iScience, Volume *26*

Supplemental information

A quinolin-8-ol sub-millimolar inhibitor of UGGT, the ER glycoprotein folding quality control checkpoint

Kevin P. Guay, Roberta Ibba, J.L. Kiappes, Snežana Vasiljević, Francesco Bonì, Maria De Benedictis, Ilaria Zeni, James D. Le Cornu, Mario Hensen, Anu V. Chandran, Anastassia L. Kantsadi, Alessandro T. Caputo, Juan I. Blanco Capurro, Yusupha Bayo, Johan C. Hill, Kieran Hudson, Andrea Lia, Juliane Brun, Stephen G. Withers, Marcelo Martí, Emiliano Biasini, Angelo Santino, Matteo De Rosa, Mario Milani, Carlos P. Modenutti, Daniel N. Hebert, Nicole Zitzmann, and Pietro Roversi

Supplementary Tables and Figures

Supplementary Table 1: **X-ray data collection parameters and data processing statistics for *Ct*UGGT$_{GT24}$ crystal structures.** Related to STAR Methods.

Structure	*Ct*UGGT$_{GT24}$	^{U2F}*Ct*UGGT$_{GT24}$	$^{5M\text{-}8OH\text{-}Q}$*Ct*UGGT$_{GT24}$
PDB ID	7ZKC	7ZLU	7ZLL
Beamline	I03DLS	I04DLS	I04DLS
Wavelength λ (mm, Å)	0.97960	0.97956	0.97950
Transmission %	100	100	100
Number of images	1,800	1,800	3,600
Oscillation range (°)	0.1	0.1	0.1
Exposure time (s)	0.02	0.017	0.1
Space Group (Z)	H3 (6)	P1 (3)	H3 (6)
Cell edges: a,b,c (Å)	a=b=118.82,c=62.12	a=68.16,b=72.53, c=72.39	a=b=118.858, c=68.551
Cell angles α, β, γ (°)	α=β=90, γ=120	α=110.72, β=108.33, γ=108.27	α=β=90, γ=120
Resolution Range (Å)	59.41-1.77 (1.86-1.77)	41.16 - 2.05 (2.32-2.05)	41.16-1.65 (1.74-1.65)
R_{merge}	0.14 (2.01)	0.05 (0.42)	0.05 (1.60)
R_{meas}	0.15 (2.25)	0.08 (0.60)	0.05 (1.67)
Observations	322,614 (25,624)	68,743 (3,486)	404,079 (21,046)
Unique observations	35,287 (5,115)	39,166 (1,958)	38,495 (1,925)
Average I/σ(I)	7.8 (0.7)	8.4 (1.6)	20.8 (1.3)
Completeness %	99.7 (99.2)	81.1 (45.9)	88.4 (28.7)
Multiplicity	9.1 (5.0)	1.8 (1.8)	10.5 (10.9)
$CC_{1/2}$	0.99 (0.35)	0.998 (0.646)	1.000 (0.581)

Each structure was determined using a single crystal. Values in parentheses refer to the highest resolution shell.

