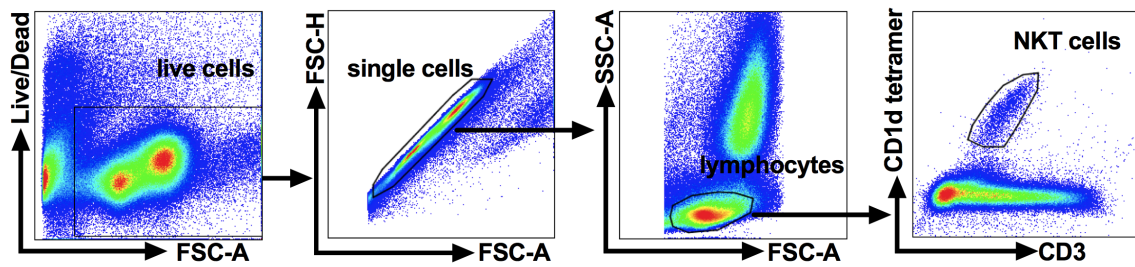
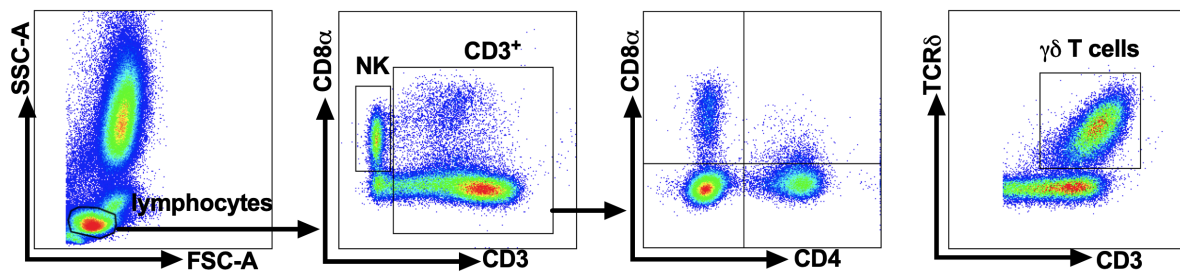


