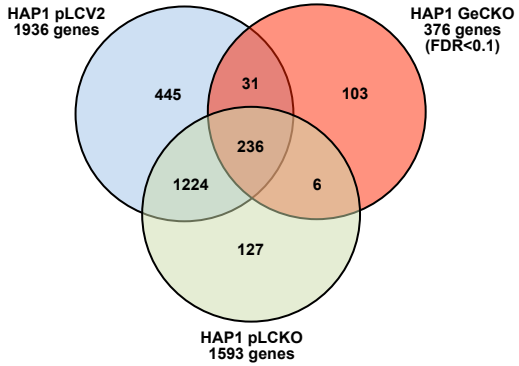
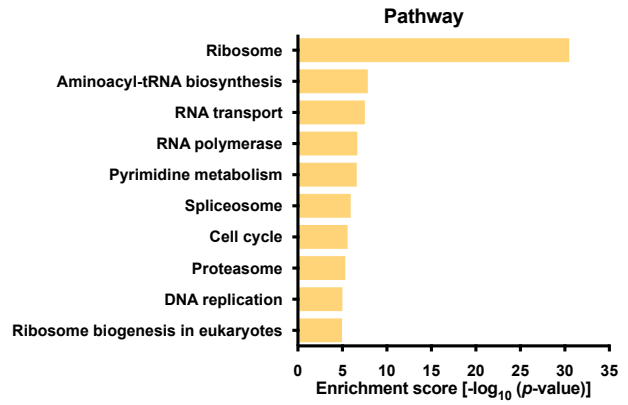
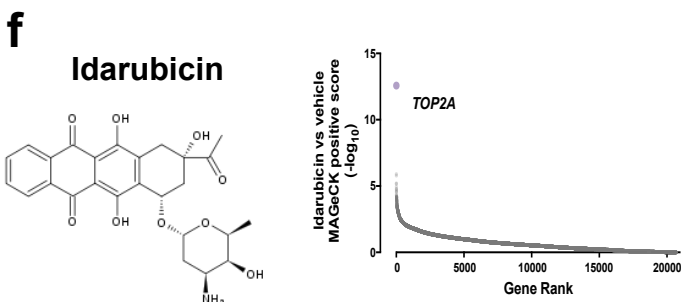
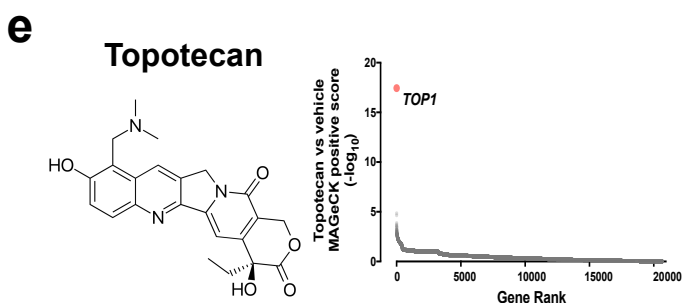
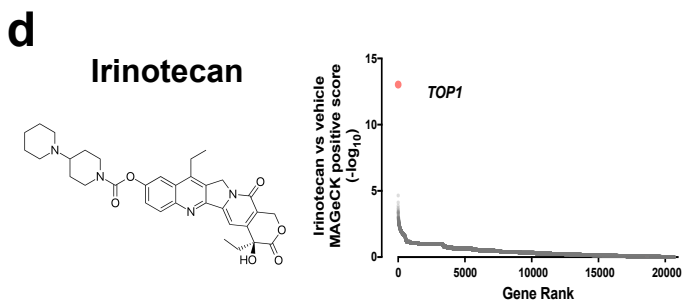
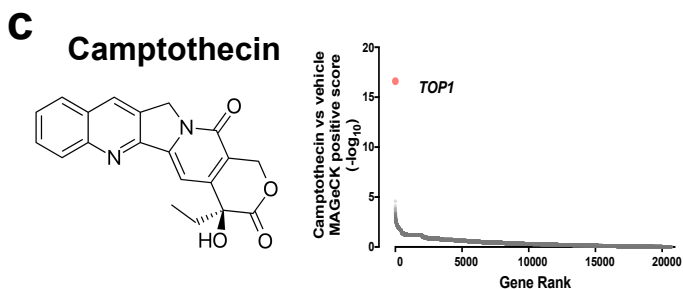
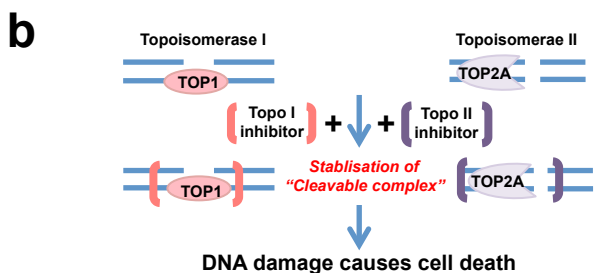


