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Supplemental information

DCcov: Repositioning of drugs and drug combinations for SARS-CoV-2 infected lung through constraint-based modeling

Ali Kishk, Maria Pires Pacheco, and Thomas Sauter

Supplementary Data

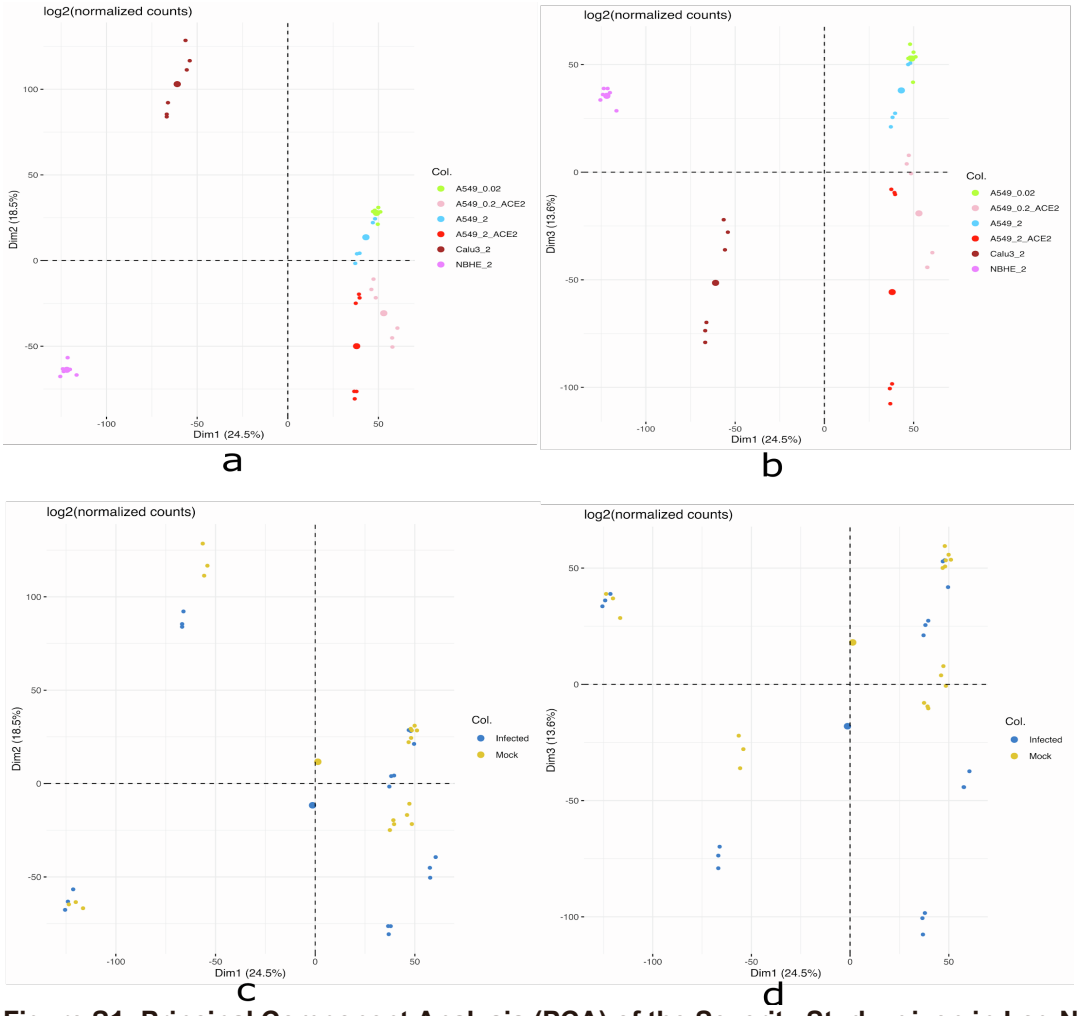

Figure S1: Principal Component Analysis (PCA) of the Severity Study, given in Log Normalized Read Counts for different principal components (PC), Related to STAR Methods (*A.1.2)*.

a) PC1 vs PC2 colored by cell line and condition.
b) PC1 vs PC3 colored by cell line and condition.
c) PC1 vs PC2 colored by infection status (infected vs. mock samples).
d) PC1 vs PC3 colored by infection status (infected vs mock samples).

