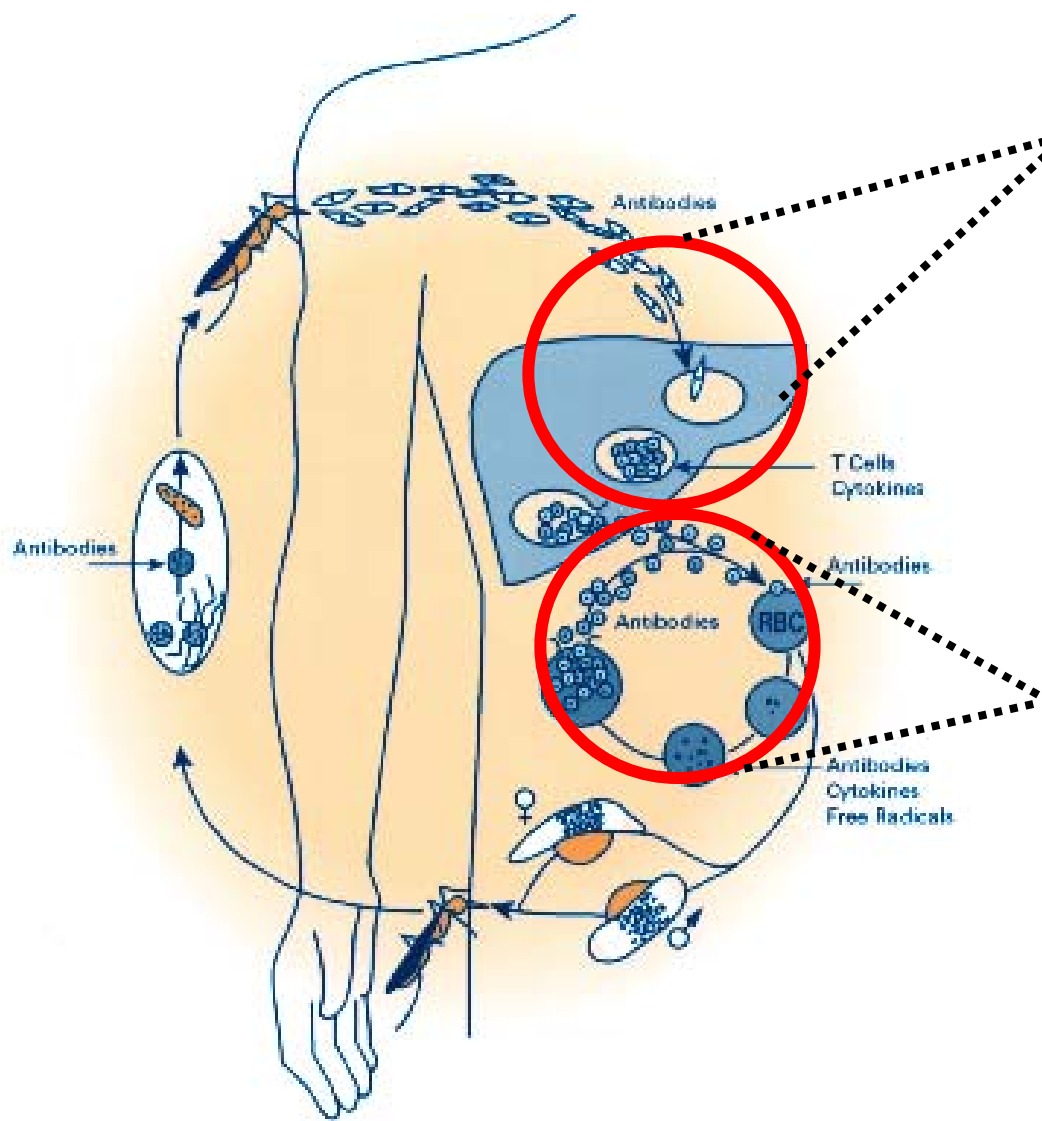


Development of a multi-antigen multi-stage adenovector-based malaria vaccine that induces robust T-cell and antibody responses

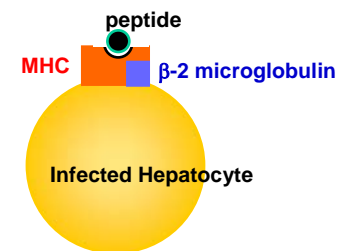
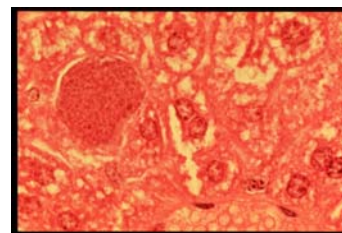
Joseph T. Bruder, Ph.D.



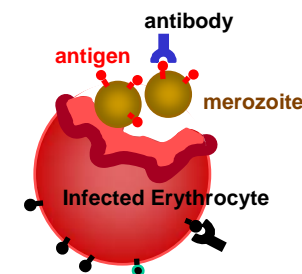
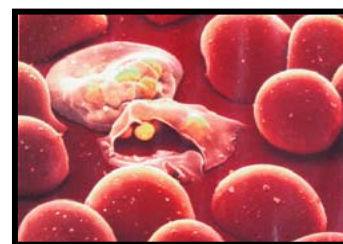
Development of a Multi-valent Multi-stage Malaria Vaccine



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



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



Adenovector–Based Vaccines May Provide Protection From *P. falciparum* Malaria in Humans

Facts -

- Ad5 vectors induce robust CD8⁺ T cell responses in humans
- Ad5 vectors induce protective responses in animal models

