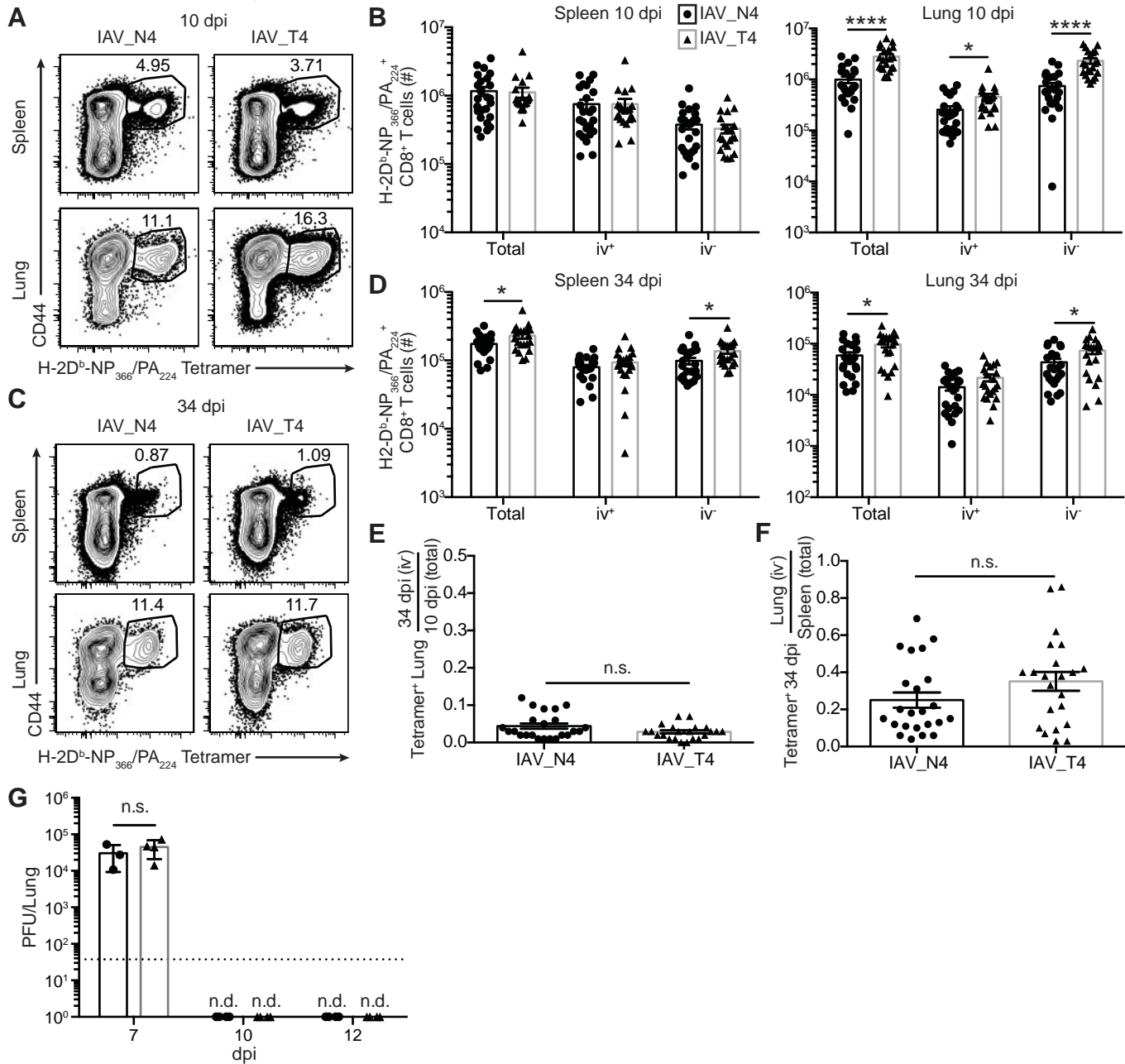


Supplemental Table 1

		Comparison	Significance	Adjusted P Value			Comparison	Significance	Adjusted P Value		
A	10 dpi spleen OT-I	Total	N4 vs. Y3	****	< 0.0001	B	10 dpi lung OT-I	Total	N4 vs. Y3	***	0.0007
			N4 vs. T4	****	< 0.0001				N4 vs. T4	****	< 0.0001
			N4 vs. N4 1000	ns	0.1714				N4 vs. N4 1000	****	< 0.0001
			Y3 vs. T4	ns	0.9088				Y3 vs. T4	ns	0.1619
			Y3 vs. N4 1000	*	0.0123				Y3 vs. N4 1000	ns	0.8948
			T4 vs. N4 1000	***	0.0003				T4 vs. N4 1000	ns	0.5397
	iv+	N4 vs. Y3	****	< 0.0001	iv+	N4 vs. Y3	****	< 0.0001			
		N4 vs. T4	****	< 0.0001		N4 vs. T4	****	< 0.0001			
		N4 vs. N4 1000	ns	0.2434		N4 vs. N4 1000	ns	0.1837			
		Y3 vs. T4	ns	0.9476		Y3 vs. T4	ns	0.8098			
		Y3 vs. N4 1000	*	0.0484		Y3 vs. N4 1000	ns	0.0928			
		T4 vs. N4 1000	**	0.0031		T4 vs. N4 1000	**	0.0029			
	iv-	N4 vs. Y3	****	< 0.0001	iv-	N4 vs. Y3	***	0.001			
		N4 vs. T4	****	< 0.0001		N4 vs. T4	****	< 0.0001			
		N4 vs. N4 1000	*	0.0391		N4 vs. N4 1000	****	< 0.0001			
		Y3 vs. T4	ns	0.9343		Y3 vs. T4	ns	0.1376			
		Y3 vs. N4 1000	*	0.0294		Y3 vs. N4 1000	ns	0.7232			
		T4 vs. N4 1000	***	0.0006		T4 vs. N4 1000	ns	0.7391			
C	34 dpi spleen OT-I	Total	N4 vs. Y3	*	0.0406	D	34 dpi lung OT-I	Total	N4 vs. Y3	ns	0.8253
			N4 vs. T4	***	0.0002				N4 vs. T4	****	< 0.0001
			N4 vs. N4 1000	*	0.046				N4 vs. N4 1000	*	0.0214
			Y3 vs. T4	ns	0.9168				Y3 vs. T4	*	0.0173
			Y3 vs. N4 1000	ns	> 0.9999				Y3 vs. N4 1000	ns	0.28
			T4 vs. N4 1000	ns	0.9453				T4 vs. N4 1000	ns	0.861
	iv+	N4 vs. Y3	ns	0.2058	iv+	N4 vs. Y3	ns	0.1399			
		N4 vs. T4	**	0.0034		N4 vs. T4	****	< 0.0001			
		N4 vs. N4 1000	ns	0.177		N4 vs. N4 1000	**	0.0045			
		Y3 vs. T4	ns	0.8822		Y3 vs. T4	*	0.0482			
		Y3 vs. N4 1000	ns	0.999		Y3 vs. N4 1000	ns	0.6265			
		T4 vs. N4 1000	ns	0.9482		T4 vs. N4 1000	ns	0.6788			
	iv-	N4 vs. Y3	*	0.0103	iv-	N4 vs. Y3	ns	0.9171			
		N4 vs. T4	****	< 0.0001		N4 vs. T4	****	< 0.0001			
		N4 vs. N4 1000	*	0.0153		N4 vs. N4 1000	*	0.0423			
		Y3 vs. T4	ns	0.9501		Y3 vs. T4	*	0.0293			
		Y3 vs. N4 1000	ns	> 0.9999		Y3 vs. N4 1000	ns	0.3029			
		T4 vs. N4 1000	ns	0.9545		T4 vs. N4 1000	ns	0.9142			

