

## SUMMARY

Patients suffering from viral respiratory diseases are often infected with several unrelated viruses, known as co-infection. However, it is unclear how viral co-infections influence disease pathogenesis. Our lab previously established a respiratory viral co-infection mouse model to better understand how viral co-existence influence pathology and severity of respiratory diseases. *The goal of this research was to test the hypothesis that mice infected with RV1B/PR8 and RV1B/PVM co-infections have lesser pathology than mice infected by individual viruses.*

Mice were inoculated with a mild respiratory virus (rhinovirus; RV1B) two days before a virus that causes severe disease (influenza A virus called PR8 or pneumonia virus of mice termed PVM). Bronchoalveolar lavage (BAL) samples were collected from lungs of mice infected with PR8 or PVM alone, or mice co-infected with RV1B and either PR8 or PVM. They were used to quantify leukocytes present in the airways of mouse lungs. In addition, lung samples were fixed in formaldehyde and embedded in paraffin. Paraffin sections were stained with Masson's tri-chrome stain and a hematoxylin and eosin (H&E) stain to evaluate inflammation and tissue damage. The results suggest that compared to single respiratory viral infections, co-infections decreases the severity of lung pathology.

## BACKGROUND

### VIRUSES IN CO-INFECTION MODEL

#### Rhinovirus (RV1B): Picornaviridae, Enterovirus

- Pathology: common colds in humans.
- RV1B is a minor serogroup human rhinovirus that infects mice.
- RV1B in high dosage can cause acute infection in airways with macrophages, neutrophils, lymphocytes, cytokines & chemokines recruitment and no outward signs of disease in Balb/c mice strain.

#### Influenza A virus (PR8): Orthomyxoviridae, Influenzavirus A

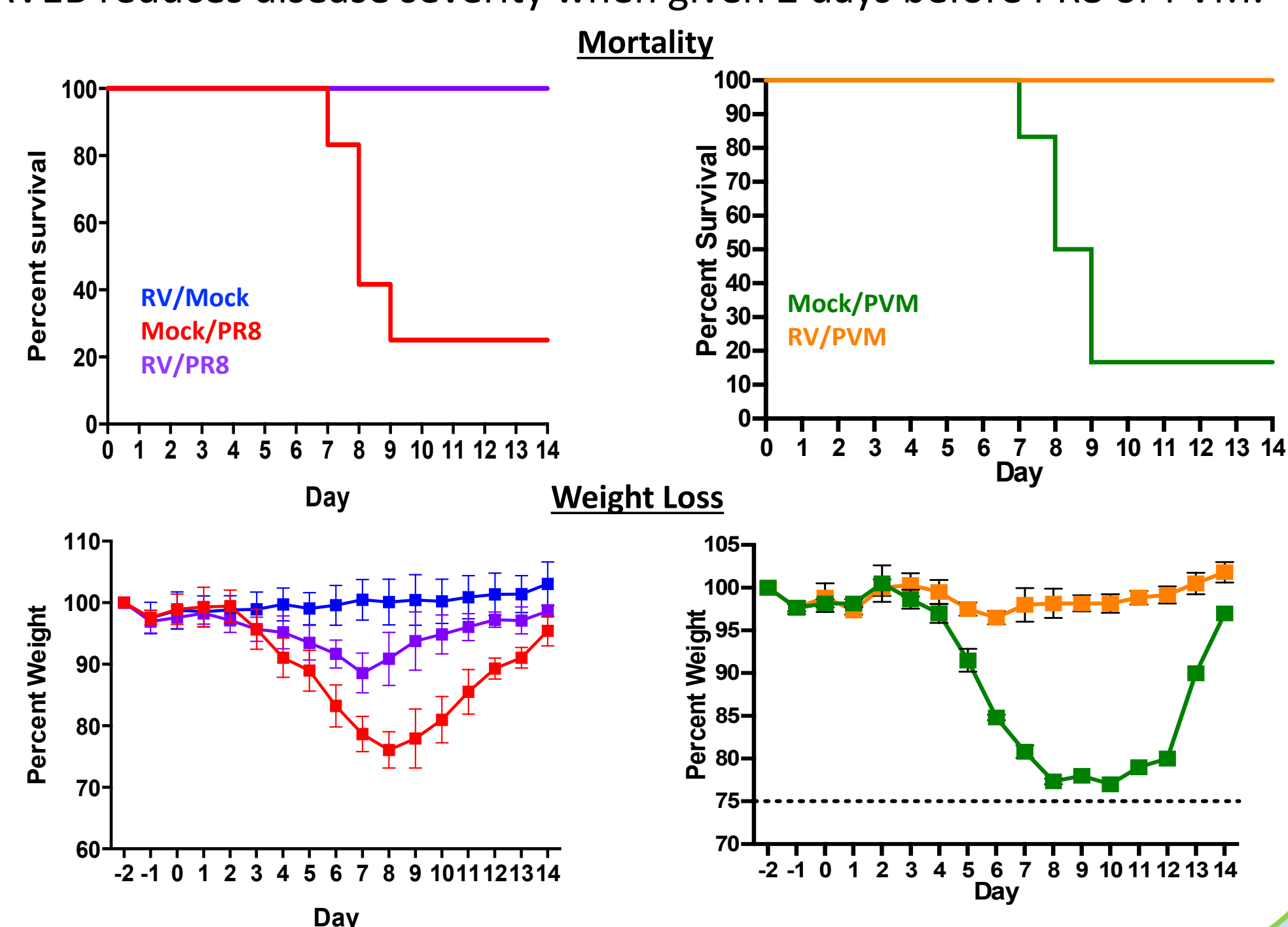
- Pathology: moderate-severe disease in humans.
- PR8 is a mouse-adapted strain of influenza A virus.
- PR8 induces dose-dependent pneumonia in Balb/c mice strain that corresponds with the spread to alveoli and excessive inflammatory response.

