Supplementary Figures

A

SoyPC DOTAP	Size (nm)	Zeta potential (mV)	PDI	Encapsulation Efficiency (%)	Drug Loading (%)
cGAMP-NP	85±27	14.8±4.6	0.124	43.1± 5.4	2.6 ± 0.3
Blank-NP	84±31	22.1±3	0.115	——	——

B

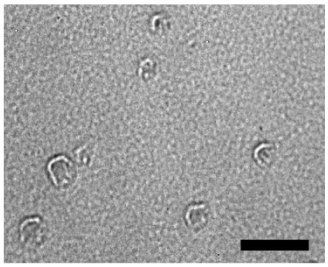


Figure S1. Liposomal nanoparticle (NP) characterization and TEM imaging. (**A**) Particle size, zeta potential, polydispersity index (PDI), cGAMP encapsulation efficiency and drug loading were measured and pooled from three experiments. (**B**) TEM images to show NPs morphology and size. Scale bar, 200nm.

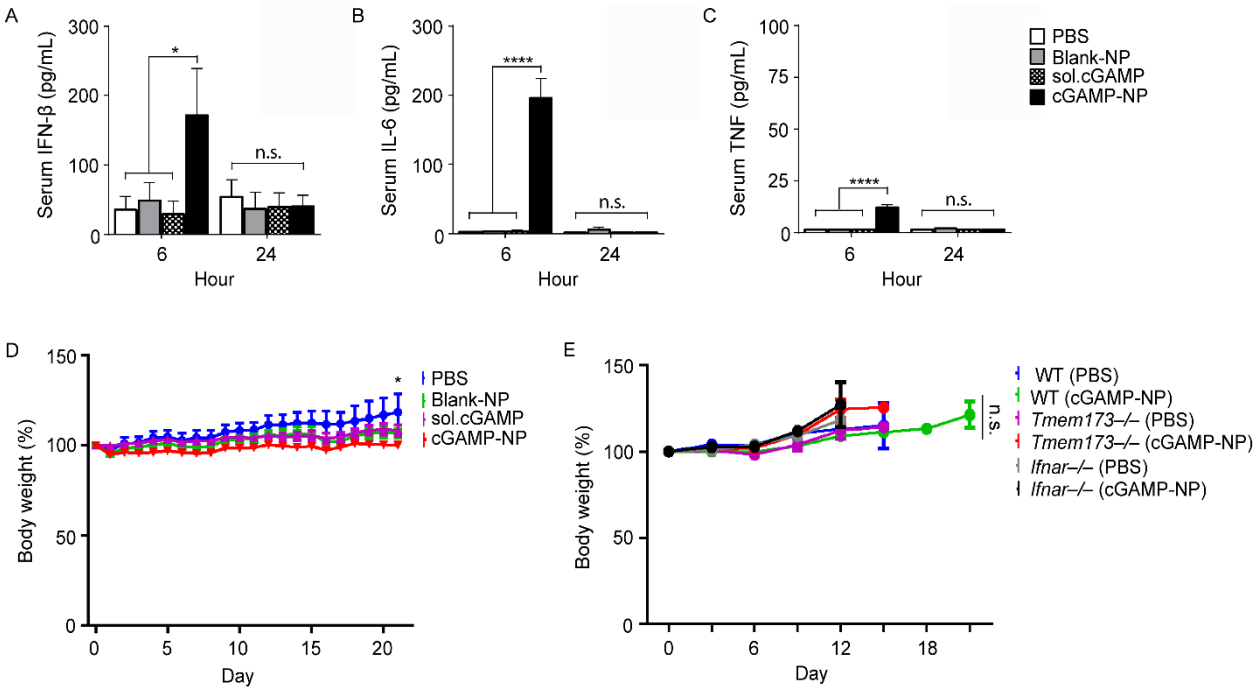
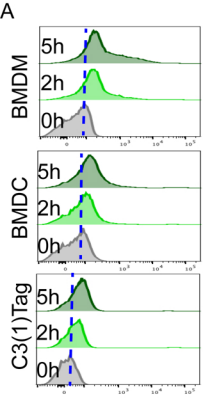


Figure S2. Body weight changes of C3(1)Tag and B16F10 tumor mice during treatments.

