



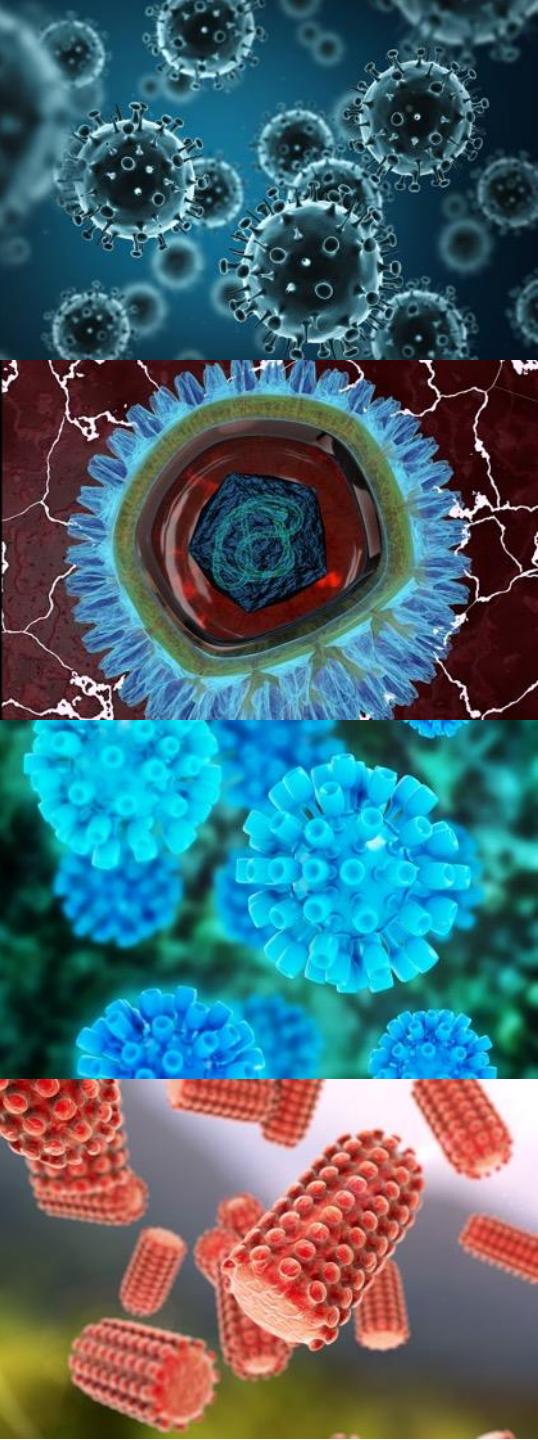
Inhibition of Sirtuins Is Broadly Effective Against Coronaviruses

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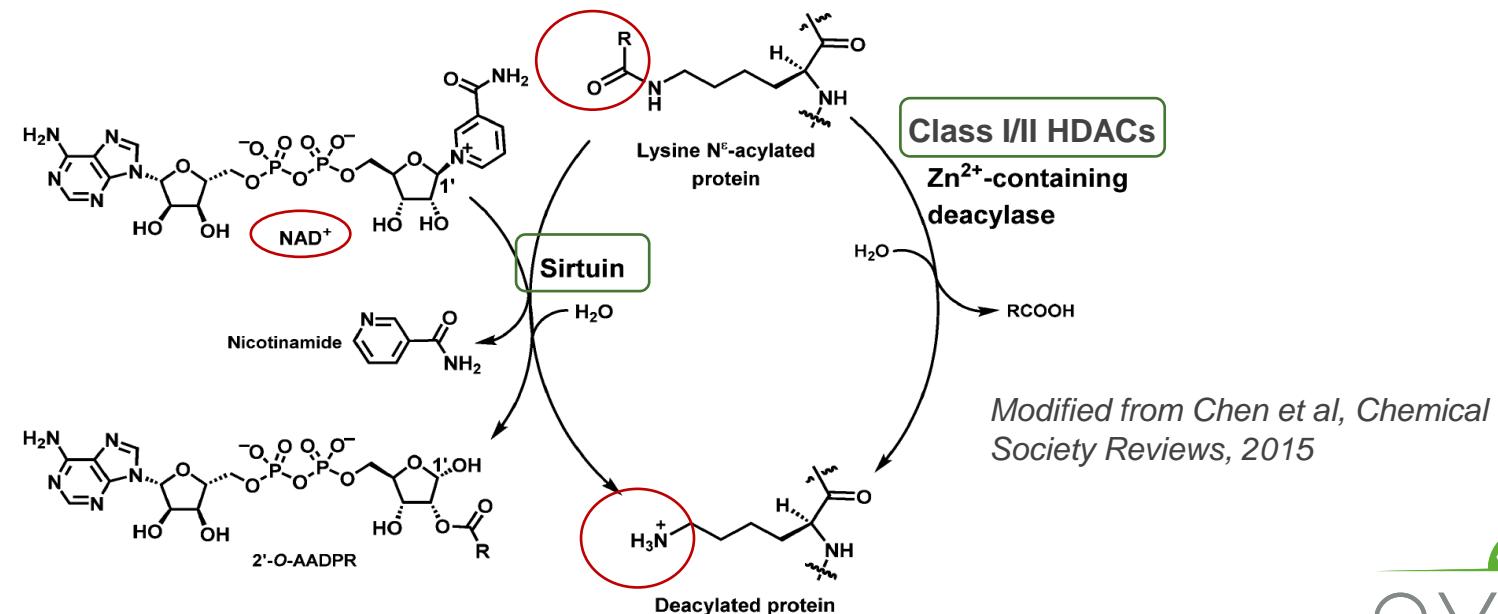
Isirv-AVG Virtual Conference on 'Therapeutics for COVID-19'

