Cell Reports, Volume *41*

Supplemental information

Mitotic phosphorylation inhibits the Golgi mannosidase MAN1A1

Shijiao Huang, Yoshimi Haga, Jie Li, Jianchao Zhang, Hye Kyong Kweon, Junichi Seino, Hiroto Hirayama, Morihisa Fujita, Kelley W. Moremen, Philip Andrews, Tadashi Suzuki, and Yanzhuang Wang

Supplemental information

The Supplemental information contains 6 supplemental figures, 4 supplemental movies and one supplemental table. Figure S1 shows that MAN1A2, but not MAN2A1, is phosphorylated in mitosis. Figure S2 demonstrates that MAN1A1 is phosphorylated at S12 by CDK1 in mitotic cells. Figure S3 shows that mitotic Golgi membranes exhibit lower mannosidase I activity, but the same mannosidase II activity compared to interphase Golgi. Figure S4 reveals that mitotic phosphorylation of Golgi membranes alters the production of different oligosaccharide isomers. Figure S5 shows that exogenously expressed MAN1A1 and its mutants are properly localized to the Golgi without disturbing the Golgi morphology. Figure S6 shows that S12 mutations do not affect MAN1A1 protein stability. Movies S1-4 display live cell videos of Rhodamine-PHA-L signals at the surface of *MAN1* QKO cells expressing GFP or GFP-tagged MAN1A1 constructs at the mitotic exit. Table S1 displays mass spectrometry data of identified phosphoproteins in mitotic Golgi membranes highlighted in Figure 1A.

