Supplemental Materials



Figure S1. Purification of native CEA. SDS PAGE (15%) profile of purified fractions of native CEA. Panel 1: Lane M: Standard protein molecular weight marker; Lane 1& 2: Purified CEA fraction; Lanes 3: CEA showing ~12 kDa band after western blot analysis with anti-CEA antibody.



Figure S2. Insect bioassay in CEA supplemented artificial diet on second instar nymphs of red cotton bug. Graph demonstrating percentage of insect survivability at different concentrations of CEA (0, 5, 10, 15, 20, 25 μ g/mL) recorded over 72 h.

		•	<u> </u>			
Target insect	LC50 Value (µg/mL)	Fiducial lim LC50 Value (μg Lower	it /mL) SE of slope Upper	Regression equation (Y)	χ^2 value	df
Dysdercus cingulatus (Red cotton bug)	15.64	14.77	16.58 0.268	2.08 + 2.440x	0.358	3
Γ	1	2	3	4		
			, , , ,			
	+ Ve	Midgut	Haemolymph	n Ovary		
		RCB fed	tured CEA			

Table S1. Toxicity assay of native CEA determined against *Dysdercus cingulatus*.

Figure S3. Western blotting with the midgut, haemolymph and ovary of *D. cingulatus* nymph fed with heat denatured CEA supplemented diet. Lane 1: CEA (0.5 μ g), positive control. Lane 2, 3 & 4: total protein extract from heat denatured CEA fed RCB ovary, haemolymph and midgut showing no CEA band at ~12 kDa.





Spot 3: Result obtained by PMF and MS/MS analysis . (MATRIX) Mascot Search Results **Protein View** Match to: Q16E72_AEDAE Score: 118 Expect: 2.3e-07 Cytochrome P450. - Aedes aegypti (Yellowfever mosquito). Nominal mass (Mr): 48360; Calculated pI value: 6.40 Taxonomy: Aedes aegypti Fixed modifications: Carbamidomethyl (C) Variable modifications: Oxidation (M) Cleavage by Trypsin: cuts C-term side of KR unless next residue is P Number of mass values searched: 16 Number of mass values matched: 10 Sequence Coverage: 23% Matched peptides shown in Bold Red Mascot Score Histogram 1 MQQVLCCNEL YDKPFLYDFF RLGNGILTER SGERWLQARK LVNPAFNTRM Protein scores greater than 64 are significant (p<0.05). 51 LTAFLPIMDS EAKNLCDKLE PLADGNTEID IFSHLSSCTL STTFGTTMGQ 101 NAKEIPEQHD YIRNVEIFLK AVGERLVNVY YFIEPIYKLS KAYKIHDEAR 151 RICNEFTHRI VSKRRFEIQS LGEDFQQKDE YIKQHLNALD QIITMKRPDG 201 TGFSDPEVNE HLYTLIGAGT DTSALTVAYT CLYLAMYPEV QEKVLTEINQ 251 VFYSPEVEVN IENLKQLEYT EMVIKEILRL FPAGPLGARQ TMSAIELDGI 301 RIPKDOIIIF SMFTLHRRKD IWGPDPEOFD PERFRPEAIE ARHPFAYLPF 351 SCGLRNCIGH RYAMNVMRII LLRIMQKFEI QTNMKPTDLK LKFEVTLKLD 100 125 Protein Score 401 GPHRVWLVRR NK Show predicted peptides also Sort Peptides By
 Residue Number
 Increasing Mass
 Decreasing Mass Start - End Observed Mr (expt) Mr (calc) ppm Miss Sequence I K.LVNPAFNTRMLTAFLPIMDSEAK.N Oxidation (M) 0 R.MLTAFLPIMDSEAK.N 2594.3236 41 - 63 2595.2430 2594.2357 -34 50 - 63 15 1566.8143 1565.8070 1565.7833 50 - 63 0 R.MLTAFLPIMDSEAK.N 2 Oxidation (M) 1598.7943 1597.7871 1597.7731 9 50 - 68 2213.1385 2212.1313 2212.0578 33 1 R.MLTAFLPIMDSEAKNLCDK.L Oxidation (M) 1373.6674 114 - 125 1374.6747 1373.7667 -72 1 R.NVEIFLKAVGER.L 152 - 159 1076.4974 1075.4901 1075.4869 3 0 R.ICNEFTHR.I 276 - 279530.3109 529.3037 529.3224 -35 0 K.EILR.L 305 - 317 0 K.DQIIIFSMFTLHR.R Oxidation (M) 1636.8240 1635.8167 1635.8443 -17 343 - 361 1 R.HPFAYLPFSGGLRNCIGHR.Y 2199.0897 2198.0824 2198.0956 -6 378 - 390 1564.7280 1563.7207 1563.7967 -49 0 K.FEIQTNMKPTDLK.L No match to: 842.5051, 1383.6950, 1490.7330, 1645.8514, 1994.0907, 2072.4496 MS/ MS matched peptides : Start - End Observed Mr (expt) Mr (calc) ppm Miss Sequence (Ions score 25) 152 - 1591076.4974 1075.4901 1075.4869 R. ICNEFTHR. I 7 0 114 - 125 1374.6747 1373.6674 1373.7667 -7 1 R.NVEIFLKAVGER.L (Ions score 42) 305 - 317 1636.8240 1635.8167 1635.8443 -13 0 K.DQIIIFSMFTLHR.R (Ions score 58) 378 - 390 1564.7280 1563.7207 1563.7967 -29 0 K.FEIQTNMKPTDLK.L (<u>Ions score 57</u>)





