Supplemental information

Supplemental tables

Table S1. Enzyme names and DNA primers used in PCR amplification and cloning

Gene name	EC Number	Enzyme	Oligo name	DNA sequence
ST6GAL1	EC:2.4.99.1	ST6Gal-I	Forward-*Hind*III	AAAAA AAGCTT ATGATTCACACCAACCTG
			Reverse-*Xba*I	AAAAA TCTAGA GCAGTGAATGGTCCG
B4GALT1	EC:2.4.1.22	GalT-I	Forward-*Hind*III	AAAAA AAGCTT ATGAGGCTTCGGGAG
			Reverse-*Xba*I	AAAAA TCTAGA CTTGCTGGTGTCCC
MGAT1	EC:2.4.1.101	GlcNAcT-I	Forward-*Bam*HI	AAAAA GGATCC ATGCTGAAGAAGCAGTCTG
			Reverse-*Xba*I	AAAAA TCTAGA ATTCCAGCTAGGATCATAGC
MGAT2	EC:2.4.1.143	GlcNAcT-II	Forward-*Eco*RI	AAAAA GAATTC CATGAGGTTCCGCATCTAC
			Reverse-*Xba*I	AAAAA TCTAGA CTGTGATTTCCACTGCAGTC
ST3GAL3	EC:2.4.99.6	ST3Gal-III	Forward-*Hind*III	AAAAA AAGCTT ATGGACTCTTGGTATTTGTG
			Reverse-*Xba*I	AAAAA TCTAGA GATGCCACTGCTTAGATCAG
GALNT6	EC:2.4.1.41	ppGalNAcT-6	Forward-*Bam*HI	AAAAA GGATCC ATGAGGCTCCTCCGCAGACG
			Reverse-*Xho*I	AAAAA CTCGAG GACAAAGAGCCACAACTGATGGGGG
GCNT1	EC:2.4.1.102	C2GNT-1	Forward-*Bam*HI	AAAAA GGATCC ATGGCTTTTCCCTGCCGCAGGTCCC
			Reverse-*Xba*I	AAAAA CTCGAG GGAGACCCGGTGTCCCCGG
GCNT3	EC:2.4.1.102	C2GNT-3	Forward-*Eco*RI	ATTA GAATTC ATGGGTCAATTGAAGAGACTCTGC
			Reverse-*Xho*I	ATTA CTCGAG AAAGGTCAGTCCCATAGATTGCC
GCNT4	EC:2.4.1.102	C2GNT-4	Forward-*Eco*RI	ATTA GAATTC ATGAAGATATTCAAAGGTTATTTTAAACATACCC
			Reverse-*Xho*I	ATTA CTCGAG TGATGTGGTAGTGAGATTTCTATCC
B3GNT6	EC:2.4.1.147	C3GNT-1	Forward-*Bam*HI	AAAAA GGATCC ATGGCTTTTCCCTGCCGCAGGTCCC
			Reverse-*Xho*I	AAAAA CTCGAG GGAGACCCGGTGTCCCCGG
B4GALT5	EC:2.4.1.38	GalT-V	Forward-*Eco*RI	ATTA GAATTC ATGCGCGCCCGC
			Reverse-*Xho*I	ATTA CTCGAG GTACTCGTTCACCTGAGCCAGC
SIAT4A	EC:2.4.99.4	ST3Gal-I	Forward-*Bam*HI	AAAAA GGATCC ATGGTGACCCTGCGGAAGAG
			Reverse-*Xho*I	AAAAA CTCGAG TCTCCCCTTGAAGATCCGGATTTTATTG
ST6GALNAC1	EC:2.4.99.3	ST6GalNAc-I	Forward-*Bam*HI	AAAAA GGATCC ATGGGTCCTGCCTGTGGAGATGC
			Reverse-*Xho*I	AAAAA CTCGAG GTTCTTGGCTTTGGCAGTTCCGGG
C1GALT1	EC:2.4.1.122	C1GalT-1	Forward-*Bam*HI	AAAAA GGATCC ATGGCCTCTAAATCCTGGCTG
			Reverse-*Xho*I	AAAAA CTCGAG AGGATTTCCTAACTTCACTTTTGTATCTTC

