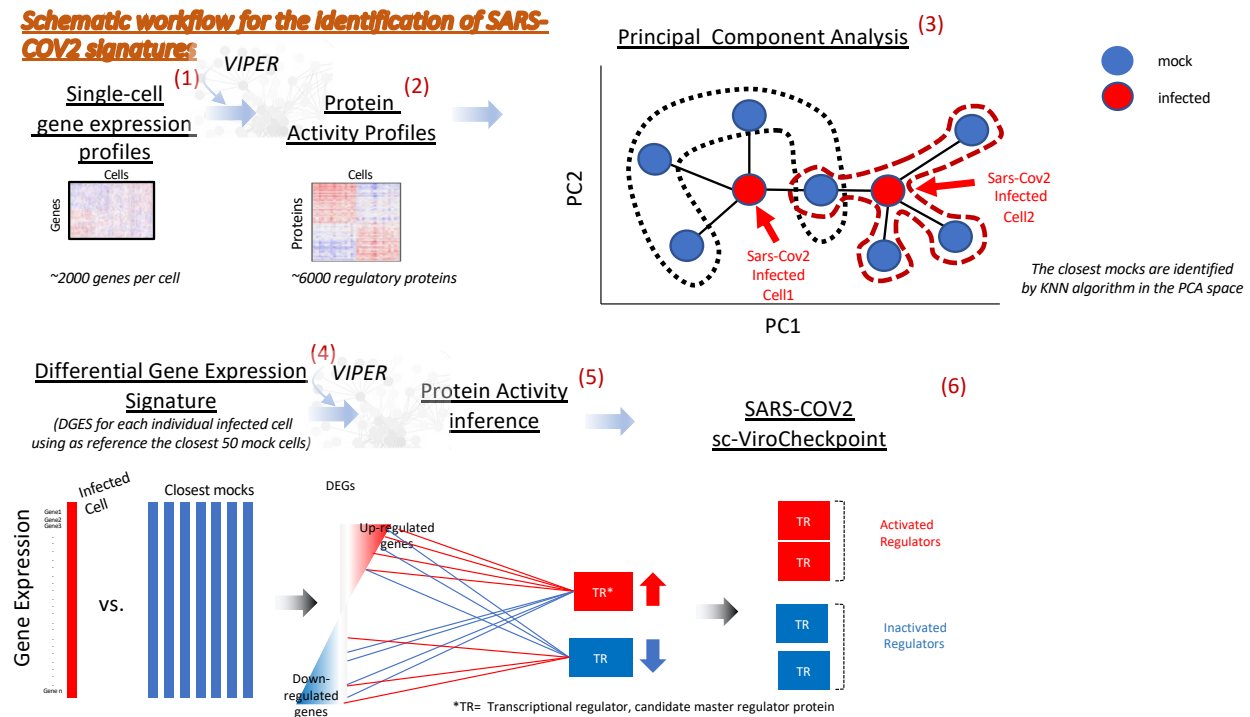


**Schematic workflow for the Identification of SARS-COV2 signatures**

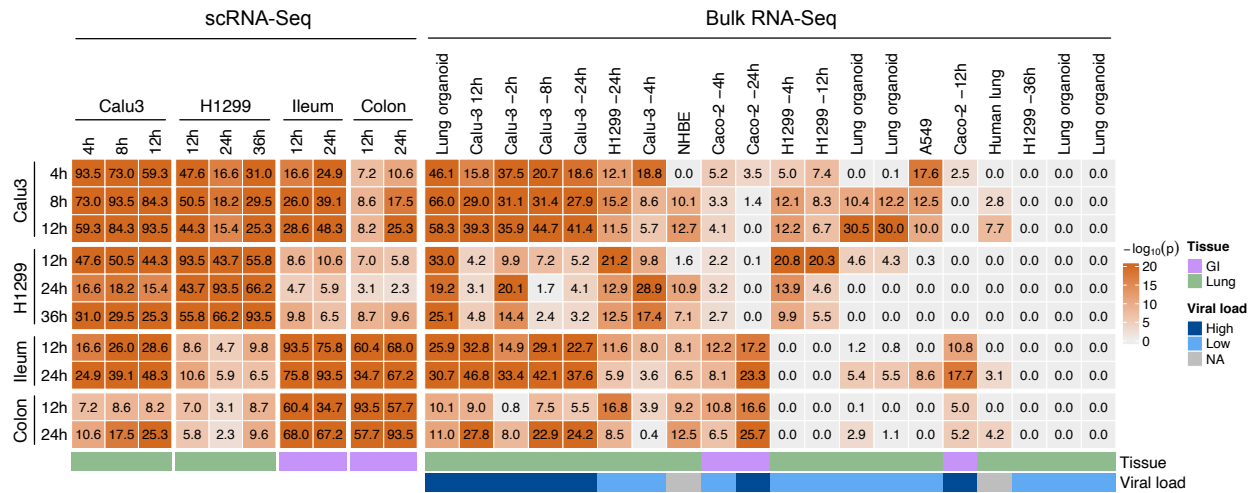


849

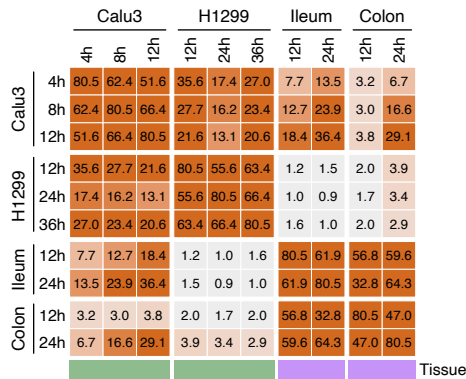
850 **Supplementary Figure 1. Diagram showing the workflow used to compute the protein**  
 851 **activity signatures induced by SARS-CoV-2 infection from scRNA-Seq data. Related to**  
 852 **Figure 2 and methods.** Normalized single-cell gene expression profiles for all cells of the same  
 853 model (i.e. Calu3, H1299, colon and ileum) were transformed to differential gene expression  
 854 signatures by applying the z-score procedure. Single-cell differential gene expression signatures  
 855 were then transformed to protein activity profiles by applying the VIPER algorithm with context-  
 856 specific regulatory networks. A principal component analysis (PCA) was performed on these  
 857 VIPER-inferred protein activity profiles. For each infected cell the closest 50 mock cells in the  
