Inhibition of Sirtuins Is Broadly Effective Against Coronaviruses

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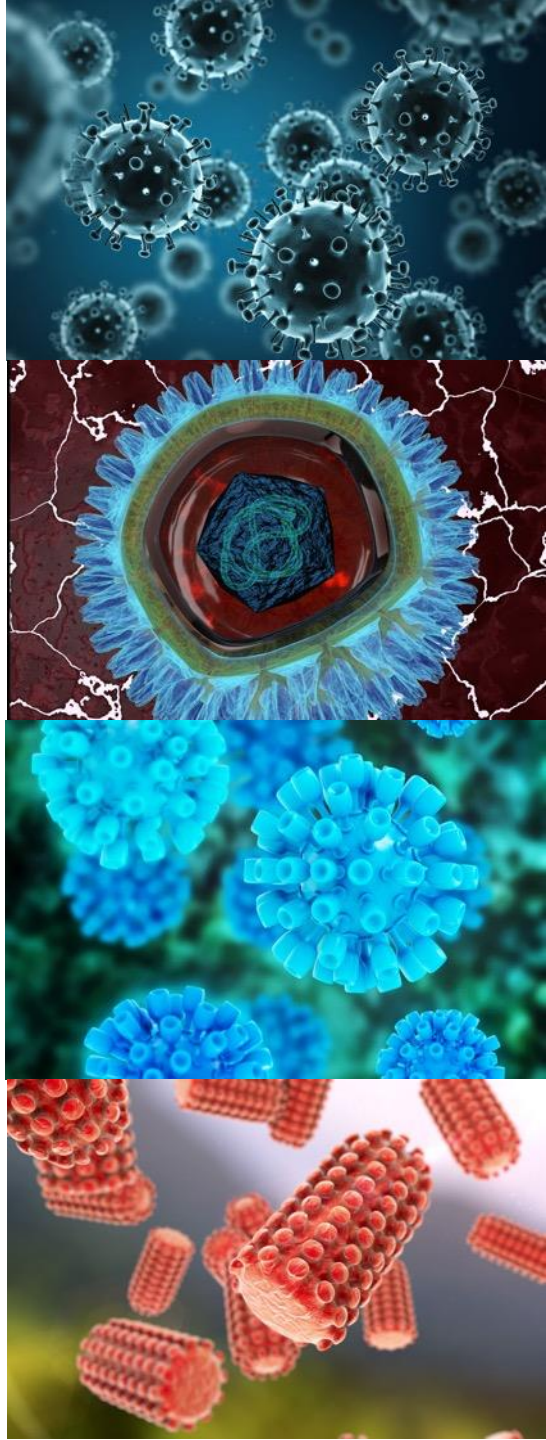
Evrys Bio, LLC

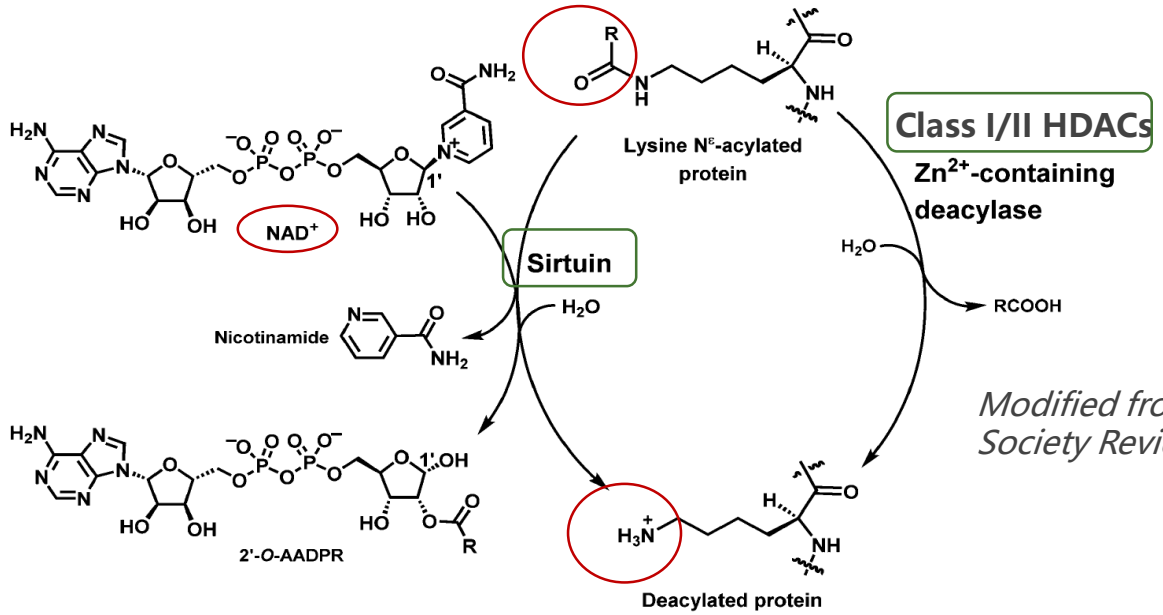
Isirv-AVG Virtual Conference on 'Therapeutics for COVID-19'

10/06/2020



Sirtuins: a Target for restoring host cell metabolism

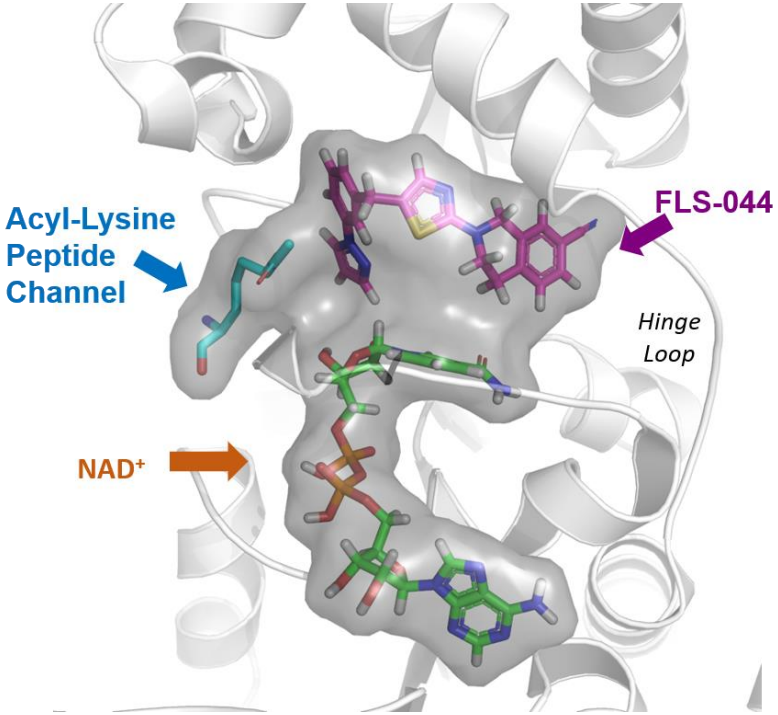
- Sirtuins (SIRT1-7) are a family of NAD^+ dependent deacylases
- SIRT2 expression and function is induced during low-energy status (*Gomes, 2015, Trends Pharmacol Sci)*
- Viral infection often ramps-up host cell metabolism to provide viral building blocks
- SIRT2 inhibitors have been shown to inhibit HCMV[1,2], HAV[2,3], HBV[4,5], *Listeria*[6,7], *Salmonella*[8] *etc*



