nature research

https://doi.org/10.1038/s41596-020-00403-2

Supplementary information

Genetically modified mouse models to help fight COVID-19

General notes

- The information presented in the first two columns of the Table 1 (included in the main article) is copied here for the convenience to the reader.

Model #	Name
1	B6.mAce2$^{\text{KO}}$-hACE2$^{\text{KI}}$
2	B6.mAce2$^{\text{KO}}$-hACE2-P2A-EGFP$^{\text{KI}}$
3	NSG.mAce2$^{\text{KO}}$-hACE2$^{\text{KI}}$
4	NSG.mAce2$^{\text{KO}}$-hACE2-P2A-EGFP$^{\text{KI}}$
5	BALB/c.mAce2$^{\text{KO}}$-hACE2$^{\text{KI}}$
6	BALB/c.mAce2$^{\text{KO}}$-hACE2-P2A-EGFP$^{\text{KI}}$
7	B6.mAce2$^{\text{KI-31K,82M,353K}}$
8	NSG.mAce2$^{\text{KI-31K,82M,353K}}$
9	BALB/c.mAce2$^{\text{KI-31K,82M,353K}}$
10	B6.mAce2$^{\text{KO}}$-hACE2-P2A-hTMPRSS2$^{\text{KI}}$
11	NSG.mAce2$^{\text{KO}}$-hACE2-P2A-hTMPRSS2$^{\text{KI}}$
12	BALB/c.mAce2$^{\text{KO}}$-hACE2-P2A-hTMPRSS2$^{\text{KI}}$
13	B6.mAce2$^{\text{KO}}$-hACE2-IRES-hTMPRSS2$^{\text{KI}}$
14	NSG.mAce2$^{\text{KO}}$-hACE2-IRES-hTMPRSS2$^{\text{KI}}$
15	BALB/c.mAce2$^{\text{KO}}$-hACE2-IRES-hTMPRSS2$^{\text{KI}}$
16	ROSA26$^{\text{(ACTB-Lox-hACE2-P2A-tdT-Lox-EGFP)}}$
17	ROSA26$^{\text{(ACTB-Lox-hACE2-IRES-tdT-Lox-EGFP)}}$
18	ROSA26$^{\text{(ACTB-Lox-tdT-Lox-hACE2-P2A-EGFP)}}$
19	ROSA26$^{\text{(ACTB-Lox-tdT-Lox-hACE2-IRES-EGFP)}}$
20	Ai63-TIGRE-TRE-hACE2-P2A-tdT
21	Ai63-TIGRE-TRE-hACE2-IRES-tdT
22	B6.mAce2$^{\text{cKO}}$-hACE2$^{\text{cKI}}$
23	NSG.mAce2$^{\text{cKO}}$-hACE2$^{\text{cKI}}$
24	BALB/c.mAce2$^{\text{cKO}}$-hACE2$^{\text{cKI}}$
25	B6.mAce2$^{\text{cKO}}$-hACE2-P2A-hTMPRSS2$^{\text{cKI}}$
26	NSG.mAce2$^{\text{cKO}}$-hACE2-P2A-hTMPRSS2$^{\text{cKI}}$
27	BALB/c.mAce2$^{\text{cKO}}$-hACE2-P2A-hTMPRSS2$^{\text{cKI}}$
28	B6.mAce2$^{\text{cKO}}$-hACE2-IRES-hTMPRSS2$^{\text{cKI}}$
29	NSG.mAce2$^{\text{cKO}}$-hACE2-IRES-hTMPRSS2$^{\text{cKI}}$
30	BALB/c.mAce2$^{\text{cKO}}$-hACE2-IRES-hTMPRSS2$^{\text{cKI}}$

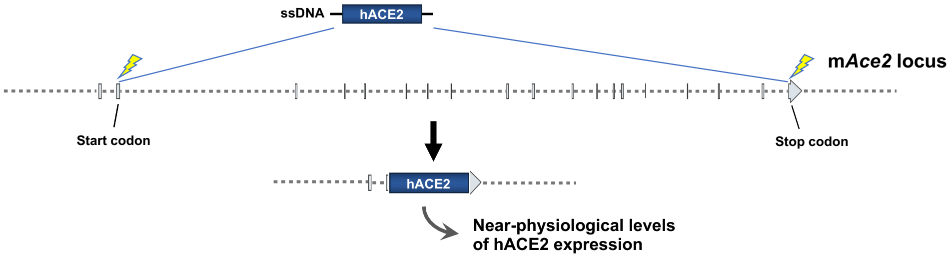
- The rationale for creating these models, and their advantages/disadvantages are described in the main article, wherever possible. Additional information relevant to specific models is included as ‘notes’ at the beginning the description for each model.
- For convenience, we have included schematics and parts of the figures that are presented in the main article.
- The nucleotide sequences of all the designs and the constructs were derived from the sequences available from ensemble.org web portal. Specifically, all sequences used for guide design are from the C57BL/6 mouse strain, even though some proposed models are meant to be created in other strains (NSG/NOG and BALB/c). We strongly recommend that researchers double-check the accuracy of the sequences and designs before proceeding with generating the model(s).
- Various elements in the schematics, and the nucleotide sequences, are displayed with matching colors.

Models #1, #3 and #5

1) B6.mAce2^{KO}-hACE2^{KI}
3) NSG.mAce2^{KO}-hACE2^{KI}
5) BALB/c.mAce2^{KO}-hACE2^{KI}

Notes:

a. The goal is to swap h*ACE2* cDNA into the m*Ace2* genomic locus between the start and stop codons. All the mouse introns between the coding exons will be deleted.
b. The mouse sequence was derived from the transcript, https://uswest.ensembl.org/Mus_musculus/Transcript/Exons?db=core;g=ENSMUSG00000015405;r=X:164139332-164188420;t=ENSMUST00000112271. Exon 1 is noncoding.



Sequence near the insertion site (the cleavage location expected for the chosen guide RNA is marked as '/').

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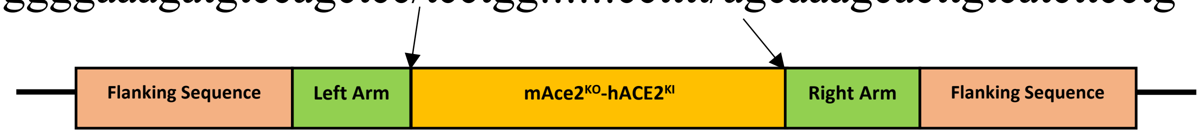

