

# **Vaccine strain affects seroconversion after influenza vaccination in COPD patients and healthy older people**

## **Supplementary Methods**

### ***Study population***

Eligible participants 50 years of age or over, were recruited through the respiratory outpatients' clinic at Princess Alexandra Hospital and Mater Hospital (Brisbane), and the established Royal Melbourne Hospital COPD cohort between 2015 and 2017. Patients were eligible for enrolment if co-morbidities were stable and well controlled, and use of inhaled or continuous low-dose oral corticosteroid was stable in the 28 days preceding vaccination and remained stable for the study duration. Study exclusions included; invasive malignancy within the past two years, renal impairment (eGFR < 40 mL/min), acute febrile illness with fever >38.5°C, hypersensitivity to egg proteins, use of oral prednisolone or equivalent  $\geq 10$  mg/day, or use of other immunosuppressive therapy. 76 COPD patients of median age 69 years (range: 66 - 71) and 72 healthy participants, median age 63 years (range: 60 - 68) were analysed in this study.

Demographic, clinical assessment and spirometry were recorded at the initial clinic visit. Blood samples taken at baseline (day 0) and 28 days p.i. ( $\pm 3$  days), were collected in either lithium-heparin tubes or serum tubes. Seasonal approved vaccine was administered to each participant by a clinical research nurse, at day 0 clinic visit.

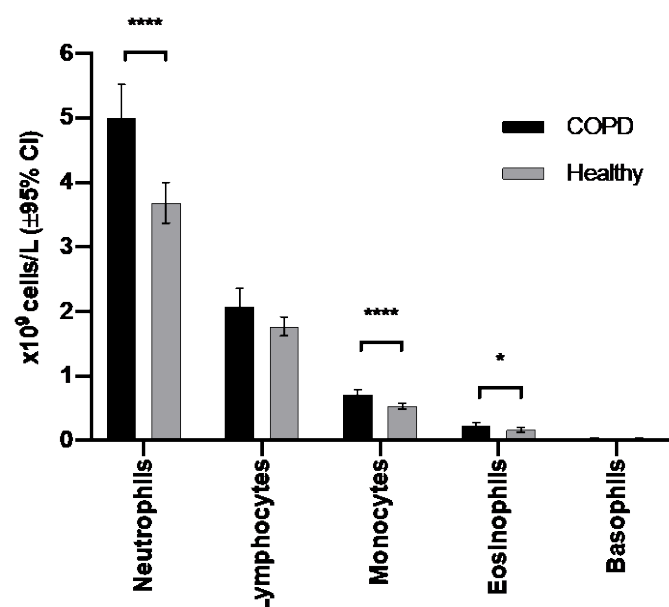
### ***Immunogenicity***

HI assays were conducted by the WHO Collaborating Centre for Reference and Research on Influenza, Victorian Infectious Diseases Reference Laboratory, Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Victoria, Australia. Briefly, sera were serially diluted two-fold in PBS (1:10 to 1:1280), incubated with 4 haemagglutinating units of vaccine strain-specific influenza antigen, and 1% turkey erythrocytes for H1N1 and B strains, or 1% guinea pig erythrocytes in the presence of oseltamivir for H3N2 strains. Titres below the assay's limit of detection (<10 or

<20 respectively) were arbitrarily designated a value half the threshold of detection. Titres were read as the reciprocal of the highest dilution that completely inhibited haemagglutination. Bloods were collected at each sample day in either: lithium-heparin tubes for isolation of peripheral blood mononuclear cells (PBMCs), plasma and granulocytes, using ficoll-paque™ density gradient centrifugation; or serum tubes, for serum separation. Blood samples were processed on the day of collection, and blood components stored at -80°C or in gaseous phase liquid nitrogen, as required.

### ***Statistical analysis***

Chi Square tests with Yates' correction assessed differences in sero-conversion and sero-protection between groups. Differences in GMT between strains were determined with Wilcoxon matched-pairs signed-rank tests, and considered significant if confidence intervals (CI) did not overlap. Odds ratios (OR) were adjusted for gender, age, year and sampling site. Binomial logistic regression models were generated to identify variables associated with either sero-conversion or sero-protection. Separate models were used for each combination of these two outcomes and for each vaccine strain. Stepwise regression modelling was performed to assess the weight of variables associated with these responses.



