Supporting Information

Nanoscale reduced graphene oxide-mediated photothermal therapy together with IDO inhibition and PD-L1 blockade synergistically promote antitumor immunity

Mengmeng Yan[#], Yijia Liu[#], Xianghui Zhu, Xiaoli Wang, Lanxia Liu, Hongfan Sun, Chun Wang, Deling Kong, Guilei Ma[*]

[#] Mengmeng Yan and Yijia Liu contributed equally to this work.

The Tianjin Key Laboratory of Biomaterials, Institute of Biomedical Engineering, Peking Union Medical College & Chinese Academy of Medical Sciences, Tianjin, China

[*]***Correspondence to****:* Guilei Ma (E-mail: bmemgl@126.com)

Materials and reagents

Epacadostat (100% purity, TargetMol), Poly (maleic anhydride-alt-1-octadecene) (Sigma-Aldrich), mPEG-NH_2 (molecular weight 2000, Beijing KaiZheng United Medical Technology Co. Ltd), Folic acid (100% purity, Beijing Bailingwei Technology Co. Ltd), anti-PD-L1 antibody (BioXcell), fluorochrome-labelled CD11c, CD80, CD86, CD3, CD4, CD8, CD45, FoxP3 and CD335(NKρ46) monoclonal antibodies (eBioscience), graphene oxide (GO) (gifted by Professor Jiafu Shi from Tianjin University).

Experimental section

Quantification of IDOi in organs

The organs were collected and washed twice with ice-cold NaCl 0.9%. Then, the organs were weighed, cut up and transferred into Eppendorf centrifuge tubes. Then RIPA buffer and ceramic beads (2.0mm in diameter) were added to the tubes. The organs fragments were homogenized using the Homogenizer (TissuePrep TP-24, Gering, China), then centrifuged for 10 min to obtain the supernatant of tissues homogenate. Finally, the fluorescence intensity of FITC-IDOi was determined using thermos-svarioskan flash multifunction microplate reader.

Histopathological analysis

Mice were injected via the tail vein with 100 μl of PEG-rGO, PEG-rGO-FA or PEG-rGO-FA-IDOi (at the same GO concentration of 200 μg/100 μL) and sacrificed at different time points after treatment. PBS treated mice were used as control group. Major organs including liver, spleen, kidney, heart and lung were collected, and conducted with cryostat section. The sections were stained with hematoxylin and eosin (H&E), and then examined under a digital microscope.

Results

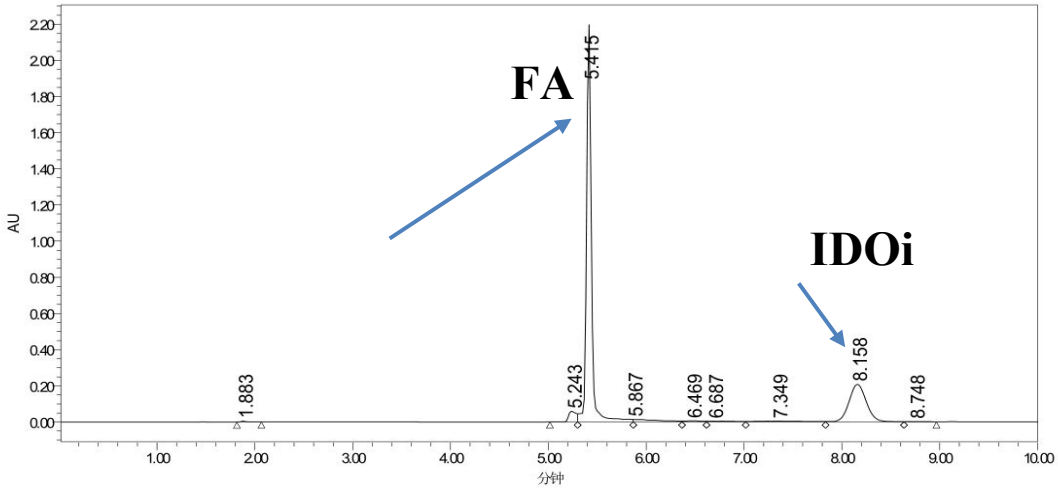

Figure S1. The HPLC spectrum of FA and IDOi (Epacadostat).

