Comparative “prime-boost” immunization and the role of neutralizing antibodies in protection against mucosal $SHIV_{SF162\ P4}$ infection

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“Prime-Boost” Immunization

- Live recombinant virus for priming
  - “Adjuvant” activity of live virus infection
  - Generate long-term memory
  - Present immunogens in native conformation
  - Induce both antibody and cell-mediated immunities
- Subunit protein for boosting
  - Minimize effects of pre-existing vector immunity
  - Augment specific B- and T-cell responses

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T-cell responses to human AIDS virus in macaques immunized with recombinant vaccinia viruses

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Table 1 LAV-induced proliferative responses of PBL from macaques after immunization with a vaccinia recombinant virus expressing LAV envelope glycoproteins

Macaque No.	Immunization	Virus used for PBL stimulation: None	LAV	Recombinant vaccinia v-env5
		c.p.m. ^{3}H-TdR incorporated (stimulation index)		
67	v-env5	1,586	7,465(4.7)	60,415(38.1)
68	v-env5	2,245	9,075(4.0)	28,638(12.8)
74	v-env5	1,585	4,732(3.0)	21,487(13.6)
75	v-env5	581	8,645(14.9)	37,847(14.1)
76	v-env5	2,479	5,987(2.5)	36,657(14.8)
80	v-env5	1,077	13,922(12.9)	24,752(23.0)

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Proliferative and Cytotoxic T Cells to AIDS Virus Glycoproteins in Chimpanzees Immunized with a Recombinant Vaccinia Virus Expressing AIDS Virus Envelope Glycoproteins[1]

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Neutralizing Antibodies Against HIV-1 BRU and SF2 Isolates Generated in Mice Immunized with Recombinant Vaccinia Virus Expressing HIV-1 (BRU) Envelope Glycoproteins and Boosted with Homologous gp160

SHIU-LOK HU, JIM KLANIECKI, TRACY DYKERS, PENNATHUR SRIDHAR, and BRUCE M. TRAVIS

TABLE 1. HIV-1 ANTIBODY RESPONSE IN MICE IMMUNIZED WITH RECOMBINANT VACCINIA VIRUS AND/OR gp160

	Immunization scheme		*Mean ELISA titer*		*Neutralizing titer*[a]	
Animal group	*Week 0*	*Week 8*	*Week 8*	*Week 10*	*BRU*	*SF2*
1	v-*env*5[b]	v-*env*5	800	1600	0	0
2	v-*env*5	gp160	800	>51200	>400	200
3	gp160	v-*env*5	1600	1600	0	0
4	gp160	gp160	800	6400	0	0
5	v-NY	v-NY	<50	<50	0	0
6	CFA[c]	IFA	<50	<50	0	0
Monoclonal antibody 110.4 (refs. 34,35)					>3200	0
Hyperimmune rabbit serum against the principal neutralizing determinant of SF2					0	400

Objectives

- To compare immune responses generated by recombinant viral vector, DNA plasmid and protein immunogens in different “prime-boost” combinations
- To evaluate their protective efficacy against a pathogenic R5 SHIV infection

Overall experimental plan

Expt	Prime	Boost
I	Vaccinia virus	DNA
		Protein
		VEE/SIN
		Controls
II	DNA	DNA
		Protein
		Vaccinia virus
		Controls
III	Protein	DNA
		Protein
		Vaccinia virus
		Controls

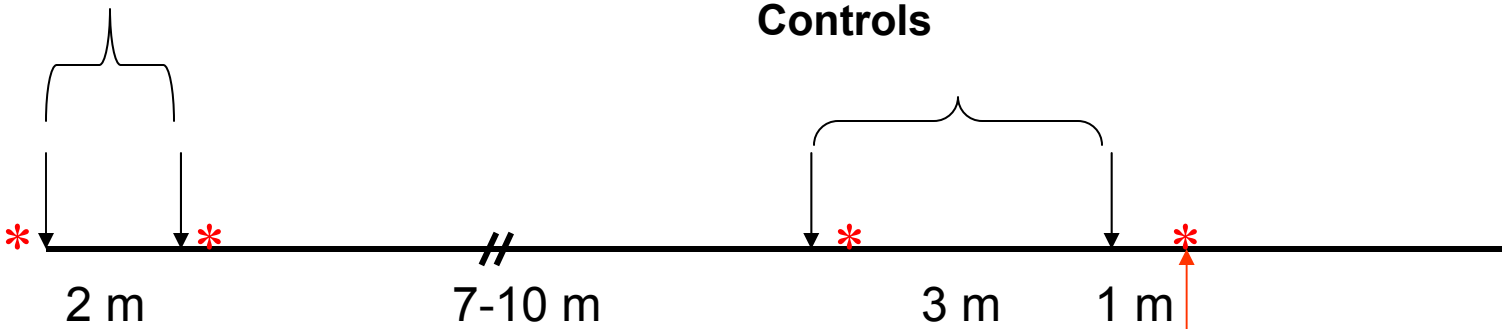
Immunogens, dose and route

- DNA:
  - pCMV SF162 Env gp140; pCMV SIVmac239 Gag-Pol
  - 2 mg/dose in normal saline
  - IM injection
- Virus vectors:
  - Replication competent vaccinia virus (v-NY) expressing:
    - SF162 Env gp160 & SIVmac239 Gag-Pol on separate vectors
    - 10^8 plaque-forming units/dose
    - Skin scarification
  - Chimeric alphavirus (VEE/SIN) replicon expressing
    - SF162 Env gp160 & SIVmac239 Gag-Pol on separate replicons
    - 10^8 particles/dose
    - IM injection
- Proteins:
  - SF162 Env gp140 (CHO cells); SIVmac239 Gag-Pol particles (recombinant vaccinia virus infected BSC cells)
  - 0.1 mg each/dose formulated in MF59 adjuvant
  - IM injection

