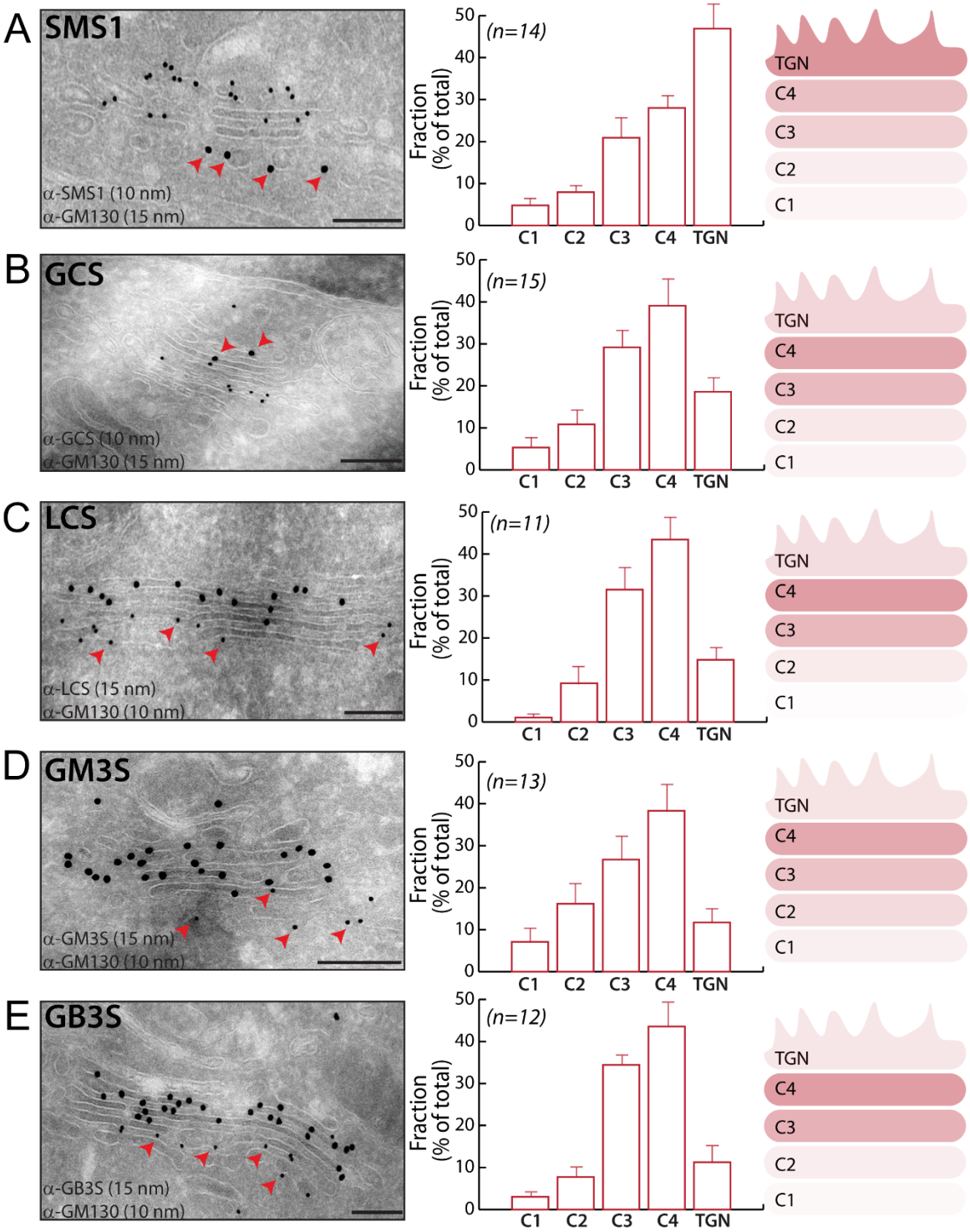
Appendix Information:

Title: **GRASP55 regulates intra-Golgi localization of glycosylation enzymes to control glycosphingolipid biosynthesis**

Table of Contents

Appendix Figure S1……2
Appendix Figure S2……4
Appendix Figure S3……6
Appendix Figure S4……8
Appendix Figure S5……10
Appendix Figure S6……12
Appendix Figure S7……14
Appendix Figure S8……16
Appendix Figure S9……18
Appendix Figure S10……20
Appendix Figure S11……22
Appendix Table S1……24
Appendix Table S2……24
Appendix Table S3……25
Appendix Table S4……28
Appendix Table S5……31
Appendix Table S6……32
Appendix Table S7……33
Appendix Table S8……34

Appendix Figure S1

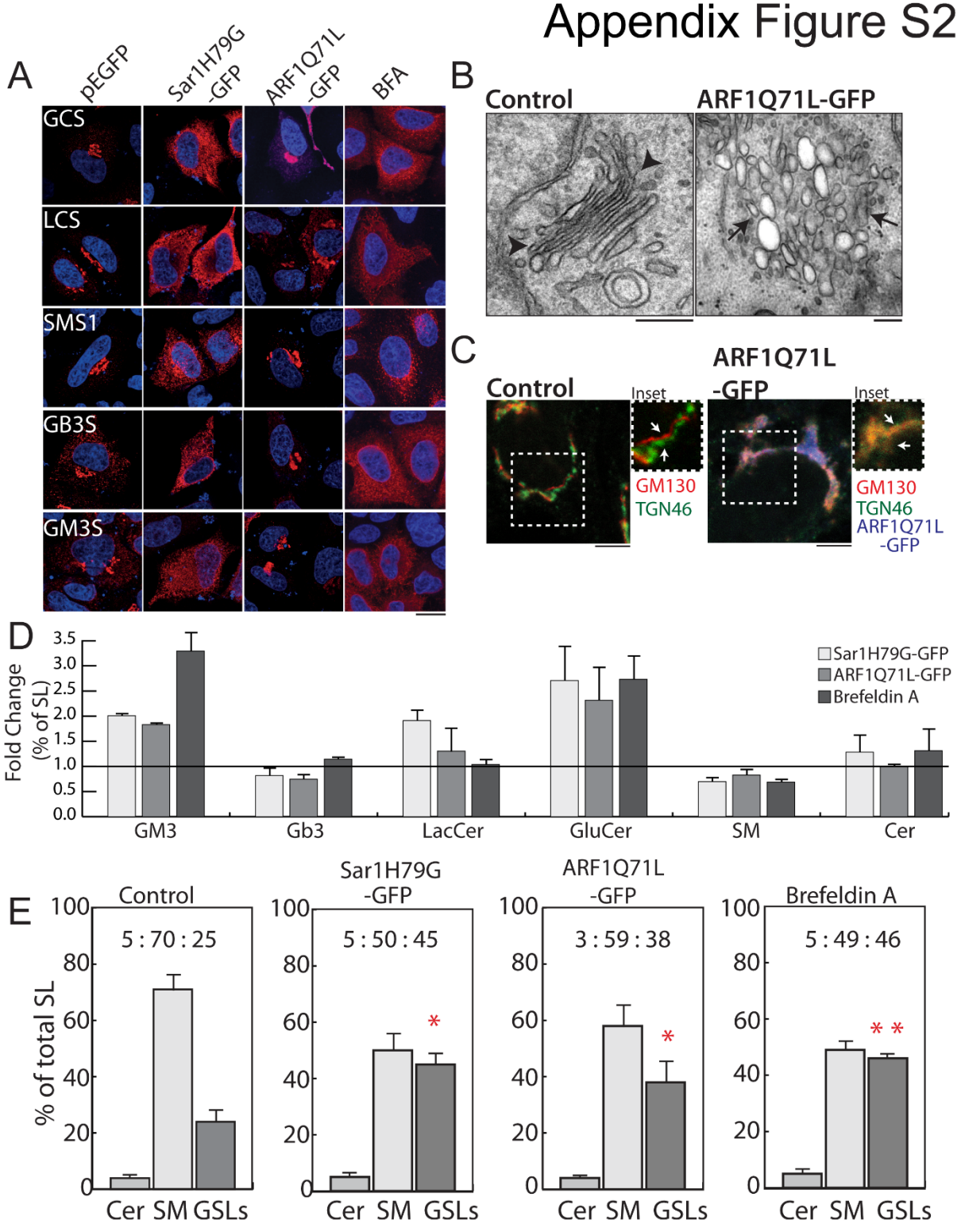


Appendix Figure S1. Distribution of SL biosynthetic enzymes in Golgi:

(A-E) HeLa cells were transfected with indicated HA-tagged SL biosynthetic enzymes for 16 hours, fixed and processed for cryoimmunolabeling with anti-HA and anti-GM130 antibodies followed by Protein A-gold. HA and GM 130 are represented by 10-

and 15-nm gold particles in case of SMS1 and GCS while in case of LCS, GB3S and GM3S they correspond to 15- and 10-nm gold particles respectively. Red arrow heads indicate cis face of Golgi marked by GM130 labelling. Enzyme distribution expressed as fraction of total gold particles per Golgi stack including TGN. (n indicated in the graph); data are mean ± SEM, Scale Bar, 200nm.

