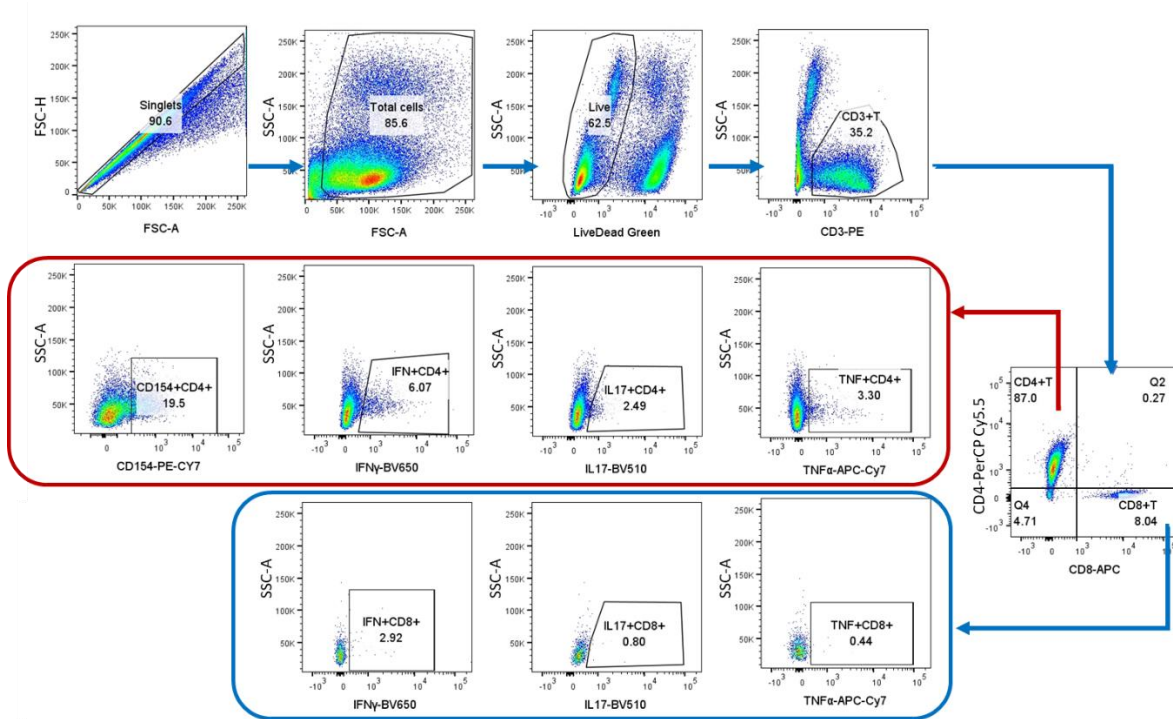
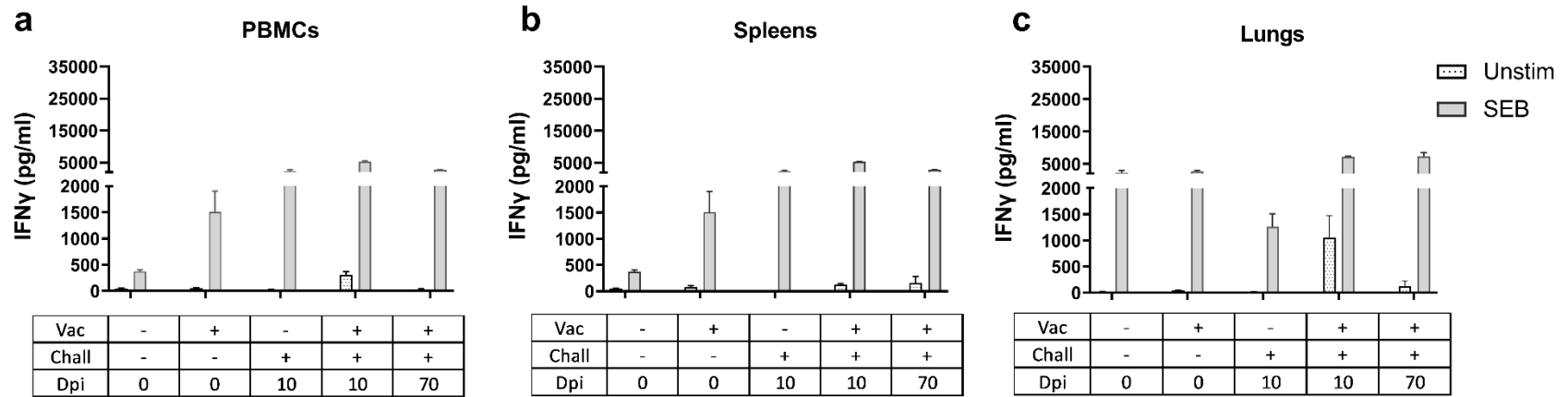




