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Supplemental information

SARS-CoV-2 variants of concern display enhanced intrinsic pathogenic properties and expanded organ tropism in mouse models

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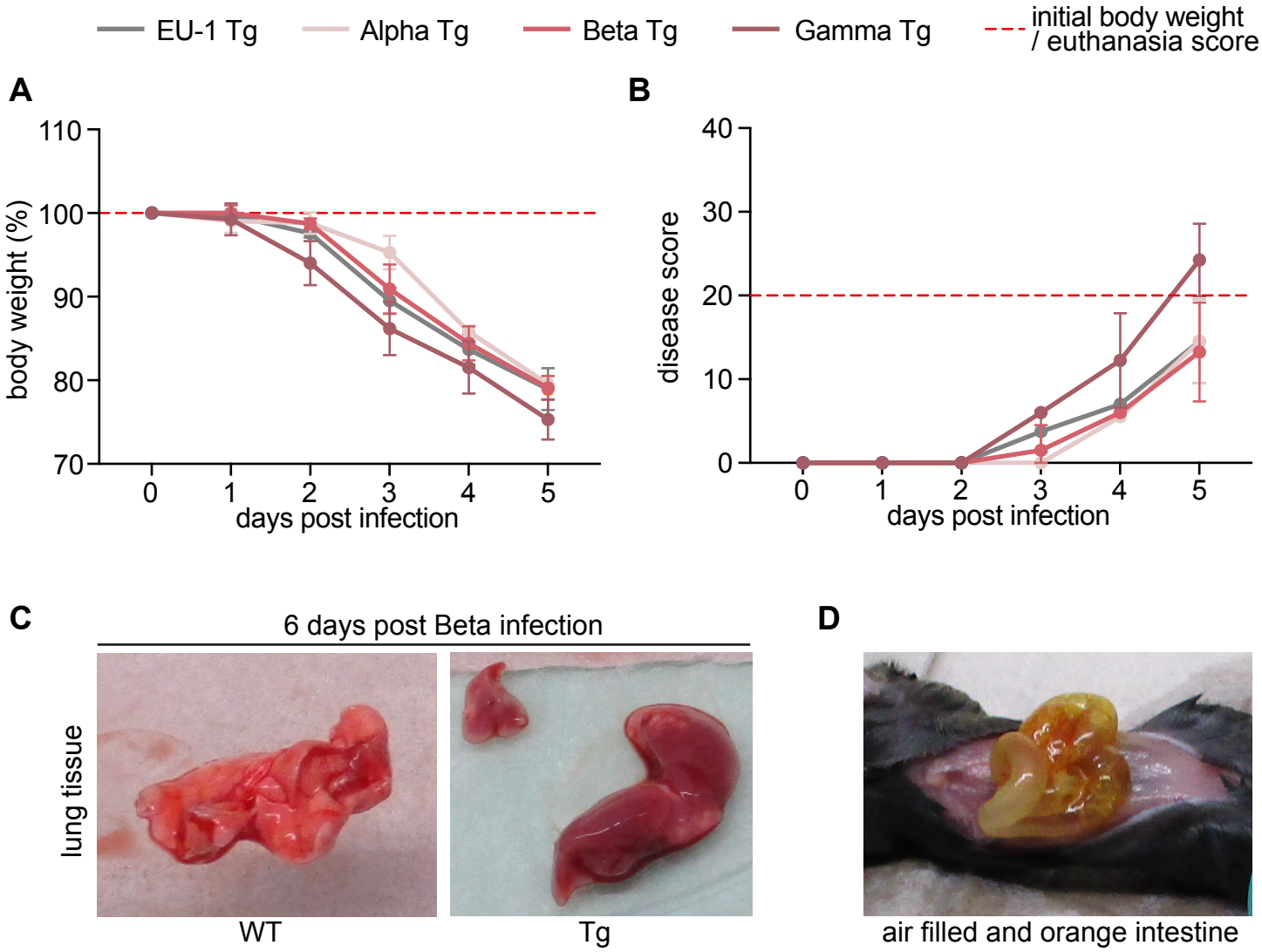
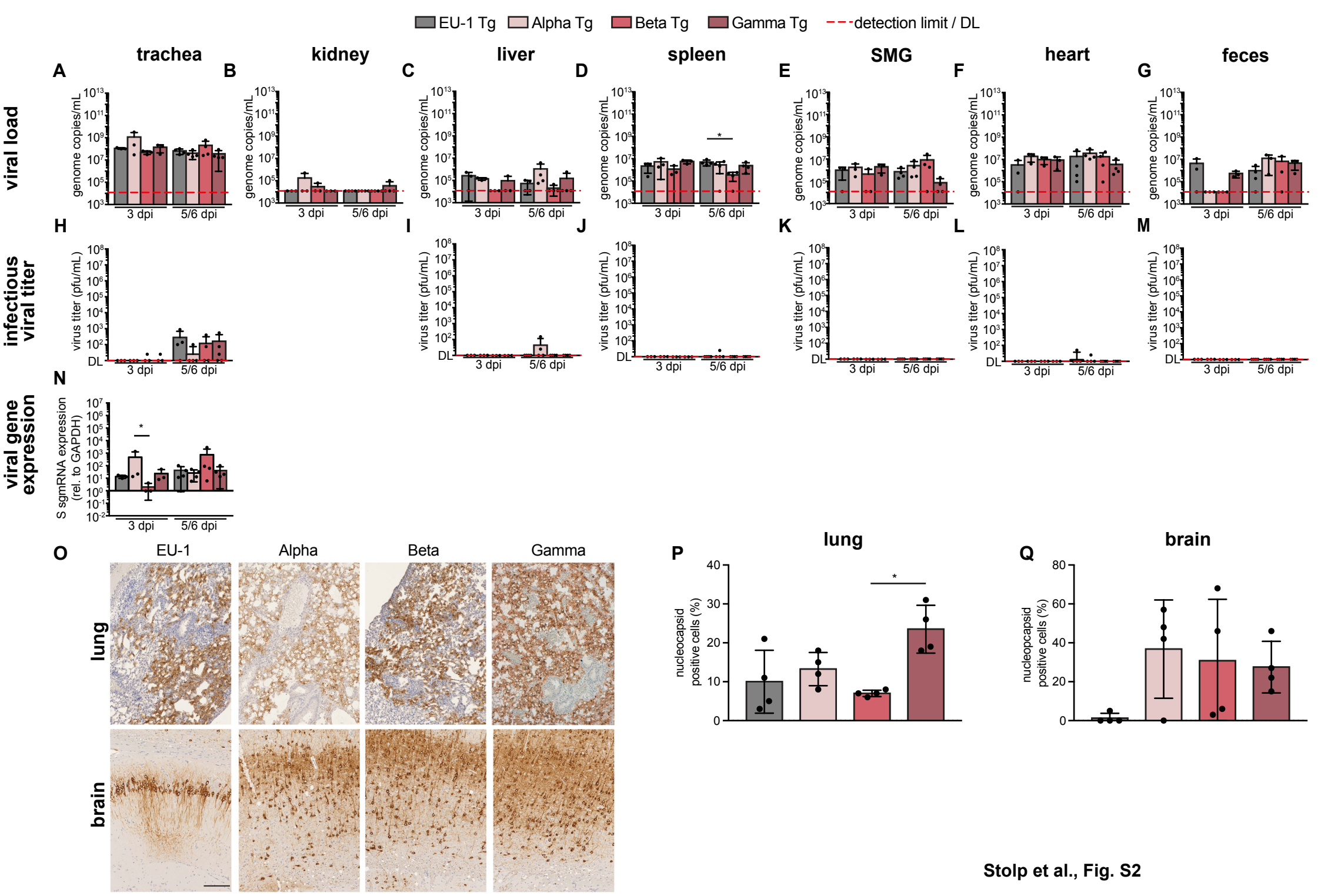