10/06/2020

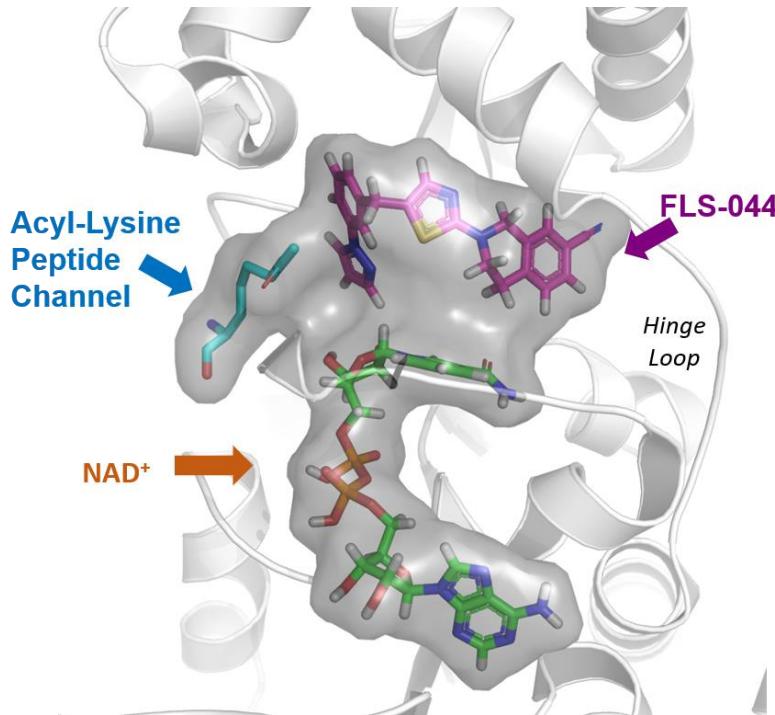


Sirtuins: a Target for restoring host cell metabolism

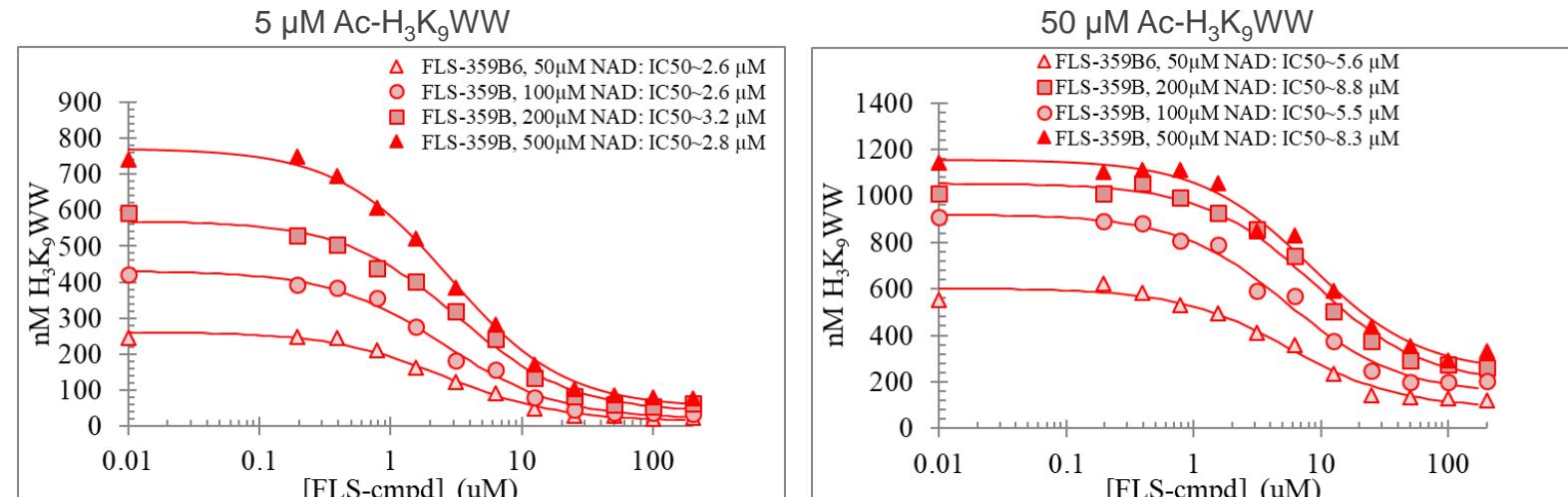
- Sirtuins (SIRT1-7) are a family of NAD⁺ dependent deacetylases
- SIRT2 expression and function is induced during low-energy status (Gomes, 2015, *Trends Pharmacol Sci*)
- Viral infection often ramps-up host cell metabolism to provide viral building blocks
- SIRT2 inhibitors have been shown to inhibit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



FLS-359: an early lead SIRT2 inhibitor



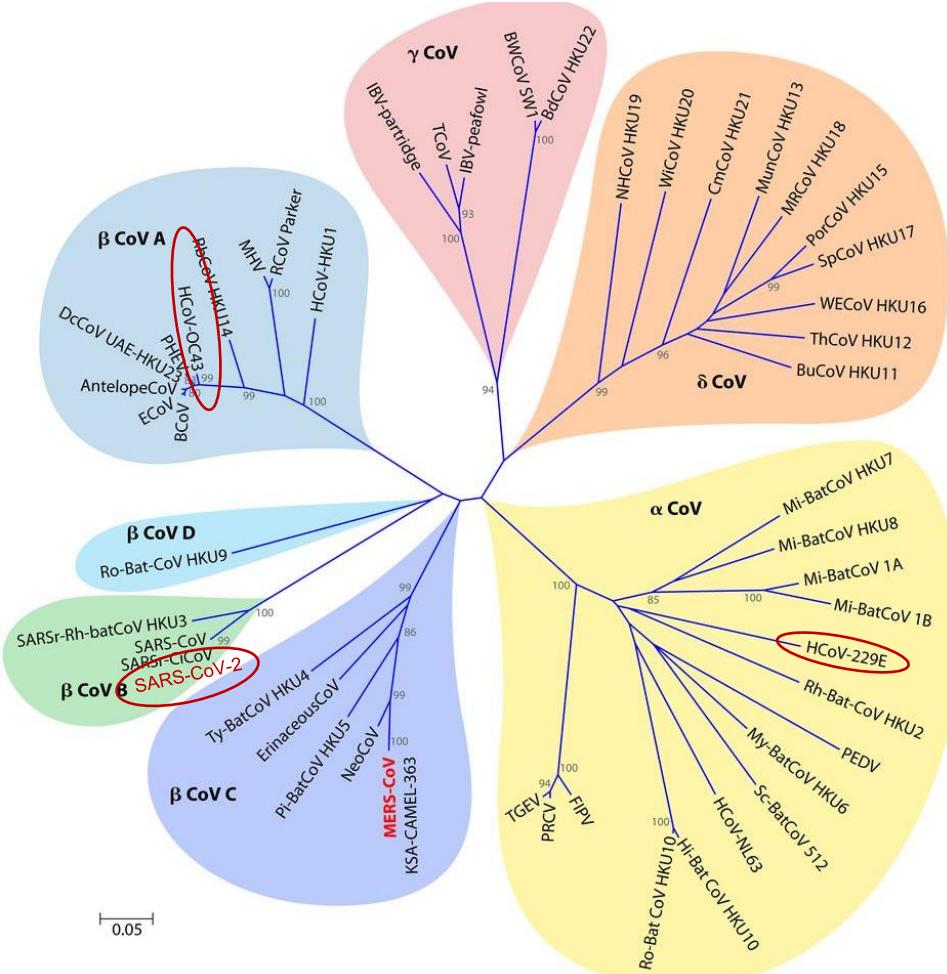
SIRT2 deacetylation activity with FLS-359



