Immunity, Volume *54*

Supplemental information

A modified vaccinia Ankara vector-based vaccine protects macaques from SARS-CoV-2 infection, immune pathology, and dysfunction in the lungs

Nanda Kishore Routhu, Narayanaiah Cheedarla, Sailaja Gangadhara, Venkata Satish Bollimpelli, Arun K. Boddapati, Ayalnesh Shiferaw, Sheikh Abdul Rahman, Anusmita Sahoo, Venkata Viswanadh Edara, Lilin Lai, Katharine Floyd, Shelly Wang, Stephanie Fischinger, Caroline Atyeo, Sally A. Shin, Sanjeev Gumber, Shannon Kirejczyk, Joyce Cohen, Sherrie M. Jean, Jennifer S. Wood, Fawn Connor-Stroud, Rachelle L. Stammen, Amit A. Upadhyay, Kathryn Pellegrini, David Montefiori, Pei-Yong Shi, Vineet D. Menachery, Galit Alter, Thomas H. Vanderford, Steven E. Bosinger, Mehul S. Suthar, and Rama Rao Amara

Supplemental item titles and legends

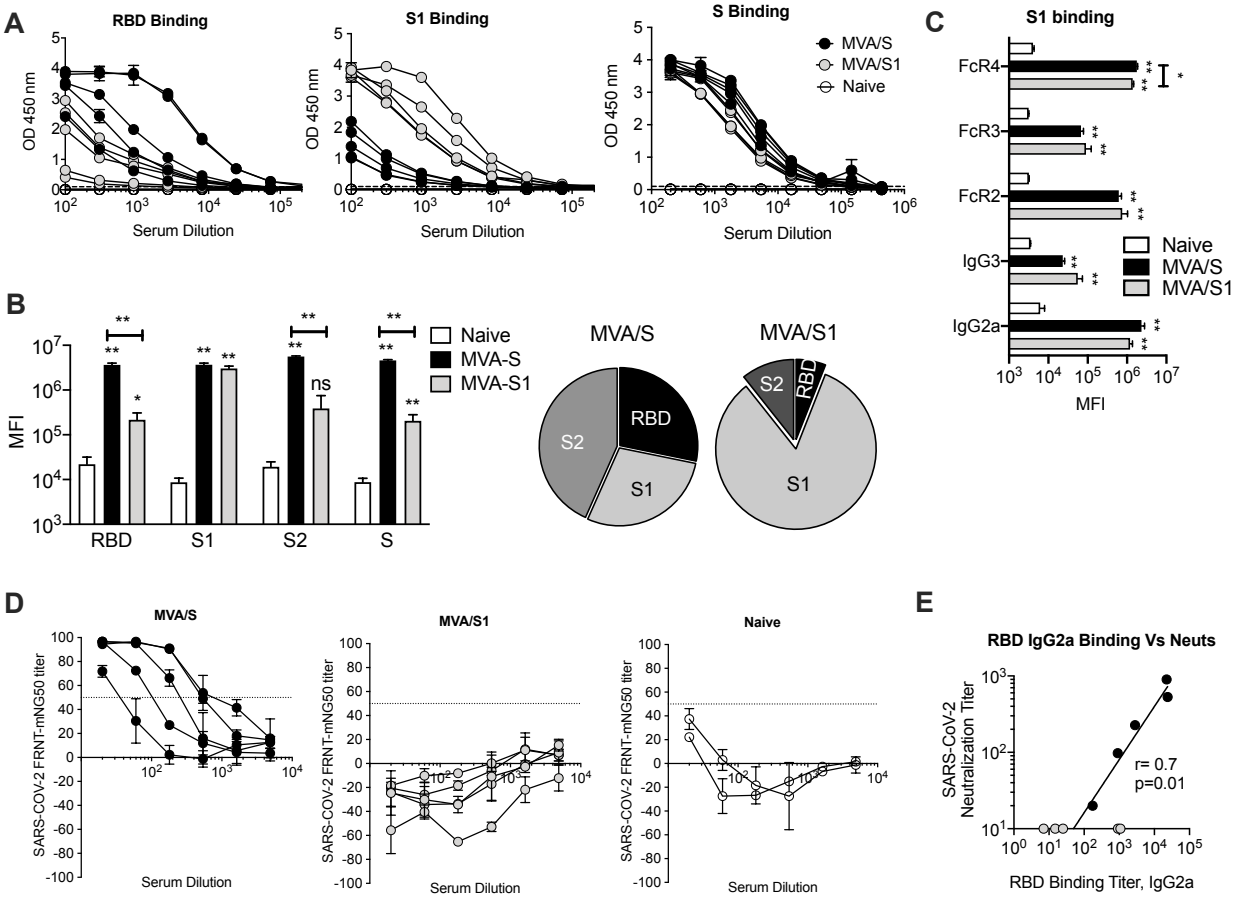


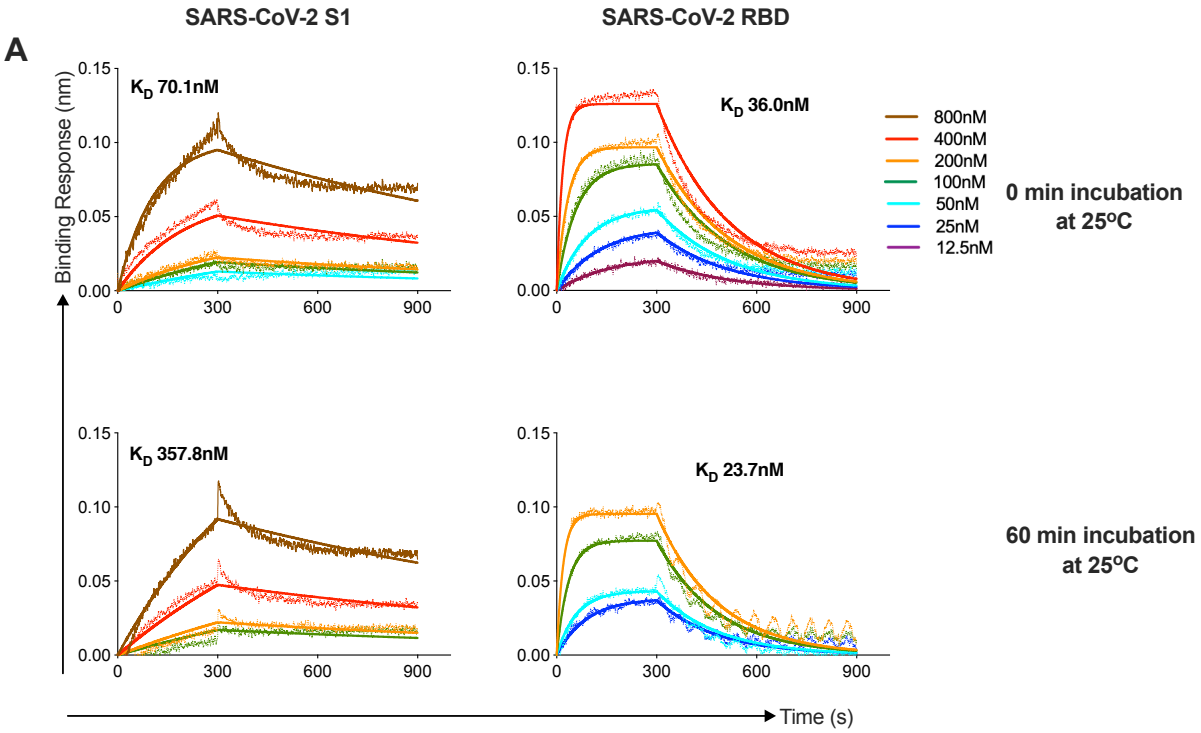
Figure S1. Related to Figure 2. Antibody responses induced by MVA/S or MVA/S1 in mice.