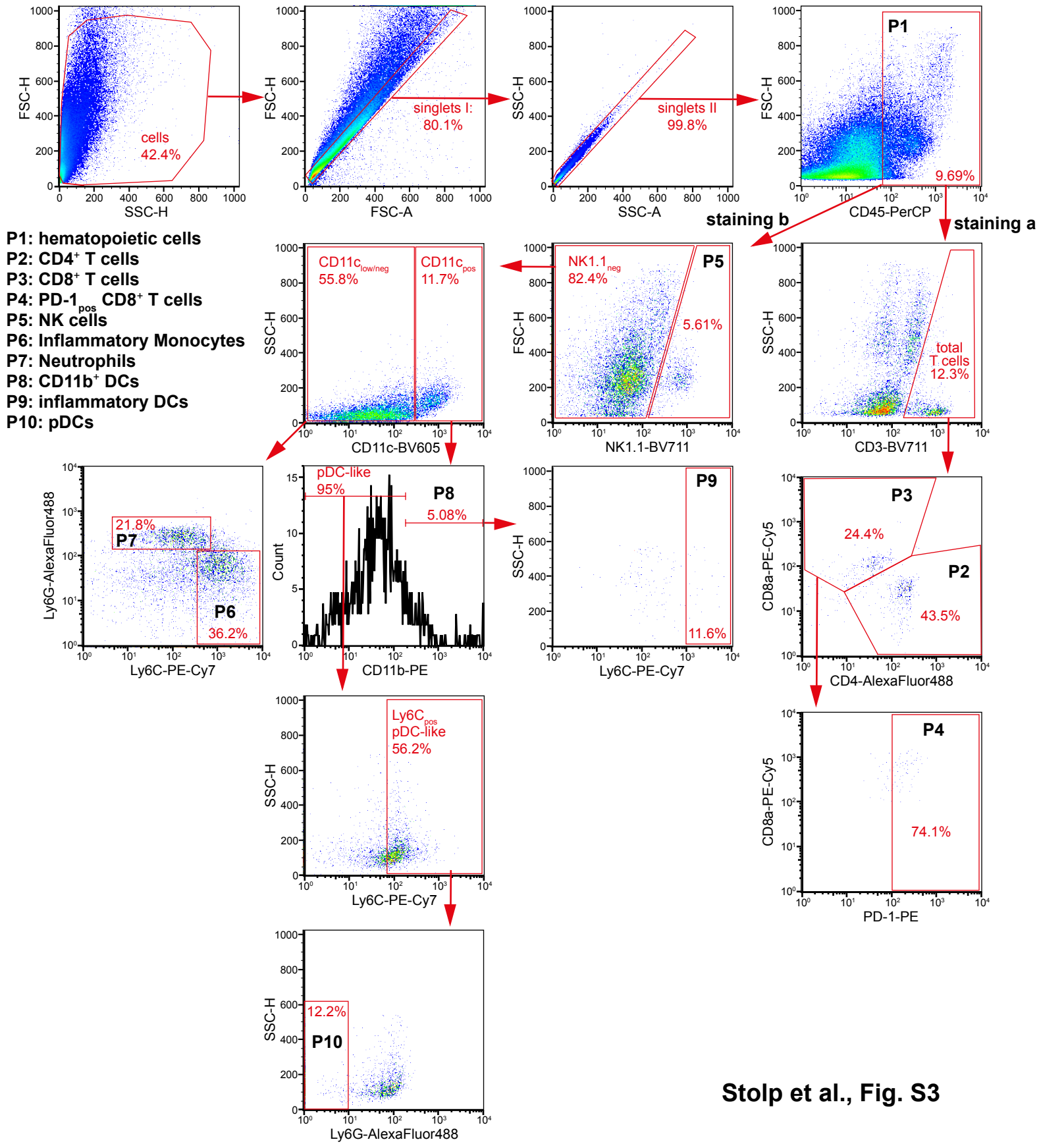
Stolp et al., Fig. S1

Figure S1: Body weight/ disease score curves of SARS-CoV-2 infected hACE2 Tg mice and examples of organ pathology upon harvest. Related to Figure 1. (A) Body weight and (B) disease score curves as used for calculation of the AUC, shown in Figure 1C, D. Shown are mean values ±SD of 4 mice per group from days 0 to 5 post infection. Dashed red line indicates initial body weight (100%) (A) or indicates the score at which animals become eligible for euthanasia (B). (C) Lung tissue directly post harvesting of a WT mouse (left panel) and a hACE2 Tg mouse (right panel) 6 d.p.i. with Beta. (D) Example of an air filled and orange colored intestine, as observed for some hACE2 Tg mice, as taken as pathological parameter in Figure 1.