Left gRNA Sequence - GAAAGATGTCCAGCTCCTCC [*TGG*]
Right gRNA Sequence - ATGACAAGTGCTTTGCTAAA [*AGG*]
Sequence of the **mAce2^{KO}-hACE2^{KI}** ssDNA:

```
TTTCTAGGATTAATGAATAACATGTCTTTATTTAGTTTACTTAAAAAAATCATTCTAAAATATCTGTTTACATATCTGTCCTCTCCAGGATTAACTTCATAT
TGGTCCAGCAGCTTGTTTACTGTTCTCTTCTGTTTCTTCTTCTGCTTTTTTTTTCTTCTCTTCTCAGTGCCCAACCCAAGTTCAAAGGCTGATGAGAGAGA
AAAACTCATGAAGAGATTTTACTCTAGGGAAAGTTGCTCAGTGGATGGGATCTTGGCGCACGGGGAAAGATGTCCAGCTCaTCtTGGCTCCTTCTCAGCC
TTGTTGCTGTAACTGCTGCTCAGTCCACCATTGAGGAACAGGCCAAGACATTTTTGGACAAGTTTAACCACGAAGCCGAAGACCTGTTCTATCAAAGTTC
ACTTGCTTCTTGGAATTATAACACCAATATTACTGAAGAGAATGTCCAAAACATGAATAATGCTGGGGACAAATGGTCTGCCTTTTTAAAGGAACAGTCC
ACACTTGCCCAAATGTATCCACTACAAGAAATTCAGAATCTCACAGTCAAGCTTCAGCTGCAGGCTCTTCAGCAAAATGGGTCTTCAGTGCTCTCAGAAG
ACAAGAGCAAACGGTTGAACACAATTCTAAATACAATGAGCACCATCTACAGTACTGGAAAAGTTTGTAACCCAGATAATCCACAAGAATGCTTATTACT
TGAACCAGGTTTGAATGAAATAATGGCAAACAGTTTAGACTACAATGAGAGGCTCTGGGCTTGGGAAAGCTGGAGATCTGAGGTCGGCAAGCAGCTGA
GGCCATTATATGAAGAGTATGTGGTCTTGAAAAATGAGATGGCAAGAGCAAATCATTATGAGGACTATGGGGATTATTGGAGAGGAGACTATGAAGTAA
ATGGGGTAGATGGCTATGACTACAGCCGCGGCCAGTTGATTGAAGATGTGGAACATACCTTTGAAGAGATTAAACCATTATATGAACATCTTCATGCCT
ATGTGAGGGCAAAGTTGATGAATGCCTATCCTTCCTATATCAGTCCAATTGGATGCCTCCCTGCTCATTTGCTTGGTGATATGTGGGGTAGATTTTGGAC
AAATCTGTACTCTTTGACAGTTCCCTTTGGACAGAAACCAAACATAGATGTTACTGATGCAATGGTGGACCAGGCCTGGGATGCACAGAGAATATTCAA
GGAGGCCGAGAAGTTCTTTGTATCTGTTGGTCTTCCTAATATGACTCAAGGATTCTGGGAAAATTCCATGCTAACGGACCCAGGAAATGTTCAGAAAGC
AGTCTGCCATCCCACAGCTTGGGACCTGGGGAAGGGCGACTTCAGGATCCTTATGTGCACAAAGGTGACAATGGACGACTTCCTGACAGCTCATCATGA
GATGGGGCATATCCAGTATGATATGGCATATGCTGCACAACCTTTTCTGCTAAGAAATGGAGCTAATGAAGGATTCCATGAAGCTGTTGGGGAAATCAT
GTCACTTTCTGCAGCCACACCTAAGCATTTAAAATCCATTGGTCTTCTGTCACCCGATTTTCAAGAAGACAATGAAACAGAAATAAACTTCCTGCTCAAA
CAAGCACTCACGATTGTTGGGACTCTGCCATTTACTTACATGTTAGAGAAGTGGAGGTGGATGGTCTTTAAAGGGGAAATTCCCAAAGACCAGTGGATG
AAAAAGTGGTGGGAGATGAAGCGAGAGATAGTTGGGGTGGTGGAACCTGTGCCCCATGATGAAACATACTGTGACCCCGCATCTCTGTTCCATGTTTCT
AATGATTACTCATTCATTCGATATTACACAAGGACCCTTTACCAATTCCAGTTTCAAGAAGCACTTTGTCAAGCAGCTAAACATGAAGGCCCTCTGCACA
AATGTGACATCTCAAACTCTACAGAAGCTGGACAGAAACTGTTCAATATGCTGAGGCTTGGAAAATCAGAACCCTGGACCCTAGCATTGGAAAATGTTG
TAGGAGCAAAGAACATGAATGTAAGGCCACTGCTCAACTACTTTGAGCCCTTATTTACCTGGCTGAAAGACCAGAACAAGAATTCTTTTGTGGGATGGA
GTACCGACTGGAGTCCATATGCAGACCAAAGCATCAAAGTGAGGATAAGCCTAAAATCAGCTCTTGGAGATAAAGCATATGAATGGAACGACAATGAAA
TGTACCTGTTCCGATCATCTGTTGCATATGCTATGAGGCAGTACTTTTTAAAAGTAAAAAATCAGATGATTCTTTTTGGGGAGGAGGATGTGCGAGTGGC
TAATTTGAAACCAAGAATCTCCTTTAATTTCTTTGTCACTGCACCTAAAAATGTGTCTGATATCATTCCTAGAACTGAAGTTGAAAAGGCCATCAGGATG
TCCCGGAGCCGTATCAATGATGCTTTCCGTCTGAATGACAACAGCCTAGAGTTTCTGGGGATACAGCCAACACTTGGACCTCCTAACCAGCCCCCTGTT
TCCATATGGCTGATTGTTTTTGGAGTTGTGATGGGAGTGATAGTGGTTGGCATTGTCATCCTGATCTTCACTGGGATCAGAGATCGGAAGAAGAAAAAT
AAAGCAAGAAGTGGAGAAAATCCTTATGCCTCCATCGATATTAGCAAAGGAGAAAATAATCCAGGATTCCAAAACACTGATGATGTTCAGACCTCaTTTT
AGCAAAGCACTTGTCATCTTCCTGTATGTAAATGCTAACTTCATAGTACACAAAATATGAGAGTATACACATGTCATTAGCTATCAAAACTATGATCTGTT
CAGTAAACGTTGTCCAAAGAGCATCAGACTTGAGTGGACATCTTCACTGACATTGCTTTCAGTATTTATTTCTGCCTAAGGATTTGACATCTCTTCTGTTT
ATTAATAGAGATGTTTATCTTAGCATA
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Additional notes:

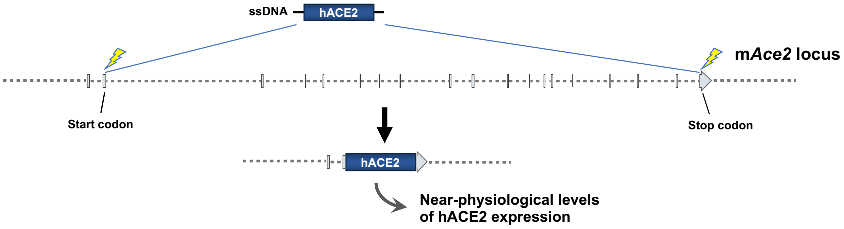
Lower case letters at the beginning and end of the h*ACE2* cDNA are silent mutations introduced to prevent Cas9 re-cleavage.

Models #2 #4 and #6

2) B6.mAce2^{KO}-hACE2-P2A-EGFP^{KI}
4) NSG.mAce2^{KO}-hACE2-P2A-EGFP^{KI}
6) BALB/c.mAce2^{KO}-hACE2-P2A-EGFP^{KI}

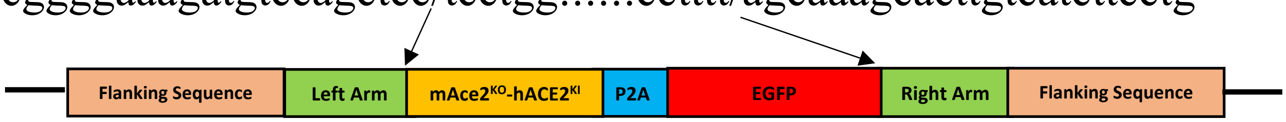
Notes:

a. The goal is to swap the insertion cassette into the m*Ace2* genomic locus between the start and stop codons. All the mouse introns between the coding exons will be deleted.
b. The mouse sequence was derived from the transcript, https://uswest.ensembl.org/Mus_musculus/Transcript/Exons?db=core;g=ENSMUSG00000015405;r=X:164139332-164188420;t=ENSMUST00000112271. Exon 1 is noncoding.



Sequence near the insertion site (the cleavage location expected for the chosen guide RNA is marked as '/').

cggggaaagatgtccagctcc/tcctgg::::::cctttt/agcaaagcacttgtcatcttcctg



Left gRNA Sequence - GAAAGATGTCCAGCTCCTCC [*TGG*]
Right gRNA Sequence - ATGACAAGTGCTTTGCTAAA [*AGG*]
Sequence of the **mAce2^{KO}-hACE2-P2A-EGFP^{KI}** ssDNA donor:

**TTTCTAGGATTAATGAATAACATGTCTTTATTTAGTTTACTTAAAAAAATCATTCTAAAATATCTGTTTACATATCTGTCCTCTCCAGGATTAACTTCATAT
TGGTCCAGCAGCTTGTTTACTGTTCTCTTCTGTTTCTTCTTCTGCTTTTTTTTTCTTCTCTTCTCAGTGCCCAACCCAAGTTCAAAGGCTGATGAGAGAGA
AAAACTCATGAAGAGATTTTACTCTAGGGAAAGTTGCTCAGTGGATGGGATCTTGGCGCACGGGGAAAGATGTCCAGCTCaTCtTGGCTCCTTCTCAGCC
TTGTTGCTGTAACTGCTGCTCAGTCCACCATTGAGGAACAGGCCAAGACATTTTTGGACAAGTTTAACCACGAAGCCGAAGACCTGTTCTATCAAAGTTC
ACTTGCTTCTTGGAATTATAACACCAATATTACTGAAGAGAATGTCCAAAACATGAATAATGCTGGGGACAAATGGTCTGCCTTTTTAAAGGAACAGTCC
ACACTTGCCCAAATGTATCCACTACAAGAAATTCAGAATCTCACAGTCAAGCTTCAGCTGCAGGCTCTTCAGCAAAATGGGTCTTCAGTGCTCTCAGAAG
ACAAGAGCAAACGGTTGAACACAATTCTAAATACAATGAGCACCATCTACAGTACTGGAAAAGTTTGTAACCCAGATAATCCACAAGAATGCTTATTACT
