**Supplemental Methods and Materials**

**mtDNA isolation, enrichment and ultra-deep sequencing**

mtDNA Extraction

*p53-/-;KrasG12D/+;Atg7+/+*or *p53-/-;KrasG12D/+;Atg7-/-*lung tumor samples from GEMMs for NSCLC were used for mitochondria genome sequencing (Guo et al. 2013). Eight tumor samples were used for each genotype. Two normal lung tissues were obtained from *p53F/F;KrasG12D/+;Atg7+/+*or *p53F/F;KrasG12D/+;Atg7F/F* micewithout adenovirus-Cre infection for controls (Guo et al. 2013). mtDNA was isolated from tumors and normal lung tissues using DNeasy blood and Tissue kit (QIAGEN) kit according to the manufacturer’s instructions. The concentration of mtDNA was measured using Qubit 2.0 Fluorometer (Invitrogen) and the quality was assessed with Agarose gel electrophoresis.

mtDNA sequencing

Library preparation was carried out using the Agilent SureSelectXT Mouse Mitochondrial Custom Enrichment protocol (Agilent Technologies, Santa Clara, CA). Extracted DNA from each sample was fragmented and then enriched, and enriched fragments were amplified using PCR. The barcoded libraries were assessed on the Agilent Bioanalyzer for proper sizing, and then quantified using the KAPA Library Quantification Kit for Illumina Sequencing Platforms (KAPA BioSystems, Boston, MA). Libraries were individually diluted to a 10nM concentration, and then symmetrically pooled for sequencing run preparation. Each pool of 16 samples was clustered and sequenced on the Illumina MiSeq instrument using 2x150bp paired-end reads, following the manufacturer’s protocols. Each library was then loaded to obtain ~2.6 million reads per sample (Table S1)**.** Raw sequencing data was processed into read sequences and base quality scores using the standard Illumina pipeline.

**Bioinformatics data analysis**

Quality control and pre-processing on sequenced reads were done using FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>) followed by alignment to mtDNA reference genome (mm10) using default BWA-MEM (bwa-0.7.5a (Li and Durbin 2009)). There was an average of 2.6 million reads per DNA sample (ranging from 910802 to 4065612 paired reads) with ~95% of 150bp paired reads aligned to the mouse mitochondrial genome (mm10) with an average coverage of ~24,000x across chrM genome (except for nuclear mitochondrial pseudogenes regions, NuMTs)(Table S4). Post-processing of alignments results e.g., sam to bam file, sorting, creating mpileup files etc. were done using Picard tool v1.100 (<https://github.com/broadinstitute/picard>) and Samtools (Li et al. 2009) (*mpileup* with –q 1, -Q 20, -d 1000000 and –L 1000000). Final processed bam files from tumor and normal samples were used to call somatic point mutations and indels using VarScan2 (v2.3.9 (Koboldt et al. 2012)). We considered uniquely mapped mtDNA reads to detect somatic variations.

VarScan2 *somatic* for variant calling with following parameter were used: --strand-filter 1 (removes variants with >90% strand bias), --min-var-freq 0.03 (minimum Variant allele frequency of 3%), --min-avg-qual 20 (minimum base quality 20) with rest parameters as default. With regard to --strand-filter option, it effectively removes >90% strand bias mismatches which could result from PCR or sequencing error. Due to presence of known nuclear mitochondrial DNA (NuMTs) in the mouse genome, we used >3% minimum variant allele frequency cutoff to detect variants and to filter false positive events.

All variations were visually screened using IGV for read depth, variation status and alignments. Somatic variants were annotated using VEP (McLaren et al. 2010) to classify/predict effect of these variants on coding mtDNA genes. We used Barnard's test (one-tail) to find the enrichment of somatic variations in *Atg7* null verses WT tumors.

**Supplemental figure legends**

Fig. S1. Utilization of glucose is independent of autophagy status in nutrient rich conditions.

1. Glucose uptake rate (Top) and lactate secretion rate (Bottom) of *Kras*-driven tumor cells in RPMI. Error bar: +/- SEM (n=3); \* p<0.05.
2. Left: schematic of carbon atom transitions and tracers used to detect carbon contribution from [U13C6]-Glc to glycolytic intermediates. Right: kinetic carbon incorporation from [U13C6]-Glc to glycolytic intermediates. Error bar: +/- SEM (n=3).
3. Left: schematic of carbon atom transitions and tracers used to show carbon contribution from [U13C6]-Glc to TCA cycle intermediates. Right: kinetic carbon incorporation from [U13C6]-Glc to TCA cycle intermediates. Error bar: +/- SEM (n=3).
4. Left: schematic of carbon atom transitions and tracers used to show carbon contribution from glucose to the intermediates of the TCA cycle in steady state in RPMI. Right: [U13C6]-Glc flux to the substrates of the TCA cycle in steady state in RPMI. Error bar: +/- SEM (n=3).

Fig. S2. Utilization of glutamine is independent of autophagy status in nutrient replete conditions.

1. Glutamine uptake rate of *Kras*-driven tumor cells in RPMI.
2. Left: schematic of carbon atom transitions and tracers used to show carbon contribution from [U13C5]-Gln to TCA cycle intermediates. Right: kinetic carbon incorporation from [U13C5]-Gln to TCA cycle intermediates. Error bar: +/- SEM (n=3).
3. Left: schematic of carbon atom transitions and tracers used to show carbon contribution from [U13C5]-Gln to the intermediates of TCA cycle in steady state. Right: [U13C5]-Gln flux to the substrates of TCA cycle in steady state in RPMI. Error bar: +/- SEM (n=3).

Fig. S3. Glutamine and its derivatives enable *Atg7*-deficient TDCLs to survive starvation.

1. *Atg7* WT and deficient cells were treated with HBSS or HBSS supplemented with different amino acids for 2 days, which was then replaced with normal RPMI medium for 2 days, and stained with Giemsa to assess clonogenic survival. Individual amino acid concentrations used in the assay was the same as the concentration in RPMI.
2. Clonogenic survival assays show that proline (2mM) supplementation rescued *Atg7*-deficient cell death in HBSS. Treated cells were allowed to recover for 1 day in RPMI following 1 day of HBSS.

Fig. S4. OCR of *Atg7* WT and deficient tumor cells in RPMI, HBSS and HBSS with glutamine (Q) (2mM) supplementation conditions. Error bar: +/- SEM (n=4).

Fig. S5. Cellular concentrations of nucleoside phosphates in RPMI and HBSS (4hours). Error bar: +/- SEM (n=3); \* p<0.05, \*\* p<0.01, \*\*\* p<0.0001 (t-test).

Fig. S6. Nucleoside supplementation does not restore levels of TCA intermediates.

1. Pools sizes of TCA cycle intermediates in RPMI, HBSS and HBSS supplemented with nucleosides (2mM each) for 4 hours. Error bar: +/- SEM (n=3).
2. OCR of *Atg7* WT and deficient tumor cells in RPMI, HBSS and HBSS with nucleosides supplementation (2mM each). Error bar: +/- SEM (n=4).

**Supplemental references**

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