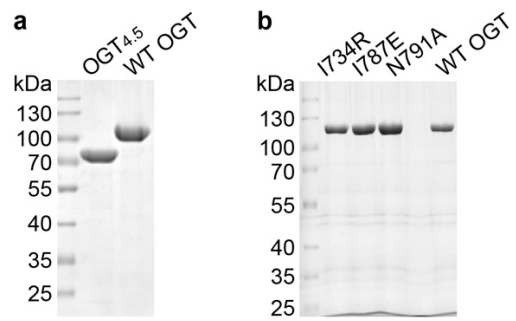
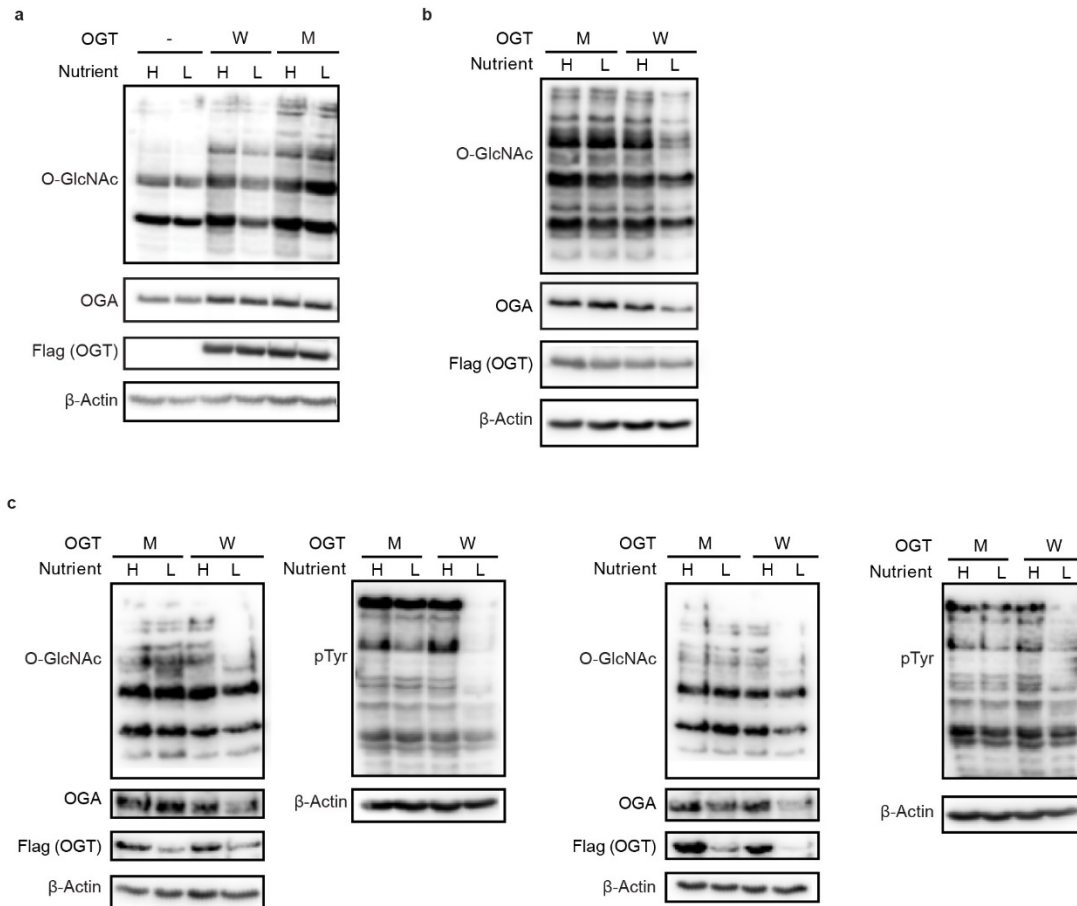


Supplementary Figures



Supplementary Figure 1. (a) OGT_{4.5} and full-length wild-type (WT) OGT purified from *E. coli*. (b) Full-length wild-type (WT) OGT and point mutants OGT-I734R, OGT-I787E, and OGT-N791A purified from *E. coli* for *in vitro* fluorescence polarization assays.



