

## Aberystwyth University

### *Protective human IgE responses are promoted by comparable life-cycle dependent Tegument Allergen-Like expression in Schistosoma haematobium and Schistosoma mansoni infection*

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## S1 Text

### Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) methodology

#### Sample preparation

Samples were subjected to enzymatic digestion with trypsin overnight at 37°C. After digestion, the supernatant was pipetted into a sample vial and loaded onto an autosampler for automated LC-MS/MS analysis.

#### LC-MS/MS

All LC-MS/MS experiments were performed using a Dionex Ultimate 3000 RSLC nanoUPLC (Thermo Fisher Scientific Inc, Waltham, MA, USA) system and a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific Inc, Waltham, MA, USA). Separation of peptides was performed by reverse-phase chromatography at a flow rate of 300 nL/min and a Thermo Scientific reverse-phase nano Easy-spray column (Thermo Scientific PepMap C18, 2 µm particle size, 100Å pore size, 75 µm i.d. x 50 cm length). Peptides were loaded onto a pre-column (Thermo Scientific PepMap 100 C18, 5 µm particle size, 100Å pore size, 300 µm i.d. x 5mm length) from the Ultimate 3000 autosampler with 0.1% formic acid for 3 minutes at a flow rate of 15 µL/min. After this period, the column valve was switched to allow elution of peptides from the pre-column onto the analytical column. Solvent A was water + 0.1% formic acid and solvent B was 80% acetonitrile, 20% water + 0.1% formic acid. The linear gradient employed was 2-40% B in 40 minutes. Further wash and equilibration steps gave a total run time of 60 minutes.

