Schistosomiasis is among the most prevalent human parasitic diseases, affecting more than 200 million people worldwide. The aetiological agents of this disease are trematode flatworms (Schistosoma) that live and lay eggs within the vasculature of the host. These eggs lodge in host tissues, causing inflammatory responses that are the primary cause of morbidity. Because these parasites can live and reproduce within human hosts for decades, elucidating the mechanisms that promote their longevity is of fundamental importance. Although adult pluripotent stem cells, called neoblasts, drive long-term homeostatic tissue maintenance in long-lived free-living flatworms (for example, planarians), and neoblast-like cells have been described in some parasitic tapeworms, little is known about whether similar cell types exist in any trematode species. Here we describe a population of neoblast-like cells in the trematode Schistosoma mansoni. These cells resemble planarian neoblasts morphologically and share their ability to proliferate and differentiate into derivatives of multiple germ layers. Capitalizing on available genomic resources and RNA-seq-based gene expression profiling, we find that these schistosome neoblast-like cells express a fibroblast growth factor receptor orthologue. Using RNA interference we demonstrate that torne orthologue. Using RNA interference we demonstrate that

Neoblasts are the only proliferating somatic cells in planarians and they possess a distinct morphology: they are round-to-ovoid mesenchymal cells with a high nuclear-to-cytoplasmic ratio, a large nucleolus, and they often extend a cytoplasmic projection. To determine whether PSCs share similarities with planarian neoblasts, we examined these cells by dissociating male tissues devoid of germ cells (Fig. 1e). In these preparations we observed a number of distinct differentiated cell types that failed to incorporate EdU, including cells with a low nuclear-to-cytoplasmic ratio, neuron-like cells, and ciliated cells.

Figure 1 | Proliferation of somatic cells in adult schistosomes. a, b, EdU labelling in male (a) and female (b) parasites. c, d, Distribution of mesenchymal PSCs in male (c) and female (d) parasites. Phalloidin staining for actin shows male enteric and dorso-ventral muscles and female enteric and uterine muscles. e, Strategy to characterize PSC morphology. f, The morphology of EdU− and EdU+ cells. The arrowhead indicates a nucleolus. g, FISH for histone h2b with EdU labelling. The arrowhead indicates a cytoplasmic projection. a–d, g are confocal projections; a, b, ×500; c, d, 20 μm; f, g, 5 μm. Inset magnifications, relative to original: a, g, ×2.2; b, ×2.6; c, ×1.6; d, ×1.7.
cells (Fig. 1f). By contrast, we found that EdU incorporation was restricted to a neoblast-like population of cells with scant cytoplasm (n = 136 out of 137 cells) and often a prominent nucleolus (Fig. 1f). We also inspected PSCs within the mesenchyme using EdU to label nuclei and fluorescent in situ hybridization (FISH) to detect histone h2b messenger RNA in the cytoplasm of proliferative cells. Consistent with our results from tissue macerates, EdU+ cells possess a narrow rim of cytoplasm surrounding their nucleus, and these cells often display a cytoplasmic projection (Fig. 1g). These observations highlight morphological similarities between proliferating cells in schistosomes and planarian neoblasts.

Previous studies have exploited the sensitivity of planarian neoblasts to γ-irradiation as a means to identify neoblast-enriched transcripts. Using this strategy to identify PSC-expressed genes, we exposed parasites to various dosages of γ-irradiation and determined that 100–200 Gy were sufficient to block EdU incorporation (Fig. 2a). Because of their high ratio of somatic tissue to reproductive tissue and their large number of PSCs relative to female worms (compare insets in Fig. 1a and b), our remaining studies, unless otherwise noted, focused on male parasites. By comparing the transcriptional profiles of irradiated and non-irradiated parasites by RNA-seq (Fig. 2b), we identified 128 genes with significantly downregulated expression (≥2-fold, P < 0.05) 48 h after irradiation (Fig. 2c and Supplementary Table 1). Highlighting the efficacy of this approach to identify transcripts specific to proliferating cells, we found that genes expressed in differentiated tissues, such as the intestine (S. mansoni cathepsin B (ref. 17)), were unaffected by irradiation. By contrast, our list of downregulated genes was enriched for factors involved in the cell cycle (Supplementary Fig. 4 and Supplementary Table 1).