Fig. S1

**a****b**

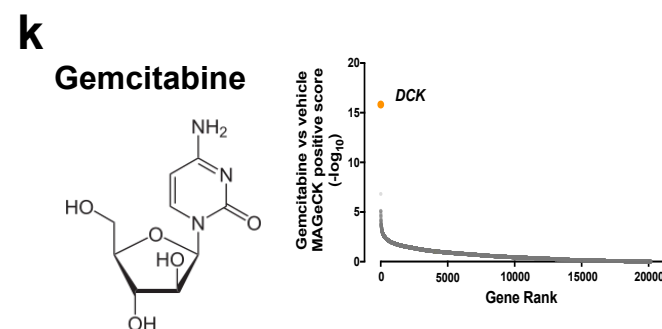
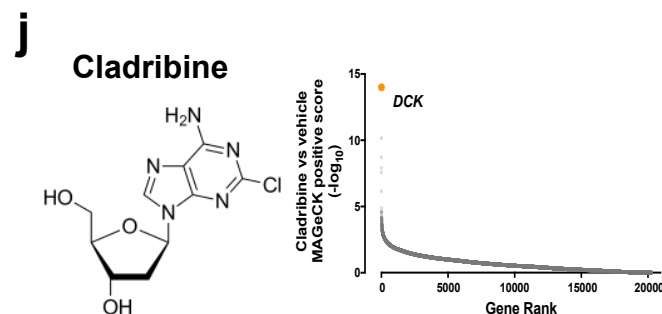
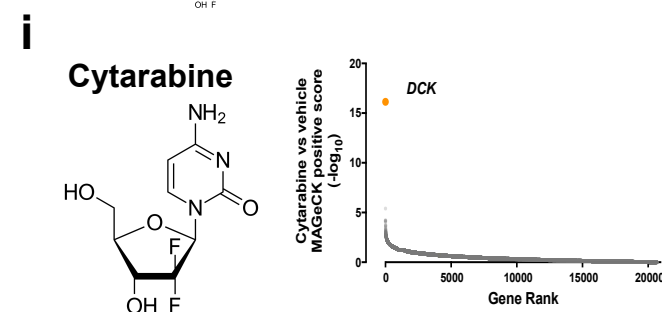
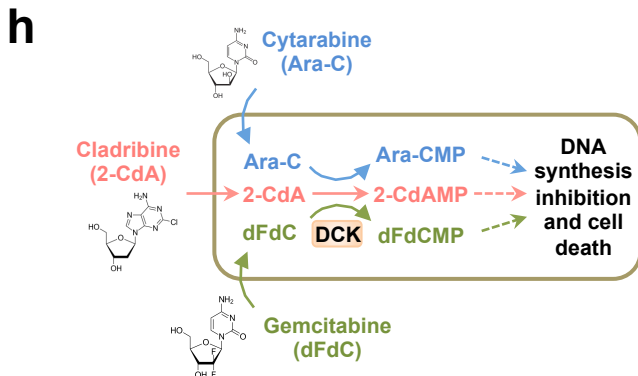
**a**

| Drug name    | Drug Target      | Gene Hit     | Rank |
|--------------|------------------|--------------|------|
| Camptothecin | Topoisomerase I  | <i>TOP1</i>  | 1    |
| Irinotecan   | Topoisomerase I  | <i>TOP1</i>  | 1    |
| Topotecan    | Topoisomerase I  | <i>TOP1</i>  | 1    |
| Idarubicin   | Topoisomerase II | <i>TOP2A</i> | 1    |



