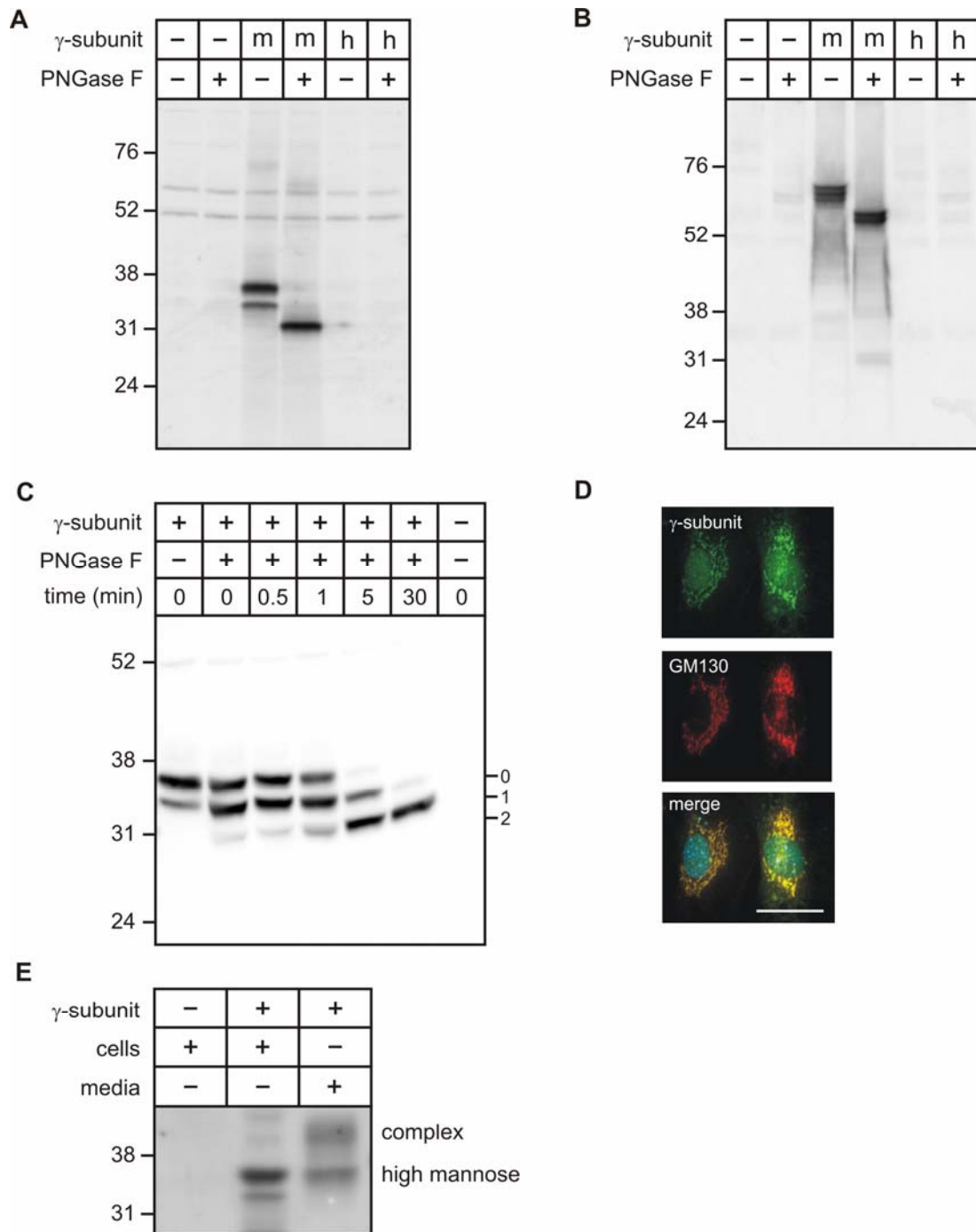


**POSTTRANSLATIONAL MODIFICATIONS OF THE γ -SUBUNIT AFFECT
INTRACELLULAR TRAFFICKING AND COMPLEX ASSEMBLY OF THE GLcNAC-1-
PHOSPHOTRANSFERASE**

