

# **Cell-mediated immune responses in the eye associated lymphoid tissues of chickens during vaccination or infection with Infectious laryngotracheitis virus (ILTV)**

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4<sup>th</sup> Annual PRD-CAP Meeting Kansas City, Kansas

October 24, 2018

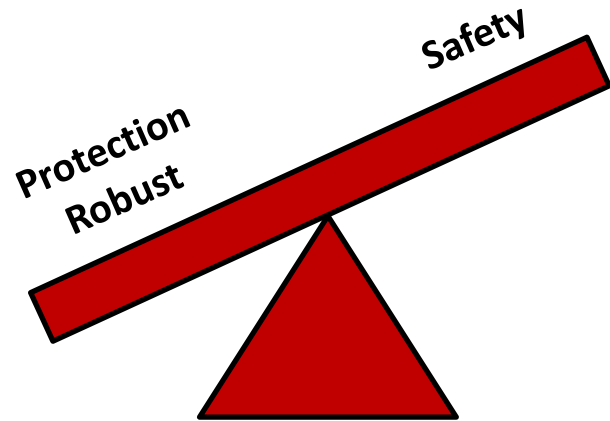
# Components of cell mediated immunity

- **Objective:** Unmask key components of the local cell immune response that contribute to disease protection and/or influence the outcome of disease
- **Approach:** Assessment of lymphocyte populations and cytokines that favors cell mediated immune responses in CALT and HG after ocular inoculation of 5 week old SPF chickens with the chicken embryo (CEO) vaccine and 63140 virulent strain



# Why is this important?

## Live attenuated Vaccines



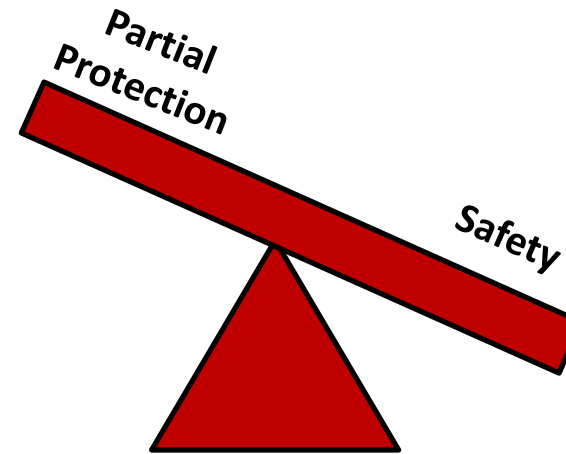
### KNOW

Establish latency  
Inherent virulence  
Regain virulence

### UNKNOWN

Sites of latency?  
Are immune cells infected?  
Virus Immune evasion?  
Protective immunity?

## Recombinant vaccines



### KNOW

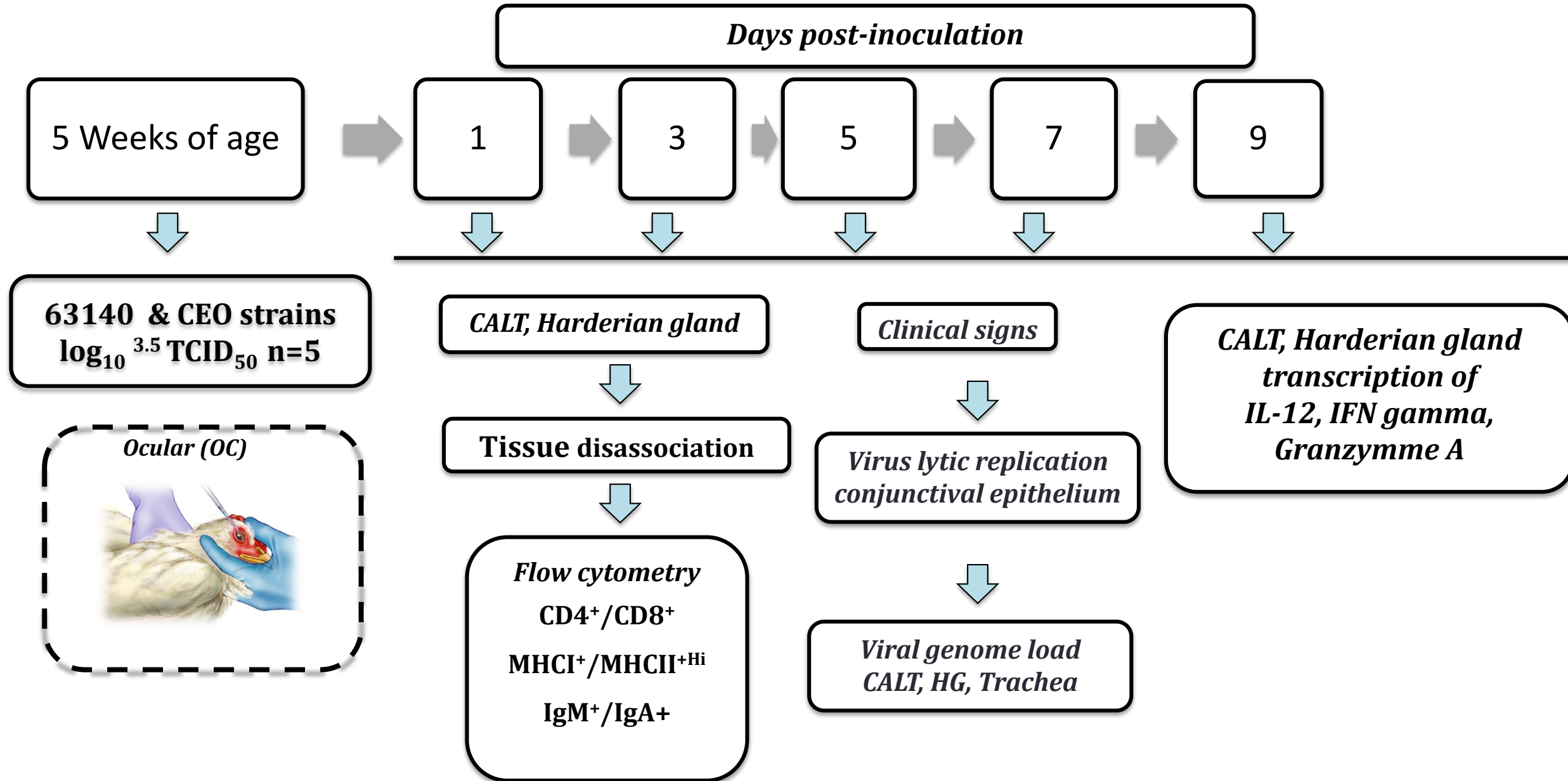
Reduction of disease  
Field virus shedding