The LC eluant was sprayed into the mass spectrometer by means of an Easy-Spray source (Thermo Fisher Scientific Inc.). All  $m/z$  values of eluting ions were measured in an Orbitrap mass analyzer, set at a resolution of 35000 and was scanned between  $m/z$  380-1500. Data dependent scans (Top 20) were employed to automatically isolate and generate fragment ions by higher energy collisional dissociation (HCD, NCE:26%) in the HCD collision cell and measurement of the resulting fragment ions was performed in the orbitrap analyser, set at a resolution of 17500. Singly charged ions and ions with unassigned charge states were excluded from being selected for MS/MS and a dynamic exclusion window of 20 seconds was employed.

## Sequencing and multiple sequence alignment of ShTAL5 expression vector Sanger sequence results

Multiple sequence alignment of ShTAL5 (version 1 and version 3 genome annotation) cDNA sequences with SmTAL5, SmTAL6 and results from Sanger sequencing of ShTAL5-pGEX expression vector, performed in CLUSTAL O (1.2.4). ShTAL5 V3.0 sequence truncated at 567 nucleotides.

```

ShTAL5_v3_MS3_00002091 -----ATGTATCAACAAAATGAGACAATTACA 27
SmTAL5_Smp_195090 ATGGAACCATTTGTTAATATTTTTTTGCTATAGATGAGCAACAGAAATGAAACAATTACA 60
