

# Supplementary Materials For

## GalNAc-T isozyme surface charge governs charge substrate preferences to modulate mucin type O-glycosylation

**Collin Ballard<sup>2</sup>, Miya Paserba<sup>2</sup>, Earnest James Paul Daniel<sup>2,3</sup>, Ramon Hurtado-Guerrero<sup>4,5,6,7</sup>, Thomas A Gerken<sup>2,1</sup>**

<sup>2</sup>Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106, USA,

<sup>3</sup>present address Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA, <sup>4</sup>BIFI and Laboratorio de Microscopías Avanzada (LMA), University of Zaragoza, Mariano Esquillor s/n, Campus Rio Ebro, Edificio I+D, Zaragoza, 50018, Spain, <sup>5</sup>Department of Cellular and Molecular Medicine, <sup>6</sup>Department of Dentistry, Faculty of Health Sciences, Copenhagen Center for Glycomics (CCG), University of Copenhagen, Copenhagen N DK-2200, Denmark, <sup>7</sup>Fundación ARAID, Zaragoza, 50018, Spain

<sup>1</sup>To whom correspondence should be addressed: Tel: 216-368-4556; Fax: 216-368-4223; e-mail: [txg2@cwru.edu](mailto:txg2@cwru.edu)

### **Supplementary Figures:**

Supplementary Figure S1: Template Structures and peptides used for homology modeling of the GalNAc-Ts

Supplementary Figure S2: GalNAc-T surface electrostatic potentials for all template models

Supplementary Figure S3: GalNAc-T surface electrostatic potentials for all template models

Supplementary Figure S4: Electrostatics of the LA repeat regions of the LDL receptor reveal defined regions of negative flanking charge.

Supplementary Figure S5: PGANT surface electrostatic potentials for all template models

Supplementary Figure S6: Structures showing *in vivo* GalNAc-T N- & O-glycosylation sites.

Supplementary Figure S7: Comparison of GalNAc-T11 and PGANT35A Specificity

Supplementary Figure S8: Comparison of GalNAc-T3 and GalNAc-T6 Specificity

## **Supplementary Tables**

Supplementary Table SI: Sequences of peptides bound to GalNAc-Ts in the presence of  $Mn^{2+}$  and UDP

Supplementary Table SII: In vivo GalNAc-T N-glycosylation sites

Supplementary Table SIII: In vivo GalNAc-T O-glycosylation sites

Supplementary Table SIV: Previously unpublished enhancement values (EV) for hGalNAc-T11, PGANT35A (dT1) and hGalNAc-T6.

## **Supplementary Movies (as separate files)**

Movies of the 250ns MD simulations for tgGalNAc-T3, hGalNAc-T12, and T2 bounds to charged peptides. Charged peptide residues are color coded blue for the positive Arg residues, red for the negative Asp residues, and white/green for the neutral Gly and Ala residues.

**Supplementary Movie 1:** 250nSec MD trajectory of the most active RR peptide bound to tgGalNAc-T3 (PDB 6S24)

**Supplementary Movie 2:** 250nSec MD trajectory of the least active DD peptide bound to tgGalNAc-T3 (PDB 6S24)

**Supplementary Movie 3:** 250nSec MD trajectory of the most active DR peptide bound to tgGalNAc-T12 (PDB 6XPU)

**Supplementary Movie 4:** 250nSec MD trajectory of the least active RD peptide bound to tgGalNAc-T12 (PDB 6XPU)

**Supplementary Movie 5:** 250nSec MD trajectory of the most active DD peptide bound to tgGalNAc-T2 (PDB5AJP)

**Supplementary Movie 6:** 250nSec MD trajectory of intermediately active GG peptide bound to tgGalNAc-T2 (PDB5AJP)

**Supplementary Movie 7:** 250nSec MD trajectory of least active RR peptide bound to tgGalNAc-T2 (PDB5AJP)

### **Supplementary Figure S1:**

GalNAc-T template structures used for homology modeling of the GalNAc-Ts studied in this work (see Materials and Methods). A) Template structures, with the bound peptides used to model the GalNAc-Ts left-compact lectin structure (tgGalNAc-T3, PDB: 6S24), the extended lectin structure (hGalNAc-T2, PDB: 2FFU) and the right compact lectin structure (hGalNAc-T2 PDB: 5AJP). B) Sequences of the bound peptides in part A that were aligned onto each of our GalNAc-T homology structures. T\* represents Thr-O-GalNAc.

### **Supplementary Figure S2:**

Electrostatic surface potentials for all GalNAc-Ts studied. Each transferase was homology modeled against the three lectin domain orientations given in **Supplementary Figure S1** and are listed in the order given in **Figure 2**. The left column represents the lectin left compact structure (tgGalNAc-T3 PDB 6S24), middle column, extended lectin orientation (hGalNAc-T2 PDB: 2FFU), and right column, lectin right compact orientation (hGalNAc-T2 PDB: 5AJP). Bound peptides were aligned around the T/S-PXP sequence motif in each structure after superimposing the catalytic domains with bound peptides in PyMOL.

### **Supplementary Figure S3:**

Electrostatic surface potentials for all GalNAc-Ts studied. Each transferase was homology modeled against the three lectin domain orientations given in **Supplementary Figure S1** and are listed in the order given in **Figure 3**. The left column represents the lectin left compact structure (tgGalNAc-T3 PDB 6S24), middle column, extended lectin orientation (human GalNAc-T2 PDB: 2FFU), and right column, lectin right compact orientation (human GalNAc-T2 PDB:

5AJP). Bound peptides were aligned around the T/S-PXP sequence motif in each structure after superimposing the catalytic domains with bound peptides in PyMOL.

#### **Supplementary Figure S4:**

Electrostatics of the LA repeat regions of the LDL receptor reveal defined regions of negative flanking charge. A) The electrostatic surface potential (blue) of GalNAc-T11. B) The location of the Thr residues of the low-density-lipoprotein receptor (LDLR) glycosylated by this isozyme. C) Electrostatic distributions of the LDLR surrounding these residues (black circles) showing areas of highly negative flanking charge (red) likely to enhance GalNAc-T11 binding (C).

#### **Supplementary Figure S5:**

Homology Models and electrostatic surface potentials of the PGANT based on the templates in **Supplementary Figure S1**. Left column *tg*GalNAc-T3 (PDB 6S24), second column *h*GalNAc-T2 (PDB: 2FFU), and third column *h*GalNAc-T2 (PDB: 5AJP) templates respectfully. The rightmost column gives the electrostatic surface potentials of the *h*GalNAc-T orthologues that were studied showing partial conservation of surface charge.

#### **Supplementary Figure S6:**

*In-Vivo* reported O-glycosylation sites and reported and predicted N-glycosylation sites for the studied GalNAc-Ts. Shown are the structures of *h*GalNAc-T1, -T3, -T4, -T5, -T6, -T7, -T11, -T12, -T13 & -T16 and *tg*GalNAc-T3 showing reported O-glycosylation sites (orange), reported (red) and/or predicted (blue) N-glycosylation sites, where the Thr or Asn residues are shown as spheres (see **Supplemental Table SII and SIII**). The shown bound peptide structures (green, blue and yellow), <sup>2+</sup>Mn (purple) and UDP (green) were obtained from the homology model templates as described **Figure 2**. The N-terminal stem regions have been omitted in the

structures while the structures are shown to maximize visualizing the glycosites relative to the peptide binding site. Note the C-terminal Thr554 O-glycosite identified for GalNAc-T1 is absent in the PDB 6S24 template structure.