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

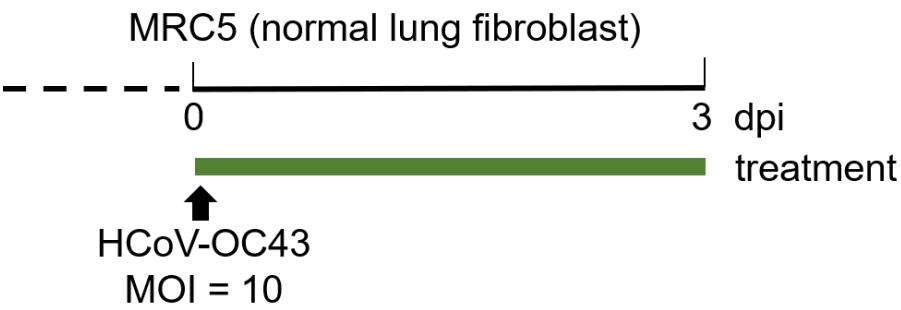
- FLS-044 is docked into the SIRT2 peptide binding channel, separating the acyl-lysine substrate away from NAD⁺
- *In vitro* deacetylation assay demonstrates a partially non-competitive manner
- FLS-359 shows moderate SIRT2 and weak SIRT1, SIRT3 inhibition

FLS-359 inhibits multiple coronaviruses

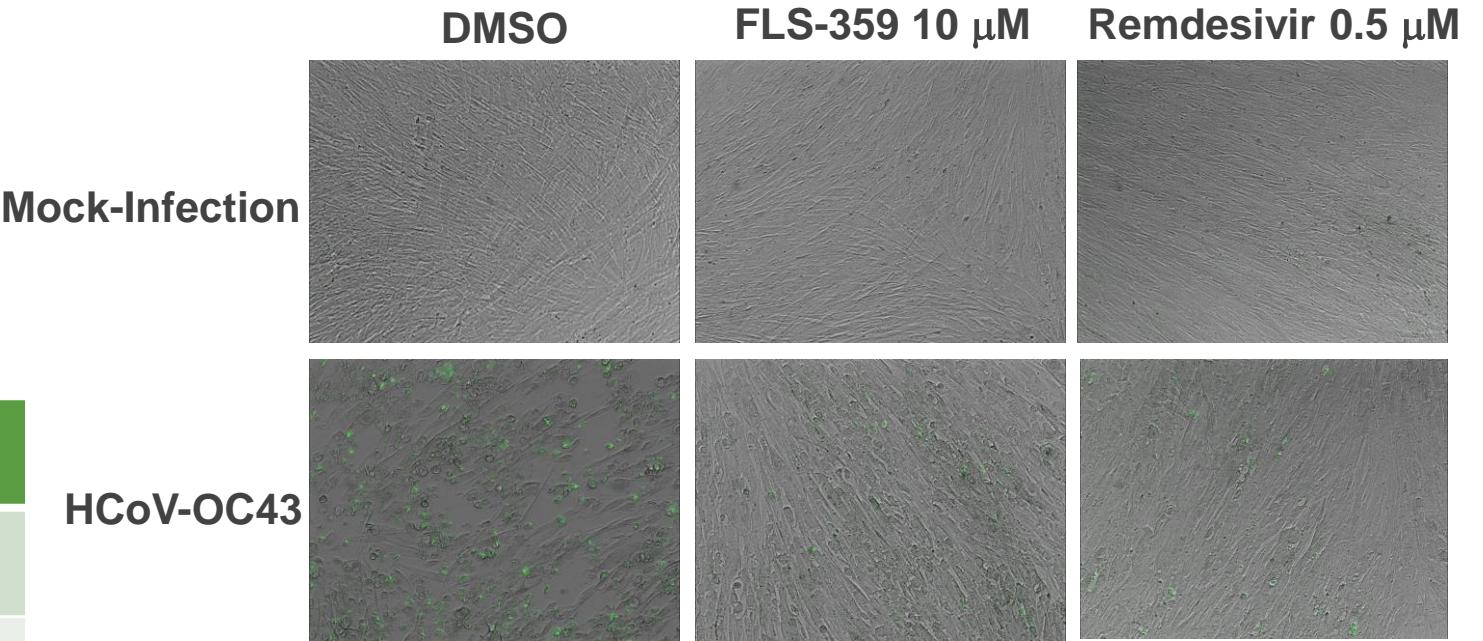


Coronaviruses	FLS-359 IC ₅₀ (μM)
SARS-CoV-2	1.12
HCoV-OC43	1.51
HCoV-229E	1.66

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-Coronavirus	1.51	0.10
HCoV-229E	Alpha-Coronavirus	1.66	0.04



