Supporting Information

Ascaris suum Informs Extrasynaptic Volume Transmission in Nematodes

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Table S1. The modulatory effects of As-PCF on *Ascaris suum* ovijector tissue preparations.

Test	Time (p.a.)	Contraction freq. (% 0 min, 2 min prior to min p.a.)	Contraction ampl. (% 0 min, 2 min prior to min p.a.)	Change in tension (mg/mm, 5 min p.a.)
4 µl As-PCF n=7	10 secs	N/A	N/A	Sht 0.002±0.01
	20 secs	N/A	N/A	Sht 0.001±0.01
	30 secs	N/A	N/A	Lng -0.02±0.01
	50 secs	N/A	N/A	Lng -0.02±0.01
	70 secs	N/A	N/A	Lng -0.001±0.02
	2 min	↓ 58.16±12.33 ✓	↓ 96.25±7.177 ×	Sht 0.04±0.05
	5 min	↓ 10.57±7.699 ✓	↓ 23.85±17.36 ✓	Sht 0.08±0.05
	10 min	↓ 3.810±3.810 ✓	↓ 16.21±16.21 ✓	Sht 0.11±0.06
	OVERALL	**P<0.0001, F=43.61**	**P=0.0151, F=3.647**	**NS**
40 µl As-PCF n=7	10 secs	N/A	N/A	Lng -0.02±0.04
	20 secs	N/A	N/A	Lng -0.05±0.05
	30 secs	N/A	N/A	Lng -0.08±0.08
	50 secs	N/A	N/A	Lng -0.08±0.10
	70 secs	N/A	N/A	Sht 0.05±0.09
	2 min	↓ 46.48±9.588 ✓	↑ 131.1±23.72 ×	Sht 0.10±0.07
	5 min	↓ Ceased ✓	↓ Ceased ✓	Sht 0.15±0.05
	10 min	↓ Ceased ✓	↓ Ceased ✓	Sht 0.20±0.06
	OVERALL	**P<0.0001, F=98.56**	**P<0.0001, F=32.80**	**NS**
4 µl SB (As-FLP-18A) n=6	10 secs	N/A	N/A	0.00±0.00
	20 secs	N/A	N/A	Lng -0.01±0.01
	30 secs	N/A	N/A	Lng -0.06±0.04
	50 secs	N/A	N/A	Lng -0.13±0.07
	70 secs	N/A	N/A	Lng -0.11±0.07
	2 min	↓ 43.00±5.223 ✓	↑ 154.5±19.98	Lng -0.04±0.06
	5 min	↓ 8.730±7.817 ✓	↓ 81.52±70.6	Sht 0.03±0.04
	10 min	↓ Ceased ✓	↓ Ceased	Sht 0.05±0.04
	OVERALL	**P<0.0001, F=91.35**	**NS**	**NS**
40 µl SB (As-FLP-18A) n=3	10 secs	N/A	N/A	Lng -0.26±0.08
	20 secs	N/A	N/A	Lng -0.26±0.08
	30 secs	N/A	N/A	Lng -0.23±0.09
	50 secs	N/A	N/A	Lng -0.22±0.09
	70 secs	N/A	N/A	Lng -0.20±0.10
