Autophagy maintains tumour growth through circulating arginine

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Autophagy captures intracellular components and delivers them to lysosomes, where they are degraded and recycled to sustain metabolism and to enable survival during starvation1-8. Acute, whole-body deletion of the essential autophagy gene Atg7 in adult mice causes a systemic metabolic defect that manifests as starvation intolerance and gradual loss of white adipose tissue, liver glycogen and muscle mass1. Cancer cells also benefit from autophagy. Deletion of essential autophagy genes impairs the metabolism, proliferation, survival and malignancy of spontaneous tumours in models of autophagous cancer6,7. Acute, systemic deletion of Atg7 or acute, systemic expression of a dominant-negative ATG4b in mice induces greater regression of KRAS-driven cancers than does tumour-specific autophagy deletion, which suggests that host autophagy promotes tumour growth1,8. Here we show that host-specific deletion of Atg7 impairs the growth of multiple allografted tumours, although not all tumour lines were sensitive to host autophagy status. Loss of autophagy in the host was associated with a reduction in circulating arginine, and the sensitive tumour cell lines were arginine auxotrophs owing to the lack of expression of the enzyme argininosuccinate synthase 1. Serum proteomic analysis identified the arginine-degrading enzyme arginase I (ARG1) in the circulation of Atg7-deficient hosts, and in vivo arginine metabolic tracing demonstrated that serum arginine was degraded to ornithine. ARG1 is predominantly expressed in the liver and can be released from hepatocytes into the circulation. Liver-specific deletion of Atg7 produced circulating ARG1, and reduced both serum arginine and tumour growth. Deletion of Atg5 in the host similarly regulated circulating arginine and suppressed tumorigenesis, which demonstrates that this phenotype is specific to autophagy function rather than to deletion of Atg7. Dietary supplementation of Atg7-deficient hosts with arginine partially restored levels of circulating arginine and tumour growth. Thus, defective autophagy in the host leads to the release of ARG1 from the liver and the degradation of circulating arginine, which is essential for tumour growth; this identifies a metabolic vulnerability of cancer.

To validate whether host autophagy promotes tumour growth, we tested the growth of an autophagy-competent C57Bl/6J isogenic BrαfV600E+/Pten−/−Cdkn2a−/− mouse melanoma cell line (termed YUMM 1.1) in C57Bl/6J host mice, without (Atg7+/+) and with (Atg7−/−) conditional whole-body Atg7 deficiency (Fig. 1a). YUMM 1.1 tumours were significantly smaller when grown in Atg7−/− hosts compared to Atg7+/+ hosts (Fig. 1b), demonstrating that host autophagy promoted tumour growth. The examination of additional cell lines—autophagy-competent isogenic C57Bl/6J BrαfV600E+/Pten−/−Cdkn2a−/− YUMM 1.3 melanoma—confirmed the findings in autophagy-deficient hosts (Extended Data Fig. 1). To further test the role of host autophagy specifically, we generated Atg7−/− YUMM 1.1 tumours in Pten+/−, KrasG12D+/− and p53−/− backgrounds (p53 is also known as Trp53) 71.8 non-small-cell lung cancer cells—revealed a similar requirement for host autophagy for tumour growth (Extended Data Fig. 1a, c, e). The decreased tumour growth observed in Atg7−/− hosts was associated with decreased proliferation. In some tumour types, there was also increased apoptosis (Fig. 1c, Extended Data Fig. 1b, d, f). However, host autophagy was not required for the growth of autophagy-competent androgen-insensitive C57Bl/6J BrαfV600E+/Pten−/−Cdkn2a−/− YUMM 1.7 and 1.9 melanoma cell lines (Extended Data Fig. 2a–d), which indicates that—although dependency on host autophagy is common—there are tumour-specific adaptation mechanisms.

The melanoma cell lines that are dependent on host autophagy for tumour growth are derived from genetically engineered mouse models of cancer, and therefore have a low mutation burden, low neoantigen load and fail to provoke an efficient T cell response9. Nonetheless, autophagy modulates a variety of immune mechanisms that could underlie defective tumour growth in autophagy-deficient hosts. Atg7−/− hosts did not modify infiltration of YUMM 1.1 tumours with CD3+, CD4+ or CD8+ cells (Extended Data Fig. 2e). Depletion of CD4+ and CD8+ T cells modestly increased tumour growth in Atg7−/− hosts but did not significantly rescue growth in Atg7−/− hosts (Extended Data Fig. 2f). Thus, despite the relative increase in the fraction of myeloid-derived suppressor cells and CD8+ T cells in Atg7−/− hosts (Extended Data Fig. 2g), the decreased tumour growth in Atg7−/− hosts was not due to the induction of an anti-tumour T cell response.

Autophagy supports metabolism by recycling cargo to provide anabolic and catabolic substrates6. This metabolic recycling function of autophagy promotes mammalian survival during fasting1-3, and tumour cell survival under conditions of nutrient limitation10,11. One major source of tumour nutrients is the host blood supply. Accordingly, we tested whether circulating nutrients provided by host autophagy were required for tumour growth. Metabolite profiling of serum from Atg7−/− and Atg7−/− hosts identified 12 metabolites that were decreased and 7 that were increased with autophagy knockout (Fig. 1d, Supplementary Tables 1, 2). Serum arginine was notably downregulated in Atg7−/− compared to Atg7+/+ hosts (−2.37 fold change (log2(serum arginine in Atg7−/− serum arginine in Atg7+/+)) (Fig. 1d), confirming previous results1.