(A-C) Sera were collected from C3(1)Tag mice at 6 and 24 hours after cGAMP-NP treatment. Serum IFN-β, IL-6 and TNF levels were detected by ELISA assay (n=7-8/group)**.** Body weight of orthotopic C3(1)Tag mice (**D**) and B16F10 melanoma mice **(E**) were measured during cGAMP treatments (n=10/group). Data in (**A-E**) were pooled from two independent experiments (n=10 mice/group). The statistical significance was determined by one-way ANOVA with a Tukey's post hoc test.


A
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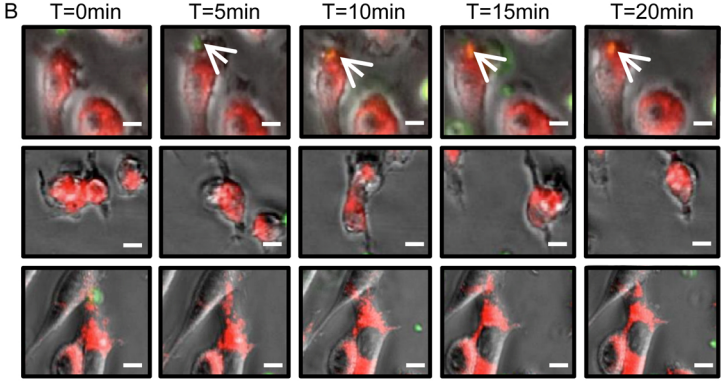
5h
2h
0h
BMDC
5h
2h
0h
C3(1)Tag
5h
2h
0h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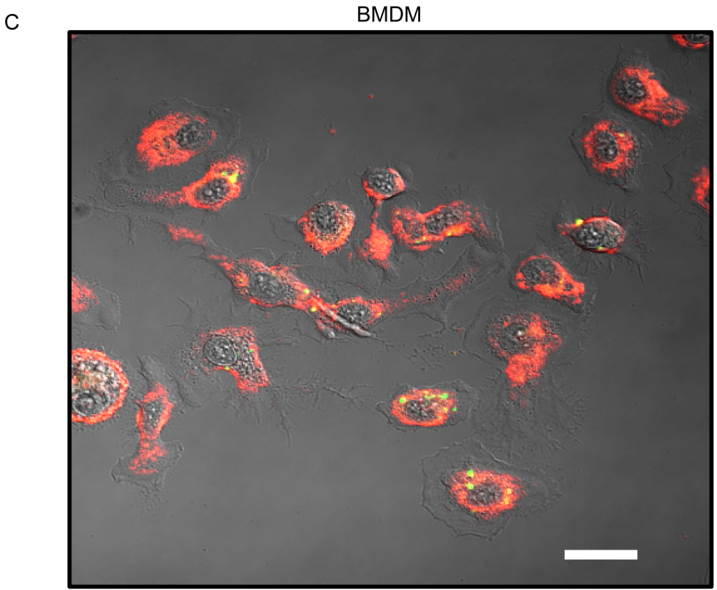
C
BMDM