**Supplementary Figure S7:**

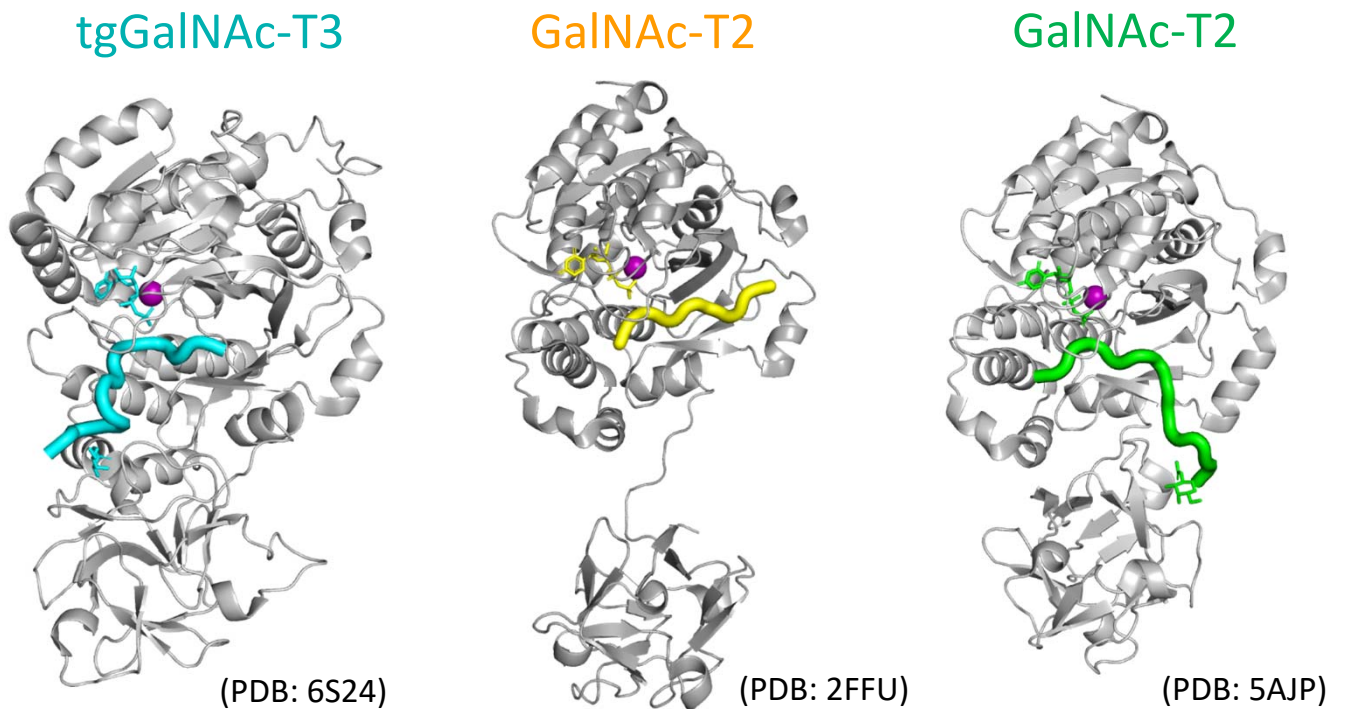
Comparison of the peptide substrate specificity of hGalNAc-T11 and its fly orthologue PGANT35A. A) Bar graph plot comparing hydrophobic residue enhancement values (EV) of hGalNAc-T11 (T11) and PGANT35A (T35A). B) Bar graph plot comparing hydrophilic residue enhancement values (EV) of hGalNAc-T11 (T11) and PGANT35A (T35A). C) Scatter plots of EVs for hGalNAc-T11 and PGANT35A showing their relative correlation. EV values are given in **Supplementary Table SIV**.

**Supplementary Figure S8**

Comparison of the peptide substrate specificity of subfamily Ic members hGalNAc-T3 and T6. A) Bar graph plot comparing hydrophobic residue enhancement values (EV) of hGalNAc-T3 (T3) and hGalNAc-T6 (T6). B) Bar graph plot comparing hydrophilic residue enhancement values (EV) of hGalNAc-T3 (T3) and hGalNAc-T6 (T6). C) Scatter plots of EVs for hGalNAc-T3 and T6 showing their relative correlation. EV values for hGalNAc-T6 are given in **Supplementary Table SIV**, EV values for hGalNAc-T3 have previously been reported (Gerken et al 2011).