	2 min	↓ 6.754±0.8874 ✓	↑ 389.2±171.3	Lng -0.18±0.12
	5 min	↓ Ceased ✓	↓ Ceased	Lng -0.10±0.10
	10 min	↓ Ceased ✓	↓ Ceased	Lng -0.04±0.07
	OVERALL	**P<0.0001, F=12186**	**NS**	**NS**
4 µl SA (As-FLP-18A) n=5	10 secs	N/A	N/A	Lng -0.01±0.01
	20 secs	N/A	N/A	Lng -0.02±0.01
	30 secs	N/A	N/A	Lng -0.03±0.02
	50 secs	N/A	N/A	Lng -0.04±0.04
	70 secs	N/A	N/A	Lng -0.002±0.05
	2 min	↓ 84.28±15.31 ×	↑ 119.3±15.54 ×	Sht 0.008±0.06
	5 min	↓ 52.98±25.80 ×	↓ 68.72±19.68 ×	Sht 0.04±0.06

	10 min	↓ 34.48±21.11 ✓	↓ 35.89±22.41 ✓	Sht 0.05±0.04
	OVERALL	**P=0.0149, F=5.285**	**P=0.0070, F=6.595**	**NS**
40 µl SA (As-FLP-18A) n=4	10 secs	N/A	N/A	Sht 0.03±0.06
	20 secs	N/A	N/A	Sht 0.02±0.07
	30 secs	N/A	N/A	Lng -0.001±0.09
	50 secs	N/A	N/A	Sht 0.05±0.07
	70 secs	N/A	N/A	Sht 0.07±0.05
	2 min	↓ 37.85±22.51 ✓	↓ 41.31±23.90 ✓	Sht 0.09±0.04
	5 min	↓ Ceased ✓	↓ Ceased ✓	Sht 0.11±0.02
	10 min	↓ 3.125±3.125 ✓	↓ 16.90±16.90 ✓	Sht 0.10±0.02
	OVERALL	**P=0.0004, F=17.29**	**P=0.0025, F=10.68**	**NS**
Negative control (n=3)	10 secs	N/A	N/A	N/A
	20 secs	N/A	N/A	N/A
	30 secs	N/A	N/A	N/A
	50 secs	N/A	N/A	N/A
	70 secs	N/A	N/A	N/A
	2 min	↑ 100.36±8.567	↑ 103.70±4.436	0
	5 min	↑ 112.02±5.802	↓ 92.63±5.689	0
	10 min	↑ 116.97±14.66	↓ 88.94±8.705	Lng -0.011±0.01
	OVERALL	**NS**	**NS**	**NS**

% 0 min, values presented as percentage of that recorded at time 0; p.a., post-addition; ↓, denotes decrease; ↑, denotes increase; ×, denotes non-significant Dunnett's post hoc test; ✓, denotes significant Dunnett's post hoc test; NS, not significant; Sht, shortening of tissue; Lng, lengthening of tissue**.**

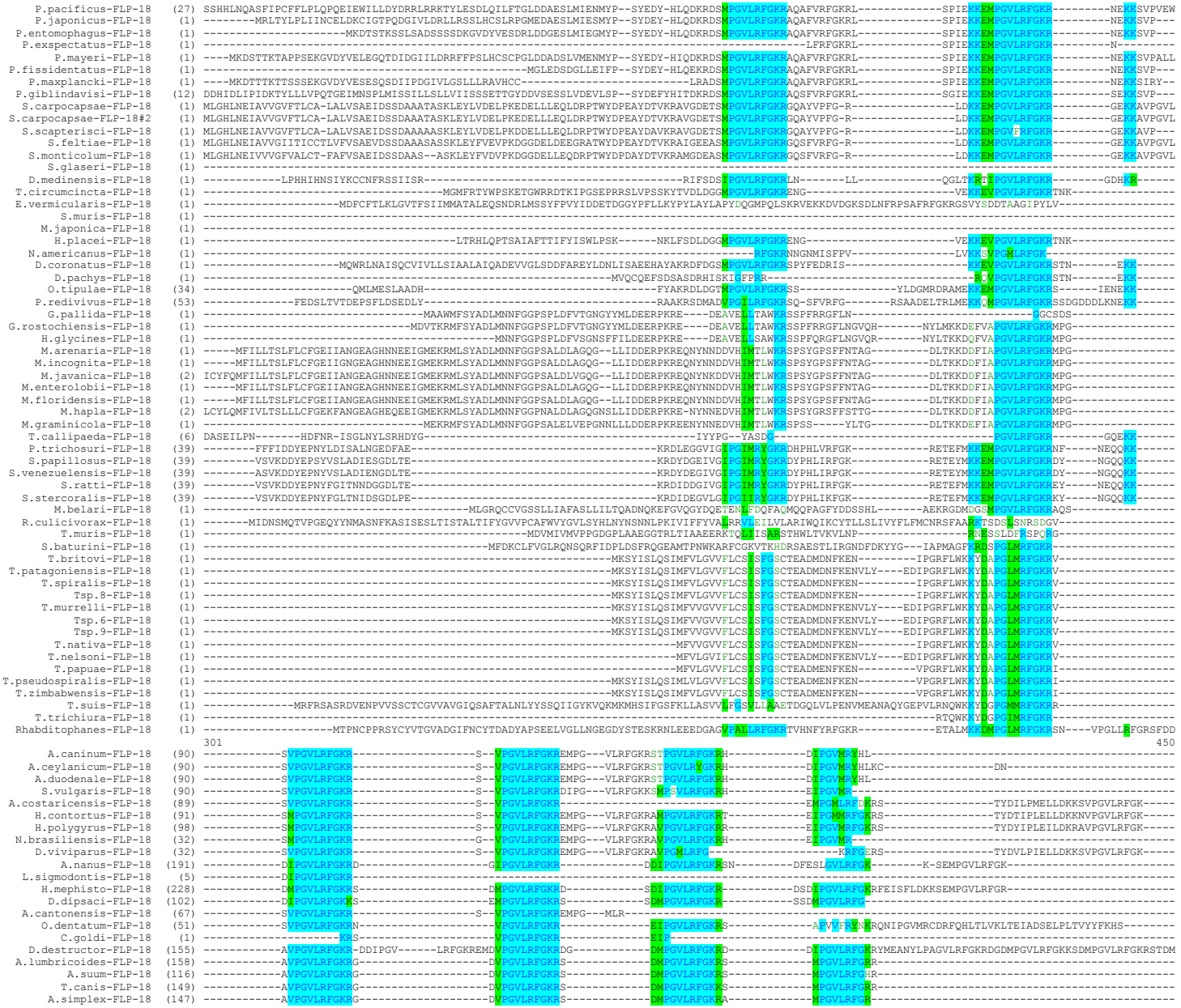
Table S2. The modulatory effects of synthetic As-FLP-18A on *Ascaris suum* ovijector tissue preparations.

