

Respiratory Syncytial Virus (RSV) Pre-fusogenic F Nanoparticle Vaccine Compared to Pre-fusion F and Post-fusion F Proteins

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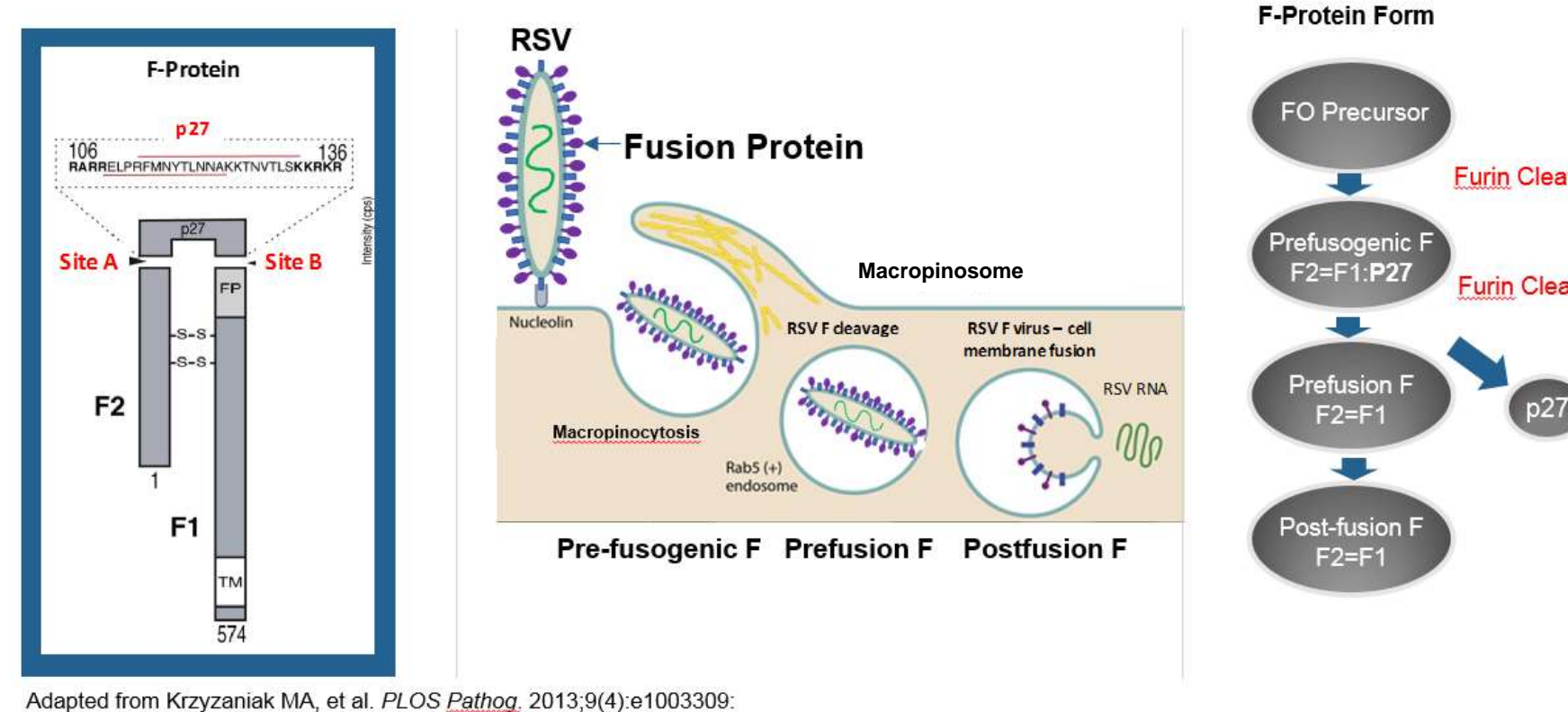
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BACKGROUND

Respiratory syncytial virus (RSV) is the leading viral cause of severe lower respiratory tract disease in infants and young children worldwide. RSV fusion (F) envelope glycoprotein is a major target for vaccine development. RSV F is subject to significant rearrangements; precursor (F₀) is cleaved by furin forming a fusion inactive F₂-p27F₁ intermediate (Pre-fusogenic F). A second furin cleavage releases a p27 peptide to form metastable pre-fusion F (Pre-F), which upon rearrangement from a 3-helix to 6-helix bundle post-fusion F configuration (Post-F) drives fusion of viral and cellular membranes. Novavax has developed a near full-length RSV Pre-fusogenic F vaccine composed of purified F₂-p27F₁ trimers formulated with polysorbate 80 (PS80) as stable 40nm protein – detergent nanoparticles. ResVax (Pre-fusogenic RSV F nanoparticle vaccine) is currently being assessed in the Prepare™ (Phase 3) trial for the protection of infants via maternal immunization in healthy third trimester pregnant women.



