Balance of Cellular and Humoral Immunity Determines the Level of Protection by HIV Vaccines in Rhesus Macaque Models of HIV Infection

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Profectus BioSciences, Inc.

Profectus is a clinical-stage biotechnology company developing vaccines for Ebola/Marburg, and other biodefense targets, as well as HIV, HPV, HSV, HCV and Chikungunya.

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6411 Beckley Street
Baltimore, MD

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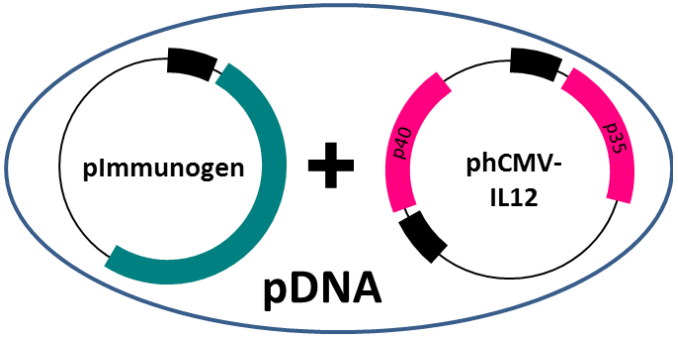
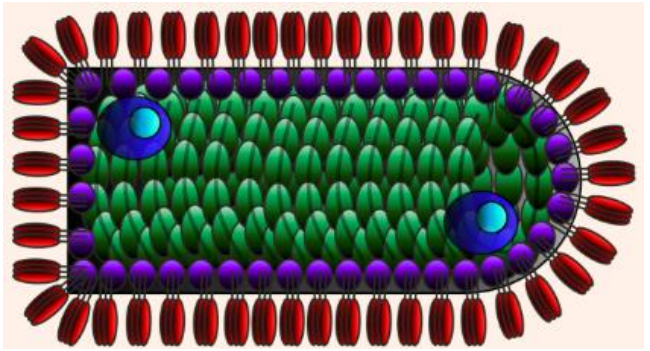
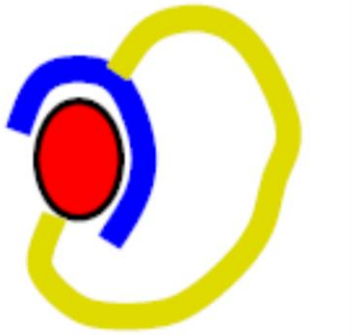
Technology to tailor the vaccine response to the target







Transition State Vaccines

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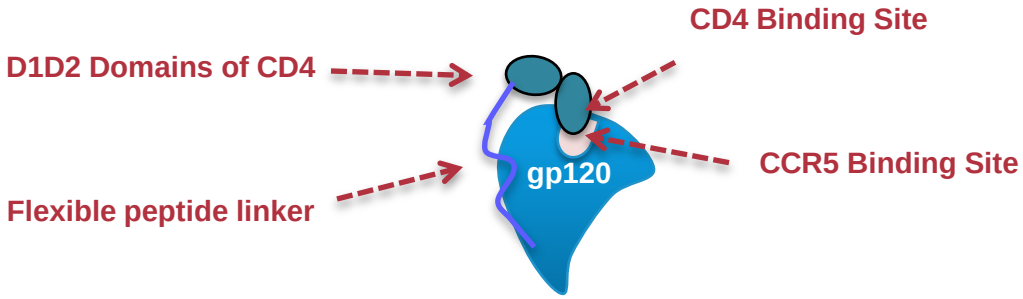
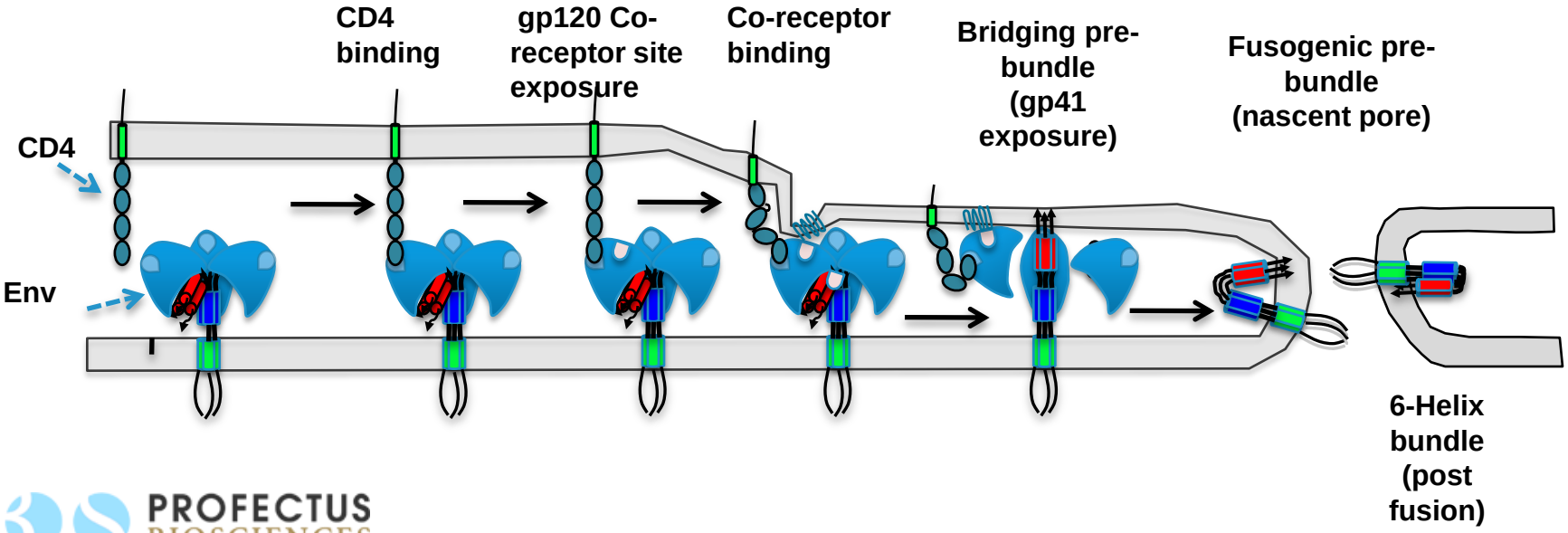
The FLSC Subunit Mimics an Early Conformational Change in the HIV-1 Env Trimer Induced During Viral Entry



Conformational Change in the HIV-1 Env Trimer During Viral Entry



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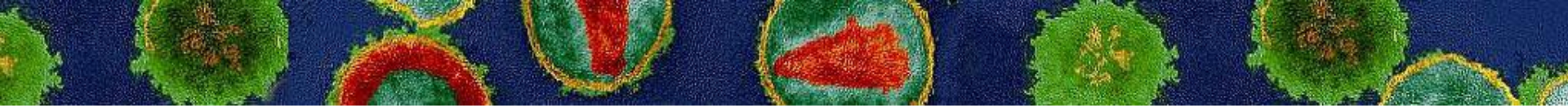
How the FLSC works?

- Chronic viruses hide their vulnerable parts
- Altering envelope structure (envelope-receptor)
  - changes how the antigen is presented to the immune system
  - enhances the immunogenicity of the conserved “vulnerable parts”
  - expands epitope diversity in Ab responses



Are transition state/CD4i epitopes ever immunoreactive during infection?

- Structural analyses and molecular models of soluble antigens were interpreted to indicate “No”…

- But-
  - Antibodies to transition state epitopes mediate potent ADCC activity against cell-bound virions and against chronically infected cells

    Guan, Lewis et al., PNAS 2013, Ferrari et al., J. Virol. 2012; Bonsignori et al., J. Virol. 2012; Veillette et al. J. Virol. 2014

  - A number of nonhuman primate studies link protection from infection with antibody specificities (including CD4i) and functions that lie outside the sphere of direct virion neutralization.

    Fouts et al, PNAS 2015; Barouch, Michael et al Nature 2012; Alpert et al Plos Pathogen 2012; Sun, Letvin et al, J Virol 2011; Xiao, Robert-Guroff et al J Virol 2010; Florese, Robert-Guroff et al, J Immunol 2009; Hidajat, Robert-Guroff et al J Virol 2009; DeVico et al, PNAS 2007, Hessel, Burton et al Nature 2007; Gomez-Roman, Robert-Guroff et al, JAIDS 2006; Banks et al, AIDS Res Hum Retroviruses 2002

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Envelope targets for antiviral Abs

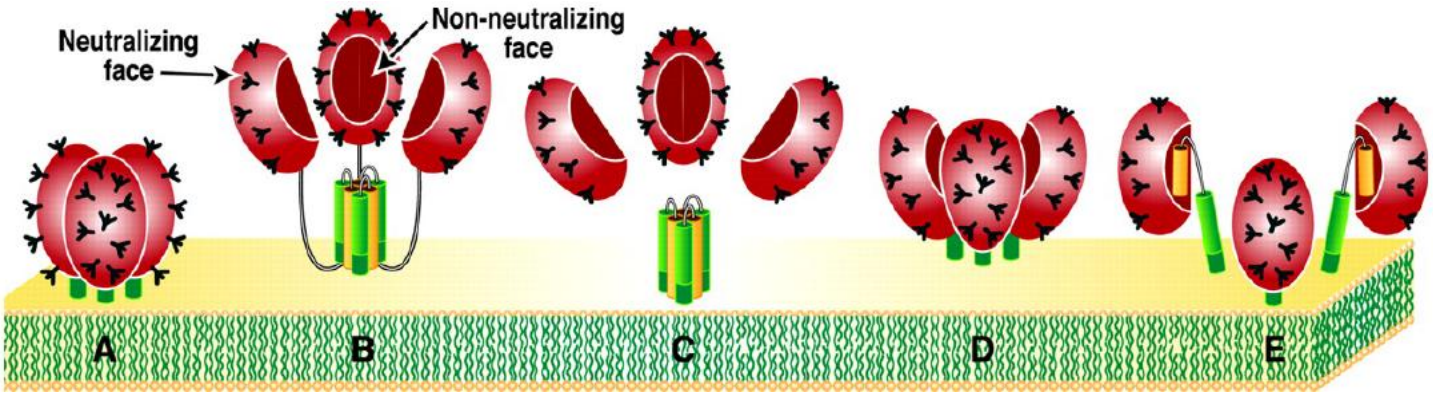

Figure 1. Potential forms of Env on the HIV-1 membrane. The gp120 moiety is shown in red with the outer neutralizing face in light shading and the inner non-neutralizing face in darker shading. Carbohydrate moieties are depicted as black tree-like structures. The gp41 transmembrane subunit contains N-terminal (yellow) and C-terminal (green) helical domains along with a membrane-proximal region (shown in dark green). Figure 1A: functional Env trimer; Figure 1B: uncleaved gp160 precursor (depicted here as a trimer; however, it may also exist as other oligomeric forms); Figure 1C: gp120 shedding; Figure 1D: an alternative trimer form exposing the non-neutralizing face of gp120; Figure 1E: gp120/gp41 monomers. The gp41 ectodomain is shown in the six-helix bundle configuration in Figure 1B and C. (Taken from ref. 31)

