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Supplementary Information for

Selective tumor antigen vaccine delivery to human CD169^{+} antigen presenting cells using ganglioside-liposomes.

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This PDF file includes:

Tables S1 to S2
Figures S1 to S6

Supplementary Tables

Table S1

Liposome	Mean diameter (nm) ± S.D. (*n*=2)	PDI (0-1) ± S.D. (*n*=2)	Zeta potential (mV)
Control	159 ± 1	0.11 ± 0.02	-54.1
GM3	164 ± 1	0.13 ± 0.03	-51.8
GD3	176 ± 8	0.09 ± 0.08	-53.5
GM1	170 ± 0	0.1 ± 0.01	-56.8
GD1a	177 ± 0	0.1 ± 0.02	-51.2
GT1b	168 ± 1	0.1 ± 0.02	-52.2

Physical properties of liposomes determined by dynamic light scattering. PDI: polydispersity index

Table S2

Antigen	Fluorochrome	Clone	Company	Dilution
CD169	PE	7-239	BD	1/50
CD123	PE-Dazzle594	6H6	Biolegend	1/50
CD56	PE-Dazzle594	QA17A16	Biolegend	1/100
CD3	PE-Cy5	UCHT1	BD	1/100
CD19	PE-Cy5	B159	BD	1/50
CD56	PE-Cy5	HIB19	BD	1/50
CD141	PE-Cy7	M80	Biolegend	1/50
CD163	BV421	GHI/61	Biolegend	1/100
CD8	BV421	RPA-T8	BD	1/100
CD3	BV480	UCHT1	BD	1/100
CD14	BV605	M5E2	Biolegend	1/100
CD11c	BV650	B-ly6	BD	1/100
HLA-DR	BV711	L243	Biolegend	1/50
CD16	BV786	3G8	Biolegend	1/100
CD4	BV786	RPA-T4	Biolegend	1/100
Siglec-6	AF700	767329	R&D	1/50
Axl	AF488	108724R	R&D	1/50
CD19	FITC	LT19	Immunotools	1/100
CD1c	PerCP-eF710	L161	eBioscience	1/50
CD16	PerCP-Cy5.5	3G8	Biolegend	1/100
TNFα	BV750	MAb11	BD	1/50

