The American Journal of Human Genetics, Volume *108*

Supplemental information

A mutation in *SLC37A4* causes a dominantly inherited congenital disorder of glycosylation characterized by liver dysfunction

Bobby G. Ng, Paulina Sosicka, François Fenaille, Annie Harroche, Sandrine Vuillaumier-Barrot, Mindy Porterfield, Zhi-Jie Xia, Shannon Wagner, Michael J. Bamshad, Marie-Christine Vergnes-Boiteux, Sophie Cholet, Stephen Dalton, Anne Dell, Thierry Dupré, Mathieu Fiore, Stuart M. Haslam, Yohann Huguenin, Tadahiro Kumagai, Michael Kulik, Katherine McGoogan, Caroline Michot, Deborah A. Nickerson, Tiffany Pascreau, Delphine Borgel, Kimiyo Raymond, Deepti Warad, University of Washington Center for Mendelian Genomics (UW-CMG), Heather Flanagan-Steet, Richard Steet, Michael Tiemeyer, Nathalie Seta, Arnaud Bruneel, and Hudson H. Freeze

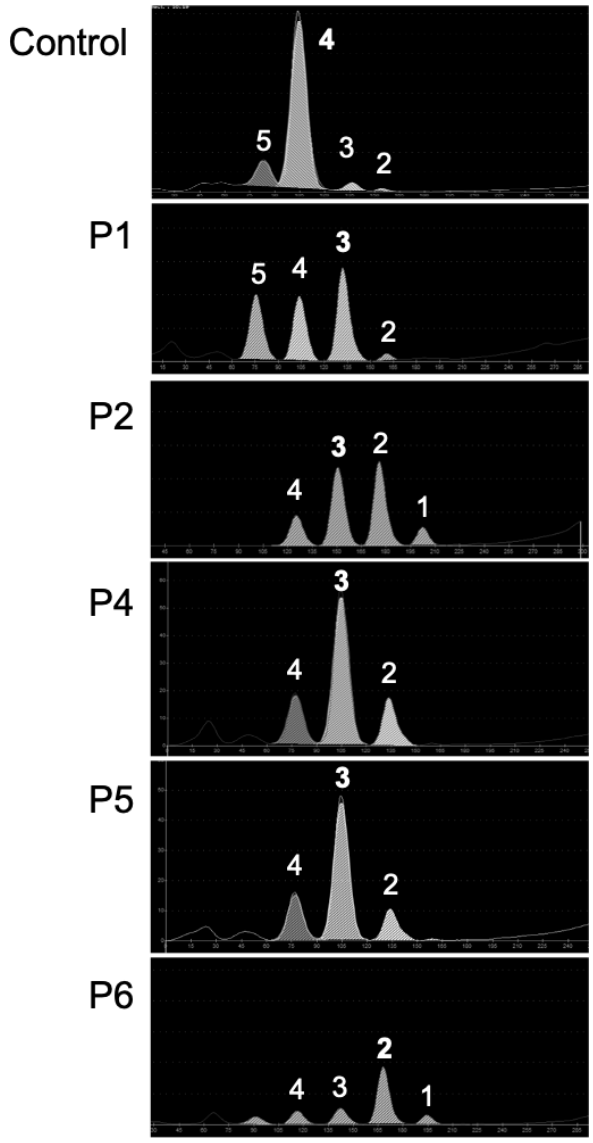


Figure S1: Capillary zone electrophoresis of transferrin for Control, P1, P2, P4, P5, P6. Glycosylated transferrin isoforms were separated by capillary zone electrophoresis with each peak labeled with the corresponding number of sialic acids. 0-Asiaolo, 1-Monosialo, 2-Disialo, 3-Trisialo, 4-Tetrasialo, 5-Pentasialo.

