

# **A Novel Concept for Antiviral Vaccines and Therapies**

Luis J. Sigal  
Fox Chase Cancer Center  
Philadelphia

# Orthopoxvirus (OPV)

- DNA viruses,  $\approx 200$  Kb,  $\approx 200$  proteins
- The amino acid sequences among all OPV are highly conserved (90% overall)  $\rightarrow$  strong antigenic cross reactivity
- Host specificity  $\rightarrow$  very pathogenic only in the host species
- Variola virus, humans  $\rightarrow$  smallpox
- **Ectromelia** virus (ECTV), laboratory mouse  $\rightarrow$  mousepox
- Vaccinia virus (VACV). Poorly or non pathogenic in humans and mice  $\rightarrow$  Vaccine against both, smallpox and mousepox
- Cowpox virus  $\rightarrow$  First vaccine

# Advantages of the ECTV model

- ECTV is a mouse pathogen
- Mousepox is very similar to smallpox
- Mousepox can be prevented by immunization with VACV
  
- *C57BL/6 (resistant, when young)*  
Mechanism of natural resistance:  
Role of T cells, Abs, NK cells, aging etc.
  
- *Balb/c (susceptible)*  
Mechanisms of acquired resistance  
Role of Immune Response Modifiers in virulence.

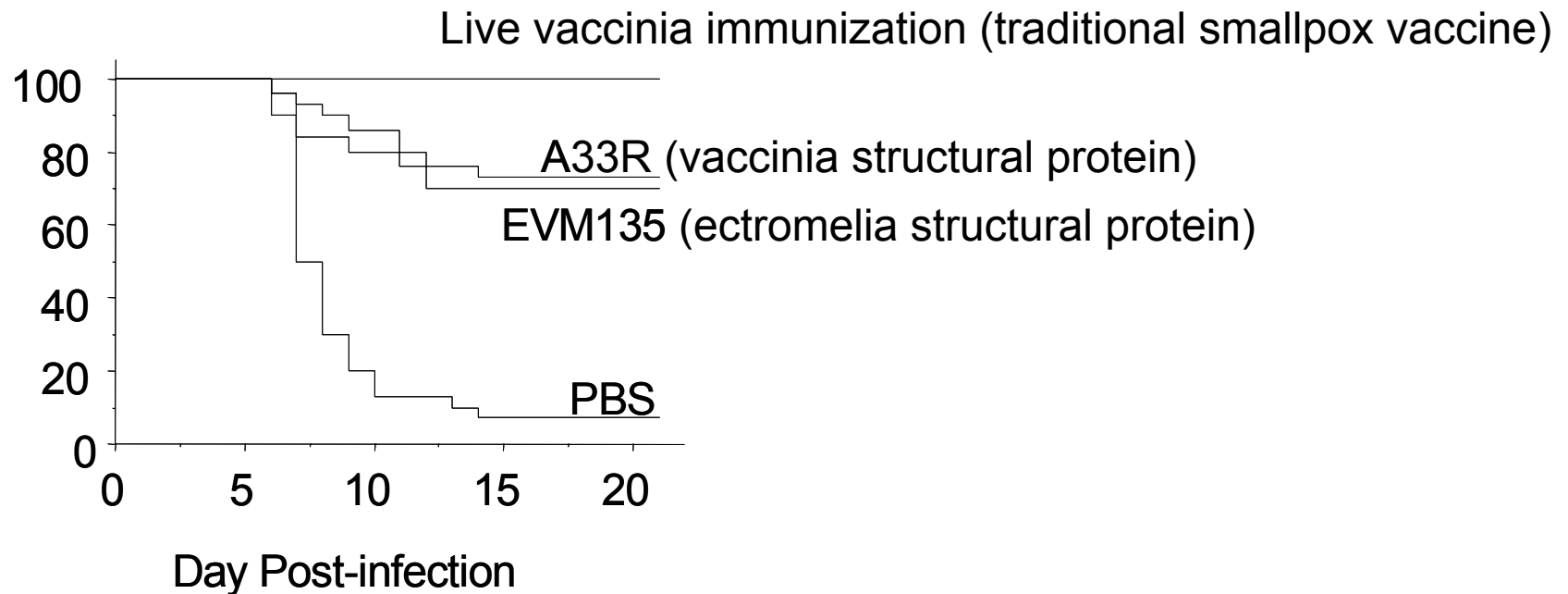
# Interferons (anti-viral cytokines)

