

**Supplementary information**

**Supplementary Table 1. Global statistics of the dataset used in the analysis.**

| Dataset                                   | TPI           | SRA<br>accession | Total reads          | Selected<br>transcripts <sup>1</sup> | Transcripts<br>per GEM | Selected<br>GEMs <sup>3</sup> | Selected<br>genes <sup>4</sup> |
|-------------------------------------------|---------------|------------------|----------------------|--------------------------------------|------------------------|-------------------------------|--------------------------------|
| <b>Bronchial<br/>epithelial<br/>cells</b> | <i>Mock</i>   | SRR13711613      | 604,127,582          | 92,488,540                           | 1,878-16,810           | 16,531                        |                                |
|                                           | <i>1 dpi</i>  | SRR13711614      | 589,830,183          | 75,836,207                           | 1,232-30,555           | 9,345                         |                                |
|                                           | <i>2 dpi</i>  | SRR13711615      | 653,390,536          | 90,806,792                           | 1,752-20,120           | 12,472                        |                                |
|                                           | <i>3 dpi</i>  | SRR13711616      | 573,563,158          | 91,799,408                           | 1,781-13,181           | 17,820                        |                                |
|                                           |               |                  | <b>2,420,911,459</b> | <b>350,930,947</b>                   |                        | <b>56,168</b>                 | <b>9,913</b>                   |
| <b>Colon*</b><br><b>organoids</b>         | <i>Mock</i>   | SRR12508049      | 294,735,427          | 71,838,718                           | 1,030-47,489           | 4,024                         |                                |
|                                           |               | SRR12508050      | 107,844,234          | 30,338,763                           |                        |                               |                                |
|                                           | <i>12 hpi</i> | SRR12508052      | 304,892,652          | 79,187,463                           | 1,149-46,952           | 3,992                         |                                |
|                                           |               | SRR12508053      | 123,149,496          | 37,510,656                           |                        |                               |                                |
|                                           | <i>24 hpi</i> | SRR12508055      | 307,339,187          | 79,260,961                           | 1,232-31,513           | 4,407                         |                                |
|                                           |               | SRR12508056      | 121,191,449          | 35,611,648                           |                        |                               |                                |
|                                           |               |                  | <b>1,259,152,445</b> | <b>333,748,209</b>                   |                        | <b>12,423</b>                 | <b>7,582</b>                   |
| <b>Ileum*</b><br><b>organoids</b>         | <i>Mock</i>   | SRR12508058      | 310,516,022          | 77,784,527                           | 968-40,234             | 3,457                         |                                |
|                                           |               | SRR12508059      | 115,263,635          | 35,651,119                           |                        |                               |                                |
|                                           | <i>12 hpi</i> | SRR12508061      | 308,565,909          | 73,011,266                           | 1,100-40,163           | 4,115                         |                                |
|                                           |               | SRR12508062      | 116,802,746          | 33,699,484                           |                        |                               |                                |
|                                           | <i>24 hpi</i> | SRR12508064      | 304,036,624          | 69,478,009                           | 908-42,604             | 3,629                         |                                |
|                                           |               | SRR12508065      | 115,167,592          | 33,137,814                           |                        |                               |                                |
|                                           |               |                  | <b>1,270,352,528</b> | <b>322,762,219</b>                   |                        | <b>12,201</b>                 | <b>8,334</b>                   |

<sup>1</sup>Only transcripts unambiguously attributed to a single gene and a single unique molecular identifier (UMI) were included in the analysis.

<sup>2</sup>Specifies the minimum and maximum number of transcripts required for a cell in the corresponding dataset to be included in the analysis.

