

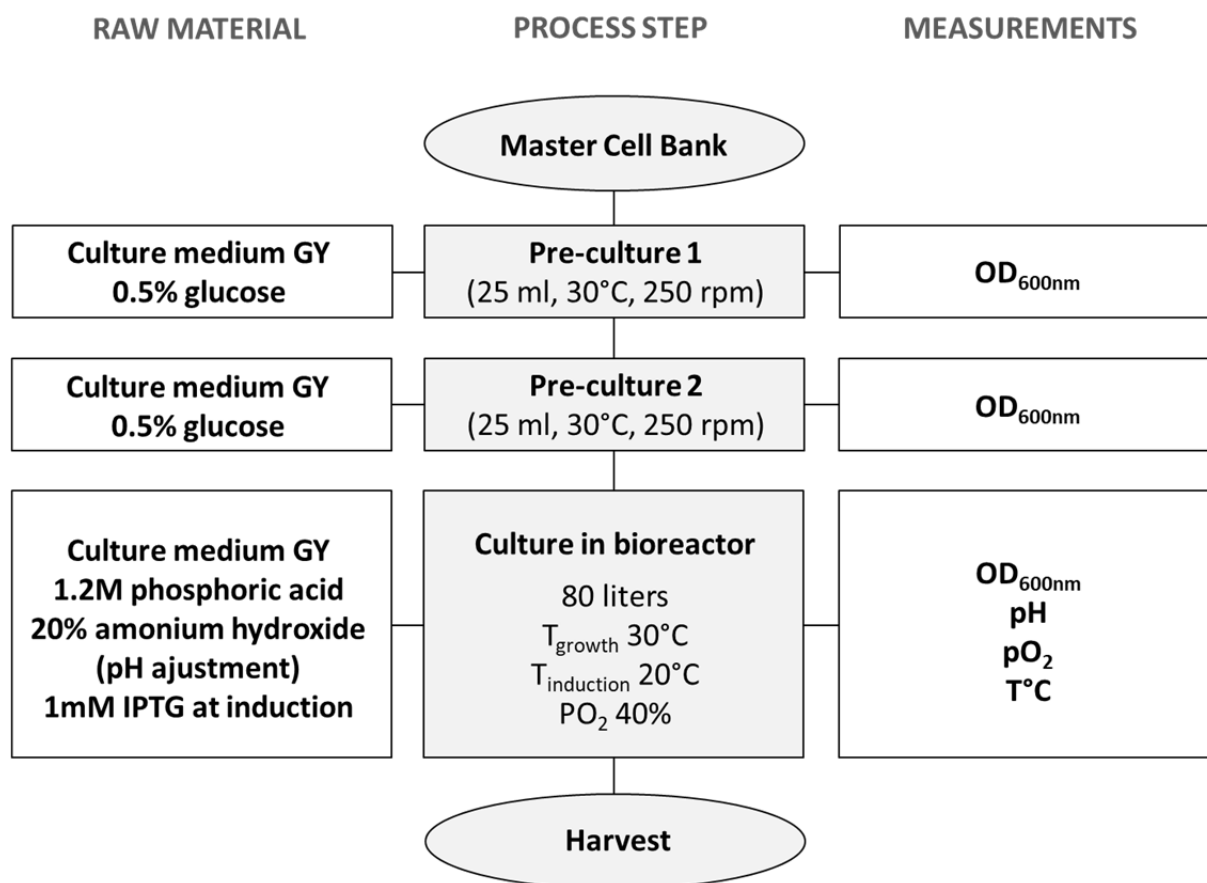
SUPPLEMENTARY TABLE 1

Quality control data of the Master Cell Bank

	RELEASE TESTS	SPECIFICATIONS	RESULTS
Stability	Numeration (before freezing)	> 1×10^8 cfu/ml	Complies
	Viability (Numeration after freezing)	> 1×10^8 cfu/ml	Complies
	Plasmid retention	> 80%	Complies
Purity	Bacterial and fungal contamination	Aerobic contaminants: < 1 contaminant / 10^7 <i>E. coli</i>	Complies
	Culture on Columbia CAP agar	< 1 contaminant / 10^7 <i>E. coli</i>	Complies
	Bacteriophage detection	Abscence	Complies
Identity	Microscopic examination and GRAM staining	GRAM negative bacillus	Complies
	Biochemical characterisation	Positive for <i>E. coli</i>	Complies
	Other biochemical parameter	Resistant to kanamycin	Complies
	Protein expression	At least one specific band at 100 kDa after induction (Western Blot anti-DBL1x)	Complies
		At least one band at 100 kDa after induction (SDS-PAGE)	Complies
Genetic characterisation	Sequence conform	Complies	

