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Supplemental information

Pre-infection antiviral innate immunity contributes to sex differences in SARS-CoV-2 infection

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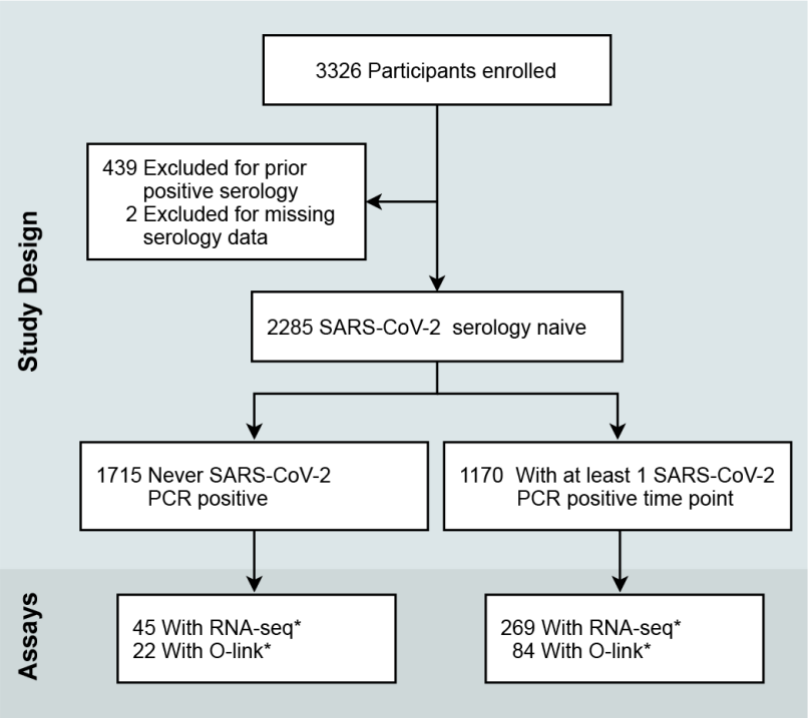
Supplementary Materials: Pre-infection antiviral innate immunity contributes to sex differences in SARS-CoV-2 infection



	Total	Male	Female
Initial study cohort	3326	3029	297
Excluded for prior positive serology	439	387	52
Excluded for missing serology data	2	1	1
Serologically naive	2885	2641	244
PCR never positive	1715	1608	107
RNA-seq*	45	32	13
O-link (serial)*	22	18	4
PCR positive at least once	1170	1033	137
RNA-seq*	269	226	43
Pre-infection†	222	197	25
First-infection†	89	58	31
Mid-infection†	203	177	26
O-link (serial)*	84	75	9

Fig. S1.

Study description.

* Numbers refer to unique participants with at least one sample, not total sample counts.

† Categories are non-exclusive, one subject can have samples across multiple time points.

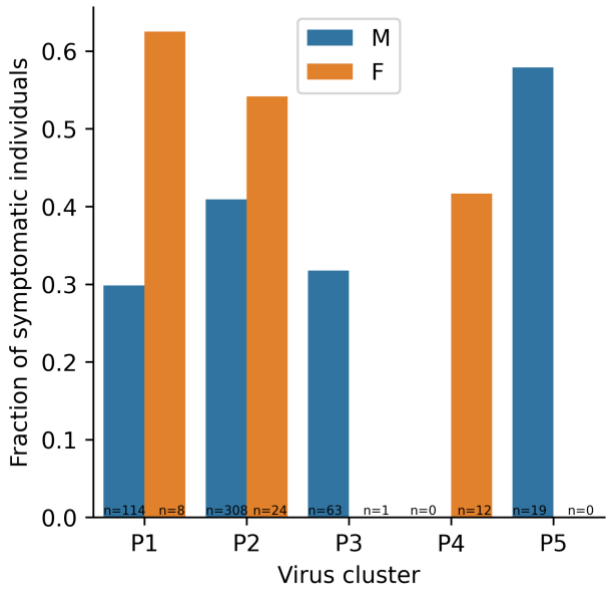


Fig. S2.

Symptomatic rate across sequenced virus clusters.

Phylogenetic analysis of over 600 infections revealed 5 main virus clusters that mostly infected individuals in the same company. For the only two clusters with multiple infections in both males and females, we observe higher symptomatic rates among females than males.

