Supplemental Information

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Figure S1

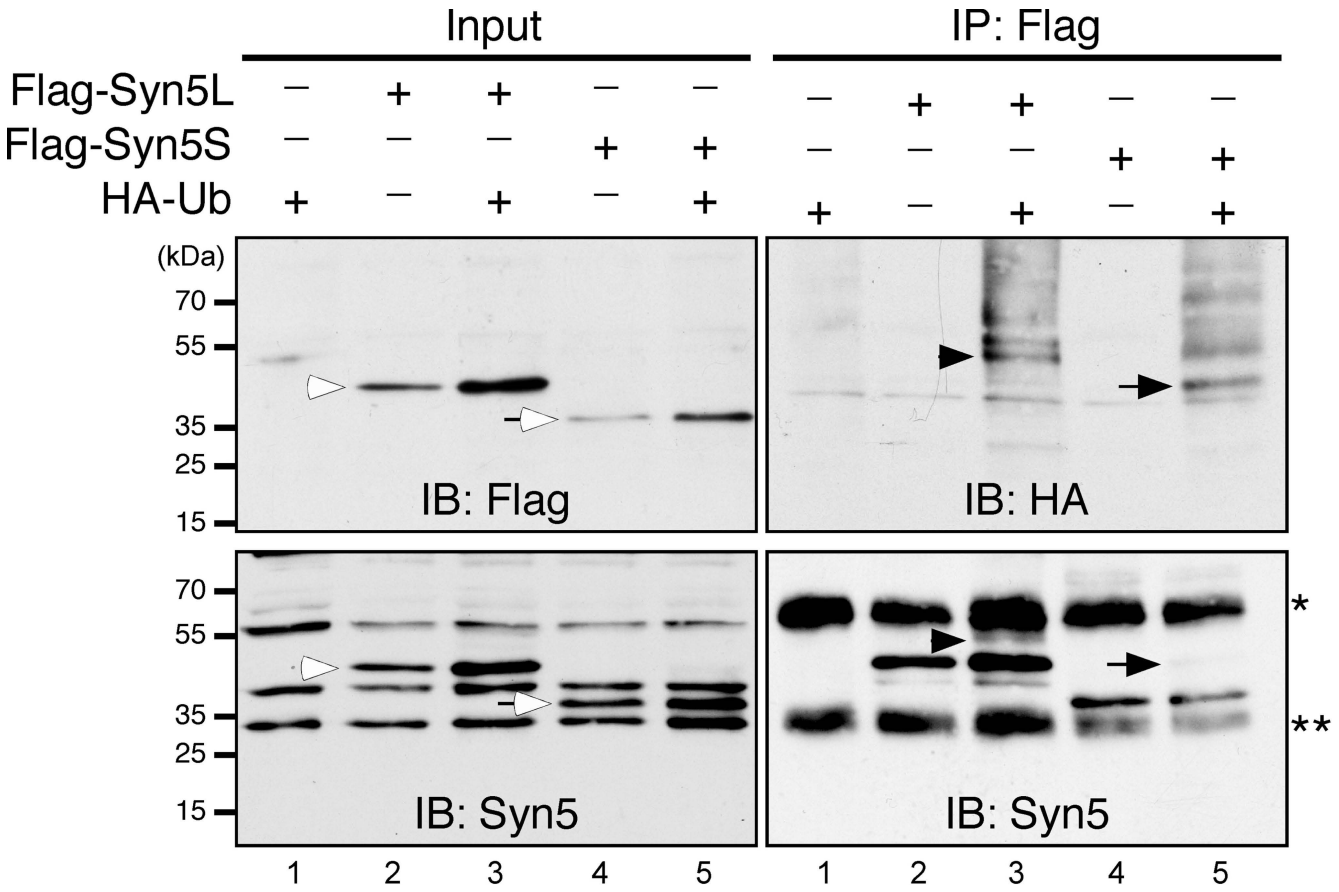


Figure S1. Exogenously expressed Syn5 is ubiquitinated in mitosis. Related to Figure 1.
HeLa cells were co-transfected with Flag-Syn5 long (Flag-Syn5L) or short form (Flag-Syn5S) and HA-Ubiquitin (HA-Ub) as indicated and synchronized in mitosis, followed by immunoprecipitation of Flag-Syn5 by an anti-Flag antibody and Western blot of the indicated proteins. Note the 8 kD shift of Syn5L-ub (lane 3, solid arrowhead) and Syn5S-ub (lane 5, solid arrow) in the right panels compared to Syn5L (lane 3, empty arrowhead) and Syn5S (lane 5, empty arrow) in the left panels. Asterisks indicate IgG heavy chain (*) and light chain (**), respectively.

