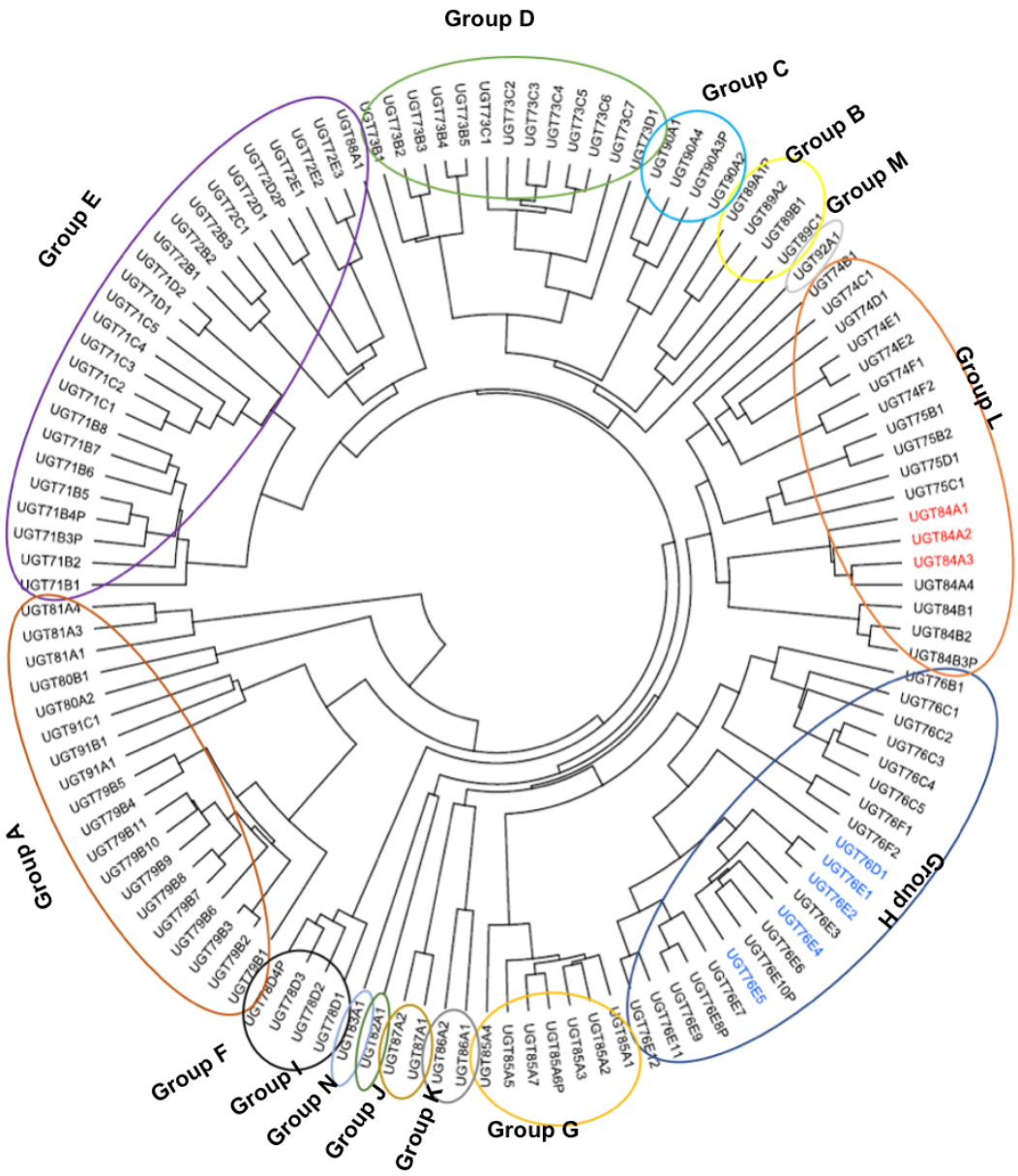



**Figure S1:** Phylogenetic tree showing 122 Arabidopsis thaliana UGT genes grouped into A-N (circled). Eight UGTs from groups H are printed in blue.

