Expanded View Figures

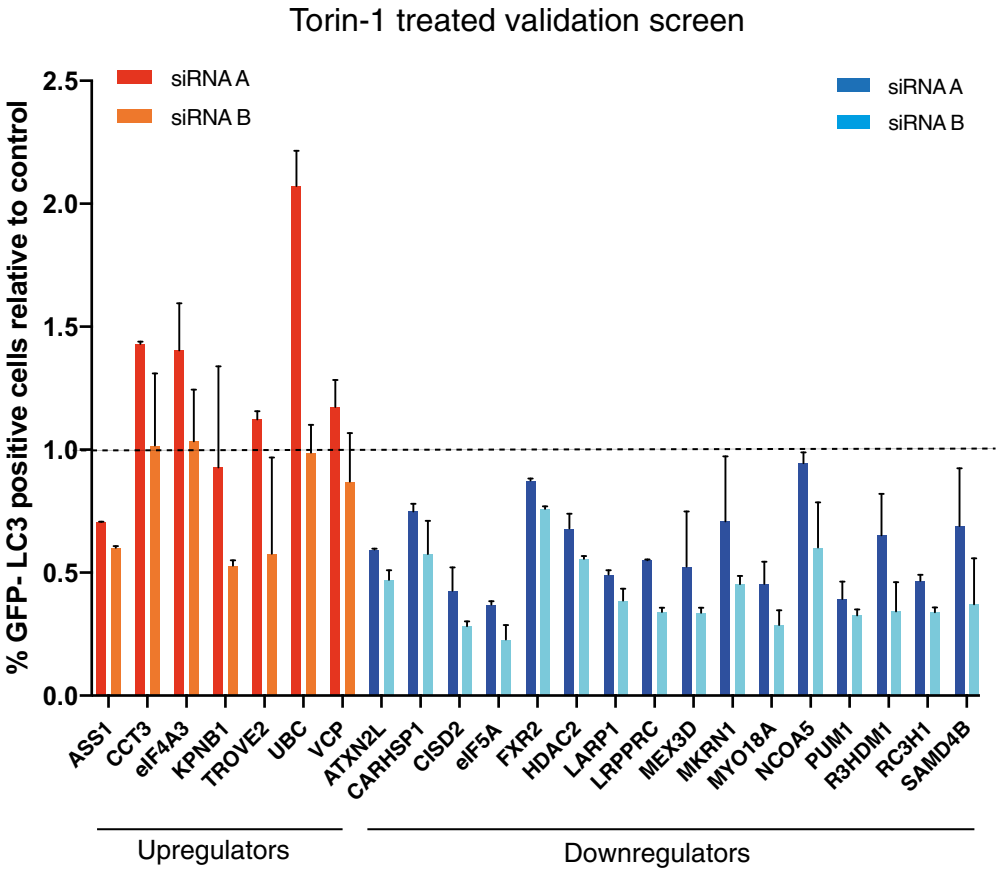

Figure EV1. Secondary validation screen upon Torin-1 induction of autophagy.

Secondary validation screen performed as in Fig 1C, but treated for 2 h with Torin-1 prior to fixation. Data shown are the percentage of GFP-LC3B puncta-positive cells relative to the scramble siRNA control (indicated by dashed line) and represent the mean + SD from one representative experiment (n = 2).

Figure EV2. eIF5A depletion effects on specific autophagy markers.

A Western blotting analysis of p-mTOR (Ser 2448) in MCF-7 GFP-LC3B cells after indicated treatments (2 h) and siRNA transfections (72 h). A representative experiment is shown (n = 2).

B Western blot of MCF-7 GFP-LC3B cells after 72-h transfection with indicated siRNAs. A representative experiment is shown (n = 3).

C qRT–PCR of LC3B, GABARAP and GATE-16 mRNA levels in MCF-7 GFP-LC3B cells of indicated transcripts after 72-h transfection with indicated siRNAs. Data represent the mean + SD (n = 3).

D Western blots of LC3B in indicated cells lines and siRNA transfections (72 h). A representative experiment is shown (n = 3).

E Western blot in ATG5 WT or ATG5 KO HeLa cells with indicated siRNAs transfections for 72 h. A representative experiment is shown (n = 3).

F Western blot of MCF-7 cells with indicated siRNAs for 72 h. Bafilomycin treatment for the last 2 h prior to harvest. A representative experiment is shown (n = 3).

G Mature autophagosomes identified from TEM images were scored as having diameter of > 300 or < 300 nm, and their percentage-wise distribution in cntrl or eIF5A siRNA-treated cells is shown (n = 2).

