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Ribosome profiling reveals a functional role for autophagy in mRNA translational control

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Autophagy promotes protein degradation, and therefore has been proposed to maintain amino acid pools to sustain protein synthesis during metabolic stress. To date, how autophagy influences the protein synthesis landscape in mammalian cells remains unclear. Here, we utilize ribosome profiling to delineate the effects of genetic ablation of the autophagy regulator, ATG12, on translational control. In mammalian cells, genetic loss of autophagy does not impact global rates of cap dependent translation, even under starvation conditions. Instead, autophagy supports the translation of a subset of mRNAs enriched for cell cycle control and DNA damage repair. In particular, we demonstrate that autophagy enables the translation of the DNA damage repair protein BRCA2, which is functionally required to attenuate DNA damage and promote cell survival in response to PARP inhibition. Overall, our findings illuminate that autophagy impacts protein translation and shapes the protein landscape.
Autophagy is a cellular recycling system that degrades proteins and organelles by delivery to the lysosome and promotes cell survival in response to metabolic and oxidative stress. As such, autophagy is rapidly upregulated, both post-translationally and transcriptionally, during nutrient starvation. At the same time, mRNA translation is tightly regulated by the metabolic state of the cell. The Rag complex senses lysosomal amino acid levels and signals through mTORC1 to regulate cap-dependent mRNA translation. Upon amino acid starvation, reduced mTOR signaling attenuates global cap-dependent mRNA translation, while concurrently inducing autophagy. Accordingly, in starving cells and tissues, autophagy-mediated recycling of amino acids is proposed to sustain residual translation of proteins, in particular those necessary for survival and metabolic adaptation during starvation or stress. In support, studies in Saccharomyces cerevisiae demonstrate that autophagy is crucial to maintain protein synthesis during nitrogen starvation. However, in mammalian cells, it remains unclear whether autophagy similarly impacts protein synthesis, either in nutrient replete or starvation conditions.

Here, we utilize ribosome profiling to dissect how the autophagy pathway impacts the mRNA translation landscape, both at baseline and in response to starvation. We uncover indirect roles for autophagy in regulating the translation of specific mRNAs, distinct from tuning protein synthesis rates in mammalian cells. In contrast to previous results from Saccharomyces cerevisiae, genetically abolishing autophagy in mammalian cells does not dramatically impair amino acid recycling, nor does it globally suppress protein synthesis rates, or cap-dependent or internal ribosome-entry site (IRES)-dependent mRNA translation during nutrient stress. Instead, autophagy inhibition leads to specific changes in the translation of a group of mRNAs involved in DNA repair, centrosome clustering and cell-cycle control. To further understand the mechanisms controlling autophagy-regulated mRNA translation, we specifically focus on how autophagy enables translation of the DNA damage repair gene Brca2. Loss of autophagy results in diminished levels of Brca2 and increased DNA damage, which can be partly rescued upon ectopically enforcing BRCA2 expression. Furthermore, the 5′UTR sequence and structure of Brca2 is an important determinant of the sensitivity to translational control in response to autophagy inhibition. We propose that autophagy-dependent regulation contributes to the efficient translation of proteins necessary for DNA damage repair and cell-cycle fidelity.

**Results**

**Autophagy loss does not affect translation rates.** ATG12 is an autophagy regulator required for the elongation of the double-membrane structure during autophagosome formation. To limit the effects of long-term adaptation due to the lack of autophagy in mammalian cells, we created a cell culture model for autophagy in mammalian cells. We developed immortalized mouse embryonic fibroblasts (MEFs) heterozygous for Atg12 floxed alleles and heterozygous for the CreER allele driven from the ubiquitous Cag promoter (Atg12<sup>f/f</sup>; Cag-Cre<sup>ER</sup>), which were treated with 4-hydroxytamoxifen (4OHT), resulting in Atg12Ablation and robust autophagy inhibition. Within 2d, the null allele was detectable by PCR (Supplementary Fig. 1a), and after 5d, no detectable Atg12 protein was found by immunoblotting. Lipidation and lysosomal turnover of LC3 (LC3-II) was profoundly attenuated in Atg12<sup>KO</sup> cells, resulting in the accumulation of the autophagy cargo receptor, p62/SQSTM1 (Supplementary Fig. 1b). For subsequent studies, we analyzed Atg12<sup>KO</sup> cells at 5d following 4OHT treatment.

First, we assessed the effect of autophagy loss on overall global protein synthesis rates, using a 35S methionine incorporation assay and a puromycin incorporation assay. Cells were starved for 2 h in Hanks buffered saline solution (HBSS), a serum free, amino acid-free saline solution containing glucose. This brief starvation period induces autophagy but precedes major transcriptional changes associated with starvation. Interestingly, we consistently observed robustly increased 35S methionine incorporation during HBSS starvation, in contrast to the removal of single nutrients, such as glucose or glutamine (Fig. 1a, b). However, upon alternatively assay protein synthesis rates using puromycin incorporation, we observed a trend decreasing in HBSS-starved cells (Fig. 1c, d). Most importantly, upon evaluating the effects of genetic autophagy inhibition on the rates of de novo protein synthesis, we found no significant differences in either 35S methionine incorporation or puromycin incorporation between Atg12<sup>KO</sup> and control (Atg12<sup>f/f</sup>) cells, in either fed or starved conditions (Fig. 1b, d). Similar results were observed in a broader array of immortalized MEFs lacking various autophagy regulators, including Atg12, ATG5, ATG7, or ATG3 (Supplementary Fig. 1d) as well as in primary MEFs lacking ATG12 (Supplementary Fig. 1e). Hence, the genetic loss of autophagy does not acutely impact the rate of de novo protein synthesis under either fed or starved conditions in mammalian cells, in contrast to previous results in Saccharomyces cerevisiae.

The availability of translation initiation factors or variant isoforms can regulate the rate of translation and impact which mRNAs are translated. Although phosphorylation of translation initiation factor 2-α (p-eIF2α), which represses cap-dependent global translation, was slightly increased, these changes were not statistically significant (Fig. 1e, f). There was no significant difference in the ratio of IRES-dependent to cap-dependent translation between Atg12<sup>f/f</sup> and Atg12<sup>KO</sup> cells using well-characterized viral IRES motifs from cricket paralysis virus (CrPV) (Fig. 1g) and Hepatitis C virus (HCV) (Supplementary Fig. 1f). In addition, using a m7GTP cap-puromycin assay, we observed no significant differences in the cap-binding abilities of key initiation factors eIF4E, eIF4G1, or their variants eIF4E2 or eIF4G2 between Atg12<sup>eff</sup> and Atg12<sup>KO</sup> cells (Fig. 1h), nor any differences in the binding of the inhibitory factor 4E binding protein (4EBP1) to the m7GTP cap (Fig. 1i).

