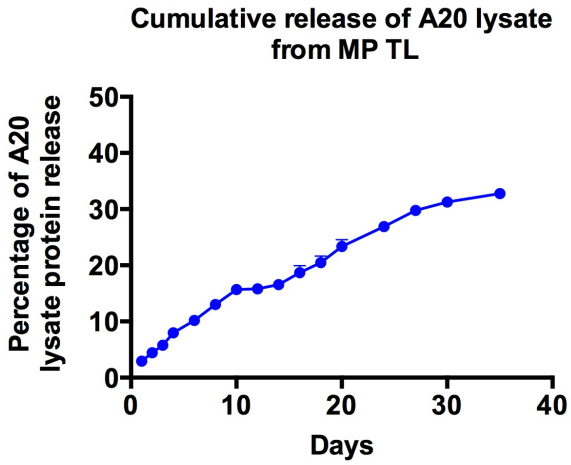
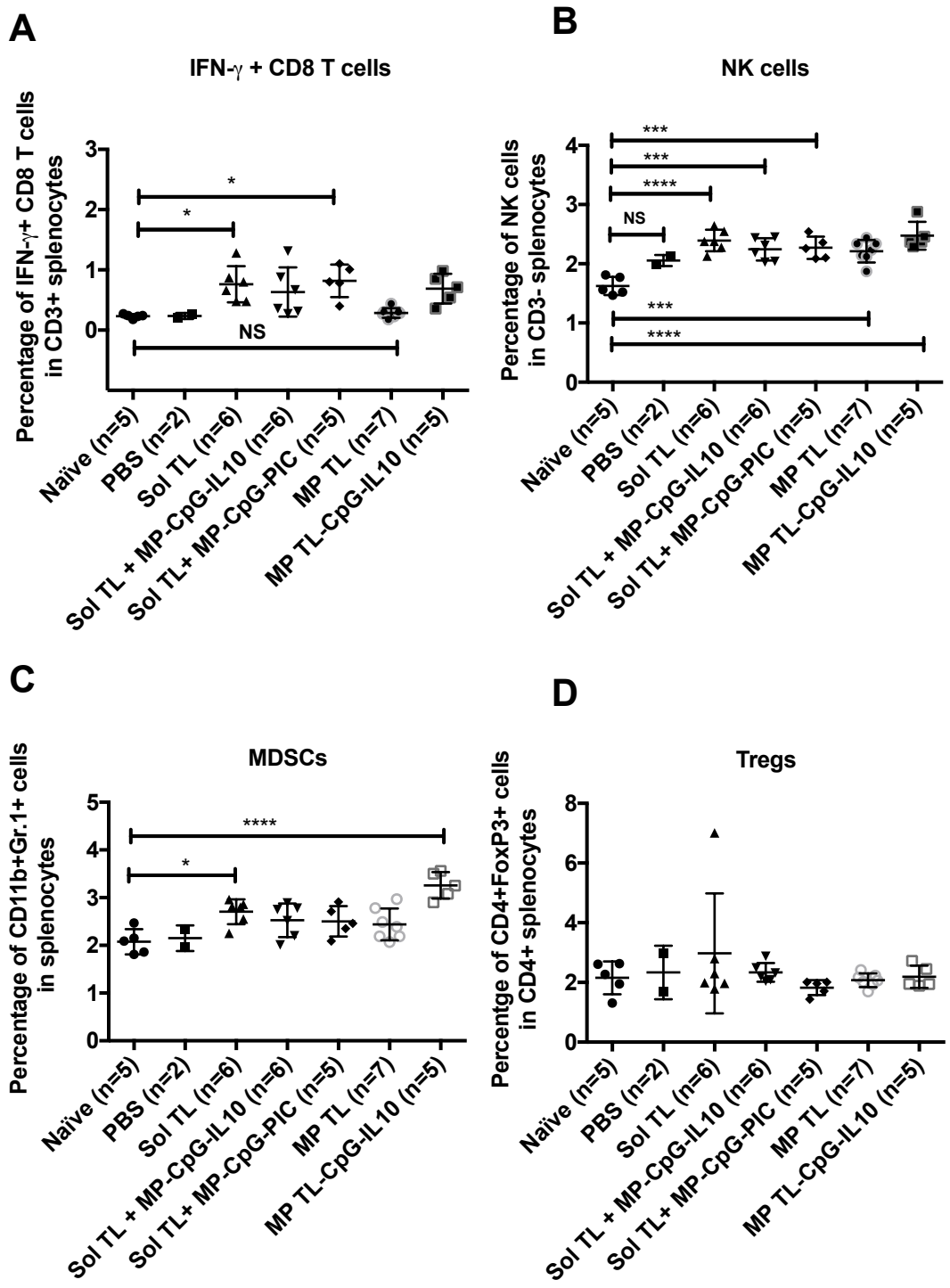
A

Formulation (n=4)	Size	PDI	Zeta	Encapsulation efficiency (%)
Microparticle encapsulated tumor lysate (MP TL)	1.84 ± 0.50	0.41 ± 0.17	0.61 ± 3.18	74.9 ± 7.6

