Supplementary Materials for

Efficient Lymph Node-Targeted Delivery of Personalized Cancer Vaccines with Reactive Oxygen Species-Inducing Reduced Graphene Oxide Nanosheets.

Cheng Xu,[†,‡] Hao Hong,[§] Yonghyun Lee,[†,‡,¶,∥] Kyung Soo Park,[‡,⫴] Mingjiao Sun,[†] Tianrui Wang,[†] Marisa E. Aikins,[†,‡] Yao Xu,[†,‡] and James J. Moon[†,‡,⫴,*]

[†] Department of Pharmaceutical Sciences, University of Michigan, Ann Arbor, MI 48109, United States.

[‡] Biointerfaces Institute, University of Michigan, Ann Arbor, MI 48109, United States.

[§] Medical School of Nanjing University, 22 Hankou Road, Nanjing, Jiangsu 210093, China.

[¶] Department of Pharmacy, College of Pharmacy, Ewha Womans University, Seoul, 03760, South Korea.

[∥] Pharmacy of Graduate School, Ewha Womans University, Seoul, 03760, South Korea.

[⫴]Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109, United States.

*. Correspondence should be addressed to J.J.M. (moonjj@umich.edu).

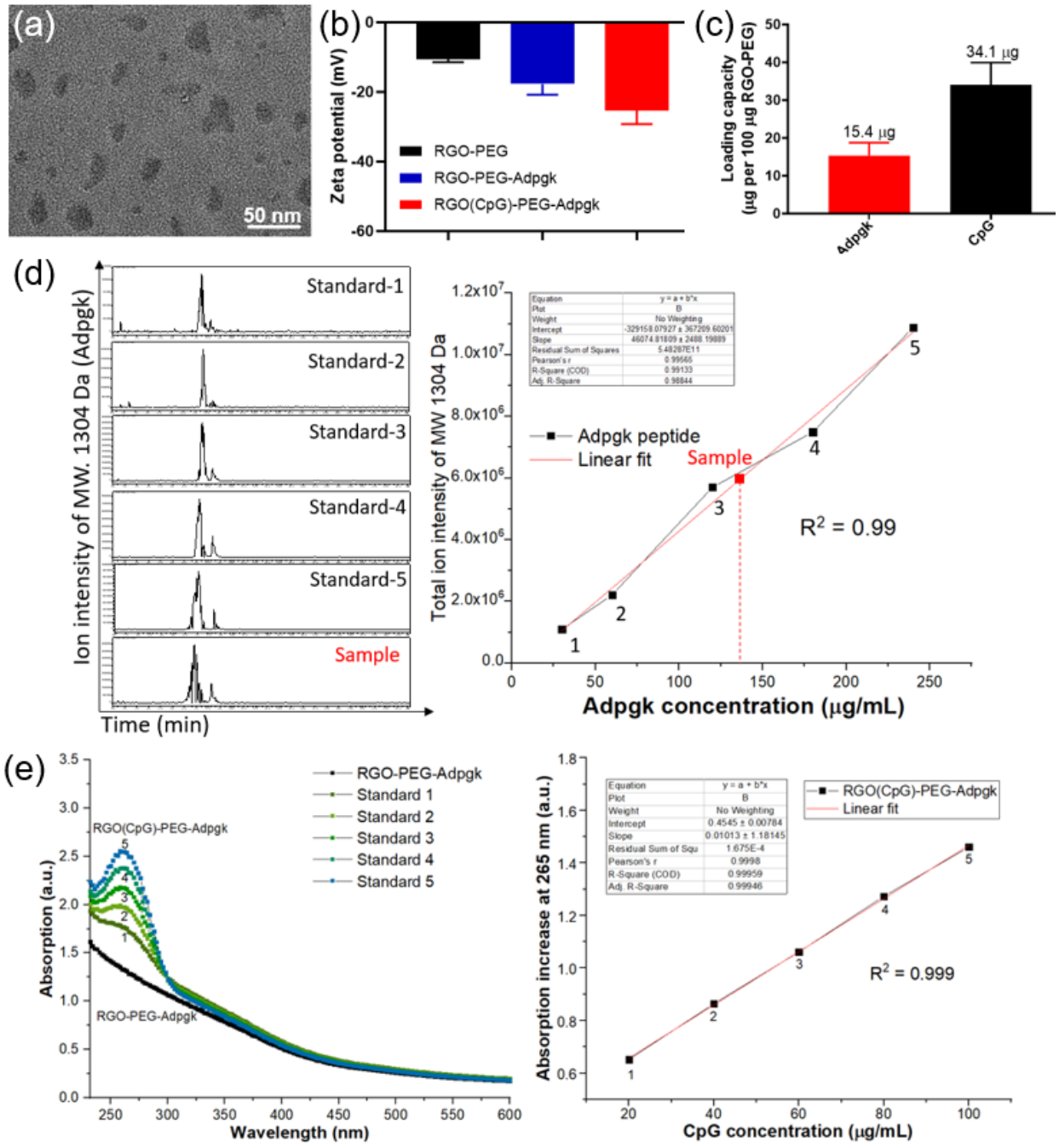

Figure S1. a, TEM of RGO-PEG. **b**, Surface zeta potential of RGO-PEG, RGO-PEG-Adpgk, and RGO(CpG)-PEG-Adpgk. **c**, The amount of CpG and Adpgk neoantigen peptide loaded per 100 μg of RGO-PEG in PBS at RT. **d**, mass spectrum and standard curve of Adpgk peptide (concentration *vs*. ion intensity of molecular weight of 1304 Da) for the quantification of Adpgk peptide. excess glutathione (100 mM) was added to cleavage and release Adpgk peptide from RGO(CpG)-PEG. **e**, UV-vis spectrum of RGO-PEG-Adpgk and RGO(CpG)-PEG-Adpgk with various CpG concentrations. Inset: the standard curve for CpG quantification (concentrations *vs*. absorption increase at 265 nm between RGO-PEG-Adpgk and RGO(CpG)-PEG-Adpgk).

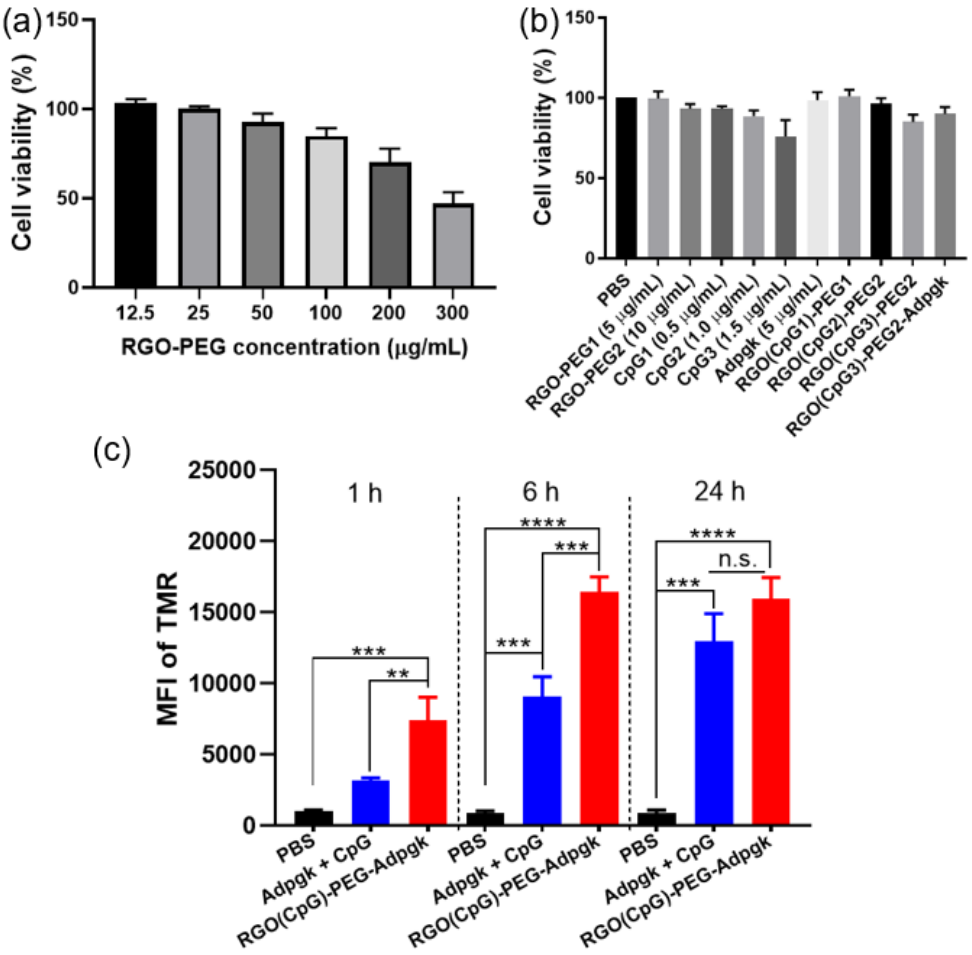

Figure S2. a, Viability of BMDCs after 24 h incubation with varying concentrations of RGO-PEG. **b**, Viability of BMDCs after 24 h incubation with varying concentrations of RGO-PEG, soluble CpG, soluble Adpgk, RGO(CpG)-PEG, and RGO(CpG)-PEG-Adpgk. **c,** BMDC uptake of free Adpgk-tetramethylrhodamine (TMR) (4 μg/mL) with CpG (0.5 μg/mL) or RGO(CpG)-PEG-Adpgk-TMR (4 μg/mL) after incubation with BMDCs for 1 h, 6 h, or 24 h. The concentrations of CpG and Adpgk in RGO(CpG)-PEG or RGO(CpG)-PEG-Adpgk were the same with the corresponding soluble CpG and Adpgk group. Data represent mean ± SEM from a representative experiment (n = 3). Data was analyzed by one-way ANOVA with Tukey's HSD multiple comparison post hoc test, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