| Plant UGT | PDB code | In complex with | Reference |
|---|---|---|---|
| ***At***UGT74F2 | 5V2K<br>5U6M<br>5U6N<br>5U6S<br>5V2J | (T15A) with UDP and 2-bromobenzoic acid<br>UDP and salicylic acid<br>(T15S) with UDP and salicylic acid<br>UDP and 2-bromobenzoic acid<br>(T15S) with UDP and 2-bromobenzoic acid | George-Thompson et al. (2017) |
| ***At***UGT72B1 | 2VCE<br>2VCH<br>2VG8 | UDP<br>UDP<br>UDP-2-fluoro-glucose and TCP | Brazier-Hicks et al. (2007) |
| ***Mt***UGT78G1 | 3HBF<br>3HBJ | UDP and myricetin<br>UDP | Modolo et al. (2007) |
| ***Mt***UGT71G1 | 2ACV<br>2ACW | UDP<br>UDP-glucose | Shao et al. (2005) |
| ***Mt***UGT85H2 | 2PQ6 | None | Li et al. (2007) |
| ***Ct***UGT78K6 | 3WC4<br>4REL<br>4REM<br>4REN<br>4WHM | None<br>Kaempferol<br>Delphinidin<br>Petunidin<br>UDP | Hiromoto et al. (2013), (2015) |
| ***Vv***GT1 | 2C1X<br>2C1Z<br>2C9Z | UDP<br>UDP-2-fluoro-glucose and kaempferol<br>UDP and quercetin | Offen et al. (2006) |
| ***Os***79 | 5TMB<br>5TMD<br>5TME<br>6BK0<br>6BK1<br>6BK2 | UDP<br>U2F and trichothecene<br>UDP<br>Q202A with UDP<br>T291V with UDP<br>H122A/L123A with UDP | Wetterhorn et al. (2016), (2017) |
| ***P***tigS | 5NLM | Magnesium ion and 3-sulfooxy-1H-indole | Hsu et al. (2018) |
| ***At***UGT89C1 | 6IJ7<br>6IJ9<br>6IJA<br>6IJD | None<br>UDP<br>UDP-rhamnose<br>Quercetin | Zong et al., (2019) |

