Phosphate dysregulation via the XPR1–KIDINS220 protein complex is a therapeutic vulnerability in ovarian cancer

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Despite advances in precision medicine, the clinical prospects for patients with ovarian and uterine cancers have not substantially improved. Here, we analyzed genome-scale CRISPR–Cas9 loss-of-function screens across 851 human cancer cell lines and found that frequent overexpression of SLC34A2—encoding a phosphate importer—is correlated with sensitivity to loss of the phosphate exporter XPR1, both in vitro and in vivo. In patient-derived tumor samples, we observed frequent PAX8-dependent overexpression of SLC34A2, XPR1 copy number amplifications and XPR1 messenger RNA overexpression. Mechanistically, in SLC34A2-high cancer cell lines, genetic or pharmacologic inhibition of XPR1-dependent phosphate efflux leads to the toxic accumulation of intracellular phosphate. Finally, we show that XPR1 requires the novel partner protein KIDINS220 for proper cellular localization and activity, and that disruption of this protein complex results in acidic “vacuolar” structures preceding cell death. These data point to the XPR1–KIDINS220 complex and phosphate dysregulation as a therapeutic vulnerability in ovarian cancer.

A n emerging paradigm in cancer medicine is the tailoring of a therapeutic strategy to the specific molecular profile of a patient’s tumor. Despite remarkable advances in ‘precision medicine’ in other cancer types, outcomes for ovarian and uterine cancers have not improved substantially in the past 20 years1–3. Accordingly, ovarian and uterine cancers remain among the most deadly, globally killing over 300,000 women in 2020 alone4. New therapeutic strategies are needed.

We and others have demonstrated that novel cancer vulnerabilities can be discovered from genome-scale, loss-of-function cell viability screens5. From these data, both biological insights6,7 and therapeutic hypotheses8,9 can be developed. Here, we systematically analyze CRISPR–Cas9 loss-of-function screens across 851 human cancer cell lines to identify novel candidate therapeutic targets in ovarian cancer. We identified an unexpected synthetic lethal relationship between increased expression of the phosphate importer SLC34A2 and loss of the phosphate exporter XPR1. We provide compelling evidence for the therapeutic development of XPR1 inhibitors through extensive in vitro and in vivo validation, analysis of patient samples and proof-of-concept pharmacologic inhibition. The relationship between increased phosphate import and reliance on phosphate export suggests that intracellular phosphate accumulation is toxic to cancer cells, and we leverage coessentiality data to elucidate XPR1 as a member of a phosphate efflux protein complex also containing KIDINS220. Together, these data highlight the power of functional genomics screens to identify compelling therapeutic targets and elucidate their biological function.

Results

Loss of XPR1 is toxic to gynecological cancers that express SLC34A2. To identify novel therapeutic targets for ovarian and uterine cancers, we analyzed genome-scale, pooled CRISPR–Cas9 loss-of-viability screens in 851 genomically characterized human cancer cell lines as part of the Cancer Dependency Map10–12. We focused on genes that, when inactivated, selectively lead to loss of viability in ovarian or uterine cancer cell lines, since a broad killing pattern is more likely to represent mechanisms that would be poorly tolerated if pharmacologically inhibited. This analysis (Fig. 1a) yielded ‘selective dependencies’ such as the transcription factor PAX8 (ref. 13), a known lineage-restricted transcription factor. This analysis also revealed that inactivation of the phosphate exporter XPR1 has a cell-killing pattern that is highly selective and enriched in ovarian and uterine cancers (Extended Data Fig. 1a). XPR1 is a transmembrane protein13 and the only phosphate exporter annotated in the human genome14.

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Fig. 1 | Functional genomics identifies XPR1 loss as a cancer vulnerability in SLC34A2-high ovarian and uterine cancers. a, For the >18,000 genes tested in CRISPR-Cas9 loss-of-viability screens, the selectivity of the killing profile across all 851 cell lines (x axis, likelihood ratio test; Methods) and the enrichment of that gene’s dependency (Chronos score) in ovarian and uterine cancers (y axis) are plotted. The top 5% most predictable dependencies are highlighted in teal, where a random forest model using the genomic and molecular features of cancer cell lines can predict the strength of dependency. b, Heatmap indicating XPR1 and SLC34A2 expression (log2(TPM + 1)) and dependency (CERES) values across all cell lines, ranked approximately by decreasing dependency on XPR1. The Pearson correlation (cor.) coefficient across all 851 cell lines is indicated. c, Across a panel of ovarian and uterine cancer cell lines, viability effects after inactivation of XPR1 were evaluated by comparison with negative control sgRNA and sgRNA targeting pan-essential genes (n = 3 independent transductions representative of at least n = 2 independent experiments). Note that A2780 is not considered to be ovarian cancer despite its historical annotation. Data are scaled such that a value of 0 represents the viability effect of CRISPR–Cas9 genome editing and −1 represents loss of an essential gene. High SLC34A2 expression indicates mRNA expression >3 TPM. d, Viability assessment 7 days after suppression of XPR1 using the indicated shRNA or seed-matched controls (shSeed; n = 5 technical replicates representative of at least n = 3 independent experiments per cell line). e, Six days after induction of shXPR1 in the indicated cell lines, cells were stained with DAPI to distinguish live and dead cells, and with Annexin V (AnnV) to distinguish non- and pre-apoptotic cells (n = 1 flow cytometric analyses of at least 10,000 cells, representative of n = 2 independent experiments). Right, quantification of percentage of cells in the indicated quadrants. f, Analysis of cell death pathways in OVISE and IGROV1 5 days after suppression of XPR1 by shRNA, using protein arrays (n = 1). Note that OVISE has wild-type (WT) TP53 while IGROV1 has an inactivating mutation in TP53.
We next pursued the molecular basis of the selective dependency on XPR1. Using $>100,000$ molecular features of cancer cell lines\(^ {15}\), we built multivariate models—potential ‘biomarkers’ of response—to predict XPR1 dependency\(^ {16,17}\). Remarkably, the feature that most robustly predicted XPR1 dependency was expression of the phosphate importer SLC34A2 (Fig. 1b; Pearson coefficient = –0.42 in all cell lines). SLC34A2 overexpression in ovarian cancer is well documented\(^ {16,19}\) and was highly correlated with XPR1 dependency in cell lines from the ovarian clear cell, high-grade serous and endometrial adenocarcinoma lineages (Extended Data Fig. 1b,c).

We initially validated the pooled screening results in a total of seven SLC34A2-high- and four -low-expressing cancer cell lines, confirming that XPR1 is a selective and strong dependency in the context of SLC34A2 overexpression (Fig. 1c,d and Extended Data Fig. 2). Loss of XPR1 profoundly slows cell growth and leads to an increase in growth-inhibitory and proapoptotic markers (Fig. 1e,f and Extended Data Fig. 2e,f). We next assessed XPR1 dependency in a CRISPR–Cas9-based tumor formation competition assay with 74 single-guide RNAs (sgRNA), and observed XPR1 sgRNA depletion in SLC34A2-high tumors (Fig. 2 and Extended Data Fig. 3). In contrast, sgRNAs targeting other metabolic dependencies, such as the ferroptosis regulator GPX4, were depleted in vitro but not in vivo, as previously reported\(^ {20,21}\). These results indicate that XPR1 dependency is retained in vivo.

**Evidence of phosphate dysregulation in primary patient samples.** To extend the relevance of XPR1 and SLC34A2 beyond cell lines, we evaluated the relationship between XPR1 and other cancer vulnerabilities in a tumor formation competition assay, as described in a for OVISE (squares) and SNGM (circles) cancer cell lines ($n = 2–3$ independent tumors derived from the same transduction per cell line per time point). GPX4, glutathione peroxidase 4, a metabolic dependency reliant on the amount of peroxidized lipids in the metabolic environment of cancer cells; PAX8, paired box 8, a transcription factor dependency in many ovarian cancer cell lines; POLR2D, RNA polymerase II subunit D, a pan-essential gene used as a positive control. Bottom, the significance (sig.) of depletion of three sgRNAs targeting XPR1 relative to seven control sgRNAs was calculated via t-test and corrected for multiple comparisons with the Holm–Sidak method.