Arginine is a non-essential amino acid derived from the diet, de novo synthesis and protein turnover, and is important for mTOR activation12, ammonia detoxification through the urea cycle as well as the synthesis of proteins, creatine, polyamines and nitric oxide13. It has long been known that some human cancers silence expression of ASS1, the gene that encodes argininosuccinate synthase 1 (ASS1), which results in arginine auxotrophy14. Without ASS1, cancer cells are unable to synthesize arginine from citrulline and are dependent
Fig. 1 | Host autophagy promotes growth of arginine auxotrophic tumours. a, Experimental design to induce host mice with conditional whole-body Atg7 deletion (Atg7Δ/Δ) and wild-type controls (Atg7+/+) with which to assess tumour growth. Ubc-creERT2T2/2; Atg7Δ/Δ and Ubc-creERT2T2/2; Atg7+/+ mice were injected with tamoxifen (TAM) to delete Atg7 and were then injected subcutaneously with tumour cells. Tumour growth was monitored over three weeks. b, Comparison of tumour weight between Atg7Δ/Δ (n = 5) and Atg7Δ/Δ+ (n = 8) hosts. Data are mean ± s.e.m. ***P < 0.0001. c, Immunohistochemistry quantification of Ki-67+ and active caspase-3+ cells in tumours from Atg7Δ/Δ+ and Atg7Δ/Δ hosts. Data are mean ± s.e.m. **P < 0.001. d, Serum metabolites with fold-change (log2[metabolite in Atg7Δ/Δ+]/metabolite in Atg7Δ/Δ) cut-offs of >1 or <−1 between Atg7Δ/Δ+ (n = 17) and Atg7Δ/Δ hosts obtained by liquid chromatography mass spectrometry (LC–MS), with P < 0.05. e, Illustration of the arginine metabolism. NO, nitric oxide; NOS, nitric oxide synthetase. f, YUMM 1.1 proliferation in vitro, in medium containing different percentages of arginine. Cell density was measured every 2 h using IncuCyte. Data are representative of three independent experiments performed in duplicate. g, Western blotting showing expression of ASS1, ASL and OTC in tumours from Atg7Δ/Δ+ and Atg7Δ/Δ hosts (n = 4 each), representative of three independent experiments. The kidney was used as a control tissue for ASS1 and ASL, and the liver was used for OTC. Actin was used a loading control. In all figures, n represents the number of mice.

Fig. 2 | Levels of ARG1 in serum increase in Atg7Δ/Δ hosts and deplete circulating arginine. a, Comparison of serum proteins between Atg7Δ/Δ+ and Atg7Δ/Δ hosts (n = 5 each) obtained by nano LC–MS/MS with corrected P < 0.05. b, Proteins with fold-change (log2[protein in Atg7Δ/Δ+]/protein in Atg7Δ/Δ]) cut-offs of >1 or <−1 between Atg7Δ/Δ+ and Atg7Δ/Δ hosts. c, Western blotting showing expression of ARG1 in serum and liver from Atg7Δ/Δ+ and Atg7Δ/Δ hosts. *P < 0.05 compared to Atg7Δ/Δ+ hosts. Data are representative of two independent experiments. Actin and transferrin were used as loading controls. d, Illustration of the labelling pattern of the 13C/15N arginine-tracer. e, Concentration (in μM) of arginine, citrulline and ornithine in serum from Atg7Δ/Δ+ and Atg7Δ/Δ hosts (n = 3 and 4, respectively) after infusion with 13C6/15N4 arginine. Data are mean ± s.e.m. f, Concentration (in nmol g−1) of arginine, citrulline and ornithine in tumours from Atg7Δ/Δ+ and Atg7Δ/Δ hosts (n = 2 each) after infusion with 13C6/15N4-arginine. Data are mean. ***P < 0.001 by two-way ANOVA test.
To determine their requirement for exogenous arginine, YUMM 1.1, 1.3, 1.7, 1.9, MB49 and 71.8 cells were tested for growth without and with arginine. Proliferation was blocked in vitro in complete medium with the sole absence of arginine, and this was not associated with cell death. Growth rates increased with an increased percentage of arginine in the medium, which demonstrates arginine auxotrophy (Fig. 1f, Extended Data Fig. 3a). YUMM 1.1 tumours were tested for the lack of expression of enzymes involved in arginine biosynthesis: ASS1, argininosuccinate lyase (ASL)—which converts citrulline to arginine—and ornithine transcarbamylase (OTC), which converts ornithine to citrulline (Fig. 1e). As previously shown for melanoma9,20 and irrespective of the use of Atg7+/+ and Atg7−/− hosts, tumours lacked ASS1 and OTC expression, which explains their arginine auxotrophy (Fig. 1g). In contrast to tumours, both Atg7+/+ and Atg7−/− hosts express ASS1, ASL and OTC in liver and ASS1 and ASL in kidney, which suggests that they are capable of arginine synthesis (Extended Data Fig. 3b). Consistent with findings from the YUMM 1.1 line and the literature14–16, YUMM 1.7 tumours that grew on Atg7−/− hosts also lacked expression of ASS1 and OTC, which suggests that—in a subset of tumour cell lines—there is a mechanism of intrinsic resistance that is independent of arginine auxotrophy (Extended Data Fig. 3c).

To determine how circulating arginine is depleted in Atg7−/− hosts, we examined the serum proteome by nanoscale liquid chromatography coupled to tandem mass spectrometry (nano LC–MS/MS), which identified 19 proteins that were downregulated and 32 that were upregulated upon loss of Atg7 (Fig. 2a, Supplementary Table 3). ARG1 was among the proteins that were upregulated in the serum of Atg7−/− hosts (2.43 fold change (log2 [ARG1 in Atg7−/−/ARG1 in Atg7+/+]) (Fig. 2b). ARG1 is expressed in the liver, where it degrades arginine to ornithine. The appearance of ARG1 in serum, without altered levels in the liver, in Atg7−/− hosts was confirmed by western blotting (Fig. 2c). Levels of arginine, ornithine and citrulline in serum from Atg7−/− hosts and hosts with liver-specific deletion of Atg7 (n = 18 each), obtained by LC–MS. Data are mean ± s.e.m. **P < 0.01, ***P < 0.001.

