

Adenovector-Based Malaria Vaccine Development

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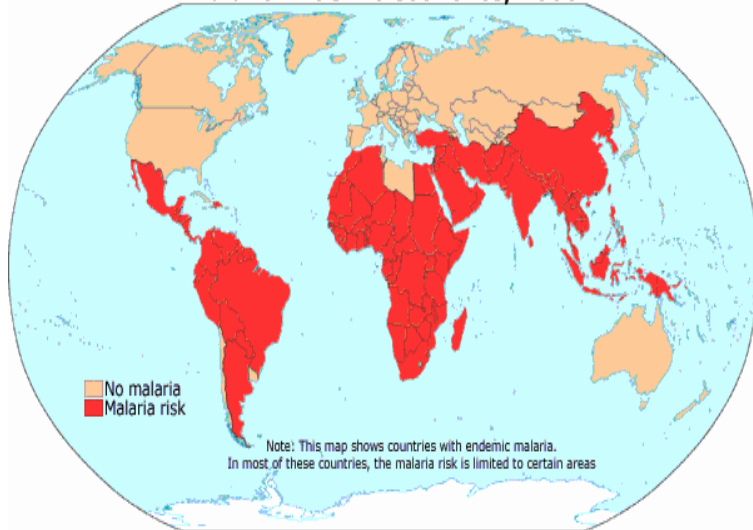


Malaria: World Health Problem

- >1 million deaths/year
- No licensed malaria vaccine
- Drug resistance spreading



Malaria Endemic Countries, 2000



Product Opportunities

- Military vaccine
- Travelers vaccine
- Vaccine for developing world

Human Experimental Sporozoite Challenge Model for Malaria



Huge advantage for vaccine development

- Rapid evaluation of vaccine efficacy in people
- Enables early stage go / no go decision making

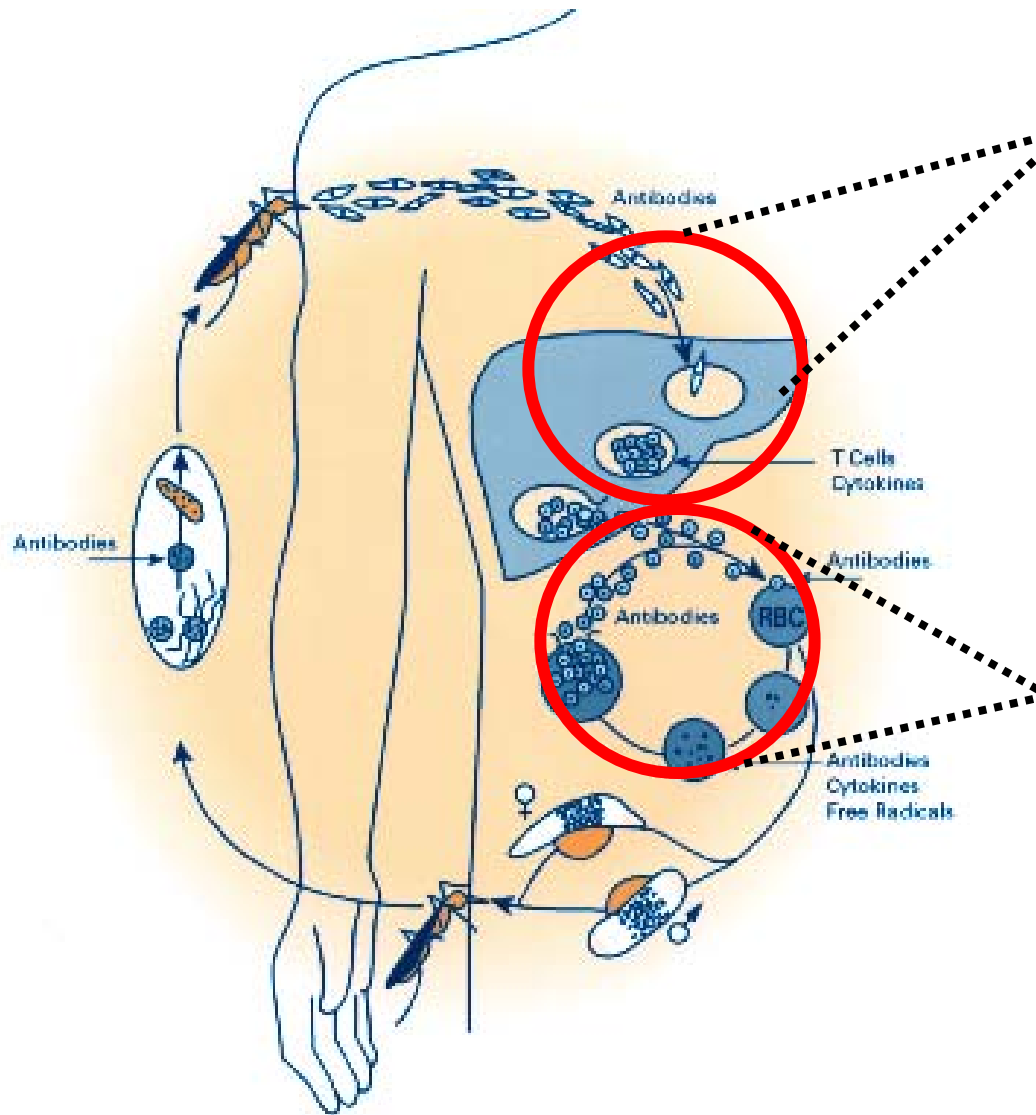
Two vaccine candidates have substantial protective efficacy in this model

- RTS,S (CSP protein in HepB surface antigen VLP)
- Attenuated sporozoites

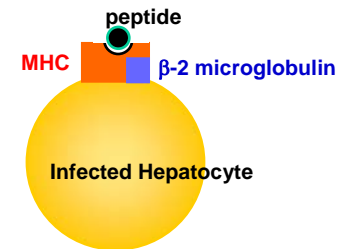
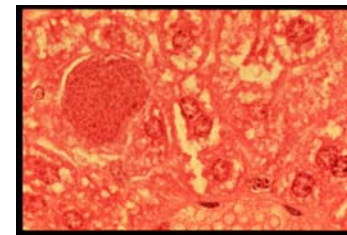
Malaria Vaccine Development Strategy

- **Evaluate Ad5 vectors expressing CSP and AMA1 in clinic**
 - Human experimental challenge model
 - Adenovector alone and prime-boost regimens
- **Develop adenovector platform for multi-antigen expression**
 - Target multiple antigens expressed at multiple stages of the parasite life-cycle
 - Increase the breadth of the immune response
 - Reduce cost of vaccine for developing world
- **Avoid prevalent adenovector specific neutralizing antibodies**
 - Modify the capsid
 - Develop rare serotypes
 - Develop non-human serotypes

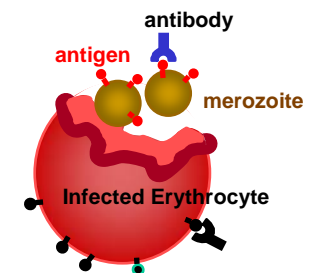
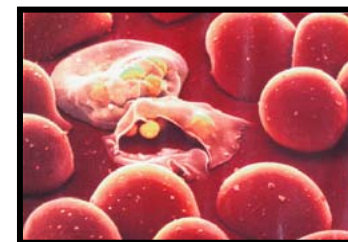
Development of a Multi-Valent Multi-Stage Malaria Vaccine



Target: Liver-stage
Immunity: T Cell responses
Antigens: *PfCSP*, *PfLSA1*, *PfAg2*



