

Supplementary Materials for

Hamsters immunized with formalin-inactivated SARS-CoV-2 develop accelerated lung histopathological lesions and Th2-biased response following infection

Supplementary Table S1 (VAED model development study). Group mean and 95% confidence intervals (95% CI) of the viral loads in lungs AT 5 DPI, as evaluated by subgenomic (sg)RNA RT-qPCR; n=4 per group.

Group	RNA copies per gram tissue [Log10]	95% CI
mock-vaccinated	9.32	7.98; 10.66
2x high dose	6.18	0; 13.06
2x low dose	8.53	7.66; 9.39
1x high dose	8.10	6.52; 9.68
1x low dose	8.13	6.22; 10.03

Supplementary Table S2 (VAED model development study). Geometric mean titers (GMT) and 95% confidence intervals of the neutralizing antibodies measured in the treatment groups; n=4 per group per DPI except for group 1x high dose on 13 DPI where n=3.

Group	GMT 5 DPI	95% CI	GMT 13 DPI	95% CI
mock-vaccinated	140	88; 211	225	86; 492
2x high dose	332	53; 1047	291	132; 551
2x low dose	140	88; 211	360	65; 1125
1x high dose	106	67; 160	516	120; 1831
1x low dose	135	49; 284	725	456; 1093

Supplementary Table S3 (VAED model development study). Group means and 95% confidence intervals of the extent of histopathological lesions, expressed as percentage of the total lung area of a section of the whole left lung lobe; n=4 per group per DPI except for group 1x high dose on 13 DPI where n=3.

Group	Affected lung tissue [%], 5 DPI	95% CI	Affected lung tissue [%], 13 DPI	95% CI
mock-vaccinated	32	2; 62	9	3; 15
2x high dose	40	24; 57	8	4; 12
2x low dose	50	39; 61	16	8; 24
1x high dose	64	36; 91	14	11; 17
1x low dose	57	37; 77	18	13; 23

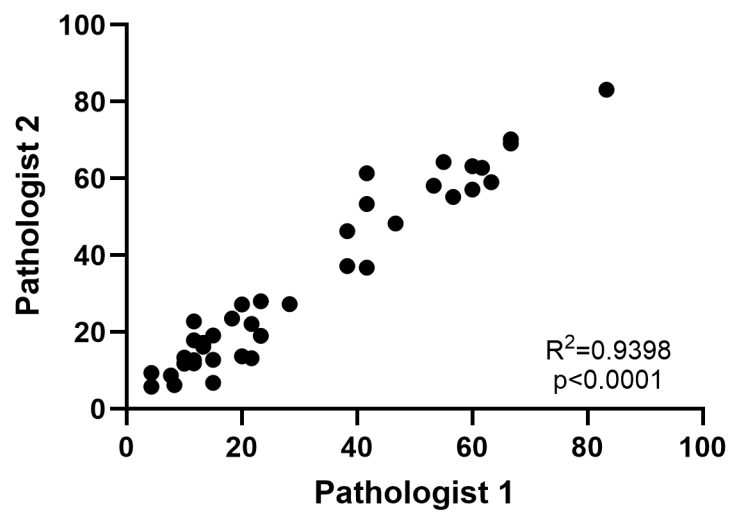
Supplementary Table S4 (VAED kinetic study). Group mean and 95% confidence intervals (95% CI) of the viral loads in lungs per necropsy day, as evaluated by total E-gene RNA RT-qPCR; n=3 per group.

Group	RNA copies per gram tissue [Log10] (95% CI)				
	2 DPI	4 DPI	6 DPI	8 DPI	10 DPI
Re-challenge	5.80 (4.28 – 7.32)	5.22 (3.42 – 7.02)	5.12 (3.21 – 7.02)	5.86 (5.29 – 6.44)	5.28 (3.91 – 6.65)
Mock-vaccinated	10.93 (10.66 – 11.19)	10.18 (9.56 – 10.79)	8.49 (7.00 – 9.97)	7.20 (6.13 – 8.28)	6.69 (5.95 – 7.42)
Vaccinated	10.13 (8.64 – 11.63)	9.72 (8.46 – 10.98)	6.97 (6.54 – 7.40)	6.47 (5.06 – 7.88)	5.89 (4.27 – 7.51)

