SUPPLEMENTARY DATA

Table S 1: Predicted MHC class I and class II binding epitopes from PyAMA-1.

MHC-Peptide#	Sequence	Length	Position	H-2 Kd score*	H-2 Dd score*
I-1	YYIFILCSI	9	5	0.1	12.4
I-2	SLCAKHTSL	9	160	0.9	41.0
I-3	QYIAENNDV	9	455	1.1	82.1
I-4	VYDKSNSTC	9	184	1.3	57.5
I-5	KNFIATTAL	9	368	1.4	68.1
I-6	EGPNQVISE	9	23	95.0	0.5
I-7	DKPVRSGGL	9	118	40.8	0.7
I-8	IGSYKSGQI	9	327	26.4	0.8
I-9	ISPITITNL	9	135	11.9	1.2
I-10	FTPEKIENY	9	221	91.4	1.2
I-11	ENPKQKLLI	9	474	48.6	1.4
I-12	MGPRYCSND	9	203	88.0	1.9
I-13	AFPETDVHI	9	127	8.1	2.2
I-14	KFPRIFIST	9	416	34.0	2.7
I-15	GKGYNWGNY	9	339	83.7	3.6
				H2-IAd median	**H2-IEd median**
II-1	LIGVGIIIVILLVAY	15	486	1.4	86.5
II-2	LIIIVLIGVGIIIVI	15	481	1.8	80.6
II-3	PKQKLLIIIVLIGVG	15	476	5.4	81.5
II-4	NDKNFIATTALSSTE	15	366	6.0	72.6
II-5	LLVAYYFKSGKKGEN	15	496	65.0	7.2

* Putative $CD4^+$ and $CD8^+$ T cell epitopes were predicted from the complete PyAMA-1 protein sequence using computerised MHC-binding prediction algorithms available at the Immune Epitope Database (IEDB, www.iedb.org; (46)). The top scoring peptides predicted to bind with high affinity to MHC class I alleles H-$2K^d$ and H-$2D^d$ (1^{st} percentile of predicted peptides) (top panel) and the top scoring peptides predicted to bind with high affinity to MHC class II alleles IA^d and IE^d(2^{nd} percentile of predicted peptides) (Bottom panel) were selected for further study. Highlighted sections show allele-specific affinity of these peptides.

Figure S 1.