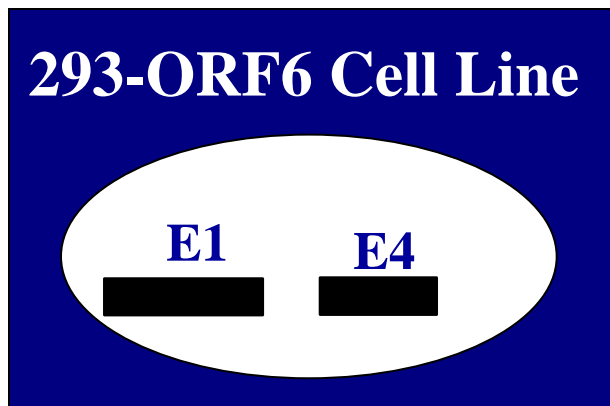
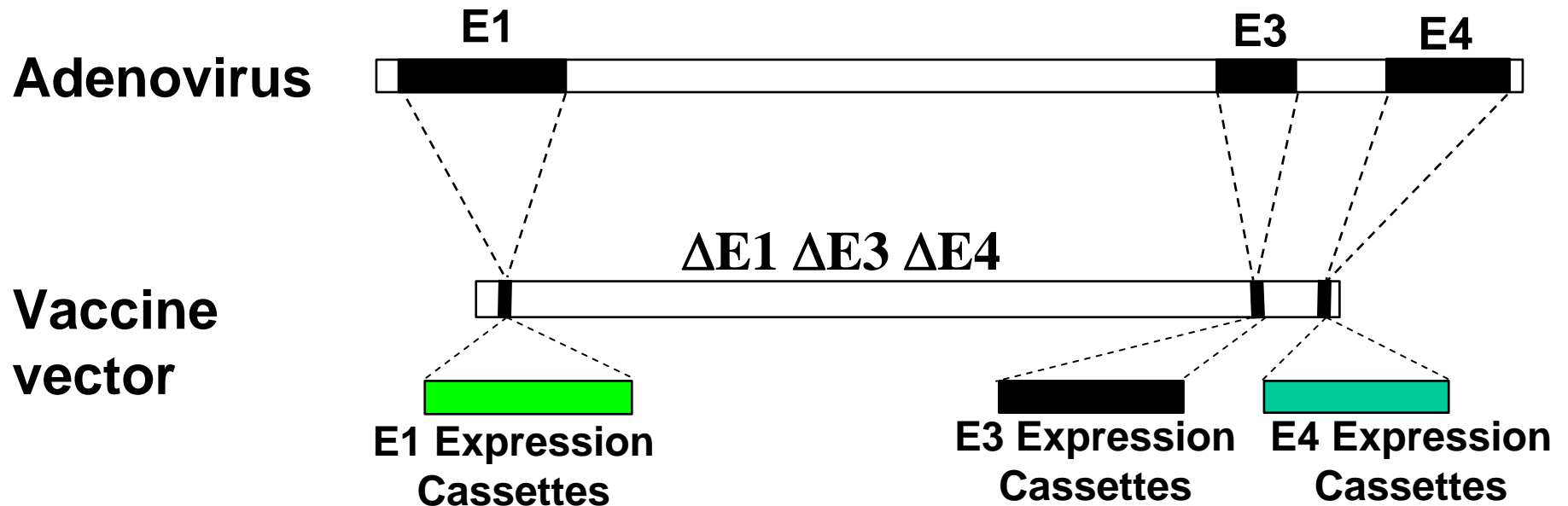
Target: Blood-stage
Immunity: Antibody response
Antigens: *PfAMA1*, *PfMSP1-42*



Rationale for Multi-Valent Adenovector Vaccines for Malaria

- Adenovectors induce robust CD8⁺ T cell responses in humans
- CD8⁺ T cells are associated with protection in humans immunized with irradiated sporozoite vaccine
- CD8⁺ T cells are required for protection in *P. yoelii* model following vaccination with irradiated sporozoites or AdPyCSP
- **Multiple antigens will:**
 - Increase the breadth of the immune response
 - overcome genetic restriction of host immune responses
 - sequence polymorphism of critical T and B cell epitopes
- **Multiple antigens expressed by one vector will:**
 - Reduce the cost of the vaccine
 - Increase potency of the vaccine

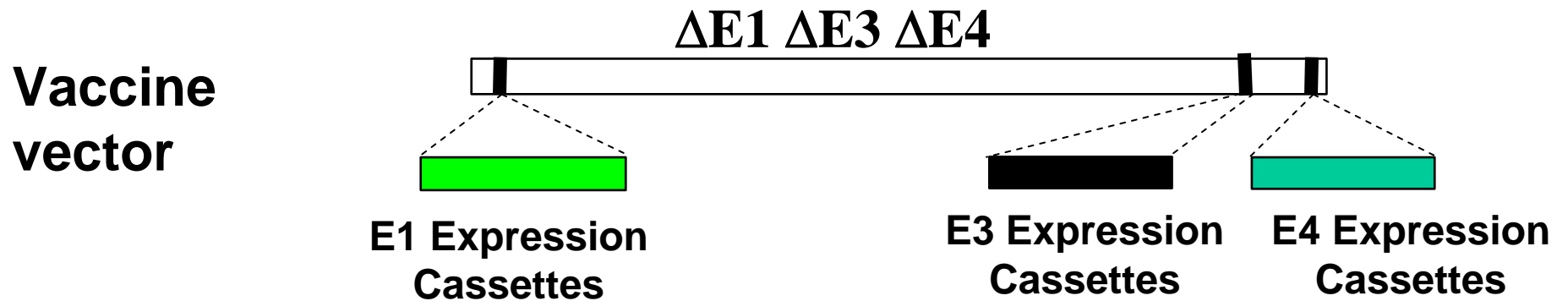
Multi-Valent Adenovector Platform



Advantages of the 293-ORF6 cell line

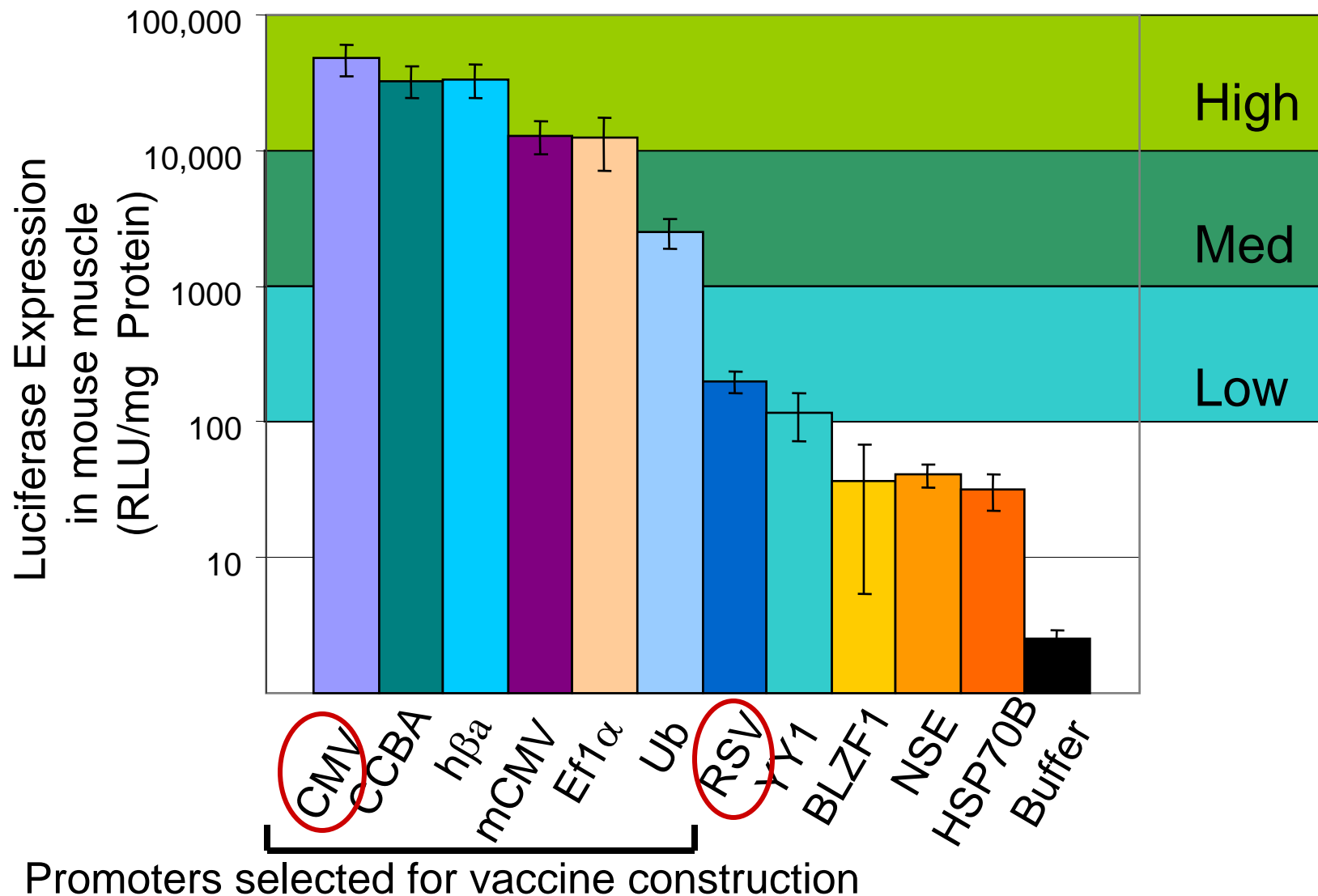
- Robust RCA free production of E1/E3/E4-deleted adenovectors
- Extensively characterized cell line
- Biological Master File at FDA
- Serum free bioreactor process
- Efficient growth of alternative serotype vectors
- Potential for production of other viral vectors

