**Supplementary Figure and Table Legends**

**Supplementary Figure 1.**

**Identification of autophagy-dependent and independent cell lines**

PDAC cell lines were infected with NTC control, Atg5 and Atg7 sgRNAs. At indicated time-points after infection, cells were counted using Casy cell counter and represented as relative cell number. Atg5 and Atg7 protein level depletion was confirmed by immunoblot analysis.

**Supplementary Figure 2.**

**Validation summary for 11 top autophagy regulators**

The PDAC cell lines PA-TU-8902, PK-1, SUIT-2 and KP-4 were treated with the indicated siRNAs for 72 hours, and Baf A1 for the final 2 hours, followed by immunoblotting analysis (n=1). qPCR was performed alongside to indicate knockdown efficiency.

**Supplementary Figure 3.**

**Levels of MDH1, MPP7 and downstream autophagy regulators ULK1 and YAP1 affect PDAC cell survival**

**A)** A high-throughput long-term growth assay was performed in the autophagy-dependent PK-1 and PA-TU-8902 and the autophagy-independent PDAC cell lines KP-4 and SUIT-2. Cells were reverse transfected with deconvoluted siRNA, 4 96-wells/condition, and allowed to grow for 7 days prior to fixation and nuclear Hoechst staining. Using TTP’s Labtech acumen Cellista, cell number/96-well was determined and normalised to RISC-free siRNA transfected cells. Represenative example of n=2 experiments is presented. **B)** Cells from (A) analysed by immunoblotting to confirm target knockdown. **C)** PK-1 and KP-4 cells were treated for 6 days with the indicated siRNAs followed by flow-cytometry Annexin V analysis. **D)** Cells from (C) analysed by immunoblotting to confirm target knockdown. **E)** Indirect interactions uncovered by using network information between the selected target MDH1 and core autophagy proteins. **F**) A model showing the proposed mechanism of autophagy regulation by MDH1 through ULK1**.**

**Supplementary Table 1.**

Table presenting LC3 (ch2) parameters for top 200 LC3 decreasers, ranked by “weighted decrease ch2”. Hits with a mean Z-score ≤ -2 for the autophagy-related parameters mean and the %low Z-scores of total spot count, total spot area, average spot area, total spot intensity and average spot intensity were awarded a binary score of 2. Hits with a %low Z-score ≥ 2 in the remaining parameters were given a binary score of 1. The sum of these scores over all autophagy-related parameters produces a total weighted binary score with a maximum of 15. Some hits were excluded based on biology-dependent criteria, e.g. low expression levels in a relevant cell line/tissue or competitive scientific landscape.

**Supplementary Table 2.**

Table presenting deconvolution data for the 200 hits tested in deconvolution screen. Ranking is based on score in deconvolution combined with expression in pancreatic cancer and existing interactions with the ARN (autophagy regulatory network).

**Supplementary Table 3.**

Table showing complete growth assay and qPCR data for 116 siRNAs targeting 56 hits, mean of N=2 experiments. Values are presented as % relative to RISC-free control. This data is also presented in Figure 2A, and a subset of the data (11 selected hits only) is shown in Figure 2B.

**Supplementary Table 4.**

Table showing LC3 parameter data in the PK-1 cell line for the top 50 siGENOME KP-4 screen hits. Reverse siRNA transfection with deconvoluted siRNA was performed in 384-well format using a protocol identical to that of the siGENOME KP-4 screen. LC3 parameter data is presented as a percentage of RF-transfected cells. A subset of the data (11 selected hits only) is shown in Figure 2C.