Supplemental figures

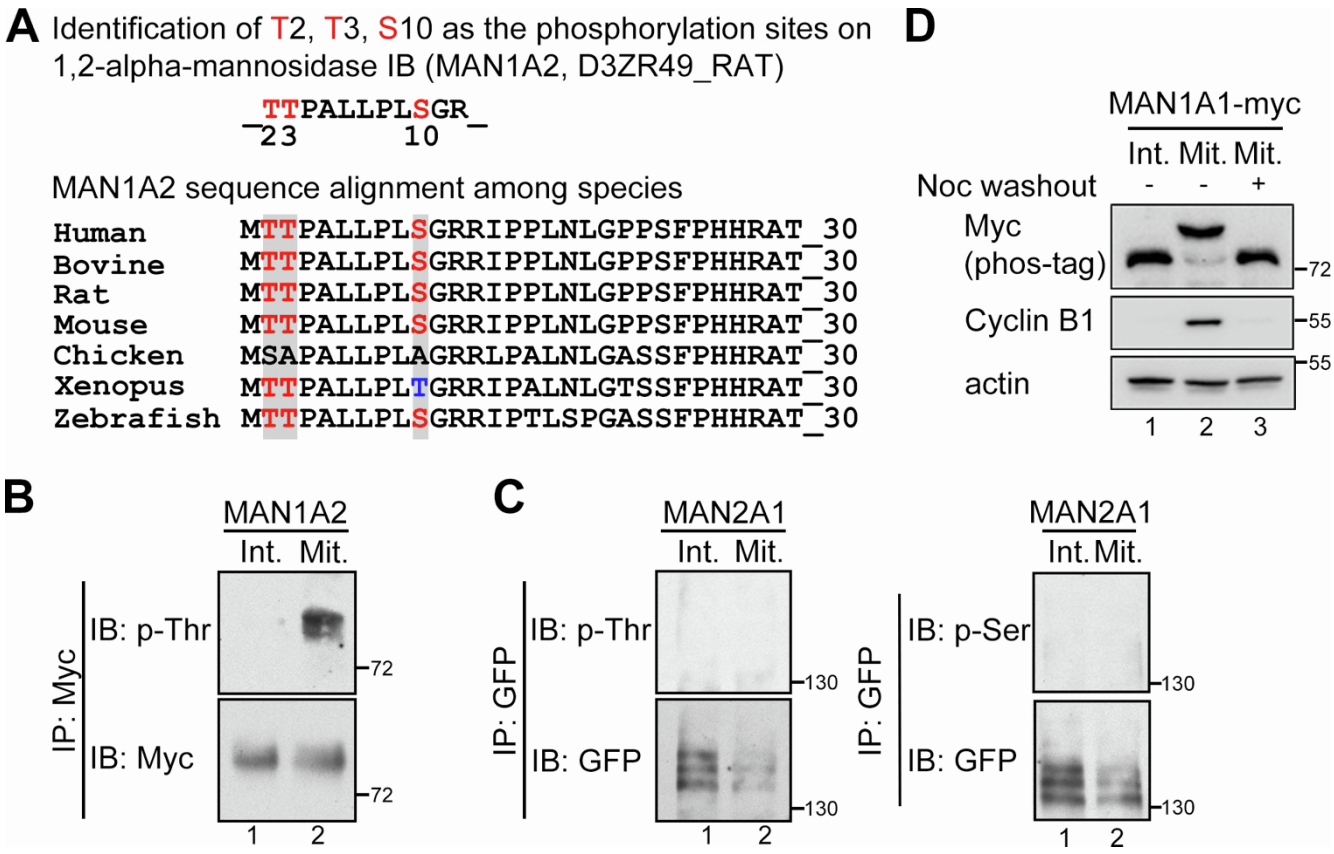

Figure S1. MAN1A2, but not MAN2A1, is phosphorylated in mitosis. Related to Figure 1.

A. Sequence alignment shows conserved T2, T3, and S10 on MAN1A2 across species.

B. MAN1A2 is phosphorylated in mitotic cells. Interphase (Int.) and mitotic (Mit.) HeLa cells transfected with a MAN1A2-myc were immunoprecipitated with an anti-myc antibody followed by western blot for p-Thr and myc. Shown are representative results from three independent experiments.

C. MAN2A1 is not phosphorylated. Interphase (Int.) and mitotic (Mit.) HeLa cells transfected with a MAN2A1-GFP construct were immunoprecipitated with an anti-GFP antibody followed by western blot for p-Thr, p-Ser, and GFP.

D. MAN1A1 phosphorylation in mitosis is reversible. Unsynchronized interphase (Int.) and nocodazole-synchronized mitotic (Mit.) HeLa cells expressing MAN1A1-myc with or without nocodazole washout for 4 h were analyzed by normal SDS-PAGE or phos-tag gels as indicated. Cyclin B1 was used as a mitotic marker.

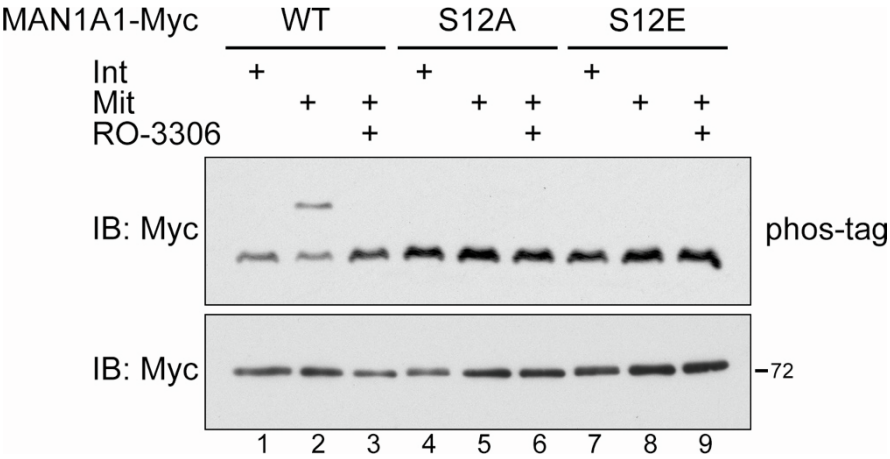

Figure S2. MAN1A1 is phosphorylated at S12 by CDK1 in mitotic cells. Related to Figure 2.

Immunoblots of interphase or mitotic HeLa cells expressing WT MAN1A1 or its S12 mutants treated with or without 10 μM RO-3306 for 30 min using phos-tag and normal gels. Cyclin B1 was used as a mitotic marker.

