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ADVANCED MATERIALS

Supporting Information

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Large-Sized Graphene Oxide Nanosheets Increase DC–T-Cell Synaptic Contact and the Efficacy of DC Vaccines against SARS-CoV-2

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Supporting Information

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Keywords: dendritic cells, DC-T cell synapse, ICAM-1, SARS-CoV-2

Methods

Reagents and antibodies

Murine GM-CSF and IL-4 were purchased from Peprotech Asia (Rehovot, Israel). Lipopolysaccharide (LPS) and D-luciferin were purchased from Sigma Aldrich (Missouri, USA) and Promega Corporation (Madison, WI, USA), respectively. Rhodamine-conjugated phalloidin were purchased from Thermo Fisher (Massachusetts, USA). The following antibodies were used for FACS analysis: phycoerythrin (PE)-conjugated anti-CD86, peridinin-chlorophyll-protein complex (PerCP)-conjugated anti-CD80, fluorescein isothiocyanate (FITC)-conjugated anti-MHC II, allophycocyanin fluorescence 750 (APC-FL750)-conjugated CD40, FITC -conjugated anti-CD8α, APC-conjugated anti-CD69, PE-conjugated anti-CD107a, PerCP-conjugated anti-CD25，PE-conjugated anti-ICAM-1, PE-conjugated anti-Plexin A2, PE-conjugated anti-Neurophilin-1, PE-conjugated anti-CD69, Percp-cy5.5-conjugated anti-CD44, PE-conjugated OVA257-264 tetramer, PE-conjugated IFN-γ, APC-conjugated TNF-α, and PE-conjugated anti-H2Kb-binding OVA257-264 were purchased from BioLegend (San Diego, CA, USA). Rabbit anti-murine antibodies of AKT, p-AKT, P56, p-P56, RhoA, p-RhoA, p-MLC and GAPDH, CD8α, ICAM-1, β-tubulin, 488-conjugated goat anti-rabbit IgG and ICAM-1 blocking antibody were purchased from Abcam (Cambridge, UK).

Characterization of graphene oxide (GO) nanosheets

GO nanosheets, prepared from flake graphite using the modified Hummers' method, were purchased from Nanjing XFNANO Materials Tech Co., Ltd . China. After oxidation, the GO nanosheets were washed at least three times and filtrated. Low-speed centrifugation was performed to remove the multi-layered GO and increase the purity of the single-layered GOs. The supernatant was collected for freeze-drying overnight to obtain GO powder. Furthermore, the GO powder was re-suspended in deionized water and sonicated in a bath sonicator (Kunshan, 300W) for several hours to acquire GO particles of different sizes; the longer the sonication time, the smaller lateral size of GO particles were obtained. X-ray photoelectron

spectroscopy (XPS) was performed using an Escalab 250Xi spectrometer with monochromated Al Kα (hυ = 1436.6 eV), and survey scans (0-1350 eV) for each sample were recorded at a pass energy (constant analyzer energy mode) of 200 and step size 1 eV. Narrow scans for carbon 1S and oxygen 1S were recorded at a pass energy (constant analyzer energy mode) of 50 eV and step size 0.1 eV. Fourier transform infrared (FT-IR) spectra of the GO powder samples were acquired using a Nicolet-460 FT-IR spectrophotometer, and the samples were prepared by combining powdered GO with KBr and compressing to form pellets. All data were recorded and analyzed in the spectral region of 4000 to 500 cm-1. X-ray diffraction (XRD) patterns of the GO sheets were collected using a Bruker D8 Phaser equipment with a Cu-Kα radiation source (λ=1.5418Å, 40 KV, 40 mA). Raman spectroscopy was performed on a Thermo Fisher DXR Raman spectrometer, using an He-Ne laser operating at 532 nm as the excitation source.

Production of dendritic cells from bone marrow in mice

The femurs were collected after euthanizing the C57BL/6J mice, and the bone marrow monocytes were cultured in RPMI-1640 medium (Life Technologies Corporation, California, USA) containing 10% fetal bovine serum (PAN-Biotech, Aidenbach, Germany), 10 ng/mL recombinant mouse GM-CSF, and 5 ng/mL recombinant mouse IL-4, at a density of 2 × 106 cells. On Day 3 and 5, the used medium was replaced with 2 mL fresh medium containing GM-CSF and IL-4. The cells cultured on Day 6 were used as immature DCs.

Mice

Male wild-type C57BL/6J and Balb/c mice (6-8 weeks old) were procured from Beijing Vital River Laboratory Animal Technology Co., Ltd. tdTomato, enhanced GFP, and OT-I TCR Tg mice which express a TCR specific for ovalbumin 257-264 on H-2Kb were procured from the Model Animal Research Center of Nanjing University. LTD.L2G85 (FVB) mice expressing firefly luciferase (Fluc) were backcrossed with C57BL/6J mice and used in phase N7 (L2G85.C57BL/6J). All experiments were approved by the Committee on Animal Care

and Use of the Academy of Military Medical Sciences (Approval No.: AMMS-09-2019-004). All animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Pretreatment of DCs with GOs

1×10^6 DCs in 2 mL of complete RPMI 1640 were incubated in six-well plates with S-GO or L-GO at different concentrations for 48 hours. To phenotype the DCs and detect apoptosis, the DCs were washed twice with cold PBS and re-suspended in a cell staining buffer. CD11c, CD40/80/86, and MHCII antibodies were added to the cell suspension and incubated at room temperature for 15 min. For flow cytometric assays, the cells were washed twice with cold PBS. Next, the cells were stained with the APC-anti-CD11c antibody and re-suspended in a binding buffer to detect apoptosis. Annexin V-FITC and propidium iodide were added, incubated at 4 ℃ for 30 mins, and 300 μL binding buffer was then added for fluorescence-activated cell sorting (FACS). All data were analyzed using the FlowJo software. The isotype control antibody was used for each antibody to define the specific binding, and CD11c + cell population was gated as DCs.

Transmission electron microscopy (TEM)

For TEM, the GO-treated DCs were centrifuged at 400 g, washed with PBS, and fixed with 2.5% glutaraldehyde (Sigma, CA) for 30 mins. The cells were then washed with PBS for 3 times, fixed with 1% osmic acid (Merck, Schwalbach, Germany) for 30 mins, and dehydrated using an ethanol gradient. After dehydration by epoxy resin (Sigma, CA), the cells were dried in an embedded template of porous rubber and sliced. Finally, the phagocytosis of GO by DCs was observed by TEM at a working voltage of 80 kV.

Raman mapping

We imaged the GO powder or GO-treated DCs (seeded in a coverglass bottom dish) using a DXR Raman microscope (Thermo Fisher, USA) with an excitation laser of 633 nm (10 mW). The laser spot size was ~ 1μm, and images were obtained by scanning a 70 μm × 70

μm area in 10 μm × 10 μm steps and collecting the Raman spectrum at each spot (10 s integration time). The G-peak signal was set at 1,596 cm-1 to generate the cellular mapping image.

MTT analysis

For MTT analysis, DCs were treated with varying doses of GOs. collected, and reseeded in 96-well plates with 100 μL of fresh culture medium. 10 μL of the 12 mM MTT stock solution (Vybrant® MTT Cell Proliferation Assay Kit, Thermo Scientific) was then added to the cells and incubated at 37°C for 4 hours. Next, 100 μL of the SDS-HCl solution was added, and the microplates were incubated at 37°C for 4 h. The absorbance was measured at 570 nm.