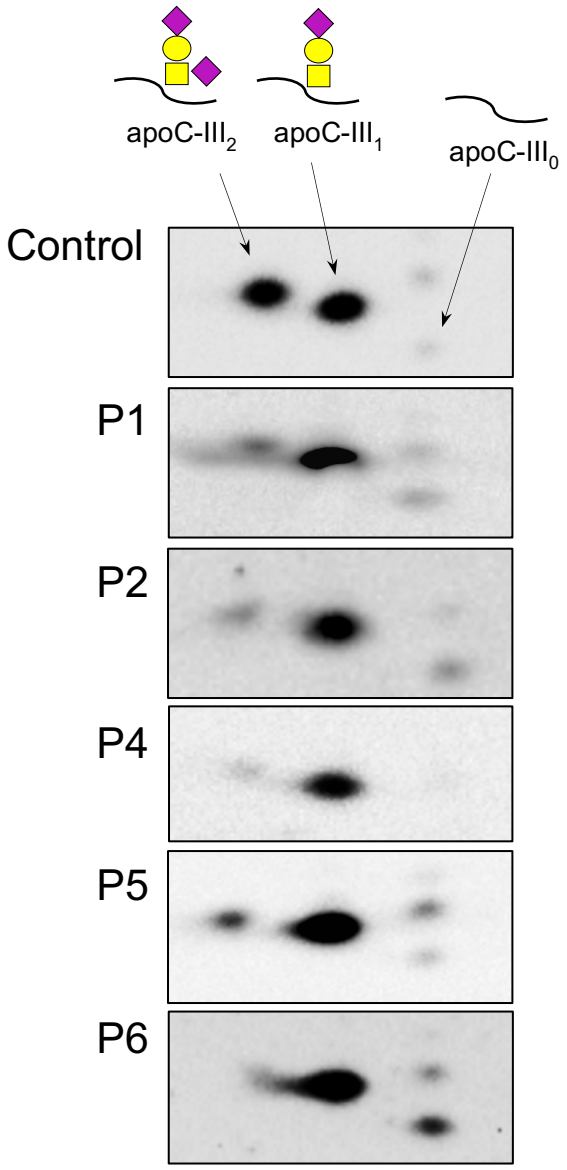
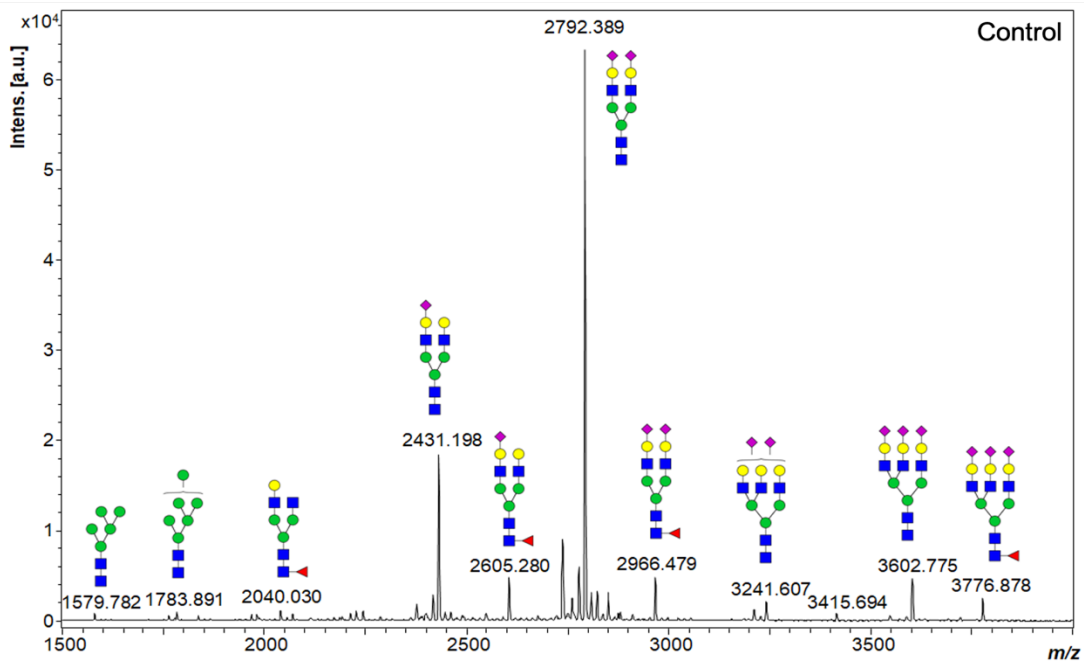
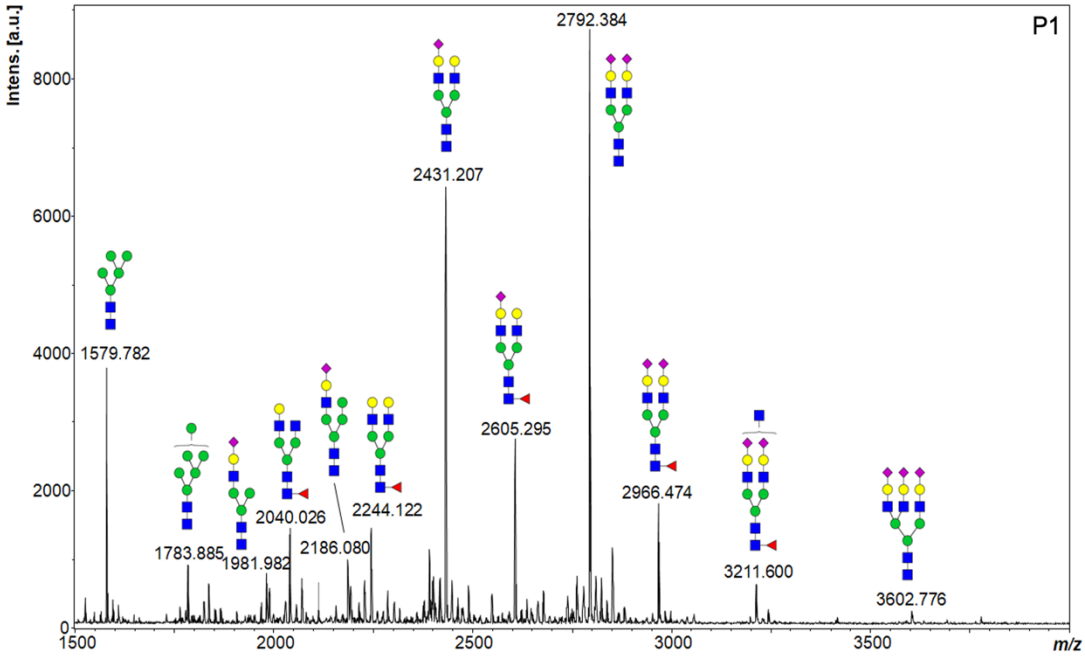
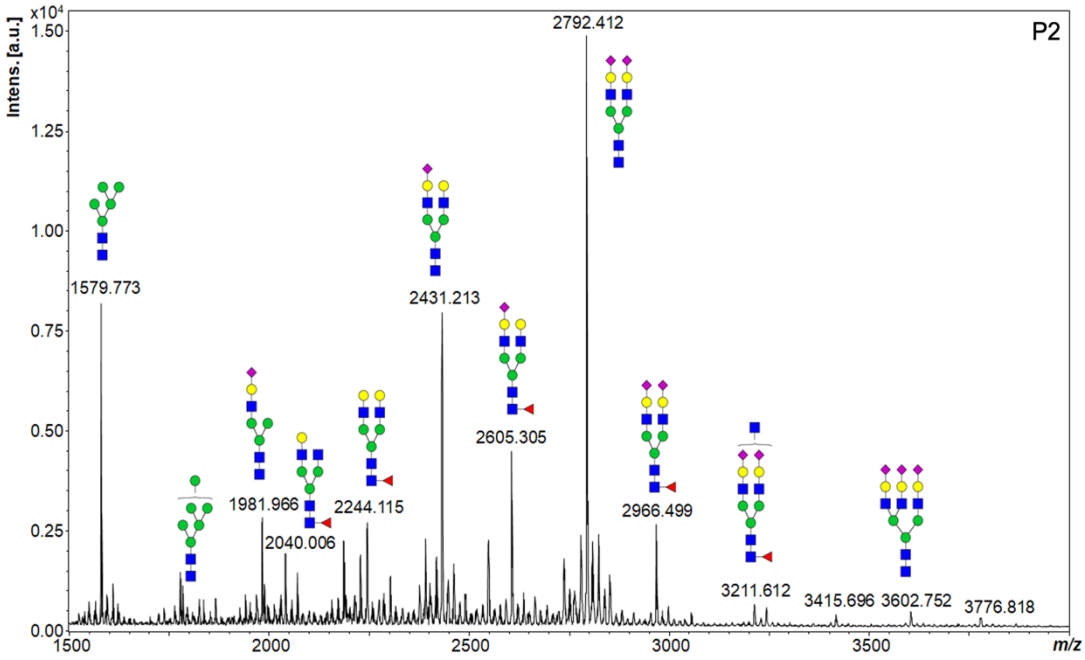
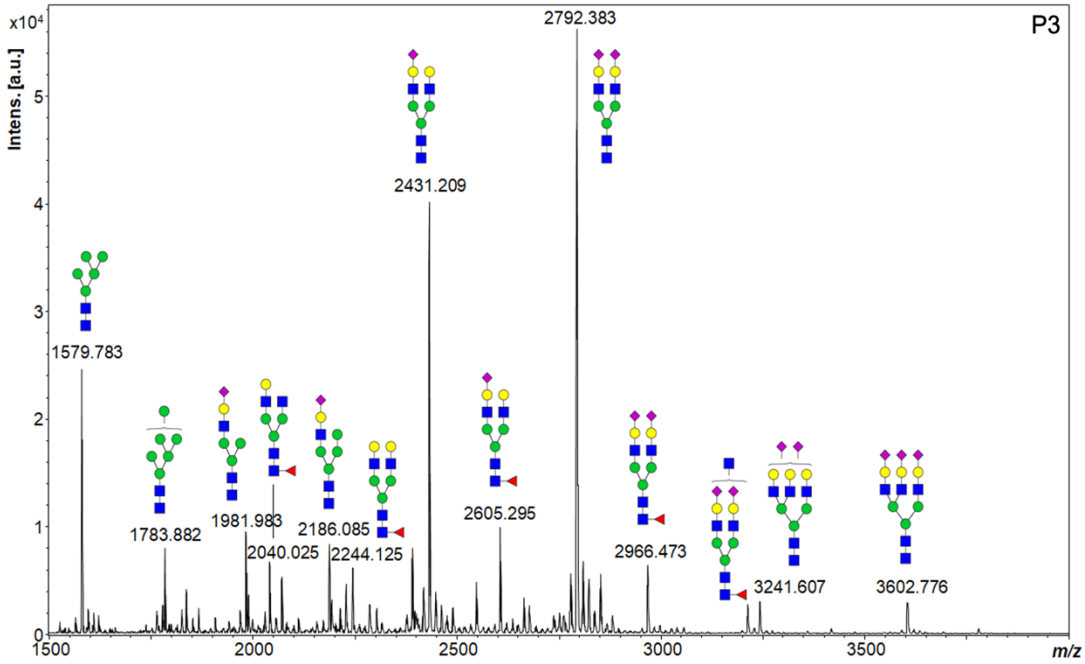