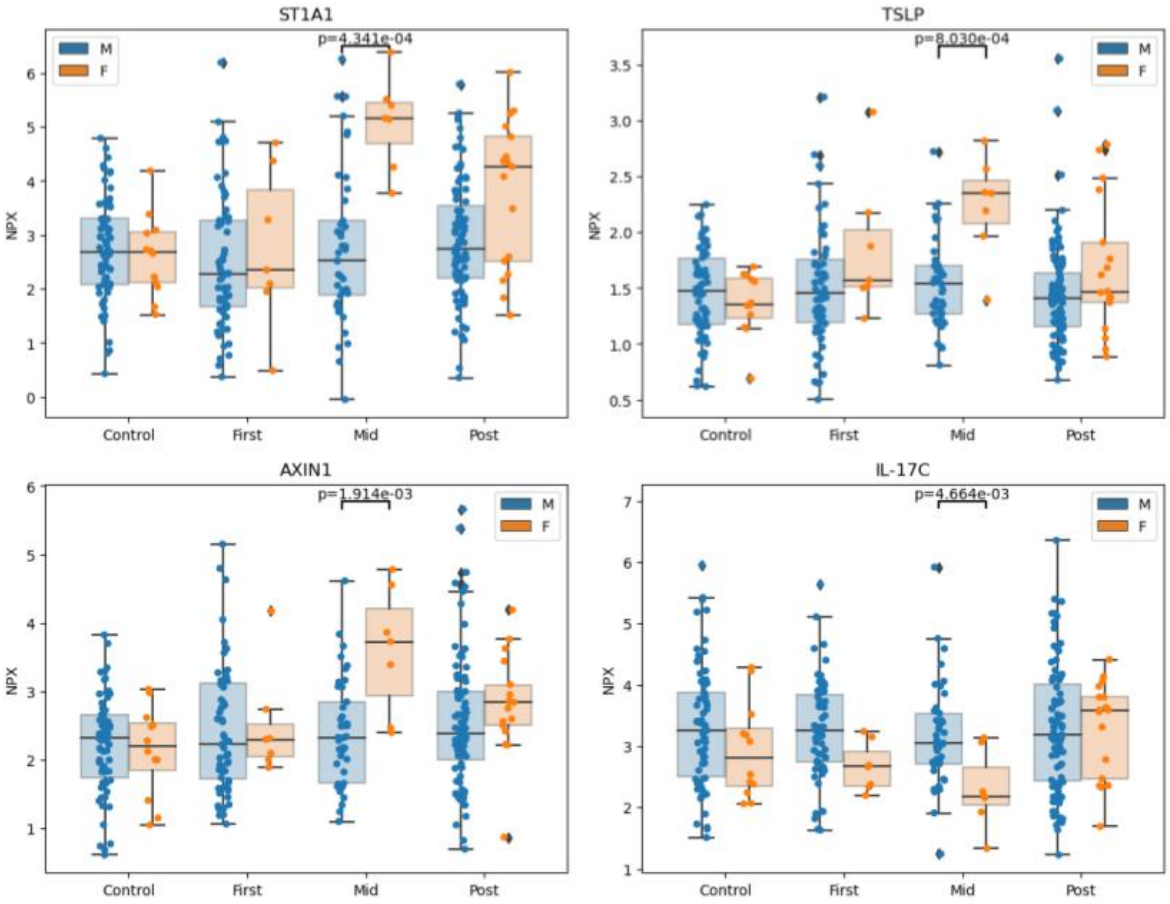


Fig. S3.

Examples of sex-specific inflammatory proteins. Boxplots show the normalized protein expression (NPX) levels of ST1A1 (upper left), TSLP (upper right), IL-17C (lower right), and AXIN1 (lower left). ST1A1, TSLP, and AXIN1 all show statistically significantly higher protein levels in Mid samples from females, while IL-17C shows lower levels in Mid female samples as compared to Mid male samples.