OBJECTIVES

- Characterization of RSV Pre-fusogenic F nanoparticle vaccine, RSV Pre-F, and Post-F produced in Sf9 cells:
 - Hydrodynamic properties AUC and DLS.
 - Single particle CryoEM at 5 Å resolution.
 - Monoclonal antibody binding to antigenic sites Ø and VIII (pre-fusion specific) and sites II, IV, II/IV, and p27.
- FACS analyses of recombinant, membrane associated RSV F:
 - Insect Sf9 cells: RSV Pre-fusogenic F and Pre-F.
 - Human 293T: RSV Pre-fusogenic F and wild type F.
- RSV Pre-fusogenic, Pre-F, and Post-F immunogenicity in mice.

METHODS

- RSV Pre-fusogenic F (GMP drug substance), Pre-F (single-chain triple mutant; SCTM), and Post-F were produced in Sf9 cells.
- Hydrodynamic properties purified RSV F antigens were measured using analytical ultracentrifugation (AUC) and dynamic light scattering (DLS).
- Single particle CryoEM of a 72-aa C-terminal truncated Pre-fusogenic F was compared to Pre-F and Post-F at 5 Å resolution.
- Antigen binding of mAbs specific for pre-fusion sites Ø and VIII and sites II, IV, II/IV and p27 using Octet 384 Bio-Layer Interferometry and dot blot.
- FACS cell surface expressed RSV Pre-fusogenic F and Pre-F on Sf9 cells and Pre-fusogenic F and wild type RSV/A2 F on human 293T cells.
- Immunogenicity 1µg and 10µg antigens w/w/o AIPO₄ adjuvant in mice.