### UNKNOWN

Generation of Systemic immunity?  
Lack of local immunity?

This knowledge is necessary for the development of better attenuated live vaccines and/or viral vector, subunits vaccines, and adjuvants that could effectively trigger local specific T cell responses after mucosal administration (eye-drop, spray, drinking water)

# Experimental Design



# Lytic replication conjunctiva

*Days Post-inoculation*

1

3

5

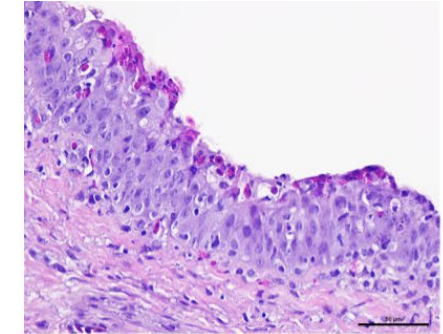
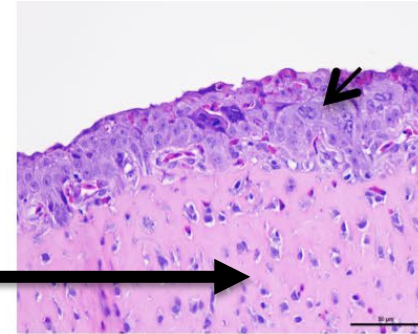
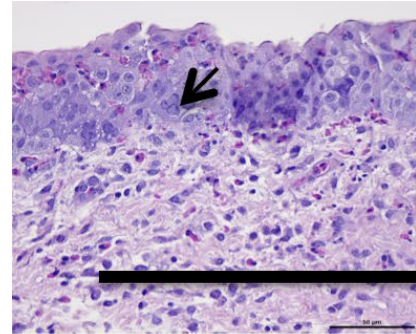
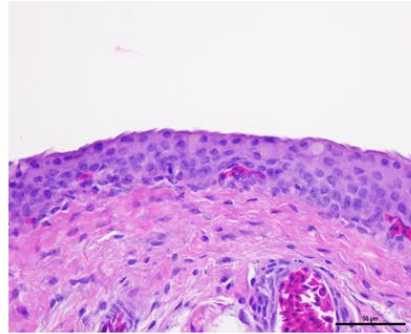
7

a)

c)

e)

g)

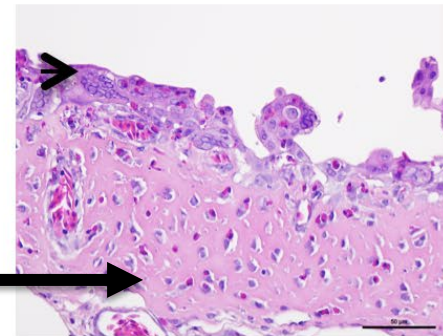
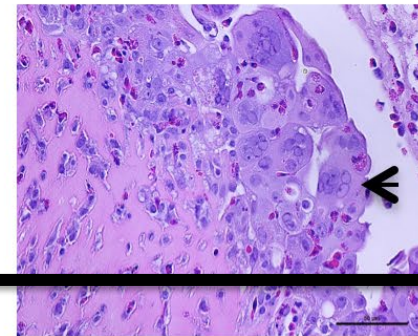
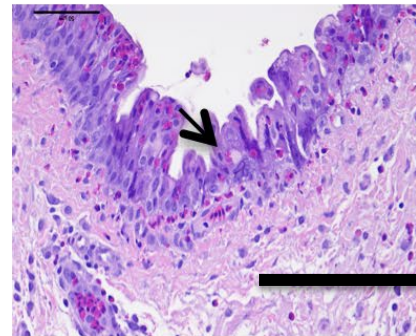
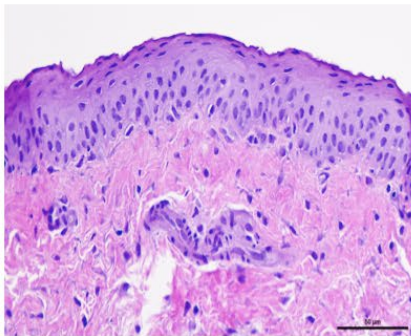


b)

d)

f)

h)



