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# NIR-triggered on-site NO/ROS/RNS nanoreactor: Cascade-amplified photodynamic/photothermal therapy with local and systemic immune responses activation

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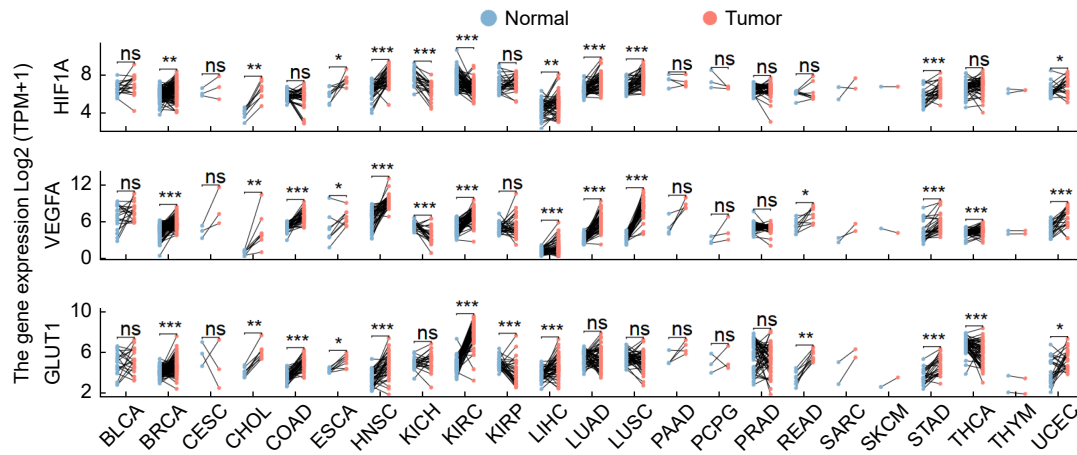
Supplementary information for this paper is available at <https://doi.org/10.29026/oea.2024.240013>