46     **Supplementary Figure 1. Comparison of baseline whole blood cell populations between**  
47     **COPD and healthy participants collected over three seasonal vaccination years**  
48     **2015/2016/2017. Displayed as number of cells x 10<sup>9</sup> per litre of whole blood ± 95% confidence**  
49     **intervals (\*\*\*\* p≤0.0001, \* p≤0.05, Wilcoxon ranked sum analysis).**

50 **Supplementary Table 1. Demographic and clinical data: Comparative between participants at**  
51 **both sample sites, Brisbane and Melbourne.**

| Demographic and Clinical Characteristics        | Total            | Brisbane         | Melbourne         | p-value |
|-------------------------------------------------|------------------|------------------|-------------------|---------|
| <b>N</b>                                        | <b>147</b>       | <b>94</b>        | <b>53</b>         |         |
| <b>Female - n (%)</b>                           | 60 (40.8)        | 38 (40.4)        | 22 (41.5)         | 0.963   |
| <b>Male - n (%)</b>                             | 87 (59.2)        | 56 (59.6)        | 31 (58.5)         | 0.963   |
| <b>Healthy</b>                                  | 72 (48.6)        | 46 (49)          | 26 (49)           | 0.875   |
| <b>COPD</b>                                     | 75 (51)          | 48 (51)          | 27 (51)           | 0.875   |
| <b>2016 Returns from 2015 (%)</b>               | 9 (6.1)          |                  |                   |         |
| <b>2017 Returns from 2016 (%)</b>               | 9 (6.1)          |                  |                   |         |
| <b>AGE (95%CI)</b>                              |                  |                  |                   |         |
| Mean                                            | 66.8 (65.3-68.3) | 65.9 (64.1-67.7) | 68.49 (65.8-71.2) | 0.114   |
| Median                                          | 67 (64-69)       | 65 (63-69)       | 69 (63-74)        |         |
| Range                                           | 50-90            | 50-87            | 53-90             |         |
| <b>BMI (95%CI)</b>                              |                  |                  |                   |         |
| Mean                                            | 28.1 (27.0-29.2) | 28.8 (27.3-30.2) | 27 (25.4-28.5)    | 0.174   |
| Median                                          | 26.7 (26.1-27.7) | 27.2 (26.1-29.4) | 26.6 (24.7-27.5)  |         |
| Range                                           | 18.2 - 52.9      | 18.2-53          | 18.9-46           |         |
| <b>Smoking status n (%)</b>                     |                  |                  |                   |         |
| Never                                           | 40 (27.2)        | 25 (26.6)        | 15 (28.3)         | 0.976   |
| Former                                          | 84 (57.1)        | 53 (56.4)        | 31 (58.5)         | 0.940   |
| Current                                         | 23 (15.6)        | 16 (17)          | 7 (13.2)          | 0.707   |
| <b>Pack Years (95%CI)</b>                       |                  |                  |                   |         |
| Mean                                            | 31.3 (25.6-37.1) | 30 (22.8-37.1)   | 33.8 (23.8-43.9)  | 0.574   |
| Median                                          | 21 (14.0-30.0)   | 21.5 (9.5-31)    | 18 (9-41)         |         |
| Range                                           | 0 - 168          | 0-168            | 0-120             |         |
| <b>Diabetes - n (%)</b>                         | 20 (13.6)        | 14 (14.9)        | 6 (11.3)          | 0.721   |
| <b>Heart condition - n (%)</b>                  | 35 (23.8)        | 18 (19.1)        | 17 (32)           | 0.117   |
| <b>Asthma - n (%)</b>                           | 23 (15.6)        | 13 (13.8)        | 10 (18.9)         | 0.568   |
| <b>Bronchiectasis - n (%)</b>                   | 6 (4.1)          | 3 (3.2)          | 3 (5.7)           | 0.770   |
| <b>High blood pressue - n (%)</b>               | 57 (38.8)        | 36 (38.3)        | 21 (39.6)         | 0.985   |
| <b>High cholesterol - n (%)</b>                 | 52 (35.4)        | 30 (31.9)        | 22 (41.5)         | 0.323   |
| <b>Mean FEV<sub>1</sub> predicted % (95%CI)</b> | 74 (68.6-79.5)   | 70.1 (64-76.3)   | 80.9 (70.4-91.5)  | 0.104   |
| <b>Mean FEV<sub>1</sub>/FVC % (95%CI)</b>       | 62.6 (59.1-66)   | 64.6 (60.5-68.7) | 58.9 (52.7-65.1)  | 0.098   |
| <b>Vaccine History n (%)</b>                    |                  |                  |                   |         |
| Never                                           | 5 (3.4)          | 3 (3.2)          | 2 (3.8)           | 0.774   |
| Previous 2 years (both)                         | 120 (81.6)       | 78 (83)          | 42 (79.2)         | 0.734   |
| Previous year (only)                            | 11 (7.5)         | 6 (6.4)          | 5 (9.4)           | 0.727   |
| Year before previous (only)                     | 4 (2.7)          | 2 (2.1)          | 2 (3.8)           | 0.951   |
| Ever before (except previous 2 years)           | 7 (4.8)          | 5 (5.3)          | 2 (3.8)           | 0.985   |