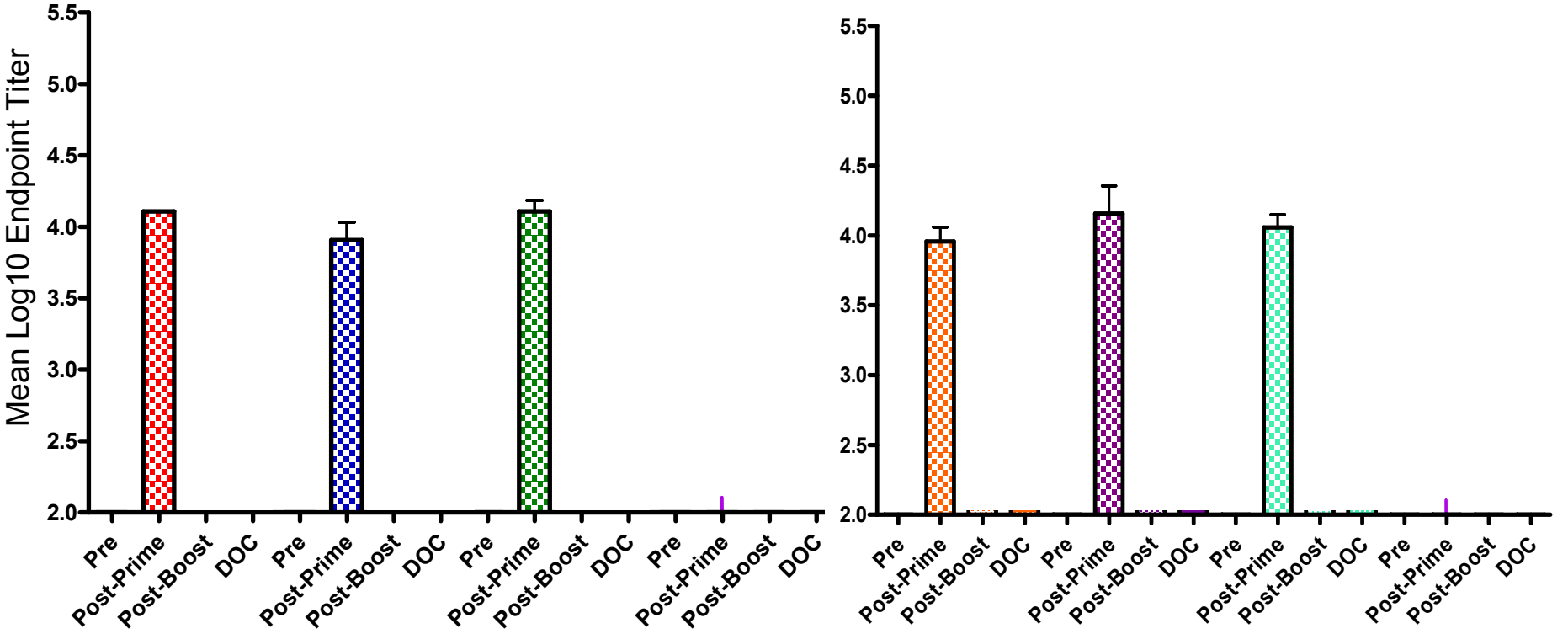
Immunization schedule

Expt	Prime	Boost
I	Vaccinia virus	DNA Protein VEE/SIN Controls

N = 6/group
Outbred pig-tailed macaques

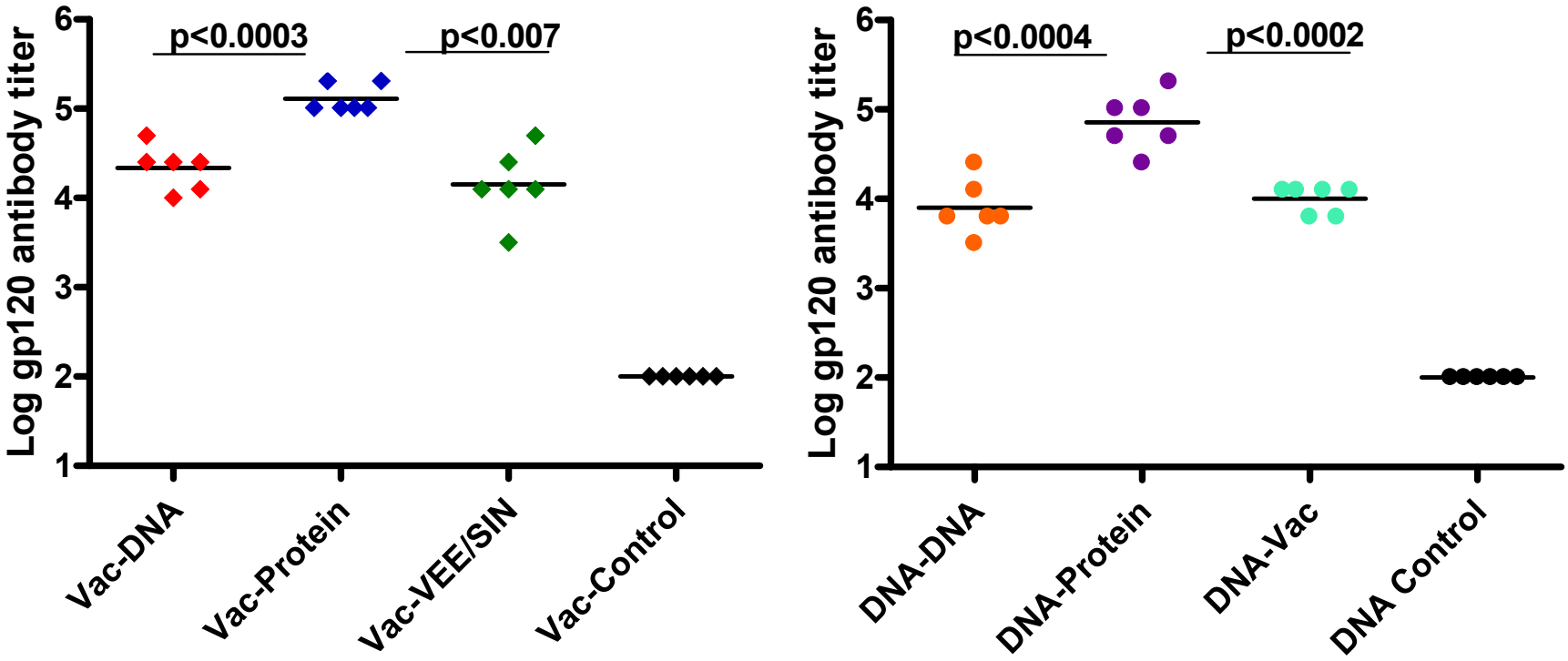


HIV-1 gp120-specific antibody response to "prime-boost" immunization

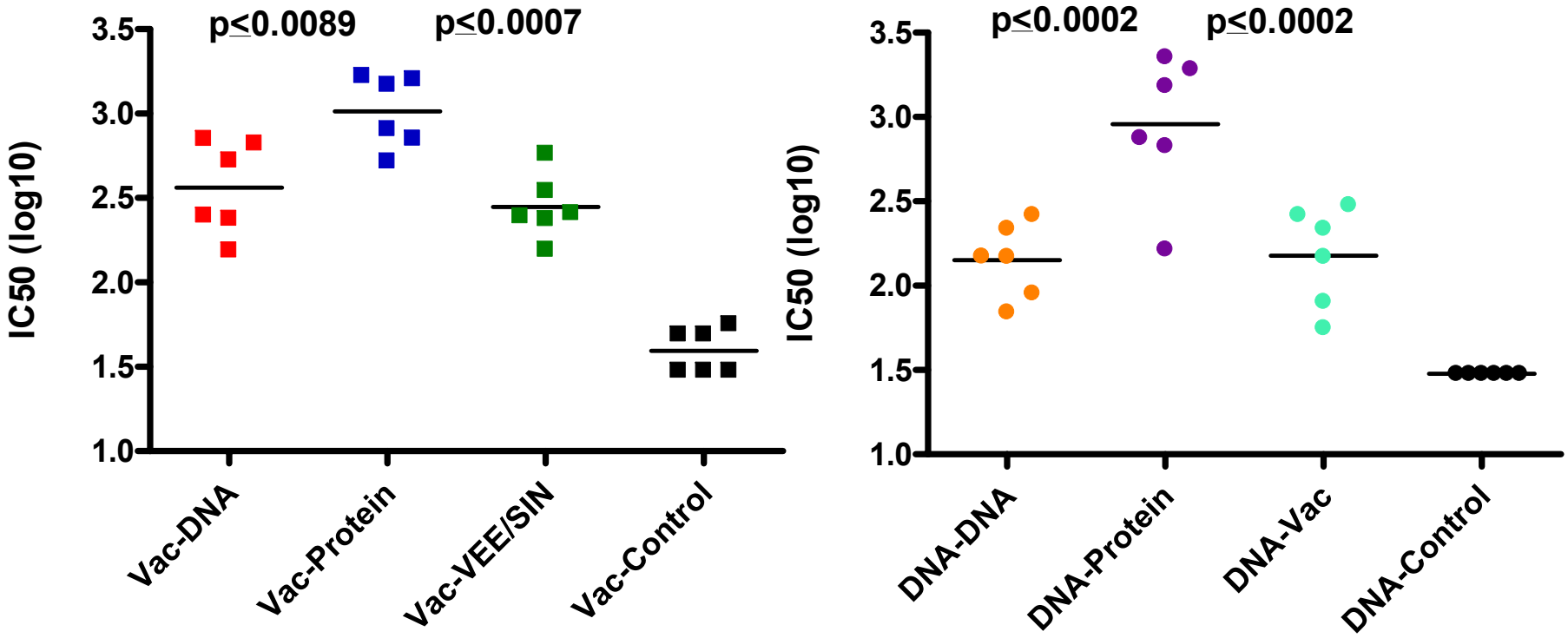


