

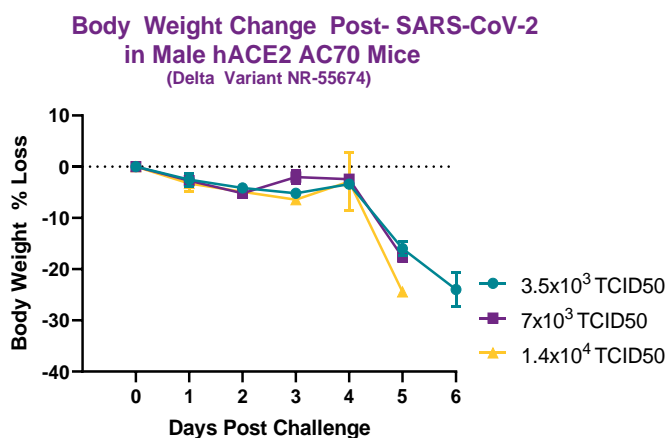
## Collaboration on hACE2 AC70 Mouse Model

Taconic Biosciences worked in collaboration with Bioqual, Inc., a preclinical testing CRO, to obtain validation data on Taconic's hACE2 AC70 model (Model # 18222) for research pertaining to COVID-19. The hACE2 AC70 mouse model is a great resource to study SARS-CoV-2. This model overexpresses the human Angiotensin converting enzyme 2 (ACE2) gene, making it susceptible to infection by SARS-CoV viruses. ACE2 is an enzyme found on the outer surface of a wide variety of cells, and it is the primary host cell target with which the S protein of SARS-CoV and SARS-CoV-2 associates (Ali & Vijayan, 2020).

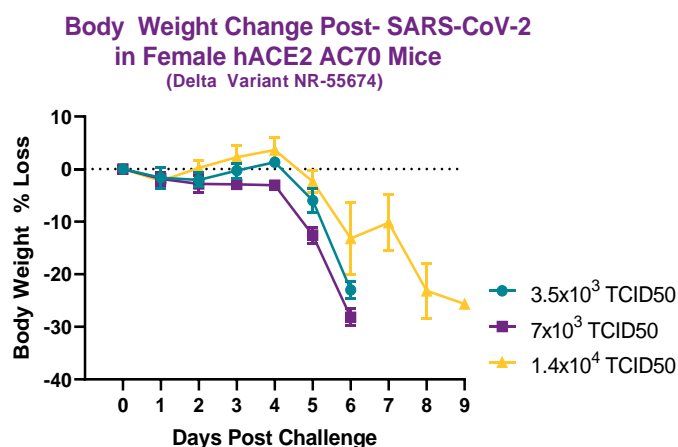
In this study we determined the virulence of several SARS-CoV-2 variants [Washington (US\_WA-1/2020); Delta (B.1.617.2); and Omicron sub-variants (BA.4/.5) in the hACE2 transgenic murine model (AC70).

In short, viral isolates from the Delta Variant NR-55674 were titrated from a stock solution, and hACE2 AC70 mice received different doses, subsequently, they were challenged intranasally with 50 $\mu$ L of high titer virus and two additional dilutions of the high titer stock. Body weight, clinical scores, and survival were recorded for these mice. hACE2 AC70 mice were challenged with SARS-CoV-2: Delta Variant NR-55674 at different doses (Figure 1). Male and female hACE2 AC70 mice are susceptible to SARS-CoV-2 infection, and there is an increased trend in body weight loss seen male mice compared to females (Figures 1A and 1B).

