*These predicted genes are absent from new genome assembly, however PCR data have confirmed their presence and expression in juvenile and adult worms.

### Supplementary Table 1

| Designation | F. hepatica ID (Predicted Protein Dataset) | Forward Primer | Reverse Primer | Product Size |
|-------------|-------------------------------------------|----------------|---------------|--------------|
| drasha      | maker-scaffold10x_157_pilon-snap-sne-0.203 | GCGATCAGTGCACCAATAGC | GCCATAGCTGCTACCAATTGC | 745 |
| dprcr8      | maker-scaffold10x_503_pilon-snap-gene-0.75 | GCCCATACTATCGGTCGATCCA | ATCCAGGCGCTTGATCAATC | 200 |
| dcr-1       | maker-scaffold10x_432_pilon-snap-gene-0.93 | TTACCAGCCGGAGAGATGAC | TCTCCTGATAGGCTGCTC | 204 |
| dcr-2.1     | maker-scaffold10x_1080_pilon-juvenile-gene-0.2 | ACCGATCAGGGGATGCAATC | ACCAAGGCTTCACGACTAC | 119 |
| dcr-2.2     | maker-scaffold10x_1080_pilon-snap-gene-0.12 | CCAAGGATATCGGGTGTTG | GGGTGGAGGCGGCTACTTA | 250 |
| trbp        | marker-scaffold10x_1115_pilon-snap-gene-0.4 | TTTGCTTGGTTCGATTTTC | TGCACTGATCCTCGACT | 197 |
| ago-1       | maker-scaffold10x_919_pilon-snap-gene-0.104 | GAATCAGGCGATCGAAGTC | CCCAGCAAATCGAGGAGAG | 599 |
| ago-2       | maker-scaffold10x_145_pilon-juvenile-gene-2.246 | AATCGGATCATTGCGATGAG | TGAATCTGCGGACGATGGA | 249 |
| ago-3       | maker-scaffold10x_979_pilon-snap-gene-0.4 | AATCAGGGCGGCAATACG | TGGTTTGGAGCAACCGTGTC | 578 |
| tsn-1       | maker-scaffold10x_485_pilon-snap-gene-0.80 | GTTGTCGACATCGCGATCT | TGCAAGTCGAAAGGTAAG | 200 |
| fmr1        | maker-scaffold10x_889_pilon-snap-gene-0.31 | CGCGATCCGTTGAGAAAAG | ATTCATCCTACGCAACCAC | 190 |
| maker-scaffold10x_2403_pilon-snap-gene-0.22 | CATACCGTGGTTCGAAACC | GCTGATGCGGTTGTTGATAA | 211 |
| maker-scaffold10x_142_pilon-snap-gene-0.83 | TACCAGCGATGCTTCGCTC | CTCAAGGATCAGGACACCAG | 216 |
| snap_masked-scaffold10x_1280_pilon-processed-gene-0.18/0.19 | GTGACAGCGGCAGTAAGTG | TCGTGAGGCGAGACACTAT | 202 |
| sid-1       | maker-scaffold10x_566_pilon-snap-gene-0.12 | CGGATTTGGGAAGTAATGTC | GTCAGCGCCGAGCTGTTTC | 636 |
| exportin-1  | maker-scaffold10x_1096_pilon-snap-gene-0.68 | GCCAACCGCAAGTACGTCA | ACGACGAGTCAGCAGTAG | 570 |
| exportin-2  | maker-scaffold10x_1061_pilon-snap-gene-0.108 | TCTCACGATCAGCTTCCCT | TTGGTTGCTGCGCTTTTC | 551 |
| exportin-5  | snap_masked-scaffold10x_1656_pilon-processed-gene-0.32 | TGCCTGACGTTGCTTATTAC | ACAGACGAGTACGAGTCA | 209 |
| eri1        | maker-scaffold10x_1695_pilon-processed-gff-StringTie-gene-0.47 | TTGAAACGAGTTGGTGCTCC | ACAGATCGACGACGTTCC | 501 |
| eri2        | maker-scaffold10x_48_pilon-snap-gene-0.20 | ACCGCTTGTGAGGCTACC | TGTCCCTGCGGCTGCTCAC | 441 |
| eri3*       | BN1106_s1255B000315.1 | TGGACTGCTGGATGTGAT | AAGCAATTCAATGTCGAGTAGA | 591 |
| BN1106_s1255B000315.2 | GGATCGCTGACGTTGCTTCC | TCCAGTATGCGTGCAATG | 410 |
| smg-2       | maker-scaffold10x_513_pilon-snap-gene-1.141 | GCCACCGAGCGATCCAGT | CATGAGCTCCGCTGATC | 588 |
| smg-4       | maker-scaffold10x_637_pilon-snap-gene-0.146 | GCAACAGGCTCAACACACC | ATTCGGCTCGACCCGATC | 503 |
| smg-5       | maker-scaffold10x_503_pilon-snap-gene-0.67 | CGGGTCAAGGCCCTCTAT | TCCGTCGATACGGGTC | 562 |
| smg-6       | maker-scaffold10x_102_pilon-juvenile-gene-0.93 | GTCTAGGCTATAGGCACAC | GGCAACGATGACGGTAC | 573 |
## Supplementary Table 2