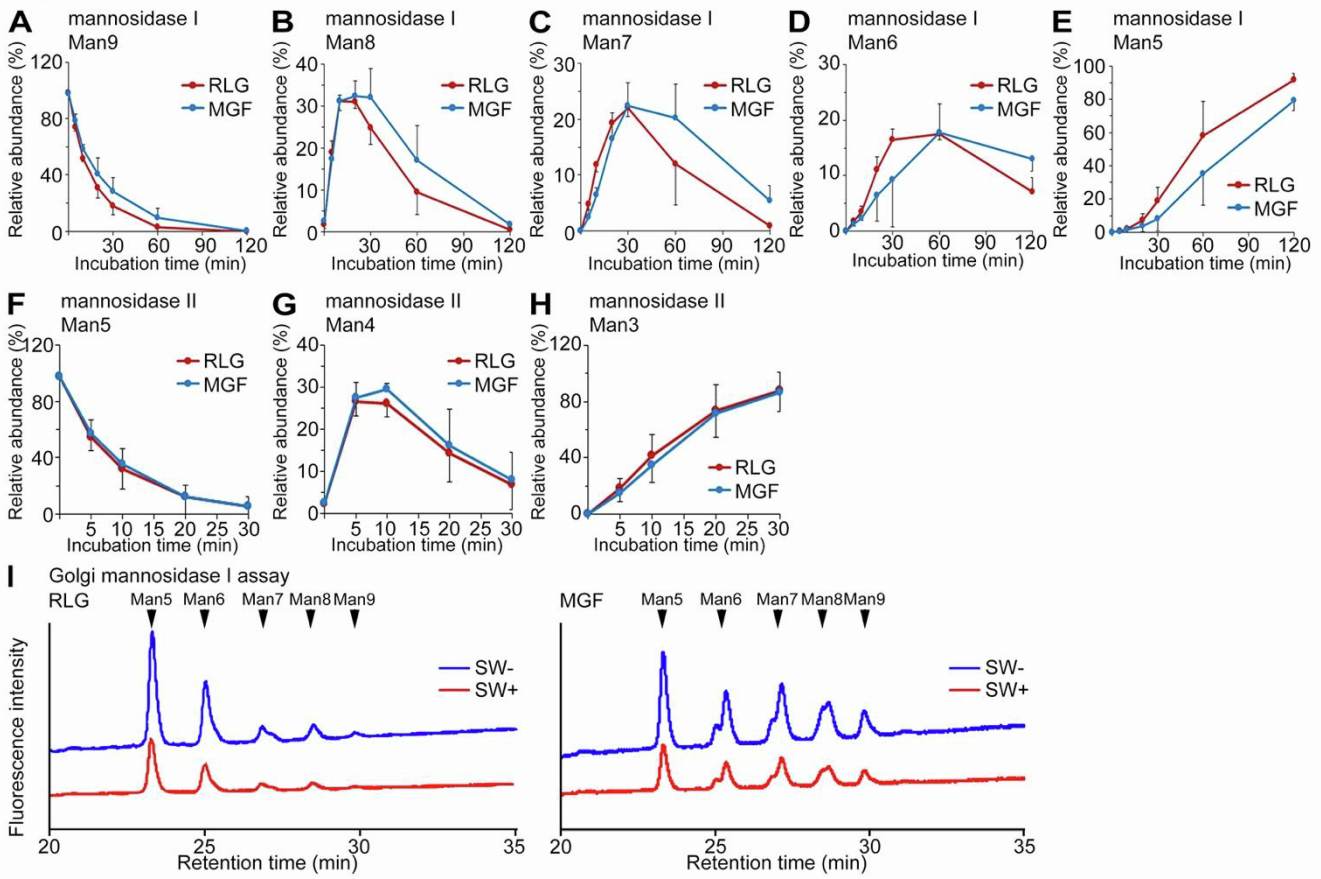

Figure S3. Mitotic Golgi membranes exhibit lower mannosidase I activity but same mannosidase II activity compared to interphase Golgi. Related to Figure 3.

A-E. Mitotic Golgi membranes (MGF) exhibit lower mannosidase I activity than interphase Golgi (RLG). RLG and MGF were incubated with a high-mannose substrate (Man9) for 5, 10, 20, 30, 60 and 120 min. The substrate and products were quantified by HPLC and shown in each panel (A-E). Data represent the mean ± SD from three independent experiments.

F-H. Golgi mannosidase II exhibits similar activity in interphase and mitotic Golgi membranes. RLG and MGF were incubated with a Man5 substrate for 5, 10, 20 and 30 min. The substrate and products were quantified by HPLC and shown in each panel (F-H).

