Haploinsufficiency networks identify targetable patterns of allelic deficiency in low mutation ovarian cancer

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Identification of specific oncogenic gene changes has enabled the modern generation of targeted cancer therapeutics. In high-grade serous ovarian cancer (OV), the bulk of genetic changes is not somatic point mutations, but rather somatic copy-number alterations (SCNAs). The impact of SCNAs on tumour biology remains poorly understood. Here we build haploinsufficiency network analyses to identify which SCNA patterns are most disruptive in OV. Of all KEGG pathways (N=187), autophagy is the most significantly disrupted by coincident gene deletions. Compared with 20 other cancer types, OV is most severely disrupted in autophagy and in compensatory proteostasis pathways. Network analysis prioritizes MAP1LC3B (LC3) and BECN1 as most impactful. Knockdown of LC3 and BECN1 expression confers sensitivity to cells undergoing autophagic stress independent of platinum resistance status. The results support the use of pathway network tools to evaluate how the copy-number landscape of a tumour may guide therapy.
Characterization of specific cancer mutations has yielded a map of which oncogenes and tumour suppressors that may be chemically or biologically targetable\(^1,2\) and guided immunotherapy\(^3\). However, single-nucleotide variants and short insertion–deletion mutations (here referred to simply as ‘mutations’) are not the sole drivers of oncogenesis. High-grade serous ovarian cancer (OV) is uniquely low in mutation and high in somatic copy-number alterations (SCNAs). SCNAs drive cancer through losses of tumour suppressors or amplifications of oncogenes, often by large SCNAs encompasing hundreds of genes\(^4\).

Homoygous deletion occurs rarely (1–2% of SCNAs) due to deletion of essential genes. On a gene-to-gene basis, SCNAs are more common than mutations even in highly mutated cancer types and \(\approx 95\%\) of SCNAs observed in tumours are monoallelic changes. However, with \(\approx 16,000\) genes with SCNAs in the average OV tumour (Fig. 1d), statistical modelling of driver SCNAs is complicated by pervasive ‘background’ SCNAs, which may not drive tumour progression. Previous analyses of SCNAs via chromosome arm alterations identified correlated pairs\(^5,6\), but lack a consideration of collaborative monoallelic SCNAs altering entire molecular pathways. Pathway analysis can improve an understanding of which molecular processes are altered when multiple genes contribute to cellular function, since different gene deletion combinations can yield identical phenotypes.

We developed a new tool to analyse highly variable SCNA tumours to determine significantly altered pathways and the gene-level SCNAs, which most likely contribute to pathway disruption. The tool is designed to incorporate known pathway concepts of genetic bottlenecking\(^2\), and is found to correctly prioritize known tumour suppressors and oncogenes as impactful genes in OV. By this analysis, the most suppressed pathways in OV is autophagy. Many other proteostasis pathways, such as the proteasome, endoplasmic reticulum (ER) stress and the lysosome are suppressed in OV. In validation of these computational findings, treatment of multiple OV in vivo models by autophagy- and proteostasis-disrupting drugs abolishes tumour growth. Knockdown of BECN1 and LC3B sensitizes OV to the autophagy halting drug chloroquine. These results implicate autophagy as a major disrupted pathway in OV, which is also amenable to therapy.

**Results**

Half of ovarian tumours lack clear driver mutations. OV tumours have been characterized\(^8\) as being uniquely low in mutations and high in SCNAs (Fig. 1a). However, it is possible that despite relatively low mutation rates, each OV tumour nonetheless contains multiple tumour suppressor or oncogene mutations that drive cancer formation. To investigate this possibility, we analysed The Cancer Genome Atlas (TCGA) OV data for mutations in well-known tumour-driver genes\(^8\). Interestingly, 48% of studied tumours have no mutations in these oncogenes or tumour suppressors, other than TP53 (Fig. 1b). Since mutant p53 alone is insufficient for tumour formation\(^9,10\), these tumours likely contain SCNA drivers\(^5\) which aid in tumorigenesis. Given the high ratio of SCNAs to mutations in OV (Fig. 1c,d), we sought a new method to better understand potential SCNA drivers.