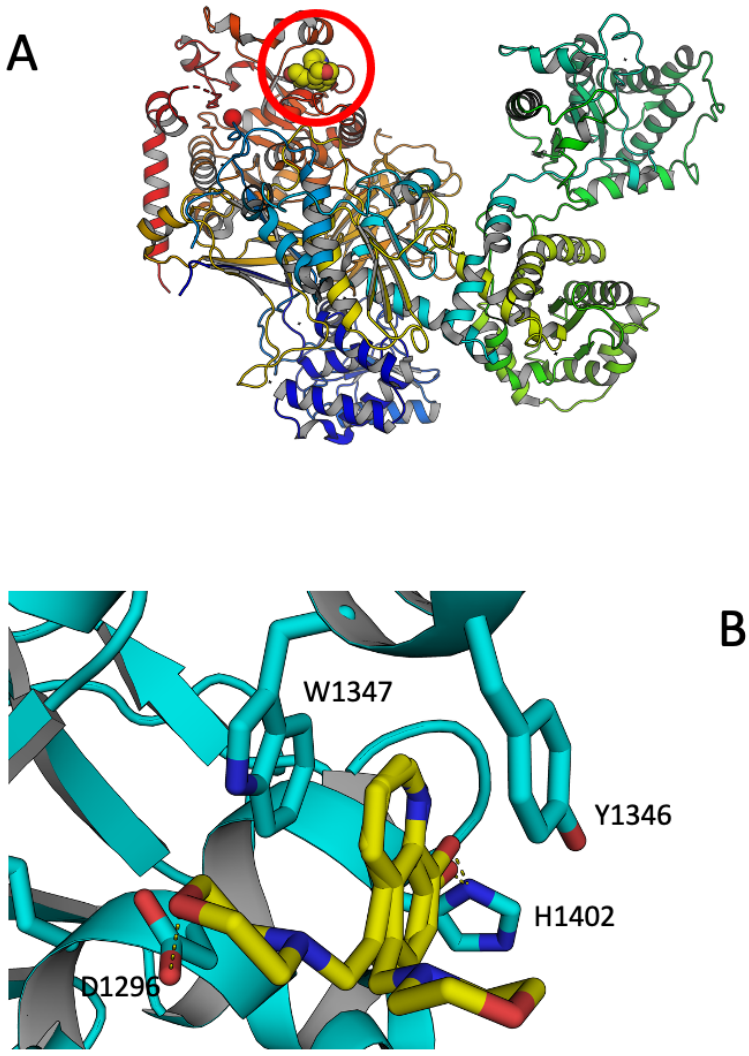
Supplementary Table 2: **Refinement statistics for *Ct*UGGT$_{GT24}$ crystal structures.** Related to STAR Methods.

Structure	*Ct*UGGT$_{GT24}$	^{U2F}*Ct*UGGT$_{GT24}$	$^{5M\text{-}8OH\text{-}Q}$*Ct*UGGT$_{GT24}$
PDB ID	7ZKC	7ZLU	7ZLL
Ligand	-	U2F	5M-8OH-Q
Wavelength λ (Å)	0.97960	0.97956	0.97950
Space Group (Z)	H3 (6)	P1 (3)	H3 (6)
Resolution Range (Å)	59.41-1.77 (1.86-1.77)	41.16 - 2.05 (2.32-2.05)	59.27-2.53 (2.67-2.53)
R_{work},R_{free}	0.205, 0.226 (0.317,0.331)	0.226, 0.270 (0.322, 0.445)	0.209, 0.235 (0.310, 0.301)
Protein atoms (<B factor>, Å^2)	2,432 (43.64)	7,137 (36.44)	2,448 (38.34)
Water molecules (<B factor>, Å^2)	227 (48.48)	289 (34.65)	226 (48.00)
Ligands (<B factor>, Å^2)	Ca^{2+} (30.34)	3 × (Ca^{2+}, U2F) (42.62)	Ca^{2+}, 5M-8OH-Q (49.56)
$rmsd_{bonds}$ (Å), $rmsd_{angles}$ (°)	0.008, 0.90	0.008, 0.94	0.008, 0.93
Number of Ramachandran favoured (%)	268 (100.0)	824 (98.0)	287 (99.0)
Number of Ramachandran allowed (%)	1 (0.0)	15 (2.0)	3 (1.0)
Number of Ramachandran outliers (%)	0 (0.0)	0 (0.0)	0 (0.0)