Moore, et al, 2006, ***JV,*** 80:2515

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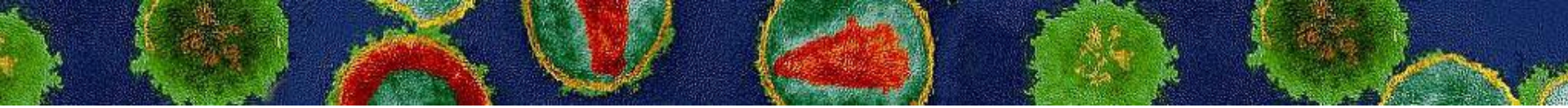

Evidence that FLSC can protect

Antibodies to CD4-induced sites in HIV gp120 correlate with the control of SHIV challenge in macaques vaccinated with subunit immunogens

Anthony DeVico*†, Timothy Fouts‡, George K. Lewis*, Robert C. Gallo*†, Karla Godfrey*, Manhattan Charurat*, Ilia Harris‡, Lindsey Galmin§, and Ranajit Pal§

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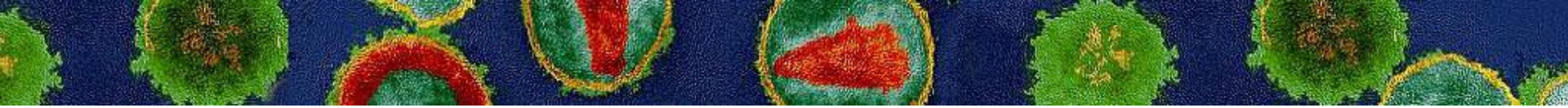
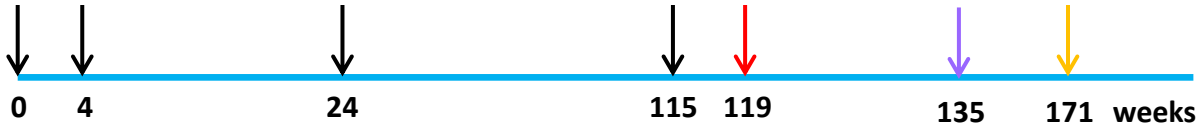


Group	#	Immunogen	Dose	Adjuvant	Dose
1	4	Gp120(BaL)	300 ug	QS21	100 ug
2	4	Chemically crosslinked gp120(BaL)-shCD4	300 ug	QS21	100 ug
3	4	rhFLSC	300 ug	QS21	100 ug
4	4	Soluble human CD4 (D1-D4)	300 ug	QS21	100 ug
5	4	Naive			



= i.m. immunization (subunit/adjuvant)

= single i.r. challenge, 600 $TCID_{50}$ $SHIV_{162P3}$

= stop tracking viral load

= sacrifice

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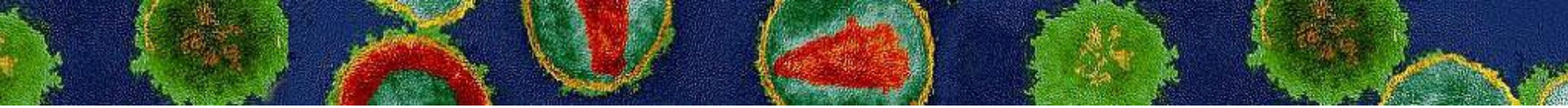
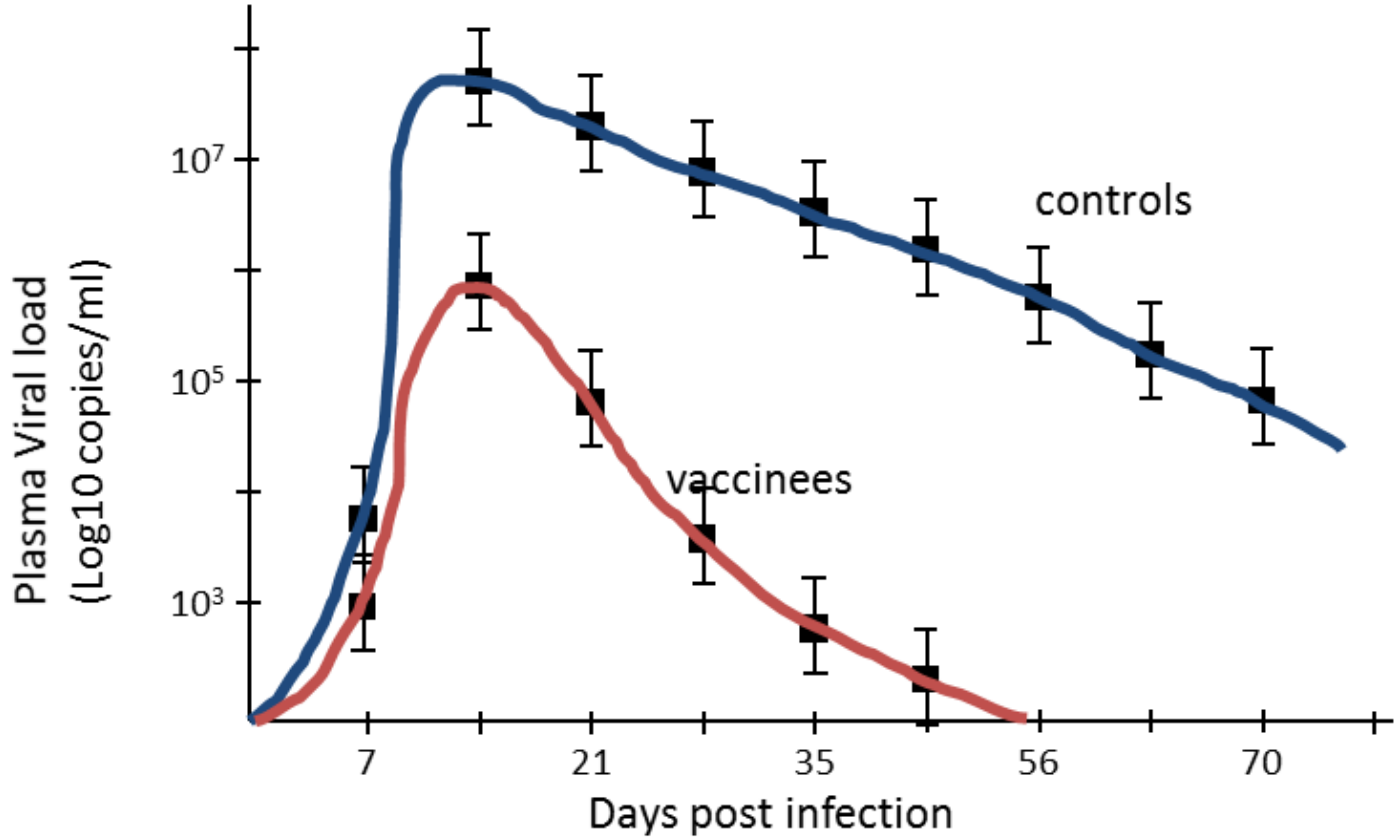


Vaccine efficacy in high dose challenge model



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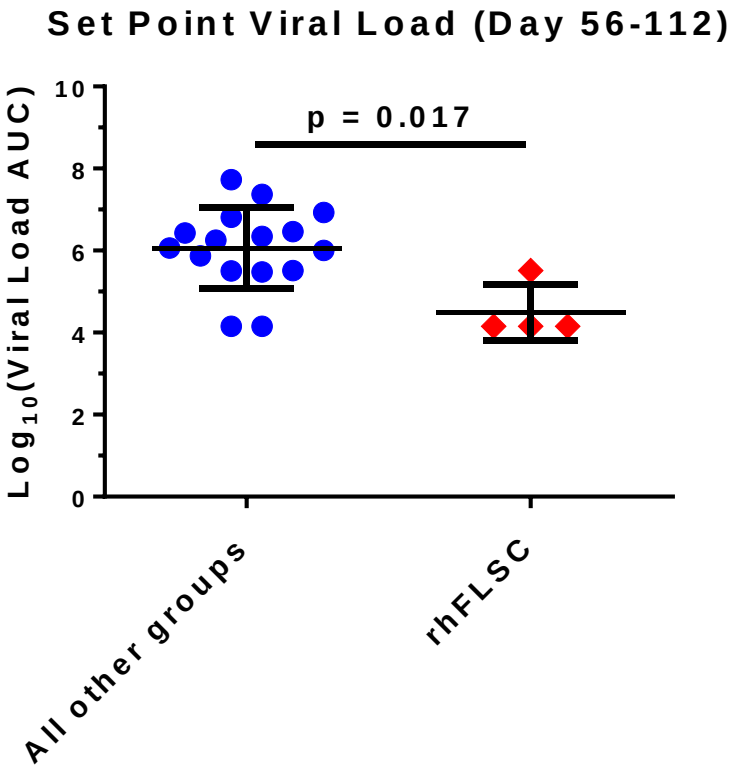
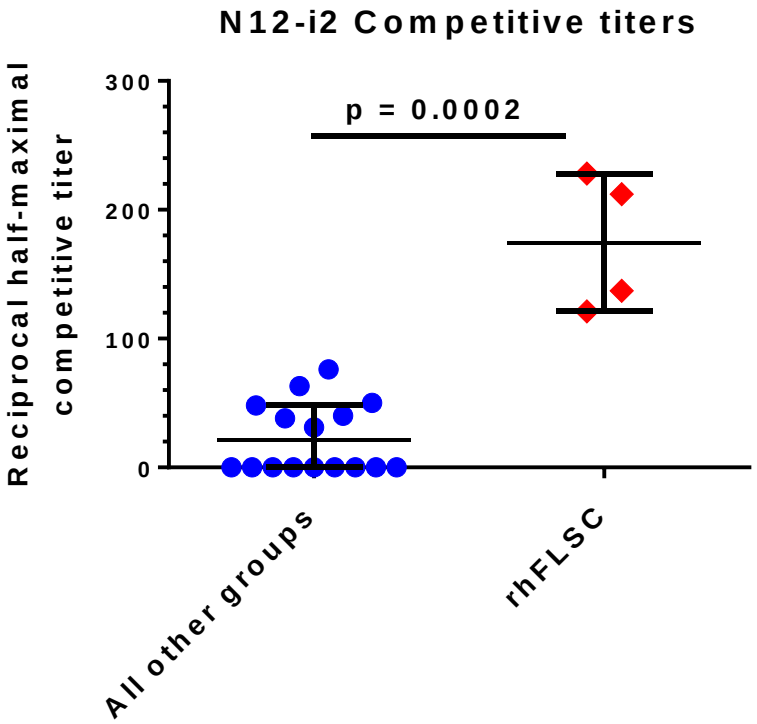
Identified relationship between lower viral set point and higher CD4i Ab titers