**Design of the HAPTRIG SCNA analysis tool**. We developed a computational tool to identify pathways significantly disrupted by SCNAs in the highly noisy genetic background of OV tumours. The program was designed to analyse diverse genetic backgrounds which all yield at least one similar phenotype (Fig. 2a). Many biological pathways have multiple bottleneck\(^7\) or regulatory points\(^11\), any of which can equivalently affect pathway phenotype\(^12\). While Gene Set Enrichment Analysis (GSEA) also looks at multiple genes within a pathway to determine statistical significance at the cohort level\(^13\), we designed our tool to incorporate two additional pieces of information to better characterize genetic disturbance of pathway biology.
phenotypic changes (to prioritize genes that modulate other genes within the same pathway) and haploinsufficiency data (to prioritize genes that are known to affect biology when only a single gene copy is altered).

This Haploinsufficient/Triposensitive Gene (HAPTRIG) tool generates network scores by (1) building protein–protein interaction networks of pathway proteins from BioGRID, (2) prioritizing interactions that contain a haploinsufficient or triposensitive gene, (3) negatively scoring interactions containing gene deletion SCNAs and positively scoring interactions containing gene amplification SCNAs, and (4) summing all interaction scores within a molecular pathway. For statistical significance, pathway scores from observed tumours were compared with control data of 1000 tumour-paired randomly permuted SCNAs to derive a P value of observed tumour pathway changes compared with what would be expected by chance (for a schematic, see Supplementary Fig. 1). This design enables statistically significant pathway changes in a cohort of tumours to be detected in a high noise background. In addition, the HAPTRIG pipeline scores the contribution of each gene within a pathway to allow for ranking the biological importance of each gene within a pathway. For example, since TP53 is highly interactive and often deleted, it is ranked by the HAPTRIG tool as the most impactful deletion within the p53 pathway for most OV tumours.

To test the robustness of the HAPTRIG approach, we queried HAPTRIG for its ability to prioritize known tumour suppressor genes and oncogenes, as most affecting deleted or amplified gene sets, respectively, and similarly tested for ‘STOP’ and ‘GO’ gene prioritization. Using the full HAPTRIG approach as a reference, we measured how its sensitivity is affected by the following parameters: (1) removal of haploinsufficient orthologue data from mice and yeast, (2) inclusion of only intrinsic (within gene set only) interactions or primary/secondary interactions as well, and (3) when gene ontology (GO) pathways were used in place of comparable Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Fig. 2b, Supplementary Fig. 2A). All components altered HAPTRIG efficiency in the range of 10–60%. While we predict many GO pathways to be useful in HAPTRIG analysis, GO pathways are typically much larger and contain many genes with tangential relation to core pathway function. The most accurate view of SCNA-altered pathways within OV was thus found by using all distinct, human KEGG pathways (N = 187 pathways) scored for intrinsic and haploinsufficient interactions.

HAPTRIG pathway analysis of OV identifies autophagy loss. In TCGA OV cohort, we observed the most statistically unlikely disrupted deletion-enriched pathway to be autophagy
Drugs24; redundant losses may underlie the severely pervasive deletions in protein and organelle quality control such as p53 and focal adhesion were also significantly altered, albeit at lower significance. This pattern persisted in an independent OV cohort16 but did not reach statistical significance in an endometrioid OV cohort, perhaps due to small sample size (Supplementary Fig. 2; Supplementary Data 2 and 3). While we focus on KEGG pathways here, HAPTRIG functions on any pathway set (Hallmark pathway set results shown in Supplementary Data 4). HAPTRIG improves on GSEA to identify these significantly disrupted pathways: only two KEGG pathways reached statistical significance using GSEA (Supplementary Table 1; Supplementary Fig. 3). We release the code for HAPTRIG as Supplementary Software 1, and provide example input data sets as Supplementary Data 5.