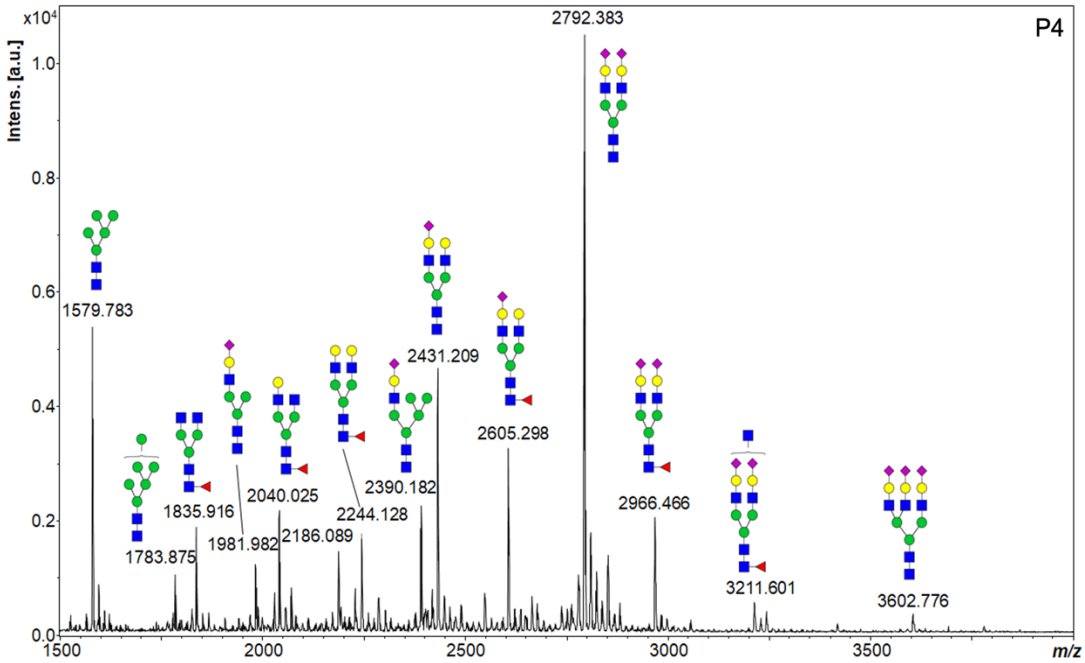
Figure S2: 2-Dimensional gel electrophoresis of ApoCIII for Control, P1, P2, P4, P5, P6. 2-DG electrophoresis of the serum O-linked glycoprotein, ApoCIII showing changes in the ratio of di-sialo ($ApoCIII_2$), mono-sialo ($ApoCIII_1$) and Asialo ($ApoCIII_0$).

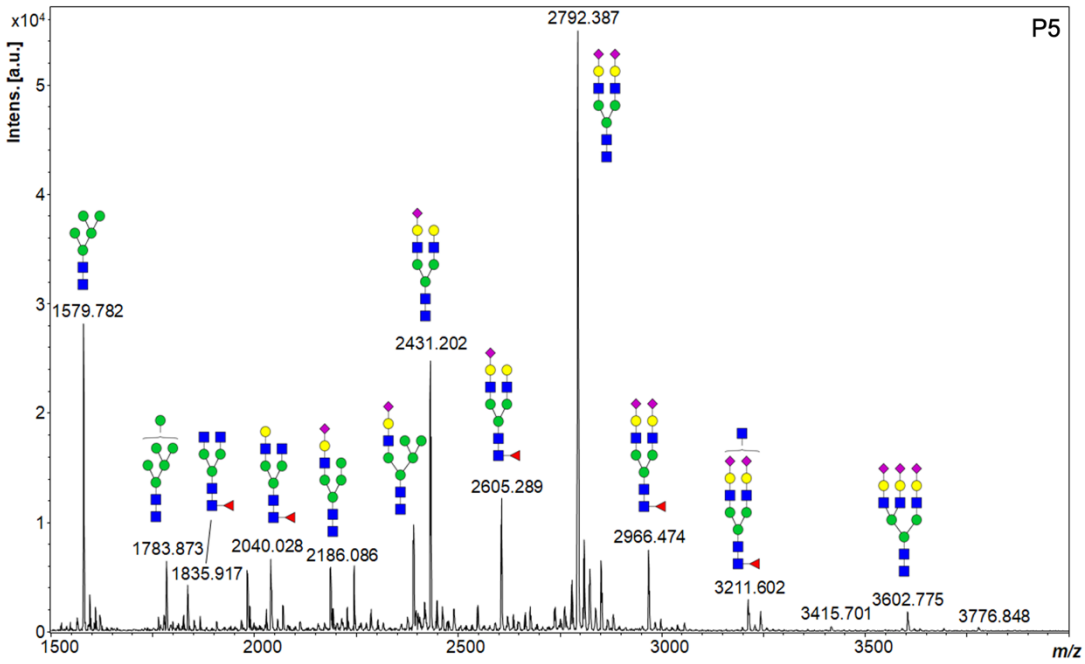

Control
x10⁴
Intens. [a.u.]
2792.389
2431.198
2605.280
2966.479
3241.607
3415.694
3602.775
3776.878
1579.782
1783.891
2040.030
m/z