**Fig. 2** | **XPR1 inactivation prevents tumor formation in vivo.** **a.** Experimental design for in vivo competition assays. Using a rapid infection-and-selection protocol, pooled sgRNA can be introduced via lentivirus into cancer cell lines and inoculated as subcutaneous xenografts, and the effect of gene inactivation can then be evaluated in an environment more physiologically relevant than tissue culture (TC). **b.** Following rapid infection with pooled sgRNA, 8 million SNGM or OVISE cells were grown in TC and in parallel were inoculated as subcutaneous xenografts and allowed to grow. Tumor tissue was harvested at the indicated time points. **c.** Evaluation of sgRNA targeting XPR1 and other cancer vulnerabilities in a tumor formation competition assay, as described in a for OVISE (squares) and SNGM (circles) cancer cell lines ($n = 2–3$ independent tumors derived from the same transduction per cell line per time point). GPX4, glutathione peroxidase 4, a metabolic dependency reliant on the amount of peroxidized lipids in the metabolic environment of cancer cells; PAX8, paired box 8, a transcription factor dependency in many ovarian cancer cell lines; POLR2D, RNA polymerase II subunit D, a pan-essential gene used as a positive control. Bottom, the significance (sig.) of depletion of three sgRNAs targeting XPR1 relative to seven control sgRNAs was calculated via t-test and corrected for multiple comparisons with the Holm–Sidak method.
Fig. 3 | Expression of XPR1 and SLC34A2 in patient samples indicates cancer-specific phosphate dysregulation caused by lineage survival transcription factor PAX8. a, SLC34A2 is expressed in ovarian and uterine tumor samples at levels sufficient to predict dependency on XPR1. RNA expression values for SLC34A2 were compared within the indicated lineages. XPR1 dependency status is indicated by color where applicable (CERES < −0.5). Q-values indicate the likelihood of the indicated populations having the same level of SLC34A2 expression according to two-sided Wilcoxon ranked-sum test with Bonferroni correction for multiple comparisons. Boxplots are drawn indicating the first and third quartiles, and whiskers span to the largest value within 1.5 × interquartile range. b, Expression of SLC34A2 was measured using RNA-seq (n = 1) after stable overexpression of PAX8 as indicated (PAX8 O/E), and induction of a PAX8-targeting (sg4) or control (sg9) sgRNA and dCas9-KRAB. c, Seven days after transduction with the indicated sgRNA (n = 2, separate transductions), RNA was extracted, converted to cDNA and the expression of SLC34A2 measured using RT–PCR. Significance was assessed by comparison of expression relative to sgChr2-2 across two cell lines with a one-tailed t-test, and corrected for multiple comparisons using the Bonferroni method. Data are representative of n = 2 independent experiments. d, XPR1 copy number heatmap for a ~2.5-Mb region of chromosome 1 indicating XPR1 amplification in TCGA serous ovarian cancer22. Each patient sample is represented by a horizontal line. Red and blue indicate copy gain and loss, respectively. Dashed vertical lines represent the location of indicated genes. Data are a subset of 489 samples, rank ordered by highest copy gain to indicate both focal and chromosome arm variants. e, XPR1 mRNA expression is increased in ovarian and uterine cancer. XPR1 mRNA expression values from the same sources as in a are compared for the indicated tissues, with TCGA OV and TCGA UCEC color coded by XPR1 copy number status as determined by GISTIC analysis. Boxplots are drawn as in a. Statistical differences between tissues were determined as in a. Correlation of XPR1 copy number and expression was performed using Spearman’s ranked correlation test. See Methods for exact n values. Del, deletion; amp, amplification; exn, expression; FKPM, fragments per kilobase million.
In contrast to the more restricted pattern of expression of SLC34A2 and PAIX8, XPR1 is widely expressed in both normal and cancer tissues (Extended Data Fig. 4f). Nevertheless, we found strong evidence for positive selection of XPR1 copy number amplifications and enhanced mRNA expression in ovarian and uterine cancer, consistent with its dependency in these tissues (Fig. 3d,e). In ovarian cancer these amplifications were often focal, involving only the XPR1 gene (Fig. 3d; \( q = 0.0015 \) (ref. \(^3\)) whereas in uterine cancer, broader and less significant amplifications were observed (Extended Data Fig. 4g; \( q = 0.568 \)). XPR1 mRNA expression levels were correlated with XPR1 copy number alterations, but other mechanisms probably also contribute to XPR1 mRNA expression (Fig. 3e). Thus, the dysregulated expression of the essential transcription factor PAIX8 results in enhanced expression of SLC34A2, thereby creating dependency on XPR1.

XPR1 loss causes toxic phosphate accumulation. To further understand the mechanism by which XPR1 loss of function results in cancer cell death, we performed a genome-wide CRISPR–Cas9 rescue screen to determine which genes, when inactivated, were capable of rescuing XPR1-mediated loss of viability (Fig. 4a and Extended Data Fig. 5). Remarkably, the top-rescuing gene among >18,000 tested was also the top predictive biomarker: SLC34A2. We further demonstrated that SLC34A2 is both necessary and sufficient to confer XPR1 dependency in three ovarian and uterine cell lines (Fig. 4b).

The observation that high expression of the phosphate importer SLC34A2 is required for loss of cell viability after inactivation of the phosphate exporter XPR1 led us to hypothesize that accumulation of intracellular phosphate is toxic to ovarian and uterine cancer cells (Fig. 4c). XPR1 is the only known phosphate exporter in humans\(^4\), suggesting that, in the context of increased phosphate import, XPR1-dependent phosphate efflux would be in higher demand, consistent with frequent copy number amplifications observed in patient samples (Fig. 3e).

Although the extracellular availability of phosphate in typical tissue culture medium far exceeds what is physiologically relevant, we found no correlation between the phosphate content of growth medium and XPR1 dependency across the Cancer Dependency Map dataset (Extended Data Fig. 6a). Furthermore, XPR1 dependency was retained when cells were adapted to growth medium with near-physiological phosphate concentrations (reduced by \( \sim 90\% \) from 72.8 to 7.8 mg dl\(^{-1}\); Extended Data Fig. 6b,c), indicating that XPR1 dependency is not an artifact of high concentrations of extracellular phosphate.

Consistent with the phosphate accumulation hypothesis, we observed two-to-four-fold increased intracellular phosphate following XPR1 suppression (Fig. 4d). These large fluctuations in intracellular phosphate co-occur with loss of cell viability (Extended Data Fig. 6d). To understand the cellular response to phosphate accumulation, we used single-cell RNA sequencing (RNA-seq) of 2,501 cells across eight ovarian and uterine cancer cell lines at an early time point following XPR1 inactivation (Fig. 4e and Extended Data Fig. 7a–g). The resulting transcriptional signature reflected cellular attempts to restore phosphate homeostasis, including the upregulation of FGF23. This critical phosphate homeostatic hormone is typically expressed in osteogenic bone cells, and its expression in ovarian cancer cells—although not represented at the protein level (Extended Data Fig. 7h)—is consistent with sensing of elevated phosphate\(^5\). We also observed downregulation of phosphate importers at both the mRNA (Fig. 4e) and protein level (Extended Data Figs. 2c and 7i) following XPR1 inactivation or suppression.

Phosphate efflux activity of XPR1 is required for cancer cell survival. We next confirmed that the phosphate efflux function of XPR1 is critical for cell survival. Expression of a naturally occurring hypomorphic XPR1 mutation (L218S), associated with a rare brain calcification disorder\(^6\),\(^7\), failed to rescue endogenous XPR1 inactivation whereas WT XPR1 fully restored cell viability (Fig. 5a and Extended Data Fig. 8). In addition, we pharmacologically inhibited XPR1 using a previously reported protein inhibitor (XRBD; Fig. 5b,c) and found that its cancer cell line growth-inhibitory effects paralleled inactivation of XPR1 (Fig. 5d). Together, these results indicate that inhibition of the phosphate efflux capacity of XPR1 in SLC34A2-high cells is sufficient for loss of cancer cell viability.

The activity of XPR1 is entirely dependent on a novel partner protein, KIDINS220. To gain further insight into the mechanism by which XPR1 regulates phosphate homeostasis, we analyzed the Cancer Dependency Map for genes with dependency profiles highly correlated to XPR1. These ‘codependencies’ often indicate proteins that are part of the same protein complex\(^8\)\(^–\)\(^10\). Of the \( \sim 18,000 \) genes analyzed, XPR1 dependency is most strongly correlated with that of KIDINS220, a gene with no known connection to phosphate homeostasis\(^11\)\(^–\)\(^14\). (Pearson correlation = 0.81; Fig. 6a and Extended Data Fig. 9a). Given the strength of this correlation, we extensively validated KIDINS220 dependency (Extended Data Fig. 9b,c) and hypothesized that KIDINS220 might be part of an XPR1 phosphate export complex.

In support of an XPR1–KIDINS220 protein complex, protein interaction databases indicate that XPR1 and KIDINS220 interact with each other (Extended Data Fig. 9d). Further, their gene expression is highly correlated across diverse tissues (Extended Data Fig. 9e), suggesting cofunction and coregulation. To confirm this interaction, we performed coimmunoprecipitation experiments and found that XPR1 and KIDINS220 indeed interact with each other and with several other partner proteins (Fig. 6b,c and Extended Data Fig. 9f,g). Native XPR1–KIDINS220 is consistent with a highly oligomerized protein complex (Fig. 6d). We mapped the XPR1–KIDINS220 interaction to the C terminus of XPR1 containing the EXS domain (Fig. 6b and Extended Data Fig. 9g), an evolutionarily conserved domain known to be required for XPR1 trafficking between the Golgi apparatus and plasma membrane to achieve phosphate efflux\(^15\)\(^–\)\(^17\). In contrast, the N-terminal SPX domain of XPR1, which has been implicated in phosphate efflux and regulation\(^18\)\(^–\)\(^20\), was neither necessary nor sufficient to bind KIDINS220 (Fig. 6b).