Enzyme name as listed in KEGG (see Http://ww.genome.jp/kegg/pathway.html

Table S2. DNA primers used for PCR amplification in domain swapping experiments

Gene Name	Enzyme/Oligo name	DNA sequence
ST6GAL1	5' XhoI ST6GAL-I-cat	ATTA CTCGAG GGGCCAGGACCAGGC
	3' XbaI ST6Gal-I-cat	ATTA TCTAGA GCAGTGAATGGTCCGGAAG
	5' HindIII ST6Gal-Itm	ATTA AAGCTT ATGATTCACACCAACCTGAAGAAAAAG
	3' XhoI ST6Gal-Itm	ATTA CTCGAG GTTCATGCTTAGGTAATTCTTCCAGATC
ST3GAL3	5' XhoI ST3Gal-IIIcat	ATTA CTCGAG CGGGAGTTCGTGCCG
	3' XbaI ST3Gal-IIIcat	ATTA TCTAGA GATGCCACTGCTTAGATCAGTG
	5' HindIII ST3Gal-IIItm	ATTA AAGCTT ATGGGACTCTTGGTATTTGTGC
	3' XhoI ST3Gal-IIItm	ATTA CTCGAG CAGGAACATGGGTGCTGG
B4GALT1	5' XhoI GalT-Icat	ATTA CTCGAG ACCACCGCACTGTCGC
	3' XbaI GalT-Icat	ATTA TCTAGA GCTCGGTGTCCCGATG
	5' HindIII GalT-Itm	ATTA AAGCTT ATGAGGCTTCGGGAGCC
	3' XhoI GalT-Itm	ATTA CTCGAG GTGGGGCACTGGGACC
MGAT1	5' XhoI GlcNAcT-Icat	ATTA CTCGAG TGCCTGGACAAGCTGC
	3' XbaI GlcNAcT-Icat	ATTA TCTAGA ATTCCAGCTAGGATCATAGCC
	5' HindIII GlcNAcT-Itm	ATTA AAGCTT ATGCTGAAGAAGCAGTCTGC
	3' XhoI GlcNAcT-Itm	ATTA CTCGAG GCGAACAGTGC
MGAT2	5' XhoI GlcNAcT-IIcat	ATTA CTCGAG GTGGTCCAGGTGCATAACC
	3' XbaI GlcNAcT-IIcat	ATTA TCTAGA CTGCAGTCTTCTATAACTTTTACAGAGTTC
	5' HindIII GlcNAcT-IItm	ATTA AAGCTT ATGAGGTTCCGCATCTACAAAC
	3' XhoI GlcNAcT-IItm	ATTA CTCGAG CAGCACCAGCTCCCG
GalNT6	5' XhoI ppGalNT-6cat	ATTA CTCGAG CAGAAGAGCAAGTGGACCC
	3' XbaI ppGalNT-6cat	ATTA TCTAGA GACAAAGAGCCACAACTGATG
	5' HindIII ppGalNT-6tm	ATTA AAGCTT ATGAGGCTCCTCCGCAG
	3' XhoI ppGalNT-6tm	ATTA CTCGAG AAATGCTTTTCCATCTGCC
C1GALT1	5' XhoI C1GalT-1cat	ATTA CTCGAG ATGAGTTCAGAAGAAAATAAAGACTTC
	3' XbaI C1GalT-1cat	ATTA TCTAGA AGGATTTCCTAACTTCACTTTTGTATC
	5' HindIII C1GalT-1tm	ATTA AAGCTT ATGGCCTCTAAATCCTGGC
	3' XhoI C1GalT-1tm	ATTA CTCGAG AAACAACACTTTGTTACAACGC
GCNT1	5' XhoI C2GCNT-1cat	ATTA CTCGAG TTTCCAATAGCATATTCTATAGTGGTTC
	3' XbaI C2GCNT-1cat	ATTA TCTAGA GTGTTTTAATGTCTCCAAAGCTTTG
	5' BamHI C2GCNT-1tm	ATTA GGATCC ATGCTGAGGACGTTGCTG
	3' XhoI C2GCNT-1tm	ATTA CTCGAG CTCCGCCTCTTCTTTACTAAGG

