

# **Protein *N*-glycosylation and *N*-glycan trimming are required for postembryonic development of the pest beetle**

***Tribolium castaneum***

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




















**Supplementary information**

**Supplementary Table 1. Developmental expression changes of genes putatively involved in N-glycan attachment and processing in *T. castaneum***

Gene	ANOVA P	Stage with the highest expression vs 4 <sup>th</sup> instar larvae			Mature adults vs 4 <sup>th</sup> instar larvae	
		Stage	Expression ratio (95% CI)	t-test P	Expression ratio (95% CI)	t-test P
<i>TcDad1</i>	<0.001	Early pupae	1.87 (1.34-2.56)	<0.001	1.08 (0.78-1.49)	0.262
<i>TcStt3A</i>	0.011	Early pupae	2.35 (1.11-4.35)	0.029	1.15 (0.58-2.26)	0.123
<i>TcStt3B</i>	0.547	Late pupae	1.32 (0.53-3.13)	0.998	1.01 (0.42-2.45)	0.472
<i>TcGCS1</i>	0.001	Late pupae, pharate adults	2.86 (1.34-6.45)	0.018	2.25 (1.07-5.15)	0.032
<i>TcGCS2a</i>	0.013	Early pupae	3.03 (1.21-11.76)	0.018	2.30 (0.91-8.85)	0.029
<i>Tca-Man-Ia</i>	<0.001	Late pupae	1.74 (1.21-2.49)	0.002	1.46 (1.01-2.07)	0.031
<i>Tca-Man-Ib</i>	<0.001	Pharate adults	2.97 (1.66-5.85)	0.001	2.53 (1.39-4.90)	0.012
<i>TcMgat1</i>	0.025	Late pupae	1.86 (0.92-4.24)	0.111	1.35 (0.66-3.06)	0.128
<i>Tca-Man-IIa</i>	<0.001	Early pupae	3.88 (1.80-8.26)	0.004	2.33 (5.18-1.12)	0.008
<i>Tca-Man-IIb</i>	<0.001	Early pupae	3.59 (1.71-7.94)	0.002	2.23 (1.05-4.90)	0.022
<i>Tcfdl</i>	0.009	Early pupae	4.86 (1.21-23.26)	<0.001	1.37 (0.26-5.05)	0.316
<i>TcFucT6</i>	0.003	Early pupae	4.14 (1.63-16.39)	<0.001	2.55 (1.03-10.31)	0.011
<i>TcFucTA</i>	0.001	4 <sup>th</sup> inst. larvae	1.00	-	0.74 (0.23-2.37)	0.101
<i>TcFucTA/C</i>	<0.001	Late pupae	8.11 (3.75-15.63)	<0.001	2.55 (1.24-5.21)	0.006
<i>TcFucTB</i>	<0.001	Early pupae	4.56 (2.59-8.26)	<0.001	2.35 (1.31-4.20)	0.012
<i>TcFucTC</i>	<0.001	Prepupae	2.68 (1.64-4.59)	<0.001	1.63 (0.97-2.71)	0.059
<i>TcMgat2</i>	<0.001	Early pupae	4.77 (1.87-16.13)	0.004	2.65 (1.07-9.26)	0.007
<i>TcMgat4</i>	0.004	Late pupae	9.21 (2.18-142.86)	0.003	4.08 (1.01-62.50)	0.006
<i>TcβGalNAcTA</i>	<0.000	Early pupae	10.24 (2.74-38.46)	0.003	2.57 (0.75-10.31)	0.037
<i>TcβGalNAcTB</i>	<0.000	Early pupae	2.15 (1.41-3.28)	0.001	1.60 (1.05-2.44)	0.016
<i>TcST6Gal</i>	<0.000	Late pupae	3.55 (2.18-6.06)	<0.001	2.77 (1.71-4.76)	0.001
<i>TcCsas</i>	<0.000	Early pupae	6.60 (2.96-16.13)	0.001	5.65 (2.61-14.29)	0.002

ANOVA P – p-value obtained using ANOVA in qbase+ software to reveal life stage dependent changes in expression. T-test P – p-value obtained via independent sample t-test to compare expression between 4<sup>th</sup> instar larvae and life stage with highest expression or between 4<sup>th</sup> instar larvae and mature adults. All values are averages of 3 biological replications. 95% CI are 95% confidence intervals for expression ratio between indicated life stages.

**Supplementary Table 2. N-glycan content**

	Measured m/z [M+Na <sup>+</sup> ]	Larva	Adult	Glycan content [% total profile]			Deduced structure	Composition
				L vs. A P	$\alpha$ -Man-Ia RNAi Adult	A vs. A <sup>RNAi</sup> P		
1.	891.3	3.5±0.7	6.1±0.9	0.004	3.8±0.1	0.007		HexNAc2Hex2
2.	1021.4	0.2±0.4	1.2±0.2	0.005	0.1±0.1	0.001		HexNAc2HexdHex2
3.	1037.4	11.5±3.1	21.9±5.8	0.013	4.6±0.3	0.003		HexNAc2Hex2dHex
4.	1053.3	3.4±0.7	4.7±0.4	0.015	4.8±0.2	0.294		HexNAc2Hex3
5.	1183.5	7.3±1.3	11±3.8	0.063	1.8±0.2	0.007		HexNAc2Hex2dHex2
6.	1199.4	4.0±0.8	8.4±1.1	0.001	1.8±0.2	0.000		HexNAc2Hex3dHex
7.	1215.5	2.4±0.5	3.5±0.2	0.010	4.7±0.2	0.001		HexNAc2Hex4
8.	1256.5	0.8±0.3	0.7±0.4	0.297	0.1±0	0.034		HexNAc3Hex3
9.	1345.5	1.2±0.4	1.2±0.2	0.391	0.2±0	0.000		HexNAc2Hex3dHex2
10.	1361.5	0.2±0.2	0.7±0.3	0.029	0.1±0	0.023		HexNAc2Hex4dHex
11.	1377.5	3.3±0.4	4.5±0.3	0.003	5.5±0.3	0.013		HexNAc2Hex5
12.	1402.5	1.0±0.3	1.4±0.3	0.105	0.4±0	0.003		HexNAc3Hex3dHex
13.	1418.4	0.3±0.1	0.1±0.1	0.016	<0.1	0.082		HexNAc3Hex4
14.	1539.5	2.9±0.3	2.6±0.4	0.231	5.6±0.3	0.000		HexNAc2Hex6
15.	1548.5	0.2±0.2	0.4±0.3	0.139	<0.1	0.049		HexNAc3Hex3dHex2
16.	1580.5	0.3±0.1	0.2±0.1	0.161	n.d.	-		HexNAc3Hex5
17.	1605.5	0.2±0.1	0.3±0.2	0.160	<0.1	0.039		HexNAc4Hex3dHex
18.	1701.6	3.9±0.4	2.6±0.5	0.005	11.0±0.4	<0.001		HexNAc2Hex7
19.	1863.6	6.9±0.7	3.8±0.9	0.002	15.9±0.6	<0.001		HexNAc2Hex8
20.	2025.7	40.7±7.4	22.0±6.0	0.008	35.5±1.5	0.010		HexNAc2Hex9
21.	2187.7	5.8±1.4	2.2±0.4	0.004	4.0±0.5	0.005		HexNAc2Hex10

[M+Na]<sup>+</sup> indicate measured masses of sodiated 2-AB-glycan ions. Glycan content values are means of glycan abundance relative to all glycans detected ± standard deviation (n=4). P's were calculated using independent sample t-test (SPSS 22).