Supplementary Figure 1. Representative flow cytometry plots illustrating the gating strategy for experiments examining T cell activation and intracellular cytokine production in cultured lung leukocytes. Singlet cells were gated based on forward scatter (FSC) height (FSC-H) vs. area (FSC-A). Debris was excluded based on FSC-A and side scatter area (SSC-A). Dead cells were excluded based on LIVE/DEAD green staining. T cells were selected based on CD3⁺ staining. The CD4⁺CD8⁻ population was selected from the CD3⁺ population. Finally, the intracellular expression of IFN γ , IL-17A, TNF α and CD154 by the live CD3⁺CD4⁺CD8⁻ gated population (shown in red box) was examined. An identical gating strategy was used to examine CD8⁺ T cells except the CD4⁻CD8⁺ population (shown in blue box) was selected from the CD3⁺ population. CD154 expression was not analyzed in the CD4⁻CD8⁺ population. The plots are from lung cells of a GP-Cda1/Cda2 vaccinated mouse 10d post infection stimulated *ex vivo* with SEB.



Supplementary Figure 2. IFN γ production by PBMCs, splenocytes, and lung leukocytes following SEB stimulation. Experiments were designed and conducted as indicated in the Fig. 5 legend. Supernatants of unstimulated (Unstim) or SEB-stimulated PBMCs (**a**), spleens (**b**) and lung (**c**) were collected and analyzed for IFN γ by ELISA. Each group had 5 mice. Vac, vaccinated with GP-Cda1/Cda2. Chall, challenged with *C. neoformans* strain KN99. Dpi, days post infection. The results of the statistical comparisons between unstimulated and SEB-stimulated groups are shown in Supplementary Figure 3.

a. PBMCs

Group	Unstim vs Cda1		Unstim vs Cda2		Unstim vs KN99		Unstim vs SEB	
Vac- / Chall- / Dpi 0	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac+ / Chall- / Dpi 0	*	0.0268	****	<0.0001	ns	>0.9999	****	<0.0001
Vac- / Chall+ / Dpi 10	ns	>0.9999	**	0.0061	ns	>0.9999	****	<0.0001
Vac+ / Chall+ / Dpi 10	****	<0.0001	****	<0.0001	**	0.0019	****	<0.0001
Vac+ / Chall+ / Dpi 70	ns	0.7014	ns	0.0769	***	0.0003	****	<0.0001