## Supplementary Figure S1: Template Structures and peptides used for homology modeling of the GalNAc-Ts Studied

### A) PDB Structure Templates from X-Ray Crystallography



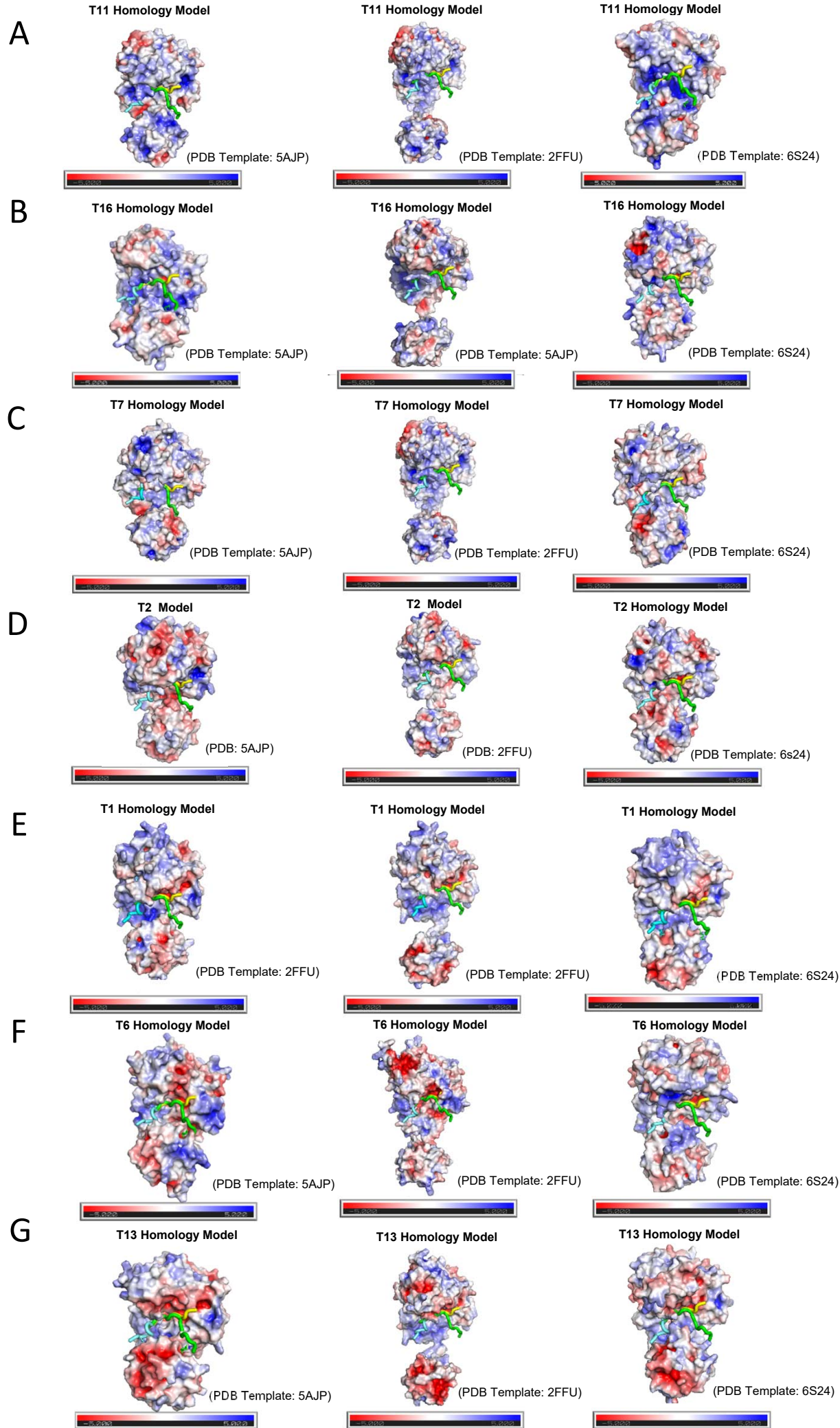
### B) Bound Substrate Sequences

**P3: GAT\*GAGAGAGTTPGPG (PDB: 6S24)**

**EA2: PTTDSTTPAPTTK (PDB: 2FFU)**

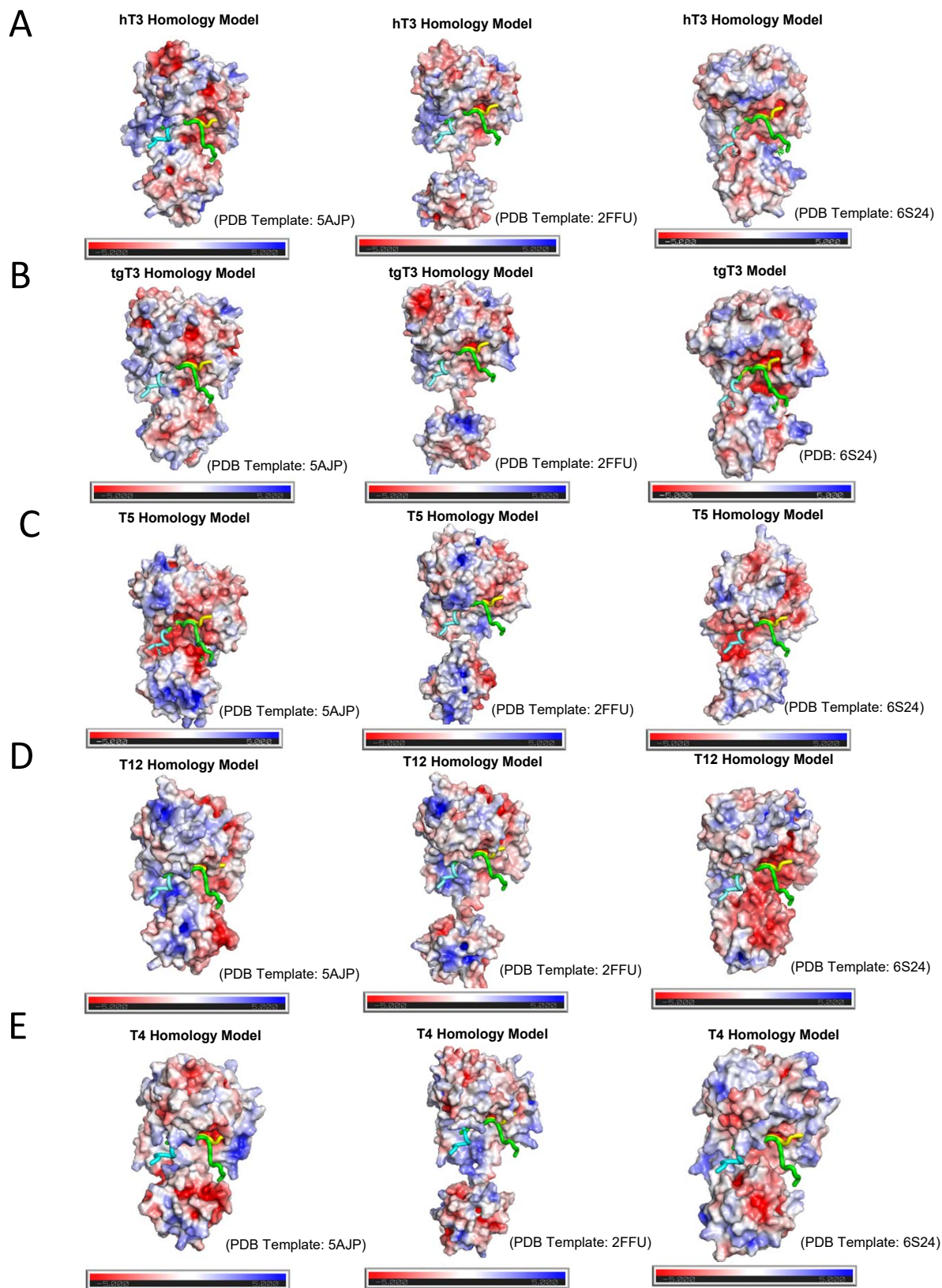
**AC13: GTTPSPVPTTSTT\*SAP (PDB: 5AJP)**

# Supplementary Figure S2: Electrostatic Surface Potentials for the GalNAc-Ts in Figure 2





# Supplementary Figure S2: Electrostatic Surface Potentials for the GalNAc-Ts in Figure 3

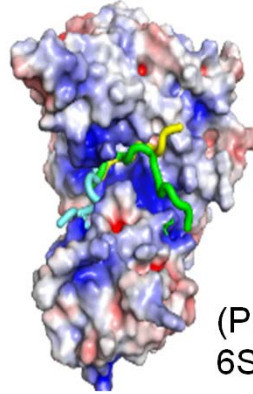




# Supplementary Figure S4: Electrostatics of the LA Repeat Regions of the LDL Receptor Reveal Defined Regions of Negative Flanking Charge

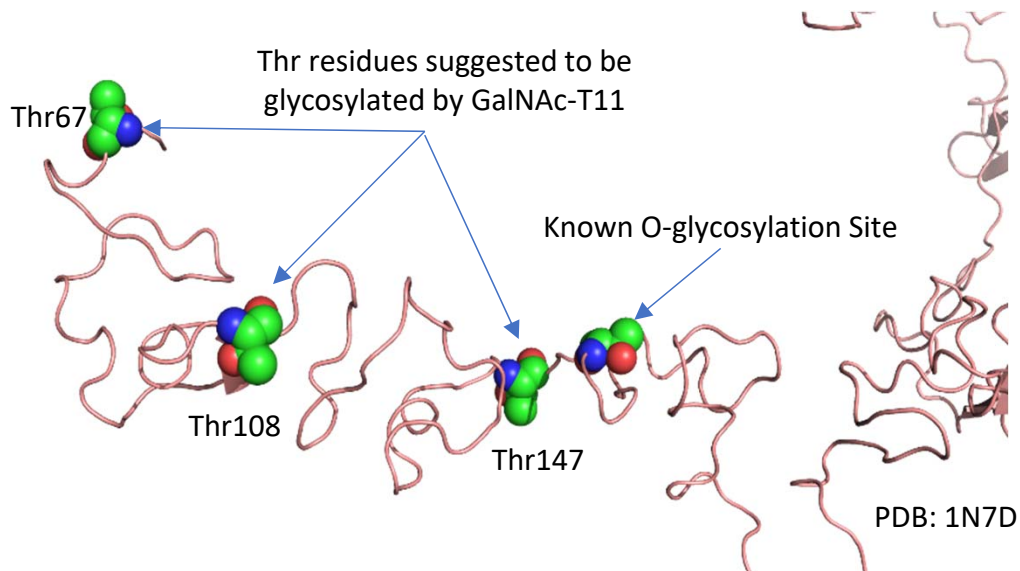
A

T11 Homology Model

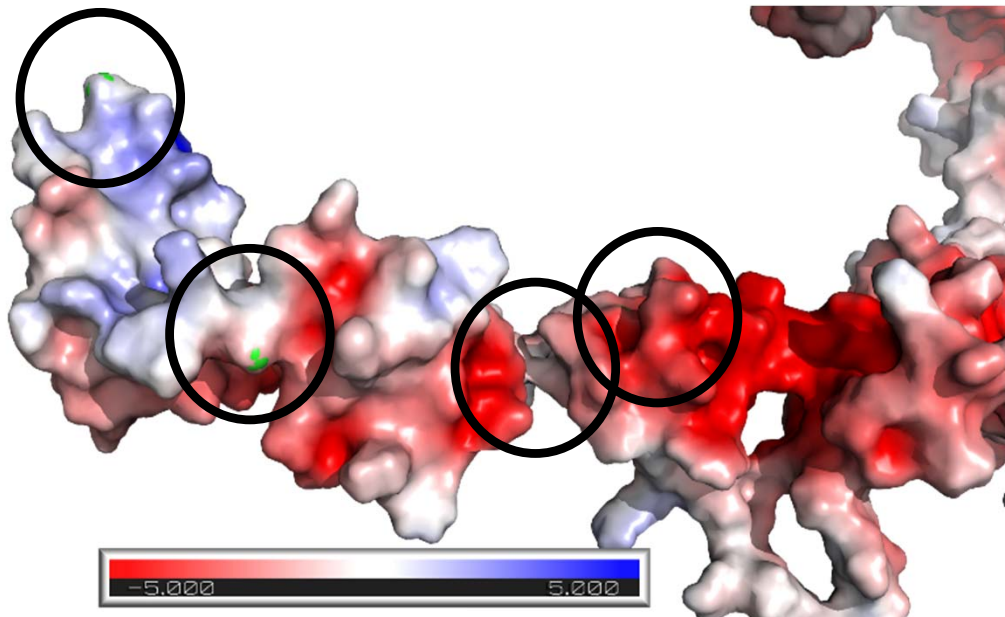


(PDB Template:  
6S24)

