Supplemental Material

Age-Specific Effects of Vaccine Egg adaptation And Immune Priming on A(H3N2) Antibody Responses Following Influenza vaccination

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Supplemental Methods

Generation of reassortant viruses by reverse genetics

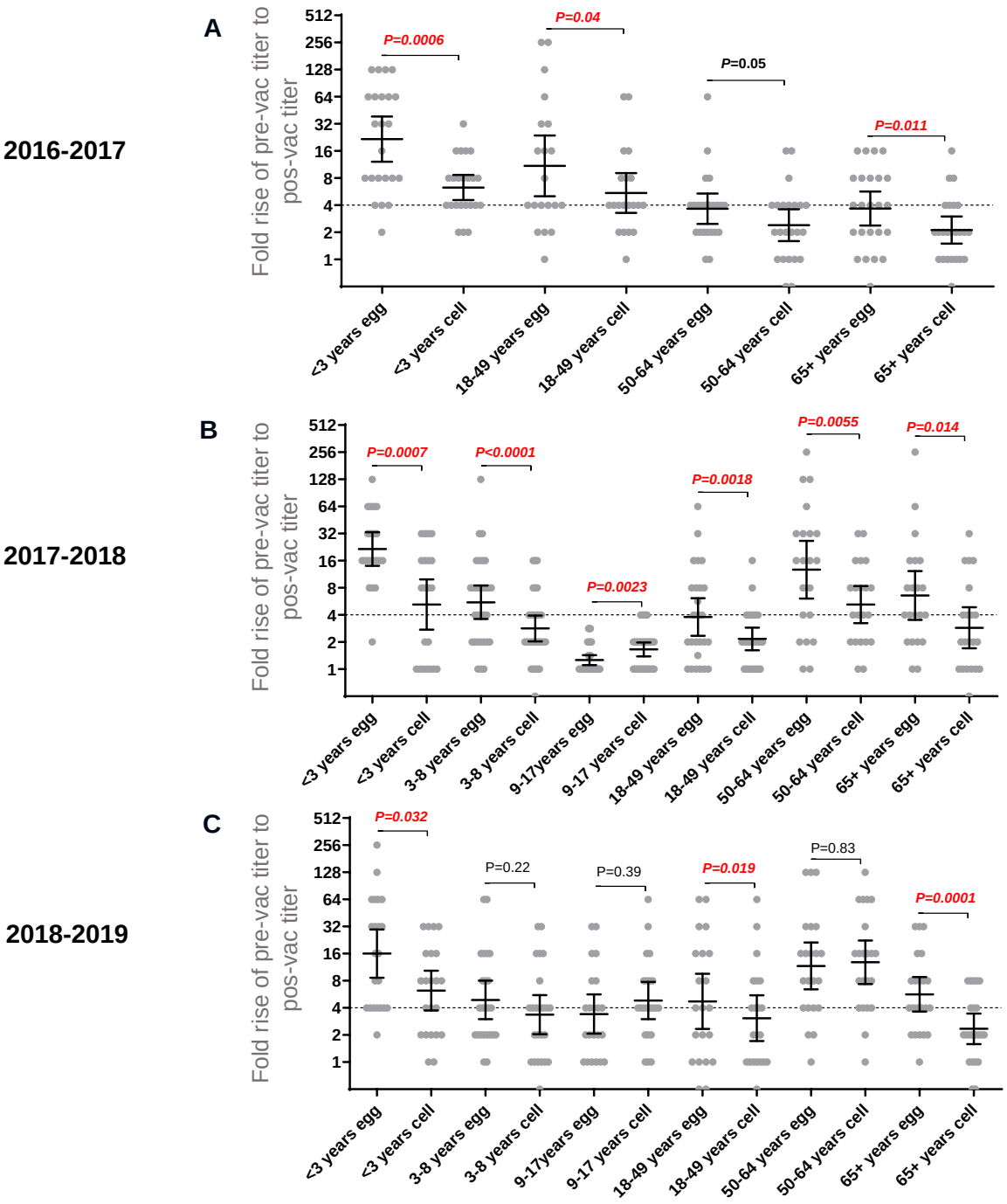
The generation of the reassortant virus were based on the procedures previously described (Ridenour C et al. 2015). Briefly, the hemagglutinin (HA) and neuraminidase (NA) genes derived from A/Singapore/INFIMH-16-0019/2016 H3N2 cell-propagated virus, were respectively cloned into a reverse genetics vector flanked by human polymerase I promoter and mouse RNA polymerase I terminator elements. Mutagenesis encoding T160K, or D225G, or L194P substitution was engineered into the HA gene respectively. Then, reverse genetics plasmids encoding the HA and NA surface genes of A/Singapore/INFIMH-16-0019/2016 H3N2 cell-propagated virus, as well as plasmids containing the six internal genes from A/Puerto Rico/8/1934 virus were transfected into 293T cells (American Type Culture Collection) and MDCK-SIAT1 cells co-culture system. Viruses from transfected 293T and MDCK-SIAT1 co-culture cells were inoculated into MDCK-SIAT1 cells and passaged two times to prepare virus stocks. The reassortant virus were sequenced to confirm there were no additional amino acid substitutions other than the T160K, or D225G, or L194P introduced into the HA gene. The infectivity of reassortant virus stocks was verified by a $TCID_{50}$ assay (Gross FL et al., 2017).