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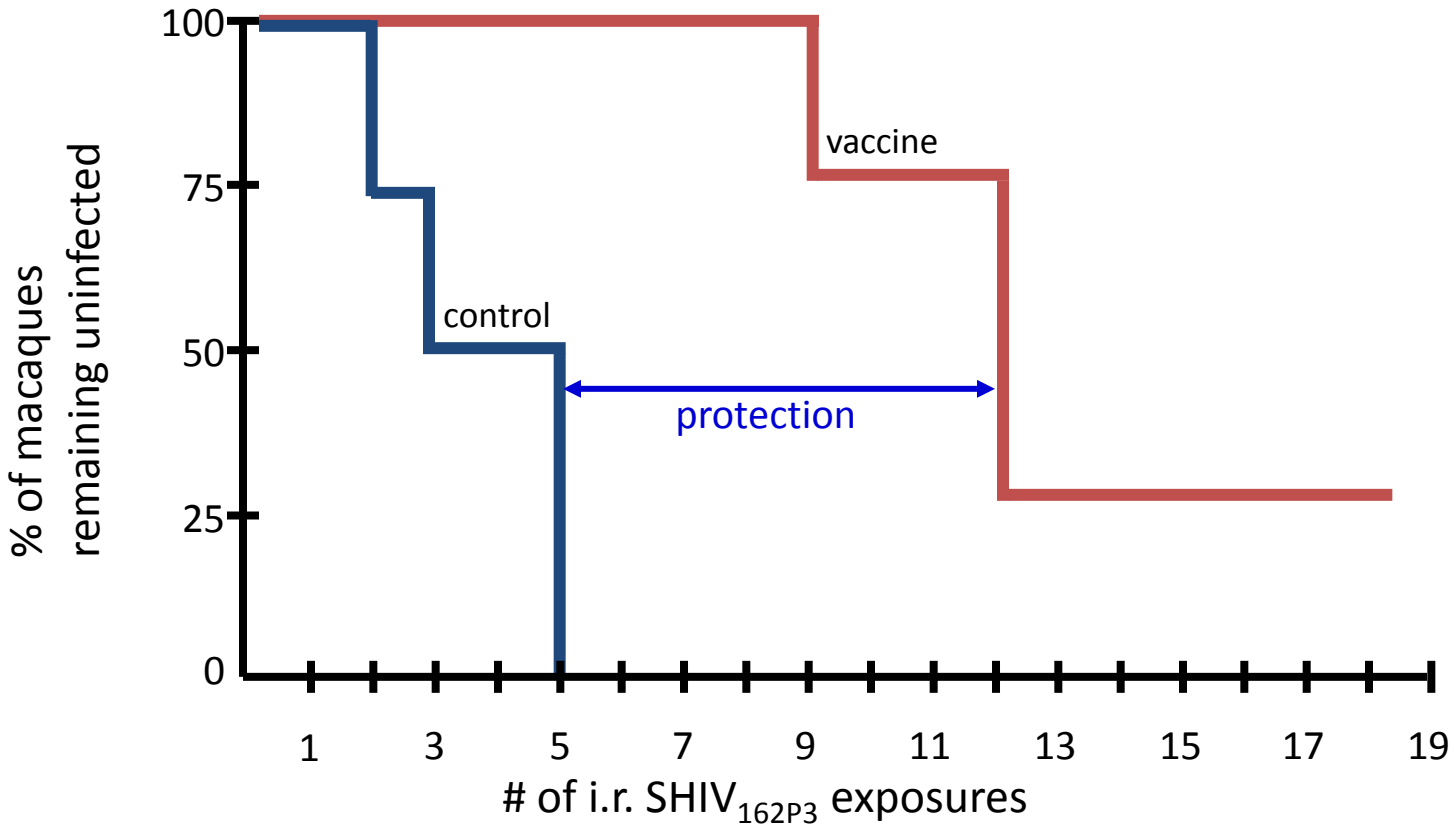
Survival analysis using the repeat exposure macaque model.



Adapted from Kim et al., J. Med. Primatology 35:210-216 (2006).

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Study 1 Design (powered to detect >82% protection i.e. “slam dunk”)

Grp	N	Immunogen	Adjuvant
1	6	Naïve	Naïve
2	6	RhFLSC (300 ug)	RC529-SE (50 ug)
3	6	Gp120 (300 ug)	RC529-SE (50 ug)
4	6	RhFLSC (300 ug)	Iscomatrix (100 ug)
5	6	Gp120 (300 ug)	Iscomatrix (100 ug)

RC529: Squalene, glycerol, phosphatidyl choline and synthetic monophosphoryl lipid A emulsion

Iscomatrix: Saponin-ISCOM

↓ = i.m. immunization

↓ = weekly i.r. challenge, 50 $TCID_{50}$ $SHIV_{162P3}$

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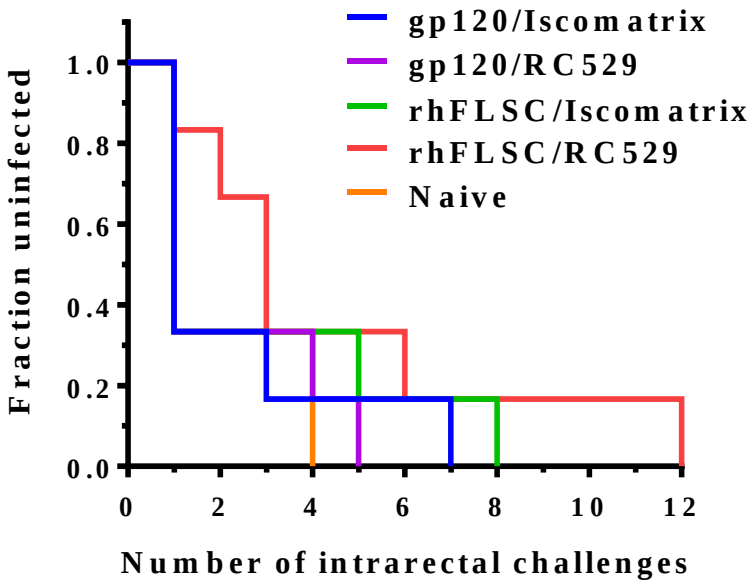
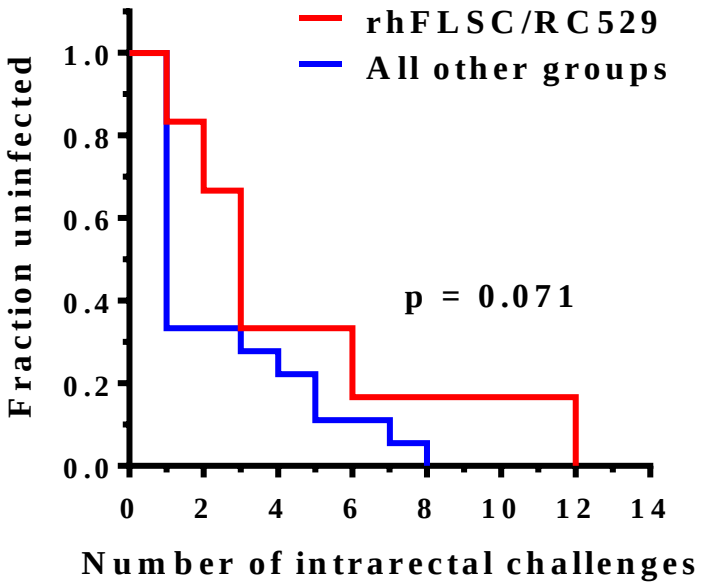
One simple formulation, rhFLSC/RC529, trended towards better protection, but not a “slam dunk”

Challenge outcome all groups



Challenge outcome rhFLSC/RC529 group vs all other groups



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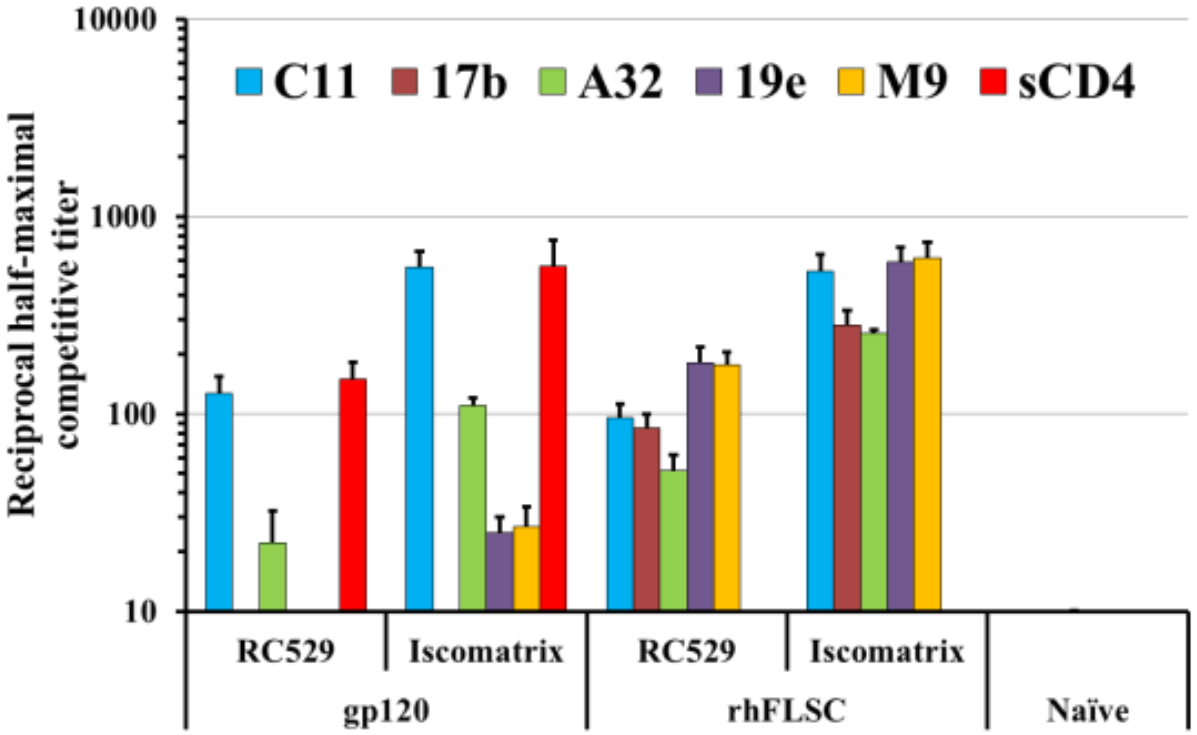
Immunization with rhFLSC induces antibodies to CD4i epitopes



CD4i MAbs = 17b, A32, 19e, M9 and N12-i2 (next slide)