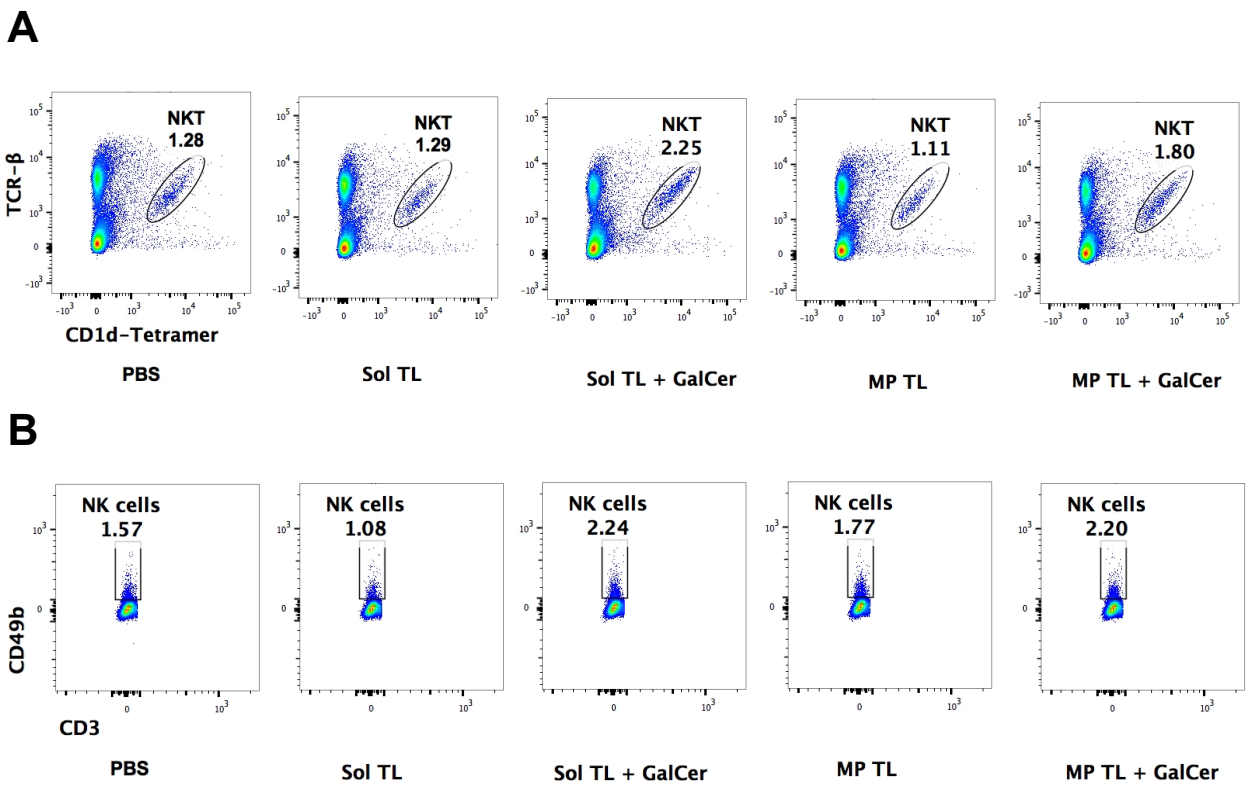
B



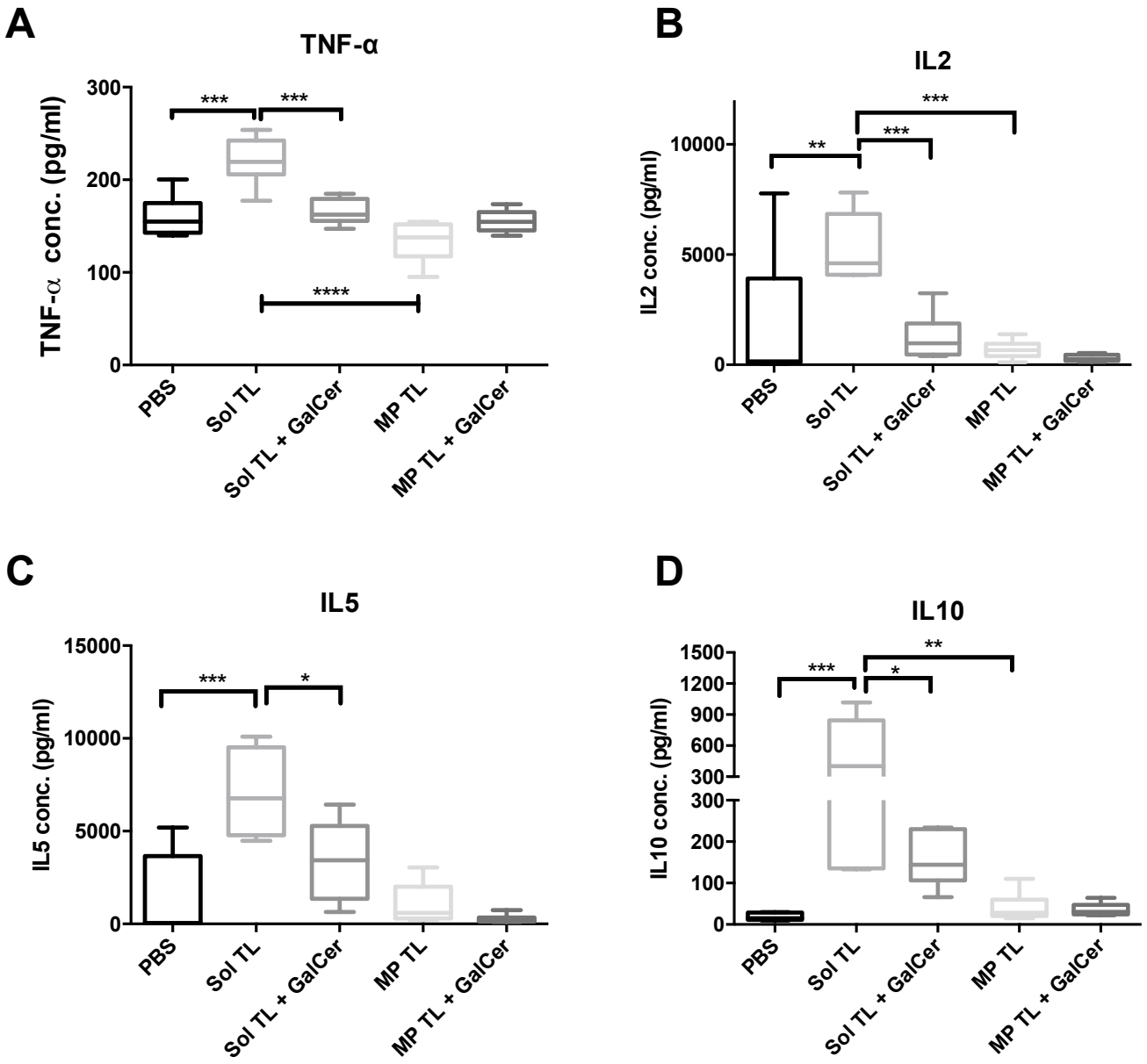
Supplementary Figure 1 (A) Characterization of A20 lysate encapsulated PLGA microparticle formulation (described as MP TL; Size- µm, Zeta-mV) and (B) Cumulative release of A20 lysate protein from PLGA MP (n=4). Data represent Mean ± S.D.



