Appendix

Non-autophagic Golgi-LC3 lipidation facilitates TFE3 stress response against Golgi dysfunction

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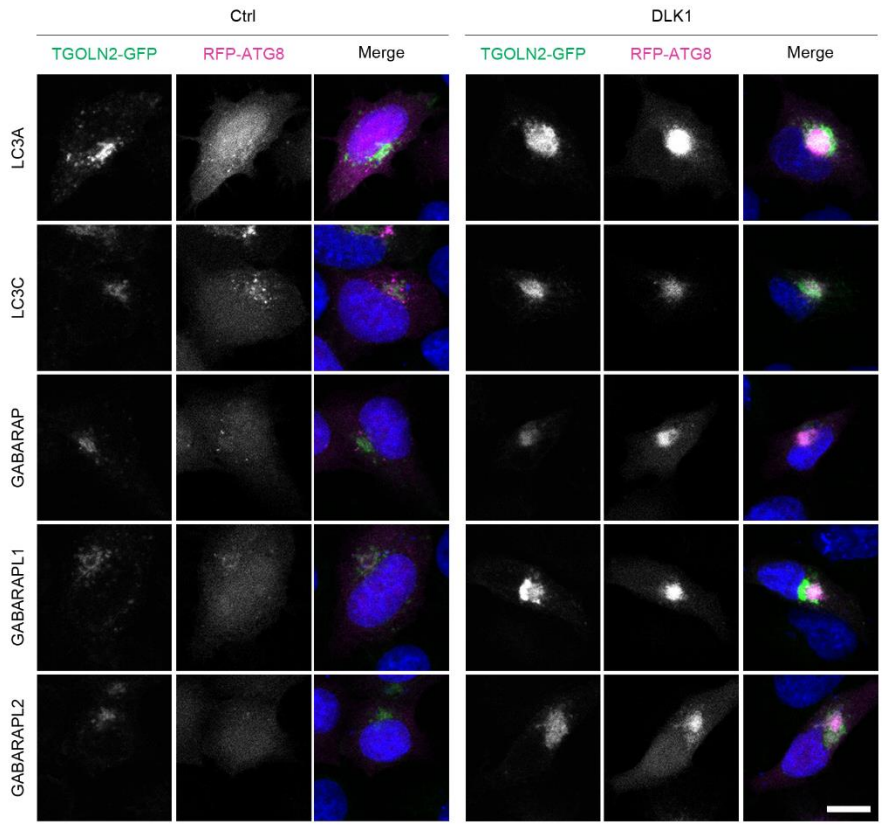
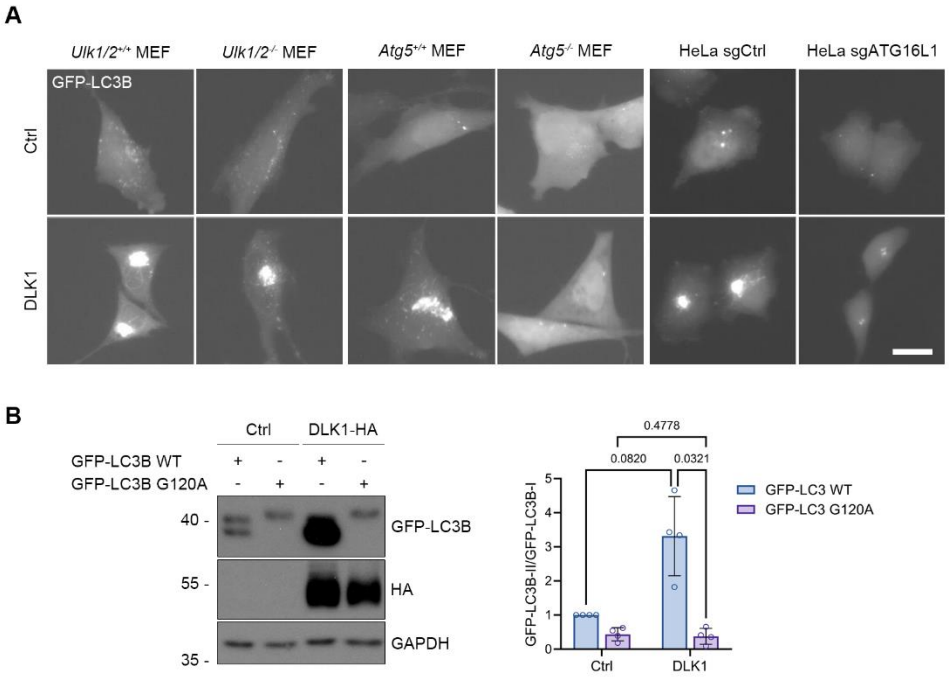