Detection of cytokines secreted by DCs

The co-culture supernatants were collected by centrifuging at 400 g/min for 6 min. IL-6, IL-12p70, IL-1β, and TNF-α were tested according to the instructions of the ELISA kit (Dakewe, Shenzhen, China).

Western blot analysis

Total cellular protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis using 10% gels and transferred to polyvinylidene difluoride membranes (Millipore, UK). The membranes were incubated first with the primary antibodies overnight, and then the corresponding secondary antibodies for 1 h at room temperature. GAPDH was used as a loading control, and the signals were detected using an ECL western blotting detection kit (Amersham, Pharmacia Biotech).

Homing ability of DCs in vivo

To test the homing ability of DCs to drain the lymph nodes, 3 × 106 DCs derived from Fluc or GFP transgenic mice were incubated with GO for 48 h, and injected subcutaneously into the footpad of mice. The LN-homing of the DCs was directly detected by the IVIS Spectrum System (PerkinElmer, Massachusetts, USA) or by detecting the GFP signal in tissue slices of PLNs using a fluorescence confocal microscope (PerkinElmer, Massachusetts, USA).

Immunofluorescence staining

DCs cultured in glass bottom cell culture dishes (NEST, Jiangsu, China) were fixed with 4% paraformaldehyde (Merck, Schwalbach, Germany) for 1 h, and incubated with 1% Triton X-100 (Sigma, Missouri, USA) for 15 min. The cells were then washed thrice and incubated with the primary antibodies for 12 hours at 4 °C. Next, the cells were washed with PBS and incubated with fluorescence-conjugated secondary antibodies for 60 mins at room temperate. After washing, the cells were incubated with DAPI to stain the nuclei, and PerkinElmer (Massachusetts, USA) or Nikon A1 (Japan) confocal laser-scanning microscopes were used to view the cells.

Imaging cytoskeletal organization and ICAM-1 location

DCs of different treatments were adhered on cover-glass bottom dish for 4 h, and were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 in PBS. For actin staining, permeabilized cells were labeled with 300 μL rhodamine-conjugated phalloidin (50 μg/mL) for 2 h. For β-tubulin staining, permeabilized DCs were stained with 300 μL anti-β-tubulin (10 μg/mL) overnight, followed by FITC-Goat Anti-Rabbit IgG for 2 h at room temperate. For paxillin staining, permeabilized DCs were stained with 300 μL anti-paxillin (1 μg/mL, Abcam) followed by 300 μL 488-Alex-Goat Anti-Rabbit IgG (10 μg/mL) for 2 h. For ICAM-1 staining, permeabilized DCs were stained with 300 μL anti-ICAM-1 (ab222736, Abcam) at 1/50 dilution followed by 300 μL 488-Alex-Goat Anti-Rabbit IgG (10 μg/mL) antibody at 1/1000 dilution for 2 h at room temperature. The coverslips were then mounted using fluoroshield mounting medium with DAPI (Abcam) and viewed on a confocal microscope (PerkinElmer, Massachusetts, USA).

To inhibit the Rho/Rock signal pathway, DCs were treated with Y-27632 2HCL (20 μM, Selleckchem, USA) for 12 h. Thereafter, 9 μg/mL GOs was added into the culture medium

and incubated for another 48 h. Then, the DCs were collected for staining the cytoskeleton or for measuring ICAM-1 membrane expression by FACS.

Antigen loading

To prepare antigen-bearing DCs, $OVA_{257-264}$ (1μg/ml) or SARS-CoV-2 spike 1 (5 μg/ml) were added into the culture medium and incubated with DCs for 48 h. Thereafter, DCs were collected and washed twice to remove the free antigens before use.

DC-T cell interaction

DCs from tdTomato transgenic mice were incubated with GOs (9 μg/mL) and $OVA_{257-264}$ for 48 h and then collected by centrifuging at 400 g/min for 6 min. CD8+ T cells from OT-I transgenic mice were purified by CD8+ T Cell Isolation Kit (Miltenyi Biotec, Germany) and were labeled with the CellTracker™ Blue kit (Thermo Scientific, USA). Thereafter, tdTomato + DCs were cocultured with the labeled T cells at the ratio of 1:3 (DC: T cell). The interaction between DCs and T cells were monitored using a live cell imaging system (PerkinElmer, Massachusetts, USA), and the colocalized areas visualized using light channels 561 and 405 in dynamically pictured micrographs were analyzed using Volocity software to calculate the contact area of DC-T cells. Their dynamic contact tracing further summarized the durations of the DC-T cell interaction.

To detect L-GO-induced DC-T cell clustering, tdTomato + DCs bearing $OVA_{257-264}$ were cocultured with the CellTracker™ Blue-labeled T cells at the ratio of 1:3 (DC:T cell), and FITC-labeled L-GOs were then added into the co-culture at a dose of 9 μg/mL. Thereafter, the mutual interactions among L-GOs, DCs, and T cells were dynamically imaged under the live cell imaging system. The colocalized areas visualized using light channel 488 with 561 and 405 reflected the adherence of GOs on DCs and T cells, respectively.

To detect the ex vivo activation of T cells, GO-pretreated and OVA-loaded DCs were collected and cocultured with CFSE (5 μM, Selleckchem, USA)-labeled CD8α+ T cells from OT-I mice at the ratio of 1:3 (DC : T cell) for 72 h; thereafter, the cells were collected by

centrifuging at 400 g/min for 6 min and T cell activation was detected by co-staining for the markers CD8α, CD69, CD107a, and CD25. T cell proliferation was detected by measuring the CFSE dilution in daughter cells. All samples were detected by FACS and were analyzed by FlowJo. The isotype control antibody was used for each antibody to define the specific binding, and CD8α+ cell population was gated as T cells.

ICAM-1 blocking

DCs, with or without Y-27632 pretreatment (described above), were co-incubated with GOs (9 μg/mL) for 48 h, and were collected by centrifuging at 400 g/min for 6 min. LPS (100 ng/mL)-treated DCs were set as a positive control. Then, all DCs were stained by FAST DiI™ kit (Thermo Fisher, USA). CD8+ T cells from OT-I mice were stained by CFSE (Selleck Chemicals, USA) and mixed with DCs at a ratio of 3:1 with or without 1 μg/mL ICAM-1 blocking antibody. Cells were seeded in cover-glass bottom dish and cultured for 12 h. Then, the culture medium was removed carefully and the adhered DC-T cells were monitored under the confocal microscope (PerkinElmer, Massachusetts, USA).

In vivo T cell priming

First, 9 μg/mL GOs and 1 μg/mL $OVA_{257-264}$ were added into the culture medium (Day 0) and co-incubated with DCs for 48 h. A cytokine-cocktail composed of 1000 U/mL IL-1β, 1000 U/mL IL-6, 1000 U/mL TNF-α, and 1 μg/mLPGE_2 (Pepro Tech, Rehovot, Israel), the mostly used DCs adjuvant, was used as a control. Then DCs of the different treatment groups were collected and injected into the footpad of C57BL/6J recipient mice twice (Day 3 and Day 9) at a dose of 2×10^6 per mouse. On Day 12, the PLNs of mice were isolated and grinded on a 200-mesh filter to obtain 1 mL of a single-cell suspension. The antigen-specific T cell activation was detected by double staining the lymphoid cells with 1 μg/mL FITC-CD8α and T-Select H-2Kb OVA Tetramer-SIINFEKL-PE (MBL, Nagoya, Japan) and FACS analysis. Furthermore, the lymphoid cells were stained with a panel of antibodies, namely

anti-CD8α-FITC, anti-CD69-PE, and anti-CD44-Percp-cy5.5, to detect CD8+ T cell activation. For intracellular cytokine detection, cells were stained with FITC-anti-CD8α antibody and then re-suspended in a fixation/permeabilization solution (BD, New Jersey, USA). After incubation at 4 °C for 20 min, the cells were washed and then labeled by APC-anti-TNF-α and PE-anti-IFN-γ antibodies for 30 min at 4 °C. Cells were washed twice before FACS analysis.

