Supporting Information

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SI Materials and Methods

Fly Strains. RNA interference (RNAi) lines containing UAS-inducible inverted repeats from the Vienna *Drosophila* RNAi Center (VDRC) (1) were stocks #7286 (*pgant4IR*), 21594 (*Tango1IR*), 22853 (*Dfur1IR*), and 101242 (*Dfur2IR*). Additionally, the w^{1118}; *UAS-pgant4IR#7* stock was constructed as described previously (2, 3). Bloomington stock #5138 (y^1; *w*; P{tubP-GAL4}LL7/TM3,* Sb^1) is referred to as the *Tub-Gal4* driver and was used to drive dsRNA expression throughout the larvae. Bloomington stock #6978 (w^{1118};*P{GawB}c135*) is referred to as the *c135-Gal4* driver and was used to drive dsRNA expression in the proventriculus. Bloomington stocks #4533 [*w*; In(2LR)noc*^{4L}*Sco*^{rv9R}, b^1*/CyO, P{ActGFP}JMR1*] and #6502 [*Df(2L)tim-02/CyO*] were also used. The *pgant4* transposon insertion is stock #18521 (*PBac{WH}pgant4*^{f02186}) from the Exelixis Collection at Harvard Medical School. The *w; Dr/ TM3,* Sb^1*, twi-2XGFP* stock was the kind gift of D. Andrew (Johns Hopkins University, Baltimore). The *w; TM6C, cu, Sb, e, ca/* $Su(Tpl)^{s1}$*, red, e* stock was the kind gift of J. Kennison (National Institutes of Health). The wild-type stocks used were either Oregon R or VDRC #60000 (w^{1118}).

Antibody Preparation. The keyhole limpet hemocyanin-conjugated N-terminal peptide (EQIDQKEFPKQVLDA-C) and C-terminal peptide (C-HRGSYSHSPRTYRSL) of Tango1 (Transport and Golgi organization 1) were synthesized and used to produce N-terminal and C-terminal antibodies, respectively, in rabbits. To verify the specificity of each antibody, protein extracts from third-instar larval proventriculi of *Tango1* RNAi and RNAi negative control (VDRC#60000) (under the control of the *c135-Gal4* driver) were used for Western blotting. Membranes were blocked and incubated with Tango1 antibodies (1:500), and developed with HRP-conjugated rabbit IgG (Cell Signaling Technology; 1:2,000) secondary antibody. One Muc26B antibody was raised in rabbits using the keyhole limpet hemocyanin-conjugated peptide RPVRPAVRPALEIDE-C (amino acids 82–96). Antibodies were affinity purified on a peptide column (Covance).

Quantitative Real-Time PCR. To examine *pgant4* gene expression levels, proventriculi from third instar larvae were used to isolate RNA and perform real-time PCR. Briefly, RNA was isolated using the FastRNA Pro Green kit (Q-BIOgene). cDNA synthesis was performed using the iScript cDNA Synthesis Kit (Bio-Rad). Quantitative RT-PCR was performed on a MyiQ real time PCR thermocycler (Bio-Rad) using the SYBR-Green PCR Master Mix (Bio-Rad). Analyzed products were assayed in triplicate and in multiple independent experiments. Primers used are as described previously (2).

***pgant4*^{m2} Point Mutation.** A histidine at amino acid position 289 in the conserved "DXH" motif of the catalytic region of PGANT4 was changed to an aspartic acid using the QuikChange II XL site-directed mutagenesis kit (Agilent Technologies) to construct the *pgant4* mutant, *pgant4*^{m2}. Briefly, *pgant4* cDNA was digested with EcoRI and NotI and cloned into pIB/V5-His vector (Invitrogen). Mutagenic primers were designed using QuikChange Primer Design Program available online at www.agilent.com/genomics/qcpd. The sense primer sequence is 5′-gacaagtgatggtgttctttgattctgacatagaagtcaac-3′ and antisense primer sequence is 5′-gttgacttctatgtcagaatcaaagaacaccatcacttgtc-3′. Reactions and transformation were performed according to the manufacturer's instructions. DNA sequencing was performed to verify the mutation. The mutant *pgant4* cDNA was then excised from pIB/V5-His vector and subcloned into pUAST vector (3). Transgenic flies were produced by Genetic Services.

