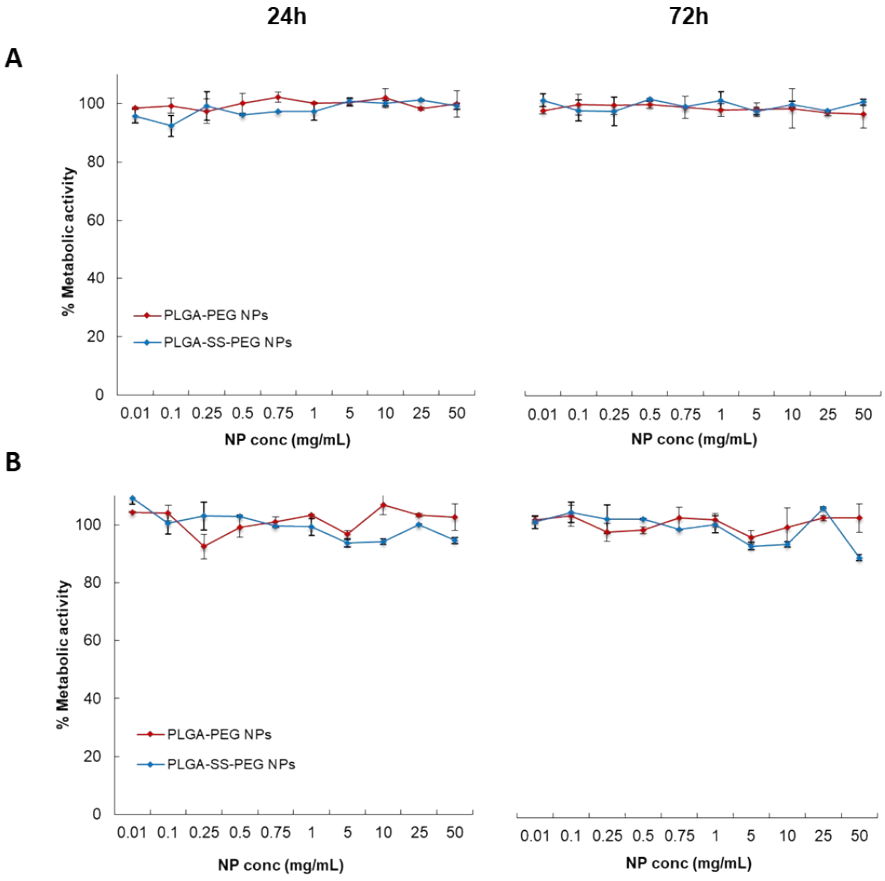


Figure S5. Cell metabolic activity of PLGA-PEG NPs and PLGA-SS-PEG NPs toward (A) A549 and (B) H1299 cells after 24 h and 72 h of incubation. Results are expressed as mean ± SD of three experiments.

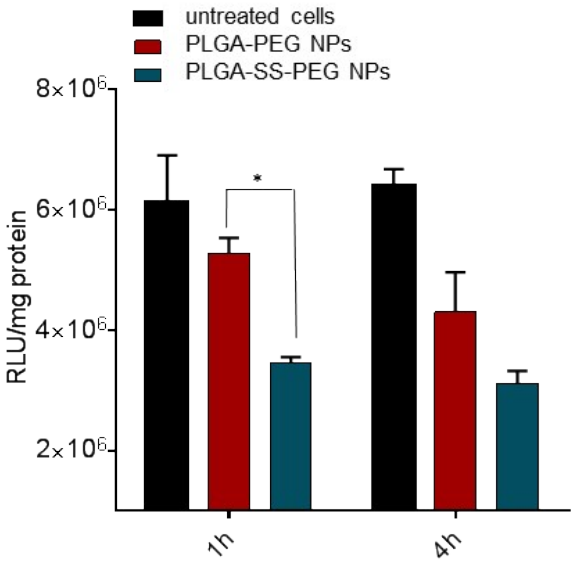


Figure S6. In vitro luciferase siRNA transfection efficacy of NPs in A549-luciferase expressing cells reported as relative light units (RLU/mg protein) of Luciferase activity. Results are expressed as mean ± SD of three experiments. **P<0.01, *P<0.05 two-way ANOVA test.