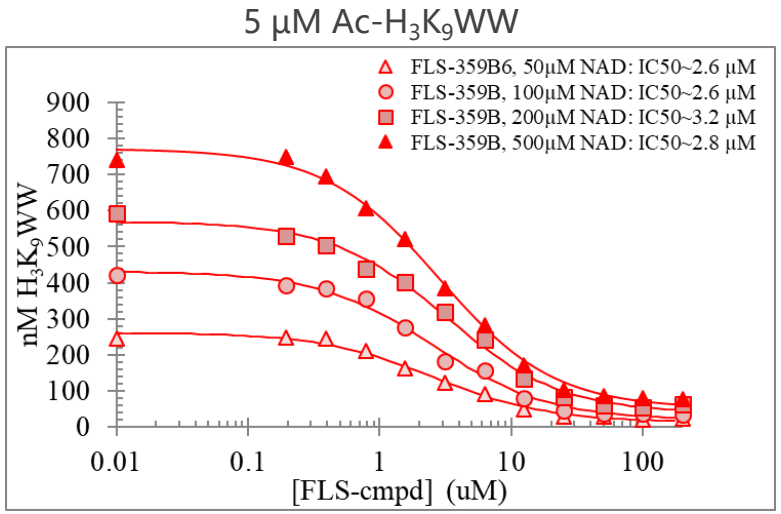
Modified from Chen et al, Chemical Society Reviews, 2015

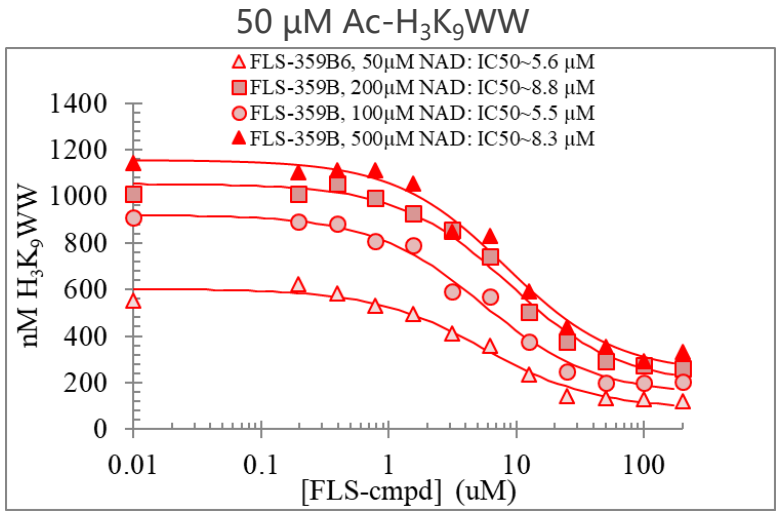
[1,2]*Mao 2016, Koyuncu 2014,* [3]*Kanda 2015,* [4,5]*Piracha 2018, Yu 2018,* [6,7]*Eskandarian 2013, Pereira 2018,* [8]*Gogoi 2018*

FLS-359: an early lead SIRT2 inhibitor



SIRT2 deacetylation activity with FLS-359

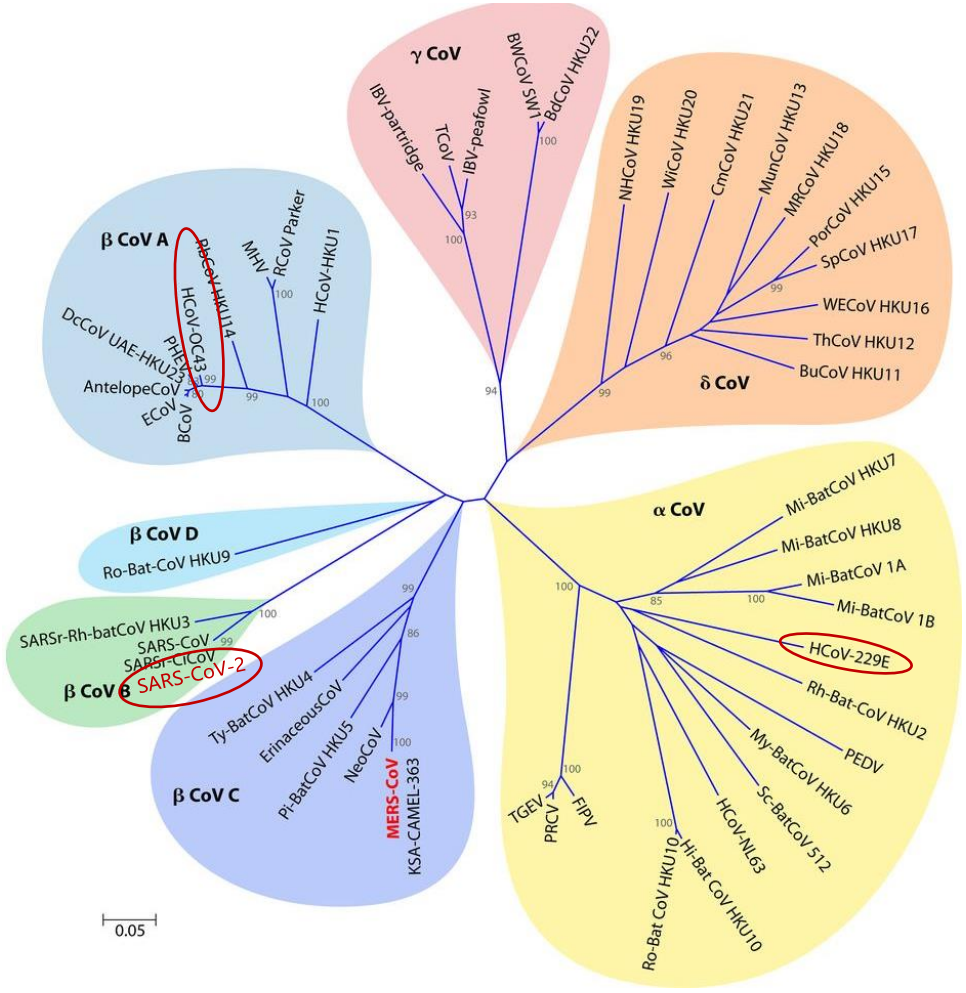




Drug (µM)	SIRT1 dAcetyl IC_{50}	SIRT2 dAcetyl IC_{50}	SIRT3 dAcetyl IC_{50}	SIRT5 dSucc IC_{50}	SIRT6 dMyr IC_{50}
FLS-359	33	3.2	12.3	>100	>100

- FLS-044 is docked into the SIRT2 peptide binding channel, separating the acyl-lysine substrate away from NAD^+
- *In vitro* deacetylation assay demonstrates a partially non-competitive manner
- FLS-359 shows moderate SIRT2 and weak SIRT1, SIRT3 inhibition