TGAACCAGGTTTGAATGAAATAATGGCAAACAGTTTAGACTACAATGAGAGGCTCTGGGCTTGGGAAAGCTGGAGATCTGAGGTCGGCAAGCAGCTGA
GGCCATTATATGAAGAGTATGTGGTCTTGAAAAATGAGATGGCAAGAGCAAATCATTATGAGGACTATGGGGATTATTGGAGAGGAGACTATGAAGTAA
ATGGGGTAGATGGCTATGACTACAGCCGCGGCCAGTTGATTGAAGATGTGGAACATACCTTTGAAGAGATTAAACCATTATATGAACATCTTCATGCCT
ATGTGAGGGCAAAGTTGATGAATGCCTATCCTTCCTATATCAGTCCAATTGGATGCCTCCCTGCTCATTTGCTTGGTGATATGTGGGGTAGATTTTGGAC
AAATCTGTACTCTTTGACAGTTCCCTTTGGACAGAAACCAAACATAGATGTTACTGATGCAATGGTGGACCAGGCCTGGGATGCACAGAGAATATTCAA
GGAGGCCGAGAAGTTCTTTGTATCTGTTGGTCTTCCTAATATGACTCAAGGATTCTGGGAAAATTCCATGCTAACGGACCCAGGAAATGTTCAGAAAGC
AGTCTGCCATCCCACAGCTTGGGACCTGGGGAAGGGCGACTTCAGGATCCTTATGTGCACAAAGGTGACAATGGACGACTTCCTGACAGCTCATCATGA
GATGGGGCATATCCAGTATGATATGGCATATGCTGCACAACCTTTTCTGCTAAGAAATGGAGCTAATGAAGGATTCCATGAAGCTGTTGGGGAAATCAT
GTCACTTTCTGCAGCCACACCTAAGCATTTAAAATCCATTGGTCTTCTGTCACCCGATTTTCAAGAAGACAATGAAACAGAAATAAACTTCCTGCTCAAA
CAAGCACTCACGATTGTTGGGACTCTGCCATTTACTTACATGTTAGAGAAGTGGAGGTGGATGGTCTTTAAAGGGGAAATTCCCAAAGACCAGTGGATG
AAAAAGTGGTGGGAGATGAAGCGAGAGATAGTTGGGGTGGTGGAACCTGTGCCCCATGATGAAACATACTGTGACCCCGCATCTCTGTTCCATGTTTCT
AATGATTACTCATTCATTCGATATTACACAAGGACCCTTTACCAATTCCAGTTTCAAGAAGCACTTTGTCAAGCAGCTAAACATGAAGGCCCTCTGCACA
AATGTGACATCTCAAACTCTACAGAAGCTGGACAGAAACTGTTCAATATGCTGAGGCTTGGAAAATCAGAACCCTGGACCCTAGCATTGGAAAATGTTG
TAGGAGCAAAGAACATGAATGTAAGGCCACTGCTCAACTACTTTGAGCCCTTATTTACCTGGCTGAAAGACCAGAACAAGAATTCTTTTGTGGGATGGA
GTACCGACTGGAGTCCATATGCAGACCAAAGCATCAAAGTGAGGATAAGCCTAAAATCAGCTCTTGGAGATAAAGCATATGAATGGAACGACAATGAAA
TGTACCTGTTCCGATCATCTGTTGCATATGCTATGAGGCAGTACTTTTTAAAAGTAAAAAATCAGATGATTCTTTTTGGGGAGGAGGATGTGCGAGTGGC
TAATTTGAAACCAAGAATCTCCTTTAATTTCTTTGTCACTGCACCTAAAAATGTGTCTGATATCATTCCTAGAACTGAAGTTGAAAAGGCCATCAGGATG
TCCCGGAGCCGTATCAATGATGCTTTCCGTCTGAATGACAACAGCCTAGAGTTTCTGGGGATACAGCCAACACTTGGACCTCCTAACCAGCCCCCTGTT
TCCATATGGCTGATTGTTTTTGGAGTTGTGATGGGAGTGATAGTGGTTGGCATTGTCATCCTGATCTTCACTGGGATCAGAGATCGGAAGAAGAAAAAT
AAAGCAAGAAGTGGAGAAAATCCTTATGCCTCCATCGATATTAGCAAAGGAGAAAATAATCCAGGATTCCAAAACACTGATGATGTTCAGACCTCaTTTG
GAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGTGAGCAAGGGCGAGGAGCTGTTCACCGG
GGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTG
ACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCC
GACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACC
CGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCT
GGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACG
GCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCC
CTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTA
ATAGCAAAGCACTTGTCATCTTCCTGTATGTAAATGCTAACTTCATAGTACACAAAATATGAGAGTATACACATGTCATTAGCTATCAAAACTATGATCT
GTTCAGTAAACGTTGTCCAAAGAGCATCAGACTTGAGTGGACATCTTCACTGACATTGCTTTCAGTATTTATTTCTGCCTAAGGATTTGACATCTCTTCT
GTTTATTAATAGAGATGTTTATCTTAGCATA**

Models #7, #8 and #9

7) B6.mAce2$^{KI\text{-}31K,\ 82M,\ 353K}$

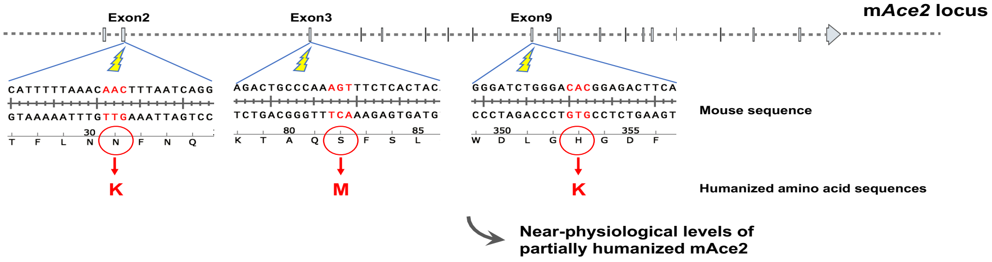
8) NSG.mAce2$^{KI\text{-}31K,\ 82M,\ 353K}$

9) BALB/c.mAce2$^{KI\text{-}31K,\ 82M,\ 353K}$

Notes:

a. The goal is to create point mutations changing the coding sequence of m*Ace2* to that of h*ACE2*.

b. There are three amino acid differences between mACE2 and hACE2 that are thought to be important for binding to the SARS-CoV-2 spike protein: N31K, S82M and H353K (Luan, et al., 2020. *Biochem. Biophys. Res. Commun.* **526**, 165–169).



mACE2$^{KI\text{-}31K}$

Additional note for the 31K mutation: Human and mouse ACE2 differ in this exonic region by three amino acids; 30, 31 and 34. Because they are close to each other, and because there is no gRNA site close to codon 31, we propose changing codons 30 (N to D) and 34 (Q to D) in addition to 31 (N to K) by using two flanking guide RNAs to remove the entire region and replace it with the complete humanized sequence.

Wild type Sequence (codons 30, 31 and 34 are shown in bold)

GGAAAGATGTCCAGCTCCTCCTGGCTCCTTCTCAGCCTTGTTGCTGTTACTACTGCTCAGTCCCTCACCGAGGAAAATGCCAAGACATTTTTA**AACAAC**TTTAAT**CAG**GAAGCTGAAGACCTGTCTTATCAAAGTTCACTTGCTTCTTGGAATTATAATACTAACATTACTGAAGAAAATGCCCA

Left gRNA Sequence - TACTGCTCAGTCCCTCACCG [*AGG*]

Right gRNA Sequence - CAAGTGAACTTTGATAAGAC [*AGG*]

31K Donor Sequence

GGAAAGATGTCCAGCTCCTCCTGGCTCCTTCTCAGCCTTGTTGCTGTTACTACTGCTCAGTCCCTCACCGA***a***GAAAATGCCAAGACATTTTTA***gAC*AAa**TTTAAT***gAc***GAAGCTGAAGA***t***CTGTCTTATCAAAGTTCACTTGCTTCTTGGAATTATAATACTAACATTACTGAAGAAAATGCCCA

Note: Yellow highlighted, lower case letters are the silent mutations introduced to prevent Cas9 re-cleavage. The codons that are changed are highlighted in red.

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mACE2$^{KI\text{-}82M}$

Wild type Sequence (codon 82 is shown in bold)

AGTGAGGCTGCAGCCAAATGGTCTGCCTTTTATGAAGAACAGTCTAAGACTGCCCAA**AGT**TTCTCACTACAAGAAATCCAGACTCCGATCATCAAGCGTCAACTACAGGCCCTTCAGCAA

gRNA Sequence - TTCTTGTAGTGAGAAACTTT [*GGG*]

82M Donor Sequence

AGTGAGGCTGCAGCCAAATGGTCTGCCTTTTATGAAGAACAGTCTAAGACTGCCCAA**Atg**TTCTCACTACAAGAAATCCAGACTCCGATCATCAAGCGTCAACTACAGGCCCTTCAGCAA

