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Pharmaceuticals  
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## **EDP-323, a Small Molecule L-Protein Inhibitor in Development Against Respiratory Syncytial Virus**

M. H. J. Rhodin, N. V. McAllister, N. Bisht, R. E. Levene, J. S. Gibbons, A. Balakrishnan, J. Yu, A. A. Szymaniak, K. P. McGrath, T. J. Mann, I. J. Kim, B. Ludeke, R. Fearn, Y. S. Or, and B. Goodwin



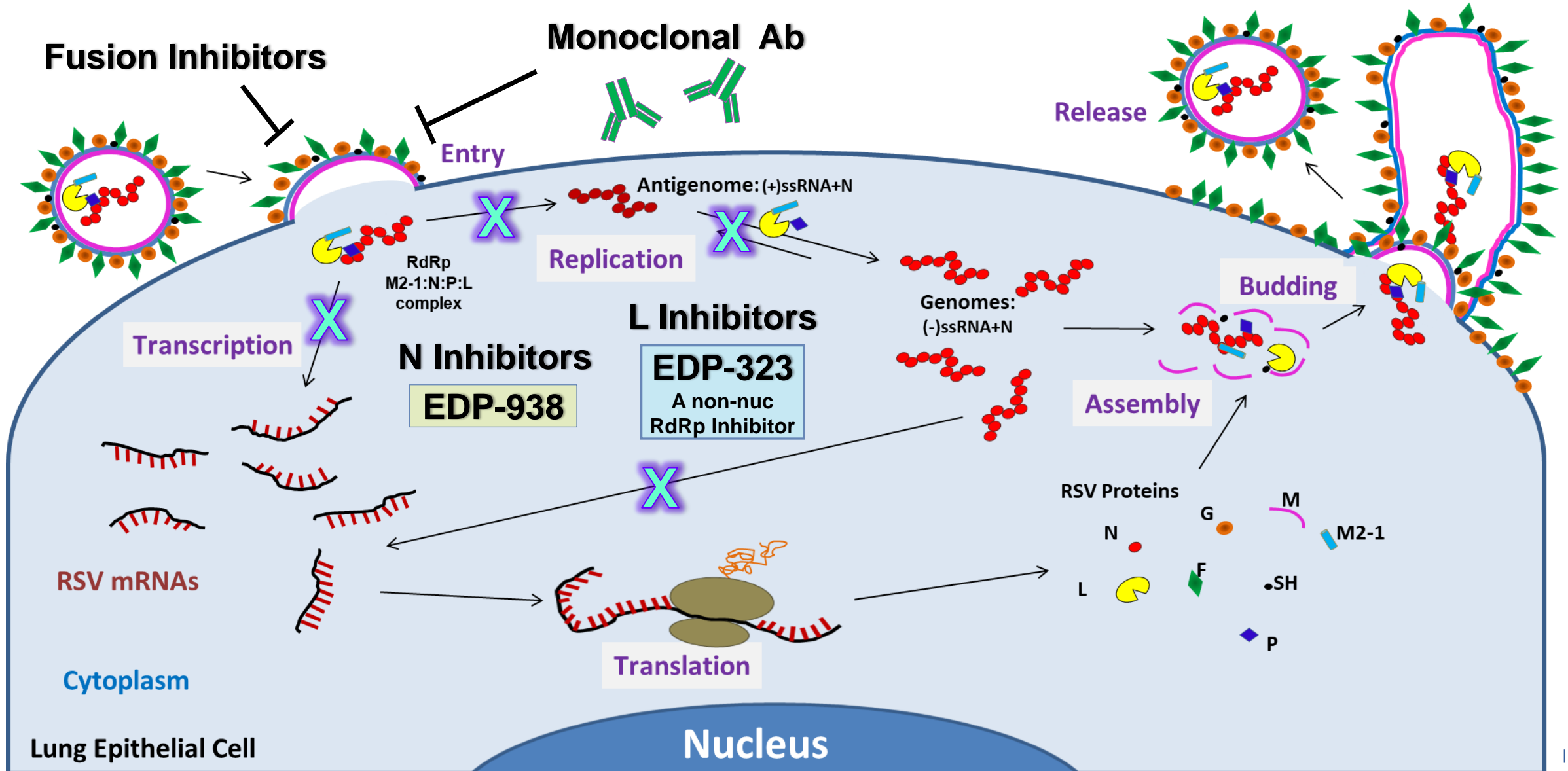
**Disclosures:** Authors M. H. J. Rhodin, N. V. McAllister, N. Bisht, R. E. Levene, J. S. Gibbons, A. Balakrishnan, J. Yu, A. A. Szymaniak, K. P. McGrath, T. J. Mann, I. J. Kim, Y. S. Or, and B. Goodwin are employees of Enanta Pharmaceuticals

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Pharmaceuticals

# RSV Life Cycle and Antiviral Targets



# EDP-323 Displays Potent Antiviral Activity *In Vitro*

### EDP-323 Antiviral Activity: EC<sub>50/90</sub> [nM]

Cell Type	Virus	Readout	EC <sub>50</sub>	EC <sub>90</sub>
HEp-2	RSV-A Long	CPE	0.44	0.52
		RT-qPCR	0.84	1.10
	RSV-B VR-955	CPE	0.40	0.57
		RT-qPCR	0.55	0.64
	RSV-A M37	CPE	0.32	0.36
		RT-qPCR	0.34	0.69
	RSV-A2	CPE	0.15	0.19
HBEC	RSV-A Long	RT-qPCR	0.09	0.15
3D pHAEC ALI Culture	RSV-A Long	RT-qPCR	0.16	0.27
	RSV-B VR-955	RT-qPCR	0.09	0.33

### EDP-323 Cytotoxicity: CC<sub>50</sub>

Cells	5 days
HEp-2	18 μM

### EDP-323 Activity vs Clinical Isolates EC<sub>50/90</sub> [nM]

Virus	Isolate	EC <sub>50</sub>	EC <sub>90</sub>
RSV-A HEp-2 Cells CPE Readout	80189	0.33	0.45
	Tracy	0.36	0.61
	79365	0.28	0.45
	37425	0.19	0.28
	121301018	0.19	0.21
	79309	0.36	0.68
	79223	0.21	4.20
	121301343	0.18	0.56
	61245	0.20	0.22
	RSV-B HEp-2 Cells CPE Readout	57097	0.15
60188		0.11	0.31
79222		0.30	0.75
65848		0.11	0.30
65859		0.10	0.31
121301314		0.04	0.05
<b>Mean</b>		<b>0.21</b>	<b>0.67</b>