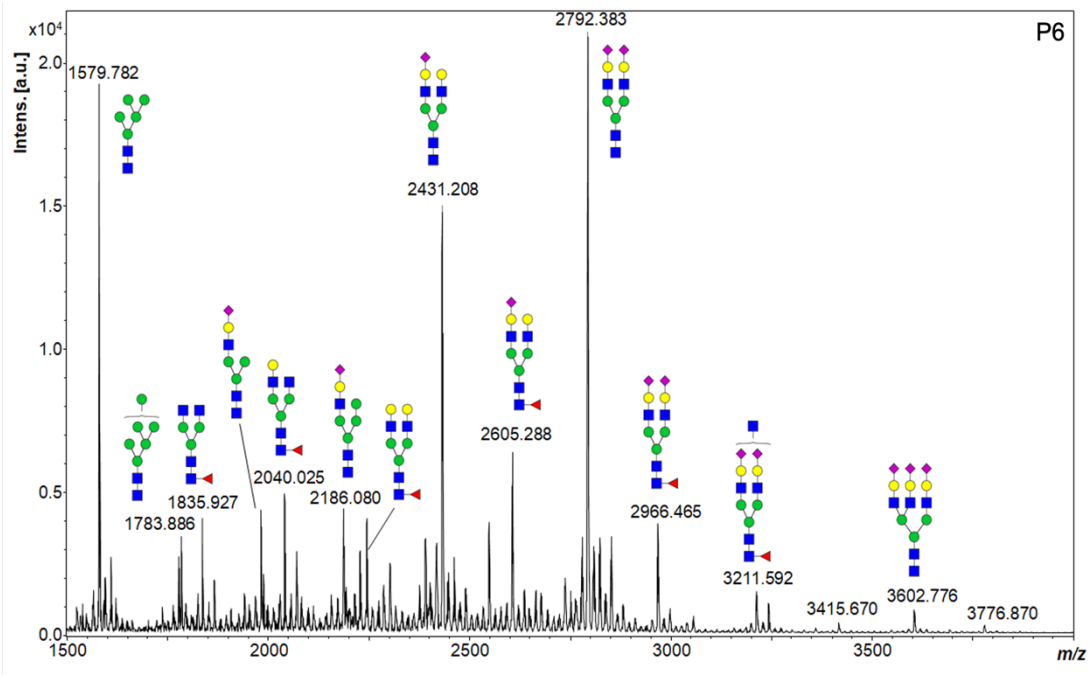

P1
Intens. [a.u.]
2792.384
2431.207
2605.295
2966.474
3211.600
3602.776
1579.782
1783.885
1981.982
2040.026
2186.080
2244.122
m/z


P2
Intens. [a.u.]
x10^4
1.50
1.25
1.00
0.75
0.50
0.25
0.00
1500
2000
2500
3000
3500
m/z
2792.412
1579.773
2431.213
2605.305
1981.966
2244.115
2040.006
2966.499
3211.612
3415.696
3602.752
3776.818


P3
Intens. [a.u.]
x10^4
5
4
3
2
1
0
1500
2000
2500
3000
3500
m/z
2792.383
2431.209
1579.783
1783.882
1981.983
2040.025
2186.085
2244.125
2605.295
2966.473
3241.607
3602.776


P4
x10^4
Intens. [a.u.]
1.0
0.8
0.6
0.4
0.2
0.0
1500
2000
2500
3000
3500
m/z
2792.383
1579.783
2431.209
2605.298
2966.466
3211.601
3602.776
1783.875
1835.916
1981.982
2040.025
2186.089
2244.128
2390.182


P5
x10^4
Intens. [a.u.]
5
4
3
2
1
0
1500
2000
2500
3000
3500
m/z
2792.387
1579.782
2431.202
2605.289
2966.474
3211.602
3602.775
3415.701
3776.848
1783.873
1835.917
2040.028
2186.086


P6
x10^4
Intens. [a.u.]
2.0
1.5
1.0
0.5
0.0
1579.782
2792.383
2431.208
2605.288
2040.025
2186.080
1835.927
1783.886
2966.465
3211.592
3602.776
3415.670
3776.870
1500
2000
2500
3000
3500
m/z

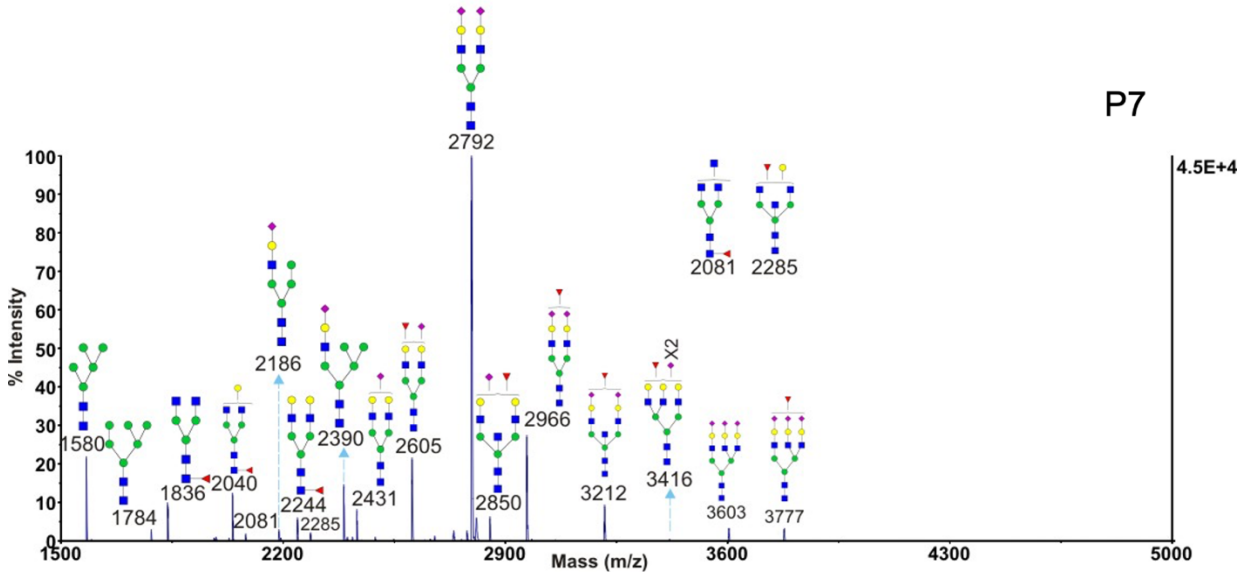

P7
2792
4.5E+4
% Intensity
100
90
80
70
60
50
40
30
20
10
0
2081
2285
2186
X2
1580
2966
2390
2605
1836
2040
3212
3416
1784
2244
2431
2850
3603
3777
2081
2285
1500
2200
2900
3600
4300
5000
Mass (m/z)

Figure S3: Characterization of N-glycan abnormalities in serum from affected individuals. MS spectra of N-glycans from serum glycoproteins for control and all seven affected unrelated individuals showing N-glycan abnormalities in serum. Specifically, the accumulation of multiple species of both high mannose (peaks 1579.8,1783.9m/z) and hybrid type N-glycans was observed (peaks 1981.9, 2186.1, 2390.2m/z).