#### Pneumonia virus of mice (PVM): Paramyxoviridae, Pneumovirus

- Natural rodent pathogen related to respiratory syncytial virus.
- Respiratory syncytial virus causes bronchiolitis, severe in human newborns and elderly.
- PVM induces severe pneumonia in Balb/c mice strain with early and excessive inflammatory response.

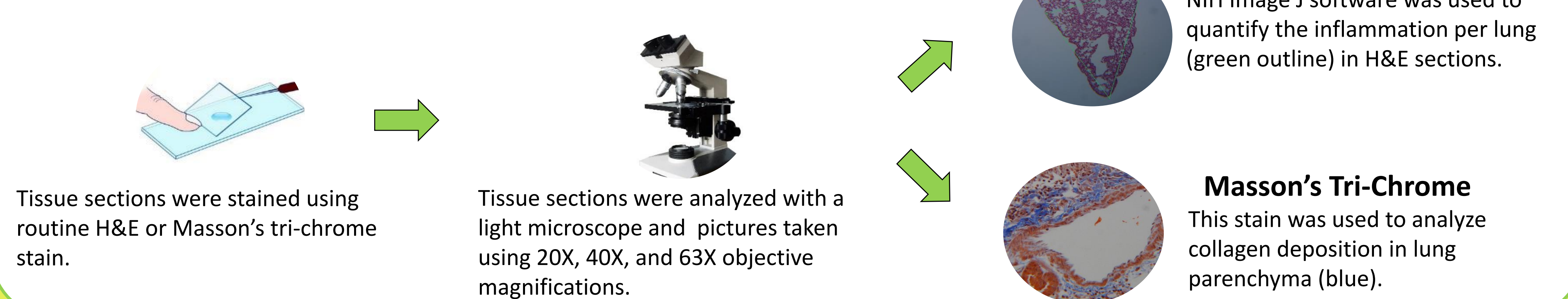
### CURRENT CO-INFECTION MODEL

RV1B reduces disease severity when given 2 days before PR8 or PVM.



## METHODS & ANALYSIS

### HISTOPATHOLOGY ANALYSIS



### H&E

NIH Image J software was used to quantify the inflammation per lung (green outline) in H&E sections.

### Masson's Tri-Chrome

This stain was used to analyze collagen deposition in lung parenchyma (blue).