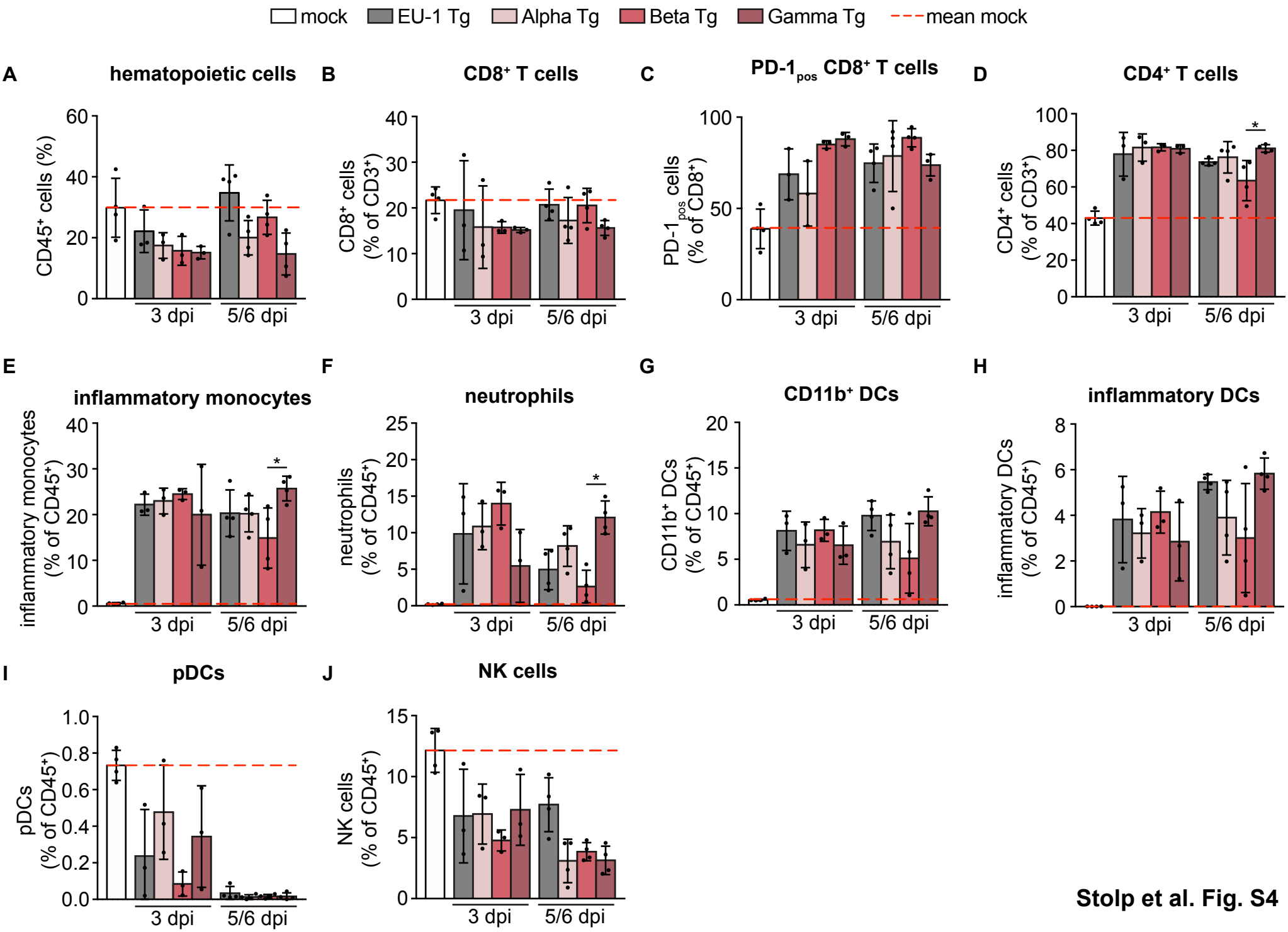
Stolp et al., Fig. S2

Figure S2: SARS-CoV-2 variants differ in organ tropism and organ viral load upon infection of hACE2 Tg mice. Related to Figure 2. hACE2 Tg mice were infected i.n. with 10^4 pfu of the indicated SARS-CoV-2 variants on day 0 p.i.. In addition to the organs displayed in Figure 2, (A, H, N) trachea, (B) kidney. (C, I) liver, (D, J) spleen, (E, K) submandibular salivary gland (SMG), (F, L) heart and (G, M) feces were harvested on days 3 and 5 (Gamma) or 6 (EU-1, Alpha, Beta) p.i. and analyzed for viral load by qPCR for SARS-CoV-2 N1 gene (A-G), infectious viral titer by plaque assay on Vero E6 cells (H-M) and viral gene expression by qPCR for SARS-CoV-2 S gene sgmRNA (N). Shown are mean values ±SD from 3 (3 d.p.i.) to 4 (5/6 d.p.i.) mice. Dashed red line indicates detection limit (DL), which is 12042 viral genome copies/mL for the qPCR and 0 plaques/mL for the plaque assay. S gene sgmRNA is presented as $2^{-\Delta\Delta CT}$ with 10^0 representing mock; down error bars are omitted in case of negative values, due to the logarithmic scale. (O) Representative SARS-CoV-2 immunohistochemistry of lung (upper panels) and brain (lower panels) tissue infected with EU-1, Alpha, Beta and Gamma at day 5 or 6 p.i. (scale bar 100 µm). (P,Q) Percentages of nucleocapsid protein positive cells as quantified from three high power fields per section of lung (P) and brain (Q). Shown are mean values ±SD. Each black dot represents one individual animal. P-values were calculated performing a Kruskal-Wallis test with a Dunn's multiple comparison test (*, $p \leq 0.05$).



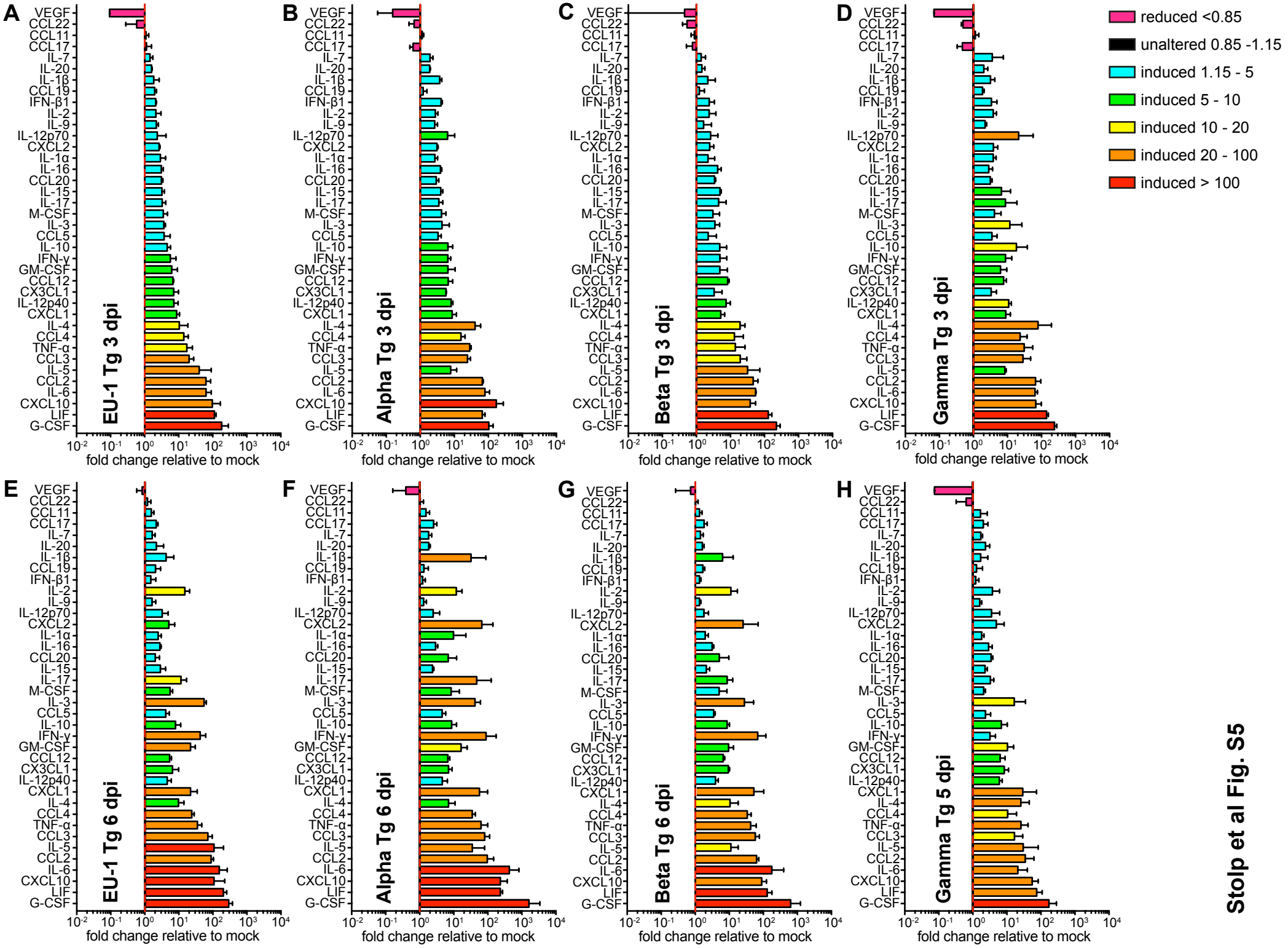
Stolp et al., Fig. S3

Figure S3: Gating strategy of the flow cytometric analysis of lung single cell suspensions. Related to Figures 3 and 6. Mice were infected i.n. with 10^4 pfu of the indicated SARS-CoV-2 variants on day 0 p.i.. 0 (mock), 3 and 5 (Gamma) or 6 (EU-1, Alpha, Beta) d.p.i. lungs were harvested, minced and collagenase/dispase digested. Single cell populations were stained for flow cytometric analysis using two different staining mixtures: staining a (anti-CD45, -CD3, -CD4, -CD8, -PD-1) and staining b (anti-CD45, -NK1.1, -CD11c, -CD11b, -Ly6C, -Ly6G). Gating strategy is indicated by arrows. P1 to P10 indicate the gates used to plot the data in Figures 3, 6, S4 and S9.