Figure S2

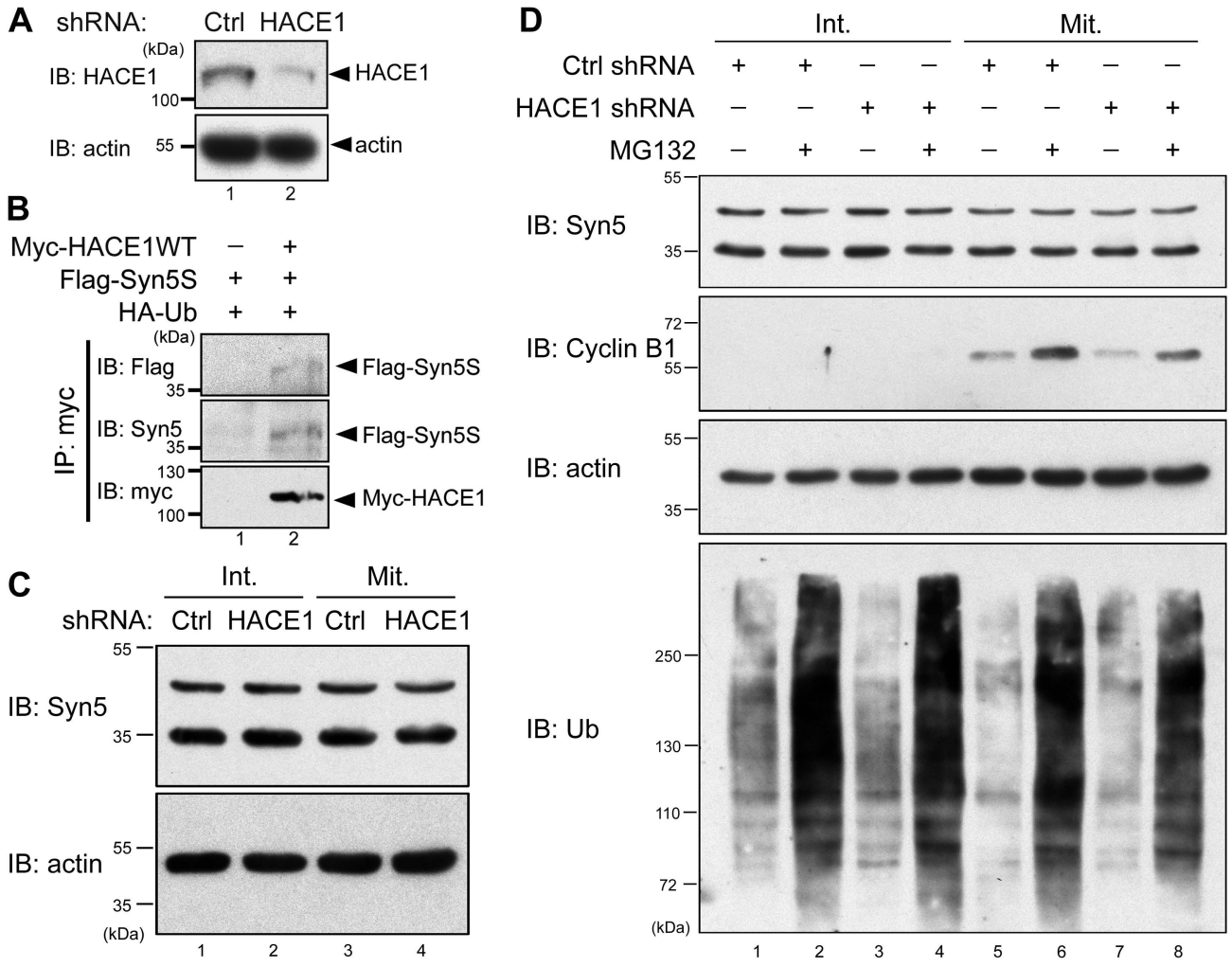


Figure S2. Syn5 ubiquitination does not affect Syn5 protein level in interphase and mitotic cells. Related to Figure 2.

(A) HeLa cells were infected by control (Ctrl) or HACE1 shRNA lentivirus to establish stable cell lines (Tang et al., 2011). The depletion efficiency of HACE1 shRNA was shown by Western blotting with HACE1 and actin antibodies.

(B) Exogenously expressed Syn5 (Flag-Syn5S) interacts with HACE1. HeLa cells were co-transfected with the indicated constructs and synchronized in mitosis, followed by immunoprecipitation of Flag-Syn5 and Western blotting with indicated antibodies.

(C) Syn5 protein level does not change upon HACE1 depletion in interphase and mitotic cells. Untreated interphase cells (Int.) or nocodazole-arrested mitotic cells (Mit.) stably expressing Ctrl shRNA or HACE1 shRNA were solubilized and blotted with Syn5 and actin antibodies.

(D) Syn5 level does not change after MG132 treatment (20 μM for 6 h) or upon HACE1 depletion in interphase (Int.) and mitotic (Mit.) cells.

