

Expanded View Figures

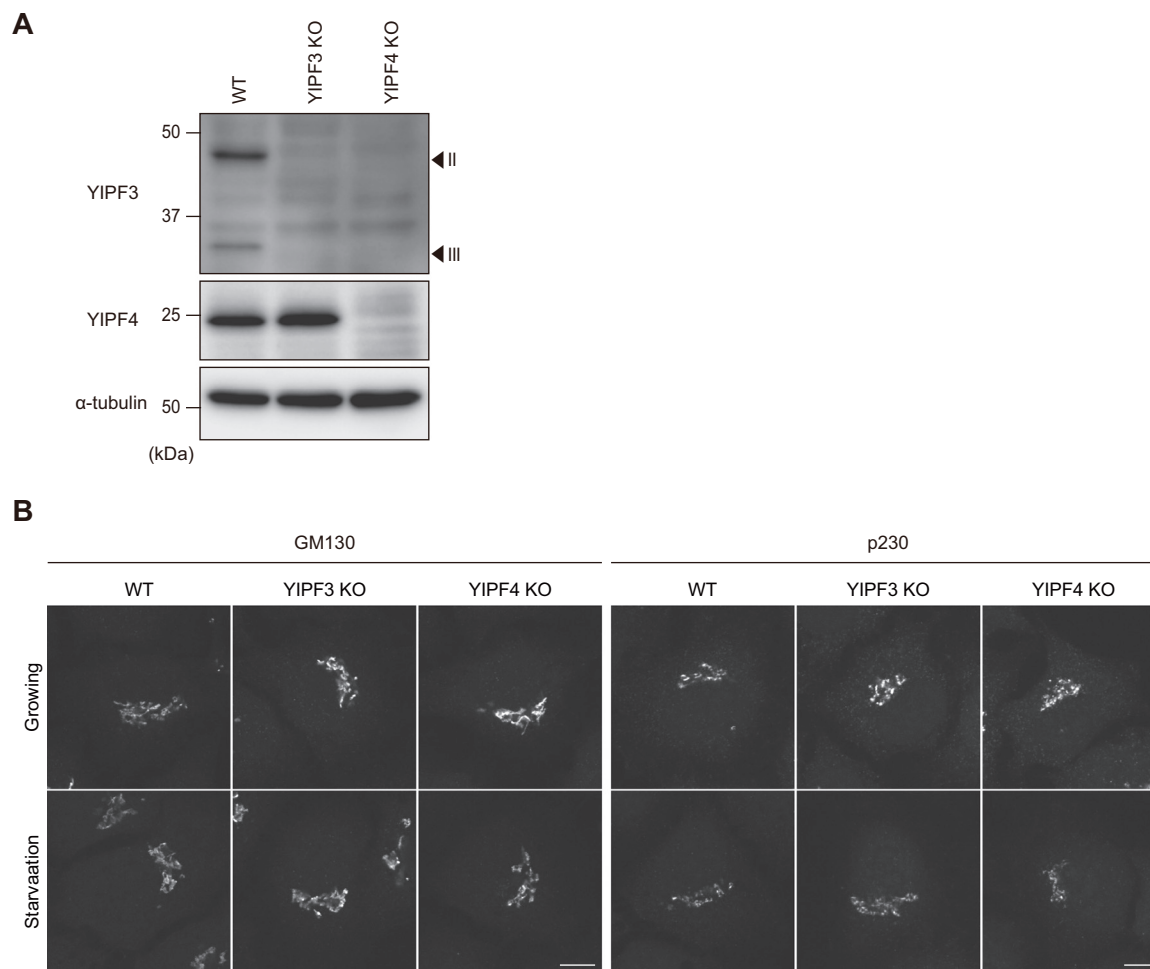


Figure EV1. Loss of YIPF4 suppresses YIPF3 protein levels.

(A) Immunoblotting of indicated proteins in WT, YIPF3-KO, and YIPF4-KO HeLa cells. (B) Immunofluorescence image of WT, YIPF3-KO and YIPF4-KO HeLa cells. Cells were cultured in DMEM or EBSS (9 h) and stained with anti-GM130 or anti-p230 antibodies. Scale bars, 10 μ m. Source data are available online for this figure.

