

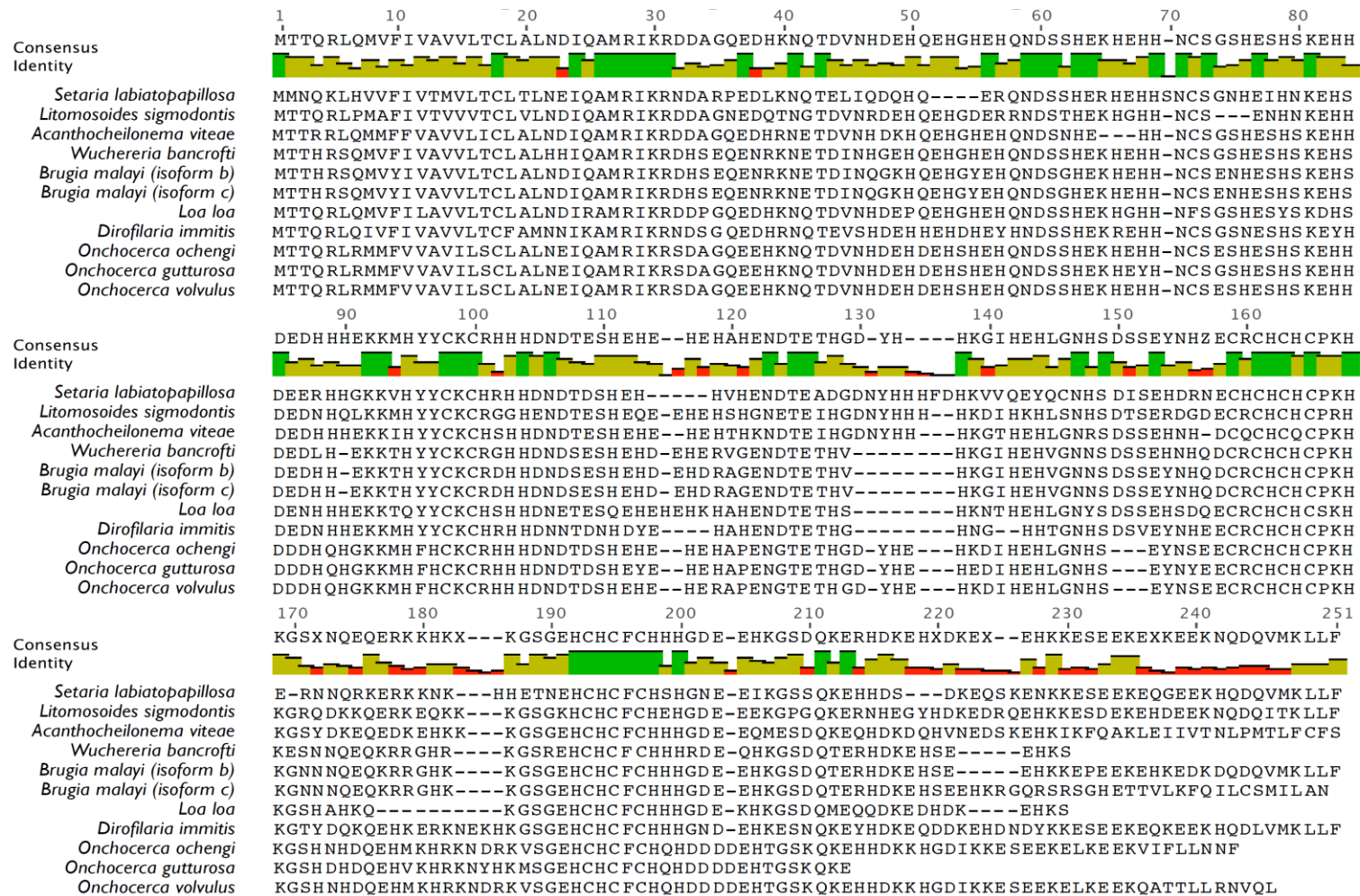
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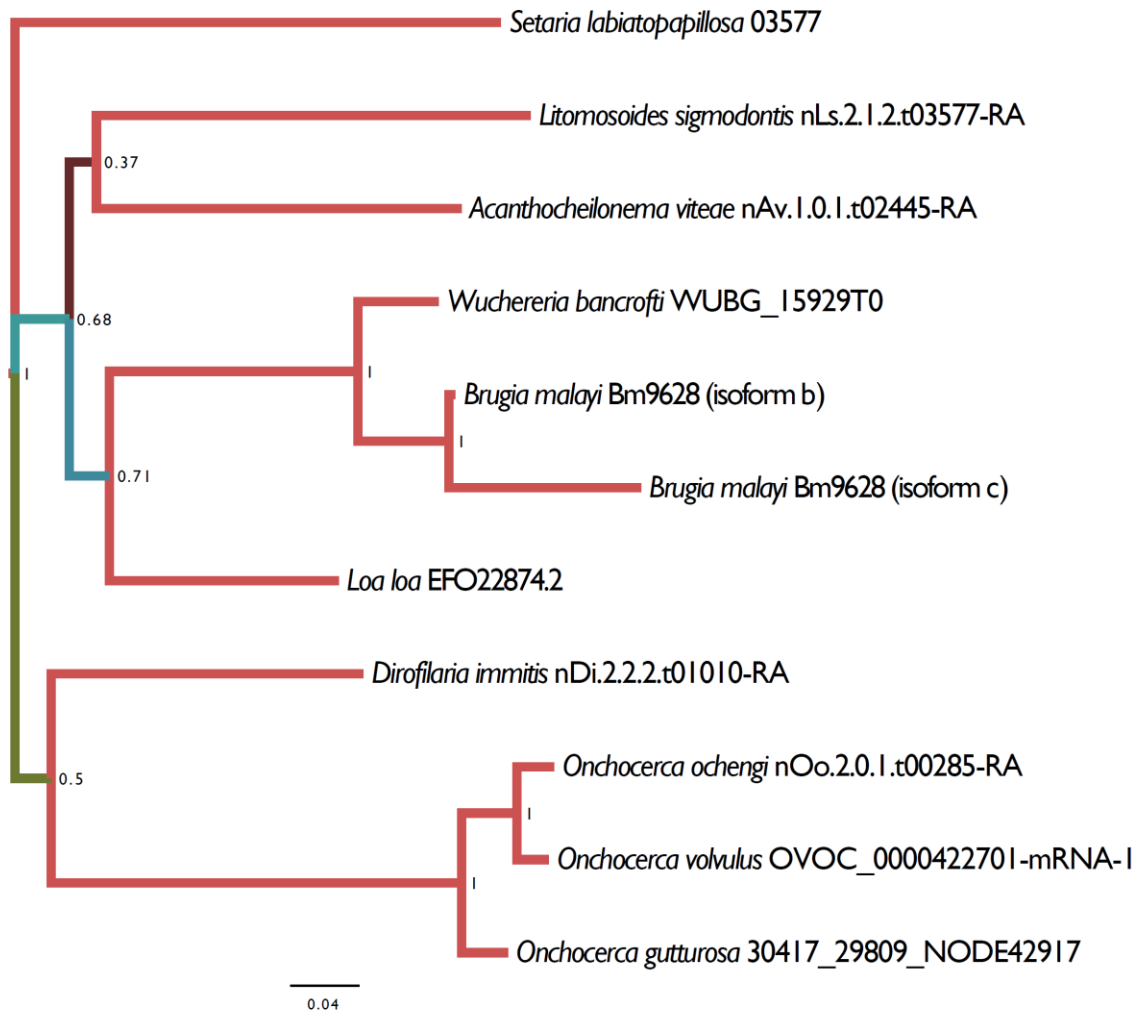
SUPPLEMENTAL FIG. S1 [see file Supplemental-figure-S1.xlsx]. **Distribution of ESP proteins between life stages of *L. sigmodontis*.** Interactive Venn diagram of the shared and stage-specific ESP proteins in each of the life stages examined.