Prime	Vaccinia			Vaccinia Control	DNA			DNA Control
Boost	DNA	Protein	VEE/SIN		DNA	Protein	Vaccinia	

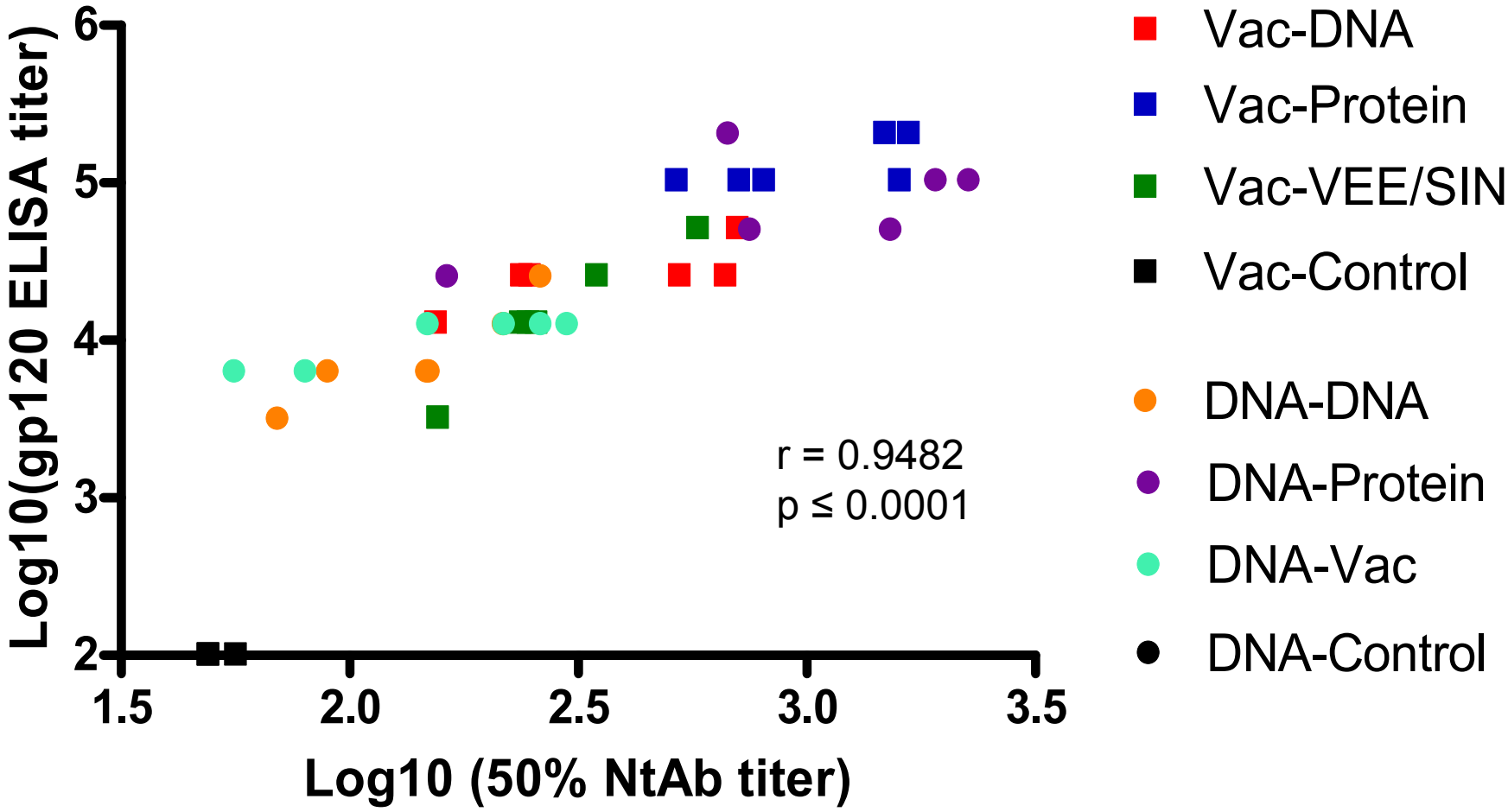
HIV gp120-specific antibody titer on day of challenge (DOC)



SF162 neutralization titer on DOC



DOC gp120-specific Ab titer correlates with NtAb titer against the homologous virus



