SUPPLEMENTARY INFORMATION

Lethality of SARS-CoV-2 infection in K18 human angiotensin converting enzyme 2 transgenic mice

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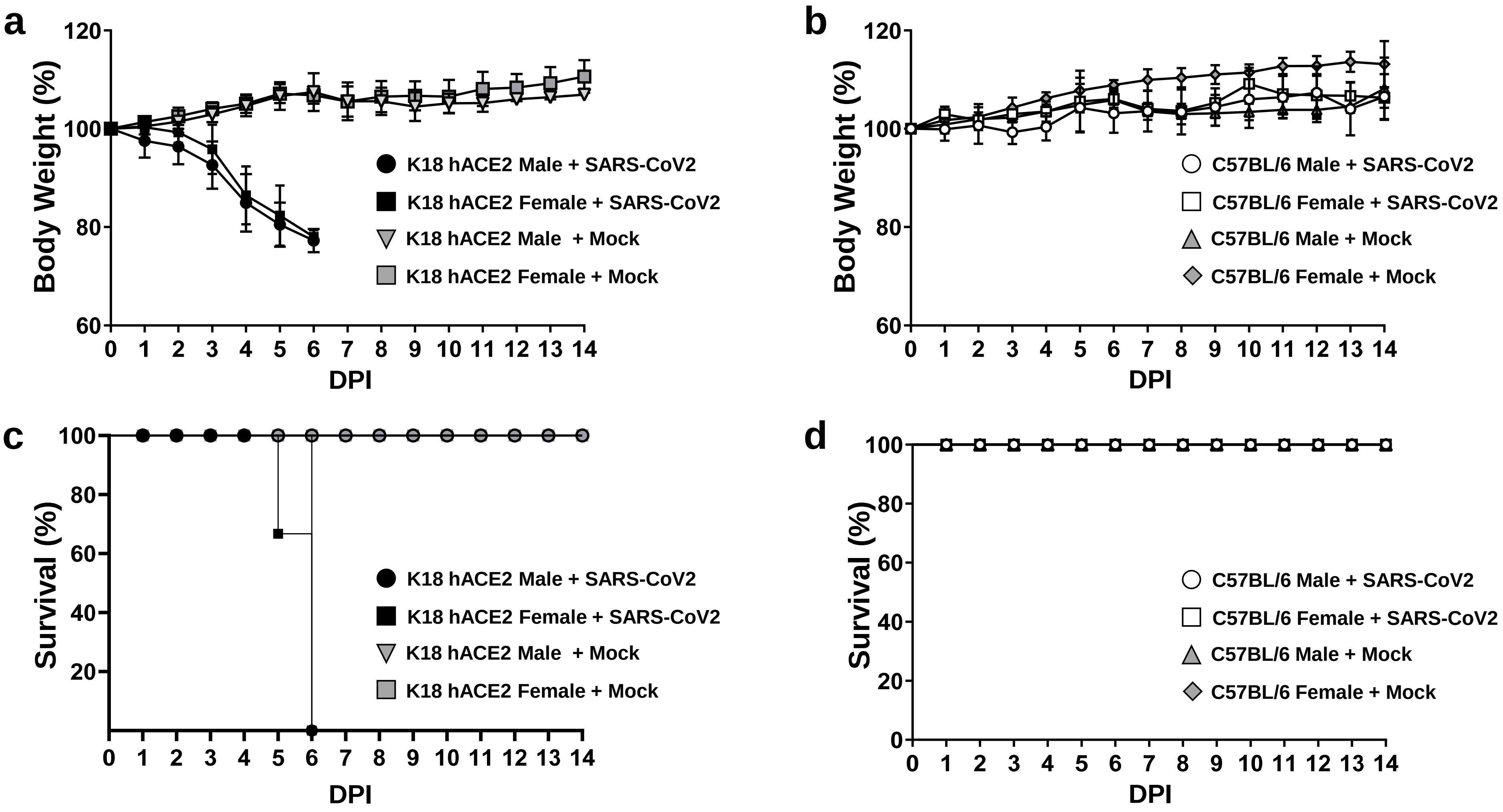
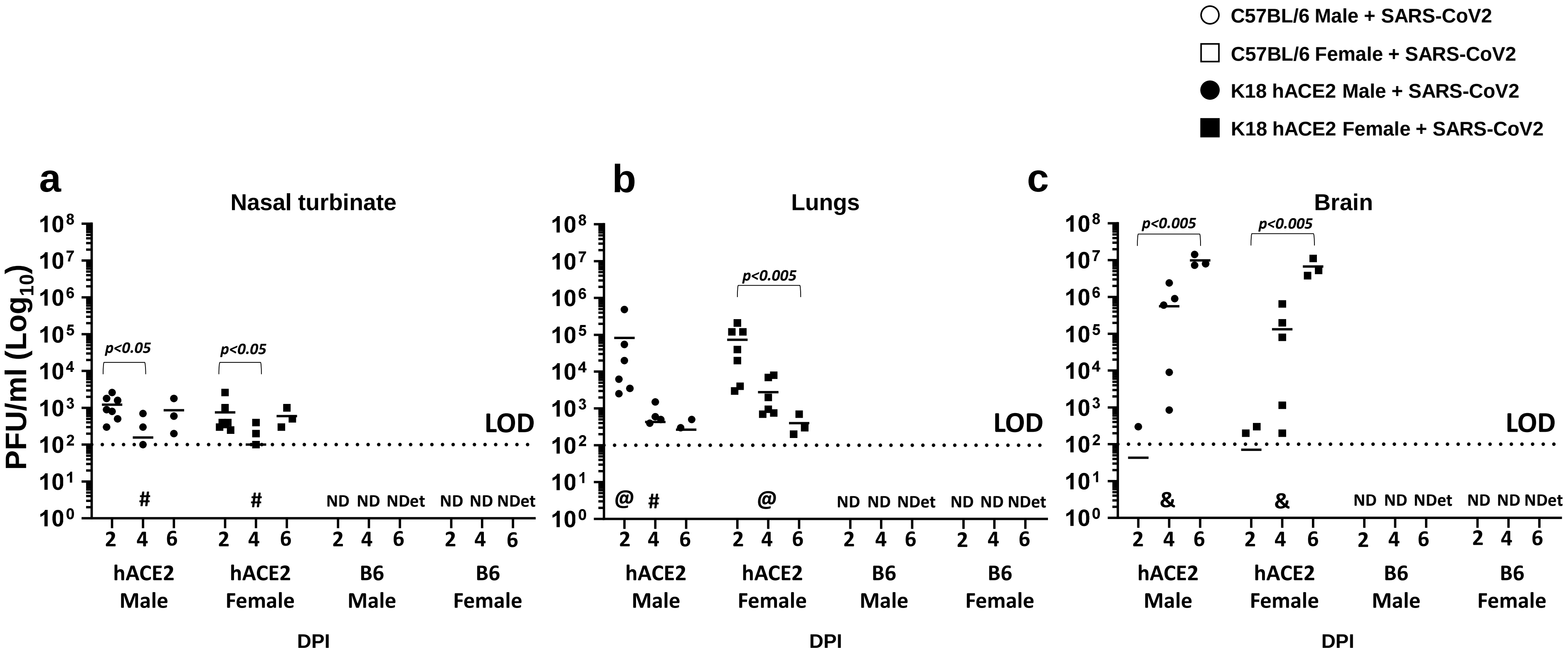
Supplementary Table 1. Evaluation of SARS-CoV-2 infected K18 hACE2 transgenic C57BL/6 mice in Lung and Brain Severity Scored. When not indicated, observed in the majority of females and males

Mouse	DPI**	Lung	Brain	Mouse	DPI**	Lung	Brain	Mouse	DPI**	Lung	Brain
1	2	2	0	1	4	1	0	1	6	1	1
2	2	1	0	2	4	2	0	2	6	1	1
3	2	1	0	3	4	1	1	3	6	2	2
4	2	2	0	4	4	2	0	4	6	3	2
5	2	1	0	5	4	3	0	5	6	3	3
6	2	1	0	6	4	3	1	6	6	4	2

