

# Supplementary material

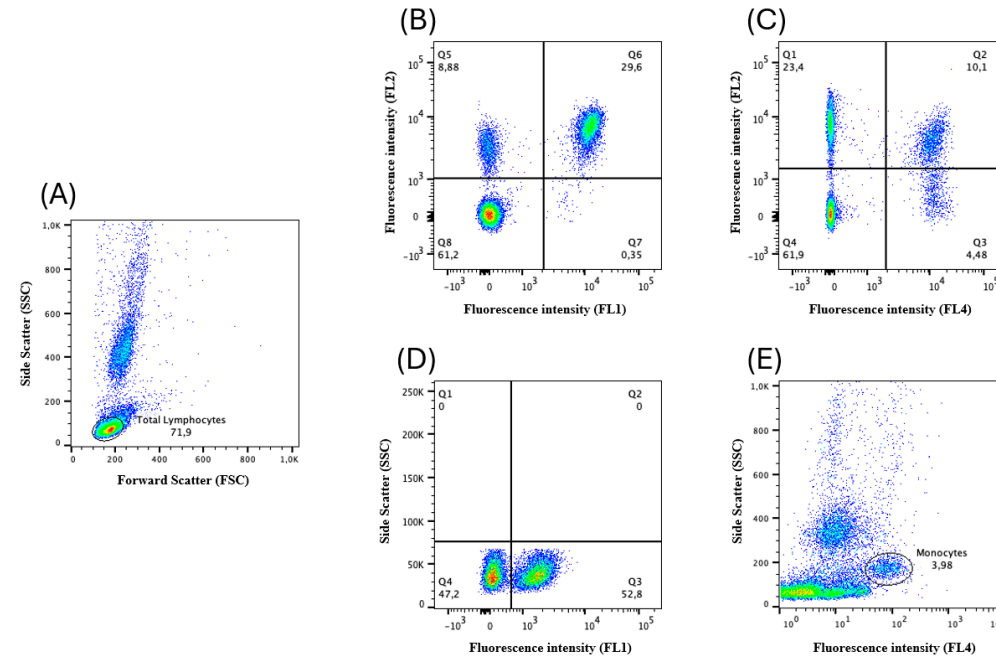


Figure S1. Representative dot plots illustrating the immunophenotyping analysis in peripheral blood from mice. CD4 T cells, CD8 T cells, B cells, and monocytes were characterized by flow cytometry using the following antibodies: anti-CD3 PE (FL2), anti-CD4 FITC (FL1), anti-CD8 APC (FL4), anti-CD19 FITC (FL1) and anti-CD14 APC (FL4). (A) Total lymphocytes were gated based on low size and granularity (FSC vs SSC). CD4 and CD8 T cells were selected as CD3+CD4+ (B) and CD3+CD8+ (C) double-positive populations within total leukocytes. B cells were identified as CD19+ cells within total leukocytes (D). Monocytes were selected based on CD14+ expression and intermediate granularity (E).

Table S1. Kidney and liver function of mice immunized with rAs8.9kDa (8.9), rAsBasicTail (BT), rAsKunitz (Kn) and rAsChimera (Ch) combinations.

Parameter	Urea (mg/dL)		ALT(U/L) <sup>1</sup>		AST(U/L) <sup>2</sup>	
Experimental Day	D-2	D40	D-2	D40	D-2	D40
Ct - Control	62.4 ± 4.6	63.3 ± 4.8	76.0 ± 7.2	77.8 ± 6.0	94.1 ± 11.6	124.0 ± 9.5
V1 - 8.9Ch	65.5 ± 3.7	65.3 ± 3.9	61.4 ± 4.8	67.0 ± 3.8	81.6 ± 5.6	141.8 ± 11.3**
V2 - BtCh	62.4 ± 2.6	63.0 ± 3.6	53.9 ± 4.0	55.8 ± 4.6	83.6 ± 11.5	121.7 ± 5.4*
V3 - KnCh	58.9 ± 6.0	59.0 ± 6.2	72.1 ± 6.5	72.9 ± 5.9	139.6 ± 10.6	139.4 ± 10.7
V4 - Kn8.9Ch	54.5 ± 13.2	63.6 ± 5.4	57.6 ± 10.4	64.7 ± 4.0	124.6 ± 24.4	155.8 ± 14.1
V5- KnBt8.9Ch	45.7 ± 7.0	52.5 ± 2.3	46.4 ± 3.8	47.3 ± 2.8	86.6 ± 7.4	116.3 ± 8.7*

<sup>1</sup>ALT - alanine aminotransferase; <sup>2</sup>AST - aspartate aminotransferase; Data is shown as mean ± SD; Blood from five animals of each group were used in the analysis; Statistical analysis: the T test was used to detect differences between D-2 and D40, \*p<0.05, \*\*p<0.01.