Supplementary Figures

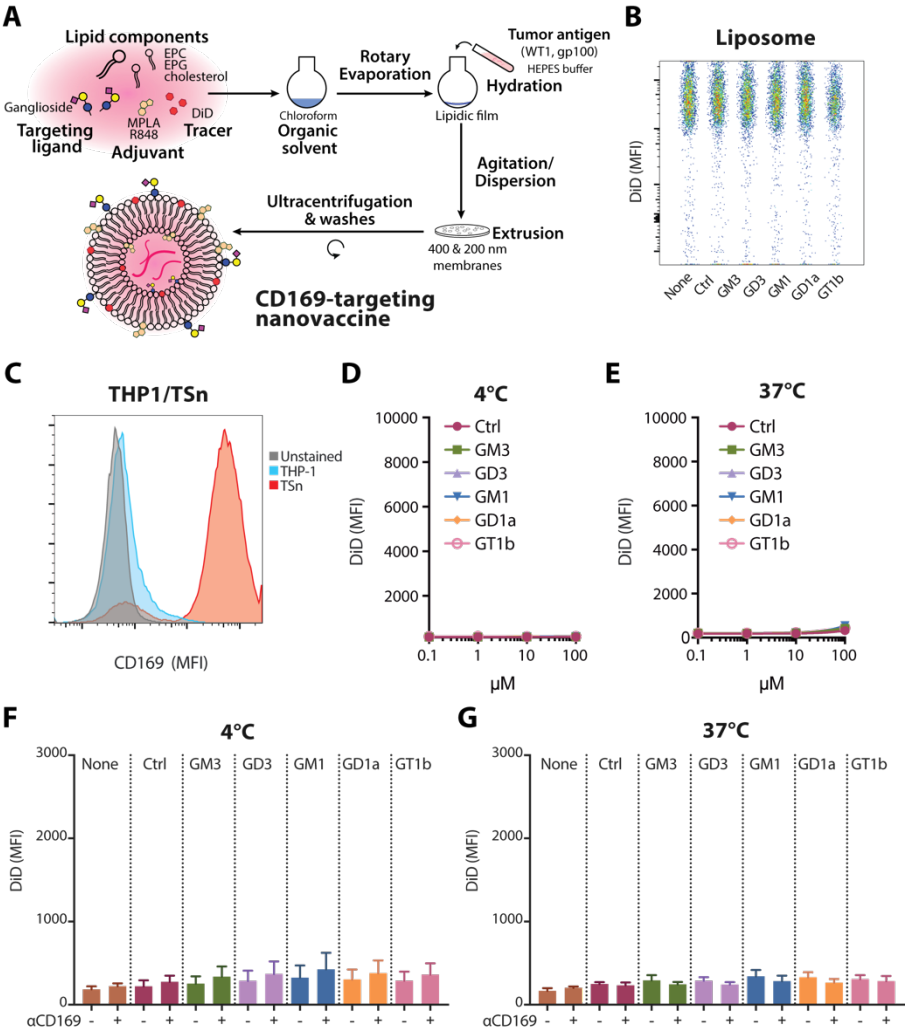

Figure S1. Ganglioside-liposomes specifically bind to and are taken up via CD169. (**A**) Schematic overview of the production of ganglioside-liposomes containing cancer antigens. (**B**) Liposomes containing gangliosides GM3, GD3, GM1, GD1a, or GT1b showed no differences in DiD-tracer intensity as measured by flow cytometry. (**C**) The expression of CD169 in THP-1 cells or CD169-overexpressing TSn cells shown by flow cytometry. (**D-G**) DiD-labeled ganglioside-liposomes were incubated with THP-1 cells and binding at 4°C or uptake at 37°C were determined by flow cytometry. (**D**,**E**) Binding or uptake of ganglioside-liposomes at different concentrations from one representative experiment out of two is shown. (**F**,**G**) THP-1 cells were pre-incubated with anti-CD169 blocking antibody at 4°C for 15 min prior to ganglioside-liposomes binding or uptake. Data are mean ± SEM from three independent experiments.

