a unique series of humanized-ACE2 and Ace2-KO mouse models available for your COVID-19 research needs

Lily – Aurelie Jory
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Mouse Genome Engineering Facility
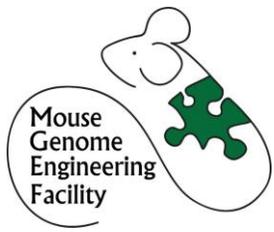
- Aurelie Jory (Lily)
- Suba Soundarya
- Saumya Mary Mathew
- Yogesh Chandra

TIGS — Tata Institute for Genetics and Society

- Baskar Bakthavachalu

(Tata Institute for Genetics and Society – Center at InStem & School of Basic Sciences, Indian Institute of Technology Mandi)

- Divij Kinger

ncbs — National Centre for Biological Sciences, Tata Institute of Fundamental Research

- Raj Ladher
- Mona Hosny

inStem

- Sai Manoz
- Aditya Deshpande
- Tanay Bhatt
- Arjun Guha
- Colin Jamora

Mynvax

- Randhir Singh
- Madhuraj Bhat

IISc

- Raghavan Varadarajan
- Raju S Rajmani

birac — Ignite Innovate Incubate

Department of Biotechnology, Government of India

Generating novel mouse models at MGEF

© Ravi Kumar Boyapati

Number of Transgenic & Genome Engineered Animal models generated per year @ MGEF

| Year	Value
2016	3
2017	2
2018	2
2019	10
2020	19
2021	13
Jan-May 2022	3 / 5 / 4
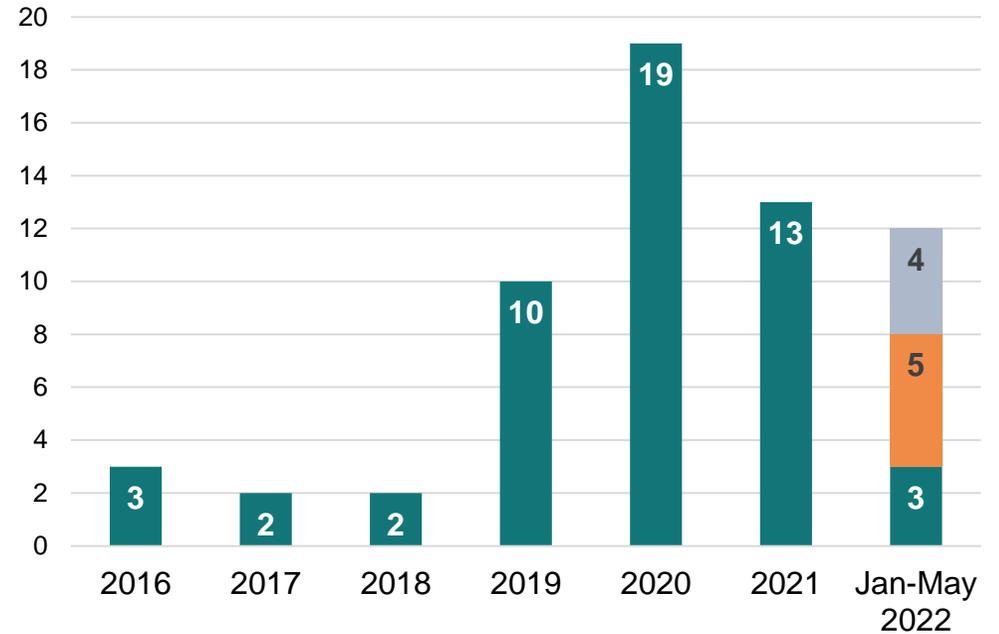
- # of Tg/Crispr projects in queue / under review
- # of Tg/Crispr ongoing projects
- Nb of Tg/KO/KI founder animals generated

mgef@ncbs.res.in

BLiSC ACE2 mouse models for COVID-19 research

1) – 3 different K18-hACE2 transgene copy number variants

Tg(K18-hACE2)/Blisc

HpaI | hK18 5prime | hACE2 | hK18 3prime | XbaI

3.3 Kb | 2.4 Kb | 880bp

K18-hACE2 plasmid generously donated by Paul McCray - doi:10.1128/JVI.02012-06

➤ Zygote pronuclear μ-injection

2) – KO-1

ext m5prime | mLHA/5'UTR | mRHA/3'UTR | ext m3prime

Ace2 KO-1/Blisc
Exon2 deletion – frame shift mutation

ext m5prime | mLHA/5'UTR | 47 Kb | mRHA/3'UTR | ext m3prime

Mouse Ace2 (WT)

3) – KO-2

Ace2 KO-2/Blisc
Full locus 47Kb deletion

ext m5prime | mLHA/5'UTR | mRHA/3'UTR | ext m3prime
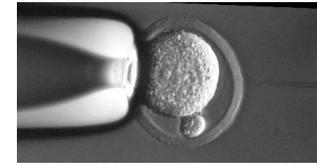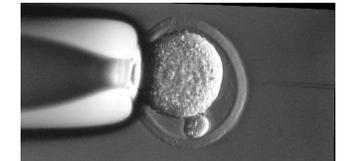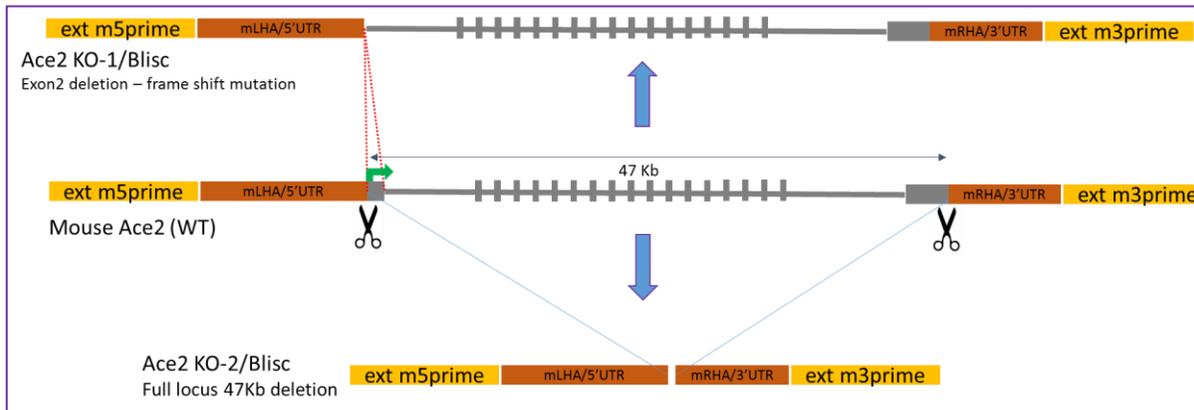
Crispr/Cas μ-injection in zygote

