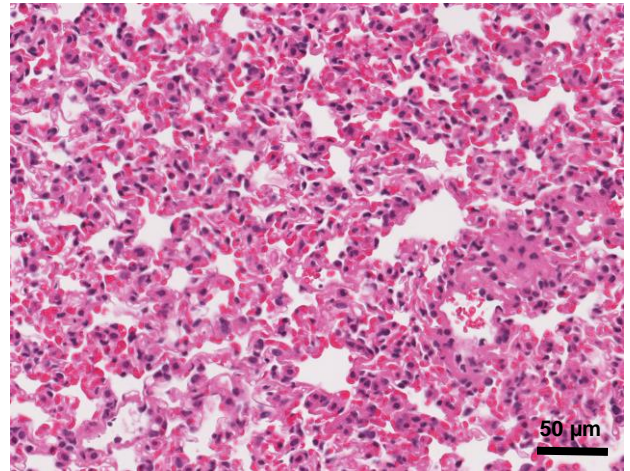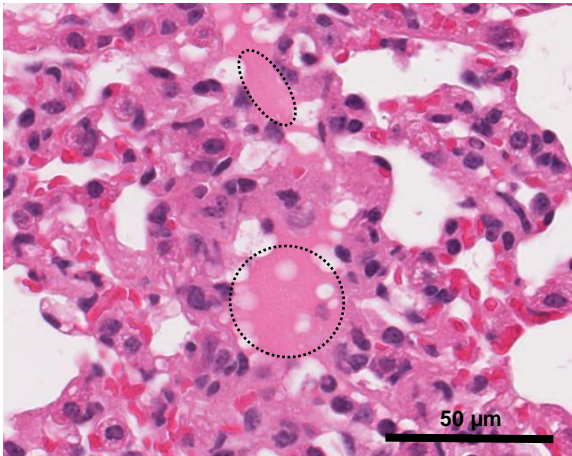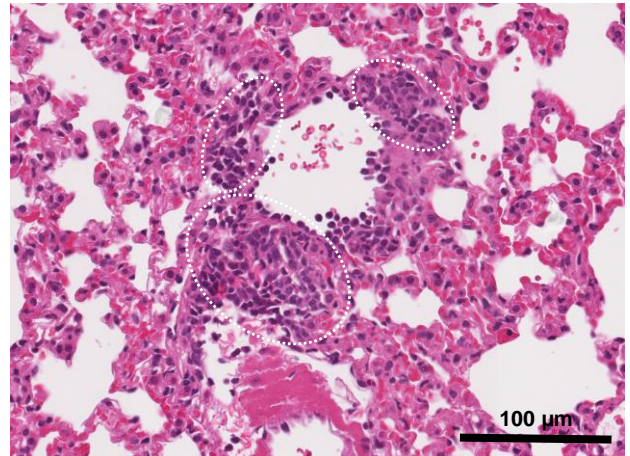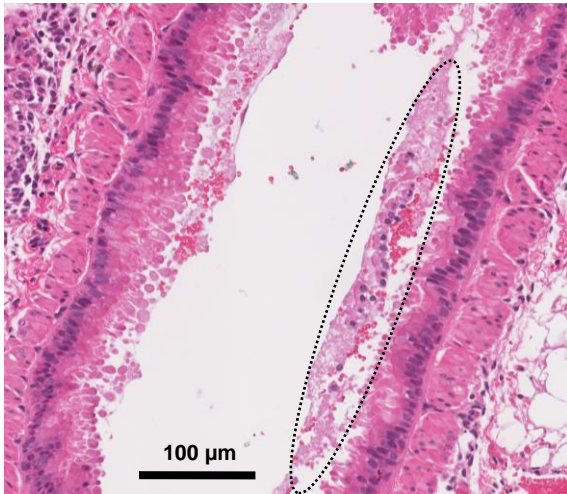Focal alveolar haemorrhage

