Supporting Information

Title: Sex differences in antibody responses to influenza A/H3N2 across the life course

Siyu Chen[1,2], Bernardo García-Carreras[3,4], James A. Hay[5], Huachen Zhu[6], Chao Qiang Jiang[7], Kin On Kwok[8,9], Steven Riley[10,11], Jonathan Read[12], Justin Lessler[13,14,15], Derek A.T. Cummings[16,17*], C. Jessica E. Metcalf[18,19*]

[1]High Meadows Environmental Institute, Princeton University, Princeton, NJ, USA
[2]Department of Public and Ecosystem Health, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA
[3]Department of Biology, University of Florida, Gainesville, FL 32611, USA
[4]Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA
[5]Pandemic Sciences Institute, Nuffield Department of Medicine, University of Oxford, Oxford, UK
[6]School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong Special Administrative Region, China
[7]Guangzhou No. 13 Hospital, Guangzhou, Guangdong, 510620, China
[8]The Jockey Club School of Public Health and Primary Care, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China
[9]Hong Kong Institute of Asia-Pacific Studies, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China
[10]United Kingdom Health Security Agency, London, E14 4PU, United Kingdom
[11]MRC Centre for Global Infectious Disease Analysis, Jameel Institute for Disease and Emergency Analytics, Imperial College London, London, UK
[12]Lancaster Medical School, Lancaster University, Lancaster, LA1 4YW, United Kingdom
[13]Carolina Population Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[14]Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[15]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[16]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
[17]Department of Biomedical Engineering, Johns Hopkins Whiting School of Engineering, Baltimore, MD, USA
[18]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ, USA
[19]School of Public and International Affairs, Princeton University, Princeton, NJ, USA

*Corresponding author: C. Jessica E. Metcalf; cmetcalf@princeton.edu

Summary of Antibody Diversity Metrics

Yang and colleagues [1] developed a number of statistical metrics to quantify individual antibody profiles of HI titers against 21 tested A/H3N2 strains. In this study, we used three of them to assess sex differences, they are: the area under the antigenic landscape surface (Area Under the Curve, AUC), the breadth of antibody profile (Width, W), where the breadth is calculated above either detective (1:10) or protective threshold (1:40). The statistics are calculated as follows:

- Area Under the Curve (AUC) estimated the area under the curve of antibody profile as

$$AUC_{j,v} = \sum_{i=1}^{M-1} \frac{y_{i,j,v} + y_{i+1,j,v}}{2}(t_{i+1} - t_i)$$

Equation (1)

Where $AUC_{j,v}$ is the area-under the curve of titers by time for person j and visit v, M is the total number of included strains, $y_{i,j,v}$ is participant j's log-titer against strain i at visit v, and t_i is the time of isolation of strain i.

- Width (W) defines the proportion of time during which the antibody profile that is greater than or equal to some predefined antibody titer cutoff, Z. Here, we focus on the commonly used cutoff $Z = 1{:}40$. When performing the calculation, we transformed the threshold to log-scale based on the formula $z = \log_2(\frac{Z}{5})$. Hence,

$$W_{Z,j,v} = \sum_{i=1}^{M-1} W_{Z,j,v}(t_i, t_{i+1})$$

Equation (2)

where,

$$W_{Z,j,v} = \begin{cases} t_{i+1} - t_i, & y_{i+1,j,v} > z \text{ and } y_{i,j,v} \leq Z \\ 0, & y_{i+1,j,v} < z \text{ and } y_{i,j,v} < Z \\ \dfrac{(y_{i+1,j,v} - z)(t_{i+1} - t_i)}{y_{i+1,j,v} - y_{i,j,v}}, & y_{i+1,j,v} \geq z \text{ and } y_{i,j,v} < Z \\ \dfrac{(z - y_{i,j,v})(t_{i+1} - t_i)}{y_{i+1,j,v} - y_{i,j,v}}, & y_{i+1,j,v} < z \text{ and } y_{i,j,v} \geq Z \end{cases}$$

Equation (3)

When titers to all strains are above the threshold Z, the width for an individual given the tested strains is at its maximum:

$$\max\left(W_{Z,j,v}\right) = \sum_{i=1}^{M-1}\left(t_{i+1} - t_i\right) = t_M - t_1$$