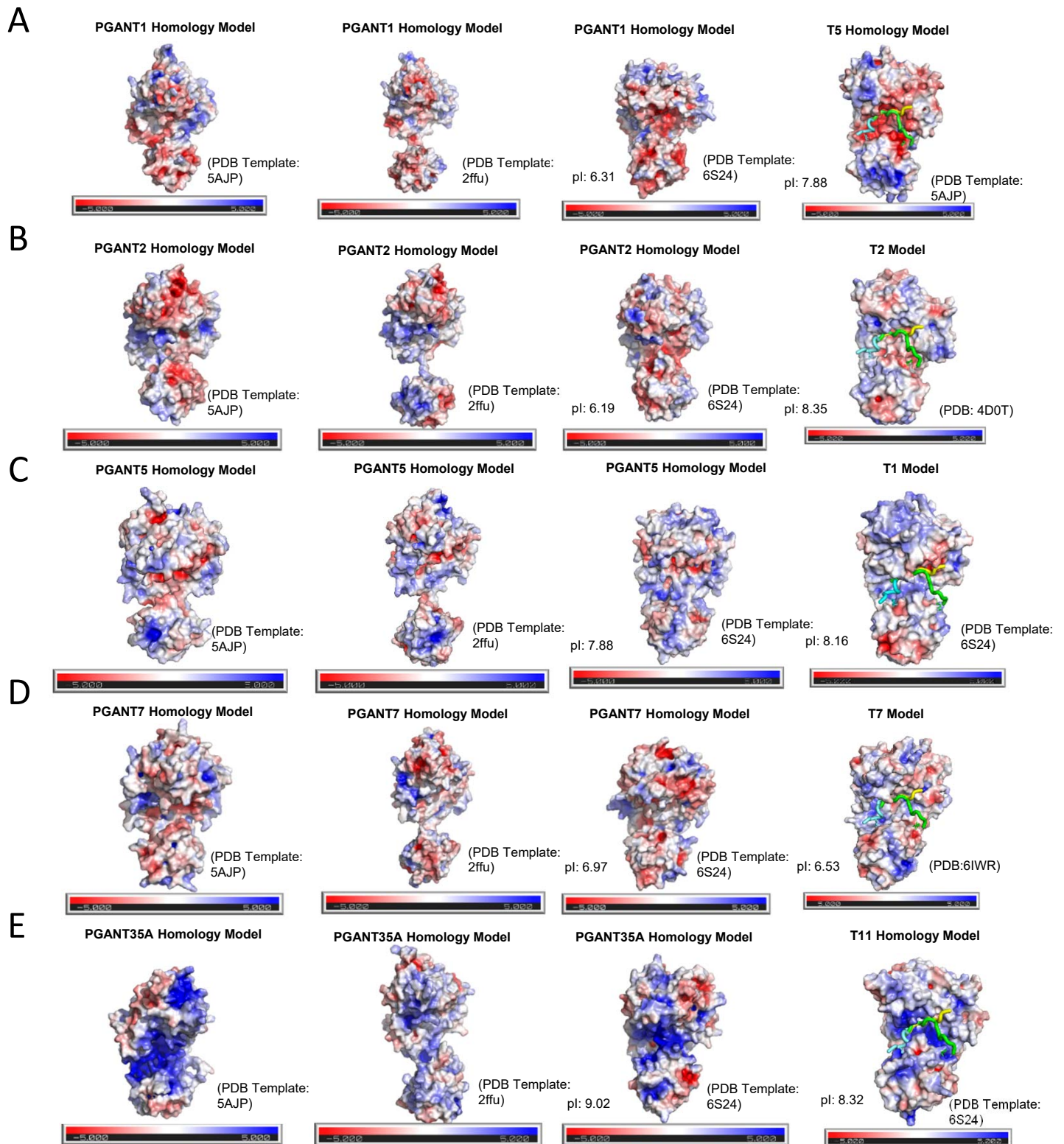
B



C

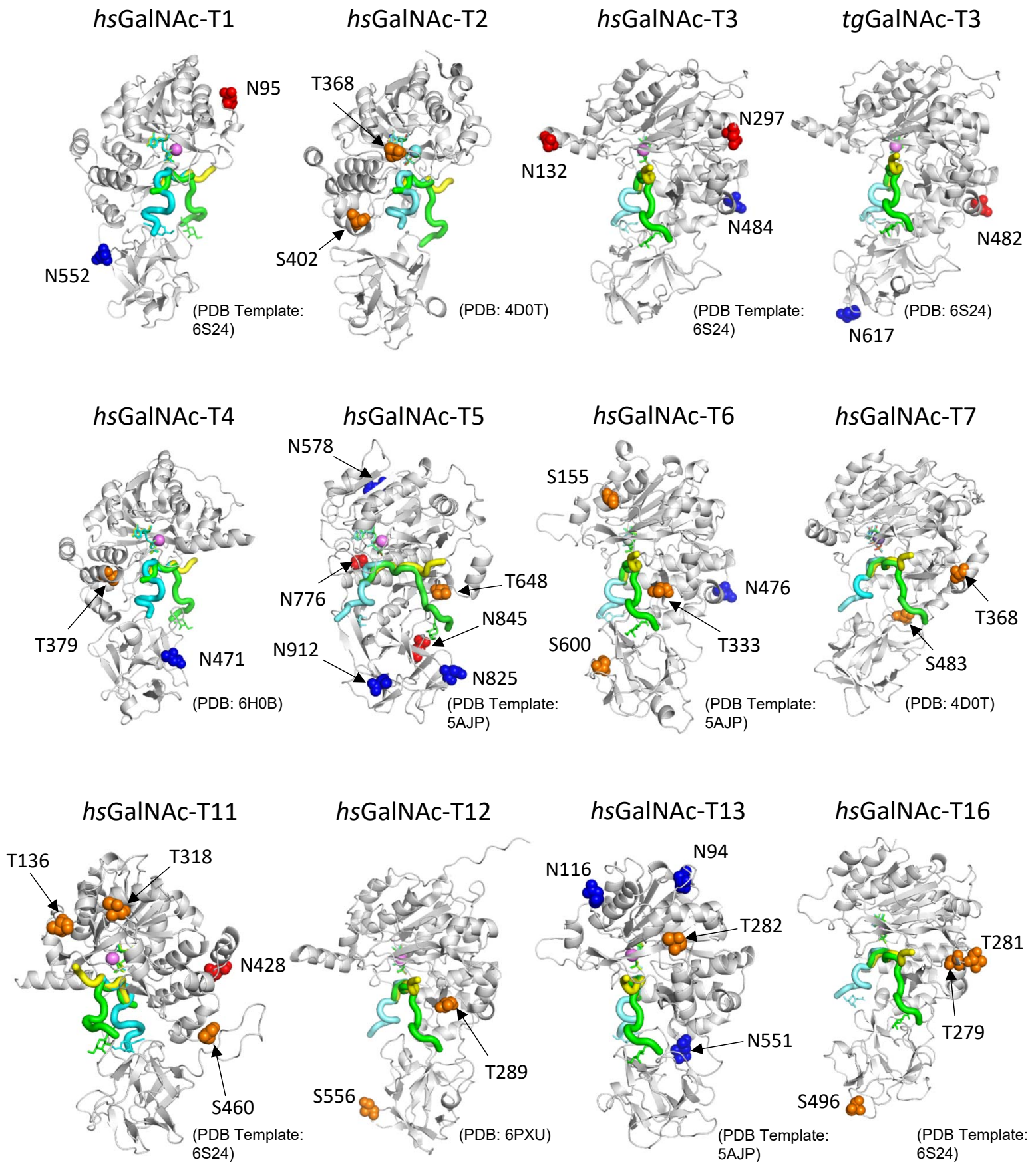


# Supplementary Figure S5: PGANT and Orthologue Surface Electrostatic Potentials



## Supplementary Figure S6:

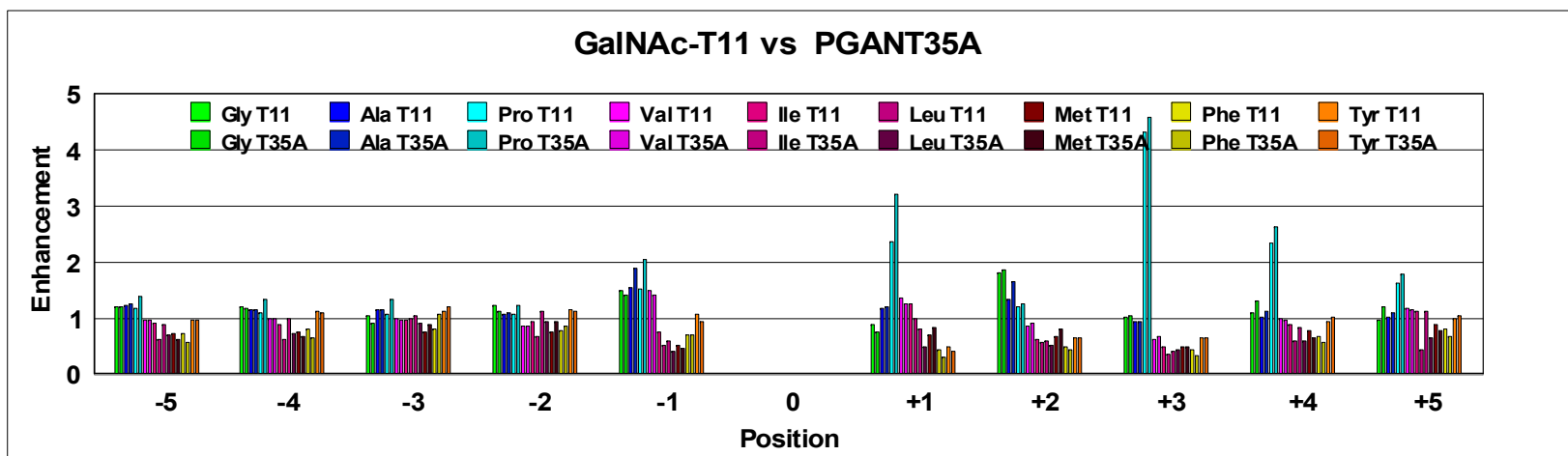
### *In-Vivo* Reported O-Glycosylation and Reported and Predicted N-Glycosylation Sites on the Characterized GalNAc-Ts