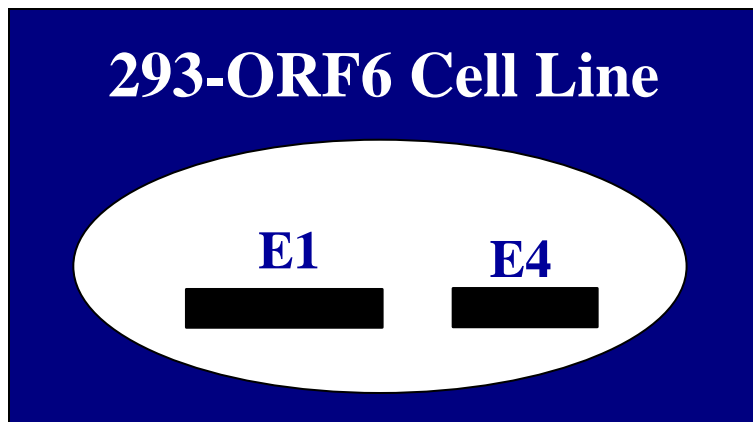
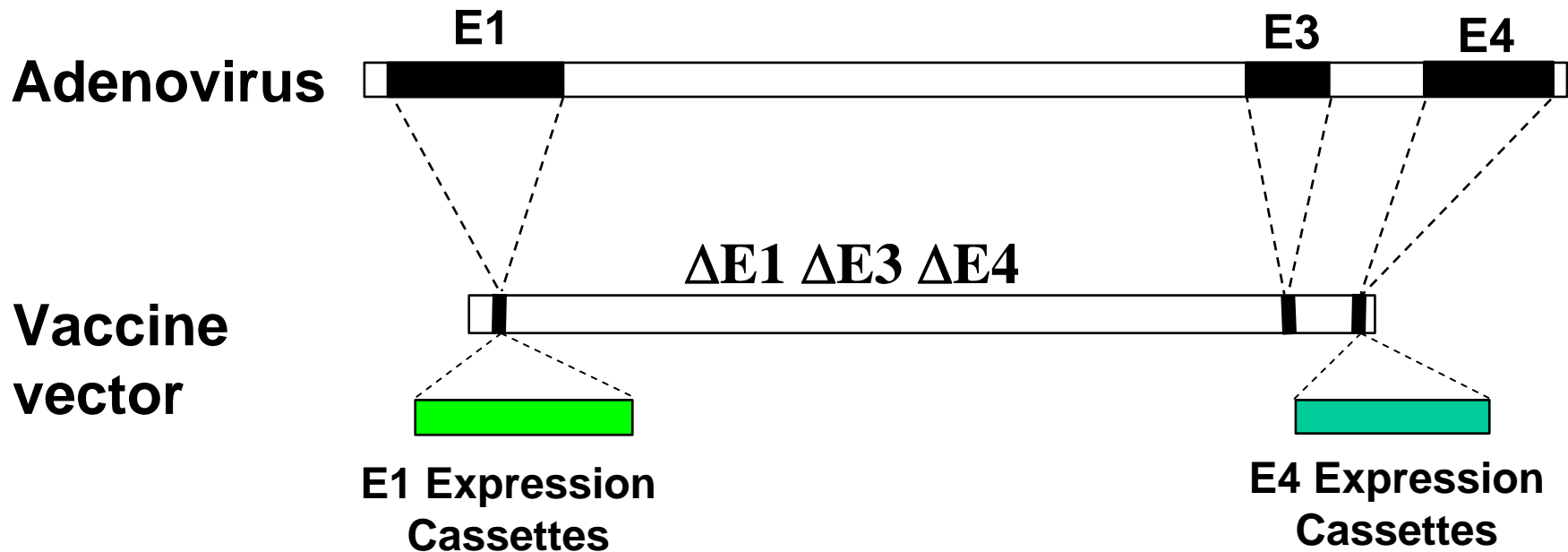
Rationale –

- IFN γ expressing 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

Opportunity -

- Ad5 vectors that express multiple *P. falciparum* antigens should be evaluated for protective efficacy in the human experimental challenge model