Figure S2: Reactions per Pathway Heatmap for Pathway Analysis of All Conditions of Differentially expressed genes in the Severity Study, Including the ACE2 Transfection Analyses, Related to Figure 2.

Differentially expressed genes (DEGs) were computed with DESeq2. The Down- and up-regulated were mapped to the pathways (subSystems) of Recon3D_01. The number of up and down-regulated reactions was then summed up to identify the top altered pathways in the infected lung cell lines in the severity study (related to STAR Methods *A.1.2*) but without filters on the number of reactions nor the Reactions per Pathway. The color code "Reactions per Pathway [%]" represents the number of enriched metabolic reactions in a pathway divided by the overall number of reactions in this pathway. Removing filters increased the number of differentially expressed pathways and allowed including the NBHE_2 condition. Additionally, the figure includes the DEG analysis of the effect of ACE2 transfection in ACE2_Mock and ACE2_Infected (related to STAR Methods *A.1.2*).

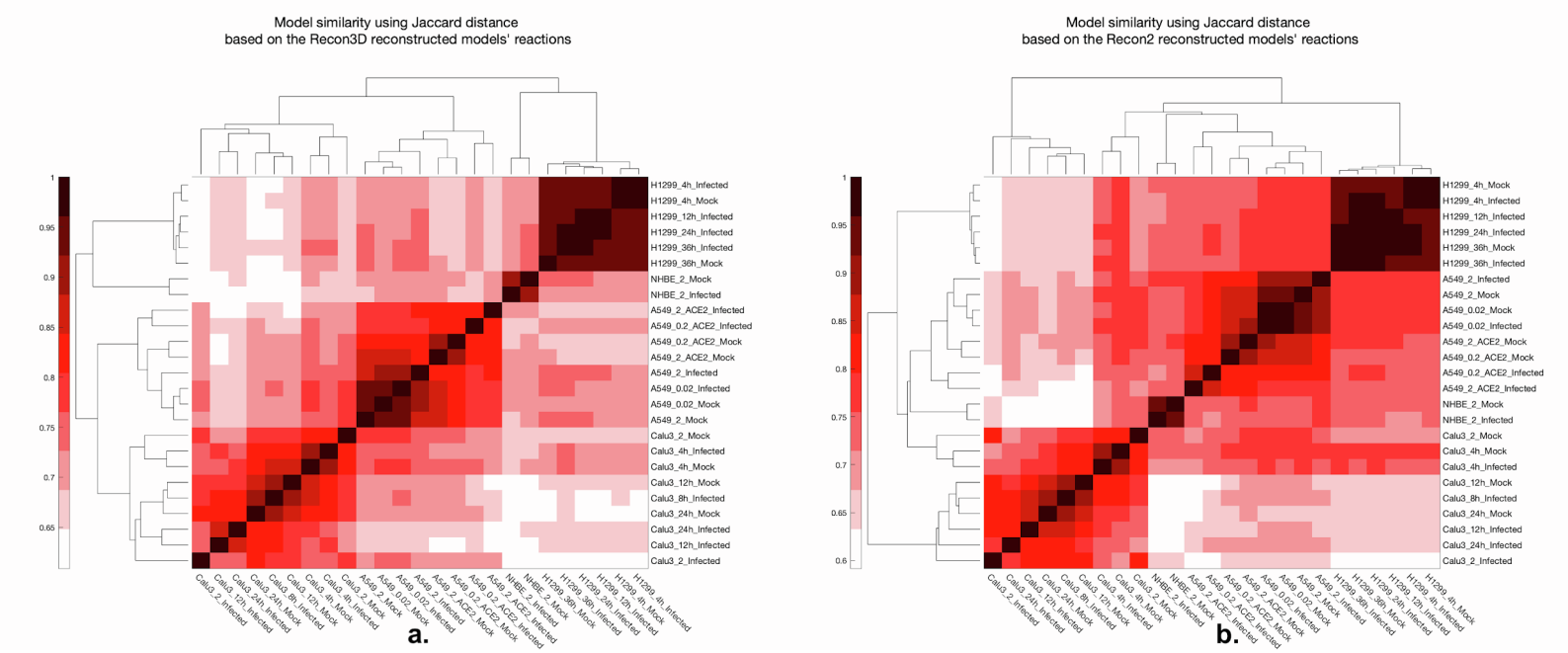

Figure S3: Clustergrams of the Reconstructed Models' Similarity using Reactions Presence with Jaccard Similarity Metrics of Both Recon3D_01 Models (a) and Recon2.04 Models (b), Related to Figure 4.