Neutralization of heterologous primary viruses by DOC sera

Expt I	Animal	SS1196 (B)	SVPB6 (B)	Q461d1 (A)	Expt II	Animal	SS1196 (B)	SVPB6 (B)	Q461d1 (A)
Vac-DNA	L02320	51.98	23.48	46.05	DNA-DNA	T03172	0.00	11.27	0.00
	K03099	27.05	3.97	59.28		M03360	0.00	5.11	0.00
	A03181	42.61	4.53	68.19		M03242	12.87	12.50	11.63
	L01211	37.44	42.95	72.29		M03358	20.16	27.70	7.92
	J02411	47.24	13.52	45.13		M02044	7.00	0.00	0.00
	A03180	34.88	17.05	40.06		K02280	37.35	24.61	7.61
Vac-Protein	M02181	79.76	65.47	95.72	DNA-Protein	M03182	13.20	46.26	52.85
	M02156	52.83	58.83	89.09		A03188	54.81	50.54	66.63
	T02238	71.14	67.52	95.16		M03240	59.73	64.21	76.60
	L02393	75.51	66.56	89.89		M03126	46.34	54.12	73.50
	M02408	54.77	54.50	76.21		M02383	63.81	63.10	84.87
	Z02384	72.56	66.14	90.70		M02298	51.79	57.30	72.42
Vac-VEE/SIN	L03014	57.21	8.32	57.70	DNA-Vac	M03396	50.43	26.00	2.87
	J02263	1.99	0.00	26.08		J03189	37.24	28.55	25.20
	M02240	67.49	40.60	84.05		M03311	30.21	19.68	19.99
	A03176	32.20	22.42	44.81		M03299	22.88	19.71	19.58
	T02142	21.50	0.00	20.54		K02170	22.12	1.57	20.24
	L01283	57.24	37.17	63.13		K02144	43.51	10.82	31.40
Vac-Control	J02303	15.19	0.00	0.00	DNA-Control	K03258	0.00	0.00	1.61
	K02305	21.16	0.00	0.00		M03186	0.00	0.00	0.00
	L02143	0.00	0.00	0.00		M03053	20.48	7.04	0.00
	M02127	14.82	0.00	29.18		M04082	29.71	39.16	24.02
	K02264	17.12	0.00	21.85		K02290	1.00	17.29	6.82
	M02295	0.00	0.00	0.00		M02294	6.10	7.68	22.80

% inhibition @ 1:30 dilution

<50	50-69	70-89	≥90

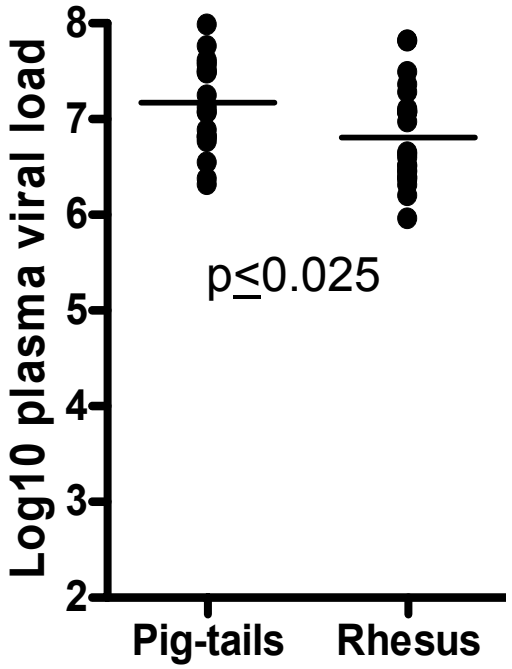
$SHIV_{SF162\ P4}$ challenge stock

- A macaque-passaged stock $SHIV_{SF162\ P3}$, was established in Dr. Cecilia Cheng-Mayer's lab (Harouse *et al*., *Science* 1999). Lymph node and PBMC from a macaque infected with $SHIVSF_{162\ P3}$ collected at 2 weeks after infection were amplified in human PBMC to generate a $SHIV_{SF162\ P4}$ stock.
- Plasma from $SHIV_{SF162\ P4}$-infected macaque was passaged twice by successive IV injection in naive Indian rhesus macaques.
- PBMC from the second infected macaque were collected at peak viremia and expanded in vitro by co-culture with PBMC from naïve macaques.
- The 50% tissue culture infectious dose ($TCID_{50}$) of this stock was 3.6×10^3/ml determined on rhesus PBMC.

$SHIV_{SF162\ P4}$ has similar infectivity in rhesus and pig-tailed macaques

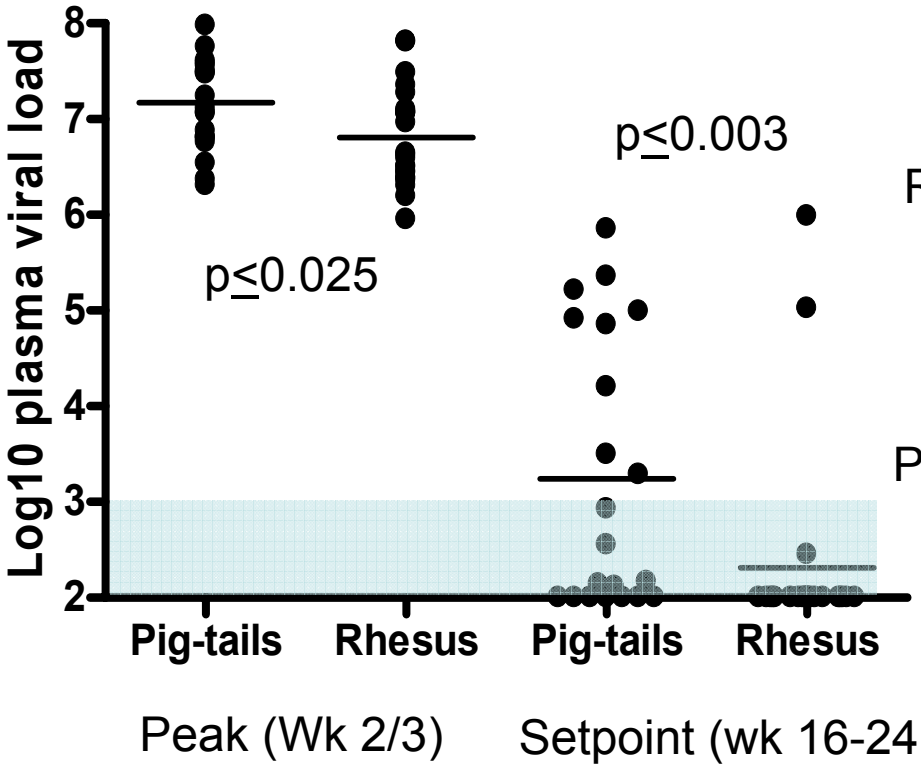
Route	Rhesus macaques		Pig-tailed macaques	
	$TCID_{50}$	Inf./total	$TCID_{50}$	Inf./total
IV	36 – 3.6	4/4	360-0.036	10/10
IR	1800 360	6/6 5/6	1800 360	6/6 5/6
IVag	3600 1800	6/7 3/4		
Total Infected		24		21