In addition to identifying cell-cycle-associated factors, systematic comparison of irradiation-sensitive genes with neoblast-enriched transcripts uncovered a number of interesting similarities (Supplementary Table 1). For instance, homologues of genes known to regulate planarian neoblasts such as p53, a sex-family transcription factor, fibroblast growth factor receptors, and argonaute 2 (ago2) 14–22, were significantly downregulated in irradiated schistosomes (Supplementary Fig. 4 and Supplementary Table 1). Another distinctive feature of neoblasts 14–22, and the somatic stem cells of other invertebrates 23, is that they often express post-transcriptional regulators associated with germline development (for example, vasa, piwi, tudor and nanos). Although vasa-like genes have been reported in Schistosoma, no true vasa orthologue has been identified 24. Similarly, piwi and tudor genes seem to be absent from schistosomes (data not shown). However, we identified a nanos orthologue (S. mansoni nanos-2 (Smnanos-2)) that was downregulated in somatic tissue after irradiation (Supplementary Fig. 4 and Supplementary Table 1). Because these genes represented potential regulators of PSC behaviour and could serve as useful markers for these cells, we examined their expression by whole-mount in situ hybridization. We detected Smago2-1, Smnanos-2 and SmfgfrA transcripts in cells scattered throughout the mesenchyme (Fig. 2d and Supplementary Fig. 5) in a pattern similar to that of cells incorporating EdU (Fig. 1a). This mesenchymal expression was radiation sensitive (Fig. 2d), indicating that these genes are expressed in proliferating cells. Consistent with

Figure 2 | Transcriptional profiling identifies genes expressed in proliferative cells. a, EdU incorporation is abrogated at d3 after irradiation. b, Strategy to identify PSC-expressed genes. c, Volcano plot showing expression differences in control versus irradiated parasites. n = 3 for each group. d, Whole-mount in situ hybridization for various transcripts in unirradiated and d5 post-irradiation parasites. n > 3 parasites. e, EdU labelling and FISH for SmfgfrA. A total of 1.988 out of 2,000 EdU+ PSCs were SmfgfrA+ after a 20–22 h pulse (n = 20 male parasites). a, e are confocal projections. Scale bars: a, e, 20 μm; d, 100 μm. Inset magnification, relative to original: e, ×2.6.

Figure 3 | PSCs self-renew and differentiate. a, EdU and BrdU double labelling. Arrowheads indicate EdU−/BrdU+ nuclei. Arrows indicate EdU+/BrdU− ‘doublets’. b, Percentage (±s.d.) EdU−/BrdU+ doublets (green) that are BrdU−/BrdU+ (top), BrdU−/BrdU− (middle, BrdU is magenta), or BrdU+/BrdU− (bottom). n = 21 parasites. c, Strategy to monitor cellular differentiation. d–g, EdU and succinylated wheat germ agglutinin (sWGA) labelling showing EdU+ cells in male intestine (d, e) or dorsal musculature (f, g) at d1 (d, f) and d7 (e, g) following a pulse. Insets in d, e show intestinal basal surface (dashed lines) and lumen (green). Arrowheads in e and g show EdU+ intestinal cells (e) or muscle cells (g). Images are confocal projections. Scale bars: a, d–g, 20 μm; b, 5 μm. Inset magnifications, relative to original: d, e, ×1.5.
this idea, we found that after an EdU pulse, >99% of EdU-incorporating somatic cells also expressed SmfgfrA (Fig. 2e).

