| SLC genes and pseudogenes | Positive controls |
|---------------------------|-------------------|
| SLC1A1 | SLC6A1 | SLC9A7 | SLC16A10 | SLC22A12 | SLC25A26 | SLC27A6 | SLC35F2 | SLC41A1 | CEP85 |
| SLC1A2 | SLC6A2 | SLC9A8 | SLC16A11 | SLC22A13 | SLC25A27 | SLC28A1 | SLC35F3 | SLC41A2 | CTCF |
| SLC1A3 | SLC6A3 | SLC9A9 | SLC16A12 | SLC22A14 | SLC25A28 | SLC28A2 | SLC35F4 | SLC41A3 | CTNNB1 |
| SLC1A4 | SLC6A4 | SLC9B1 | SLC16A13 | SLC22A15 | SLC25A29 | SLC28A3 | SLC35F5 | SLC41A4 | DYSK1A |
| SLC1A5 | SLC6A5 | SLC9B2 | SLC16A14 | SLC22A16 | SLC25A30 | SLC29A1 | SLC35F6 | SLC41A5 | KANSL1 |
| SLC1A6 | SLC6A6 | SLC9C1 | SLC17A1 | SLC22A17 | SLC25A31 | SLC29A2 | SLC35G1 | SLC43A1 | MED26 |
| SLC1A7 | SLC6A7 | SLC9C2 | SLC17A2 | SLC22A18 | SLC25A32 | SLC29A3 | SLC35G2 | SLC43A2 | NBAS |
| SLC1A8 | SLC6A8 | SLC10A1 | SLC17A3 | SLC22A20 | SLC25A33 | SLC29A4 | SLC35G3 | SLC43A3 | NFYC |
| SLC1A9 | SLC6A9 | SLC10A2 | SLC17A4 | SLC22A23 | SLC25A34 | SLC30A1 | SLC35G4 | SLC43A4 | NIPBL |
| SLC1A10 | SLC6A11 | SLC10A3 | SLC17A5 | SLC22A24 | SLC25A35 | SLC30A2 | SLC35G5 | SLC44A1 | NUP153 |
| SLC1A31 | SLC6A12 | SLC10A4 | SLC17A6 | SLC22A25 | SLC25A36 | SLC30A3 | SLC35G6 | SLC44A2 | PFAH1B1 |
| SLC1A12 | SLC6A13 | SLC10A5 | SLC17A7 | SLC22A31 | SLC25A37 | SLC30A4 | SLC36A1 | SLC44A3 | RCC1 |
| SLC1A13 | SLC6A14 | SLC10A6 | SLC17A8 | SLC23A1 | SLC25A38 | SLC30A5 | SLC36A2 | SLC44A4 | RPL13A |
| SLC1A14 | SLC6A15 | SLC10A7 | SLC17A9 | SLC23A2 | SLC25A39 | SLC30A6 | SLC36A3 | SLC44A5 | RPTOR |
| SLC1A15 | SLC6A16 | SLC11A1 | SLC18A1 | SLC23A3 | SLC25A40 | SLC30A7 | SLC36A4 | SLC45A1 | SAEB1 |
| SLC1A16 | SLC6A17 | SLC11A2 | SLC18A2 | SLC24A1 | SLC25A41 | SLC30A8 | SLC37A1 | SLC45A2 | SRBD1 |
| SLC1A17 | SLC6A18 | SLC12A1 | SLC18A3 | SLC24A2 | SLC25A42 | SLC30A9 | SLC37A2 | SLC45A3 | U2AF2 |
| SLC1A18 | SLC6A19 | SLC12A2 | SLC18B1 | SLC24A3 | SLC25A43 | SLC30A10 | SLC37A3 | SLC45A4 | URI1 |
| SLC1A19 | SLC6A20 | SLC12A3 | SLC19A1 | SLC24A4 | SLC25A44 | SLC31A1 | SLC37A4 | SLC46A1 | VMP1 |
| SLC1A20 | SLC6A21 | SLC12A4 | SLC19A2 | SLC24A5 | SLC25A45 | SLC31A2 | SLC38A1 | SLC46A2 | VPS13D |
| SLC1A21 | SLC7A1 | SLC12A5 | SLC19A3 | SLC25A46 | SLC32A1 | SLC38A2 | SLC46A3 | SLC47A1 | |
| SLC1A22 | SLC7A2 | SLC12A5 | SLC19A4 | SLC25A47 | SLC33A1 | SLC38A3 | SLC47A2 | SLC47A3 | |
| SLC1A23 | SLC7A3 | SLC12A6 | SLC20A1 | SLC47A4 | SLC34A1 | SLC38A4 | SLC47A4 | SLC48A1 | |
| SLC1A24 | SLC7A4 | SLC12A7 | SLC20A2 | SLC25A5 | SLC34A2 | SLC38A5 | SLC48A2 | SLC48A3 | |
| SLC1A25 | SLC7A5 | SLC12A8 | SLC20A3 | SLC25A6 | SLC34A3 | SLC38A6 | SLC48A4 | SLC48A5 | |
| SLC1A26 | SLC7A6P1 | SLC12A9 | SLC20A4 | SLC25A7 | SLC35A1 | SLC38A7 | SLC48A6 | SLC50A1 | |
| SLC1A27 | SLC7A7 | SLC12A10 | SLC20A5 | SLC25A8 | SLC35A2 | SLC38A8 | SLC48A7 | SLC50A2 | |
| SLC1A28 | SLC7A8 | SLC12A11 | SLC20A6 | SLC25A9 | SLC35A3 | SLC38A9 | SLC48A8 | SLC50A3 | |
| SLC1A29 | SLC7A9 | SLC12A12 | SLC20A7 | SLC25A10 | SLC38A10 | SLC50A4 | SLC48A9 | SLC50A5 | |
| SLC1A30 | SLC7A10 | SLC14A1 | SLC20A8 | SLC25A11 | SLC38A11 | SLC50A6 | SLC48A10 | SLC50A7 | |
| SLC1A31 | SLC7A11 | SLC14A2 | SLC20A11 | SLC25A12 | SLC38A12 | SLC50A8 | SLC48A11 | SLC50A9 | |
| SLC1A32 | SLC7A12 | SLC14A3 | SLC20A12 | SLC25A13 | SLC38A13 | SLC50A10 | SLC50A10 | |
| SLC1A33 | SLC7A13 | SLC14A4 | SLC20A13 | SLC25A14 | SLC38A14 | SLC50A11 | SLC50A11 | |
| SLC1A34 | SLC7A14 | SLC14A5 | SLC20A14 | SLC25A15 | SLC38A12 | SLC50A12 | SLC50A12 | |
| SLC1A35 | SLC7A15 | SLC14A6 | SLC20A15 | SLC25A16 | SLC38A13 | SLC50A13 | SLC50A13 | |
| SLC1A36 | SLC7A16 | SLC14A7 | SLC20A16 | SLC25A17 | SLC38A14 | SLC50A14 | SLC50A14 | |
| SLC1A37 | SLC7A17 | SLC14A8 | SLC20A17 | SLC25A18 | SLC38A15 | SLC50A15 | SLC50A15 | |
**Supplementary Table 2.** Screened compounds.