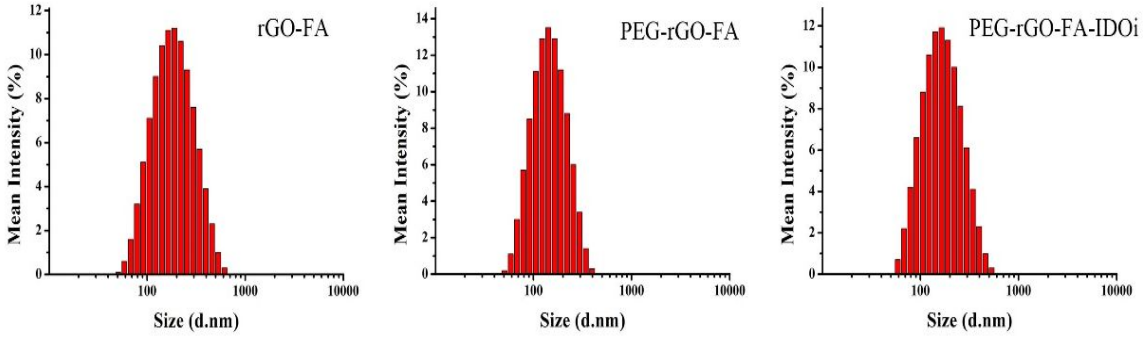

Figure S2. The size distribution of the prepared rGO nanosheets.

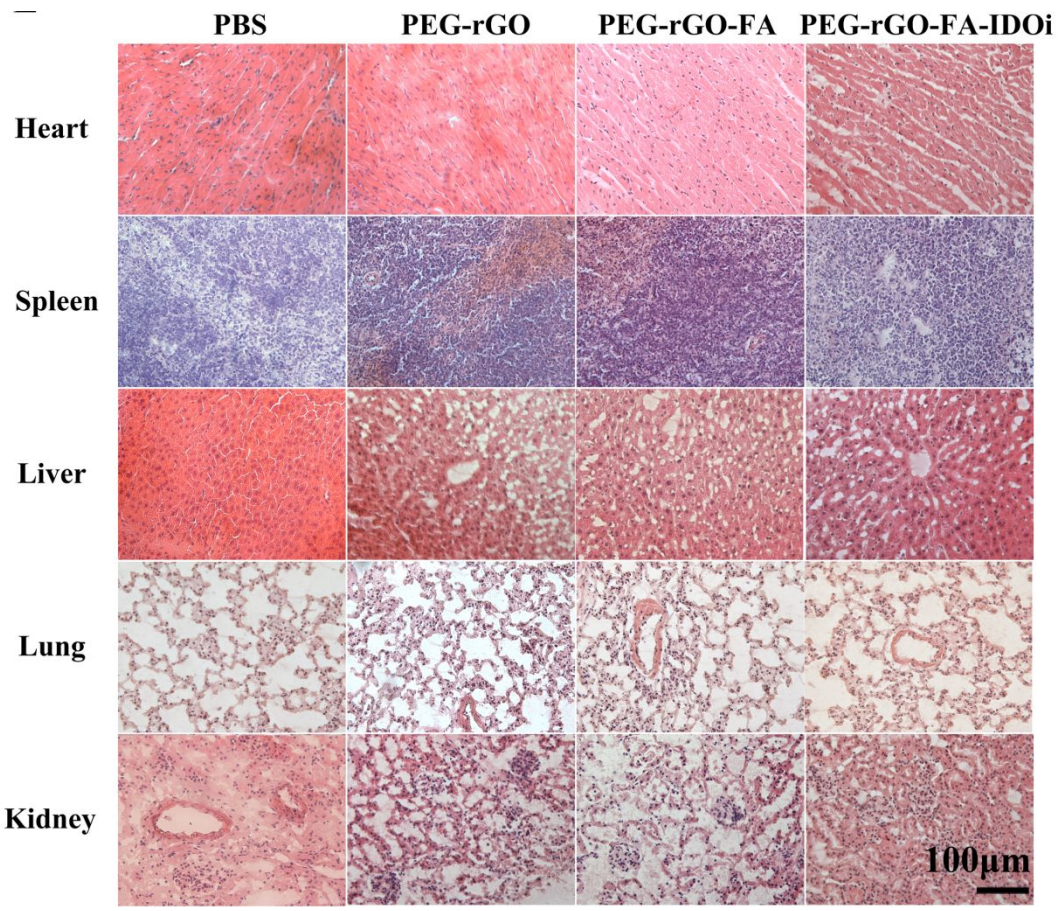

Figure S3. H&E stained images of major organs slices collected from the treated mice 7 days post-injection.

