

GAPDH inhibits intracellular pathways during starvation for cellular energy homeostasis

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Starvation poses a fundamental challenge to cell survival. Whereas the role of autophagy in promoting energy homeostasis in this setting has been extensively characterized¹, other mechanisms are less well understood. Here we reveal that glyceraldehyde 3-phosphate dehydrogenase (GAPDH) inhibits coat protein I (COPI) transport by targeting a GTPase-activating protein (GAP) towards ADP-ribosylation factor 1 (ARF1) to suppress COPI vesicle fission. GAPDH inhibits multiple other transport pathways, also by targeting ARF GAPs. Further characterization suggests that this broad inhibition is activated by the cell during starvation to reduce energy consumption. These findings reveal a remarkable level of coordination among the intracellular transport pathways that underlies a critical mechanism of cellular energy homeostasis.

The activation of ADP-ribosylation factor (ARF) small GTPases initiates vesicular transport by recruiting coat proteins to intracellular membrane compartments for vesicle formation². In the case of vesicle formation by the COPI complex, the GAP that de-activates ARF1—known as ARFGAP1—is also an ARF effector, acting as a coat component^{3,4}. To gain further insight into how ARFGAP1 acts in COPI transport, we sought to identify new interacting proteins. By incubating ARFGAP1 with cytosol in a pull-down experiment, we identified GAPDH as one such protein (Extended Data Table 1). Although it is well known for acting in glycolysis, GAPDH is also known to have multiple non-glycolytic roles⁵. Therefore, to investigate whether it has a role in COPI transport, we initially performed a COPI transport assay as described^{6–8}. In cells treated with small interfering RNA (siRNA) against *GAPDH*, we observed enhanced COPI transport (Fig. 1a, Extended Data Fig. 1a). Targeting specificity was confirmed by a rescue experiment (Fig. 1a, Extended Data Fig. 1b), and by treatment with an unrelated siRNA sequence (Extended Data Fig. 1c). The siRNA treatments were limited to two days to maintain cell viability (Extended Data Fig. 1d). Consistent with the effect of reducing GAPDH level, GAPDH overexpression had the opposite effect of inhibiting COPI transport (Fig. 1b, Extended Data Fig. 1e). The expression levels of GAPDH in the different treatment conditions were also documented (Extended Data Fig. 1f). These initial results suggested that GAPDH acts as a negative regulator of COPI transport.

We next investigated COPI vesicle formation from Golgi membrane using a reconstitution system^{6–8}. The addition of purified GAPDH to this *in vitro* assay inhibited vesicle formation (Fig. 1c). This effect was specific to GAPDH, as several other metabolic enzymes did not have a similar effect (Fig. 1c). We also performed electron microscopy, and found that GAPDH induced the accumulation of buds with constricted necks on the Golgi membrane (Fig. 1d). These results suggested that GAPDH inhibits COPI transport by targeting the fission stage of vesicle formation.

We next found that GAPDH binds directly to ARFGAP1 (Extended Data Fig. 1g) and interacts with ARFGAP1 in cells (Extended Data Fig. 1h). Thus, we performed a GAP assay, which showed that GAPDH—but not other metabolic enzymes—inhibits the catalytic

activity of ARFGAP1 (Fig. 1e). Complementing this finding, we found that a mutant ARFGAP1 that is deficient in catalytic activity could not promote COPI vesicle fission (Fig. 1f). ARFGAP1 also acts as a coat component by promoting coat polymerization and cargo sorting^{3,4}. However, GAPDH did not affect the interaction of ARFGAP1 with coatomer (Extended Data Fig. 1i) or with COPI cargo proteins (Extended Data Fig. 1j). Therefore, we concluded that GAPDH inhibits COPI vesicle fission by targeting the GAP activity of ARFGAP1. This role of GAPDH did not require its catalytic activity (Supplementary Information, and Extended Data Fig. 1k, l).

We then sought to understand how GAPDH could be recruited to the Golgi to inhibit COPI transport. Prompted by the observation that starvation redistributes GAPDH from the cytosol to the nucleus⁹, we performed a subcellular fractionation experiment and found that starvation also redistributed GAPDH to cytoplasmic membranes (Extended Data Fig. 2a). Confocal microscopy revealed that this redistribution involved the Golgi (Extended Data Fig. 2b), the trans-Golgi network (TGN) (Extended Data Fig. 2c), early endosomes (Extended Data Fig. 2d) and the late endocytic compartments (Extended Data Fig. 2e). We also detected a pool of GAPDH at the endoplasmic reticulum (ER), but the level of this ER pool was not affected by starvation (Extended Data Fig. 2f).

We next examined whether GAPDH also inhibits other intracellular pathways. Performing a quantitative screen of the major pathways, as previously described¹⁰, we found that siRNA against *GAPDH* did not affect transport from the ER to the Golgi (Fig. 2a, Extended Data Fig. 2g), but enhanced transport from the Golgi to the plasma membrane (Fig. 2b, Extended Data Fig. 2h). All three major endocytic routes were also enhanced, including endocytic recycling (Fig. 2c, Extended Data Fig. 2i), endocytic transport to the Golgi (Fig. 2d, Extended Data Fig. 2j) and endocytic transport to the lysosome (Fig. 2e, Extended Data Fig. 2k). Fluid-phase uptake was also enhanced by the siRNA treatment against *GAPDH* (Fig. 2f, Extended Data Fig. 2l). However, clathrin-mediated endocytosis (Fig. 2g) and two types of non-clathrin endocytosis (Extended Data Fig. 2m, n) were not affected. Moreover, similar to the case of COPI transport, we found that GAPDH overexpression had the opposite effect of inhibiting the affected pathways (Fig. 2a–g). The specificity of our detection of GAPDH and organelle markers were also confirmed (Supplementary Information and Extended Data Figs. 2o–r, 3a–l).

We then examined whether GAPDH inhibits these additional pathways also by targeting ARF GAPs. ARF6 and its cognate GAP, ACAP1, act in endocytic recycling¹¹; GAPDH inhibited the GAP activity of ACAP1 towards ARF6 (Fig. 2h). ARF1 and its cognate GAP, AGAP1, act in endocytic transport to the lysosome¹²; GAPDH also inhibited the GAP activity of AGAP1 towards ARF1 (Fig. 2i). Moreover, other metabolic enzymes did not have similar effects (Extended Data Fig. 3m, n). In the case of exit from the ER, Sec23p is the cognate GAP for the ARF-like small GTPase Sar1p¹³. Consistent with our finding above that GAPDH does not inhibit this pathway, we found that GAPDH also did

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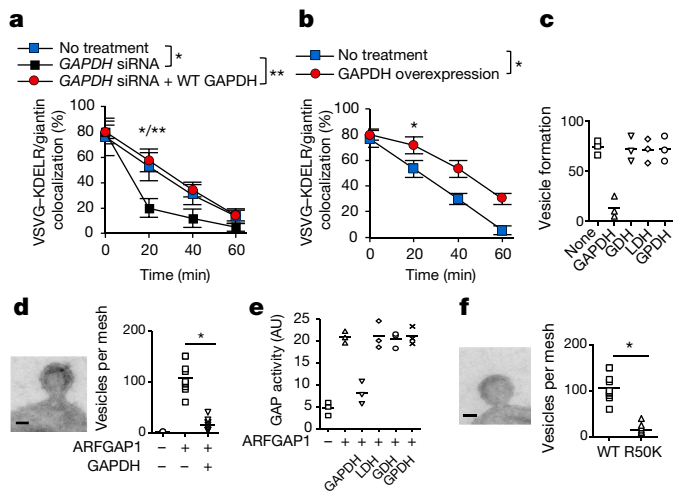


Fig. 1 | GAPDH inhibits COPI vesicle fission by targeting the GAP activity of ARFGAP1. **a, b**, COPI transport in HeLa cells. * $P = 9.8 \times 10^{-7}$, ** $P = 9.2 \times 10^{-9}$ (**a**); * $P = 6.8 \times 10^{-6}$ (**b**). **c**, Vesicle reconstitution system using Golgi membrane and purified proteins, with added metabolic enzymes as indicated. GPDH, glutamate dehydrogenase; LDH, lactate dehydrogenase; GAPDH, glyceral-3-phosphate dehydrogenase. **d**, Vesicle reconstitution system, with added purified proteins as indicated. Left, electron microscopy image of a Golgi membrane. Right, vesicle quantification. * $P = 8.9 \times 10^{-7}$. **e**, GAP assay using ARF1 and ARFGAP1, with added metabolic enzymes as indicated. **f**, Vesicle reconstitution system, with added ARFGAP1 form as indicated. Left, electron microscopy image of Golgi membrane. Right, vesicle quantification. * $P = 6.2 \times 10^{-6}$. In **a** and **b**, a representative experiment of three independent experiments is shown, data are mean $\pm$ s.d. of 10 fields of cells examined. In **c** and **e**, data are mean $\pm$ s.d. from a representative experiment of three independent experiments. In **d** and **f**, a representative experiment of three independent experiments is shown, data are mean $\pm$ s.d. of 10 electron microscopy meshes. P values determined by two-tailed t -test. Scale bars, 50 nm.

not inhibit the GAP activity of Sec23p towards Sar1p (Fig. 2j). GAPDH bound directly to ACAP1 (Extended Data Fig. 3o) and AGAP1 (Extended Data Fig. 3p), but not to Sec23p (Extended Data Fig. 3q). Therefore, these results suggest that GAPDH inhibits the transport

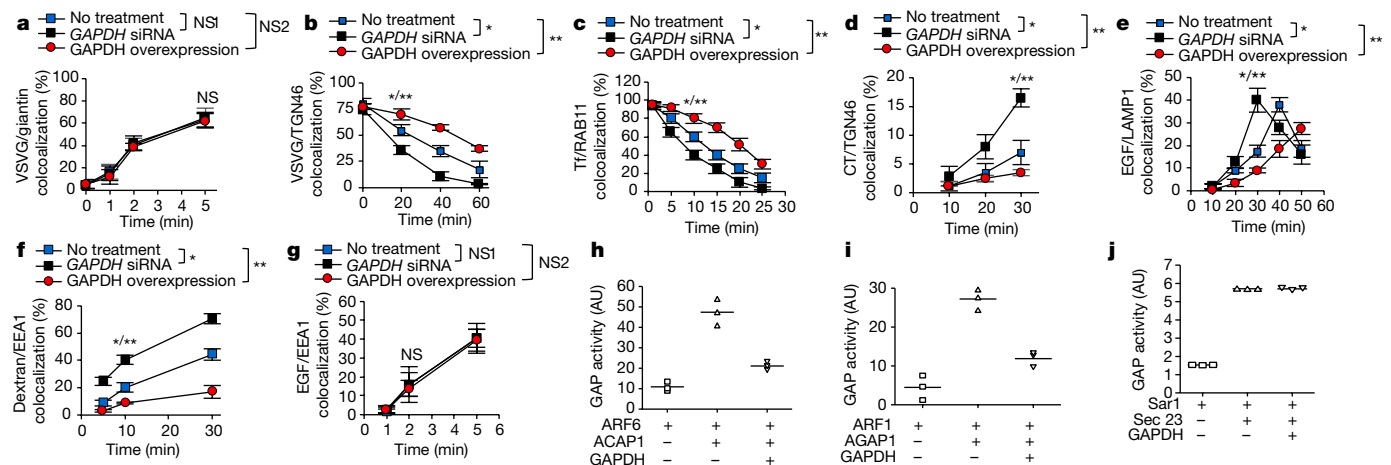


Fig. 2 | GAPDH inhibits other intracellular pathways also by targeting ARF GAPs. **a–g**, Transport assays in HeLa cells. **a**, Transport of vesicular stomatitis virus G protein (VSVG) from the ER to the Golgi. $P = 0.625$ (NS1); $P = 0.438$ (NS2). **b**, Transport of VSVG from the Golgi to the plasma membrane. * $P = 7.6 \times 10^{-8}$, ** $P = 5.4 \times 10^{-6}$. **c**, Endocytic recycling of transferrin (Tf) from the early endosome to the plasma membrane. * $P = 1.05 \times 10^{-7}$, ** $P = 1.05 \times 10^{-7}$. **d**, Endocytic transport of cholera toxin (CT) from the plasma membrane to the Golgi. * $P = 2.7 \times 10^{-9}$, ** $P = 4.5 \times 10^{-4}$. **e**, Endocytic transport of

pathways by targeting ARF GAPs. Moreover, led by the consideration that the catalytic domain in ARFGAP1 is conserved across many ARF GAPs, but not for Sec23p, we found that GAPDH bound to the catalytic domain of ARFGAP1 (Extended Data Fig. 3r), which suggests why GAPDH does not interact with Sec23p, and therefore does not inhibit exit from the ER. We also examined transferrin endocytosis and the results further supported that GAPDH inhibits the transport pathways by targeting ARF GAPs (Supplementary Information and Extended Data Figs. 3s–v).

We then examined the effect of starvation on the transport pathways. Cells were incubated with a general starvation medium (Fig. 3a–h), which lacks glucose and amino acids, Hank's medium (Extended Data Fig. 4a–h), which lacks amino acids and serum, or media that lack either glucose (Extended Data Fig. 4i–p) or amino acids (Extended Data Fig. 4q–x). In all cases, the same pathways were inhibited, and notably, these effects were reversed by siRNA against GAPDH (Fig. 3a–h and Extended Data Fig. 4a–x).

We then considered that vesicular transport consumes substantial cellular energy. We therefore hypothesized that the GAPDH-mediated transport inhibition enables the cell to conserve energy during starvation. In support of this hypothesis, we found that siRNA against GAPDH exacerbated the starvation-induced reduction in total ATP level, whereas GAPDH overexpression had the opposite effect (Fig. 3i, Extended Data Fig. 5a–c). Moreover, siRNA against GAPDH enhanced starvation-induced cell death, whereas GAPDH overexpression had the opposite effect (Fig. 3j). We ruled out that these effects could be attributed to the conventional role of GAPDH in glycolysis (Supplementary Information and Extended Data Fig. 5d–f). We also confirmed the key findings regarding how GAPDH acts in another cell type (Supplementary Information and Extended Data Fig. 5g–x).

We pursued another line of investigation that further supported GAPDH-mediated transport inhibition acting in energy homeostasis. AMPK has a central role in energy homeostasis, acting by sensing an energy deficit and then coordinating a wide range of cellular processes to restore energy homeostasis¹⁴. Treating cells with siRNA against AMPK α 1 (also known as PRKAA1), we found that starvation could no longer inhibit the transport pathways (Extended Data Fig. 6a–h); this was confirmed with a rescue experiment (Extended Data Fig. 6i, j), and with treatment using a different siRNA sequence (Extended Data Fig. 6k–s). Complementing these findings, we found that siRNA against

epidermal growth factor (EGF) from the plasma membrane to the lysosome. * $P = 1.6 \times 10^{-8}$, ** $P = 7.2 \times 10^{-7}$. **f**, Endocytosis of dextran. * $P = 1.1 \times 10^{-10}$, ** $P = 1.8 \times 10^{-6}$. **g**, EGF endocytosis. $P = 0.9997$ (NS1); $P = 0.418$ (NS2). **h–j**, GAP assays using ARF6 and ACAP1 (**h**), ARF1 and AGAP1 (**i**), or Sar1p and Sec23p (**j**). In **a–g**, one representative experiment of three independent experiments is shown, data are mean $\pm$ s.d. of 10 fields of cells examined. In **h–j**, data are mean $\pm$ s.d. from a representative experiment of three independent experiments. P values determined by two-tailed t -test. NS, not significant.