| Class                  | Subclass                  | Name                   | Status*  | IC50 in HAP1 cells (µM) |
|------------------------|---------------------------|------------------------|----------|-------------------------|
| antineoplastic         | purine analogs           | 6-mercaptopurine       | A        | 3,176                   |
|                        | nucleoside analogs       | 5-azacitidine          | A        | 11,07                   |
|                        |                           | clofarabine            | A        | 0,1129                  |
|                        |                           | cytarabine             | A        | 0,3032                  |
|                        |                           | decitabine             | A        | 0,7                     |
|                        |                           | gemcitabine            | A        | 0,0075                  |
|                        | antifolates              | methotrexate           | A        | 0,1                     |
|                        |                           | pralatrexate           | A        | 8,3                     |
|                        |                           | raltitrexed            | A        | 0,03902                 |
|                        | HDAC inhibitors          | belinostat             | A        | 0,3215                  |
|                        |                           | chidamide (tucidinostat)| I        | 3,39                    |
|                        |                           | entinostat             | I        | 1,95                    |
|                        |                           | panobinostat           | A        | 0,01022                 |
|                        |                           | pracinostat            | I        | 0,3094                  |
|                        |                           | resminostat            | I        | 2,478                   |
|                        |                           | romidepsin             | A        | 10,5                    |
|                        |                           | vorinostat             | A        | 2,894                   |
|                        | microtubule inhibitors   | vinblastine            | A        | 9,8                     |
|                        | (destabilizing)          | vincristine            | A        | 28                      |
|                        |                           | vindesine              | A        | 0,00543                 |
|                        |                           | vinorelbine            | A        | 0,1012                  |
|                        | microtubule inhibitors   | docetaxel              | A        | 0,02                    |
|                        | (stabilizing)            | paclitaxel             | A        | 6,3                     |
|                        | proteasome inhibitors    | bortezomib             | A        | 0,00649                 |
|                        |                           | carfilzomib            | A        | 3,6                     |
|                        | RTK inhibitors           | crizotinib             | A        | 3,6                     |
|                        |                           | ponatinib              | A        | 0,46                    |
|                        |                           | sunitinib              | A        | 2,891                   |
|                        | topoisomerase I inhibitors| topotecan              | A        | 0,006                   |
|                        |                           | irinotecan             | A        | 0,7631                  |