Figure S3

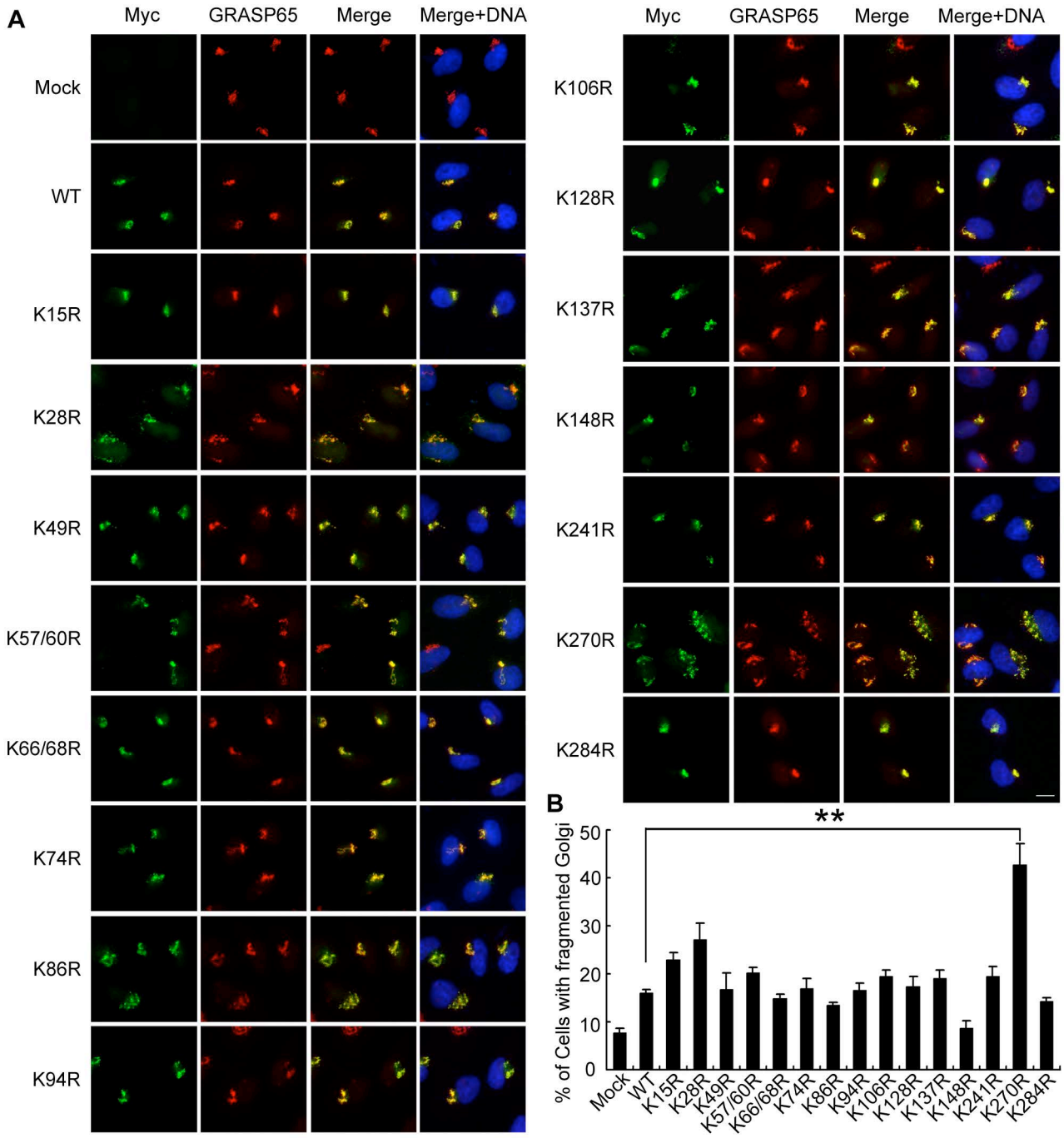


Figure S3. Expression of Syn5 K270R mutant results in Golgi fragmentation. Related to Figure 4.

(A) Representative fluorescence images of HeLa cells transfected with Syn5 WT or KR mutant and stained for Myc (Syn5) and GRASP65. Note the fragmented Golgi in K270R transfected HeLa cells. Scale bar, 10 μm.

(B) Quantitation results of (A) from three independent experiments. Data are represented as mean ± s.e.m. **, $p < 0.01$.

