

Supplemental Material to:

Carole Couillault, Patrick Fourquet, Matthieu Pophillat and Jonathan J. Ewbank. A UPR-independent infection-specific role for a BiP/GRP78 protein in the control of the antimicrobial peptide expression in *C. elegans* epidermis. *Virulence* 2012; 3(3);

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Supplementary Text

Primers sequences (5'-3'):

Fw *gpa-12** JEP1976:

ggggacaagtttgtaaaaaagcaggcttaATGGTATGCTGTTTCGGGAA

Rv *gpa-12** JEP1977:

ggggaccactttgtacaagaaagctgggtaTTACTGCATCATGAGAGTCT

Fw *xbp-1* JEP1594: CGTCGTCTACGAAGAAGAAGTCGTC

Rv *xbp-1* JEP1595: GATGATAGTTAGATACATATCCCACTG

Fw *eft-2* JEP1596: TCGAAATTCAATGCCCAGAAG

Rv *eft-2* JEP1597: CTCCTCGAAAACGTGTCCTCTT

Worms analyzed in each Biosort experiment (from left to right)

Figure 2A: 63; 150; 105; 144; 109; 137; 144; 210; 143; 182; 146; 154; 152; 143; 151; 165

Figure 2B: 217; 136; 50; 20; 47; 265; 282; 91; 66; 39; 44; 56; 30; 25; 23

Figure 3A: 41; 84; 58; 80

Figure 3B: 15; 31; 14; 30; 25; 29; 13; 55

Figure S1: 61; 78; 36; 41; 133; 86; 193; 159

Bibliographic references for EASE class lists in Tables 4 and S2.

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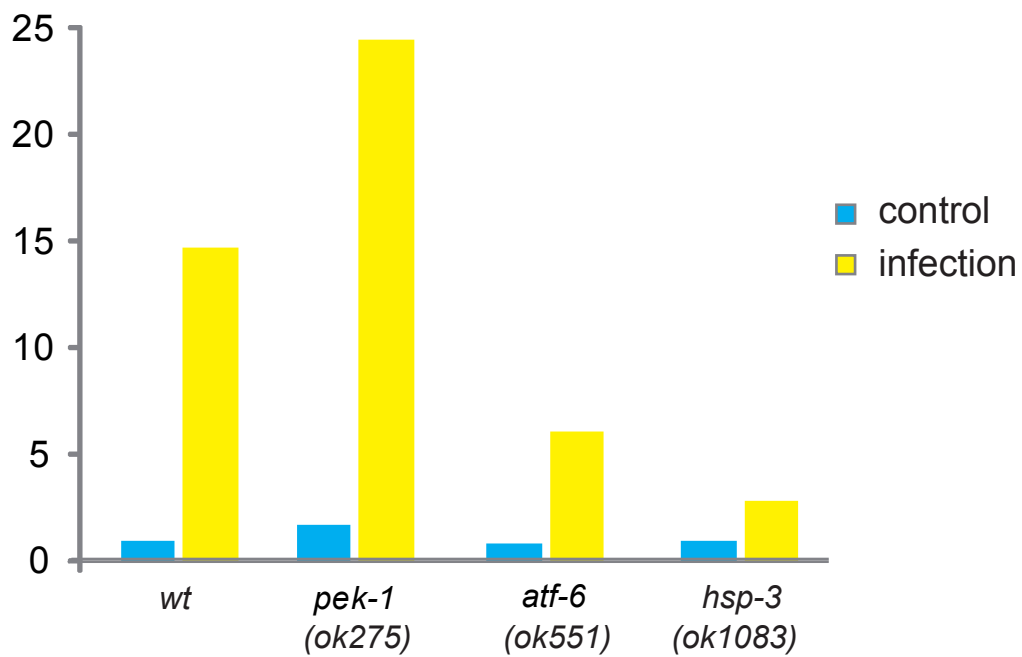
Table S1. Descriptions of the genes corresponding to the 68 differentially represented proteins. The information is derived from WormMart WS220.

