ADVANCED MATERIALS

Supporting Information

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Porous Silicon Nanoparticle Delivery of Tandem Peptide Anti-Infectives for the Treatment of *Pseudomonas aeruginosa* Lung Infections

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Name	Sequence	Mw	AA	+AA (%)	-AA (%)	pI	AI
Lactoferrin	KCFQWQRNMRKVRGPPVSCIKR	2718.3	22	32	0	11.6	44
S413-PV	ALWKTLLKKVLKAPKKKRKV	2377.0	20	45	0	11.5	117
CecroA+Mel	KWKLFKKIGIGAVLKVLTTGLPALIS	2795.5	26	19	0	10.6	150
Buforin 2	TRSSRAGLQFPVGRVHRLLRK	2434.8	21	29	0	12.6	88
Magainin	GIGKWLHSAKKFGKAFVGEIMNS	2505.9	23	17	4	10.0	72
Pep1	KETWWETWWTEWSQPKKKRKV	2848.2	21	29	14	9.8	14
Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ	2847.4	26	19	0	12.0	135
GALA	WEAALAEALAEALAEHLAEALAEALEALAA	3032.4	30	0	23	3.8	138
Apidaecins	GNNRPVYIPQPRPPHPRL	2108.4	18	17	0	11.7	59
Tat	YGRKKRRQRRRG	1616.9	12	67	0	12.3	0
KFFKFFKFFK	KFFKFFKFFK	1413.7	10	40	0	10.5	0
YTA4	IAWVKAFIRKLRKGPLG	1953.4	17	29	0	12.0	121
M918	MVTVLFRRLRIRRACGPPRVRV	2652.3	22	32	0	12.4	110
Penetratin	RQIKIWFQNRRMKWKK	2246.7	16	44	0	12.3	49
VP22	NAATATRGRSAASRPTQRPRAPARSASRPRRPVQ	3656.0	34	26	0	12.9	32
HGP	LLGRRGWEVLKYWWNLLQYWSQEL	3137.6	24	13	8	8.5	110
Bac7	RRIRPRPPRLPRPRPRPLPFPRPG	2938.5	24	38	0	12.9	49
6R	RRRRRR	955.1	6	100	0	12.7	0
HuT-cell CPP	YARVRRRGPRGYARVRRRGPRR	2768.2	22	50	0	12.4	35
pVEC	LLIILRRRIRKQAHAHSK	2209.7	18	33	0	12.5	141
CADY	GLWRALWRLLRSLWRLLWRA	2622.2	13	40	0	12.6	147
MAP	KLALKLALKALKAALKLA	1877.4	18	28	0	10.6	185

Table S1. Tandem peptide library

A library of membrane-active peptides were selected and are listed with their molecular weight (Mw), number of amino acids (AA), percentage of positive amino acids (+AA), percentage of negative amino acids (-AA), isoelectric point (pI), and aliphatic index (AI) calculated from ExPASy - ProtParam. All peptides were synthesized in the format from N- to C-terminus: membrane-active peptide, FAM fluorophore, and dKK antibiotic cargo.

