Supplemental materials

Fig. S1. Golgi targeting of C2GnT-M and C1GalT1 did not require Sar1a or Sar1b. (A,B) Confocal immunofluorescence images of Panc1-bC2GnT-M-c-Myc cells labeled with green (anti-c-Myc or anti-C1GalT1 Abs) and red (anti-Sar1a and anti-Sar1b Abs) fluorescence in cells treated with scramble siRNA. (C¬-F) Images of cells labeled with green (anti-c-Myc or anti-C1GalT1 Abs) and red (anti-Giantin and anti-Sar1 Abs) fluorescence in cells treated with Sar1a or Sar1b siRNA. Scale bar, 10 μm. (G) Sar1a and Sar1b western blots of the lysates of cells treated with scramble, Sar1a, or Sar1b siRNA, respectively. β-actin was used as a loading control. (H) Quantification of C2GnT-M and C1GalT1 immunofluorescence signal of non-Golgi vs. Golgi (=100%) in cells treated with scramble or protein-specific siRNA.

Fig. S2. β-COP depletion did not prevent Golgi targeting of C1GalT1. Panc1-bC2GnT-M-c-Myc cells were transfected with scramble or β-COP-specific siRNA. After 3 d, confocal immunofluorescence microscopy images were analyzed for colocalization of C1GalT1 with Giantin (A) or with β-COP (B). White boxes indicate areas enlarged and shown in the inset. Scale bar, 10 μm. (C) Quantification of C1GalT1 immunofluorescence signal of non-Golgi vs. Golgi (=100%) in cells treated with scramble or β-COP-siRNA. (D) β-COP western blots of the lysates of cells treated with scramble or β-COP siRNA. β-actin was used as a loading control.

Movie S1. Time lapse images of C2GnT-M-GFP and C1GalT1-RFP in live HEK293 cells. The C2GnT-M-GFP and C1GalT1-RFP spots were originated from opposite sides of cell periphery, and moved toward and fused with the Golgi at 73.67 s. For simplicity, the speed of movie has been increased.

Movie S2. The fluorescence recovery of C2GnT-M-GFP after photobleaching. This movie shows the images of fluorescence signal recorded every 8 s. Complete restoration of C2GnT-M-GFP fluorescence was detected at 168 s. For simplicity, the speed of movie has been increased.

Movie S3. The fluorescence recovery of C1GalT1-RFP after photobleaching. This movie shows the images of fluorescence signal recorded every 8 s. Complete restoration of C1GalT1-RFP fluorescence was detected at 240 s. For simplicity, the speed of movie has been increased.

Figure S1.

