

The P-glycoprotein repertoire of the equine parasitic nematode *Parascaris univalens*

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Supplementary Material

Supplementary Figure S1 Comparison of annotation of *Parascaris univalens* P-glycoproteins

Supplementary Figure S2 Tissue expression levels

Supplementary Figure S3 RT-PCR *PunPgp-2* and *PunPgp-9*

Supplementary Table S1 Contig Coverage

Supplementary Table S2 Primers and sequences

Supplementary Table S3 Accession numbers

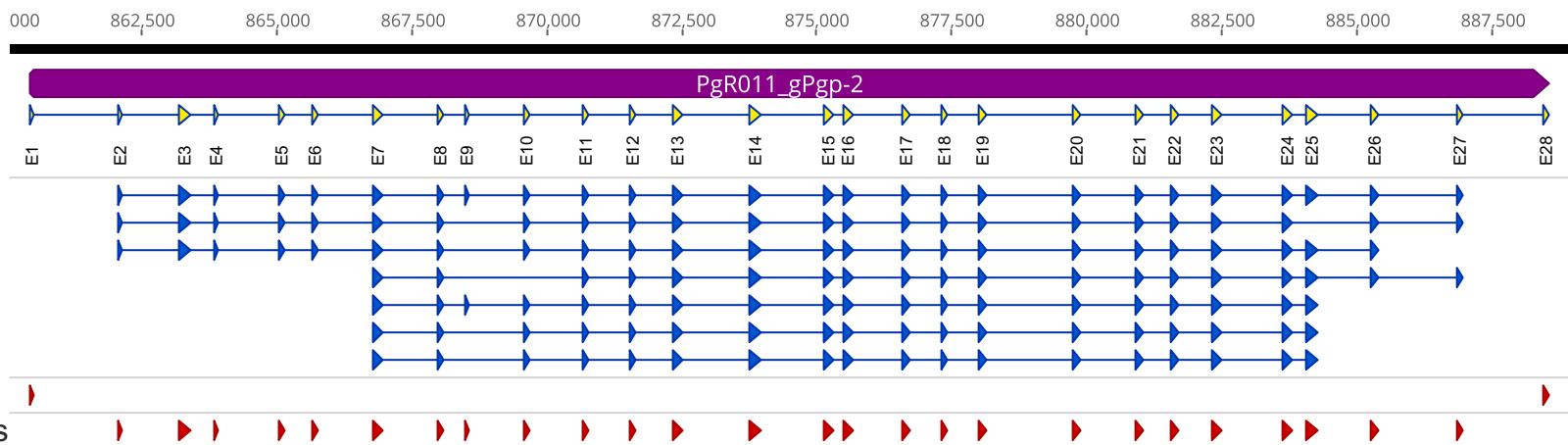
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cDNA annotation

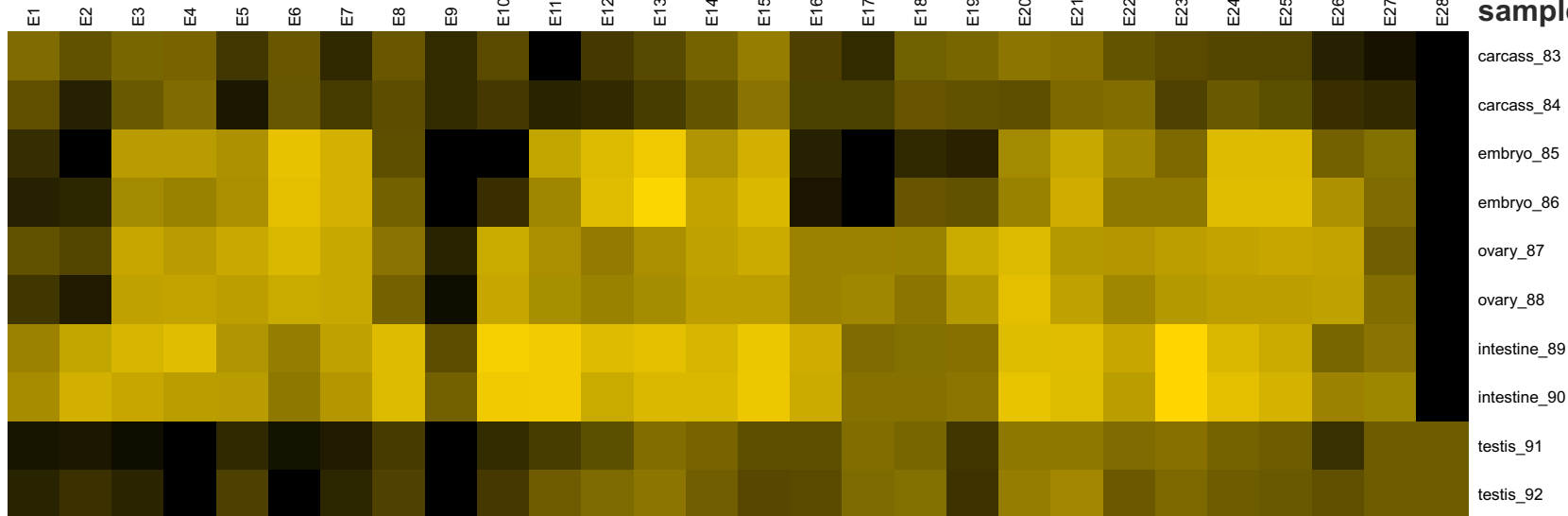
WormBase ParaSite annotation
version WBPS14

new cDNA exons

exons common to both annotations



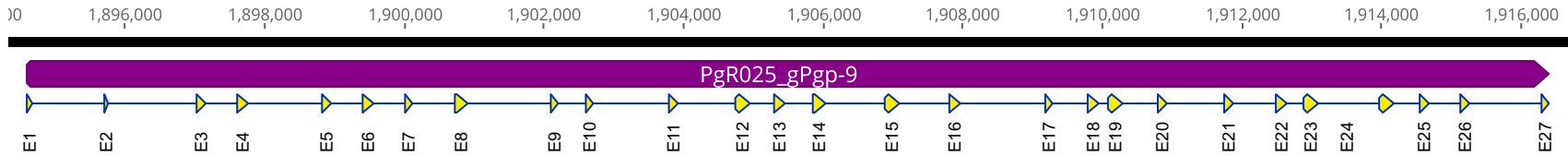
transcriptome
sample (tissue)



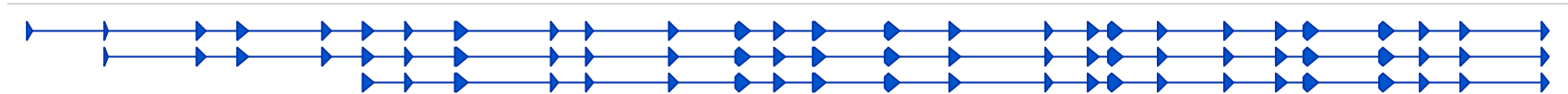
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cdDNA annotation



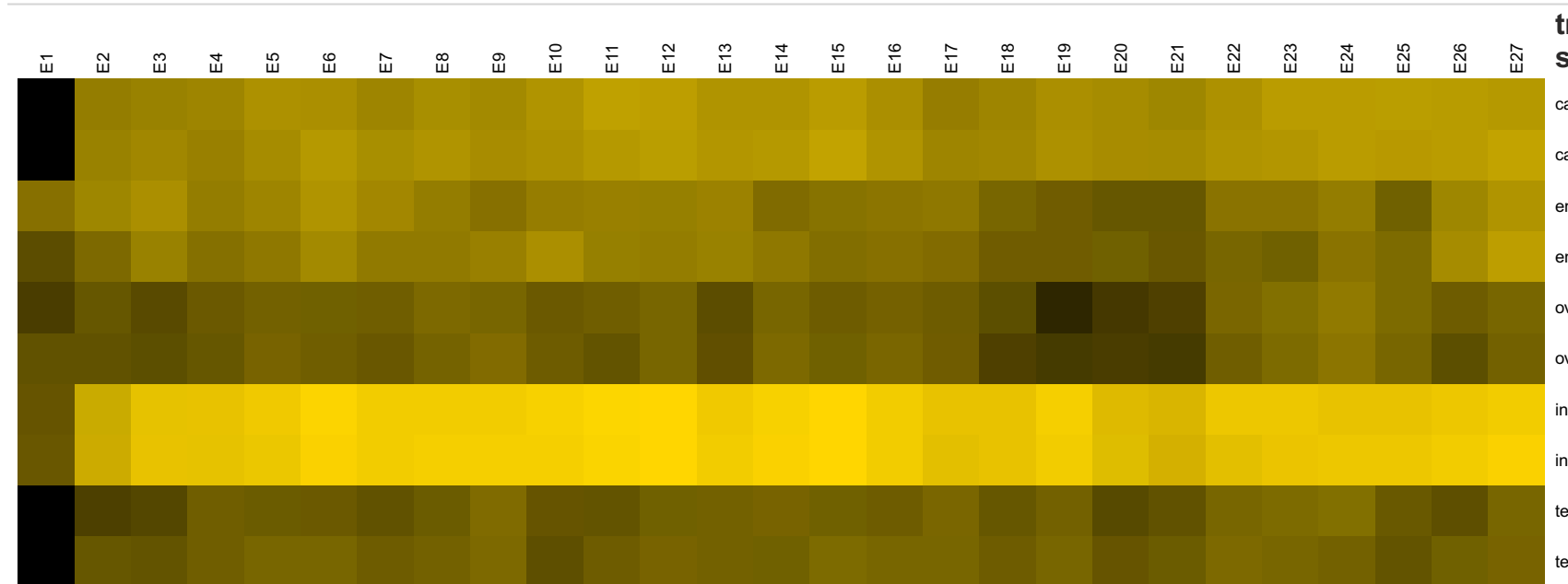
WormBase ParaSite annotation
version WBPS14



new cDNA exons



exons common to both annotations



transcriptome
sample (tissue)