I. The mannosidase I assay is specific in detecting mannosidase I activity. RLG and MGF were incubated with a high-mannose substrate (Man9) for 1 h with or without 10 μM swainsonine (SW) to inhibit MAN2 and the products were analyzed by HPLC. Shown are representative results from three independent experiments.

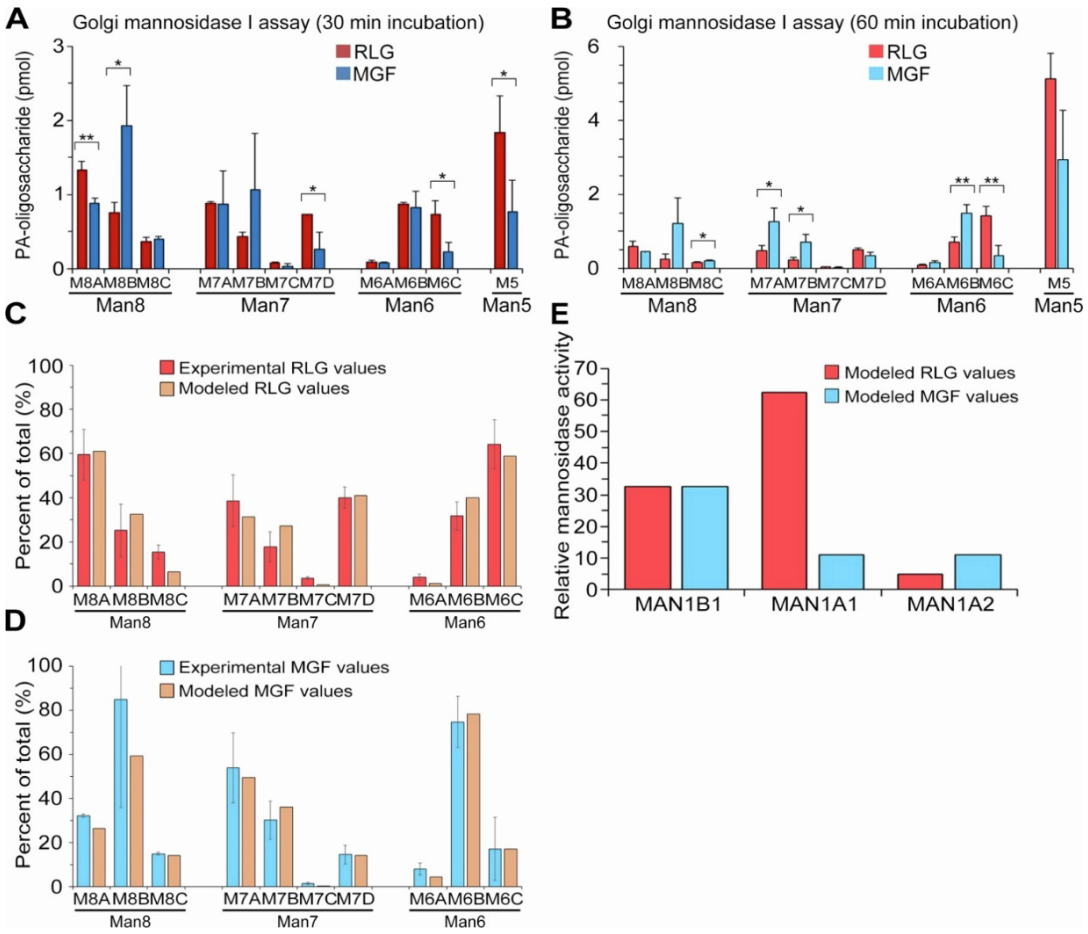

Figure S4. Mitotic phosphorylation of Golgi membranes alters the production of different oligosaccharide isomers. Related to Figure 4.

A-B. Quantitation of indicated isomers after Man9-PA was incubated with RLG and MGF for 30 min (A) or 60 min (B) based on the dual-gradient, reversed-phase HPLC results. Data represent the mean ± SD of three independent experiments. The *p* value was determined by Student *t*-test. *, $p < 0.05$; **, $p < 0.01$.