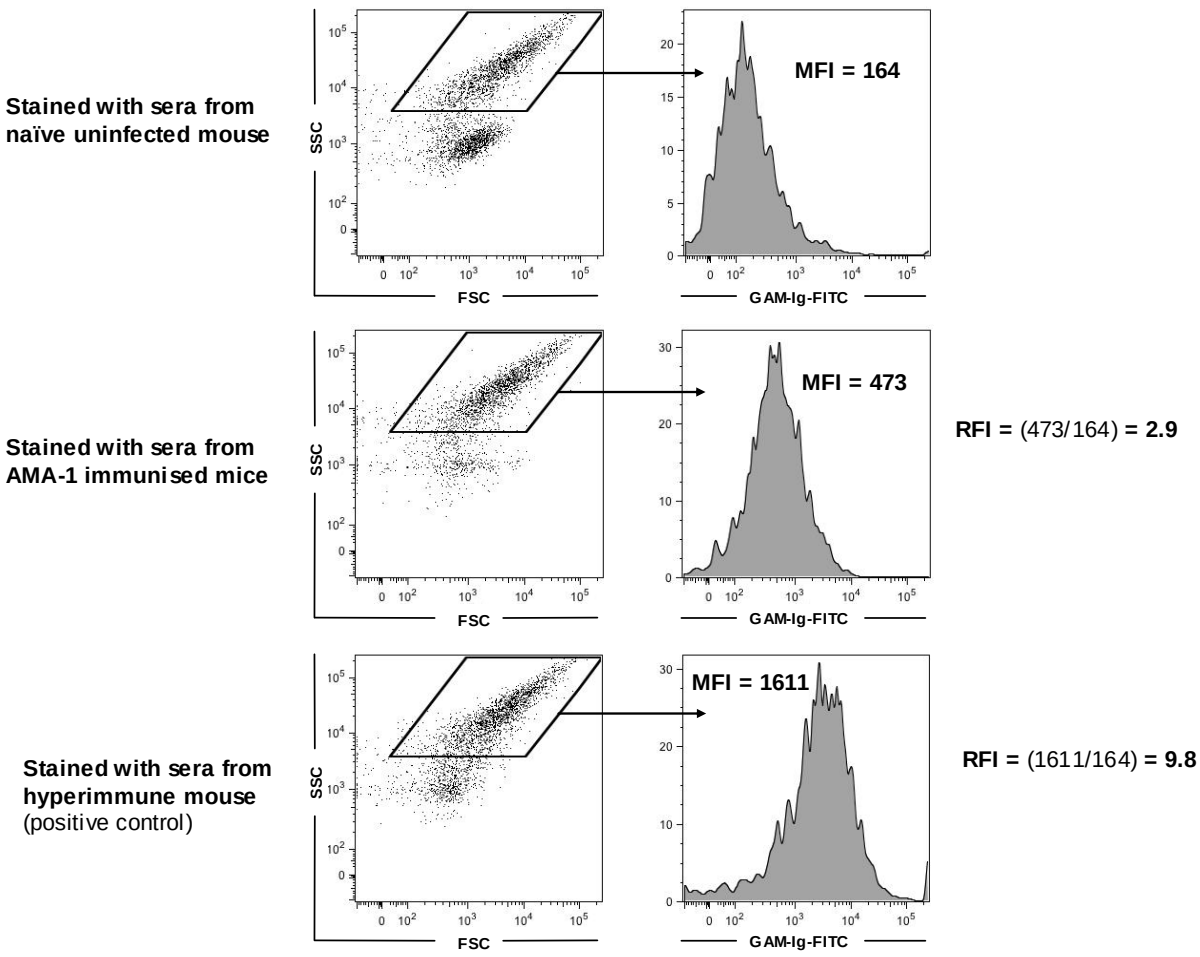

Figure S 1: Indirect Fluorescence Antibody Test (IFAT) using whole blood-stage parasite extract. Flow cytometric analysis of IgG levels binding to P. yoelii blood stage extract in the serum of a naive uninfected mouse (top), an AMA-1 immunised mouse (middle) and a hyperimmune mouse (bottom). Parasite extract was incubated with mouse sera and stained with fluorescently labelled anti-mouse IgG antibody. The median fluorescence intensity (MFI) of the parasite extract (gate on the left) correlates with the antibody titre in mouse sera. The relative fluorescence intensity (RFI) of immunised or hyperimmune mice compared to naive uninfected mice is displayed for comparison.

Figure S 2.

