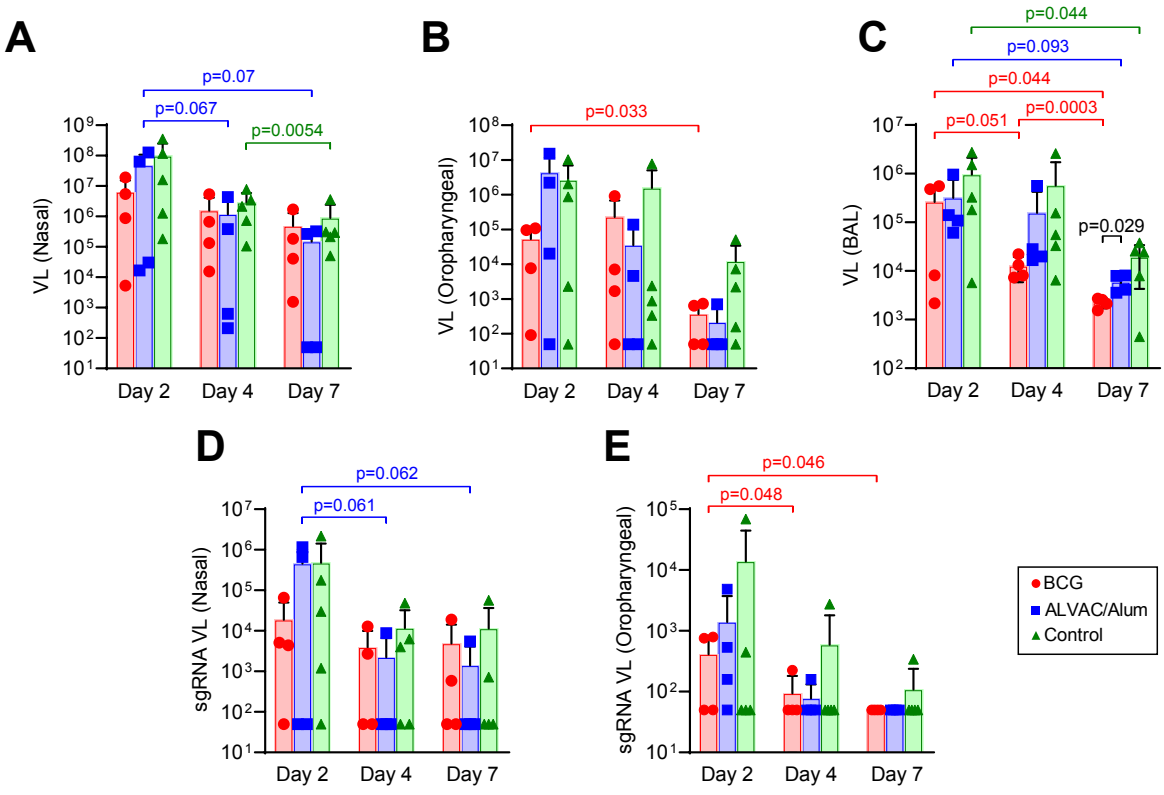
# Supplemental Material

| *JCI Insight* |
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| | |
|---|---|
| **Article Title:** | **BCG immunization mitigates SARS-CoV-2 replication in macaques via monocyte efferocytosis and neutrophil recruitment in lung** |
| **Corresponding Author:** | Mohammad Arif Rahman Ph.D. mohammadarif.rahman@nih.gov<br>Genoveffa Franchini, franchig@mail.nih.gov |