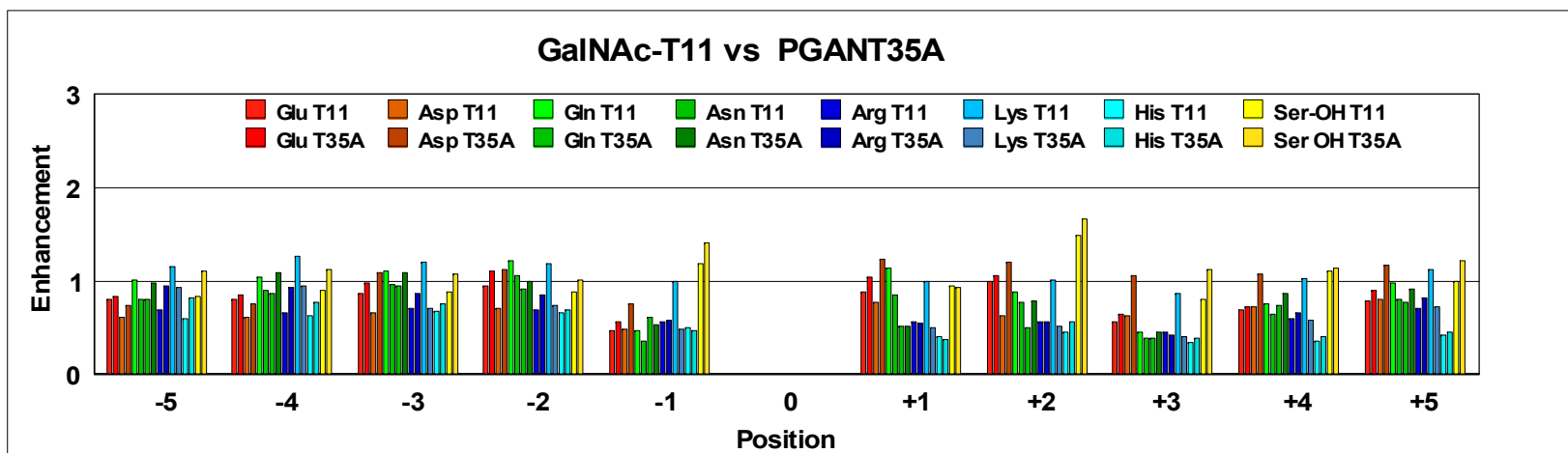


# Supplementary Fig. S7: Comparison of GalNAc-T11 and PGANT35A Specificity

A



B



C

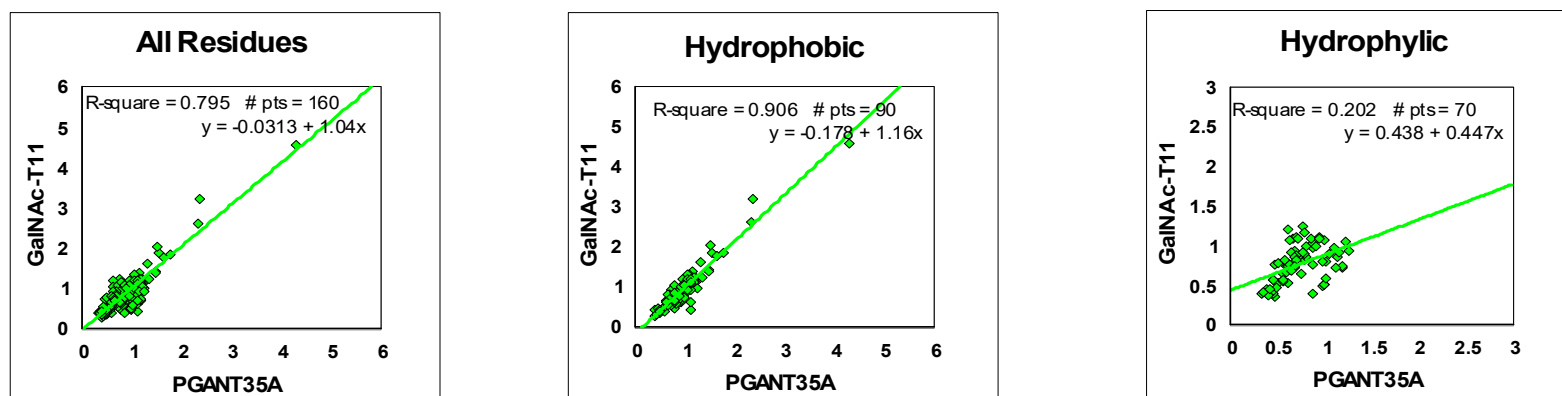


Figure S7

# Supplementary Fig. S8: Comparison of GalNAc-T3 and GalNAc-T6 Specificity

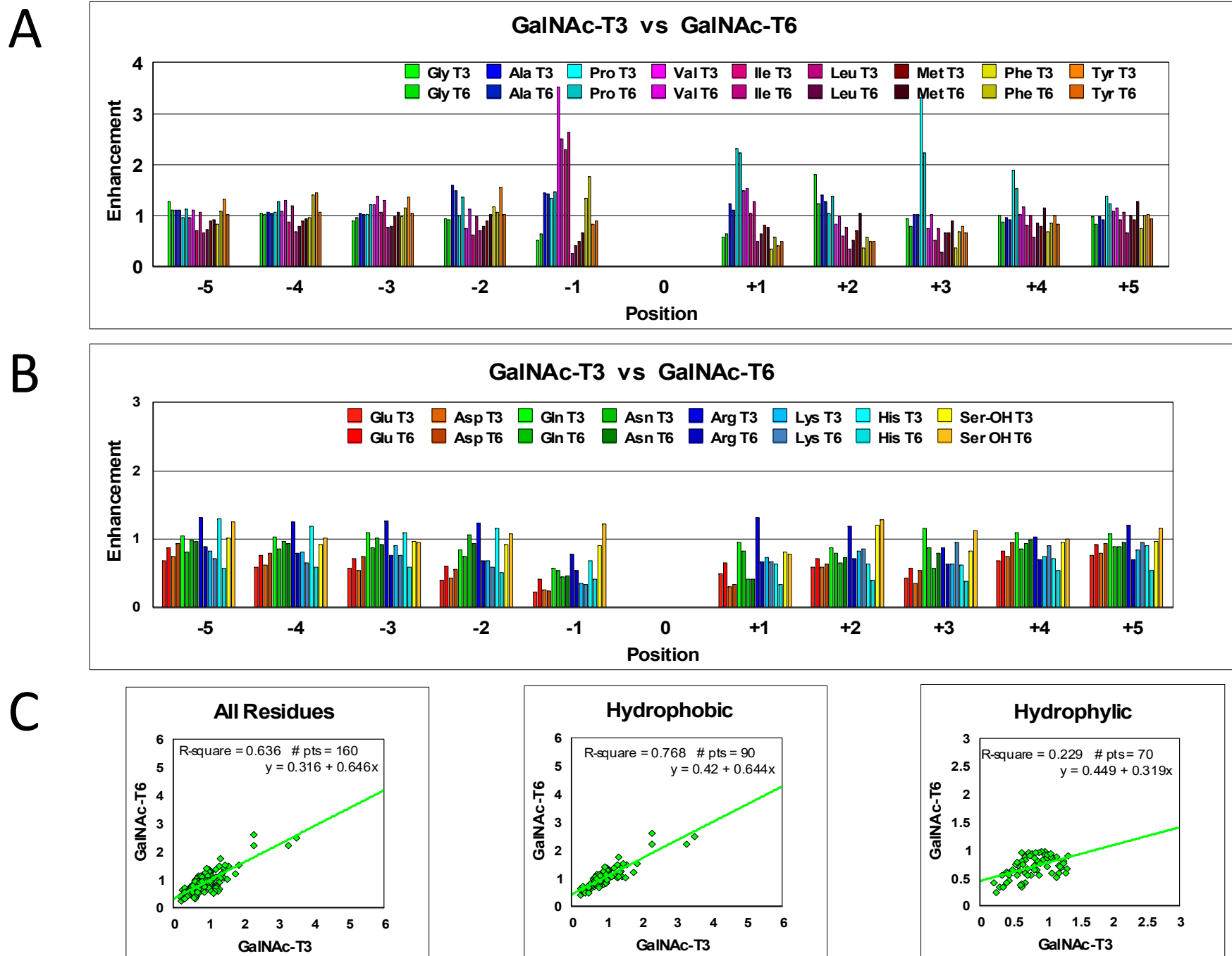


Figure S8

## Supplementary Table S1

Peptide sequences of peptides bound to GalNAc-T crystal structures in the presence of Mn<sup>2+</sup> and UDP shown in Fig 1.

<u>GalNAc-T</u>	<u>PDB</u>	<u>Peptide</u>	<u>Sequence<sup>a</sup></u>
T2	2FFU	EA2	ST <u>T</u> PAPTTK
T2	5AJP	AC13	TTP <u>S</u> PVPTTSTT*SAA
T2	4D0Z	mEA2	S <u>T</u> CPAA
T3	6S24	P3	AT*GAGAGAGT <u>T</u> PGP
T3	6S22	FGF23c	NT*PIPRRH <u>T</u> RSA
T4	6H0B	DGP6	GAT*GAGAGAGT <u>T</u> PGPG
T12	6PXU	DPG-5-17	GAGAT*GAGAGYYI <u>T</u> PRT*GAGA

<sup>a</sup>Acceptor site underlined and in bold, T\* represents Thr-O-GalNAc.

## Supplementary Table SII

### *In-Vivo* reported and predicted N-glycosylation sites on the characterized GalNAc-Ts

Transferase	N-Linked Glycan Residue (Reported)	N-Linked Glycan Residue (Predicted)
<i>HsGalNAc-T1</i>	N95 <sup>C</sup>	N552 <sup>L</sup>
<i>HsGalNAc-T2</i>	None	None
<i>HsGalNAc-T3</i>	N132 <sup>C</sup> , N297 <sup>C</sup>	N484 <sup>C</sup>
<i>TgGalNAc-T3</i>	N482 <sup>C</sup>	N617 <sup>L</sup>
<i>HsGalNAc-T4</i>	None	N471 <sup>L</sup>
<i>HsGalNAc-T5</i>	N776 <sup>C</sup> , N845 <sup>L</sup>	N217 <sup>S</sup> , N256 <sup>S</sup> , N273 <sup>S</sup> , N316 <sup>S</sup> , N362 <sup>S</sup> , N395 <sup>S</sup> , N406 <sup>S</sup> , N578 <sup>C</sup> , N827 <sup>L</sup> , N912 <sup>L</sup>
<i>HsGalNAc-T6</i>	None	N86 <sup>S</sup> , N476 <sup>C</sup>
<i>HsGalNAc-T7</i>	None	None
<i>HsGalNAc-T11</i>	N428 <sup>C</sup>	None
<i>HsGalNAc-T12</i>	None	None
<i>HsGalNAc-T13</i>	None	N94 <sup>C/S</sup> , N116 <sup>C</sup> , N551 <sup>L</sup>
<i>HsGalNAc-T16</i>	None	None

<sup>S</sup> Residue located on N-terminal stem.

<sup>C</sup> Residue located on catalytic domain, in red.

<sup>L</sup> Residue located on the lectin domain, in red.



## Supplementary Table SIII

### *In-Vivo* reported O-glycosylation sites on the characterized GalNAc-Ts

Transferase	O-Linked Glycan Residue (Reported)
<i>HsGalNAc-T1</i>	<b>T554<sup>L</sup></b>
<i>HsGalNAc-T2</i>	S5 <sup>S</sup> , S29 <sup>S</sup> , S59 <sup>S</sup> , S67 <sup>S</sup> , T70 <sup>S</sup> S94 <sup>S</sup> , <b>T368<sup>Cnp</sup></b> , <b>S402<sup>Cnp</sup></b>