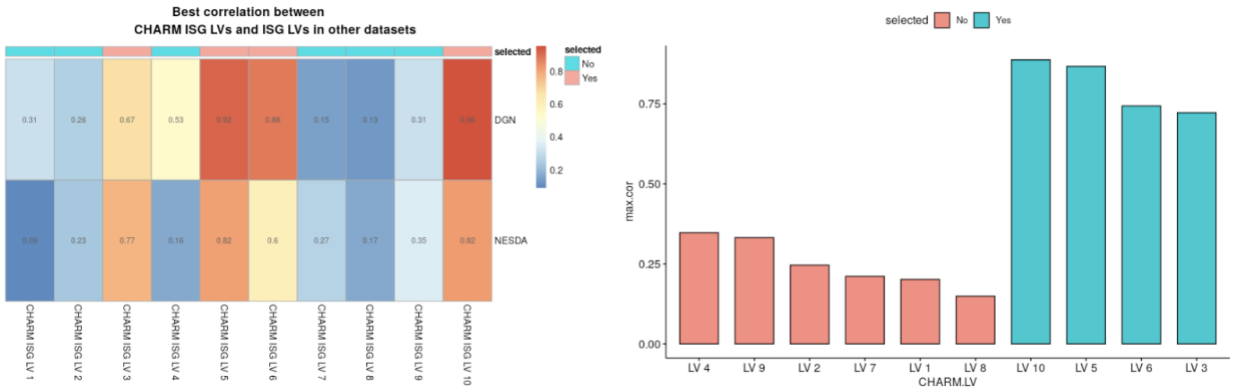


Fig. S4.

Factor analysis of ISG latent variables in CHARM and two independent cohorts. Matrix factorization resulted in 10 latent variables from the ISG levels of CHARM control samples. Four of these (LVs 3, 5, 6, and 10) showed significantly high correlations across all three data sets, as shown in the heatmap on the left, suggesting that these are biologically consistent across a broad population base. The bar plot on the right shows the maximum correlation values between data sets for all 10 LVs, highlighting the four functionally relevant that were selected for further analysis.

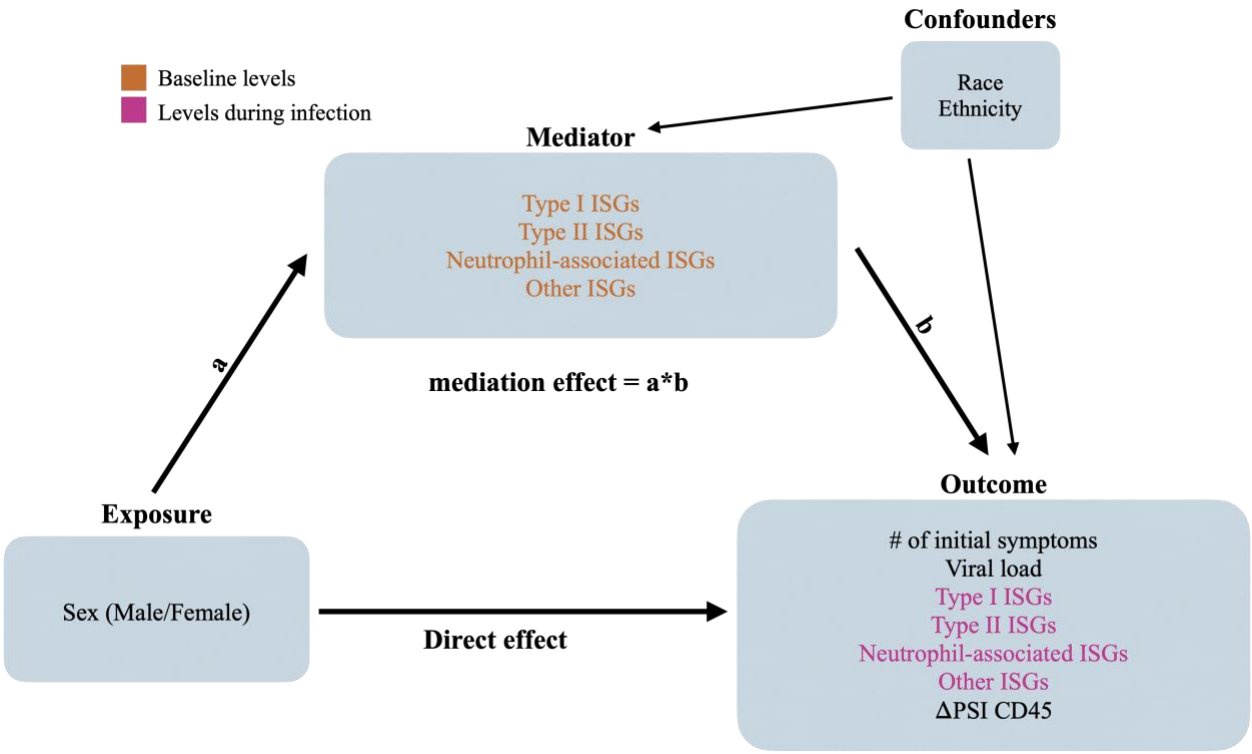


Fig. S5.
Causal mediation diagram shows the analytical framework for the causal mediation analysis performed. Each mediator was tested for significant mediation effect of the sex exposure for each outcome listed, while controlling for race and ethnicity. Additionally, we tested the mediation of all four ISG LVs during infection on the symptoms measured throughout infection.