### A) Male

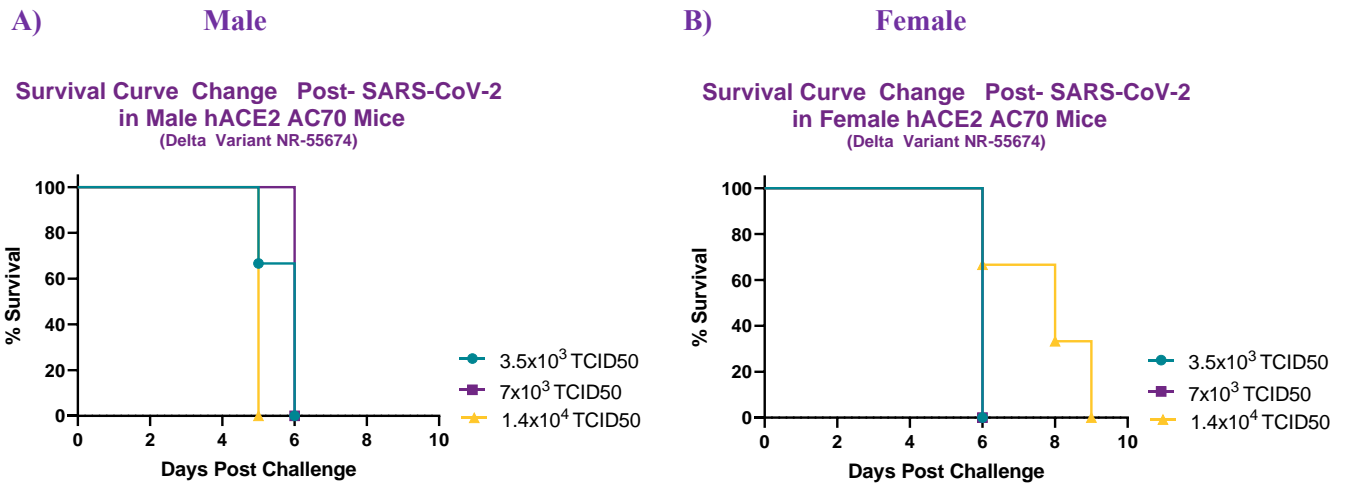


### B) Female



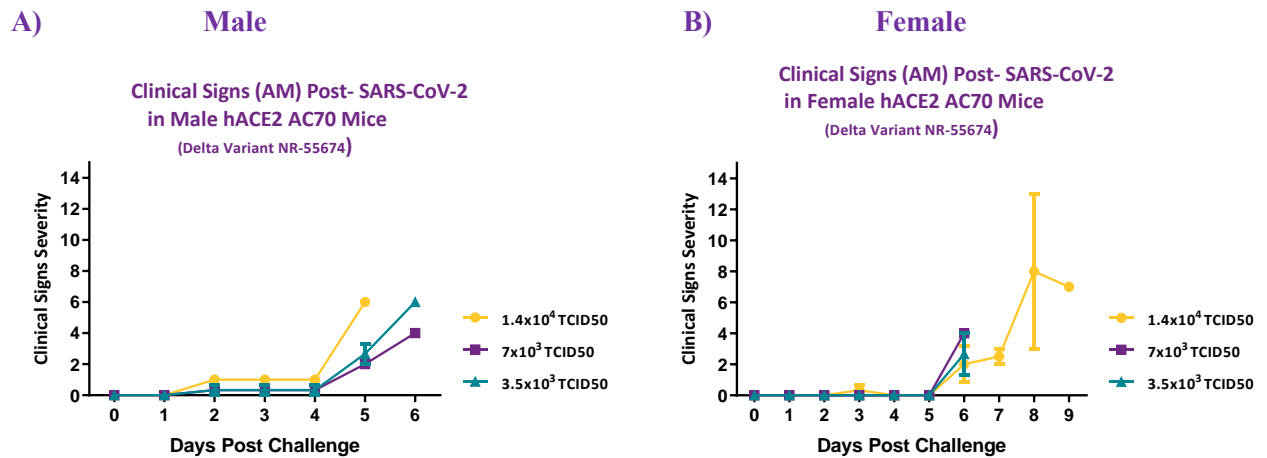
**Figure 1:** Body weight in hACE2 AC70 mice challenged with SARS-CoV-2 (Delta Variant NR-55674) at different doses. A) Body weight change for male hACE2 AC70 mice. B) Body weight change for female hACE2 AC70 mice.

