

SARS-CoV-2 RBD Trimer Protein Adjuvanted with Alum-3M-052 Protects from SARS-CoV-2 infection and Immune Pathology in the Lung

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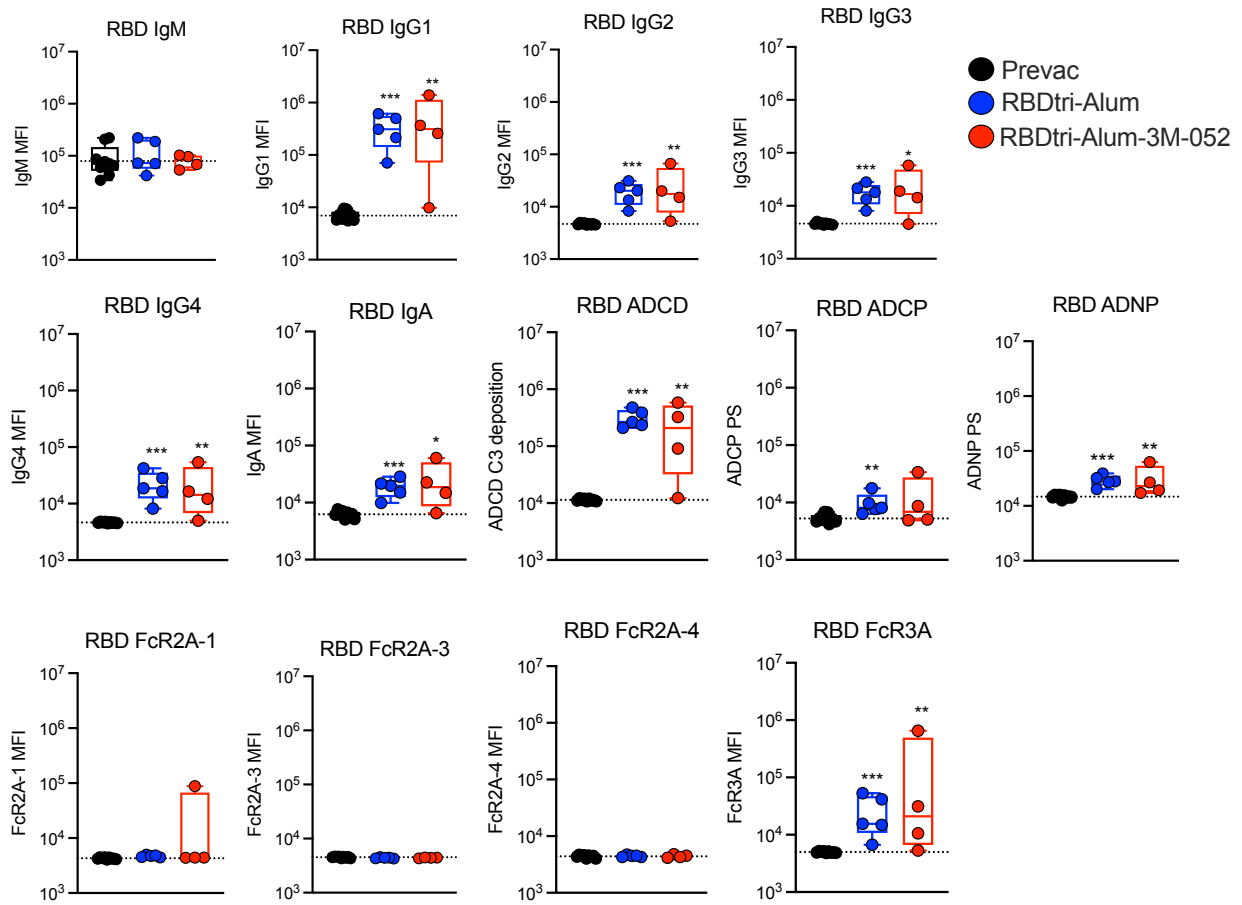
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Footnotes