For the antigen-recall experiment, PLN cells were plated in triplicate at a dose of 1×10^5 per well with 1 μg/mL $OVA_{257-264}$ supplemented in the medium. Then, 48 h later, 2 μg/mL monensin (BD Pharmingen, SanDiego, CA) was added into the culture medium, and was co-incubated with cells for another 4-6 h. Then, cells were collected for intracellular cytokine staining.

Recombinant virus generation and mouse infection

Recombinant adenovirus (AdFLO) expressing fusion proteins of OVA and Fluc were generated according to standard protocols. The immunized mice followed the schedule shown in Fig. 5F and were challenged with virus at 1×10^9 Pfu/mouse via tail vein injection. The liver Fluc activity was detected at 12 h and 48 h to quantify hepatocellular infection. The CTL infiltration into the liver was detected by immunohistochemical staining of liver sections with CD8α antibody (Abcam, Cambridge, UK).

Imaging of SARS-CoV-2 Spike 1 expression in the lung

Recombinant pcDNA3.1 vector carrying Spike1-IRES-Fluc was constructed at Gene Universal Inc. (AnHui, China). The recombinant vectors were mixed with the transfection reagent of in vivo-jetPEI® kit (Polyplus-transfection®, New York, USA) and directionally delivered to the lungs of recipient mice via tail vein injection. Hence, calculating the deduction of Fluc light intensity could quantify anti-spike 1 CTL functions. The infiltration of CTLs into the lungs was detected by immunohistochemical staining of lung sections with CD8α antibody (Abcam, Cambridge, UK). The TdT-mediated dUTP nick-end labeling

(TUNEL) Apoptosis Assay Kit (Abcam, Cambridge, UK) was used to detect the apoptosis of lung epithelium cells.

SARS-CoV-2 viral challenge

All animal experiments were conducted in Biosafety Level 3 laboratory (BSL-3) and approved by the Animal Experiment Committee of Laboratory Animal Center, Beijing Institute of Microbiology and Epidemiology (approval number: IACUC-DWZX-2020-002). SARS-CoV-2 strain IME-BJ05 (BetaCov/huma n/CHN/Beijing_IME- BJOS/2020) was originally isolated from a patient with COVID-19. Adaptation of SARS-CoV-2 was achieved by serial passage through lungs of BALB/c mice, which has been described in detail previously (reference). This was followed by animal immunization and viral challenge (Fig. 7A). To quantify viral RNA, lung homogenates were collected, and total RNA was extracted from them using an RNA extraction kit (QIAamp Viral RNA Mini Kit, Qiagen, Germany). The following primers and probes targeting the S gene of SARS-CoV-2 were used to quantify viral RNA by quantitative reverse transcription PCR:

CoV-F3 (5'-TCCTGGTGATTCTTCTTCAGGT-3');

CoV-R3 (5'-TCTGAGAGAGGGTCAAGTGC-3');

CoV-P3 (5'-AGCTGCAGCACCAGCTGTCCA-3').

Antibodies against SARS-CoV-2 S (Sino biological, China, 1:2000) and CC10 (Millipore, USA, 1:500) were used to visualize the SARS-CoV-2 Spike protein and the lung club cells.

Figures and Tables:

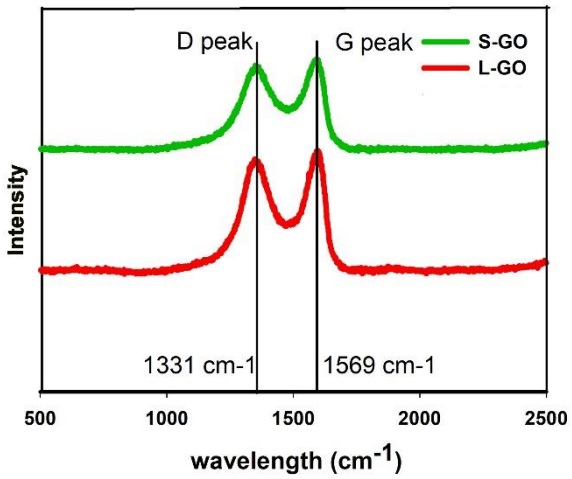

Figure S1. Raman spectrum of GOs.

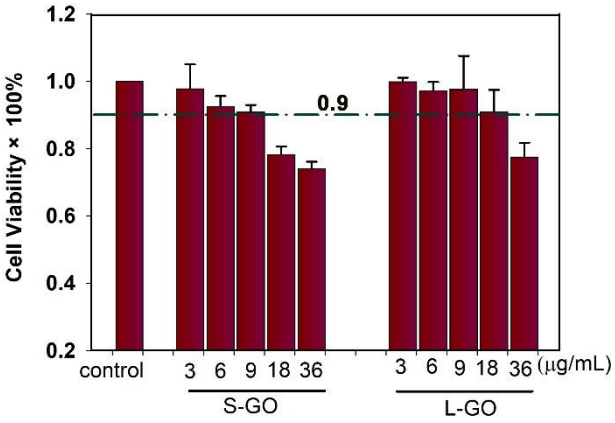

Figure S2. The MTT analysis for DC viability; Data are mean ± s.d; n=5.

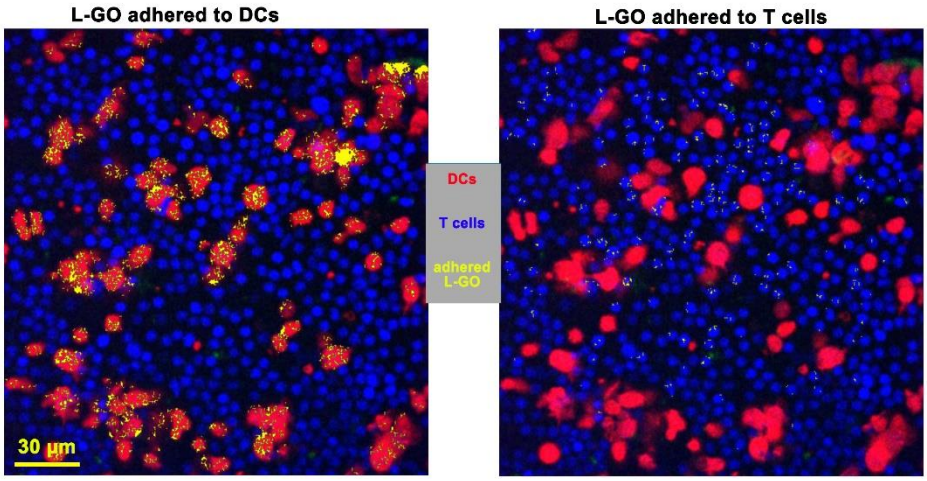

Figure S3. L-GOs absorbed on respective DCs (left panel) and T cells (right panel). Red: DCs; blue: T cells; yellow: adhered L-GOs.