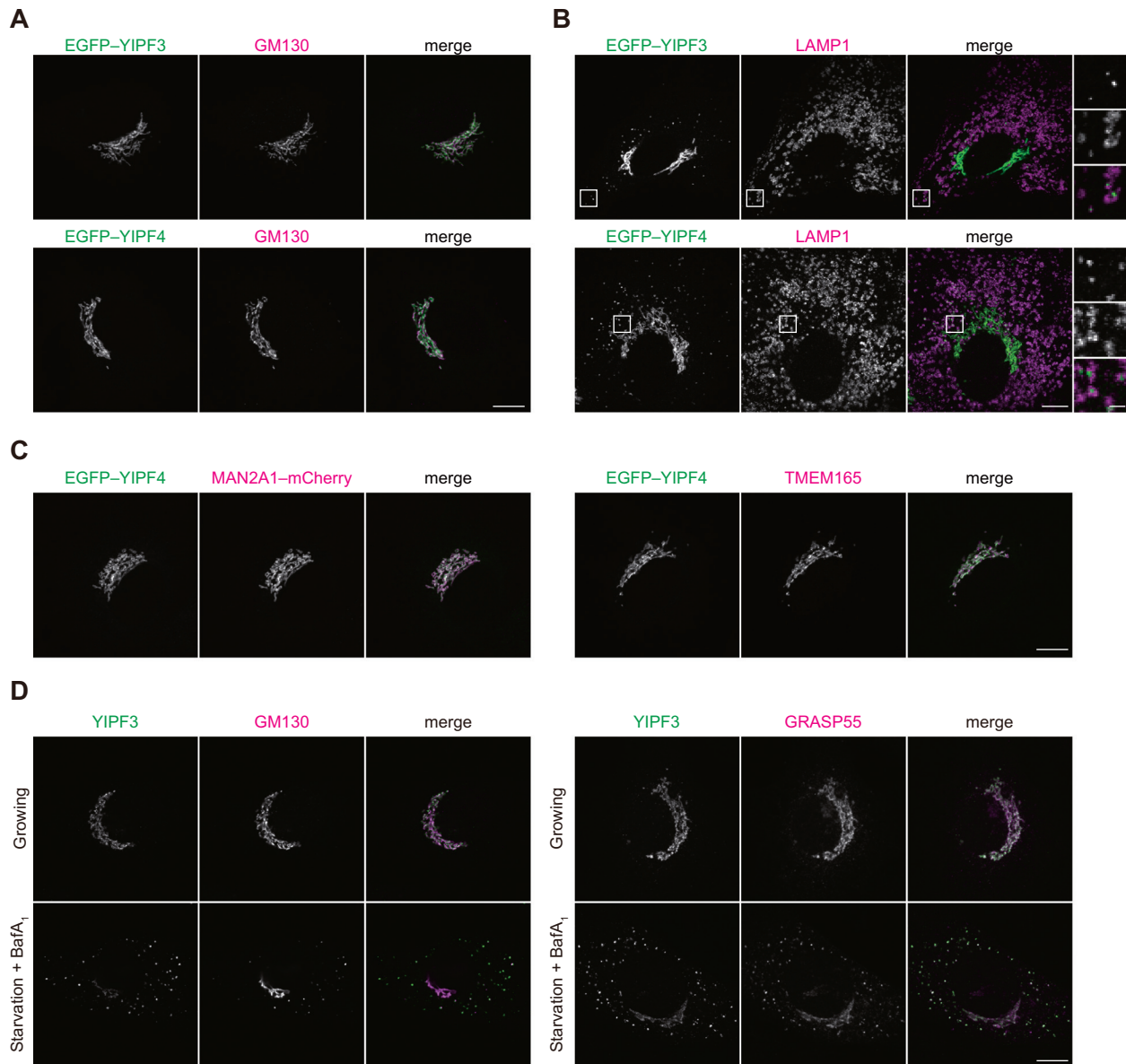


Figure EV2. EGFP-YIPF3 and EGFP-YIPF4 are localized to the Golgi apparatus under growing conditions, related to Fig. 2.

(A) Immunofluorescence image of MEFs stably expressing EGFP-YIPF3 or EGFP-YIPF4. Cells were cultured in DMEM and stained with anti-GM130 antibody. Scale bars, 10 μm and 1 μm (insets). (B) Immunofluorescence image of MEFs stably expressing EGFP-YIPF3 or EGFP-YIPF4. Cells were cultured in EBSS (2 h) in the presence of 125 nM Baf A₁ and stained with anti-LAMP1 antibodies. Scale bars, 10 μm and 1 μm (insets). (C) Immunofluorescence image of MEFs stably expressing EGFP-YIPF4 or coexpressing EGFP-YIPF4 and MAN2A1-mCherry. Cells were cultured in DMEM. The indicated cells were stained with anti-TMEM165 antibody. Scale bars, 10 μm. (D) Immunofluorescence image of MEFs cultured in DMEM or EBSS (6 h) in the presence of 125 nM Baf A₁. Each cell was stained with anti-YIPF3 antibody and the indicated cells were stained with anti-GM130 (*cis*-Golgi) or anti-GRASP55 (*medial*-Golgi) antibodies. Scale bars, 10 μm. The ratio of YIPF3 puncta positive for each Golgi protein to total YIPF3 puncta was quantified in three independent experiments (mean ± SD). Ten cells were analyzed per condition in each experiment. Source data are available online for this figure.