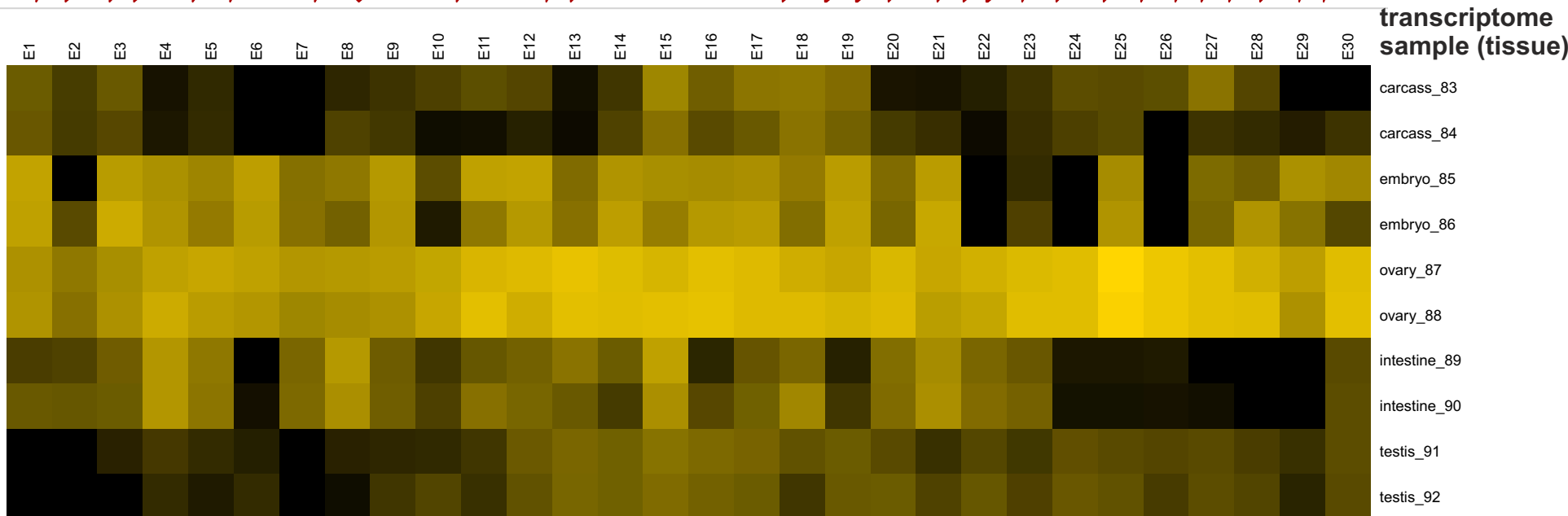
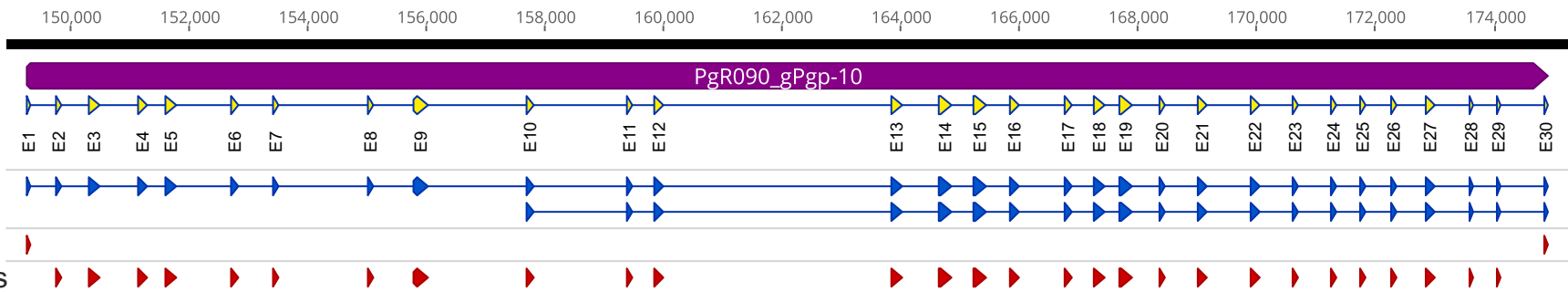
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cDNA annotation

WormBase ParaSite annotation
version WBPS14

new cDNA exons

exons common to both annotations



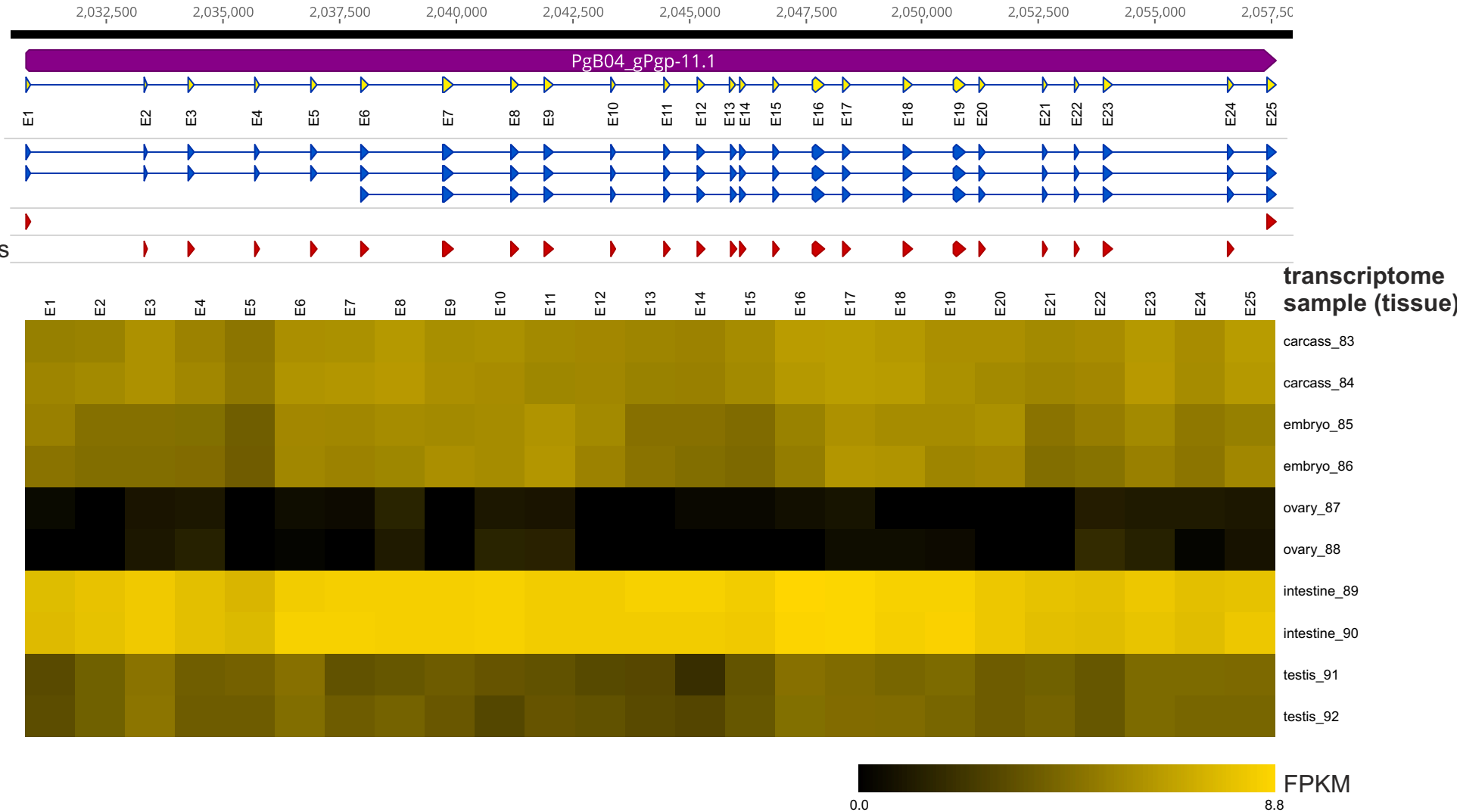
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cDNA annotation