Cat: primers corresponding to the sequence of amino acids starting from 121

Tm: primers corresponding to the 120 first amino acids of the corresponding protein

Table S3. Heteromeric interactions between O- and N-Glycosyltransferases

		N-glycosyltransferases				
		GlcNAcT-I	**GlcNAcT-II**	**GalT-I**	**ST6Gal-I**	**ST3Gal-III**
O-glycosyltransferases	**ppGalNAcT-6**	4,52% ± 2,63	2,23% ± 0.24	3,58% ± 1,64	3,02% ± 1,72	3,12% ± 1,86
	C1GalT-1	1,40% ± 1,32	4,02% ± 1,99	0,92% ± 0,42	1,98% ± 0,68	1,72% ± 0,23
	C2GNT-1	3,02% ± 0,90	3,84% ± 1,32	2,29% ± 2,12	4,28% ± 2,39	2,13% ± 1,05
	C2GNT-3	0,81% ± 0,28	0,84% ± 0,32	0,67% ± 0,56	1,23% ± 1,12	1,45% ± 0,39
	C2GNT-4	2,35% ± 0,87	4,73% ± 3,52	1,24% ± 0,21	4,01% ± 2,45	3,75% ± 1,29
	ST3Gal-I	1,32% ± 0,67	3,33% ± 2,38	4,32% ± 0,28	2,32% ± 1,16	2,54% ± 1,53
	ST6GalNAc-I	2,80% ± 0,88	3,27% ± 0,89	0,92% ± 0,43	3,89% ± 2,57	2,12% ± 0,75
	C3GNT-1	1,20% ± 0,28	0,47% ± 0,31	1,17% ± 0,17	0,61% ± 0,55	3,67% ± 1,62
	GalT-V	1,73% ± 1,12	2,53% ± 1,04	6,12% ± 4,17	3,00% ± 1,67	3,23% ± 1,35

