# The Pentameric Complex is Not Required in a Live Virus Vaccine for Protection Against Maternal Viremia and Congenital Transmission of Guinea Pig Cytomegalovirus (GPCMV)

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CMV
CONGENITAL CYTOMEGALOVIRUS
PUBLIC HEALTH & POLICY
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# Congenital Cytomegalovirus (CMV)

- 0.5 - 1% cCMV birth prevalence USA[1]
- 15-20% of congenital infections lead to permanent injury[2]
- Risk is greatest with symptomatic congenital infection in context of primary maternal infection[3]
- Most pregnant women have no symptoms that would trigger concern about cCMV

[1] Kenneson 2007 Rev Med Virol
[2] Cannon 2009, J Clin Virol
[3] Permar et al 2018, J Virol

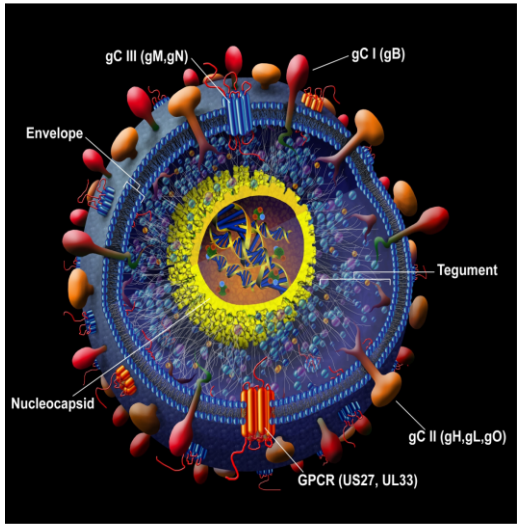
# Congenital Cytomegalovirus (CMV)

Adverse outcomes include:

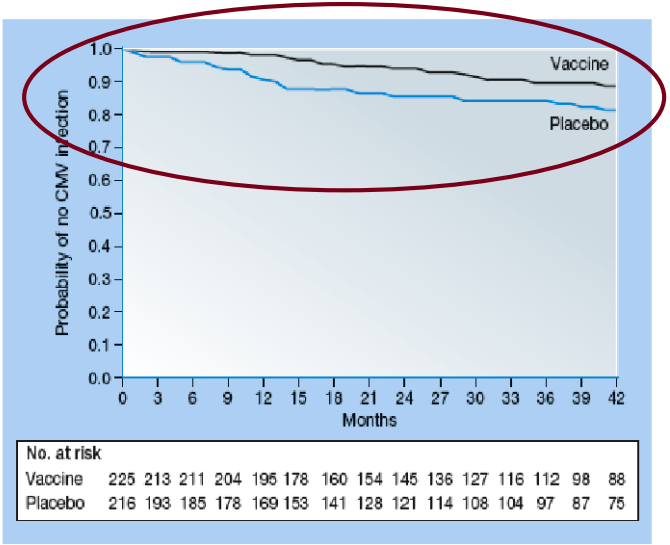
- hearing loss
- microcephaly
- developmental disabilities
- vision loss
- cerebral palsy

American Academy of Pediatrics 2018

# Development of a CMV Vaccine is a Major Public Health Priority…

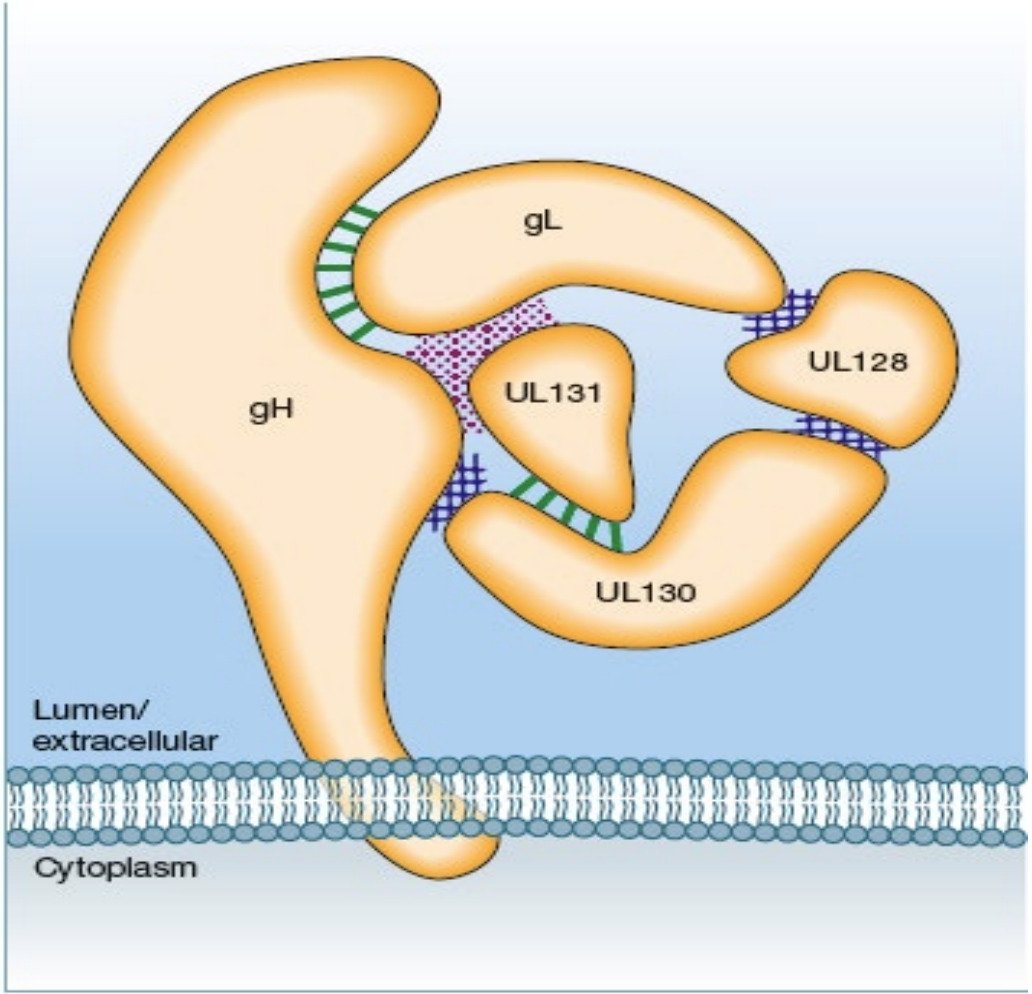


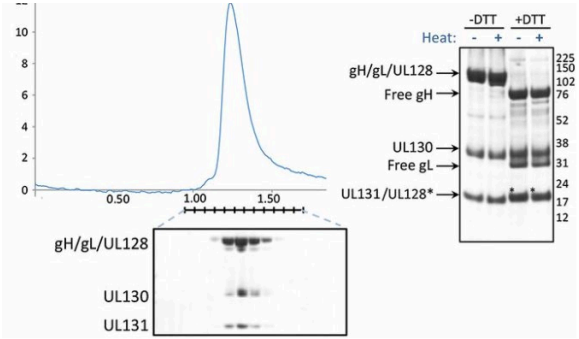

| CMV Gene Product | Host Immune Response |
|---|---|
| **Envelope Glycoproteins** | |
| gB | Major target of neutralizing antibodies; target of CTLs |
| gH | Important target of neutralizing antibodies; target of CTLs |
| UL128-131 | Associated with gH on viral envelope; target of neutralizing antibodies; important target for antibodies that neutralize infection at epithelial surfaces |
| gM/gN | Target of antibody neutralizing antibody responses |
| **Structural proteins** | |
| pp65 | Major target of CTLs; target of non-neutralizing antibody responses |
| pp150, pp28 | Target of CTLs and antibody responses |
| pp50 | Target of CTLs |
| pp71, pp52 | Targets of antibody responses |
| **Nonstructural proteins** | |
| IE1 | Important target of CTLs; target of non-neutralizing antibody responses |

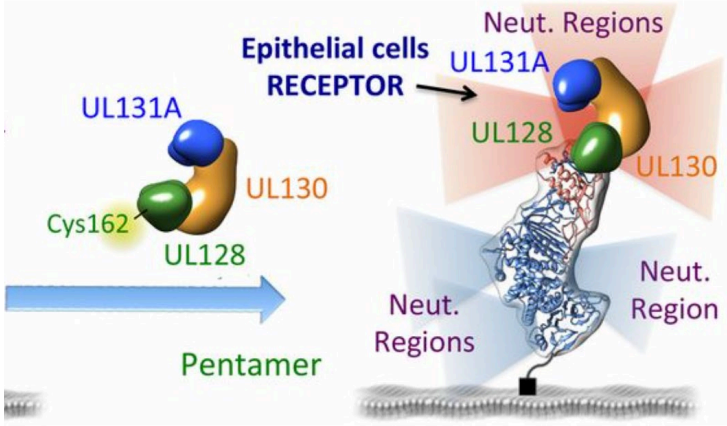



Pass RF, et al. *N Engl J Med*. 2009;360:1191-1199

Schleiss and Plotkin, *Vaccines*, 7th Edition, 2017, Chapter 16




Ryckman B J et al. *J. Virol* 2008;82:60-70

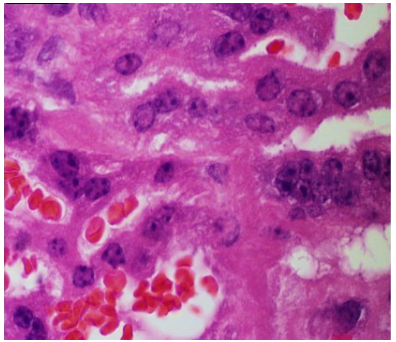
Ciferi et al. PNAS, https://doi.org/10.1073/pnas.1424818112