1 mm | 10 mm
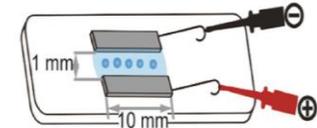
Zygote electroporation

4) - KI humanized

Mouse Ace2 (WT)

ext m5prime | mLHA/5'UTR | 47 Kb | mRHA/3'UTR | ext m3prime

hACE2-KI/Blisc =
Mouse Ace2 replaced
by Human ACE2 coding sequences

ext m5prime | mLHA/5'UTR | hACE2 | mRHA/3'UTR | ext m3prime

5.8 Kb
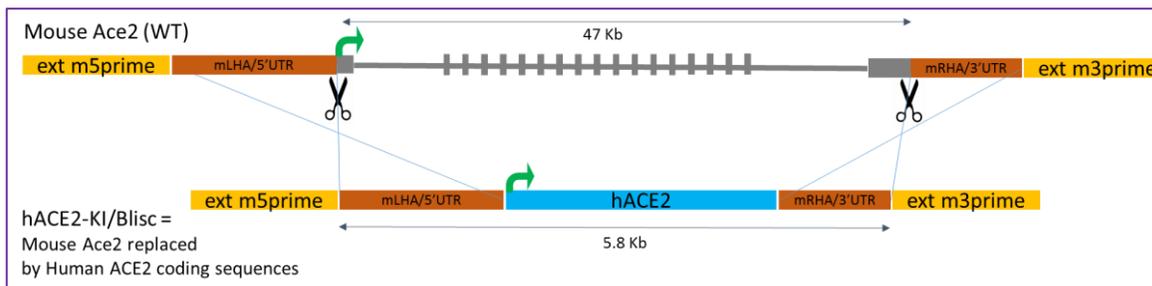
2 cell nuclear & cytoplasmic μ-injection
(2C-HR)-CRISPR

hACE2/Blisc mouse models for COVID-19 research

K18-hACE2 transgene copy number in different Tg lines
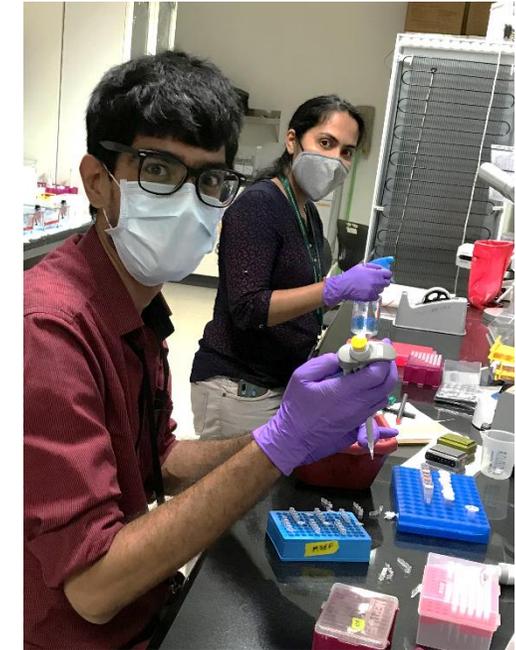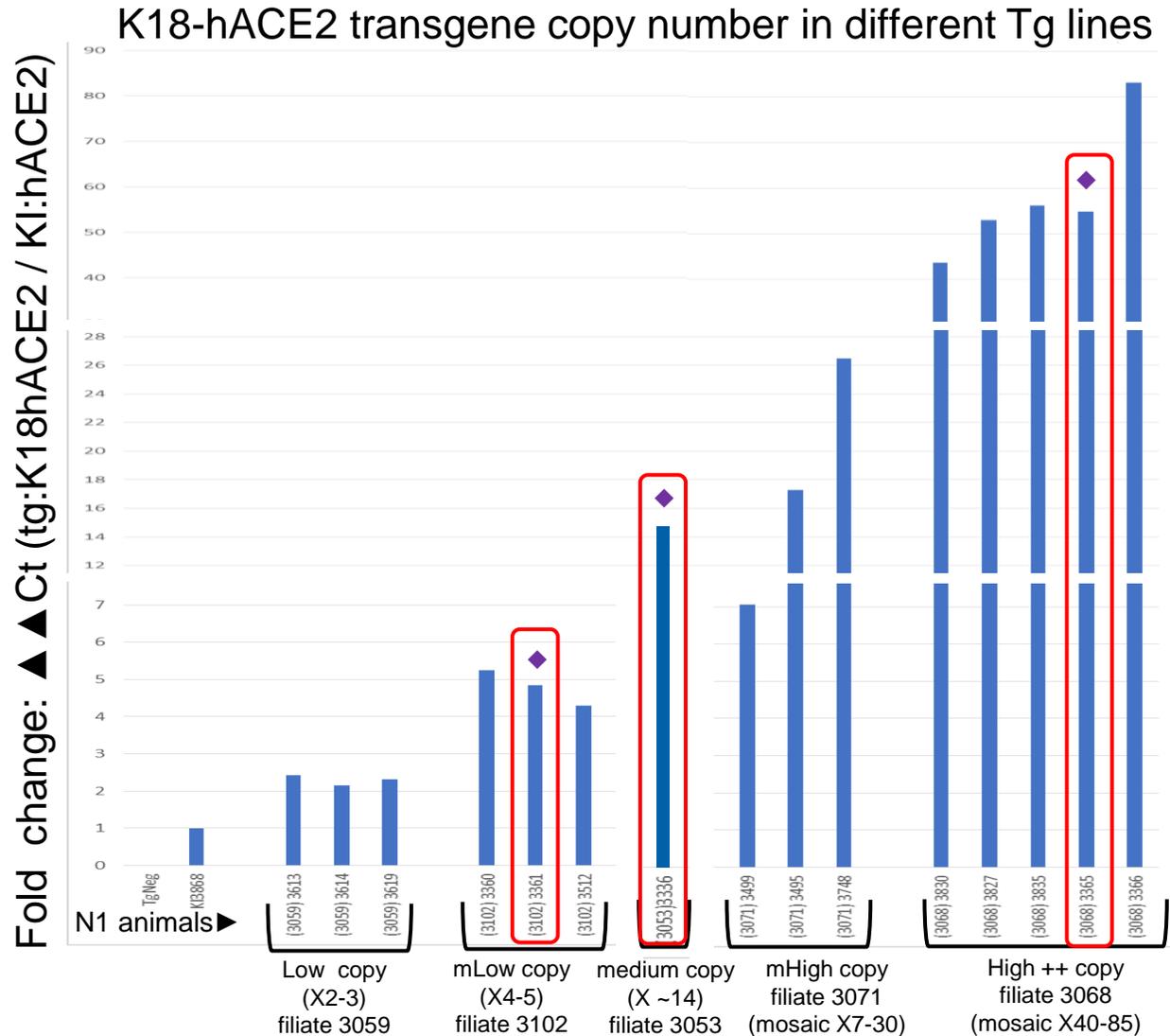
Divij Kinger &
Saumya M Mathew
(August 2020)

