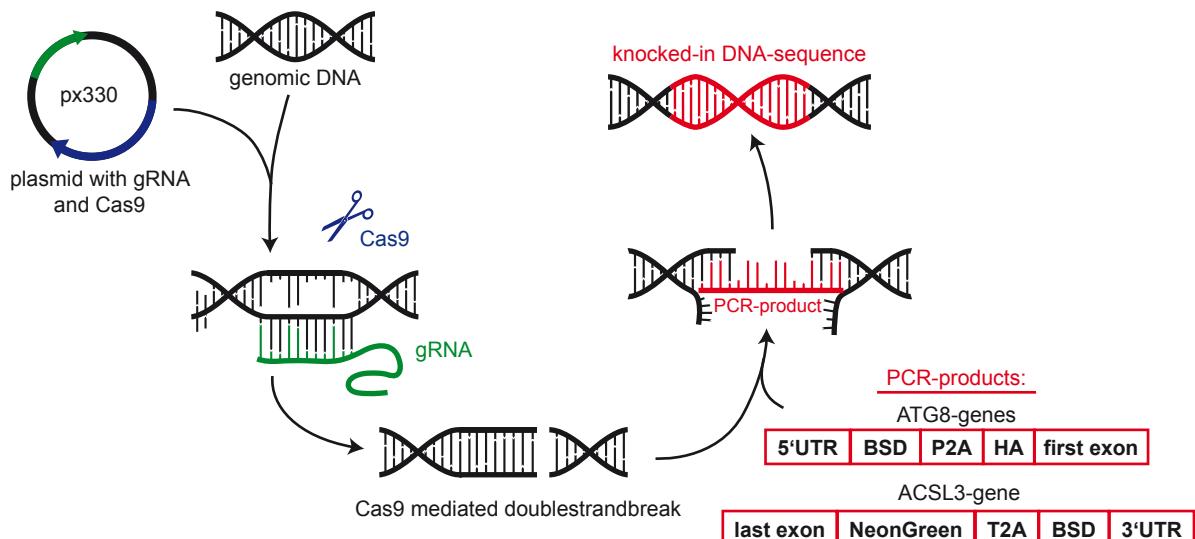


A



B

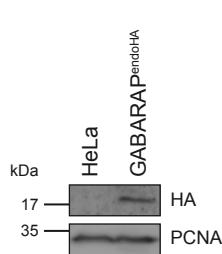
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C

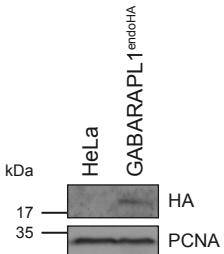
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CGGGATGTCGCCCTTCCAGGCCATGGTAGATGGTCCGGATACCAAGTCCATCGCACAATGCAAGTTGAAGATGGTCC

**Fig. S1. Endogenous epitope tagging of hATG8 and ACSL3 genes.** (A) Experimental CRISPR/Cas9 workflow. (B,C) Sequence data from PCR products of the tagged GABARAP<sub>endoHA</sub>, GABARAPL1<sub>endoHA</sub>, GABARAPL2<sub>endoHA</sub>, LC3B<sub>endoHA</sub> cell lines (B) and the GABARAPL2<sub>endoHA</sub>/ACSL3<sub>endoNeonGreen</sub> cell line (C). Introduced CRISPR sequences are indicated in bold.