Table S2. Structural and functional categories for genes corresponding to the 68 differentially represented proteins. An edited output from EASE, generated using an in-house database⁴ is shown. Results are restricted to those with a probability > 0.01 (Fisher exact test). The categories derived from 2D gel electrophoresis-based studies are highlighted in green; those related to infection are in blue, and those related to stress are in red.

Gene WB ID	Gene Public ID	Sequence Na	Gene Description (Concise)	KOG Title
WBGene00009583	aagr-3	F40F9.6		Glucosidase II catalytic (alpha) subunit and related enzymes, glycosyl hydrolase family 31
WBGene00000040	aco-1	ZK455.1	aco-1 encodes an aconitase that is homologous to mammalian iron regulatory protein-1 (IRP1); aco-1 activity is required for normal RNA-binding translational regulator IRP (aconitase superfamily)	
WBGene000000066	act-4	M03F4.2	act-4 encodes an actin isoform that is most similar to act-2 in amino acid sequence; an act-4 reporter gene is expressed in body wall muscle	Actin and related proteins
WBGene000000067	act-5	T25C8.2	an ortholog of human cytoplasmic actin; and is expressed only in microvillous intestinal cells and excretory cell.	Actin and related proteins
WBGene000000229	atp-2	C34E10.6	atp-2 encodes the beta subunit of the soluble, catalytic F1 portion of ATP synthase (mitochondrial respiratory chain [MRC] complex F0F1-type ATP synthase, beta subunit)	
WBGene000000479	cgh-1	C07H6.5	cgh-1 encodes a putative DEAD-box RNA helicase, orthologous to budding yeast Dhh1p, fission yeast Ste13p, Drosophila ME31B, ATP-dependent RNA helicase	
WBGene000000776	cpl-1	T03E6.7	cpl-1 encodes a member of the cathepsin L-like cysteine protease family required for embryonic viability and normal growth; encodes Cysteine proteinase Cathepsin L	
WBGene000000802	crt-1	Y38A10A.5	crt-1 encodes an ortholog of calreticulin (a calcium-binding molecular chaperone of the endoplasmic reticulum); crt-1 is dispensable for embryonic viability	Calreticulin
WBGene00010266	dct-18	F58G1.4		Unnamed protein
WBGene00001000	dim-1	C18A11.7	dim-1 encodes novel proteins containing three immunoglobulin-like repeats in the carboxy terminus; dim-1 was originally identified in Drosophila	Immunoglobulin and related proteins
WBGene00018846	eef-1B.1	F54H12.6		Elongation factor 1 beta/delta chain
WBGene00012768	eef-1B.2	Y41E3.10		Elongation factor 1 beta/delta chain
WBGene00001167	eef-2	F25H5.4	eef-2 encodes a homolog of translation elongation factor 2 (EF-2), a GTP-binding protein essential for the elongation phase of protein synthesis	Elongation factor 2
WBGene00001598	glh-1	T21G5.3	glh-1 encodes a putative DEAD-box RNA helicase that contains four CCHC zinc fingers and is homologous to Drosophila VASA, a GTP-dependent RNA helicase	
WBGene00001685	gpd-3	K10B3.7	gpd-3 encodes a predicted glyceraldehyde 3-phosphate dehydrogenase that affects embryonic viability; GPD-3 interacts with LIN-10	Glyceraldehyde 3-phosphate dehydrogenase
WBGene00001686	gpd-4	F33H1.2	gpd-4 encodes one of four C. elegans glyceraldehyde-3-phosphate dehydrogenases (GAPDHs); by homology, GPD-4 is predicted to be a GTP-dependent RNA helicase	Glyceraldehyde 3-phosphate dehydrogenase
WBGene00001840	hel-1	C26D10.2	hel-1 encodes an ortholog of human 56-kD U2AF65 associated protein (UAP56) that is required for exporting mRNAs from the nucleus	ATP-dependent RNA helicase