H MCF-7 cells were transfected for 72 h as indicated, fixed and immunostained for ATG16L1. Cells containing more that 5 puncta were defined as ATG16L1 puncta-positive cells. Data represent the mean + SD (n = 3). Student's t-test: $^{**}P < 0.01$.

Source data are available online for this figure.

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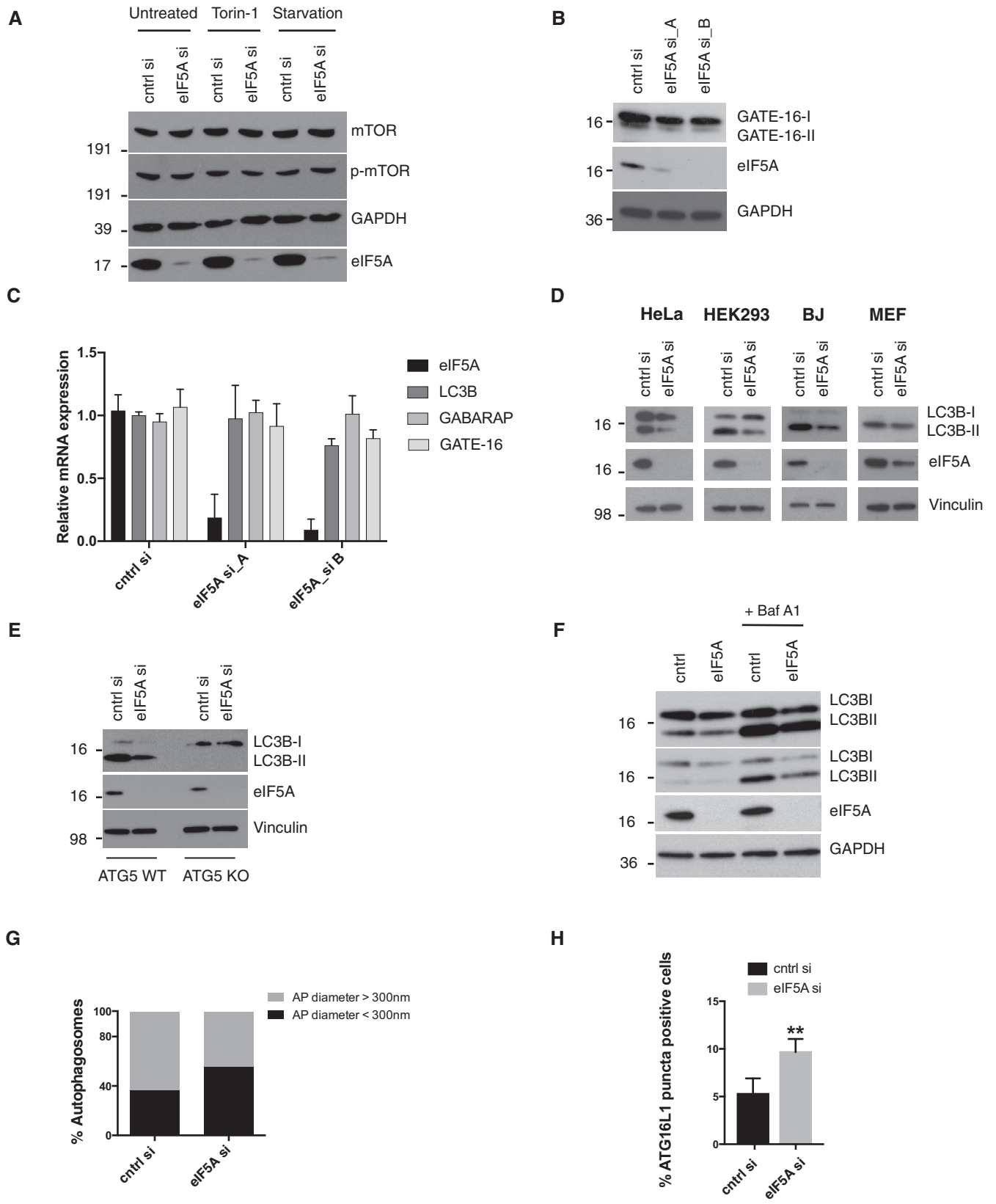
A
Untreated
Torin-1
Starvation
cntrl si
eIF5A si
mTOR
p-mTOR
GAPDH
eIF5A
191
191
39
17
B
cntrl si
eIF5A si_A
eIF5A si_B
GATE-16-I
GATE-16-II
eIF5A
GAPDH
16
16
36
C
Relative mRNA expression
eIF5A
LC3B
GABARAP
GATE-16
cntrl si
eIF5A si_A
eIF5A_si B
D
HeLa
HEK293
BJ
MEF
LC3B-I
LC3B-II
eIF5A
Vinculin
E
LC3B-I
LC3B-II
eIF5A
Vinculin
ATG5 WT
ATG5 KO
F
+ Baf A1
cntrl
eIF5A
LC3BI
LC3BII
eIF5A
GAPDH
G
% Autophagosomes
AP diameter > 300nm
AP diameter < 300nm
H
% ATG16L1 puncta positive cells
cntrl si
eIF5A si
**