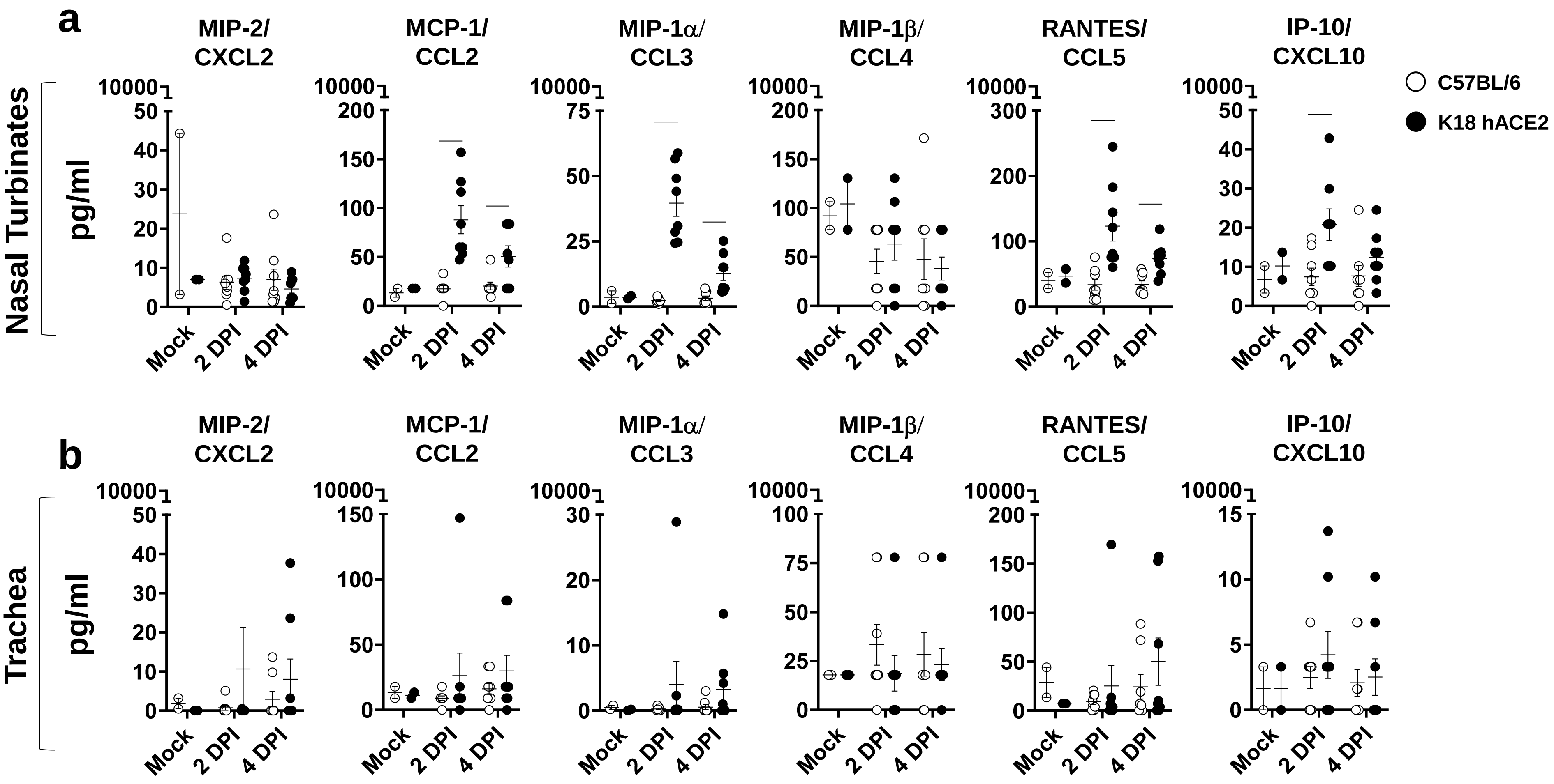
**Severity score for lung and brain overtime. 0 = normal; 1 = minimal (≤10%); 2 = mild (11-30%), 3 = moderate (30-60%), 4 = marked (60-80%), 5+ severe (>80%). DPI: Days post-infection. Different individual mice assessed (#1 to #6)



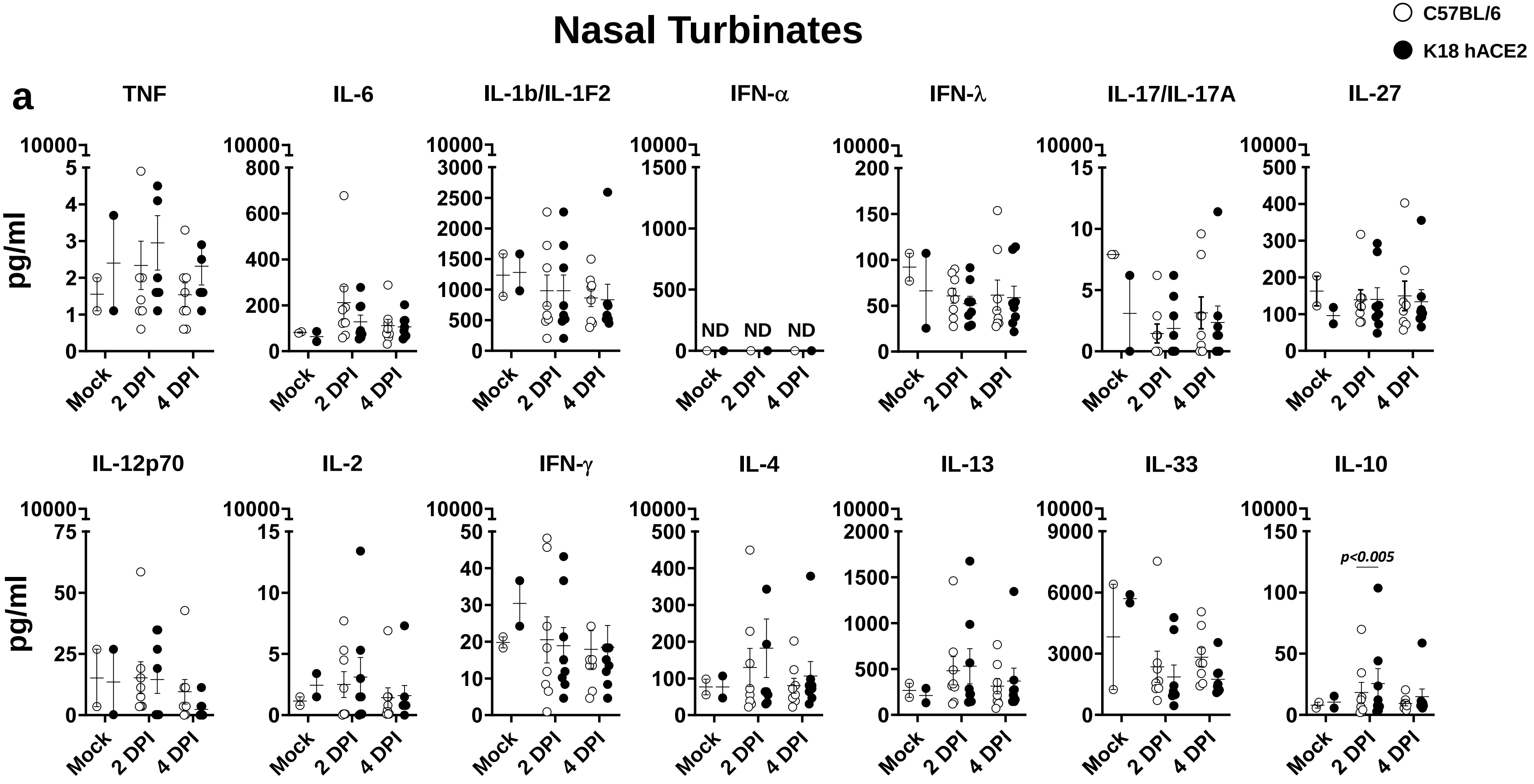
Supplementary Figure 1. Infection of K18 hACE2 transgenic and WT C57BL/6 mice with SARS-CoV-2. Male (n=7) and female (n=7) K18 hACE2 transgenic, and female (n=4) and male (n=4) WT C57BL/6 mice were infected or mock-infected (n=3/mouse strain/sex) i.n. with 1 x 10^5 PFU of SARS-CoV-2. Body weight (**a**, **b**) and survival (**c**, **d**) were evaluated at the indicated DPI. Mice that loss more than 25% of their initial body weight were humanely euthanized. Error bars represent standard deviations (SD) of the mean for each group of mice. DPI: Days post-infection. Data are combined from 2 independent experiments. Source data are provided as a Source Data file.