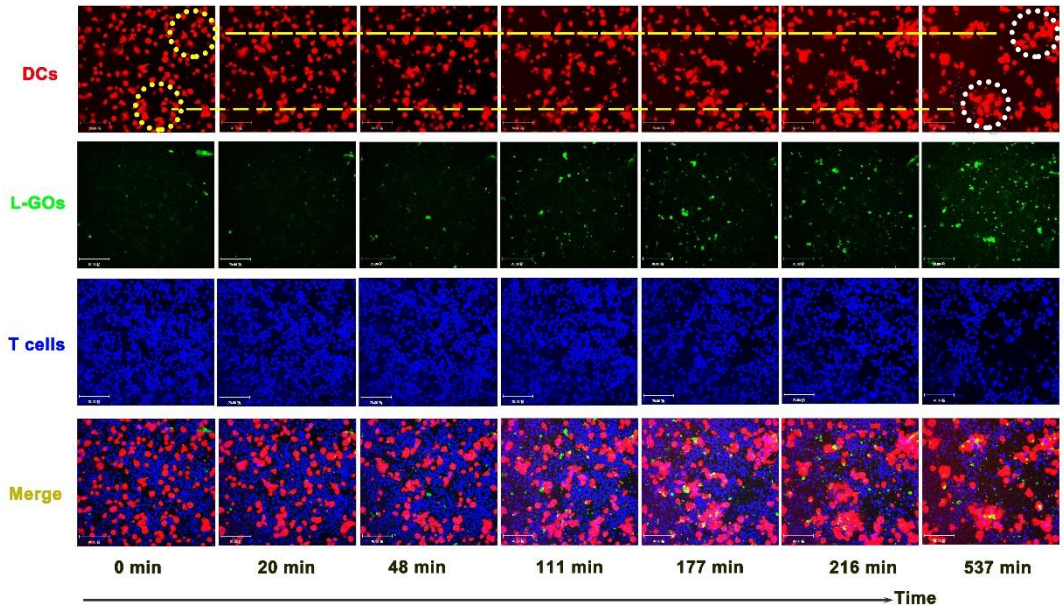

Figure S4. L-GOs mediated DC-T cell clustering by promoting DC aggregation. Yellow circle: scattered DCs; white circle: aggregated DCs. Red: DCs; green: L-GOs; blue: T cells.

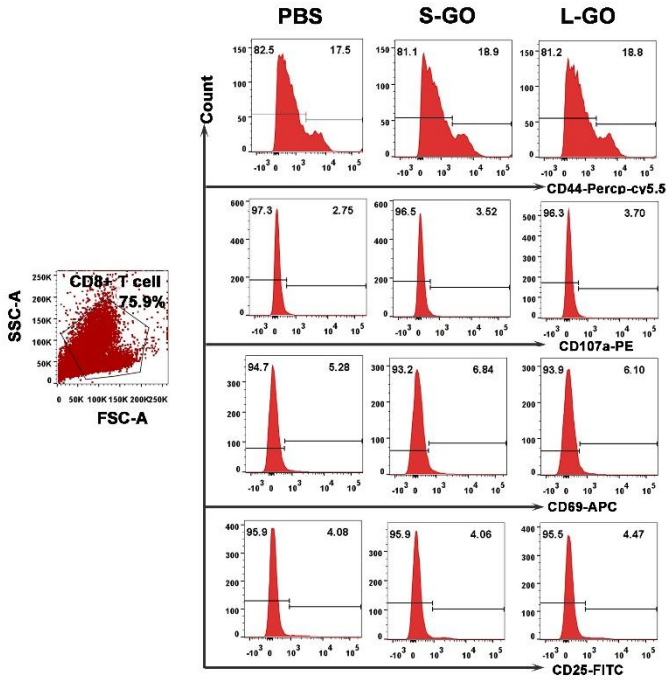

Figure S5. GOs have limited effect on T cell activation. CD8+ T cells from OT-1 transgenic mice and incubated with 9 μg/mL GOs for 72 h. Then cells were collected for detecting the activating markers of CD44, CD107a, CD69 and CD25 by FACS.

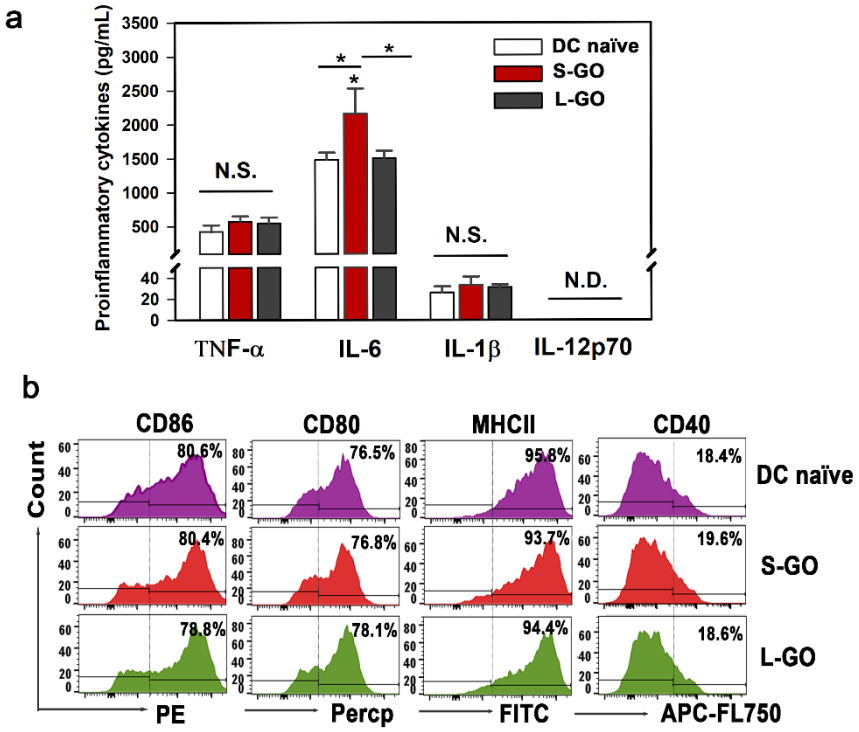

Figure S6. The effect of GO treatment on DC cytokine secretion a) and allo-stimulatory molecular expression b). Data are mean ± s.d; n=4; p* < 0.05 by Holm-Sidak method.