 858 PCA space were selected as reference to compute a SARS-CoV-2 induced differential gene  
 859 expression signature. The VIPER algorithm was then applied to these SARS-CoV-2 induced  
 860 differential gene expression signatures to infer SARS-CoV-2 induced protein activity signatures.

861

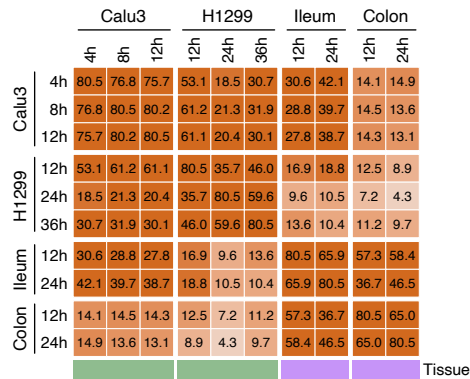
**a** Top 25 most inactivated and top 25 most activated proteins



**b** Top 50 most inactivated proteins



**c** Top 50 most activated proteins



862

863 **Supplementary Figure 2. Conservation of VIPER-inferred Viral Checkpoint. Related to**

864 **Figure 2. a.** Heatmap showing the conservation across single-cell and bulk-tissue samples.

865 Results are expressed as  $-\log_{10}(\text{p-value})$ , estimated by the reciprocal enrichment of the 25 most

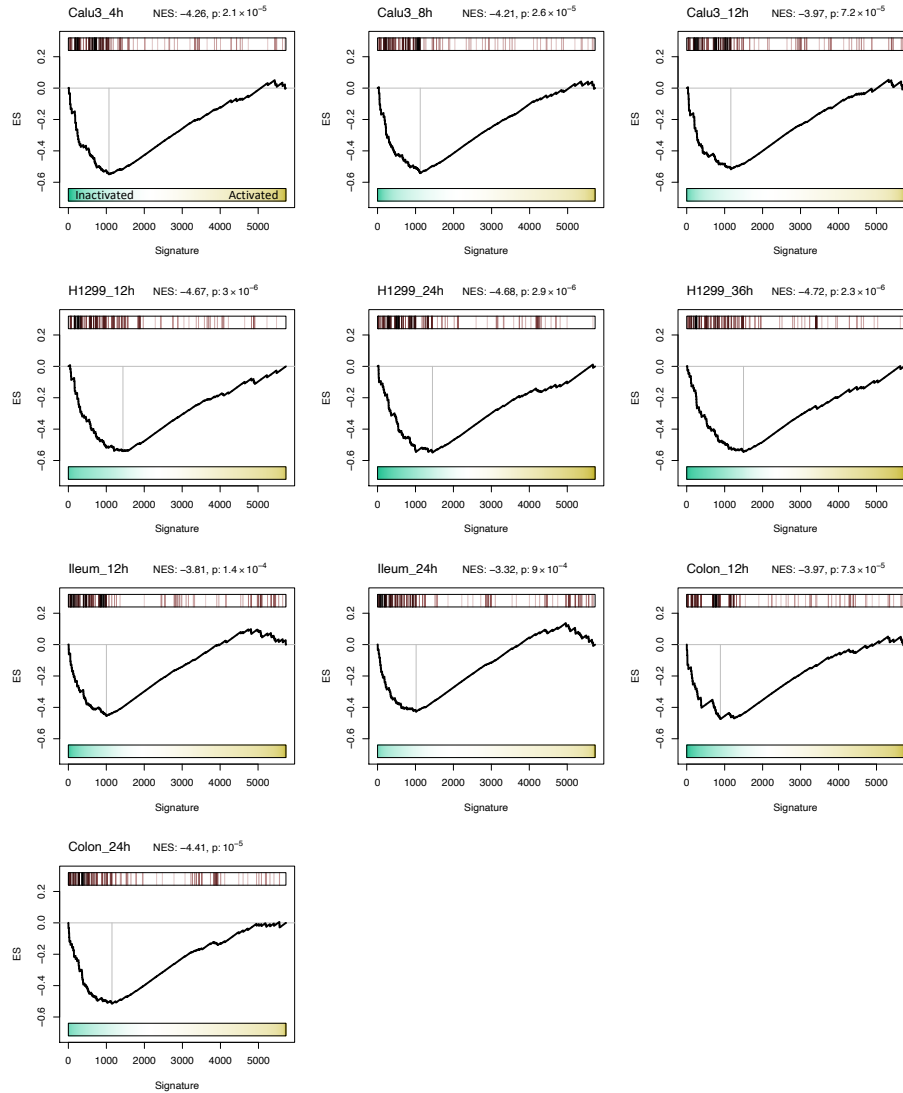
866 activated and 25 most inactivated proteins in each signature using the aREA algorithm as