MSPFILLALLINAPANCRPDNGISRSDASSAC^CYDKDPD^CSSDI^CKNYPYTAKER^CPKF^CGGL^CSDTV
GSSARPSSQFLPSSSQRQSLALTS^CGAVEKERKSLTS^CTDKSD^CTAEI^CRNYPFTARER^CAKT^CGR^C
DDVAIGSGSTTAAHRSTAFGVEKFKGGSASSLS^CPRIGNALISGSL^CFDRKFD^CSREI^CCRDFPFTARQ
E^CAKT^CGF^CSVDTSISSSSSNATLRVMSPSVEIGGSSGGTSSHRTAKQDSYEANHNI^CPAYPRLSRGEE
LE^CVVDNID^CTQQT^CCKDYPFTARER^CAKT^CGF^CCRKGSVVEERHSSLPAAQGNKATAI^CTKE^CCKEDDSQ^C
SERS^CLEHPYKASRK^CAKT^CGF^CGEKSSYGSVIELESPIAASSDEGSVIALDSDGNDGSST^RS^CTMTSE
RRLTSGSGDTMSMQPKHSSIRGRTPIRSSSSASTAHIQQP^CTNKQYLGTQRYPGRTGP^CTDANQL^CE
KAD^CY^CKY^CPNFSQ^CKY^CCEKT^CNY^C

SUPPLEMENTAL FIG. S3. Domain organisation of protein nLs_04059 from *Litomosoides sigmodontis*.
Linear representation of the amino-acid sequence highlighting the signal peptide (indigo box), six ShK toxin-like domains (open rectangles) containing six cysteine residues each (yellow), and a predicted propeptide cleavage site (red). Domain six at the C-terminus is unique in containing two lyslytyrosine dyads (cyan).

