West et al., Supplemental Data

Primer	Sequence
N95Q-F	5'-GGGTGGCCTCTTCCTCACCATCTACCAGAGCACCACACG-3'
N95Q-R	5'-CGTGTGGTGCTCTGGTAGATGGTGAGGAAGAGGCCACC -3'
N120Q-F	5'-GCCACCATGTTCCAGAGCTCGGAGCAGTCCC-3'
N120Q-R	5'-GGGACTGCTCCGAGCTCTGGAACATGGTGGC-3'
N230Q-F	5'-CCCAGGCCTTCTACCAGGGCAGCCTCACGGC-3'
N230Q-R	5'-GCCGTGAGGCTGCCCTGGTAGAAGGCCTGGG-3'
N266Q-F	5'-CGAGCACCCGCTGCAGATCAGCCTGGGAG-3'
N266Q-R	5'-CTCCCAGGCTGATCTGCAGCGGGTGCTCG -3'
N297Q-F	5'-CCTCAAAGGGTACCAGTTCTCCCGGGAGAGCG -3'
N297Q-R	5'-CGCTCTCCCGGGAGAACTGGTACCCTTTGAGG-3'
N344Q-F	5'-GACTGAGGTGGTCCGCCAGATGACCTCCGAG -3'
N344Q-R	5'-CTCGGAGGTCATCTGGCGGACCACCTCAGTC -3'
N511Q-F	5'-CCAGCTTCTGCCCCAGGTCACGACAGTGGAG-3'
N511Q-R	5'-CTCCACTGTCGTGACCTGGGGCAGAAGCTGG-3'
G61L-F	5'-GCACTGCGGGACCTGGGCTCTGCGGTGGATGCAG-3
G61L-R	5'-CTGCATCCACCGCAGAGCCCAGGTCCCGCAGTGC-3'
G62L-F	5'-GCACTGCGGGACGGTCTGTCTGCGGTGGATGCAG-3
G62L-R	5'-CTGCATCCACCGCAGACAGACCGTCCCGCAGTGC-3'
G61L,G62L-R	5'-GGGATGCACTGCGGGACCTGCTGTCTGCGGTGGATGCAGCC-3'
G61L,G62L-F	5'-GGCTGCATCCACCGCAGACAGCAGGTCCCGCAGTGCATCCC-3'
MBW-P1	5'-CACACAGAATTCTCAGCCTCCAAGGAACCT-3'
MBW-P2	5'-CACACAGCGGCCGCGTAGCCGGCAGGCTCCCC-3'

Supplemental Table 1. Primers used for site-directed mutagenesis of human GC	ЗТ.
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Supplemental Figure 1. Iterative human GGT propeptide homology model building with MODELLER. Five hundred initial models of human GGT (see Methods Section) were built in iteration A followed by three additional iterations (B-D), comprised of 200 models each, which refined loop regions. The DOPE score of each model was used to evaluate the model quality.

Supplemental Figure 2. Additional SDS-PAGE analyses of GGT *N*-glycosylation mutants. (A.) Western analysis against the deglycosylated small subunit of wild-type human GGT and single *N*-glycosylation site mutants to determine relative expression levels. Total cell homogenates $(1.5\mu g)$ were incubated with 20U of PNGaseF for 18 h at 37°C and then resolved on a 10% SDS-PAGE followed by immunoblotting against the small subunit of GGT (left panel). Band densities (listed below immunoblot) were defined relative to the wild-type control (1 unit of GGT/1.5 μ g of protein) as described in the Methods section. An immunoblot against GAPDH is included as a loading control (lower left panel). An immunoblot of an eight-fold (12 μ g) increase in the sample load from a cell extract expressing the N95Q mutant is included to demonstrate the relative ratio of the expression levels of the mature heteordimer from this allele relative to wild-type GGT (right panel). (B.) Western analysis against the large subunit of GGT within whole cell lysates from HEK293 cells transfected with either empty vector (lane 1), wild-type GGT (lane 2), or mutant GGT alleles (lanes 3-9) were a resolved by SDS-PAGE and electroblotted onto nitrocellulose. Immunoblotting was conducted using an antibody specific to the large subunit of GGT.

Supplemental Figure 3. Comparative sequence analysis of the N-glycosylation sites on human GGT. Amino acid sequence alignment of residues surrounding each of the N-glycosylation sites from human (Homo sapiens) GGT with the corresponding sequences from pig (Sus scrofa), mouse (Mus novegicus), nematode musculis), rat (Rattus (Caenorhabiditis elegans), fission yeast (Schizosaccharomyces pombe), and the bacteria Bacillus subtilis, Escherichia coli, and Helicobacter pylori with the ClustalW program (52). Shading is used to indicate the occurrence of similar amino acids (identical residues, magenta; conserved substitutions, aquamarine) or putative N-glycosylation sites (green shading and arrowheads). The alignment of residues surrounding the universally-conserved catalytic threonine residue at the N-terminus of the small subunit (T381 in human GGT) are also included (red shading) for reference.