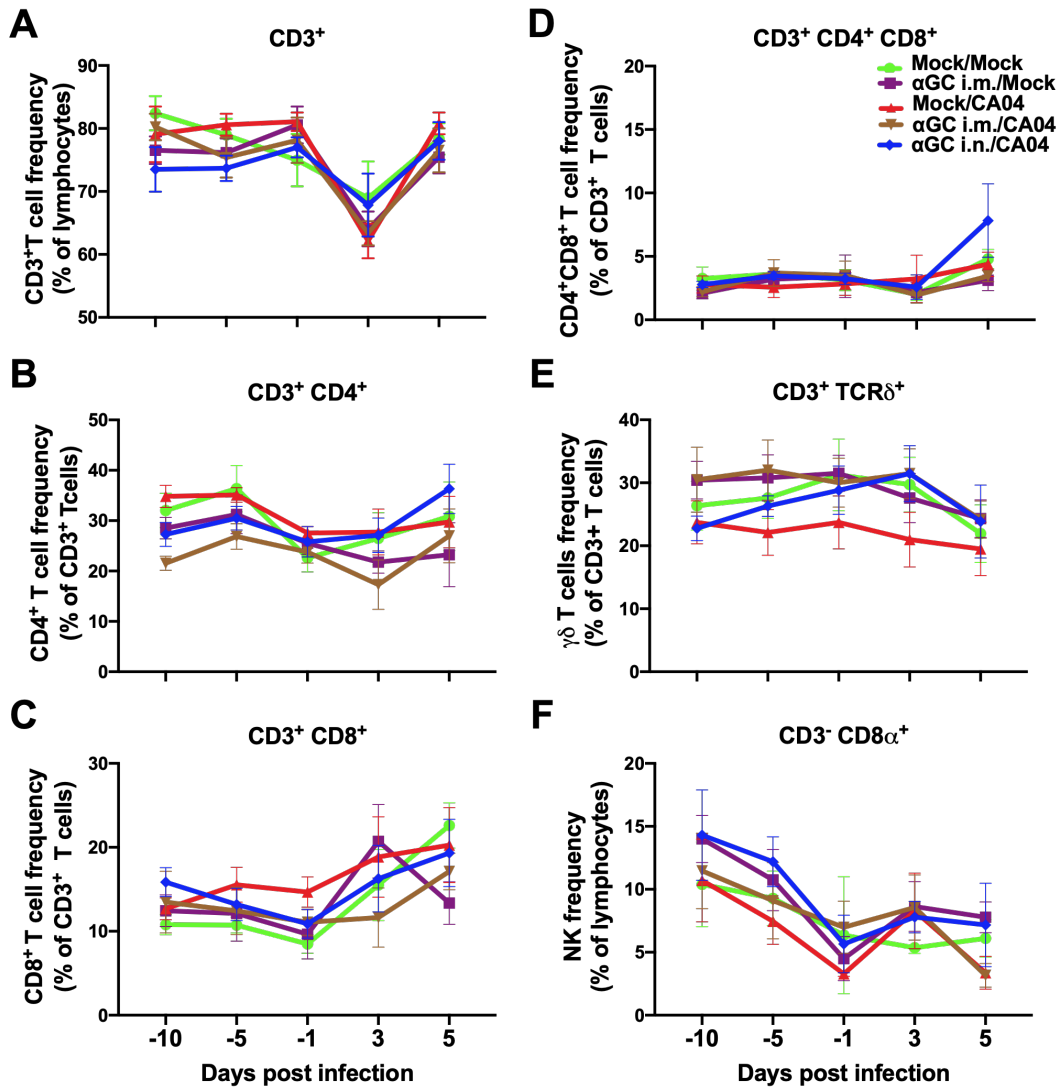
Supplementary Material



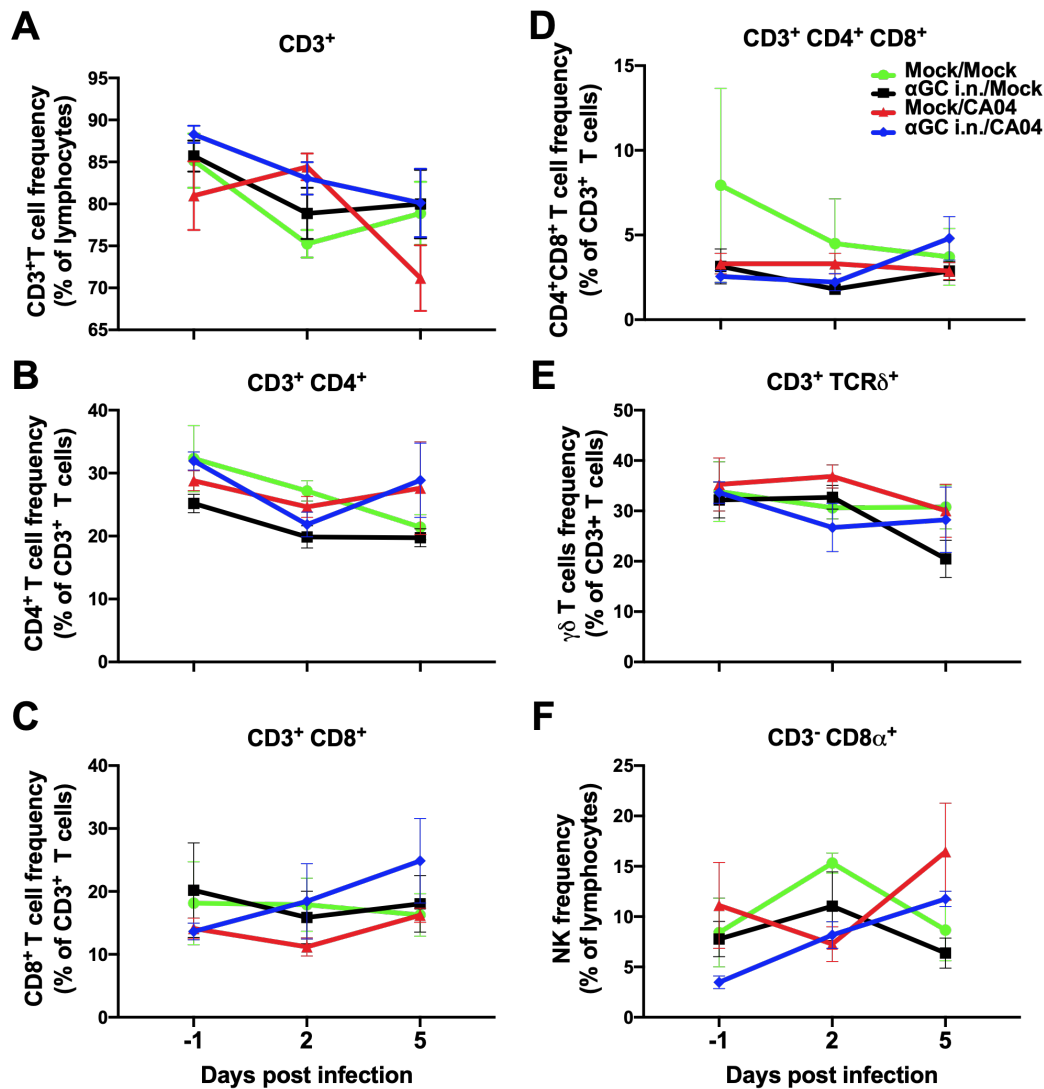
Supplemental Figure 1. Representative blood sample showing gating strategy used to identify iNKT cells. iNKT cells were identified by sequentially gating on Live/Dead negative cells, the lymphocyte singlet population, and then CD3⁺ and CD1d tetramer⁺ cells. FSC, forward scatter; SSC, side scatter; A, signal area; H, signal height.