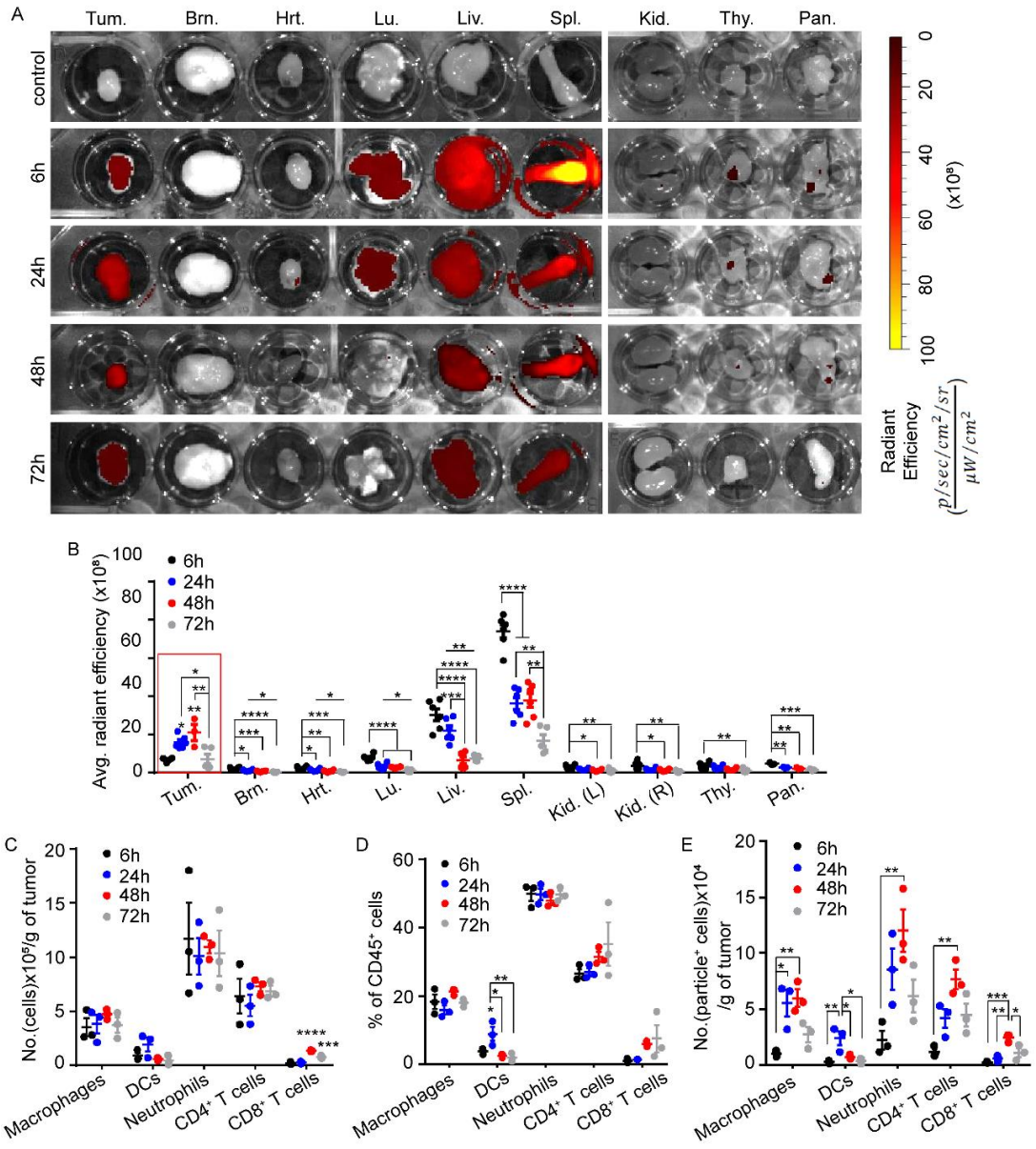

Figure S4. Liposomal NPs tissue biodistribution and trafficking in C3(1)Tag tumor bearing mice. (**A**) Orthotopic C3(1)Tag tumor mice were injected with DiD labeled liposomes (i.v.). Tumor (Tum.), brain (Brn.), heart (Hrt.), lung (Lu.), liver (Liv.), spleen (Spl.), kidney (Kid.), thymus (Thy.) and pancreas (Pan.) were harvested for IVIS optical imaging at 6, 24, 48 and 72hr (n=6 mice/group), using untreated tumor bearing mice (n=4) as background control. **(B)** Quantification for radiance efficiency in (**A**) (n=6 mice/group). (**C-E**) Tumors (n=3/time point) were processed for FACS staining to characterize macrophages ($CD45^+F4/80^+CD11b^+$), DCs ($CD45^+CD11b^+CD11c^+$), neutrophils ($CD45^+CD11b^+Ly6G^+$), $CD4^+$ T cells ($CD45^+CD3^+CD4^+$) and $CD8^+$ T cells ($CD45^+CD3^+CD8^+$). Data in (**B**) were pooled from two individual experiments. Flow cytometry analysis was performed to quantify the number of indicated cells per gram of tumor (**C**) and the percentage of each cell subpopulation in total leukocytes ($CD45^+$) (**D**). In vivo NPs association was determined as the number of $particles^+$ cells per gram of tumor (**E**). Control mice were used to establish appropriate gating. (**B-E**) The statistical significance was determined by one-way ANOVA with a Tukey's post hoc test.

