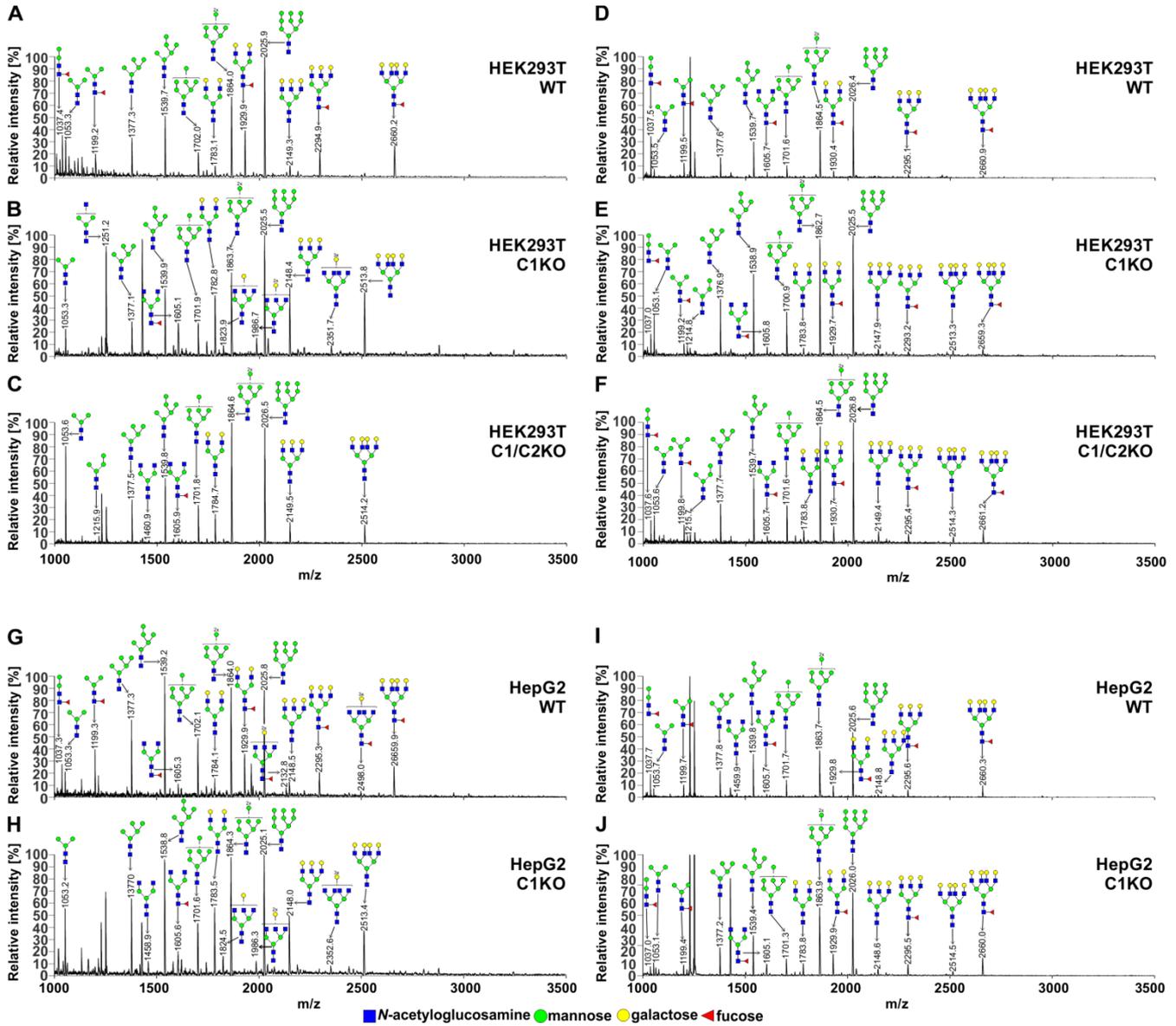
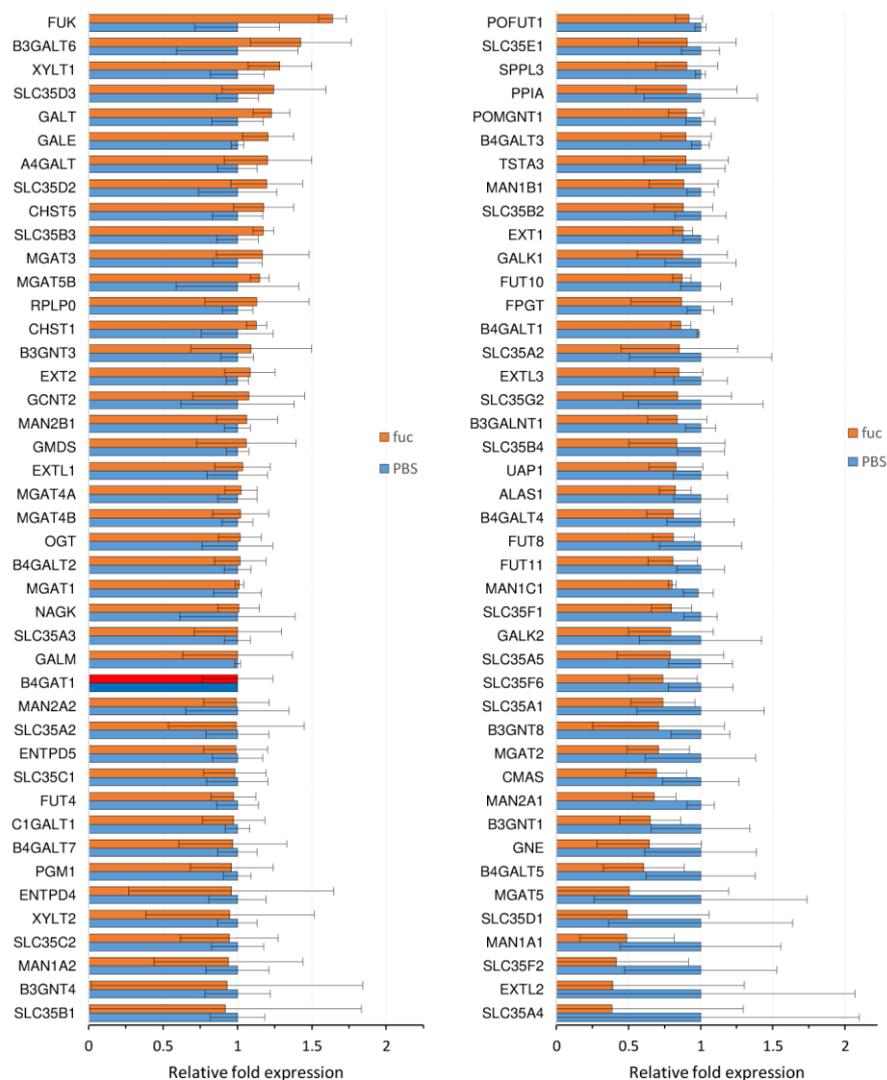