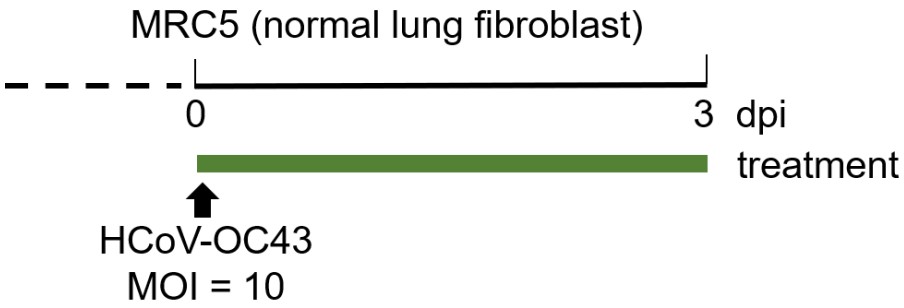
FLS-359 inhibits multiple coronaviruses



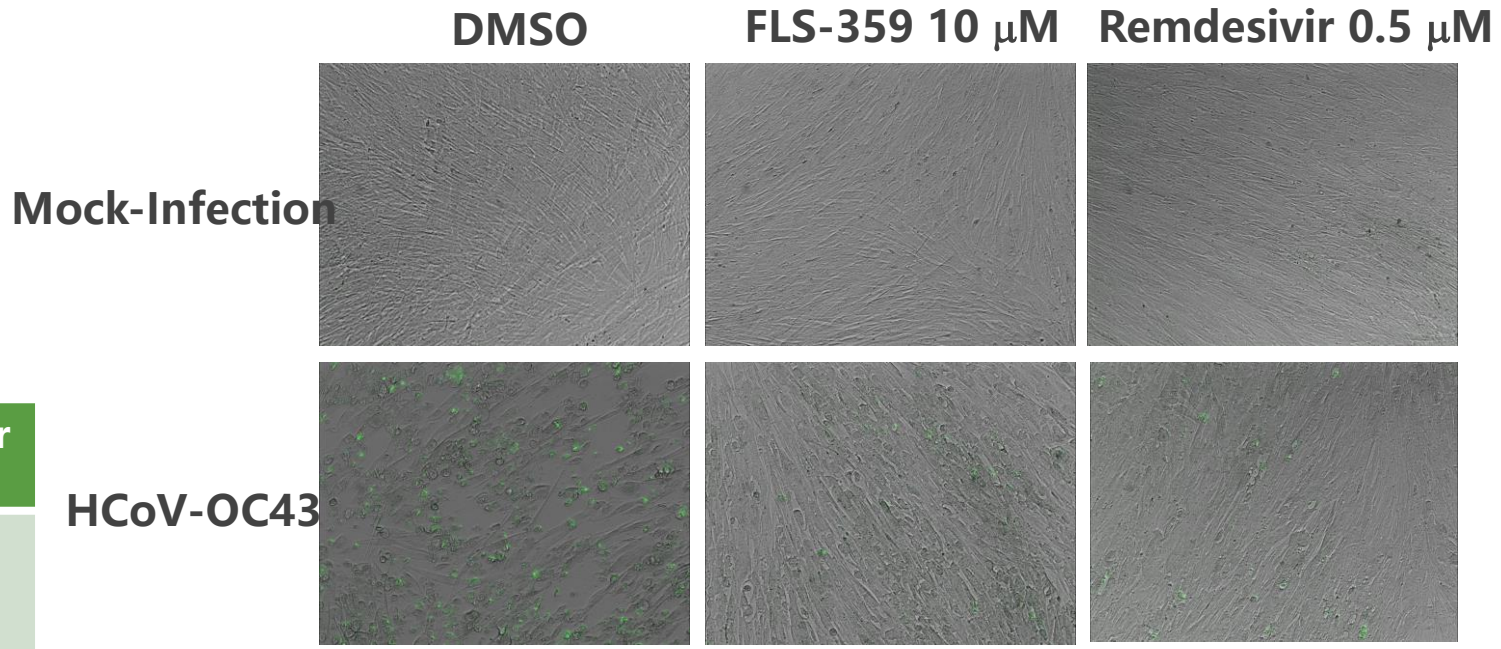
Coronaviruses	FLS-359 IC_{50} (μM)
SARS-CoV-2	1.12
HCoV-OC43	1.51
HCoV-229E	1.66

Modified from Clinical Microbiology Reviews

FLS-359 potently rescues the death of MRC5 cells upon infection with human coronaviruses



HCoVs/ IC_{50}	Genus	FLS-359 (μM)	Remdesivir (μM)
HCoV-OC43	Beta-Coronaviruses	1.51	0.10
HCoV-229E	Alpha-Coronaviruses	1.66	0.04



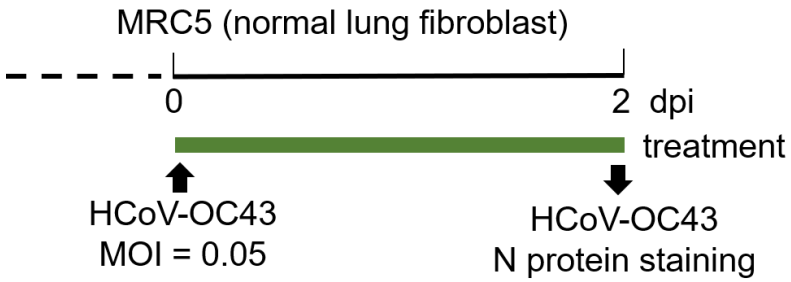
***Green cells indicate the ones with activated Caspase-3/**

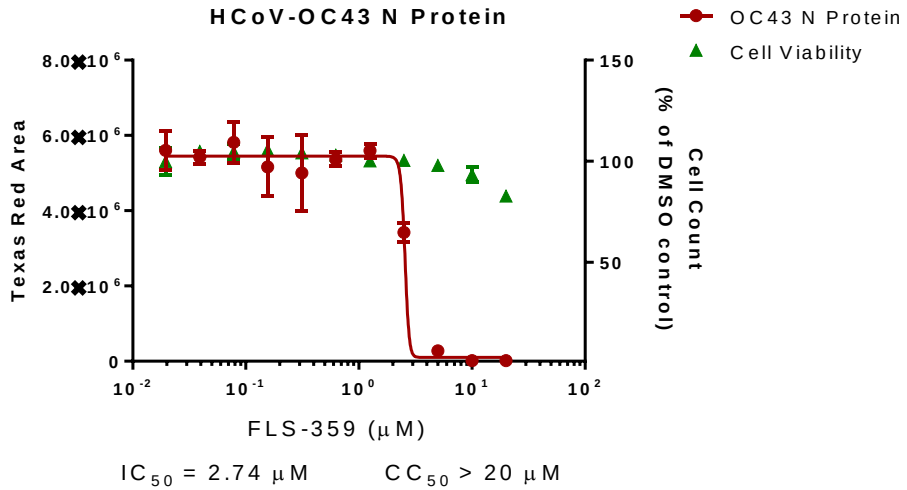
Anti-HCoV-OC43 compound screening

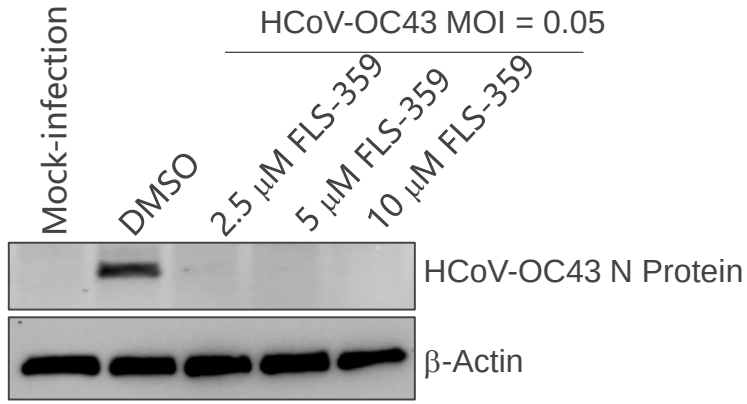
Evrys Bio Compounds	IC_{50} (μM)	CC_{50} (μM)		Selectivity Index	Precipitation (μM)
CoV-1	0.095	>10	*	>109	15.8
Remdesivir (Gilead)	0.103	>25		>243	50
CoV-2	0.124	>16	*	>127	25
CoV-3	0.380	>7	*	>18	10.4
CoV-4	0.451	>10	*	>23	15.8
CoV-5	0.692	>10	*	>15	15.8
CoV-6	0.697	>4	*	>6	6.77
CoV-7	0.716	>10	*	>15	15.8
CoV-8	0.753	>2	*	>3	2.89
CoV-9	1.06	>50		>47	none
FLS-359	1.51	>16	*	>10	15.8
CoV-10	1.97	>25		>13	50
CoV-11	2.11	>25		>12	50
CoV-12	3.34	>25		>7	50
CoV-13	5.79	>7	*	>1	10.4