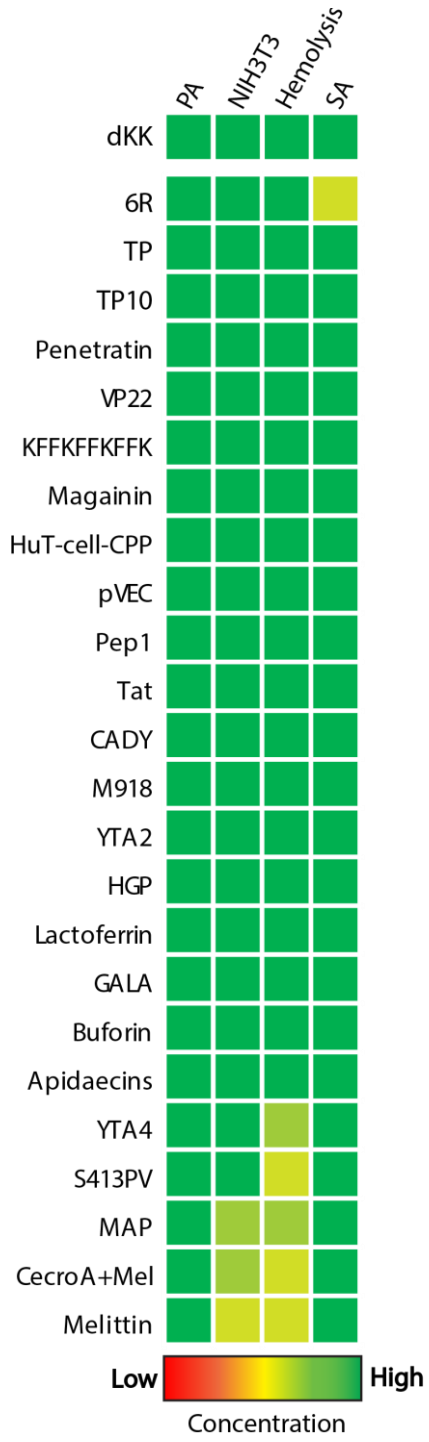

Figure S1. Pseudomonas killing, NIH3T3 toxicity, Blood hemolysis and S. aureus killing of single peptides. All single peptides displayed lower efficacy relative to the tandem peptides (Figure 1).

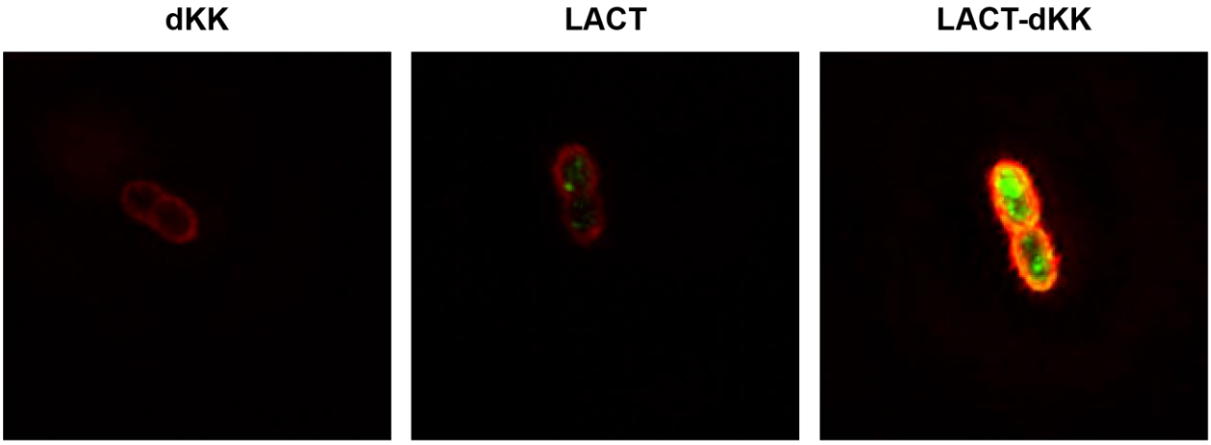

Figure S2. Three dimensional structured illumination microscope images of PAO1 incubated with dKK, LACT, or LACT-dKK peptides (FAM labeled; green). Membranes were stained with a lipophilic dye (FM 4-64FX; red). 1x108 CFU/sample were incubated with 1 μM of LACT, dKK, or LACT-dKK peptide for 90 minutes at 37 ˚C and stained with the membrane dye FM 4-64FX (ThermoFisher) for 10 minutes at 5 μg/mL. After washing and fixation, samples were mounted with VectaShield (VectorLabs) and imaged on an Applied Precision DeltaVision-OMX Super-Resolution Microscope (GE Life Sciences).