To determine whether PSCs are stem cells, we assessed their ability both to self-renew and to produce differentiated cell types. To examine self-renewal, we administered sequential pulses of EdU and 5-bromo-2′-deoxyuridine (BrdU) to parasites in vitro. Because nearly all PSCs that incorporate EdU are SmfgfrA−, the ability of EdU+ cells to incorporate BrdU in subsequent cell cycles would suggest that fgfrA+ PSCs self-renew (that is, divide and produce more fgfrA+ PSCs). For these experiments we chose a chase period of 44 h, as this time frame should give many EdU+ PSCs sufficient time to divide (Supplementary Fig. 3d). Consistent with PSCs possessing the capacity for self-renewal, we found that 41% of cells that initially incorporate EdU are BrdU+ 3 days after an initial EdU pulse (Fig. 3a). Furthermore, we observed that many EdU+ cells were distributed in pairs, or ‘doublets’ (Fig. 3a); we suggest that most of these doublets are the products of cell division (Supplementary Discussion and Supplementary Fig. 6). In these EdU+ doublets, a disproportionately large fraction displayed asymmetric BrdU incorporation (that is, one nucleus is EdU+/BrdU−, whereas the other is EdU−/BrdU+) (Fig. 3b and Supplementary Discussion). This observation suggests that division progeny have an asymmetric capacity to proliferate. Whether this represents stem-cell-like asymmetric division or temporal differences in the ability of these cells to re-enter the cell cycle requires further experimentation. Nevertheless, these data are consistent with PSCs (or some PSC subpopulation) being capable of self-renewal.

To examine the capacity of PSCs to differentiate, we performed EdU pulse–chase experiments in vivo. For these experiments, schistosome-infected mice were injected with EdU and the distribution of EdU+ cells was monitored at early (day d1) and late (d7) time points (Fig. 3c). We successfully used this pulse–chase approach to monitor the differentiation of schistosome germ cells (Supplementary Fig. 7). Visualizing the syncytial epithelium of the schistosome intestine at d1, we did not observe EdU+ intestinal nuclei in male or female parasites (Fig. 3d, 0 EdU+/3,151 DAPI+ nuclei, 14 mixed sex parasites, n = 5 mice), confirming that cells in the intestine do not proliferate. After a 7-day chase, however, ~2.5% of the intestinal nuclei were EdU+ (Fig. 3e, 56 EdU+/2,189 DAPI+ nuclei, 10 mixed sex parasites, n = 3 mice). This observation suggests that cells initially labelled with EdU have the capacity to migrate into the intestine and differentiate into new intestinal cells. Similarly, we were able to monitor the differentiation of new cells in the body wall muscles. At d1 no EdU+ nuclei were observed in the male body wall musculature (Fig. 3f, 0 EdU+/1,882 DAPI+ nuclei, 13 male parasites, n = 6 mice), whereas at d7 ~10% of the muscle cell nuclei were EdU+ (Fig. 3g, 55 EdU+/584 DAPI+ nuclei, 6 male parasites, n = 3 mice). Because virtually all cells that initially incorporate EdU are SmfgfrA+, we suggest that these double-positive cells probably represent the only source of new intestinal and muscle cells and, thus, represent a collectively multipotent population of neoblast-like stem cells. Whether all SmfgfrA+ PSCs are multipotent or whether they exist as lineage-restricted progenitors remains unclear.

Although progress has been made in identifying transcriptional and post-transcriptional regulators of planarian neoblasts, little is known about the signal transduction networks functioning within these cells. Because the expression of FGFR receptor family members in proliferative cells is conserved between planarians and schistosomes, we speculated that FGFR signalling could regulate these cells in S. mansoni. To examine this idea, we disrupted SmfgfrA in vitro-cultured adult parasites using RNA interference (RNAi) (Supplementary Fig. 8). We found that inhibition of SmfgfrA resulted in reduced EdU incorporation (Fig. 4a, b and Supplementary Table 2) and down-regulation of cell-cycle-associated transcripts (Fig. 4c and Supplementary Fig. 8). To resolve whether this effect is due to reduced cell proliferation or a failure to maintain neoblast-like cells, we monitored the expression of PSC markers Smago-2 and Smmanos-2 in SmfgfrA(RNAi) parasites. SmfgfrA RNAi treatment resulted in a marked reduction in the number of cells expressing Smmanos-2 (Fig. 4c) as well as significantly reduced mRNA levels for Smago-2 and Smmanos-2 (Supplementary Fig. 8b). Together, these results indicate that SmfgfrA promotes the long-term maintenance of neoblast-like cells in S. mansoni. FGFR signalling is known to influence multiple processes, such as cell proliferation, differentiation and survival; furthermore, it has key roles in various stem-cell populations. Our results suggest a conserved role for FGFR signalling in controlling stem-cell behaviour in these parasites and demonstrate the feasibility of using RNAi to abrogate adult gene expression and manipulate neoblast-like cells in S. mansoni.