**Table S1:** Crystal structures of plant UGTs available in the protein data bank.

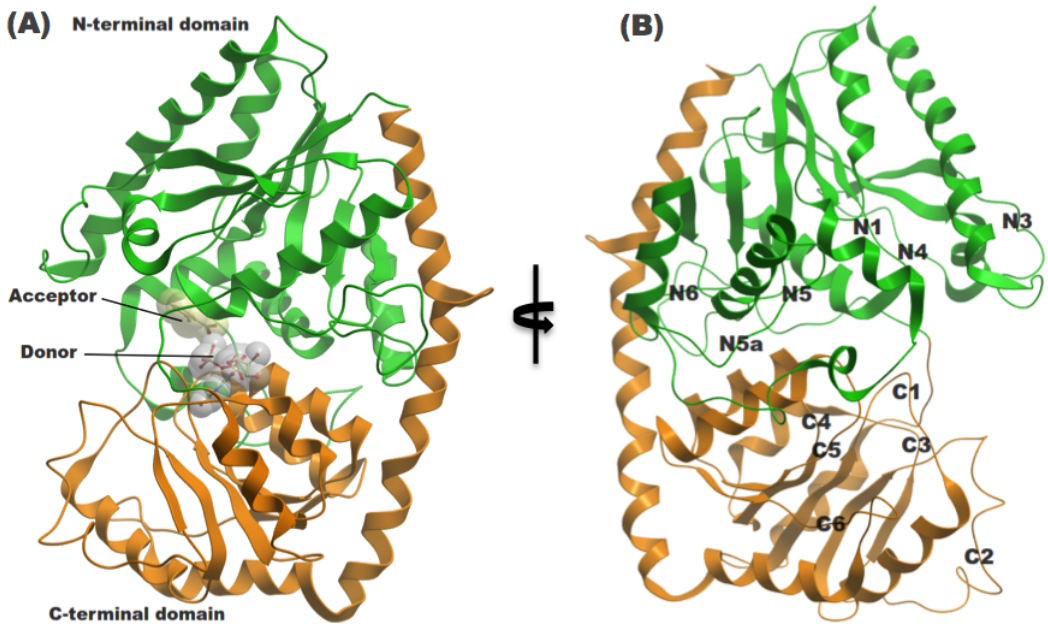



**Figure S2:** (A) Conserved GT-B fold of a representative of ten currently solved plant UGT crystal structures; The N- and C-terminal domains are (shown in green and orange respectively) with acceptor and donor substrates bound; (B) Loop regions of the N- and C-terminal domains in AtUGT74F2. These loop regions offer vital interactions with both acceptor and donor substrates in plant UGTs.

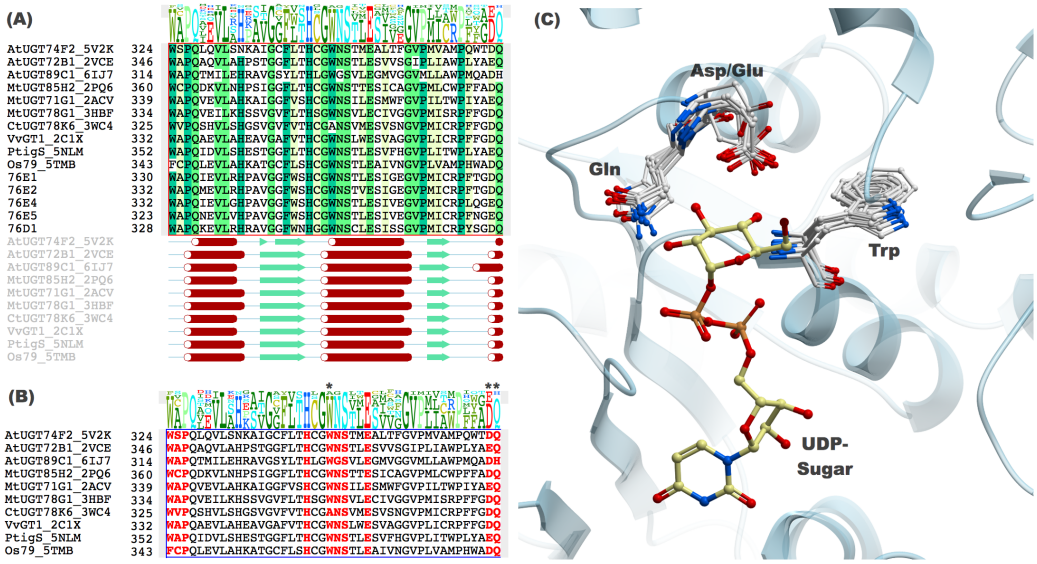



**Figure S3:** (A) Sequence alignment of the Plant Secondary Product Glycosyltransferase (PSPG) box, a characteristic structural feature of plant UGTs, from all ten crystal structures of plant UGTs solved and the five group H ***At***UGTs reported in this study; (B) Conservation of key amino acid residues in the PSPG box motif in the crystal structures that interact with the sugar; (C) Structural superimposition of conserved residues (Tryptophan, Aspartic acid/Glutamic acid and Glutamine) from the ten plant crystal structures which interact with the sugar part of the UDP- donor sugar.