References

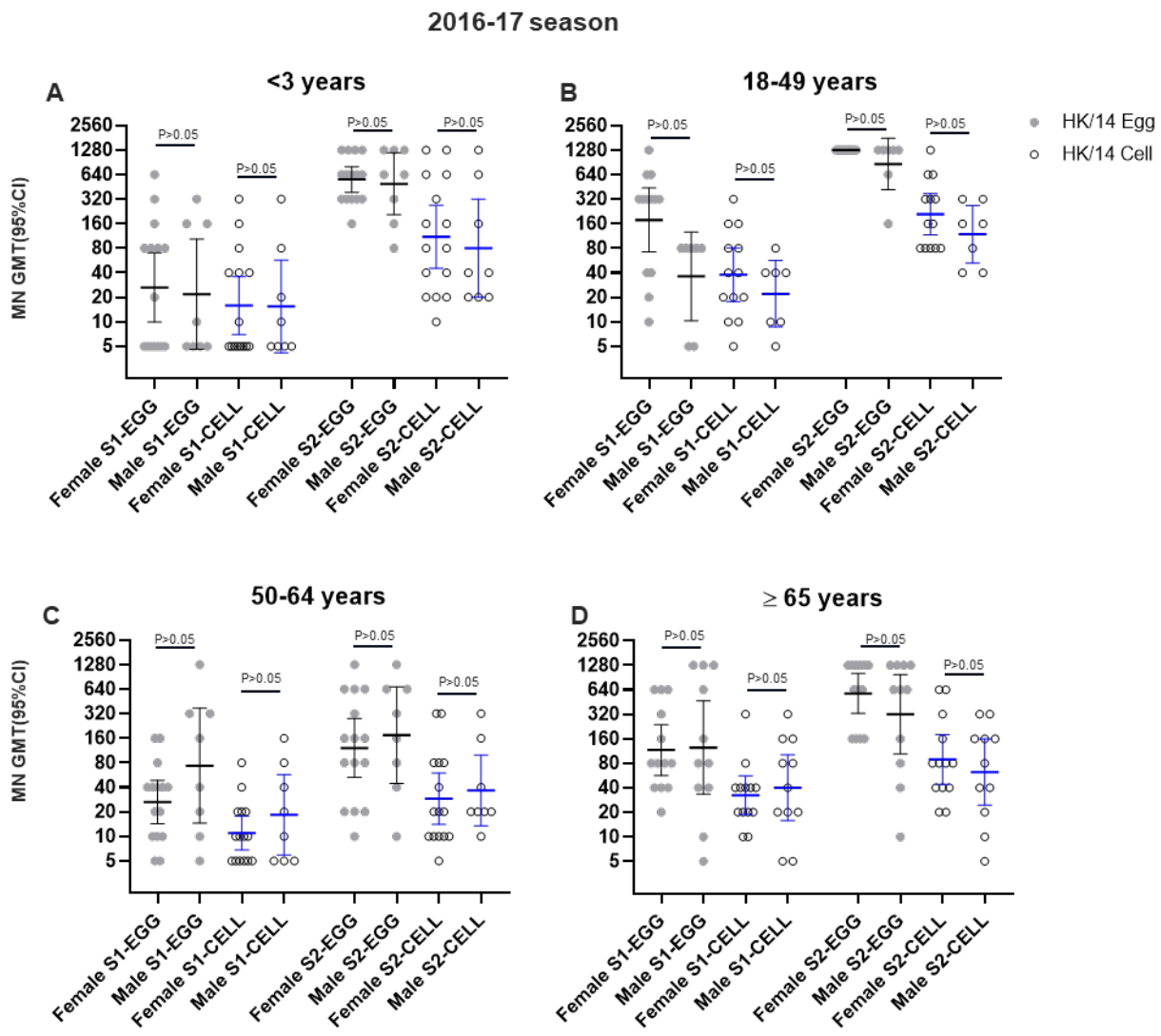
Ridenour C et al. Development of influenza A(H7N9) candidate vaccine viruses with improved hemagglutinin antigen yield in eggs. Influenza Other Respir Viruses. 2015 Sep; 9(5): 263–270

Gross FL et al. Measuring Influenza Neutralizing Antibody Responses to A(H3N2) Viruses in Human Sera by Microneutralization Assays Using MDCK-SIAT1 Cells. J Vis Exp. 2017; (129): 56448.