Appendix Figure S2



Appendix Figure S2. Golgi organization determines faithful SL output:

(A) HeLa cells were transfected with indicated HA-tagged SL biosynthetic enzymes and either empty vector (PEGFP) or a plasmid encoding Sar1H79G-GFP or ARF1Q71L-GFP for 16 hours or treated with brefeldin A (BFA) (5μg/ml) for 30 min,

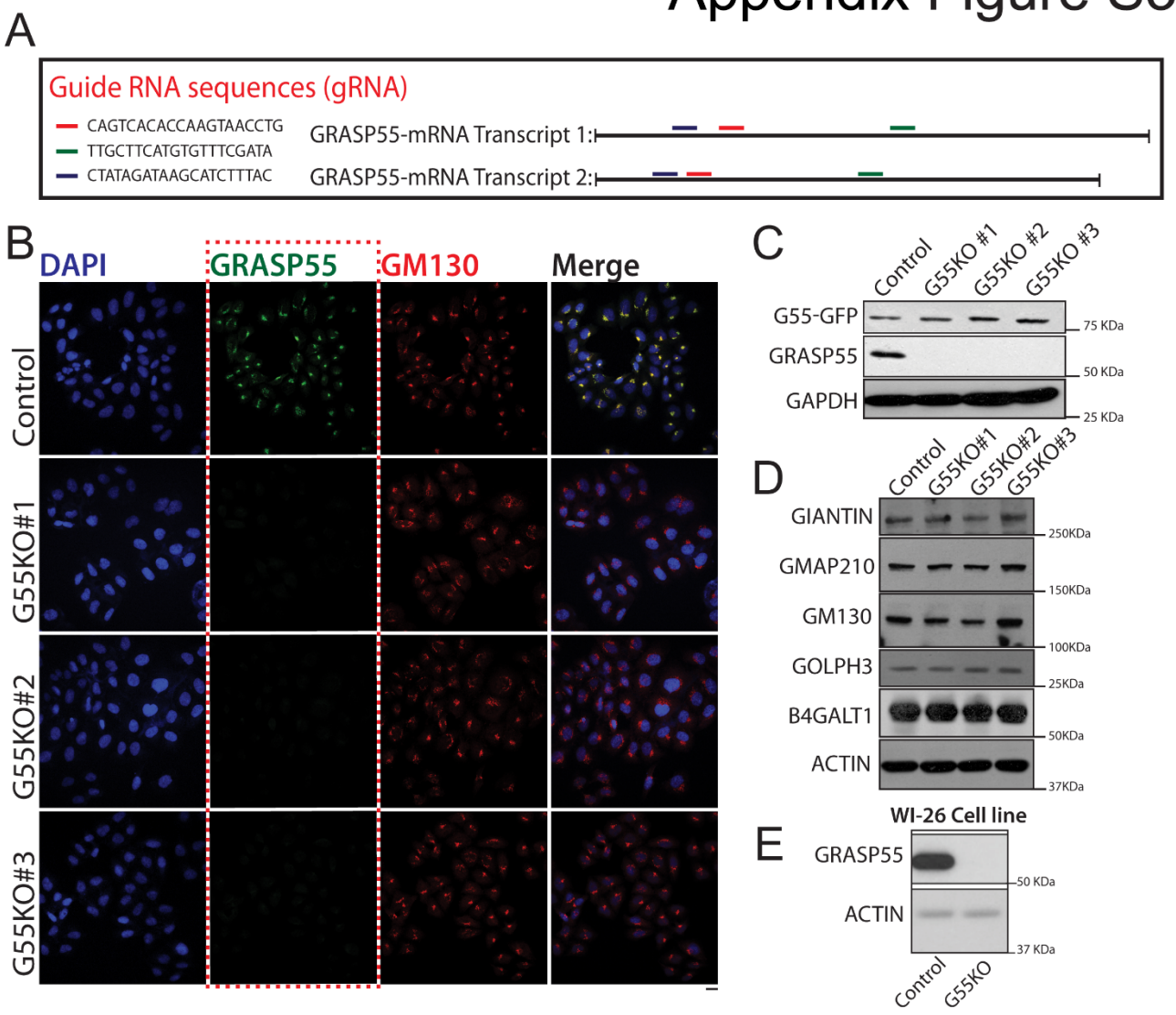
fixed, permeabilized and stained with DAPI (blue) and anti-HA antibody (red). Scale bar, 10 μm.

(B) HeLa cells transfected with PEGFP or ARF1Q71L-GFP for 16 hours were fixed and processed for electron microscopy. Black arrow heads indicate the intact Golgi and black arrows represent the tubulo-vesicular clusters. Scale Bar, 200nm.

(C) HeLa cells were transfected with ARF1Q71L-GFP for 16 hours, fixed, permeabilized and stained with anti-GM130 and anti-TGN46 antibodies. GM130 is represented in red, TGN46 in green, and GFP in blue. White arrows indicate the separation of cis and trans markers of the Golgi in control cells and their overlap in ARF1Q71L-GFP expressing cells. Scale bar, 10 μm.

(D-E) SL species quantified by radioactive pulse chase assay in HeLa cells transfected with Sar1H79G-GFP or ARF1Q71L-GFP or treated with BFA and represented as fold change with respect to control (**D**) or as relative percentage of Cer, SM and GSLs **(E)**. For BFA treated cells, the SL output was measured 8h after a pulse. Data represented as mean ± SD of 2 independent experiments. *P< 0.05, **P<0.01 (Student's t test).

Appendix Figure S3



Appendix Figure S3. Generation of GRASP55KO cell lines:

(A) Specific guide RNA oligonucleotides for human GRASP55 gRNA #1, #2 and #3 used for CRISPR/Cas9-mediated deletion of GRASP55 expression. The sequences of oligonucleotides are shown and the sites in GRASP55 transcripts to which the gRNA correspond are indicated.

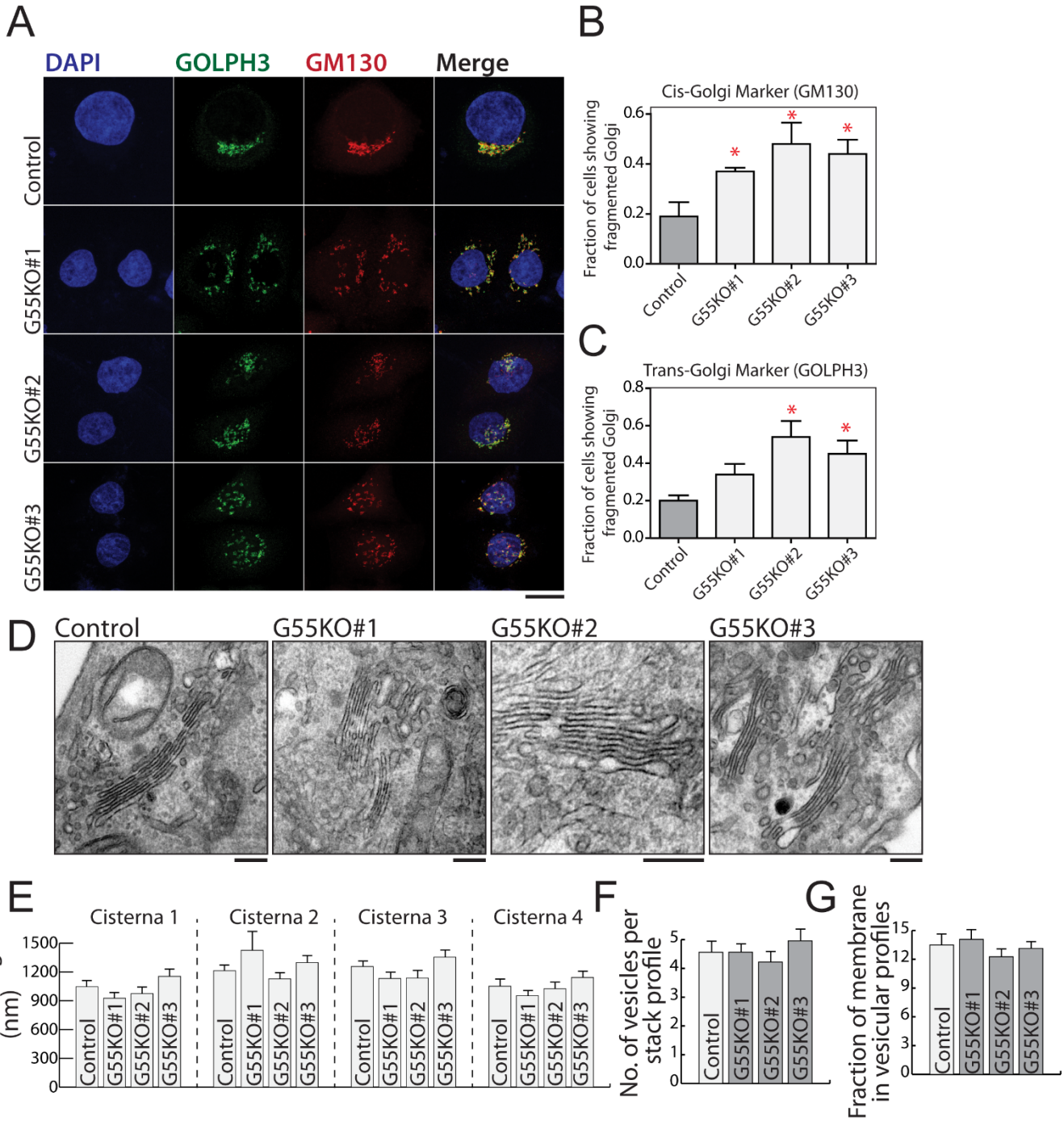
(B) The control and GRASP55 KO clones were fixed, permeabilized and stained with DAPI (blue), anti-GM130 antibody (red) and anti-GRASP55 antibody (green). Scale bar, 10 μm.