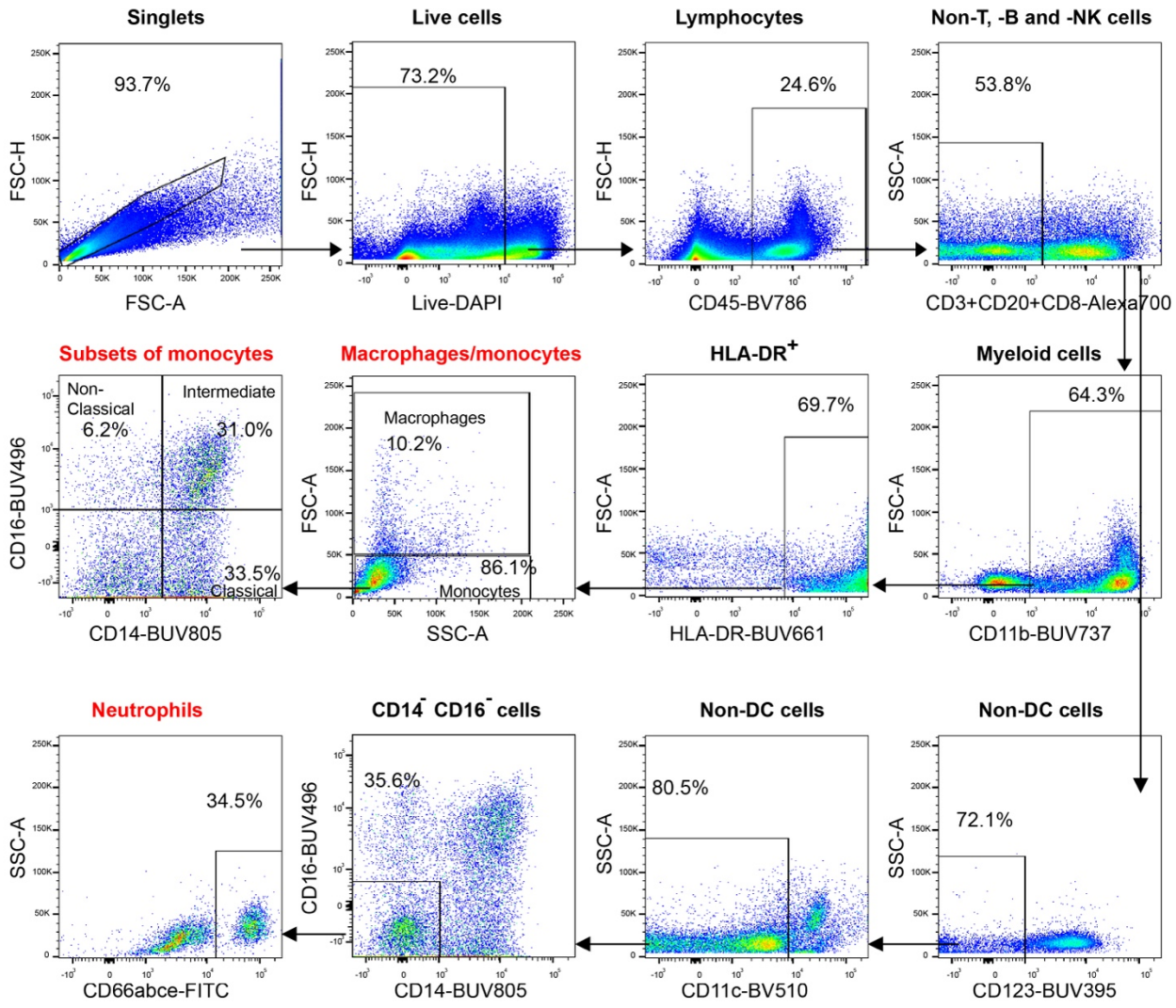
| Supplemental Item & Number | Title or Caption |
|---|---|
| Supplemental Figure 1 | Input virus viral load (VL) and replicating sgRNA VL in different respiratory tract compartments |
| Supplemental Figure 2 | Representative flow cytometry gating strategy of neutrophils and macrophages/monocytes |
| Supplemental Figure 3 | Association between replicating viral load (VL) in bronchoalveolar lavage (BAL) at day 7 and vaccine-induced immune parameters |
| Supplemental Figure 4 | Correlations between immune parameters, replicating viral load at day 7 in bronchoalveolar lavage (BAL), and efferocytosis in peripheral blood |
| Supplemental Figure 5 | Association of immune parameters with replicating viral load in bronchoalveolar lavage (BAL) at Day 7 |
| Supplemental Figure 6 | T cell gating strategy |
| Supplemental Figure 7 | Binding antibody responses against spike and receptor-binding domain (RBD) protein |
| Supplemental Figure 8 | Summary of immune responses associated with reduced replicating SARS-CoV-2 in bronchoalveolar lavage (BAL) |
| Supplemental Figure 9 | Summary of immune responses associated with increased replicating SARS-CoV-2 in bronchoalveolar lavage (BAL) |
| Supplemental Table 1 | Plasma neutralizing antibodies to SARS-CoV-2 |