(A-E) Six-week-old female BALB/c mice (n=5 per group) were immunized via intramuscular route with MVA/S or MVA/S1 on weeks 0 and 3. The mouse immunization study was repeated twice and representative data are shown.

(A) Binding IgG antibody response serial dilution for individual proteins measured against SARS-CoV-2 RBD, S1 and S using ELISA at two weeks after boost. The data show responses from individual mice.

(B) Binding antibody response determined against SARS-CoV-2 RBD, S1, S2 and S proteins using Luminex assay at 3 weeks post boost. The pie graphs show the relative proportions of binding to three proteins in each group. Data come from one experiment.

(C) IgG subclass and soluble Fc receptor binding analysis of S1 specific IgG measured using the Luminex assay. Raw values are presented as in mean fluorescence intensity (MFI) in bar graph. Data come from one experiment.
(D) Percent neutralization of SARS-CoV-2 virus expressing GFP. Serum collected from the naïve animals used as negative controls. Each sample analyzed in duplicates and data come from one experiment.
(E) Correlations between SARS-CoV-2 neutralization titer and RBD-binding IgG2a titers ELISA binding titer.
Bars and columns show mean responses in each group ± SEM; Mann-Whitney test: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. Correlation analysis was performed using Spearman rank test. See Figure 2 also for details.



B

		Binding affinity estimated against Fc-huACE2 by BLI		
	SARS-CoV-2	K_D (nM)	k_{on}(1/Ms)	k_{dis}(1/s)
After 0 min incubation at 25°C	S1	70.1	1.1E+04	7.5E-04
	RBD	36.0	1.3E+05	4.6E-03
After 60 min incubation at 25°C	S1	357.8	1.8E+03	6.5E-04
	RBD	23.7	2.3E+05	5.5E-03

Figure S2. Related to Figure 1. Analyzing SARS-CoV-2 RBD and S1 proteins affinities to human ACE2 (hACE2) proteins using biolayer interferometry (BLI).

(A) Bio-Layer Interferometry sensograms of the binding of SARS-CoV-2 S1 and RBD proteins to immobilized Fc-human ACE2, after incubation of the analytes at 25°C for 0and 60 minutes. The traces represent BLI response curves for SARS-CoV-2 proteins serially diluted from 800nM to 12.5nM, as indicated.

Dotted lines show raw response values, while bold solid lines show the fitted trace. Association and dissociation phases were monitored for 300s and 600s, respectively. The data was globally fit using a 1:1 binding model to estimate binding affinity.

(B) Binding affinity specifications of S1 and RBD proteins against hu-ACE2. This experiment was performed twice and representative data are reported.