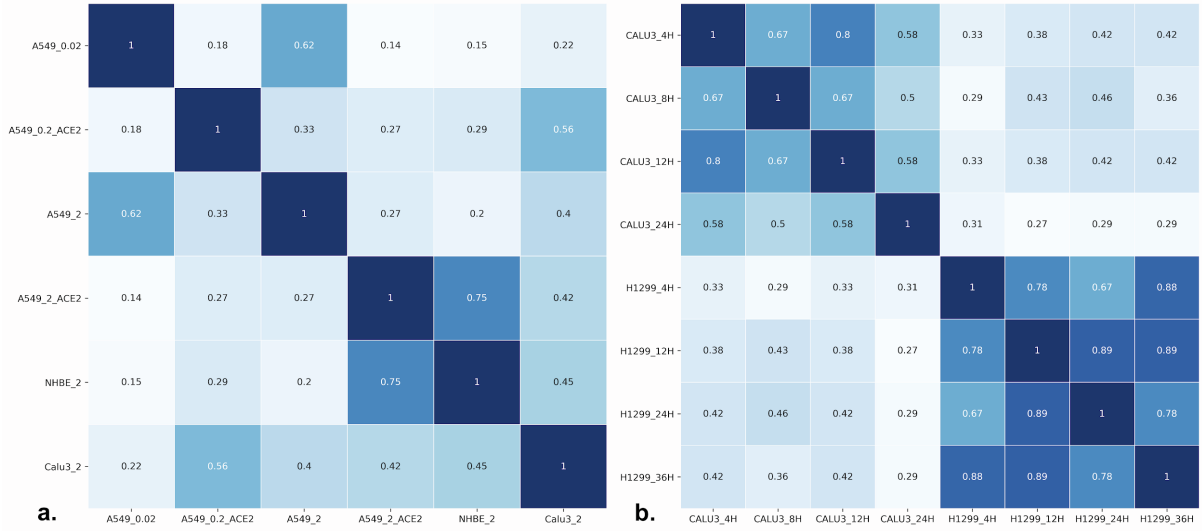

Figure S4: Jaccard Similarity of the Essential Genes Derived from the Different Conditions of the Two Lung Studies (Severity Study & Time-series Study), Related to STAR Methods (*A.2.4*).