- Type I ( $\alpha$  and  $\beta$ ): produced by all cells.  
Essential for the initiation of innate and adaptive immune response and for resistance to viral infections
- Type II ( $\gamma$ ) produced by immune cells  
Essential anti-viral effector arm of NK and T cells

# Subunit anti-viral vaccines

- Classic strategy: use structural proteins involved in virus entry

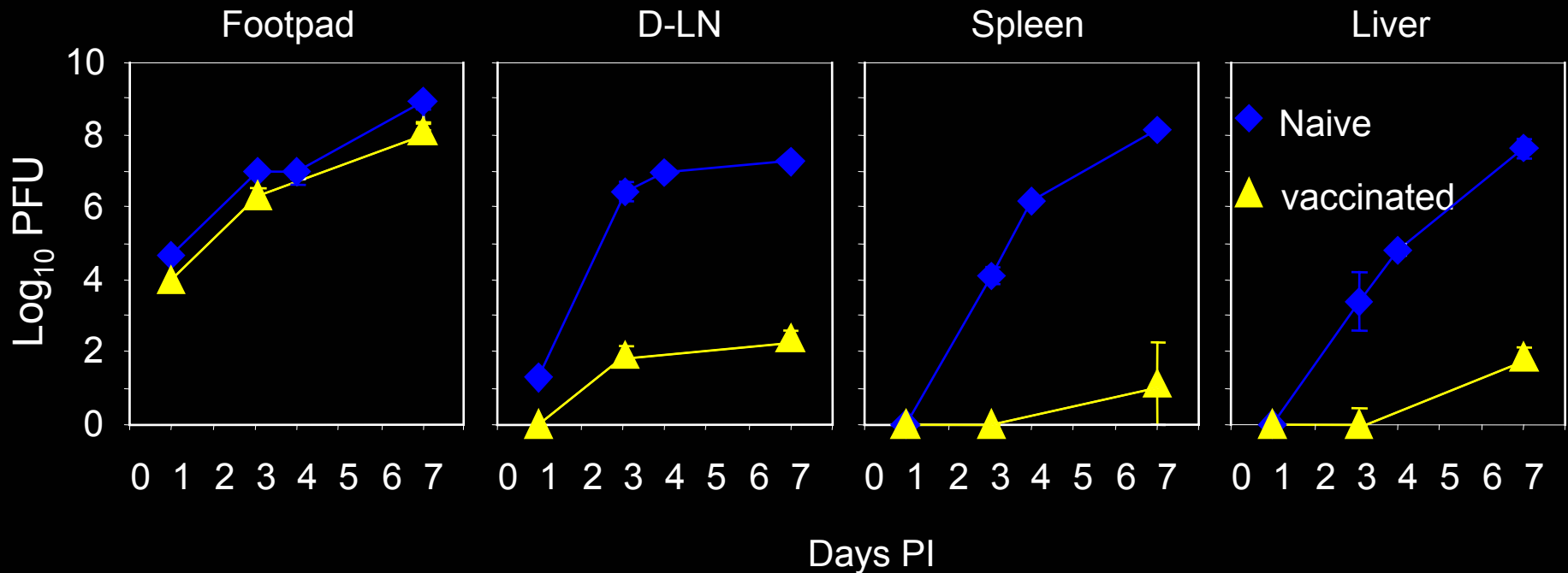
# Immunization with recombinant structural protein protects susceptible mice from lethal ECTV infection