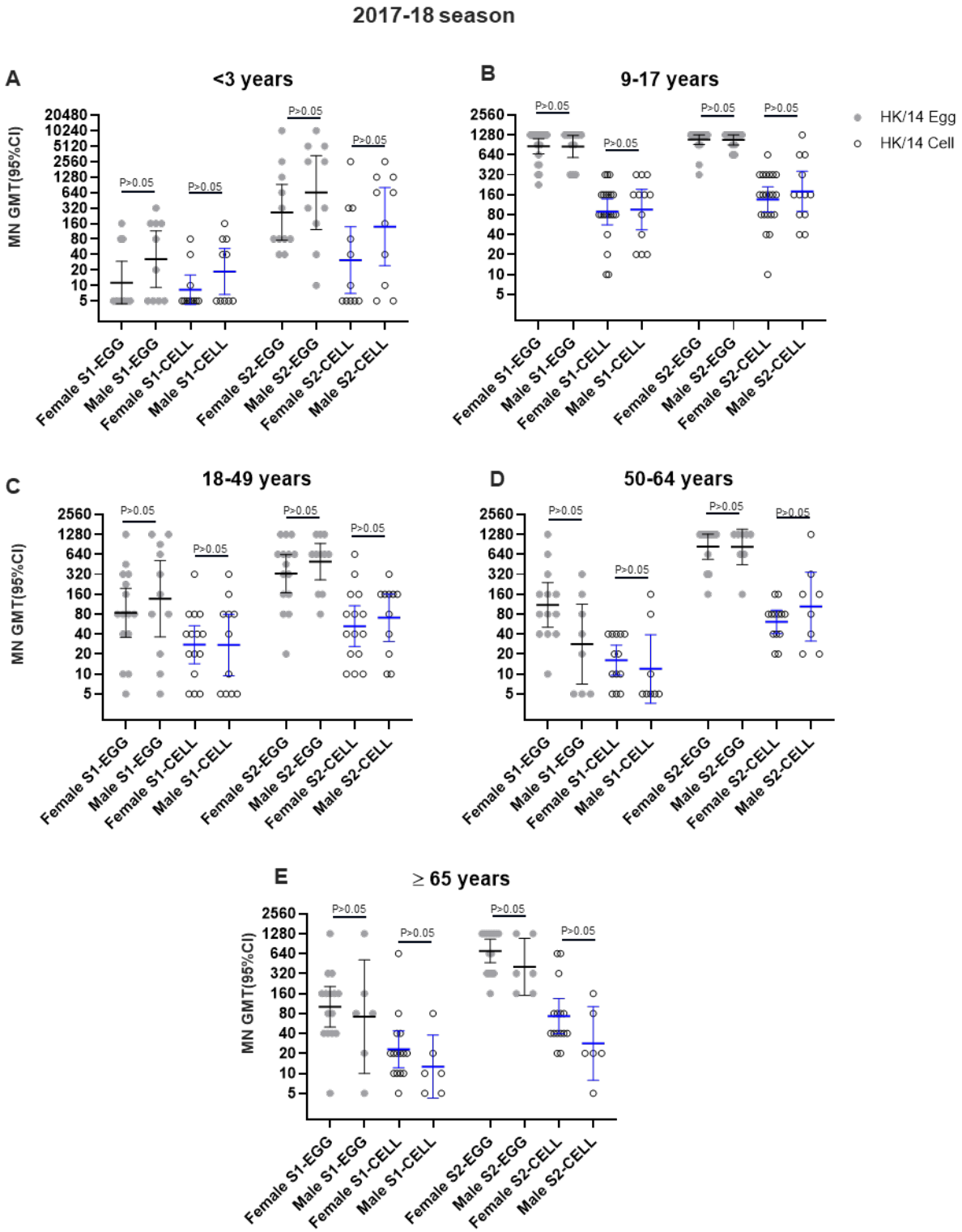
Supplemental Results



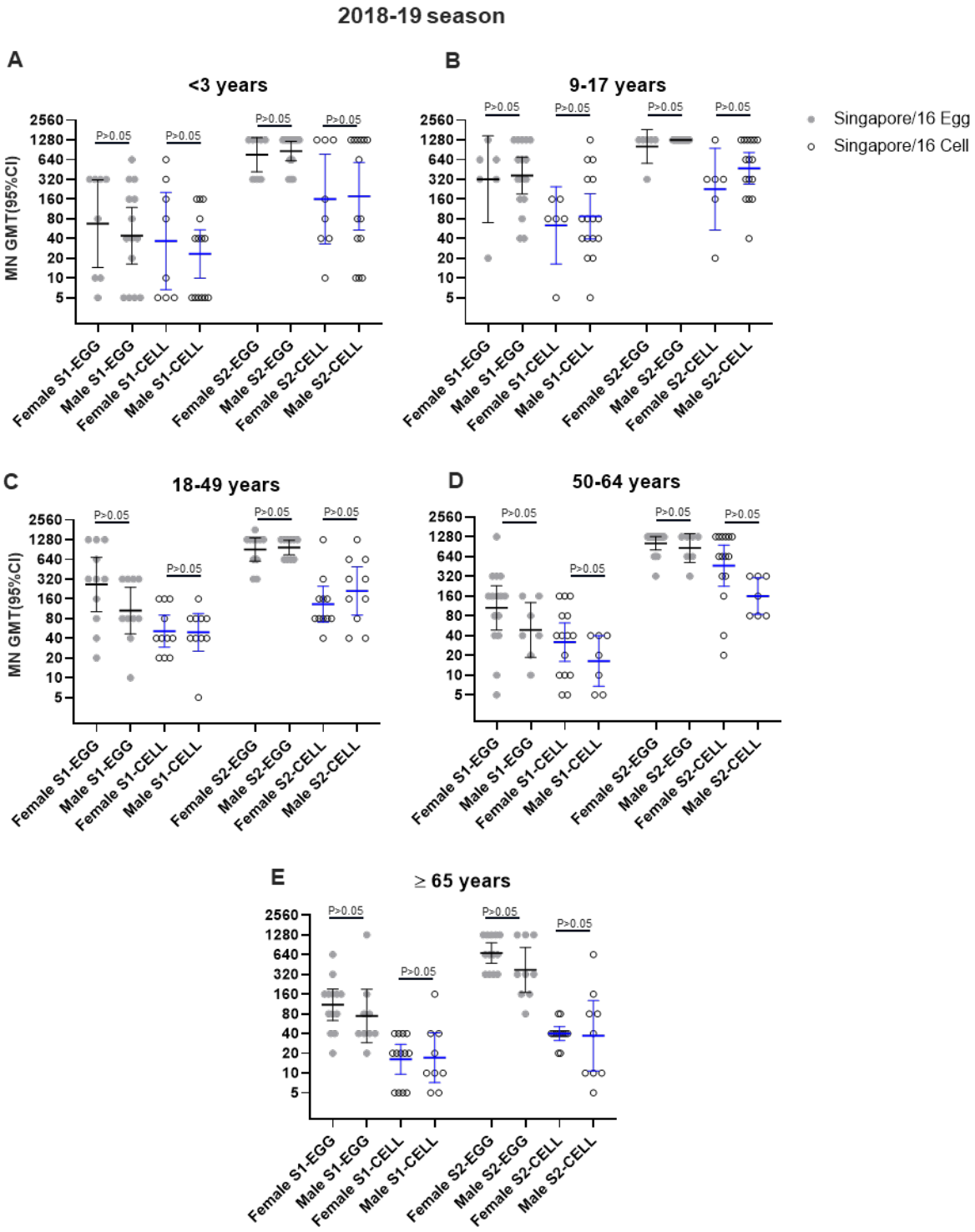
Supplemental Figure 1. Fold rise of pre-vaccination MN titer to post-vaccination MN titer across age groups in 2016-17, 2017-18, and 2018-19 seasons. Fold rise was calculated by the ratio of post/pre- vaccination MN titers for each individual against egg- and cell- propagated wild type A(H3N2) vaccine virus respectively. Each dot represents individual data. Black bars are geometric mean fold rises with 95% confidence interval. Dashed lines denote 4-fold rise. Wilcoxon matched-pairs signed rank test was used for comparison of fold rises between egg- and cell- propagated viruses in the same age group. *P*<0.05 (in red) is considered significant.