Appendix Figure S1. DLK1 overexpression accumulates ATG8 family proteins on the Golgi apparatus.

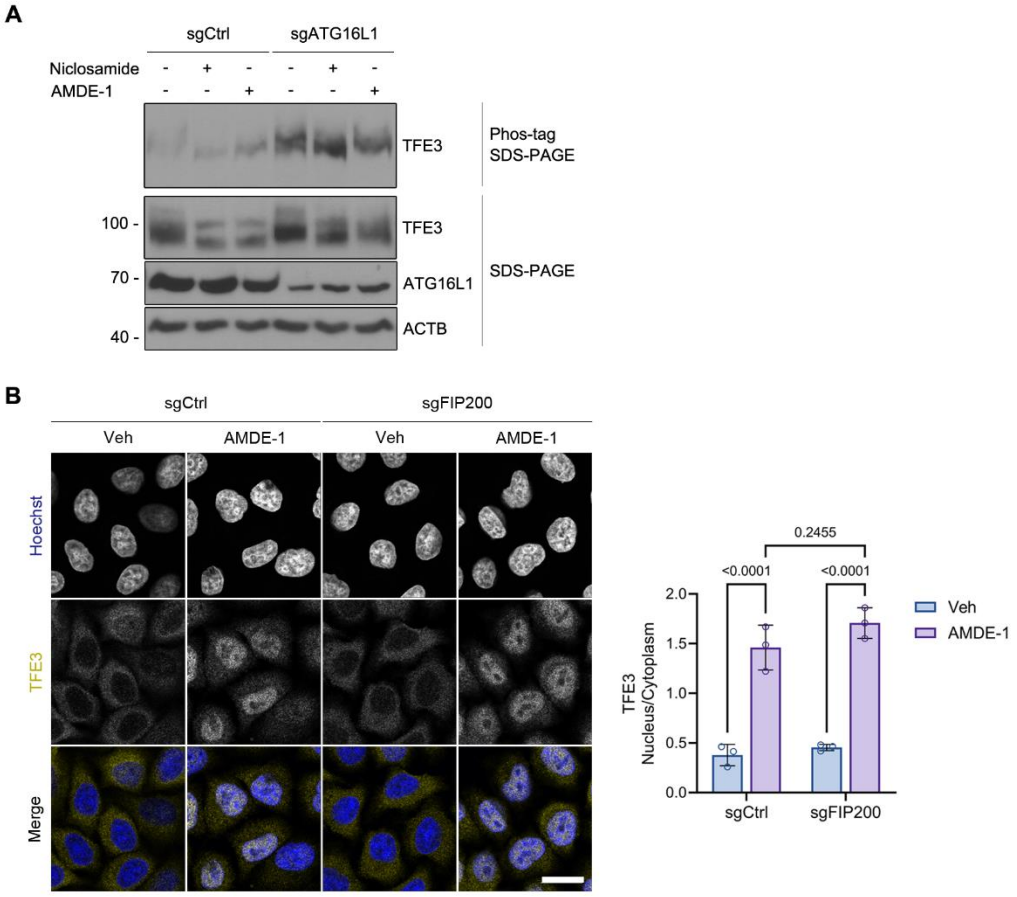
Confocal images of HeLa cells expressing DLK1-HA together with TGOLN2-GFP and one of the RFP-ATG8 family proteins. Nuclei were stained by Hoechst dye 33342. Scale bar, 10 μm.