When looking at survival (Figures 2A and 2B) at the two lowest doses, both males and females responded similarly. Conversely, at the highest dose, there is trend for increased survival in females, emphasizing that dose selection is critical, as a lower dose, yet lethal, would be preferred.



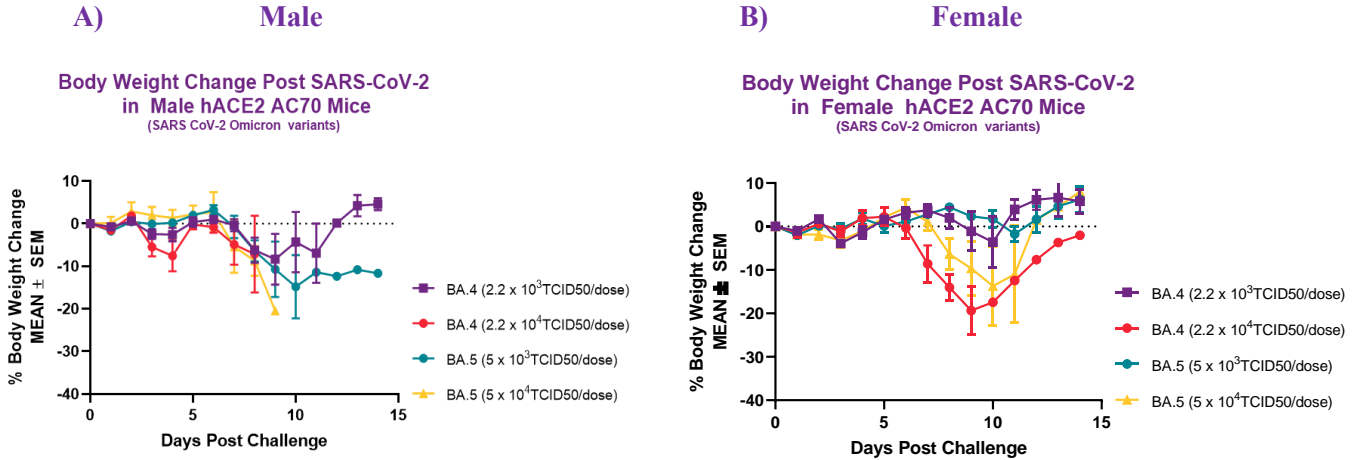
**Figure 2:** Survival curve for hACE2 AC70 mice challenged with SARS-CoV-2 (Delta Variant NR-55674) at different doses. **A)** Survival curve for male hACE2 AC70 mice. **B)** Survival curve for female hACE2 AC70 mice.

Clinical Severity was also scored, ranging from 0-15 as disease severity intensified. As shown in Figure 3, male hACE2 AC70 mice start showing worse severity score at an earlier timepoint compared to females.