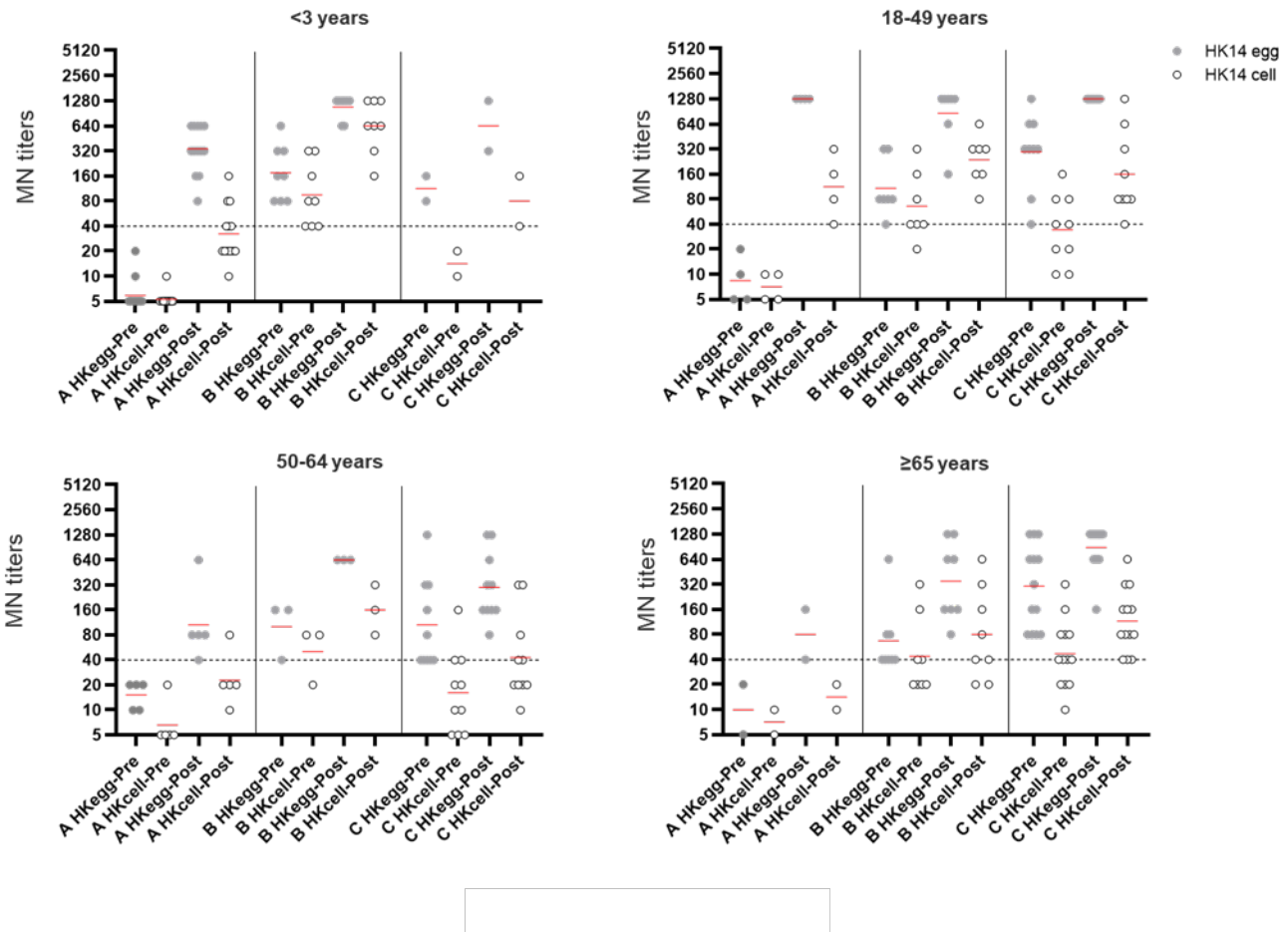
Supplemental Figure 2. Neutralizing antibody responses in male versus female vaccinees in 2016-17. Difference in neutralizing antibody responses between male versus female vaccinees per age group pre- and post-vaccination were compared by Mann Whitney unpaired t test. P values are indicated on graph. P<0.05 is considered statistically significant.



