

Supplemental Material

Impact of Hypermannosylation on the Structure and Functionality of the ER and the Golgi Complex

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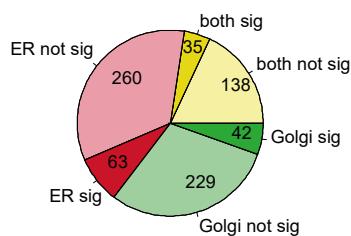
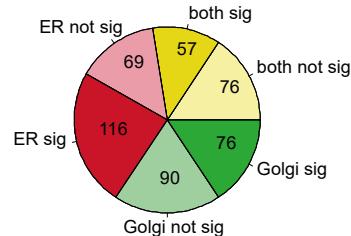
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Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1. Figure S1: Quantitative changes of proteins necessary for ER and Golgi organization and function in GMPPA KO skeletal muscle and brain compared to WT upon Con A pulldown; S2: Significantly altered ER- and Golgi-resident proteins in GMPPA KO mice upon Con A pulldown; S3: Protein interaction network of Con A enriched proteins necessary for ER and Golgi function and organization; Figure S4: Example for ComDet analysis in ImageJ; Figure S5: Mannose levels are increased in the supernatant of GMPPA KO MEFs; Figure S6: Increased mannosylation in WT MEFs treated with mannose; Table S1: ER- and Golgi-resident proteins.

Raw figures can be found at: <https://cloud.med.uni-jena.de/index.php/s/E775K6h9zsGOzr1>

A**Golgi and ER changes in skm****Golgi and ER changes in brain****B** ER skm

Pgrmc1
Abod
Syncrip
Fkbp10
Akab1
Osg9
Cybr5r3
Tmem119
Cnpy3
Pip4k2b
Rcn2
Erie1
Pdia4
Ero21
Sdf2l1
Dig4
Rpn1
Ago4
St6
Tor1b
Erap1
Cnbp
Slc27a1
Colgat1
Cst3
Nt5c3a
Fkbp7
Cept1
Sec63
Minpp1
Ces1c
Esy2t
Asph
Alg9
Sfum1
Sumf2
Aup1
Tgm2
Crat
Tecr
Prkcd
Nsdhl
Hadhb
Alt2
Plcd4
Park7
Kcnj11
Sqstm1
Sppapdc3
Hhatl
Tmed4
Ryr2
Epm2a
Hif4
H6pd
Joh1
Msrb3
Ryr1
Adipoq
Ces1d
Thbs4
Col1a2
Rtn2

Golgi skm

Spp1
Mink1
Cltb
Oclr
Syap1
Dennnd4b
Ppt1
Copb2
Fut9
Itm2b
Hs2st1
Cln1
Clasp2
Abxn2
Cand1
Pacsin1
Dmip2
Ar13
Cita
Neo1
Glyr1
H1f0
Rp2
Akr1e2
Sod3
M6pr
Scyl2
Gpc1
Itgb10
Igf2r
Cnpad1
Cnrb
Kif1c
Casq1
Dmd
Ahsg
Scyl1m1
Ryr1
Hspg2
Aplp1
Pdxd1c
Cd36
Man2a2

ER brain

Sez6l2
Pgs1
Ryr2
Adipoq
Dnase1l1
Rpn2
Brsk2
Kenj11
Rcn3
St3b
Erp1
Usp9
Ces1c
Pomk
Scps1
Hhatl
Sts
Ces2c
Atp13a1
Pgap1
P4ha1
Esy1t
For1b
Renxm3
Ron1
Ncn
Cst3
Mlec
Clu
Minp1
Thbs4
H6pd
Klh41
Tmed4
Slc27a1
Emc1
Scamp3
Kif1c
Lamp2
Golm4
Cttn
Atp6v0a1
Tcp1
Arl2
Scamp3
Akr7a2
Ap1g1
Gtb2
Cand1
Ap1m1
Syn1
Gnas
Arf3
Ece1
Mapre1
Cltc
Ppt1
Ap1b1
Rab7a
Map6
Snx1
Raf1
Ap3d1
Lap3
Ap2b1
Casc4
Cdc42
Gpc6
Riffox2
Riffox2
Rac1
Clasp2
Syt1
Vamp2
Coq6
Pak1
Mapk1
Rph3a
Ap3b2
Rab6a
Glb1
Dbnl
Hras
Rasip1
Ocr1
Gga3
Gdi1
Scamp1
Mapk3
Cntnap2