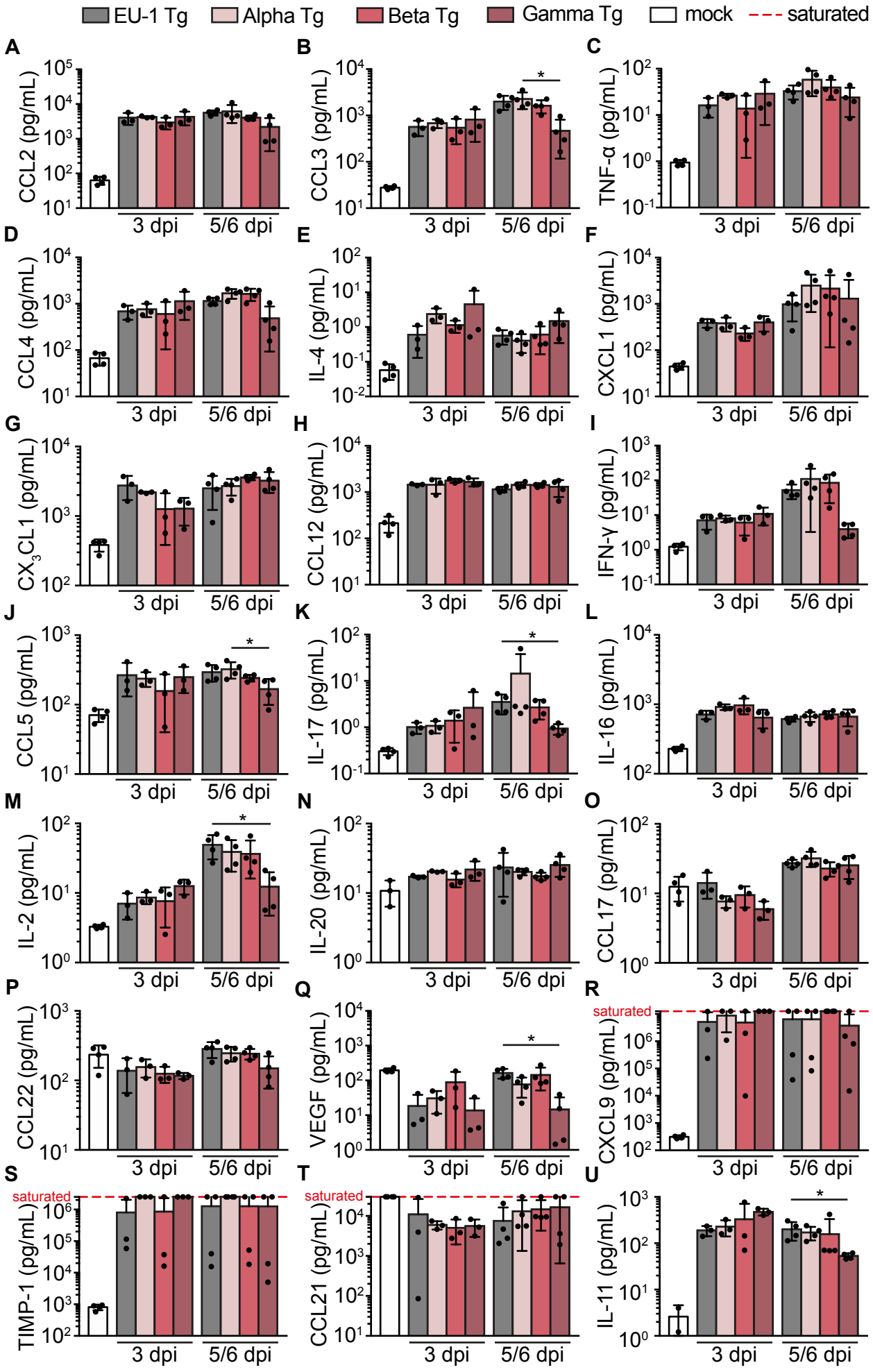
Stolp et al. Fig. S4

Figure S4: Infection of hACE2 Tg mice with variants of SARS-CoV-2 induces changes in immune cell composition of the lung, most pronounced for Gamma. Related to Figure 3. hACE2 Tg mice were infected i.n. with 10^4 pfu of the indicated SARS-CoV-2 variants on day 0 p.i.. 0 (mock, n=4), 3 (n=3) and 5 (Gamma, n=4)) or 6 (EU-1, Alpha, Beta; n=4) d.p.i. lungs were harvested, minced and collagenase/dispase digested. Single cell populations were stained for flow cytometry analysis as shown in Figure S3. Shown are mean percentages with standard deviation of (A) $CD45^+$ hematopoietic cells as fraction of singlets, (B) $CD8^+$ T cells as fraction of $CD3^+$ total T cells, (C) $PD\text{-}1_{pos}$ cells as fraction of $CD8^+$ T cells, (D) $CD4^+$ T cells as fraction of $CD3^+$ total T cells, (E) inflammatory monocytes as fraction of $CD45^+$ cells, (F) neutrophils as fraction of $CD45^+$ cells, (G) $CD11b^+$ DCs as fraction of $CD45^+$ cells, (H) inflammatory DCs as fraction of $CD45^+$ cells, (I) pDCs as fraction of $CD45^+$ cells and (J) NK cells as fraction of $CD45^+$ cells. Red dashed line indicates the mean of mock. Each black dot represents one individual animal. P-values were calculated performing a Kruskal-Wallis test with a Dunn's multiple comparison test (*, $p \leq 0.05$).



