# Construction and Characterization of Adenovirus Vectors Expressing Optimized Blood Stage Antigens of *Plasmodium falciparum*

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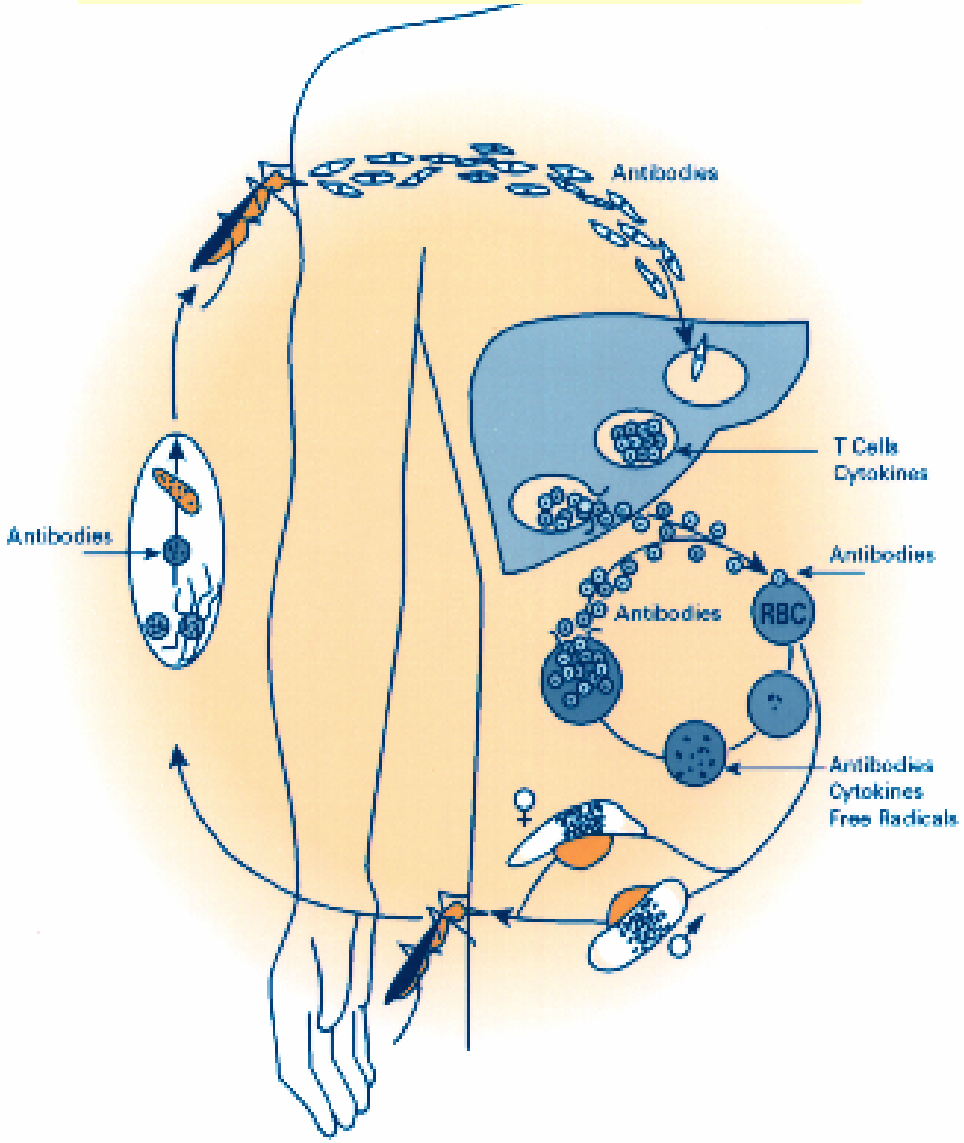
# MALARIA: World Health Problem

- 300-500 million cases / year
- 1 million deaths / year

# Malaria Vaccine Development

***Plasmodium Falciparum*** **Life Cycle**




**Goals of vaccine**

**1. Pre-erythrocytic Stage Vaccine**

**•Vaccine induces T cell responses against liver-stage parasite**

**2. Blood-Stage Vaccine**

**•Vaccine induces antibody responses against blood stage parasites**

**GenVec Vaccine Development**

**1.Advancing adenovector-based vaccine to clinic (two antigens)**

**2. Pre-clinical development of 2nd generation multivalent adenovirus vaccine**

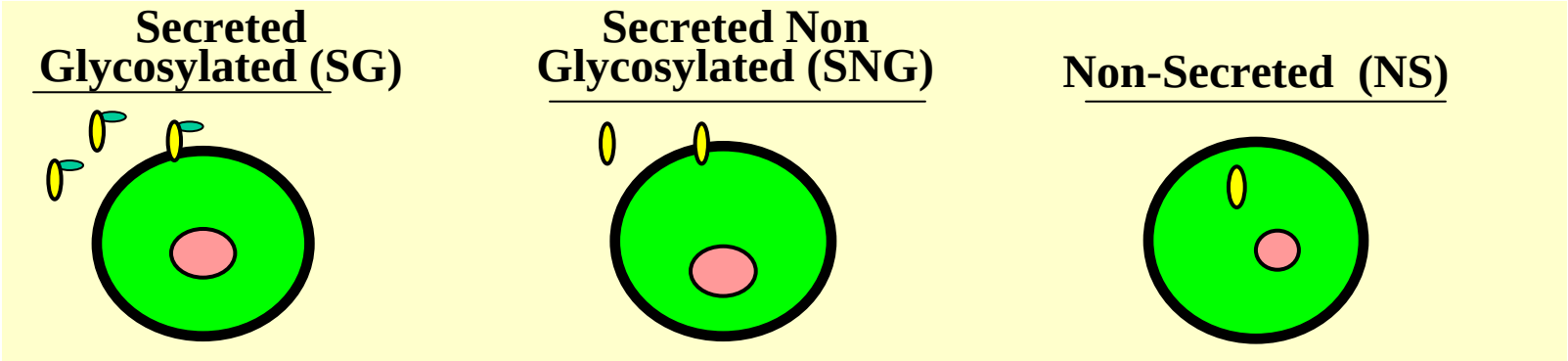
**3. Improving adeno-vaccine technology**