(C) Control and GRASP55 KO clones transfected with GRASP55-GFP were analysed by Western blotting with anti-GRASP55 and anti-GFP antibody.

(D) Cell lysates from control and GRASP55 KO clones were analysed for the expression of indicated Golgi-Matrix proteins by western blotting.

(E) Cell lysates of wild type and GRASP55 KO fibroblasts were analysed by western blotting for GRASP55 expression.

Appendix Figure S4



Appendix Figure S4. Characterization of Grasp55 KO clones:

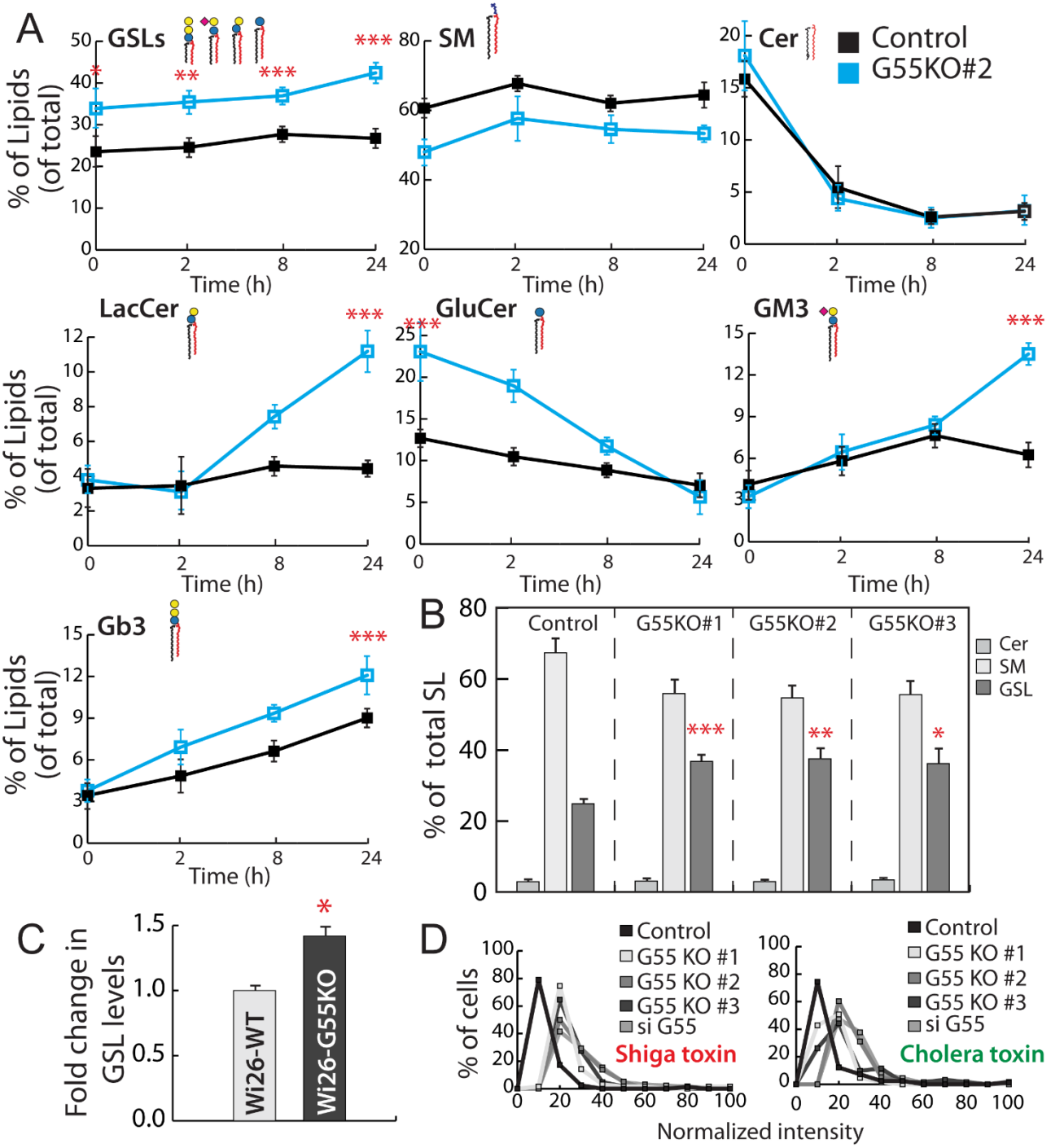
(A) Control and GRASP55 KO clones were fixed, permeabilized and stained with DAPI (blue), anti-GM130 antibody (red) and anti-GOLPH3 antibody (green). The images represent the maximum intensity projection along the z-axis of 7 consecutive confocal sections. Scale bar, 10 μm.

(B-C) The graphs represent the fraction of cells displaying fragmented Golgi in control and GRASP55 KO clones. Values are mean ± SD of 2 independent experiments. *p <0.05 (Student's t test).

(D) Control and GRASP55 KO cells were fixed and prepared for EM. The Golgi profile shown suggests no significant alterations in the stack architecture of Golgi. Scale bar corresponds to 200nm.

(E-G) Control and GRASP55 KO micrographs were subjected for morphological evaluation of the Golgi stacks. (E) Quantification of the length of cisternae of Golgi stacks in control and GRASP55 KO clones. (F-G) The number of vesicles associated with Golgi stacks and fraction of membrane in vesicular profiles were quantified and represented. All the EM micrographs, E-G, at least 30 Golgi stacks across three biological replicates were quantified. Error bars represent the SEM.

Appendix Figure S5



Appendix Figure S5. GRASP55 regulates GSL biosynthesis:

(A) Control and GRASP55 KO (#2) cells were subjected to radioactive pulse chase assay as described earlier, the indicated SL species were quantified and are represented as percentage of total SLs. X-axis represents chase time. Data