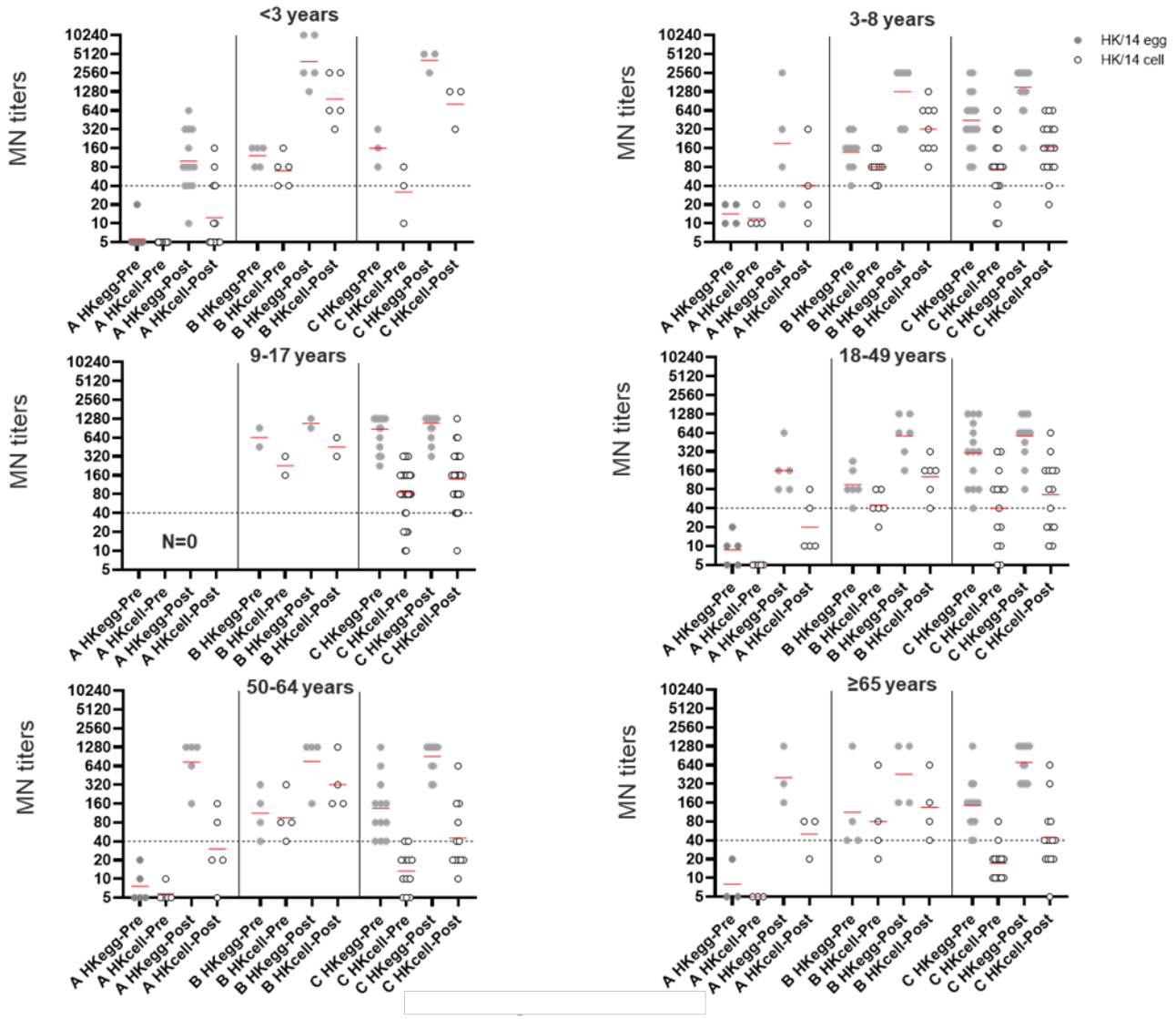
Supplemental Figure 3. Neutralizing antibody responses in male versus female vaccinees in 2017-18. Difference in neutralizing antibody responses between male versus female vaccinees per age group pre- and post-vaccination were compared by Mann Whitney unpaired t test. P values are indicated on graph. P<0.05 is considered statistically significant.