SUPPLEMENTARY FIGURE 1

Flow chart of the fermentation process



SUPPLEMENTARY TABLE 2

Mean body temperature 4 and 24 hours after the first and the last immunizations

DAY	SEX	TIME		TREATMENT				
				Group 1 0.9% NaCl	Group 2 GLA-SE	Group 3 PRIMVAC	Group 4 PRIMVAC + Alhydrogel®	Group 5 PRIMVAC + GLA-SE
1	M	0	N	16	15	15	17	15
			mean	37.7	37.7	38.0	37.5	37.6
		+4h	mean ²	37.4	38.4 **	37.3	37.8	38.4 **
		+24h	mean	37.8	37.9	37.6	37.4	37.9
	F	0	N	15	15	15	15	15
			mean	38.8	38.9	38.5	38.3	38.6
		+4h	mean ¹	38.3	39.0 **	38.2	38.0	38.5
		+24h	mean ¹	38.7	38.7	37.9 **	38.4 *	38.3 *
43	M	0	N	15	15	15	15	15
			mean ¹	37.2	36.8	37.5	37.5	37.0
		+4h	mean	37.4	37.8	37.2	37.1	37.8
		+24h	mean ¹	37.7	36.5 **	37.7	37.6	37.1 **
	F	0	N	15	15	15	15	15
			mean ¹	39.1	38.5 **	38.9	38.7 *	38.6 *
		+4h	mean ¹	38.7	38.6	38.1 *	38.4	38.6
		+24h	mean ²	38.8	38.4 *	38.4	38.7	38.3

*: (p<0.05), **: (p<0.01) by ¹ Dunnett test or ² Dunn test with respect to Group 1; numbers in bold are statistically significant from pre-dose values (time 0) in the same group

SUPPLEMENTARY TABLE 3

Incidence of oedema, erythema and nodosities after injection

LOCAL REACTION/ TIME	SEX	TREATMENT				
		Group 1 0.9% NaCl	Group 2 GLA-SE	Group 3 PRIMVAC	Group 4 PRIMVAC + Alhydrogel®	Group 5 PRIMVAC + GLA-SE
Increase in size (oedema)						
1st inj. (Day 1)	M	-	2/30 (1-8)	-	5/34 (3.5-3)	4/30 (1-4)
	F	-	2/30 (1.5-5)	-	-	-
2nd inj. (Day 15)	M	-	-	-	1/34 (1-6)	3/30 (1-3.5)
	F	-	-	-	6/30 (1-2)	1/30 (1-1)
3rd inj. (Day 29)	M	-	-	-	4/34 (1-3)	-
	F	-	-	-	1/30 (1-1)	-
4th inj. (Day 43)	M	1/32 ^a (0-2)	7/30 ^a (0.5-2.5)	1/30 (0-4)	10/34 ^a (0-3)	17/30 ^a (1.5-2)
	F	1/30 ^a (0-3)	7/30 ^a (1.5-1.5)	6/30 ^a (0.5-2)	5/30 ^a (0-3)	22/30 ^a (1.5-2)
Erythema						
1st inj. (Day 1)	M	-	-	1/30 (2-1)	2/34 (1-1.5)	-
	F	-	1/30 (2-1)	-	3/30 (1-2.5)	-
2nd inj. (Day 15)	M	-	1/30 (0-3)	1/30 (1-1)	1/34 (2-1)	2/30 (0.5-2)
	F	-	2/30 (0-2.5)	-	-	1/30 (0-1)
3rd inj. (Day 29)	M	-	3/30 (1-2.5)	-	1/34 (1-2)	3/30 (1-2)
	F	-	-	-	-	1/30 (0-3)
4th inj. (Day 43)	M	-	-	-	-	-
	F	-	-	-	-	-
Nodosities						
1st inj. (Day 1)	M	-	-	-	-	-
	F	-	-	-	6/30 (11-18)	-
2nd inj. (Day 15)	M	-	-	-	-	-
	F	-	-	-	-	-
3rd inj. (Day 29)	M	-	-	-	-	-
	F	-	-	-	1/30 (0-3)	-
4th inj. (Day 43)	M	-	-	-	1/34 (7-7)	-
	F	-	-	-	2/30 (0-3)	-

-: no findings

In brackets: mean latency – mean duration (in days)

Latency: Number of days between the injection day and the occurrence of the finding

