Relative cell viability (%) vs. Docetaxel (nM)

Relative cell viability (%) vs. Pacitaxel (nM)

Relative cell viability (%) vs. Vincristine (nM)

Relative cell viability (%) vs. Vinorelbine (nM)

Relative cell viability (%) vs. Gemcitabine (nM)

Relative cell viability (%) vs. Cytarabine (µM)

Relative cell viability (%) vs. Cladribine (µM)

Relative cell viability (%) vs. Mercaptopurine (µM)

Relative cell viability (%) vs. Thioguanine (µM)

Relative cell viability (%) vs. Cisplatin (µM)

Relative cell viability (%) vs. Oxaliplatin (µM)

Fig. S1
HAP1 pLCV2 1936 genes
HAP1 GeCKO 376 genes (FDR<0.1)
HAP1 pLCKO 1593 genes

445 31 103
1224 236 6
127

Ribosome biosynthesis
Pyrimidine metabolism
Spliceosome
Cell cycle
Proteasome
DNA replication
Ribosome biogenesis in eukaryotes

Pathway
Enrichment score [-log_{10}(p-value)]

Fig. S2
### Table: Drug Targets and Gene Hits

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Drug Target</th>
<th>Gene Hit</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camptothecin</td>
<td>Topoisomerase I</td>
<td>TOP1</td>
<td>1</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>Topoisomerase I</td>
<td>TOP1</td>
<td>1</td>
</tr>
<tr>
<td>Topotecan</td>
<td>Topoisomerase I</td>
<td>TOP1</td>
<td>1</td>
</tr>
<tr>
<td>Idarubicin</td>
<td>Topoisomerase II</td>
<td>TOP2A</td>
<td>1</td>
</tr>
</tbody>
</table>

### Diagrams:

- **b**: Stabilisation of "Cleavable complex" leading to DNA damage causing cell death.
- **c**: Camptothecin structure and its gene rank.
- **d**: Irinotecan structure and its gene rank.
- **e**: Topotecan structure and its gene rank.
- **f**: Idarubicin structure and its gene rank.
- **g**: Cladribine, Cytarabine, Gemcitabine with their gene hits and ranks.
- **h**: Mechanism of action for Cladribine (2-CdA) and Cytarabine (Ara-C) involving DNA synthesis inhibition and cell death.
- **i**: Cytarabine structure and its gene rank.
- **j**: Cladribine structure and its gene rank.
- **k**: Gemcitabine structure and its gene rank.
Fig. S4

Mismatch repair processing and cell death

Methotrexate (MTX)
MTX → DHFR → DNA synthesis inhibition and cell death

6-MP vs vehicle
MAGeCK p-value

Methotrexate (MTX)
MTX → DHFR

Thioguanine vs vehicle
MAGeCK p-value

Methotrexate vs vehicle
MAGeCK p-value

Mercaptopurine vs vehicle
MAGeCK p-value

Thioguanine vs vehicle
MAGeCK p-value

6-MP
Metabolism
6-meTG

6-TG
**a** Tasisulam

![Tasisulam molecule]

**b** Tasisulam

![Scatter plot showing Tasisulam vs vehicle MAGeCK p-value](scatter_plot.png)

**c** HAP1

![Graph showing Relative cell viability (%) vs Tasisulam (µM)](graph_hap1.png)

**d** HeLa

![Graph showing Relative cell viability (%) vs Tasisulam (µM)](graph_hela.png)

**e**

![Diagram showing Ubc, RBM39, Tasisulam, E2, DCAF15, RBX1, DDB1, DDA1, CUL4A/B](diagram.png)
Cladribine vs vehicle
MAGECK p-value

Vincristine vs vehicle
MAGECK p-value

Imatinib vs vehicle
MAGECK p-value

Paclitaxel vs vehicle
MAGECK p-value

Vinorelbine vs vehicle
MAGECK p-value

Genes

C1orf115

Cladribine
Vincristine
Imatinib
Paclitaxel
Vinorelbine

Fig. S6
Over-expression
Under-expression

-5
-4
-3
-2
-1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Normalised expression units

1. Curtis Breast
2. Gaedcke Colorectal
3. Landi Lung
4. Sun Brain
5. Barretina Sarcoma
6. Beroukhim Renal
7. Ghen Gastric
8. Talantov Melanoma
9. TCGA Ovarian
10. Giordano Thyroid
11. Choi Leukemia
12. Ye Head-Neck
13. Jacobuzio Donahue Pancreas 2
14. Skotheim Testis

Fig. S9
Ovarian cancer with paclitaxel treatment (I & II)

High (n=656)  
Low (n=288)

HR = 0.7257  
*p = 0.0003

Ovarian cancer with paclitaxel treatment (II)

High (n=484)  
Low (n=231)

HR = 0.7963  
*p = 0.0186
Fig. S1 Drug response curve for all the 27 screened drugs in HAP1 cells
Trypsinized HAP1 cells (7.2 x 10^4 cells; seeding density 225,000 cells/cm^2) were seeded in each well of a 96-well plate. After 24hrs, various concentrations of anti-cancer drugs were added, and the cells were incubated for an additional 72hrs. Cell viability was analyzed using a resazurin-based assay. The concentration used for screening is indicated by the blue dotted line.