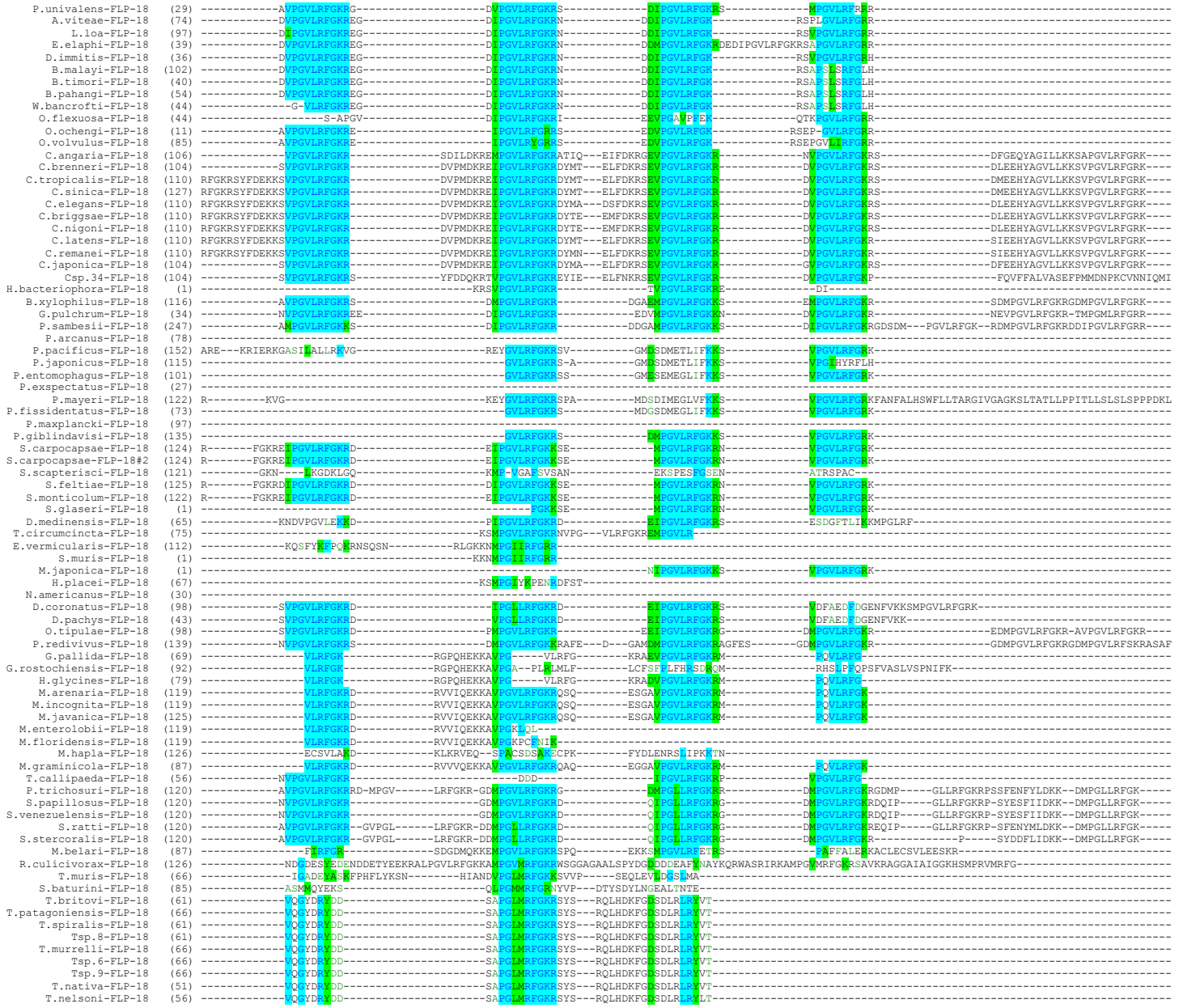
Test	Time (p.a.)	Contraction freq. (% 0 min, 2 min prior to min p.a.)	Contraction ampl. (% 0 min, 2 min prior to min p.a.)	Change in tension (mg/mm, 5 min p.a.)
10 µM As-FLP-18A n=13	10 secs	N/A	N/A	0.00±0.00×
	20 secs	N/A	N/A	Sht +0.01±0.01 ×
	30 secs	N/A	N/A	Lng -0.07±0.04 ×
	50 secs	N/A	N/A	Sht +0.05±0.02 ✓
	70 secs	N/A	N/A	Sht +0.09±0.03 ✓
	2 min	↓ 52.32±9.931 ✓	↑ 111.4±11.55 ×	Sht +0.12±0.02 ✓
	5 min	↓ 5.015±3.097 ✓	↓ 24.33±14.28 ✓	Sht +0.13±0.01 ✓
	10 min	↓ 4.872±3.897 ✓	↓ 11.51±8.354 ✓	Lng -0.03±0.02 ✓
	OVERALL	**P<0.0001, F=67.76**	**P<0.0001, F=25.54**	**P<0.0001, F=12.26**
1 µM As-FLP-18A n=6	10 secs	N/A	N/A	0.00±0.00
	20 secs	N/A	N/A	0.00±0.00
	30 secs	N/A	N/A	Lng -0.11±0.07
	50 secs	N/A	N/A	Lng -0.13±0.08
	70 secs	N/A	N/A	Lng -0.14±0.08
	2 min	↓ 83.01±16.35 ×	↑ 126.2±18.34	Sht +0.02±0.06
	5 min	↓ 47.66±18.09 ×	↑ 102.7±30.34	Sht +0.03±0.03
	10 min	↓ 34.08±16.39 ✓	↑ 119.0±34.18	Sht +0.002±0.02
	OVERALL	**P=0.0489, F=3.521**	**NS**	**NS**
0.1 µM As-FLP-18A n=6	30 secs	N/A	N/A	Lng -0.002±0.002 ×
	2 min	↓ 95.42±21.06 ×	↑ 130.3±12.89 ×	Lng -0.27±0.002 ✓
	5 min	↑128.8±11.92 ×	↑ 167.7±26.43 ×	Lng -0.28±0.07 ✓
	10 min	↑104.1± 18.30 ×	↑ 224.5±56.79 ✓	Lng -0.20±0.07 ×
	OVERALL	**P=0.3250, F=1.282**	**P=0.0340, F=4.025**	**P=0.0044, F=8.701**
0.01 µM As-FLP-18A n=6	30 secs	N/A	N/A	Lng -0.04±0.03
	2 min	↑ 116.3±11.68	↑ 111.4±13.26	Lng -0.07±0.03
	5 min	↑ 109.6±11.67	↑ 152.8±41.98	Lng -0.08±0.04
	10 min	↓ 92.57±10.62	↑ 154.2±45.03	Lng -0.05±0.05
	OVERALL	**NS**	**NS**	**NS**
1 nM As-FLP-18A n=6	30 secs	N/A	N/A	Lng -0.01±0.01
	2 min	↑ 104.3±6.685	↓ 92.52±7.007	Sht +0.01±0.01
	5 min	↓ 90.13±6.395	↓ 97.22±5.835	Sht +0.01±0.01