Further supporting an XPR1–KIDINS220 protein complex, we found dramatically decreased KIDINS220 protein levels following XPR1 genetic inactivation or suppression, but not after inhibition by XRBD (Extended Data Figs. 2c, 7i and 9h). In addition, KIDINS220 inactivation decreased XPR1 cell surface localization and dramatically changed the sedimentation pattern of XPR1, indicating that native localization and conformation of XPR1 requires KIDINS220 (Fig. 6d–f and Extended Data Fig. 9h). Finally, we measured phosphate efflux directly and found that inactivation of either XPR1 or KIDINS220 impaired it to a similar degree (Fig. 6g), and resulted in increased intracellular phosphate (Extended Data Fig. 9i). These results, taken together, indicate that phosphate efflux is achieved by the XPR1–KIDINS220 protein complex and that loss of either complex member leads to a disruption in the phosphate efflux required for cancer cell survival.
**Fig. 4 | XPR1 inactivation in SLC34A2-high ovarian cancer causes loss of cell viability via dysregulation of intracellular phosphate homeostasis.**

a, Identification of rescue genes that protect OVISE ovarian cancer cells from XPR1 dependency. Beta-scores (determined by MAGeCK MLE) represent the change in representation for each gene from the initial library to the final time point for either the control condition (x-axis) or in combination with XPR1 inactivation (y-axis). See Methods for full experimental and analytical details. *n* = 1 transduction per cell line, expanded and cultured as *n* = 2 independent cultures. b, SLC34A2 status of normally XPR1-resistant (ES2, SLC34A2-low (lo)) or XPR1-sensitive (EMTOKA and OVISE, SLC34A2-high (hi)) cell lines was modified by overexpression (O/E) or inactivation (KO) of SLC34A2, and XPR1 dependency was evaluated as in Fig. 1c (*n* = 3 separate transductions, representative of at least *n* = 2 independent experiments per cell line). c, Because of their relative directionalities of phosphate transport, we hypothesize that XPR1 perturbation is toxic because of intracellular phosphate accumulation in SLC34A2-high ovarian and uterine cancers. d, At various time points after treatment with doxycycline and induction of shRNA, intracellular phosphate was measured in OVISE and IGROV1 cell lines (*n* = 3 separate measurements per condition, representative of at least *n* = 3 separate experiments). e, A pool of eight cancer cell lines was transduced with lentivirus to inactivate XPR1, and 4 days later cells were subjected to 10x single-cell transcriptomic measurement (*n* = 1 transduction). The measured transcriptional change (relative to control sgRna infection) in the indicated genes is plotted on the left for the three cell lines with the largest and most correlated transcriptional change (Extended Data Fig. 7), and for the other five on the right. Blue and red lines connect cell lines displaying decreased or reduced expression, respectively, upon XPR1 inactivation. f, XPR1 perturbation causes compensatory inhibition of phosphate uptake, measured by incubation of OVISE ovarian cancer cells in medium supplemented with 32PO4− phosphate for 30 min before washing off excess medium and cell lysis (*n* = 1 transduction measured in technical duplicate, representative of *n* = 2 independent experiments). Significance was assessed by one-way analysis of variance and corrected for multiple comparisons using Bonferroni’s method.
A striking feature of XPR1- or KIDINS220-mediated loss of cell viability is the formation of large, cytoplasmic, vacuolar structures preceding loss of cell viability (Fig. 7 and Supplementary Video 1). Colocalization with the acidic dye LysoTracker and the lysosomal marker LAMPI (Fig. 7b,c) suggested they may be related to the lysosomal system. Ultrastructural analysis by transmission electron microscopy (TEM) found these structures to be bound by a double membrane that was often fenestrated (Fig. 7d,e). Although they lack the electron-dense appearance typical of lysosomes, we did note their fusion with lysosomes (Fig. 7d,c).

**Discussion**

This study highlights a previously unappreciated strategy used to kill cancer cells: the disruption of phosphate homeostatic mechanisms that are normally tightly regulated. Interestingly, antibody–drug conjugates targeting the phosphate importer SLC34A2 (ref. 51) have been explored for the past decade, but this approach exploits SLC34A2 simply as a biomarker of ovarian cancer rather than serving as a mechanism to disrupt phosphate homeostasis. Furthermore, these strategies may have alternate mechanisms of resistance and/or on-target toxicity due to the physiological roles for SLC34A2 in intestinal54 and lung 38,39 tissues. Nevertheless, the expression and regulation of SLC34A2 within fallopian tube epithelial cells—the probable cell of origin of ovarian and uterine cancers—has not been extensively studied. We observed elevated SLC34A2 expression in normal fallopian tube samples (Fig. 3a), and hypothesize that SLC34A2 may play a similar role in the lung regarding uptake of inorganic phosphate derived from surfactant metabolism.

In the context of cancer, it is not clear whether enhanced SLC34A2 expression is required for ovarian and uterine carcinogenesis or survival in vivo. Our data clearly indicate that SLC34A2 is not required for cancer cell survival in vitro (Figs. 1b and 4a,b) and that these cell lines display a profound ability to downregulate SLC34A2 in response to XPR1 inhibition (Extended Data Figs. 2c and 7i), suggesting that strong overexpression of SLC34A2 is not necessarily required for ovarian cancer cell survival. We hypothesize...
Fig. 6 | KIDINS220 is a critical component of the phosphate efflux protein complex. **a**, Across 851 cancer cell lines, the viability defects of XPR1 and KIDINS220 inactivation in each cell line were plotted and the Pearson correlation is indicated. A Chronos value of –1 is the median viability defect of inactivating pan-essential genes in a given cell line. **b**, The interaction between the V5-tagged XPR1 mutant and KIDINS220 was evaluated using coimmunoprecipitation. XPR1 WT corresponds to isoform nM_004736, while XPR1 (Short) corresponds to isoform nM_001135669. Green arrows indicate expected molecular weight; \( n = 1 \) experiment representative of \( n = 3 \) independent transfections. **c**, After XPR1-V5 immunoprecipitation, interacting proteins were identified using in-gel tryptic digestion followed by mass spectrometry. The \( x \) and \( y \) axes show the total number of peptides per protein detected specifically in XPR1 immunoprecipitation for \( n = 2 \) independent transfections and immunoprecipitations. Higher-abundance proteins (more than ten peptides detected in IP:XPR1) are highlighted in teal. **d**, Glycerol gradient sedimentation analysis of XPR1-containing native protein complexes with or without KIDINS220 inactivation. The crude lysate of the indicated cell lines was layered onto 10–30% glycerol gradients and centrifuged to fractionate protein complexes by molecular weight, followed by immunoblot analysis (\( n = 1 \) centrifugation representative of \( n = 3 \) independent experiments). The elution profile of protein standards is indicated below the immunoblot. **e**, Localization of XPR1-V5 proteins after inactivation of KIDINS220. Scale bars, 100 \( \mu \)m (\( n = 1 \) technical replicate of \( n = 2 \) independent experiments). **f**, Evaluation of XPR1 cell surface localization after KIDINS220 inactivation. \( n = 1 \) flow cytometric analysis of 10,000 cells, representative of \( n = 4 \) independent experiments. **g**, Cellular phosphate efflux after KIDINS220 inactivation. Three days after genetic inactivation of XPR1 or KIDINS220, cellular phosphate efflux was assessed. Cells were loaded with \( { }^{32} \)P-labeled phosphate, washed extensively to remove excess \( { }^{32} \)P and then phosphate efflux was measured at the indicated times by isolation of conditioned medium and cellular lysates (\( n = 3 \) technical replicates of the same transduction, representative of \( n = 3 \) independent transductions). Phosphate efflux is calculated as the percentage of \( { }^{32} \)P in the conditioned medium relative to total \( { }^{32} \)P measured for that sample. Note that medium without phosphate does not stimulate phosphate efflux, and was used as a control.
that elevated activity of the lineage survival transcription factor PAX8 drives SLC34A2 expression, and that PAX8-driven SLC34A2 expression is sufficient to engender XPR1 sensitivity. Further work is needed to elucidate the many mechanisms by which SLC34A2 is regulated in both normal and cancer cells.

This study also highlights fundamental gaps in our understanding of how intracellular phosphate is sensed, regulated and stored. Although excessive phosphate has previously been shown to be toxic55,56, the exact mechanism of this toxicity is unknown. Our transcriptional profiling experiments failed to identify a clear ‘phosphate stress response’, and yet XPR1 perturbation causes a profound delay in cell growth. Along with cell cycle arrest, we also observed large, acidic, vacuolar structures (Fig. 7). Whether these structures reflect a compensatory cell survival mechanism involving the sequestration of potentially toxic phospho-metabolites57,58, or are themselves the cause of cell death, remains to be determined.

Finally, this study uses the power of large-scale functional genomics to elucidate biological processes: we show that the phosphate efflux activity of XPR1 is entirely dependent on KIDINS220. The KIDINS220 gene essentiality profile (Fig. 6a) is both selective and highly correlated with XPR1, suggesting a specific role for these two proteins in phosphate efflux as opposed to a more general or pleiotropic role. We show that the XPR1–KIDINS220 protein complex is probably oligomeric (Fig. 6d) and trafficks between multiple subcellular compartments, an activity previously attributed to both proteins45,50. Interestingly, we found that XPR1 inactivation or suppression invariably caused loss of KIDINS220 protein; nevertheless, KIDINS220 protein loss is not required for cell death because the XRBD phosphate efflux inhibitor did not decrease KIDINS220 protein levels (Extended Data Fig. 7i). The exact mechanism of the XPR1–KIDINS220 phosphate efflux complex requires further study, as does reconciliation of cellular phosphate efflux with other activities attributed to KIDINS220, such as neurotrophin signaling47,48 and genetic associations between XPR1, KIDINS220 and various diseases59.