Autophagy has long been implicated in tumour development and may have dual roles of autophagy genes including BECN1 leads to early oncogenesis in mouse models17,18; however, KRAS mutant cancers are addicted to elevated autophagy19. Interestingly, most proteostasis pathways in our pan-pathway analysis were enriched for deletions, including ER stress, ubiquitin-mediated proteolysis and the lysosome, although the peroxisome pathway was enriched for amplifications. Haploinsufficiency in model organism screens is associated with an inability to form adequately proportioned protein–quality control complexes20, suggesting single allele SCNAs disrupt these pathways. To determine whether proteostasis disruption was specific to OV, we ran HAPTRIG analyses across 20 other cancer types studied by TCGA. Alterations ranged from minimal among acute myeloid leukaemia and thyroid cancers, a strongly suppressed network of proteostasis genes in invasive breast (BRCA) and serous ovarian (OV) cancers, to a uniquely amplified autophagy network in renal papillary cell carcinoma (KIRC; Fig. 3a). Many genes were frequently altered in OV, and HAPTRIG ranked known biologically impactful genes (for example, BRCA1, TP53, BECN1 and CASP3) as most altered for OV (Fig. 3b, full OV networks in Supplementary Fig. 4), as well as some genes uncommonly associated with cancer (for example, CTSD for lysosomal function and PEX5 for peroxisomal function, full summary in Supplementary Table 2). OV was clearly the most disrupted for proteostasis amongst these 21 tumour types. We next evaluated whether these SCNA network alterations contribute to cancer phenotypes as mutations do, and whether they might be predictably targeted.

Targeting autophagy and proteostasis in vivo halts OV growth. Well-controlled single-allele losses reduce messenger RNA (mRNA) expression up to 90% of the time, even in a single unstrressed experimental condition21. In OV, protein expression correlated with mRNA expression for 80–90% of genes22. Autophagy depends on mRNA induction for full function23. TCGA OV tumours exhibit decreased mRNA expression of core autophagy genes upon heterozygous loss and often contain several core autophagy gene deletions (Supplementary Fig. 5). Such pervasive deletions in protein and organelle quality control genes may sensitize OV to proteotoxic, autophagy-stressing drugs24; redundant losses may underlie the severely compromised capacity of these tumours to compensate for proteotoxic treatment combinations (Supplementary Fig. 6). To investigate this possibility, we treated OVCA3 cells with chloroquine, to prevent autophagy resolution25, and nelfinavir, to promote ER stress26. Protein aggregates increased by 3–6-fold (Supplementary Fig. 7), concurrent with the accumulation of autophagolysosomes (Supplementary Fig. 8). The phenotype was further amplified when chloroquine/nelfinavir was combined with rapamycin and/or dasatinib24, which we term Combination Of Autophagy Selective Therapeutics (COAST; Supplementary Fig. 8). Proteasomal inhibitors also stress autophagy, and bortezomib exhibited cytotoxicity in the low nanomolar range. However, bortezomib was not OV selective and risks high clinical toxicity (Supplementary Fig. 9). Cytotoxic concentrations required for the OV tumour cells were low for other proteostasis-targeting agents (Supplementary Figs 10 and 11). Chloroquine and nelfinavir within the concentration range found in patients’ blood24 was sufficient to prevent single-cell colony formation, cell growth in suspension, and to promote cytotoxicity (Supplementary Fig. 12) in OV cells. Higher-order combinations (COAST) were selective across six different OV tumour cell lines (Supplementary Fig. 11) with autophagy gene deletions (Supplementary Table 3), and no drug or combination reduced the effects of any other drug.

We next evaluated whether this HAPTRIG-informed choice of drugs would ameliorate disease in preclinical models of OV. Cisplatin and docetaxel did not alter the growth of a patient-derived xenograft model derived from a recurrent chemotherapy-resistant patient (Fig. 4a), while the proteostasis-targeted cocktail resulted in a striking complete abatement of tumour growth. Given the lack of any macroscopic disease, we next used an ID8-IP-mCherry labelled tumour model27 to allow detection of persistent microscopic disease. Again, mice treated with COAST showed eradication of tumours, although microscopic nests of cells were still detected in 2/8 mice. Interestingly, chloroquine and nelfinavir alone did not result in statistically significant inhibition (Fig. 4b), despite having the best efficacy of two drugs in vitro (Supplementary Fig. 10), possibly reflecting the complexity of the tumour microenvironment and other forms of heterogeneity in syngeneic models. This five drug cocktail was remarkably well tolerated in mice24, in which we tested up to 8 weeks of COAST therapy, long after all control mice perished (Supplementary Fig. 13). COAST also arrested tumour growth in a subcutaneous OVCA3 model (Fig. 4c), with residual tumour showing accumulation of autophagosomal LC3-II and the ER stress marker Grp78 (Fig. 4d).

