

Supplementary Information

PEBP balances apoptosis and autophagy in whitefly upon arbovirus infection

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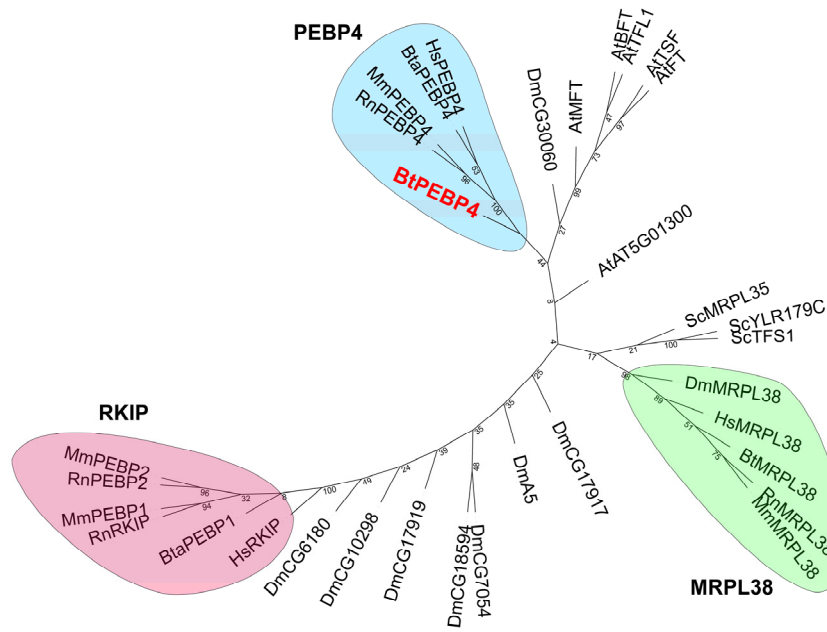
Supplementary Figure 5. PEBP4 interacts with ATG8.

Supplementary Figure 6. Comprehensive interaction of apoptosis and autophagy verified by RNA interference.

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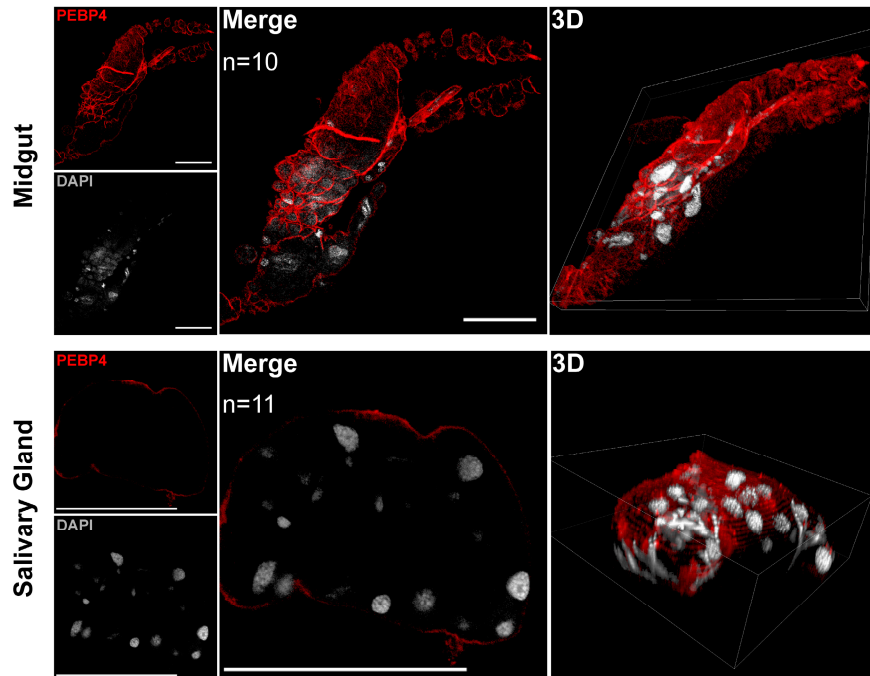
A