Expression of PGANT4, PGANT3, and Tango1 in *Drosophila* Cells. cDNAs encoding PGANT4, PGANT3, or Tango1 were digested with EcoRI and NotI, and subcloned into pIB/V5-His vector (Invitrogen) to form a C-terminal V5 or Myc fusion. S2R$^+$ cells were transfected with plasmids (or vector alone) using Effectene transfection reagent (Qiagen) according to manufacturer's instructions. After 3 d, cells were collected and lysed with RIPA buffer (Sigma). V5-agarose beads (Bethyl) were used to purify the proteins with V5 tag. Purified proteins were run on westerns and also used for mass spectroscopy.

In Situ Hybridization. *pgant4* RNA probes were prepared as described previously (4) for in situ hybridization. Briefly, *pgant4* cDNA (AT25481; Invitrogen) was linearized with EcoRI and transcribed by T3 RNA polymerase (antisense probe) or linearized with NotI and transcribed by T7 RNA polymerase (sense probe). RNA probes were then labeled using DIG labeling kit (Roche). *Drosophila* embryos were collected at 18 h after egg lay on apple juice agar plates. Embryos were dechorionated and fixed as described previously (4). After fixation, the whole-mount embryo in situ hybridization was performed. Briefly, fixed embryos were rinsed in methanol/PBST (PBS with 0.1% Tween 20), then treated with proteinase K for 2–3 min and on ice for 30 min, then postfixed in 4% (vol/vol) formaldehyde/PBS for 20 min. Prehybridization was performed by incubating with hybridization buffer at 60 °C for 1 h. Hybridization was performed by incubating with denatured 100 ng/mL DIG RNA probes at 60 °C overnight. The next day, embryos were washed and incubated with anti-DIG-POD antibody (Roche; 1:100) and Fasciclin III antibody (Developmental Studies Hybridoma Bank; 1:10) at 4 °C overnight. Embryos were washed and incubated with FITC-conjugated anti-mouse IgG antibody (Jackson ImmunoResearch Laboratories; 1:100) and Tyramide Signal Amplification (TSA, plus Cy3, PerkinElmer, 1:50 in amplification buffer) for 1 h. Finally, embryos were washed with PBST and mounted in aqueous mounting medium with antifading agents (Biomeda). Tissue in situ hybridization was performed as previously described (4). Briefly, the third-instar larval proventriculi were dissected and fixed, then incubated with DIG RNA probes. After washing, proventriculi were incubated with anti–DIG-AP antibody (Roche; 1:2,000 in PBST) at 4 °C overnight. Wash and color development was performed using BM Purple AP substrate (Roche). The staining reaction was stopped by washing in PBST. Samples were then transferred to 70% (vol/vol) glycerol/PBST and stored at 4 °C until mounted.

Staining of Paraffin Sections. The third-instar larvae were prepared by cutting off the posterior spiracles and immersing in 4% (vol/vol) formaldehyde in PBS. Fixation was continued on a shaker overnight at room temperature. Larvae were then moved to 70% (vol/vol) ethanol. Paraffin sections were performed by Histoserv. After deparaffinization and hydration, sections were blocked in 2% (wt/vol) BSA in PBS at room temperature for 1 h. Sections were incubated with primary antibody in blocking buffer at 4 °C overnight. Primary antibodies used were anti-GM130 antibody (Abcam; 1:50) and anti-Papilin antibody (5) (a kind gift of L. and J. Fessler, University of California, Los Angeles; 1:500). Alexa 488-and 568-conjugated secondary antibodies (Invitrogen; 1:100) were used. Nuclei were stained with SYBR Green (Invitrogen; 1:1,000). Sections were mounted in aqueous mounting medium with anti-fading agents (Biomeda) and imaged on a Zeiss LSM 510 confocal laser

scanning microscope. Images were processed using the LSM Imager Browser and Photoshop.

Whole-Mount Staining of Proventriculi. Proventriculi were dissected from third-instar larvae and fixed in 4% (vol/vol) formaldehyde in PBS. Samples were washed in PBST (PBS-0.3% Triton X-100) and transferred to blocking buffer [2% (wt/vol) BSA-PBS-0.3% Triton X-100] for 1 h on a shaker. Primary antibodies used were anti-Muc26B antibody (1:250) and anti-Rab8 antibody (BD Transduction Lab; 1:100). FITC-conjugated Chitin-Binding Probe (New England Biolabs; 1:100) was used to detect chitin. Samples were incubated with primary antibodies overnight at 4 °C in blocking buffer and incubated with FITC-conjugated anti-rabbit or mouse IgG antibody (Jackson ImmunoResearch Laboratories; 1:100) at room temperature for 1 h. Counterstaining was performed using TRITC-phalloidin (Sigma; 1:100) and DAPI (Sigma; 1:100). Samples were mounted in aqueous mounting medium with antifading agents (Biomeda) on slides with a spacer (Electron Microscopy Sciences) and imaged on a Zeiss LSM 510 confocal laser scanning microscope. Images were processed using the LSM Imager Browser and Photoshop.

