

Supplementary Material

Figure S1. Comparison of the immune responses in rabbits vaccinated with standard DNA immunization regimen (n = 3), DNA immunization with “intensive” priming (n = 2) and protein immunization (n = 3). DNA immunization contained the same *env* gp140Bx08 and the protein immunization was with rgp120IIIb in CAF01 adjuvant. Immunization time points are indicated with arrows. Specific IgG response was measured against strain IIIb gp120.

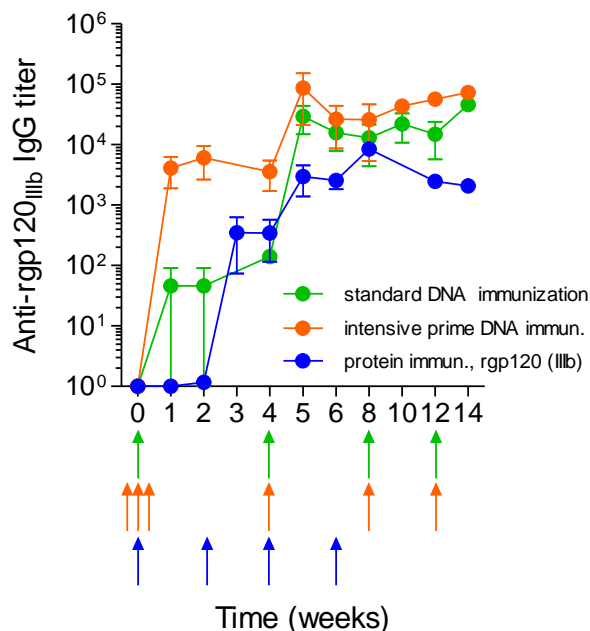


Figure S2. Average neutralization kinetics of rabbit IgG (green) and macaque sera (red) of virus strain SF162. Rabbit IgG was tested at 250 μ g/mL and macaque sera was diluted 30 times. Both groups of animals included 4 animals, immunized with the same plasmid DNA encoding syn.gp140_{mix}. Immunization time points are indicated with arrows.

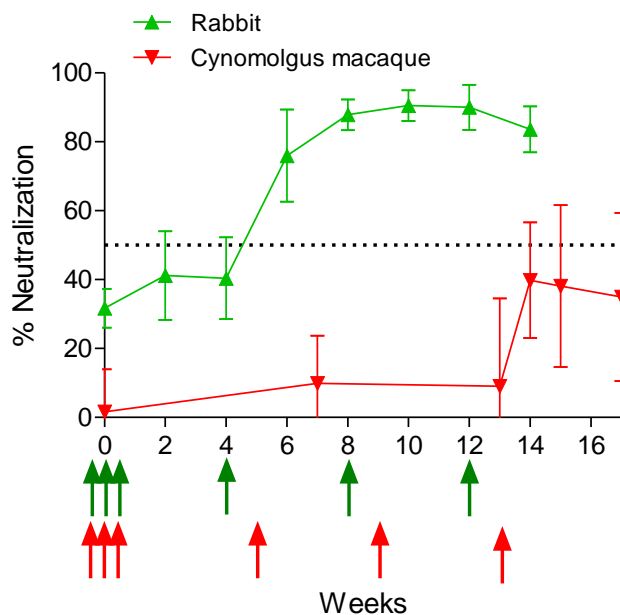


Table S1. Neutralizing activity in serum from immunized guinea pigs and rabbits. Pre-immune sera and 14 weeks post vaccination was tested. Darker color corresponds to higher neutralizing activity. For guinea pigs reciprocal titers correspond to 1/dilution of serum giving 50% inhibitory concentration (IC50) are described. For rabbits purified IgG corresponding to 50% inhibitory concentration (IC50) are described.