*Contributed equally to this work

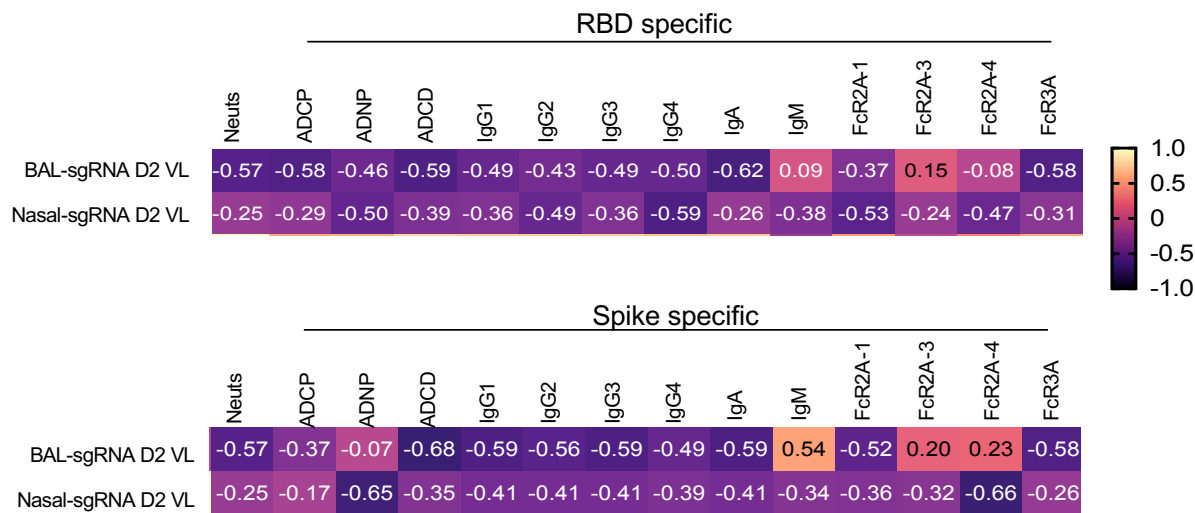
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Supplementary Fig. 1