**Atg12 loss minimally impacts mTORC1 activity.** In parallel, we measured whether Atg12 deletion impacts intracellular free amino acid levels, and found minimal differences between Atg12<sup>f/f</sup> and Atg12<sup>KO</sup> MEFs in both nutrient-rich conditions and following HBSS starvation for up to 2 h (Fig. 2a, Supplementary Fig. 2a). While glutamine may have lower levels in Atg12<sup>KO</sup> cells (Supplementary Fig. 2b), only glycine was significantly decreased in Atg12<sup>KO</sup> cells compared with controls grown in nutrient-rich full media conditions (Supplementary Fig. 2c), and upon starvation, the only amino acid lost more rapidly in Atg12<sup>KO</sup> cells was glutamic acid (Supplementary Fig. 2d). Notably, Atg12<sup>KO</sup> cells exhibited increased levels of oxoproline during starvation (Supplementary Fig. 2e), suggesting low glutamine levels may be due to reduced extracellular glutamine uptake through the gamma-glutamyl cycle. Notably, essential amino acids, including the branched chain amino acids (leucine, isoleucine, and valine), serine and threonine all exhibited higher measured levels in Atg12<sup>KO</sup> cells compared with controls at baseline (Supplementary Fig. 2f–j). Arginine is not discernible by this technique, however hydroxymethyl levels were higher in the Atg12<sup>KO</sup> starved cells, suggesting arginine metabolism in the autophagy-deleted cells may be enhanced (Supplementary Fig. 2k).

Although autophagy is proposed to sustain de novo protein synthesis by recycling...
Amino acid levels affect the activity of mTORC1, which regulates the translation of mRNAs containing terminal oligopyrimidine (TOP) motifs including translational machinery\(^\text{16}\), and is considered a master regulator of cell growth and protein synthesis\(^\text{17}\). Therefore, we investigated the effects of autophagy inhibition on mTORC1. Downstream markers of mTORC1 activation, phosphorylation of 4EBP1 at Ser65 and ribosomal protein S6 at Ser240 and 244, demonstrated that mTORC1 activity was robustly inhibited following HBSS starvation in both autophagy competent and deficient cells (Fig. 2b–e)\(^\text{18}\). No significant differences in key markers of mTORC1 activity were observed between \textit{Atg12\textsuperscript{ff}} and \textit{Atg12\textsuperscript{ko}} cells, either in full media or upon HBSS starvation (Fig. 2c, d).

Overall, these results indicate that the genetic loss of autophagy in both nutrient replete and short-term starvation conditions does not impact mTORC1 signaling or global protein synthesis.

**Autophagy modulates the translation of specific mRNAs.** To more thoroughly understand the impact of autophagy on mRNA translation, we employed ribosome profiling (RP), a sensitive and unbiased technique to identify the changes in the rate of translation of all expressed mRNAs in the context of autophagy deficiency\(^\text{19}\). Briefly, translating ribosomes are fixed onto mRNAs by treatment with cycloheximide so that ribosome protected
footprints (RPFs) can be isolated, amplified, deep-sequenced, mapped to the transcriptome and normalized to total mRNA levels measured by parallel RNA sequencing. Because the ribosome counts are normalized to mRNA levels, confounding gene expression changes are factored into the statistical analysis, hence allowing evaluation of ribosome occupancy, and by inference, translation rates at a particular snapshot in time.

Atg12f/f and Atg12KO cells were compared to each other in full media conditions and after 2 h HBSS starvation. Verifying experimental quality and reproducibility between replicates, we found generally equal and low levels of contaminating rRNA reads, RPF versus mRNA count plots were similar, and found a statistically significant correlation (Pearson’s) of both the raw values of RNA and RPF counts and the calculated p values (Supplementary Fig. 3a, b, Supplementary Table 1). Minimal changes in the numbers of RPF counts per mRNA were found between Atg12f/f and Atg12KO cells, while RPF counts in starved versus fed Atg12f/f cells decreased over all biological replicates (Fig. 3a–c). Analysis of the fold change of RPF counts vs. fold change of mRNA counts per gene revealed general changes in the transcriptional and translational landscape (Fig. 3d–f, Supplementary Fig. 3c). Statistical significance of ribosome occupancy changes normalized to mRNA changes was assessed at the gene level using Babel20,21; Supplementary Data 2 lists the 30 most significant genes from each comparison. ATG12 affected ribosome occupancy on a small subset of mRNAs, both positively and negatively.

We grouped genes with significant ribosome occupancy changes between Atg12f/f and Atg12KO cells into two cohorts: those exhibiting increased ribosome occupancy in Atg12KO compared with Atg12f/f (Fig. 3g, p < 0.01), and those exhibiting reduced ribosome occupancy in Atg12KO cell compared with Atg12f/f (Fig. 3h, p < 0.005). Among the cohort of mRNAs exhibiting reduced ribosome occupancy in Atg12KO cells, gene ontology (GO) analysis revealed an observed enrichment of genes involved in cell-cycle control and chromosome organization (Supplementary Fig. 3d). In contrast, no significant enrichment in biological processes were evident in the cohort displaying increased ribosome occupancy in Atg12KO cells. Notably, this GO enrichment correlated with slowed cell-cycle progression in autophagy-deficient cells. Atg12KO cells exhibited slower growth rates compared with the Atg12f/f cells in full media (Supplementary Fig. 3e) and higher percentage of phospho-Histone H3 (pH3) positive cells (Supplementary Fig. 3f), indicating slower progression through mitosis. We observed a significantly decreased percentage of cells in G1 in an unsynchronized population (Supplementary Fig. 3g). We propose that the translation of cell-cycle control mRNAs may represent an important consequence of enhanced autophagic flux observed during early mitosis and S phase.22

**Autophagy enables the translation of Brca2.** Several genes with significantly lower ribosome occupancy in Atg12KO cells regulate the DNA damage repair response, a process previously linked to autophagy.23 We focused on the function of autophagy to enable the translation of Brca2. BRCA2, which is commonly deleted in heritable breast cancer, functions in DNA double strand break repair and centrosome clustering. We labeled newly synthesized protein in cells with azidohomoalanine (AHA), a methionine analog that can be conjugated to biotin, and pulled down BRCA2 protein in cells with azidohomoalanine (AHA), a methionine analog that can be conjugated to biotin, and pulled down BRCA2 protein in cells with azidohomoalanine (AHA). We observed impaired label incorporation in the Atg12KO cells compared with control cells (Fig. 4a, b), demonstrating a reduced rate of BRCA2 synthesis in Atg12KO cells and consistent with reduced ribosome occupancy on Brca2 mRNA.