- Picomolar EC<sub>90</sub>s in primary human cells, with a CC<sub>50</sub>/EC<sub>50</sub> selectivity index >30,000

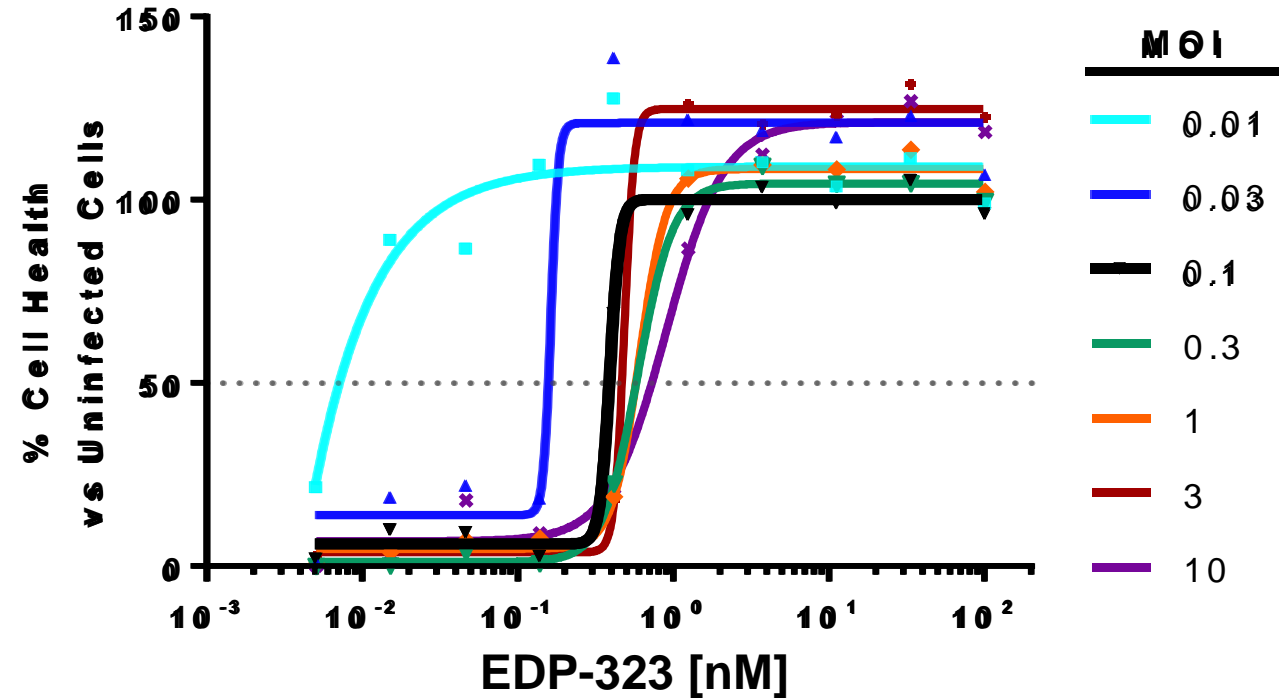
# EDP-323's Potency is Resilient to Increases in Viral Load

## EDP-323 Activity vs Viral Load

MOI	RSV-A Long	
	EC <sub>50</sub> [nM]	EC <sub>90</sub> [nM]
10	0.70	1.30
3	0.47	0.53
1	0.56	0.79
0.3	0.56	0.92
0.1	0.38	0.46
0.03	0.16	0.18
0.01	0.01	0.02

Viral Load

## Viral Load Effect on EDP-323 Activity

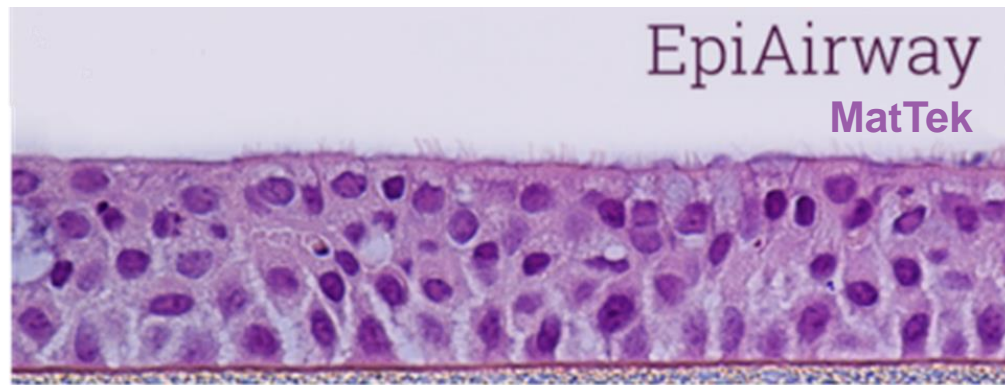


- Improved potency when MOI drops below 0.1
- Only minor potency reduction when viral load increases over MOI 0.1