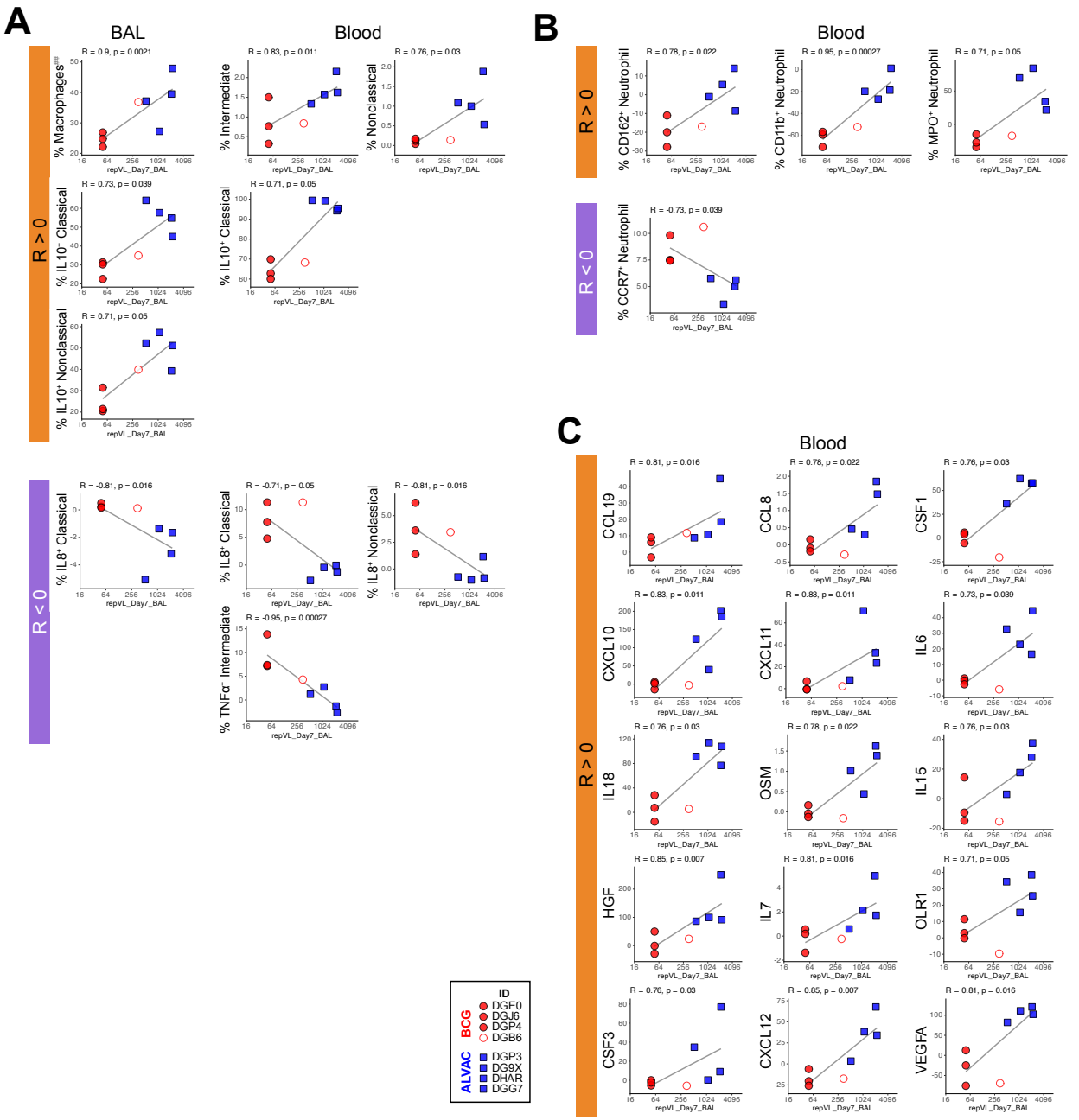

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| Group or Timepoint | Comparison | VL | | | | | | repVL | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nasal | | Oropharengeal | | BAL | | Nasal | | Oropharengeal | | BAL | |
| | | p_raw | p_adj | p_raw | p_adj | p_raw | p_adj | p_raw | p_adj | p_raw | p_adj | p_raw | p_adj |
| BCG | Day4vsDay2 | 0.24 | 0.24 | 0.34 | 0.34 | 0.051 | 0.051 | 0.31 | 0.53 | 0.048 | 0.072 | 0.038 | 0.053 |
| BCG | Day7vsDay2 | 0.13 | 0.24 | 0.033 | 0.099 | 0.044 | 0.051 | 0.37 | 0.53 | 0.046 | 0.072 | 0.039 | 0.053 |
| BCG | Day7vsDay4 | 0.16 | 0.24 | 0.24 | 0.34 | 0.00025 | 0.00075 | 0.53 | 0.53 | 0.25 | 0.25 | 0.053 | 0.053 |
| ALVAC | Day4vsDay2 | 0.067 | 0.105 | 0.17 | 0.23 | 0.52 | 0.52 | 0.061 | 0.093 | 0.18 | 0.25 | 0.15 | 0.15 |
| ALVAC | Day7vsDay2 | 0.07 | 0.105 | 0.17 | 0.23 | 0.093 | 0.279 | 0.062 | 0.093 | 0.18 | 0.25 | 0.13 | 0.15 |
| ALVAC | Day7vsDay4 | 0.23 | 0.23 | 0.23 | 0.23 | 0.2 | 0.3 | 0.25 | 0.25 | 0.25 | 0.25 | 0.15 | 0.15 |
| Control | Day4vsDay2 | 0.12 | 0.12 | 0.68 | 0.68 | 0.48 | 0.48 | 0.22 | 0.345 | 0.26 | 0.33 | 0.18 | 0.25 |
| Control | Day7vsDay2 | 0.11 | 0.12 | 0.13 | 0.39 | 0.044 | 0.132 | 0.23 | 0.345 | 0.26 | 0.33 | 0.11 | 0.25 |
| Control | Day7vsDay4 | 0.0054 | 0.0162 | 0.27 | 0.405 | 0.23 | 0.345 | 0.99 | 0.99 | 0.33 | 0.33 | 0.25 | 0.25 |
| Day2 | BCGvsALVAC | 0.69 | 0.69 | 0.69 | 0.81 | 0.69 | 0.69 | 0.88 | 0.9 | 0.88 | 0.89 | 0.69 | 0.73 |
| Day4 | BCGvsALVAC | 0.49 | 0.49 | 0.66 | 0.9 | 0.11 | 0.285 | 0.62 | 0.7 | 1 | 1 | 0.46 | 0.69 |
| Day7 | BCGvsALVAC | 0.66 | 0.66 | 0.62 | 0.62 | 0.029 | 0.087 | 0.62 | 1 | n/v | n/v | 0.026 | 0.078 |
| Day2 | BCGvsControl | 0.41 | 0.69 | 0.56 | 0.81 | 0.56 | 0.69 | 0.62 | 0.9 | 0.89 | 0.89 | 0.56 | 0.73 |
| Day4 | BCGvsControl | 0.41 | 0.49 | 0.81 | 0.9 | 0.19 | 0.285 | 0.7 | 0.7 | 1 | 1 | 0.26 | 0.69 |
| Day7 | BCGvsControl | 0.29 | 0.585 | 0.26 | 0.39 | 0.19 | 0.285 | 1 | 1 | 0.5 | 0.5 | 0.073 | 0.109 |
| Day2 | ALVACvsControl | 0.56 | 0.69 | 0.81 | 0.81 | 0.41 | 0.69 | 0.9 | 0.9 | 0.61 | 0.89 | 0.73 | 0.73 |
| Day4 | ALVACvsControl | 0.29 | 0.49 | 0.9 | 0.9 | 0.56 | 0.56 | 0.5 | 0.7 | 1 | 1 | 0.81 | 0.81 |
| Day7 | ALVACvsControl | 0.39 | 0.585 | 0.12 | 0.36 | 0.29 | 0.29 | 0.77 | 1 | 0.5 | 0.5 | 0.41 | 0.41 |