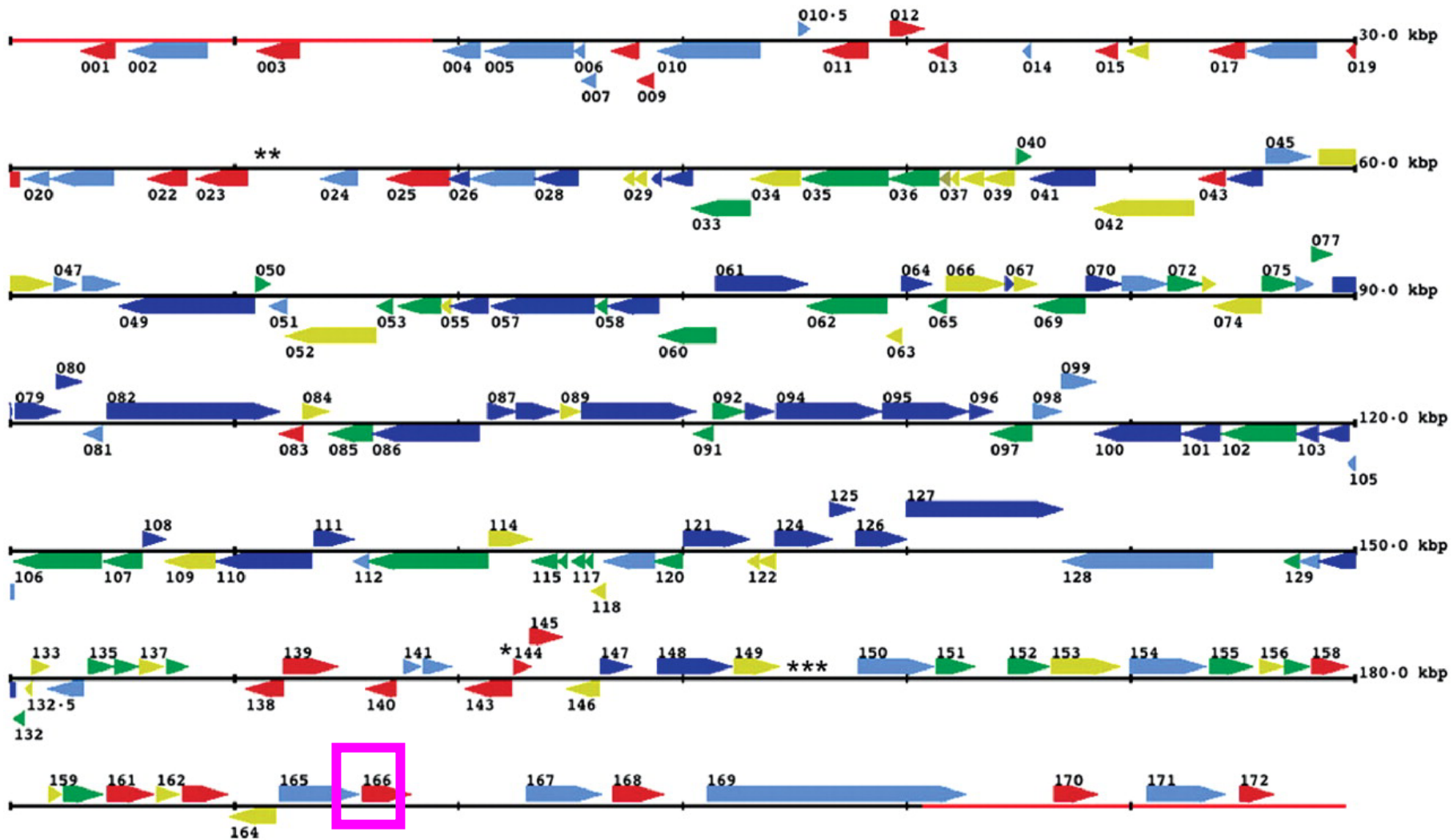


# Why structural proteins?

- Hope to induce sterilizing immunity
- But is this possible?