**g**

| Drug name   | Drug Target   | Gene Hit   | Rank |
|-------------|---------------|------------|------|
| Cladribine  | DNA synthesis | <i>DCK</i> | 1    |
| Cytarabine  | DNA synthesis | <i>DCK</i> | 1    |
| Gemcitabine | DNA synthesis | <i>DCK</i> | 1    |



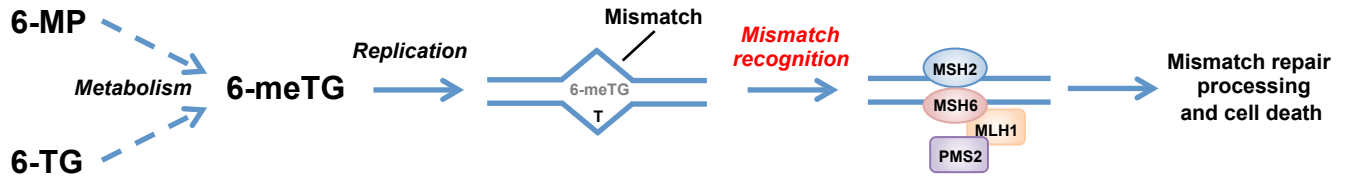
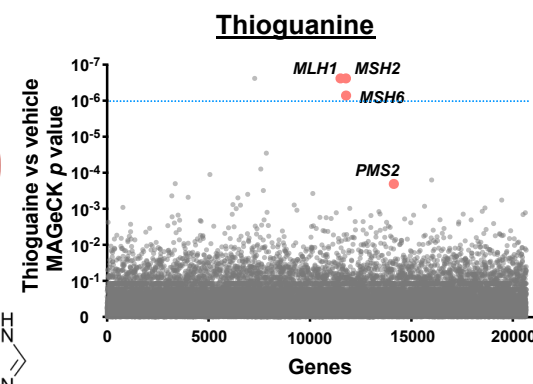
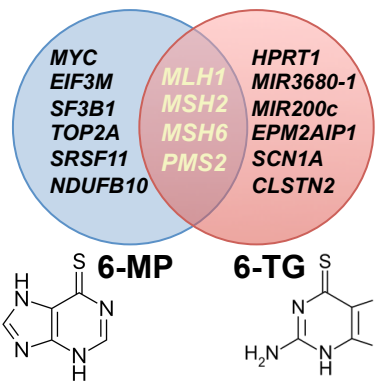
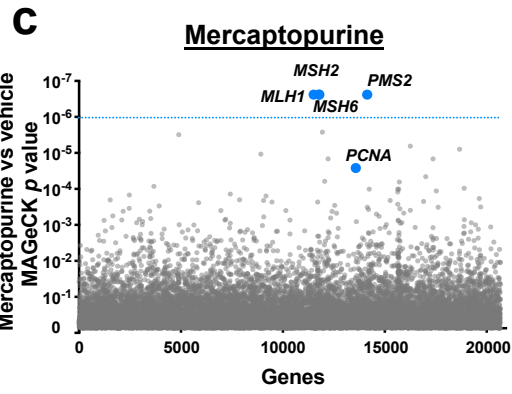