Supplementary Table S3. Primers used for qRT-PCR analysis of gene expression.

gene	<i>Tribolium</i> accession number		qRT-PCR primer sequence	Amplicon length	Efficiency (%)
<i>TcDad1</i>	TC016366	F	CCACAGGTGACTTCAGTTATCG	147	92.5
		R	AAAAGTGCCCAACAAGACAGC		
<i>TcStt3A</i>	TC010433	F	TGTGCTACTACCGCTTTGGG	148	94.7
		R	ATACGCACGAGCCAGTGTTT		
<i>TcStt3B</i>	TC009183	F	CCCCTTCATGTGTTTGTCC	141	101.1
		R	TCGCTGGTACGAATGGGTTG		
<i>TcGCS1</i>	TC011354	F	ATCGGCCCTCAGTAACATGC	132	95.5
		R	AAACTTCGAGACGGGACTGC		
<i>TcGCS2a</i>	TC032148	F	GGGAAGGGGAACAAGAGGTG	103	97.6
		R	TCAACACCCACTGCTTCAGG		
<i>Tca-Man-Ia</i>	TC011089	F	AATGCAAGCTGTCCTCCAAC	124	97.6
		R	CAGACCACCCGAAAAACAAC		
<i>Tca-Man-Ib</i>	TC014283	F	ACGTGCTATTTGCCTGGAAC	83	98.9
		R	TTCGGGTGCTAGAAATGTGG		
<i>TcMgat1</i>	TC009001	F	ACCAGGAGTTGAAAGAGGGC	147	94.1
		R	TACCCTTGACGCAGTTCGG		
<i>Tca-Man-IIa</i>	TC009186	F	CAAAGCGAAGAAAGGAGTGG	104	97.2
		R	ACGATCGGTCTTGAATCAC		
<i>Tca-Man-IIb</i>	TC002991	F	GGAGTTCCCCTGTGATACGC	124	95.2
		R	ACTCACAGCACAGTCGTAGC		
<i>Tcfdl</i>	TC009779	F	CCCAGAGTTTTCTTTGGTG	123	91.6
		R	CTCTGATTCGGGCATATTCG		
<i>TcFucT6</i>	TC008521	F	ATTGCTTGCCTGGAGACCAG	117	103.5
		R	TGGGCTCTTCTTTCGCACTC		
<i>TcFucTA/C</i>	TC005825	F	ACGTCACGGAGAAGCTTTGG	83	94.2
		R	CCTGTAATCGCCACGGAAG		
<i>TcFucTA</i>	TC014343	F	CACCCTCCAGTTTCCAGACC	136	92.0
		R	CGACCAGATCGCTGTCTCTG		
<i>TcFucTB</i>	TC007385	F	ACAACGAACAACCTGCTCCAC	80	91.6
		R	GATGTAGTCGTCGCAAATGG		
<i>TcFucTC<sup>§</sup></i>	TC008651/2	F	CGACAAACCAACCTTTCTGG	71	90.6
		R	GGCTTTGGAACATTGGTGTC		
<i>TcMgat2</i>	TC001867	F	CCCATACTCGATCCAAACACATCC	112	97.7
		R	GAGGGCATTGTTGCATTTCTGG		
<i>TcMgat4</i>	TC003870	F	GGTGCCTACAAACCTTCGC	147	94.3
		R	ATTCTGAGACGACTTCCGC		
<i>Tcβ4GalNAcTA</i>	TC006388	F	GCAAGATACACGATGCTGACG	92	91.0
		R	TCCTTGTCGAAGCGTTTGGG		
<i>Tcβ4GalNAcTB</i>	TC006987	F	CGTGTGTGAGCAAACCAAG	103	97.1
		R	CGATGAAATCGCCACAACCTCC		
<i>TcST6Gal</i>	TC014265	F	CTCACCGAGCAGACTTAGGAG	124	94.0
		R	GCGTTTAGTGACCCTGGTTG		
<i>TcCsas</i>	TC015995	F	CAAGGGAAAGGAGTGTGTCTTC	119	92.5
		R	CTTGTCTTCGTGGTCGATTG		
<i>TcRpS6</i>	TC010830	F	GAATATCGGGCGGGAACGAC	150	95.7
		R	TGCACCCTCTAACTGACTTGC		
<i>TcRpL32</i>	TC006106	F	ACCAGTCTGACCGTTATGGC	114	94.2
		R	CGTAGCCAATGTTGGGCATC		