ShTAL5_v1_MS3_05957 ATGGAACCATTTGTTAATATATTTTTTTGCGATAGATGAGCAACAAAATGAGACAATTACA 60
ShTAL5_expression_vector ATGGAACCATTTGTTAATATATTTTTTTGCGATAGATGAGCAACAAAATGAGACAATTACA 60
                                     .: * *****.*****.*****

ShTAL5_v3_MS3_00002091 AGAGATGAATTGAGACGTTATGTAAAGCACAATCATTAGATGAAGGGATGATAACGAGA 87
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ShTAL5_v1_MS3_05957 AGAGATGAATTGAGACGTTATGTAAAGCACAATCATTAGATGAAGGGATGATAACGAGA 120
ShTAL5_expression_vector AGAGATGAATTGAGACGTTATGTAAAGCACAATCATTAGATGAAGGGATGATAACGAGA 120
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ShTAL5_v1_MS3_05957 TGGCAAGCATTATTTGATCCAACAAATACTGGTATTATAACATTTCAAAAATTTTGTGAT 180
ShTAL5_expression_vector TGGCAAGCATTATTTGATCCAACAAATACTGGTATTATAACATTTCAAAAATTTTGTGAT 180
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ShTAL5_v1_MS3_05957 GTCCTCGGTGTA AAAACCGGAACAAGCACGTA CTCTCGGAAGAGTGTGTCAATAACCGA 240
ShTAL5_expression_vector GTCCTCGGTGTA AAAACCGGAACAAGCACGTA CTCTCGGAAGAGTGTGTCAATAACCGA 240
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ShTAL5_v3_MS3_00002091 CCACTGCCGAAAGATTTACAAATAATCTCCCAAATATGTCACCAGAAGATCAATTCCAA 267
SmTAL5_Smp_195090 CCACTGCCGAAAGATTTACAAATAATCTCCCAAATATGTCATCAGAAAGATCAATTCCAA 300
ShTAL5_v1_MS3_05957 CCACTGCCGAAAGATTTACAAATAATCTCCCAAATATGTCACCAGAAGATCAATTCCAA 300
ShTAL5_expression_vector CCACTGCCGAAAGATTTACAAATAATCTCCCAAATATGTCACCAGAAGATCAATTCCAA 300
                                     *****.*****.*****