Supplementary Figure 2. Further replicates of low nutrient response assay in both TRex-293 and HeLa cell lines. Western blot of O-GlcNAcylation under high (H) and low (L) nutrient conditions in TRex-293 cells with OGT (W) or OGT-N791A (M) overexpression (**a**, **b**). High nutrient conditions include DMEM with 4.5 g/L glucose and 10% FBS, low nutrient conditions include DMEM with 0.45 g/L glucose and 1% FBS. (**c**) Western blot of O-GlcNAcylation and phosphotyrosine in HeLa cells overexpressing OGT (W) or OGT-N791A (M) under high (H) and low (L) nutrient conditions.

Supplementary Table 1. Primer table

| Primer name | Primer sequence |
|----------------------------|--|
| SMG9-BamHI-F | atatatGGATCCATGCCAACTTTGTACAAAAAAGC |
| SMG9-XhoI-R | atatatCTCGAGTCAGGCCAGCAGGCGGC |
| OGT-F | cggGGTACCATGGCGTCTTCCGTGGGCA |
| OGT-R1 | ATCTTTATAATCACCGTCATGGTCTTTGTAGTCTCCGCTGCCTGCTGACTCAGTGACTTC |
| OGT-R2 | CATCCTTGTAGTCGATGTCATGATCTTTATAATCACCGTCATGGTCT |
| OGT-R3 | cgcGGGCCCTACTTGTGCATCGTCATCCTTGTAGTCGATGTCATG |
| OGA-F1 | GGTACCATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGACTACAAG |
| OGA-F2 | CATGACATCGACTACAAGGATGACGATGACAAGGGCAGCGGAGTGCAGAAGGAGAGTCAA |
| OGA-R | gcgCTCGAGTCACAGGCTCCGACCAAGTATAAC |
| pLenti-OGT-F | atatatGCTAGCATGGCGTCTTCCGTGGGCAACG |
| pLenti-OGT-R | atatatGCGGCCGCTACTTGTGCATCGTCATCCTTGTAGTCG |
| <i>E. coli</i> OGT-I787R-F | ACACCATCGCTGAAGCTGTTcgcGAAATGATCAACCGTGGTCAG |
| <i>E. coli</i> OGT-I787R-R | CTGACCACGGTTGATCATTTCgcgAACAGCTTCAGCGATGGTGT |
| <i>E. coli</i> OGT-I787E-F | ATGAACACCATCGCTGAAGCTGTTgaaGAAATGATCAACCGTGGTCAGATC |
| <i>E. coli</i> OGT-I787E-R | GATCTGACCACGGTTGATCATTTCttcAACAGCTTCAGCGATGGTGTTTCAT |
| <i>E. coli</i> OGT-I734R-F | CGGTCACATCTACGACAACCGTcgcGTTCTGAACGGTATCGACCTG |
| <i>E. coli</i> OGT-I734R-R | CAGGTCGATACCGTTCAGAACgcgACGGTTGTCGTAGATGTGACCG |
| <i>E. coli</i> OGT-I734E-F | CGGTCACATCTACGACAACCGTgaaGTTCTGAACGGTATCGACCTGA |
| <i>E. coli</i> OGT-I734E-R | TCAGGTCGATACCGTTCAGAACttcACGGTTGTCGTAGATGTGACCG |
| <i>E. coli</i> OGT-F723R-F | AAAAAAAAGCTGTTATCGACcgcAAATCTAACGG |
| <i>E. coli</i> OGT-F723R-R | AGATGTGACCGTTAGATTTgcgGTCGATAACAGC |
| <i>E. coli</i> OGT-F723E-F | GAAAAAAAAGCTGTTATCGACgaaAAATCTAACGGTCACATCTACG |
| <i>E. coli</i> OGT-F723E-R | CGTAGATGTGACCGTTAGATTTtctGTCGATAACAGCTTTTTTTTTTC |
| <i>E. coli</i> OGT-N791A-F | GCTGTTATCGAAATGATCgccCGTGGTCAGATCCAGAT |
| <i>E. coli</i> OGT-N791A-R | ATCTGGATCTGACCACGggcGATCATTTCGATAACAGC |
| OGT-I724R-F | GGCACATTTATGACAATCGGcgaGTTCTGAATGGCATCGACC |
| OGT-I724R-R | GGTCGATGCCATTCAGAActcgCCGATTGTCATAAATGTGCC |
| OGT-I777E-F | GAATACTATTGCAGAAGCAGTTgagGAAATGATTAACCGAGGACAGA |
| OGT-I777E-R | TCTGTCTCGGTTAATCATTTCctcAACTGCTTCTGCAATAGTATTC |
| OGT-N791A-F | GCAGAAGCAGTTATTGAAATGATTgccCGAGGACAGATTCAAATAACAAT |
| OGT-N791A-R | ATTGTTATTTGAATCTGTCTCGggcAATCATTTCATAAAGCTTCTGCTG |
| OGT-N791A-I787E-F | GTTgagGAAATGATTgccCGAGGACAGATTCA |
| OGT-N791A-I787E-R | TGAATCTGTCTCGggcAATCATTTCctcAAC |
| OGA-D175N-F | GTGCAGATCATTGCTTTGCTTTTTGATaATATAGACCATAATATGTGTG |
| OGA-D175N-R | CACACATATTATGGTCTATATtATCAAAAAGCAAAGCAAATGATCTGCAC |
| SMG9-Y147F-F | AGAGACCCACACAGCCTGTGTtCCAGATCCAGAACCGGGGCAT |
| SMG9-Y147F-R | ATGCCCCGGTCTGGATCTGGaACACAGGCTGTGTGGGTCTCT |
| shOGT-F | CTAGCTGTTGCAGATGGGTGATATATTACTAGTATATATCACCCATCTGCAACATTTTTG |
| shOGT-R | AATTCAAAAATGTTGCAGATGGGTGATATATACTAGTAATATATCACCCATCTGCAACAG |