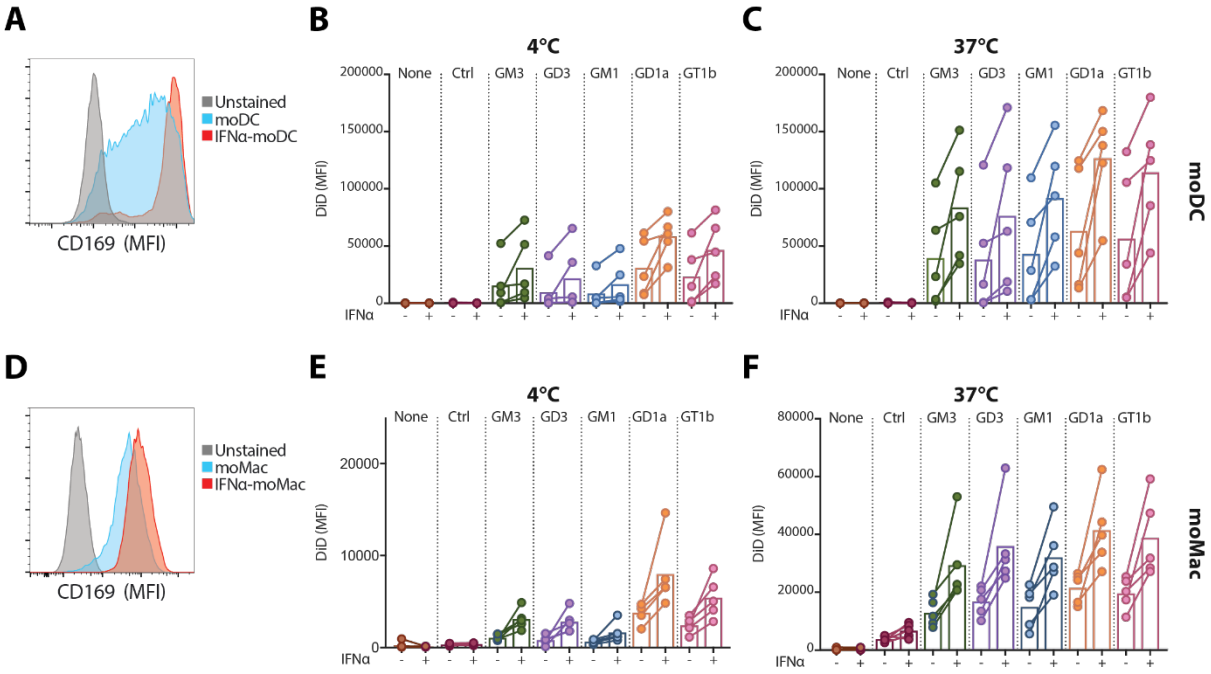

Figure S2. IFN-I enhances ganglioside-liposome binding and uptake by monocyte-derived dendritic cells (moDCs) and monocyte-derived macrophages (moMacs). (**A-C**) moDCs and (**D-F**) moMacs were incubated with DiD-labeled ganglioside-liposomes and binding (**B,E**) and uptake (**C,F**) was determined by flow cytometry. Means (bars) and values from each donor are shown (*n* = 4-5). When indicated, cells were treated for 48 h with recombinant human IFNα.

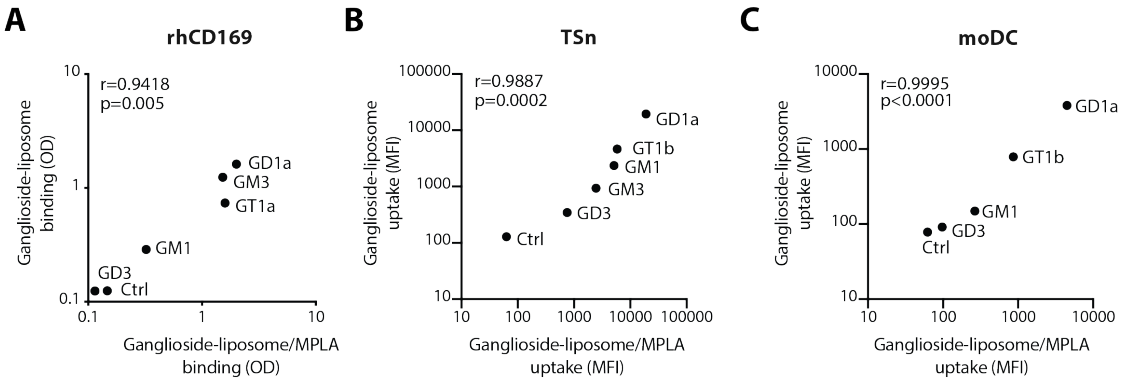

Figure S3. TLR-ligand incorporation does not interfere with ganglioside-liposome binding to CD169 and uptake by CD169-expressing cells. Gangliosides GM3, GD3, GM1, GD1a, and GT1b, were incorporated into liposomes and binding to CD169 was determined by (**A**) recombinant-CD169 ELISA or (**B,C**) cell-based flow cytometry. (**B**) Ganglioside-liposomes with or without MPLA-incorporation were coated onto plate, fixed and incubated with recombinant human CD169-6x His to determine binding using ELISA. (**B**) TSn and (**C**) monocyte-derived dendritic cells (moDCs) were incubated with DiD-labeled ganglioside-liposomes or MPLA-incorporated ganglioside-liposomes and uptake was evaluated by flow cytometry. Pearson correlation was performed to show correlation.