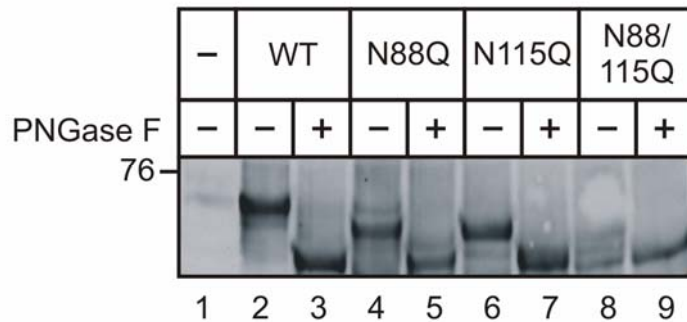
Marisa Encarnação, Katrin Kollmann, Maria Trusch, Thomas Braulke, and Sandra Pohl

Supplemental Figures and Table

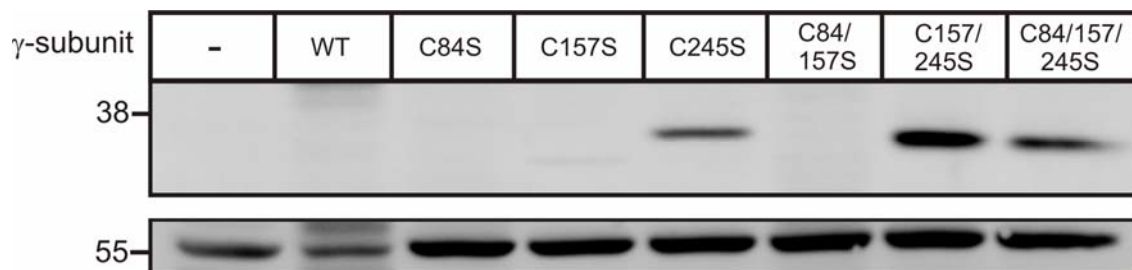


SUPPLEMENTAL FIGURE S1. Expression analysis of mouse γ -subunits. Cell extracts (75 μ g) from mouse (m) and human (h) γ -subunit overexpressing COS7 cells were analyzed by SDS-PAGE under reducing (A) and non-reducing (B) conditions and western blot analysis using anti mouse γ -subunit antibodies (dilution 1:250). Non-transfected COS7 cells were used as negative control. Cell extracts

(50 μ g) from mouse γ -subunit overexpressing COS7 cells were incubated for different time points in the presence (+) or absence (-) of PNGase F followed by SDS-PAGE under reducing conditions and western blot analysis using anti mouse γ -subunit antibodies (C). Cultured MEFs were fixed and stained with antibodies against mouse γ -subunit (dilution 1:100) and the *cis*-Golgi marker protein GM130 (red, dilution 1:200). Merged images indicate co-localization (yellow). Nuclei were visualized with DAPI (blue) (D). Cell extracts and conditioned media from COS7 cells overexpressing the mouse γ -subunit were analyzed by SDS-PAGE under reducing conditions followed by western blot analysis against mouse γ -subunit (E). The positions of the molecular mass marker proteins in kDa are indicated.



SUPPLEMENTAL FIGURE S2. Dimerization of glycosylation-defective γ -subunits Cell extracts (75 μ g) from COS7 cells overexpressing the wildtype (WT) or mutant (N88Q, N115Q, N88/115Q) γ -subunits were incubated for 1 h in the presence (+) or absence (-) of PNGase F. The proteins were analyzed by SDS-PAGE (10% acrylamide) under non-reducing conditions and human γ -subunit western blot analysis. The position of the molecular mass marker protein is indicated.