867 implemented in the viperSimilarity function of the VIPER package. **b-c.** Conservation specifically

868 for the top 50 most activated proteins (b) and most inactivated proteins (c) in response to SARS-

869 CoV-2 infection between time points and models profiled at the single-cell level.

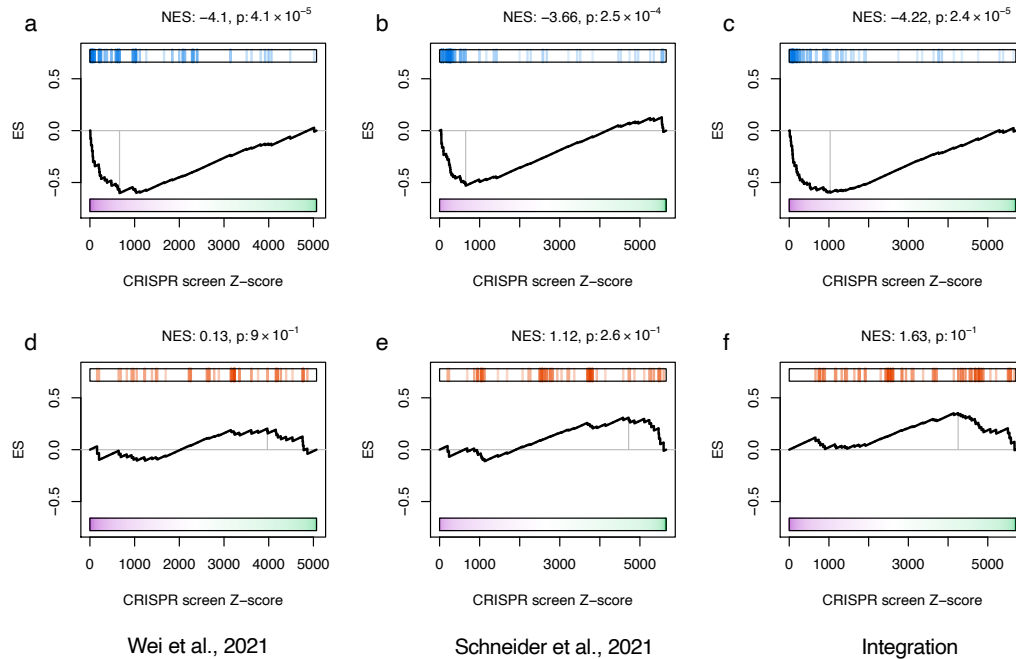
870



871

872 **Supplementary Figure 3. Enrichment of host factors known to physically interact with**  
 873 **SARS-CoV-2 proteins on the host proteins differentially active in response to viral**  
 874 **infection. Related to Figure 2. GSEA showing the enrichment for the SARS-CoV-2 interacting**  
 875 **proteins in the individual SARS-CoV-2 induced protein activity signatures. NES and p-values were**  
 876 **estimated by one-tailed test and 1,000 permutations.**