ShTAL5_v3_MS3_00002091 ATATTTGAATTTGTTTCGATCACTGTTAGATAAAAATTTATCAGGTCAAGATATGACACAA 327
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ShTAL5_v1_MS3_05957 ATATTTGAATTTGTTTCGATCACTGTTAGATAAAAATTTATCAGGTCAAGATATGACACAA 360
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ShTAL5_v1_MS3_05957 ATGATAAAAACAATGGCTTGATAAGACATTCGACCCTTCATGGCATGTTGTTATAATCGAT 420
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SmTAL5_Smp_195090 GGTTTCATATTGGATATCATATTCACATTTACCTGAACAATCTTTACAATTTTCGATTGAAA 480
ShTAL5_v1_MS3_05957 GGTTTCATATTGGATATCGTATTCACATTTACCTGAACAATCTCTACAATTTTCGATTGAAA 480
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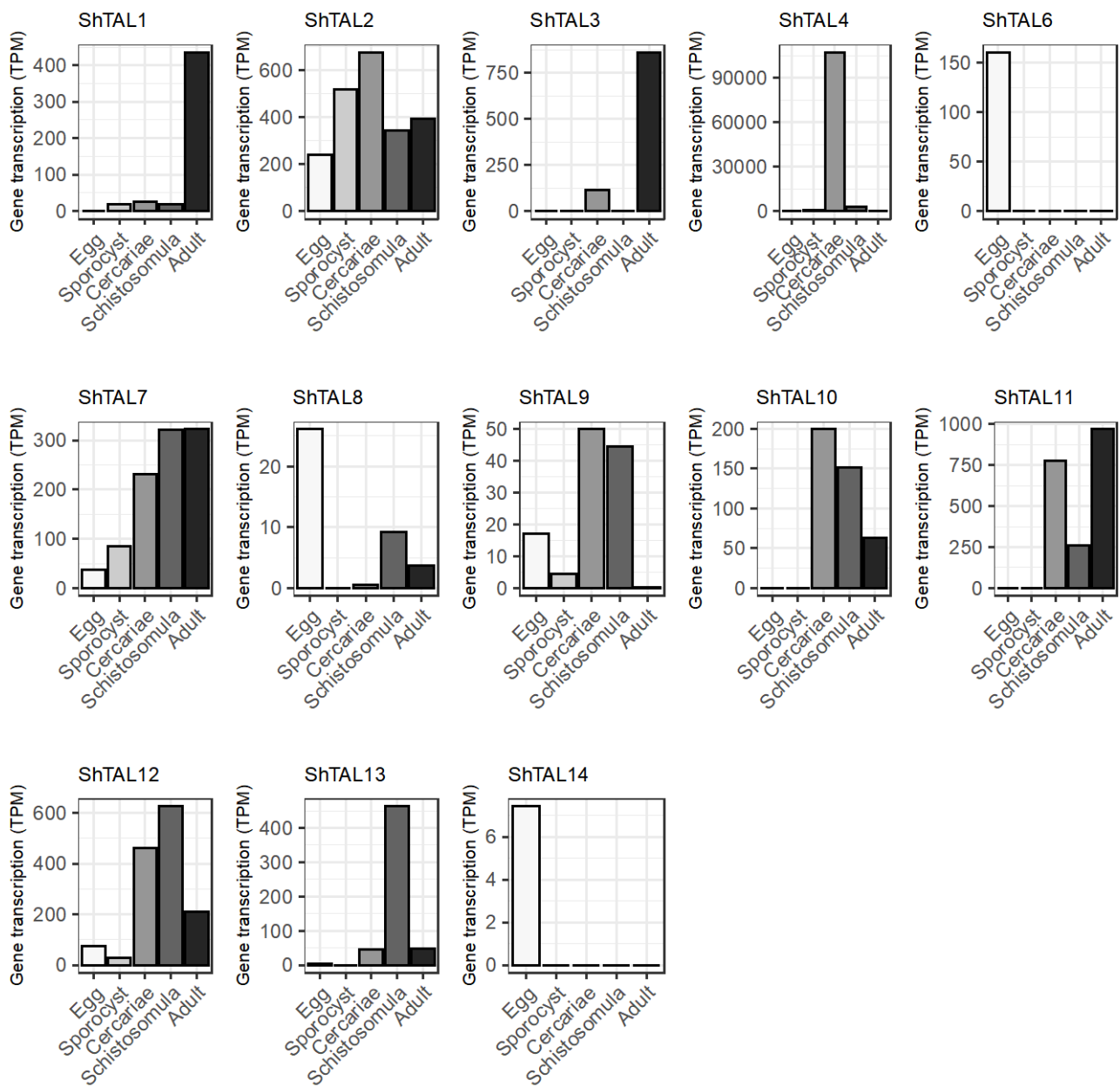
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ShTAL5_v1_MS3_05957 GAAAAGTGTATTATTAGTTT-GGCGCACAC----CTA-AATATTGA----- 519
ShTAL5_expression_vector GAAAAGTGTATTATTAGTTT-GGCGCACAC----CTA-AATATTGA----- 519
                                     *****.***** * * .***.* * .:. ****

ShTAL5_v3_MS3_00002091 CAACAACCTACTGTAAGAGAGAAGAACTAGATCCAGAGGAGGAAGAAATCAGGAAGAA 567
SmTAL5_Smp_195090 ----- 519
ShTAL5_v1_MS3_05957 ----- 519
ShTAL5_expression_vector ----- 519

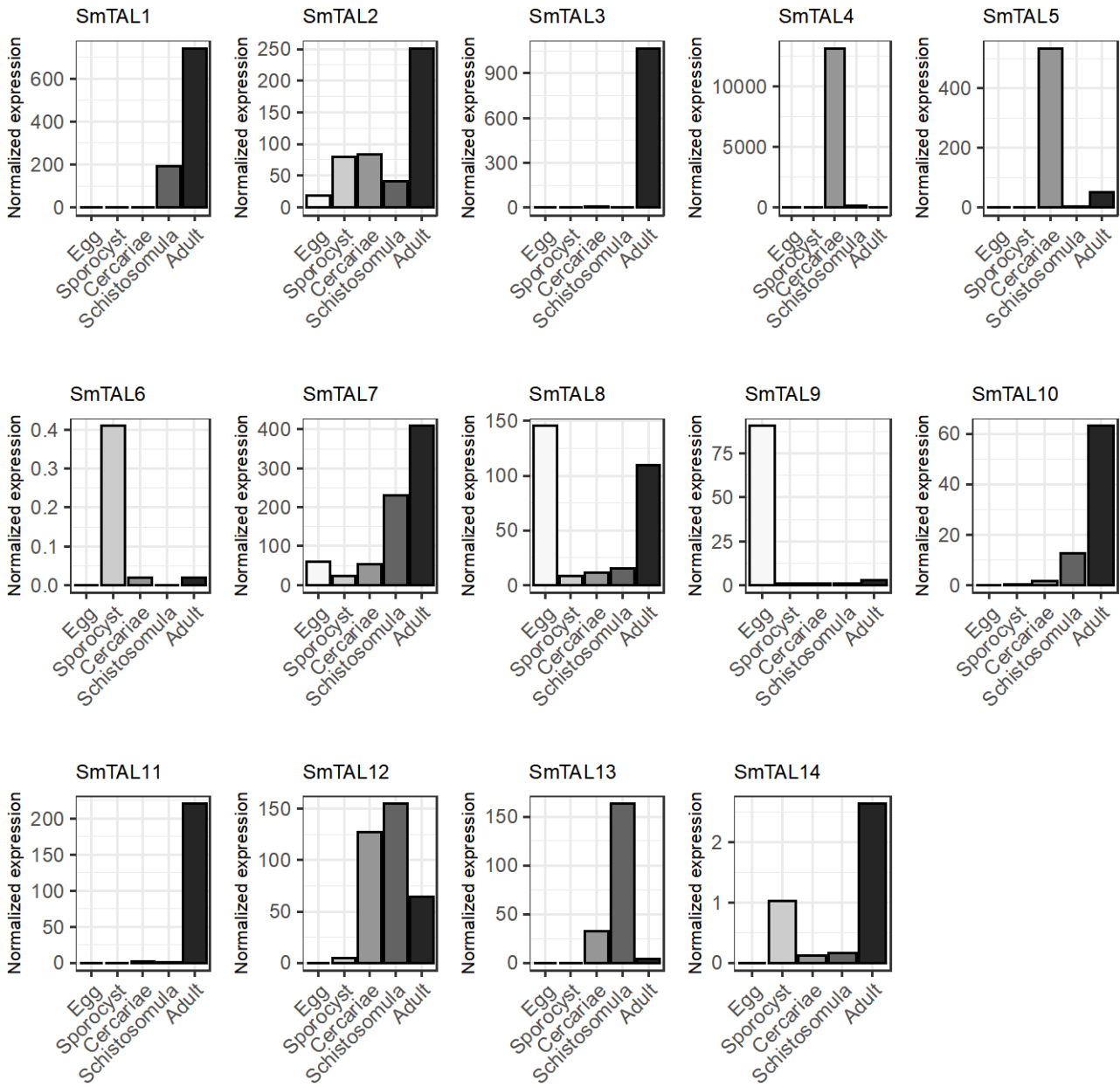
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Multiple sequence alignment of ShTAL6 version 1 and ShTAL5 version 3 genome annotation cDNA sequences with SmTAL6, performed in CLUSTAL O (1.2.4). ShTAL5 V3.0 sequence truncated, showing nucleotides 1201–1697 only.

SmTAL6_Smp_072620	-----ATGTTTAAT---GTTTGTAAATTTTCCA	25