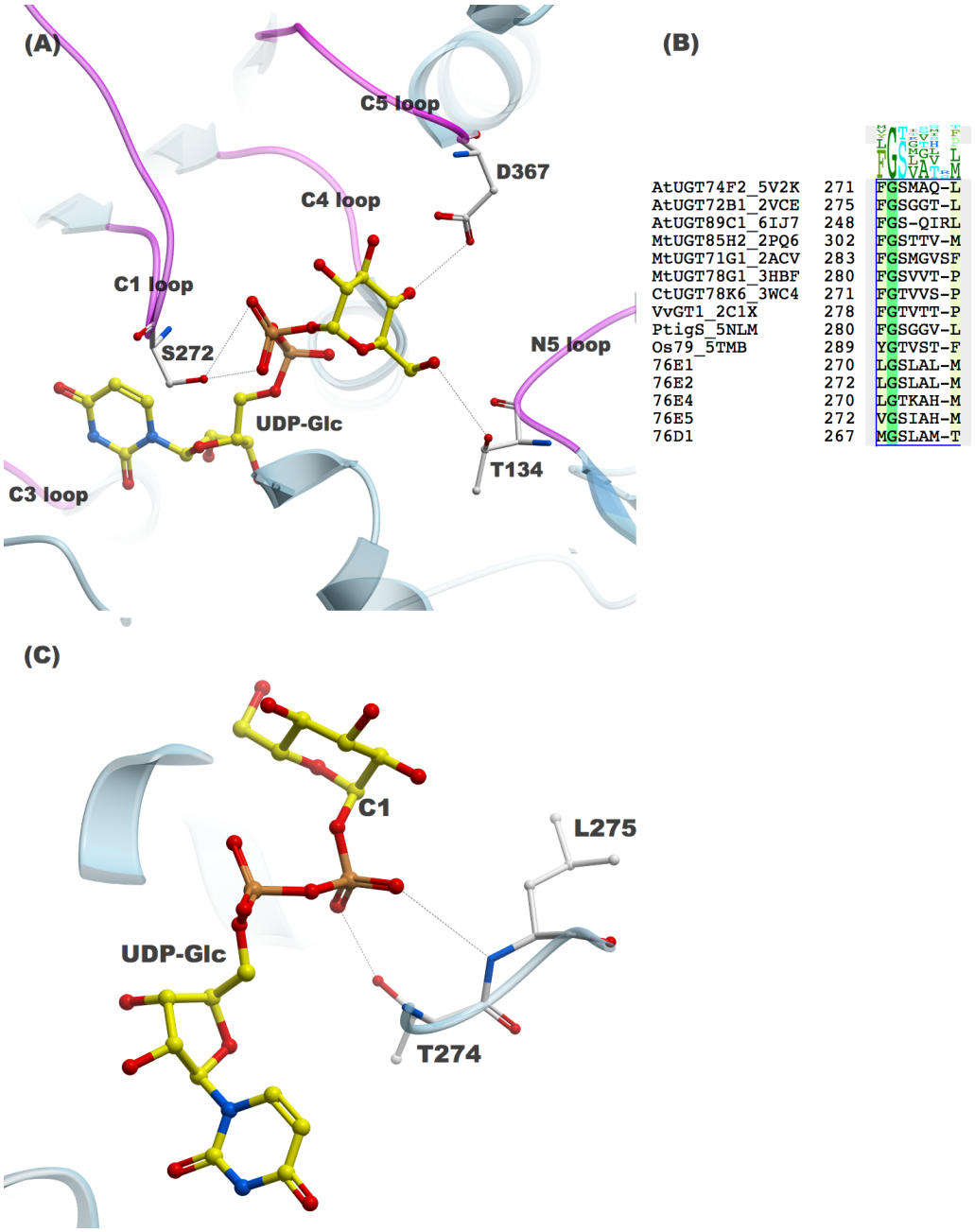



**Figure S4:** (A) Residues in loops from both C-terminal and N-terminal domain surround the UDP-sugar binding site and contribute to direct structural interactions; (B) Multiple sequence alignment highlighting C1 loop residues potentially involved in substrate recognition; (C) The spatial position of L275 in ***At***UGT76E4 K275L mutant, highlighting the proximity to the reaction centre of UDP-Glucose, which may have helped improved donor specificity.

