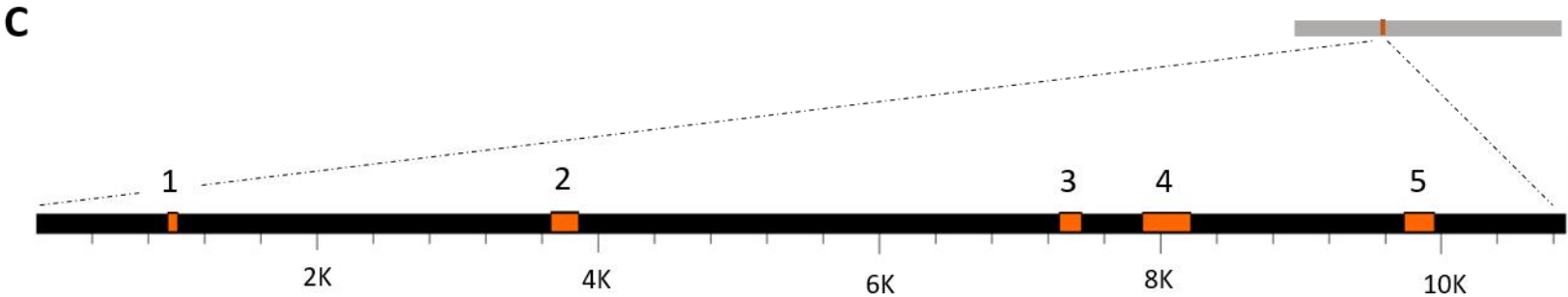
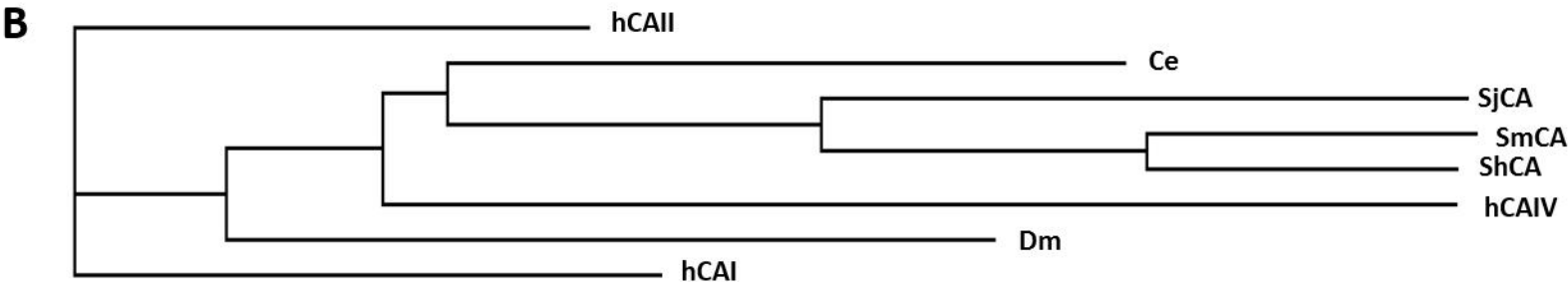
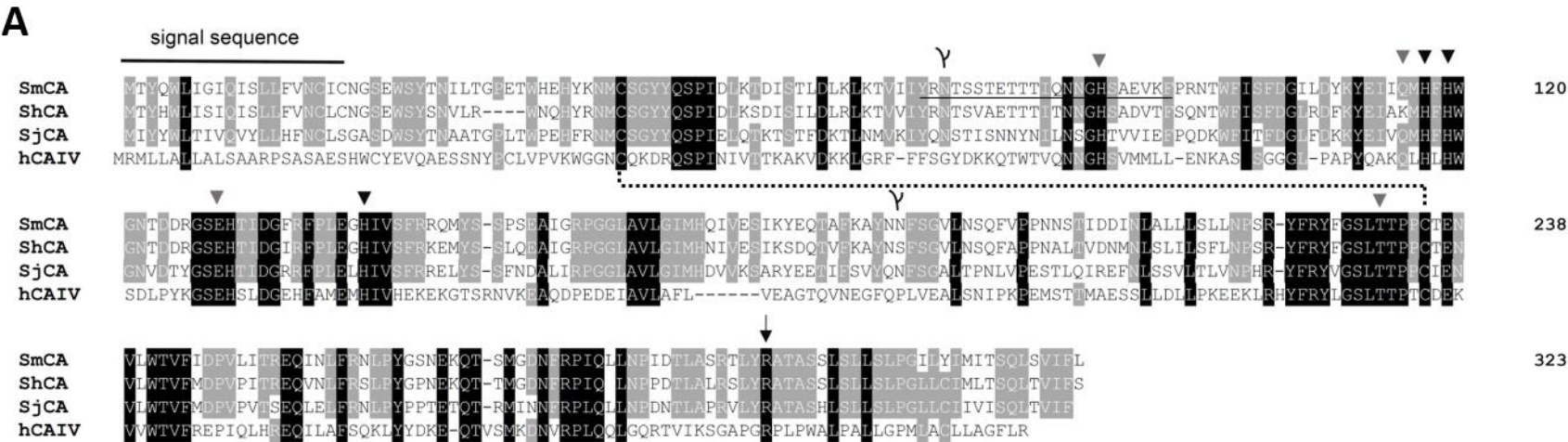