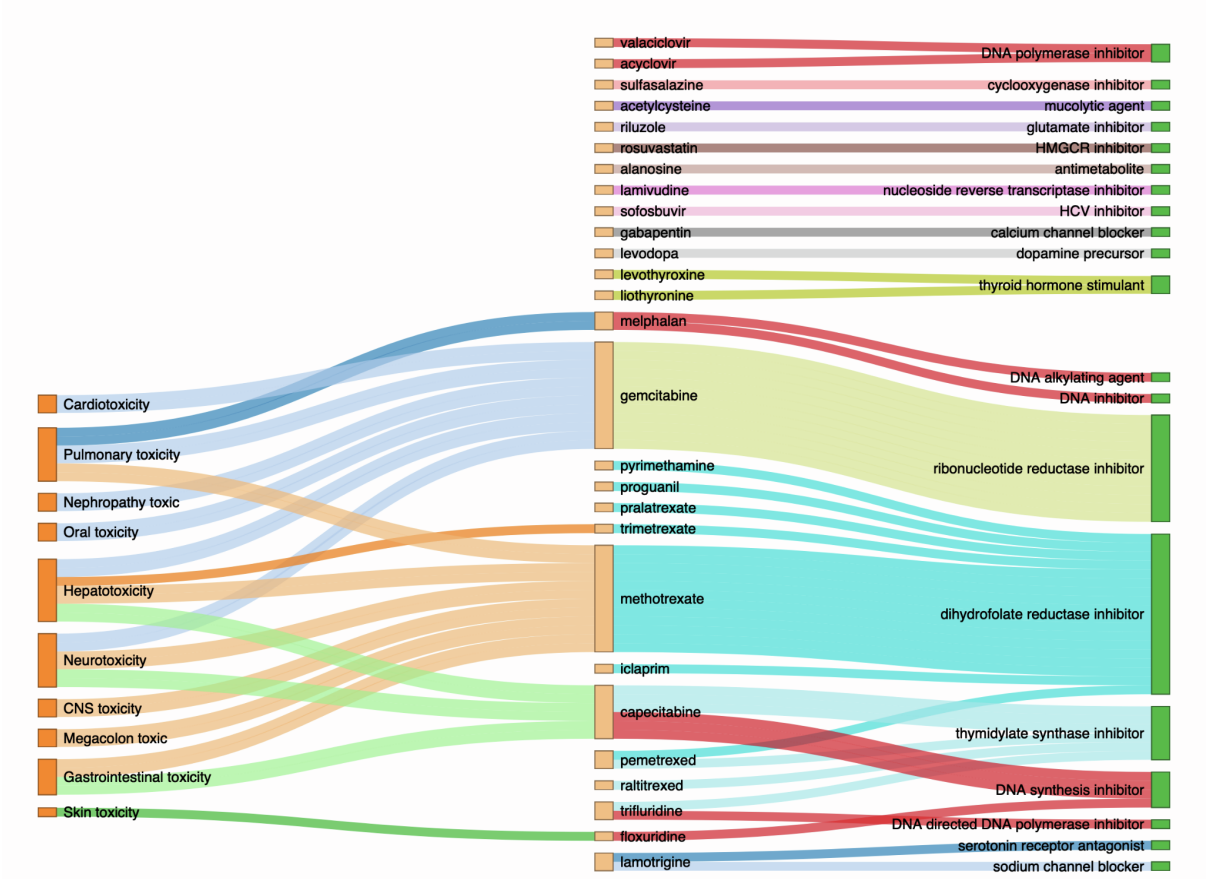

Figure S5: Tripartite Network of the Candidate Drugs and their Mode of Action and Toxicity, Related to STAR Methods (*C*). Drug mode of actions were extracted from the Drug Repurposing Hub database. While the drug toxicity was extracted from the MedDRA database for side effects containing “toxic” words.

Table S1: Essential Metabolic Genes of the Severity Study, Related to STAR Methods (*A.2.4*). The essential genes of each condition of the infected model are aggregated from the Recon2.04 and Recon3D_01 based reconstructions. Genes are classified according to the predicted toxicity into toxic or safe, based on the presence or absence from the essential genes of the mock model. A gene is considered with unknown toxicity if there is no mock model corresponding to the infected model, or the essential gene was filtered from the context mock model.

Condition	Safe Essential	Toxic Essential	Unknown Safety Essential
A549_0.02	SLC3A2; SLC7A11	CRLS1; ISYNA1; SGMS1	
A549_0.2_ACE2	AGXT; DHFR; SLC27A4; TYMS	CRLS1; GUK1; PEPD; SGMS1	
NHBE_2	SLC3A1; SLC7A9	CMPK1; CRLS1; CTH; GUK1; PTDSS1; SGMS1; SLC27A4; SLC5A3	SLC5A3
A549_2	DTYMK; GUK1; PEPD	CRLS1; GUK1; ISYNA1; SGMS1; SLC3A2; SLC7A11	
A549_2_ACE2	CMPK1; CTH; GUK1; PTDSS1; SLC3A1; SLC7A9	CRLS1; PEPD; SGMS1; SLC2A13	SLC5A3
Calu3_2	CMPK1; SLC27A4	CRLS1; GUK1; PEPD; SGMS1	

Table S2: Essential Metabolic Genes of the Time-series Study, Related to STAR Methods (*A.2.4*). The essential genes of each condition of the infected model are aggregated from the Recon2.04 and Recon3D based reconstructions. Gene gain and gene loss are genes gained or lost from the previous time point in the same cell line. Time points are in hours. Genes are classified according to the predicted toxicity into toxic or safe, based on the presence or absence from the essential genes of the mock model. A gene is considered with unknown toxicity if there is no mock model corresponding to the infected model, or the essential gene was filtered from the context mock model.