ShTAL5_v3_MS3_00002091	TTCGCCAAAGAGAATCATCTGGGTAATCGAATGGTTCAAAGATGGTTTGAGCTATTTTCG	1260
ShTAL6_v1_MS3_05176	-----ATGGTTCAAAGATGGTTTGAGCTATTTTCG	30
	: *** **: *****.:*:*** *	
SmTAL6_Smp_072620	CTAATGTCATATGTACCAGTATCATTCTTCTTAATCCATACAAACCAT--ACTTTTAA	83
ShTAL5_v3_MS3_00002091	GAAGAGACAACAA-AC-----CAAATTACACTTAATA-----	1291
ShTAL6_v1_MS3_05176	GAAGAGACAACAA-AC-----CAAATTACACTTAATAAGTTTTTAAGTGTCTAGTGT	83
	:*.:*:**:.:. ** **: *:**:*****.	
SmTAL6_Smp_072620	TATATATTTTTATAGTGAATAATACGAAGAAATACAACCCACAGAATGCAGCATTGTT	143
ShTAL5_v3_MS3_00002091	-----ATGAAAAAATGCGAAGAAATACAATTCACACACATTTCAGCATTGTT	1337
ShTAL6_v1_MS3_05176	TGCAAAAGAGGAATATGAAAAAATGCGAAGAAATACAATTCACACACATTTCAGCATTGTT	143
	.*****:***.***** *****.* ** *****	
SmTAL6_Smp_072620	TAAACTTGGTTCAGATATTGAATACATTCTGGTGATATGATGTTATCACACAACAAATCAA	203
ShTAL5_v3_MS3_00002091	TAAACTTGGCTCAGACATTGAATACATTCTGGCGATATGATGTTACCACAACAAATCAA	1397
ShTAL6_v1_MS3_05176	TAAACTTGGCTCAGACATTGAATACATTCTGGCGATATGATGTTACCACAACAAATCAA	203
	***** ** ** ***** ***** ***** ***** ***** *****	
SmTAL6_Smp_072620	TGTCACCAATGAGGCTAGAAAAATATATCAAGAATATAAATCTGAAAAATAAGCTGTTGAT	263
ShTAL5_v3_MS3_00002091	TGTCAGCAATGAAGCCAGAAAAATATATCAAGAATATGAATGCGACAATAAAATATCGAT	1457
ShTAL6_v1_MS3_05176	TGTCAGCAATGAAGCCAGAAAAATATATCAAGAATATGAATGCGACAATAAAATATCGAT	263
	***** *****.* ***** ***** ***** ***** ***** *****	
SmTAL6_Smp_072620	TGCTACAAAGCTTAAAGAAATTTCTAGATAAAACATTTGGCAGAGCGTGGCATGTTACGGT	323