Supplemental Figure 2. Representative blood sample showing gating strategy to identify immune cells. T cells were identified as CD3⁺ cells after first gating on live single-celled, lymphocytes. NK cells were identified as CD8α⁺ CD3⁻ cells after gating single live lymphocytes. T cell subsets were distinguished according to surface expression of CD4, CD8α. γδ T cells were identified as TCRδ⁺ after gating on CD3⁺ T cells.



Supplemental Figure 3. Frequencies of immune cell populations in peripheral blood during Experiment 1 (A-F). (A) CD3⁺ cells as a proportion of lymphocytes, (B) CD4⁺ single positive T cells as a proportion of CD3⁺ lymphocytes, (C) CD8 α ⁺ single positive T cells as a proportion of CD3⁺ lymphocytes, (D) double positive CD4⁺ CD8 α ⁺ T cells as a proportion of CD3⁺ lymphocytes, (E) $\gamma\delta$ T cells as a proportion of CD3⁺ lymphocytes, (F) NK cells (CD3⁻CD8 α ⁺) as a proportion of lymphocytes. Data are presented as mean \pm SEM. CD3⁺, CD4⁺, and $\gamma\delta$ T cell frequencies were analyzed using a two-way ANOVA. Other cell types were compared at each time point using the non-parametric Kruskal-Wallis test. No statistical differences were identified.



Supplemental Figure 4. Frequencies of immune cell populations in peripheral blood during Experiment 2 (A-F). (A) CD3⁺ cells as a proportion of lymphocytes, (B) CD4⁺ single positive T cells as a proportion of CD3⁺ lymphocytes, (C) CD8α⁺ single positive T cells as a proportion of CD3⁺ lymphocytes, (D) double positive CD4⁺ CD8α⁺ T cells as a proportion of CD3⁺ lymphocytes, (E) γδ T cells as a proportion of CD3⁺ lymphocytes, (F) NK cells (CD3⁻CD8α⁺) as a proportion of lymphocytes. Data are presented as mean ± SEM. CD3⁺ and γδ T cell frequencies were analyzed using a two-way ANOVA. Other cell types were compared at each time point using the non-parametric Kruskal-Wallis test. No statistical differences were identified.

Supplemental Table 1. Reagents used for flow cytometric analysis

Antigen	Clone	Isotype	Conjugation	Source
Live/Dead	N/A	N/A	APC-Cy7	Invitrogen
mCD1d tetramer	N/A	N/A	PE	NIH Tetramer Core
Anti-CD3 ϵ	BB23-8E6-8C8	Mouse IgG2a κ	PE-Cy7	BD Biosciences
Anti-CD8 α	76-2-11	Mouse IgG2a κ	Alexa488	Southern Biotech
Anti-CD4	74-12-4	Mouse IgG2b κ	PE	Southern Biotech
Anti-TCR δ	PGBL22A	Mouse IgG1	Alexa647	WSU mAb Center
Anti-CD14	MIL2	Mouse IgG2b	Alexa488	Bio-Rad
Anti-CD172 α	74-22-15A	Mouse IgG2b κ	PerCP	BD Biosciences
Anti-MHC class II	H42A	Mouse IgG2a κ	Alexa647	WSU mAb Center
Anti-CD11b	M1/70	Rat IgG2b κ	BV421	Biolegend

Supplemental Table 2. Frequency of leukocyte populations in BALF in pigs administered α -GalCer 9 days before infection.