The decrease in the rate of BRCA2 production in Atg12KO cells correlated with reduced BRCA2 protein levels compared with controls in fed and starved conditions (Fig. 4c, d). In addition, CRISPR engineered HEK293T cells lacking ATG12 exhibited lower steady state BRCA2 protein levels (Fig. 4e, f), indicating that autophagy-dependent control of BRCA2 protein levels is not
Fig. 3 Ribosome profiling reveals that autophagy supports the translation of proteins involved in DNA damage response and cell-cycle control.

a–c Violin plots of number of read counts of ribosome protected footprints (RPFs) per gene per biological replicate (above) and histogram of the mean of the number of read counts of ribosome protected footprints per gene (below) in 

(a) Atg12\textsuperscript{ff} and Atg12\textsuperscript{KO} MEFs in control media

(b) Atg12\textsuperscript{ff} and Atg12\textsuperscript{KO} MEFs following 2 h HBSS starvation or

(c) Atg12\textsuperscript{ff} MEFs in control media or following 2 h HBSS starvation, p = 0.005 by t test.

d–f Fold change of RPF counts versus fold change of mRNA counts. Labeled points in orange are mRNAs whose change in ribosome occupancy was significant, and protein level changes confirmed by immunoblotting (see Supplementary Fig. 3c).

g, h Molecular functions of mRNAs whose ribosome occupancy is increased (p < 0.01, n = 36 significant genes) or decreased (p < 0.005, n = 60 significant genes) in Atg12\textsuperscript{KO} cells versus Atg12\textsuperscript{ff} cells in either fed or starved conditions.
limited to fibroblasts. To assess whether reduced levels of BRCA2 arose from defective autophagy, we probed for BRCA2 levels in Atg5-deleted and Bafilomycin-treated MEFs and CRISPR engineered HEK293T cells lacking ATG7 or ATG14 (Supplementary Fig. 4a–f). Steady state BRCA2 levels were decreased autophagy-inhibited MEFs. ATG7- and ATG14-knockout HEK293T cells had variable expression of BRCA2; we hypothesize that a compensatory upregulation of BRCA2 occurred to promote cellular health during the single-cell clone selection, or that due to variability, the statistical analysis was underpowered.

To confirm that decreased BRCA2 steady state protein levels were due to reduced translation, we assessed Brca2 transcription and BRCA2 stability and turnover in Atg12KO cells. We found no significant difference between Atg12f/f and Atg12KO MEFs in

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Fig. 4 Reduced BRCA2 protein translation in autophagy-deficient cells. a, b Newly synthesized BRCA2 levels were assessed following 8 h AHA incorporation and BRCA2 immunoprecipitation from control or Atg12KO HEK293T cells. a Representative immunoblot is shown. b Quantification (boxplot with dotplot overlay for each independent biological replicate) of newly synthesized BRCA2 (StrepHRP levels), collected from the 5′UTR of Brca2 directs autophagy-enabled translation. The untranslated regions (UTRs) of mRNAs function as important regulators of translational efficiency; 5′UTRs can contain upstream open reading frames and motifs to interact with various RNA-binding proteins (RBPs), while the 3′UTR harbors RBPs binding and miRNA motifs. We investigated whether the Brca2 5′UTR mediated translational control downstream of autophagy. A green fluorescent protein (GFP) reporter was transiently overexpressed in Atg12f/f or Atg12KO MEFs alone or flanked by the Brca2 5′UTR, 3′UTR, or both UTRs. GFP protein levels were decreased in the presence of the 5′UTR of Brca2, but not the 3′UTR, in Atg12KO compared with Atg12f/f cells, despite equivalent Gfp expression (Fig. 5a, Supplementary Fig. 5a). Moreover, utilizing nano-luciferase reporters to quantitatively measure the effects of Brca2 UTRs, we observed significantly decreased luciferase activity in the Atg12KO MEFS compared with Atg12f/f when the 5′UTR of Brca2 preceded luciferase (Fig. 5b). Therefore, the 5′UTR of Brca2 contains the region that mediates autophagy-dependent translation of this mRNA.

Remarkably, the 5′UTRs of the cohort of mRNAs exhibiting lower RP occupancy in Atg12KO cells had significantly lower folding energies compared with the 5′UTRs of a random sampling of mouse genes (Supplementary Fig. 5b). To adjust for length of the UTRs, the minimum free energy (MFE) within the 5′UTRs was predicted by RNALfold; significant differences were detected between the two groups (Fig. 5c, Supplementary Fig. 5c, d). These results indicate that mRNAs whose translation efficiency is enhanced in autophagy competent cells possess above average 5′UTR secondary structure complexity. Indeed, Ifi7, another hit from our ribosome profiling screen is notable for a secondary structure that facilitates the loading and reading of ribosomes. We investigated whether the helicase elf4A1, part of the elf4F initiation complex that recruits ribosomes to mRNA, exhibited impaired binding to Brca2 in Atg12KO cells. RNA immunoprecipitation (RIP) confirmed decreased interaction between endogenous elf4A1 and Brca2 mRNA in Atg12KO versus Atg12f/f cells

either Brca2 mRNA levels (Fig. 4g) or in BRCA2 protein stability or turnover following cycloheximide treatment (Fig. 4h, i), which did not impact autophagic flux (Supplementary Fig. 4g), indicating that the lower levels of BRCA2 were not due to changes in Brca2 expression levels, nor enhanced degradation of BRCA2. Overall, these results demonstrate efficient Brca2 translation and maintenance of normal BRCA2 protein levels requires an intact autophagy pathway.

The accumulation of p62 sequesters elf4A1 from Brca2. To further understand elf4A1 sequestration in Atg12KO cells, we tested the interaction between elf4A1 and known autophagy cargo receptors (ACRs), mediators of selective autophagy that accumulate upon autophagy inhibition. We observed increased co-location of elf4A1 within puncta of the ACR p62/SQSTM1 in autophagy-deficient cells (Fig. 6a, b) and immunoprecipitation studies indicated that endogenous elf4A1 interacts with endogenous p62/SQSTM1 in Atg12KO but not Atg12f/f cells (Fig. 6c, Supplementary Figs. 6a, 5f). In contrast, autophagy deficiency did not significantly enhance the interaction of elf4A1 with NBR1, a similar ACR (Fig. 6b, c). p62/SQSTM1 depletion rescued the ability of elf4A1 to interact with the cap, and overexpression of a mutant p62/SQSTM1 that cannot be degraded by autophagy (p62ΔLIR) reduced elf4A1 binding to the cap (Fig. 6d, c). Furthermore, p62/SQSTM1 depletion was able to enhance elf4A1 binding to Hnrnpc, whose translation is dependent on elf4A1 (Supplementary Fig. 6b). However, p62/SQSTM1 knockdown was not sufficient to restore BRCA2 levels in Atg12f/f cells (Fig. 6f–h). In addition, although elf4A1 interaction with the m7GTP cap was impaired in Atg12KO cells, there was not significant overlap in the mRNAs whose translation is affected by autophagy deletion compared with mRNAs whose translation is affected by elf4A1 inhibitors (Supplementary Fig. 6c). Notably, p62/SQSTM1 has been previously implicated in the sequestration of the E3 ligase KEAP1 away from its target substrate NRF2 in autophagy-deficient cells. Based on our results, we propose a similar model in which the accumulated p62/SQSTM1 in Atg12KO cells sequesters elf4A1 away from the translation initiation complex. We postulate that elf4A1 availability is determined by p62/SQSTM1 levels, which contributes to autophagy-enabled translation, but this not the sole determinant of the sensitivity of Brca2 translation to autophagy inhibition.

Autophagy impacts the availability of RBPs to bind to Brca2. We investigated whether other RBPs that bind to the Brca2 5′UTR of Brca2 are lost in autophagy-deficient conditions.

