Supplementary materials and methods

PCR primers sequences:

POMP1 FW AAGGCAGTGCAGCAGGTTCAGC and RV TGTGGCTCTCCCATGACTTCGC, *STMN1* FW CCTCTGTTTGGCGCTTTTGTGCG and RV GGCACGCTTCTCCAGTTCTTTCACC, *RAB5A* FW ACAGAGGAGCACAAGCAGCCA and RV AGGTCGGCCTTGTTTCCCGAT, *STAMBP* FW AAGGGTCCCGGGAACGTGGT and RV CACCTCCCAAGGCCAGGAAGC, *MCL-1* FW TGAAGATGGTAGGGTGGAAAG and RV TAATGAATTCGGCGGGTAAT, *ATG4D* FW AGCTCCTCCTCAGCCACA and RV GGAGCAGAGGTCGTCCAG, *EZH2* FW GAGCAAAGCTTACACTCCTTTCA and RV CCGCTTATAAGTGTTGGGTGTT, *PDCD4* FW TATGATGTGGAGGAGGTGGATGTGA and RV CCTTTCATCCAAAGGCAAAACTACAC, *CAPN2* CTGCTCCATCGACATCAC and RV CCCTGTCCACTCCACTTC, *ASAP1* FW CTCACATGCCACATCTAACAG and RV GCTACATAATCCTGCTCATCTTC, *MTMR2* FW CTTGTCCAGTGCCTCCAC and RV AAGGGTGGTTCTTCCATTTC, *PEBP1* FW CCACCCAGGTTAAGAATAGAC and RV ATGCCATTCTCTGTATTTGG, *TGFBR1* FW AGAGCTGTGAAGCCTTGAG and RV AAAGGAGAGTTCAGGCAAAG, *RAPTOR* FW TCCACCCGCACTGGCCTCA RV CGTGTACTCGGTGGAGAAGCGTG, *CDKN1A* FW GGCAGACCAGCATGACAGATTTC and RV CGGATTAGGGCTTCCTCTTGG.

Vector construction:

pGL3-STMN1, pGL3-RAB5A and pGL3-ATG4D reporter vectors were cloned by PCR amplification of relevant 3'UTR fragments from human genomic DNA and insertion into the pGL3 control vector (Invitrogen). Primers used for PCR amplification are as follows (restriction sites underlined): STMN1 FW: ggg-agatct-atatccaaagactgtactgg and RV: ggg-ctcgag-gtcactgccaccaacagcac, RAB5A FW: ggg-agatct-aaatgcaccttaatggtcag and RV: ggg-ctcgag-tacaaattacgggtacattg, ATG4D FW: ggg-agatct-tgagctctggcagtgatgat and RV: ggg-ctcgag-caagttacacagctttattg. The quickchange site-directed mutagenesis kit (Stratagene) was used for introducing 3 point mutations into the miR-101 binding sites. The mutagenesis primer used for pGL3-STMN1MUT was gggttaatggctactagtctattggctctgtg and for pGL3-ATG4DMUT was ggcctgactcacctagtctagtttgcactggacgcc. For pGL3-RAB5AMUT, 2 step mutation was

done for the 8-mer using: cgatcttcagctctagtctaaatagggtagtgcattgtagtctc and for the 7-mer using: gcttaaaagcatacaaaatctagtcttactaaaacagctaattatttctctctccc.
pBabe-HA-STMN1 was cloned by PCR amplifying the human STMN1 gene and introducing it into the pCR8/GW/TOPO gateway entry vector (Invitrogen) according to the manufacturer's protocol. The sequence was verified and STMN1 was then further cloned into the pBabe-HA gateway destination vector by gateway technology (Invitrogen).