Impactful HAPTRIG genes influence OV drug targeting. Since genetic targeting is an important consideration of new therapies, we next utilized HAPTRIG network information to determine gene SCNAs most likely to have an impact on autophagy in OV. These most ‘impactful’ genes were identified by summing the score contribution of each gene within HAPTRIG networks across all tumours. We ranked impactful suppressive and oncogenic genes for all pathways in OV (Supplementary Table 2). For autophagy, the two highest impact genes were MAP1LC3B (LC3) and BECN1. These two genes were also commonly lost in OV, along with ATG10, ULK2 and GAPARAP12 (Fig. 5a). LC3 and/or BECN1 are monoallelically deleted in 94% of OV (Supplementary Fig. 5C). Mechanistically, this may explain the sensitivity of OV tumours to drugs pressuring the autophagy network, since orthologues of each gene confer haploinsufficiency in yeast20 or mice18. These losses occur early in the evolution of OV28 and have an associated defect in expression when monoallelically lost (Supplementary Fig. 5), consistent with previous reports29. OV cell lines that differ in LC3 and BECN1 gene dose (Fig. 5b) were next tested for differences in autophagy.

OVCA3 is a cisplatin-resistant tumour cell genetically similar to TCGA assayed OV30, exhibiting monoallelic deletions
evaluated IGROV1 or SKOV3 cells stably expressing lentiviral was sufficient to confer a proteostasis bottleneck, we next represented as overlaid circles of size proportional to the log10 different pathways. The chart displays pathway network scores as blue fill if deletion-enriched, red fill if gain-enriched, and white fill for neither. Significance and the p53 pathway. Since these pathways are functionally interdependent, HAPTRIG scored both intrinsic and primary interactions from within these (Fig. 5c,d).25,32. Similar results were found when autophagy was chloroquine, as measured using complementary assays relative to IGROV1 and SKOV3 following treatment with Flux through autophagy showed a delayed response in OVCAR3 ovarian, cancer30 cell lines that have lost neither allele (Fig. 5b). characterized as ovarian, but not serous (nor high SCNA) with losses in autophagy genes such as LC3 and BECN1, and forming appropriate high-grade histology in mice31. In contrast, IGROV1 and SKOV3 are characterized as ovarian, but not serous (nor high SCNA) ovarian, cancer30 cell lines that have lost neither allele (Fig. 5b). Flux through autophagy showed a delayed response in OVCAR3 relative to IGROV1 and SKOV3 following treatment with chloroquine, as measured using complementary assays (Fig. 5c,d).25,32. Similar results were found when autophagy was perturbed with rapamycin, nelfinavir or combination (COAST) treatments (Supplementary Fig. 14). While few OV cell lines are currently well established and also contain common OV genetics31,33,34, we additionally studied OVCAR5, OVCAR8, the patient-derived xenograft model cells LPPDOV and A2780 for autophagic response to chloroquine and again found cell lines with low HAPTRIG scores to poorly induce autophagy upon chloroquine stress (Supplementary Fig. 15), which correlated with increased cell death. Taken together, although OV cells are not completely lacking autophagy, a maximized response to stress is compromised among cells with losses in autophagy genes such as LC3 and BECN1.

To test directly whether suppression of LC3/BECN1 was sufficient to confer a proteostasis bottleneck, we next evaluated IGROV1 or SKOV3 cells stably expressing lentiviral shRNA selected for modest suppression (~35–70%) of LC3 or Beclin. Slowed autophagosome accumulation was clearly observed with shLC3, although not significantly with shBECN1 (Fig. 5e,f). Cells with reduced LC3 or BECN1 showed compromised survival following treatment with chloroquine, which prevents clearance of autophagosomes35 (Fig. 5g). This survival defect was observed with multiple cell types, including IGROV1 and a glioblastoma (U373) resistant to autophagy drugs (Supplementary Figs 10, 11 and 15). Resistance to cisplatin, a standard of care agent used to treat OV, was not indicative of response to COAST drugs including chloroquine (Fig. 5g; Supplementary Figs 10 and 15). Rather, autophagy-stressing drugs compromised cell survival selectively among lines with autophagy gene losses, regardless of single or combined drug treatment (Supplementary Figs 10, 11 and 15). The results support a model implicating haploinsufficiency, at a minimum for LC3 and BECN1, in the sensitivity of OV to agents targeting autophagy.