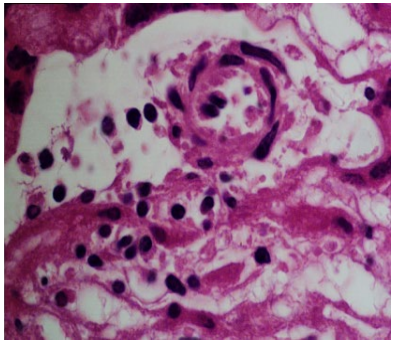
# Guinea Pig Model of Congenital CMV Infection

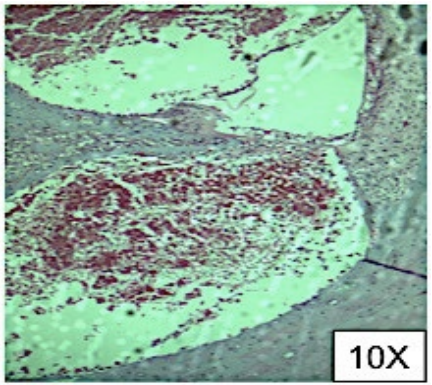
- Guinea pigs have histological/structural similarities to human placenta
- Mid-gestation viral challenge results in fetal infection, IUGR, demise, sequelae
- Vaccines attenuate disease, prevent infection
- Guinea pig is an excellent model to study maternal-placental-fetal transmission

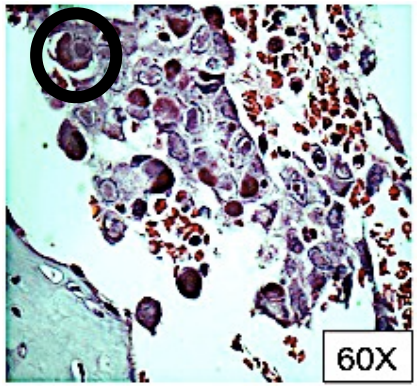
Schleiss Future Virology, 2013;8:1161-1182
Auerbach et al. PLoS Pathog, 2014;10(4):e1004060
Yamada et al., J Gen Virol, 2014, doi: 10.1099

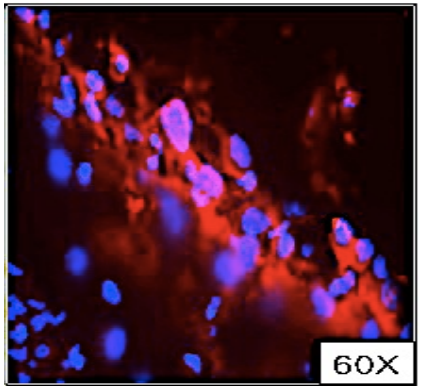
# GPCMV-Infected Guinea Pigs Demonstrate IUGR, Morbidity, Mortality, Labyrinthitis, Virolactia