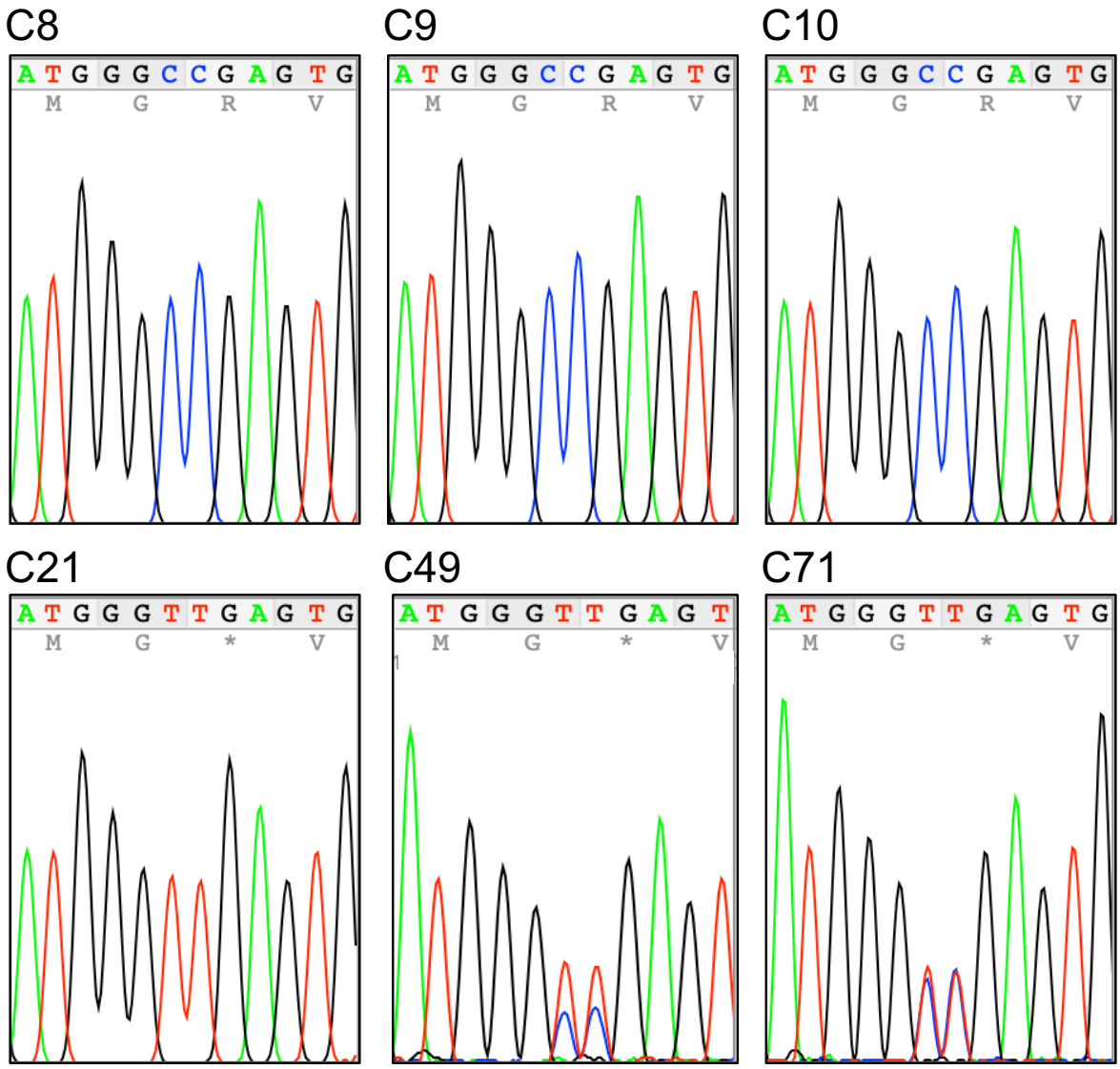


Figure S4: Sanger sequencing of Huh7 base edited clones. Sanger sequencing isogenic Huh7 base edited clones for the c.1267C>T [p.Arg423*] in *SLC37A4.* Clones C8, C9, C10 underwent the same editing process as C21, C49, C71 but no edit was performed.

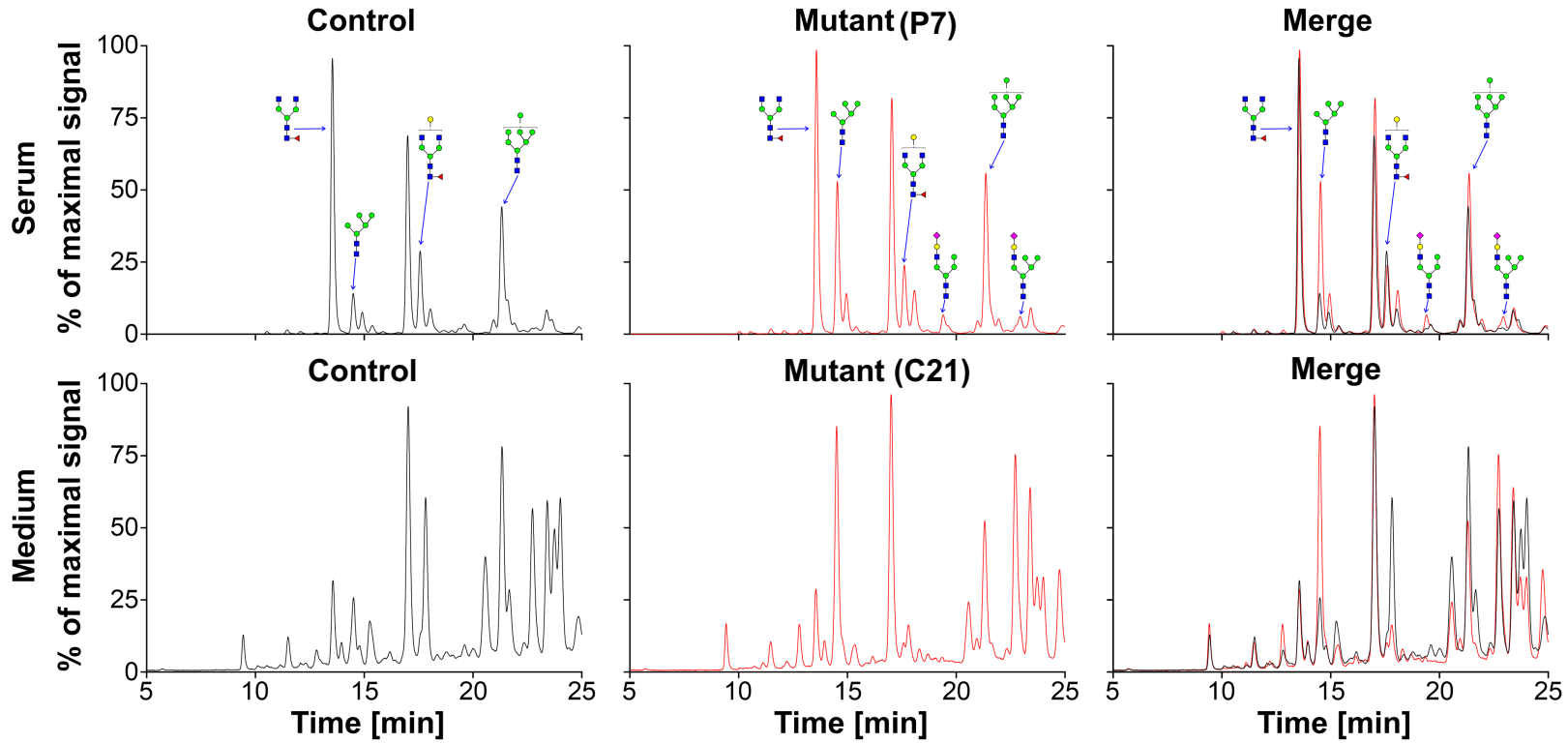


Figure S5: HPLC analysis of N-glycans. N-glycans were purified from Control and P7 serum (upper panel) as well as Huh7 Control and C21 medium (lower panel). Samples were run on HPLC and detected by fluorescence.