SUPPLEMENTAL FIGURE S3. Dimerization of cysteine mutant γ -subunits. Cell extracts (75 μ g) from BHK cells overexpressing the wildtype (WT) or mutant (C84S, C157S, C245S, C84/157S, C157/245S, C84/157/245S) γ -subunits were analyzed by SDS-PAGE (10% acrylamide) under reducing conditions and human γ -subunit western blot analysis. A 55 kDa band from the same blot interacting unspecifically with the anti γ -subunit antibody in COS7 cells were presented as loading control. The position of the molecular mass marker protein in kDa is indicated.

	20	40	60
<i>Homo sapiens</i>	MAAGLARLLLLLGLSAGGPAPAGAAMKMKVVEEPNAFGVNPPFLPQASRLQAKRDPSPVSG		
<i>Pan troglodytes</i>	MAAGLARLLLLLGLSARGPAPAGAAMKMKVVEEPNSFGVNNPFLPQASRLQAKRDPSPVSG		
<i>Mus musculus</i>	MAGRLAGFLMLLLGLASQGPAPACAGKMKVVEEPNTFGLNPPFLPQASRLQPKREPSAVSG		
<i>Rattus norvegicus</i>	MAGRLTGFLLMLLGLAAQGPAPTHAGKMKVVEEPNTFGLNPPFLPQASRLQPKREPSAVSG		
<i>Gallus gallus</i>	MAA--ARLLLLAVFVGAALGVLSASGKMKIVVEEPNTFGLNPPFLPQTNRLOPKMLPSAISG		
	80	100	120
<i>Homo sapiens</i>	PVHLFRLSGKCFSLVESTYKYEF CPFHNV TQHEQTFRWNAYS SGIKGIWHEWEI ANNTFTG		
<i>Pan troglodytes</i>	PVHLFRLSGKCFSLVESTYKYEF CPFHNV TQHEQTFRWNAYS SGIKGIWHEWEI ANNTFTG		
<i>Mus musculus</i>	PLHLFRLAGKCFSLVESTYKYEF CPFHNV TQHEQTFRWNAYS SGIKGIWHEWEI INNTFKG		
<i>Rattus norvegicus</i>	PPLHLFRLAGKCFSLVESTYKYEF CPFHNV TQHEQTFRWNAYS SGIKGIWHEWEI VNNTFKG		
<i>Gallus gallus</i>	PVHLFRLAGKCFSEVESTYKYEF CPFHNV TQHEQTFRWNAYS SGIKGIWHEWEI DNNTFVG		
	140	160	180
<i>Homo sapiens</i>	MWMRDGDACRSRSRQSKVELACGKSNRLAHVSE PSTCV YALTFETPLV CHPHALLVYPTL		
<i>Pan troglodytes</i>	MWMRDGDACRSRSRQSKVELACGKSNRLAHVSE PRTCV YALTFETPLV CHPHALLVYPTL		
<i>Mus musculus</i>	MWMTDGDSCHRSRSRQSKVELT CGK INRLAHVSE PSTCV YALTFETPLV CHPSALLVYPTL		
<i>Rattus norvegicus</i>	MWMTDGDSCHRSRSRQSKVELT CGK TNRLAHVSE PSTCV YALTFETPLV CHPSALLVYPTL		
<i>Gallus gallus</i>	MWMREGDS CE TKSRQTKVHLV CGK SNKLA Y VSE PSTCV YSLTFETPLV CHPSALLVYPTL		
	200	220	240
<i>Homo sapiens</i>	PEALQRWDQVEQDLADELIT POGHEKLLR TLFEDAGY LKTP EENE PTQ LEGGPDS LGFE		
<i>Pan troglodytes</i>	PEALQRWDQVEQDLADELIT POGHEKLLR TLFEDAGY LKTP EENE PTQ LEGGPDS LGFE		
<i>Mus musculus</i>	SEALQRWDQVEQDLADELIT POGYEKLLR VLFE DAGY LKVP GETHPTQ LAGGSKGLGLE		
<i>Rattus norvegicus</i>	SEALQRWDQVEQDLADELIT POGYEKLLR ALFE DAGY LKVP GETHPTQ LAGGSKGLGLE		
<i>Gallus gallus</i>	TEALQRKWD EAEQ SLYD QLITE QGY KKILKEI FEEAGL LKATEEKE -AEKQNM KTSLQFE		
	260	280	300
<i>Homo sapiens</i>	TLE NC RKAH KEL SKEIKR LKGL L TOHG IPYTRP-TETS NLEHLGH ETPR- AKSPEQLR GD		
<i>Pan troglodytes</i>	TLE NC RKAH KEL SKEIKR LKGL L TOHG IPYTRP-TETS NLEHLGH KTPR- AKSPEQLR GD		
<i>Mus musculus</i>	TLD NC RKAH AEL SQ EVKRL KS LLEOHG IPHT Q P-TET HSQHLGH K TP IG AI A EH LRSD		
<i>Rattus norvegicus</i>	TLD IC RKAH AEL S REVKRL KS LLEOHG IPHT Q P-TET HSQHLGH K TP VGE IS -EQLR GD		
<i>Gallus gallus</i>	TV DKCR KEY KKLSE ET KLK DL LNQ HN TAYQR SSA ENTSV EH VN HK WATA ETT V LN GSTN		
	309		
<i>Homo sapiens</i>	PGLRGS-I--		
<i>Pan troglodytes</i>	PGLRGS-I--		
<i>Mus musculus</i>	PGLRCNII--		
<i>Rattus norvegicus</i>	PGLRCNII--		
<i>Gallus gallus</i>	AERLHG DAGI		