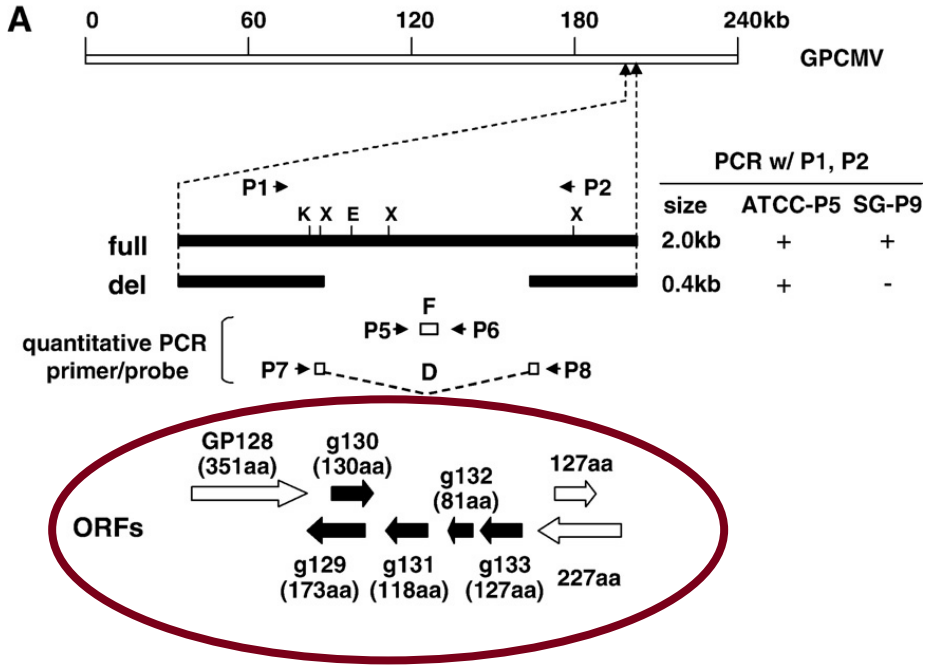


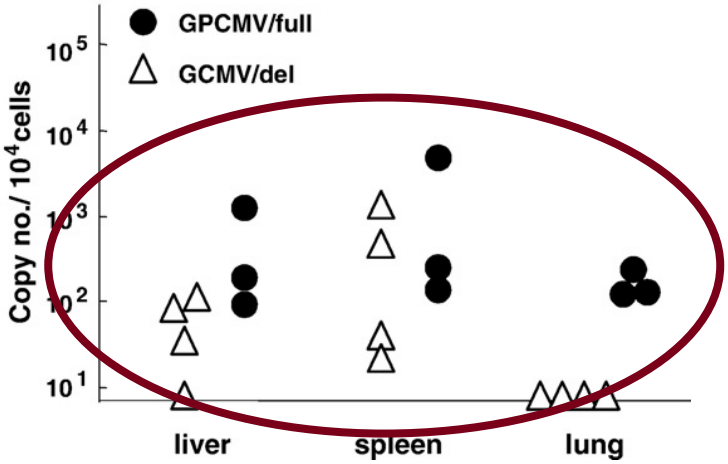


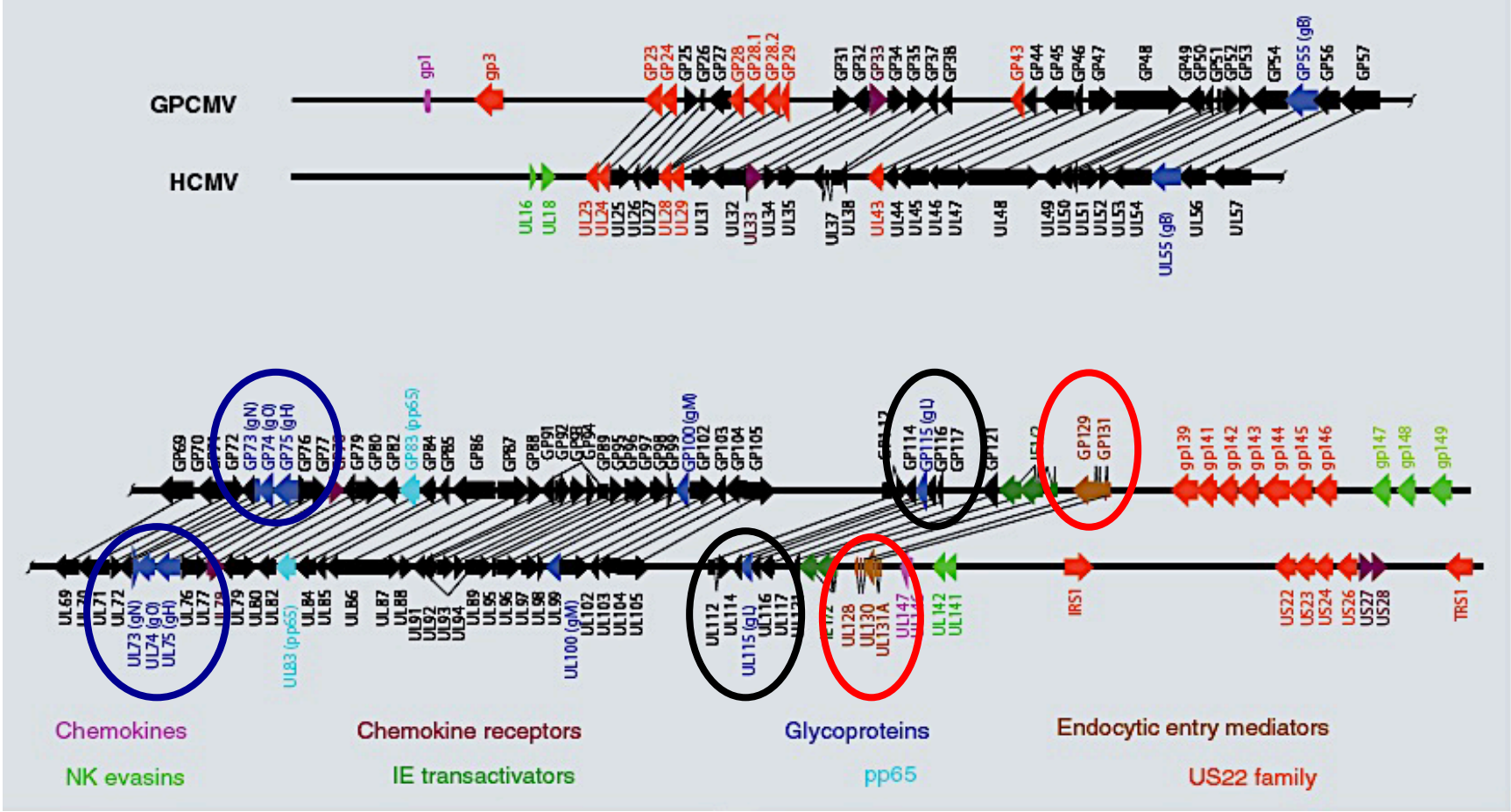




Schleiss and McVoy *Future Virology* 5:207-17

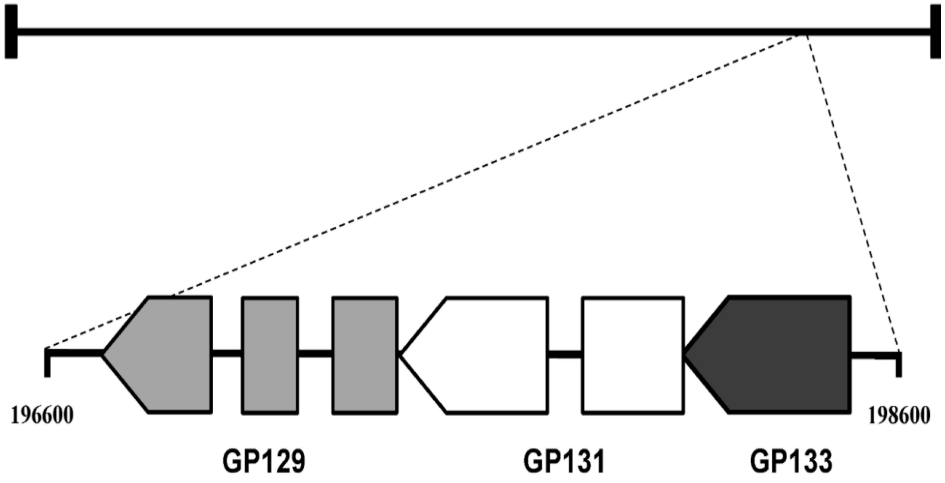



*Virology*. 2008;379(1):45-54.
*J Gen Virol*. 2014;95(Pt 6):1376-82.
*Virology*. 2013;441(1):75-8.
*PLoS Pathog*. 2016;12(7):e1005755.
*Virology*. 2017;509:205-221.




Schleiss and McVoy, Future Virology 2010;5:207-17

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SLEENLFESLALNVSRQELHVLAPESKFGPVEVGINKQVRCIRYPRMPSVQSKPEKPSILGVTYRV
DYTVMIPTPHFPRDFNGLLCTFLEKNDTFYNTTVDVCGSEFYSVDGNGK*

129

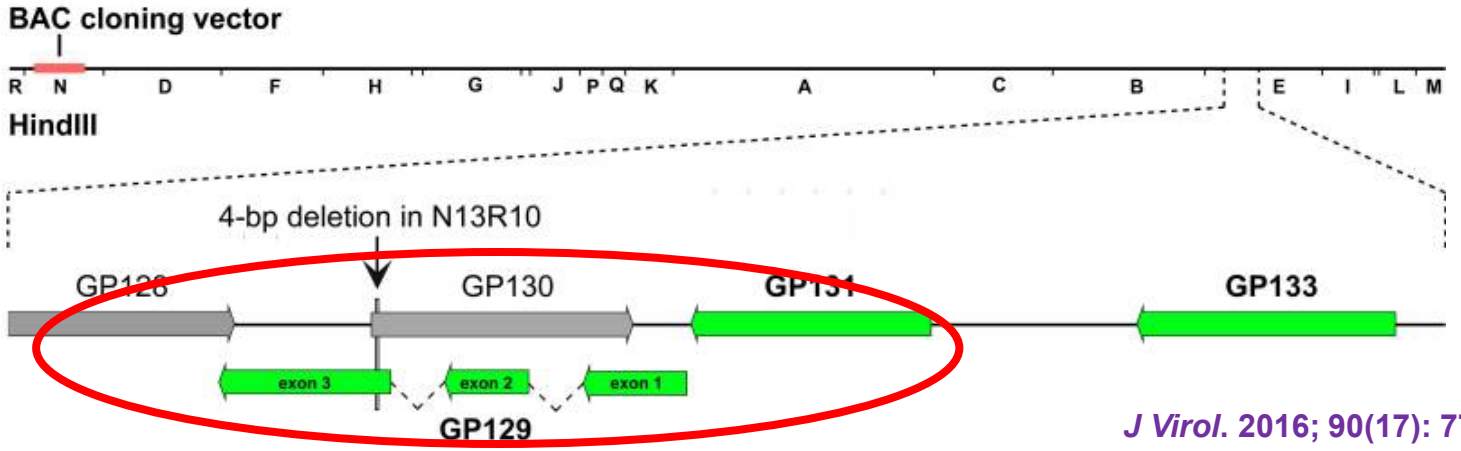
MMKRYLVLLPWIMFYASFGRA GRCYYPSTPIPKSFVKHVDTTRSLPECENDTVAVLTLTNGAK
LYVNMLNTWIDGYITTLQYAIPPTLSDIFAFIKRRIDYGSTGTAASTLPSLTSLRTYFGDRDSSFLW
HYTIRMKDGAKTLDCDVYVTSRVHFVLNSYEAVQTVLFEGGVVISRHPADSIACLLINWNWT*

131

MFWRLVYVYLVSLLLSIGA EDEGIDTWWLGGVTDNTRVKKENQLAHYILKTIVLTHHRRLRT
GDECTEQLSNDLDIHSVHTLADSIRRLRGRYRKGLVSIDGIRISIQQSTRTQQKGLWISARIDRA*

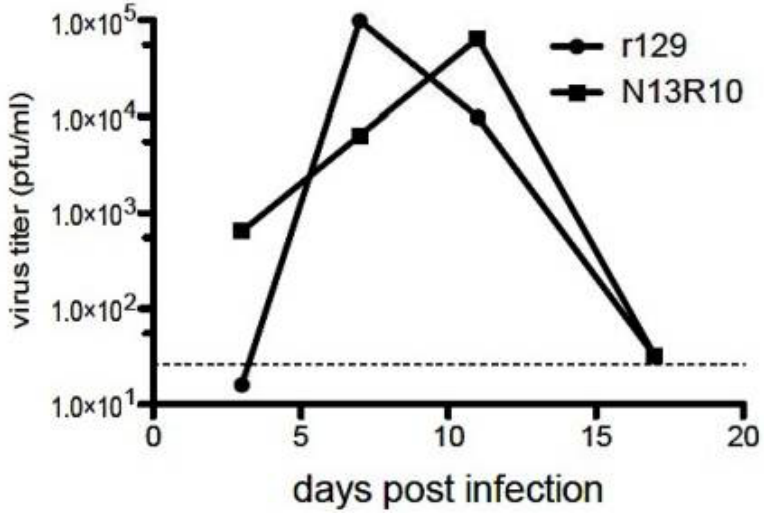
133

A

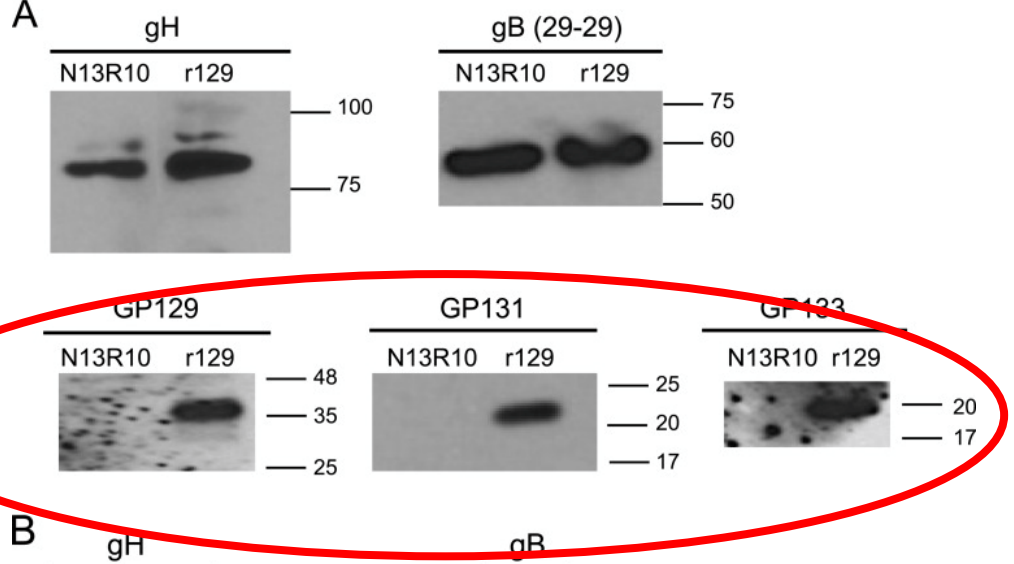


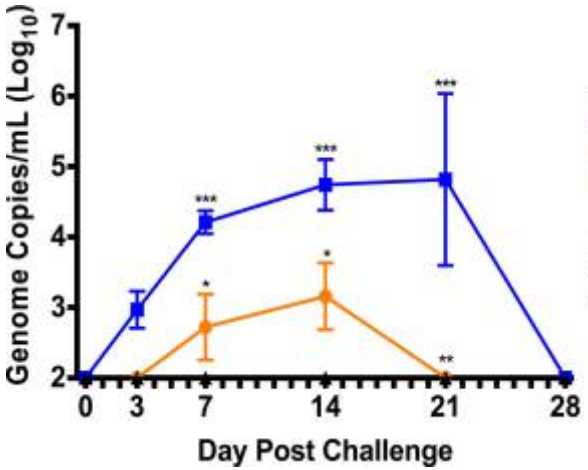

*J Virol*. 2016; 90(17): 7715–7727

B




A deletion/frame-shift mutation in *GP129* abrogates PC expression and attenuates GPCMV pathogenesis