Equation (4)

we present the width (ranges from 0 to 1) standardized by the maximum width in the results:

$$sW_{Z,j,v} = \frac{1}{t_M - t_1} W_{Z,j,v}$$

Equation (5)

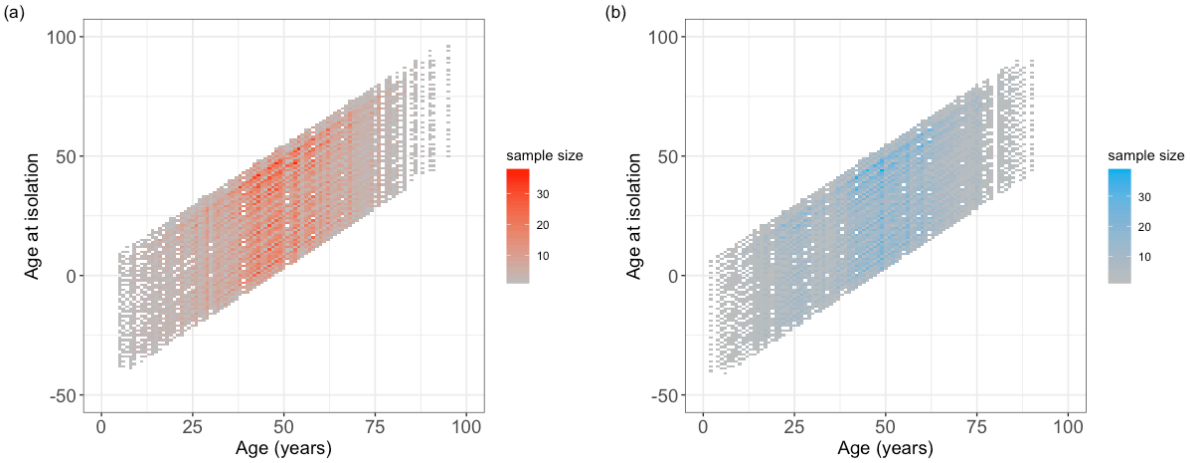

Figure S1. Number of observed titers (sample size) by age and age at isolation in females (left) and males (right).