# EDP-323 Maintains *In Vitro* Efficacy Post-Infection in 3D Cell Culture

## 3D pHAEC-ALI Culture

primary human airway epithelium cells  
grown at an air-liquid interface



Ciliated apical surface

- **Infect at apical surface**

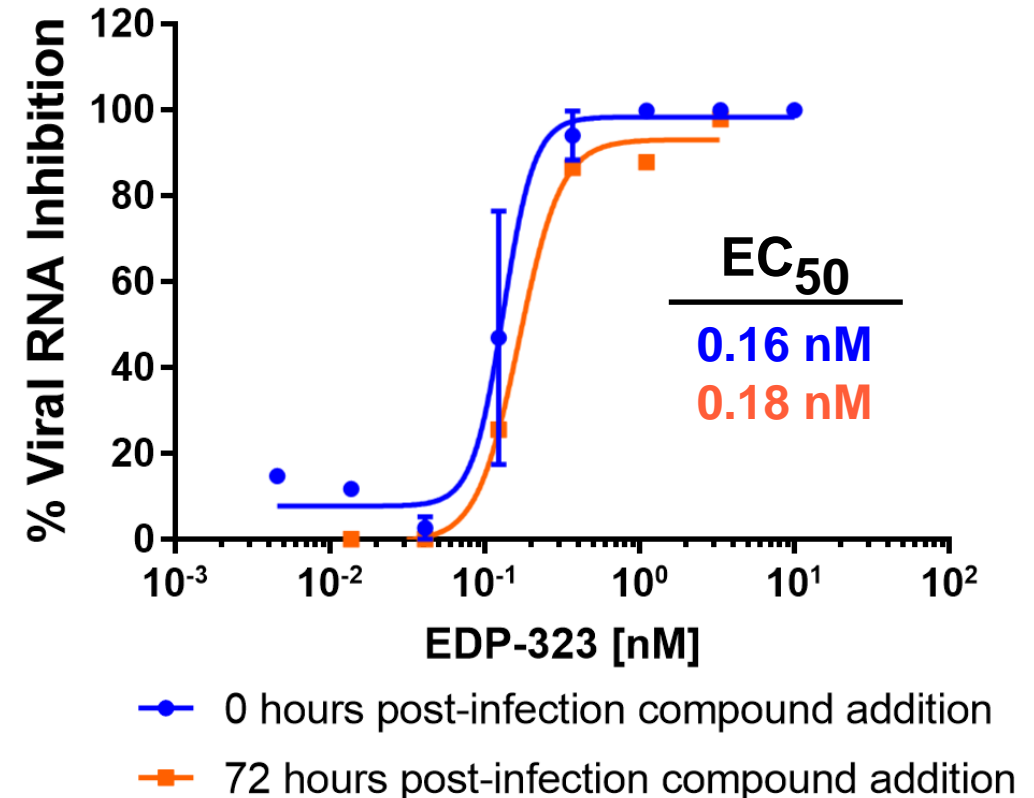
Mucociliary epithelium

Microporous membrane

- **EDP-323 added to basal media**

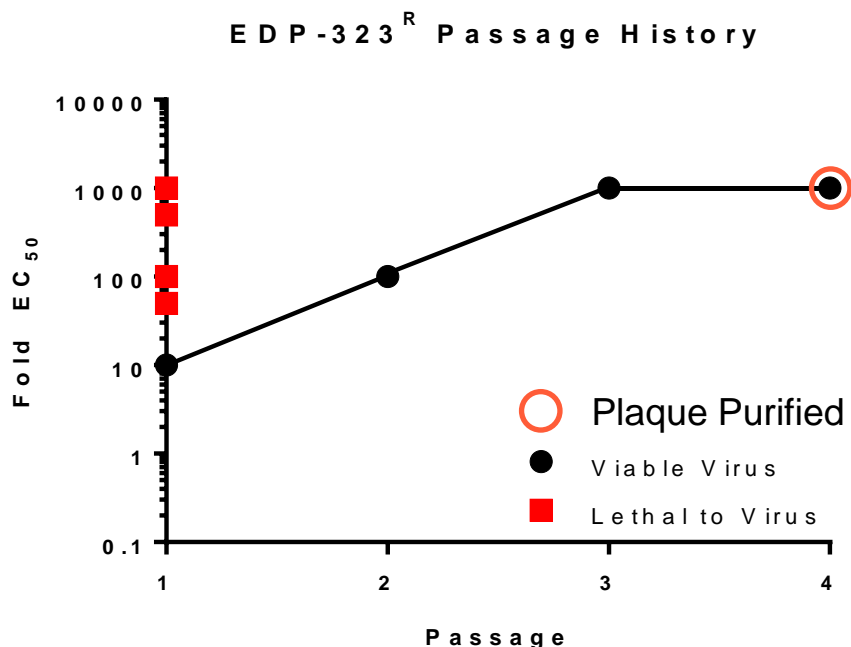
Basal media supply

## RSV-A Long in pHAEC-ALI Culture



- EDP-323 maintains activity 72 hours post-infection in the pHAEC-ALI culture

# EDP-323 Resistance Mapping Indicates Binding to Capping Domain of RdRp



## Plaque Purified and Sequenced

- 18/18 plaques: L: L1372V
- 18/18 plaques: L: C1388G
- 3/18 plaques: L: I392L
- 3/18 plaques: L: K1532E

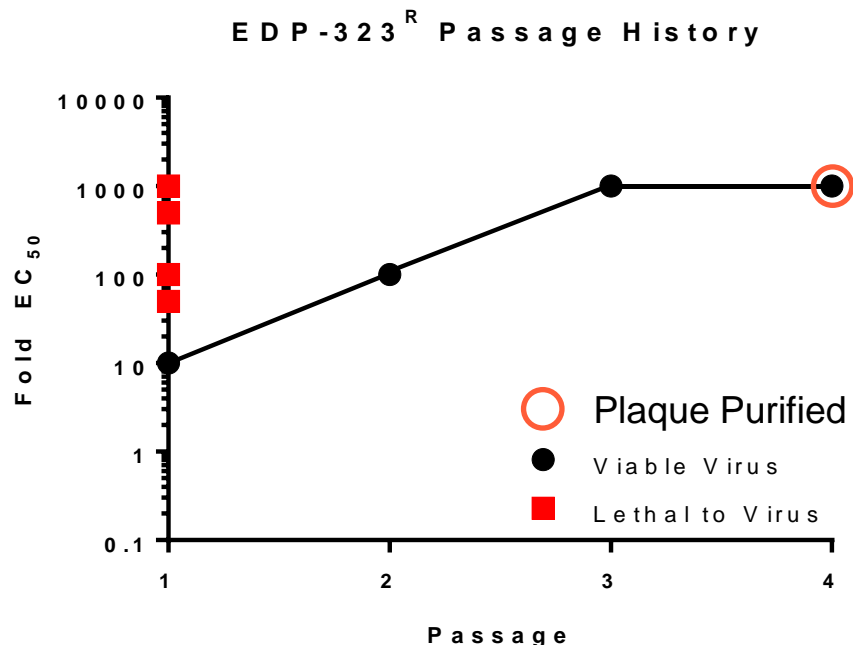
## EDP-323 Cross Resistance Profiling

Compound	Target	WT	L1372V, C1388G	I392L, L1372V, C1388G	L1372V, C1388G, K1532E
		EC <sub>50</sub> nM	Fold-shift	Fold-shift	Fold-shift
<b>EDP-323</b>	<b>L (non-nuc.)</b>	<b>0.3</b>	<b>10,453</b>	<b>7,963</b>	<b>7,834</b>
EDP-938	Nucleoprotein	21	4	3	4
AZ-27	L (non-nuc.)	15	2	1	2
PC-786	L (non-nuc.)	1.9	3	2	3
BI-D	L (non-nuc.)	56	2	3	2
ALS-8176	L (nuc.)	1,209	6	4	7
Remdesivir	L (nuc.)	54	2	1	1
GS-5806	Fusion	1.2	1	1	1
RV-521	Fusion	2.8	1	1	2

Note: WT and mutant strains are in an RSV-A Long background, CPE readout  
 Wild-type RSV-A Long was unable to survive initial treatment ≥50X EDP-323's EC<sub>50</sub>