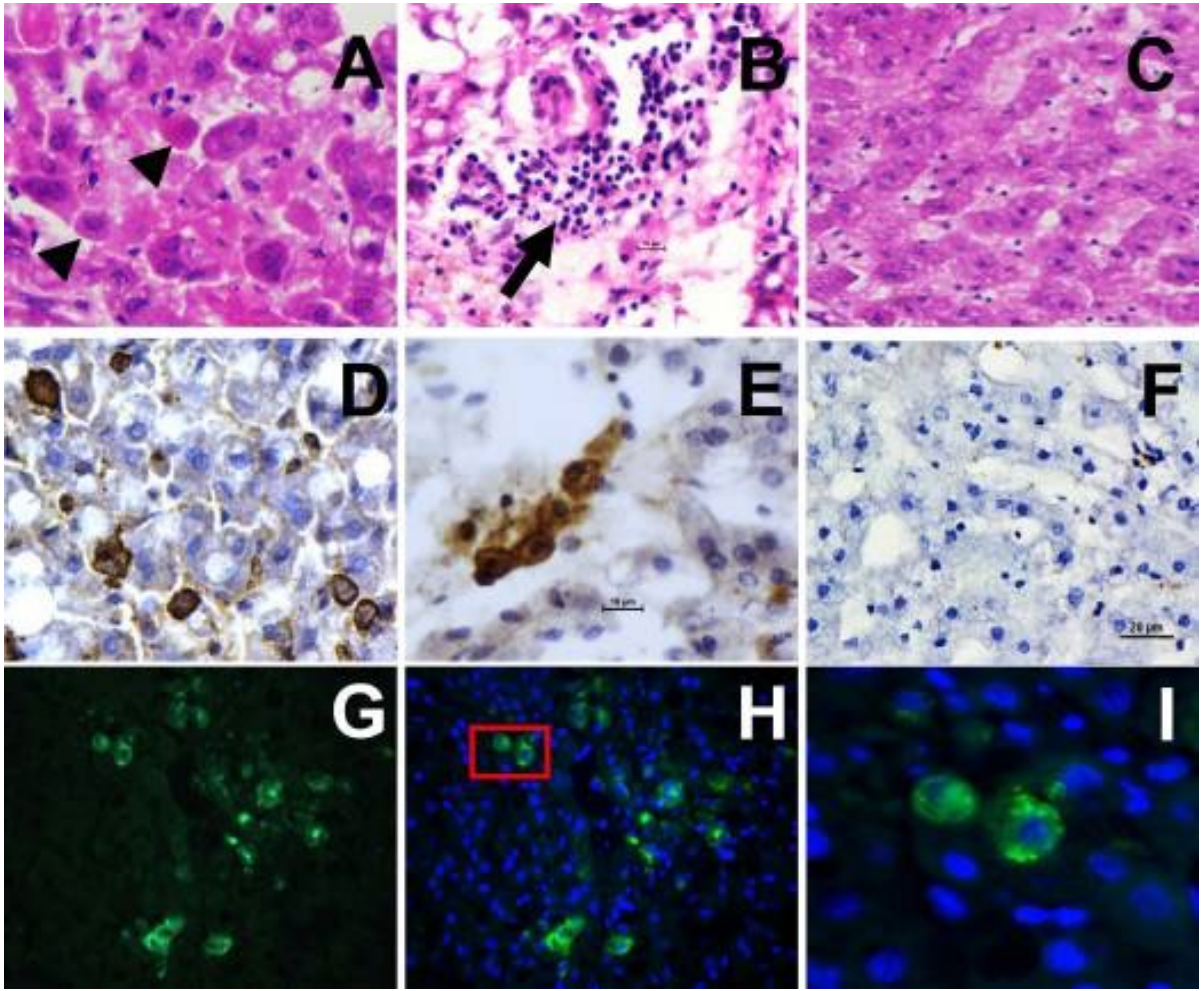


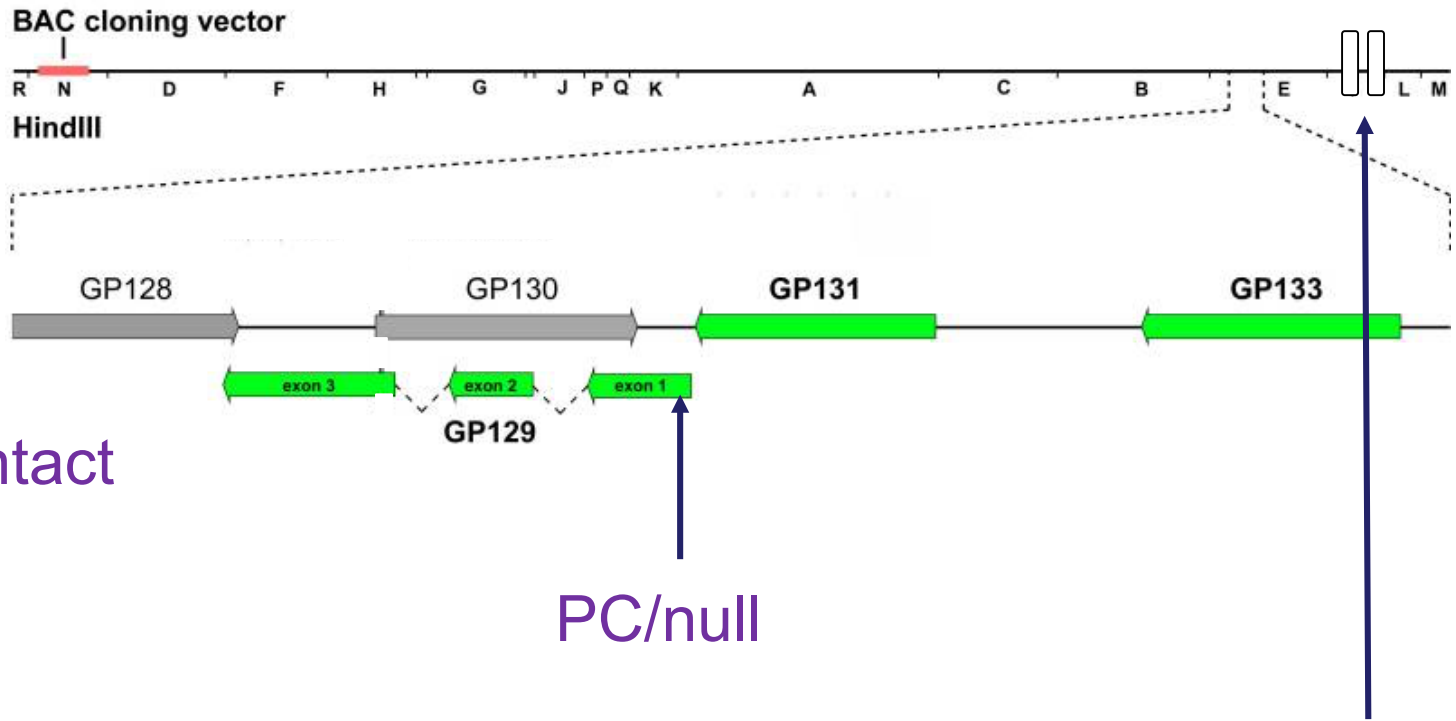

*J Virol*. 2016; 90(17): 7715–7727

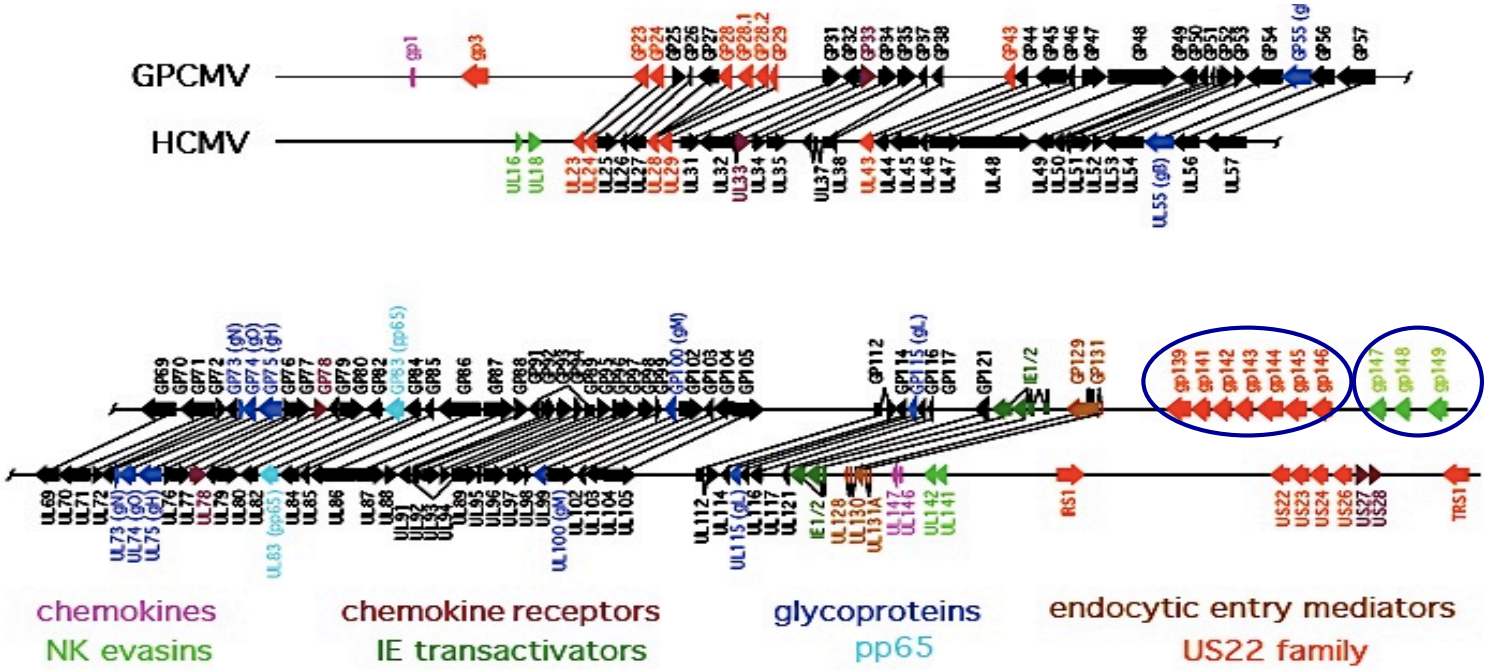
PBS
r129
N13R10

*** p <0.0001 r129 v control
** p <0.0001 r129 v N13R10
* p <0.02 r129 v N13R10

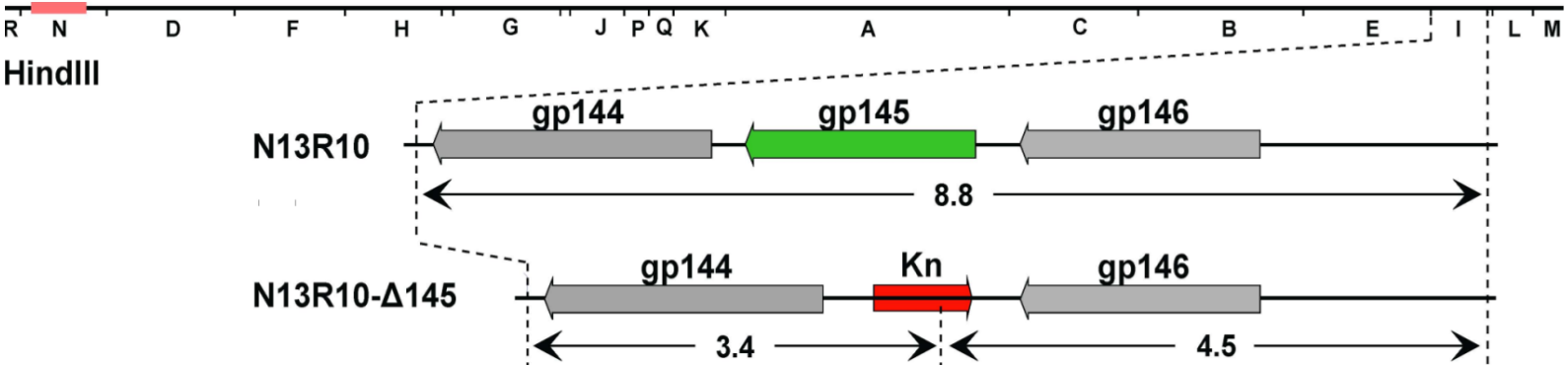


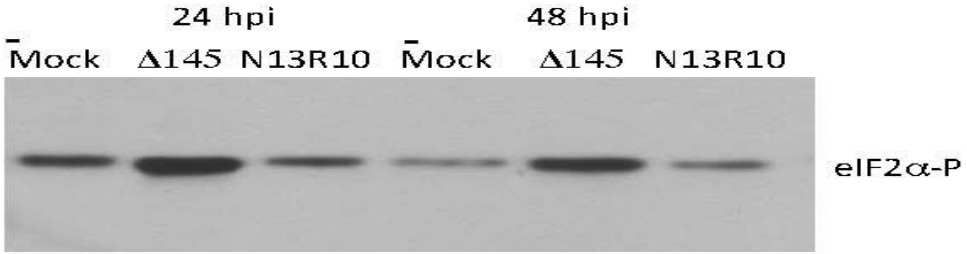

*J Virol*. 2016; 90(17): 7715–7727


BAC cloning vector
R N D F H G J P Q K A C B E L M
HindIII
GP128
GP130
GP131