**Fig. 3 | Atg7 deletion in liver increases serum ARG1, and decreases serum arginine and tumour growth.** a. Experimental design to induce liver-specific deletion of Atg7. Atg7flox/flox mice were injected in the tail vein with AAV–TBG–GFP or AAV–TBG–iCre to delete Atg7 in the liver, and were injected subcutaneously with tumour cells. Tumour growth was monitored over three weeks. b. Western blotting showing expression of ARG1 in serum from Atg7−/− hosts and hosts with liver-specific deletion of Atg7 (n = 11 each). *P < 0.05 compared to Atg7+/− hosts. Data are representative of two independent experiments. Transferrin was used as a loading control. c. Levels of arginine, ornithine and citrulline in serum in Atg7−/− hosts and hosts with liver-specific deletion of Atg7 (n = 18 each), obtained by LC–MS. Data are mean ± s.e.m. **P < 0.01, ***P < 0.001.

d. Serum metabolites with fold-change (log2 [metabolite in liver-specific Atg7−/−/metabolite in Atg7+/+]) cut-offs of >1 or <−1 between Atg7−/− hosts and hosts with liver-specific deletion of Atg7 (n = 17 each) obtained by LC–MS, with P < 0.05. Data are mean ± s.e.m. e. Comparison of tumour volume and weight between Atg7+/− hosts (n = 17) and hosts with liver-specific deletion of Atg7 (n = 19). Data are mean ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001. f. Immunohistochemistry quantification of Ki-67 and active caspase-3+ cells in tumours from Atg7−/− and liver-specific Atg7−/− hosts. Data are mean ± s.e.m. *P < 0.05. g. Levels of arginine, ornithine and citrulline in tumours in Atg7−/− hosts (n = 16) and hosts with liver-specific deletion of Atg7 (n = 16), obtained by LC–MS. Data are mean ± s.e.m. **P < 0.01.
observed in levels of arginine, citrulline or ornithine in the livers of Atg7−/−Δ hosts compared to Atg7+/+ hosts (Extended Data Fig. 4c, d). Tumours from Atg7−/−Δ hosts also displayed decreased levels of arginine (13C15N2) and 13C15N2 and increased levels of ornithine (13C and 13C15N2) (Fig. 2f). These results confirm that arginine is depleted in liver-specific deletion of Atg7 and mice were supplied with supplementary dietary arginine in serum; tumour growth was also decreased in these hosts, compared to Atg7+/+Δ hosts, which suggests that deletion of Atg7 in the liver is responsible for ARG1 release into tumours 25,26. These results suggest that deletion of Atg7 in the liver is responsible for ARG1 release into the circulation, which leads to depletion of circulating arginine and decreased tumour growth.

To determine whether the degradation of circulating arginine by ARG1 in Atg7−/−Δ hosts was due to loss of autophagy, we examined mice with conditional deletion of Atg5. Whole-body conditional deletion of Atg5 also introduced ARG1 into circulation and decreased the level of arginine in serum; tumour growth was also decreased in these Atg5−/−Δ hosts (Extended Data Fig. 6). Similar to liver-specific deletion of Atg7, liver-specific deletion of Atg5 led to histopathologic changes in the liver with increased circulating ARG1 and reduced arginine (Extended Data Fig. 7), which confirms that the modulation of circulating arginine and tumorigenesis was dependent on autophagy.

We next tested whether dietary arginine supplementation can rescue tumour growth in Atg7−/−Δ hosts (Fig. 4a). Dietary arginine supplementation was able to partially increase levels of arginine in serum in Atg7−/−Δ hosts, and did not modify levels of ornithine or citrulline (Fig. 4b). This increased circulating arginine promoted growth and proliferation of the YUMM 1.1 and 1.3 melanoma cell lines in Atg7−/−Δ hosts, compared to Atg7−/−Δ hosts (Fig. 4c, d). In hosts with liver-specific deletion of Atg7, autophagy in the microenvironment may locally feed the tumour with amino acids, as has previously been shown in pancreatic cancer and Drosophila tumours 25,26. These results suggest that deletion of Atg7 in the liver is responsible for ARG1 release into the circulation, which leads to depletion of circulating arginine and decreased tumour growth.

During inflammation, injury and liver disease, ARG1 is released from hepatocytes into circulation, which leads to arginine depletion 27. Atg7−/−Δ hosts have steatosis, and liver-specific deletion of Atg5 or Atg7 is associated with liver damage 22,23. Accordingly, we hypothesized that ARG1 is released into circulation after deletion of Atg7 in the liver. To test this hypothesis, we deleted Atg7 specifically in the liver and examined levels of arginine and ARG1 in circulation, and tumour growth (Fig. 3a). Injection of an AAV–TBG–iCre vector efficiently deleted Atg7 in the liver, but not in other organs such as the brain and kidney (Extended Data Fig. 5a–c). As expected, liver-specific deletion of Atg7 led to histopathologic changes in liver cells without affecting other tissues (Extended Data Fig. 5d). As seen in Atg7−/−Δ hosts, serum from hosts with liver-specific deletion of Atg7 showed increased levels of ARG1 (Fig. 3b), a reduced level of arginine, an increased level of ornithine (Fig. 3c) and no change in the level of nitric oxide (Extended Data Fig. 5e). Liver-specific deletion of Atg7 also modified levels of other circulating metabolites, with 18 increased and 4 decreased compared to Atg7+/+ hosts (Supplementary Tables 4, 5). Some of these metabolites showed increased levels of ornithine (4-pyridoxic acid, d-glutonate and glucaric acid) were also altered in Atg7−/−Δ hosts, which suggests that the dysregulation of these metabolites has a liver-specific origin (Fig. 1d, Extended Data Fig. 5f). The level of arginine in serum was downregulated in hosts with liver-specific deletion of Atg7 compared to Atg7+/+ hosts (Fig. 3d), as shown in Atg7−/−Δ hosts (Fig. 1d). The weight and volume of YUMM 1.1 melanoma tumours were significantly decreased in hosts with liver-specific deletion of Atg7, compared to Atg7+/+ hosts (Fig. 3e); this decrease in weight and volume was associated with decreased proliferation and no change in apoptosis (Fig. 3f). Tumours from hosts with liver-specific deletion of Atg7 had decreased levels of arginine and increased levels of ornithine, but to a lesser extent than the tumours from Atg7−/−Δ hosts—this may explain why the decreased tumour growth in hosts with liver-specific deletion of Atg7 was not as marked as with Atg7−/−Δ hosts (Fig. 3g). In hosts with liver-specific deletion of Atg7, autophagy in the microenvironment may locally feed the tumour with amino acids, as has previously been shown in pancreatic cancer and Drosophila tumours 25,26. These results suggest that deletion of Atg7 in the liver is responsible for ARG1 release into the circulation, which leads to depletion of circulating arginine and decreased tumour growth.

