Having a pair: the key to immune evasion for the diploid pathogen *Schistosoma japonicum*

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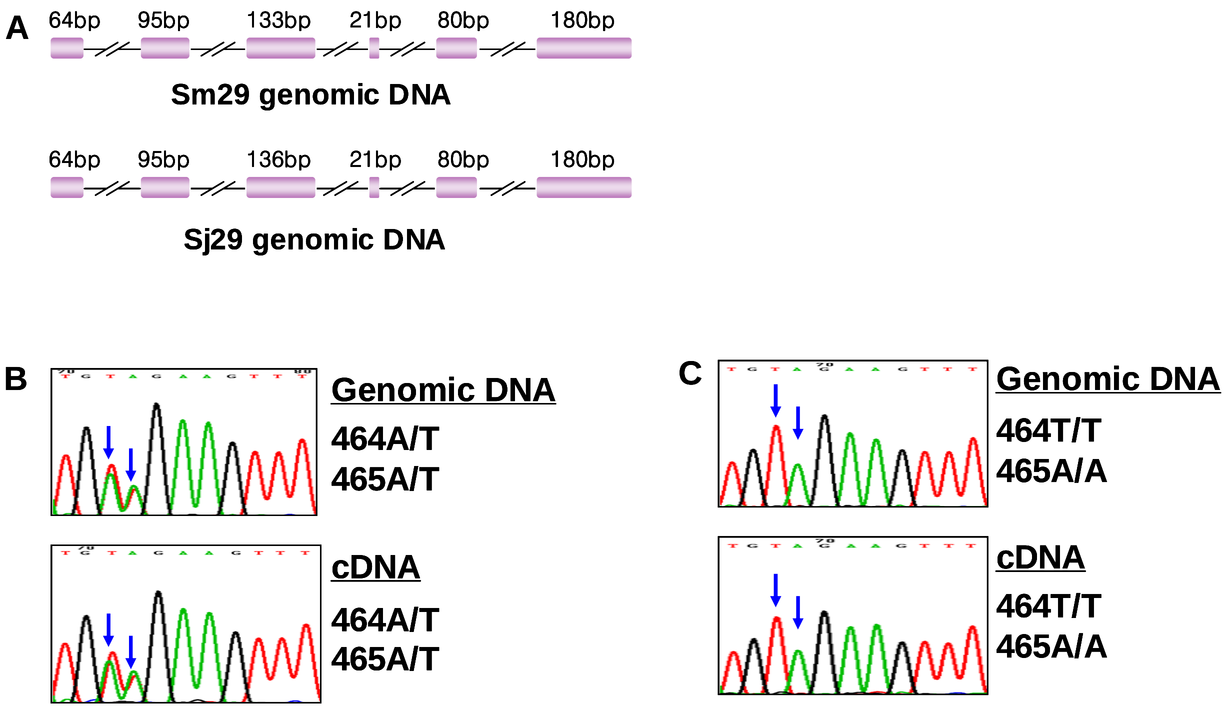
Supplementary Information

Supplementary Figure 1 to 7

Supplementary Table 1 to 3

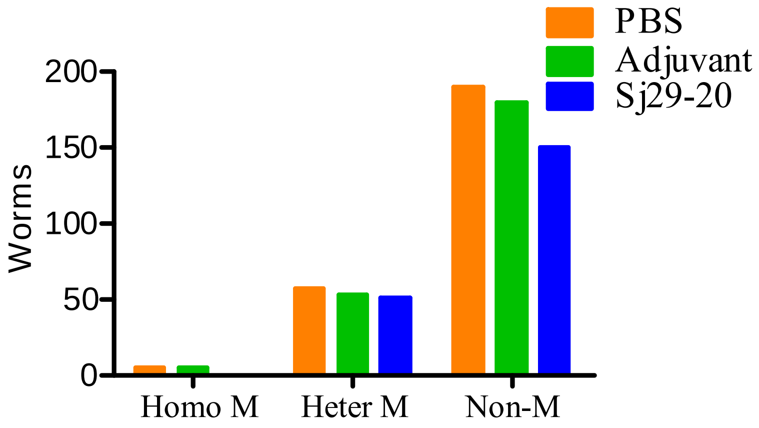


Supplementary Figure 1. Genomic structure and allelic expression pattern of Sj29.