Spot 6: Result obtained after Pertide mass fingerprinting (PMF) (MATRIX) Mascot Search Results **Protein View** Match to: gi|170040984 Score: 106 Expect: 2.2e-05 26S proteasome non-ATPase regulatory subunit 1 [Culex guinguefasciatus] Nominal mass (M_): 112988; Calculated pI value: 5.50 Taxonomy: Culex guinguefasciatus Fixed modifications: Carbamidomethyl (C) Variable modifications: Oxidation (M) Cleavage by Trypsin: cuts C-term side of KR unless next residue is P Number of mass values searched: 14 Number of mass values matched: 10 Sequence Coverage: 14% Matched peptides shown in Bold Red 1 MKLNITSAAG IICLLDEPIQ ELKVFALKKL DTIVDEFWPE ISEAVEKIEI Mascot Score Histogram 51 LHEDKGFOOH DLAALVASKV YYHLGSFEDS LTYALGAGDL FDVNARNEYV 101 DIIIAKCIDH YTOLRVOLAE NPAKAKPIDA RLEAIVNRMI ORCLDDGOYR Ions score is -10*Log(P), where P is the probability that the observed match is a random event. 151 QALGIALETR RMDVVESSIM KADDIAGMLA YAFQVTMSLI QNRAFRNTVL Individual ions scores > 45 indicate identity or extensive homology (p<0.05). 201 RCLVGLYRNL GVPDYVNMCO CLIFLEDPLA VAELLDNLTK GGEHSVLMAY Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits. 251 QIAFDLYESA TQQFLGQVLQ ALKATAPIPS ALISNLKPQG TNAAGAAAAP 301 EVKQEPKEVG EESEGGDVKI ERTVDSLNES EKTHQANIEK LAGILSGEIT



Figure S4. (Spot 1) MALDI TOF/TOF identification of ATP synthase α subunit from brown citrus aphid. (Spot 2) MALDI TOF/TOF identification of mitochondrial inorganic phosphate carrier from silk moth. (Spot 3) MALDI TOF/TOF identification of Cyt P450 from Yellow fever mosquito. (Spot 4) MALDI TOF/TOF identification of RNA binding protein from Yellow fever mosquito. (Spot 5) MALDI TOF/TOF identification of actin from Yellow fever mosquito. (Spot 6) MALDI TOF/TOF identification of 26s proteasome non-ATPase regulatory subunit from *Culex* mosquito.



Figure S5. Immunoprecipitation and subsequent ligand blot analysis of Red cotton bug BBMV with CEA. Lane M: Standard protein molecular weight marker; Lane 1: ligand blotting with total BBMV immunoprecipitated with anti-ATP synthase antibody. Lane 2: ligand blotting with total BBMV immunoprecipitated with anti-actin antibody. Lane 3 & 4: immunoprecipitation without anti ATP synthase and anti actin antibody incubation (negative control).