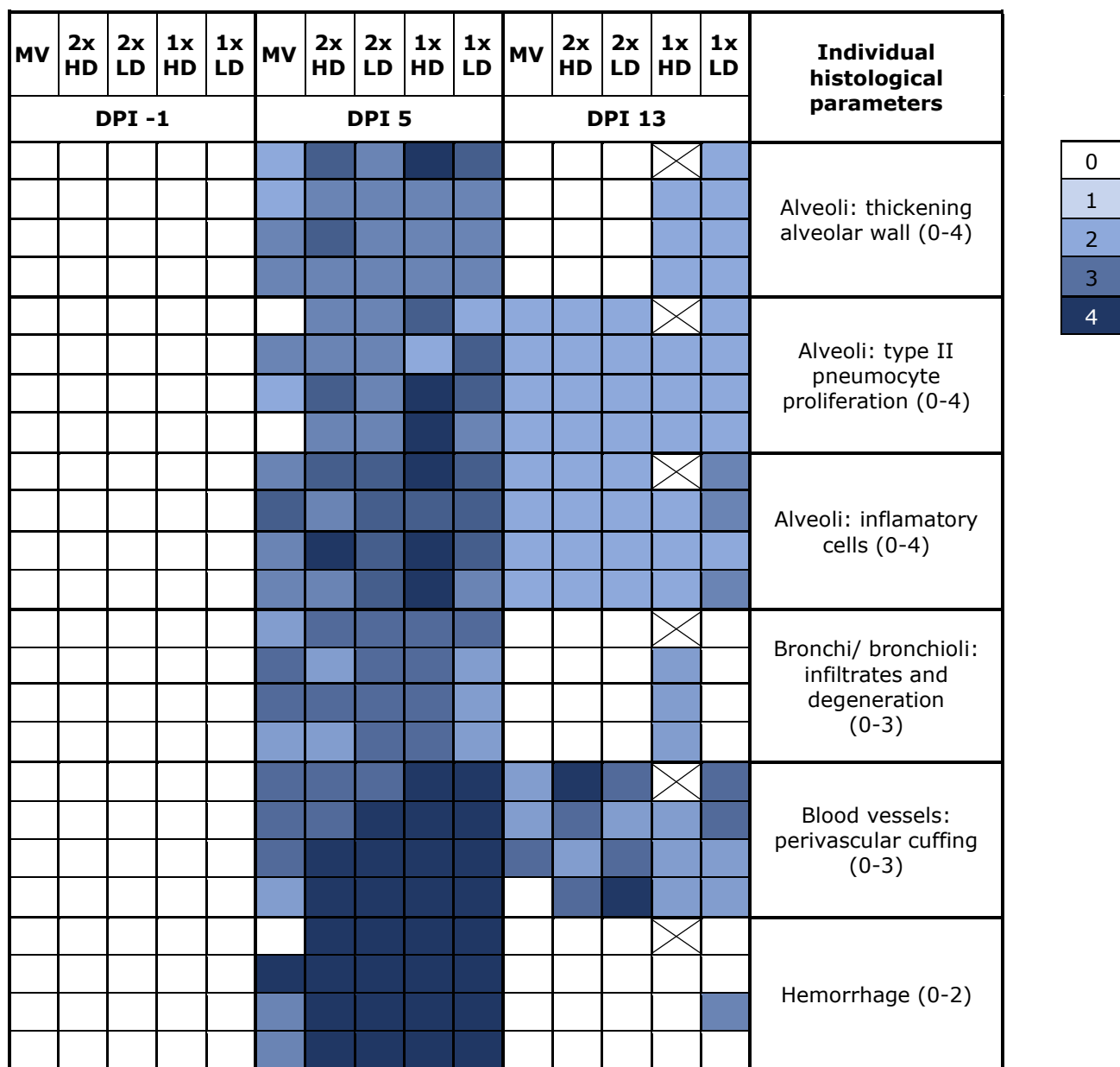
Supplementary Table S5 (VAED kinetic study). List of gene identifiers in the panel used for gene expression analysis. Gene annotations associated with Th-pathways are based on human and mouse. Annotations for specific immune cell populations are based on predominant gene expression patterns in mice.