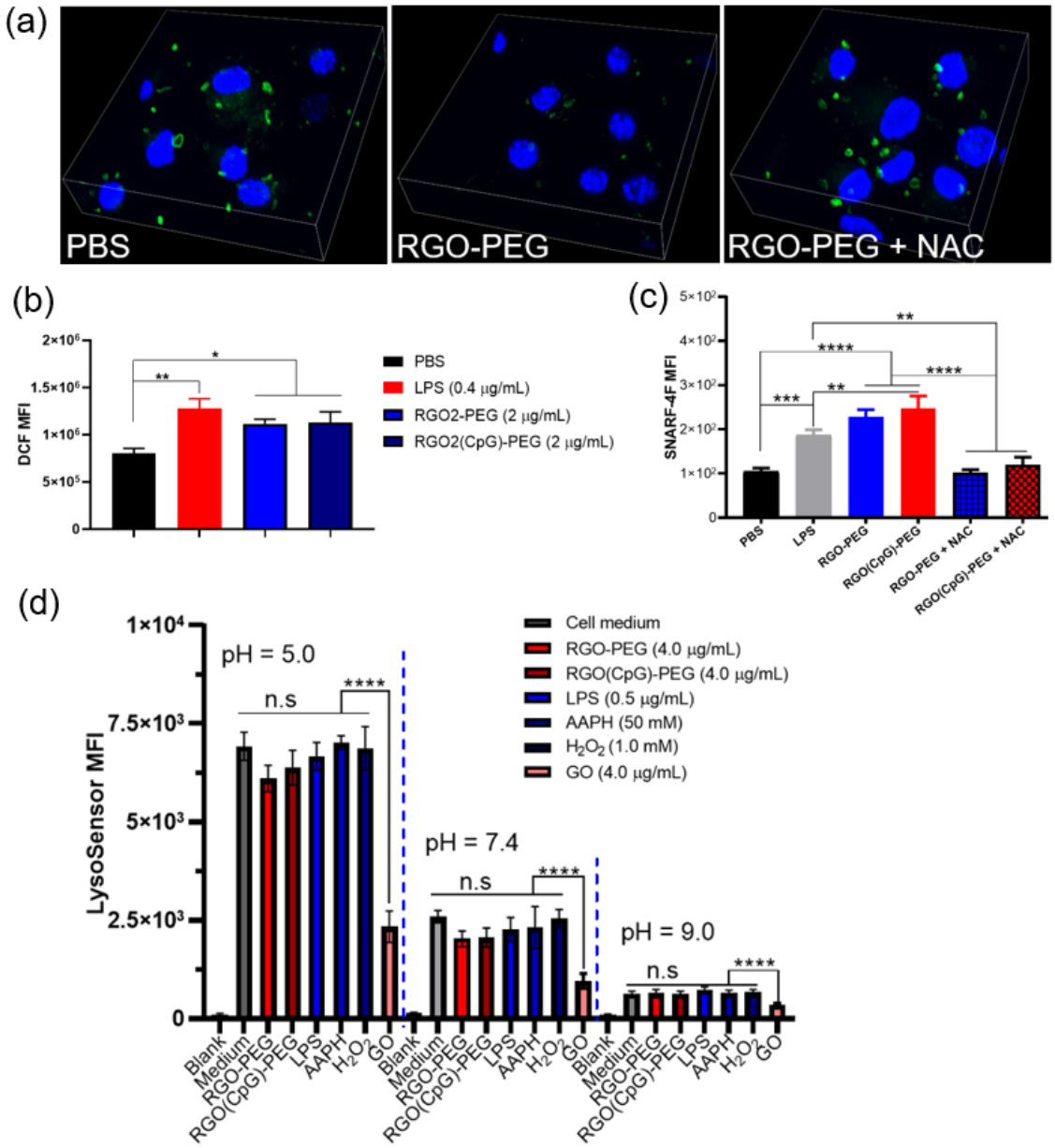

Figure S3. a, Confocal microscopy images of BMDCs incubated for 4 h with PBS, RGO-PEG (4 μg/mL), or RGO-PEG (4 μg/mL) + NAC (5 mM), followed by staining with LysoSensor (green). **b**, Intracellular DCF fluorescence level in BMDMs after 24 h incubation with LPS, RGO-PEG, or RGO(CpG)-PEG. **c**, SNARF-4F-5-(and 6-) carboxylic acid fluorescence intensity in BMDCs after incubation with PBS, LPS (0.4 μg/mL), RGO-PEG (4 μg/mL), RGO(CpG)-PEG (4 μg/mL), RGO-PEG + NAC (5 mM), RGO(CpG)-PEG + NAC (5 mM) for 4 h. **d**, Changes in LysoSensor fluorescence signal after incubation with RGO-PEG, RGO(CpG)-PEG, LPS, AAPH (2,2'-Azobis(2-amidinopropane) dihydrochloride), H_2O_2, or GO in cell medium with different pH conditions. Data represent mean ± SEM from a representative experiment (n = 4). Data was analyzed by one-way ANOVA with Tukey's HSD multiple comparison post hoc test, * $P < 0.05$. ** $P < 0.01$, *** $P < 0.0001$, **** $P < 0.0001$.

