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Supplementary Materials for

Age-dependent acquisition of pathogenicity by SARS-CoV-2 Omicron BA.5

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Supplemental Figure 1

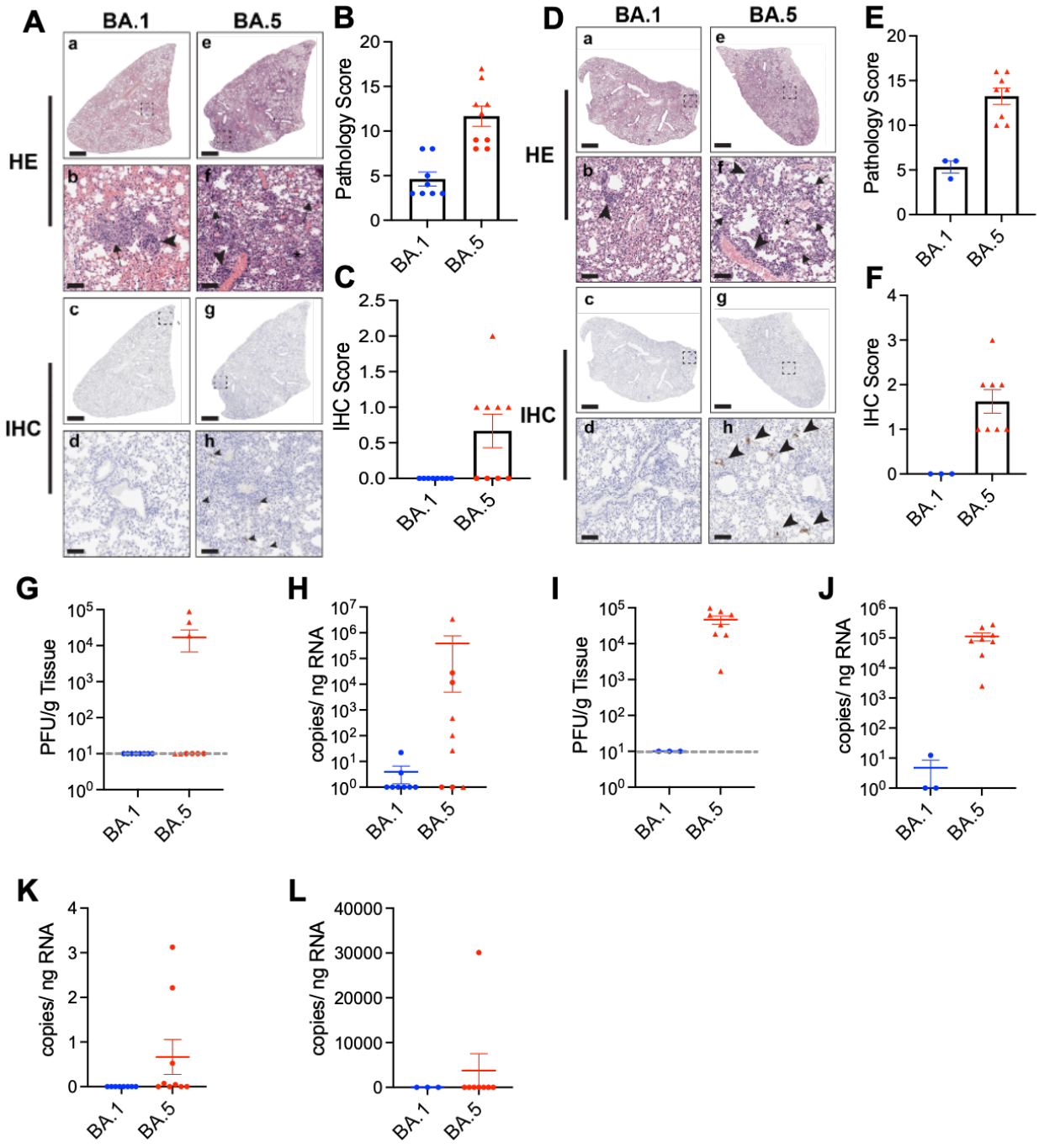


Figure S1: SARS-CoV-2 Omicron BA.5 induces increased virus titers and pathology following infection. A-F. K18-hACE2 mice were infected with 3.25x10^4 PFU SARS-CoV-2 Omicron BA.1and BA.5. Lungs were collected for H&E and IHC. **A.** Representative H&E staining (a,b,e,f)) of lung histopathology or IHC for SARS-CoV-2 N protein (c,d,g,h) (one section analyzed per mouse) in Omicron BA.1 infected 3 month old mice at 14 dpi (a-d) or Omicron BA.5 (e-h) (BA.1 *n* = 8, BA.5 *n* = 9). Mice infected with Omicron BA.5 had more significant lesions with increased perivascular infiltrates (arrowheads), interstitial infiltrates (asterisk), inflammatory cells in alveoli (arrow), and mild alveolar edema compared to mice infected with Omicron BA.1. Scale bar a,c,e,g = 1 mm ; b,d,f,h = 100 µm. For histopathology and IHC analyses, representative images were selected based on the mean pathological score. **B.** Histopathology scores at death or endpoint. Triangle represents mice euthanized before 14 dpi and circle represent mice euthanized 14 dpi (BA.1 *n* = 8, BA.5 *n* = 9). **C.** IHC score at death or endpoint. Triangles represent mice euthanized