WormBase ParaSite annotation
version WBPS14

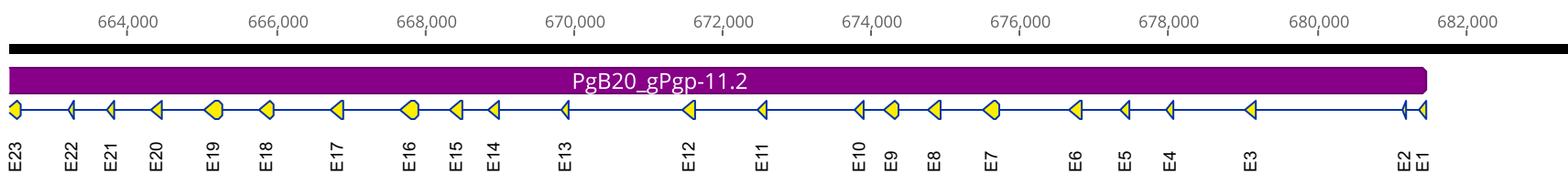
new cDNA exons

exons common to both annotations



(F) PGR011

cDNA annotation

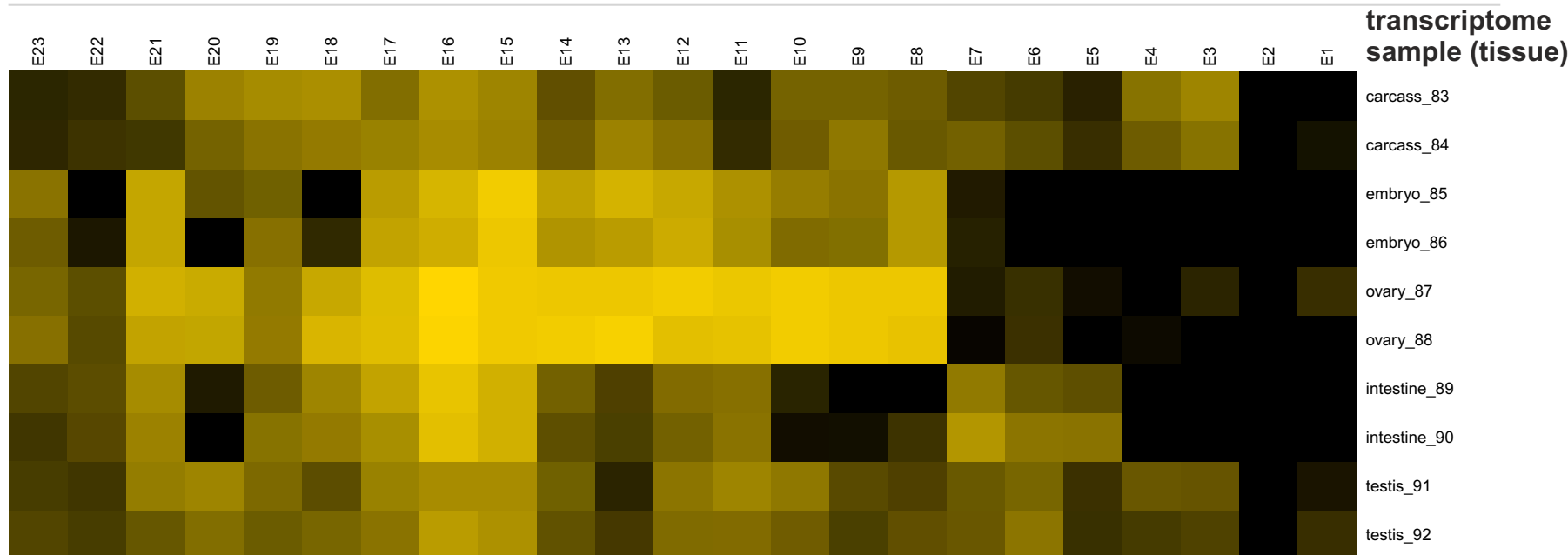


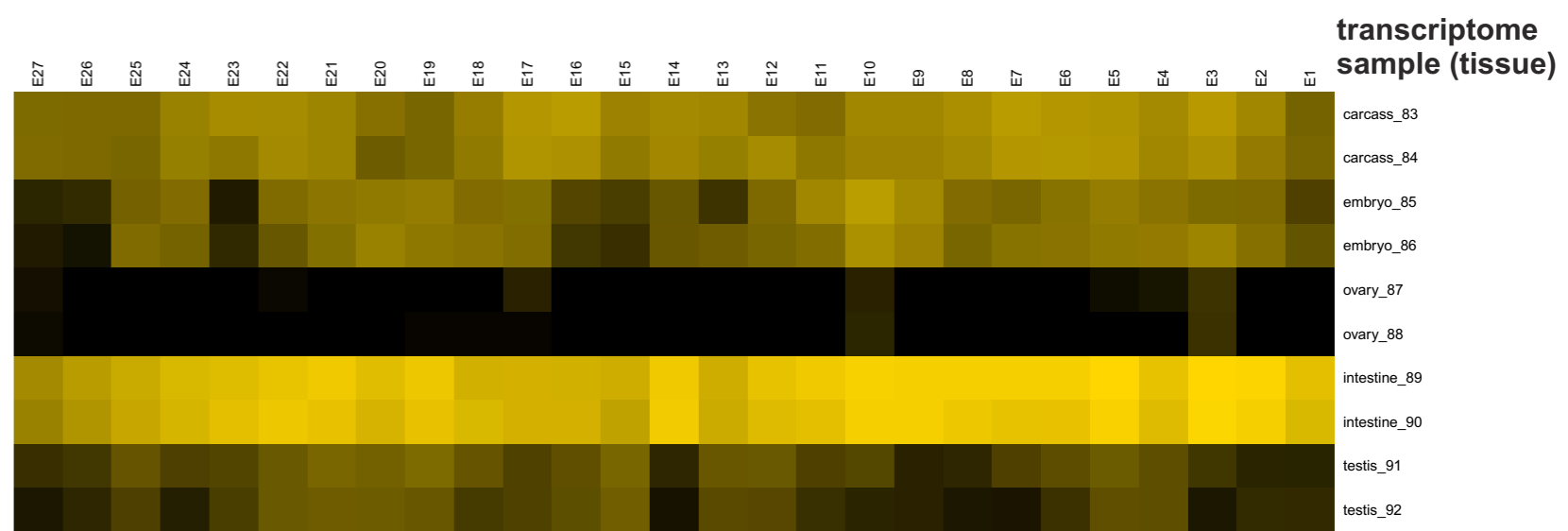
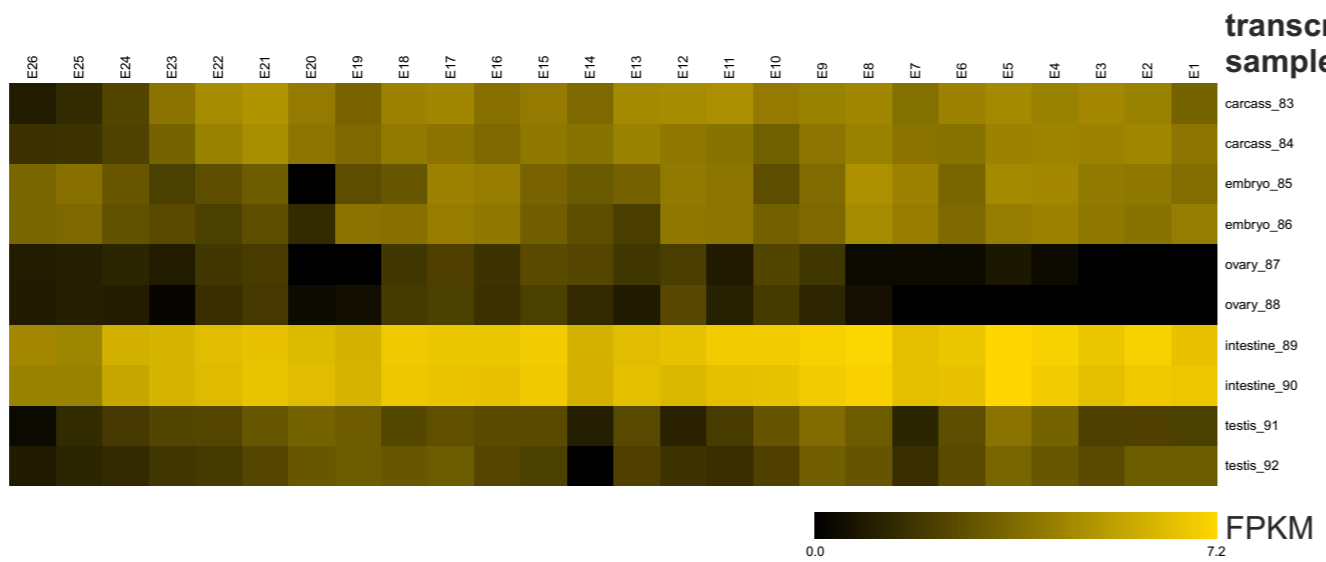
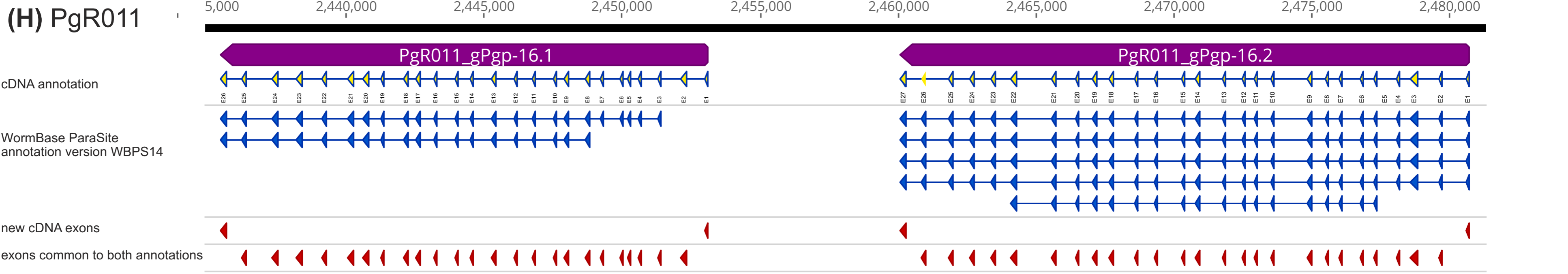
WormBase ParaSite annotation
version WBPS14



new cDNA exons

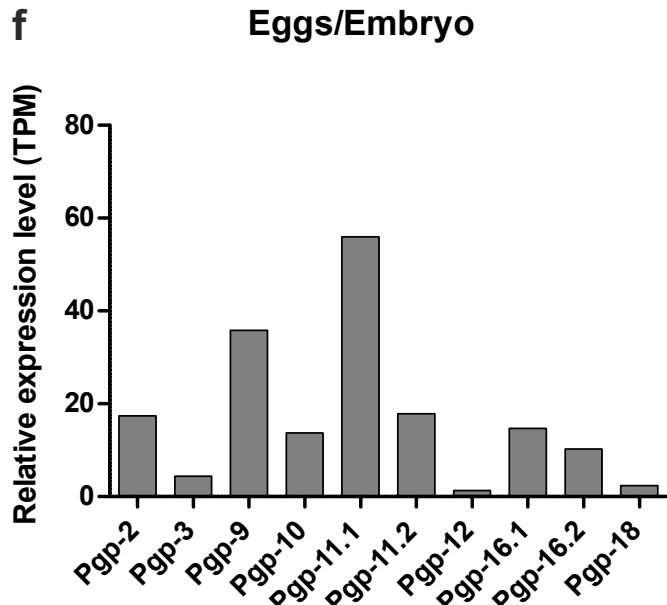
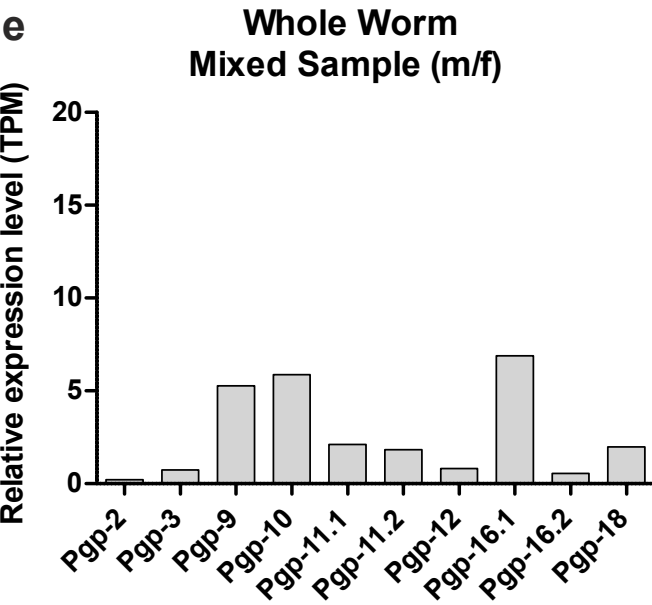
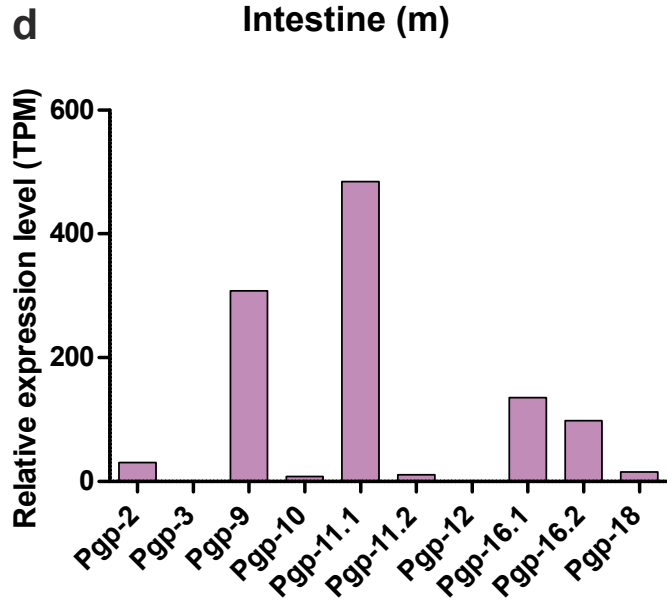
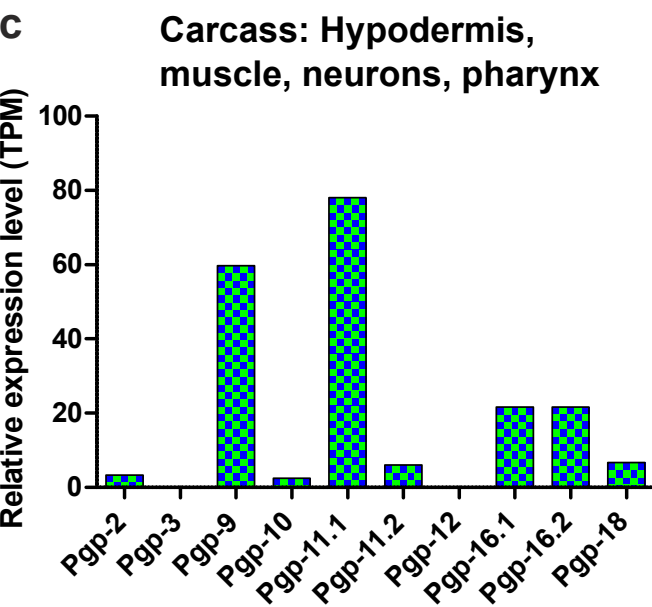
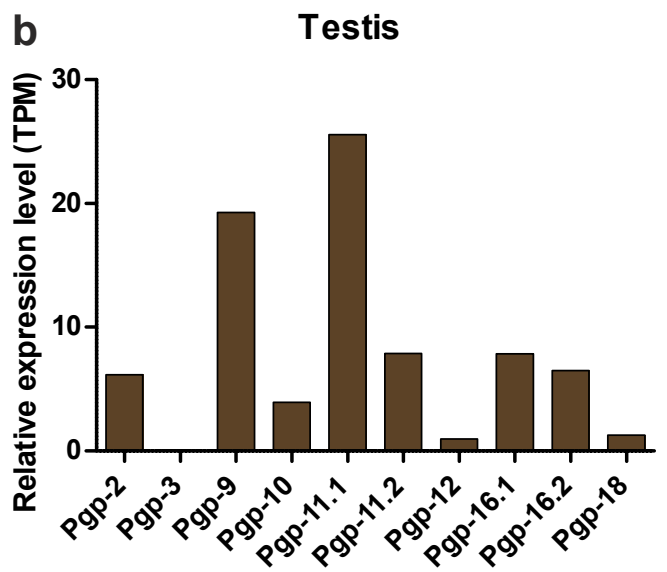
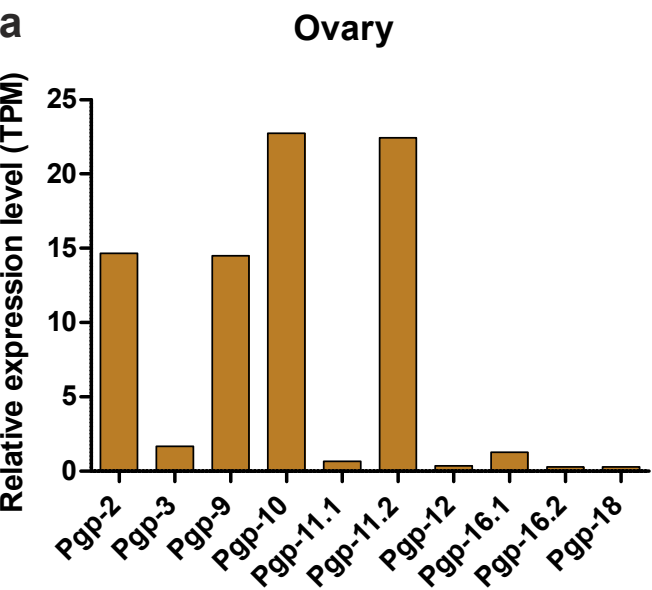
exons common to both annotations