Th pathway or immune cell type	Gene identifier
Th1	<i>Ifngr1, Ifngr2, Il2, Stat4, Il12rb2, Bcl6, Jak1, Jak2, Gata3, Stat1, Il12b, Il12a, Rela, Il12rb1, Tbx21, Tyk2, Ifng, Maml2, Maml3, Dll1, Runx3</i>
Th2	<i>Stat6, Stat5B, Il4, Maf, Il2rg, Ikzf2, Il6, Tbx21, Stat5A, Gata3, Il2ra, Il2rb, Il4R, Notch1, Il2, Il5, Ptgdr2, Notch2, Il13, Jag1</i>
Th17	<i>Il6st, Il17a, Il1b, Foxp3, Jak2, Ahr, Il4, Il1r1, Tyk2, Tgfbr1, Il21r, Tgfbr2, Rorc, Rora, Il22, Smad2, Il2rg, Tnf, Il21, Il6, Mapk12, Il12rb1, Tbx21, Mtor, Tgfb1, Jak1, Il23r, Il6r, Il17f, Il23a, Irf8, Stat3, Hif1a, Il12b, Irf4, Smad3</i>
Treg	<i>Foxp3, Il10, Tyk2, Tgfbr1, Tgfbr2, Smad2, Foxo1, Entbd1, Il10ra, Ikzf4, Stat5A, Cd69, Tgfb1, Nt5e, Jak1, Prdm1, Stat3, Irf4, Smad3</i>
Cd8 T-cells	<i>Cd8A, Cd8B, Ly49</i>
Cytotoxic cells	<i>Ctsw, Gzma, Gzmb, Gzmh, Gzmk, Klrb1, Klrd1, Klrk1, Nkg7, Prf1, Xcl1</i>
T-cells	<i>Cd3d, Cd3e, Cd3g, Cd6, Trat1, Il1a, Cd80, Itga4</i>
NK-cells	<i>Ncr1, Eomes, Ly49, Fcar</i>
Neutrophils	<i>Csf3r, Fpr1, Cxcr2, Itgam, Ly6g, S100a8/9, Clec7a, Ly49, Ym1</i>
Macrophages	<i>Csf2 (alveolar macrophages), Nos2, Arg1, Ccl22, Cd163, Clec7a, Retnla, Ym1, Adgre1, Cd68, Msr1, S100A8/9, H2-Ab1, Ly49, Siglecf (alveolar macrophages), Cd80</i>
Eosinophils	<i>Ccr3 (also basophils and Th2 lymphocytes), Il5ra (also basophils and B-cells), Siglecf</i>
DCs	<i>Flt3, H2-Ab1, Cd209, Ccl22, Clec7a, Cd8A, Cd8B, Adgre1, Ly49, Cd80, Itga4</i>
IFN I or III	<i>Ifna1, Ifnb1, Ifnl1</i>