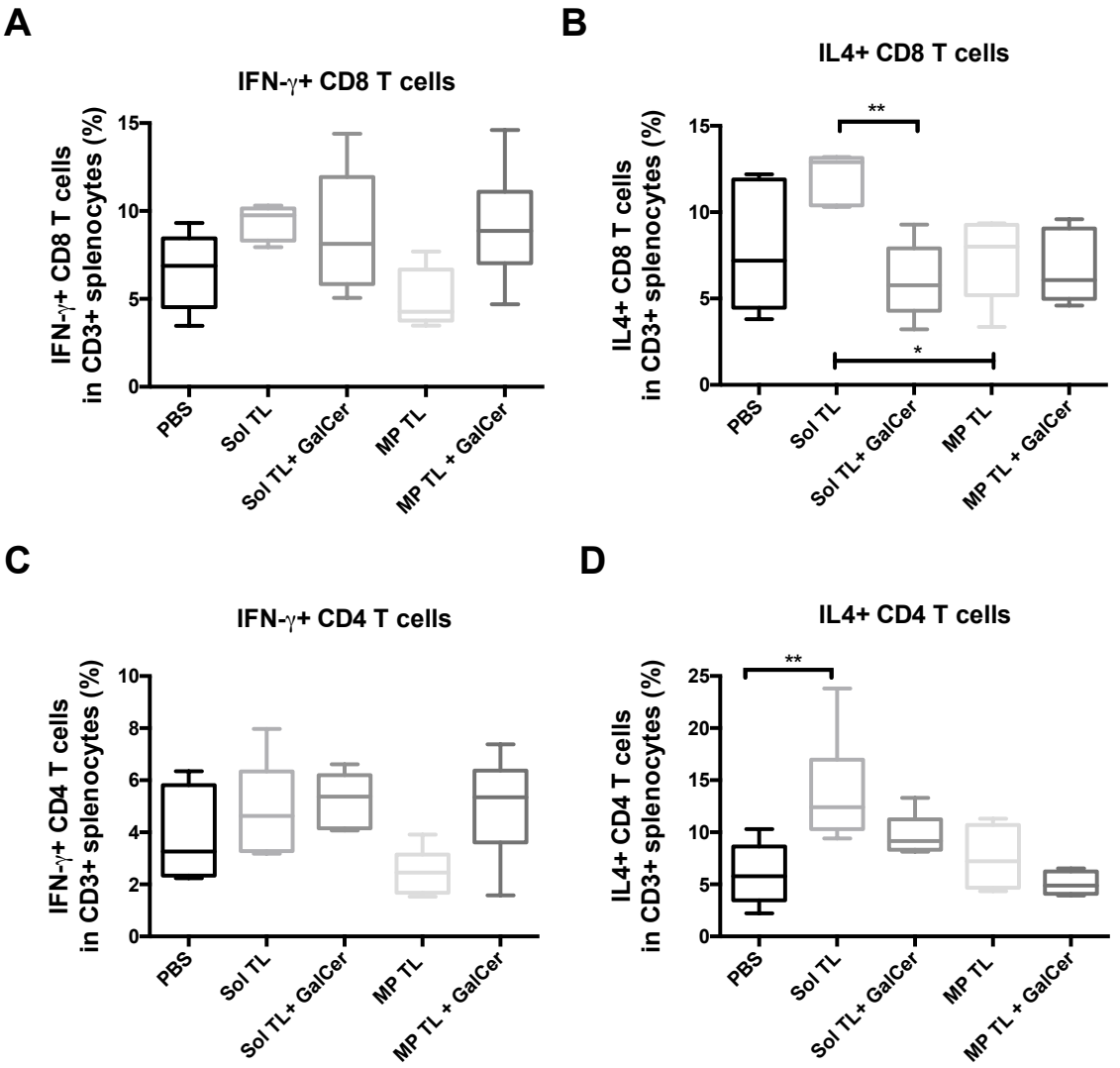
Supplementary Figure 2. (A) IFN-γ secreting CD8+ T cells percentage following re-stimulation of splenocytes with A20 tumor lysate ex vivo. Percentage of (B) NK cells, (C) MDSCs, and (D) Tregs in splenocytes. Naïve Balb/C mice were first injected with a lethal dose of A20 cells ($2x10^5$ cells/mice, IP) followed by 3 immunizations at days 8, 10, 14 with various tumor lysate formulations (100 μg lysate protein per mice, SC) and survival was tracked upto 56 days. At day 56, splenocytes were analyzed for IFN-γ secreting CD8+ T cells (after restimulation with A20 tumor lysate in vitro for 72 hrs) and various immune cell populations by flow cytometry. *P<0.05, **P<0.01, ***P<0.001; ****P<0.0001; One way ANOVA with Tukey multiple comparison test.