Duration: Number of days for which the finding is present

^a Finding still present in one or several animals at the time of sacrificed

SUPPLEMENTARY TABLE 4

Haematology parameters in male rats

TIME/PARAMETER	TREATMENT				
	Group 1 0.9% NaCl	Group 2 GLA-SE	Group 3 PRIMVAC	Group 4 PRIMVAC + Alhydrogel®	Group 5 PRIMVAC + GLA-SE
Day 3					
Leucocyte (G/L)	12.83	13.92	13.51	15.19	14.97
Neutrophil (G/L)	1.15	1.37	1.15	1.75**	1.61*
Fibrinogen (g/L)	3.24	5.60**	3.44	4.15	5.78**
Day 45					
Leucocyte (G/L)	12.32	14.17	11.80	16.48**	14.26
Neutrophil (G/L)	1.22	1.76*	1.32	4.93**	2.33**
Fibrinogen (g/L)	3.36	4.72**	3.42	4.71**	5.67**
Day 65					
Leucocyte (G/L)	15.29	17.91	15.04	9.67	14.90
Neutrophil (G/L)	1.36	2.25	1.39	1.15	1.46
Fibrinogen (g/L)	2.99	3.05	3.12	3.11	3.03

Statistically significant from controls: *: (p<0.05) or **: (p<0.01)

SUPPLEMENTARY TABLE 5

Haematology parameters in female rats

TIME/PARAMETER	TREATMENT				
	Group 1 0.9% NaCl	Group 2 GLA-SE	Group 3 PRIMVAC	Group 4 PRIMVAC + Alhydrogel®	Group 5 PRIMVAC + GLA-SE
Day 3					
Leucocyte (G/L)	6.76	10.42**	7.86	10.01**	10.13**
Neutrophil (G/L)	0.70	1.10**	0.66	1.36**	1.46**
Eosinophil (G/L)	0.11	0.20**	0.12	0.13	0.19**
Basophil (G/L)	0.02	0.05**	0.02	0.03	0.04**
Lymphocyte (G/L)	5.77	8.65**	6.88	8.12*	7.92
Large unstained cell (G/L)	0.03	0.13**	0.05	0.09**	0.18**
Monocyte (G/L)	0.12	0.30**	0.13	0.29**	0.34**
Fibrinogen (g/L)	2.79	5.10**	3.03	3.40**	5.44**
Day 45					
Leucocyte (G/L)	7.40	10.88**	9.42	11.76**	14.08**
Neutrophil (G/L)	0.60	1.73**	0.84	3.65**	3.44**
Eosinophil (G/L)	0.16	0.28**	0.19	0.22	0.43**
Basophil (G/L)	0.02	0.05*	0.03	0.03	0.07**
Lymphocyte (G/L)	6.30	8.19*	7.94*	7.14	9.12**
Large unstained cell (G/L)	0.06	0.17**	0.11*	0.14**	0.36**
Monocyte (G/L)	0.25	0.47**	0.30	0.57**	0.66**
Fibrinogen (g/L)	2.54	4.37**	3.12**	3.45**	5.85**
Day 65					
Leucocyte (G/L)	8.86	7.83	9.72	4.77	7.84
Neutrophil (G/L)	0.67	0.84	0.68	0.49	0.77
Eosinophil (G/L)	0.20	0.14	0.19	0.10	0.19
Basophil (G/L)	0.03	0.03	0.04	0.01	0.03
Lymphocyte (G/L)	7.56	6.47	8.45	4.06	6.53
Large unstained cell (G/L)	0.11	0.06	0.11	0.02	0.06
Monocyte (G/L)	0.29	0.29	0.26	0.10	0.27
Fibrinogen (g/L)	2.05	2.08	2.19	1.68	2.16

Statistically significant from controls: *: (p<0.05) or **: (p<0.01)