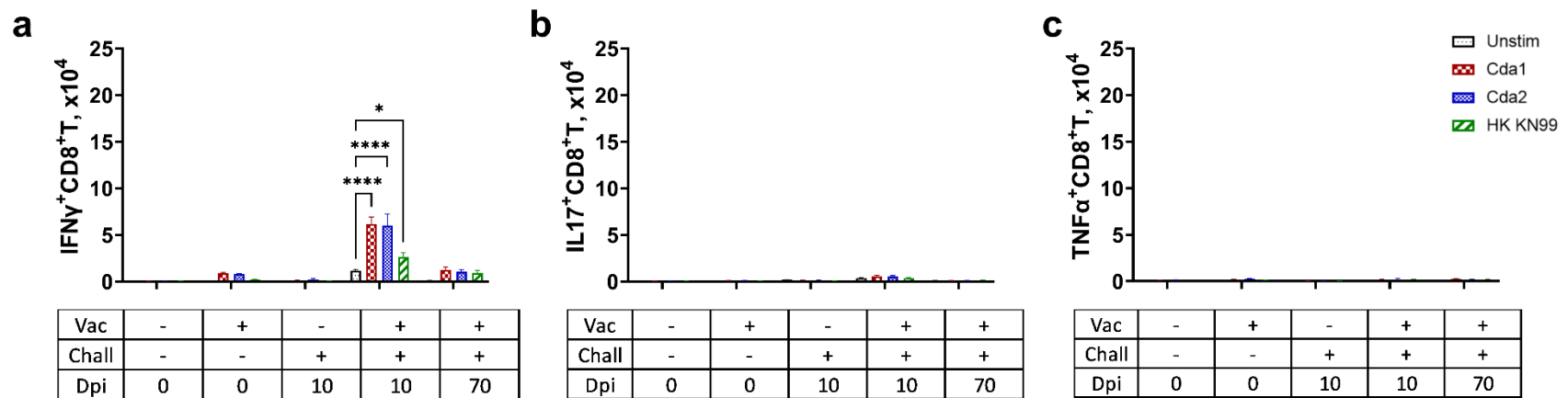
b. Spleens

Group	Unstim vs Cda1		Unstim vs Cda2		Unstim vs KN99		Unstim vs SEB	
Vac- / Chall- / Dpi 0	ns	>0.9999	ns	>0.9999	ns	>0.9999	****	<0.0001
Vac+ / Chall- / Dpi 0	***	0.0005	****	<0.0001	**	0.0011	****	<0.0001
Vac- / Chall+ / Dpi 10	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	0.1228
Vac+ / Chall+ / Dpi 10	****	<0.0001	**	0.0034	ns	>0.9999	****	<0.0001
Vac+ / Chall+ / Dpi 70	****	<0.0001	****	<0.0001	****	<0.0001	****	<0.0001

c. Lungs

Group	Unstim vs Cda1		Unstim vs Cda2		Unstim vs KN99		Unstim vs SEB	
Vac- / Chall- / Dpi 0	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	0.0958
Vac+ / Chall- / Dpi 0	*	0.0186	***	0.0001	ns	>0.9999	**	0.0052
Vac- / Chall+ / Dpi 10	ns	>0.9999	ns	>0.9999	ns	>0.9999	ns	0.4789
Vac+ / Chall+ / Dpi 10	****	<0.0001	****	<0.0001	****	<0.0001	****	<0.0001
Vac+ / Chall+ / Dpi 70	***	0.0001	****	<0.0001	***	0.0004	****	<0.0001

Supplementary Figure 3. Statistical comparisons between groups in Fig. 5 and Supplementary Figure 2. Experiments were designed and conducted as indicated in the Fig. 5 and Supplementary Figure 2 legends. IFN γ production of unstimulated cells and cells stimulated with the indicated antigens was compared using two-way ANOVA with Bonferroni's correction. Statistics of comparison were shown in **a** for PBMCs, **b** for Spleens and **c** for Lungs. Vac, vaccinated with GP-Cda1/Cda2. Chall, challenged with *C. neoformans* strain KN99. Dpi, days post infection. Unstim, unstimulated. ns (not significant), $P > 0.05$. *, $P < 0.05$. **, $P < 0.005$. ***, $P < 0.0005$. ****, $P < 0.0001$. Comparisons with statistical differences are shown in red font.



Supplementary Figure 4. Intracellular cytokine production by pulmonary CD8⁺ T cells in GP-Cda1/Cda2-vaccinated and/or infected mice. BALB/c mice were vaccinated thrice at biweekly intervals with GP-Cda1/Cda2. Two weeks after last boost, the mice received a pulmonary challenge with *C. neoformans*. Mice were euthanized at 0 dpi (uninfected), 10 dpi or 70 dpi. Controls included unvaccinated mice euthanized at 0 dpi or 10 dpi. Lungs were harvested and single cell suspensions were prepared. Leukocytes were cultured in complete media supplemented with amphotericin B and stimulated with the indicated antigens or left unstimulated (Unstim) for 18h. Then the cells were collected, stained, and analyzed by polychromatic FACS, as described in Methods. From left to right shows the numbers of lung CD8⁺ T cells producing the intracellular cytokines IFN γ (a), IL-17 (b), and TNF α (c) following *ex vivo* stimulation. Each group had 5 mice. Vac = vaccinated with GP-Cda1/Cda2, Chall = challenged with *C. neoformans*, Dpi = days post infection, HK = heat killed. Two-way ANOVA with Bonferroni's correction was used for comparisons. *, $P < 0.05$. ****, $P < 0.0001$.