| Designation | ID | NCBI/Wormbase Parasite ID | Forward dsRNA Primer* | Reverse dsRNA Primer* | Forward qPCR Primer | Reverse qPCR Primer |
|-------------|----|---------------------------|-----------------------|-----------------------|--------------------|---------------------|
| fhcam2      | Calmodulin 2 | AM412547/maker-scaffold10x_520_pilon-snap-gene-1.36 | AGATGCCACGGAACTGAAAG | AAATGTACCCGTCGCCATTA | ATCCGGGCAATCTTGTGATAAT | TTGCGCAGAAGAGTCAAGAA |
| fhgσgst     | σ Glutathione s-transferase | DQ974116/maker-scaffold10x_1043_pilon-snap-gene-0.18 | AATTCGCTTCTGCTCACTTG | TACTCTGCTTGTTTCCACC | AATTCGCTTCTGCTCACTTG | TCTTCACACTCCAAATGATCG |
| fhparamyo   | Paramyosin | augustus_masked-scaffold10x_377_pilon-processed-gene-0.188 | CTCTGGCATCTGCACTTGA | TTCCGATCTGCTGTTTG | TTGAAATCCCGGAAATTACC | ATCGACGTGATTCACCACAT |
| fhmap3k1    | Mitogen Activated Protein 3k1 | maker-scaffold10x_208_pilon-snap-gene-0.24 | AGCCCCACACAATACCAGAG | AATTTGGTGACTGGGTTGG | AGCCCCACACAATACCAGAG | TGTACCTGCTACACGTCGTG |
| fhcatl      | Cathepsin L | See McVeigh et al. 2014 | TKRTTATGTGACGGGTGTA | GCCBKRTAHGGRTAAT | TKRTTATGTGACGGGTGTA | GTATAGAAGCCACGCTTTG |
| fhgapdh     | GAPDH | AY005475/maker-scaffold10x_2706_pilon-snap-gene-0.15 | GGTGGAGAGGGTATTCGCT | GGTGGAGAGGGGCAATTCGTC | GGTGGAGAGGGGCAATTCGTC | AGATCCAGACGGAAACATC |
| neo         | Bacterial Neomycin | U55762 | GGTGGAGAGGCTATTCGCT | CTTCCCGTCTAGTACAGA | CTTCCCGTCTAGTACAGA | CTTCCCGTCTAGTACAGA |

*dsRNA constructs were amplified with primers labelled at the 5’ end with T7 RNA polymerase promoter sequence (taatacgactcactatagggt) to make T7 labelled templates in each direction.
## Supplementary Table 3