before 14 dpi and circles represent mice euthanized 14 dpi (BA.1 n = 8, BA.5 n = 9). **D.** Representative H&E staining (a,b,e,f) of lung histopathology or IHC staining with an anti-SARS-CoV-2 N protein antibody (c,d,g,h) (one section analyzed per mouse) at 14 dpi in 5-8 month old mice infected with Omicron BA.1 (a-d) or Omicron BA.5 (e-h) (BA.1 n = 3, BA.5 n = 8). Mice infected with Omicron BA.5 had more significant lesions with increased perivascular infiltrates (arrowheads), interstitial infiltrates (arrows), edema in alveoli (asterisk), and inflammatory cells in alveoli compared to mice infected with Omicron BA.1. Scale bar a,c,e,g = 1 mm ; b,d,f,h = 100 μm. For histopathology and IHC analyses, representative images were selected based on the mean pathological score. **E.** Histopathological score at death or endpoint. Triangle represents mice euthanized before 14 dpi and circle represent mice euthanized 14 dpi (BA.1 n = 3, BA.5 n = 8). **F.** IHC scores at death or endpoint. Triangles represent mice euthanized before 14 dpi and circles represent mice euthanized 14 dpi (BA.1 n = 3, BA.5 n = 8). Data are means ± S.E.M. **G.** Virus titers in the lungs of 3-month old mice (BA.1 n = 8, BA.5 n = 9). Triangles represent mice euthanized before 14 dpi and circles represent mice euthanized 14 dpi. Data are means ± S.E.M. **H.** Viral RNA in the lungs of 3-month old mice at endpoint (BA.1 n = 8, BA.5 n = 9). Data are means ± S.E.M. **I.** Virus titers in the lungs of 5-8 month old mice (BA.1 n = 3, BA.5 n = 8). Data are means ± S.E.M. **J.** Viral RNA levels in the lung of 5-8 month old mice (BA.1 n = 3, BA.5 n = 8). Data are means ± S.E.M. **K.** Viral RNA in the brains of 3-month old mice at endpoint (BA.1 n = 8, BA.5 n = 9). Data are means ± S.E.M. **L.** Viral RNA in the brains of 5-8 month old mice at endpoint (BA.1 n = 3, BA.5 n = 8). Data are means ± S.E.M.