Western Blotting and Analysis. Protein extracts were prepared from cells or from ∼40 third-instar larval proventriculi. Samples were electrophoresed under reducing conditions in a 4–12% SDS/PAGE gradient gel and transferred to nitrocellulose membranes. Membranes were blocked and incubated with anti-Tango1 antibody (1:500), Tn antibody (a kind gift of Richard Cummings, Emory University, Atlanta; 1:500) (6), anti-V5 antibody (Invitrogen; 1:1,000) or anti–α-Tubulin antibody (Cell Signaling Technology; 1:1,000). Blots were then developed with HRP-conjugated mouse IgG (Cell Signaling Technology; 1:2,000), HRP-conjugated rabbit IgG (Cell Signaling Technology; 1:2,000) or HRP-conjugated mouse IgM (Enzolifesciences; 1:2,000) secondary antibodies. The signal was detected using a chemiluminescent substrate (Thermo Scientific) and analyzed on a Fuji Imager. Band intensity was measured using National Institutes of Health ImageJ software. For westerns shown in Fig. 3*B*, membranes were blocked with Odyssey Blocking Buffer (LI-COR) and incubated with anti-V5 antibody (Invitrogen; 1:1,000). After washing, membranes were incubated with IRDye 800CW goat anti-mouse antibody (1:20,000; LI-COR) and IRDye 680LT-conjugated lectin helix pomatia agglutinin (HPA) (1:5,000). After washing, the signal was detected with LI-COR Odyssey Imager and the band intensity was measured using Image Studio Lite.

Immunoprecipitation. Dynabeads protein G (Invitrogen) was used for immunoprecipitation according to the manufacturer's instructions. Briefly, protein extracts were prepared from ∼100 wild-type third-instar larval proventriculi. After removing the supernatant, 50 μL of Dynabeads Protein G was resuspended in 200 μL Ab binding and washing buffer with 10 μg Tango1 N-terminal antibody and incubated with slow-tilt rotation mixing at 4 °C for 8 h. Beads were then collected using a magnet and the supernatant was removed. Beads were then washed with Ab Binding and Washing Buffer (Invitrogen). Beads were resuspended in 200 μL proventriculi lysate and incubated with slow-tilt rotation mixing at 4 °C overnight. Beads were collected and washed with Washing Buffer (Invitrogen). After washing, beads were resuspended in Elution Buffer (Invitrogen) with sample loading buffer and heated to 95 °C for 5 min. This mixture was then cooled on ice for 5 min. Tubes were then put on the magnet to trap beads and the supernatant was removed. The supernatant (containing the immunoprecipitated protein) was analyzed by SDS/PAGE, followed by immunoblotting with the anti-Tango1 C-terminal antibody (1:500) and Tn antibody (1:500).

Bioinformatic Predictions. Potential O-glycosylation sites of Tango1 were predicted using the NetOGlyc4.0 server (www.cbs.dtu.dk/services/NetOGlyc) and the UTEP ISOGlyP server (http://isoglyp.utep.edu). Potential PC cleavage sites of Tango1 were predicted using ProP1.0 Server (www.cbs.dtu.dk/services/ProP).

Statistics. All experiments were performed two or more times. Average values obtained for experiments shown in Figs. 3 *C* and *E* and 4*B* were averaged over multiple experiments and error bars were calculated as SEM. Error bars shown in Fig. 2*H* and Figs. S2 *C* and *D*, S3*A*, S4, and S6 *A* and *B* are standard deviation. Significance values were calculated using the Student *t* test.

Sample Preparation for Analysis of Mass Spectrometry. $S2R^+$ cells were transfected with either *Tango1-V5+pIB* (empty vector) or *Tango1-V5+pgant4* expression vectors and then Tango1-V5 protein was purified as described above. The eluted samples were reduced with 10 mM DTT (DTT) for 1 h at 56 °C; carboxyamidomethylated with 55 mM iodoacetamide (ICH_2CONH_2; Sigma) in the dark for 45 min; and then digested with 3 μg of modified trypsin (Promega) in 40 mM ammonium bicarbonate (NH_4HCO_3) overnight at 37 °C. After digestion, the peptides were acidified with 1% trifluoroacetic acid. Desalting was subsequently performed with C18 spin columns (Vydac Silica C18; The Nest Group) and the resulting peptides were lyophilized and stored at −20 °C until analysis.