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UTR were affected by autophagy deficiency. The 5′ UTR of Brca2 contains two predicted binding sites for the RBP MS11, which represses the translation of p21 and Numb34. We therefore assayed MS11 binding to Brca2 by RNA immunoprecipitation and observed increased MS11 associated with Brca2 in the Atg12KO cells (Fig. 7a). Atg12KO MEFs in both fed and starved conditions demonstrated a modest, albeit not significant, accumulation of MS11 of a similar pattern and magnitude that inversely correlated with BRCA2 decrease (Fig. 7b). MS11 possesses four putative LC3 interacting domains (LIRs)35 and we found that MS11 interacts with multiple LC3/ATG8 orthologues (Fig. 7c), suggesting that MS11 is selectively targeted by autophagy. To further define how MS11 affected Brca2 translation, we depleted MS11 in Atg12+/f and Atg12KO MEFs by shRNA (Fig. 7d), which reduced the steady state levels of BRCA2 compared with nontargeting control shRNA in both cell types (p = 0.23 by ANOVA) (Fig. 7e, f). Nonetheless, upon MS11 depletion, the reduction in BRCA2 protein levels in Atg12KO compared with Atg12+/f MEFs was significantly less pronounced (Fig. 7g).

Furthermore, the analysis of two published data sets of the autophagy interactome36 and a comprehensive list of RNA-binding proteins37 revealed that nearly one quarter of the autophagy interactors are RBPs (Supplementary Fig. 7a). We therefore questioned whether common RNA-binding motifs were more prevalent in the UTRs of significant RP hits. Using RBPDB38, we analyzed the UTRs of the significant hits from our RP analysis compared with a randomly generated gene list of equivalent length, revealing that multiple RBP motif sequences located at both 5′ and 3′ UTRs of the significant RP hits were enriched above the control set (Supplementary Fig. 7b). We specifically identified changes in the number of motifs for the RNA-binding proteins YBX1 and MATR3 (Supplementary Fig. 7c) and corroborated that both of these RBPs interacted with various LC3/ATG8 orthologues (Supplementary Fig. 7d). However, we did not observe their accumulation in Atg12-deleted cells, either in full media or HBSS starvation conditions, suggesting these RBPs did not undergo significant turnover via starvation-induced autophagy (Supplementary Fig. 7e). Based on our results, we postulate that Brca2 translation is partly controlled by the autophagic turnover of MS11, and that the regulation of other mRNAs in an autophagy sensitive manner likely arises from the coordinate regulation of multiple LC3/ATG8-interacting RBPs, including elf4A1, YBX1, and MATR3.

Atg12KO cells accumulate DNA damage and centrosome defects. We next dissected the functional consequences of reduced Brca2 translation in autophagy-deficient cells. BRCA2-deficient cells are impaired in homologous recombination and accumulate DNA damage39. We observed increased levels of DNA damage in Atg12KO MEFs (independent of Cre expression), evidenced by increased levels of γH2AX, a marker of double strand DNA damage, by immunofluorescence and immunoblotting, as well as increased puncta double positive for γH2AX and 53BP1 by immunofluorescence (Fig. 8a, b, Supplementary Fig. 8a). Similar increases in γH2AX were observed in Atg5KO MEFs and autophagy-deficient 293T cells (Supplementary Fig. 8b).

Previous work attributes increased DNA damage in autophagy-deficient mammalian cells to reactive oxygen species (ROS) produced from accumulated defective mitochondria40,41. However, we observed no significant differences in ROS levels, mitochondrial mass, or membrane potential between Atg12+/f and Atg12KO cells (Fig. 8c, Supplementary Fig. 8c). To determine the functional contributions of ROS versus BRCA2 levels to DNA damage accumulation in Atg12KO MEFs, we measured γH2AX levels in cells treated with the ROS scavenger N-acetyl cysteine (NAC), or ectopically expressing the human Brca2 cDNA (Supplementary
Fig. 6 elf4A1 is sequestered by accumulated p62 in autophagy-deficient cells. a Representative immunofluorescence images for elf4A1 (red in merged imaged) and p62/SQSTM1 (green in merged imaged) in Atg12fl/fl and Atg12KO MEFs, nuclei were counterstained with Hoechst (blue). Yellow box indicates region of inset panel in the top left corner. Far right panels show the points of colocalization (white) of elf4A1 in p62/SQSTM1. Bars was performed with three biologically independent replicates. An outlier (Dixon test p = 0.03) was excluded from statistical analysis and the p values between Atg12fl/fl and Atg12KO were calculated by t test. b Representative immunoprecipitation of elf4A1 and immunoblot for the autophagy cargo receptors p62/SQSTM1 and NBR1. Arrow indicates p62/SQSTM1, asterisk indicates immunoglobulin heavy chain. Immunoprecipitation was performed with three biologically independent replicates. c Protein lysate from Atg12fl/fl and Atg12KO MEFs that were knocked down for p62/SQSTM1 or treated with nontargeting shRNA was captured by cap pulldown, and the ratio of elf4A1 to eIF4G1 was quantified (mean ± SD, n = 4 biologically independent replicates). d Protein lysate from Atg12fl/fl and Atg12KO MEFs that were knocked down for p62/SQSTM1 or treated with nontargeting shRNA or HEK293Ts that overexpress p62ΔLIR or empty vector control was captured by cap pulldown, and immunoblotted as indicated. e-h Protein lysate from Atg12fl/fl or Atg12KO MEFs stably infected with shRNA to p62/SQSTM1 or nontargeting control was collected. Relative quantification from immunoblots for elf4A1, elf4G1 in p62/SQSTM1 and NBR1. Arrow indicates p62/SQSTM1, asterisk indicates immunoglobulin heavy chain. Immunoprecipitation was performed with three biologically independent replicates. f Protein lysate from Atg12fl/fl and Atg12KO MEFs that were knocked down for p62/SQSTM1 or treated with nontargeting shRNA or HEK293Ts that overexpress p62ΔLIR or empty vector control was captured by cap pulldown, and immunoblotted as indicated. Relative quantification from immunoblots for elf4A1, elf4G1 in p62/SQSTM1 and NBR1. Arrow indicates p62/SQSTM1, asterisk indicates immunoglobulin heavy chain. Immunoprecipitation was performed with three biologically independent replicates. g Protein lysate from Atg12fl/fl or Atg12KO MEFs stably infected with shRNA to p62/SQSTM1 or nontargeting control was collected. Relative quantification from immunoblots for elf4A1, elf4G1 in p62/SQSTM1 and NBR1. Arrow indicates p62/SQSTM1, asterisk indicates immunoglobulin heavy chain. Immunoprecipitation was performed with three biologically independent replicates. h The change in BRCA2 levels between Atg12fl/fl and Atg12KO in shNT-treated cells or shp62-treated cells is plotted, p = 0.76 between shNT and shp62 for ∆BRCA2, calculated by t test.

