Supplementary Information

Identification of new drug treatments to combat COVID19: A signature-based approach using iLINCS

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Supplemental Figure Legends

Figure S1. Tanimoto coefficient correlogram. Heat map showing the Tanimoto coefficients of the 9 drugs with antiviral indications that comprise drug groupings 1-5 (x-axis) and the 14 candidate repurposable drugs identified in this study (y-axis). The Tanimoto coefficient, which represents the degree of structural similarity between two compounds, was computed using binary chemical fingerprints for each of the drugs. Coefficients range from 0 (no structural similarity) to 1 (exact structural similarity). In this analysis, Tanimoto coefficients ranged from 0.1 - 0.59, suggesting low to moderate structural similarity between antiviral treatments currently in use for treating coronavirus family pathogens and the identified candidate drugs.

Figure S2. Heat map of disease signatures. Unsupervised clustering of Library of Integrated Network-Based Cellular Signature (LINCS) L1000 genes, extracted from RNAseq datasets of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome-related coronavirus (MERS) (GSE56192). L1000 genes were also extracted from an Influenza A (INFL) microarray dataset (GSE47963) as a non-coronavirus pathogen comparison group. This heat map shows clustering of coronavirus pathogens SARS and MERS in contrast to INFL.

Figure S3. **Heat map of drug groupings 1-5 (MCF7 cell line).** Unsupervised clustering of Library of Integrated Network-Based Cellular Signature (LINCS) L1000 genes, extracted from RNAseq datasets of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome-related coronavirus (MERS) (GSE56192). L1000 genes were also extracted from an Influenza A (INFL) microarray dataset (GSE47963) as a non-coronavirus pathogen comparison group. L1000 genes were also extracted from five drug groupings (drug clusters 1-5) which represent drugs currently in use to treat coronavirus pathogens. Drug clusters signatures where extracted from the MCF7 cell line. Drugs are clustered based on mechanism of action, ATC

classification and/or structural similarity. Furosemide (FUR), a drug which is not utilized in the treatment of viral pathogens, was utilized as a comparison group. Drug group signatures were calculated by taking the average logFoldChange of each of the drugs within the cluster to represent the gene expression. This heat map provides an overview of the difference in patterns of gene expression between drug target groupings and disease in the MCF7 cell line, demonstrating discordance between drugs used as antivirals and disease signatures. Drug cluster 1: chloroquine and hydroxychloroquine, Drug cluster 2: Lopinavir and Ritonavir, Drug cluster 3: Fedratinib, Ruxolinitib, and Bariticinib. Drug cluster 4: azithromycin, and Drug cluster 5: Losartan.

Figure S4. **Heat map of drug target groupings 1-5 (HA1E cell line).** Unsupervised clustering of Library of Integrated Network-Based Cellular Signature (LINCS) L1000 genes, extracted from RNAseq datasets of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome-related coronavirus (MERS) (GSE56192). L1000 genes were also extracted from an Influenza A (INFL) microarray dataset (GSE47963) as a non-coronavirus pathogen comparison group. L1000 genes were also extracted from five drug groupings (drug clusters 1-5) which represent drugs currently in use to treat coronavirus pathogens. Drug clusters signatures where extracted from the HA1E cell line. Drugs are clustered based on mechanism of action, ATC classification and/or structural similarity. Furosemide (FUR), a drug which is not utilized in the treatment of viral pathogens, was utilized as a comparison group. Drug group signatures were calculated by taking the average logFoldChange of each of the drugs within the cluster to represent the gene expression. This heat map provides an overview of the difference in patterns of gene expression between drug target groupings and disease in the HA1E cell line, demonstrating discordance between drugs used as antivirals and disease signatures. Drug cluster 1: chloroquine and hydroxychloroquine, Drug cluster 2: Lopinavir and Ritonavir, Drug

cluster 3: Fedratinib, Ruxolinitib, and Bariticinib. Drug cluster 4: azithromycin, and Drug cluster 5: Losartan.

Figure S5. **Biological Pathway analysis of disease and drug target grouping signatures from MCF7 cell line.** Gene lists for drug target groupings 1-5 consisting of L1000 genes at LFC +/- 0.85 were searched in Reactome. Pathways for drug groupings 1 - 3 were determined by p-value < 0.05, and "Entities Found" ≥ 10, and groupings 4 and 5 were determined by p-value < 0.05, and "Entities Found" ≥ 3. Gene lists for disease signatures consisting of L1000 genes at LFC ≥ 0.5 and ≤ -0.5 were searched in Reactome. Pathways were determined by p-value < 0.05, and "Entities Found" ≥ 6. Drug cluster 1: chloroquine and hydroxychloroquine, Drug cluster 2: Lopinavir and Ritonavir, Drug cluster 3: Fedratinib, Ruxolinitib, and Bariticinib. Drug cluster 4: azithromycin, and Drug cluster 5: Losartan.

Figure S6. Biological Pathway analysis of disease and drug target grouping signatures from HA1E cell line. Gene lists for drug target groupings 1-5 consisting of L1000 genes at LFC +/- 0.85 were searched in Reactome. Pathways for drug groupings 1 - 3 were determined by p-value < 0.05, and "Entities Found" ≥ 10, and groupings 4 and 5 were determined by p-value < 0.05, and "Entities Found" ≥ 3. Gene lists for disease signatures consisting of L1000 genes at LFC ≥ 0.5 and ≤ -0.5 were searched in Reactome. Pathways were determined by p-value < 0.05, and "Entities Found" ≥ 6. Drug cluster 1: chloroquine and hydroxychloroquine, Drug cluster 2: Lopinavir and Ritonavir, Drug cluster 3: Fedratinib, Ruxolinitib, and Bariticinib. Drug cluster 4: azithromycin, and Drug cluster 5: Losartan.

Figure S7. **Heat map of 14 candidate drugs (MCF7 cell line).** Unsupervised clustering of Library of Integrated Network-Based Cellular Signature (LINCS) L1000 genes, extracted from RNAseq datasets of severe acute respiratory syndrome (SARS) and Middle East respiratory

syndrome-related coronavirus (MERS) (GSE56192). L1000 genes were also extracted from an Influenza A (INFL) microarray dataset (GSE47963) as a non-coronavirus pathogen comparison group. L1000 gene signatures for the fourteen candidate drugs were generated from MCF7 cell line. Furosemide (FUR), a drug which is not utilized in the treatment of viral pathogens, was utilized as a comparison group. A heat map was constructed using the logFoldChange from LINCS. This heat map provides an overview of the difference in patterns of gene expression between the candidate drugs and disease in the MCF7 cell line, demonstrating discordance between these putative repurposable drugs and coronoavirus disease signatures. AC1MJ3VH (AC1), AT-9283 (AT), Alvocidib (ALV), BRD-K54343811 (BRD), Broad-Sai (BRO), CHEMBL2136735 (CHE), COT-10B (COT), Genistein (GEN), GSK-1059615 (GSK), GSK3 Inhibitor-IX (GIX), Idebenone (IDB), Idelalisib (IDL), Ivermectin (IVE), Pencillin V (PEN).