Together, this study establishes the XPR1–KIDINS220 protein complex as a previously unrecognized therapeutic target in ovarian cancer.
and uterine cancer. Moreover, the work highlights disruption of phosphate homeostasis as a potential new anticancer strategy.

**Methods**

In vivo studies were carried out under the Institutional Animal Care and Use Committee (IACUC) of the Broad Institute under animal protocol no. 0194-01-18.

**Statistics and reproducibility.** In general, tissue culture experiments were conducted with cells grown in parallel vessels to evaluate the reproducibility of numeric data (for example, the two wells treated with an identical dose of XRBD in Fig. 5d). In most cases, experiments were always conducted at least twice to confirm effect size; in most situations only representative experiments are shown. This is the case for data from each cell line in Figs. 1c–e, 3c, 5d, 6b, c, d, e, f, g, h (all replicates are shown), and in Extended Data Figs. 2a–f, 4b–d, 5a, b, 6a, b, c, d, e, f, 7a, b, c, 8a, b, c, d, e, f, 9a, b, c, d, e, f, 10a, b, c, d, e, f, g, h. The experiment presented in Extended Data Fig. 2a was conducted once only. The cell lines presented in Fig. 6f and Extended Data Fig. 9h are representative of at least two clones derived by single-cell isolation. For the in vivo competition assay (Fig. 2), cells were infected with the small library of sgRNA only once, and parallel cultures of different subcutaneous xenografts were treated as technical replicates. Sample sizes were chosen (eight mice, two tumors per mouse) to account for the penetrance of tumor development, to evaluate multiple time points and to have at least two animals per time point. Those animals with the largest tumor burden were euthanized at the indicated time points (that is, no randomization was employed). No statistical methods were used to determine sample size. For cell death pathway profiling after XPR1 suppression (Fig. 1f), ratios were calculated. For immunohistochemistry (Fig. 4e), experiments were conducted once only. Statistical significance testing is discussed in more detail in Methods.

**Genetic dependency data.** The dependency data used in this manuscript were derived from the Public Avana 21Q2 dataset, consisting of dependency data for 18,025 genes across 859 cancer cell lines from 26 lineages. Expression data from the Cancer Cell Line Encyclopedia (CCLE) were also used. These data are available online, at https://depmap.org/portal/download/sil/.

**Cell lines.** Cancer cell lines ES2, HeyA8, A2780, 59 M, SNU8, OVK18, SNGM, OVISE, EMTOKA, IGROV1, OVCA4, KURAMOCHI, RMGI, COV143a, HJO54, HE6C and JHUEM1 were collected by the CCLE before distribution for our use. The source of the aforementioned cell lines can be found at DepMap.org. All cell lines were adapted to growth in RPMI 1640 (Corning) +10% fetal bovine serum (FBS) before use. All cell lines are routinely validated using short-tandem repeat profiling.

**sgRNA sequences.** The negative control guides sgChr2 and sgAVS1 were designed to cleave a gene desert and an intronic region in PPP1R1C, respectively, to control for the effects of DNA double-strand breaks. sgLaCZ targets a sequence not found in the human genome. Positive-control sgRNA targets common essential splicing factors (SF3B1), ribosomal subunits (POLR2D) or kinesin motor proteins (KIF11). The 20-base-pair (bp) targeting sequences can be found in Supplementary Table 2.

**Lentiviral production.** Lentiviral production was performed using HEK293T cells as described on the Broad Institute Genetic Perturbation Web Portal (https://portals.broadinstitute.org/gpp/public/).

**Plasmids, overexpression constructs and site-directed mutagenesis.** Open reading frames (ORFs) of the following genes were obtained from a genome-scale library of annotated genes46. SLC34A2 (NM_006424) was isolated from this library in pDONR223 and was transferred into the expression vector plX-TRC313 (similar to Addgene, no. 118001) using gateway cloning. The resultant construct has a C-terminal 3x-myc tag, which was removed after stable integration of plX-TRC313 into cell lines using lentiviral infection, the proper protein product with a V5 tag was detected using immunoblot (not shown). XPR1 constructs (both isoforms, NM_004736 and NM_001135669) were obtained in a similar way. Only the NM_004736 isoform was observed using isoform-agnostic PCR primers and complementary DNA generated from the OVISE cancer cell line. Mutations were introduced with PCR-based methods, either the Q5 Site-directed Mutagenesis kit (NEB, no. M0554S) for large deletions or the QuickChange II XL (Agilent, no. 200521) for point mutations, and were confirmed using Sanger sequencing.

**Immunohistochemistry.** Cell pellets were fixed using paraformaldehyde (PFA) and then paraffin embedded. Immunohistochemistry was performed on the Leica Bond RX automated staining platform using the Leica Biosystems Retina Detection Kit. The antibody for SLC34A2/MX35 (Creative Biolabs, no. TAB-467MZ, recombinant) was run at 1:400 dilution with citrate antigen retrieval.

**CRISPR viability assays.** CRISPR viability assays were performed in 96-well plates with cells seeded at a low density to allow for logarithmic growth throughout the entire assay. For 7-day assays, cells were seeded and infected with lentiviruses expressing the sgRNA in pXPR-BRD003 on day 0. The next day, infection medium was replaced with fresh medium and puromycin postinfection. Viability was evaluated by the addition of 25µl per well of Cell Titer Glo (Promega) reagent and luminescence measured. Infection efficiency was determined by comparing the viability of cells with and without puromycin after infection, and assays were repeated if <80% of cells were infected with every sgRNA. The data shown normalized such that (shRNA (targeting the common essential genes KIF11, SF3B1 and POLR2D)) was ~1.0. For 10-day assays, infections were carried out in six-well plates. Three days postinfection, cells were extracted and seeded into replicate 96-well plates. On days 3, 7 and 10 postinfection, viability was evaluated by the addition of 50µl per well of Cell Titer Glo (Promega) reagent and monitoring of luminescence. Fold change (FC) viability was calculated by comparison of days 7 or 10 to day 3, and data were normalized as above.

**Short hairpin RNA sequences.** Short hairpin RNA sequences for XPR1 were selected from project DRIVES's subgenome-scale shRNA library47 using DEMETER2 estimates for on- and off-target seed effects48. A detailed protocol for selection of shRNA using these datasets is available online (https://protocols.io/ view/shrna-selection-and-quality-control-for-cancer-tar-bfmmjk5e). Doxycycline (dox)-inducible shRNA expression was accomplished by cloning these sequences into the pSRT3P-UC6-Tet (shRNA)-EF1-TetRep-2A-Puro vector (Cellecta, no. SVSHU/6TEP-L). shRNA seed-matched negative control sequences were generated by substitution of complementary by sequences into positions 9–11 bp of the target shRNA using a web-based tool (https://web.archive.org/web/201806051430/http://rmi.nih.gov/haystack/C911Calc2.html).

**CRISPR and seed-matched control sequences were rigorously tested for both on-target XPR1 suppression and off-target cell viability effects. Off-target cell viability effects were determined when a given shXPR1 construct knocked down XPR1 but produced strong loss of cell viability, regardless of whether a cell line was predicted to be XPR1 dependent or nondependent. shRNA target sequences are provided below.

**Antibodies.** A full list of antibodies and their respective dilutions is included in the Reporting summary accompanying this paper.

**Protein analysis of cell lysates by immunoblotting.** For protein analyses, cells were grown in six-well dishes and harvested by washing with PBS and incubation with 0.5ml of Tryple until all cells had lifted, followed by dilution to 1.5ml with PBS. Cells were then centrifuged and washed once with PBS and lysed with radioimmunoprecipitation assay buffer (150mM NaCl 1.0% IGEPA, CA 630, 0.5% sodium deoxycholate, 0.1% SDS, 50mM Tris pH 8.0) supplemented with Complete, Mini Protease and Phosphatase Inhibitor Cocktail Tablets (Roche).

**For quantitative determination of cellular proteins (Fig. 6b), lysates were analyzed using Protein Simple, an automated capillary-based protein separation and immunoblotting assay. Lysates were prepared as above and diluted with sample buffer and 40mM DTT before loading 3 µl of sample onto each plate.**

**Foci formation.** Cells stably expressing doxycycline-inducible short hairpins against XPR1 or the corresponding seed controls were plated at three different densities (18,000, 12,000 and 6,000 cells per well) to determine optimal seeding.
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were selected with 4 × staining, 10% acetic acid was incubated in each well for 20 min, diluted 1:3 with to remove residual crystal violet and left to dry overnight. To quantify crystal violet to achieve roughly 30–50% infection efficiency and nearly 100% selection 50 h after minimize time in tissue culture, we optimized infection and puromycin conditions lentiviral library of 74 total sgRNAs (including nontargeting, negative controls consistent with more than one cell per droplet. Then washed, and 10,000 individual cells analyzed by flow cytometry. Data were with DAPI, washed with Annexin Staining Buffer and incubated for 15 min at room temperature with Annexin V-APC (ThermoFisher, no. A35110). Cells were then washed, and >10,000 individual cells analyzed by flow cytometry. Data were analyzed using FlowJo, gating against smaller cellular debris as well as events consistent with more than one cell per droplet.