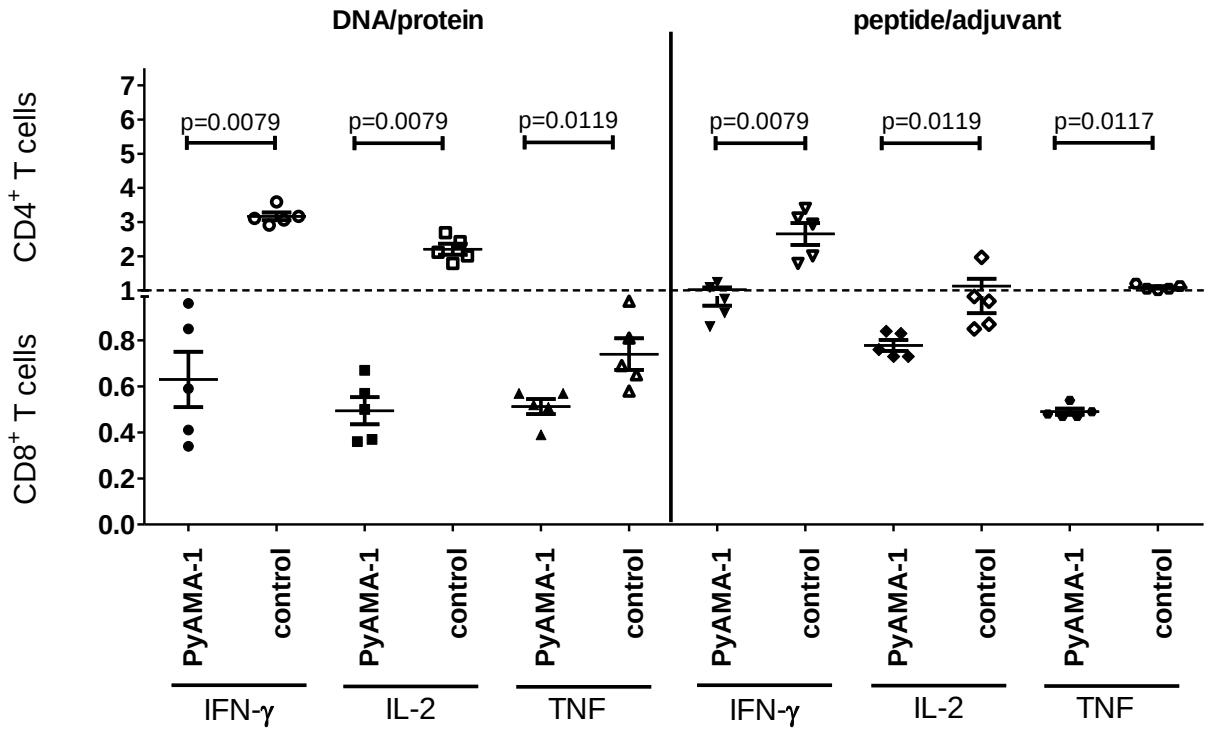

Figure S 2: Comparative analysis of Th1 cytokine production in CD4$^+$ and CD8$^+$ T cells. The frequencies of CD4+ and CD8+ T cell population producing IFN-γ, IL-2 or TNF were measured by intracellular cytokine staining and the CD4+/ CD8+ ratio was calculated for each cytokine secreting population. Statistical comparison of PyAMA-1 immunised versus no-vaccine or adjuvant only control groups was performed using two-tailed Mann-Whitney test; significance was defined as $p<0.05$.

Figure S 3.