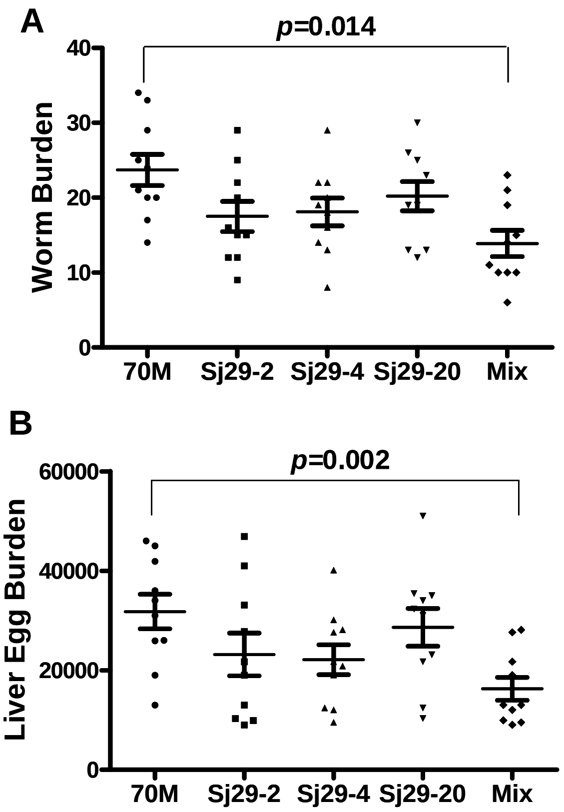
(A) Genomic structure of Sj29 and Sm29. The size of exons is indicated.

(B) Chromatograms of exon 6 sequence of heterozygous worm.

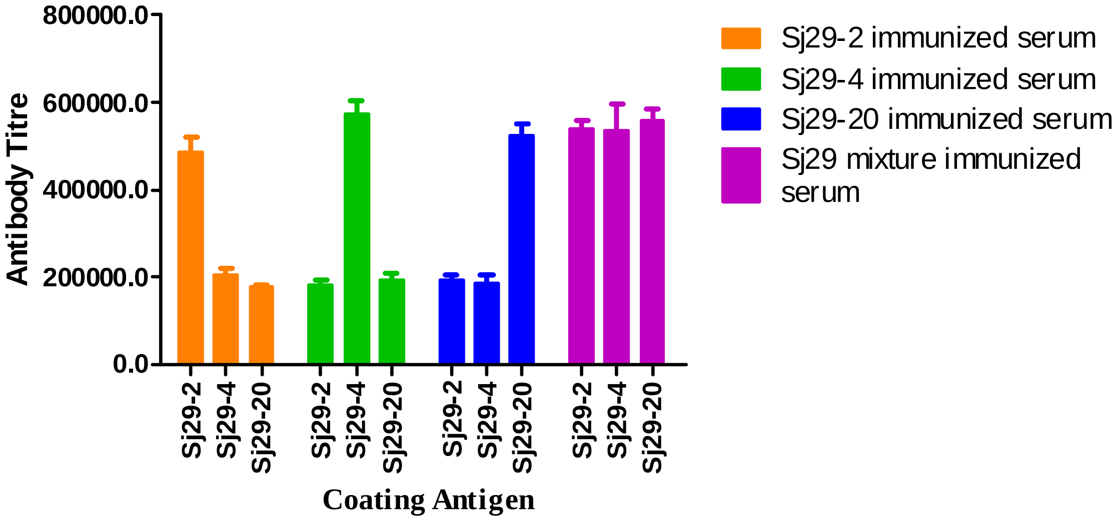
(C) Chromatograms of exon 6 sequence of homologous worm.