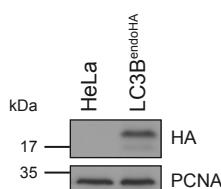
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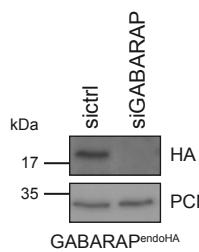
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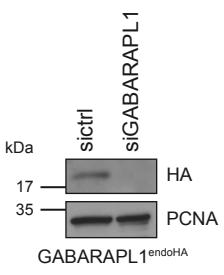
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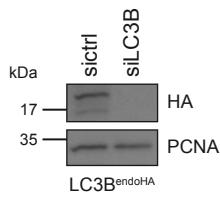
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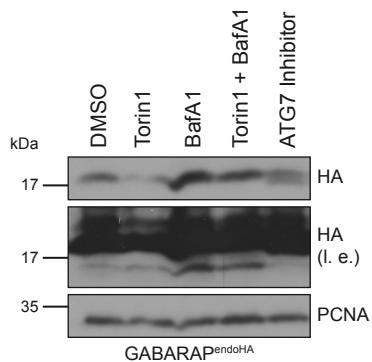
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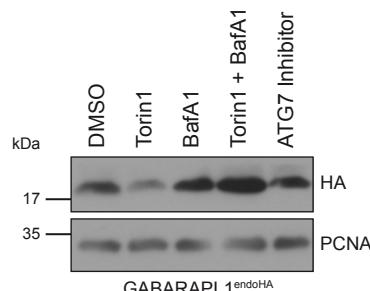
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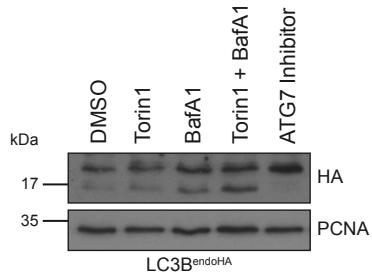
G



H



I



J

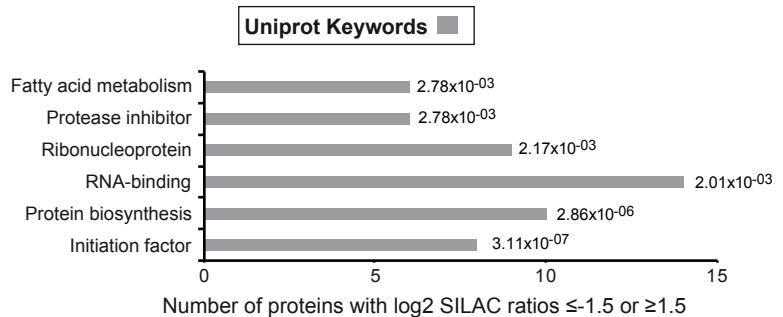
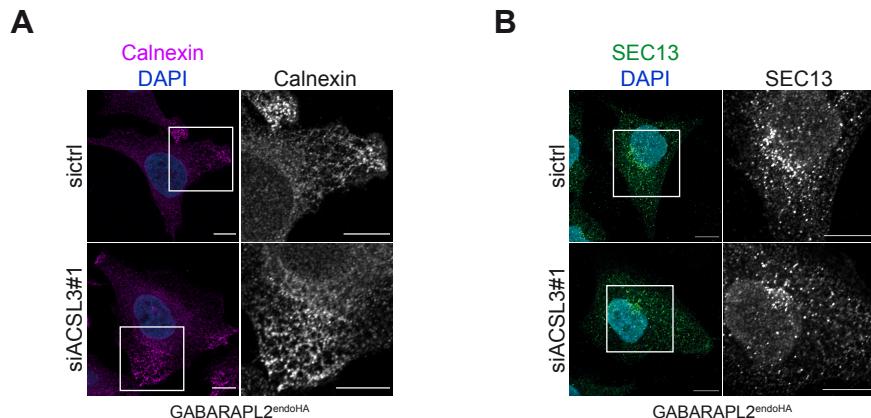


Figure S2

**Fig. S2. Validation of endogenously HA-tagged hATG8 proteins.** (A-C) GABARAP<sub>endoHA</sub> (A) GABARAPL1<sub>endoHA</sub> (B) LC3B<sub>endoHA</sub> (C) and parental HeLa (A-C) cells were lysed followed by immunoblotting and analysis with indicated antibodies. (D-F) GABARAP<sub>endoHA</sub> (D), GABARAPL1<sub>endoHA</sub> (E), LC3B<sub>endoHA</sub> (F) cell lines were reversely transfected with indicated siRNAs prior to immunoblot analysis. (G-I) GABARAP<sub>endoHA</sub> (G), GABARAPL1<sub>endoHA</sub> (H), LC3B<sub>endoHA</sub> (I) were treated as indicated followed by lysis and immunoblotting. (J) Annotation enrichment analysis of candidate GABARAPL2-interacting proteins with log<sub>2</sub> SILAC H/L ratios  $\geq 1.5$  or  $\leq -1.5$ . The bar graphs show significantly overrepresented UniProt keywords.