Adult schistosomes can modulate growth in response to host immune signals and male–female pairing status and they can regenerate damaged tissues after sublethal doses of the anti-schistosomal drug praziquantel. These observations reveal the developmental plasticity of schistosomes, and suggest that these parasites can use distinct developmental programs in response to a range of external stimuli. Future studies characterizing the role of neoblast-like cells in diverse contexts could address long-standing gaps in our knowledge of schistosome biology and may reveal novel therapeutic strategies for treating and eliminating schistosomiasis.

METHODS SUMMARY

EdU detection in adult parasites was performed using Alexa 488 azide (Invitrogen) as described previously. To observe the morphology of PSCs, single-cell suspensions were obtained by incubating minced male parasites in trypsin-EDTA (Sigma); cell suspensions were fixed in 4% formaldehyde and spread on microscope slides. For in situ hybridizations, parasites were fixed in 4% formaldehyde for 4.5 h and processed essentially as described previously. Immunofluorescence and histological staining were performed similar to previous studies. For transcriptional profiling, male parasites were γ-irradiated, cultured in vitro for 48 h and processed for RNA-seq (Illumina). Transcriptional differences between irradiated and mock-irradiated controls were assessed using CLC Genomics Workbench (version 4, CLC Bio). For RNAi, in vitro cultured parasites were soaked with dsRNA (20–30 μg ml−1) freshly added on days 1–3 and every 5–6 days thereafter. A 1.5-kb dsRNA derived from an irrelevant bacterial sequence was used as a negative control. Experiments with and care of vertebrate animals were performed in accordance with protocols approved by the Institutional Animal Care and Use
Committee (IACUC) of the University of Illinois at Urbana-Champaign (protocol approval number 10035).

Full Methods and any associated references are available in the online version of the paper.

Received 3 September 2012; accepted 18 January 2013.

Published online 20 February 2013.