Figure S4

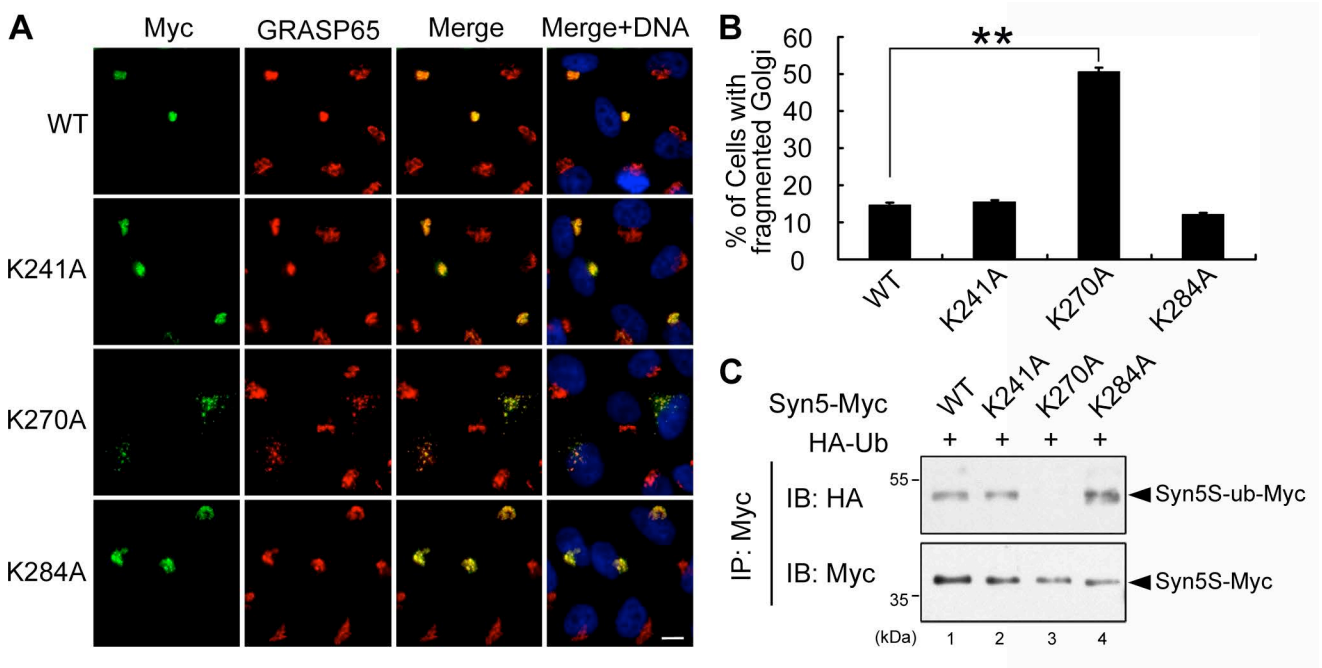


Figure S4. Expression of Syn5 K270A mutant results in Golgi fragmentation. Related to Figure 4.

(A) Representative fluorescence images of HeLa cells transfected with Myc-tagged Syn5 WT, K241A, K270A, or K284A mutant and stained for Myc (Syn5) and GRASP65. Note the fragmented Golgi in K270A transfected HeLa cells. Scale bar, 10 μm.

(B) Quantitation of cells with fragmented Golgi in (A) from three independent experiments. Data are represented as mean ± s.e.m. **, $p < 0.01$.

(C) Mutation of K270A reduces Syn5 monoubiquitination. HeLa cells were co-transfected with Myc-tagged Syn5 WT, K241A, K270A, or K284A mutant and HA-Ub, synchronized to mitosis, immunoprecipitated by anti-Myc and blotted for both HA-Ub and Syn5-Myc.