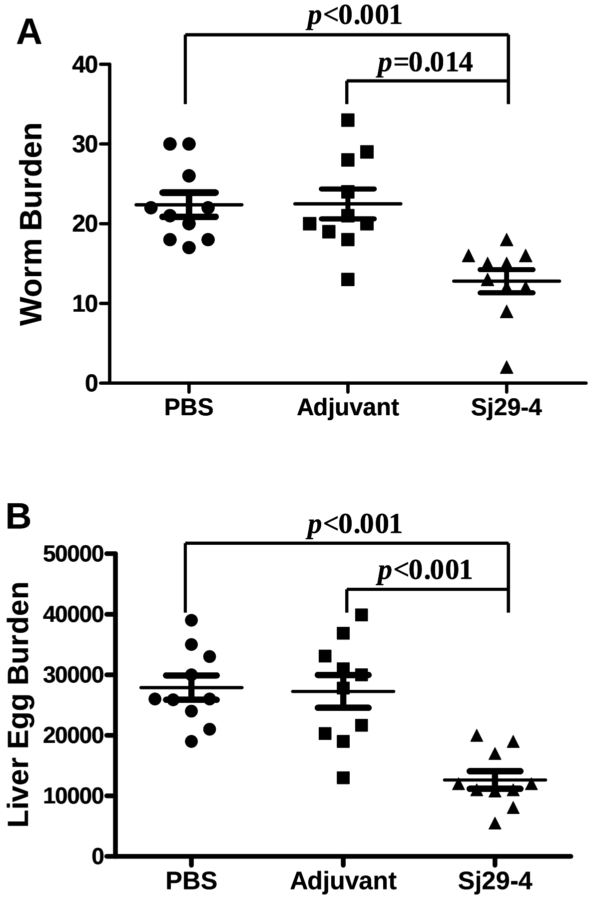
Supplementary Figure 2. Comparasion of the distribution of homozygotes and heterozygotes in Sj29-20 immunized and control groups.



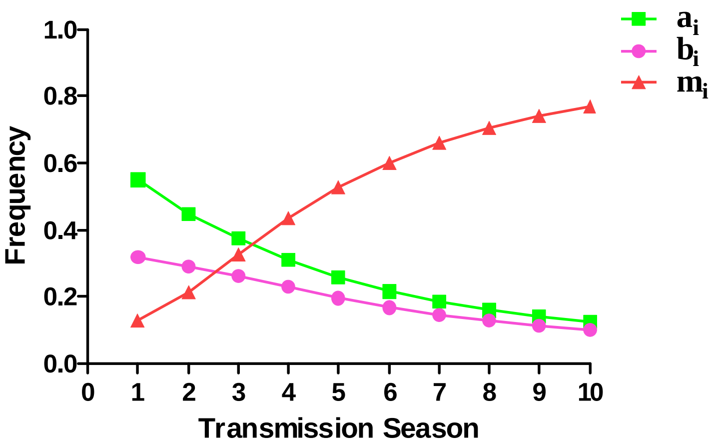
Supplementary Figure 3. Comparison of protection efficacy against field isolates in adjuvant, individual alleles (Sj29-2, Sj29-4, and Sj29-20) and an allele mixture (Mix) immunized groups. (A) Worm burdens. (B) Liver egg burdens.