Supplemental Figure 4. Homology models of human GGT alleles bearing Loop 3 mutations. (A.-D.) Comparative homology modeling of amino acid substitutions that are predicted to disrupt hydrogen bonding (dashed lines) between the Loop 3 (L3) structure (see Fig. 6) and the common oligosaccharide precursor. (A.) Homology model of the hydrogen bonding interactions predicted to occur between L3 and mannose 6 of the oligosaccharide precursor in the wild-type GGT propeptide. (B.) Homology model of the G61L mutant propeptide, depicting the predicted loss of hydrogen bonding between the oligosaccharide precursor and G62. (C.) Homology model of the G62L mutant propeptide, depicting the predicted loss of hydrogen bonding between the oligosaccharide precursor and L58. (D.) Homology model of the G61L,G62L double mutant that is predicted to disrupt both of the hydrogen bonds between L3 and the oligosaccharide precursor. Leucine was selected as the substituted amino acid at G61 and G62 in order to maximize disruption of L3-glycan interaction while minimizing perturbations to the relative orientation of α -helices 2 and 3. Direct substitutions at L58 that were predicted to disrupt hydrogen

bonding with the *N*-glycan at N95 were also predicted to significantly distort the disulfide-bonded alpha helices and were, thus, avoided.

Supplemental Figure 5. Effects of the Loop 3 mutations on the autocatalytic cleavage of human GGT. (A.) L3 mutations disrupt GGT's enzymatic activity. Total cell lysates from HEK293 cells transfected with wild-type GGT, GGT(N95Q), GGT(G61L), GGT(G62L), or GGT(G61L,G62L) were assayed at 37°C for transpeptidation activity in the presence of 3mM L-GpNA and 40mM glycylglycine, using 2µg of total protein from each extract. Data are the mean +/- SD from triplicate assays. Data are presented as the percent transpeptidation activity relative to wild-type GGT. (B.) L3 mutations inhibit propeptide cleavage. Total cell lysates from HEK293 cells transfected with either wild-type GGT (lane 1), or mutant GGT alleles (lanes 2-5) were resolved by SDS-PAGE and electroblotted onto nitrocellulose, using 4µg of total protein from each extract. Immunoblotting was conducted using an antibody specific to the small subunit of GGT (upper and middle panels). The upper panel depicts the simultaneous detection of both the propeptide and small subunit in the same immunoblot, while the middle panel depicts a prolonged exposure of the region of the blot where the mature small subunit migrated. The faster migration of the propeptide from the GGT(N95Q) mutant is consistent with the loss of glycosylation at N95. An immunoblot against GAPDH is included as a loading control (lower panel). MW, molecular weight markers.



