**Supplementary methods**

***Cell lines***

Cell lines were obtained from the Cancer Cell Line Encyclopedia (1) at the Broad Institute. Cell lines were genotyped using short tandem repeats (DFCI Molecular Diagnostics Core) or single nucleotide polymorphism panels (Broad Institute Genomics Platform) prior to each screen and once during validation studies. Cells were assayed and negative for *Myclopasma* using the MycoAlert PLUS mycoplasma detection kit (Lonza LT07-710) prior to each screen and every 2-4 months during validation. Cell lines were maintained in appropriate medium and passaged once or twice per week depending on density, for a maximum of 10-15 passages.

***Open reading frame overexpression screen***

A pooled lentiviral open reading frame overexpression screen was performed to identify chemotherapy resistance genes (2). Optimization experiments determined the optimal cell density, puromycin concentration, and polybrene concentration for each cell line to support effective viral transduction without nonspecific toxicity. Drug doses were selected as the lowest dose that killed the majority (80-90%) of parental cells over 2-3 weeks of exposure. Plating densities were optimized to ensure that the drug-treated cells maintained a minimum cell density despite cell death due to drug treatment.

Kuramochi and OVSAHO cell lines were infected with a library of 17,255 ORF clones; of these, 12,952 clones - representing 10,135 distinct human genes - have at least a 99% nucleotide and protein identity to a human reference sequence (2,3). Each ORF clone was expressed in lentiviral vector pLX317 under the control of the EF1a promoter (3). The ORF clones are individually barcoded, enabling screening in a pooled format. The clones were sequenced during library generation as previously described (3) and the sequences and any identified nucleotide or protein mutations are available at the Broad Institute Genomics Perturbation Platform web portal (https://portals.broadinstitute.org/gpp/public/).

Cells were spin-infected with pooled lentivirus library titrated to achieve 30-50% infection efficiency, corresponding to a multiplicity of infection (MOI) ~ 0.5-1 and a library representation of at least 1000 cells per ORF construct. Following one week of puromycin selection, a sample of the pooled cells was collected for an early time point to establish initial representation of each ORF construct. The remaining cells were divided into 4 drug arms with 4 replicates each. Cells were treated with DMSO, cisplatin (0.5 µM), paclitaxel (10 nM), or cisplatin + paclitaxel combination (0.5 µM + 10 nM, respectively) every 3-4 days for 21 days. Cells were passaged in drug or fresh media containing drugs every 3-7 days. and counted to obtain growth curves. The majority of cells were killed at these drug concentrations. Surviving cells were harvested 21 days after initiation of treatment.

Genomic DNA was isolated from the pooled cells using DNA isolation kits according to the manufacturer’s protocol (Qiagen). PCR amplification was performed as previously described (4). Samples were sequenced on a HiSeq2000 (Illumina). Sequencing data were deconvoluted to quantitate the frequency of each ORF clone barcode. Adequate sequencing depth (goal ~1000 average reads/ORF) with a high proportion of matched reads (>85%) and high replicate reproducibility were observed. The total number of sequencing reads for each clone was determined and normalized to reads per million using the following formula: reads per ORF/total reads per sample × 106. Reads per million was then log2-transformed by first adding 1 to all values. The log2 fold-change of each ORFs was determined relative to the initial early time point for each biological replicate. In addition to this “basic” method to calculate log-fold change which was used for selection of neutral and sensitizing genes for the overexpression mini-pool and analysis of the secondary overexpression screen mini-pool data, further computational analysis of the primary overexpression screen data was performed (next section) to identify enriched resistance genes.