1. Chitsulo, L., Engels, D., Montresor, A. & Savioli, L. The global status of schistosomiasis and its control. Acta Trop. 77, 41–51 (2000).
2. Basch, P. F. Schistosomes: Development, Reproduction, and Host Relations (Oxford Univ. Press, 1991).
3. Wagner, D. E., Wang, I. E. & Reddien, P. W. Clonogenic neoblasts are pluripotent adult stem cells that underlie planarian regeneration. Science 322, 811–816 (2011).
4. Newmark, P. A. & Sánchez Alvarado, A. Not your father’s planarian: a classic model enters the era of functional genomics. Nature Rev. Genet. 3, 210–219 (2002).
5. Brehm, K. Echinococcus multilocularis as an experimental model in stem cell research and molecular host-parasite interaction. Parasitol. Res. 107, 537–555 (2010).
6. Protaisio, A. V. et al. A systematically improved high quality genome and transcriptome of the human blood fluke Schistosoma mansoni. PLoS Negl. Trop. Dis. 6, e1455 (2012).
7. Berriman, M. et al. The genome of the blood fluke Schistosoma mansoni. Nature 460, 352–358 (2009).
8. Den Hollander, J. E. & Erasmus, D. A. Schistosoma mansoni: DNA synthesis in males and females from mixed and single-sex infections. Parasitology 88, 463–476 (1984).
9. Nollen, P. M., Floyd, R. D., Kolzow, R. G. & Deter, D. L. The timing of reproductive cell development and movement in Schistosoma mansoni, S. japonicum, and S. haematobium, using techniques of autoradiography and transplantation. J. Parasitol. 62, 227–231 (1976).
10. Salic, A. & Mitchison, T. J. A chemical method for fast and sensitive detection of DNA synthesis in vivo. Proc. Natl Acad. Sci. USA 105, 2415–2420 (2008).
11. Newmark, P. A. & Sánchez Alvarado, A. Bromodeoxyuridine specifically labels the regenerative stem cells of planarians. Dev. Biol. 226, 142–153 (2000).
12. Forsthoefel, D. J., Park, A. E. & Newmark, P. A. Stem cell-based growth, regeneration, and remodeling of the planarian intestine. Dev. Biol. 356, 445–459 (2011).
13. Baguñà, J. & Romero, R. Quantitative analysis of cell types during growth, degrowth and regeneration in the planarians Dugesia mediterranea and Dugesia tigrina. Hydrobiologia 184, 181–194 (1981).
14. Wagner, D. E., Ho, J. J. & Reddien, P. W. Genetic regulators of a pluripotent adult stem cell system in planarians identified by RNAi and clonal analysis. Cell Stem Cell 10, 299–311 (2012).
15. Solana, J. et al. Defining the molecular profile of planarian pluripotent stem cells using a combinatorial RNAseq, RNA interference and irradiation approach. Genome Biol. 13, R19 (2012).
16. Eisenhover, G. T., Kang, H. & Sánchez Alvarado, A. Molecular analysis of stem cells and their descendants during cell turnover and regeneration in the planarian Schmidtea mediterranea. Cell Stem Cell 3, 327–339 (2008).
17. Caffrey, C. R., McKerrrow, J. H., Salt, P. J. & Sajid, M. Blood ‘n’ guts: an update on schistosomiasis digestive peptidases. Trends Parasitol. 20, 241–248 (2004).
18. Onali, P. et al. Gene expression of pluripotency determinants is conserved between mammalian and planarian stem cells. Dev. Growth Differ. 44, 191–204 (2002).
19. Labbé, R. M. et al. A comparative transcriptomic analysis reveals conserved features of stem cell pluripotency in planarians and mammals. Stem Cells 30, 1734–1745 (2012).
20. Pearson, B. J. & Sánchez Alvarado, A. A planarian p53 homolog regulates proliferation and self-renewal in adult stem cell lineages. Development 137, 213–221 (2010).
21. Ogawa, K. et al. Planarian fibroblast growth factor receptor homologs expressed in stem cells and cephalic gangliaons. Dev. Growth Differ. 44, 191–204 (2002).
22. Rouhana, L., Shibata, N., Nishimura, O. & Agata, K. Different requirements for conserved post-transcriptional regulators in planarian regeneration and stem cell maintenance. Dev. Biol. 341, 429–443 (2010).
23. Juliano, C. & Wessel, G. Versatile germline genes. Nature Rev. Genet. 3, 41–51 (2002).
24. Skinner, D. E. et al. Vasa-likedeAD-box RNA helicases of Schistosoma mansoni. PLoS Negl. Trop. Dis. 6, e1686 (2012).
25. Lanner, F. & Rossant, J. The role of FGF/Erk signaling in pluripotent cells. Development 137, 3351–3360 (2010).
26. Davies, S. J. et al. Modulation of blood fluke development in the liver by hepatic CD44 lymphocytes. Science 294, 1358–1361 (2001).
27. Severinghaus, A. E. Sex studies on Schistosoma japonicum. Q. J. Microsc. Sci. 71, 653–702 (1928).
28. Shaw, M. K. & Erasmus, D. A. Schistosoma mansoni: structural damage and tegumental repair after in vivo treatment with praziquantel. Parasitology 94, 243–254 (1987).
29. Collins, J. J. III et al. Genome-wide analyses reveal a role for peptide hormones in planarian germline development. PLoS Biol. 8, e1000509 (2010).
30. Collins, J. J. III, King, R. S., Cogswell, A., Williams, D. L. & Newmark, P. A. An atlas for Schistosoma mansoni organs and life-cycle stages using cell type-specific markers and confocal microscopy. PLoS Negl. Trop. Dis. 5, e1009 (2011).