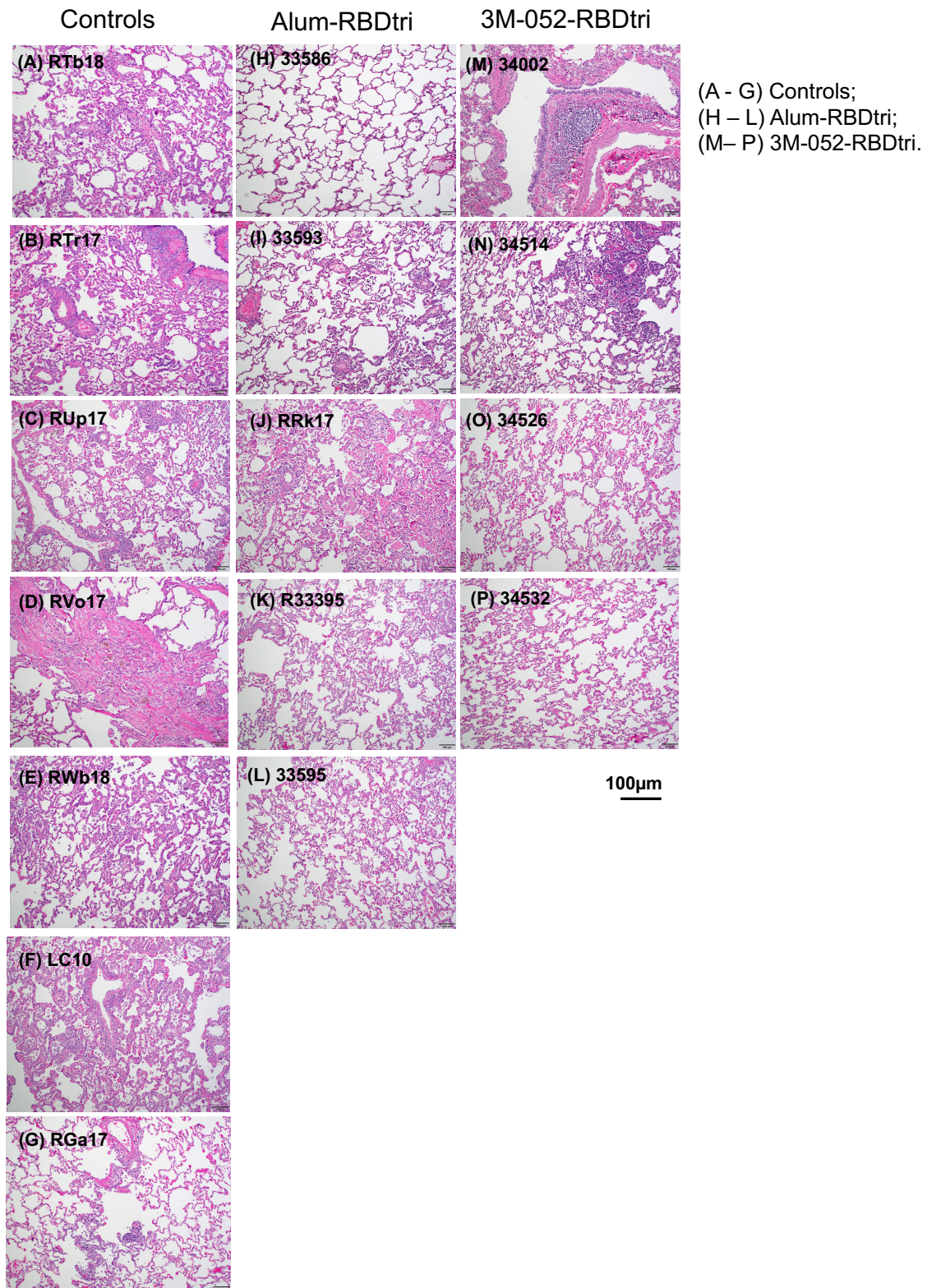
Supplementary Fig. 1: Antibody subclass, non-neutralizing antibody effector functions and FcγR binding activities of antibody induced after RBD trimer vaccination in macaques. Box plots showing SARS-CoV-2 RBD-specific systems serology measurements (antibody isotypes, antibody-dependent effector functions and FcγR binding) pre vaccination (prevac) and week 6 post-vaccination in alum (blue) or alum-3M-052 (red)) groups. Data for pre vaccination time point from both groups is shown together (black). Each filled circle represents an individual animal. Error bars show minimum to maximum. *p*-values indicate difference between prevac and post