GP133
exon 3
exon 2
exon 1
GP129
PC/Intact
PC/null
Δ145/3DX
PC/intact




Schleiss and McVoy, Future Virology 2010;5:207-17



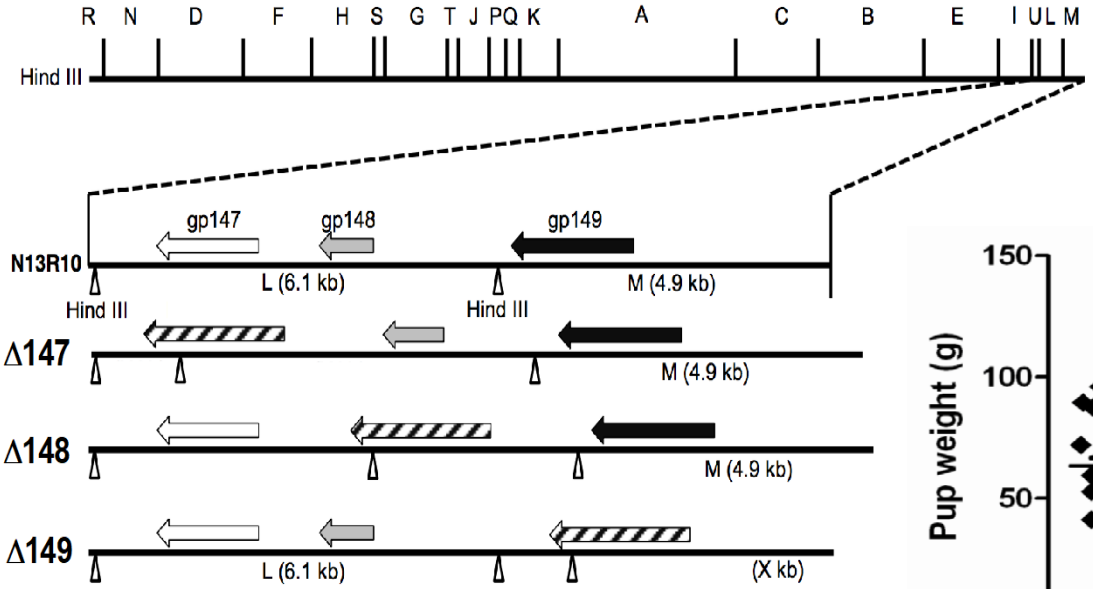



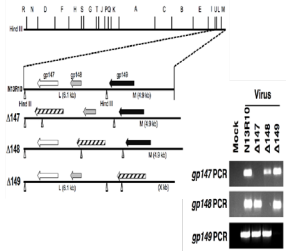

| Group | Transmission (live-born) | Transmission (still-born) | Overall Transmission Rate (%) |
|---|---|---|---|
| Control | 3/5 | 17/26 | 20/31 (65%) |
| ! 145 ($10^5$) | 17/25 | 2/4 | 19/29 (66%) |
| ! 145 ($10^6$) | 8/23 | 0/1 | 8/24 (33%)* |