| Name   | S. mansoni ID             | F. hepatica ID                  | Length (aa) | % Identity with S. mansoni | Domains/Pfam IDs                                                                 | Signature Motifs Identified                        | Endonuclease 1 | Endonuclease 2 |
|--------|---------------------------|--------------------------------|-------------|---------------------------|---------------------------------------------------------------------------------|---------------------------------------------------|----------------|----------------|
| Drosha | Smp_142510 (1531aa)       | maker-scaffold10x_157_pilon-snap-| 1640        | 45.00                     | Ribonuclease III domain (IPR002099)                                             | Endonuclease 1                                      | ENFLEFDQ        | CAGLQGID_DLE   |
|        |                           | gene-0.203                      |             |                           | Double-stranded RNA-binding domain (IPR014720)                                   |                                                   |                |                |
| DGC8   | Smp_087220 (761aa)        | maker-scaffold10x_503_pilon-snap-| 457         | 37.60                     | Double-stranded RNA-binding domain (IPR014720)                                   |                                                   |                |                |
|        |                           | gene-0.75                       |             |                           |                                                                                  |                                                   |                |                |
| Dicer-1| Smp_169750 (2646aa)       | maker-scaffold10x_432_pilon-snap-| 2422        | 46.10                     | P-loop containing nucleoside triphosphate hydrolase (IPR027417)                |                                                   | 3’ End Pocket  | 5’ End Pocket  |
|        |                           | gene-0.93                       |             |                           | Dicer dimersiation domain (IPR005034)                                           |                                                   | SFPFTSTY...NSAAYYM | RPSPY...SGLQMTV...TV/MHL|SVLP| YRPN        |
|        |                           |                                 |             |                           | Ribonuclease III domain (IPR002099)                                             |                                                   |                |                |
|        |                           |                                 |             |                           | Double-stranded RNA-binding domain (IPR014720)                                   |                                                   |                |                |
| Dicer-2| Smp_033600 (954aa)        | maker-scaffold10x_1080_pilon-augustus-gene-0.2 | 601         | 30.83                    | Ribonuclease III domain (IPR002099)                                             | Endonuclease 1                                      | ENFLEFDQ        | CAGLQGID_DLE   |
|        |                           |                                 |             |                           |                                                                                  |                                                   |                |                |
|        |                           |                                 |             |                           |                                                                                  |                                                   |                |                |
|        |                           | maker-scaffold10x_1080_pilon-snap-gene-0.12 | 1091        | 41.50                    | Ribonuclease III domain (IPR002099)                                             | Endonuclease 1                                      | ENFLEFDQ        | CAGLQGID_DLE   |
| TRBP   | Smp_023670 (956aa)        | maker-scaffold10x_1115_pilon-snap-gene-0.4 | 357         | 56.10                    | Double-stranded RNA-binding domain (IPR014720)                                   |                                                   |                |                |
| Argonaute-1 | Smp_198380 (928aa) | maker-scaffold10x_919_pilon-snap-gene-0.104 | 675         | 17.00                     | Argonaute, linker 1 domain (IPR014811)                                           | Piwi Magnesium Ion Interacting Residues            | GADVTHP...YRGDV |                |
| Argonaute-2 | Smp_179320 (932aa) | maker-scaffold10x_145_pilon-augustus-gene-2.246 (BN1106_s11928000304) | 355 (1024)  | 16.8 (28.3)             | Protein argonaute, N-terminal (IPR032474)                                        | Piwi Magnesium Ion Interacting Residues            | GADVTHP...YRGDV |                |
|        |                           |                                 |             |                           | Argonaute, linker 1 domain (IPR014811)                                           | Piwi domain (IPR003165)                           |                |                |
| Argonaute-3 | Smp_102690 (854aa) | maker-scaffold10x_979_pilon-snap-gene-0.4 | 898         | 36.60                     | Argonaute, linker 1 domain (IPR014811), Ribonuclease H-like domain (IPR012337)  | Piwi Magnesium Ion Interacting Residues            | GADVTHP...YRGDV |                |
|        |                           |                                 |             |                           | Piwi domain (IPR003165)                                                         |                                                   |                |                |
| Tudor-SN | Smp_246840 (992aa) | maker-scaffold10x_485_pilon-snap-gene-0.80 | 1098        | 50.40                     | Staphylococcal nuclease (Snase-like), OB-fold (IPR016071),                      |                                                   |                |                |
|        |                           |                                 |             |                           |                                                                                  |                                                   |                |                |
|        | Smp_246850 (378aa)       | maker-scaffold10x_1508_pilon-snap-gene-0.1 (BN1106_s3758000233) | 1220 (518)  | 8.0 (22.22)             | Staphylococcal nuclease (Snase-like), OB-fold (IPR016071), Tudor domain (IPR002099) |                                                   |                |                |
| FMR1   | Smp_099630 (598aa)       | BN1106_s29328000172             | 690         | 20.40                    | K Homology domain (IPR004087)                                                   |                                                   |                |                |
|        |                           | maker-scaffold10x_2403_pilon-snap-gene-0.22 (BN1106_s60678000089) | 1015 (460)  | 24.1 (43.7)             | K Homology domain, type 1 (IPR004088)                                           |                                                   |                |                |
|        |                           |                                 |             |                           | K Homology domain, type 1 (IPR004088)                                           |                                                   |                |                |
|        | Smp_058170 (827aa)       | maker-scaffold10x_142_pilon-snap-gene-0.83 | 812         | 44.70                    | K Homology domain (IPR004088)                                                   |                                                   |                |                |
| **SID-1** | Smp\_152020 (933aa) | maker\_scaffold10x\_566\_pilon\_snap\_gene\_0.12 | 864 | 44.80 | PF13965 (SID-1\_RNA\_chan) |
| **Exportin-1** | Smp\_124820 (1076aa) | maker\_scaffold10x\_1096\_pilon\_snap\_gene\_0.68 | 1211 | 72.30 | Armmidil\-like helical (IPR011989) |
| **Exportin-2** | Smp\_128050 (1049aa) | maker\_scaffold10x\_1061\_pilon\_snap\_gene\_0.108 | 1061 | 60.00 | Armmidil\-like helical (IPR011989) |
| **Exportin-5** | Smp\_152800 (1286aa) | snap\_masked\_scaffold10x\_1656\_pilon\_processed\_gene\_0.32 | 786 | 25.10 | Armmidil\-like helical (IPR011989) |
| **ER1** | Smp\_029850 (562aa) | maker\_scaffold10x\_1695\_pilon\_pred\_gff\_StringTie\_gene\_0.47 | 788 | 38.70 | Ribonuclease H-like domain (IPR012337) |
| **ER2** | Smp\_051420 (232aa) | maker\_scaffold10x\_48\_pilon\_snap\_gene\_0.20 | 240 | 57.45 | Ribonuclease H-like domain (IPR012337) |
| **ER3** | Smp\_17760 (191aa) | BN1106\_s1255\_b000315.1 | 211 | 54.30 | Ribonuclease H-like domain (IPR012337) |
| **SMG2** | Smp\_061590 (1325aa) | maker\_scaffold10x\_513\_pilon\_snap\_gene\_1.141 | 1336 | 74.10 | RNA helicase UPF1, UPF2-interacting domain (IPR018999) |
| **SMG4** | Smp\_102240 (560aa) | maker\_scaffold10x\_637\_pilon\_snap\_gene\_0.146 | 456 | 12.90 | Pi-loop containing nucleoside triphosphate hydrolase (IPR027417) |
| **SMG5** | Smp\_169530 (1479aa) | maker\_scaffold10x\_503\_pilon\_snap\_gene\_0.67 | 1658 | 31.50 | Tetratricopeptide-like helical domain (IPR011990) |
| **SMG6** | Smp\_131650 (1409aa) | maker\_scaffold10x\_102\_pilon\_augustus\_gene\_0.93 (BN1106\_s2800\_b000128) | 224 (1381) | 3.3 (54.2) | Tetratricopeptide-like helical domain (IPR011990) |