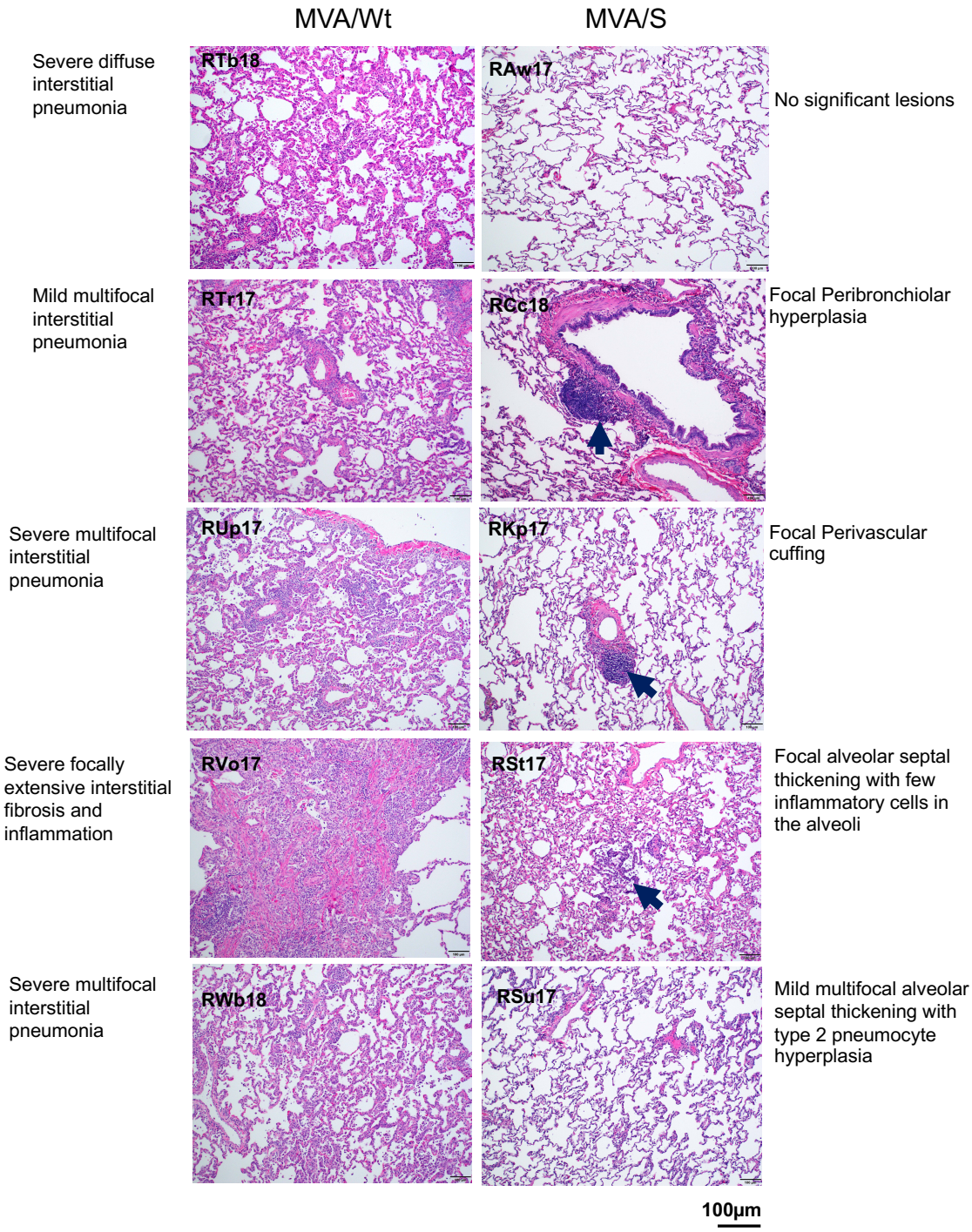


Figure S3. Related to Figure 5. Post-challenge lung pathology of MVA/S vaccinated and MVA/Wt immunized rhesus macaque. Hematoxylin and eosin (H&E) staining of lung sections (10X magnifications) of individual control (MVA/Wt) (n=5) (left) and MVA/S (n=5) (right) rhesus macaques after SARS-CoV-2 challenge at euthanizations (Day 10 post-infection).

Arrows indicate pathological observations such as interstitial pneumonia, type 2 pneumocytes hyperplasia, alveolar septal thickening, syncytia formation, neutrophils and macrophages infiltrations. See also Figure 5 and Table S1 for details.