# The Smallpox Vaccine does not Protect from Systemic Infection

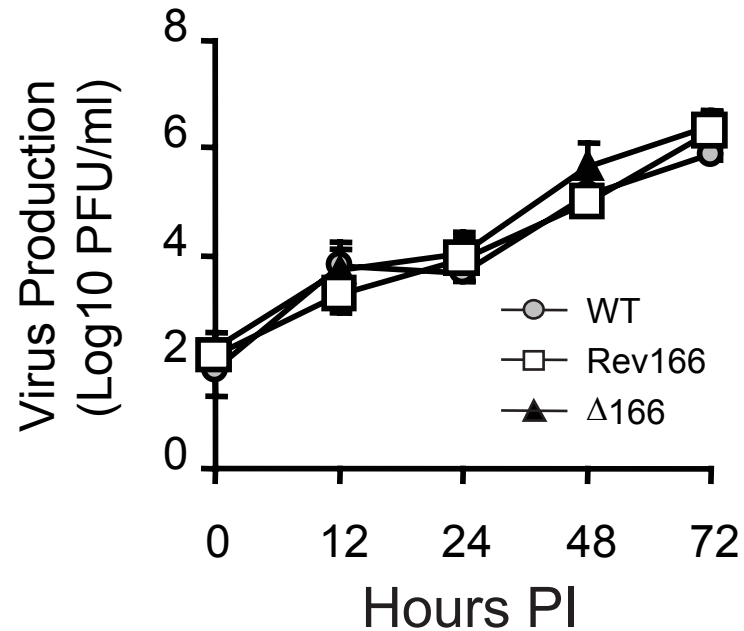




IFN- $\alpha$  bp

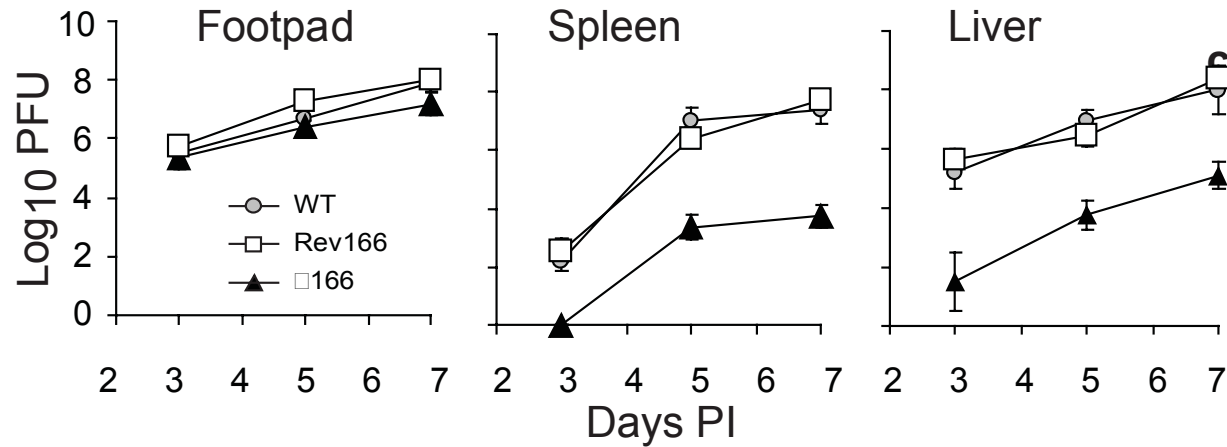
IL-1b bp

# In vitro characterization of $\Delta 166$ and Rev166

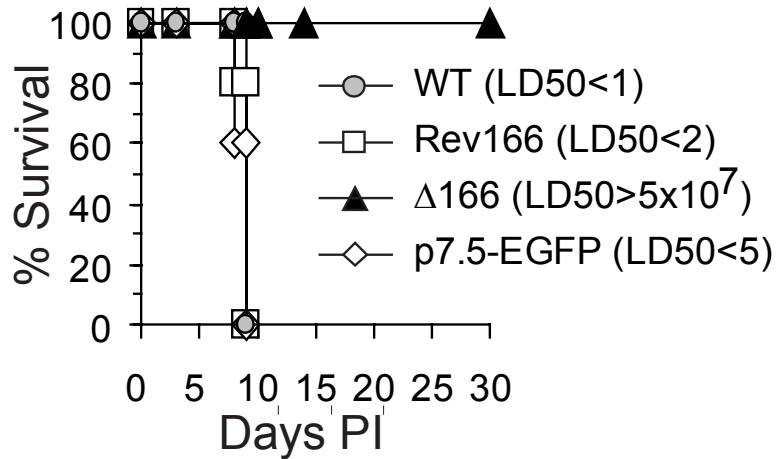