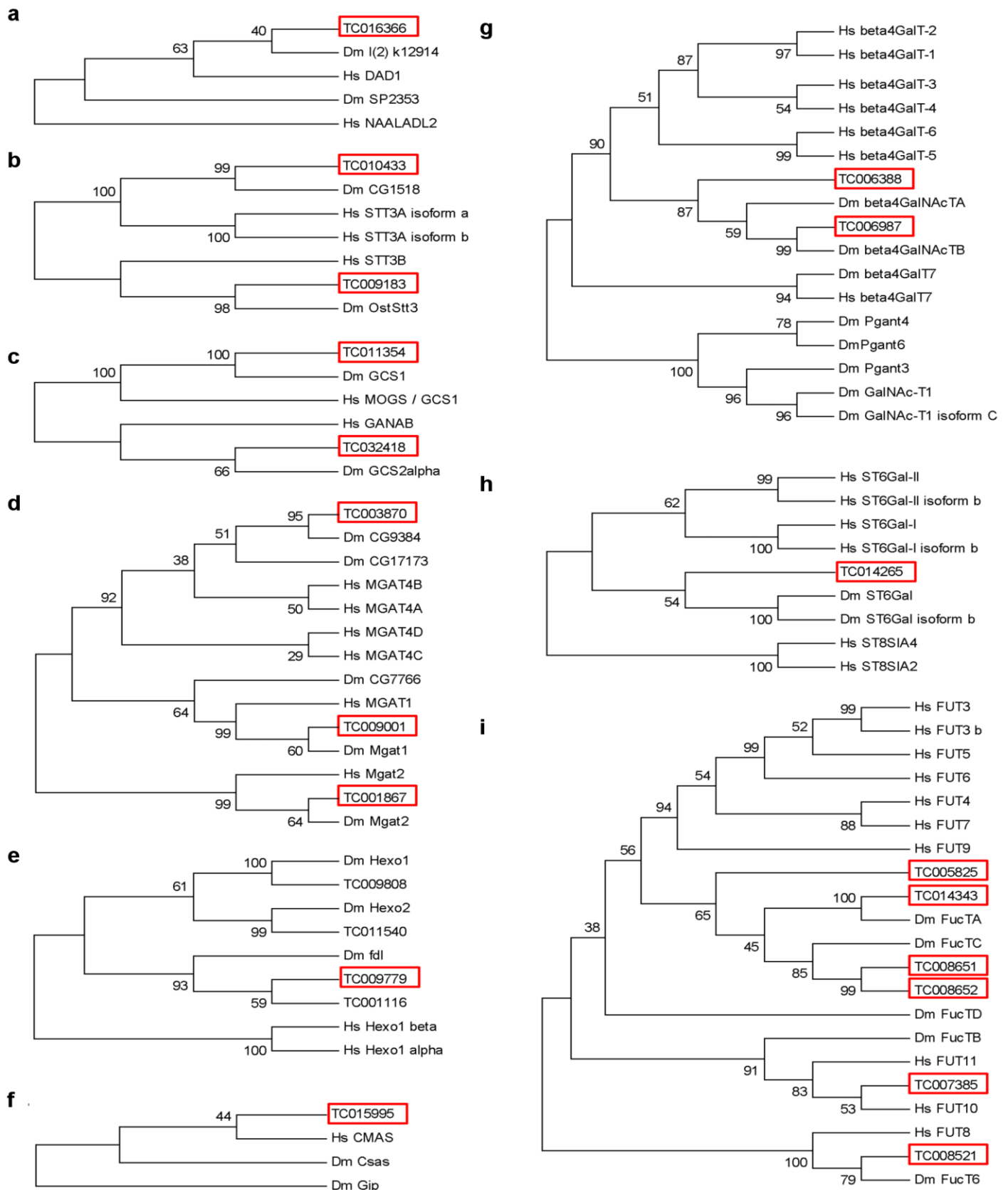
§ - primer for FucTC was designed to detect both FucTC paralogs TC008651 and TC008652. The paralogs share 87% identical aminoacids.

Supplementary Table S4. Primers used for dsRNA synthesis and dsRNAs obtained from *Eupheria*

Target gene	<i>Tribolium</i> accession number		Primer sequence	dsRNA length
<i>TcDad1</i>	TC016366	F	taatacgactcactatagggTGCCACAGGTGACTTCAGTT	367
		R	taatacgactcactatagggATGTAGGACCACATGGGCG	
<i>TcGCS1</i>	TC011354	F	taatacgactcactatagggCAACAACCAATCGTGAGCTG	374
		R	taatacgactcactatagggGACCAATCGGTATTCGGGAT	
<i>Tca-Man-Ia</i>	TC011089	F	taatacgactcactatagggCTTGGGGCAAGAACGAACT	381
		R	taatacgactcactatagggCCAGTCTGTGTCTGAAACGC	
<i>Tca-Man-Ib</i>	TC014283	F	taatacgactcactatagggCTAGTCTCGACCGAATCCGA	385
		R	taatacgactcactatagggTGTCAATCGAGTCAACAATGC	
<i>Tca-Man-IIa</i>	TC009186	F	taatacgactcactatagggTGTCCATGGAAAGTGCCC	365
		R	taatacgactcactatagggAAAAATCGCCCGATAACGA	
<i>Tca-Man-IIb</i>	TC002991	F	taatacgactcactatagggGGCCATAGGCCTCACAAATA	423
		R	taatacgactcactatagggTTCATTATCTGGCGGGAGAC	
<i>TcFucTA/C</i>	TC005825	F	taatacgactcactatagggAATCGAAATTTTCAGCGTCCG	435
		R	taatacgactcactatagggCACAAATTCGAGCCCATGAT	
<i>TcFucTA</i>	TC014343	F	taatacgactcactatagggATGCCTCCGCGACTCTCT	378
		R	taatacgactcactatagggGGCAAGAATCACTTTCTCCG	
<i>Tcβ4GalNAcTA</i>	TC006388	F	taatacgactcactatagggACGCTCCATCTCAAGAAAGC	500
		R	taatacgactcactatagggTCTTCCGCAACAAATCAAT	
<i>TcST6Gal</i>	TC014265	F	taatacgactcactatagggACGCTCATGATTTGGTTTTG	374
		R	taatacgactcactatagggCAAAAACCCCGACGATAGTG	
<i>TcCsas</i>	TC015995	F	taatacgactcactatagggTCTTTTTTCATGCAAATCCAGC	395
		R	taatacgactcactatagggACGTACTGCACCAAAGCA	
<b>Eupheria catalog number</b>				
<i>TcStt3A</i>	TC010433		iB_01689	479
<i>TcStt3B</i>	TC009183		iB_01492	497
<i>TcGCS2a</i>	TC032148		iB_00458	501
<i>TcMgat1</i>	TC009001		iB_01454	489
<i>Tcfdl</i>	TC009779		iB_04695	490
<i>TcFucT6</i>	TC008521		iB_04509	481
<i>TcMgat2</i>	TC001867		iB_03261	482
<i>TcMgat4</i>	TC003870		iB_00631	483
<i>Tcβ4GalNAcTB</i>	TC006987		iB_06724	391

taatacgactcactataggg – is a T7 promoter sequence. F- forward, R - reverse.