# Design of Blood Stage Antigens

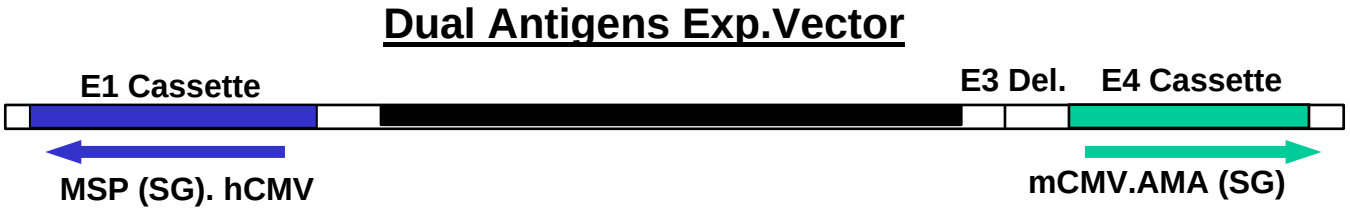
**MSP-1**: Merozoite Surface Protein-1; **AMA-1**: Apical Membrane Antigen-1

## I. Hypothesis

1. **Secreted or cell surface associated antigen will induce better immune response.**
2. **Non-glycosylated antigen will induce better immune responses**