	10 min	↓ 83.74±7.552	↑ 105.5±5.656	Sht +0.03±0.01
	OVERALL	**NS**	**NS**	**NS**
-ve control (n=3)	30 secs	N/A	N/A	N/A
	2 min	↓ 96.97±3.030	↓ 97.72±3.249	0±0.00
	5 min	↑ 101.96±1.961	↓ 91.04±2.207	0±0.00
	10 min	↑ 109.16±1.911	↓ 82.40±5.482	0±0.00
	OVERALL	**NS**	**NS**	**NS**

% 0 min, values presented as percentage of that recorded at time 0; p.a., post-addition; ↓, denotes decrease; ↑, denotes increase; ×, denotes non-significant Dunnett's post hoc test; ✓, denotes significant Dunnett's post hoc test; NS, not significant; Sht, shortening of tissue; Lng, lengthening of tissue.

Figure S1. Alignment of FMRF-amide like peptide 18 (FLP-18) sequelogues


M.arenaria-FLP-18 (1)
M.incognita-FLP-18 (1)
M.javanica-FLP-18 (1)
M.enterolobii-FLP-18 (1)
M.floridensis-FLP-18 (1)
M.hapla-FLP-18 (1)
M.graminicola-FLP-18 (1)
T.callipaeda-FLP-18 (1)
P.trichosuri-FLP-18 (1)
S.papillosus-FLP-18 (1)
S.venezuelensis-FLP-18 (1)
S.ratti-FLP-18 (1)
S.stercoralis-FLP-18 (1)
M.belari-FLP-18 (1)
R.culicivorax-FLP-18 (1)
T.muris-FLP-18 (1)
S.baturini-FLP-18 (1)
T.britovi-FLP-18 (1)
T.patagoniensis-FLP-18 (1)
T.spiralis-FLP-18 (1)
Tsp.8-FLP-18 (1)
T.murrelli-FLP-18 (1)
Tsp.6-FLP-18 (1)
Tsp.9-FLP-18 (1)
T.nativa-FLP-18 (1)
T.nelsoni-FLP-18 (1)
T.papuae-FLP-18 (1)
T.pseudospiralis-FLP-18 (1)
T.zimbabwensis-FLP-18 (1)
T.suis-FLP-18 (1)
T.trichiura-FLP-18 (1)
Rhabditophanes-FLP-18 (1)
151
300
A.caninum-FLP-18 (1)
A.ceylanicum-FLP-18 (1)
A.duodenale-FLP-18 (1)
S.vulgaris-FLP-18 (1)
A.costaricensis-FLP-18 (1)
H.contortus-FLP-18 (1)
H.polygyrus-FLP-18 (1)
N.brasiliensis-FLP-18 (1)
D.viviparus-FLP-18 (1)
A.nanus-FLP-18 (73)
L.sigmodontis-FLP-18 (1)
H.mephisto-FLP-18 (124)
D.dipsaci-FLP-18 (1)
A.cantonensis-FLP-18 (1)