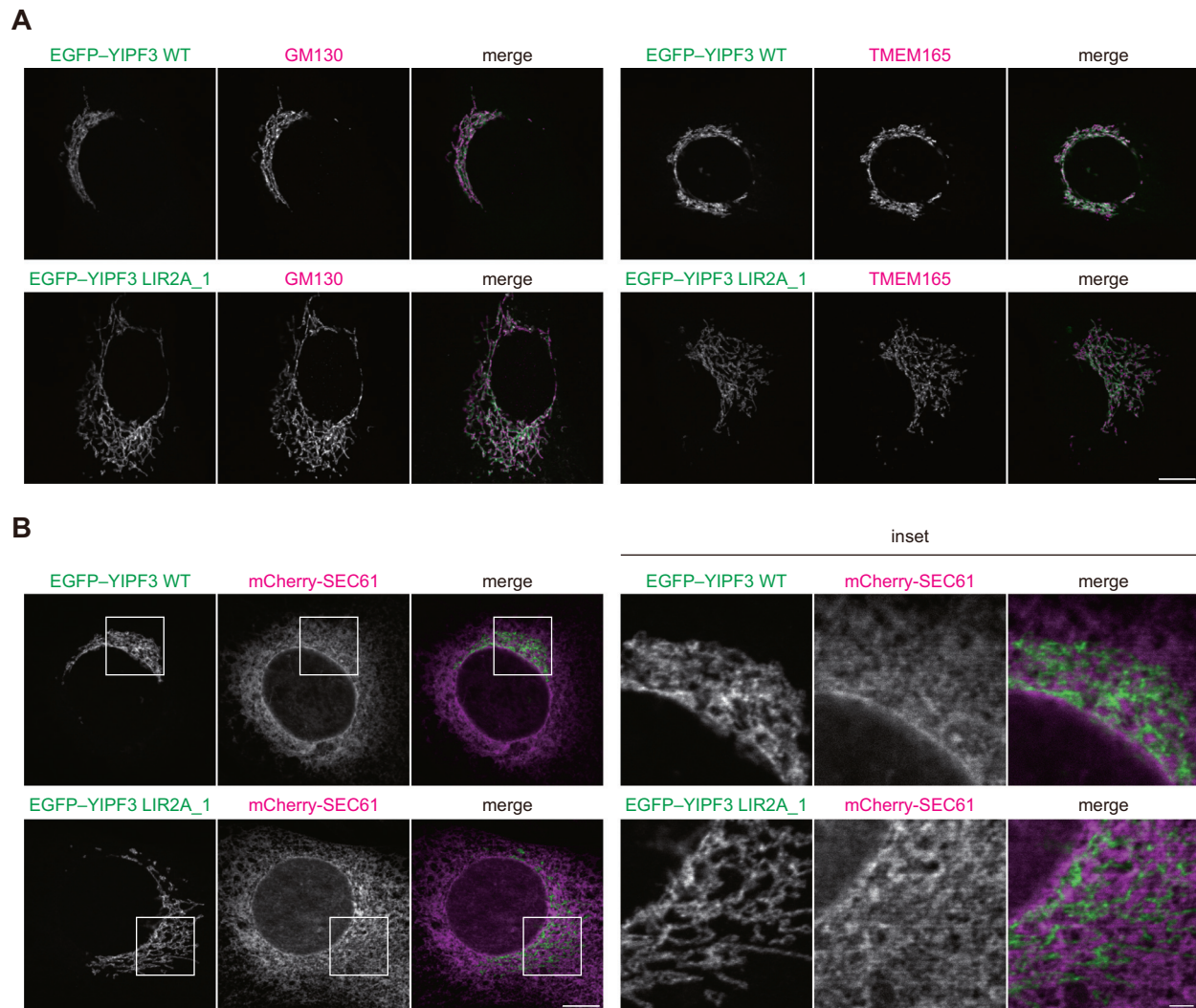
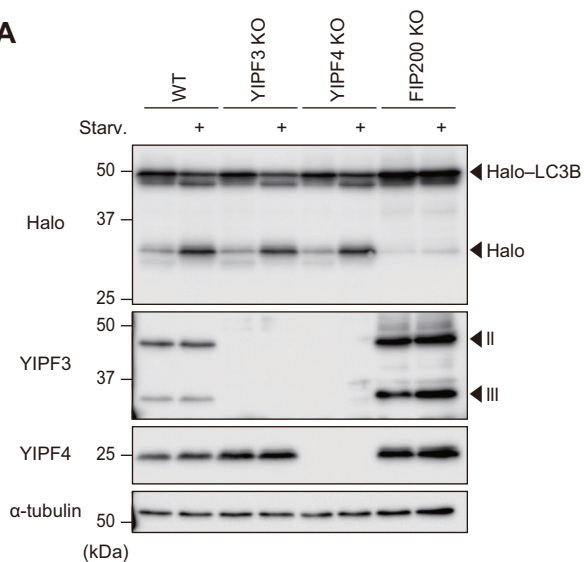