Fig. 8d). Whereas treatment with NAC had minimal effects on γH2AX levels in Atg12KO cells, overexpression of Brca2 decreased the levels of γH2AX in Atg12KO cells (Fig. 8d) and in ATG-deleted HEK293T cells (Supplementary Fig. 8e). Overall, these results indicate that the impaired translation of Brca2 functionally contributes to DNA damage in autophagy-deficient cells.

We next treated Atg12KO cells with the Poly ADP-ribose polymerase (PARP) inhibitors rucaparib, olaparib, and BMN to assess if reduced BRCA2 protein levels conferred sensitivity to inhibition of single-strand DNA damage repair, as previously observed in the context of BRCA2 genetic deficiency. Indeed, Atg12KO cells exhibited increased sensitivity to PARP inhibitors, evidenced by increased γH2AX and cleaved caspase 3 levels (Fig. 8e, Supplementary Fig. 8f), as well as impaired colony-replating efficiency following PARP inhibitor treatment (Fig. 8f).
lysate was collected from family members interaction with MSI1 in HEK293T cells. Immunoprecipitation was performed with three biologically independent replicates. Immunoblotting. Statistical analysis was performed by p-value (Dixon test with 4OHT or control, and assayed by immunoblotting.

BRCA2 contributes to clustering of mother and daughter centrosomes following duplication. We observed similar impairments in centrosome clustering in Atg12KO cells. The distance between the two centrosomes in non-mitotic cells was increased (Fig. 8g, h) and there was a significant increase in percentage of cells with more than two centrosomes in non-mitotic cells compared with wild-type controls (Fig. 8g, i). These defects in centrosome organization may further exacerbate DNA damage in Atg12KO cells. In addition to BRCA2, our ribosome profiling analysis identified other mRNAs involved in centrosome function, including Haus3 and Cntln, that exhibited reduced ribosome occupancy in Atg12KO cells (Supplementary Data 2), suggesting that autophagy-dependent translation of multiple mRNAs may functionally contribute to centrosome organization.

**Fig. 7 Increased MSI1 in autophagy-deficient cells impairs Brca2 translation efficiency.** a Quantification (mean ± SD, n = 4 biologically independent replicates) of the fold enrichment of Brca2 interaction with MSI1 over IgG control in Atg12f/f or Atg12KO MEFs by RNA immunoprecipitation. An outlier (Dixon test p = 0.007) was excluded from statistical analysis, and the p value by t test is shown. b Boxplot, with dotplot overlay for each biological replicate, of relative MS11 protein levels normalized to loading control and representative immunoblots from autophagy-inhibited MEFs, assayed by immunoblotting. Statistical analysis was performed by t test. c Representative immunoblot of immunoprecipitation of myc-tagged overexpressed LC3 family members interaction with MS11 in HEK293T cells. Immunoprecipitation was performed with three biologically independent replicates. d Protein lysate was collected from Atg12f/f MEFs treated with shRNA to MS11, and immunoblotted as indicated. Knockdown of ~50% was consistent among three independent biological replicates. e-g Protein lysate was collected from Atg12f/f MEFs that were stably knocked down for MS11 and subsequently treated with 4OHT or control, and assayed by immunoblotting. e Representative immunoblot is shown. f Quantification of relative BRCA2 protein levels normalized to loading control, are plotted as a boxplot with dotplot overlay for each independent biological replicate, p values by ANOVA with Tukey’s post hoc test. g Difference in BRCA2 steady state protein levels (ΔBRCA2) between Atg12f/f and Atg12KO MEFs following MS1 depletion, p value by t test.

Autophagy deletion in vivo reduces BRCA2. We assessed the effects of autophagy ablation on BRCA2 protein levels in vivo following systemic acute genetic deletion of Atg12 in adult mice. At 6 weeks of age, Atg12f/f Cag-CreER mice were subject to treatment with tamoxifen or vehicle control for 5 consecutive days (Fig. 9a). Loss of ATG12 correlated with accumulation of the autophagy substrate p62/SQSTM1 and the absence of LC3-II at 2 weeks following tamoxifen administration (Fig. 9b). Atg12KO animals survived for 10 weeks following the acute loss of autophagy. Similar to acute systemic genetic deletion of Atg7 in adult mice, systematically deleted Atg12 mice were smaller and failed to gain weight following deletion (Fig. 9c, d). Immunoblotting revealed decreased BRCA2 protein levels in the cerebral cortex of Atg12KO mice compared with autophagy competent Atg12f/f controls, and a non-significant decrease in the kidney (Fig. 9e, f). This correlated with increased levels of DNA damage, evidenced by a twofold increase in γH2AX-positive nuclei in the kidney, cerebral cortex, and small intestine of Atg12KO mice (Fig. 9g, h). These in vivo findings are consistent with our in vitro results that an autophagy pathway supports the production of BRCA2.

**Discussion**

Using ribosome profiling, we have uncovered that autophagy is functionally required for the efficient translation of specific proteins, and in particular BRCA2, in mammalian cells. Strikingly, our results show important differences in the starvation response between mammalian cells and Saccharomyces cerevisiae, which rely heavily on autophagy to maintain amino acid availability and...
protein synthesis during starvation. In contrast, global protein synthesis and the availability of intracellular amino acids remains largely intact in mammalian cells following autophagy ablation, including cells undergoing short-term starvation, suggesting that other proteolytic pathways, such as direct delivery of ER and plasma membrane components to the lysosome, likely compensate to maintain amino acid levels in response to acute stress. Because of both the specific regulation of a small subset of mRNAs, and the minimal changes observed in amino acid availability in autophagy-deficient cells, we propose that the effects of autophagy deficiency on the translational control are unlikely due to altered cellular metabolism. In contrast to our work, previous studies in several cancer models demonstrate that autophagy supports local and serum amino acids levels necessary to fuel tumor growth. These dependencies may be specific to tumor cells, which are highly reliant on glutamine levels, or indirectly arise from the autophagy-regulated secretion of specific factors in certain cells and tissues, such as arginase or alanine.
Fig. 8 Decreased BRCA2 levels in Atg12-deleted cells result in increased DNA damage and centrosome abnormalities. a Immunofluorescence for γH2AX (red) in Atg12f/f and Atg12KO MEFs; nuclei are counterstained with Hoechst (blue). Bar = 50 μm. Percent of γH2AX-positive cells was quantified in Atg12f/f and Atg12KO MEFs; three random fields were counted over three independent biological replicates, p = 0.002 by t test. b Immunofluorescence for γH2AX (red) and 53BP1 (green) in Atg12f/f and Atg12KO MEF; nuclei are counterstained with Hoechst (blue). Bar = 50 μm. Three independently replicated experiments were performed. c ROS-glo assay (Promega) in Atg12f/f and Atg12KO MEFs treated with vehicle control or menadione (50 μM for 2 h, positive control) (mean ± SEM; n = 2 biologically independent replicates). d Protein lyte was collected from Atg12f/f and Atg12KO MEFs treated with vehicle control or NAC (5 mM for 8 h), or ectopically overexpressing either GFP (pGFP) or human BRCA2 (huBRCA2). A representative immunoblot for γH2AX is shown, as well as boxplots with independent biological replicates, for γH2AX levels normalized to loading control. Statistical analysis was performed by t test. e Protein lyte was collected from Atg12f/f and Atg12KO MEFs treated for 16 h with vehicle control, rucaparib (100 nM), olaparib (100 nM), or BMN (2 nM), and immunoblotted as shown. Three independent biological replicates were performed. A clonogenic retening assay was performed on Atg12f/f or Atg12KO MEFs treated for 16 h with vehicle control, rucaparib (100 nM), olaparib (100 nM), or BMN (2 nM). Colony size is shown as a boxplot including biological replicates, p value by t test. g Immunofluorescence of centrosomes (γ-tubulin, red) and mitotic cells (ph3, green), nuclei counterstained by Hoechst (blue), in Atg12f/f and Atg12KO MEFs. Yellow box indicates magnified region below. White arrows indicate non-mitotic cells with multiple centrosomes (3+) or non-clustered centrosomes. Bar = 100 μm. h Quantification (mean ± SEM; n = 3 biologically independent replicates) of distance between mother and daughter centrosomes measured on immunofluorescence of γ-tubulin in non-ph3 positive cells, p value by t test. i Quantification of Atg12f/f and Atg12KO MEFs with abnormal numbers (3+) of centrosomes from immunofluorescence images (n = 4 biologically independent replicates). p values by t test on log transformed percent per replicate. Fraction above the bar plots indicates number of cells with 3+ centrosomes out of number of cells enumerated.