| System | Donor Sugar | Cα RMSD (Å) |
|---|---|---|
| 76E1 | Glc | 4.01 |
| | Gal | 4.36 |
| | GlcNAc | 4.69 |
| 76E2 | Glc | 3.89 |
| | Gal | 4.59 |
| | GlcNAc | 3.87 |
| 76E2 D374E | Gal | 4.52 |
| | GlcNAc | 4.13 |
| 76E4 | Glc | 4.93 |
| | Gal | 4.82 |
| | GlcNAc | 4.35 |
| 76E4 K275L | Glc | 4.72 |
| | Gal | 4.58 |
| | GlcNAc | 4.22 |
| 76E5 | Glc | 4.23 |
| | Gal | 3.72 |
| | GlcNAc | 3.89 |
| 76D1 | Glc | 4.98 |
| | Gal | 3.89 |
| | GlcNAc | 4.28 |
| 76D1 P129T | Glc | 4.27 |
| | Gal | 4.20 |
| | GlcNAc | 4.42 |

**Table S2:** Summary of simulations. All simulations were of 1μs each (see methods for details). RMSD of Cα were calculated from their positions in the starting structure and averaged over the total number of simulations.

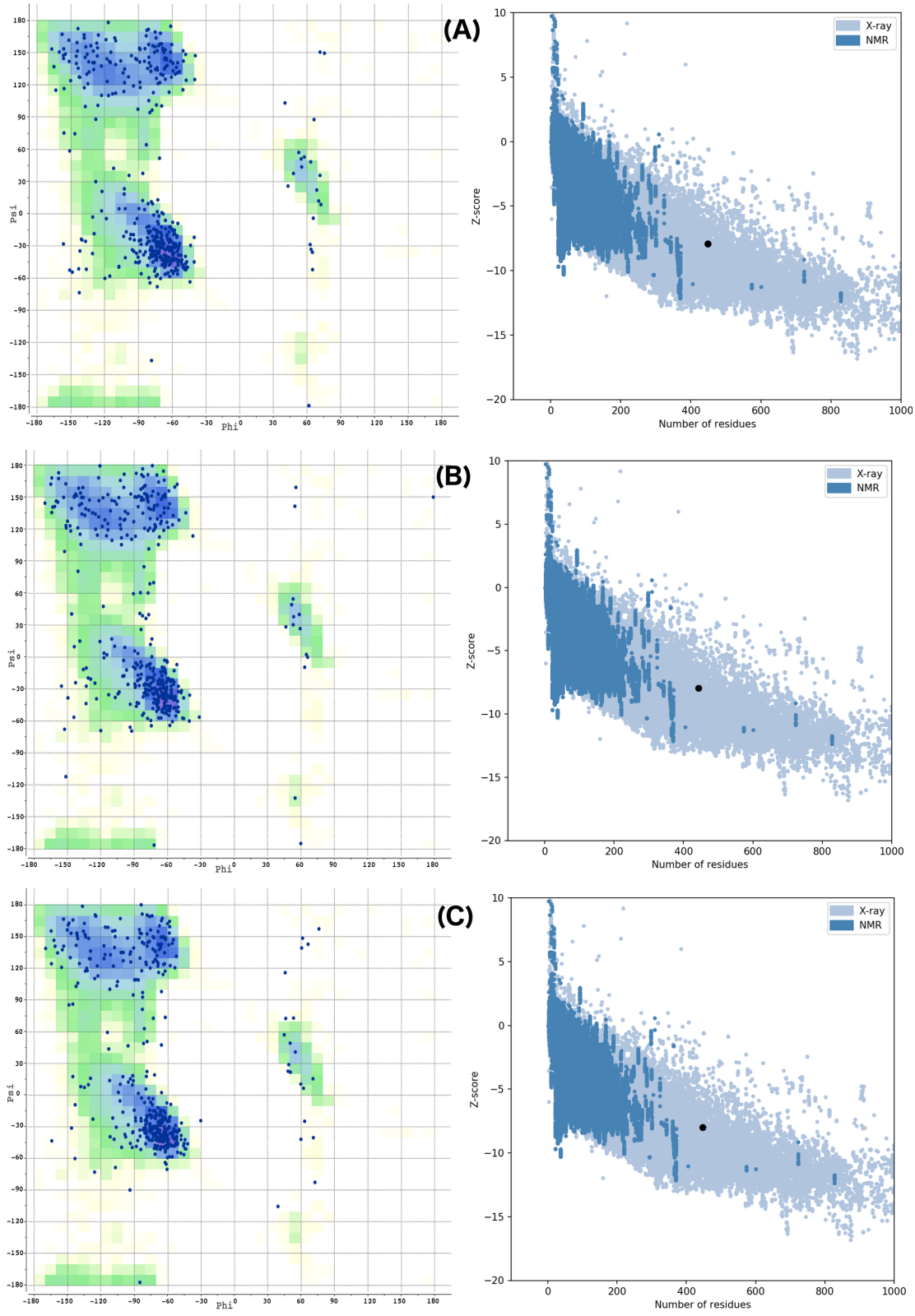



**Figure S5:** Stereochemical checks of the models. Ramachandran plots and PROSA analysis for models (A) 76E1, (B) 76E2, (C) 76E4. The black dot in each PROSA plot represents the Z-score. The Z-score is -7.91, -7.96 and -7.99 respectively.