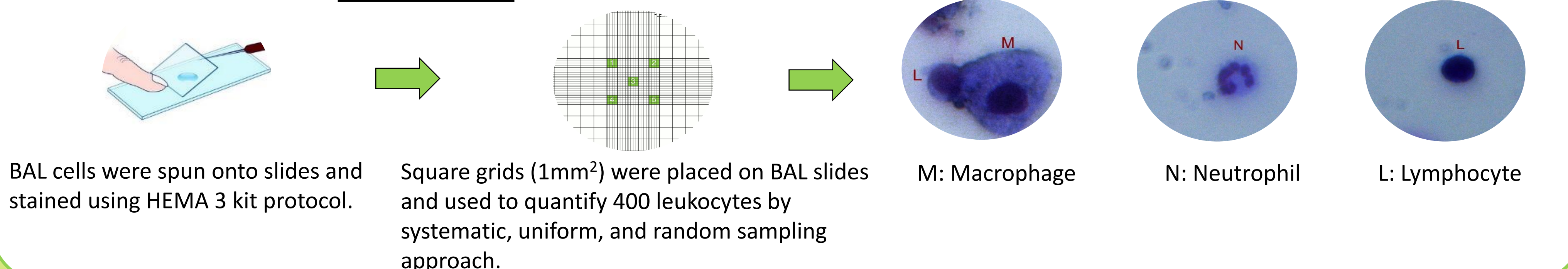
### HISTOLOGY STAINS

- All mice lung samples were collected at 6 days post infection.
- Paraffin embedded tissues were sectioned and stained with:
- Hematoxylin & eosin (H&E): nucleus (dark blue); cytoplasm and red blood cells (pink).
- Masson's tri-chrome: collagen (blue); nucleus (dark brown); hyaline membrane (light purple).

### LEUKOCYTE COUNT IN BRONCHOALVEOLAR LAVAGE (BAL) FLUID

- Macrophages: phagocytic innate immune cells.
- Neutrophils: inflammatory innate immune cells.
- Lymphocytes: pathogen specific adaptive immune cells.