Supplemental Figure S1 Annotation of *Parascaris univalens* Pgp

Improved annotation of experimentally verified *Parascaris univalens* Pgp exons (yellow) numbered 5' to 3' end were compared to original automatic annotation (WormBase ParaSite annotation version WBPS14, blue) and differences of new exons and exons common to both annotation highlighted (red). cDNA sequences of *Parascaris* Pgps were mapped onto the *P. univalens* genome with Splign version 2.1.0 to identify genomic coordinates of exons. Visualisation of original annotation and the newly generated annotation was made using Geneious Prime 2019.2.3. Coverage of individual exons of the new experimental cDNA exons was calculated with R package SGSeq and visualized as a heatmap (FPKM, yellow) for each tissue sample of the tissue specific transcriptome (GeoDataset accession: GSE99524). For each Pgp (A) Pgp-2, (B) Pgp-3, (C) Pgp-9, (D) Pgp-10, (E) Pgp-11.1, (F) Pgp-11.2, (G) Pgp-12, (H) Pgp-16.1 and Pgp-16.2, (I) Pgp-18 were visualized on the respective genomic locus of the respective genomic scaffold (genome assembly ASM225920v1, version WBPS14). Pgp: P-glycoprotein

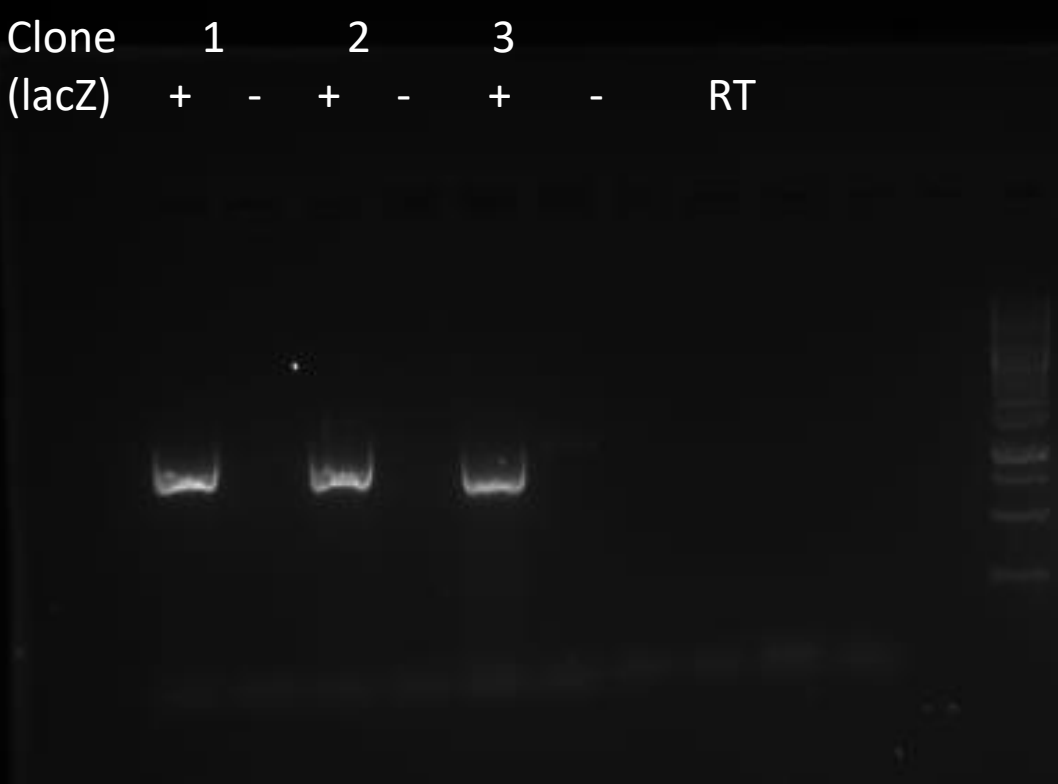


Supplementary Figure S2 Tissue expression levels of *Parascaris univalens* P-glycoproteins

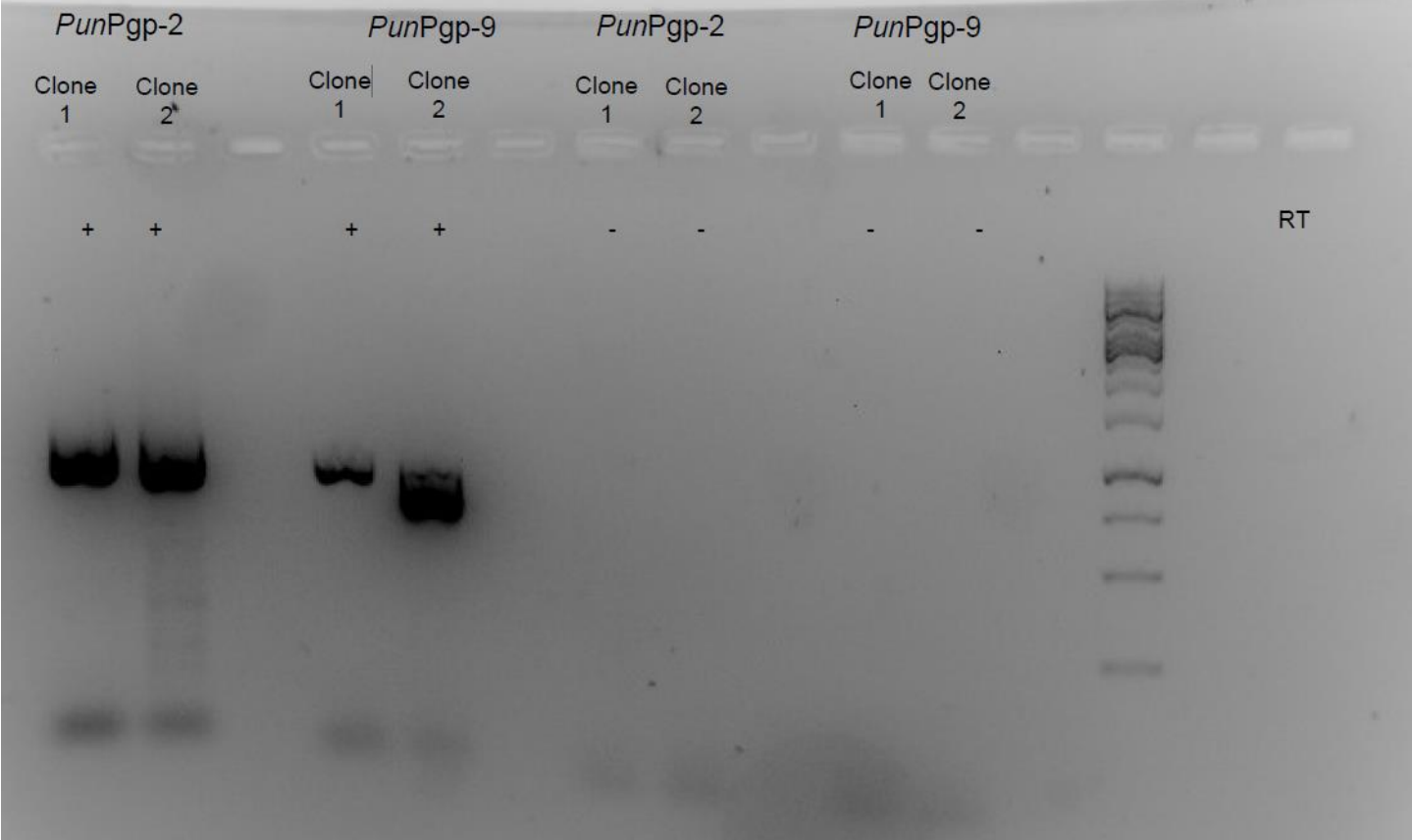
Expression levels of *Parascaris univalens* Pgp were calculated based on transcriptome raw reads data sets (Geo Accession GSE99524, samples GSM2645460 to GSM2645464) mapped onto the augmented (with *P. univalens* Pgp cDNA sequences) *P. univalens* genome (WormBase Parasite genome assembly ASM225920v1, version WBPS14) using STAR. Expression levels were normalised as transcripts per million (TPM) from raw read counts obtained with featureCounts. Then expression levels were visualised by tissue of (a) the ovary (light brown), (b) the testis (brown), (c) carcass tissue including the epidermis, neurons, muscles and the pharynx (blue and green), (d) intestine (purple), (e) a whole worm sample (5'-TeloPrime library), (f) eggs. Pgp: P-glycoprotein; m: Male; f: female; TPM: transcripts per million

Supplementary Figure S3 RT-PCR of *Saccharomyces cerevisiae* AD1-7 expressing *PunPgp-2*, *PunPgp-9* or *lacZ*

a



b



Supplementary Figure S3 RT-PCR of *Saccharomyces cerevisiae* AD1-7 expressing *PunPgp-2*, *PunPgp-9* or *lacZ*

Saccharomyces cerevisiae AD1-7 expressing *lacZ*, *PunPgp-2* or *PunPgp-9* were generated through transformation. Following total RNA extraction and cDNA synthesis including a no-reverse transcriptase (RT) control, RT-PCRs were performed. PCR products were visualised with Gel-electrophoresis. Several clones were screened but only clone 1 for all genes was used for analyses. Pgp: P-glycoprotein. RT: Reverse transcriptase