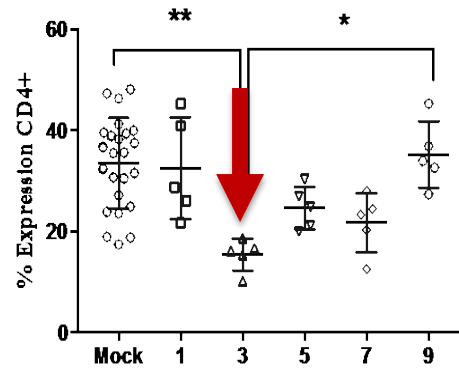
CEO

63140

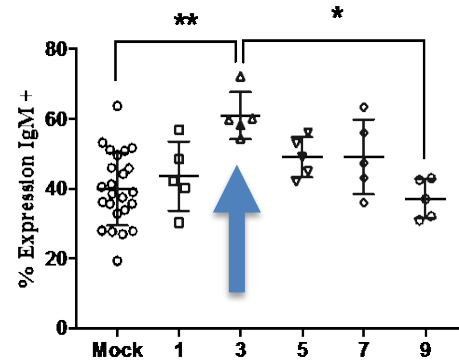
# CALT

CEO

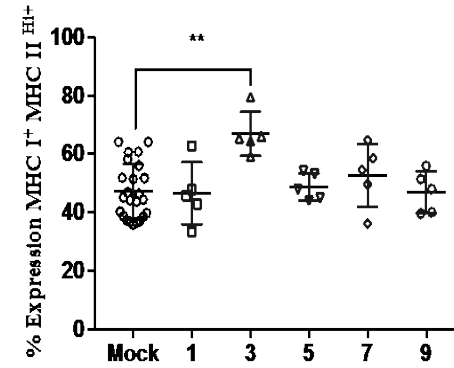
CD4+



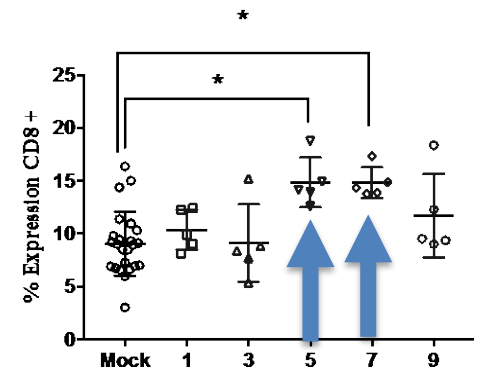
IgM+



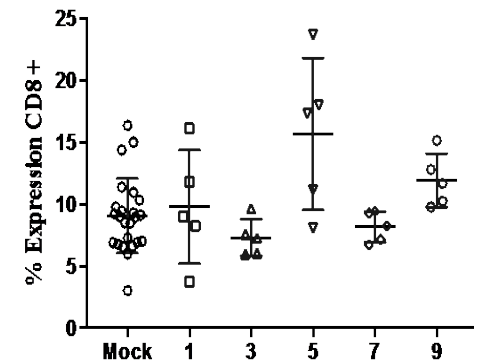
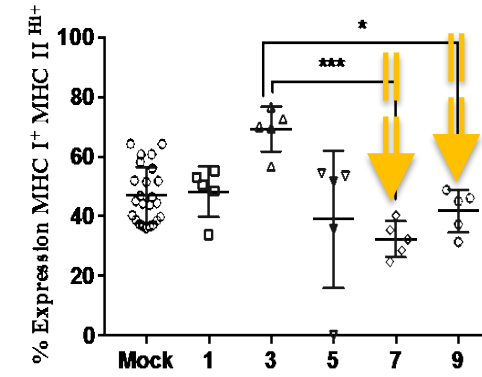
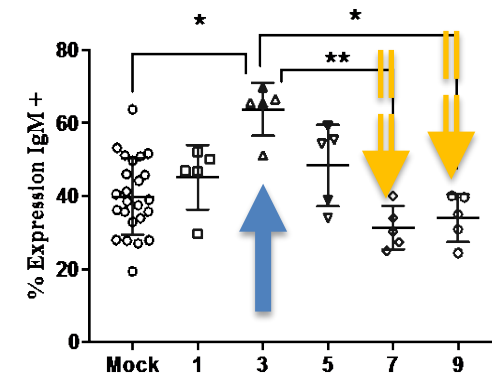
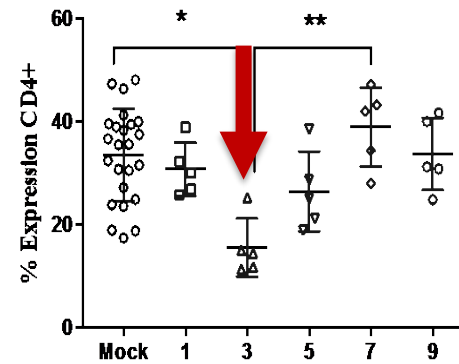
MHCI+/MHCII<sup>Hi</sup>