Lung consolidation, thickening of alveolar septa
(loss of alveolar airspaces)
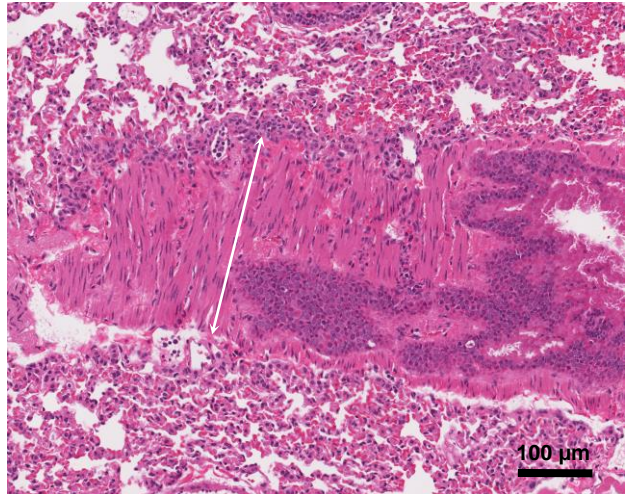

Alveolar edema

Focal leukocyte infiltration

Serum and red blood cells in airways
(mild sloughing of bronchial epithelium)

Smooth muscle hyperplasia/hypertrophy

Supplementary Figure 1. Images of H&E stained sections from lungs of mACE2-hACE mice infected with omicron BA.5, 6 dpi. Histopathological features described herein have been reported previously for mouse models of COVID-19.
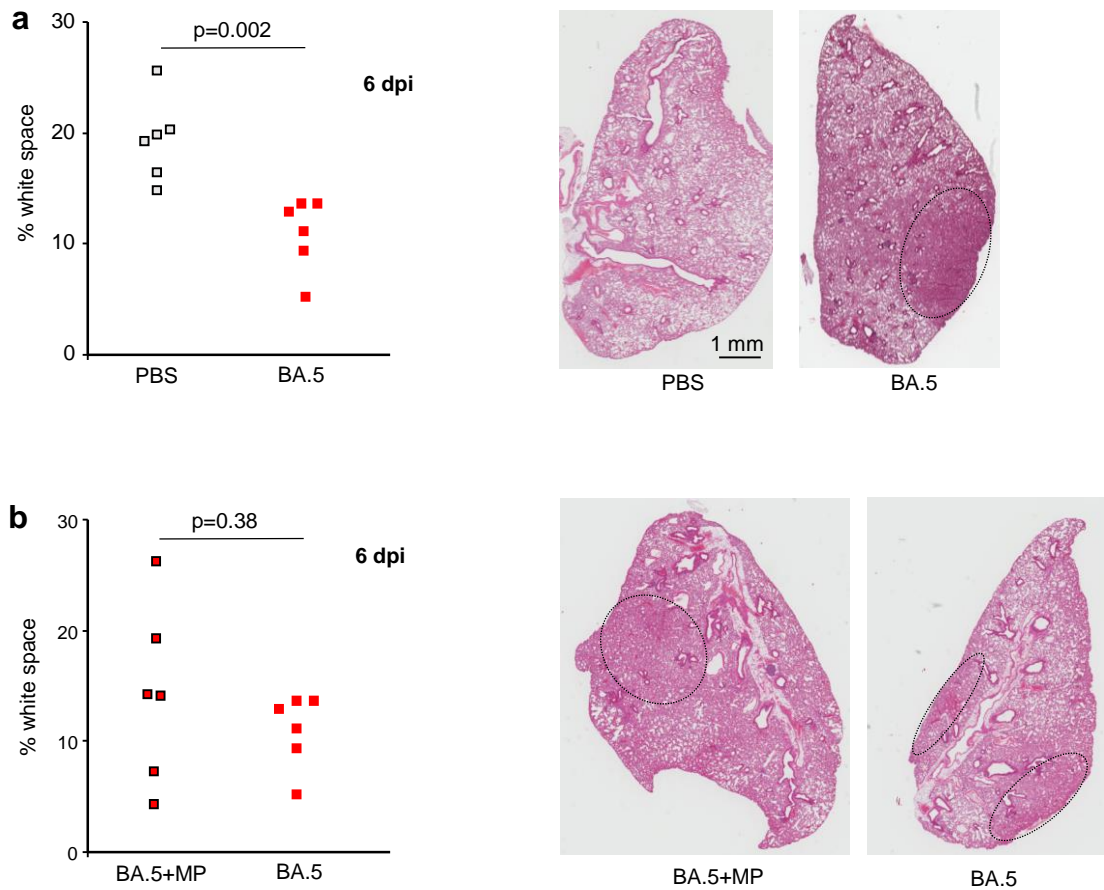
Supplementary Figure 2. **a** H&E stained sections of lungs of mACE2-hACE mice inoculated with PBS or infected with BA.5 (6 dpi), where analysed for white space; the area within the lung that remains unstained (white) as a percentage of the total lung area is shown for each mouse.   