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## Legends

**Appendix Table S1. Indel analysis and sequencing of *GALNT* KO cell lines.** Sequencing results for *GALNT* KO cell lines on WT or *COSMC* KO background. The table also contains information regarding the first predicted amino acid to be altered from indel formation. The clone suffixes A1-A3 refer to alleles 1-3, if heterozygotic indel formation is found.

**Appendix Figure S1. Overlap between different O-glycoproteomic approaches.** Venn diagrams depict overlap between proteins identified using the two O-glycoproteomic approaches for individual *GALNT* isoform KOs.

**Appendix Figure S2. Expression of *GALNT* genes (> 1 RPKM) in *GALNT* KO cell lines.** Bar graphs show average RPKM + SD.

**Appendix Table S1. Indel analysis and sequencing of *GALNT* KO cell lines.**

Gene	Clone	Sequence	gRNA/ZFN	Exon	Indel	First a.a.	
<i>GALNT1</i>	REF	TTCCTGGATGCCCATTTGTgagtgtACAGTGGGATGGCTGGAGCCTC	ZFN nuclease CTGGATGCCCATTTGTgagtgtACAGT GGGATGGCTG	Total 11		559	
	12C9.A1	TTCCTGGATGCCCA-----CAGTGGGATGGCTGGAGCCTC		4	-11	211	
	12C9.A2	TTCCTGG-----		4	-129+12	208	
	3E4.A1	-----TGGAGCCTC		4	-108	185	
	3E4.A2	-----ATGGCTGGAGCCTC		4	-104	184	
	12G9.A1	TTCCTGGATGCCCA-----CAGTGGGATGGCTGGAGCCTC		4	-11	211	
	12G9.A2	TTCCTGG-----		4	-129+12	208	
	10G6	TTCCTGGATGCCCATTTGT-ag-----		4	-73	212	
	(In <i>COSMC</i> KO)	2E9.A1	-----TGGGATGGCTGGAGCCTC		4	-42	202
	2E9.A2	TTCCTGGATGCCCA-----		4	-41	211	
<i>GALNT2</i>	REF	GGTCGGCCCTACTCAGGACcgg--tggtCAGGTGAGGCCAGGAGATG	ZFN nuclease GTCGGCCCTACTCAGGACcgtggtCA GGTGGGCCAGGAGAT	Total 16		571	
	2A2	GGTCGGCCCT-ACTCAGGA-----GATG		4	-22	155	
	2D3.A1	GGTCGGCCCTACTCAGGACcgg-tggtCAGGTGAGGCCAGGAGATG		4	+1	156	
	2D3.A2	GTCGG-----		4	-53	151	
	2D6.A1	GG-----AGATG		4	-37	149	
	2D6.A2	GGTCGGCC-T-----		4	-43	152	
	10G5.A1	GGTCGGCCCTCACTCAG-----GTGAGGCCAGGAGATG		4	-10	154	
	10G5.A2	GGTCGGCCCT-ACTCT-----GGCCAGGAGATG		4	-16	152	

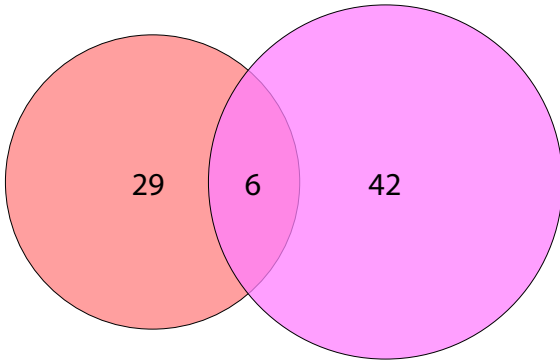
(In COSMC KO)	1G8.A1	GGTCGGCCCT-CTCAGGACcgTAtggtCAGGTGAGGCCAGGAGATG		4	+2	157
	1G8.A2	-----cg--tggtCAGGTGAGGCCAGGAGATG		4	-117	125

