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Supplemental Information

Heterogeneity of Antiviral Responses in the Upper Respiratory Tract Mediates Differential Non-lytic Clearance of Influenza Viruses

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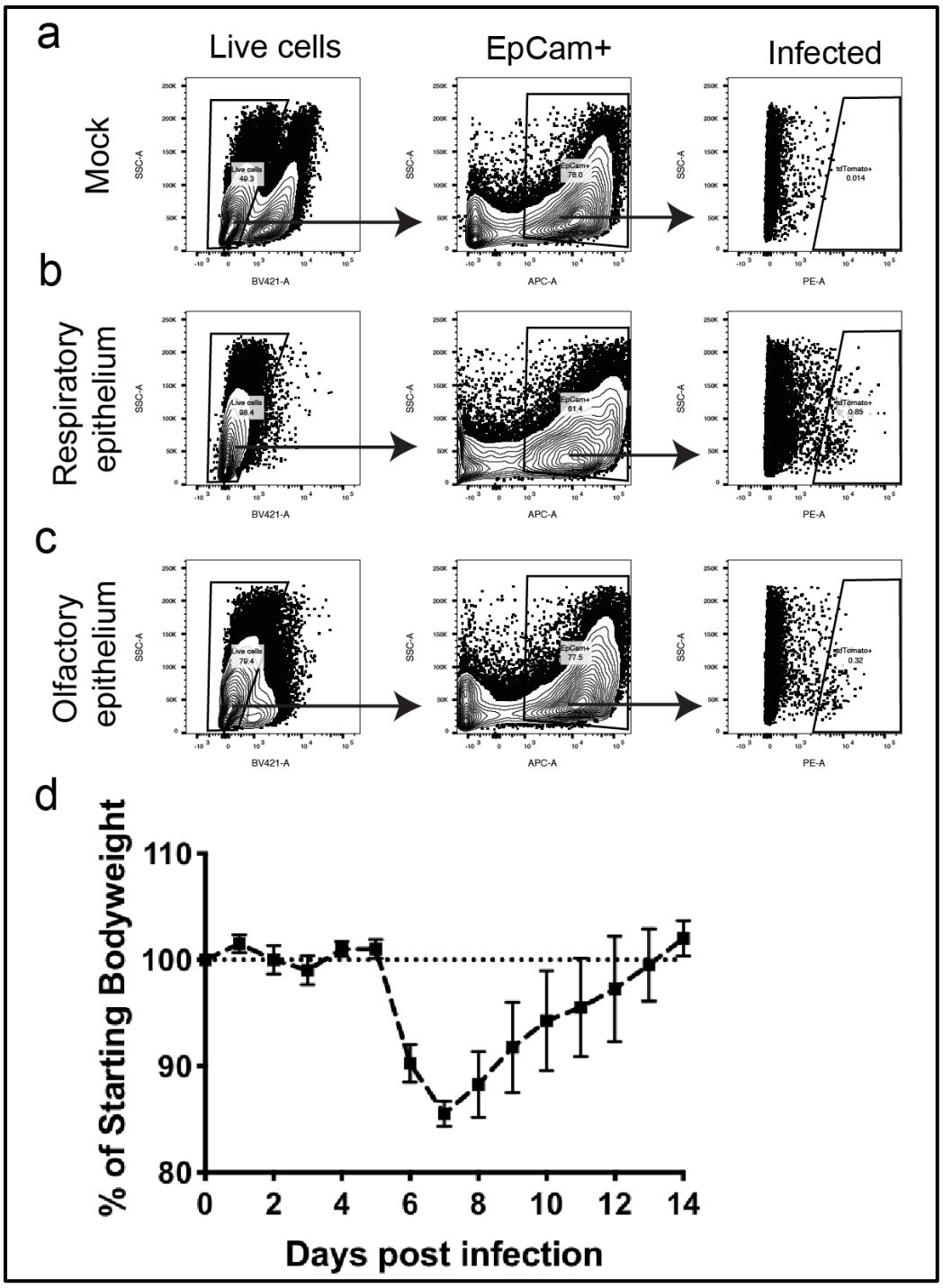
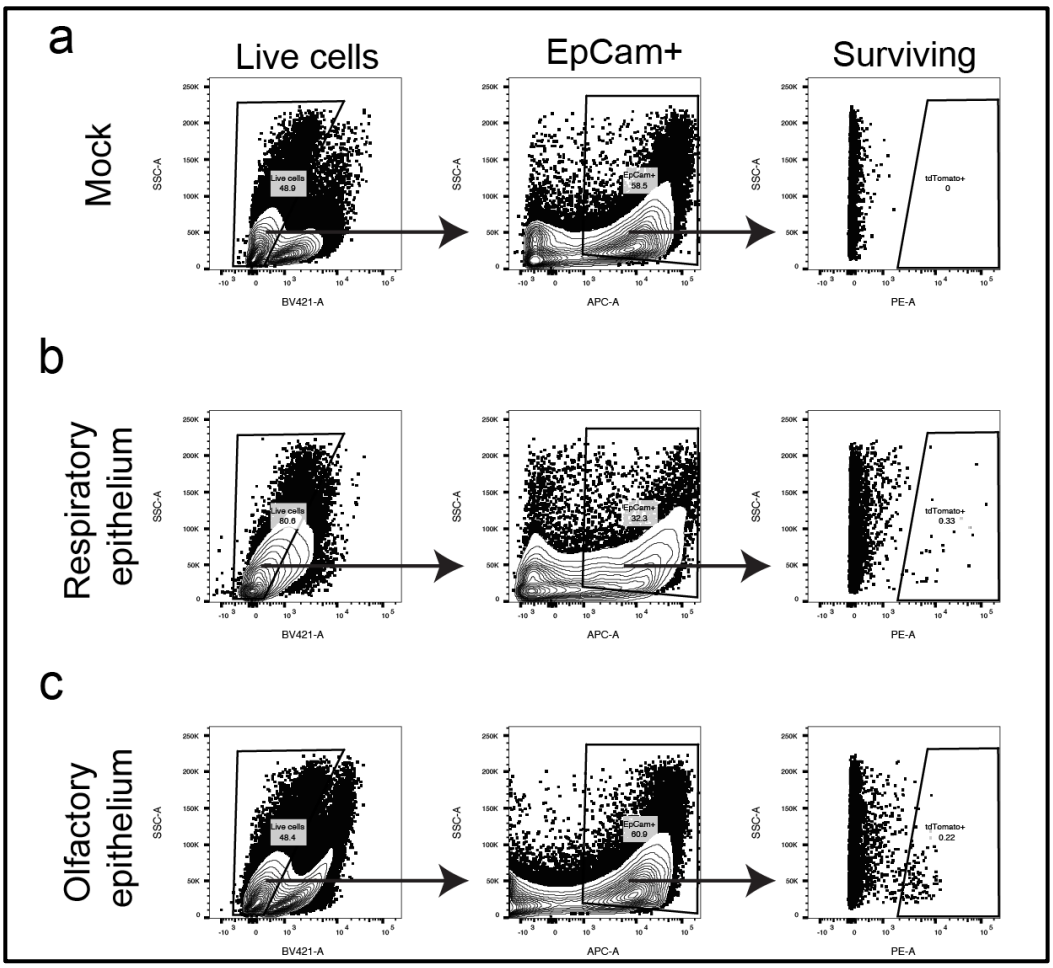
Supplemental Figures and Legends, Dumm *et al*.