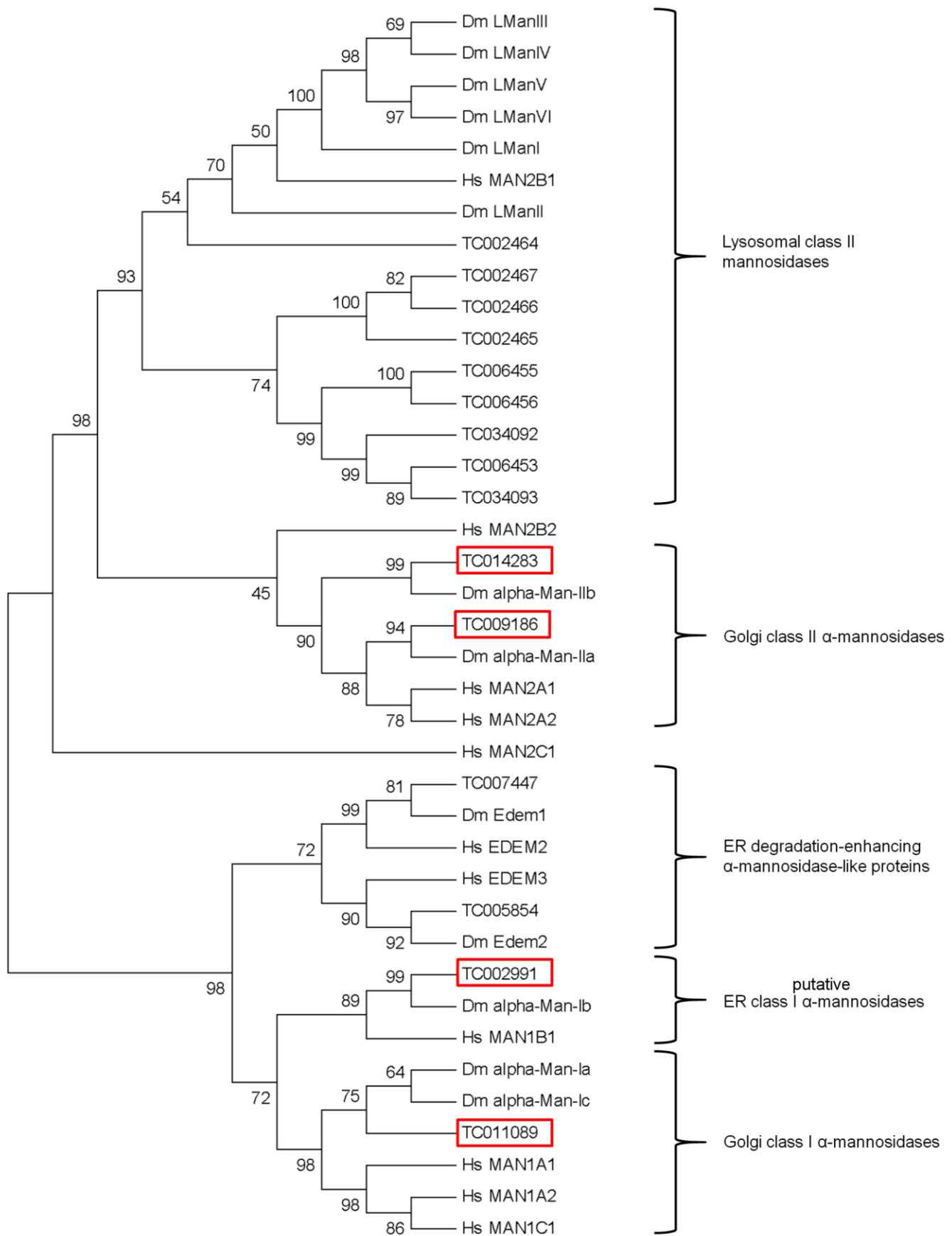
**Supplementary Figure 1. Phylogeny of proteins putatively involved in N-glycan attachment and processing**



**Fig. S1. Phylogeny of proteins putatively involved in N-glycan attachment and processing in *Tribolium castaneum*.** The evolutionary history was inferred by using the Maximum Likelihood method based on the Poisson correction model. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Hs - *homo sapiens*, Dm -

*Drosophila melanogaster*, TC0##### are putative *Tribolium castaneum* orthologs obtained from <http://bioinf.uni-greifswald.de/gb2/gbrowse/tcas4>. *Tribolium* orthologs studied in this paper are in brackets. **(a)** DAD1 orthologs. RefSeq accessions: Hs DAD1 - NP 001335.1; Dm lethal (2) k12914 - NP 609222.1; Dm SP2353 - NP 611082.2; Hs NAALDL2 - XP 011510919.1. The latter two sequences are remotely related and are used as outgroups to improve the quality of tree. **(b)** Orthologs of STT3 subunits. RefSeq accessions: Hs STT3A - NP 689926.1, NP 001265433.1; Hs STT3B - NP 849193.1; Dm CG1518 - NP 608425.1; Dm OstStt3 - NP 524494.1. **(c)** Orthologs of  $\alpha$ -glucosidases. RefSeq accessions: Hs MOGS/GCS1 - NP\_001139630.1; Hs GANAB - NP\_001265121.1; Dm GCS1 - NP\_001245621.1; Dm GCS2 $\alpha$  - NP\_652145.1. **(d)** Orthologs of GlcNAc transferases, RefSeq accessions: Hs MGAT1 - NP 002397.2; Hs MGAT2 - NP 002399.1; Hs MGAT4A - NP 036346.1; Hs MGAT4B - NP 055090.1; Hs MGAT4C - NP 037376.2; Hs MGAT4D - NP 001264282.1; Dm Mgat1 - NP 525117.2; Dm Mgat2 - NP 001263096.1; Dm CG9384 - NP 648720.3; Dm CG17173 - NP 648721.1; Dm CG7766 - NP 001138180.1. **(e)** Orthologs of N-acetylglucosaminidases, RefSeq accessions: Hs Hexo1 $\alpha$  - NP 000511.1; Hs Hexo1 $\beta$  - NP 000512.1; Dm Hexo1 - NP 728975.1; Dm Hexo2 - NP 525081.1; Dm fdl - NP 001286351.1. **(f)** Orthologs of CMP-sialic acid synthase, RefSeq accessions: Hs CMAS - NP 061156.1; Dm Csas - ACH92492.1; Dm Gip - NP 511106.1 (distantly related sequence used as an outgroup for tree construction). **(g)** Orthologs of Gal/GalNAc transferases RefSeq accessions: Hs beta4GalT-2 isoform b - NP 003771.1; Hs beta4GalT-2 - NP 085076.2; Hs beta4GalT-1 - NP 001488.2; Hs beta4GalT-3 - NP 003770.1; Hs beta4GalT-4 - NP 003769.1; Hs beta4GalT-6 - NP 004766.2; Hs beta4GalT-5 - NP 004767.1; Hs beta4GalT7 - NP 651319.2; Dm beta4GalNAcTA - NP 610946.1; Dm beta4GalNAcTB - NP 651657.1; Dm beta4GalT7 - NP 009186.1; Dm Pgant4 - NP 001137779.1; Dm Pgant6 - NP 647749.2; Dm GalNAc-T1 - NP 611043.1; Dm GalNAc-T1 isoform C - NP 001286461.1. **(h)** Orthologs of sialic acid transferases, RefSeq accessions: Hs ST6Gal-II - NP 115917.1; Hs ST6Gal-II isoform b - NP 001135824.1; Hs ST6Gal-I - NP 003023.1; Hs ST6Gal-I isoform b - NP 775324.1; Hs ST8SIA4 - NP 005659.1; Hs ST8SIA2 - NP 006002.1; DmSt6Gal - NP 726474.1; DmSt6Gal isoform b - NP 523853.1. **(i)** Orthologs of Fucosyltransferases, RefSeq accessions: Hs FUT3 - NP 000140.1; Hs FUT5 - NP 002025.2; Hs FUT6 - NP 000141.1; Hs FUT4 - NP 002024.1; Hs FUT7 - NP 004470.1; Hs FUT9 - NP 006572.2; Hs FUT11 - NP 775811.2; Hs FUT10 - ref|NP 116053.3; Hs FUT8 - NP 835368.1; Dm FucTA - NP 648754.2; Dm FucTC - NP 001036320.3; Dm FucTD - NP 612107.1; Dm FucTB - |NP 609288.4; Dm FucT6 - NP 572740.1.