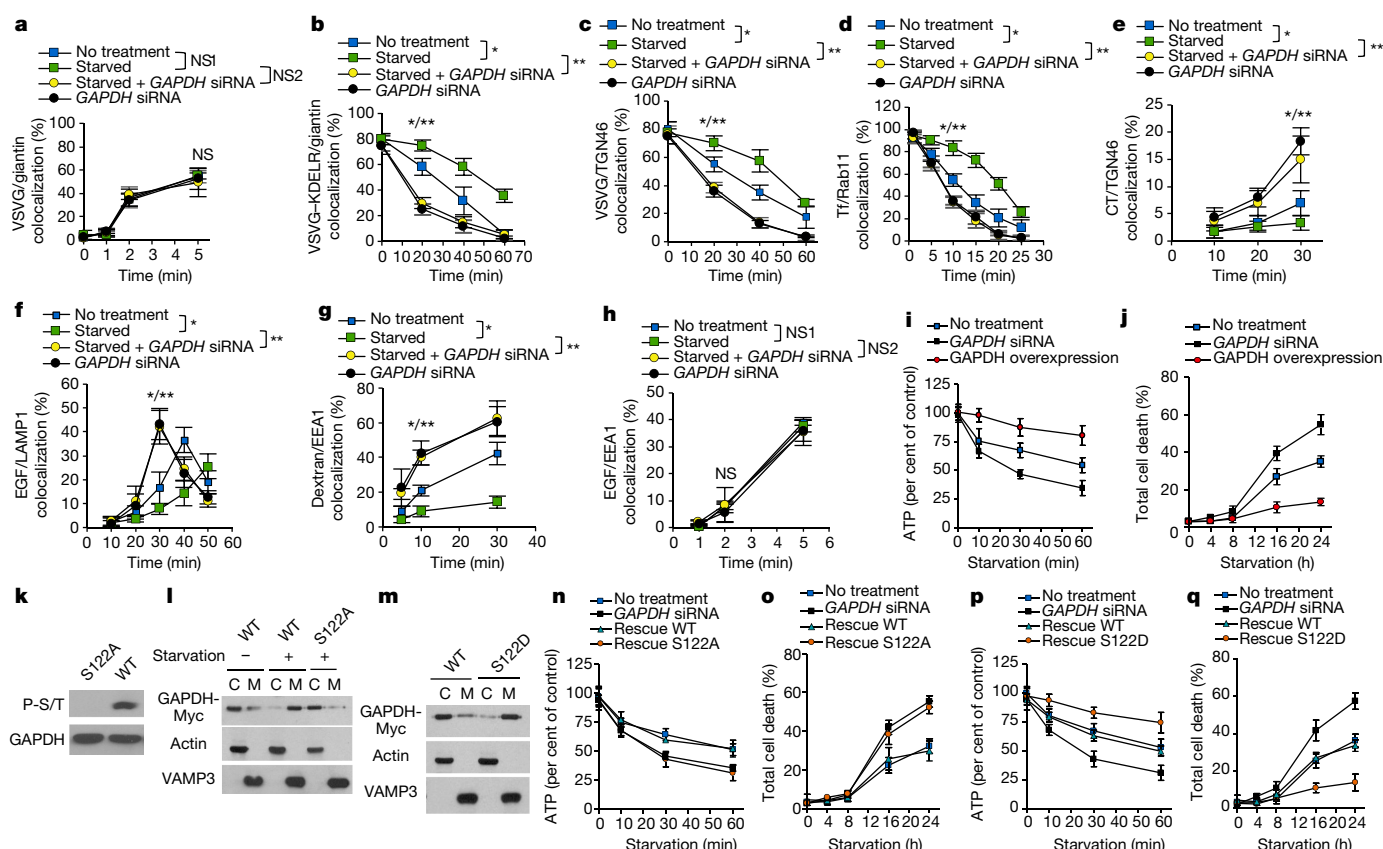


Fig. 3 | AMPK phosphorylates GAPDH to inhibit the transport pathways for energy homeostasis. Treatments as indicated. **a–h**, Transport assays in HeLa cells. **a**, ER to Golgi. $P = 0.7084$ (NS1), $P = 0.175$ (NS2). **b**, Golgi to ER. $*P = 5.7 \times 10^{-6}$, $**P = 6.3 \times 10^{-15}$. **c**, Golgi to plasma membrane. $*P = 5.4 \times 10^{-6}$, $**P = 4.3 \times 10^{-11}$. **d**, Early endosome to plasma membrane. $*P = 5.5 \times 10^{-9}$, $**P = 1.2 \times 10^{-12}$. **e**, Plasma membrane to Golgi. $*P = 5.4 \times 10^{-4}$, $**P = 6.2 \times 10^{-6}$. **f**, Plasma membrane to lysosome. $*P = 1.7 \times 10^{-3}$, $**P = 1.9 \times 10^{-8}$. **g**, Dextran endocytosis. $*P = 7.8 \times 10^{-8}$, $**P = 2.1 \times 10^{-12}$. **h**, EGF endocytosis. $P = 0.9994$ (NS1), $P = 0.7437$ (NS2). **i**, Total ATP level. **j**, Cell death.

AMPK α 1 prevented starvation from redistributing GAPDH to membrane compartments (Extended Data Figs. 7a–e).

We also activated AMPK in the normal (non-starved) condition by treating cells with 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), and found that it reproduced the effect of starvation in suppressing the identical transport pathways, and this effect was also reversed by siRNA against *GAPDH* (Extended Data Fig. 7f–m). This result was further confirmed using A769662, a more specific activator of AMPK (Extended Data Fig. 7n–u). Moreover, AICAR treatment in the normal condition was sufficient to redistribute GAPDH to membrane compartments (Extended Data Fig. 8a–e). Thus, the collective results suggested that starvation acts through AMPK and then GAPDH to inhibit the transport pathways.

To examine how AMPK affects GAPDH, we were initially led by a previous observation that AMPK phosphorylates GAPDH at serine 122⁹. We confirmed this finding, observing that AMPK did not phosphorylate GAPDH(S122A) (Fig. 3k, Extended Data Fig. 8f, g). GAPDH was phosphorylated less efficiently when compared to acetyl-CoA carboxylase, a well-established substrate of AMPK (Extended Data Fig. 8h–k), possibly because the sequence surrounding the S122 residue does not represent an optimal consensus site for AMPK phosphorylation¹⁵. We also pursued cell-based studies, confirming that starvation activates AMPK in cells (Extended Data Fig. 8l). Starvation also increased the phosphorylation of GAPDH S122, and this effect was prevented by the S122A mutation (Extended Data Fig. 8m). Moreover, this phosphorylation was prevented by siRNA against *AMPK α 1*

(Extended Data Fig. 8n). Similar results were also seen in HEK293 cells (Extended Data Fig. 8o–q). Starvation also did not redistribute cytosolic GAPDH(S122A) to membranes (Fig. 3l). Consistent with this result, GAPDH(S122A) also prevented starvation from inhibiting the transport pathways (Extended Data Fig. 9a–h). When GAPDH S122 was mutated to aspartate (S122D), which mimics constitutive phosphorylation at this site⁹, we found that simply expressing GAPDH(S122D) in the non-starved condition was sufficient to redistribute cytosolic GAPDH to membranes (Fig. 3m). The expression of this mutant in the normal condition was also sufficient to inhibit the transport pathways (Extended Data Fig. 9i–p).

The effects of mutating the S122 residue in GAPDH suggested yet another way of confirming that GAPDH-mediated inhibition of transport acts in energy homeostasis. Replacing the endogenous GAPDH with the S122A mutant in cells exacerbated the decline in both the total ATP level and cell viability induced by starvation (Fig. 3n, o). By contrast, replacing endogenous GAPDH with the S122D mutant had the opposite effect of increasing the ATP level (Fig. 3p) and cell viability (Fig. 3q) during starvation.

AMPK phosphorylation of the S122 residue in GAPDH has previously been found to induce the translocation of GAPDH to the nucleus, where it stimulates sirtuin 1 (SIRT1) activity to activate a transcription program that promotes autophagy⁹. We confirmed that our general starvation medium (which lacks glucose and amino acids) activated autophagy (Fig. 4a, b), and found that the recruitment of GAPDH to the Golgi—which is needed to inhibit COPI transport—began immediately

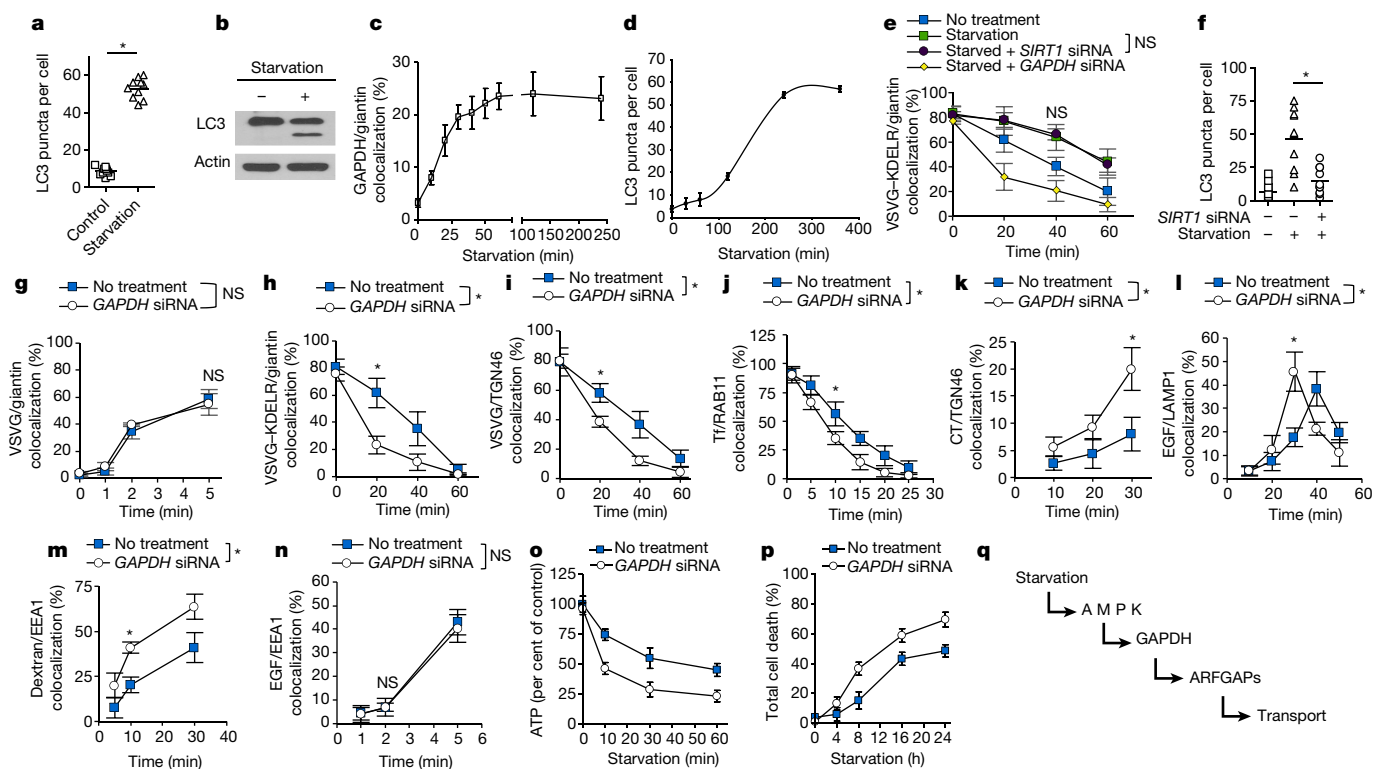


Fig. 4 | The roles of GAPDH in transport and autophagy are distinct. **a, b**, Autophagy in HeLa cells following starvation, assessed by LC3 puncta formation (**a**; $*P = 0.000152$) or LC3 gel shift (**b**). **c**, GAPDH recruitment to the Golgi in HeLa cells with the indicated conditions. **d**, LC3 puncta formation in HeLa cells. **e**, COPI transport assay in HeLa cells. $P = 0.8490$ (NS). **f**, LC3 puncta formation in HeLa cells. $*P = 0.00174$. **g–n**, Transport assays in ATG5-deficient MEFs. **g**, ER to Golgi. $P = 0.25$ (NS). **h**, Golgi to ER. $*P = 9.9 \times 10^{-8}$. **i**, Golgi to plasma membrane. $*P = 5.9 \times 10^{-7}$. **j**, Early endosome to plasma membrane. $*P = 6.4 \times 10^{-5}$. **k**, Plasma membrane to Golgi. $*P = 7.6 \times 10^{-7}$. **l**, Plasma membrane to lysosome. $*P = 3.2 \times 10^{-7}$.

upon starvation (Fig. 4c). By contrast, LC3 puncta formation, which tracks autophagosome formation, started more slowly (Fig. 4d). Further distinguishing between the roles of GAPDH in transport inhibition and autophagy, we found that siRNA against *SIRT1* did not have an appreciable effect on starvation-induced inhibition of COPI transport (Fig. 4e), but prevented starvation from inducing autophagy (Fig. 4f).

We also examined mouse embryonic fibroblasts (MEFs) derived from Atg5-deficient mice, which cannot undergo autophagy¹⁶, and found that GAPDH could still inhibit the identical transport pathways as those seen in autophagy-competent cells (Fig. 4g–n). Moreover, siRNA against *GAPDH* could still exacerbate the decline of total ATP (Fig. 4o) and cell viability (Fig. 4p) during starvation. In another approach, we modified GAPDH so that its redistribution to the nucleus was prevented, but its recruitment to cytoplasmic membrane compartments was preserved. This form of GAPDH could still inhibit the transport pathways, and increased ATP level and cell viability during starvation (Supplementary Information and Extended Data Fig. 10a–u). Therefore, multiple lines of evidence all point to the roles of GAPDH in transport inhibition and autophagy being distinct.

In summary, we have defined a new mechanism of energy homeostasis that is critical for the cell to survive during starvation. Mechanistically, this involves the activation of AMPK to induce the redistribution of cytosolic GAPDH to multiple membrane compartments, which then targets ARF GAPs to inhibit different transport pathways (Fig. 4q). As the cell encounters starvation in many settings, considerable effort has been devoted to elucidating mechanisms of cell survival in this condition. Autophagy has emerged as a major mechanism¹. In the overall energy equation, autophagy acts to increase energy availability. By contrast, transport inhibition by GAPDH acts to reduce

energy consumption. These fundamentally distinct mechanisms complement each other to promote energy homeostasis during starvation. Another notable distinction between the two mechanisms is that the transport inhibition by GAPDH occurs more rapidly than autophagy. This more rapid mechanism is likely to enable the cell to stave off the lethal consequences of starvation while longer-term solutions, such as autophagy, are being mobilized.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at <https://doi.org/10.1038/s41586-018-0475-6>.