Multi-valent Adenovector Platform



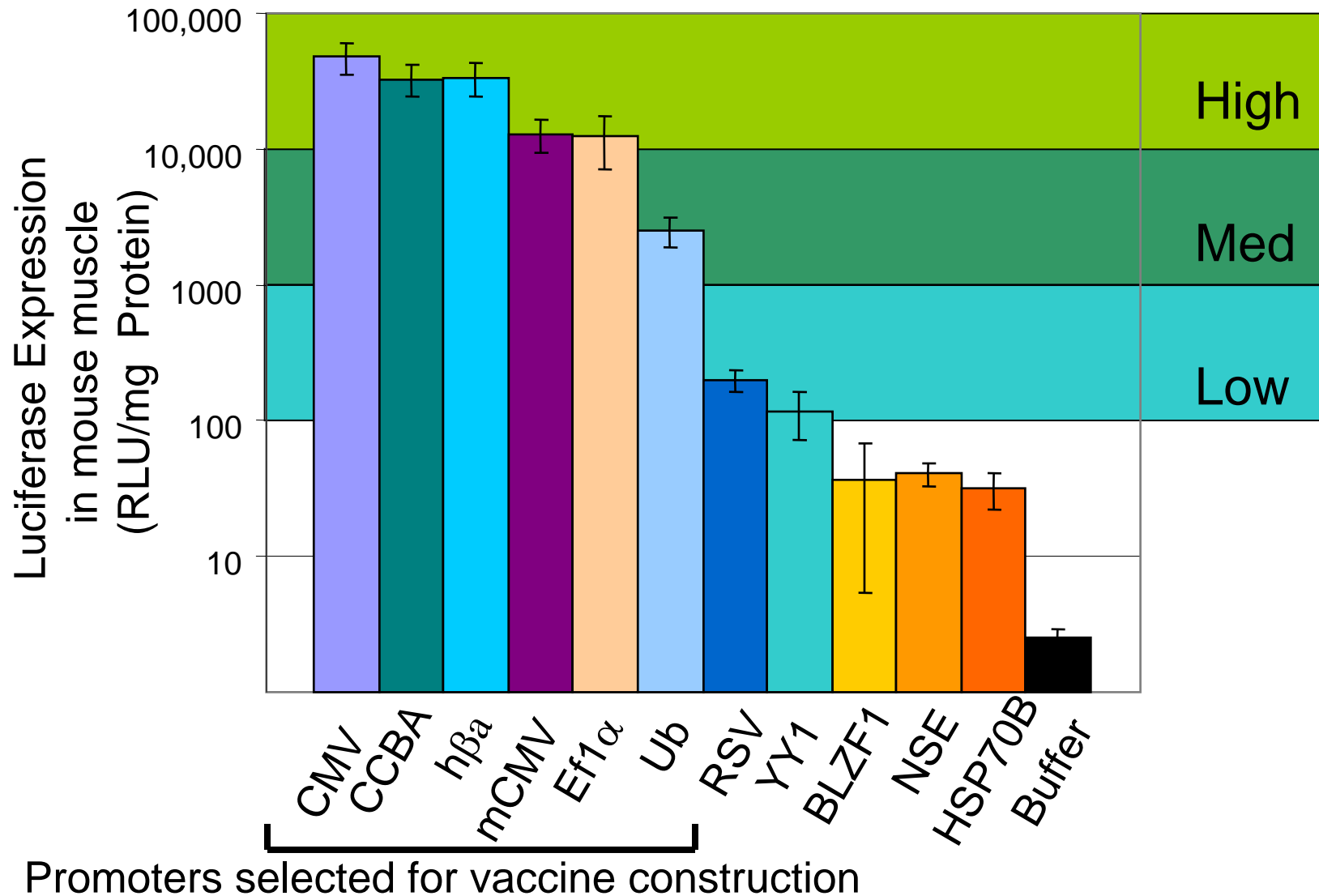
Advantages of the 293-ORF6 cell line

- 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

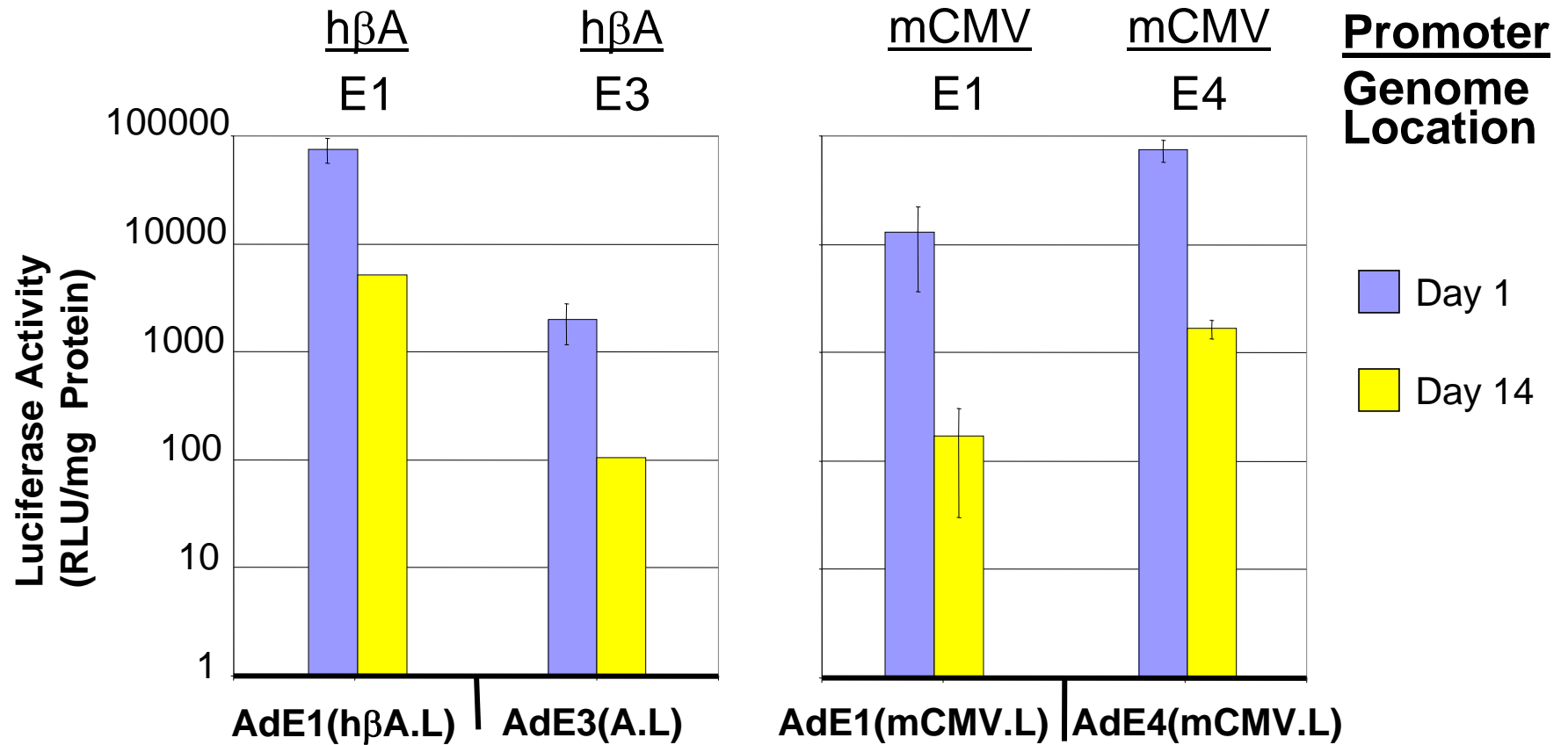
Requirements of Multivalent Adenovector

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

Identified Multiple Promoters Capable of High Level Antigen Expression

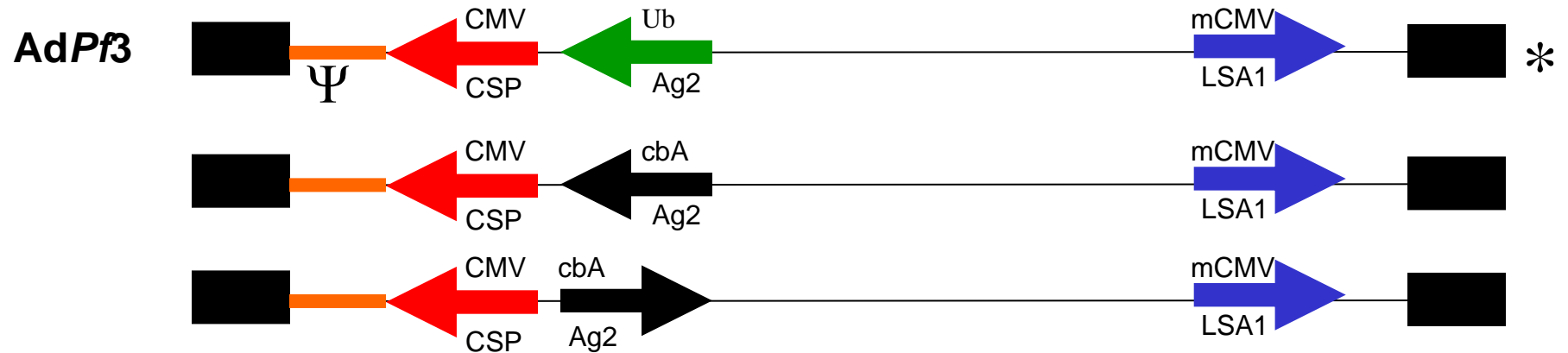


Multiple Locations in Genome for Insertion of Antigen Expression Cassettes



Conclusion: E4 and E1 locations are preferred for high level antigen expression

Selection of Trivalent PES Vector for Clinical Development

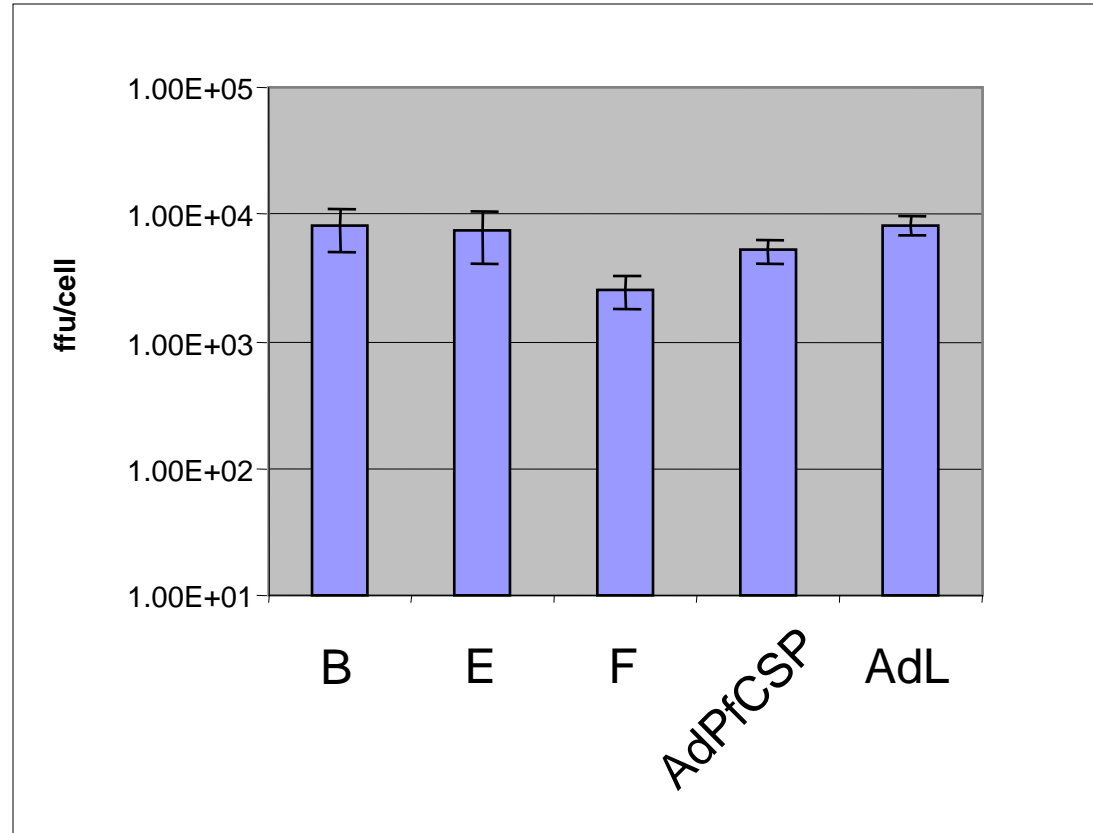
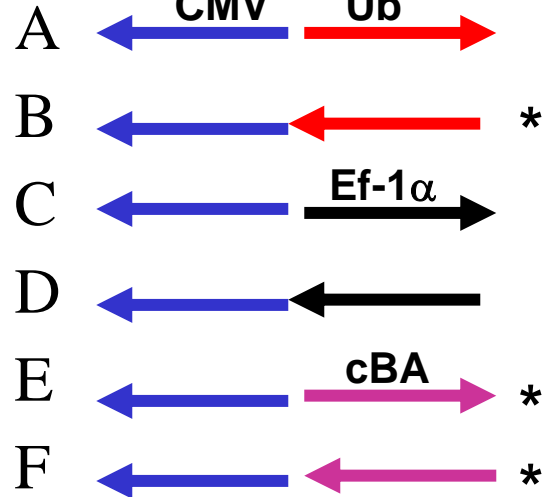