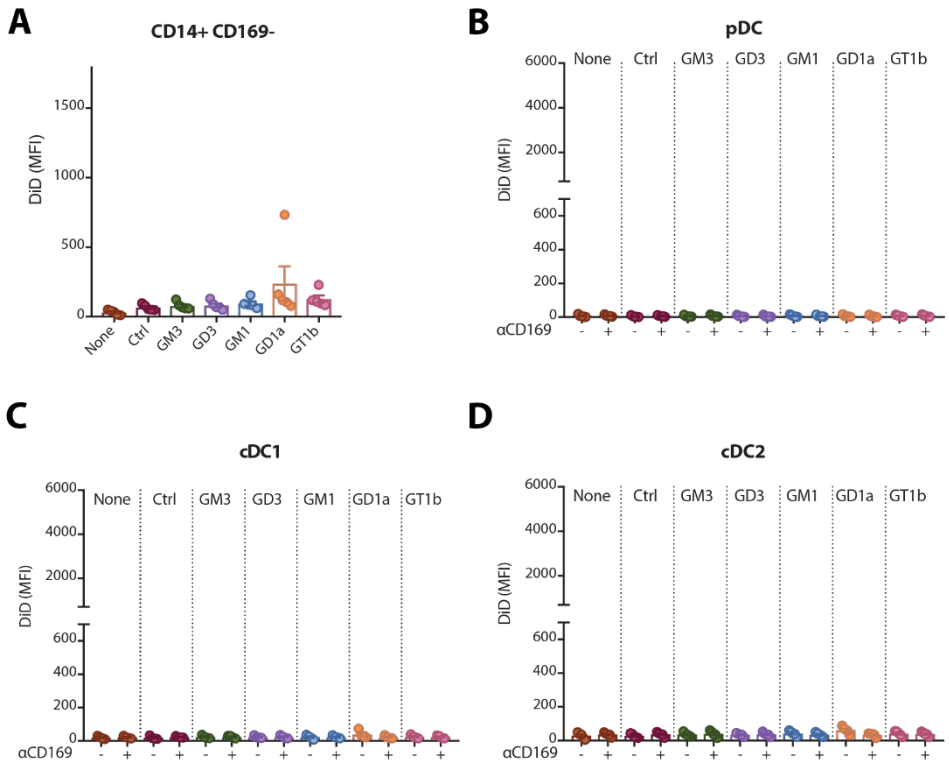

Figure S4. Ganglioside-liposomes do not target CD169$^-$ monocytes or CD169$^-$ dendritic cells. (**A**) Uptake of DiD-labeled ganglioside-liposomes by (**A**) CD14$^+$ CD169$^-$ monocytes, (**B**) plasmacytoid DCs (pDCs), (**C**) conventional DC1 (cDC1), and (**D**) conventional DC2 (cDC2) as measured by flow cytometry. When indicated, cells were pre-incubated with anti-CD169 blocking antibody at 4°C for 15 min prior to liposomes uptake. Data are mean ± SEM from five donors.