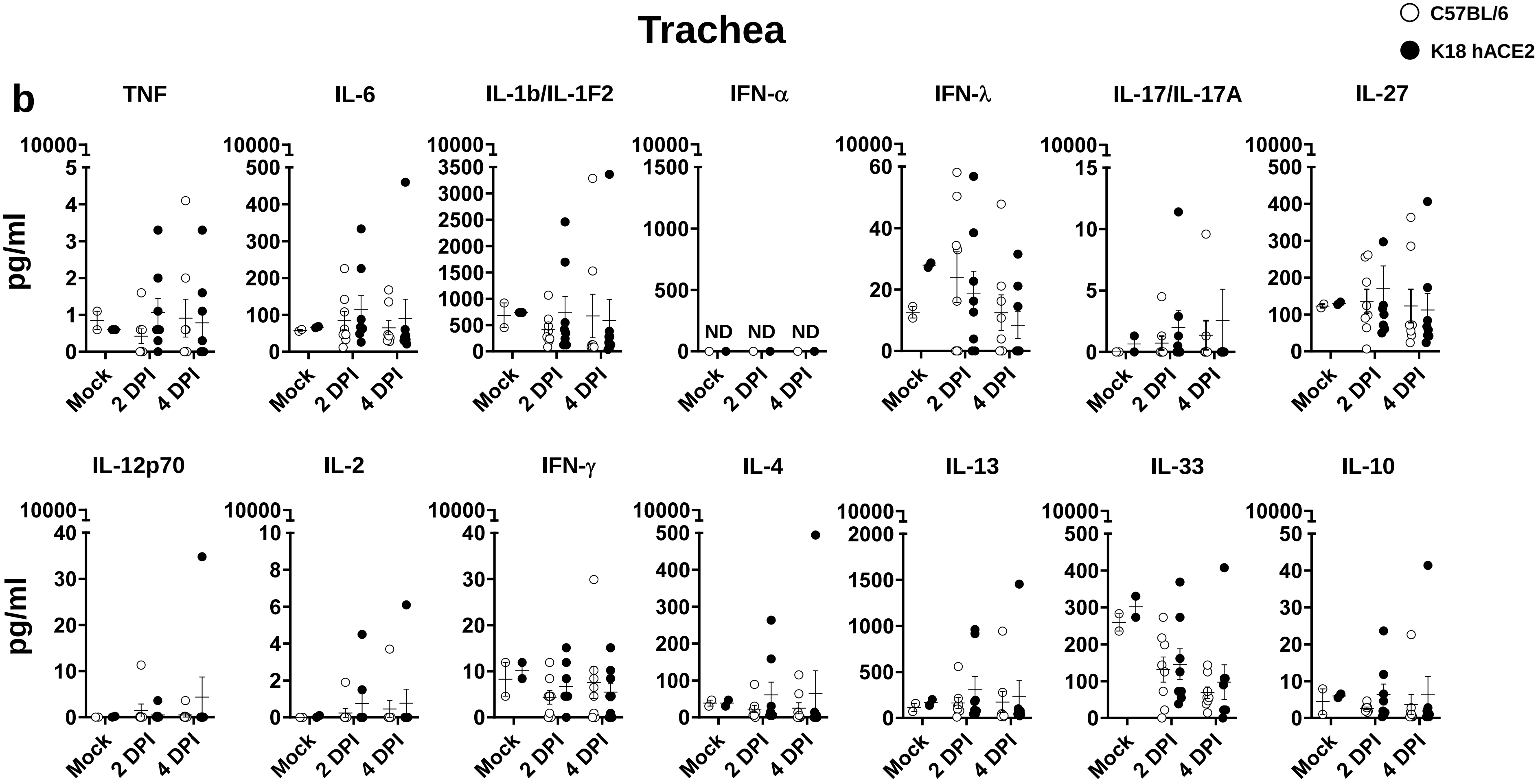
Supplementary Figure 2. Viral loads in male and female K18 hACE2 transgenic and WT C57BL/6 mice infected with SARS-CoV-2. K18 transgenic hACE2 male (n=17 mice distributed as n=7 for 2-DPI, n=7 for 4-DPI and n=3 for 6-DPI) and female (n=17 mice, distributed identically as the males) and WT C57BL/6 male (n=8 mice distributed as n=4 for 2-DPI and n=4 for 4-DPI) and female (n=8 mice distributed identically as the males) infected as in Figure S1 and sacrificed at 2- and 4-DPI and viral titers in different organs (nasal turbinate, trachea, lung, brain, heart, kidney, liver, spleen, small intestine, and large intestine) were determined by plaque assay (PFU/ml). Only data from virus containing organs and/or tissue samples are shown: nasal turbinate (**a**), lungs (**b**) and brain (**c**). Error bars represent standard deviations (SD) of the mean for each group of mice. Symbols represent data from individual mouse, and bars the geometric means of viral titers, $p < 0.05$; $p < 0.005$. @, virus not detected in one mouse; &, virus not detected in two mice; #, virus not detected in three mice; ND, not detected; NDet, non-determined. Dotted black lines indicate the limit of detection (10^2 PFU/ml). DPI: Days post-infection. Data are combined from of 2 independent experiments. Source data are provided as a Source Data file.



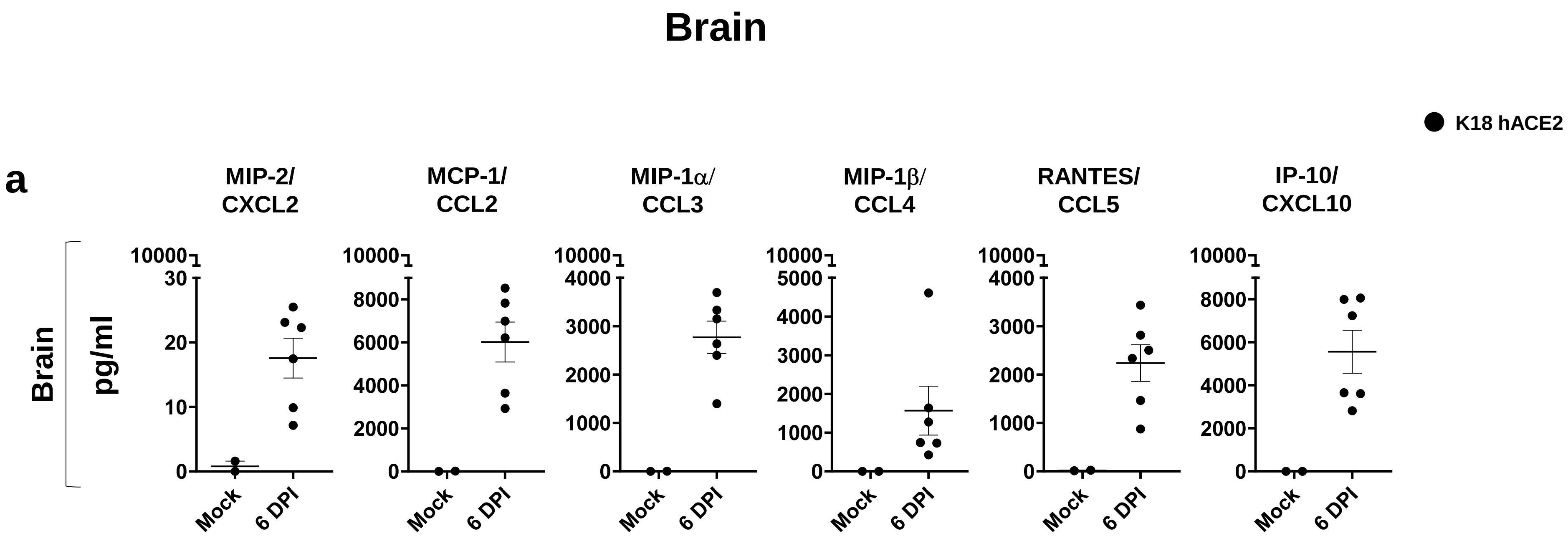
Supplementary Figure 3. Chemokine profile in selected tissues from SARS-CoV-2 infected K18 hACE2 transgenic mice. (**a**) Nasal turbinates and (**b**) Trachea. Student's *t*-test, two-tailed, C57BL/6 *vs.* K18 hACE2 $p< 0.05$; $p<0.005$; $p<0.0005$; 2-WAY ANOVA C57BL/6 or K18 hACE2 transgenic mice over time, $p< 0.05$; $p<0.005$; $p<0.0005$, M±SEM, n= 8 (per time-point studied, except mock n=2). DPI: Days post-infection. Data are representative over 2 independent experiments. Source data are provided as a Source Data file.