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Competing interests The authors declare no competing interests.

Additional information

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METHODS

Chemicals, proteins and cells. AICAR, chloroquine diphosphate, methyl pyruvate, protease inhibitor cocktail and phosphatase inhibitor cocktail were obtained from Sigma. AMPK activator A-769662 was obtained from Cayman. Alexa Fluor 546-conjugated transferrin, and Alexa Fluor 555-conjugated forms of EGF, dextran and cholera toxin were obtained from Invitrogen.

Purification of coatomer, ARF1, ARFGAP1, BARS, Golgi-enriched membrane and cytosol have been described^{3,17}. Purification of ARF6, ACAP1, and AGAP1 have also been described^{11,12}. Purified mammalian Sar1p and Sec23p were obtained from J. Goldberg, Memorial Sloan-Kettering Cancer Center. Purified forms of GAPDH, glutamate dehydrogenase (GDH), glycerol-3-phosphate dehydrogenase (GPDH), and lactate dehydrogenase (LDH) were obtained from Sigma. AMPK and the SAMS peptide (HMRSAMSGHLVKRR) were obtained from (Promega).

For the purification of recombinant GAPDH, the pET30-2-GAPDH plasmid was transformed into BL21 competent cells. Isopropyl β -D-1-thiogalactopyranoside (0.1 mM) was added to induce protein expression. Bacteria were then resuspended in 5 ml lysis buffer (20 mM Tris pH 8.0, 100 mM NaCl, 10 mM imidazole, 1 mg/ml lysozyme, and protease inhibitors cocktail). After gentle agitation at 4°C for 30 min, the suspension was sonicated on ice 6 times for 10 s each, and then centrifuged at 12,000g for 20 min. The supernatant was recovered and then added to 0.5 ml Ni-beads (50% slurry), followed by incubation at 4°C for 1 h. Beads were then washed with 10 ml washing buffer (20 mM Tris pH 8.0, 100 mM NaCl, 10 mM imidazole). Bound proteins were then eluted with elution buffer (20 mM Tris pH 8.0, 100 mM NaCl, 250 mM imidazole).

HeLa, BSC-1, COS-7, CHO and HEK293 cells have previously been described^{10,18,19}. Wild-type and ATG5-deficient MEF cells were obtained from N. Mizushima, University of Tokyo. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum and supplemented with glutamine. Cells had been free of mycoplasma contamination upon their receipt, but were not tested immediately prior to use. Unless stated otherwise, starvation involves incubating cells in a medium consisting of phosphate-buffered saline (PBS) with 10% dialysed bovine serum.

Mass spectrometry. Cytosol was purified from rat liver by homogenization in lysis buffer (1 mM Tris pH 7.4, 800 mM sucrose, 5 mM EDTA and protease inhibitors) at 4°C, followed by centrifugation at 80,000g for 90 min. The resulting supernatant was dialysed against dialysis buffer (25 mM Tris at pH 8.0, 50 mM KCl and 1 mM DTT), followed by centrifugation at 150,000g for 90 min at 4°C. Aliquots were stored at -80°C. Pull-down experiments were performed by incubating glutathione S-transferase (GST) or GST-ARFGAP1 on beads (5 μ g) with 50 μ g rat liver cytosol in traffic buffer (25 mM HEPES pH 7.2, 50 mM KCl and 2.5 mM Mg(OAc)₂) for 1 h followed by extensive washing. Samples were separated by SDS-PAGE and then stained with Coomassie blue, followed by gel excision for protein identification by mass spectrometry (Taplin Mass Spectrometry Facility, Harvard Medical School). Those proteins (having at least five identified peptides) that interacted with GST-ARFGAP1 but not GST were considered specific.

Plasmids and antibodies. Mammalian expression vectors that contain temperature-sensitive mutants (ts-045) of VSVG and VSVG-KDEL have been described⁷. GAPDH for mammalian expression was subcloned into pcDNA3.1 and pEGFP-N1. GAPDH for bacterial expression (pET30-2-GAPDH) was obtained from Addgene (83910). Mutant forms of GAPDH were generated using QuikChange Site-Directed-Mutagenesis (Stratagene). The sequences of oligonucleotides used for mutagenesis are listed in Supplementary Table 1. The pEGFP-NES-GAPDH construct was obtained from W. Liu (Zhejiang University). IL2R- β tagged with haemagglutinin (HA) and Venus (HA-Venus-IL2R- β) was obtained from T. Waldman and O. Hurtado (NIH). GST-ARFGAP1, GST-Wbp1 and GST-KDEL have been described^{3,17}.

Antibodies against ARF1, ARFGAP1, β -COP, calnexin, cellubrevin, coatomer, giantin, GM130, LAMP1, Sec61p, TGN46 VSVG, TGN46, HA tag and Myc tag have previously been described^{10,17,19}. The following antibodies were also used: AMPK α (Cell Signaling, 2603S), β -actin (Ambion, AM4302), GFP (Invitrogen, GF28R), LC3 (Sigma, L7543), p62 (Santa Cruz, 28359), p172-AMPK α (Cell Signaling, 2531S), RAB11 (BD Biosciences, 610656), SIRT1 (Cell Signaling, 2493S), and Flag tag M2 (Sigma, F1804). Antibodies against GAPDH were obtained from three sources: Ambion (AM4300), Santa Cruz (FL335), and Sigma (G9545). Anti-pS/pT was obtained from Abcam (117253) and conjugated antibodies were obtained from Jackson ImmunoResearch, including Cy2- or Cy3-conjugated donkey antibodies against mouse or rabbit IgG, and horseradish peroxidase-conjugated donkey antibodies against mouse or rabbit IgG.

Transfections and siRNA. Transfection of DNA plasmids was performed using FuGene6 (Roche). Transfection of siRNA was performed using Lipofectamine RNAiMAX (Invitrogen). Sequences used for siRNA against GAPDH and AMPK α 1, and primers used to generate siRNA-resistant forms of GAPDH and AMPK α 1 are listed in Supplementary Table 1. Rescue plasmids were generated using QuikChange Site-Directed Mutagenesis (Stratagene).

We found that siRNA against GAPDH for 2 days was optimal for reducing the endogenous level while maintaining cell viability. Rescue of siRNA against GAPDH involved the transfection of siRNA-resistant GAPDH for 12 h to achieve limited GAPDH expression. GAPDH overexpression involved the transfection of GAPDH for 48 h.

In vivo transport assays. A quantitative microscopy-based approach, which involves the colocalization of model cargoes with organelle markers coupled with kinetic analysis was performed as previously described^{8,10}.

For anterograde transport from ER to Golgi, cells were transfected with pROSE-VSVG-ts045-Myc for 1 day, and then incubated at 39°C for 4 h to accumulate VSVG in the ER. Cells were then shifted to 32°C for different times as indicated in figures. Cells were then stained for giantin, followed by confocal microscopy to assess the arrival of VSVG to the Golgi.

For retrograde transport from the Golgi to the ER, cells were transfected with pROSE-VSVG-ts045-KDEL-Myc for 1 day, and then incubated at 32°C for 8 h to achieve steady-state distribution at the Golgi. Cells were then shifted to 39°C for different times as indicated in the figures. Cells were then stained for giantin, followed by confocal microscopy to assess the exit of VSVG-KDEL from the Golgi.

For anterograde transport from the Golgi to the plasma membrane, cells were transfected with pROSE-VSVG-ts045-Myc for 1 day, and then incubated at 20°C for 2 h to accumulate VSVG at the TGN. Cells were then shifted to 32°C for different times as indicated in the figures. Cells were then stained for TGN46, followed by confocal microscopy to assess the exit of VSVG from the Golgi.

For the recycling of transferrin from the early recycling endosome to the plasma membrane, cells were incubated with Alexa 546-conjugated transferrin (5 μ g/ml in DMEM) at 37°C for 2 h to allow the steady-state accumulation of transferrin in endosomes. Subsequently, cells were incubated with medium without transferrin for different times as indicated in the figures. Cells were then stained for RAB11, followed by confocal microscopy to assess the exit of transferrin from the early recycling endosome.

For the retrograde transport of cholera toxin from the plasma membrane to the Golgi, cells were incubated with Alexa 555-conjugated cholera toxin for 30 min at 4°C (0.5 μ g/ml in DMEM). After washing to clear unbound cholera toxin, cells were shifted to 37°C for different times as indicated in the figures. Cells were then stained for TGN46, followed by confocal microscopy to assess the arrival of cholera toxin to the Golgi.

For endocytic transport of EGF to the lysosome, cells were incubated with Alexa 555-conjugated EGF (1 μ g/ml in DMEM) for 1 h at 4°C. Cells were then washed to clear unbound EGF, followed by shifting to 37°C for times indicated in the figures. Cells were stained for LAMP1, followed by confocal microscopy to assess the arrival of EGF to the lysosome.

For the fluid-phase uptake of dextran, cells were incubated with Alexa 555-conjugated dextran (0.2 mg/ml) at 37°C for different times as indicated in the figures. Cells were then stained for EEA1, followed by confocal microscopy to assess the arrival of dextran to the early endosome.

For the endocytosis of EGF, cells were incubated with Alexa 555-conjugated EGF (1 μ g/ml in DMEM) for 1 h at 4°C. Cells were then washed to clear unbound EGF, followed by shifting to 37°C for times indicated in the figures. Cells were stained for EEA1, followed by confocal microscopy to assess the arrival of EGF to the early endosome.

For the endocytosis of transferrin, cells were incubated with Alexa 546-conjugated transferrin (5 μ g/ml in DMEM) for 1 h at 4°C. Cells were then washed to clear unbound transferrin, followed by shifting to 37°C for times indicated in the figures. Cells were stained for EEA1, followed by confocal microscopy to assess the arrival of transferrin to the early endosome.

For the endocytosis of cholera toxin, cells were incubated with Alexa 555-conjugated cholera toxin for 30 min at 4°C (0.5 μ g/ml in DMEM). Cells were then washed to clear unbound cholera toxin, followed by shifting to 37°C for different times as indicated in the figures. Cells were stained for EEA1, followed by confocal microscopy to assess the arrival of cholera toxin to the early endosome.

For the endocytosis of IL2R- β , cells were transfected with pCDH-HA-Venus-IL2R- β for 1 day at 37°C. Cells were then incubated with anti-HA antibody at 4°C for 1 h to label the surface pool of the receptor. Cells were washed to clear unbound antibody, followed by shifting to 37°C for different times as indicated in the figures. Cells were then stained for EEA1, followed by confocal microscopy to assess the arrival of IL2R- β to the early endosome.

Confocal microscopy. Colocalization studies were performed with either a Nikon or a Zeiss system. The Nikon system is equipped with the Nikon Eclipse TE2000U Inverted Microscope having a Plan Apo 60 $\times$ /1.40 oil objective, Nikon D-Eclipse C1 confocal package with a 488 Laser (having 515/30 emission filter) and a 543 Laser (having 590/50 emission filter), and Nikon EZ-C1 v.3.90 acquisition software. The Zeiss system is equipped with the Zeiss Axio Observer Z1 Inverted Microscope having a Plan-Apochromat 63 $\times$ objective, the Zeiss LSM 800 with Airyscan confocal package with Zeiss URG (488- and 561-nm) laser lines, and Zen 2.3 blue edition confocal acquisition software.

For quantification of colocalization, ten fields of cells were examined, with each field typically containing about 5 cells. Images were imported into the NIH ImageJ v.1.50e software, and then analysed through a plugin software (https://imagej.net/Coloc_2). Under the 'image' tab, the 'split channels' option was selected. Under the 'plugins' tab, 'colocalization analysis' option was selected, and within this option, the 'colocalization threshold' option was selected. Colocalization values were then calculated by the software, and expressed as the fraction of protein of interest (cargo or GAPDH) colocalized with an organelle marker.

In vitro reconstitution of COPI vesicle formation. The reconstitution was performed essentially as described¹⁷. In brief, Golgi membrane (0.2 mg/ml) was washed with 3 M KCl, and then incubated with ARF1 (6 µg/ml) and coatamer (6 µg/ml) for the first-stage incubation that reproduces the ARF-dependent recruitment of coatamer onto Golgi membrane. The Golgi membrane was re-isolated and then incubated with ARFGAP1 (2 µg/ml), BARS (2 µg/ml) for the second stage that results in vesicle formation. GAPDH (1 µg/ml) was added at the second stage. Electron microscopy examination of Golgi membrane using the whole-mount technique has previously been described³. Quantification involves the examination of 10 meshes per condition.

Fractionation of the cytoplasm to obtain total membrane versus cytosol. Cells were washed with PBS, resuspended in homogenization buffer (0.25 M sucrose, 1 mM EDTA, and 20 mM HEPES-KOH, pH 7.4 and protease inhibitor cocktail) and then disrupted by passing through 28-gauge needles. After low-speed centrifugation (800g for 6 min) to spin out nuclei and unbroken cells, the resulting post-nuclear supernatant was centrifuged at 100,000g for 1 h to obtain cytosol and total membrane fractions.

Other in vitro assays. For pull-down assays, GST fusion proteins were bound to glutathione beads, then incubated with purified proteins. For the GAP assay, recombinant forms of ARF small GTPase were first loaded with GTP and then incubated with different ARF GAPs in the presence of liposomes. GTP hydrolysis was then quantified by detecting the generation of free phosphate (ATPase/GTPase Activity Assay Kit, Sigma). The in vitro kinase assay was performed by incubating AMPK (0.1 µg) with the SAMS peptide (a sequence derived from acetyl-CoA carboxylase) (3 µg) or different GAPDH proteins (40 µg) in 50 µl reaction buffer (25 mM MOPS, pH 7.2, 12.5 mM β-glycero-phosphate, 25 mM MgCl₂, 5 mM EGTA, 2 mM EDTA, 0.25 mM DTT, 100 µM AMP and 50 µM ATP) at 30 °C for times as indicated. Phosphorylation of the substrate was then quantified using the ADP-Glo Kinase assay (Promega), which detects ADP generated from ATP in the kinase reaction.

Other in vivo assays. Cellular ATP level was detected using ATPlite Luminescence Detection Assay System (Perkin Elmer), and was performed according to the

manufacturer's instructions, with final values normalized for cell number. Cell death was assessed by incubating cells with propidium iodide (1 ng/µl), and then quantifying for positively stained cells by flow cytometry. Lactate level was measured using a colorimetric assay kit (Biovision). Oxygen consumption rate (OCR) was measured using an XFe24 Extracellular Flux Analyzer (Seahorse Biosciences), as previously described²⁰. SIRT1 activity was measured using SIRT1 Direct Fluorescent Screening Assay Kit (Cayman), as previously described⁹. Autophagy was assessed through LC3 lipidation, using either confocal microscopy to quantify LC3 puncta formation, or western blotting to detect LC3 mobility shift, as previously described⁹.