*based on DrugBank: A (approved), A, W (approved, withdrawn), I (investigational), E (experimental)
**Supplementary Table 2 (cont).** Screened compounds.

| Class                  | Subclass                             | Name                                | Status* | IC50 in HAP1 cells (µM) |
|------------------------|--------------------------------------|-------------------------------------|---------|------------------------|
| antineoplastic         | topoisomerase II inhibitors          | doxorubicin                         | A       | 0,007                  |
|                        |                                     | epirubicin                          | A       | 0,017                  |
|                        |                                     | etoposide                           | A       | 0,238                  |
|                        |                                     | idarubicin                          | A       | 0,022                  |
|                        |                                     | mitoxantrone                        | A       | 0,007                  |
|                        | protein translation inhibitors       | homoharringtonine (omacetaxine mepesuccinate) | A       | 0,025                  |
|                        | transcription inhibitors              | dactinomycin                        | A       | 0,039                  |
|                        | alkylation                           | cisplatin                           | A       | 1                      |
|                        |                                     | methyl methanesulfonate             | A       | 24                     |
|                        |                                     | mitomycin C                         | A       | 15                     |
|                        |                                     | temozolomide                        | A       | 5                      |
|                        | other                                | triptolide                          | I       | 0,005                  |
| antiparasitic          | antimalarial                         | artesunate                          | A       | 1,786                  |
|                        |                                     | dihydroartemisinin (artemimol)      | I       | 6,3                    |
|                        |                                     | mefloquine                          | A       | 7,914                  |
|                        | antihelmintic                        | albendazole                         | A       | 0,452                  |
|                        | antiprotozoal                        | pentamidine                         | A       | 6,206                  |
| antiarrhythmic         | type III: K-channel blocker          | amiodarone                          | A       | ~10                    |
|                        |                                     | dronedarone                         | A       | 6,3                    |
|                        | type V                               | digitoxin                           | A       | 0,001                  |
| antihypertensive       | Ca-channel blocker                   | nisoldipine                         | A       | 9,8                    |
| anti-inflammatory      | NSAID                                | oxyphenbutazone                     | A, W    | ~10                    |
| immunosuppressant      | HMG-CoA reductase inhibitor          | cerivastatin                        | A, W    | 0,15                   |
| hypolipidemic          |                                     | chlorzoxazone                       | A       | ~10                    |
|                        |                                     | metaxalone                          | A       | ~10                    |
| antipasmodic           | prokinetic                           | serotonin agonist                   | A, W    | 5,856                  |
| mineralocorticoid      |                                     | desoxycorticosterone pivalate       | E       | ~10                    |
| uricosuric             |                                     | sulfipyrazone                       | A       | 10                     |
| alcohol deterrent      |                                     | disulfiram                          | A       | 28                     |