Supplementary Figure legends

Supplementary Figure S1: Controls for the luciferase-based screening assay

(A) Western blot showing knock-down efficiency of Beclin-1 siRNA. Cells were lysed 48 h after transfection. **(B)** Beclin-1 siRNA controls from screen 1. Representative example, from 42 h timepoint of screen 1, showing efficiency of Beclin-1 siRNA in inhibiting autophagic flux. The Y-axis depicts the $LC3^{WT}/LC3^{G120A}$ ratios while the X-axis depicts the individual wells across all 18 plates of the screen. Highlighted in red are the wells transfected with the Beclin-1 siRNA, generally with relatively high $LC3^{WT}/LC3^{G120A}$ ratios. **(C)** Renilla siRNA controls from screen 1. Representative example, from the 42 h timepoint of screen 1, showing efficiency of the renilla siRNA. The Y-axis depicts the $LC3^{WT}$ raw luciferase values while the X-axis depicts the individual wells across all 18 plates of the screen. Highlighted in red are the wells transfected with the renilla siRNA. **(D)** Average $LC3^{WT}/LC3^{G120A}$ ratios from screen 1 at 42, 54 and 66 h for all scramble controls and for the entire miRNA precursor library.

Supplementary Figure S2: Induction of autophagy increases endogenous miR-101 expression levels

(A) miR-101 expression is increased during starvation. Top: miR-101 qPCR after indicated timepoints of starvation in hanks buffered salt solution (HBSS). Data are normalized relative to housekeeping *RNU6B* and 0 h sample and error bars represent ±S.D. of three replicates. Bottom: Western blot showing p-S6K and total S6K levels at indicated timepoints after starvation in HBSS. **(B)** miR-101 expression is increased by rapamycin and etoposide treatment. Top: miR-101 qpcr after 16 h treatment with 200 nM rapamycin, 50 μM etoposide or DMSO

control. Data are normalized relative to housekeeping *RNU6B* and DMSO sample and error bars represent ±S.D. of three replicates. Bottom: Western blot showing p-S6K and total S6K levels after indicated treatments.

Supplementary Figure S3: miR-101 inhibits autophagy in other cell lines

miR-101 overexpression leads to p62 accumulation. Western blot analysis in T47D, HEK and HeLa cells 72 h after transfection with miR-101 or scramble control. p62 bands were quantified relative to the GAPDH/Vinculin loading control using ImageJ software and the relative quantifications are shown.

Supplementary Figure S4: Effect of LNA 101 on MCF-7 cell growth

Cystal violet assay measuring cell density at 1, 2 and 3 days after transfection with LNA SC or LNA2 101, showing that knock-down of endogenous miR-101 does not affect MCF-7 cell growth ($p>0.61$ for all three timepoints).

Supplementary Figure S5: Knockdown efficiency of siRNAs

(A,B) Knockdown efficiency of indicated genes measured by qpcr. Data shown is the mean ±S.D. from three replicates normalized relative to the housekeeping gene and to the scramble control.

Supplementary Figure S6: miR-101 inhibits siRaptor-induced eGFP-LC3 translocation

MCF-7 eGFP-LC3 cells were transfected with indicated miRNAs and/or siRNAs and fixed 72 h post-transfection. Percentage of eGFP-LC3 puncta-positive cells was quantified using a threshold of >5 dots per cell. Data is shown as the mean ±S.D. of 5 replicates and is representative of 3 independent experiments.

Supplementary Figure S7: Transmission electron microscopy - multiple image alignment

(A-C) Multiple image alignment of 24,500X images showing the same representative cells as shown in Figure 7. This was done for 25-30 cells per sample and allowed for accurate identification and counting of autophagosomes per cellular cross-section (denoted by white arrowheads).

Supplementary Figure S8: miR-101 collaborates with 4-OHT in T47D cells

Combined miR-101 and 4-OHT reduces cell viability. The experiment was performed essentially as described for MCF-7 cells in Figure 9C, instead using 2 μM 4-OHT. The data is normalized to the Etoh control and shows the mean ±S.D. of 3 replicates, representative of 3 independent experiments.