Fig. 4 | Dietary arginine supplementation rescues tumour growth in Atg7−/−Δ hosts. a, Experimental design to perform arginine supplementation and induce conditional whole-body deletion of Atg7 to assess YUMM 1.1 tumour growth. Ubc–creERT2;Atg7flx/flx and Ubc–creERT2;Atg7flx/flx mice were supplied with supplementary dietary arginine (0 or 1%). Seven days later, tamoxifen was injected to delete Atg7 and mice were injected subcutaneously with tumour cells. Tumour growth was monitored over three weeks. b, Serum arginine, ornithine and citrulline in Atg7−/−Δ (n = 5) and Atg7+/+Δ (n = 6) hosts with or without arginine supplementation, obtained by LC–MS. Data are mean ± s.e.m. c, Comparison of tumour weight between Atg7−/−Δ (n = 13), Atg7+/+Δ + 1% arginine (n = 13), Atg7−/−Δ (n = 13) and Atg7+/+Δ + 1% arginine (n = 14) hosts. Data are mean ± s.e.m. **P < 0.01, ***P < 0.001, ****P < 0.0001. d, Immunohistochemistry quantification of Ki-67+ and active caspase-3+ cells in tumours from Atg7−/−Δ and Atg7Δ/Δ hosts, with or without arginine supplementation. Data are mean ± s.e.m. **P < 0.01, ***P < 0.001. e, Model of host autophagy promoting tumour growth. Illustrations are by Servier (https://smart.servier.com/), CC-BY-3.0.
work has demonstrated that autophagy in the local tumour microenvironment can provide amino acids that promote tumour growth. Our work demonstrates that host autophagy also sustains a circulating amino acid—arginine—that is essential for tumour growth. This finding underscores the importance of understanding the sensitivity of ASS1-deficient tumours to arginine deprivation therapy, with or without autophagy inhibition. As tumour nutrients are mainly derived from host circulation, restricting essential tumour nutrients in the circulation—such as asparaginase treatment—is a form of cancer therapy that is ripe for further exploitation.

**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/s41586-018-0697-7.

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**Author contributions**

L.P.-P. performed the majority of the experimental work and wrote the manuscript. L.Z. performed surgery and infusion with labelled arginine. Y.Y. developed the methods and provided the mice required for generating Atg5Δ/Δ and hosts with liver-specific deletion of Atg5. A.M. and C.J. assisted with in vitro experiments. X.X. and J.Y.G. performed some of the tumour growth experiments. J.M.M. provided melanoma expertise. D.W.S. and E.L. assisted with CD4 and CD8 depletion. Z.S.H. assisted with mouse husbandry. H.Z. performed proteomics processing and analysis. X.S., W.L. and J.D.R. performed metabolomics processing and analysis. M.W.B. provided YUMM 1.1, 1.3, 1.7 and 1.9 melanoma cells. E.W. is the leading principal investigator who conceived the project, supervised research and edited the paper.

**Competing interests**

E.W. is co-founder of Vescor Therapeutics. The other authors declare no competing interests.

**Additional information**

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METHODS

Mice. All animal care and treatments were carried out in compliance with Rutgers University Institutional Animal Care and Use Committee guidelines (IACUC). Mice for conditional whole-body deletion of Atg7 (C57BL/6J Ubc-creERT2+/−;Atg7fl/fl) were engineered with floxed alleles of Atg7 (Atg7fl/fl) and a transgene expressing the TAM-regulated Cre recombinase fusion protein under the control of the ubiquitously expressed ubiquitin C promoter (Ubc)31, as previously described32. Acute deletion of Atg7 throughout the mouse is obtained after TAM injection. TAM (T9848, Sigma) was suspended at a concentration of 20 mg/ml in a mixture of 98% sunflower seed oil and 2% ethanol and 250 μl per 25 g of body weight was injected intraperitoneally into 8–10-week-old male Ubc-creERT2+/− or Ubc-creERT2−/−;Atg7fl/fl mice once per day for four generations to delete Atg7-deleted (Atg7−/−Δ) and wild-type (Atg7+/+) control host mice. To assess the consequence of acute deletion of Atg7 on tumorigenesis of C57BL/6J isogenic male tumour cells, one week after TAM treatment, YUMM 1.1 (1 × 106 cells), 1.3 (2 × 106 cells), 1.7 (1.0 × 106 cells), 1.9 (1.1 × 106 cells), 7.18 (1.1 × 106 cells) or MB49 (0.25 × 106 cells) cells were resuspended in 100 μl PBS and injected subcutaneously into the dorsal flanks of mice. Three weeks after cell injection, mice were killed and serum and tumours were collected. The maximal tumour volume (1,700 mm3) permitted by Rutgers University IACUC was never exceeded. For arginine supplementation, 1% arginine (A8094, Sigma) in drinking water was given to the mice a week before TAM and throughout the experiment.