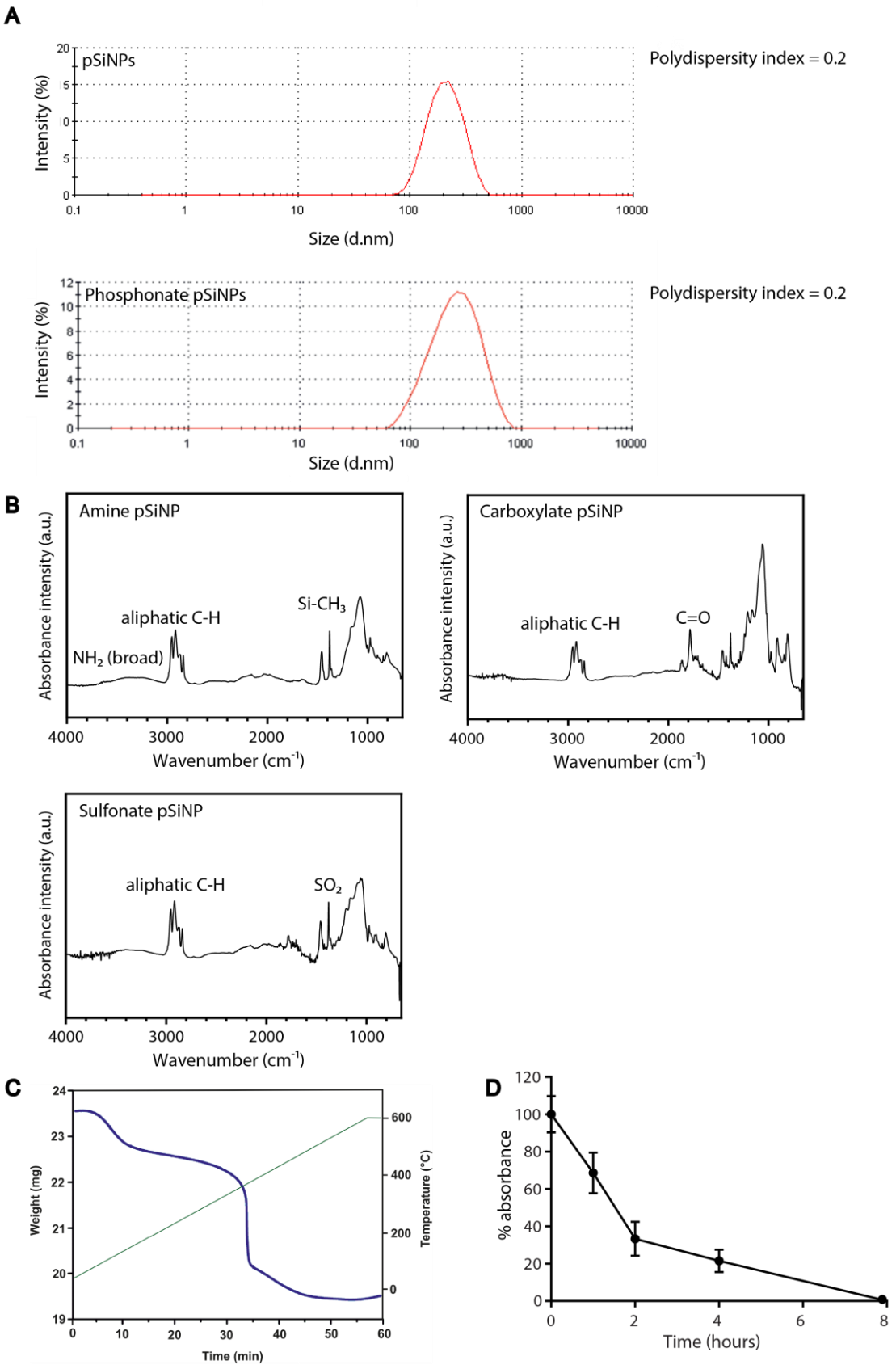

Figure S3. (**A**) Representative size distribution (hydrodynamic diameter) of porous silicon nanoparticles (top; pSiNPs) and phosphonate pSiNPs (bottom) used in this study, measured by dynamic light scattering. Polydispersity indices are indicated on the right. (**B**) Fourier transform infrared (FTIR) spectroscopy of amine, carboxylate, and sulfonate pSiNPs. (**C**)

Thermogravimetric analysis (TGA) of phosphonate pSiNPs. Calculations from the TGA curve shows a weight loss of 14%, indicating there are 140 μg of alkyl-phosphanate chains per mg of pSiNPs. (**D**) Degradation of phosphonate pSiNPs in PBS, pH 7.4, as measured by loss of absorbance signal at 405 nm. Monitoring of degradation using this method has been previously established (J. Kang, J. Joo, E. J. Kwon, M. Skalak, S. Hussain, Z. G. She, E. Ruoslahti, S. N. Bhatia, M. J. Sailor, Adv Mater 2016, 28, 7962.) The loss of absorbance over time is ascribed to the degradation of the elemental Si core of the nanoparticles; silicon absorbs strongly at λ = 405 nm, whereas SiO_2 and released silicic acid are transparent at this wavelength.