*Green cells indicate the ones with activated Caspase-3/7

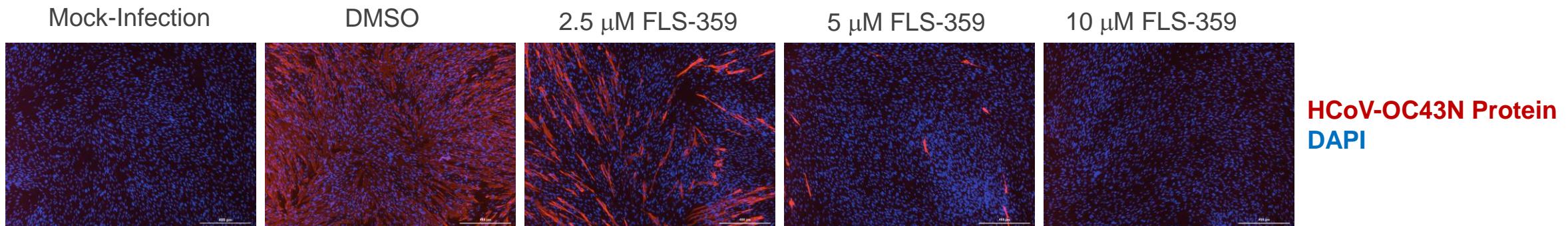
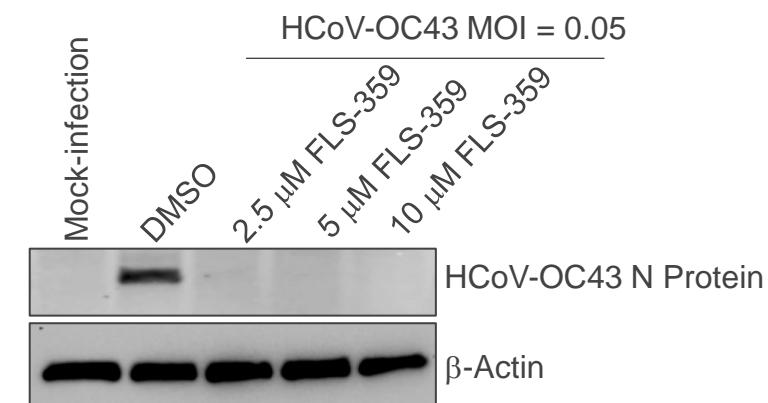
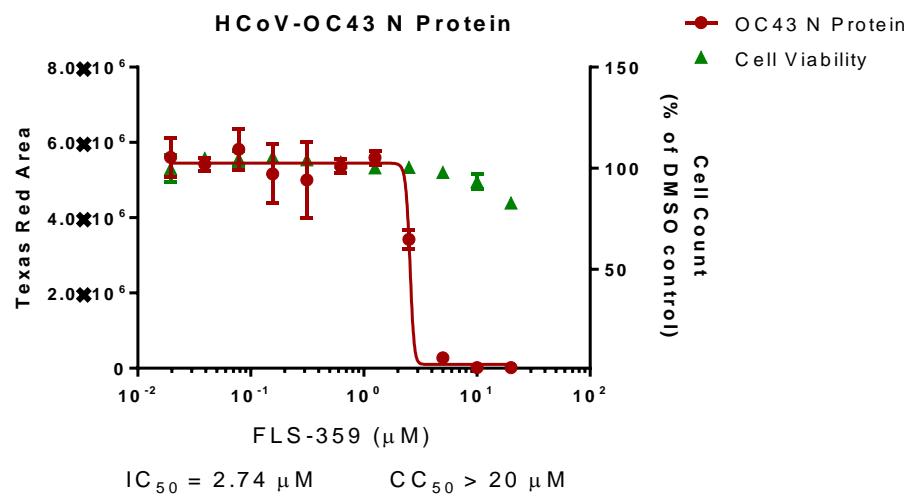
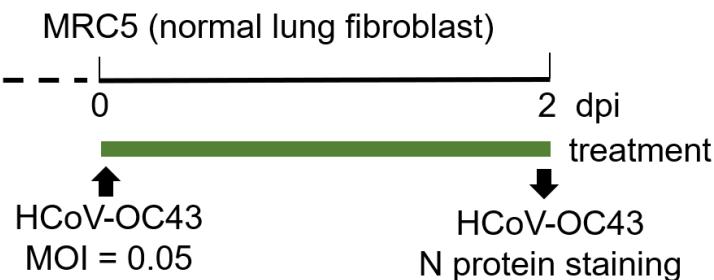
Anti-HCoV-OC43 compound screening

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

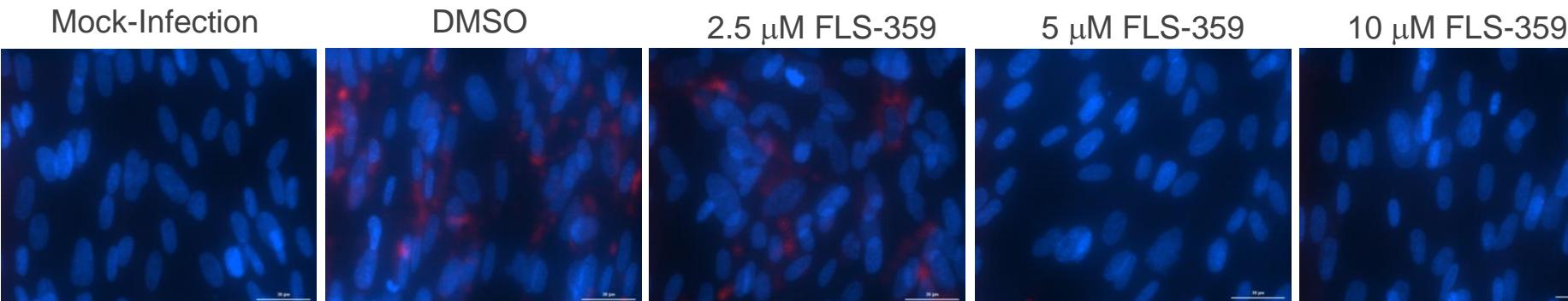
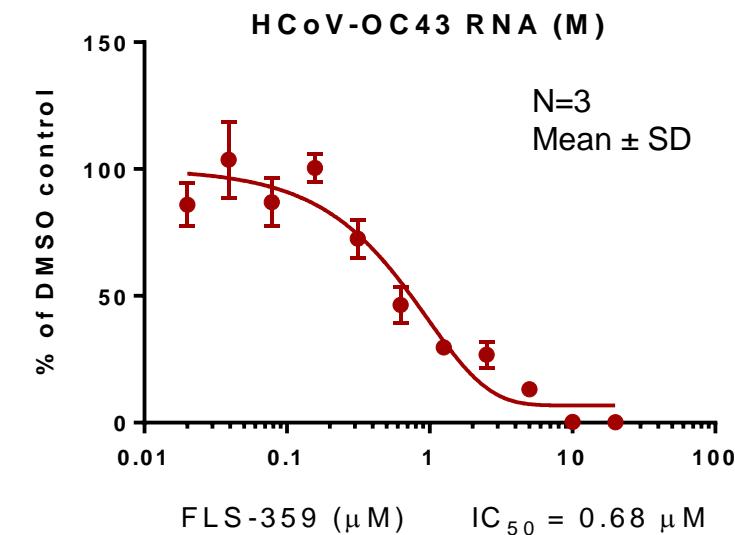
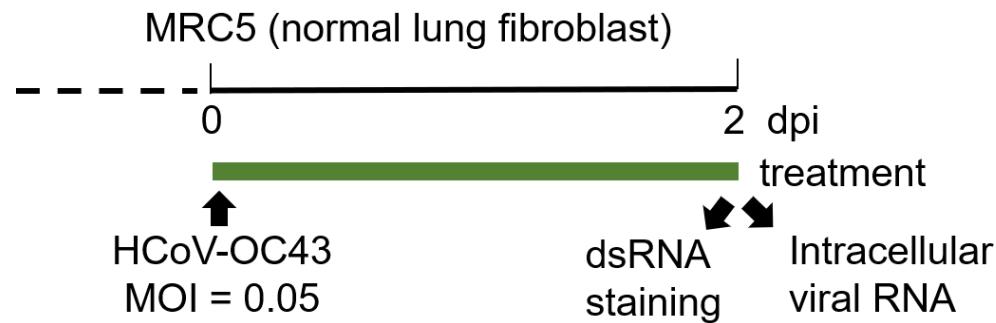
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* Compound precipitated at higher concentrations, limiting the estimate of SI