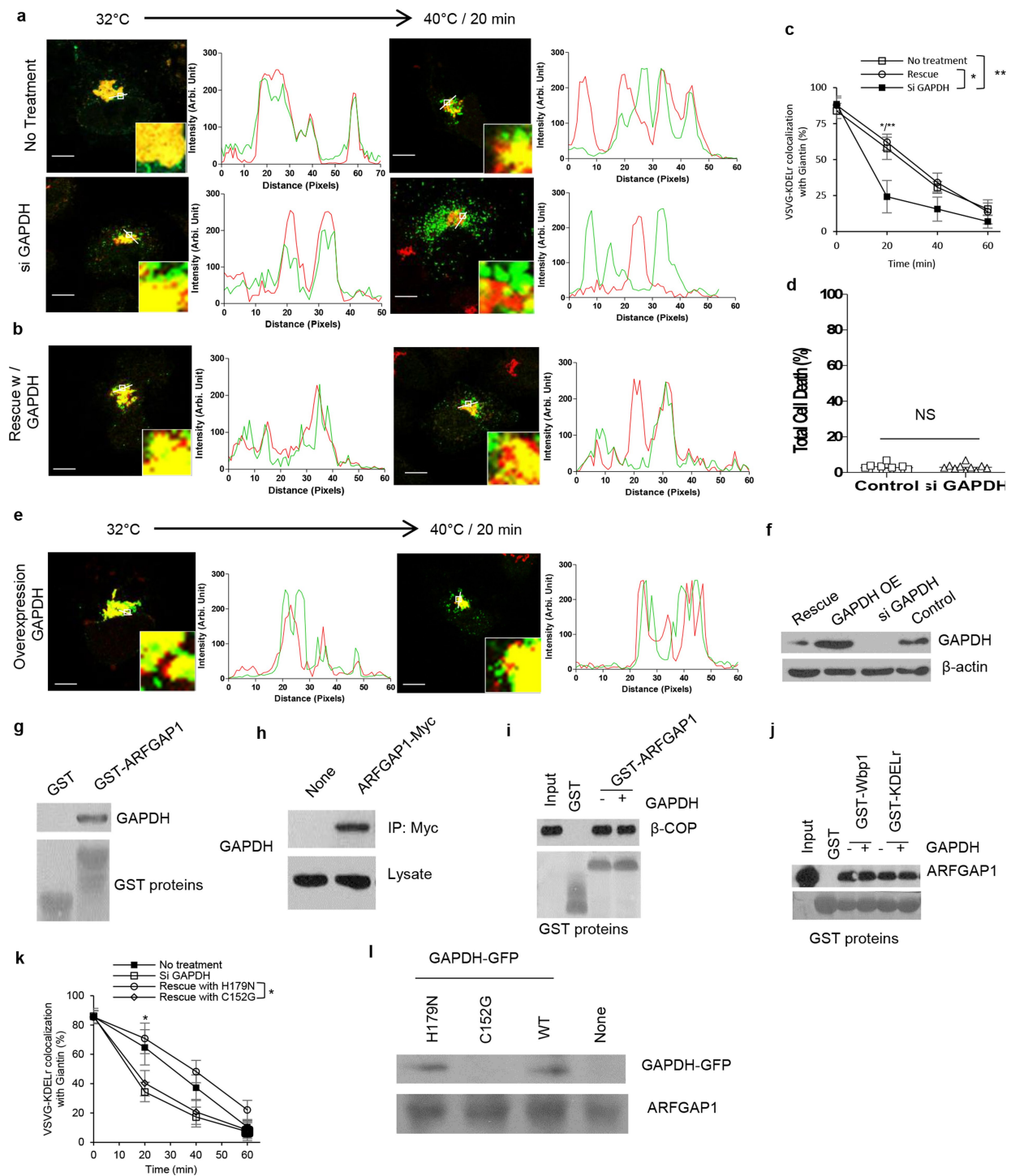
Experimental replicates. All colocalization studies are shown as 10 technical replicates from a representative experiment, with 3 independent experiments having been performed. Metabolic studies involving oxygen consumption rate are performed as 5 technical replicates, with 2 independent experiments having been performed. The result from a representative replicate is shown. All other studies show a representative experiment with the number of independent experiments indicated in the figure legend.

Statistical analysis. Sample size is noted in the figure legends. No statistical methods were used to predetermine sample size. Statistical significance was determined using Excel or Prism software for the two-tailed Student's *t*-test. No inclusion or exclusion criteria were pre-established. The experiments were not randomized. The investigators were not blinded to the group allocation during experiments and in outcome assessment.

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

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request. The following figures have associated raw data: Figs. 1a, b, 2a–g, 3a–j, n–q, 4c–e, g–p; Extended Data Figs. 1c, k, 2m,n, s–v, 3 s–v, 4a–x, 5a–c, g–x, 6a–h, j, l–s, 7f–u, 9a–p, 10h–u. For gel source data, see Supplementary Fig. 1.

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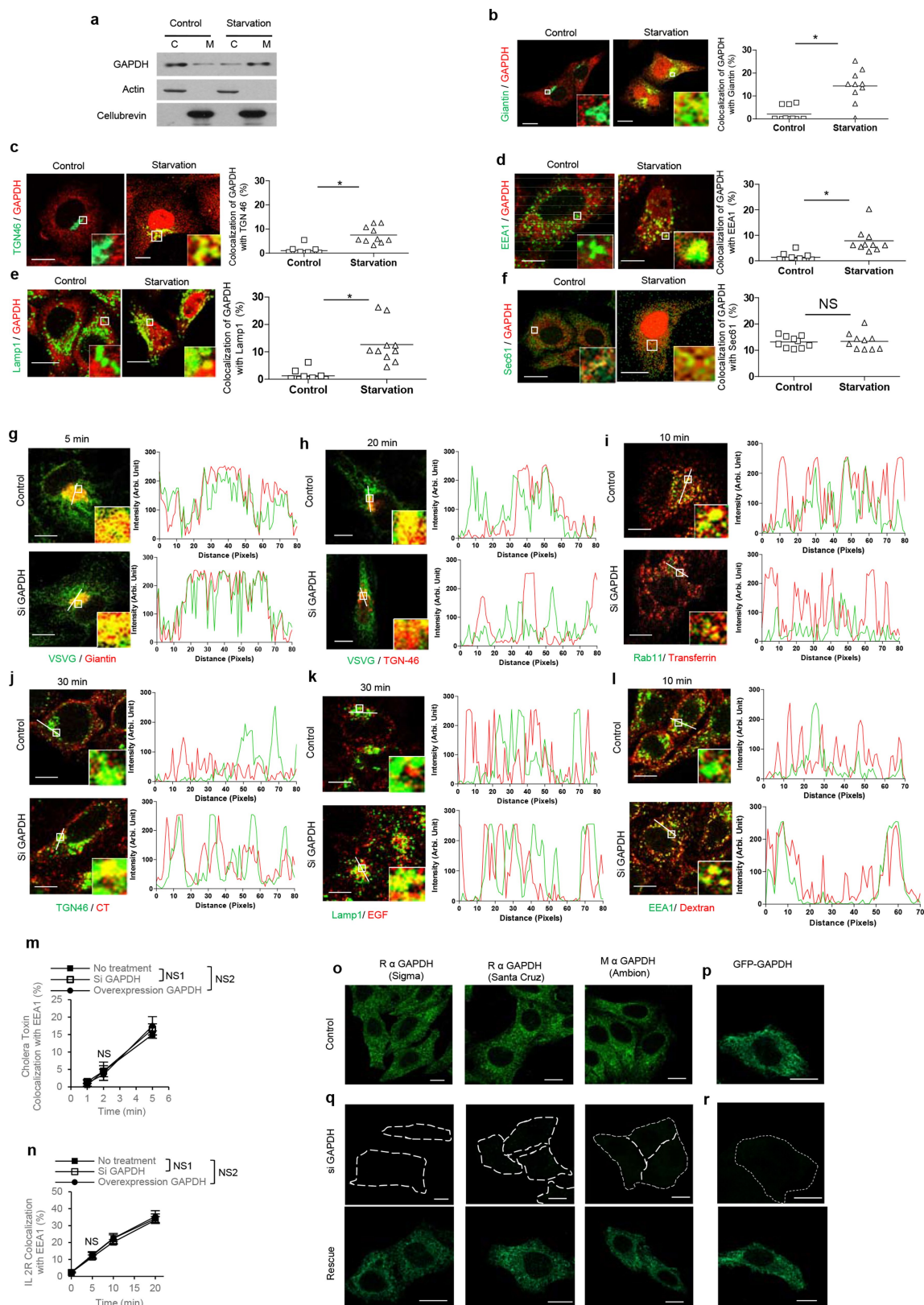


Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Further characterization of GAPDH inhibition of COPI transport. **a, b**, The COPI transport assay was performed on HeLa cells that were treated as indicated. A confocal image from a representative experiment (out of three) is shown. Green, VSVG-KDEL; red, giantin. Scale bar, 10 μ m. Line-scan analysis for each image is also shown. **c**, The COPI transport assay was performed on HeLa cells that were treated as indicated. Quantification of a representative experiment (out of three) is shown, $n = 10$ fields of cells examined. Data are mean $\pm$ s.d. $*P = 2.8 \times 10^{-7}$, $**P = 8.2 \times 10^{-7}$. **d**, HeLa cells were treated as indicated, and cell death was quantified by flow cytometry. Data are mean $\pm$ s.d. from $n = 10$ independent experiments. $P = 0.7314$ (NS). **e**, HeLa cells were treated as indicated, and the COPI transport assay was performed. A confocal image from a representative experiment (out of three) is shown. Green, VSVG-KDEL; red, giantin. Scale bar, 10 μ m. Line-scan analysis for each image is also shown. **f**, HeLa cells were treated as indicated, and whole-cell lysates were immunoblotted for proteins as indicated. Representative blot from three independent experiments. **g**, GST fusion proteins were incubated with purified GAPDH in a pull-down experiment, followed by immunoblotting as indicated. Representative blot from three independent experiments. **h**, HeLa

cells were transfected with constructs as indicated, followed by immunoprecipitation using the Myc tag and then immunoblotting for GAPDH. Representative blot from two independent experiments. **i**, GST fusion proteins as indicated were bound to beads and then incubated with purified coatamer in a pull-down experiment, followed by immunoblotting to detect β -COP or Coomassie staining to detect GST fusion proteins. Representative results from three independent experiments. **j**, The indicated cytoplasmic tails of cargo proteins were fused to GST, bound to beads, and then incubated with ARFGAP1, followed by immunoblotting with antibody against ARFGAP1 or Coomassie staining to detect GST fusion proteins. Representative results from two independent experiments. **k**, HeLa cells were treated as indicated, and assayed for COPI transport. Quantification of a representative experiment (of three) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. $*P = 1.4 \times 10^{-6}$. **l**, HeLa cells were transfected with GAPDH constructs as indicated. ARFGAP1 immunoprecipitates were immunoblotted for the different forms of GAPDH as indicated. Representative blot from two experiments. P values determined by two-tailed t -test.

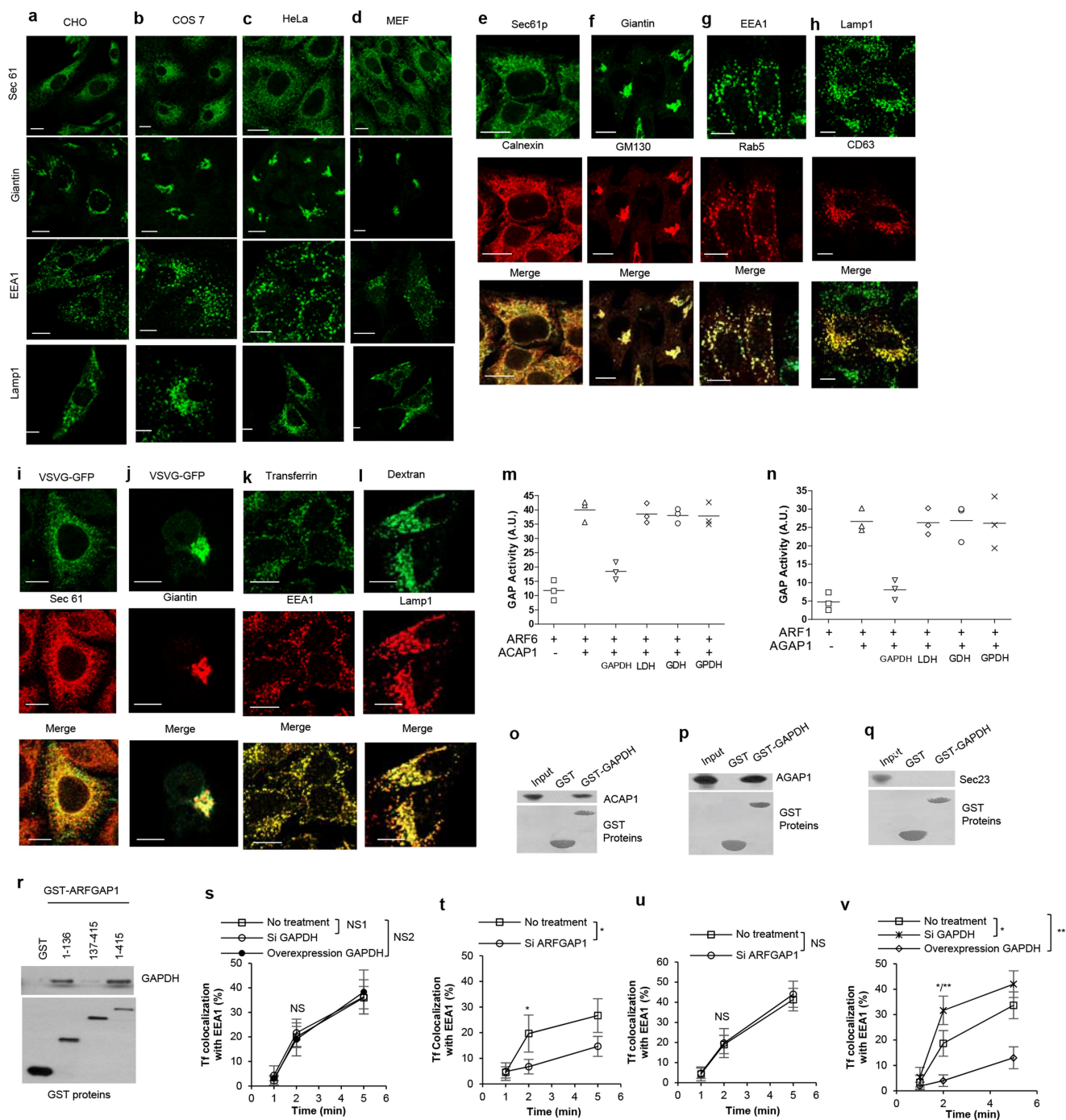
cells were transfected with constructs as indicated, followed by immunoprecipitation using the Myc tag and then immunoblotting for GAPDH. Representative blot from two independent experiments. **i**, GST fusion proteins as indicated were bound to beads and then incubated with purified coatamer in a pull-down experiment, followed by immunoblotting to detect β -COP or Coomassie staining to detect GST fusion proteins. Representative results from three independent experiments. **j**, The indicated cytoplasmic tails of cargo proteins were fused to GST, bound to beads, and then incubated with ARFGAP1, followed by immunoblotting with antibody against ARFGAP1 or Coomassie staining to detect GST fusion proteins. Representative results from two independent experiments. **k**, HeLa cells were treated as indicated, and assayed for COPI transport. Quantification of a representative experiment (of three) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. $*P = 1.4 \times 10^{-6}$. **l**, HeLa cells were transfected with GAPDH constructs as indicated. ARFGAP1 immunoprecipitates were immunoblotted for the different forms of GAPDH as indicated. Representative blot from two experiments. P values determined by two-tailed t -test.