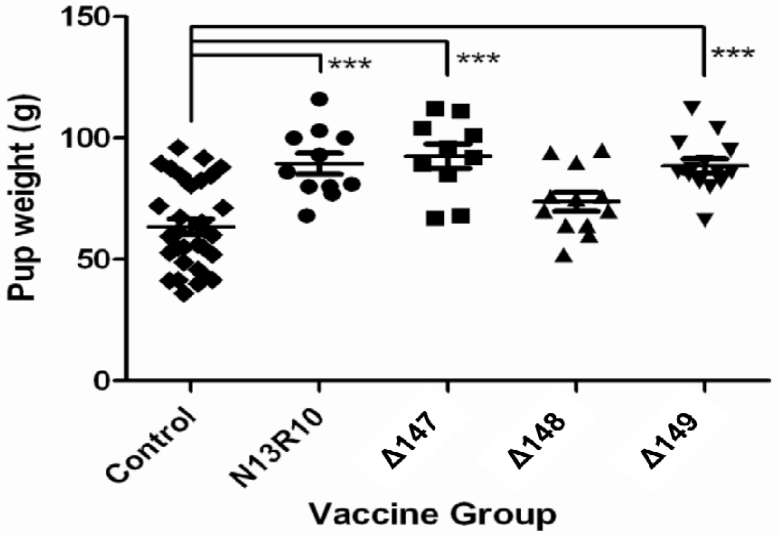
*p < 0.05 vs. control

*J Virol*. 2015;89(19):9727-38

# Construction of Deletion Mutants
# Δ147, Δ148, and Δ149 from Parental "WT" N13R10

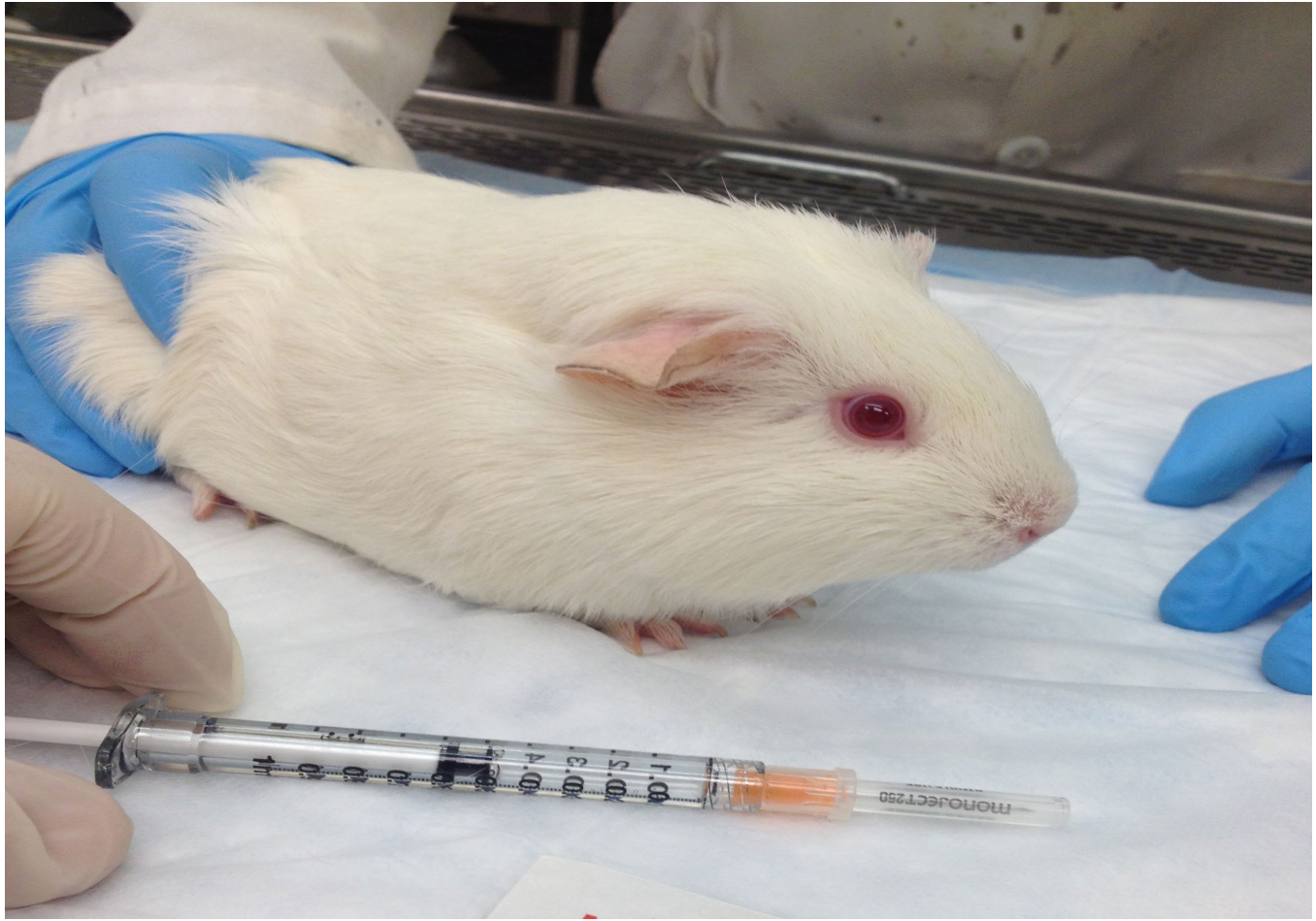


# Questions...

- In an otherwise “wild-type” vaccine, how much does inclusion of the intact pentameric complex region contribute to protective immunity?
- In an attenuated (hence, safe) but incompletely protective vaccine, how much does inclusion of the intact pentameric complex region enhance protection?


monoject250

## Vaccination

Group 1 – PBS
Group 2 – 3DX/Δ145
Group 3 – PC/null
Group 4 – PC/intact

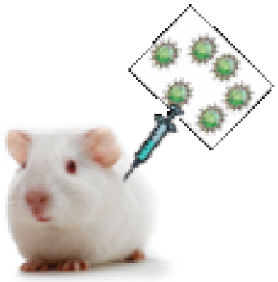
Vaccine series q30 days, 2 doses,

1X10$^5$ PFU/dose

## Pregnancy

30 days post vaccine series: mating & pregnancy

## SG GPCMV Challenge



30-35 days gestation: SG-adapted GPCMV 1x10$^5$ PFU

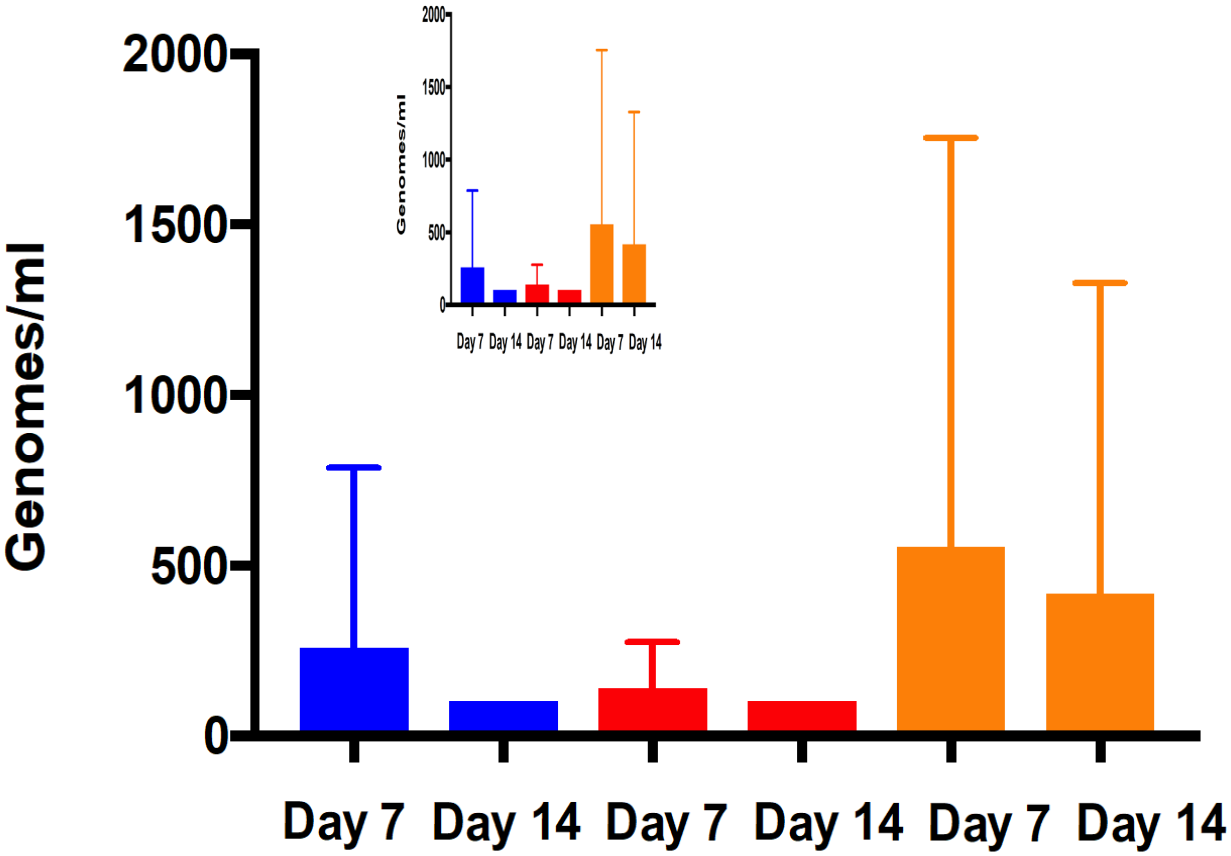
## Delivery

~30 days post challenge: delivery

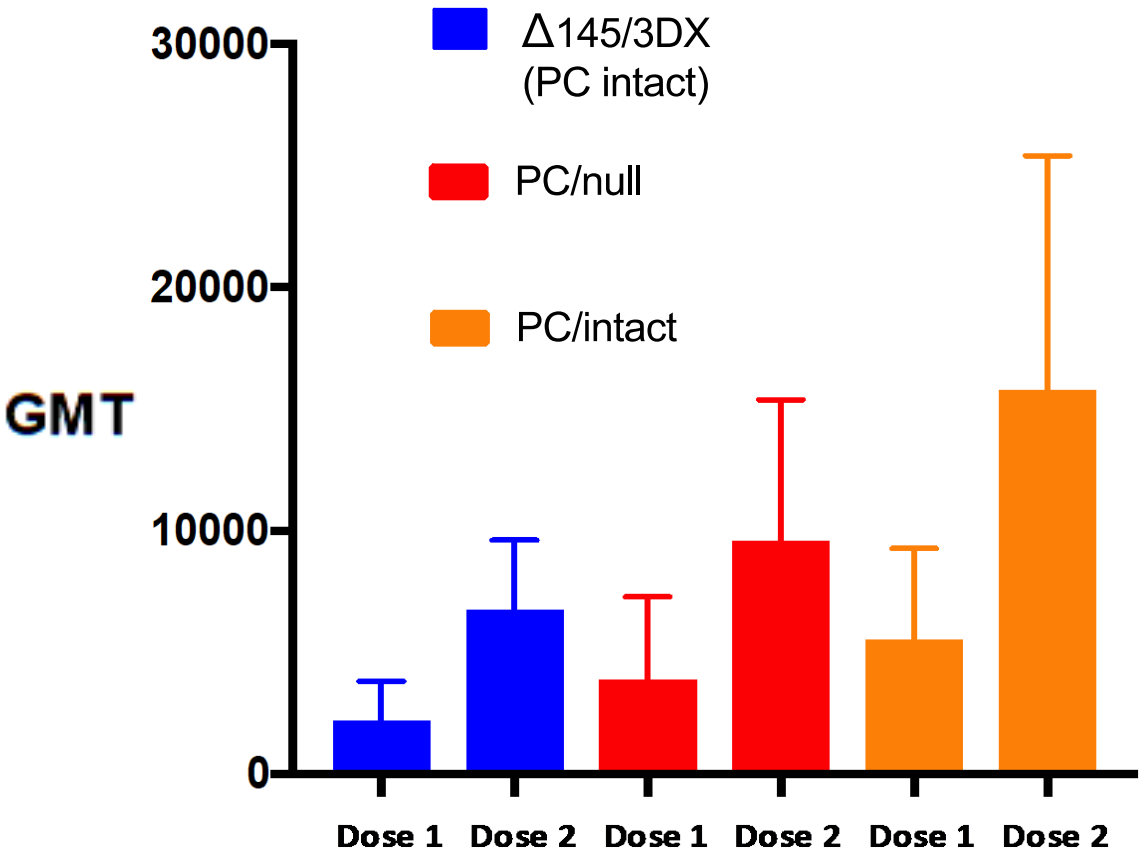
**Dams bled for qPCR (test of vaccine attenuation), ELISA (dose 1 and 2) and neutralization titers (dose 2)**