◆ = backcrossed & N6 animals now available

Organ specific hACE2 relative mRNA levels

# K18-hACE2/Blisc mice respond to SARS-Cov2 infection
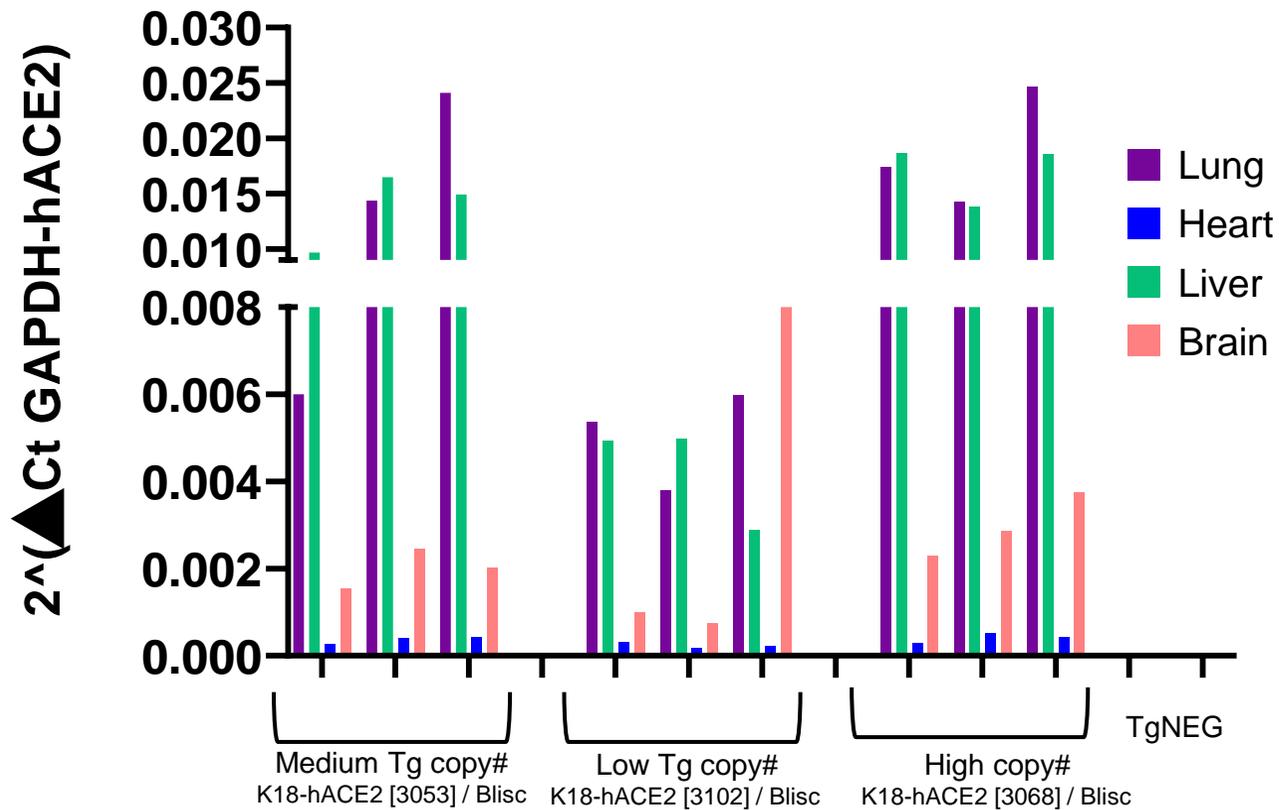
the different K18-hACE2 / BLISC mice challenged with 1e3-1e6 PFU
of SARSCov-2 US strain (USA-WA1/2020 obtained from BEI resources)
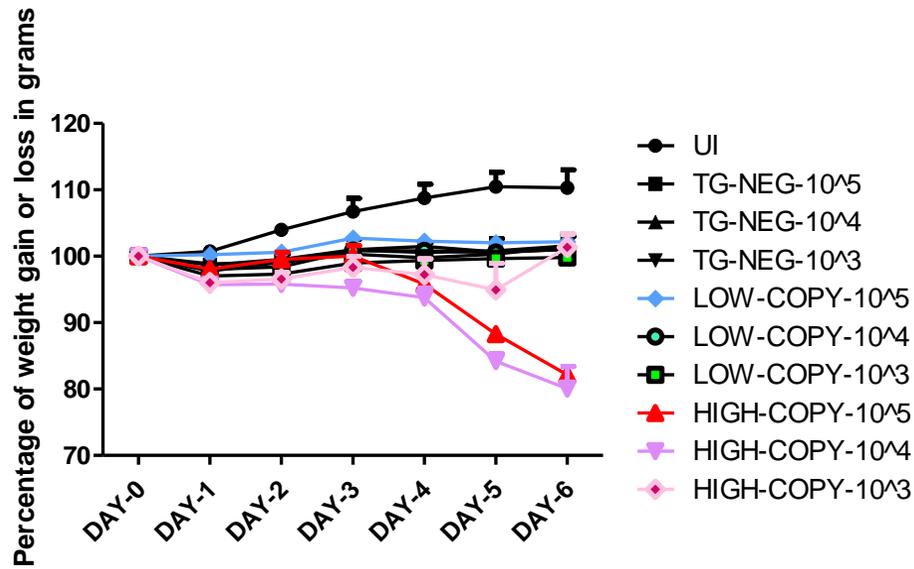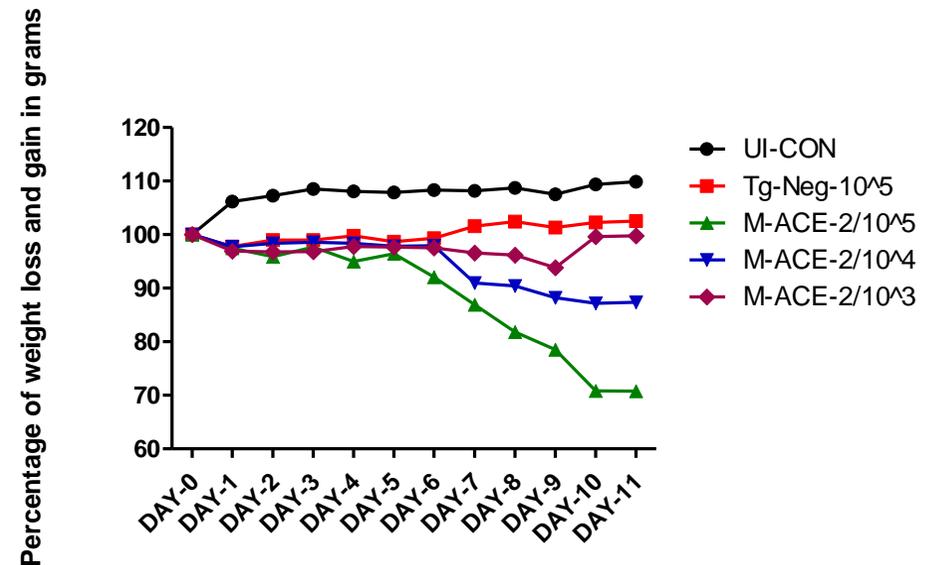