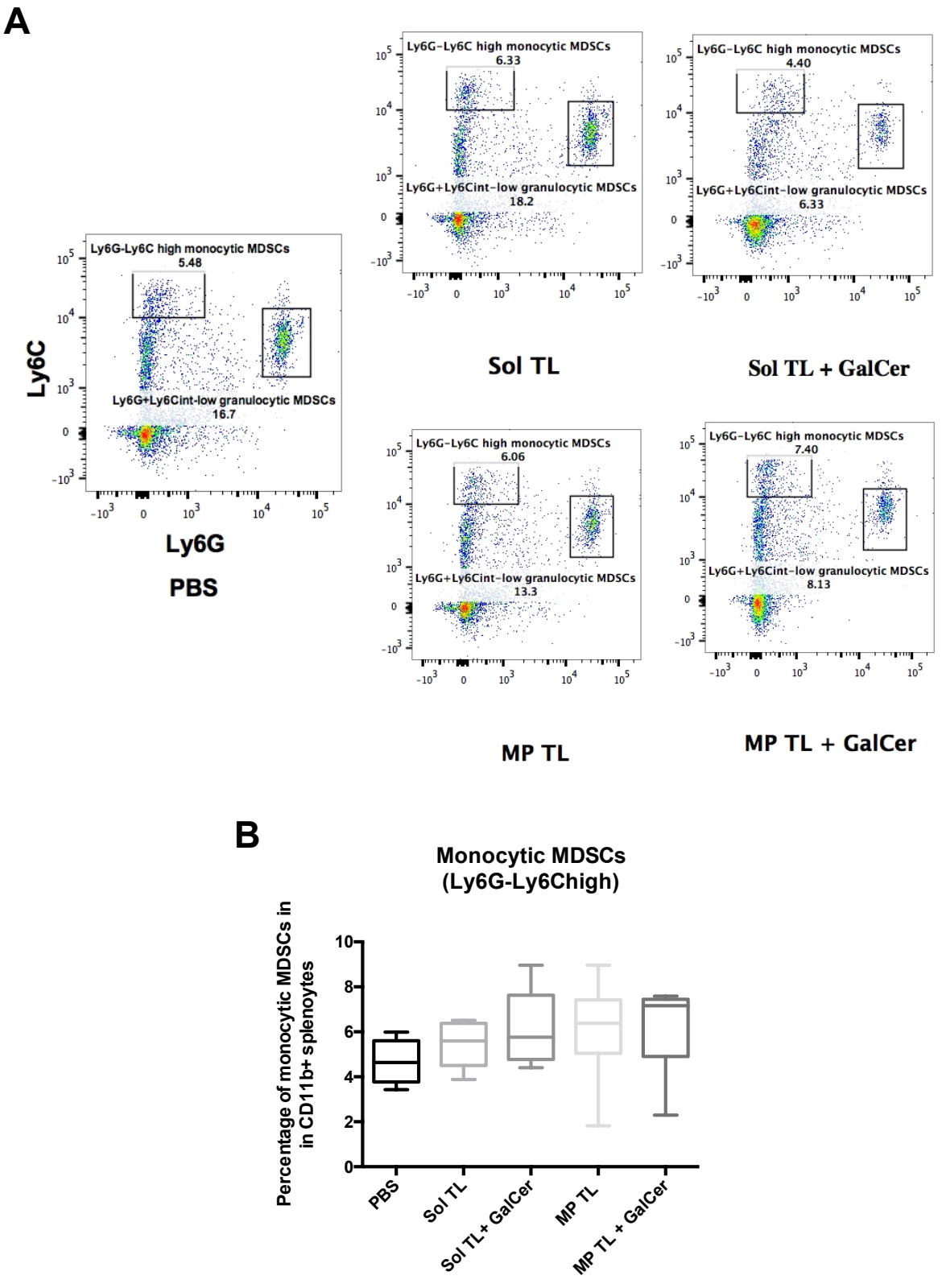
Supplementary Figure 3. Representative histogram showing (A) NKT cells (TCR-β+ CD1d-Tetramer+), and (B) NK (CD3-CD49b+) population in lymph node at day 21 of mechanistic (time line shown in Figure 3A).



