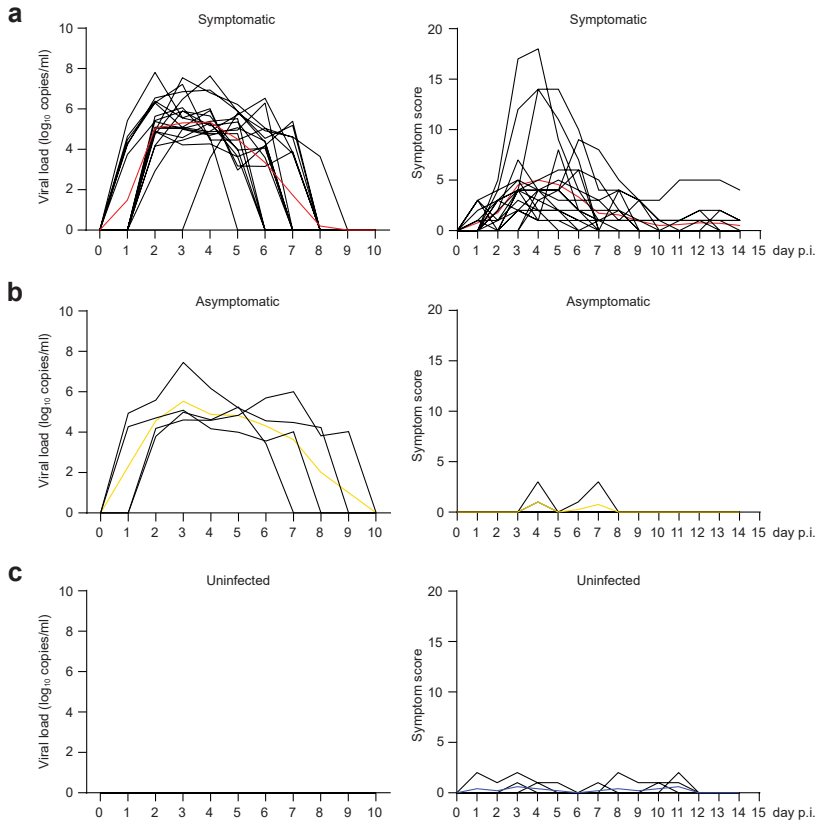
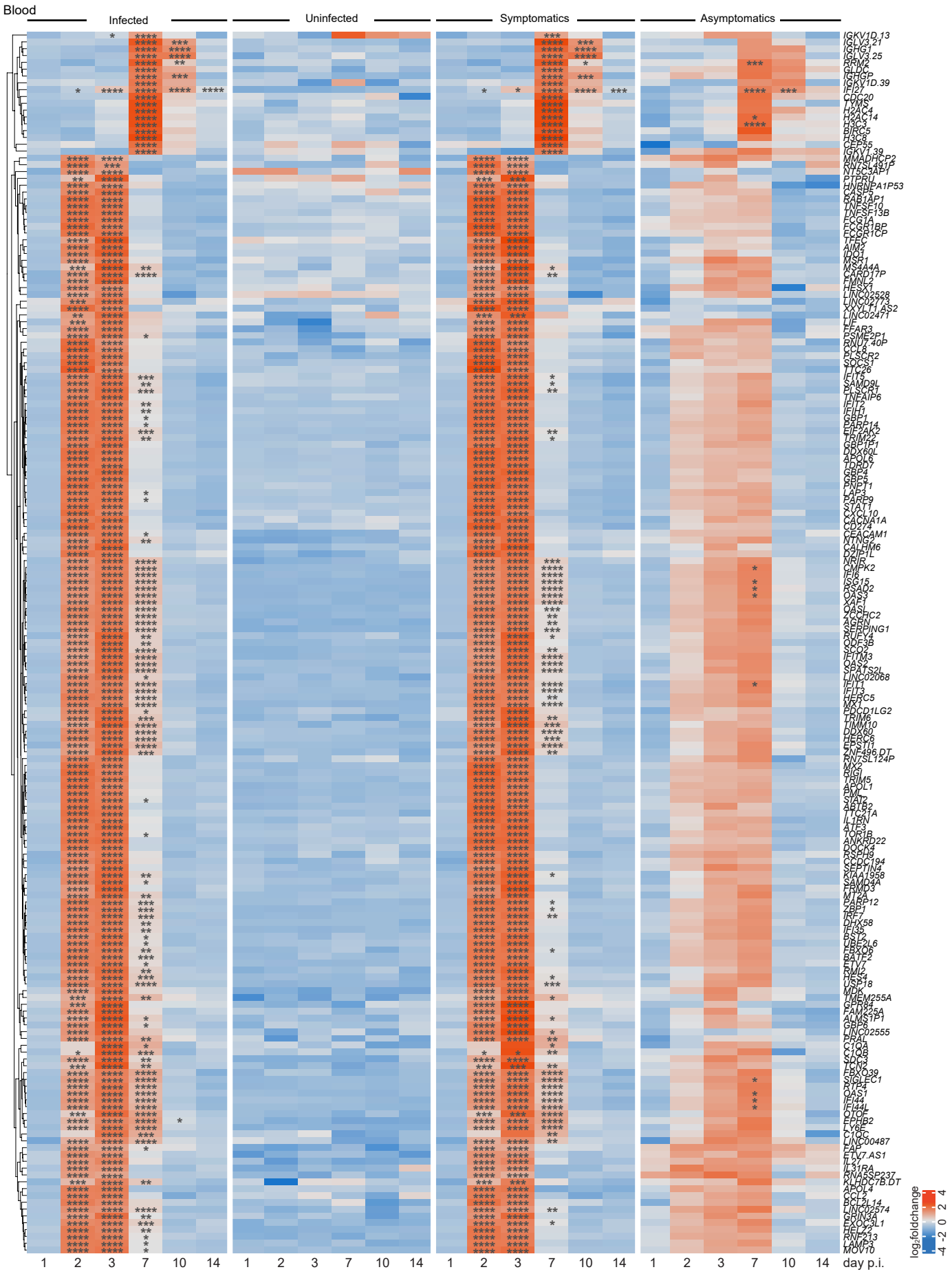


