

Figure S1. Validation of the RNAi-mediated knockdown efficiency by quantitative real-time PCR (qPCR). Relative abundance of mRNA levels for Nasrat, Closca, Polehole, and Nudel was analyzed in dissected mosquito ovaries at 36 h PBM. Mosquitoes were microinjected with each dsRNA at four days prior to blood feeding, as shown in Fig. 1. dsRNA-Fluc-injected mosquitoes were used as controls. A single mosquito analysis was performed to isolate total RNA, synthesize cDNA, and monitor silencing efficiency by qPCR. mRNA levels were normalized according to transcript levels of ribosomal S7 protein. Data are presented as MEAN ± SEM of 12 individual mosquitoes. *** $P < 0.001$ compared to RNAi-Fluc. Vectorbase ID: Nasrat (AAEL008829), Closca (AAEL000961), Polehole (AAEL022628), and Nudel (AAEL016971). qPCR Primers used are shown in [SI Appendix, Table S4](#).

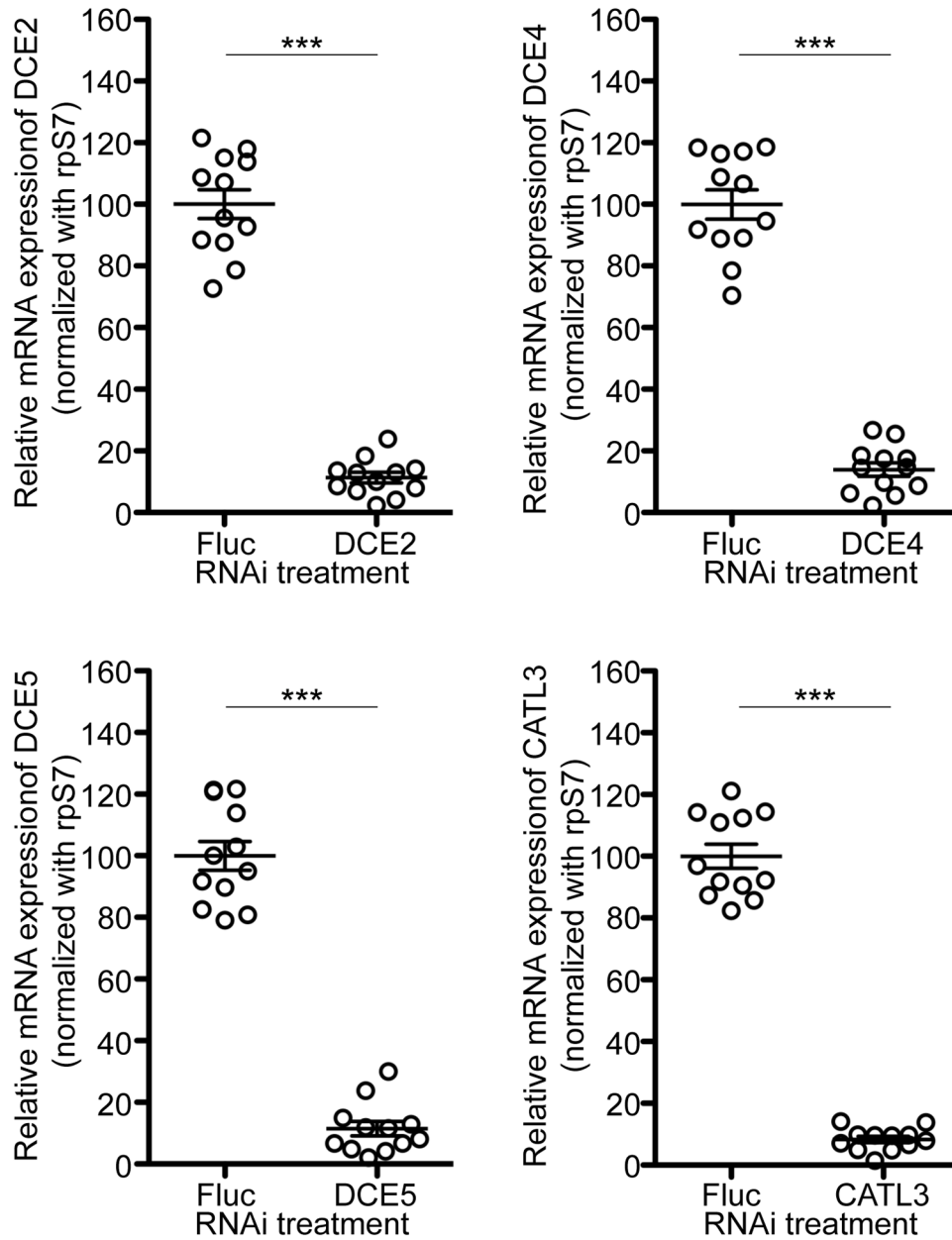


Figure S2. Validation of the RNAi-mediated knockdown efficiency by quantitative real-time PCR (qPCR). Relative abundance of mRNA levels for DCE2, DCE4, DCE5, and CATL3 was analyzed in dissected mosquito ovaries at 36 h PBM. Mosquitoes were microinjected with each dsRNA at four days prior to blood feeding, as shown in Fig. 1. dsRNA-Fluc-injected mosquitoes were used as controls. A single mosquito analysis was performed to isolate total RNA, synthesize cDNA, and monitor silencing efficiency by qPCR. mRNA levels were normalized according to transcript levels of ribosomal S7 protein. Data are presented as MEAN \pm SEM of 12 individual mosquitoes. *** $P < 0.001$ compared to RNAi-Fluc. Vectorbase ID: DCE2 (AAEL006830), DCE4 (AAEL007096), DCE5 (AAEL010848), and CATL3 (AAEL002196). qPCR Primers used are shown in [SI Appendix, Table S4](#).

Table S1. RNAi screening of *Aedes aegypti* eggshell proteins.