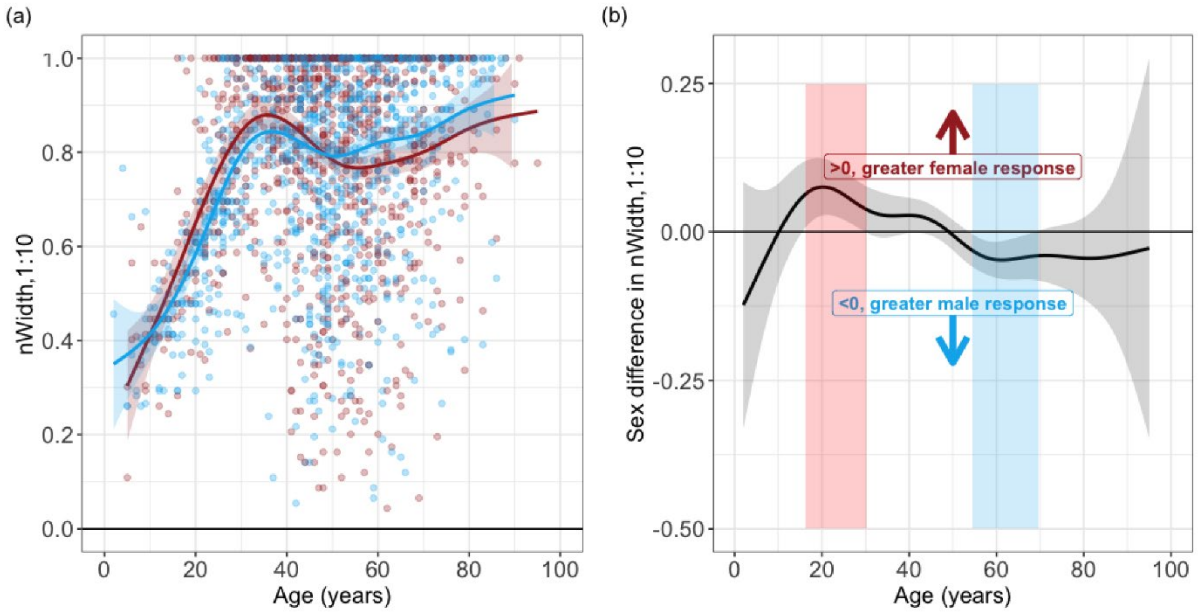

Figure S2. Summary metrics of antibody profiles by age and sex. (a) shows the width above a titer of 1:10 by sex. Metrics developed by Yang et al. 2021 [18] were calculated using pre-birth strains and post-birth strains and normalized by the total number of strains tested by the individual. Blue and red represent the metrics measured for serum collected from males and females, respectively. Solid lines are predictions of sex differences from a generalized additive model, and the grey band represents the corresponding 95% confidence band. (b) shows the model-predicted difference between sexes of width for titers ≥1:10. Lines with associated 95% confidence intervals above zero indicate a female bias while below zero represent a male bias. Significant differences e.g., the confidence intervals do not include 0, are marked with red areas when females have higher responses and blue areas when males have higher responses.

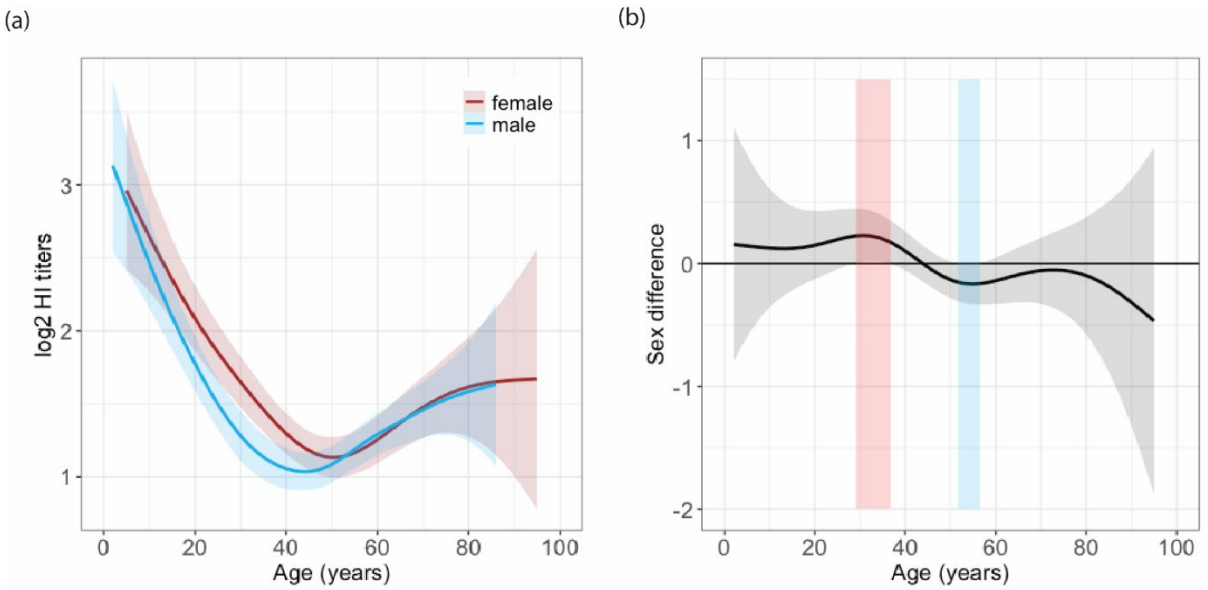

Figure S3. Marginal effects of age at sampling on antibody responses and its associated sex difference for F group. (a) shows fitted mean and 95% confidence intervals from the generalized additive model for log HI titers against the effect of age at time of testing for the only two strains, HK14 and TX12 in F group. (b) shows the fitted mean and 95% confidence intervals for sex difference of log HI titers shown in (a). Significant differences are marked with red areas when females have higher responses and blue when males have higher responses.

