

violet (in 30% methanol) for 15 min. After extensively washing with H<sub>2</sub>O, DNA-bound dye was extracted with 1 mL of 10% acetic acid and measured for optical density (OD) at 590 nm. The cell numbers at different days were normalized to the cell number from the first measurement. The results were shown as the mean  $\pm$  SEM,  $n = 3$ .

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1: Expression of non-regulatable GRASP65 mutants enhances Golgi stacking in interphase cells.** Bar charts of Figure 2K indicating the actual frequency of each value of number of cisternae per stack in HeLa cells expressing GFP or indicated GFP-tagged GRASP65 constructs. Note that the expression of non-regulatable GRASP65 mutants, but not the wild-type protein, increased the number of cisternae in the stacks.

**Figure S2: Knockdown of GRASP65 reduces the number of cisternae per stack, which can be rescued by expression of exogenous GRASP65.** A) Bar charts of Figure 5Q showing the actual frequency of Golgi cisternal numbers in the stacks in HeLa cells transfected with control or GRASP65 siRNA. Note the shift of the bars to lower numbers when GRASP65 was depleted (lower panel) compared with the control siRNA-transfected cells (upper panel). B) Bar charts of Figure 5R showing the actual frequency of Golgi cisternal numbers in the stacks in HeLa cells in which endogenous GRASP65 was replaced by GFP, rat GRASP65 or its mutants, as indicated. Note that the expression of GRASP65 and its mutant increased the number of cisternae in the stacks compared to the GFP cell line.

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