Condition	Safe Essential	Toxic Essential	Unknown Safety Essential	Gene Gain	Gene Loss
Calu3_4H	GLTP	CRLS1; GUK1; ISYNA1; PEPD; SGMS1; SLC27A4; SLC3A2; SLC7A11			
Calu3_8H			CRLS1; GLTP; GUK1; ISYNA1; PEPD; SGMS1; SLC27A4; SLC3A2; SLC7A11; SLC7A5	SLC7A5	
Calu3_12H	ISYNA1; SLC27A4; SLC7A5	CRLS1; GUK1; ISYNA1; PEPD; SGMS1; SLC3A2; SLC7A11; SLC7A5			GLTP
Calu3_24H	PTDSS1	CRLS1; GLTP; GUK1; ISYNA1; SGMS1; SLC27A4; SLC3A2; SLC7A11; SLC7A5		GLTP; PTDSS1	
H1299_4H	CTH	CRLS1; ISYNA1; PLD2; SGMS1; SLC27A4; SLC7A6			
H1299_12H			CRLS1; CTH; ISYNA1; PEPD; PLD2; SGMS1; SLC27A4; SLC7A6	PEPD	
H1299_24H			CRLS1; ISYNA1; PEPD; PLD2; SGMS1; SLC27A4; SLC7A6		CTH
H1299_36H	PEPD; SLC7A6	CRLS1; CTH; ISYNA1; PLD2; SGMS1; SLC27A4		CTH	

Table S3: Candidate Drugs for the Essential Genes Given by their Gene Targets, Related to Figure 4.

Drug-target interactions were downloaded from DrugBank and filtered (related to STAR Methods C). Essential genes were determined using single-gene knockouts. eight essential genes are targeted by 45 drugs.

Gene Symbol	Drugs	Essentiality Score	Safety Score
GUK1	Valaciclovir, Acyclovir	16	3
ISYNA1	Glycerin	9	1
SLC7A11	Riluzole, Taurocholic acid, Alanosine, Thimerosal, Tauroursodeoxycholic acid, Rosuvastatin, Acetylcysteine, Sulfasalazine	5	1
CMPK1	Lamivudine, Gemcitabine, Sofosbuvir	4	3
SLC7A5	Dextrothyroxine, Pregabalin, Levothyroxine, Liothyronine, Melphalan, Gabapentin, Levodopa, Thyroid, porcine	3	1
GLTP	Oleic Acid, Lactose, Lauric acid	2	1
DHFR	Methotrexate, Pyrimethamine, Pemetrexed, Lamotrigine, Piritrexim, Pralatrexate, Trimetrexate, Proguanil, Iclaprim, Gentamicin	1	1
TYMS	ANX-510, Fluorouracil, Capecitabine, Methotrexate, Floxuridine, Thymectacin, Trifluridine, Gemcitabine, Pemetrexed, Pralatrexate, OSI-7904L, Tegafur, Tegafur-uracil, Raltitrexed	1	1

Table S4: Single Drugs with Two Paired Targets of Double Gene Deletion, Related to Figure 5.B. Candidate gene-pairs with possible essentiality to the viral biomass were determined by double gene knockout (related to STAR Methods *A.2.3*). Gene-pairs that have one drug for both gene targets were selected. Essentiality and safety scores were calculated (related to STAR Methods *A.2.4)*. As many gene-pairs share the same drug, gene-pairs were clustered by their drugs. Essentiality and safety scores were averaged between the different gene-pairs. Drugs were sorted by the number of gene-pairs they are targeting.