Appendix Figure S2. LC3 lipidation of Golgi by DLK1 is ATG12-ATG5-ATG16L1 complex-dependent.

(A) Fluorescence microscopy images of *Ulk1/2*$^{+/+}$, *Ulk1/2*$^{-/-}$, *Atg5*$^{+/+}$, *Atg5*$^{-/-}$ MEFs and HeLa sgCtrl, sgATG16L1 cells expressing GFP-LC3B and either pcDNA3-HA (Ctrl) or DLK1-HA. Scale bar, 20 μm.

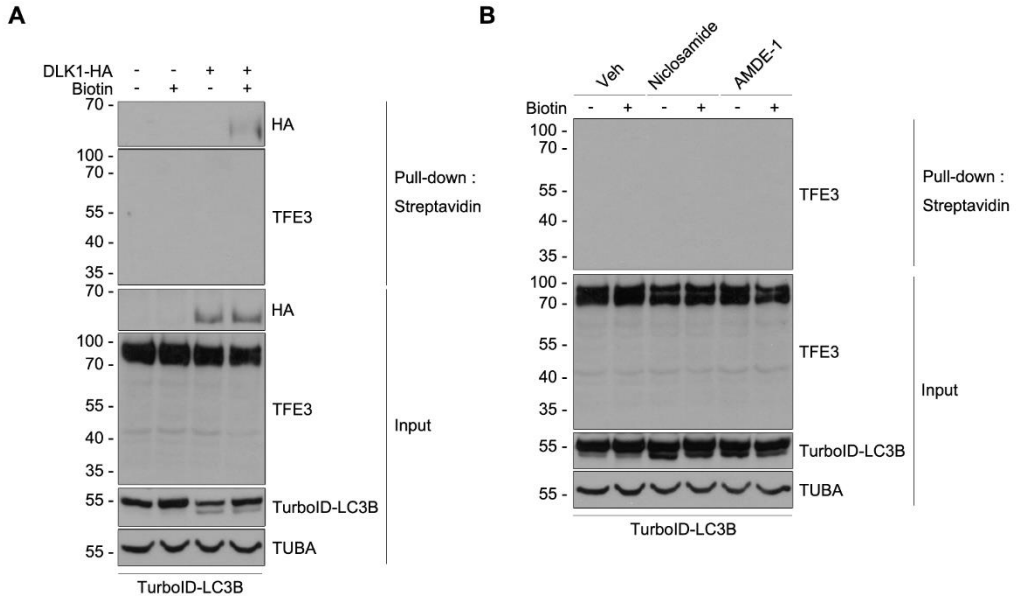
(B) Immunoblot analysis of HeLa cells expressing DLK1-HA and either GFP-LC3B WT or GFP-LC3B G120A (left). Relative signals of GFP-LC3B-II and GFP-LC3B-I on the blots are represented as mean ± s.d. (n = 4, two-way ANOVA followed by Tukey's multiple comparisons test) (right).



Appendix Figure S3. FIP200-independent TFE3 regulation under Golgi-stress.

(A) Phos-tag SDS-PAGE analysis of TFE3 in HeLa sgCtrl and sgATG16L1 cells after incubation with 10 μM niclosamide or 10 μM AMDE-1 for 6 h.