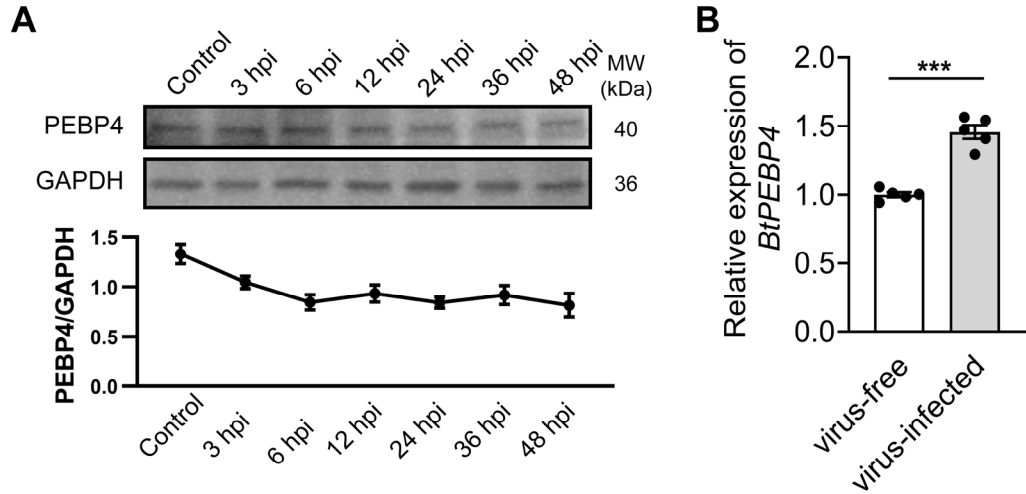
B

BtPEBP4	MASVSVMLSMVLQTYSVSVVSMASHLLHLLGFPPrKVRSRKNKENMERTDSPESMEMESTKGTSTKTKTRRTKSTKTKSTK	80
HsPEBP4	0
MmPEBP4	0
BtaPEBP4	0
RnPEBP4	0
BtPEBP4	SKKPKSTKSTKTKSTKARSTKITKTKKTPPKIKKSTRNTRRPRISKNTTESTKISKTEVEIGSTEILTTTRHGKTRSRPK	160
HsPEBP4MGWTMRLVTAALLGLMMVVTGDEDE.....N	27
MmPEBP4MTMKLVAAALCLSLLAAGLWVGLSLTAESIEEGKPGGKPGGKPGGGSG	49
BtaPEBP4MGWTMRLVTAALLGLAVAVTGEED.....A	27
RnPEBP4MKLVAAAFGLSILVAGLWVGLSITNSNDGGKPGG...GGG...GGGG	42
BtPEBP4	NISPFDAIFRENEIVPQIVNNTPTMKCEVEYGEKEIHFGNNMTADQIAEKPTLVKWPQSGAFYTLIMFGPDVETREKS	239
HsPEBP4	SPCAHEALDED..TLFCQGLEVFPELGNIGCKVVPDCNNYRQKITSWMEPIVKFPGAVDGATYILVMVDPDAPSRAP	105
MmPEBP4	RGCFLLPPLPKED..VSLCRNLEVFYMEMGNISCKIVPKCNLYRQKITAWQAPIVKFHTALDGLYLLVMVDPDAPSRSNP	127
BtaPEBP4	DLCVYEALPDND..AVLCKGLKVFYPELGNIGCMIVPEQNNYRQKITTWPEPIVKFPOALDDAAYILVMVDPDAPSRSSP	105
RnPEBP4	RGCFLLPPLPKED..VSLCRNLEVFYMEMGNISCKVVPKCNLYRQKITWQSPIVKPHGALD.....	101
BtPEBP4	CEMMWRHWAVGNIPKND...IHRGDTLVEYENIEPFYRAGFHRILFFIYKQPGRRRLNFTSSKGVIKKAKWEVGVVY	315
HsPEBP4	RQRFWRHWLVTDIKGADLKKGKIQQELSAVQAFSPPAHSGFHRYQFFVYLQEGKV..ISLLPKENKTRG.....SWK	176
MmPEBP4	VMKYWRHWLVSNITGADMKSISRGNVLSYSPPTPPPETGLHRYQFFVYLQGGDRD..ISLSVEEKADLG.....GWN	198
BtaPEBP4	KARFWRHWLVSDIKGSDMKIGKIQQELSPYHPPSPPAHSGLHRYQFFVYLQEGRT..ISLSPKENKTRG.....SWK	176
RnPEBP4VSELG.....WLVKENVGPQLAFSSSGP.....VFLLTN...IKWPAFENVSL.....	140
BtPEBP4	IERFLAKFNLTBEPIAANFFFLYDLNPHPEIPQVTVDPPLSLIR.....	357
HsPEBP4	MDRFLNRFHLGPEASTQFMTONYQDSPTLQAPRERASEPKHKNAEIIAAC	227
MmPEBP4	LDKFLQQYGLRDEDTSTQFMTOFDEELSSEFGRINDDQEQFNQK.....	242
BtaPEBP4	MDKFLSHFHLTEPEASTQFMTOYYLDGLSRQNPVVSNETTDPKPEPK....	223
RnPEBP4	...FSSHRKIKSEKERLD.....	155