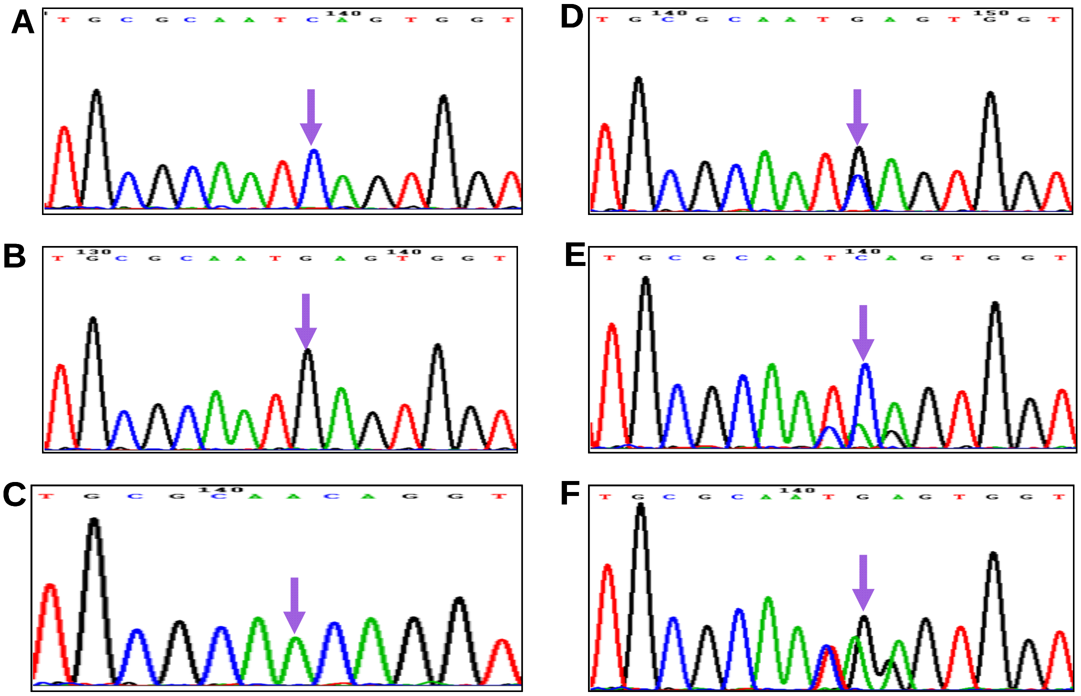
Supplementary Figure 4. The antibody titres against different antigens (Sj29-2, Sj29-4 and Sj29-20) in mice immunized with single protein or multiple protein.



Supplementary Figure 5. Comparison of protection efficacy against a clonal isolate Sj6M8 in PBS, adjuvant and Sj29-2 immunized groups . (A) Worm burdens. (B) Liver egg burdens.



Supplementary Figure 6. Prediction of the change of the clades A, B and M frequencies (a_i, b_i and m_i) under immune pressure induced by multiple antigens Sj29-2 and Sj29-4.



Supplementary Figure 7. Chromatogram traces around the nucleotide site 162 of different genotypes. The nucleotide site 162 was indicated by arrow. (A) AA genotype; (B) BB genotype; (C) MM genotype; (D) AB genotype; (E) AM genotype; (F) BM genotype.

Supplementary Table 1. Distribution of Sj29 alleles. Vertical numbers indicate the position of variable nucleotides within the sequence. Dots indicate the same nucleotides as is present in Sj29-1. No., number of alleles.