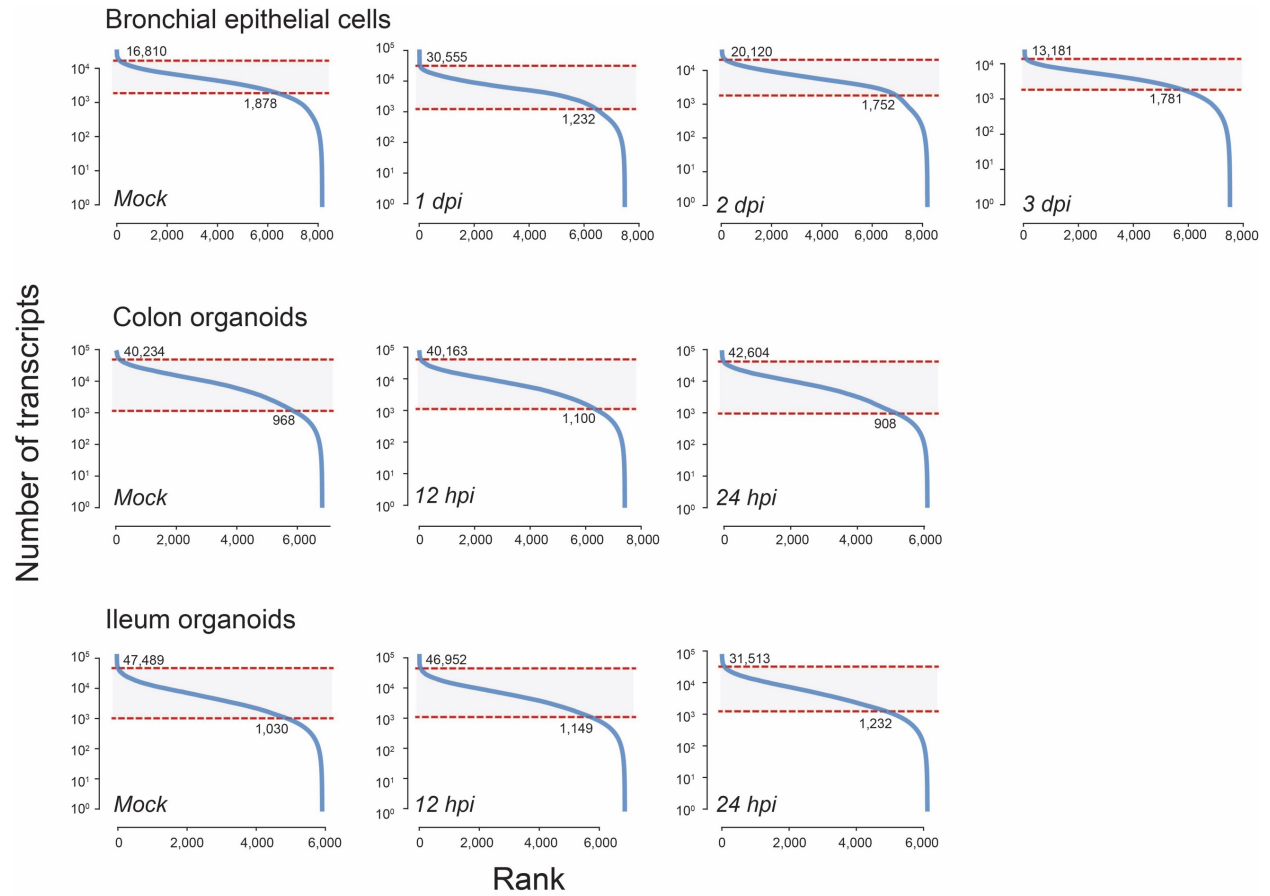
<sup>3</sup>Total number of cells selected based on the number of transcripts.

<sup>4</sup>Total number of genes included in the analysis for each cell type. Selected genes required to expressed in a minimum of one thousand cells.