Requirements of Multi-Valent Adenovector

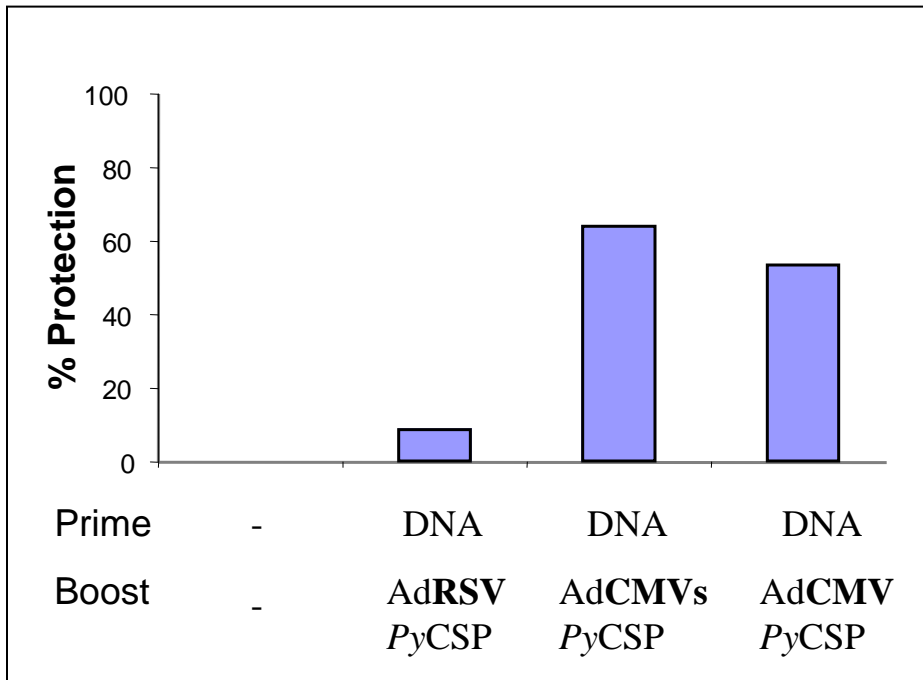
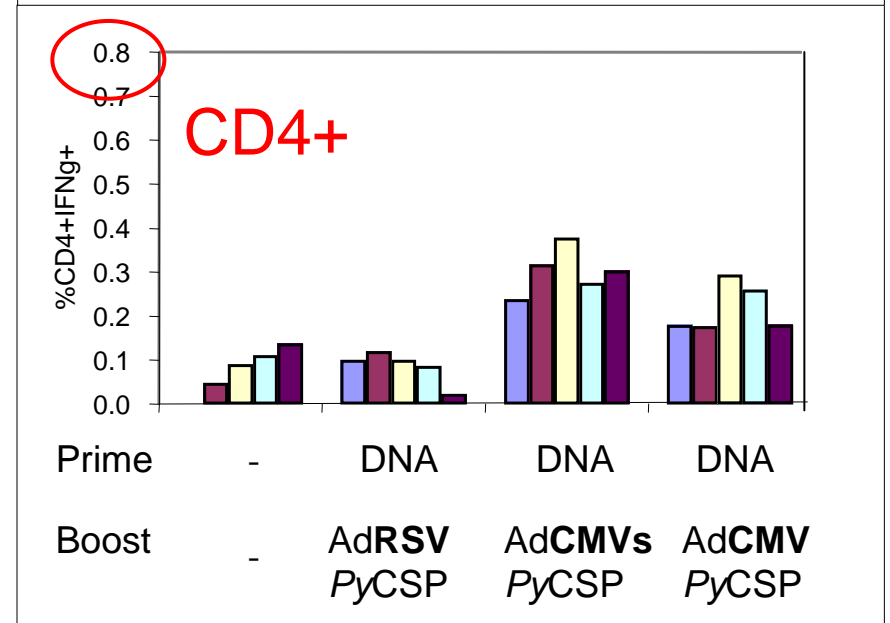
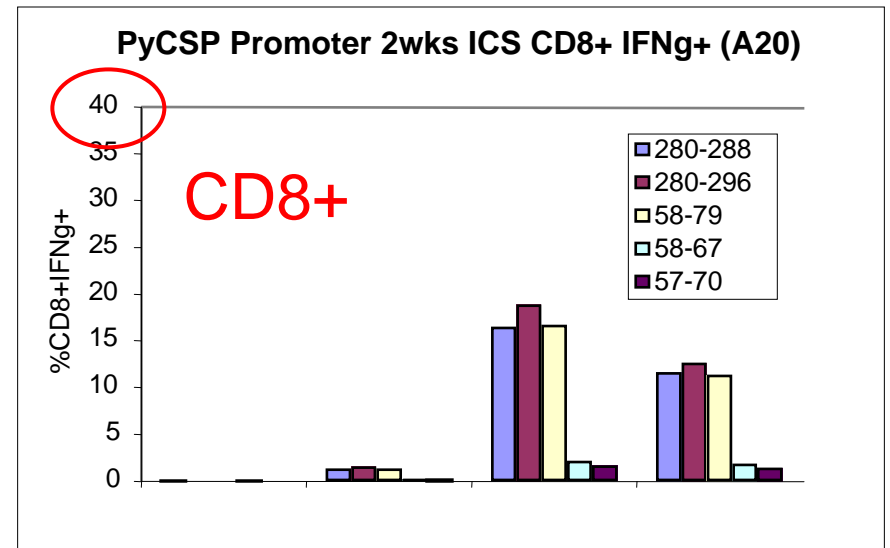
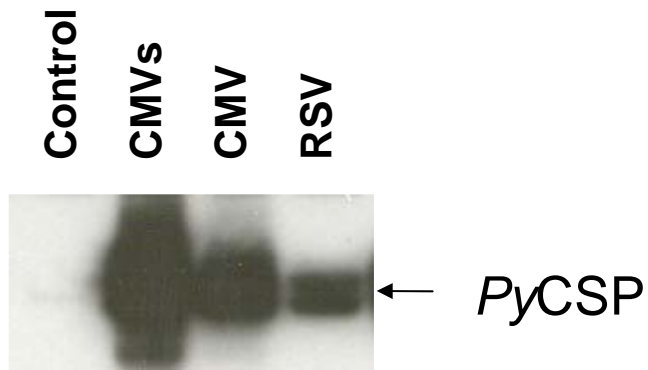


- **Sufficient capacity** to insert multiple antigens and expression cassettes
- **Multiple promoters** and poly A sequences to prevent vector rearrangements
- **Sufficient antigen expression** to induce appropriate immune responses
- **Multiple locations** in genome for insertion of antigen expression cassettes
- **High vector yields**
- **Genetic stability**