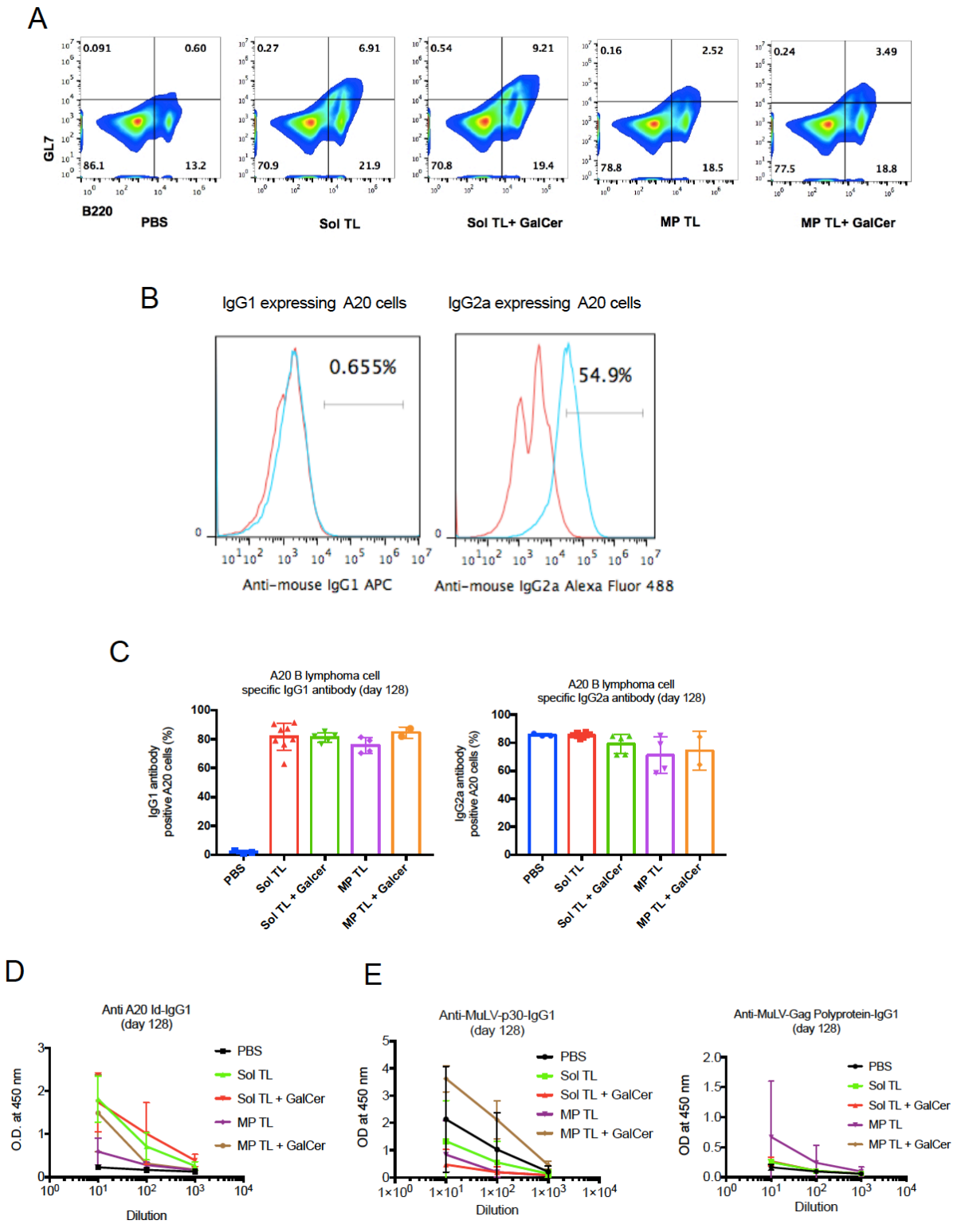
Supplementary Figure 4. Th1 and Th2 cytokines secreted by splenocytes following restimulation by A20 tumor lysate ex vivo. Balb/C mice (n=6) was first injected with 2 x10^5 A20 tumor cells on day 1 and then immunized with the formulations on days 8, 10, and 14. Then, a single IP injection of 2 μg of α-GalCer was done at day 17 only in α-GalCer treatment groups. On day 21, all the mice were sacrificed and splenocytes (10^6/well of 96 well plate) were restimulated with A20 lysate (100 ug/well) for 72 hrs in vitro and the culture media was analyzed for various Th1/Th2 cytokines using a multiplex kit. Data represent Mean ± S.D. *P<0.05, **P<0.01, ***P<0.001; One way ANOVA with Tukey multiple comparison test.



Supplementary Figure 5. CD4+ and CD8+ T cell responses of therapeutic A20 lysate vaccines. (A-B) IFN-γ and IL4 secreting CD8 T cell percentage following restimulation of the splenoctyes with A20 tumor lysate for 72 hrs; (C-D) IFN-γ and IL4 secreting CD4 T cell percentage following restimulation of splenoctyes with A20 tumor lysate; Balb/C mice (n=6) was first injected with 2 x10^5 A20 tumor cells on day 1 and then immunized with the formulations on days 8, 10, and 14. Then, a single IP injection of 2 μg of α-GalCer was done at day 17 only in α-GalCer treatment groups. On day 21, splenocytes (10^6/well of 96 well plate) were restimulated with A20 lysate (100 ug/well) for 72 hrs and stained and analyzed for IFN-γ+/IL4+, CD3+CD8+ and CD3+CD4+T cells using a flow cytometer. Data represent Mean (n=5-6) ± S.D. *P<0.05, **P<0.01; One way ANOVA with Tukey multiple comparison test.



Supplementary Figure 6. (A) Representative histogram showing monocytic (Ly6G-Ly6C high) and granulocytic (Ly6G+Ly6C int-low) populations, and (B) Box plot showing percentage of monocytic MDSCs in CD11b+ splenocytes of lymph nodes at day 21 of mechanistic studies (time line shown in Figure 3A). Data represent Mean (n=6) ± S.D.