CD8+



63140

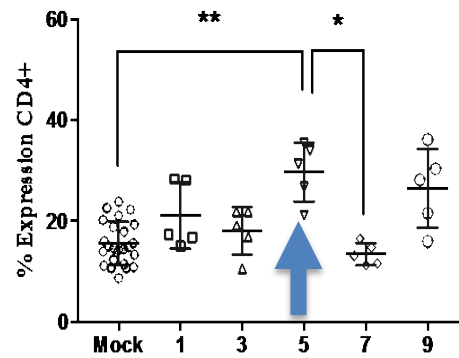


Days post-inoculation

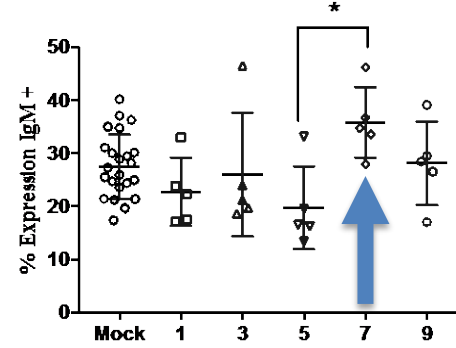
# Harderian Gland

CEO

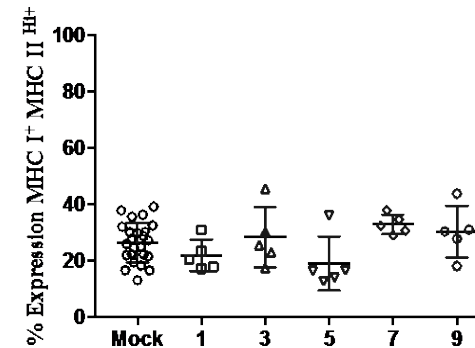
CD4+



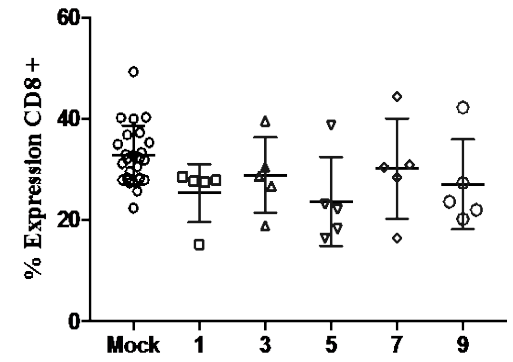
IgM+



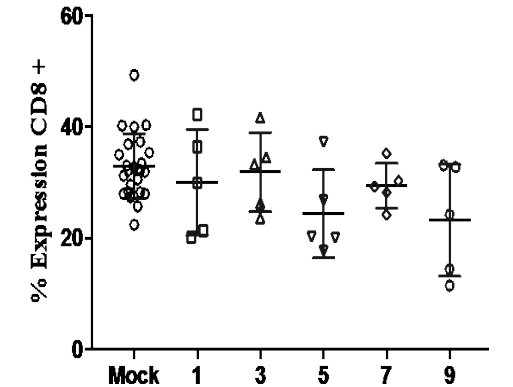
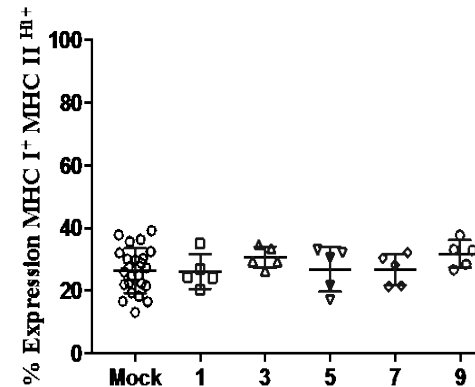
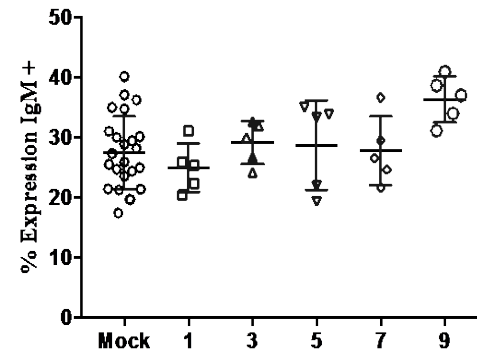
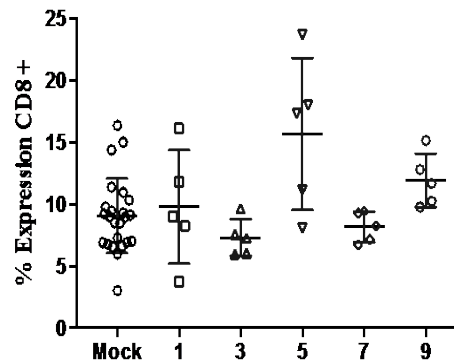
MHCI+/MHCII<sup>Hi</sup>