Preferred Criteria for Clinical Lead Decision

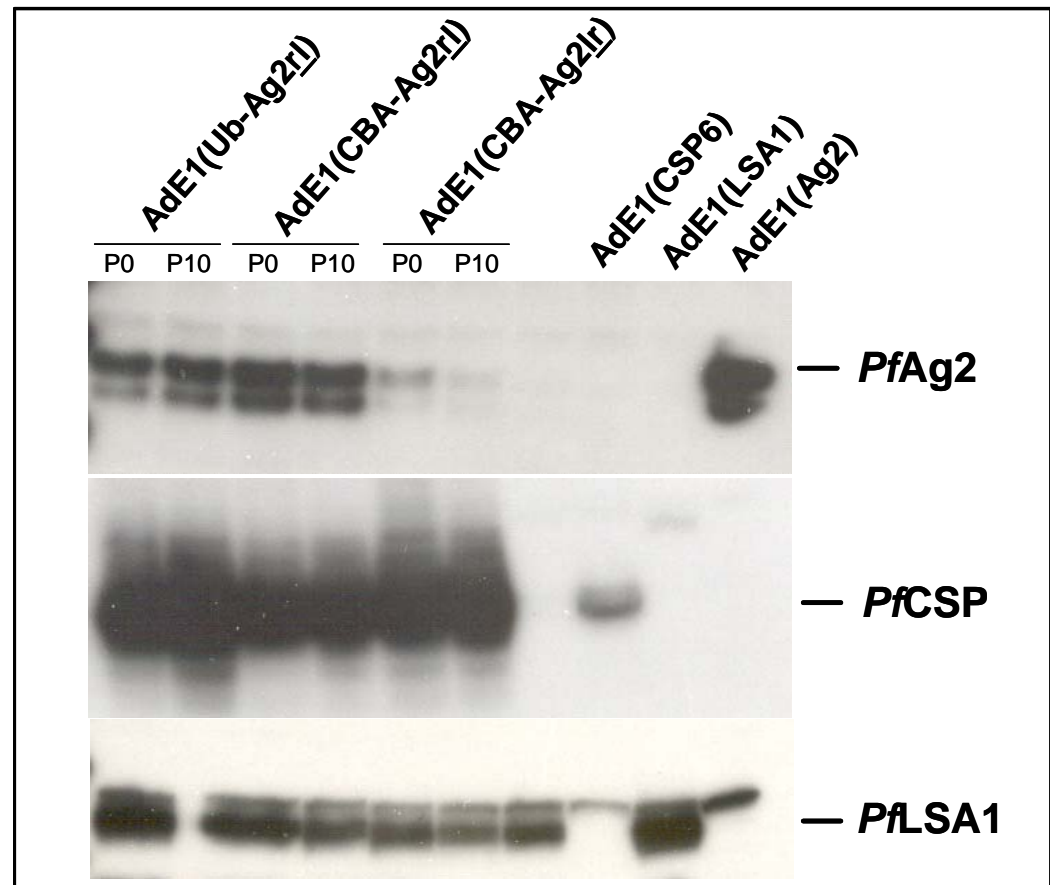
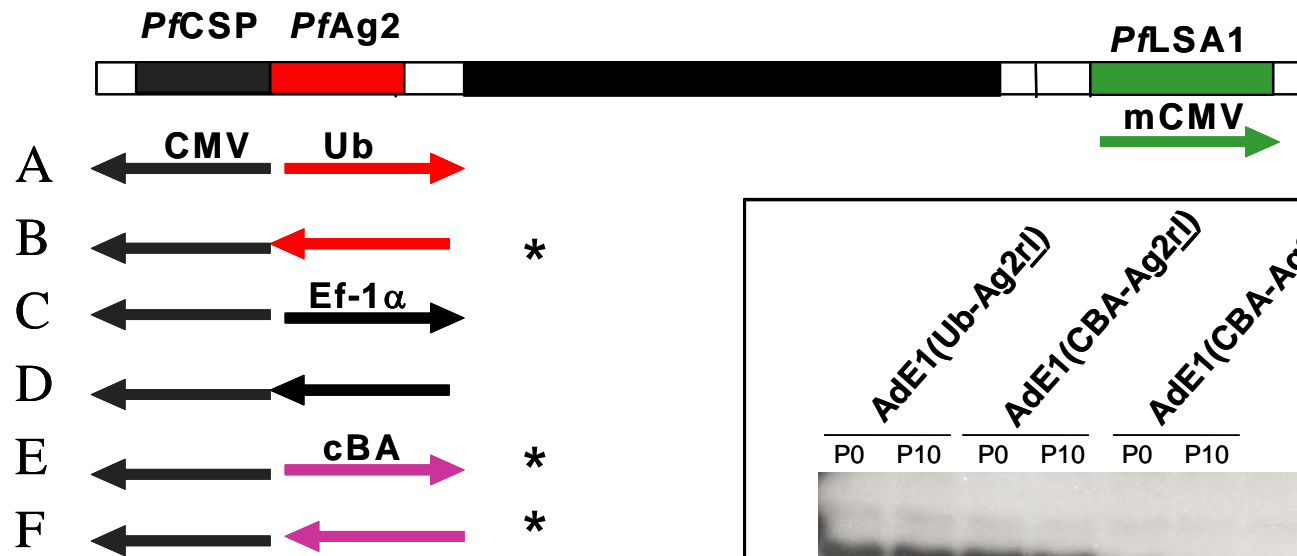
- Expression of all three antigens *in vitro*
- Robust T cell responses against all three antigens in mice
- Production growth analysis showing high yield vector production
- Genetic stability of vectors after multiple passages