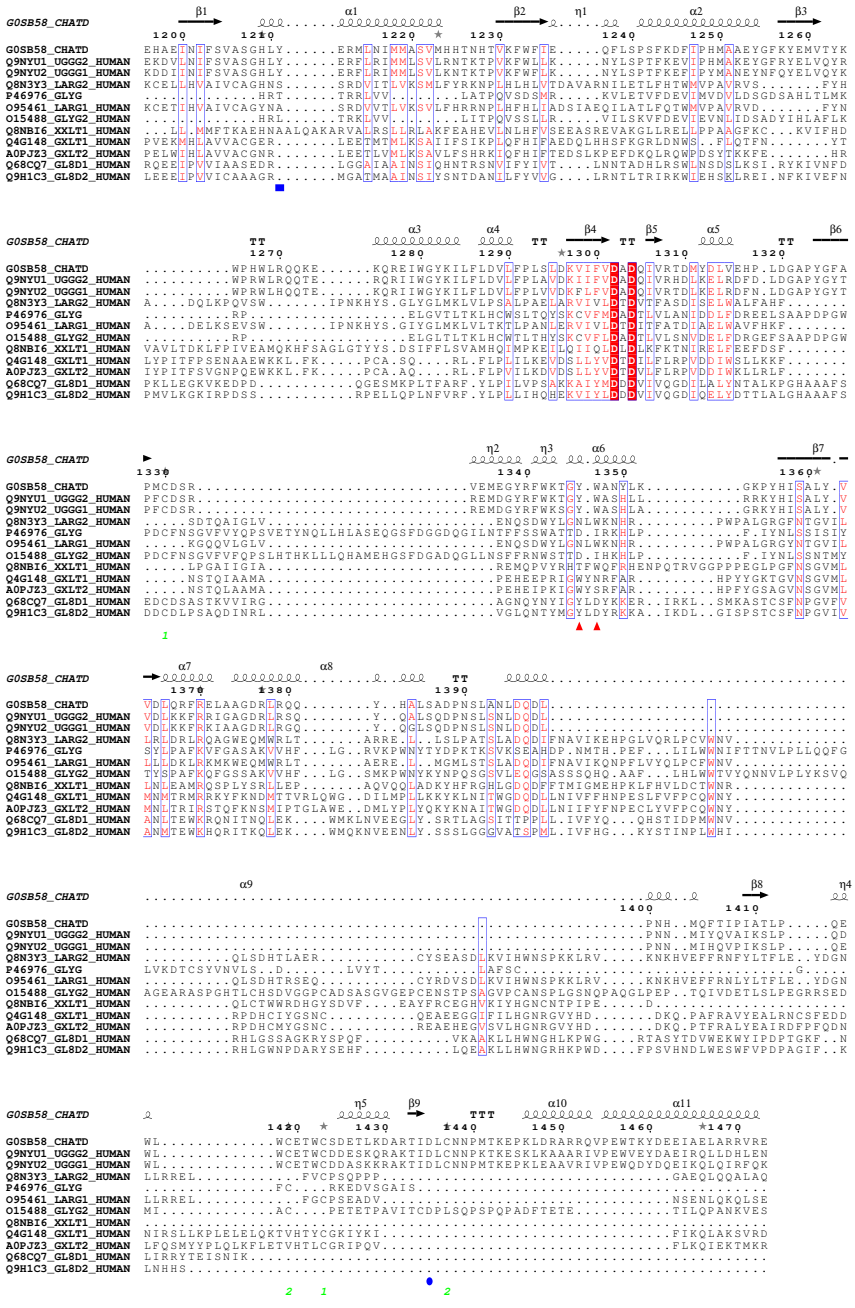
Each structure was determined using a single crystal. All structures contain a Ca^+ ion coming from the protein solution. Values in parentheses refer to the highest resolution shell.

Supplementary Table 3: **Protein structures with 8-OH-quinoline ligands.** Related to Figures 1 and 2.