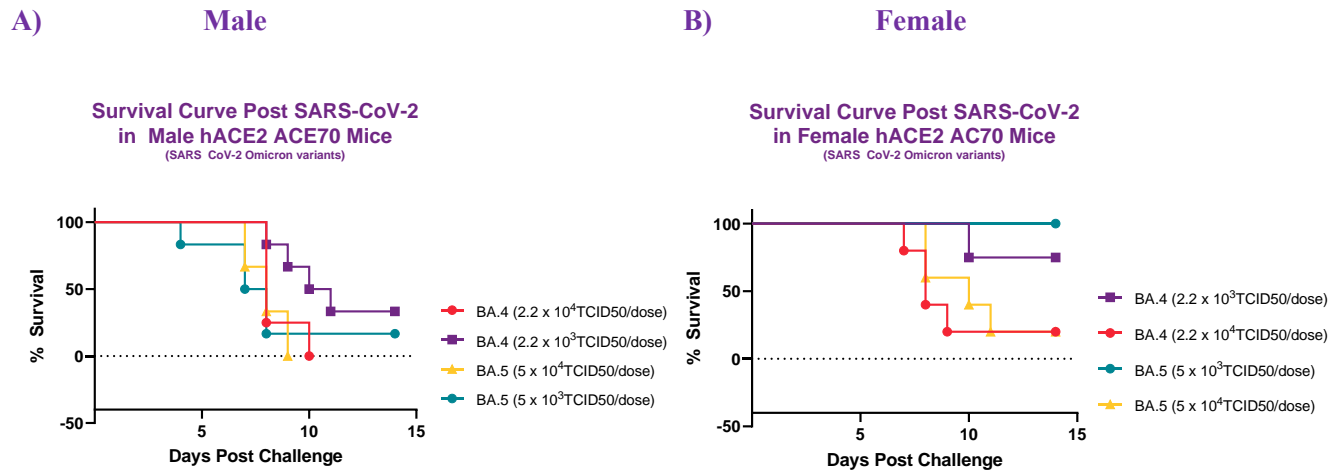
**Figure 3:** Clinical Signs Severity for hACE2 AC70 mice challenged with SARS-CoV-2 (Delta Variant NR-55674) at different doses. **A)** Clinical Signs severity for male hACE2 AC70 mice. **B)** Clinical Signs severity for female hACE2 AC70 mice.

Similarly, hACE2 AC70 mice were challenged with two SARS CoV-2 Omicron Sub-variants (BA.4) NR-56806 Lot# 70053068 and SARS CoV-2 Omicron Variant (BA.5) NR-58620 (Lot# 70053504) at different doses (Figure 4). While both males and females lost weight at higher doses of viral challenge, male mice were more sensitive to lower doses than females.



**Figure 4:** Body weight in hACE2 AC70 mice challenged with SARS CoV-2 Omicron Sub- Variants: (BA.4) NR-56806 (Lot# 70053068) and SARS CoV-2 Omicron Variant (BA.5) NR-58620 (Lot# 70053504) at different doses. **A)** Body weight change for male hACE2 AC70 mice. **B)** Body weight change for female hACE2 AC70 mice.

When looking at survival curve, BA.5 appears to be more lethal at the tested doses in males, as shown in Figures 5A and 5B. Both BA.4/.5 appear to be sub-lethal in females, but the survival outcome, and the overall response is dose dependent.

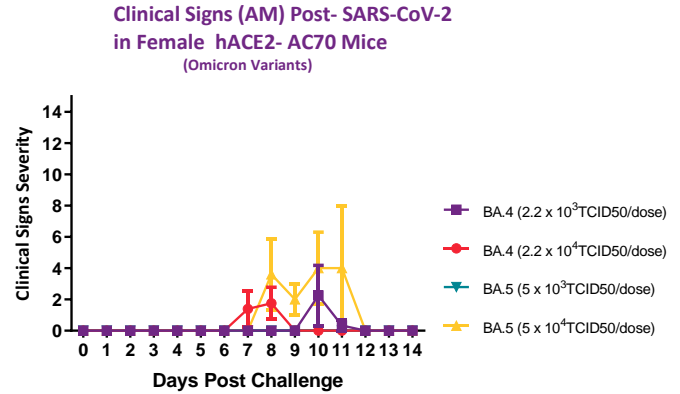
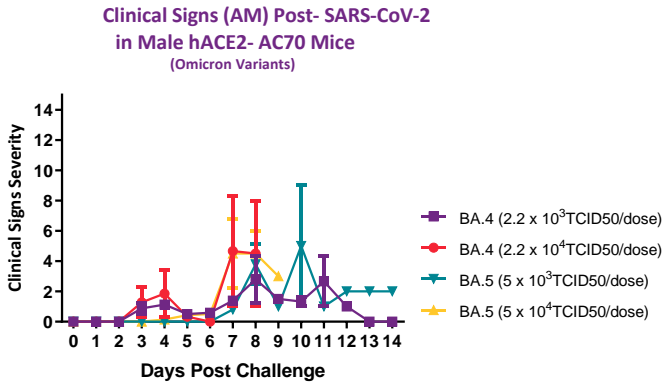