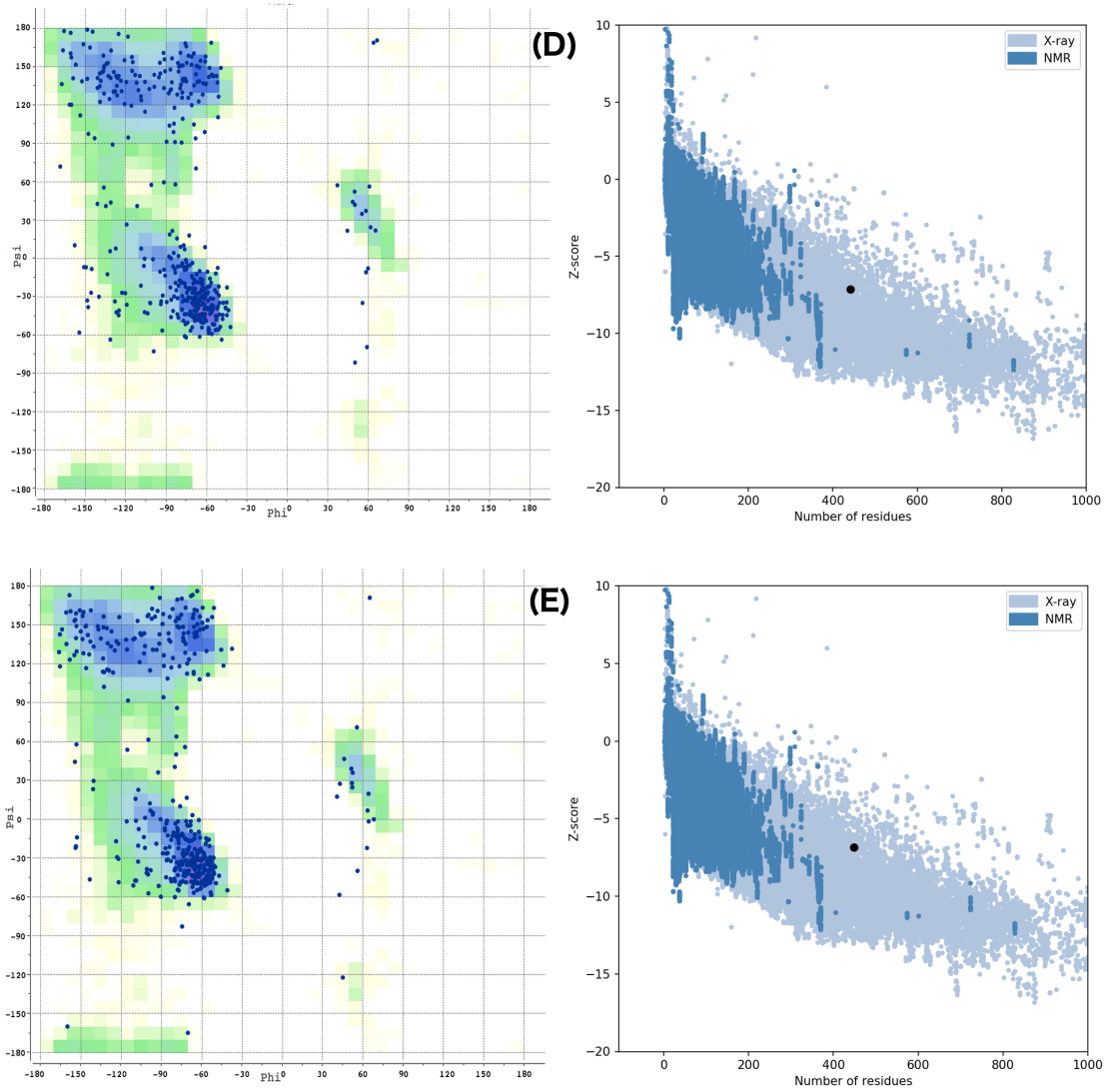



**Figure S5 (contd):** Stereochemical checks of the models. Ramachandran plots and PROSA analysis for models (D) 76E5 and (E) 76D1. The black dot in each PROSA plot represents the Z-score. The Z-score is -7.13 and -6.92 respectively.

| *At*UGT | Residues within 5Å of sugar |
|---|---|
| 76E1 | H20, Y114, T134, L273,G371, D372, Q373, N376, H348, C349, G350, S351, S352, S353 |
| 76E2 | K275, Y116,H21, T136, G373, D374, Q375, N378, H350, C351, G352, W353, N354, S355 |
| 76E4 | K275, L115, H20, T135, G373, F374, H350, C351, G352, W353, N354, S355, Q375, N378 |
| 76E5 | I273, L115, H20, T143, G364, E365, Q366, N369, H341, C342, G343, W344, N345, S346 |
| 76D1 | L270, F109, H19, P129, G369, D370, Q371, N374, H346, G347, G348, W349, N350, S351 |