Figure S8. Heat map of top 8 candidate drugs (MCF7 cell line). Unsupervised clustering of Library of Integrated Network-Based Cellular Signature (LINCS) L1000 genes, extracted from RNAseq datasets of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome-related coronavirus (MERS) (GSE56192). L1000 genes were also extracted from an Influenza A (INFL) microarray dataset (GSE47963) as a non-coronavirus pathogen comparison group. L1000 gene signatures for the 8 candidate drugs that are currently approved for use in humans were generated from MCF7 cell line. Furosemide (FUR), a drug which is not utilized in the treatment of viral pathogens, was utilized as a comparison group. A heat map was constructed using the logFoldChange from LINCS. This heat map provides an overview of the difference in patterns of gene expression between the candidate drugs and disease in the MCF7 cell line, demonstrating discordance between these putative repurposable drugs and coronoavirus disease signatures. AT-9283 (AT), Alvocidib (ALV), Genistein (GEN), GSK-1059615 (GSK), Idebenone (IDB), Idelalisib (IDL), Ivermectin (IVE), Pencillin V (PEN)

Figure S9. Heat map of 14 candidate drugs (HA1E cell line). Unsupervised clustering of Library of Integrated Network-Based Cellular Signature (LINCS) L1000 genes, extracted from RNAseq datasets of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome-related coronavirus (MERS) (GSE56192). L1000 genes were also extracted from an Influenza A (INFL) microarray dataset (GSE47963) as a non-coronavirus pathogen comparison group. L1000 gene signatures for the fourteen candidate drugs were generated from HA1E cell line. Furosemide (FUR), a drug which is not utilized in the treatment of viral pathogens, was utilized as a comparison group. A heat map was constructed using the logFoldChange from LINCS. This heat map provides an overview of the difference in patterns of gene expression between the candidate drugs and disease in the HA1E cell line, demonstrating discordance between these putative repurposable drugs and coronoavirus disease signatures. AC1MJ3VH (AC1), AT-9283 (AT), Alvocidib (ALV), BRD-K54343811 (BRD), Broad-Sai (BRO), CHEMBL2136735 (CHE), COT-10B (COT), Genistein (GEN), GSK-1059615 (GSK), GSK3 Inhibitor-IX (GIX), Idebenone (IDB), Idelalisib (IDL), Ivermectin (IVE), Pencillin V (PEN).

Figure S10. Heat map of top 8 candidate drugs (HA1E cell line). Unsupervised clustering of Library of Integrated Network-Based Cellular Signature (LINCS) L1000 genes, extracted from RNAseq datasets of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome-related coronavirus (MERS) (GSE56192). L1000 genes were also extracted from an Influenza A (INFL) microarray dataset (GSE47963) as a non-coronavirus pathogen comparison group. L1000 gene signatures for the 8 candidate drugs that are currently approved for use in humans were generated from HA1E cell line. Furosemide (FUR), a drug which is not utilized in the treatment of viral pathogens, was utilized as a comparison group. A heat map was constructed using the logFoldChange from LINCS. This heat map provides an overview of the difference in patterns of gene expression between the candidate drugs and disease in the HA1E

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Figure S11. Biological Pathway analysis of disease and candidate repurposable drug signatures from MCF7 cell line. Gene lists for candidate drugs consisting of L1000 genes at LFC +/- 0.85 were searched in Reactome. Pathways were determined by p-value < 0.05, and "Entities Found" ≥ 30. Gene lists for disease signatures consisting of L1000 genes at LFC ≥ 0.5 and ≤ -0.5 were searched in Reactome. Pathways were determined by p-value < 0.05, and "Entities Found" ≥ 6. AC1MJ3VH (AC1), AT-9283 (AT), Alvocidib (ALV), BRD-K54343811 (BRD), Broad-Sai (BRO), CHEMBL2136735 (CHE), COT-10B (COT), Genistein (GEN), GSK-1059615 (GSK), GSK3 Inhibitor-IX (GIX), Idebenone (IDB), Idelalisib (IDL), Ivermectin (IVE), Pencillin V (PEN).

Figure S12. Biological Pathway analysis of disease and candidate repurposable drug signatures from HA1E cell line. Gene lists for candidate drugs consisting of L1000 genes at LFC +/- 0.85 were searched in Reactome. Pathways were determined by p-value < 0.05, and "Entities Found" ≥ 30. Gene lists for disease signatures consisting of L1000 genes at LFC ≥ 0.5 and ≤ -0.5 were searched in Reactome. Pathways were determined by p-value < 0.05, and "Entities Found" ≥ 6. AC1MJ3VH (AC1), AT-9283 (AT), Alvocidib (ALV), BRD-K54343811 (BRD), Broad-Sai (BRO), CHEMBL2136735 (CHE), COT-10B (COT), Genistein (GEN), GSK-1059615 (GSK), GSK3 Inhibitor-IX (GIX), Idebenone (IDB), Idelalisib (IDL), Ivermectin (IVE), Pencillin V (PEN).

Figure S1

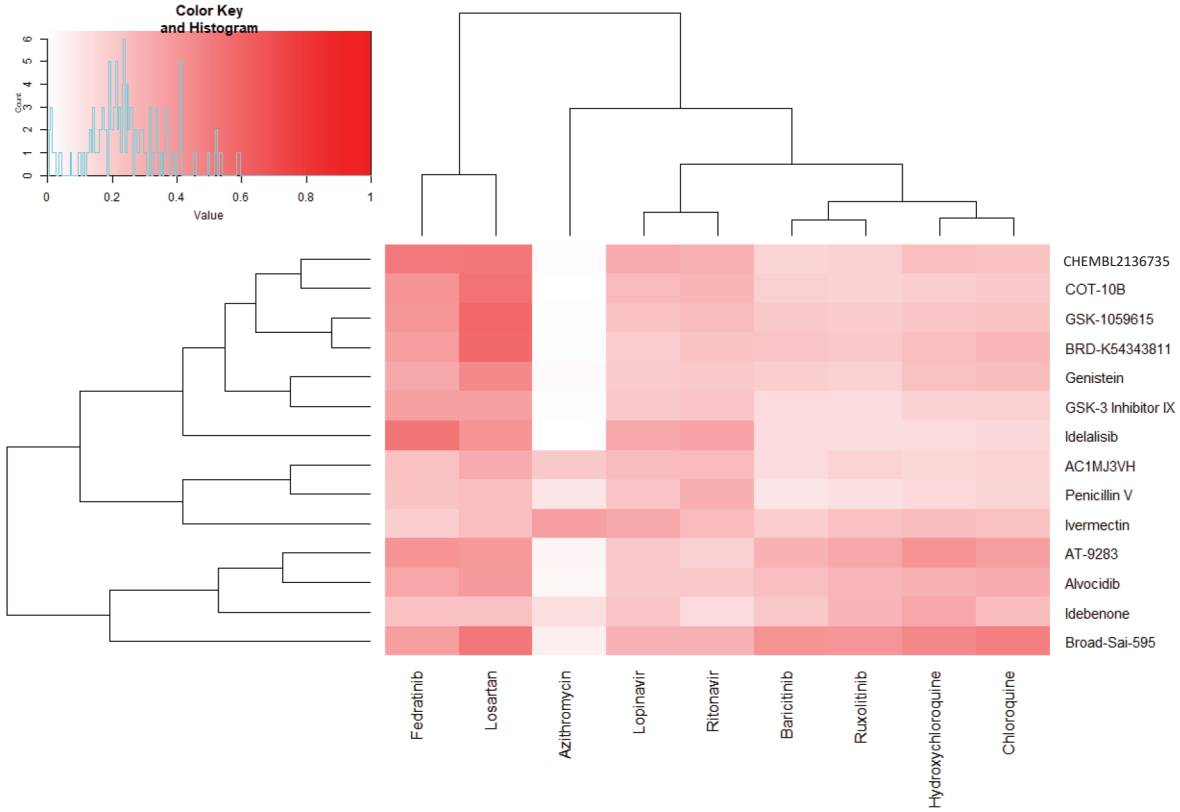
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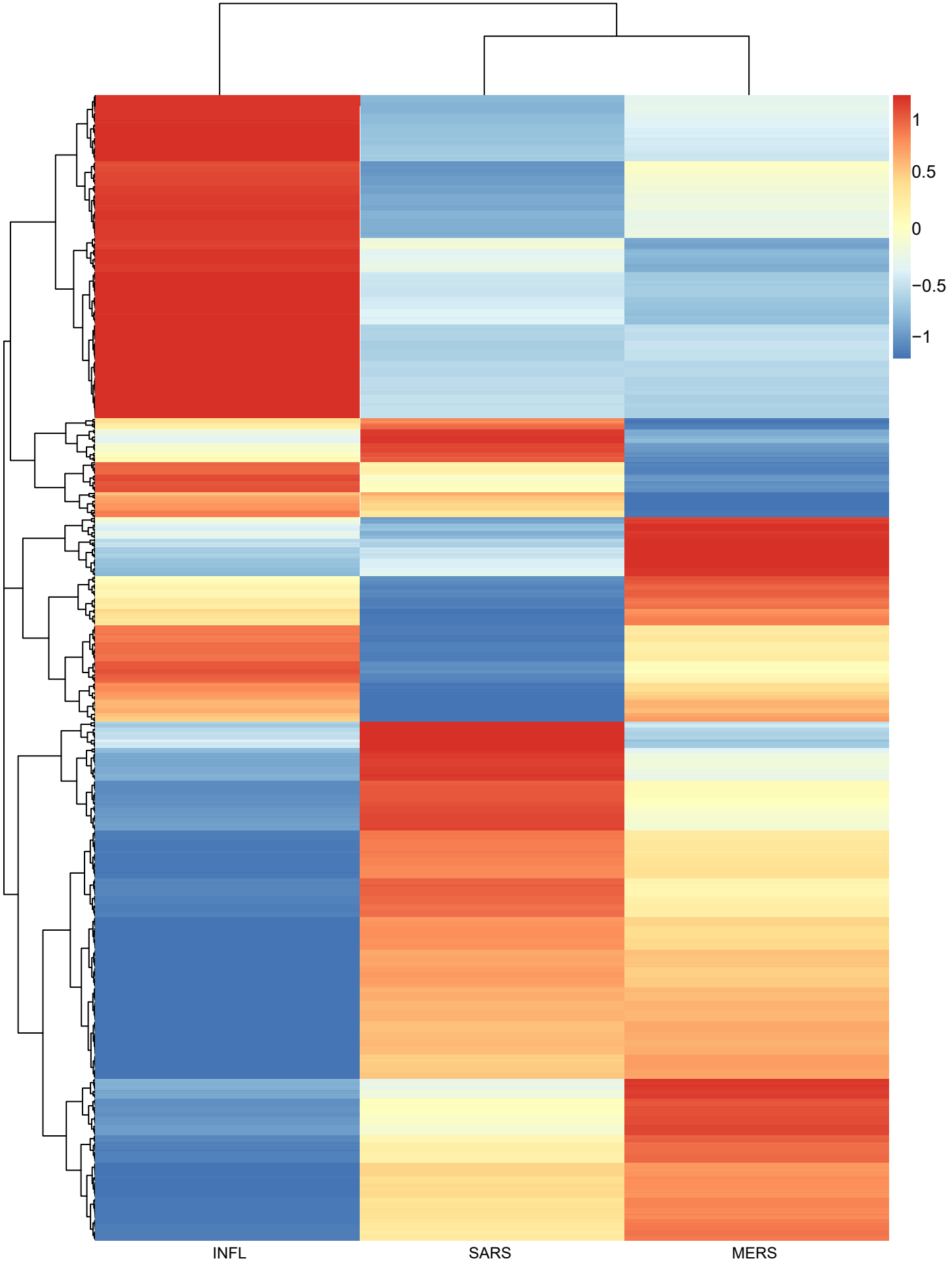
Figure S2