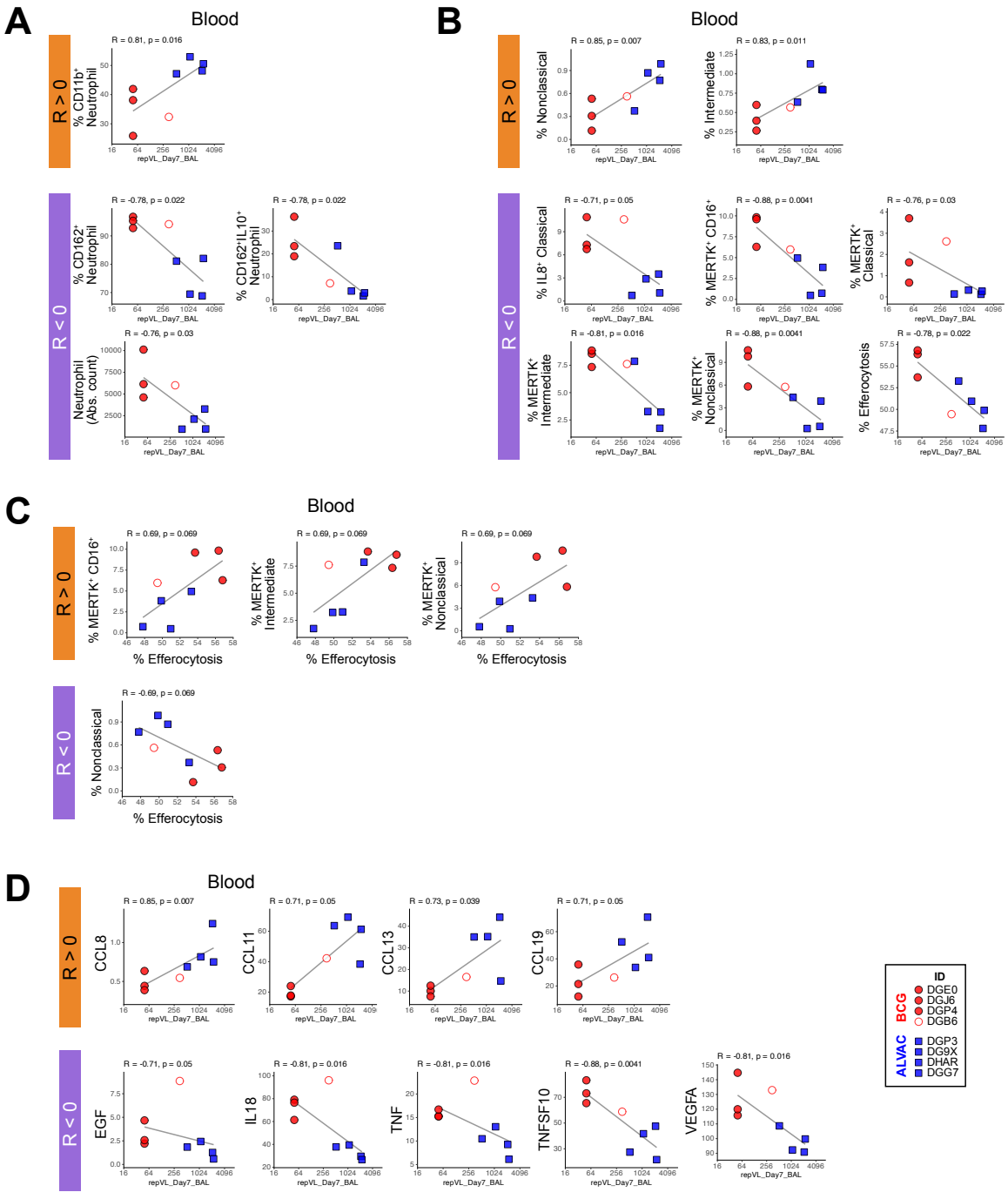
**Supplemental Figure 1. Input virus viral load (VL) and replicating sgRNA VL in different respiratory tract compartments.** (**A, B, C**) VL was determined in (**A**) nasal swab, (**B**) oropharyngeal swab, and (**C**) bronchoalveolar lavage (BAL) post SARS-CoV-2 viral challenge. sgRNA replicating VL was determined in (**D**) nasal swab and (**E**) oropharyngeal swab post SARS-CoV-2 viral challenge. (**F**) summary p-value table with raw and adjusted p values for each category/comparison/analyte. p-values that are labeled in the plots are also colored in this table and calculated by fitting generalized estimating equations (between timepoints for each group separately) or two-tailed Mann-Whitney test (between groups at each timepoint). Barplots denote mean and error bars are SD.