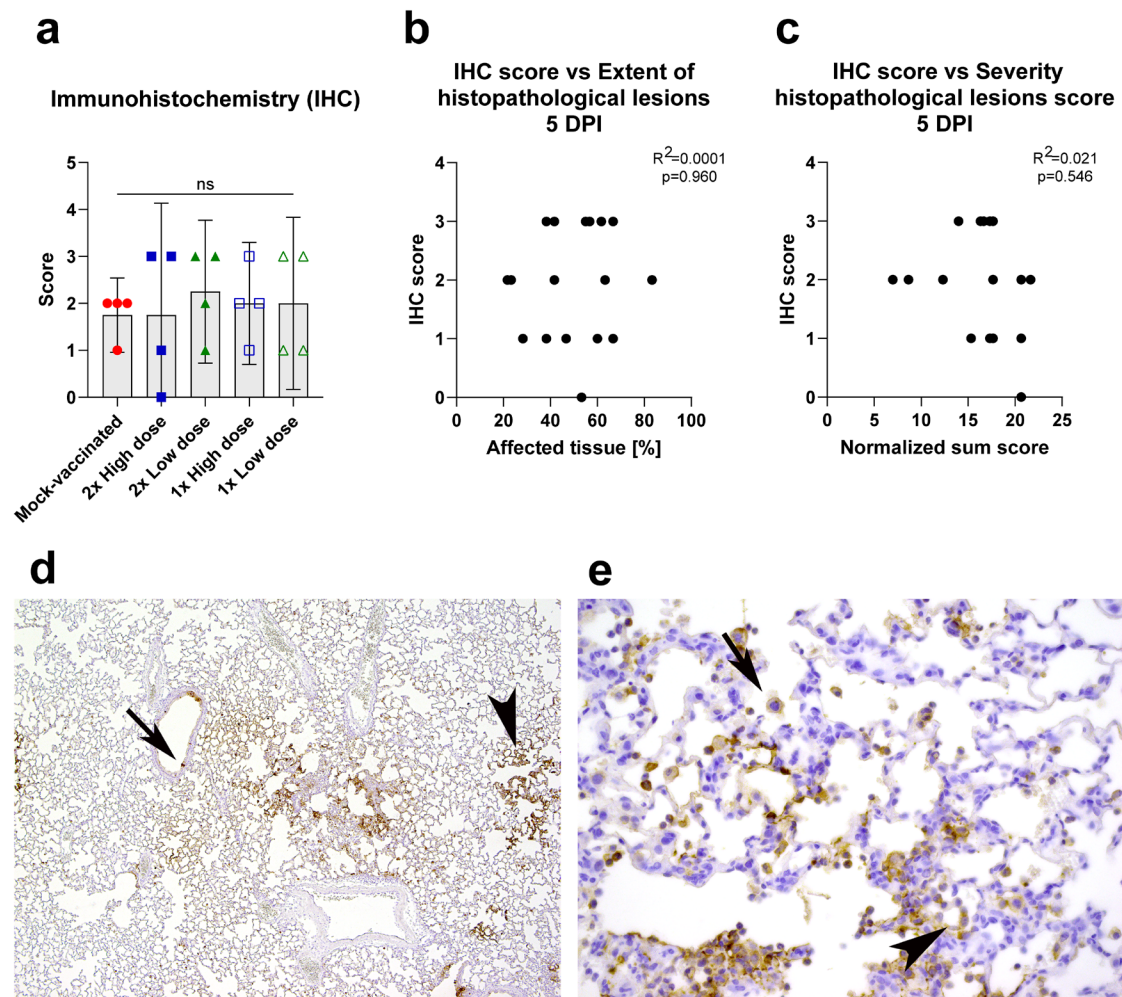
Extent of lung histopathological lesions [%]



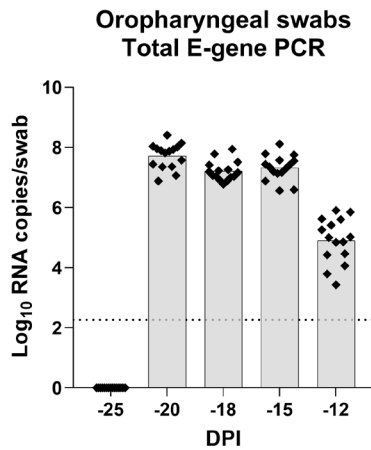
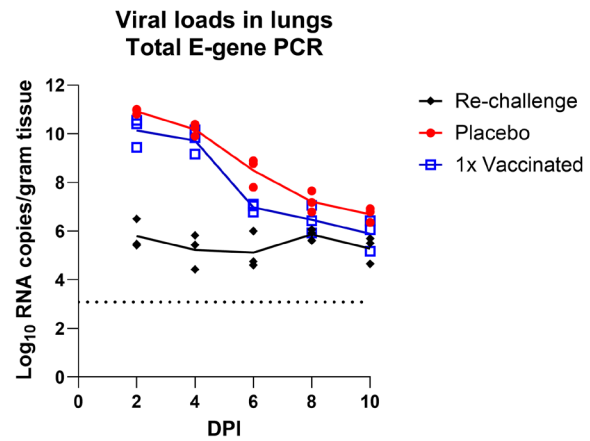
Supplementary Figure S1 (VAED model development study) Correlation analysis between the two independent evaluations of the extent of pathological lesions present in lungs of hamsters vaccinated with different regimens of FIWV vaccine. Lungs obtained on 5 DPI and 13 DPI were scored. Correlation analysis was performed with GraphPad Prism v. 8.3.0; n=39.