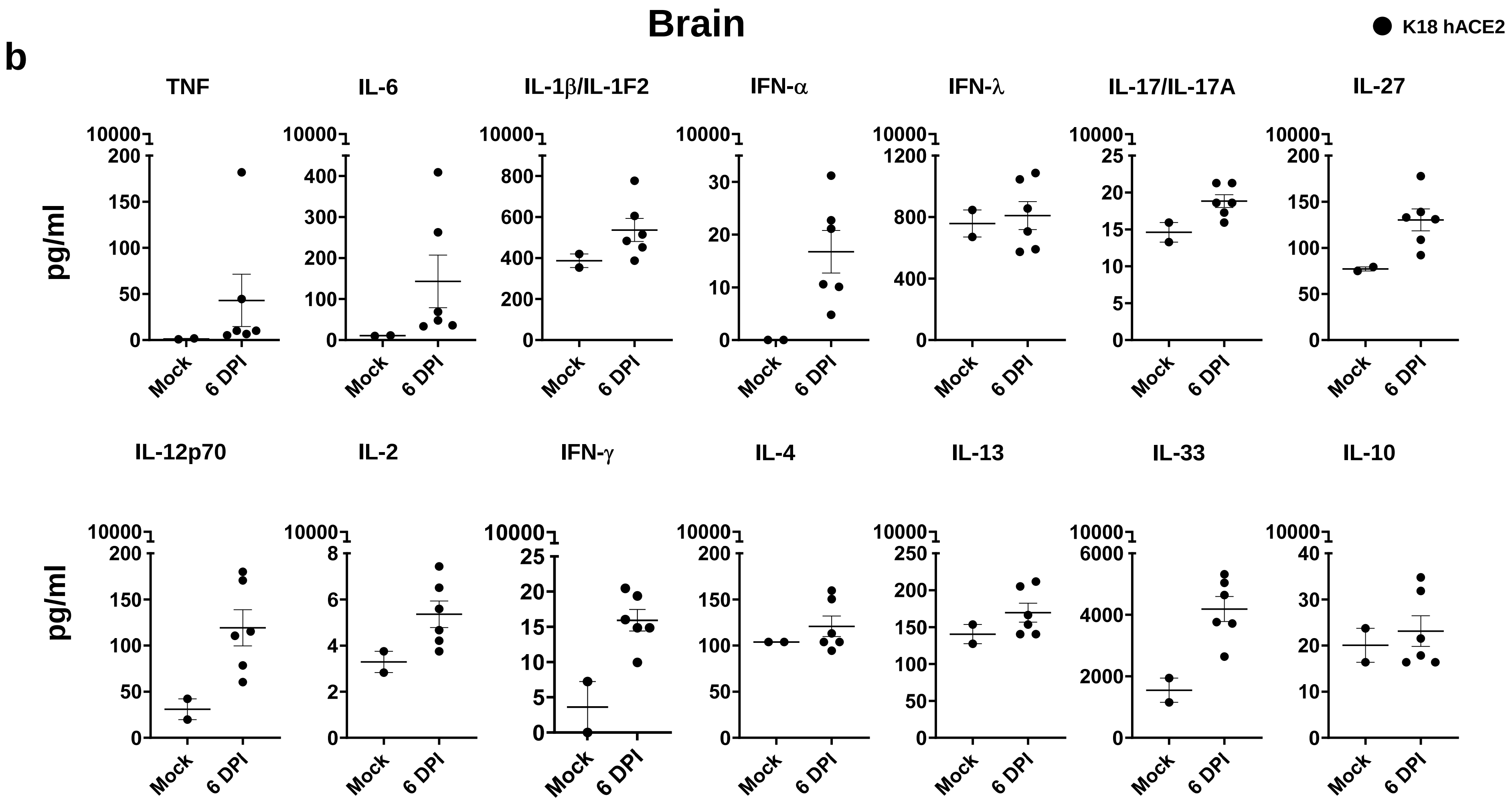
Supplementary Figure 4. Cytokine profile in selected tissues from SARS-CoV-2 infected K18 hACE2 transgenic mice. (**a**) Nasal turbinates and (**b**) Trachea. Student's t-test, two-tailed, C57BL/6 *vs.* K18 transgenic hACE2 $p < 0.05$; $p<0.005$; $p<0.0005$; 2-WAY ANOVA C57BL/6 or K18 hACE2 transgenic mice over time, $p < 0.05$; $p<0.005$; $p<0.0005$, M±SEM, n= 8 (per time-point studied, except mock n=2). DPI: Days post-infection. Data are representative over 2 independent experiments. Source data are provided as a Source Data file.



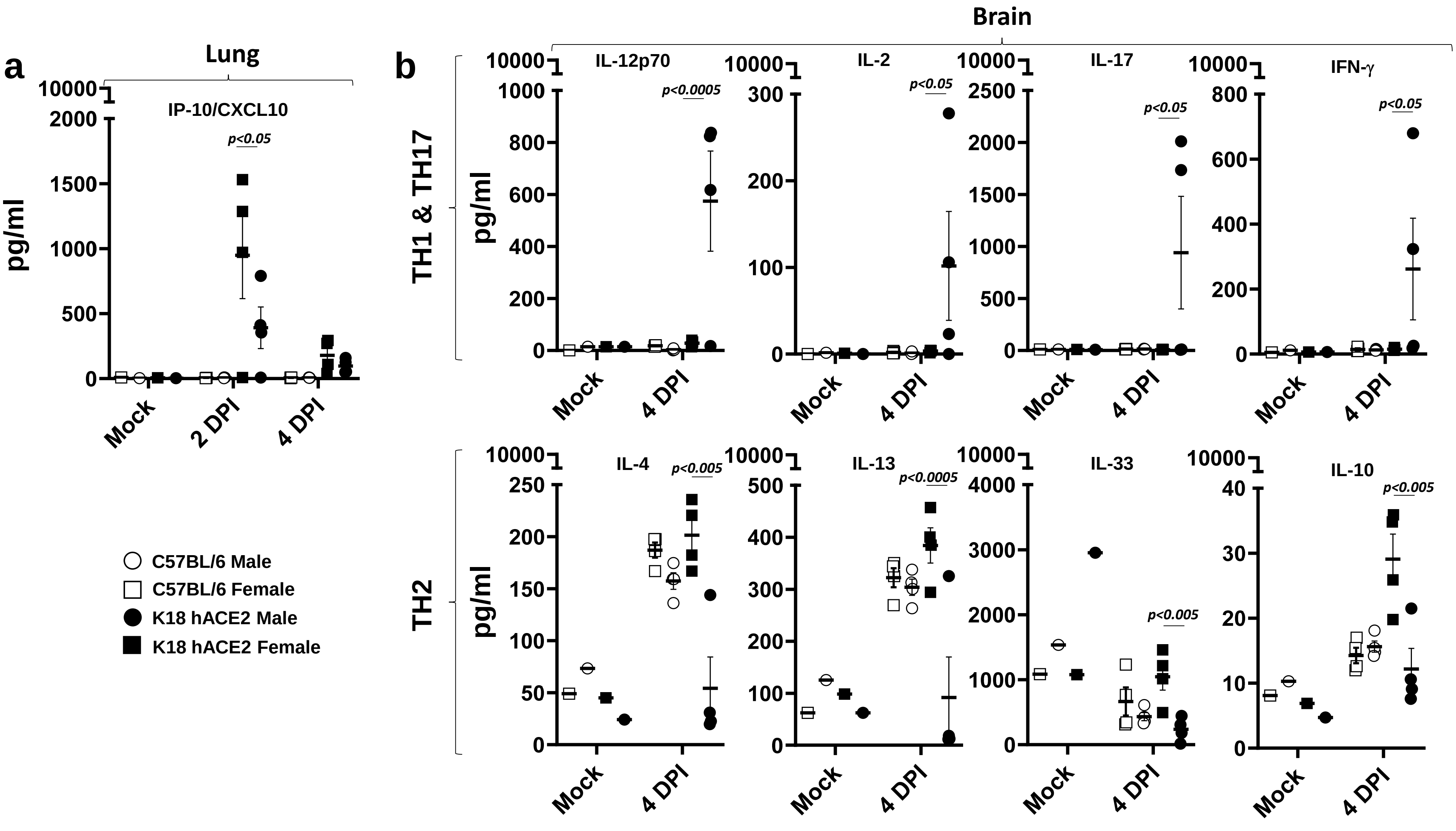
Supplementary Figure 4. Continuation.