Vectorbase ID	GenBank ID	Putative functions	<i>RNAi phenotypic effects</i>	
			Eggs	Hatching
AAEL000361	EAT48607	Trypsin inhibitor-like/serpin	NO	NO
AAEL000363	EAT48611	Trypsin inhibitor-like/serpin	NO	NO
AAEL000375	EAT48605	Trypsin inhibitor-like/serpin	NO	NO
AAEL000507	EAT48446	Chorion peroxidase, CP4	NO	NO
AAEL000961	EAT47957	Closca	YES	YES
AAEL002196	EAT46597	Cysteine proteinase L-like, CATL3	YES	YES
AAEL002382	EAT46452	Unknown	NO	NO
AAEL003110	EAT45649	Chitinase domain	NO	NO
AAEL004202	EAT44412	Unknown	NO	NO
AAEL004386	EAT44219	Chorion peroxidase, CP1	NO	NO
AAEL004390	EAT44218	Chorion peroxidase, CP2	NO	NO
AAEL004401	EAT44216	Chorion peroxidase, CP7	NO	NO
AAEL005098	EAT43477	Trypsin inhibitor-like/serpin	NO	NO
AAEL005648	EAT42848	Clip-domain serine protease	NO	NO
AAEL005861	EAT42645	Vacuolar sorting protein	NO	NO
AAEL006830	EAT41553	Dopachrome converting enzyme, DCE2	YES	YES
AAEL006985	EAT41324	Dopachrome converting enzyme, DCE3	NO	NO
AAEL007096	EAT41240	Dopachrome converting enzyme, DCE4	YES	NO
AAEL007415	EAT40867	Laccase-like multicopper oxidases	NO	NO
AAEL007641	EAT40646	Transglutaminase	NO	NO
AAEL008829	EAT39370	Nasrat	YES	YES
AAEL009290	EAT38853	Unknown	NO	NO
AAEL009452	EAT38674	Unknown	NO	NO
AAEL009746	EAT38349	Unknown	NO	NO
AAEL010544	EAT37465	Unknown	NO	NO
AAEL010848	EAT37145	Dopachrome converting enzyme, DCE5	YES	NO
AAEL011238	EAT36701	Trypsin inhibitor-like/serpin	NO	NO
AAEL012586	EAT35235	Unknown	NO	NO
AAEL013027	EAT34764	Vitelline membrane protein, 15a1	NO	NO
AAEL013936	EAT33799	Trypsin inhibitor-like/serpin	NO	NO
AAEL014561	EAT33176	Vitelline membrane protein, 15a3	NO	NO
AAEL015203	EAT32616	Unknown	NO	NO
AAEL017403	EJY58008	Vitelline membrane protein, 15a2	NO	NO
AAEL017467	EJY57339	Chorion peroxidase, CP6	NO	NO

Table S2. Primers used for RNAi screening.