Different hACE2 mouse models for different purposes:
- K18-hACE2 / BLISC [3068] HIGH copy = COVID-19 Vaccine assays
- K18-hACE2 / BLISC [3053] MEDIUM copy = COVID-19 Therapeutics

In collaboration with: Raghavan Varadarajan, Raju S Rajmani (IISc) & Randhir Singh, Madhuraj Bhat (Mynvax, IISc)

# SARS-CoV2 infection induces acute lesions in K18-hACE2/Blisc mouse lungs
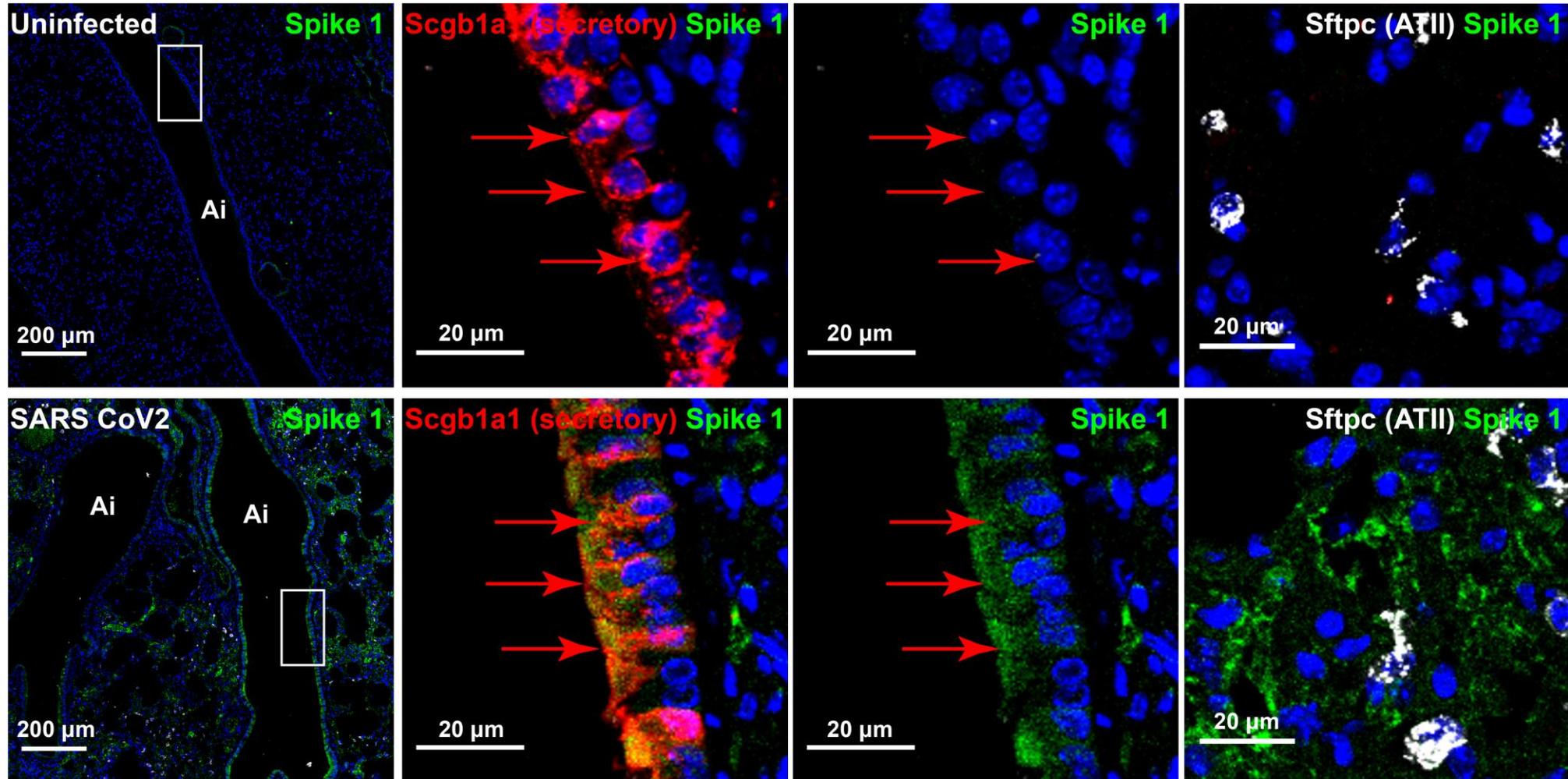
➤ K18-hACE2 / BLISC [3068] HIGH copy animals were infected with hCov19/USA/MD-HP01542/2021 (lineage.1.351) of SARS CoV2 @ 10^5 PFU and sacrificed 4 days post infection