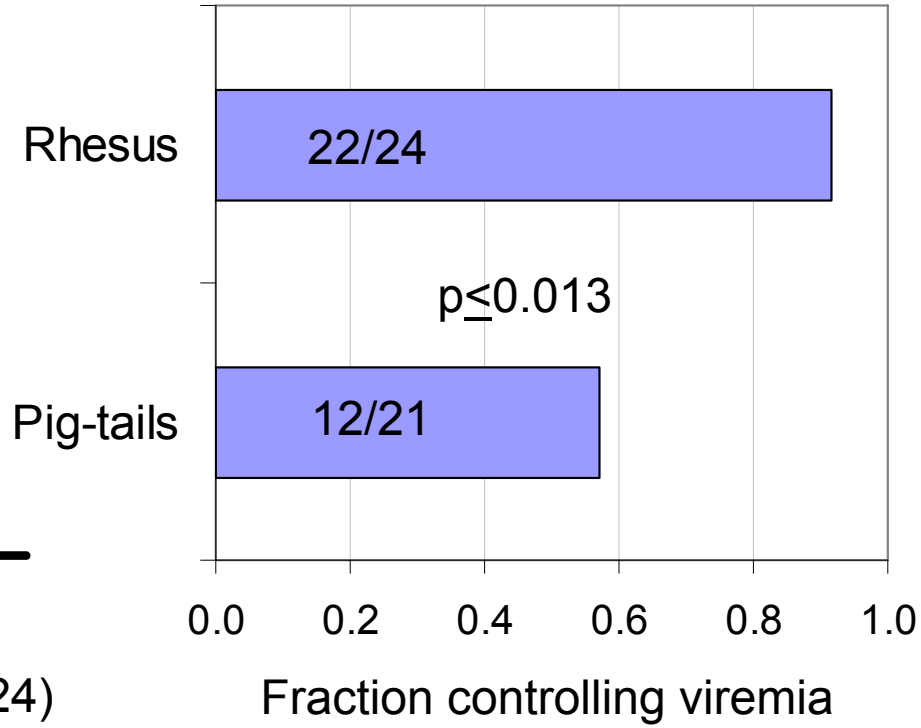
But, infected pig-tail macaques had higher peak viral load …



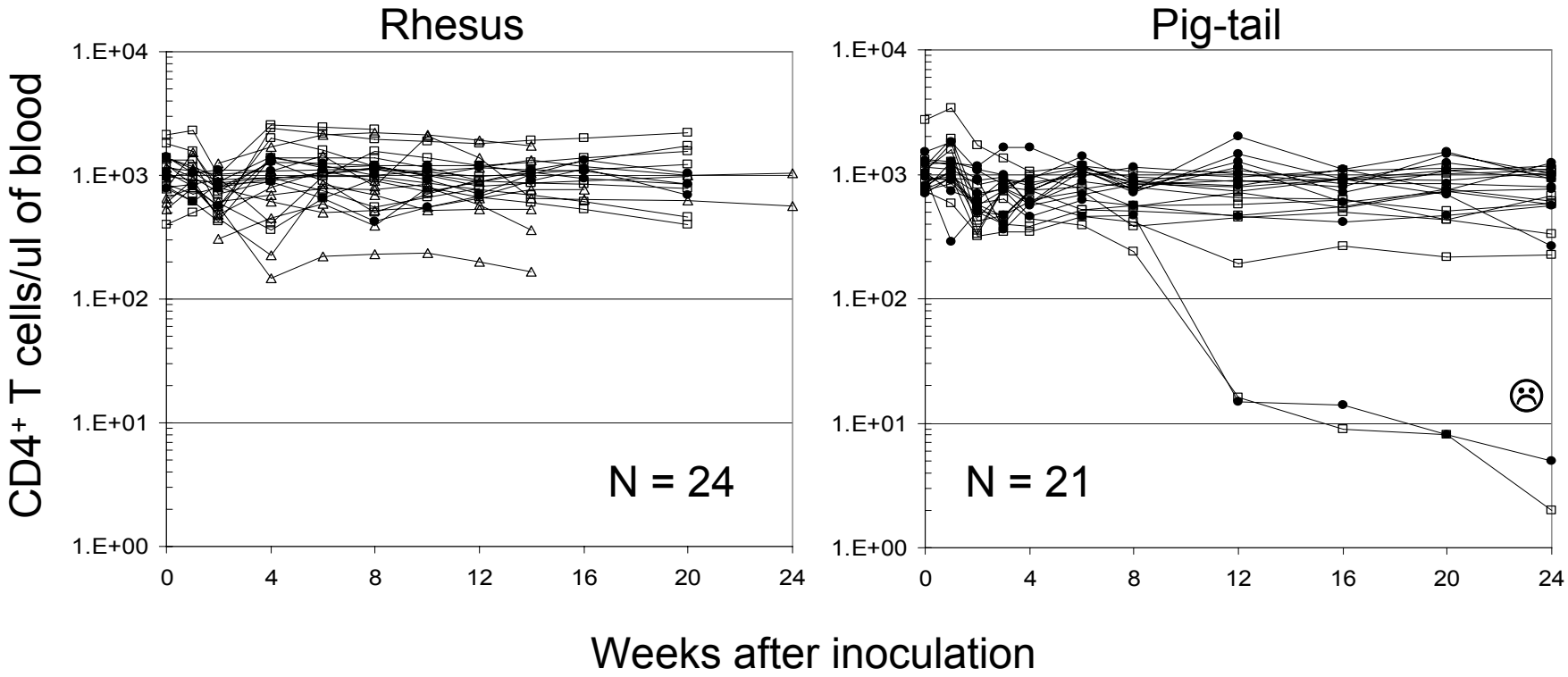
Peak (Wk 2/3)

…and maintained higher setpoint viremia than rhesus macaques





Peripheral blood $CD4^+$ T-cell depletion in $SHIV_{SF162\ P4}$-infected pig-tailed macaques