Golgi brain

Snap25
Dpp7
Cryab
Mmg1t
Spp1
Prkka2
Gpc4
Casq1
St3gal6
Sod3
Tgoln1
Slc2a4
Cd36
Dnm3
Impad1
B2m
Ahsg
Gpc1
Hspq2
Man2a2
H1f0
Cltb
Man2a2
H1f0
Cttn
Atp6v0a1
Tcp1
Arl2
Scamp3
Akr7a2
Ap1g1
Gtb2
Cand1
Ap1m1
Syn1
Gnas
Arf3
Ece1
Mapre1
Cltc
Ppt1
Ap1b1
Rab7a
Map6
Snx1
Raf1
Ap3d1
Lap3
Ap2b1
Casc4
Cdc42
Gpc6
Riffox2
Riffox2
Rac1
Clasp2
Syt1
Vamp2
Coq6
Pak1
Mapk1
Rph3a
Ap3b2
Rab6a
Glb1
Dbnl
Hras
Rasip1
Ocr1
Gga3
Gdi1
Scamp1
Mapk3
Cntnap2

ER and Golgi skm

App
Tmem30a
Tmem132a
Crel2
Dhd2
Svip
Atp6ap1
Lrpap1
Aspcr1
Ugg1
Tapbp
Preb
Lman2
Tmc01
Serpinh1
Anpep
Tmed10
Fn1
Tmed9
Myo18a
P4hb
Ctsc
Tapbp
Capn2
P4hb
Fn1
Cpq
Rab1A
Pofut2
Npc1
Serpina1b
Ecm29
Sh3glb1
Ptpn2
Atp8a1
Sar1a
Vapb
App
Cat
Rtn3
Dhd2
Pnpla8
Tmem30a
Sort1
Pdd6
Trappc12
Rab3gap1
Sor1
Acsgb1
Giyf2
Gja1
Dlg1
Myo5a
Svip
Alt1
Atp1a3
Pde2a