Supplementary Information is available in the online version of the paper.

Acknowledgements We thank R. Roberts-Galbraith, M. Iggins and L. Rouhana for comments on the manuscript; R. King for sharing the castahespin B plasmid and unpublished protocols; and A. Hernandez for assistance with Illumina sequencing. Schistosome-infected mice were provided by the NIAID Schistosomiasis Resource Center and the Biomedical Research Institute through NIAID contract no. HHSN2720110000051. This work was supported by: NIH F32 HD062124 (J.J.C.) and NIH R21 A099642 (P.A.N.). P.A.N. is an investigator of the Howard Hughes Medical Institute.

Author Contributions J.J.C., B.W., B.G.L., M.E.T. and H.I. performed experiments. J.J.C., B.W., B.G.L., M.E.T. and H.I. wrote the paper. J.J.C., B.W., B.G.L., M.E.T. and H.I. contributed to data interpretation. J.J.C., B.W., B.G.L., M.E.T. and H.I. prepared the figures. J.J.C., B.W., B.G.L., M.E.T. and H.I. performed experiments. J.J.C., B.W., B.G.L., M.E.T. and H.I. performed experiments. J.J.C., B.W., B.G.L., M.E.T. and H.I. contributed to data interpretation. J.J.C., B.W., B.G.L., M.E.T. and H.I. prepared the figures.

Author Information RNA-seq analyses have been deposited in the NCBI Gene Expression Omnibus under accession number GSE42757. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to P.A.N. (pnewmark@life.illinois.edu).
METHODS

Parasite acquisition and culture. Adult S. mansoni (6–8 weeks after infection) were obtained from infected mice by hepatic portal vein perfusion with 37 °C DMEM (Mediatech) plus 5% fetal calf serum (FBS, HyClone/Thermo Scientific Logan). Parasites were rinsed several times in DMEM + 5% FBS and cultured (37 °C/5% CO₂) in Basch’s Medium 169 (ref. 32) and 1× antibiotic-antimycotic (Gibco/Life Technologies). Media was changed every 1–3 days.

EDU labelling. For in vitro labelling, parasites were cultured in Basch’s Medium 169 supplemented with 10 μM EdU (Invitrogen) diluted from a 10 mM stock in DMSO. Unless otherwise noted, animals were pulsed for 18–24 h. For in vivo labelling, schistosome-infected mice (6–8 weeks after infection) were given a single intraperitoneal injection (100–200 μg EdU per kg body weight) with 5 mg/ml EdU dissolved in PBS and then collected at various time points after injection.

In situ hybridization. Male and female parasites were separated by incubation (2–3 min) in a 0.25% solution of the anesthetic ethyl 3-aminobenzoate methanesulfonate (Sigma-Aldrich) dissolved in Basch’s Medium 169 or phosphate buffered saline (PBS). Relaxed parasites were then killed in a 0.6 M solution of MgCl₂ and fixed for 4.5 h in 4% formaldehyde dissolved in PBSTx (PBS + 0.3% Triton X-100). After fixation, parasites were dehydrated in MeOH and stored at −20 °C. Samples were rehydrated by incubation in 1:1 MeOH/PBSTx followed by incubation in PBSTx. Rehydrated samples were bleached for 1–2 h in formamide bleaching solution (0.5% Formaldehyde, 0.5% SSC, and 1.2% H₂O₂), rinsed with PBSTx, treated with proteinase K (2–10 μg/ml), Invitrogen) for 20–30 min at room temperature and post-fixed for 10–15 min in 4% formaldehyde in PBSTx. Samples were hybridized at 52–55 °C and otherwise processed as previously described24–26. Plasmids used for riboprobe synthesis were generated as described previously29 using oligonucleotide primers listed in Supplementary Table 3.