**Supplementary Figure 2. Phylogeny of putative *Tribolium*  $\alpha$ -mannosidases**

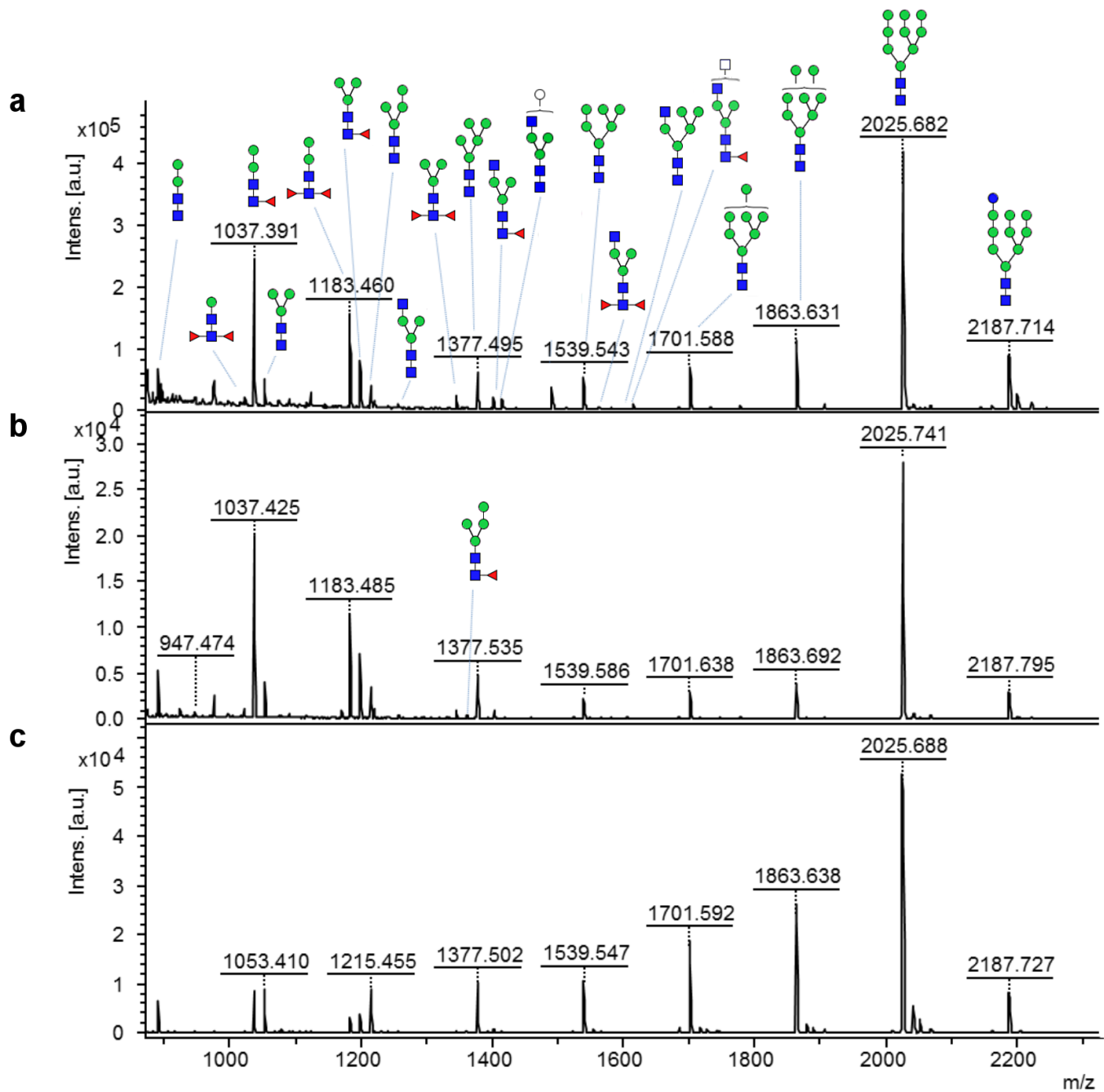


**Fig. S2. Phylogeny of putative *Tribolium*  $\alpha$ -mannosidases.** The evolutionary history was inferred by using the Maximum Likelihood method based on the Poisson correction model. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Hs - *homo sapiens*, Dm - *Drosophila melanogaster*, TC0##### are putative *Tribolium castaneum*