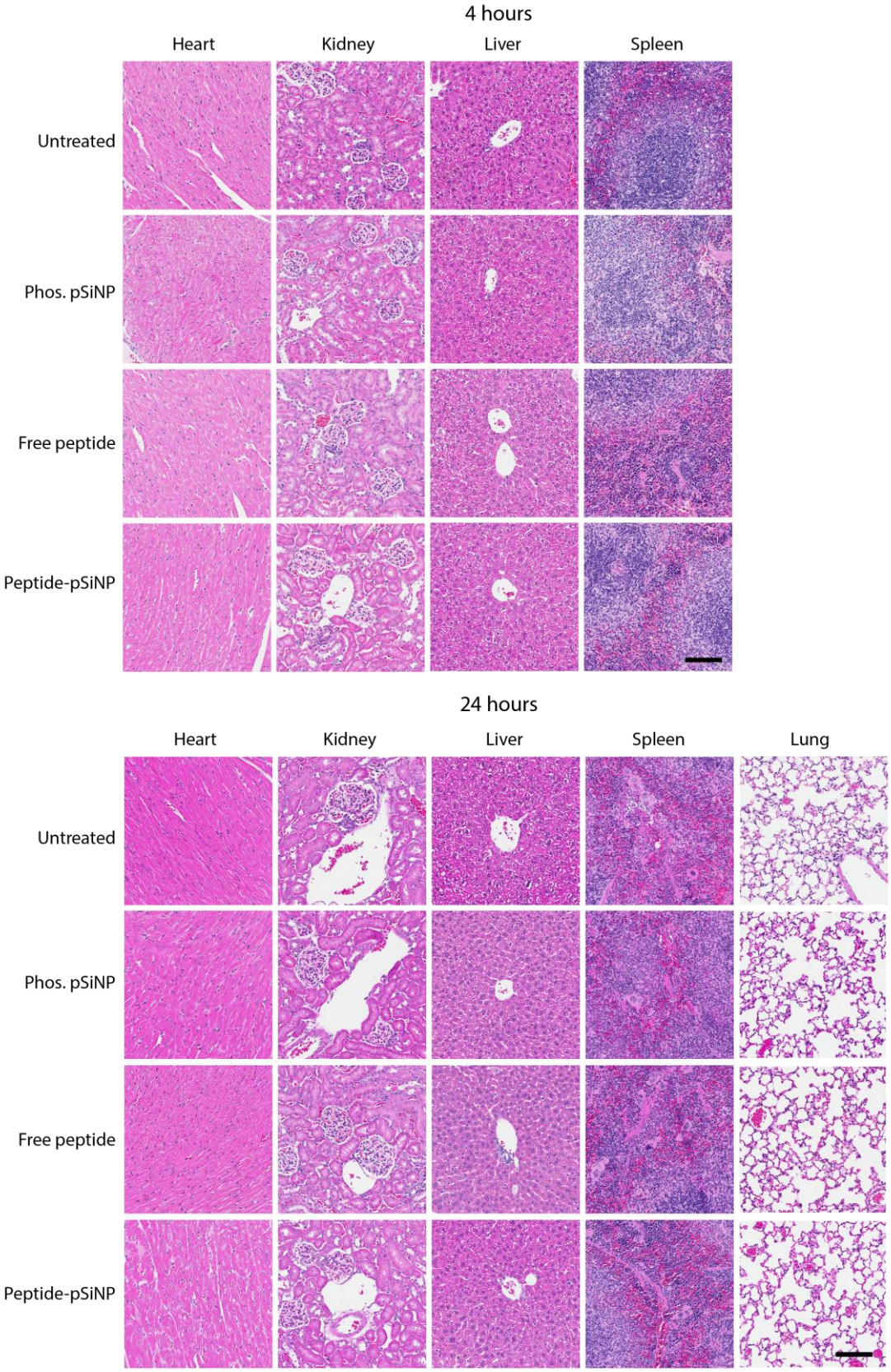

Figure S4. H&E staining of organs at 4 hours (top) and 24 hours (bottom) after administration of treatments. All organs appear normal at 24 hours. All organs except for lungs appear normal at 4 hours (examples of lung toxicity shown in Figure 3C). Scale bar indicates 100 μm.