O.dentatum-FLP-18 (1)
C.goldi-FLP-18 (1)
D.destructor-FLP-18 (63)
A.lumbricoides-FLP-18 (93)
A.suum-FLP-18 (51)
T.canis-FLP-18 (82)
A.simplex-FLP-18 (43)
P.univalens-FLP-18 (1)
A.viteae-FLP-18 (1)
L.loa-FLP-18 (1)
E.elaphi-FLP-18 (1)
D.immitis-FLP-18 (1)
B.malayi-FLP-18 (1)
B.timori-FLP-18 (1)
B.pahangi-FLP-18 (1)
W.bancrofti-FLP-18 (1)
O.flexuosa-FLP-18 (1)
O.ochengi-FLP-18 (1)
O.volvulus-FLP-18 (1)
C.angaria-FLP-18 (1)
C.brenneri-FLP-18 (1)
C.tropicalis-FLP-18 (1)
C.sinica-FLP-18 (8)
C.elegans-FLP-18 (1)
C.briggsae-FLP-18 (1)
C.nigoni-FLP-18 (1)
C.latens-FLP-18 (1)
C.remanei-FLP-18 (1)
C.japonica-FLP-18 (1)
Csp.34-FLP-18 (1)
H.bacteriophora-FLP-18 (1)
B.xylophilus-FLP-18 (45)
G.pulchrum-FLP-18 (1)
P.sambesii-FLP-18 (151)
P.arcanus-FLP-18 (1)






T.papuae-FLP-18 (51)
T.pseudospiralis-FLP-18 (61)
T.zimbabwensis-FLP-18 (61)
T.suis-FLP-18 (119)
T.trichiura-FLP-18 (19)
Rhabditophanes-FLP-18 (115)
451
498
A.caninum-FLP-18 (145)
A.ceylanicum-FLP-18 (149)
A.duodenale-FLP-18 (145)
S.vulgaris-FLP-18 (142)
A.costaricensis-FLP-18 (146)
H.contortus-FLP-18 (171)
H.polygyrus-FLP-18 (178)
N.brasiliensis-FLP-18 (84)
D.viviparus-FLP-18 (104)
A.nanus-FLP-18 (252)
L.sigmodontis-FLP-18 (16)
H.mephisto-FLP-18 (297)
D.dipsaci-FLP-18 (149)
A.cantonensis-FLP-18 (96)
O.dentatum-FLP-18 (133)
C.goldi-FLP-18 (17)
D.destructor-FLP-18 (262)
A.lumbricoides-FLP-18 (204)
A.suum-FLP-18 (162)
T.canis-FLP-18 (195)
A.simplex-FLP-18 (192)
P.univalens-FLP-18 (75)
A.viteae-FLP-18 (122)
L.loa-FLP-18 (145)
E.elaphi-FLP-18 (100)
D.immitis-FLP-18 (84)
B.malayi-FLP-18 (150)
B.timori-FLP-18 (88)
B.pahangi-FLP-18 (102)
W.bancrofti-FLP-18 (89)
O.flexuosa-FLP-18 (84)
O.ochengi-FLP-18 (57)
O.volvulus-FLP-18 (132)
C.angaria-FLP-18 (192)