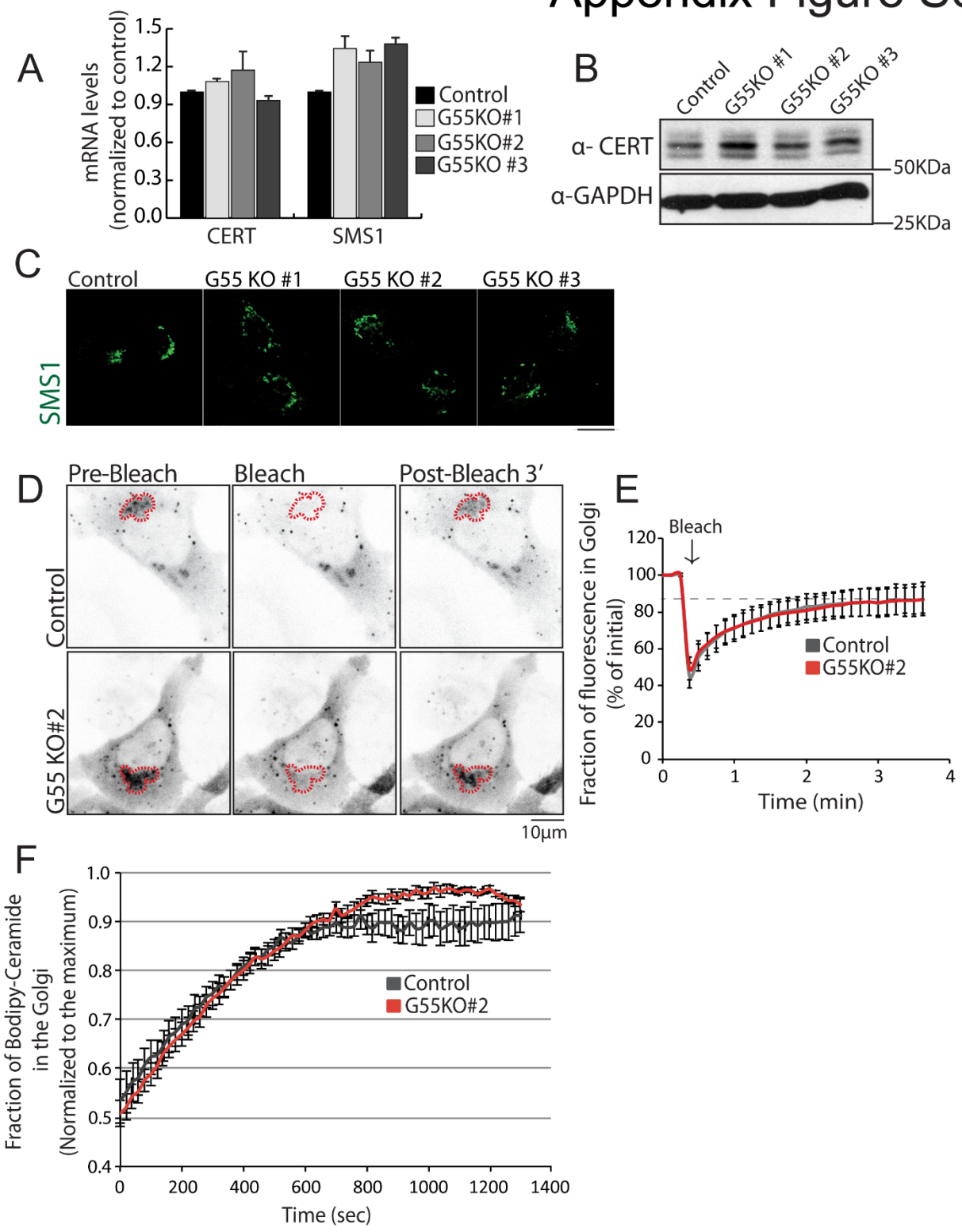
represented as mean ± SD of 3 independent experiments. *p <0.05, **p <0.01, ***p <0.001 (Student's t test).

(B) Control and GRASP55 KO cells were subjected to radioactive pulse chase assay as described earlier. SL levels were quantified after 24h of chase and represented as percentage of total SLs. Data represented as mean ± SD of 3 independent experiments. Data represented as mean ± SD of 2 independent experiments. *p <0.05, **p <0.01, ***p <0.001 (Student's t test).

(C) SL production in control or GRASP55 KO fibroblasts was measured by [^{3}H] - sphingosine pulse-chase assay, and total GSL levels are expressed as fold changes with respect to control. Data represented as mean ± SD of 2 independent experiments. *p <0.05 (Student's t test).

(D) Effect of GRASP55 depletion on GSL levels measured by Cy3-conjugated ShTxB (Shiga Toxin) and Alexa488-conjugated ChTxB (Cholera Toxin) staining in control and GRASP 55 KO cells or cells treated with GRASP55 siRNA. The cells were imaged by epifluorescence microscopy and intensity of the fluorescence staining quantitated. Frequency distribution of level of STxB and CTxB staining is represented with intensities normalized to maximum intensity for each condition (>100 cells per condition were analysed).

Appendix Figure S6



Appendix Figure S6. GRASP55 does not regulate SM biosynthetic arm of SL biosynthetic pathway:

(A) The mRNA levels of CERT and SMS1 were evaluated by qRT-PCR (values are mean ± SD; n=3).

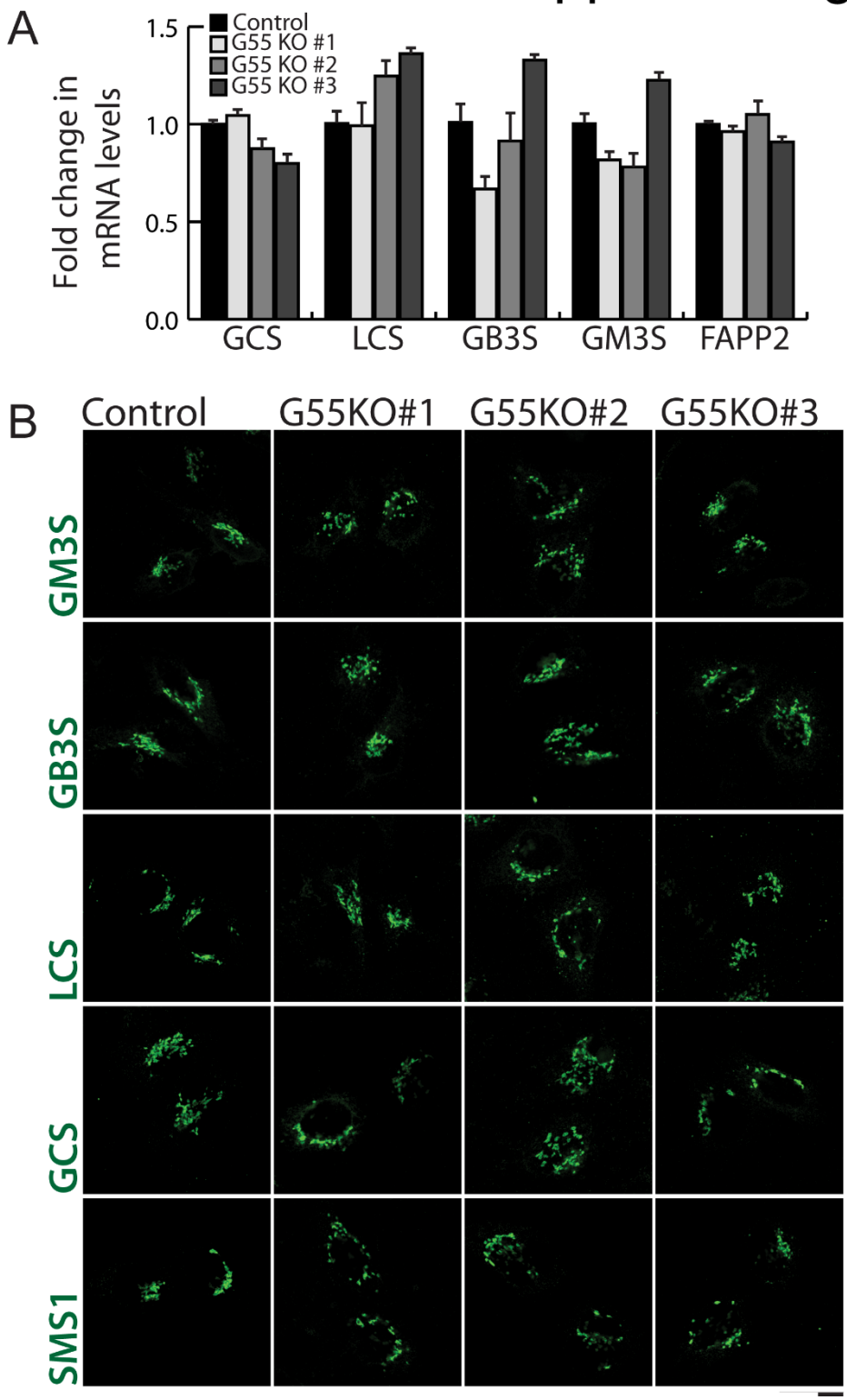
(B) Control and GRASP55 KO cell protein lysates were analysed by western blotting for CERT expression.

(C) Control and GRASP55 KO cells expressing SMS1-HA were fixed, and processed for immunofluorescence with anti-HA antibody (green). Scale Bar, 10 μm.

(D-E) Control cells and GRASP55 KO (#2) cells were transfected with CERT-GFP for 16 hours, the area of Golgi indicated by the red dotted line was bleached and the recovery of fluorescence was observed by live epifluorescence imaging. Representative images of indicated times are shown (**D**). The ratio of fluorescence of the bleached area to an adjacent unbleached area was measured for each time point, normalized to initial values and plotted in the indicated graph (**E**). Scale bar, 10 μm.

