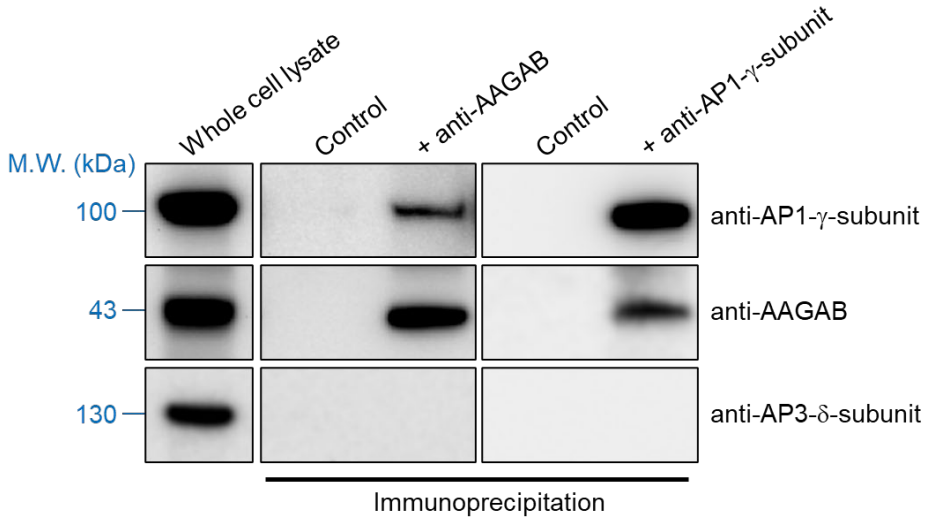

Fig. S1. Interactions of endogenous AAGAB and AP1. Endogenous AAGAB and AP1 γ subunit were immunoprecipitated from extracts of wild-type (WT) HeLa cells using protein G agarose beads with anti-AAGAB or anti-AP1-γ antibodies. The presence of AAGAB, AP1 γ subunit, and AP3 δ subunit in the immunoprecipitates was detected using anti-AAGAB, anti-AP1-γ, and anti-AP3-δ antibodies, respectively. Protein G agarose beads were added without antibodies as a control.