West et al., Supplemental Figure 1

West et al., Supplemental Figure 2





West et al., Supplemental Figure 3

	DISULFIDE BRIDGE											
							V		V			
н.	sapiens	48	KQ <mark>CSKIG</mark> RDA <mark>L</mark> RD <mark>GG</mark> SAVI	DAAIAALLCV	GLMNAHSMG	I <mark>GGG</mark> L <mark>F</mark> LT <mark>I</mark> Y	NSTTRKAEV <mark>IN</mark> A	106 111	PRLAFATMENSSEQ	2-S 125		
s.	scrofa	47	LR <mark>CSEIG</mark> RDT <mark>L</mark> RD <mark>GG</mark> SAVI	DAAIAALLCV	GLMNAHSMG	I <mark>GGG</mark> L <mark>F</mark> LT <mark>I</mark> Y	NSTTRKAEI <mark>IN</mark> A	105 110	PRLASASMENSSEQ	2-S 124		
м.	musculus	47	KR <mark>CSEI</mark> GRDILQEGGSVVI	DAAIASLLCM	GLMNAHSMG	I <mark>GGG</mark> L <mark>F</mark> FT <mark>I</mark> Y	NSTTGKVEV <mark>IN</mark> A	105 110	PRLANTTMENNSKI)-S 124		
R.	norvegicus	47	KR <mark>CSEIG</mark> RDM <mark>L</mark> QE <mark>GG</mark> SVVI	DAAIASLLCM	GLINAHSMG	I <mark>GGG</mark> L <mark>F</mark> FT <mark>I</mark> Y	NSTTRKAEV <mark>IN</mark> A	105 110	PRLANTS <mark>MEN</mark> NSKI)-S 124		
c.	elegans	104	EI <mark>CSEIG</mark> RNI <mark>L</mark> LK <mark>GG</mark> NAVI	D <mark>SAIA</mark> ALF <mark>CI</mark>	GVMDTHSAG	I <mark>GGG</mark> HFMT I Y	NATTKECTVIDA	161 174	PLAATEE <mark>MY</mark> RDKWN	IQS 189		
s.	pombe	107	ET <mark>CSQIG</mark> VGI <mark>LKAGG</mark> NAVI	DAAIASGI <mark>CI</mark>	GAVNSFSSG	I <mark>GGG</mark> GFMLIF	-HPNGTAHSLNF	164 169	PAGASKNMFHGNS1	LS 184		
в.	subtilis	54	PLASEICADVLKKGGNAI	D <mark>A</mark> AVAIQFAL	NVTEPMMSG	I <mark>GGG</mark> GFMMVY	DGKTKDTTI <mark>ID</mark> S	112 117	PAGATPDMFLDENG	KA 132		
E.	coli	53	ATATOV GVDILKE GGNAVI	D <mark>A</mark> AVAVGYAL	AVTHPQAGN	L <mark>GGG</mark> GFML I F	RS-KNGNTTAIDF	110 115	PAKATRDMFLDDQG	INP 130		
н.	pylori	45	PLATEICQKILEDCCNAI	D <mark>AA</mark> VAMGFAL	AVVHPAAGN	I <mark>GGG</mark> G <mark>F</mark> AVIH	il-Angenva <mark>ld</mark> f	102 107	PLKATKNMFLDKQ0	NV 122		
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			•					•				
		225	ACA EVALOPET TAOTUKDTO	. 242	265 1 1 1910	DUUTYMD 2	77 295 63	SPOD-POUPO 3	05			
п. с	sapiens	223	ACARCOSSINGSING	E 242	264 10191	EDAOLYAD 2	76 294 G	NESP-ASVET 3	04			
ы.	scrora	224	AKA FYNCSLTAOTVKDIO	E 242	264 MSTGI	DATINUP 2	76 294 G	NESP-KSVAT 3	04			
D.	nascurus	224	ARAFYNCSLTAOTVKDIO	E 242	264 MSTGI	DSTLYVP 2	76 294 G	NESP-KSVAT 3	04			
ĉ.	alegans	295	TADEYTORLAFOLAKEFE	A 313	337 TYTKI	NGROVCG 3	49 368 GI	TYNM-KSFN- 3	77			
e.	nombe	285	PEVEYTCKTAERLVKEVO	0 303	324 IYGNE	DREVITC 3	36 354 K	DLSEGTSILG 3	65			
в.	subtilis	237	TDAFYECKFAKALSDTVO	D 255	277 WGDYO	SYOIATTP 2	89 307 NE	NLSOYDVR 3	16			
E.	coli	236	PDEFYKGTIAEOIAOEMO	K 254	276 SGDYR	YOVYSMP 2	88 306 NE	DMKKYGFG 3	15			
н.	pylori	228	AKGEYOCOVAELTEKDMK	K 246	268 IGSYR	YKIISMS 2	80 298 NZ	DLSALGYG 3	07			
••••	PILOLL		** * .: ::					: .				
			•					-				
		240										
п. с	sapiens	342	VRIMTSEFFAAQLRAQ 3	5/ 381	TA LSVVAE	DGSAVSA	399 • • • 502	PREHNQLEPNVI	TVERN 518			
э.	sciora	341	VRIMSSEFFADQLRAR 3	56 380	A LSVVSX	DGSAVSA		PREHNQLEPNT	TLEKG 517			
D.	nuscurus	341	IR MSSEFIATQLRAR 3	56 380	TA LSAVSE	DGSAVAA	398 501	PREHNOLLPNT	TVERD 517			
c.	norvegicus	341	IRMSSEFIATQLRAR 3	56 380	LSVVSE	DGSAVAA	398 • • 501	PREHNQLEPNT	TVERN 517			
с. с	eregans	413	ARNITSREWADWVRSK 4	28 452	TT VSIIDA	DGNAVSVIS	470 573	PRMHNQLQPNY	WYEPN 589			
э. р	pombe	402	VEQLESLETADEIRNN 4.		LSVIDK	DNMA GLIA	459 • • 561	PREHHQLMPNI	YIDET 577			
D.	subtills	349	LIGHLHPDIIKEKQQL 3	64 403	FTVADR	WGNVVSIAT	421 • • • 514	PRITTNSMS-S	RIEDG 529			
ц.	nulori	348	WORT THEAT ARTICON 2	55	ISVVDR	DGNAVAV	406 509	PREHHOWLPDEI	RVEKG 525			
п.	PATOLI	540	VDRUINKAIAKKIFDT 3	55 380	I SVADR	WGNAVSVAY	398 501	PRC HMQWLPDEI	RIERF 517			
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West et al., Supplemental Figure 5