Importantly, we demonstrate that basal autophagy enables the efficient translation of a cohort of mRNAs by regulating the availability of certain RBPs. In particular, we show that autophagy enables the efficient production of the DNA damage repair protein BRCA2. As a result, when autophagy is inhibited, reduced levels of BRCA2 lead to increases in DNA damage and centrosome defects. We propose multiple indirect mechanisms of translational control, including the ability of autophagy to modulate the interaction of MSI1 with the 5’UTR of Brca2, which impairs efficient translation and production of BRCA2. In addition to these autophagy-dependent effects on the 5’UTR of Brca2, we recognize that a broader repertoire of translation control mechanisms are impacted by autophagy, including the modulation of additional LC3/ATG8-binding RBPs, such as elf4A1, MATR3, and YBX1. Moreover, although we found no role for autophagy in controlling cap versus HCV or CrPV IRES translation initiation, we cannot rule out that autophagy may regulate translation from IRES-like or IRES motifs distinct from the viral motif driven reporter systems we employed. Notably, we observed a trending increase in the levels of p-eIF2α in autophagy-deficient cells, which may tune attenuated translation in response to stresses other than nutrient starvation. Overall, identifying the diverse array of molecular mechanisms by which autophagy impacts mRNA translation, both directly and indirectly, remains an important area for further study.

Autophagy-enhanced Brca2 translation may have particular relevance for human health and disease. We found in vivo reductions in BRCA2 protein levels and increases in DNA damage, including a twofold reduction in BRCA2 levels and a threefold increase in γH2AX levels in the kidney, in Atg12-deleted mice. Polycystic kidney disease has been linked independently to both defects in autophagy and defects in centrosome organization that disrupt primary cilia formation45–57. Our results broach centrosome disorganization as a potential mechanism by which defective autophagy contributes to this disease phenotype. Furthermore, because intestinal stem cells and hematopoietic stem cells are highly dependent on autophagy to maintain genome integrity58–69, our data suggest a previously unrecognized mechanism by which autophagy maintains the genome in stem cells. While ROS has been primarily implicated as the DNA damaging driver in autophagy-deficient intestinal stem cells, our results identify reduced Brca2 translation as an aggravating factor contributing to DNA damage in autophagy-deficient cells. With regard to cancer, one can speculate autophagy mitigates genomic damage by enabling the translation of Brca2, thereby suppressing early tumorigenesis. In support, a polymorphism in the 5’UTR of Brca2 that decreases the secondary structure and promotes translation is protective against breast cancer in patients81. Because autophagy inhibitors are being tested as adjuvant chemotherapies62, further defining the effects of autophagy on protein synthesis in cancer cells will help refine the proper contexts to effectively employ such strategies. Overall, our findings illuminate roles for autophagy in directing the protein synthesis landscape in mammalian cells, which maintains genome integrity.

Methods

Experimental models and subject details

Mouse maintenance. Compound transgenic C57Bl/6 mice harboring Atg12LOF and Cag-CreER were generated by cross-breeding of Atg12LoF and Cag-CreERm animals (obtained via the UCSC mouse database). Offspring were genotyped with polymerase chain reaction (PCR) primers (msAtg12LOF primers: FRT in/h sense 1: ATG TGA ATC AGT CCT TTG GCC; FRT-FRT as 2: ATG TGA ATC AGT CCT TTG GCC; WT-FRT as 2: CTC TGA AGG CGT TCA ACG GC; WT-FRT as 2: CTC TGA AGG CGT TCA ACG GC; WT-FRT as 2: CTC TGA AGG CGT TCA ACG GC; Cag-CreER primers: forward: GCCCTGCATTACCGGTCGATGC, reverse: CAGGCTGTATTAAAGC AAACTCC; msAtg12-null allele: forward: CACCCCTGTTTTACGAAAGCCA, reverse: ATCTGCAAGGGGTCTACG GCC). At 6 weeks of age, animals of indicated genotypes received either Tamoxifen (0.2 mg/gram mouse; Sigma-Aldrich T5648-1G) or vehicle (peanut oil) via oral gavage for 5 consecutive days. At 10 weeks after the first tamoxifen treatment, animals were sacrificed and tissues were collected for biochemistry and histological analysis. Both male and female animals were used in roughly equal numbers for all experiments. All experimental procedures and treatments were conducted in compliance with UCSF Institutional Animal Care and Use Committee guidelines.

Isolation of mouse embryonic fibroblasts. Mouse embryonic fibroblasts were generated from E13.5 mice described above following the protocol from Robertson81. Briefly, embryos were collected, heart, liver, and head were removed and fibroblasts were minced, digested in trypsin for 30 min at 37 °C and plated in DMEM with 10% serum and Pen/Strep. Cells were genotyped and Atg12f/f:Cag-CreERm cells were immortalized by infection with SV40 large T antigen (SV40 1: pBSSVD2005, Addgene #21826, deposited by David Ron). Cells were plasmocin treated prior to use (Invivogen, anti-mpt). Following immortalization and plasmocin treatment, cells were maintained in DMEM 1x (Gibco) supplemented with 10% FBS (Atlas).

Genetic deletion of MEFs. Cells were treated with 2 μM 4-hydroxy-tamoxifen (4OH-T; Sigma-Aldrich H7904) or vehicle (100% ethanol) for 3 consecutive days. Genetic recombination was achieved following 2 days of 4OH-T treatment, and confirmed by PCR.