***Computational methods for ORF screen data analysis***

To compute the magnitude (log-fold change, LFC) and statistical significance (q-value), after correcting for multiple hypotheses across the entire ORFome, we compiled ORF-specific barcode read counts across all replicates in the test condition (post-drug) and the control (early time point), modelling the barcode representation with negative binomial distribution as implemented to measure differential expression of genes (5,6). Our evaluation is based on the consensus of two widely used methods to compute differential expression – edgeR (6) and DEseq (5). We run both edgeR and DEseq with quantile factor normalization and otherwise, default parameters. Our consensus LFC is based on mean values and consensus q-value based on geometric means of the two methods, thus balancing between DEseq which tends to be more powerful and edgeR which tends to be more sensitive to outliers (7). ORFs with overall read count below 100 in all arms were discarded from further analysis. ORF constructs with a high LFC relative to the early time point were considered candidate resistance “hits,” as these ORFs were most substantially enriched in the surviving cells after drug treatment, compared to the start of the screen in the absence of drug. Genes with a LFC from this analysis of greater than 0.5 and a q-value for significance of less than 0.001 were considered top candidate hits.

***Mini-pool secondary overexpression screen***

The mini-pool consisted of a) 191 “candidate resistance hit” ORFs with LFC>0.5 and q<.0.001 using the differential expression methods (see above) (limited to 2 ORFs per gene, which removed 6 ORFs for a total of 185); b) 48 “consistent hit” ORFs with LFC>0.5 and q<0.1 in at least 2 screen arms; c) 77 “neutral” ORFs with a Z-score of the “basic” LFC between -0.1 and 0.1 in all 3 drugs for both cell lines; d) 42 “negative” ORFs, a combined set of the 10 ORFs with the most negative “basic” LFC in each arm and duplicates removed; e) 9 selected “lethal” ORFs which have generalized toxicity in the absence of drug (DMSO arm); f) 11 “control” ORFs encoding fluorescent proteins or luciferase, and g) 4 individually selected ORFs related to top hits from the screen (*MCL1*, *PLAC1,* and a 2nd ORF for each of *BCL2L1* and *BCL2;* the second *BCL2* ORF BRDN0000990058 was later omitted from analysis due to a large deletion within the construct).

A lentivirus pool consisting of all ORFs was generated using a similar method to the primary screen, and sufficient infection efficiency of the lentiviral pool in Kuramochi and OVSAHO cells was confirmed prior to the mini-pool experiment. Kuramochi and OVSAHO cells were infected with the lentivirus pool and selected with puromycin. Following selection, cells were collected for the early time point and the remaining cells were divided into drug treatment arms in duplicate. Treatment conditions were DMSO, cisplatin (0.5 µM), paclitaxel (10 nM), and cisplatin + paclitaxel (0.3 µM + 5 nM) (lower doses than original screen to enable comparable cell killing with single agents). Drug was refreshed every 3-4 days. Cells were passaged with cell counts every 4-6 days for 10-14 days. At the end of the screen the remaining cells were collected and genomic DNA was extracted using a Qiagen DNA mini kit. PCR and sequencing were performed as described in the primary ORF screen. To analyze the data, basic log2-fold change compared to the early time point was calculated for each ORF construct.

***CRISPR-Cas9 knockout screen***

We performed an unbiased genome-wide CRISPR-Cas9 rescue screen using the same treatment conditions used in the overexpression screen. To generate stable cell lines expressing Cas9, OVSAHO and Kuramochi were infected with a Cas9 expressing vector, pLX-311-Cas9, and selected with blasticidin. Cas9 activity was verified. Cells were then spin-infected with the Avana4 barcoded library containing 73,687 barcoded sgRNAs targeting 18,454 genes and 1000 non-targeting guides (4). For each screen, four infection replicates were performed with a sufficient number of cells per replicate that achieved 500 cells per guide following puromycin selection (4 × 107 surviving cells). After selection, 4 × 107 cells were spun down for the gDNA extraction at Day 0 (early time point sample), and 4 × 107 cells were treated with drugs: cisplatin (1 µM), paclitaxel (10 nM), or two combination doses (cisplatin 0.5 µM + paclitaxel 10 nM (“high”); cisplatin 0.3 µM + paclitaxel 5 nM (“low”)). Cells were passaged or fresh drug-containing media was added every 3-4 days. Cells were harvested 14 days after initiation of treatment. Genomic DNA was isolated from the pooled cells using Mini or Maxi Kits according to the manufacturer’s protocol (Qiagen). PCR and sequencing were performed as previously described (4). Samples were sequenced on a HiSeq2000 (Illumina). For analysis, the read counts were normalized to reads per million and then log2 transformed. The log2 fold-change of each sgRNA was determined relative to the initial time point for each biological replicate. The top 2% of candidate resistance genes were also ranked using the STARS algorithm (http://portals.broadinstitute.org/gpp/public/software/stars).