Innate immune responsiveness predicts enhanced cellular immunity and symptomatic disease after controlled human influenza infection

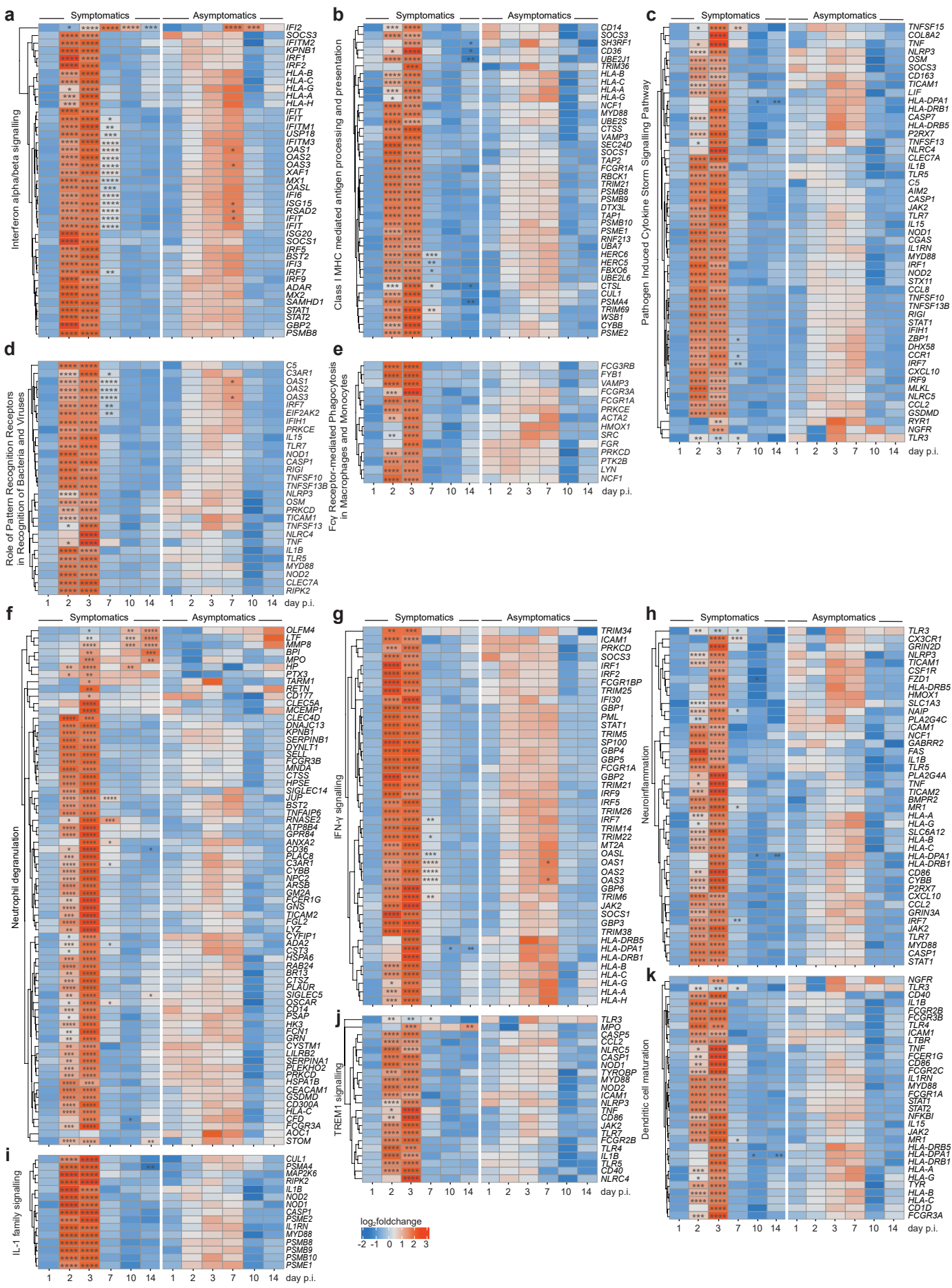
In the format provided by the authors and unedited



Supplementary Fig. 1. Individual viral loads and symptom scores. a-c, Individual viral shedding and symptom scores for symptomatic (n = 22) (a), asymptomatic (n = 4) (b), and uninfected (n = 5) participants (c). Coloured lines connect mean values per day.



Supplementary Fig. 2. Heatmap representation of the top DEGs in the blood post-inoculation. High-definition heatmap representation of the top DEGs ($\log_2$ fold-change > 2 and $P_{\text{adj}} < 0.01$) in the blood ($n = 179$) post-inoculation. The P -values reported are derived from DESeq2 analysis and indicate the statistical significance of differences in gene expression levels (normalised mean counts) between each post-inoculation timepoint and baseline (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).



Supplementary Fig. 3. Heatmaps of highly DEG-enriched pathways in blood associated with symptomatic and asymptomatic disease. a-k, Heatmap representation of DEGs involved in highly-enriched pathways in the blood of symptomatic (n = 13) and asymptomatic (n = 3) participants post-inoculation. The *P*-values reported are derived from DESeq2 analysis and indicate the statistical significance of differences in gene expression levels (normalised mean counts) between each post-inoculation timepoint and baseline (**P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001).