Supplemental Figure 2

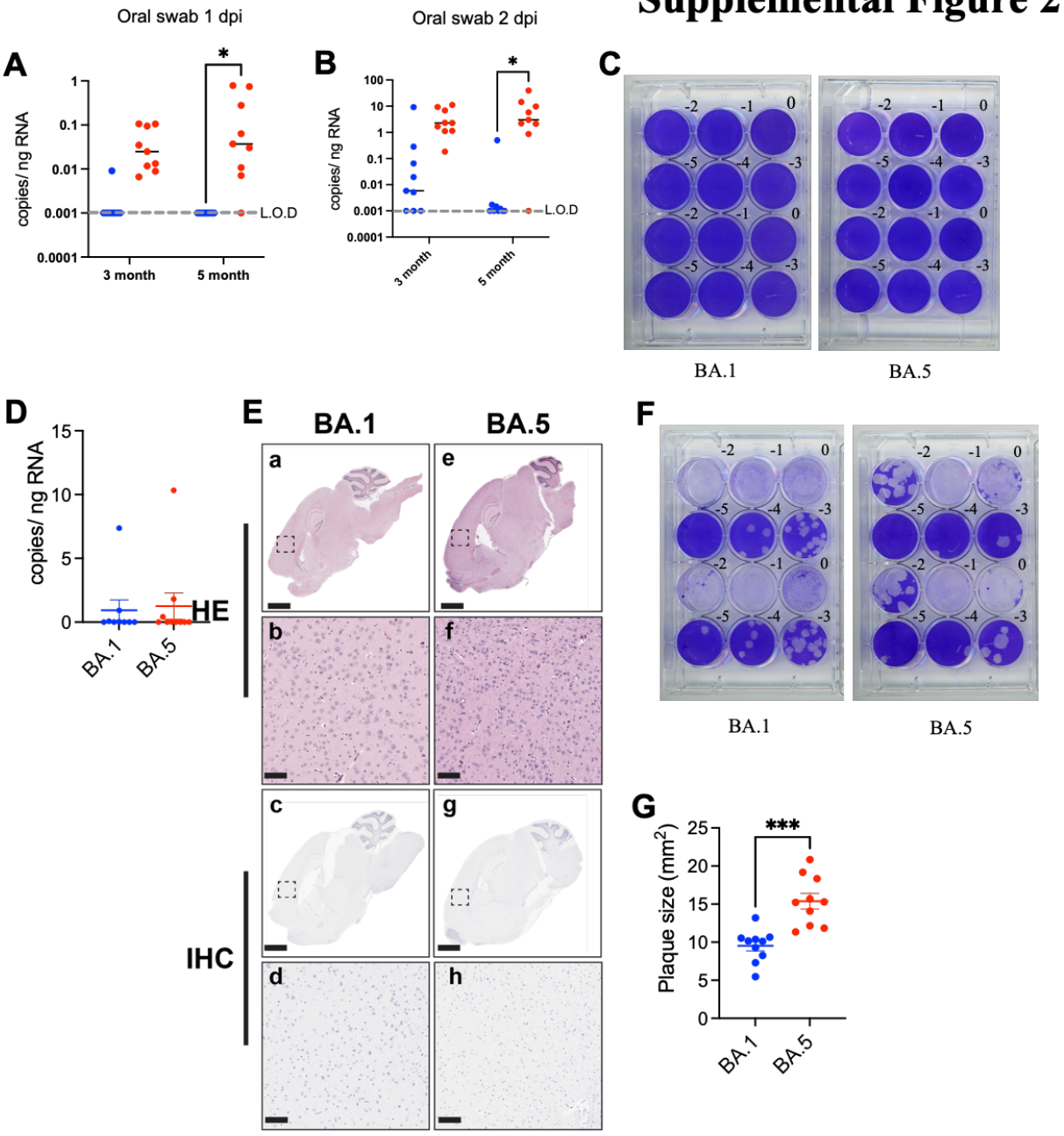


Figure S2: Viral titers 1, 2 and 5 dpi. A. Viral RNA quantification by RT-qPCR in the oral cavity of 3 and 5 month old K18-hACE2 mice infected with Omicron BA.1 and Omicron BA.5 (BA.1 $n = 9$, BA.5 $n = 9$) at 1 dpi, or **B.** 2 dpi. **C.** Brain plaques from 2 dpi. **D.** Brain viral RNA quantification by RT-qPCR from 5 dpi (BA.1 $n = 9$, BA.5 $n = 10$). **E.** Representative H&E staining (a,b,e,f) or IHC staining with an anti-SARS-CoV-2 N protein antibody (c,d,g,h) (one section analyzed per mouse) of brain tissue at death or study endpoint in K18-hACE2 mice infected with Omicron BA.1 (a-d) or Omicron BA.5 (e-h). The brain of all mice infected with either strain is histologically normal with no evidence of immunolabeling. Scale bar a,c,e,g = 2 mm ; b,d,f,h = 100 µm. **F.** Lung plaques, and **G.** Lung plaque area from 5 dpi (BA.1 $n = 10$, BA.5 $n = 10$). One-way ANOVA with Tukey's multiple comparison test.