***Individual ORF constructs***

DNA from individual ORF constructs in the pLX317 vector was obtained from the Broad Genetic Perturbation Platform and transformed into competent cells for bacterial culture and DNA midiprep or maxiprep. ORF sequences were confirmed by PCR using flanking primers for the pLX317 vector, and/or by full-length plasmid sequencing at the Massachusetts General Hospital DNA Core facility. All ORF sequences matched the expected NCBI reference sequence. The FLAG-BCL2 and FLAG-MCL1 constructs were kind gifts from Guo Wei and were generated by Genescript synthesis and verified by sequencing. FLAG-BCL2 was expressed in the pLX307 vector and FLAG-MCL1 was expressed in the pLX311 vector; both are functionally equivalent to PLX317 (307 is the same as 317 except without barcodes, and 311 is the same as 307 except with blasticidin instead of puromycin resistance).

***Lentiviral infection***

For lentivirus production, 293T cells were seeded and then transfected with lentivirus construct DNA using FuGene 6 (Promega E2692), packaging plasmid pCMV-dR8.74psPAX2 and envelope plasmid VSV-G. Viral supernatant was harvested 24-48h later, filtered through a 0.45 µm filter (VWR 4604), and stored at -80C until use. For viral transduction for the screen and mini-pool, cells in suspension were treated with viral supernatant and polybrene (EMD Millipore TR-1003-G) and spin-infected by centrifugation, incubated overnight, then collected and plated for selection in puromycin (2 µg/mL). For viral transduction for the individual validation experiments, cells were plated 24 hours prior to viral transduction; the next day adherent cells were treated with viral supernatant and polybrene (8 µg/mL), and centrifuged for 30 minutes at 2250 rpm at 37°C. 24 hours later, puromycin selection was initiated. After puromycin selection for 4-5 days, infection efficiency was determined and the complete death of uninfected cells treated with puromycin was confirmed. Selected cells were maintained in puromycin between experiments but changed to non-puromycin media at the start of each experiment.

***Western blotting***

Following ORF transduction and selection, protein overexpression was confirmed by Western blotting. Cells were lysed as frozen pellets or in a tissue culture dish using cOmplete Lysis-M buffer (Roche 4719956001) with cOmplete mini EDTA-free protease inhibitor tablets (Sigma 11836170001) and PhosSTOP phosphatase inhibitor tablets (PHOSS-RO, Roche). Protein was quantitated using a Pierce BCA protein assay kit (Thermo Fisher Scientific 23227) and prepared for Western blotting using NuPAGE LDS sample buffer and reducing agent (Thermo Fisher Scientific NP007, NP009). Proteins were resolved on Bolt 4-12% Bis-Tris Plus Gels (Thermo Fisher Scientific NWO4120) using NuPAGE MOPS SDS running buffer 20X (NP001) and transferred using the iBlot 2 system and iBlot 2 nitrocellulose membrane transfer stacks (IB23001). Membranes were blocked and incubated with primary antibodies at 4°C overnight followed by incubation with conjugated secondary antibodies (LiCOr) for at least one hour and detection with the LiCOr system. Primary antibodies included β-Actin (8H10D10) Mouse mAb (Cell Signaling Technologies (CST) 3700S), vinculin (Sigma-Aldrich V9131), MDR1/ABCB1 (D3H1Q) Rabbit mAb (CST 12683S), Bcl-xL (54H6) Rabbit mAb (CST 2764S), Bcl-w (31H4) Rabbit mAb (CST 2724S), Mcl-1 (D2W9E) Rabbit mAb (CST 94296S), Purified Mouse Anti-Human Bcl-2 (BD Biosciences 551107), Anti-V5 Antibody (Invitrogen R96025), Anti-Flag DYKDDDDK Tag (D6W5B) Rabbit mAb (CST 14793S). Secondary antibodies included IRDye® 680RD Goat anti-Mouse or anti-Rabbit IgG (H + L) (LiCOr 926-68070, 68071) and IRDye® 800CW Goat anti-Mouse or anti-Rabbit IgG (H + L) (LiCOr 926-32210, 32211).

