

Supplementary material

Table S1 Description of pool sera used in ELISA inhibition

Code	Gender	Age	Origin	Diagnosis	IgE to rAsc l 5 ¹	IgE to <i>Ascaris</i> spp ²
ASC235	M	12	ASA cohort	Asthma	3.20	119.6
ASC188	F	42	ASA cohort	Asthma	0.85	9.08
ASC115	F	49	ASA cohort	Asthma	0.55	1.22
ASC084	M	9	ASA cohort	Asthma	0.65	2.49
ASC064	M	10	ASA cohort	Asthma	0.50	3.61
ASC055	M	28	ASA cohort	Asthma	0.39	3.10
ASC147	F	27	ASA cohort	Asthma	0.29	5.66
ASC081	F	9	ASA cohort	Asthma	0.27	60.2

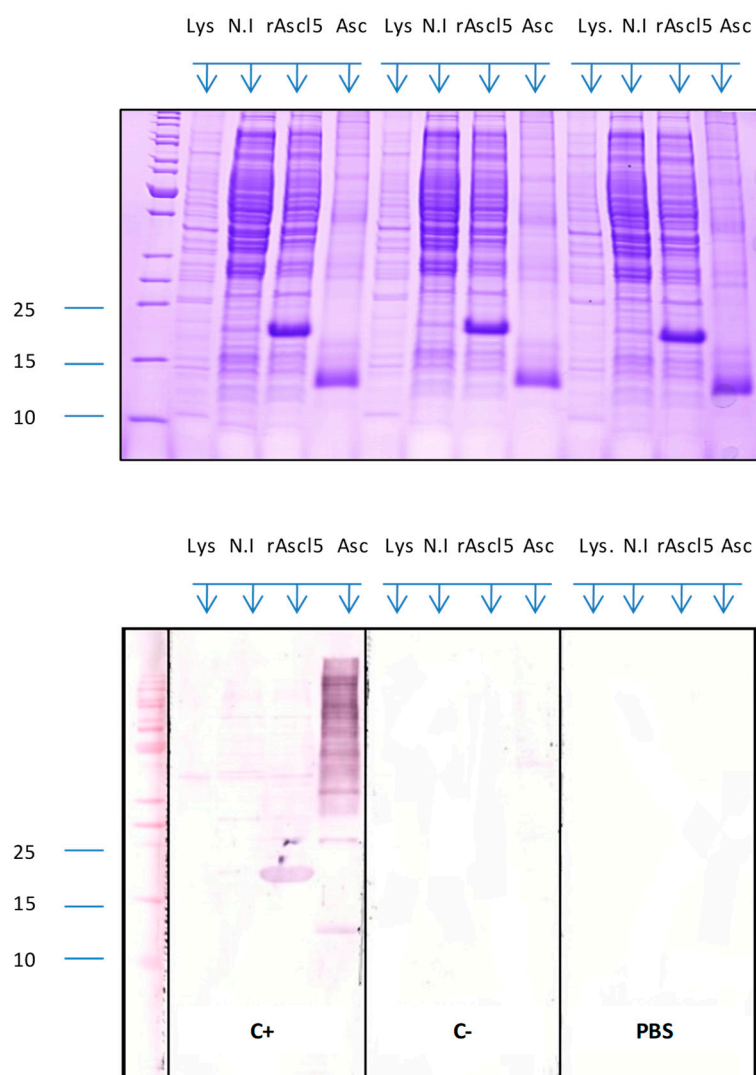
¹ OD: Optical density units. As determined by ELISA

² kU/l as determined by ImmunoCAP.

Table S2 Peptide List of natural Asc l 5. Tryptic peptides coinciding with Asc l 5 sequence after nano-LC-MS/MS of the *A. lumbricoides* extract.