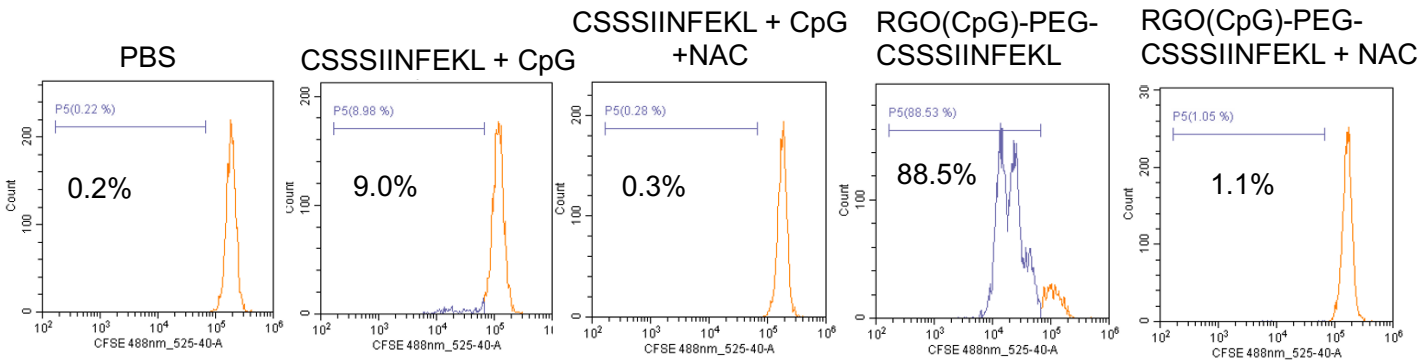

Figure S4. CFSE dilution assay. BMDCs were incubated with PBS, soluble CSSSIINFEKL + CpG, CSSSIINFEKL + CpG with NAC (5 mM), RGO(CpG)-PEG-CSSSIINFEKL, or RGO(CpG)-PEG-CSSSIINFEKL with NAC (5 mM). All groups had equivalent concentration of peptide at 5 μg/mL and CpG at 1 μg/mL. After 24 h, BMDCs were co-cultured with CFSE-labeled OT-1 T cells. After 48 h, CFSE dilution was quantified by flow cytometric analysis.