C.brenneri-FLP-18 (191)
C.tropicalis-FLP-18 (209)
C.sinica-FLP-18 (226)
C.elegans-FLP-18 (209)
C.briggsae-FLP-18 (209)
C.nigoni-FLP-18 (209)
C.latens-FLP-18 (208)
C.remanei-FLP-18 (208)
C.japonica-FLP-18 (191)
Csp.34-FLP-18 (194)
H.bacteriophora-FLP-18 (28)
B.xylophilus-FLP-18 (189)
G.pulchrum-FLP-18 (106)
P.sambesii-FLP-18 (333)
P.arcanus-FLP-18 (78)
P.pacificus-FLP-18 (210)
P.japonicus-FLP-18 (149)
P.entomophagus-FLP-18 (135)
P.exspectatus-FLP-18 (27)
P.mayeri-FLP-18 (210)
P.fissidentatus-FLP-18 (106)
P.maxplancki-FLP-18 (97)
P.giblindavisi-FLP-18 (166)
S.carpocapsae-FLP-18 (175)
S.carpocapsae-FLP-18#2 (175)
S.scapterisci-FLP-18 (162)
S.feltiae-FLP-18 (176)
S.monticolum-FLP-18 (173)
S.glaseri-FLP-18 (28)
D.medinensis-FLP-18 (117)
T.circumcincta-FLP-18 (105)
E.vermicularis-FLP-18 (144)
S.muris-FLP-18 (14)
M.japonica-FLP-18 (23)
H.placei-FLP-18 (83)
N.americanus-FLP-18 (30)
D.coronatus-FLP-18 (159)
D.pachys-FLP-18 (93)
O.tipulae-FLP-18 (168)
P.redivivus-FLP-18 (226)
G.pallida-FLP-18 (114)

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 G.rostochiensis-FLP-18  (154) ------------------------------------------------
      H.glycines-FLP-18  (124) ------------------------------------------------
      M.arenaria-FLP-18  (172) ------------------------------------------------
     M.incognita-FLP-18  (172) ------------------------------------------------
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   M.enterolobii-FLP-18  (143) ------------------------------------------------
   M.floridensis-FLP-18  (146) ------------------------------------------------
        M.hapla-FLP-18  (169) ------------------------------------------------
