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Supplemental information

Subtractive CRISPR screen identifies the ATG16L1/vacuolar ATPase axis as required for non-canonical LC3 lipidation

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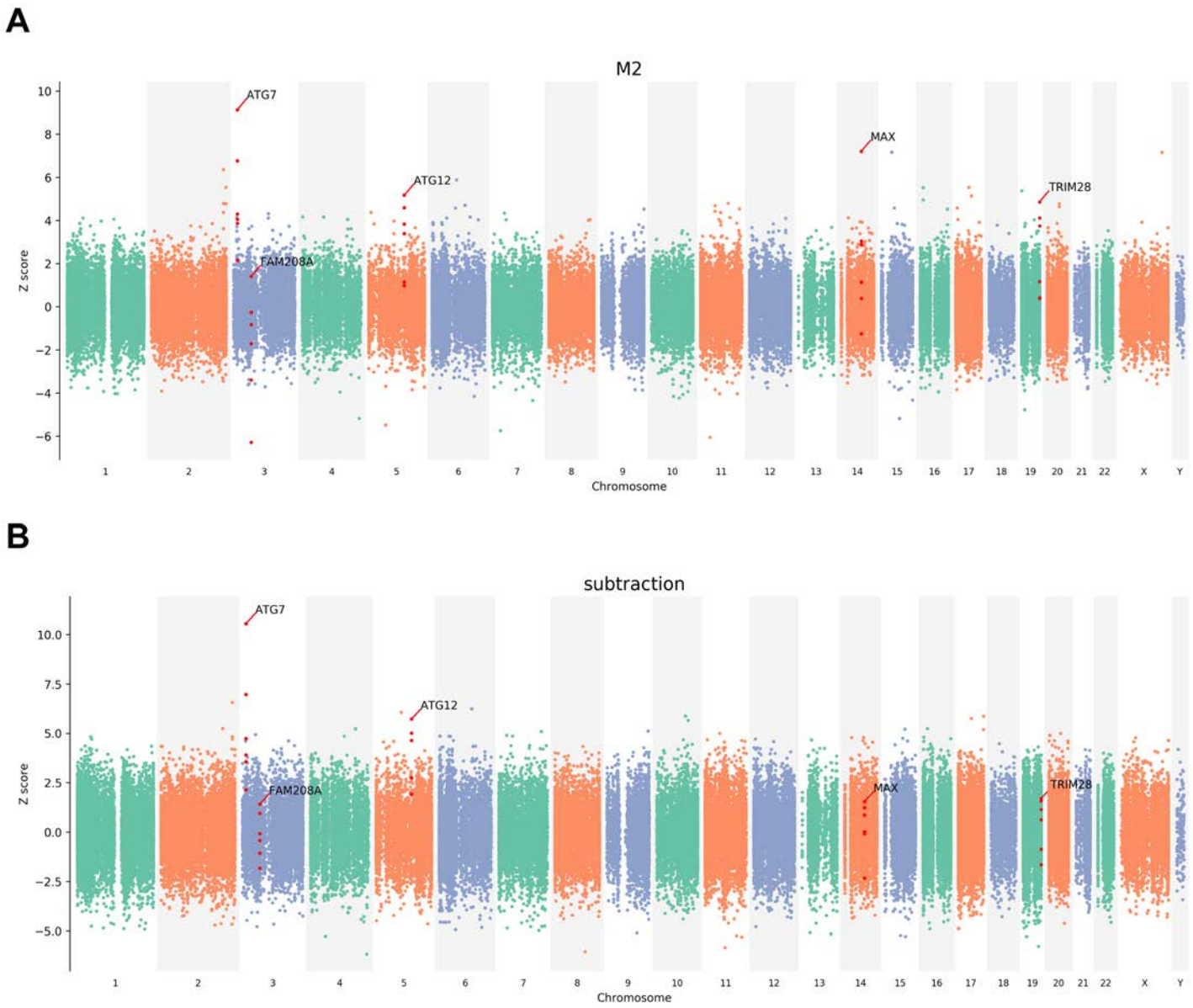

Figure S1, related to figure 1: Spatial distribution on the genome of the z-score of sgRNAs the A) permeabilised FACS sort of EGFP-LC3B levels and B) the subtractive analysis of permeabilised and unpermeabilised FACS sorts.