CD8+



63140

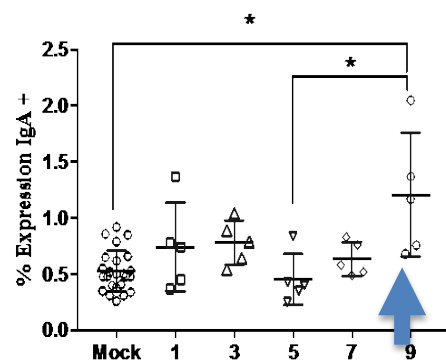


Days post-inoculation

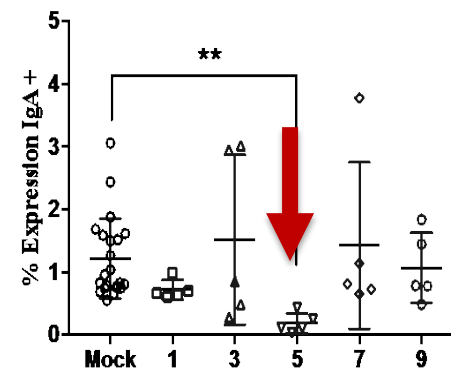
# IgA<sup>+</sup> B cells

CEO

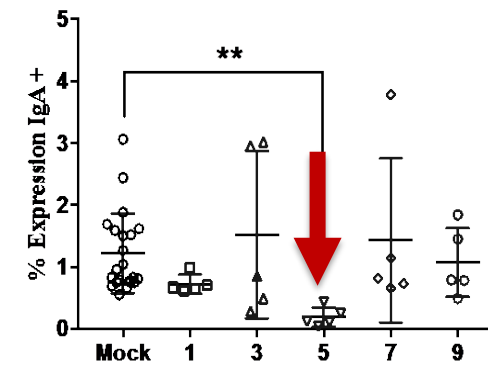
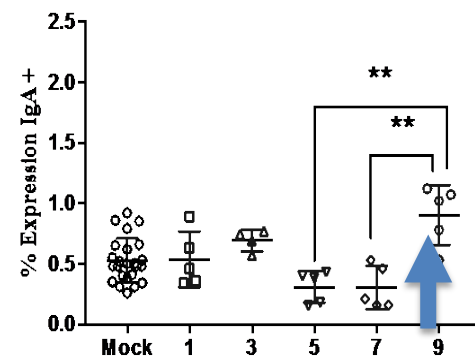
CALT