Supplementary figures and legends

Supplementary figure 1

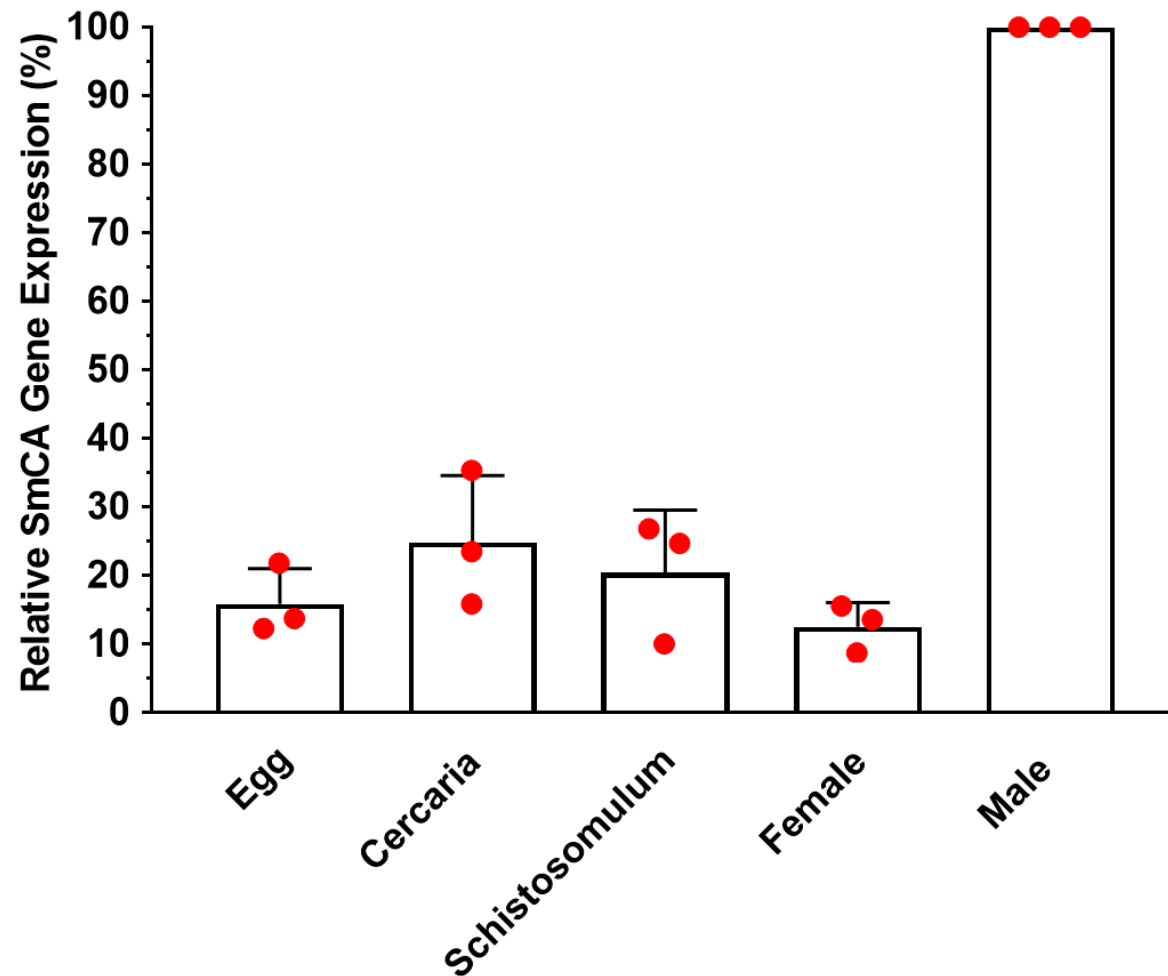


Supplementary figure 1 legend.

SmCA protein and gene organization. A. Alignment of the predicted amino acid sequence of SmCA with other selected CAs using CLUSTAL. Dashes indicate gaps introduced to maximize matching. Identical amino acids across all four sequences are white on a black background and amino acids found in three of four are white on a grey background. A predicted amino terminal 20 amino acid signal sequence in SmCA (¹MTYQWLIGIQISLLFVNCIC²⁰) is indicated by the solid line in the upper left. Three conserved zinc binding histidine residues (H¹¹⁷, H¹¹⁹, H¹⁴²) are indicated by black arrowheads and four highly conserved, critical active site residues (H⁸⁸, Q¹¹⁵, E¹²⁹, T²³¹) are indicated by grey arrowheads. A conserved, potential GPI-modification site (R²⁹⁵) is indicated by an arrow.