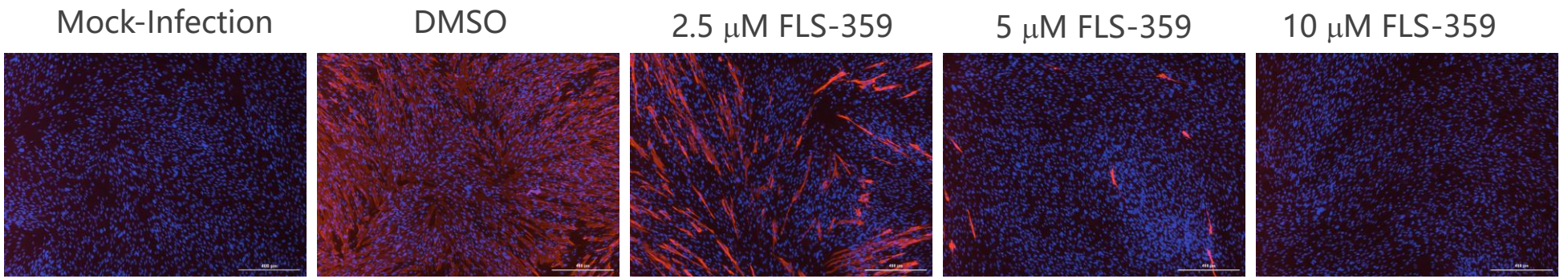
* Compound precipitated at higher concentrations, limiting the estimate of SI

FLS-359 reduces HCoV-OC43 cell-to-cell spread



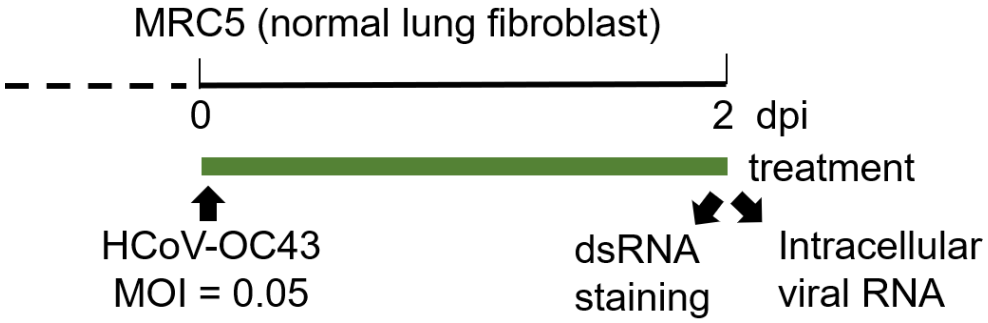


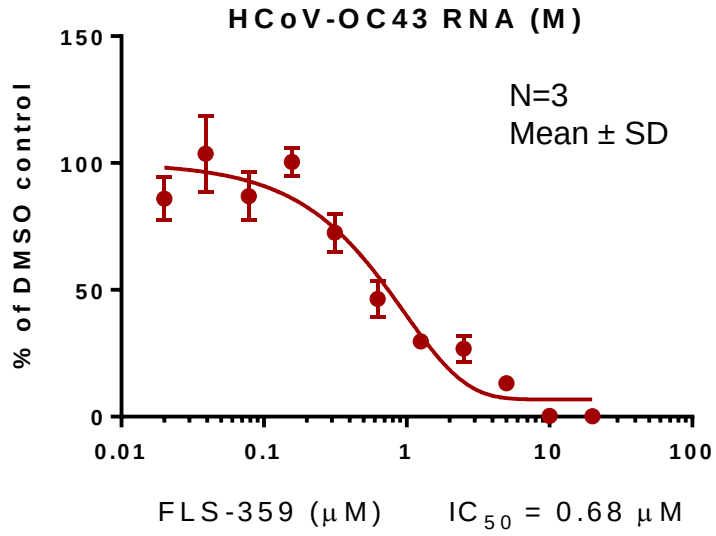


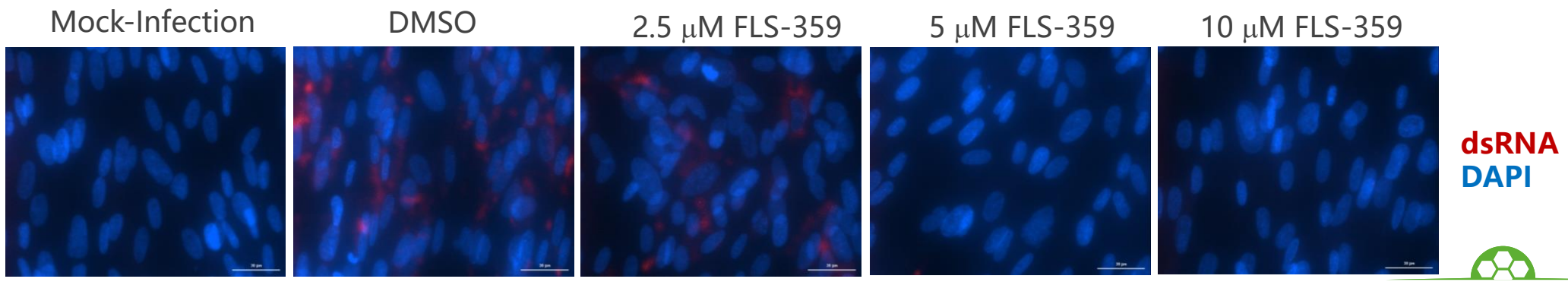


HCoV-OC43N Protein
DAPI

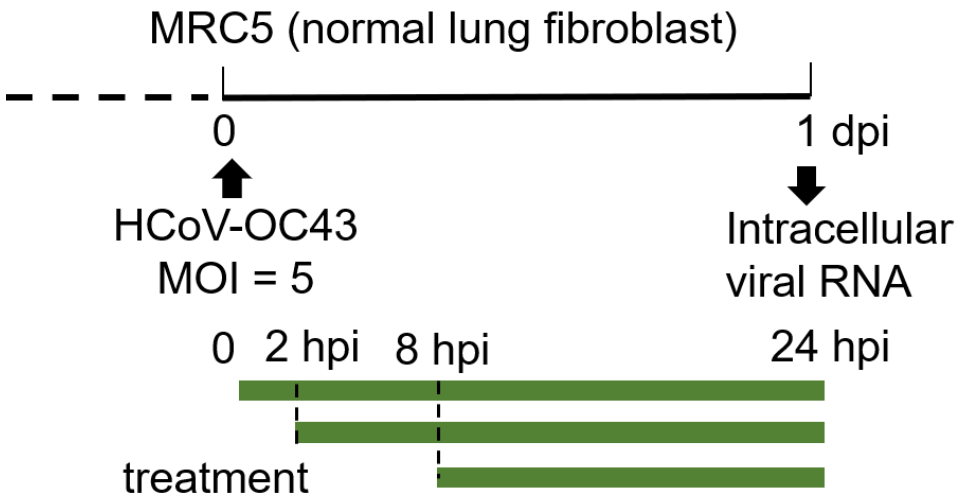
FLS-359 leads to the reduction of HCoV-OC43 RNA