Figure S5

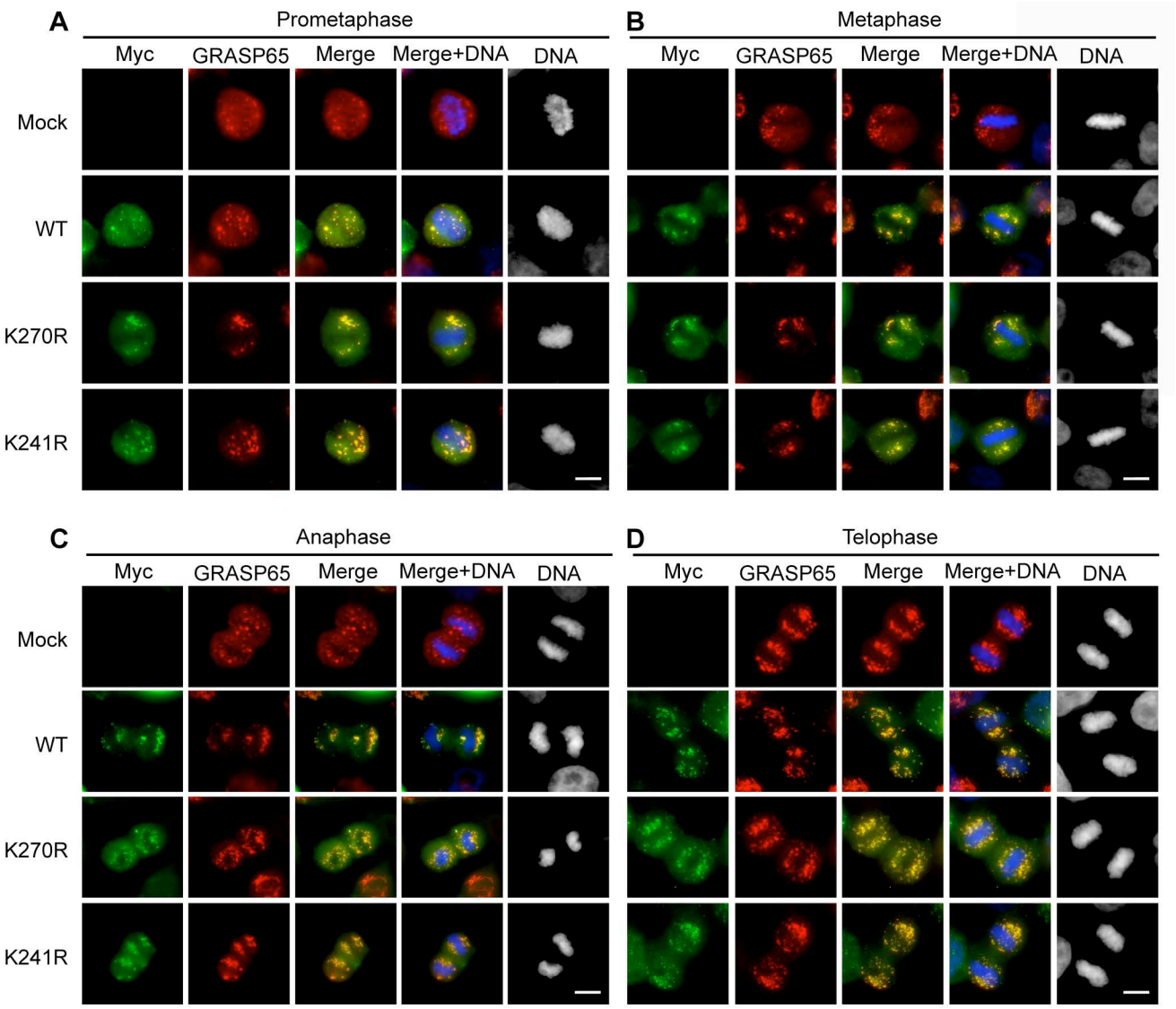