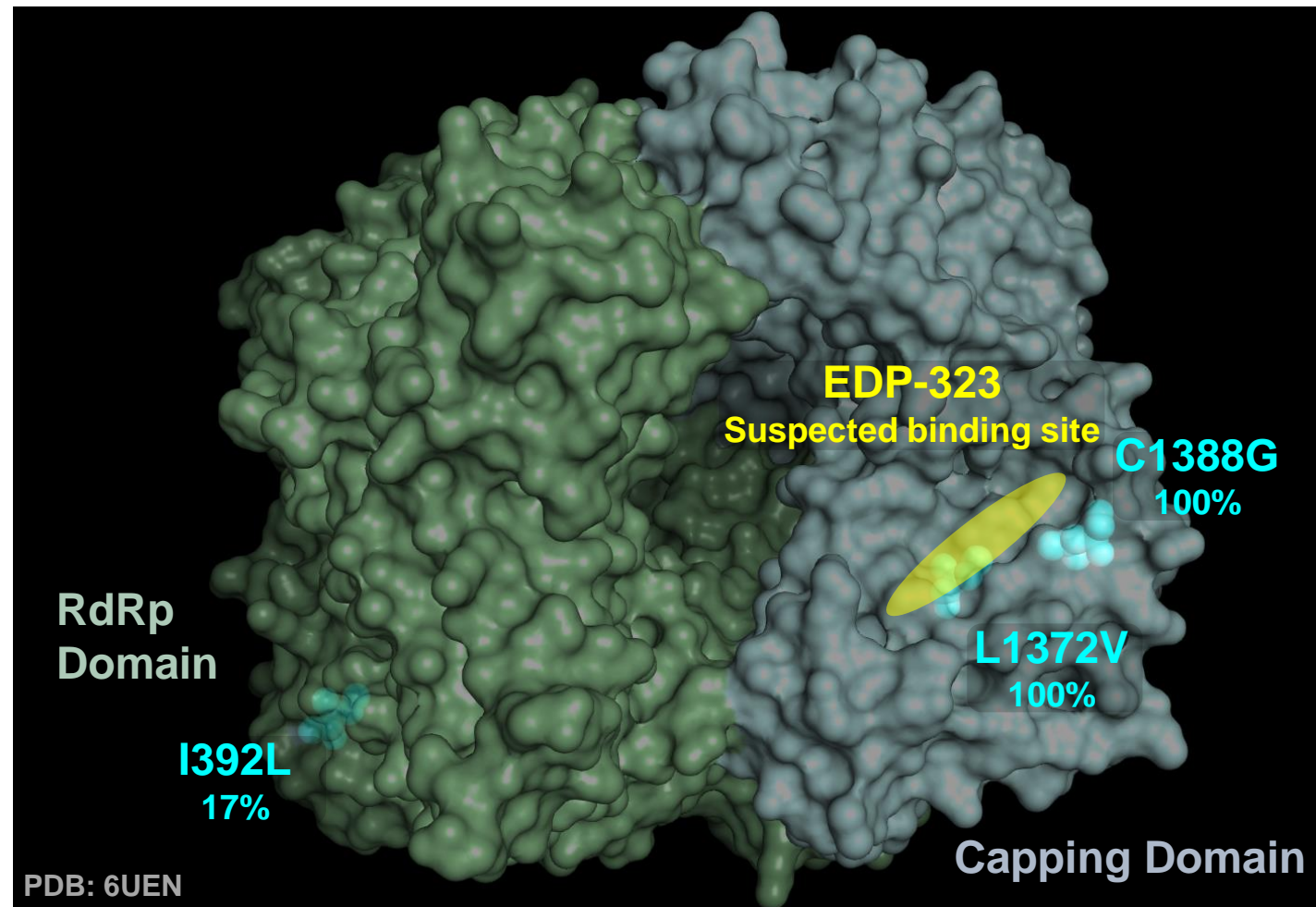


# EDP-323 Resistance Mapping Indicates Binding to Capping Domain of RdRp



## Plaque Purified and Sequenced

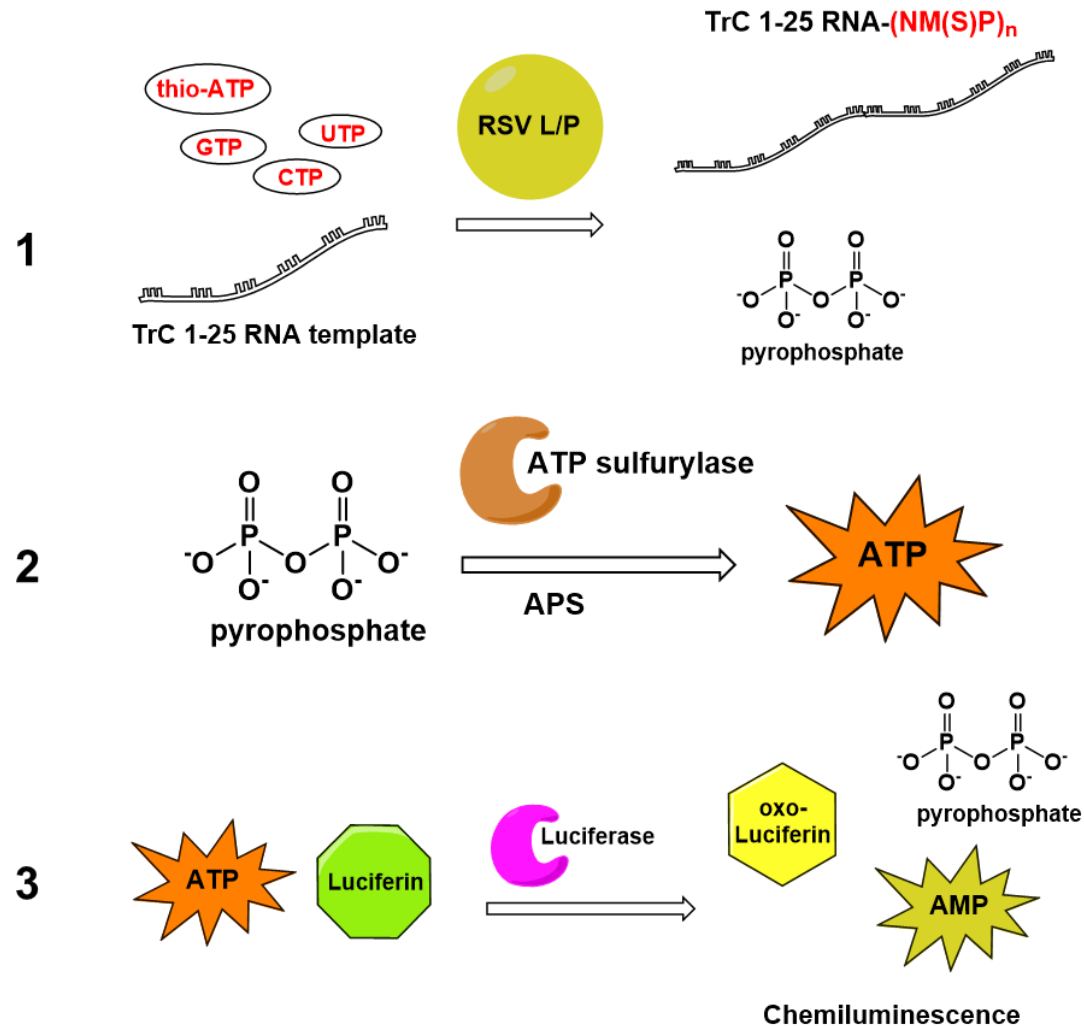
- 18/18 plaques: L: L1372V
- 18/18 plaques: L: C1388G
- 3/18 plaques: L: I392L
- 3/18 plaques: L: K1532E