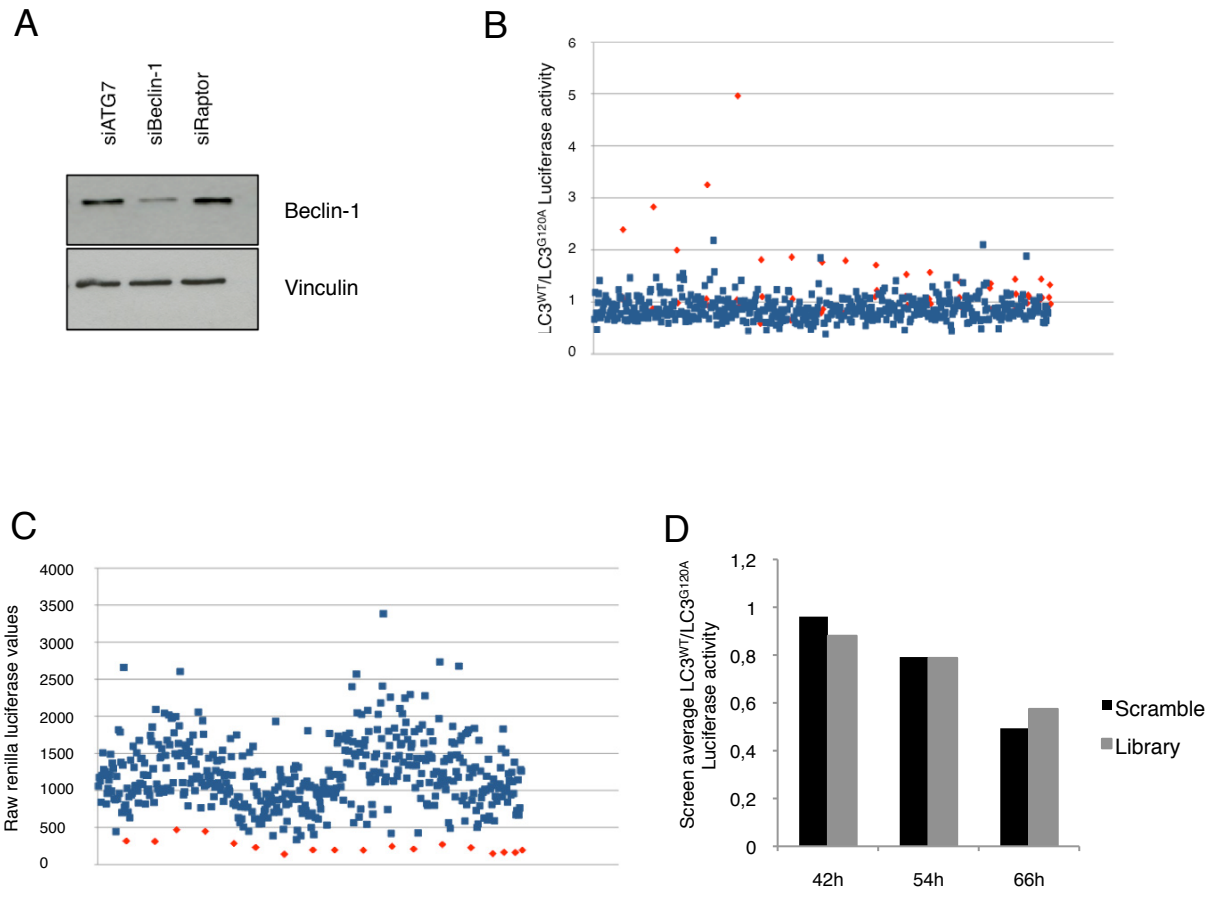
Supplementary Table I:

List of differentially expressed genes upon miR-101 overexpression. See Materials and Methods for details.

