frontiers

Supplementary Material

TLR7 promotes chronic airway disease in RSV-infected mice

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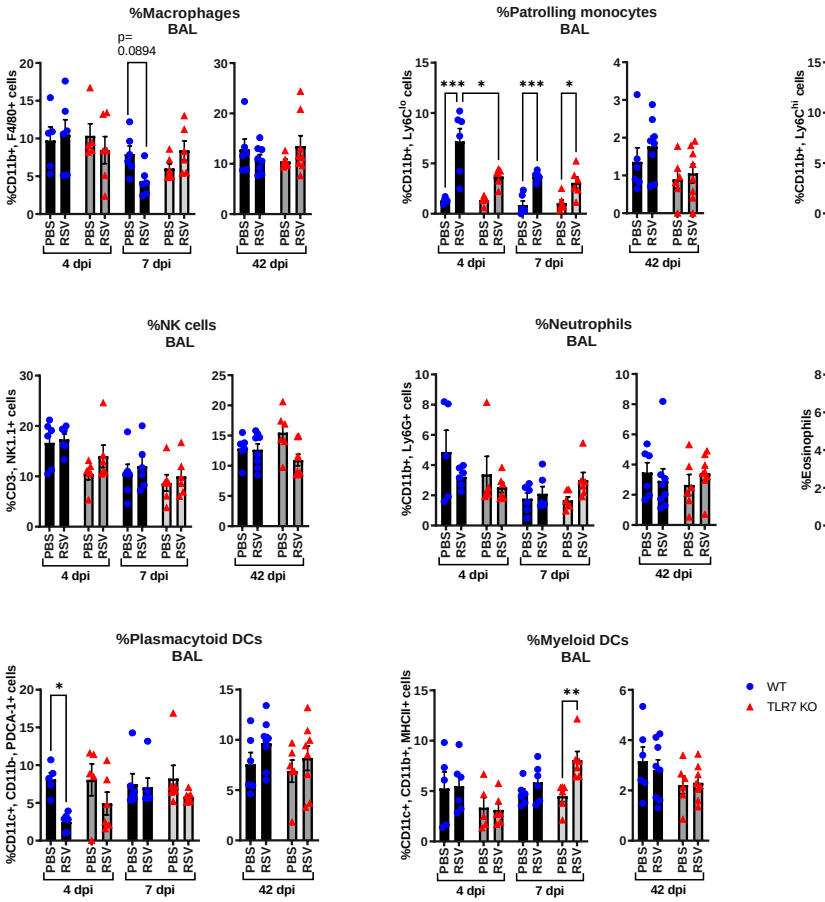
A



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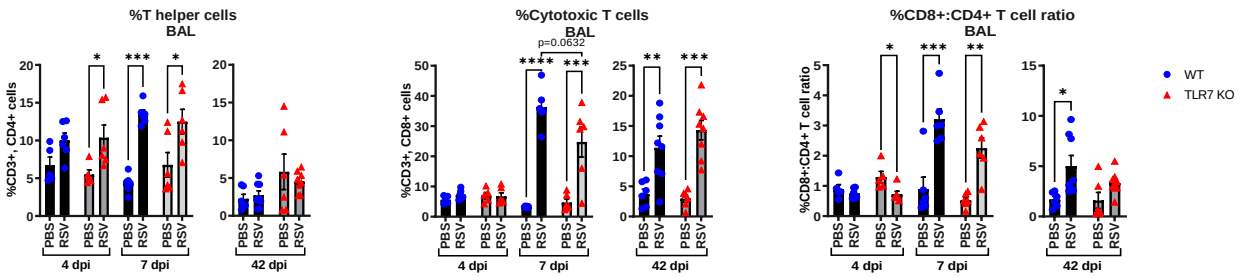
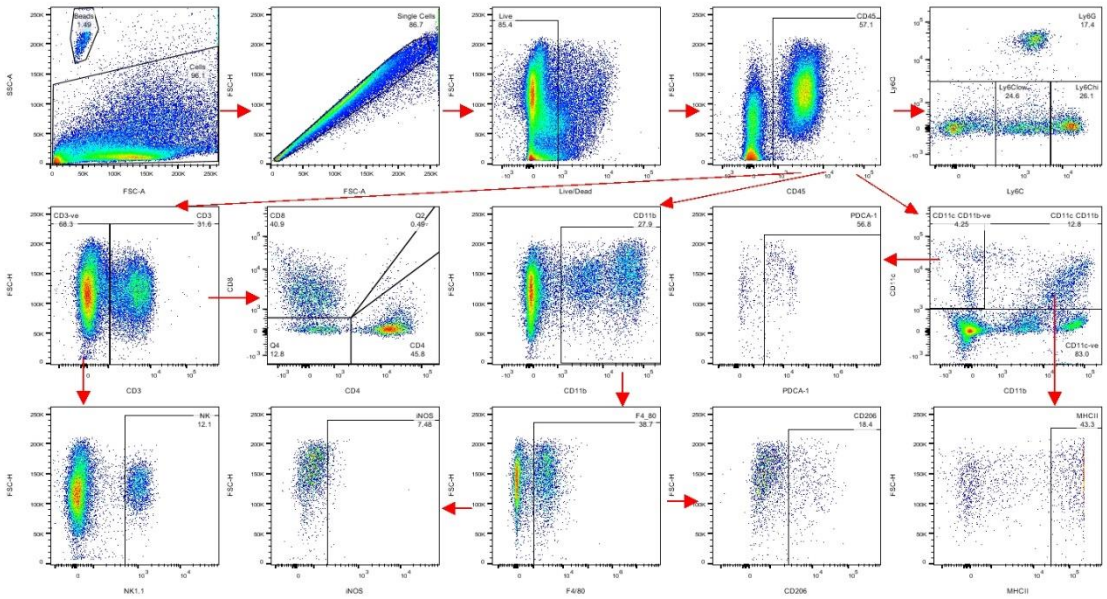