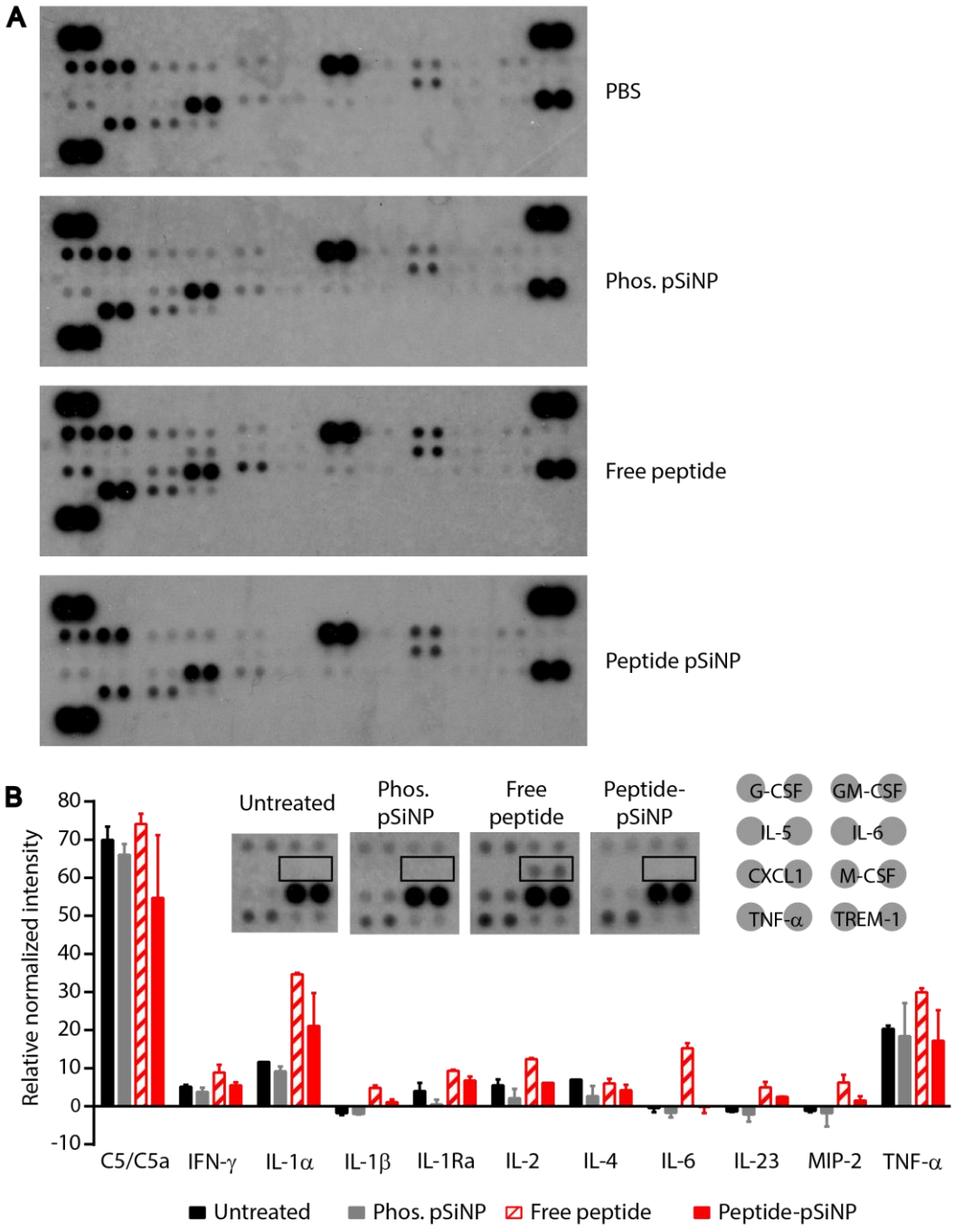

Figure S5. (A) Serum collected from healthy mice 4 hours after lung delivery of pSiNP, free peptide, or peptide-pSiNP was applied to a mouse cytokine array, 2 mice per group. Full representative blot from Mouse Cytokine Antibody Array Panel A (R&D Systems). (B) Quantification of a subset of cytokines important in acute responses are shown. Inset shows a portion of representative blots, with a box around IL-6. Map of cytokines showed in the inset is shown on the right.