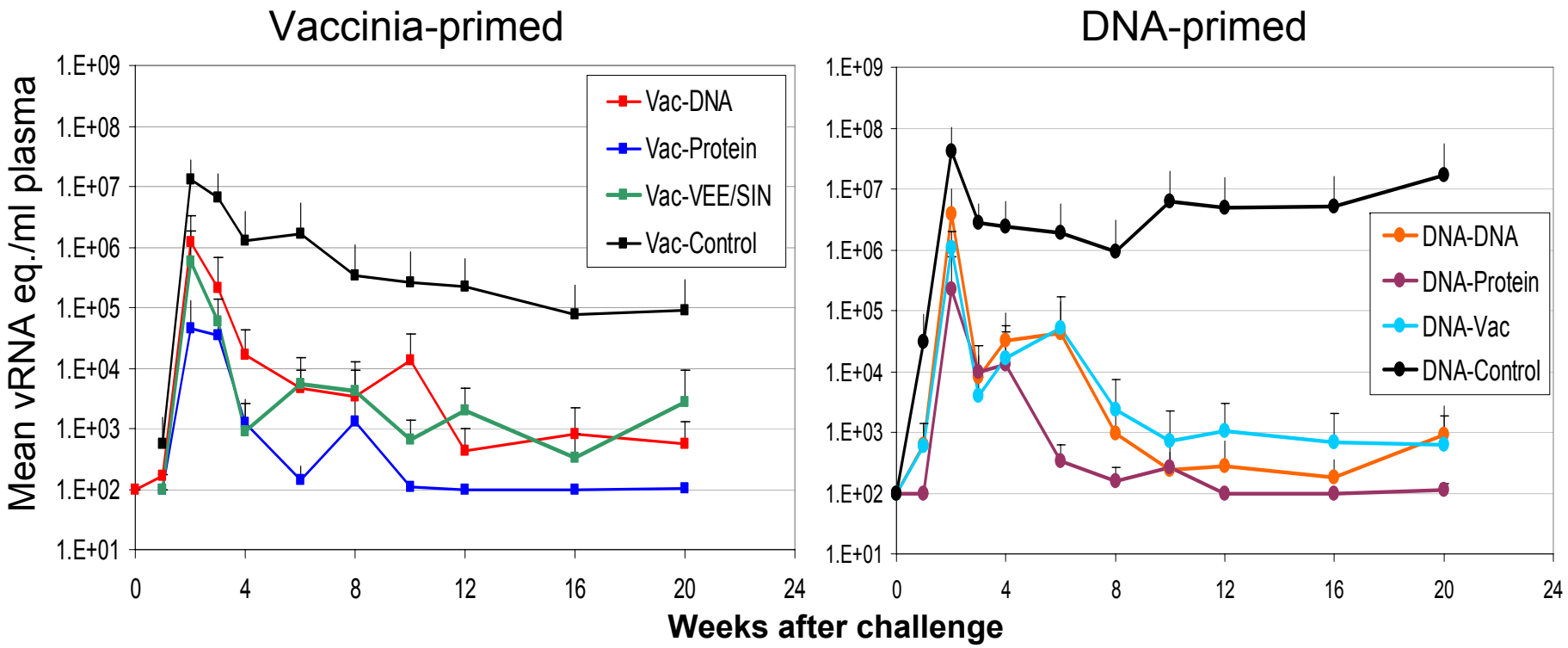


Pig-tailed macaques offer a more robust model than rhesus macaques for a pathogenic R5 SHIV infection

$SHIV_{SF162\ P4}$ Challenge

- Four weeks after the last immunization
- Route: Intrarectal inoculation
- Dose: Single dose of 1,800 $TCID_{50}$

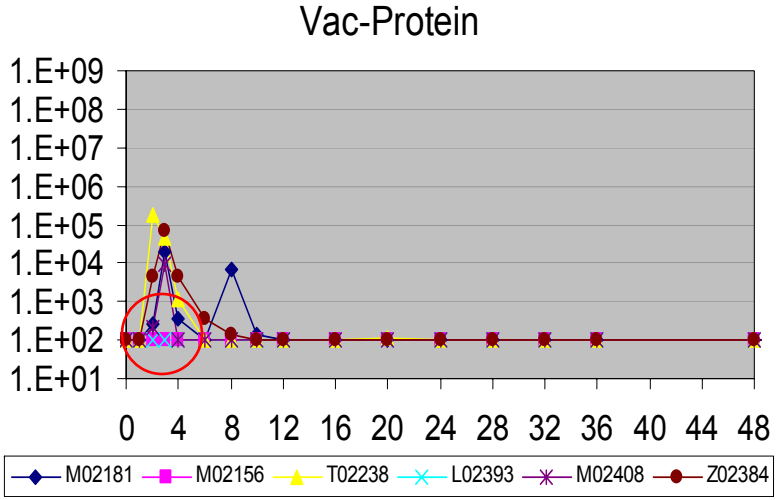
Reduction of plasma viral load in immunized macaques after intrarectal $SHIV_{SF162\ P4}$ challenge

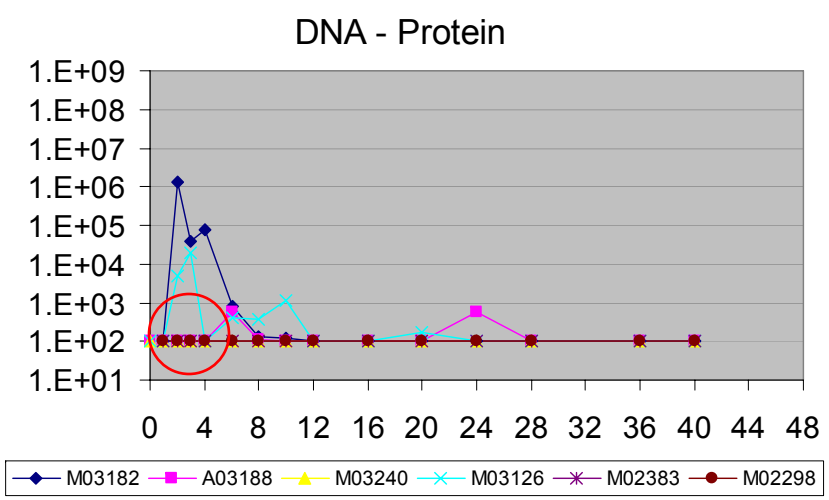


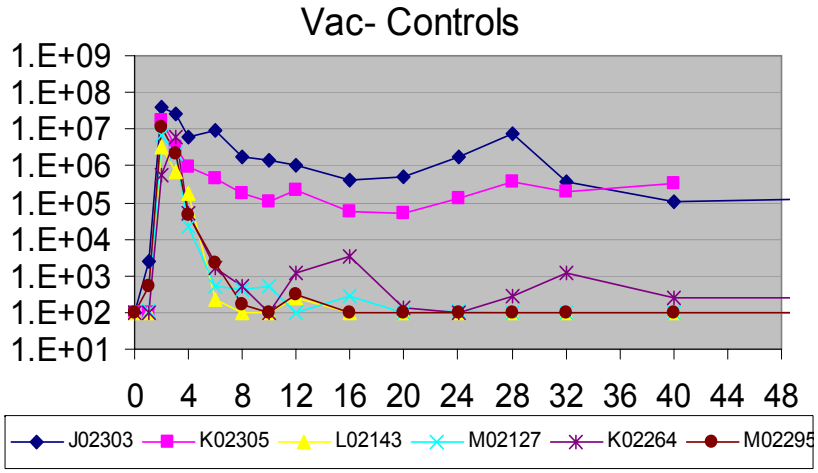
Control vs.	Peak VL	Wk 12 VL
Vac-DNA	$p \leq 0.0124$	ns
Vac-Protein	$p \leq 0.0002$	$p \leq 0.047$
Vac-VEE/SIN	$p \leq 0.0004$	ns

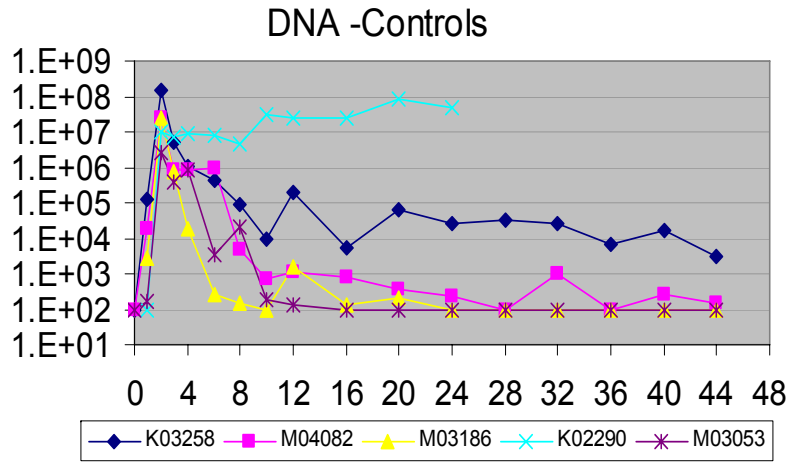
Control vs.	Peak VL	Wk 12 VL
DNA-DNA	$p \leq 0.0183$	$p \leq 0.047$
DNA-Protein	$p \leq 0.0005$	$p \leq 0.030$
DNA-Vac	$p \leq 0.0169$	ns