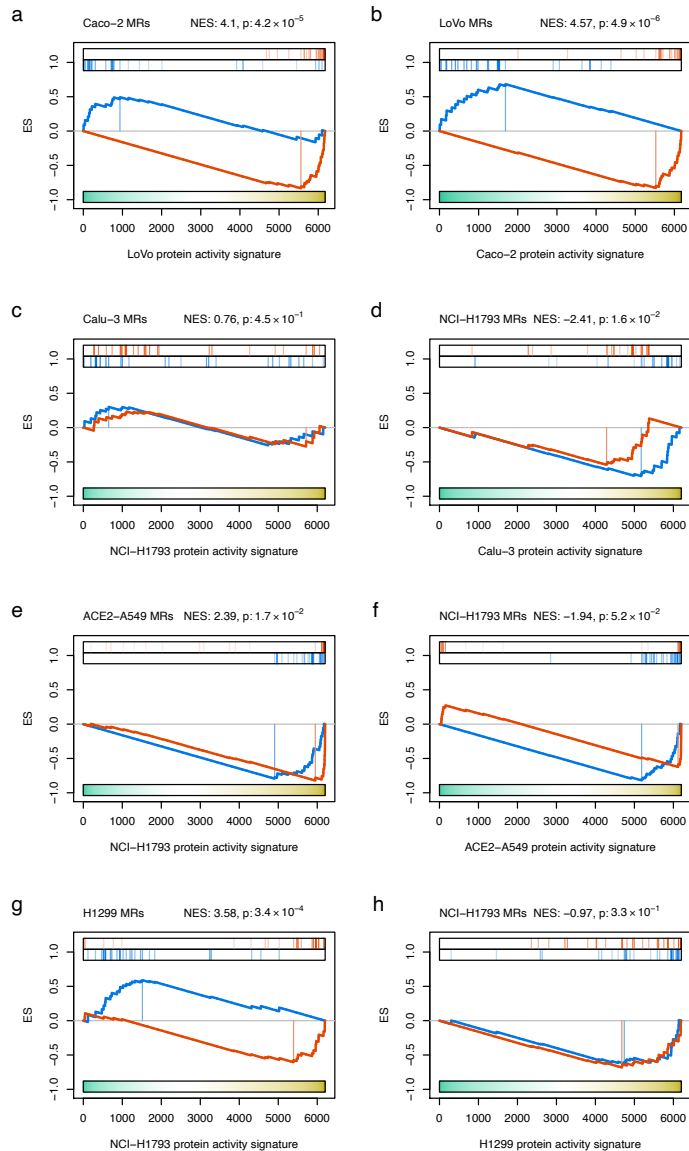
877



878

879 **Supplementary Figure 4. Enrichment of candidate SARS-CoV-2 infection MR proteins on**  
 880 **host factors essential for SARS-CoV-2 infectivity. Related to Figure 2.** GSEA showing the  
 881 enrichment of the top 50 most inactivated proteins in response to SARS-CoV-2 infection  
 882 (inactivated candidate MR proteins) on the antiviral essential genes (a-c), but no enrichment of  
 883 the top 50 most activated proteins in response to SARS-CoV-2 infection (activated candidate MR  
 884 proteins) on the pro-viral essential genes (d-f), identified by 2 CRISPR screens (a, b, d and e) and  
 885 their integration (c and f).