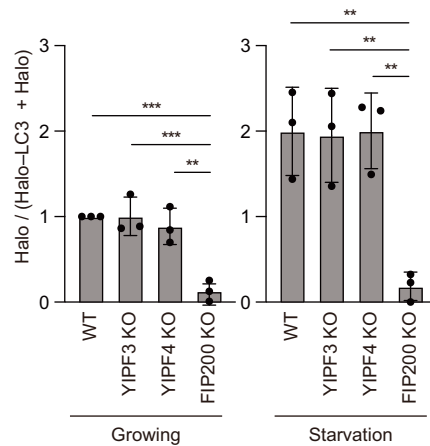
Figure EV3. The Golgi apparatus is partially discontinuous and expanded in cells stably expressing YIPF3 LIR2A mutant.

(A) Immunofluorescence image of MEFs stably expressing EGFP-YIPF3 WT or EGFP-YIPF3 LIR2A_1 under growing conditions. Cells were stained with anti-GM130 or anti-TMEM165 antibodies. Scale bars, 10 μ m. (B) Immunofluorescence image of MEFs stably coexpressing EGFP-YIPF3 WT or EGFP-YIPF3 LIR2A_1 and mCherry-SEC61 under growing conditions. Scale bars, 10 μ m and 2 μ m (insets). Source data are available online for this figure.