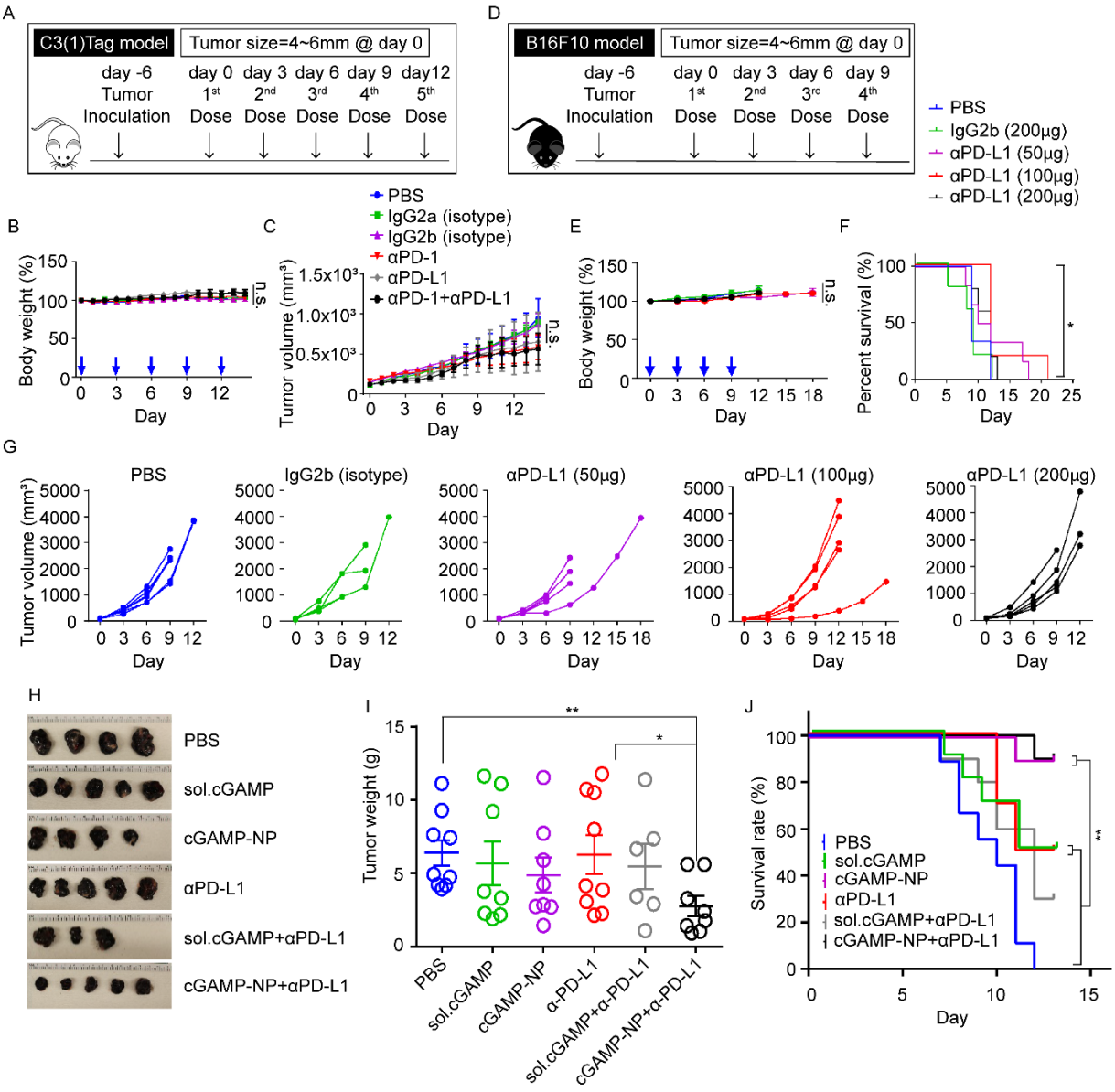

Figure S5. The limited anti-tumor response of α-PD-1/PD-L1 in established tumor models. (A-C) C3(1)Tag tumor cells ($5x10^5$/mouse) were inoculated in FVB/NJ mice (n=3-5 mice/group). On day 0, mice received PBS, IgG2a (α-PD-1 isotype control), IgG2b (α-PD-L1 isotype control), α-PD-1, α-PD-L1 and combination of α-PD-1+α-PD-L1. α-PD-1 (100 μg/mouse) or α-PD-L1 (100 μg/mouse) were administered (i.p.) every 3 days for 5 doses. Body weight (**B**) and tumor volume (**C**) were monitored. **(D-G)** B16F10 tumor cells ($2.5x10^5$/mouse) were inoculated in C57BL/6J mice (n=5-6 mice/group). On day 0, mice received PBS, IgG2b (200 μg/mouse) and α-PD-L1 (50 μg, 100 μg and 200 μg/mouse) were administered (i.p.) every 3 days. Body weight (**E**), survival rate (**F**) and tumor volume (**G**) were measured. Each line in (**G**) indicates tumor growth in a single mouse. **(H-J)** B16F10 cells were inoculated in C57BL/6J mice (n=10 mice/group). Six days later (designated as day 0), mice received PBS, soluble cGAMP, cGAMP-NP, α-PD-L1, soluble cGAMP+α-PD-L1, or cGAMP-NP+α-PD-L1. cGAMP-NPs and soluble cGAMP controls were administered (i.v.) for 4 times. (**H**) Representative images of tumors were taken upon sacrifice on day 12 (n=3-5/group) and (**I**) tumor weight was measured thereafter. (**J**) Survival rate was monitored daily. Data in (I-J) was pooled from two independent experiments. The statistical significance was determined by one-way ANOVA with a Tukey's post hoc test (**B**, **C**, **E**, **G** and **I**) and by the log-rank test (**F** and **J**). *P<0.05 (**F**; α-PD-L1 (100 μg) vs. PBS or IgG2b).