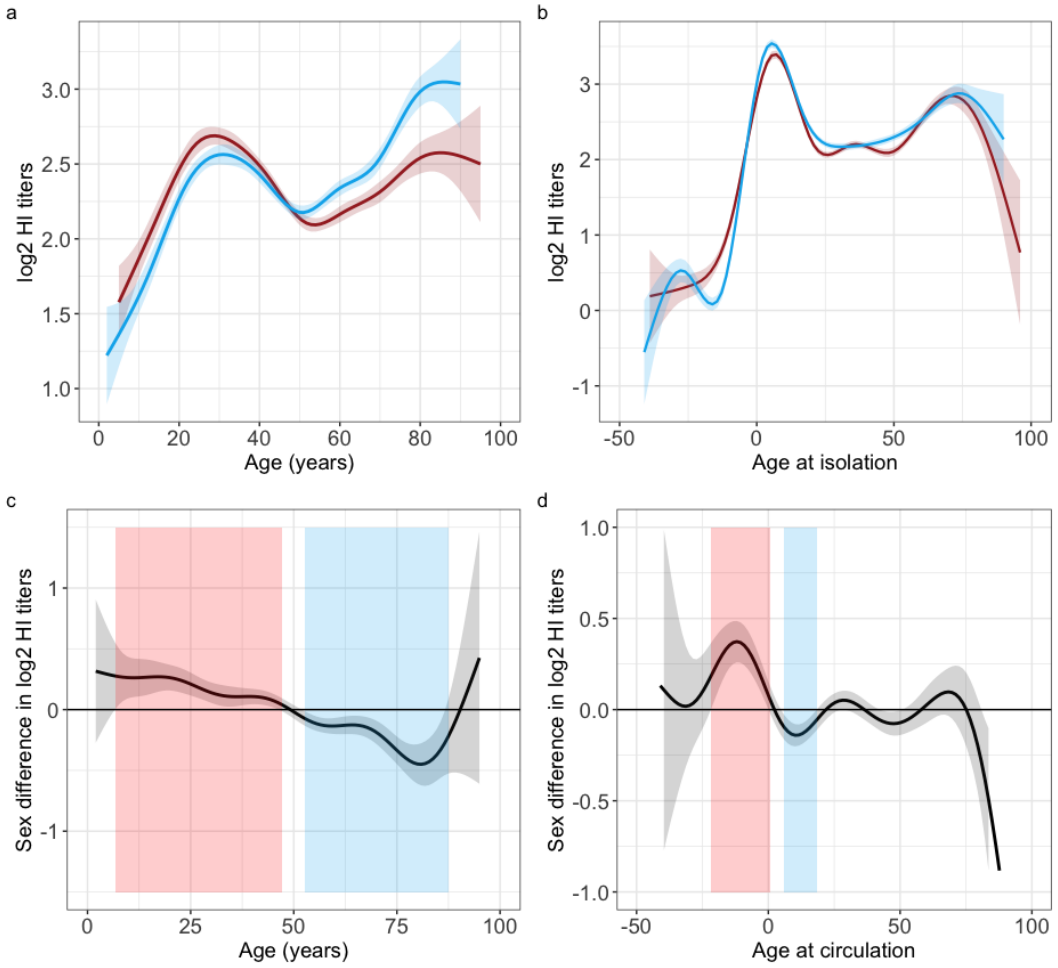

Figure S4. Marginal effects of age and age at strain isolation on antibody responses and its associated sex difference for all three groups defined in Figure 1. (a-b) show the model-predicted log HI titers against all studied strains against age and age at isolation. Blue and red represent the model-predicted log HI titers for males and females, respectively. (c-d) represent the sex difference in the corresponding predictions in (a) and (b). Solid lines are predictions of sex differences, and the grey band represents the corresponding 95% confidence band. Significant differences are marked with red areas when females have higher responses and blue areas when males have higher responses.