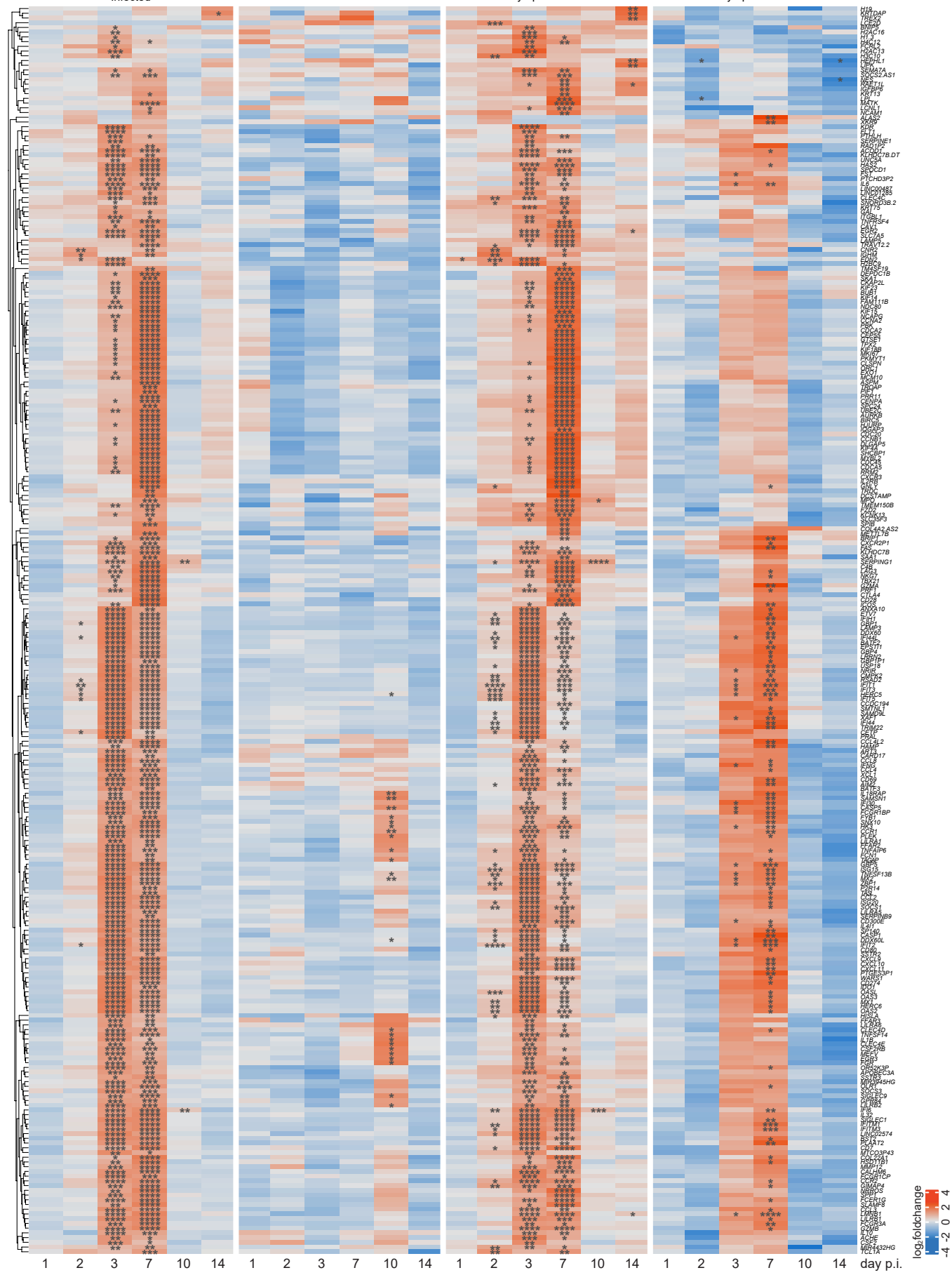
Nasal mucosa

Infected

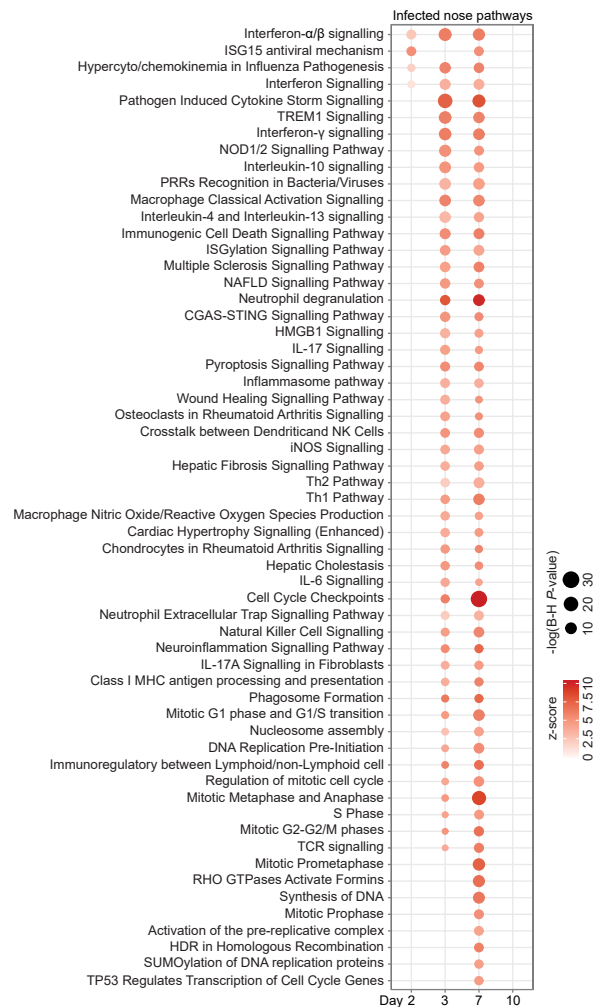
Uninfected