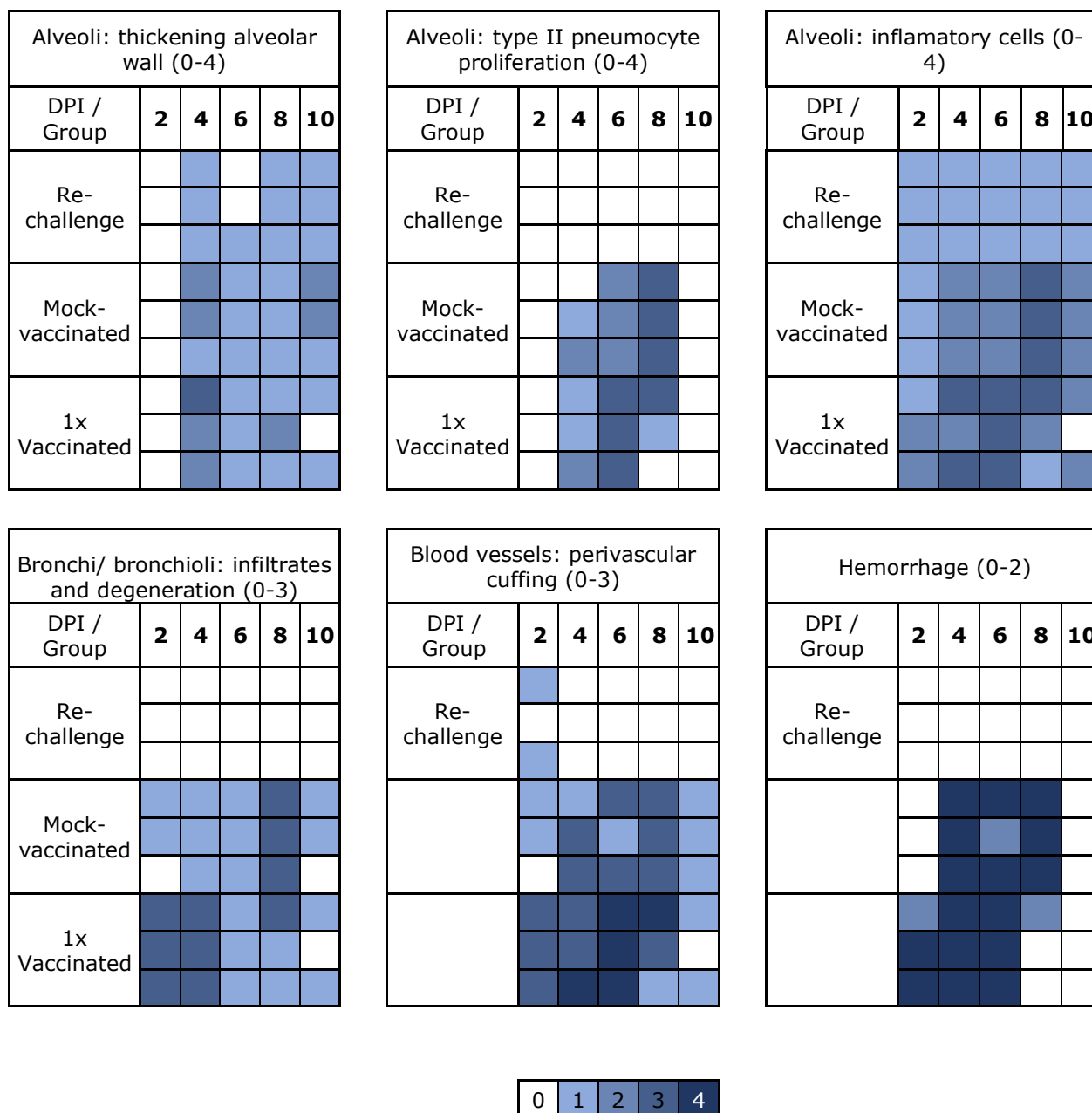
Supplementary Figure S2 (VAED model development study). Heatmap, showing scores for 6 individual parameters, per group and per DPI. The scale of the parameters with range between 0-3 and 0-2 was normalized to the highest range (0-4), so that the highest severity for all parameters is comparable (detailed in materials and methods section). Treatment groups are shown on top of the heatmap (MV = mock-vaccinated; 2xHD = 2x high dose; 2xLD = 2x low dose; 1xHD = 1x high dose; 1xLD = 2x low dose). Within one parameter and DPI, each box represents one animal. The animals from group 1x high dose that was excluded from scoring is shown with crossed box. A color map is shown on the right.