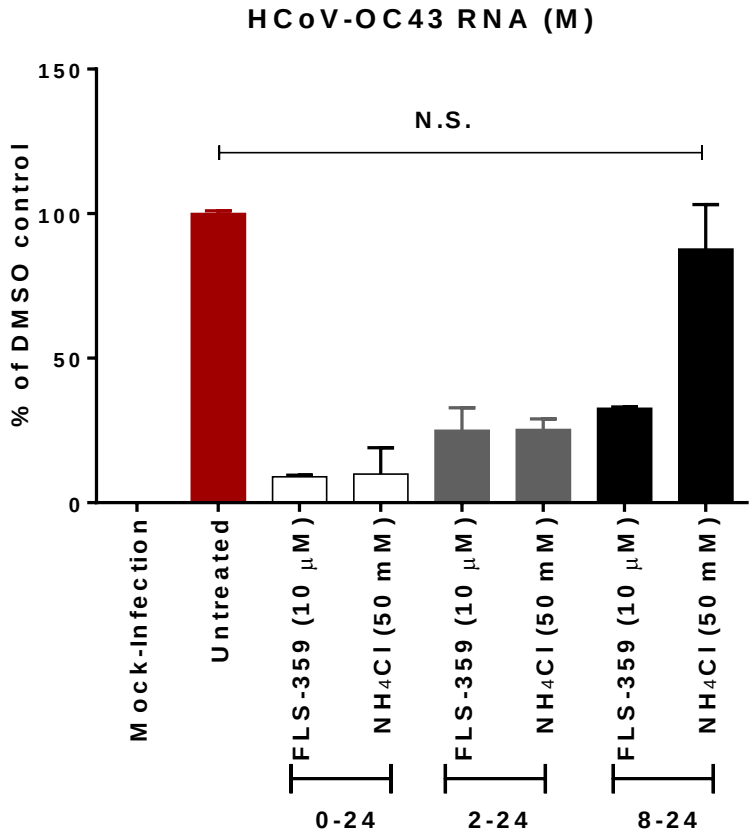






FLS-359 inhibits a post-entry step of HCoV-OC43

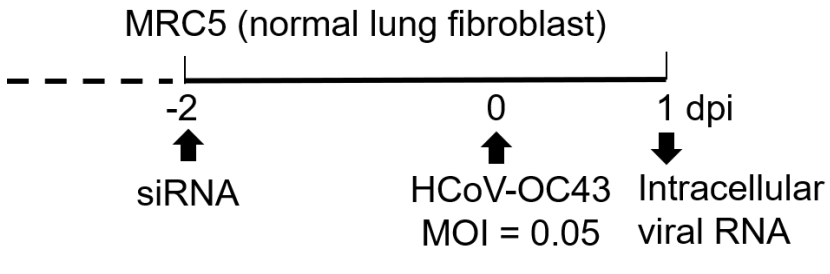


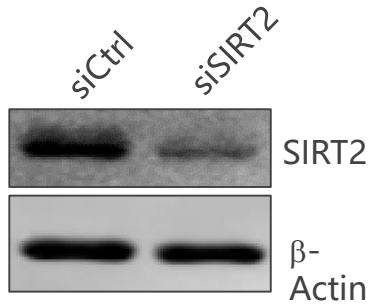


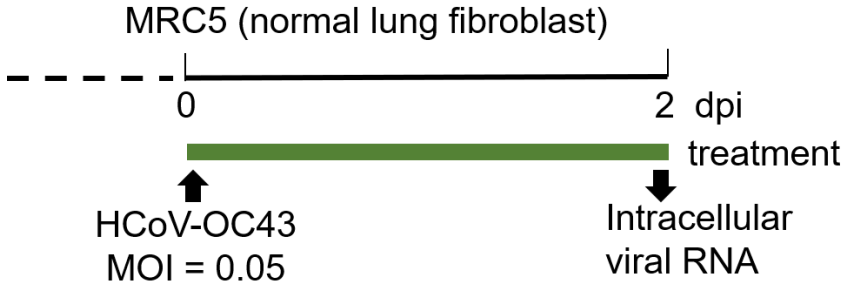
N=2
Mean ± SD

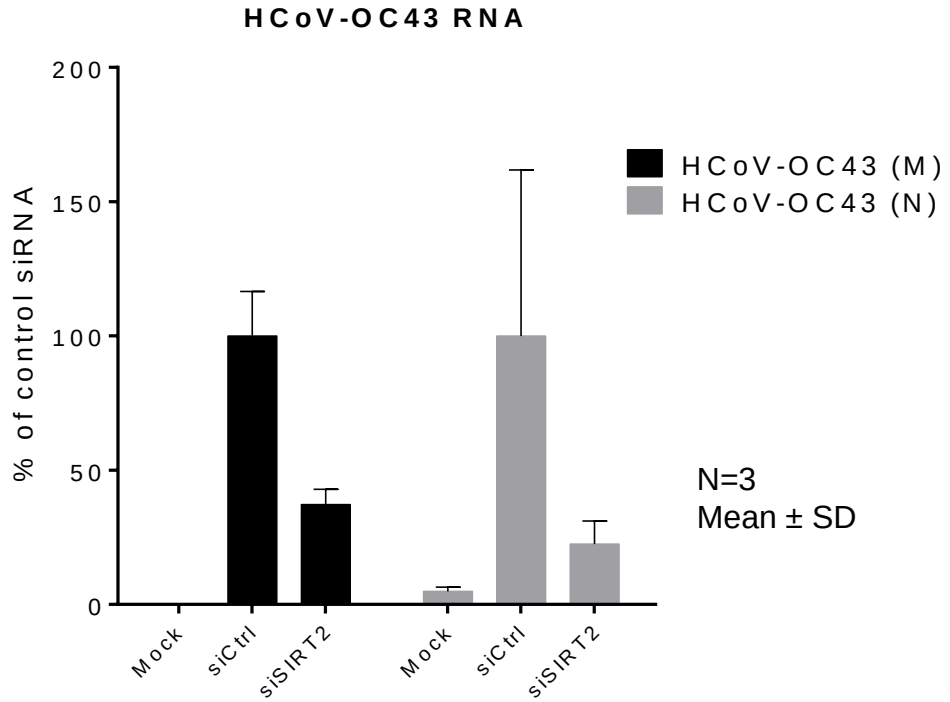
- NH_4Cl inhibits HCoV-OC43 entry through disrupting the endosome pH. *(Owczarek, 2018, Sci Rep)*