Vectorbase ID	Gene-specific RNAi primers (5' - 3')	Vectorbase ID	Gene-specific RNAi primers (5' - 3')
AAEL000361	Forward GTAGCGATTGTTGTTCTAGCG Reverse GCGTAAGTAACTTGCACACGG	AAEL007415	Forward TCAGTGCCGACCAGCAAGT Reverse CTGAGATTGTCTTGTGGACTTC
AAEL000363	Forward CCCTGTGCCGACCCAAACGA Reverse CGGTGTGGTCGTAGTAATACA	AAEL007641	Forward CGCTCGCAACGTGCATTGG Reverse GGACACCACGCACGCTGCAA
AAEL000375	Forward ATGCAGCTTCCAATATGTGCTAT Reverse GCGGCTTCGGCGTAGGCTT	AAEL008829	Forward GAGCCCATTGAGAACCTCCT Reverse AGCGTAACTCCGTTGACGTA
AAEL000507	Forward TACAGCTCTGCGTGCATCTG Reverse CCACAATCGGTCTTCGTGTCAG	AAEL009290	Forward ATCGAGGGATTGATGGAAGG Reverse CCGTCCGAGTAGTGGATCGC
AAEL000961	Forward GGCAAGGGCTTCTACAACGT Reverse CCGTTCAAAGTATGCTCCAC	AAEL009452	Forward GTCCTCCATCTCTTTGGTGA Reverse TCACCAACCAGCTCTTCTCG
AAEL002196	Forward TGAAGAAACAACCTGCTGTGG Reverse CCATCTGCTGGGTACTGAC	AAEL009746	Forward CAGCCTACATCGTTGACCTA Reverse TGTCGAAGCACAACCGATGGTT
AAEL002382	Forward GCCCATGTGAACTTCCCTTGCC Reverse GATACCTCGCCCTGTTGAAC	AAEL010544	Forward CAGCGGGATCAGAACCAGGAT Reverse CATAAGATCCGTCAGACCGTC
AAEL003110	Forward TCCAACCAGGAGTGCAGTGA Reverse CGCCAATTCCACCGAGTTG	AAEL010848	Forward GCCCTCGACTCAGGCATTTG Reverse TGCGTGCTCAAGCGACACTC
AAEL004202	Forward TCCTACGGCGAAGCTGGTTC Reverse GACTCTCGTTTGTCTGCTTC	AAEL011238	Forward CAACCAGTTGATGGCAGGATAC Reverse CCGTTGTGCTTCACATAACC
AAEL004386	Forward TGAGGGAACACAACCGACTA Reverse TAAACCTGTGCCAAGAGTGC	AAEL012586	Forward GCCGACAGGGACCGATGATG Reverse GCCGAAATGTTGATCTTGTGTAC
AAEL004390	Forward TGAGGGAACACAACCGACTA Reverse TAAACTCGCGCCAGAAGAGC	AAEL013027	Forward TTCCCATCCAACCTCAGTAACCAT Reverse TTCCGCTGCATCTTCAAGAG
AAEL004401	Forward CCACACTGGTCTGACGACAT Reverse CGCCTACGTAAAGATCGACGT	AAEL013936	Forward TTAGCAATAGTTTCTCACTGCCA Reverse GCCTGTGGGCTTCGATTGG
AAEL005098	Forward ATGAAGTTGGCAATCATTTGTGT Reverse GCTCCAGGACAATCGCACAG	AAEL014561	Forward CGGAAGGAATCCATCCAACCTT Reverse CAGTCCAATCGATGATCCGC
AAEL005648	Forward GCCAAAGCCGATAGCCATC Reverse CTAGGCATGTTGAGAGCACC	AAEL015203	Forward GTGTTGGTGCCGAAGAAGAG Reverse TAGCACTTCAACTCGGATGACTT
AAEL005861	Forward TGTGATGGCGATGACGACTG Reverse CTCATCACTTCCATCCTTGCA	AAEL017403	Forward CCAGCGTGGTACAACAGTAAATC Reverse CCGTTCCTTGGTCTCGGTTTC
AAEL006830	Forward TGTGGAAATCGTCGGTGGT Reverse TGTAGGCGAAGGTGTCTC	AAEL017467	Forward TACAGCTCTGCGTGCATCTG Reverse CCACAATCGGTCTTCGTCAG
AAEL006985	Forward CTCCCGGTTGGAATCGAAAAG Reverse GTCCGTAGTCCAGTTCATTGG	AAEL012336	Forward AGCCCGTCCAAGAGGAAGTT (EOF1) Reverse CTCGGATGGTACTCACACAA
AAEL007096	Forward GCAAGAAGTGCGACAAGAC Reverse CGTCCACCCAGATAGGTGAA	U47295	Forward AGCACTCTGATTGACAAATACGA (Luciferase) Reverse AGTTCACCGGCGTCATCGTC

T7 promoter sequence (5' TAATACGACTCACTATAGGGAGA 3') was added in 5' of each RNAi primer.

Table S3. Reproductive phenotypes associated with RNAi in *Aedes aegypti*.