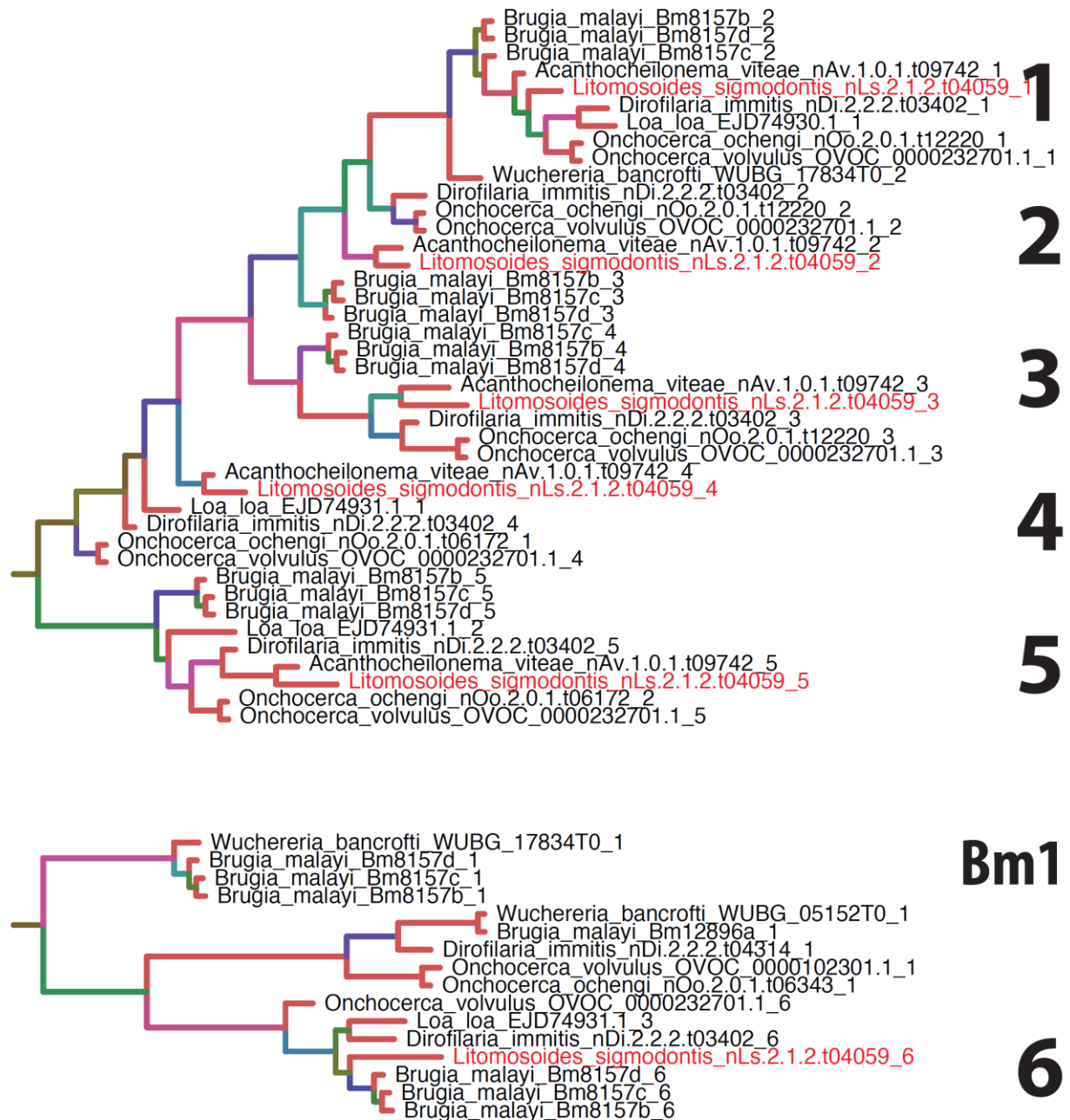


SUPPLEMENTAL FIG. S4. Amino acid sequence alignment of *L. sigmodontis* protein nLs_03577 and its orthologues in other filarial nematodes. Homologues of nLs_03577 were identified by BLASTp search of protein databases from sequenced nematode genomes and a transcriptome assembly for *Setaria labiatopapillosa* (G. Koutsovoulos, B. Makepeace, M. Blaxter; unpublished). No homologues were found outside the filarial nematodes. The protein sequences were aligned with ClustalOmega, and identity is indicated by a coloured scale (green, high; yellow, moderate; red, low).

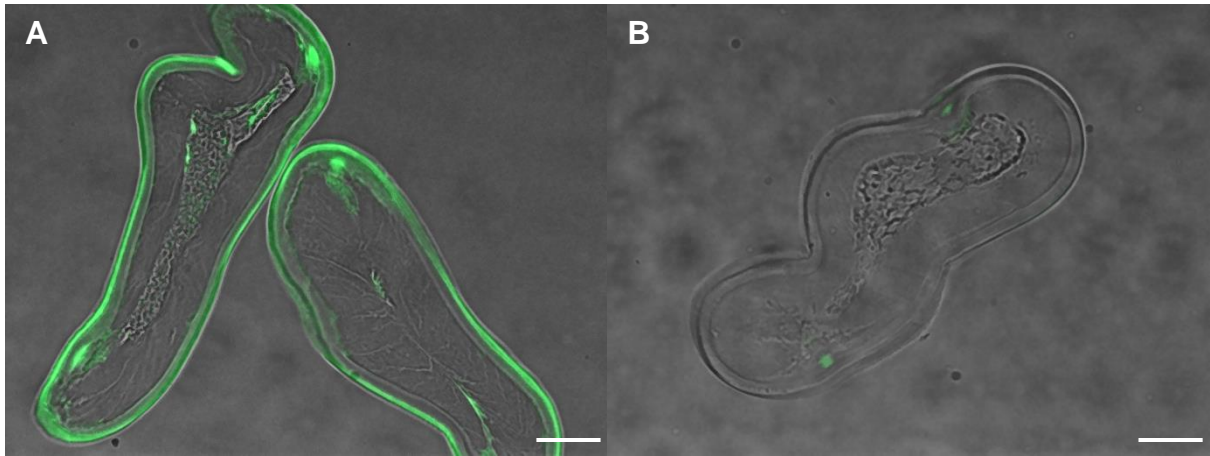


SUPPLEMENTAL FIG. S5. **Rooted phylogenetic tree of *L. sigmodontis* protein nLs_03577 and its orthologues in other filarial nematodes.** Homologues of nLs_03577 were identified by BLASTp search of protein databases from sequenced nematode genomes and a transcriptome assembly for *Setaria labiatopapillosa* (G. Koutsovoulos, B. Makepeace, M. Blaxter; unpublished). No homologues were found outside the filarial nematodes. The protein sequences were aligned with ClustalOmega and the alignment subjected to phylogenetic analysis using MrBayes version 3.2. Every 100th generation from the final 1 million generations of a 2 million generation analysis were combined to derive the consensus shown. Posterior probabilities are indicated by branch colouring (red: pp = 1) The tree is rooted with *S. labiatopapillosa*, in accordance with accepted systematics, and nuclear small subunit ribosomal RNA phylogeny.

SUPPLEMENTAL FIG. S6. **Rooted phylogenetic tree of ShK domains among predicted proteins in filarial nematodes.** The rooted subtrees for the six ShK domains from the nLs_04059 orthologues are shown. Node support is indicated by colour on the branches (red: posterior probability = 1). In *B. malayi*, domain 1 is represented by two distinct isoform clusters, one of which (Bm1) is found only in this species and in *W. bancrofti*.



SUPPLEMENTAL FIG. S7 [see file Supplemental-figure-S7.pdf]. **Unrooted phylogenetic tree of ShK domains among predicted proteins in filarial nematodes and *Ascaris suum*.** The unrooted cladogram is the consensus of the last 1 million of 2 million generations of the analysis, sampled every 100 generations. Node supports are indicated by dots (width is proportional to support) and colour (red: posterior probability of 1). The clades containing the six nLs_04059-like domains are highlighted.



SUPPLEMENTAL FIG. S8. **Distribution of biotin in labelled and unlabelled specimens of adult *Litomosoides sigmodontis*.** Fixed worm sections were incubated with streptavidin-FITC. *A*, Biotin-labelled worms. *B*, An unlabelled control specimen. Scale bars represent 20 μm .

SUPPLEMENTAL TABLE S1 [see file “Supplemental-table-S1.xlsx”]. **Protein predictions from the *Litomosoides sigmodontis* genome, including *Brugia malayi* and *Dirofilaria immitis* orthologues and MS evidence.** Tab 1, summary overview of nuclear-encoded proteins, including annotations, *Brugia* and *Dirofilaria* orthologues, and presence in ESP and/or WBE preparations; tab 2, summary overview of detected wLs-encoded proteins; tabs 3 – 7, protein inference data from ESP and WBE preparations for gAF, pgAF, AM, iMf and vL3, respectively; tab 8, protein inference data for adult nematode surface extracts; tabs 9 – 13, peptide assignment data from ESP and WBE preparations for gAF, pgAF, AM, iMf and vL3, respectively; tab 14, peptide assignment data for adult nematode surface extracts. On tab 1, “Updated description” in column P includes revised annotations for some ESP proteins following manual curation.