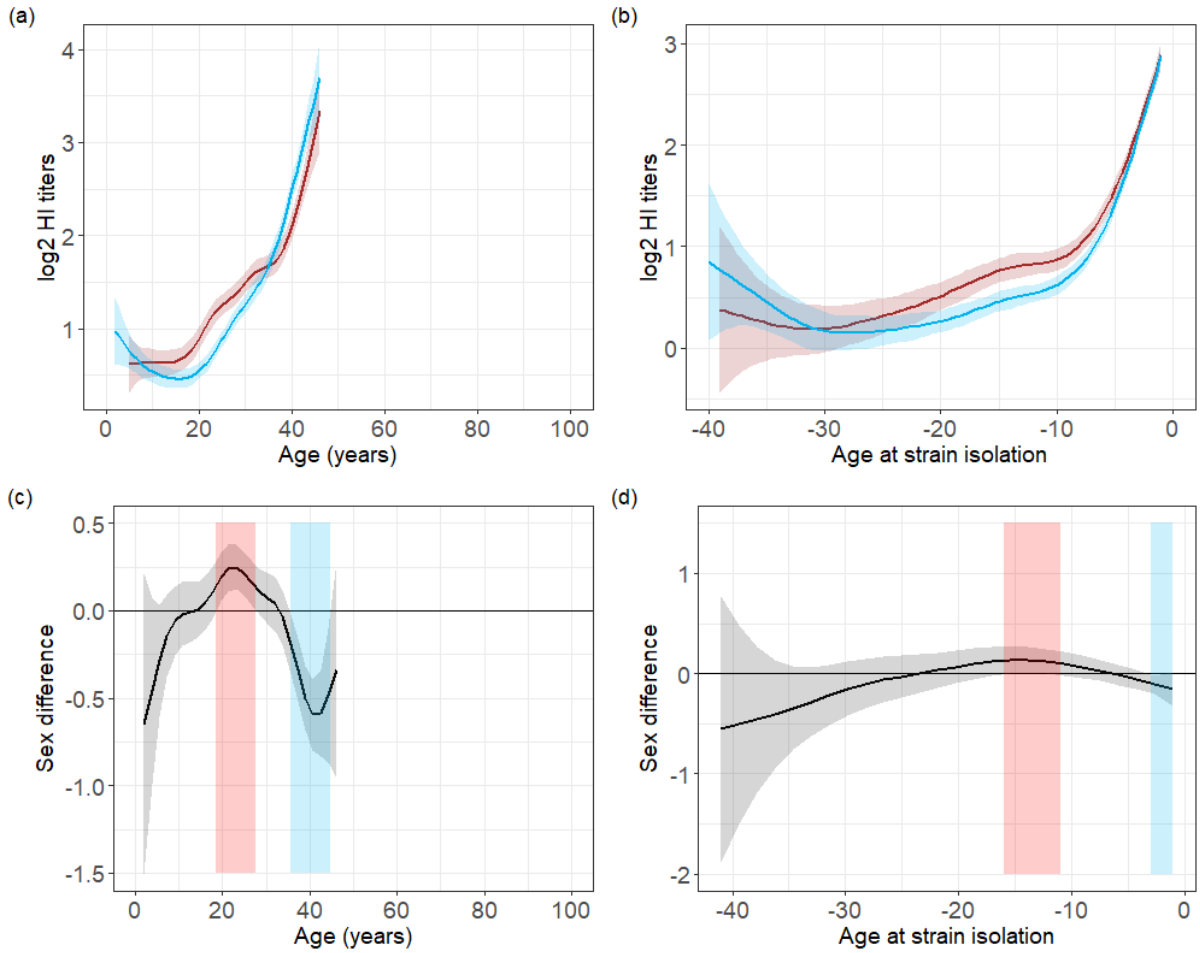

Figure S5. Marginal effects of age and age at strain isolation on antibody responses and its associated sex difference for B group. (a-b) show the model-predicted log2 HI titers against statins isolating before birth against age and age at strain isolation. Blue and red represent the model-predicted log HI titers for males and females, respectively. (c-d) represent the sex difference in the corresponding predictions in (a-b). Solid lines are predictions of sex differences, and the grey band represents the corresponding 95% confidence intervals. Significant differences are marked with red areas when females have higher responses and blue areas when males have higher responses.