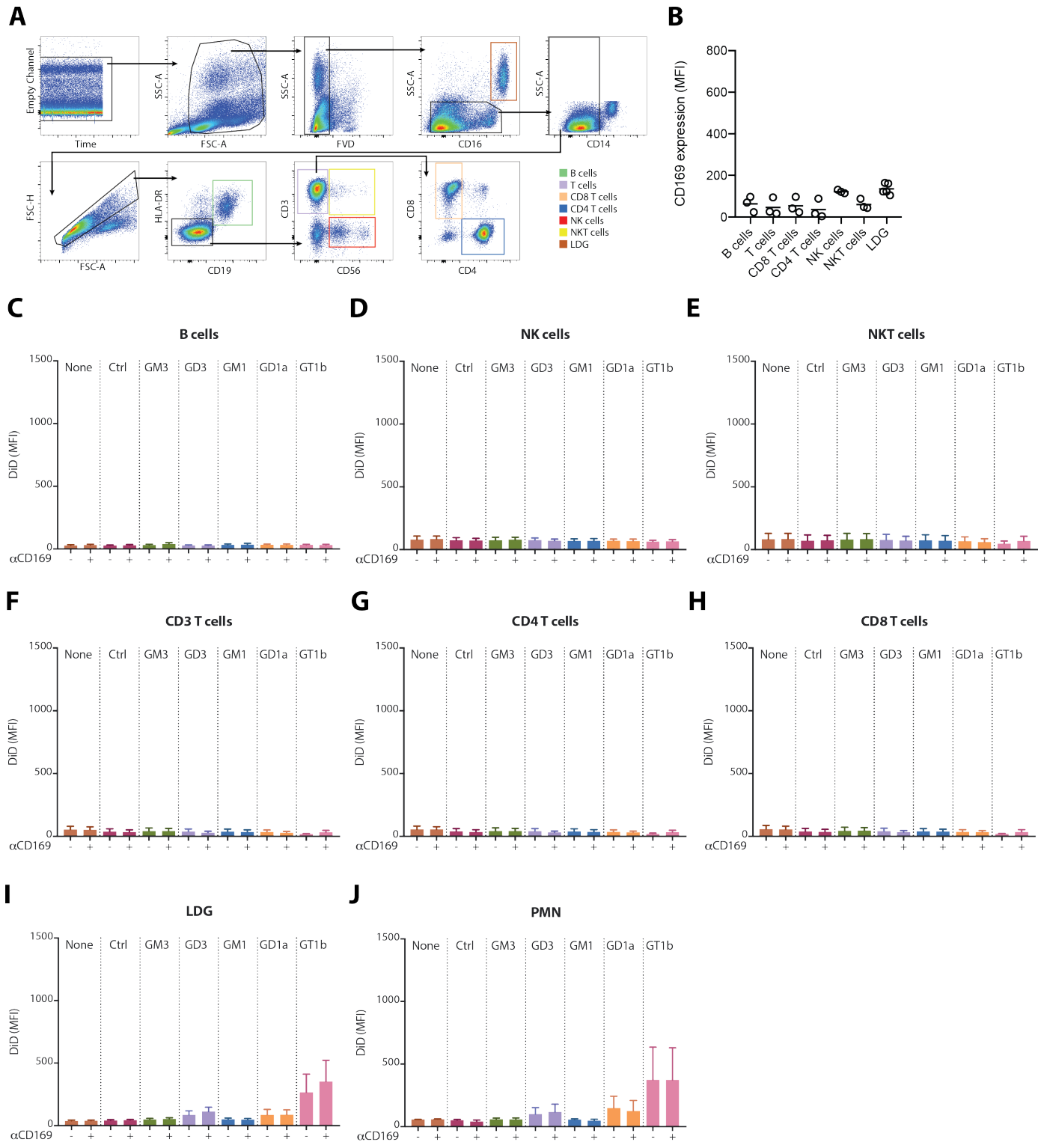

Figure S5. Ganglioside-liposomes do not target human lymphocytes or granulocytes. (**A**) Gating strategy identifies lymphocyte subsets and granulocytes after density gradient centrifugation of peripheral blood mononuclear cells. (**B**) The expression of CD169 as determined by flow cytometry is shown. Means and value of each donor are shown (*n* = 3-4). (**C-J**) Uptake of DiD-labeled ganglioside-liposomes on different (**C**) B cells, (**D**) NK cells, (**E**) NKT cells, (**F**) total T cells, (**G**) $CD4^+$ T cells, (**H**) $CD8^+$ T cells, (**I**) low-density granulocytes (LDG), and (**J**) normal-density polymorphonuclear cells (PMN) as measured by flow cytometry. When indicated, cells were pre-incubated with anti-CD169 blocking antibody at 4°C for 15 min prior to liposomes uptake. Data are mean ± SEM from three to four donors.