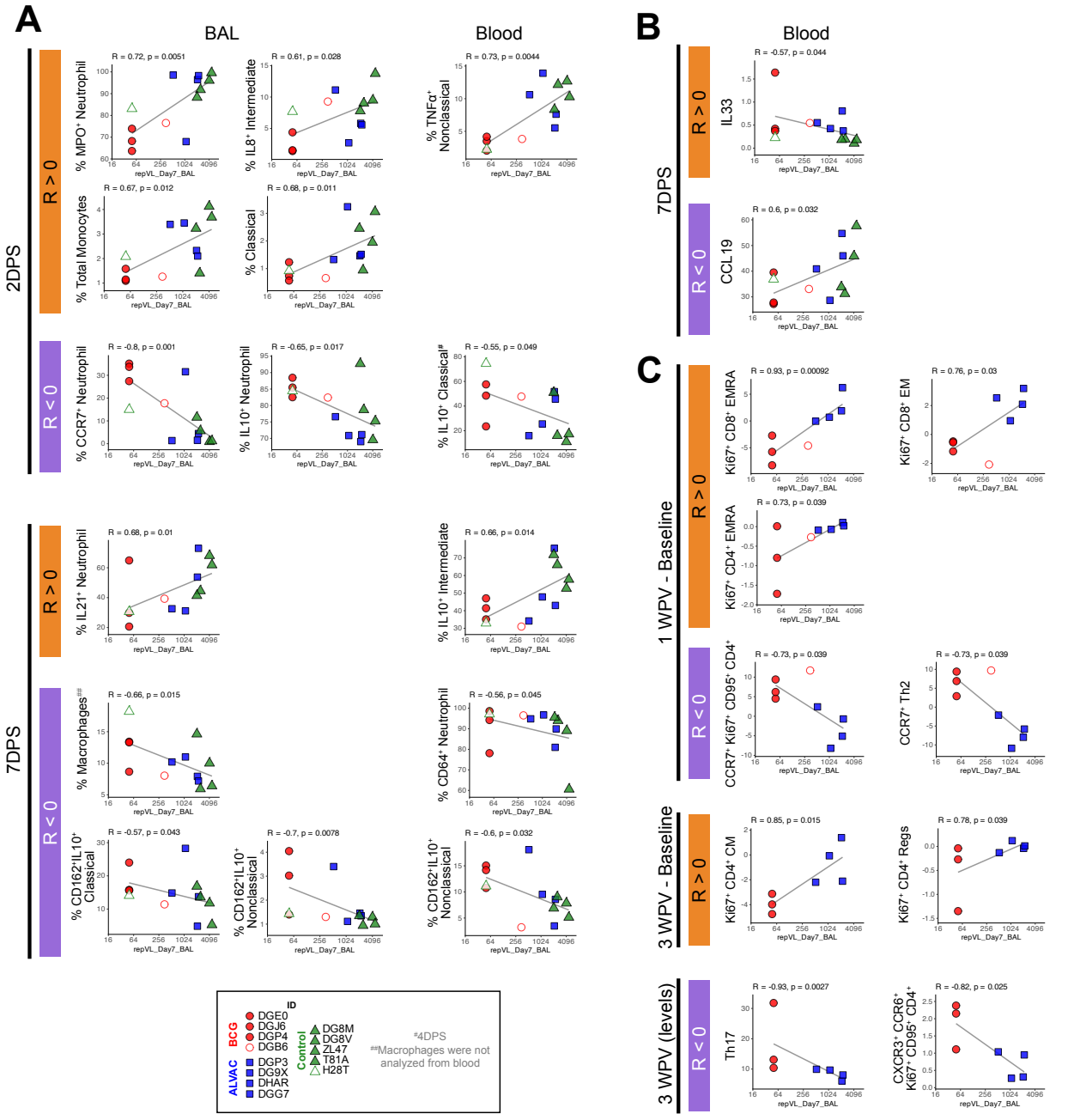

**Supplemental Figure 2. Representative Flow Cytometry Gating Strategy for Identification of Neutrophils and Macrophages/Monocytes.** The figure illustrates the sequential gating strategy employed to identify neutrophils and macrophages/monocytes in peripheral blood and bronchoalveolar lavage (BAL) samples.