# ECTV $\Delta$ 166 is completely avirulent

a



c



# ECTV $\Delta$ 166 does not cause organ necrosis

Naïve

$\Delta$ 166

Rev166

D-LN

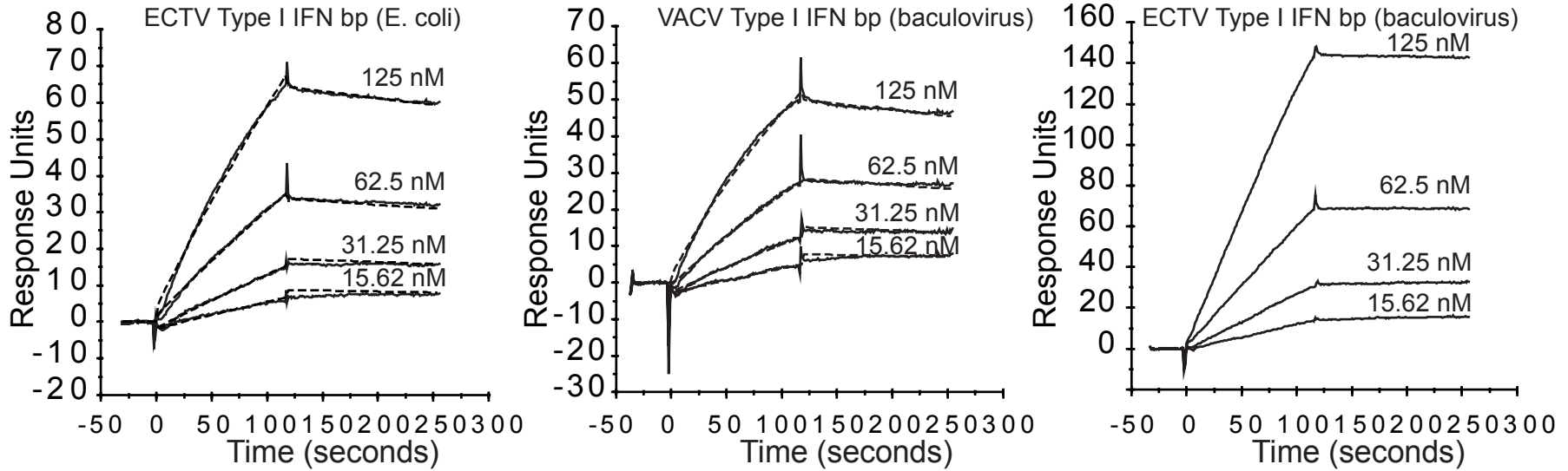
Spleen

Liver

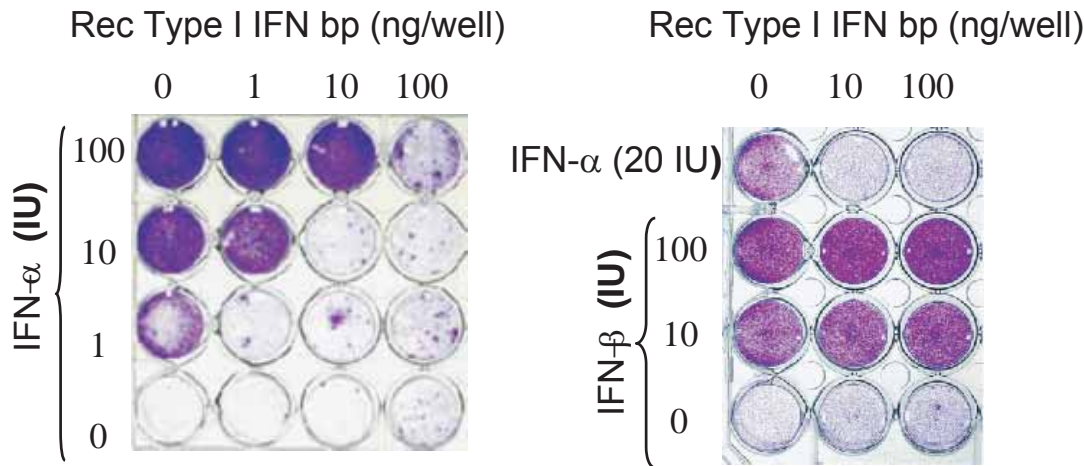


# Recombinant IFN- $\alpha$ bp is biologically active *in vitro* and *in