Uninfected — Nucleocapsid / Scgb1a1 — Ai — 200 μm

SARS CoV 2 — Nucleocapsid / Scgb1a1 — Ai — 200 μm

Uninfected — Scgb1a1 (Secretory cells) — Ai — 200 μm

SARS CoV 2 — Scgb1a1 (Secretory cells) — Ai — 200 μm

Uninfected — Sftpc (Alveolar Type II cells) — Ai — 200 μm

SARS CoV 2 — Sftpc (Alveolar Type II cells) — Ai — 200 μm
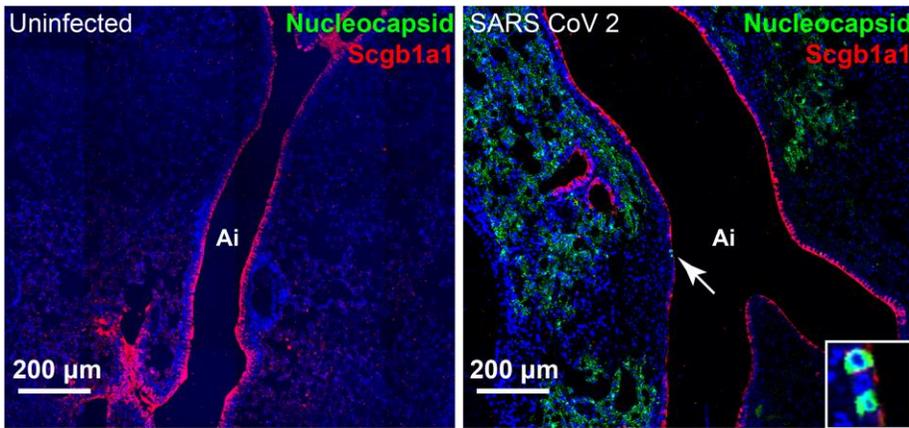
Aditya Deshpande

Sai Manoz
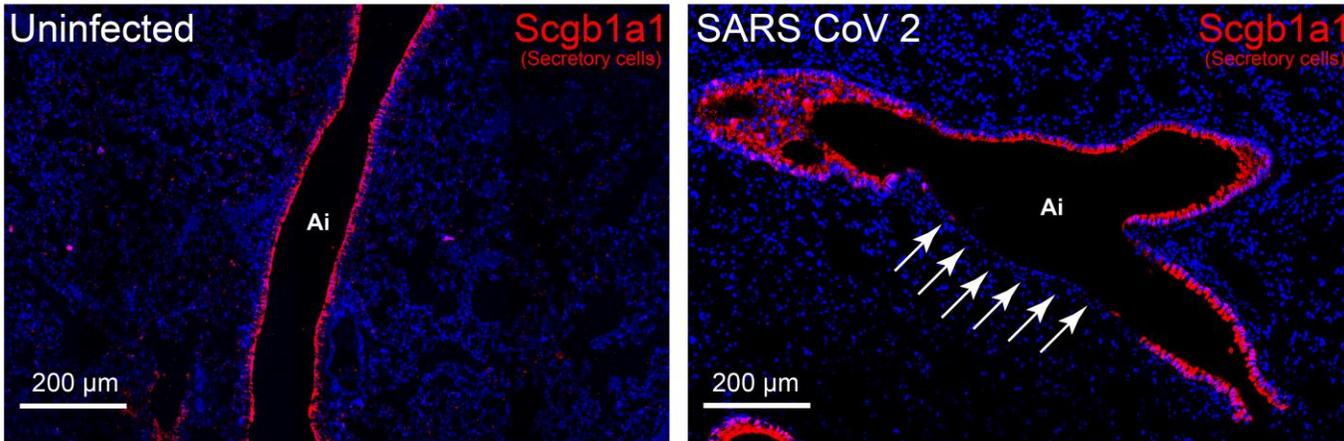
SARS-CoV2 infection induces acute lesions and cell loss in K18-hACE2/Blisc mouse lungs
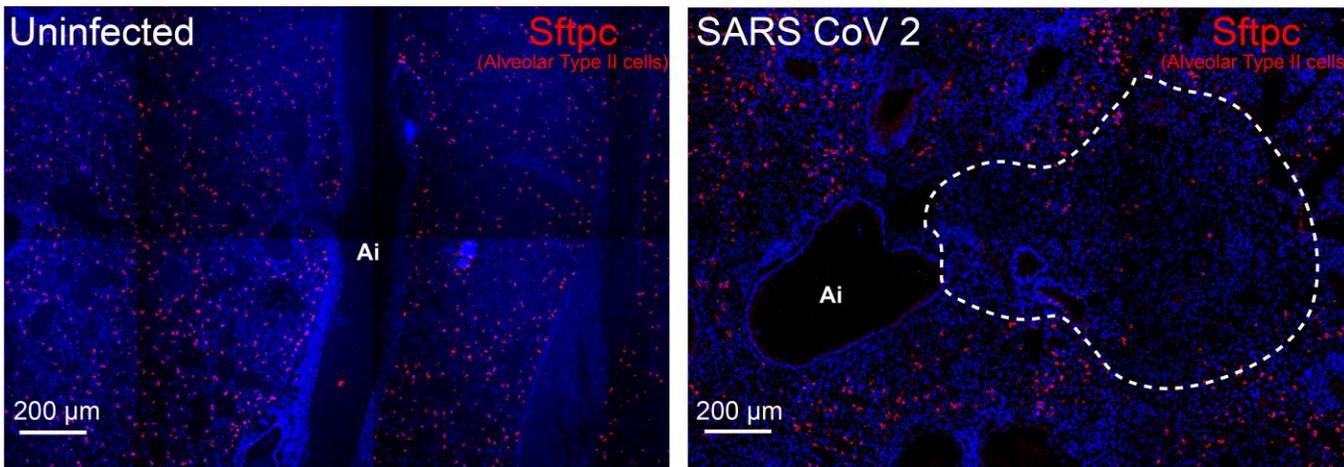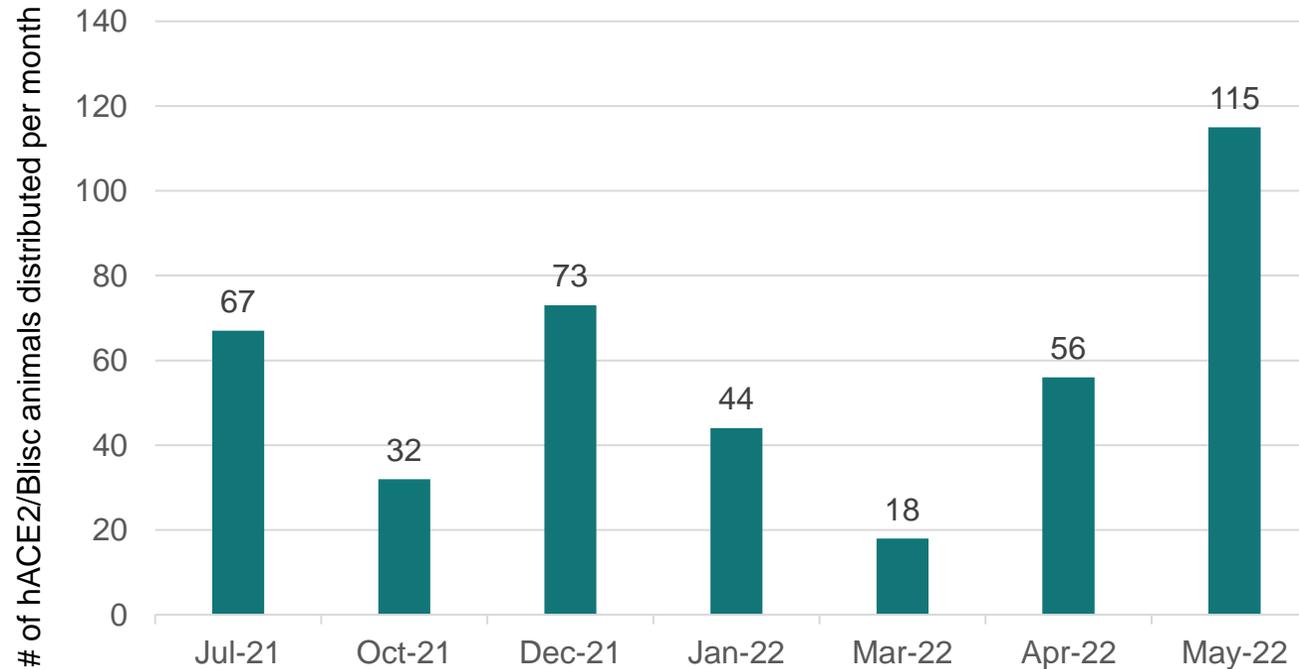
BLiSC — Bangalore Life Science Cluster