C-D. The abundances of the Man8, Man7, and Man6 isomers were quantified for the 60 min digestion of Man9-PA by RLG (C) or MGF (D) membrane preparations based on B. Values for each isomer abundance were normalized as a percent of total for that size fraction. Results represent mean ± SD from triplicate analyses (light red and light blue bars). The data was then modeled based on the established substrate specificities of MAN1B1, MAN1A1 and MAN1A2 (Fig. 4F) and the abundances of the three enzymes were adjusted to result in a modeled data set (tan bars) that best matched the profiles of the respective experimental isomer intermediates.

E. The modeled activities of MAN1B1, MAN1A1 and MAN1A2 for 60 min digestion of Man9-PA by RLG (C) and MGF (D) fractions were used to display changes in relative enzyme activities, with the assumption that MAN1B1 did not change.

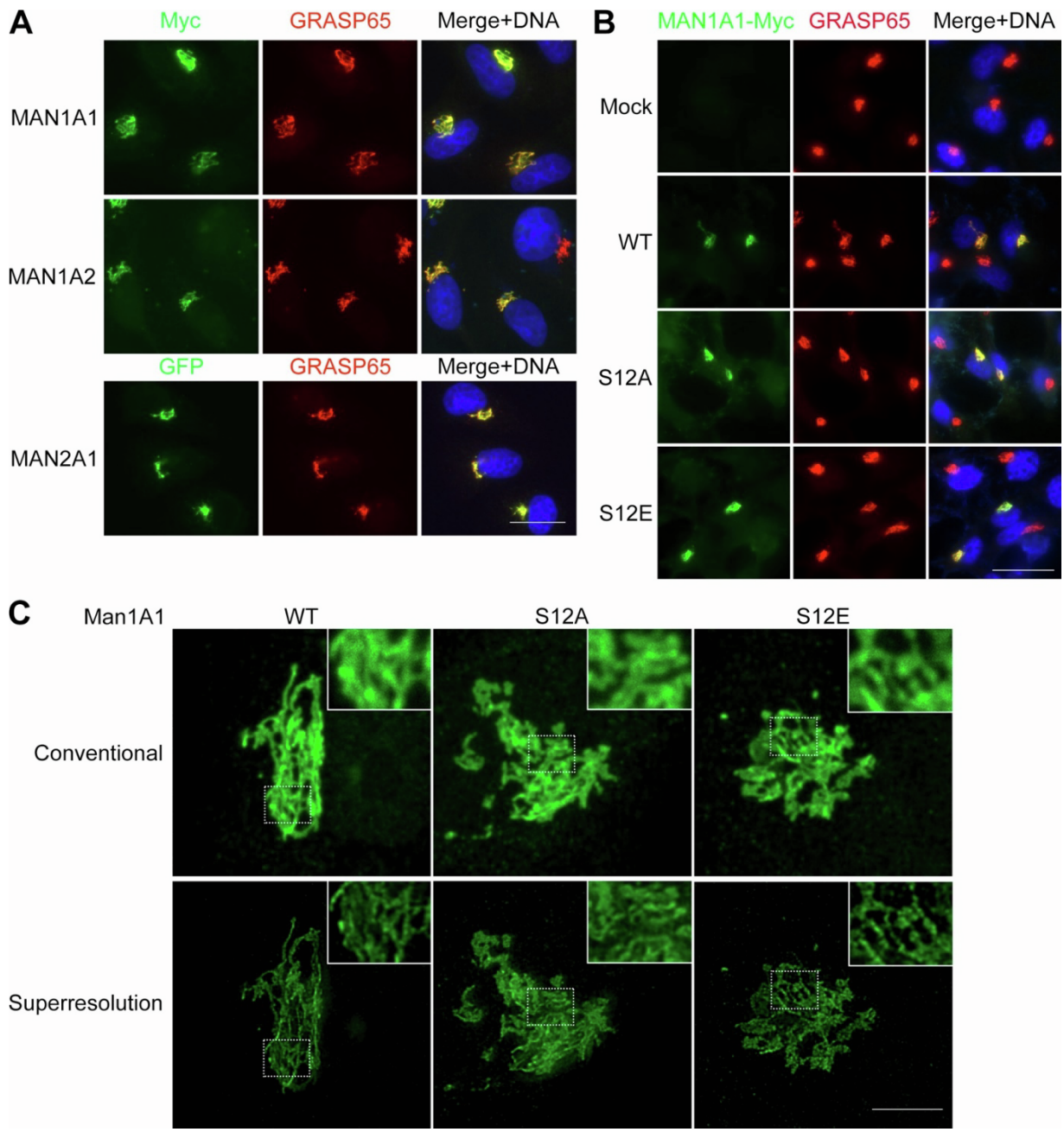

Figure S5. Exogenously expressed MAN1A1 and its mutants are localized to the Golgi without impacting the Golgi morphology. Related to Figure 7.