WBGene00002007	hsp-3	C15H9.6	hsp-3 encodes one of two C. elegans heat shock response 70 (hsp70) proteins homologous to mammalian grp78/BiP (glucose regulated protein 78/BiP/KAR2, HSP70 superfamily)	Molecular chaperones GRP78/BiP/KAR2, HSP70 superfamily
WBGene00002025	hsp-60	Y22D7AL.5	hsp-60 encodes a mitochondrial-specific chaperone that is a member of the GroE/Hsp10/60 superfamily of molecular chaperone Mitochondrial chaperonin, Cpn60/Hsp60p	Mitochondrial chaperonin, Cpn60/Hsp60p
WBGene00002053	ifb-1	F10C1.2	ifb-1 (also known as vab-21) encodes two isoforms of an essential intermediate filament protein that is coexpressed with the essential nuclear envelope protein lamin, intermediate filament superfamily	Nuclear envelope protein lamin, intermediate filament superfamily
WBGene00002056	ifc-2	M6.1	ifc-2 encodes three isoforms of an intermediate filament protein dispensable for viability but required for normal movement, germline development and sex determination	Nuclear envelope protein lamin, intermediate filament superfamily
WBGene00002067	ifp-1	C43C3.1	ifp-1 encodes a nonessential intermediate filament protein; IFP-1 is predicted to function as a structural component of the cytoskeleton	Nuclear envelope protein lamin, intermediate filament superfamily
WBGene00002083	inf-1	F57B9.6	inf-1 encodes a protein with high similarity to eukaryotic initiation factor 4A.	Translation initiation factor 4F, helicase subunit (eIF-4A) and related helicases
WBGene00002244	laf-1	Y71H2AM.19	laf-1 encodes a DEAD-box RNA helicase; laf-1 is required for embryonic development and sex determination; laf-1 was identified in screens for dominant suppressors of the sterility produced by gain-of-function fem-3 mutations; when homozygous, laf-1 mutations result in embryonic and larval lethality and when heterozygous, feminization of XX and XO animals; reporter gene analyses indicate that wild-type laf-1 activity is required for proper translational repression of tra-2 mRNA via its 3'UTR; when expressed in embryos, LAF-1 localizes predominantly to the cytoplasm with additional localization seen in P granules.	
WBGene00002263	lea-1	K08H10.1	The lea-1 gene encodes a protein that is predicted to be hydrophilic and heat-resistant, and that might participate in anhydrobiosis	Uncharacterized conserved protein
WBGene00002269	lec-6	Y55B1AR.1	lec-6 encodes a 'proto' type galectin (beta-galactosyl-binding lectin) containing a single carbohydrate recognition domain; by homology, Lec-6 is predicted to be a beta-galactosyl-binding lectin	Galectin, galactose-binding lectin
WBGene00003052	lmn-1	DY3.2	lmn-1 encodes the sole C. elegans nuclear lamin; lmn-1 is an essential gene that is required for a number of nuclear processes, including transcription, DNA replication, and cell division	Nuclear envelope protein lamin, intermediate filament superfamily
WBGene00003119	mac-1	Y48C3A.7	mac-1 encodes a AAA ATPase (ATPase associated with a variety of cellular activities) most closely related to the ATPase subfamily Nuclear AAA ATPase (VCP subfamily)	Nuclear AAA ATPase (VCP subfamily)
WBGene00003161	mdf-2	Y69A2AR.30	mdf-2 encodes the C. elegans ortholog of the Mad2p spindle assembly checkpoint protein; mdf-2 activity is essential for regulating the spindle assembly checkpoint	Spindle assembly checkpoint protein