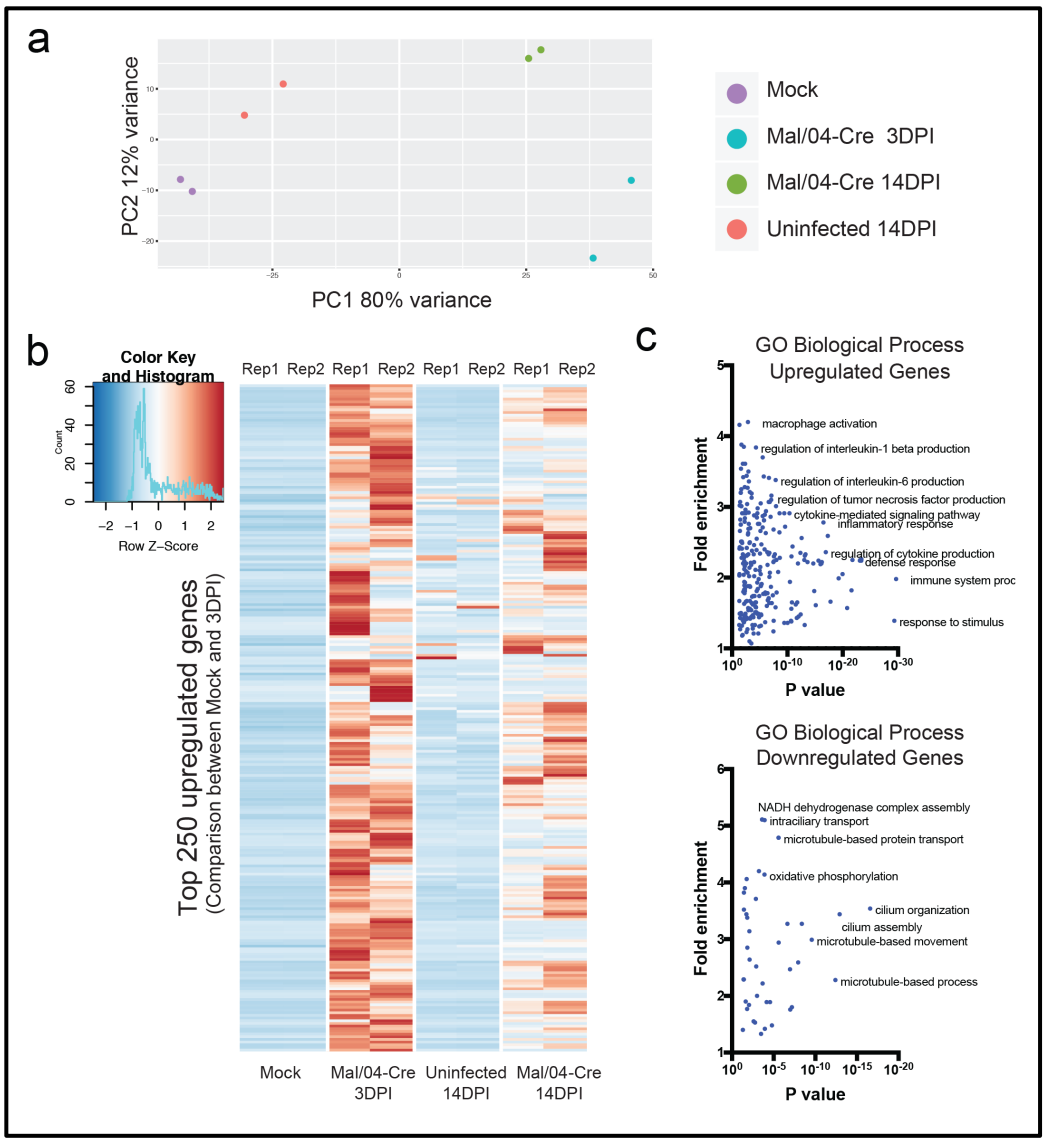


Supplemental Figure 1: Representative gating strategy for cells collected at 3 days post-infection and infected animal morbidity. Related to Figure 1. A) Cells from Mock infected animals were stained with Live/Dead and EpCam with endogenous tdTomato

used as a marker of infection. B) Respiratory epithelial cells from Mal/04-Cre infected animals were stained with Live/Dead and EpCam with endogenous tdTomato used as a marker of infection. C) Olfactory epithelial cells from Mal/04-Cre infected animals were stained with Live/Dead and EpCam with endogenous tdTomato used as a marker of infection. D) Time course of bodyweight of mice infected with Mal04-Cre, shown as percentage of starting bodyweight. n=4 mice, error bars indicate the S.E.M. Data are representative of multiple independent experiments.