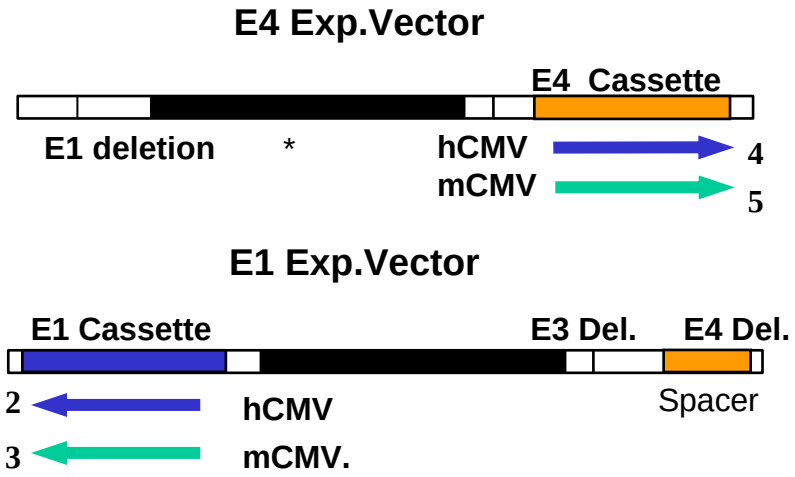


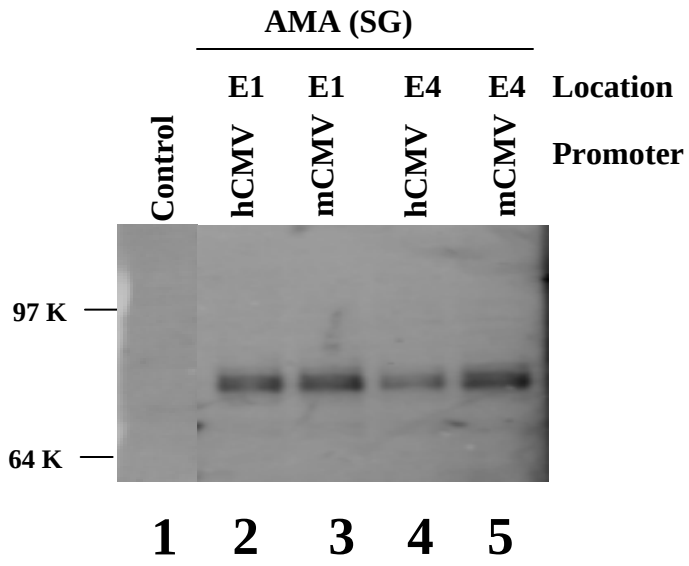

## II. Developing new adenovectors for two antigens



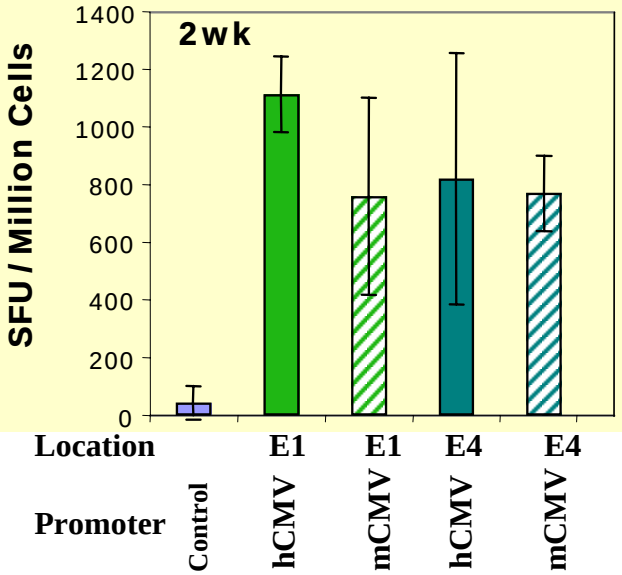
# Optimization of the Vector

## Vector Optimization

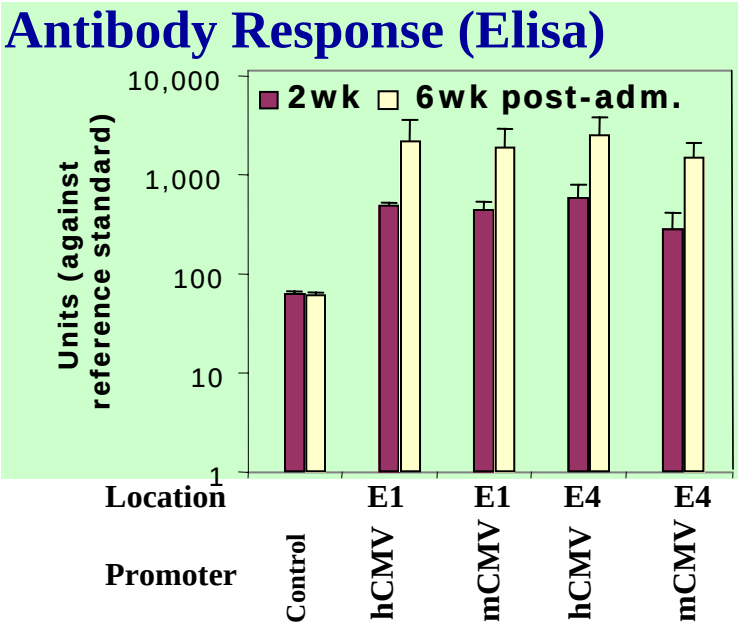