“Sterilizing immunity” in DNA- or vaccinia-primed animals boosted with protein immunogens





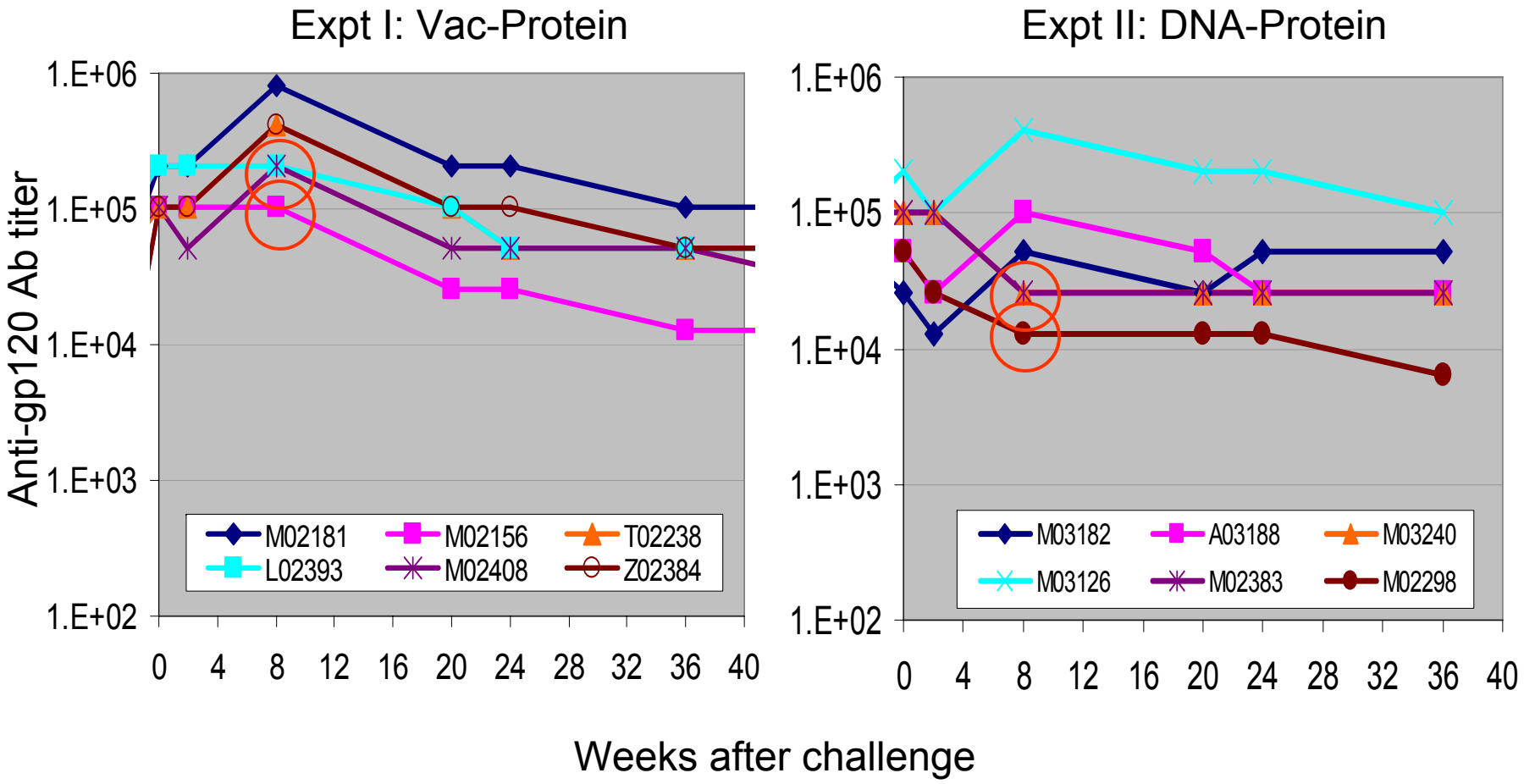




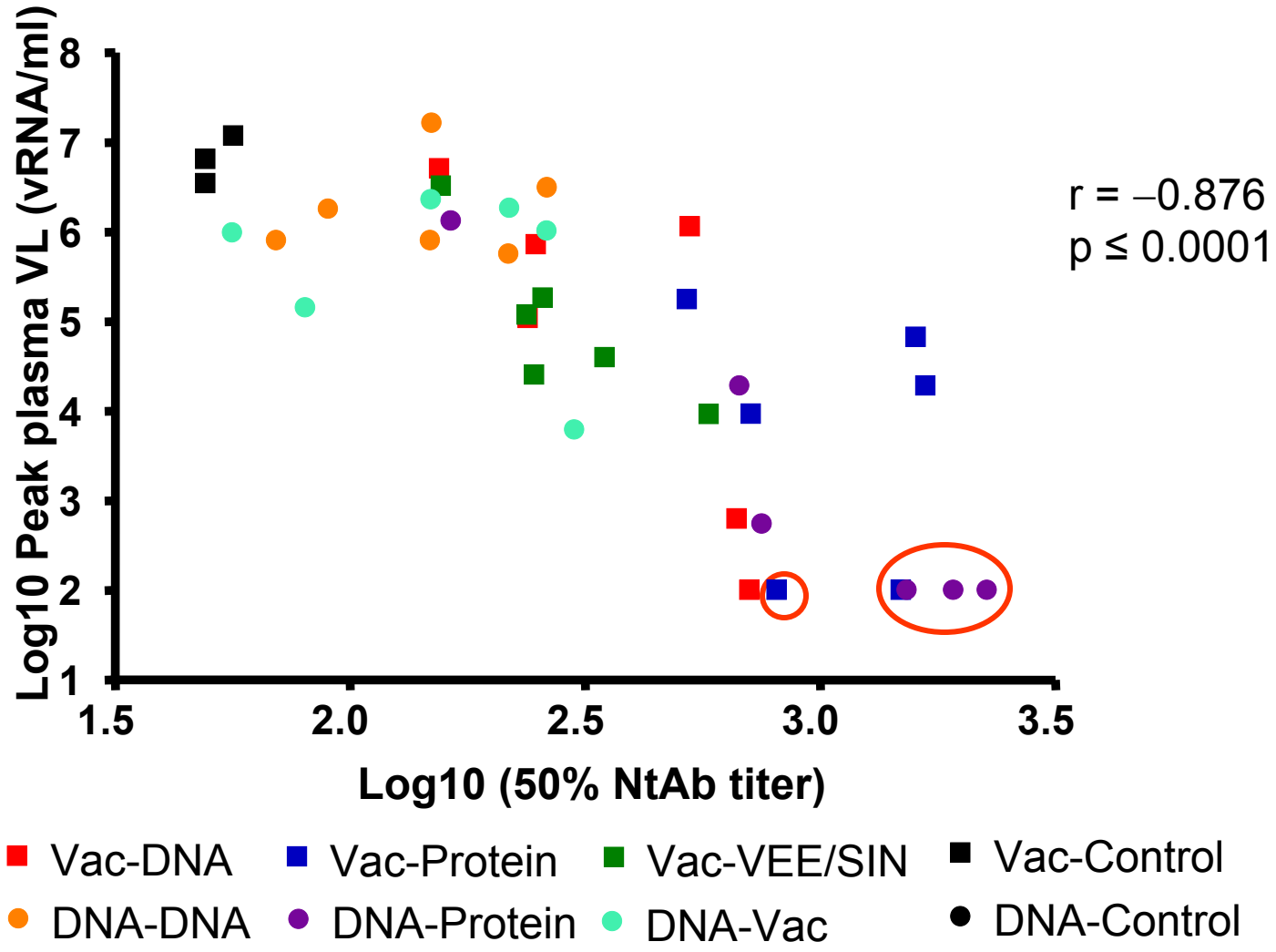
Animal	Plasma VL (all time)	PBMC VL (w6)
M02156	<10 vRNA eq./ml	< 0.3 copy/ug DNA
L02393	<10 vRNA eq./ml	< 0.3 copy/ug DNA