In vivo sgRNA competition assay. These studies were used under the IACUC of the Broad Institute under animal protocol no. 0194-01-18. A detailed protocol for the tumor formation competition assay is available online (https://www.protocol.hcb.io/view/in-vivo-nano-pooled-sgRNA-competition-assays-t-bishman). A small lentiviral library of 74 total sgRNAs (including nontargeting, negative controls targeting gene deserts or introns, positive controls targeting pan-essential genes and experimental sgRNA) was made in an arrayed format and then pooled. To minimize time in tissue culture, we optimized infection and puromycin conditions to achieve roughly 30–50% infection efficiency and nearly 100% selection 36 h after infection. OVISE cells were selected with 8 μg/ml puromycin while SNGM cells were selected with 4 μg/ml puromycin.

Fifty hours after infection, cells were lifted, counted and diluted in 50% matrigel to a final concentration of 8 million cells 100 μl. Some cells were frozen to determine the early representation of the library. For in vitro experiments, cells were replated and grown under standard conditions for 2 weeks. In total, the study used 26 female, 7-week-old, Rag1−/−IL2rg−/− mice obtained from The Jackson Laboratories. For in vivo experiments, mice were anesthetized under isoflurane gas and two bilateral subcutaneous xenografts inoculated in each of five mice (ten tumors per experiment). Tumors were measured twice weekly with calipers, and tumor volumes calculated using the formula: 4/3 × π × width × length x 2 (mm3). Body weights were recorded once or twice weekly during the course of all studies. Mice were euthanized and tumors harvested on days 14, 21 and 28 after inoculation, ensuring they had not reached endpoint (tumor size >2,500 mm3; no visual distress and no evidence of ascites development). After harvesting and weighing, tumors were flash-frozen in liquid nitrogen until required for genomic DNA isolation. At the end of the study (28 days postinoculation), some of the tumors were thawed and minced and genomic DNA extracted from all samples using Qiagen DNeasy Blood and Tissue kits. Sample barcodes were sequenced by Illumina Next-Generation sequencing then deconvoluted with Broad Genetic Perturbation Platform’s PoolQ software for sgRNA read counts. Data from all animals included in the study are provided.

Statistical analysis for in vivo competition assay. The gene-level effect was determined by comparison of normalized sgRNA read counts at the early time point (2 days after infection, the day of inoculation) with those at the indicated time points. If normalized read counts at the early time points were significantly different for a particular sgRNA, that sgRNA was not included in downstream analyses (an indication that the plasmid had not produced lentivirus). sgRNA read counts were normalized such that the seven cutting-control sgRNAs (representing viability effects from CRISPR-Cas9 genome editing) had a median depletion of 0. Next the median fold change of all sgRNAs targeting a particular gene was calculated for those genes with a relative to control sgRNA are reported per replicate in Extended Data Fig. 3. The different time-point replicates were median averaged and are reported in Fig. 2c. The statistical significance of XPR1 depletion was calculated by comparison of the gene-level depletion of XPR1 (three different sgRNAs per replicate) with cutting-control sgRNA (seven different sgRNAs per replicate), using a t-test to compare all replicates at each time point. The test was conducted in GraphPad Prism 8.0 with Holm– Sidak multiple comparisons correction. Corrected Pvalues (q-values) are reported in Fig. 3c. Test statistics are as follows: OVISE TC 2 weeks (n = 2, t-value = 29.6, 14 degrees of freedom); OVISE tumor 2 weeks (n = 4, t-value = 8.9, 30 degrees of freedom); OVISE tumor 3 weeks (n = 4, t-value = 7.1, 30 degrees of freedom); OVISE tumor 4 weeks (n = 2, t-value = 4.6, 30 degrees of freedom); SNGM TC 2 weeks (n = 2, t-value = 19.1, 14 degrees of freedom); SNGM tumor 2 weeks (n = 3, t-value = 5.6, 16 degrees of freedom); SNGM tumor 3 weeks (n = 4, t-value = 2.5, 28 degrees of freedom); SNGM tumor 4 weeks (n = 4, t-value = 5.0, 22 degrees of freedom).

Comparing expression of genes across normal and tumor tissues. We compiled transcripts per million (TPM) gene expression data for normal fallopian tube (GTEx, n = 5), normal ovary (GTEx, n = 88), normal uterus (GTEx, n = 78), ovarian cancer (TCGA, OV, n = 426) and uterine cancer (TCGA UCEC, n = 2380) from the TOIL RSEM log(TPM+0.01) data at Xena Browser (https://xenabrowser.net/) and then converted the data to log(TPM + 1). RNA-seq gene expression for ovarian cancer cell lines (CCLE, n = 40), and uterine cancer cell lines (CCLE, n = 22) was downloaded from the CCLE (https://depmap.org) as log(TPM + 1). Because most TCGA ovarian and uterine samples have relatively high purity (>80%), we used these data directly for the following comparisons. In Fig. 3a and Extended Data Fig. 4c, boxplots were drawn using the ‘geom_boxplot’ command in the R package ggplot2, such that boxes span the first and third quartiles of values with the median indicated by a line; whiskers extend 1.5× interquartile range, with outliers plotted as individual points. Statistical analysis for in vitro experiments, cells were treated with doxycycline for 5 days with DMSO as a control, and then washed, and reseeded at 40% confluence. Time-course analysis of cell growth. To measure cell growth over time after XPR1 suppression, cells were seeded in a 96-well plate, treated with doxycycline to induce shXPR1 and imaged every 6 h (Essen Incucyte S3). Images were quantified using built-in software.

Proteme for cell death markers. To evaluate markers associated with different cell death pathways, cells were treated with doxycycline for 5 days to induce shXPR1, then both nonattached and adherent cells were collected, pooled and stained for 40 min at room temperature with DAPI, washed with Annexin Staining Buffer and incubated for 15 min at room temperature with Annexin V-APC (ThermoFisher, no. A35110). Cells were then washed, and >10,000 individual cells analyzed by flow cytometry. Data were analyzed using FlowJo, gating against smaller cellular debris as well as events consistent with more than one cell per droplet.

PCR with reverse transcription. After the indicated perturbations, cells were lysed and RNA extracted using Qiazol and phenol-chloroform. Total RNA was determined spectrophotometrically, and normalized amounts of RNA converted to cDNA using the iScript kit (Bio-Rad, no.1708890). Diluted cDNA was then mixed with gene-specific primers and Power Sybr Green Master Mix (Thermo Fisher, no. 4367659) and analyzed on a Quant Studio 7 PCR with reverse transcription (RT–PCR) instrument. A full list of RT–PCR primers used can be found in Supplementary Table 3. The quality of RNA extraction was evaluated by comparison of CT values of cDNA samples with control samples treated in the same way but without the addition of reverse transcriptase. Gene expression values were corrected for loading with a housekeeping gene (VCL). In Fig. 3, SLC34A2 gene expression values were compared using a one-tailed t-test correction for multiple comparisons with Bonferroni’s method and are reported on the y-axis. The difference in median SLC34A2 expression (tissue expression across all tissues) is plotted on the x axis of Extended Data Fig. 4a. In Extended Data Fig. 4b, the correlation between SLC34A2 and PAOX8 mRNA was tested using a two-tailed Pearson correlation test across the indicated tissues (n = 897). In Extended Data Fig. 9e, the correlation between XPR1 and KIDINS220 mRNA was tested using a two-tailed Pearson correlation test for the indicated tissue groups (all 60 tissues, n = 17,194; top 15 correlated, n = 2,799; and for all other tissues, listed from top to bottom, n = 337, 55, 172, 173, 36, 47, 182, 520, 66, 182, 496, 154, 119, 181, and 79, respectively).

Analysis of XPR1 copy number in TCGA. To evaluate the frequency of XPR1 amplification, we evaluated precomputed GISTIC2 (ref. 2) analyses for recurrent copy number alterations in ovarian and uterine TCGA datasets. To compare the expression of XPR1 with its copy number status, XPR1 copy number thresholds as determined by GISTIC—were downloaded from CBIoPortal65,66 and then matched to the corresponding TCGA samples. In total, 410 ovarian cancer and 171 uterine cancer samples were included in this analysis. In Fig. 3d and Extended Data Fig. 4g, each patient sample is represented by a horizontal line; red indicates copy gain and blue indicates copy loss, and dashed vertical lines represent the locations of indicated genes. The samples are rank ordered by highest copy gain, to indicate both focal and chromosome arm variants. Figure 3e, shows testing of whether there was a significantly nonzero correlation between XPR1 copy number and XPR1 mRNA expression using a two-tailed Spearman correlation test, and reports Spearman’s rho and Pvalue. Also in Fig. 3e, the expression of XPR1 by tissue was performed using a Wilcoxon ranked-sum test with Bonferroni correction. Modified screen. The anchor modifier screen was performed as described previously. OVISE cells stably expressing sgRNA targeting Chr2-2, XPR1-1 or XPR1-2 in the lentiviral guide-only vector pXPR-BRD016 were infected with the
Brunello all-in-one vector (pXPR-BRD023), in a format such that each cell received their depletion observed in every arm of the experiment is probably not due to a change in representation was converted to gene-level beta-values, representing the average of phosphate concentrations of each component (Supplementary Table 4 and ref. 19). The XPR1 dependency score was then compared to the concentration of phosphate using a one-tailed Pearson correlation test, in which we expected to find was designed using isogenic cell lines expressing either Firefly or Renilla luciferase; Cell competition assays in low-phosphate media. We thank J. L. Battini for providing the sequence of 300,000 cells in 50 µl filter and diluted to ~1,500 cells µl–1. The detailed protocol can be found through the following changes. SNGM and OVISE Cas9 stable cell lines were plated at 10,000–20,000 cells per well on µ-Slide eight-well coated chamber slides (IBIDI, no. 80826) and simultaneously infected with lentivirus-expressing sgRNAs. The next day, transfected cells were selected with 2 µg ml–1 puromycin for 24 h, removed from puromycin selection and fixed 6 days after infection.