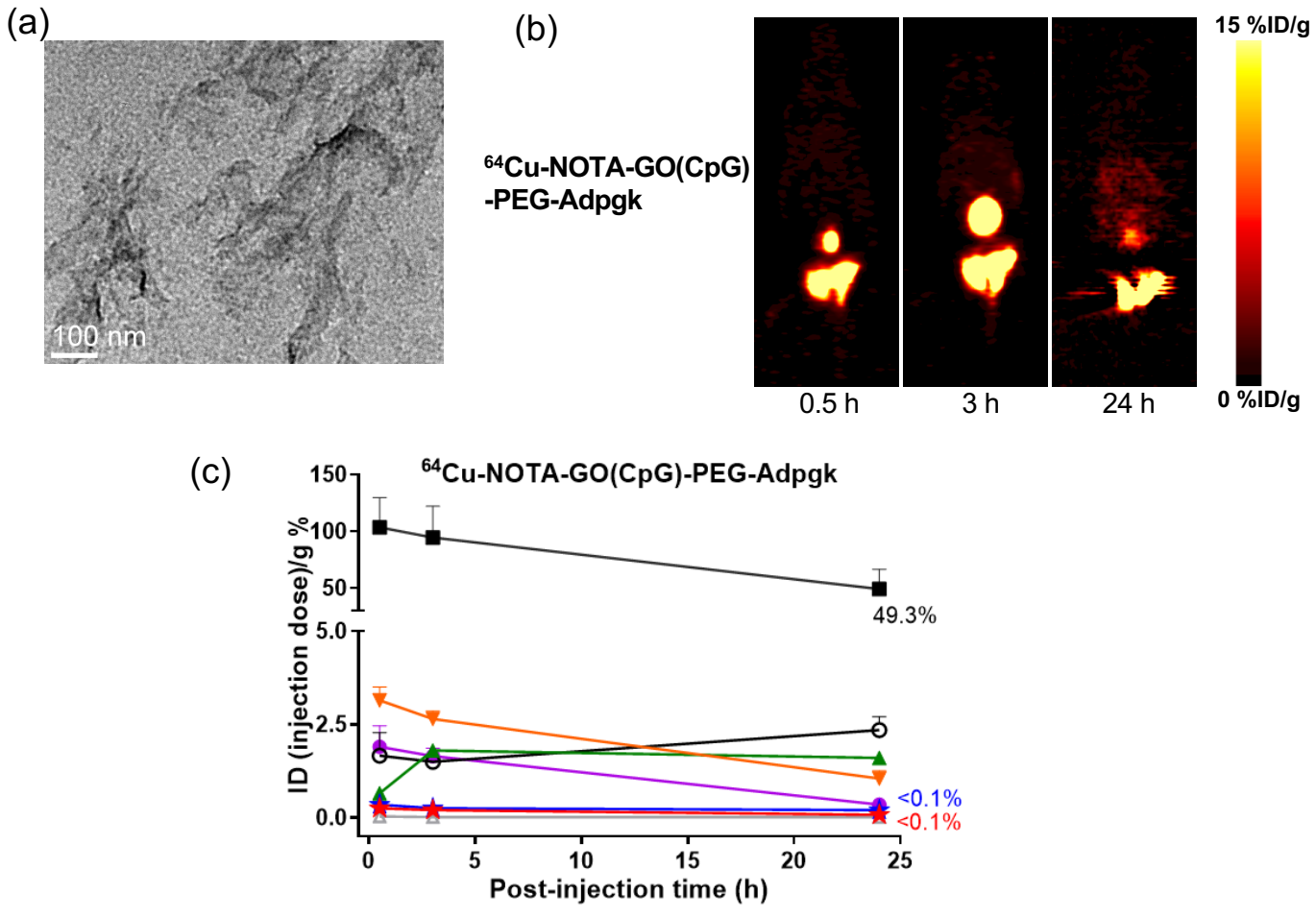

Figure S5. a, TEM image of GO-PEG, showing ~200-300 nm in size. **b**, Serial PET images of C57BL/6 mice administered SC with ^{64}Cu-NOTA-GO(CpG)-PEG-Adpgk. **c**, Time-radioactivity curves of injection site, inguinal and axillary LNs, kidney, intestine, liver, blood, and muscle after SC administration of ^{64}Cu-NOTA-GO(CpG)-PEG-Adpgk.

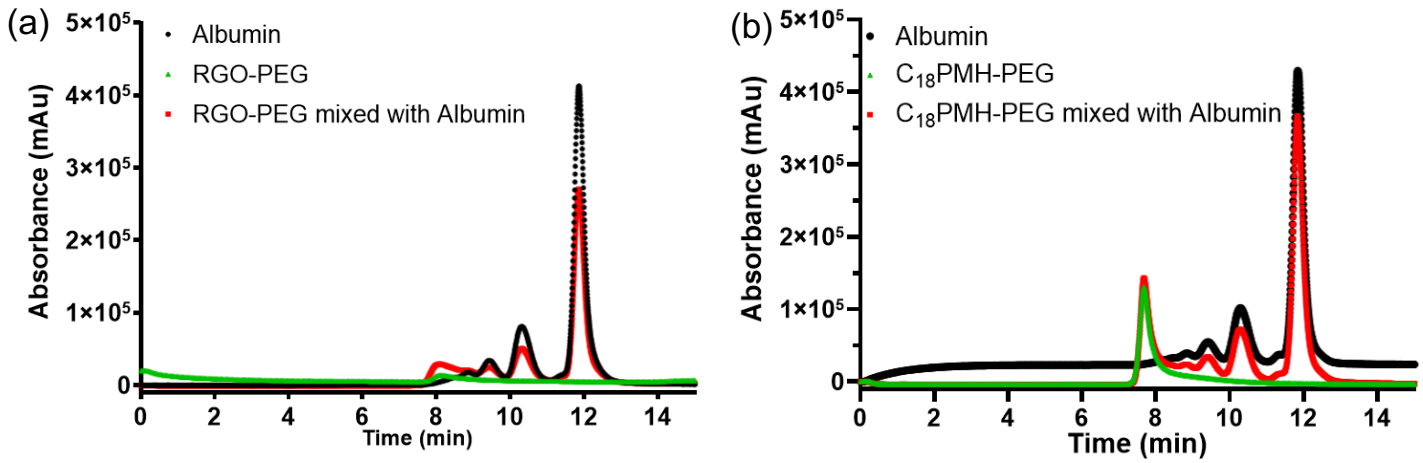

Figure S6. Gel permeation chromatography (GPC) of mouse albumin (0.5 mg/mL) with (**a**) RGO-PEG (0.5 mg/mL) or (**b**) C_{18}PMH-PEG (0.5 mg/mL) after incubation of 4 h at 37 °C.