Symptomatic

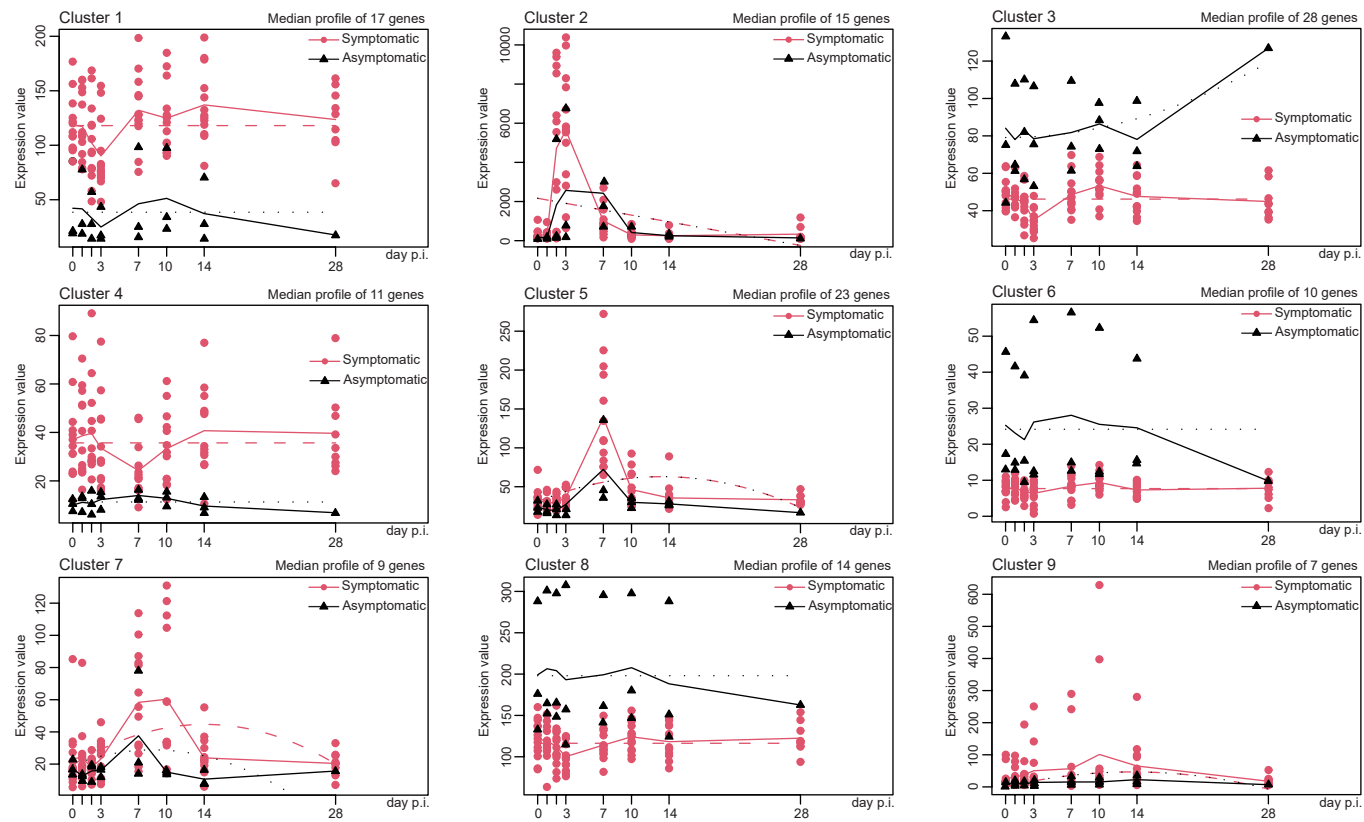
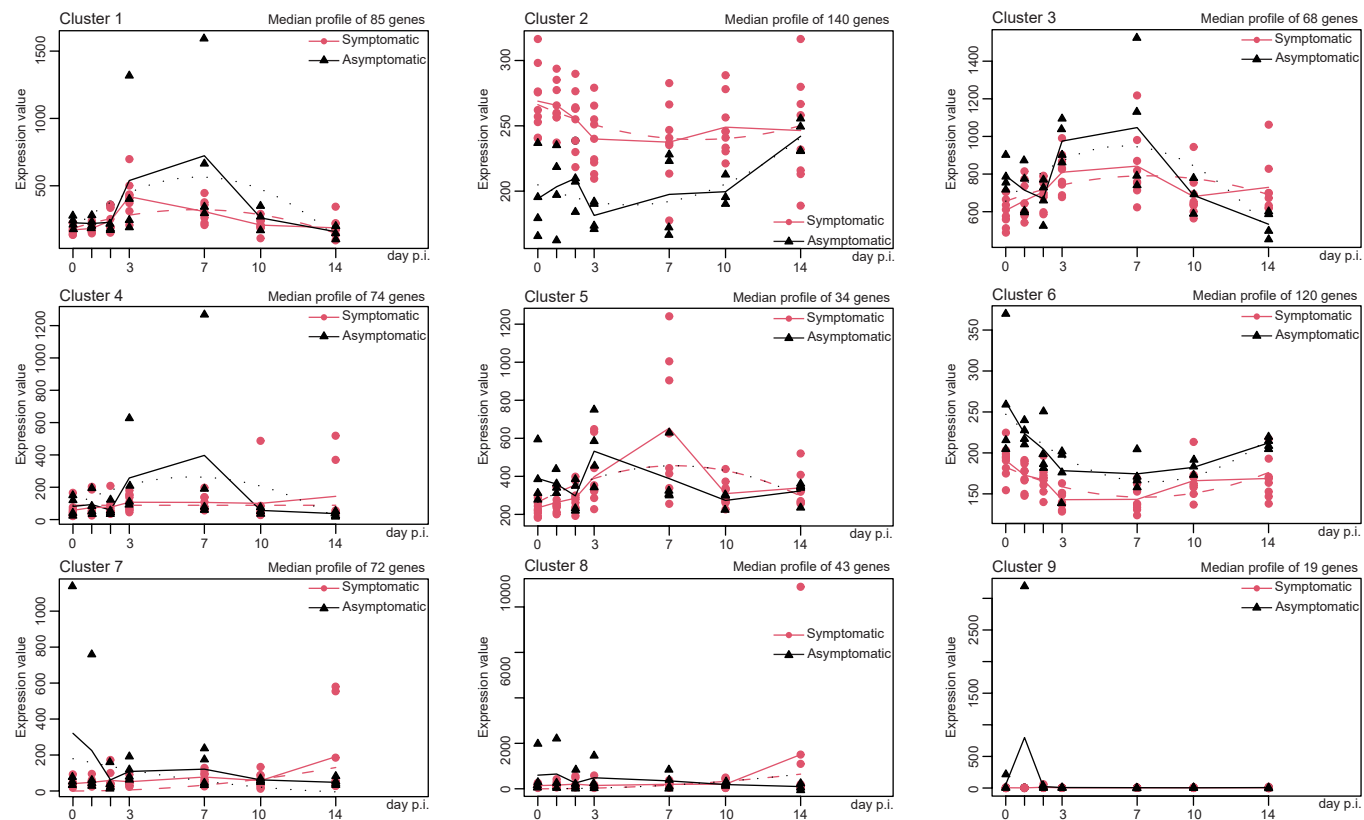
Asymptomatic