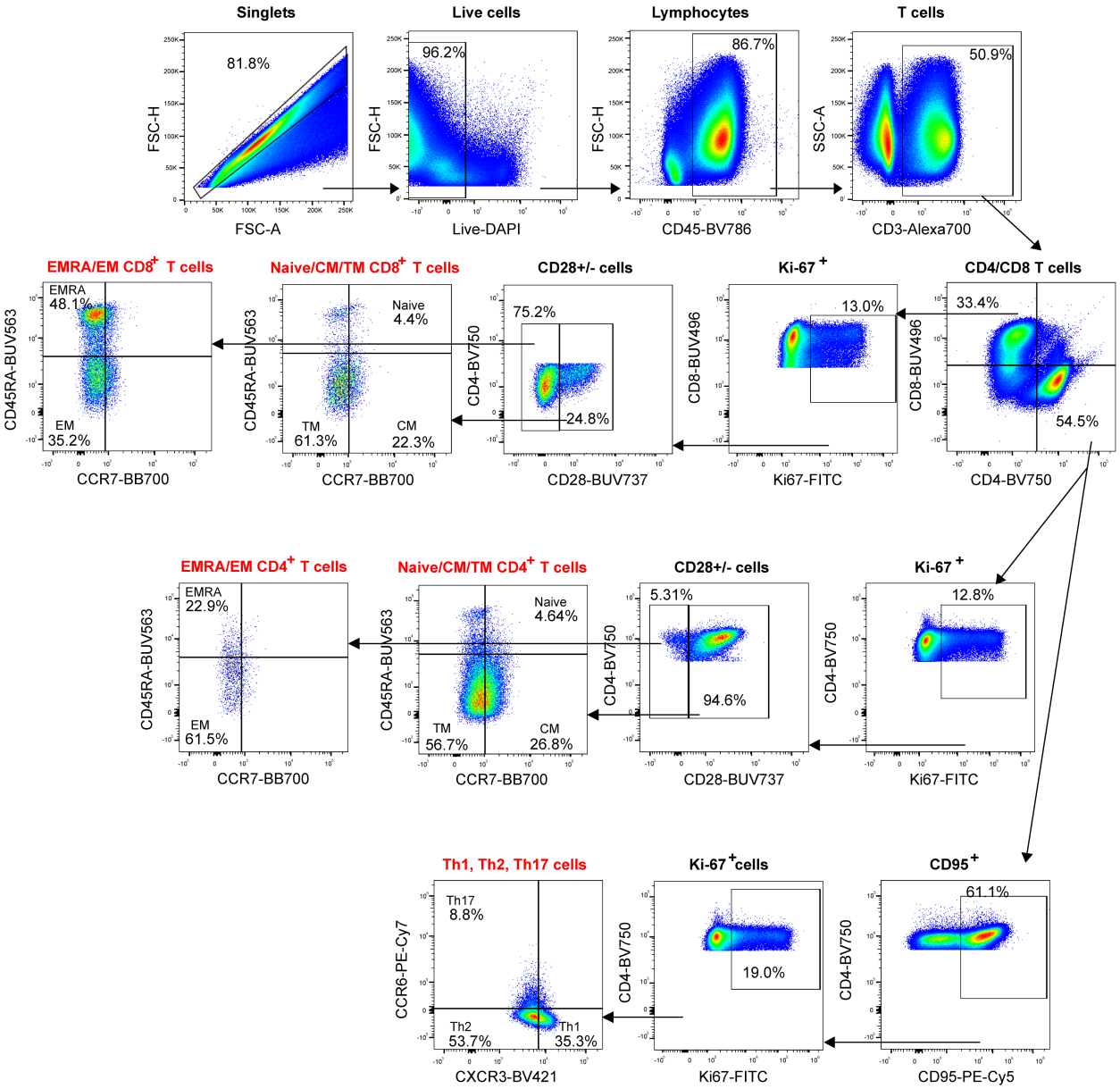

**Supplemental Figure 3. Association between replicating viral load (VL) in bronchoalveolar lavage (BAL) at day 7 and vaccine-induced immune parameters. (A–C)** Correlation analyses were performed between replicating VL in BAL at day 7 post-challenge and vaccine-induced immune responses, calculated as the change from pre-vaccination baseline to 2 days post-vaccination for each parameter. Data points represent individual animals; corresponding Spearman correlation coefficients (R) and two-tailed p-values are indicated in each panel. Only analytes with two-tailed Spearman $p < 0.05$ are shown. Hollow circle designates BCG-vaccinated animal that did not control replicating VL.



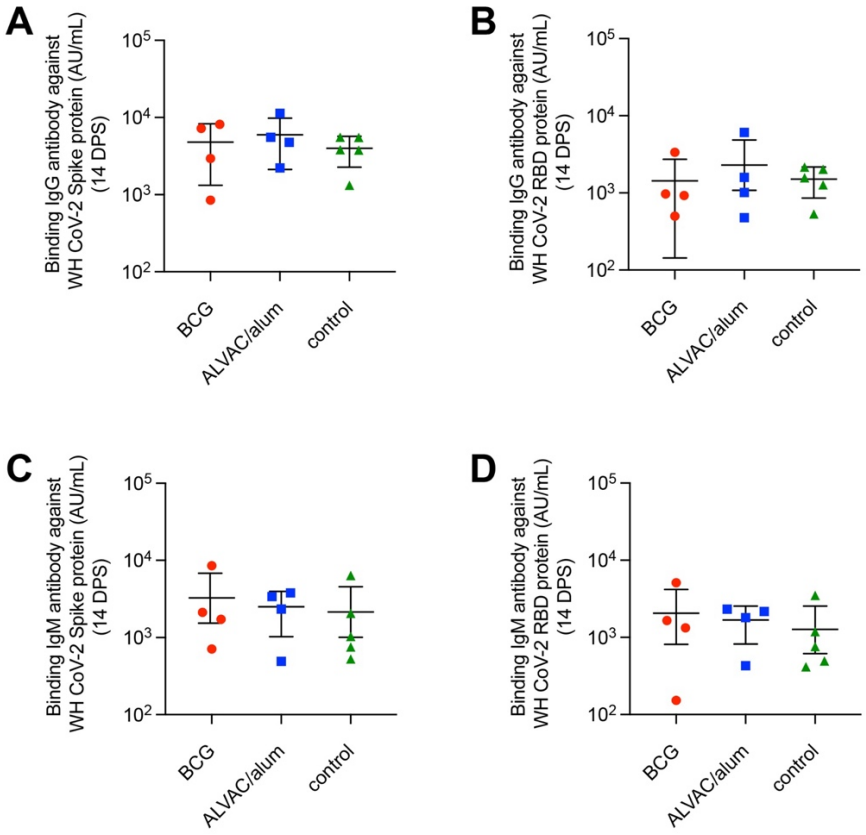

**Supplemental Figure 4. Correlations between immune parameters, replicating viral load at day 7 in bronchoalveolar lavage (BAL), and efferocytosis in peripheral blood. (A, B, D)** Correlation of selected immune parameters with replicating viral load (VL) in BAL at day 7 post-infection. **(C)** Correlation of immune parameters with levels of efferocytosis in blood at pre-SARS-CoV-2 infection (Pre-S). Data points represent individual animals; corresponding Spearman correlation coefficients (R) and two-tailed p-values are indicated in each panel. Only analytes with two-tailed Spearman $p < 0.05$ are shown. Hollow circle designates BCG-vaccinated animal that did not control replicating VL.