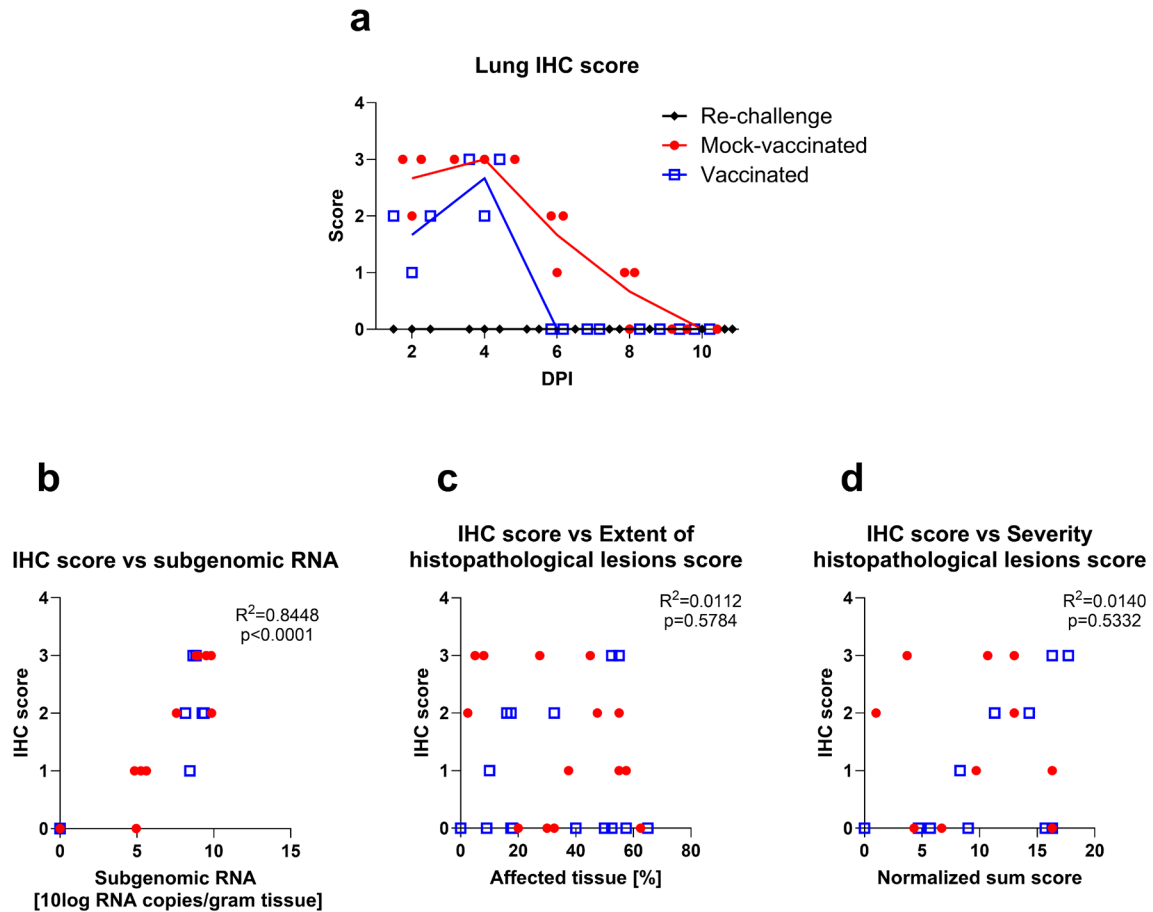
Supplementary Figure S3 (VAED model development study) Immunohistochemistry (IHC) evaluation of lungs. **a)** IHC scores of hamster lungs from all treatment groups at 5 DPI; n=4 per group. Bars illustrate group means, error bars – 95% CI; ns—not significant (≥ 0.05). **b)** Correlation analysis between the IHC scores and the extent of the histopathological lesions at 5 DPI. **c)** Correlation analysis between the IHC scores and the cumulative (sum) score of the severity of histopathological lesions at 5 DPI. **b) and c)** Correlation analysis was performed with GraphPad Prism v. 8.3.0; n=20. **a to c)** Symbols show individual values. **d and e)** Immunohistochemistry staining of SARS-CoV nucleoprotein in lung tissue. Two representative photo showing different cell types that harbour virus nucleoprotein. **d)** Bronchiolar epithelial cells (arrow) and multifocal expression in pneumocytes (arrowhead), objective 2.5; **e)** Alveolar macrophages (arrow) and pneumocytes (arrowhead), objective 40.