Statistics by t test. H&E images show examples of whole lung sections stained with H&E; an area with overt loss of white space (lung consolidation) is indicated by the dotted oval. **b** As for "a" but comparing lungs receiving BA.5+MP vs. BA.5.
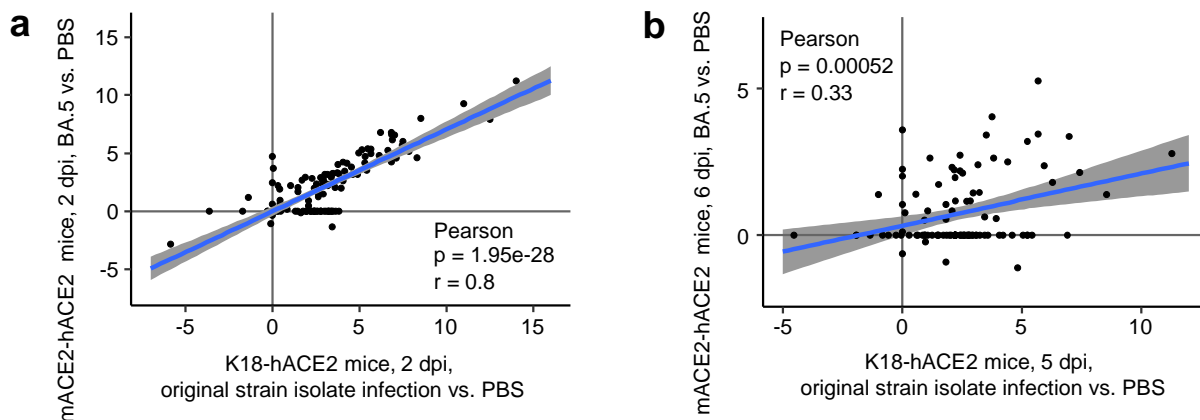
Supplementary Figure 3. **a** Pearson correlation of IPA USR z-score for Cytokine annotations for lungs at 2 dpi for the two indicated mouse models.   **b** As for 'a' but at 5/6 dpi. More cytokine USR annotations with higher z scores were evident in the K18-hACE2 model consistent with the lower level of disease severity in the BA.5 mACE2-hACE2 model.
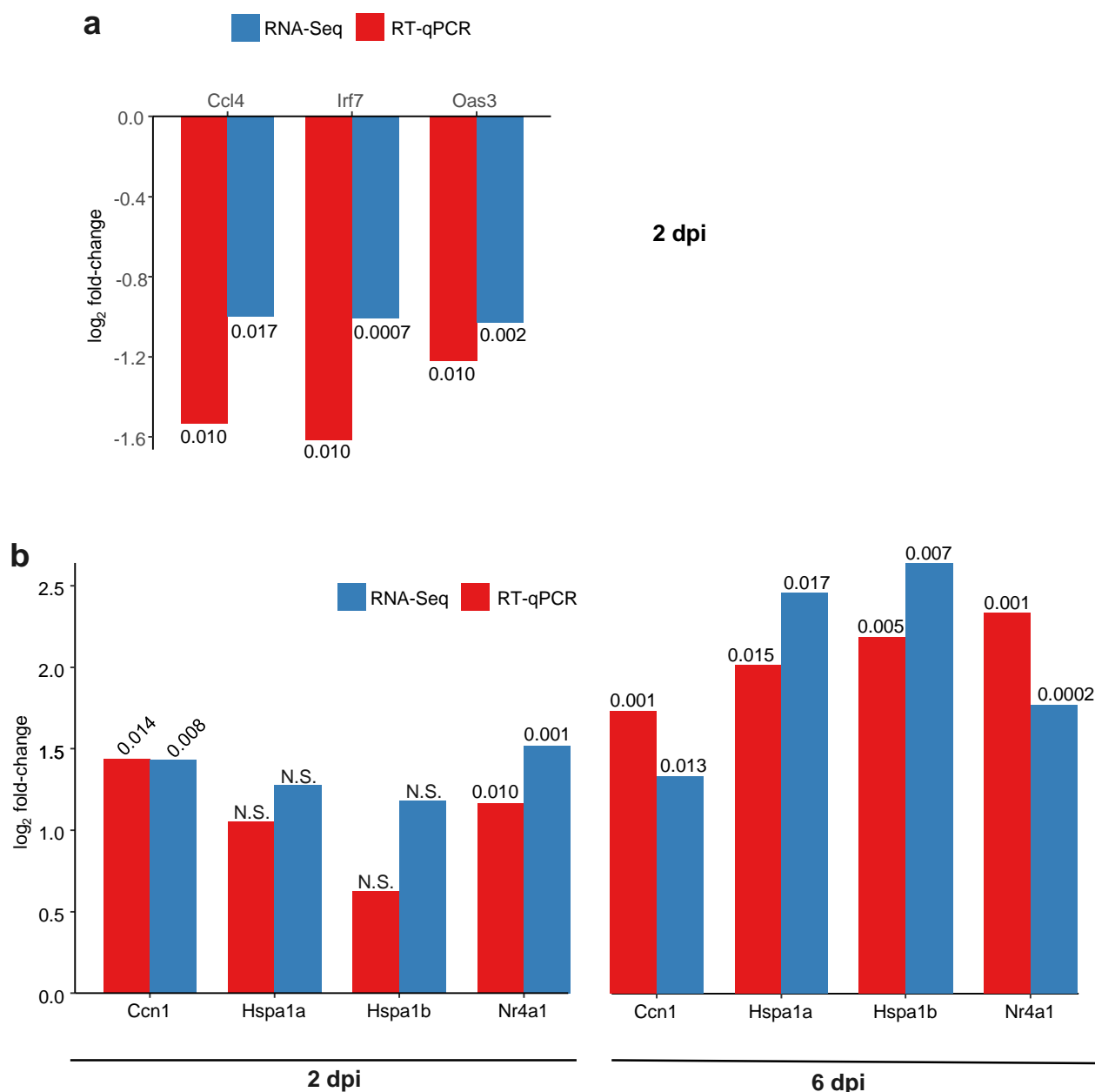