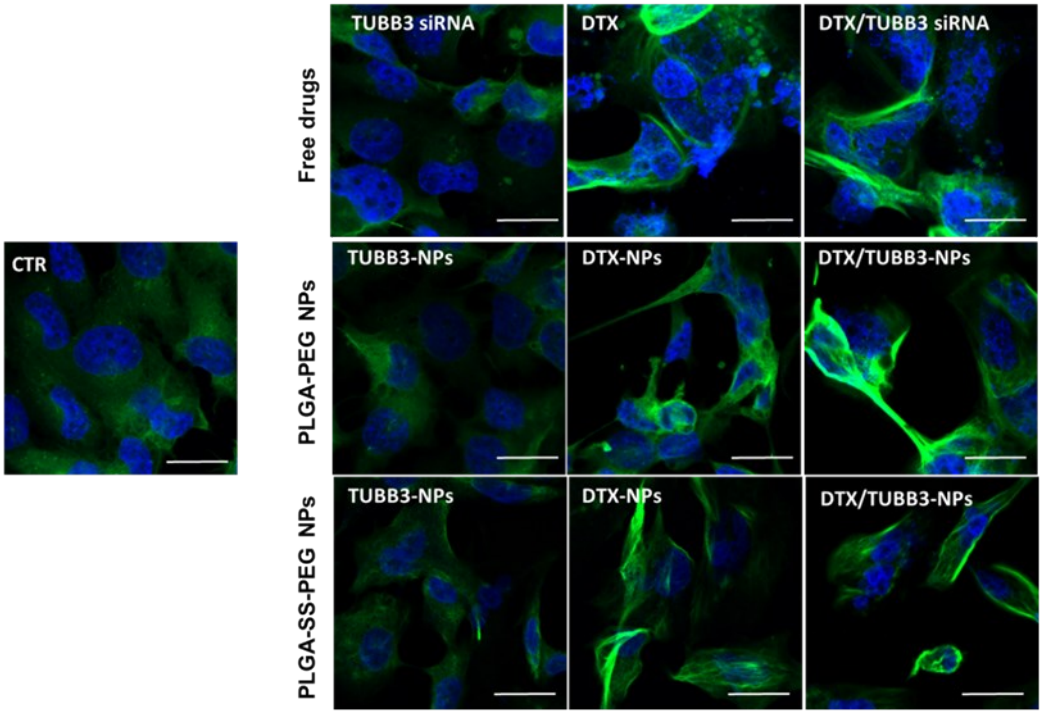


Figure S7. Immunofluorescence microscopy of H1299 cells treated with NPs for 72h. βIII-tubulin was stained in green whereas nuclei were stained with Hoechst 33258 in blue. Scale bar: 10 μm. Zen 2009 image Software was utilized for image processing.

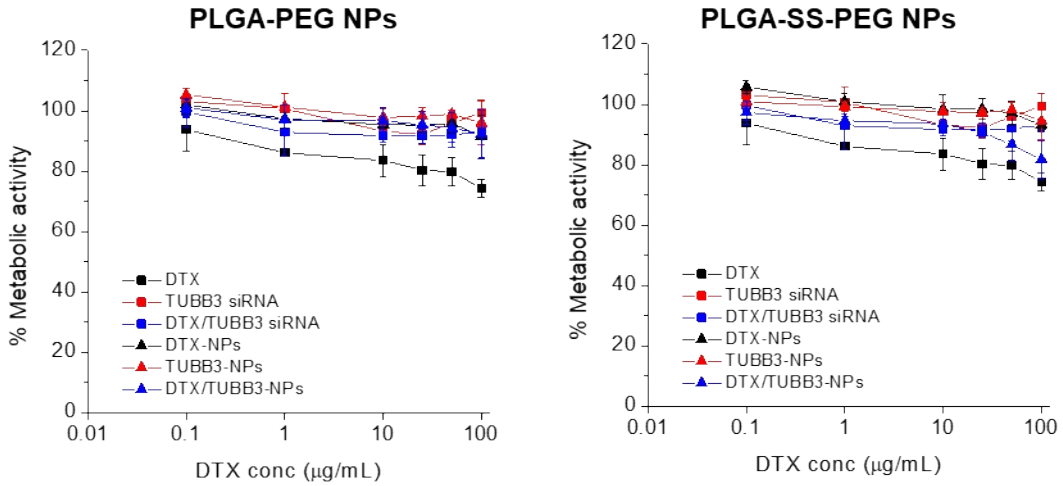


Figure S8. Cell metabolic activity of PLGA-PEG NPs and PLGA-SS-PEG NPs toward A549 cells after 24 h of incubation. Results are expressed as mean ± SD of three experiments.