HG

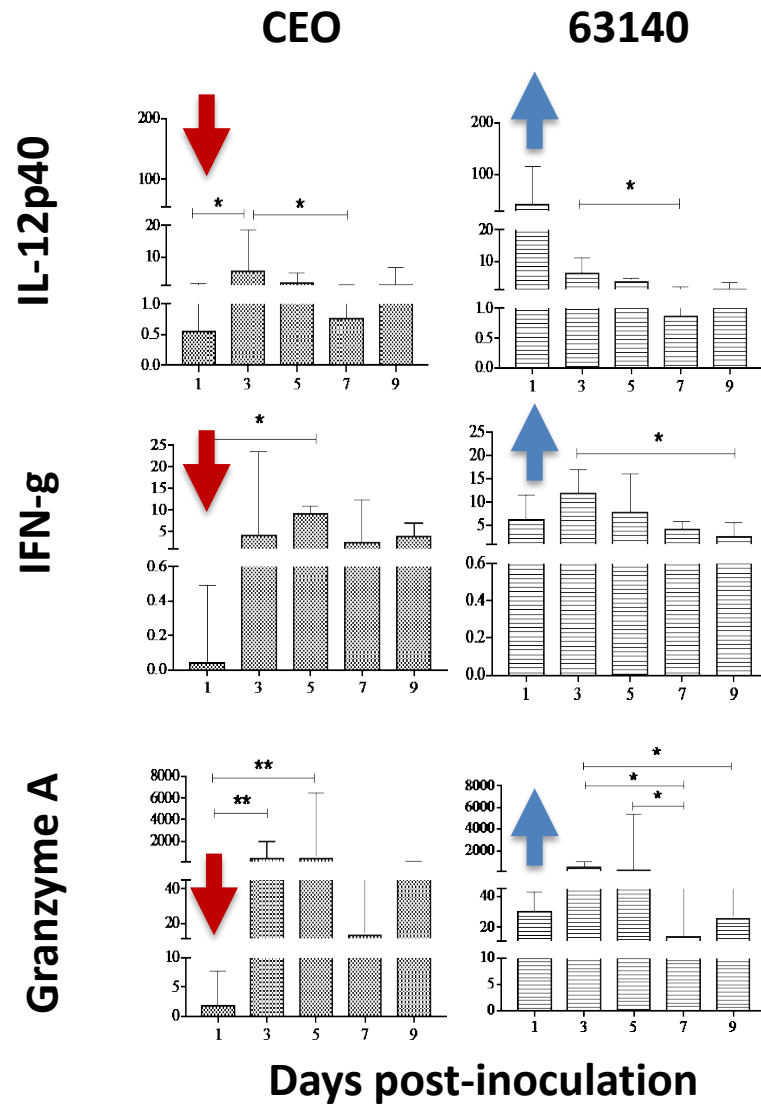


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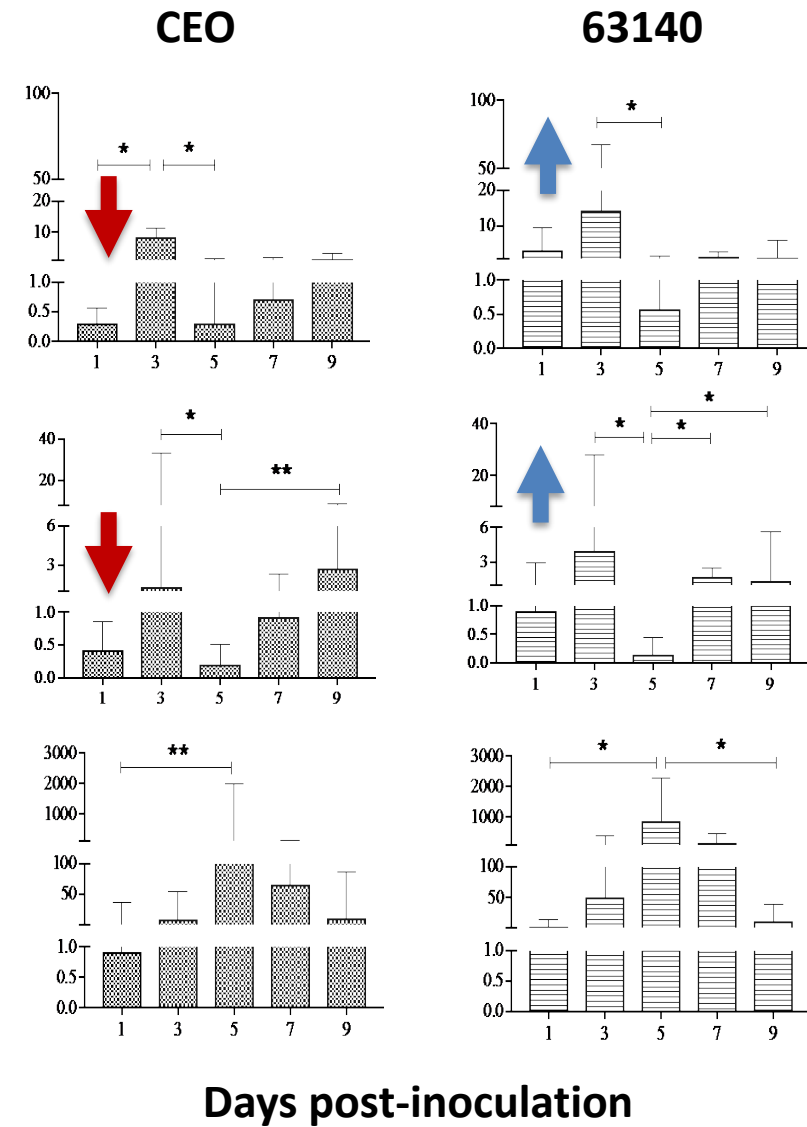


# Cytokine Transcription

## CALT



## HG





# Components of cell mediated immunity

- **Objective:** Unmask key components of the local cell immune response that contribute to disease protection and/or influence the outcome of disease
- **Approach:** Assessment of **mononuclear phagocyte (MNPs) populations** and cytokines that favors cell mediated immune responses in CALT and HG after ocular inoculation of 5 week old SPF chickens with the chicken embryo (CEO) vaccine and 63140 virulent strain

# Mononuclear phagocytes (MNP)s

CALT

HG

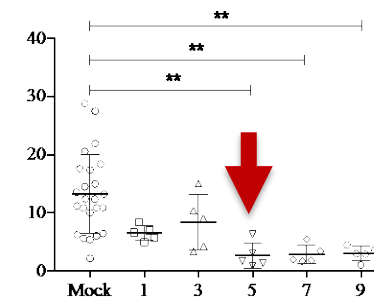
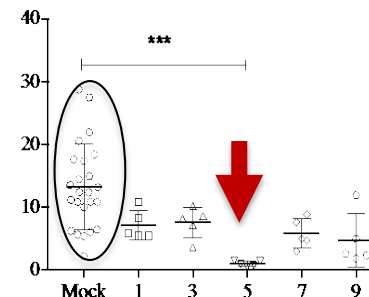
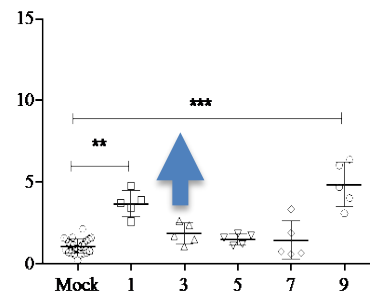
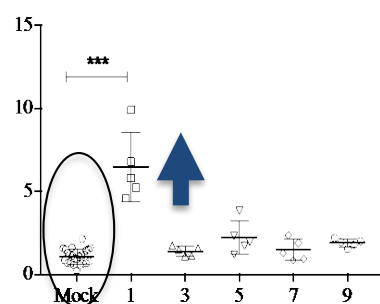
CEO

63140

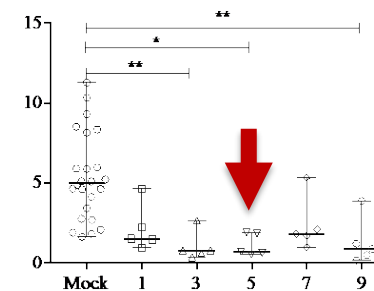
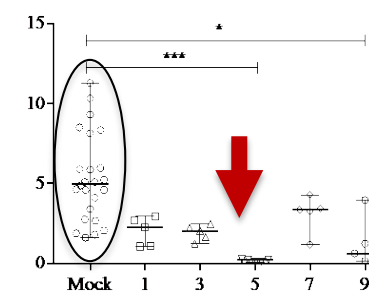
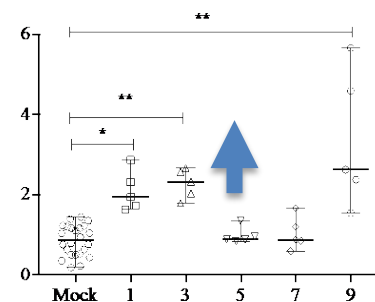
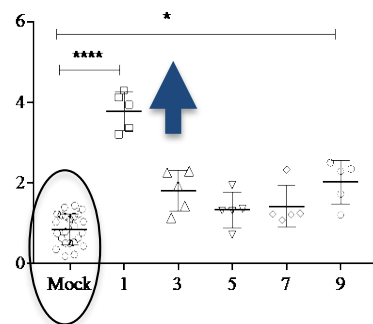
CEO