Supplementary Table S1

Contig Coverage of ORFs in the tissue specific transcriptome assembly

Contig	Lenth (bp)	From (cDNA)	To (cDNA)	From (contig)	To (contig)	Gaps	Identities	Identities (%)	ORF Coverage (%)	
										Number of contigs:
PunPgp-2	cDNA ORF length:	3864		7		1		3864	Full ORF Coverage:	100.00%
	Contig	Lenth (bp)	From (cDNA)	To (cDNA)	From (contig)	To (contig)	Gaps	Identities	Identities (%)	ORF Coverage (%)
	hub_106215_PG_hub_106215_all_transcripts_PgR011_g057_t01	3653	1	3658	21	3653	25	3622	99.0%	94.7%
	hub_106215_PG_hub_106215_all_transcripts_PgR011_g057_t02	4339	1	3864	21	3857	27	3825	99.0%	100.0%
	hub_106215_PG_hub_106215_all_transcripts_PgR011_g057_t03	3833	1	3841	21	3833	28	3803	99.7%	99.4%
	hub_106215_PG_hub_106215_all_transcripts_PgR011_g057_t04	3638	407	3583	114	3269	21	3148	99.1%	82.2%
			3376	3511	3271	3406	0	136	100.0%	3.5%
	hub_106215_PG_hub_106215_all_transcripts_PgR011_g057_t05	3552	407	3583	114	3269	21	3148	99.1%	82.2%
			3376	3658	3271	3552	1	281	99.3%	7.3%
	hub_106215_PG_hub_106215_all_transcripts_PgR011_g057_t06	4009	407	3864	114	3527	44	3404	99.7%	89.5%
	hub_106215_PG_hub_106215_all_transcripts_PgR011_g057_t07	3525	407	3583	1	3156	21	3148	99.1%	82.2%
			3376	3511	3158	3293	0	136	100.0%	3.5%
PunPgp-3	cDNA ORF length:	3723		5		591		3971	Overall ORF Coverage (%)	90.8%
	Contig	Lenth (bp)	From (cDNA)	To (cDNA)	From (contig)	To (contig)	Gaps	Identities	Identities (%)	ORF Coverage (%)
	hub_106215_PG_hub_106215_all_transcripts_PgR059_g034_t01	3248	2113	3723	1604	3205	9	1602	100.0%	43.3%
			591	2112	1	1512	10	1509	99.8%	40.9%
PunPgp-9	cDNA ORF length:	3948		3		1		3948	Full ORF Coverage:	100.00%
	Contig	Lenth (bp)	From (cDNA)	To (cDNA)	From (contig)	To (contig)	Gaps	Identities	Identities (%)	ORF Coverage (%)
	hub_106215_PG_hub_106215_all_transcripts_PgR025_g103_t03	5506	1	3948	793	4714	26	3922	99.3%	100.0%
	hub_106215_PG_hub_106215_all_transcripts_PgR025_g103_t02	5620	76	3948	981	4828	25	3848	99.4%	98.1%
	hub_106215_PG_hub_106215_all_transcripts_PgR025_g103_t01	6823	276	3948	2382	6031	23	3650	100.0%	93.0%
			76	278	1749	1950	1	202	100.0%	5.1%
PunPgp-10	cDNA ORF length:	3924		6		1		3924	Overall ORF Coverage (%)	100.00%
	Contig	Lenth (bp)	From (cDNA)	To (cDNA)	From (contig)	To (contig)	Gaps	Identities	Identities (%)	ORF Coverage (%)
	hub_106215_PG_hub_106215_all_transcripts_PgR090_g012_t01	4387	1	3924	266	4160	29	3888	99.8%	100.0%
	hub_106215_PG_hub_106215_all_transcripts_PgR090_g012_t02	2987	1144	3924	1	2760	21	2757	99.9%	70.9%
PunPgp-11.1	cDNA ORF length:	3858		3		1		3858	Full ORF Coverage:	100.00%
	Contig	Lenth (bp)	From (cDNA)	To (cDNA)	From (contig)	To (contig)	Gaps	Identities	Identities (%)	ORF Coverage (%)
	hub_106215_PG_hub_106215_all_transcripts_PgB04_g139_t01	5657	1	3858	1574	5430	24	3825	99.1%	100.0%
	hub_106215_PG_hub_106215_all_transcripts_PgB04_g139_t02	4968	1	3858	885	4718	24	3825	99.8%	100.0%
	hub_106215_PG_hub_106215_all_transcripts_PgB04_g139_t03	4359	388	3858	659	4109	20	3442	99.7%	90.0%
PunPgp-11.2	cDNA ORF length:	3909		4		1		3909	Overall ORF Coverage (%)	100.00%
	Contig	Lenth (bp)	From (cDNA)	To (cDNA)	From (contig)	To (contig)	Gaps	Identities	Identities (%)	ORF Coverage (%)
	hub_106215_PG_hub_106215_all_transcripts_PgB20_g052_t01	4103	1	3909	5	3889	24	3876	99.8%	100.0%
	hub_106215_PG_hub_106215_all_transcripts_PgB20_g052_t02	4097	1	3909	5	3883	30	3870	99.8%	100.0%
	hub_106215_PG_hub_106215_all_transcripts_PgB20_g052_t03	620	448	1070	1	620	3	618	99.7%	15.9%
	hub_106215_PG_hub_106215_all_transcripts_PgB20_g052_t04	338	166	386	119	338	1	219	99.5%	5.7%
PunPgp-12	cDNA ORF length:	4056		3		1464		3971	Overall ORF Coverage (%)	61.83%

	Contig	Lenth (bp)	From (cDNA)	To (cDNA)	From (contig)	To (contig)	Gaps	Identities	Identities (%)	ORF Coverage (%)	
PunPgp-16.1	hub_106215_PG_hub_106215_all_transcripts_PgR063X_g008_t01	1038	2929	3971	1	1038	5	1037	99.9%	28.0%	
	hub_106215_PG_hub_106215_all_transcripts_PgR063X_g007_t01	650	1917	2418	152	650	3	499	100.0%	13.5%	
	hub_106215_PG_hub_106215_all_transcripts_PgR063X_g006_t01	264	1464	1728	1	264	1	264	100.0%	7.1%	
	cDNA ORF length:	3855	Number of contigs:	2	Coverage Start:	84	Coverage End:	3855	Overall ORF Coverage (%)	97.85%	
	Contig	Lenth (bp)	From (cDNA)	To (cDNA)	From (contig)	To (contig)	Gaps	Identities	Identities (%)	ORF Coverage (%)	
	hub_106215_PG_hub_106215_all_transcripts_PgR011_g140_t01	5610	84	833	1165	1909	5	744	99.2%	19.5%	
	hub_106215_PG_hub_106215_all_transcripts_PgR011_g140_t02	5024	829	3855	2490	5497	19	3004	99.2%	78.5%	
PunPgp-16.2	hub_106215_PG_hub_106215_all_transcripts_PgR011_g140_t02	5024	84	3855	1165	4911	25	3742	99.9%	97.8%	
	cDNA ORF length:	3864	Number of contigs:	5	Coverage Start:	1	Coverage End:	3864	Overall ORF Coverage (%)	100.00%	
		Contig	Lenth (bp)	From (cDNA)	To (cDNA)	From (contig)	To (contig)	Gaps	Identities	Identities (%)	ORF Coverage (%)
		hub_106215_PG_hub_106215_all_transcripts_PgR011_g141_t01	5174	1	3864	625	4462	26	3835	99.2%	100.0%
	hub_106215_PG_hub_106215_all_transcripts_PgR011_g141_t02	5170	1	3864	621	4458	26	3835	99.2%	100.0%	
	hub_106215_PG_hub_106215_all_transcripts_PgR011_g141_t03	6530	1	3864	1981	5818	26	3835	99.9%	100.0%	
	hub_106215_PG_hub_106215_all_transcripts_PgR011_g141_t04	7109	1	3864	2560	6397	26	3835	99.9%	100.0%	
	hub_106215_PG_hub_106215_all_transcripts_PgR011_g141_t05	3529	411	3124	810	3505	20	2692	99.9%	70.2%	
PunPgp-18	cDNA ORF length:	3888	Number of contigs:	1	Coverage Start:	1	Coverage End:	3888	Overall ORF Coverage (%)	100.00%	
		Contig	Lenth (bp)	From (cDNA)	To (cDNA)	From (contig)	To (contig)	Gaps	Identities	Identities (%)	ORF Coverage (%)
	hub_106215_PG_hub_106215_all_transcripts_PgB01_g089_t01	4070	1	3888	26	3892	37	3858	99.8%	100.0%	

Supplementary Table S1. Contigs encoding P-glycoproteins of the tissue specific transcriptome (GEO accession number: GSE99524). For each P-glycoprotein ORF and for each contig length, ORF coordinates (from/to cDNA) as well as the corresponding contig coordinates (from/to contig), gaps, identities, identity percentage and ORF coverage (ignoring gaps) is listed. Number of contigs vary strongly between Pgp and do not correspond to their expression level. Complete ORF coverage was not available for several Pgp. Pgp: P-glycoprotein

Supplementary Table S2

a) Full Length P-glycoprotein Open Reading Frame Primers

Gene	Direction	contig of origin	Sequence (5'-3')	Primer length	Tm (°C)	Fragment Size (bp)
<i>PunPgp-2</i>	Forward	hub_106215_PG_hub_106215_al l_transcripts_PgR011_g057_t02	ACTGGTGGTTATCAA CGTG	19	71.4	4065
	Reverse	hub_106215_PG_hub_106215_al l_transcripts_PgR011_g057_t02	ATCCACACTACAAAA CGTGA	20		
<i>PunPgp-10</i>	Forward	hub_106215_PG_hub_106215_al l_transcripts_PgR090_g012_t01	CAACTTGTTCCGGCA GCTTCAGTTTACCGA CAA	32	55.0	4547
	Reverse	hub_106215_PG_hub_106215_al l_transcripts_PgR090_g012_t01	GAGAAGTGGTAGAA CAAAGGATGAGGTG ATAA	32		
<i>PunPgp-3</i>	Forward	comp75122_c0_seq1	TGAGAGATGTCTTCA CGAAGAAAGG	25	55.0	4091
	Reverse	comp75122_c0_seq1	CAACTTAAAAGATCT TGTTTCCTGA	25		
<i>PunPgp-18</i>	Forward	hub_106215_PG_hub_106215_al l_transcripts_PgB01_g089_t01	CTATCAGGTAAAGT GAATCCAAGAAATG GGT	31	65.0	4120
	Reverse	hub_106215_PG_hub_106215_al l_transcripts_PgB01_g089_t01	ATTGATTAAGAGATG AAATGAATGATAGC GAA	32		
<i>PunPgp-11.2</i>	Forward	hub_106215_PG_hub_106215_al l_transcripts_PgB20_g052_t01	AAAATGACCGACAA CGACAAGTCAAAAT GCAG	32	60.0	4105