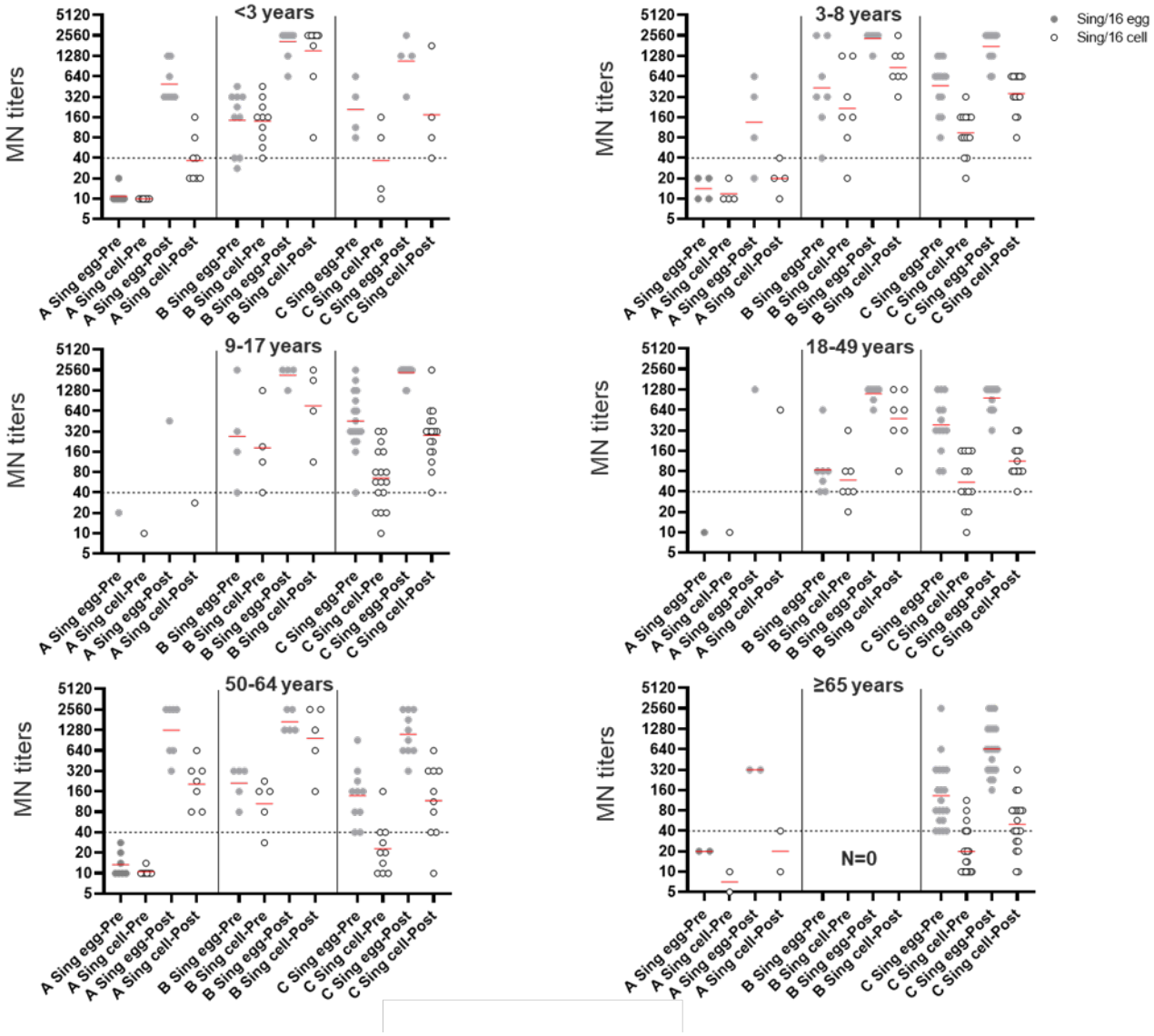
Supplemental Figure 4. Neutralizing antibody responses in male versus female vaccinees in 2018-19. Difference in neutralizing antibody responses between male versus female vaccinees per age group pre- and post-vaccination were compared by Mann Whitney unpaired t test. P values are indicated on graph. P<0.05 is considered statistically significant.