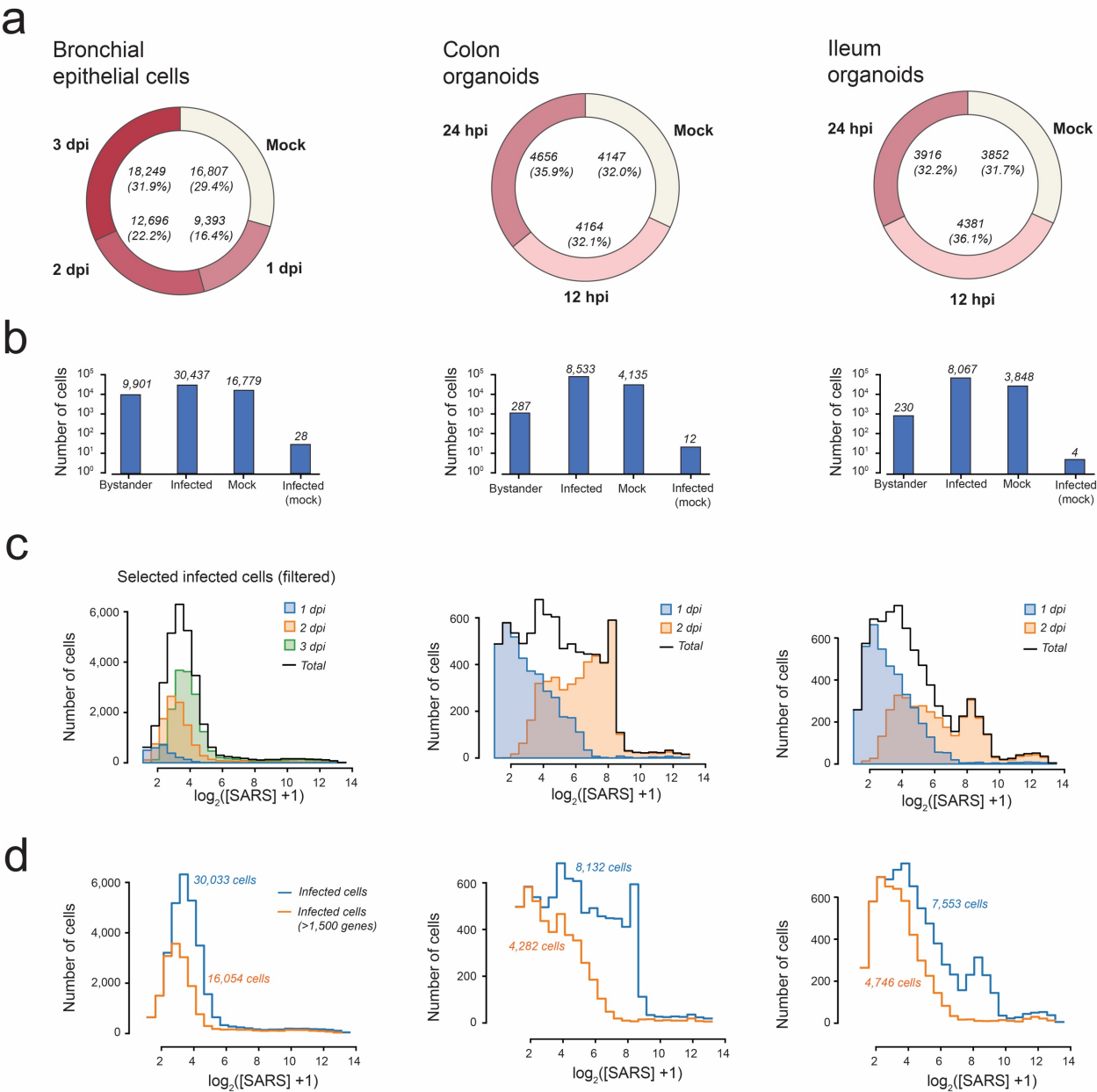
\*Datasets from colon and ileum organoids comprised two separate files per experiment that were merged for subsequent analyses.

Abbreviations: GEM: Gel Bead-in Emulsion, SRA: Sequence read archive, TPI: time post-inoculation, dpi: days post-infection, hpi: hours post-infection.

Aggregated values for selected columns are shown in bold.