*based on DrugBank: A (approved), A, W (approved, withdrawn), I (investigational), E (experimental)
| Gene   | Drug                  | Concentration (IC50) |
|--------|-----------------------|----------------------|
| SLC1A5 | Mitoxantrone          | 3                    |
|        | Vinorelbine           | 3                    |
|        | Homoharringtonine     | 1                    |
|        | Panobinostat          | 3                    |
|        | Entinostat            | 3                    |
| SLC1A2 | Artesunate            | 3                    |
|        | Dihydroartemisinin    | 3                    |
| SLC1A1 | Artesunate            | 3                    |
|        | Dihydroartemisinin    | 3                    |
|        | Nisoldipine           | 3                    |
| SLC1A2 | Pralatraxate          | 3                    |
|        | Raltitrexed           | 3                    |
|        | Pentamidine           | 3                    |
|        | Methotrexate          | 3                    |
| SLC2A1 | Pentamidine           | 3                    |
|        | Methotrexate          | 3                    |
| SLC2A3 | Decitabine            | 3                    |
|        | Cytarabine            | 1                    |
| MTCH2  | Decitabine            | 3                    |
|        | Cytarabine            | 3                    |
|        | Nisoldipine           | 3                    |
|        | Belinostat            | 3                    |
| SLC2A5 | Gemcitabine           | 1                    |
|        | Topotecan             | 1                    |
|        | Decitabine            | 3                    |
|        | Cytarabine            | 3                    |
|        | 5-azacytidine         | 3                    |
| SLC3A1 | Sulfinpyrazone        | 3                    |
|        | Digitoxin             | 1                    |
| SLC3A2 | Cisplatin             | 1                    |
|        | 5-azacytidine         | 1                    |
| SLC3A5 | Cisplatin             | 1                    |
| SLC4A1 | Mitoxantrone          | 3                    |
| SLC4A2 | Mitoxantrone          | 3                    |
Supplementary Table 4. sgRNAs used to generate MCA cell lines and corresponding editing efficiencies as assessed by Tide-seq.

| Gene   | KO | %editing | sgRNA                      |
|--------|----|----------|----------------------------|
| SLC1A5 | 1  | 83,8     | GCCGCTGATGATGAAGTGCG       |
|        | 2  | 67,8     | CAGCGCCACACCAAGACGAG       |
| SLC11A2| 1  | 39,1     | ATCAGCCACAGTGACTCG         |
|        | 2  | 52,1     | ATGAGAAGGCCACCCACAG        |
| SLC16A1| 1  | 54       | ACAGAGTATAGTGTGCTGA        |
|        | 2  | 42,8     | TATCCATGACACTCGTGG         |
| SLC19A1| 1  | 40,3     | GGCACGACAAGACTTCACG        |
|        | 2  | 42,8     | CGACTACCTGGCCTACGACG       |
| SLC20A1| 1  | 51,1     | TTGGCAGGAAATGACTCCAG       |
|        | 2  | 42,4     | CAGGCCGAATCTTATGCA         |
| SLC25A3| 1  | NA       | TCAAACAGTGCTCAGTAAAGC      |
|        | 2  | 57       | TCTGACCTGACCTCCAGG         |
| MTCH2  | 1  | 35,5     | ACATTGCAATATCTATCGG        |
|        | 2  | 10,7     | AGCAGTTTCAGTACGAG          |
| SLC29A1| 1  | 49,2     | GCTCAAGCTGAGGACCACG        |
|        | 2  | 40,7     | GCTCAAGCTGAGGACCACG        |
| SLC35A1| 1  | 32,6     | TGAACGCTACATAACCGA         |
|        | 2  | 19,2     | ACACGGATCTTCAAACGGT         |
| SLC35A2| 1  | 29,6     | TAGAGATGGCCACATACCCTGA     |
|        | 2  | 13,5     | CTACGCCCAGCTGGCCCA         |
| SLC38A5| 1  | 44,5     | CTATGCCATGGCCACACG         |
|        | 2  | 32,4     | TATCGCCACC TTCCCTGACA      |
| SLC47A1| 1  | 17,7     | AGCCAGACCTGAAGCAGT         |
|        | 2  | 42,9     | GCAACTCCAGTTACGATCTG       |
| SLC47A2| 1  | 36,5     | GGCATCGGTAGCCCTCGG         |
|        | 2  | 33,4     | GCTGGCATCG GTGACCCTCG      |
**Supplementary Table 5.** Descriptors used in the chemical space analysis