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**Supplementary Table 2. Vaccine response pre- (D0) and post- (D28) vaccine comparison between sites (Brisbane and Melbourne). Sero-protection rate: proportion of subjects with HI titre  $\geq 1:40$ . Sero-conversion rate: proportion of subjects eliciting a  $\geq 4$ -fold rise in Ab HI titre from baseline to D28 p.i. Differences in GMT calculated by Wilcoxon ranked sum test. Differences in proportions calculated by Chi Square test with Yates' correction. OR calculated from GLM adjusted for gender, age year and disease status.  $p$ -values of  $\leq 0.05$  is considered significant. Ab: antibody; GMT: geometric mean titre; GLM: generalised linear model; HI: haemagglutination inhibition; OR: odds ratio; Ref: reference.**

| Vaccine Strain                       | Stie | No. Subjects | Pre- Seroprotection rate (percentage of subjects with Ab titres $\geq 1:40$ ) |               |                      |                   | Post- Seroprotection rate (percentage of subjects with Ab titres $\geq 1:40$ ) |              |                      |                   | Seroconverted (percentage of subjects with 4-fold Ab increase) |               |                      |                   | Pre- GMT |               | Post- GMT |                   |
|--------------------------------------|------|--------------|-------------------------------------------------------------------------------|---------------|----------------------|-------------------|--------------------------------------------------------------------------------|--------------|----------------------|-------------------|----------------------------------------------------------------|---------------|----------------------|-------------------|----------|---------------|-----------|-------------------|
|                                      |      |              | % Positive                                                                    | $p$ -value    | adjusted OR (95% CI) | adj OR $p$ -value | % Positiv                                                                      | $p$ -value   | adjusted OR (95% CI) | adj OR $p$ -value | % Positiv                                                      | $p$ -value    | adjusted OR (95% CI) | adj OR $p$ -value | GMT      | $p$ -value    | GMT       | $p$ -value        |
| H1N1_A/CALIFORNIA/07/2009-like       | Bris | 94           | 39.4                                                                          |               | Ref                  |                   | 67                                                                             |              | Ref                  |                   | 20.2                                                           |               | Ref                  |                   | 19.7     |               | 40.9      |                   |
|                                      | Melb | 53           | 49                                                                            | 0.333         | 1.56 (0.75, 3.24)    | 0.231             | 84.9                                                                           | <b>0.03*</b> | 2.78 (1.15, 7.32)    | <b>0.029*</b>     | 39.6                                                           | <b>0.019*</b> | 2.78 (1.26, 6.3)     | <b>0.012*</b>     | 26       | 0.223         | 90        | <b>&lt;0.001*</b> |
| B/PHUKET/3073/2013-like              | Bris | 94           | 70.2                                                                          |               | Ref                  |                   | 87.2                                                                           |              | Ref                  |                   | 29.8                                                           |               | Ref                  |                   | 39.4     |               | 97.6      |                   |
|                                      | Melb | 53           | 86.8                                                                          | <b>0.038*</b> | 1.66 (0.63, 4.72)    | 0.320             | 98.1                                                                           | 0.054        | 4.65 (0.75, 90)      | 0.162             | 35.8                                                           | 0.567         | 1.66 (0.76, 3.65)    | 0.204             | 59.2     | 0.064         | 150       | 0.059             |