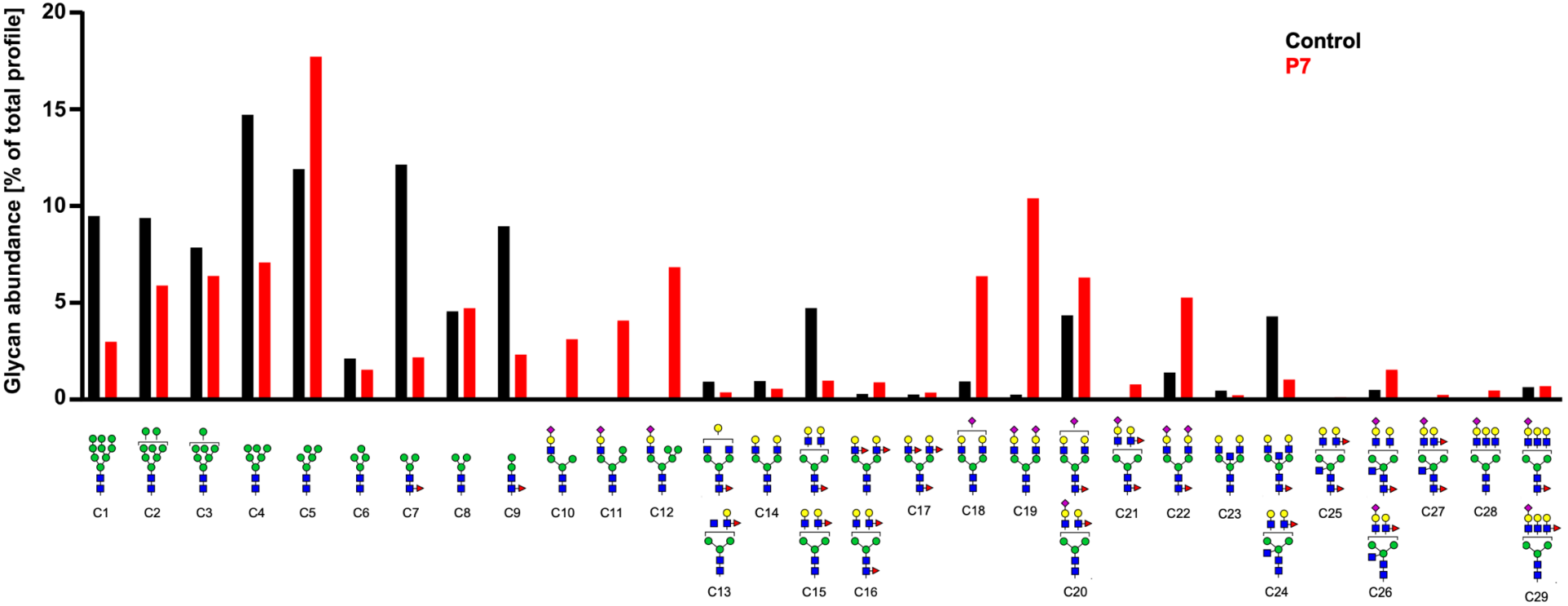

Glycan abundance [% of total profile]
20
15
10
5
0
Control
P7
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
C11
C12
C13
C14
C15
C16
C17
C18
C19
C20
C21
C22
C23
C24
C25
C26
C27
C28
C29

Figure S6: Nanospray ionization mass spectrometry (NSI-MSn) analysis of N-glycans released from iPSC-derived hepatocytes. Control and P7 iPSCs were differentiated to hepatocytes. N-glycans were released from total cell-associated proteins and analyzed after permethylation.

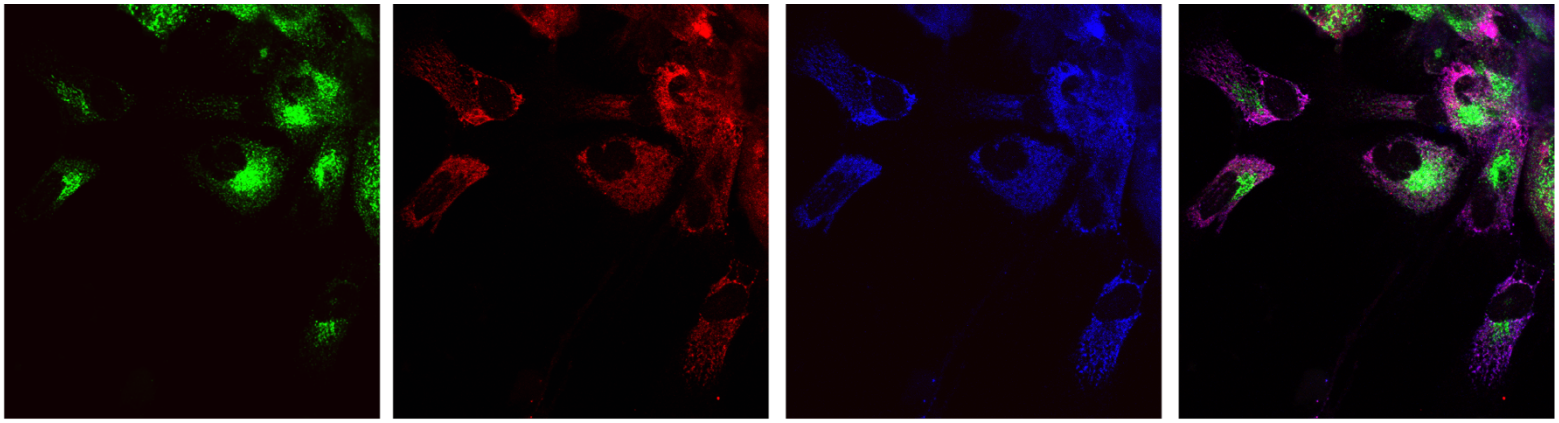


Figure S7: ERGIC53 and SLC37A4 do not colocalize. Immunofluorescence staining showing no colocalization of SLC37A4 with ERGIC53 in IPSC differentiated hepatocytes from P7.