Extended Data Fig. 2 | See next page for caption.

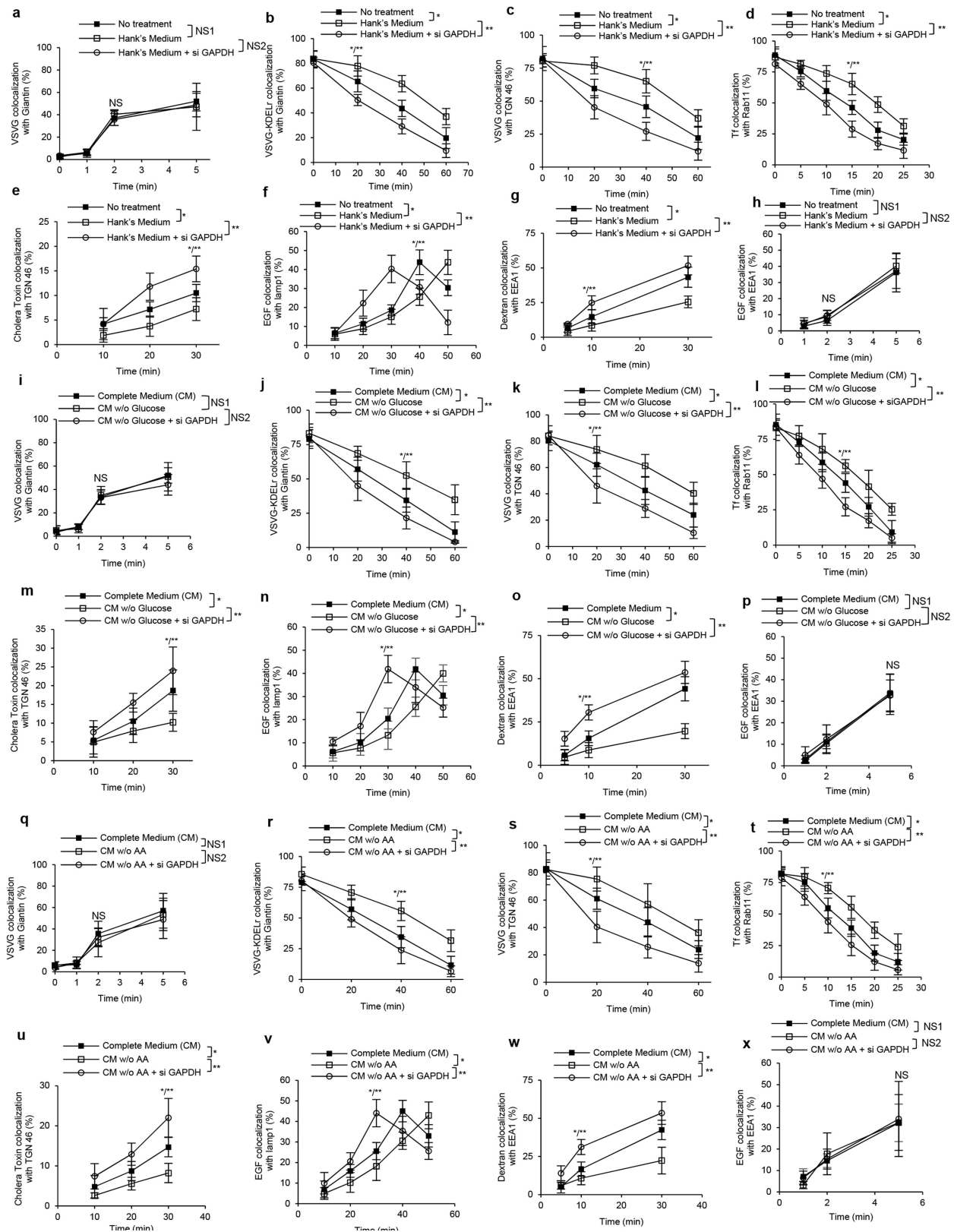
Extended Data Fig. 2 | Further characterizing how GAPDH affects other pathways. **a**, HeLa cells were treated as indicated, fractionated into cytosol (C) and total membrane (M), and immunoblotted for proteins as indicated. Representative blot from two independent experiments. **b–f**, HeLa cells were treated as indicated, and examined for the colocalization between GAPDH and different organelle markers. A confocal image from a representative experiment (out of three) is shown. Scale bar, 10 μm (left); quantification of colocalization (right). Data are mean $\pm$ s.d. of $n = 10$ fields of cells examined. Colocalization of GAPDH (red) with giantin (green), $*P = 2.9 \times 10^{-4}$ (**b**); colocalization of GAPDH (red) with TGN46 (green), $*P = 1.5 \times 10^{-4}$ (**c**); colocalization of GAPDH (red) with EEA1 (green), $*P = 1.9 \times 10^{-3}$ (**d**); colocalization of GAPDH (red) with LAMP1 (green), $*P = 7.2 \times 10^{-4}$ (**e**); colocalization of GAPDH (red) with Sec61p (green), $P = 0.8531$ (**f**). **g–l**, Transport assays were performed in HeLa cells. A confocal image from a representative experiment (out of three) is shown. Scale bar, 10 μm . Line-scan analysis

for the representative image is also shown. Transport from the ER to the Golgi (**g**; green, VSVG; red, giantin), transport from the Golgi to the plasma membrane (**h**; green, VSVG; red, TGN46), transport from the early endosome to the plasma membrane (**i**; red, transferrin; green, RAB11), transport from the plasma membrane to the Golgi (**j**; red, cholera toxin; green, TGN46); transport from the plasma membrane to the lysosome (**k**; red, EGF; green, LAMP1), transport from the plasma membrane to the early endosome (**l**; red, dextran; green, EEA1). **m, n**, Transport assays in HeLa cells; a representative experiment (out of three) is shown, $n = 10$ fields of cells examined. Data are mean $\pm$ s.d. Cholera toxin endocytosis (**m**), $P = 1$ (NS1); $P = 0.3205$ (NS2); interleukin-2 receptor beta subunit (IL2R- β) endocytosis (**n**), $P = 0.063$ (NS1), $P = 0.9264$ (NS2). **o–r**, HeLa cells were treated as indicated followed by immunofluorescence microscopy using different antibodies against GAPDH (**o, q**) or imaging of GFP-tagged GAPDH (**p, r**). Image from a representative experiment of two is shown. Scale bar, 10 μm . P values determined by two-tailed t -test.



Extended Data Fig. 3 | Additional characterization of GAPDH and its effects on the transport pathways. **a–d**, The distribution of different organelle markers in CHO (**a**), COS-7 (**b**), HeLa (**c**) or MEF (**d**) cells were assessed by immunofluorescence microscopy. Image from a representative experiment of two experiments is shown. Scale bar, 10 μ m. **e–h**, Comparison of the distribution of two markers against the same intracellular compartment using confocal microscopy. ER markers Sec61p (green) and calnexin (red) (**e**); Golgi markers giantin (green) and GM130 (red) (**f**); early endosome markers EEA1 (green) and RAB5 (red) (**g**); lysosome markers LAMP1 (green) and CD63 (red) (**h**). Images are representative of two experiments. Scale bar, 10 μ m. **i–l**, Confirming the staining specificity of organelle markers using model cargoes that reside at specific intracellular compartments using confocal microscopy: GFP-tagged VSVG at the ER (green) and Sec61p (red) (**i**); GFP-tagged VSVG at the Golgi (green) and giantin (red) (**j**); fluorescently labelled transferrin

(green) and EEA (red) (**k**); fluorescently labelled dextran (green) and LAMP1 (red) (**l**). Images are representative of two experiments. Scale bar, 10 μ m. **m, n**, The GAP assay was performed using ARF6 and ACAP1 (**m**) or ARF1 and AGAP1 (**n**), in the presence of different metabolic enzymes as indicated. One experiment of three independent experiments is shown. **o–r**, Pull-down studies to detect GAPDH binding directly to: ACAP1 (**o**), AGAP1 (**p**), Sec23p (**q**) or different portions of ARFGAP1 as indicated (**r**). Representative blot from three experiments. **s–v**, HeLa (**s, u**) or BSC-1 (**t, v**) cells were treated as indicated, followed by transport assay for transferrin endocytosis. Quantification of a representative experiment (out of three) is shown, $n = 10$ fields of cells examined. Data are mean $\pm$ s.d. In **s**: $P = 0.4646$ (NS1); $P = 0.6973$ (NS2). In **t**: $*P = 1.8 \times 10^{-4}$. In **u**: $P = 0.8073$ (NS). In **v**: $*P = 3.6 \times 10^{-5}$, $**P = 1.7 \times 10^{-6}$. P values determined by two-tailed t -test.

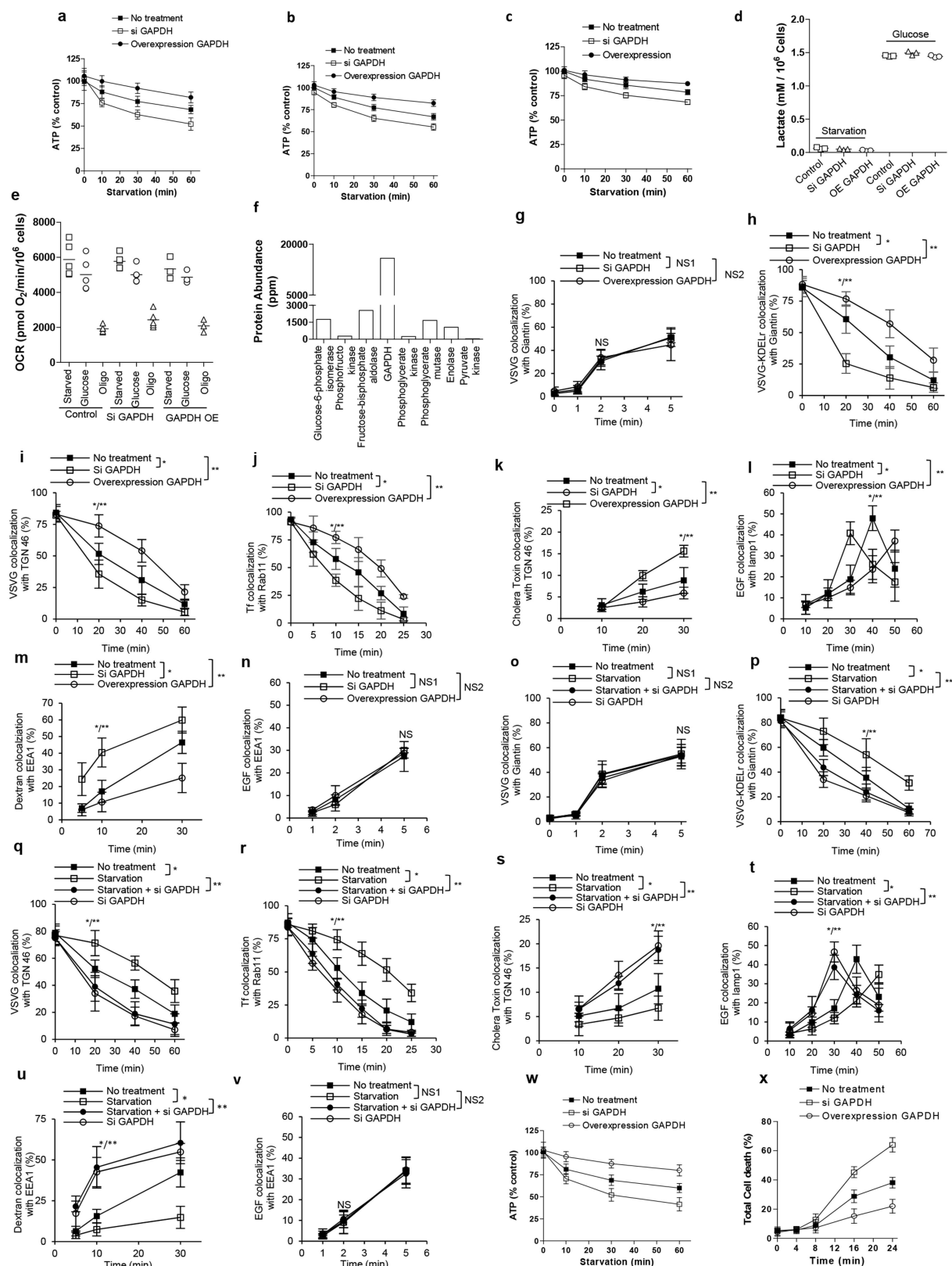


Extended Data Fig. 4 | See next page for caption.