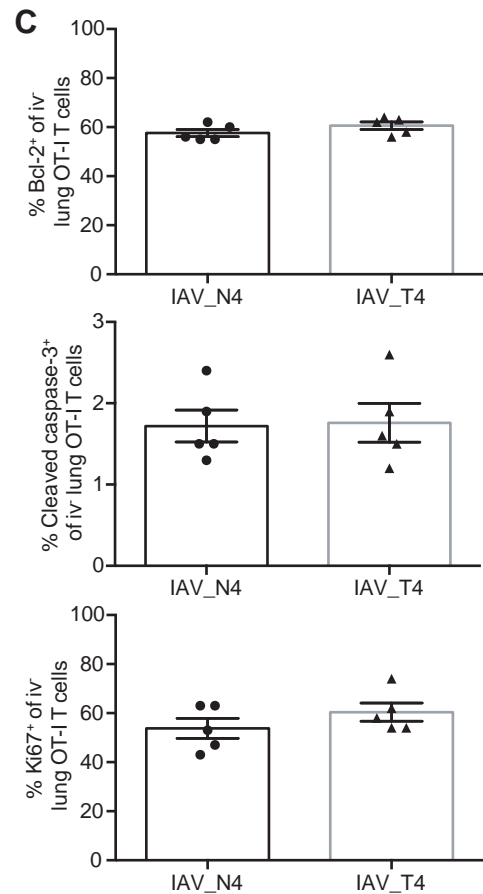
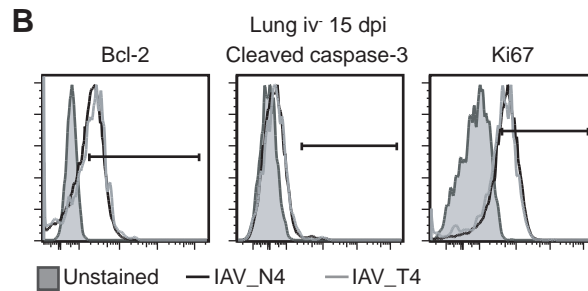
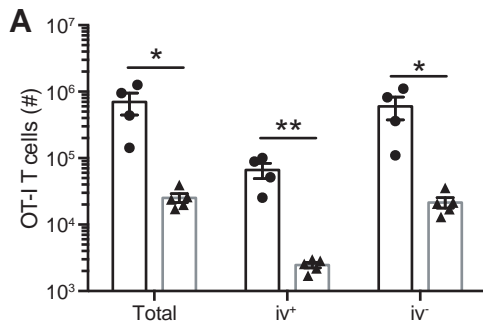
S. Table 1: One-way ANOVA statistical analysis of data sets from Fig. 1 A-D.

Supplemental Figure 1



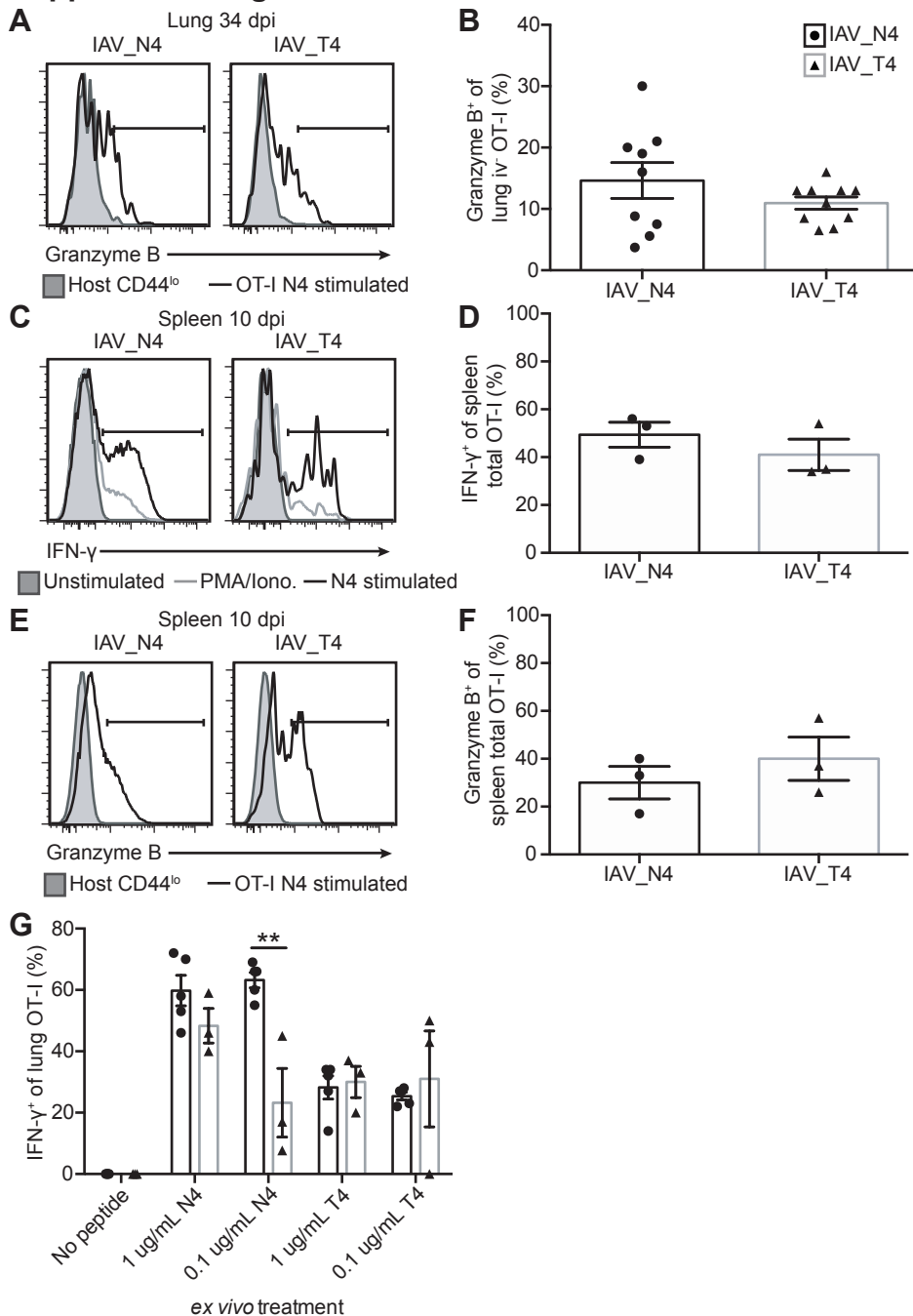
S. Figure 1 Endogenous virus-specific CD8⁺ T cells are equivalently stimulated by influenza A viruses expressing high and low affinity OT-I epitopes. (A-F) CD45.1⁺ OT-I T cells were transferred into CD45.2⁺ hosts and were infected with 40 PFUs of IAV_N4 or IAV_T4. On indicated dpi, spleen and lungs were harvested after anti-CD8 α iv injections. **(A and C)** Representative flow plots of total host CD8⁺ T cells for H-2D^b-PA₂₂₄/NP₃₆₆ tetramers and CD44, on 10 **(A)** or 34 **(C)** dpi. Numbers represent the percentage of cells in the gate. **(B and D)** The number of endogenous CD8⁺ H-2D^b-PA₂₂₄/NP₃₆₆ tetramer⁺ CD44^{hi} cells, either total (iv⁺ and iv⁻ combined), iv⁺, or iv⁻ cells at 10 **(B)** or 34 **(D)** dpi. **(E)** The number of iv⁻ tetramer⁺ CD8⁺ T cells in the lung at 34 dpi was divided by the average number of total tetramer⁺ CD8⁺ T cells in the lung at 10 dpi. **(F)** The number of iv⁻ tetramer⁺ CD8⁺ T cells in the lung at 34 dpi was divided by the average number of total tetramer⁺ CD8⁺ T cells in the spleen at 34 dpi. **(G)** CD45.2⁺ mice were infected with 40 PFUs of IAV_N4 or IAV_T4. Lungs were harvested at indicated dpi and virus titered by plaque assay. Dotted line represents the limit of detection. The results **(B and D)** are compiled from at least 5 independent experiments with at least 4 mice per group, per experiment (\pm SEM). The results **(G)** are from 1 experiment with 3 mice per group (\pm SEM). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (unpaired t test).