----

mACE2$^{KI\text{-}353K}$

Wild type Sequence (codon 353 is shown in bold)

GCTGACTGAGCCAGCAGATGGCCGGAAAGTTGTCTGCCACCCCACAGCTTGGGATCTGGGA**CAC**GGAGACTTCAGGTAGTTGGGTTGTCACTTACAGTGCAGCTACTAAGTGAGAAACAG

gRNA Sequence - ACAGCTTGGGATCTGGGACA [*CGG*]

353K Donor Sequence

GCTGACTGAGCCAGCAGATGGCCGGAAAGTTGTCTGCCACCCCACAGCTTGGGATCTGGGA**aAg**GGAGACTTCAGGTAGTTGGGTTGTCACTTACAGTGCAGCTACTAAGTGAGAAACAG

Models #10, #11 and #12

10) B6.mAce2^{KO}-hACE2-P2A-hTMPRSS2^{KI}

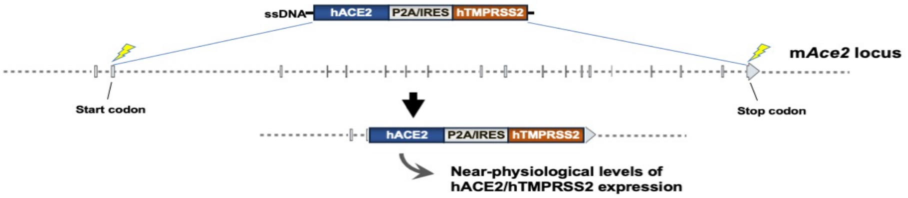
11) NSG.mAce2^{KO}-hACE2 -P2A-hTMPRSS2^{KI}

12) BALB/c.mAce2^{KO}-hACE2-P2A-hTMPRSS2^{KI}

Notes:

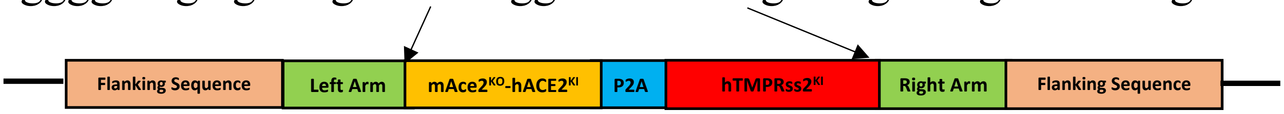
a. The goal is to swap a hACE2-P2A-hTMPRSS2 cassette into the m*Ace2* locus between the start and stop codons in three different genetic backgrounds. All the mouse introns between the coding exons will be deleted.

b. The mouse sequence was derived from the transcript; https://uswest.ensembl.org/Mus_musculus/Transcript/Exons?db=core;g=ENSMUSG00000015405;r=X:164139332-164188420;t=ENSMUST00000112271. Exon 1 is noncoding.



Sequence near the insertion site (the cleavage location expected for the chosen guide RNA is marked as '/').

cggggaaagatgtccagctcc/tcctgg::::::cctttt/agcaaagcacttgtcatcttcctg



Left gRNA Sequence - GAAAGATGTCCAGCTCCTCC [*TGG*]

Right gRNA Sequence - ATGACAAGTGCTTTGCTAAA [*AGG*]

Sequence of the **mAce2^{KO}-hACE2 -P2A-hTMPRSS2^{KI}** ssDNA donor:

TATAGTAAAATCATAGTACACCCTCCTCCTCCAGTGTATCTTTAACAGCTTTTAAGGAACATATTAACTAAATGTCCAGGTTTTGATTTGGCCATAAAATGTTAGCAAA
GCTAAGGTTTTCTAGGATTAATGAATAACATGTCTTTATTTAGTTTACTTAAAAAAATCATTCTAAAATATCTGTTTACATATCTGTCCTCTCCAGGATTAACTTCATAT
TGGTCCAGCAGCTTGTTTACTGTTCTCTTCTGTTTCTTCTTCTGCTTTTTTTTTCTTCTCTTCTCAGTGCCCAACCCAAGTTCAAAGGCTGATGAGAGAGAAAAACTCA
TGAAGAGATTTTACTCTAGGGAAAGTTGCTCAGTGGATGGGATCTTGGCGCACGGGGAAAGATGTCCAGCTCaTCtTGGCTCCTTCTCAGCCTTGTTGCTGTAACTGC
TGCTCAGTCCACCATTGAGGAACAGGCCAAGACATTTTTGGACAAGTTTAACCACGAAGCCGAAGACCTGTTCTATCAAAGTTCACTTGCTTCTTGGAATTATAACAC
CAATATTACTGAAGAGAATGTCCAAAACATGAATAATGCTGGGGACAAATGGTCTGCCTTTTTAAAGGAACAGTCCACACTTGCCCAAATGTATCCACTACAAGAAAT
TCAGAATCTCACAGTCAAGCTTCAGCTGCAGGCTCTTCAGCAAAATGGGTCTTCAGTGCTCTCAGAAGACAAGAGCAAACGGTTGAACACAATTCTAAATACAATGAG
CACCATCTACAGTACTGGAAAAGTTTGTAACCCAGATAATCCACAAGAATGCTTATTACTTGAACCAGGTTTGAATGAAATAATGGCAAACAGTTTAGACTACAATGA
GAGGCTCTGGGCTTGGGAAAGCTGGAGATCTGAGGTCGGCAAGCAGCTGAGGCCATTATATGAAGAGTATGTGGTCTTGAAAAATGAGATGGCAAGAGCAAATCATT
ATGAGGACTATGGGGATTATTGGAGAGGAGACTATGAAGTAAATGGGGTAGATGGCTATGACTACAGCCGCGGCCAGTTGATTGAAGATGTGGAACATACCTTTGAA
GAGATTAAACCATTATATGAACATCTTCATGCCTATGTGAGGGCAAAGTTGATGAATGCCTATCCTTCCTATATCAGTCCAATTGGATGCCTCCCTGCTCATTTGCTTG
GTGATATGTGGGGTAGATTTTGGACAAATCTGTACTCTTTGACAGTTCCCTTTGGACAGAAACCAAACATAGATGTTACTGATGCAATGGTGGACCAGGCCTGGGATG
CACAGAGAATATTCAAGGAGGCCGAGAAGTTCTTTGTATCTGTTGGTCTTCCTAATATGACTCAAGGATTCTGGGAAAATTCCATGCTAACGGACCCAGGAAATGTTC
AGAAAGCAGTCTGCCATCCCACAGCTTGGGACCTGGGGAAGGGCGACTTCAGGATCCTTATGTGCACAAAGGTGACAATGGACGACTTCCTGACAGCTCATCATGAG
ATGGGGCATATCCAGTATGATATGGCATATGCTGCACAACCTTTTCTGCTAAGAAATGGAGCTAATGAAGGATTCCATGAAGCTGTTGGGGAAATCATGTCACTTTCT
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GGGACTCTGCCATTTACTTACATGTTAGAGAAGTGGAGGTGGATGGTCTTTAAAGGGGAAATTCCCAAAGACCAGTGGATGAAAAAGTGGTGGGAGATGAAGCGAGA
GATAGTTGGGGTGGTGGAACCTGTGCCCCATGATGAAACATACTGTGACCCCGCATCTCTGTTCCATGTTTCTAATGATTACTCATTCATTCGATATTACACAAGGAC
CCTTTACCAATTCCAGTTTCAAGAAGCACTTTGTCAAGCAGCTAAACATGAAGGCCCTCTGCACAAATGTGACATCTCAAACTCTACAGAAGCTGGACAGAAACTGTT
CAATATGCTGAGGCTTGGAAAATCAGAACCCTGGACCCTAGCATTGGAAAATGTTGTAGGAGCAAAGAACATGAATGTAAGGCCACTGCTCAACTACTTTGAGCCCT
TATTTACCTGGCTGAAAGACCAGAACAAGAATTCTTTTGTGGGATGGAGTACCGACTGGAGTCCATATGCAGACCAAAGCATCAAAGTGAGGATAAGCCTAAAATCA
GCTCTTGGAGATAAAGCATATGAATGGAACGACAATGAAATGTACCTGTTCCGATCATCTGTTGCATATGCTATGAGGCAGTACTTTTTAAAAGTAAAAAATCAGATG
ATTCTTTTTGGGGAGGAGGATGTGCGAGTGGCTAATTTGAAACCAAGAATCTCCTTTAATTTCTTTGTCACTGCACCTAAAAATGTGTCTGATATCATTCCTAGAACTG
AAGTTGAAAAGGCCATCAGGATGTCCCGGAGCCGTATCAATGATGCTTTCCGTCTGAATGACAACAGCCTAGAGTTTCTGGGGATACAGCCAACACTTGGACCTCCT
AACCAGCCCCCTGTTTCCATATGGCTGATTGTTTTTGGAGTTGTGATGGGAGTGATAGTGGTTGGCATTGTCATCCTGATCTTCACTGGGATCAGAGATCGGAAGAAG
AAAAATAAAGCAAGAAGTGGAGAAAATCCTTATGCCTCCATCGATATTAGCAAAGGAGAAAATAATCCAGGATTCCAAAACACTGATGATGTTCAGACCTCCTTTGGA
AGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGCTTTGAACTCAGGGTCACCACCAGCTATTGGACCTTACTA
TGAAAACCATGGATACCAACCGGAAAACCCCTATCCCGCACAGCCCACTGTGGTCCCCACTGTCTACGAGGTGCATCCGGCTCAGTACTACCCGTCCCCCGTGCCCC
AGTACGCCCCGAGGGTCCTGACGCAGGCTTCCAACCCCGTCGTCTGCACGCAGCCCAAATCCCCATCCGGGACAGTGTGCACCTCAAAGACTAAGAAAGCACTGTGC
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CTCAGGTACCTGCATCAACCCCTCTAACTGGTGTGATGGCGTGTCACACTGCCCCGGCGGGGAGGACGAGAATCGGTGTGTTCGCCTCTACGGACCAAACTTCATCC
TTCAGGTGTACTCATCTCAGAGGAAGTCCTGGCACCCTGTGTGCCAAGACGACTGGAACGAGAACTACGGGCGGGCGGCCTGCAGGGACATGGGCTATAAGAATAA
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GCCTAAGGATTTGACATCTCTTCTGTTTATTAATAGAGATGTTTATCTTAGCATAAAAGAGGGAAATGTGCCTTTGGCCTCACAGTCTATCCAGGGTGATATGGTTGG