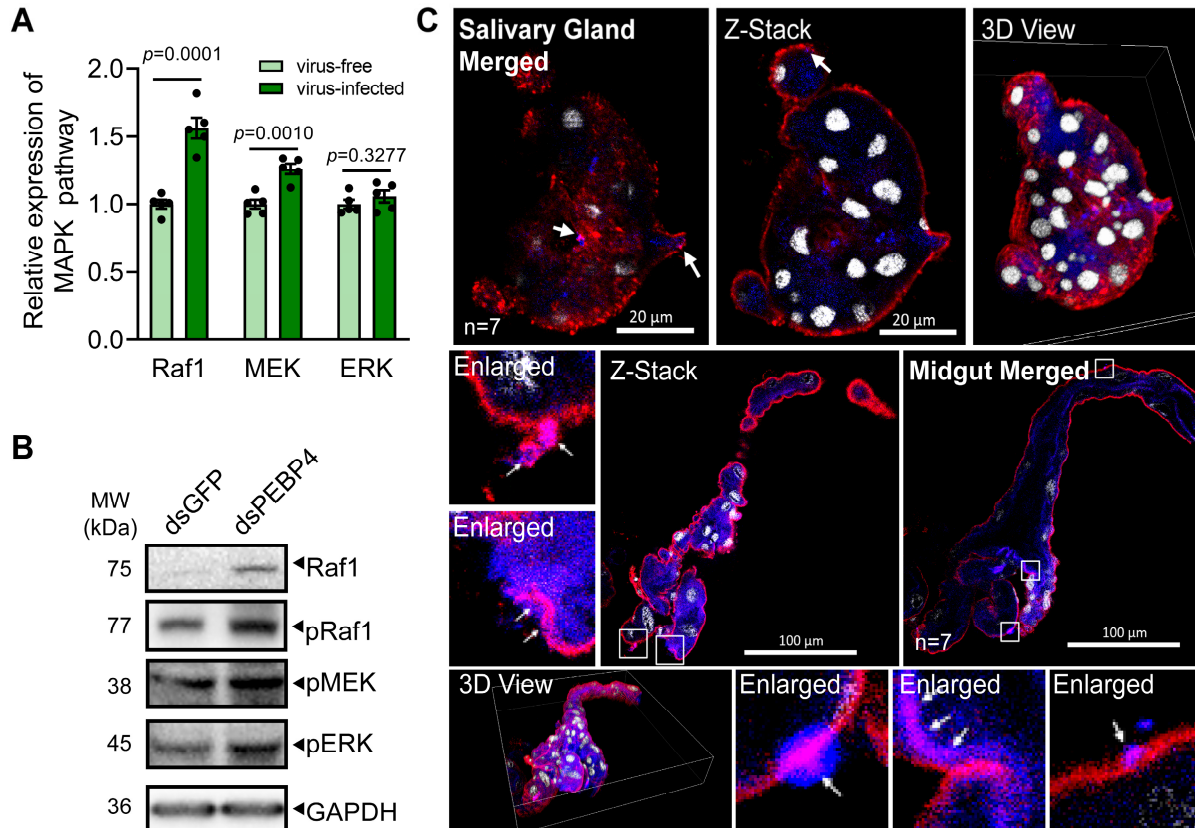
Supplementary Figure 1. Homology analysis of the candidate whitefly PEBP. (A) The PEBP protein sequences that identified from 7 species and the candidate PEBP in whitefly were taken as input sequences, and the phylogenetic tree was constructed through the neighbor-joining (N-J) method with 1000 bootstrap replicates. Three major clusters were named according to the dominate proteins. (B) The candidate PEBP in whitefly was named as BtPEBP4, and the amino acid sequence was aligned with other PEBP4s in mammals.



Supplementary Figure 2. Whitefly PEBP4 is primarily located in cytoplasmic membrane. PEBP4 (red), and nuclei (white) were labeled with immunofluorescence. Sample sizes (n) for statistical tests indicated in the panels refer to biologically independent whitefly. Midgut and salivary gland were dissected from untreated whitefly. 3D images were simulated based on Z-Stack scanning. Scale bar represents 50 μ m. Sample sizes (n) for statistical tests indicated in the panels refer to biologically independent whitefly.