SUPPLEMENTAL TABLE S2

Homologues of abundant *Litomosoides sigmodontis* excretory-secretory proteins identified by DELTA-BLAST (National Centre for Biotechnology Information)

Query	Filter ^a	Top annotated hit ^b [species] and accession	Max. score	Identity (%)	Query cover (%)	E-value
nLs_00113	AT	PAN domain containing protein [<i>Brugia malayi</i>] XP_001900239.1	652	37	77	0.0
	FE	Flagellin [<i>Salmonella enterica</i>] WP_023208887.1	134	15	12	2 ⁻²⁸
	CO	Protein SRAP-1, isoform a [<i>C. elegans</i>] NP_496398.3	114	26	56	3 ⁻²⁴
nLs_01398	AT	Protein UNC-52, isoform m [<i>Caenorhabditis elegans</i>] NP_001254444.1	1848	52	97	0.0
nLs_02001	AT	KH domain-containing protein [<i>Loa loa</i>] EFO27012.2	513	75	58	2 ⁻¹⁷⁴
	FE	Far upstream element-binding protein 1-like [<i>Setaria italica</i>] XP_004972470.1	97.4	18	65	5 ⁻¹⁸
	CO	RNA helicase GLH-2 [<i>C. elegans</i>] AAB03337.1	72.0	25	33	2 ⁻¹²
nLs_03577	AT	Hypothetical protein Bm1_38495 [<i>Brugia malayi</i>] XP_001899152.1	128	60	100	2 ⁻³²
	FE	Heavy metal translocating P-type ATPase [<i>Dorea</i> sp. 5-2] WP_016217557.1	63.5	29	74	2 ⁻⁰⁸
	CO	Protein THOC-2 [<i>C. elegans</i>] NP_498392.2	42.0	27	55	1 ⁻⁰³
nLs_04059	AT	Hypothetical protein LOAG_17826 [<i>Loa loa</i>] EJD74931.1	262	51	87	7 ⁻⁸⁰
	FE	A disintegrin and metalloproteinase with thrombospondin motifs 3-like [<i>Aplysia californica</i>] XP_005091919.1	52.0	29	68	7 ⁻⁰⁴
	CO	- ^c	-	-	-	-
nLs_05850	AT	Hypothetical protein LOAG_04060 [<i>Loa loa</i>] XP_003139645.1	269	54	94	1 ⁻⁸⁰
	FE	Chondroitin proteoglycan 2 [<i>Ascaris suum</i>] ERG86992.1	247	25	98	1 ⁻⁶⁸
	CO	CBR-CPG-2 protein [<i>C. briggsae</i>] XP_002633936.1	218	20	93	8 ⁻⁶³
nLs_08836	AT	Apolipoprotein [<i>Ascaris suum</i>] ERG86007.1	1535	42	99	0.0
	FE	Zonadhesin-like [<i>Saccoglossus kowalevskii</i>] XP_002738323.1	256	19	44	4 ⁻⁶⁵
	CO	Protein VIT-4 [<i>C. elegans</i>] NP_508612.1	97.1	21	8	9 ⁻²⁰
nLs_01626	AT	Animal heme peroxidase [<i>Loa loa</i>] XP_003141164.1	1367	84	98	0.0
	FE	Peroxidase-like protein [<i>Ascaris suum</i>] ERG87495.1	1308	72	98	0.0
	CO	CBR-PXN-2 protein [<i>C. briggsae</i>] XP_002644069.1	1093	47	99	0.0

AT, all taxa; FE, Filarioidea excluded; CO, *Caenorhabditis* only.

^a Filters were applied only where the top hit was to taxa other than *Caenorhabditis* spp.

^b Only annotated hits are shown for non-filarial proteins.

^c The only hits were to hypothetical proteins containing ShK domains.

SUPPLEMENTAL TABLE S3

Homologues of abundant Litomosoides sigmodontis excretory-secretory proteins identified by PSI-BLAST (Phyre²)

Query	Top annotated hit [species]	UniRef50 ID	Bits	Normalised identity (%)	E-value
nLs_02001	Transcription elongation factor SPT5 [<i>Cryptococcus neoformans</i> var. <i>neoformans</i> serotype D]	POCR70	135	14.9	3 ⁻³⁰
nLs_04059	Sortilin-related receptor [<i>Homo sapiens</i>]	Q92673	210	10.0	1 ⁻⁵²
nLs_08836	SCO-spondin [<i>Danio rerio</i>]	B3LF39	351	10.4	1 ⁻⁹⁴

Extended narrative - Abundant proteins released by larval parasites

The dominant serum components identified in bMf ESP were fibronectin, complement C3, serum albumin, hemopexin, plasminogen and ceruloplasmin; while lower amounts of IgM were also detected (supplemental Table S4). Of the five quantifiable parasite-derived molecules, three were TTL proteins. Interestingly, the two most abundant parasite ESP proteins observed in bMf, a TTL protein and a nematode-specific uncharacterised protein (nLs_03443), were not present in iMf ESP (supplemental Table S4). Non-unique but proportionally enriched proteins in iMf included two galectins (β -galactoside-binding proteins 1 and 2), a fatty acid and retinoid-binding protein (FAR-1), and a nucleoside diphosphate kinase (supplemental Fig. S2b), all of which are known to be expressed throughout the filarial lifecycle (1, 2). In addition, Ls110, which is secreted from the uterine epithelium during embryonic development (3), was detected in iMf ESP but not iMf WBE. Conversely, the major sheath proteins Shp1a and Shp4 were found in iMf WBE but were not secreted (supplemental Table S1). Another distinctive feature of the iMf ESP was the overrepresentation of two proteoglycan core proteins: a perlecan-like protein that exhibited moderate similarity to UNC-52 from *C. elegans* (supplemental Table S2, supplemental Fig. S2b) (4); and a chondroitin proteoglycan (CPG) containing six peritrophin-A chitin-binding domains, which was distantly related to *C. elegans* CPG-2 (5) (supplemental Tables S1 and S2). However, a large (~250 kDa predicted mass) plasminogen-apple-nematode (PAN) domain protein, which displayed weak similarity (supplemental Table S2) to the predicted mucin SRAP-1 from *C. elegans* (6), was more abundant than either of the proteoglycans in iMf ESP (supplemental Fig. 2b). Finally, an apparently novel peroxidase-like protein with orthologues in other filarial nematodes and more distant relatives in *A. suum* and *Caenorhabditis briggsae* (supplemental Table S2) was also identified in iMf ESP (supplemental Fig. 2b).