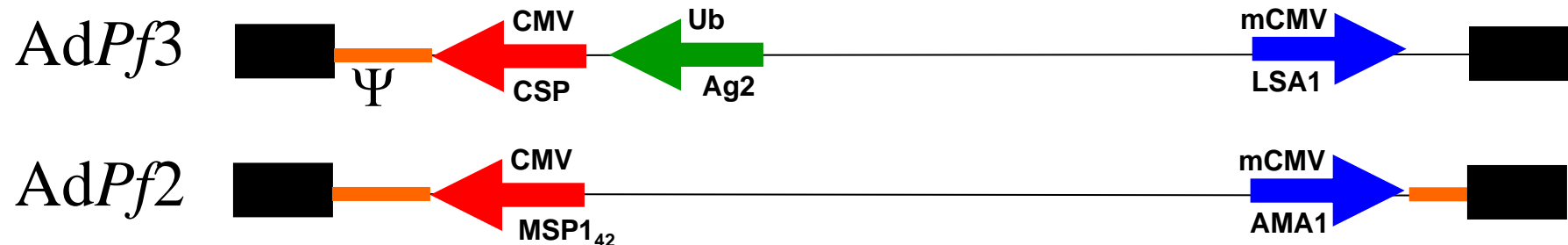
Identified Multiple Promoters Capable of High Level Antigen Expression



Promoter Strength Correlates with Protective Immune Responses



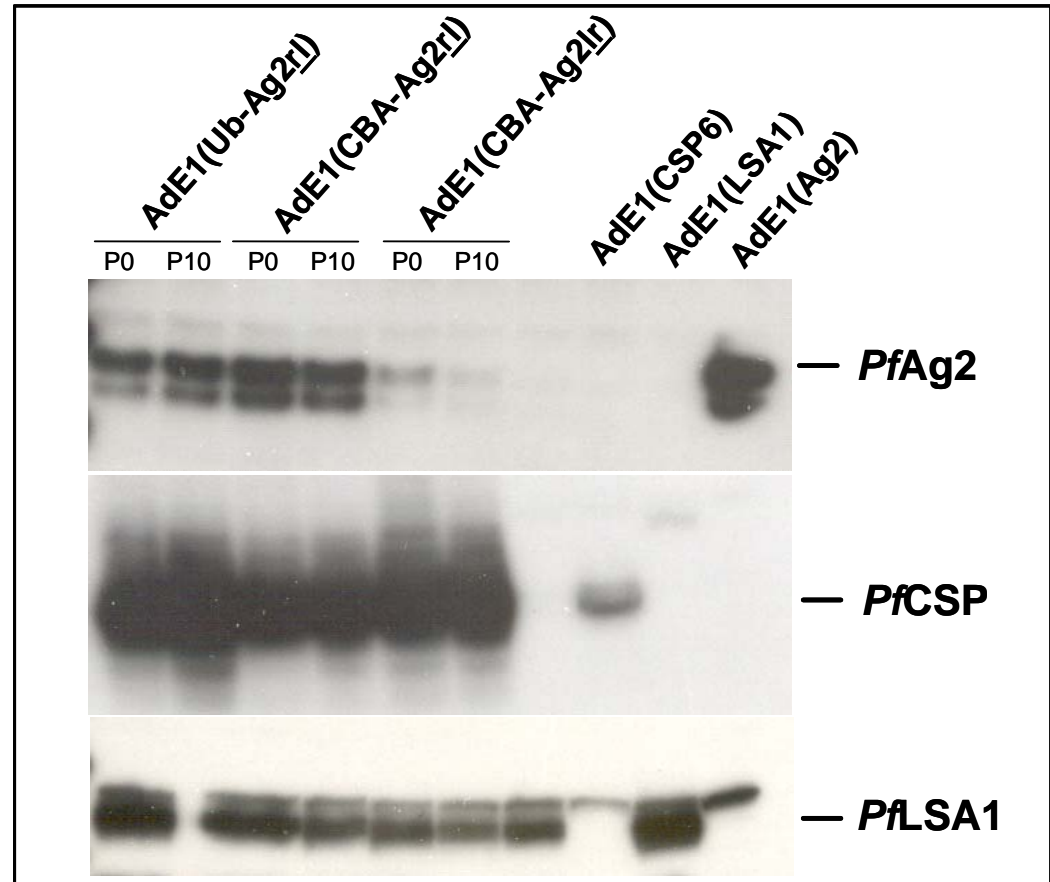
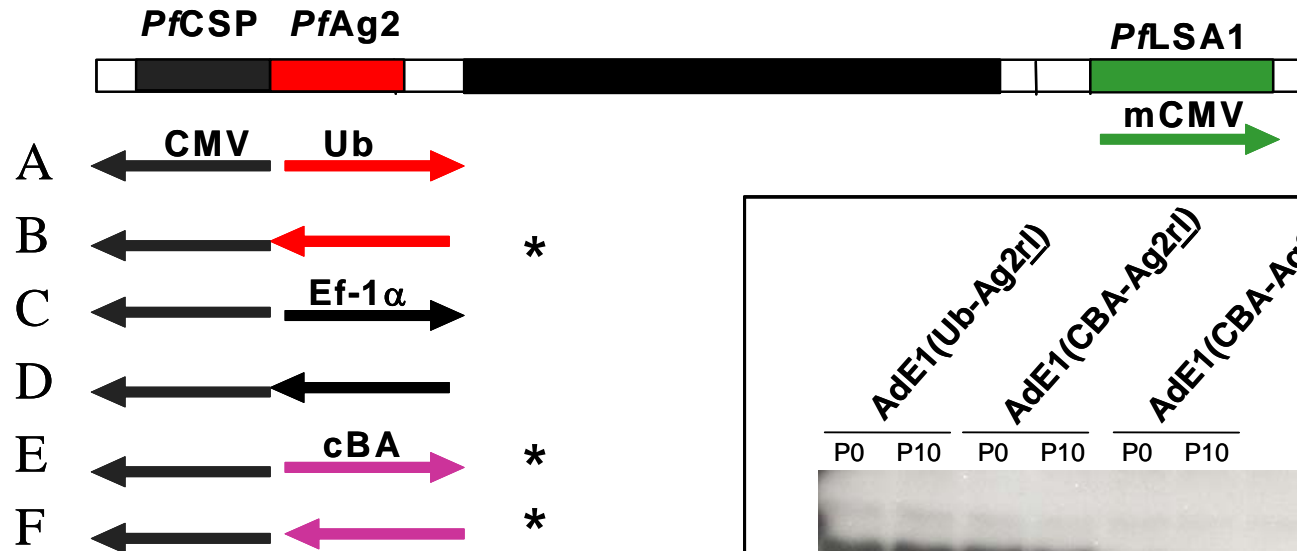
Multi-Valent, Multi-Stage Adenovector Malaria Vaccine Candidates



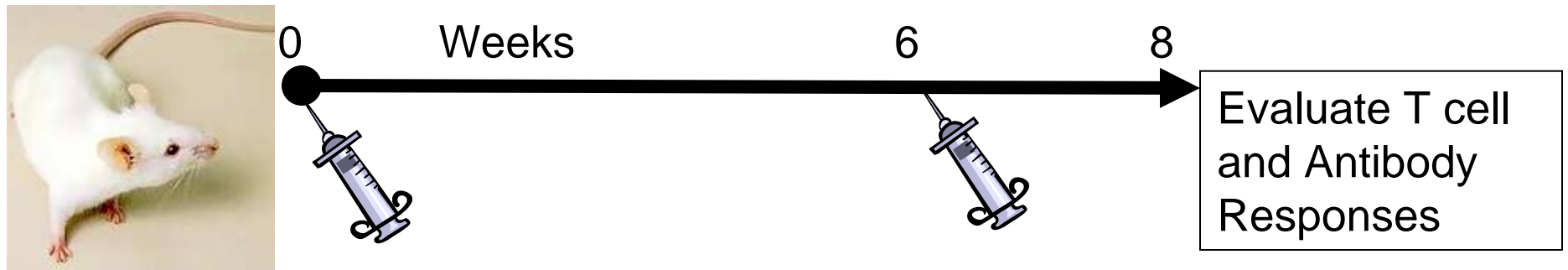
Predetermined criteria for advancement of multi-valent leads:

- High level of expression of two or three different antigens from a single vector
- High yield, genetically stable after multiple passages
- Robust immunogenicity
 - Ag-specific T cell responses (ELIspot, ICS) (mice)
 - Ag-specific Ab responses (ELISA) (mice, rabbits)
 - Functional Ab responses (GIA) (rabbits)

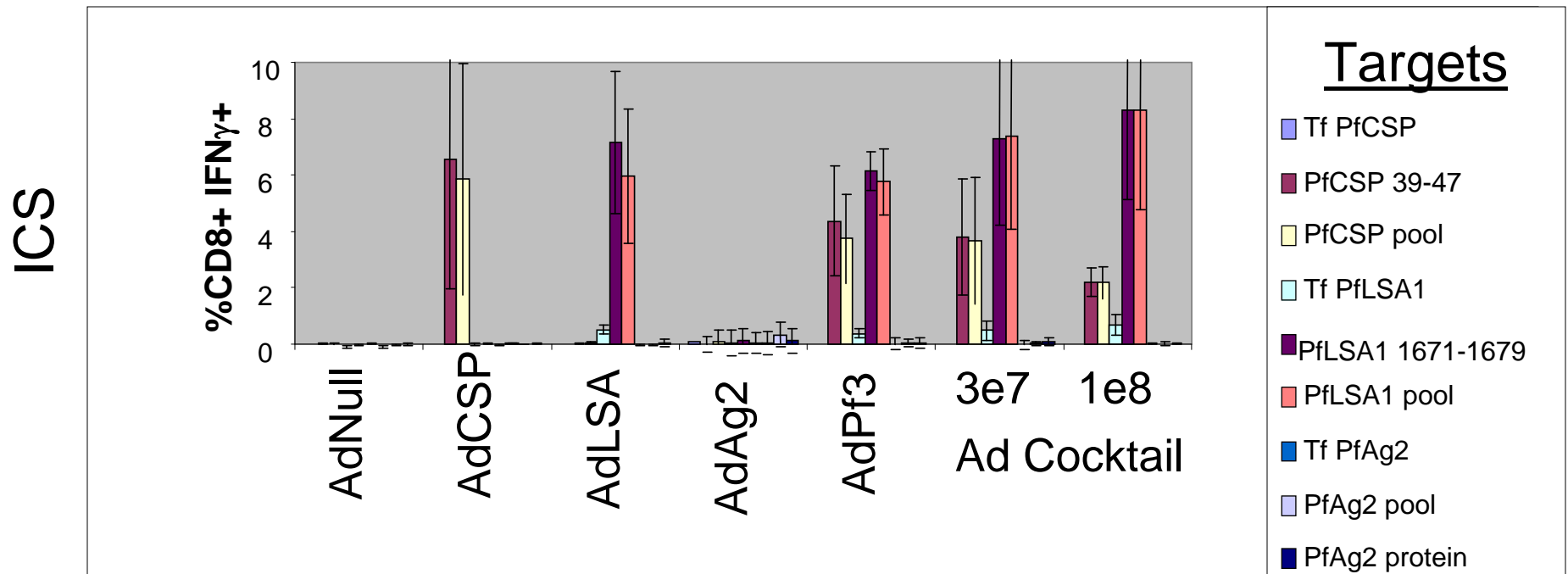
Trivalent Pre-erythrocytic Stage Vector: High Level Antigen Expression and Genetic Stability



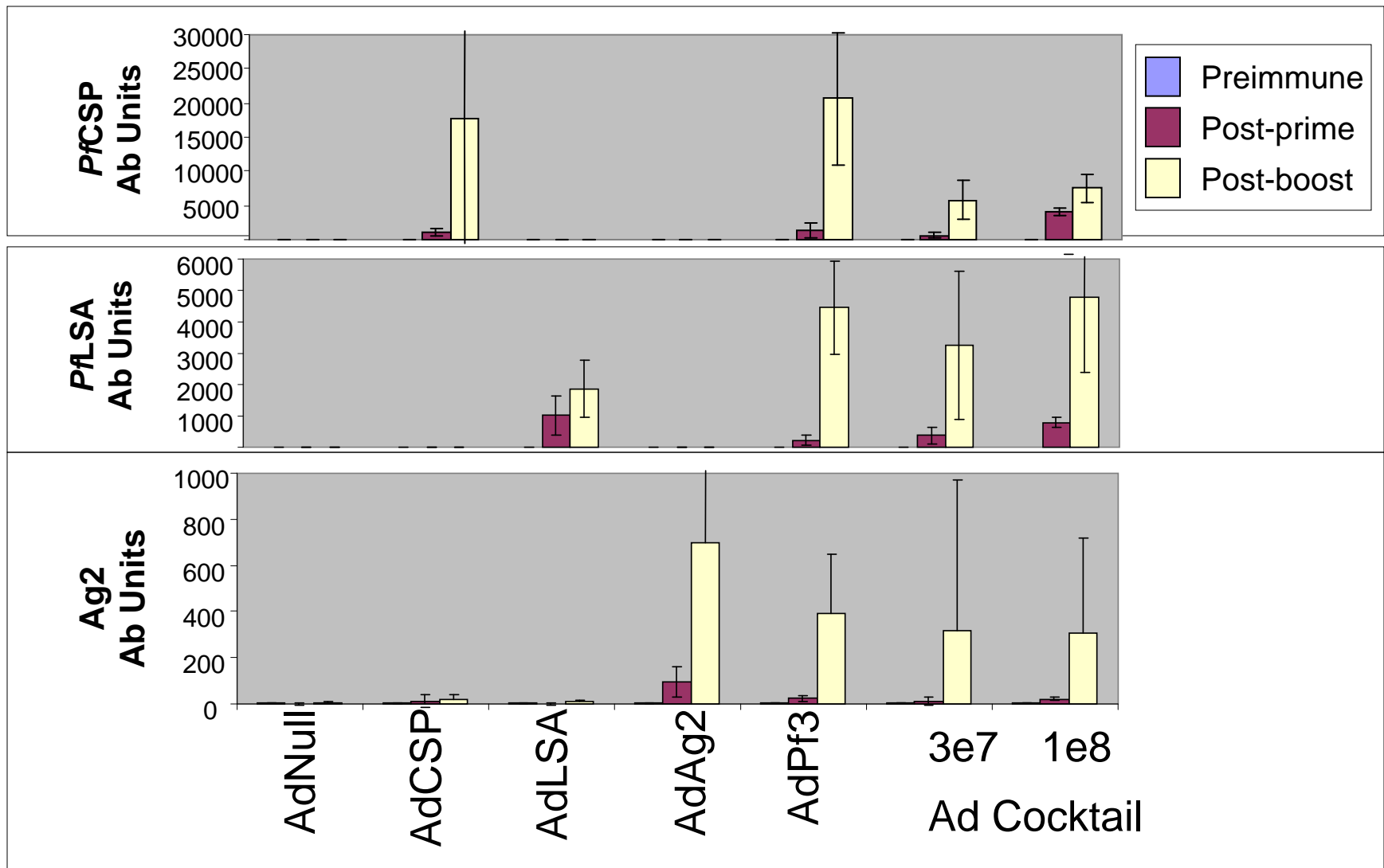
Selected AdPf3 for Mouse Immunogenicity Studies