Animal	Vaccine construct	Animal ID	Sample	MLV	Tier 2											Subtype Strain				
					B	B	B	B	A	C	CRF02_AG CRF02_AG D; (X4)			B	B		C			
					Bx08	SF162	BaL	JR-FL	92RW009	92Br025	VII090	DJ263.8	92UG024.2	QH0692	AC10.0.29		DU151.2			
Guinea pigs	gp140x08	5622	Preimmune	74	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	nd		
				5623	34	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	
				5624	26	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
				5625	33	<20	<20	<20	<20	<20	<20	<20	24	<20	<20	<20	<20	23	<20	<20
	Gp140Mix	5634	Preimmune	26	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	21	<20	<20	<20	
				5635	24	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	24	<20	<20	<20	
				5636	22	<20	<20	<20	<20	<20	<20	<20	21	<20	27	<20	23	<20	<20	<20
				5637	21	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
	Gp140Mix SOSIP-	6460	Preimmune	30	30	36	38	23	<20	25	<20	<20	<20	<20	28	36	35	<20	<20	
				6461	26	41	24	32	<20	<20	25	<20	<20	<20	29	32	29	<20	<20	
				6462	28	29	<20	37	23	23	27	<20	<20	<20	31	33	26	<20	<20	
				6463	29	<20	<20	23	<20	21	27	<20	<20	<20	30	31	26	<20	<20	
	R6-IZ-H8	5622	Preimmune	26	>80	27	<20	<20	37	58	38	24	32	60	76	62	<20	<20		
				5623	<20	>80	>80	<20	25	69	35	38	30	27	127	>160	>160	<20	<20	
				5624	<20	74	<20	<20	<20	35	28	28	23	23	39	52	37	<20	<20	
				5625	22	>80	<20	<20	<20	31	37	26	25	28	61	54	55	<20	<20	
	Gp140Bx08	5634	Preimmune	34	>80	>80	<20	<20	38	46	50	35	33	94	95	40	<20	<20		
				5635	28	>80	>80	<20	<20	30	36	34	35	23	68	72	42	<20	<20	
				5636	32	>80	>80	<20	<20	59	74	63	39	38	134	151	79	<20	<20	
				5637	<20	>80	30	<20	<20	39	32	25	24	26	63	49	34	<20	<20	
Gp140Mix	6460	Preimmune	70	>80	55	68	40	132	>160	56	36	40	>160	>160	>160	<20	<20			
			6461	>160	>80	57	45	40	86	>160	39	34	35	>160	>160	138	<20	<20		
			6462	117	>80	>80	58	55	56	76	38	31	29	>160	>160	>160	<20	<20		
			6463	74	>80	70	66	46	37	0	27	<20	25	>160	>160	127	<20	<20		
Gp140Mix SOSIP-	5622	Week 14 post vaccination	26	>80	27	<20	<20	37	58	38	24	32	60	76	62	<20	<20			
			5623	<20	>80	>80	<20	25	69	35	38	30	27	127	>160	>160	<20	<20		
			5624	<20	74	<20	<20	<20	35	28	28	23	23	39	52	37	<20	<20		
			5625	22	>80	<20	<20	<20	31	37	26	25	28	61	54	55	<20	<20		
Gp140Mix	5634	Week 14 post vaccination	34	>80	>80	<20	<20	38	46	50	35	33	94	95	40	<20	<20			
			5635	28	>80	>80	<20	<20	30	36	34	35	23	68	72	42	<20	<20		
			5636	32	>80	>80	<20	<20	59	74	63	39	38	134	151	79	<20	<20		
			5637	<20	>80	30	<20	<20	39	32	25	24	26	63	49	34	<20	<20		
Gp140Mix SOSIP-	6460	Week 14 post vaccination	70	>80	55	68	40	132	>160	56	36	40	>160	>160	>160	<20	<20			
			6461	>160	>80	57	45	40	86	>160	39	34	35	>160	>160	138	<20	<20		
			6462	117	>80	>80	58	55	56	76	38	31	29	>160	>160	>160	<20	<20		
			6463	74	>80	70	66	46	37	0	27	<20	25	>160	>160	127	<20	<20		
R6-IZ-H8	5622	Week 14 post vaccination	26	>80	27	<20	<20	37	58	38	24	32	60	76	62	<20	<20			
			5623	<20	>80	>80	<20	25	69	35	38	30	27	127	>160	>160	<20	<20		
			5624	<20	74	<20	<20	<20	35	28	28	23	23	39	52	37	<20	<20		
			5625	22	>80	<20	<20	<20	31	37	26	25	28	61	54	55	<20	<20		

Table S2. Cont.

Animal	Vaccine construct	Animal ID	B Sample	B SF162	B Bx08	B BaL	B JR-FL	C 92Br025	A 92RW009	Subtype Strain	
Rabbits	gp140Bx08	116036	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
		116157	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
		116064	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
		116069	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
	gp150Bx08	116087	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
		116115	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
		116126	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
		116130	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
	gp140Mix	134040	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
		134114	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
		134126	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
		134132	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
	gp140Mix SOSIP-R6-IZ-H8	946369	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
		946387	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
		946376	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
		946370	>250	>250	n. d.	n. d.	n. d.	n. d.	n. d.		
	gp140Bx08	116036	<31	35	239	>400	>400	>400	>400		
		116157	<31	174	>400	>400	343	>400	>400		
		116064	58	143	393	>400	>400	>400	>400		
		116069	<31	<31	154	>400	370	>400	>400		
		gp150Bx08	116087	197	>250	>400	>400	391	>400	>400	
			116115	72	<31	180	>400	273	>400	>400	
			116126	<31	36	>400	>400	373	>400	>400	
			116130	129	148	>400	>400	>400	>400	>400	
		gp140Mix	134040	102	84	>400	>400	309	>400	>400	
			134114	170	193	>400	>400	329	>400	>400	
			134126	110	218	>400	>400	307	>400	>400	
			134132	<31	81	>400	>400	323	>400	>400	
gp140Mix SOSIP-R6-IZ-H8	946369	128	>250	>400	>400	367	>400	>400			
	946387	55	<31	>400	>400	>400	>400	>400			
	946376	31	82	>400	>400	369	>400	>400			
	946370	64	136	>400	>400	374	>400	>400			