WBGene00003369	mlc-1	C36E6.3	mlc-1 encodes a muscle regulatory myosin light chain that is nearly identical to MLC-2 and functions redundantly with MLC-2 in muscle	Myosin regulatory light chain, EF-Hand protein superfamily
WBGene00019940	npp-21	R07G3.3		
WBGene00021562	nuo-5	Y45G12B.1	nuo-5 is orthologous to human NADH-UBIQUINONE OXIDOREDUCTASE Fe-S PROTEIN 1 (NDUFS1; OMIM:157655), which when mutated causes NADH-ubiquinone oxidoreductase deficiency	NADH-ubiquinone oxidoreductase, NDUFS1/75 kDa subunit
WBGene00003929	pat-2	F54F2.1	pat-2 encodes an alpha integrin subunit; during embryogenesis, pat-2 is essential for body wall muscle assembly and function	Integrin alpha subunit
WBGene00003963	pdi-2	C07A12.4		Protein disulfide isomerase (prolyl 4-hydroxylase beta subunit)
WBGene00004408	rfa-0	F25H2.10	rfa-0 encodes an acidic ribosomal subunit protein P0.	60S acidic ribosomal protein P0
WBGene00004415	rpl-4	B0041.4	rpl-4 encodes a large ribosomal subunit L4 protein.	Ribosomal protein RPL1/RPL2/RL4L4
WBGene00004754	sec-23	Y113G7A.3		Vesicle coat complex COPII, subunit SEC23
WBGene00010790	sodh-1	K12G11.3		Alcohol dehydrogenase, class V
WBGene00006536	tbb-1	K01G5.7	This gene encodes a homolog of mammalian beta-tubulin (TUBB) that is expressed at high levels in the germline; TBB-1 is redundant with TBB-2	Beta tubulin
WBGene00006537	tbb-2	C36E8.5	tbb-2 encodes a homolog of mammalian beta-tubulin (TUBB) that is expressed at high levels in the germline; TBB-2 is redundant with TBB-1	Beta tubulin
WBGene00007000	tufm-1	Y71H2AM.23		Mitochondrial translation elongation factor Tu
WBGene00006715	ubc-20	F40G9.3		Ubiquitin-protein ligase
WBGene00006789	unc-54	F11C3.3	unc-54 encodes a muscle myosin class II heavy chain (MHC B); UNC-54 is the major myosin heavy chain expressed in C. elegans muscle	Myosin class II heavy chain
WBGene00006819	unc-87	F08B6.4	unc-87 encodes, through alternative splicing, two proteins that are required to maintain the structure of myofilaments in body wall muscle	Unnamed protein
WBGene00006888	vbh-1	Y54E10A.9	vbh-1 encodes a DEAD-box RNA helicase with similarity to Drosophila Vasa and Belle; vbh-1 activity is required for the proper timing of germline development	ATP-dependent RNA helicase
WBGene00013025	vha-13	Y49A3A.2	vha-13 encodes an ortholog of subunit A of the cytoplasmic (V1) domain of vacuolar proton-translocating ATPase (V-ATPase); V1-ATPase is involved in vacuolar acidification	Vacuolar H ⁺ -ATPase V1 sector, subunit A
WBGene00006925	vit-1	K09F5.2		Predicted lipoprotein
WBGene00006926	vit-2	C42D8.2	The vit-2 gene encodes the vitellogenin homolog YP170.	Predicted lipoprotein
WBGene00006927	vit-3	F59D8.1	vit-3 encodes a vitellogenin, a precursor of the lipid-binding protein related to vertebrate vitellogenins and mammalian ApoB-10	Predicted lipoprotein
WBGene00006928	vit-4	F59D8.2		Predicted lipoprotein
WBGene00006930	vit-6	K07H8.6	vit-6 encodes a 180 kd vitellogenin precursor protein that is cleaved into two smaller yolk proteins, YP115 and YP88.	Predicted lipoprotein
WBGene00007143	B0334.3	B0334.3		Thiamine pyrophosphate-requiring enzyme
WBGene00007463	C08H9.2	C08H9.2		Vigilin
WBGene00016630	C44B7.10	C44B7.10		Acetyl-CoA hydrolase
WBGene00008607	F09B12.3	F09B12.3		Uncharacterized conserved protein Lama
WBGene00009812	F47B10.1	F47B10.1		Succinyl-CoA synthetase, beta subunit
WBGene00019017	F57F4.4	F57F4.4		Unnamed protein