ShTAL5_v3_MS3_00002091	TGCTACAAAGCTTAAAGAAATTTCTAGATAGAGCATTGGTAGGTCATGGCATGTTACGGT	1517
ShTAL6_v1_MS3_05176	TGCTACAAAGCTTAAAGAAATTTCTAGATAGAGCATTGGTAGGTCATGGCATGTTACGGT	323
	***** *****.* ***** ***** ***** ***** ***** *****	
SmTAL6_Smp_072620	GGTGGATGGTTCGTTTGGCCAGTGCACATACAGAAGAAGTTAATACTTCATTCACATTTCAA	383
ShTAL5_v3_MS3_00002091	AGTGGATGGTTCCTTTGCCAGTTCATATACACAAGAAGTTAATACTTCATTTCACTTTAA	1577
ShTAL6_v1_MS3_05176	AGTGGATGGTTCCTTTGCCAGTTCATATACACAAGAAGTTAATACTTCATTTCACTTTAA	383
	.***** ***** ** ***** ***** ***** ***** ***** *****	
SmTAL6_Smp_072620	AATGAAAAATCTTTGTTTTATTATATGGAAAACACCAGATTGTAGAAATGAATAA-----	438
ShTAL5_v3_MS3_00002091	AATGAAAAATCTTTGTTATCTTATATGGAAAACACCAGAGTACATAGATGAATAATTGAT	1637
ShTAL6_v1_MS3_05176	AATGAAAAATCTTTGTTATCTTATATGGAAAACACCAGAGTACATAGATGAATAA-----	438
	***** *****.* ***** ***** ***** ***** ***** *****	
SmTAL6_Smp_072620	-----	438
ShTAL5_v3_MS3_00002091	AAAAATGATTTTCAGTAATAAATGTATTCAAGCAGTAAAAACGTGTTTTAAATATTCAAAG	1697
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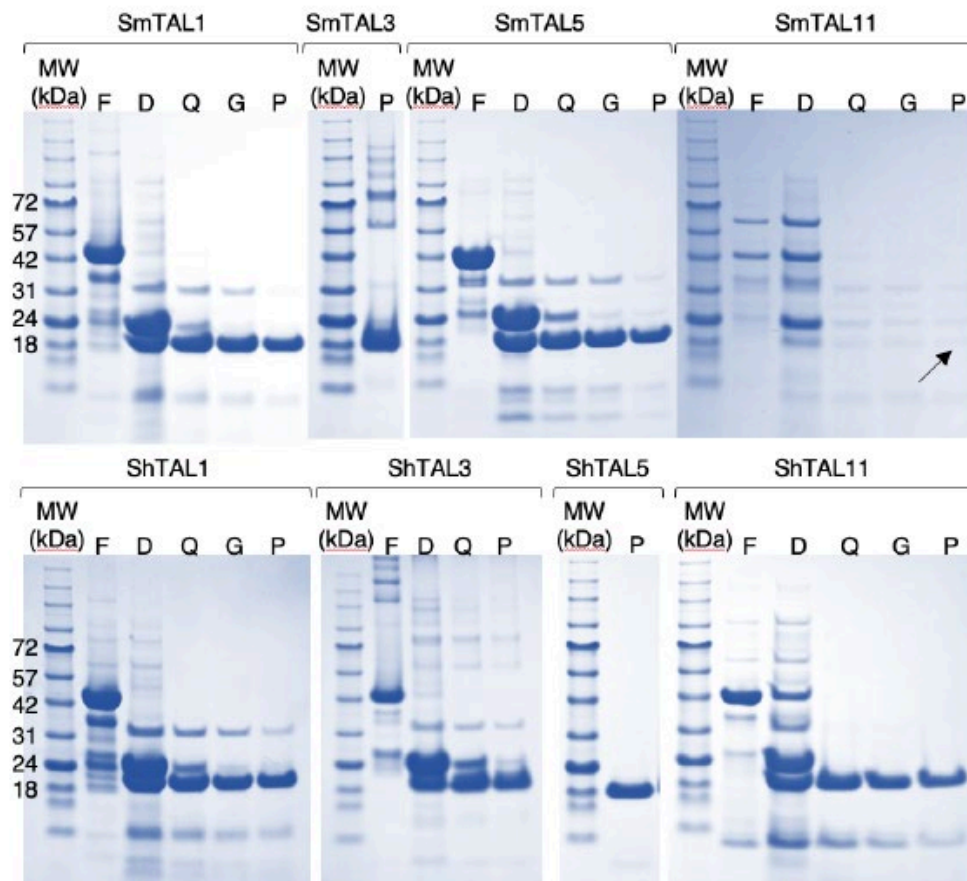
**Fig A. Lifecycle transcription profiles of *S. haematobium* TAL proteins (Data from Stroehlein *et al.* (1)).**



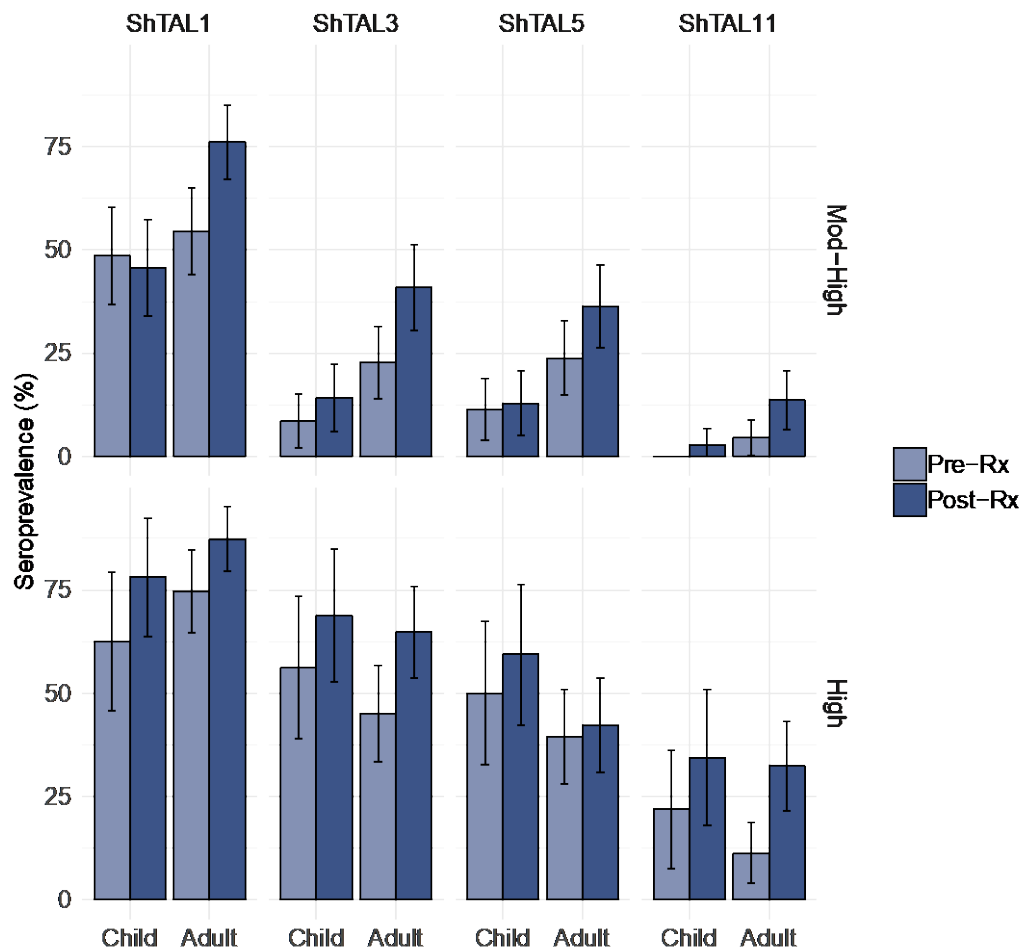
**Fig B. Lifecycle transcription profiles of *S. mansoni* TAL proteins** (Data from meta-analysis by Lu *et al.* (2)).

## Gel electrophoresis of ShTAL proteins

Recombinant TAL proteins of an appropriate molecular weight were expressed in an *E. coli* expression system.