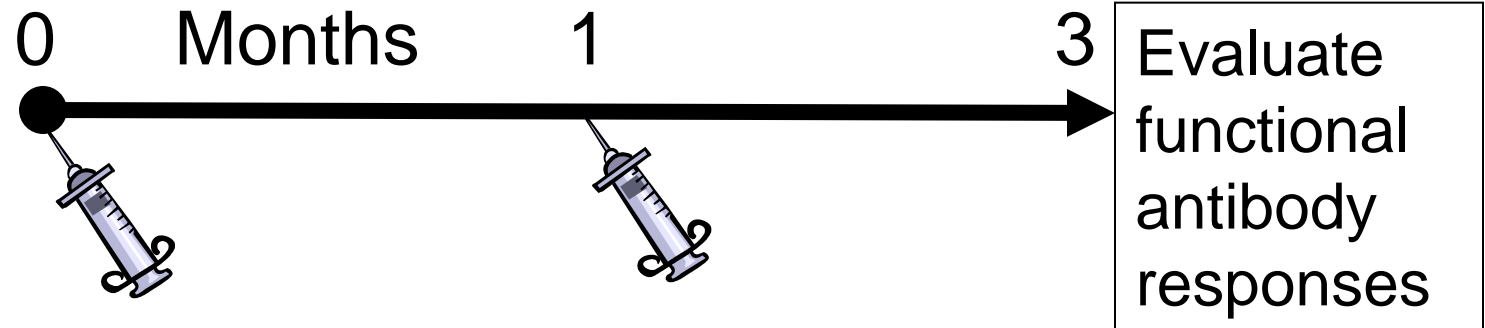
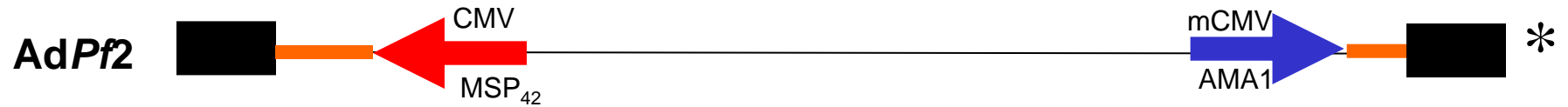
AdPf3 Induces T Cell Responses to All Three Antigens



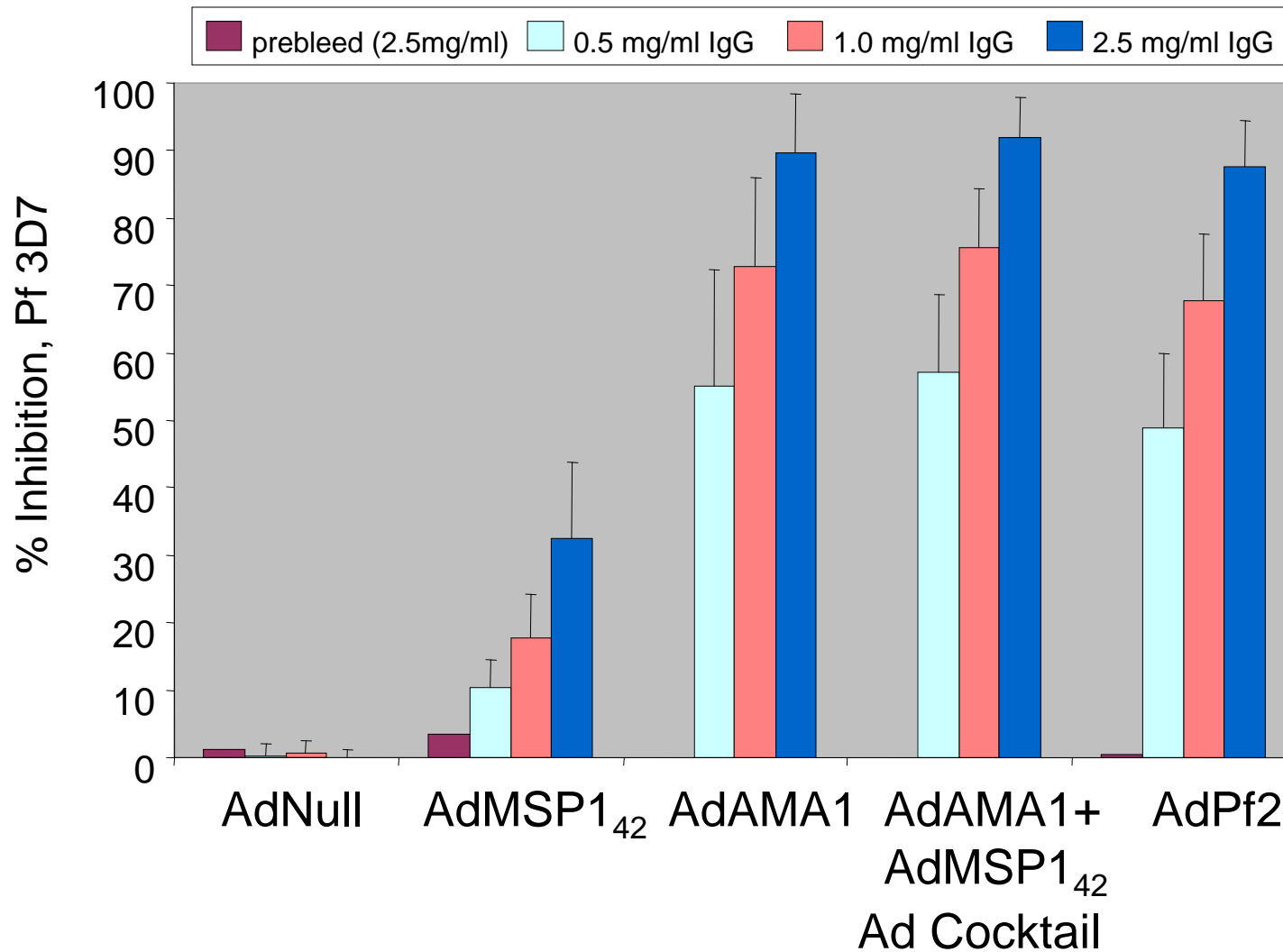
AdPf3 Induces Antibody Responses to All Three Antigens in Mice



AdPf2: Bivalent Blood Stage Vector



AdPf2 Induces Robust Functional Antibody Responses in Rabbits



Conclusions

- Generated Ad5 vectors that express multiple malaria antigens (Ad5Pf3 and Ad5Pf2)
- Induce robust T cell and antibody responses to five malaria antigens
- Pf2 induces unprecedented functional antibody responses
- Genetically stable, grow to high yields

Next Steps

- Develop Ad28-based multivalent vaccine (see poster 961)