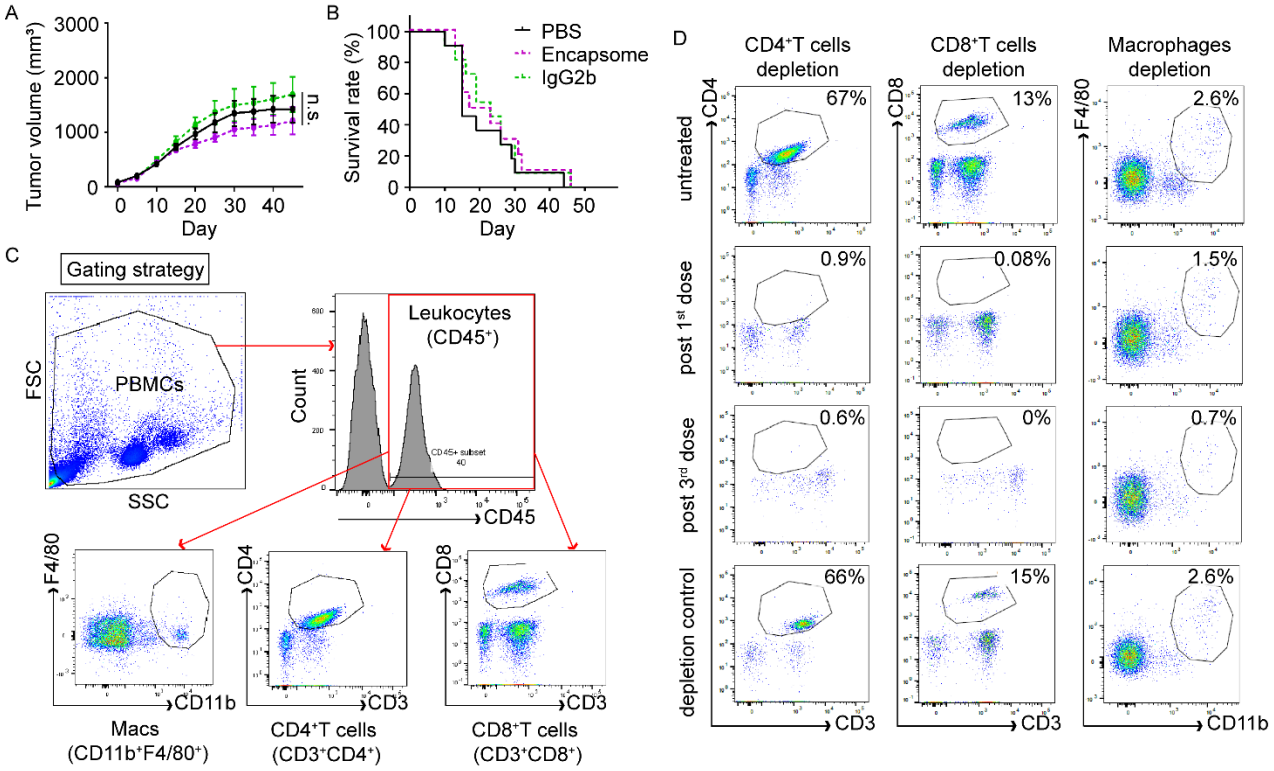

Figure S6. C3(1)Tag tumor burden in cell depletion control mice and measurement of cell depletion efficiency by FACS. Prior to tumor inoculation, mice were subjected to Encapsome® (empty liposomes for macrophage depletion control; i.v.) twice per week for 2 weeks, or IgG2b antibody (isotype control for both $CD4^+$ and $CD8^+$ T cells depletions) every day for 3 days (n=10/group). Cell depletion was maintained as two injections per week thereafter. (**A**) Tumor volume and (**B**) survival rate were monitored. (**C-D**) Depletion efficiency was determined in mice (n=3/group) depleted of macrophages (Clodrosome®; i.v.), CD4 (GK1.5; i.p.) or CD8 (clone 2.43; i.p.) T cells after 1 and 3 doses, using mice left untreated or administered with Encapsome® or IgG2b antibody as depletion controls. (**C**) Peripheral blood mononuclear cells (PBMCs) were isolated and stained for flow cytometry. PBMCs were gated on live cells (FSC versus SSC) to remove debris. Leukocytes were gated on $CD45^+$ population and used to sub-gate macrophages ($CD11b^+F4/80^+$), $CD4^+$ T cells ($CD3^+CD4^+$) and $CD8^+$ T cells ($CD3^+CD8^+$). The percentage of macrophages, $CD4^+$ and $CD8^+$ T cells were indicated in (**D**). Data in (**A, B**) were pooled from two individual experiments. Data in (**C, D**) were representative of two individual experiments. The statistical significance was determined using one-way ANOVA with post-hoc Tukey (**A**, vs. PBS) and log-rank test (**B**, vs. PBS).

Table S1. Antibody information

Antibody name	Clone	Source	Catalogue number	Vendor	Dose/ Dilution	Application
PD-L1	10F.9G2	Rat	BE0101	BioXCell	50, 100, 200 μg/mouse	Checkpoint blockade
PD-1	RMP1-14	Rat	BE0146	BioXCell	100 μg/mouse	Checkpoint blockade