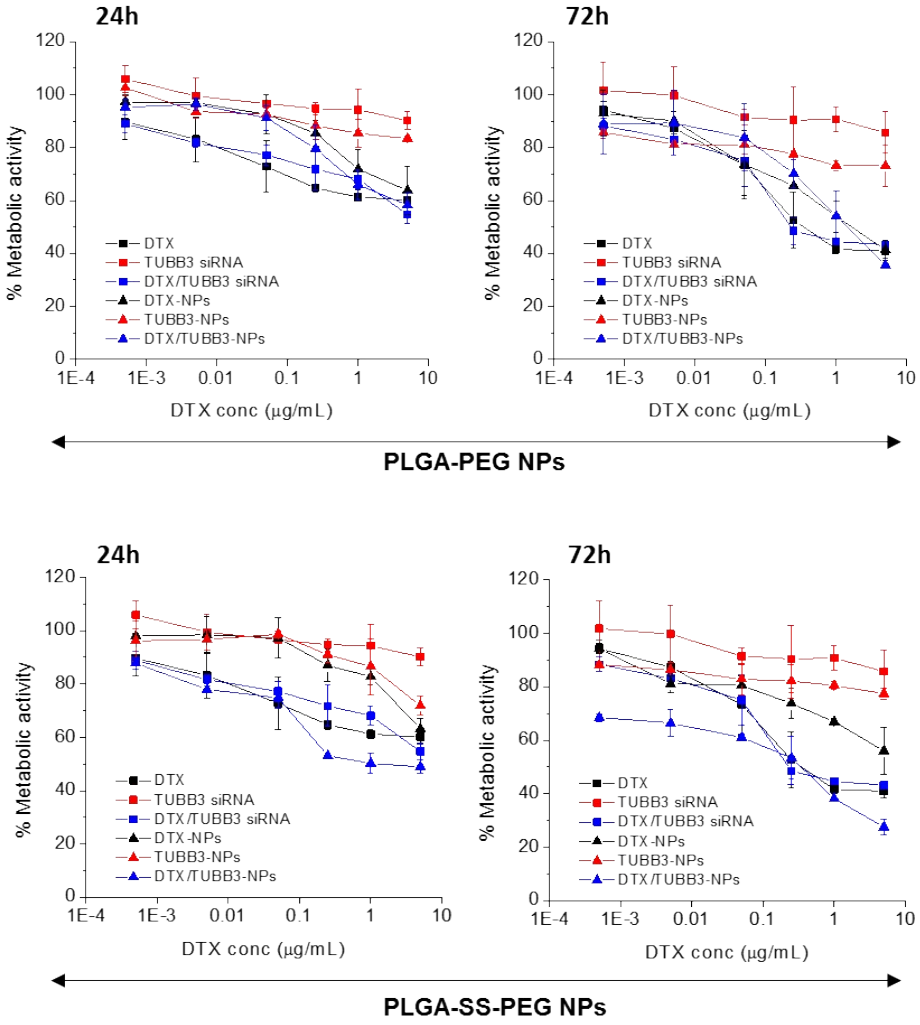


Figure S9. Cell metabolic activity of PLGA-PEG NPs and PLGA-SS-PEG NPs toward H1299 cells after 24 h and 72 h of incubation. Results are expressed as mean ± SD of three experiments.