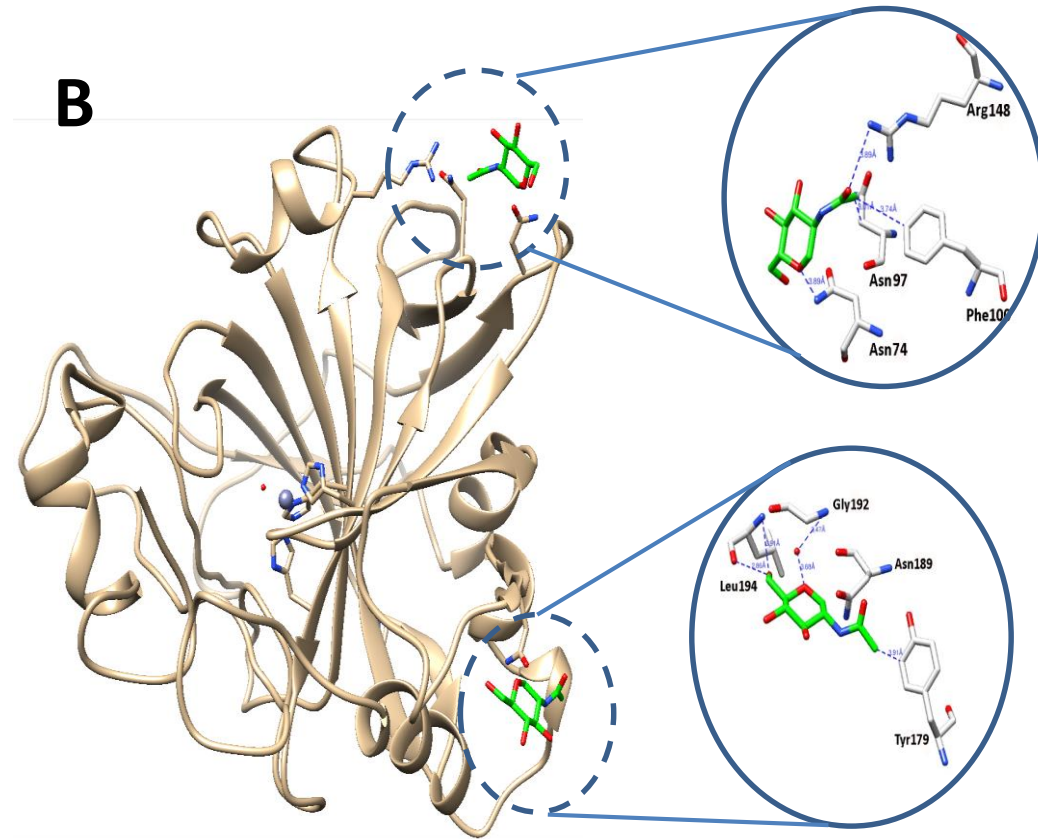
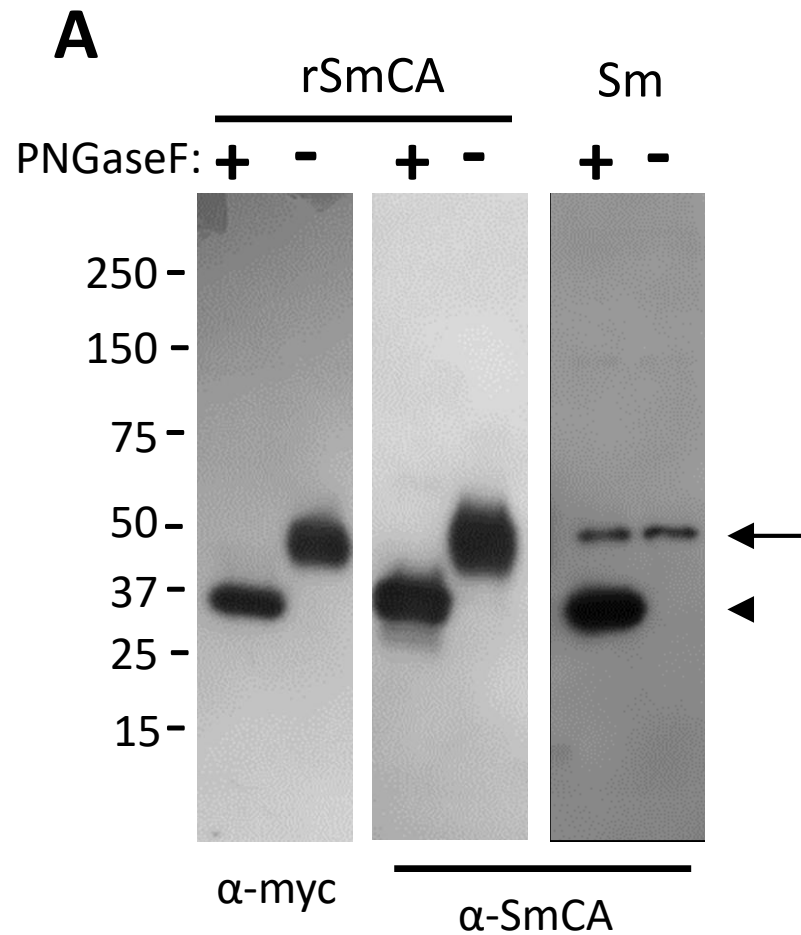
γ signifies verified glycosylation sites (at residues N⁷⁴ and N¹⁸⁹). The dotted line connects C⁴⁵ and C²³⁵ signifying the presence of a disulfide linkage. Designations (and accession numbers) are as follows: *S. mansoni* CA (SmCA, MK611932); *S. haematobium* ShCA (MK611933); *S. japonicum* SjCA, (CAX74423); Human isoform IV (hCA IV, AAA35625.1). The sequence of the peptide used to generate anti-SmCA antibodies (⁷²YRNTSSTETTTIQNNGHSAEVKF⁹⁴) is underlined. **B.** Unrooted phylogenetic tree of selected CAs generated by neighbor joining with Accelrys Gene software. The scale bar represents the number of amino acid differences per unit length. Designations (and accession numbers) additional to those given in A, above, are as follows: Ce (*Caenorhabditis elegans*) NP_510674.1, Dm (*Drosophila melanogaster*) NP_523561.1. hCAI (Human isoform 1) NP_001729.1, hCAII (Human isoform 2) AAH14949.1. **C.** Top: Diagrammatic representation of *S. mansoni* chromosome 6 showing the location of the SmCA gene. Bottom: Diagrammatic representation of the SmCA gene. Numbered exons are depicted in orange. Scale numbers at bottom represent kilobases (K).