### BAL ANALYSIS



M: Macrophage

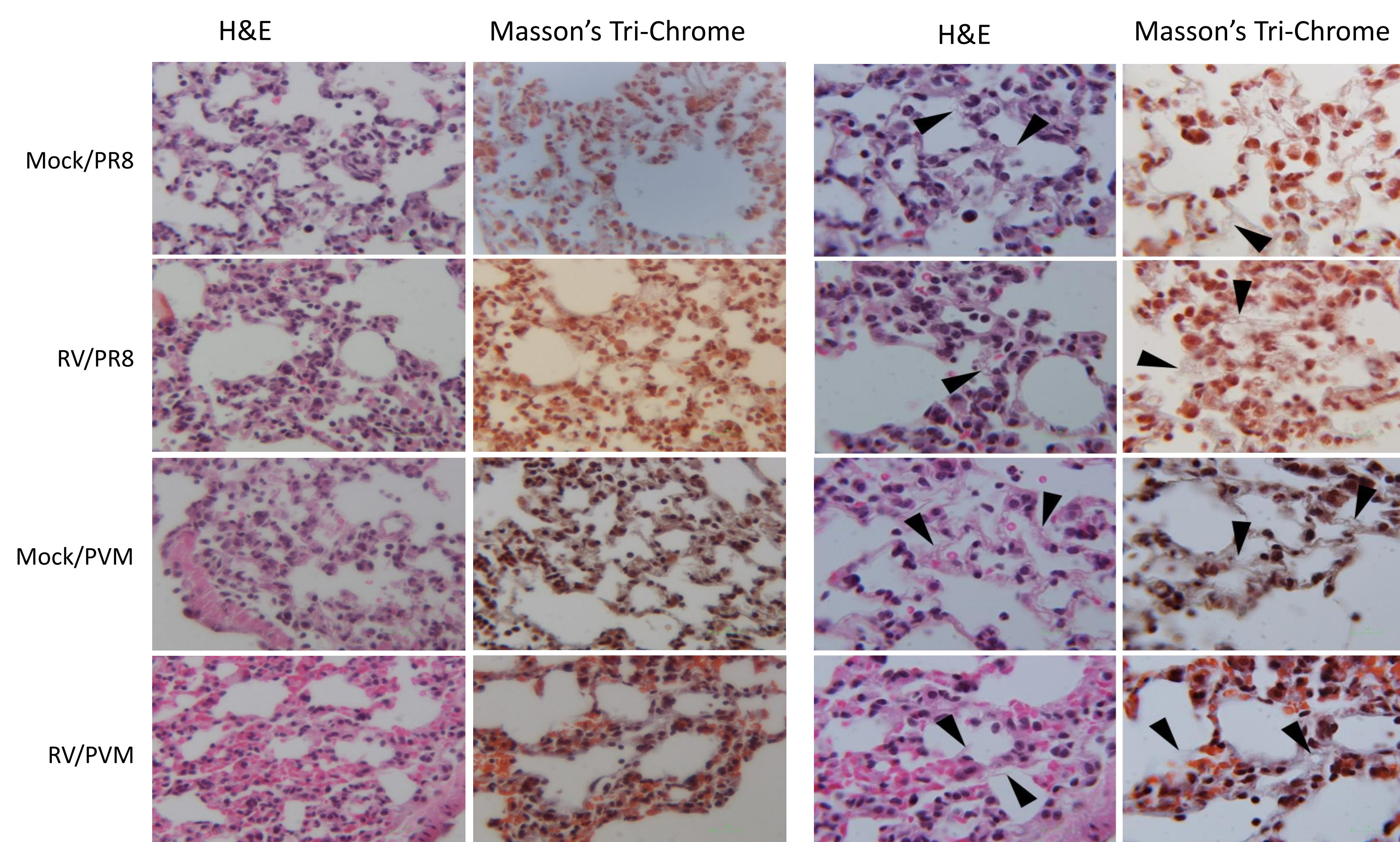
N: Neutrophil

L: Lymphocyte

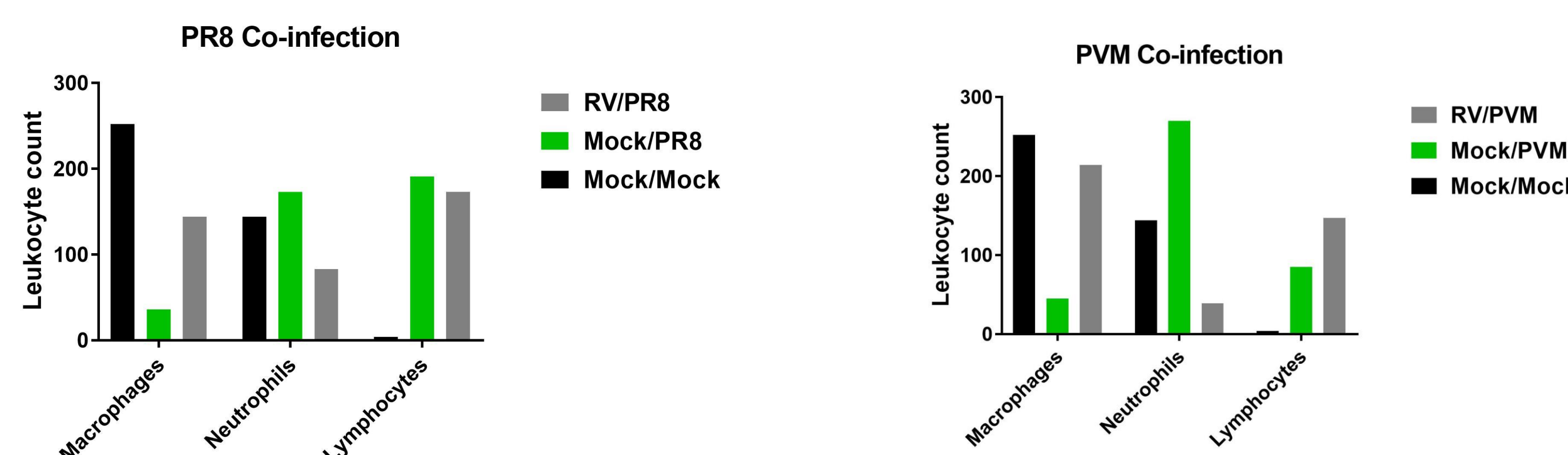
## RESULTS

### 40x Magnification

### 63x Magnification



Black arrows indicate hyaline membrane formation



	Mock/Mock	Mock/PR8	RV/PR8	Mock/PVM	RV/PVM
<b>Inflammation</b>	3.99%	85.70%	71.58%	78.23%	94.81%
<b>Hemorrhage</b>	None	Severe	Mild	Severe	Very Severe

## DISCUSSION & CONCLUSION

- Infections caused epithelial necrosis and sloughing, and accumulation of mucopurulent discharge in the alveoli and bronchioles.
- Lesions were typical multifocal bronchioalveolitis characterized by neutrophils, macrophages, and lymphocytes infiltration; capillary congestion, hemorrhage, extravasation of fluid and proteins, and formation of hyaline membranes (black arrows). Other changes were interalveolar septae thickening and alveoli collapse.
- Co-infecting RV1B with PR8 or PVM reduced disease severity as determined by lessened mortality and weight loss.
- Reduced inflammation in Balb/c mouse lungs co-infected with rhinovirus and influenza.

## FUTURE DIRECTIONS

- Increase sample size
- Immunohistochemistry staining in order to visualize viral antigens and neutrophil extracellular traps.
- Investigate change in pathology on different days post infection.

## ACKNOWLEDGEMENTS

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