Supplementary Figure 4. RT-qPCR validation. **a** Three interferon regulated genes (IRGs) that were among the most down-regulated DEGs at 2 dpi were validated using RT-qPCR. Red columns are annotated with p-values of t-tests comparing normalised expression (ΔC_t) of indicated genes for BA.5+MP vs. BA.5. Blue bars are annotated with FDR-corrected p-values of corresponding EdgeR differential expression tests from the RNA-Seq data. **b** RT-qPCR validation of selected top DEGs associated with stress and/or inhalation of smoke or diesel at 2 dpi and 6 dpi. Bars are annotated with p-values as in a.
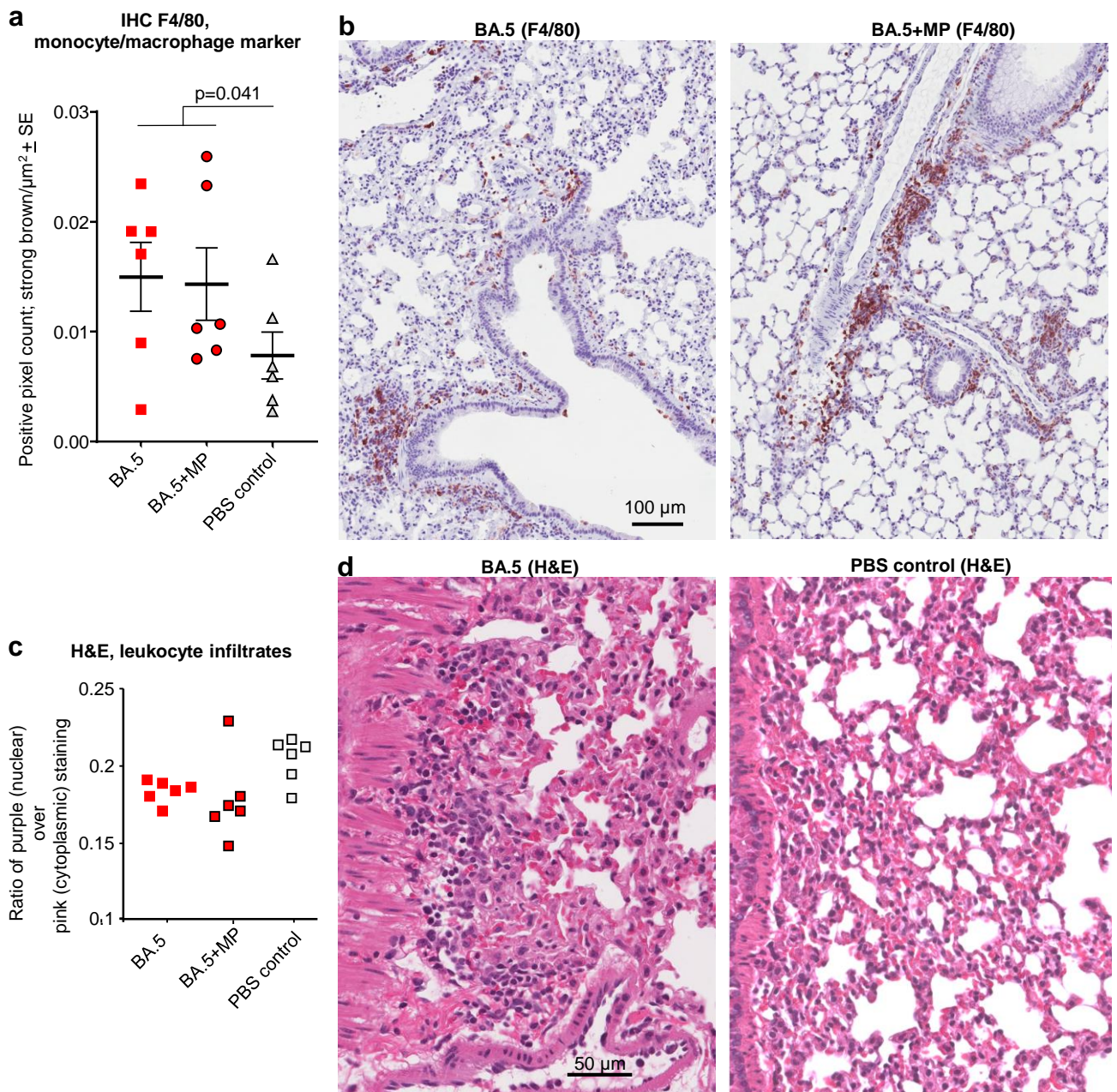
Supplementary Figure 5. **a** Positive pixel count analysis of IHC stained (F4/80) sections of lungs of mACE2-hACE mice infected with BA.5 (BA.5), infected with BA.5 and inoculated with MP (BA.5+MP), or