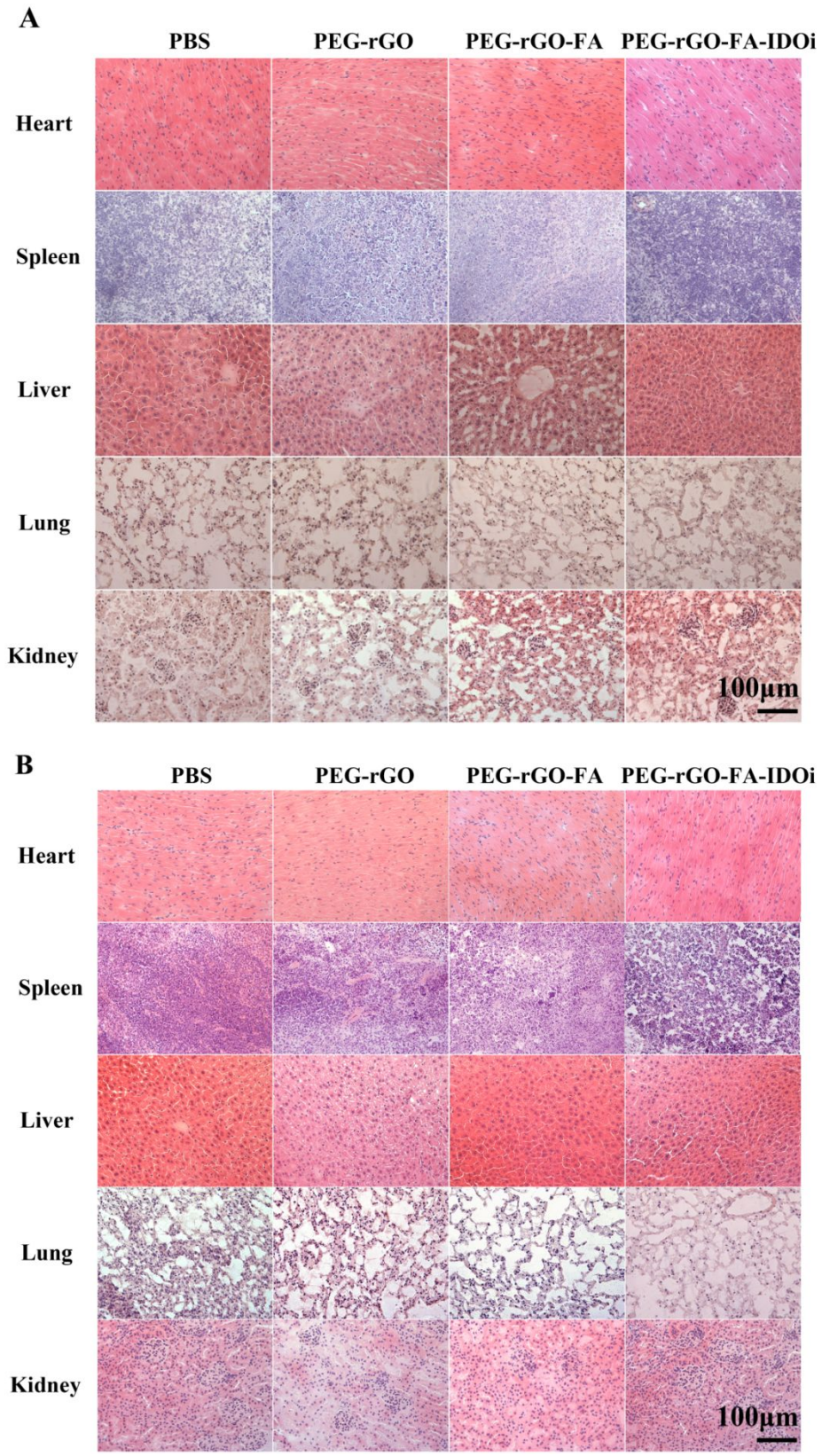

Figure S4. H&E stained images of major organs slices collected from the treated mice (A) 1 day and (B) 18 days post-injection.