**Discussion**

The HAPTRIG tool represents an initial haploinsufficiency network-based analysis program that can be applied genome
wide for any cancer. Sequencing of mutations has identified potentially targetable genes in minorities of OV patients. However, given the excessive (two-third of the genome) SCNAs present in OV, we undertook a strategy to identify pathways that are uniquely and perhaps unexpectedly disrupted by SCNAs. Our permutation strategy enabled identification of significant pathways despite a potentially passenger-filled SCNA landscape. Critically, aside from merely identifying known altered genetics such as suppression of the p53 pathway, enhancement of the focal adhesion pathway

Figure 4 | OV tumours are sensitive to disruption of proteostasis. (a) Low passage patient-derived OV (LPPDOV) ascites cells from a patient who failed cisplatin-docetaxel chemotherapy were injected i.p. into Nu/nu mice, allowed to disseminate and grow for 10 days, and then treated with control 50% PEG400 or with COAST (Combination of Autophagy Selective Therapeutics: chloroquine 30 mg kg⁻¹, nelfinavir 250 mg kg⁻¹, rapamycin 2.24 mg kg⁻¹, dasatinib 4 mg kg⁻¹, and metformin 150 mg kg⁻¹ in 50% PEG400) daily for 15 days. An additional control group was treated with cisplatin/docetaxel chemotherapy (injected i.p. with 1 mg kg⁻¹ cisplatin and 2.5 mg kg⁻¹ docetaxel once per week starting at the first control treatment day for 2 weeks). Upon harvest, all visible and palpable tumours in the peritoneum space were dissected, counted and weighed, as were mouse spleens. (b) C57BL/6 immunocompetent mice were injected i.p. with ID8-IP-mCherry cells (N = 8 per group). After 2 weeks to permit tumour establishment, mice were orally gavaged daily with control 50% PEG400, with COAST, or chloroquine and nelfinavir alone. At 14 days, control mice developed ascites. All groups were killed, ascites were measured and tumour burden assayed by native mCherry fluorescence. Ovaries are displayed for all mice, and any additional tumor fluorescence observed is displayed on the right panel with labels ‘P’ for peritoneal wall growth and ‘L’ for liver. (c) Nu/nu mice with 100 mm³ subcutaneous OVCAR3 tumours were gavaged with COAST or control and tumour growth monitored by digital calipers for 7 days. Tumours were then dissected, weighed and (d) subjected to immunoblotting for autophagosomal Lc3-II and the ER stress marker Grp78 (mean ± s.e.m. N = 7 mice per group). *P < 0.05, **P < 0.01, ***P < 0.001 by Wilcoxon rank-sum test.
and disruption of homologous recombination repair pathways\(^{37}\), our top hits are not currently considered to be canonical OV driver pathways. Yet, using \textit{in vivo} and \textit{in vitro} models, we validated that autophagy was suppressed in OV and moreover that by targeting this suppression by drugs that disrupt proteostasis we achieved remarkable tumour remission independent of platinum resistance.

Given the strong autophagy phenotypes we found in OV, it is curious why the autophagy pathway has not been emphasized in prior integrative analysis publications. Previous publications have supported the finding that OV is deficient in DNA repair pathways, dysregulated in cell cycle control and often overexpress MYC and ERBB2 (Supplementary Table 4). HAPTRIG confirms these disruptions in KEGG pathways and in MSigDB (Molecular Signature Database) Hallmark pathways. Interestingly, GSEA\(^{13}\) of copy-number data also highly ranks these pathways and autophagy, albeit at a lower rank than HAPTRIG. This is likely because GSEA does not incorporate interaction or haploinsufficiency data, resulting in an altered spectrum of prioritized genes relative to HAPTRIG. A second significant
reason that autophagy has not received further exposure in the context of OV is that very few pathway sets include autophagy. In the many thousands of pathways annotated in MSigDB38, autophagy is only included in KEGG and GO pathways, as assayed here. Many genes remain to be annotated within pathways39, and improved pathway curation will certainly advance pathway analysis tools such as HAPTRIG.