For endoplasmic reticulum labeling, cells were transfused with 24 µl of CellLight ER-GFP, BacMam 2.0 in 200 µl of culture medium 24 h before imaging (ThermoFisher, no. C10590), with no permeabilization step performed. For mitochondrial imaging, cells were treated with 100 nM Mitotracker Red CMXRos (ThermoFisher, no. M7512) in serum-free RPMI for 30 min at 37°C, then Mitotracker dye medium was replaced with normal growth medium (RPMI 1640 with 10% FBS) and incubated for 1 h at 37°C before fixation. All other antibody-based stains were treated as described above. Information on the antibodies used and their concentrations for staining are provided above. Multiplexed transcripational profiling. Multiplexed transcripational profiling (MixSeq)15 was performed using custom pools of ovarian and uterine cancer cell lines. Cancer cell lines were pooled (five to seven cell lines per minipool) based on doublet time, and then frozen. To initiate the experiment, cells were thawed and plated in 12-well dishes. The next day, virus-encoding mixtures of sgRNAs (sgLaCZ/sgChr2-2 or sgXPR1, 1/sgXPR1_2) were prepared under conditions in which each cell received both sgRNAs to discriminate between cell lines (mixture). Cells were treated with 2 µg ml–1 puromycin 24 h after infection. Four days after infection, cells were extracted with TrypLE, spun down, resuspended in cell-staining buffer (PBS + 2% BSA + 0.02% Tween) and counted.

Perturbations were multiplexed for 10X sequencing using Cell Hashing16. Equal numbers of each minipool were then pooled, blocked with 2X blocking buffer (BioLegend) for 10 min on ice and incubated with hash-antibodies (TotalSeq, BioLegend) for 30 min on ice. Cells were then washed three times with cell-staining buffer and resuspended in Cell Capture buffer (PBS + 0.04% BSA), filtered through a 40-µm filter and diluted to ~1,500 cells µl–1. The detailed protocol can be found online (https://www.protocolscience.com/view/cell-hashing-zn56b). Approximately 40,000 cells were then loaded onto a 10X Chromium controller using 3′ single-cell 3′ reagent chemistries. Library preparation and next-generation sequencing were performed as previously reported17.

Sequencing data were processed using 10X Cell Ranger software (v3, hg19 reference genome) run with the Cumulus cloud-based analysis framework. Single-cell methylome polymorphisms (SNP)–based cell line classification and quality control was performed according to the methods described in ref. 17. In brief, for each cell the allelic fractions across a predefined 100,000–SNP reference set were estimated from bulk RNA-seq data using Freebayes18. A logistic regression model was then used to estimate the likelihood of the observed SNP reads for an individual cell having come from each cell line given the allelic fractions across
Phosphate uptake and efflux assays. To determine phosphate uptake or efflux, OVISE cells were infected with lentiviruses expressing Tropinin and the membrane potential dye Rhodamine 123. After 4 days to induce shRNA-targeting XPR1. The conditioned medium was then collected, centrifuged to remove any debris and FGF23 levels were measured according to the manufacturer's instructions (R&D Systems, no. DY2604-05). Similar results were obtained using a tandem mass spectrometry of specific fragment ions for each peptide. Peptide sequences (and hence protein identity) were determined by matching protein database with the acquired fragmentation pattern using the software program Sequest (Thermo Fisher Scientific). Peptides were detected, isolated and fragmented to produce a tandem mass spectrum of specific fragment ions for each peptide. Peptide sequences (and hence protein identity) were determined by matching protein database with the acquired fragmentation pattern using the software program Sequest (Thermo Fisher Scientific).
Article: Zou, Y. et al. A GPX4-dependent cancer cell state underlies the clear-cell phenotype of ovarian cancer. Nature Cancer 20, 415–456 (2021).

Received: 17 July 2020; Accepted: 4 March 2022; Published online: 18 April 2022

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Acknowledgements

This work was funded in part by the Slim Initiative in Genomic Medicine for the Americas, a joint United States–Mexico project funded by the Carlos Slim Foundation (to T.R.G.), and grants from the National Cancer Institute (nos. CA242457 to T.R.G. and CA212229 to D.P.B.). We thank the Dana-Farber/Harvard Cancer Center in Boston, MA, for the use of the Specialized Histopathology Core, which provided histology and immunohistochemistry services and is supported in part by a NCI Cancer Center Support Grant (no. CA063166). We thank J. Barnett, B. Buckley, M. Veneskey, M. Cecilia-Saberi and D. Tennen for technical support.

Author contributions

D.P.B., B.R.P., F.V. and T.R.G. initiated the project and oversaw the research plan. J.K.B, D.P.B. and D.P.B. analyzed genetic dependency data under the supervision of W.C.H., D.E.R., J.B., F.V. and T.R.G. and with support from I.F. and E.S.C. M.V.R., A.A., T.A.S., B.R.P. and D.P.B. conducted viability experiments and immunoblotting. A.A., B.R.P. and D.P.B. conducted the genome-scale modifier screen with analysis support from M.K., J.M.D. and J.M.M. and supervision from J.M.D. In vivo experiments were conducted by A.G. and N.D. under the supervision of F.P. Intracellular phosphate assays were performed by M.V.R. and D.P.B. PAX8 RNA-seq experiments were conducted by K.I. with analytical support from W.N.C. D.P.B. analyzed TGEs, TCGA and CCLE expression datasets with supervision from J.M.M. Multiplexed transcriptional profiling was conducted by B.R.P. and D.P.B. and was analyzed by A.W. and W.N.C. Phosphate uptake and efflux assays were conducted by D.P.B. and L.E.S. with supervision from M.M. A.A. and D.P.B. conducted coimmunoprecipitation experiments. B.R.P. conducted cellular imaging studies. D.P.B. and M.E. conducted ultrastructural analysis. D.P.B., B.R.P., F.V. and T.R.G. wrote the manuscript, and all authors edited and approved the manuscript.

Competing interests

F.V. receives research funding from Novo Holdings. D.E.R. receives research funding from the Functional Genomics Consortium (Abbvie, Janssen, Merck and 1Vir) and is a director of Addgene. T.R.G. previously received cash and/or equity compensation for consulting to GlaxoSmithKline, Sherlock Biosciences and FORMA Therapeutics, is currently a paid consultant for Anji Pharmaceuticals and receives research funding from Bayer HealthCare, Calico Life Sciences and Novo Holdings. W.C.H. is a consultant for ThermoFisher, Solasta, MPM Capital, Ifos, Frontier Medicines and Paraxel and is a Scientific Founder and serves on the Scientific Advisory Board for KSQ Therapeutics. All other authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s43018-022-00360-7.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s43018-022-00360-7.

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Peer review information Nature Cancer thanks Ronny Drapkin and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Extended Data Fig. 1 | XPR1 dependency is observed selectively in SLC34A2-high cancer cell lines. a) For every cell line profiled in the Cancer Dependency Map dataset (N = 851 cancer cell lines), the degree of XPR1 essentiality is plotted on the Y-axis. The Chronos score is a scaled value of gene essentiality, where 0 is the effect of CRISPR/Cas9 genome editing and -1 is the effect of inactivation of pan-essential genes. Note that the ovarian lineage is separated into cancer subtypes. b) For every tissue type, the 10 highest SLC34A2 expressing cell lines were analyzed for their median expression of SLC34A2 (X-axis) and dependency on XPR1 (Y-axis). Note that some lineages may have less than 10 cell lines. Color encodes the correlation of SLC34A2 expression and XPR1 dependency across all cell lines within that lineage. c) Comparison of analytical methods for CRISPR/Cas9 genome-scale loss of function screens.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Validation of SLC34A2 and XPR1 protein levels and viability defects upon shRNA induction. a) Validation of SLC34A2 in cell lines using immunohistochemistry. N=1 experiment. b) Five days after viral transduction of the indicated sgRNA in the indicated cell lines stably expressing Cas9, cells were harvested and XPR1 levels were analyzed by immunoblotting. Note that irrelevant lanes were cropped out for clarity, but that this image represents a single blot at a single exposure. N=1 technical replicate of at least N=5 representative experiments. c) Three days after induction of shRNA, protein levels were measured in cellular lysates by protein simple. Protein levels normalized to vinculin and the untreated (-Dox) conditions are expressed below each band. Note that shXPR1 reagents effectively suppress XPR1 protein levels but shSeed reagents do not. N=1 technical replicate of at least N=5 representative experiments. d) Colony formation assay to measure the long-term (14 day) penetrance and viability effect of suppression of XPR1 using shRNA in IGROV1 and OVISE cells. N=3 technical replicates of at least N=2 representative experiments. e) Growth curves of SLC34A2-expressing cell lines after suppression of XPR1. In 96-well plates, confluency of the indicated cell lines was assessed every 4 hours. N=3 technical replicates of at least N=2 representative experiments. f) Six days after induction of shXPR1 in the indicated cell lines, cells were stained with DAPI to distinguish live and dead cells and Annexin V to distinguish non- and pre-apoptotic cells. N=2 flow cytometric analyses of at least 10,000 cells, representative of N=2 experiments.
Extended Data Fig. 3 | In vivo CRISPR/Cas9 competition assays for target validation in mouse xenografts. 