SUPPLEMENTARY TABLE 6

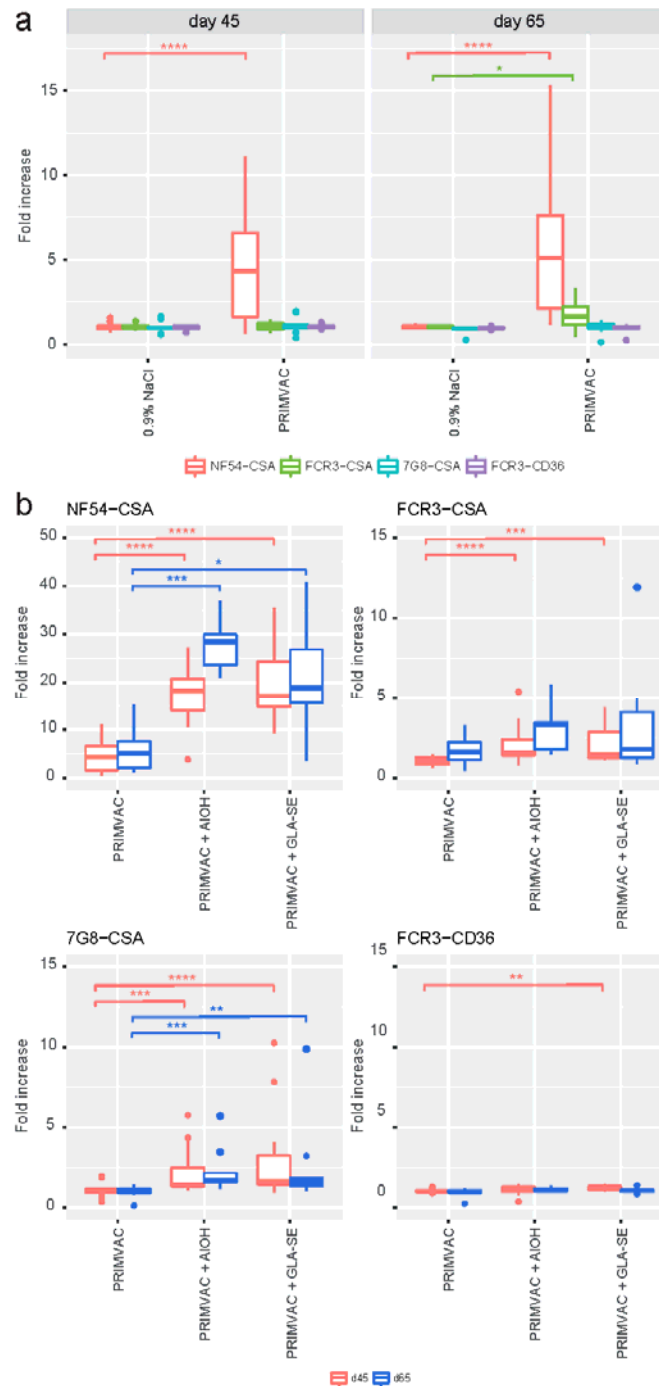
Plasma alpha-2 macroglobulin levels

TIMING	SEX	Group 1 0.9% NaCl	Group 2 GLA-SE	Group 3 PRIMVAC	Group 4 PRIMVAC + Alhydrogel®	Group 5 PRIMVAC + GLA-SE
Pre-dose	M	101.7	84.0	93.7	96.1	77.6
	F	55.1	38.0	55.9	49.6	50.0
Day 3	M	52.4	2055.5** (x25)	43.1	80.5	3281.3** (X42)
	F	36.0	1511** (x40)	38.0	43.0	2161** (x43)
Day 45	M	23.3	464.5** (x6)	19.0	31.8	924.2** (x12)
	F	41.4	349.5* (x9)	30.5	31.0	1064.1** (x21)
Day 65	M	6.8	20.9*	3.5	17.2	19.0
	F	20.5	24.7	26.8	24.5	47.7**

Statistically significant from controls: *: (p<0.05) or **: (p<0.01)

SUPPLEMENTARY FIGURE 3

Immune recognition of erythrocytes infected by different parasite strains (NF54, FCR3, and 7G8) selected for different adhesive phenotypes (CSA and CD36) by vaccination-induced antibodies directed towards native VAR2CSA



(A) Wilcoxon rank sum test was performed for each of the 8 parasite strain/time of sacrifice combinations separately. *, $p \leq 0.05$; **, $p \leq 0.01$; ****, $p \leq 0.0001$ (45-day groups: 0.9% NaCl, $n=19$; PRIMVAC, $n=20$. 65-day groups, $n=10$).

(B) Kruskal-Wallis one-way ANOVA on ranks test was performed for each of the 8 parasite strain/time of sacrifice combinations separately, followed by Dunn's multiple comparison procedure in case of showing differences at a 0.05 significance level. Significant differences against the PRIMVAC group are shown: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$ (45-day groups: PRIMVAC, $n=20$; PRIMVAC + AIOH (Alhydrogel[®]), $n=18$; PRIMVAC + GLA-SE, $n=19$. 65-day groups, $n=10$).

SUPPLEMENTARY TABLE 7

Sera pools used in inhibitions assays

GROUPS	TREATMENTS	SERA POOLS	
1	0.9% NaCl	pool 1M	16 males
		pool 1F	15 females
3	PRIMVAC	pool 3M	15 males
		pool 3F	15 females
4	PRIMVAC + Alhydrogel [®]	pool 4M	17males
		pool 4F	15 females
5	PRIMVAC + GLA-SE	pool 5M	15 males
		pool 5F	15 females