<i>HsGalNAc-T3</i>	S124 <sup>S</sup> , T130 <sup>S</sup> , T131 <sup>S</sup>
<i>TgGalNAc-T3</i>	N.D
<i>HsGalNAc-T4</i>	S46 <sup>S</sup> , S50 <sup>S</sup> , T56 <sup>S</sup> , S60 <sup>S</sup> , <b>T379<sup>Cnp</sup></b>
<i>HsGalNAc-T5</i>	S69 <sup>S</sup> , S70 <sup>S</sup> , T94 <sup>S</sup> , T108 <sup>S</sup> , T134 <sup>S</sup> , T138 <sup>S</sup> , T144 <sup>S</sup> , T149 <sup>S</sup> , S154 <sup>S</sup> , S155 <sup>S</sup> , S159 <sup>S</sup> , T163 <sup>S</sup> , T164 <sup>S</sup> , S172 <sup>S</sup> , S192 <sup>S</sup> , S200 <sup>S</sup> , S202 <sup>S</sup> , S204 <sup>S</sup> , S205 <sup>S</sup> , S207 <sup>S</sup> , S208 <sup>S</sup> , S221 <sup>S</sup> , T224 <sup>S</sup> , S231 <sup>S</sup> , S243 <sup>S</sup> , T244 <sup>S</sup> , S249 <sup>S</sup> , S262 <sup>S</sup> , T274 <sup>S</sup> , S275 <sup>S</sup> , T282 <sup>S</sup> , S292 <sup>S</sup> , T296 <sup>S</sup> , S300 <sup>S</sup> , S302 <sup>S</sup> , S320 <sup>S</sup> , T327 <sup>S</sup> , S356 <sup>S</sup> , S360 <sup>S</sup> , S367 <sup>S</sup> , S368 <sup>S</sup> , S369 <sup>S</sup> , S370 <sup>S</sup> , S379 <sup>S</sup> , T381 <sup>S</sup> , S402 <sup>S</sup> , S408 <sup>S</sup> , S418 <sup>S</sup> , T420 <sup>S</sup> , T429 <sup>S</sup> , S431 <sup>S</sup> , <b>T648<sup>Ccp</sup></b>
<i>HsGalNAc-T6</i>	T38 <sup>S</sup> , S48 <sup>S</sup> , S67 <sup>S</sup> , T81 <sup>S</sup> , S84 <sup>S</sup> , S88 <sup>S</sup> , T95 <sup>S</sup> , S123 <sup>S</sup> , <b>S155<sup>C</sup></b> , <b>T333<sup>Ccp</sup></b> , <b>S600<sup>L</sup></b>
<i>HsGalNAc-T7</i>	T29 <sup>S</sup> , S36 <sup>S</sup> , S39 <sup>S</sup> , S103 <sup>S</sup> , T114 <sup>S</sup> , T119 <sup>S</sup> , T121 <sup>S</sup> , <b>T368<sup>C</sup></b> , <b>S483<sup>Cp</sup></b>
<i>HsGalNAc-T11</i>	S45 <sup>S</sup> , S51 <sup>S</sup> , T60 <sup>S</sup> , S64 <sup>S</sup> , S96 <sup>S</sup> , <b>T136<sup>Ccp</sup></b> , <b>T318<sup>C</sup></b> , <b>S460<sup>CL</sup></b>
<i>HsGalNAc-T12</i>	T55 <sup>S</sup> , <b>T289<sup>Ccp</sup></b> , <b>S556<sup>L</sup></b>
<i>HsGalNAc-T13</i>	S51 <sup>S</sup> , <b>T282<sup>C</sup></b>
<i>HsGalNAc-T16</i>	S34 <sup>S</sup> , S35 <sup>S</sup> , S47 <sup>S</sup> , T55 <sup>S</sup> , T61 <sup>S</sup> , T63 <sup>S</sup> , S65 <sup>S</sup> , S112 <sup>S</sup> , <b>T279<sup>C</sup></b> , <b>T281<sup>C</sup></b> , <b>S496<sup>L</sup></b>