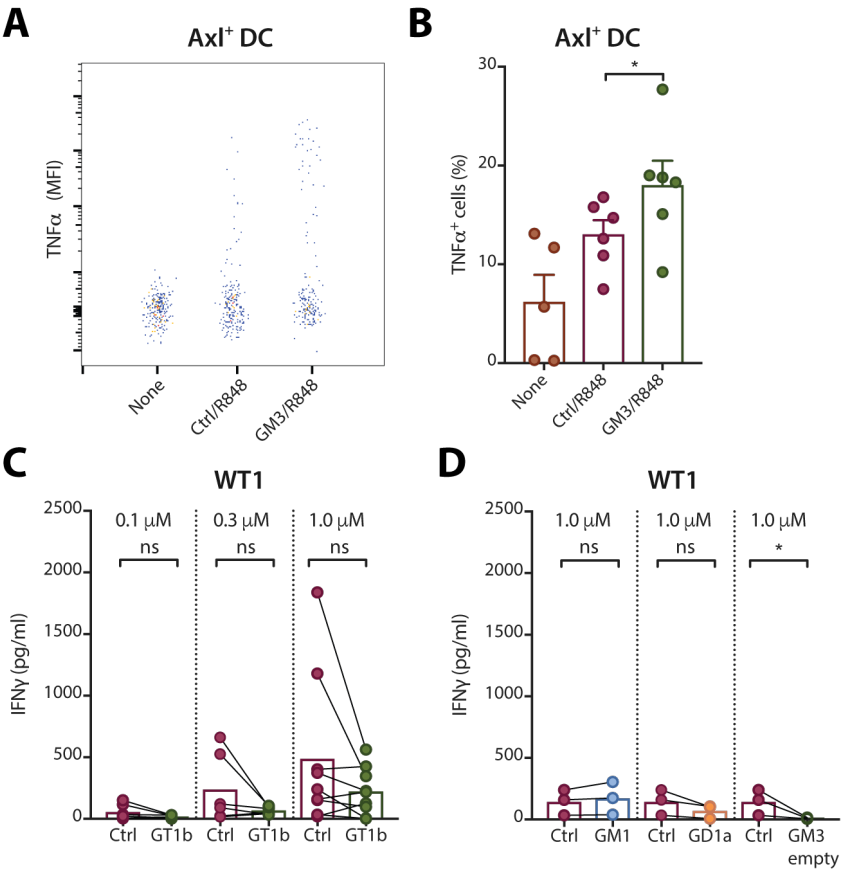

Figure S6. Ganglioside-liposomes activate human Axl$^+$ DCs and GM1, GD1a, GT1b liposomes do not result in stimulation of CD8$^+$ T cells. (**A-B**) PBMCs were incubated with 0.1 μM R848-containing ganglioside-liposomes at 37°C for 45 min, washed, and cultured for four hours in complete medium, with the addition of brefeldin-A for the final three hours. TNFα production by Axl$^+$ DCs was measured by intracellular flow cytometry, gated on Axl$^+$ Siglec6$^+$ HLA-DR$^+$ Lin(CD3/CD19/CD56/CD14/CD16)$^-$ cells, (**A**) representative plot from one donor and (**B**) quantification are shown. Data are mean ± SEM from 5-6 donors. (**C-D**) After (CD3/CD14/CD16/CD19/CD56) lineage depletion, enriched DCs were incubated with ganglioside/WT1/R848 liposome or control liposomes at 37°C, washed, and WT1-specific CD8$^+$ T cells were added. GM3 empty contained no WT1 peptide. IFNγ secretion after 24h was determined by ELISA. Data are mean from three to six donors. Paired t-test was used for analysis. * $P < 0.05$, *** $P < 0.001$, ns not significant.