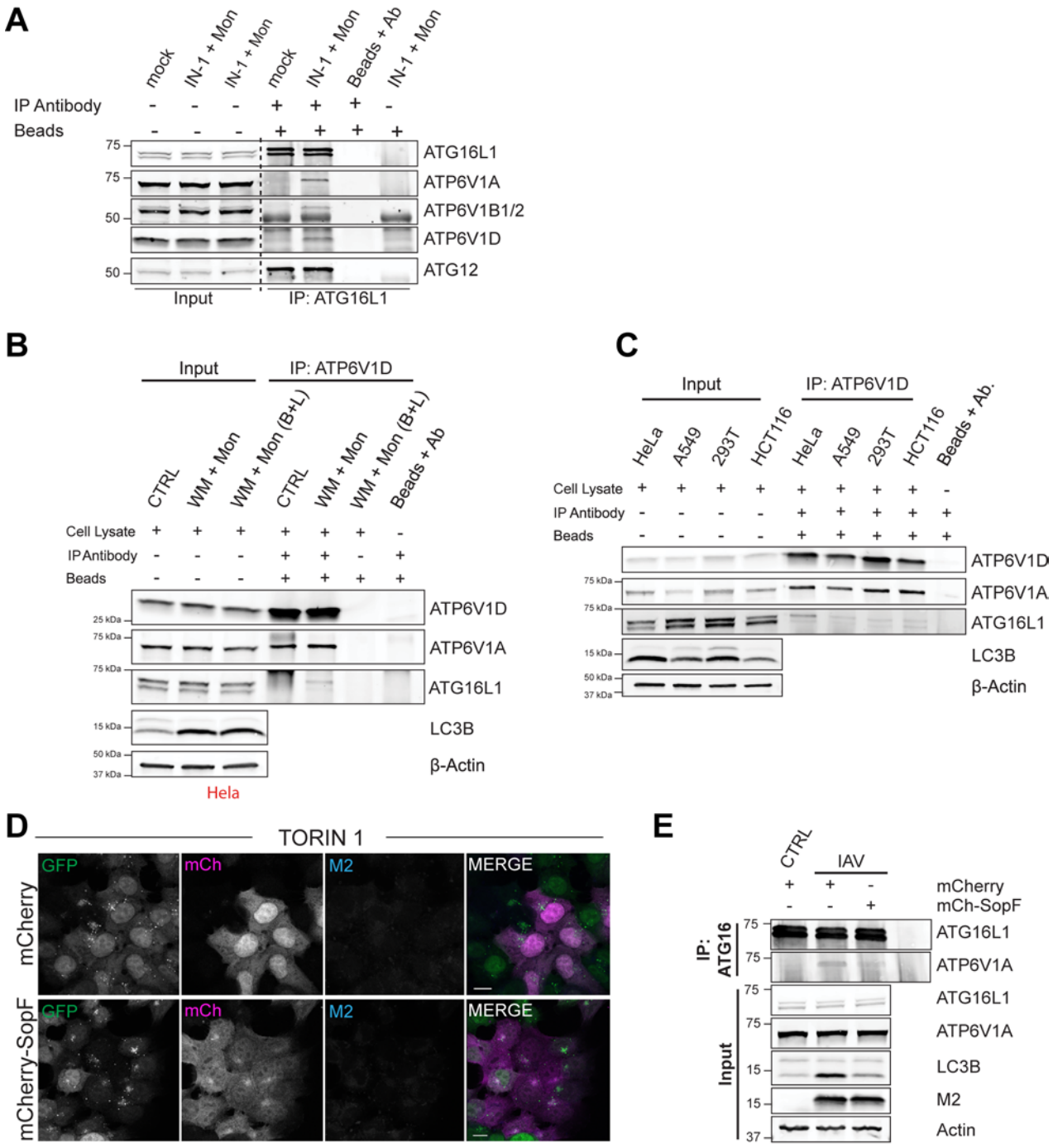

Figure S2, related to figure 2: A) Immunoprecipitation analysis of endogenous ATG16L1 in HeLa cells treated with VPS34 IN-1 (pretreatment: 1 μM for 30 minutes) followed by monensin (100 μM for 1 h). B) Immunoprecipitation analysis of endogenous of ATP6V1D in HeLa cells treated with wortmannin (IN-1 substitute; pretreatment: 100 nM for 30 minutes) followed by monensin (100 μM for 1h). C) Immunoprecipitation analysis of endogenous of ATP6V1D in the indicated cell lines following treatment with VPS34 IN-1 (pretreatment: 1 μM for 30 minutes) followed by monensin (100 μM for 1h). D) IF analysis of EGFP-LC3B relocalisation in HCT116 EGFP-LC3B TetON-M2 cells stably expressing mCherry or mCherry-SopF after treatment with Torin 1 (250 nM for 3 h). scale bar 10 μm F) Pull down analysis of ATG16L1 interaction with the vATPase in HCT116 EGFP-LC3B cells stably expressing mCherry or mCherry-SopF after infection with PR8 for 16 h.