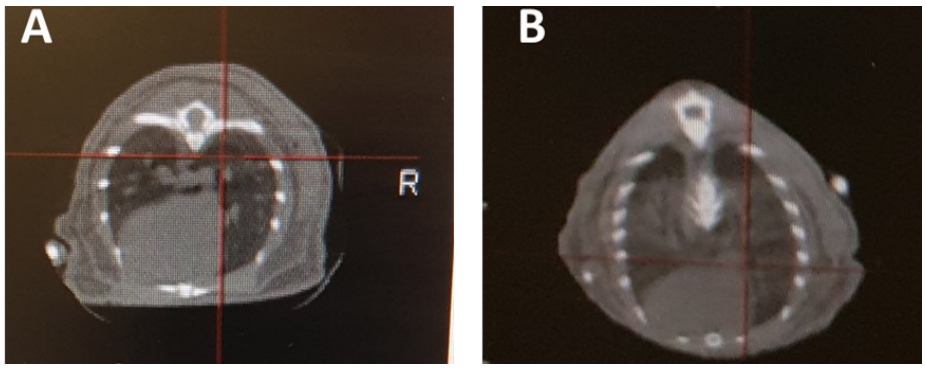
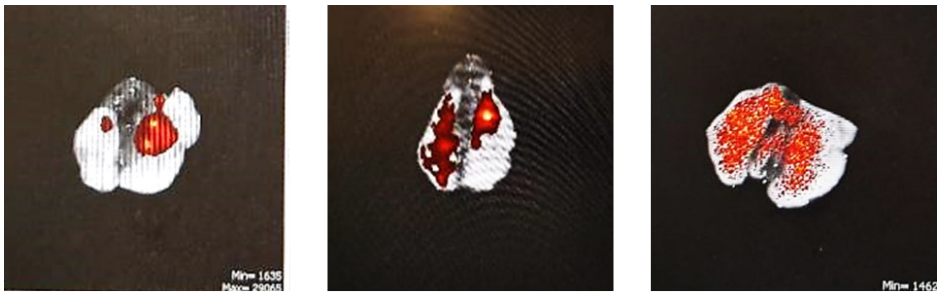
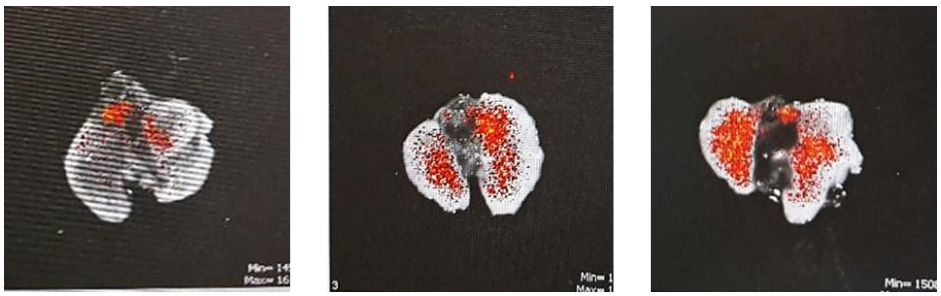


Figure S10. In vivo X-ray of lungs in A) healthy mice and B) tumor-bearing mice.



PLGA-PEG



PLGA-SS-PEG

Figure S11. Live DiR fluorescence in the lungs 24 h after intratracheal administration of NPs. Each figure corresponds to 1 mouse. Ex/Em = 710/780 nm.

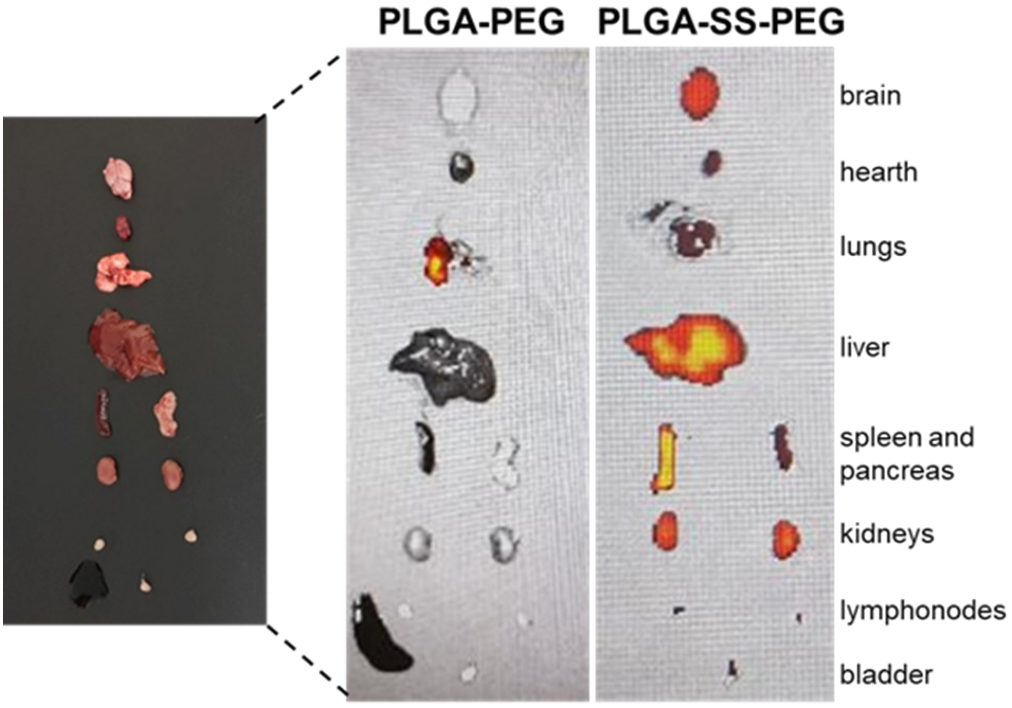


Figure S12. Ex vivo DiR fluorescence images of mice organs 24 h after intratracheal administration of NPs.