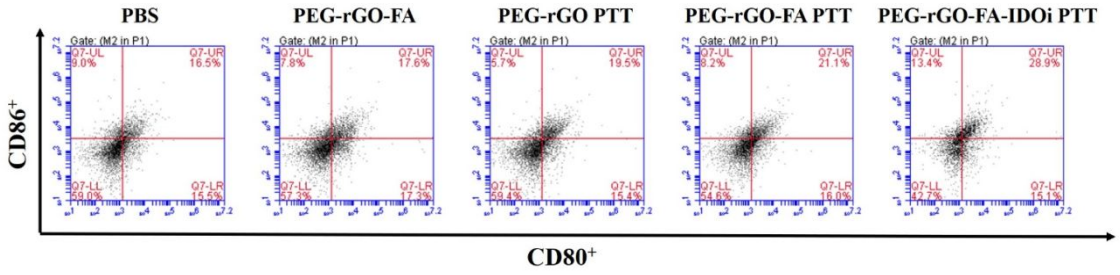

Figure S5. Expression of the co-stimulatory molecules CD86 and CD80 on $CD11c^+$ DCs in lymph node determined by flow cytometry. Representative FACS plots are shown.

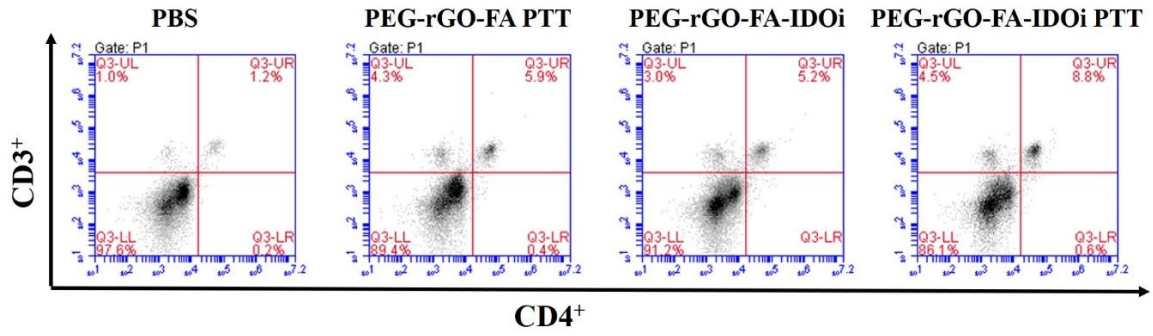

Figure S6. PEG-rGO-FA-IDOi-mediated PTT activates systematic antitumor immune response. Proportion of $CD3^+$ $CD4^+$ T cells in the distant tumor of the treated mice was determined by flow cytometry. Representative FACS plots are shown.

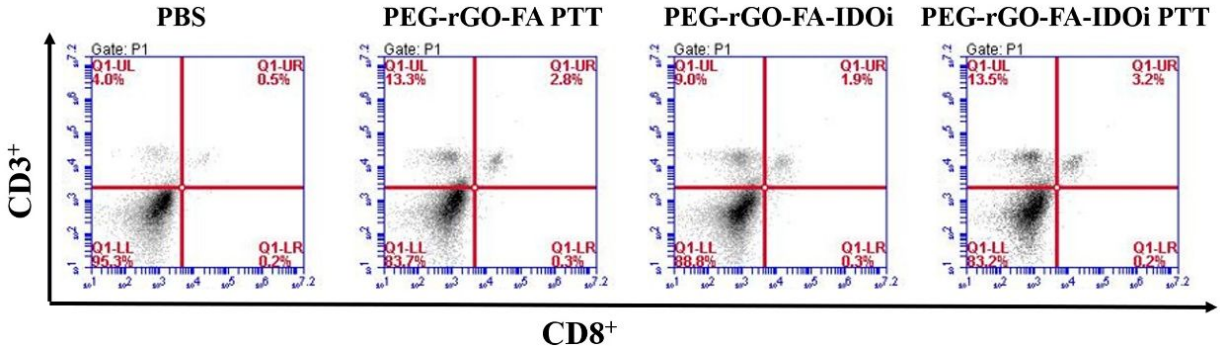

Figure S7. PEG-rGO-FA-IDOi-mediated PTT activates systematic antitumor immune response. Proportion of $CD3^+$ $CD8^+$ T cells in the distant tumor of the treated mice was determined by flow cytometry. Representative FACS plots are shown.