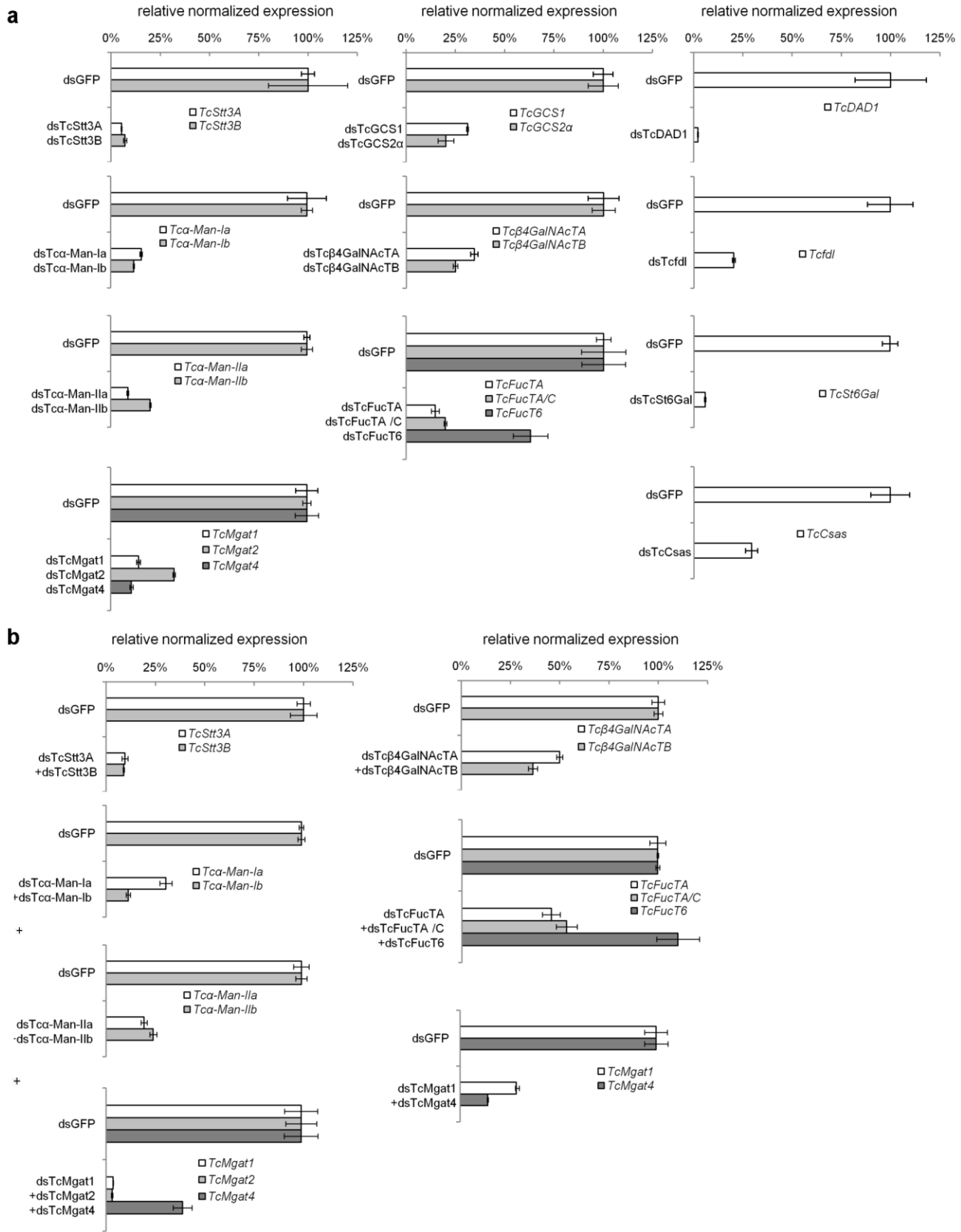
orthologs obtained from <http://bioinf.uni-greifswald.de/gb2/gbrowse/tcas4>. Tribolium orthologs studied in this paper are in brackets. RefSeq accessions: Hs MAN2B1 - NP 000519.2; Hs MAN2B2 - AAH94773.1; Hs MAN2A1 - NP 002363.2; Hs MAN2A2 - NP 006113.2; Hs MAN2C1 - NP 006706.2; Hs MAN1B1 - NP 057303.2; Hs MAN1A1 - NP 065112.1; Hs MAN1A2 - NP 006690.1; Hs MAN1C1 - NP 005898.2; Hs EDEM2 - NP 060687.2; Hs EDEM3 - XP 005245556.1; Dm LManIII NP 609250.2; Dm LManIV - NP 609251.2; Dm LManV - NP 609252.1; Dm LManVI - NP 609253.1; Dm LManI - NP 609407.1; Dm LManII - NP 609408.1; Dm  $\alpha$ -Man-IIb - NP 650494.2; Dm  $\alpha$ -Man-IIa - NP 524291.2; Dm Edem1 - NP 001259152.1; Dm Edem2 - NP 609611.1; Dm  $\alpha$ -Man-Ia - NP 727408.2; Dm  $\alpha$ -Man-Ib - NP 651667.1; Dm  $\alpha$ -Man-Ic - NP\_733331.1.

Supplementary Figure 3. Examples of MALDI-MS spectra of *Tribolium* N-glycans.



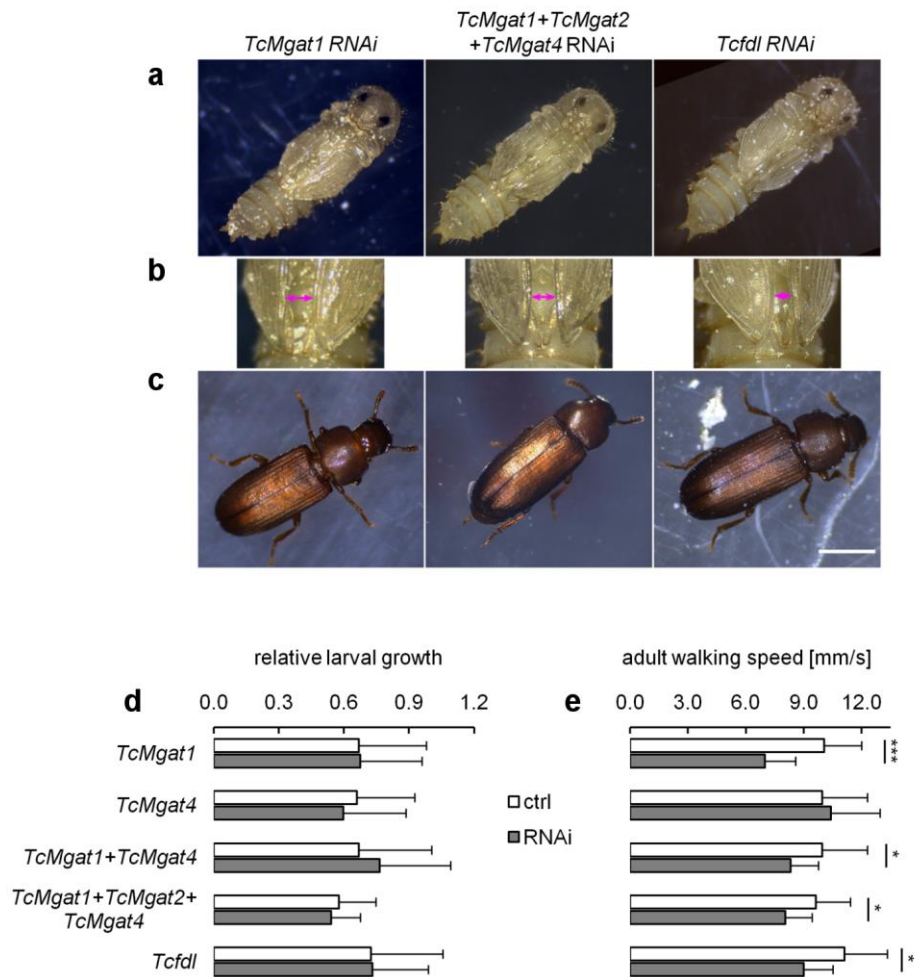
**Fig. S3. Examples of MALDI-MS spectra of *Tribolium* N-glycans.** Proteins were extracted from whole 4th instar larvae or mature adults. N-glycans were released using PNGaseA, labeled with 2-AB and analyzed by MALDI-MS using DHB matrix. **(a)** larval N-glycans. **(b)** adult N-glycans. **(c)** *Tca-Man-1a* RNAi adult N-glycans. Source files for the representative spectra are included in the Supplementary Files online.