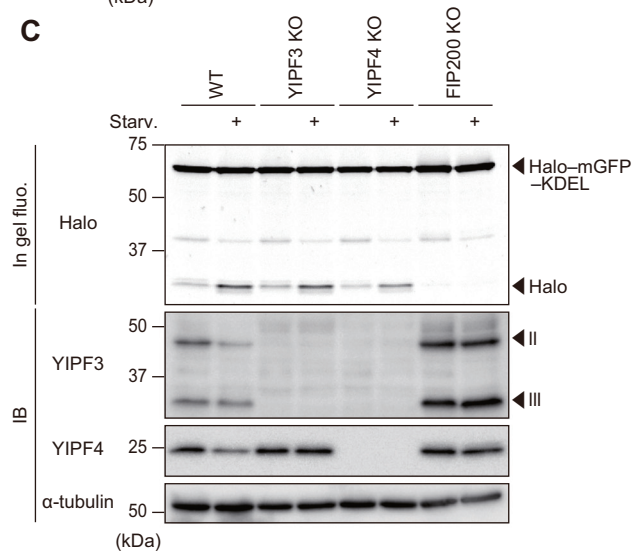
A



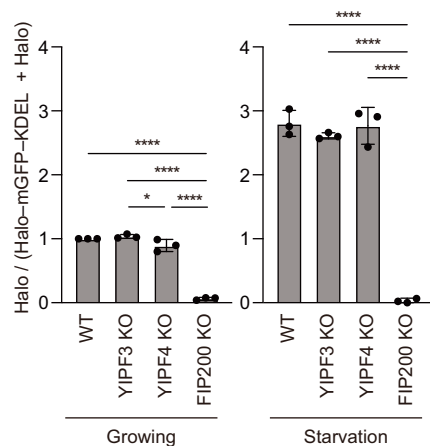
B



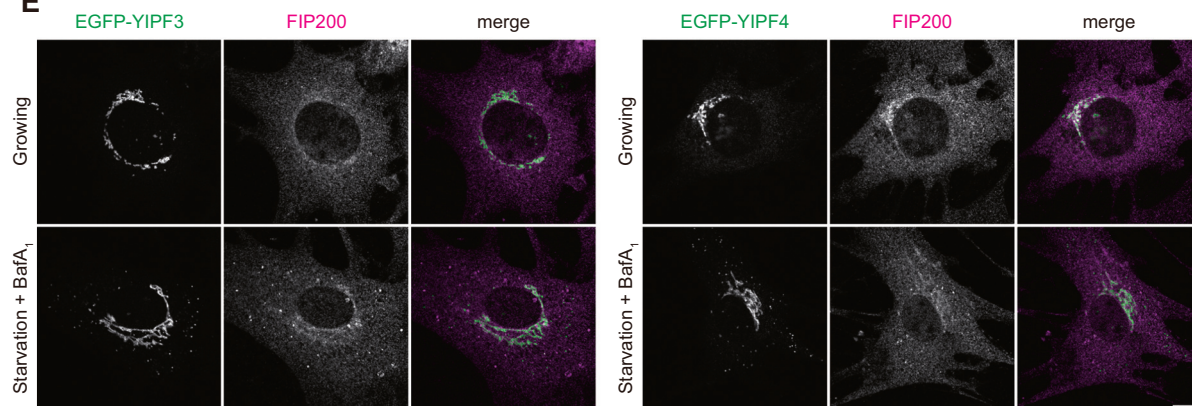
C



D



E



◀ Figure EV4. YIPF3 and YIPF4 are not involved in nonselective bulk autophagy and ER-phagy.

(A) Immunoblotting of indicated proteins in WT, YIPF3-KO, YIPF4-KO, and FIP200-KO HeLa cells stably expressing Halo-LC3B. After cells were cultured in the presence of TMR-conjugated ligands for 20 min and washed out, they were cultured in DMEM or EBSS for 4 h. (B) Quantification of results shown in (A). The ratio of Halo:Halo-LC3B is shown. Each ratio is normalized to WT cultured in nutrient-rich medium. Error bars indicate mean \pm SD ($n = 3$ independent experiments). (C) In-gel fluorescence image of Halo and immunoblotting of indicated proteins in WT, YIPF3-KO, YIPF4-KO, and FIP200-KO HeLa cells stably expressing Halo-mGFP-KDEL. After cells were cultured in the presence of TMR-conjugated ligands for 20 min and washed out, they were cultured in DMEM or EBSS for 9 h. (D) Quantification of results shown in (C). The ratio of Halo:Halo-mGFP-KDEL is shown. Each ratio is normalized to WT cultured in nutrient-rich medium. Error bars indicate mean \pm SD ($n = 3$ independent experiments). (E) Immunofluorescence image of MEFs stably expressing EGFP-YIPF3 or EGFP-YIPF4 cultured in DMEM or EBSS in the presence of 125 nM Baf A₁ for 2 h. Cells were stained with anti-FIP200 antibodies. Scale bars, 10 μ m. Data information: P values were determined using one-way ANOVA with Tukey's multiple comparisons test (B, D). Symbols indicate: * $P \leq 0.05$; ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$. Source data are available online for this figure.

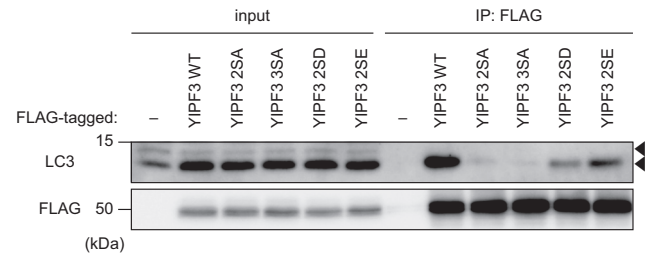


Figure EV5. YIPF3 phosphomimetic mutants show reductions in their affinity to LC3.

Immunoprecipitation of FLAG-YIPF3, FLAG-YIPF3^{S45A, S46A} (2SA), FLAG-YIPF3^{S43A, S45A, S46A} (3SA), FLAG-YIPF3^{S45D, S46D} (2SD) or FLAG-YIPF3^{S45E, S46E} (2SE) in HEK293T cells. Cells were transfected with FLAG-YIPF3 WT or each serine mutants. The lysates were immunoprecipitated with anti-FLAG antibody and detected with anti-LC3 and anti-FLAG antibodies. Source data are available online for this figure.