	Reverse	hub_106215_PG_hub_106215_al l_transcripts_PgB20_g052_t01	TTTCCTTGTTAGATA TAACGAAACGCTCAT GG	32		
<i>PunPgp-3</i>	Forward	comp75122_c0_seq1	ATGGCACGATTCAT GTCAAGGTTCTATC	28	55.0	3765
	Reverse	comp75122_c0_seq1	GATGGTTTCTGTGAT ATATAGCGTTTCTCT CT	32		
<i>PunPgp-16.2</i>	Forward	hub_106215_PG_hub_106215_al l_transcripts_PgR011_g141_t01	GCTACTAATCGTCTA GCTCTATTGGTCATC AA	32	60.0	4332
	Reverse	hub_106215_PG_hub_106215_al l_transcripts_PgR011_g141_t01	TTTCTAGGCCTGCT GCTATTCCTTTATTG CAA	32		
<i>PunPgp-9</i>	Forward	hub_106215_PG_hub_106215_al l_transcripts_PgB04_g139_t01	TCTCTCGTCGATGTT CACACGTT	23	59.0	4110
	Reverse	hub_106215_PG_hub_106215_al l_transcripts_PgB04_g139_t01	TGGTGATTATCGCA GCAACAGG	22		

b) Additional Primers

Gene	Direction	contig of origin	Sequence (5'-3')	Primer length	T _m (°C)	Fragment Size (bp)	Purpose
<i>PunPgp-12</i>	Forward	hub_106215_PG_hub_106215_al l_transcripts_PgR063X_g008_t01	TCAACTTCTAAGCAGAG AGGCACG	24	65	634	Verification of fragment
	Reverse	hub_106215_PG_hub_106215_al l_transcripts_PgR063X_g008_t01	ATCGTGGTTCTTGTCCC ACCAATG	24			

Forward	hub_106215_PG_hub_106215_al l_transcripts_PgR063X_g008_t01	AGGTCTCGACACAGAAG TTGGAGA	24	62	226	
Reverse	hub_106215_PG_hub_106215_al l_transcripts_PgR063X_g008_t01	TGCTGAATTG ACGATAGTCG ATGTGC	28			
Forward	comp29735_c0_seq1	TCTTcACGAAGAAAGGT TCAAGATGAGCA	29	65	421	
Reverse	comp29735_c0_seq1	GATAGGCGGCAAGGAG AAATTCG	23			
Forward	comp29735_c0_seq1	CGGCAGGTTTCATAACA ATTTATCG	25	65	270	
Reverse	comp29735_c0_seq1	TAACATACGAGCGTCGA TTACCGC	24			
Forward	hub_106215_PG_hub_106215_al l_transcripts_PgR063X_g008_t01	TCAACTTCTAAGCAGAG AGGCACG	24	65		Verification of g008/29735 as one Pgp
Reverse	comp29735_c0_seq1	TAACATACGAGCGTCGA TTACCGC	24			
Forward	Splice Leader (SL) <i>Ascaris suum</i>	GGTTTAATTACCCAAGT TTGAG	22			Amplificatio n of 5' end
Reverse	Sequencing product of PgR063X_g008_t01	AGTGATACTGGTTTTGC CTCG	21	60	unspecific	Reverse for SL
Reverse 2	Sequencing product of PgR063X_g008_t01	TTGTTTCGCCAATCAA GCC	20	60	~220bp	Nested SL reverse
Forward	Sequencing product of comp29735_c0_seq1	GTGCGTTTCGATGGACG TGAC	22	70		3'RACE
Forward	Sequencing product of comp29735_c0_seq1	AGAAATCAGATGGCATT GGCGG	23		700bp	nested_1 (3'RACE)
Forward	Sequencing product of 3' RACE SP6	GGGTCTCGACACAGAA GTTGGAGA	24		600bp	nested_2 (3'RACE)

c) Full Length P-glycoprotein Sequencing Primers

Gene	Forward Primers	Sequence (5'-3')	Reverse Primers	Sequence (5'-3')
<i>PunPgp-2</i>	M13-24F-BLUE	GTAAAACGACGGCCAGTGAGCGCG	M13-24R-BLUE	AACAGCTATGACCATGATTACGCC
	F1	CGTCAGAAATATCTGAAAGCAATA	R1	GTGTATGTTGGCTAATTTGGCG
	F2	GCTGGATCGAGAAGTTTCACAC	R2	CATCAGTGAGACGAGCGGTAAG
	F3	TATTCGATGGAACCATCGAGAGTA	R3	TCCCGGTGGAATGTCTTTGATCAT
	F4	TTGTGCACTCGATTTGCGACCGAT	R4	CGGTTCTTGCGATACGATTCCGAT
	F5	GTGCAGCAATCGTCAAAGAACT		
<i>PunPgp-10</i>	M13-24F-BLUE	GTAAAACGACGGCCAGTGAGCGCG	M13-24R-BLUE	AACAGCTATGACCATGATTACGCC
	F1	TCTCGTTGGCGGTTTGTTTC	R1	AGGTAAAATCGGCCTTGAAGG
	F2	TCCAATGGGCGTTTAATGCT G	R2	ACATCACCCAGTGTCACATC
	F3	TCTGGTGGTCAAAGCAACG	R3	ACCGGAAGAGCTATTCCAAG
	F4	TCGTTACCCGGTAAGGATAC	R4	ACGTATAACAGCTCGGGCGA
<i>PunPgp-18</i>	M13-24F-BLUE	ACGATGATCGATCGATACTCG	M13-24R-BLUE	AACAGCTATGACCATGATTACGCC

	F1	TCGATTCTCACAGGTGCAAC	R1	TGCGAAGTCCATTGCTACTG
	F2	ATGGGCGAGTGGTAGAGACG	R2	AGTGACATCTGCCAACCGAAG
	F3	ATGTCACTCGTTACTCTTGTA	R3	AGATCGACTGTCGTCCATGT
	F4	GATGACGATCCAGATGACGGG	R4	CAGGTTCTATCTCACCTGAGC
<i>PunPgp-12</i>	M13-24F-BLUE	ACGATGATCGATCGATACTCG	M13-24R-BLUE	AACAGCTATGACCATGATTACGCC
	F1	AGGTGCTATTGCCGAGGAATC	R1	TAGCCCAGCAGCAGTTCTTG
	F2	TCCTGAGGGATATCAGACTC	R2	ATATGCACCCGCAAGTGCTC
	F3	ATGTCTTTTCGAGCACTTGCG	R3	GTCTGATATCCCTCAGGAAG
	F4	CAAGAACTGCTGCTGGGCTA	R4	ATTCCTCGGCAATAGCACCTG
<i>PunPgp-11.2</i>	M13-24F-BLUE	ACGATGATCGATCGATACTCG	M13-24R-BLUE	AACAGCTATGACCATGATTACGCC
	F1	TGCTACAGGC GATATTGACG	R1	ATCGCCTGTAGCACTCTCTG
	F2	TGCTACAGGC GATATTGACG	R2	TCGAGTATCGATCGATCATCG
	F3	TACTGGATGAAGCGACTAGC	R3	TGCTCTGGGTTCTCTTGTT C
	F4	TTCACTTTTCGGCATCGAGC	R4	AGAGAACCCAGAGCATGGAA C
<i>PunPgp-3</i>	M13-24F-BLUE	ACGATGATCGATCGATACTCG	M13-24R-BLUE	AACAGCTATGACCATGATTACGCC

	F1	TGAAGAAGGCTCGTAAGATGGG	R1	AGCACCGGTAAATATTCCCGTC
	F2	TTCAAAGGCTCTTGACCAGGC	R2	TCGCTTTCTGTGTCCAACGCAC
	F3	TGATAGCAGGAGTTGGAATCGC	R3	GATAGCAGGAGTTGGAATCGCA
	F4	TATCTGAAGCGGGTCTACGTCAG	R4	TTGTCGTCGATTGGGATAAGCG
<i>PunPgp-16.2</i>	M13-24F-BLUE	ACGATGATCGATCGATACTCG	M13-24R-BLUE	AACAGCTATGACCATGATTACGCC
	F1	ACATCTCGTGAAGCGACGTTG	R1	GTACAAGGTCAGCTCGATTG
	F2	CAATCGAGCTGACCTTGTAC	R2	TCTTCCAACGTCGCTTCACG A
	F3	GATTGCAAGACGTCCATCAG	R2-1	TTGGTGACAACTGGGCAAG
	F4	TAGTTCCTCTGATGCAGGAC	R3	CCACTTATAGTCGTCGCAAC
	F5	GATGCGGCTATTTTGTCGAG	R4	GTGTCTGTCTG CATTCTTCTT C
	F6	AAGTGGCCAATAAACCGGCAC	R5	TCATCGAGCGTTCCGAATTG
	F7	TCGCATTTCGCCTATTGTGCAG	R6	TGGTGCACAATTTCCGCAAC
<i>PunPgp-9</i>	M13-24F-BLUE	GTAAAACGACGGCCAGTGAGCGCG	M13-24R-BLUE	AACAGCTATGACCATGATTACGCC
	F1	GAGTCAGTTCTTTGCCGATT	R1	GTGAGAGTGACATCATAATCAA
	F2	TTGGACCAAGTGGATGTGGTAA	R2	ACAGTTCCATCAATTGGGTCAT

	F3	GGAAACGAGGATGTCACTGAG	R3	CGCTCCTACGACAAGAAACATG
	F4	GATCAGATGC TCAGCAATGG TC	R4	CTCAGTGACATCCTCGTTTC C
	F5	TTCGCATATCCAGAACGACCAG		

d) Codon optimized constructs for expression in *Saccharomyces cerevisiae* of *PunPgp-2* and *PunPgp-9*