| B/BRISBANE/60/2008-like              | Bris | 65           | 76.9                                                                          |               | Ref                  |                   | 93.8                                                                           |              | Ref                  |                   | 33.8                                                           |               | Ref                  |                   | 44.8     |               | 130.2     |                   |
|                                      | Melb | 53           | 75.5                                                                          | 0.973         | 1.29 (0.45, 3.85)    | 0.630             | 94.3                                                                           | 0.780        | 1.79 (0.32, 12.2)    | 0.517             | 30.2                                                           | 0.386         | 0.72 (0.29, 1.72)    | 0.46              | 53.3     | 0.561         | 146       | 0.370             |
| H3N2_A/SWITZERLAND/9715293/2013-like | Bris | 29           | 17.2                                                                          |               |                      |                   | 65.5                                                                           |              |                      |                   | 48.3                                                           |               |                      |                   | 13       |               | 72.7      |                   |
|                                      | Melb | 0            | –                                                                             | –             | –                    | –                 | –                                                                              | –            | –                    | –                 | –                                                              | –             | –                    | –                 | –        | –             | –         | –                 |
| H3N2_A/HONG KONG/4801/2014-like      | Bris | 30           | 50                                                                            |               | Ref                  |                   | 86.7                                                                           |              | Ref                  |                   | 60                                                             |               | Ref                  |                   | 34.8     |               | 136.1     |                   |
|                                      | Melb | 29           | 72.4                                                                          | 0.134         | 2.72 (0.85, 9.43)    | 0.1               | 93.1                                                                           | 0.699        | 2.4 (0.39, 20.35)    | 0.365             | 28.6                                                           | 0.520         | 0.61 (0.20, 1.81)    | 0.38              | 81.9     | <b>0.031*</b> | 312.4     | 0.057             |
| H1N1_A/MICHIGAN/45/2015-like         | Bris | 34           | 55.9                                                                          |               | Ref                  |                   | 73.5                                                                           |              | Ref                  |                   | 29.4                                                           |               | Ref                  |                   | 29.5     |               | 69.4      |                   |
|                                      | Melb | 24           | 45.8                                                                          | 0.626         | 0.69 (0.23, 2.06)    | 0.510             | 79.2                                                                           | 0.855        | 1.22 (0.34, 4.68)    | 0.759             | 45.8                                                           | 0.315         | 2.06 (0.67, 6.52)    | 0.206             | 28.3     | 0.834         | 120       | 0.168             |

**Supplementary Table 3. Pre- and Post-vaccine response (D28 p.i.) comparing female and male subjects. Sero-protection rate: proportion of subjects with HI titre  $\geq 1:40$ . Sero-conversion rate: proportion of subjects eliciting a  $\geq 4$ -fold rise in Ab HI titre from baseline to D28 p.i. Differences in GMT calculated by Wilcoxon ranked sum test. Differences in proportions calculated by Chi Square test with Yates' correction. OR calculated from GLM adjusted for site, age year and disease status.  $p$ -values of  $\leq 0.05$  is considered significant. Ab: antibody; GMT: geometric mean titre; GLM: generalised linear model; HI: haemagglutination inhibition; OR: odds ratio; Ref: reference.**