	No.	Nucleotides position									
			111111111	1111111111	1111111222	2222222222	2222222222	2222233333	3333344444	4444444455	5
		2246677789	9112233333	4444556666	7777789000	1111344455	5555677778	8888912355	7788912444	5556666902	2
		3736901270	3182734789	0134031239	0238906278	1239645834	5678115782	4569770917	3404988589	0580145252	8
Sj29-1	6	CGTCAATTTC	GGGTACCGAA	GCGTGTTCAG	TGAGACAATA	AATGTAATAT	TGGCTGAGTC	GGAGGCGCCT	CGCTCATGGC	TGGGATATGA	C
Sj29-2	49	..C.......	....	....	....	....	........C.	....	....T.....	........A.	T
Sj29-3	2	..C.......	....	....	....	....	........C.	....	....T.....	....	T
Sj29-4	122	.A........	.A...T....	AT.....G..	...CGA...G	C.AAA...TC	.AA.....C.	....AAAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-5	2	.A........	.A...T....	AT.....G..	...CGA...G	C.AAA...TC	.AA.....C.	....AAAATG	AATC.CAACA	GTAC.ATCA.	.
Sj29-6	3	.A........	.A...T....	AT.....G..	...CGA...G	C.AAA...TC	.AA.......	....AAAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-7	1	.A........	.A...T....	AT.....G..	...CGA...G	C.AAA...TC	.AA.....C.	....AAAATG	AATC.CAACA	GTAC.AT.A.	T
Sj29-8	2	.A........	.A.A.T....	AT.....G..	...CGA...G	C.AAA...TC	.AA.....C.	....AAAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-9	1	.A........	.A...T....	AT.....G..	...CGA...G	C.AAA....C	.AA.....C.	....AAAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-10	1	.A........	.A...T....	AT.....G..	...CGA...G	C.AAA...TA	.AA.....C.	....AAAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-11	2	.A........	.A...T....	AT.....G..	...CGA...G	CCAAA...TC	.AA.....C.	....AAAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-12	2	.AC.......	.A...T....	AT.....G..	...CGA...G	C.AAA...TC	.AA.....C.	....AAAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-13	2	TAC.......	.A...T....	AT.....G..	...CGA...G	C.AAA...TC	.AA.....C.	....AAAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-14	1	....	.A...T....	AT.....G..	...CGA...G	C.AAA...TC	.AA.....C.	....AAAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-15	1	..C.......	.A...T....	AT.....G..	...CGA...G	C.AAA...TC	.AA.....C.	....AAAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-16	3	.A........	.A...T....	AT.....G..	...CGA...G	C.AAA...TC	.AA.....C.	.....AAATG	AATC..A.AA	GTACG..CA.	.
Sj29-17	1	.A........	.A...T....	AT.....G..	...CGA...G	C.AAA...TC	.AA.....C.	....AAAATG	AATC.CA.AA	GTACG..CA.	.
Sj29-18	2	.A........	.A...T....	AT.....G..	...CGA...G	C.AAA...TC	.AA...CACG	TTG..AAATG	AATC..A.AA	GTACG..CAG	.
Sj29-19	2	.A........	.A...T....	AT........	........A.	........TA	.AA.....C.	.....AAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-20	32	.A.TGTACCT	.AAAG.GATG	A.TAACCAGA	CAG.CAG...	..A.AGTA.A	AA.TA.CACG	TTG.AAAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-21	2	.A.TGTACCT	.AAAG.GATG	A.TAACCAGA	CAG.CAG...	..A.AGTA.A	AA.TA.CACG	TTG..AAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-22	2	.A.TGTACCT	.AAAG.GATG	A.TAACCAGA	CAG.CAG...	..A.AGTA.A	AA.TA.CACG	TTG..AAATG	AATC..A.AA	GTACG..CA.	.
Sj29-23	2	..CTGTACCT	.AAAG.GATG	A.TAACCAGA	CAG.CAG...	..A.AGTA.A	AA.TA.CACG	TTG..AAATG	AATC..A.AA	GTACG..CAG	.
Sj29-24	1	...TGTACCT	.AAAG.GATG	A.TAACCAGA	CAG.GAG...	..A.AGTA.A	AA.TA.CACG	TTG..AAATG	AATC..A.AA	GTACG..CA.	.
Sj29-25	2	...TGTACCT	.AAAG.GATG	A.TAACCAGA	CAG.GAG...	..A.AGTA.A	AA.TA.CACG	TTG..AAATG	AATC..A.AA	GTACG..CAG	.
Sj29-26	2	TACTGTACCT	.AAAG.GATG	A.TAACCAGA	CAG.CAG...	..A.AGTA.A	AA.TA.CACG	TTG..AAATG	AATC..A.AA	GTACG..CA.	.
Sj29-27	2	...TGTACCT	.AAAG.GATG	A.TAACCAGA	CAG.GAGG..	...AAG...A	AA.TACCAC.	TTGT.AAATG	AATC..A.AA	GTACG..CA.	.
Sj29-28	2	TACT.TACCT	CA....GAT.	A.ACACCAGA	CAG.GAG...	...AAG...A	AA.TACCAC.	TTGT.AAATG	AATC..A.AA	GTACG..CA.	.
Sj29-29	1	TACT.TACCT	CA....GAT.	A.ACACCAGA	CAG.GAGG..	...AAG...A	AA.TACCAC.	TTGT.AAATG	AATC..A.AA	GTACG..CA.	.
Sj29-30	2	TACT.TACCT	CA....GAT.	A.ACACCAGA	CAG.GAGG..	.C.AAG...A	AA.TACCAC.	TTGT.AAATG	AATC..A.AA	GTACG..CA.	.
Sj29-31	6	TACT.TACCT	CA....GAT.	A.ACACCAGA	CAG.GAG...	...AAG...A	AA.TACCAC.	TTGT.AAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-32	1	.ACT.TACCT	CA....GAT.	A.ACACCAGA	CAG.GAG...	...AAG...A	AA.TACCAC.	TTGT.AAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-33	1	..C.......	....	......CAGA	CAG.GAG...	...AAG...A	AA.TACCAC.	TTGT.AAATG	AATC..A.AA	GTACG..CA.	.
Sj29-34	1	..C.......	....	....	....	....	........C.	....AAAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-35	1	..C.......	....	....	....	........TC	.AA.....C.	....AAAATG	AATC.CAACA	GTAC.ATCA.	T
Sj29-36	2	.A........	.A...T....	AT........	........A.	........TA	.AA.....C.	.....AAATG	AATC......	........A.	.
Sj29-37	1	.A........	.A...T....	AT........	........A.	........TA	.AA.....C.	.....AAATG	AATC......	....	.