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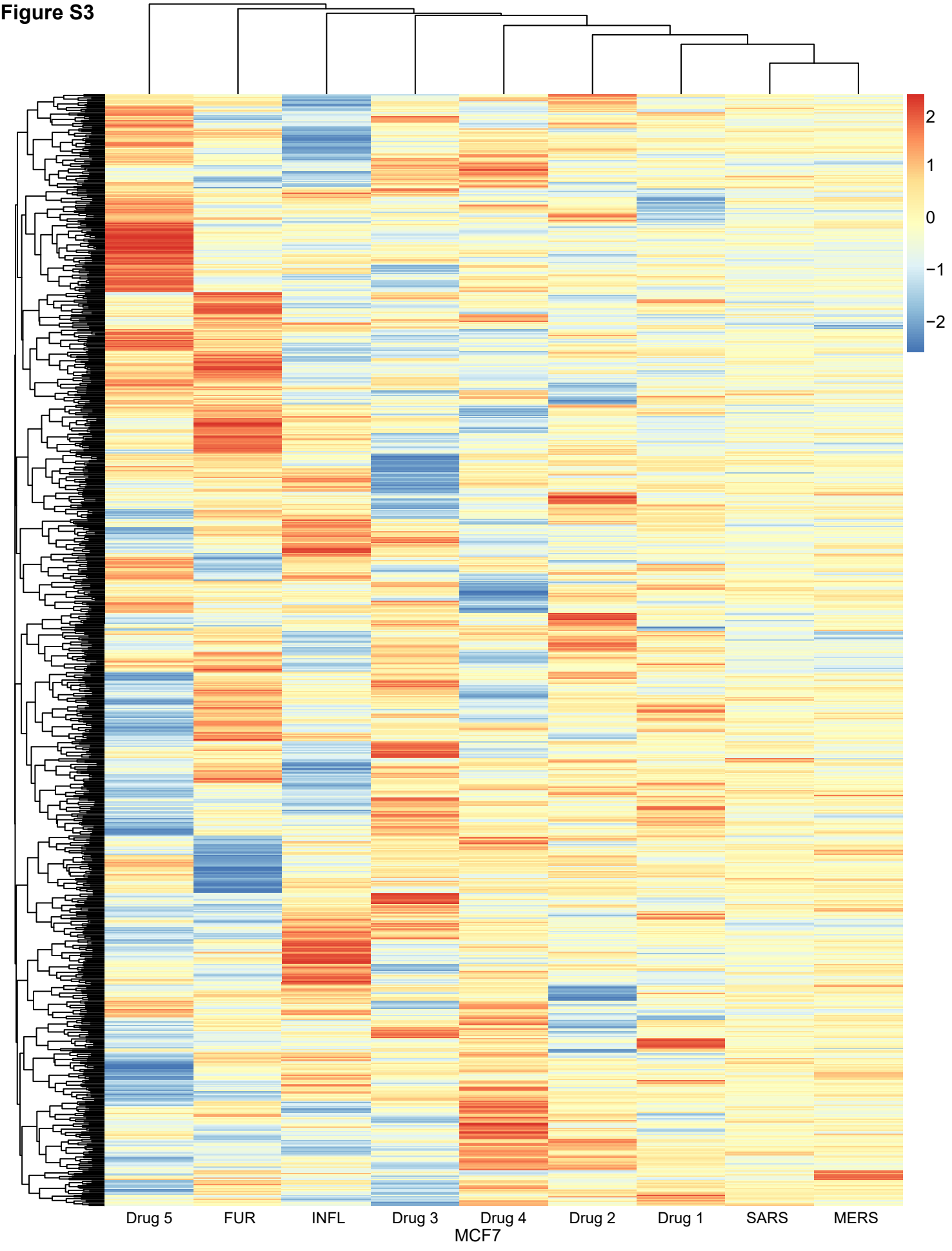