Mice for conditional whole-body deletion of Atg5 (C57BL/6J Ubc-creERT2+/−;Atg5fl/fl) were engineered with floxed alleles of Atg5 (Atg5fl/fl)32 and a transgene expressing the TAM-regulated Cre recombinase fusion protein under the control of the Ubc promoter31. Acute deletion of Atg5 throughout the mouse was obtained after TAM injection (250 μl of TAM per 25 g of body weight) were injected intraperitoneally into 8–10-week-old male Ubc-creERT2+/−;Atg5+/+ or Ubc-creERT2−/−;Atg5fl/fl mice once a week for four weeks) to generate cohorts of Atg5-deleted (Atg5−/−Δ) and wild-type (Atg5+/+) control host mice. Liver-specific deletion of Atg5 and Atg7 was achieved by injecting an adenovirus-associated virus (AAV)–thymine binding globulin (TBG) promoter–Cre recombinase vector (AAV–TBG–Cre, Vector Biosials) into Atg5fl/fl and Atg7fl/fl mice. An AAV–TBG promoter–GPV vector (AAV–TBG–GPV, Vector Biosials) was injected into Atg5fl/fl and Atg7fl/fl mice as a control. I.5 × 1011 genome copies of either AAV–TBG–Cre or AAV–TBG–GPV vectors in 100 μl PBS were injected into the tail vein of 8–10-week-old male Atg5fl/fl and Atg7fl/fl mice to generate liver-specific Atg5−/−Δ and Atg7−/−Δ and Atg5+/−Δ or Atg7+/−Δ control mice, respectively. Three weeks post injection, YUMM 1.1 cells (1 × 106 cells) were resuspended in 100 μl PBS and injected subcutaneously into the dorsal flanks of the liver-specific Atg5−/−Δ and Atg7−/−Δ control mice. Tumour growth was monitored daily. Tumour volume was calculated with the following formula: volume = 1/2 × L × W × H. Three weeks after cell injection, mice were killed and liver, kidney, brain, serum, and 0.1 l of blood were collected. For jugular vein catheterization, the procedure was modified from work previously described33. In brief, Atg5−/−Δ and Atg7+/+ mice were anaesthetized using isoflurane carried by oxygen, followed by placement of a central venous catheter (polyurethane tubing. 1 F in OD) (SAI Infusion Technologies) into the jugular vein of mice. A minimal amount of blood was carefully withdrawn to verify the catheter patency. Afterwards, the saline solution in the catheter was replaced by heparin–glycerol catheter lock solution (SAI Infusion Technologies). The proximal end of the catheter was then tunneled subcutaneously, exited between the shoulder blades and properly secured. A fully recovered surgical mouse was placed in a plastic harness (SAI Infusion Technologies), and the catheter was connected to an infusion pump (New Era Pump System) through a mouse tether and swivel system (Instech Laboratories). Arginine arginate tracer (15N-Arg, C57Bl/6J; Atg7fl/fl; Atg5fl/fl) was dissolved in sterile saline and infused at a rate of 3.5 mmol/min (0.1 μg/ml/min) for 3 h. Infusion rate was determined using turnover flux calculations32. Mice were killed after infusion for serum, tumour, liver and kidney analysis by LC–MS. The isotope natural abundance and impurity of labelled substrate was corrected using a matrix-based algorithm.

Labelled arginine infusion. For jugular vein catheterization, the procedure was modified from work previously described33. In brief, Atg5Δ−Δ and Atg7+/+ mice were anaesthetized using isoflurane carried by oxygen, followed by placement of a central venous catheter (polyurethane tubing. 1 F in OD) (SAI Infusion Technologies) into the jugular vein of mice. A minimal amount of blood was carefully withdrawn to verify the catheter patency. Afterwards, the saline solution in the catheter was replaced by heparin–glycerol catheter lock solution (SAI Infusion Technologies). The proximal end of the catheter was then tunneled subcutaneously, exited between the shoulder blades and properly secured. A fully recovered surgical mouse was placed in a plastic harness (SAI Infusion Technologies), and the catheter was connected to an infusion pump (New Era Pump System) through a mouse tether and swivel system (Instech Laboratories). Arginine arginate tracer (15N-Arg, C57Bl/6J; Atg7fl/fl; Atg5fl/fl) was dissolved in sterile saline and infused at a rate of 3.5 mmol/min (0.1 μg/ml/min) for 3 h. Infusion rate was determined using turnover flux calculations32. Mice were killed after infusion for serum, tumour, liver and kidney analysis by LC–MS. The isotope natural abundance and impurity of labelled substrate was corrected using a matrix-based algorithm.
acid solution were added to stop the reaction; the mixture was allowed to sit on ice for 10 min. The extract was neutralized with 40 μl of 15% NH₄HCO₃ solution and centrifuged at 16,000g for 10 min at 4°C. Then, 500 μl of supernatant was removed to clean tubes and stored at −80°C until analysis by LC–MS. The LC–MS analysis was performed on the Q Exactive PLUS mass spectrometer coupled to UltiMate 3000 UHPLC system with an XBridge BEH Amide column (150 mm × 2.1 mm, 2.5 μm particle size, Waters) with the corresponding XF VanGuard Cartridge. The liquid chromatography used a 6-min isocratic elution of 28% solvent A (95.5:3% H₂O:acetonitrile with 20 mM ammonium acetate, 20 mM ammonium hydroxide, pH 9.4) and 72% solvent B (20:80% H₂O:acetonitrile with 20 mM ammonium acetate, 20 mM ammonium hydroxide, pH 9.4). The flow rate was 300 μl/min. Injection volume was 5 μl and column temperature 25°C. The mass spectrometry scans were in negative-ion mode with a resolution of 70,000 at m/z 200. The automatic gain control target was 3 × 10⁶ and the scan range was 75–1,000. Metabolite features were extracted in MAVEN v.7.07 with the labelled isotope specified and a mass accuracy window of 5 p.p.m.

Proteomic analysis by LC–MS. Technical duplicates of pooled serum samples from both Atg7−/− (n = 5) and Atg7−/−Δ (n = 5) mice were processed in parallel. Two different methods were also used to reduce the amount of major serum proteins, to allow detection of rarer components: AlbuVoid (Biotech Support Group) was used to deplete albumin, and the Agilent multiple affinity removal spin cartridge mouse system (Mars3) was used to remove albumin, IgG and transferrin following the manufacturer’s protocol. Untreated or depleted sera were loaded onto NuPage 10% Bis-Tris Gel (Invitrogen), run a short distance into the gel, and proteins reduced, alkylated and digested with trypsin as described. Digests were analysed by nano LC–MS/MS using a Dionex UltiMate 3000 RSLC nano system interfaced with Q Exactive HF (ThermoFisher). Peptides were loaded onto a self-packed 100 μm × 2 cm trap (Magic C18AQ, 5 μm 200 Å, Michrom Bio resources) and washed with buffer A (0.1% trifluoroacetic acid) for 5 min with a flow rate of 10 μl/min. The trap was brought in-line with the analytical column (self-packed Magic C18AQ, 3 μm 200 Å, 75 μm × 50 cm) and fractionated at 300 nl/min using a segmented linear gradient of 4–15% B in 30 min (A, 0.2% formic acid; B, 0.16% formic acid/80% acetonitrile), 15–25% B in 40 min, 25–50% in 44 min and 50–90% B in 11 min. Mass spectrometry data were acquired using a data-dependent acquisition procedure with each cycle consisting of a MS1 scan (resolution 120,000) followed by MS2/MS3 scans (HCD relative collision energy 27%, resolution 30,000) of the 20 most intense ions using a dynamic exclusion duration of 20 s. The raw data were converted into MASCOT generic format using Protologue Discover 2.1 (Thermo Fisher) and searched against the Ensemble mouse database and a database of common laboratory contaminants (http://www.theegpm.org/crap/) using a local implementation of the global proteome machine (GPM Fury). Peptide spectrum matches were assigned to genes using BioMart Ensembl tables. To estimate differential abundances of proteins, data from all LC–MS runs were combined (neat, AlbuVoid-depleted and Mars3-depleted for each of the four samples). For mouse enzymatic assays, data from all LC–MS runs were validated with the use of positive and negative controls, following the manufacturer’s protocol.