| Vaccine Strain                       | Disease status | No. Subject | Pre- Seroprotection rate (percentage of subjects with Ab titres $\geq 1:40$ ) |            |                      |                   | Post- Seroprotection rate (percentage of subjects with Ab titres $\geq 1:40$ ) |            |                      |                   | Seroconverted (percentage of subjects with 4-fold Ab increase) |            |                      |                   | Pre- GMT |               | Post- GMT |            |
|--------------------------------------|----------------|-------------|-------------------------------------------------------------------------------|------------|----------------------|-------------------|--------------------------------------------------------------------------------|------------|----------------------|-------------------|----------------------------------------------------------------|------------|----------------------|-------------------|----------|---------------|-----------|------------|
|                                      |                |             | % Positive                                                                    | $p$ -value | adjusted OR (95% CI) | adj OR $p$ -value | % Positive                                                                     | $p$ -value | adjusted OR (95% CI) | adj OR $p$ -value | % Positiv                                                      | $p$ -value | adjusted OR (95% CI) | adj OR $p$ -value | GMT      | $p$ -value    | GMT       | $p$ -value |
| H1N1_A/CALIFORNIA/07/2009-like       | Female         | 60          | 33.3                                                                          |            | Ref                  |                   | 76.6                                                                           |            | Ref                  |                   | 31.7                                                           |            | Ref                  |                   | 16.8     |               | 47.6      |            |
|                                      | Male           | 87          | 49.4                                                                          | 0.077      | 2.16 (1.07, 4.46)    | <b>0.033*</b>     | 71.3                                                                           | 0.590      | 0.772 (0.34, 1.71)   | 0.528             | 24.1                                                           | 0.415      | 0.671 (0.31, 1.46)   | 0.312             | 26       | <b>0.032*</b> | 59.6      | 0.19       |
| B/PHUKET/3073/2013-like              | Female         | 60          | 75                                                                            |            | Ref                  |                   | 93.3                                                                           |            | Ref                  |                   | 38.3                                                           |            | Ref                  |                   | 41.4     |               | 116       |            |
|                                      | Male           | 87          | 77                                                                            | 0.932      | 1.22 (0.51, 2.94)    | 0.65              | 89.7                                                                           | 0.634      | 0.498 (0.11, 1.84)   | 0.316             | 27.6                                                           | 0.232      | 0.477 (0.22, 1.01)   | 0.056             | 48.8     | 0.624         | 113       | 0.938      |
| B/BRISBANE/60/2008-like              | Female         | 47          | 72.3                                                                          |            | Ref                  |                   | 95.7                                                                           |            | Ref                  |                   | 29.8                                                           |            | Ref                  |                   | 41.8     |               | 125       |            |
|                                      | Male           | 71          | 78.9                                                                          | 0.551      | 1.99 (0.67, 6.12)    | 0.217             | 92.9                                                                           | 0.818      | 0.442 (0.05, 2.54)   | 0.389             | 33.8                                                           | 0.798      | 0.976 (0.39, 2.43)   | 0.958             | 53.4     | 0.598         | 146       | 0.279      |
| H3N2_A/SWITZERLAND/9715293/2013-like | Female         | 13          | 23.1                                                                          |            | Ref                  |                   | 69.2                                                                           |            | Ref                  |                   | 46.1                                                           |            | Ref                  |                   | 15.3     |               | 68.2      |            |
|                                      | Male           | 16          | 12.5                                                                          | 0.798      | 1.45 (0.50, 4.28)    | 0.495             | 62.5                                                                           | 0.989      | 0.528 (0.14, 1.81)   | 0.321             | 50                                                             | 0.867      | 1.45 (0.30, 7.69)    | 0.644             | 11.4     | 0.603         | 76.6      | 1          |
| H3N2_A/HONG KONG/4801/2014-like      | Female         | 20          | 50                                                                            |            | Ref                  |                   | 85                                                                             |            | Ref                  |                   | 55                                                             |            | Ref                  |                   | 36.1     |               | 135       |            |
|                                      | Male           | 39          | 66.6                                                                          | 0.337      | 1.35 (0.38, 4.58)    | 0.634             | 92.3                                                                           | 0.671      | 2.77 (0.4, 19.8)     | 0.29              | 53.8                                                           | 0.848      | 1.06 (0.33, 3.47)    | 0.918             | 64.6     | 0.189         | 254       | 0.155      |
| H1N1_A/MICHIGAN/45/2015-like         | Female         | 27          | 48.1                                                                          |            | Ref                  |                   | 81.5                                                                           |            | Ref                  |                   | 37                                                             |            | Ref                  |                   | 27.2     |               | 84.2      |            |
|                                      | Male           | 31          | 54.8                                                                          | 0.806      | 1.35 (0.46, 4.07)    | 0.587             | 70.9                                                                           | 0.531      | 0.548 (0.14, 1.92)   | 0.358             | 35.5                                                           | 0.88       | 0.897 (0.29, 2.72)   | 0.847             | 30.6     | 0.727         | 89.5      | 0.747      |