Targeting SIRT2 inhibits HCoV-OC43

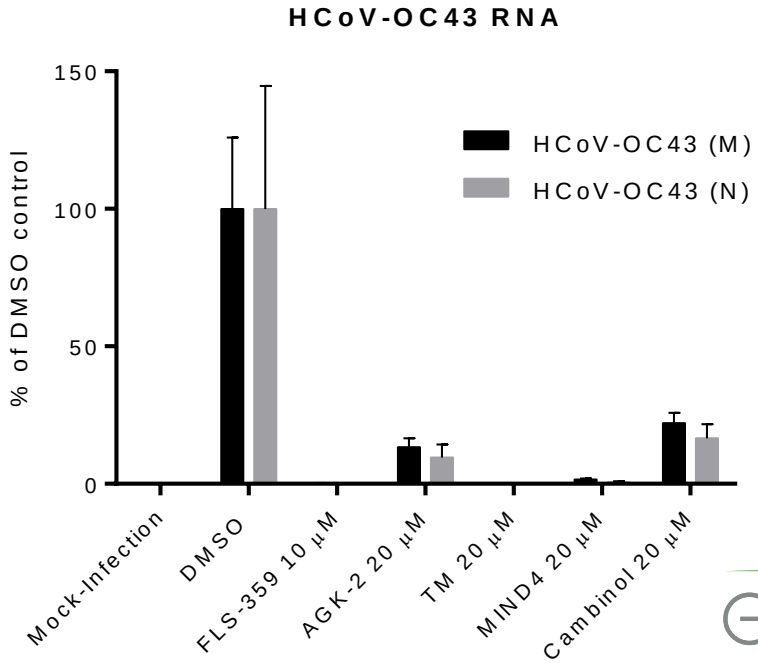






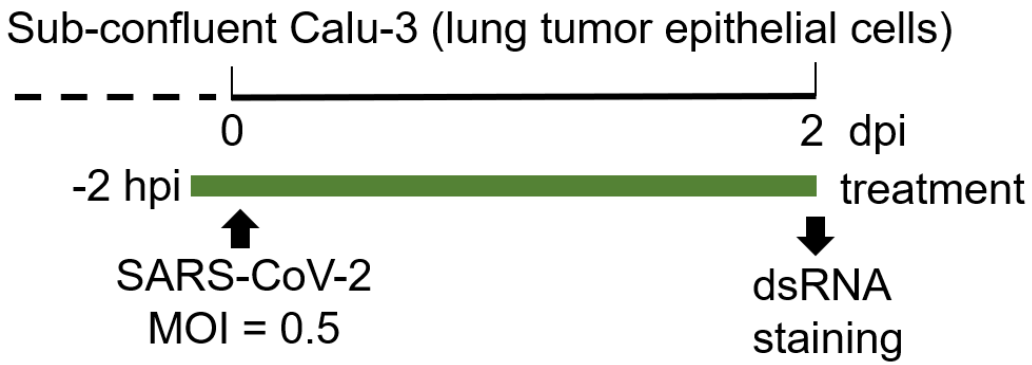


N=3
Mean ± SD

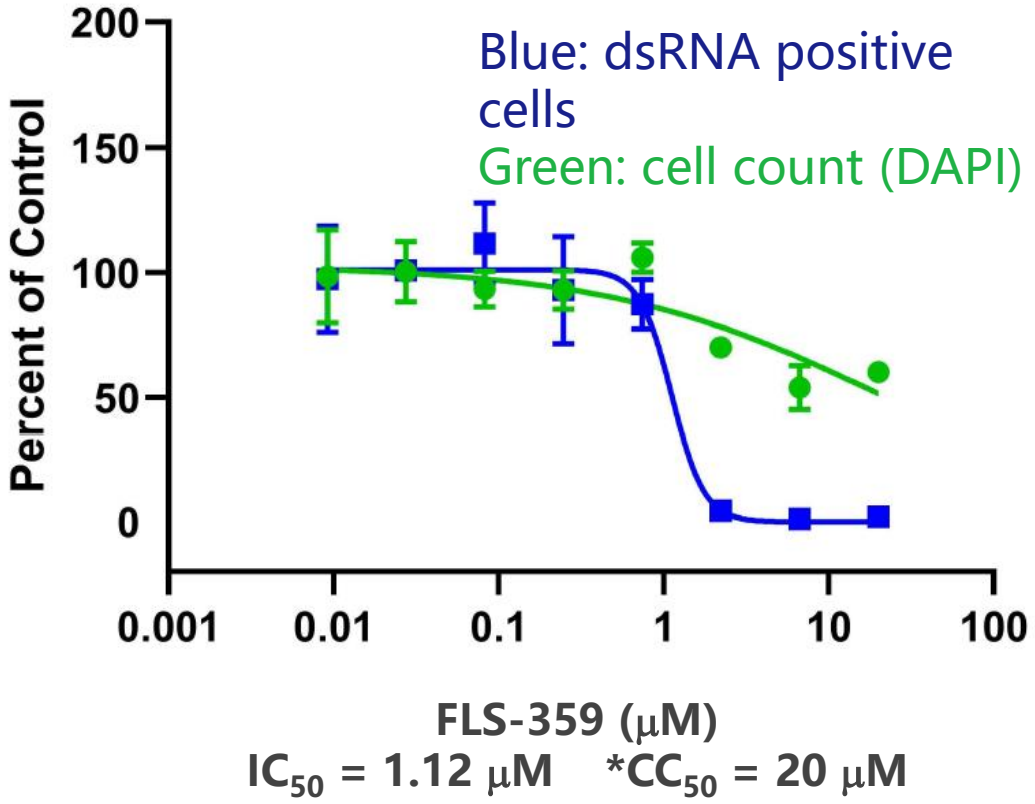


N=3
Mean ± SD

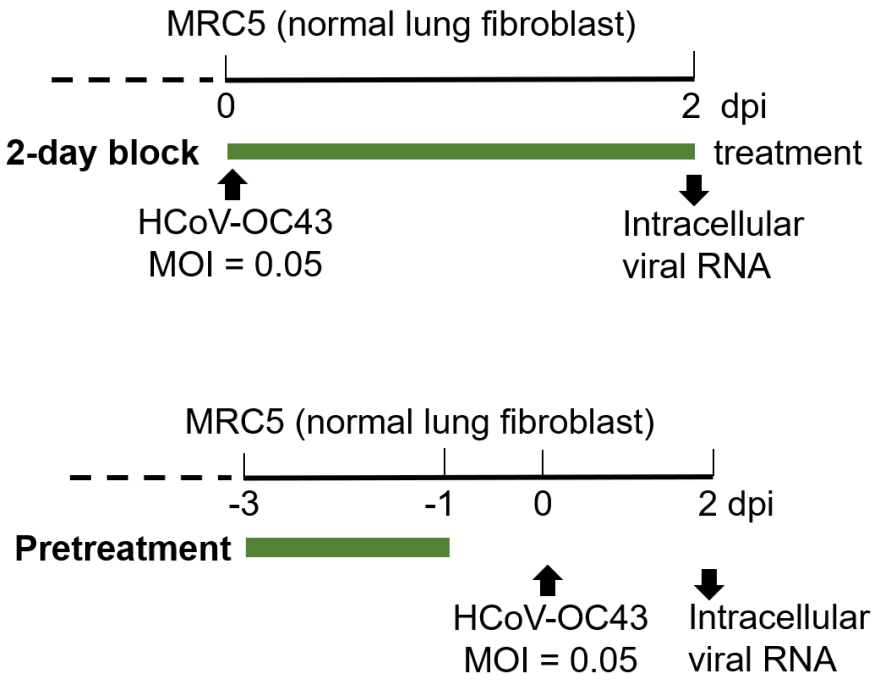
FLS-359 is active against the SARS-CoV-2