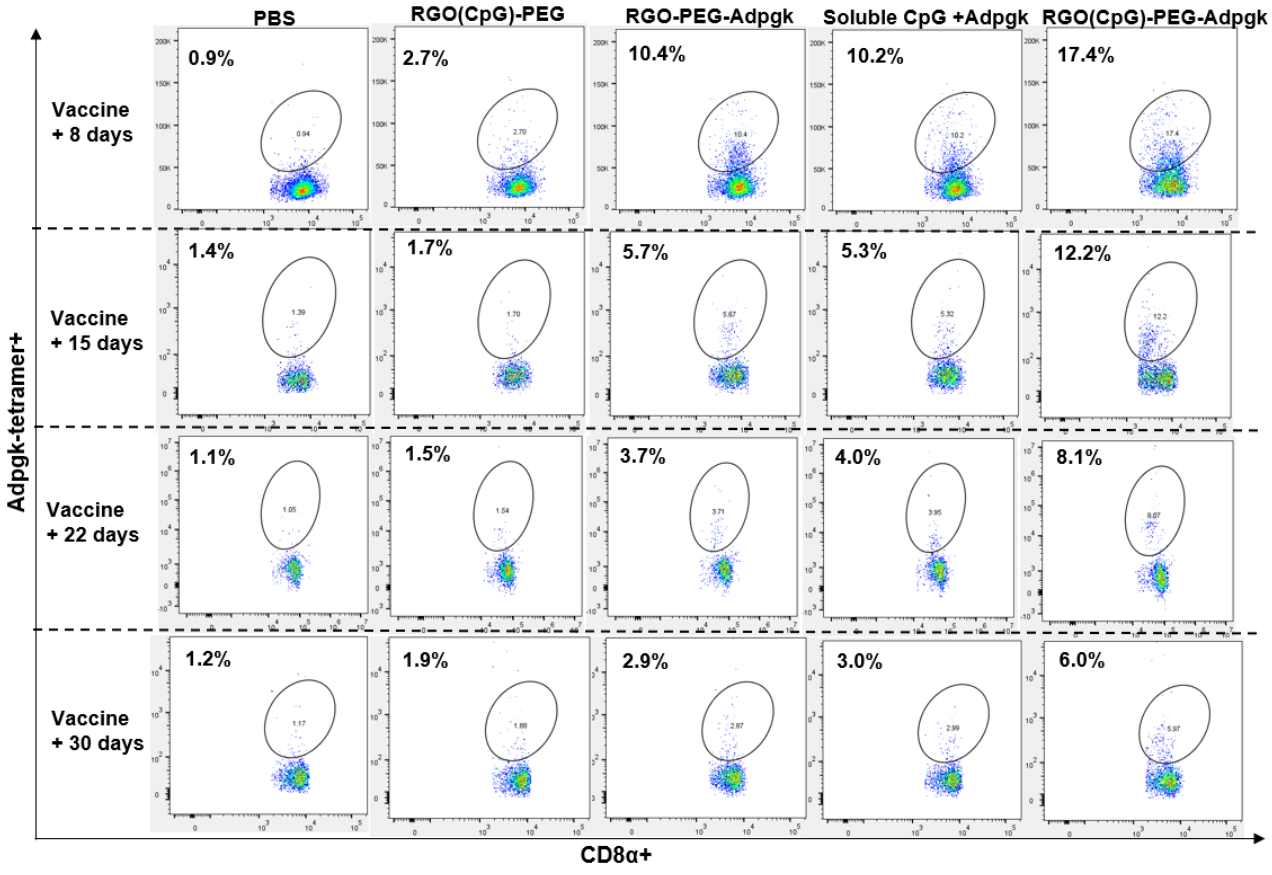

Figure S7. The representative scatter plots and the frequencies of Adpgk-specific CD8α$^+$ T-cells in peripheral blood in animals treated as in **Figure 4a**.