WBGene00010419	H28O16.1	H28O16.1		F0F1-type ATP synthase, alpha subunit
WBGene00010695	K08H10.2	K08H10.2	The K08H10.2 gene encodes a protein (K08H10.2A) that is predicted to be hydrophilic and heat-resistant, and that might participate in	Uncharacterized conserved protein
WBGene00019900	R05G6.7	R05G6.7		Porin/voltage-dependent anion-selective channel protein
WBGene00011480	T05E11.3	T05E11.3	T05E11.3 encodes the C. elegans ortholog of the Hsp90 family member and endoplasmic reticulum (ER) chaperone GRP94/GP96	Endoplasmic reticulum glucose-regulated protein (GRP94/endoplasmic reticulum chaperone), HSP90 family
WBGene00011771	T14G8.3	T14G8.3	T14G8.3 encodes an ortholog of hypoxia-upregulated vertebrate proteins such as human HYOU1; T14G8.3, its C. elegans paralog	Molecular chaperones GRP170/SIL1, HSP70 superfamily
WBGene00020696	T22F3.3	T22F3.3	T22F3.3 is orthologous to the human gene MUSCLE GLYCOGEN PHOSPHORYLASE (PYGM; OMIM:232600), which when mutated in	Glycogen phosphorylase
WBGene00020781	T24H7.2	T24H7.2	T24H7.2 encodes an ortholog of hypoxia-upregulated vertebrate proteins such as human HYOU1; T24H7.2, its C. elegans paralog	Molecular chaperones GRP170/SIL1, HSP70 superfamily
WBGene00012722	Y39G8B.1	Y39G8B.1		Aldo/keto reductase family proteins
WBGene00012964	Y48A6B.3	Y48A6B.3		Box H/ACA snoRNP component, involved in ribosomal RNA pseudouridylation
WBGene00022089	Y69A2AR.18	Y69A2AR.18		F0F1-type ATP synthase, gamma subunit
WBGene00014095	ZK829.4	ZK829.4	ZK829.4 encodes a putative glutamate dehydrogenase, required in mass RNAi assays for normal fertility and rapid growth, that is	Glutamate/leucine/phenylalanine/valine dehydrogenases