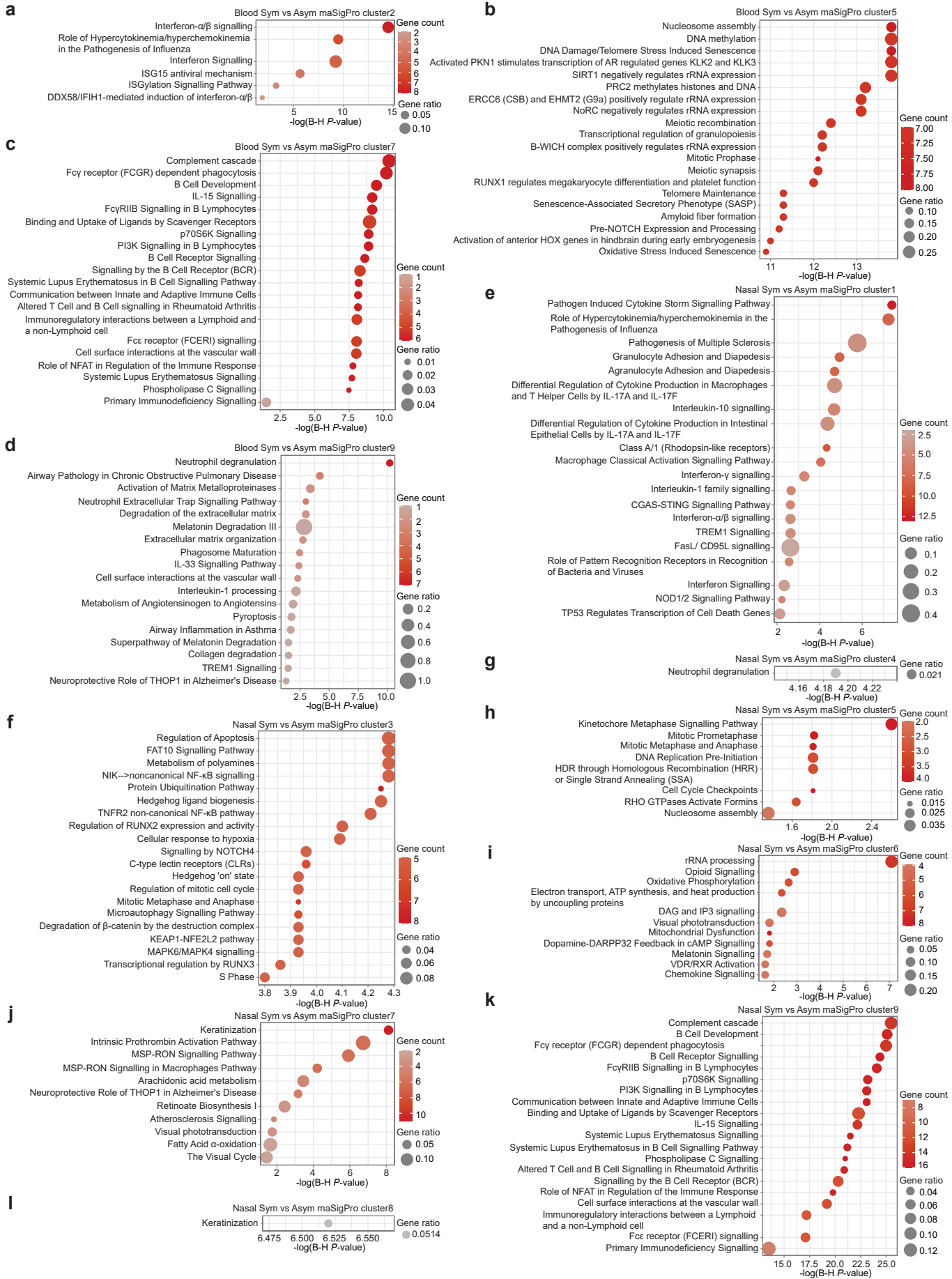
Supplementary Fig. 4. Heatmap representation of the top DEGs in the nasal mucosa post-inoculation. High-definition heatmap representation of the top DEGs ($\log_2$ fold-change > 2 and $P_{\text{adj}} < 0.01$) the nasal mucosa ($n = 264$) post-inoculation. The P -values reported are derived from DESeq2 analysis and indicate the statistical significance of differences in gene expression levels (normalised mean counts) between each post-inoculation timepoint and baseline (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).