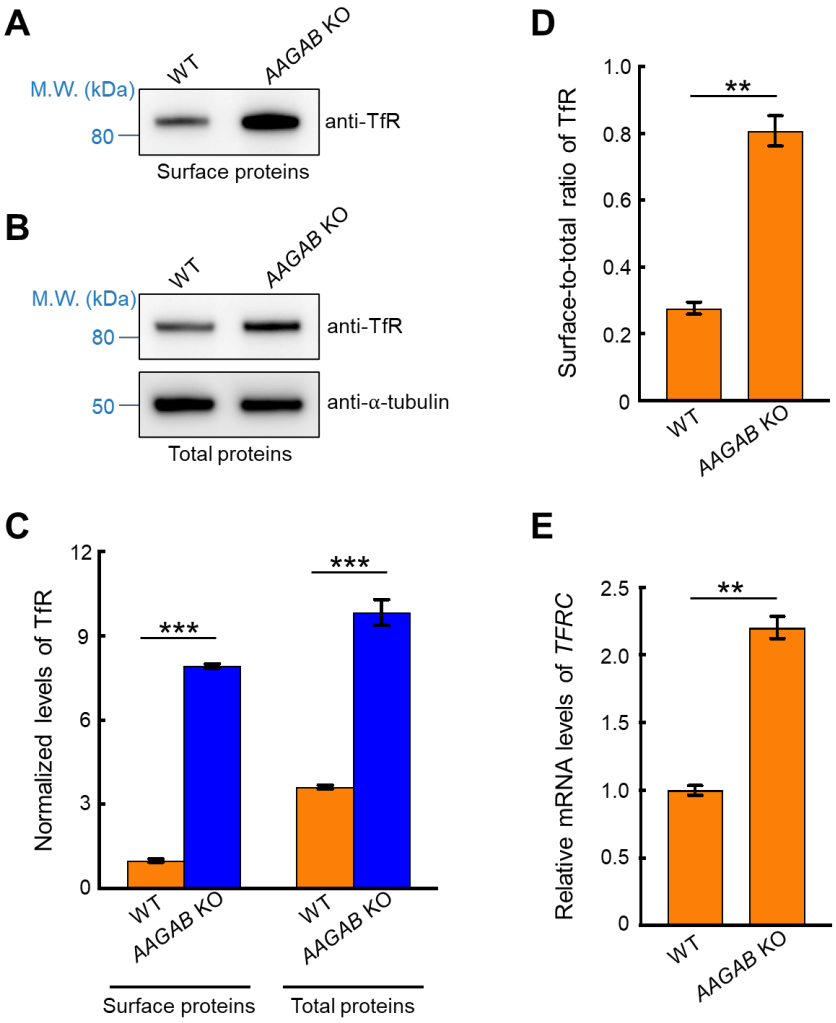

Fig. S2. Surface and total transferrin receptor (TfR) levels in WT and *AAGAB* knockout (KO) cells. (A) Representative immunoblot showing surface TfR in WT and *AAGAB* KO HeLa cells. Surface proteins were biotinylated using Sulfo-NHS-Biotin and isolated by NeutrAvidin agarose. **(B)** Representative immunoblots showing total TfR and α-tubulin in WT and *AAGAB* KO HeLa cells. **(C)** Flow cytometry measurements showing normalized surface and total levels of TfR in WT and *AAGAB* KO HeLa cells. To measure total TfR, cells were disassociated by accutase, fixed using 2% paraformaldehyde, permeabilized using 0.1% saponin, and stained with monoclonal anti-TfR antibodies and APC-conjugated secondary antibodies. To measure surface TfR, cells were processed and analyzed in a similar way except that saponin was omitted. APC fluorescence of ~5,000 cells was measured on a CyAN ADP analyzer. Mean APC fluorescence of a sample was normalized to that of surface staining in WT cells. Data are presented as mean ± SD, n = 3. *** $p < 0.001$. *P* values were calculated using one-way ANOVA. **(D)** The surface-to-total ratio of TfR was calculated from data in C. A Student's t-test was used to calculate statistical significance using three independent datasets. ** $p < 0.01$. **(E)** Relative mRNA levels of *TFRC*, which encodes human TfR, were calculated by normalizing the threshold cycles of *TFRC* to those of *GAPDH*, a gene whose expression remained unchanged in *AAGAB* KO HeLa cells. The fold change was determined by comparing the normalized threshold cycles of *AAGAB* KO cells to those of WT cells. A Student's t-test was used to calculate statistical significance using three independent datasets. ** $p < 0.01$.

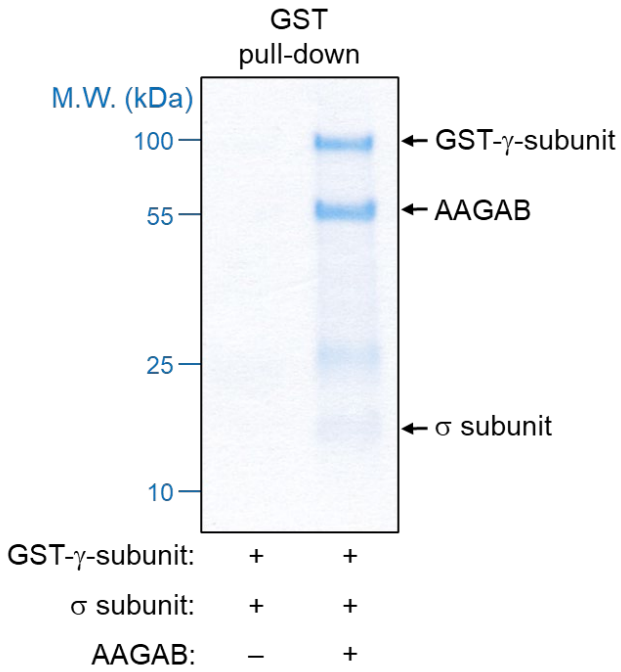

Fig. S3. AP1 γ and σ subunits cannot be expressed in *E. coli* unless AAGAB is co-expressed. GST-γ-subunit (trunk domain) and untagged σ subunit were co-expressed in *E. coli* at 37 °C with or without His_6-SUMO-AAGAB. GST-γ-subunit and associated proteins were isolated as described in Figure 6, resolved on SDS-PAGE, and stained with coomassie blue.

Table S1. Whole-cell proteomes of WT and *AAGAB* KO HeLa cells. To calculate relative expression levels, values of *AAGAB* KO HeLa cells were divided by those of WT cells.

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Table S2. Surface proteomic analysis of WT and *AP1G1* KO HeLa cells. To calculate relative surface levels in Tables S2-S4, values of a HeLa KO cell line were divided by those of WT cells. Data are presented as relative expression levels to WT. *P* values were calculated using Student's t-test by comparing relative surface levels of a protein with those of ACTR2, which remained unchanged in all KO cell lines.

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Table S3. Surface proteomic analysis of WT and *AP2S1* KO HeLa cells.

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Table S4. Surface proteomic analysis of WT and *AAGAB* KO HeLa cells.

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Table S5. Ranking of genes based on CRISPR scores (essentiality) in HeLa cells. The reads in the passage control were divided by those in the initial plasmid library to calculate fold changes. Predicted essential genes are highlighted in bold with a CRISPR score cutoff of -0.25. Genes encoding AAGAB, AP1 and AP2 are highlighted in boxes.

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