Extended Data Fig. 4 | Different ways of starving cells lead to identical pathways being inhibited and inhibition requires GAPDH.

a–h, Transport assays were performed following incubation of HeLa cells in Hank's medium. Quantification of one experiment (out of three) is shown. Data are mean $\pm$ s.d. of $n = 10$ fields of cells examined. Transport of VSVG from the ER to the Golgi, $P = 0.1944$ (NS1), $P = 0.05$ (NS2) (**a**); COPI transport of VSVG–KDEL from the Golgi to the ER, $*P = 2.4 \times 10^{-6}$, $**P = 4.2 \times 10^{-10}$ (**b**); transport of VSVG from the Golgi to the plasma membrane, $*P = 1.7 \times 10^{-5}$, $**P = 6.7 \times 10^{-8}$ (**c**); transport of transferrin from the early endosome to the plasma membrane, $*P = 2.9 \times 10^{-5}$, $**P = 6.9 \times 10^{-9}$ (**d**); transport of cholera toxin from the plasma membrane to the Golgi, $*P = 2.4 \times 10^{-3}$, $**P = 7.8 \times 10^{-7}$ (**e**); transport of EGF from the plasma membrane to the lysosome, $*P = 1.9 \times 10^{-6}$, $**P = 1.5 \times 10^{-2}$ (**f**); transport of dextran from the plasma membrane to the early endosome, $*P = 7.8 \times 10^{-3}$, $**P = 3.4 \times 10^{-7}$ (**g**); transport of EGF from the plasma membrane to the early endosome, $P = 0.1485$ (NS1), $P = 0.6378$ (NS2) (**h**). **i–p**, HeLa cells were incubated in medium without glucose, and then transport assays were performed. Quantification of one experiment (out of three) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. Transport of VSVG from the ER to the Golgi, $P = 0.6921$ (NS1), $P = 0.5648$ (NS2) (**i**); COPI transport of VSVG–KDEL from the Golgi to the ER, $*P = 4.4 \times 10^{-4}$, $**P = 6.5 \times 10^{-7}$ (**j**); transport of VSVG from the Golgi to the plasma membrane, $*P = 1.6 \times 10^{-2}$, $**P = 6.7 \times 10^{-5}$ (**k**); transport of transferrin

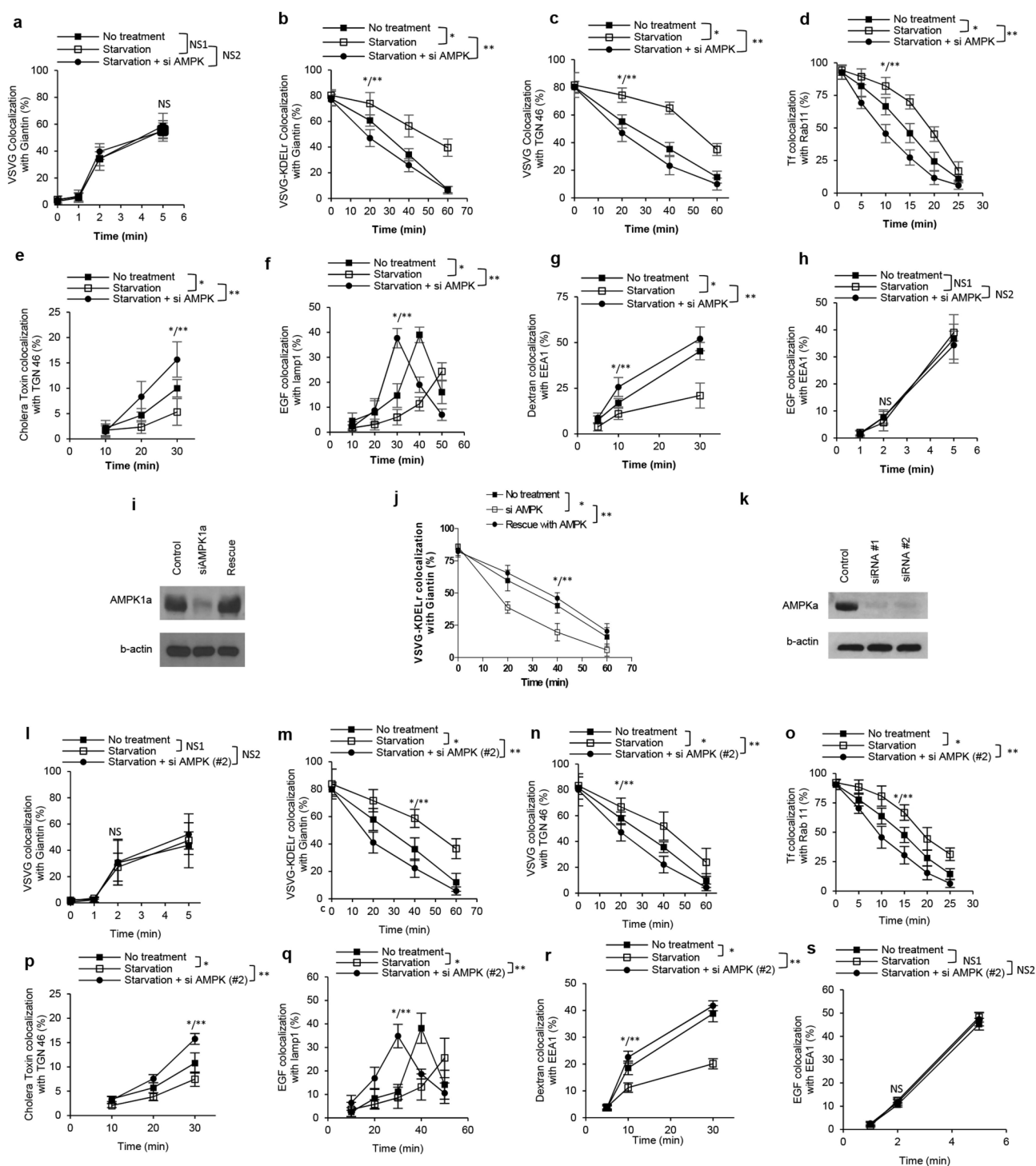
from the early endosome to the plasma membrane, $*P = 1.9 \times 10^{-4}$, $**P = 2.8 \times 10^{-9}$ (**l**); transport of cholera toxin from the plasma membrane to the Golgi, $*P = 7.4 \times 10^{-4}$, $**P = 4.2 \times 10^{-5}$ (**m**); transport of EGF from the plasma membrane to the lysosome, $*P = 7.6 \times 10^{-3}$, $**P = 3.2 \times 10^{-9}$ (**n**); transport of dextran from the plasma membrane to the early endosome, $*P = 2.2 \times 10^{-3}$, $**P = 1.6 \times 10^{-5}$ (**o**); transport of EGF from the plasma membrane to the early endosome, $P = 0.8648$ (NS1), $P = 0.8946$ (NS2) (**p**). **q–x**, HeLa cells were incubated in medium without amino acids, and transport assays were performed. Quantification of a representative experiment (out of three) is shown. Data are mean $\pm$ s.d. of $n = 10$ fields of cells examined. Transport of VSVG from the ER to the Golgi, $P = 0.1419$ (NS1), $P = 0.3379$ (NS2) (**q**); COPI transport of VSVG–KDEL from the Golgi to the ER, $*P = 1.9 \times 10^{-5}$, $**P = 1.1 \times 10^{-6}$ (**r**); transport of VSVG from the Golgi to the plasma membrane, $*P = 1.1 \times 10^{-3}$, $**P = 6.6 \times 10^{-7}$ (**s**); transport of transferrin from the early endosome to the plasma membrane, $*P = 7.2 \times 10^{-5}$, $**P = 8.4 \times 10^{-7}$ (**t**); transport of cholera toxin from the plasma membrane to the Golgi, $*P = 1.1 \times 10^{-5}$, $**P = 1.8 \times 10^{-6}$ (**u**); transport of EGF from the plasma membrane to the lysosome, $*P = 1.1 \times 10^{-2}$, $**P = 9.3 \times 10^{-8}$ (**v**); transport of dextran from the plasma membrane to the early endosome, $*P = 7.8 \times 10^{-3}$, $**P = 1.2 \times 10^{-8}$ (**w**); transport of EGF from the plasma membrane to the early endosome, $P = 0.9731$ (NS1), $P = 0.8159$ (NS2) (**x**). P values determined by two-tailed t -test.



Extended Data Fig. 5 | See next page for caption.

Extended Data Fig. 5 | Further characterizing the effects of starvation and GAPDH. **a–c**, HeLa cells were starved using Hank's medium (**a**), medium lacking glucose (**b**), or medium lacking amino acids (**c**), and the total ATP level was measured. Results of one experiment representative of three independent experiments are shown. **d**, Lactate production, measured in HeLa cells treated as indicated. Results of one experiment representative of three independent experiments are shown. **e**, Oxygen consumption rate in HeLa cells treated as indicated. Results of one experiment representative of three independent experiments is shown. **f**, Relative abundance of glycolytic enzymes in HeLa cells. Data are derived from <https://pax-db.org>; glucose-6-phosphate isomerase (<https://pax-db.org/protein/1860535>); phosphofructokinase (<https://pax-db.org/protein/1852972>); fructose-bisphosphate aldolase (<https://pax-db.org/protein/1851945>); GAPDH (<https://pax-db.org/protein/1843185>); phosphoglycerate kinase (<https://pax-db.org/protein/1848799>); phosphoglycerate mutase (<https://pax-db.org/protein/1855378>); enolase (<https://pax-db.org/protein/1850612>); pyruvate kinase (<https://pax-db.org/protein/1852273>). **g–n**, Transport assays were performed on HEK293 cells that were treated as indicated. Quantification of one representative experiment (out of three) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. Transport of VSVG from the ER to the Golgi, $P = 0.6468$ (NS1), $P = 0.2133$ (NS2) (**g**); COPI transport of VSVG–KDEL from the Golgi to the ER, $*P = 4.3 \times 10^{-7}$, $**P = 1.3 \times 10^{-3}$ (**h**); transport of VSVG from the Golgi to the plasma membrane, $*P = 2.1 \times 10^{-3}$, $**P = 1.9 \times 10^{-5}$ (**i**); transport of transferrin from the early endosome to

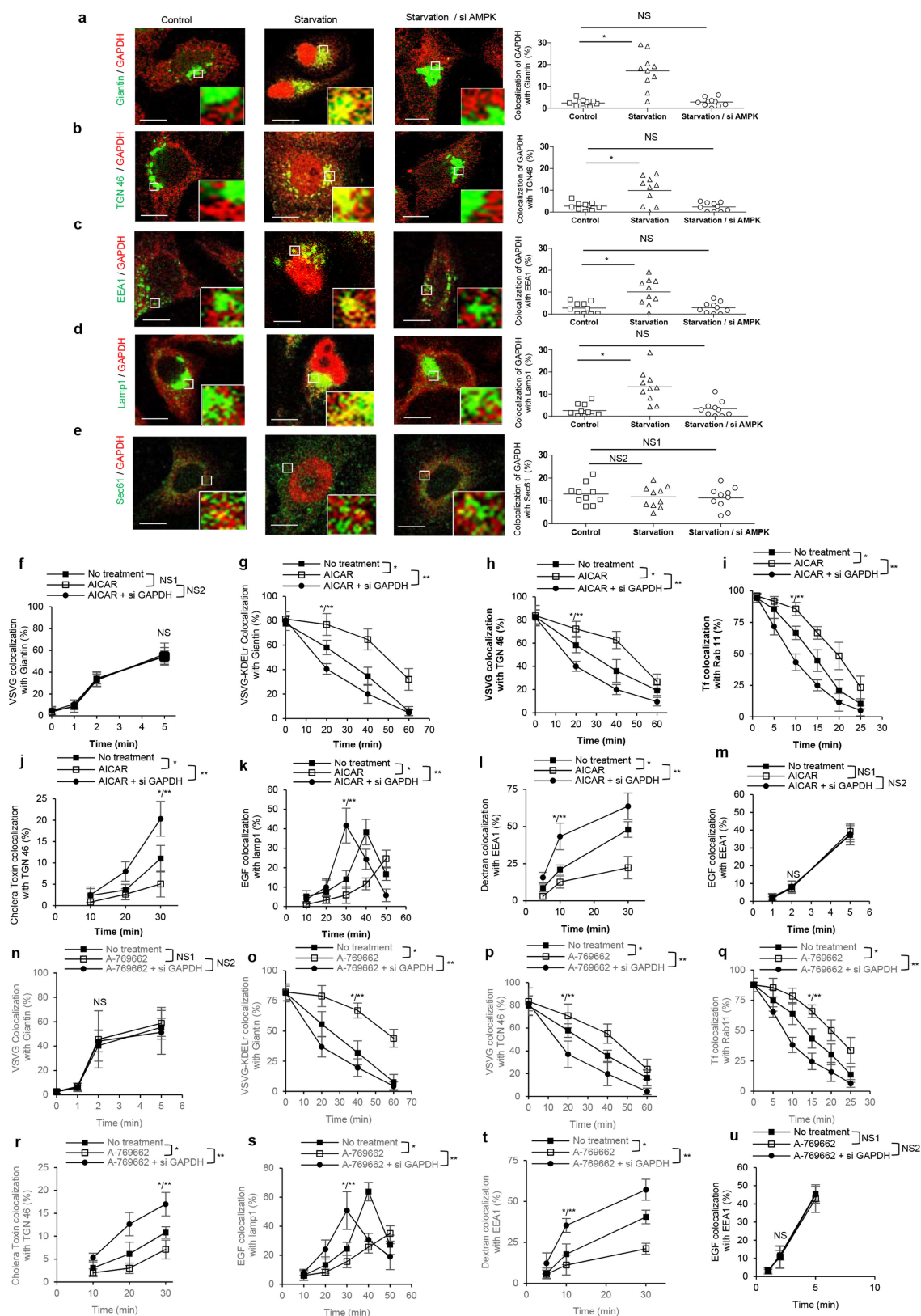
the plasma membrane, $*P = 6.3 \times 10^{-5}$, $**P = 4.7 \times 10^{-4}$ (**j**); transport of cholera toxin from the plasma membrane to the Golgi, $*P = 6.5 \times 10^{-4}$, $**P = 1.5 \times 10^{-2}$ (**k**); transport of EGF from the plasma membrane to the lysosome, $*P = 7.8 \times 10^{-7}$, $**P = 6.5 \times 10^{-8}$ (**l**); transport of dextran from the plasma membrane to the early endosome, $*P = 3.6 \times 10^{-6}$, $**P = 3.4 \times 10^{-2}$ (**m**); transport of EGF from the plasma membrane to the early endosome, $P = 0.2375$ (NS1), $P = 0.5291$ (NS2) (**n**). **o–v**, Transport assays were performed on HEK293 cells that were treated as indicated. Quantification of a representative experiment (out of three) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. Transport of VSVG from the ER to the Golgi, $P = 0.6649$ (NS1), $P = 0.84$ (NS2) (**o**); COPI transport of VSVG–KDEL from the Golgi to the ER, $*P = 4.6 \times 10^{-3}$, $**P = 2.5 \times 10^{-6}$ (**p**); transport of VSVG from the Golgi to the plasma membrane, $*P = 7.1 \times 10^{-5}$, $**P = 1.1 \times 10^{-6}$ (**q**); transport of transferrin from the early endosome to the plasma membrane, $*P = 7.2 \times 10^{-6}$, $**P = 6.5 \times 10^{-9}$ (**r**); transport of cholera toxin from the plasma membrane to the Golgi, $*P = 5.6 \times 10^{-3}$, $**P = 8.8 \times 10^{-9}$ (**s**); transport of EGF from the plasma membrane to the lysosome, $*P = 1.1 \times 10^{-2}$, $**P = 2.9 \times 10^{-8}$ (**t**); transport of dextran from the plasma membrane to the early endosome, $*P = 2.4 \times 10^{-4}$, $**P = 2.4 \times 10^{-6}$ (**u**); transport of EGF from the plasma membrane to the early endosome, $P = 0.7924$ (NS1), $P = 0.4675$ (NS2) (**v**). **w, x**, HEK293 cells were treated as indicated, followed by starvation and quantification of total ATP level (**w**) or cell death (**x**). Data are mean $\pm$ s.d. of $n = 3$ experiments. P values determined by two-tailed t -test.



Extended Data Fig. 6 | See next page for caption.

Extended Data Fig. 6 | Inhibition of transport pathways by starvation and AMPK. **a–h**, HeLa cells were starved using a general starvation medium (lacking glucose and amino acids), and transport assays were performed. Quantification of a representative experiment (out of three) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. Transport of VSVG from the ER to the Golgi, $P = 0.3506$ (NS1), $P = 0.9126$ (NS2) (**a**); COPI transport of VSVG–KDEL from the Golgi to the ER, $*P = 8.6 \times 10^{-4}$, $**P = 5.8 \times 10^{-7}$ (**b**); transport of VSVG from the Golgi to the plasma membrane, $*P = 1.0 \times 10^{-7}$, $**P = 4.4 \times 10^{-9}$ (**c**); transport of transferrin from the early endosome to the plasma membrane, $*P = 4.2 \times 10^{-5}$, $**P = 4.4 \times 10^{-10}$ (**d**); transport of cholera toxin from the plasma membrane to the Golgi, $*P = 2.6 \times 10^{-4}$, $**P = 9.5 \times 10^{-7}$ (**e**); transport of EGF from the plasma membrane to the lysosome, $*P = 2.0 \times 10^{-4}$, $**P = 2.2 \times 10^{-13}$ (**f**); transport of dextran from the plasma membrane to the early endosome, $*P = 1.4 \times 10^{-4}$, $**P = 1.6 \times 10^{-6}$ (**g**); transport of EGF from the plasma membrane to the early endosome, $P = 0.1085$ (NS1), $P = 0.1408$ (NS2) (**h**). **i**, HeLa cells were treated as indicated and cell lysate was immunoblotted for proteins as indicated. Blot is representative of two independent experiments. **j**, HeLa cells were starved, and the COPI transport assay was performed.