886

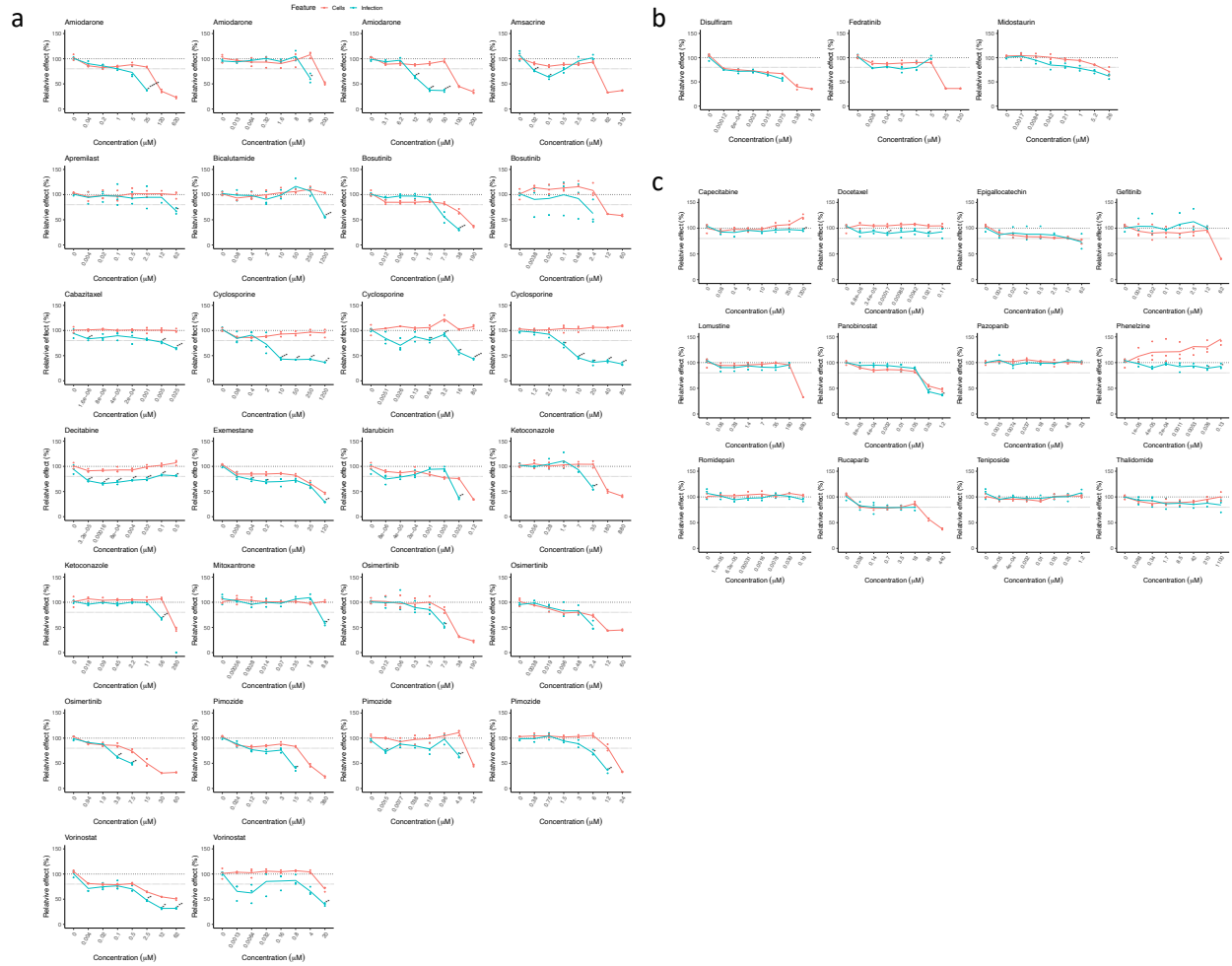


887

888 **Supplementary Figure 5. Conserved activity of MR proteins between cell line models**  
 889 **susceptible to SARS-CoV-2 infection (Caco-2, Calu-3, ACE2-A549 and H1299) and the**  
 890 **lineage context-matched cell lines included in the drug perturbation PANACEA resource**  
 891 **(LoVo and NCI-H1793). Related to Figure 2-3. a. GSEA for the enrichment of the Caco-2 top**  
 892 **25 most activated and top 25 most inactivated proteins in the LoVo protein activity signature. b.**  
 893 **GSEA for the enrichment of the LoVo top 25 most activated and top 25 most inactivated proteins**  
 894 **in the Caco-2 protein activity signature. c. GSEA for the enrichment of the Calu-3 top 25 most**  
 895 **activated and top 25 most inactivated proteins in the NCI-H1793 protein activity signature. d.**  
 896 **GSEA for the enrichment of the NCI-H1793 top 25 most activated and top 25 most inactivated**  
 897 **proteins in the Calu-3 protein activity signature. e. GSEA for the enrichment of the ACE2-A549**

898 top 25 most activated and top 25 most inactivated proteins in the NCI-H1793 protein activity  
899 signature. **f.** GSEA for the enrichment of the NCI-H1793 top 25 most activated and top 25 most  
900 inactivated proteins in the ACE2-A549 protein activity signature. **g.** GSEA for the enrichment of  
901 the H1299 top 25 most activated and top 25 most inactivated proteins in the NCI-H1793 protein  
902 activity signature. **h.** GSEA for the enrichment of the NCI-H1793 top 25 most activated and top  
903 25 most inactivated proteins in the H1299 protein activity signature. Normalized enrichment score  
904 (NES) and p-value were estimated by two-tailed test and 1,000 permutations.