(F) Kinetics of Ceramide transport to Golgi was studied using BODIPY labelled-C6 ceramide in GRASP55 KO cells. Cells were labelled with BODIPY C6 ceramide (10 μM) for 30 min at 4ºC. The cells were then washed and shifted to 37ºC in the microscope and analysed by epifluorescence microscopy. The perinuclear concentration of fluorescence signal was quantified and plotted.

Appendix Figure S7

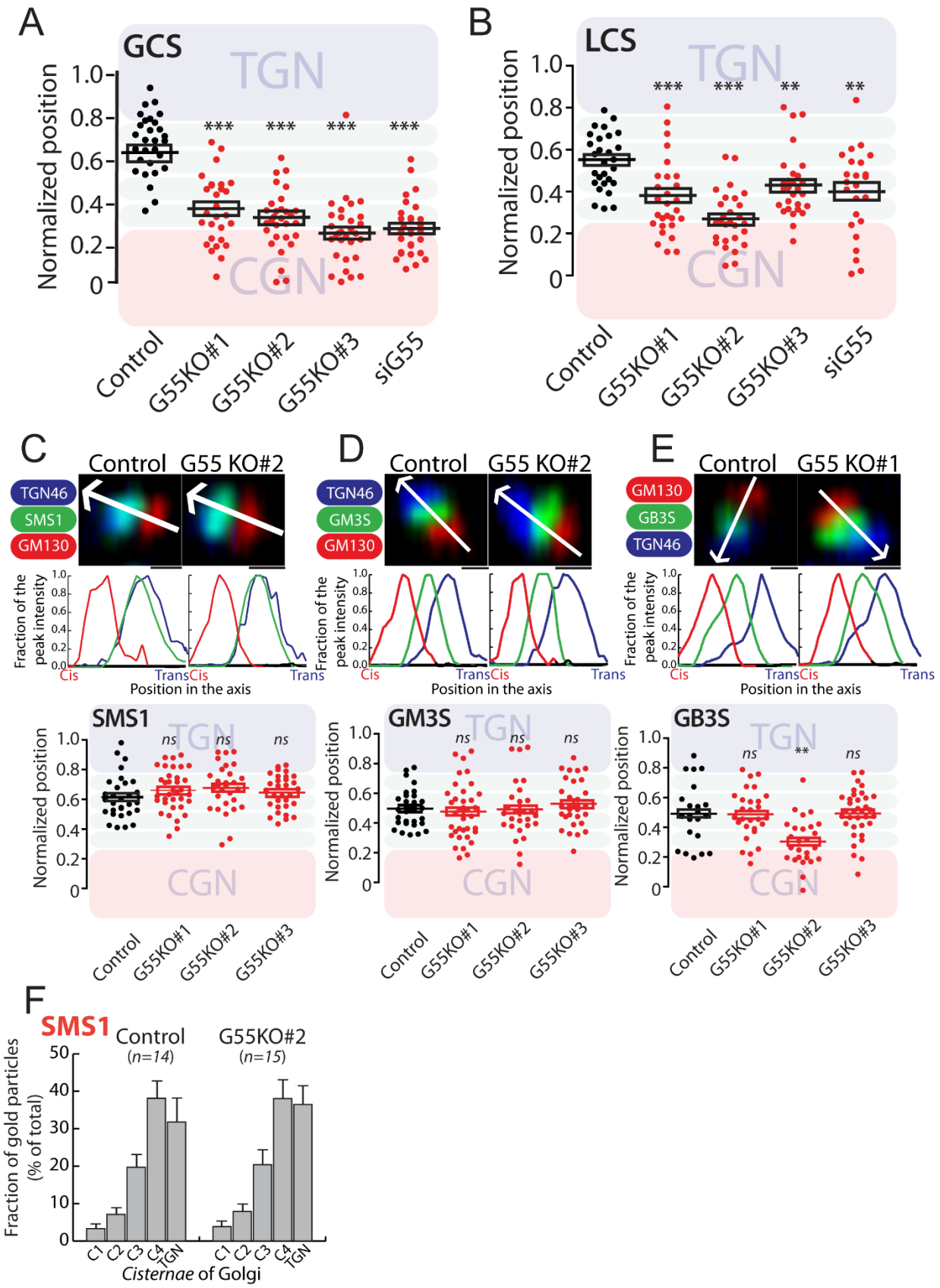


Appendix Figure S7. GRASP55 does not regulate the levels or cellular localization of GSL biosynthetic enzymes:

(A) Expression of GSL biosynthetic enzymes in control and GRASP55 KO clones were analysed by qRT-PCR. Values are mean ± SD; n=3

(B) Control and GRASP55 KO clones were transfected with the indicated HA-tagged GSL biosynthetic enzymes for 16 hours, fixed, permeabilized and stained with anti-HA antibody (Green). Scale bar, 10 μm. Note the image corresponding to SMS1 is the same as in **Fig.S6C**.

Appendix Figure S8

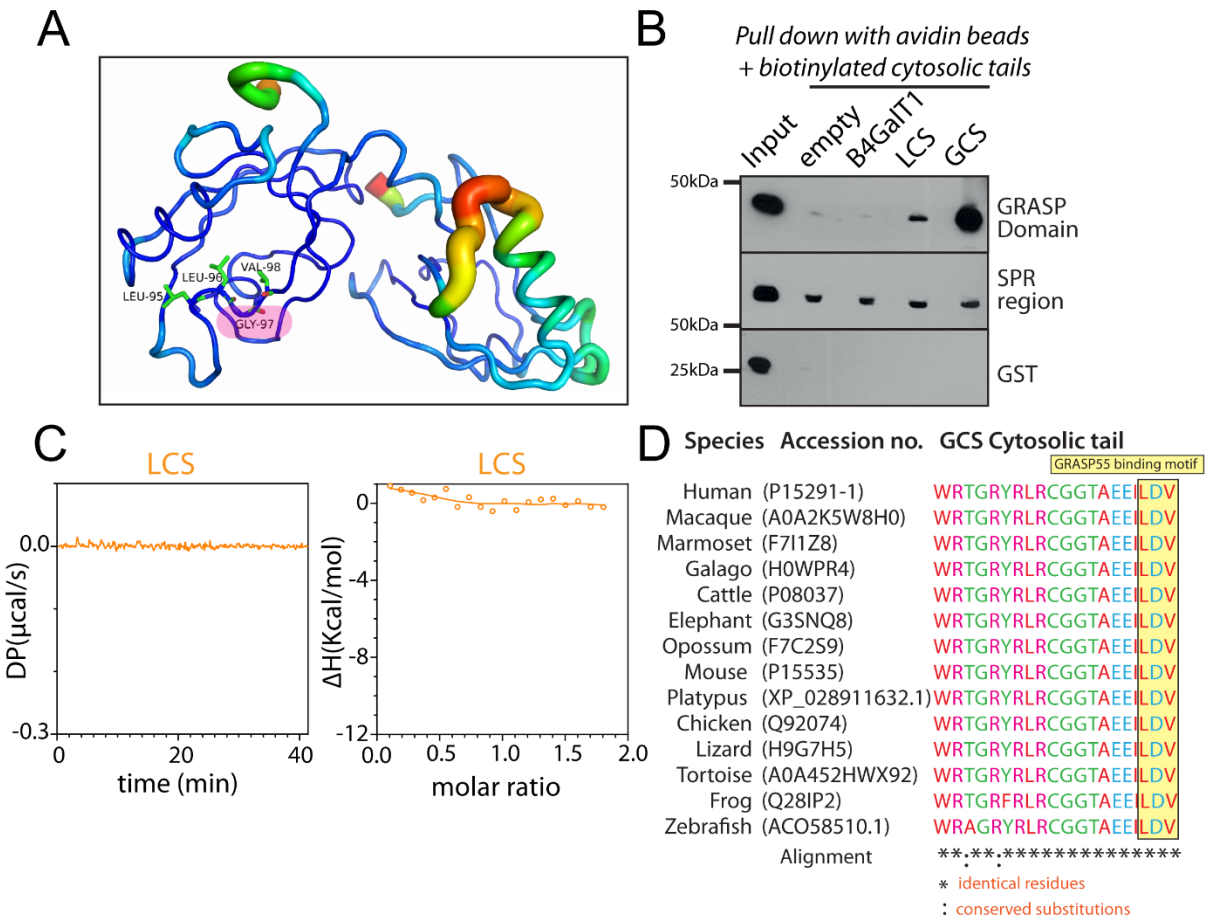


Appendix Figure S8. GRASP55 regulates intra-Golgi localization of GCS and LCS:

(A-E) Control cells treated with or without GRASP55 siRNA and GRASP55 KO clones were transfected with HA-tagged GSL biosynthetic enzymes, treated with nocodazole (33 μM) for 3 hours, and processed for immunofluorescence with anti-HA, anti-GM130, and anti-TGN46 antibodies. The relative position of HA-tagged enzymes with respect to GM130 and TGN46 was measured by line scanning and expressed as normalized positions of the peak intensity with peak of GM130 set to 0 and that of TGN46 to 1. The images are representative of >30 stacks analysed for each condition from 3 independent experiments. The data are mean ± SD (n=30) representative of 3 experiments. **p <0.01, ***p <0.001(Student's t test) and *ns* signifies not statistically significant.