vivo*

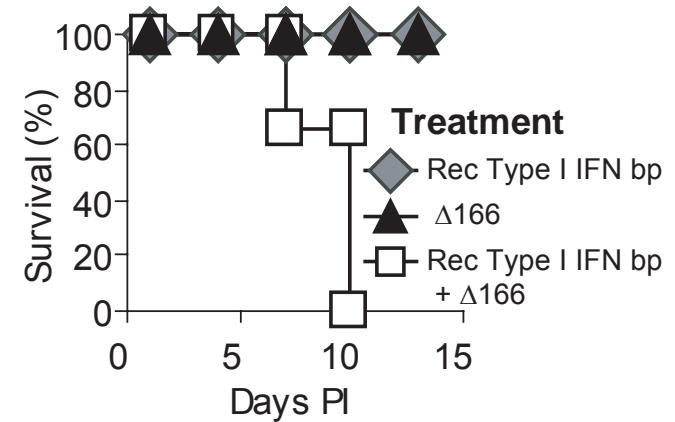
**a**



**b**

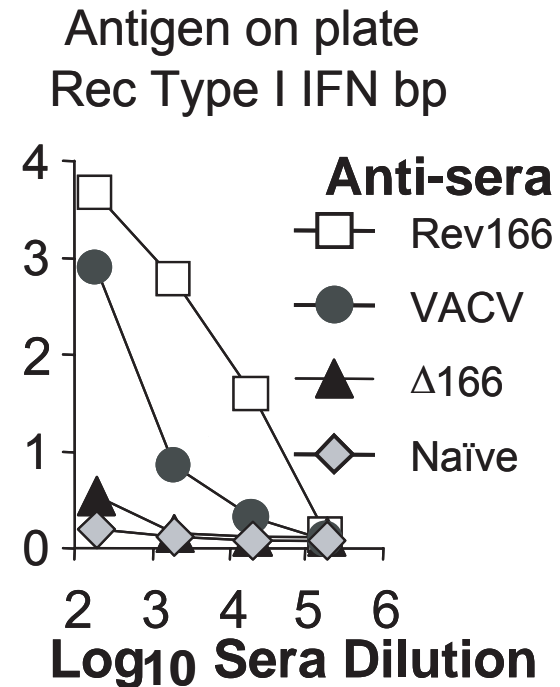
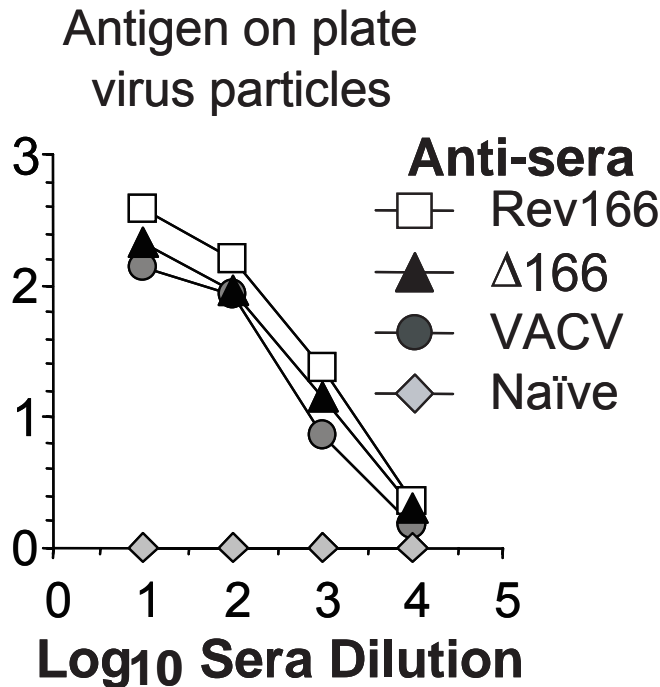


**c**



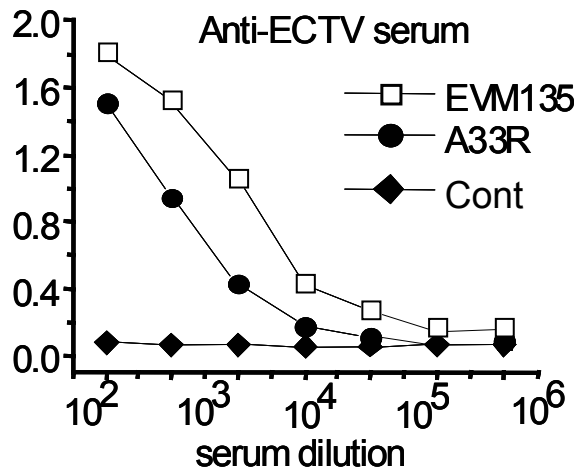
A recombinant protein that can  
be used to inactivate IFN- $\alpha$  *in*  
*vitro* and *in vivo*