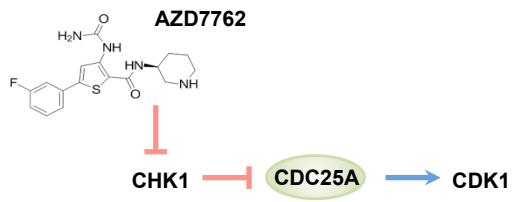
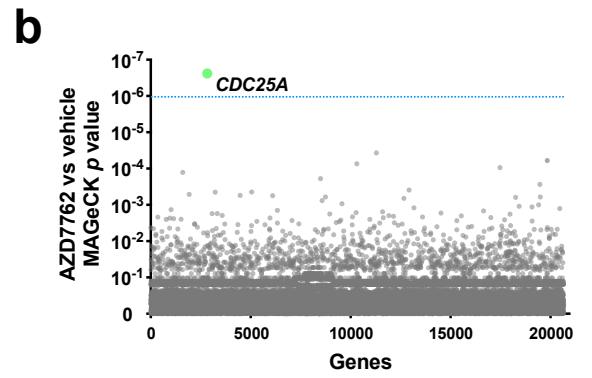
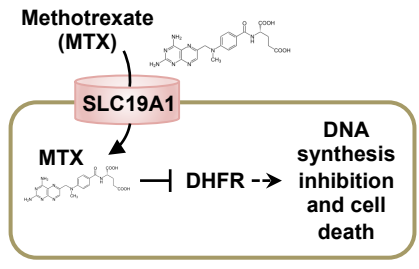
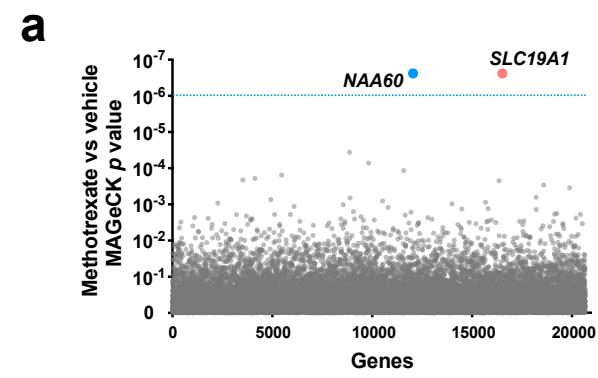
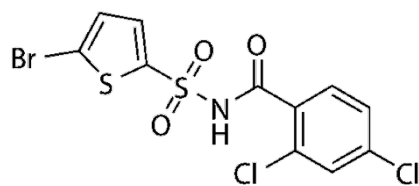
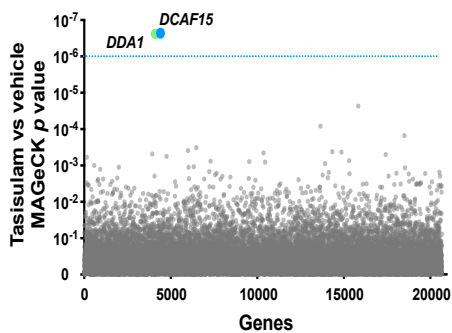
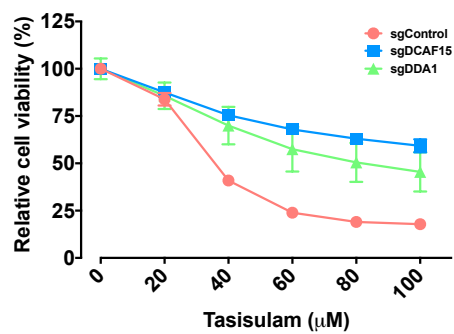
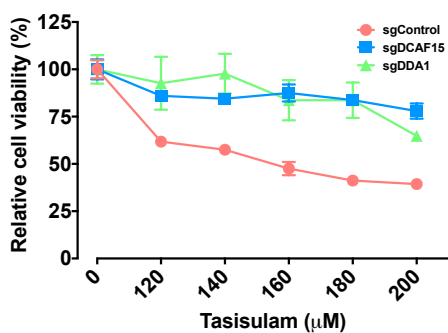
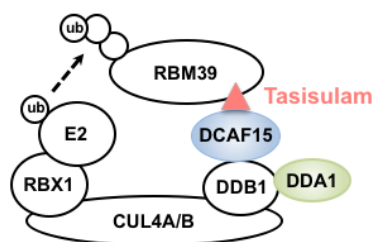
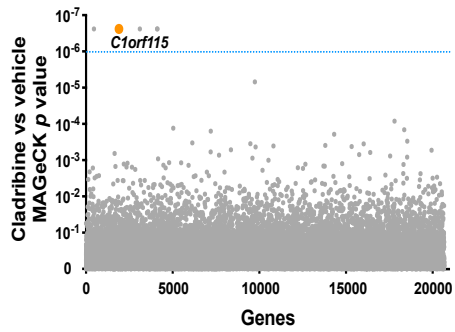