## T Cell Response (Elispot)

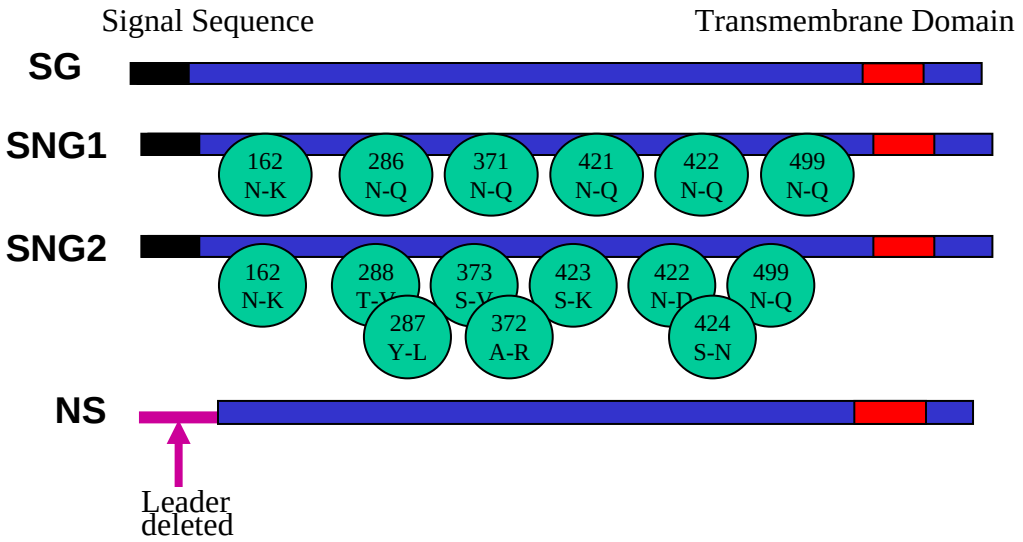


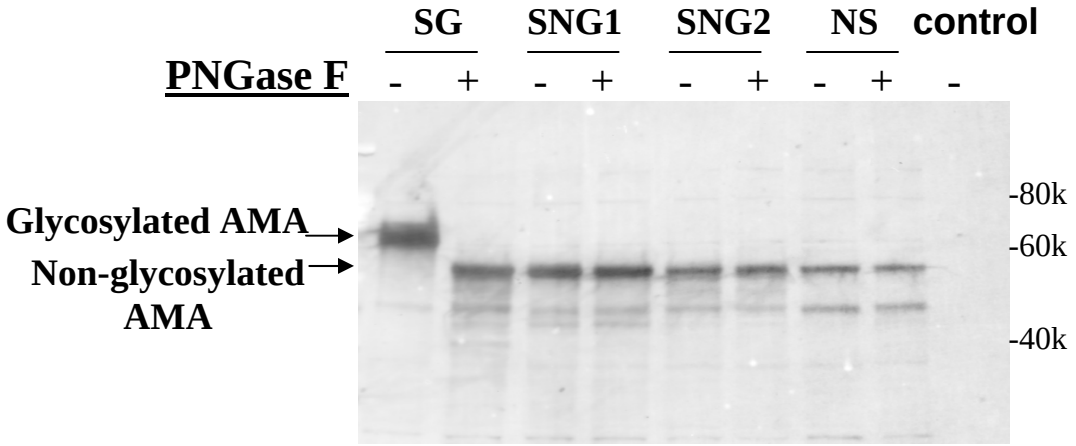

## Antibody Response (Elisa)



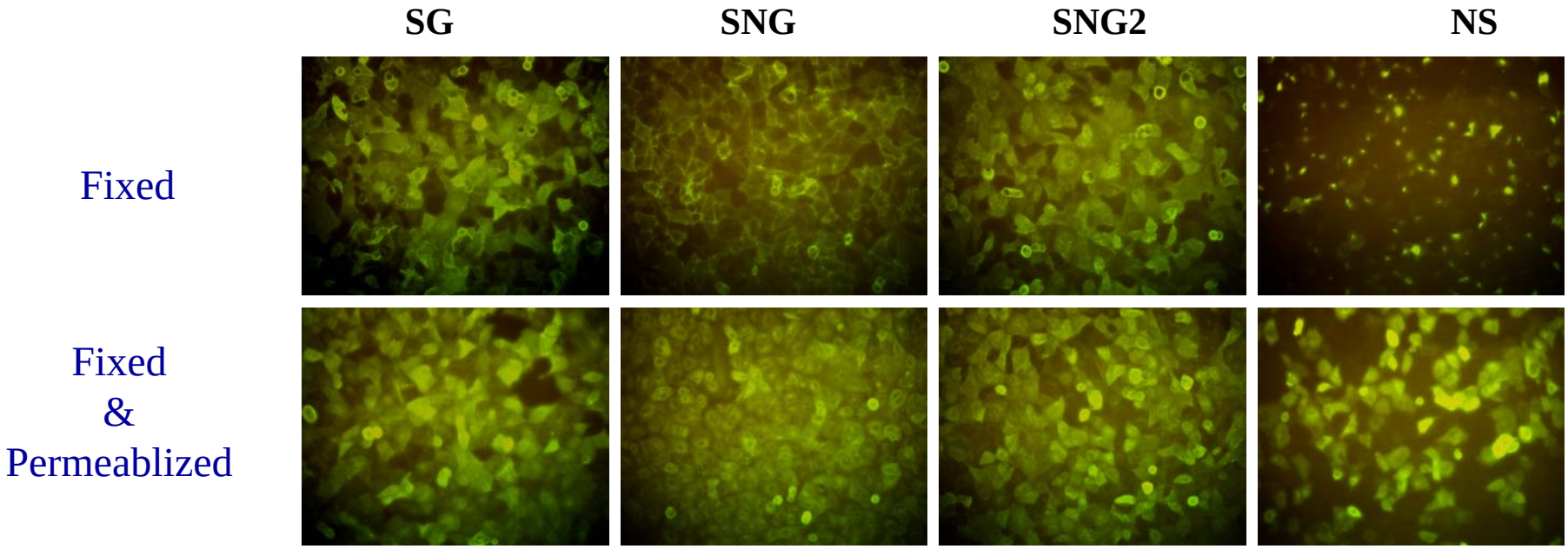
# *Pf*AMA Antigen Glycosylation Analysis