Supplementary figure 2



Relative developmental expression of SmCA as measured by RT-qPCR in the parasite life stages listed. Expression in adult males is set at 100%. Red symbols represent source data (i.e. mean values from 3 biological replicates).

Supplementary figure 3



C

MTYQWLIGIQISLLFVNCIC**NG**SEWSYTNILTGPETWHEHYKNMCSGYYQSP
 IDLKTDISTLDLKLKTVIIYR**NTSS**TETTTIQNNGHSAEVKFPRNTWFISFDGILD
 YKYEIIQMHHFWGNTDDRGSEHTIDGFRFPLEGHIVSFRRQMYSSPSEAIGR
 PGGLAVLGIMHQIVESIKYEQTAFKAY**N****NFSS**VLNSQFVPP**NN**STIDDINLAL
 LLSLL**N**PSRYFRYFGSLTTPPCTENVLWTVFIDPVLITREQINLFRNLPYGSNEK
 QTSMGDNFRPIQLLNPIDTLASRTLYRATASSLSLLSLPGILYIMITSQLSVIFL

Supplementary figure 3 legend.

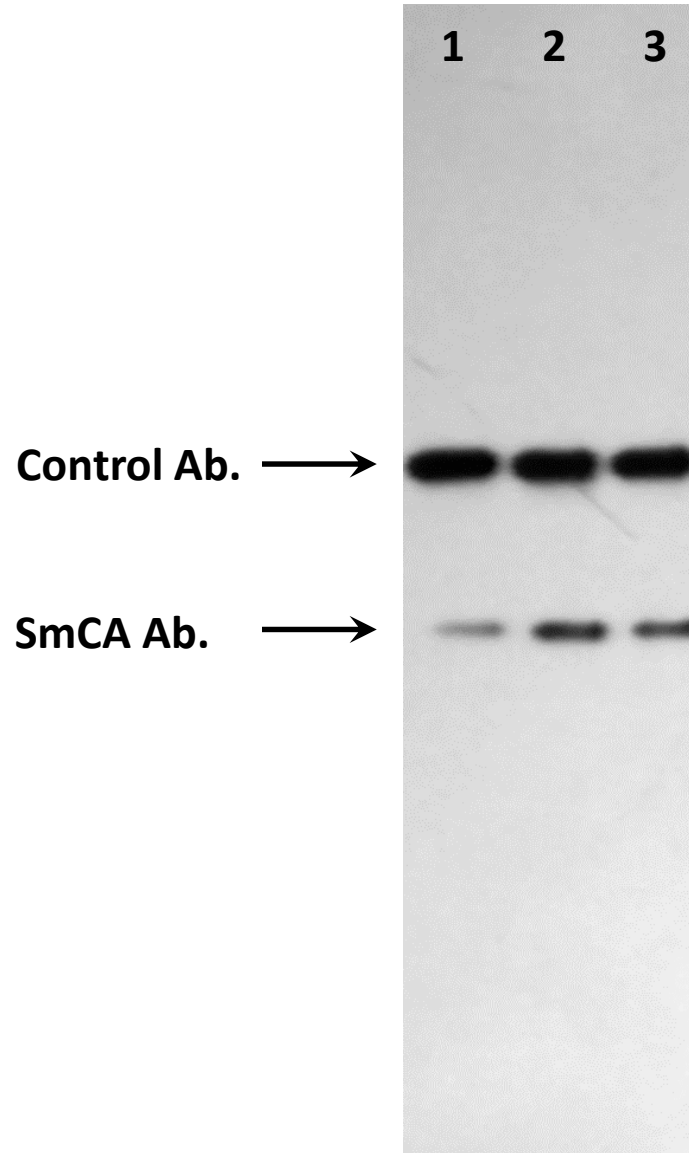
Supplementary figure 3. A. Western blot analysis of SmCA glycosylation status. Recombinant SmCA (rSmCA) or adult worm lysate (Sm) was resolved by SDS-PAGE following treatment with PNGase F (+) or after no treatment (-), as indicated. In both cases, untreated protein resolves at ~50kDa (arrow). Following treatment with PNGase F, a new, smaller band (at ~37 kDa) appears (arrowhead). The recombinant protein is detected with α -myc tag antibody (left panel) or anti-SmCA antibody (center panel) and the native protein is detected with anti-SmCA antibody (right panel) **B. Crystal structure determination of the N-glycosylation sites of SmCA.** Ribbon diagram of SmCA structure with circles highlighting sites of N-glycosylation. N-acetyl-D-glucosamine residues (green) are shown covalently bound to Asn⁷⁴ (upper circle) and Asn¹⁸⁹ (lower circle). Surrounding amino acids are also depicted. **C. Primary sequence of SmCA** with potential N-linked glycosylation sites highlighted. Sites shown by crystal structure analysis to bind carbohydrate (N⁷⁴ and N¹⁸⁹) are denoted by red dotted boxes.