Although loss-of-heterozygosity accompanied by mutations is a recognized phenomenon in breast, ovary, and other cancer, 99.8% of gene deletions in OV show no mutation in the opposing allele. For autophagy genes, mutations in the remaining allele for tumours with heterozygous deletion were not observed. Rather, cumulative gene expression changes from SCNAs contribute to biological phenotypes40–42. Reduced gene expression is observed much more commonly than no change in controlled heterozygous deletions39, and mRNA correlates with protein expression in ~80–90% of OV mRNAs22. Losses of proteostasis genes are likely oncogenic; multiple studies implicate BECN1 as a haploinsufficient tumour suppressor in mice39,77, possibly related to roles in chromosomal segregation during cell division43,44. Chromosome instability in human cancers such as OV and BRCA may be further exacerbated by loss of BRCA1, a functionally independent tumour suppressor neighbouring BECN1 (ref. 45) on cytoband 17q21. Early losses in autophagy genes may contribute to the extreme SCNA heterogeneity of OV, but as we have shown here, also provide opportunity for network-targeted therapy.

The prevalence of such monoallelic changes has been largely unappreciated. In all cancer types, more genes are affected by single gene-dose changes than by biallelic deletions, but as we have shown here, also provide opportunity for network-targeted therapy.

HAPTRIG analysis construction. HAPTRIG proteostasis networks were built from the KEGG pathways autophagy (hsa04140), Lysosome (hsa04142), endoplasmic reticulum processing (hsa04141), ubiquitin-mediated proteolysis (hsa04120), peroxisome (hsa04146) and the p53 (hsa015115) pathway. The KEGG autophagy pathway was further curated using current knowledge by adding MAP1LC3R, encoding the protein most commonly used to define autophagosomes.25 We used protein–protein interactions (PPIs) from the BioGRID curated database14 to connect input pathway genes. For the pan-pathway analysis and in quality control networks, all human KEGG pathways were used. The full list of 187 KEGG pathways tested is included in Supplementary Data 1. For quality control, a table of the top 10 ranked genes (as in the gene-impact prioritization) for each of the 187 KEGG molecular pathways was generated and compared to the permutation analysis corresponding to the random data to increase the confidence in the output P value. For example, if our HAPTRIG code creates 1,000 control network scores for each tumour and output P values are generated from the average log10(P value) resulting from these 1,000 control network comparisons. HAPTRIG score distributions were compared by Student’s t-test and multiple hypothesis testing corrected for the Bonferroni method (for 6 pathways and 21 cancer types = 126 comparisons in Fig. 3, 187 comparisons—all KEGG pathways—for Fig. 2) to generate a q value. Visual networks were drawn using Cytoscape 3.3 (ref. 90). To produce a representative network for the entire OV cohort, the EdgeScores were recomputed at the cohort level using mean GISTIC scores across all tumours. If a node had an SCNA alteration in >33% of patients, an edge was drawn to its PPI partner (blue: loss, red: gain, purple: antagonistic). To accommodate lower numbers of mutations relative to SCNA events, if a gene reached a mutation rate of above 10%, PPI edges were represented as disrupted by mutations (green edge visualization). Node size and colour represent their frequency of loss or gain of a pathway than a relatively SCNA stable cancer, we compared the distribution of observed HAPTRIG module scores to that of the distribution of random/gain score suppression (blue) or enhancement (red) in Fig. 3a.

Each cancer type has a unique distribution of chromosome losses and gains. Since a highly copy-number variable cancer may have a higher chance of a random loss or gain of a pathway than a relatively SCNA stable cancer, we compared the distribution of observed HAPTRIG module scores to that of the distribution of random/gain score suppression (blue) or enhancement (red) in Fig. 3a.

For gene-impact prioritization, EdgeScores were summed among all tumours using mean GISTIC scores across all tumours. If a node had an SCNA alteration in >33% of patients, an edge was drawn to its PPI partner (blue: loss, red: gain, purple: antagonistic). To accommodate lower numbers of mutations relative to SCNA events, if a gene reached a mutation rate of above 10%, PPI edges were represented as disrupted by mutations (green edge visualization). Node size and colour represent their frequency of loss or gain of a pathway than a relatively SCNA stable cancer, we compared the distribution of observed HAPTRIG module scores to that of the distribution of random/gain score suppression (blue) or enhancement (red) in Fig. 3a.
gene set. Efficiency was calculated as the per cent of possible hits that were found to be present in the quality control table.

**Code availability.** Complete HAPTRIG code is available as Supplementary Software 1. Demo data for input are provided as a convenience as Supplementary Data 5.