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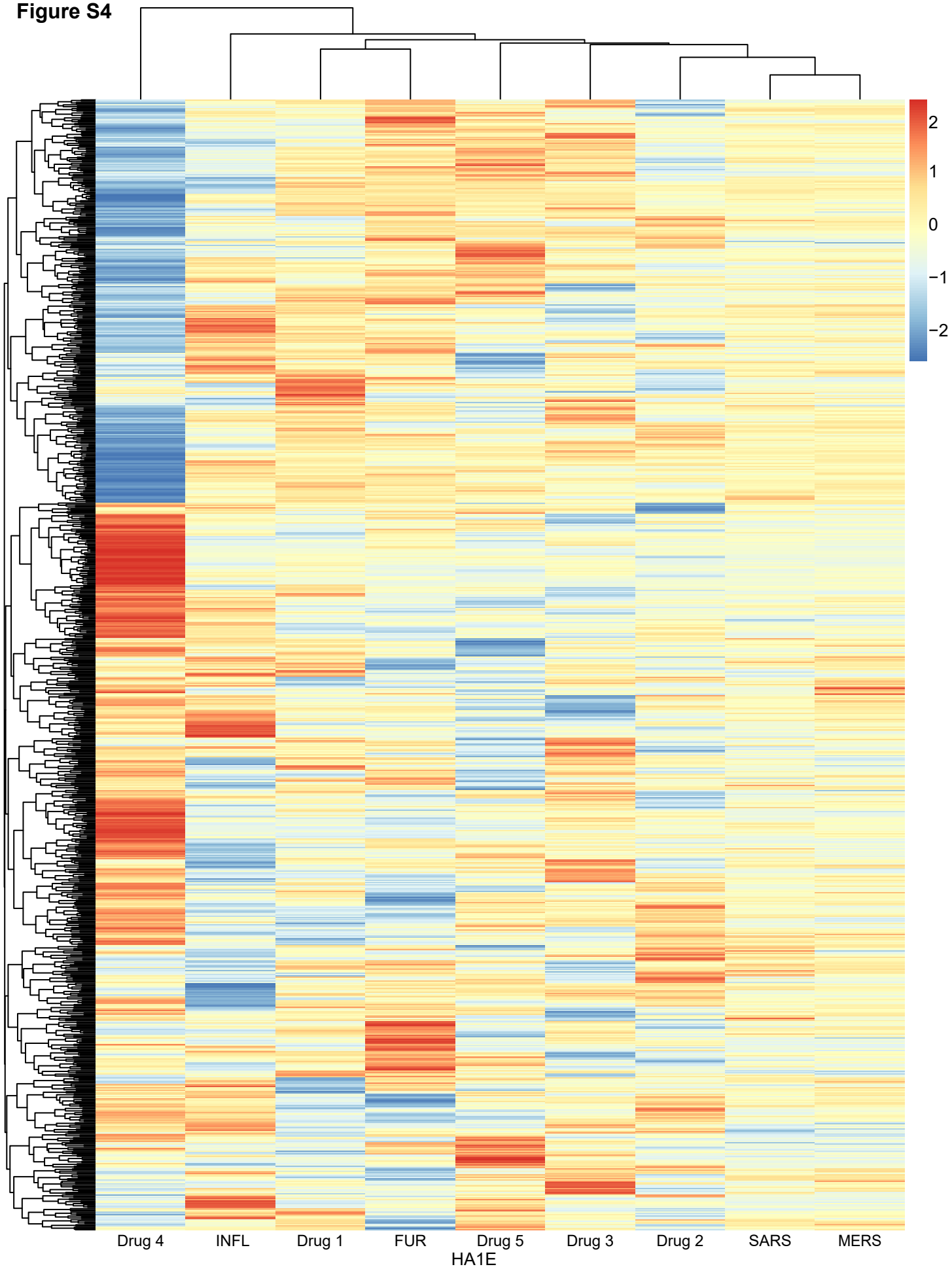

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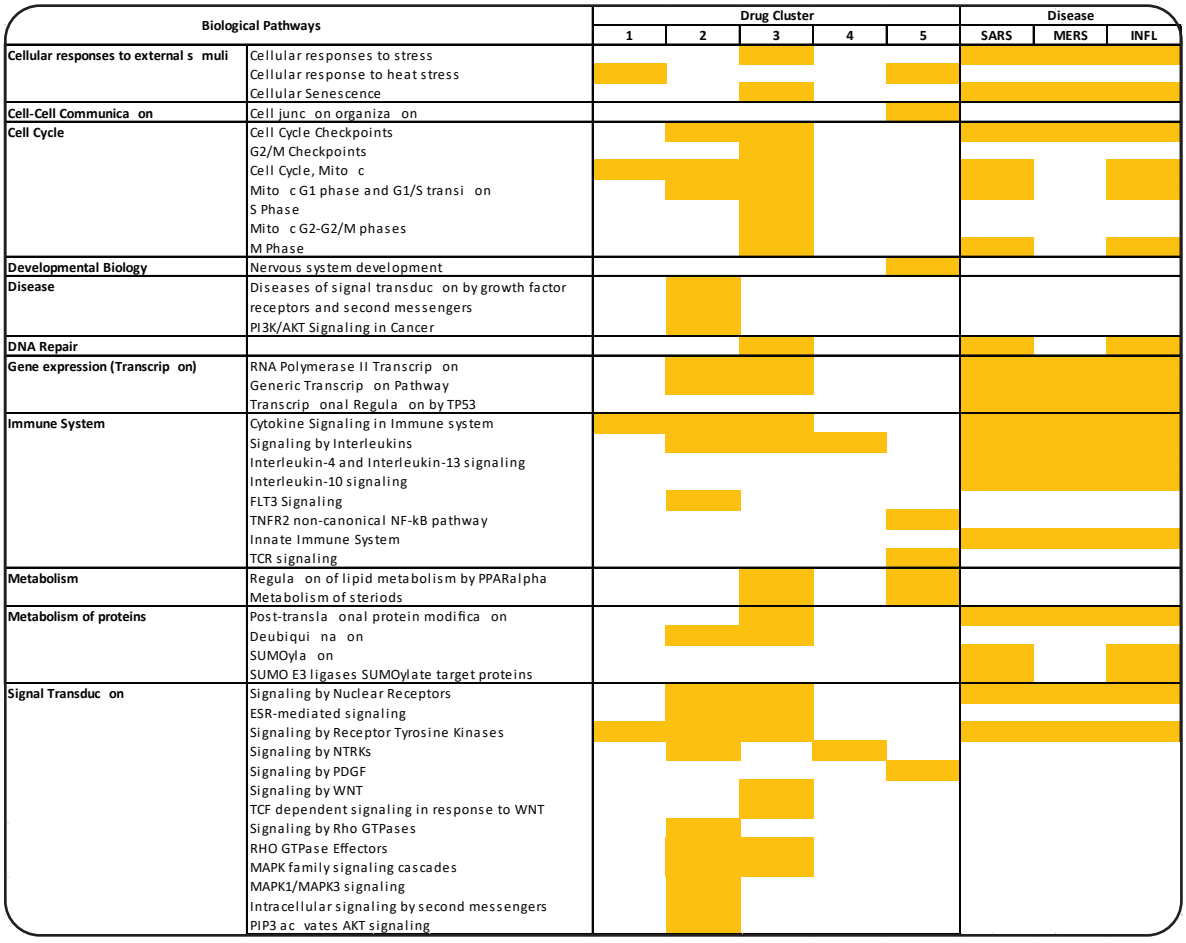
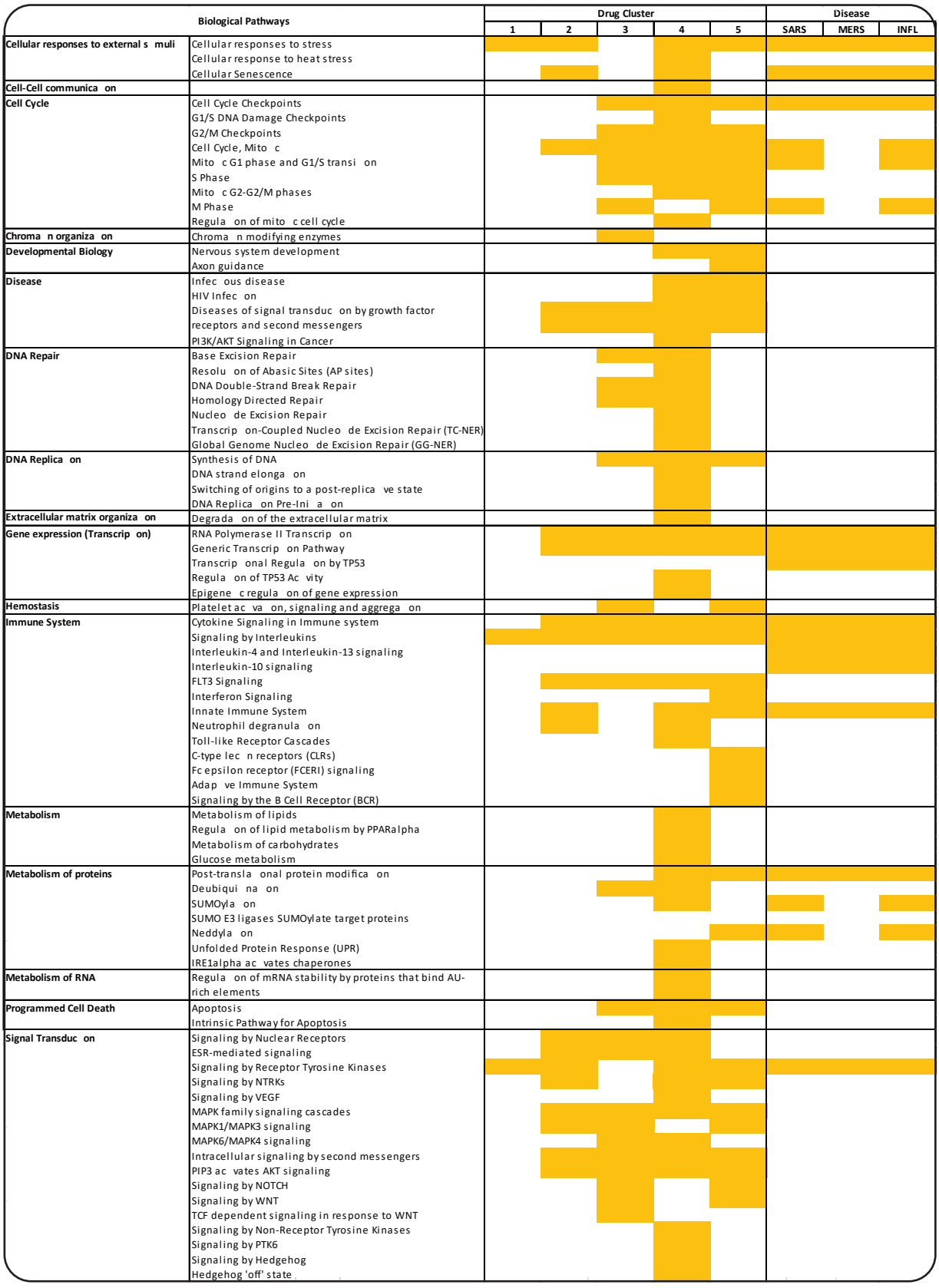