#### Supplementary Table 4: Binomial GLM determining which variables influence Ab response:

a) Dependent variable = antibody sero-conversion (Fold change of  $\geq 4$ ) at day 28 p.i. Year, Strain, Site and Age are significant variables effecting ability to attain a 4-fold increase in Ab titre.

```
glmfold <- glm(fold2 ~ Disease2 + sex2 + YEAR + Strain + site2 + AGE + BMI
data = data, family = "binomial")
```

| summary(glmfold) | Estimate | Std. Error | z value | Pr(> z ) |
|------------------|----------|------------|---------|----------|
| Intercept        | 825.3109 | 331.9323   | 2.4860  | 0.0129*  |
| Disease2healthy  | -0.1751  | 0.1966     | -1.8910 | 0.3730   |
| sex2m            | -0.2026  | 0.1936     | -1.0460 | 0.2954   |
| YEAR             | -0.4096  | 0.1646     | -2.4880 | 0.0128*  |
| StrainA_HK       | 1.0549   | 0.3322     | 3.1750  | 0.0015** |
| StrainA_MICH     | 0.7234   | 0.3638     | 1.9880  | 0.0468*  |
| StrainA_SWZ      | 0.5693   | 0.4580     | 1.2430  | 0.2139   |
| StrainB_BRIS     | 0.2660   | 0.2837     | 0.9380  | 0.3485   |
| StrainB_PHU      | 0.2053   | 0.2619     | 0.7840  | 0.4332   |
| site2Melb        | 0.4802   | 0.2026     | 2.3700  | 0.0178*  |
| AGE              | -0.0158  | 0.0108     | -1.4670 | 0.1425   |
| BMI              | 0.0185   | 0.0145     | 1.2750  | 0.2022   |

b) Dependent variable = antibody sero-protection (Ab titre of  $\geq 1:40$ ) at day 28 p.i.

```
glmsero <- glm(t2sero ~ Disease2 + sex2 + YEAR + Strain + site2 + AGE + BMI
data = data, family = "binomial")
```

| summary(glmfold) | Estimate   | Std. Error | z value | Pr(> z )  |
|------------------|------------|------------|---------|-----------|
| Intercept        | -1218.0000 | 425.3000   | -2.8630 | 0.0042*   |
| Disease2healthy  | -0.1108    | 0.2637     | -0.4200 | 0.6743    |
| sex2m            | -0.3249    | 0.2654     | -1.2240 | 0.2209    |
| YEAR             | 0.6052     | 0.2109     | 2.8690  | 0.0041*   |
| StrainA_HK       | 1.0430     | 0.4809     | 2.1680  | 0.0302*   |
| StrainA_MICH     | -0.4923    | 0.4212     | -1.1690 | 0.2425    |
| StrainA_SWZ      | 0.4694     | 0.4919     | 0.9540  | 0.3399    |
| StrainB_BRIS     | 1.4250     | 0.4467     | 3.1910  | 0.0014**  |
| StrainB_PHU      | 1.3270     | 0.3590     | 3.6960  | 0.0002*** |
| site2Melb        | 0.7547     | 0.3039     | 2.4840  | 0.0130*   |
| AGE              | -0.0208    | 0.0144     | -1.4490 | 0.1475    |
| BMI              | 0.0094     | 0.0195     | 0.4850  | 0.6274    |