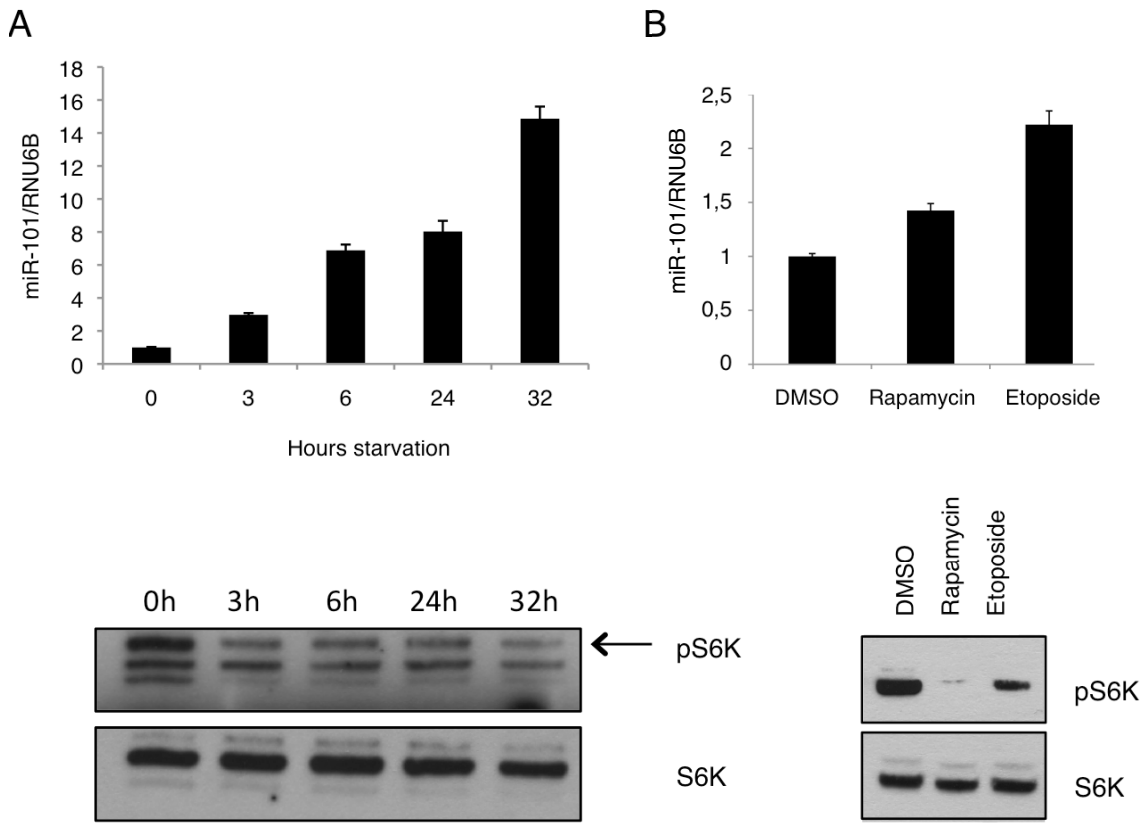
Supplementary Table II:

Gene Ontology analysis of genes downregulated upon miR-101 overexpression. The table shows the 25 most significantly enriched GO terms in the molecular function category.