	RNAi	Fluc	Nasrat	Closca	Polehole	Nudel
<i>Fecundity</i>						
Number of mosquitoes examined		24	26	25	27	24
Total number of eggs oviposited		2273	2128	2073	2359	1955
Mean number of eggs oviposited		94.7	81.8	82.9	87.4	81.5
<i>Eggshell melanization</i>						
Number of eggs examined		2273	2128	2073	2359	1955
Incompletely tanned eggs oviposited		23	1520	1749	1955	1938
Incomplete eggshell melanization (%)		1.01%	79.89%	84.37%	82.87%	99.13%
<i>Egg viability</i>						
Number of eggs examined		666	2128	2073	2359	1955
Number of eggs hatched		626	231	184	226	5
Egg viability (%)		93.99%	10.86%	8.88%	9.58%	0.26%

Egg phenotypes are shown in Figure 2.

dsRNA was microinjected 4 days prior to blood feeding as shown in Figure 1.

Table S4. Gene-specific primers used for RNAi and qPCR in *Aedes aegypti*.

Genes		Primer sequence (5' to 3')
<i>Gene-specific primers used for RNAi</i>		
Polehole, AAEL022628	Forward	TGTACGGAAGGCAGGATTC
	Reverse	GGGTAGAGTTTGGTCAGGTT
Nudel, AAEL016971	Forward	GCAAGCTGATGAAGTTCCACA
	Reverse	CCACGTCTTGTTCTCGGTTTC
<i>Gene-specific primers used for qPCR</i>		
Nasrat	Forward	CTGAACACCGATCAGACGAT
	Reverse	TGAGCGTATTCTTGCGTTTCGTA
Closca	Forward	GCCACCGACGTGCTGTTGAA
	Reverse	TCCCGTAGTTTAGCGTAGTTC
Polehole	Forward	ATAGTTCATCAGTTTCGATGCC
	Reverse	AACATCACATCGAACAAGAGTGT
Nudel	Forward	CACTTCGAGAACCAACATAAGG
	Reverse	GGAAGGTGATGTGCGTTAG
DCE2	Forward	GCTAACATTGCCATCGACATG
	Reverse	GCCAGGACTTGTTCTTCTCAA
DCE4	Forward	TGTAGATTCCGCGACGTTCT
	Reverse	CGTAGGTGATCCGTAGCAAT
DCE5	Forward	TGGAACACTGATCAACCGTACAA
	Reverse	GACAATGAGTACATCAGAGCATC
CATL3	Forward	GCCCTCAATGGACAGATTATG
	Reverse	GATCCTCCAGCACATCCCTT
Ribosomal protein S7	Forward	ACCGCCGTCTACGATGCCA
	Reverse	ATGGTGGTCTGCTGGTTCTT

T7 promoter sequence (5' TAATACGACTCACTATAGGGAGA 3') was added in 5' of each RNAi primer.

Table S5. Reproductive phenotypes associated with RNAi in *Aedes aegypti*

	RNAi	Fluc	Nasrat	Closca	Polehole	Nudel
<i>Fecundity</i>						
Number of mosquitoes examined		12	12	12	12	12
Total number of eggs oviposited		1076	1012	1022	1024	976
Mean number of eggs oviposited		89.7	84.3	85.2	85.3	81.3
<i>Eggshell melanization</i>						
Number of eggs examined		1076	1012	1022	1024	976
Incompletely tanned eggs oviposited		14	30	22	31	964
Incomplete eggshell melanization (%)		1.30%	2.96%	2.15%	3.03%	98.77%
<i>Egg viability</i>						
Number of eggs examined		364	368	348	342	976
Number of eggs hatched		333	332	312	302	8
Egg viability (%)		91.48%	90.22%	89.66%	88.30%	0.82%

Egg phenotypes are shown in Fig. 4.

dsRNA was microinjected immediately after blood feeding as shown in Fig. 4.

Table S6. Reproductive phenotypes associated with RNAi in two gonotrophic cycles.

RNAi	First gonotrophic cycle		Second gonotrophic cycle	
	Fluc	Nudel	Fluc	Nudel
<i>Fecundity</i>				
Number of mosquitoes examined	12	12	12	12
Total number of eggs oviposited	1007	902	807	731
Mean number of eggs oviposited	83.9	75.2	67.3	60.9
<i>Eggshell melanization</i>				
Number of eggs examined	1007	902	807	731
Incompletely tanned eggs oviposited	11	890	22	21
Incomplete eggshell melanization (%)	1.09%	98.67%	2.73%	2.87%
<i>Egg viability</i>				
Number of eggs examined	365	902	383	393
Number of eggs hatched	335	7	338	337
Egg viability (%)	91.78%	0.78%	88.25%	85.75%

Egg phenotypes are shown in Fig. 5.

dsRNA was microinjected immediately after blood feeding as shown in Fig. 5.

Table S7. An *in vitro* follicle melanization assay in *Aedes aegypti*.