Supplementary Table 2. Comparison of Sj29 genotype distribution in protein immunized groups, adjuvant immunized control group and PBS control group.

	Groups	**Survived Worms**	Genotypes					
			AA	**BB**	**MM**	**AB**	**AM**	**BM**
EXP. 1	PBS	238	25	53	22	72	30	36
	Adjuvant	247	34	61	27	61	44	20
	Sj29-2	174	4	54	22	49	16	29
EXP. 2	PBS	252	104	37	5	49	39	18
	Adjuvant	237	95	43	5	41	29	24
	Sj29-2	175	32	22	5	54	35	27
	Sj29-4	181	57	13	5	58	25	23
	Sj29-20	202	68	35	0	47	40	12
	Mix	139	49	28	2	15	28	17

Supplementary Table 3. Nucleotide diversity at different region of the Sj29 gene

Region	Residues	π(S.D)[a]	S[b]	A[c]	*d*N(s.e)[d]	*d*S(s.e)[e]	*d*N/*d*S
Entire gene	1-192	0.060(0.040)	91	37	0.059(0.009)	0.082(0.015)	0.72
Signal peptides	1- 26	0.044(0.025)	8	11	0.020(0.017)	0.155(0.082)	0.13
Extracellular domain	27-170	0.071(0.048)	81	28	0.076(0.012)	0.078(0.018)	0.97
Transmembrane domain	171-192	0.010(0.007)	2	2	0.000(0.000)	0.040(0.038)	0.00

a. Nucleotide diversity (all sits-synonymous and nonsynonymous mutations- are considered in the calculation) and its standard deviation in parentheses.

b. Number of polymorphism sites.

c. Number of alleles.

d. Number of nonsynonymous mutations sites per nonsynonymous site using the Nei-Gojobori method with the jukes-cantor correction and standard error in parentheses.

e. Number of synonymous mutations sites per synonymous site using the Nei-Gojobori method with the jukes-cantor correction and standard error in parentheses.