Detection of O-Linked Glycosylation Sites in Tango1 by LC-MS/MS. Peptides were resuspended with 19.5 μL of mobile phase A (0.1% formic acid, FA, in water) and 0.5 μL of mobile phase B [80% (vol/vol) acetonitrile, ACN, and 0.1% formic acid in water] and filtered with 0.2-μm filters (Nanosep; PALL). The samples were loaded off-line onto a nanospray tapered capillary column/emitter (360 × 75 × 15 μm, PicoFrit, New Objective, 15-cm column) that was self-packed with C18 reverse phase resin (Waters) in a nitrogen pressure bomb for 10 min at 1,000 psi (∼5 μL load). The peptides were separated using the Dionex UltiMate 3000 nano-LC system (ThermoFisher) with a 180-min linear gradient of increasing mobile phase B at a flow rate of 120 nL/min. The LC-MS/MS analysis was performed using the Orbitrap Fusion Tribrid MS (ThermoFisher) equipped with a Nanospray Flex Ion Source at 2.2-kV spray voltage and 280 °C ion transfer tube temperature. The full Fourier transform mass spectrometry spectrum, typically recorded at 120,000 of resolution in positive ion and profile mode, was acquired at 300–2000 *m/z* followed by the MS/MS spectra of ion trap mass spectrometry on the 15 most intense ions from the targeted mass lists or data-dependent MS/MS spectra on the most intense ion with dynamic exclusion at 30-s duration time. The targeted ions were isolated by the quadruple at 1.5 *m/z* isolation window for collision-induced dissociation (CID) and 3.0 *m/z* for electron-transfer dissociation (ETD) and fragmented by decision-tree algorithm by alternating between CID at 38% normalized collision energy and ETD at 80 ms of reaction time for above triply charged and 150 ms of reaction time with 40% of supplemental activation for doubly charged.

The raw files were searched against the Tango1 database, including a contaminant database (along with reversed proteins as decoys) using Proteomic Discoverer (Thermo Scientific) with a peptide tolerance of 30 ppm, a MS/MS tolerance of 0.8 Da, the carbamidomethylated cysteine, oxidation of methionine and O-linked glycosylation (HexNAc, HexNAc + Hex, HexNAc + Hex + HexA) of serine and threonine as variable modifications. The peptide sequences were identified by Proteomic Discoverer from the CID and ETD spectra and verified manually. The glycosylations on the peptides were verified by the presence of corresponding neutral loss fragment ions of sugar, such as the HexNAc at 203.0794 Da, Hex at 162.0528 Da, and HexA 176.0321 Da calculating charge states in CID spectra. The representative MS and MS/MS spectra are shown in Fig. S5 *B* and *C*.

1. Dietzl G, et al. (2007) A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. *Nature* 448(7150):151–156.
2. Tran DT, et al. (2012) Multiple members of the UDP-GalNAc: Polypeptide N-acetylgalactosaminyltransferase family are essential for viability in *Drosophila*. *J Biol Chem* 287(8):5243–5252.
3. Brand AH, Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118(2):401–415.
4. Tian E, Ten Hagen KG (2006) Expression of the UDP-GalNAc: Polypeptide N-acetylgalactosaminyltransferase family is spatially and temporally regulated during *Drosophila* development. *Glycobiology* 16(2):83–95.
5. Kramerova IA, et al. (2000) Papilin in development; a pericellular protein with a homology to the ADAMTS metalloproteinases. *Development* 127(24):5475–5485.
6. Avichezer D, Springer GF, Schechter B, Arnon R (1997) Immunoreactivities of polyclonal and monoclonal anti-T and anti-Tn antibodies with human carcinoma cells, grown in vitro and in a xenograft model. *Int J Cancer* 72(1):119–127.