**Supplementary Figure 4. Efficiency of RNAi mediated gene knockdown.**



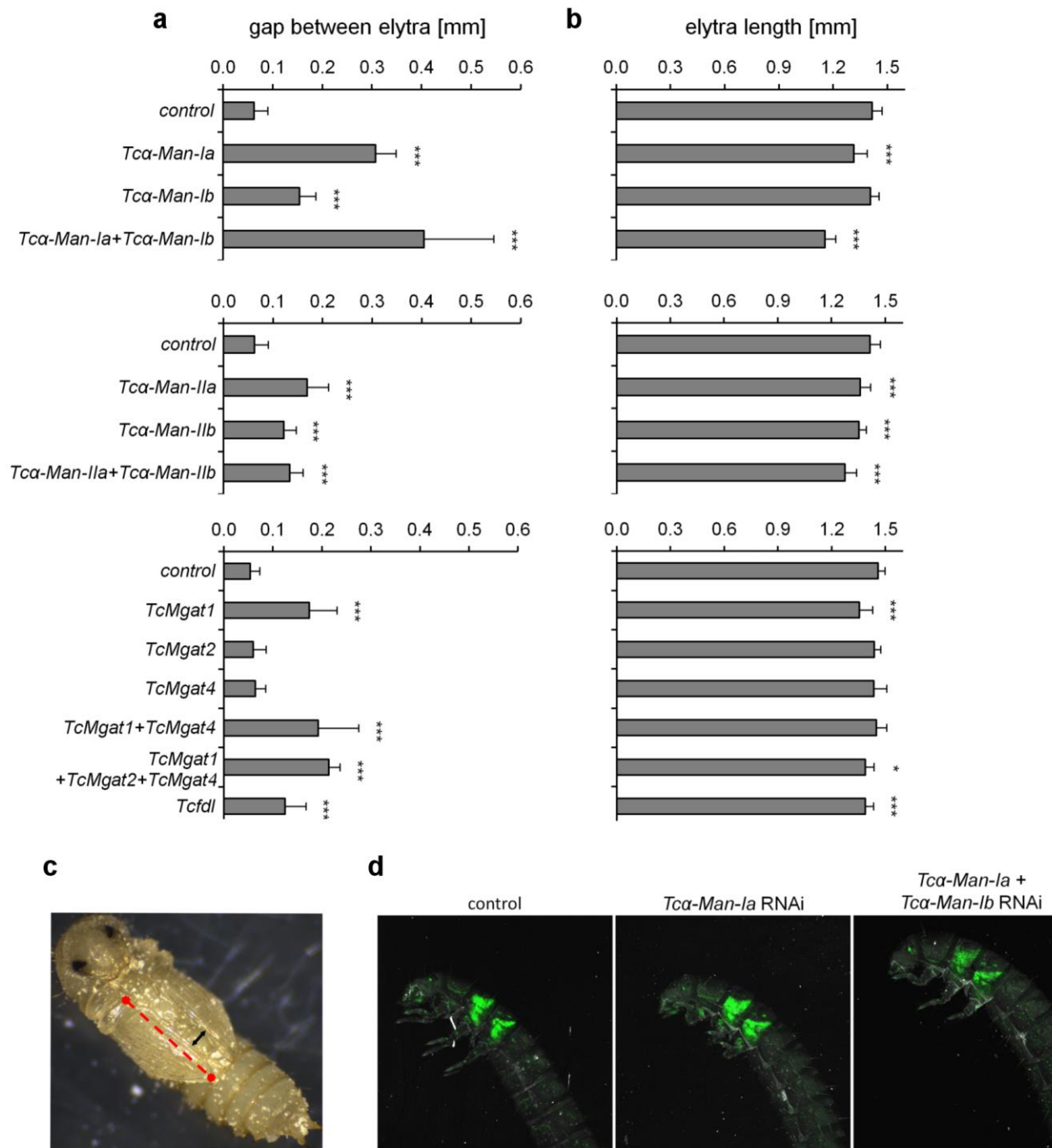
**Fig. S4. Efficiency of RNAi mediated gene knockdown.** 4<sup>th</sup> instar larvae were injected with appropriate dsRNAs to induce gene silencing or with dsGFP in case of control. Total RNA was isolated 6 days post injection. Relative gene expression was quantified with qRT-PCR using *TcRpL32* and *TcRpS6* as reference genes and normalized to control. Data are averages  $\pm$  SD (n=3). (a) Single knockdown experiments. (b) Multiple knockdown experiments performed by injection of mixture of indicated dsRNA's.

Supplementary Figure 5. RNAi of genes involved in GlcNAc attachment and removal cause minor developmental defects.



