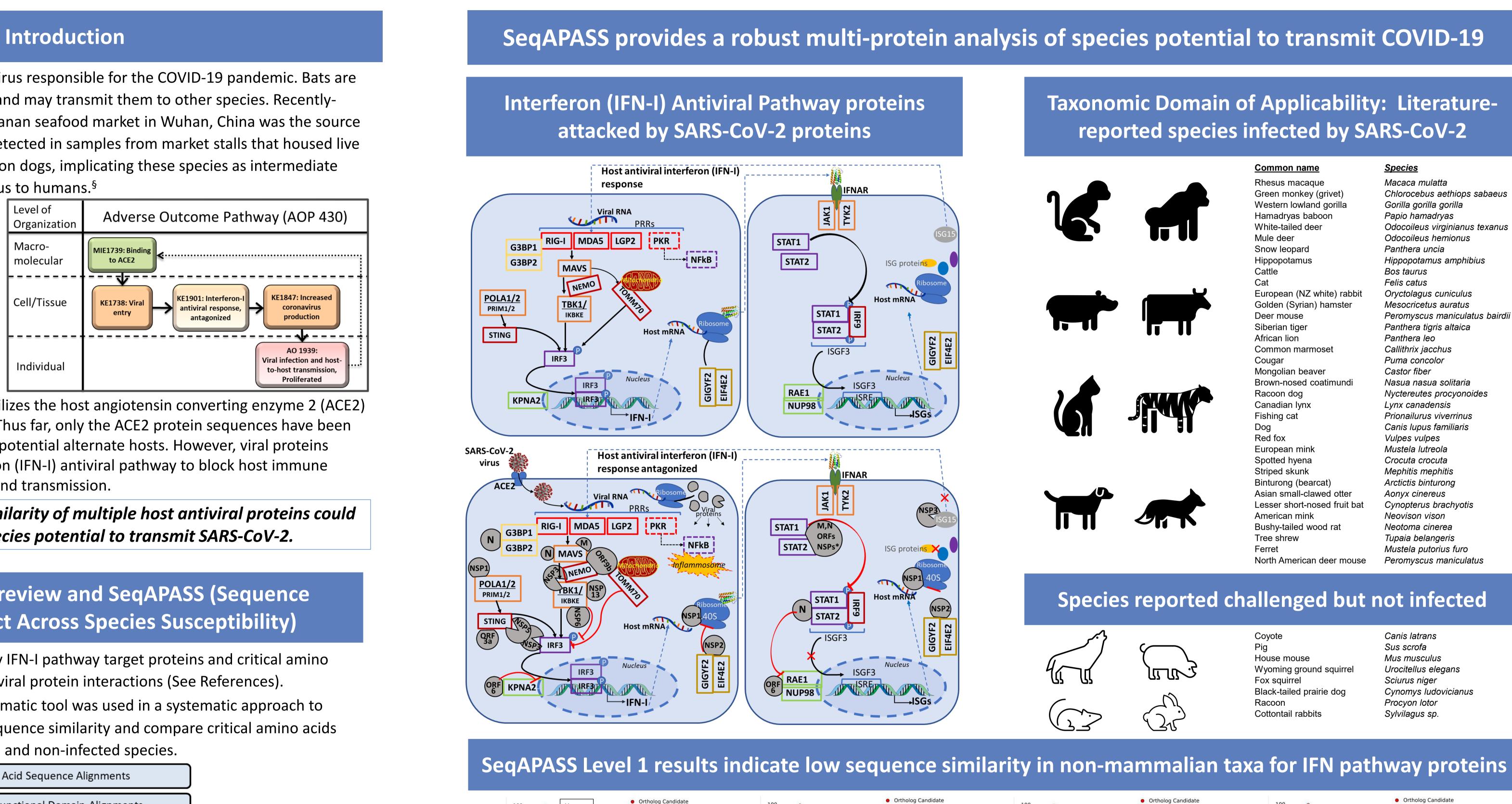


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SARS-CoV-2 is a zoonotic coronavirus responsible for the COVID-19 pandemic. Bats are known reservoirs for coronaviruses and may transmit them to other species. Recentlypublished evidence indicates the Huanan seafood market in Wuhan, China was the source of the pandemic. SARS-CoV-2 was detected in samples from market stalls that housed live animals including red foxes and racoon dogs, implicating these species as intermediate hosts that potentially carried the virus to humans.§

The virus has been proven to be transmitted from humans to other mammals and back to humans, which could result in mutations that increase virulence or transmissibility.