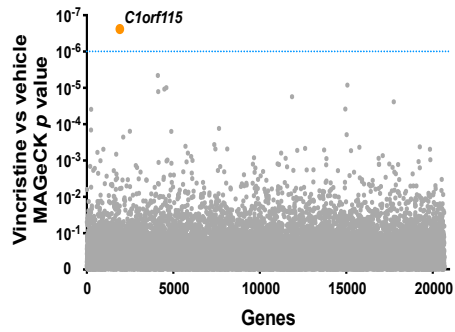
Fig. S4

**a****Tasisulam****b****Tasisulam****c****HAP1****d****HeLa****e**

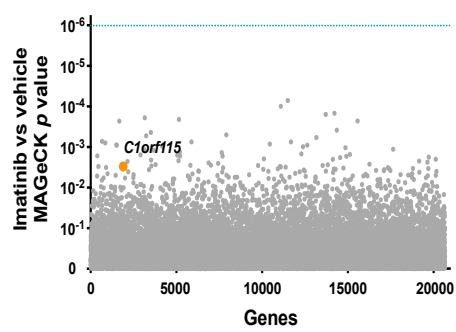
### Cladribine



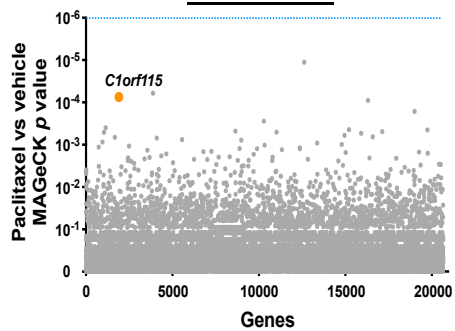
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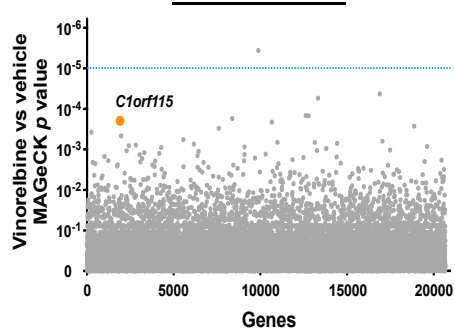
### Imatinib