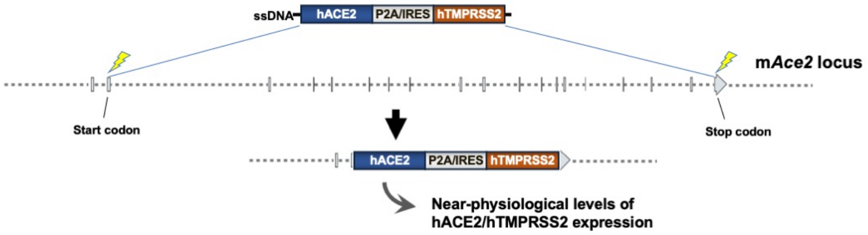
Additional note: Lower case letters in the beginning of h*ACE2* cDNA are the silent mutations introduced to prevent Cas9 re-cleavage.

Models #13, #14 and#15

13) B6.mAce2^{KO}-hACE2-IRES-hTMPRSS2^{KI}
14) NSG.mAce2^{KO}-hACE2-IRES-hTMPRSS2^{KI}
15) BALB/c.mAce2^{KO}-hACE2-IRES-hTMPRSS2^{KI}

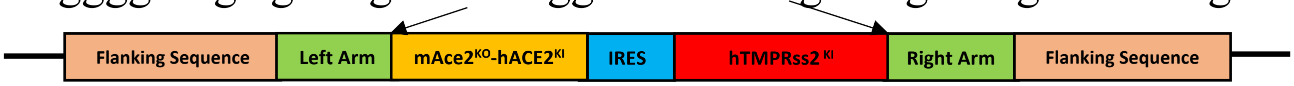
Notes:

a. The goal is to swap hACE2-IRES-hTMPRSS2 into the m*Ace2* locus between the start and stop codons in three different genetic backgrounds. All the mouse introns between its coding exons will be deleted.
b. The mouse sequence was derived from the transcript; https://uswest.ensembl.org/Mus_musculus/Transcript/Exons?db=core;g=ENSMUSG00000015405;r=X:164139332-164188420;t=ENSMUST00000112271. Exon 1 is noncoding.



Sequence near the insertion site (the cleavage location expected for the chosen guide RNA is marked as ‘/’).

cggggaaagatgtccagctcc/tcctgg:::::cctttt/agcaaagcacttgtcatcttcctg



Left gRNA Sequence- GAAAGATGTCCAGCTCCTCC [*TGG*]
Right gRNA Sequence - ATGACAAGTGCTTTGCTAAA [*AGG*]
Sequence of the **mAce2^{KO}-hACE2-IRES-hTMPRSS2^{KI}** ssDNA donor:

TATAGTAAAATCATAGTACACCCTCCTCCTCCAGTGTATCTTTAACAGCTTTTAAGGAACATATTAACTAAATGTCCAGGTTTTGATTTGGCCATAAAATGTTAGCAAAGCTAAGGTTT
TCTAGGATTAATGAATAACATGTCTTTATTTAGTTTACTTAAAAAAATCATTCTAAAATATCTGTTTACATATCTGTCCTCTCCAGGATTAACTTCATATTGGTCCAGCAGCTTGTTTAC
TGTTCTCTTCTGTTTCTTCTTCTGCTTTTTTTTCTTCTCTTCTCAGTGCCCAACCCAAGTTCAAAGGCTGATGAGAGAGAAAAACTCATGAAGAGATTTTACTCTAGGGAAAGTTGCT
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GACAAGTTTAACCACGAAGCCGAAGACCTGTTCTATCAAAGTTCACTTGCTTCTTGGAATTATAACACCAATATTACTGAAGAGAATGTCCAAAACATGAATAATGCTGGGGACAAAT
GGTCTGCCTTTTTAAAGGAACAGTCCACACTTGCCCAAATGTATCCACTACAAGAAATTCAGAATCTCACAGTCAAGCTTCAGCTGCAGGCTCTTCAGCAAAATGGGTCTTCAGTGCT
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TTGAATGAAATAATGGCAAACAGTTTAGACTACAATGAGAGGCTCTGGGCTTGGGAAAGCTGGAGATCTGAGGTCGGCAAGCAGCTGAGGCCATTATATGAAGAGTATGTGGTCTTG
AAAAATGAGATGGCAAGAGCAAATCATTATGAGGACTATGGGGATTATTGGAGAGGAGACTATGAAGTAAATGGGGTAGATGGCTATGACTACAGCCGCGGCCAGTTGATTGAAGA
TGTGGAACATACCTTTGAAGAGATTAAACCATTATATGAACATCTTCATGCCTATGTGAGGGCAAAGTTGATGAATGCCTATCCTTCCTATATCAGTCCAATTGGATGCCTCCCTGCT
CATTTGCTTGGTGATATGTGGGGTAGATTTTGGACAAATCTGTACTCTTTGACAGTTCCCTTTGGACAGAAACCAAACATAGATGTTACTGATGCAATGGTGGACCAGGCCTGGGATG
CACAGAGAATATTCAAGGAGGCCGAGAAGTTCTTTGTATCTGTTGGTCTTCCTAATATGACTCAAGGATTCTGGGAAAATTCCATGCTAACGGACCCAGGAAATGTTCAGAAAGCAGT
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GGTCTTCTGTCACCCGATTTTCAAGAAGACAATGAAACAGAAATAAACTTCCTGCTCAAACAAGCACTCACGATTGTTGGGACTCTGCCATTTACTTACATGTTAGAGAAGTGGAGGT
GGATGGTCTTTAAAGGGGAAATTCCCAAAGACCAGTGGATGAAAAAGTGGTGGGAGATGAAGCGAGAGATAGTTGGGGTGGTGGAACCTGTGCCCCATGATGAAACATACTGTGAC
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GACCCTGGGGACCTTCCTCGTGGGAGCTGCGCTGGCCGCTGGCCTACTCTGGAAGTTCATGGGCAGCAAGTGCTCCAACTCTGGGATAGAGTGCGACTCCTCAGGTACCTGCATCA
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GGGTCAACTTGAACTCAAGCCGCCAGAGCAGGATTGTGGGCGGCGAGAGCGCGCTCCCGGGGGCCTGGCCCTGGCAGGTCAGCCTGCACGTCCAGAACGTCCACGTGTGCGGAGG
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GGAGCCGGATACCAAGTAGAAAAAGTGATTTCTCATCCAAATTATGACTCCAAGACCAAGAACAATGACATTGCGCTGATGAAGCTGCAGAAGCCTCTGACTTTCAACGACCTAGTG
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TGACAGTGGAGGGCCTCTGGTCACTTCGAAGAACAATATCTGGTGGCTGATAGGGGATACAAGCTGGGGTTCTGGCTGTGCCAAAGCTTACAGACCAGGAGTGTACGGGAATGTGA
TGGTATTCACGGACTGGATTTATCGACAAATGAGGGCAGACGGCTAACAAAGCACTTGTCATCTTCCTGTATGTAAATGCTAACTTCATAGTACACAAAATATGAGAGTATACACATG
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TCTTCTGTTTATTAATAGAGATGTTTATCTTAGCATAAAAGAGGGAAATGTGCCTTTGGCCTCACAGTCTATCCAGGGTGATATGGTTGG

Note: Lower case letters at the beginning and the end of h*ACE2* cDNA are silent mutations introduced to prevent Cas9 re-cleavage.

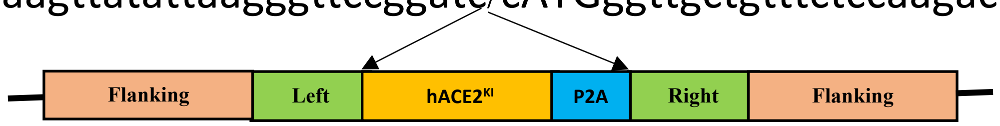
Model #16: *ROSA26*$^{(ACTB\text{-}Lox\text{-}hACE2\text{-}P2A\text{-}tdT\text{-}Lox\text{-}EGFP)}$

Notes:

a. The purpose is to create a constitutive expression model for hACE2 with reporter capability.
b. The design strategy is to insert a 'hACE2-P2A' cassette upstream of the tdTomato sequence by re-engineering the mTmG locus of mouse strain; https://www.jax.org/strain/007576) Muzumdar et al. 2007, A global double-fluorescent Cre reporter mouse Genesis;45(9):593-605. doi: 10.1002/dvg.20335). The source of the mTmG cassette sequence is from the Addgene plasmid 17787 (https://www.addgene.org/17787/sequences/).

Sequence near the start codon of the mT region (ATG shown in uppercase) and the cleavage location of the chosen guide RNA is marked as '/'.

tatacgaagttatattaagggttccggatc/cATGggttgctgtttctccaagaccatg



gRNA sequence: ATTAAGGGTTCCGGATCCAT [*GGG*]. It cleaves 1bp upstream of the ATG.

Sequence of the ROSA26$^{(ACTB\text{-}Lox\text{-}hACE2\text{-}P2A\text{-}tdT\text{-}Lox\text{-}EGFP)KI}$ ssDNA:

```
GGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGC
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GCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTT
TTCCTACAGCTCCTGGGCAACGTGCTGGTTATTGTGCTGTCTCATCATTTTGGCAAAGAATTGATTTGATACCGCGGGCTCGACACTAGTGAACCTCTTC
GAGGGACCTAATAACTTCGTATAGCATACATTATACGAAGTTATATTAAGGGTTCCGGATCCATGTCAAGCTCTTCCTGGCTCCTTCTCAGCCTTGTTGC
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AGGTTTGAATGAAATAATGGCAAACAGTTTAGACTACAATGAGAGGCTCTGGGCTTGGGAAAGCTGGAGATCTGAGGTCGGCAAGCAGCTGAGGCCAT
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AGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGCCTGTACCCCCGCGACGGCG
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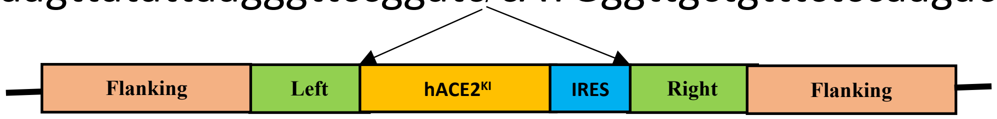
Model #17: *ROSA26*$^{\textit{(ACTB-Lox-hACE2-IRES-tdT-Lox-EGFP)}}$