* = lead selected for clinical development

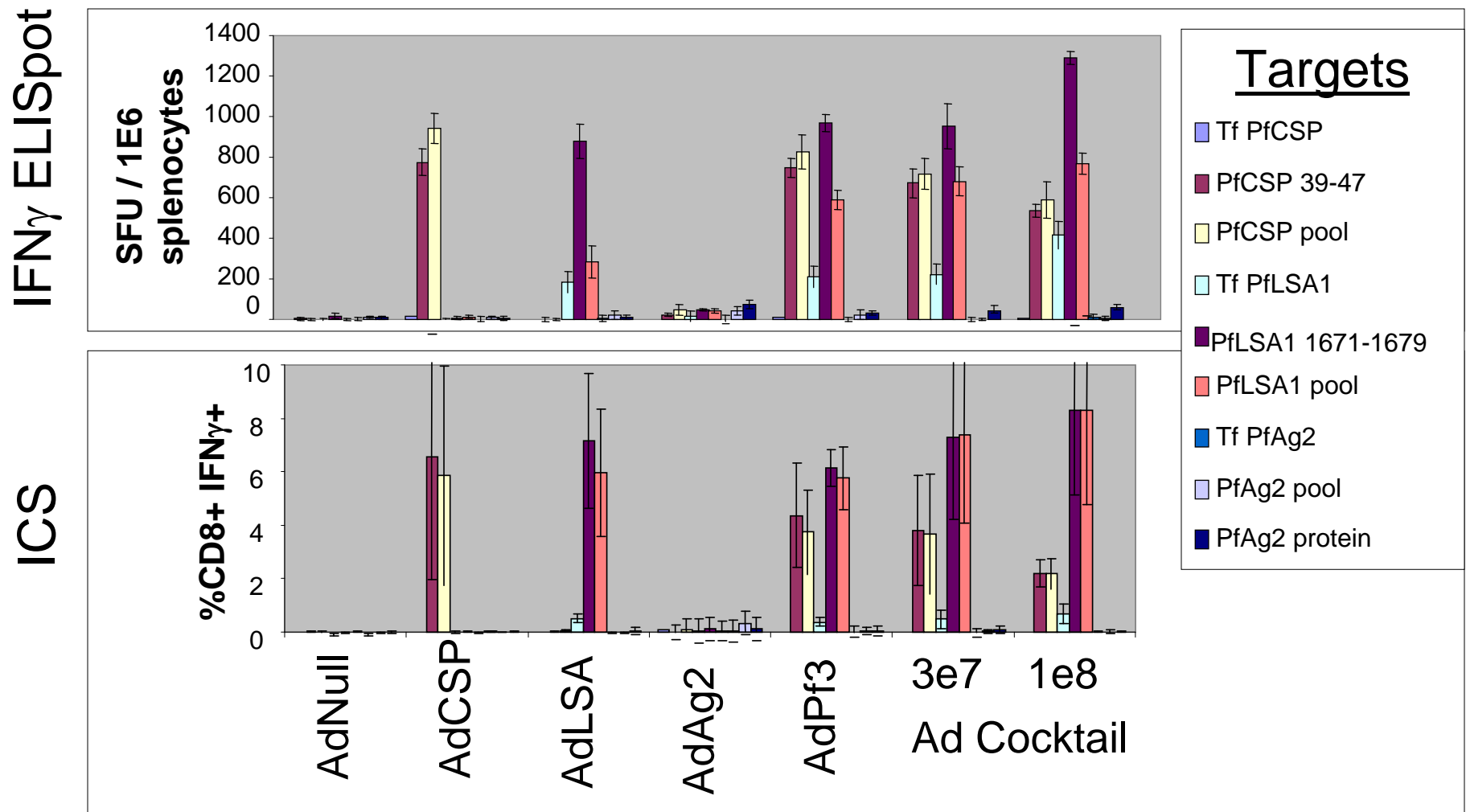
Trivalent Vectors Grow Well



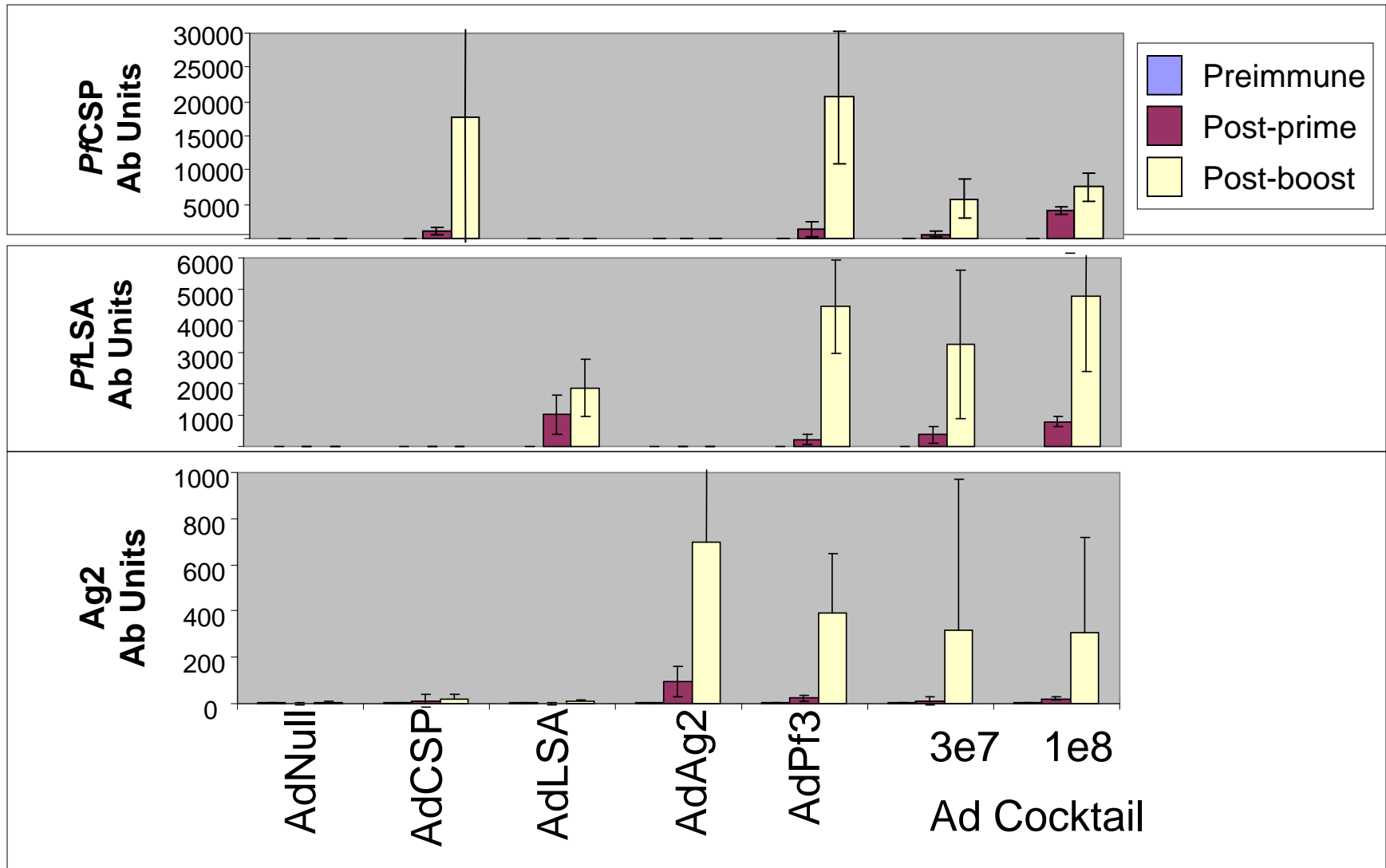
High Level Antigen Expression and Genetic Stability