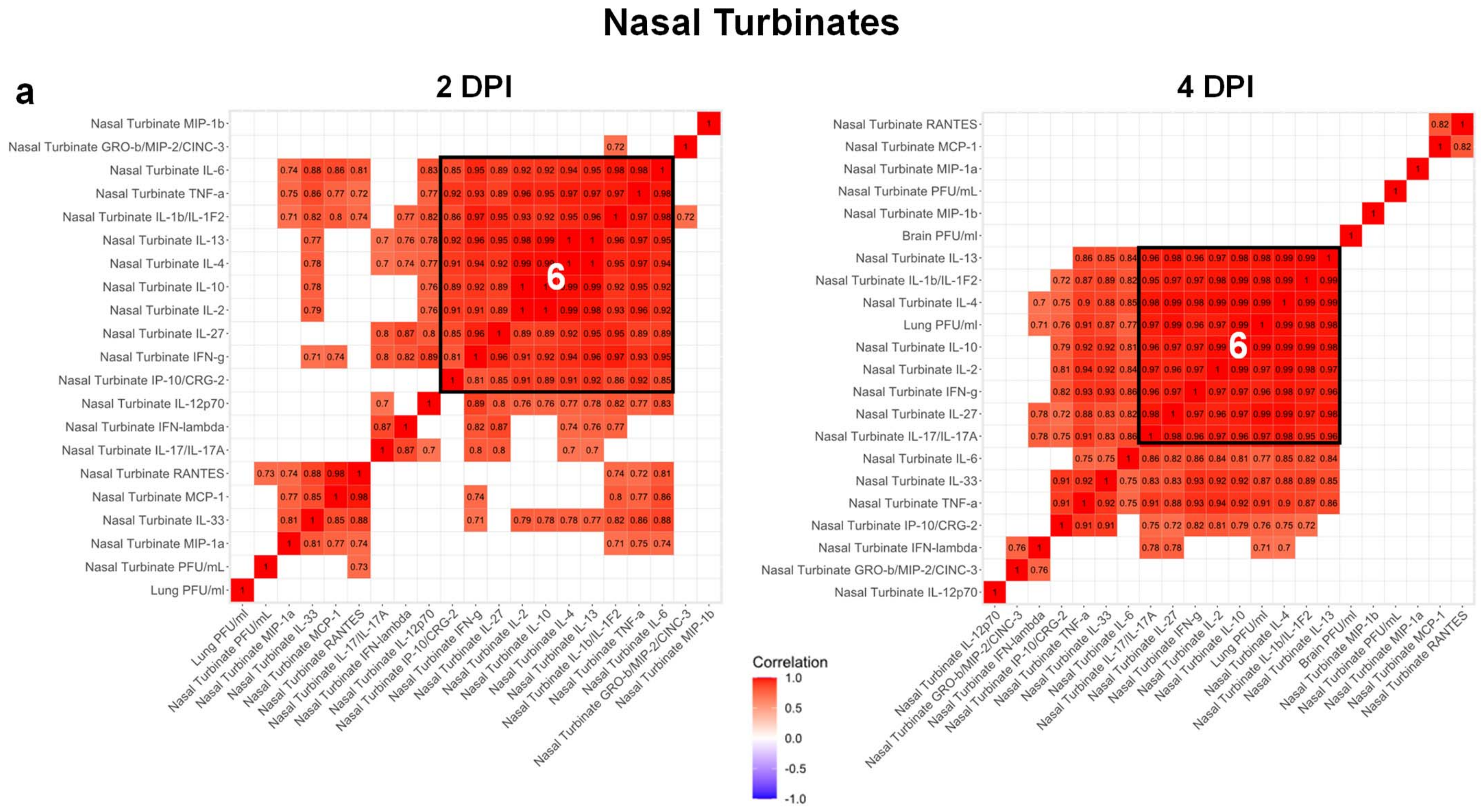
Supplementary Figure 5. Chemokines and cytokine profiles in brain tissues from SARS-CoV-2 infected K18 hACE2 transgenic mice at 6-DPI. (**a**) Chemokines and (**b**) Cytokines. M±SEM, n=6 (per time-point studied, except mock n=2). DPI: Days post-infection. Data are representative over 2 independent experiments. Source data are provided as a Source Data file.



Supplementary Figure 5. Chemokines and cytokine profiles in brain tissues from SARS-CoV-2 infected K18 hACE2 transgenic mice at 6-DPI. (**a**) Chemokines and (**b**) Cytokines. M±SEM, n=6 (per time-point studied, except mock n=2). DPI: Days post-infection. Data are representative over 2 independent experiments. Source data are provided as a Source Data file.