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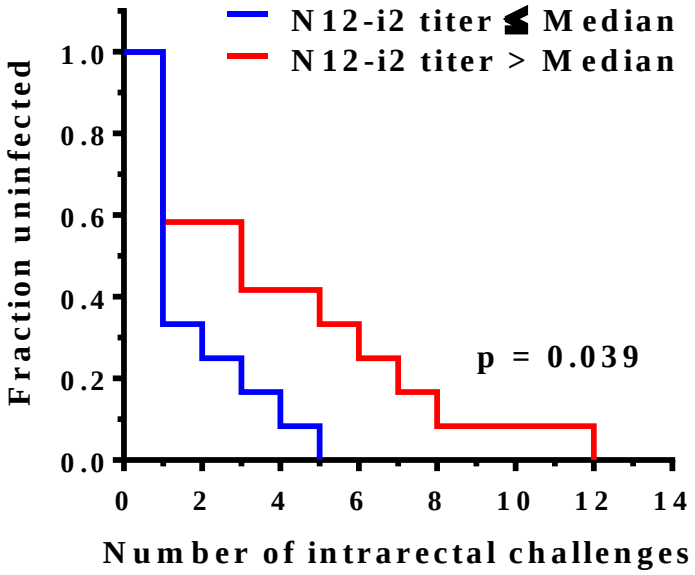
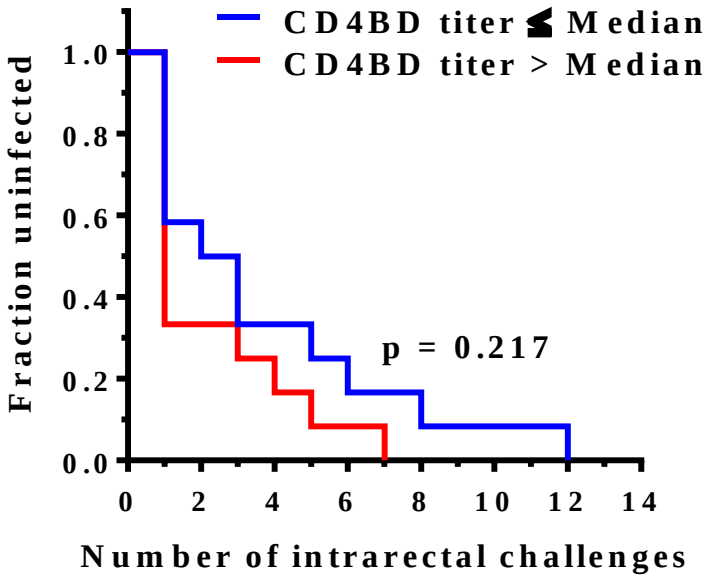
Animals with higher anti-CD4i antibody titers resisted infection…..





Meta analysis of all 24 vaccinated animals where 100% of naïve animals became infected within four challenges

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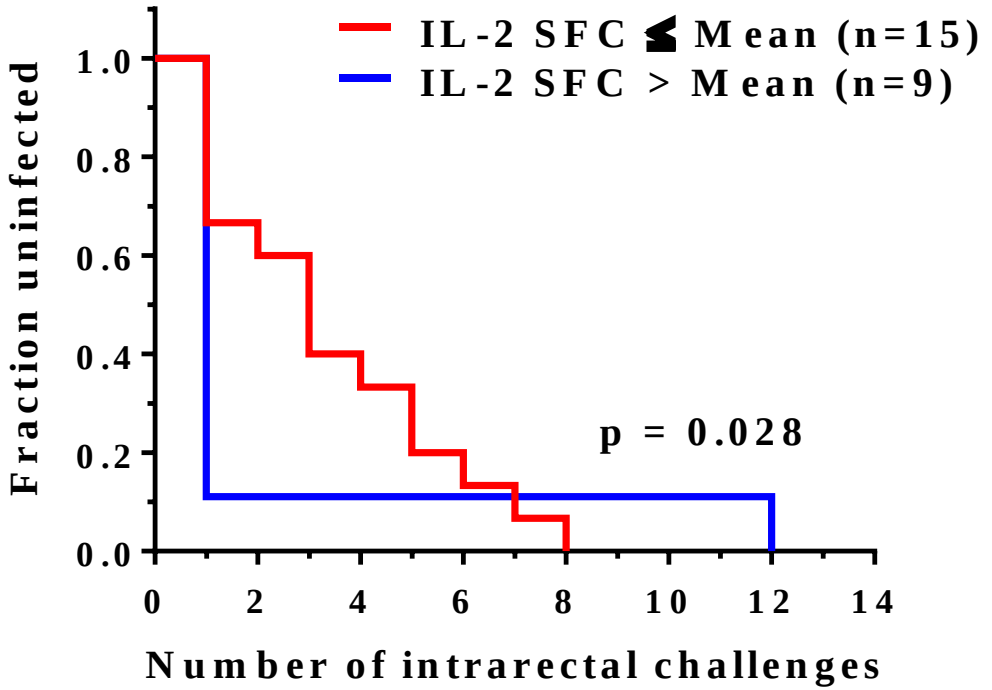
But protection was lost if there were too many T cells…



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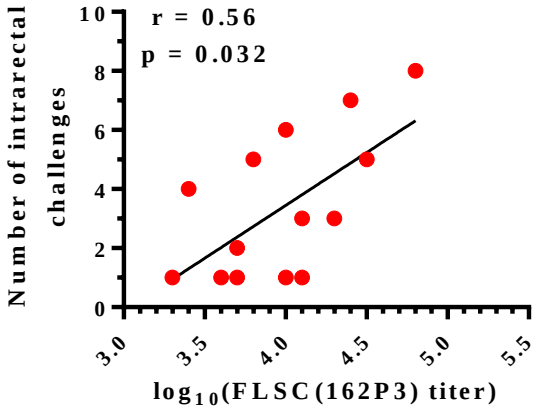
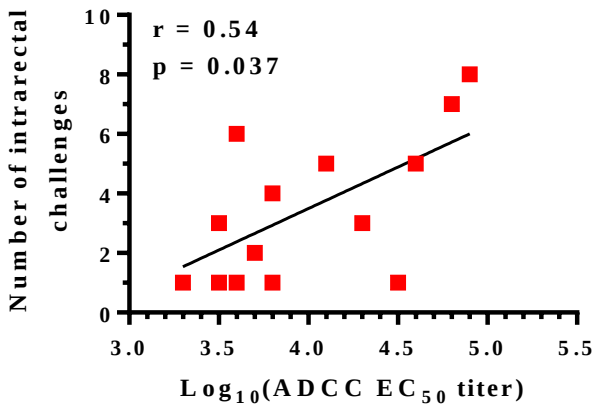
Removing animals with high T cell responses reveals that protection tracks with….

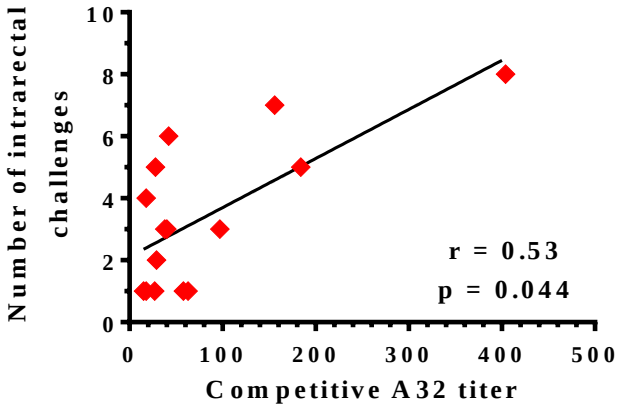
FLSC titer



ADCC



Competitive A32 titer



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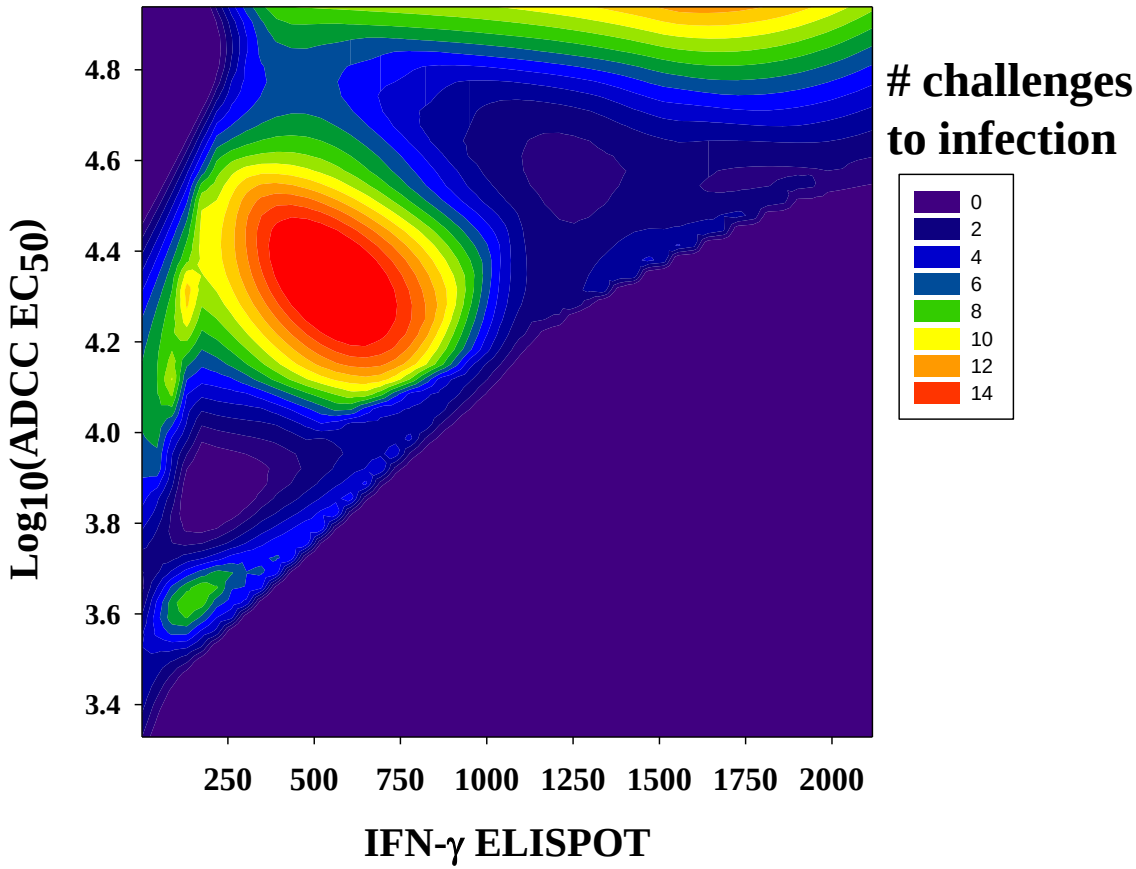
Suggesting a relationship between ADCC, IFN-γ Elispots, and protection: A zone of immune balance



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Study 2: Different Study, same story

Grp	N	Immunogen	Adjuvant
1	6	rhFLSC (1.2 mg)	GPI-0100 (500 ug) + ODN2006 (200 ug)
2	6	rhFLSC (1.2 mg) + Tat toxoid (100 ug)	GPI-0100 (500 ug) + ODN2006 (200 ug)
3	6	None	GPI-0100 (500 ug) + ODN2006 (200 ug)