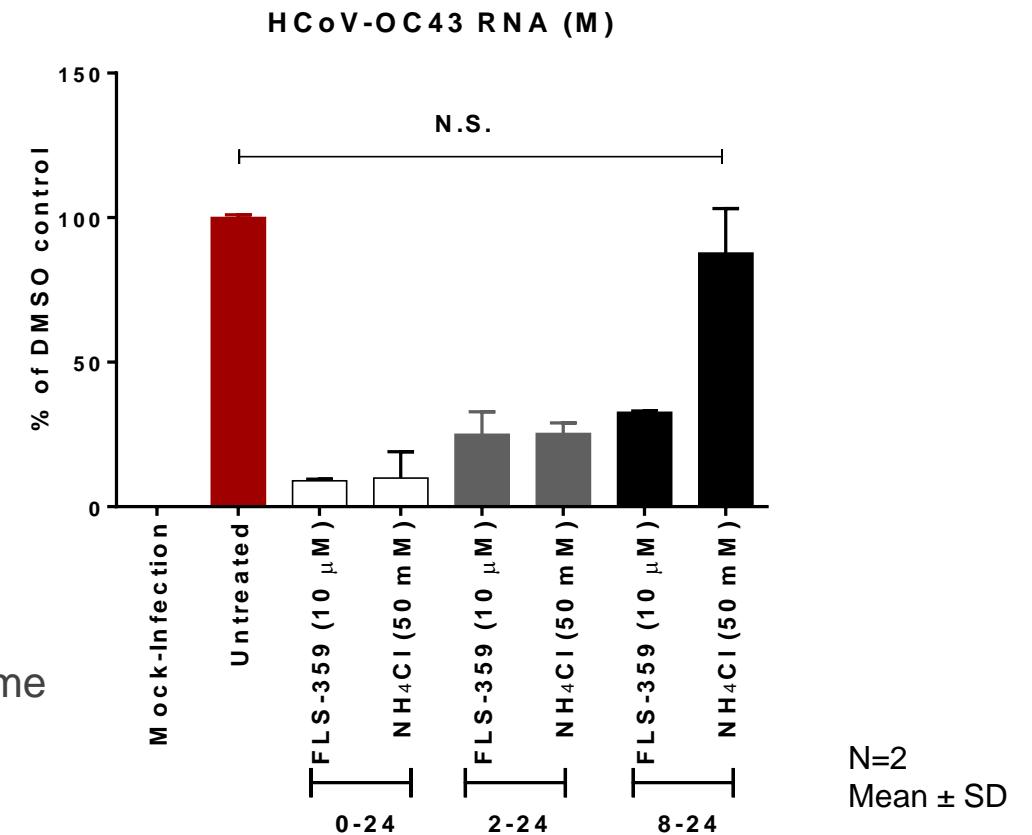
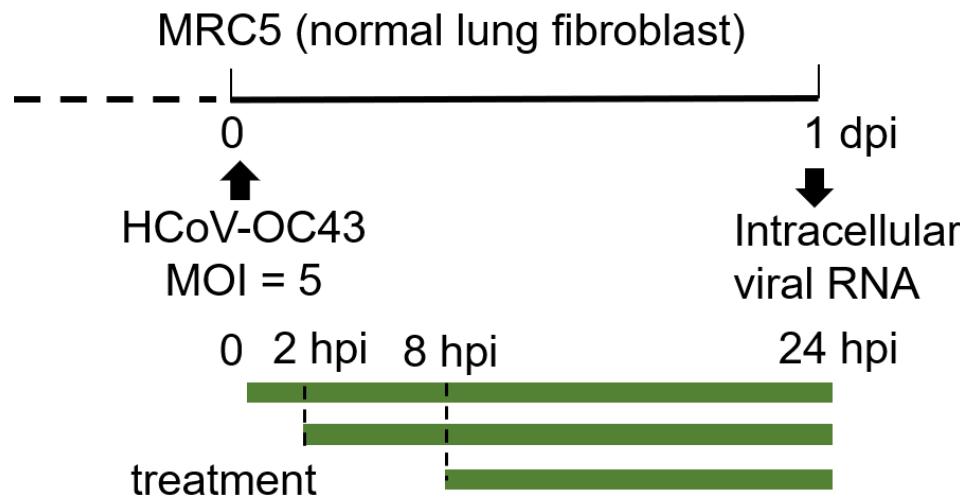
FLS-359 reduces HCoV-OC43 cell-to-cell spread