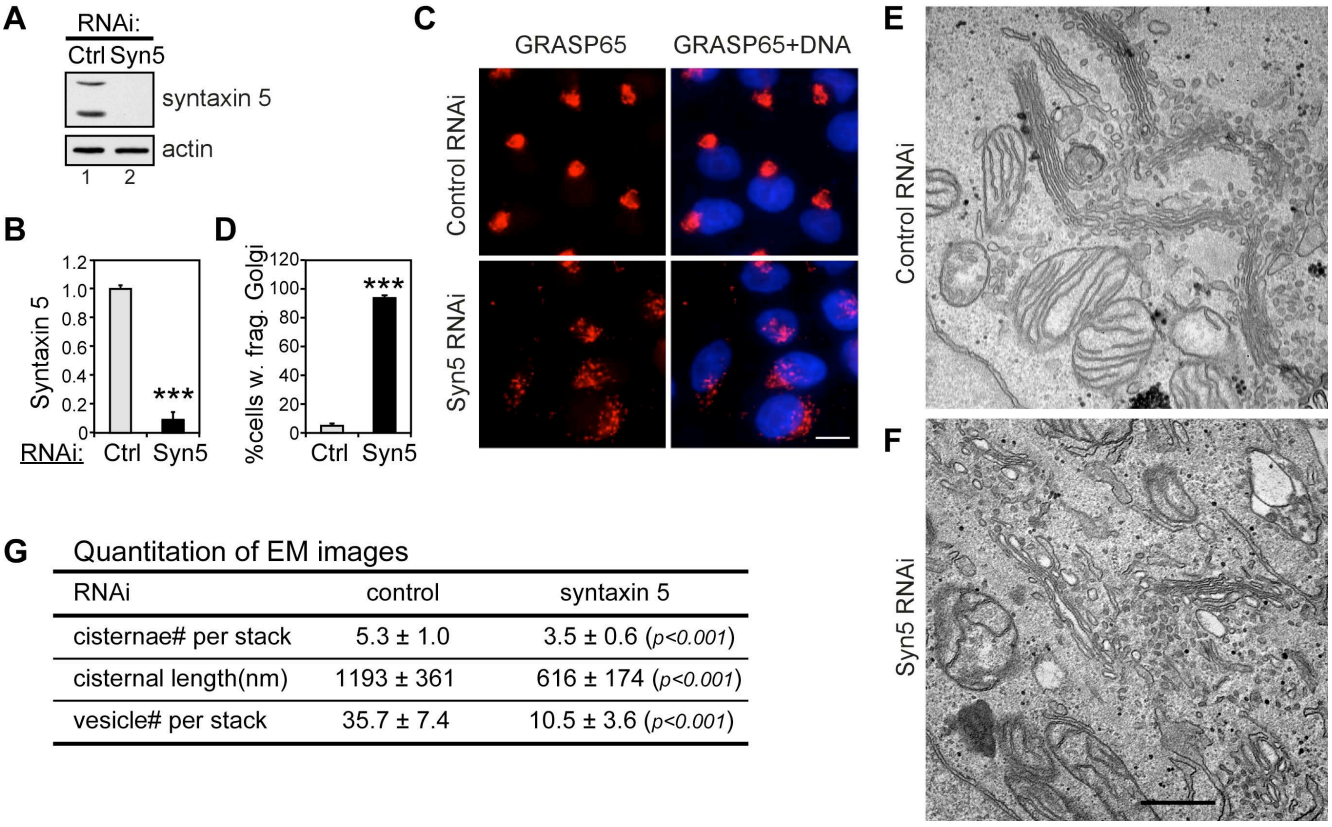