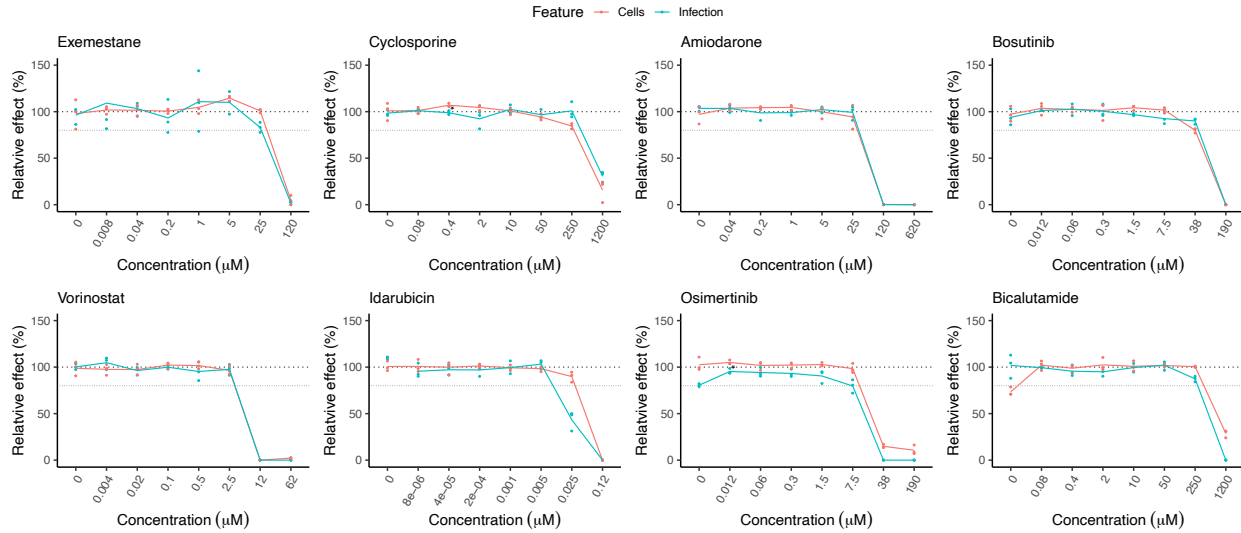
905



906

907 **Supplementary Figure 6. Experimental evaluation of the antiviral effect of FDA-approved**  
 908 **drugs in Caco-2 cells. Related to Figure 4 and Supplementary Table 2. a.** 15 of the 18 drugs  
 909 predicted by ViroTreat showing significant antiviral effect (FDR < 0.05 and  $\geq 20\%$  viral replication  
 910 decrease). **b.** 3 of the 18 drugs predicted by ViroTreat showing no significant antiviral effect. **c.**  
 911 12 drugs not significant by ViroTreat ( $p \geq 0.01$ ) selected as putative negative controls. The scatter-  
 912 plots show the effect of each drug—SARS-CoV-2 replication shown in cyan and cell viability in  
 913 red—relative to vehicle control (y-axis), assayed at different concentrations (x-axis) in triplicate.  
 914 The lines indicate the average across replicates. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 10^{-4}$ ,  
 915 \*\*\*\*\*  $p < 10^{-6}$ , 1-tailed Student's t-test, BC.