RESULTS

Fig 1. RSV Pre-fusogenic, Pre-F, Post-F Proteins

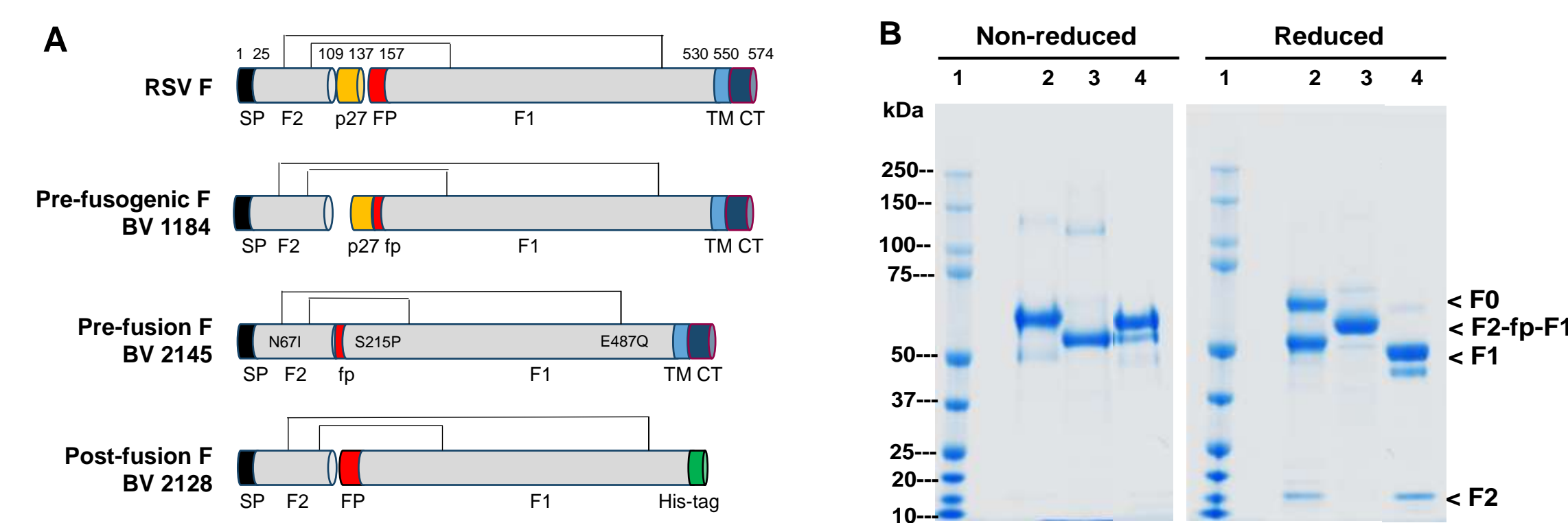
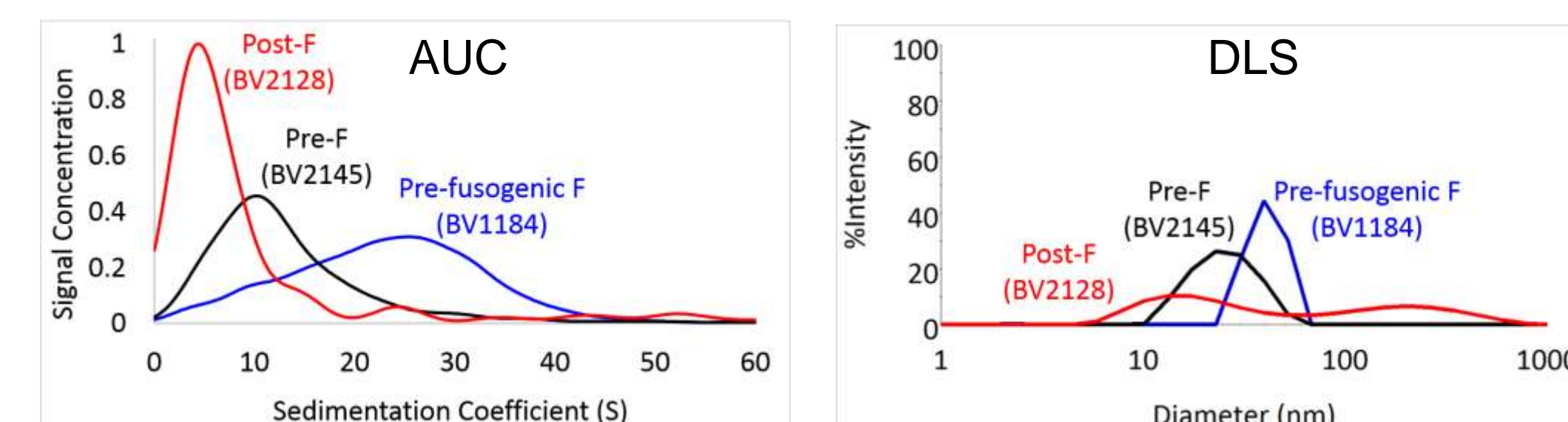


Fig 2. Hydrodynamic parameters RSV F constructs: AUC and DLS



Hydrodynamic parameters of RSV fusion glycoprotein constructs

RSV F proteins	SV-AUC		DLS		
	S ⁰ 20°C,w _v Svedberg	M _w app kDa ²	f/f ₀ 20°C,w	Z-average (nm)	PDI
Pre-fusogenic F	23.3 S	1,146	1.11	40	0.17
Pre-fusion F	13.7 S	710	1.12	23	0.15
Post-fusion F	5.0 S	123	1.24	28	Multi-modal