Supplemental figures

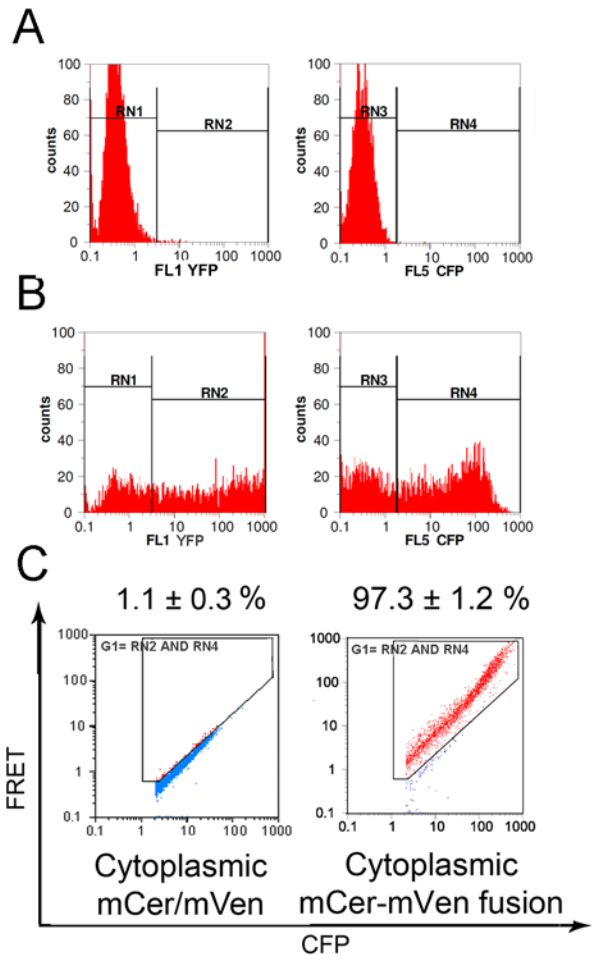

Figure S1. Validation of FRET settings for the flow cytometric quantification. Validation of the gates were performed according to Banning et al. and Thyrock et al. (14, 15). Briefly, COS7 cells were transfected with either mCer or mVen encoding plasmids, detached, and analyzed by flow cytometry using appropriate filter sets for mCer (405 nm excitation, 425-475 nm emission) or mVen (488 nm excitation, 515-540 nm emission). (**A)**. The gates RN1 and RN3 were set to remove autofluorescence and cross-over between the channels; CFP in the YFP channel (left) and YFP in the CFP channel (right), **B)** Cells expressing both mCer and mVen were selected using the RN2 and RN4 gates. **C)** FRET+ gate was determined by using the cytoplasmic mCer and mVen constructs (negative controls, blue dots), and their fusion protein (mCer-mVen) as a positive control (red dots, right). The fusion protein was prepared by PCR cloning of mVen in frame with mCer using *Hind*III and *Bam*HI restriction cleavage sites. This created a 33 amino acid long helical linker region (ADPLVTAASVLEFCRYPSHWRPLEHASRRSIAT) between the two fluorescent proteins. FRET signals are plotted as the ratio between mVen and mCer intensities after excitation with the 405 nm laser only (405ex, 515-540em). In each measurement, 2-5 $x10^3$ cells expressing both mCer and mVen were counted.

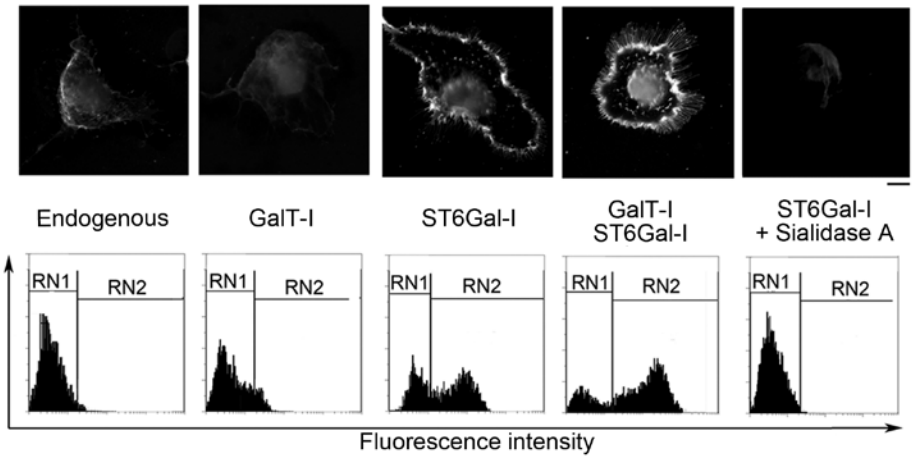

Figure S2. Cell surface sialic acid content in live cells. COS7 cells transfected with the plasmids that encode the indicated glycosyltransferase constructs were prepared either for fluorescence microscopy or quantification of FITC-fluorescence intensity by flow cytometry. Staining of cell surface sialic acids was performed using aminooxy-biotin and FITC-conjugated streptavidin (20). Sialidase A treatment was used to verify the specificity of the staining. RN1 gate denotes autofluorescence and RN2 gate FITC-positive cells.