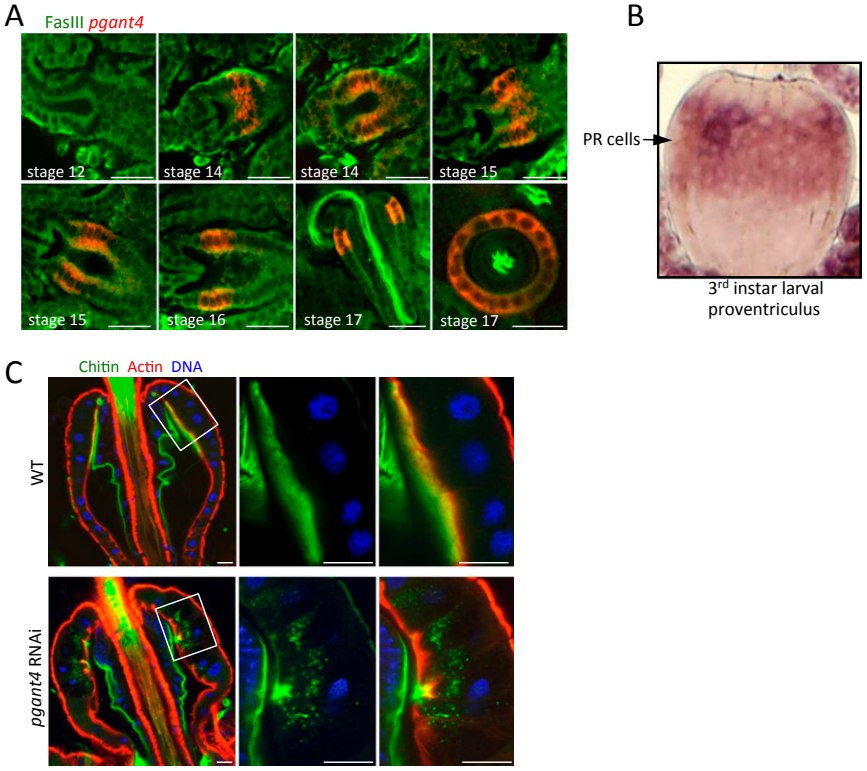
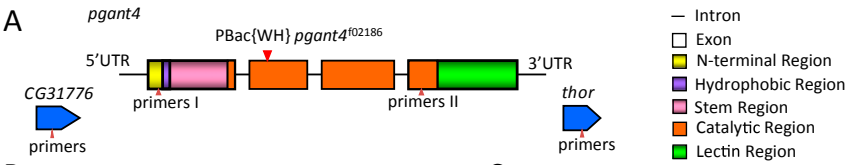


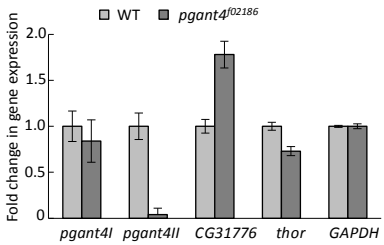
Fig. S1. Expression of *pgant4* in PR (secretory) cells of the developing embryonic and larval proventriculus. (*A*) RNA in situ hybridization shows *pgant4* expression (red) during embryonic stages 12–17. Fasciclin III (FasIII) staining (detected by the anti-FasIII antibody) is shown in green to outline the proventriculus (PV). Views are sagittal sections with the exception of the final stage 17 image, which is transverse across the PR cells. (Scale bar, 20 μm.) (*B*) *pgant4* expression (by RNA in situ hybridization) in the PR cells of a whole mount third-instar larval PV. (*C*) Chitin and actin staining in wild-type (WT) and *pgant4* RNAi third instar larval proventriculi by immunofluorescence. *Inset* in each panel is shown magnified to the right. Chitin secretion to the apical surface and apical actin localization are disrupted upon loss of *pgant4*. (Scale bar, 20 μm.)

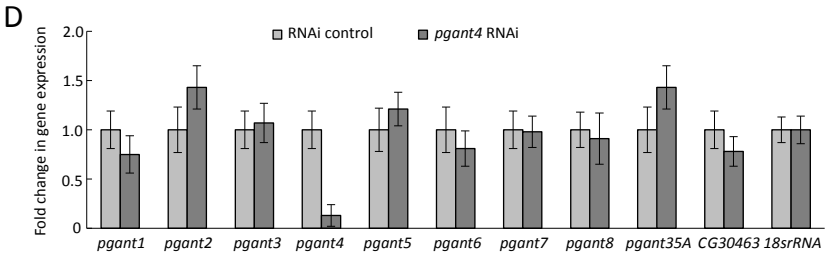


B

Genotype	Lethality (%)	n*
pgant4^{f02186}/+	0	112
Df2L/+	0	147
pgant4^{f02186}/*pgant4*^{f02186}	72	122
pgant4^{f02186}/*Df2L*	79	180
Tub-Gal4 > pgant4 IR #7286	100	115
c135-Gal4 > pgant4 IR #7286	88	169

* Total number scored.