↓ = i.m. immunization

↓ = wks 45-53, i.r. challenge 50 $TCID_{50}$ $SHIV_{162P3}$

↓ = wks 54-56, i.r. challenge 100 $TCID_{50}$ $SHIV_{162P3}$

↓ = wk 59, i.r. challenge 200 $TCID_{50}$ $SHIV_{162P3}$

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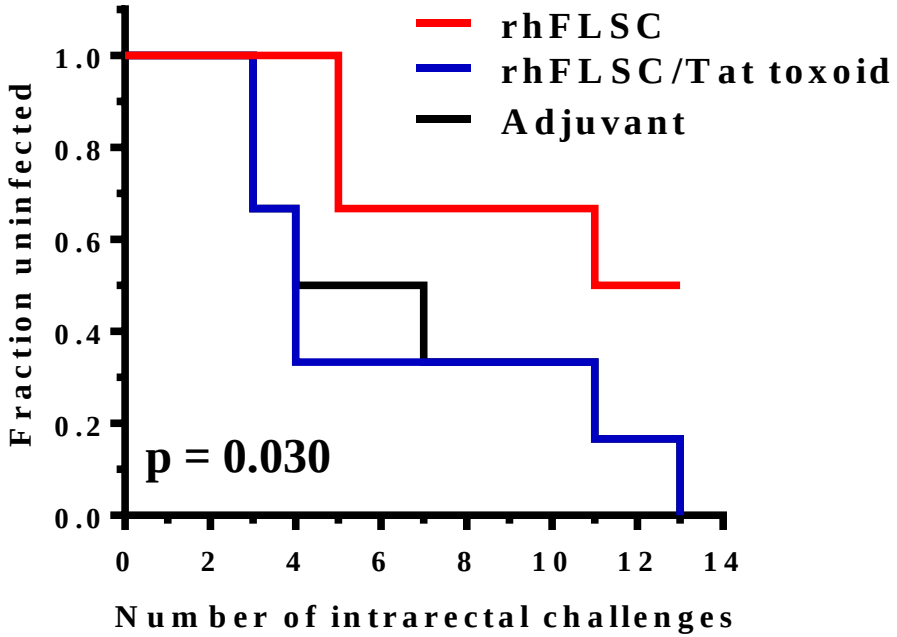
Key differences from 1st study

- included Tat in one of the immunogen formulations
- used higher rhFLSC vaccine dose (1200 ug)
- used a different adjuvant (GPI-0100)
- employed multiple dosing throughout the challenge phase to prolong durability
- increased the challenge dose over time

Addition of Tat toxoid eliminated protection conferred by rhFLSC



Kaplan-Meier Log Rank p = 0.030

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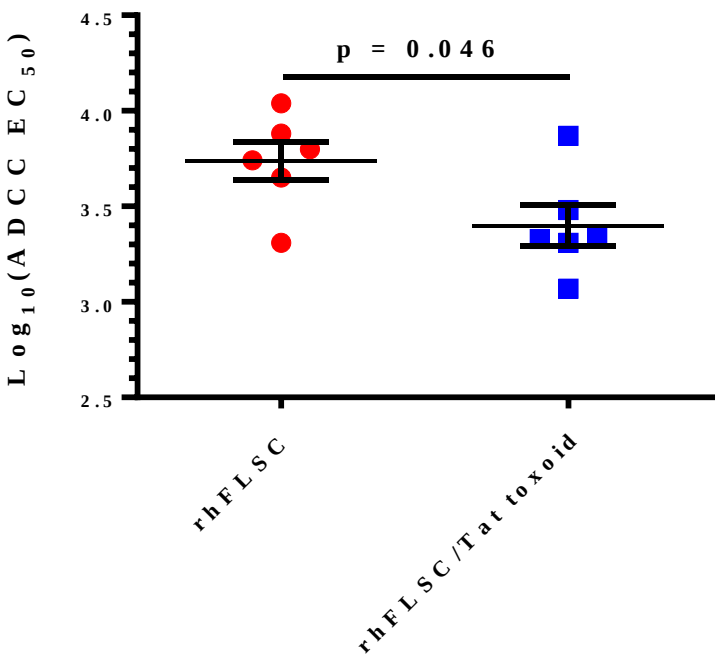
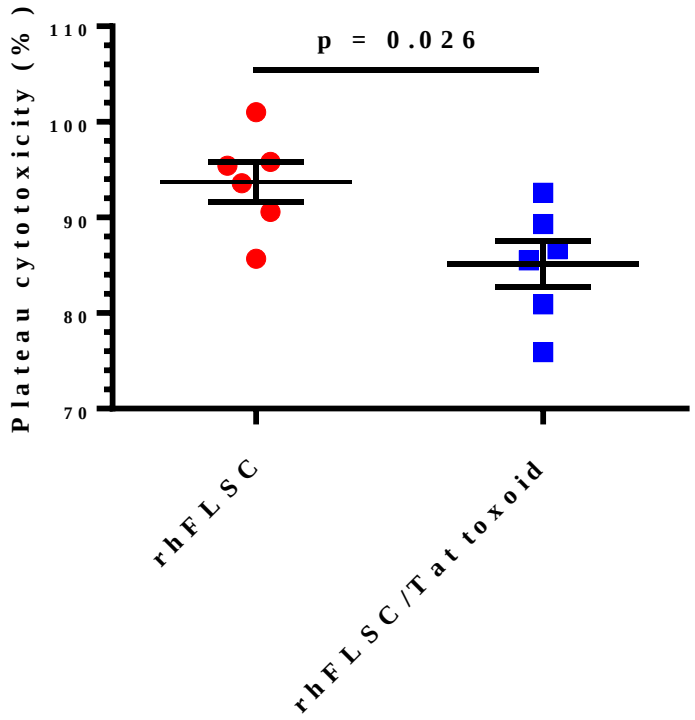
ADCC highest in rhFLSC (protected) group





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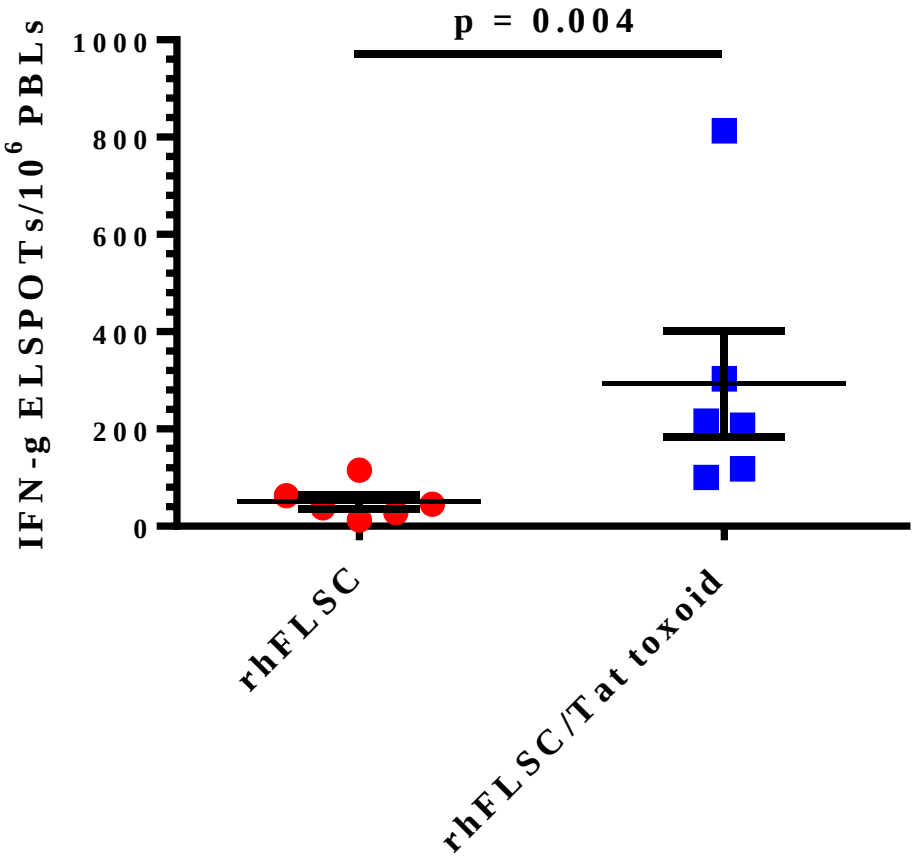
Tat toxoid enhanced T cell responses



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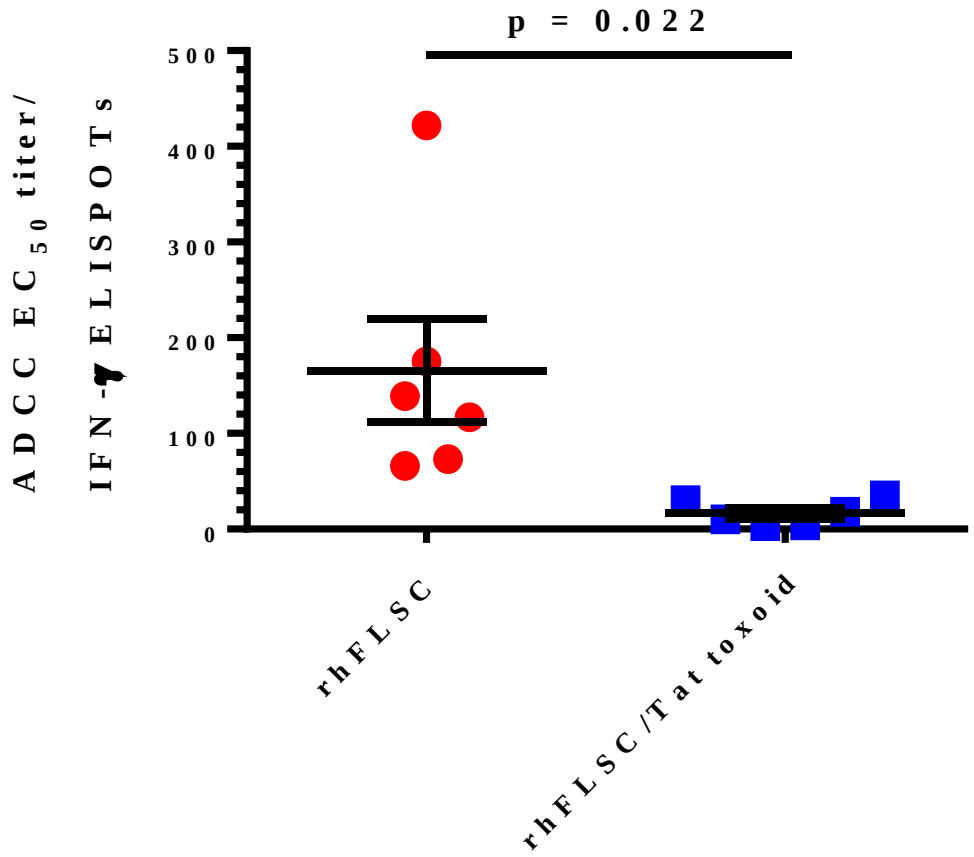
Immune balance in protected group