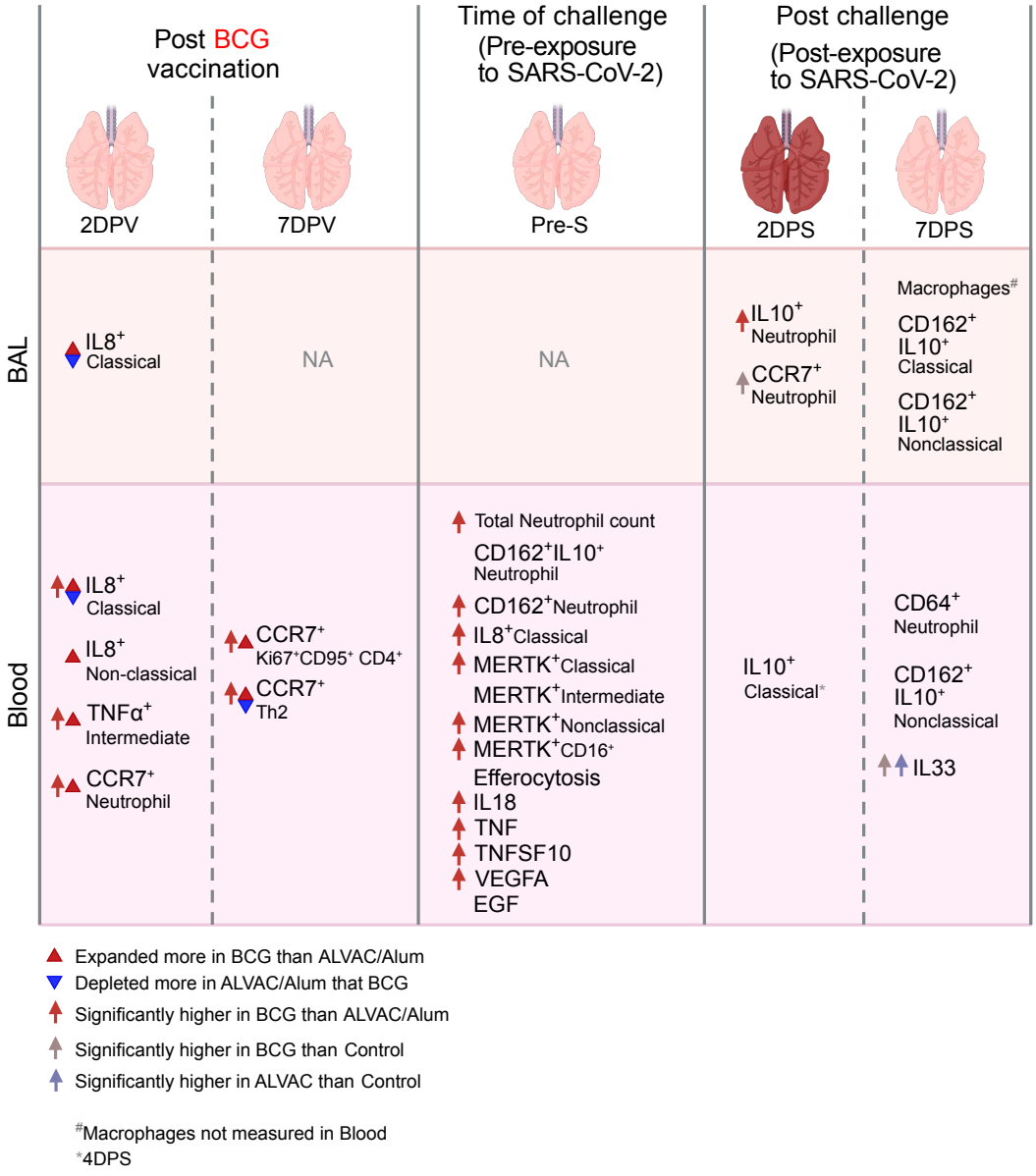

**Supplemental Figure 5. Association of Immune Parameters with Replicating Viral Load (VL) in bronchoalveolar lavage (BAL) at Day 7. (A–C)** Association between selected immune parameters and replicating VL in BAL samples at day 7 post-infection. Data points represent individual animals; corresponding Spearman correlation coefficients (R) and two-tailed p-values are indicated in each panel. Only analytes with two-tailed Spearman $p < 0.05$ are shown. Hollow circle designates BCG-vaccinated animal that did not control replicating VL, hollow triangle highlights the nonvaccinated animal that controlled replicating VL.