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

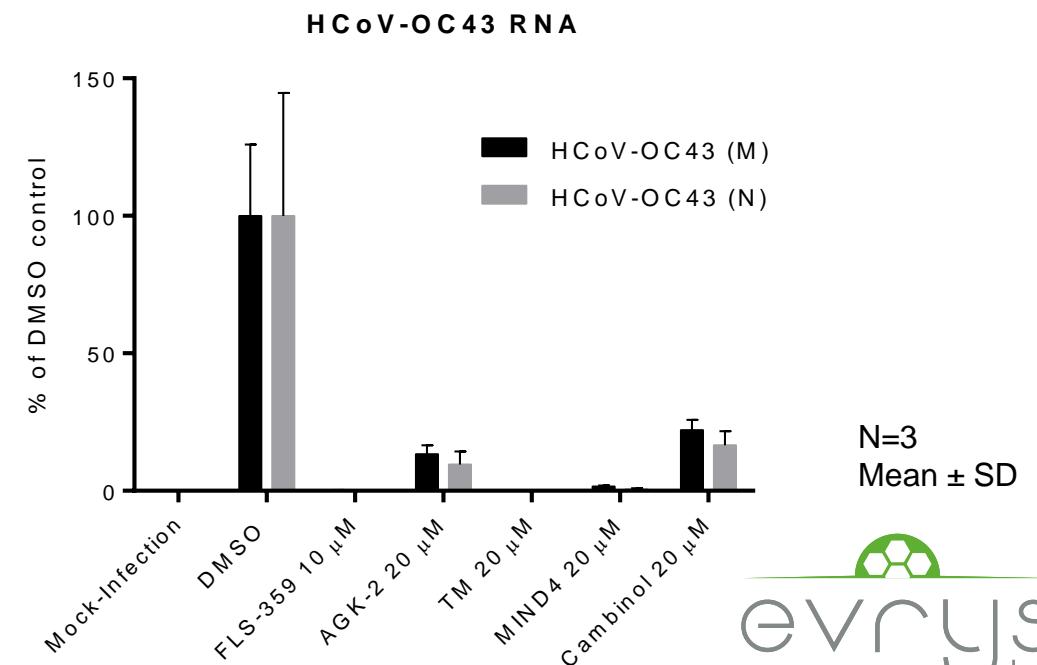
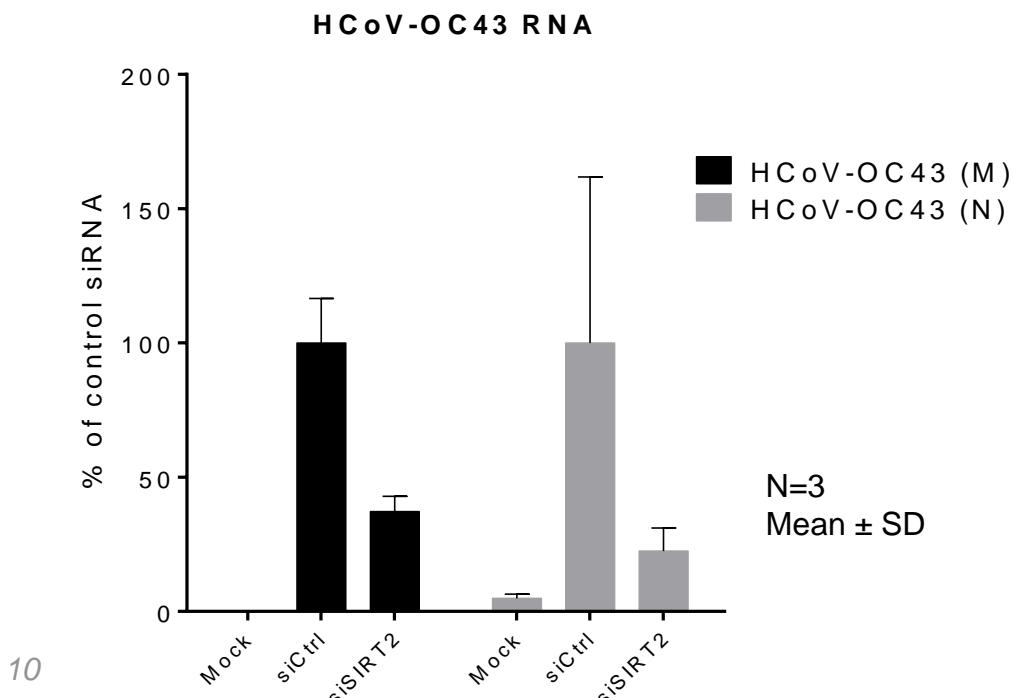
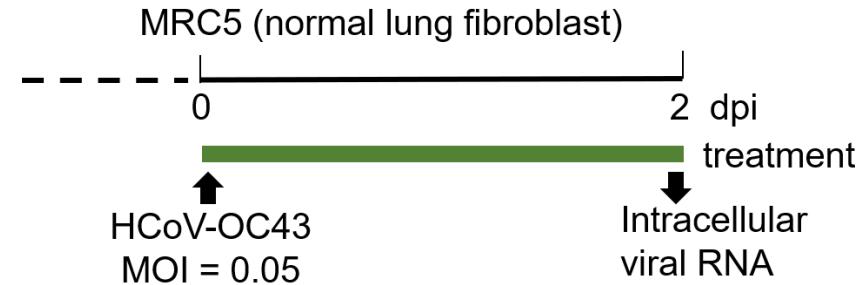
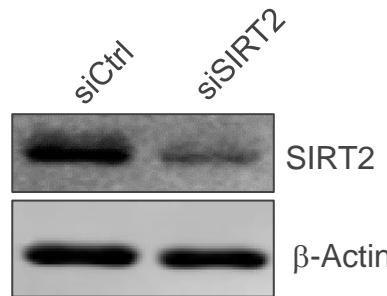
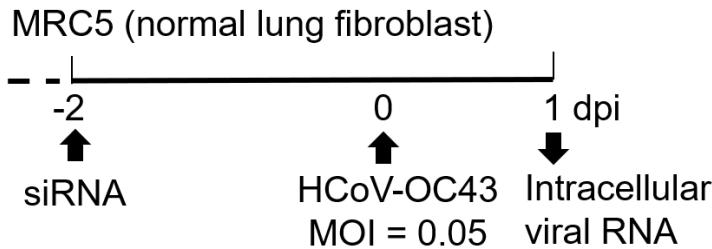


FLS-359 inhibits a post-entry step of HCoV-OC43

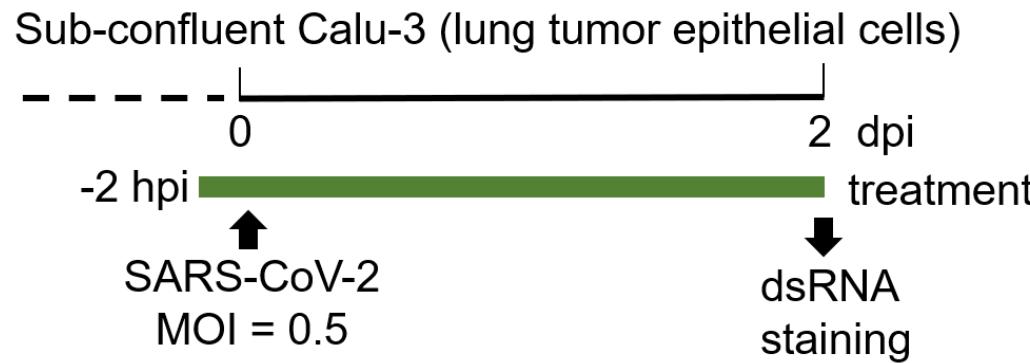


- NH₄Cl inhibits HCoV-OC43 entry through disrupting the endosome pH. (Owczarek, 2018, Sci Rep)