Improving Immunogenicity and Safety of Adenovectors for Malaria Vaccine

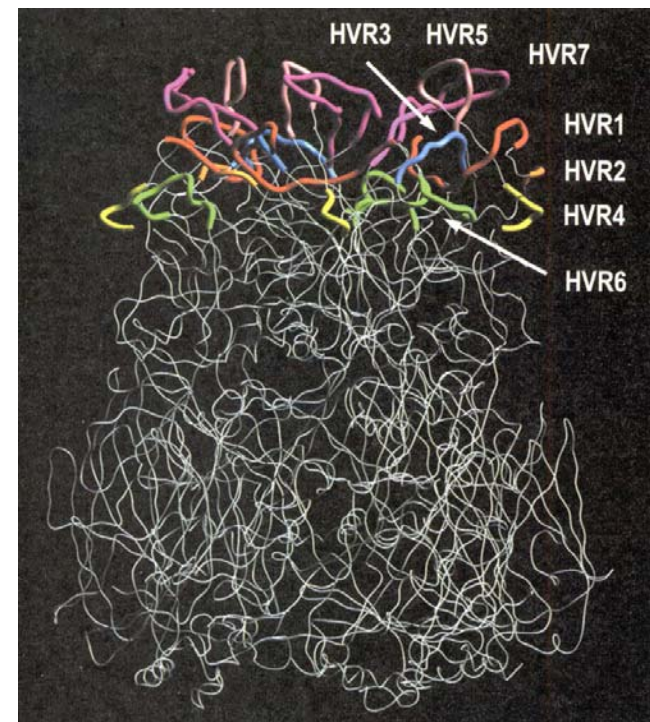
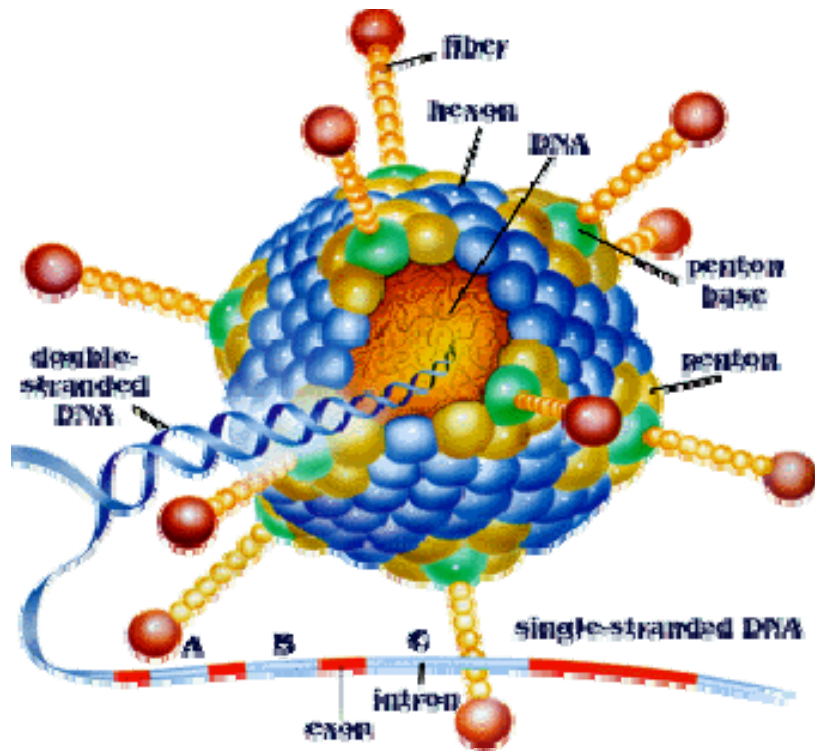
Avoid pre-existing Ad5 neutralizing antibodies

- Ad5 neutralizing antibody (NAb) are prevalent in sub-Saharan Africa
- Animal data indicate greatly reduced T cell responses in mice and NHP in presence of Ad5 NAb
- Data from HIV trial suggest reduced magnitude of T cell responses in people with Ad5 NAb
- STEP trial data suggest increased HIV acquisition in uncircumcised volunteers with high pre-existing Ad5 NAb titers

Strategy

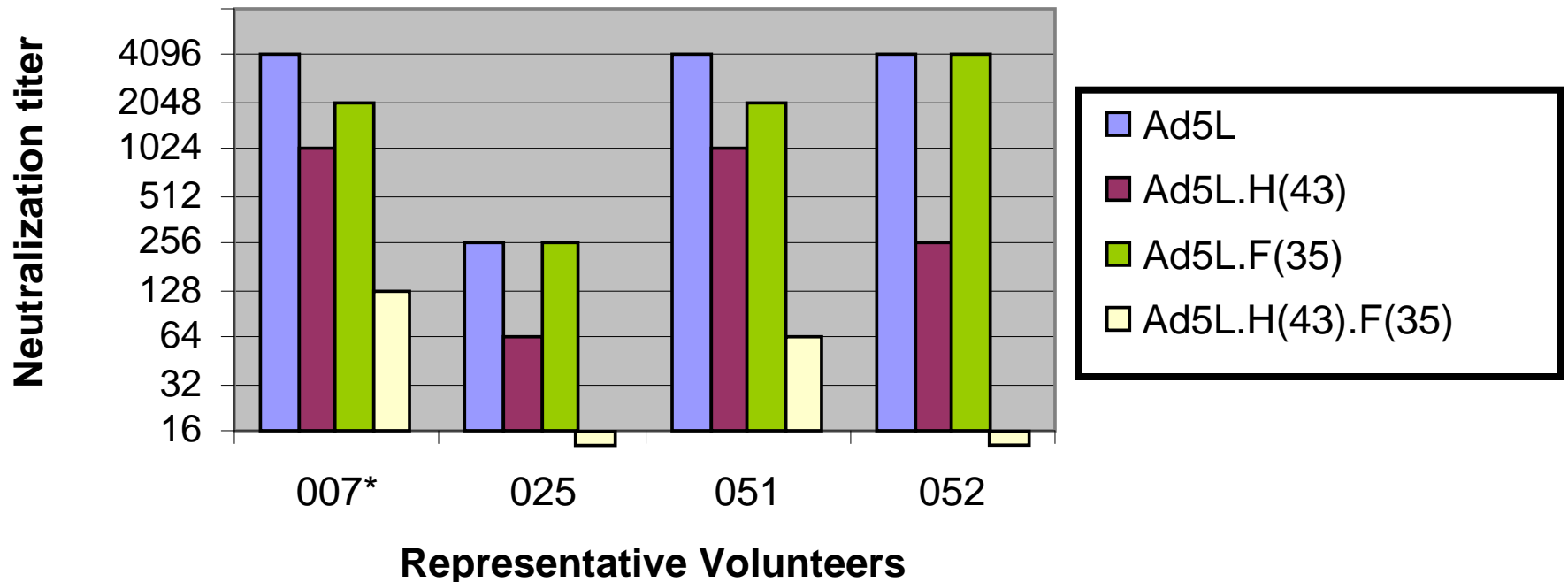
- Develop alternative adenovectors based on less prevalent serotypes
- Modify Ad5 capsid components to avoid pre-existing Ad5 NAb

Hexon Modifications to Avoid Pre-Existing Neutralizing Antibody



- The HVR on hexon are the main determinants of NAb
- The HVR can be modified to circumvent pre-existing NAb

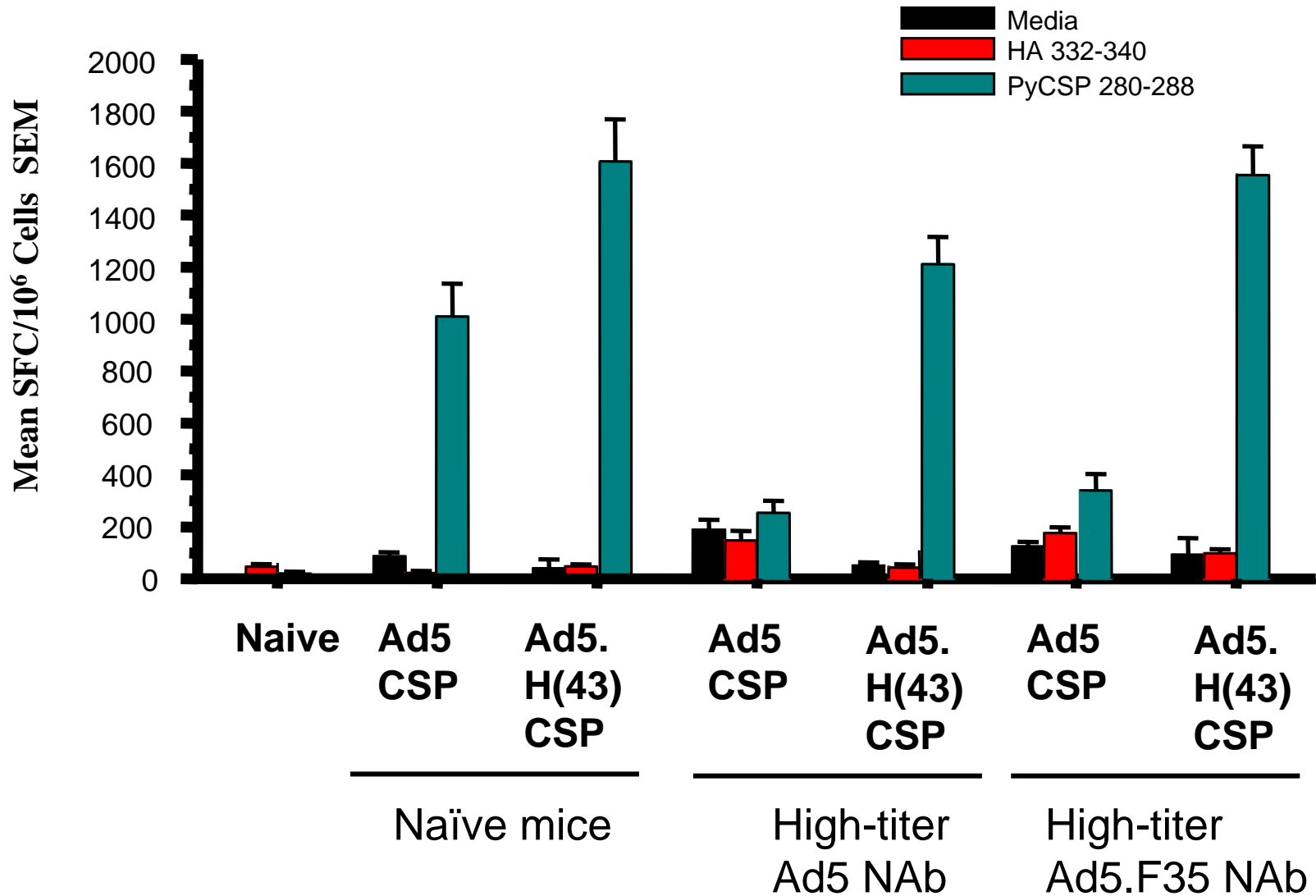
Hexon/Fiber-Modified Vector Avoids Ad5 NAb