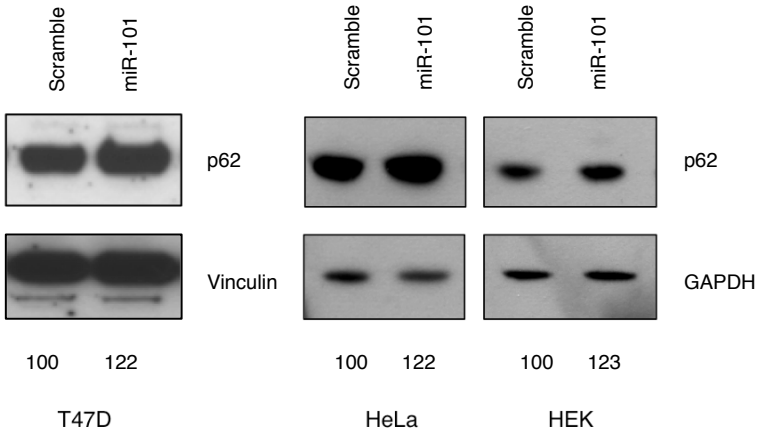
Frankel et al. Supplementary Figure S1



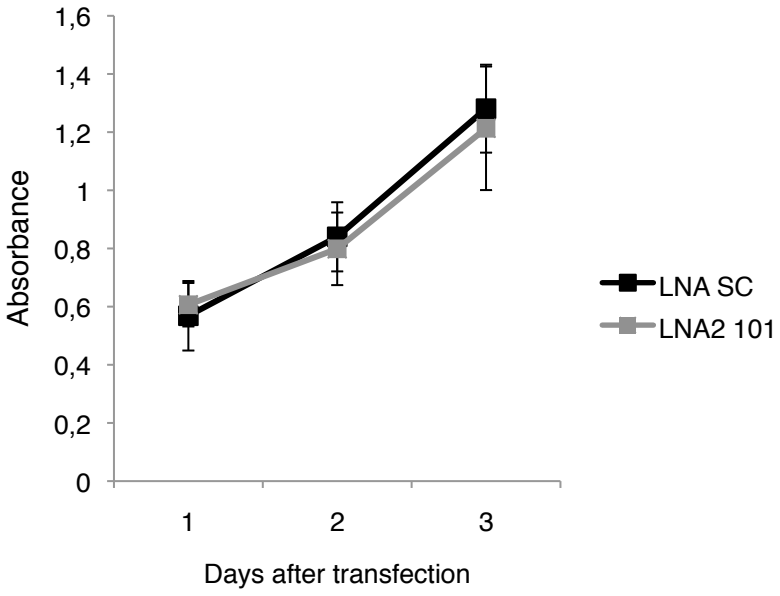
Frankel et al. Supplementary Figure S2



Frankel et al. Supplementary Figure S3

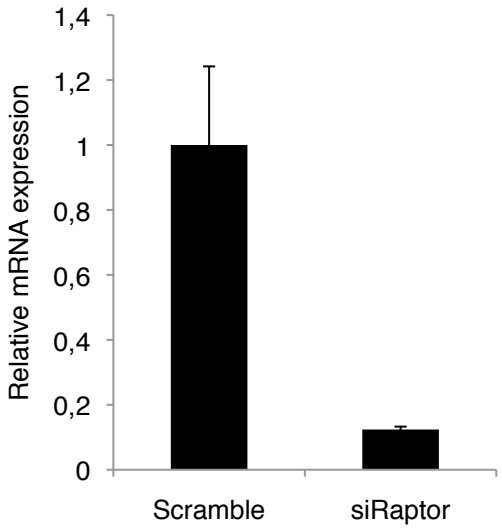