In many filarial nematodes, microfilariae are enclosed in a proteinaceous sheath comprising an inner layer that originates from the eggshell and an outer layer that is produced by secretions in the distal portion of the uterus. Five major structural proteins have been identified in the *L. sigmodontis* sheath, some of which are synthesised in the developing embryo and others in the uterine epithelium (7), but none of these were found in iMf ESP, indicating that they are stable components. Many host serum proteins were released from bMf in culture. These are likely to derive from specific interactions with the parasite surface, perhaps reflecting a tension between the nematode exploiting the host and the host immune system recognising the parasite. The finding of host material at the Mf surface is not new, as five serum components were the only proteins released by SDS extraction of *L. sigmodontis* Mf sheaths (8), and human serum albumin has been detected on the sheath surface of *W. bancrofti* Mf (9), but is generally not found on *Brugia* spp. Mf (10). The *L. sigmodontis* sheath is permeable to molecules of up to 70 kDa (11), and therefore might retain some host proteins after transfer to culture. However, several abundant serum proteins that we detected in bMf ESP are considerably larger than this (for

example, ceruloplasmin and fibronectin); therefore, they must be either adsorbed onto the sheath surface or proteolytically processed prior to uptake. Hemopexin and ceruloplasmin have roles in heme and copper transport (12), respectively. Hence, they might be exploited as a source of these essential cofactors by the parasite.

Several parasite-derived products were identified as secreted by iMf, including Ls110 [a protein localised in the uterine lumen and variably present on iMf, but absent from bMf (3)] and two putative proteoglycan core proteins. Accordingly, large glycoproteins (~200 kDa) have been described from *B. malayi* ESP (13). The closest *C. elegans* homologue of the perlecan-like proteoglycan, UNC-52, is a major component of the basement membrane of contractile tissues, including the pharynx and anus in developing embryos and subsequent stages (4). The *L. sigmodontis* iMf-derived CPG-like protein is predicted to have chitin-binding domains and may function in eggshell and sheath development. In *C. elegans*, CPGs form an inner layer that binds to the central chitinous layer of the eggshell, maintaining the perivitelline space around the embryo (14) and forming a barrier to prevent polyspermy (15). In *L. sigmodontis*, chitin has been detected in the oocytes and zygotes, although it is absent from the iMf sheath (16). The degradation of chitin during Mf sheath development *in utero* may release the underlying CPG, which is highly soluble (14), into the surrounding milieu. The origin and roles two of the other novel proteins that were enriched in iMf ESP is less clear. The closest homologue in *C. elegans* of the PAN domain protein is SRAP-1, which is expressed in the hypodermis, central nervous system and vulva of developing larvae and is secreted onto the cuticle surface during moulting (17). In *C. elegans*, peroxidase PXN-2 is located in the extracellular matrix and is required for late embryonic elongation, muscle attachment, and motoneuron axon guidance choice (18).

The vL3 ESP was composed of previously characterised filarial proteins that are known to be uniquely expressed or enriched in this stage [such as ASP-1 (19), ALT-1 (20), and cathepsin-L-like protease (21)], alongside other antigens that were well represented in ESP from other stages (RAL-2, CPI-2, Ov16 and β -galactoside-binding proteins) (supplemental Fig. 2a). The nematode secreted protein 22U was moderately abundant in the *L. sigmodontis* vL3 ESP preparations (supplemental Fig. 2a), but apparently is not expressed in vL3 of other filarial species (22). This stage may be relatively quiescent in terms of secretory activity until they adapt to the mammalian host and undergo the third moult. Indeed, analysis of ESP from moulting L3 identified fivefold more proteins than from vL3 ESP in *B. malayi* (23).

SUPPLEMENTAL TABLE S4

Quantifiable proteins present in the excretory-secretory products of blood-derived microfilariae

Accession Gene name	Peptides used for quantification	Confidence score	Description (species)	Normalised iBAQ
Q91X72 HEMO_MOUSE	7	898.19	Hemopexin (<i>Mus musculus</i>)	1.44 ⁻⁰¹
Q8VCM7 FIBG_MOUSE	8	1040.62	Fibrinogen γ chain (<i>Mus musculus</i>)	1.15 ⁻⁰¹
Q8K0E8 FIBB_MOUSE	9	1594.25	Fibrinogen β chain (<i>Mus musculus</i>)	9.56 ⁻⁰²
O35090 ALBU_MERUN	29	5013.66	Serum albumin (<i>Meriones unguiculatus</i>)	6.09 ⁻⁰²
P70274 SEPP1_MOUSE	3	203.64	Selenoprotein P (<i>Mus musculus</i>)	5.76 ⁻⁰²
Q61147 CERU_MOUSE	12	2215.19	Ceruloplasmin (<i>Mus musculus</i>)	5.56 ⁻⁰²
P29788 VTNC_MOUSE	6	1035.62	Vitronectin (<i>Mus musculus</i>)	5.43 ⁻⁰²
Q61702 ITIH1_MOUSE	7	1236.34	Inter- α -trypsin inhibitor heavy chain H1 (<i>Mus musculus</i>)	5.32 ⁻⁰²
P11276 FINC_MOUSE	49	8184.33	Fibronectin (<i>Mus musculus</i>)	3.80 ⁻⁰²
P01027 CO3_MOUSE	28	3368.39	Complement C3 (<i>Mus musculus</i>)	3.63 ⁻⁰²
P01942 HBA_MOUSE	2	149.76	Hemoglobin subunit α (<i>Mus musculus</i>)	3.60 ⁻⁰²
P97515 FETUA_MERUN	6	665.57	α -2-HS-glycoprotein (<i>Meriones unguiculatus</i>)	3.24 ⁻⁰²
P20918 PLMN_MOUSE	13	2062.8	Plasminogen (<i>Mus musculus</i>)	3.13 ⁻⁰²
P13020 GELS_MOUSE	5	1587.22	Gelsolin (<i>Mus musculus</i>)	2.83 ⁻⁰²
Q62577 AMBP_MERUN	6	1120.89	Protein AMBP (<i>Meriones unguiculatus</i>)	2.33 ⁻⁰²
P01029 CO4B_MOUSE	11	1814.14	Complement C4-B (<i>Mus musculus</i>)	1.52 ⁻⁰²
P05367 SAA2_MOUSE	4	820.91	Serum amyloid A-2 protein (<i>Mus musculus</i>)	1.34 ⁻⁰²
Q61703 ITIH2_MOUSE	7	1223.42	Inter- α -trypsin inhibitor heavy chain H2 (<i>Mus musculus</i>)	9.20 ⁻⁰³
nLs.2.1.2.t10069-RA	4	264.21	Transthyretin-like protein, partial (<i>Litomosoides sigmodontis</i>)	9.11 ⁻⁰³
P04186 CFAB_MOUSE	6	361.74	Complement factor B (<i>Mus musculus</i>)	8.08 ⁻⁰³
P52430 PON1_MOUSE	2	166.19	Serum paraoxonase/arylesterase 1 (<i>Mus musculus</i>)	7.36 ⁻⁰³
Q02105 C1QC_MOUSE	2	292.88	Complement C1q subcomponent subunit C (<i>Mus musculus</i>)	6.31 ⁻⁰³
P06909 CFAH_MOUSE	2	156.16	Complement factor H (<i>Mus musculus</i>)	5.81 ⁻⁰³
P05017 IGF1_MOUSE	2	458.52	Insulin-like growth factor I (<i>Mus musculus</i>)	5.69 ⁻⁰³
P14106 C1QB_MOUSE	2	62.86	Complement C1q subcomponent subunit B (<i>Mus musculus</i>)	3.94 ⁻⁰³
A6X935 ITIH4_MOUSE	4	301.36	Inter α -trypsin inhibitor, heavy chain 4 (<i>Mus musculus</i>)	3.94 ⁻⁰³
E7D4P4 E7D4P4_MERUN	9	1172.7	Apolipoprotein E (<i>Meriones unguiculatus</i>)	3.78 ⁻⁰³
P97298 PEDF_MOUSE	5	420.63	Pigment epithelium-derived factor (<i>Mus musculus</i>)	3.31 ⁻⁰³