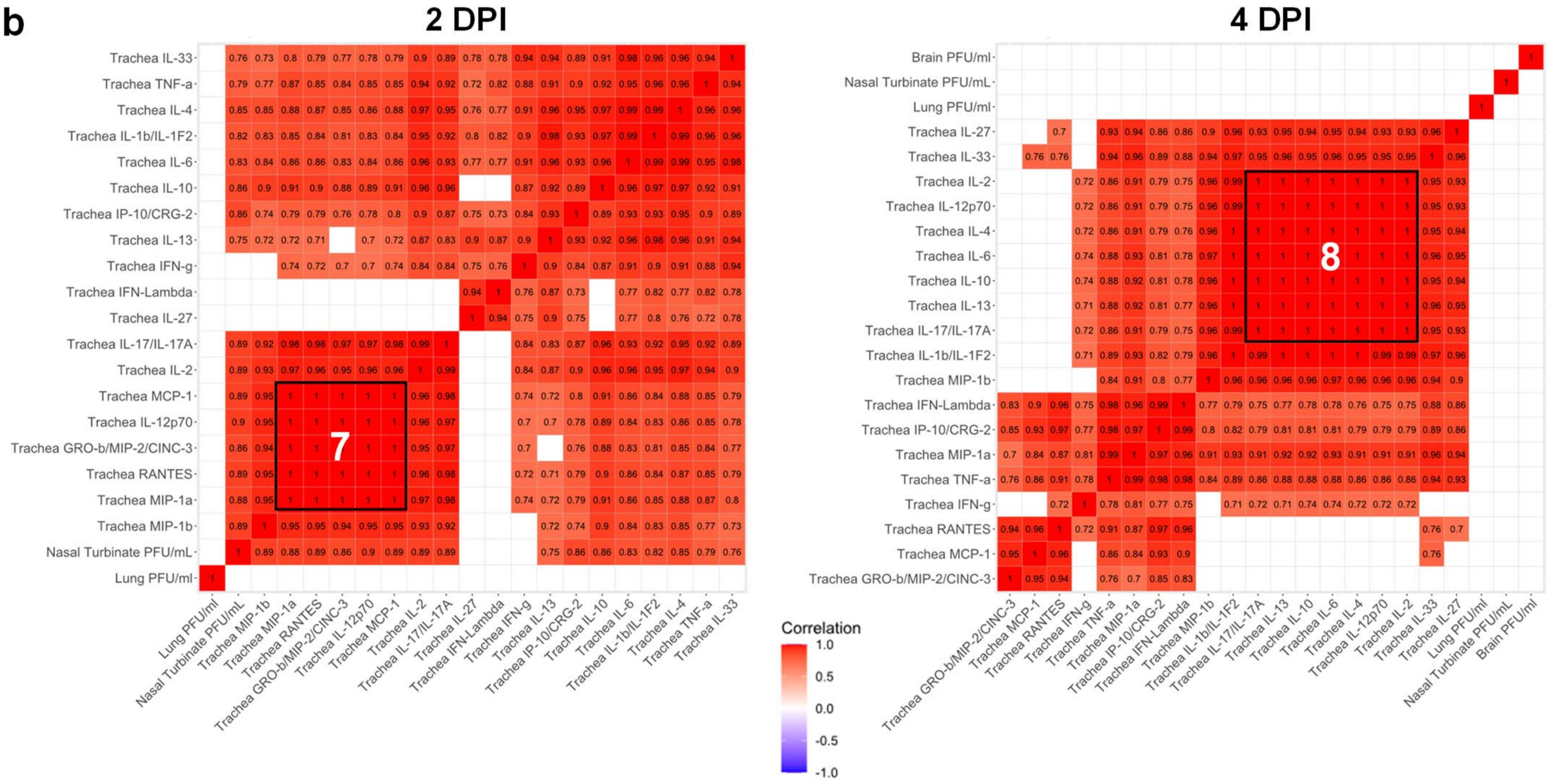


Supplementary Figure 6. Chemokine and cytokine profile in selected tissues from SARS-CoV-2 infected K18 hACE2 transgenic mice by sex. Chemokines and cytokine differences in the (**a**) lung and (**b**) brain in K18 hACE2 transgenic and WT C57BL/6 male and female mice. Student's t-test, two-tailed, C57BL/6 *vs.* K18 hACE2 *p*< 0.05; *p*<0.005; *p*<0.0005; 2-WAY ANOVA C57BL/6 or K18 hACE2 transgenic mice over time, *p*< 0.05; *p*<0.005; *p*<0.0005, M±SEM, n=4/group/sex (per time-point studied, except mock n=2). DPI: Days post-infection. Data are representative of 2 over independent experiments. Source data are provided as a Source Data file.

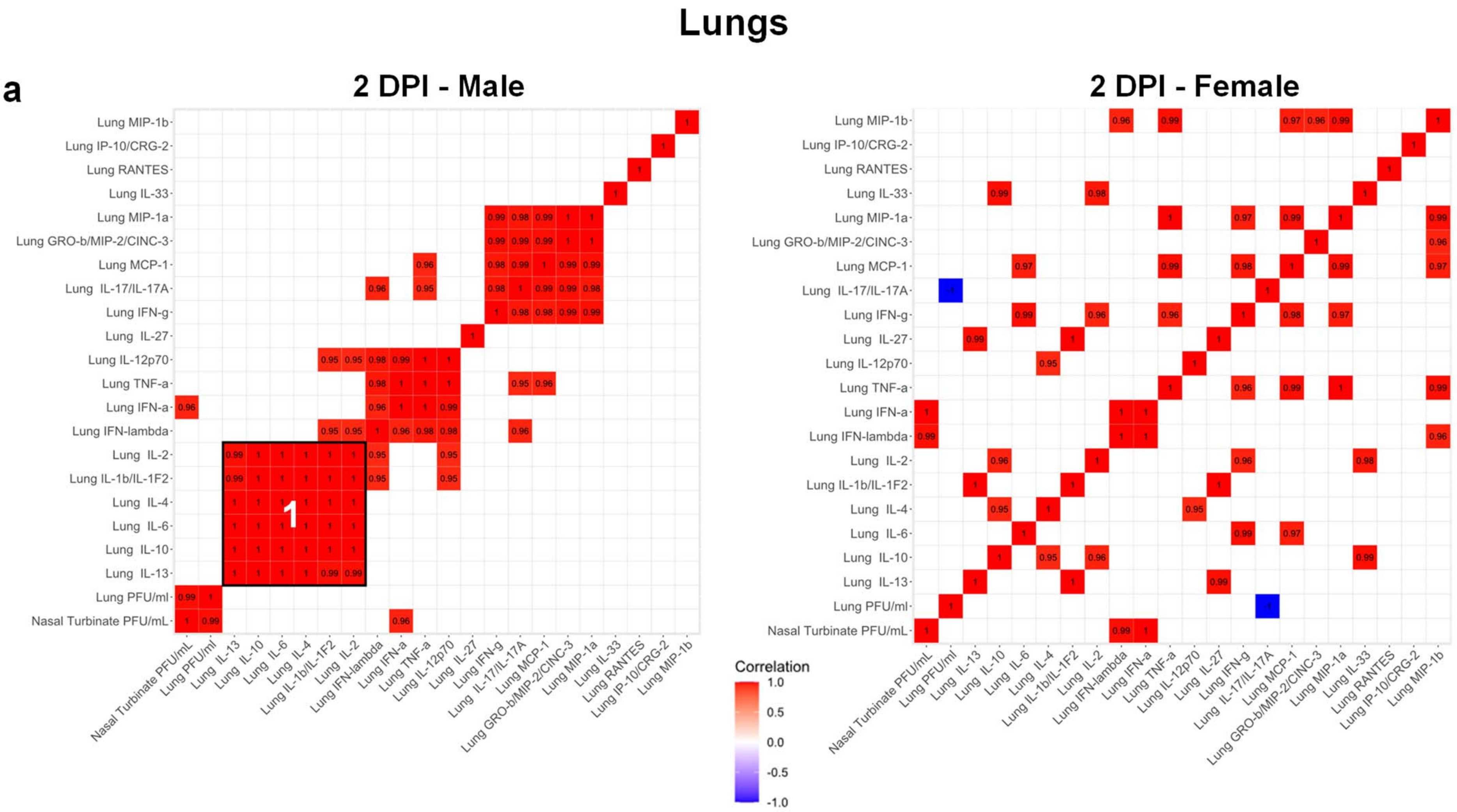


Supplementary Figure 7. SARS-CoV-2 infected K18 hACE2 transgenic mice reveal differentiated clusters of chemokine and cytokine correlations with clinical symptoms progression. Hierarchically clustered Pearson correlations of measurements in (**a**) nasal turbinate and (**b**) trachea of SARS-CoV-2 infected K18 hACE2 transgenic mice (Left: 2-DPI, Right: 4-DPI). Positive correlation (Red = 1) and negative correlations (Blue = -1), with clusters (Black outlined boxes with cluster number). Non-significant values ($p > 0.05$ measured by Pearson's correlation t-test) left blank. DPI: Days post-infection. MIP-2/CXCL2; MCP-1/CCL2; MIP-1α/CCL3; MIP-1β/CCL4; RANTES/CCL5; IP-10/CXCL10. Data are representative over 2 independent experiments. Source data are provided as a Source Data file.