# The ECTV and VACV IFN bp are natural targets of the antibody response and can be used to determine previous exposure

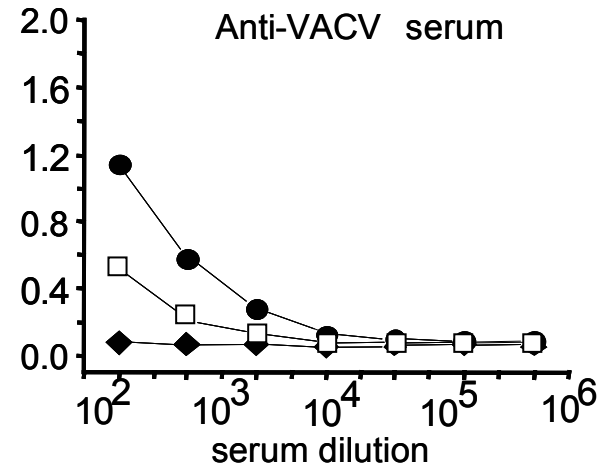


# And the same for the structural EVM135

A

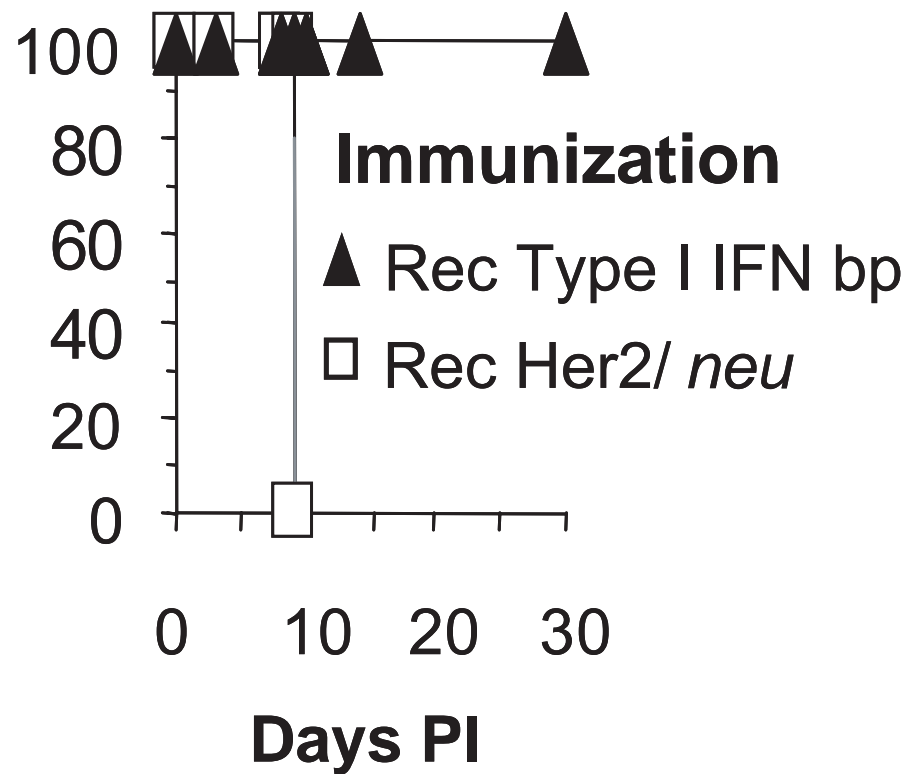


B



Recombinant proteins can be used to diagnose OPV exposure and determine species

# The OPV type I IFN bp can be used as an effective vaccine



**A recombinant virulence factor  
can be used as a vaccine**

# Summary

- Recombinant IFN- $\alpha$  bp from ECTV can be used to block IFN- $\alpha$  activity *in vitro* and *in vivo*
- Previous exposure to ECTV and other OPVs can be determined by ELISA using recombinant proteins rather than infected cell lysates (no BSL2 or vaccination required)
- Stronger reactivity of antiserum with proteins from the homologous virus can be used to identify the infecting OPV
- Virulence factors can be used as anti-OPV vaccines
- Virulence factors are excellent candidates for anti-OPV drugs