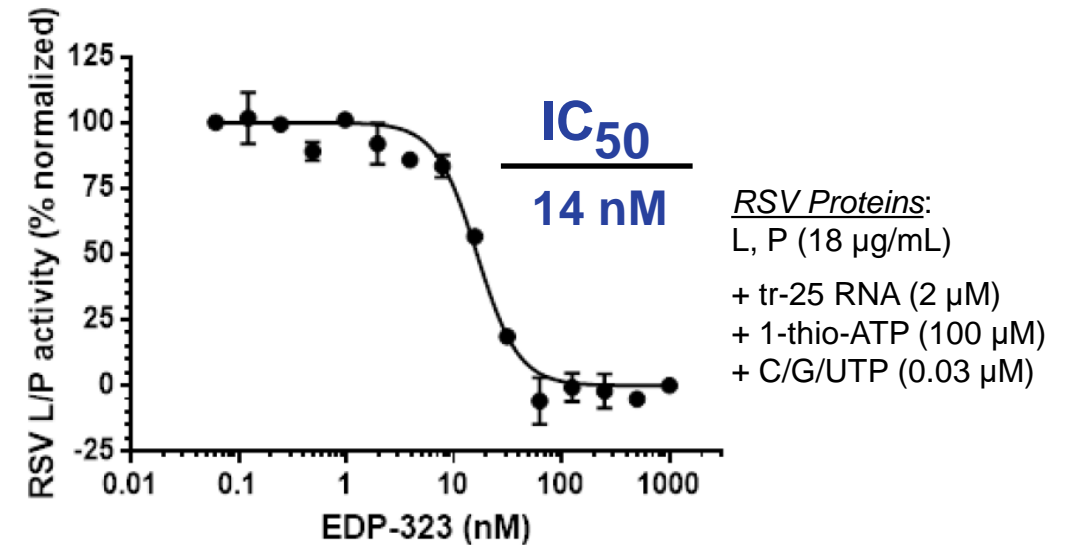
# EDP-323 Directly Inhibits L Protein Function

## Biochemical Assay



## Inhibition of RSV L/P RdRp Activity by EDP-323

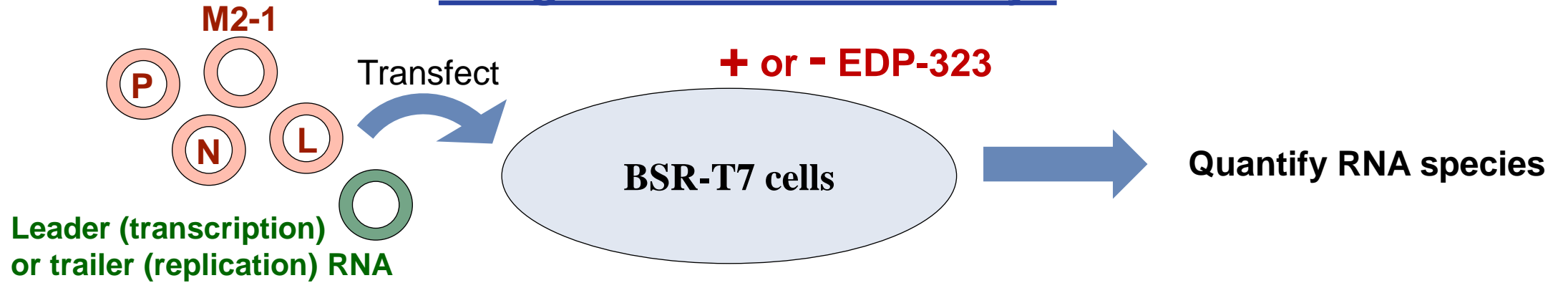
Pyrophosphate detection by enzyme-coupled luminescence assay



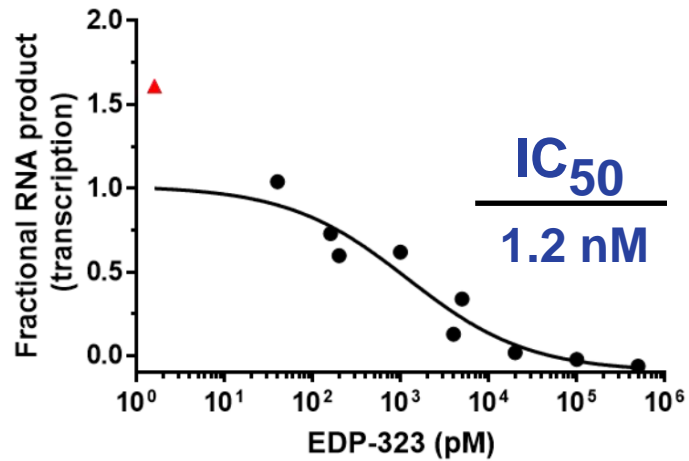
- EDP-323 directly interferes with L protein functionality