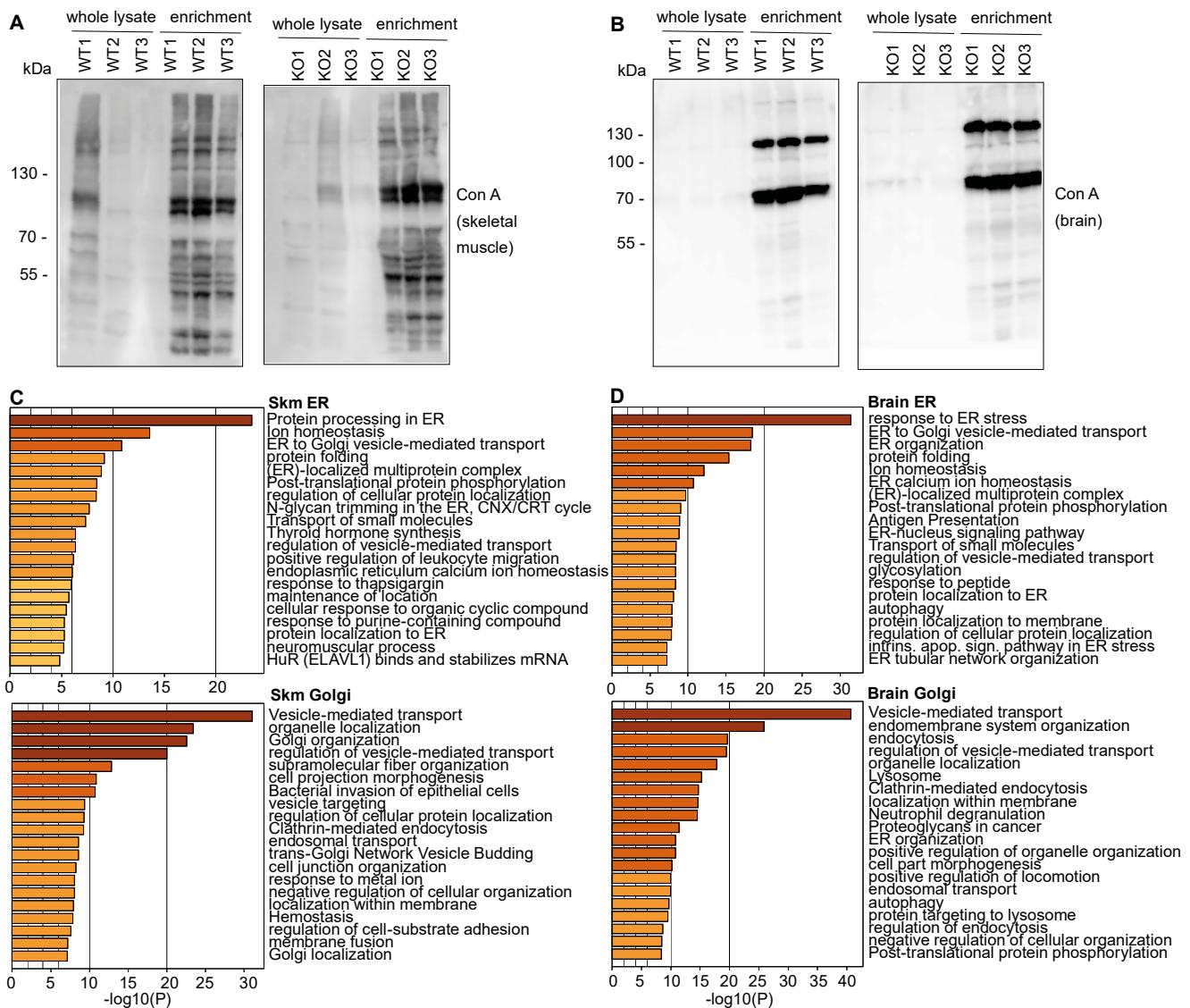
ER and Golgi brain

H2-K1
Tmed5
Aoc3
Prkce
Crel2
Pebp1
Rab1b
Plod3
Cln5
Vtn
Tmed9
Serpinh1
Tmed10
Surf4
Hspa5
Anpep
Ugg1
Lman2l
Calu
Myoc
Ctsc
Tapbp
Capn2
P4hb
Fn1
Cpq
Rab1A
Pofut2
Npc1
Serpina1b
Ecm29
Sh3glb1
Ptpn2
Atp8a1
Sar1a
Vapb
App
Cat
Rtn3
Dhd2
Pnpla8
Tmem30a
Sort1
Pdd6
Trappc12
Rab3gap1
Sor1
Acsgb1
Giyf2
Gja1
Dlg1
Myo5a
Svip
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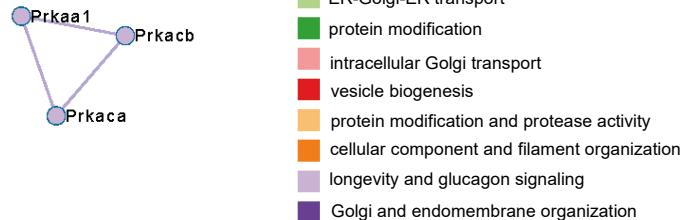
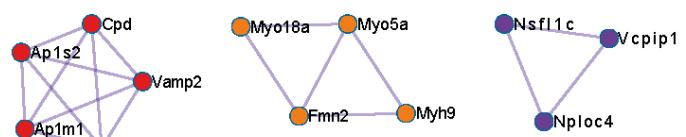
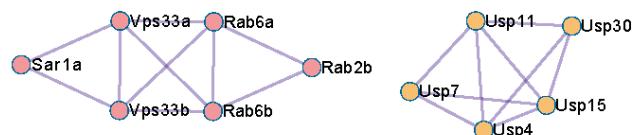
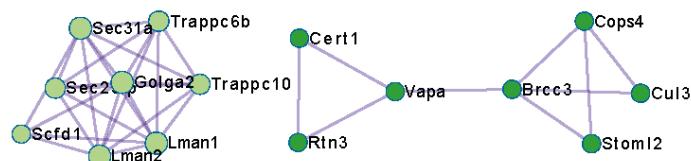
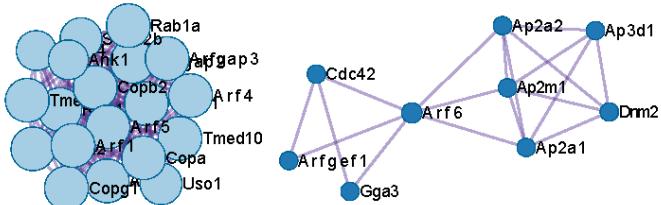
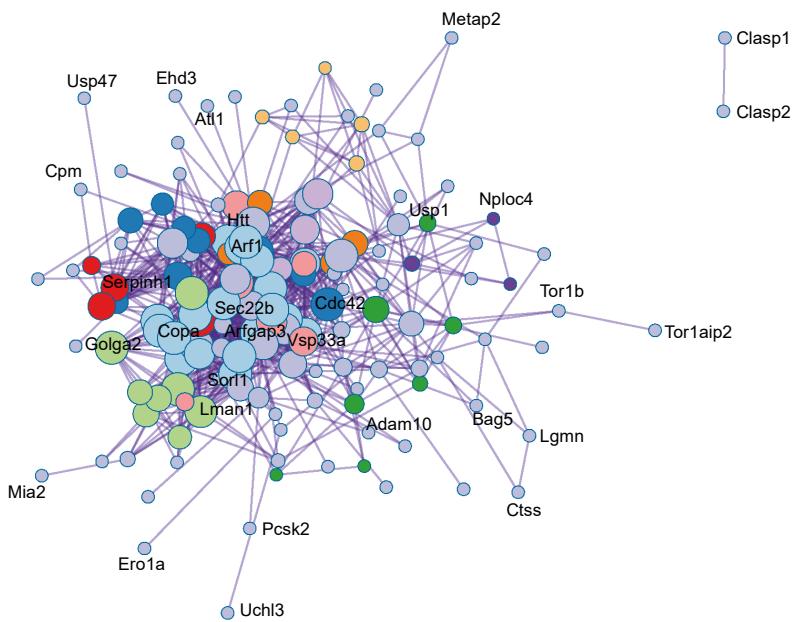


Supplementary Figure S1. Quantitative changes of proteins necessary for ER and Golgi organization and function in GMPAA KO skeletal muscle and brain compared to WT upon Con A pulldown. A) Pie chart for altered ER- and Golgi-resident proteins

in skeletal muscle (skm) and brain of GMPPA KO mice after Con A enrichment. Significant (sig) values show a q-value below 0.05 and an absolute fold change (Log2 FC) above 0.5. Proteins significantly up-regulated between WT and GMPPA KO are shown in red and those down-regulated in blue ($n = 3$ mice per group). **B**) Depicted are heatmaps for all significantly altered (q-value below 0.05 and fold change above 0.5 or below -0.5) proteins resident in ER and/or Golgi apparatus for skm and brain tissue of GMPPA KO mice after Con A pulldown ($n=3$ mice per group).