Supplemental Figure 3

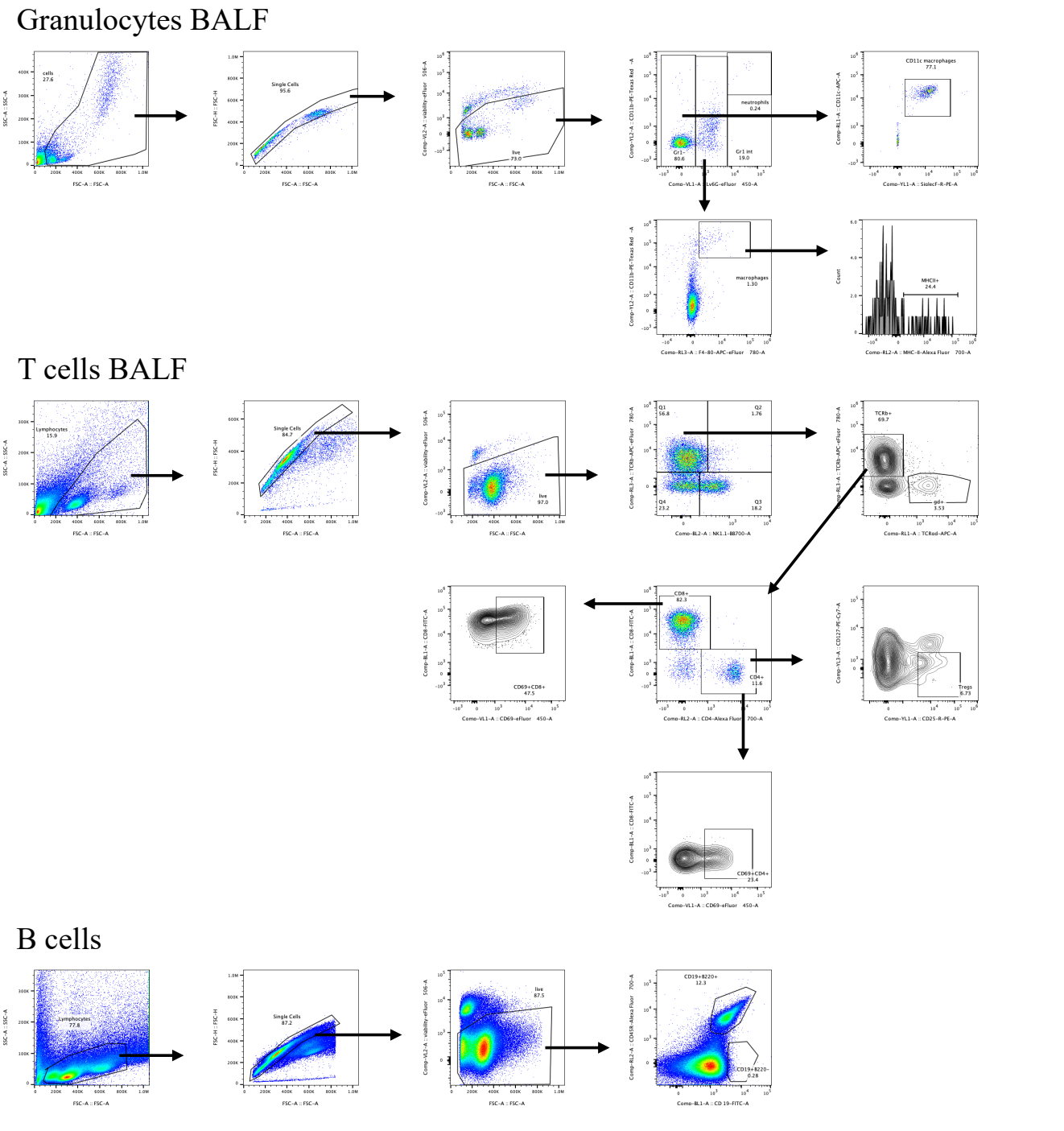


Figure S3: Gating strategy for analysis of lung immune cells. Cells were stained and analyzed via flow cytometry. Representative images are shown to demonstrate the gating strategy for the indicated populations.