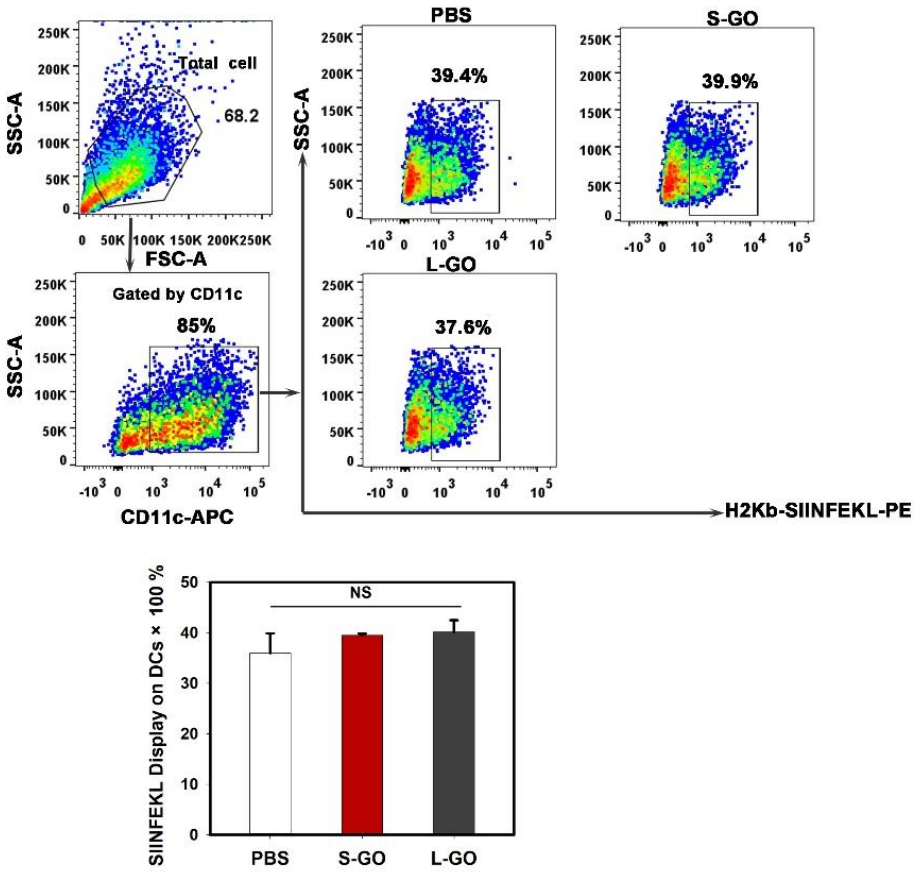

Figure S7. $OVA_{257-264}$ presented on DCs. The H-2Kb binding $OVA_{257-264}$ on DC surface was detected by PE-conjugated antibody for FACS analysis. NS: not significant; Data are mean ± s.d; n=3-4.

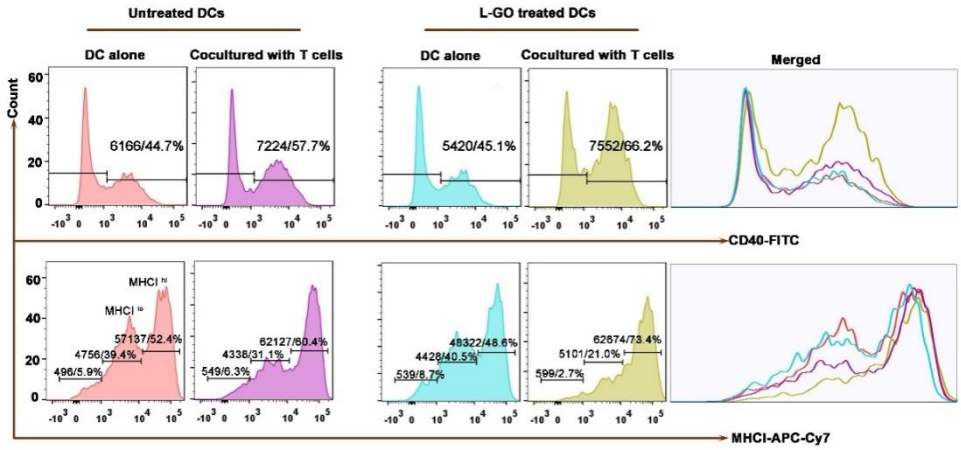

Figure S8. The expression of CD40 (up panel) and MHC I (below panel) on DCs before and after being incubated with CD8+ T cells. DCs were treated with L-GOs (9 μg/mL) in the presence of 100 μg/mL $OVA_{257-264}$ for 48 h. The untreated DCs were set as the control. Then they were collected and cocultured with CD8+ T cells from OT I mice at the ratio of 1:3 (DCs:T cells). Forty eight hours later, the expressions of CD40 and MHCI in DCs were detected by FACS.

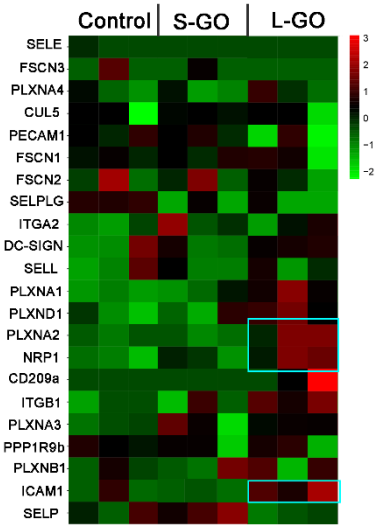

Figure S9. Transcriptional level of adhesion molecules in DCs by RNA sequencing.

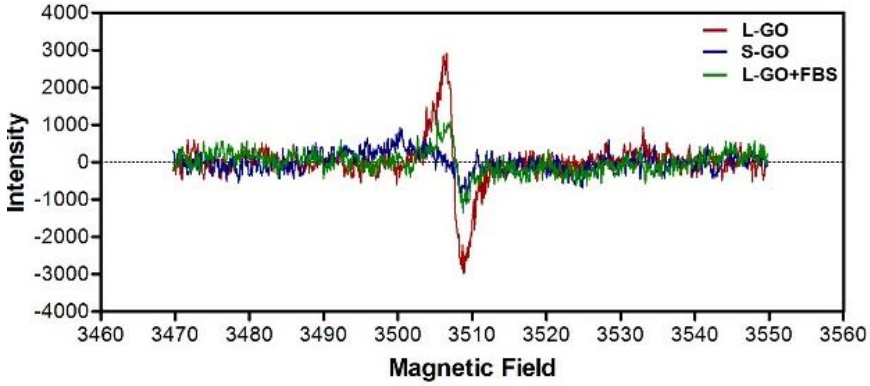

Figure S10. Assessment of carbon radical formation quantification by an X-band Bruker ELEXYS 580 electron paramagnetic resonance (EPR) spectrometer.

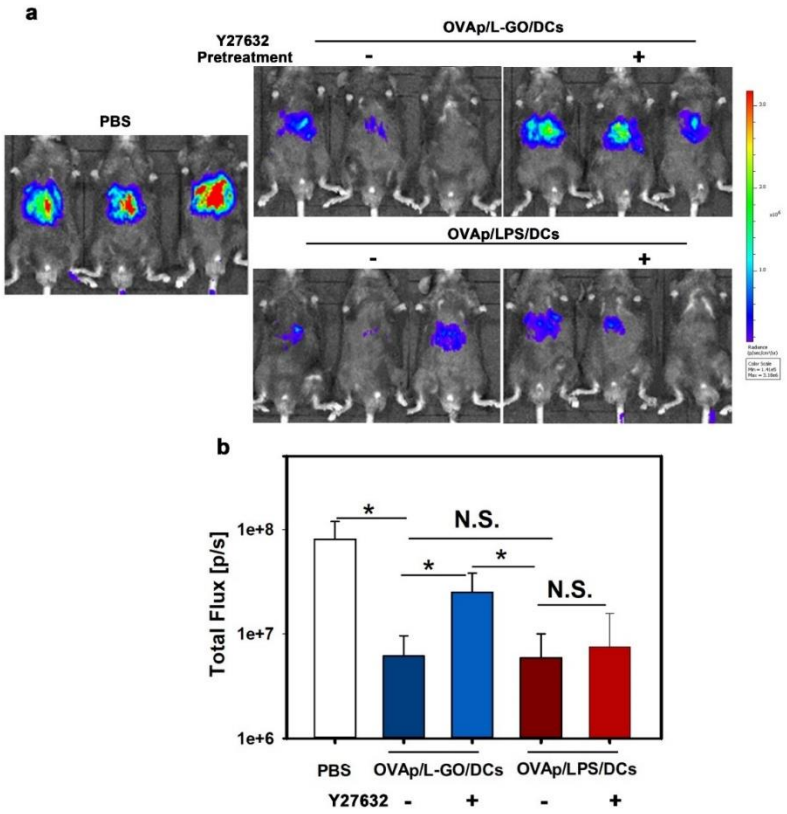

Figure S11. Inhibiting Rho-ROCK-MLC pathway of L-GO adjuvanted DCs greatly impaired their protective efficiency against viral infection. In the presence of $OVA_{257-264}$ (1μg/mL), DCs was stimulated by L-GO and LPS with or without Y27632 pretreated for 48 h. In the untreated group, PBS was added as a control. After incubation, DCs were collected and injected to the footpad of mice following the schematic diagram in Figure 4a. Three days after the second vaccination, the immunized mice were challenged AdFLO. Then mice were imaged by BLI for detecting the viral clearance 48 hours after viral challenge. Bioluminescence imaging a) and statistical analysis b) of the AdFLO infection in the liver. n = 3-5.