Frankel et al. Supplementary Figure S4



Frankel et al. Supplementary Figure S5

A



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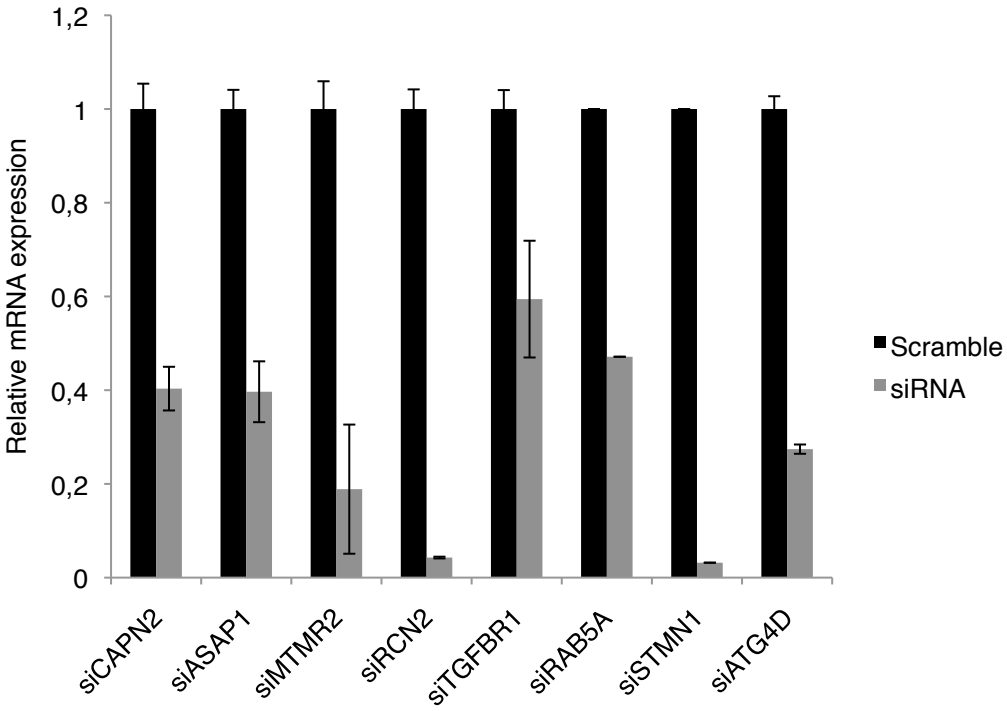
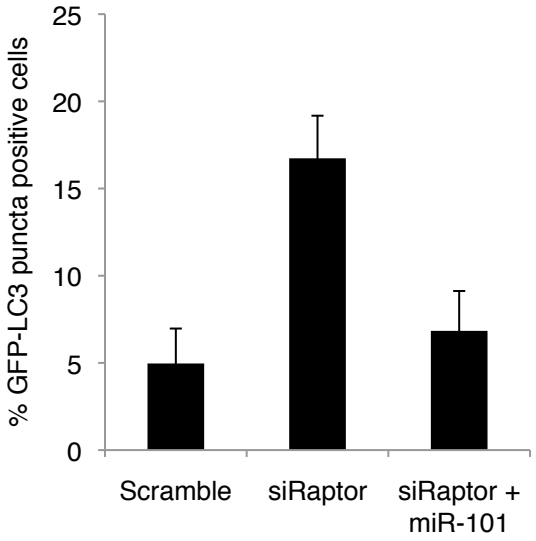
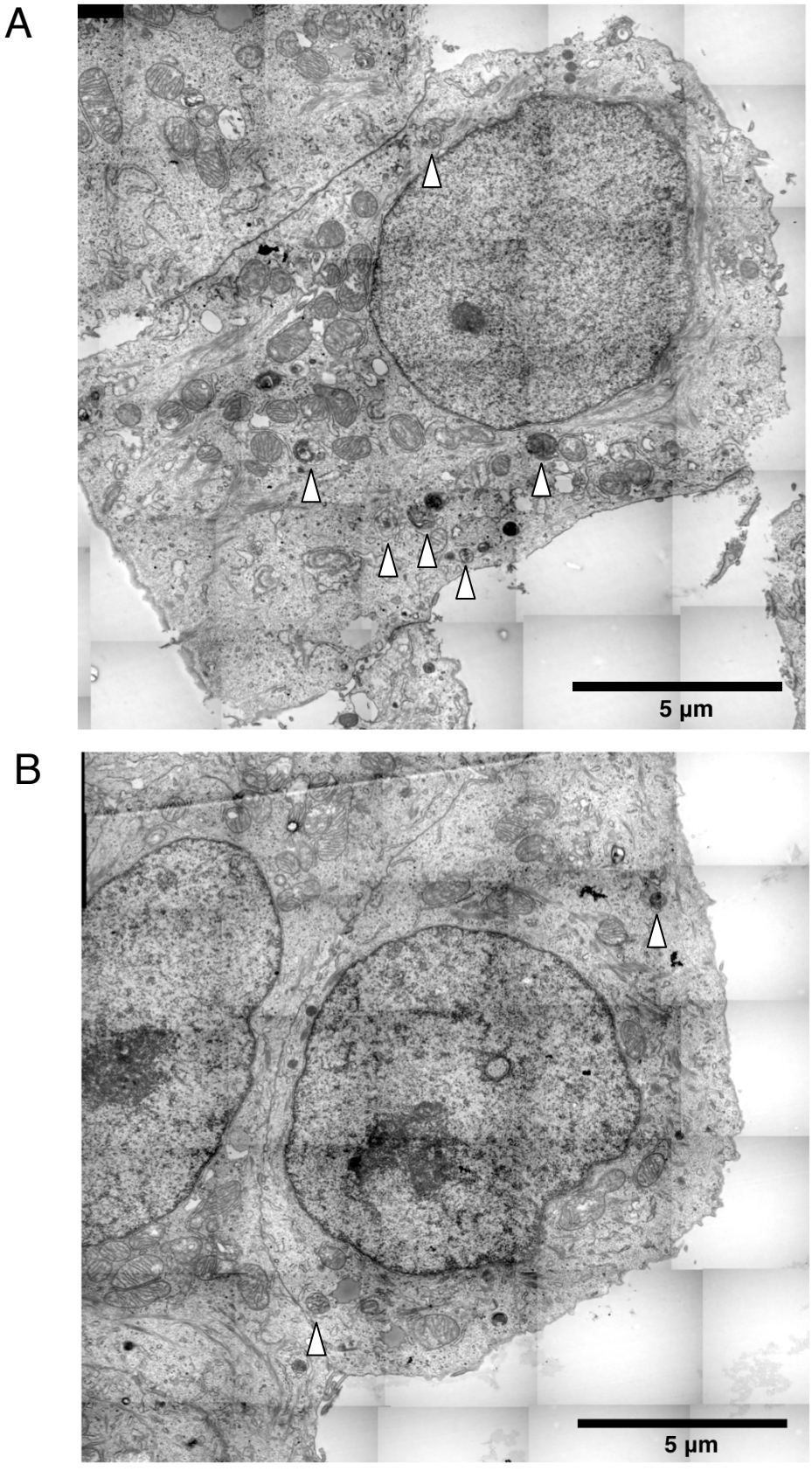