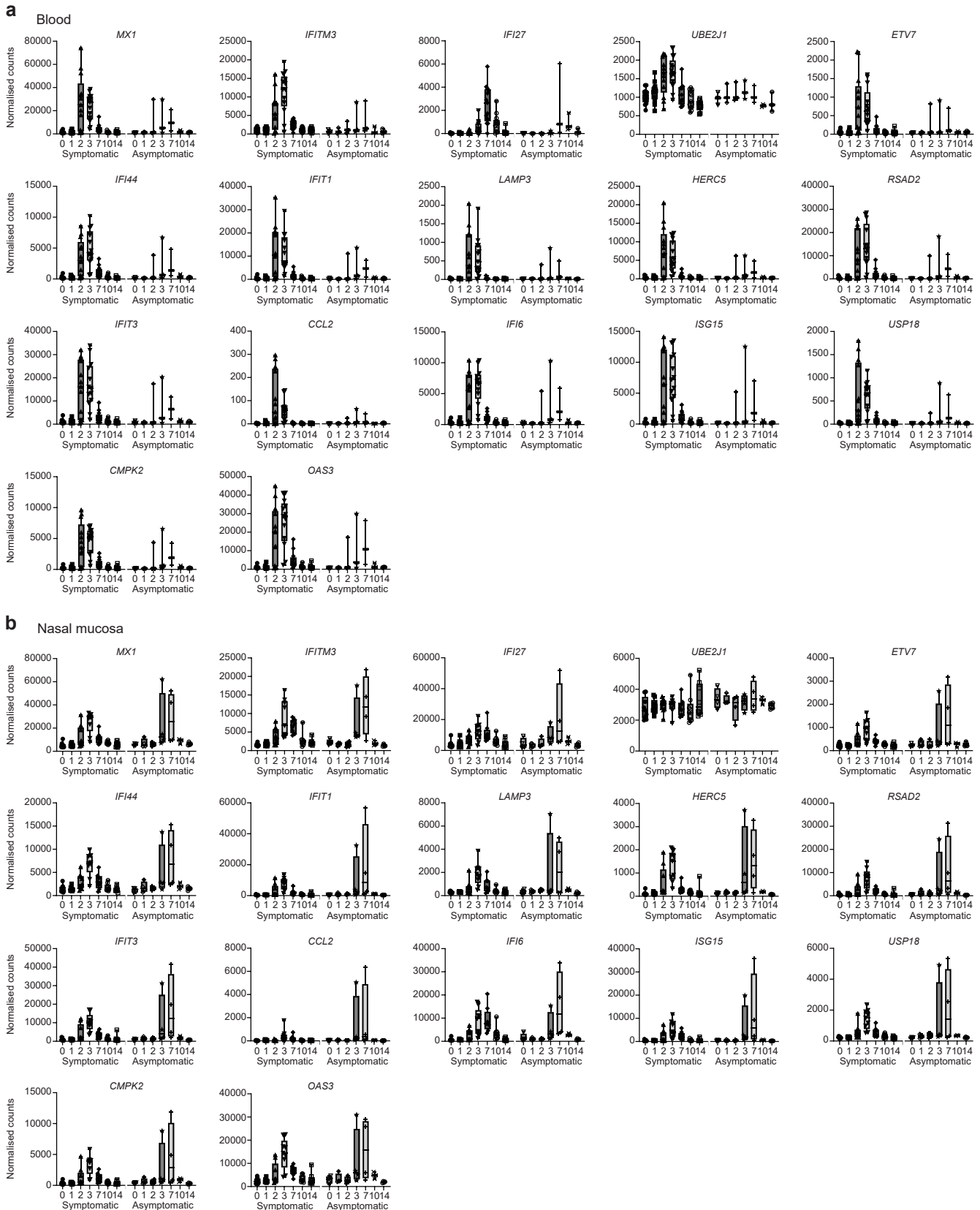
Supplementary Fig. 5. Extended analysis of the most significantly DEG-enriched pathways in the nasal mucosa post-inoculation. The most significantly DEG-enriched pathways ($B-H P_{adj} < 10^{-5}$, $z\text{-score} > 3.5$) in the nasal mucosa of infected ($n = 9$, symptomatic; $n = 4$, asymptomatic) participants post-inoculation are shown by IPA. Significance was assessed using a right-tailed Fisher's Exact Test. The P -values were adjusted using the Benjamini-Hochberg method for multiple hypothesis test correction.

a Blood**b** Nasal mucosa

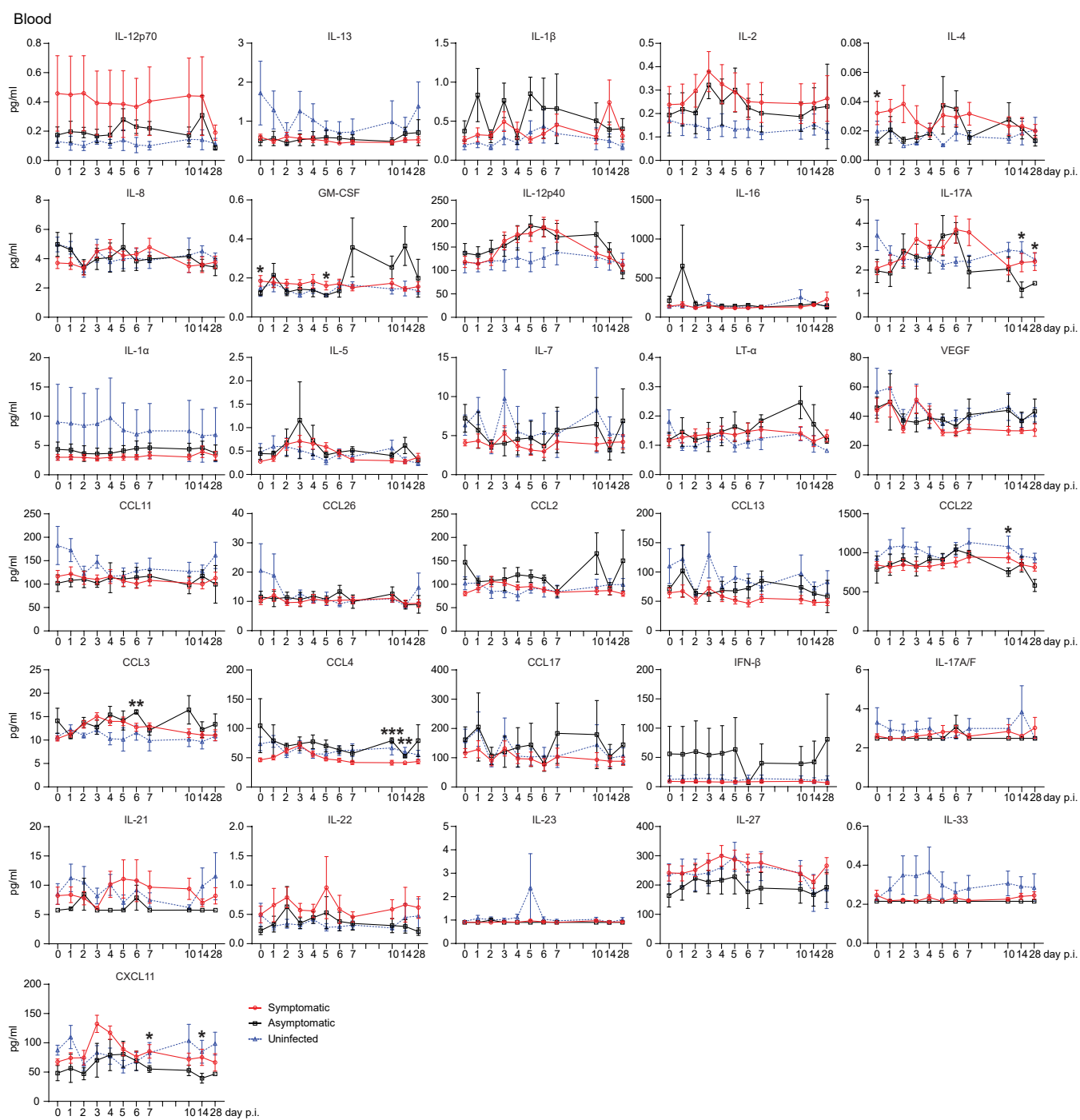
Supplementary Fig. 6. Gene clusters and temporal expression patterns of DEGs identified by maSigPro. a,b, Gene clusters and the temporal expression patterns were identified in the blood (**a**) of n = 13 symptomatic and n = 3 asymptomatic participants, and the nasal mucosa (**b**) of n = 9 symptomatic and n = 4 asymptomatic participants. Solid lines connect the median DEG expression values for each group of participants and the dashed lines show the regression curves fitted to the data.