**Figure S3**

**Fig. S3. ACSL3 is not an autophagy substrate.** (A,B) GABARAPL2<sub>endoHA</sub>/ACSL3<sub>endoNeonGreen</sub> cells were transfected with indicated siRNAs prior to immunolabeling with Calnexin (A) or SEC13 (B). Scale bar: 10 μm.

**A**



■ potential LIR ■ potential UIM

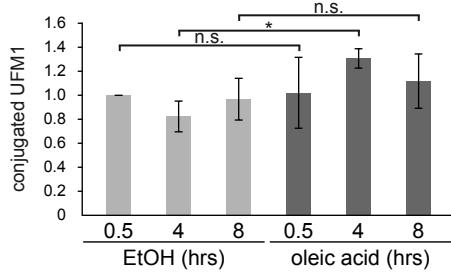
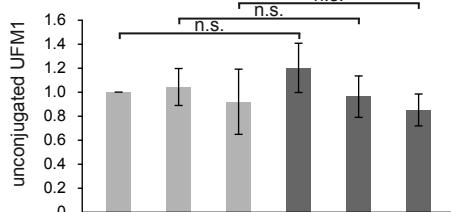
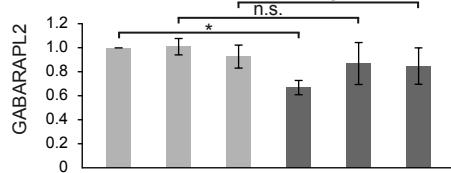
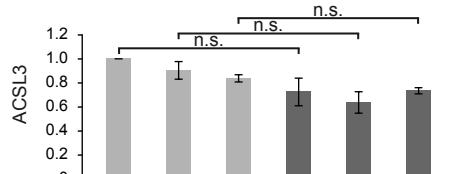
LIR-2 (135-140): LSYEDV

LIR-3 (589-594): GEYVSL

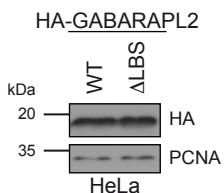
LIR-4 (643-648): GTWEEL

UIM-A (72-81): SLDGLASLV

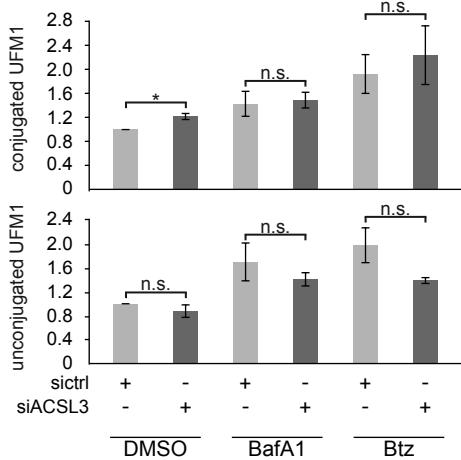
**D**



**B**



**C**



**Figure S4**

**Fig. S4. Effects of ACSL3 depletion and LD induction on ufmylation.** (A) Amino acid sequences of potential LIRs and UIM in ACSL3. (B) Immunoblot analysis of HeLa cells stably expressing wild-type (WT) and LIR-binding deficient ( $\Delta$ LBS) GABARAPL2. (C) Quantitative analysis from Fig. 7A. Data represents mean  $\pm$ SEM. Statistical analysis ( $n = 3$ ) of the indicated protein/PCNA ratio normalized to sctrl-DMSO was performed using Student's t-test (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). (D) Quantitative analysis of Fig. 7C. Data represents mean  $\pm$ SEM. Statistical analysis ( $n = 3$ ) of the indicated protein/PCNA ratio normalized to 0.5 hrs EtOH was performed using Student's t-test (\* $p < 0.05$ , \*\* $p < 0.01$ ).