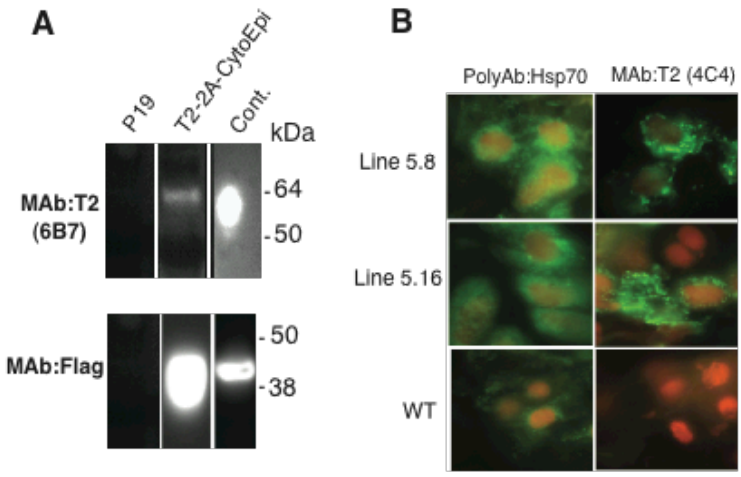
Table S1. Primers used in cloning

PBY7For	5’-GAGCTCATGGATTACAAGGACGACGACGACAAGCACGTGGAATT CGCCATGGTTATGATGAGTCGTTATGAAGA-3’
PBY7Rev	5’-AGCGCTAGGCCTGAGCTCTCATTTCAAAAACATGATGTA-3’
HAT2For	5’GGCTTAAUATGTACCCATACGACGTCCCAGACTACGCCCGGCGGCG CTCGCGGATGCTGCT-3’
HAT2Rev	5’ACTTAAGCAAAUCAAAATTCAAAGTTTGACCAGAACCCTGCTGCAG TGTGAGCGTGAAC-3’
PFwbppFor	5’-ATTTGCTTAAGUTGGCAGGAGATGTGGAATCTAACCCAGGAC CTATGGATTACAAGGACGACGACG-3’
PFwbppRev	5’-GGTTTAAUTCATTTCAAAAACATGAT-3’
PwbppFor	5’-TCTAGAATGATGAGTCGTTATGAAGAGC-3’
PT2Rev	5’-GAGCTCCTACTGCTGCAGGTTGAGCGT-3’
PFlagFor	5’-TCTAGAATGGATTACAAGGACGACGACGACAAG-3’
PBY9For	5’-CTGCAGATGAAGACCGCCGCTCTTGCACCGCTCTTCTTCCTCCC CTCTGCCCTCGCCACTACTCACGTGCATCATCATCATCATCACAGTAG CGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCATGACTGGTGGAC AGCAAATGGGTCGGGATCCGAATTCTGTCACCTCGGCCCCGGACACC AGGCCGGC-3′
PBY9Rev	5′-GAGCTCCTAGGTGTCCGGGGCCGAGGT -3′
SPMUC1For	5’-GGCTTAAUATGGGGGCATCGAGGA-3’
MUC1Rev	5’-GGTTTAAUACTGTATCCGGTGCGGAAGTGA-3’
ERWbpPfor	5′GAGCTCATGAAGACTGCTGCTTTGGCTCCTTTGTTTTTTTTGCCTTCT GCTTTGGCTGATTACAAGGACGACGACGA-3′
ERWbpPrev	5’AGCGCTAGGCCTGAGCTCTCATAGCTCATCTTTCAAAAACATGATG TACC-3’

Supplemental Fig. S1



Supplemental Fig. S1. **Expression and rough localization of O-glycosylation machinery comprising human GalNAc-transferase T2 (GalNAc-T2) and Flag-tagged *P. aeruginosa* cytosolic C4-epimerase WbpP (CytoEpi).** *A*, Western blot analysis of Golgi targeted GalNAc-T2 expressed alone or from the 2A linked glycosylation machinery GalNAc-T2-2A-CytoEpi in leaves of *N. benthamiana* demonstrated that both the epimerase and GalNAc-T2 were expressed as their expected molecular sizes 39.7 & 64.7 kDa, respectively. Total proteinacious extracts of 5-6 days Argrobacterium mediated transient expression of the silencing inhibitor construct P19 (empty vector control) and GalNAc-T2-2A-CytoEpi. MAbs 6B7 (upper panel) and anti-Flag M2 (Sigma, lower panel), were used for detection of GalNAc-T2 and Flag-Tagged WbpP (CytoEpi), respectively. Approximately 30 µg total protein was loaded in each lane. Positive controls (Cont.): *Bv*T2 (upper panel) and *Ec*WbpP (lower panel), were GalNAc-T2 (200 ng) and Flag-Tagged epimerase (50 ng) expressed and purified from *Baculo virus* Sf9 cells and *E. coli*, respectively. *B*, Immunostainings of GalNAc-T2 in protoplasts of *Nicotiana tabacum* L. cv Bright Yellow 2 (BY2) suspension cells (another tobacco species) stably transformed with GalNAc-T2 (lines 5.8 & 5.16) revealed sub-cellular localization similar to Golgi-like structures using MAb 4C4 (human GalNAc-T2) (1) and as a control HSP70 ER localization using a anti HSP70 polyclonal Ab

(2). Nuclei were visualised using PI stain (orange). Western blots and immunostainings were done as described in Supplemental Experimental Procedures.

Supplemental Experimental Procedures.

Preparation and Immunostaining of BY-2 Protoplasts - Tobacco Bright Yellow 2 (BY-2) suspension cells were cultivated, transformed and fermented in accordance to Mayo et al. (2006) (3). 1 ml of BY-2 cells in exponential phase was pellet gently (150 × *g*) then resuspended in 1 ml EB (25 mM MES, pH 5.5, 2 mM $CaCl_2$, 600 mM mannitol) containing 0.25% macerozyme (Yakult Honsha Co. Ltd., Japan) and 1% cellulase (Yakult Honsha Co. Ltd., Japan) and incubated for ≥ 2 h with mild shaking (45 rpm). The suspension was then pelleted (150 × *g*, RT) and resuspended in 0.5 ml EB. Generation of intact protoplast was checked - either visually (round spherical cells) or with 0,01% Calco flour white (which stains the cell wall) in distilled water for 2 sec to 2 min, then washed briefly in water. Protoplasts were then dried onto Teflon printed diagnosyic slides (Immuno-Cell Int., USA). Dried protoplasts were acetone fixed at -20°C for 8 min's and airdried 1 h at room temperature. Protoplast permeabilization was done with 5 % BSA incl. 0.2 % saponin for 20 min's at room temperature. Slides were overlaid with primary antibody over night at 4°C. Slides were incubated with secondary FITC labeled secondary antibodies for 45 min's at room temperature. All washing steps between procedures were done with 1 × PBS. Finally slides were mounted with fluoromount anti fade and imaged.

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