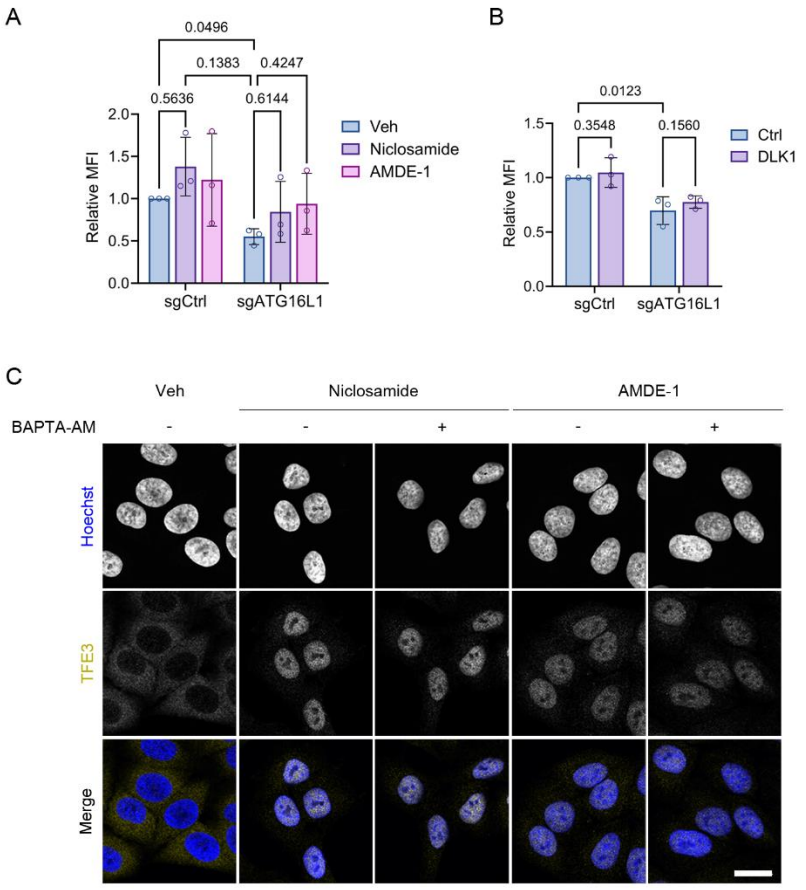
(B) Confocal images of HeLa sgCtrl and sgFIP200 cells incubated with 10 μM AMDE-1 for 6 h and immunostained with anti-TFE3 antibody. Nuclei were stained by Hoechst dye 33342. Scale bar, 20 μm (left). The nucleus/cytoplasm ratio of TFE3 fluorescence intensity is represented as mean ± s.d. (n = 3, 154 ~ 216 cells per experiment, two-way ANOVA followed by Tukey's multiple comparisons test) (right).



Appendix Figure S4. LC3 does not directly bind to TFE3 under Golgi stress.

(A) HeLa cells expressing DLK1-HA and TurboID-LC3B were treated with 100 μM biotin for 6 h, lysed and incubated with streptavidin-agarose beads. Enriched biotinylated proteins (Pull-down: Streptavidin) and whole cell lysates (Input) were analyzed by immunoblotting.

(B) HeLa cells expressing TurboID-LC3B were treated with 10 μM niclosamide or 10 μM AMDE-1 together with 100 μM biotin for 6 h, lysed and incubated with streptavidin-agarose beads. Enriched biotinylated proteins (Pull-down: Streptavidin) and whole cell lysates (Input) were analyzed by immunoblotting.



Appendix Figure S5. Calcium-independent TFE3 regulation by LC3 lipidation under Golgi stress.

(A, B) HeLa sgCtrl and sgATG16L1 cells exposed to 10 μM niclosamide or 10 μM AMDE-1 for 6 h **(A)** or HeLa sgCtrl and sgATG16L1 cells expressing pcDNA3-HA (Ctrl) or DLK1-HA **(B)** were treated with 1 μM Fluo-4 AM and subjected to flow cytometry analysis. Median values of the excitation ratios are represented as mean ± s.d. (n = 3, two-way ANOVA followed by Tukey's multiple comparisons test).

(C) HeLa cells were treated with 10 μM niclosamide or 10 μM AMDE-1 for 6 h in the presence or absence of 10 μM BAPTA-AM. Cells were immunostained with anti-TFE3 antibody and

observed by confocal microscopy. Nuclei were stained by Hoechst dye 33342. Scale bar, 20 μm.