**T cell depletion and flow cytometry.** A week after TAM, and every 5 days, 200 μg of CD4 (clone GK1.5; BE003-1, BioXcell) and CD8 (clone 2.43; BE0061, BioXcell) antibodies were injected intraperitoneally into Atg7−/− and Atg7−/−Δ mice. Two days after the first antibody injection, YUMM 1.1 (1 × 10⁶ cells) were resuspended in 100 ml PBS and injected subcutaneously into the dorsal flanks of the mice. Three weeks after cell injection, mice were killed and tumours and spleen were collected. Tumours were homogenized in PBS in a gentleMACS Octo Dissociator (Miltenyi Biotec), according to the manufacturer’s protocol, and passed through a 70 mm cell strainer. Spleens were ground with a rubber grinder through steel mesh, treated with ACK Lysis Buffer to remove erythrocytes and passed through a 70 mm cell strainer. Nonspecific binding of antibodies to cell Fc receptors was blocked using 20 ml per 10⁶ cells of FcR blocker (Miltenyi Biotec). Cell surface immunostaining was performed with the following antibodies (1:200): CD11c-PE-eFluor610 (clone N418, 61-0114-82), CD4-APC (clone GK1.5, 17-0041-82) CD3-AF700 (clone 17A2, 56-0032-82) and CD11b-APC-Cy7 (clone M1/70, 15390) (eBioscience); and CD45-FITC (clone 30-F11, 103107), MHC-II-BV605 (clone MS1/145.12, 107639), Ly6G-BV650 (clone 1A8, 127641) and CD8-BV785 (clone 53.67, 100749) (BioLegend). Aqua Live/Dead (Invitrogen) was included to determine live cells. After staining of surface markers, cells were fixed and permeabilized using transcribing factor staining kit and stained with FoxP3-eFluor450 (eBioscience). Cell staining data were acquired using a LSR-II flow cytometer (BD Biosciences, BD FACS Diva v2 software) and analysed with FlowJo v.10 software (Tree Star). Live lymphocytes were gated using forward scatter area (FSC-A) versus side scatter area (SSC-A), followed by FSC-A versus forward scatter height (FSC-H), SSC-A versus side scatter height (SSC-W) plots, forward scatter width (FSC-W) versus side scatter width (SSC-W), and Aqua Live/Dead. Populations were gated as follows: CD45 (percentage CD45 of total live lymphocytes), CD13 (percentage CD13 of CD11b−CD11c−CD45+), CD8 (percentage CD8+ of CD3), CD4 (percentage CD4+ of CD3), Trig (percentage FoxP3+ of CD4), DC (percentage CD11c+ of MHC-II+CD45+) and MDSC (percentage Ly6G, CD11b+ of MHC-II+ CD45+). Antibodies for western blotting, flow cytometry and immunohistochemistry were validated with the use of positive and negative controls (gene knockouts and through the use of control tissues and cell lines), and following the manufacturer’s protocol.

**Statistical analysis.** All statistical analyses were performed with GraphPad Prism v.7 software using two-sided Student’s t-test, unless specified otherwise. The sample size was chosen in advance on the basis of common practice of the described experiment and is mentioned for each experiment. No statistical methods were used to pre-determine sample size. Each experiment was conducted with biological replicates and repeated multiple times. All attempts at replication were successful and no data were excluded. Mice were randomly allocated to experimental groups and the investigators were not blinded during the experiments and outcome assessment. Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

**Data availability.** All data are available from the authors upon reasonable request. Source Data for Figs. 1a, 2h, 3d are provided with the paper. The raw mass spectrometry data have been deposited in the MassIVE repository, entry MSV000082879.

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Extended Data Fig. 1 | Host autophagy promotes growth of different tumour cell types. a, c, e. Comparison of tumour weight between Atg7^{+/+} (n = 5) and Atg7^{Δ/Δ} (a, n = 4; c, n = 5; e, n = 4) hosts after injection of 1.3 (a), MB49 (c) or 71.8 (e) cells. Data are mean ± s.e.m. *P < 0.05, **P < 0.01. b, d, f. Immunohistochemistry quantification of Ki-67+ and active caspase-3+ cells in tumours from Atg7^{+/+} and Atg7^{Δ/Δ} hosts. Data are mean ± s.e.m. *P < 0.05, ***P < 0.001, ****P < 0.0001.
Extended Data Fig. 2  | Immune response is not involved in decreased tumour growth observed in Atg7Δ/Δ hosts. a, c, Comparison of tumour weight between Atg7Δ/Δ (n = 5) and Atg7Δ/Δ (a, n = 5; c, n = 6) hosts after injection of 1.7 (a) or 1.9 (c) cells. Data are mean ± s.e.m. b, d, Immunohistochemistry quantification of Ki-67+ and active caspase-3+ in 1.7 (b) and 1.9 (d) tumours from Atg7Δ/Δ and Atg7Δ/Δ hosts. Data are mean ± s.e.m. e, Representative immunohistochemistry images and quantification of CD3+, CD4+ and CD8+ cells in tumours from Atg7Δ/Δ and Atg7Δ/Δ hosts. Data are mean ± s.e.m. f, Comparison of tumour volume and weight between Atg7Δ/Δ (n = 10), Atg7Δ/Δ + CD4 and CD8 antibody depletion (n = 15), Atg7Δ/Δ (n = 7) and Atg7Δ/Δ + CD4 and CD8 antibody depletion (n = 8) hosts. Data are mean ± s.e.m. *P < 0.05, ****P < 0.0001. g, Fold change in immune components between Atg7Δ/Δ and Atg7Δ/Δ, with or without antibody depletion (n = 5 each). Treg, T regulatory cells; DC, dendritic cells; MDSC, myeloid-derived suppressor cells. Data are mean ± s.e.m. ***P < 0.001, ****P < 0.0001, by two-way ANOVA test.
Extended Data Fig. 3  Tumour cells are arginine auxotrophs.