Figure S5. Syn5 K270R mutation does not affect mitotic Golgi disassembly. Related to Figure 5. HeLa cells were transfected with Myc-tagged Syn5 WT, K270R, or K241R mutant and stained for Myc (Syn5) and GRASP65. Representative fluorescence images of prometaphase (A), metaphase (B), anaphase (C) and telophase (D) cells. Scale bar, 10 μm.

Figure S6



G Quantitation of EM images

RNAi	control	syntaxin 5
cisternae# per stack	5.3 ± 1.0	3.5 ± 0.6 (*p<0.001*)
cisternal length(nm)	1193 ± 361	616 ± 174 (*p<0.001*)
vesicle# per stack	35.7 ± 7.4	10.5 ± 3.6 (*p<0.001*)

Figure S6. Depletion of Syn5 leads to Golgi fragmentation. Related to Figure 5.
(A) Western blot of control or Syn5 RNAi treated HeLa cells with indicated antibodies.
(B) Quantitation of Syn5 levels in control or Syn5 RNAi treated cells from three independent experiments.
(C) Representative fluorescence images of Syn5 knockdown cells stained for GRASP65. Scale bars, 10 μm.
(D) Quantitation of cells with fragmented Golgi in (C) from three independent experiments. Data are represented as mean ± s.e.m. ***, $p < 0.001$.
(E-F) Representative EM micrographs of control or Syn5 depleted cells. Scale bar, 0.5 μm. Note that the Golgi ribbon in Syn5-depleted cells is disorganized, and the cisternae are shorter compared to control RNAi treated cells.
(G) Quantitation of the EM images in (E and F) from three independent experiments. Note that cisterna number per stack, cisternal length and vesicle per stack are reduced by Syn5 depletion compared to control cells.

Supplemental Experimental Procedures

Chemicals, plasmids, recombinant proteins, antibodies and RNAi

Protease inhibitor was purchased from Roche and used according to the manufacturer's instructions. N-Ethylmaleimide (NEM) was purchased from Fisher, resolved in ethanol, and used at a final concentration of 50 mM. Epoxomicin was purchased from Calbiochem and used at 0.5 μM. MG132 was purchased from Calbiochem and used at 2 μM for 6 h.