<i>RNAi treatment</i>	Fluc	Nasrat	Closca	Polehole	Nudel
Number of mosquitoes examined	5	5	5	5	5
Total number of follicles examined	118	121	105	107	113
Total number of follicles melanized	114	6	13	15	4
Follicle melanized (%)	96.6%	5.0%	12.4%	14.0%	3.5%
<i>Protease inhibitor (PI) treatment on wildtype mosquitoes</i>		PI added minutes after follicle dissection			
	Untreated	0	10	20	
Number of mosquitoes examined	5	5	5	5	
Total number of follicles examined	122	146	130	132	
Total number of follicles melanized	118	3	125	126	
Follicle melanized (%)	96.7%	2.1%	96.2%	95.5%	

Follicle phenotypes are shown in Figure 6.

dsRNA was microinjected 4 days prior to blood feeding as shown in Figure 1.

Table S8. An *in vitro* follicle melanization assay in *Aedes aegypti*.

<i>RNAi treatment</i>	Fluc	Nasrat	Closca	Polehole	Nudel
Number of mosquitoes examined	5	5	5	5	5
Total number of follicles examined	127	133	123	123	117
Total number of follicles stained	3	126	119	118	113
Follicles stained (%)	2.4%	94.7%	96.7%	95.9%	96.6%

Follicle stained phenotypes are shown in Figure 6.

dsRNA was microinjected 4 days prior to blood feeding as shown in Figure 1.

Table S9. Reproductive phenotypes associated with RNAi in *Aedes aegypti*.

	RNAi	Fluc	DCE2	DCE4	DCE5	CATL3
<i>Fecundity</i>						
Number of mosquitoes examined		23	24	25	25	26
Total number of eggs oviposited		2022	2128	2144	2130	2195
Mean number of eggs oviposited		87.9	88.7	85.8	85.2	84.4
<i>Eggshell melanization</i>						
Number of eggs examined		2022	2128	2144	2130	2195
Incompletely tanned eggs oviposited		19	2036	27	36	952
Incomplete eggshell melanization (%)		0.94%	95.68%	1.26%	1.69%	43.37%
<i>Egg viability</i>						
Number of eggs examined		605	751	685	714	767
Number of eggs hatched		557	45	623	630	33
Egg viability (%)		92.07%	5.99%	90.95%	88.24%	4.30%

Egg phenotypes are shown in Figure 7.

dsRNA was microinjected 4 days prior to blood feeding as shown in Figure 1.

Table S10. Raw data on eggshell proteomic analyses in *Aedes aegypti*.

Vectorbase ID	GenBank ID	Putative functions	# peptide hits	
			RNAi-Fluc	RNAi-EOF1
AAEL010434	AAA18221	Vitellogenin	244	323
AAEL006126	EAT42292	Vitellogenin	281	222
AAEL006138	XP_001657509	Vitellogenin	278	219
AAEL006830	XP_001658066	Dopachrome converting enzyme	180	230
AAEL004390	XP_001649029	Chorion peroxidase	153	170
AAEL010872	XP_001661124	Odorant binding protein	172	141
AAEL026038	XP_021692994	Chorion peroxidase	160	146
AAEL000961	XP_021708553	Closca	143	161
AAEL013492	EAT34242	Phenoloxidase	156	147
AAEL008829	XP_001659577	Nasrat	112	128
AAEL007415	EAT40867	Multicopper oxidase	87	106
AAEL004386	P82600	Chorion peroxidase	100	82
AAEL011763	XP_001661890	Phenoloxidase	93	78
AAEL022628	XP_021707815	Polehole	67	88
AAEL003110	EAT45649	Chitinase	86	61
AAEL003511	XP_001656921	Odorant binding protein	108	33
AAEL009433	XP_001649879	Odorant binding protein	95	42
AAEL009599	XP_001660278	Odorant binding protein	98	37
AAEL022918	XP_021710656	Glucose dehydrogenase	65	61
AAEL006396	EAT42032	Odorant binding protein	90	35
AAEL002851	ABH03477	Tubulin beta	56	63
AAEL026563	XP_021695347	Odorant binding protein	66	42
AAEL005752	XP_001651411	Lysosomal alpha-mannosidase	61	43
AAEL006563	P42660	Vitellogenic carboxypeptidase	42	55
AAEL006393	EAT42030	Odorant binding protein	74	23
AAEL008797	XP_001659527	Titin	61	33
AAEL005325	EAT43230	Dopachrome converting enzyme	53	40
AAEL006642	XP_001652144	Tubulin alpha-1	43	47
AAEL000796	EAT48134	Odorant binding protein	55	31
AAEL006398	EAT42033	Odorant binding protein	57	28
AAEL005198	EAT43356	Juvenile hormone esterase	49	35
AAEL016971	EJY57924	Nudel	54	26
AAEL009642	XP_001653891	Cathepsin B-like cysteine proteinase 3	57	23
AAEL007096	XP_001658111	Dopachrome converting enzyme	42	35
AAEL013338	XP_001663495	Lethal(2)essential for life	43	31
AAEL017467	EJY57339	Chorion peroxidase	48	25
AAEL011758	ABF18058	Cyclophylin	42	27
AAEL011197	AAY81972	Actin	34	31
AAEL011116	XP_001655111	14-3-3 protein epsilon	32	27
AAEL019403	ABF18332	Heat shock 70	40	14
AAEL022697	XP_021706511	Odorant binding protein	40	14
AAEL007339	XP_001652677	Heat shock protein 67b2	32	22
AAEL006328	XP_021710623	Chitinase	38	14
AAEL000889	XP_001651634	Carboxylic ester hydrolase	34	18
AAEL003315	EAT45429	Odorant binding protein	30	21
AAEL005733	XP_021701099	Myosin heavy chain	12	38
AAEL009895	EAT38188	Neprilysin	36	14
AAEL017096	XP_011493435	Elongation factor 1-alpha	30	19
AAEL000144	EAT48853	Chitinase	39	10
AAEL000377	EAT48658	Odorant binding protein	30	18
AAEL015116	AAG02219	Prophenoloxidase	15	31
AAEL012062	XP_021693479	Na ⁺ /K ⁺ ATPase alpha subunit	14	32
AAEL013719	EAT34018	Odorant binding protein	29	16
AAEL015289	EAT32570	Uncharacterized protein	18	25
AAEL004434	XP_001649141	Transketolase-like protein 2	18	24