Supplemental Figure 4

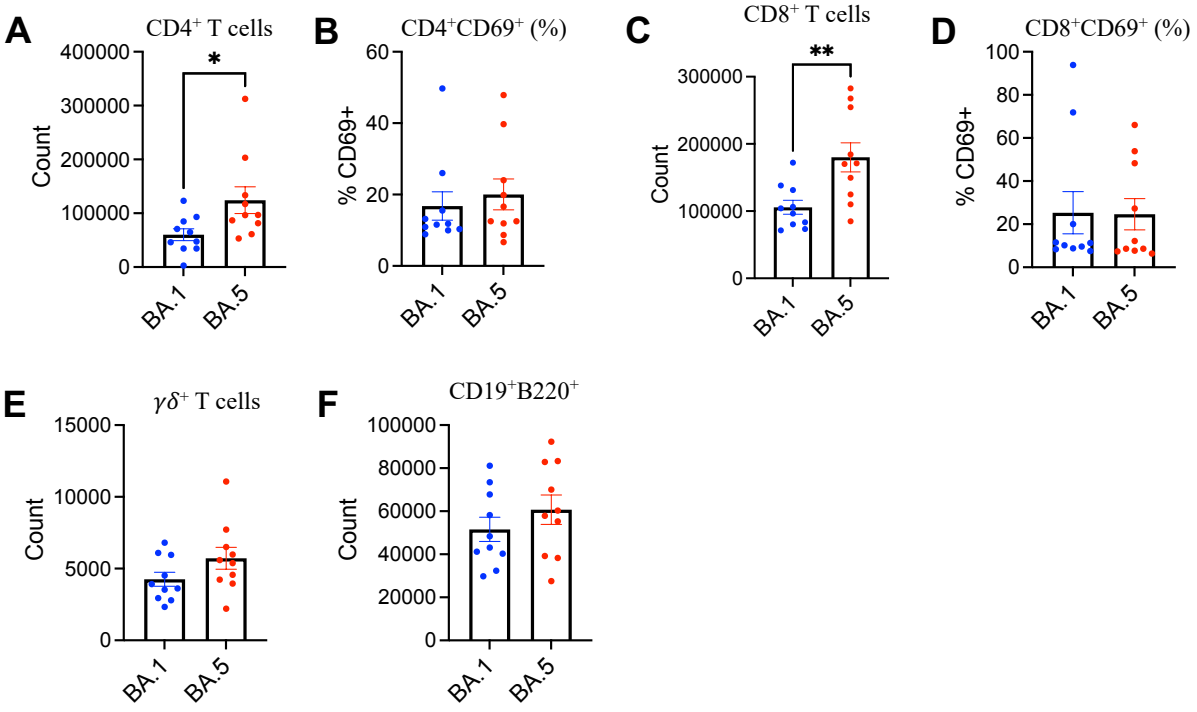
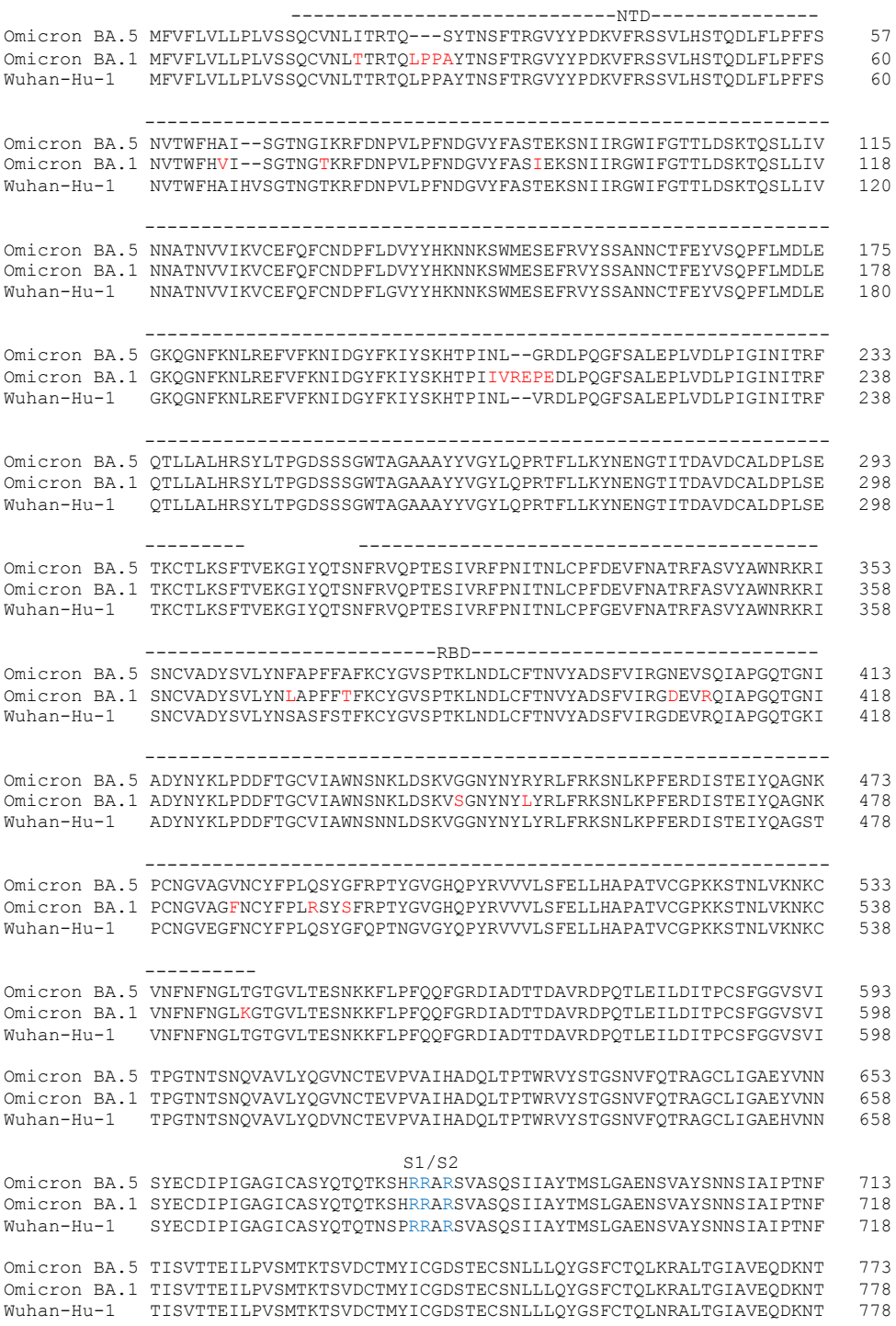


Figure S4: Lymph node immune cell profile following SARS-CoV-2 Omicron infection. K18-hACE2 mice were infected with SARS-CoV-2 Omicron BA.1and BA.5 (BA.1 $n = 10$, BA.5 $n = 10$). At 5 dpi, mediastinal lymph nodes were collected and immune cells were analyzed by flow cytometry. **A.** Total $CD4^+$ cells in the lymph nodes. **B.** Percent activated $CD4^+$ T cells. **C.** $CD8^+$ T cells in the lymph node. **D.** Percent activated $CD8^+$ T cells. **E.** γδ T cells in the lymph node. **F.** Total B cells in the lymph node. All data are means ± S.E.M.