916

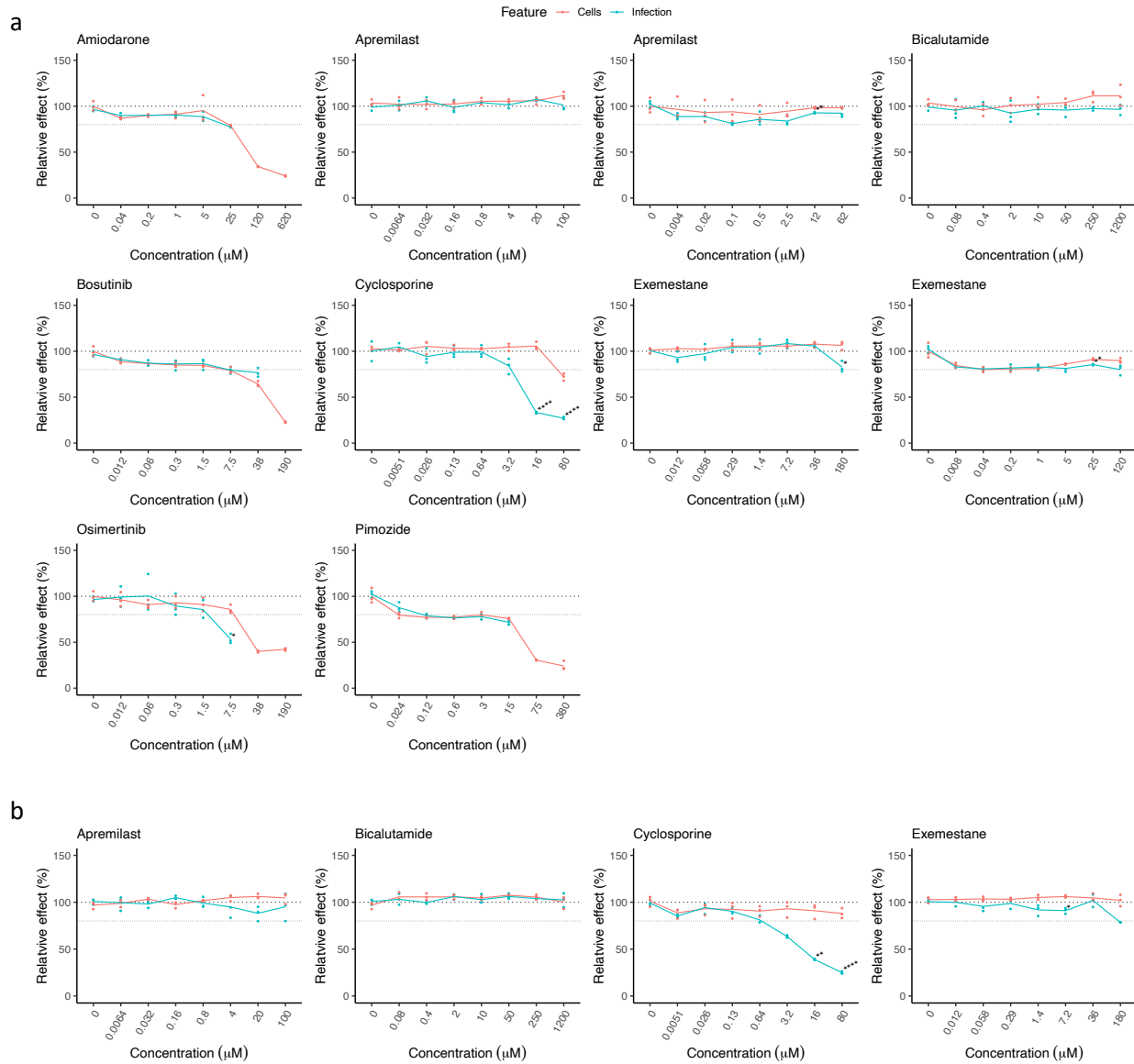


917

918 **Supplementary Figure 7. Experimental evaluation of 8 drugs, predicted by ViroTreat and**  
 919 **showing the strongest SARS-CoV-2 antiviral effect in Caco-2 cells, for their effect on**  
 920 **rotavirus replication. Related to Figure 4 and Supplementary Table 2.** The scatter-plots show  
 921 the effect of each drug—rotavirus replication shown in cyan and cell viability in red—relative to  
 922 vehicle control (y-axis), assayed at different concentrations (x-axis) in triplicate. The lines indicate  
 923 the average across replicates. \*  $p < 0.05$ , 1-tailed Student's t-test, BC.

924



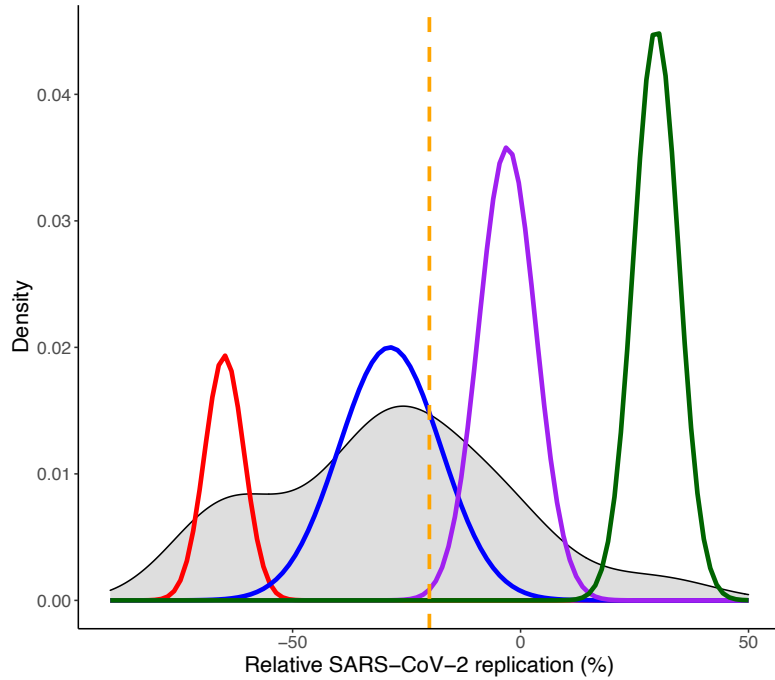


925

926 **Supplementary Figure 8. Experimental evaluation of the antiviral effect of FDA-approved**  
 927 **drugs in lung adenocarcinoma cell lines. Related to Figure 4 and Supplementary Table 2.**

928 A set of drugs, predicted by ViroTreat for the GI context and with validated antiviral effect in Caco-  
 929 2 cells were evaluated in Calu-3 (a) and A549-ACE2 (b) cells. The scatter-plots show the effect  
 930 of each drug—SARS-CoV-2 replication shown in cyan and cell viability in red—relative to vehicle  
 931 control (y-axis), assayed at different concentrations (x-axis) in triplicate. The lines indicate the  
 932 average across replicates. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*\*  $p < 10^{-4}$ , 1-tailed Student's t-test, BC.

933



934

935 **Supplementary Figure 9. Distribution for the relative effect of the evaluated drugs on**  
 936 **SARS-CoV-2 replication. Related to Figure 4.** Histogram and Gaussian Mixture Model (GMM)  
 937 fitted to the relative effect of the drugs, expressed as percentage, on SARS-CoV-2 replication  
 938 in Caco-2 cells. The dashed orange vertical line represents the threshold of 20% used as  
 939 additional criteria when considering the antiviral effect of a drug.