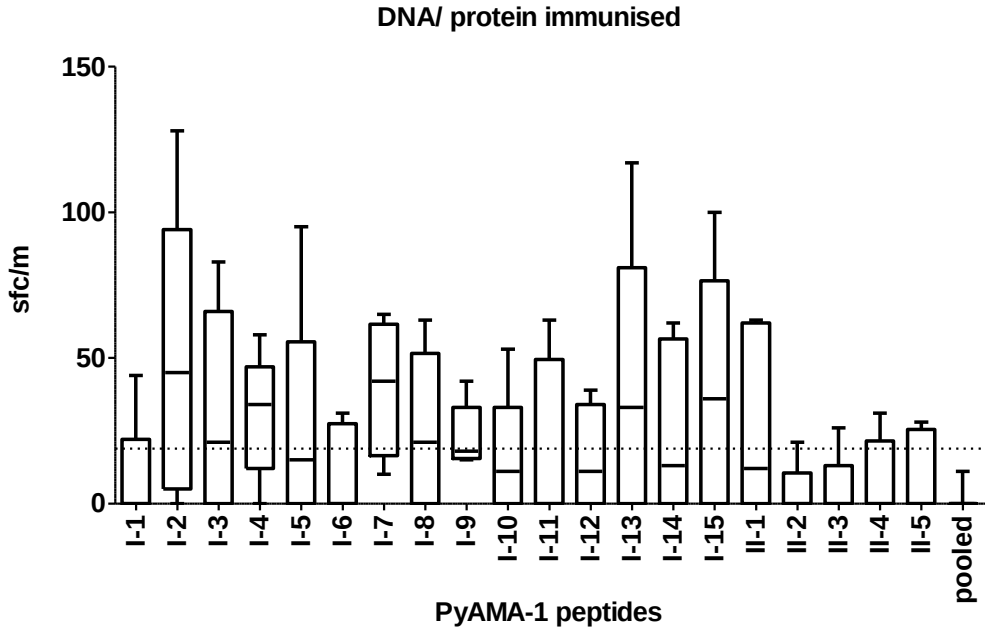

Figure S 3: PyAMA-1 peptide reactivity after DNA/protein immunisation. IFN-γ production of splenocytes harvested from mice immunised with DNA/protein at 10 days after the third immunisation and restimulated with individual peptides or PyAMA-1 peptide pool *in vitro*. Data are presented as mean spot forming cells (SFCs) for individual mice (n=5) with error bars representing the total range of values. The dotted line represents the mean background reactivity of unstimulated cells. Statistical significance was determined by one-way ANOVA and two-tailed Mann-Whitney test, *p<0.05.*

Figure S 4.

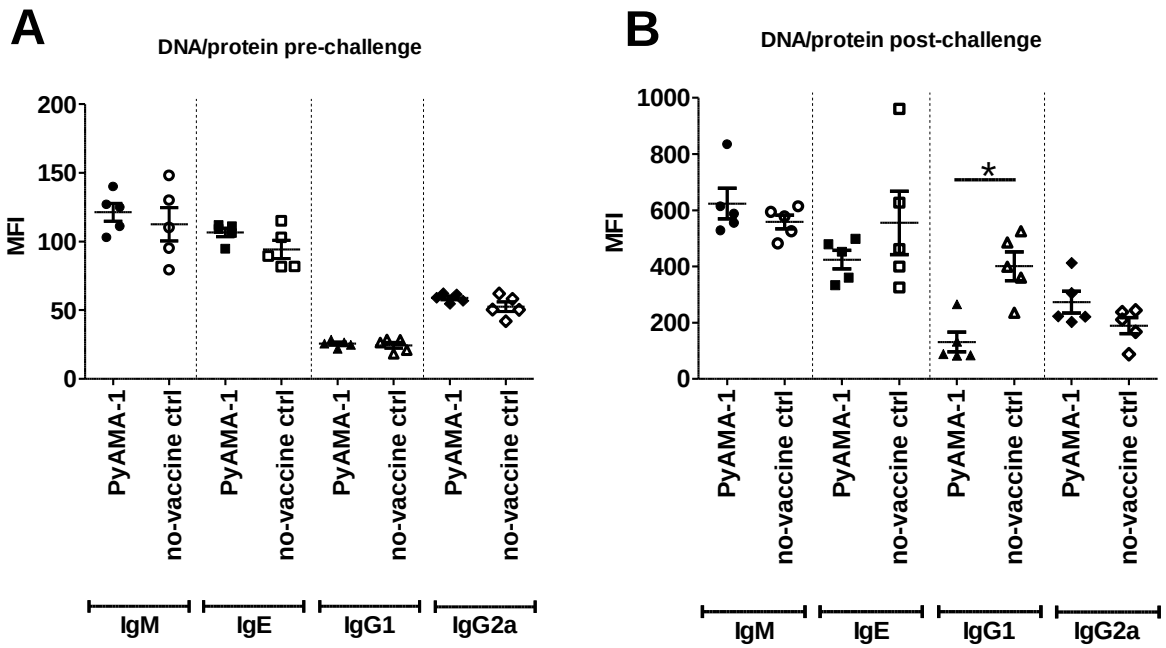

Figure S 4: Antibody subtypes induced by PyAMA-1 DNA/protein immunisation. Flow cytometric analysis of mIgM, mIgG1, IgG2a and mIgE levels binding to *P. yoelii* blood stage extract in the serum of mice **(A)** before and **(B)** after sporozoite challenge. Statistical significance was determined by two-tailed Mann-Whitney test; * *p<0.05.*