<sup>S</sup> Residue located on N-terminal stem.

<sup>C</sup> Residue located on catalytic domain, in red.

<sup>Cp</sup> Residue located on catalytic domain potentially affecting peptide substrate binding, in bold red.

<sup>Cnp</sup> Residue located on catalytic domain potentially affecting N-terminal substrate binding, in bold red.

<sup>Ccp</sup> Residue located on catalytic domain potentially affecting C-terminal substrate binding, in bold red.

<sup>L</sup> Residue located on the lectin domain, in red.

<sup>CL</sup> Residue located on the flexible linker connecting the catalytic and lectin domains, in red.

## **Notes for Supplementary Table SIV**

Previously unpublished enhancement values (EV) for hGalNAc-T11, PGANT35A (dT1) and hGalNAc-T6.

EV values were obtained using 3 different random peptides, PVI, PVII and PVIII as described in the Materials and methods. EV values (and their standard deviations, SD) are given for -/+ 5 residues of the acceptor site at position 0. “n” represents the number of experimental determinations obtained for each amino acid residue, which range from 2 to 21 determinations depending on transferase.

## Supplementary Table SIV: Previously Unpublished Enhancement Values

### hGalNAc-T11

n=5-21 unpublished		POSITION										
		EV -5	EV -4	EV -3	EV -2	EV -1	EV 0	EV +1	EV +2	EV +3	EV +4	EV +5
<b>G</b>	Avg	1.20	1.18	0.92	1.13	1.42	0.75	1.86	1.04	1.30	1.20	
	SD	0.20	0.17	0.25	0.21	0.29	0.32	0.31	0.29	0.24	0.21	
<b>A</b>	Avg	1.25	1.14	1.15	1.09	1.88	1.21	1.64	0.93	1.12	1.10	
	SD	0.14	0.10	0.16	0.13	0.35	0.23	0.43	0.23	0.11	0.15	
<b>P</b>	Avg	1.39	1.33	1.34	1.22	2.06	3.22	1.26	4.58	2.62	1.79	
	SD	0.31	0.26	0.29	0.28	0.63	0.88	0.18	1.14	0.59	0.25	
<b>V</b>	Avg	0.96	0.99	0.96	0.87	1.40	1.26	0.91	0.67	0.96	1.14	
	SD	0.06	0.07	0.16	0.10	0.23	0.18	0.11	0.14	0.11	0.15	
<b>I</b>	Avg	0.62	0.62	0.99	0.68	0.51	1.00	0.57	0.35	0.60	0.44	
	SD	0.11	0.08	0.53	0.12	0.10	0.21	0.19	0.07	0.05	0.37	
<b>L</b>	Avg	0.70	0.74	0.92	0.94	0.41	0.49	0.51	0.45	0.61	0.64	
	SD	0.11	0.10	0.23	0.24	0.13	0.07	0.12	0.30	0.10	0.40	
<b>M</b>	Avg	0.63	0.67	0.88	0.93	0.45	0.84	0.82	0.49	0.65	0.78	
	SD	0.05	0.06	0.21	0.25	0.08	0.20	0.28	0.15	0.05	0.06	
<b>F</b>	Avg	0.57	0.64	1.07	0.87	0.70	0.29	0.44	0.32	0.57	0.68	
	SD	0.14	0.11	0.53	0.40	0.26	0.09	0.18	0.07	0.10	0.14	
<b>Y</b>	Avg	0.97	1.09	1.21	1.12	0.94	0.41	0.65	0.65	1.01	1.04	
	SD	0.17	0.20	0.24	0.22	0.16	0.13	0.17	0.21	0.08	0.08	
<b>E</b>	Avg	0.83	0.86	0.98	1.10	0.57	1.04	1.07	0.65	0.73	0.90	
	SD	0.14	0.15	0.36	0.37	0.37	0.33	0.33	0.33	0.15	0.20	
<b>D</b>	Avg	0.74	0.75	1.10	1.12	0.76	1.24	1.20	1.07	1.08	1.17	
	SD	0.13	0.12	0.41	0.32	0.31	0.44	0.37	0.39	0.42	0.42	
<b>Q</b>	Avg	0.80	0.89	0.97	1.06	0.36	0.86	0.77	0.40	0.65	0.81	
	SD	0.19	0.22	0.20	0.20	0.13	0.18	0.15	0.06	0.09	0.18	
<b>N</b>	Avg	0.99	1.09	1.09	1.00	0.54	0.52	0.79	0.45	0.88	0.92	
	SD	0.17	0.16	0.17	0.16	0.13	0.13	0.13	0.05	0.07	0.06	
<b>R</b>	Avg	0.95	0.93	0.87	0.85	0.58	0.54	0.57	0.43	0.67	0.82	
	SD	0.44	0.36	0.34	0.27	0.21	0.18	0.15	0.16	0.12	0.13	
<b>K</b>	Avg	0.93	0.94	0.72	0.74	0.49	0.51	0.52	0.41	0.59	0.72	
	SD	0.27	0.30	0.35	0.27	0.30	0.28	0.26	0.25	0.30	0.29	
<b>H</b>	Avg	0.82	0.77	0.75	0.70	0.47	0.37	0.57	0.39	0.41	0.46	
	SD	0.28	0.20	0.15	0.13	0.13	0.13	0.13	0.10	0.09	0.09	
<b>S</b>	Avg	1.19	1.19	1.22	1.15	1.50	1.27	1.81	1.30	1.24	1.32	
	SD	0.13	0.10	0.13	0.13	0.11	0.36	0.28	0.19	0.11	0.14	