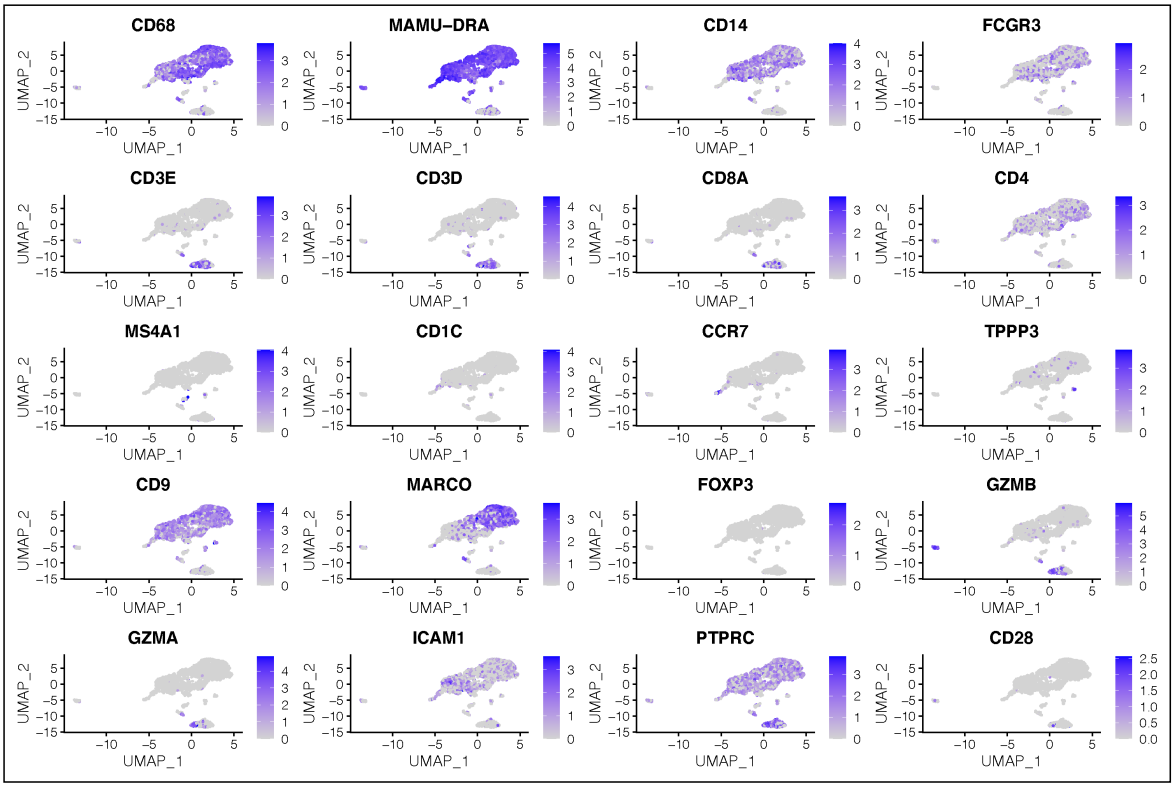


Figure S4. Related to Figure 7. Expression of canonical markers in clusters. Cells were classified into subtypes using SingleR (Aran et al., 2019), and confirmed by expression of canonical markers as described in the Methods. See also Figure 7 for details.