CD4	GK1.5	Rat	BE0003-1	BioXCell	500 μg/mouse	Cell depletion
CD8	2.43	Rat	BE0061	BioXCell	500 μg/mouse	Cell depletion
IgG2a	2A3	Rat	BE0089	BioXCell	100 μg/mouse	Isotype control
IgG2b	LTF-2	Rat	BE0090	BioXCell	200, 500 μg/mouse	Isotype control
CD45	30-F11	Rat	103133	Biolegend	1:100 dilution	FACS staining
CD11b	M1/70	Rat	101224	Biolegend	1:100 dilution	FACS staining
F4/80	BM8	Rat	123108	Biolegend	1:100 dilution	FACS staining
H2Kb	AF6-88.5	Mouse (BALB/c)	116518	Biolegend	1:100 dilution	FACS staining
IA/IE	M5/114.15.2	Rat	107635	Biolegend	1:100 dilution	FACS staining
CD86	GL-1	Rat	105007	Biolegend	1:100 dilution	FACS staining
CD206	MR5D3	Rat	MCA2235	BioRad	1:100 dilution	FACS staining
IL-4R	I015F8	Rat	144806	Biolegend	1:100 dilution	FACS staining
CD3	17A2	Rat	100219	Biolegend	1:100 dilution	FACS staining
CD4	RM4-4	Rat	116005	Biolegend	1:100 dilution	FACS staining
CD8	53-6.7	Rat	100741	Biolegend	1:100 dilution	FACS staining
CD8	4SM15	Rat	14-0808-80	eBioscience	1:100 dilution	IF staining
IFNγ	ab9918	Rabbit	ab9918	Abcam	1:750 dilution	IF staining
Caspase3	5A1E	Rabbit	#9664	Cell signaling	1:200 dilution	IF staining

Movie S1. Compiled video for the real time liposomal NPs uptake by live cell microscopy. Compiled video taken by live cell microscopy of mouse BMDMs (**A**), BMDCs (**B**) and C3(1)Tag tumor cells (**C**) during 24hrs incubation with 100μg/mL liposomal NPs at 37°C. Scale bar, 10μm. Green, NBD-PC labeled liposomal NPs; red, cells labeled with LysoTracker Red DND-99.

Movie S2. Three-dimensional (3D) reconstruction of liposomal NPs uptake by the live cell confocal microscopy. Representative 3D reconstructed confocal microscopy images show that NPs located and accumulated in the cytosol of mouse BMDM. Scale bar, 20μm. Green, NBD-PC labeled liposomal NPs; red, cells labeled with LysoTracker Red DND-99.