Supplementary Figure S1. Airway immune composition is not dramatically altered in TLR7 KO mice following RSV infection. WT C57BL/6 or TLR7 KO mice were infected with RSV (0.5-2x10^7 PFUs) or PBS via intranasal administration and analysis performed after 4, 7 or 42 dpi. The percentage of immune cell populations in the airways collected in the BAL fluid was determined by flow cytometry. (A) Innate immune cell types: interstitial macrophages ($CD11b^+$ $F4/80^+$), patrolling monocytes ($CD11b^+$ $Ly6C^{lo}$), inflammatory monocytes ($CD11b^+$ $Ly6C^{hi}$), NK cells ($CD3^-$ $NK1.1^+$), neutrophils ($CD11b^+$ $Ly6G^+$), plasmacytoid DCs ($CD11c^+$ $CD11b^-$ $PDCA\text{-}1^+$) and myeloid DCs ($CD11c^+$ $CD11b^+$ $MHCII^+$). Numbers of differentially stained eosinophils was determined by counting 500 cells from random fields by standard morphological criteria relative to the total number of isolated cells. (B) Adaptive T cell subtypes: T helper ($CD3^+$ $CD4^+$) and cytotoxic T cells ($CD3^+$ $CD8^+$). Cell populations were measured as percentage of $CD45^+$ cells. Data are expressed as mean ± SEM, n = 5-8 mice per experimental group from two independent experiments. Statistical analysis was conducted using two-way ANOVA test followed by Tukey's post hoc test for multiple comparison test (*$p < 0.05$, **$p < 0.01$, ***$p<0.001$, ****$p<0.0001$).



Supplementary Figure S2. Flow cytometry gating strategy. Lymphocytes and myeloid cells were gated as $CD3^+$ or $CD11b^+$ respectively from $CD45^+$ leukocytes. $CD3^+$ T cells were further divided into subsets T helper ($CD4^+$) and cytotoxic ($CD8^+$) T cells. NK cells ($NK1.1^+$) were gated from $CD3^-$ populations. Patrolling $Ly6C^{lo}$, pro-inflammatory $Ly6C^{hi}$ monocytes and inflammatory $Ly6G^+$ neutrophils were identified within $CD11b^+$ myeloid cells. $F4/80^+$ macrophages were identified from $CD11b^+$ cells then further divided into subsets M1 proinflammatory ($iNOS^+$) and M2 anti-inflammatory ($CD206^+$). Dendritic cell subsets were gated from $CD11b^+/CD11c^-$ populations (plasmacytoid, $PDCA\text{-}1^+$) or $CD11b^+/CD11c^+$ populations (myeloid, $MHCII^+$).

Supplementary Table S1. RSV Fusion gene and RPS18 housekeeping cycle threshold (Ct) values of lung and nasal tissue from PBS control and infected mice from 4, 7 and 42 dpi study timepoints.

Study endpoint	Genotype	Infection status	Lung tissue [b]			Nasal tissue [c]		
			Ct RSV	Ct RPS18	ΔCt (x 10^4) [a]	Ct RSV	Ct RPS18	ΔCt (x 10^4) [a]
4 dpi	WT	PBS	31.21	21.04	10.23	38.47	22.73	0.15
		RSV	18.69	20.98	55679.65	27.95	24.28	831.10
	TLR7 KO	PBS	28.88	21.03	63.29	37.45	24.45	1.56
		RSV	18.7	21.04	55866.14	29.83	25.28	705.97
7 dpi	WT	PBS	33.73	21.13	2.97	38.78	26.64	3.12
		RSV	24.31	21.23	1454.06	31.42	23.31	41.53
	TLR7 KO	PBS	35.04	21.27	1.28	36.46	25.06	4.21
		RSV	24.35	20.97	1309.66	29.99	23.36	77.94
42 dpi	WT	PBS	36.5	21.46	0.35	37.16	22.26	0.48
		RSV	33.62	20.92	2.22	35.77	23.47	5.35
	TLR7 KO	PBS	36.36	21.51	0.41	38.88	22.63	0.25
		RSV	33.22	21.67	3.97	37.34	24.43	4.18

[a] Cycle threshold values for RSV Fusion gene amplification relative to RPS18 housekeeping

[b] *n = 6-8* in both PBS controls and RSV infected groups

[c] *n = 5-8* in both PBS controls and RSV infected groups