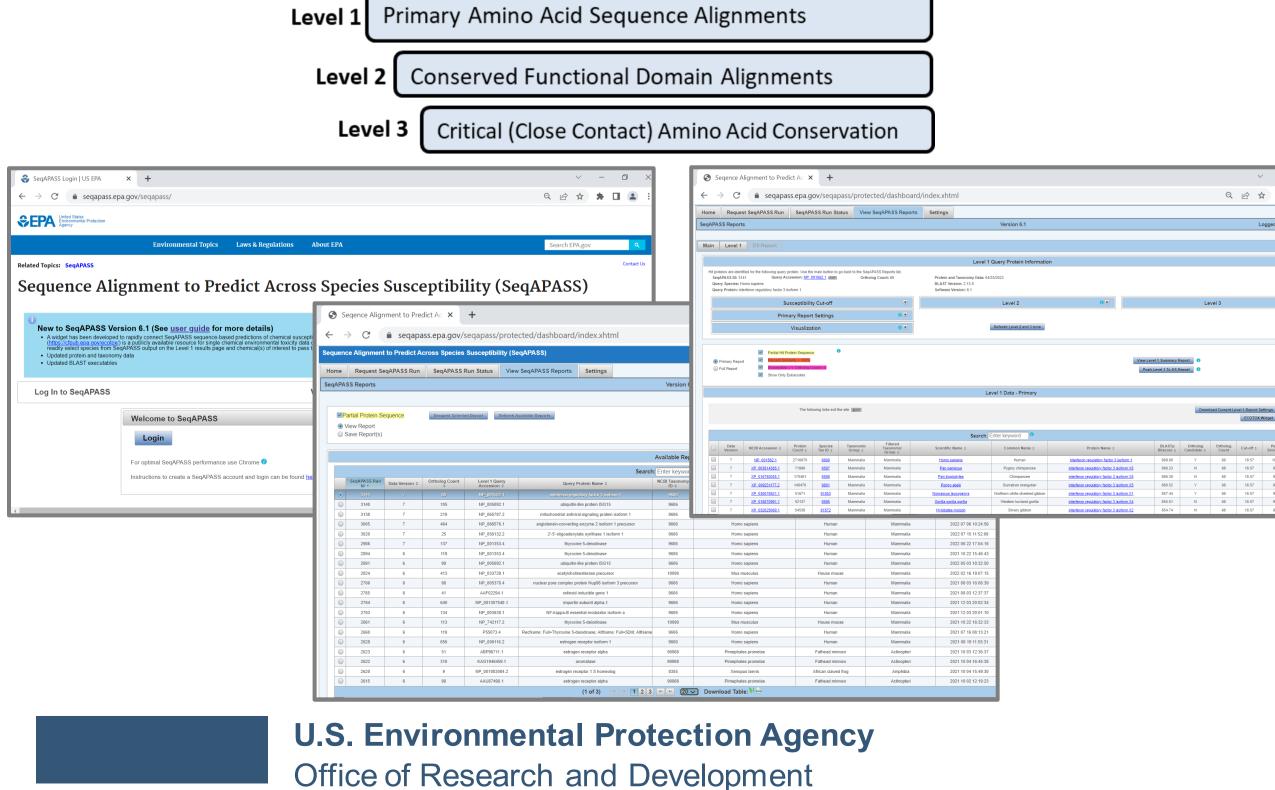
The SARS-CoV-2 spike protein utilizes the host angiotensin converting enzyme 2 (ACE2) as a receptor to enter the host cell. Thus far, only the ACE2 protein sequences have been compared across species to identify potential alternate hosts. However, viral proteins attack many proteins in the Interferon (IFN-I) antiviral pathway to block host immune response, allowing viral replication and transmission.

Determining cross-species similarity of multiple host antiviral proteins could provide stronger evidence of species potential to transmit SARS-CoV-2.

§ Worobey et al., Science 10.1126/science.abp8715 (2022).