T Cell Responses to Trivalent Vaccine

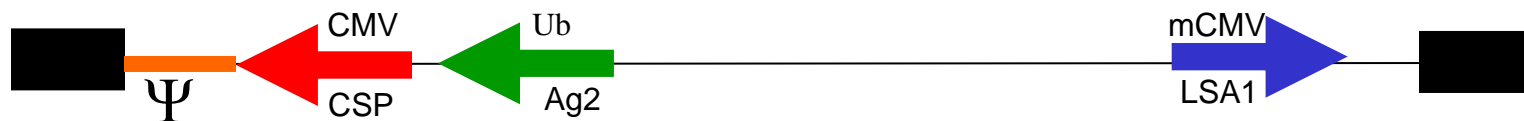


AdPf3 Induces Antibody Responses to All Three Antigens in Mice

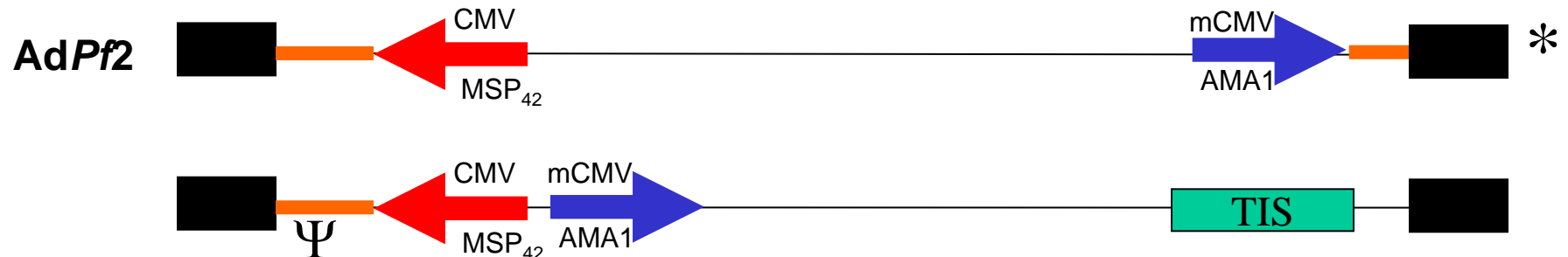


Conclusions - Trivalent Pre-erythrocytic Stage Adenovectors

- Locations
 - E1 and E4 regions are preferred for antigen expression
- Promoters
 - Strong correlation between promoter strength *in vitro* and *in vivo*
 - Threshold levels of promoter activity / antigen expression required for robust (and protective) antibody and T cell responses
 - Several promoters that drive expression \geq threshold level
- Trivalent adenovectors
 - Good growth characteristics & genetic stability after multiple passages
 - High level gene expression *in vitro*
 - Robust T cell and antibody responses to each antigen *in vivo*, similar to single antigen expressing vector
 - No apparent antigen interference



Selection of Bivalent Blood Stage Vector for Clinical Development

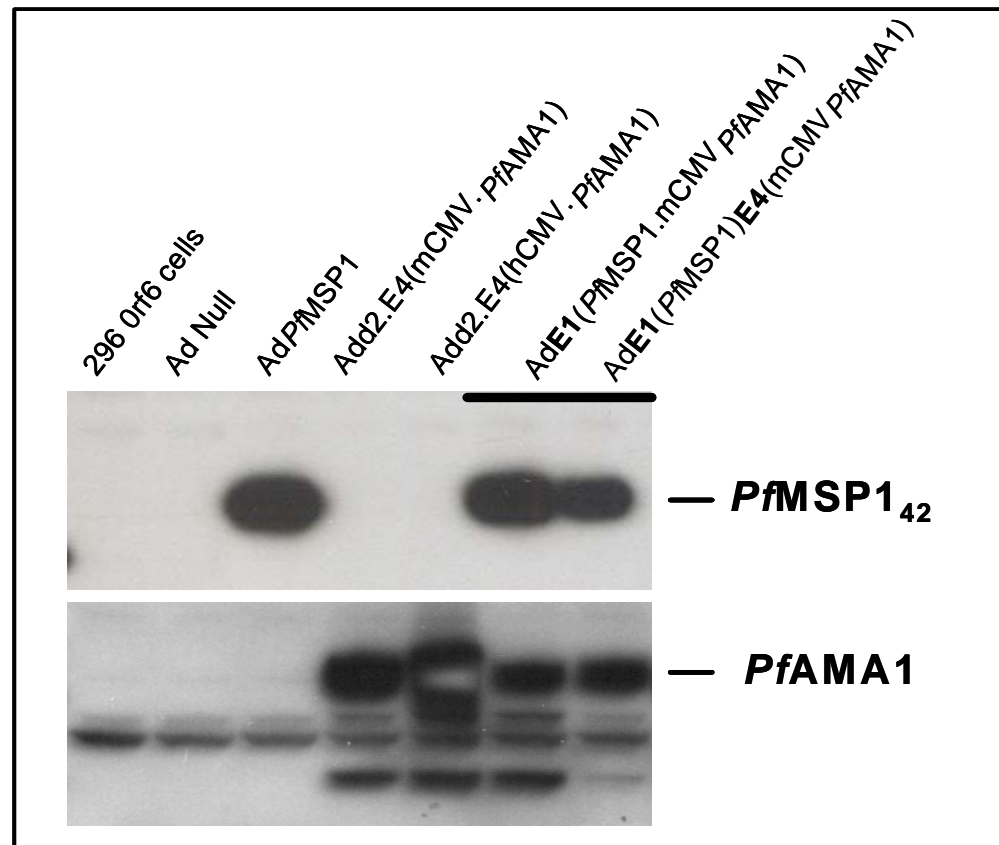
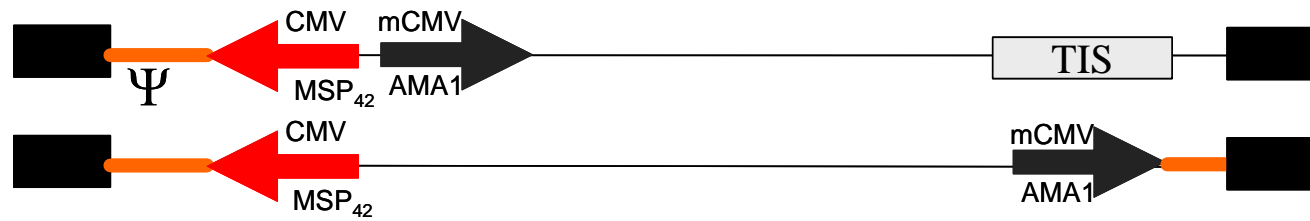