Animal	Plasma VL (all time)	PBMC VL (w12)
M03240	<10 vRNA eq./ml	< 0.3 copy/ug DNA
M02383	<10 vRNA eq./ml	< 0.3 copy/ug DNA
M02298	<10 vRNA eq./ml	< 0.3 copy/ug DNA

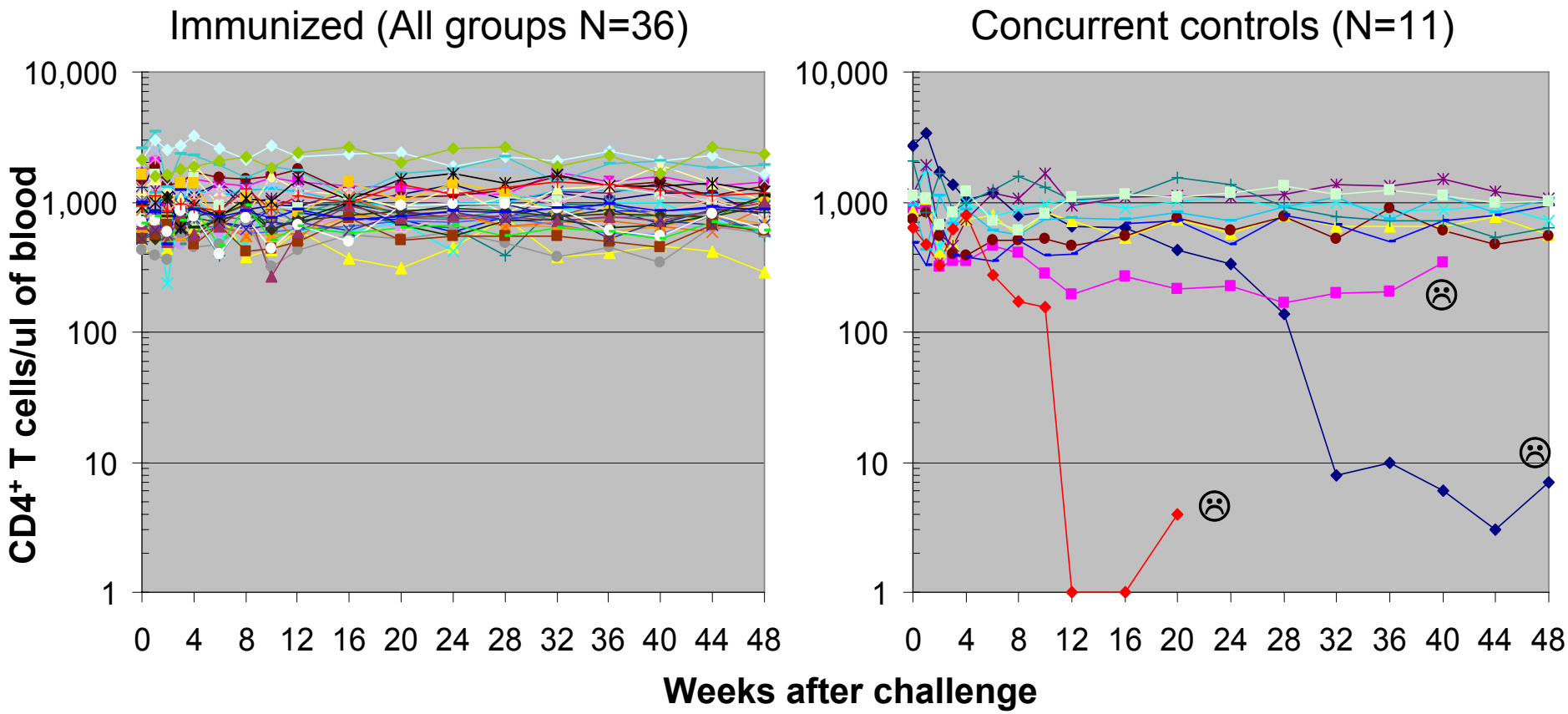
No anamnestic response after SHIV challenge in animals with “sterilizing immunity”



Neutralizing antibody titer on DOC inversely correlates with peak plasma viral load



Maintenance of peripheral blood CD4$^+$ T cell levels in immunized macaques challenged with SHIV$_{SF162\ P4}$



CD4$^+$ T cells	<200/ul	Normal	p ≤ 0.083
Immunized (all)	0	36	
Naïve (conc. & historic)	6	26	

Summary and Conclusions

- Vaccinia or DNA prime, followed by protein boost, generated significant better antibody responses than boosting with DNA or heterologous viral vector
- "Sterilizing immunity" was achieved against a high-dose intrarectal challenge by $SHIV_{SF162\ P4}$ in 5/12 (>40%) of animals that were primed with the vaccinia or DNA and boosted with protein immunogens
- Homologous neutralizing antibody titers (NtAb) on DOC correlated inversely with plasma viral load after challenge
- Heterologous NtAb were generated against a typical subtype B and two easy-to-neutralize subtype A & B primary isolates
- "Prime-boost" immunization protected animals against peripheral $CD4^+$ T-cell depletion induced by a relatively easy-to-neutralize, but moderately pathogenic R5 SHIV
- Protein vaccines may be an essential component in "prime-boost" strategies aimed at the generation of both cell-mediated and antibody responses

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