**Dams bled for viral load post-challenge by qPCR on days 7, 14**

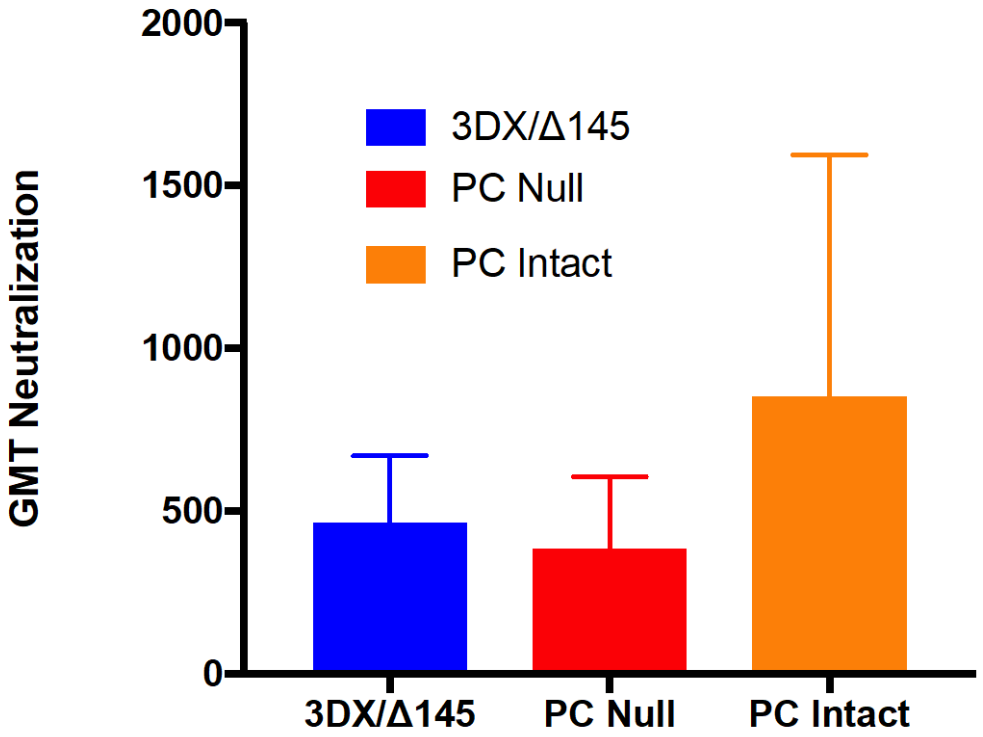
**Pup weights, mortality analysis, tissue harvested for qPCR**



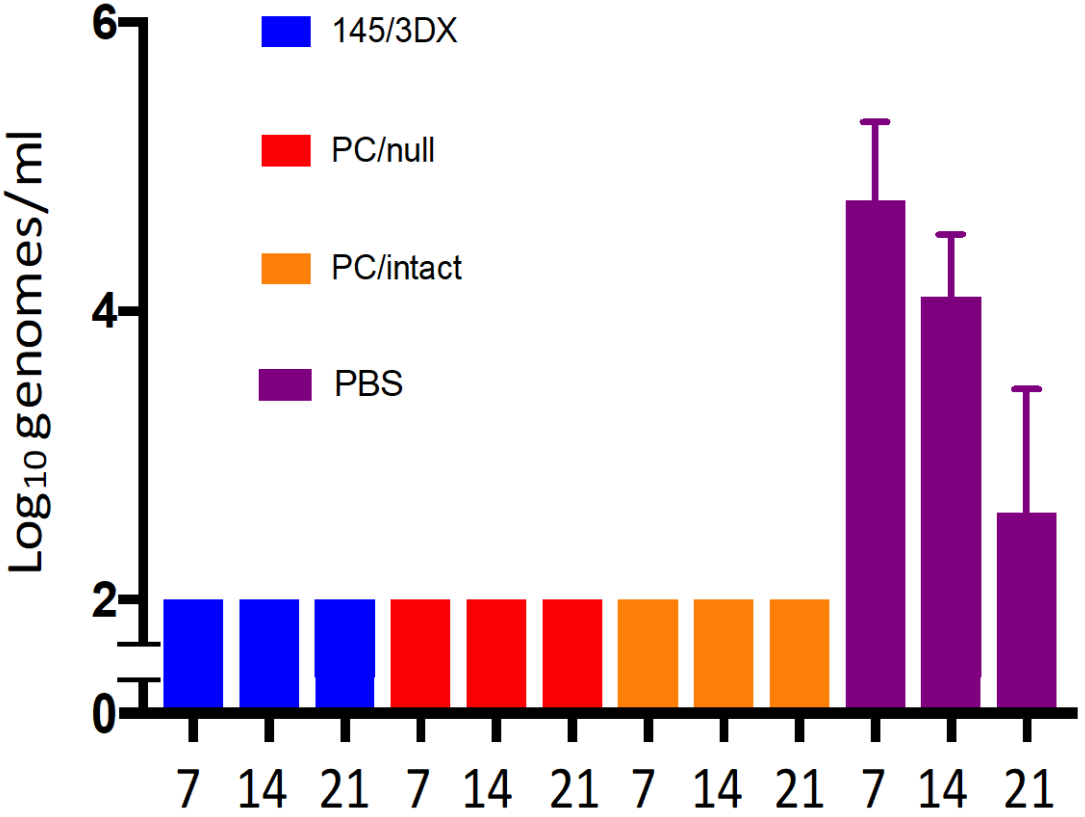

**Vaccine** viremia in 5/12 of PC/intact-vaccinated, 2/12 of PC/null-vaccinated, and 1/11 of PC/intact-3DX/Δ145-vaccinated animals ($p<0.05$).




Total ELISA antibody titer higher (P<0.01) in PC/intact immunized dams compared to the other groups.



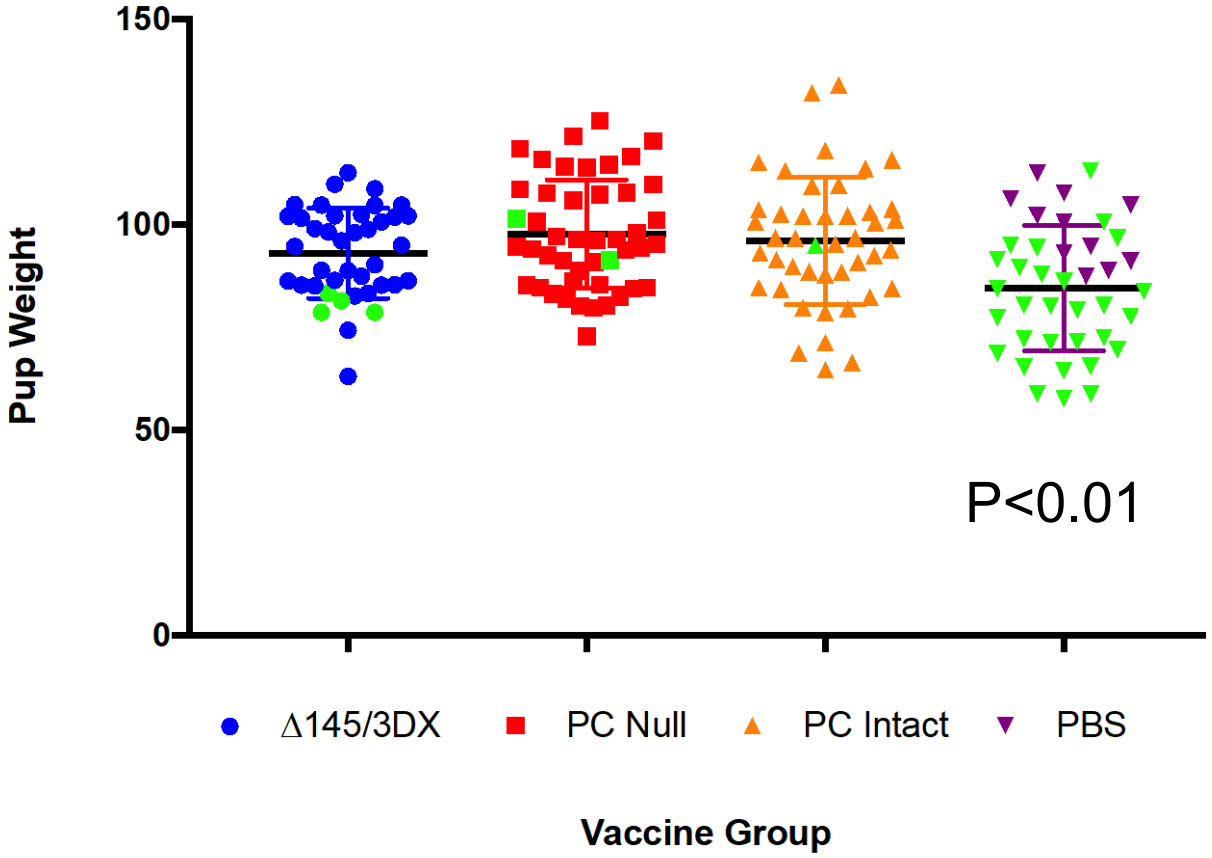

Total neutralizing antibody (fibroblast cells) titer higher ($P<0.01$) in PC/intact immunized dams compared to the other groups.