Fig. S2 CRISPR dropout screening identifies HAP1 essential genes
a, Overlap of published HAP1 essential gene sets (pLCV2 and pLCKO) and our HAP1 GeCKO screen.
b, Enrichment pathway analysis of dropout screen hits based on the KEGG database.

Fig. S3 Functional resistance profiles identify known MoA
a, Top enriched genes identified from screens encoding direct therapeutic targets.
b, Schematic of MoA for inhibitors of topoisomerase I and II.
c-f, Enriched genes identified from screens for (c) camptothecin, (d) irinotecan, (e) topotecan and (f) idarubicin.
g, Top enriched genes identified from screens encoding enzyme involved in drug metabolism.
h, Schematic of MoA for nucleoside analogues- cladribine, cytarabine and gemcitabine.
i-k, Enriched genes identified from screens for (i) cytarabine, (j) cladribine and (k) gemcitabine.

Fig. S4 Functional resistance profiles reveal known MoA
a and b, Top enriched genes identified from screens encoding (a) drug transporter, and (b) a downstream mediator of drug action. FDR=0.1 (blue dotted line).
c, Top, enriched genes identified from screens for mercaptopurine (6MP; left) and thioguanine (6TG; right) related to mismatch repair machinery (MMR). FDR=0.1 (blue dotted line). Common enriched genes across two screens within the top 10 (middle), the genes involved in MMR are highlighted in yellow.

Fig. S5 Loss of DCAF15 or DDA1 leads to tasisulam resistance
a, Chemical structure of tasisulam.
b, Enriched genes identified from screens for tasisulam. FDR=0.1 (blue dotted line).
c and d, CRISPR-Cas9 targeted cells decreased their sensitivity to tasisulam in (c) HAP1 and (d) HeLa cells.
e, Schematic of the drug MoA for tasisulam.

Fig. S6 Identification of novel multi-drug resistance gene C1orf115/ RDD1
C1orf115/ RDD1 gene identified from screens including cladribine, imatinib, paclitaxel, tasisulam, vincristine and vinorelbine. FDR=0.1 (blue dotted line).
Fig. S7 Loss of RDD1 results in multiple drug resistance

a, RDD1 mRNA levels were analyzed by RT-qPCR in HAP1 RDD1 KO cells. All data represented as mean ± S.E.M (n=6). One-way ANOVA followed by Tukey’s post hoc test, **, p <0.01.

b and c, CRISPR-Cas9 targeted cells decreased their sensitivity to drugs in (c) HeLa and (d) HEK293T cells.

d, RDD1 mRNA levels were analyzed by RT-qPCR in siRNA targeting RDD1 in HAP1 cells. All data represented as mean ± S.E.M (n=3). One-way ANOVA followed by Tukey’s post hoc test, **, p <0.01.

e, siRNA targeting RDD1 cells decreased their sensitivity to drugs in HEK293T cells.

f, Overexpression of sgRNA resistant RDD1 (OE_RDD1) rescues the loss of RDD1-induced drug resistance in HEK293T cells. HEK293T control cells (sgControl) and clonal CRISPR-Cas9 targeted cells (sgRDD1_B_#1) were transiently transfected with pLenti-C-mGFP and gRNA-resistant constructs (OE_RDD1) for 72hrs and subjected to drug treatments and cell viability assays.

G, CRISPR-Cas9 RDD1 targeting HAP1 cells decreased their sensitivity to vincristine (VCR) and paclitaxel (PTX) in xenograft tumor model. Overall survival is shown. All data represented as mean ± S.E.M (n = 4-6 mice per group). P values were calculated using the log-rank (Mantel-Cox) test.

Fig. S8 CRISPR-Cas9 targeted RDD1 cells do not display broad resistance in HeLa cells

Fig. S9 RDD1 expression in human cancers

a, RDD1 expression in different cancers using oncomine database (www.oncomine.org). Cell color is determined by the best gene rank percentile for the analyses within the cell. NOTE: An analysis may be counted in more than once cancer type.

b, Oncomine database analysis of gene expression of RDD1 are significantly downregulated in different cancers (T) relative to normal tissue (N). Only the most significant representative data set in the particular cancers are shown.

Fig. S10 Low RDD1 expression is significantly associated with poor patient survival a and b,

Kaplan-Meier survival plots of patient overall survival in ovarian cancer with paclitaxel treatment in (a) second patient cohort and (b) combined cohorts using the Kaplan-Meier Plotter.