• Preferred Criteria for Clinical Lead Decision

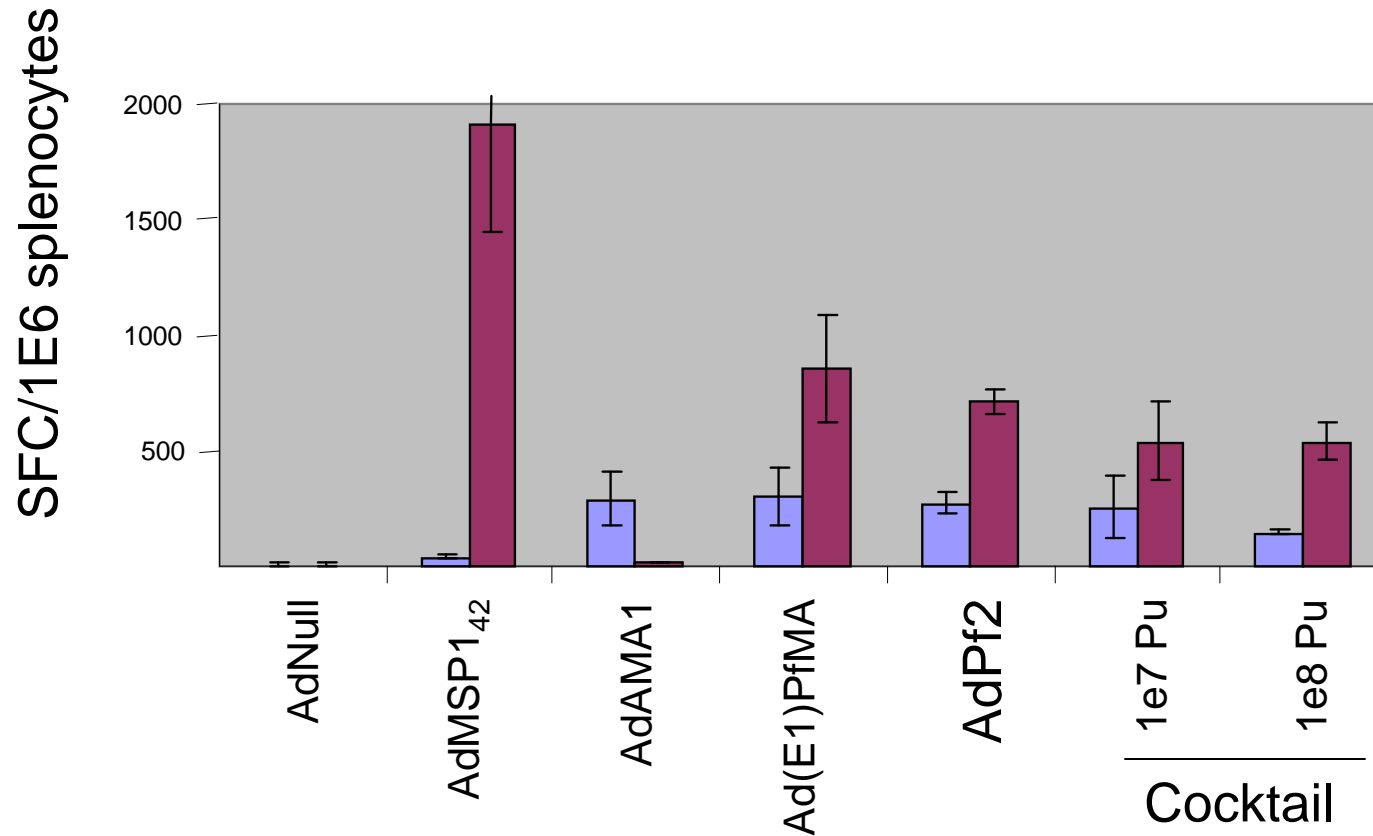
- Expression of both antigens *in vitro*
- Functional antibody responses (positive GIA) against both antigens in rabbits
- Antibody responses against both antigens in mice and rabbits
- T cell responses against both antigens in mice
- Production growth analysis showing high yield vector production
- Genetic stability of vectors after multiple passages