PDB ID (Ref.)	Protein	Uniprot ID	8-OH-Q ligand (PDB name)	Binding site
3KCY [89]	HIF1AN	Q9NWT6	”	Fe^{++}
4BIO, 3OD4 [65]	”	”	8-hydroxyquinoline-5-carboxylic acid (8XQ)	Zn^{++} or Fe^{++}
2XXZ	KDM6B	O15054	”	Ni^{++} instead of physiological Fe^{++}
3NJY [64]	KDM4A	O75164	”	”
6FUK [90]	UTX	O15550	”	Fe^{++}
4IE4 [66]	FTO	Q9C0B1	”	Zn^{++}
4JHT [65]	*E. coli* AlkB	P05050	”	Mn^{++}
6RBJ	KDM3B	H0Y946	5-(1 H-1,2,3,4-tetrazol-5-yl)quinolin-8-ol (JX8)	Mn^{++}
6RBI	KDM5B	Q9UGL1	”	Mn^{++}
6AFR [91]	BRD4	O60885	5-[(4-fluoranylimidazol-1-yl)methyl]quinolin-8-ol (9E3)	quinolin-8-ol in hydrophobic pocket
5Z5T [92]	”	”	2-amino-4-(1H-imidazol-1-yl)quinolin-8-ol (96R)	”
6LG7	”	”	2-azanyl-6-fluoranyl-4-imidazol-1-yl-quinolin-8-ol (ECF)	H-bond and hydrophobic interactions
6LG8	”	”	2-azanyl-5-fluoranyl-4-imidazol-1-yl-quinolin-8-ol (ECR)	”
6LG9	”	”	2-azanyl-7-bromanyl-4-imidazol-1-yl-quinolin-8-ol (ECU)	”
4E26 [93]	BRAF	P15056	5-chloro-7-[(R)-furan-2-yl(pyridin-2-ylamino)methyl]quinolin-8-ol (734)	quinolin-8-ol sandwiched between Trp and Phe rings
5PA1	*Rn*Comt	P15056	6-(4-fluorophenyl)quinolin-8-ol (7JS)	Mg^{++}
5PA7	”	”	6-(4-fluorophenyl)-8-oxidanyl-3 H-quinazolin-4-one (7JD)	Mg^{++}
6GY1 [94]	”	”	7-fluoranyl-5-(4-methylphenyl)sulfonyl-quinolin-8-ol (FGQ)	Mg^{++}
3JSF [95]	MIF	P14174	7-(2-fluorobenzyl)quinolin-8-ol (XV1)	quinolin-8-ol sandwiched between Tyr and Phe rings
3JSG [95]	”	”	7-(pyridin-3-ylmethyl)quinolin-8-ol (0IN)	”
3JTU [95]	”	”	7-(pyridin-2-ylmethyl)quinolin-8-ol (ZIN)	”

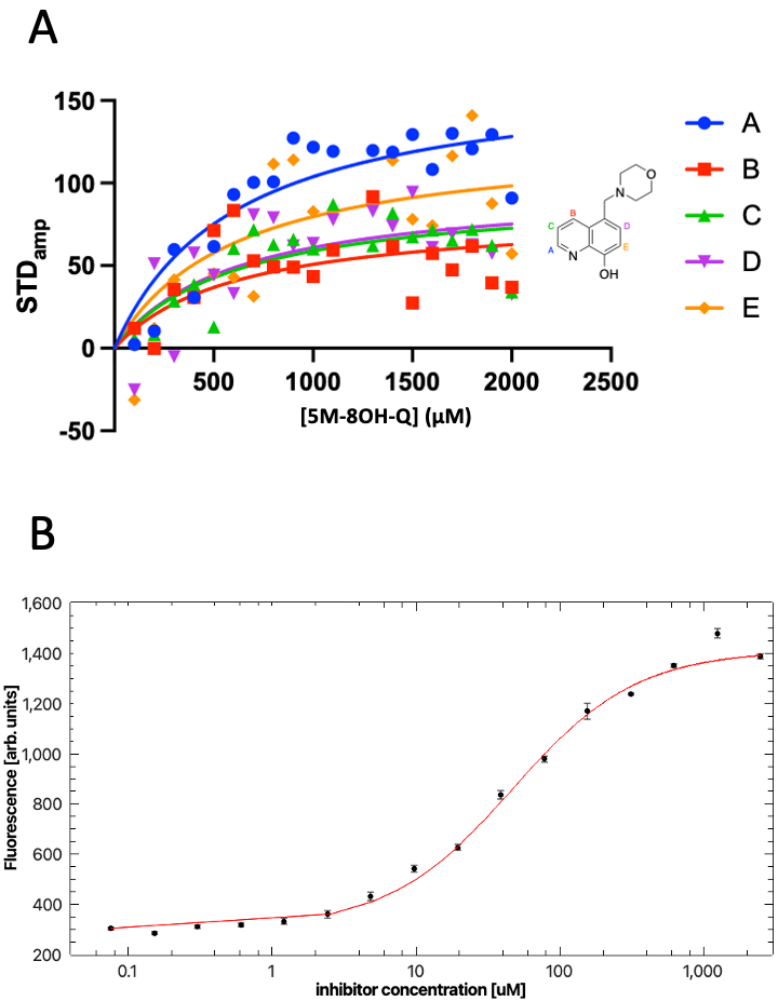


Supplementary Figure 1: **5M-8OH-Q binding site.** Related to Figure 1. **A**: the structure of $^{5M\text{-}8OH\text{-}Q}$*Ct*UGGT$_{GT24}$ (PDB ID 7ZLL) in complex with 5M-8OH-Q, superposed onto the structure of *Ct*UGGT (PDB ID 5MZO) in order to illustrate the binding site of the inhibitor in the context of the whole structure. The protein is in cartoon representation coloured blue-to-red from N- to C-terminus; the 5M-8OH-Q is inside a red circle, in spheres representations (C atoms in yellow). **B**: Zoom onto the *Ct*UGGT ^{1346}YW^{1347} clamp (C atoms in green) binding 5M-8OH-Q (C atoms in cyan) Representative distances to interacting residues are in dashed lines. Only two of the many morpholine ring placements are shown. PDB ID: 7ZLL.