**Gene set enrichment analysis.** TCGA OV data were used as the expression data set, with tumour copy number compared with normal tissue control copy number. Gene sets were the same as HAPTRIG. Gene set permutations were set at 1,000. To find the best comparison, the comparison was TUMOR versus NORMAL, verss TUMOR, and verss NORMAL. Leading edge analysis was performed and the top 10 genes for each pathway were input as benchmarking genes for quality control analysis, as described above. GSEA version used was 2.2.2.

**Cell culture and reagents.** Established cell lines were purchased from the American Type Culture Collection and validated by short tandem repeat profiling (Promega). Routine morphological morphology tests were performed before each experiment. Cells were verified to be mycoplasma negative by a PCR assay (Agilent Technologies (Stratagene), cat# 302008). Patient consent was obtained for scientific use and publication of the LPPDOV patient-derived OVs, as previously described4. All cells were grown in RPMI (Life Technologies) supplemented with 2% glucose, nonessential amino acids (Mediatech #45000-700), sodium pyruvate (Mediatech #45000-710), antibiotics (penicillin, streptomycin and amphotericin, Mediatech #30-004-CL) and 10% fetal bovine serum (Omega Scientific #FB-11). Cells were cultured at 37°C with 5% CO2.

**Antibodies.** All primary antibodies were used at 1:1,000 dilution. LC3B (Novus Biologicals #NB100-2220), p62 (BD Biosciences #610382), β-actin (Sigma-Aldrich #A5441-2ML), GRP78 (BioLegend #644402), BECN1 (Creative Dynamics Inc, special order, or for in vivo studies Viracept, Agouron Pharmaceuticals) were purchased in powder form. Anti-p62 (VWR, cat# 395571) and 680 nm for anti-luteinizing hormone (VWR # P355158). Secondary horseradish peroxidase antibodies were anti-rabbit (Jackson ImmunoResearch #211-037-171) anti-lactate (Life Technologies #195201) or anti- mouse (Jackson ImmunoResearch #113-035-003).

**Drugs.** Docetaxel (Winthrop, US, 20 mg ml-1 concentration) and cisplatin, US, 1 mg ml-1 obtained by the Moores Cancer Center pharmacy. Metformin (VWR, cat# 89147-892), rapamycin (R, 10 nM) and dasatinib (D, 50 nM)). Cells were then imaged live by a Olympus XI-51 spinning disc microscope fitted with an environmental chamber set to standard 5% CO2 37°C conditions.

**Autophagic flux microscopy.** OVCAR3 cells with mCherry-GFP-LC3B virally integrated were seeded on a glass bottom 12-well plate to 5,000 cells per well and treated with COAST drugs (chloroquine (10 μM), C), nelfinavir (10 μM, N), rapamycin (R, 10 nM) and dasatinib (D, 50 nM)). Cells were then imaged live by a Olympus XI-51 spinning disc microscope fitted with an environmental chamber set to standard 5% CO2 37°C conditions.

**Western blotting.** Cells were grown to 50% confluence on 10 cm plates and treated with drugs or control for 24 h at 37°C. Media was collected, cells washed in PBS and the supernatant was spun 500 g. Iced RIPA buffer (supplemented with a protease inhibitor cocktail (Sigma-Aldrich), 2 mM sodium orthovanadate and 50 mM NaF) was added to solubilize the cells (15 min, room temperature) at which point cell pellets were collected using a cell lifter (Fisher Scientific). Supernatant pellets were added to the RIPA buffer and combined with adherent cell fraction. Lysates were spun at 100,000g for 10 min at 4°C, and supernatant was saved and quantified by bicinchoninic acid (BCA) assay (Pierce #23235). A measure of 30 μg of protein was loaded per well of a 15% SDS-polyacrylamide gel electrophoresis gel and transferred onto polyvinylidene difluoride membrane. The membrane was blocked in 5% dry milk (Genesee Scientific #20-241) or 0.1% casein (Sigma #S5990-500G). Primary antibodies were used at 1:1,000 dilution, and secondary horseradish peroxidase antibodies were used at 1:5,000 dilution or secondary fluorescent antibodies were used at 1:1,500. Fluorescent secondary antibodies were visualized using a LI-COR Odyssey scanner. Quantification of band intensity was performed in ImageJ and all normalizations were to the shown loading control. For uncropped western blots, refer to Supplementary Fig. 17.