Figure S6. Confocal microscopic images showing the partial inhibition of binding of CEA to the midgut epithelial cells of *D. cingulatus* due to feeding the artificial diet supplemented with anti-actin antibody. (A) Fluorescence image showing significantly reduced binding of CEA to midgut epithelial cells of insects fed with anti-actin supplemented diet; (B) Merged image; (C) Phase contrast image. [Bar = 25 µm]

NetNglyco 1.0 server prediction of N glycosylation:

ATP synthase

Sequence Name: tr Q5XUA1 Q5XUA1 TOXCI Length: 551

>tr|Q5XUA1|Q5XUA1 TOXCI ATP synthase subunit alpha OS=Toxoptera citricida PE=2 SV=1 MANLSLRLATKIGKQLNNTLPQISKASWTTSKLISRSLHITCSARAAEISSILEERILGA PPKADLEETGRVLSIGDGIARVYGLKNVQAEEMVEFSSGLKGMALNLEPDNVGVVVFGND KLIKEGDVVKRTGAIVDVPVGEDLLGRVVDALGNTIDGKGPLTSKLRYRVGIKAPGIIPR VSVREPMQTGIKAVDSLVPIGRGQRELIIGDRQTGKTALAIDTIINQKRFNDAQDEKKKL YCIYVAIGQKRSTVAQIVKRLTDTGAIKYTIIVSATASDAAPLQYLAPYSGCAMGEFFRD NGKHALIIYDDLSKQAVAYRQMSLLLRRPPGREAYPGDVFYLHSRLLERAAKMSETLGGG SLTALPVIETQAGDVSAYIPTNVISITDGQIFLETELFYKGVRPAINVGLSVSRVGSAAQ TKAMKQVAGSMKLELAQYREVAAFAQFGSDLDAATQQLLNRGVRLTELLKQGQYVPMAIE EQVAVVYCGVRGFLDKMEPSKITTFEKEFLQHIKTSEKSLLESIAKEGKITDETDAKLKS **VVTNFLASFNA**

(Threshold=0.5)

SeqName	Position	Potential	Jury	N-Glyc agreement	result
tr_Q5XUA1	_Q5XUA	1_TOXCI	3 NLSI	. 0.7272	(9/9) ++
tr_Q5XUA1	_Q5XUA	1_TOXCI	17 NNT	L 0.7161	(9/9) ++



NetNGlyc 1.0: predicted N-glycosylation sites in tr-Q5XUA1-Q5XUA1-T0XCI

Figure S7. Cont.

NetCGlyc 1.0 produces neural network predictions of C-mannosylation sites

ATP synthase

YinOYang 1.2 Prediction Results: The predictions for O-GlcNAc sites

ATP synthase

SeqName	Res res	idue ult	0-	-Glel	NAc Poten (1) (2)	itial Thr	esh. Thresh
tr_Q5XUA	1_Q	24	S	++	0.5384	0.4258	0.5243
tr_Q5XUA	1_Q	30	Т	+	0.5384	0.4514	0.5589
tr_Q5XUA	1_Q	31	S	+	0.5018	0.4657	0.5781
tr_Q5XUA	1_Q	253	Т	+	0.4742	0.4416	0.5457
tr_Q5XUA	1_Q	270	Т	+	0.6203	0.5213	0.6531
tr_Q5XUA	$1\bar{Q}$	274	S	+	0.5187	0.5099	0.6377
tr Q5XUA	1 Q	354	S	+	0.4868	0.4398	0.5432
tr Q5XUA	$1\bar{Q}$	370	Т	+	0.5066	0.4591	0.5692
tr Q5XUA	1 Q	381	Т	+	0.5280	0.4985	0.6224
tr Q5XUA	1° Q	413	S	+	0.5276	0.4878	0.6079
tr_Q5XUA	1_Q	421	Т	+	0.5316	0.4408	0.5445





Figure S7. Cont.

NetNglyco 1.0 server prediction of N glycosylation:

ACTIN

>tr|Q16VS2|Q16VS2_AEDAE AAEL009451-PA OS=Aedes aegypti GN=AAEL009451 PE=3 SV=1 MCDDDQGALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDAYVGDE AQ

SKRGILTLKYPIEHGIITNWDDMEKIWHHTFYNELRVAPEEHPILLTEAPLNPKSNREKM TQIMFETFAAPAAYVAIQAVLSLYASGRTTGVVLDSGDGVSHTVPIYEGYALPHAILRMD LAGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMQAAAASSSSEKS YELPDGQVITIGNERFRAPEALFQPSFLGMEATGVHETVYNSIMRCDVDIRKDLYANSVL SGGTTMYPGIADRMQKEITSLAPSTIKIKIIAPPERKYSVWIGGSILASLSTFQTMWISK HEYDEGGPGIVHRKCF

(Threshold=0.5)

SeqName	Position	Potential	Jury	N-Glyc agreement result

tr_Q16VS2_Q16VS2_AEDAE 13 NGSG 0.6679 (9/9) ++



NetNGlyc 1.0: predicted N-glycosylation sites in tr-Q16VS2-Q16VS2-AEDAE

NetCGlyc 1.0 produces neural network predictions of C-mannosylation sites

Actin

##gff-version 2 ##source-version netCglyc-1.0b ##date 2014-10-02 ##Type Protein # seqname feature start end score +/- ? source # -----tr Q16VS2 Q16VS2 AEDAE netCglyc-1.0b 80 80 0.173 . . C-manno tr Q16VS2 Q16VS2 AEDAE netCglyc-1.0b 87 0.235 . . C-manno 87 C-manno tr Q16VS2 Q16VS2 AEDAE netCglyc-1.0b 341 341 0.224 . . tr Q16VS2 Q16VS2 AEDAE netCglyc-1.0b 357 357 0.154 . . C-manno # _____ _____

Figure S7. Cont.