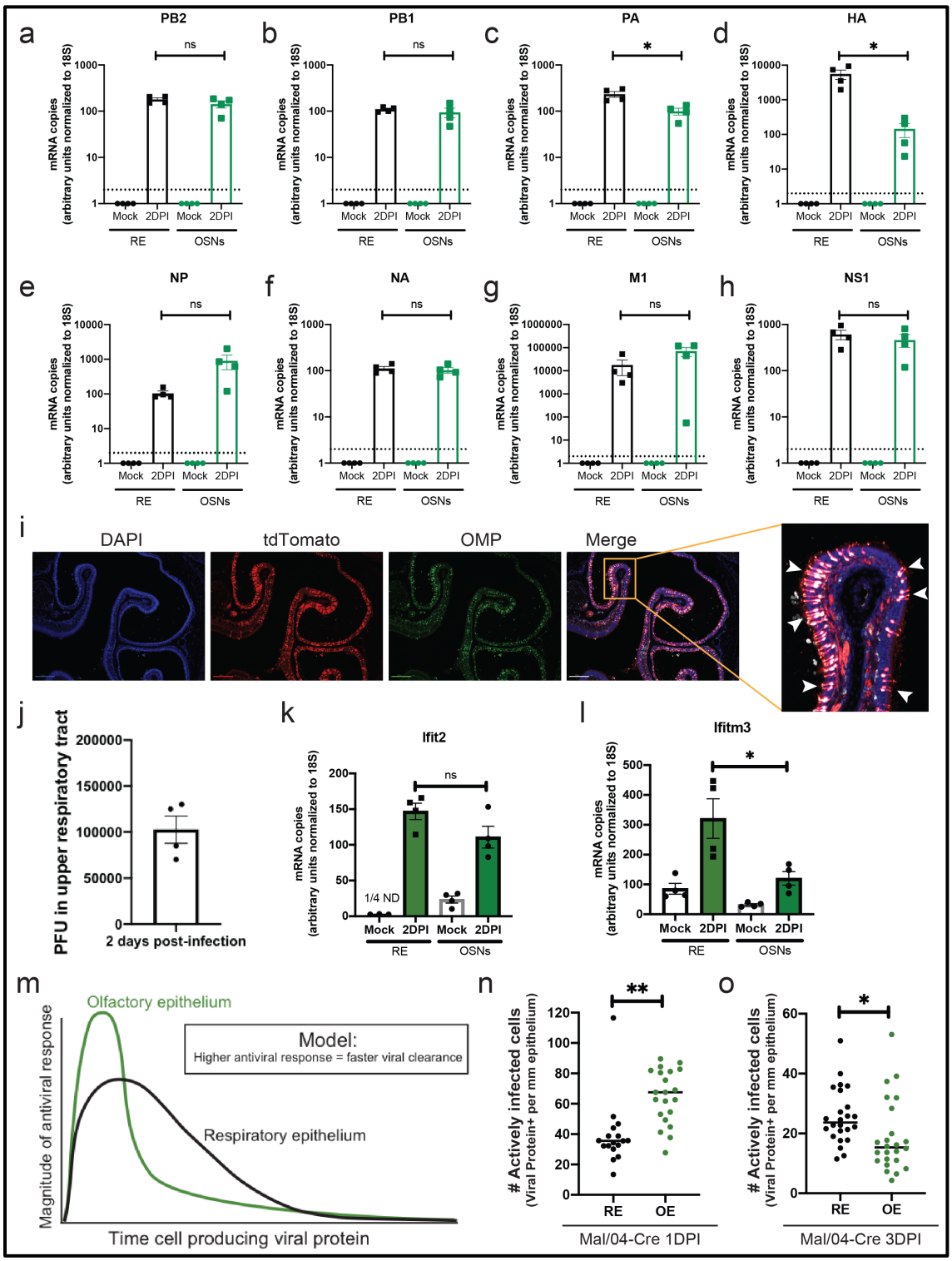


Supplemental Figure 2: Representative gating strategy for cells collected at 14 days post-infection. Related to Figure 1. A) Cells from Mock infected animals were stained with Live/Dead and EpCam with endogenous tdTomato used as a marker of previous infection. B) Respiratory epithelial cells from Mal/04-Cre infected animals were stained with Live/Dead and EpCam with endogenous tdTomato used as a marker of previous infection. C) Olfactory epithelial cells from Mal/04-Cre infected animals were stained with Live/Dead and EpCam with endogenous tdTomato used as a marker of previous infection.