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Table S10. Raw data on eggshell proteomic analyses in *Aedes aegypti* (continued).

Vectorbase ID	GenBank ID	Putative functions	# peptide hits	
			RNAi-Fluc	RNAi-EOF1
AAEL010848	XP_021698010	Dopachrome converting enzyme	32	10
AAEL013501	EAT34239	Prophenoloxidase	20	22
AAEL000507	EAT48446	Chorion peroxidase	33	9
AAEL017501	EJY57337	NA-vitelline membrane	17	24
AAEL011708	EAT36186	Heat shock protein 83	28	13
AAEL014876	EAT32887	Odorant binding protein	28	12
AAEL003393	EAT45330	ATP synthase beta subunit	26	13
AAEL022484	XP_021706407	Odorant binding protein	25	14
AAEL000318	EAT48660	Odorant binding protein	20	19
AAEL000833	XP_001651310	Odorant binding protein	23	16
AAEL015312	EAT32556	Cysteine proteinase-1	22	16
AAEL004172	EAT44445	Alpha-Tubulin	16	22
AAEL014431	EAT33288	Odorant binding protein	22	15
AAEL004028	XP_001648297	Glucose dehydrogenase	25	12
AAEL001174	EAT47749	Odorant binding protein	28	8
AAEL009955	XP_021707150	Apolipoporphins	6	29
AAEL000344	XP_001655549	Odorant binding protein	20	15
AAEL009038	XP_021697410	Serine protease/prolylcarboxypeptidase	23	12
AAEL007599	EAT40702	Cathepsin B	27	7
AAEL002023	EAT46826	Chitinase	19	14
AAEL014430	EAT33287	Odorant binding protein	20	11
AAEL006820	XP_001658058	Lipid storage droplets-binding protein 2	24	7
AAEL011300	EAT36638	Uncharacterized protein	17	14
AAEL004500	AAK01430	Eukaryotic translation elongation	17	13
AAEL006336	EAT42103	Chitinase	28	2
AAEL008787	O16109	V-ATPase subunit A	15	15
AAEL014222	EAT33503	Vitellogenin receptor	17	12
AAEL024387	XP_021712253	Serine protease	19	10
AAEL018219	XP_021705132	BM-specific heparan sulfate proteoglycan	11	17
AAEL005766	XP_001651423	Fructose-bisphosphate aldolase	21	7
AAEL001593	EAT47332	GAPDH 1	14	13
AAEL006271	EAT42157	Superoxide dismutase 3	15	11
AAEL005756	EAT42731	Uncharacterized protein	20	6
AAEL012175	XP_001655906	ATP synthase alpha subunit	16	9
AAEL001061	XP_011493351	Glutathione S transferase	14	11
AAEL005759	EAT42734	Uncharacterized protein	21	4
AAEL013359	XP_001656676	DEAD box ATP-dependent RNA helicase	11	14
AAEL000496	XP_001656955	Chorion peroxidase	15	10
AAEL006322	EAT42110	Odorant binding protein	11	14
AAEL001189	EAT47748	Uncharacterized protein	13	11
AAEL010912	EAT37053	DPPIV-SP, Omega	12	12
AAEL013279	XP_001663442	Peptidyl-prolyl cis-trans isomerase	14	9
AAEL017502	EJY57472	Uncharacterized protein	20	3
AAEL010874	EAT37093	Odorant binding protein	20	2
AAEL007097	XP_001658126	4-nitrophenylphosphatase	15	7
AAEL022038	XP_021695232	Uncharacterized protein	15	6
AAEL000703	XP_001650265	Glycogen phosphorylase	17	4
AAEL003513	EAT45173	Uncharacterized protein	16	4
AAEL000179	XP_001658903	Ubiquitin-conjugating enzyme E2	11	9
AAEL027038	XP_021710733	Uncharacterized protein	11	9
AAEL008799	XP_001659528	Uncharacterized protein	15	5
AAEL010097	XP_001654240	Maternal protein exuperantia	12	7
AAEL023983	ABF18051	40S ribosomal protein S4	9	10
AAEL012054	EAT35810	Uncharacterized protein	11	8
AAEL013353	XP_001656670	Chicadee/profilin	7	12