# EDP-323 Directly Inhibits L Protein Function

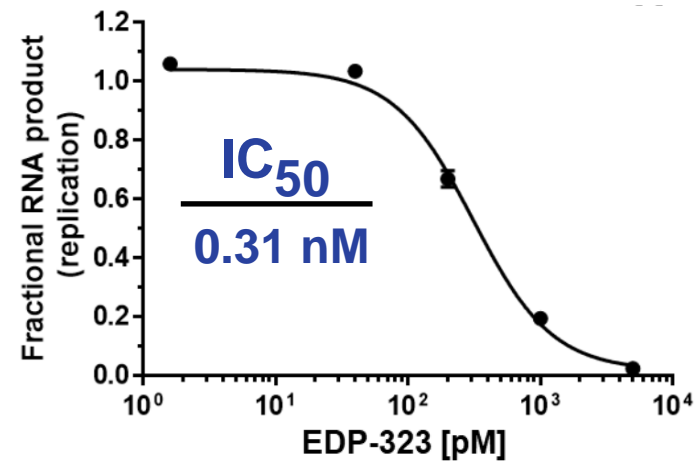
## Minigenome Cellular Assays



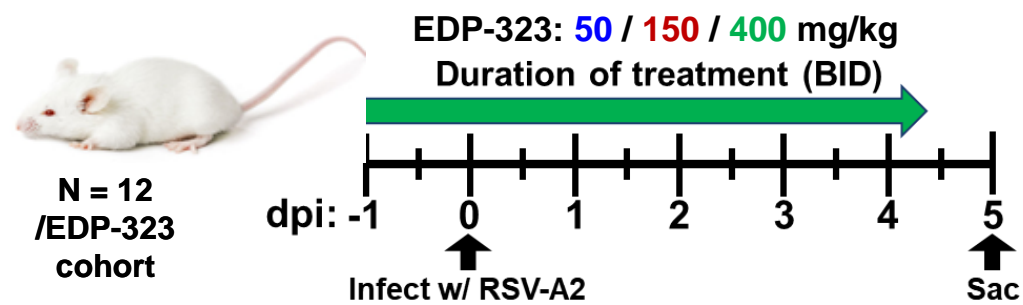
### RNA Transcription Inhibition



### RNA Replication Inhibition

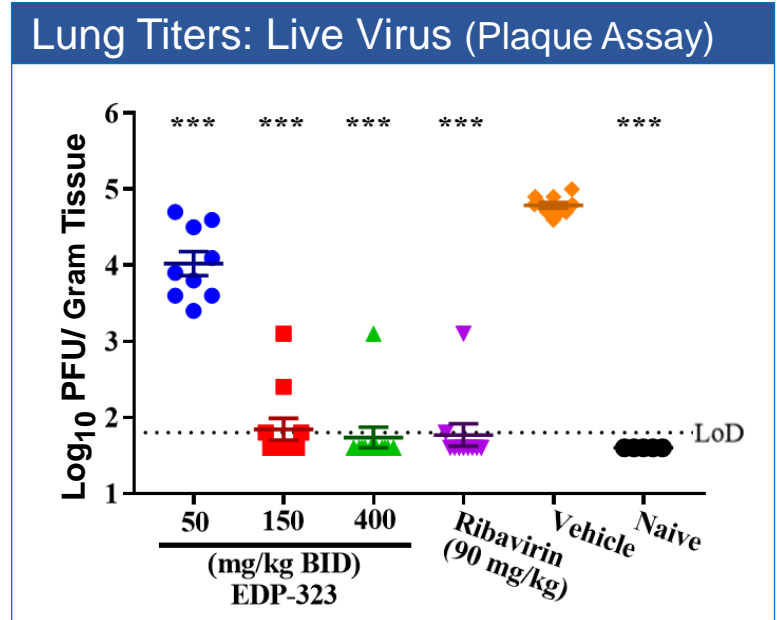
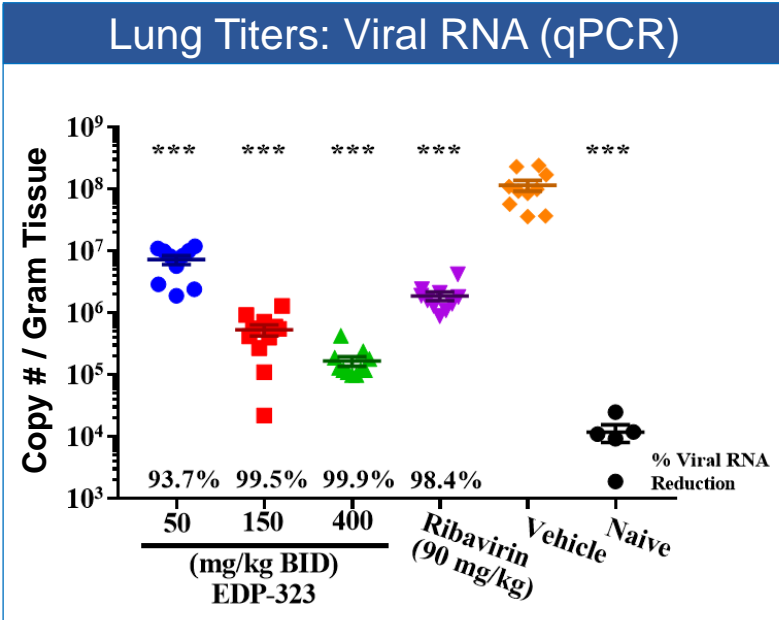
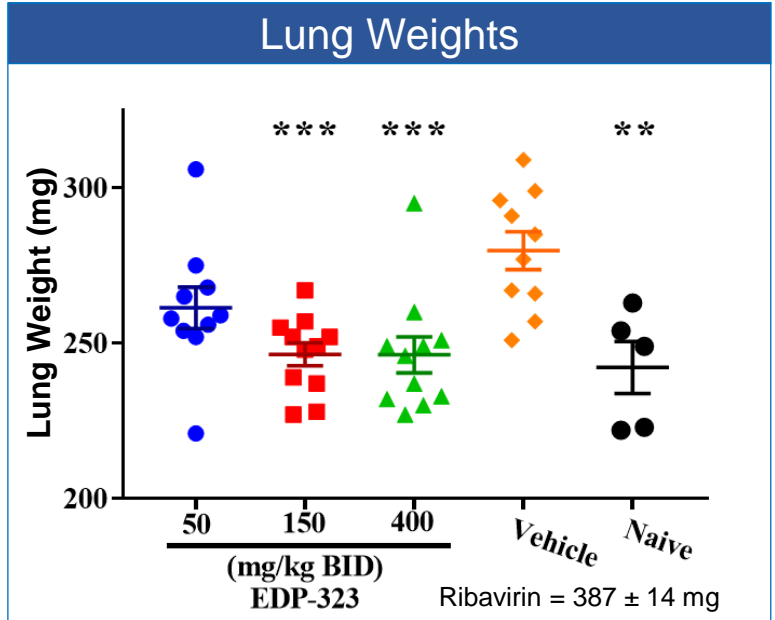