Table S2. Hematological parameters of mice immunized with rAs8.9kDa (8.9), rAsBasicTail (BT), rAsKunitz (Kn) and rAsChimera (Ch) combinations.

Parameter	Eritrocytes ( $\times 10^6/\mu\text{L}$ )		Platelets ( $\times 10^3/\mu\text{L}$ )		Hemoglobin (g/dL)		MCH <sup>1</sup> (pg)		MCV <sup>2</sup> (fL)		MCHC <sup>3</sup> (g/dL)		Leucocytes ( $\times 10^3/\text{mm}^3$ )	
Experimental day	D-2	D40	D-2	D40	D-2	D40	D-2	D40	D-2	D40	D-2	D40	D-2	D40
Ct - Control	8.1 $\pm$ 0.4	8.6 $\pm$ 0.1	1.5 $\pm$ 0.3	1.3 $\pm$ 0.1	14.2 $\pm$ 0.5	14.7 $\pm$ 0.2	17.6 $\pm$ 0.3	17.4 $\pm$ 0.3	53.4 $\pm$ 2.5	56.5 $\pm$ 0.9	33.0 $\pm$ 1.1	30.6 $\pm$ 0.4	5.8 $\pm$ 0.6	11.6 $\pm$ 6.2
V1 - 8.9Ch	8.3 $\pm$ 0.1	8.7 $\pm$ 0.1	1.3 $\pm$ 0.3	1.4 $\pm$ 0.2	14.3 $\pm$ 0.3	14.8 $\pm$ 0.2	17.2 $\pm$ 0.5	17.0 $\pm$ 0.3	54.4 $\pm$ 2.3	56.8 $\pm$ 1.4	31.8 $\pm$ 1.5	30.0 $\pm$ 0.4	5.9 $\pm$ 0.6	7.9 $\pm$ 1.5
V2 - BtCh	8.3 $\pm$ 0.1	8.3 $\pm$ 0.2	1.4 $\pm$ 0.3	1.2 $\pm$ 0.2	14.5 $\pm$ 0.2	14.5 $\pm$ 0.7	17.6 $\pm$ 0.3	17.5 $\pm$ 0.5	57.2 $\pm$ 2.8	59.9 $\pm$ 1.9	29.1 $\pm$ 0.5	30.8 $\pm$ 1.6	6.7 $\pm$ 0.6	8.6 $\pm$ 1.3
V3 - KnCh	8.4 $\pm$ 0.1	8.8 $\pm$ 0.2	1.9 $\pm$ 0.4	1.3 $\pm$ 0.1	14.7 $\pm$ 0.4	15.1 $\pm$ 0.5	17.5 $\pm$ 0.2	17.1 $\pm$ 0.3	49.7 $\pm$ 0.1	56.1 $\pm$ 0.5	35.3 $\pm$ 0.3	30.5 $\pm$ 0.2*	7.9 $\pm$ 2.9	9.5 $\pm$ 1.7
V4 - Kn8.9Ch	7.1 $\pm$ 0.5	8.7 $\pm$ 0.3	1.1 $\pm$ 0.2	1.5 $\pm$ 0.3	13.2 $\pm$ 1.4	15.0 $\pm$ 0.4	16.2 $\pm$ 0.5	17.2 $\pm$ 0.2	55.2 $\pm$ 0.9	57.5 $\pm$ 1.6	29.1 $\pm$ 1.3	31.2 $\pm$ 1.3	7.7 $\pm$ 0.4	7.9 $\pm$ 0.4
V5- KnBt8.9Ch	8.7 $\pm$ 0.2	8.8 $\pm$ 0.2	1.3 $\pm$ 0.1	1.3 $\pm$ 0.1	15.5 $\pm$ 0.2	15.4 $\pm$ 0.1	17.9 $\pm$ 0.3	17.6 $\pm$ 0.2	55.6 $\pm$ 2.1	57.6 $\pm$ 0.9	32.4 $\pm$ 1.1	30.6 $\pm$ 0.6	7.9 $\pm$ 1.4	8.8 $\pm$ 1.1

<sup>1</sup>HCM - Mean corpuscular hemoglobin; <sup>2</sup>MCV- Mean corpuscular volume; <sup>3</sup>MCHC - Mean corpuscular hemoglobin concentration; Data is shown as mean  $\pm$  SD; Blood from five animals of each group were used in the analysis; Statistical analysis: the T test was used to detect differences between D-2 and D40, \*p<0.05.