**Table S3:** Residues that lie within 5 Å of donor sugar

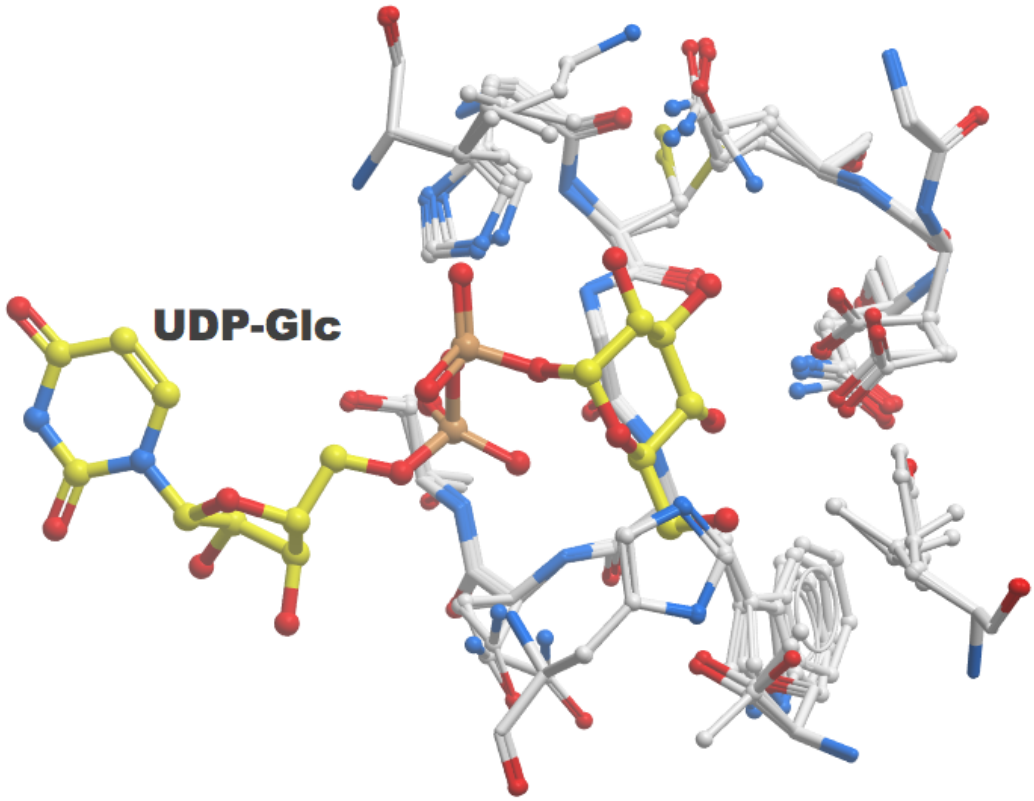
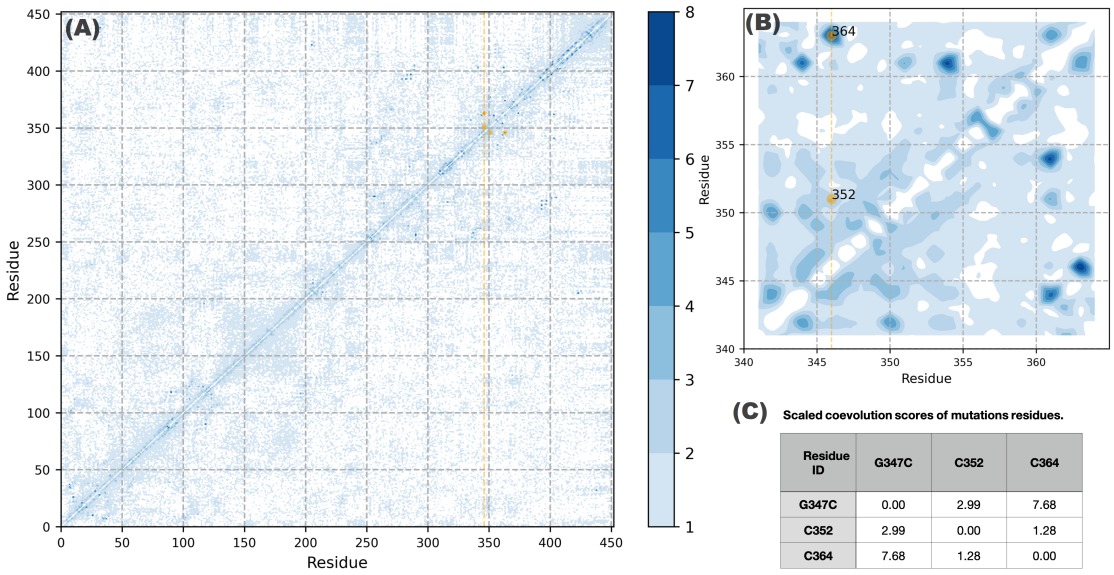



**Figure S6:** Side chains of 14 residues lie within 5Å of the donor sugar. All 5 ***At***UGTs have been superimposed on 76E1-UDP-Glc complex.




| Residue ID | G347C | C352 | C364 |
|---|---|---|---|
| G347C | 0.00 | 2.99 | 7.68 |
| C352 | 2.99 | 0.00 | 1.28 |
| C364 | 7.68 | 1.28 | 0.00 |

**Figure S7:** Scaled coevolution score matrix for UDP-glycosyltransferase 76D1 highlighting (A) full length sequence and (B) residues 340-365. Residue pairs with non-significant score (<1) are shown in white. Residue 347 is shown by an orange line and its pairings with 352 and 364 are highlighted by orange circles; (C) Pairwise coevolution scores between residue pairs. G347 shows strongest coevolution signal with C364. The maximum scaled coevolution score was 8.3