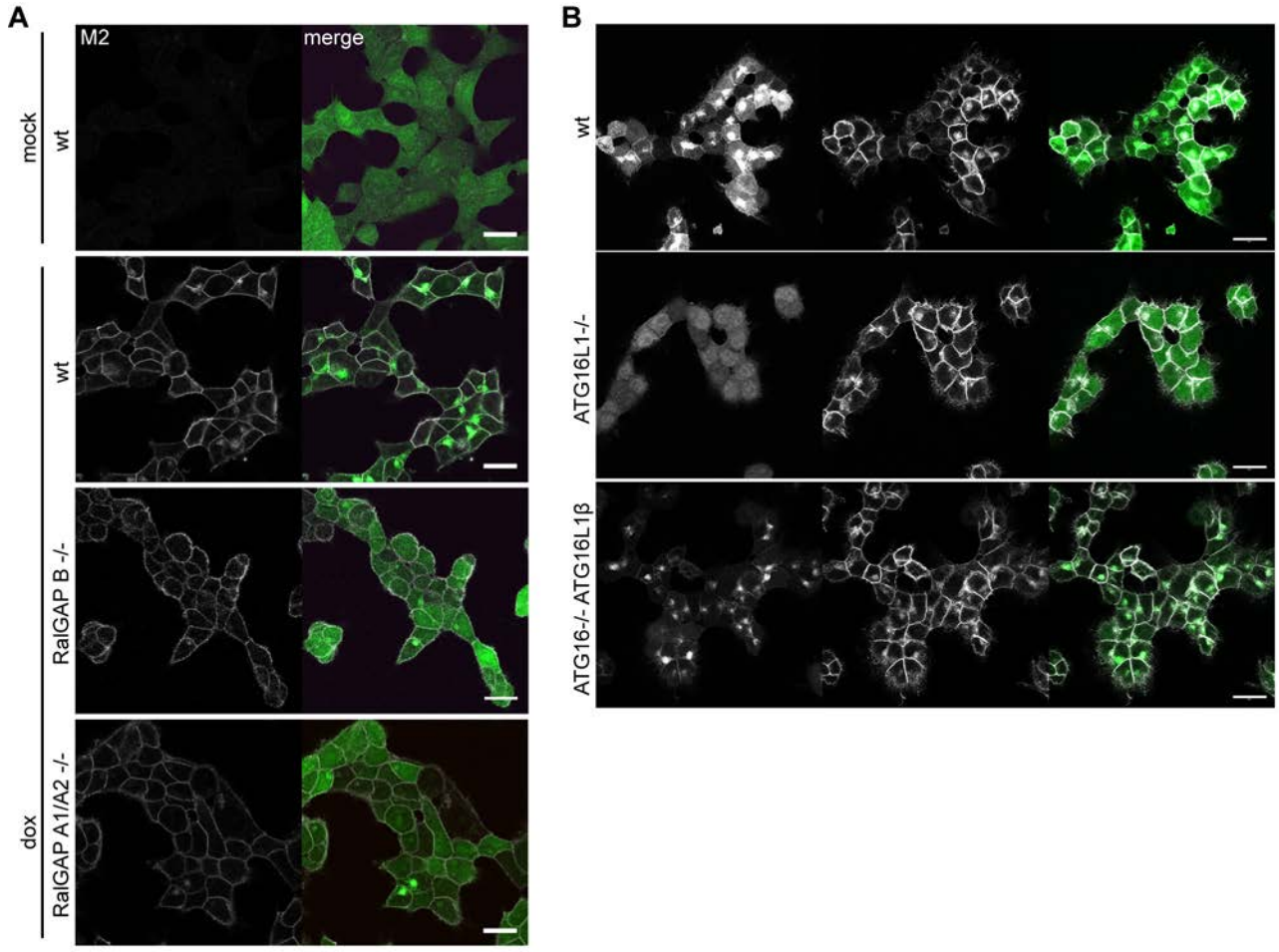

Figure S3, related to figure 3: A) IF analysis of M2 localisation in HCT116 EGFP-LC3B TetON-M2 wt or RalGAPA1A2-/- or RalGAPB -/- cells. M2 expression was induced with 3 µg/ml dox for 16 h followed by staining for M2. Scale bar 10 µm. B) IF analysis of M2 localisation in HCT116 EGFP-LC3B TetON-M2 wt, ATG16L1-/- or ATG16L1-/- reconstituted with hATG16L1β cells treated as in A. Scale bar 10 µm.