A. MAN1A1, MAN1A2, and MAN2A1 are correctly localized to the Golgi, as indicated by the colocalization with GRASP65. Scale bar, 20 µm.

B. MAN1A1 and its mutants are localized to the Golgi. Scale bar, 20 µm.

C. MAN1A1 mutation does not impact the Golgi structure. HeLa cells were transfected with WT MAN1A1-Myc or its S12 mutants and analyzed by conventional or super resolution confocal microscopy. Note the tubular connections between the Golgi membranes. Scale bar, 5 µm.

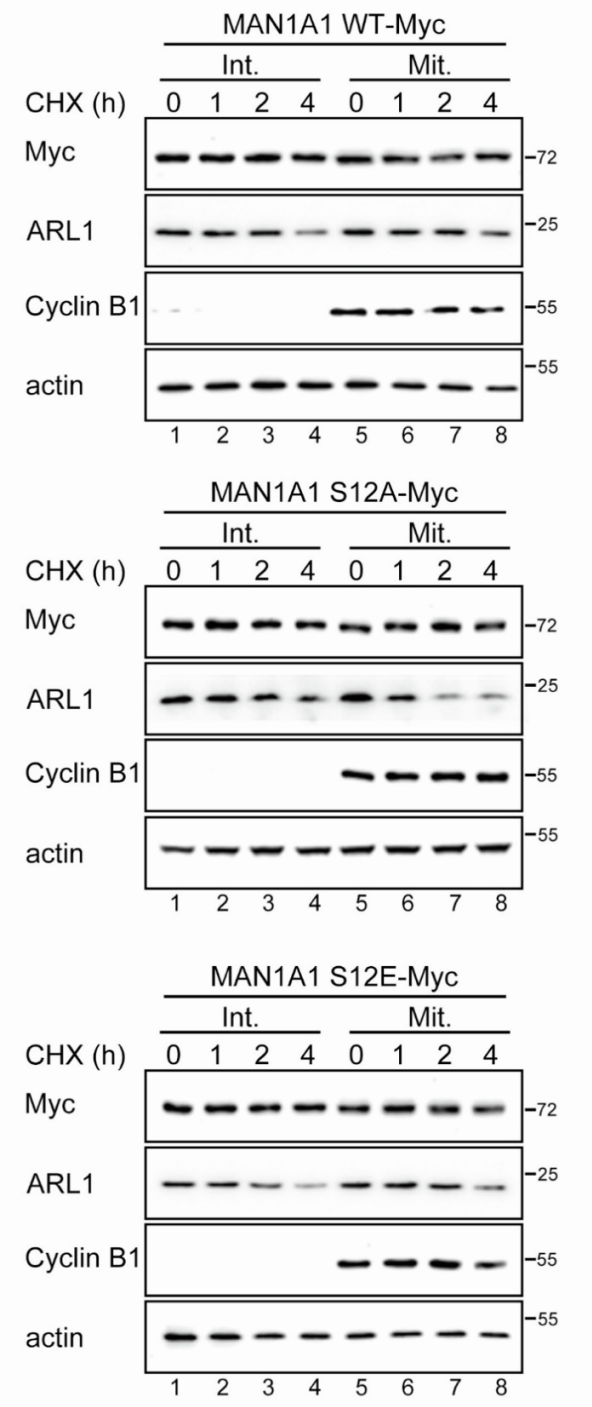

Figure S6. S12 mutation does not affect MAN1A1 stability in interphase and mitotic cells. Related to Figure 7.

Interphase (Int.) and mitotic (Mit.) HeLa cells expressing WT, S12A, or S12E MAN1A1-myc were treated with cycloheximide (CHX) for indicated times and analyzed by western blotting of myc, cyclin B1, actin, and a Golgi protein ADP-ribosylation factor-like protein 1 (ARL1). Note that MAN1A1 and its mutants are all stable, while ARL1 reduces its protein level over time upon protein synthesis inhibition by CHX.