***Drug treatment***

Cells were treated with the following drug stocks diluted in media to the final concentrations indicated in each experiment: cisplatin clinical solution (APP Fresnius Kabi) 1 mg/mL in 0.9% NaCl (from the DFCI research pharmacy); paclitaxel solid (Life Technologies P3456) dissolved to 10 mM in DMSO; olaparib (Selleck S1060) 10 mM in DMSO. Anti-apoptotic drugs included ABT-199/venetoclax (Selleck S8048); ABT-263/navitoclax (Selleck S1001); WEHI-539 hydrochloride (Med Chem Express HY-15607A); S63845 (ChemieTek CT-S63845); A1331852 (ChemieTek CT-A133); all were prepared as 10 mM stock solutions in DMSO. Chemotherapeutic doses were selected to be in ranges considered to be clinically relevant and achievable in patients. Cells were treated with a single dose of drug unless otherwise indicated. Vehicle controls used DMSO or 0.9% NaCl as appropriate. Drugs were added manually or using an HP/Tecan d300e digital dispenser. Tween-20 at 0.3% in the stock drug solution was added for aqueous drugs dispensed by the digital dispenser to enable accurate dispensing and did not have a detectable effect on cell viability.

***CellTiterGlo viability assay***

Because drug sensitivity assays are affected by density, and the HGSOC cell lines have variable doubling times, we adjusted the initial cell number individually for each cell line. Starting cell number was determined empirically such that cells were 10-20% confluent at the time of drug addition and 80-90% confluent at the end of the assay after 5 days. Approximate starting cell densities were 2500 cells/well for Kuramochi and 3000 cells/well for OVSAHO in 96-well plates and 1500 cells/well for each in 384-well plates. Cells were seeded at the given density 24 hours prior to drug addition. Drugs or vehicle were added using an HP/Tecan d300e digital dispenser in 8- to 10-point dose ranges in duplicate or triplicate. After 5 days or as indicated, CellTiterGlo luminescent cell viability reagent (Promega G7571) was added at a 1:10 dilution and incubated for 15-30 minutes after which luminescence was read on an Envision instrument. After subtracting background from media, luminescence signal in drug-treated cells was normalized to vehicle-treated cells. Data were analyzed using GraphPad Prism.

***CaspaseGlo caspase 3/7 activity assay***

Cells were seeded at 2500-3000 cells/well in 384-well plates and treated with drugs 24 hours later using an HP/Tecan d300e digital dispenser. After 24-72 hours of treatment, cells were incubated with CaspaseGlo reagent from the Caspase-Glo 3/7 assay kit (Promega G8091) at a 1:4 dilution for 1-2 hours and luminescence was read on an Envision instrument. Luminescence values were normalized to untreated cells and analyzed using GraphPad Prism.