**Fig C. Gel electrophoresis of purified SmTAL and ShTAL proteins.** Coomassie blue-stained SDS-PAGE gel of recombinant *S. mansoni* and *S. haematobium* TAL1, TAL3, Tal5 and TAL11, including fusion protein (F); thrombin digest (D); and subsequent purification steps (Q: Q-sepharose bead purification; G: glutathione Sepharose bead purification; P: purified protein following aminobenzamide agarose bead purification).



**Fig D. Pre- and post-treatment (Rx) seroprevalence of IgE responses to *S. haematobium* TAL proteins by age and village, as a proxy for force of transmission.** Error bars represent 95% confidence intervals around the seroprevalence. Children represent individuals less than 10 years old; ‘adults’ represent individuals aged 10 years and older, based on age of peak CAA across whole cohort. Number of individuals within each demographic group: Children (n = 70) and adults (n = 88) in moderate intensity village; Children (n = 32) and adults (n = 71) in high intensity villages.



**Table A. Recombinant antigen coating concentration**

Antigen	Stock concentration (mg/ml)	Coating dilution
SmTAL1	0.87	1:100
SmTAL3	0.70	1:100
SmTAL5	0.66	1:100
SmTAL11	0.21	1:40
ShTAL1	0.42	1:80
ShTAL3	0.48	1:80
ShTAL5	0.36	1:100
ShTAL11	0.57	1:100

**Table B. Percent identity matrix between ShTAL1, 3, 5 and 11 and SmTAL1, 3, 5 and 11. Alignment performed in CLUSTAL O (1.2.4)**

	ShTAL1	ShTAL3	ShTAL5	ShTAL11
ShTAL1	100.00	32.43	33.72	33.52
ShTAL3	32.43	100.00	45.35	43.18
ShTAL5	33.72	45.35	100.00	46.51
ShTAL11	33.52	43.18	46.51	100.00
	SmTAL1	SmTAL3	SmTAL5	SmTAL11
SmTAL1	100.00	29.31	30.59	26.44
SmTAL3	29.31	100.00	44.77	40.56
SmTAL5	30.59	44.77	100.00	44.19
SmTAL11	26.44	40.56	44.19	100.00

**Table C. Association between pre-treatment ShTAL-specific IgE responses (seropositivity) and baseline CAA, as a measure of worm burden. Results for the reduced linear regression model, adjusted for age and village are displayed, for total cohort (n = 261).**

	ShTAL1		ShTAL3		ShTAL5		ShTAL11	
	GM ratio (95%CI)	P-value	GM ratio (95%CI)	P-value	GM ratio (95%CI)	P-value	GM ratio (95%CI)	P-value
Age (years) †								
7-9	11.48 (3.91-33.74)	***	12.56 (4.31-36.62)	***	11.4 (4.09-34.80)	***	11.95 (4.11-34.71)	***
10-14	33.83 (10.16-112.7)	***	36.46 (10.93-121.6)	***	33.75 (10.08-112.9)	***	33.12 (9.97-110.0)	***
15 - 24	25.99 (7.20-93.88)	***	29.05 (7.99-105.67)	***	26.01 (7.18- 94.15)	***	26.30 (7.31-94.56)	***
25 - 29	16.34 (5.38-49.60)	***	18.65 (6.19-56.23)	***	16.98 (5.65-51.06)	***	15.93 (5.30-47.84)	***
30+	10.18 (2.81-36.89)	***	11.34 (3.13-41.09)	***	10.38 (2.87-37.58)	***	10.50 (2.92-37.75)	***
Village‡	175.0 (44.1-693.9)	***	225.01 (55.25-916.3)	***	180.1 (45.49-712.9)	***	166.3 (42.19-655.3)	***
TALx-IgE§	1.19 (0.66-2.14)	ns	0.67 (0.35-1.30)	ns	1.10 (0.57-2.13)	ns	2.14 (0.70-6.56)	ns
Interaction terms								
7-9 * village	0.066 (0.010-0.45)	**	0.061 (0.009-0.41)	**	0.061 (0.009-0.42)	**	0.055 (0.008-0.38)	**
10-14 * village	0.030 (0.004-0.21)	***	0.028 (0.004-0.19)	***	0.030 (0.004-0.21)	***	0.030 (0.004-0.21)	***
15 - 24 * village	0.015 (0.002-0.11)	***	0.013 (0.002-0.09)	***	0.014 (0.002-0.10)	***	0.015 (0.002-0.11)	***
25 - 29 * village	0.008 (0.001-0.05)	***	0.007 (0.001-0.04)	***	0.008 (0.001-0.05)	***	0.009 (0.001-0.06)	***
30+ * village	0.008 (0.001-0.07)	***	0.007 (0.001-0.06)	***	0.008 (0.001-0.08)	***	0.007 (0.001-0.07)	***

\*\*\*p<0.001, \*\*p<0.01, \*p<0.05, † p<0.1, ns non-significant; ‡ values are compared to 5- to 6-year-old age group; § values are for high intensity villages compared to moderate infection intensity village; § where TALx represents the indicated combination of TAL1, TAL3, TAL5 and TAL11 responses.