**AdPfAMA1 Vectors**



# Cell Surface Expression of *Pf*AMA

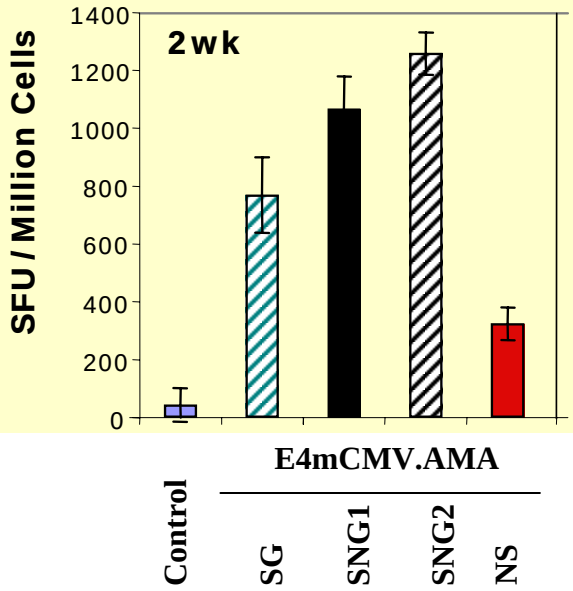



## Conclusion:

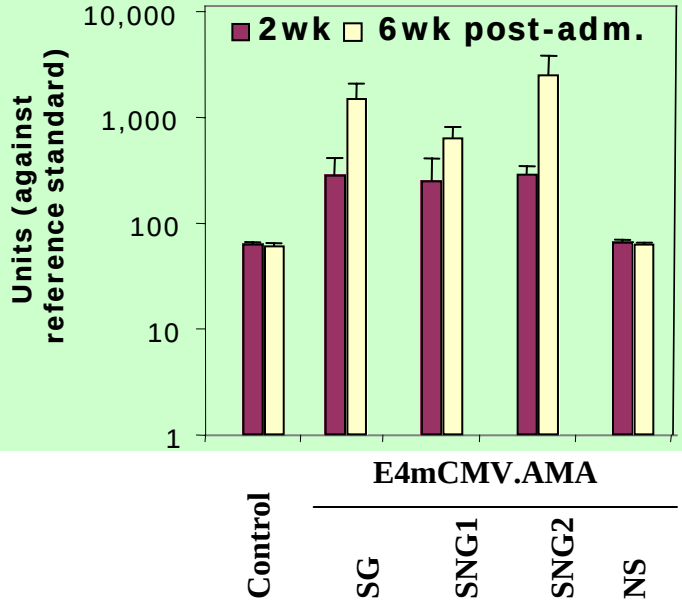
**All *Pf*AMA1 versions with the signal sequence appear to be located at the cell surface**

# *Pf*AMA1 Immunogenicity

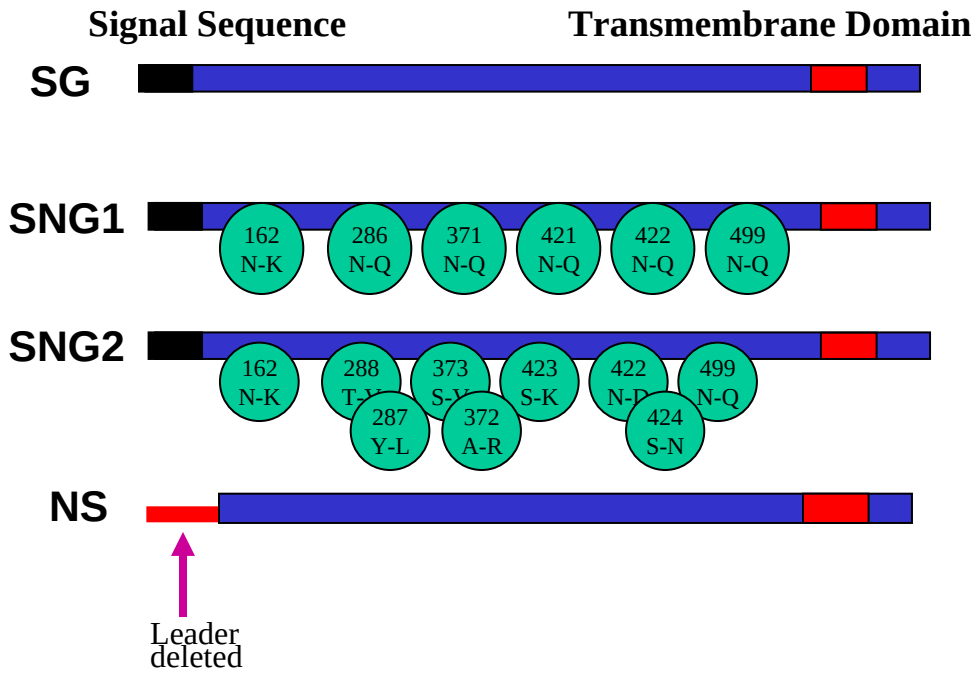
## T Cell Response (Elispot)