Figure EV2.

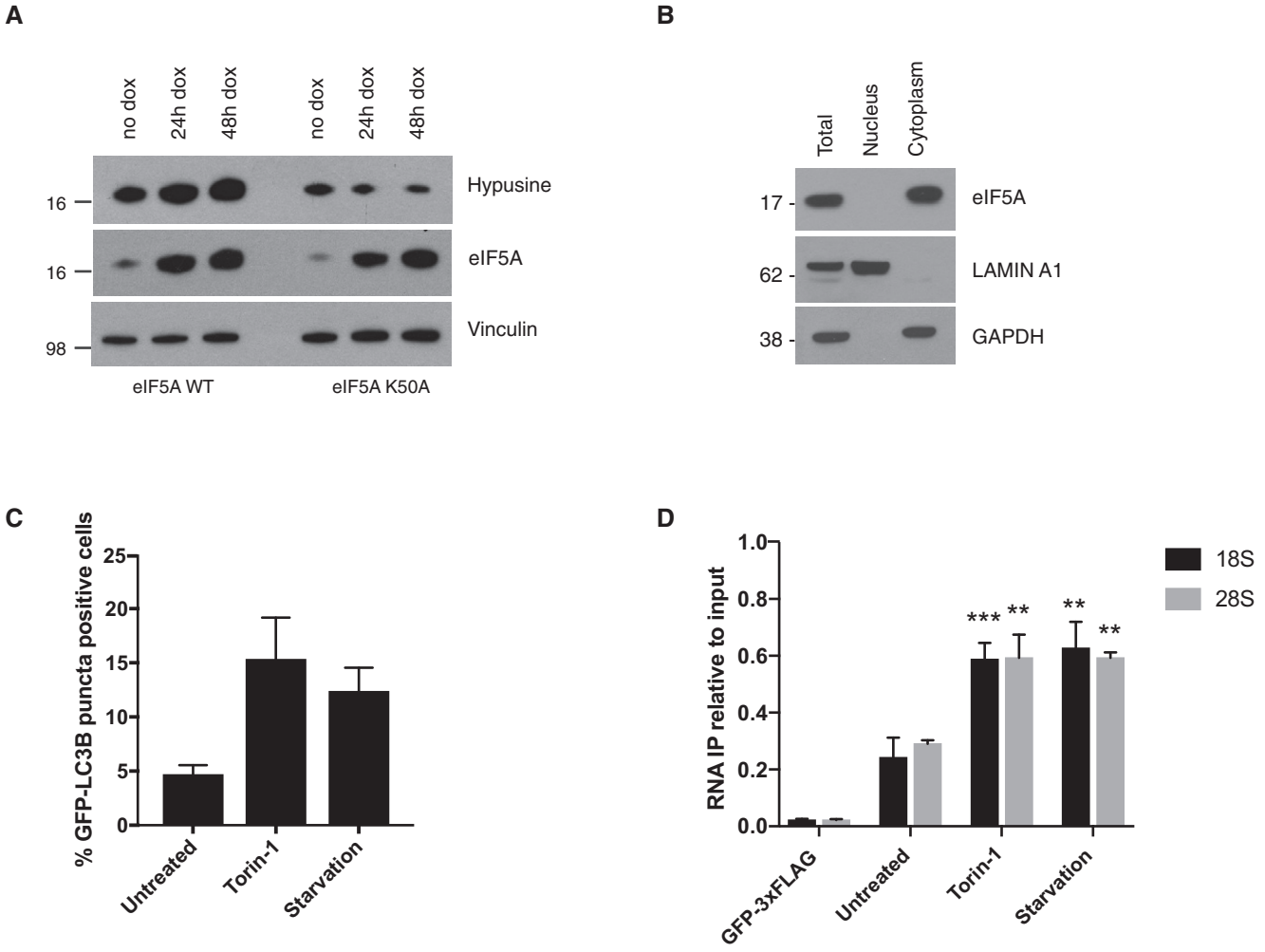

Figure EV3. eIF5A hypusination, localization and ribosome binding.