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	PTM
KDEEKTDPEIEADIDAFVAK	103.00	22.620.903	20	1.1	11.320.537	84.50	
DEEKTDPEIEADIDAFVAK	102.93	21.339.954	19	-0.5	10.680.044	102.04	
VPPFLVGAPESAVKDFFELIKK	88.26	24.303.562	22	-1.3	6.085.955	147.46	
Q(-17.03)TPSRVPPFLVGAPESAVKDFFELIK	88.16	28.545.269	26	-0.6	9.525.156	165.32	Pyro-glu from Q
Q(-17.03)TPSRVPPFLVGAPESAVKDFFELIKK	87.25	29.826.218	27	-0.6	7.466.623	151.29	Pyro-glu from Q
TDPEIEADIDAFVAK	84.46	16.327.882	15	-0.2	8.174.012	108.49	
VPPFLVGAPESAVKDFFELIK	80.50	23.022.612	21	0.0	7.684.277	167.40	
DDFELIKKDEEKTDPEIEADIDAFVAK	76.78	31.545.596	27	2.3	7.896.490	136.15	
VPPFLVGAPESAVK	72.46	14.097.917	14	0.1	7.059.032	86.62	
PEIEADIDAFVAK	68.23	14.167.136	13	-0.2	7.093.640	96.59	
IKETMESLPK	55.06	11.746.267	10	-1.0	5.883.201	29.24	
LTAIAEDAKLNGIQK	53.43	15.838.882	15	-1.0	7.929.506	49.78	
D(+226.08)EEKTDPEIEADIDAFVAK	52.41	23.600.728	19	-1.5	11.810.419	83.81	Biotinylation
EEKTDPEIEADIDAFVAK	51.93	20.189.684	18	-2.2	6.739.952	94.30	
DDFELIKKDEEK	51.27	15.397.820	12	-1.4	7.708.972	72.54	
IKETM(+15.99)ESLPK	50.40	11.906.217	10	-0.5	5.963.178	28.79	Oxidation (M)
DDFELIKK	48.67	10.385.750	8	0.4	5.202.950	78.50	
LTAIAEDAKLNGIQKR	48.55	17.399.894	16	-0.2	5.810.036	36.85	
LTAIAEDAK	48.18	9.305.022	9	-0.6	4.662.581	29.93	
EVVRDELEKAIAGGA	43.40	14.567.521	14	1.3	7.293.843	67.37	
DDFELIK	42.43	9.104.800	7	-0.7	4.562.469	105.59	
IKETMESLPKEVRDELEK	42.29	21.731.299	18	-0.9	5.442.892	64.63	
EVVRDELEK	42.19	10.165.138	8	-0.4	5.092.640	25.88	
AHEAEYEK	41.48	9.754.297	8	-0.3	4.887.220	24.37	
AHAAAIK	37.29	7.514.340	8	-0.8	3.767.240	27.50	
KDEEKTDPEIQ(sub E)ADIDAFVAK	36.41	22.611.062	20	3.0	5.662.855	92.13	
total 26 peptides							

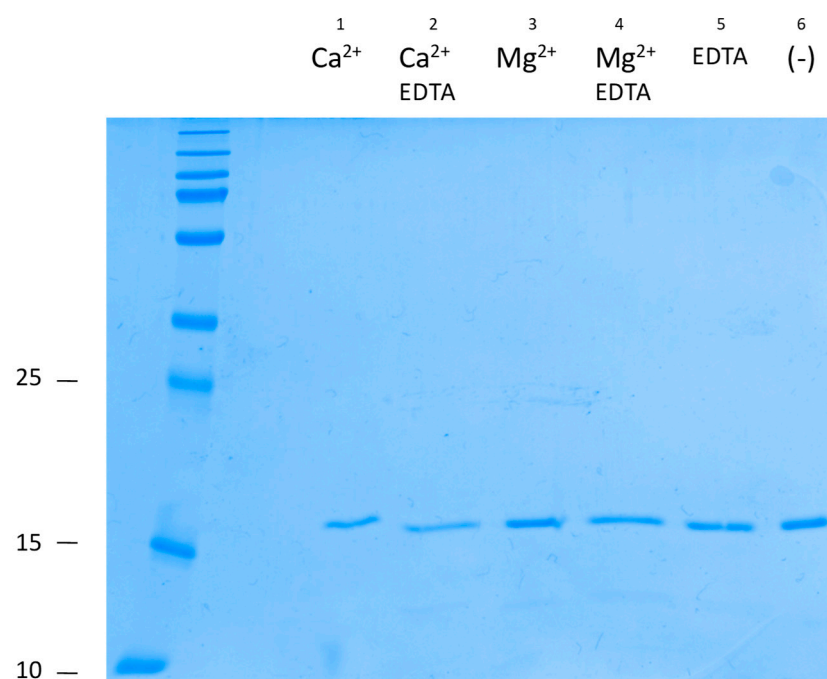
Figure S1 SDS-PAGE of samples used for Western blot probed with sera pool from individuals with positive IgE to *Ascaris*.