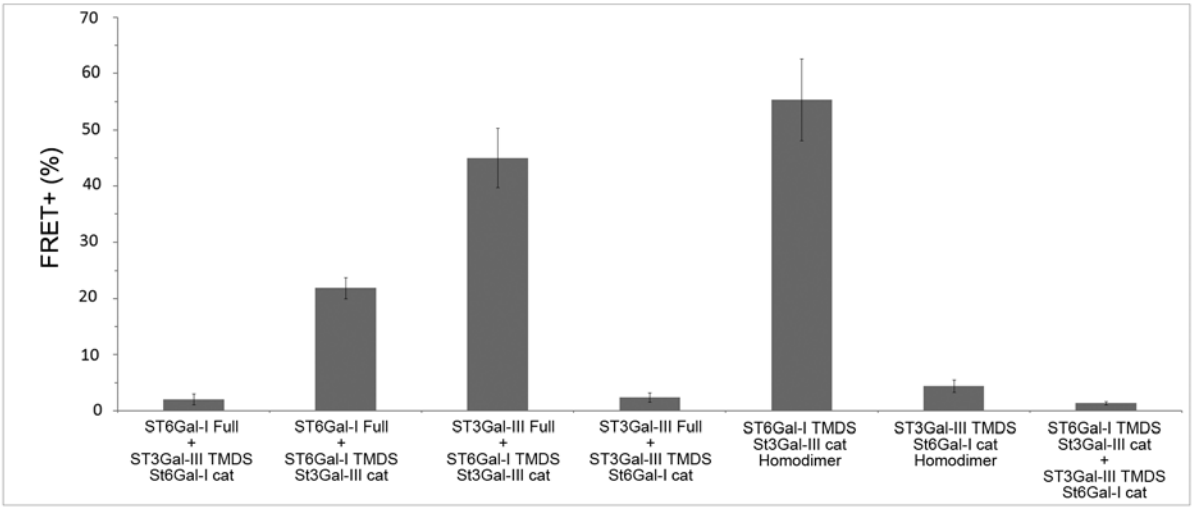

Figure S3. Preliminary screening of the interaction domains of ST3Gal-III and ST6Gal-I homomers. We selected these enzymes for the preliminary screening of their homomeric interaction domains because the earlier data (shown in Fig. 1) showed that these enzymes do not interact with each other. To identify the homomeric interaction domains we constructed chimeric enzymes by linking the TMDS of ST3Gal-III with the catalytic domain (cat) of ST6Gal-I, and vice versa. FRET measurements between the full length and the chimeric constructs showed that ST6Gal-I interacts only via its TMDS (the first two bars), whereas ST3Gal-III interacts only via its catalytic domain (the third and fourth bars). Cross-testing of the chimeric constructs validated these results, as the combined interacting domains gave the highest signal (the fifth bar), whereas the non-interacting domains of these two proteins gave no signal (the last two bars). The non-interacting domains of these enzymes (ST6Gal-Icat and ST3Gal-IIITMDS were used for the screening of interaction domains in other N- and O-glycosyltransferases.

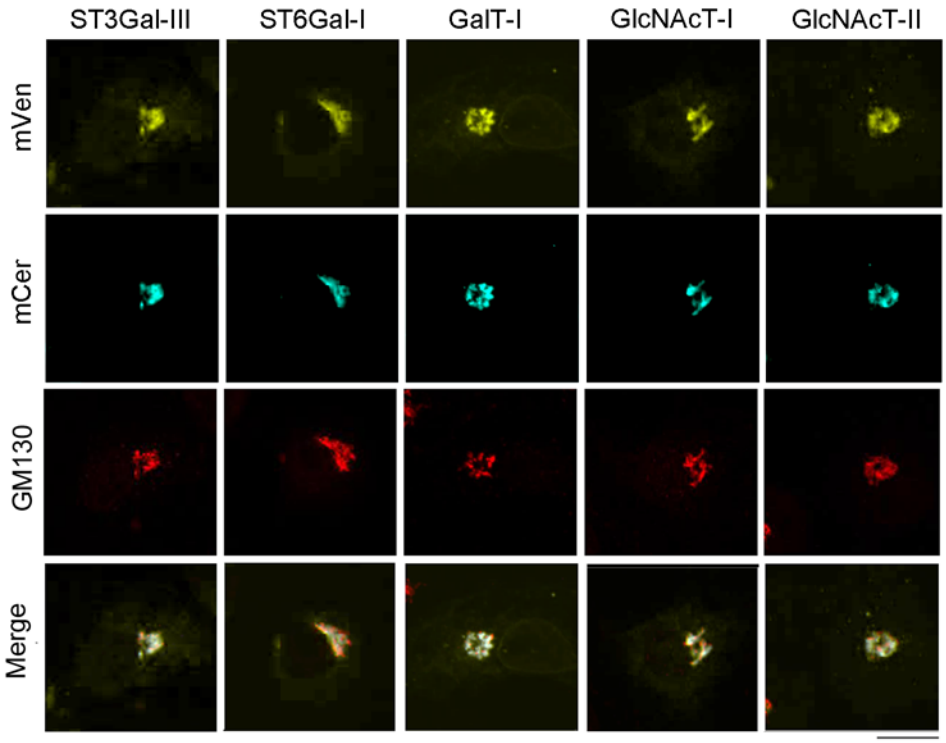

Figure S4. Localization of the N-glycosyltransferase FRET constructs in COS7 cells. Cells were transfected with the indicated plasmids, grown overnight, and fixed prior to staining cells with the monoclonal Golgi marker (GM130) antibody. Stained cells were visualized using appropriate filter set for mVen, mCer and Alexa Fluor 594-conjugated anti-mouse antibodies (for GM130). Both mCer and mVen fusion proteins co-localized with the Golgi marker. Scale bar 10 μm.