**Figure 5:** Survival curve for hACE2 AC70 mice challenged with SARS CoV-2 Omicron Sub- Variants: (BA.4) NR-56806 (Lot# 70053068) and SARS CoV-2 Omicron Sub-variant (BA.5) NR-58620 (Lot# 70053504) **A)** Survival curve for male hACE2 AC70 mice. **B)** Survival curve for female hACE2 AC70 mice.

Clinical Severity was also scored for the Omicron sub-variants, ranging from 0-15 as disease severity intensified. As shown in Figure 6, male hACE2 AC70 mice challenged with the lower doses of BA.4 and BA.5 start showing worse severity score at an earlier timepoint. And both males and females started showing higher clinical severity scores at Day 7 when challenged with the higher doses.

**A) Male**

**B) Female**



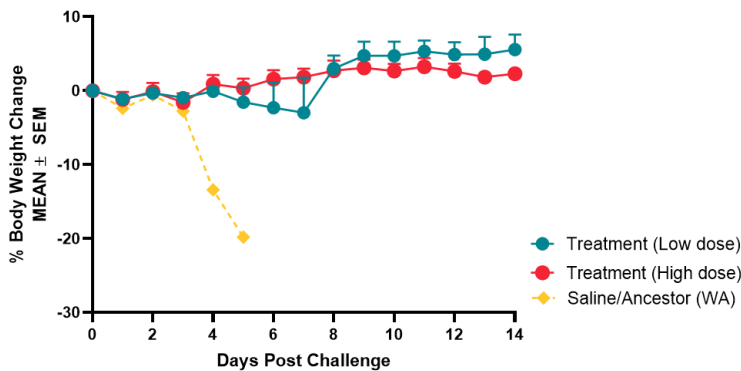
**Figure 6:** Clinical Signs Severity for hACE2 AC70 mice challenged with two SARS CoV-2 Omicron Sub-Variants, (BA.4) NR-56806 Lot# 70053068 and SARS CoV-2 Omicron Variant (BA.5) NR-58620 (Lot# 70053504). **A)** Clinical Signs severity for male hACE2 AC70 mice. **B)** Clinical Signs severity for female hACE2 AC70 mice.

The hACE2 AC70 model was also used in a treatment study. In summary, animals were treated with a test article, followed by intranasal (IN) challenge of Wild Type (WA) SARS-CoV-2 ( $2.8 \times 10^3$  PFU/mouse). Mice had their weights taken and recorded, and they were also observed for clinical signs until the study was terminated. Oral swab was collected during the in-life phase for viral load analysis using PCR assay. The control group that received saline, had greater weight loss, and they only survived 4-5 days (Figures 7A and 7B). Conversely, mice that were treated with a high or low dose, survived the challenge. Moreover, in the treated group, a significant decrease in genomic RNA was seen by Day 4, and the sub genomic RNA was undetectable by Day 4 (Figures 7C and 7B).