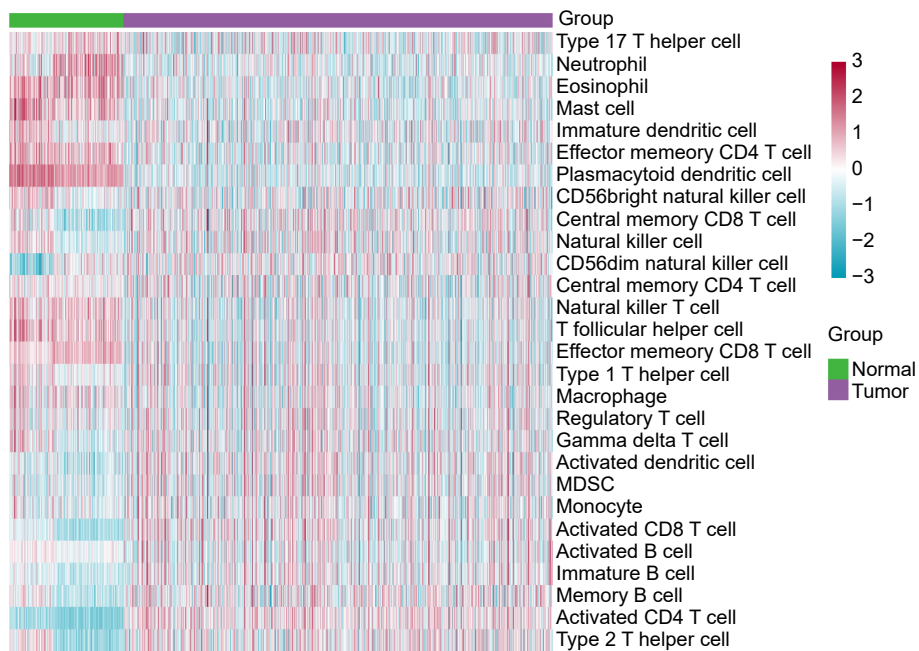
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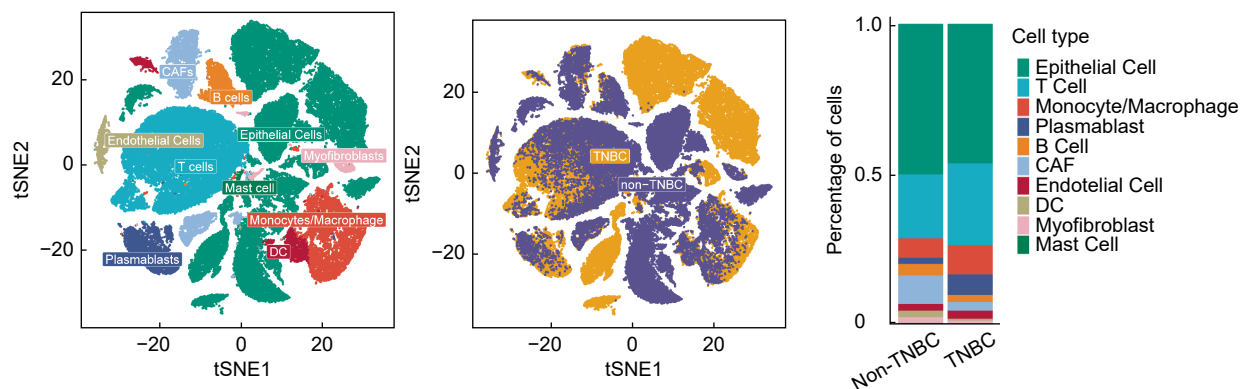
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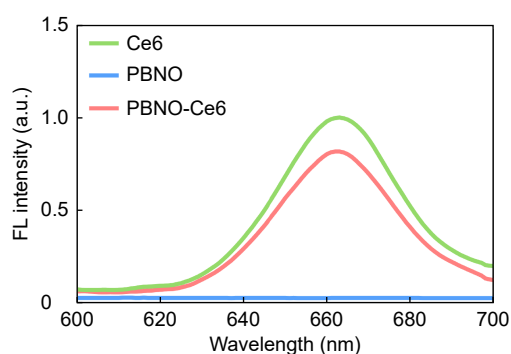
**Fig. S1 | Expression levels of hypoxia-related genes HIF1A, VEGFA, and GLUT1 across TCGA and GTEx data from multiple cancer types.** ns: Not significant. (BLCA: Bladder Urothelial Carcinoma; BRCA: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; ESCA: Esophageal carcinoma; HNSC: Head and Neck squamous cell carcinoma; KICH: Kidney Chromophobe; KIRP: Kidney renal papillary cell carcinoma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and Paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectum adenocarcinoma; SARC: Sarcoma; SKCM: Skin Cutaneous Melanoma; STAD: Stomach adenocarcinoma; THCA: Thyroid carcinoma; THYM: Thymoma; UCEC: Uterine Corpus Endometrial Carcinoma. )



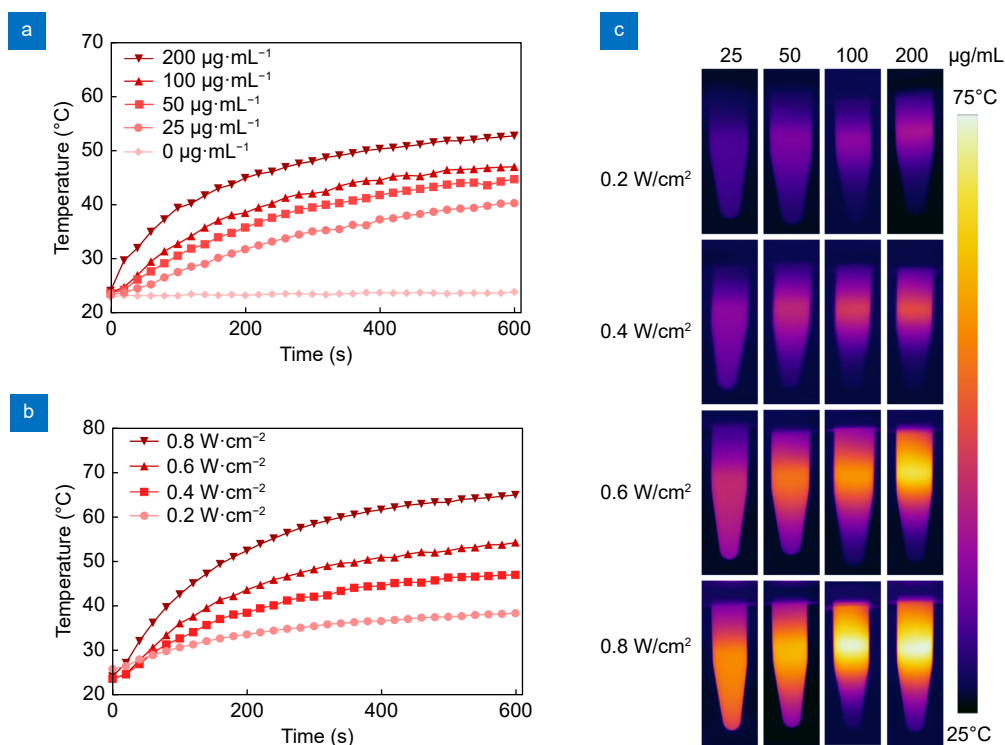
**Fig. S2 | Heatmap illustrating immune cell subtype distribution in normal breast tissue and breast tumors based on single-sample Gene Set Enrichment Analysis (ssGSEA) results.** (MDSC: Myeloid-derived suppressor cells)