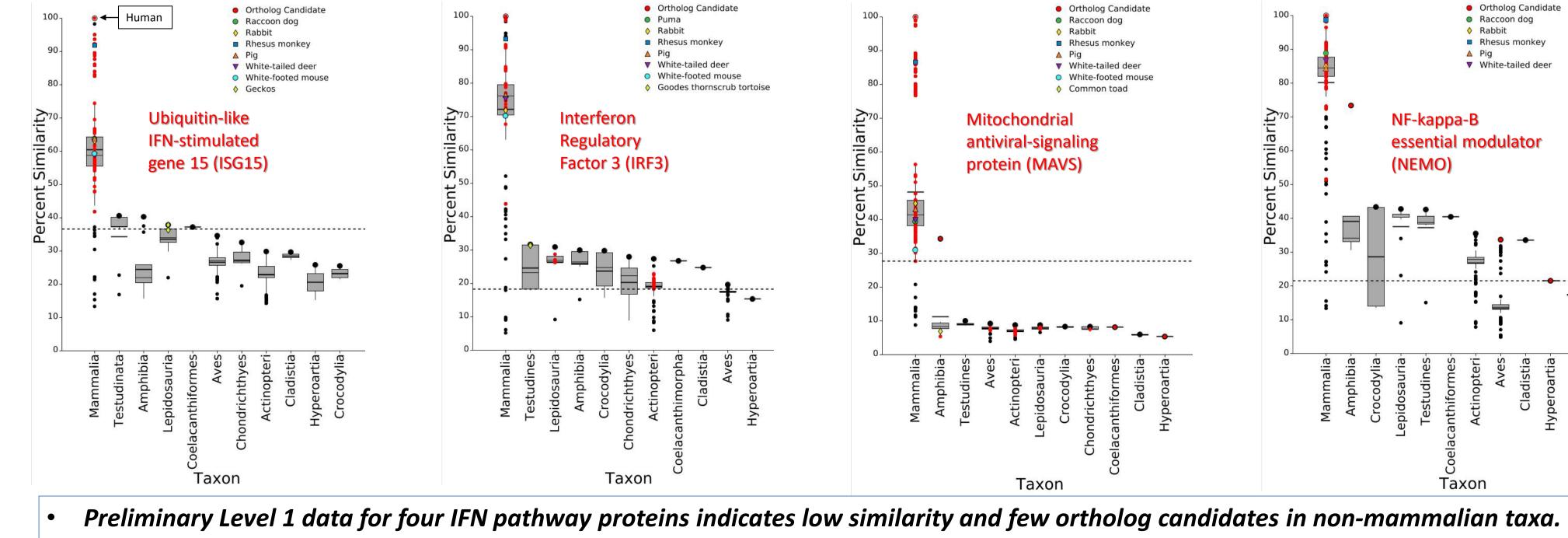
Methods: Literature review and SeqAPASS (Sequence Alignment to Predict Across Species Susceptibility)

- Literature was searched to identify IFN-I pathway target proteins and critical amino acids involved in the host protein-viral protein interactions (See References).
- The U.S. EPA's **SeqAPASS** bioinformatic tool was used in a systematic approach to evaluate IFN-I pathway protein sequence similarity and compare critical amino acids across literature-reported infected and non-infected species.



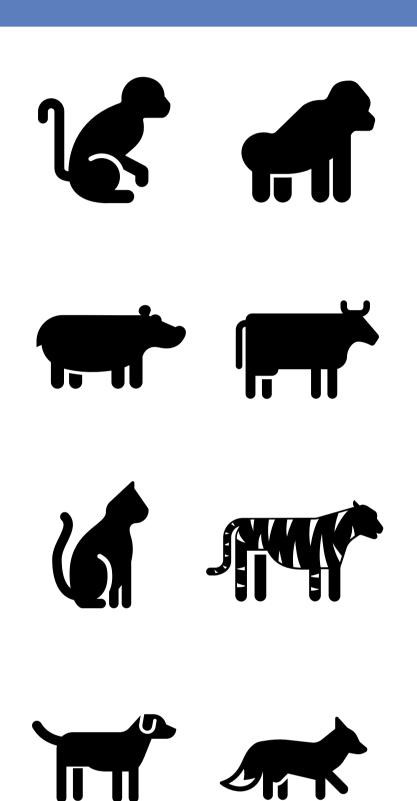
Identification of potential SARS-CoV-2 intermediate host species using SeqAPASS