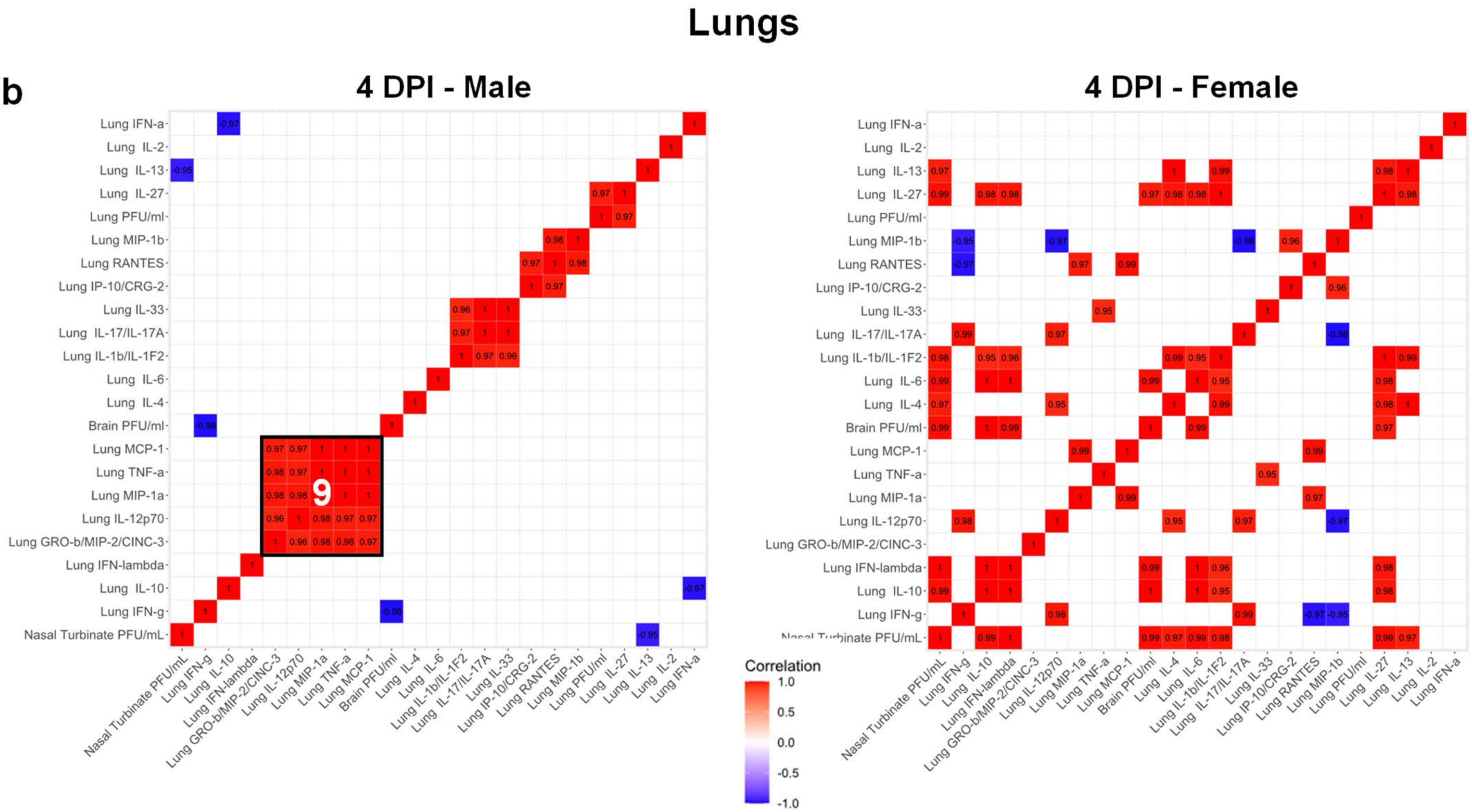
Trachea



Supplementary Figure 7. Continuation.



Supplementary Figure 8. **Immune response of SARS-CoV-2 infected K18 hACE2 transgenic mice is dependent on mouse sex.** Hierarchically clustered Pearson correlations of measurements in lung at (**a**) 2- and (**b**) 4-DPI and brain at (**c**) 2- and (**d**) 4-DPI of SARS-CoV-2 infected K18 hACE2 transgenic mice (Left: Males, Right: Females). Positive correlation (Red = 1) and negative correlations (Blue = -1), with clusters (Black outlined boxes with cluster number). Non-significant values (p > 0.05 measured by Pearson's correlation t-test) left blank. DPI: Days post-infection. MIP-2/CXCL2; MCP-1/CCL2; MIP-1α/CCL3; MIP-1β/CCL4; RANTES/CCL5; IP-10/CXCL10. Data are representative over 2 independent experiments. Source data are provided as a Source Data file.



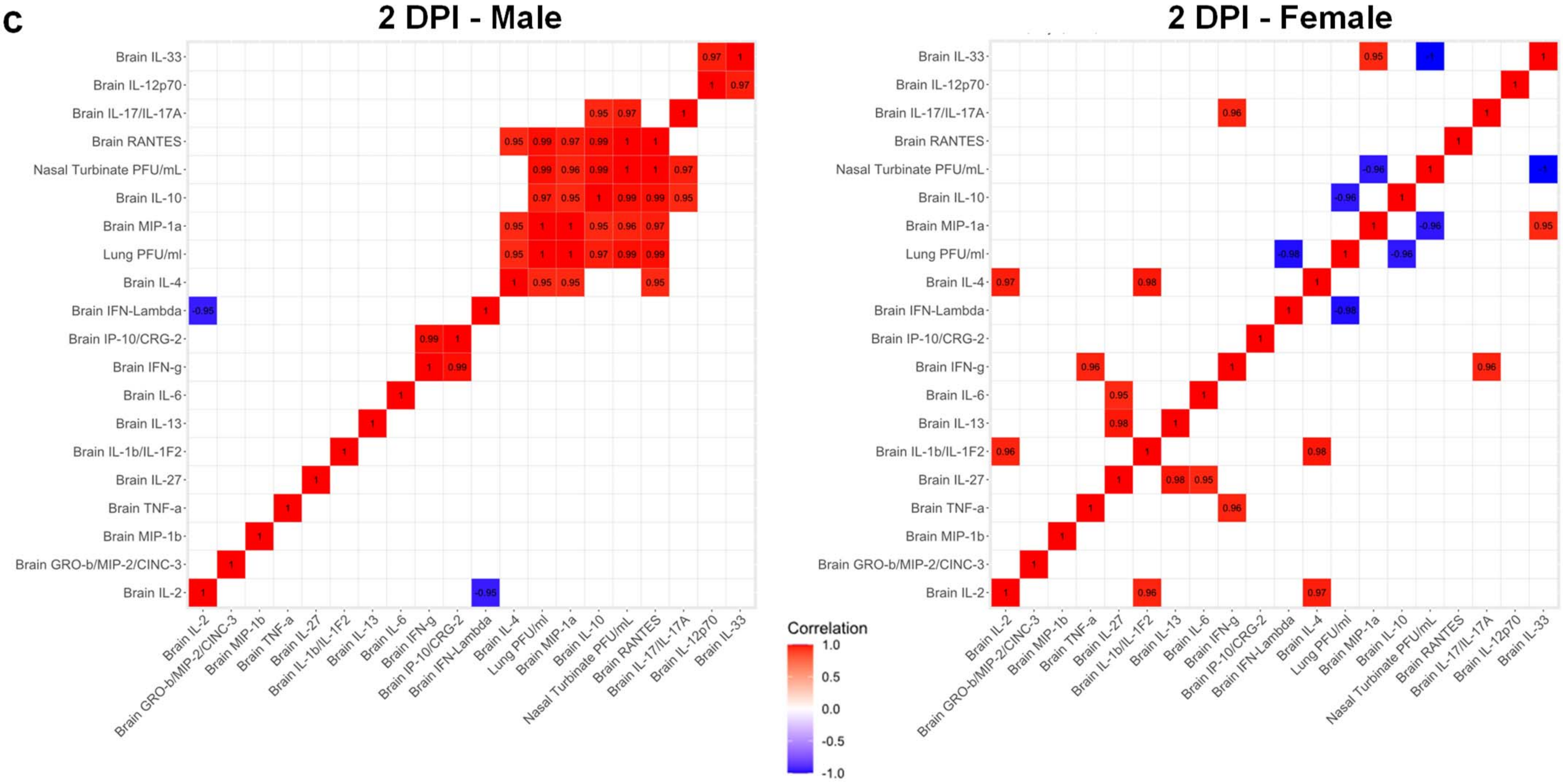
Lungs

4 DPI - Male

4 DPI - Female

Supplementary Figure 8. Continuation.