## Antibody Response (Elisa)

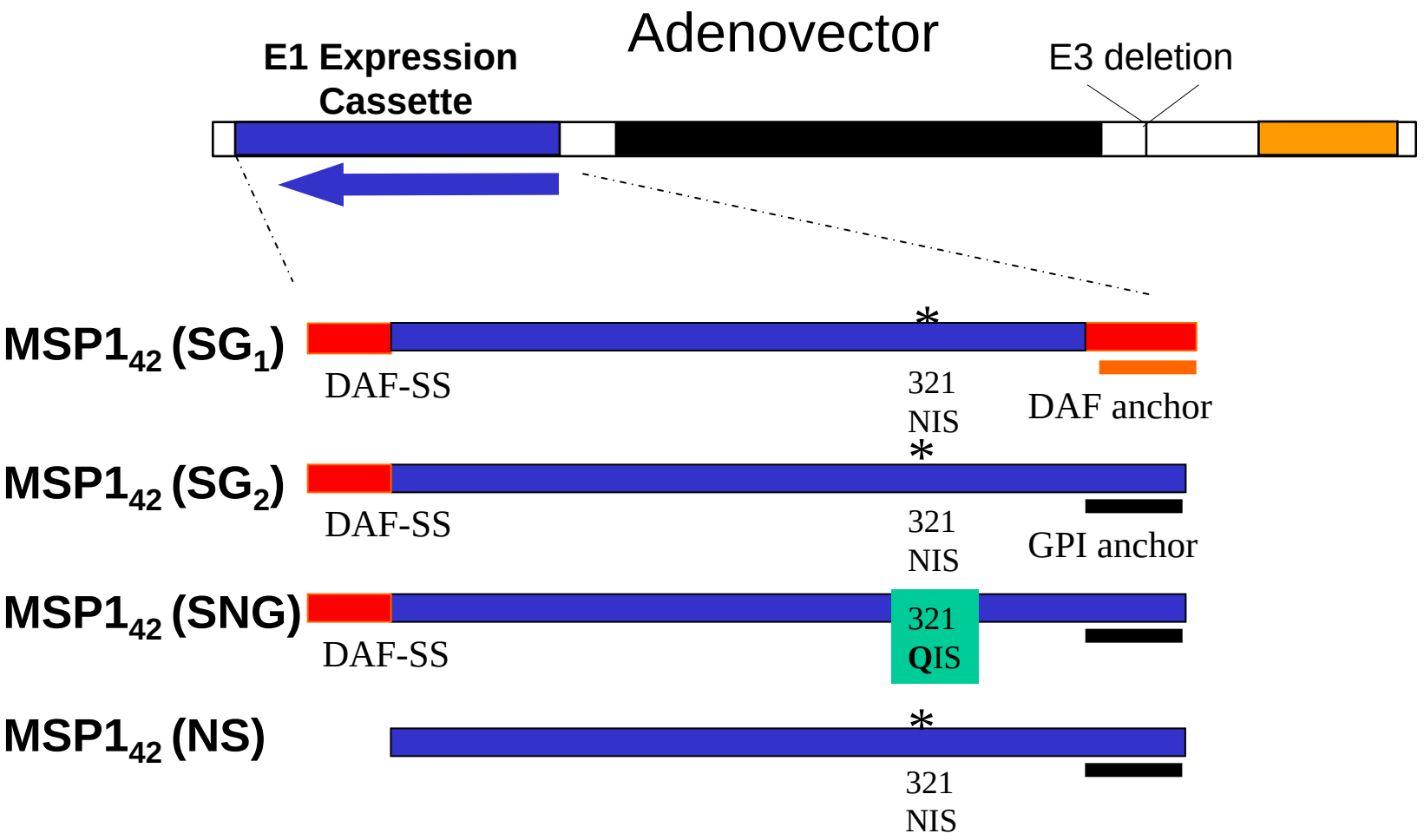


# Summary: Evaluation of *Pf*AMA1 Constructs

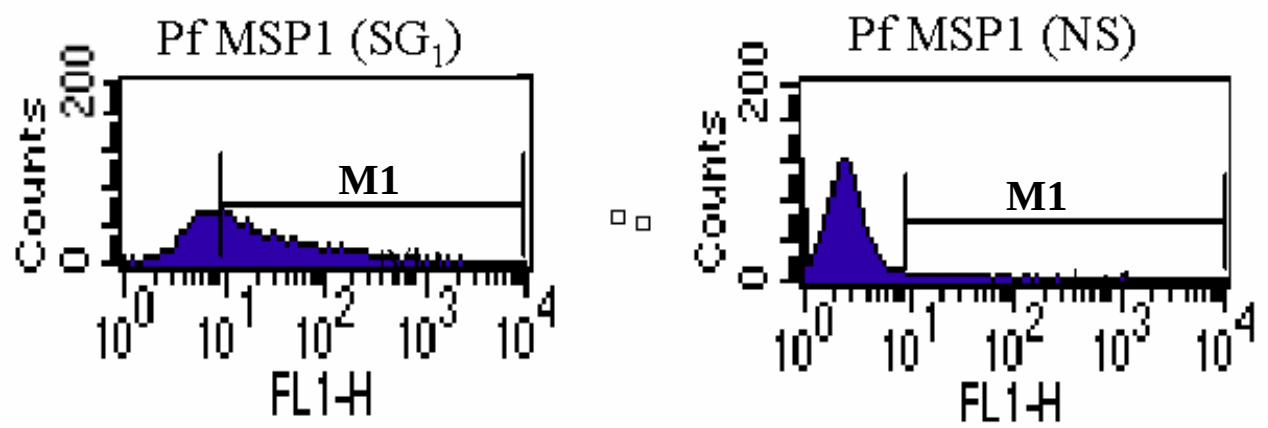



| N-Glycosylation | Cell surface | T cell Response | Ab Response |
|---|---|---|---|
| + | + | + | + |
| - | + | + | + |
| - | + | + | + |
| - | - | - | - |