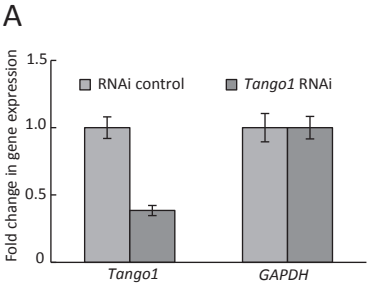


E

Genotype	Normal proventriculus (%)	n*
pgant4^{f02186}/*Df2L*; *c135-Gal4 > pgant4OE*	71	53
pgant4^{f02186}/*Df2L*; *c135-Gal4 > pgant4*^{m2}	0	19
pgant4^{f02186}/*Df2L*; *c135-Gal4*	38	34

* As determined by morphology.

Fig. S2. *pgant4* transposon mutation and RNAi to *pgant4* result in a specific decrease in *pgant4* transcripts and loss of viability. (*A*) PiggyBac insertion (PBac{WH}*pgant4*^{f02186}) in the coding region of *pgant4*. Position of the primers used to perform quantitative PCR for *pgant4* and flanking genes are shown. (*B*) Loss of *pgant4*, via in vivo RNA interference (RNAi) or conventional mutations (*pgant4*^{f02186}/*Df2L*) results in lethality. (*C*) Quantitative PCR to quantitate the level of *pgant4* transcripts in flies containing the transposon insertion mutation. (*D*) Quantitative PCR to quantitate the level of *pgant4* transcripts in flies expressing dsRNA to *pgant4* under the control of the *c135-Gal4* driver in the proventriculus. (*E*) Rescue of PR cell phenotype in *pgant4*^{f02186}/*Df2L* flies that also express a catalytically active version of *pgant4* in the proventriculus using the *c135-Gal4* driver.



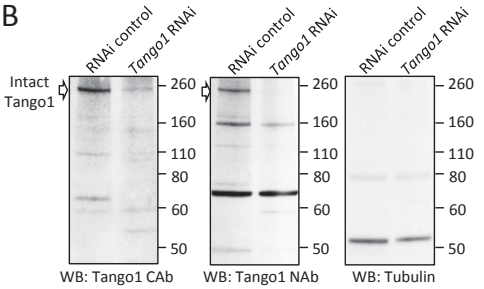


Fig. S3. *Tango1* is efficiently knocked down in the PV of animals expressing dsRNA to *Tango1*. (*A*) Quantitataive PCR to quantitate levels of *Tango1* expression in PV of wild-type larvae and larvae expressing dsRNA to *Tango1* under the control of the *c135-Gal4* driver. (*B*) Western blots of PV extracts from control or *Tango1* RNAi larvae probed with an antibody to the C-terminal (Tango1 CAb) or N-terminal region of Tango1 (Tango1 NAb) to demonstrate the loss of Tango1-specific bands (denoted by arrows). Control blot probed with the tubulin antibody is shown to the right.

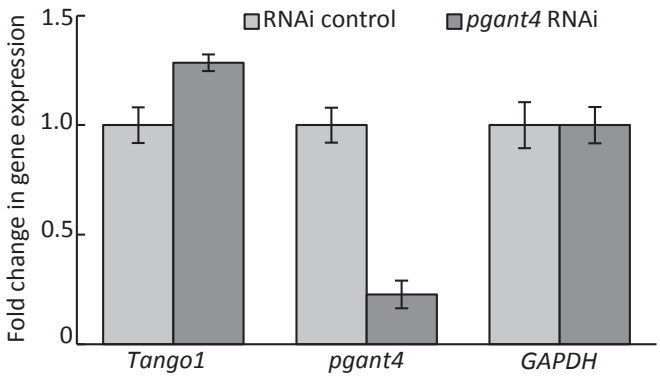


Fig. S4. *Tango1* gene expression is unaffected upon knock down of *pgant4* expression. Quantitative PCR to quantitate levels of *Tango1* and *pgant4* expression in PV of wild-type larvae and larvae expressing dsRNA to *pgant4* under the control of the *c135-Gal4* driver.