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Table S10. Raw data on eggshell proteomic analyses in *Aedes aegypti* (continued).

Vectorbase ID	GenBank ID	Putative functions	# peptide hits	
			RNAi-Fluc	RNAi-EOF1
AAEL002160	XP_001654823	GTP-binding protein	11	8
AAEL003094	EAT45648	Chitinase	15	4
AAEL026426	XP_021695306	Vitelline membrane protein-like	10	8
AAEL009207	EAT38962	Uncharacterized protein	13	5
AAEL02734	XP_021694355	Uncharacterized protein	11	7
AAEL012960	XP_001663145	Pendulin (NLS-receptor)	14	4
AAEL007432	EAT40884	Serine protease	18	0
AAEL008862	XP_001653480	Metalloprotease	14	3
AAEL001863	AAT36732	Zinc carboxypeptidase	12	5
AAEL001153	EAT47747	Uncharacterized protein	14	3
AAEL005216	EAT43331	Uncharacterized protein	14	3
AAEL001588	XP_001653563	Glutamate carboxypeptidase	12	5
AAEL011808	EAT36073	Gglucose dehydrogenase	11	6
AAEL008640	EAT39566	Odorant binding protein	13	4
AAEL016984	XP_011493026	Glyceraldehyde-3-phosphate dehydrogenase	11	5
AAEL004388	EAT44220	Chorion peroxidase	0	16
AAEL004516	XP_001649345	Odorant binding protein	9	7
AAEL015288	EAT32569	Uncharacterized protein	8	8
AAEL006885	XP_001652301	14-3-3 zeta	9	7
AAEL019604	XP_021698211	Uncharacterized protein	10	5
AAEL010585	XP_001654680	TER94	15	0
AAEL010821	XP_001655016	60S ribosomal protein LP0	8	7
AAEL012996	XP_021709703	Rho guanine dissociation factor	10	5
AAEL008500	XP_001659287	DEAD box ATP-dependent RNA helicase	11	3
AAEL001965	ABF18180	Chitinase/imaginal disc growth factor	5	9
AAEL005901	XP_001663344	40S ribosomal protein S3a	8	6
AAEL020238	Q16ZR8	40S ribosomal protein SA	10	4
AAEL001845	EAT47019	Sepiapterin reductase	8	6
AAEL003525	EAT45172	Odorant binding protein	14	0
AAEL005422	XP_001650875	Pyrroline-5-carboxylate dehydrogenase	11	3
AAEL004984	XP_001650136	Cullin-associated NEDD8-dissociated protein	14	0
AAEL000827	EAT48139	Odorant binding protein	10	4
NA	CAF02084	Odorant binding protein	12	2
AAEL003872	XP_001664282	Translationally-controlled tumor protein	8	6
AAEL001179	EAT47746	Odorant binding protein	10	4
AAEL009994	XP_001660544	60S ribosomal protein L4	9	4
AAEL012897	XP_001663037	Aconitase, mitochondrial	10	3
AAEL009496	XP_001660169	40S ribosomal protein S7	6	7
AAEL014548	EAT33191	Thioredoxin peroxidase	6	7
AAEL019408	AAL37254	2-Cys thioredoxin peroxidase	9	4
AAEL028058	XP_021695267	Odorant binding protein	9	4
AAEL012035	XP_001655825	Vacuolar ATP synthase subunit e	7	6
AAEL014274	XP_001648333	Uncharacterized protein	7	6
AAEL000821	EAT48127	Odorant binding protein	11	2
AAEL017349	XP_011493320	HSP70	8	4
AAEL019579	XP_021704200	Furin-like protease 2	7	5
AAEL001432	EAT47483	Protein disulfide isomerase	9	3
AAEL009097	EAT39077	Cathepsin	7	5
AAEL004978	XP_001650127	DEAD box ATP-dependent RNA helicase	9	3
AAEL003820	EAT44826	Histone H2A-like	6	6
AAEL000758	EAT48168	Ubiquitin activating enzyme 1	11	1
AAEL017451	XP_011493087	Angiotensin converting enzyme	9	3
AAEL009077	EAT39089	Alkaline phosphatase	9	3
AAEL008280	EAT39971	Uncharacterized protein	5	6
AAEL010168	XP_001654299	40S ribosomal protein S2	6	5

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Table S10. Raw data on eggshell proteomic analyses in *Aedes aegypti* (continued).