MCF7 Cell Line

Figure S6



HA1E Cell Line

Figure S7

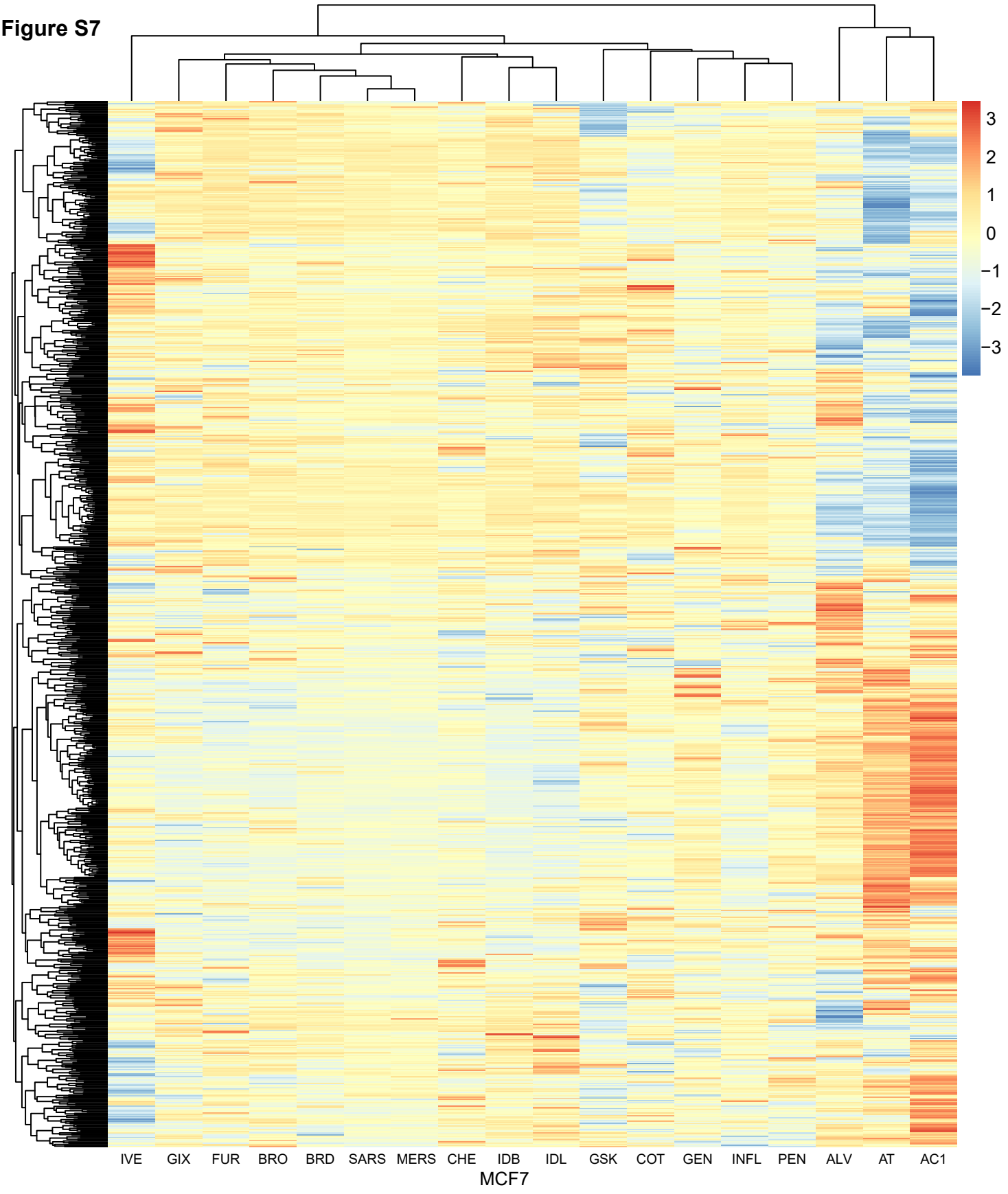

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Figure S8