A Western blotting analysis of eIF5A and hypusine levels in cell lines expressing WT and K50A eIF5A at indicated time points after doxycycline treatment. A representative experiment is shown (n = 2).

B Nucleus/cytoplasmic fractionations were performed in MCF-7 cells and subsequently analysed by Western blotting for eIF5A. Lamin A1 and GAPDH were used as nuclear and cytoplasmatic markers, respectively. A representative experiment is shown (n = 3).

C Quantification of GFP-LC3B puncta after indicated treatments (2 h) in MCF-7 GFP-LC3B eIF5A WT inducible cells (used for ribosome purifications in Fig 3E). Data shown are mean + SD (n = 2).

D RNA inputs and eluates obtained from co-purification of GFP-eIF5A were analysed by qRT–PCR for 18S and 28S rRNA. A cell line expressing GFP-3xFLAG was used as a negative control. Data are shown as eluates (RNA IP) relative to input and represent the mean + SEM (n = 4). Student's t-test comparing treatments to untreated: $^{**}P < 0.01$, $^{***}P < 0.001$.

Source data are available online for this figure.

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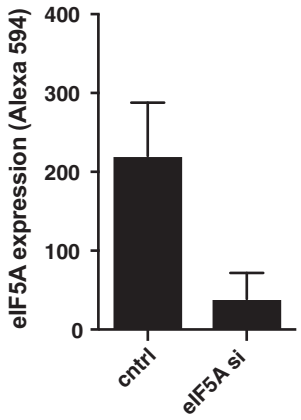

Figure EV4. eIF5A depletion from OPP assay.

Quantification of eIF5A knockdown in MCF-7 cells from Fig 4A by immunostaining using Alexa 594-coupled secondary antibody. Data are mean + SEM from four independent experiments.

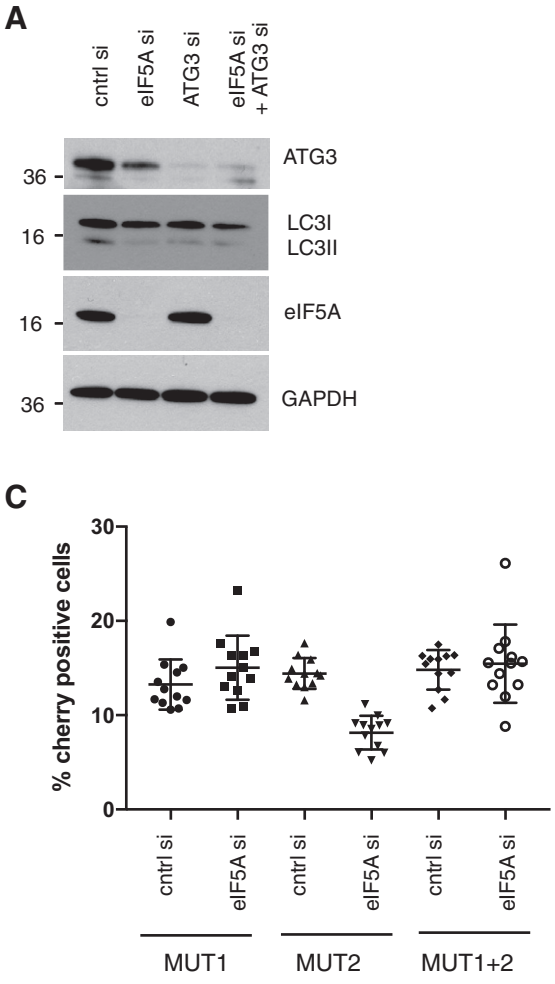

Figure EV5. eIF5A affects autophagy via ATG3.

A Western blotting analysis of LC3B and ATG3 protein levels after 72-h transfection with indicated siRNAs. Representative experiment is shown (n = 3).

B Western blotting analysis of effects of p62 depletion on LC3B lipidation in MCF-7 cells after 72-h transfection with indicated siRNAs. Representative experiment is shown (n = 2).

C Analysis of eIF5A effect on single or combined mutations of ATG3-mCherry constructs assessed by imaging and automated quantification of mCherry fluorescent signal. HEK 293 cells were transfected with indicated siRNAs for 72 h. For the last 48 h, cells were transfected with indicated ATG3-mCherry constructs. Data show quantification of mean mCherry fluorescence + SD from one representative experiment (n = 2).

Source data are available online for this figure.

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