63140

CSF1R+/MHC II<sup>Lo</sup>

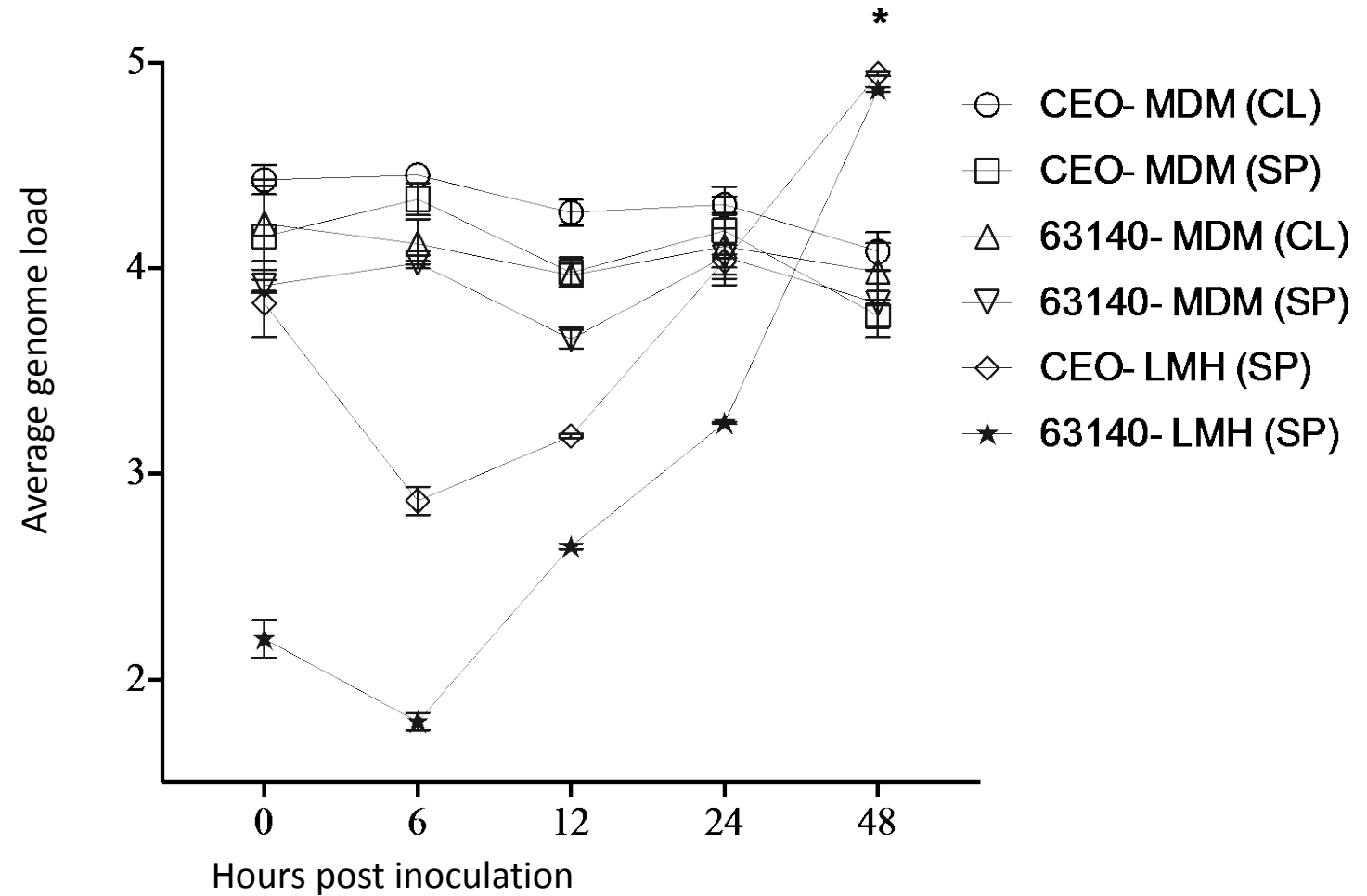
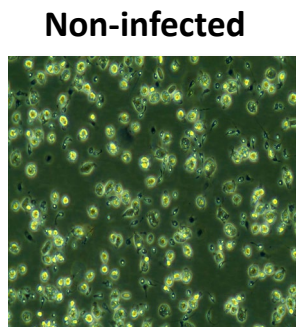
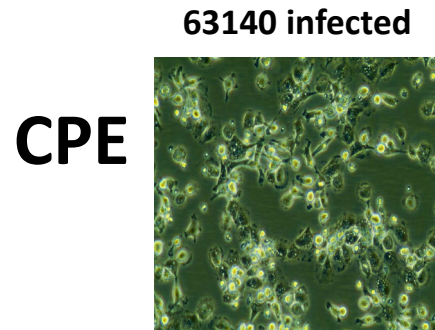
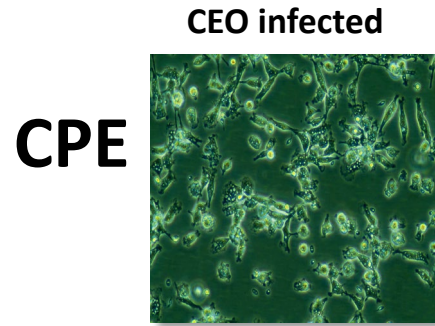