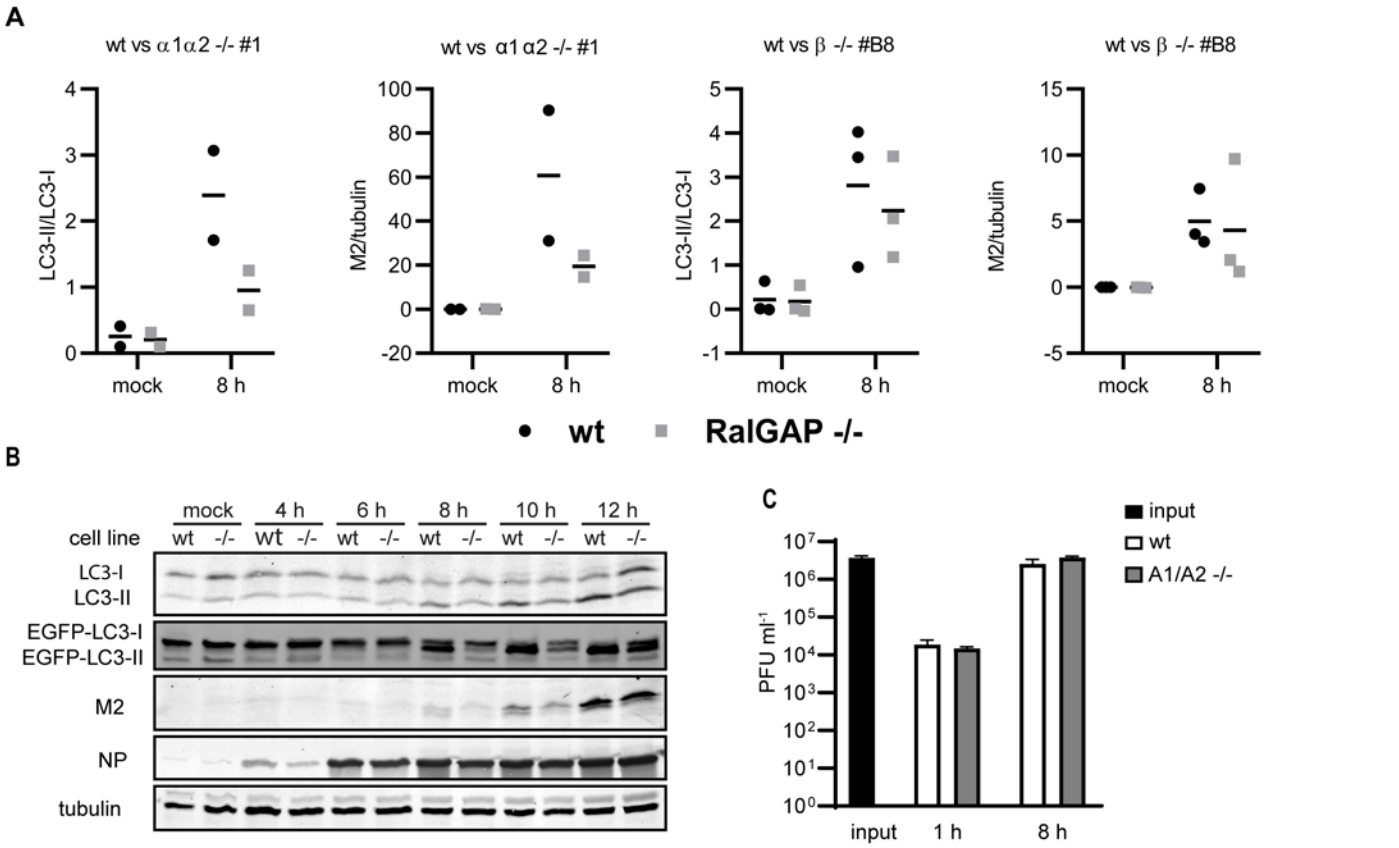

Figure S4, related to figure 3: A) Quantification of LC3-lipidation analysis by western blot of HCT116 EGFP-LC3B TetON-M2 cells depleted for RalGAPA1A2 (panel 1 and 2) or RalGAPB (clone B8) after infection with PR8 at an MOI of 10 PFU per cell for 8 h. Graphs show mean of LC3II/LC3I ratio (panels 1 and 3) and M2 expression normalised to tubulin (panels 2 and 4) from two or three independent experiments. B) LC3-lipidation analysis of HCT116 EGFP-LC3B TetON-M2 wt and cells depleted for RalGAPA1A2 infected with MUd at an MOI of 10 PFU per cell and lysed at the indicated time point p.i.. C) HCT116 EGFP-LC3B TetON-M2 wt or cells depleted for RalGAPA1A2 were infected at an MOI of 10 PFU per cell. Supernatants were harvested at 1 or 8 h p.i. and titres determined by plaque assay.