Drug	Gene Pairs	Average Essentiality Score	Average Safety Score	Number of Gene Pairs
Gemcitabine	CMPK1;SLC29A2, TYMS;SLC29A2, SLC29A2;SLC29A1	1.66	1	3
Trifluridine	TYMS;TYMP, TYMS;SLC29A2, SLC29A2;SLC29A1	1.33	1	3
Mercaptopurine	PPAT;HPRT1, SLC29A2;SLC29A1	3.5	1	2
Tegafur-uracil	TYMS;SLC29A2, SLC29A2;SLC29A1	1.5	1	2
Valaciclovir	SLC15A2;SLC15A1	13	0	1
Moexipril	SLC15A2;SLC15A1	13	0	1
Nateglinide	SLC15A2;SLC15A1	13	0	1
Oxacillin	SLC15A2;SLC15A1	13	0	1
Perindopril	SLC15A2;SLC15A1	13	0	1
Lisinopril	SLC15A2;SLC15A1	13	0	1
Valganciclovir	SLC15A2;SLC15A1	13	0	1
Spirapril	SLC15A2;SLC15A1	13	0	1
Tolbutamide	SLC15A2;SLC15A1	13	0	1
Trandolapril	SLC15A2;SLC15A1	13	0	1
Ubenimex	SLC15A2;SLC15A1	13	0	1
Ramipril	SLC15A2;SLC15A1	13	0	1
Quinapril	SLC15A2;SLC15A1	13	0	1
Cefepime	SLC15A2;SLC15A1	13	0	1
Cefixime	SLC15A2;SLC15A1	13	0	1
Cefalotin	SLC15A2;SLC15A1	13	0	1
Cefdinir	SLC15A2;SLC15A1	13	0	1
Ceftazidime	SLC15A2;SLC15A1	13	0	1
Ceftriaxone	SLC15A2;SLC15A1	13	0	1
Ceftibuten	SLC15A2;SLC15A1	13	0	1
Cefmetazole	SLC15A2;SLC15A1	13	0	1
Cefradine	SLC15A2;SLC15A1	13	0	1
Cefotaxime	SLC15A2;SLC15A1	13	0	1
Amoxicillin	SLC15A2;SLC15A1	13	0	1
Aminolevulinic acid	SLC15A2;SLC15A1	13	0	1
Cefaclor	SLC15A2;SLC15A1	13	0	1
Benzylpenicillin	SLC15A2;SLC15A1	13	0	1

Ampicillin	SLC15A2;SLC15A1	13	0	1
Benazepril	SLC15A2;SLC15A1	13	0	1
Fosinopril	SLC15A2;SLC15A1	13	0	1
Glyburide	SLC15A2;SLC15A1	13	0	1
Chlorpropamide	SLC15A2;SLC15A1	13	0	1
Cilazapril	SLC15A2;SLC15A1	13	0	1
Cephalexin	SLC15A2;SLC15A1	13	0	1
Cefuroxime	SLC15A2;SLC15A1	13	0	1
Dicloxacillin	SLC15A2;SLC15A1	13	0	1
Cloxacillin	SLC15A2;SLC15A1	13	0	1
Cyclacillin	SLC15A2;SLC15A1	13	0	1
Zalcitabine	SLC29A2;SLC29A1	2	2	1
Didanosine	SLC29A2;SLC29A1	2	2	1
Capecitabine	TYMS;TYMP	1	1	1
Fluorouracil	TYMS;TYMP	1	1	1
Floxuridine	TYMS;TYMP	1	1	1

Table S5: Reduced list of Double Gene Deletion Drug Pairs, Related to Figure 5.A. Candidate gene-pairs with possible essentiality to the viral biomass were determined by double gene knockout (DKO) (related to STAR Methods *A.2.3*). Gene-pairs that have one drug for both genes were excluded. Essentiality and safety scores and the number of gene-pairs were calculated such as in (**Table S4**). The reduced DKO drug dataset was filtered by selecting drug pairs with more than two in essentiality scores, and more than one in either the number of gene-pairs or safety scores.