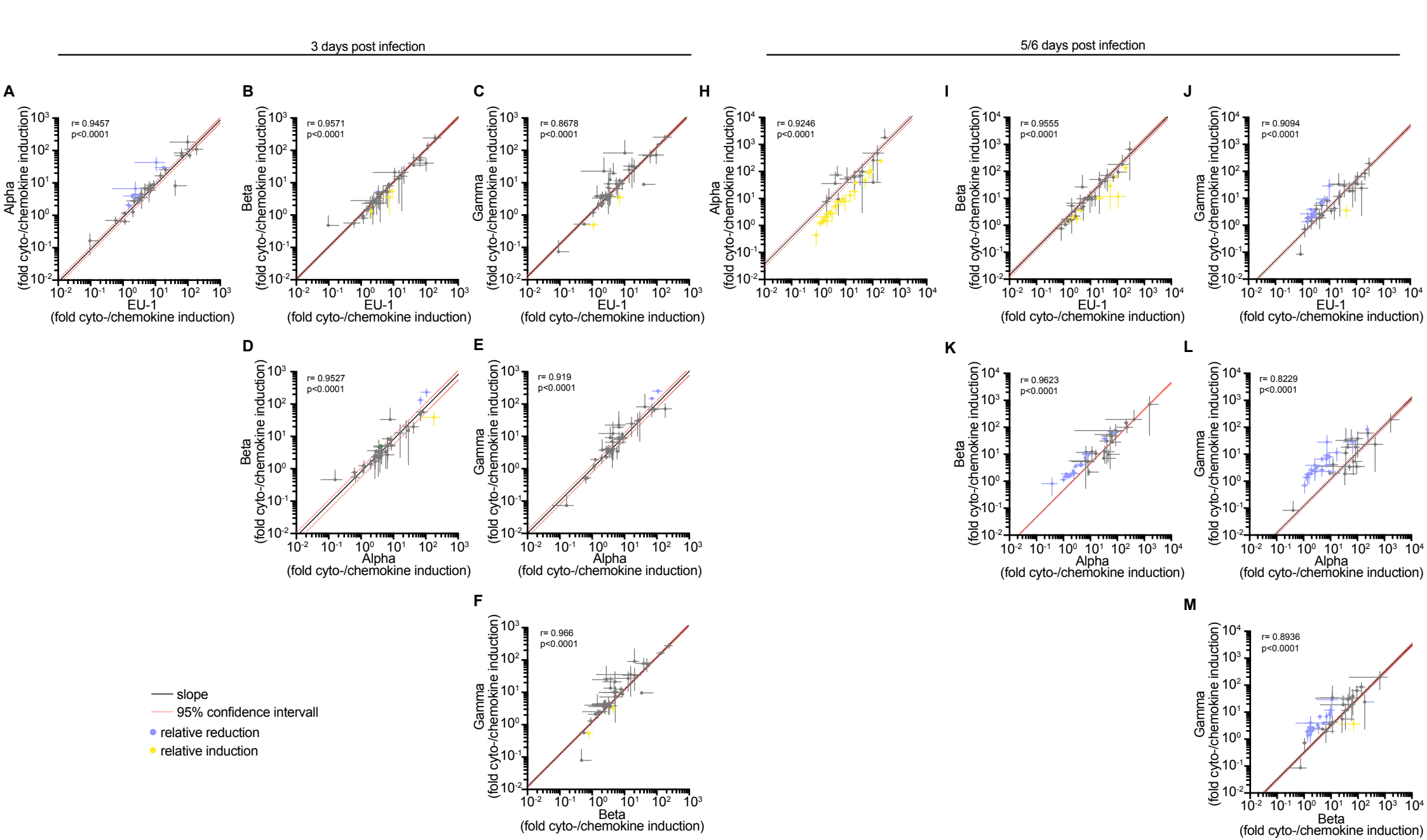
Stolp et al Fig. S5

Figure S5: Cytokine and chemokine expression in lungs, induced by infection of hACE2 Tg mice with SARS-CoV-2 variants. **Related to Figure 4.** hACE2 Tg mice were infected i.n. with 10^4 pfu of the indicated SARS-CoV-2 variants on day 0 p.i.. 3 (n=3) and 5 (Gamma, n=4) or 6 (EU-1, Alpha, Beta; n=4) d.p.i. lungs were harvested, homogenized and analyzed for cytokines and chemokines present, performing a Mouse Cytokine Array / Chemokine Array 44-Plex. Mean values were normalized to the mean of mock samples (n=4) and fold changes in cytokine/chemokine levels are plotted relative to mock infected control mice. Some cytokines/chemokines were excluded from this graph: signals of CXCL9 and TIMP-1 were frequently saturated for hACE2 Tg mice, Erythropoietin was too close to background, CCL21 was saturated for most mock samples, IL-13 and IL-11 could not be normalized as they were not detectable in the majority of mock samples. Shown are mean values ±SD relative to mock samples for lung homogenates of hACE2 Tg mice infected with SARS-CoV-2 (A) EU-1 3 d.p.i., (B) Alpha 3 d.p.i., (C) Beta 3 d.p.i., (D) Gamma 3 d.p.i., (E) EU-1 6 d.p.i., (F) Alpha 6 d.p.i., (G) Beta 6 d.p.i., (H) Gamma 5 d.p.i. Cytokines and chemokines are ordered according to their strength of induction in the EU-1 3 d.p.i. sample (A), relative to mock. Cytokines/chemokines reduced to less than 0.85 times of the mock value are shown in pink, unaltered cytokines/chemokines in black (fold change of 0.85-1.15), cytokines/chemokines induced 1.15 to 5 times relative to mock in cyan, 5 to 10 times induction relative to mock in green, 10 to 20 times induction relative to mock in yellow, 20 to 100 times induction relative to mock in orange and more than 100 times induction relative to mock in red. Dashed red line indicates mock.



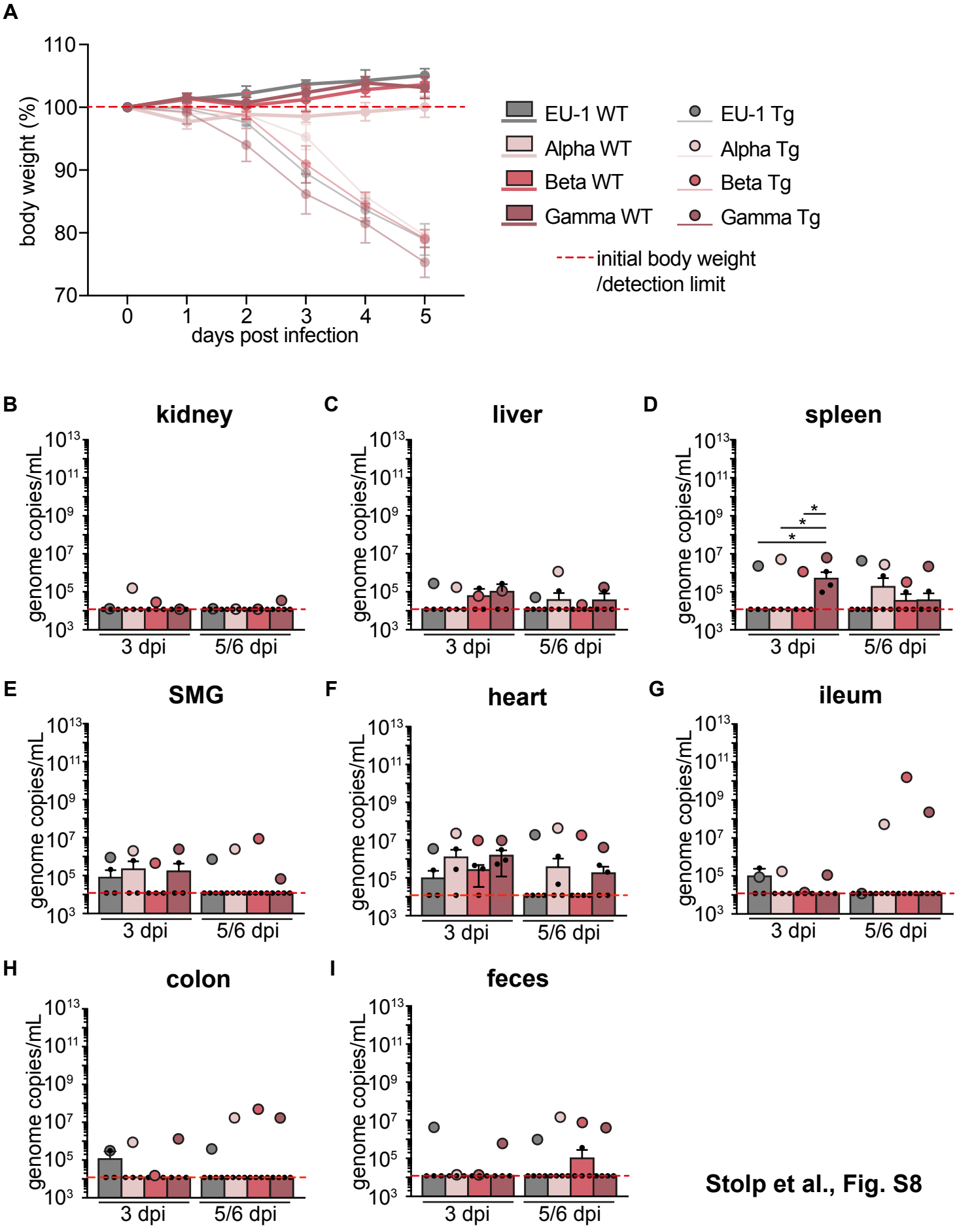
Stolp et al Fig. S6

Figure S6: Cytokine and chemokine expression induced by infection of hACE2 Tg mice with SARS-CoV-2 variants. **Related to Figure 4.** hACE2 Tg mice were infected i.n. with 10^4 pfu of the indicated SARS-CoV-2 variants on day 0 p.i.. 3 and 5 (Gamma) or 6 (EU-1, Alpha, Beta) d.p.i. lungs were harvested, homogenized and analyzed for cytokines and chemokines present, performing a Mouse Cytokine Array / Chemokine Array 44-Plex. Shown are mean values ±SD of the absolute amounts of Cytokines/Chemokines detected in lung homogenates in pg/mL. Each black dot represents one individual animal. (A) CCL2, (B) CCL3, (C) TNFα, (D) CCL4, (E) IL-4, (F) CXCL1, (G) CX_3CL1, (H) CCL12, (I) IFNγ, (J) CCL5, (K) IL-17, (L) IL-16, (M) IL-2, (N) IL-20, (O) CCL17, (P) CCL22, (Q) VEGF, (R) CXCL9, (S) TIMP-1, (T) CCL21, (U) IL-11. Cytokines are ordered as in Figure S5A. Shown are Cytokines/Chemokines that display statistically significant differences in expression for the different VOCs upon infection of either hACE2 Tg or WT mice or that are not included in Figure S5: (R-T) display saturated values that could not be normalized, (U) less than 3 mock samples contained measurable amounts of the cytokine, precluding meaningful normalization. Dashed red lines indicate saturation. P-values were calculated performing a Kruskal-Wallis test with a Dunn's multiple comparison test (*, $p \leq 0.05$).