Additional tissue culture cells. N. Mizushima (University of Tokyo, Japan) provided Atg5+/−, Atg3+/−, Atg7+/−, and Atg12−/− MEFS and M. Komatsu (Tokyo Metropolitan Institute, Japan) provided Atg5−/− and Atg3−/− MEFS, Atg12+/− and Atg12−/− MEFS were originally generated in Malhotra et al.76. HEK293Ts were cultured in DMEM 1x (Gibco) supplemented with 10% FBS (Atlas) and Pen/Strep. HEK293Ts were purchased from ATCC (CRL-3216). HEK293T knockout cell lines lacking ATG7, ATG12, or ATG14 were generated by CRISPR/Cas9. Human guide sequences (scramble: gcataccagcgacta; huAtg12: CGGCTCTCCGCGTCGAGT...
TTC; huAtg7: ACACACTCGAGTCTTTCAAG; huAtg14: CTACTTCGACGGCCGCGACC) were ligated into pSpCas9(BB)-2A-Puro (PX459) plasmid using the BbsI site. HEK293T cells were transfected with plasmid DNA using Lipofectamine 3000. Cells were selected 48–72 h post-transfection with 1 mg/ml puromycin for 48 h. Polyclonal populations were collected for Surveyor analysis (IDT, 706020) and were sorted into single-cell populations by limiting dilution at 1.5 cells/well per 96-well plate. Monoclonal wells were identified, expanded, and analyzed. For DNA analysis, genomic DNA samples were prepared using QuickExtract (Epicentre). The PCR products were column purified and analyzed with Surveyor Mutation Detection Kit (IDT). For genotyping of single-sorted cells, PCR amplified products encompassing the edited region were cloned into pCR-TOPO TA vector using the TOPO-TA cloning kit (Thermo Fisher #450030) and sequence verified (primers for genotyping CRISPR deleted HEK293T cells: Atg12: forward: AGCCGGGAACACCAAGTTT, reverse: GTGGCAGCCAAGTATCAGGC; Atg7: forward: TGGGGGACAGTAGAACAGCA, reverse: CCTGGATGTCCTCTCCCTGA; Atg14: forward: AAAATCCCACGTGACTGGCT, reverse: AATGGCAGCA...
**Fig. 9** Atg12 deletion in vivo leads to reduced BRC2A2 and DNA damage repair. 

**a.** Diagram of Atg12<sup>fl</sup>/Cop-Cre<sup>ER</sup> mouse treatment and tissue collection. 

**b.** Protein lysate was collected from tissues two weeks following vehicle or 0.2 mg/g tamoxifen treatment and immunoblotted for markers of autophagic flux (p62/SQSTM1, LC3b). 

**c.** Representative images of male and female Atg12<sup>fl</sup> and Atg12<sup>2KO</sup> littermates. 

**d.** Body weights of male mice following Atg12 deletion (Atg12<sup>fl</sup> (N = 17), Atg12<sup>2KO</sup> (N = 14)). 

**e.** Protein lysate was collected from mouse tissues 10 weeks following vehicle or tamoxifen treatment, and immunoblotted for BRC2A2. 

**f.** Boxplot with dotplot overlay for biological replicates of BRC2A2 protein levels, normalized to total protein levels, assayed by immunoblotting. 

p = 0.02 by t test. 

**g.** Boxplot with dotplot overlay for biological replicates of percent of yH2AX-positive nuclei by immunofluorescence, counted over four randomly selected fields of stained tissue per a minimum of four mice. 

**h.** Representative immunofluorescence for yH2AX (red) in mouse tissues from the cerebral cortex and small intestine, with nuclei counterstained by Hoechst (blue). 

Bar = 50 µm.

**A C G G G G A A A A A C .** Sequencing is available upon request. Cells were routinely tested for mycoplasma contamination and authenticated using short- tandem repeat (STR) profiling for human cell lines and immunoblotting for Atg expression in mouse cells.

**In vitro drug treatments.** Cells were treated with the following drugs at concentrations and times as indicated in the figure legends: 

- Cycloheximide (Sigma-Aldrich, C6798). 
- Ribosome profiling (Epicentre RPHMR12126), with RNA extraction by Trizol LS (Ambion), RNA deproteinization via RiboZero Gold (Epicentre MRGZ126), and quality and quantity of small RNA and DNA assayed using Agilent High Sensitivity Small RNA kit and DNA kit, respectively (Agilent 5067-1548, 5067-4626).

**Stable RNA interference.** plKO.1 basicidin or plKO.1 purimycin lentiviral plasmids with non-targeting shRNA, which targets no known mammalian genes (SHC-002, CAA CAA GAT GAA GAG CAC CAA), or shRNA against mouse Atg7 (TRC N000092163, CCA CTT GCT AAC TCA ATA ATA), or mouse Msi1 (TRCN00003850). CCGG CCGCTTGGACAGCTTCGTCTTCAGAAACAGTGGCTGAAGCCAGGGACAGTTGTGATGGGTGGTGGAGGTTTGG

**Immunoblotting.** For immunoblot analysis, 200,000 cells were lysed in RIPA buffer (1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 25 mM Tris, pH 7.6, and 150 mM NaCl) plus protease inhibitor cocktail (Sigma-Aldrich), 10 mM N-acetyl cysteine, 1 mM Na2VO4, 0.5 mM sodium orthovanadate, plus protease inhibitor cocktail. RNA immunoprecipitation (RIP) buffer: 10 mM Tris HCl pH 7.4, 150 mM NaCl, 1% NP-40, 5% glycerol, 1 mM EDTA, 1 mM EGTA, 1 mM β-glycerophosphate, 10 mM NaF, 2.5 mM Na3PO₄, 1 µM sodium orthovanadate, plus protease inhibitor cocktail. RNA immunoprecipitation (RIP) buffer: 100 mM NaCl, 25 mM Tris HCl pH 7.4, 5 mM EDTA, 5% glycerol, 1 mM DTT, 1% NP-40, plus protease inhibitor cocktail and RNase inhibitors. 

**Immunoprecipitation, RNA immunoprecipitation, cap-pulldown assays.** Antibodies used for immunoprecipitation are anti-BRC2A2 rabbit pAb Abcam ab123491, anti-Msi1 EMD Millipore AB5977, anti-elf4fA1 Cell Signaling #2490, anti-c-Myc clone 9E10 Sigma-Aldrich M5546, and normal rabbit IgG Santa Cruz Biotechnology sc-2027. Cells were lysed in the following buffers: immunoprecipitation (IP) buffer: 25 mM Tris HCl pH 7.4, 150 mM NaCl, 1% NP-40, 5% glycerol, 1 mM EDTA, 1 mM EGTA, 1 mM β-glycerophosphate, 10 mM NaF, 2.5 mM Na3PO₄, 1 µM sodium orthovanadate, plus protease inhibitor cocktail. RNA immunoprecipitation (RIP) buffer: 200 mM NaCl, 25 mM Tris HCl pH 7.4, 5 mM EDTA, 5% glycerol, 1 mM DTT, 1% NP-40, plus protease inhibitor cocktail and RNase inhibitors. Cap-pulldown (CPD) buffer: 10 mM Tris HCl pH 7.6, 140 mM KCl, 4 mM MgCl₂, 1 mM DTT, 1 mM EDTA, 1% NP-40, 1 mM PMSF, protease inhibitor cocktail, 0.2 mM sodium orthovanadate.