P47878 IBP3_MOUSE	5	520.36	Insulin-like growth factor-binding protein 3 (<i>Mus musculus</i>)	3.10 ⁻⁰³
P46412 GPX3_MOUSE	3	275.46	Glutathione peroxidase 3 (<i>Mus musculus</i>)	2.99 ⁻⁰³
Q8BH35 CO8B_MOUSE	4	586.84	Complement component C8 β chain (<i>Mus musculus</i>)	2.99 ⁻⁰³
Q64118 A1AT_MERUN	3	183.98	α -1-antitrypsin (<i>Meriones unguiculatus</i>)	2.89 ⁻⁰³
Q06890 CLUS_MOUSE	5	427.41	Clusterin (<i>Mus musculus</i>)	1.91 ⁻⁰³
P70389 ALS_MOUSE	3	471.58	Insulin-like growth factor-binding protein complex acid labile subunit (<i>Mus musculus</i>)	1.88 ⁻⁰³
P35441 TSP1_MOUSE	8	853.9	Thrombospondin-1 (<i>Mus musculus</i>)	1.81 ⁻⁰³
P68033 ACTC_MOUSE	2	978.66	Actin, α cardiac muscle 1 (<i>Mus musculus</i>)	1.64 ⁻⁰³
Q00724 RET4_MOUSE	4	335.66	Retinol-binding protein 4 (<i>Mus musculus</i>)	1.63 ⁻⁰³
P26262 KLKB1_MOUSE	5	625.92	Plasma kallikrein (<i>Mus musculus</i>)	1.52 ⁻⁰³
Q61704 ITIH3_MOUSE	4	422.55	Inter- α -trypsin inhibitor heavy chain H3 (<i>Mus musculus</i>)	1.52 ⁻⁰³
P19221 THRB_MOUSE	7	587.54	Prothrombin (<i>Mus musculus</i>)	1.45 ⁻⁰³
P33434 MMP2_MOUSE	3	283.7	72 kDa type IV collagenase (<i>Mus musculus</i>)	1.42 ⁻⁰³
Q9JHH6 CBPB2_MOUSE	3	367.6	Carboxypeptidase B2 (<i>Mus musculus</i>)	1.38 ⁻⁰³
P32261 ANT3_MOUSE	2	306.33	Antithrombin-III (<i>Mus musculus</i>)	1.30 ⁻⁰³
nLs.2.1.2.t03443-RA	3	366.35	Hypothetical protein, Bm1_50630 homolog (<i>Litomosoides sigmodontis</i>)	1.18 ⁻⁰³
nLs.2.1.2.t01366-RA	2	269.78	Transthyretin-like protein, partial (<i>Litomosoides sigmodontis</i>)	9.56 ⁻⁰⁴
P11680 PROP_MOUSE	2	36.41	Properdin (<i>Mus musculus</i>)	9.27 ⁻⁰⁴
Q61646 HPT_MOUSE	3	236.91	Haptoglobin (<i>Mus musculus</i>)	8.65 ⁻⁰⁴
Q9JM99 PRG4_MOUSE	5	683.96	Proteoglycan 4 (<i>Mus musculus</i>)	8.57 ⁻⁰⁴
Q92111 TRFE_MOUSE	4	728.55	Serotransferrin (<i>Mus musculus</i>)	8.09 ⁻⁰⁴
P28798 GRN_MOUSE	2	300.7	Granulins (<i>Mus musculus</i>)	7.86 ⁻⁰⁴
P26928 HGFL_MOUSE	4	375.93	Hepatocyte growth factor-like protein (<i>Mus musculus</i>)	7.43 ⁻⁰⁴
Q9JJN5 CBPN_MOUSE	3	219.53	Carboxypeptidase N catalytic chain (<i>Mus musculus</i>)	7.01 ⁻⁰⁴
P47879 IBP4_MOUSE	2	211.08	Insulin-like growth factor-binding protein 4 (<i>Mus musculus</i>)	6.96 ⁻⁰⁴
Q07968 F13B_MOUSE	3	410.66	Coagulation factor XIII B chain (<i>Mus musculus</i>)	6.89 ⁻⁰⁴
Q9DBD0 ICA_MOUSE	6	787.37	Inhibitor of carbonic anhydrase (<i>Mus musculus</i>)	5.99 ⁻⁰⁴
P97290 IC1_MOUSE	4	387.11	Plasma protease C1 inhibitor (<i>Mus musculus</i>)	5.94 ⁻⁰⁴
Q8K182 CO8A_MOUSE	2	332.91	Complement component C8 α chain (<i>Mus musculus</i>)	5.78 ⁻⁰⁴
O70362 PHLD_MOUSE	2	100.85	Phosphatidylinositol-glycan-specific phospholipase D (<i>Mus musculus</i>)	4.92 ⁻⁰⁴
P01872 IGHM_MOUSE	2	174.92	Ig μ chain C region secreted form (<i>Mus musculus</i>)	4.48 ⁻⁰⁴