**Flow cytometry.** Flow cytometry was performed on a BD FACSAria Calibur cytometer and analysed with BD CellQuest Pro.

**Propidium iodide viability staining.** A total of 100,000 cells were grown in a six-well TC dish with 3 ml media containing drug or control solution for 48 h. Media was collected, cells were washed with 1 ml PBS, which was pooled with the media, and then cells were trypsinized for 5 min in 1 ml Tryspin-EDETA. Trypsinized cells were then combined with 1 ml iced RPMI and centrifuged for 5 min at 500g. Supernatant was aspirated and cells were resuspended in 400 μl iced PBS. Cells were then analysed on the flow cytometer.

**Mouse models.** All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of University of California: San Diego (UCSD), and all rules and regulations were followed during experimentation on animals. Experiments were timed to detect differences of 30% (http://homepage.divms.uiowa.edu/~relnth/Power/). No blinding was performed, since drug and control solutions were visually distinguishable. All mice were...
female, and COAST doses (250 mg kg$^{-1}$; 1 nelfinavir, 30 mg kg$^{-1}$) chloroquine, 2.24 mg kg$^{-1}$ rapamycin, 150 mg kg$^{-1}$ mefloquin and 4 mg kg$^{-1}$ dasatinib, daily by gavage). In 50% PEG400 (water) were determined using clinically safe doses as determined from a previous study. All mice were included for the following experiments if above 18 g starting weight and with a healthy disposition before any injections. No mice were censored in these experiments.

In the subcutaneous model, 5 $\times$ 10$^5$ OVCA3 cells were injected into the right flank of 8–10-week-old female Nu/nu mice (N = 7 per group). Mice were randomized when tumours were palpable. Treatment with control (gavage, daily, 50% PEG400) or COAST began when tumours reached 100 mm$^3$, which was 14–20 days after cell injection. Mice were treated for 7 days and then killed 5 hours following the last treatment. Tumours were removed and weighed as additional confirmation of the caliper size measurements.

For the chemo-resistant model, 5 $\times$ 10$^3$ early passage LPPDOV cells were injected intraperitoneal (i.p.) into a female Nu/nu mouse, allowed to develop visible tumours, and ascites were collected and plated in complete RPMI on a TC-treated plate. The surviving cells were cultured in RPMI. After subculturing, the xenografted tumours were injected i.p. into syngeneic C57BL/6 mice at 10 weeks of age. Mice of equal mean weights were used in each group (N = 8 per group), randomized pre-injection, and are the same cohort summarized in a previous study of ours. Fourteen days after injection, one group received daily (seven times a week) vehicle gavage injections (50% PEG400), the C group received daily chloroquine and nelfinavir gavage (30 and 250 mg kg$^{-1}$, respectively), and the COAST group received daily COAST gavage. Mice were monitored daily for distended abdomens following the first treatment injections. All mice were killed when ascites formation produced visible discomfort to control animals, which occurred after 14 days of treatment (28 days since cell injection). The peritoneum of the mice was exposed and any visible nodules on the peritoneum wall were surgically dissected out with the liver and ovaries. These tissues were then imaged with the OV100 Small Animal Imaging System (Olympus). Bright-field, GFP and mCherry channel information were collected and only red fluorescent (but not green autofluorescent) punctae area was quantified in ImageJ. Fluorescent area was mathematically converted into tumour volume assuming spherical shape of the tumour and circular shape of the fluorescent area. Any bloody ascites present upon initial opening of the peritoneum was transferred by P1000 micropipette into a 15 ml conical tube and volume determined by micropipette. In the longer-term safety experiment, the experiment was performed identically, except mice were treated by COAST for a period of 8 weeks with 5 days daily doses (excluding weekends).

Data availability. All the data that support the findings of this study are available in Supplementary Files, or available from the authors upon request.

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Author contributions

J.R.D. and D.G.S. conceived the experiments and wrote the manuscript. D.D.S., O.H., C.S. and I.T. conceived experiments. J.R.D. built the HAPTRIG tool. J.R.D., S.H. and O.H. performed pilot TCGA data analysis. All other authors and J.R.D. performed experiments.

Additional information

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