a) sgRNA abundance in SNGM tumor xenografts was evaluated by PCR and next-generation sequencing analysis, and the fold change compared to the early time point is shown as a heatmap for all of the negative control genes as well as any gene with a > 4 fold change in abundance in any of the screens. Each tumor/replicate is shown as an individual column, N = 1 transduction. 

b) Same as in d, but with the OVISE cancer cell line.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | SLC34A2 in ovarian cancer is likely driven by PAX8. a) Using the combined GTEx, TCGA, and CCLE dataset, the differential expression of SLC34A2 in each tissue relative to the average of all tissues is compared. The relevant gynecological tissues (fallopian tube, ovary, and uterus) are highlighted in teal. The false discovery rate (FDR) was calculated using a two-sided Wilcoxon ranked sum test comparing each group to the average expression across all groups and correcting for multiple comparisons using Bonferroni’s method. The Cancer Genome Atlas abbreviations used include: LUAD = Lung adenocarcinoma; THCA = Thyroid carcinoma; KRP = Kidney renal cell papillary carcinoma; LUSC = Lung squamous cell carcinoma; OV = Ovarian serous cystadenocarcinoma; UCEC = Uterine corpus endometrial carcinoma. b) The expression of PAX8 and SLC34A2 mRNA in the indicated tissues is plotted. The Pearson correlation within these samples is indicated. c) Expression of PAX8 across the indicated tissues was compared as in Fig. 3a. See methods for exact N values. Boxplots are drawn indicating the first and third quartiles, and whiskers span to the largest value within 1.5x the interquartile range. d) Immunoblot validation of CRISPR-interference mediated suppression of PAX8. N = 1 technical replicate, representative of N = 2 independent experiments. e) Gene expression - relative to un-perturbed, parental cell lines profiled in parallel - of reported PAX8 target genes (see main text) after stable overexpression of WT-PAX8 (‘PAX8 O/E’) and/or induction of PAX8-target (sg4) or control (sg9) sgRNA and dCas9-KRAB. Data represents a single experiment. Boxplots are drawn as in b. f) XPR1 expression across all tissues in TCGA and GTEx, with ovarian and uterine tissues highlighted in teal. Boxplots are drawn as in b. g) XPR1 copy number heatmap for a ~2.5 Mb region of chromosome 1 indicating XPR1 amplification in TCGA Uterine Corpus Endometrial Carcinoma20. Each patient sample is represented by a horizontal line. Red indicates copy gain and blue indicates copy loss. Data are a subset of the samples rank-ordered by highest copy gain to indicate both focal and chromosome arm-level gains.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | A genome-scale CRISPR/Cas9 screen validates the relationship between XPR1 dependency in the context of high expression of SLC34A2. a) Outline of the experimental method and analysis for a genome-scale, dual-knock-out modifier screen. OVISE (without Cas9 expression) is engineered to stably express sgRNA targeting XPR1 (the ‘anchor’ sgRNA). Upon introduction of ‘all-in-one’ lentivirus, containing both Cas9 ORF and a second sgRNA, both genes are simultaneously inactivated by Cas9. We used three anchor sgRNA: one targeting a gene desert on chromosome 2 (sgChr2-2) and two targeting XPR1 (sgXPR1_1 and sgXPR1_2) and infecting the cells with the Brunello genome-scale sgRNA library. 15 days after infection, cells were harvested, genomic DNA was isolated, and sgRNA barcodes were quantified with next generation sequencing. See methods for full experimental and analytical details. b) Western confirmation of dual-knock-out of XPR1 and SLC34A2. The three cell lines used in the genome-scale screen were infected with ‘all-in-one’ lentivirus expressing control-, XPR1-, or SLC34A2-targeting sgRNA. Note that in the sgXPR1 ‘anchor’ cell lines, XPR1 is suppressed with the control virus, indicating that the first infection provides XPR1-targeting sgRNA and the second infection provides Cas9 protein. NIC stands for ‘no-infection control’. n = 1 technical replicate representative of n = 3 independent transductions. c) Arm-level results of the genome-scale modifier screen. See methods for full analysis details. Beta-scores represent the extent to which a gene was enriched or depleted relative to the initial plasmid representation. An XPR1-positive and control-neutral score represents a likely rescue gene (that is SLC34A2 and ARNT). XPR1-positive and control-positive scores represent genes with profound viability defects without specificity for XPR1 (for example RANBP17). N = 1 transduction per anchor condition, expanded and cultured as N = 2 independent replicates.
Extended Data Fig. 6 | The XPR1 dependency is not affected by phosphate levels in the tissue culture medium. a) The concentration of phosphate in the growth medium of DepMap cell lines does not determine the extent of XPR1 dependency. Concentrations of phosphate were estimated from manufacturer formulations (see methods) and the pearson correlation between growth medium phosphate and XPR1 dependency is indicated. b) Experimental procedure for manipulating tissue culture medium and assessing its effect on XPR1 dependency. The same parental cancer cell line was engineered to express firefly luciferase and Cas9, or Renilla luciferase alone. After a one-week adaptation to lowered phosphate, the two variants were mixed together and infected with sgRNA-encoding lentivirus. After selection for lentivirus-infected cell lines, the initial representation of Cas9:parental cells was determined by measuring the ratio of Firefly:Renilla luciferase using a DualGlo assay (Promega). One week after infection (Day 16 of the protocol), the extent to which genetic perturbation was detrimental to cell viability was determined using the DualGlo assay. c) The XPR1 dependency is maintained in a low phosphate medium condition. SNGM and ES2 were profiled in the assay outlined in panel b. Note that the CERES score displayed below the plot represents the viability defect of the cell line 21 days after knock-out of XPR1 and growth in the indicated growth medium. n = 5 technical replicates representative of n = 2 experiments. d) The viability of cells (as measured by total protein content) was measured in parallel with total phosphate as in Fig. 4d. n = 3 technical replicates representative of n = 4 independent experiments per cell line.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Transcriptional profiling reveals a phosphate-related homeostatic response after XPR1 inactivation. a) Experimental workflow to determine the transcriptional profile of XPR1 inactivation across many different cancer cell lines. See methods for full details; N = 1 transduction event per panel b–g. b) The total number of cells per cell line de-multiplexed from the 10X scRNAseq library. c) The total number of unique transcripts measured for each cell, as measured by unique molecular identifiers (UMIs). Box plots represent the 1st – 3rd quartiles, with whiskers representing the minimum and the maximum. The exact N for each sample in c is indicated in panel b. d) UMAP projection of the 2,501 cells from the indicated cell lines (determined by SNP profiles) and perturbations (indicated by cell-surface antibody ‘hash-tag’ labeling). Arrows indicate the degree to which the average transcriptional profile changes between the control sgRNA and the sgXPR1 infection condition. e) Summary of transcriptional effects across cell lines after inactivation of XPR1. Middle, the log-fold change of the top 500 differentially expressed genes after regressing out the effect of cell cycle. Left, summary annotations for each cell line include XPR1 dependency (XPR1 CERES), the overall transcriptional change (average log2 fold-change), and the degree of cell cycle arrest observed in the single-cell data (ΔG0/G1). The pearson correlation of these values is shown above the heatmap. Right, the pearson correlation of the top 500 differentially expressed genes between each cell line. f) Differentially expressed genes - after correcting for cell cycle - in the less sensitive cell lines (COV413a, JHOS4, OVCAR4, HEC6, and JHUEM1). Significance was assessed by the limma-voom pipeline using a two-tailed statistical test (see methods). g) Same as in f, but for the highly correlated cell lines RMG1, IGROV1, and OVISì. h) Four days after induction of shXPR1_2 (IGROV1) or shXPR1_4 (OVISE) using doxycycline, the amount of secreted FGF23 was measured in the conditioned medium using ELISA. N = 2 technical replicates representative of N = 3 independent experiments. i) 72 hours after treatment with the XPR1 inhibitor XRBD, the indicated proteins were detected using immunoblot. N = 1 technical replicate representative of N = 2 independent experiments. j) Top, western blot analysis of SLC34A2 and XPR1 in the SLC34A2-high yet XPR1-insensitive lung cancer cell lines, five days after infection with lentivirus expressing the indicated sgRNA. Bottom, viability of the indicated cancer cell lines was assessed using Cell Titer Glo after a five day XRBD treatment to inhibit XPR1. Points represent the mean of N = 3 technical replicates; error bars represent standard error of the mean. Data are representative of N = 2 independent experiments.
Extended Data Fig. 8 | Open-reading frames of XPR1 resistant to CRISPR/Cas9-mediated gene editing. a) Immunofluorescent localization of XPR1 mutants using the V5 epitope tag. Left, WT XPR1 localizes to the secretory pathways as well as puncta within the cytoplasm. Middle, XPR1 (short) staining appears more diffuse. Note similar localization patterns between L218S and wildtype XPR1 alleles. Scale bar = 200 µm. N = 1 experiment representative of N = 2 independent transductions. b) Western blot validation of guide-resistant ORF. OVISE.Cas9 cells (parental, left, or overexpressing the WT XPR1 ORF, right, used in Fig. 3e) were infected with the indicated sgRNA and harvested 5 days after infection. The XPR1 ORF includes a mutation to block binding of sgXPR1_2 but not sgXPR1_1. Note the inactivation of both endogenous and overexpression ORF with sgXPR1_1 and only endogenous XPR1 with sgXPR1_2. N = 1 experiment representative of N = 2 independent transductions.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | KIDINS220 is a unique partner protein of the XPR1 phosphate efflux complex. a) Genetic dependency correlations to XPR1 dependency across 851 cancer cell lines was assessed by pearson correlation test and corrected for multiple comparisons using the Benjamini-Hochberg method. Genes with significantly correlated dependency profiles are highlighted, as are proteins with known connection to XPR1 regulation. b) The viability defect of the indicated cancer cell lines after KIDINS220 inactivation was evaluated as in Fig. 1c. N = 3 technical replicates representative of at least N = 2 independent transductions per cell line. c) SLC34A2 was inactivated in EMTOKA and OVISE, and the KIDINS220 dependency was evaluated as in b. N = 3 technical replicates representative of at least N = 2 independent transductions per cell line. d) The interacting partners of XPR1 and KIDINS220 were downloaded from the BioGrid and Bioplex databases and compared. Proteins present in the XPR1 or KIDINS220 interactomes are highlighted as text. e) Left, the mRNA expression of XPR1 and KIDINS220 is shown for the fifteen tissues with the highest correlation in expression. The line represents linear regression for these samples (N = 2,799). Right, the Pearson correlation for those tissues, highlighting the diverse tissues in which there is a high correlation between XPR1 and KIDINS220 expression. f) Mutants of XPR1 used in this manuscript. XPR1 WT refers to the 696 amino acid protein produced by NM_004736 (the only isoform detected by RT-PCR of OVISE mRNA), while XPR1 (short) refers to the 631 amino acid product of NM_001135669. All constructs have C-terminal V5 tags for immunoprecipitation, western blotting, and immunofluorescent detection. g) XPR1 or Luciferase (Luc) were overexpressed in 293T cells and immunoprecipitated using the V5 tag and analyzed by targeted immunoblot or for total protein. Proteins were extracted from this gel and identified using mass spectrometry, the results of which are shown in Fig. 6c. N = 1 replicate of N = 2 independent transfections. h) Cas9 + sgRNA targeting XPR1 or KIDINS220 were transfected into 293T cells, and clones were isolated that lacked expression of the target proteins. For XPR1 inactivated cells, the XPR1 ORF was re-expressed, and the relative levels of the indicated proteins were detected by immunoblot. At least N = 2 clonal populations were profiled for each inactivation condition. i) Five days after infection with the indicated sgRNA targeting XPR1 or KIDINS220, free inorganic intracellular phosphate was determined as in Fig. 4d. N = 3 technical replicates of N = 3 independent transductions.
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Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.