Supplementary Fig. 7. maSigPro-clustered DEG-enriched pathways post-inoculation. a-l, Up to 20 most upregulated maSigPro-clustered DEG-enriched pathways in the blood (**a-d**) and the nasal mucosa (**e-l**) are visualised by IPA. Significance was assessed using right-tailed Fisher's Exact Tests. The *P*-values were adjusted using the Benjamini-Hochberg method for multiple hypothesis test correction. Only clusters that resulted in significantly enriched pathways (B-H $P_{\text{adj}} < 0.05$) are shown.

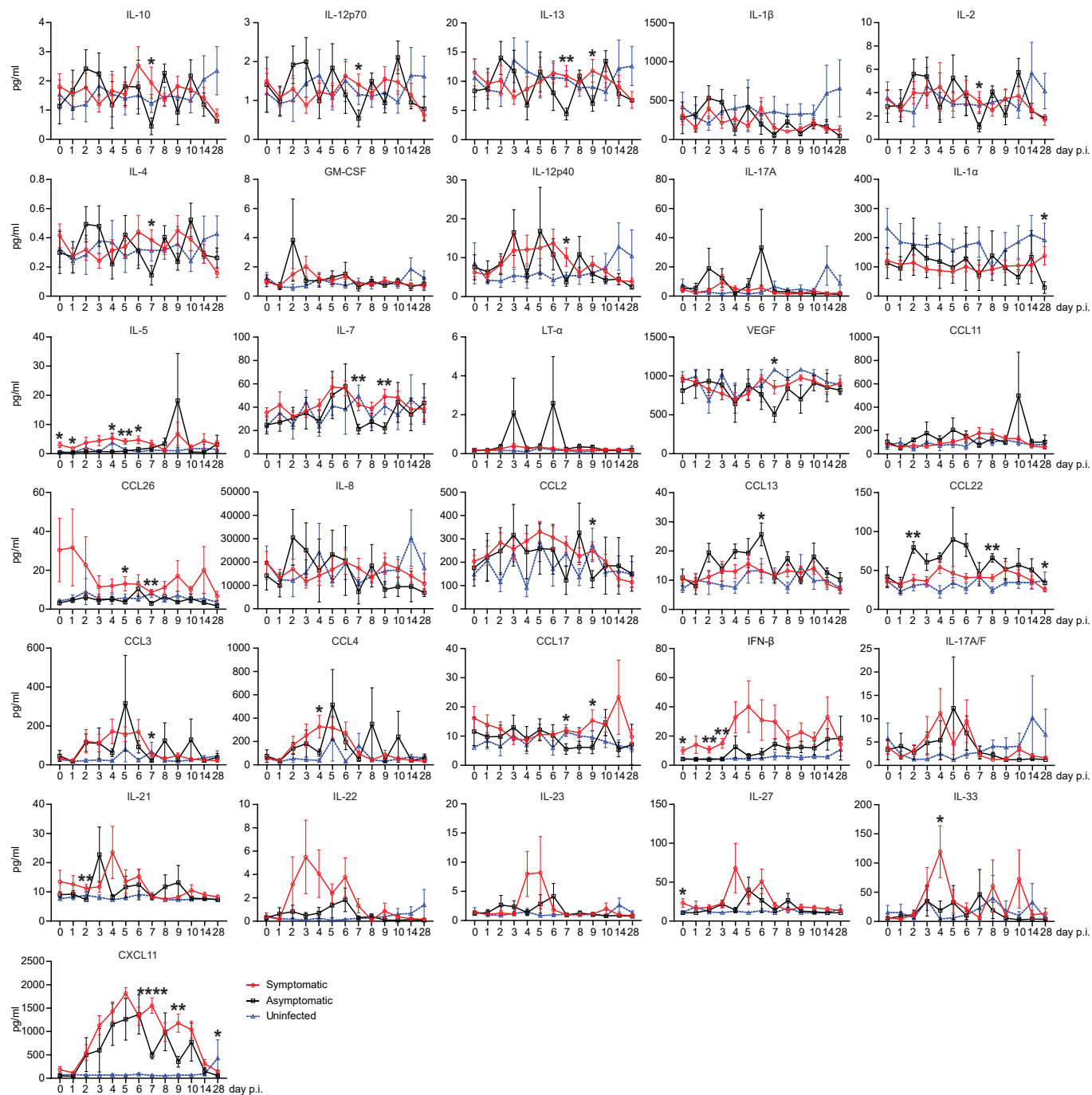


Supplementary Fig. 8. Longitudinal expression levels of canonical ISGs in the blood and the nasal mucosa. a,b, Gene counts of canonical ISGs on baseline (day 0 and day -14 pre-inoculation for blood [a] and nasal mucosa [b], respectively) and at days 1, 2, 3, 7, 10 and 14 p.i. for symptomatic (n = 13, blood; n = 9, nasal mucosa) and asymptomatic (n = 3, blood; n = 4, nasal mucosa) participants measured as described in Extended Data Fig. 1a. Data are min to max, showing all points.