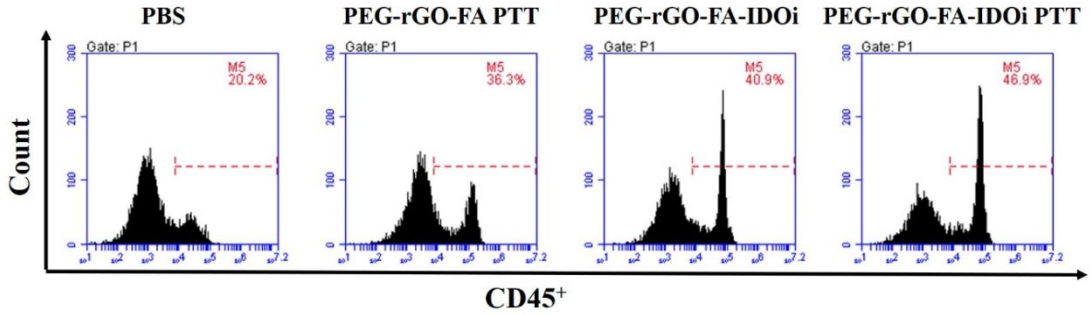

Figure S8. PEG-rGO-FA-IDOi-mediated PTT activates systematic antitumor immune response. Proportion of $CD45^+$ leukocytes in the distant tumor of the treated mice was determined by flow cytometry. Representative FACS plots are shown.

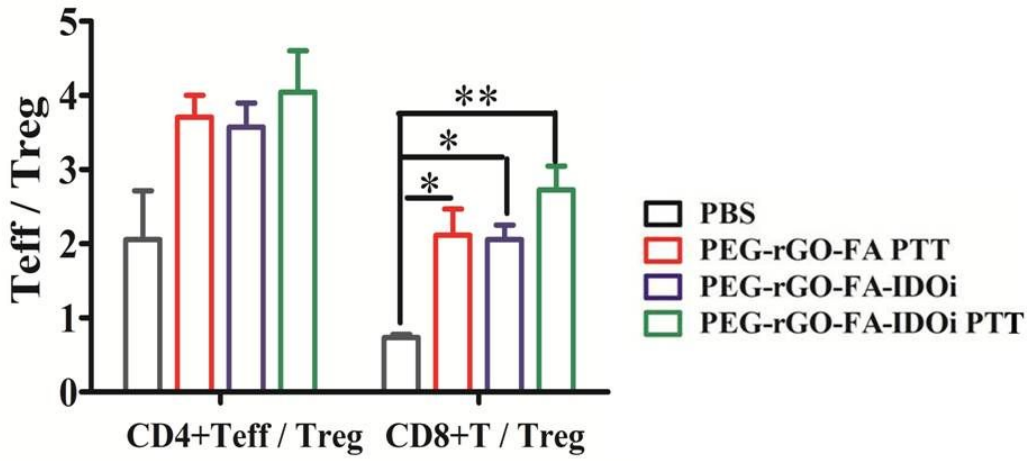

Figure S9. PEG-rGO-FA-IDOi-mediated PTT activates systematic antitumor immune response. $CD3^+$ $CD4^+$ $Foxp^+$ effector T cells ($CD4^+$ T_{eff}): $CD3^+$ $CD4^+$ $Foxp^-$ regulator T cells (T_{reg}) ratios and $CD3^+$ $CD8^+$ T cells: T_{reg} ratios in the distant tumor. The asterisks indicate that differences between groups are statistically significant: $*p < 0.05$; $**p < 0.01$.

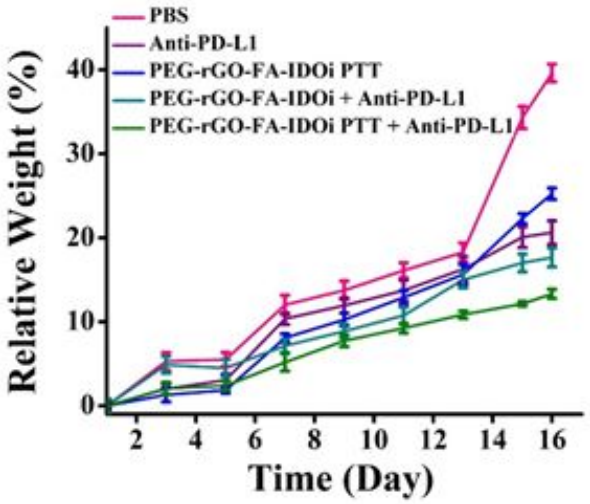

Figure S10. Relative weight during the treatment course (n = 5).