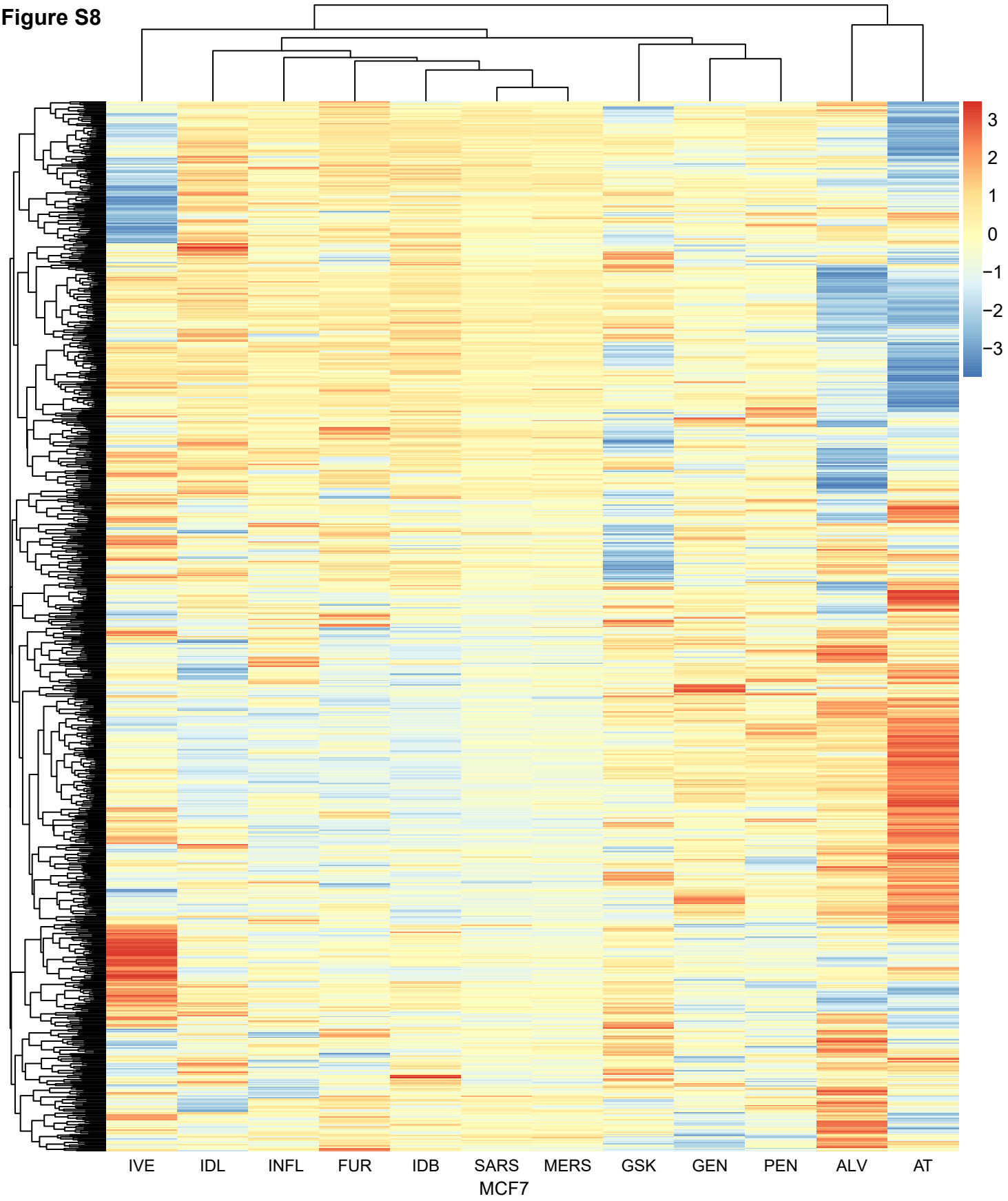

Figure S9

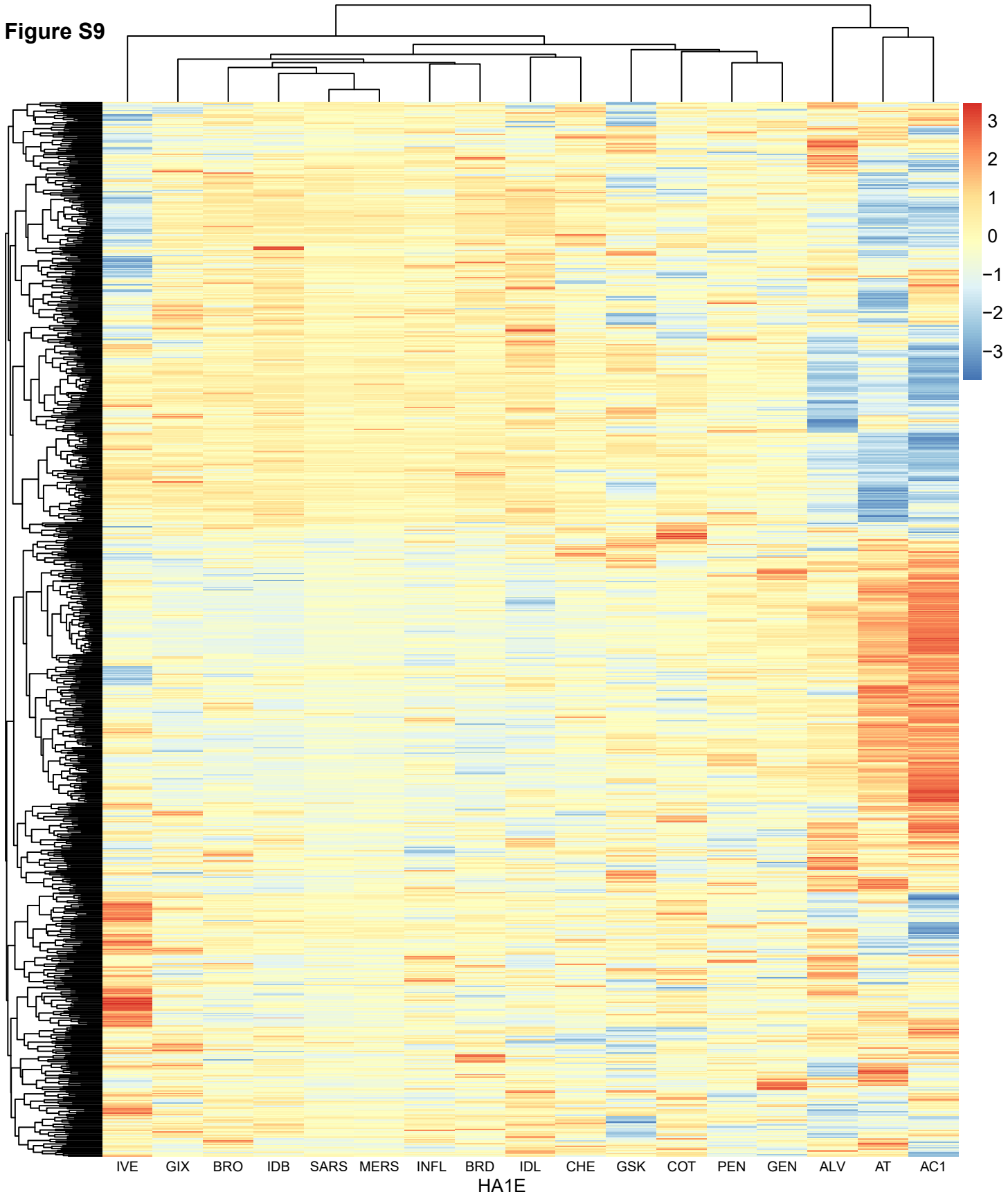
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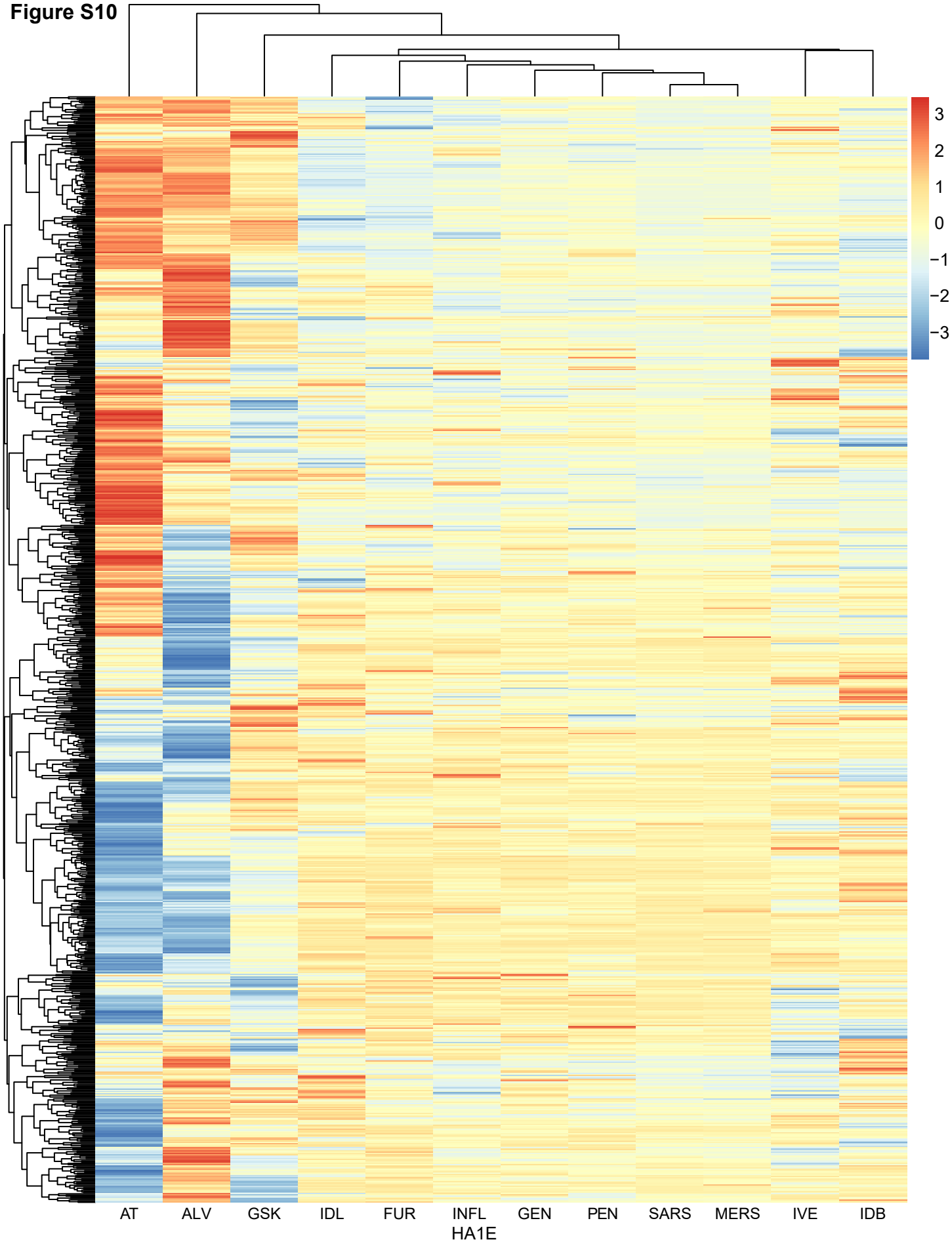
Figure S10