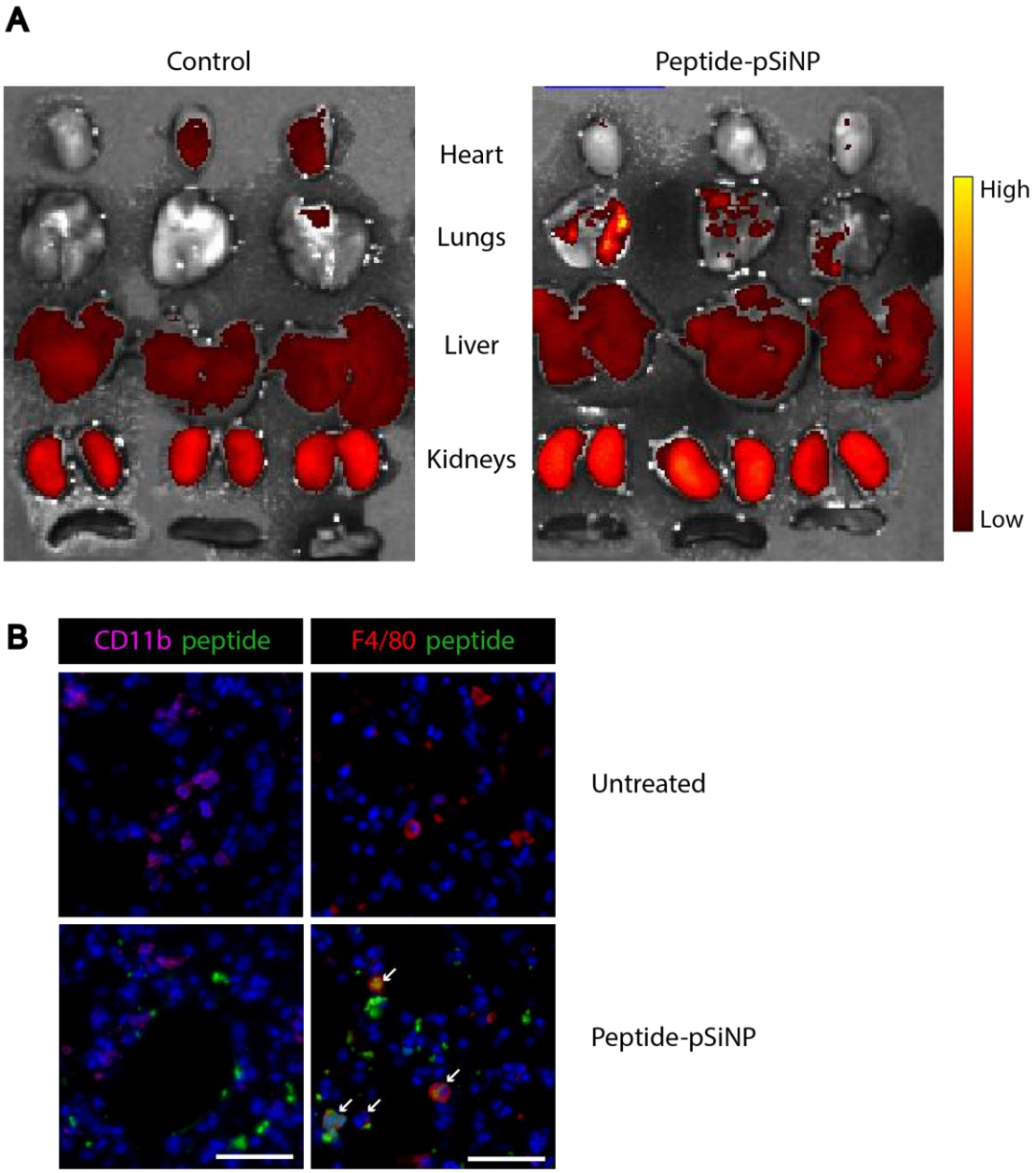

Figure S6. Biodistribution of pSiNP-tandem peptides. (A) IVIS images of organs from mice administered PBS (control) or peptide-pSiNP. A filter set for FAM was used to measure fluorescence and overlayed with the photograph of the organs. Figure 5B is quantified by tracing each organ and measuring the average fluorescence intensity. (B) Lung sections from untreated mice and mice delivered peptide-pSiNP (peptide-FAM; green) were stained for monocytes (magenta, CD11b; Abcam 1:1000) or alveolar macrophages (red, F4/80; Abcam 1:50). White arrows indicate peptide co-localization with F4/80. Scale bar indicates 40 μm.