Gene Category	List Hits	List Total	Population I	Population †	Probability	Bonferroni	Gene Sequence Name
>=2 down daf-2 (D6) ; Halaschek-Wiener ; 2005	25	68	234	19057	7.64E-31	1.21E-28	B0041.4; C07A12.4; C07H6.5; C15H9.6; C26D10.2; C34E10.6; C36E8.5; C42D8.2; DY3.2; F25H5.4; F33H1.2; F59D8.1; F59D8.2; H28O16.1; K01G5.7; K07H8.6; K08H10.1; K09F5.2; M03F4.2; T03E6.7; T05E11.3; T25C8.2; Y41E3.10; Y69A2AR.18; ZK829.4
Proteome changes <i>S. aureus</i> ; Bogaerts ; 2010	17	68	109	19057	7.22E-24	1.14E-21	T05E11.3; T25C8.2; Y113G7A.3; Y38A10A.5; Y49A3A.2
Differentially expressed proteins in crt-1;cnx-1 vs N2 at 20C ; Lee ; 2006	7	68	13	19057	9.04E-15	1.43E-12	C07A12.4; C15H9.6; C42D8.2; F58G1.4; K12G11.3; M03F4.2; Y49A3A.2
Protein expression ; Kim ; 2001	14	68	446	19057	4.70E-10	7.42E-08	Y41E3.10; Y48A6B.3
Proteome changes <i>Aeromonas h.</i> ; Bogaerts ; 2010	7	68	64	19057	2.84E-09	4.48E-07	F25H2.10; K07H8.6; K12G11.3; M6.1; Y22D7AL.5; Y41E3.10; Y45G12B.1
>=2 down dauer ; Halaschek-Wiener ; 2005	6	68	36	19057	2.95E-09	4.66E-07	C42D8.2; F59D8.1; F59D8.2; K01G5.7; K07H8.6; K09F5.2
Ortholog_Worm,Fly ; McCarroll ; 2004	35	68	3707	19057	3.37E-09	5.33E-07	ZK829.4
828 genes marked by O-GlcNAc ; Love ; 2009	16	68	828	19057	2.46E-08	3.89E-06	T22F3.3; Y22D7AL.5; Y38A10A.5; ZK829.4
Differentially expressed proteins in crt-1;cnx-1 vs N2 at 25C ; Lee ; 2006	4	68	12	19057	7.18E-08	1.13E-05	C07A12.4; C15H9.6; C42D8.2; M03F4.2
up >1,75 fold in <i>M. luteus</i> versus <i>Pseudomonas sp</i> ; Coolon ; 2009	6	68	69	19057	1.66E-07	2.62E-05	F25H2.10; F54H12.6; K09F5.2; M03F4.2; T03E6.7; Y55B1AR.1
Glycoproteins Gal6 binding ; Kaji ; 2007	9	68	287	19057	7.96E-07	1.26E-04	C42D8.2; F09B12.3; F54F2.1; F57F4.4; F59D8.1; F59D8.2; K09F5.2; T14G8.3; T24H7.2
Heat shock ; Kim ; 2001	4	68	25	19057	1.77E-06	2.80E-04	C15H9.6; T05E11.3; T14G8.3; Y22D7AL.5
Cell structural,muscle ; Kim ; 2001	9	68	332	19057	2.65E-06	4.18E-04	C36E8.5; C43C3.1; DY3.2; F08B6.4; F11C3.3; K01G5.7; M03F4.2; M6.1; T25C8.2
down after Organophosphorus pesticide chlorpyrifos + diazinon ; Vinuela ; 2010	5	68	65	19057	3.49E-06	5.51E-04	C42D8.2; F59D8.1; F59D8.2; K07H8.6; K09F5.2
Glycoproteins Con A binding ; Kaji ; 2007	11	68	587	19057	6.72E-06	1.06E-03	C07A12.4; C42D8.2; F09B12.3; F54F2.1; F57F4.4; F59D8.1; F59D8.2; K07H8.6; T03E6.7; T14G8.3; T24H7.2
Regulated DOWN_daf2 mutant&RNAi,Class2,-IGF1 ; Murphy ; 2003	7	68	222	19057	1.40E-05	2.22E-03	C07H6.5; C42D8.2; F57F4.4; F58G1.4; F59D8.1; F59D8.2; T03E6.7
down >1,75 fold in <i>Pseudomonas sp</i> vs <i>E. coli</i> ; Coolon ; 2009	4	68	44	19057	1.81E-05	2.86E-03	F25H2.10; F54H12.6; M03F4.2; Y69A2AR.18
down after Organophosphorus pesticide diazinon ; Vinuela ; 2010	5	68	121	19057	7.19E-05	1.14E-02	C42D8.2; F59D8.1; F59D8.2; K07H8.6; K09F5.2
Regulated DOWN_Bt toxin,Cry5B ; Huffman ; 2004	8	68	442	19057	0.00017	2.72E-02	F09B12.3; F57F4.4; F58G1.4; K09F5.2; M6.1; R05G6.7; Y48A6B.3; ZK455.1
Differentially expressed proteins in crt-1vs N2 at 20C ; Lee ; 2006	2	68	7	19057	0.00026	4.11E-02	C07A12.4; C15H9.6