Quantification of a representative experiment (out of three) is shown. Data are mean $\pm$ s.d. of $n = 10$ fields of cells examined. $*P = 1.1 \times 10^{-4}$, $**P = 3.5 \times 10^{-6}$. **k**, HeLa cells were treated as indicated and the whole-cell lysate was immunoblotted for proteins as indicated. Representative blot of two independent experiments. **l–s**, HeLa cells were starved, and transport assays were performed. Quantification of a representative experiment (out of three) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. Transport of VSVG from the ER to the Golgi, $P = 0.6077$ (NS1), $P = 0.5535$ (NS2) (**l**); COPI transport of VSVG–KDEL from the Golgi to the ER, $*P = 3.5 \times 10^{-6}$, $**P = 3.6 \times 10^{-10}$ (**m**); transport of VSVG from the Golgi to the plasma membrane, $*P = 4.1 \times 10^{-3}$, $**P = 4.4 \times 10^{-6}$ (**n**); transport of transferrin from the early endosome to the plasma membrane, $*P = 3.7 \times 10^{-6}$, $**P = 1.1 \times 10^{-9}$ (**o**); transport of cholera toxin from the plasma membrane to the Golgi, $*P = 7.6 \times 10^{-4}$, $**P = 8.7 \times 10^{-11}$ (**p**); transport of EGF from the plasma membrane to the lysosome, $*P = 3.1 \times 10^{-8}$, $**P = 1.3 \times 10^{-2}$ (**q**); transport of dextran from the plasma membrane to the early endosome, $*P = 1.1 \times 10^{-6}$, $**P = 3.7 \times 10^{-10}$ (**r**); transport of EGF from the plasma membrane to the early endosome, $P = 0.228$ (NS1), $P = 0.1738$ (NS2) (**s**). P values determined by two-tailed t -test.

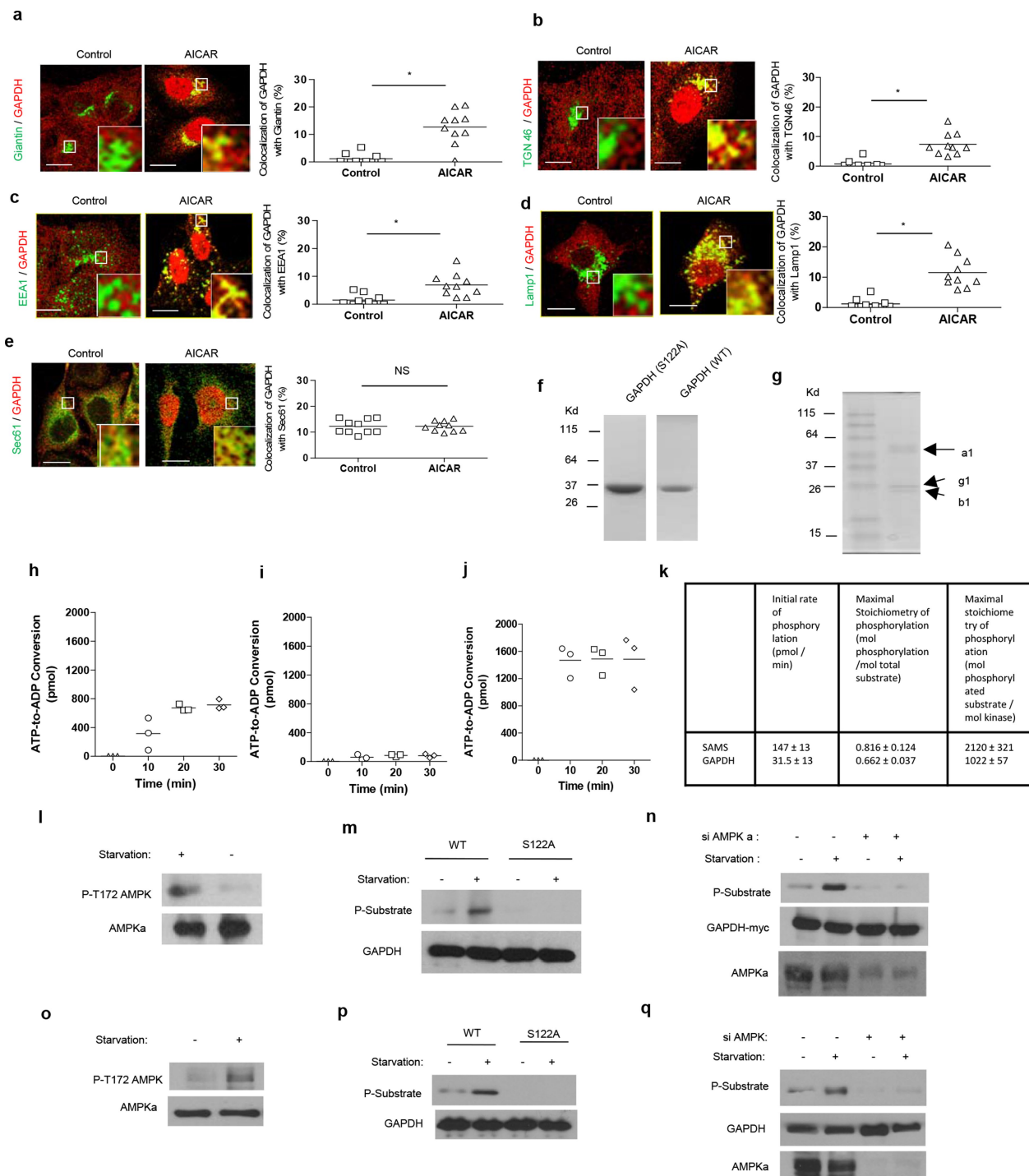


Extended Data Fig. 7 | See next page for caption.

Extended Data Fig. 7 | Effects of AMPK on GAPDH distribution and transport pathways. a–e.

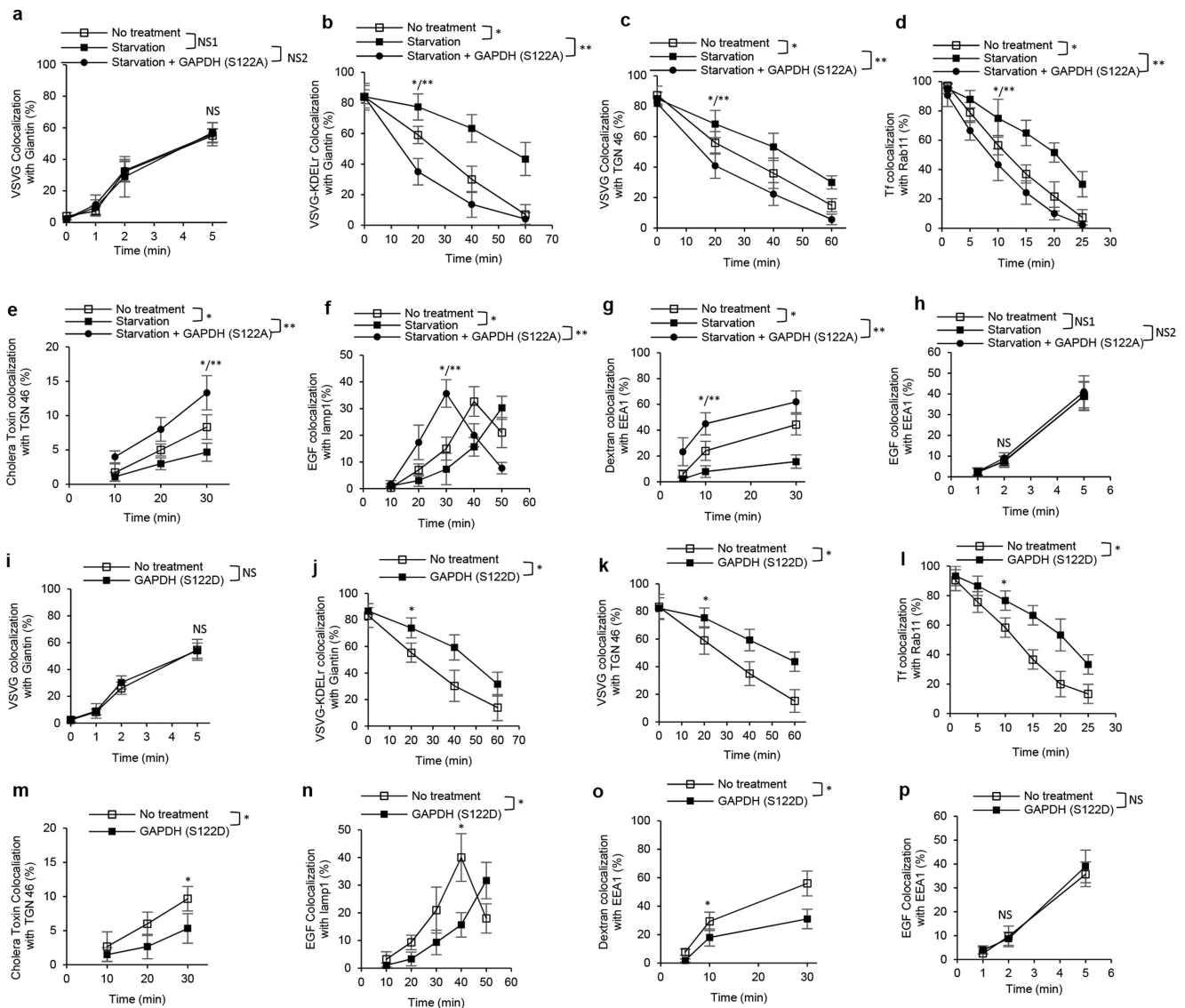
HeLa cells were treated as indicated, and then examined for colocalization between GAPDH and different organelle markers. A confocal image from a representative experiment (out of three) is shown. Scale bar, 10 μ m (left). Quantification is also shown (right). Data are mean $\pm$ s.d. of $n = 10$ fields of cells examined. Colocalization of GAPDH (red) with giantin (green), $*P = 2.9 \times 10^{-3}$, $P = 0.6397$ (NS) (a); colocalization of GAPDH (red) with TGN46 (green), $*P = 6.5 \times 10^{-3}$, $P = 0.6413$ (NS) (b); colocalization of GAPDH (red) with EEA1 (green), $*P = 3.5 \times 10^{-3}$, $P = 0.8793$ (NS) (c); colocalization of GAPDH (red) with LAMP1 (green), $*P = 9.4 \times 10^{-4}$, $P = 0.551$ (NS) (d); colocalization of GAPDH (red) with Sec61p (green), $P = 0.5361$ (NS1), $P = 0.4243$ (NS2) (e). **f–m**, HeLa cells were treated as indicated, and transport assays were performed. Quantification of a representative experiment (out of three) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. Transport of VSVG from the ER to the Golgi, $P = 0.5250$ (NS1), $P = 0.8291$ (NS2) (f); COPI transport of VSVG–KDEL from the Golgi to the ER, $*P = 4.6 \times 10^{-5}$, $**P = 2.9 \times 10^{-8}$ (g); transport of VSVG from the Golgi to the plasma membrane, $*P = 1.4 \times 10^{-4}$, $**P = 6.9 \times 10^{-10}$ (h); transport of transferrin from the early endosome to the plasma membrane, $*P = 2.0 \times 10^{-7}$, $**P = 1.0 \times 10^{-11}$ (i) transport

of cholera toxin from the plasma membrane to the Golgi, $*P = 4.0 \times 10^{-4}$, $**P = 3.9 \times 10^{-8}$ (j); transport of EGF from the plasma membrane to the lysosome, $*P = 7.1 \times 10^{-4}$, $**P = 4.2 \times 10^{-8}$ (k); transport of dextran from the plasma membrane to the early endosome, $*P = 1.4 \times 10^{-7}$, $**P = 1.9 \times 10^{-9}$ (l); transport of EGF from the plasma membrane to the early endosome, $P = 0.6088$ (NS1), $P = 0.4061$ (NS2) (m). **n–u**, HeLa cells were treated as indicated, and transport assays were performed. Quantification of a representative experiment (out of three) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. Transport of VSVG from the ER to the Golgi, $P = 0.5559$ (NS1), $P = 0.8576$ (NS2) (n); COPI transport of VSVG–KDEL from the Golgi to the ER, $*P = 4.1 \times 10^{-5}$, $**P = 2.3 \times 10^{-9}$ (o); transport of VSVG from the Golgi to the plasma membrane, $*P = 4.2 \times 10^{-3}$, $**P = 6.3 \times 10^{-5}$ (p); transport of transferrin from the early endosome to the plasma membrane, $*P = 7.2 \times 10^{-4}$, $**P = 3.7 \times 10^{-11}$ (q) transport of cholera toxin from the plasma membrane to the Golgi, $*P = 2.3 \times 10^{-4}$, $**P = 3.0 \times 10^{-8}$ (r); transport of EGF from the plasma membrane to the lysosome, $*P = 2.9 \times 10^{-3}$, $**P = 5.8 \times 10^{-6}$ (s); transport of dextran from the plasma membrane to the early endosome, $*P = 2.9 \times 10^{-2}$, $**P = 1.6 \times 10^{-8}$ (t) transport of EGF from the plasma membrane to the early endosome, $P = 0.6856$ (NS1), $P = 0.9248$ (NS2) (u). *P* values determined by two-tailed *t*-test.