Fig 3. Single Particle CryoEM RSV Pre-fusogenic F 5 Å Resolution

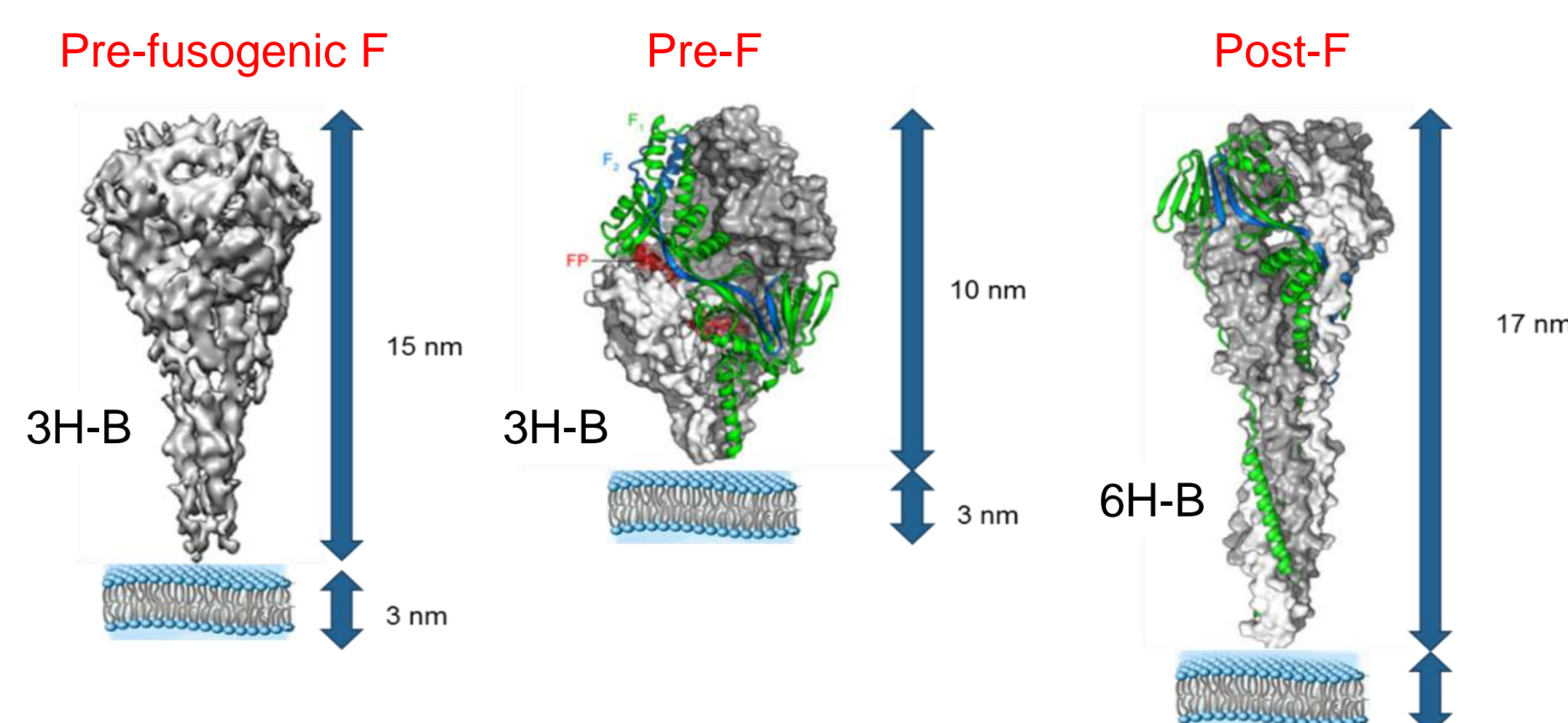
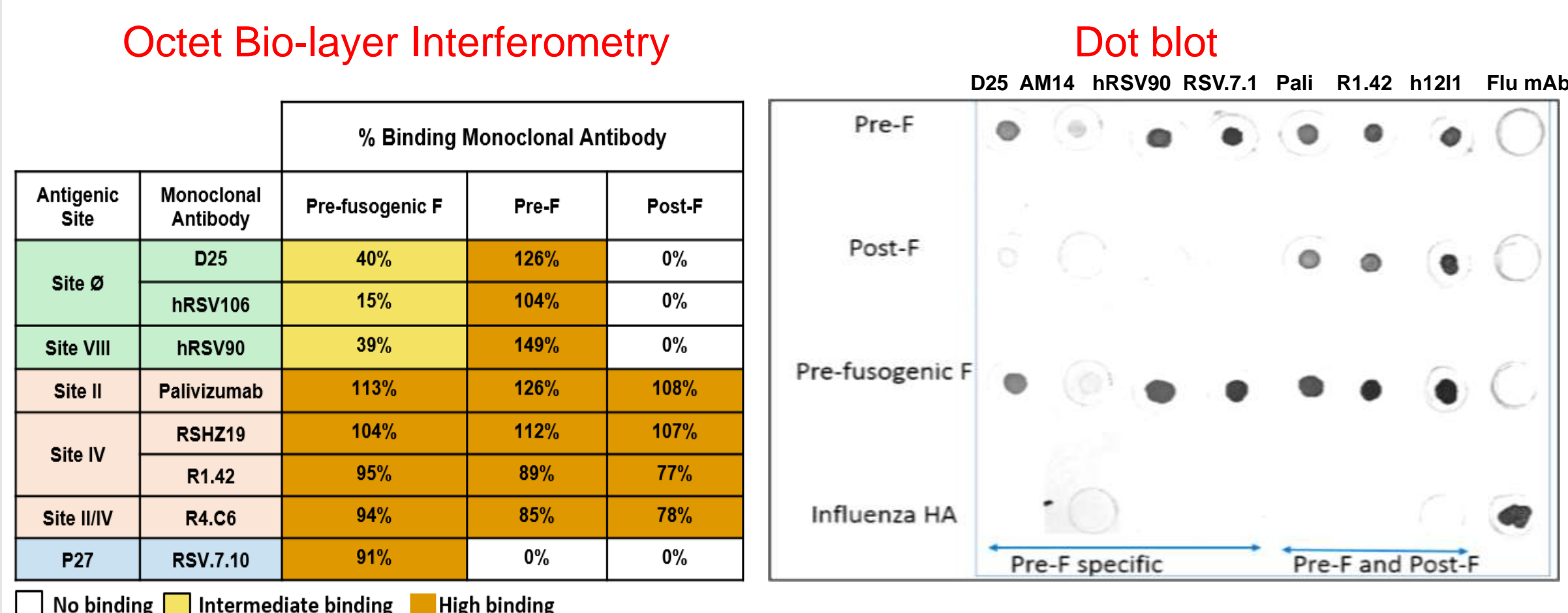