>*PunPgp-2_codon_optimized*

ATGATGCGTTCAAGTCAGGATAATGAAAAAGTTCCGCTGATTTTCAGCCAGTATTGCGTCATACGAGTCTTTTAACTCA
AGCAATTGCAAGCGTCCTTCATCCCTTAAGGTCAAGGAGAAAGGGCTTTTCTCATACACGACGTGTCTAGACTATTG
TCTAATCCTTATGGGCACCTTCGCAGGTATAGCACACGGAACCGGCTTTCCCTCTTTTAAGTATAGTGCTAGGGGGGA
TGACAACGATATTCCTGAGAGCCGAGAACTCCGACTTTGTTTCGTGGTTATTCTGTTATAGTCAACAATTCAGCCCTTA
GCCAGATTACGAAGGAAGAGTTCGACGCTTCTGTTACCACCTATTGCCTTTACTACCTATTGATAGGCGTATTTATGT
TTATATCCAGCTATATACAGATCGCTTGCTGGGAGTCTTTTAGTGAGAGAACCACTCATAGGATAAGACAAAAATATT
TAAAAGCGATACTGAGGCAGCAAATTGCCTGGTTCGATACCCAACAGAGTGGCAATCTTACGGCCCGTTTGACTGA
CGACCTGGAACGTGTTAGAGAGGGGTTAGGCGATAAGCTAAGCATGATGATTCAACTGATGGCCGCTTCATCGCG
GGATTTATAGTCGGCTTTATATATAATTGGCGTATGACTCTAGTGATGATGGCTTTCGCCCCATTGAACGCCCTGACA
GGCGCATGGATGTCTCGTATGGCGGCGACGAGAACACAGGTCGAGCAAGAGAAGTATGCTGTGGCCGGGGCTATA
GCAGAAGAAACCTTTAGTAGCATAAGAACCGTTCATAGTCTGAACGGAGCAACCAGAGAAATCGCAAGATATGAGAA
GGCCTTAGAGGATGGGAGGCGTACTGGGCGTTTGAAATACTTATATCTTGGGACCGGAATGGCGTTGAATTACCTT
ATTGTGTATGCCAGCTACGCAGTGGCCTTTTGGTACGGCAGTCTGATCATAATCGGTGATCCGACTTTCGACAGAGG
CTCCGTGTTACGGTATTCTTTAGTGTGATGAGTGGAAGCATGGCGCTAGGTGGTGGCCTTCCAAACATGGCTACTT
TTGCTATGGCGAGAGGTGCAGCAAGAAAAGTACTGAGTGTCAACAGTGTGCCTATTATTGACCCTTACTCCAGC
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ATTCAAATTTTGGACCGTATTACTTTTATATTAGTCCCGGTAGAAAAATCGCGTTGGTGGGAAGCAGTGGTTGTGG
GAAATCTACCGTGATGCACCTGCTTTTGGAGGTTTTATGATCCGGACCTTGGTATGGTACTCTTATGATGGTTATGACAT
TCGTAGTCTTAATGTCCGTCGTCTGAGGGATGCAATAGGGATAGTGAGCCAAGAGCCGATACTGTTTATGATGGCAGC
ATAGAATCAAATATCCGTCTTGGGTGGGAGAAAGCTACGAGAGAGGACATTGTCAGGGCATGTAAACAAGCTAACG
CTTGGGAATTTATTCAGCTTCTGCCCCGACGGGCTTAGTACCAGAGTAGGCGAGCGTGGCGTGCAACTATCAGGGGG
CCAGAAACAAAGAATTGCCATTGCACGTGCACTAATAAAGGATCCCCAAATTTGCTTCTGGACGAGGCAACCAGCG
CACTTGACACGGAGAGCGAATCCGTAGTACAAAAGGCTCTTGAACAGGCTCAGATCGGTAGAACTACAATCACGATT
GCACACCGTCTGTCAACTATCAGGGACGTGGATGAGATCCTAGTCTTTAAAACGGATCCATCGTCAAGGGGGAA
CGCACATAGAGTTAATTGCCGCGCGTGGACTATACTACGGAATGGTGTCTTGGCGAAGATATAAACCAACAGACTGAA

ATGATCGATGATGAAATGGACGAGGTCGACGATGTAGACGACCGTAGTTCTAATTTGGATGTCGTTTCGTAAGAAAAG
 GTCAATTGCGAATTCTTACCACAGATCCGTGTCCGACCCTTCAGAATTATCTCTTAGGTCTTCAGCCGCAATCGTAAA
 AGAGCTTCAGGACGCCGCGGAGGAGAGCTCAGTAAGACCTACACCGATGTCACGTATTTTTCTTATGAATAGAGAAA
 CTTGGCCATATCTTTTCGTGGGACTGGTCGGTTGCTGCTTAAGTGGTATCGTCCCGCCCTTCTTTGCATTGGTTTATA
 GTCAGATATTCAGCGTCTTCTCCGAACCGGTAGACAGACTTGGGAGCGACGCTCGTTTCTGGTCCCTTATGTTCTTA
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 TTTTCGCTTCTCACAATCCTTAATCTTCTTCATGTATGCGTTAGCATTTTGGTTAGGTTCTTTATTTGTGACAATGCG
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 CCTTTATTCCGGACGTGGTTAAAGCACGTTTAGCCGCAAGTTTGGTCTTTCACCTAAGCGAGTATCCGACGGCCATA
 GACTCATTGAGCGATCAAGGTAGTAGAATAACTATAAAGGGTGCTATCCAGTTAAAGAATGTTTTCTTTTCCTACCCG
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 CTCTGGCTGTGGGAAATCCACAGTTATGGGTCTGCTTGTGAGCGTTTTTACGACCCTAACAGAGGGCAACATACGTTG
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 TTTGATTGCACGATTGAGGAGAACATAATGTACGGACTGGACAGGGAAGTGAGCCATGAGGAGGTGGTCAACGCTG
 CCAAACCTGGCTAATATCCATAAATTTATCTTAAGCTTGCCCCTGGGGTATGAAACTAGGGTCCGGTGAAAAAGGGACC
 CAGTTGAGCGGCGGACAAAAGCAGCGTATAGCGATAGCAAGGGCATTGATAAGAAATCCAAGCATTCTACTTCTAG
 ACGAAGCAACCTCCGCTCTTGATACAGAATCTGAACAGGTGCTTCAAGAAGCTTTAGAAAACGCGCGTAAAGGTAG
 GACTTGTCTTGTGATAGCCCATAGACTGAGCACAATTCAAATTTCAAATTTGATTGTTGTAATCAACGAGGGTAAGGT
 TGCTGAGAAGGGCACCCATGCACAGTTGCTAGAGGCCAGCGGCATTTACAAAACACTGTGTGAGACGCAGACACTG
 GTAGCTGCCAGTTATCCATACGATGTCCCTGATTATGCATGA

>*PunPgp-9_codon_optimized*

ATGTCCATTCGTAGCTCTCATAAGCGTAGAGATAACCTTTTTGTCAGTCCCCTTATTGAGATCATTGAGAAAACGTCAG
 AGCGAGGAACCGAAGGAGACCCCGCCGCTAAGGCCTCCCTGAAACAACCTGTTGCGTTATACCTCAACTTTTGACC
 GTGCTTTGTTTTCGGTGGGCTCTAGTTGCCATTGGTACAGGCTGTGGGTTTCCGCTTCTGAGTATTCTAATGGGG
 TCTATGTCCCAGTCCTTTGTGACGCGCAGACCGCGTATTACGCTGGCTTTCGCTCCGCATCTTAACACTACTATCGA
 TAATGTAGAAGTCCCGCCTAGCTTGGAGAATTTACCTGGGATGGCTTTAGTGACCAGGTGATTACATACTGTCTAG
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TGAACACAGAATGAGAAAGGAGTTCTTCAAGGCGGTTATCAGGCAAGATATCGGGTGGTTTGACGAAAATCAATCA
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CCAGTTTATGAGTCAATTCTTCGCCGGCTTTATCATCGCATTTACATACGATTGGAGATTGACACTGATTATGATGAG
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AGGCCCTCAATTCGCCGTCTTTGGGAGCGCTTTAGGCGCAGCCGGTGCATTGTTTGAATAATTGATAGGGTGCCC
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CAGTTACCCGACGAGACCGGATATCAAATACTAAACGGAATTTCTTTTTCCGTTAATCCTGGGGAAACAGTTGCGTT
GGTAGGCACTAGCGGCTGTGGCAAGTCTACCATAGTGTCTCTTTTGTACGTTATTACAACCCGGAGTATGGAAACA
TCCTGATTGACGGCCATGAGATTTCTTCACTGAACTTGGCGTATCTGCGTAAGATGATCGGCGTTGTATCCCAGGAA
CCAGTTCTGTTTAATATGACGATTAAGGAGAACATCGAAATGGGCAACGAGGATGTTACCGAGGGAGAGATCTTGG
CCGCTTGCAGACGTGCTAACGCTACAACTTCATTAACCAACTTCCGAATAAGTACGAAACGATAGTGGGTGACAGA
GGTACACAATAAGTGGAGGGCAAAAACAAAGAATCGCTATAGCGCGTGCCTAGTTAGGAACCCAAAAATACTTCT
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AGGCGTGACAGAGTCTAATTTGATAGAAATACTTCAGTACGCTAGACCTGAGTGGATATATATAATATTAGGCTTGT
CGCATGCCTTATAACAAGGTAGTGTATATCCTGTCTTTAGTTTACTATTTAACGAGATTCTGGCGATCTTCGCGAAACC
TAGGGATCAGATGCTAAGTAATGGACACTTTTACTCCCTGATGTTCTTGGTGGTGGGTGCGGTATCTGCGACTACAC
TACTAATTCAGGCCTTTTTCTTTGGTATGTCCGCCGAGCGTCTTACGAGAAGGTTAAGGTCACGTATATTTCAGAACG
TACTTAGGATGGATATTGCGTACTTCGACATGCCTAACCAATTCAAGCGGCAAGATTTCCACGAGGCTGGCCACGGA
CACACCAAATGTGAAGTCTGCCATAGACTATAGACTGGGCTCAGTTTTAAGCAGCCTGGTTTCAGTAGGTTTCGGGA
TAGGCATTGCTTTTTATTTGGTTGGCAGATGGCACTTCTTGTATCGCTATATCCCGCTTGCAGGTGTCCGGCCAG
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GGAAGCGATTGAAAGTATCAGGACGGTTCATGCCCTAACCTAGAGCGTAAATTCTATGAGAAATTCTGCCATTTCT
TAGAGCGTCCTCATGAAACATCAACGCGTAAGGCGATAGCCCAGGGCATGGCTTACGGTTTTGCGAATTCTATATTC
TACTTCTGTACGCCTCTGCTTTCAGGTTTGGGTTATTTTAATTTTAGAGGAGATCAGCAGTCCAATAAATGTAATGA
AGGTGTTATTTGCCATATCTTTTACAGCCGGTACGTTAGGGTTCGCGTCAGCGTACTTTCCAGAGTATGCGAAAGCA

AAATTCGCTGCCGCAATAATTTTTAAAATGTTGAAGGAGGAACCTAAGATCGACTCTATGAAAACCTGATGGCGAGAA
 GCCGGAGATTTCCGGGTCCGTCGATTTTTCCAAGATCTATTTTCGCATATCCCAGCGTCCTGAAGTCGGCGTCCTAA
 AGGGCCTGGATTTACATGTAGACGCCGGGCAGACCCTGGCAATAGTAGGACCGAGCGGGTGTGGAAAGAGTACTG
 TCGTCAGCCTACTAGAACGTTTTTACGATCCGATTGACGGTACTGTCCGTGTGCACGGGAACGACATAAGACTTATC
 AATCCGACATATTTGAGATCACAACCTGGCTCTTGTCTCACAAGAGCCCATCTTGTTGATTGCAGCATCAGGGAAAA
 TATCATCTACGGCCTTCAGACGGAGGAATTTCCGATGAAGACATCGTGAATGTGGCGAGGTTGGCAAATATCGACA
 AATTTATTAAGAGCTGCCAGATGGTTATGAGACCAGAGTCGGGGAAAAGGGTACCCAATAAGCGGGGGACAGAA
 GCAACGTATAGCGATAGCGCGTGC GTTAATTAGGCAACCCAAAATACTATTGCTTGATGAAGCTACAAGTGCTCTAG
 ATACAGAGTCAGAGAAAGTGGTACAGGAGGCACTAGATAGGGCCGGGAAAGGTAGGACGTGTATAATAATAGCTCA
 CAGGCTGTCAACAGTAGTGAATGCAAATTGCATTGCCGTGGTGAAGAACGGCATTGTTCTGGAAAAGGGACCCAC
 GCAGAATAATGGATAAGCGTGGTGCCTATTACTACTAACACAAAACAGAATTTGAAGGAGACTGACGACGATGA
 CGATGATTTTGACGCAAGAATGTACCCATACGACGTTCCGGACTATGCTTGA

e) Primers for validation of constructs by sequencing

Gene	Forward Primers	Sequence (5'-3')	Reverse Primers	Sequence (5'-3')
<i>PunPgp-2</i> codon optimized	M13-24F-BLUE	GTAAAACGACGGCCAGTGAGCGCG	M13-24R-BLUE	AACAGCTATGACCATGATTACGCC
	Pgp-2_fw_1	TGATGCGTTCAAGTCAGGATAATG		
	Pgp-2_fw_2	AACATGCTTGGGCTATGTGGAG		
Gene	Forward Primers	Sequence (5'-3')	Reverse Primers	Sequence (5'-3')
<i>PunPgp-9</i> codon optimized	M13-24F-BLUE	GTAAAACGACGGCCAGTGAGCGCG	M13-24R-BLUE	AACAGCTATGACCATGATTACGCC
	Pgp-9_fw_1	ATGTCCATTCGTAGCTCTCATAAG		
	Pgp-9_fw_2	AACGAGATTCTGGCGATCTTCG		

f) Primers for RT-PCR for validation of transgene expression in yeast

Gene	Direction	Sequence (5'-3')	Tm (°C)	Fragment Size (bp)
<i>PunPgp-2</i> Codon optimized	Forward	GCATAATCAGGGACAT CGTATGG	63.9	962
	Reverse	GCGGTGATGCAACCCA TCAAC		
<i>PunPgp-9</i> Codon optimized	Forward	TGGCCACGGACACACC AAATGTG or TAGCCCAGGGCATGG CTTACGGTTT (used for RT-PCR in Supplemental Fig. 3)		1441 or 1085
	Reverse	GCATAGTCCGGAACGT CGTATGG		
lacZ	Forward	CTTTTGACACCAGACC AACTGG	63.1	1141
	Reverse	TGTTCCGTCATAGCGA TAACG		