vaccination time points. Statistical differences were calculated using unpaired Mann-Whitney (two-tailed) test. *, *p*<0.05; **, *p*<0.01 and ***, *p*<0.001.

Supplementary Fig. 2



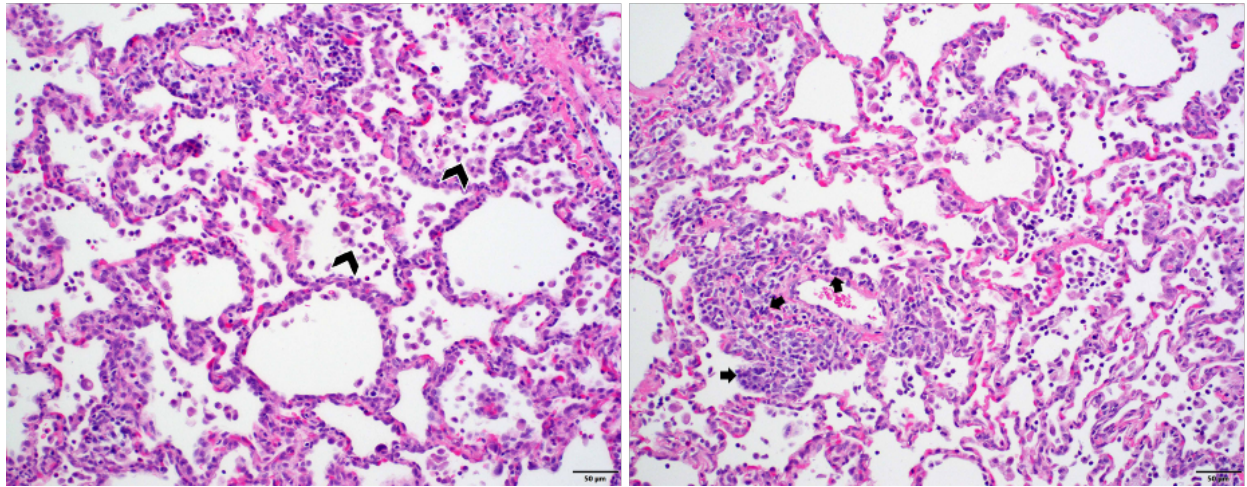
Supplementary Fig. 2: Correlations between Day 2 viral loads (BAL and Nasal) and antibody specificity and function. The correlation matrix was generated between Day 2 viral loads (BAL and Nasal) and respective antibody specificity or function. The color refers to r value scale shown on the right. The number in each cell indicate the actual r value. The Spearman rank test was used to perform correlation analysis. None of the correlations were statistically significant.