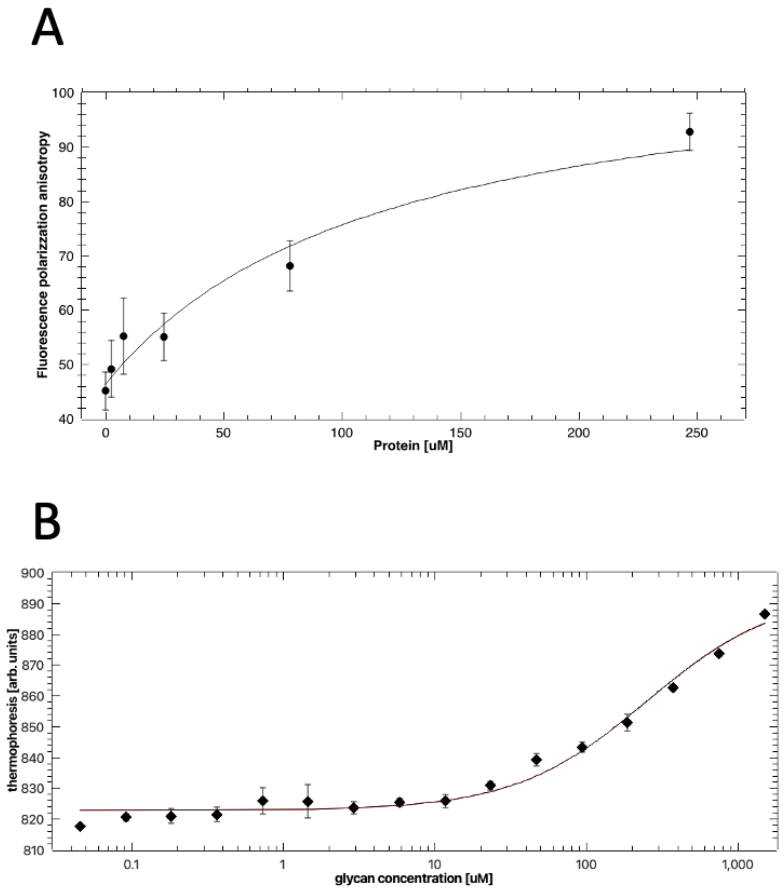


Supplementary Figure 2: **Alignment of GT-24 and GT-8 domains in human proteins**. Caption follows in the next page.