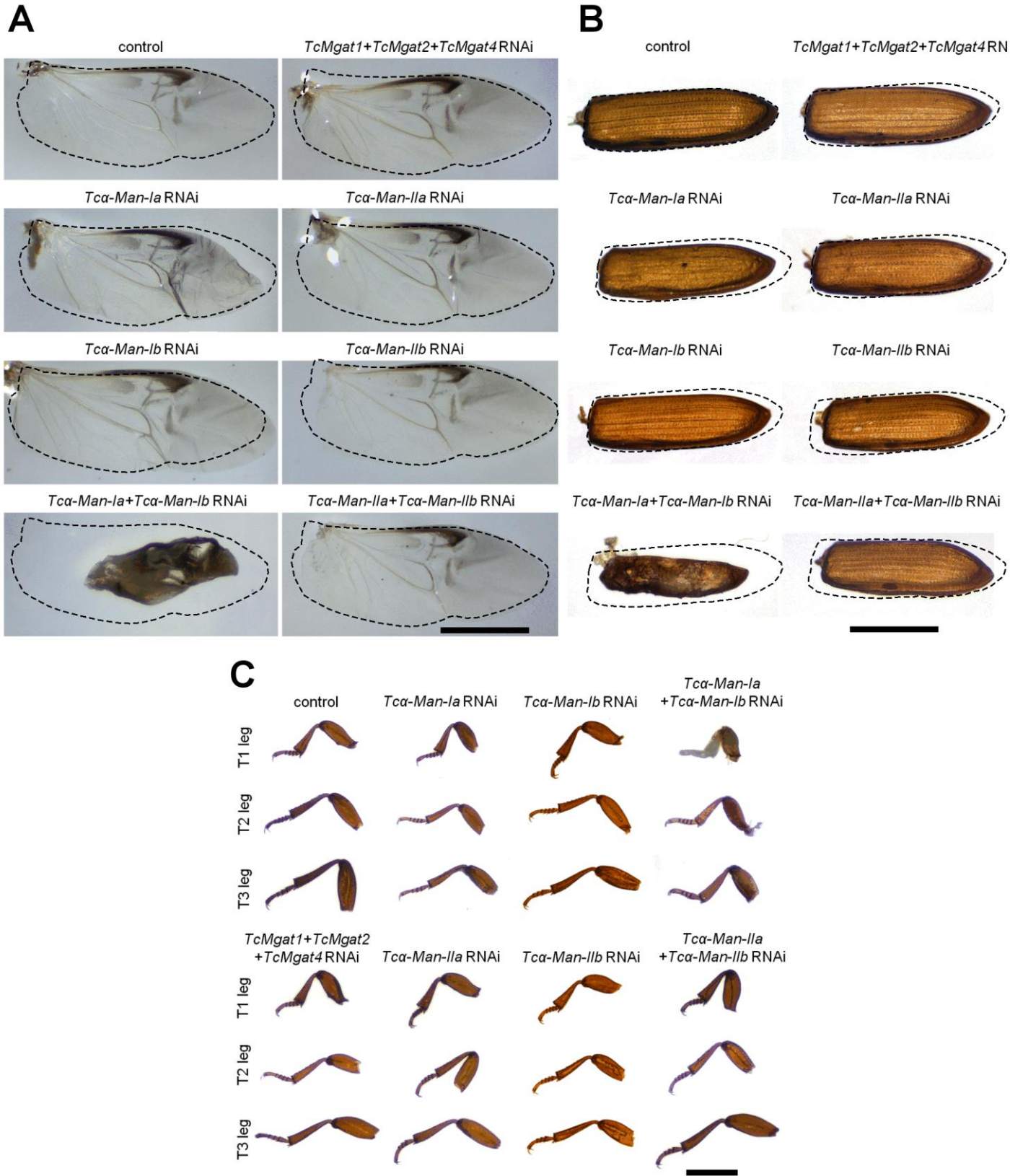
**Fig. S5. RNAi of genes involved in GlcNAc attachment and removal cause minor developmental defects.** 4<sup>th</sup> instar larvae were injected with appropriate dsRNAs to induce gene silencing or with dsGFP in case of control. (a) and (b) RNAi of *TcMgat1* and *Tcfdl* caused minor disruption of pupal elytra but not of (c) adult morphology and (d) larval growth (n=57, 57, 58, 58, 69). (e) Silencing of *TcMgat1* and *Tcfdl* reduced adult mobility (n=20, 12, 12, 12, 12). Double RNAi of *TcMgat1* and *TcMgat4* as well as triple RNAi of *TcMgat1*, *TcMgat2* and *TcMgat4* did not cause any increase in phenotype severity. Scale bar is 1 mm in (a) and (c) and 0.5 mm in (b). Error bars are SD, asterisks indicate statistically significant differences: \*\*\* P<0.001, \*\* P<0.01, \* P<0.05 (t-test).

**Supplementary Figure 6. RNAi of genes involved in N-glycan processing affect pupal elytra morphology.**



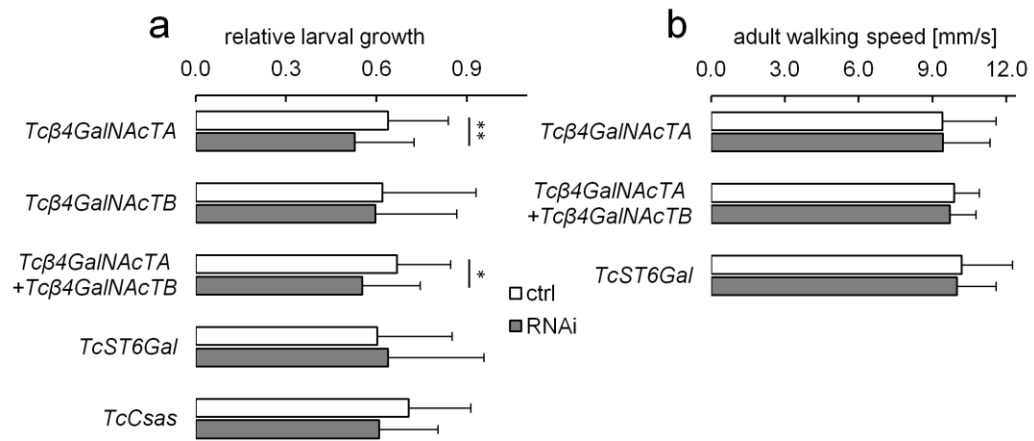
**Fig. S6. RNAi of genes involved in N-glycan processing affect pupal elytra morphology.** (a) Knockdown effects on elytra position. Elytra gap is enlarged in case of RNAi of each of the genes involved in N-glycan processing towards paucimannose glycans (all four  $\alpha$ -mannosidases, *TcMgat1* and *Tcfdl*) and not after RNAi of *TcMgat2* and *4* which are involved in complex glycan synthesis. (b) Effects of N-glycan processing genes RNAi on pupal wing length (n=11-17). (c) Scheme of elytra length (red dashed line) and gap (black double-headed arrow) measurement. To avoid errors caused by elytra curvature, the length was measured as distance from the joint between T1 tibia and tarsus to the tip of elytra. The gap between elytra was measured at 0.5 mm from the elytra tip. Error bars are SD, asterisks indicate statistically significant differences compared to control: \*\*\* P<0.001, \*\* P<0.01, \* P<0.05 (t-test). (d) RNAi of class I  $\alpha$ -mannosidases had no clear effect on development of wing and elytra wing discs in *pu11* strain. This strain expresses EYFP in wing and elytron primordia, eyes and neurons. Maximum projection confocal images are shown: green - EYFP, white - cuticle autofluorescence.

Supplementary Figure 7. RNAi of class I  $\alpha$ -mannosidases affect adult appendage development.



**Fig. S7. RNAi of class I  $\alpha$ -mannosidases affect adult appendage development.** (a) RNAi effects on adult wings, (b) adult elytra and (c) adult legs. Knockdown of *Tcα-Man-Ia* caused minor malformations: wrinkled and smaller wings and elytra. Double knockdown of *Tcα-Man-Ia* and *Tcα-Man-Ib* completely blocked wing and elytra development and interfered with proper leg sclerotization (especially visible on T1 legs). Other knockdowns caused no obvious effects. Dashed line indicate shape of wing and elytron of control beetles. Scale bars in (A) and (B) are 1 mm. Scale bar in (C) is 0.5 mm.

**Supplementary Figure 8. Silencing of genes potentially involved in complex glycan synthesis and sialylation caused minor or no effects.**



**Fig. S8. Silencing of genes potentially involved in complex glycan synthesis and sialylation caused minor or no effects. (a)** Only RNAi of *Tcβ4GalNAcTA* caused minor reduction of larval growth (n=49-63). **(b)** There was no effect on adult movement speed (n= 15-25). Error bars are SD, asterisks indicate P<0.001 (\*\*\*), P<0.01 (\*\*), P<0.05 (\*).