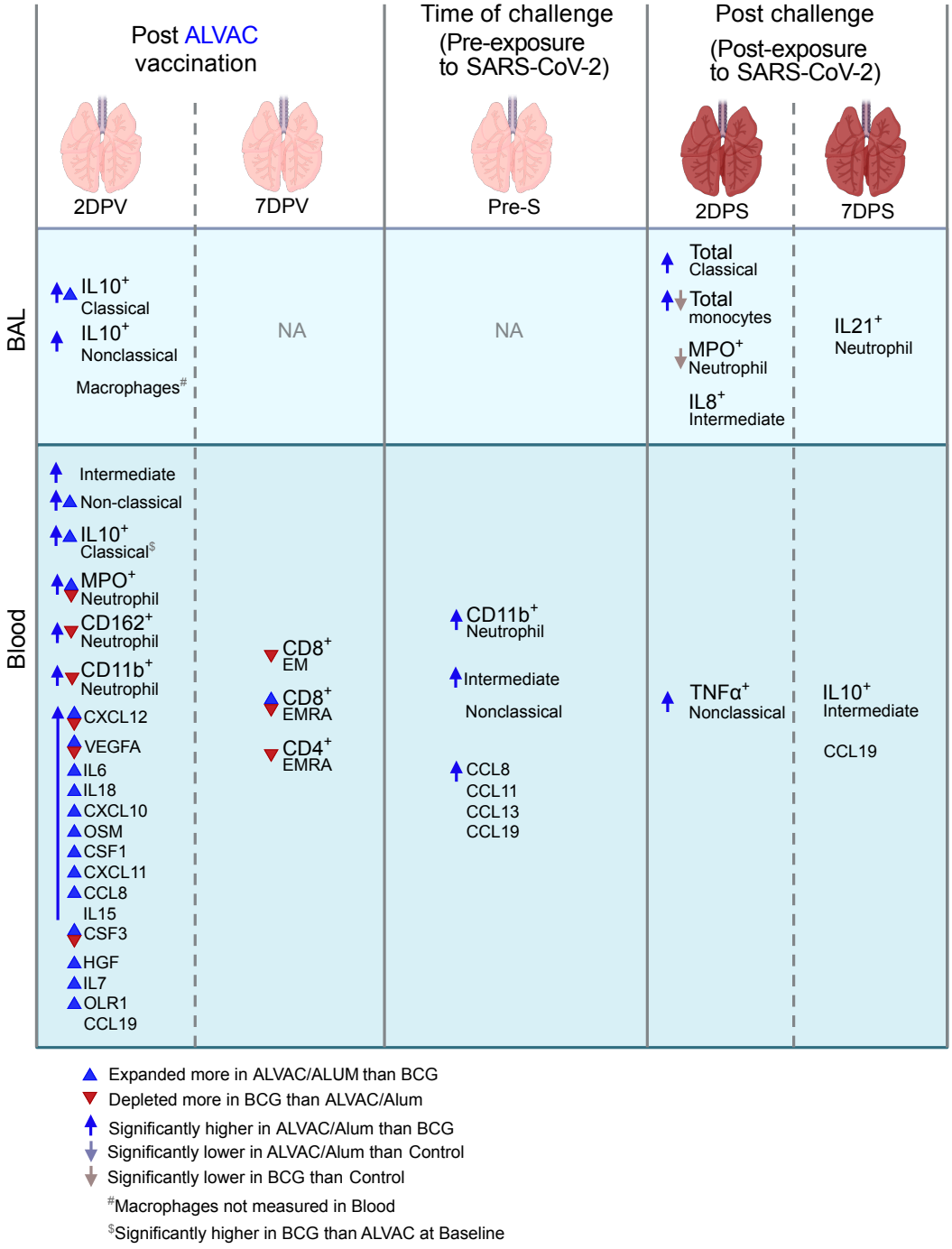

**Supplemental Figure 6. T Cell Gating Strategy in blood.** Representative flow cytometry gating strategy used to identify $CD4^+$/ $CD8^+$ T cell subsets of naïve T cells, central memory (CM), effector memory (EM), transitional memory (TM), $RA^+$ effector memory (EMRA), as well as Th1, Th2, Th17 cells.




**Supplemental Figure 7. Binding antibody responses against spike and receptor-binding domain (RBD) protein in plasma.** Comparison of (**A**) IgG binding against spike, (**B**) IgG binding against RBD, (**C**) IgM binding against spike, and (**D**) IgM binding against RBD among different groups of animals at 14 days post SARS-CoV-2. Data shown in (**A-D**) were analyzed with two-tailed Mann-Whitney U test. Horizontal and vertical bars denote mean and SD.




**Supplemental Figure 8. Summary of immune responses associated with reduced replicating SARS-CoV-2 in bronchoalveolar lavage (BAL).** Cell populations and cytokines associated with reduced replicating SARS-CoV-2 are summarized by time (left to right) and compartment (top to bottom). Triangles indicate analytes expanded more over time in BCG than ALVAC/Alum (up, red) or depleted more over time in ALVAC/Alum than BCG (down, blue). Arrows indicate analyte levels that are higher in BCG vs ALVAC/Alum (red), BCG vs non-vaccinated control (muted red), or ALVAC/Alum vs non-vaccinated control (muted blue).




**Supplemental Figure 9. Summary of immune responses associated with increased replicating SARS-CoV-2 in bronchoalveolar lavage (BAL).** Cell populations and cytokines associated with increased replicating SARS-CoV-2 are summarized by time (left to right) and compartment (top to bottom). Triangles indicate analytes expanded more over time in ALVAC/Alum than BCG (up, blue) or depleted more over time in BCG than ALVAC/Alum (down, red). Arrows indicate analyte levels that are higher in ALVAC/Alum vs BCG (up, blue), or BCG vs non-vaccinated control (down, muted red).

| | Animal ID | Experimental Control | Pre | 7 days post vaccine | Pre-exposure | 7 days post exposure | 14 days post exposure |
|---|---|---|---|---|---|---|---|
| BCG | DGE0 | | <20 | <20 | <20 | <20 | <20 |
| BCG | DGJ6 | | <20 | <20 | <20 | <20 | <20 |
| BCG | DGB6 | | <20 | <20 | <20 | <20 | <20 |
| BCG | DGP4 | | <20 | <20 | <20 | <20 | <20 |
| ALVAC/Alum | DGP3 | | <20 | <20 | <20 | <20 | <20 |
| ALVAC/Alum | DHAR | | <20 | <20 | <20 | <20 | <20 |
| ALVAC/Alum | DG9X | | <20 | <20 | <20 | <20 | <20 |
| ALVAC/Alum | DGG7 | | <20 | <20 | <20 | <20 | <20 |
| Naïve control | DG8M | | | | <20 | <20 | <20 |
| Naïve control | DG8V | | | | <20 | <20 | <20 |
| Naïve control | T81A | | | | <20 | <20 | <20 |
| Naïve control | ZL47 | | | | <20 | <20 | <20 |
| Naïve control | H28T | | | | <20 | <20 | <20 |
| Positive Control | Rabbit reference serum | 1,620 | | | | | |

**Supplemental Table 1. Plasma neutralizing antibodies to SARS-CoV-2.** Neutralization assay was performed against 2019-nCoV/USA-WA1/2020 SARS-CoV-2 strain in plasma samples collected at baseline (Pre), 7 days following last vaccination, 14 days prior (Pre-exposure), and 7- and 14-days following SARS-CoV-2 exposure.