   M.graminicola-FLP-18  (140) ------------------------------------------------
    T.callipaeda-FLP-18   (89) ------------------------------------------------
    P.trichosuri-FLP-18  (213) ------------------------------------------------
    S.papillosus-FLP-18  (200) ------------------------------------------------
 S.venezuelensis-FLP-18  (200) ------------------------------------------------
         S.ratti-FLP-18  (211) ------------------------------------------------
   S.stercoralis-FLP-18  (199) ------------------------------------------------
       M.belari-FLP-18  (152) ------------------------------------------------
   R.culicivorax-FLP-18  (239) ------------------------------------------------
        T.muris-FLP-18  (116) ------------------------------------------------
      S.baturini-FLP-18  (124) ------------------------------------------------
       T.britovi-FLP-18  (102) ------------------------------------------------
 T.patagoniensis-FLP-18  (107) ------------------------------------------------
      T.spiralis-FLP-18  (102) ------------------------------------------------
           Tsp.8-FLP-18  (102) ------------------------------------------------
      T.murrelli-FLP-18  (107) ------------------------------------------------
           Tsp.6-FLP-18  (107) ------------------------------------------------
           Tsp.9-FLP-18  (107) ------------------------------------------------
       T.nativa-FLP-18   (92) ------------------------------------------------
       T.nelsoni-FLP-18   (97) ------------------------------------------------
       T.papuae-FLP-18   (92) ------------------------------------------------
T.pseudospiralis-FLP-18  (102) ------------------------------------------------