## Supplementary Table SIV continued:

<b>PGANT35A (dT1)</b>		<b>POSITION</b>										
<b>n=2-6</b>		<b>EV</b>	<b>EV</b>	<b>EV</b>	<b>EV</b>	<b>EV</b>	<b>EV</b>	<b>EV</b>	<b>EV</b>	<b>EV</b>	<b>EV</b>	
<b>Ref: unpublished</b>		<b>-5</b>	<b>-4</b>	<b>-3</b>	<b>-2</b>	<b>-1</b>	<b>0</b>	<b>+1</b>	<b>+2</b>	<b>+3</b>	<b>+4</b>	<b>+5</b>
<b>G</b>	Avg	1.20	1.19	1.06	1.22	1.50		0.88	1.80	1.03	1.10	0.98
	SD	0.16	0.11	0.15	0.10	0.22		0.15	0.26	0.11	0.10	0.12
<b>A</b>	Avg	1.24	1.15	1.16	1.06	1.54		1.17	1.33	0.94	1.02	1.02
	SD	0.12	0.08	0.10	0.06	0.24		0.05	0.10	0.03	0.04	0.04
<b>P</b>	Avg	1.17	1.11	1.07	1.07	1.51		2.36	1.20	4.32	2.33	1.64
	SD	0.33	0.25	0.19	0.20	0.29		0.48	0.11	1.18	0.50	0.27
<b>V</b>	Avg	0.96	1.00	1.00	0.85	1.48		1.37	0.85	0.63	1.00	1.18
	SD	0.06	0.11	0.11	0.08	0.40		0.16	0.09	0.05	0.19	0.19
<b>I</b>	Avg	0.92	0.88	0.96	0.93	0.75		1.26	0.62	0.50	0.88	1.12
	SD	0.01	0.00	0.02	0.01	0.02		0.14	0.01	0.02	0.05	0.02
<b>L</b>	Avg	0.90	1.00	1.04	1.12	0.60		0.79	0.59	0.42	0.83	1.13
	SD	0.04	0.01	0.02	0.01	0.08		0.04	0.08	0.09	0.08	0.08
<b>M</b>	Avg	0.72	0.75	0.76	0.75	0.51		0.69	0.66	0.48	0.77	0.89
	SD	0.01	0.01	0.00	0.00	0.01		0.03	0.01	0.02	0.02	0.04
<b>F</b>	Avg	0.74	0.82	0.80	0.79	0.69		0.43	0.50	0.44	0.67	0.81
	SD	0.01	0.01	0.01	0.00	0.02		0.01	0.02	0.03	0.04	0.01
<b>Y</b>	Avg	0.98	1.13	1.12	1.14	1.07		0.50	0.65	0.65	0.94	1.00
	SD	0.10	0.12	0.10	0.06	0.16		0.02	0.05	0.11	0.10	0.07
<b>E</b>	Avg	0.80	0.81	0.88	0.95	0.46		0.88	1.00	0.56	0.69	0.80
	SD	0.15	0.13	0.09	0.04	0.07		0.07	0.04	0.04	0.03	0.04
<b>D</b>	Avg	0.61	0.62	0.66	0.71	0.48		0.78	0.62	0.63	0.72	0.80
	SD	0.03	0.01	0.01	0.02	0.04		0.02	0.01	0.05	0.01	0.03
<b>Q</b>	Avg	1.02	1.05	1.11	1.23	0.48		1.14	0.88	0.46	0.75	0.98
	SD	0.12	0.09	0.07	0.04	0.00		0.05	0.00	0.01	0.01	0.04
<b>N</b>	Avg	0.81	0.87	0.96	0.92	0.61		0.52	0.51	0.38	0.75	0.77
	SD	0.06	0.07	0.04	0.09	0.01		0.01	0.03	0.03	0.02	0.02
<b>R</b>	Avg	0.69	0.66	0.71	0.70	0.57		0.56	0.57	0.46	0.59	0.71
	SD	0.15	0.14	0.14	0.15	0.13		0.13	0.14	0.18	0.14	0.15
<b>K</b>	Avg	1.16	1.27	1.20	1.19	0.99		1.00	1.01	0.87	1.03	1.13
	SD	0.08	0.09	0.13	0.14	0.22		0.18	0.15	0.15	0.12	0.10
<b>H</b>	Avg	0.59	0.64	0.68	0.66	0.50		0.41	0.46	0.34	0.36	0.43
	SD	0.10	0.12	0.12	0.14	0.06		0.05	0.06	0.02	0.03	0.06
<b>S</b>	Avg	0.83	0.90	0.89	0.89	1.18		0.95	1.48	0.81	1.11	0.99
	SD	0.01	0.05	0.05	0.06	0.07		0.09	0.09	0.05	0.15	0.09

## Supplementary Table SIV continued:

### hGalNAc-T6

n=2-11		POSITION										
Ref: unpublished		EV	EV	EV	EV	EV	EV	EV	EV	EV	EV	
		-5	-4	-3	-2	-1	0	+1	+2	+3	+4	+5
<b>G</b>	Avg	1.12	1.03	0.96	0.93	0.65	0.65	1.23	0.80	0.87	0.83	
	SD	0.08	0.07	0.05	0.12	0.11	0.17	0.16	0.20	0.19	0.19	
<b>A</b>	Avg	1.11	1.04	1.03	1.50	1.42	1.10	1.28	1.03	0.92	0.91	
	SD	0.13	0.07	0.12	0.23	0.15	0.08	0.08	0.15	0.15	0.12	
<b>P</b>	Avg	1.12	1.27	1.22	1.37	1.48	2.23	1.38	2.22	1.53	1.23	
	SD	0.10	0.15	0.09	0.09	0.32	0.53	0.15	0.35	0.17	0.21	
<b>V</b>	Avg	1.12	1.30	1.38	1.13	2.50	1.53	0.98	1.03	1.17	1.15	
	SD	0.11	0.12	0.17	0.19	0.21	0.21	0.10	0.11	0.11	0.09	
<b>I</b>	Avg	1.07	1.19	1.30	0.99	2.63	1.28	0.77	0.76	1.01	1.07	
	SD	0.12	0.03	0.04	0.06	0.35	0.16	0.18	0.08	0.09	0.08	
<b>L</b>	Avg	0.72	0.79	0.80	0.79	0.41	0.64	0.52	0.66	0.85	1.01	
	SD	0.17	0.14	0.15	0.14	0.06	0.11	0.04	0.10	0.19	0.26	
<b>M</b>	Avg	0.93	0.94	1.07	1.02	0.67	0.77	1.05	0.90	1.15	1.27	
	SD	0.11	0.04	0.12	0.14	0.17	0.16	0.19	0.19	0.30	0.36	
<b>F</b>	Avg	1.09	1.40	1.15	1.07	1.76	0.58	0.58	0.70	0.85	1.00	
	SD	0.06	0.31	0.09	0.04	0.16	0.04	0.05	0.03	0.04	0.03	
<b>Y</b>	Avg	1.04	1.07	1.05	1.02	0.89	0.49	0.49	0.66	0.84	0.93	
	SD	0.14	0.14	0.12	0.11	0.04	0.05	0.09	0.12	0.08	0.11	
<b>E</b>	Avg	0.88	0.77	0.72	0.61	0.42	0.65	0.72	0.58	0.83	0.93	
	SD	0.19	0.14	0.11	0.14	0.12	0.14	0.11	0.11	0.14	0.16	
<b>D</b>	Avg	0.94	0.80	0.75	0.56	0.24	0.34	0.63	0.55	0.95	0.94	
	SD	0.12	0.09	0.11	0.08	0.07	0.07	0.07	0.09	0.07	0.07	
<b>Q</b>	Avg	0.81	0.85	0.87	0.75	0.54	0.83	0.80	0.88	0.85	0.89	
	SD	0.19	0.17	0.17	0.05	0.09	0.07	0.06	0.23	0.19	0.12	
<b>N</b>	Avg	0.97	0.94	0.93	0.93	0.46	0.42	0.74	0.79	0.99	0.96	
	SD	0.07	0.08	0.05	0.08	0.02	0.04	0.06	0.05	0.08	0.04	
<b>R</b>	Avg	0.89	0.80	0.77	0.68	0.54	0.68	0.72	0.64	0.70	0.70	
	SD	0.51	0.45	0.38	0.27	0.18	0.19	0.14	0.16	0.16	0.14	
<b>K</b>	Avg	0.72	0.66	0.77	0.59	0.34	0.66	0.86	0.95	0.91	0.96	
	SD	0.05	0.06	0.04	0.05	0.09	0.09	0.14	0.14	0.14	0.21	
<b>H</b>	Avg	0.58	0.58	0.60	0.52	0.42	0.33	0.40	0.38	0.55	0.54	
	SD	0.11	0.10	0.10	0.12	0.09	0.08	0.05	0.05	0.10	0.07	
<b>S</b>	Avg	1.25	1.03	0.96	1.08	1.22	0.78	1.28	1.13	1.01	1.16	
	SD	0.23	0.02	0.18	0.09	0.21	0.09	0.06	0.10	0.05	0.19	