Supplementary Figure 3. PEBP4 is continuously consumed during TYLCV infection. (A) The dynamic of the PEBP4 protein abundance in whitefly after virus acquisition was analyzed by immunoblotting, and the grey value of PEBP4 was quantified by ImageJ software compared to GAPDH, six independent samples were used for each treatment. (B) The transcriptional expression of *BtPEBP4* was analyzed by qPCR after 48 hours TYLCV acquisition, five independent samples were used for each treatment. Values in bar or line plots represent mean \pm SEM. All data were checked for normality by the Wilk-Shapiro test. Two-sided paired t-test was used to separate the means of normally distributed data.



Supplementary Figure 4. PEBP4 mediates the MAPK pathway by interacting with Raf1. (A) Transcriptional expression of *Raf1*, *MEK*, and *ERK* after 48 hours TYLCV acquisition, five independent samples were used for each treatment. Values in bar plot represent mean \pm SEM. All data were checked for normality by the Wilk-Shapiro test. Two-sided paired t-test was used to separate the means of normally distributed data. (B) The phosphorylation of MAPK pathway after PEPBP4 knockdown was analyzed by immunoblotting. (C) Raf1 (blue) and PEBP4 (red) of nonviruliferous whitefly were labeled and localized by immunofluorescence. The nuclei were stained by DAPI (white). Z-Stack image showed another layer of the same sample. 3D images were simulated from all scanned Z-Stack layers. Sample sizes (n) for statistical tests indicated in the panels refer to biologically independent whitefly.

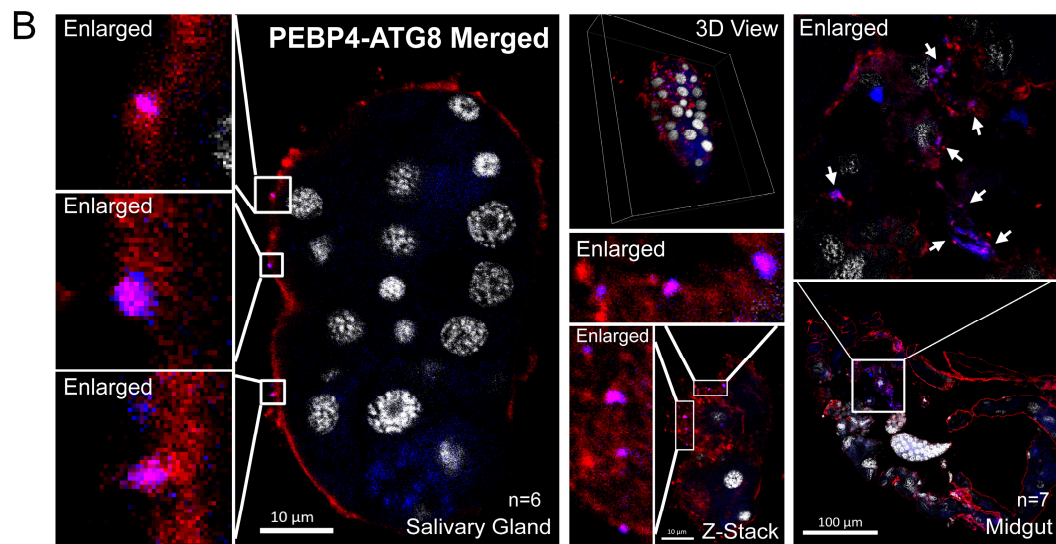
A Query: >PEBP4

Motif	Start	End	Pattern	PSSM Score	LIR in Anchor
WxxL	163	168	SPFDAI	9	Yes
WxxL	221	226	GAFYTL	5	No
WxxL	264	269	VEYENI	9	No
WxxL	275	280	AGFHRI	6	No
WxxL	331	336	ANFFFL	7	No