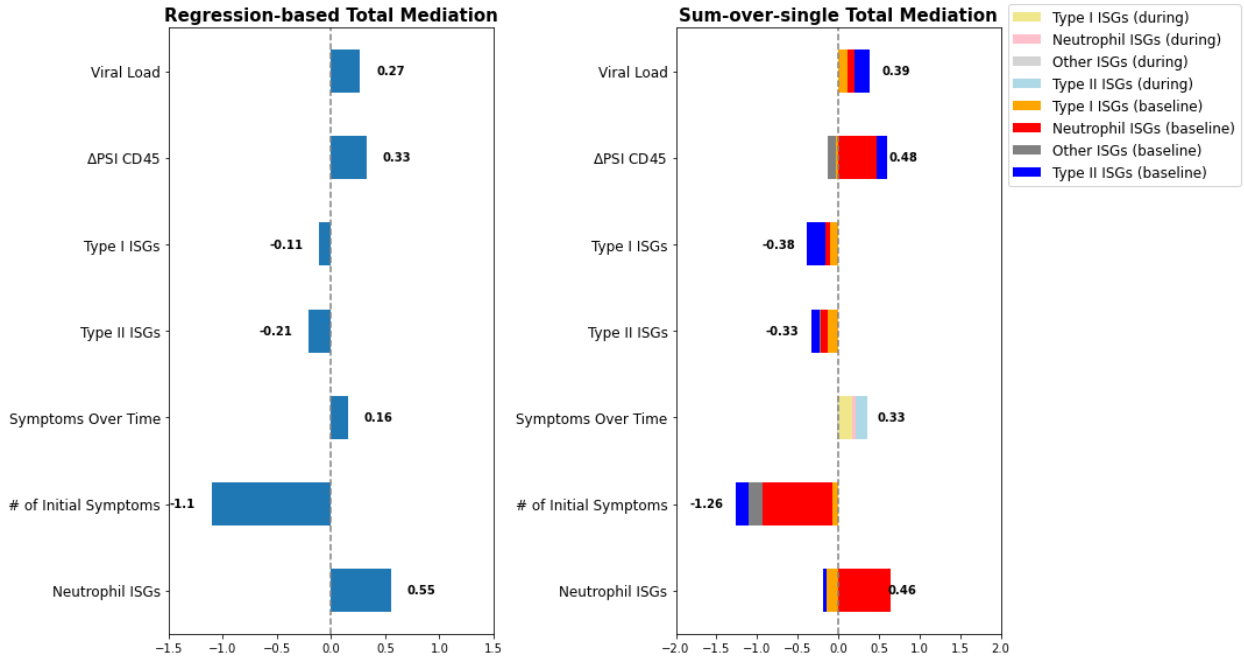


Fig. S6.

Multiple-mediator analysis largely agrees with the results from testing each mediator individually. In consideration of the possible correlations between mediators, multiple-mediator analysis was performed to compute the total mediation effect of each variable (left). These results are consistent with the sums of each individual mediation effect shown in the stacked bar chart (right). For both graphs, the x-axis shows the proportion of mediation effect.

Ethnicity	Number of participants
Non-Hispanic	1457
Hispanic	630
Not reported	798

Race	Number of participants
White	2121
Black	398
Multi-racial	80
Asian	69
Other	44
AI/AN	30
Hawaiian/OPI	12
Not reported	131

Age	Number of participants
18	1622
19	606
20	238
21	133
22	97
23	63
24	45
25	23
26	13
27	18
28	19
29	4
30	1
31	2
36	1

Sex	Number of participants
Male	2641
Female	244

Table S1.
Demographics of CHARM cohort.

Protein	Sex-specific	p-value
SCF	M	0.000895
IL-17C	M	0.004664
AXIN1	F	0.001914
TSLP	F	0.000803
TNFSF14	F	0.005521
SIRT2	F	0.005999
DNER	M	0.005076
CASP-8	F	0.003018
ST1A1	F	0.000434

Table S2.

O-link proteins with significant differences between males and females.