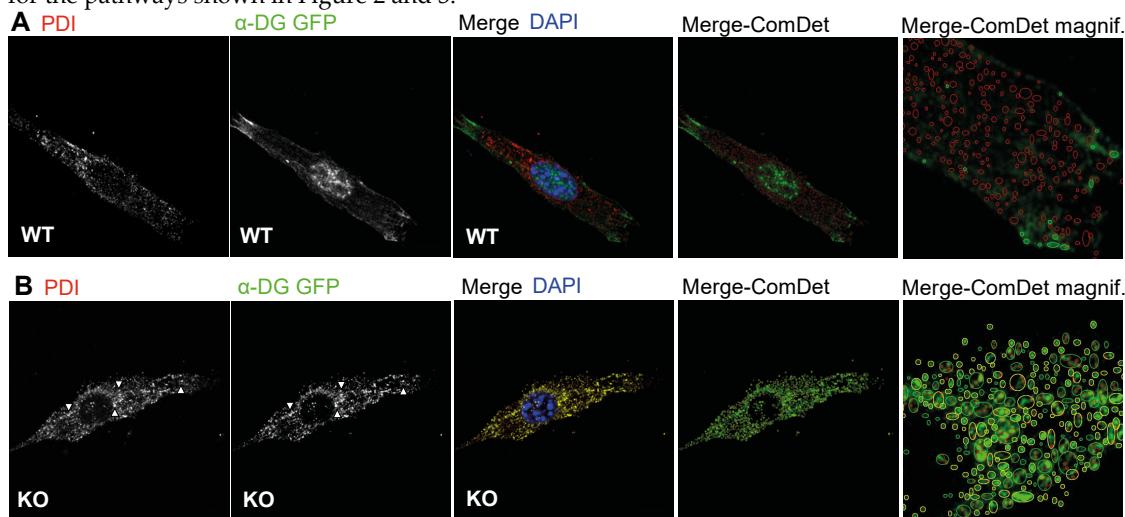


Supplementary Figure S2. Affected pathways upon Con A pulldown in GMPPA KO mice. **A-B**) Western Blot membranes probed for Con A for whole tissue samples as well as after Con A enrichment. **A)** Membranes showing skeletal muscle samples. **B)** Membranes showing brain samples. **C-D**) Metascape analysis for involved biological processes for all in the Con A pulldown identified proteins with a q-value below 0.05 for: **C)** ER-resident and Golgi-resident proteins in skm. **D)** ER-resident and Golgi-resident proteins in brain.

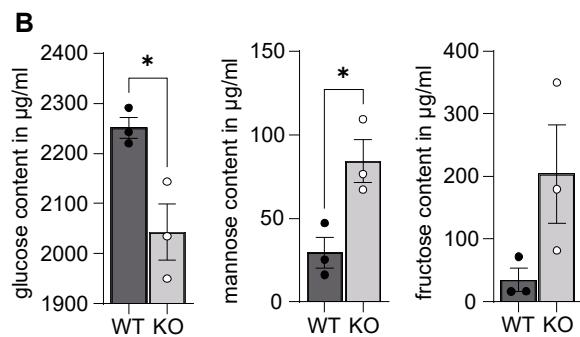
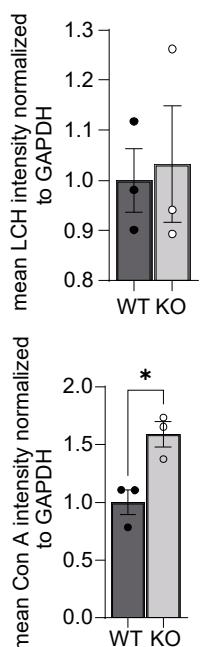
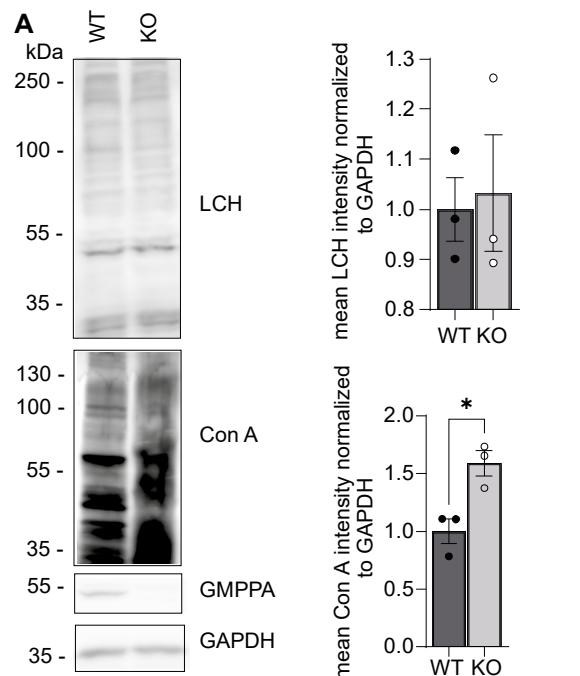