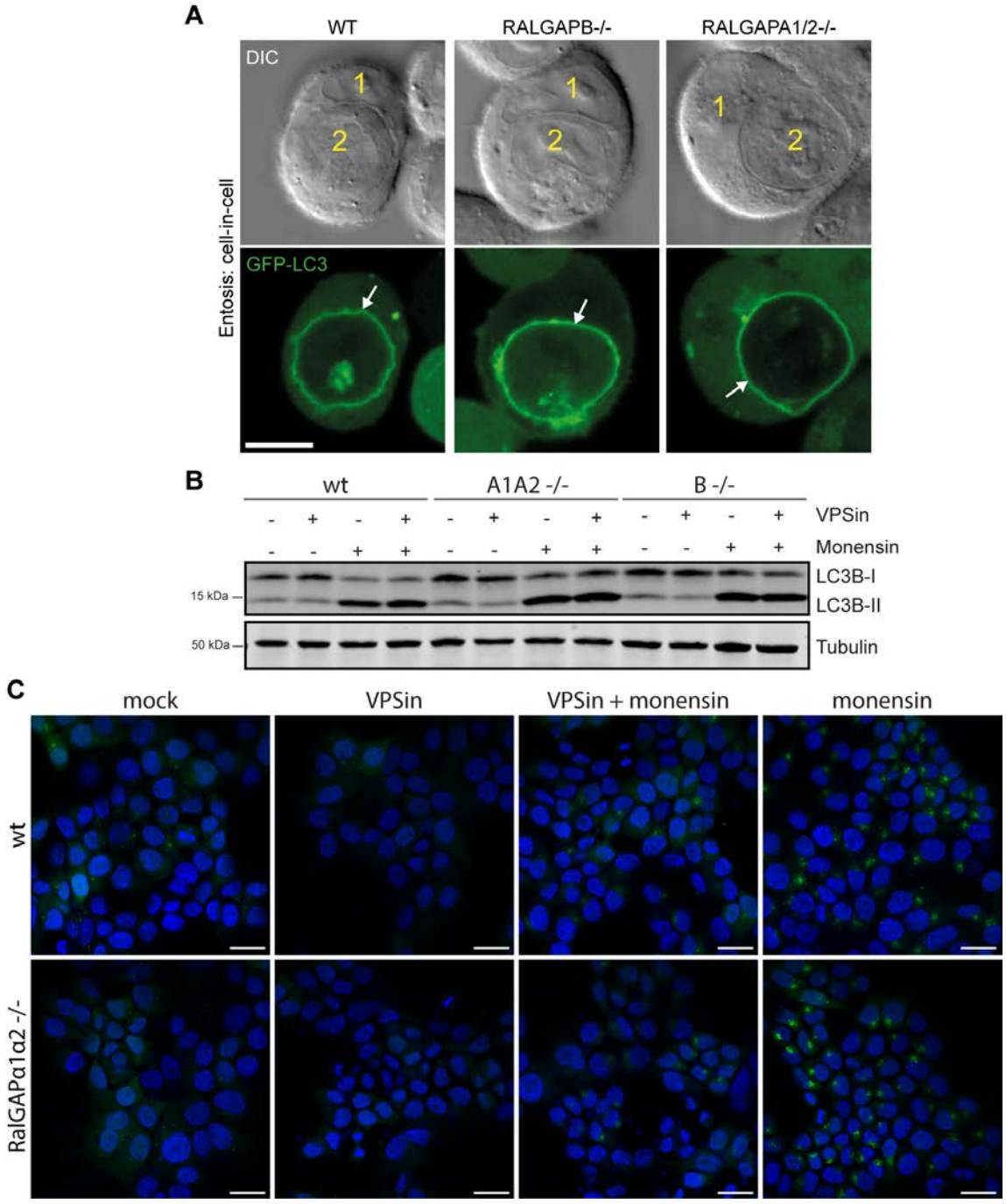

Figure S5, related to figure 3: A) EGFP-LC3B recruitment to entotic vesicles in HCT116 EFGP-LC3B TetON M2 wt, RalGAPα1α2-/- and RalGAPβ-/- cells analysed by fluorescence (top panel) and bright field (bottom panel) live cell microscopy. Scale bar 10 μM. B) LC3 lipidation analysis of HCT116 EFGP-LC3B TetON M2 wt and RalGAPα1α2-/- cells after 30 min treatment with 10 μM monensin. Where indicated cells were pretreated with 1 μM VPSin for 20 min prior to addition of 10 μM monensin to inhibit canonical LC3-lipidation. C) Representative