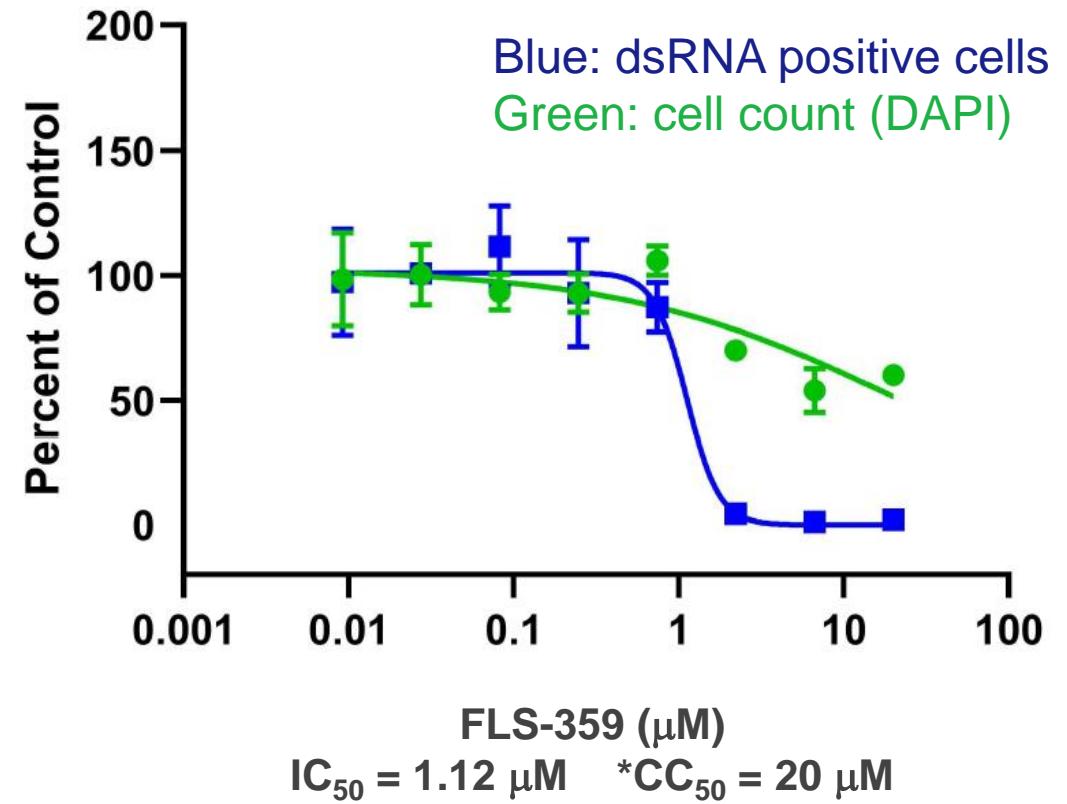
Targeting SIRT2 inhibits HCoV-OC43



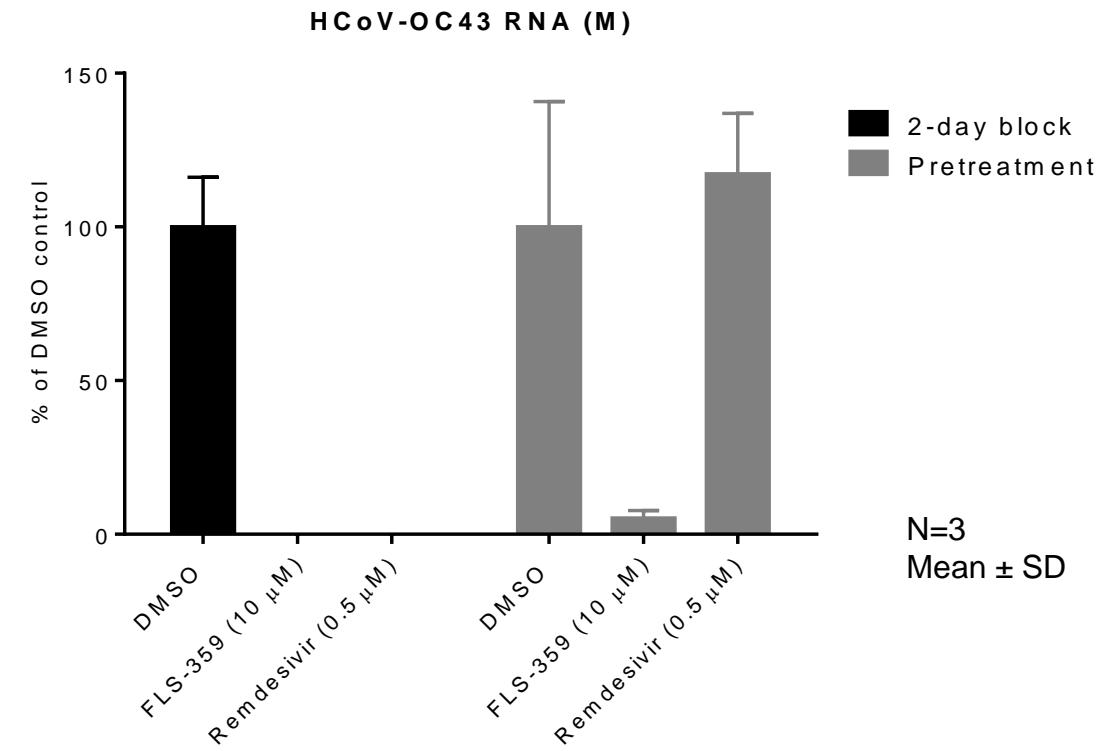
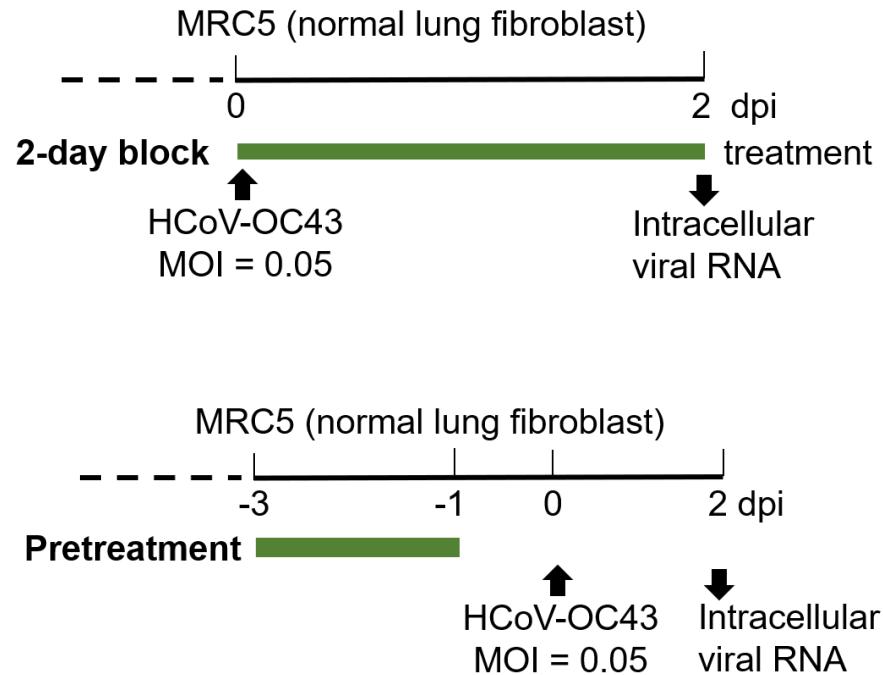
FLS-359 is active against the SARS-CoV-2