SUPPLEMENTAL FIGURE S4. Sequence alignment of γ -subunit orthologues. Identical amino acids are shown in yellow. Conserved *N*-glycosylation consensus sequences and cysteine residues are marked in red.

SUPPLEMENTAL TABLE 1: Sequences of primers used in this study

Primer	5'-3' Sequence
hy-HindIII-for	GTACA <u>AAGCTT</u> GCAATGGCGGGCGGGGCTGGCGCGG
hy-NotI-rev	GTAGCGGCCGCTCACAAACTCCCACGCAGTCC
hy-RGSHis6-NotI-rev	GTAGCGGCCGCTTAGTGATGGTGATGGTGATGCGATCCTCTTCCCA AACTCCCACGCAGTCCTGG
my-HindIII-for	GTACA <u>AAGCTT</u> GACCCTAGGAGCAATGGCGG
my-NotI-rev	GTAGCGGCCGCTCACAGGATGTTCCCACGTAG
my-RGSHis6-NotI-rev	GTAGCGGCCGCTTAGTGATGGTGATGGTGATGCGATCCTCTTC
my-NcoI pET-for	GTACA <u>AAGCTT</u> GACCCTAGGAGCAATGGCGG
hy-N88Q-for	TTCTGCCC GTTCCACCAAGTGACCCAGCACGA
hy-N88Q-rev	TCGTGCTGGGTCACTTGGTGGAACGGGCAG
hy-N115Q-for	TGGGAGATCGCCCAAACACCTTCAC
hy-N115Q-rev	GTGAAGGTGTTTTGGGCGATCTCCCA
hy-C142S-for	GTGGAGCTGGCGTCTGGAAAAAGCAAC
hy-C142S-rev	GTTGCTTTTTCCAGACGCCAGCTCCAC
hy-C157S-for	GAGCCGAGCACCTCCGTCTACGCGCTG
hy-C157S-rev	CAGCGCGTAGACGGAGGTGCTCGGCTC
hy-C169S-for	ACCCCCCTCGTCTCCCACCCCCACGCC
hy-C169S-rev	GGCGTGGGGGTGGGAGACGAGGGGGGT
hy-C245S-for	ACCCTGGAAA ACTCCAGGAAGGCTCAT
hy-C245S-rev	ATGAGCCTTCCTGGAGTTTTCCAGGGT

hy: human γ -subunit; my: mouse γ -subunit

The restriction sites are indicated by underlined nucleotides.

Substituted amino acid is introduced by bold nucleotides.