Brain



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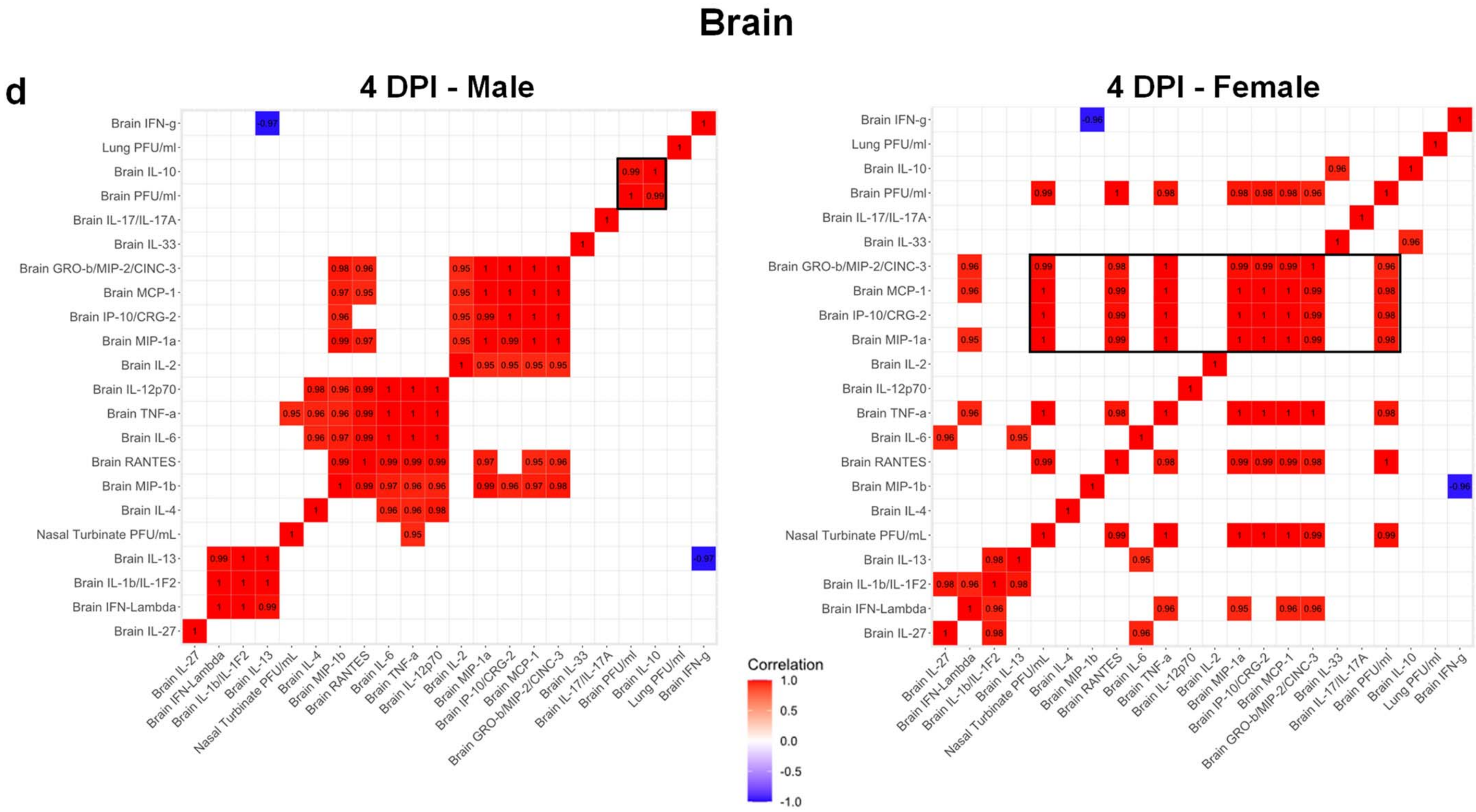

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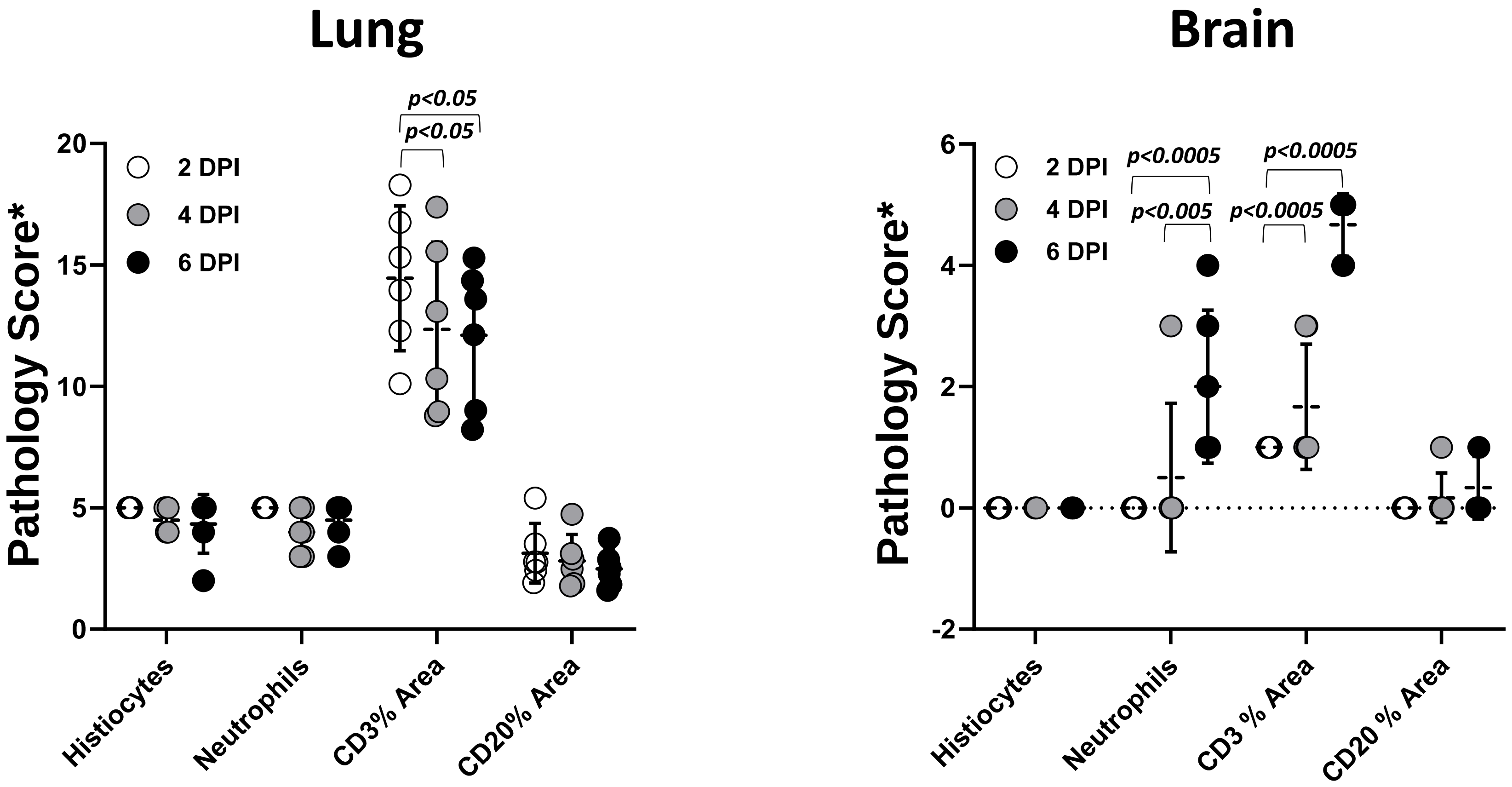

Supplementary Figure 9. Pathology score of immune cell infiltration into lung and brain of SARS-CoV-2 infected K18 hACE2 transgenic mice. Histiocytes and neutrophils were graded for severity according to the follow scores: 0=normal, 1=<10 cells, 2= <25 cells, 3= <50 cells, 4= >100 cells, 5=too many to count and plotted as a pathology score. $CD3^+$ and $CD20^+$ cells were graded by area of lung tissue involvement. 2-WAY ANOVA K18 hACE2 transgenic mice over time, $p < 0.05$; $p<0.005$; $p<0.0005$, M±SD, n = 6 (50:50 male:female per time-point and group) randomly chosen. Data are combined from of 2 independent experiments. Source data are provided as a Source Data file.