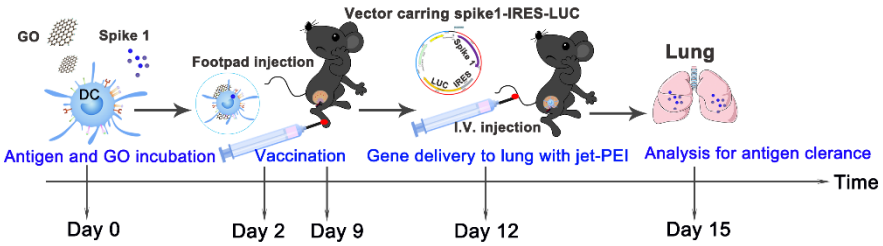

Figure S12. Schematic diagram of SARS-CoV-2 spike 1- bearing DC vaccination process.

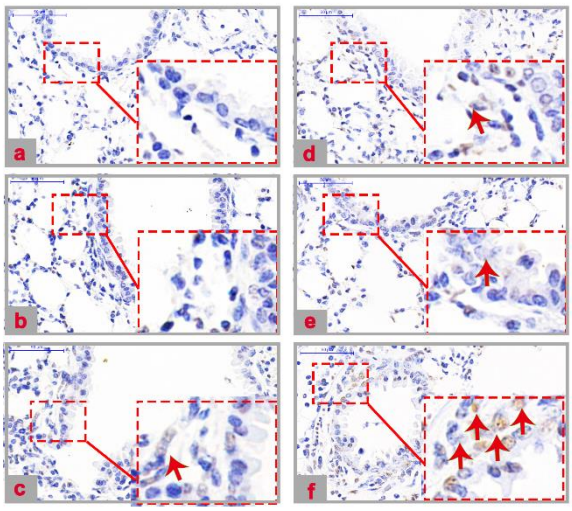

Figure S13. Apoptosis of lung epithelial cells by TUNEL staining. Red arrow points to the apoptotic cell.

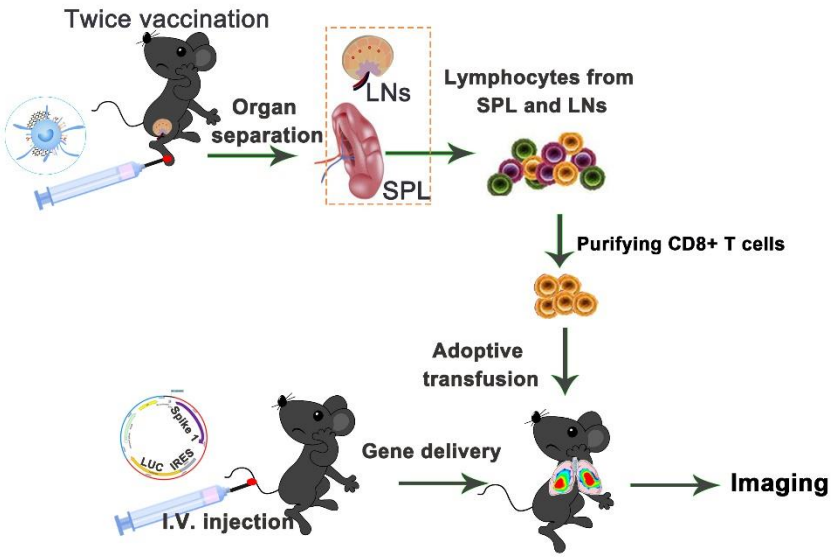

Figure S14. Flow chart of purified CD8+ T cell transfer experiment.

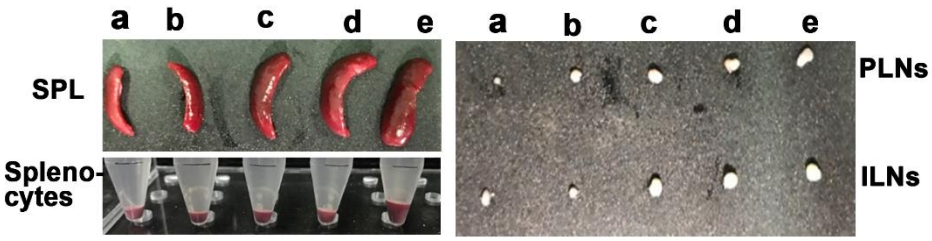

Figure S15. Pictures of spleen, splenocytes, PLNs, and ILNs of immunized mice. a: PBS; b: naïve DCs; c: spike 1/C-C/DCs; d: spike 1/S-GO/DCs; e: spike 1/L-GO/DCs.

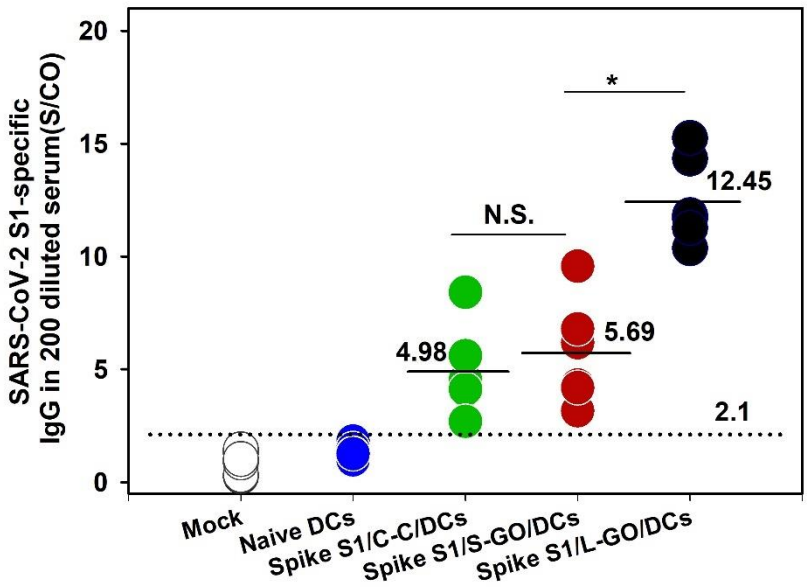

Figure S16. SARS-CoV-2 spike1 specific IgG detected in the serum of immunized mice. Data are mean ± s.d.; n=5; Holm-Sidak method was used for multiple comparisons; *p < 0.05. N.S.: not significant.