Expressed in the epidermis ; Wormbase WS220	11	68	888	19057	0.00028	4.49E-02	C07A12.4; C34E10.6; F10C1.2; H28O16.1; M6.1; R05G6.7; T03E6.7; Y113G7A.3; Y38A10A.5; Y69A2AR.18; ZK455.1
Age-Regulated Genes ; Budovskaya ; 2008	12	68	1084	19057	0.00041	6.45E-02	C18A11.7; C42D8.2; F09B12.3; F10C1.2; F47B10.1; F57F4.4; K07H8.6; K09F5.2; K10B3.7; K12G11.3; T03E6.7; Y49A3A.2
Regulated DOWN_Cadmium ; Huffman ; 2004	7	68	388	19057	0.00046	7.23E-02	C42D8.2; F09B12.3; F57F4.4; R05G6.7; Y48A6B.3; Y49A3A.2; ZK455.1
Energy generation ; Kim ; 2001	4	68	104	19057	0.00052	8.24E-02	C34E10.6; F47B10.1; H28O16.1; K10B3.7
up >1,75 fold in <i>B. megaterium</i> versus <i>Pseudomonas sp</i> ; Coolon ; 2009	3	68	45	19057	0.00055	8.75E-02	F25H2.10; F54H12.6; M03F4.2
>=2 down oxidative stress ; Park ; 2009	2	68	13	19057	0.00095	1.51E-01	F58G1.4; T21G5.3
DNA synthesis ; Kim ; 2001	7	68	440	19057	0.00096	1.52E-01	C34E10.6; F47B10.1; H28O16.1; K10B3.7; T22F3.3; ZK455.1; ZK829.4
UP>=2 by PA14 8h ; Troemel ; 2006	5	68	233	19057	0.00146	2.30E-01	F09B12.3; F59D8.1; F59D8.2; K09F5.2; Y39G8B.1
Overlap Between Oxidative Stress and Aging- DOWN-regulated genes by oxidative stress ; Park ; 2009	3	68	66	19057	0.00169	2.68E-01	F10C1.2; F47B10.1; Y49A3A.2
Infection-Upregulated P.luminescens ; Wong ; 2007	8	68	652	19057	0.00218	3.44E-01	F40F9.6; F57F4.4; F58G1.4; K08H10.1; K12G11.3; Y38A10A.5; Y49A3A.2; Y55B1AR.1
down after Organophosphorus pesticide chlorpyrifos ; Vinuela ; 2010	6	68	389	19057	0.00262	4.15E-01	C42D8.2; F10C1.2; F59D8.1; K07H8.6; K09F5.2; K12G11.3
Regulated DOWN_>2fold,Dauer/[Dauer+12hrs] ; McElwee ; 2004	11	68	1175	19057	0.00280	4.42E-01	C07A12.4; C18A11.7; C36E8.5; C43C3.1; F09B12.3; F10C1.2; F47B10.1; F54F2.1; F57B9.6; Y48A6B.3; ZK455.1
Genes Downregulated in ets-4(ok165) ; Thyagarajan ; 2010	3	68	88	19057	0.00384	6.07E-01	C42D8.2; F59D8.1; F59D8.2
>=2 up from D6 to D10 ub daf-2 ; Halaschek-Wiener ; 2005	2	68	27	19057	0.00416	6.57E-01	B0041.4; C15H9.6
>=2 down after 8 h infection with <i>S. aureus</i> ; Irazoqui ; 2010	4	68	198	19057	0.00548	8.66E-01	C18A11.7; F09B12.3; F59D8.1; K09F5.2
Down >0.5x after <i>B. thuringiensis</i> infection in N2 ; Boehnisch ; 2011	2	68	35	19114	0.00688	1.00E+00	F59D8.1; K09F5.2
>=2 down from D1 to D6 ub daf-2 ; Halaschek-Wiener ; 2005	2	68	35	19057	0.00692	1.00E+00	B0041.4; C15H9.6
Regulated DOWN_various genes,xenobiotics ; Reichert ; 2005	2	68	39	19057	0.00854	1.00E+00	K07H8.6; K09F5.2
Regulated UP_in dys1 mutants ; Towers ; 2006	2	68	41	19057	0.00940	1.00E+00	F59D8.1; M6.1
down skn-1 mutant, normal culture condition ; Oliveira ; 2009	4	68	232	19057	0.00949	1.00E+00	C43C3.1; F09B12.3; T25C8.2; ZK455.1



Supplementary Figure 1. Quantification of *pnlp-29::GFP* expression in different mutant backgrounds following infection. The normalized average ratio of green fluorescence to TOF is shown. The analysis was restricted to worms with a TOF above 450. The results shown are representative of several independent experiments. It should be noted that for reasons that are currently unclear, the decreased fluorescence ratio seen in the *atf-6* mutant was not always (3/7 experiments) obvious.