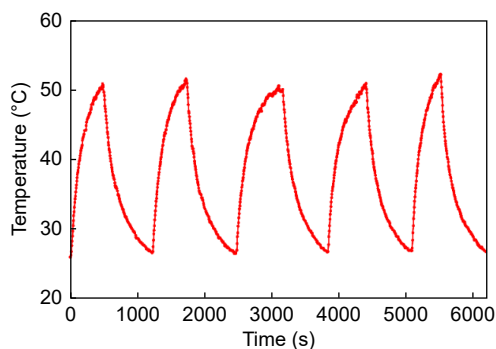
**Fig. S3 | t-SNE visualization of single cell RNA-seq data from TNBC and non-TNBC patient samples.** Cells are clustered by their identified cell types based on expression profiles.



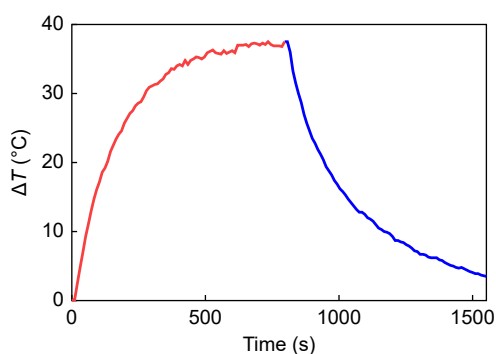
**Fig. S4 | Fluorescence spectra of Ce6, PBNO and PBNO-Ce6.**



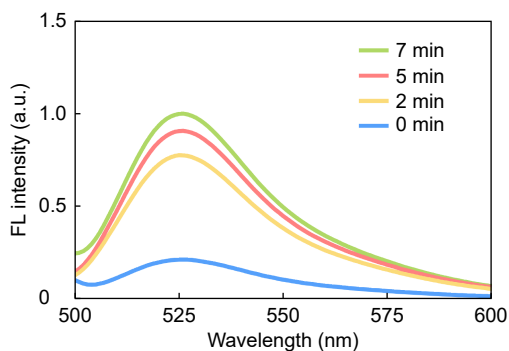
**Fig. S5 | (a)** Photothermal curves of different concentration of PBNO irradiated (660 nm 0.4 W·cm<sup>-2</sup>) for 10 min. **(b)** Photothermal curves of 0.10 mg/mL PBNO irradiated with different power densities of 660-nm laser for 10 min. **(c)** Thermal images of PBNO at the end of NIR irradiation.