YinOYang 1.2 Prediction Results: The predictions for O-GlcNAc sites

Actin

$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	SeqName	Residue result	O-GlcNA (1	Ac Potent	ial Thres	h. Thresh.
	tr_Q16VS2_ tr_Q16VS2_ tr_Q16VS2_ tr_Q16VS2_ tr_Q16VS2_ tr_Q16VS2_	Q 234 Q 235 Q 236 Q 320 Q 325	S ++ S +++ S +++ S + T +	0.5698 0.5950 0.5557 0.5354 0.4977	0.4073 0.3888 0.3712 0.4416 0.4495	$\begin{array}{c} 0.4994 \\ 0.4745 \\ 0.4507 \\ 0.5456 \\ 0.5562 \end{array}$



YinOYang 1.2: predicted O-(beta)-GlcNAc sites in tr-Q16VS2-Q

Cytocrome P450

>tr|Q16E72|Q16E72 AEDAE AAEL015361-PA OS=Aedes aegypti GN=AAEL015361 PE=3 SV=1 MQQVLCCNELYDKPFLYDFFRLGNGILTERSGERWLQARKLVNPAFNTRMLTAFLPIMDS EAKNLCDKLEPLADGNTEIDIFSHLSSCTLSTTFGTTMGQNAKEIPEQHDYIRNVEIFLK AVGERLVNVYYFIEPIYKLSKAYKIHDEARRICNEFTHRIVSKRRFEIQSLGEDFQQKDE YIKQHLNALDQIITMKRPDGTGFSDPEVNEHLYTLIGAGTDTSALTVAYTCLYLAMYPEV QEKVLTEINQVFYSPEVEVNIENLKQLEYTEMVIKEILRLFPAGPLGARQTMSAIELDGI RIPKDQIIIFSMFTLHRRKDIWGPDPEQFDPERFRPEAIEARHPFAYLPFSGGLRNCIGH RYAMNVMRIILLRIMQKFEIQTNMKPTDLKLKFEVTLKLDGPHRVWLVRRNK

[Note: CytP450 does not have any N-glycosylation site according to the prediction of NetNglyco 1.0 server]

NetCGlyc 1.0 produces neural network predictions of C-mannosylation sites

Cytocrome P450

##gff-version 2 ##source-version netCglyc-1.0b ##date 2014-10-02 ##Type Protein # seqname feature start end score +/-? source # -----tr Q16E72 Q16E72 AEDAE netCglyc-1.0b C-manno 35 35 0.166 . . tr Q16E72 Q16E72 AEDAE netCglyc-1.0b C-manno 322 322 0.179 . . tr Q16E72 Q16E72 AEDAE netCglyc-1.0b 406 C-manno 406 0.207 . . # -----

YinOYang 1.2 Prediction Results: The predictions for O-GlcNAc sites

Cytocrome P450

 SeqName
 Residue
 O-GlcNAc
 Potential
 Thresh.

 result
 (1)
 (2)

 tr_Q16E72_Q
 222
 T
 +
 0.5128
 0.4363
 0.5385



Figure S7. Glycosylation and mannosylation site prediction of putative RCB interactive partners.



Figure S8. Insect bioassay in mannose presaturated CEA supplemented artificial diet on second instar nymphs of red cotton bug. Insects fed with or without mannose presaturated CEA ($25 \mu g/mL$) supplemented artificial diet. Insect survivability graph recorded over 72 h showing the improved survivability percentage (approximately double) when fed with CEA, presaturated with 1 M Mannose. Only diet and water served as positive and negative control, respectively.

References

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- 2. Gupta, R.; Brunak, S. Prediction of glycosylation across the human proteome and the correlation to protein function. *Pac. Symp. Biocomput.* **2002**, *7*, 310–312.
- 3. Julenius, K. NetCglyc 1.0: Prediction of mammalian C-mannosylation sites. *Glycobiology* **2007**, *17*, 868–876.

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