* = *lead selected for clinical development*

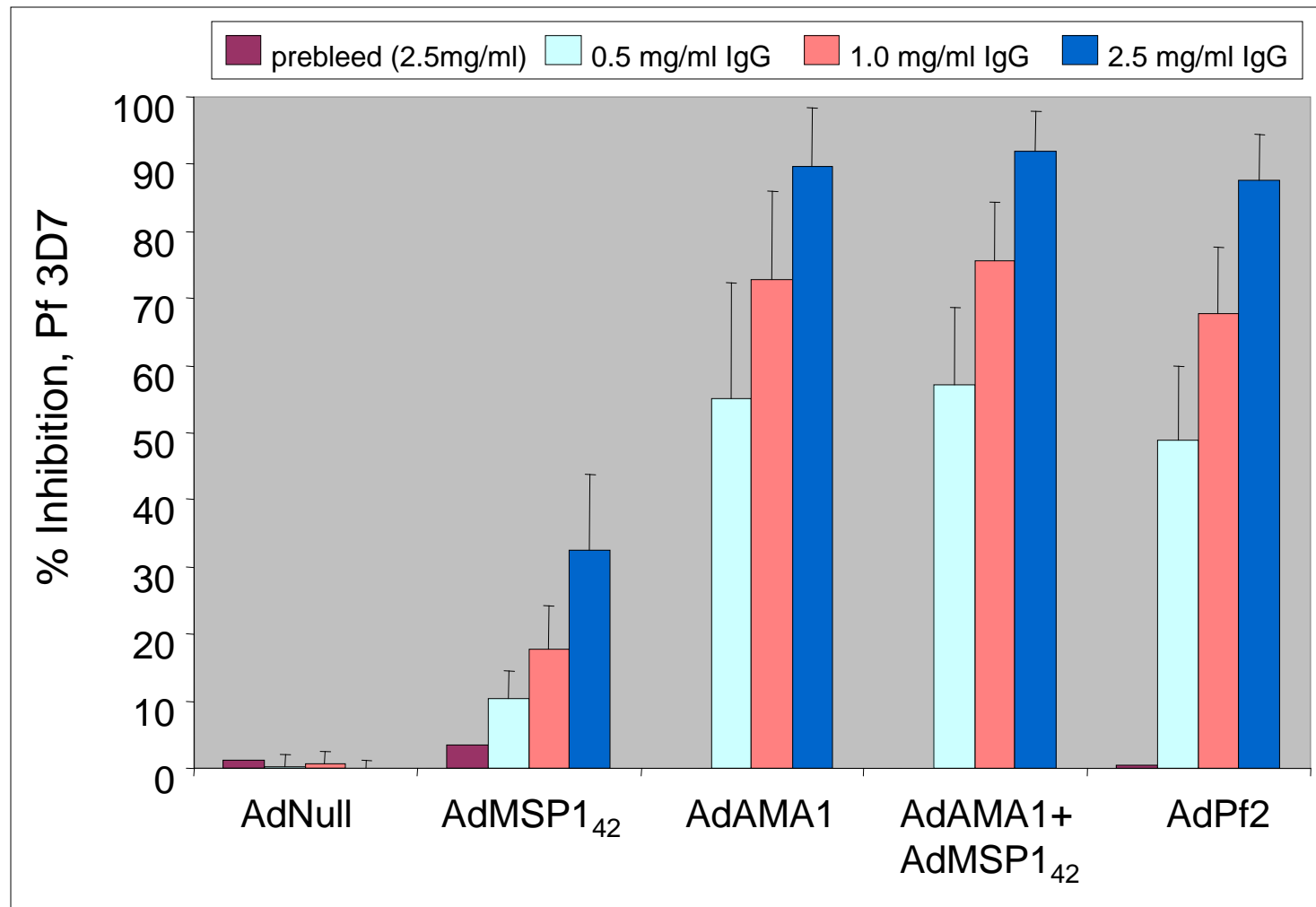
High Level Target Gene Expression



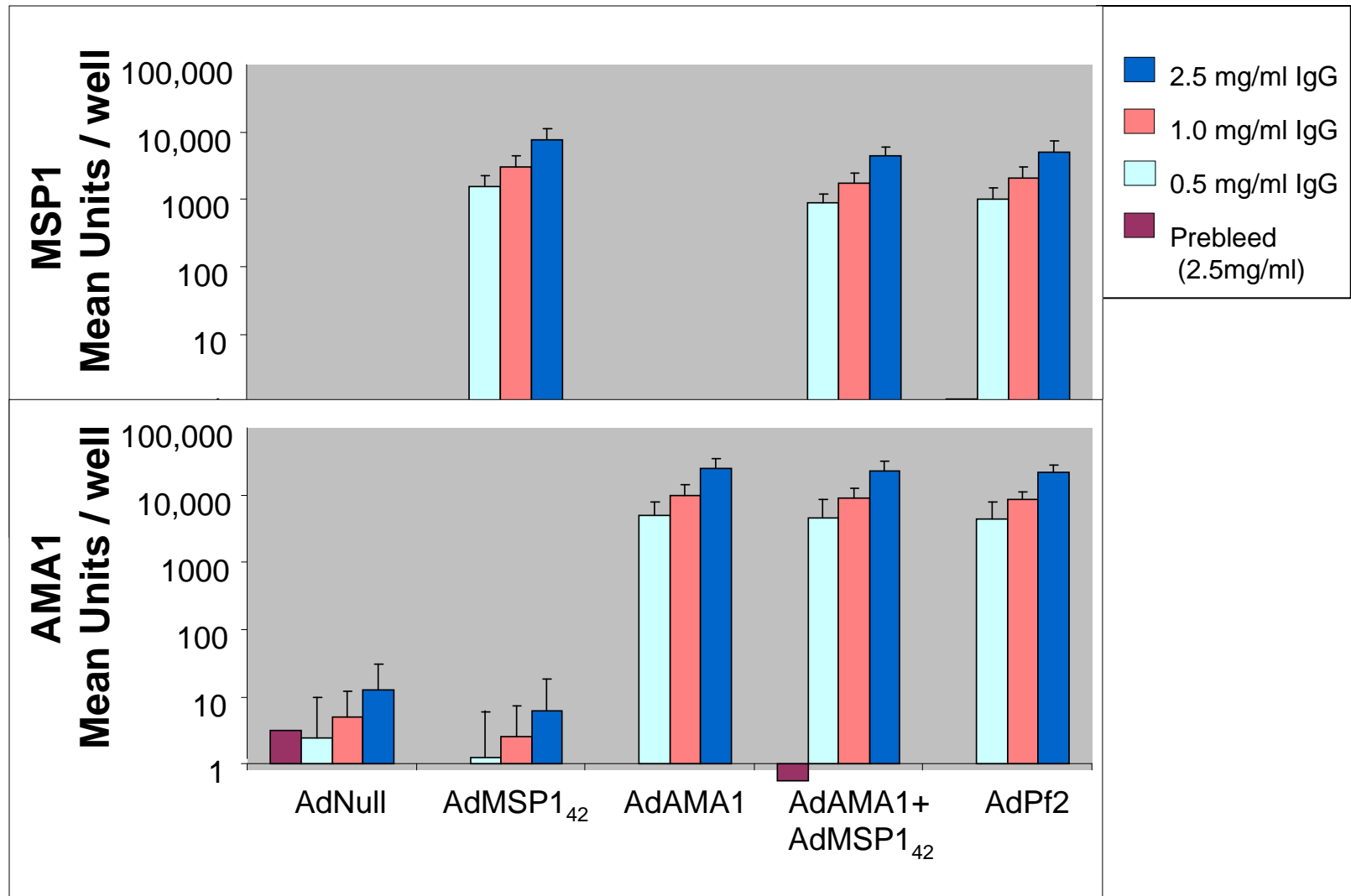
Blood Stage Vector (Pf2) Induces T cell Responses Specific for AMA1 and MSP1₄₂



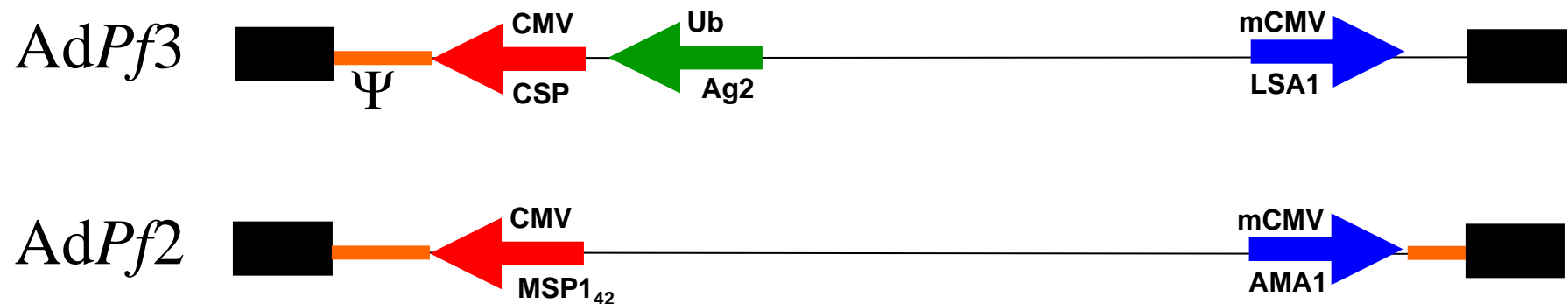
AdPf2 Induces Robust Functional Antibody Responses in Rabbits



AdPf2 Induces AMA1 and MSP1₄₂-Specific Antibodies in Rabbits



Multi-valent, Multi-stage Adenovector Malaria Vaccine Advancing to Clinical Development



- **High level of expression of 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)

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