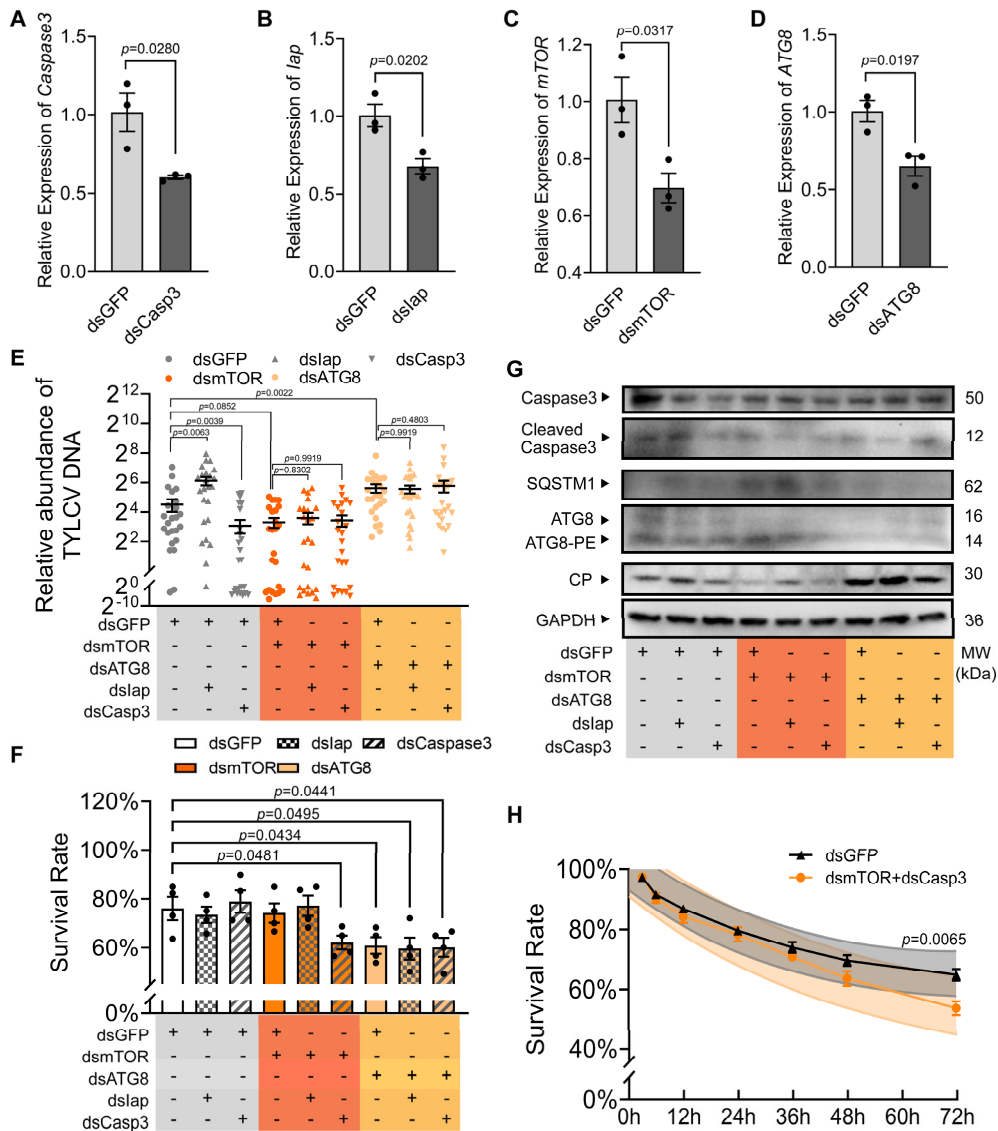
ANCHOR Positions - Start and End

Anchor	Start	End
1	1	38
2	96	103
3	137	148
4	162	175
5	179	189

iLIR Autophagy Database



Supplementary Figure 5. PEBP4 interacts with ATG8. (A) LIR motifs are necessary for direct interaction with the autophagy-related ATG8 family. The amino acid sequence of whitefly PEBP4 was analyzed and predicted by the online tool of LIR motif searching in iLIR Autophagy Database (<https://ilir.warwick.ac.uk/index.php>). (B) ATG8 (blue) and PEBP4 (red) of nonviruliferous whitefly were labeled and localized by immunofluorescence. The nuclei were stained by DAPI (white). Z-Stack image showed another layer of the same sample. 3D images were simulated from all scanned Z-Stack layers. Sample sizes (n) for statistical tests indicated in the panels refer to biologically independent whitefly.



Supplementary Figure 6. Comprehensive interaction of apoptosis and autophagy verified by RNA interference. (A-D) RNAi efficiency of ds*lap*, dsCaspase3, dsdTOR and dsATG8 feeding (dsGFP as control), three independent samples were used for each treatment. (E-G) After feeding on combinations of two different dsRNAs as indicated, whiteflies were collected and transferred to infected plants for 24 hours TYLCV acquisition. (E) Relative virus DNA content per whitefly was determined by qPCR, 24 independent whiteflies were sampled for each treatment. (F) Whitefly survival rate was recorded after dsRNA feeding and virus acquisition, four independent samples were used for each treatment. (G) 100 whiteflies per replicate from the same treatment as shown above was sampled for monitoring dsRNA combination effects on apoptosis, autophagy and CP abundance. (H) Nonviruliferous whiteflies fed with both dsdTOR (autophagy activation) and dsCaspase3 (relieve autophagy limitation of apoptosis) were sampled for survival rate measurement, dsRNA-contained diet was replaced every 24 h, prediction bands (95% confidential interval) of whitefly survival rate were shown based on linear quadratic survival model, five independent samples were used for each treatment. Values in all bars or dot plots represent mean \pm SEM. All data were checked for normality by the Wilk-Shapiro test. Two-sided paired t-test was used to separate the means of normally distributed data, while