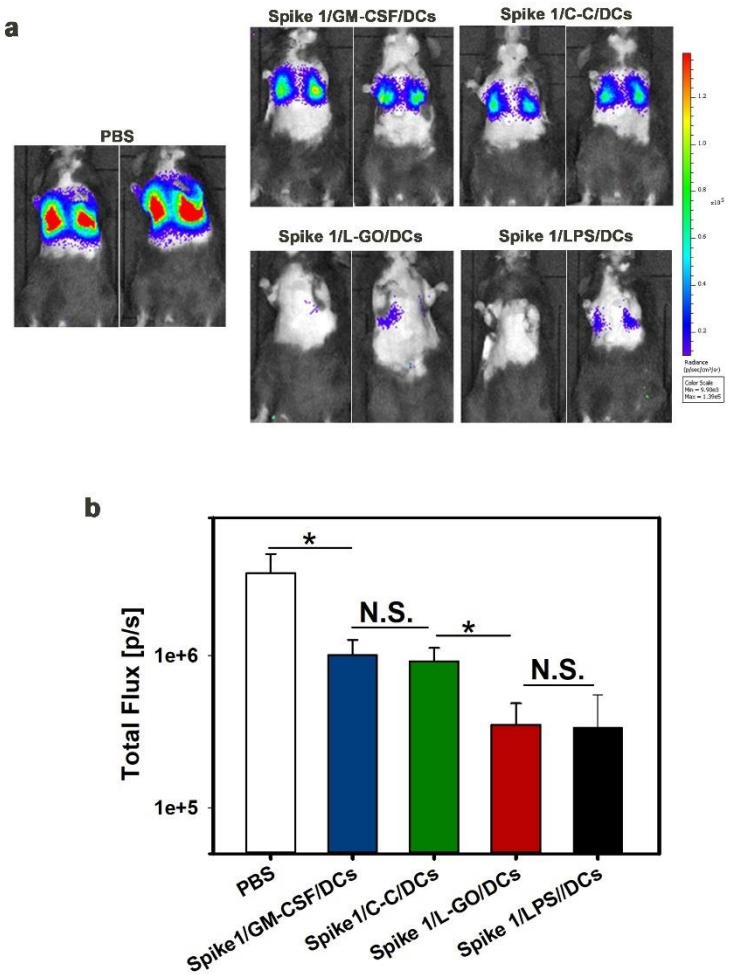

Figure S17. Bioluminescence imaging a) and statistical analysis b) the clearance of Spike 1 expression in the lungs. DCs adjuvanted by GM-CSF,cytokine-cocktail,L-GOs or LPS were ex vivo pulsed with spike 1 proteins from SARS-CoV-2, and then were adoptively injected into the recipient mice following the vaccination procedure shown in Figure S12, Supporting Information. After two immunizations, recombinant vectors carrying Spike1-IRES-Fluc were directionally delivered to the lungs of the recipient mice. Data are mean ± s.d. n = 4.*p <0.05 ;N.S.: not significant.

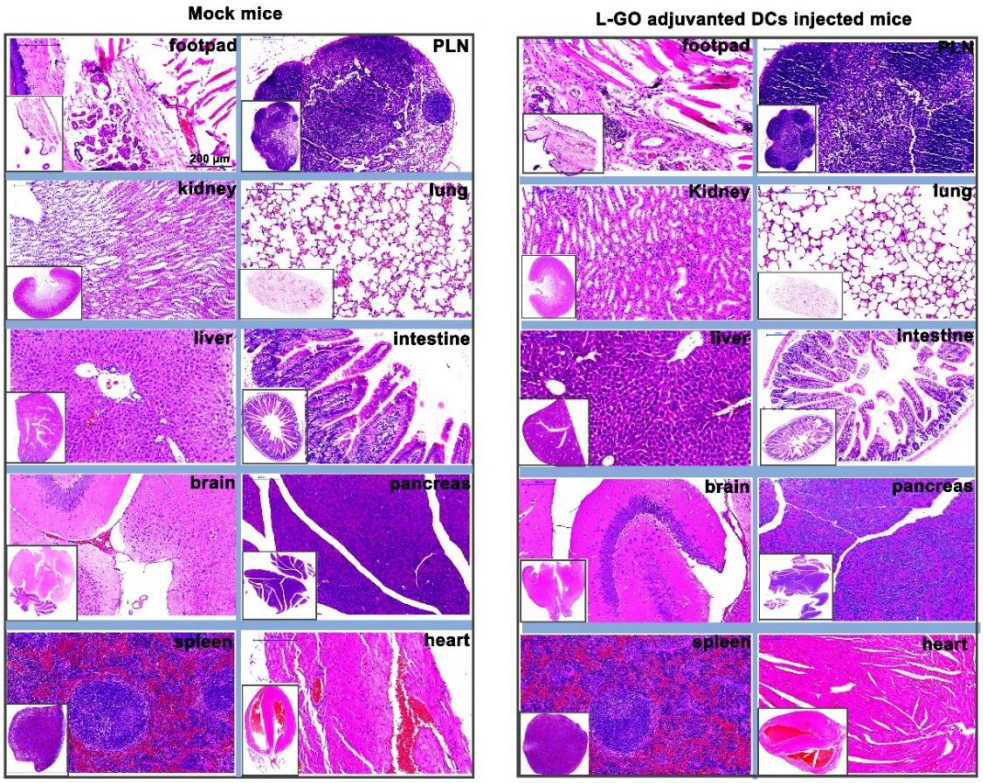

Figure S18. H&E staining of major organs of mice. A representative dataset from n=5.

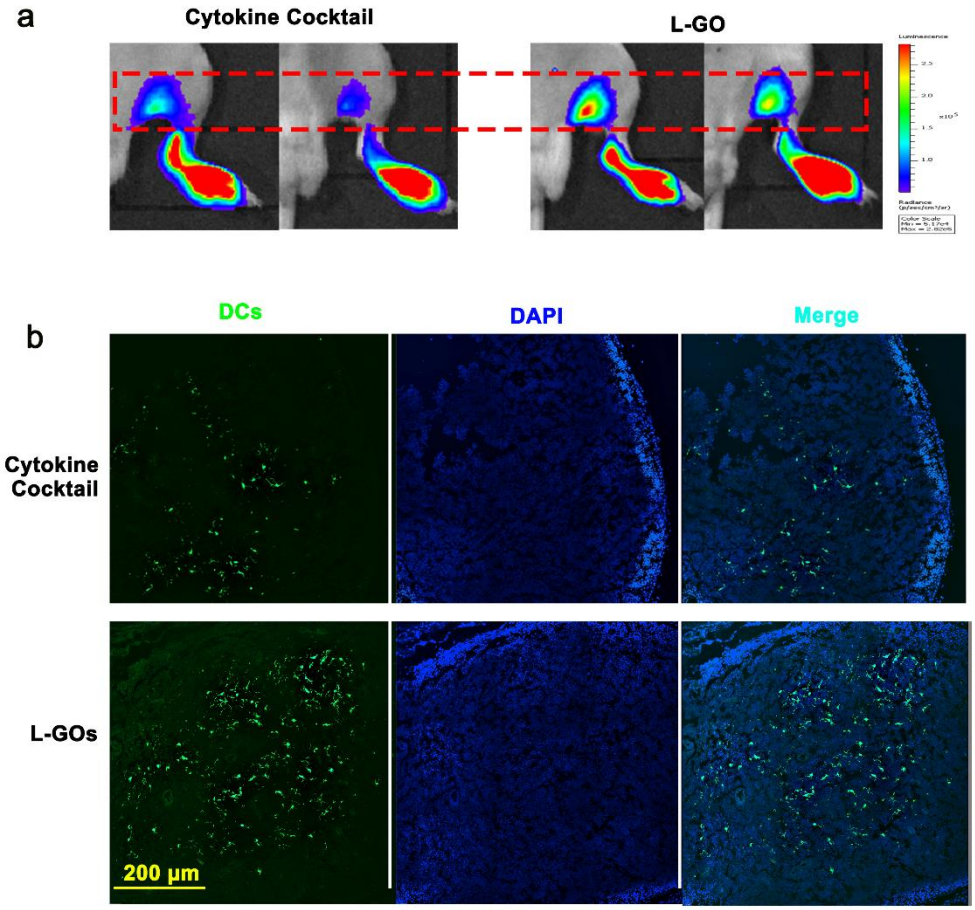

Figure S19. Contrastive analysis of the in vivo homing ability of cytokine cocktail- and L-GOs- adjuvated DCs by bioluminescence imaging a) and immunofluorescence staining b). The red rectangle frame in A represented DCs homing to PLNs.