a, YUMM 1.3, 71.8, MB49 and YUMM 1.7, 1.9 proliferation in vitro, in medium containing different percentages of arginine. Cell density was measured every 2 h using the IncuCyte. Data are representative of three independent experiments performed in duplicate.

b, Western blotting showing expression of ASS1, ASL and OTC in kidneys and livers from \( \text{Atg7}^{+/+} \) and \( \text{Atg7}^{-/-} \) hosts. *\( P < 0.05 \) compared to \( \text{Atg7}^{+/+} \) hosts. Data are representative of three independent experiments. Actin was used as a loading (kidney ASL and liver OTC) and processing (kidney ASS1, liver ASS1 and ASL) control.

c, Western blotting showing expression of ASS1, ASL and OTC in YUMM 1.7 tumours from \( \text{Atg7}^{+/+} \) and \( \text{Atg7}^{-/-} \) hosts. Data are representative of two independent experiments. Actin was used as a loading (OTC) and processing (ASS1 and ASL) control.

d, Analysis of levels of nitric oxide in serum in \( \text{Atg7}^{+/+} \) (\( n = 11 \)) and \( \text{Atg7}^{-/-} \) (\( n = 9 \)) hosts. Data are mean ± s.e.m.
Extended Data Fig. 4 | Atg7 deletion increases serum arginine degradation but does not modify arginine metabolism in kidney and liver. a: Serum $^{13}$C$_6$-arginine and $^{13}$C$_5$-ornithine in Atg7$^{+/+}$ and Atg7$^{\Delta\Delta}$ hosts (n = 3 each) over time. Data are mean ± s.e.m. b, Concentration (in μM) of arginine, citrulline and ornithine in serum from Atg7$^{+/+}$ (n = 3) and Atg7$^{\Delta\Delta}$ hosts (n = 4), after infusion with $^{13}$C$_6^{15}$N$_4$-arginine. c, d, Concentration (in nmol g$^{-1}$) of arginine, citrulline and ornithine in kidneys (c) and livers (d) from Atg7$^{+/+}$ and Atg7$^{\Delta\Delta}$ hosts (n = 2 each) after infusion with $^{13}$C$_6^{15}$N$_4$-arginine. Data are mean. **P < 0.01 by two-way ANOVA test.
Liver-specific deletion of \( \text{Atg7} \) leads to liver-cell enlargement without affecting other tissues. a–c, Western blotting showing expression of \( \text{Atg7} \) in livers (\( n = 11 \) each) (a), brains (\( n = 9 \) and 11, respectively) (b) and kidneys (\( n = 10 \) each) (c) from \( \text{Atg7}^{+/+} \) hosts and hosts with liver-specific deletion of \( \text{Atg7} \). \(* P < 0.05\) compared to \( \text{Atg7}^{+/+} \) hosts. Data are representative of two independent experiments. Actin was used as a loading control. d, Representative haematoxylin and eosin tissue staining from \( \text{Atg7}^{+/+} \) hosts and hosts with liver-specific deletion of \( \text{Atg7} \). Images are representative of two independent experiments. e, Analysis of levels of nitric oxide in serum, in \( \text{Atg7}^{+/+} \) hosts (\( n = 13 \)) and hosts with liver-specific deletion of \( \text{Atg7} (n = 15) \). Data are mean ± s.e.m. f, Comparison of serum metabolites that are significantly regulated in \( \text{Atg7}^{\Delta\Delta} \) hosts and hosts with liver-specific deletion of \( \text{Atg7} (n = 17 \text{ each, } P < 0.05)\).
Extended Data Fig. 6 | Atg5 deletion increased serum ARG1, decreased serum arginine and tumour growth. a, Experimental design to induce host mice with conditional whole-body deletion of Atg5 (Atg5Δ/Δ) and wild-type controls (Atg5+/+) with which to assess tumour growth. Ubc-CreERT2;Atg5Δ/Δ and Ubc-CreERT2;Atg5+/+ mice were injected with TAM at 8 to 10 weeks of age to delete Atg5 and create Atg5Δ/Δ hosts. Mice were then injected subcutaneously with tumour cells and tumour growth was monitored over three weeks. b, Comparison of tumour weight between Atg5+/+ (n = 4) and Atg5Δ/Δ (n = 3) hosts. c, Immunohistochemistry quantification of Ki-67+ and active caspase-3+ cells in tumours from Atg5+/+ and Atg5Δ/Δ hosts. d, Western blotting showing expression of ARG1 in serum from Atg5+/+ (n = 3), Atg5Δ/Δ (n = 4) and Atg7Δ/Δ (n = 3) hosts. Transferrin was used as a loading control. e, Levels of arginine, ornithine and citrulline in serum in Atg5+/+ (n = 4) and Atg5Δ/Δ (n = 3) hosts, obtained by LC–MS. Data are mean ± s.e.m. *P < 0.05, **P < 0.01. © 2018 Springer Nature Limited. All rights reserved.
Liver-specific Atg5-deleted hosts present liver-cell enlargement, increased serum ARG1 and decreased serum arginine. a, Experimental design to induce liver-specific deletion of Atg5. Atg5<sup>flx/flx</sup> mice were injected in the tail vein with AAV–TBG–GFP or AAV–TBG–iCre at 8 to 10 weeks of age to delete Atg5 in the liver and create Atg5<sup>+/+</sup> hosts and hosts with liver-specific deletion of Atg5, respectively. b, Western blotting showing expression of Atg5 in the livers, brains and kidneys of Atg5<sup>+/+</sup> hosts and hosts with liver-specific deletion of Atg5 (n = 6 each). *P < 0.05 compared to Atg5<sup>+/+</sup> hosts. Actin was used as a loading control. c, Haematoxylin and eosin tissue staining from Atg5<sup>+/+</sup> hosts and hosts with liver-specific deletion of Atg5 (n = 6 each). d, Western blotting showing expression of ARG1 in serum from Atg5<sup>+/+</sup> hosts and hosts with liver-specific deletion of Atg5 (n = 6 each). *P < 0.05 compared to Atg5<sup>+/+</sup> hosts. Transferrin was used as a loading control. e, Levels of arginine, ornithine and citrulline in serum in Atg5<sup>+/+</sup> hosts and hosts with liver-specific deletion of Atg5 (n = 6 each), obtained by LC–MS. Data are mean ± s.e.m. ****P < 0.0001.
Extended Data Fig. 8 | Dietary arginine supplementation rescues YUMM 1.3 tumour growth in Atg7\(\Delta/-\Delta\) hosts. a, Serum arginine, ornithine and citrulline in Atg7\(+/-\) (n = 5), Atg7\(+/-\) + 1% arginine (n = 5), Atg7\(-/-\) (n = 6) and Atg7\(-/-\) + 1% arginine (n = 6) hosts, obtained by LC–MS. Data are mean ± s.e.m. *P < 0.05, **P < 0.01. b, Comparison of YUMM 1.3 tumour weight between Atg7\(+/-\) and Atg7\(-/-\) (n = 5 each) hosts, with or without arginine supplementation. Data are mean ± s.e.m. **P < 0.01, ****P < 0.0001. c, Immunohistochemistry quantification of Ki-67\(^+\) and active caspase-3\(^+\) cells in tumours from Atg7\(+/-\) and Atg7\(-/-\) hosts, with or without arginine supplementation. Data are mean ± s.e.m. **P < 0.01, ****P < 0.0001.
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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).