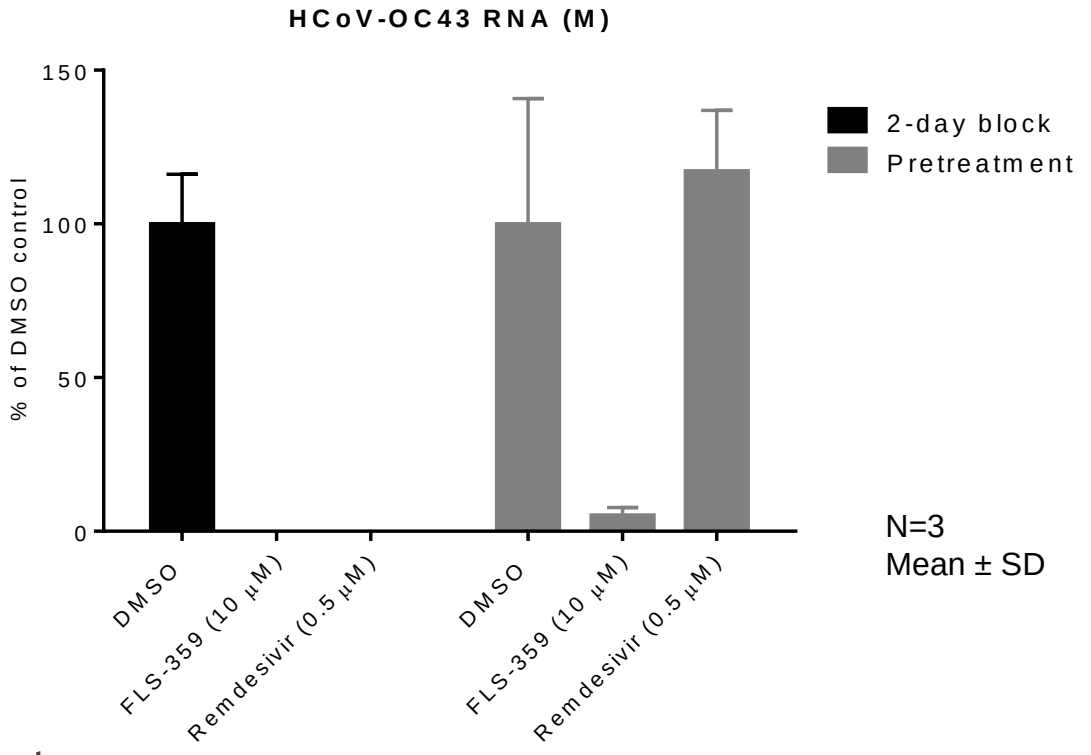


- Inhibition of SIRT2 has broad anti-cancer activity due to c-Myc degradation *(Jing, 2016, Cancer Cell)*
- *20 μM CC_{50} is due to reduced cell division of Calu-3, not cell killing
- FLS-359 anti-SARS-CoV-2 activity is currently being tested in iPSC induced alveolar epithelial type II cell model



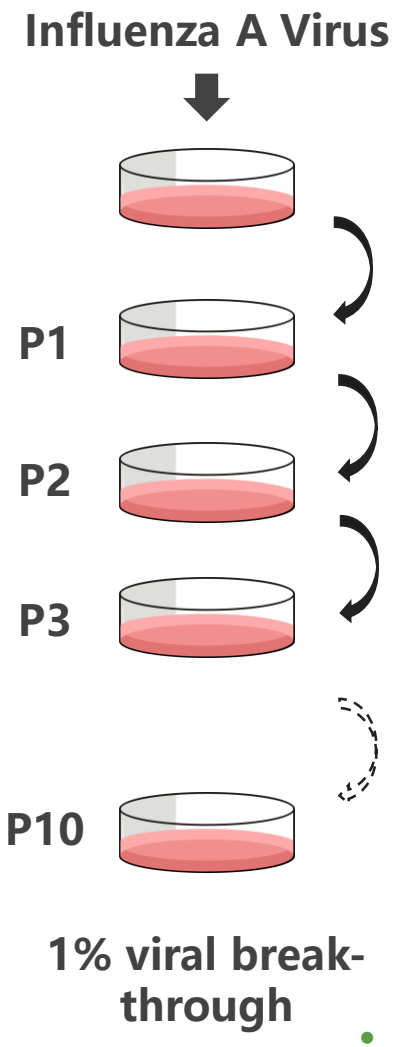
Pretreatment with FLS-359 is protective against HCoV-OC43

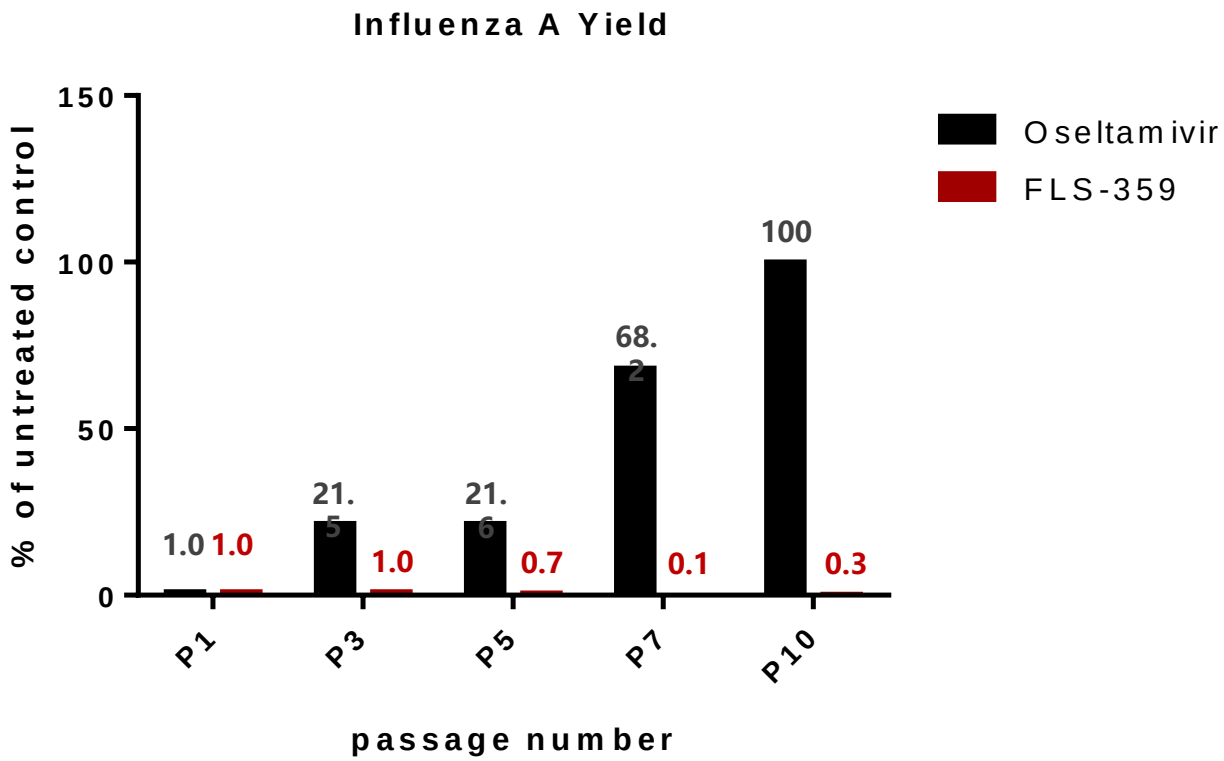




- 10 μM FLS-359 and 0.5 μM Remdesivir lead to equivalent fold of viral RNA reduction in the 2-day block assay

Host-targeting prevents drug resistance





- Viral passage experiment for HCoV-OC43 is ongoing

Host-targeting FLS-359 has broad spectrum antiviral activity against DNA and RNA viruses