a**b**

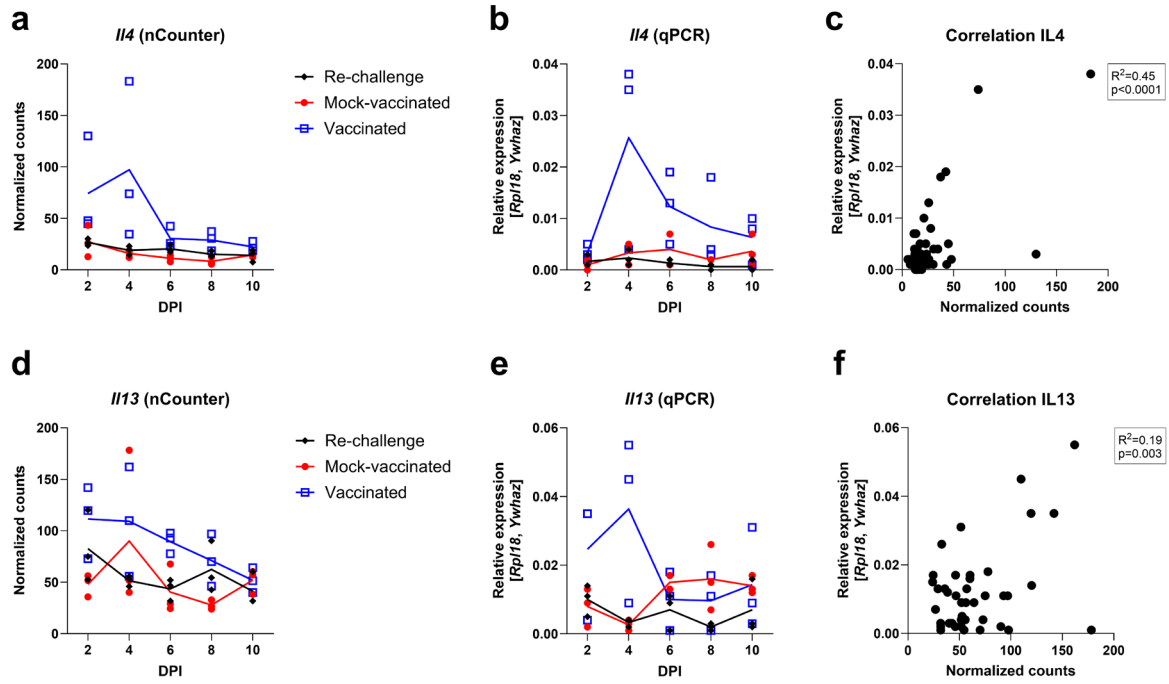
Supplementary Figure S4 (VAED kinetic study). Viral loads as detected by total E-gene RT-qPCR. **a)** Confirmation of the primary SARS-CoV-2 infection in the Re-challenge group of hamsters. Viral RNA loads in oropharyngeal swabs (n=15), expressed as log₁₀ RNA copies per swab. Bars show group means and symbols – individual values. **b)** Viral RNA loads in lungs. **a and b)** The 50% limit of detection of the PCR assay is indicated by the dotted line. DPI – days post infection.