| Descriptor name            | Description                                                                 |
|----------------------------|-----------------------------------------------------------------------------|
| SlogP                      | Smarts LogP, Octanol Water Partition Coefficient                           |
| LabuteASA                  | Labute’s Approximate Surface Area, approximated surface area of a molecule (J. Mol. Graph. Mod. 18, 464-77 (2000)) |
| TPSA                       | Total Polar surface area                                                    |
| ExactMW                    | Molecular weight                                                           |
| NumRotatableBonds          | Number of rotatable bonds                                                  |
| NumHBD                     | Number of hydrogen bond donors                                             |
| NumHBA                     | Number of hydrogen bond acceptors                                          |
| NumAmideBonds              | Number of amide bonds                                                      |
| NumHeteroAtoms             | Number of hetero atoms                                                     |
| NumHeavyAtoms              | Number of heavy atoms                                                      |
| NumAtoms                   | Number of atoms                                                            |
| NumRings                   | Number of rings                                                            |
| NumAromaticRings           | Number of aromatic rings                                                   |
| NumSaturatedRings          | Number of saturated rings                                                  |
| NumAliphaticRings          | Number of aliphatic rings                                                  |
| NumAromaticHeterocycles    | Number of aromatic heterocycles                                            |
| NumSaturatedHeterocycles   | Number of saturated heterocycles                                           |
| NumAliphaticHeterocycles   | Number of aliphatic heterocycles                                           |
| NumAromaticCarbocycles     | Number of aromatic carbocycles                                             |
| NumSaturatedCarbocycles    | Number of saturated carbocycles                                            |
| NumAliphaticCarbocycles    | Number of aliphatic carbocycles                                            |
| FractionCSP3               | Fraction of sp3 hybridized Carbons                                         |
Supplementary Figure 1: a. Violin plots of sgRNA count distributions in the SLC library plasmid samples (left-hand two plots) and in the 9 days post-infection (p.i., two right-hand plots) samples (n=2). b. Volcano plot (FDR vs. log2 fold change) for the differential representation of sgRNAs in samples collected 9 days p.i. vs. the plasmid library. FDRs correspond to a two-tailed Wald test (DESeq2). sgRNA corresponding to the set of 20 essential control genes are shown in green (n=2). c. Same as b., but in this case sgRNAs corresponding to the set of 120 non-target negative control sequences are shown in red (n=2). d. Gene-level enrichment in 9 days p.i. vs. plasmid library (n=2) determined by DESeq2 and GSEA analysis. e. YM155 benchmarking screen. Read counts for the samples at day0, DMSO-treated and YM155-treated samples are shown (n=1). f. Circular plot showing SLCs expressed in HAP1 cells according to RNAseq data from Brockmann et al.60 SLC families are indicated in the inner circle while transcript expression level (log2 counts per 10^7 reads) is shown as blue bars. SLCs with an expression level above 9 are labeled.
Supplementary Figure 2 - continued

C  

aLFC distribution for all (black), FDR=<10 (red), FDR=<1 (green)

\[ \text{aLFC} \]

\[ \text{density} \]

\[ \text{aLFC} \]

\[ \text{expression level} \]

\[ \text{HAP1 wt lines} \]

D  

\[ \text{aLFC} \rightarrow 0.5 \]

\[ \text{All} \]

\[ \text{HAP1 wt lines} \]

E  

\[ \text{HAP1 wt lines} \]

\[ \text{expression level} \]

\[ \text{HAP1 wt lines} \]

F  

Localizations:
- ER
- Golgi
- Lysosome
- Mitochondria
- Peroxisome
- PM
- PM, Endosome, Mitochondria
- PM, Golgi
- PM, Lysosome
- Unknown
**Supplementary Figure 2:** a. Overview of significantly enriched SLCs (FDR≤1%) identified for drug treatments at different concentrations. SLC genes are ordered by name, and treatments are ordered by hierarchical clustering based on the gene-level results (n=2). b. Overview of significantly enriched SLCs (FDR≤1%) identified upon treatment with different compounds. Significant enrichments for all different doses of the same compound are merged together in order to ease interpretation (union), always selecting the most significant value for repeats. x- and y-axis dendrograms display the hierarchical clustering of SLCs and treatments, respectively, calculated using the complete-linkage method with Euclidean distances based on gene-level adjusted p-values. c. Distribution of average log2(fold change) values for all SLC-drug associations (black line), associations with FDR≤10% (red line) and FDR≤1% (green line). d. Plot showing the number of SLCs associated to the tested drugs at average log2(fold change) above 0.5 (left panel) and for all associations at FDR≤1%. e. Expression levels (log2 counts per 10^7 reads) in HAP1 cells for SLCs significantly enriched in our screen. f. Localization of the SLCs significantly enriched in our screen. Data was assembled from the UniProt and Compartments databases followed by manual curation and annotation.
Supplementary Figure 3