CSF1R+/MHC II<sup>Hi</sup>

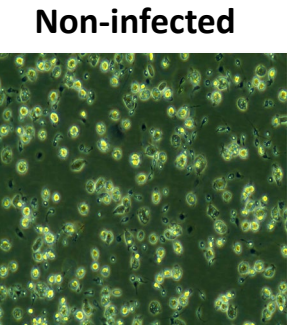
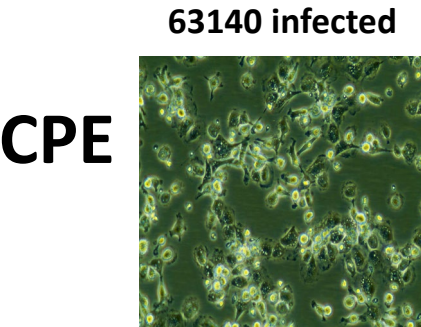
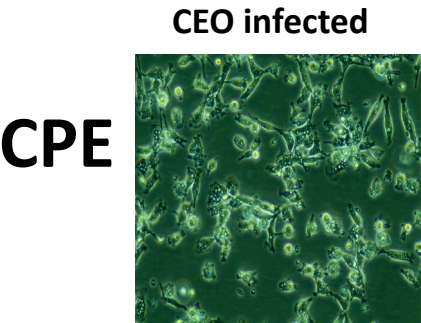


Days post-inoculation

# Macrophage derived monocytes (MDMs)



# Macrophage derived monocytes (MDMs)



MDMs CSF			
	Non-infected	CEO infected	63140 infected
Early apoptosis CSF1R+ (AnnV+/7-AA-)	32.4%	8.8%	18.8%
Late apoptosis & Necrosis CSFR1R+ (AnnV+/7-AA+) (AnnV-/7-AA+)	18.8%	2.8%	4.5%

# Highlights

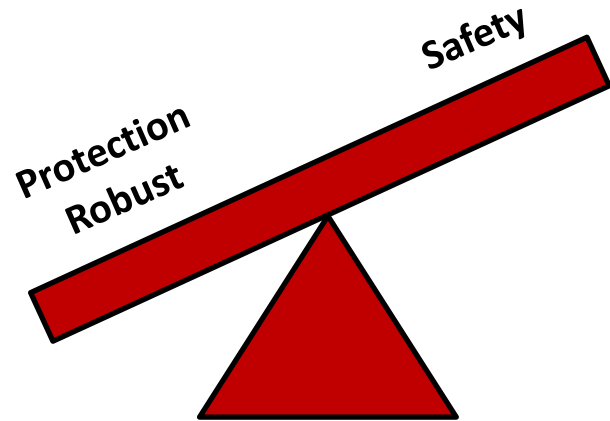
- Essential role of the local cell mediated immunity (increases in CD8+ cells, interferon gamma and granzyme A in CALT occurred in parallel to CEO vaccine clearance from the conjunctiva epithelium)
- Increase in IgA+ B cells occurred late after acute replication indicating that local antibody response did not played a role in clearance of the virus
- Upon ILTV stimulation HG site of B cell proliferation also resident CD8+ plays a role in the cytotoxic response (increase IFN gamma and granzyme A transcription)
- The virulent strain 63140 circumvent the immune response by delaying transcription of IL-12 and downregulating expression of MHCII<sup>Hi</sup>

# Highlights

- Two distinct **mononuclear phagocytes (MNP)** cell populations were identified in unstimulated birds (higher percentages in the HG than in the CALT) after stimulation abrupt decrease in the HG and increase in CALT by day 1 (higher for CEO than 63140)
- MNPs : Macrophage (CSF1R<sup>+</sup>/MHC II <sup>+Lo</sup>) and Dendritic cells (**CSF1R<sup>+</sup>/MHC II <sup>+Hi</sup>**)
- **Macrophage derived monocytes (MDMs)**
  - ILTV established non-productive infection
  - Both vaccine and virulent strain inhibit apoptosis in CSF1R<sup>+</sup> cells
- Can depletion of MNPs populations in the HG of CEO vaccine stimulated chickens can serve as a source of infected MNPs and facilitate virus spread while evading the host immune response to ultimately establish latency?

# Why is this important?

## Live attenuated Vaccines



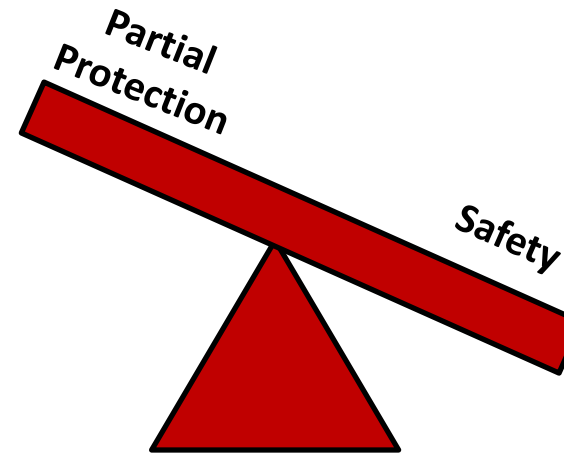
### KNOW

Establish latency  
Inherent virulence  
Regain virulence

### UNKNOWN

Sites of latency?  
Are immune cells infected?  
Virus Immune evasion?  
Protective immunity?

## Recombinant vaccines



### KNOW

Reduction of disease  
Field virus shedding

### UNKNOWN

Generation of Systemic immunity?  
Lack of local immunity?

Questions??