All vaccine strategies induced sterilizing immunity against maternal DNAemia irrespective of the presence or absence of the PC ORFs.

| Vaccine Group | Vaccinated | Pregnant | Total Pups | Live Pups | Pup Mortality | Pregnancy Duration Post-GPCMV Challenge |
|---|---|---|---|---|---|---|
| PBS (Sham) | 12 | 11 | 40 | 11 | 73% | 24.1±1.2 days |
| PC/intact | 12 | 12 | 44 | 43 | 2.3%* | 20.8±1.2 days |
| PC/null | 12 | 12 | 46 | 44 | 4.3%* | 21.3±1.3 days |
| Δ145/3DX (PC/intact) | 12 | 11 | 40 | 36 | 10%* | 20.9±1.3 days |

*P<0.001 v. control group

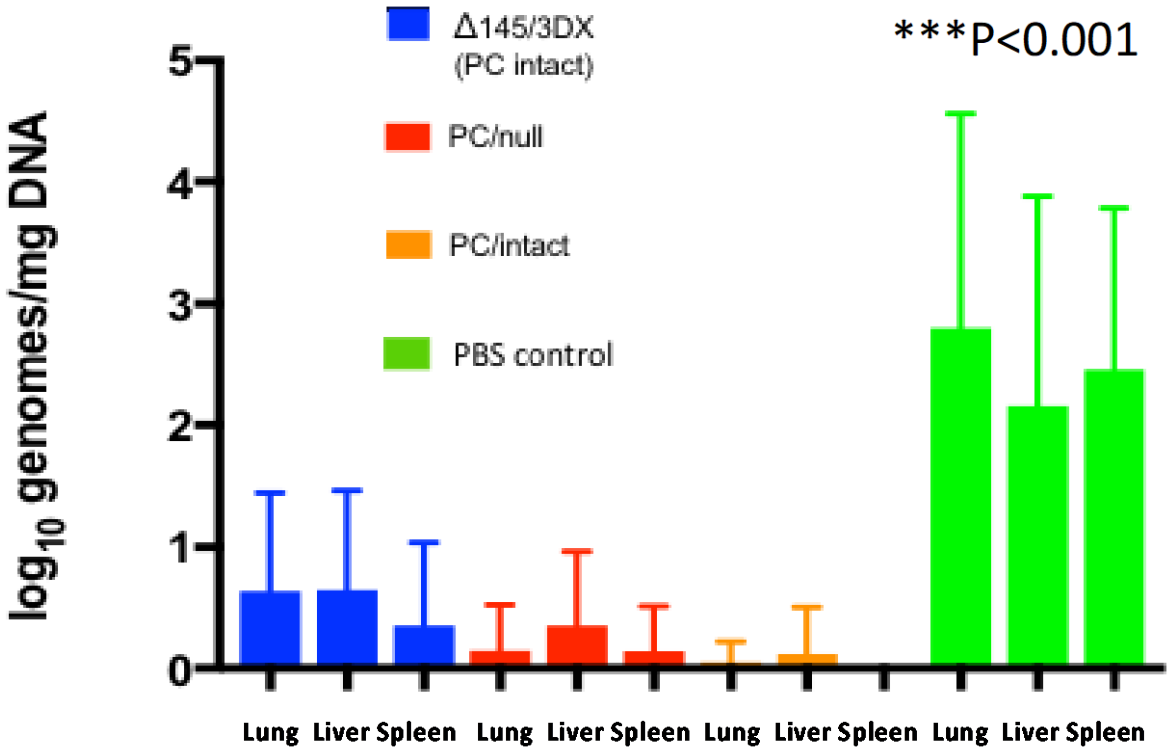



All vaccine strategies resulted in improved pup weights in addition to decreased pup mortality...

| Group | Transmission |
| --- | --- |
| PC/intact | 5/44 (11%)* |
| PC/null | 16/46 (35%) |
| 3DX/DPKR (PC intact) | 29/38 (76%) |
| PBS Control | 36/40 (90%) |

*P=0.01 v. PC/null

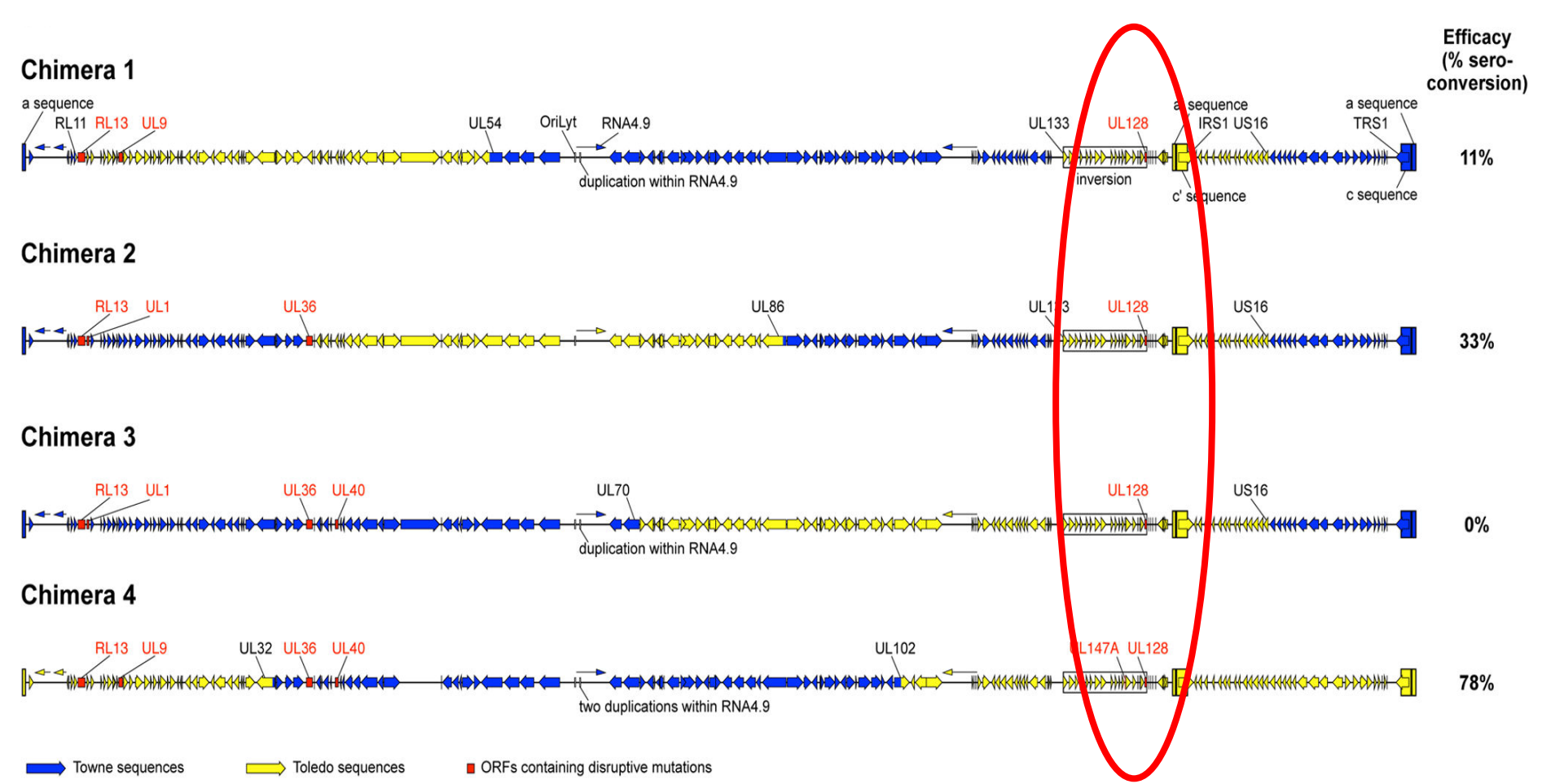
Although there were differences among vaccine groups with respect to congenital infection rate...




Viral loads in congenitally infected pups were in many cases barely above the limit of detection...

# Summary

- Modifications of the GPCMV genome mediated by BAC mutagenesis can provide insights into the roles of immune modulation genes in pathogenesis and protection.
- Targeted modification of the GPCMV PC ORFs results in virus that is impaired in pathogenesis (entry/tropism versus other functions in viral life cycle).
- In the GPCMV model the PC is **dispensable** for a protective vaccine.
- In the GPCMV model the PC is **required** for a protective vaccine.




*Virus Genes* 2017 Aug;53(4):650-655

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- Adam Geballe

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