Mann-Whitney test was used to analyze nonparametric distributed data, no multiple comparisons were performed in each test.

Supplementary Table 1. Putative MED *Bemisia tabaci* proteins that interacted with coat protein of tomato yellow leaf curl virus in yeast two-hybrid screening.

Query No.	Score ^a	E-value ^a	Identity(%) ^a	Genome ID	Score ^b	E-value ^b	Identity(%) ^b	Accession No.	Description ^c
1	25.0	5.4	43.3	BTA008214.1	180	5.00E-47	27.72%	XP_018897595.1	PREDICTED: uncharacterized protein LOC109030865 isoform X1 [Bemisia tabaci]
					120	3.00E-27	24.76%	XP_018915628.1	PREDICTED: glutamate receptor U1-like [Bemisia tabaci]
2	94.4	4.80E-25	93.9	BTA012003.1	263	9.00E-91	98.43%	XP_018901274.1	PREDICTED: NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 5, mitochondrial [Bemisia tabaci]
3	149.0	1.82E-42	97.3	BTA000233.1	516	0	96.73%	XP_018900154.1	PREDICTED: uncharacterized protein LOC109032456 isoform X2 [Bemisia tabaci]
					33.5	1.7	25.76%	RZF41284.1	obscurin isoform X3 [Sipha flava]
4	523.0	0	99.2	BTA010352.1	1000	0	99.59%	XP_018911064.1	PREDICTED: esterase E4-like [Bemisia tabaci]
5	33.1	0.017	31.5	BTA017005.1	564	0	96.50%	XP_018915181.1	PREDICTED: probable dimethyladenosine transferase [Bemisia tabaci]
6	26.2	6.5	71.4	BTA006720.1	564	0	96.50%	XP_018915181.1	PREDICTED: rho GTPase-activating protein 21 isoform X8 [Bemisia tabaci]
7	265.0	2.89E-85	100.0	BTA007379.1	567	0	99.62%	XP_018901717.1	PREDICTED: alpha-N-acetylgalactosaminidase-like [Bemisia tabaci]
8	227.0	4.41E-77	93.0	BTA023875.1	267	4.00E-93	100.00%	XP_018910818.1	PREDICTED: 40S ribosomal protein S15Aa-like [Bemisia tabaci]
9	38.5	0.006	50.0	BTA015065.1	286	4.00E-87	34.67%	XP_025417167.1	ATP-binding cassette sub-family B member 10, mitochondrial [Sipha flava]
10	157.0	2.10E-46	85.4	BTA024002.1	293	2.00E-102	93.79%	XP_018899372.1	PREDICTED: WAS/WASL-interacting protein family member 1-like [Bemisia tabaci]
11	311.0	2.15E-97	99.3	BTA011564.1	1976	0	99.36%	XP_018904225.1	PREDICTED: C-1-tetrahydrofolate synthase, cytoplasmic [Bemisia tabaci]
12	223.0	6.24E-69	94.1	BTA012527.1	913	0	84.84%	XP_018917484.1	PREDICTED: uncharacterized protein LOC109044293 [Bemisia tabaci]
					34.7	1.9	27.63%	XP_027848922.1	ethylmalonyl-CoA decarboxylase-like isoform X1 [Aphis gossypii]
13	28.9	3.5	44.4	BTA029690.1	2754	0	99.85%	XP_018903654.1	PREDICTED: uncharacterized protein LOC109034784 [Bemisia tabaci]
					1675	0	98.90%	XP_018903657.1	PREDICTED: PAX3- and PAX7-binding protein 1 [Bemisia tabaci]
14	106.0	2.57E-27	99.0	BTA014874.1	1469	0	98.28%	XP_018901035.1	PREDICTED: proteoglycan 4-like [Bemisia tabaci]
15	145.0	1.14E-43	97.5	BTA017133.1	518	0	99.21%	XP_018902549.1	PREDICTED: probable 39S ribosomal protein L24, mitochondrial [Bemisia tabaci]
16	306.0	1.08E-104	99.3	BTA013106.1	722	0	97.51%	XP_018910109.1	PREDICTED: uncharacterized protein LOC109039182 isoform X1 [Bemisia tabaci]
					336	3.00E-116	98.08%	XP_018910114.1	PREDICTED: phosphatidylethanolamine-binding protein homolog F40A3.3-like isoform X4 [Bemisia tabaci]
17	26.9	0.43	44.4	BTA020855.1	459	9.00E-166	99.55%	XP_018897453.1	PREDICTED: uncharacterized protein LOC109030777 [Bemisia tabaci]
					126	1.00E-34	39.41%	VVC29685.1	Immunoglobulin subtype,Immunoglobulin-like domain,Immunoglobulin-like fold [Cinara cedri]
18	62.4	2.41E-10	34.4	BTA027805.1	347	3.00E-108	41.13%	QEP09172.1	cytochrome c oxidase subunit I [Aphaena discolor nigrotibata]
19	27.3	0.53	39.3	BTA004184.1	4908	0	98.87%	XP_018899018.1	PREDICTED: uncharacterized protein LOC109031748 [Bemisia tabaci]
					957	3.00E-173	65.36%	XP_025414375.1	protein FAM135A [Sipha flava]
20	26.2	1.7	41.9	BTA000356.1	1333	0	98.76%	XP_018901482.1	PREDICTED: glucose dehydrogenase [FAD, quinone]-like [Bemisia tabaci]
21	25.4	1.8	39.1	BTA026158.1	623	0	99.35%	XP_018915405.1	PREDICTED: facilitated trehalose transporter Tret1-like isoform X1 [Bemisia tabaci]
22	29.6	4	34.5	BTA021511.2	1143	0	96.05%	XP_018915012.1	PREDICTED: cytochrome P450 4g15-like isoform X1 [Bemisia tabaci]
23	27.3	0.41	41.9	BTA019316.1	1060	0	100.00%	XP_018913374.1	PREDICTED: kelch-like protein 30 [Bemisia tabaci]