Mouse Genome Engineering Facility

Over 400 K18-hACE2/Blisc mice have been redistributed across India For various COVID-19 vaccine & Therapeutics development assays.

Suba

➢ **MGEF now setup to provide aged match cohorts of up to 50TgPOS animals + tgNEG littermate control animals**

Standardized Multiplex Genotyping for K18-hACE2 /Blisc colonies
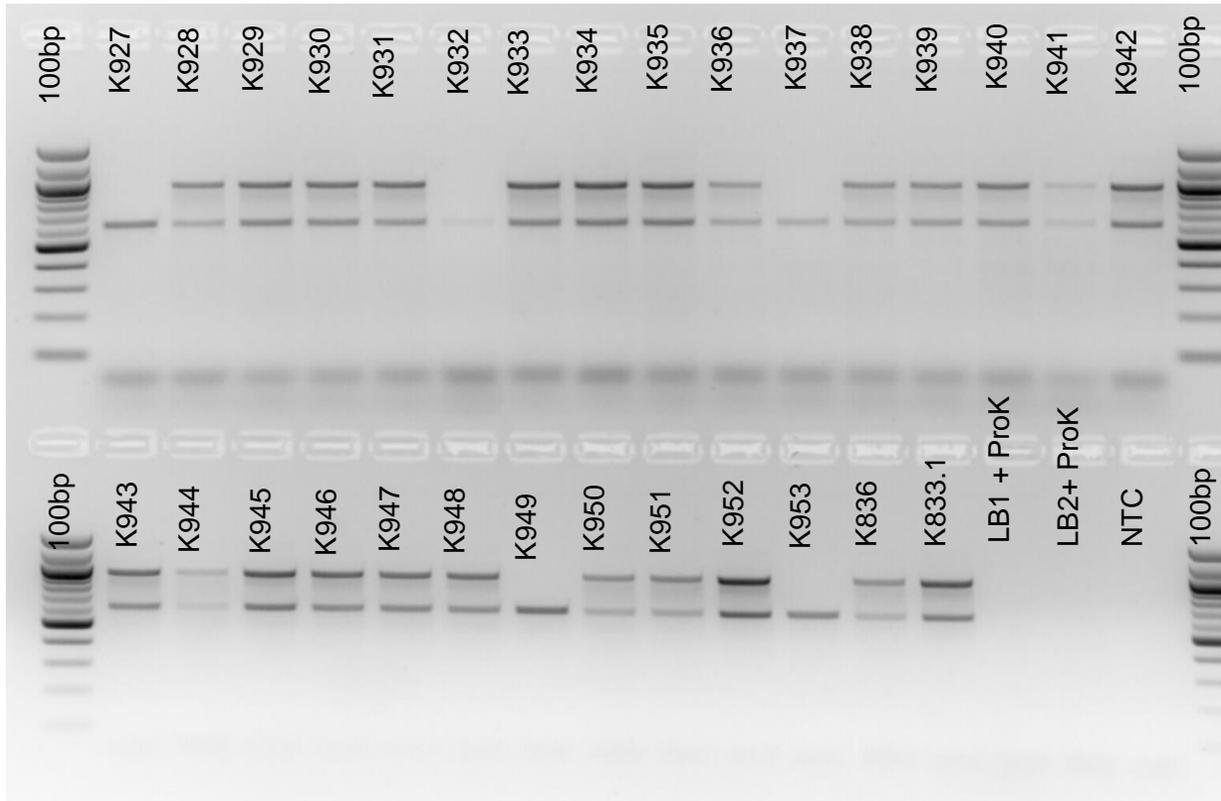
K18-hACE2 band @ 934bp
Internal control band @ 610bp

Suba

➤ **MGEF now setup to provide aged match cohorts of up to 50TgPOS animals + tgNEG littermate control animals**