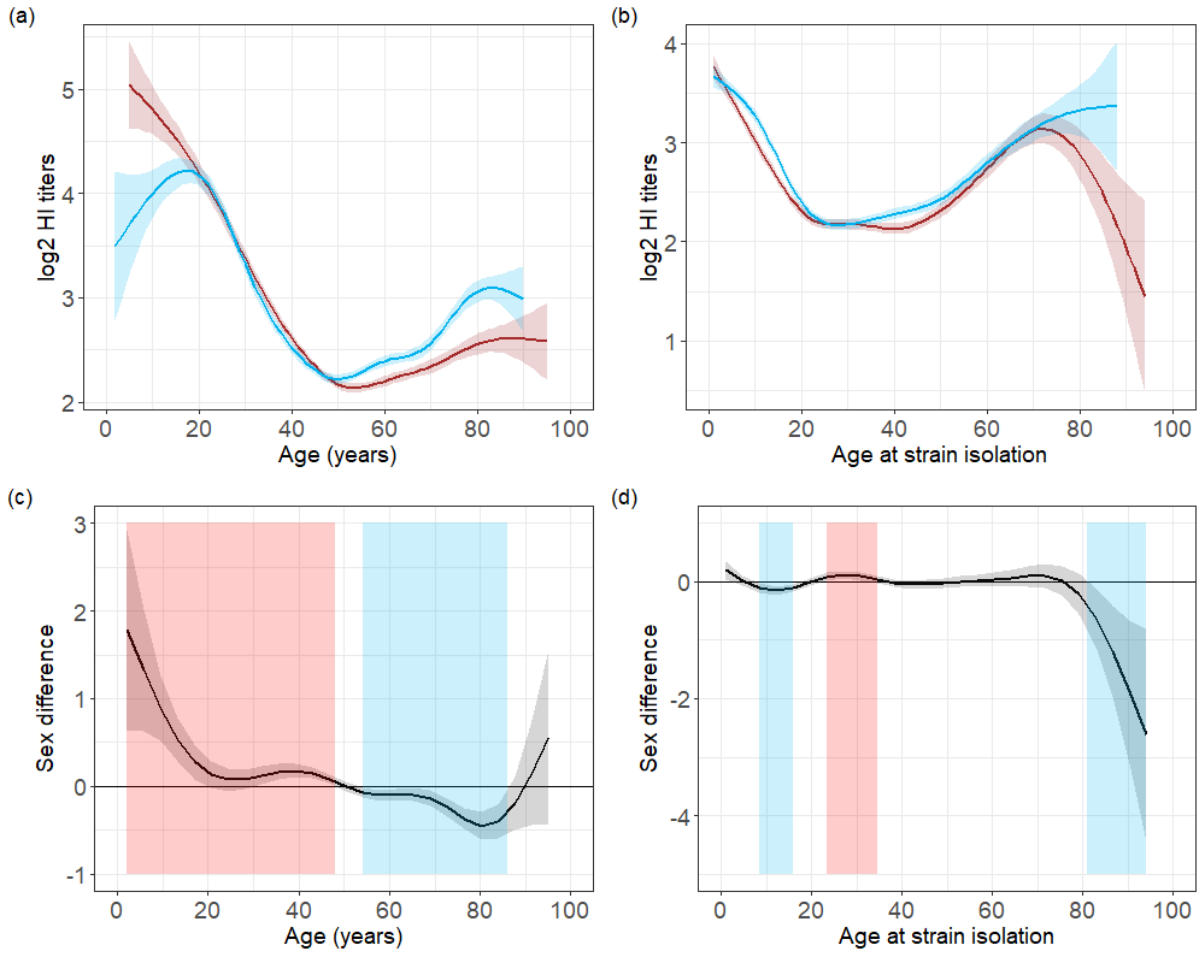

Figure S6. Marginal effects of age and age at strain isolation on antibody responses and its associated sex difference for L group. (a-b) show the model-predicted log2 HI titers against statins isolating during participants' lifetime against age and age at strain isolation. Blue and red represent the model-predicted log HI titers for males and females, respectively. (c-d) represent the sex difference in the corresponding predictions in (a-b). Solid lines are predictions of sex differences, and the grey band represents the corresponding 95% confidence intervals. Significant differences are marked with red areas when females have higher responses and blue areas when males have higher responses.

SI References

[1] Yang B, Lessler J, Zhu H, Jiang CQ, Read JM, Hay JA, Kwok KO, Shen R, Guan Y, Riley S, Cummings DA. Life course exposures continually shape antibody profiles and risk of seroconversion to influenza. PLoS Pathogens. 2020 Jul 23;16(7):e1008635.