Cell type	Mock/ Mock	α GC i.m./ Mock	Mock/ CA04	α GC i.m./ CA04	α GC i.n./ CA04
CD3 ⁺ T cells	75.9 \pm 4.3	78.2 \pm 1.4	68.8 \pm 4.9	70.1 \pm 4.5	74.7 \pm 5.8
CD4 ⁺ T cells	13.4 \pm 1.2	8.4 \pm 0.9	8.9 \pm 1.8	3.0 \pm 5.9	9.0 \pm 1.4
CD8 ⁺ T cells	58.1 \pm 4.5	41.0 \pm 6.8	48.1 \pm 3.5	54.2 \pm 4.5	48.1 \pm 6.1
CD4 ⁺ CD8 ⁺ T cells	4.1 \pm 0.9	0.8 \pm 0.1	1.4 \pm 0.3	3.2 \pm 0.8	2.8 \pm 0.5
$\gamma\delta$ T cells	10.8 \pm 1.5	17.9 \pm 2.9	12.3 \pm 2.1	12.9 \pm 0.9	13.1 \pm 0.6
NK cells	2.9 \pm 0.9	0.7 \pm 0.1	1.4 \pm 0.4	3.6 \pm 2.6	2.4 \pm 1.6

Supplemental Table 3. Frequency of leukocyte populations in lung in pigs administered α -GalCer 9 days before infection.

Cell type	Mock/ Mock	α GC i.m./ Mock	Mock/ CA04	α GC i.m./ CA04	α GC i.n./ CA04
CD3 ⁺ T cells	69.2±1.6	65.5±1.9	67.5±1.7	69.0±2.1	66.0±3.3
CD4 ⁺ T cells	13.7±2.4	10.9±1.5	15.9±1.8	16.3±2.0	16.3±2.7
CD8 ⁺ T cells	68.0±5.1	65.2±4.7	55.1±4.3	58.7±2.3	58.9±6.1
CD4 ⁺ CD8 ⁺ T cells	7.2±1.2	5.6±1.7	8.1±2.3	10.2±1.4	7.6±1.0
$\gamma\delta$ T cells	11.7±1.0	14.4±1.9	15.0±3.0	16.0±1.6	19.1±4.7
NK cells	10.6±2.2	8.3±2.2	7.2±1.7	10.2±0.8	10.9±2.6

Supplemental Table 4. Frequency of leukocyte populations in spleen in pigs administered α -GalCer 9 days before infection.

Cell type	Mock/ Mock	α GC i.m./ Mock	Mock/ CA04	α GC i.m./ CA04	α GC i.n./ CA04
CD3 ⁺ T cells	74.4 \pm 1.6	71.9 \pm 2.5	75.2 \pm 1.5	74.6 \pm 3.6	75.6 \pm 4.1
CD4 ⁺ T cells	13.7 \pm 2.4	10.9 \pm 1.5	15.9 \pm 1.8	16.3 \pm 2.0	16.3 \pm 2.7
CD8 ⁺ T cells	25.7 \pm 2.5	27.7 \pm 3.6	18.5 \pm 3.4	20.2 \pm 2.3	23.4 \pm 5.2
CD4 ⁺ CD8 ⁺ T cells	0.9 \pm 0.1	1.0 \pm 0.2	1.9 \pm 0.6	1.8 \pm 0.2	1.6 \pm 0.2
$\gamma\delta$ T cells	15.3 \pm 2.2	15.7 \pm 1.4	17.7 \pm 0.8	22.1 \pm 4.2	21.5 \pm 3.5
NK cells	1.7 \pm 0.2	2.7 \pm 0.5	1.3 \pm 0.5	1.1 \pm 0.3	1.7 \pm 0.4

Supplemental Table 5. Frequency of leukocyte populations in TBLN in pigs administered α -GalCer 9 days before infection.

Cell type	Mock/ Mock	α GC i.m./ Mock	Mock/ CA04	α GC i.m./ CA04	α GC i.n./ CA04
CD3 ⁺ T cells	86.4 \pm 1.8	82.2 \pm 3.8	75.0 \pm 2.2	79.7 \pm 2.2	73.5 \pm 2.5
CD4 ⁺ T cells	45.0 \pm 1.3	52.4 \pm 4.6	53.4 \pm 2.2	51.4 \pm 3.3	54.4 \pm 2.5
CD8 ⁺ T cells	25.4 \pm 2.3	24.9 \pm 1.6	21.6 \pm 1.0	21.3 \pm 1.3	19.9 \pm 1.9
CD4 ⁺ CD8 ⁺ T cells	4.1 \pm 1.0	2.9 \pm 0.4	1.5 \pm 0.2	3.1 \pm 0.8	2.1 \pm 0.2
$\gamma\delta$ T cells	3.8 \pm 0.8	5.0 \pm 1.3	2.1 \pm 0.3	5.1 \pm 1.2	2.9 \pm 0.6
NK cells	1.5 \pm 0.2	1.4 \pm 0.2	0.8 \pm 0.2	1.0 \pm 0.2	0.7 \pm 0.1

Supplemental Table 6. Frequency of leukocyte populations in BALF in pigs administered α -GalCer 2 days before infection.