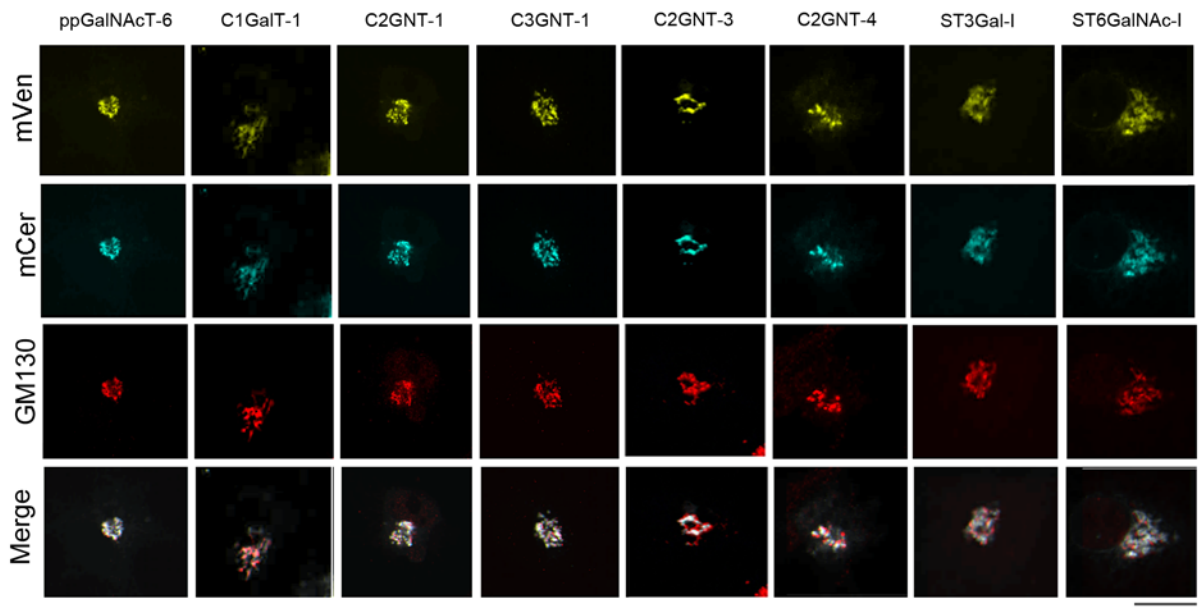

Figure S5. Localization of the O-glycosyltransferase FRET constructs in COS7 cells. Cells were transfected with the indicated plasmids, grown overnight, and fixed prior to staining with the monoclonal Golgi marker (GM130) antibody. Stained cells were visualized using appropriate filter set for mVen, mCer and Alexa Fluor 594-conjugated anti-mouse antibodies (for GM130). Both mCer and mVen fusion proteins co-localized with the Golgi marker. Scale bar 10 μm.