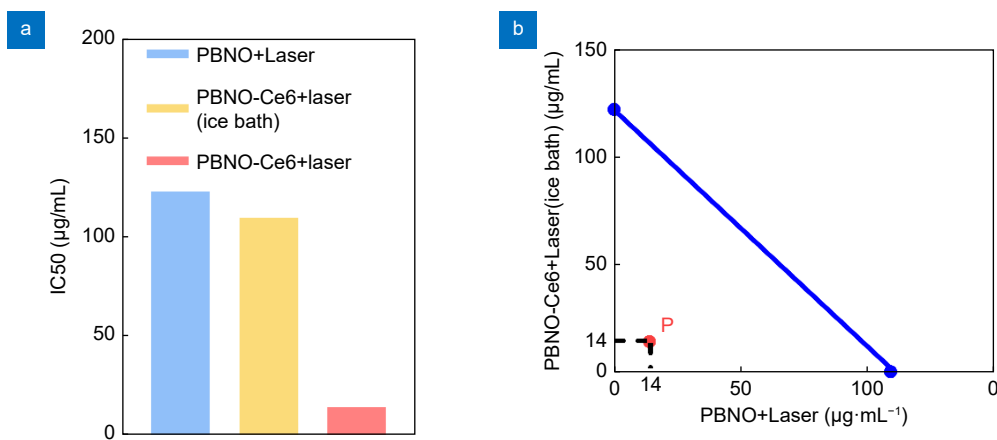
**Fig. S6 |** Photothermal stability of PBNO within five cycles of 660-nm laser irradiation.



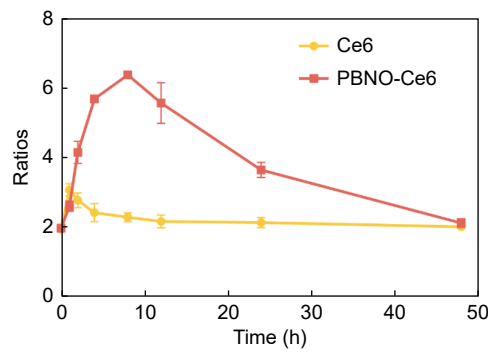
**Fig. S7 |** Heating and cooling curves of 200 µg·mL<sup>-1</sup> PBNO under laser irradiation (0.4 W·cm<sup>-2</sup>).



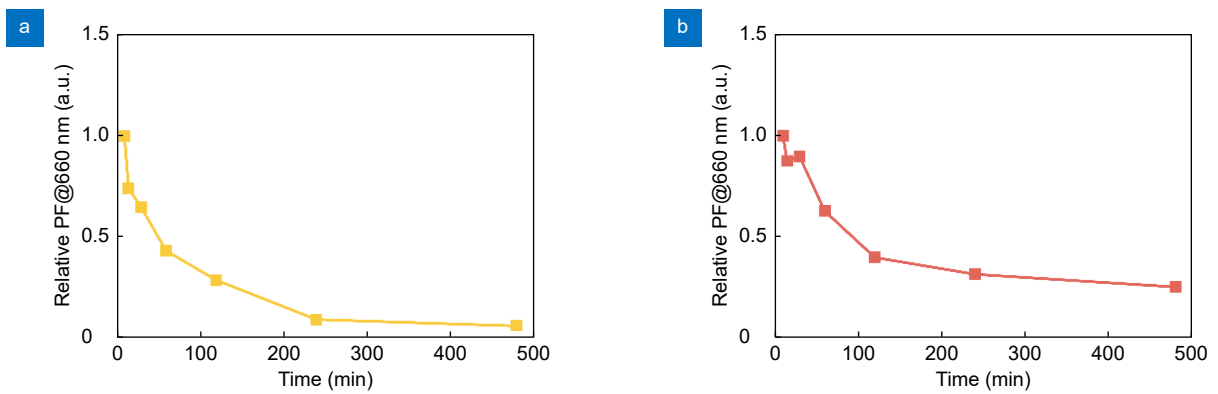
**Fig. S8 |** The fluorescence spectra of PBNO-Ce6 during laser irradiation. The solutions are mixed with BBoxiProbe® O56.