Supplemental Figure 2



S. Figure 2 TCR signal strength does not alter the phenotype of cells during contraction. Challenged mice were generated as in Figure 1. **(A)** The number total, iv^- or iv^+ lung OT-I T cells at 15 dpi. **(B)** Representative Bcl-2, cleaved caspase-3 and Ki67 staining of IAV_N4 or IAV_T4 challenged iv^- lung OT-I T cells at 15 dpi. **(C)** The percentage of iv^- lung OT-I T cells that Bcl-2⁺, cleaved caspase-3⁺ or Ki67⁺ at 15 dpi. The results **(A, C)** are of 1 experiment with 5 mice per group (\pm SEM). * $p < 0.05$, ** $p < 0.01$ (unpaired t test).

Supplemental Figure 3



S. Figure 3 TCR affinity does not impact the production of effector molecules. Challenged mice were generated as in Figure 1. On indicated dpi, lungs and spleens were harvested and cells were stimulated *ex vivo* with 1 μ g/mL N4 peptide. **(A,E)** Representative granzyme B staining of *iv* lung **(A)** or total spleen **(E)** OT-I T cells and CD44^{lo} host cells stimulated with N4 peptide. **(C)** Representative IFN- γ ⁺ staining of total spleen OT-I T cells unstimulated, stimulated with PMA/Ionomycin or N4 peptide. **(B)** Percentage of *iv* lung granzyme B⁺ OT-I T cells after *ex vivo* N4 stimulation at 34 dpi. **(D, F)** Percentage of total spleen IFN- γ ⁺ **(D)** or granzyme B⁺ **(F)** OT-I T cells after *ex vivo* N4 stimulation at 10 dpi. **(G)** Percentage of lung IFN- γ ⁺ OT-I T cells after *ex vivo* stimulation with 1 or 0.1 μ g/mL of N4 or T4 peptides at 34 dpi. The results **(B)** are compiled from 2 independent experiments with at least 4 mice per group, per experiment (\pm SEM). The results **(D, F, G)** are from 1 experiment with at least 3 mice per group (\pm SEM). * p < 0.05 (unpaired t test).