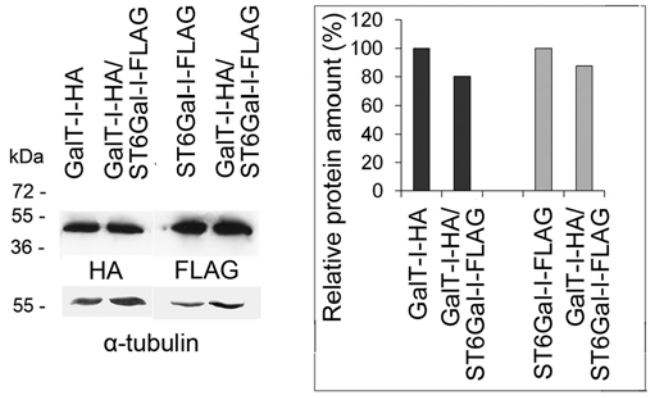

Figure S6. Immunoblot analyses of enzyme protein levels in the transfected cells. Cells were transfected with GalT-I-HA and ST6Gal-I-FLAG encoding plasmids either alone or together. After 24 h cells were solubilized in SDS sample buffer, subjected to SDS-PAGE and transferred to nitrocellulose sheet before immunoblotting with the anti-HA and FLAG antibodies. Quantification of the protein bands was done using ImageJ 1.43 software. α-tubulin staining was used for normalization of the protein levels. Note that the protein levels rather decrease slightly than increase upon co-expression of the two constructs.

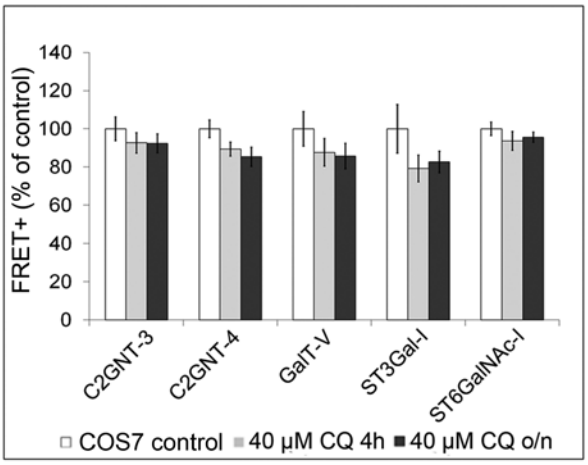

Figure S7. pH sensitivity of the homomeric O-glycosyltransferases. Cells were single transfected with the indicated enzyme constructs and treated or not with 40 µM CQ for 4 h or overnight. 24 h after transfection, FRET+ cells were quantified by flow cytometry as described in Fig. 1. All the results are presented as percentages of nontreated COS7 cells (mean ± SD, n = 3).