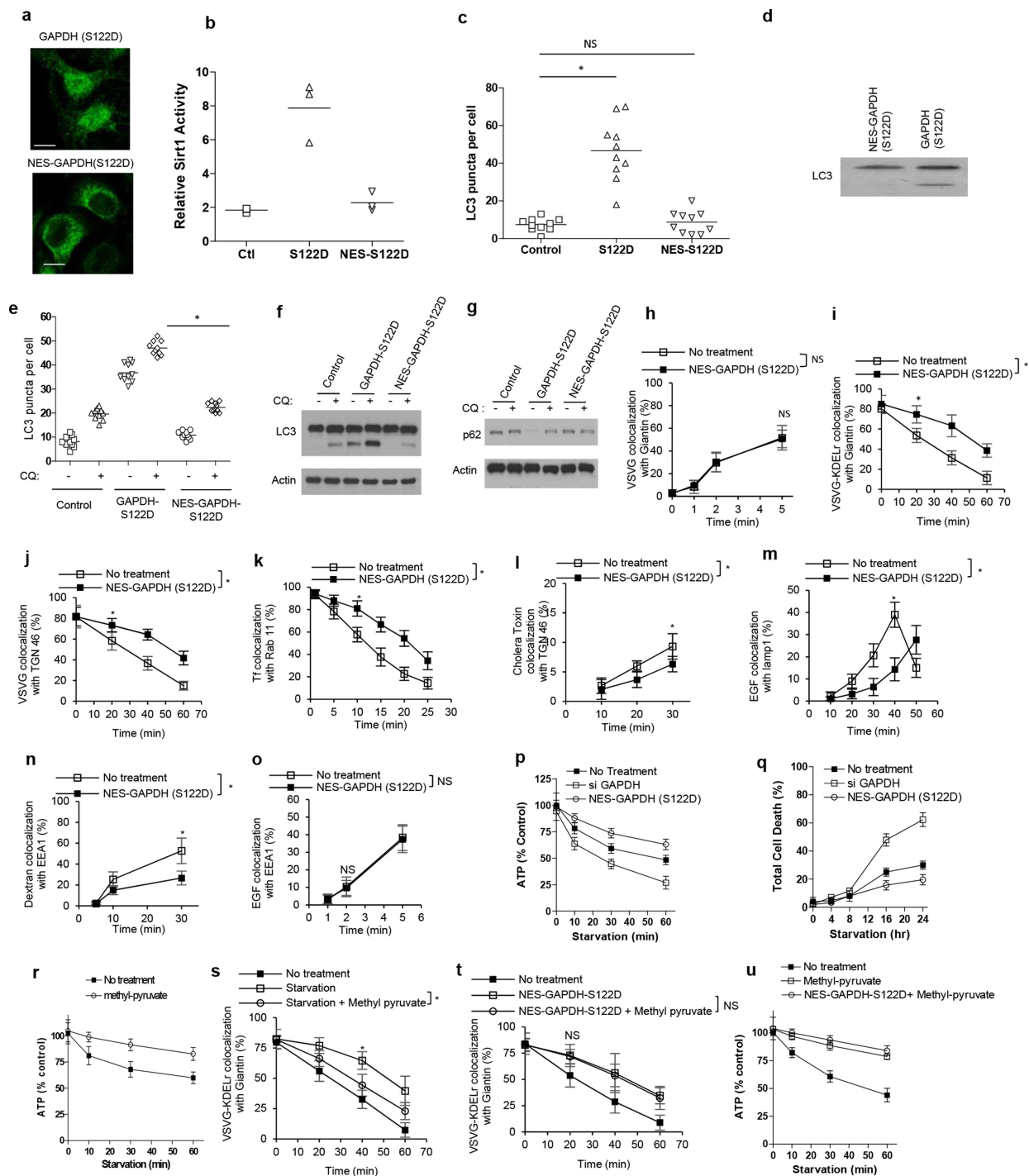
Extended Data Fig. 8 | Further characterizing how AMPK acts on GAPDH. **a–e**, HeLa cells were treated as indicated, and then examined for colocalization between GAPDH and different organelle markers. A confocal image from a representative experiment (out of three) is shown (left) and quantified (right). Scale bar, 10 μ m. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. Colocalization of GAPDH (red) with giantin (green), $*P = 1.5 \times 10^{-4}$ (**a**); colocalization of GAPDH (red) with TGN46 (green), $*P = 2.2 \times 10^{-4}$ (**b**); colocalization of GAPDH (red) with EEA1 (green), $*P = 2.1 \times 10^{-3}$ (**c**); colocalization of GAPDH (red) with LAMP1 (green), $*P = 7.1 \times 10^{-5}$ (**d**); colocalization of GAPDH (red) with Sec61p (green), $P = 0.9933$ (NS) (**e**). **f**, Purity of GAPDH forms assessed by Coomassie gel staining. Representative gel from two independent

experiments. **g**, Purity of AMPK complex assessed by Coomassie gel staining. Representative gel from two independent experiments. **h–j**, AMPK was incubated with wild-type GAPDH (**h**), GAPDH(S122A) (**i**), or SAMS, a peptide derived from acetyl-CoA carboxylase (**j**) in the *in vitro* kinase assay, and phosphorylation was measured over time. Data are mean $\pm$ s.d. from a representative experiment of three independent experiments. **k**, Calculation of the stoichiometry of phosphorylation from the results shown in **h–j**. **l–q**, Whole-cell lysates, from HeLa (**l–n**) or HEK293 (**o–q**) cells, were treated as indicated and immunoblotted for proteins as indicated. Blots are representative of two independent experiments. P values determined by two-tailed *t*-test.



Extended Data Fig. 9 | Further characterizing how GAPDH mediates the ability of starvation to inhibit the transport pathways. a–h, HeLa cells were transfected with GAPDH(S122A), followed by starvation. A representative transport assay (of three experiments) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. Transport of VSVG from the ER to the Golgi, $P = 0.4144$ (NS1), $P = 0.7463$ (NS2) (a); COPI transport of VSVG–KDEL from the Golgi to the ER, $*P = 3.8 \times 10^{-5}$, $**P = 1.9 \times 10^{-9}$ (b); transport of VSVG from the Golgi to the plasma membrane, $*P = 3.6 \times 10^{-3}$, $**P = 1.4 \times 10^{-6}$ (c); transport of transferrin from the early endosome to the plasma membrane, $*P = 1.4 \times 10^{-3}$, $**P = 1.4 \times 10^{-5}$ (d); transport of cholera toxin from the plasma membrane to the Golgi, $*P = 7.9 \times 10^{-5}$, $**P = 1.5 \times 10^{-7}$ (e); transport of EGF from the plasma membrane to the lysosome, $*P = 4.0 \times 10^{-3}$, $**P = 1.3 \times 10^{-9}$ (f); transport of dextran from the plasma membrane to the early endosome, $*P = 3.1 \times 10^{-5}$, $**P = 1.2 \times 10^{-8}$ (g); transport

of EGF from the plasma membrane to the early endosome, $P = 0.7549$ (NS1), $P = 0.2279$ (NS2) (h). i–p, HeLa cells were transfected with GAPDH(S122D). A representative transport assay (of three experiments) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. Transport of VSVG from the ER to the Golgi, $P = 0.8289$ (NS) (i); COPI transport of VSVG–KDEL from the Golgi to the ER, $*P = 2.2 \times 10^{-5}$ (j); transport of VSVG from the Golgi to the plasma membrane, $*P = 6.3 \times 10^{-4}$ (k); transport of transferrin from the early endosome to the plasma membrane, $*P = 6.9 \times 10^{-6}$ (l); transport of cholera toxin from the plasma membrane to the Golgi, $*P = 1.3 \times 10^{-4}$ (m); transport of EGF from the plasma membrane to the lysosome, $*P = 1.6 \times 10^{-3}$ (n); transport of dextran from the plasma membrane to the early endosome, $*P = 7.4 \times 10^{-4}$ (o); transport of EGF from the plasma membrane to the early endosome, $P = 0.5473$ (NS) (p). P values determined by two-tailed t -test.



Extended Data Fig. 10 | See next page for caption.

Extended Data Fig. 10 | Further confirmation that the roles of GAPDH in transport and autophagy are distinct. **a**, HeLa cells were transfected with GFP-tagged forms of GAPDH as indicated and examined by immunofluorescence microscopy. A representative image from two independent experiments is shown. Scale bar, 10 μ m. **b**, SIRT1 activity in HeLa cells transfected with GFP-tagged forms of GAPDH as indicated. Data are mean $\pm$ s.d. from three independent experiments. **c**, LC3 puncta formation in HeLa cells transfected with GFP-tagged forms of GAPDH as indicated. Quantification of a representative experiment (of three) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. $*P = 2.3 \times 10^{-5}$, $P = 0.5598$ (NS). **d**, LC3 lipidation was assessed by immunoblotting of lysates from HeLa cells transfected with GFP-tagged forms of GAPDH as indicated. A representative blot from two independent experiments is shown. **e**, LC3 puncta formation in HeLa cells treated as indicated. Quantification of a representative experiment (of three) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. $*P = 1.7 \times 10^{-5}$. **f**, LC3 lipidation was assessed by immunoblotting of lysates from HeLa cells treated as indicated. A representative blot from two independent experiments is shown. **g**, p62 level was assessed by immunoblotting of lysates from HeLa cells treated as indicated. A representative blot from two independent experiments is shown.

h–o, Transport assays in HeLa cells treated as indicated. Quantification of a representative experiment (of three) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. Transport of VSVG from the ER to the Golgi, $P = 0.8155$ (NS) (**h**); COPI transport of VSVG-KDEL from the Golgi to the ER, $*P = 1.4 \times 10^{-5}$ (**i**); transport of VSVG from the Golgi to the plasma membrane, $*P = 5.5 \times 10^{-4}$ (**j**); transport of transferrin from the early endosome to the plasma membrane, $*P = 3.3 \times 10^{-7}$ (**k**); transport of cholera toxin from the plasma membrane to the Golgi, $*P = 2.0 \times 10^{-3}$ (**l**); transport of EGF from the plasma membrane to the lysosome, $*P = 2.4 \times 10^{-6}$ (**m**); transport of dextran from the plasma membrane to the early endosome, $*P = 3.9 \times 10^{-5}$ (**n**); transport of EGF from the plasma membrane to the early endosome, $P = 0.7725$ (NS) (**o**). **p, q**, Total ATP level (**p**) and cell death (**q**) in HeLa cells treated as indicated. Data are mean $\pm$ s.d. from three independent experiments. **r**, Total ATP level in HeLa cells treated as indicated. Data are mean $\pm$ s.d. from three independent experiments. **s, t**, COPI transport in HeLa cells treated as indicated. Quantification of a representative experiment (of three) is shown. Data are mean $\pm$ s.d. from $n = 10$ fields of cells examined. $*P = 3.5 \times 10^{-5}$ (**s**), $P = 0.7907$ (NS) (**t**). **u**, Total ATP level in HeLa cells treated as indicated. Data are mean $\pm$ s.d. from three independent experiments. P values determined by two-tailed t -test.

Extended Data Table 1 | Proteins that interact with ARFGAP1

Abbreviation	Protein Name	MWT(kDa)
Tln1	Protein Tln1	270
Copa	Coatomer subunit alpha	138
Ap2b1	AP-2 complex subunit beta	105
Ap2a2	AP-2 complex subunit alpha-2	104
Copb2	Coatomer subunit beta'	102
Copg1	Coatomer subunit gamma-1	98
Arcn1	Coatomer subunit delta	57
Aldh2	Aldehyde dehydrogenase, mitochondrial	56
Fh	Fumarate hydratase, mitochondrial	54
Ctbp1	C-terminal-binding protein 1	47
Cth	Cystathionine gamma-lyase	44
Akr7a2	Aflatoxin B1 aldehyde reductase member 2	41
Taldo1	Transaldolase	37
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	36
Apoe	Apolipoprotein E	36
Mdh2	Malate dehydrogenase	36
Grhpr	Grhpr protein	36
Eif2s1	Eukaryotic translation initiation factor 2 subunit 1	36
Hadh	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	34
Hao	3-hydroxyanthranilate 3,4-dioxygenase	33
Gnmt	Glycine N-methyltransferase	33
Qprt	Nicotinate-nucleotide pyrophosphorylase	31
Psma3	Proteasome subunit alpha type-3	28
Psme2	Proteasome activator complex subunit 2	27

Mass spectrometry was used to identify interacting proteins following a pull-down experiment using a GST fusion of ARFGAP1 and cytosol.

Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated
- ☐ ☒ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection

Colocalization was performed using the Nikon EZ-C1 version 3.90 acquisition software or the Zeiss Zen 2.3 blue edition confocal acquisition software.

Data analysis

Quantitation of colocalization used Image J. Statistical analysis used Prism or Excel.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. The following figures have associated raw

data: Figures 1a,b, 2a-g, 3a-j, 3n-q, 4c-e, 4g-p; Extended Data Figures 1c, 1k, 2m,n, 2s-v, 3s-v, 4a-x, 5a-c, 5g-x, 6a-h, 6j, 6l-s, 7f-u, 9a-p, 10h-u. For gel source data, see Supplementary Figure 1.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

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

Sample size	Sample size was based on our previous experience in the experimental approach, and also taking into account feasibility, in obtaining reliable results.
Data exclusions	No data were excluded.
Replication	Findings were reliably reproduced.
Randomization	The experimental approaches did not require samples to be randomized. Samples, and the subsequent data collection and analysis, were handled the same way in all experiments.
Blinding	The investigators were not blinded to group allocation during data collection or subsequent analysis. This approach is considered standard for biochemical and microscopy experiments done in this study.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Antibodies against ARF1 (WB: 1:1000), ARFGAP1 (WB: 1:1000), beta-COP (WB:1:10), calnexin (IF: 1:200), cellubrevin (WB: 1:1000), coatmer (IF: 1:10), giantin (IF: 1:2000), GM130 (IF: 1:500), Lamp1 (IF: 1:200), Sec61p (IF: 1:500), TGN46 (IF: 1:200), VSVG (IF: 1:10), HA epitope tag (WB: 1:200), and Myc epitope tag (WB: 1:200) have been validated in our previous studies. See next section below for their specific listing.

The following antibodies were obtained from commercial sources: AMPK-alpha (WB: 1:500, Cell Signaling, 2603S), beta-actin (WB: 1:1000, Ambion, AM4302), GFP (WB: 1:500, Invitrogen, GF28R), LC3 (WB: 1:500, Sigma, L7543), p62 (WB: 1:500, Santa Cruz, 28359), phospho-substrate-AMPK antibody (WB: 1:500, Cell Signaling, 5759S), p172-AMPK-alpha (WB: 1:500, Cell Signaling, 2531S), pS/pT (WB: 1:500, Abcam, 117253), Rab11 (IF: 1:200, BD Biosciences, 610656), Sirt1 (WB: 1:500, Cell Signaling, 2493S), and Flag epitope tag M2 (WB: 1:1000, Sigma, F1804). Antibody against GAPDH was obtained from three sources: Ambion (AM4300, IF: 1:500, WB: 1:1000), Santa Cruz (FL335, IF: 1:500), and Sigma (G9545, IF: 1:500). Conjugated antibodies were also obtained (Jackson ImmunoResearch), which include Cy2- or Cy3-conjugated donkey antibodies against mouse or rabbit IgG (IF: 1:200), and horseradish peroxidase-conjugated donkey antibodies against mouse or rabbit IgG (WB: 1:20,000).

Validation

For antibodies that have been validated in our previous studies, see Bai, M. et al. Nature Cell Biol 13, 559-567 (2011), Dai, J. et al. Dev Cell 7, 771-776 (2004), Yang, J.S. et al. EMBO J 24, 4133-4143 (2005). For antibodies obtained from commercial sources listed in the above section, validation was performed by the vendors.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HeLa and HEK293 were obtained from ATCC. Atg5-deficient MEFs were from N. Mizushima, University of Tokyo, Japan.
Authentication	HeLa and HEK293 cells were not further authenticated. Atg5-deficient MEFs were confirmed by functional tests for autophagy.
Mycoplasma contamination	Cells were not tested.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	MEFs from Atg5-deficient mice were obtained (from N. Mizushima, University of Tokyo, Japan), and have been previously described (Kuma, A. et al. Nature 2004, vol. 432, pp. 1032-1036).
Wild animals	<i>Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.</i>
Field-collected samples	<i>For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.</i>