940

941

942

943 **Supplementary Table 1: SARS-CoV-2 host cell RNA-Seq and scRNA-Seq datasets.**

| <i>Model</i>   | <i>Type of Data</i> | <i>Publication</i>              | <i>Source</i>      |
|----------------|---------------------|---------------------------------|--------------------|
| Calu3          | Bulk RNASeq         | Wyler et al. <sup>1</sup>       | (GEO) GSE148729    |
| H1299          | Bulk RNASeq         | Wyler et al. <sup>1</sup>       | (GEO) GSE148729    |
| Caco2          | Bulk RNASeq         | Wyler et al. <sup>1</sup>       | (GEO) GSE148729    |
| A549           | Bulk RNASeq         | Blanco Melo et al. <sup>2</sup> | (GEO) GSE147507    |
| Lung Organoids | Bulk RNASeq         |                                 | (GEO) GSE160435    |
| NHBE           | Bulk RNASeq         | Blanco Melo et al. <sup>2</sup> | (GEO) GSE147507    |
| Human lung     | Bulk RNASeq         | Blanco Melo et al. <sup>2</sup> | (GEO) GSE147507    |
| Calu3          | scRNASeq            | Wyler et al. <sup>1</sup>       | (GEO) GSE148729    |
| H1299          | scRNASeq            | Wyler et al. <sup>1</sup>       | (GEO) GSE148729    |
| Ileum          | scRNASeq            | Triana et al. <sup>3</sup>      | Boulant Lab        |
| Colon          | scRNASeq            | Triana et al. <sup>3</sup>      | Boulant Lab        |
| Vero6          | CRISPRcas9          | Wei et al. <sup>4</sup>         | Supplementary Data |
| A549           | CRISPRcas9          | Daniloski et al. <sup>5</sup>   | Supplementary Data |
| Huh-7.5        | CRISPRcas9          | Wang et al. <sup>6</sup>        | Supplementary Data |
| Huh-7.5        | CRISPRcas9          | Schneider et al. <sup>7</sup>   | Supplementary Data |

944

945 <sup>1</sup>Wyler, E., et al. (2021). *iScience* **24**(3): 102151.

946 <sup>2</sup>Blanco-Melo, D., et al. (2020). *Cell* **181**(5): 1036-1045 e1039.

947 <sup>3</sup>Triana, S., et al. (2021). *Mol Syst Biol* **17**(4): e10232.

948 <sup>4</sup>Wei, J., et al. (2021). *Cell* **184**(1): 76-91 e13.

949 <sup>5</sup>Daniloski, Z., et al. (2021). *Cell* **184**(1): 92-105 e116.

950 <sup>6</sup>Wang, R., et al. (2021). *Cell* **184**(1): 106-119 e114.

951 <sup>7</sup>Schneider, W. M., et al. (2021). *Cell* **184**(1): 120-132 e114.

952

953 **Supplementary Table 2:** Drugs library, ViroTreat and focused validation screen results.

954 < See supplementary file Table-S2.xlsx >

955

956 **Supplementary Table 3:** Organoids' culture media.

| <i>Compound</i>              | <i>Final concentration</i> |
|------------------------------|----------------------------|
| <b>Basal media</b>           |                            |
| Ad DMEM/F12                  |                            |
| +GlutaMAX                    |                            |
| +HEPES                       |                            |
| +P/S                         |                            |
| L-WRN                        | 50% by volume              |
| B27                          | 1:50                       |
| N-acetyl-cysteine            | 1 mM                       |
| EGF                          | 50 ng/mL                   |
| A83-01                       | 500 nM                     |
| IGF-1                        | 100 ng/mL                  |
| FGF basic                    | 50 ng/mL                   |
| Gastrin                      | 10 mM                      |
| <b>Differentiation Media</b> |                            |
| Ad DMEM/F12                  |                            |
| +GlutaMAX                    |                            |
| +HEPES                       |                            |
| +P/S                         |                            |
| B27                          | 1:50                       |
| N-acetyl-cysteine            | 1 mM                       |
| R-spondin                    | 5% by volume               |
| Noggin                       | 50 ng/mL                   |
| EGF                          | 50 ng/mL                   |
| Gastrin                      | 10 mM                      |
| A83-01                       | 500 nM                     |

957

958 **Supplementary Table 4:** PCR primers.

| <i>Gene name</i> | <i>Species</i> | <i>Forward sequence</i>     | <i>Reverse sequence</i>       |
|------------------|----------------|-----------------------------|-------------------------------|
| HPRT1            | Human          | cct ggc gtc gtg att agt gat | aga cgt tca gtc ctg tcc ata a |
| COV1             | SARS-CoV-2     | gcc tct tct gtt cct cat cac | aga cag cat cac cgc cat tg    |

959

960