The following antibodies were used: monoclonal antibodies against β-actin (AC-15, Sigma), Bet1 (B32320, Transduction Lab), GS28 (611184, Transduction Lab), Flag (M1804, Sigma), HA (16B12, Covance), Myc (9E10, The University of Michigan Hybridoma Core Facility), and ubiquitin (VU101, Lifesensors; 13-1600, Invitrogen); polyclonal antibodies against GFP (sc-8834, Santa Cruz), rat Syn5 (Graham Warren, Max F. Perutz Laboratories, Vienna), human Syn5 (Vladimir Lupashin, University of Arkansas for Medical Sciences), ubiquitin (U5379, Sigma), HACE1 (SAB2101010, Sigma), VCIP135 (Graham Warren, Max F. Perutz Laboratories, Vienna, Austria), p47 (Graham Warren) and Cyclin B1 (Mark Jackman, University of Cambridge).

Myc-tagged long and short forms of Syn5 cDNAs were cloned from a construct of pFLAG-CMV-6-Syn5 long and short forms (a gift from Mitsuo Tagaya, Tokyo University) (Miyazaki et al., 2012) and inserted into the XhoI/BamHI site of pcDNA™3.1/Myc-His (-) A vector (Invitrogen) and expressed in HeLa cells by transfection using Polyethylenimine (Linear, MW 25,000, Polysciences). Long and short forms of Syn5 lacking the transmembrane domain were cloned in pMal-C2X vector and the MBP fusion proteins were expressed in BL21. The KR mutants of Syn5 were generated by site-directed mutagenesis. Constructs of Myc-tagged HACE1 and C876A mutant, and preparation of recombinant GST-tagged HACE1, ΔC and C876A proteins, were expressed and purified as previously described (Tang et al., 2011). GFP-tagged VCIP135 and C218S, and recombinant streptavidin-binding peptide (SBP)-tagged VCIP135 WT and C218S mutant proteins, were prepared and used as previously described (Zhang and Wang, 2015; Zhang et al., 2014). His-tagged ubiquitin was expressed and purified as previously described (Wang et al., 2004). Construct for GST-tagged Bet1 was kindly provided by Wanjin Hong (Institute of Molecular and Cell Biology, A*STAR, Singapore) and expressed in BL21. His-tagged p47 was kindly provided by Tsui-Fen Chou (Los Angeles Biomedical Research Institute) (Zhang et al., 2015). HA-tagged p47 was cloned from a construct of pTrcHisB-p47 (a gift from Hemmo Meyer, University of Duisburg-Essen) (Bruderer et al., 2004) and inserted into pcDNA3.1+/N-HA Vector. All constructs were confirmed by DNA sequencing.

Control non-specific RNAi was purchased from Ambion. Syn5 specific RNAi was synthesized by Invitrogen (sense sequence: AAGCUGGAGAAGCUGACAAUC) (Diao et al., 2008). RNAi-resistant mutant of Syn5 was generated by mutating the RNAi targeting sequence from 5'-AAGCTGGAGAAGCTGACAATC-3' to 5'-AAACTAGAAAAGCTCACGATC-3'. Syn5 K241R and K270R RNAi-resistant mutants were constructed based on the RNAi-resistant form of Syn5-Myc.

Cell culture and microscopy

HeLa cells were cultured in DMEM supplemented with 10% donor bovine serum (Gibco/Invitrogen), 2 mM L-glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml).

For immunofluorescence microscopy, HeLa cells were fixed in 3.7% paraformaldehyde, permeabilized with 0.3% Triton X-100 and processed for immunofluorescence microscopy with indicated antibodies. Images were taken with a Zeiss Observer Z1 epifluorescence microscope with a 63X oil lens.

Supplemental References

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