**Table D. Association between pre-treatment ShTAL-specific IgE responses (seropositivity) and baseline CAA, as a measure of worm burden.** Results for the reduced linear regression model, adjusted for age, sex, village and ShTAL-specific IgG<sub>4</sub> seropositivity are displayed, for total cohort (n = 261).

	ShTAL1		ShTAL1, 3		ShTAL1, 3, 5		ShTAL1, 3, 5, 11	
	GM ratio (95%CI)	P-value	GM ratio (95%CI)	P-value	GM ratio (95%CI)	P-value	GM ratio (95%CI)	P-value
Age (years) †								
7–9	4.70 (1.86–11.89)	**	4.65 (1.87–11.59)	**	4.64 (1.88–11.48)	***	4.09 (1.65–10.13)	**
10–14	8.84 (3.29–23.73)	***	8.86 (3.38–23.26)	***	9.65 (3.69–25.21)	***	8.54 (3.27–22.28)	***
15 – 24	4.97 (1.82–13.56)	**	4.63 (1.71–12.48)	**	5.33 (1.97–14.41)	**	4.24 (1.53–11.74)	**
25 – 29	3.23 (1.27–8.17)	*	2.68 (1.07–6.73)	*	3.00 (1.20–7.52)	*	2.86 (1.15–7.12)	**
30+	2.02 (0.67–6.12)	ns	1.90 (0.64–5.67)	ns	2.54 (0.84–7.72)	.	2.40 (0.80–7.22)	ns
Sex	-	ns	-	ns	-	ns	-	ns
Village †	5.03 (2.66–9.50)	***	4.09 (2.09–8.00)	***	3.34 (1.69–6.59)	***	2.86 (1.45–5.64)	**
TALx-IgE §	1.06 (0.57–1.96)	ns	0.59 (0.28–1.25)	ns	0.54 (0.22–1.28)	ns	1.02 (0.28–3.73)	ns
TAL1-IgG <sub>4</sub>	2.76 (1.37–5.62)	**	-	ns	-	ns	-	ns
TAL3-IgG <sub>4</sub>			4.49 (2.22–9.08)	***	2.87 (1.29–6.45)	*	-	ns
TAL5-IgG <sub>4</sub>					3.03 (1.19–7.71)	*	2.98 (1.23–7.24)	*
TAL11-IgG <sub>4</sub>							2.97 (1.43–6.17)	**

\*\*\*p<0.001, \*\*p<0.01, \*p<0.05, . p<0.1, ns non-significant; † values are compared to 5- to 6-year-old age group; ‡ values are for high intensity villages compared to moderate intensity village; § where TALx represents the indicated combination of TAL1, TAL3, TAL5 and TAL11 responses.

## References

1. Stroehlein AJ, Korhonen PK, Lee VV, Ralph SA, Mentink-Kane M, You H, et al. Chromosome-level genome of *Schistosoma haematobium* underpins genome-wide explorations of molecular variation. *PLoS Pathog*. 2022;18(2):e1010288.
2. Lu Z, Sessler F, Holroyd N, Hahnel S, Quack T, Berriman M, et al. A gene expression atlas of adult *Schistosoma mansoni* and their gonads. *Sci Data* [Internet]. 2017;4(1):170118. Available from: <https://doi.org/10.1038/sdata.2017.118>.