P06684 CO5_MOUSE	2	338.69	Complement C5 (<i>Mus musculus</i>)	4.07 ⁻⁰⁴
Q8K0D2 HABP2_MOUSE	2	60.74	Hyaluronan-binding protein 2 (<i>Mus musculus</i>)	3.53 ⁻⁰⁴
nLs.2.1.2.t01870-RA	2	196.87	ML domain-containing protein (<i>Litomosoides sigmodontis</i>)	3.44 ⁻⁰⁴
nLs.2.1.2.t01365-RA	2	188.68	Transthyretin-like protein, partial (<i>Litomosoides sigmodontis</i>)	2.74 ⁻⁰⁴
P28665 MUG1_MOUSE	3	312.94	Murinoglobulin-1 (<i>Mus musculus</i>)	2.05 ⁻⁰⁴
Q08879 FBLN1_MOUSE	2	305.68	Fibulin-1 (<i>Mus musculus</i>)	1.90 ⁻⁰⁴
Q8CG16 C1RA_MOUSE	2	102.86	Complement C1r-A subcomponent (<i>Mus musculus</i>)	1.04 ⁻⁰⁴

iBAQ, intensity-based absolute quantification; AMBP, α -1-microglobulin/bikunin precursor.

SUPPLEMENTAL TABLE S5

Putative surface-associated proteins exhibiting >50-fold enrichment in biotin-labelled adult worm whole body extracts relative to unlabelled controls

Parasite stage	Treatment	Peptides used for quantification	Confidence score	Fold-difference	Locus tag	Annotation	Presence in ESP
AM	SDS	4	316.19	1,769.5	nLs_09715	Major sperm protein	Yes
	SDS	2	249.88	341.7	nLs_01747	Filarial antigen RAL-2	Yes
	SDS	6	873.95	62.2	nLs_06907	Adenylate kinase isoenzyme 1	No
	PBS	4	172.54	50.6	nLs_09625	Transthyretin-like protein 5	Yes
gAF	Urea	2	306.56	430.9	nLs_02969	Cysteine protease inhibitor-2	Yes
	Urea	2	180.49	149.4	nLs_08458	Filarial antigen Ov16	Yes
	Urea	2	302.26	65.2	nLs_09625	Transthyretin-like protein 5	Yes
	OG	2	233.80	60,617.8	nLs_09890	Purine nucleoside phosphorylase	Yes
	OG	2	183.22	336.5	nLs_00852	Proliferating cell nuclear antigen domain protein	No
	OG	2	224.62	271.9	nLs_04749	60S ribosomal protein L18	No
	OG	2	191.67	168.3	nLs_01364	Transthyretin-like protein, partial	Yes
	OG	3	194.25	156.3	nLs_02023	Tetratricopeptide-repeat domain protein	Yes
	OG	2	159.32	139.9	nLs_02001	KH domain-containing protein	Yes
	OG	2	188.56	118.1	nLs_08084	Type I inositol-trisphosphate 5-phosphatase	Yes
	OG	3	367.83	79.9	nLs_02969	Cysteine protease inhibitor-2	Yes
	OG	3	367.26	66.6	nLs_02463	FKBP-type peptidyl-prolyl cis-trans isomerase	Yes
	OG	3	220.00	65.6	nLs_00523	KH domain containing protein	Yes
	OG	3	376.34	59.0	wLs_3910	<i>Wolbachia</i> surface protein	No
	OG	2	258.28	52.2	nLs_05241	Tetratricopeptide-repeat domain protein	No
	SDS	2	50.67	1,059.7	nLs_07759	Cyclophilin Ovcyp-2 homologue	Yes
	SDS	6	306.80	328.9	nLs_08458	Filarial antigen Ov16	Yes
	SDS	7	488.42	304.1	nLs_01747	Filarial antigen RAL-2	Yes
	SDS	4	156.74	262.8	nLs_05279	HSP20/ α -crystallin family protein	No
	SDS	2	144.16	242.7	nLs_08696	Lysozyme protein 8, partial	Yes
SDS	2	87.95	235.6	nLs_09890	Purine nucleoside phosphorylase	Yes	
SDS	7	432.37	216.4	nLs_09625	Transthyretin-like protein 5	Yes	
SDS	2	82.06	202.7	nLs_2001	KH domain-containing protein	Yes	

SDS	6	332.26	193.2	nLs_08148	Papilin	Yes
SDS	2	122.31	162.4	nLs_06907	Adenylate kinase isoenzyme 1	Yes
SDS	2	44.96	162.1	nLs_00117	L-lactate dehydrogenase	Yes
SDS	3	144.30	148.4	nLs_05914	Pyruvate dehydrogenase E1 component, α -subunit	Yes
SDS	2	77.92	115.1	nLs_09750	Transthyretin-like protein 45	Yes
SDS	7	329.76	86.5	nLs_03034	p27 heat shock protein homologue	Yes
SDS	5	166.70	82.3	nLs_08836	von Willebrand factor type-d domain protein	Yes
SDS	3	138.70	71.7	nLs_03328	Myosin	No
SDS	2	89.16	67.5	nLs_01364	Transthyretin-like protein, partial	Yes
SDS	6	560.89	52.8	nLs_08415	Enolase	Yes
PBS	5	631.96	111.4	nLs_02378	Aldo/keto reductase family protein	No
PBS	2	309.24	104.0	nLs_03070	Atypical RIO/RIO2 protein kinase	Yes
PBS	3	372.59	93.5	nLs_00473	Aldehyde dehydrogenase 11	Yes
PBS	6	1058.57	89.2	nLs_06488	Acid phosphatase	Yes
PBS	9	1570.87	69.9	nLs_01747	Filarial antigen RAL-2	Yes
PBS	4	647.70	62.5	nLs_03174	Nematode secreted protein 22U	Yes