a. CFUs

Group	Summary	P Value
Vac- / Chall- / Dpi 10 vs Vac+ / Chall+ / Dpi 10	ns	0.0952
Vac- / Chall- / Dpi 10 vs Vac+ / Chall+ / Dpi 70	**	0.0079
Vac+ / Chall+ / Dpi 10 vs Vac+ / Chall+ / Dpi 70	**	0.0079

b. Leukocytes/CD4⁺T / CD8⁺T cell counts

Group	Leukocytes		CD4 ⁺ T		CD8 ⁺ T	
Vac- / Chall- / Dpi 0 vs Vac- / Chall+ / Dpi 10	ns	0.4785	ns	0.7759	ns	>0.9999
Vac- / Chall- / Dpi 0 vs Vac+ / Chall- / Dpi 0	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac- / Chall- / Dpi 0 vs Vac+ / Chall+ / Dpi 70	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac- / Chall- / Dpi 0 vs Vac+ / Chall+ / Dpi 10	****	<0.0001	****	<0.0001	***	0.0002
Vac- / Chall+ / Dpi 10 vs Vac+ / Chall+ / Dpi 10	****	<0.0001	****	<0.0001	**	0.0016
Vac+ / Chall- / Dpi 0 vs Vac+ / Chall+ / Dpi 10	****	<0.0001	****	<0.0001	**	0.0011
Vac+ / Chall+ / Dpi 10 vs Vac+ / Chall+ / Dpi	****	<0.0001	****	<0.0001	***	0.0004
Vac+ / Chall- / Dpi 0 vs Vac+ / Chall+ / Dpi 70	ns	>0.9999	ns	>0.9999	ns	>0.9999

c. CD154⁺CD4⁺T

Group	Unstim vs Cda1		Unstim vs Cda2		Unstim vs KN99	
Vac- / Chall- / Dpi 0	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac+ / Chall- / Dpi 0	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac- / Chall+ / Dpi 10	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac+ / Chall+ / Dpi 10	****	<0.0001	****	<0.0001	****	<0.0001
Vac+ / Chall+ / Dpi 70	ns	>0.9999	ns	>0.9999	ns	>0.9999

d. IFN γ ⁺CD4⁺T

Group	Unstim vs Cda1		Unstim vs Cda2		Unstim vs KN99	
Vac- / Chall- / Dpi 0	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac+ / Chall- / Dpi 0	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac- / Chall+ / Dpi 10	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac+ / Chall+ / Dpi 10	****	<0.0001	****	<0.0001	****	<0.0001
Vac+ / Chall+ / Dpi 70	ns	>0.9999	ns	>0.9999	ns	>0.9999

e. IL-17⁺CD4⁺T

Group	Unstim vs Cda1		Unstim vs Cda2		Unstim vs KN99	
Vac- / Chall- / Dpi 0	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac+ / Chall- / Dpi 0	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac- / Chall+ / Dpi 10	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac+ / Chall+ / Dpi 10	****	<0.0001	****	<0.0001	****	<0.0001
Vac+ / Chall+ / Dpi 70	ns	>0.9999	ns	>0.9999	ns	>0.9999

f. TNF α ⁺CD4⁺T

Group	Unstim vs Cda1		Unstim vs Cda2		Unstim vs KN99	
Vac- / Chall- / Dpi 0	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac+ / Chall- / Dpi 0	ns	0.1329	ns	>0.9999	ns	>0.9999
Vac- / Chall+ / Dpi 10	ns	>0.9999	ns	>0.9999	ns	>0.9999
Vac+ / Chall+ / Dpi 10	****	<0.0001	****	<0.0001	****	<0.0001
Vac+ / Chall+ / Dpi 70	ns	>0.9999	ns	>0.9999	ns	>0.9999

Supplementary Figure 5. Statistical comparison for lung CFU and cell numbers in Fig. 6. Experiments were designed and conducted as indicated in Fig. 6 legend. Statistics of comparison were shown in (a) for lung CFUs (Man-Whitney test); (b) for lung leukocyte / CD4⁺T / CD8⁺T numbers (One-way ANOVA with Bonferroni's correction); (c) for CD154⁺CD4⁺T numbers, (d) for IFN γ ⁺CD4⁺T numbers; (e) for IL-17⁺CD4⁺T numbers; and (f) for TNF α ⁺CD4⁺T numbers (Two-way ANOVA with Bonferroni's correction were used for c-f). Vac, vaccinated with GP-Cda1/Cda2. Chall, challenged with *C. neoformans* strain KN99. Dpi, days post infection. Unstim, unstimulated. ns (not significant), $P > 0.05$. *, $P < 0.05$. **, $P < 0.005$. ***, $P < 0.0005$. ****, $P < 0.0001$. Comparisons with statistical differences are shown in red font.