images of IF analysis of HCT116 EFGP-LC3B TetON M2 wt and RalGAPα1α2-/- treated as in B. Scale bar 20 μm.

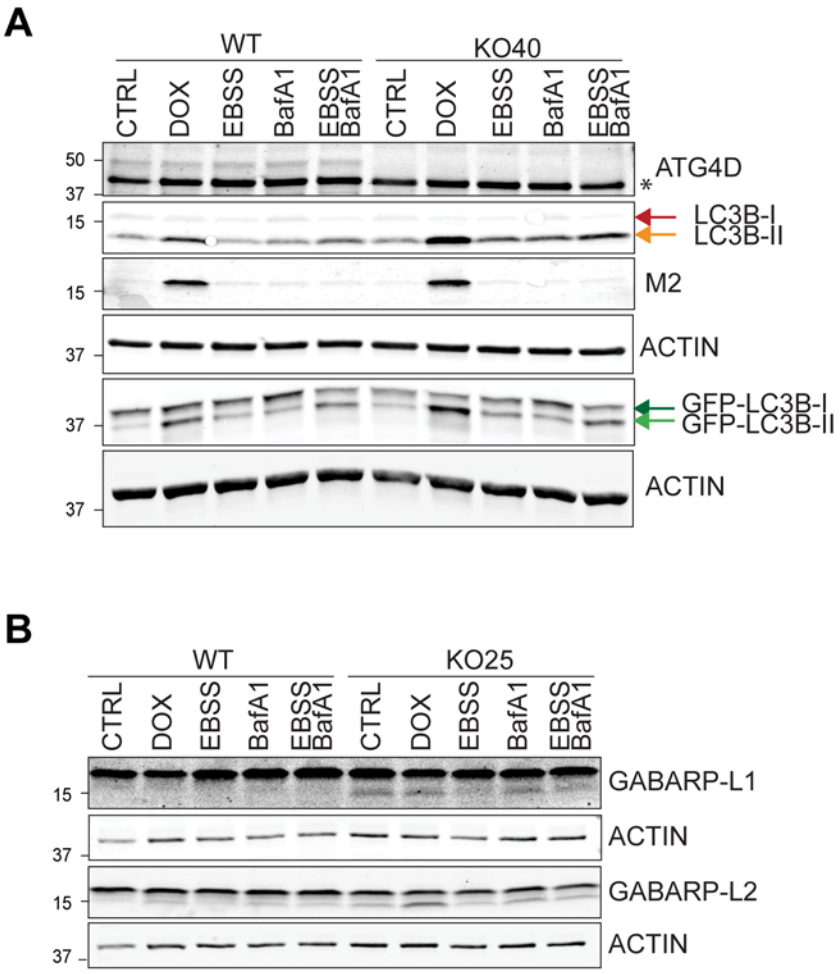

Figure S6, related to figure 4: A) HCT116 EGFP-LC3 TetOn-M2 WT and ATG4D KO clone 40 were treated with 10 µg/ml dox for 8 h, 2 hr with EBSS, 1 hr with 200 nM Bafilomycin A1 and a combination of EBSS together with Bafilomycin A1. arrowhead indicates ATG4D specific band, * background band. B) HCT116 EGFP-LC3 TetOn-M2 WT and ATG4D -/- clone 25 were treated as in B and GABARAP-L1 and GABARAP-L2-lipidation analysed by western blot.