Notes: Same as model #16 except that IRES is used instead of P2A.

Sequence near the start codon of the mT region (ATG shown in uppercase) and the cleavage location of the chosen guide RNA is marked as '/'.

tatacgaagttatattaagggttccggatc/cATGggttgctgtttctccaagaccatg



gRNA sequence: ATTAAGGGTTCCGGATCCAT [*GGG*]. It cleaves 1bp upstream of the ATG.

Sequence of the **ROSA26**$^{\textbf{(ACTB-Lox-hACE2-IRES--tdT-Lox-EGFP)KI}}$ ssDNA:

GGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCG
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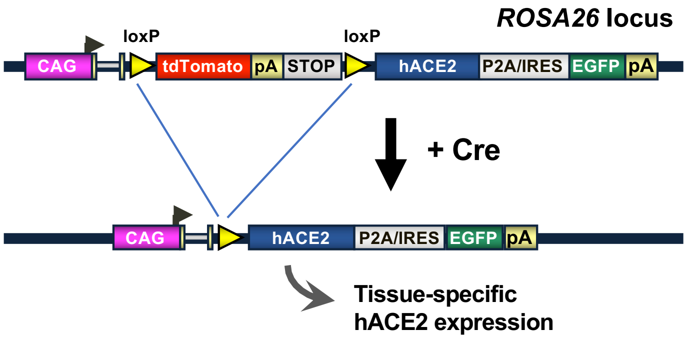
TTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCT
GGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGC
CAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCA
AATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCG
GTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAA
CACGATGATAAGGTTGCTGTTTCTCCAAGACCATGGTGAGCAAGGGAGAGGAGGTCATCAAAGAGTTCATGCGCTTCAAGGTGCG
CATGGAGGGCTCCATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAA
GCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCCAGTTCATGTACGGCTCCAAGGCGTACGTG
AAGCACCCCGCCGACATCCCCGATTACAAGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACG
GCGGTCTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCACGCTGATCTACAAGGTGAAGATGCGCGGCACCAACTTCCC
CCCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCACCGAGCGCCTGTACCCCCGCGACGGCG

Model #18: *ROSA26*$^{(ACTB\text{-}Lox\text{-}tdT\text{-}Lox\text{-}hACE2\text{-}P2A\text{-}EGFP)}$

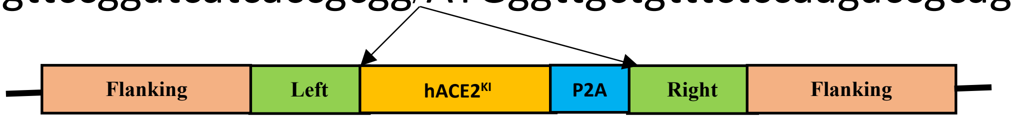
Notes:

a. The purpose is to create a CRE-activatable expression model for hACE2 with reporter capability.
b. The design strategy is to insert a 'hACE2-P2A' cassette upstream of the EGFP sequence by re-engineering the mTmG locus of the mouse strain; https://www.jax.org/strain/007576 (Muzumdar et al. 2007, A global double-fluorescent Cre reporter mouse Genesis;45(9):593-605. doi: 10.1002/dvg.20335). The source of the mTmG cassette sequence was from the Addgene plasmid 17787 (https://www.addgene.org/17787/sequences/).



Sequence near the start codon of the mG region (ATG shown in uppercase) and the cleavage location of the chosen guide RNA is marked as '/'.