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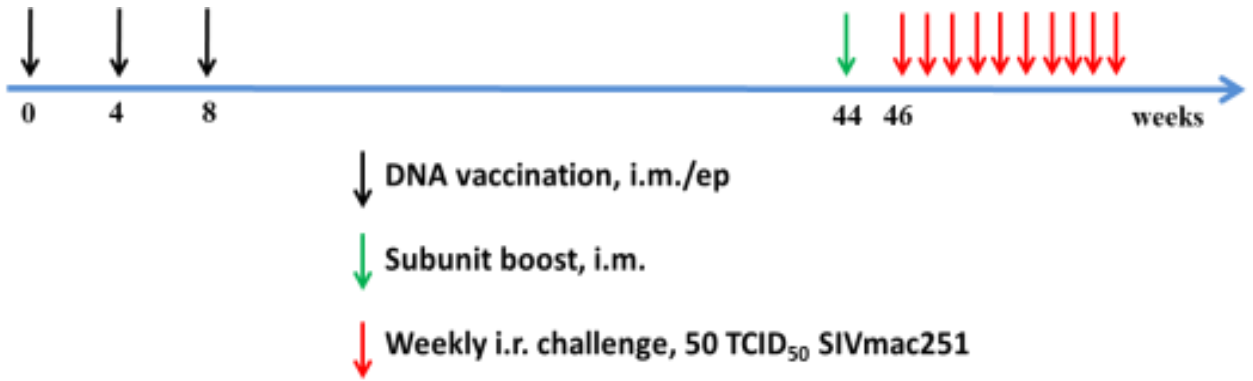
Study 3: Different model, same story (powered to detect 75% efficacy)

Grp	N	SIVsmE543 DNA Vaccine	Genetic Adjuvant	Subunit/Al(OH)$_3$
1	8	Empty	None	None
2		rhFLSC + gag/pol	Empty	rhFLSC(CCG7V)
3			LTA1	
4			Rhesus IL-12	
5			LTA1 + rhesus IL-12	



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Key differences from 1st and 2nd study

- uses SIV_{mac251} challenge (neutralization resistant batch with high quasi-species diversity (2.1%))
- Antigens derived from SIV_{smE543} (gag/pol), and SIV_{smE660} (env)
- DNA prime/protein boost protocol
- Adds IL-12 and heat labile enterotoxin (LTA1) as genetic adjuvants

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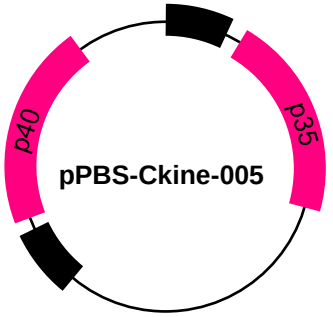
GENEVAX® IL-12

huIL-12

- Naturally produced by MΦ and DC
- Drives differentiation of Th1 cells that support $CD8^+$ CMI responses
- Drives ADCC



GeneVax® IL-12

- Induces $CD4^+$ and $CD8^+$ T_{CM}
  - ✓ Long lived
  - ✓ High expansion potential
  - ✓ PD-1, CTLA-4, and LAG-3 not up regulated – resistant to anergy
  - ✓ CD28 and CD127 up regulated – block tolerance and apoptosis

GeneVax IL-12® clinical status

- Safe and well tolerated
- Adjuvant active in humans

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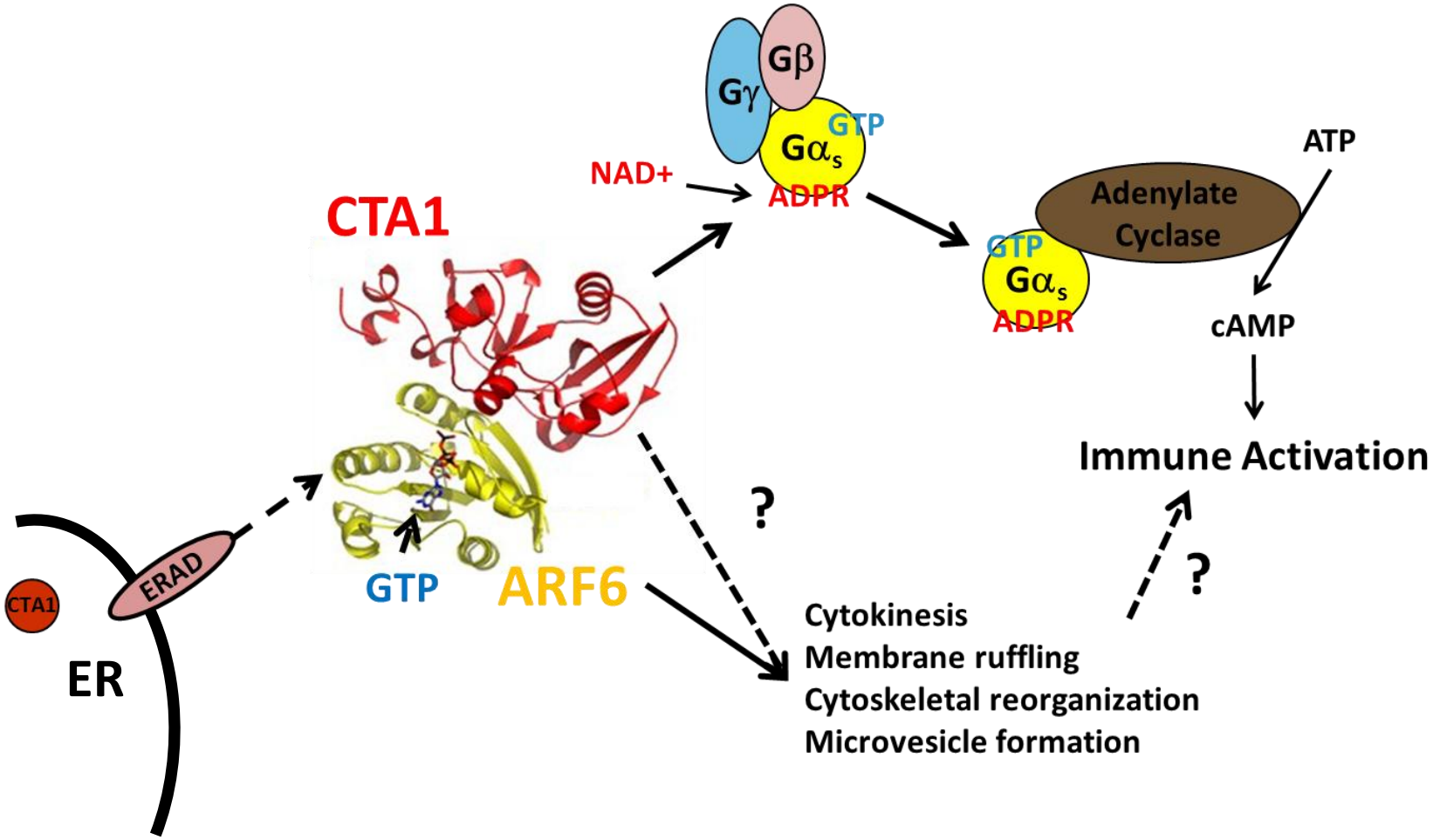
LTA1/CTA1 Mechanism of Action



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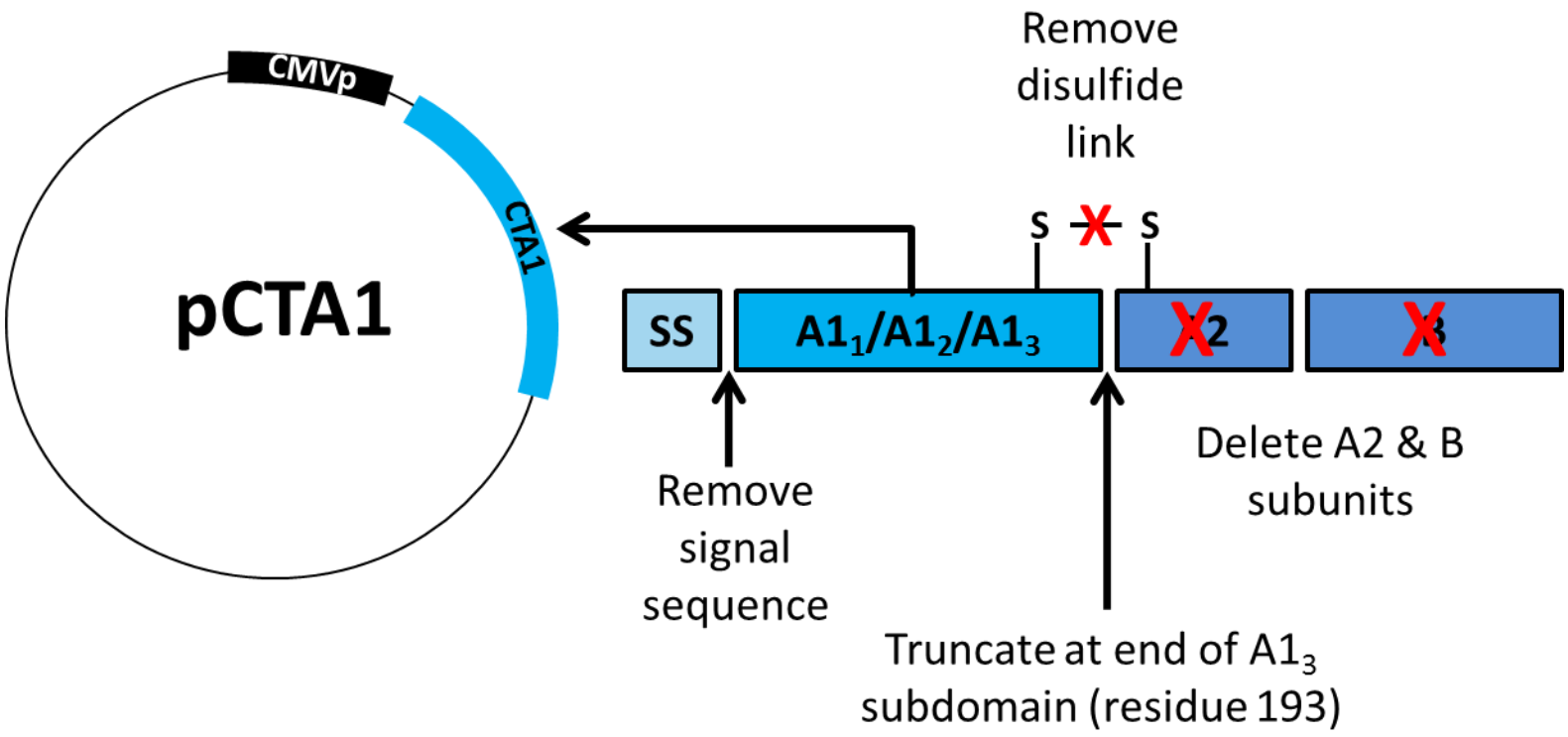
Profectus CTA1 Genetic Adjuvant