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- [ ] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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Life sciences study design

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

Sample size  The sample size was chosen in advance on the basis of common practice of the described experiment and is mentioned for each experiment. No statistical methods were used to predetermine sample size.

Data exclusions  No data were excluded

Replication  Each experiment was conducted with biological replicates and repeated multiple times. All attempts at replication were successful.

Randomization  Mice were randomly allocated to experimental groups.

Blinding  The investigators were not blinded during the experiments and outcome assessment.

Reporting for specific materials, systems and methods

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a                             | n/a     |
| ☑️ Involved in the study        | ☑️ Involved in the study |
| ☐ Unique biological materials  | ☑️ ChIP-seq |
| ☐ Antibodies                    | ☑️ Flow cytometry |
| ☑️ Eukaryotic cell lines        | ☑️ MRI-based neuroimaging |
| ☑️ Palaeontology                |         |
| ☑️ Animals and other organisms  |         |
| ☑️ Human research participants  |         |

Antibodies

Antibodies used  For immunohistochemistry: Ki67 (1:200, Ab15580, Abcam), active Caspase-3 (1:300, #9661, Cell Signaling), CD3 (1:100, Ab16669, Abcam), CD4 (1:1,000, Ab183685, Abcam) and CD8 (1:100, 14-0808-82, Invitrogen). For western blotting: ASS1 (1:1000, Ab170952, Abcam), ASL (1:500, sc-374353, Santa Cruz), OTC (1:500, sc-515791, Santa Cruz), AR1 (1:500, sc-271240, Santa Cruz), ATG7 (1:2,000, A2856, Sigma), transferrin (1:1,000, sc-22597, Santa Cruz), ATG5 (1:1,500, Ab108327, Abcam) and β-actin (1:5,000, A1978, Sigma). For antibody depletion: 200 ug of CD4 (clone GK1.5; BE003-1, BioXCell) and CD8 (clone 2.43; BE0061, BioXCell). For flow cytometry (1:200), the following antibodies purchased from eBioscience: CD11c-PE-eFluor610 (clone N418, 61-0114-82), CD4-APC (clone GK1.5, 17-0041-82) CD3-AP700 (clone 17A2, 56-0032-82), CD11b-APC-Cy7 (clone M1/70, A15390); and the following antibodies from BioLegend: CD45-FITC (clone 30-F11, 103107), MHC-II-PEV605 (clone M5/114.15.2, 107699), Ly6G-BV550 (clone 1A8, 127641), CD8-BV711 (clone 53.67, 100749).

Validation  Antibodies for western blotting, flow cytometry and immunohistochemistry were validated with the use of positive and negative control (gene knock outs and through the use of control tissues and cell lines) and following manufacturer's protocol.

Eukaryotic cell lines

Policy information about cell lines  YUMM 1.1, 1.3, 1.7 and 1.9

Cell line source(s)  YUMM 1.1, 1.3, 1.7, 1.9 and 1.9 mouse melanomas and were generated previously in the Rosenberg laboratory. Mouse lung cancer cell line 71.8 was derived from p53-/-, KrasG12D/ mouse lung tumors previously in our laboratory and the M849 cell line was provided by the Ratliff laboratory.

Authentication  YUMM 1.1, 1.3, 1.7, 1.9, 71.8 and M849 were authenticated using whole exome sequencing.
Mycoplasma contamination | Cells were tested negative for mycoplasma contamination
Commonly misidentified lines (See ITCLC register) | No misidentified lines were used

Animals and other organisms

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

| Laboratory animals | For whole-body Atg7 deletion: 8 to 10 weeks old C57Bl/6J Ubc-CreERT2/+/Atg7flox/flox and Ubc-CreERT2/+/Atg7flox/flox male mice; For liver-specific Atg7 deletion: 8 to 10 weeks old C57Bl/6J Atg7flox/flox male mice; For whole-body Atg5 deletion: 8 to 10 weeks old C57Bl/6J Ubc-CreERT2/+/Atg5flox/flox and Ubc-CreERT2/+/Atg5flox/flox male mice; For liver-specific Atg5 deletion: 8 to 10 weeks old C57Bl/6J Atg5flox/flox male mice |
| Wild animals | The study did not involve wild animals |
| Field-collected samples | The study did not involve samples collected from the field |