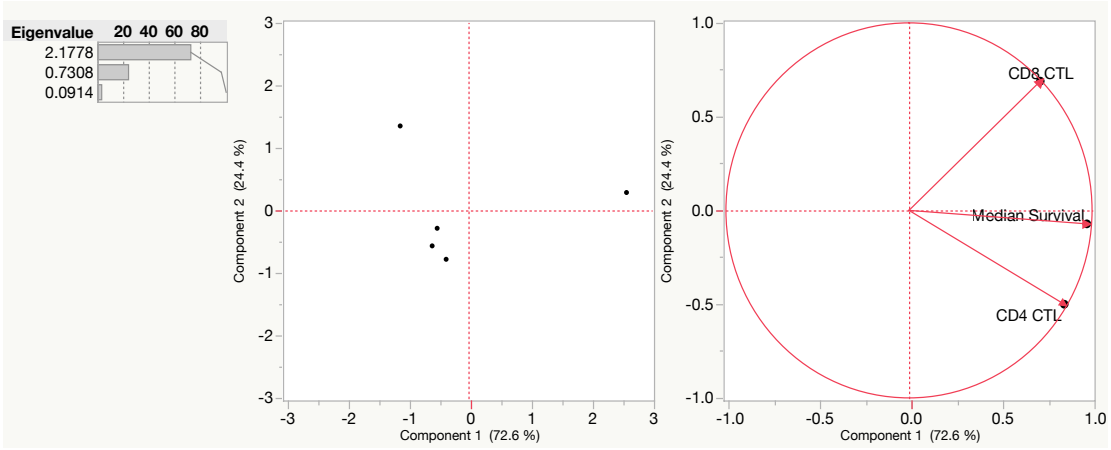


Supplementary Figure 7. (A) Representative histogram showing germinal center forming B cell (B220+GL7+) population in lymph node at day 21 of mechanistic (time line shown in Figure 3A); (B) Surface IgG1 and IgG2a expression on A20 B lymphoma cells; (C) A20 B lymphoma cell specific antibody in serum of vaccine treated mice (day 128); (D) anti A20-Id-IgG1 antibody level (day 128; n=3-8; MP TL + GalCer : n=2) and (E) Serum antibody level against moloney MuLV antigens (day 128; n=3-8; MP TL + GalCer : n=2). Data represent Mean (n=6) ± S.D.

A

Pairwise correlation

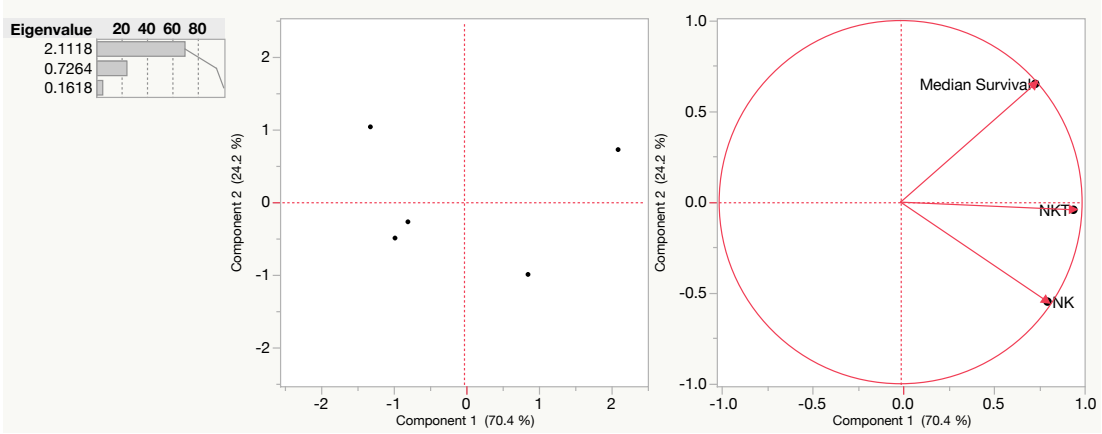
	Median Survival	CD4 CTL	CD8 CTL
Median Survival	1.0000	0.8209	0.6243
CD4 CTL	0.8209	1.0000	0.2811
CD8 CTL	0.6243	0.2811	1.0000



B

Pairwise correlation

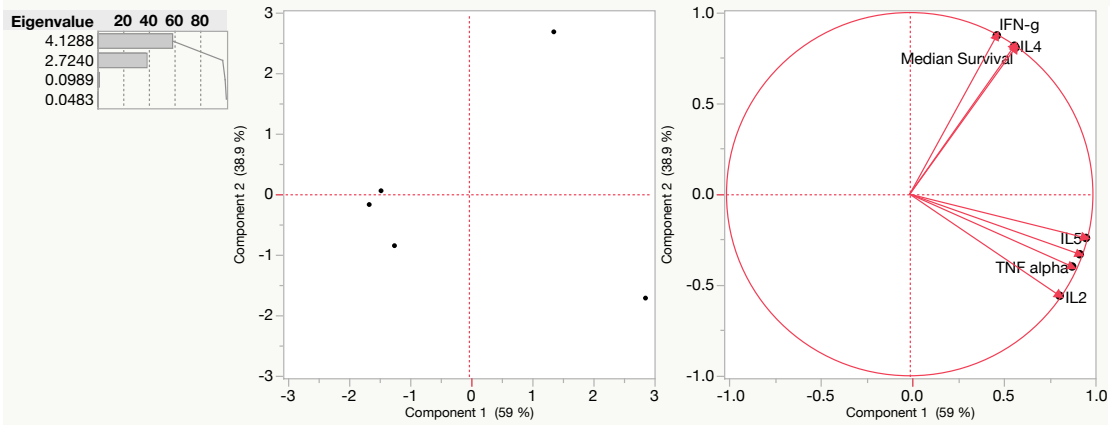
	NKT	NK	Median Survival
NKT	1.0000	0.7295	0.6308
NK	0.7295	1.0000	0.2770
Median Survival	0.6308	0.2770	1.0000