a Query sequences were used in local Blastx to identify full amino acid sequences in MED whitefly genome

data.

b Selected proteins were used Blastp and compared with the database of *Hemiptera* to predict putative function.

c If the subject protein was predicted as “uncharacterized protein”, a further PSI-Blast result with specific description would be supplied below the previous result.

Supplementary Table 2. Primer List.

Name	Primer Sequence (5'-3')	Accession No.
<i>Actin</i>	TCTTCCAGCCATCCTTCTTG	XM_019042718.1
	CGGTGATTCCTTCTGCATT	
<i>Caspase1</i>	TGTTGGAGACGGTATGGA	BTA009205.1
	ATGAAGACAGTGCTTAATGC	
<i>Caspase3</i>	CATCACGATCAACGGGACCA	BTA015946.1
	TGTCGATGTGCTGCTCGAAT	
<i>TYLCV</i>	GAAGCGACCAGGCGATATAA	AM282874.1
	GGAACATCAGGGCTTCGATA	
<i>PEBP4</i>	CCAGGAAGAAGGAGATTGAA	BTA013106.1
	GCTATTGGTTCGGTCAGAT	(Sequenced PEBP4 CDs region is appended below)
<i>lap</i>	ATGTCTCGGAATCATCTCAG	BTA000132.1
	ACCACAAGGAAGGAATGC	
<i>Bcl-2</i>	GCTAATGACACAGACTGGAT	BTA025366.1
	GAGATGAAGTTCGTGAGGAA	
<i>Raf1</i>	AAGTGCTGATGATAGTGCTA	BTA014695.1
	CATCGGTAGACAGTTCCAA	
<i>MEK</i>	TTGAGTTGCTGGACTACATAG	BTA026985.1
	TTATCCGCCTCGCACTTA	
<i>ERK</i>	CAATGATAAGGCACGCAAT	BTA017617.1
	CATAATACTGTTCCAGGTAAGG	
<i>ATG3</i>	CGTTTAAGGGAACAGCACTTG	BTA008054.1
	CCAGATTGTCTCCAGCAGCA	
<i>ATG9</i>	TTGCCATCATTAACTTTCTGCT	BTA017980.1
	AGGGTTCCTGGTTCACGC	
<i>ATG8</i>	TACACTTGAGACCAGAGGA	BTA002927.2
	CTTCTTCGTGATGTTCTTGA	
<i>ATG12</i>	TCAAAGCCACTGGTAACGC	BTA009484.1
	TCTGGTCCGGAGCAGGAGC	
dsGFP	CTCGTGACCACCCTGACCTAC	
	GTTACCTTGATGCCGTTCTT	
	T7-CTCGTGACCACCCTGACCTAC	
	T7-GTTACCTTGATGCCGTTCTT	
dsPEBP4	CAACCACAAGGCACGAA	
	TCCTTCTTCCTGGCTGTT	
	T7-CAACCACAAGGCACGAA	
	T7-TCCTTCTTCCTGGCTGTT	