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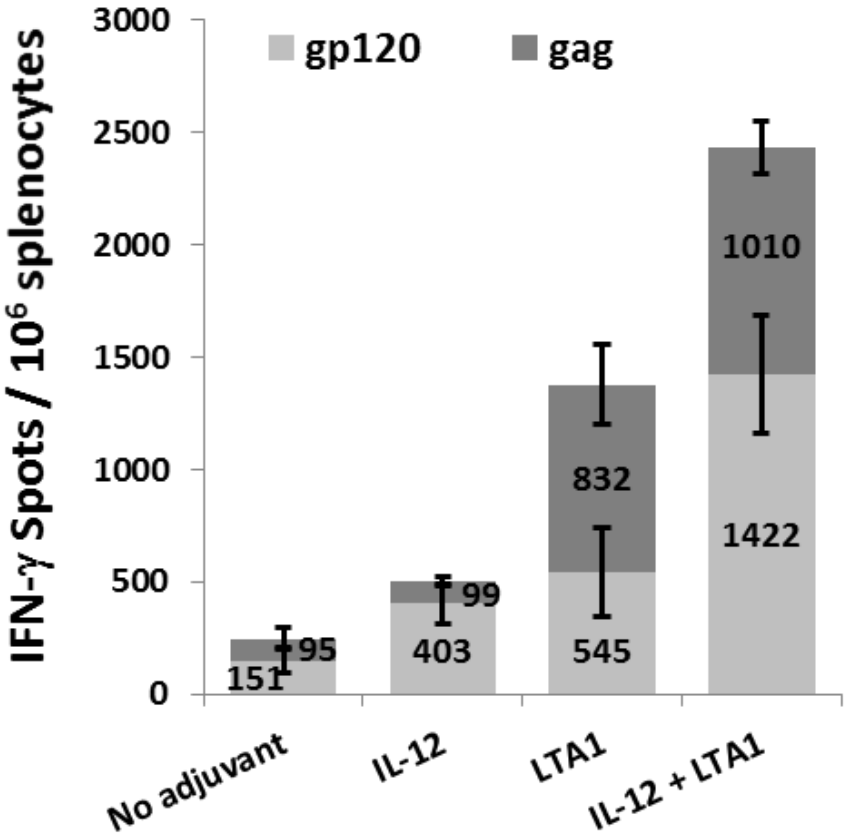
Additive adjuvant effects between IL-12 & LTA1



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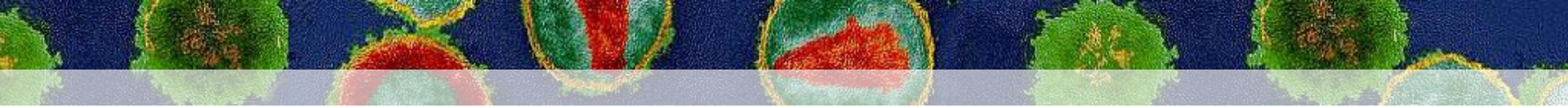

RESEARCH PAPER

Human Vaccines & Immunotherapeutics 11:9, 2228–2240; September 2015; Published with license by Taylor and Francis Group, LLC

The catalytic A1 domains of cholera toxin and heat-labile enterotoxin are potent DNA adjuvants that evoke mixed Th1/Th17 cellular immune responses

Kenneth Bagley[1,*], Rong Xu[2], Ayuko Ota-Setlik[2], Michael Egan[2], Jennifer Schwartz[1], and Timothy Fouts[1]

[1]Profectus Biosciences; Baltimore, MD USA; [2]Profectus Biosciences Tarrytown, NY USA

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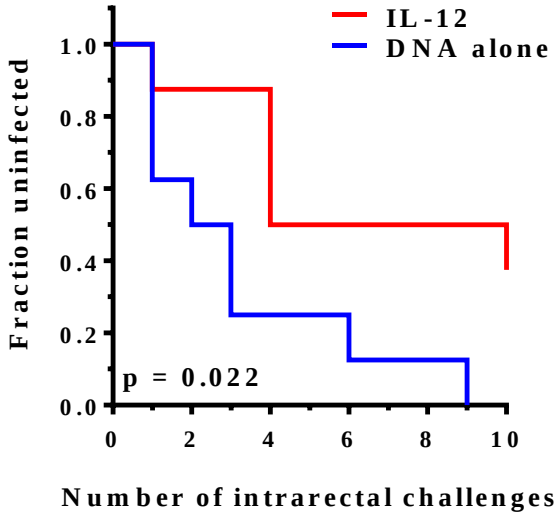
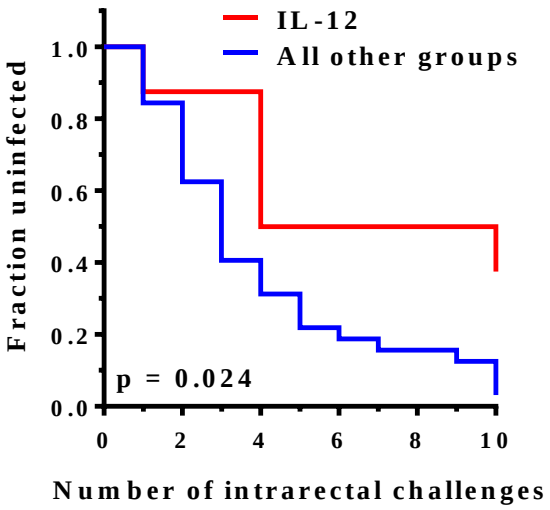
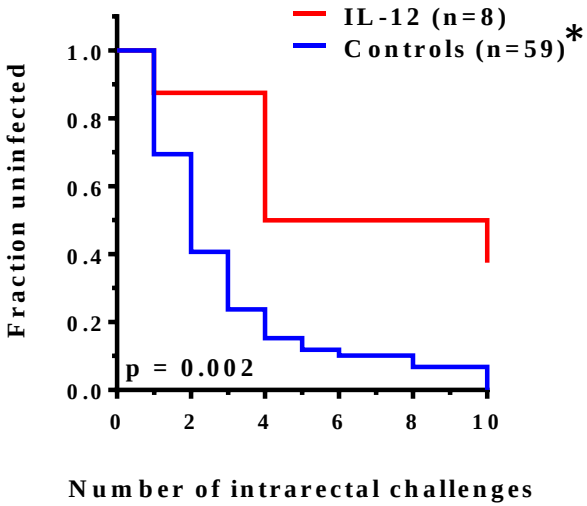
Inclusion of IL-12 in the prime yields 76% efficacy







* The addition of 51 historical controls of naïve macaques that received the same SIVmac251 challenge virus stock, at the same dose, administered by the same technical staff in the same animal facility within 1 year of the start of the challenge phase of this study

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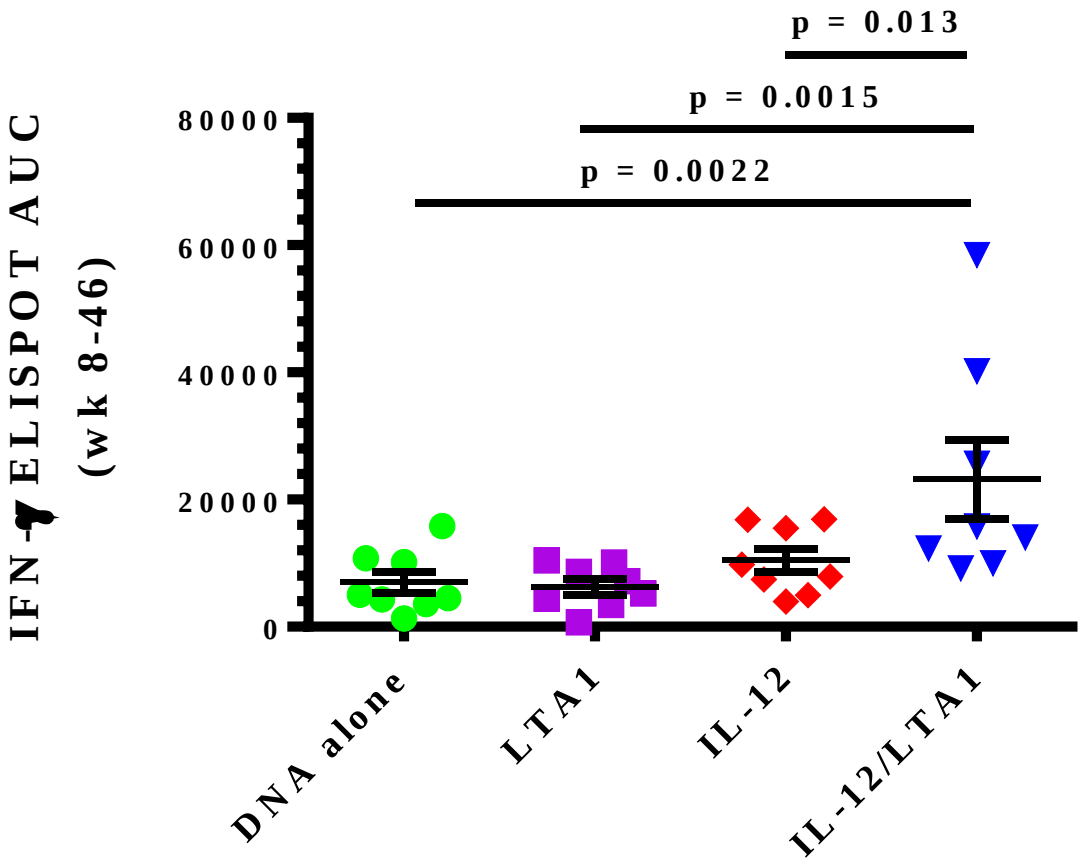
LTA1 and IL-12 combination enhances T cell responses



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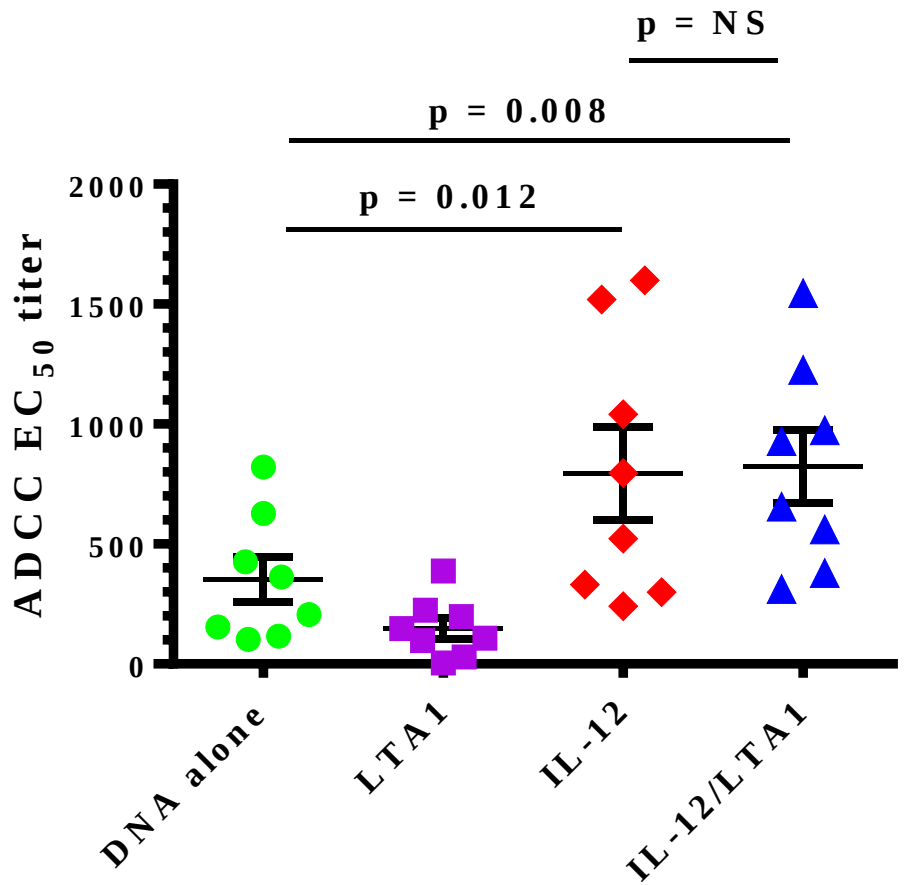
Inclusion of IL-12 during DNA prime enhances ADCC