C

Pairwise correlation

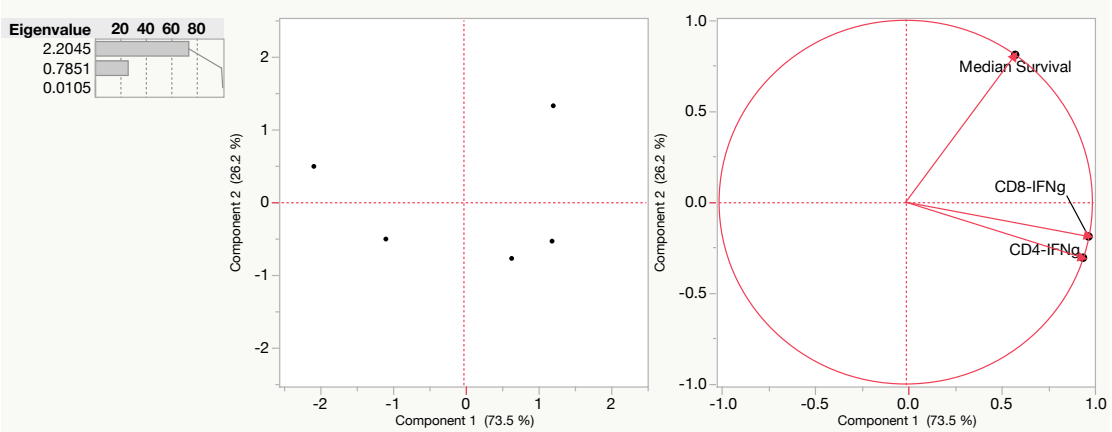
	IFN-g	TNF alpha	IL2	IL4	IL5	IL10	Median Survival
IFN-g	1.0000	0.0865	-0.0921	0.9899	0.2428	0.1358	0.9711
TNF alpha	0.0865	1.0000	0.9388	0.1976	0.9196	0.9384	0.1648
IL2	-0.0921	0.9388	1.0000	0.0068	0.9318	0.9276	0.0196
IL4	0.9899	0.1976	0.0068	1.0000	0.3438	0.2565	0.9832
IL5	0.2428	0.9196	0.9318	0.3438	1.0000	0.9750	0.3726
IL10	0.1358	0.9384	0.9276	0.2565	0.9750	1.0000	0.2836
Median Survival	0.9711	0.1648	0.0196	0.9832	0.3726	0.2836	1.0000



D

Pairwise correlation

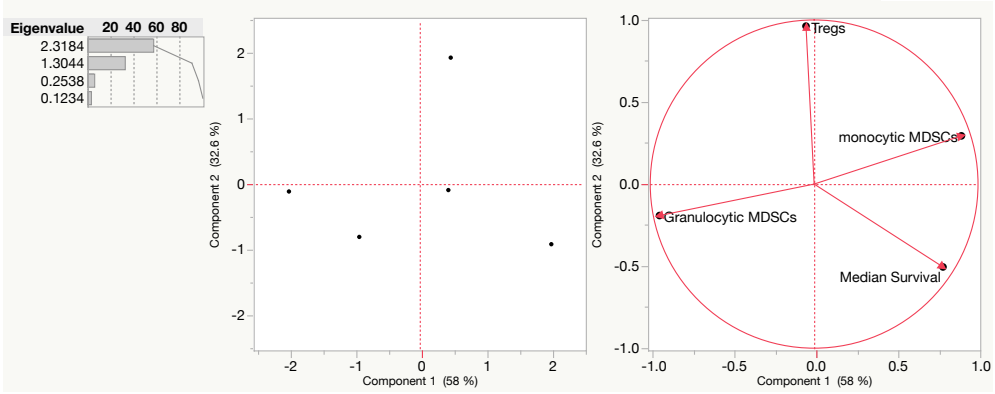
	Median Survival	CD4-IFNg	CD8-IFNg
Median Survival	1.0000	0.3099	0.4207
CD4-IFNg	0.3099	1.0000	0.9824
CD8-IFNg	0.4207	0.9824	1.0000



E

Pairwise correlation

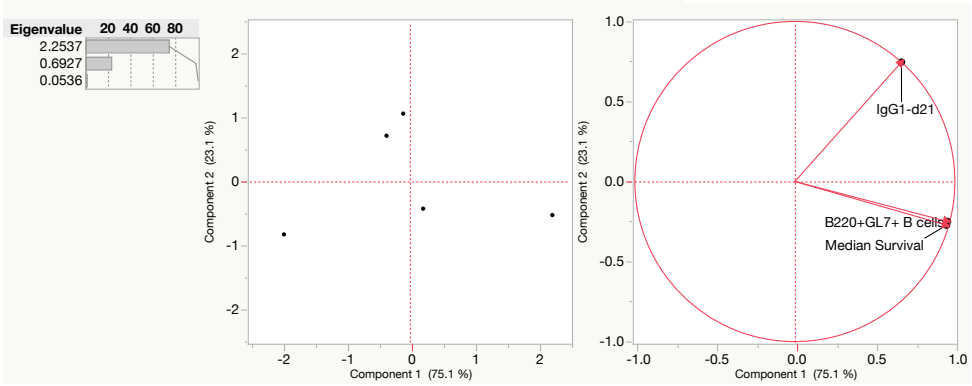
	Tregs	monocytic MDSCs	Granulocytic MDSCs	Median Survival
Tregs	1.0000	0.1789	-0.1191	-0.4307
monocytic MDSCs	0.1789	1.0000	-0.8574	0.4892
Granulocytic MDSCs	-0.1191	-0.8574	1.0000	-0.6098
Median Survival	-0.4307	0.4892	-0.6098	1.0000



F

Pairwise correlation

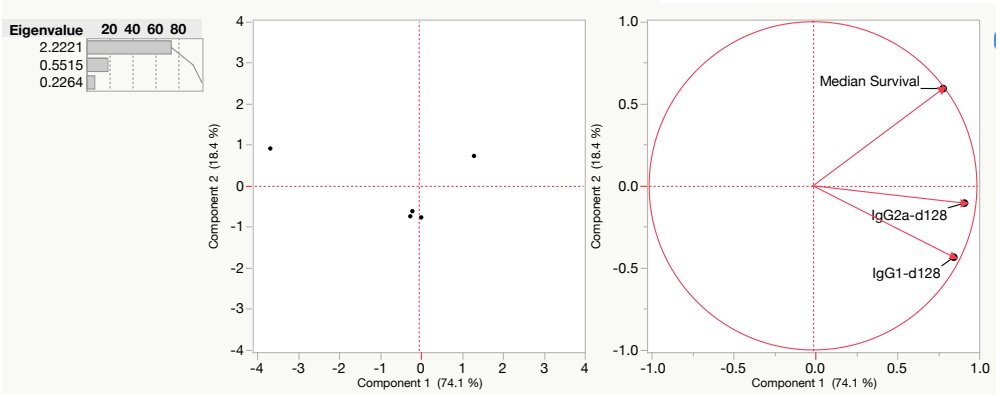
	Median Survival	B220+GL7+ B cells	IgG1-d21
Median Survival	1.0000	0.9462	0.4293
B220+GL7+ B cells	0.9462	1.0000	0.4488
IgG1-d21	0.4293	0.4488	1.0000