- COPI-dependent Golgi-to-ER retrograde traffic
- vesicle-mediated transport
- ER-Golgi-ER transport
- protein modification
- intracellular Golgi transport
- vesicle biogenesis
- protein modification and protease activity
- cellular component and filament organization
- longevity and glucagon signaling
- Golgi and endomembrane organization

Supplementary Figure S3. Protein interaction network of Con A enriched proteins necessary for ER and Golgi function and organization. Depicted are identified protein interactions via Metascape analysis of proteins from skeletal muscle and brain selected for the pathways shown in Figure 2 and 3.

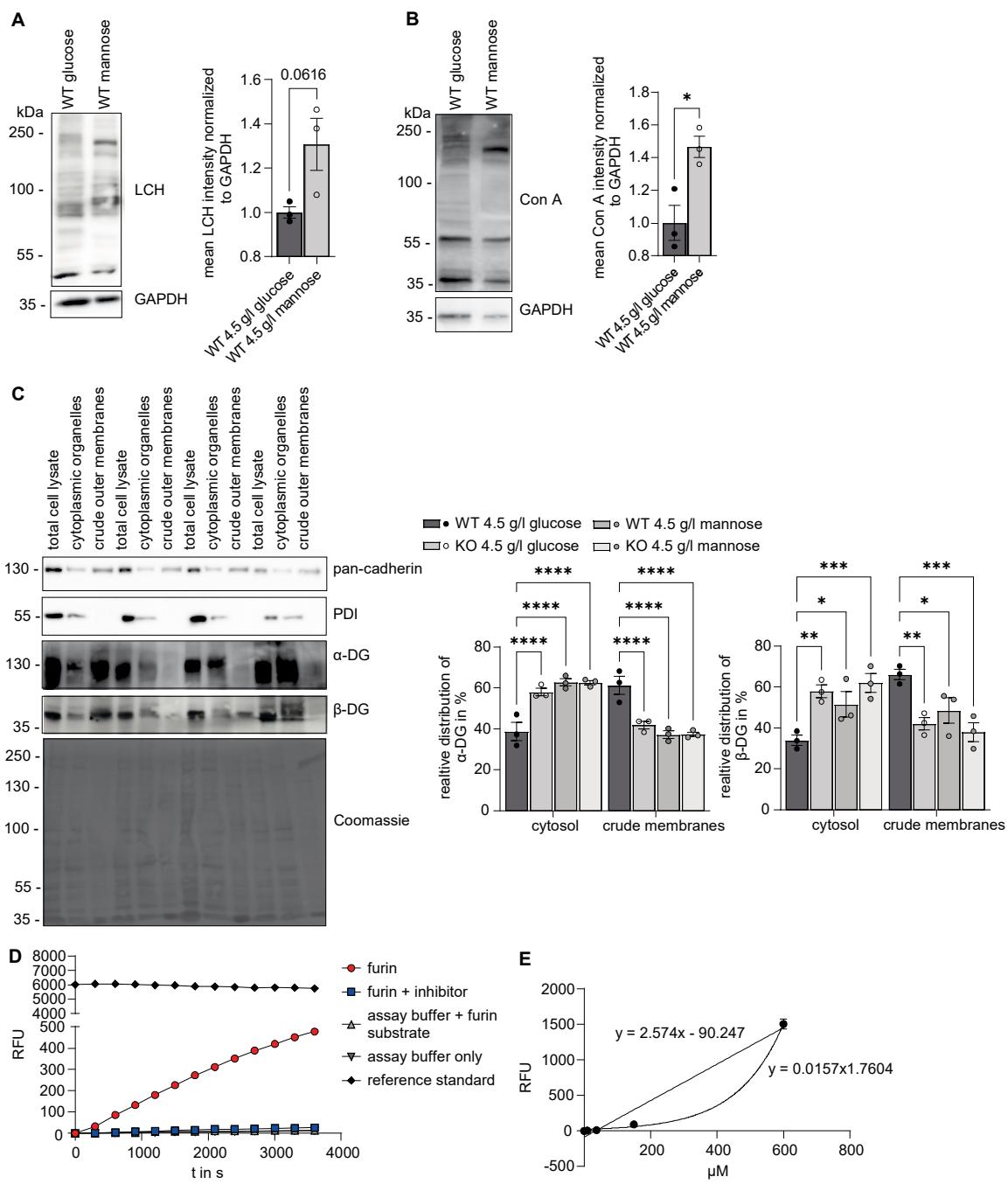


Supplementary Figure S4. Example for ComDet analysis in ImageJ. A-B) Representative images (displayed in Figure 4) and the according ComDet plugin analysis with magnifications of A) WT and B) GMPPA KO MEFs transfected with α -DG-GFP and stained for PDI (scale bars: 8 μ m). White arrows indicate colocalized structures. Green circles: α -DG-GFP detected structures. Red circles: Detected structures for PDI. Yellow circles: detected structures with colocalization of PDI and α -DG-GFP.



Supplementary Figure S5. Mannose levels are increased in the supernatant of GMPPA KO MEFs. A) Representative Western Blot images for the lens culinaris lectin (LCH) and the concanavalin A lectin (Con A) with according analysis (n=3 experiments per group).