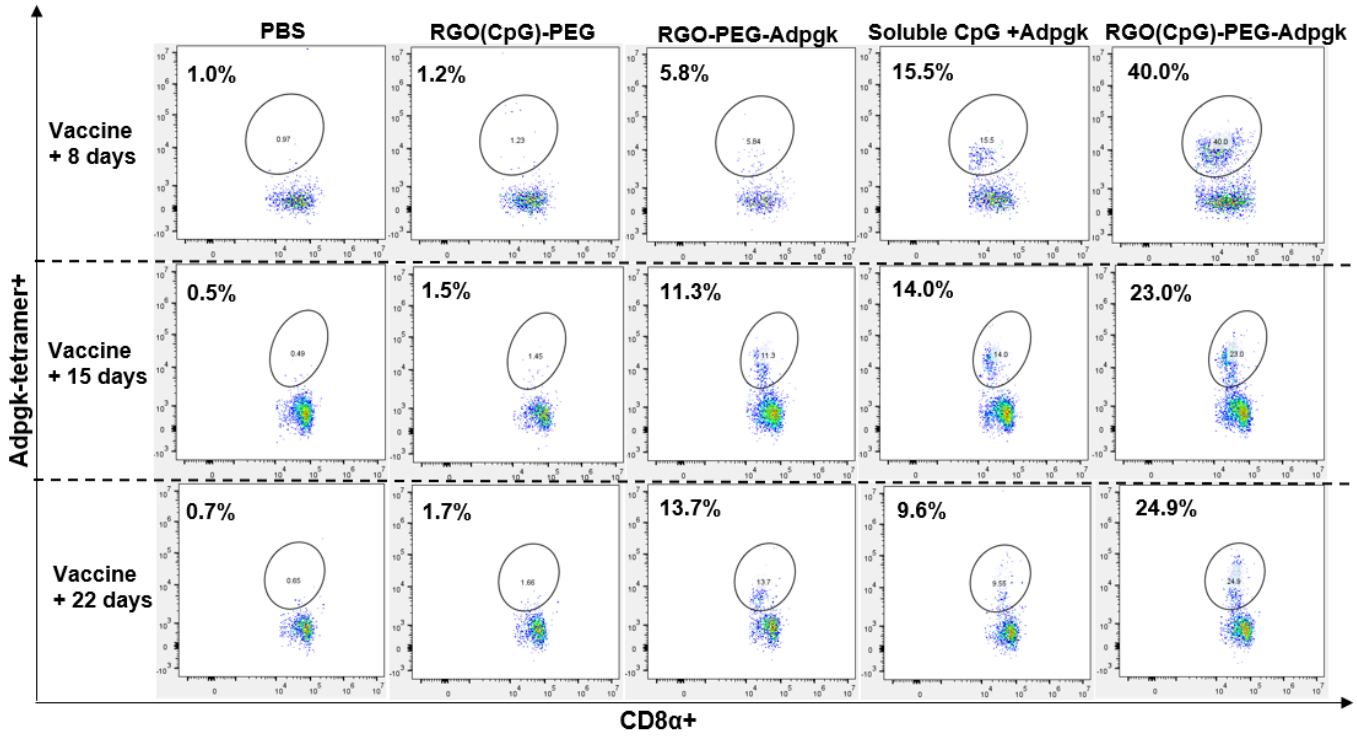

Figure S8. The representative scatter plots and frequencies of Adpgk-specific CD8α$^+$ T-cells in peripheral blood in animals treated as in **Figure 4e**.

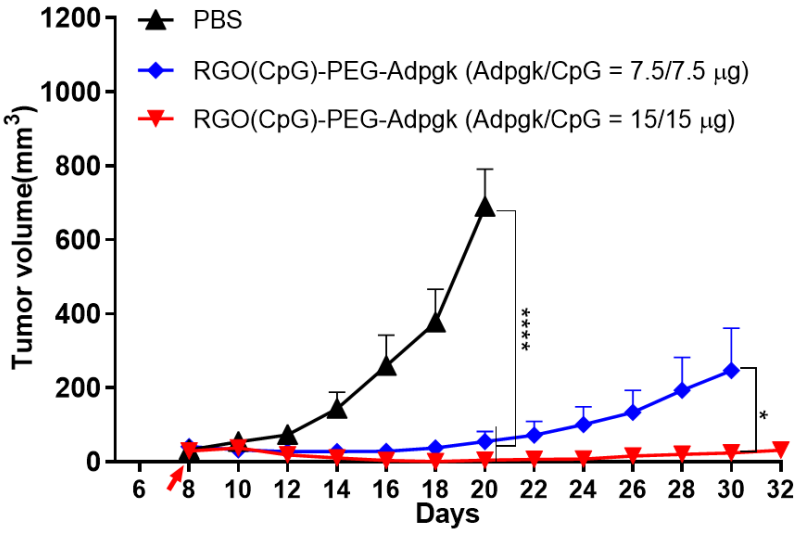

Figure S9: Dose-dependent efficacy of RGO(CpG)-PEG-Adpgk vaccine in MC-38 colon carcinoma-bearing mice. Average tumor growth curve of (1) PBS control; (2) RGO(CpG)-PEG-Adpgk treatment group (Adpgk and CpG doses = 7.5 μg each); (3) RGO(CpG)-PEG-Adpgk treatment group (Adpgk and CpG doses = 15 μg each). Mice were vaccinated by SC at tail base for once at Day 8. Data represent mean ± SEM from a representative experiment (n = 5). Data was analyzed by two-way ANOVA with Tukey's HSD multiple comparison post hoc test * $P < 0.05$, **** $P < 0.0001$.