Frankel et al. Supplementary Figure S6

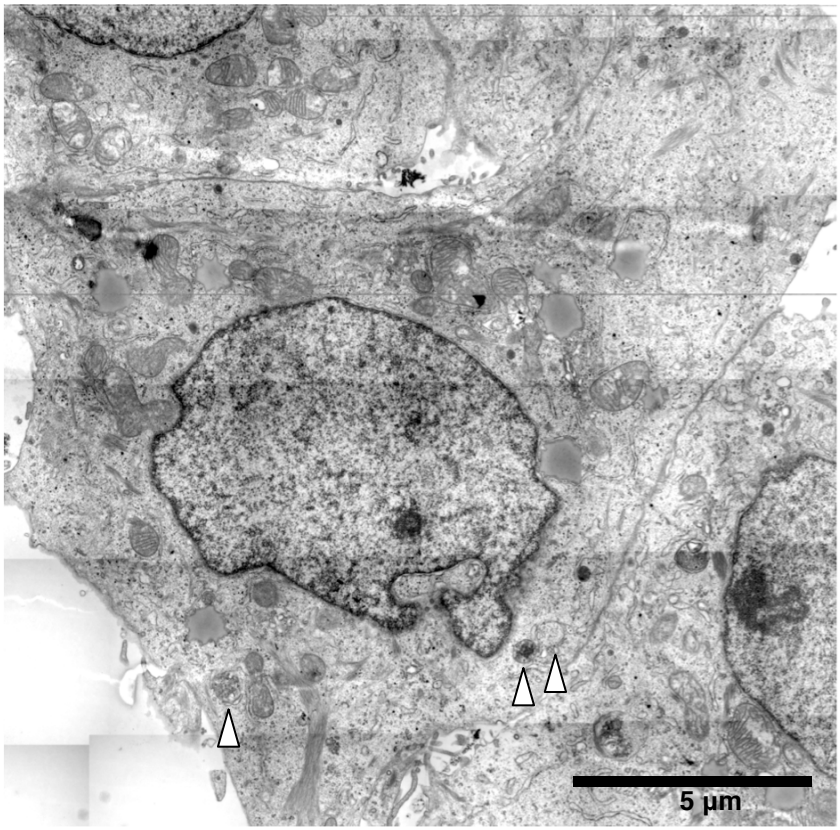


Frankel et al. Supplementary Figure S7



Frankel et al. Supplementary Figure S7

C



Frankel et al. Supplementary Figure S8