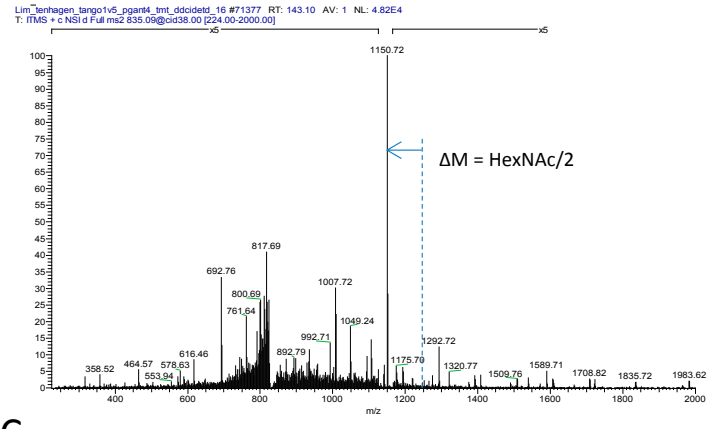
A

MRLTNEKATMQPQLSDLALVLGLLICCLPTLTWAATLSDKRLCADPKCEQIISMGIAKITYAIGGEGLISFKINSPIRVLSKSAGS
NMQLWGVDINGRRGYANKDFIMEKKILVRDKDLLYEVPVVGPGSPVQSVETTPVQSVETTVQPVLNASESTDDLATTTTSPLEIAVD
SIVVEHDKLQDQQVPDPTAASKAQVQVIEGTELPLEAIAATTEGSIVPETAADPQEATNLDSTVVDTKEPQALNSEAIKLQEEPKA
QQPATEAEKPPPLPQAINAELEDADDFDYGDDETDDDSQQGSQDNESIVEIANDNKSINESIELKPLSVAQLKKTDKVEDSKDETK
EKHAEMEVSKQEDSSLPTETTLNVTALEEQIDQKEFPKQVLDAAVELKSSDPLPVEEVTETTVAEPPRTIVEDKINEEIVPVSAKIQA
KPATTVNPTEPIVAQSDAEIKAPSESVISSTTPAPVVEEAPQKADPVGLPPLFEKKNFENPNNYYKQLQEEQEKQRLVAEAEEQKRL
QEEADQQKRLQEEAALNKRLLEEAEQQKRLQEEAEQQKRLQEEAELNKRLLEEAEKQKRLHEESEQLQRSSEEAEPQLSVQEANMQ
QLNDSVDSQSNEIVDNNNRQQPEQYQQHHHHTESAFNHPSTASHTTPTPDAESPYAAVQEETTEASQTDNHREGVGYVEPVALPAT
ASPVSEVPIKEDAAGFGLFATIVDTVNNFIGKDPQSDPADSSDELHRILYPGRPEVPPSQRKAEDFAPADVDGYCARFQAKDEHCH
RSISLDNFVEVMADKLVDHSQLLLCVVIAAISSLFFMFAYYCFCNSSQEGALLSKLNHLERSLLASHKENLIIKHDLMTTRTKLAS
IEDNSFGSNDMVADLKKQLESELYEKAKLQEQVGSLERDLDNAAEAGLELNKMLSEVLNGQNGDEAFMSTVDELQRQLNDQEKIII
EINNSLAEKSRENSELQYTFTEATTRLNSELKTLQEDNYELEMEKSKLQTRLQEIQAETESELAKALEARNYEMQKLQNQIVELTV
KWEREHGDLQTSLAKIEALEDCLKAVGKDAIHNVQELITSAKTRGELNAVHKKLVELQSKVEQEEAHKQRLESQLQQSSQDVEQLK
QDFNQSERDKLEAQTRLEVLSGYFREKENDLKKELSLQETKWLQHQGENASTVETQTLMKNEIQTLKSQNDELRAEIEAQIASHKA
QMGTLENRAHESWLAARQSERRCEEALAEAASLRRKLTTMASGGGGVGGDPGVMEAIAANGTSVLGAELKTAPSPLPLPGSPLLNM
PNPLPFLAAPFSPFMGLPPPFLPPTGAGGARPPPLGRMRSPPPSSRGDRDRERYSDYSDYDDYDDDEEDDRGMDRRRRHSGSWGRR
HRGSYSHSPRTYRSLSPSDSRYNYNDTETDFSPPPSPPPVPSGRSATSRPYSEV

Red box: potential glycosylation sites (NetOGlyc, score > 0.5; UTEP, score > 1.2)
Blue box: potential PC cleavage sites (ProP, score > 0.19)
Signal peptide: 1-34aa
Transmembrane domain: 794-817aa
Black box: region O-glycosylated in cells expressing *pgant4*
Black arrow: start position of Tango1 751-1430 construct