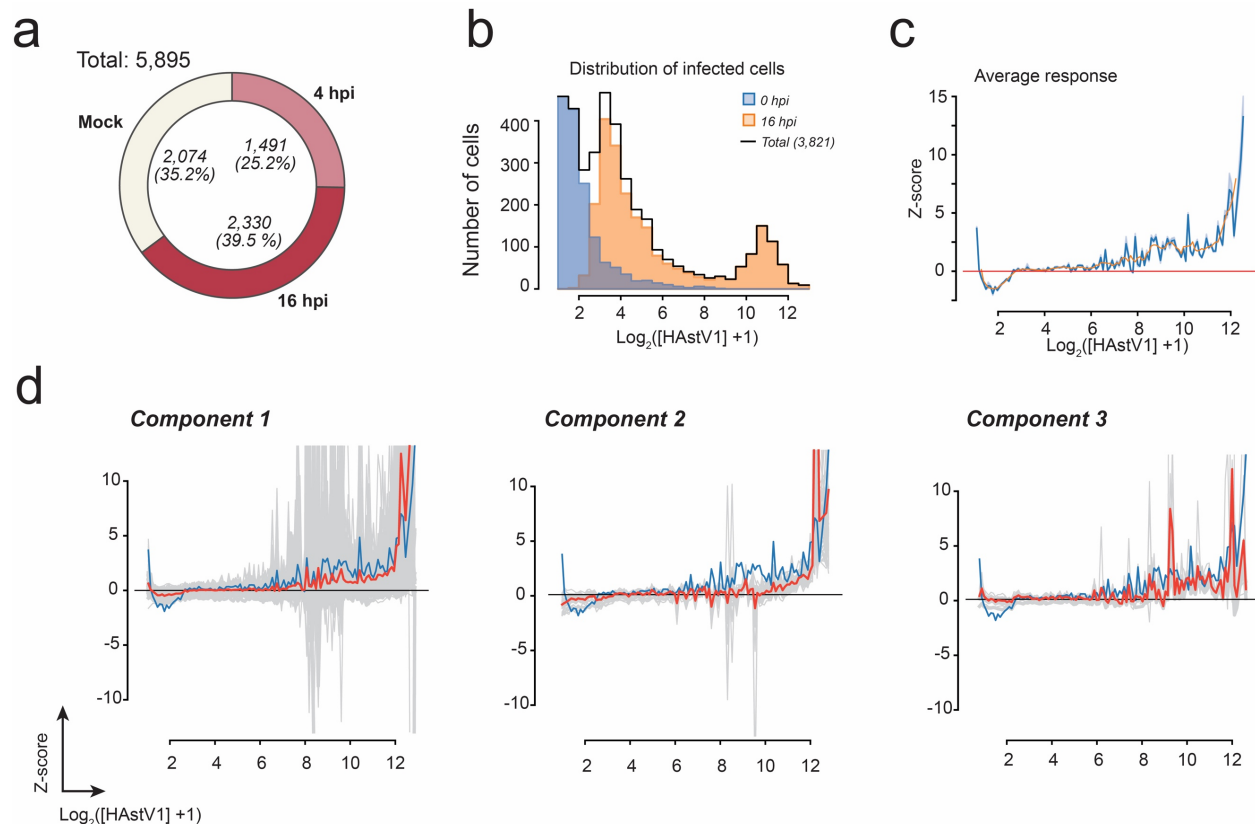


**Supplementary Figure 1. Filtering of void GEMs and multiplets.** GEMs were ordered with respect to the number of transcripts the local standard deviation of the  $\log_{10}$  using window size of five datapoints. Upper and lower thresholds delimiting multiples and voids GEMS were determined using  $1.5 \times \text{IQR}$  rule. The cells used in this study correspond to the grey area.



25 **Supplementary Figure 2. Global composition of the scRNA-seq datasets.** **a** Proportion of  
26 GEMs from mock and infected treatments from each dataset. **b** Proportion of bystanders,  
27 infected, mock, and infected mock cells in each dataset. **c** Distribution of cells with respect to  
28 viral loads for each treatment. Statistics presented in these plots correspond to observed counts.  
29 **d** Selecting cell with respect to number of individual transcripts, instead of number of detected  
30 genes increases the proportion of cells with high viral accumulations.





**Supplementary Figure 3. Transcriptional response of ileum organoid cells infected with human astrovirus 1 (HAsV1).** **a** Proportion of selected cells at each time post-infection. **b** Distribution of HAsV1 accumulations in the infected cell subset. **c** Average transcriptional response of ileum cells illustrating a very different response than SARS-CoV-2. **d** Response profiles of genes from components 1 (global SARS-CoV-2 response), 2 (genes involved in translation), and 3 (mitochondrial-encoded genes) to HAsV1 infection.

40 [Supplementary Data 1.](#) Database of reference human messenger RNAs and SARS-CoV-2  
41 genomes used for mapping reads. Raw count matrices.  
42  
43 [Supplementary Data 2.](#) Expression matrices and transcript levels in uninfected cells.  
44  
45 [Supplementary Data 3.](#) Differential Expressed Gene analysis used in the volcano plots analysis.  
46 Datasets and results used in the Gene Ontology analyses. Classification of outliers.  
47  
48 [Supplementary Data 4.](#) Transcriptional profiles for all genes in each cell type.  
49  
50 [Supplementary Data 5.](#) Networks used in the analyses presented in Figs. 5 and 6.  
51 [Supplementary Data 6.](#) Transcriptional analysis of human astrovirus 1 infection in ileum  
52 organoids.