(F) Control and GRASP55 KO cells were transfected for 16 hours with SMS1-HA and processed for cryoimmunolabeling. Distribution of indicated enzymes across the Golgi stack was quantified and represented as fraction of Gold particles in each cisterna (n indicated in the graph; data are Mean ± SEM).

Appendix Figure S9



Appendix Figure S9. GRASP55 directly interacts with GCS and LCS to promote their sub-Golgi localization:

(A) B-factor analysis of the high-resolution crystal structure of complex GRASP55:Golgin45, revealed that the cognate carboxylate binding motif is rigid in nature. Glycine is more favoured such a 'strained conformation' (left handed alpha helical), where the loop is rigid. Asp is not a favoured residue to be in left-handed α helical conformation. It can be predicted, in the absence of mutant crystal structure, that there has been reorientation in the backbone conformation, which may have led to the disruption of the characteristic hydrogen bond network between the protein and peptide.

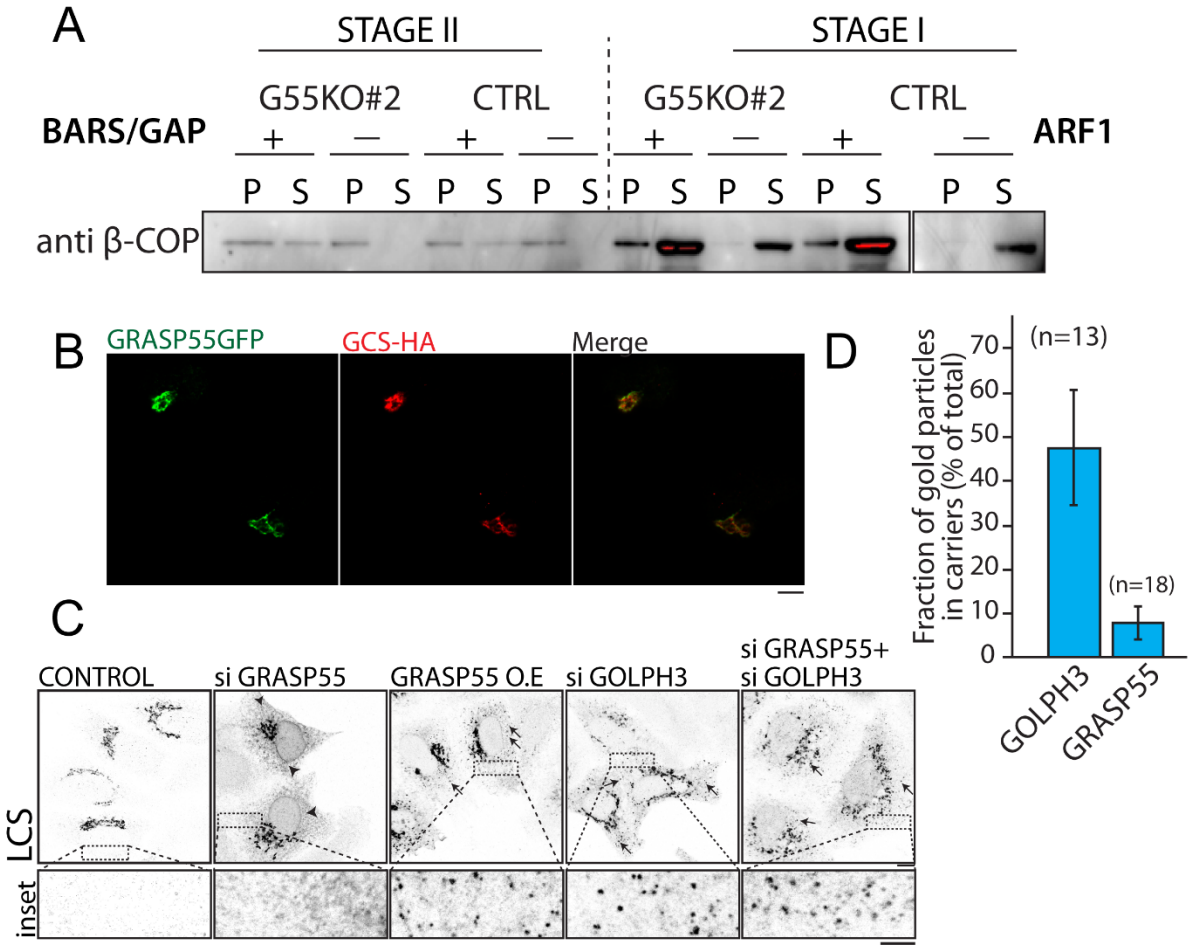
(B) Chemically synthesized biotinylated peptides corresponding to cytosolic portions of glycosylation enzymes and indicated purified GST-tagged GRASP domain or SPR region of GRASP55 were incubated together and their interaction was monitored by

pulling down the biotinylated peptides with avidin beads followed by western blotting with anti-GST tag antibody.

(C) ITC profile, representative of at least 3 independent experiments, for biotinylated LCS cytosolic tails with recombinant GRASP55.

(D) The last 20 amino acids of the cytoplasmic tail of GCS orthologs from indicated species of jawed vertebrates were retrieved from UniProt (accession numbers indicated in bracket). The alignment of the sequences was done using the web version of the Clustal Omega software (Madeira, Park et al., 2019) at http://www.ebi.ac.uk/Tools/msa/clustalo. GRASP55 binding motif is highlighted in yellow.

Appendix Figure S10



Appendix Figure S10. GRASP55 compartmentalizes the enzymes by preventing their entry into retrograde carriers:

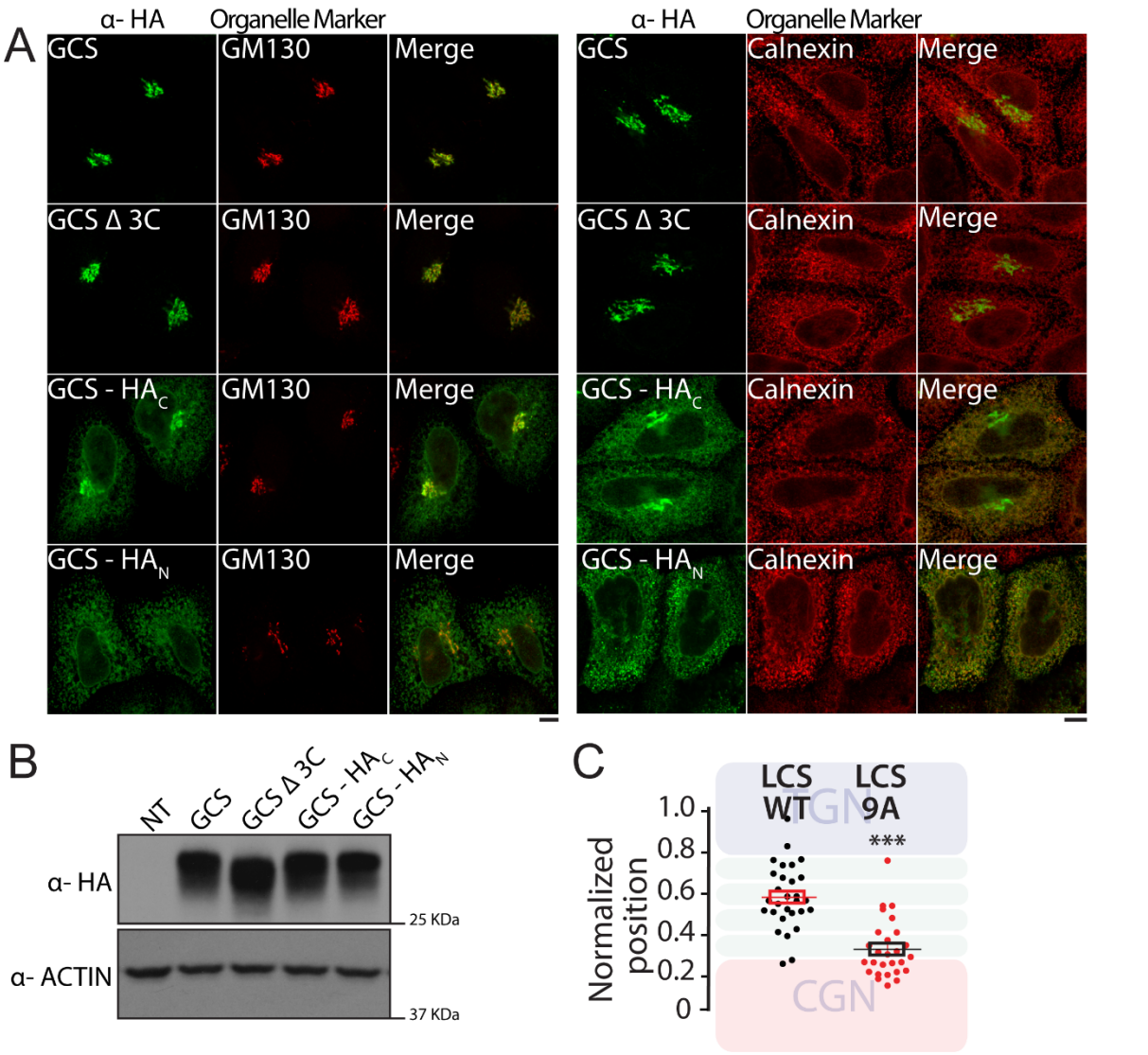
(A) The two-stage incubation system was performed to reconstitute COPI vesicles. The first-stage incubation results in the ARF1-dependent recruitment of coatomer to Golgi membrane, as reflected by β-COP being redistribution from the supernatant (soluble) fraction to the pellet (Golgi membrane) fraction. The second-stage incubation results in the generation of COPI vesicles from Golgi membrane, as reflected by β-COP being redistributed from the pellet (Golgi membrane) fraction to the supernatant (vesicular membrane) fraction.