Stolp et al Fig. S7

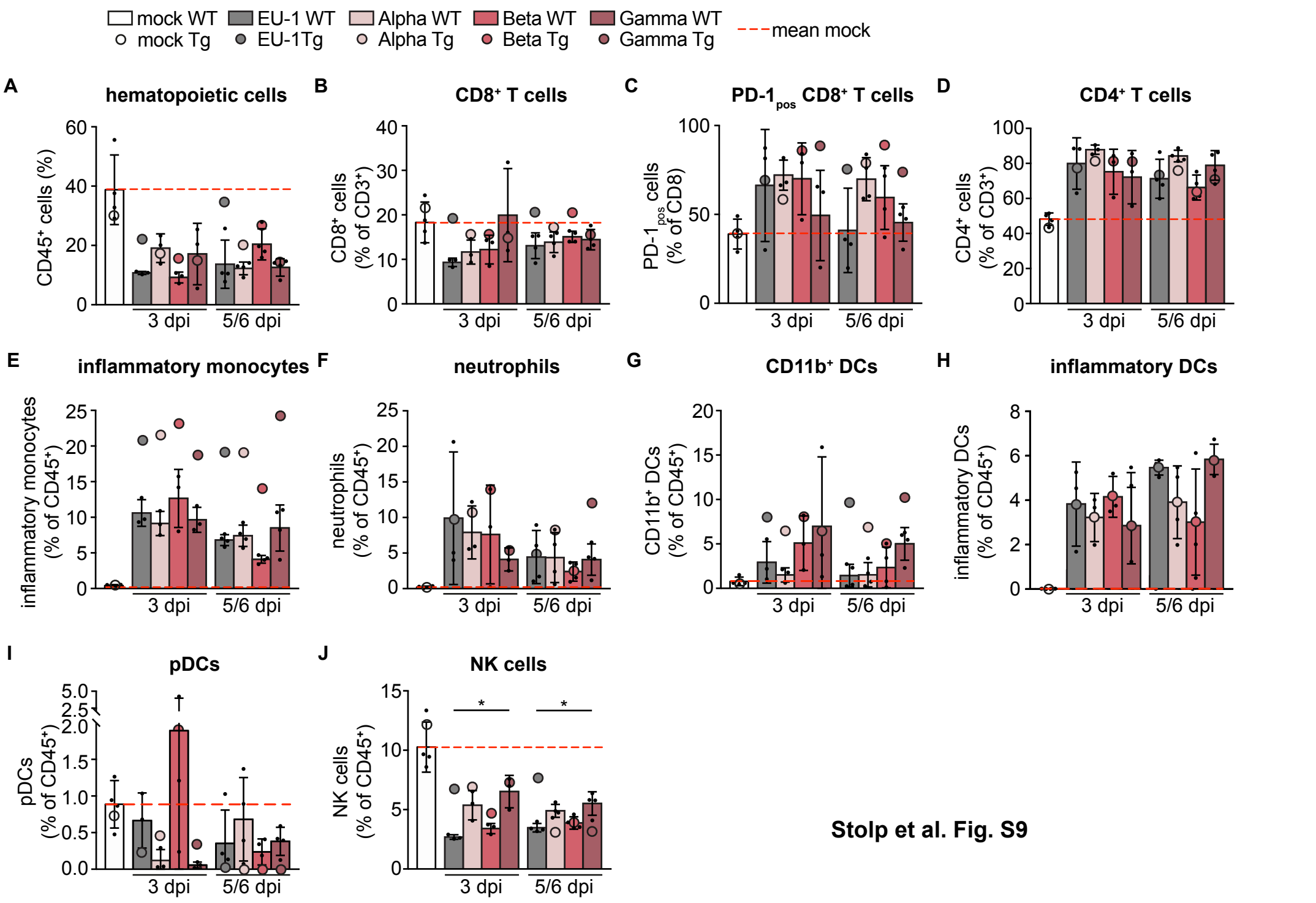
Figure S7: Induction of specific sets of cytokines and chemokines in lungs by the SARS-CoV-2 variants relative to each other in hACE2 Tg mice. Related to Figure 4. Fold cytokine and chemokine inductions in hACE2 Tg SARS-CoV-2 infected mice relative to mock, (A-F) 3 days and (H-M) 5/6 days post infection, as shown in Figure S5 are plotted in correlation plots for (A,H) EU-1 vs. Alpha, (B,I) EU-1 vs. Beta, (C,J) EU-1 vs. Gamma, (D,K) Alpha vs. Beta, (E,L) Alpha vs. Gamma and (F,M) Beta vs. Gamma. Each symbol represents the mean value ±SD of one cyto-/chemokine. Please note that due to the logarithmic scaling, some SD for cyto-/chemokines that would go beyond the limits of plotting are not visualized, some are smaller than the symbol. The black line indicates the slope of the linear regression curve with the dashed red line representing the 95% confidence intervals. r- and p-values were calculated by nonparametric Spearman correlation. Slopes and confidence intervals relative to EU-1 are plotted in Figure 4B. Cytokines and chemokines that are significantly induced or reduced relative to slope and confidence interval assuming similar expression are plotted in blue and yellow, respectively. This color code is also used in the corresponding heat maps that displays specific sets of cytokine/chemokine altered by the indicated SARS-CoV-2 variants relative to one another shown in Figure 4. This heat map is identical to Figure 4F and G.



Stolp et al., Fig. S8

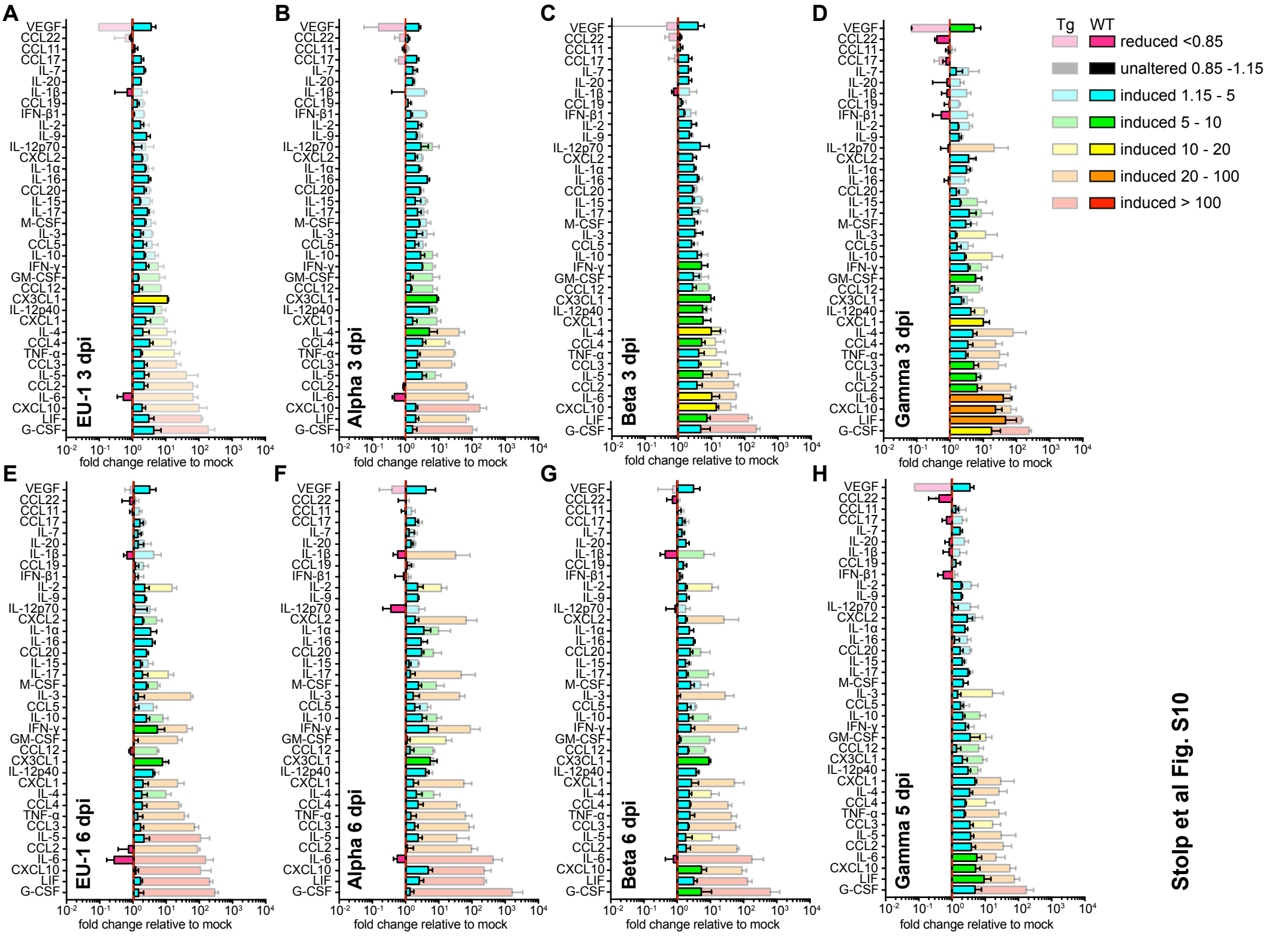
Figure S8: SARS-CoV-2 VOCs exhibit broader organ tropism and replication capacity in WT mice.