- Inhibition of SIRT2 has broad anti-cancer activity due to c-Myc degradation (*Jing, 2016, Cancer Cell*)
- *20 μM CC₅₀ 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

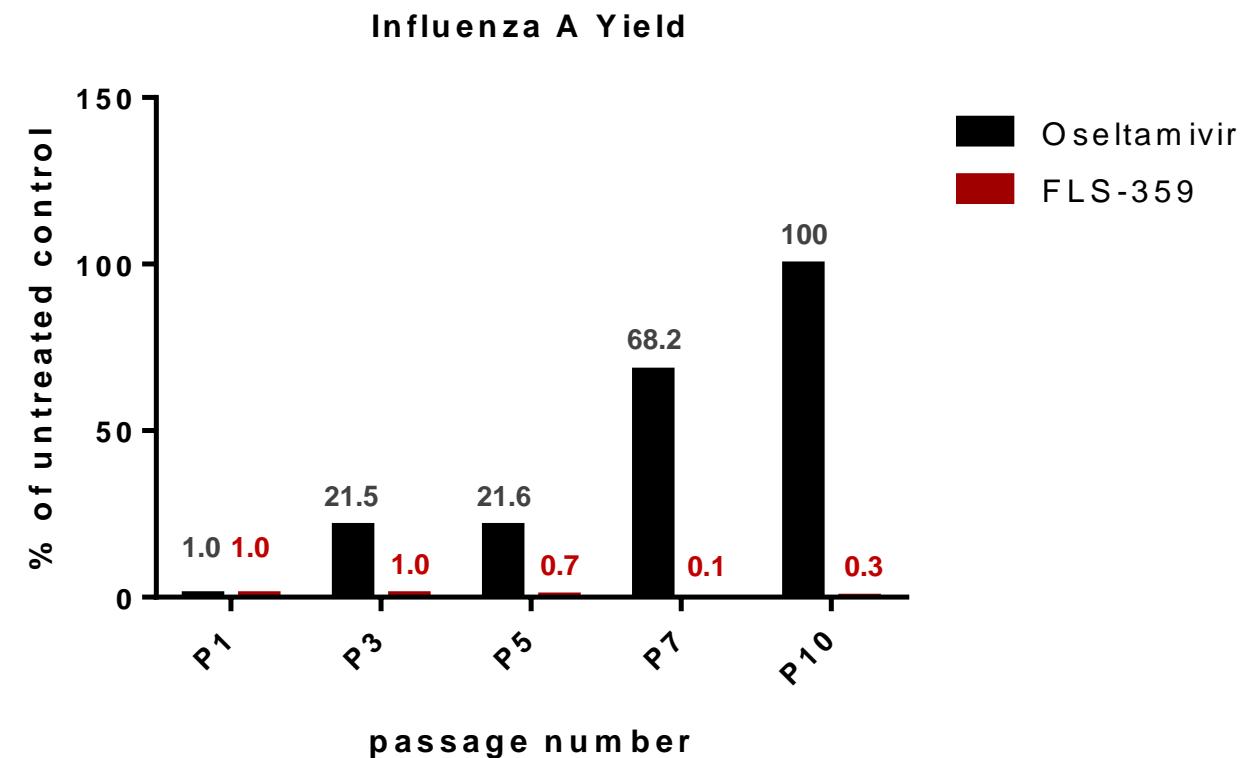
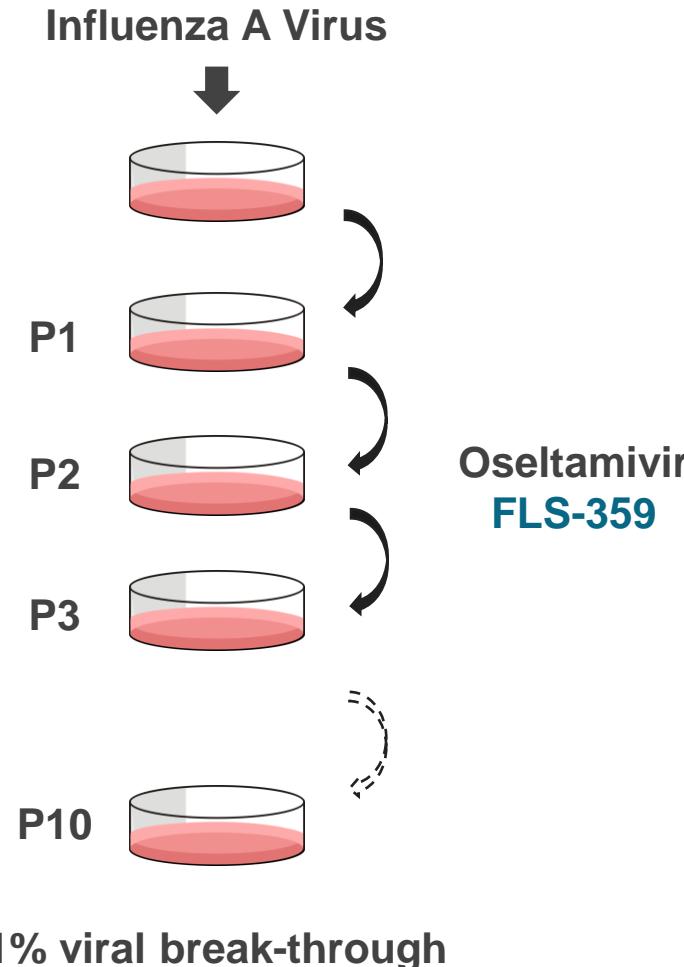


Pretreatment with FLS-359 is protective against HCoV-OC43



- 10 μ M FLS-359 and 0.5 μ M 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

¹All viruses were tested against FLS-359, except JCV and BKV, which were tested against a closely related Evrys compound; ²SOC = standard of care or comparator (C) compound; ³assayed by DMID; ⁴assayed by USAMRIID; ⁵assayed by Evrys; ⁶assayed by ImQuest; ⁷assayed by Retrovirox; ⁸IC₉₀.

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

Acknowledgement

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Thank you!



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