Supplemental Figure 3: RNA-sequencing of olfactory epithelial cells at 0-, 3- and 14-days post-infection. Related to Figure 3. A) Principal components analysis of Mock, actively infected (Mal/04-Cre 3DPI), previously infected (Mal/04-Cre 14DPI) and uninfected 14DPI. Tissues from three independent mice were pooled prior to cell isolation for each treatment group. All '*Olfr-*' genes were excluded manually prior to analysis to account for stochastic sampling variability in olfactory receptors. B) Heatmap of top 250 upregulated genes as measured with a comparison between Mock and 3DPI samples.

Rep = replicate. C) Gene ontology analysis using PANTHER of differentially expressed genes, both upregulated and downregulated in previously infected OSNs.



Supplemental Figure 4: Viral replication, antiviral responses, and clearance of viral protein from infected cells. Related to Figure 4. A-H) FACS was used to isolate live CD31-CD45-CD326+ cells from the RE and OE of Mal/04-Cre infected mice at 2 days

post-infection. Infected OSNs were identified as CD31-CD45-CD326+ OMP+tdTomato+ cells and infected RE cells were identified CD31-CD45-CD326+ OMP-tdTomato+ cells. Infected RE cells and infected OSNs were sorted then qRT-PCR was used to quantify the expression of all 8 influenza B viral genes A) *PB2* - polymerase basic protein 2 B) *PB1* - polymerase basic protein 1 C) *PA* - polymerase acidic protein D) *HA* - hemagglutinin E) *NP* - nucleoprotein F) *NA* - neuraminidase G) *M1* - matrix protein H) *NS1* - non-structural protein 1. All genes were quantified based on a standard curve and normalized to endogenous 18S expression either in the same sample or samples run on the plate. Four independent mice were used to collect matching RE and OE samples, a one-way ANOVA with Tukey's multiple test comparison was used to determine significance. Samples not detected or which amplified with a Ct value greater than that of the no template control were assigned a value of 1 for graphing purposes. Error bars indicate the S.E.M, dashed line at y=2 indicating limit of detection of assay. Data are representative of at least two independent biological replicate experiments. I) Microscopy of an infected region of the olfactory epithelium from lox-stop-lox tdTomato transgenic mice, imaged at 14 days post-infection. Scalebar = 200 μm. Blue = DAPI, Green = OMP-GFP, Red = tdTomato. White arrows = focus with 2+ cells infected. J) Quantification of infectious virus from the upper respiratory tract. Mice were infected with 10,000 PFU then tissue from upper respiratory tract infection was collected at 2 days post-infection. Infectious virus was quantified using viral plaque assays. N=4 mice, error bars indicate the S.E.M. Data are representative of at least two independent biological replicate experiments. K,L) Quantification of antiviral gene expression in infected olfactory epithelial cells as in main figure 4, C-L. FACS was used to isolate live CD31-CD45-

CD326+ cells from the RE and OE of Mal/04-Cre infected mice at 2 days post-infection. Infected OSNs were identified as CD31-CD45-CD326+ OMP+tdTomato+ cells and infected RE cells were identified CD31-CD45-CD326+ OMP-tdTomato+ cells. Infected RE cells and infected OSNs were sorted then qRT-PCR was used to quantify the expression of K) *Ifit2* and L) *Ifitm3.* All genes were quantified based on a standard curve and normalized to endogenous 18S expression either in the same sample or samples run on the plate. Four independent mice were used to collect matching RE and OE samples, a one-way ANOVA with Tukey's multiple test comparison was used to determine significance. Error bars indicate the S.E.M, ND = not detected. Data are representative of at least two independent biological replicate experiments. M) Model of antiviral gene response leading to faster clearance of viral infection. N) Quantification of microscopy of the RE and the OE from n=2 Mal/04-Cre infected mice at 1-day post-infection. Data presented represent the number of cells positive for viral protein from a total of 20 images. A total of at least 779 cells were counted for both RE and OE samples. O) Quantification of microscopy of the RE and the OE from n=3 Mal/04-Cre infected mice at three days post-infection. Data presented are the number of cells positive for viral protein from a total of 24 images. A total of at least 958 cells were counted for both RE and OE samples. The mean is shown with a black bar, and a Student's t-test was used to determine significance. For all panels, *=$p \leq 0.05$, **=$p \leq 0.001$, ns=not significant.