Supplemental Figure 5



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                                                S2' --fusion peptide----
Omicron BA.5 QEVFAQVKQIYKTPPIKYFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYG   833
Omicron BA.1 QEVFAQVKQIYKTPPIKYFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYG   838
Wuhan-Hu-1   QEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYG   838

             -----------------
Omicron BA.5 DCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPF   893
Omicron BA.1 DCLGDIAARDLICAQKFKGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPF   898
Wuhan-Hu-1   DCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPF   898

                       ------------------------------HR1---------------
Omicron BA.5 AMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNHNAQA   953
Omicron BA.1 AMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNHNAQA   958
Wuhan-Hu-1   AMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQA   958

             -------------------------- ----------------------HR2---------
Omicron BA.5 LNTLVKQLSSKFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEI  1013
Omicron BA.1 LNTLVKQLSSKFGAISSVLNDIFSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEI  1018
Wuhan-Hu-1   LNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEI  1018

             ------------------------------------------  -----------TM--
Omicron BA.5 RASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTA  1073
Omicron BA.1 RASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTA  1078
Wuhan-Hu-1   RASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTA  1078

             ------------------------------------------------------------
Omicron BA.5 PAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVY  1133
Omicron BA.1 PAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVY  1138
Wuhan-Hu-1   PAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVY  1138

             ------------------------------------------------------------
Omicron BA.5 DPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLI  1193
Omicron BA.1 DPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLI  1198
Wuhan-Hu-1   DPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLI  1198

             -------------  -------------------------CT------------------
Omicron BA.5 DLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDE  1253
Omicron BA.1 DLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDE  1258
Wuhan-Hu-1   DLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDE  1258

             ---------------
Omicron BA.5 DDSEPVLKGVKLHYT*                                              1269
Omicron BA.1 DDSEPVLKGVKLHYT*                                              1274
Wuhan-Hu-1   DDSEPVLKGVKLHYT*                                              1274
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Figure S5: Sequence alignment of SARS-CoV-2 S proteins comparing SARS-CoV-2 Wuhan to Omicron BA.1 and Omicron BA.5.

Supplemental Table 1

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Cell Markers	**Fluorochrome**	**Manufacturer and Catalog#**
CD8	fitc	invitrogen 11-0081-85
NK1.1	BB700	BD BioSciences 566503
CD25	PE	invitrogen 12-0251-83
KLRG1	pe-TexasRed	BD BioSciences 565393
CD127	PE-Cy7	invitrogen 25-1271-82
TCRgd	APC	BioLegend 118116
CD4	AF700	invitrogen 56-0041-82
TCRb	APC-cy7	BioLegend 109220
CD69	Pacific Blue	BioLegend 104524
CD62L	Pac Orange	BioLegend 104433
CD19	fitc	invitrogen 11-0193-85
IgG2a, IgG2b	BB700	BD Biosciences 745969
CD138	PE	invitrogen MA5-23527
IgM	PE-ef610	invitrogen 61-5790-82
CD23	PE-Cy7	invitrogen 25-0232-82
Fas	APC	invitrogen 17-0951-82
CD45R (B220)	AF700	invitrogen 56-0452-82
CD27	APC-AF780	invitrogen 47-0271-82
GL7	ef450	invitrogen 48-5902-82
IgD	SB600	invitrogen 63-5993-82
c-kit	Fitc	BD BioSciences 553354
CD69	*BB700*	BD BioSciences 566501
Siglec F	PE	BD BioSciences 552126
CD11b	PE-Dazzle594	BioLegend 101256
CD49b	PECy7	BioLegend 103518
CD11c	APC	BioLegend 117310
MHCII	AF700	invitrogen 56-5321-80
F4/80	APC-Cy7	BioLegend 123118
Ly6G	Pacific Blue	BioLegend 127612
viability	ef506	invitrogen 65-0866-14
CD16/32	---	invitrogen 14-0161-85

Flow cytometry antibodies.