Supplementary Fig. 3



Supplementary Fig. 3: Post-challenge lung pathology in rhesus macaque. Lung Pathology of rhesus macaques infected with SARS-CoV-2 (Panels A-G) and vaccinated with Alum-RBDtri (Panels H-L) or 3M-052-RBDtri (Panels M-P). Panels A-G show lung lesions in control SARS-CoV-2 infected rhesus macaques with multifocal interstitial pneumonia, type 2 pneumocyte hyperplasia, alveolar septal thickening, syncytia formation, neutrophils and macrophages infiltrations and severe fibrosis (RVo17). Panels I, K, and L show lesser thickening of alveolar septa, mild perivascular cuffing and decreased type 2 pneumocytes hyperplasia; Panel J shows extensive interstitial pneumonia with serofibrinous exudate. Panels H, O, and P show no significant microscopic lesions. Panels M and N show mild peribronchiolar hyperplasia and perivascular cuffing. This experiment was conducted on lung sections derived from control (n=7), alum (n=5) and alum-3M-052 (n=4) vaccinated animals following SARS-CoV-2 challenge, and multiple sections were scanned for pathological features and then the representative image with 100x magnification is shown. See also supplementary Table 1.

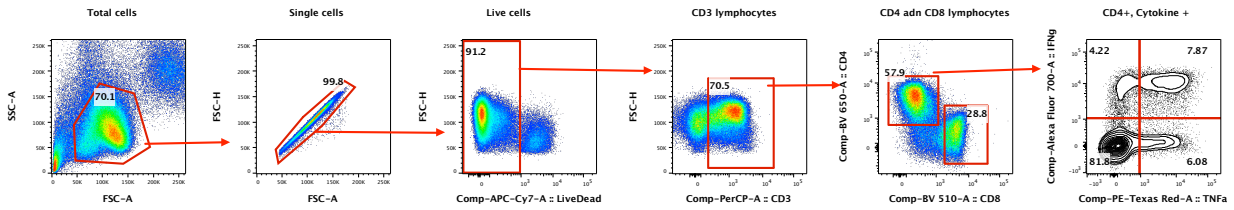
Supplementary Fig. 4



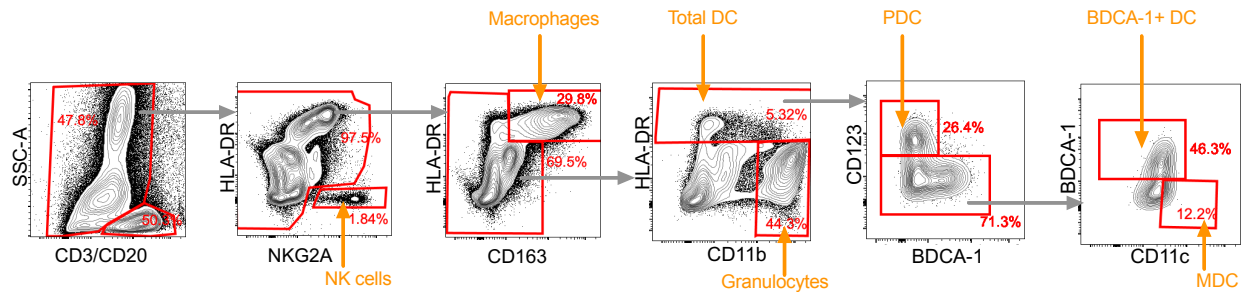
Supplementary Fig. 4: Histopathological examination of neutrophils and macrophages recruitment in lung tissue sections of rhesus macaque. Lung Pathology examination of rhesus macaques infected with SARS-CoV-2. Arrowheads on the left image show clusters of intra-alveolar macrophages for macaque Rup17 and arrows on the right image show examples of neutrophilic infiltrates for macaque RUp17. This experiment was conducted on lung sections derived from control (n=7), alum (n=5) and alum-3M-052 (n=4) vaccinated animals following SARS-CoV-2 challenge, and multiple sections were scanned for pathological features and then the representative image with 200x magnification and 20x objective is shown.

Supplementary Fig. 5

A



B



Supplementary Fig. 5: Gating strategy. A) Gating strategy for intracellular staining (ICS) assay in flow analysis. Total lymphocytes (FSC-A and SSC-A) and singlets (FSC-A and FSC-H) were gated using scatter. Live cells were selected using live/dead marker and followed CD3 expression was used to gate T cells. The T cells were then separated into CD4 versus CD8 co-receptor subsets. For CD4 subset, cytokines. Phorbol myristate acetate (PMA) and Ionomycin positive controls. B) Live cells were selected using live/dead marker and CD3⁺ and CD20⁺ cells were excluded. Then, different innate cells were defined using the following combination of markers: NK cells (HLADR⁻ NKG2A⁺), Macrophages (HLADR⁺ CD163⁺); PDCs (HLADR⁺CD163⁺CD123⁺ BDCA1⁺); BDCA1⁺ DC (HLADR⁺ CD163⁺ CD123⁺ BDCA1⁺); and MDCs (HLADR⁺CD163⁺CD123⁺ CD11C⁺). NK – Natural Killer cells; DC – Dendritic cells; PDCs (Plasmacytoid DCs); and MDCs (Myeloid DCs).

Supplementary Table 1: Lung pathology scores post infection.

	Animal ID	Type 2 pneumocyte hyperplasia	Alveolar septal thickening	Fibrosis	Perivascular cuffing	Peribronchiolar hyperplasia	Syncytia formation	Total score
MVA-Wt (Control)	RTb18	3	2	0	2	2	1	10
	RTr17	0	1	0	2	2	1	6
	RWb18	2	2	0	2	2	1	9
	RVo17	1	1	3	2	1	0	8
	RUp17	2	2	0	2	1	0	7
Control	RGa17	0	1	0	1	0	0	2
	Lc16	1	2	0	2	0	1	6
Alum (Vaccine)	33586	0	0	0	0	0	0	0
	33593	2	2	0	1	0	1	6
	RRk17	0.8	1.4	0	1.4	0	0	3.6
	R33395	1	2	0	2	0	1	6
	33595	1	2	0	2	0	1	6
Alum-3M052 (Vaccine)	34002	0	0	0	1	1	0	2
	34514	0.5	0.5	1	0.5	0	0	2.5
	34526	0	0	0	0	0	0	0
	34532	0	0	0	0	0	0	0

Supplementary Table 2: Primers used in this study.

Title	Sequences
SGMRNA-E-Fw	5'-CGATCTCTTGTAGATCTGTTCTC-3'
SGMRNA-E-Rv	5'-ATATTGCAGCAGTACGCACACA-3'
SGMRNA-E-Pr	5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-3'
RM-RPP30-Fw	5'-AGACTTGGACGTGCGAGCG-3'
RM-RPP30-Rv	5'- GAGCCGCTGTCTCCACAAGT-3'
and RPP30-Pr	5'-FAM-TTCTGACCTGAAGGCTCTGCGCG-BHQ1-3'