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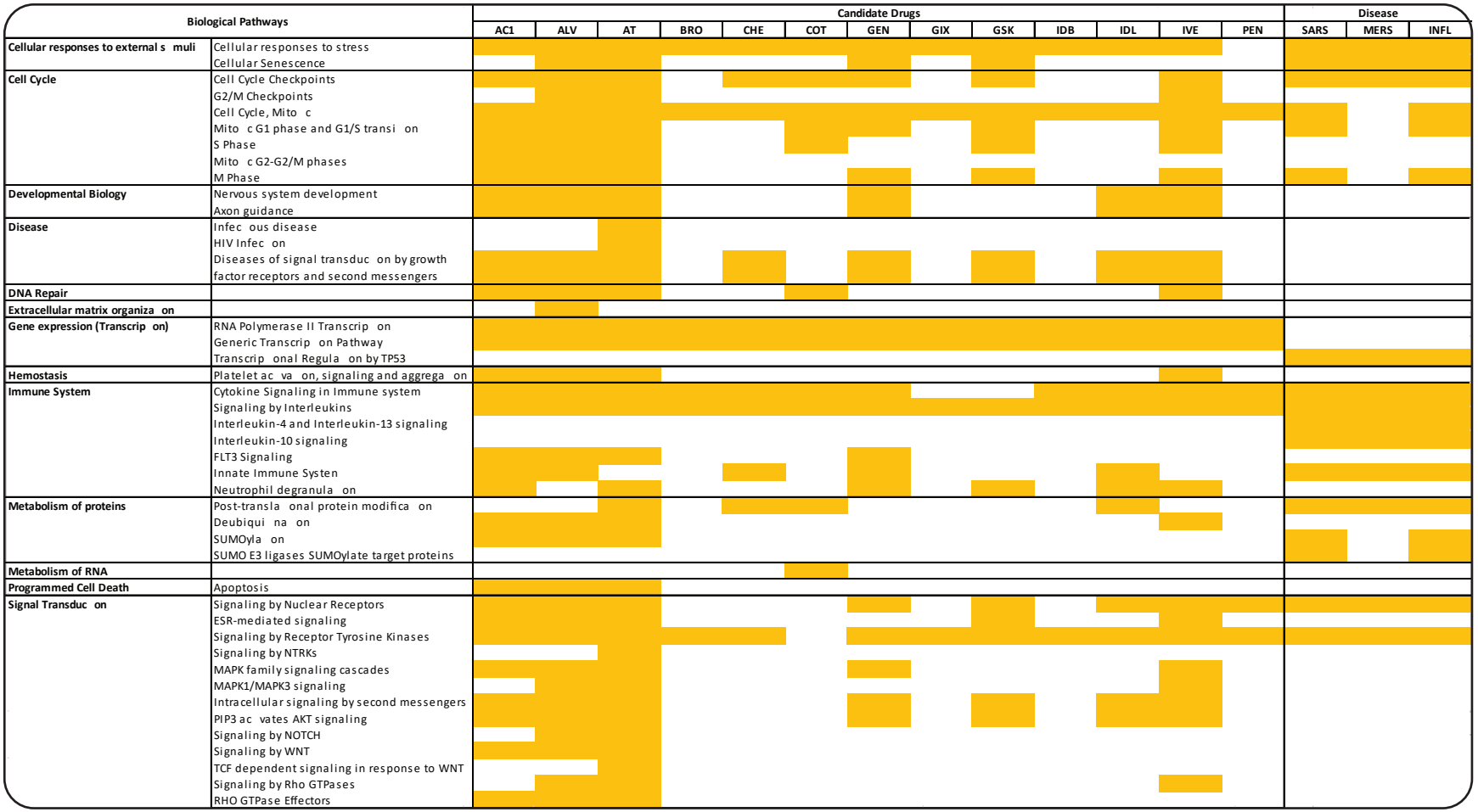
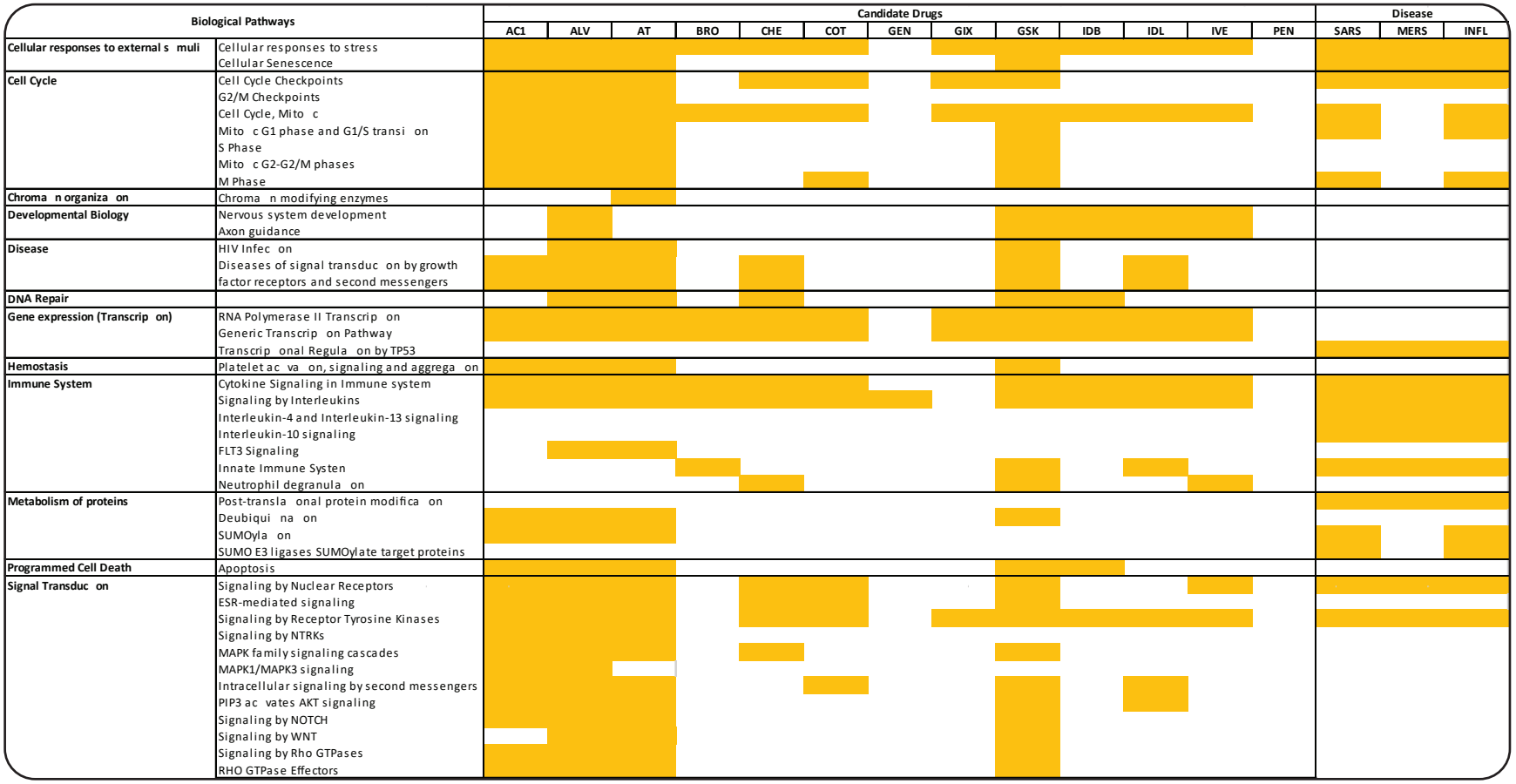