Supplementary figure 1. Confirmation of the SLC35C1 and SLC35C1/SLC35C2 knockouts and over-expression of the HA-tagged SLC35C1. (A) Verification of a knock-out of the SLC35C1 gene in HEK293T and HepG2 cell lines. Total RNA and genomic DNA (gDNA) were isolated from the wild-type (WT) and SLC35C1 knockout (C1KO) cells and either PCR (DNA) or RT-PCR (mRNA) was performed using SLC35C1 gene-specific primers. (B) Verification of a knock-out of the SLC35C2 gene in HEK293T SLC35C1 knockout cells. Total RNA and genomic DNA (gDNA) were isolated from the wild type (WT) and SLC35C1 and SLC35C2 knockouts (C1/C2KO) cells and either PCR (DNA) or RT-PCR (mRNA) was performed using SLC35C2 gene-specific primers. (C) Western blot verification of the SLC35C1 knockouts in HEK293T and HepG2 cell lines was performed using anti-SLC35C1 antibody. (D) Confirmation of the SLC35C1 knockout using AAL staining. The wild-type HEK293T and HepG2 cells were mixed with the corresponding SLC35C1 knockouts and double stained with AAL (green) and anti-SLC35C1 antibody (red). (E) Western blot verification of the over-expression of the HA-tagged SLC35C1 in the HEK293T and HepG2 SLC35C1 knockouts was performed using anti-HA antibody. HSP60 protein was immunostained to control for the amount of loaded material. (F) Localization of the over-expressed SLC35C1 in HEK293T and HepG2 cell lines using anti-HA (red) and anti-organelle markers (green). Cell nuclei were counterstained with DAPI. Scale bar = 10 μ m.