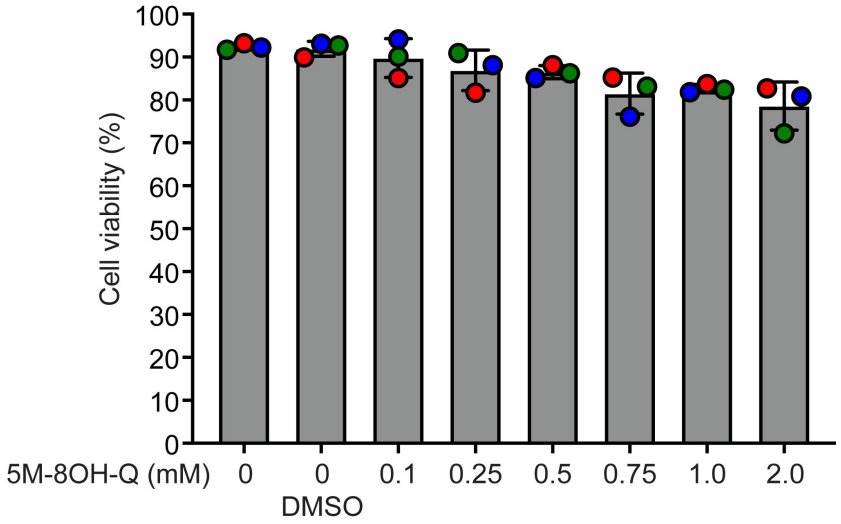
Supplementary Figure 2: **Alignment of GT-24 and GT-8 domains in human proteins**. Related to Figure 1. Caption of the figure in the previous page. The secondary structure illustrated is the one of *Ct*UGGT$_{GT24}$, Uniprot entry G0SB58_CHATD, PDB IDs 5NV4 and 6FSN[35,42]. Uniprot codes and proteins: Q8N3Y3, Xylosyl- and glucuronyltransferase LARGE2; P46976, Glycogenin 1; O95461, Xylosyl- and glucuronyl-transferase LARGE1; O15488, Glycogenin 2; Q8NBI6, Xyloside xylosyltransferase 1; Q4G148, Glucoside xylosyltransferase 1; A0PJZ3, Glucoside xylosyltransferase 2; Q68CQ7, Glycosyltransferase 8 domain-containing protein 1; Q9H1C3, Glycosyltransferase 8 domain-containing protein 2. The *Ct*UGGT D1302 and D1304 residues coordinating Ca^{2+} ion are completely conserved across these sequences. Red triangles mark the *Ct*UGGT ^{1346}WY^{1347} clamp. A blue square marks the position of *Ct*UGGT Y1211 (coordinating the U2F uracyl ring). A blue oval marks the position of *Ct*UGGT D1435 (coordinating the Ca^{2+} ion).



Supplementary Figure 3: **5M-8OH-Q binds UGGT *in vitro*.** Related to Figure 4. **A**: Measurement of the K_d dissociation constant of the complex between 5M-8OH-Q and human UGGT1 by STD NMR *in vitro*. Each curve follows the interaction of one of the H atoms of 5M-8OH-Q with the human UGGT1 protein. No measurements were possible below [5M-8OH-Q]=100 μM, so it is possible that the weak binding observed relates to a second weaker accessory site once saturation of the main one (see panel **B**) has taken place. **B**: Binding of 5M-8OH-Q to NT-RED-NHS-labelled *Ct*UGGT$_{\mathrm{GT24}}$ as measured by LEF. λ_{Excit}=650 nm. λ_{Emiss}=670 nm. NT-RED-NHS-labelled *Ct*UGGT$_{\mathrm{GT24}}$ 100 nM; 5M-8OH-Q dilution series from 2.5 mM to 76.3 nM. Three independent dilution series.



Supplementary Figure 4: **Affinity of th e *Ct* UGGT$_{\mathbf{GT24}}$ do main fo r *N*-linked glycans.** Related to Figure 4. **A:** Changes in fluorescence p olarisation anisotropy of 2AA-labelled $GlcNAc_2Man_9$ (2AA-M9) glycan binding to the *Ct*UGGT$_{GT24}$ domain: four independent dilution series of *Ct*UGGT$_{GT24}$ domain from 247 to 0 μM were mixed with 200 nM 2AA-M9 glycan and the anisotropy of fluorescence p olarisation measured (λ_{excit}=360 nm, λ_{emiss}=490 nm). Fitting the curve with an equilibrium constant a value of K_D=(117± 32) μM is obtained. **B:** Binding of 2AA-M5-9 mixture to NT-RED-NHS-labelled *Ct*UGGT$_{GT24}$ as measured by MST. λ_{Excit}=650 nm. λ_{Emiss}=670 nm. NT-RED-NHS-labelled *Ct*UGGT$_{GT24}$ 100 nM; 2AA-M5-9 dilution series from 1.5 mM to 45.8 nM. Three independent dilution series. Fitting the curve with an equilibrium constant, a value of an average K_D=(250 ± 39 μM) μM is obtained.



Supplementary Figure 5: **Viability of *ALG6*$^{-/-}$ HEK293-6E cells after 5 hr 5M-8OH-Q treatment.** Related to Figures 5 and 6. Prior to lysis, cells were dissociated and washed twice with PBS before being resuspending in 1 mL of PBS. Resuspended cells were diluted 1:1 with trypan blue and counted using a LUNA II^{TM} Automated Cell Counter. Error bars represent the standard deviation of three independent biological replicates.