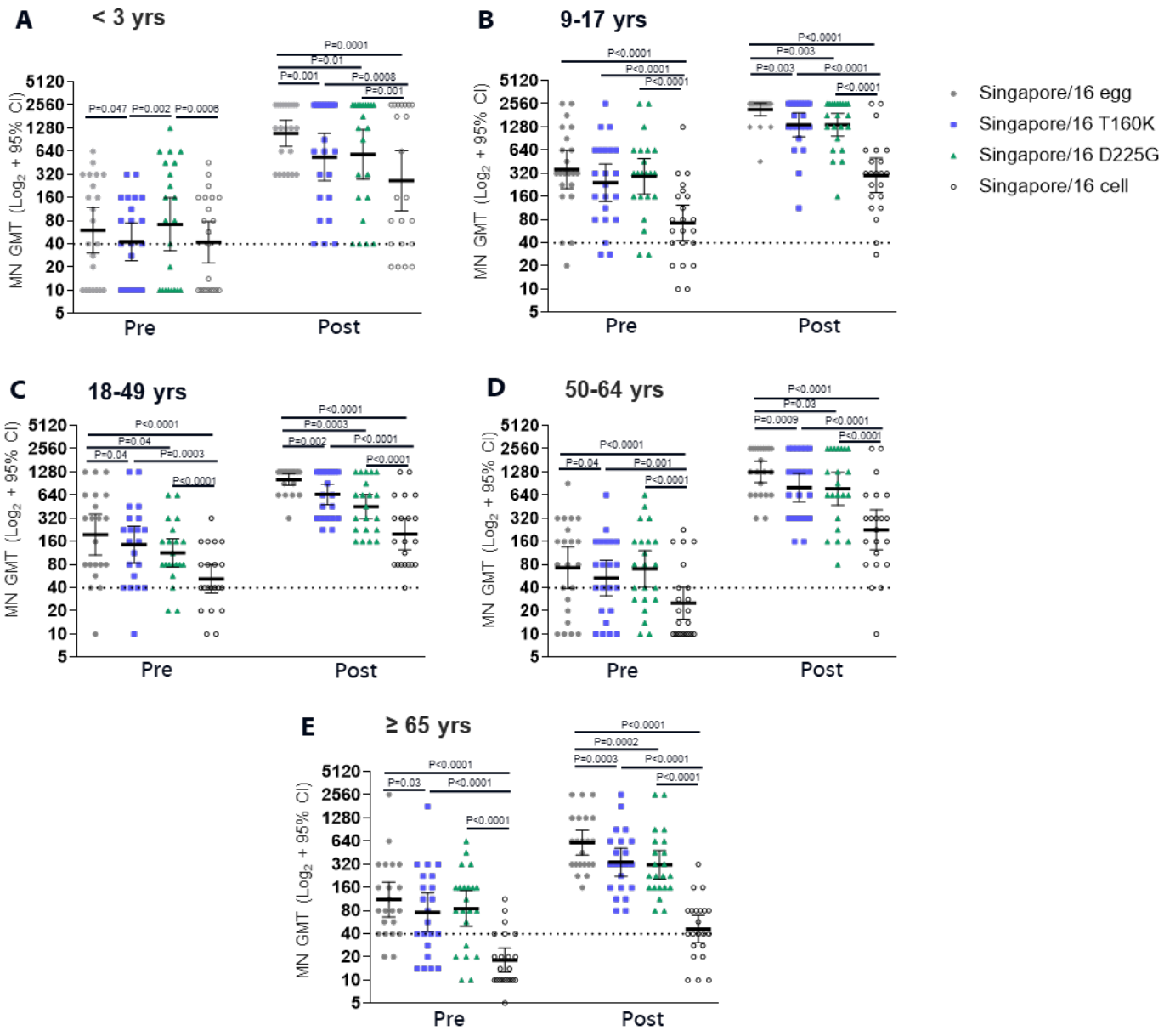
Supplemental Figure 5. Vaccine responses to egg- and cell- propagated wild type vaccine viruses across age groups with stratified pre-existing MN titers in 2016-17 season. This is the same dataset as used in Table 3. Individual MN titers to HK/14 egg virus (solid circle) and HK/14 cell virus (open circle) were plotted for each age group stratified based on pre-existing MN titers (group A, B, and C, same denotation as in Table 3). HKegg-Pre or Post stands for pre- or post- vaccination MN titers to HK/14 egg virus. HKcell-Pre or Post stands for pre- or post- vaccination MN titers to HK/14 cell virus. Red bars represent the geometric mean MN titers.