### Paclitaxel



### Vinorelbine



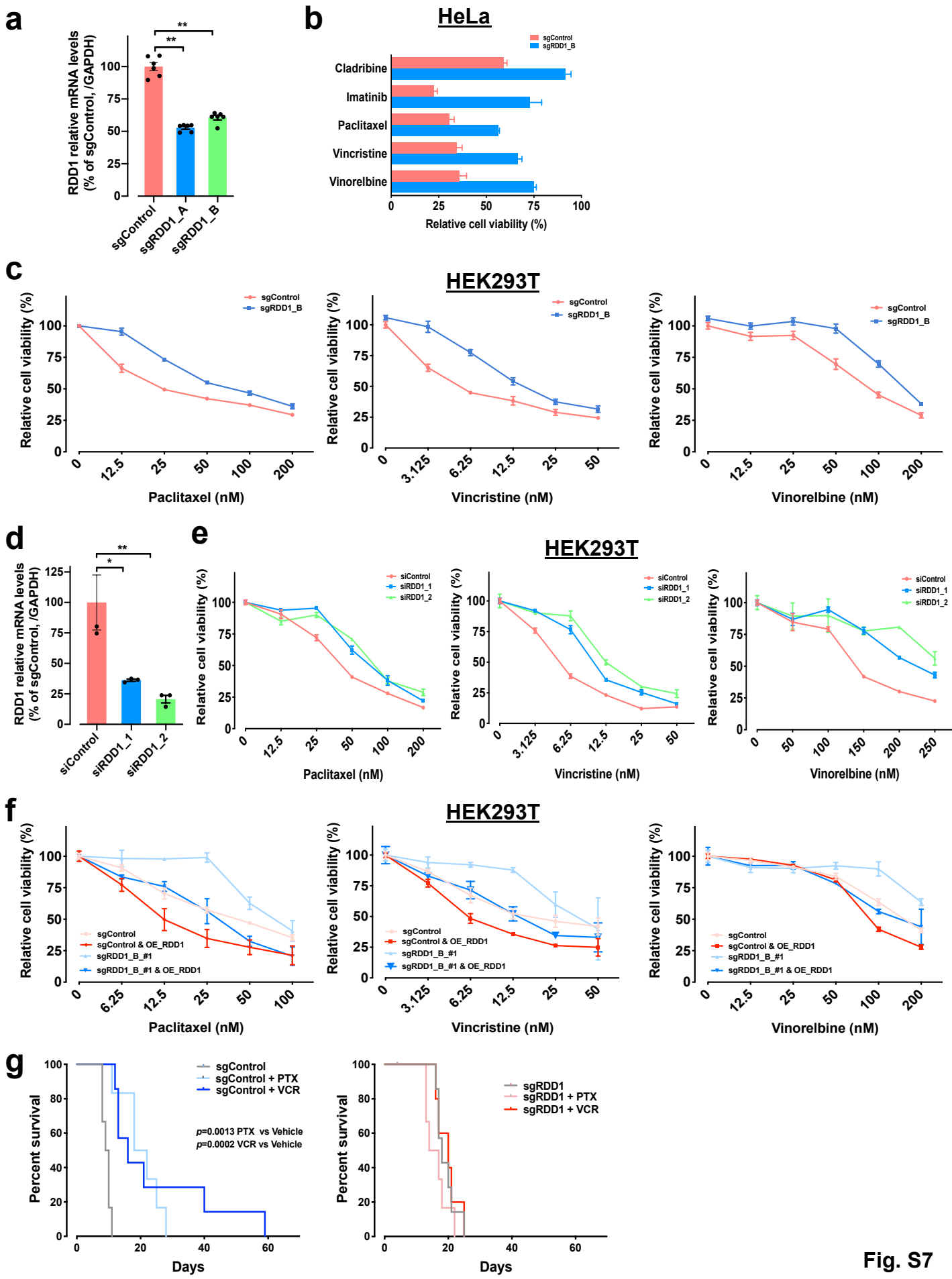


Fig. S7

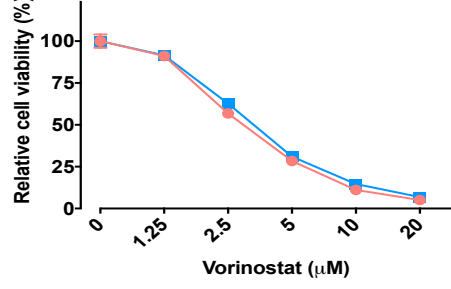
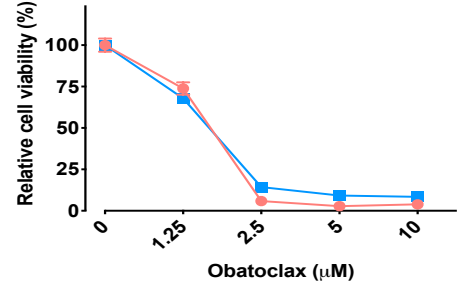
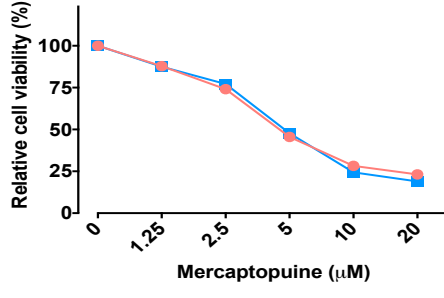
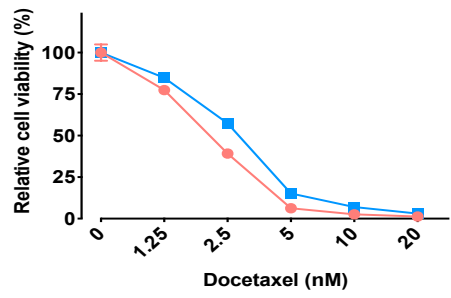
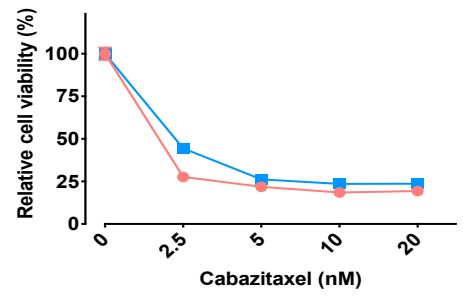
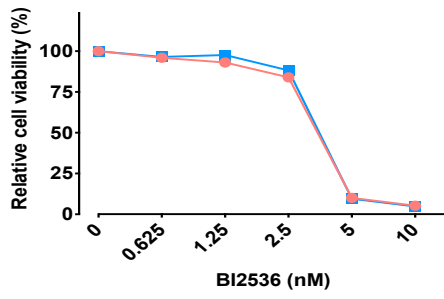
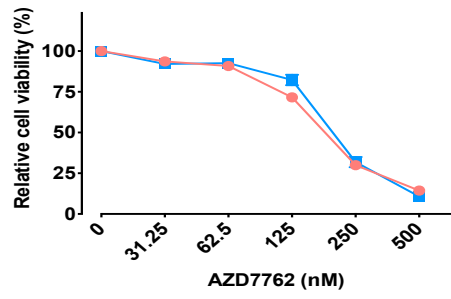
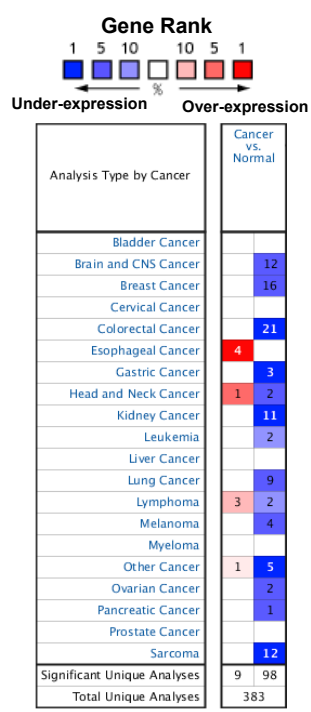
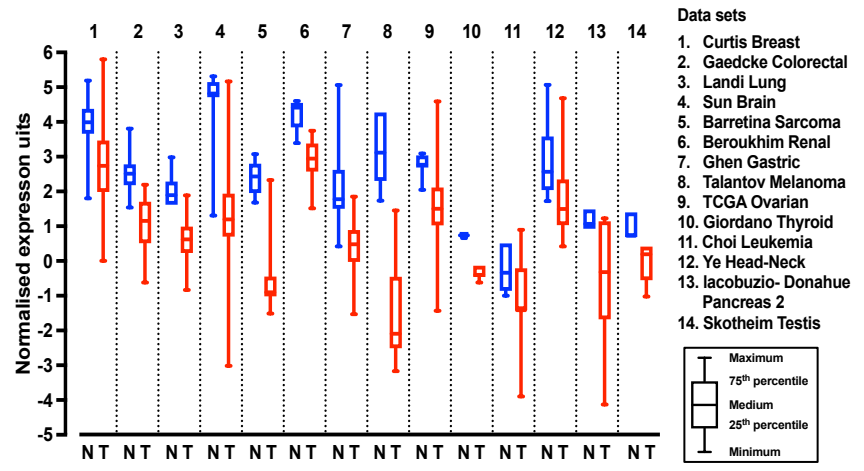
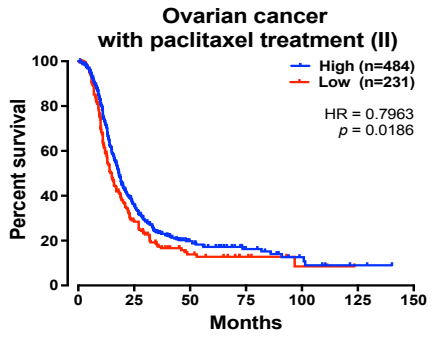
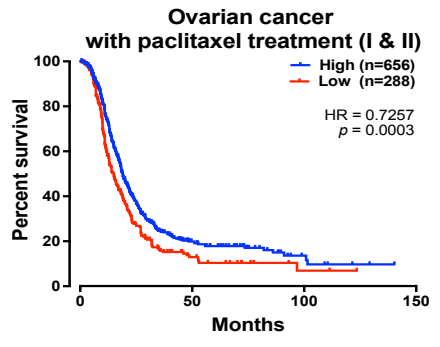


Fig. S8



**a****b****Fig. S9**

**a****b**

**Fig. S1 Drug response curve for all the 27 screened drugs in HAP1 cells**

Trypsinized HAP1 cells ( $7.2 \times 10^4$  cells; seeding density 225,000 cells/cm<sup>2</sup>) 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  $\pm$  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  $\pm$  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 (sg*RDD1*\_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  $\pm$  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](http://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.