Supplementary Table S2. Primers and sequences Sequences of a) full length open P-glycoprotein reading frame amplification (RT-PCR) primers for all *Parascaris univalens* Pgp, b) additional primers used to obtain sequences missing in both transcriptomes, verification primers, RACE PCR and spliced leader PCR) c) sequencing primers for all *Parascaris* Pgp, made by primer walking, d) Codon optimized constructs of *PunPgp-2* and *PunPgp-9* for expression in *Saccharomyces cerevisiae*, e) primers for validating correctness of codon optimized yeast expression constructs e) primers for RT-PCR for validating transgene expression at the transcription level. *Pun*: *Parascaris univalens*; Pgp: P-glycoprotein

Supplementary Table S3

Gene Name in Maximum Likely Hood Tree	Annotated Name (if applicable)	Organism	Accession Number Genbank	WormBase ID	Contig
<i>PunPgp-11,1</i>	<i>PeqPgp-11</i>	<i>Parascaris univalens</i>	AGL08022.1		
<i>PunPgp-16.1</i>	<i>PeqPgp16</i>	<i>Parascaris univalens</i>	AGL08023.1		
<i>PunPgp-2</i>		<i>Parascaris univalens</i>	MT001899		
<i>PunPgp-3</i>		<i>Parascaris univalens</i>	MT001900		
<i>PunPgp-9</i>		<i>Parascaris univalens</i>	MT001901		
<i>PunPgp-10</i>		<i>Parascaris univalens</i>	MT001902		
<i>PunPgp-11.2</i>		<i>Parascaris univalens</i>	MT001904		
<i>PunPgp-12</i>		<i>Parascaris univalens</i>	MT001905		
<i>PunPgp-16.2</i>		<i>Parascaris univalens</i>	MT001907		
<i>PunPgp-18A</i>		<i>Parascaris univalens</i>	MT001908		
<i>PunPgp-18B</i>		<i>Parascaris univalens</i>	MT001909		
<i>CbrPgp-16</i>	Hypothetical protein CBG12969	<i>Caenorhabditis briggsae</i>	CAP31851.1	BP:CBP17602	
<i>CeIPgp-1</i>		<i>Caenorhabditis elegans</i>	NP_502413	WP:CE11932	
<i>CeIPgp-2</i>		<i>Caenorhabditis elegans</i>	NP_491707	WP:CE41207	
<i>CeIPgp-3</i>		<i>Caenorhabditis elegans</i>	NP_509901	WP:CE03818	
<i>CeIPgp-4</i>		<i>Caenorhabditis elegans</i>	NP_001257143	WP:CE03308	
<i>CeIPgp-5</i>		<i>Caenorhabditis elegans</i>	NP_001257116	WP:CE43003	
<i>CeIPgp-6</i>		<i>Caenorhabditis elegans</i>	NP_001041287	WP:CE40818	

CeIPgp-7		<i>Caenorhabditis elegans</i>	NP_509812	WP:CE36668	
CeIPgp-8		<i>Caenorhabditis elegans</i>	NP_509811	WP:CE31624	
CeIPgp-9		<i>Caenorhabditis elegans</i>	NP_507487	WP:CE15714	
CeIPgp-10		<i>Caenorhabditis elegans</i>	NP_509205	WP:CE40807	
CeIPgp-11		<i>Caenorhabditis elegans</i>	NP_495674	WP:CE34788	
CeIPgp-12		<i>Caenorhabditis elegans</i>	NP_510126	WP:CE03260	
CeIPgp-13		<i>Caenorhabditis elegans</i>	NP_510127	WP:CE40253	
CeIPgp-14		<i>Caenorhabditis elegans</i>	NP_510128	WP:CE0262	
PpaPgp-1		<i>Pristionchus pacificus</i>		PP:PP30697	
PpaPgp-9		<i>Pristionchus pacificus</i>		PP:PP38391	
AsuPgp-2	Multidrug resistance protein 1	<i>Ascaris suum</i>	ADY40620		
AsuPgp-11.2	Multidrug resistance protein 3	<i>Ascaris suum</i>	ADY40644		
AsuPgp-16.2	Multidrug resistance protein 3	<i>Ascaris suum</i>	ADY40573		
AsuPgp-2_hp		<i>Ascaris suum</i>			AgR011_g279_t03
AsuPgp-3_hp		<i>Ascaris suum</i>			AgR049_g026_t04
AsuPgp-9_hp		<i>Ascaris suum</i>			AgB03_g189_t01
AsuPgp-10_hp		<i>Ascaris suum</i>			AgR054_g025_t01
AsuPgp-11.1_hp		<i>Ascaris suum</i>			AgB01_g256_t02
AsuPgp-12_hp		<i>Ascaris suum</i>			AgR009X_g036_t04
AsuPgp-16.1_hp		<i>Ascaris suum</i>			AgR016_g207_t01
AsuPgp-18A_hp		<i>Ascaris suum</i>			AgR067_g008_t02
AsuPgp-18B_hp		<i>Ascaris suum</i>			AgR067_g008_t02

HcoPgp-2		<i>Haemonchus contortus</i>	AAC38987		
HcoPgp-3		<i>Haemonchus contortus</i>	AFX93749.1		
HcoPgp-13		<i>Haemonchus contortus</i>	ARE67135.1		
HcoPgp-16		<i>Haemonchus contortus</i>	AFX93754.1		
HcoPgp-11.2		<i>Haemonchus contortus</i>	CDJ81808.1		
HcoPgp-11.1		<i>Haemonchus contortus</i>	CDJ84338.1		
HcoPgp-10	unnamed protein product	<i>Haemonchus contortus</i>	CDJ83820.1		
HcoPgp-9.1		<i>Haemonchus contortus</i>	JX430397		
ConPgp-2		<i>Cooperia oncophora</i>	AGJ71178		
ConPgp-3		<i>Cooperia oncophora</i>	AGJ71177		
OvoPgp-3 (10280)		<i>Onchocerca volvulus</i>		OV:OVP11414	
OvoPgp-10		<i>Onchocerca volvulus</i>		OV:OVP13603	
OvoPgp-11		<i>Onchocerca volvulus</i>		OV:OVP14503	
DimPgp-3		<i>Dirofilaria immitis</i>	ALI16766.1		
DimPgp-11		<i>Dirofilaria immitis</i>	ALI16773.1		
DimPgp-10		<i>Dirofilaria immitis</i>	ALI16764.1		
BmaPGP-10		<i>Brugia malayi</i>		BM:BM29899	
BmaPgp-21		<i>Brugia malayi</i>	XP_001900095		
BmaPgp-10a		<i>Brugia malayi</i>	XP_001897744		
BmaPgp11		<i>Brugia malayi</i>	CRZ23051.1		
BmaPgp-3	Bm11309	<i>Brugia malayi</i>		BM:BM42091	
BmaPgp-16	Bm2594, isoform a	<i>Brugia malayi</i>	CDP97625.1		
NamPgp-2	ABC transporter, ATP-binding protein	<i>Necator americanus</i>	XP_013305476.1		
NamPgp-13	ABC transporter, ATP-binding protein	<i>Necator americanus</i>	XP_013298646.1		

NamPgp-10	ABC transporter, ATP-binding protein	<i>Necator americanus</i>	XP_013299120.1		
NamPgp-14	hypothetical protein NECAME_00050	<i>Necator americanus</i>	XP_013309418.1		
CeloPgp-9		<i>Cylicocyclus elongatus</i>	AJM87336.1		
TciPgp-13		<i>Teladorsagia circumcincta</i>	PIO77339.1		
TciPgp-9		<i>Teladorsagia circumcincta</i>	PIO69295.1		
SmaMRP1_1 (1,2,3)		<i>Schistosoma mansonii</i>	XP_018648325.1		
SmaMRP1_2 (1,2,3)		<i>Schistosoma mansonii</i>	XP_018651783.1		
TcaPgp-2	Multidrug resistance protein 1A	<i>Toxocara canis</i>	KHN80157.1		
TcaPgp-3a		<i>Toxocara canis</i>	KHN78383.1		
TcaPgp-12	Multidrug resistance protein pgp-3	<i>Toxocara canis</i>	KHN87068.1		
TcaPgp-11.1	Multidrug resistance protein pgp-1	<i>Toxocara canis</i>	KHN73709.1		
TcaPgp-11.2	Multidrug resistance protein pgp-3	<i>Toxocara canis</i>	KHN89031.1		
TsuPgp-2	hypothetical protein M514_09538	<i>Trichuris suis</i>	KFD70158.1		
<u>Outgroup (collapsed)</u>	-				
DmePgpA		<i>Drosophila melanogaster</i>	AAA16186		
DmePgpB		<i>Drosophila melanogaster</i>	AAA28679		
DmePgpC		<i>Drosophila melanogaster</i>	AAA28680		
McaPgp-L		<i>Mytilus californianus</i>	ABS83556		
MgaPgp-L		<i>Mytilus galloprovincialis</i>	CAX46411		
MmuABCB-1b		<i>Mus musculus</i>	NP_035205		
MmuPgp		<i>Mus musculus</i>	AAA39514		

<i>PhuMRP-1</i>		<i>Pediculus humanus corporis</i>	XP_002425149		
<i>PhuMRP-2</i>		<i>Pediculus humanus corporis</i>	XP_002432260		
<i>PhuMRP-3</i>		<i>Pediculus humanus corporis</i>	XP_002426586		
<i>PhuMRP-4</i>		<i>Pediculus humanus corporis</i>	XP_002425021		
<i>HsaABCB1</i>		<i>Homo sapiens</i>	NP_000918.2		

Supplementary Table 3. NCBI Accession numbers, WormBase IDs and contigs (*Ascaris suum* transcriptome, GEO: GSE38470) of protein sequences used in the maximum likelihood tree. Annotated gene names were changed according to the result of the phylogenetic analysis in the tree and original gene names are listed.