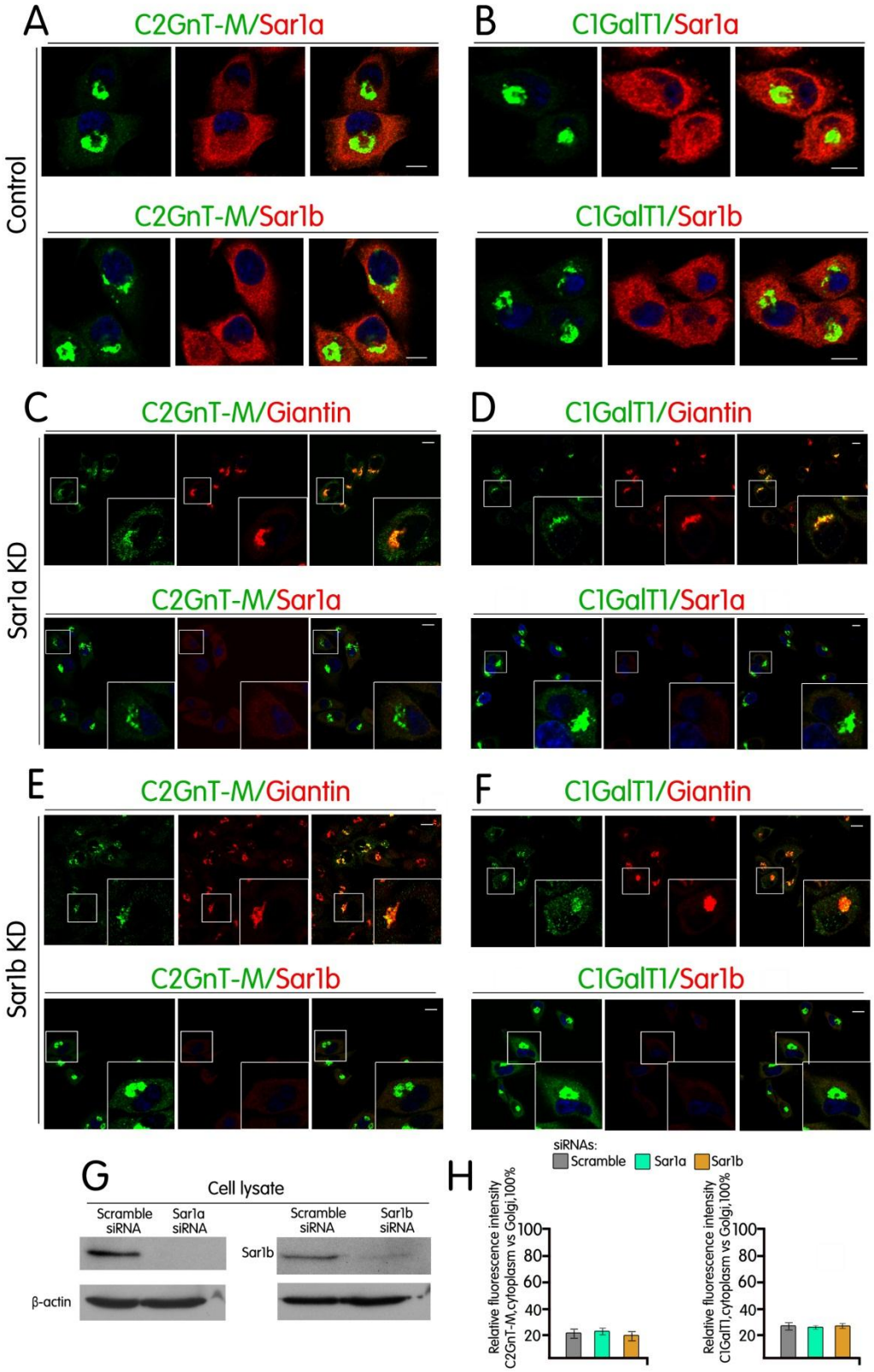
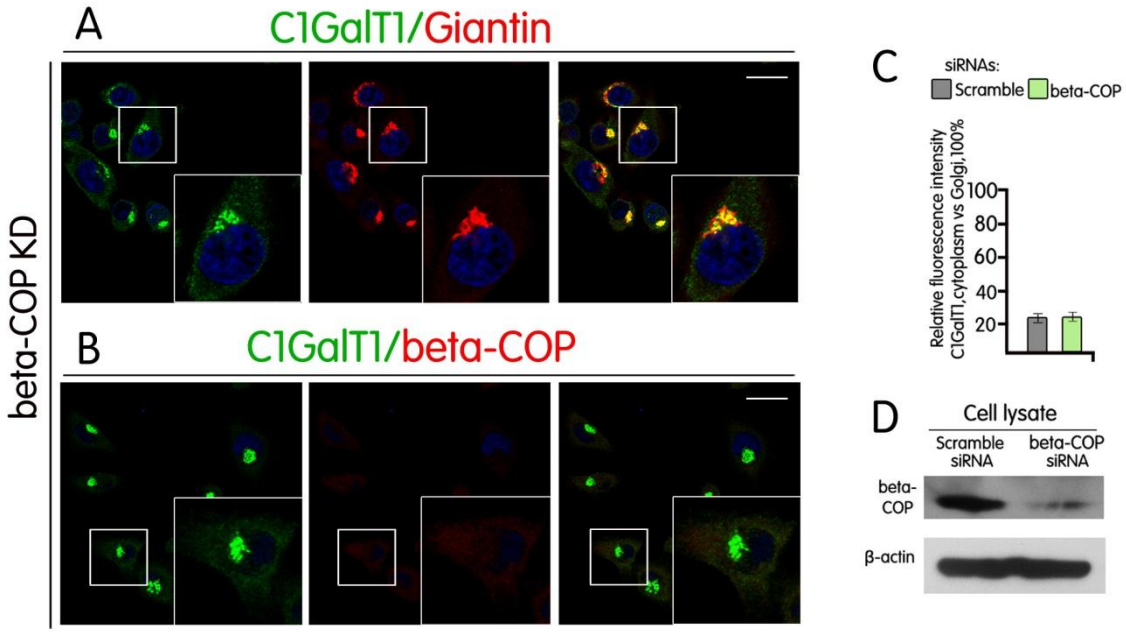

A
C2GnT-M/Sar1a
B
C1GalT1/Sar1a
Control
C2GnT-M/Sar1b
C1GalT1/Sar1b
C
C2GnT-M/Giantin
D
C1GalT1/Giantin
Sar1a KD
C2GnT-M/Sar1a
C1GalT1/Sar1a
E
C2GnT-M/Giantin
F
C1GalT1/Giantin
Sar1b KD
C2GnT-M/Sar1b
C1GalT1/Sar1b
G
Cell lysate
Scramble siRNA
Sar1a siRNA
Scramble siRNA
Sar1b siRNA
Sar1b
β-actin
H
siRNAs:
Scramble
Sar1a
Sar1b
Relative fluorescence intensity C2GnT-M,cytoplasm vs Golgi,100%
Relative fluorescence intensity C1GalT1,cytoplasm vs Golgi,100%
100
80
60
40
20

Figure S2.


A
C1GalT1/Giantin
B
C1GalT1/beta-COP
beta-COP KD
C
siRNAs:
Scramble
beta-COP
Relative fluorescence intensity
C1GalT1,cytoplasm vs Golgi,100%
100
80
60
40
20
D
Cell lysate
Scramble siRNA
beta-COP siRNA
beta-COP
β-actin