GMPPA served for genotype verification and GAPDH as loading control (n=3 experiments per group). **B)** Mannose, glucose and fructose detection in the supernatant of WT and GMPPA KO MEFs (n=3 experiments per group). Two-tailed Student's T-Test.



Supplementary Figure S6. Increased mannosylation in WT MEFs treated with mannose. **A)** Representative Western Blot analysis with the lens culinaris lectin (LCH) with according analysis (n=3 experiments per group). GAPDH served as loading control. Two-tailed Student's T-Test. **B)** Representative Western Blot membranes with the concanavalin A lectin (Con A) with according analysis (n=3 experiments per group). GAPDH served as loading control. Two-tailed Student's T-Test. **C)** Western Blot analysis of subcellular fractionation with according analysis of α - and β -DG distribution (n=3 experiments per group). 2-way-ANOVA with Bonferroni post-hoc analysis. Coomassie blue staining served as loading control, pan-cadherin served as a marker of the plasma membrane and PDI served as a marker for the cytoplasmic organelle fraction. WT and GMPPA KO cells were transfected with a dystroglycan construct, treated with either glucose (4.5 g/l) or mannose (4.5 g/l) and then fractionated. **D-E)** Furin activity reference curves. **D)** Verification of the furin activity assay: Recombinant furin shows an increase in relative fluorescence units (RFU) with increasing time, which is absent in substrate controls. A fluorescence standard that resembles cleaved furin substrate showed a slight decrease in RFU over time. **E)** Dilution series of recombinant furin enzyme after incubation with furin substrate for 40 min.