Immunofluorescence, histological staining and EDU detection. Parasites were relaxed, killed, fixed, dehydrated and rehydrated as described above and bleached in 6% H₂O₂ dissolved in PBS for 0.5–2 h. Dehydration and bleaching were omitted for samples labelled with phalloidin. Samples were then treated with Proteinase K and post-fixed as described above. Immunofluorescence, lectin and phalloidin staining were performed as described previously30. Rabbit anti-phospho-histone H3 Ser 10 (anti-pH3) (D2C8, Cell Signaling), rhodamine-conjugated sWGA (Vector Laboratories), and Alexa Fluor 568 phalloidin (Invitrogen) were used at 1:1,000, 1:100 and 1:100, respectively. EdU detection was performed essentially as previously described29. Sequences used to generate dsRNAs are provided in Supplementary Table 1. To examine similarities between proteins encoded from irradiation-sensitive transcripts in S. mansoni and genes expressed in planarian neoblasts, we compared our schistosome data set with both neoblast-enriched and ‘whole’ transcriptomes14,24–26 using standalone bLASTn. Schistosome proteins sharing no similarity to translated planarian mRNAs (e-value cutoff >1 × 10⁻⁵) were omitted from analysis. Assignment of whether protein pairs were orthologous, homologous, paralogous, or unrelated was assessed manually on an individual basis. Data and evidence supporting protein similarity are provided in Supplementary Table 1.

RNA interference. Although procedures have been previously described27,34, RNAi experiments with adult parasites were based on methods optimized for schistosomula32. Briefly, in vitro cultured parasites were soaked with 20–30 μg of dsRNA freshly added on days 1–3 and every 3–6 days thereafter. As a negative control, animals were soaked with dsRNA synthesized from the ccb and canrr containing insert of pJC53.2 (ref. 29). dsRNA synthesis was performed as previously described29. Sequence used to generate dsRNAs are provided in Supplementary Fig. 9. To measure mRNA levels, total RNA from control and knockdown parasites (~8 male posterior somatic fragments) was reverse transcribed (iScript cDNA Synthesis Kit, Bio-Rad) and quantitative real-time PCR was performed on an Applied Biosystems Step One Plus instrument using GoTaq qPCR Master Mix with SYBR green (Promega). Transcript levels were normalized to the mRNA levels of proteasome subunit beta type-4 (smp_056500). Relative quantities were calculated using the ΔΔCt calculation in the Step One Plus software. Oligonucleotide primer sequences are listed in Supplementary Table 3.

31. Lewis, F. Schistosomiasis.urr. Protoc. Immunol. 19, Unit 19 11 (2001).
32. Basch, P.F. Cultivation of Schistosoma mansoni in vitro. I. Establishment of cultures from cercariae and development until pairing. J. Parasitol. 67, 179–185 (1981).
33. Cogswell, A.A., Collins, J.J., Ill, Newmark, P.A. & Williams, D.L. Whole mount in situ hybridization methodology for Schistosoma mansoni. Mol. Biochem. Parasitol. 178, 46–50 (2011).
34. Neef, A.B. & Luedde, N.W. Dynamic metabolic labeling of DNA in vivo with arabinosyl nucleosides. Proc. Natl Acad. Sci. USA 108, 20404–20409 (2011).
35. Abramoff, M.D., Magelhaes, P.J. & Ram, S.S. Image processing with ImageJ. Biophotonics Int. 11, 36–42 (2004).
36. Falcon, S. & Genteman, R. Using GOstats to test gene lists for GO term association. Bioinformatics 23, 257–258 (2007).
37. Skelly, P.J., Da’dara, A. & Ham, D.A. Suppression of cathepsin B expression in Schistosoma mansoni by RNA interference. Int. J. Parasitol. 33, 363–369 (2003).
38. Boyle, J.P., Wu, X.J., Shoemaker, C.B. & Yoshino, T.P. Using RNA interference to manipulate endogenous gene expression in Schistosoma mansoni sporocysts. Mol. Biochem. Parasitol. 128, 205–215 (2003).
39. Štefanic, S. et al. RNA interference in Schistosoma mansoni schistosomula: selectivity, sensitivity and operation for larger-scale screening. PLoS Negl. Trop. Dis. 4, e6590 (2010).

©2013 Macmillan Publishers Limited. All rights reserved.