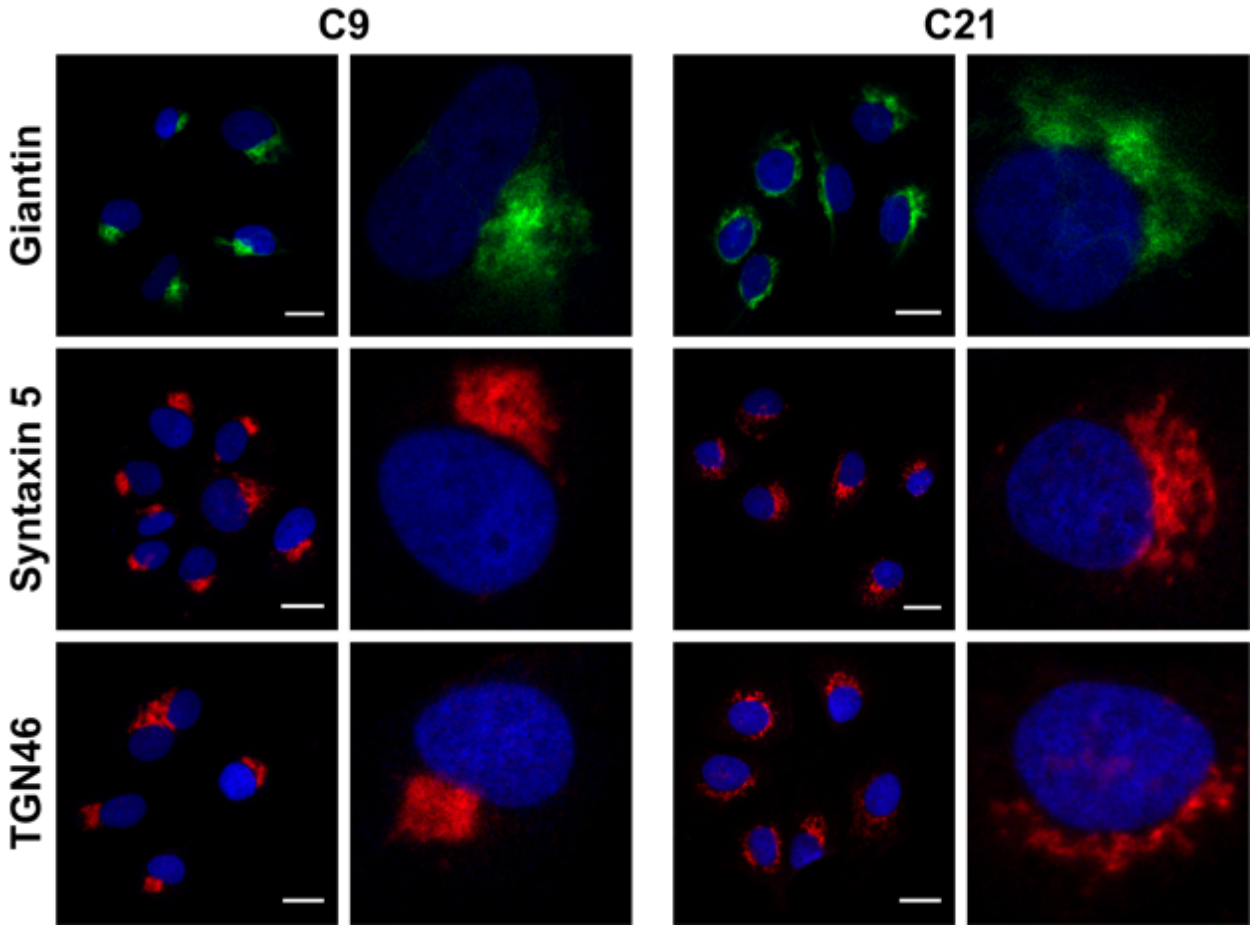


Figure S8: Immunofluorescence for the Golgi morphology. Multiple markers covering the three Golgi compartments were used to confirm abnormalities seen in the Golgi morphology. cis-Golgi/medial marker giantin, medial marker syntaxin 5 and trans marker Golgi network TGN46.

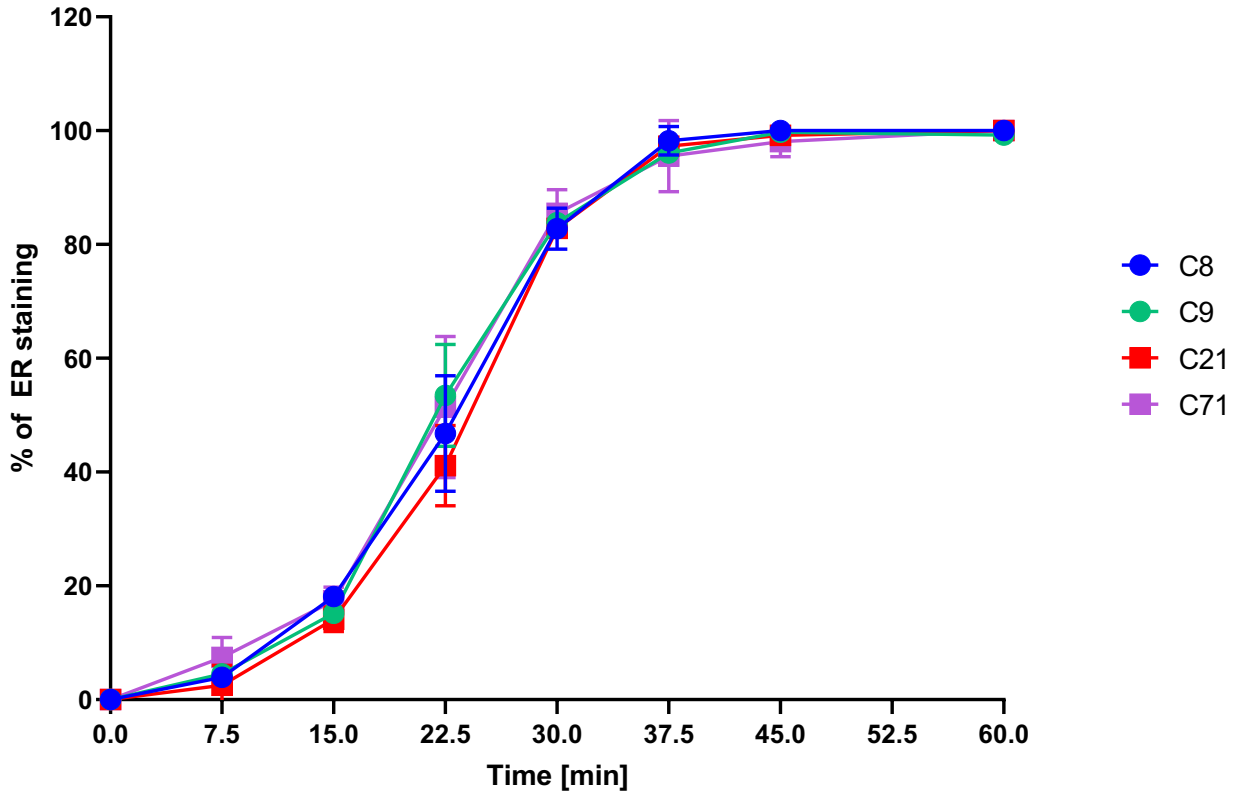


Figure S9: Brefeldin A induced retrograde transport.