# Protective Efficacy of vaccine formulations against SARS-CoV2 in K18-hACE2/BLiSC [3068] mice.

Legend:
- MMR1-DM37
- SPIKE-6P-DM37
- VC Naïve, challenged control
- UI-CON Naïve, unchallenged control
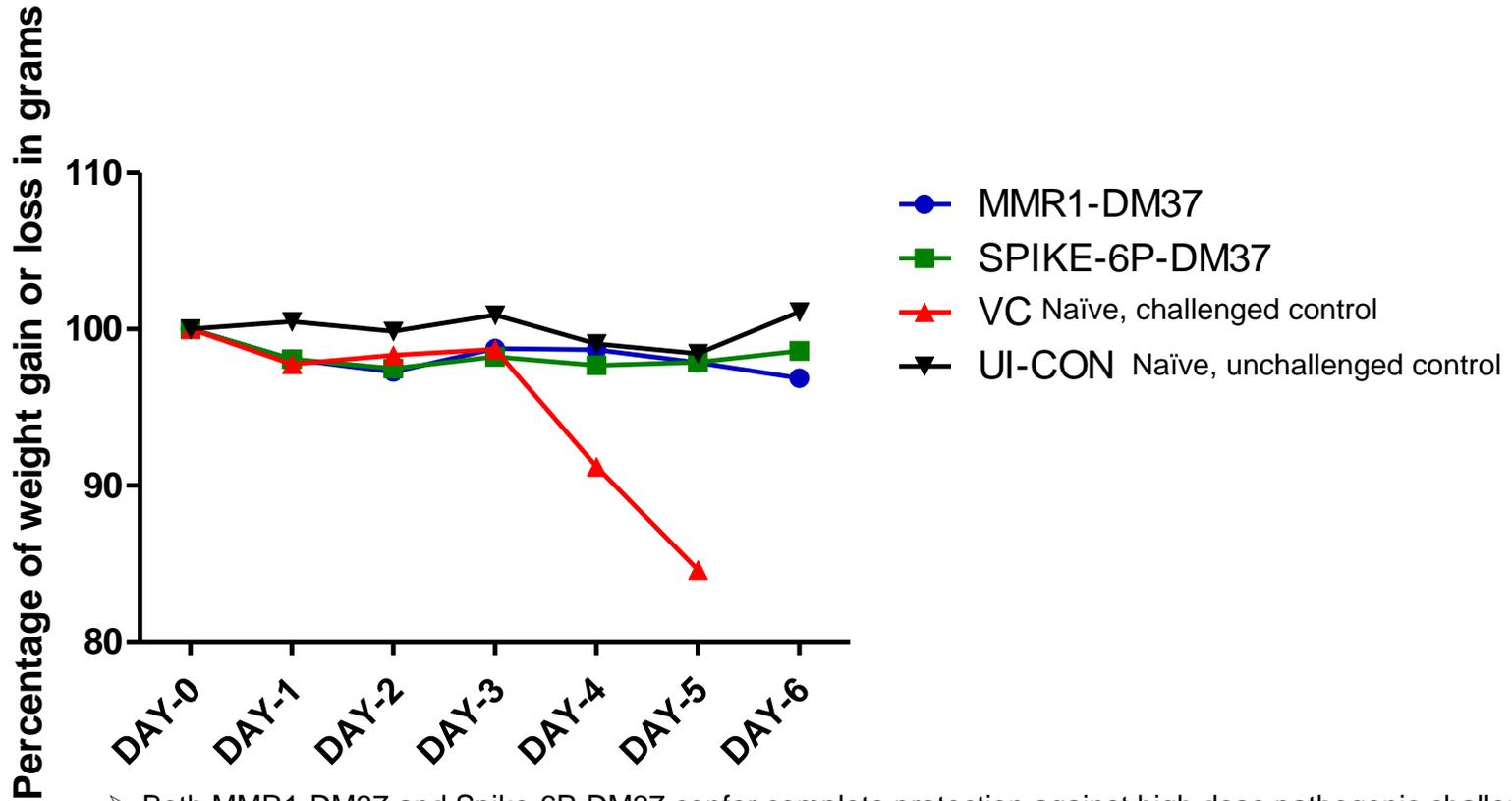
➤ Both MMR1-DM37 and Spike-6P-DM37 confer complete protection against high dose pathogenic challenge

Data from Raghavan Varadarajan, Raju S Rajmani (IISc) & Randhir Singh, Madhuraj Bhat (Mynvax, IISc)

Combining approaches:

➢ Humanization of the Immune system (huMouse or humIS mouse)