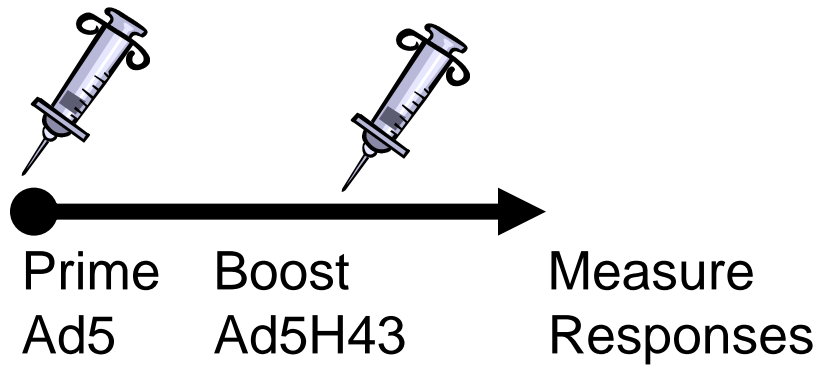
Conclusions:

- Antibodies specific for hexon HVR and fiber can neutralize Ad5 vector *in vitro*
- Hexon-fiber-modified vector avoids pre-existing Ad5 NAb
- Same results in human volunteers, rabbits, and mice

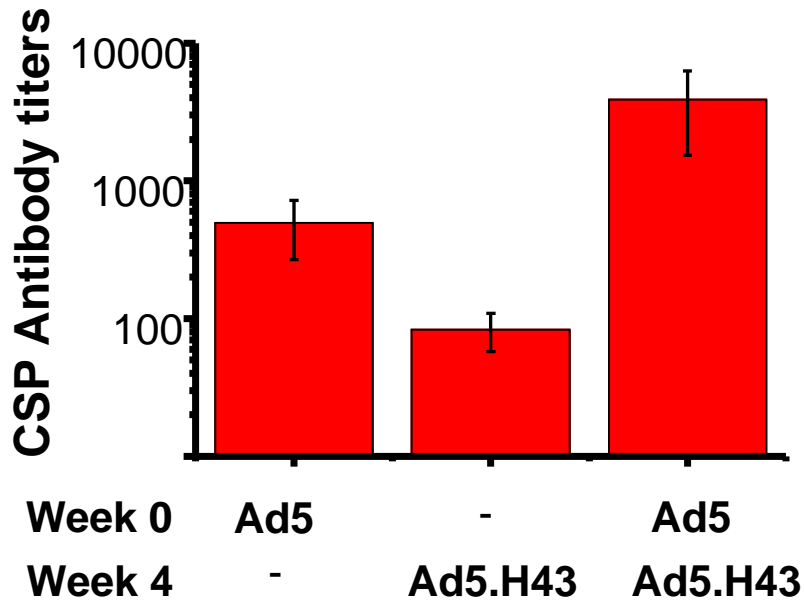
Hexon-modified Vector Induces Robust T cell Responses in Mice with High Titers of Ad5NAb



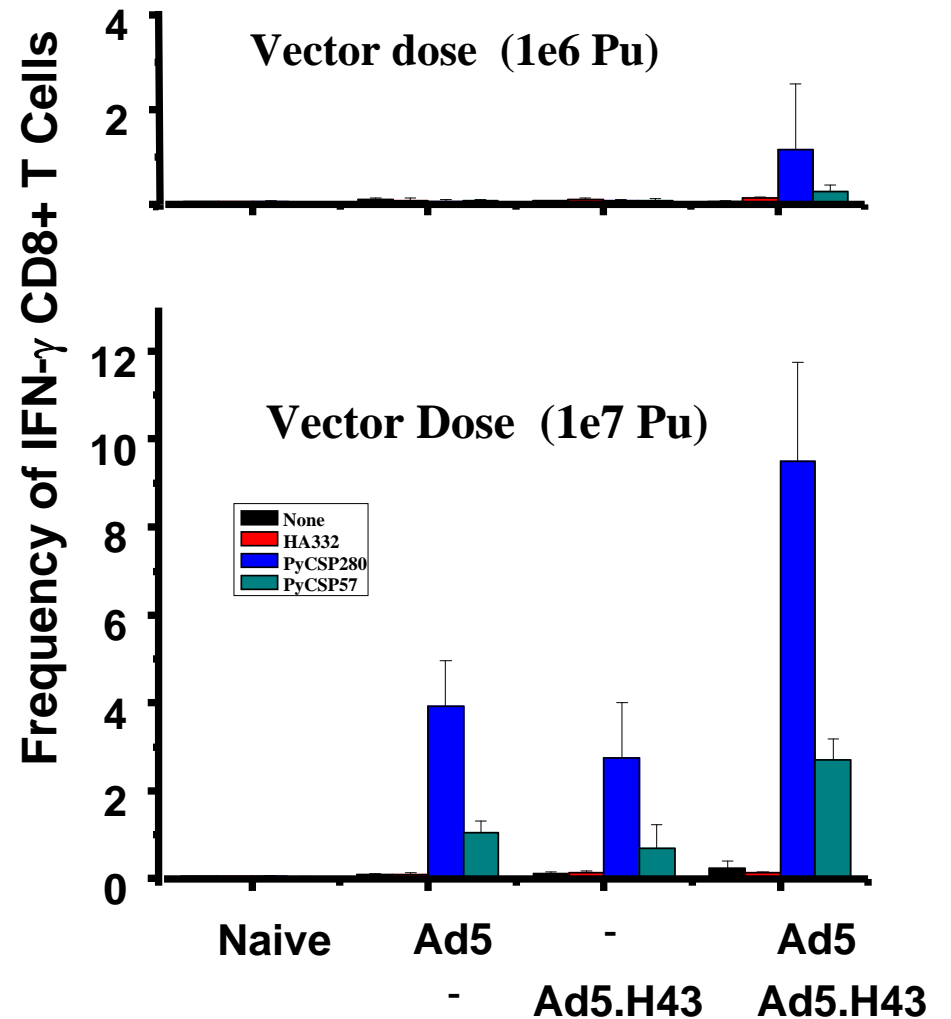
Ad5 + Ad5H43 in Prime-Boost Regimen Induce Superior T cell and Ab Responses



Antibody Responses



T cell Responses



Conclusions

- Generated hexon modified vector (Ad5HVR43)
- Avoids hexon-specific Ad5 NAb *in vitro* in mice, rabbits and humans
- Is highly immunogenic in mice with high titers of Ad5 NAb
- Can be combined with Ad5 vector in prime-boost regimens

Key Contributors



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