ESP, excretory-secretory products; AM, adult male; OG, octyl β -D-glucopyranoside; gAF, gravid adult female; FKBP, FK506-binding protein; HSP, heat-shock protein

SUPPLEMENTAL REFERENCES

1. Joseph, G. T., Huima, T., Klion, A., and Lustigman, S. (2000) A novel developmentally regulated galectin of *Onchocerca volvulus*. *Mol. Biochem. Parasitol.* 106, 187-195
2. Garofalo, A., Klager, S. L., Rowlinson, M. C., Nirmalan, N., Klion, A., Allen, J. E., Kennedy, M. W., and Bradley, J. E. (2002) The FAR proteins of filarial nematodes: secretion, glycosylation and lipid binding characteristics. *Mol. Biochem. Parasitol.* 122, 161-170
3. Dafa'alla, T. H., Taubert, A., Hobom, G., Beck, E., and Zahner, H. (2000) Molecular characterization of a *Litomosoides sigmodontis* protein involved in the development of the microfilarial sheath during embryogenesis. *Mol. Biochem. Parasitol.* 106, 37-50
4. Rogalski, T. M., Mullen, G. P., Bush, J. A., Gilchrist, E. J., and Moerman, D. G. (2001) UNC-52/perlecan isoform diversity and function in *Caenorhabditis elegans*. *Biochem. Soc. Trans.* 29, 171-176
5. Olson, S. K., Bishop, J. R., Yates, J. R., Oegema, K., and Esko, J. D. (2006) Identification of novel chondroitin proteoglycans in *Caenorhabditis elegans*: embryonic cell division depends on CPG-1 and CPG-2. *J. Cell Biol.* 173, 985-994
6. Jones, M. R., Rose, A. M., and Baillie, D. L. (2012) Oligoarray comparative genomic hybridization-mediated mapping of suppressor mutations generated in a deletion-biased mutagenesis screen. *G3 (Bethesda)* 2, 657-663
7. Zahner, H., Hobom, G., and Stirm, S. (1995) The microfilarial sheath and its proteins. *Parasitol. Today* 11, 116-120
8. Bardehle, G., Hintz, M., Linder, D., Schares, G., Schott, H. H., Stirm, S., and Zahner, H. (1992) *Litomosoides carinii*: extraction of the microfilarial sheath components and antigenicity of the sheath fractions. *Parasitol. Res.* 78, 501-508
9. Maizels, R. M., Philipp, M., Dasgupta, A., and Partoni, F. (1984) Human serum albumin is a major component on the surface of microfilariae of *Wuchereria bancrofti*. *Parasite Immunol.* 6, 185-190
10. Shenoy, R. K., Rakesh, P. G., Baldwin, C. I., and Denham, D. A. (1996) The sheath of the microfilaria of *Brugia malayi* from human infections has IgG on its surface. *Parasitol. Res.* 82, 382-384
11. Bardehle, G., Jepp-Libutzki, A., Linder, D., Moehnle, K., Schott, H. H., Zahner, H., Zahringer, U., and Stirm, S. (1992) Chemical composition of *Litomosoides carinii* microfilarial sheaths. *Acta Trop.* 50, 237-247
12. Halliwell, B., and Gutteridge, J. M. (1990) The antioxidants of human extracellular fluids. *Arch. Biochem. Biophys.* 280, 1-8

13. Lal, R. B. (1991) Monoclonal antibodies to secreted antigens of *Brugia malayi* define a cross-reactive non-phosphocholine determinant on helminth parasites. *Immunol. Cell Biol.* 69, 127-133
14. Olson, S. K., Greenan, G., Desai, A., Muller-Reichert, T., and Oegema, K. (2012) Hierarchical assembly of the eggshell and permeability barrier in *C. elegans*. *J. Cell Biol.* 198, 731-748
15. Johnston, W. L., Krizus, A., and Dennis, J. W. (2010) Eggshell chitin and chitin-interacting proteins prevent polyspermy in *C. elegans*. *Curr. Biol.* 20, 1932-1937
16. Schraermeyer, U., Peters, W., and Zahner, H. (1987) Lectin binding studies on adult filariae, intrauterine developing stages and microfilariae of *Brugia malayi* and *Litomosoides carinii*. *Parasitol. Res.* 73, 550-556
17. Jones, M. R., Rose, A. M., and Baillie, D. L. (2013) The ortholog of the human proto-oncogene ROS1 is required for epithelial development in *C. elegans*. *Genesis* 51, 545-561
18. Gotenstein, J. R., Swale, R. E., Fukuda, T., Wu, Z., Giurumescu, C. A., Goncharov, A., Jin, Y., and Chisholm, A. D. (2010) The *C. elegans* peroxidase PNX-2 is essential for embryonic morphogenesis and inhibits adult axon regeneration. *Development* 137, 3603-3613
19. Murray, J., Gregory, W. F., Gomez-Escobar, N., Atmadja, A. K., and Maizels, R. M. (2001) Expression and immune recognition of *Brugia malayi* VAL-1, a homologue of vespid venom allergens and *Ancylostoma* secreted proteins. *Mol. Biochem. Parasitol.* 118, 89-96
20. Gregory, W. F., Blaxter, M. L., and Maizels, R. M. (1997) Differentially expressed, abundant trans-spliced cDNAs from larval *Brugia malayi*. *Mol. Biochem. Parasitol.* 87, 85-95
21. Guiliano, D. B., Hong, X., McKerrow, J. H., Blaxter, M. L., Oksov, Y., Liu, J., Ghedin, E., and Lustigman, S. (2004) A gene family of cathepsin L-like proteases of filarial nematodes are associated with larval molting and cuticle and eggshell remodeling. *Mol. Biochem. Parasitol.* 136, 227-242
22. Frank, G. R., Wisnewski, N., Brandt, K. S., Carter, C. R., Jennings, N. S., and Selkirk, M. E. (1999) Molecular cloning of the 22-24 kDa excretory-secretory 22U protein of *Dirofilaria immitis* and other filarial nematode parasites. *Mol. Biochem. Parasitol.* 98, 297-302
23. Bennuru, S., Semnani, R., Meng, Z., Ribeiro, J. M., Veenstra, T. D., and Nutman, T. B. (2009) *Brugia malayi* excreted/secreted proteins at the host/parasite interface: stage- and gender-specific proteomic profiling. *PLoS Negl. Trop. Dis.* 3, e410