(a)

(b)

| Gene   | Day 3 | Day 10 |
|--------|-------|--------|
| SLC1A5 |       |        |
|        |       |        |
|        |       |        |
| MTCH2  |       |        |
|        |       |        |
|        |       |        |
| SLC47A2|       |        |
|        |       |        |
|        |       |        |
**Supplementary Figure 3:** a. Example of the gating scheme used for the MCA assay. For this experiment, Hap1-Cas9 cells infected with lentiviral particles carrying sgRNAs targeting Renilla luciferase and either eGFP or mCherry fluorescent markers were mixed at 1:1 ratio and the relative abundance of the two populations assessed by FACS. b. Validation of selected SLC/drug associations by MCA. Results are shown by gene tested, pooling data of 2-5 independent experiments (biological replicates) each performed in technical triplicates on two separate KO cell lines. Ratios of GFP+/mCherry+ populations normalized to day0 ratios are shown for the indicated SLC/drug combinations at the given timepoints, with different point shapes corresponding to separate biological replicates. Bars correspond to mean of all samples shown for a specific KO cell line. Statistical significance was calculated by ANOVA using biological replicates followed by Dunnett’s test. n.a. denotes cases where no live cells were measured. Compounds tested: NIS: Nisoldipine, PEN: Pentamidine, DAC: Decitabine, ARA-C: Cytarabine, BEL: Belinostat, MIT: Mitoxantrone, NVB: Vinorelbine, HHT: Homoharringtonine, ENT: Entinostat, PAN: Panobinostat, DGT: Digitoxin, SPZ: Sulfinpyrazone.
Supplementary Figure 4

(a) Log2 Fold Change

(b) Log2 Fold Change
**Supplementary Figure 4: a-b.** Heatmaps showing the effect of 24h drug treatment on SLC expression in WT and SLC KO cells. Differentially expressed SLC genes are shown for each of the contrasts indicated on the x-axis. \( \triangle \)SLC16A1 refers to clone \( \triangle \)SLC16A1_2; CDDP cisplatin (n=1). **c-h.** Enrichment analysis for transcription factor target genes within the contrasts indicated above each panel. Transcription factor targets enrichment analysis was performed using Fisher’s exact test and p-values were corrected for multiple testing using the Benjamini-Hochberg procedure (FDR).
Supplementary Figure 5

(a) Plot of the first two dimensions of the PCA

(b) Plot of the first two dimensions of the PCA

(c) Plot of the first two dimensions of the PCA
**Supplementary Figure 5:** a. Principal component analysis of compounds in the DrugBank set of reference as well as in the sets tested in this study based on 22 annotated 2D chemical descriptors. Compounds with a molecular weight below 900 Da (defined as “small molecule” by DrugBank) are shown as circles, the remaining compounds as crosses. The total data set size was n=9597 compounds, Drugbank n=8774, Toxic n=257, Screen n=58, 2k library n=1562. b. Principal component analysis of compounds in the DrugBank set of reference compared the SLC-associated (active) and non-SLC-associated (inactive) compounds based on 22 annotated 2D chemical descriptors. Drugbank and 2k library: n= 9539, active n=47, inactive n=11. c. Principal component analysis of compounds in the SLC-associated (active) and non-SLC-associated (inactive) sets. Active n=47, inactive n=11.
Supplementary Figure 6: Plots of individual descriptor values across the four compound sets. The total data set size was n=9597 compounds, Drugbank n=8774, Toxic n=257, Screen n=58, 2k library n=1562. Boxplots show median as bold line, mean as a triangle. The upper hinge represents the 75th percentile and the lower hinge represents the 25th percentile. The whiskers represent the 1.5 inter quartile range or the highest or lowest value within the inter quartile range. Outliers (compounds higher or lower than IQR) are represented by dots.

Supplementary Dataset 1: Table with p and FDR values used to generate Figure 2c.

Supplementary Dataset 2: Table with ratios and p values used to generate Figure 3b and Supplemental Figure 3b.