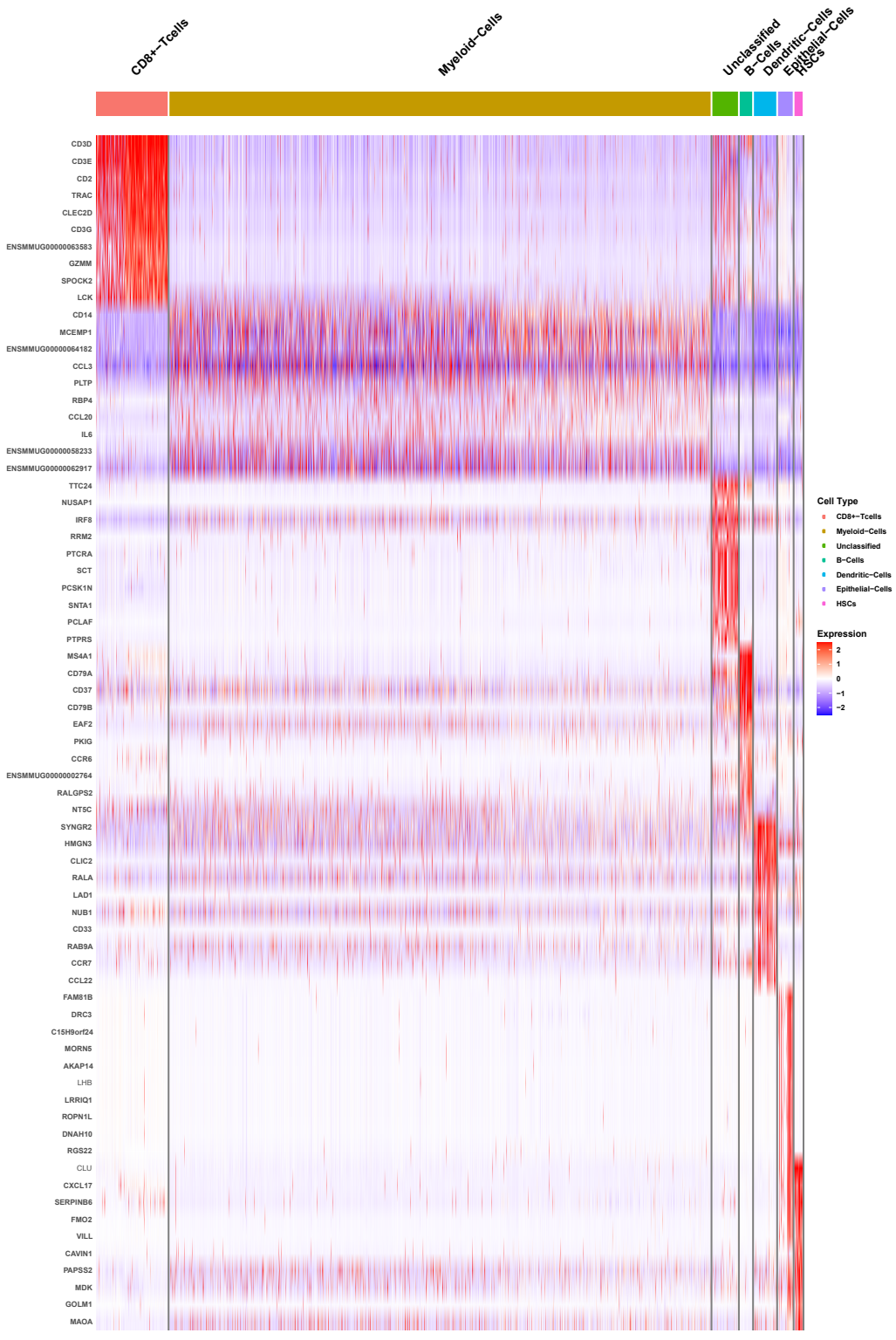

CD8+−Tcells
Myeloid−Cells
Unclassified
B−Cells
Dendritic−Cells
Epithelial−Cells
HSCs
CD3D
CD3E
CD2
TRAC
CLEC2D
CD3G
ENSMMUG00000063583
GZMM
SPOCK2
LCK
CD14
MCEMP1
ENSMMUG00000064182
CCL3
PLTP
RBP4
CCL20
IL6
ENSMMUG00000058233
ENSMMUG00000062917
TTC24
NUSAP1
IRF8
RRM2
PTCRA
SCT
PCSK1N
SNTA1
PCLAF
PTPRS
MS4A1
CD79A
CD37
CD79B
EAF2
PKIG
CCR6
ENSMMUG00000002764
RALGPS2
NT5C
SYNGR2
HMGN3
CLIC2
RALA
LAD1
NUB1
CD33
RAB9A
CCR7
CCL22
FAM81B
DRC3
C15H9orf24
MORN5
AKAP14
LHB
LRRIQ1
ROPN1L
DNAH10
RGS22
CLU
CXCL17
SERPINB6
FMO2
VILL
CAVIN1
PAPSS2
MDK
GOLM1
MAOA
Cell Type
CD8+−Tcells
Myeloid−Cells
Unclassified
B−Cells
Dendritic−Cells
Epithelial−Cells
HSCs
Expression
2
1
0
−1
−2

Figure S5. Related to Figure 7. Discriminant gene expression in sc-RNA-Seq defined cellular subsets in BAL. Cells clustering was performed using the based on PC scores using the Louvain method. The UMAP method (McInnes et al., 2018) was used for visualization of single cells in 2d embedding. See also Figure 7 for details.