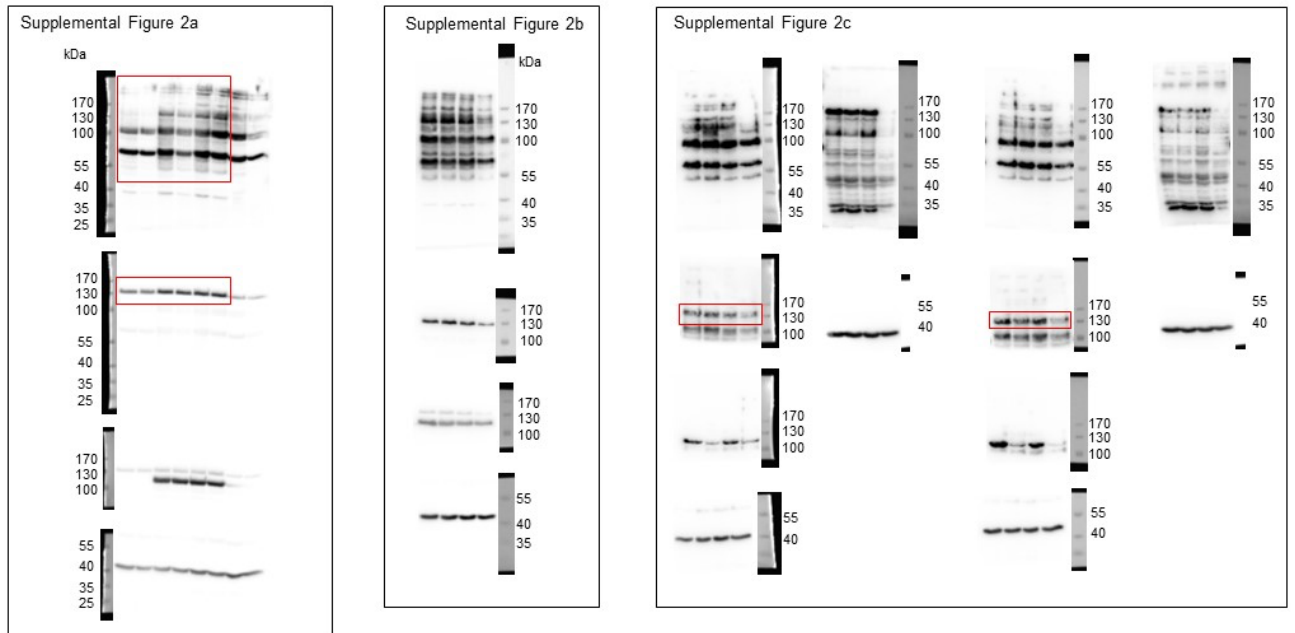
Supplementary Table 2. Data collection and refinement statistics (molecular replacement)