# *Pf*MSP1$_{42}$ Optimization: Vector Design

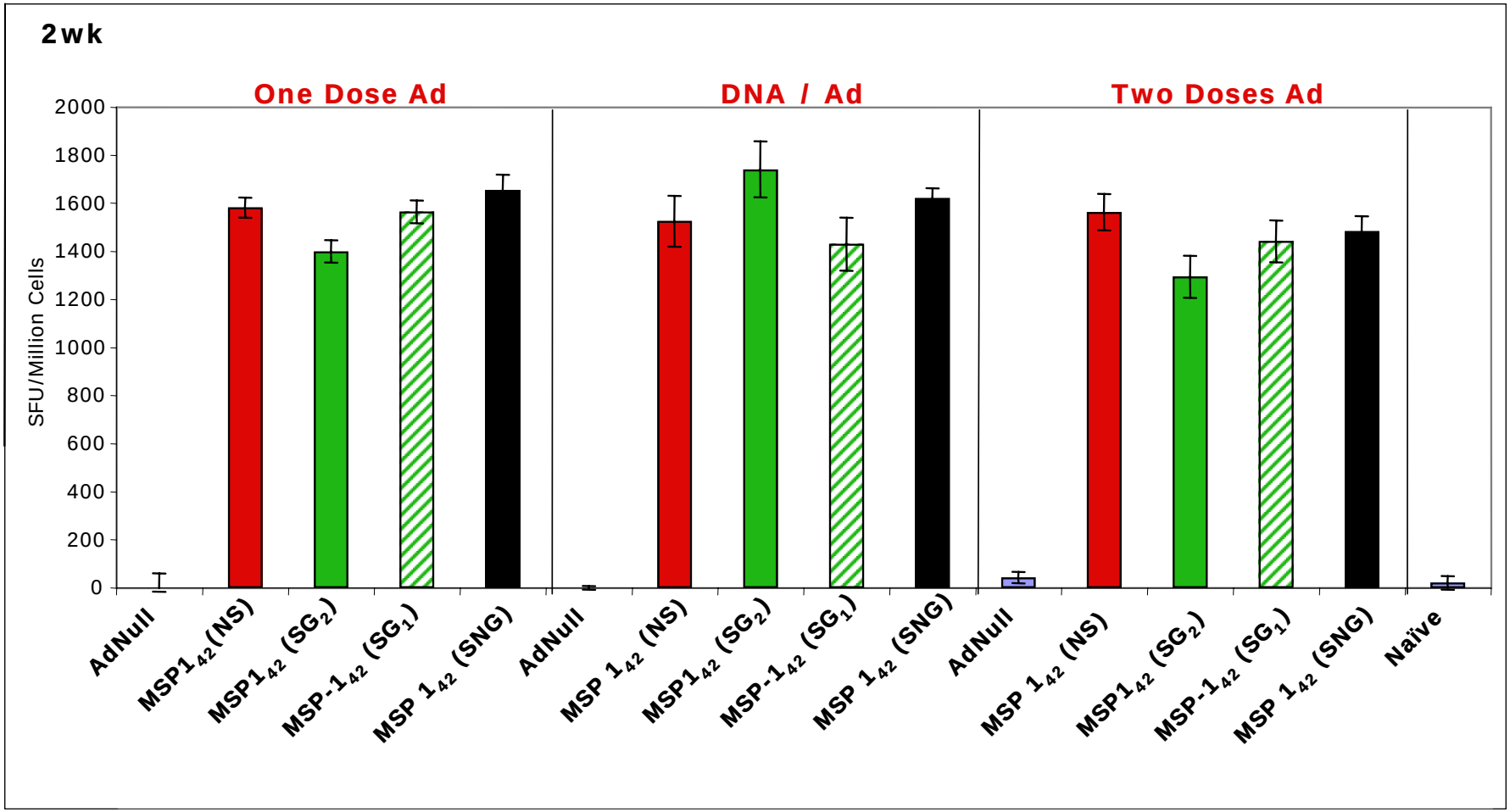


# *Pf*MSP1$_{42}$ Cell Surface Expression by FACS analysis

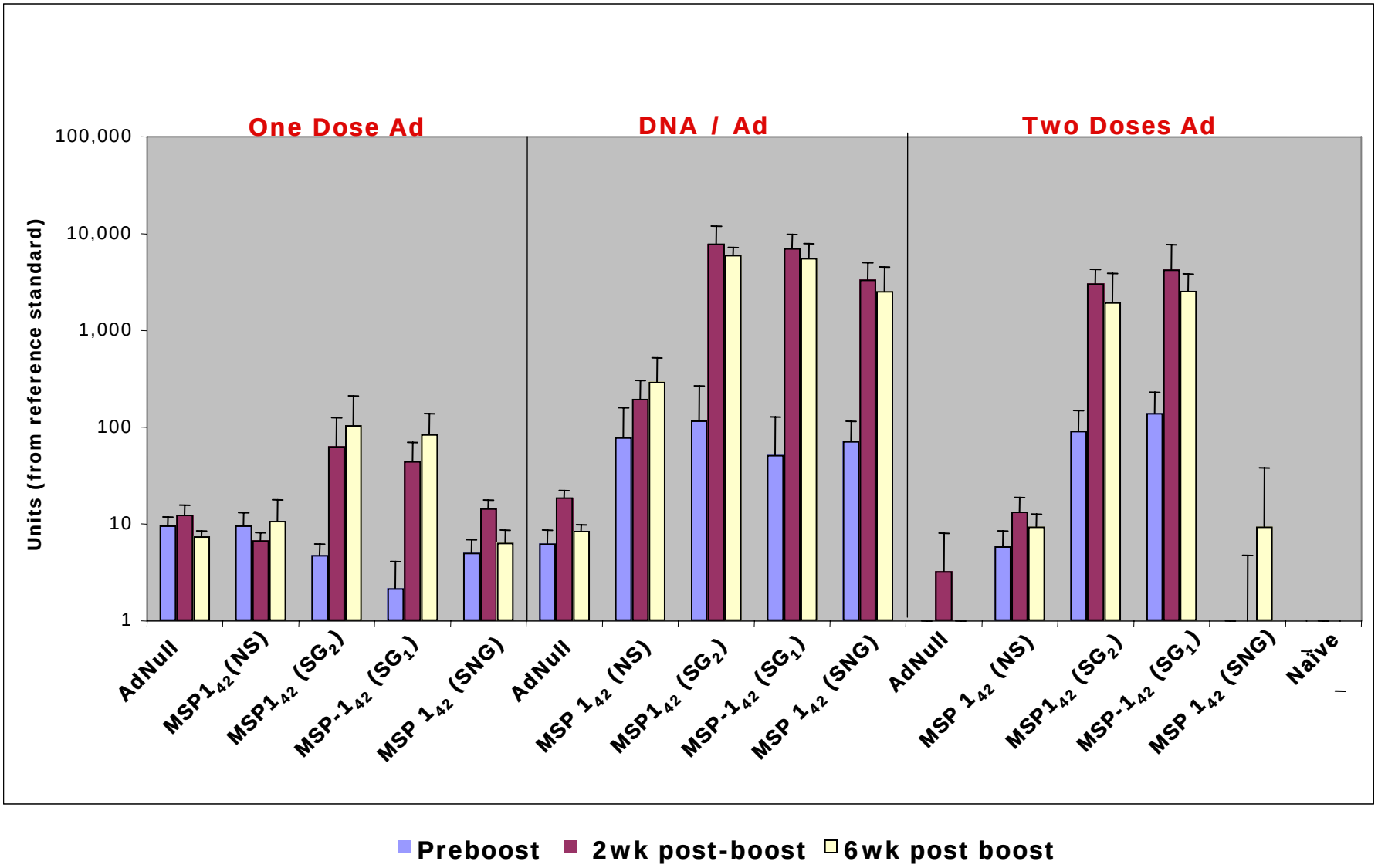



| Vector Name | M1 |
|---|---|
| Add2.11E4.PfAMA1(SG) | 0.69 |
| Adf.11D | 89.5 |
| MSP1$_{42}$ (SG$_1$) | 58.24 |
| MSP1$_{42}$ (SG$_2$) | 11.11 |
| MSP1$_{42}$(SNG) | 3.47 |
| MSP1$_{42}$(NS) | 1.75 |

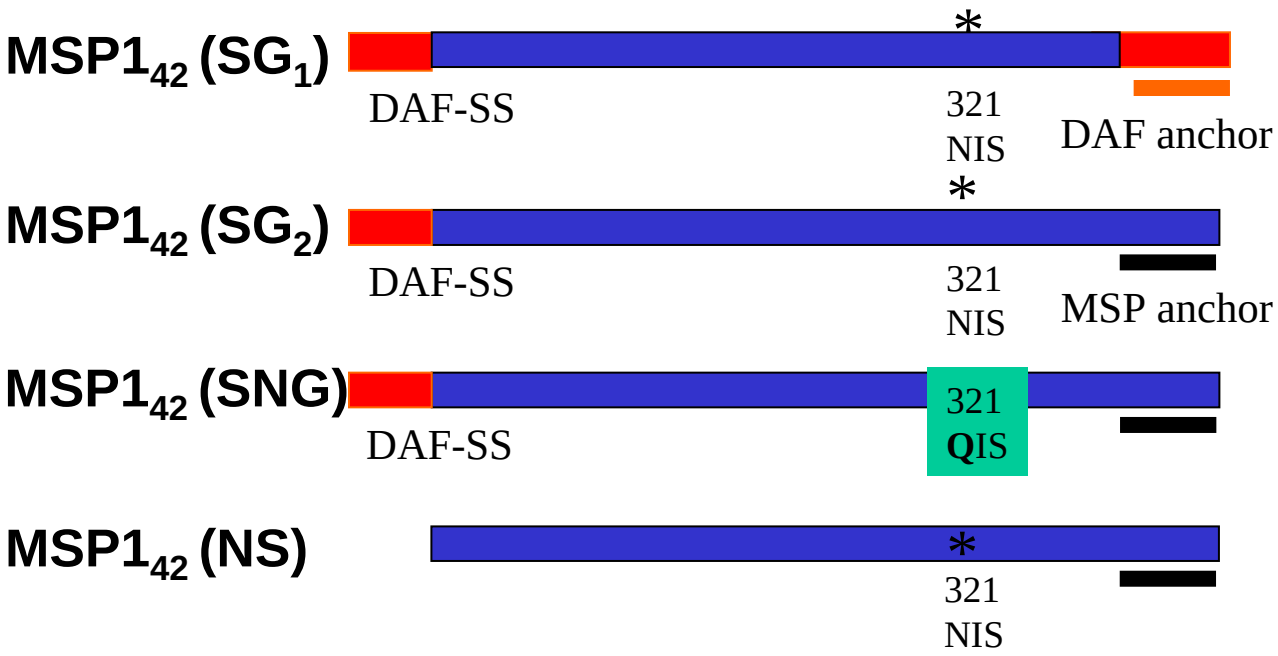
# *Pf*MSP1$_{42}$ T Cell Response



# *Pf*MSP1 Antibody Response



# Summary: Evaluation of *Pf*MSP1$_{42}$ Constructs




| | N-Glycosylation | Cell surface | T cell Response | Ab Response |
|---|---|---|---|---|
| MSP1$_{42}$ (SG$_1$) | + | + | + | + |
| MSP1$_{42}$ (SG$_2$) | + | +/- | + | +/- |
| MSP1$_{42}$ (SNG) | - | -/+ | + | -/+ |
| MSP1$_{42}$ (NS) | - | - | + | - |

# Conclusion

- Cell surface associated *vs* intracellular antigen
  - –Higher antibody responses
  - –Higher T-cell responses for *Pf*AMA1
- Glycosylation
  - –Removing glycosylation sites did not improve T cell or antibody responses
- Location of antigen genes in adenovector.
  - –Similar expression level and immunogenicity in E1 and in E4
- Human CMV promoter and mouse CMV promoter
  - –Similar expression level and immunogenicity

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