# EDP-323 Protects RSV-Infected Mice in a Dose-Dependent Manner



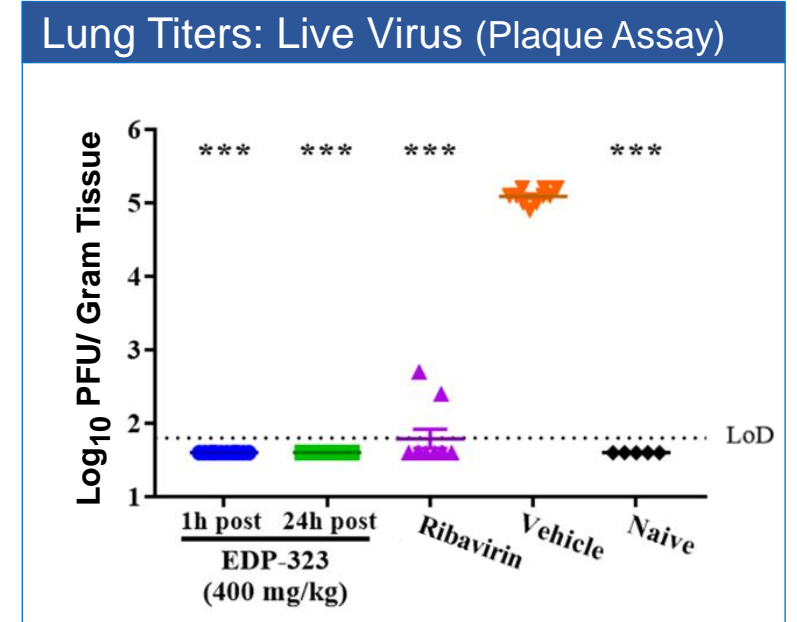
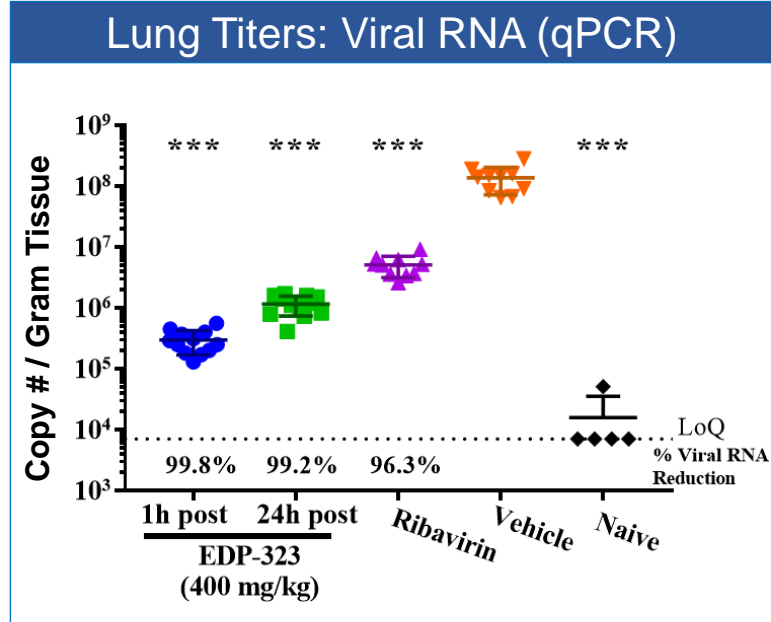
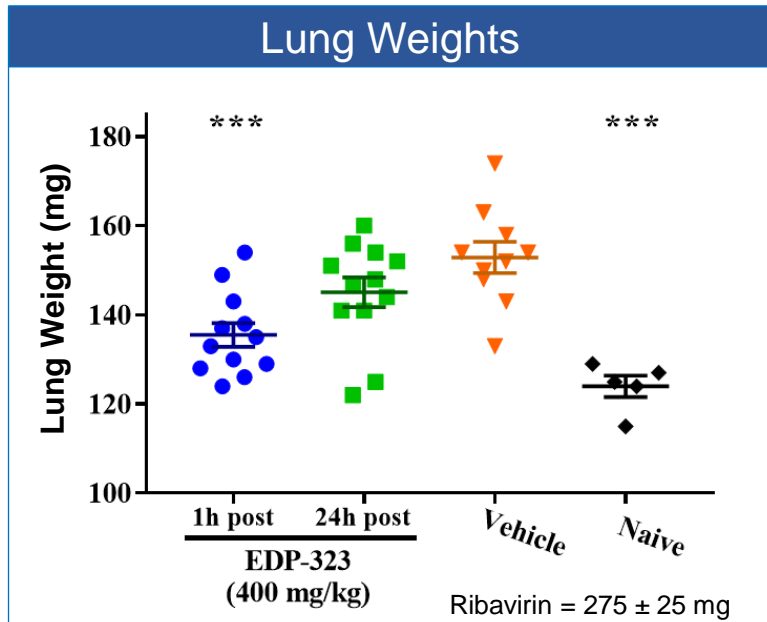
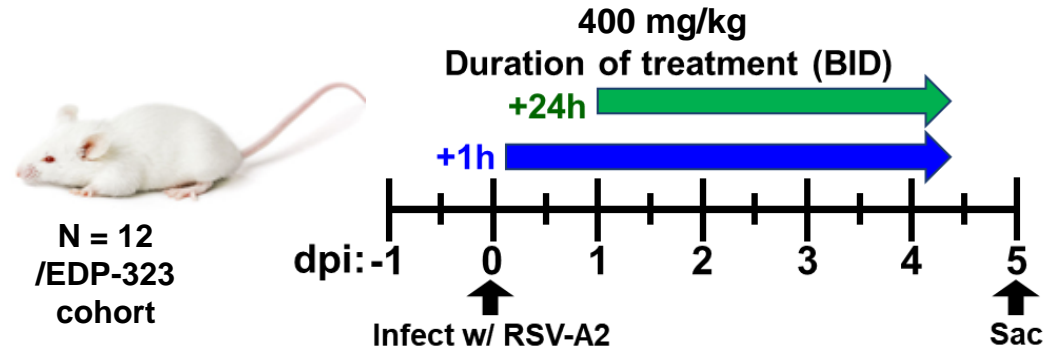
## EDP-323 Antiviral Activity: EC<sub>50/90</sub> [nM]

Cells	Virus	Readout	EC <sub>50</sub>	EC <sub>90</sub>
BALB/c Lung	RSV-A2	RT-qPCR	1.00	4.10
	RSV-A Long	RT-qPCR	1.20	5.30
3D pHAEC ALI Culture	RSV-A Long	RT-qPCR	0.16	0.27
	RSV-B VR-955	RT-qPCR	0.09	0.33