Related to Figure 5. WT mice were infected i.n. with 10^4 pfu of the indicated SARS-CoV-2 variants on day 0 p.i. (A) Body weight curves as used for calculation of the AUC, shown in Figure 5B. Shown are mean values ±SD of 4 mice per group on days 0 to 5 p.i.. Dashed red line indicates initial body weight (100%). (B) Kidney, (C) liver, (D) spleen, (E) submandibular salivary gland (SMG), (F) heart, (G) ileum, (H) colon and (I) feces were harvested 3 and 5 (Gamma) or 6 (EU-1, Alpha, Beta) d.p.i. and analyzed for viral load by qPCR for SARS-CoV-2 N1 gene. Shown are mean values ±SD from 3 (3 d.p.i.) to 4 (5/6 d.p.i.) mice. Each black dot represents an individual animal. Mean values of hACE2 Tg mice infected with the respective VOCs, as shown in Figures S1A, S2A-F and 2G,J are indicated by colored round symbols or transparent lines. Dashed red line indicates detection limit, which is 12042 genome copies/mL for the N1 qPCR. P-values were calculated performing a Kruskal-Wallis test with a Dunn's multiple comparison test (*, $p \leq 0.05$).



Stolp et al. Fig. S9

Figure S9: Infection of WT mice with variants of SARS-CoV-2 induced changes in immune cell composition of the lung. Related to Figure 6. WT mice were infected i.n. with 10^4 pfu of the indicated SARS-CoV-2 variants on day 0 p.i.. 0 (mock, n=4), 3 (n=3) and 5 (Gamma, n=4)) or 6 (EU-1, Alpha, Beta; n=4) d.p.i. lungs were harvested, minced and collagenase/dispase digested. Single cell populations were stained for flow cytometry analysis as shown in Figure S3. Shown are mean percentages with standard deviation of (A) $CD45^+$ hematopoietic cells as fraction of singlets, (B) $CD8^+$ T cells as fraction of $CD3^+$ total T cells, (C) $PD\text{-}1_{pos}$ cells as fraction of $CD8^+$ T cells, (D) $CD4^+$ T cells as fraction of $CD3^+$ total T cells, (E) inflammatory monocytes as fraction of $CD45^+$ cells, (F) neutrophils as fraction of $CD45^+$ cells, (G) $CD11b^+$ DCs as fraction of $CD45^+$ cells, (H) inflammatory DCs as fraction of $CD45^+$ cells, (I) pDCs as fraction of $CD45^+$ cells and (J) NK cells as fraction of $CD45^+$ cells. Red dashed line indicates the mean of mock. Each black dot represents one individual animal. Mean values of hACE2 Tg mice infected with the respective VOCs, as shown in Figure S4 are indicated by colored round symbols. P-values were calculated performing a Kruskal-Wallis test with a Dunn's multiple comparison test (*, $p \leq 0.05$).


A
B
C
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G
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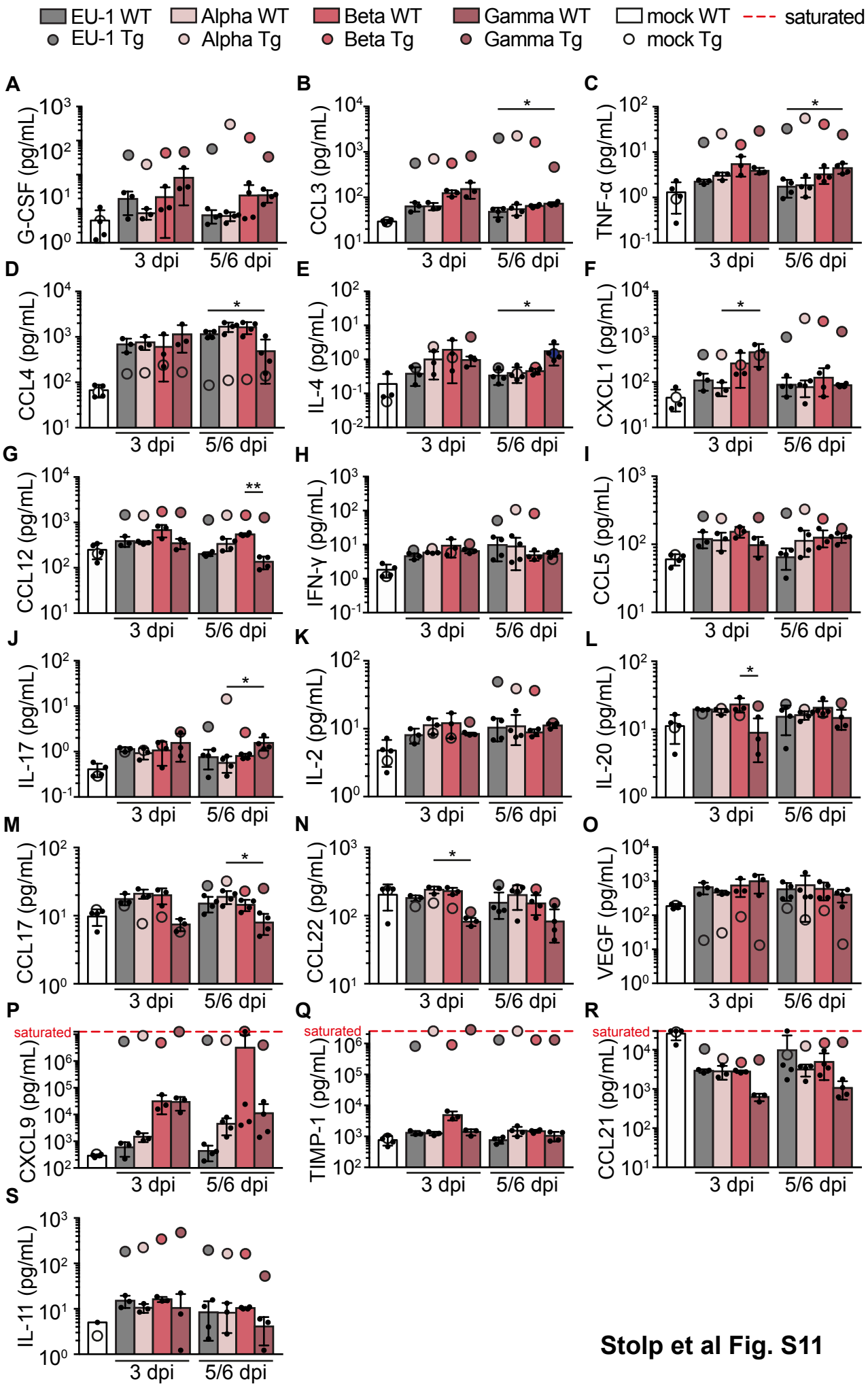
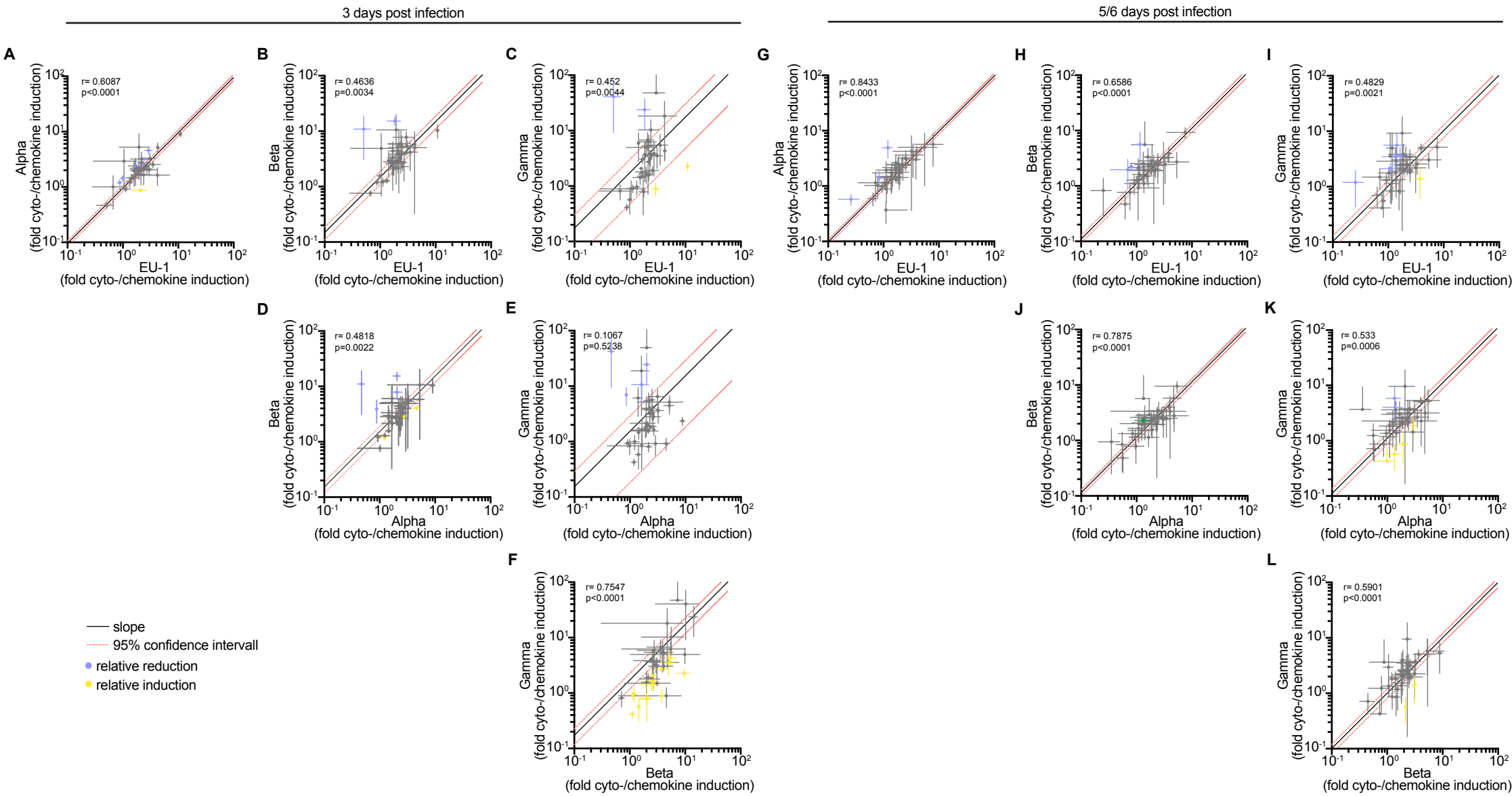
EU-1 3 dpi
Alpha 3 dpi
Beta 3 dpi
Gamma 3 dpi
EU-1 6 dpi
Alpha 6 dpi
Beta 6 dpi
Gamma 5 dpi
VEGF
CCL22
CCL11
CCL17
IL-7
IL-20
IL-1β
CCL19
IFN-β1
IL-2
IL-9
IL-12p70
CXCL2
IL-1α
IL-16
CCL20
IL-15
IL-17
M-CSF
IL-3
CCL5
IL-10
IFN-γ
GM-CSF
CCL12
CX3CL1
IL-12p40
CXCL1
IL-4
CCL4
TNF-α
CCL3
IL-5
CCL2
IL-6
CXCL10
LIF
G-CSF
fold change relative to mock
Tg
WT
reduced <0.85
unaltered 0.85 -1.15
induced 1.15 - 5
induced 5 - 10
induced 10 - 20
induced 20 - 100
induced > 100
Stolp et al Fig. S10