Sally A. Mayasich^{1,2} and Carlie A. LaLone² ¹University of Wisconsin-Madison Aquatic Sciences Center, ²U.S. EPA, Office of Research and Development, Center for Computational Toxicology and Exposure, Great Lakes Toxicology & Ecology Division (GLTED), Duluth, MN



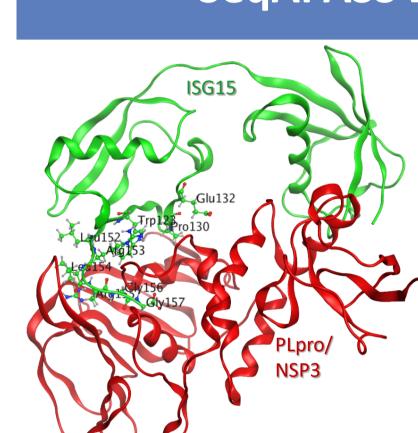
The views expressed in this poster are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.

Taxonomic Domain of Applicability: Literaturereported species infected by SARS-CoV-2

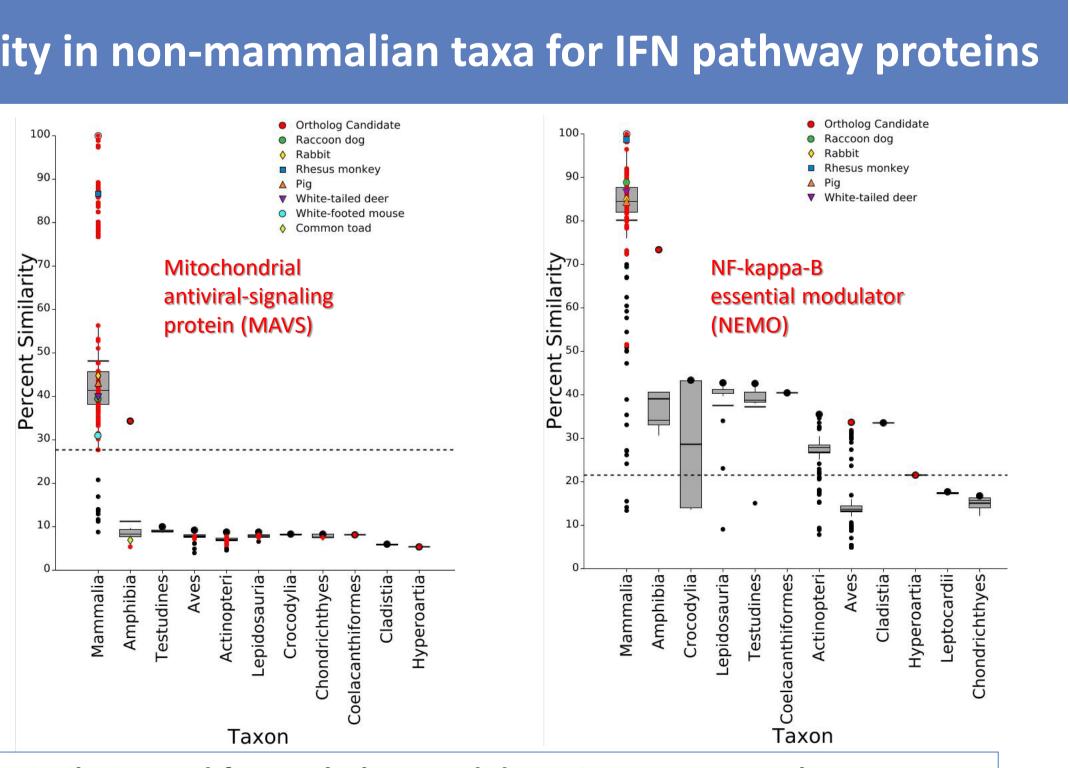


reen monkev (ari Golden (Svrian) hamst Deer mouse Siberian tiger African lion Common marmose Mongolian beaver Brown-nosed coatim Racoon dog Canadian lynx Fishing cat Doa Red fox European min Spotted hven Asian small-clawed c Lesser short-nosed fruit b American mink Bushy-tailed wood rat Tree shrew North American deer mouse