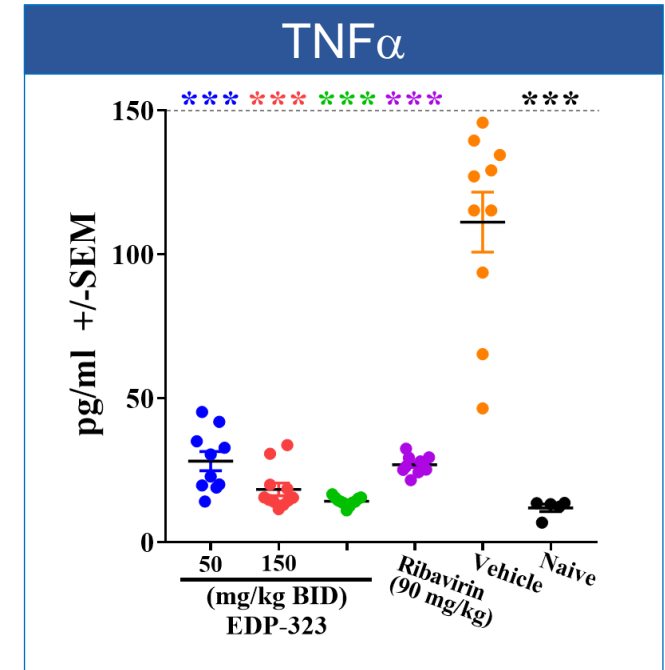
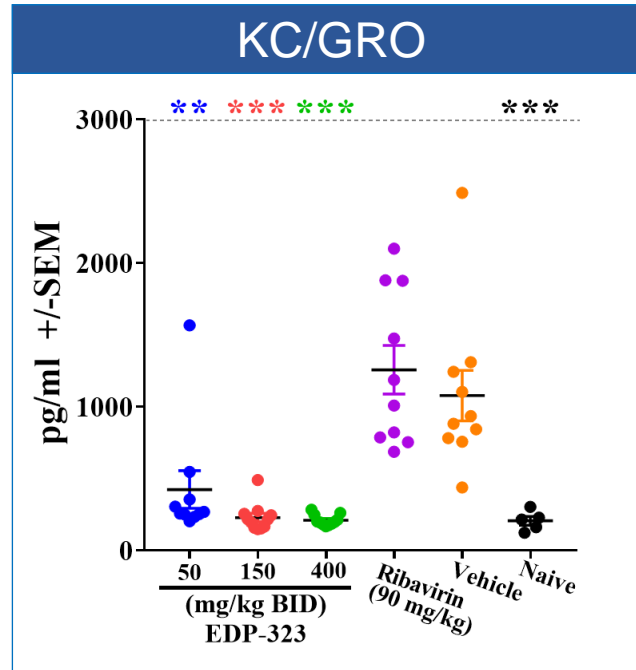
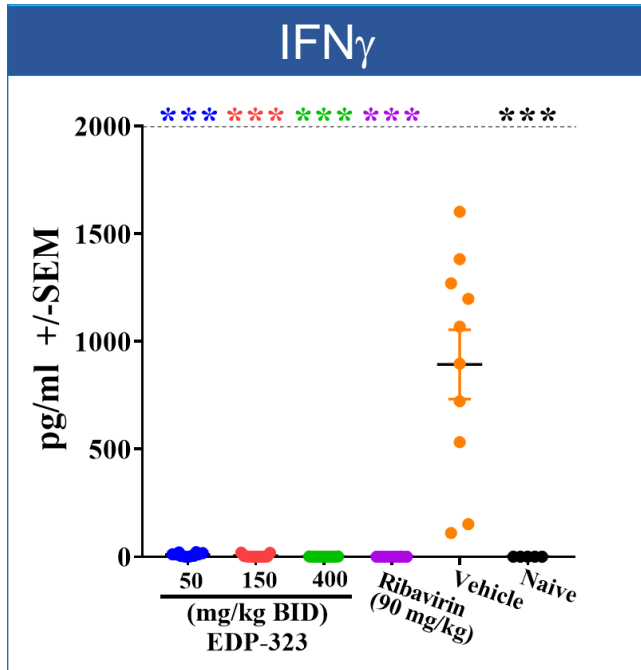
\*\* p < 0.01, \*\*\* p < 0.001 versus vehicle. LoD is 1.8 log<sub>10</sub> PFU/gram tissue. Values below LoD set arbitrary at 1.6 log<sub>10</sub> PFU/g tissue. Error bars are SEM. Ribavirin dosed SC BID from -1 - 4 dpi at 90 mg/kg/day.

# EDP-323 Therapeutically Protects RSV-Infected Mice



\*\*\*p < 0.001 versus vehicle. LoD is 1.8 log<sub>10</sub> PFU/gram tissue. Values below LoD set arbitrary at 1.6 log<sub>10</sub> PFU/g tissue. LoQ is 7 x 10<sup>3</sup> copies/gram tissue. Error bars are SEM. Ribavirin dosed SC QD from +1 hpi - 4 dpi at 90 mg/kg/day.

# EDP-323 Reduces Viral-Induced Pathology in Mice



- All EDP-323 cohorts significantly reduced viral-induced pathology vs vehicle treated infected animals

**For additional EDP-323 *in vivo* results, please visit Enanta’s poster:**

*“In Vivo Efficacy of EDP-323, A Novel L-Protein Inhibitor, for the Treatment of Respiratory Syncytial Virus.”*

Presented by Dr. Rachel Levene, Sept. 30<sup>th</sup> at 6pm

# EDP-323, A Potent L-Protein Inhibitor for the Treatment of RSV

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## EDP-323...

- is a non-nucleoside inhibitor of the RSV RdRp protein inhibiting both viral replication and transcription
- is active against both RSV-A and RSV-B subtypes with low picomolar EC<sub>50</sub>s in multiple primary human cell culture systems
- maintains efficacy and potency across a range of viral loads and times post-infection
- protects mice in a dose-dependent manner from RSV infection both prophylactically and therapeutically as quantified by both virological and pathological endpoints
- has favorable oral bioavailability and PK properties to support once-a-day oral dosing
- is targeted to initiate phase 1 human clinical trials in Q4 of 2022

# Acknowledgements

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## Enanta Pharmaceuticals, Inc.'s RSV Team:

- **Chemistry:** Yat Sun Or, In Jong Kim, Jianming Yu, Adam Szymaniak, Kevin McGrath, Tyler Mann
- **Virology:** Nicole McAllister, Nalini Bisht, Rachel Levene, Joyce Sweeney Gibbons, Nathan Manalo, Bryan Goodwin
- **DMPK:** Lijuan Jiang, Indy Zhang
- **Biochemistry:** Anand Balakrishnan, Archie Reyes

## External Collaborators/Contributors

- **Boston University MoA Studies:** Barbara Ludeke, Rachel Fearn
- **Mouse Efficacy Studies:** Aragen Life Sciences
- **3D pHAEC-ALI RSV Imaging:** Visikol



**Thank you!**

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**Questions?**