Figure S10: Differential cytokine and chemokine expression in lungs is induced by infection of WT mice with variants of SARS-CoV-2. **Related to Figure 7.** WT mice were infected i.n. with 10^4 pfu of the indicated SARS-CoV-2 variants on day 0 p.i.. 3 (n=3) and 5 (Gamma, n=4)) or 6 (EU-1, Alpha, Beta; n=4) d.p.i. lungs were harvested, homogenized and analyzed for cytokines and chemokines present, performing a Mouse Cytokine Array / Chemokine Array 44-Plex. Mean values were normalized to mock samples and fold changes in cytokine/chemokine levels are plotted relative to the mean of mock infected control mice (n=4). Some Cytokines were excluded as in Figure S5. Shown are mean values ±SD relative to mock samples for lung homogenates of WT mice infected with SARS-CoV-2 (A) EU-1 3 d.p.i., (B) Alpha 3 d.p.i., (C) Beta 3 d.p.i., (D) Gamma 3 d.p.i., (E) EU-1 6 d.p.i., (F) Alpha 6 d.p.i., (G) Beta 6 d.p.i., (H) Gamma 5 d.p.i. Cytokines and Chemokines are ordered according to their strength of induction in the EU-1 Tg 3 d.p.i. sample (Figure S5A), relative to mock. Cytokines/chemokines reduced to less than 0.85 times of the mock value are shown in pink, unaltered cytokines/chemokines in black (fold change of 0.85-1.15), cytokines/chemokines induced 1.15 to 5 times relative to mock in cyan, 5 to 10 times induction relative to mock in green, 10 to 20 times induction relative to mock in yellow and 20 to 100 times induction relative to mock in orange. Dashed red line indicates mock. Transparent bars indicate mean values ±SD of 0hACE2 Tg animals infected with the respective VOCs, as shown in Figure S5.



Stolp et al Fig. S11

Figure S11: Cytokine and chemokine expression induced by infection of WT mice with SARS-CoV-2 variants. **Related to Figure 7.** WT mice were infected i.n. with 10^4 pfu of the indicated SARS-CoV-2 variants on day 0 p.i.. 3 and 5 (Gamma) or 6 (EU-1, Alpha, Beta) d.p.i. lungs were harvested, homogenized and analyzed for cytokines and chemokines present, performing a Mouse Cytokine Array / Chemokine Array 44-Plex. Shown are mean values ±SD of the absolute amounts of Cytokines/Chemokines detected in lung homogenates in pg/mL. Each black dot represents one individual animal. Mean values of hACE2 Tg mice infected with the respective VOCs, as shown in Figure S6 are indicated by colored round symbols. (A) G-CSF, (B) CCL3, (C) TNF-α, (D) CCL4, (E) IL-4, (F) CXCL1, (G) CCL12, (H) IFN-γ, (I) CCL5 (J) IL-17, (K) IL-2, (L) IL-20, (M) CCL17, (N) CCL22, (O) VEGF, (P) CXCL9, (Q) TIMP-1, (R) CCL21, (S) IL-11. Shown are Cytokines/Chemokines that display statistically significant differences in expression for the different VOCs upon infection of either hACE2 Tg or WT mice or that are not included in Figure S10: (P-R) display saturated values that could not be normalized, (S) less than 3 mock samples contained measurable amounts of the cytokine, precluding normalization. Dashed red lines indicate saturation. p-values were calculated performing a Kruskal-Wallis test with a Dunn's multiple comparison test (*, $p \leq 0.05$).



Stolp et al Fig. S12

Figure S12: Specific sets of cytokines and chemokines induced in lungs by the SARS-CoV-2 variants relative to each other in WT mice. Related to Figure 7. Fold cytokine and chemokine inductions in WT SARS-CoV-2 infected mice, relative to mock, 3 days (A-F) and 5/6 days (G-L) post infection, as shown in Figure S10 are plotted in correlation plots for (A, G) EU-1 vs. Alpha, (B, H) EU-1 vs. Beta, (C, I) EU-1 vs. Gamma, (D, J) Alpha vs. Beta, (E, K) Alpha vs. Gamma and (F, L) Beta vs. Gamma. Each symbol represents the mean value ±SD of one cyto-/chemokine. Please note that due to the logarithmic scaling, some SD for cyto-/chemokines that would go beyond the limits of plotting are not visualized, some are smaller than the symbol. The black line indicates the slope of the linear regression curve with the dashed red line representing the 95% confidence intervals. r- and p-values were calculated by nonparametric Spearman correlation. Slopes and confidence intervals relative to EU-1 are plotted in Figure 7B. Cytokines and chemokines that are significantly induced or reduced relative to slope and confidence interval assuming similar expression are plotted in blue and yellow, respectively. This color code is also used in the corresponding heat maps that displays specific sets of cytokines/chemokines altered by the indicated SARS-CoV-2 variants relative to one another shown in Figure 7H and I.