Fig 4. Bio-layer interferometry and dot blot of RSV F mAb binding



RESULTS

Fig 5. A. FACS Pre-fusogenic F and Pre-F Sf9 cells

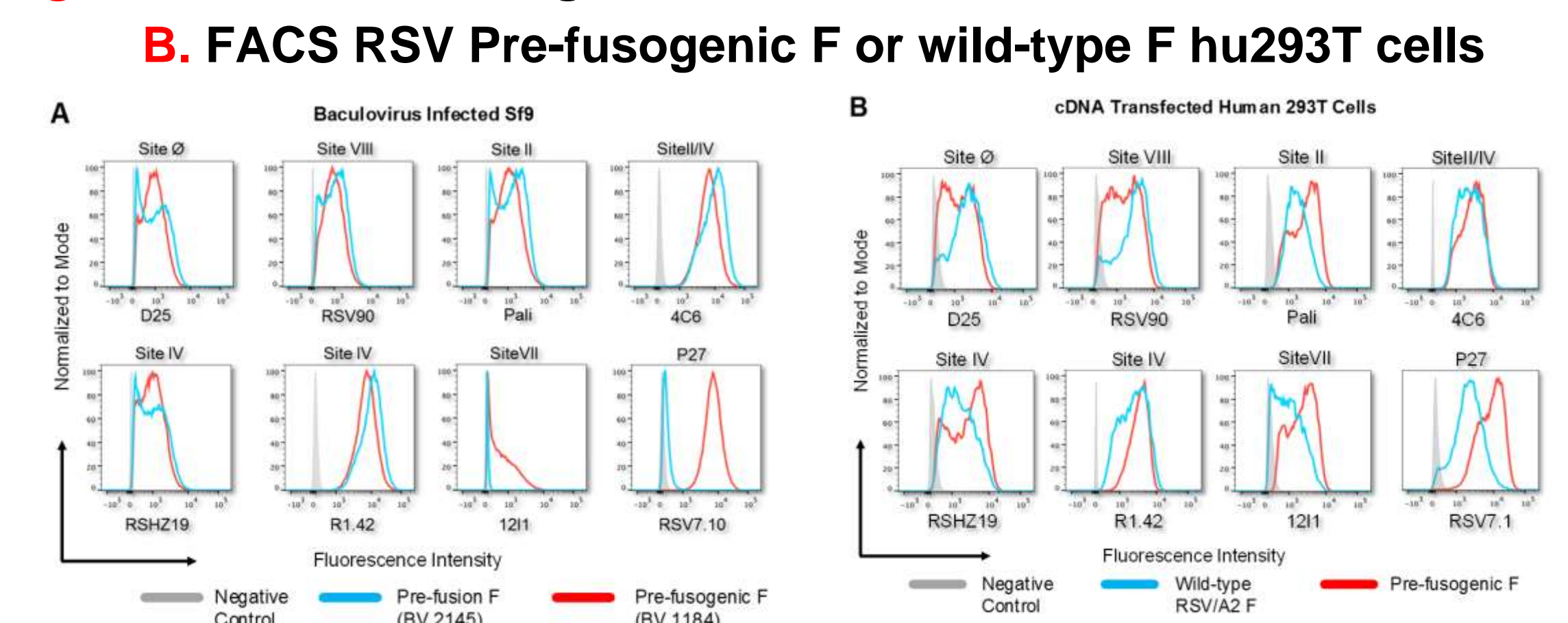
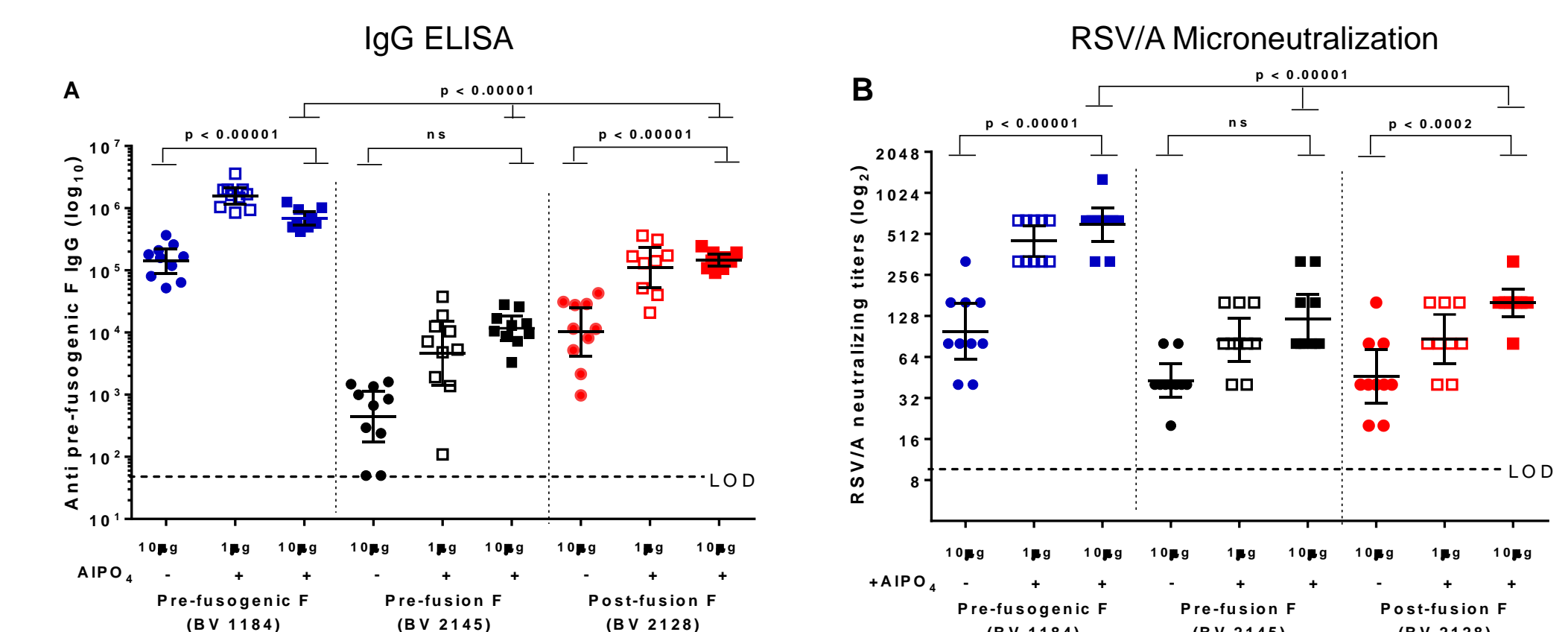


Fig 6. RSV F ELISA and Microneutralization responses in mice



CONCLUSIONS

- Pre-fusogenic F forms a distinct protein – PS80 detergent nanoparticle.
- Pre-fusogenic F with a 72aa truncation of the C-terminus form trimers with a 3helix-bundle stem and a length intermediate between Pre-F and Post-F.
- Pre-fusogenic F expressed on Sf9 and human 293T cells and when purified bind pre-fusion-specific, pre/post-fusion, and p27 monoclonal antibodies.
- Pre-fusogenic F nanoparticle vaccine without and with AIPO₄ adjuvant was significantly more immunogenic in mice than Pre-F and Post-F.

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