Cell type	Mock/ Mock	α GC i.n./ Mock	Mock/ CA04	α GC i.n./ CA04
CD3 ⁺ T cells	60.5 \pm 3.1	51.1 \pm 6.5	54.9 \pm 2.4	67.2 \pm 4.5
CD4 ⁺ T cells	19.5 \pm 4.5	15.2 \pm 3.5	18.2 \pm 2.3	19.9 \pm 2.9
CD8 ⁺ T cells	29.3 \pm 6.4	33.1 \pm 7.6	37.8 \pm 4.0	41.9 \pm 5.5
CD4 ⁺ CD8 ⁺ T cells	3.0 \pm 1.4	2.2 \pm 0.5	3.3 \pm 0.4	3.7 \pm 0.7
NK cells	9.4 \pm 2.4	6.5 \pm 0.9	10.5 \pm 2.2	5.9 \pm 1.1

Supplemental Table 7. Frequency of leukocyte populations in lung in pigs administered α -GalCer 2 days before infection.

Cell type	Mock/ Mock	α GC i.n./ Mock	Mock/ CA04	α GC i.n./ CA04
CD3 ⁺ T cells	55.5 \pm 2.8	54.2 \pm 4.3	46.7 \pm 1.9	57.6 \pm 3.9
CD4 ⁺ T cells	23.3 \pm 2.2	22.0 \pm 2.8	24.5 \pm 1.7	22.2 \pm 2.5
CD8 ⁺ T cells	42.8 \pm 10.6	42.0 \pm 6.6	41.6 \pm 5.2	51.2 \pm 8.1
CD4 ⁺ CD8 ⁺ T cells	8.6 \pm 3.8	7.7 \pm 2.1	7.8 \pm 1.5	8.5 \pm 1.7
$\gamma\delta$ T cells	38.7 \pm 8.4	40.0 \pm 4.0	40.0 \pm 2.5	33.0 \pm 5.7
NK cells	23.2 \pm 1.3	22.3 \pm 3.0	30.4 \pm 1.4	23.4 \pm 1.7

Supplemental Table 8. Frequency of leukocyte populations in spleen in pigs administered α -GalCer 2 days before infection.

Cell type	Mock/ Mock	α GC i.n./ Mock	Mock/ CA04	α GC i.n./ CA04
CD3 ⁺ T cells	68.0 \pm 1.4	66.2 \pm 2.0	65.1 \pm 3.0	67.5 \pm 2.6
CD4 ⁺ T cells	23.3 \pm 4.7	15.5 \pm 3.0	20.6 \pm 2.3	20.5 \pm 1.4
CD8 ⁺ T cells	18.1 \pm 5.2	14.3 \pm 3.7	15.4 \pm 1.5	22.7 \pm 5.3
CD4 ⁺ CD8 ⁺ T cells	2.0 \pm 1.1	1.0 \pm 0.4	1.1 \pm 0.2	1.7 \pm 0.5
$\gamma\delta$ T cells	34.9 \pm 6.7	35.0 \pm 3.1	33.0 \pm 3.6	33.0 \pm 4.5
NK cells	4.2 \pm 0.8	2.2 \pm 0.6	5.3 \pm 1.1	3.4 \pm 0.8

Supplemental Table 9. Frequency of leukocyte populations in TBLN in pigs administered α -GalCer 2 days before infection.

Cell type	Mock/ Mock	α GC i.n./ Mock	Mock/ CA04	α GC i.n./ CA04
CD3 ⁺ T cells	91.4 \pm 1.8	94.0 \pm 0.4	89.3 \pm 2.7	87.6 \pm 3.8
CD4 ⁺ T cells	60.0 \pm 5.1	48.9 \pm 10.4	51.4 \pm 6.6	50.7 \pm 5.4
CD8 ⁺ T cells	21.0 \pm 4.0	24.7 \pm 3.0	30.0 \pm 2.2	28.8 \pm 1.4
CD4 ⁺ CD8 ⁺ T cells	3.6 \pm 0.9	3.3 \pm 0.6	3.1 \pm 0.4	3.3 \pm 0.5
$\gamma\delta$ T cells	7.1 \pm 0.2	8.5 \pm 1.2	5.2 \pm 1.1	4.5 \pm 0.7
NK cells	1.4 \pm 0.4	1.5 \pm 0.3	1.0 \pm 0.4	1.0 \pm 0.1