Supplementary Figure 1. Lys) *E. coli* Origami (DE3) lysate, N.I) non-induced transformed culture lysate of *E. coli* Origami (DE3), rAsc l 5) induced transformed culture lysate of *E. coli* Origami (DE3) and Asc) *Ascaris lumbricoides* extract.

C+ (Sera pool with positive IgE to *Ascaris*); C- (Sera pool with negative IgE to *Ascaris*); PBS buffer control.

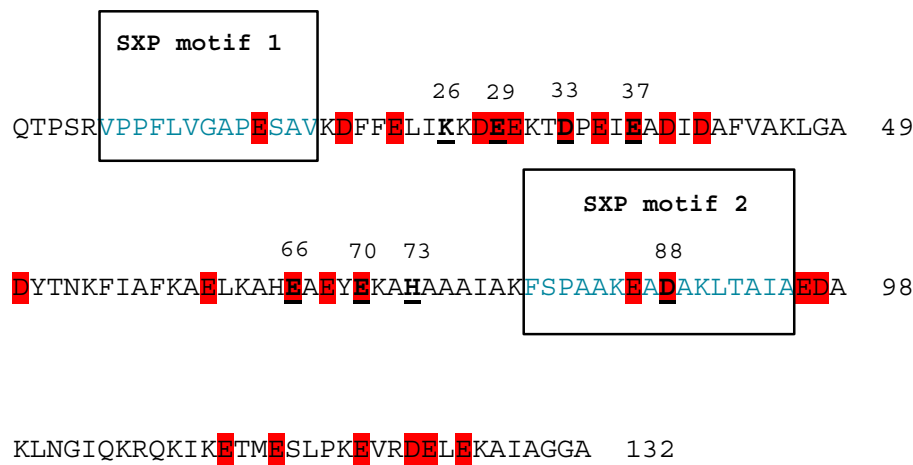
Figure S2 SDS-PAGE of rAsc l 5



Supplementary Figure 2. SDS-PAGE. Same samples from CD experiments were used for in lanes 1, 3 and 6. EDTA was added to the preparations as a control (lanes 2, 4 and 5). Concentrations were as follow: 100 $\mu\text{g/mL}$ rAsc l 5, 74 mM CaCl_2 , 221 mM MgCl_2 , or 250 mM EDTA as needed. 1) rAsc l 5 + CaCl_2 ; 2) rAsc l 5 + CaCl_2 + EDTA; 3) rAsc l 5 + MgCl_2 ; 4) rAsc l 5 + MgCl_2 + EDTA; 5) rAsc l 5 + EDTA; 6) rAsc l 5 alone. Sample volume 12 μL .

Figure S3 Calcium and magnesium binding sites in Asc I 5

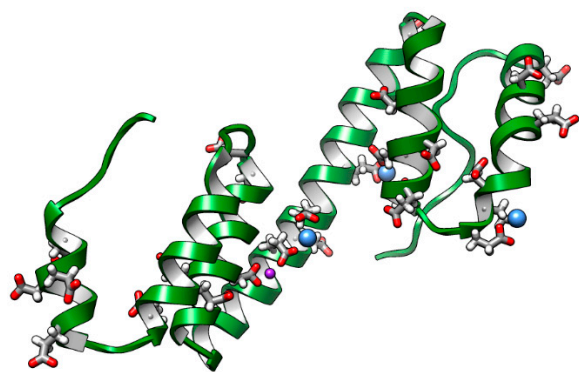
A



B

Predicted divalent cation binding sites in Asc I 5	
Ca ²⁺ binding residues	1) 26K, 29E
	2) 33D, 37E
	3) 66E, 70E
Mg ²⁺ binding residues	4) 70E, 73H, 88D

C



Supplementary Figure 3. Predicted binding sites in Asc I 5. S1A: In mature sequence of Asc I 5 (MN275230) Asp and Glu residues are in red. Predicted calcium or magnesium binding residues are numbered from 26 to 88. S1B: List of residues predicted to bind calcium or magnesium. S1C: Asc I 5 model with calcium (blue) and magnesium (magenta) ions.