➢ + Human population / Patient specific hACE2 mice models
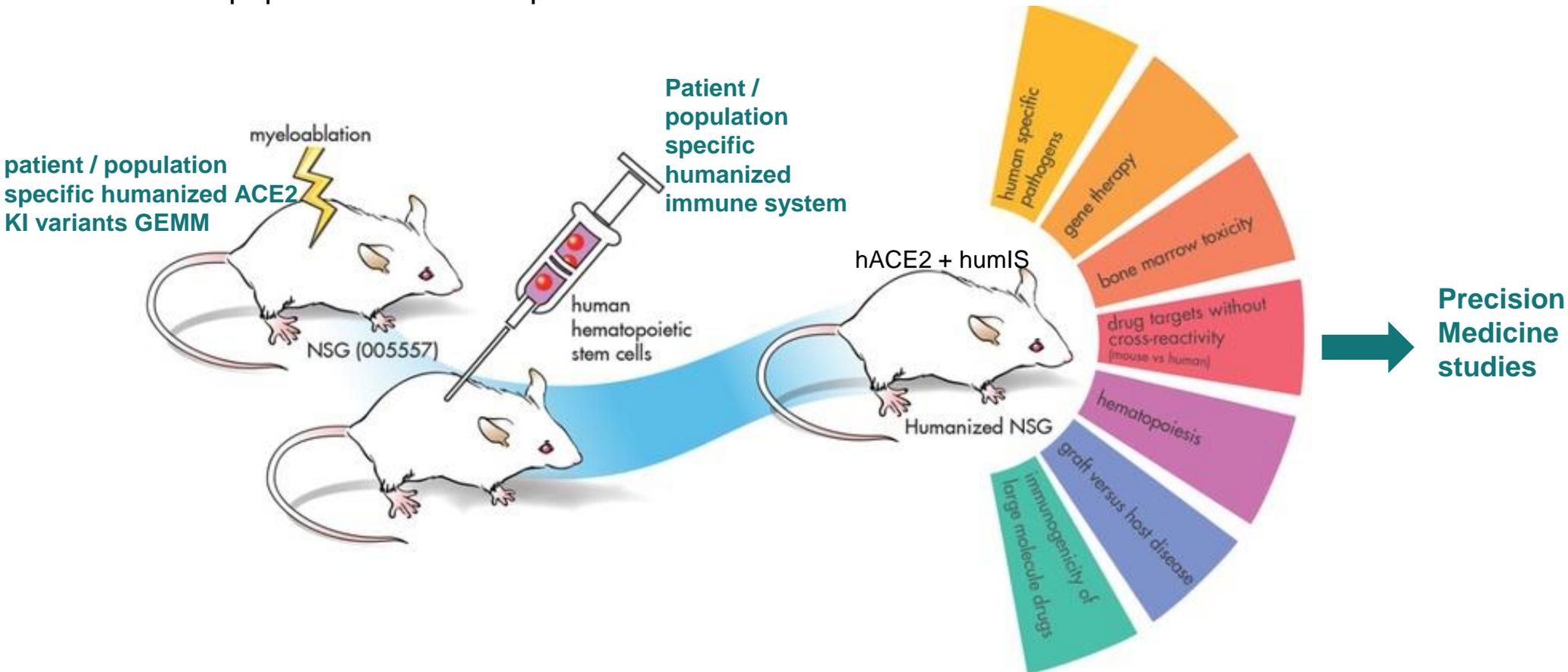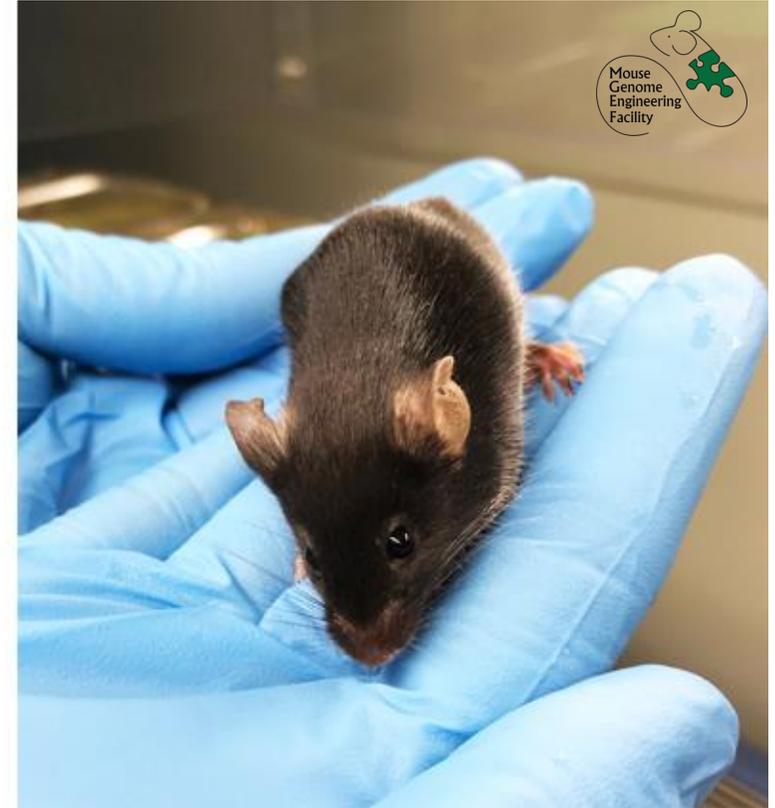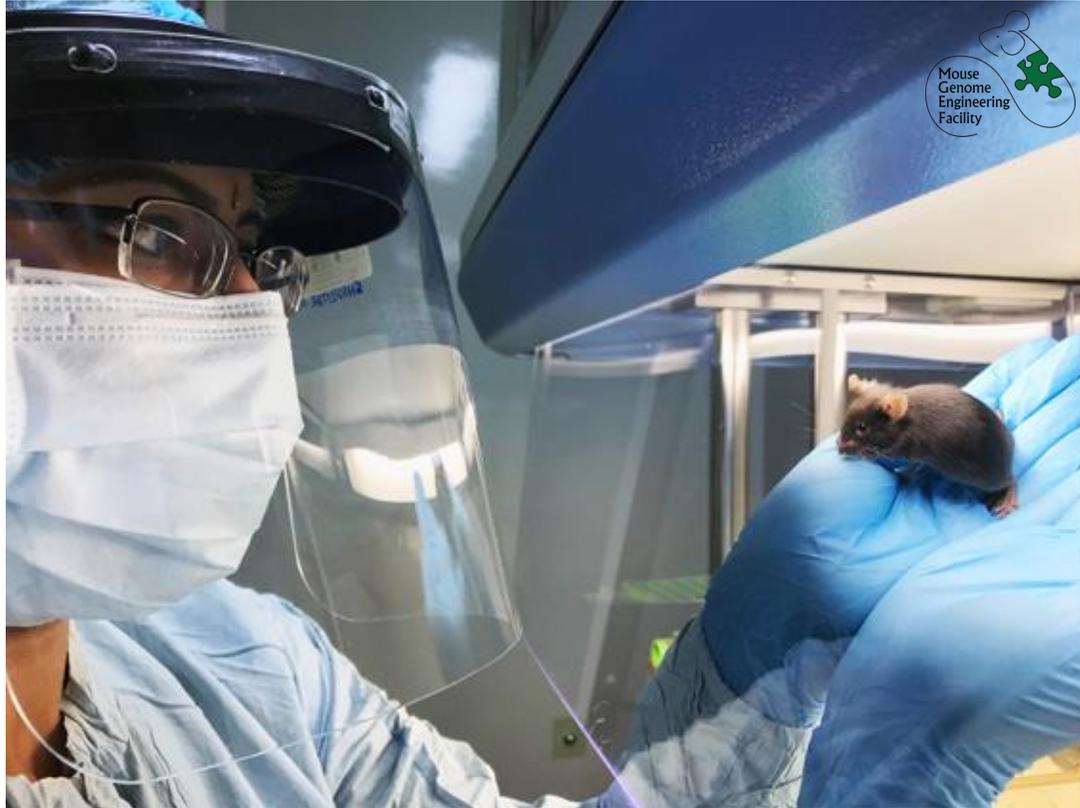
➤ hACE2 / BLiSC mice colonies maintained by dedicated staff at ACRC high barrier SPF facility
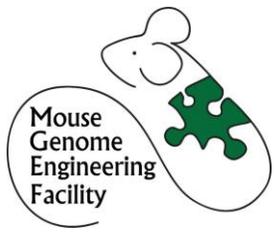
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