MCF7 Cell Line

Figure S12



HA1E Cell Line

Table S1. Selected drugs with transcriptional profiles in the Library for Integrated Network-Based Cellular Signatures database which were used for input.

Drug	Trade Names	Clinical Indication	Target	Canonical Mechanism of Action and Clusters	Anatomical Chemical Therapeutic Classification	Literature Citing Relevance to SARS-CoV-2 and/or Coronaviridae	Putative Effect on SARS-CoV-2
Drugs with iLINCS signature present.							
Hydroxychloroquine [1]	Plaquenil	Malaria; Immunosuppressant	Toll-like receptor 9; Toll-like receptor 7	Toll-like receptor antagonist	Antiparasitic products, insecticides and repellents	[2-4]	Alkalization of phagolysosome; modification of post-translation protein modifications [5, 6]
Chloroquine [7]	Aralen	Malaria; Amebiasis	Toll-like receptor 9 Glutathione S-transferase (A2); High mobility group protein B1; tumor necrosis factor				
Lopinavir [8]	Kaletra / Aluvia (with Ritonavir)	HIV/AIDS Antiviral Therapy	Human immunodeficiency virus type 1 protease	Protease inhibitor	Antiinfectives for systemic use	[9-12]	Inhibition of CoV-polyprotein processing [9, 13]
Ritonavir [14]	Norvir; Kaltera /Aluvia (with Lopinavir)						
Fedratinib [15]	Inrebic	Antineoplastic	Tyrosine-protein kinase JAK2; Receptor-type tyrosine-protein kinase FLT3	JAK Inhibitor	Antineoplastic and immunomodulating agents	[16]	Inhibition of clathrin-mediated viral endocytosis; mediation of inflammatory response, attenuation of cytokine storms from prolonged infection [16, 17]
Ruxolitinib [18]	Jakafi, Jakavi	Antineoplastic	Tyrosine-protein kinase JAK1 and JAK2				
Baricitinib [19]	Olumiant, Baricinix	Immunosuppressant	Tyrosine-protein kinase JAK1, JAK2, and JAK3; Protein-tyrosine kinase 2-beta				
Azithromycin [20]	Act Azithromycin, AzaSITE,	Bacterial infections	23S Ribosomal RNA; Protein-arginie deaminase type-4	Inhibition of bacterial protein synthesis	Antiinfectives for systemic use	[4]	Adjunct therapy with hydroxychloroquine.

	Zithromax, Zmax						
Losartan [21]	Act Losartan, Cozaar	Hypertension	Type-1 angiotensin II (AGII) receptor	AGII receptor antagonist	Cardiovascular system	[22]	Angiotensin receptor blocker.
Drugs with iLINCS signature not present.							
Remdesivir [23]	Remdesivir	Ebola	Replicase polyprotein 1ab; RNA-directed RNA polymerase L	Nucleoside analog, inhibition of viral RNA polymerase	No record	[24-27]	Inhibition of RNA dependent RNA polymerase [27]
Rocaglate	Not found in DrugBank.				No record	[28]	Inhibition of viral translation [28].
Silvesterol	Not found in DrugBank.				No record	[28, 29]	Inhibition of viral translation [28].
Umifenovir [30]	Arbidol	Viral prophylaxis; Broad spectrum antiviral	Cytochrome P450 3A4, 2E1, 1A2, 2D6, 2C9, 3A5; UDP-glucuronosyltransferase 2B7, 1-9; Dimethylaniline monooxygenase 1, 3	Aromatic rings interfere with viral endocytosis, exocytosis, intracellular trafficking, and destabilize membranes	Antiinfectives for systemic use	[31-33]	No specific purported mechanism yet published.
N10169	Not found in DrugBank.				No record	[34]	Inhibition of pyrimidine biosynthesis [34].
Interferon Alphacon-1 [35]	Infergen	Antineoplastic	Interferon alpha/beta receptor 1, 2	Interferon receptor agonist, JAK activator	Antineoplastic and immunomodulating agents	[36, 37]	Upregulation of MHC 1 to present viral antigens.
Polygonaceae spp.; Rheum palmatum L.	Not found in DrugBank.				No record	[38, 39]	Inhibition of viral cell-entry by preventing interaction of CoV Spike Protein from interacting with ACE2 receptor [39]
Camostat mesylate [40]	N/A	Pancreatitis, Japan [41, 42]	N/A	Serine protease inhibitor TMPRSS2	Blood and blood forming organs	[43-45]	Inhibition of viral cell-entry [43]

Information relating to these drugs (trade name, clinical indication, targets, and canonical mechanism of action) were referenced from DrugBank (https://www.drugbank.ca/). Anatomical Therapeutic Chemical (ATC) classification was referenced from (https://www.whocc.no/atc_ddd_index/). For simplicity, the first-level of ATC classification is indicated.

Table S2. Gene threshold cutoff of generating disease signatures**.**

Disease	Cell Line	Perturbagens Identified at LFC threshold		
		All L1000	0.26	0.5
INFL	HA1E	1	0	0
	MCF7	0	0	0
MERS	HA1E	2	28	320
	MCF7	14	142	1305
SARS	HA1E	20	172	1352
	MCF7	21	706	2181

L1000 genes with expression change at log fold change (LFC) threshold 0.5 were selected to generate severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and influenza (INFL) gene signatures. At this threshold, following connectivity analysis, a large number of chemical perturbagen signatures were identified as discordant to the SARS signature (discordance ≤ -0.321) in both MCF7 and HA1E cell lines. At the same threshold, no chemical perturbagens were identified as discordant to the INFL signature, and fewer than 50% of the number of perturbagens were identified in MERS compared to SARS.