Macaca mulatta Chlorocebus aethiops sabaeu Gorilla gorilla gorilla Papio hamadrvas ocoileus virginianus texanı docoileus hemioni Panthera uncia Felis catus Oryctolagus cuniculus Mesocricetus auratus Peromyscus maniculatus bairdii Panthera tigris altaica Panthera leo Callithrix jacchus Puma concolor Castor fiber Vasua nasua solitaria lvctereutes procvonoio Lynx canadensis Prionailurus viverrinu Canis lupus familiaris ulpes vulpes Mustela lutreola Crocuta crocuta Mephitis mephitis Arctictis binturong Aonyx cinereus Cynopterus brachyot Veovison vison Veotoma cinerea upaia belangeris Mustela putorius fure



Reported Rhesus White-ta Cattle Cat Deer m Racoon Dog Red fox America Ferret



Species reported challenged but not infected

House mouse

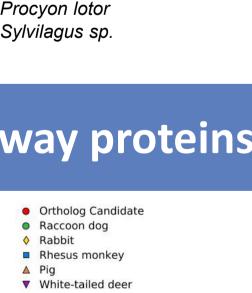
Fox squirrel

Cottontail rabbits

Wyoming ground squirre!

Black-tailed prairie dog

Level 2 results for conserved domains mirrored Level 1 results for these proteins (data not shown).



Canis latrans Sus scrofa

Mus musculus

Sciurus niger

Urocitellus elegans

Cynomys Iudovicianus



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SeqAPASS Level 3 critical amino acid evaluation

critical amino acids for protein-prote interaction with viral papain-like protease non-structural protein 3 (PLpro/NSP3 Molecular Operating Environment (MOE) software (CCG Aontreal, QC, Canada) model based on crystal structure

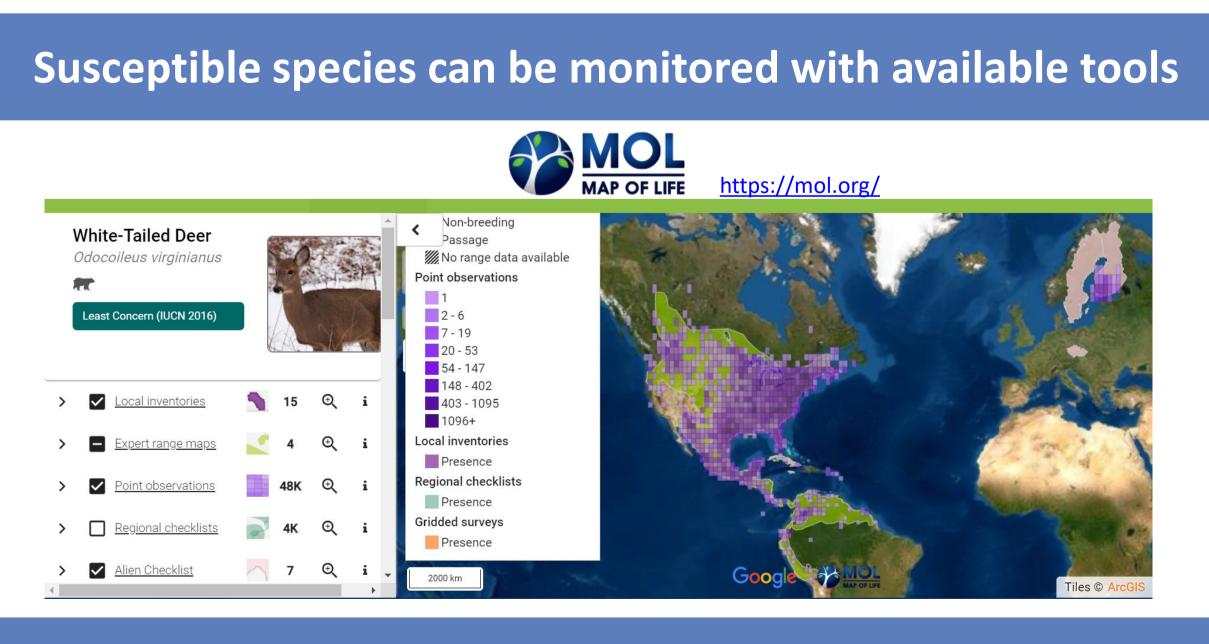
Total Match Partial Match Susceptible Yes Not a Match	SeqAPASS Level 3 heat map for IFN-stimulate gene 15 (ISG15) critical amino acids.									
Common Name	Similar Susceptibility	Amino Acid 1	Amino Acid 2	Amino Acid 3	Amino Acid 4	Amino Acid 5	Amino Acid 6	Amino Acid 7	Amino Acid 8	Amino Acid 9
Human	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
Olive baboon	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
Rhesus monkey	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
Green monkey	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
White-tufted-ear marmoset	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
Northern tree shrew	Y	123W	130D	132E	152L	153R	154L	155R	156G	157G
Tiger	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
Puma	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
Lion	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
Spotted hyena	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
Domestic cat	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
American mink	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
Fishing cat	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
Canada lynx	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
Raccoon dog	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
Pig	Y	122W	129P	131E	151L	152R	153L	154R	155G	156G
Malayan pangolin	Y	123W	1308	132E	152L	153R	154L	155R	156G	157G
Domestic ferret	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
Dog	Y	129W	136P	138E	158L	159R	160L	161R	162G	163G
Chinese pangolin	Y	141W	1485	150E	170L	171R	172L	173R	174G	175G
Red fox	Y	123W	130P	132E	152L	153R	154L	155R	156G	157G
House mouse	Y	121W	128P	130E	150L	151R	152L	153R	154G	155G
White-footed mouse	N	122W	129R	131E	151L	152H	153L	154R	155G	156G
Prairie deer mouse	N	122W	129R	131E	151L	152H	153L	154R	155G	156G
White-tailed deer	Y	125W	132P	134D	154L	155R	156L	157R	158G	159G
Cattle	Y	120W	127P	129D	149L	150R	151L	152R	153G	154G