<i>GALNT3</i>	REF	TTTGAATTCAACAAACCTTCTcct-----	ZFN nuclease TTCAACAAACCTTCTccttatGGAAG TAACCATAAC	Total 11		633
	4.6B	TTTGAATTCAACAAACCTTCTcctCCGGACCCTAATTGACTGAGCG		5	+22	322
	REF	TTTGAATTCAACAAACCTTCTccttatGGAAGTAACCATAAC				
	6.7E	T-----atGGAAGTAACCATAAC		5	-24	314
	5.6D.A1	TTTGAATTCAACAAACCTTCTc-----ATAAC		5	-15	321
	5.6D.A2	TTTGAATTCAACAAACCTTCTcctta-----ACCATAAC		5	-8	322(stop)
	5.6D.A3	TTTGAATTCAACAAACC-----		5	-26	320
	9.8B.A1	TT-----GTAACCATAAC		5	-29	314
	9.8B.A2	TTTGAATTCAACAAACCTTCT-----		5	-33	321
	REF	TTTGAATTCAACAAACCTTCTcctt-atGGAAGTAACCATAA				
	2.9B	TTTGAATTCAACAAACCTTCTccttAatGGAAGTAACCATAA		5	+1	322(stop)
gRNA	REF	GAAAAGGTCTGATCACTGCTCGGTTGCTAG	gRNA1 CTGATCACTGCTCGGTTGCT			
	2.4D.A1	GAAAAGGTCTGATCACTGC-----TGCTAG		4	-5	261
	2.4D.A2	GAAAAGGTCTGATCACTGCTCGGTT (+131) GCTAG		4	+131	263
	2.4D.A3	GAAAAGGTCTGATCACTGCTCGGTG (+206) GCTAG		4	+206	262
	REF	TAGTTTTTCATAATGAA-GCGTGGT	gRNA2 TAGTTTTTCATAATGAAGCG			
	2.2H	TAGTTTTTCATAATGAAAGCGTG		3	+1	197
	REF	CTGTCCACAGTGTGCTCTATTCTTCACCTGCAATACTG--CTGAAGG	gRNA3 TTCACCTGCAATACTGCTGA			
	11H.A1	CTGTCCACAGTGTGCTCTATTCTTCACCTGCA-----GTCTGAAGG		3	+2-6	215
	11H.A2	CT-----		3	-84	205
(In COSMC KO)	REF	TTTGAATTCAACAAACCTTCTcct-----	ZFN nuclease TTCAACAAACCTTCTccttatGGAAG TAACCATAAC			
	3.3B	TTTGAATTCAACAAACCTTCTcctCCGGATCCTAATTGACTGAGCG		5	+22	322

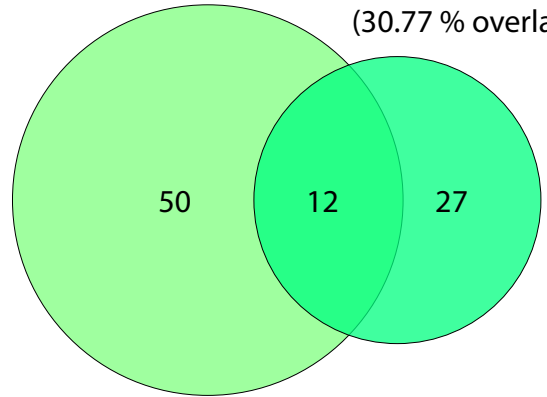
# Appendix Figure S1

## Protein identifications

WT  $\Delta$ T1 TMT Jacalin  
(12.5 % overlap)



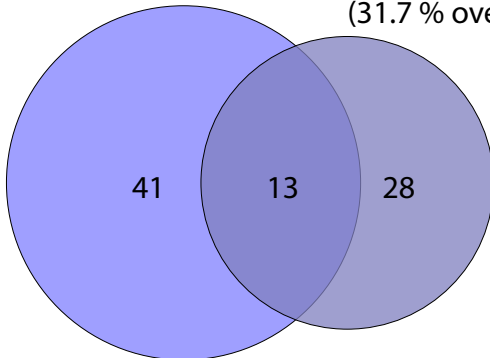
WT  $\Delta$ T2 TMT Jacalin  
(30.77 % overlap)



$\Delta$ COSMC  $\Delta$ T1 dimethyl VVA  
(17.14 % overlap)

$\Delta$ COSMC  $\Delta$ T2 dimethyl VVA  
(19.4 % overlap)

WT  $\Delta$ T3 TMT Jacalin  
(31.7 % overlap)



$\Delta$ COSMC  $\Delta$ T3 dimethyl VVA  
(24.1 % overlap)

Appendix Figure S2