Supplementary figure 4

hCAII	MSHHWGYGK-HNGPEHWHKDF-PIAKGERQSPVDIDHTAKYDPSLKPLSVSYD--QATS
4PXX	MSHHWGYGK-HNGPEHWHKDF-PICKGERQSPVDIDHTAKYDPSLKPLSVSYD--QATS
4HBA	MSHHWGYGK-HNGPEHWHKDF-PICKGERQSPVDIDHTAKYDPSLKPLSVSYD--QATS
SmCA	NGSEWSYTNILTGPETWHEHYKNMCSGYYSQSPIDLKTDISTLDLKLKTVIIYRNTSSSTET
hCAII	LRILNNGHAFNVEFDDSQDKAVLKGGPLDGTYRLIQFHFHWGSLDGQGSEHTVDKKKYAA
4PXX	LRILNNGHAFNVEFDDSQDKAVLKGGPLDGTYRLIQFHFHWGSHDGQGSEHTVDKKKYAA
4HBA	LRILNNGHAFNVEFDDSQDKAVLKGGPLDGTYRLIQFHFHWGSLDGQGSEHTVDKKKYAA
SmCA	TTIQNNGHSAEVKFPRTW-FISFDGILDYKYEIIQMHHFWGNTDDRGSEHTIDGFRFPL
hCAII	ELHLVHWNT-KYGDFGKAVQQPDGLAVLGIFLKVGSAPGLQKVV-----DVLDSIKT
4PXX	ELHLVHWNT-KYGDFGKAVQQPDGLAVLGIFLKVGSAPGLQKVV-----DVLDSIKT
4HBA	ELHLVHWNT-KYGDFGKAVQQPDGLAVLGIFLKVGSAPGLQKVV-----DVLDSIKT
SmCA	EGHIVSFRRQMYSSPSEAIGREGGLAVLGIMHQIVESIKYEQTAFKAYNNFSGVLNSQFV
hCAII	K--GKSADFTNFDPRGLLPESLDYWTYPGSLTTPPLLECVTWIVLKEPISVSSEQVLKFR
4PXX	K--GKSADFTNFDPRGLLPESLDYWTYPGSLTTPPCLESVTWIVLKEPISVSSEQVSKFR
4HBA	K--GKSADFTNFDPRGLLPESLDYWTYPGSLTTPPCLESVTWIVLKEPISVSSEQVLKFR
SmCA	PPNNSTIDDINLALLLSLLNPSRYFRYLGSLTTPCTENVLWTVFIDPVLITREQINLFR
hCAII	KLNFNGEGEPEELMVDNWRPAQPLKNRQIKASF-----
4PXX	KLNFNGEGEPEEPMVDNWRPAQPLKNRQIKASF-----
4HBA	KLNFNGEGEPEELMVDNWRPAQPLKNRQIKASF-----
SmCA	NLPYGS-NEKQTSMDGNFRPIQLLNPIDTLASRTLYRATA

SmCA sequence homology and structural comparison. Alignment of residues 21 through 298 of SmCA with that of full length hCA II and two hCA II mutants (4PXX and 4HBA) using CLUSTAL. Differences between hCA II and the mutants are highlighted in red.

Figure 3B-Full image



Parasite extracts following:

1. SmCA siRNA treatment.
2. Control siRNA treatment.
3. No treatment.

Figure 5B-Full image

Western blot showing rSmCA
(lanes a) and native SmCA (lanes
b) on the same gel