inoculated with PBS (PBS control). Lung were harvested on 6 dpi. The small increase in the infected groups reached significance if both infected groups were taken together (n=12), statistics by t test. **b** Examples of IHC staining in sections with high pixel counts. Positive cells (brown) are clearly visible, often in infiltrates adjacent to airways. (Counter staining with haematoxylin, light purple). **c** Positive pixel count analysis of H&E stained sections from the same mice as in "a". Purple (nuclear) over pink (cytoplasmic) staining is a broad (crude) measure of leukocyte infiltration, as leukocytes have a higher nuclear to cytoplasm ratio. However, no significant differences in cellular infiltrates were evident for BA.5+MP vs. BA.5. **d** Examples of H&E staining. Infiltrates in infected mice were often diffuse and associated with lung consolidation & smooth muscle hyperplasia (left), such that the purple/pink ratios were not significantly higher even than controls (right). Other mouse models of COVID-19 have more severe lung disease and are associated with more abundant and less diffuse leukocyte infiltrates, such that purple/pink ratios are significantly higher in infected lungs when compared with controls (Rawle et al., 2022; Dumenil et al., 2022).
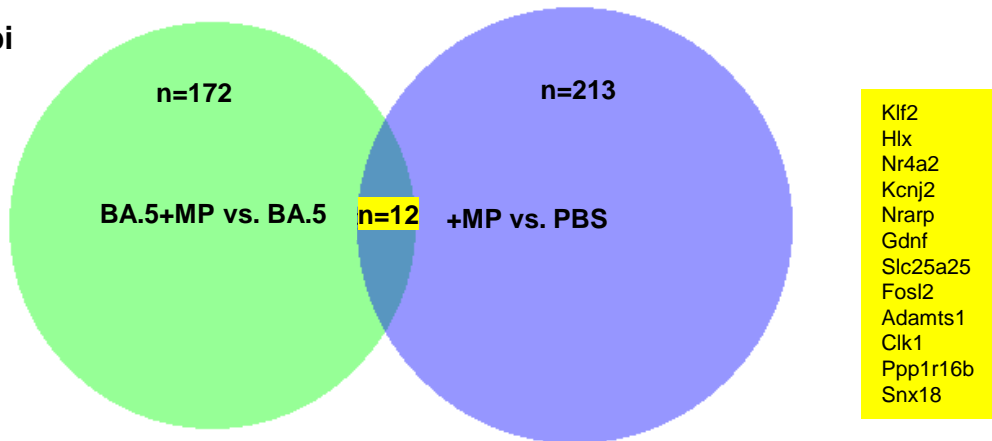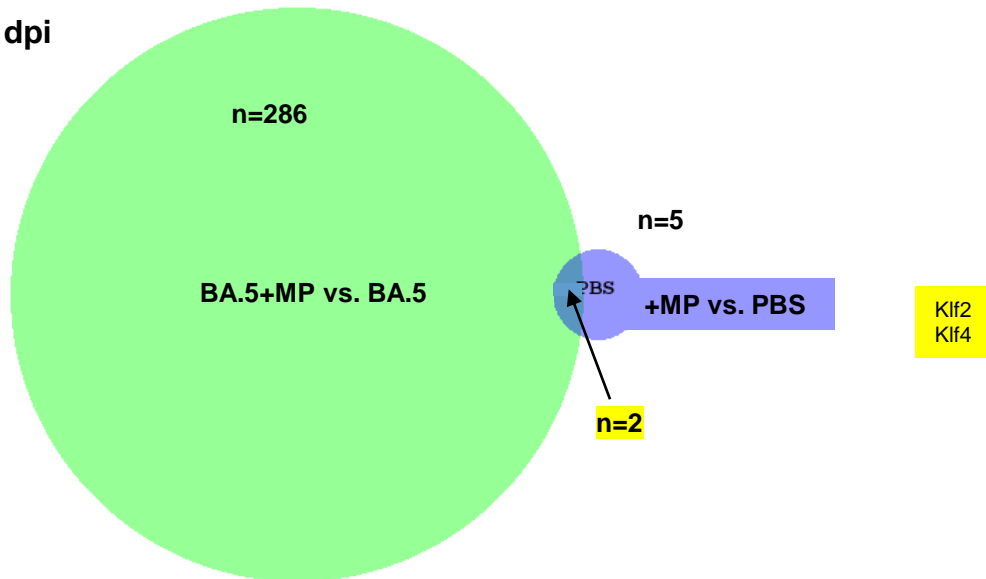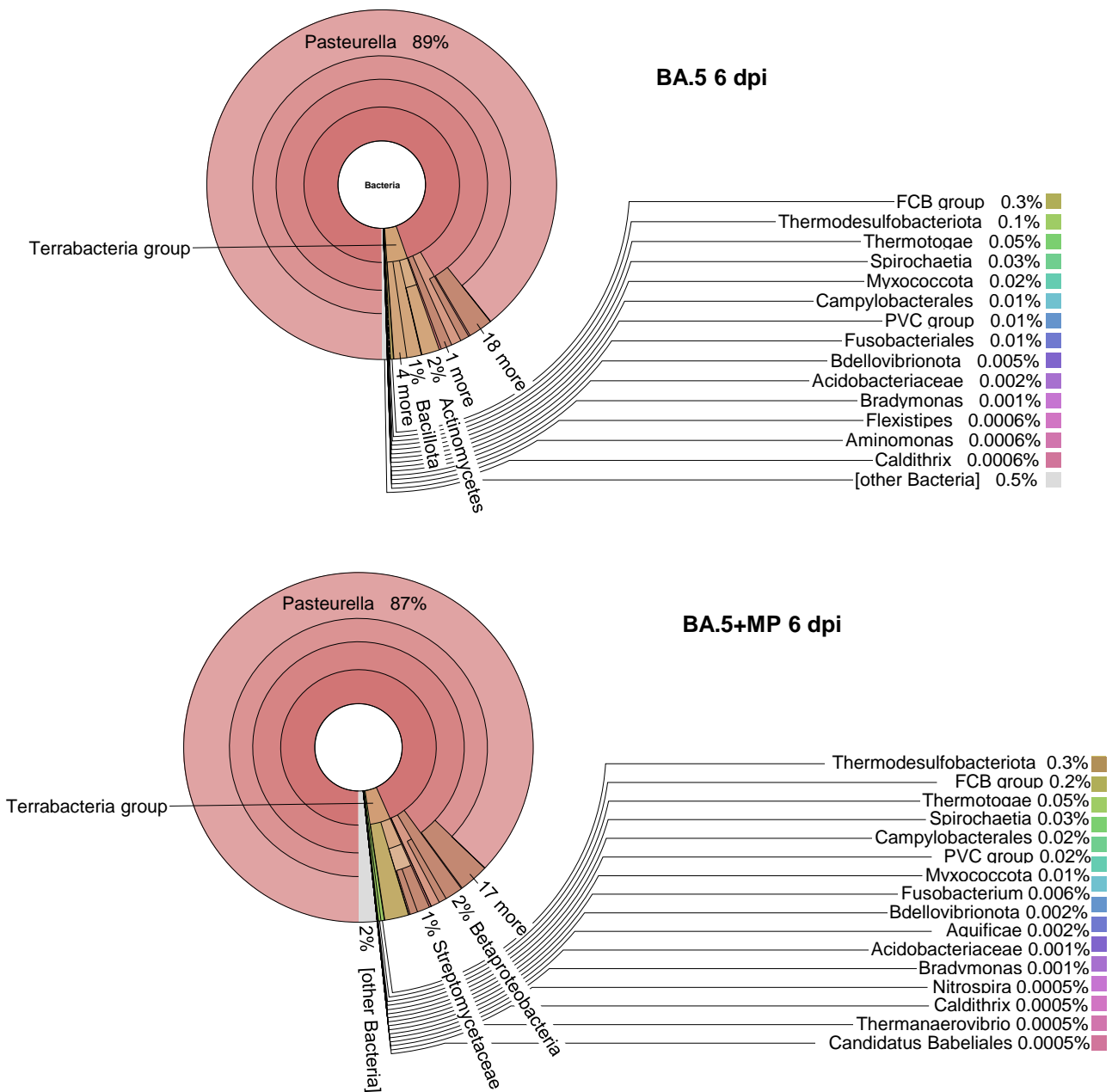
Supplementary Figure 6. Up-regulated DEGs from the indicated comparisons. Overlapping DEGs are indicated in yellow. Klf2 was an upregulated DEG in all comparisons.