74 **Supplementary Table 5: Univariate analysis using magnitude of Ab fold increase at day 28**

75 **p.i. as dependent variable**

76

77

| lm <- lm(Fold ~ log(ABT0), data = data) |          |            |         |              |
|-----------------------------------------|----------|------------|---------|--------------|
|                                         | Estimate | Std. Error | t value | Pr(> t )     |
| Intercept                               | 24.4020  | 2.5931     | 9.4100  | <2e-16 ***   |
| log(ABT0)                               | -4.6174  | 0.6828     | -6.7630 | 3.43e-11 *** |

| lm <- lm(Fold ~ BMI, data = data) |          |            |         |            |
|-----------------------------------|----------|------------|---------|------------|
|                                   | Estimate | Std. Error | t value | Pr(> t )   |
| Intercept                         | -8.8921  | 4.4026     | -2.0200 | 0.0439*    |
| BMI                               | 0.6059   | 0.1531     | 3.9570  | 0.0001 *** |

79 **Supplementary Table 6: Binomial GLM determining which smoking exposure variables are**  
80 **associated with sero-conversion (Fold change of  $\geq 4$ ) at day 28 p.i.**

81

|                   | Estimate | Std. Error | z value | Pr(> z )    |
|-------------------|----------|------------|---------|-------------|
| Intercept         | -0.9875  | 0.1588     | -6.2180 | 5.04e-10*** |
| packyr            | 0.0020   | 0.0029     | 0.7010  | 0.4831      |
| passivyr          | 0.0114   | 0.0045     | 2.5640  | 0.0103*     |
| currently smoking | 0.0518   | 0.2859     | 0.1810  | 0.8561      |

82 **Supplementary Table 7: Binomial GLM looking at comorbid diseases as variables influencing**

83 **Ab response:**

84 **a) Dependent variable = antibody sero-conversion (fold increase ≥4) at day 28 p.i.**

85

```
glmmorb1 <- glm(fold2 ~ asthma+bronch+hiblood+hichol+heartcond+diabetes+AGE, data
= data, family = "binomial")
```

|                     | Estimate | Std. Error | z value | Pr(> z ) |
|---------------------|----------|------------|---------|----------|
| Intercept           | 0.0345   | 0.6976     | 0.0490  | 0.9606   |
| Asthmatic           | -0.4084  | 0.2621     | -1.5580 | 0.1192   |
| Bronchiectasis      | 0.3648   | 0.4521     | 0.8070  | 0.4198   |
| High blood pressure | 0.4018   | 0.2042     | 1.9670  | 0.0491*  |
| High cholesterol    | -0.2234  | 0.2147     | -1.0410 | 0.2981   |
| Heart condition     | -0.2997  | 0.2338     | -1.2820 | 0.1999   |
| Diabetes            | 0.5164   | 0.2742     | 1.8830  | 0.0597   |
| Age                 | -0.0107  | 0.0107     | -1.0060 | 0.3145   |

86

87 **b) Dependent variable = antibody sero-protection (Ab titre of ≥1:40) at day 28 p.i.**

```
glmmorb1 <- glm(t2sero ~ asthma + bronch +hiblood + hichol + heartcond + diabetes
+AGE, data = data, family = "binomial")
```

|                     | Estimate | Std. Error | z value | Pr(> z )  |
|---------------------|----------|------------|---------|-----------|
| Intercept           | 2.6958   | 0.8961     | 3.0080  | 0.00263** |
| Asthmatic           | 0.2990   | 0.3513     | 0.8510  | 0.3947    |
| Bronchiectasis      | -0.0433  | 0.5748     | -0.0750 | 0.9400    |
| High blood pressure | 0.0264   | 0.2628     | 0.1000  | 0.9200    |
| High cholesterol    | -0.0448  | 0.2721     | -0.1640 | 0.8693    |
| Heart condition     | 0.3020   | 0.3013     | 1.0020  | 0.3162    |
| Diabetes            | -0.1610  | 0.3590     | -0.4490 | 0.6537    |
| Age                 | -0.0166  | 0.0135     | -1.2330 | 0.2175    |