Virus/Host Cell	Virus Family	[1]FLS-359 EC_{50} (μM)	[2]SOC EC_{50} (μM)	SOC/ Comparator (C)	Assay Performed by
JCV/Human HFF	Polyomavirus	[3]0.05	3.8	Ribavirin (C)	NIAID
Zika/Human HFF	Flavivirus	[4]0.39	3.9	Amodiaquine (C)	USAMRIID
HCoV-OC43/Human MRC5	β-coronavirus	[5]0.68	1.6	Hydroxychloroquine	Evrys Bio
HCMV/Human MRC5	β-herpesvirus	[5]0.58	1.4	Ganciclovir	Evrys Bio
BKV/Human HFF	Polyomavirus	[3]0.85	4.4	Ribavirin (C)	NIAID
SARS-CoV-2/Human Calu3	β-coronavirus	[3]1.1	0.07	Remdesivir	NIAID
Influenza A/Human HNBE	Orthomyxovirus	[3,8]1.2	0.03	Oseltamivir	NIAID
Influenza B/Canine MDCK	Orthomyxovirus	[5]1.2	>25	Oseltamivir (C)	Evrys Bio
Marburg/Human HFF	Filovirus	[4]1.5	2.4	USAMRIID (C)	USAMRIID
HCoV-229E/Human MRC5	α-coronavirus	[6]1.6	0.04	Remdesivir (C)	ImQuest
Ad5/Human MRC5	Adenovirus	[5]1.6	3.1	Cidofovir (C)	Evrys Bio
Junin/Human HFF	Arenavirus	[4]3.2	0.17	USAMRIID (C)	USAMRIID
Hepatitis B Virus/Human PHH	Hepadnavirus	[6]5.2	0.03	Tenofovir	ImQuest
RSV/Human MRC5	Orthopneumovirus	[7]6.7	16.1	Ribavirin	Retrovirox

[1]All viruses were tested against FLS-359, except JCV and BKV, which were tested against a closely related Evrys compound; [2]SOC = standard of care or comparator (C) compound; [3]assayed by DMID; [4]assayed by USAMRIID; [5]assayed by Evrys; [6]assayed by ImQuest; [7]assayed by Retrovirox; [8]IC_{90}.

Summary

- Inhibition of the fuel sensing protein SIRT2 hampers coronavirus infection
- SIRT2-targeting antiviral is broadly effective, predicting activity against newly emerging viruses
- Targeted one-virus-at-a-time approach is inadequate to tackle unknown viral outbreaks
- A safe pan-antiviral drug could be extremely beneficial to combat an early-stage viral pandemic

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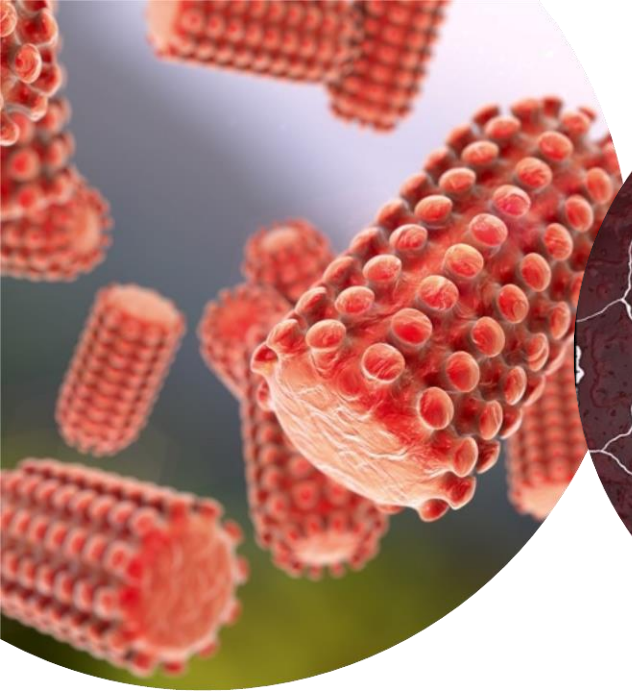
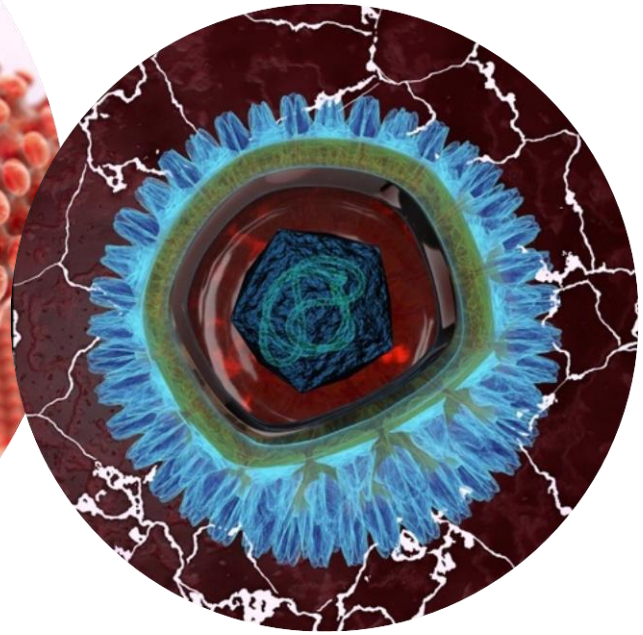
Acknowledgement

- Evrys Bio Coronavirus Team
  - Kate Sullivan
  - Chris Adam
  - Youngwok Kim
  - Matthew Todd
  - Stacy Remiszewski
  - Eain Murphy
  - Lillian Chiang
  - Thomas Shenk
- Collaborators
  - John Kulp (Conifer Point) – Molecular Docking
  - Ralph Baric (UNC) - HCoV-OC43 Strain
  - Dave Schultz and Sara Cherry (U Penn) - NIAID SARS-CoV-2 In Vitro Antiviral Screening
- Funding sources
  - National Institute of Health grants R43AI110048,R43AI114079, R44AI114079, R44AI122488
  - Department of Defense STTR Contract W911QY18P0300





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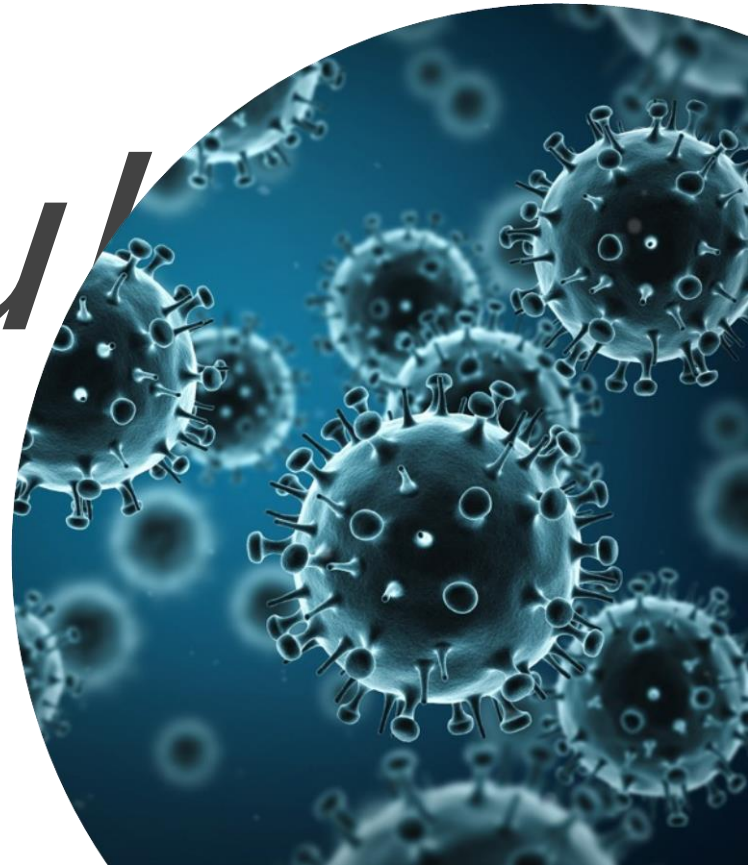

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