BA.5 6 dpi

Pasteurella 89%

Terrabacteria group

Bacteria

18 more
1 more
2%
1%
4 more
Actinomycetes
Bacillota

FCB group 0.3%
Thermodesulfobacteriota 0.1%
Thermotogae 0.05%
Spirochaetia 0.03%
Myxococcota 0.02%
Campylobacterales 0.01%
PVC group 0.01%
Fusobacteriales 0.01%
Bdellovibrionota 0.005%
Acidobacteriaceae 0.002%
Bradymonas 0.001%
Flexistipes 0.0006%
Aminomonas 0.0006%
Caldithrix 0.0006%
[other Bacteria] 0.5%

BA.5+MP 6 dpi

Pasteurella 87%

Terrabacteria group

17 more
2%
1%
2%
Betaproteobacteria
Streptomycetaceae
[other Bacteria]

Thermodesulfobacteriota 0.3%
FCB group 0.2%
Thermotogae 0.05%
Spirochaetia 0.03%
Campylobacterales 0.02%
PVC group 0.02%
Myxococcota 0.01%
Fusobacterium 0.006%
Bdellovibrionota 0.002%
Aquificae 0.002%
Acidobacteriaceae 0.001%
Bradymonas 0.001%
Nitrospira 0.0005%
Caldithrix 0.0005%
Thermanaerovibrio 0.0005%
Candidatus Babeliales 0.0005%

Supplementary Figure 7. Kraken metagenomic sequence classification undertaken as described (Hazlewood et al., 2021). Briefly, the unmapped reads were analysed using metagenomic sequence classification. Replicates were concatenated to produce one read file per treatment. Data was visualized using Krona, showing Bacteria (root).