Brefeldin A induced retrograde transport was assayed in each cell line and showed no difference in retrograde transport rate.

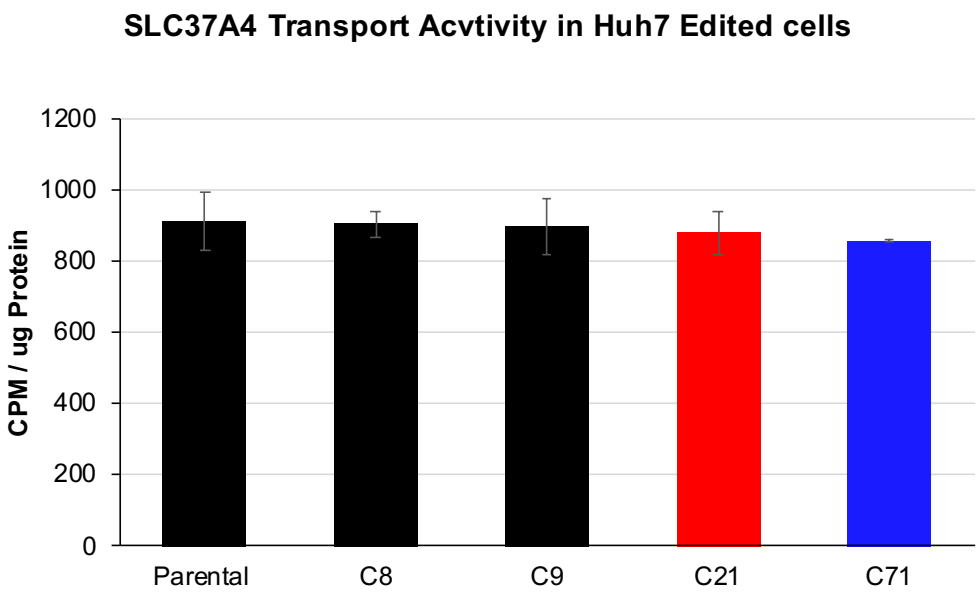


Figure S10: SLC37A4 transport activity in CRISPR edited Huh7 cells. SLC37A4 transport activity showing no difference in the ER specific uptake of ^{3}H-Glc-6P into Huh7 control or edited cells.

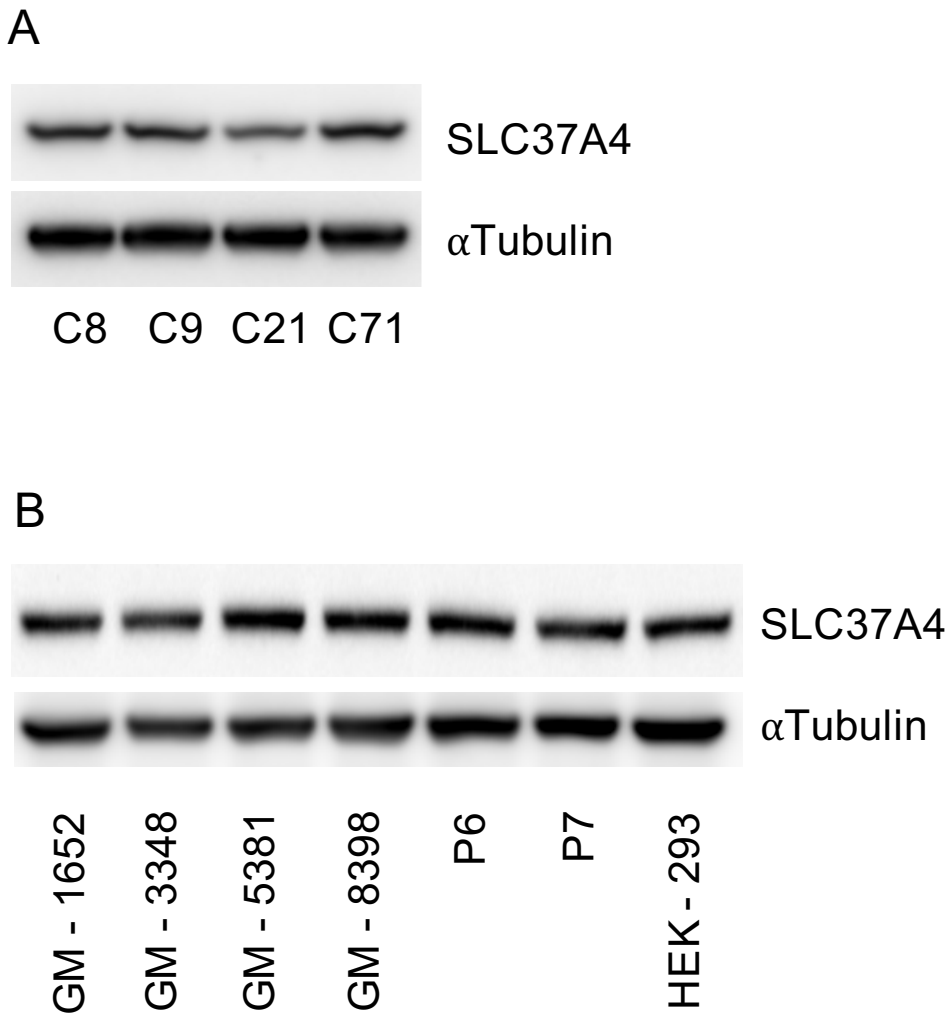


Figure S11: SLC37A4 protein expression. Western blot analysis of SLC37A4 expression in (A) Huh7 control (C8, C9) and edited (C21, C71) cells. (B) Expression of SLC37A4 in control GM – 1652, GM – 3348, GM – 5381 and P6, P7. Alpha tubulin was used to normalize protein levels.

Table S1: List of primary and secondary antibodies used in immunofluorescence.

Antibody	Cat. Number	Source	Dilution	Company
anti-SLC37A4	PA5-58599	Rabbit	1:100	Life Technologies
anti-GM130	610822	Mouse	1:250	BD Biosciences
anti-giantin Alexa Fluor 488	308701	Rabbit	1:250	BioLegend
anti-syntaxin 5	SC-365124	Mouse	1:100	Santa Cruz Biotechnology Inc.
anti-TGN46	GTX-74290	Sheep	1:100	Genetex
anti-KDEL	ADI-SPA-827-D	Mouse	1:100	Enzo Life Sciences Inc.
anti-β4-GalT1	HPA-010807-100uL	Rabbit	1:100	Sigma Aldrich
anti-Sec31A	612350	Mouse	1:100	BD Biosciences
anti-rabbit Alexa Fluor 405	A31556	Goat	1:100	Life Technologies
anti-rabbit Alexa Fluor 488	AB150077	Goat	1:500	Abcam
anti-rabbit Alexa Fluor 568	A11011	Goat	1:250	Life Technologies
anti-sheep Alexa Fluor 488	AB150178	Donkey	1:250	Abcam
anti-mouse Alexa Fluor 546	A11003	Goat	1:250	Life Technologies