Drugs1	Drugs2	Gene Pair	Average Essentiality Score	Average Safety Score	Number of Gene Pairs
Azathioprine	Pemetrexed	HPRT1;GART, SLC29A2;SLC29A1, TYMS;SLC29A2, DHFR;SLC29A2, ATIC;HPRT1	2.8	0.4	5
Mercaptopurine	Pemetrexed	HPRT1;GART, SLC29A2;SLC29A1, TYMS;SLC29A2, DHFR;SLC29A2, ATIC;HPRT1	2.8	0.4	5
Cladribine	Valaciclovir	GUK1;PNP, RRM2;GUK1, RRM1;GUK1	9.33	0	3
Cladribine	Acyclovir	GUK1;PNP, RRM2;GUK1, RRM1;GUK1	9.33	0	3
Cefradine	Methotrexate	SLC22A5;DHFR, SLC22A5;TYMS, SLC15A2;SLC15A1	5	0.66	3
Benzylpenicillin	Methotrexate	SLC22A5;DHFR, SLC22A5;TYMS, SLC15A2;SLC15A1	5	0.66	3
Cefalotin	Methotrexate	SLC22A5;DHFR, SLC22A5;TYMS, SLC15A2;SLC15A1	5	0.66	3
Ampicillin	Methotrexate	SLC22A5;DHFR, SLC22A5;TYMS, SLC15A2;SLC15A1	5	0.66	3
Ceftazidime	Methotrexate	SLC22A5;DHFR, SLC22A5;TYMS, SLC15A2;SLC15A1	5	0.66	3
Cefdinir	Methotrexate	SLC22A5;DHFR, SLC22A5;TYMS, SLC15A2;SLC15A1	5	0.66	3
Cefepime	Methotrexate	SLC22A5;DHFR, SLC22A5;TYMS, SLC15A2;SLC15A1	5	0.66	3
Cefixime	Methotrexate	SLC22A5;DHFR, SLC22A5;TYMS, SLC15A2;SLC15A1	5	0.66	3
Cyclacillin	Methotrexate	SLC22A5;DHFR, SLC22A5;TYMS, SLC15A2;SLC15A1	5	0.66	3
Cephalexin	Methotrexate	SLC22A5;DHFR, SLC22A5;TYMS, SLC15A2;SLC15A1	5	0.66	3
Azathioprine	Fluorouracil	TYMS;SLC29A2, PPAT;HPRT1, SLC29A2;SLC29A1	2.66	0.66	3
Mercaptopurine	Fluorouracil	TYMS;SLC29A2, PPAT;HPRT1, SLC29A2;SLC29A1	2.66	0.66	3
Methotrexate	Azathioprine	TYMS;SLC29A2, DHFR;SLC29A2, ATIC;HPRT1	2.33	0	3
Methotrexate	Mercaptopurine	TYMS;SLC29A2, DHFR;SLC29A2, ATIC;HPRT1	2.33	0	3
Imexon	Valaciclovir	RRM2;GUK1, RRM1;GUK1	10	0	2
Imexon	Acyclovir	RRM2;GUK1, RRM1;GUK1	10	0	2
Tioguanine	Pemetrexed	HPRT1;GART, ATIC;HPRT1	5	0	2
DB01632	Pemetrexed	HPRT1;GART, ATIC;HPRT1	5	0	2
Azathioprine	Mercaptopurine	PPAT;HPRT1, SLC29A2;SLC29A1	3.5	1	2
Mercaptopurine	Mercaptopurine	PPAT;HPRT1, SLC29A2;SLC29A1	3.5	1	2

Human calcitonin	Cystine	ANPEP;SLC3A1, ANPEP;SLC7A9	3	1	2
Lamivudine	Choline salicylate	CMPK1;SLC22A5, CMPK1;PLD2	3	1	2
Lamivudine	Choline	CMPK1;SLC22A5, CMPK1;PLD2	3	1	2
Icatibant	Cystine	ANPEP;SLC3A1, ANPEP;SLC7A9	3	1	2
Gemcitabine	Choline salicylate	CMPK1;SLC22A5, CMPK1;PLD2	3	1	2
Gemcitabine	Choline	CMPK1;SLC22A5, CMPK1;PLD2	3	1	2
Ezetimibe	Cystine	ANPEP;SLC3A1, ANPEP;SLC7A9	3	1	2
Sofosbuvir	Choline	CMPK1;SLC22A5, CMPK1;PLD2	3	1	2
Sofosbuvir	Choline salicylate	CMPK1;SLC22A5, CMPK1;PLD2	3	1	2
Gemcitabine	Phosphatidyl serine	SLC29A2;PTDSS1, CMPK1;PTDSS1	2.5	2	2
Taurocholic acid	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Nateglinide	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Lamivudine	Phosphatidyl serine	CMPK1;PTDSS1	3	2	1
Quercetin	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Probenecid	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Aminohippuric acid	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Acetic acid	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Lactic acid	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Benzoic acid	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Ampicillin	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Salicylic acid	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Arbaclofen Placarbil	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Methotrexate	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Valproic acid	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Foscarnet	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
Sofosbuvir	Phosphatidyl serine	CMPK1;PTDSS1	3	2	1
Pravastatin	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1
gamma-Hydroxybutyric acid	Phosphatidyl serine	SLC16A1;PTDSS1	3	3	1