dsCasp3	GATGGAGCGACAGATGAC	
	GGCGAGCAGAAGTTGAA	
	T7-GATGGAGCGACAGATGAC	
	T7-GGCGAGCAGAAGTTGAA	
dslap	GCATACCGTGAGCATACC	
	TCGTTGGAAGTTGATGATGA	
	T7-GCATACCGTGAGCATACC	
	T7-TCGTTGGAAGTTGATGATGA	
dsmTOR	AGGCTGTGGTTGGTCTT	
	CGGATTGTGCTCTGTCTT	
	T7-AGGCTGTGGTTGGTCTT	
	T7-CGGATTGTGCTCTGTCTT	
dsATG8	GCTGAGCATGTGTTTGA	
	AGTCTTCTTCGTGATGTTCT	
	T7-GCTGAGCATGTGTTTGA	
	T7-AGTCTTCTTCGTGATGTTCT	
PEBP4 (BL21)	<u>CGCGAATTCATGGCTTCCGTCTCCGTAAT</u> <u>CGCCTCGAGTCATCGAATTAGTGACAGTGG</u>	EcoRI/XhoI (pGEX-4T-1)
PEBP4 pep1 (BL21)	<u>CGCGAATTCATGGCTTCCGTCTCCGTAAT</u> <u>CGCTCGAGTCAAGGCCACTTGACTAACGTTG</u>	EcoRI/XhoI (pGEX-4T-1)
PEBP4 pep2 (BL21)	<u>GCGAATTCACGCAGAGTGGTGCTTTTTA</u> <u>CGCCTCGAGTCATCGAATTAGTGACAGTGG</u>	EcoRI/XhoI (pGEX-4T-1)
Raf1 (BL21(DE3))	<u>CGCGGAATTCATGTCTGTCTGAATATGACGA</u> <u>CGCCTCGAGTTAGATTATTCCACCCATTG</u>	EcoRI/XhoI (pET28a)
ATG8 (BL21(DE3))	<u>CGCGCGAATTCATGAATTTCCAATACAAAGC</u> <u>CGCTCGAGTTAAGCACAGATCTGGC</u>	EcoRI/XhoI (pET28a)

T7=5'-TAATACGACTCACTATAGG-3'

>BtPEBP4

ATGGCTTCCGTCTCCGTAATGCTATCCATGGTTTTGCAGACGTATTCGGTGTCCGTGGTATCTATGGCACAT
TCGTTGCATTTGCTCGGTTTTCCCCGAGGAAAGTGAGAAGCAGGAAAAACAAGGAAAACATGGAAAGAA
CGGATAGCCCGGAAAGTATGGAGATGGAAAGCACGAAAGGCACAGAAAGCACAAAGAAAAACAAGACGCA
CGAAAAGCACAAAACATAAAAGCACGAAAAGCAAAAAACCCAAAAGCACGAAAAGCACAAAACCAAAAAG
CACAAAAGCCAGAAGCACGAAAATCACAAAAACAAAAAACACCAACACCAAAAATCAAGAAAAGCACA
AGGAACACGAGGCGTCCGCGAATAAGTAAAAACACGAAAGCACCAAAATTTCAAAAACCACTGAAGTTAT
TGGAAGCACAGAAATTTTAAACAACCACAAGGCACGAAAGACGAGGTCAAGACCTAAAAATATATCACCTT
TTGATGCAATTTTTAGAGAAAATGAAATAGTGCCACAGATCGTTAATAACACACCAATATACAAATGTGAGGT
TGAATATGGCAAGGAGAAAATACATTTTGGGAACAACATGACGGCAGATCAAATCGCAGAAAACCAACGT
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GGAAGTGCGAGGAGTCTGGTATATTGAGCGTTTTCTCGCTAAATTCAATCTGACCGAACCAATAGCAGCGA
ACTTTTTCTTTCTTTATGATTTGAATCCTCATCCCGAAATTCACAAGTTACTGTAGATCCACTGTCACTAATT
CGATGA