Supplemental Figure 6. Vaccine responses to egg and cell -propagated wild type vaccine viruses across age groups with stratified pre-existing MN titers in 2017-18 season. This is the same dataset as used in Table 3. Individual MN titers to HK/14 egg virus (solid circle) and HK/14 cell virus (open circle) were plotted for each age group stratified based on pre-existing MN titers (group A, B, and C, same denotation as in Table 3). HKegg-Pre or Post stands for pre- or post- vaccination MN titer to HK/14 egg virus. HKcell-Pre or Post stands for pre- or post- vaccination MN titer to HK/14 cell virus. Red bars represent the geometric mean MN titers.



Supplemental Figure 7. Vaccine responses to egg and cell -propagated wild type vaccine viruses across age groups with stratified pre-existing MN titers in 2018-19 season. This is the same dataset as used in Table 3. Individual MN titers to Singapore/16 egg virus (solid circle) and Sing/16 cell virus (open circle) were plotted for each age group stratified based on pre-existing MN titers (group A, B, and C, same denotation as in Table 3). Sing egg-Pre or Post stands for pre- or post- vaccination MN titer to Singapore/16 egg virus. Sing cell-Pre or Post stands for pre- or post- vaccination MN titer to Singapore/16 cell virus. Red bars represent the geometric mean MN titers.



Supplemental Figure 8. Neutralizing antibody responses to egg- and cell-propagated vaccine viruses Singapore/16, RG Singapore/16 T160K and RG Singapore/16 D225G viruses in 5 age groups in 2018-19 season. Difference in neutralizing antibody responses pre- and postvaccination were compared by one way ANOVA. P values are indicated, $p<0.05$ is considered statistically significant.

Supplemental Table 1. Adjusted influenza vaccine effectiveness (VE) based on age groups against A(H3N2) viruses from 2016-17 to 2018-2019 seasons in the United States.

Influenza VE was estimated using a test-negative design, which compares the odds of testing positive for influenza among vaccinated versus unvaccinated persons. VE is the relative difference in influenza risk between vaccinated and unvaccinated participants, expressed as a percentage and calculated as (1 − OR) × 100, where OR is the odds ratio for influenza among vaccinated compared with unvaccinated persons, determined from logistic regression models. The 95% confidence intervals (CIs) for VE were calculated as 1 − CIOR, where CIOR is the CI of the OR estimates. A priori, estimates were adjusted for network site, sex, age, race/ethnicity, self-reported general health status, interval from illness onset to study enrollment, and biweekly interval.

	2016-17 adjusted VE [1] % (95% CI)	**2017-18 Adjusted VE [2] % (95% CI)**	**2018-19 adjusted VE [3] % (95% CI)**
All Age groups	33 (23 to 41)	22 (12-31)	9 (-4 to 20)
6 months-2 years	34(-32 to 66)	51 (20 to 70)	-12 (-189 to 43)
3-8 years	51 (27 to 67)	45 (22 to 61)	28 (2 to 47)
9-17 years	33 (7 to 52)	7 (-27 to 32)	3 (-30 to 28)
18-49 years	13 (-11 to 32)	13 (-6 to 29)	3 (-25 to 24)
50-64 years	31 (9 to 47)	22 (-4 to 41)	-20 (-74 to 18)
≥ 65 years	21 (-15 to 45)	10 (-32 to 38)	13 (-46 to 48)

CI: confidence interval.

1. Flannery B*, et al.* Influenza Vaccine Effectiveness in the United States During the 2016-2017 Season. *Clin Infect Dis* 68, 1798-1806 (2019).
2. Rolfes MA*, et al.* Effects of Influenza Vaccination in the United States During the 2017-2018 Influenza Season. *Clin Infect Dis* 69, 1845-1853 (2019).
3. Flannery B*, et al.* Spread of Antigenically Drifted Influenza A(H3N2) Viruses and Vaccine Effectiveness in the United States During the 2018-2019 Season. *J Infect Dis* 221, 8-15 (2020).