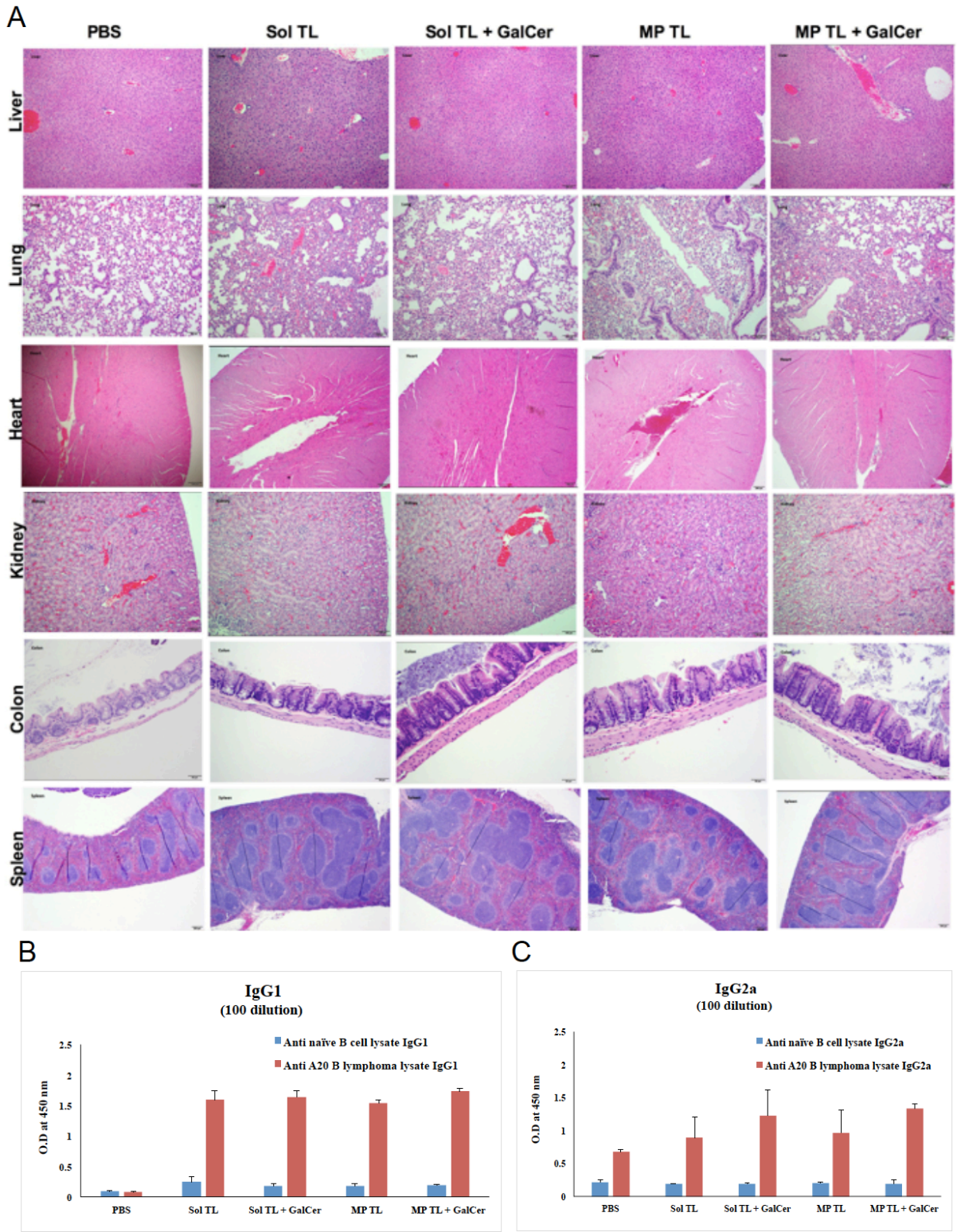
G

Pairwise correlation

	Median Survival	IgG1-d128	IgG2a-d128
Median Survival	1.0000	0.4606	0.6223
IgG1-d128	0.4606	1.0000	0.7397
IgG2a-d128	0.6223	0.7397	1.0000



Supplementary Figure 8. Principal Component Analysis (PCA) of antitumor immune response of therapeutic lysate vaccines. Median survival of various tumor lysate vaccine groups (Figure 2) and cellular (Figure 3) and humoral (Figure 4) were analyzed by PCA using JMP 12 software.



Supplementary Figure 9. (A) H&E stained tissue sections of various organs (Scale bar: Liver – 100 µm, Lung- 100 µm, Heart – 200 µm (PBS-100 µm), Kidney- 100 µm, Colon-50 µm, Spleen-200 µm), and (B-C) anti naïve B cell lysate and anti A20 tumor lysate antibody levels in serum (at 100 dilution; n=3; MP TL + GalCer: n=2) for various vaccine groups at day 128 of therapeutic immunization study as shown in Figure 2A. Data represent Mean ± S.D.