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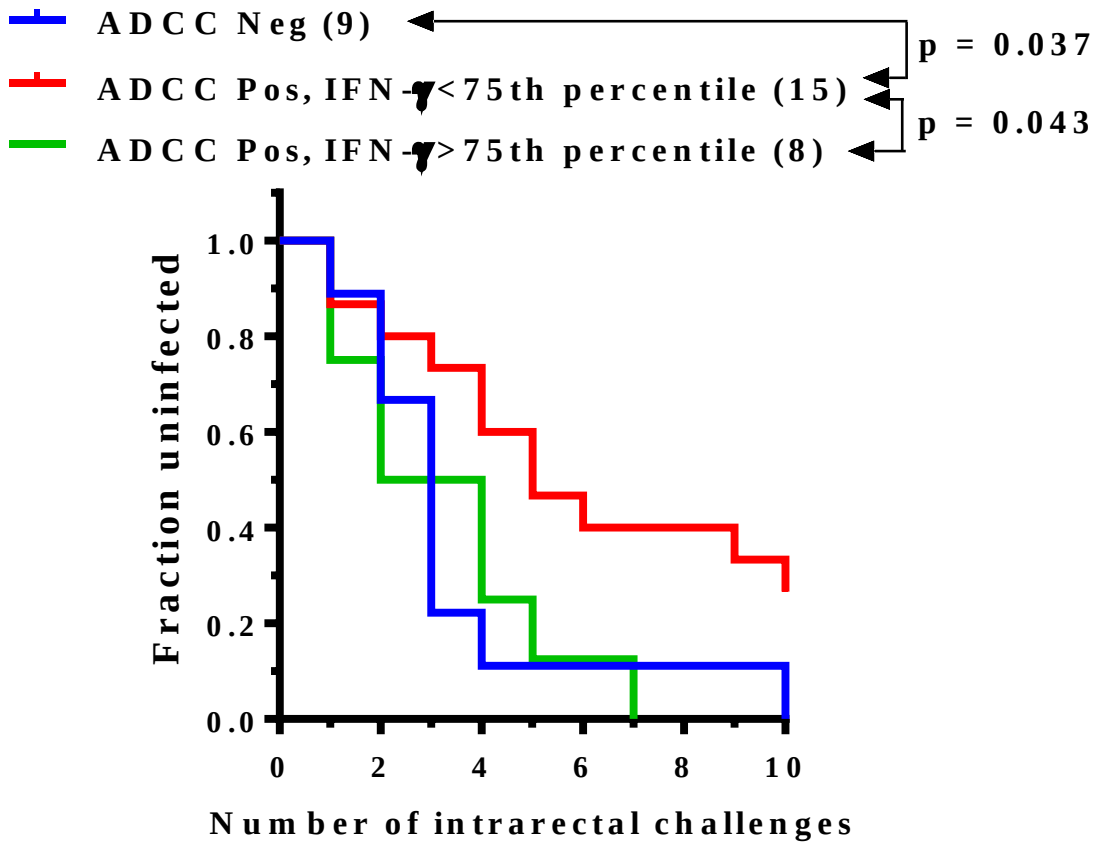
But…you need immune balance



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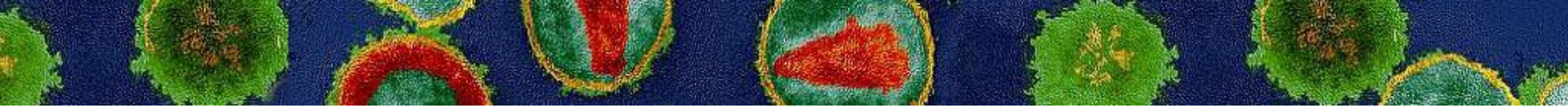
Does the FLSC induce protection?

Yes, if you induce a CD4i response above a threshold, ADCC, and without an overabundance of T cells.

(immune balance)



Balance of cellular and humoral immunity determines the level of protection by HIV vaccines in rhesus macaque models of HIV infection

Timothy R. Fouts[a,1], Kenneth Bagley[a], Ilia J. Prado[a], Kathryn L. Bobb[a], Jennifer A. Schwartz[a], Rong Xu[b], Robert J. Zagursky[c], Michael A. Egan[b], John H. Eldridge[b], Celia C. LaBranche[d], David C. Montefiori[d], Hélène Le Buanec[e], Daniel Zagury[f], Ranajit Pal[g], George N. Pavlakis[h], Barbara K. Felber[h], Genoveffa Franchini[i], Shari Gordon[i], Monica Vaccari[i], George K. Lewis[j], Anthony L. DeVico[j], and Robert C. Gallo[j,1]

[a]Profectus Biosciences, Inc., Baltimore, MD 21224; [b]Profectus Biosciences, Inc., Tarrytown, NY 10591; [c]Center for Infectious Disease and Immunology Research Institute, Rochester General Hospital, Rochester, NY 14621; [d]Duke Human Vaccine Institute, Duke University Medical Center, Durham, NC 27710; [e]INSERM U976, F-75475 Paris, France; [f]NEOVACS S.A., 75014 Paris, France; [g]Advanced Bioscience Laboratories, Rockville, MD 20850; [h]Human Retrovirus Section, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702-1201; [i]Virus Tumor Biology Section, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892-5065; and [j]Institute for Human Virology, Baltimore, MD 21201

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Our goal…..

Improve potency & durability of Fc-mediated protection with the right balance of supportive CD4+ T cells

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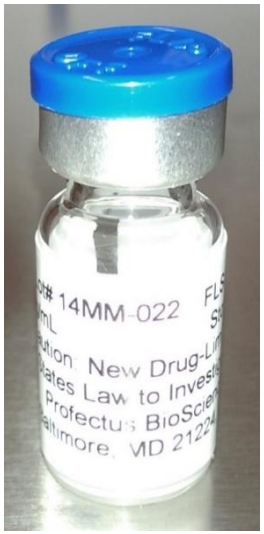
Preclinical Development of IHV01



- HEK-293 Master Cell Bank created and released
- FLSC Drug Substance –
  - cGMP manufactured and released, yield ~ 1g/L
  - 12 month DS stability at -20^{o}C
- Label Drug Product released (Alum formulated FLSC)
  - AlPO4 formulation with >95% adsorption
  - 9 month DP stability at 5^{o}C
- Standard Rabbit Toxicology – complete
- NHP Immunotoxicology Study (FDA requested) – complete
  - No evidence of deleterious auto-anti-CD4 responses induced by FLSC or rhFLSC immunization

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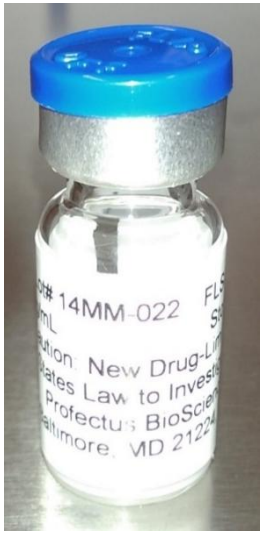
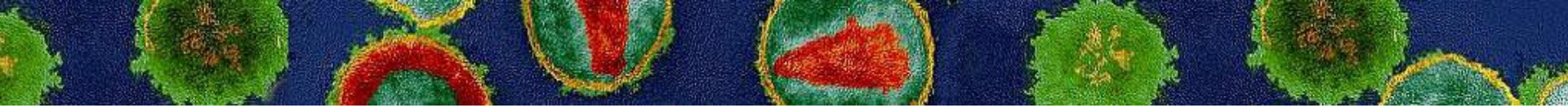
Released IHV01



- ~ 3400 vials at 300 µg/mL of formulated drug product
  - 800 labeled, 1888 unlabeled in cGMP storage at Sherpa Clinical Packaging
  - 700 set aside for release testing, clinical retains, and stability at Catalent RTP
- 125 g of unformulated FLSC [Clinical Grade] is available for future studies



Planned Phase 1 Clinical Testing of IHV01

Group	Admin Route	N** Vaccine / Control	Vaccine Dose	Vaccination Schedule in Months (Days)			
				0	1(28)	2(56)	6(168)
1	IM	**15**	**0.25 mL (75 µg)**	FLSC AIP04	FLSC AIP04	FLSC AIP04	FLSC AIP04
		5	**0.25 mL**	saline	saline	saline	saline
2	IM	**15**	**0.5 ml (150 µg)**	FLSC AIP04	FLSC AIPO4	FLSC AIPO4	FLSC AIP04
		5	**0.5 mL**	saline	saline	saline	saline
3	IM	**15**	**1.0 mL (300 µg)**	FLSC AIP04	FLSC AIP04	FLSC AIP04	FLSC AIP04
		5	**1.0 mL**	saline	saline	saline	saline
TOTALS		**60**					

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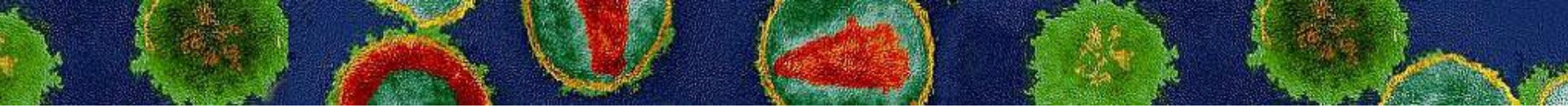

Planned Phase 1 Clinical Testing of IHV01

Primary endpoint: Safety* and Tolerability
Secondary endpoint: Immunogenicity

Immune Response Measure	Measures of an immunogenic dose of FLSC (3 or 6 months post vaccination)
Serum antibody titer to FLSC and gp120 (BaL) via ELISA	>80% of vaccinees exhibiting geometric mean titers to FLSC that are 3 SD above background, and/or
Competitive serum titers to CD4i and other epitopes (A32, 17b, 19e, N12-i2, and others) via ELISA	>60% of vaccine responders exhibiting competitive binding titers to CD4i epitopes that are 3 SD above background

*A trial is ongoing to measure fluctuations in CD4+ T cell counts over time in healthy volunteers

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Exploratory clinical trials to learn how to reach this goal

Subunit

+ New Adjuvants

+ DNA prime w/
Genetic Adjuvants
(IL-12 or IL-15)

+ rVSV (or other virus) prime

PROFECTUS BIOSCIENCES

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