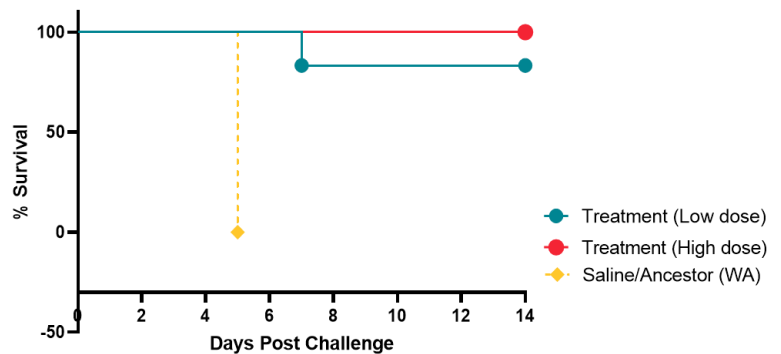
**A)**

**B)**

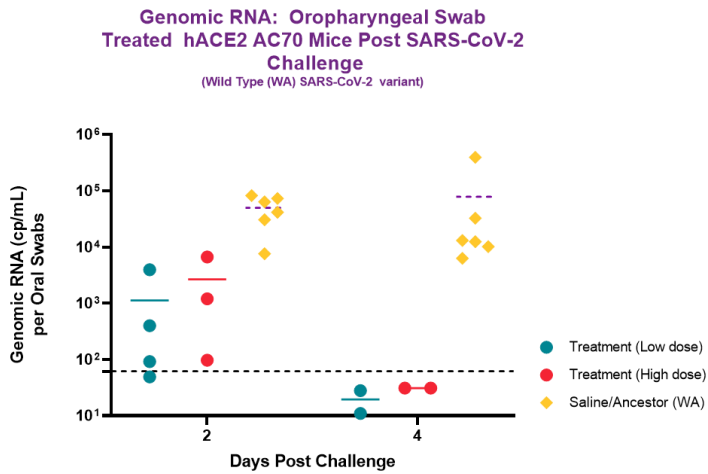
**Body Weight Change in treated hACE2 AC70 Mice Post SARS-CoV-2 Challenge**  
(Wild Type (WA) SARS-CoV-2 variant)



**Survival Curve in treated hACE2 AC70 Mice Post SARS-CoV-2 Challenge**  
(Wild Type (WA) SARS-CoV-2 variant)



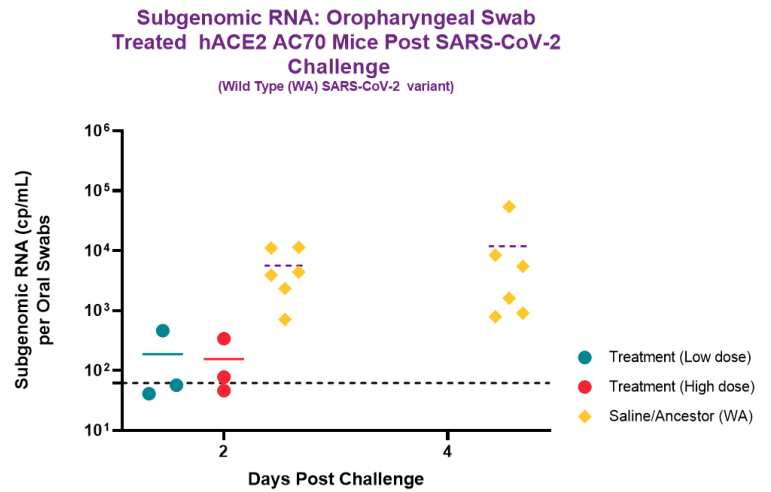
C)



Challenge stocks:

SARS-CoV-2 WRCEVA LOT TVP 23180 STOCK TITER: 1.6E+06 PFU/mL

D)



Challenge stocks:

SARS-CoV-2 WRCEVA LOT TVP 23180 STOCK TITER: 1.6E+06 PFU/mL

**Figure 7:** Data from SARS-CoV-2 hACE2 AC70 mouse treatment study. **A)** Body weight monitoring data post-challenge. Data points are group body weight change, means and errors bars represent standard error of the mean (SEM). **B)** Survival data post-challenge. Data points stand for percent survival of group. **C)** Genomic RNA level in oral swabs. **D)** Sub-Genomic RNA level in oral swabs.

In summary, both males and females hACE2 AC70 mice are susceptible to SARS-CoV-2 infection, and responses are dose and variant-dependent. The hACE2 AC70 model is an appropriate mouse model in which to study *in vivo* disease course as well as treatment and prophylactic efficacy for SARS-CoV-2 including different viral variants.