*These predicted genes are absent from new genome assembly, however PCR data have confirmed their presence and expression in juvenile and adult worms*
Supplementary File 2

fhem2 and F. hepatica cathepsin L (fhcatL) dsRNAs were generated as described in main methods (primers listed in Supplementary Table 2). Glass pipettes were pulled using a PC-10 Narishige Puller (3½” glass pipettes; 7 mm drop; Heater 1 setting: 59.1; Heater 2 setting: 58.1) and broken approximately 1 mm from the end to enable liquid to be drawn up. A Drummond “Nanoject” XV Microinjector (Drummond Scientific Company) was used to inject adult liver fluke (those that had regurgitated gut contents) with 18.2 nl of dsRNA (100 ng/µl) or RPMI at seven sites around the worm. Adult worms were maintained in RPMI+ (RPMI with gentamicin (Sigma-Aldrich) diluted 1 in 100) for 18 h before transfer to CS20 (20% chicken serum in RPMI+). Those adults that were soaked in dsRNA were soaked in 5 ml of 100 ng/µl dsRNA for 18 h prior to transfer to CS20. All adults were maintained in 30 ml media for 48 h post dsRNA exposure before being snap frozen for further analysis. All adults were maintained in groups of three.

Adult liver fluke RNA was extracted by lysing single adults in 1 ml of TRIzol® (ThermoFisher Scientific) and following kit instructions. RNA pellets were suspended in 50 µl nuclease free water with 2000 ng of nucleic acid (measured on Nanodrop 2000) DNase treated prior to reverse transcription as described in main methods. qPCRs were run on diluted cDNA (1:10) and data treated in the same way as described in main methods. Protein was extracted using RIPA buffer, as described in McVeigh et al. (2014), although 1 ml extraction buffer was used. Actin (20–33, Sigma Aldrich) was used as control band with analysis carried out as described in main methods.