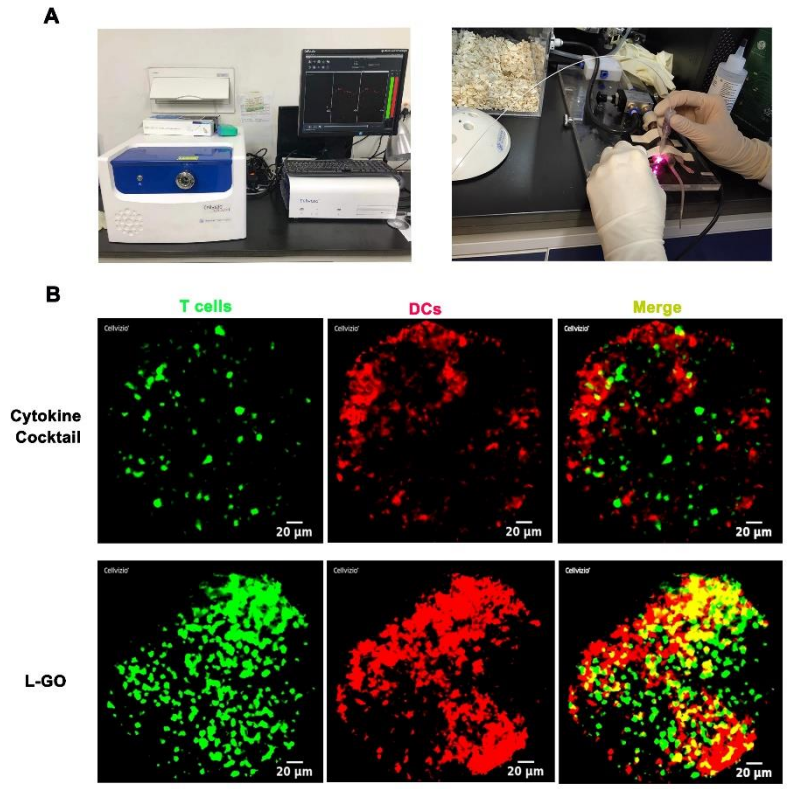

Figure S20. In vivo antigen-specific T cell capture by DCs. DCs were treated by 9 μg/mL L-GOs or cytokine-cocktail for 48 h with 100 ng/mL $OVA_{257-264}$ presence, and then were collected and labeled with MitoBright LT Deep Red (Dojindo Molecular Technologies, Inc., Rockville, MD, USA). CD8+ T cells from OT-I transgenic mice were labeled by CFSE. DCs were injected into the footpad at 2×10^6 per mice; and the labeled T cells were i.v. injected at 3×10^6 per mice. The colocalization of DC and T cells in PLN was visualized by fluorescence-based Confocal Laser Endomicroscopy (CLE) system (Cellvizio, Manua Kea Tchnologies, France). a) Detecting the fluorescence with Cellvizio. b) DC and T cells in PLN; green: CD8+ T cells; Red: DCs; yellow: colocalized DC-T cells.

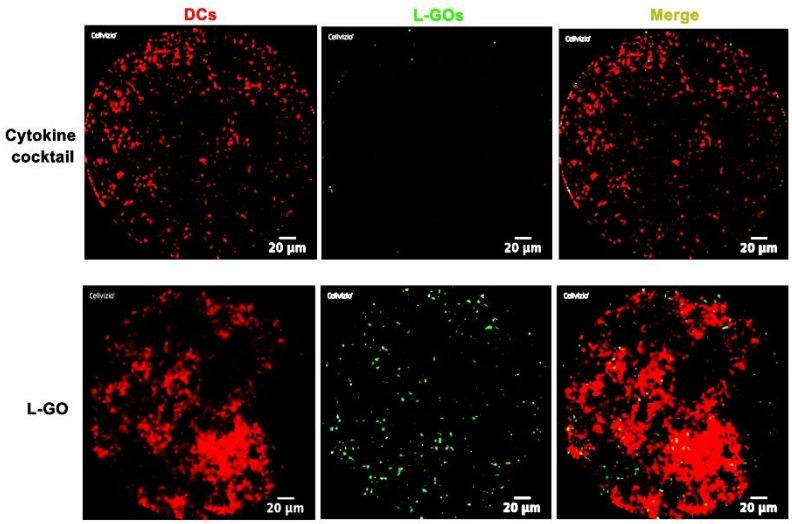

Figure S21. GOs drained to PLNs. DCs were treated by 9 μg/mL FITC-labeled L-GOs or cytokine-cocktail for 48 h, and then were collected and labeled with MitoBright LT Deep Red. Then DCs were collected and injected into the footpad at 2×10^6 per mice. The draining of L-GOs and DCs to PLN was visualized under Cellvizio. Red: DCs; green: L-GOs.

Table S1. The basic physicochemical property of two-sized GOs.

	Zeta potential (mV)[1]	Zeta Potential (mV)[2]	I_D/I_G	XPS				
				C-C/C=C	C-OH	C=O	O=C-H	C/O ratio
S-GO	-36.10±5.96	-15.4±0.35	0.98	48.54%	16.94%	29.16%	5.45%	2.19
L-GO	-30.4±2.55	-14.0±0.46	0.96	49.37%	14.31%	30.06%	6.26%	2.19

1: GOs in water; 2: GOs in cell culture medium.

Table S2. The complete blood count of mice.

	Mock-treated mice	L-GOs adjuvanted DC injected mice
WBC (10^9/L)	3.22 ± 0.779	3.540 ± 0.808
Lymph（10^9/L）	1.82 ± 0.396	2.3 ± 0.636
Mon（10^9/L）	0.10 ± 0.00	0.1 ± 0.00
Gran（10^9/L）	1.58 ± 0.466	1.420 ± 0.536
RBC (10^{12}/L)	9.266 ± 0.809	9.198± 0.411
HGB (g/L)	147.60 ± 15.742	145.8 ± 4.764
HCT (%)	39.90 ± 44.100	38.60 ± 43.700
MCV (fL)	48.10 ± 0.548	46.86 ± 1.922
MCH (pg)	15.86 ± 0.577	15.82 ± 0.311
MCHC (g/L)	330.6 ± 9.397	339 ±14.283
RDW (%)	13.18 ± 0.701	13.26 ± 0.783
PLT (109/L)	1049 ± 127.328	913.6 ± 109.869
MPV (fL)	4.74 ± 0.288	4.72 ± 0.164
PDW	16.9 ± 0.495	16.82 ± 0.286
PCT (%)	0.497 ± 0.0678	0.43 ± 0.0479

Data are mean ± s.d; n = 5.

Supplementary Video 1. T cells interact with untreated DCs; blue: T cells; red: DCs.

Supplementary Video 2. T cells interact with S-GO treated DCs; blue: T cells; red: DCs

Supplementary Video 3. T cells interact with L-GO treated DCs; blue: T cells; red: DCs

Supplementary Video 4. The interaction among L-GOs, DCs and T cells; blue: T cells; red: DCs；green: L-GOs.

Supplementary Video 5. L-GOs promoted DC-T cell clustering. blue: T cells; red: DCs；green: L-GOs.