Vectorbase ID	GenBank ID	Putative functions	# peptide hits	
			RNAi-Fluc	RNAi-EOF1
AAEL001495	EAT47408	Uncharacterized protein	2	9
AAEL019935	XP_021709761	Midline fasciclin	7	4
AAEL000837	EAT48137	Odorant binding protein	7	4
AAEL008481	XP_001659268	60S ribosomal protein L18	6	5
AAEL000846	XP_001651309	Odorant binding protein	13	8
AAEL011870	XP_001662014	Trailer hitch,protein LSM14 homolog B	9	2
AAEL010919	EAT37047	Prophenoloxidase	5	6
AAEL007003	EAT41362	Odorant binding protein	7	4
AAEL008381	EAT39841	Peptide transporter	5	6
AAEL004532	EAT44083	Glyoxylate reductase	6	5
AAEL005798	XP_001651458	ATP synthase subunit beta vacuolar	6	5
AAEL002978	EAT45789	Aminopeptidase	8	3
AAEL012904	XP_001663045	Rab gdp-dissociation inhibitor	8	3
AAEL014719	EAT33023	Uncharacterized protein	7	4
AAEL007962	XP_001658750	Glutathione transferase	7	4
AAEL004755	EAT43826	Enoyl-CoA delta isomerase	10	1
AAEL014238	AAC31639	DOPA decarboxylase	9	1
AAEL011288	ABF18271	Eukaryotic translation elongation factor 1- γ	8	2
AAEL009287	XP_001659895	Ran, GTP-binding nuclear protein	6	4
AAEL006389	ABE72972	Cathepsin L	8	2
AAEL007915	Q170J7	Moesin	7	3
AAEL004856	XP_001649919	Odorant binding protein	8	2
AAEL001487	EAT47390	Odorant binding protein	7	3
AAEL006977	XP_001652452	Ser/thr protein phosphatase 2a reg. subunit a	8	2
AAEL009882	XP_001654079	Retinoblastoma-binding protein 4	6	4
AAEL011764	EAT36127	Phenoloxidase	2	8
AAEL003404	XP_001656805	Uncharacterized protein	0	10
AAEL022214	XP_021707151	γ -interferon inducible lys. thiol reductase	7	3
AAEL007236	ABF18366	Uncharacterized protein	6	4
AAEL000987	XP_001657711	60S ribosomal protein L8	6	3
AAEL012609	EAT35209	γ -aminobutyric acid transaminase	6	3
AAEL002542	XP_001655586	Triosephosphate isomerase	7	2
AAEL006836	XP_001652256	Dihydropteridine reductase	7	2
AAEL009080	XP_021695478	Importin 7	8	1
AAEL006670	EAT41719	Vitelline membrane protein 15a-3	8	0
AAEL010403	XP_001654538	Achaete scute target 1	7	1
AAEL000641	XP_011493116	Protein disulfide isomerase	6	2
AAEL022104	ABF18250	60S ribosomal protein L3	6	2
AAEL002861	ABF18383	Saccheropin dehydrogenase 1	7	1
AAEL001112	EAT47794	Ubiquitin specific protease 5	8	0
AAEL001605	XP_001659738	Mapmodulin/microtubule binding protein	2	6
AAEL013275	XP_001663434	Female sterile (2) ketel, importin beta	6	2
AAEL017315	EJY57568	HSC70	8	0
AAEL000109	XP_001657693	Enolase-phosphatase E1-like	6	2
AAEL013857	XP_001647837	Serine protease immune response integrator	1	6
AAEL012427	XP_001662561	Uncharacterized protein	7	0
AAEL005832	EAT42655	Programmed cell death 4	6	1
AAEL010506	XP_001660884	GTP-binding protein alpha subunit, gna	1	6
AAEL001035	EAT47891	Ca ²⁺ -binding protein Regucalcin/SMP30	6	1
AAEL009142	XP_001659779	Prolyl endopeptidase	6	0
AAEL013284	AAO43403	Uncharacterized protein	6	0
AAEL010698	EAT37289	Artemis	6	0
AAEL001401	XP_001659164	Leucine-rich immune protein	0	6
AAEL009746	EAT38349	Chitinase-domain	6	0
AAEL007014	EAT41361	Odorant binding protein	6	0