| | OGT _{4.5} :UDP-GlcNAc:SMG9 PDB 8FE7 | OGT _{4.5} :UDP-GlcNAc:ZNF831 PDB 8FUF | OGT _{4.5} :UDP-GlcNAc:CP37 PDB 8FE6 |
|--|---|---|---|
| Data collection | | | |
| Wavelength (Å) | 0.97872 | 1.12717 | 1.12713 |
| Space group | P 3 2 1 | P 3 2 1 | H 3 |
| Cell dimensions | | | |
| <i>a</i> , <i>b</i> , <i>c</i> (Å) | 273.67, 273.67, 143.14 | 274.01, 274.01, 143.08 | 281.53, 281.53, 143.21 |
| α , β , γ (°) | 90.00, 90.00, 120.00 | 90.00, 90.00, 120.00 | 90.00, 90.00, 120.00 |
| Resolution (Å) | 237.00 - 2.98 (3.03 - 2.98)* | 137.01 - 3.69 (3.69 - 3.75) | 123.48 - 3.06 (3.06 - 3.11) |
| <i>R</i> _{sym} or <i>R</i> _{merge} | 0.110 (1.017) | 0.282 (2.398) | 0.183 (1.473) |
| <i>I</i> / σ <i>I</i> | 13.7 (2.2) | 12.1 (2.2) | 12.9 (2.2) |
| <i>CC</i> _{1/2} | 0.998 (0.784) | 0.997 (0.768) | 0.994 (0.492) |
| Completeness (%) | 99.5 (99.6) | 99.9 (100.0) | 99.7 (100.0) |
| Redundancy | 7.7 (7.8) | 20.7 (21.3) | 10.5 (11.0) |
| Refinement | | | |
| Resolution (Å) | 63.42 - 2.98 | 53.06 - 3.69 | 40.64 - 3.06 |
| No. reflections | 124,762 | 66,440 | 79,576 |
| <i>R</i> _{work} / <i>R</i> _{free} | 0.1880/0.2203 | 0.1977/0.2415 | 0.1791/0.2187 |
| No. atoms | | | |
| Protein | 22632 | 22336 | 22396 |
| Ligand/ion | 156 | 156 | 156 |
| Water | 0 | 0 | 0 |
| <i>B</i> -factors | | | |
| Protein | 72.46 | 135.69 | 70.10 |
| Ligand | 72.44 | 135.61 | 70.13 |
| Water | 75.74 | 147.17 | 64.91 |
| R.m.s. deviations | | | |
| Bond lengths (Å) | 0.003 | 0.002 | 0.003 |
| Bond angles (°) | 0.63 | 0.45 | 0.55 |

*Each structure was solved from one crystal.

*Values in parentheses are for highest-resolution shell.

Supplementary Figure 2 Source Data



Note: To avoid stripping the same blot for too many times that would make weak signal detection difficult, we cut the blot into two parts and probed with different antibodies for each part of the blot to detect different proteins.