**For immunoprecipitation and RNA immunoprecipitation (RIP),** lysates were preclarified with protein A/G (Santa Cruz sc-203) and incubated on a rotating shaker overnight at 4°C with protein A/G plus antibody. Four micrograms of antibody was added to 2-mg cell lysate. IP, beads were washed four times with IP buffer, eluted in 3x sample buffer and analyzed by immunoblotting. For RIP, washed beads were split into a protein fraction and an RNA fraction. The protein fraction was subject to IP as described above. The RNA fraction was extracted with Trizol LS and bound RNA was analyzed by qPCR. For cap-pulldown experiments (CPD), cells were lysed in buffer listed below. About 25–50 µl of m7GTP beads (a-amino-phenyl-m7 GTP (C10-spacer) Jena Biosciences CLK-1042-10) and the ClickIT kit (Thermo Fisher Scientific C10276) in a quarter volume, according to manufacturer’s instructions. 

**Immunofluorescence.** In total, 20,000 cells were grown on fibronectin-coated (10 µg/ml in PBS) coverslips. Cells were fixed with 4% PFA for 5 min at RT, permeabilized with 0.5% Triton X-100 in PBS, rinsed with PBS-glycine, and blocked overnight at 4°C in blocking buffer (10% goat serum and 0.2% Triton X-100 in PBS). Primary antibodies were used 2.5 µCi of exogenous 35-S L-Methionine (Perkin Elmer NE809001MC) was added for the last 30 min. Cells were washed once in ice-cold PBS, lysed in RIPA buffer, protein content was quantified by BCA assay and equal total protein per sample was run on SDS–PAGE gels and transferred to PVDF as described above. The VN was used for detection of stable cell lines. Stable pools of knockdown cells were obtained by selecting 2 ng/ml basicidin or 1–2 µg/ml puromycin for 48 h.

**A H A labeling.** Azidohomolansalane (Thermo Fisher Scientific C10102) was added to methionine-free DMEM at a final concentration of 40 µM and left to incorporate in HEK293Ts for 8 h. Subsequently, cell lysate was collected for immunoprecipitation.

**Following immunoprecipitation, the azide-alkyne conjugation reaction was performed using Diao Biotin alkyne (Jena Biosciences CLK-1042-10) and the ClickIT kit (Thermo Fisher Scientific C10276) in a quarter volume, according to manufacturer’s instructions.**
Tissues were paraffin embedded and sectioned by the UCSF Helen Diller Family Cancer Center mouse pathology core. Deparaffinization in xylene followed by antigen retrieval per manufacturer's instructions (Dako) was performed prior to immunofluorescence staining.

Epifluorescence images were obtained at ambient temperature using an Axiolver 200 microscope (Carl Zeiss) with a ×10 (NA, 0.25) or ×20 (NA, 0.4) objective, Spot RT camera (Diagnostic Instruments). High-magnification images were taken using the DeltaVision deconvolution microscope (Applied Precision) with a 60.14 NA Plan Apo objective (Olympus).

**Image analysis.** Immunoblot band intensity quantification was performed using ImageJ software. Immunofluorescence colorization was performed using ImageJ software (IACoP plugin).

**Molecular cloning.** GFP and luciferase reporters were created by cloning the UTR sequences of BRCA2 into pcDNA3.EGFP plasmid (Addgene #13031, deposited by Doug Golenbock) and pNL1.1nano-luciferase plasmid (Promega N1001), using the sequences of BRCA2 into pcDNA3.EGFP plasmid (Addgene #13031, deposited by Phil Sharp65) were transfected into A590 measured by spectrometer. Percent growth (mean ± SEM) was calculated as (Abs.124 – Abs.10)/Abs.10.

**Cell-cycle analysis.** Cells were transfected and fixed in ice-cold methanol and stored at –20 °C. Cells were stained in 3.8 mM sodium citrate, 25 μg/ml propidium iodide, and 10 μg/ml RNase A in PBS. Flow was performed on an LSRRI SOR machine and analysis of percent of cells in various cell-cycle stages was performed using FlowJo. Flow cytometry data was generated in the UCSF Parnassus Flow Cytometry Core which is supported by the Diabetes Research Center (DRC) grant, NIH P30 DK063720.

**Metabolomics.** Six million cells per condition (n = 4) were washed in PBS and pelleted before being snap frozen. Gas chromatography time-of-flight mass spectrometry with the silylation reagent N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (GC-TOF with MBSTFA) was performed at the West Coast Metabolomics Center at the University of California, Davis.

**Statistics and reproducibility.** Statistical analysis of ribosome profiling data was performed using Babel. Details of statistical analyses of experiments and number of biological replicates (n) can be found in the figure legends. Prior to statistical analysis, outliers were identified by the Dixon test and at most a single value (p < 0.05) was excluded from the statistical test, but is still represented in the plots. Wherever an outlier was excluded is stated in the figure legend. Unless otherwise stated, statistical testing was performed using the two-sample equal variance t test with a cutoff of 0.05 for significance. Normal distribution was assumed unless otherwise stated. Statistical testing was performed in Excel, R, or Prism 8. Boxplots were generated in R using ggplot2.boxplot, producing boxplots in the style of Tukey, where the center line is the median, the lower and upper hinges correspond to the first and third quartile, the upper whisker extends from the hinge to the largest value no further than 1.5*IQR from the hinge (where IQR is the interquartile range, or distance between the first and third quartiles), and the lower whisker extends from the hinge to the smallest value at most 1.5 * IQR of the hinge. Immunoblotting experiments were performed a minimum of three times with independent biological replicates. All raw data are included in Supplementary Data 1.

**Data availability**

Figure 3 has associated raw data from ribosome profiling and RNAseq. RNAseq and ribosome profiling sequencing data is available at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA634689, Accession: PRJNA634689 ID: 634689. All other figures have associated raw data available in Supplementary Data 1 (Source data for all plots) and in the Supplementary Information file (Unprocessed immunoblot images). Any additional data, hard copies of lab notebooks stored in the pathology department at UCSF and all data backed up on multiple hard drives and university cloud storage, can be made available upon request by contacting the corresponding author.

**Code availability**

All data and code used to analyze the sequencing data are available in UCSF Dash (dayhydrad.org) with the identifier https://doi.org/10.7272/Q6N87ZT.

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**Conflict of interest**

None.

**Reporting summary**

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

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**Author contributions**

Conceptualization: J.G. and J.D.; investigation: J.G.; visualization: J.G.; formal analysis: J.G.; data curation: J.G.; writing—original draft: J.G.; writing—review and editing: J.D., T.M., S.A., and A.O.; software: S.A. and A.O.; resources: T.M., D.S., A.M.L., A.O., and S.A.; funding acquisition: J.D. and A.O.; supervision: J.D.

**Competing interests**

J.D. serves on the Scientific Advisory Board for Vescor Pharmaceuticals, LLC. All other authors declare no competing interests.

**Additional information**

**Supplementary information** is available for this paper at https://doi.org/10.1038/s42003-020-1090-2.

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