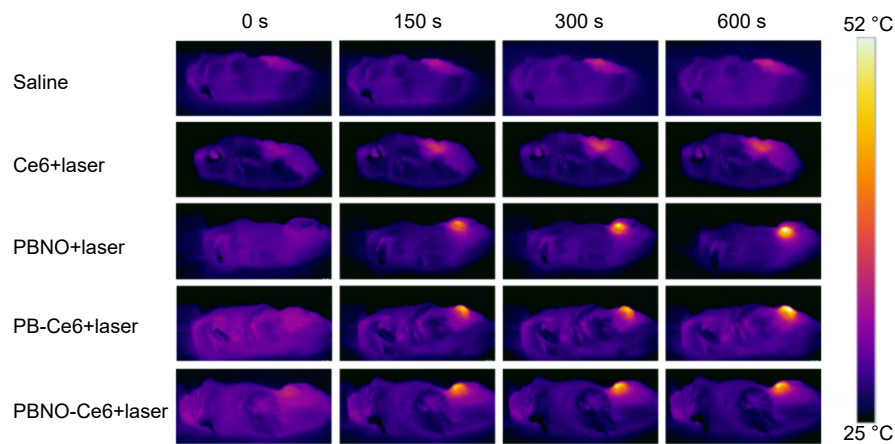
**Fig. S9 |** (a) IC<sub>50</sub> values of 4T1 cells treated with PBNO, PBNO-Ce6 with and without ice bath after laser irradiation. (b) Isobologram showing the synergy between PDT and PTT effect induced by PBNO-Ce6 upon laser irradiation. The blue line represents additivity. Mixtures that are below the blue line are synergistic, mixtures at the blue line are additive and mixtures above the line are antagonistic. P: PBNO-Ce6 + laser.



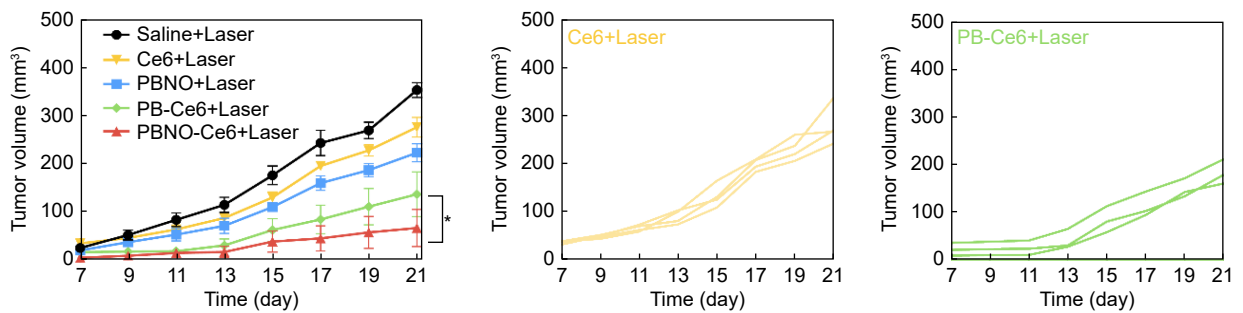
**Fig. S10 | Signal-to-signal ratio of PBNO-Ce6 and Ce6 at tumour sites in mice in *in vivo* images.**



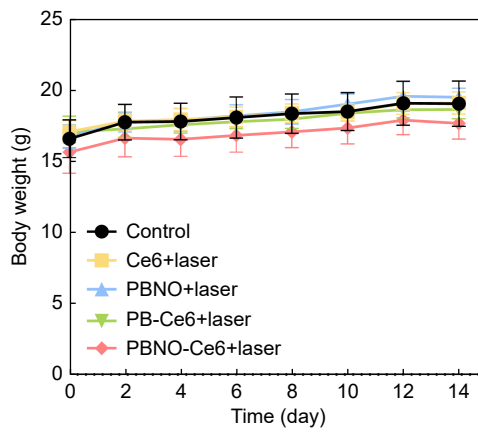
**Fig. S11 | Relative fluorescence intensity in blood at different time points after intravenous injection of Ce6 (a) and PBNO-Ce6 (b).**



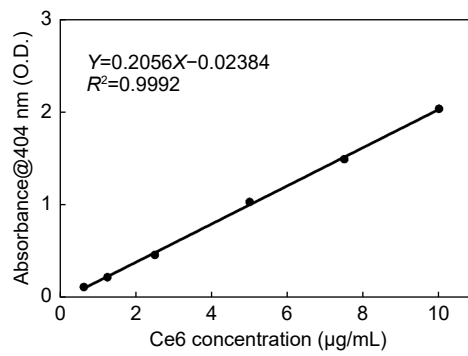
**Fig. S12 | Thermal images of mice during laser irradiation (660 nm 0.4 W·cm<sup>-2</sup>, 10 min).**