***BH3 profiling***

BH3 profiling was performed as previously described using the flow cytometry method (8). Peptide stocks were obtained from New England Peptide and resupended in DMSO. For baseline BH3 profiling, peptides were added to MEB buffer (150 mM mannitol, 10 mM HEPES-KOH pH 7.5, 50 mM KCl, 0.02 mM EGTA, 0.02 mM EDTA, 0.1% BSA, 5 mM succinate; pH 7.5) plus 0.002% digitonin, at a range of concentrations from 0-100 µM. Cells were trypsinized and, in some experiments, stained with Zombie Aqua (BioLegend 423101) as a viability marker (in other experiments, Hoechst dye added after fixation was used as a live/dead marker). Cells were resuspended in MEB buffer and added to the peptide/MEB/digitonin solution. Cells were incubated with peptide for 60 minutes at 28-30°C. Cells were fixed in 4-8% formaldehyde for 10 minutes at room temperature followed by neutralization with N2 buffer (1.7 M Tris base, 1.25 M glycine, pH 9.1) for at least 5 minutes at room temperature. Cells were stained with AlexaFluor 647 conjugated anti-cytochrome c antibody (BioLegend 612310) in cytochrome c staining buffer (10X: 10% BSA, 2% Tween-20, phosphate-buffered saline) at 4°C overnight and stored at 4°C until flow cytometry analysis. Flow cytometry analysis was performed on a BD Fortessa or a Thermo Fisher Attune NxT instrument. Single cells for analysis were selected by forward and side scatter and viability markers. Cytochrome c positive cells were gated using negative and positive controls for maximum cytochrome c retention. Negative controls for cytochrome c release included DMSO treatment (no peptide) and PUMA2A peptide. Positive controls for cytochrome c release included alamethicin (Enzo Life Sciences BML-A150-0005) and DFNA5. Unstained cells with no cytochrome c antibody served as an additional control for negative cytochrome C signal. The percentage of cytochrome c positive and negative cells, and the median fluorescence intensity for AlexaFluor647-cytochrome c, was determined for each sample. For dynamic BH3 profiling with drug treatment, the same procedure was performed but was preceded by incubation of the cells with drug, in cell culture plates and standard media, for 24 hours prior to collection. Cells killed by the drug treatment were excluded in flow cytometry analysis using the cell viability stains.

***Colony formation assay***

Cells were seeded at low density in 12-well plates (Kuramochi, 2500 cells/well; OVSAHO, 8000 cells/well). The next day, cells were treated with vehicle or with drug at 3 concentrations, in triplicate. Media and drug were refreshed approximately once per week. After 14-21 days, colonies were fixed with 4% formaldehyde and stained with 0.5% crystal violet. Photographs of colonies were taken using a Leica microscope. For quantitation, colonies were de-stained by 10% acetic acid and the absorbance was measured at 595 nM.

***Population doubling assay***

Cells were seeded at 10-20% confluence in T25 flasks or 6-well plates in duplicate. 24 hours later, cells were treated with drug or vehicle at the indicated concentrations. Cells were passaged every 3-7 days depending on confluence, with cell counting at every passage, for 14-21 days. Drug was refreshed the day after each passage. Cumulative population doublings were calculated at each time point based on the number of cells relative to the number of cells plated.

***Cell cycle analysis***

One million cells were fixed with 70% ethanol, then washed in PBS and stained with propidium iodide (PI) using Invitrogen FxCycle PI/RNase staining solution (Thermo Fisher Scientific F10797) according to the manufacturer’s protocol and analyzed by flow cytometry.

***Genomic analysis***

Data for primary HGSOC was obtained from the Cancer Genome Atlas Pan-Cancer ovarian cancer dataset (9,10). GISTIC 2.0 copy number results for ovarian cancer data from 2016\_01\_28 was downloaded from Firebrowse (http://firebrowse.org/). Thresholded and focal gene-level results were used to define states of copy number amplification (CN state=2), low-level gain (CN state=1), neutral (CN state=0), shallow loss (CN state=-1), or deep loss (CN state=-2). Copy number alterations that were also present in the focal results file were deemed focal changes. Curated clinical data was obtained from a published dataset (10) including all non-redacted patients with ovarian cancer. Statistical analyses using standard R packages were used to generate Kaplan-Meier survival curves and calculate log-rank significance values. The cBioPortal (cbioportal.org) was used to generate expression and copy number plots in which mRNA expression is displayed in log2RPKM (Reads Per kilobase of transcript per million mapped reads).

Data for sensitive and resistant HGSOC were obtained from the Australian Ovarian Cancer Study Group (11). Expression levels in counts per million were derived from RNA-sequencing data. Copy number alterations were determined by GISTIC 2.0. T-tests were performed to evaluate significant differences between groups.

Data for ovarian cancer cell lines were obtained from the Cancer Cell Line Encyclopedia (1) and analyzed with Morpheus (https://software.broadinstitute.org/morpheus) data visualization software.

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