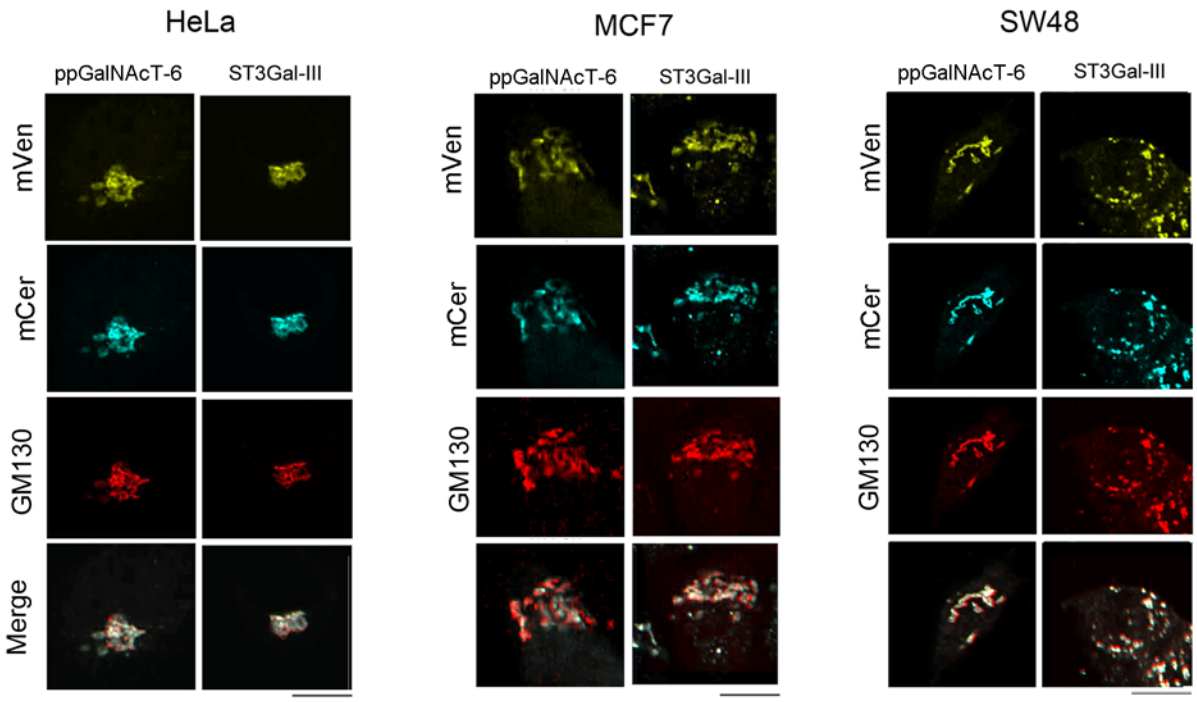

Figure S8. Localization of the N- and O-glycosyltransferase FRET constructs in cancer cell lines. Cells were transfected with the indicated plasmids, grown overnight, and fixed prior to staining cells with the monoclonal Golgi marker (GM130) antibody. Stained cells were visualized using appropriate filter set for mCer, mVen and Alexa Fluor 594-conjugated anti-mouse antibodies. Scale bar 10 µm.

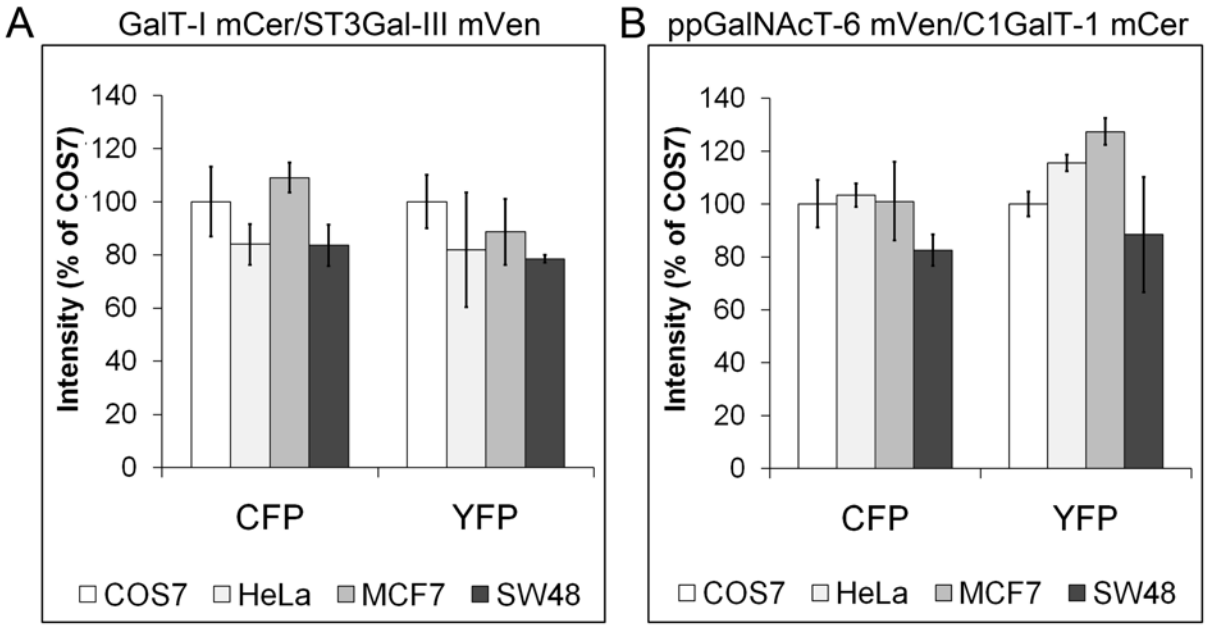

Figure S9. Relative CFP and YFP intensities of the co-expressed FRET constructs in different cell lines. **A)** GalT-I-mCer and ST3Gal-III-mVen. **B)** ppGalNAcT-6-mVen and C1GalT-I-mCer. Cells were transfected with the indicated enzyme constructs and analyzed by flow cytometry. Mean fluorescence intensity values (mean-x) for mCer and mVen were recorded in triplicate and presented as percentages of the intensities obtained with COS7 cells (mean ± SD).