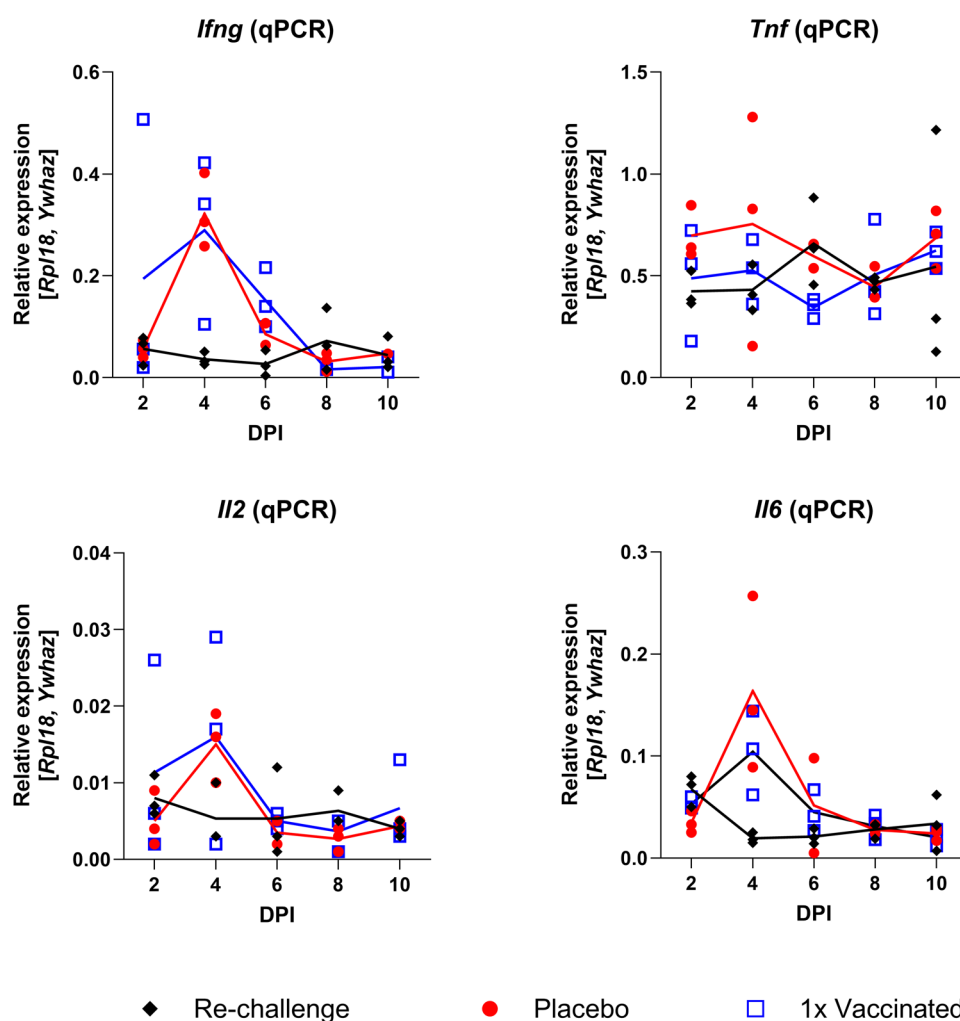
Supplementary Figure S5 (VAED kinetic study). Heatmap, showing scores for 6 individual parameters, per group and per DPI (Study 2). The scale of the parameters with range between 0-3 and 0-2 was normalized to the highest range (0-4), so that the highest severity for all parameters is comparable (detailed in materials and methods section). Treatment groups and individual parameters are shown. Within one parameter, each box represents one animal. A color map is shown at the bottom.



Supplementary Figure S6 (VAED kinetic study). Immunohistochemistry (IHC) evaluation of lungs (Study 2). **a)** IHC scores of hamster lungs from the three treatment groups; $n=3$ per group and timepoint. **b)** Correlation analysis between the IHC scores and the data generated by the sgRNA RT-qPCR over time. **c)** Correlation analysis between the IHC scores and the extent of the histopathological lesions over time. **d)** Correlation analysis between the IHC scores and the sum scores of the histopathological lesions. **a) to d)** Symbols show individual values and **a)** lines illustrate group means. **b to d)** $n=30$; Correlation analysis was performed on the whole dataset, group colors were used only for visualization. GraphPad Prism v. 8.3.0 software was used for correlation analysis.



Supplementary Figure S8 (VAED kinetic study). Expression of *Il4* and *Il13*, as determined by nCounter Technology (**a** and **d**) or RT-qPCR (**b** and **e**), and correlation between values determined by both methods (**c** and **f**). **a, b, d, e**) Symbols represent individual normalized counts or relative expression for each hamster and lines show means per group (n=3 per group and timepoint). **c** and **f**) Symbols represent individual values; n=30.



Supplementary Figure S9 (VAED kinetic study). Cytokine gene expression in lung tissue as quantified with RT-qPCR. Quantities are expressed as expression of the genes of interest relative to two housekeeping genes (*Rpl18* and *Ywhaz*) and were calculated according to the $\Delta\Delta CT$ method as described in materials and methods. Symbols show individual values and lines show means per group, $n=3$ per group and timepoint.