(B) HeLa cells co-transfected with GCS-HA and GRASP55-GFP tagged constructs were fixed, premeabilized and stained with anti-HA antibody (Green) Scale bar, 10μm.

(C) HeLa cells treated with indicated siRNAs and transfected with LCS-HA or co-transfected with GRASP55-GFP and LCS-HA were fixed, permeabilized and stained for LCS-HA (indicated in black). Spots of LCS-HA marked by black arrows and inset (shown below) indicate possible post-Golgi compartments. Scale bar, 10μm.

(D) HeLa cells were fixed and processed for cryoimmunolabeling with an anti-GRASP55 antibody and anti-GOLPH3 antibody. Quantification of the distribution (LD normalised to the Golgi stack) of GRASP55 and GOLPH3 in peri-Golgi vesicles (carriers) is shown in the graph data are means ± SEM.

Appendix Figure S11



213

214 **Appendix Figure S11. Localization of GCS WT and mutants, sub-Golgi**
215 **localization of LCS WT and mutant:**

216 **(A)** HeLa cells transfected with indicated GCS constructs were fixed, permeabilized
217 and stained with anti-HA antibody (Green) and indicated organelle markers (red).
218 Scale bar, 10μm.

219 **(B)** HeLa cells transfected with indicated GCS constructs and protein lysates were
220 analysed by western blotting for expression of indicated constructs.

221 **(C)** HeLa cells were transfected with either WT LCS, or LCS9A, treated with
222 nocodazole (33 μM) for 3 hours and labelled for enzymes, GM130, and TGN46. Line

scan analysis was performed as in Fig.3A-B and the relative position of enzymes was quantitated and plotted. The data are mean ± SD (n=30) representative of 2 experiments. **p <0.01, ***p <0.001(Student's t test) and *ns* signifies not statistically significant.

Appendix Tables

Appendix Table S1 gRNAs used in this study to generate GRASP55 knockout cell lines.

gRNA	Sequence		
#1 GUIDE RNA	CAGTCACACCAAGTAACCTG	Santa Cruz Biotechnology, Inc	Cat # SC-401106;
#2 GUIDE RNA	TTGCTTCATGTGTTTCGATA	Santa Cruz Biotechnology, Inc	Cat # SC-401106
#3 GUIDE RNA	CTATAGATAAGCATCTTTAC	Santa Cruz Biotechnology, Inc	Cat # SC-401106

Appendix Table S2 List of recombinant DNA used in this study

Recombinant DNA	Source	
GRASP55-EGFP	Kind gift from Yanzhuang Wang	*N/A*
GB3S	Kind gift from Antonella De Matteis	*N/A*
GM3S	Kind gift from Antonella De Matteis	*N/A*
LCS	Kind gift from Antonella De Matteis	*N/A*
GCS-HA_C	Kind gift from Giovanni D'angelo	*N/A*
SMS1	Kind gift from Giovanni D'angelo	*N/A*
CERT	Kind gift from Antonella De Matteis	*N/A*
PEGFP	Addgene	Cat # 6085-1
Sar1-GTP	Kind gift from Rainer Pepperkok	*N/A*
ARF1-GTP	Kind gift from Antonella De Matteis	*N/A*
Str-KDEL_B4GALT5-SBP-EGFP (referred as LCS WT)	This study.	*N/A*

Str-KDEL_B4GALT5-SBP-EGFP (referred as and LCS9A)	This study.	*N/A*
GCS-HA_N	This Study (custom made from genescript)	*N/A*
GCS	This Study	*N/A*
GCS Δ3C	This Study	*N/A*
pGEX6p-1GRASP55 N	Kind gift from Yanzhuang Wang	*N/A*
pGEX4T-1GRASP55 C	Kind gift from Yanzhuang Wang	*N/A*
pGEX6p-1GRASP55 G97D	This Study	*N/A*
GRASP55-His tag	Kind gift from Antonino Colanzi	*N/A*
GRASP65-His tag	Kind gift from Antonino Colanzi	*N/A*

Appendix Table S3 siRNA sequences used in this study to downregulate indicated human gene expression

Human Gene	**Accession Number**	**siRNA Sequence**
Giantin/GOLGB1	NM_004487	#1 5'-GAACUAGAGUCUCGGUAUA-3' #2 5'-UAAGAGAAUUGCAACCUAA-3' #3 5'-GUACACAGGUUAAGUGCUU-3' #4 5'-GAAGGUCUGUGAUACUCUA-3'
GM130/GOLGA2	NM_004486	#1 5'-GGACAAUGCUGCUACUCUA-3' #2 5'-AGAAGGAGGUGCUGCAUAA-3' #3 5'-GAAUAUCAGCAGAGGAAUA-3'

		#4 5’-UUGUAAAGCUGACUAAUGA-3’
GRASP 65/GORASP1	NM_031899	#1 5’-GAUCUCUACCACAGAAUAA-3’ #2 5’-CUGGAGGUGUUCAAUAUGA-3’ #3 5’-GAGGACUUCUUUACGCUCA-3’ #4 5’-GGACGUGUCGGGAAUUUCU-3’
GRASP 55/GORASP2	NM_015530	#1 5’-GGAGUGAGCAUUCGUUUCU-3’ #2 5’-GUAAACCAGUCCCUCACUU-3’ #3 5’-GACCACACAGUGAUUAUAU-3’ #4 5’-UGUCGAGAAGUGAUUAUUA-3’
GMAP210/TRIP11	NM_004239	#1 5’-GGACAUUACUAAAGAGUUA-3’ #2 5’-GGGCAAGACUGGAGAGUUA-3’ #3 5’-GAACUUAAGGAGCAUAUUA-3’ #4 5’-GGACUUUGGUGAUAUAAUU-3’
CERT	NM_001130105	#1 5’-GAAGAUGACUUUCCUACAAUU-3’ #2 5’-GAAGUUGGCUGAAAUGGAAUU-3’ #3 5’-GCGAGAGUAUCCUAAAUUUUU-3’ #4 5’-UCAAAGGGAUAAAGUGGUAUU-3’
Golgin 97/GOLGA1	NM_002077	#1 5’- AAGAUCACAGCCCUGGAACAA -3’ #2 5’- AAGUGCUUCUCCAGAAAGAGC -3’
Golgin 45/BLZF1	NM_003666	#1 5’-UGUGAUGUAUGGCGAAGUA-3’ #2 5’-GAAUAUUAGUUCCCAAAGC-3’ #3 5’-GAACGUCUAGCCCGUGAGA-3’