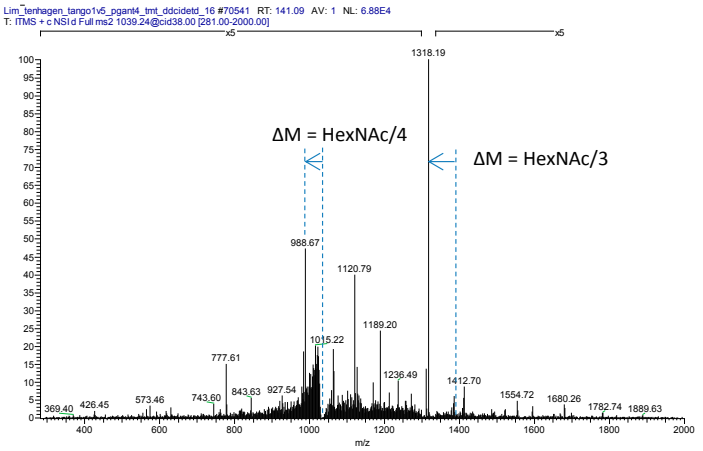
B

EDAAGFGLFATIVDTVNNFIGK + 1 HexNAc
$[M+3H]^{3+}$ = 834.7515 m/z (mono); 4.26 ppm, Xcorr=2.45



C

EDAAGFGLFATIVDTVNNFIGKDPQSDPADSSDELHR + 1 HexNAc
$[M+4H]^{4+}$ = 1038.7384 m/z (mono); 1.52 ppm, Xcorr=2.32



D

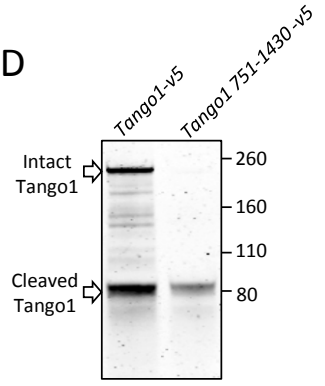
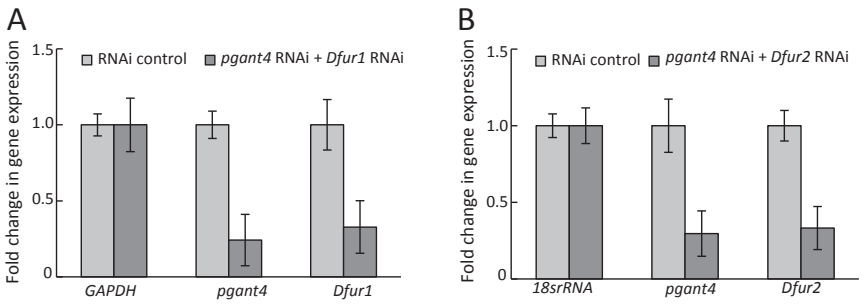


Fig. S5. Tango1 has multiple potential sites of O-glycosylation and proprotein convertase (PC) cleavage. (*A*) The entire protein sequence of Tango1 is shown with signal sequence underlined and transmembrane domain highlighted in gray. Potential sites of O-glycosylation (red) and PC cleavage (blue) are shown. Boxed region indicates region glycosylated when *pgant4* is expressed based on mass spectroscopy. (*B* and *C*) PGANT4 specific glycosylation of Tango1. MS/MS fragmentation of tryptic peptides without (*B*) and with (*C*) a missed tryptic cleavage site identify an O-GalNAc modified peptide from Tango1 only when *pgant4* is overexpressed. Note the accurate mass of the O-GalNAc modified peptides and the strong neutral loss peaks of the HexNAc in the MS/MS. Analysis of Tango1 without *pgant4* expression failed to identify either of these peptides as being glycosylated. (*D*) The Tango1 751–1430-V5 construct (comprised of the C-terminal region of Tango1 from amino acids 751–1430) has a molecular weight similar to the cleaved Tango1 fragment.



C

Genotype	% Normal PR cells*	n
RNAi control	100	102
pgant4 RNAi	0	84
Dfur1 RNAi	100	79
Dfur2 RNAi	100	73
pgant4 RNAi, D*fur1* RNAi	11	104
pgant4 RNAi, D*fur2* RNAi	100	108

* As determined by morphology.

Fig. S6. Quantitative PCR to quantitate the levels of *pgant4*, *Dfur1* and *Dfur2* transcripts in rescue experiments shown in Fig. 4. (*A*) Expression of *pgant4* and *Dfur1* in the proventriculi of flies expressing dsRNA to both *pgant4* and *Dfur1*. (*B*) Expression of *pgant4* and *Dfur2* in the proventriculi of flies expressing dsRNA to both *pgant4* and *Dfur2*. (*C*) Quantitation of PR cell rescue in *pgant4* RNAi flies that are also undergoing RNAi to *Dfur2*.