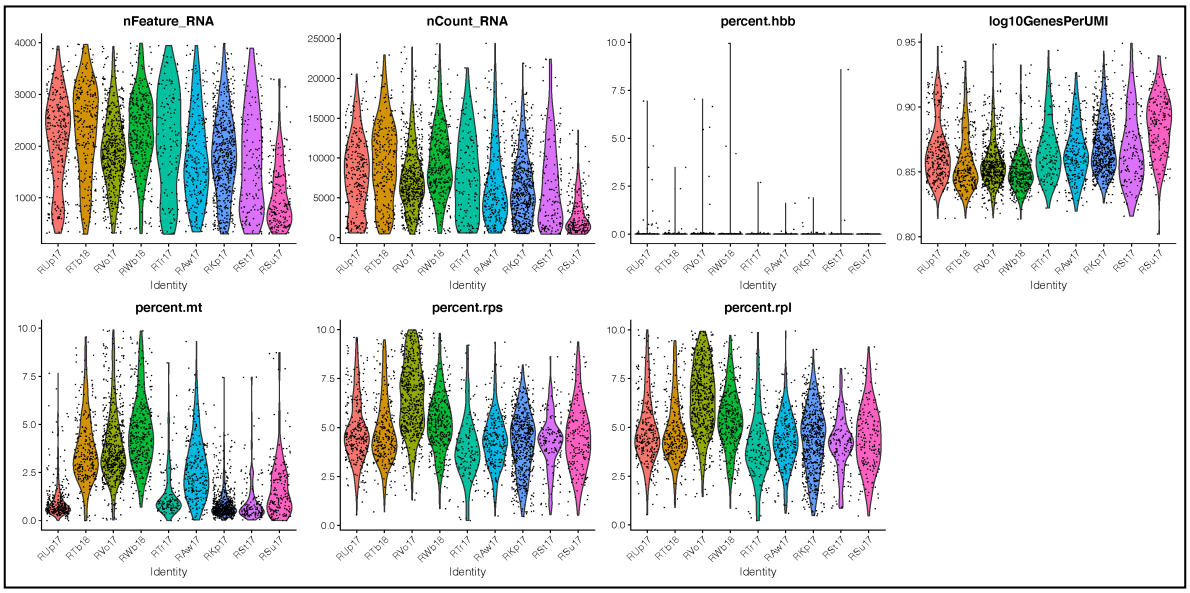


Figure S6. Related to Figure 7. **Quality control analysis of 10X single-cell RNA-Seq data from BALs of RMs challenged with SARS-CoV-2.** Count matrices for were processed with an inhouse single-cell RNA-seq pipeline that uses Seurat v3.0 (Butler et al., 2018; Stuart et al., 2019). Individual cells expressing nFeature _RNA < 300 and >10% mitochondria genes, HBB, RPS or RPL genes were filtered along with doublets. See Figure 7 for details.

	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**
MVA-S (Vaccine)	RAw17	0	0	0	0	0	0	**0**
	RCc18	0	1	0	2	1	0	**4**
	RKp17	0	1	0	2	2	0	**5**
	RSt17	1	1	0	1	1	0	**4**
	RSu17	2	2	0	2	2	0	**8**

Table S1. Related to Figure 5. Lung pathology scores of MVA/S vaccinated and MVA/Wt immunized animals. The scoring criteria used to assess the lung pathology are listed in each column. Mild – less than 6; Moderate – less than 12; Severe – less than 18. See also Figure 5 and S3 for details.