		#4 5’-GAACAGUUAGAACGUAUGU-3’
GCC1	NM_024523	#1 5’-GGACUUGGAGCUUAGGUUA-3’ #2 5’-GGAGAAGGCUACUGCACUC-3’
GCC2	NM_181453	#1 5’-UCAAAGAGAUACCAUGUUA-3’ #2 5’-GAGCAGAGUUGAUACUAUU-3’
Golgin 160/GOLGA3	NM_005895	#1 5’-GCAAACAGCCCGUGGGAAA-3’ #2 5’-GAGAUGAAGACCAAACAUA-3’
Golgin 245/GOLGA4	NM_001172713	#1 5’-GAACUAACCUGUCAGAUUU-3’ #2 5’-GAGAACAGAUUCACAAUUU-3’
GOLPH3	NM_022130	1# 5’-AAAUGAUGUGUAACCCUCGCGGUCC-3’ 2 #5’-AAUCCAGAUGAUAUACAGUCAUUCC -3’ 6 #5’-GGAGAGGAAGGUUACAACUA-3’ 7# 5’-UCAAGGACCGCGAGGGUUA -3’
GOLIM4	NM_014498	#1 5’-GAACACAGAUCAAGAUUAG-3’ #2 5’-GCAGAUGACCCUAAUAAUC-3’
GOPC	NM_020399	#1 5’-GGCCUUGGCAUUUCAAUUA-3’ #2 5’-GGACAUCGUUACCGUUUGU-3’

237

GORAB	NM_152281	#1 5'-GCAGAGACCAUGAAACUAA-3' #2 5'-CAGCAAAGCUAGAUAUACA-3'
FAPP2	NM_001197026	#1 5'-GAGAUAGACUGCAGCAUAUUU-3' #2 5'-GAAUUGAUGUGGGAACUUUUU-3' #3 5'-GAAAUCAACCUGUAAUACUUU-3' #4 5'CCUAAGAAAUCCAACAGAAUU-3'
AllStars Negative Control siRNA	Qiagen	Cat #SI03650318

238

239 **Appendix Table S4** List of antibodies used in this study for immunofluorescence
240 (IF), Western Blotting (WB), cryo-Electron Microscopy (cryo-EM).

Antibodies	**SOURCE**	**IDENTIFIER**
Monoclonal Mouse GMAP210	BD Biosciences	Clone 15 Cat # 611712 RRID:AB_399190
Monoclonal Mouse GM130	BD Biosciences	Clone 35 Cat # 610822 RRID: AB_398141
Polyclonal Rabbit GIANTIN	Abcam	Cat # ab24586 RRID: AB_448163
Monoclonal Mouse GRASP65	Santa Cruz Biotechnology	Clone D12 Cat # sc-374423 RRID: AB_10991322
Polyclonal Rabbit GOLPH3	Abcam	Clone

		Cat # ab98023 RRID: AB_10860828
Polyclonal Rabbit B4GALT1	Sigma	Cat # HPA010807 RRID: AB_1078254
Monoclonal Mouse GAPDH	Santa Cruz Biotechnology	Clone 6C5 Cat #sc-32233 RRID:AB_627679
Polyclonal Rabbit GRASP55	Novus Biologicals	Cat #NBP1-89747 RRID:AB_11024556
Monoclonal Mouse HA-Tag	Biolegend/Covance	Clone 16B12 Cat #MMS-101P RRID:AB_10063630
Monoclonal Rabbit HA-Tag	Cell Signaling Technology	Clone C29F4 Cat #3724
Polyclonal Sheep anti-human TGN46	BioRad/AbD-Serotec	Cat #AHP500G RRID:AB_323104
Monoclonal Mouse anti-β ACTIN	Sigma	Clone AC-74 Cat #A2228 RRID:AB_476697
Monoclonal Mouse Calnexin	Santa Cruz Biotechnology	Clone AF18 Cat #sc-23954; RRID: AB_626783
Monoclonal Mouse GFP	Abcam	Cat #ab6556 RRID:AB_305564
GST	This study	N/A

Anti-polyHistidine	Sigma	Clone HIS-1 Cat #H1029, RRID:AB_260015
ShTxB-CY3	Dr. Ludger Johannes	*N/A*
ChTxB-AlexaFluor 488	Invitrogen	Cat # C-22841
Anti-mouse, donkey Alexa Fluor 488	ThermoFisher Scientific	Cat #A-21202 RRID:AB_141607
Anti-mouse, donkey Alexa Fluor 568	ThermoFisher Scientific	Cat #A10037 RRID:AB_2534013
Anti-mouse, goat Alexa Fluor 633	ThermoFisher Scientific	Cat #A-21052 RRID:AB_141459
Anti-rabbit, donkey Alexa Fluor 488	ThermoFisher Scientific	Cat #A-21206 RRID:AB_141708
Anti-rabbit, donkey Alexa Fluor 568	ThermoFisher Scientific	Cat #A10042 RRID:AB_2534017
Anti-rabbit, goat Alexa Fluor 633	ThermoFisher Scientific	Cat #A-21070 RRID:AB_2535731
Anti-sheep, donkey Alexa Fluor 633	ThermoFisher Scientific	Cat #A-21100 RRID:AB_2535754
Anti-goat, donkey Alexa Fluor 568	ThermoFisher Scientific	Cat #A-11057 RRID:AB_142581

241

242

Appendix Table S5 List of cytosolic peptides used in this study for immunoprecipitation assays.

Cytosolic Tail Peptides		
B4GALT-1 cytosolic tail peptide Biotin- MRLREPLLSGSAAMPGA -COOH	This study	N/A
LCS cytosolic tail peptide Biotin- MRARRGLLRLPRRSLLA -COOH	This study	N/A
Gb3S cytosolic tail peptide Biotin- MSKPPDLLLRLLRGAPRQRVC-COOH	This study	N/A
GM3S cytosolic tail peptide Biotin- MRTKAAGCAERRPLQPRTEAAAAPAGRAMP SEYTYVKLRSDCSRPSLQWYTRAQSKMRRP S-COOH	This study	N/A
GCS Cytosolic tail peptide Biotin-DPTISWRTGRYRLRCGGTAEEILDV- COOH	This study	N/A
GCS Δ 3C cytosolic tail peptide Biotin- PTISWRTGRYRLRCGGTAEEI -COOH	This study	N/A

Appendix Table S6 Primers used in this study to determine mRNA levels of indicated gene.

Human	Forward Primer	Reverse Primer
GCS	5’-TTCGGGTTCGTCCTCTTC-3’	5’-GCTTGCTATAAGGCTGTTTGTC-3’
LCS	5’-CAATCGGTGCTCAGGTTTATG-3’	5’-GGTTTCACTGTGGTTCAAGTC-3’
GB3S	5’-GATCCCCACCTCTCTGCAAT-3’	5’-TTGGACATGGTATCCCCAGA-3’
GM3S	5’-TGGTTATTGGAAGCGGAGG-3’	5’-TCTGAATATCCCTCAACTGGT-3’
FAPP2	5’-ACATCAGGATCCGATTGAGA-3’	5’-ATGCACCTTCTGGATGTGTT-3’
CERT	5’-TGTGGATCATGACAGTGCTC-3’	5’-ATTTCCTGGTTTCCCTCTGG-3’
SMS1	5’-GCATTTCAACTGTTCTCCGAAG-3’	5’-GATAGACAAGCCACCTCCAG-3’

HPRT1	5'-AGCTTGCTGGTGAAAAGGAC-3'	5'-GTCAAGGGCATATCCAACAAC-3'

249

250 **Appendix Table S7** All the commercial kits used for cDNA extraction and mRNA
251 analysis.

Commercial assays and kits		
RNA easy mini	Qiagen	Cat #74106
QuantiTect Reverse Transcription Kit Print	Qiagen	Cat #205311
SYBR™ Green PCR Master Mix	ThermoFisher Scientific	Cat #4309155

252

253

Appendix Table S8 All the Software used for analysis and figure preparation.

Softwares		
ImageJ	NIH	https://imagej.nih.gov/ij/
MetaMorph	Molecular Devices	https://www.moleculardevices.com/systems/metamorph-research-imaging
Prism	Graphpad	https://www.graphpad.com/scientific-software/prism/
Zen Lite	Carl Zeiss	https://www.zeiss.com/microscopy/int/products/microscope-software/zen-lite.html
Adobe Illustrator	Adobe	www.adobe.com/products/illustrator/free-trial-download.htm
Adobe Photoshop	Adobe	www.adobe.com/products/photoshop.html
Soft Imaging service (Electron microscope)	Olympus	www.olympus-sis.com/corp/2256.htm
iTEM	EMSIS GmbH	https://www.emsis.eu/products/software/item/
Cell profiler	Cell Profiler Analyst	https://cellprofiler.org/releases/