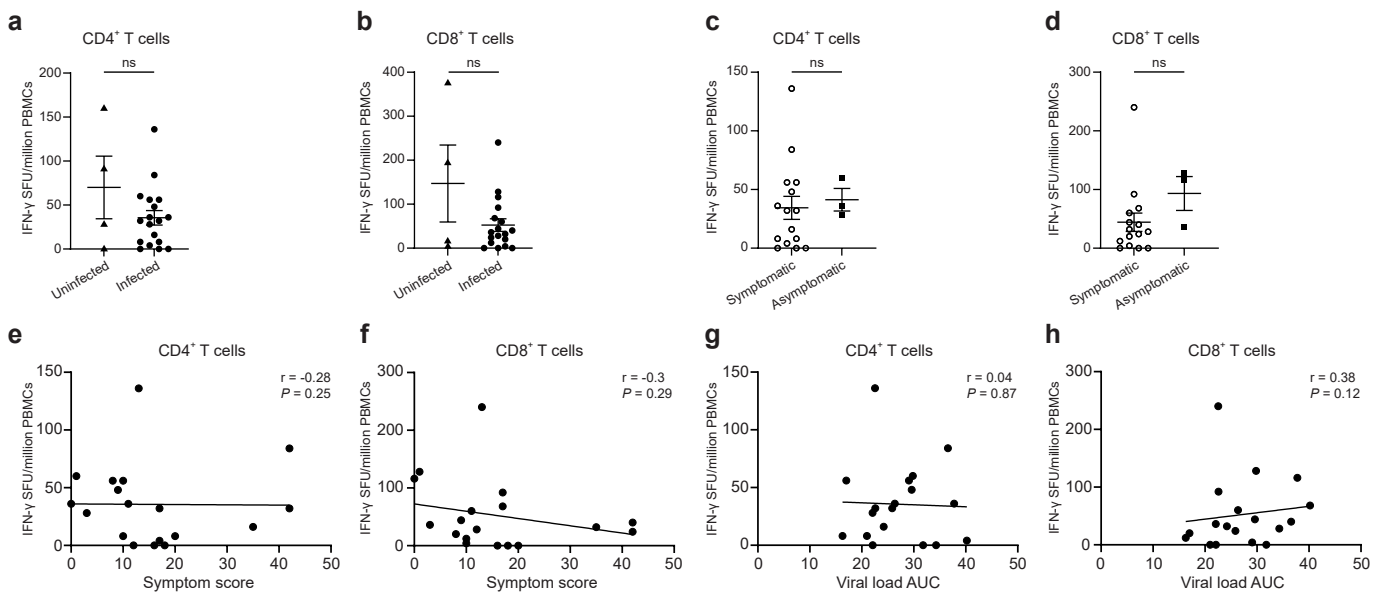


Supplementary Fig. 9. Additional soluble mediator concentrations in plasma. Cytokine and chemokine levels in plasma samples from symptomatic ($n = 18$), asymptomatic ($n = 4$) and uninfected ($n = 5$) participants as measured by Meso Scale Discovery. Data are raw concentrations at pg/ml and results are shown as mean concentrations $\pm$ S.E.M., every day from day 0 (pre-inoculation) until day 7 and at days 10, 14 and 28 p.i. Undetectable sample values were given the average lower limit of detection (LLOD) among all plates. Significance between symptomatic and asymptomatic participants was tested by a two-way linear mixed-effects model (REML) with Geisser-Greenhouse correction and post hoc pairwise comparisons were adjusted using the Holm-Šidák method (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). No statistical tests are shown for uninfected participants.

Nasal lining fluid



Supplementary Fig. 10. Additional soluble mediator concentrations in nasal lining fluid. Cytokine and chemokine levels in nasosorption samples from symptomatic (n = 18), asymptomatic (n = 4) and uninfected (n = 5) participants as measured by Meso Scale Discovery. Data are raw concentrations at pg/ml and results are shown as mean concentrations $\pm$ S.E.M., every day from day 0 (pre-inoculation) until day 10 and at days 14 and 28 p.i. Undetectable sample values were given the average lower limit of detection (LLOD) among all plates. Significance between symptomatic and asymptomatic participants was tested by a two-way linear mixed-effects model (REML) with Geisser-Greenhouse correction and post hoc pairwise comparisons were adjusted using the Holm-Šidák method (* P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001). No statistical tests are shown for uninfected participants.



Supplementary Fig. 11. Pre-existing influenza-specific T cell responses. **a-d**, CD4⁺ (**a**) and CD8⁺ (**b**) influenza-specific pre-existing T cell responses for infected (n = 18) and uninfected (n = 4) individuals assessed after Influenza peptide pool stimulation and IFN-γ ELISpot assay, and CD4⁺ (**c**) and CD8⁺ (**d**) influenza-specific pre-existing T cell responses for symptomatic (n = 15) and asymptomatic (n = 3) individuals. Influenza-specific T cell numbers are shown as spot-forming units (SFU) per 1 million PBMCs. Data are mean ± S.E.M. Significance was tested by two-sided Mann-Whitney U-tests. **e-f**, Spearman's correlation between symptom score and CD4⁺ (**e**) or CD8⁺ (**f**) Influenza-specific pre-existing T cell responses for infected participants (n = 18). **g,h**, Spearman's correlation between viral load area under curve (AUC) and CD4⁺ (**g**) or CD8⁺ (**h**) Influenza-specific pre-existing T cell responses for infected participants (n = 18). Significance was assessed using Spearman's rank correlation coefficient (two-sided).