Data analysis

As described in the Methods section, the following software was used for data analysis: Graphpad Prism v. 8.1, the publicly available PoolQ software (v. 3.2.1) from the Broad Institute Genetic Perturbation Platform, the program MAGeCK-MLE (v. 0.5.9), the program Flowjo (v. 10.8.1), the 10x Cell Ranger software (v. 3.0.2), Freebayes (v. 0.2.3), the R packages RStatix (v. 0.7.0), ggplot2 (v. 3.3.3), Seurat (v. 4.0.5), limma-voom (v. 3.44.3), and EdgeR (3.30.5). Code to reproduce figures is available upon request.

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Publicly available data used in this study include CRISPR/Cas9 loss of viability screens for 851 cancer cell lines ([https://doi.org/10.6084/m9.figshare.14541774.v2](https://doi.org/10.6084/m9.figshare.14541774.v2)), cancer cell line RNAseq expression data ([https://doi.org/10.6084/m9.figshare.14541774.v2](https://doi.org/10.6084/m9.figshare.14541774.v2)), harmonized gene expression data for GTEx and TCGA datasets ([https://doi.org/10.1101/326470](https://doi.org/10.1101/326470)), and copy number alterations for Ovarian adenocarcinoma and Uterine Corpus Endometrial Carcinoma ([https://doi.org/10.1038/](https://doi.org/10.1038/).
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
For the analysis of cancer cell line data, 859 cancer cell lines have robust gene expression and gene dependency data, and so all lines included within the analysis. In some cases, individual genes were not profiled (e.g. SC35A2 expression and XPR1 dependency in 851 cell lines) and the adjustments in sample size are noted throughout the text. Where appropriate, all samples from the GTEx and TCGA projects were analyzed. Where appropriate, technical replicates (N=2 or 3) were included. For in vivo experiments, no statistical test was used to pre-determine sample sizes.

Data exclusions
No data was excluded from the analysis.

Replication
All experiments are representative of at least two successful technical replicates.

Randomization
Randomization was not relevant to this study. For the in vivo competition assays (Figure 2), animals with the highest tumor burden were euthanized at a given timepoint; thus, no randomization was appropriate.

Blinding
Blinding was not relevant to this study, since the biases of the researcher were unlikely to influence the results of the reported experiments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☐   | Antibodies            |
| ☒   | Eukaryotic cell lines |
| ☒   | Palaeontology and archaeology |
| ☐   | Animals and other organisms |
| ☐   | Human research participants |
| ☐   | Clinical data         |
| ☒   | Dual use research of concern |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒   | Chip-seq              |
| ☒   | Flow cytometry        |
| ☒   | MRI-based neuroimaging |

Antibodies

Antibodies used

All antibodies used in this study include:

- **XPR1**
  - ProteinTech
  - 14174-1-AP
  - Immunoblot dilution of 1:2000

- **XPR1**
  - Atlas Antibodies
  - HPA015557
  - Immunoblot dilution of 1:2000
  - Protein Simple dilution of 1:37

- **KIDINS220**
  - ProteinTech
  - 21856-1-AP
  - Protein Simple dilution of 1:25
  - Immunoblot dilution of 1:2000
SLC34A2
CST
#66445
Immunoblot dilution of 1:2000
Protein Simple dilution of 1:50

SLC34A2 [MX35]
Creative Biosciences
TAB-467/M2
Immunohistochemistry dilution of 1:200

Vinculin
Sigma
#V9131
Immunoblot dilution of 1:10000

Vinculin
Abcam
ab129002
Protein Simple dilution of 1:75

V5
CST
13202
Immunoblot dilution of 1:2000

GM130
CST
12480
Immunofluorescence dilution of 1:800

LAMP1
CST
9091
Immunofluorescence dilution of 1:200

EEA1
CST
3288
Immunofluorescence dilution of 1:100

SSRM2
Abcam
ab122719
Immunoblot dilution of 1:500

Anti-Rabbit HRP
ProteinSimple
DM-001
ProteinSimple dilution of 1:1

Anti-Rabbit IgG
LiCor
926-32211
Immunoblot dilution of 1:20,000

Anti-Mouse IgG
LiCor
926-68020
Immunoblot dilution of 1:20,000

Anti-Mouse 488
ThermoFisher
# A-11004
Flow Cytometry dilution of 1:250

Validation
Each antibody (XPR1, KIDINS220, SLC34A2) was validated by detection of signal at the appropriate molecular weight with matching signal upon knockout and/or overexpression. The SRRM2 antibody only recognized samples in which an orthogonal method confirmed the presence of the protein (i.e., Mass Spectrometry). V5 antibody showed no signal in samples lacking V5-tagged proteins. GM130, LAMP1, and EEA1 were validated previously by the manufacturer.
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)  All cancer cell lines (S9M, F57, A2780, HEYA8, OVK18, SNU8, SNGM, RMG1, OVISE, KURAMOCHI, OVCA184, HCC-78, NCI-H441, NCI-H1437, RERF-LC2L2, JHUEM1, HEC6, and JHOS4 were collected by the Cancer Cell Line Encyclopedia prior to distribution for our use. The original sources are available at depmap.org. COV413A and HEK-293T were collected from ATCC.

Authentication  All cell lines were authenticated by exome sequencing and SNP profiling by the Cancer Cell Line Encyclopedia prior to distribution, and are only used for <15 passages to avoid cell line drift and potential cross contamination.

Mycoplasma contamination  All cell lines are routinely tested for mycoplasma, and only cell lines with negative results are used.

Commonly misidentified lines (See ICLAC register)  No commonly misidentified lines were used in this study.

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals  In total, this study used 26 female, 7-week-old, Rag1/-/Il2rg/- (NRG) mice obtained from The Jackson Laboratories.

Wild animals  This study did not use wild animals.

Field-collected samples  This study did not employ field collected samples.

Ethics oversight  These studies were used under the Institutional Animal Care and Use Committee (IACUC) of the Broad Institute under animal protocol 0194-0118.

Note that full information on the approval of the study protocol must also be provided in the manuscript.