89 **Supplementary Table 8: Binomial GLM determining which whole blood cells are variables**  
90 **influencing Ab response:**

91 **a) Dependent variable = antibody sero-conversion (fold increase ≥4) at day 28 p.i.**

92

| glm(formula = fold2 ~ Disease2 + neutro + lympho + mono + eosino + baso, family = "binomial", data = data) |          |            |         |          |
|------------------------------------------------------------------------------------------------------------|----------|------------|---------|----------|
|                                                                                                            | Estimate | Std. Error | z value | Pr(> z ) |
| Intercept                                                                                                  | -0.4873  | 0.3349     | -1.4550 | 0.146    |
| Disease, healthy                                                                                           | -0.1788  | 0.1964     | -0.9110 | 0.362    |
| neutro                                                                                                     | -0.0894  | 0.0584     | -1.5310 | 0.126    |
| lympho                                                                                                     | 0.1186   | 0.0955     | 1.2410  | 0.215    |
| mono                                                                                                       | 0.5252   | 0.4097     | 1.2820  | 0.200    |
| eosino                                                                                                     | -0.8861  | 0.5888     | -1.5050 | 0.132    |
| baso                                                                                                       | -2.0469  | 2.7463     | -0.7450 | 0.456    |

93

95

| glmcell2 <- glm(t2sero ~ Disease2 + neutro + lympho + mono + eosino + baso, data = data, family = "binomial") |          |            |         |          |
|---------------------------------------------------------------------------------------------------------------|----------|------------|---------|----------|
|                                                                                                               | Estimate | Std. Error | z value | Pr(> z ) |
| Intercept                                                                                                     | 1.2204   | 0.4138     | 2.9490  | 0.0032** |
| Disease, healthy                                                                                              | 0.0316   | 0.2514     | 0.1230  | 0.9001   |
| neutro                                                                                                        | -0.0309  | 0.0691     | -0.4480 | 0.6544   |
| lympho                                                                                                        | 0.2659   | 0.1582     | 1.6810  | 0.0927   |
| mono                                                                                                          | -0.0138  | 0.5510     | -0.0250 | 0.9801   |
| eosino                                                                                                        | -0.2501  | 0.7148     | -0.3500 | 0.7264   |
| baso                                                                                                          | 3.4067   | 3.7241     | 0.9150  | 0.3603   |

96 **Supplementary Table 9: Testing the interaction between Strain and Year. Year alone becomes**  
 97 **not significant, indicating all variation in fold2 is likely to be attributed to strain rather than**  
 98 **year.**

| glmfold2 <- glm(fold2 ~ YEAR* Strain, data = data, family = "binomial") |           |            |         |             |
|-------------------------------------------------------------------------|-----------|------------|---------|-------------|
|                                                                         | Estimate  | Std. Error | z value | Pr(> z )    |
| Intercept                                                               | -487.4354 | 511.9693   | -0.952  | 0.3410      |
| YEAR                                                                    | 0.2413    | 0.2539     | 0.950   | 0.3420      |
| StrainA_HK                                                              | 1.2105    | 0.3277     | 3.694   | 0.000221*** |
| StrainA_MICH                                                            | 0.2330    | 0.3794     | 0.614   | 0.5392      |
| StrainA_SWZ                                                             | 1.2129    | 0.5253     | 2.309   | 0.020952*   |
| StrainB_BRIS                                                            | 4290.221  | 1061.005   | 4.044   | 5.26e-05*** |
| StrainB_PHU                                                             | 1343.084  | 701.760    | 1.914   | 0.0556      |
| YEAR:StrainA_HK                                                         | NA        | NA         | NA      | NA          |
| YEAR:StrainA_MICH                                                       | NA        | NA         | NA      | NA          |
| YEAR:StrainA_SWZ                                                        | NA        | NA         | NA      | NA          |
| YEAR:StrainB_BRIS                                                       | -2.1276   | 0.5262     | -4.043  | 5.263-05*** |
| YEAR:StrainB_PHU                                                        | -0.6660   | 0.3481     | -1.914  | 0.0557      |

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