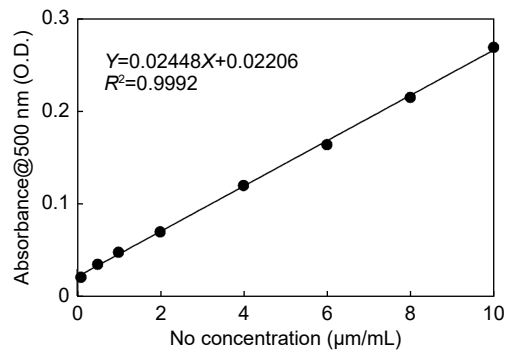
**Fig. S13 | Tumor growth curves of mice after tumors rechallenged in different treatment groups.**



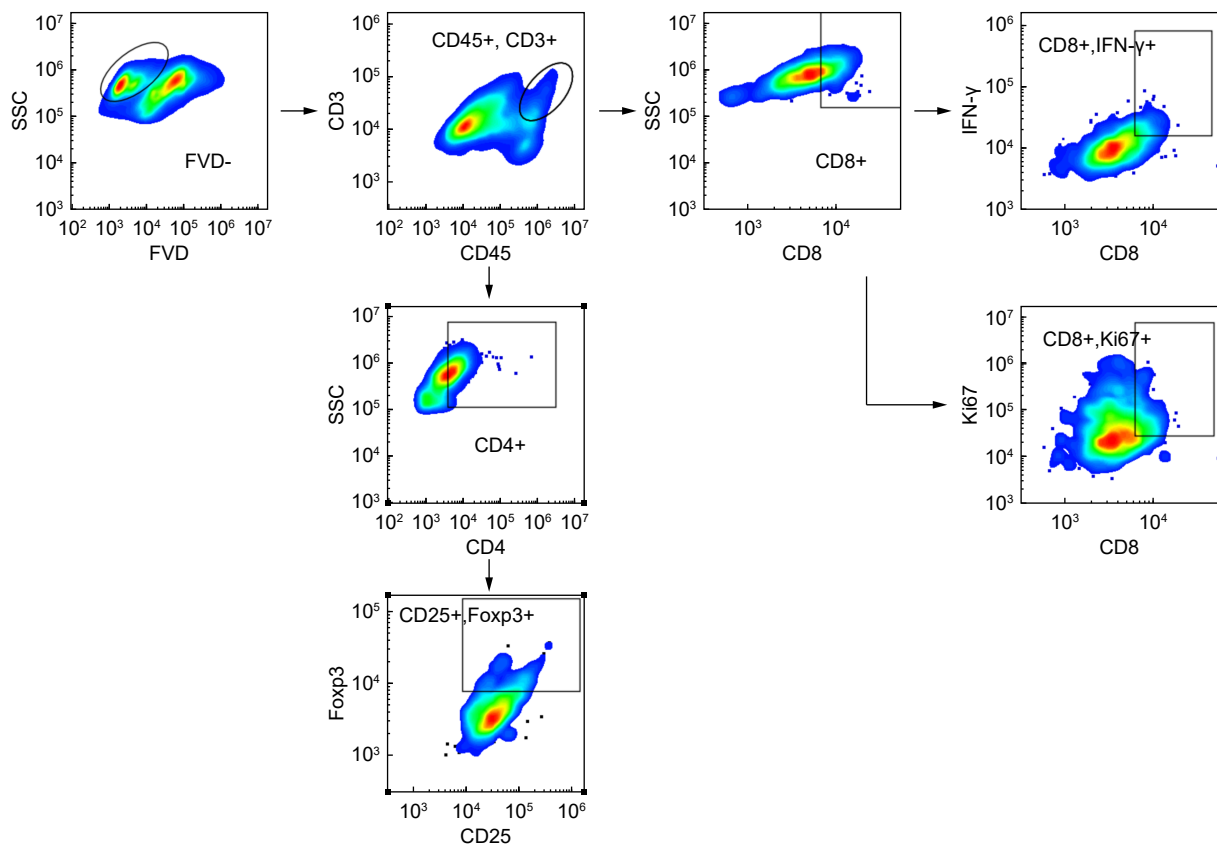
**Fig. S14 | Body weight curves of mice after treatment for 14 days.**



**Fig. S15 | The standard curve of Ce6.**



**Fig. S16 | The standard curve of NO.**



**Fig. S17 | FCM gating strategy to determine Treg cell (CD4+CD25+FcγR3+) and the function (IFN-γ+) and proliferation (Ki67+) of CD8+ T cells (CD3+CD8+).**