1 **Supplementary figure 2. N-glycosylation fingerprinting of the of HEK293T (A-F) and HepG2**
 2 **(G-J) cells. MALDI-TOF mass spectra of 2-AB-labeled and desialylated N-glycans were analyzed in**
 3 **a positive ion mode. Structural assignments based on biosynthetic knowledge were prepared using the**
 4 **GlycoWorkBench tool (2.1; EuroCarbDB).**



Red, dark blue, reference gene
 Error bars, plus/minus 1 SD (standard deviation)

Supplementary figure 3. Quantitative analysis of the gene expression levels in the non-fed and fucose-fed HEK293T SLC35C1 knockout cells. The chart represents the relative fold expression of 86 out of 91 tested glycosylation-related genes. The data for the remaining 5 genes with Cq values >35 were considered unreliable and omitted. The basal relative expression of the tested genes, i.e. the expression of the genes in the non-fed cells is set to 1 and presented as blue bars. The relative fold change of the expression in the fucose-fed cells is in orange. The error bars represent plus/minus 1 standard deviation (N=4). The genes were ordered according to the descending relative fold change expression in the fucose-fed cells. B4GAT1 gene with the expression unaffected by fucose feeding (fold change = 1) was used as a reference gene in the $\Delta\Delta Cq$ analysis (dark blue and red bars)

Supplementary table 1. Supplementary table 1. Quantitative analysis of the gene expression levels in the non-fed and fucose-fed HEK293T cell line. The results were subjected to the t-test. Data are presented as mean \pm standard deviation. ns, not significant.

<u>HEK293T</u>	Non-fed WT vs. non-fed C1KO			Non-fed WT vs. fucose-fed WT			Non-fed C1KO vs. fucose-fed C1KO		
Gene	WT	C1KO	Significant	WT	WT + fucose f	Significant	C1KO	C1KO + fucose	Significant
<i>FCL</i>	1.0 \pm 0.65	0.34 \pm 0.02	ns	1.0 \pm 0.65	0.54 \pm 0.04	ns	1.0 \pm 0.05	1.54 \pm 0.42	ns
<i>FUK</i>	1.0 \pm 0.18	1.47 \pm 0.3	ns	1.0 \pm 0.18	1.55 \pm 0.76	ns	1.0 \pm 0.2	0.47 \pm 0.87	ns
<i>FPGT1</i>	1.0 \pm 1.06	0.26 \pm 0.03	ns	1.0 \pm 1.06	0.35 \pm 0.05	ns	1.0 \pm 0.1	0.65 \pm 0.36	ns
<i>FPGT2</i>	1.0 \pm 1.32	0.38 \pm 0.12	ns	1.0 \pm 1.32	0.43 \pm 0.79	ns	1.0 \pm 0.3	0.76 \pm 0.14	ns
<i>GMDS</i>	1.0 \pm 1.21	0.35 \pm 0.1	ns	1.0 \pm 1.21	0.4 \pm 0.01	ns	1.0 \pm 0.27	1.27 \pm 0.32	ns
<i>SLC35C2</i>	1.0 \pm 1.45	0.37 \pm 0.03	ns	1.0 \pm 1.45	0.35 \pm 0.04	ns	1.0 \pm 0.08	1.3 \pm 0.29	ns

Supplementary table 2. Quantitative analysis of the gene expression levels in the non-fed and fucose-fed HepG2 cell line. The results were subjected to the t-test. Data are presented as mean \pm standard deviation. ns, not significant.

<u>HepG2</u>	Non-fed WT vs. non-fed C1KO			Non-fed WT vs. fucose-fed WT			Non-fed C1KO vs. fucose-fed C1KO		
Gene	WT	C1KO	Significant	WT	WT + fucose	Significant	C1KO	C1KO + fucose	Significant
<i>FCL</i>	1.0 \pm 0.09	1.14 \pm 0.3	ns	1.0 \pm 0.09	1.13 \pm 0.2	ns	1.0 \pm 0.27	1.17 \pm 0.11	ns
<i>FUK</i>	1.0 \pm 1.06	0.8 \pm 0.74	ns	1.0 \pm 1.06	2.68 \pm 1.27	ns	1.0 \pm 0.93	1.09 \pm 3.72	ns
<i>FPGT1</i>	1.0 \pm 0.36	1.59 \pm 0.84	ns	1.0 \pm 0.36	1.47 \pm 1.2	ns	1.0 \pm 0.53	1.37 \pm 1.87	ns
<i>FPGT2</i>	1.0 \pm 0.12	1.27 \pm 0.45	ns	1.0 \pm 0.12	1.07 \pm 0.71	ns	1.0 \pm 0.35	1.09 \pm 0.25	ns
<i>GMDS</i>	1.0 \pm 0.11	0.95 \pm 0.38	ns	1.0 \pm 0.11	1.09 \pm 0.15	ns	1.0 \pm 0.4	1.52 \pm 0.24	ns
<i>SLC35C2</i>	1.0 \pm 0.12	1.42 \pm 0.95	ns	1.0 \pm 0.12	1.43 \pm 1.33	ns	1.0 \pm 0.67	1.53 \pm 2.36	ns