  T.zimbabwensis-FLP-18  (102) ------------------------------------------------
         T.suis-FLP-18  (169) ------------------------------------------------
    T.trichiura-FLP-18   (19) ------------------------------------------------
  Rhabditophanes-FLP-18  (192) ------------------------------------------------
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Figure S2. Supplementary References

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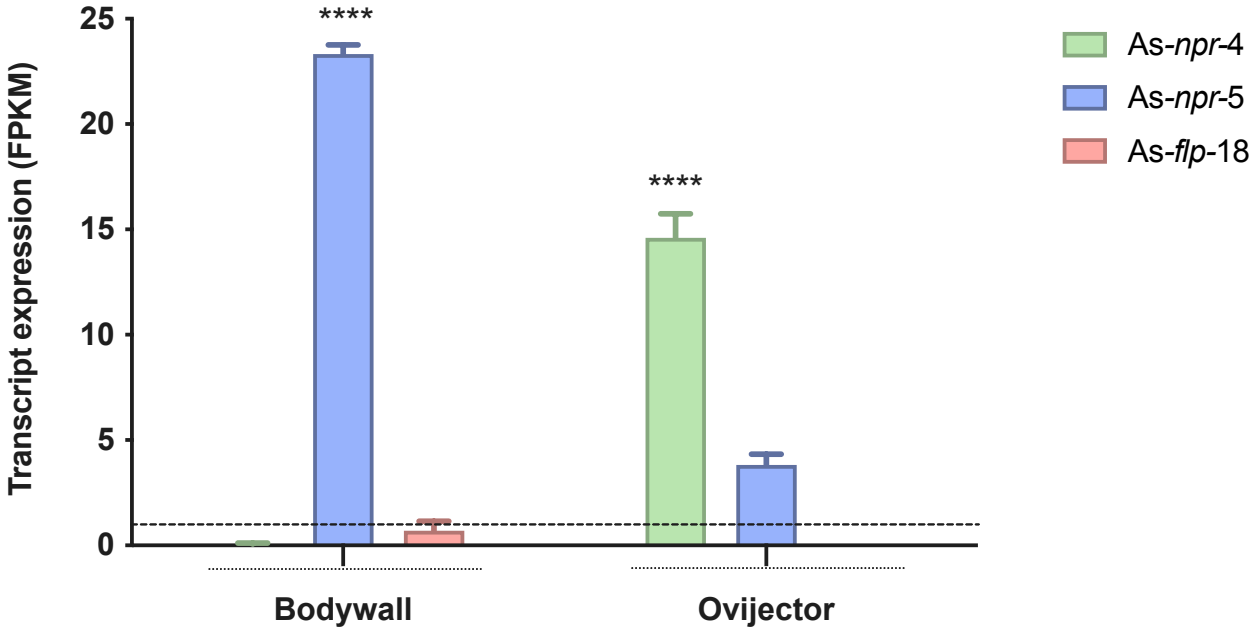
Figure S3. Differential expression of As-flp-18 and As-npr-4 and -5 in *Ascaris* body wall and ovijector tissue.



Supplementary information for Figure S3: Tissue (ovijector and body wall) was excised from adult female *Ascaris suum*. Body wall tissue contains the neuronal input to the ovijector. Ovijector tissue contains muscle based NP-GPCRs. Total RNA was extracted, quantified and assessed for integrity. The Centre for Genomic Research (Liverpool University) constructed RNAseq libraries and conducted sequencing. Six data files (n=3 for each tissue) containing 120 million, 120bp reads per file/ tissue were generated. All data were clipped, groomed and processed through a quality control pipeline (Galaxy.org) prior to analysis. For each RNA-Seq dataset, relevant adapter sequences and low quality regions were trimmed, and reads were filtered based on length and complexity. Remaining, high-quality reads were aligned to A. suum genome assembly 23 using Tophat2 (version 2.0.8, default parameters) and the genome annotation (gff3 file) as a guide. The number of reads associated with each gene was tallied using HTSeq-Count. Data are represented in FPKM (threshold for expression 1 FPKM).