		ACE2 Predicted		Transmit	SeqAPASS Results				
on name	Species	susceptibility to Spike protein ¹	Symptoms	infectious virus	ISG15 Susceptible	IRF3 Susceptible	MAVS ² Susceptible	NEMO Susceptible	
d infected									
s macaque	Macaca mulatta	Very High	Yes	Yes	\checkmark	\checkmark	\checkmark	\checkmark	
tailed deer	Odocoileus virginianus	High	Yes	Yes	\checkmark	\checkmark	X	\checkmark	
	Bos taurus	Medium	No	No	\checkmark	\checkmark	x	\checkmark	
	Felis catus	Medium	Yes	Yes	\checkmark	\checkmark	\checkmark	\checkmark	
nouse	Peromyscus maniculatus	Medium	No	Yes	x	x	x	x	
n dog	Nyctereutes procyonoides	Low	No	Yes	\checkmark	\checkmark	\checkmark	\checkmark	
-	Canis lupus familiaris	Low	No	No	\checkmark	\checkmark	\checkmark	\checkmark	
x	Vulpes vulpes	Low	Yes	Yes	\checkmark	\checkmark	\checkmark	\checkmark	
can mink	Neovison vison	Very Low	Yes	Yes	\checkmark	\checkmark	\checkmark	\checkmark	
	Mustela putorius furo	Very Low	Yes	Yes	\checkmark	X	\checkmark	\checkmark	
<u>cted</u>									
	Sus scrofa	Low	No	No	\checkmark	\checkmark	X	\checkmark	
mouse	Mus musculus ³	Very Low	No	No	\checkmark	X	X	X	

¹Damas et al. 2020. Broad host range of SARS-CoV-2 predicted by comparative and structural analysis of ACE2 in vertebrates. PNAS vol. 117 no. 36:22311–22322.

²MAVS Level 3 data based on amino acids for binding other host proteins. ³M.musculus was infectable with B.1.1.7 & other N501Y variants. See below for references and additional information

• SeqAPASS Level 3 critical amino acid preliminary evaluation indicates predicted susceptibility based on multiple IFN-I proteins is more consistent with reported infection than predicted susceptibility based on ACE2 alone.



References and additional information

References, search strategies, and a compendium of information on SARS-CoV-2/COVID-19 are available in the AOPwiki, specifically Adverse Outcome Pathway (AOP) 430, "Binding of SARS-CoV-2 to ACE2 leads to viral infection proliferation" (<u>https://aopwiki.org/aops/430</u>).