(a)

Name	Reference range	PBS	Soluble CpG + Adpgk	RGO(CpG)-PEG	RGO-PEG-Adpgk	RGO(CpG)-PEG-Adpgk
AST, U/L	39.6-386.1	33.8 ± 10.3	71.0 ± 24.9	43.4 ± 10.8	41.6 ± 7.6	45.6 ± 4.2
ALT, U/L	24.3-115.3	38.0 ± 5.1	47.5 ± 10.6	37.6 ± 9.9	39.5 ± 8.4	51.5 ± 5.7
ALP, U/L	65.5-364.2	131 ± 16.9	187 ± 17.7	140 ± 14.3	138 ± 12.8	176 ± 14.2
Triglyceride, mg/dL	72.7-303.2	79.5 ± 22.1	82.1 ± 18.6	75.1 ± 7.9	82.8 ± 16.7	70.6 ± 14.9
Cholesterol, mg/dL	60.2-167.3	72.0 ± 14.8	61.0 ± 13.7	62.4 ± 10.7	65.8 ± 9.7	61.2 ± 10.3
Glucose, mg/dL	79.4-354.7	248 ± 34.1	245 ± 27.1	198 ± 34.2	183 ± 15.9	156.8 ± 17.2
Creatine phosphokinase, U/L	22.0-198.0	95.2 ± 24.9	114 ± 30.3	93.6 ± 12.3	90.0 ± 10.6	113 ± 12.3
Blood urea nitrogen, mg/dL	5.2-30.7	29.5 ± 8.6	33.5 ± 5.4	22.5 ± 3.6	30.1 ± 8.1	26.6 ± 8.9

(b)

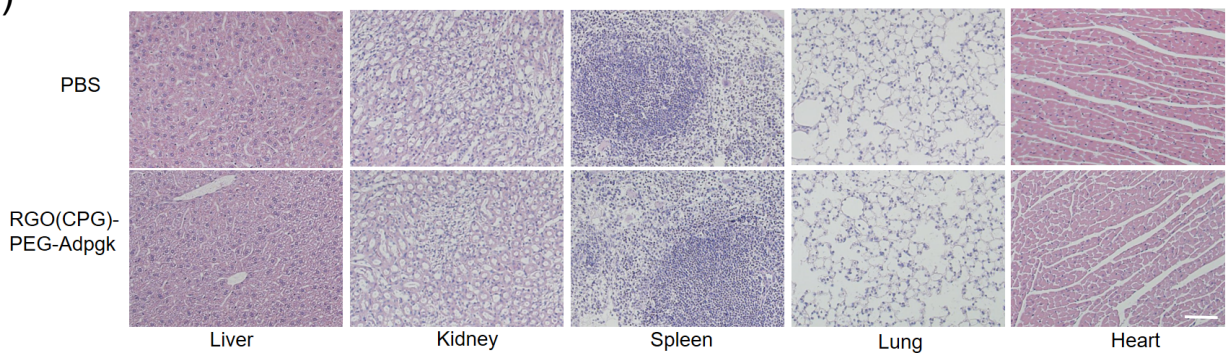

Figure S10. a, Analyses of serum biochemical markers after 7 days of vaccination with the indicated groups. **b**, Hematoxylin−eosin (H&E) staining images of major organs after 15 days of SC vaccination with PBS or RGO(CpG)-PEG-Adpgk.

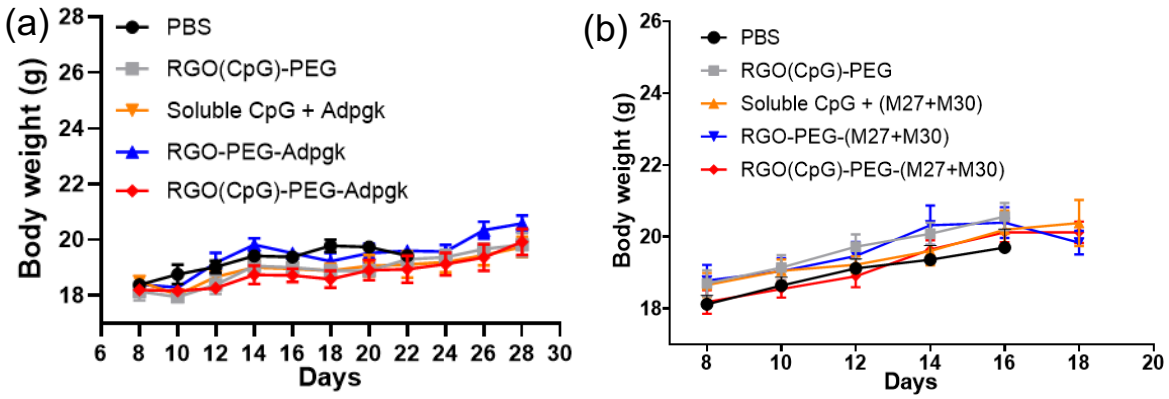

Figure S11. a, Body weight of MC-38 tumor-bearing mice treated as in **Figure 4e**. **b**, Body weight of B16F10 tumor-bearing mice treated as in **Figure 5a**.