ttaagggttccggatcatcaccgcgg/ATGggttgctgtttctccaagaccgcagcg



gRNA sequence: GAGAAACAGCAACCCATCCG [*CGG*]. It cleaves immediately upstream of the ATG sequence of the ROSA26$^{(ACTB\text{-}Lox\text{-}tdT\text{-}Lox\text{-}hACE2\text{-}P2A\text{-}EGFP)KI}$ ssDNA donor.

```
GGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCTGACATGGTAAGTAAGCTTGGGCTGCAGGTCGAGGGACCTAATAACTTCGTATAGCATACATTATA
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CAAATCTGTACTCTTTGACAGTTCCCTTTGGACAGAAACCAAACATAGATGTTACTGATGCAATGGTGGACCAGGCCTGGGATGCACAGAGAATATTCAAGGAGGCC
GAGAAGTTCTTTGTATCTGTTGGTCTTCCTAATATGACTCAAGGATTCTGGGAAAATTCCATGCTAACGGACCCAGGAAATGTTCAGAAAGCAGTCTGCCATCCCACA
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TGGAACGACAATGAAATGTACCTGTTCCGATCATCTGTTGCATATGCTATGAGGCAGTACTTTTTAAAAGTAAAAAATCAGATGATTCTTTTTGGGGAGGAGGATGTG
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CTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTATGGGTTGCTGTTTCTCCAAGACCGCAGCGAAGGGAGAAGCCGCCGCGGAGAGGCCTGGGGAGGCG
GCTGTGGCCTCGTCGCCTTCCAAAGCGAACGGACAGGAGAATGGCCACGTGAAGGTAGGATCCGTGAGCAAGGGCGAG
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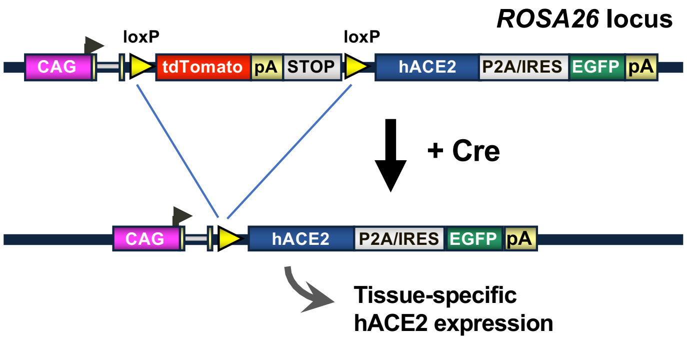
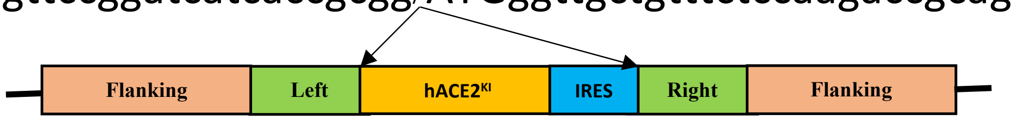
Model #19: ***ROSA26***$^{\mathbf{\mathit{(ACTB\text{-}Lox\text{-}tdT\text{-}Lox\text{-}hACE2\text{-}IRES\text{-}EGFP)}}}$

Notes: Same as model #18 except that IRES is used instead of P2A.



Sequence near the start codon of the mG region (ATG shown in uppercase) and the cleavage location of the chosen guide RNA is marked as '/'.

ttaagggttccggatcatcaccgcgg/ATGggttgctgtttctccaagaccgcagcg



gRNA sequence: GAGAAACAGCAACCCATCCG [*CGG*]. It cleaves immediately upstream of the ATG

Sequence of the **ROSA26**$^{\mathbf{(ACTB\text{-}Lox\text{-}tdT\text{-}Lox\text{-}hACE2\text{-}IRES\text{-}EGFP)KI}}$ ssDNA:

GGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCTGACATGGTAAGTAAGCTTGGGCTGCAGGTCGAGGGACCTAATA
ACTTCGTATAGCATACATTATACGAAGTTATATTAAGGGTTCCGGATCATCACCGCGGATGTCAAGCTCTTCCTGGCTCCTTCTCAG
CCTTGTTGCTGTAACTGCTGCTCAGTCCACCATTGAGGAACAGGCCAAGACATTTTTGGACAAGTTTAACCACGAAGCCGAAGACC
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TGCAGGCTCTTCAGCAAAATGGGTCTTCAGTGCTCTCAGAAGACAAGAGCAAACGGTTGAACACAATTCTAAATACAATGAGCACC
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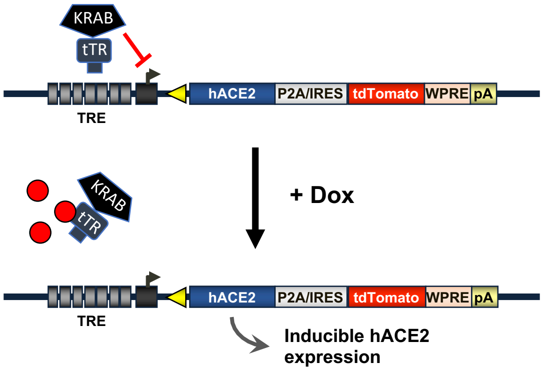
AAGGGCGAG

Model #20: *Ai63-TIGRE-TRE-hACE2-P2A-tdT*

Notes:

a. The purpose is to create a tetracycline inducible model for hACE2 expression with reporter capability.
b. The design strategy is to insert an 'hACE2-P2A' cassette upstream of the tdT sequence by re-engineering the TIGRE locus described in Daigle, T. L. et al., Cell 174, 465-480.e22 (2018).
c. The Ai63 mouse contains a TRE promoter followed by Lox-Stop-Lox cassette and tdT. The Lox-Stop-Lox cassette (1190kb region) will be excised using two guide RNAs to insert the hACE2-P2A cassette. The sequence source (for guide search) was from the Addgene plasmid 61576 https://www.addgene.org/61576/. The sequences used for guide search are as follows:



Sequence near the insertion site (the cleavage location expected for the chosen guide RNA is marked as '/')

ctatt/taaatataacttcgtatagcata::::::::gcgtgctagcgccaccA/TGgtgagc

Left gRNA Sequence- ATACGAAGTTATATTTAAAT [*AGG*]
Right gRNA Sequence - CTCGCCCTTGCTCACCATGG [*TGG*]
Sequence of the Ai63-TIGRE-TRE-hACE2-P2A-tdT ssDNA:

**GTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGTTTACTCCCTATCAGTGATAGAGAACG
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CTTTGTATCTGTTGGTCTTCCTAATATGACTCAAGGATTCTGGGAAAATTCCATGCTAACGGACCCAGGAAATGTTCAGAAAGCAGTCTGCCATCCCACA
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ACACCTAAGCATTTAAAATCCATTGGTCTTCTGTCACCCGATTTTCAAGAAGACAATGAAACAGAAATAAACTTCCTGCTCAAACAAGCACTCACGATTG
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TGAAGCGAGAGATAGTTGGGGTGGTGGAACCTGTGCCCCATGATGAAACATACTGTGACCCCGCATCTCTGTTCCATGTTTCTAATGATTACTCATTCAT
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CGCATGGAGGGCTCCATGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGA
CCAAGGGCGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCCCAGTTCATGTACGGCTCCAAGGCGTACGTGAAGCACCCCGCCGACATCCCCGATT
ACAAGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGTCTGGTGACCGTGACCCAGGACTCCTCCCTGCAG
GACGGC**

Model #21: ***Ai63-TIGRE-TRE-hACE2-IRES-tdT***

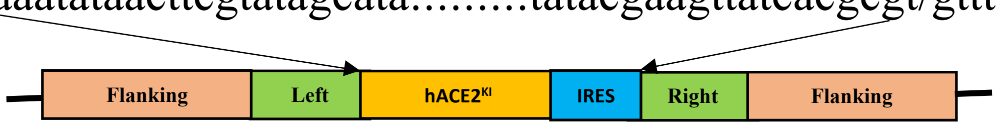
Notes:

a. The purpose is to create a tetracycline inducible model for hACE2 expression with reporter capability.
b. The design strategy is to insert an 'hACE2-IRES' cassette upstream of the tdT sequence by re-engineering the TIGRE locus described in Daigle, T. L. et al., Cell 174, 465-480.e22 (2018).

The Ai63 mouse contains a TRE promoter followed by Lox-Stop-Lox cassette and tdT. The Lox-Stop-Lox cassette (1190kb region) will be excised using two guide RNAs to insert the hACE2-P2A cassette. The sequence source (for guide search) was from the Addgene plasmid 61576 https://www.addgene.org/61576/. The sequences used for guide search are as follows：Same as model #20 except that IRES is used instead of P2A

Sequence near the insertion site (the cleavage location expected for the chosen guide RNA is marked as '/').

ctatt/taaatataacttcgtatagcata::::::::tatacgaagttatcacgcgt/gtttgggac



Left gRNA Sequence- ATACGAAGTTATATTTAAAT [*AGG*]
Right gRNA Sequence - ACGAAGTTATCACGCGTGTT [*TGG*]
Sequence of the Ai63-TIGRE-TRE-hACE2-IRES-tdT ssDNA:

**GTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGTTTACTCCCTATCA
GTGATAGAGAACGTATGTCGAGTTTATCCCTATCAGTGATAGAGAACGTATGTCGAGTTTACTCCCTATCAGTGATAGAGAACGTA
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CTCTTCCTGGCTCCTTCTCAGCCTTGTTGCTGTAACTGCTGCTCAGTCCACCATTGAGGAACAGGCCAAGACATTTTTGGACAAGTT
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CATGAATAATGCTGGGGACAAATGGTCTGCCTTTTTAAAGGAACAGTCCACACTTGCCCAAATGTATCCACTACAAGAAATTCAGA
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TCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATA
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AGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTT
GCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACA
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GCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACG
TCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAAGTTTGGGACGCGTGCTAGCGCCACCATGGT
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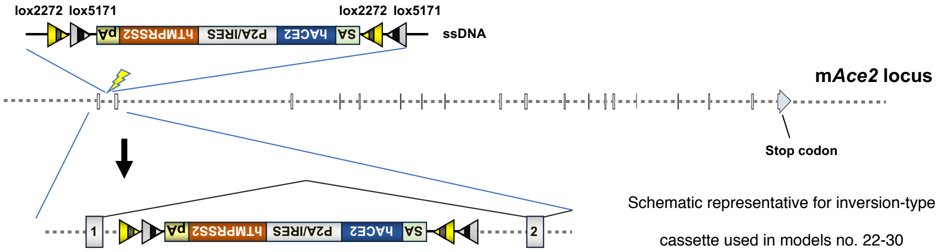
GAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGCGGCCCCCTGCCCTTCGCC
TGGGACATCCTGTCCCCCCAGTTCATGTACGGCTCCAAGGCGTACGTGAAGCACCCCGCCGACATCCCCGATTACAAGAAGCTGTC
CTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGTCTGGTGACCGTGACCCAGGACTCCTCCCTGCAG
GACGGC**

Models #22, #23 and #24

22) B6.mAce2^{cKO}-hACE2^{cKI}
23) NSG.mAce2^{cKO}-hACE2^{cKI}
24) BALB/c.mAce2^{cKO}-hACE2^{cKI}

Notes:

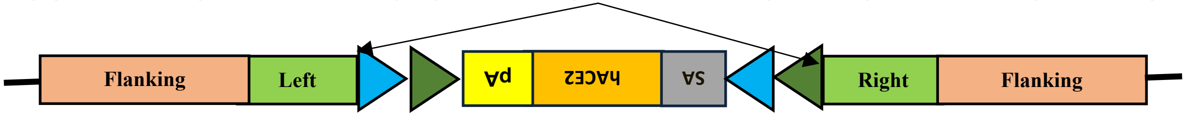
a. The purpose is to create a conditional knock-in (cKI) model for hACE2.
b. The cKI cassette is a modified version of the XTR strategy described in Camila et al., 2015 (Nat Commun. 5;6:8783.doi: 10.1038/ncomms9783). The source of the XTR cassette was from the Addgene plasmid https://www.addgene.org/browse/article/16271/
c. Exon 1 of m*Ace2* is a non-coding exon. The cKI cassette is inserted at 310 bp upstream of the beginning of exon 2 (the first coding exon). The sequence used for gRNA search was from https://uswest.ensembl.org/Mus_musculus/Transcript/Exons?db=core;g=ENSMUSG00000015405;r=X:164139332-164188420;t=ENSMUST00000112271.



Schematic representative for inversion-type cassette used in models no. 22-30

Sequence near the insertion site (the cleavage location expected for the chosen guide RNA is marked as '/').

aggctataagttccaatatgtgtcccatg/atttctaagtctagccctttctgttatagta



The gRNA sequence (in the intron 1) is GGGCTAGACTTAGAAATCAT [*GGG*]

Sequence of the mAce2^{cKO}-hACE2^{cKI} ssDNA:

AGCTCTAGCTGTCTTTGATTGGTAGACAAAGCTGTCCTCAAACTCAGAGATCTTCCTTCCTTTGTCTCCTGAGTGCTGGGATTAAAGGCATGGACCACCACTGCCCTG
CCCCATTCTCTCCATTAATTTTAAGTGAATGCTTGCAAAAGCTCACTTCTTTGGTGAACAGCTTCCTTTACAAATAAGTACCTTTGCCTTCGTTTTTATAGGATTCTTAA
AAAGAAAAAAAAAGATTCAGCCAGGTGGTTGTGGTGCACACCTTTAATCCCAGCAGTCAGGAGGCAGAGGAAAGCAGATCTCTTGAGTTTGAGGCTAGCCTAGTCTAC
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TTGGAATCCTGGATTATTTTCTCCTTTGCTAATATCGATGGAGGCATAAGGATTTTCTCCACTTCTTGCTTTATTTTTCTTCTTCCGATCTCTGATCCCAGTGAAGATCA
GGATGACAATGCCAACCACTATCACTCCCATCACAACTCCAAAAACAATCAGCCATATGGAAACAGGGGGCTGGTTAGGAGGTCCAAGTGTTGGCTGTATCCCCAGA
AACTCTAGGCTGTTGTCATTCAGACGGAAAGCATCATTGATACGGCTCCGGGACATCCTGATGGCCTTTTCAACTTCAGTTCTAGGAATGATATCAGACACATTTTTA
GGTGCAGTGACAAAGAAATTAAAGGAGATTCTTGGTTTCAAATTAGCCACTCGCACATCCTCCTCCCCAAAAAGAATCATCTGATTTTTTACTTTTAAAAAGTACTGCC
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GAAACATGGAACAGAGATGCGGGGTCACAGTATGTTTCATCATGGGGCACAGGTTCCACCACCCCAACTATCTCTCGCTTCATCTCCCACCACTTTTTCATCCACTGG
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TTGTGCACATAAGGATCCTGAAGTCGCCCTTCCCCAGGTCCCAAGCTGTGGGATGGCAGACTGCTTTCTGAACATTTCCTGGGTCCGTTAGCATGGAATTTTCCCAGA
ATCCTTGAGTCATATTAGGAAGACCAACAGATACAAAGAACTTCTCGGCCTCCTTGAATATTCTCTGTGCATCCCAGGCCTGGTCCACCATTGCATCAGTAACATCTA
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GGTTCAAGTAATAAGCATTCTTGTGGATTATCTGGGTTACAAACTTTTCCAGTACTGTAGATGGTGCTCATTGTATTTAGAATTGTGTTCAACCGTTTGCTCTTGTCTT
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TCTAGCCCTTTCTGTTATAGTAAAATCATAGTACACCCTCCTCCTCCAGTGTATCTTTAACAGCTTTTAAGGAACATATTAACTAAATGTCCAGGTTTTGATTTGGCCA
TAAAATGTTAGCAAAGCTAAGGTTTTCTAGGATTAATGAATAACATGTCTTTATTTAGTTTACTTAAAAAAATCATTCTAAAATATCTGTTACATATCTGTCCTCTCCA
GGATTAACTTCATATTGGTCCAGCAGCTTGTTTACTGTTCTCTTCTGTTTCTTCTTCTGCTTTTTTTTTCTTCTCTTCTCAGTGCCCAACCCAAGTTCAAAGGCTGATGA
GAGAGAAAAACTCATGAAGAGATTT

Models #25, #26 and #27

25) B6.mAce2^{cKO}-hACE2-P2A-hTMPRSS2^{cKI}

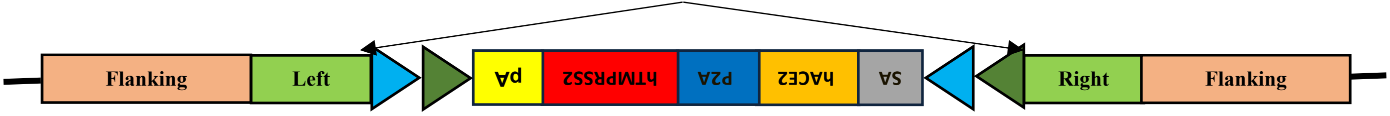
26) NSG.mAce2^{cKO}-hACE2-P2A-hTMPRSS2^{cKI}

27) BALB/c.mAce2^{cKO}-hACE2-P2A-hTMPRSS2^{cKI}

Notes: These are the same as models #22-#24 except that both hACE2 and hTMPRSS2 will be expressed instead of just hACE2.

Sequence near the insertion site (the cleavage location expected for the chosen guide RNA is marked as '/').

aggctataagttccaatatgtgtcccatg/atttctaagtctagccctttctgttatagta



The gRNA sequence (in the intron 1) is GGGCTAGACTTAGAAATCAT [*GGG*].

Sequence of the mAce2^{cKO}-hACE2-P2A-hTMPRSS2^{cKI} ssDNA:

AGCTCTAGCTGTCTTTGATTGGTAGACAAAGCTGTCCTCAAACTCAGAGATCTTCCTTCCTTTGTCTCCTGAGTGCTGGGATTAAAGGCATGGACCACCA
CTGCCCTGCCCCATTCTCTCCATTAATTTTAAGTGAATGCTTGCAAAAGCTCACTTCTTTGGTGAACAGCTTCCTTTACAAATAAGTACCTTTGCCTTCGT
TTTTATAGGATTCTTAAAAAGAAAAAAAAGATTCAGCCAGGTGGTTGTGGTGCACACCTTTAATCCCAGCAGTCAGGAGGCAGAGGAAAGCAGATCTCT
TGAGTTTGAGGCTAGCCTAGTCTACAGAGGGAGTTCCAGGACAGCCAAGGCTACAGAGAGGAACTGTCTAAAAACACCAAGAAAGAGAGAAAGGAGAG
AGGGAGAGGATGGATAGCTTATTGATAGAATTGTCAGAAAAGGCTATAAGTTCCAATATGTGTCCCATGATAACTTCGTATAGGATACCTTATACGAAGT
TATCTCAGCTAGAAGCC**ATAACTTCGTATAGTACACATTATACGAAGTTAT**GTTTATCGAATTCCTGCAGCCCGGTAGATCTTTACAATTTACGCGT**TAAGATA**
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GCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTA
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CCTGGTCTGTAAGCTTTGGCACAGCCAGAACCCCAGCTTGTATCCCCTATCAGCCACCAGATATTGTTCTTCGAAGTGACCAGAGGCCCTCCACTGTCA
CCCTGGCAAGAATCGACGTTCCCCTGCAGGAAGCCGGCACAGATCATGGCTGGTGTGATCAGGTTGTCATAGACATATCTGCTGTTGCATCTCTGTGTC
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GTCTTTGGGAATTTCCCCTTTAAAGACCATCCACCTCCACTTCTCTAACATGTAAGTAAATGGCAGAGTCCCAACAATCGTGAGTGCTTGTTTGAGCAGG
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CCCACATATCACCAAGCAAATGAGCAGGGAGGCATCCAATTGGACTGATATAGGAAGGATAGGCATTCATCAACTTTGCCCTCACATAGGCATGAAGAT
GTTCATATAATGGTTTAATCTCTTCAAAGGTATGTTCCACATCTTCAATCAACTGGCCGCGGCTGTAGTCATAGCCATCTACCCCATTTACTTCATAGTCT
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GCCCGG**ATAACTTCGTATAAGGTATCCTATACGAAGTTAT**CCATTCAGGCTGTGCTAGCATCAATGGCATGGCACAAAGCTTAGCC**ATAACTTCGTATAATGT**
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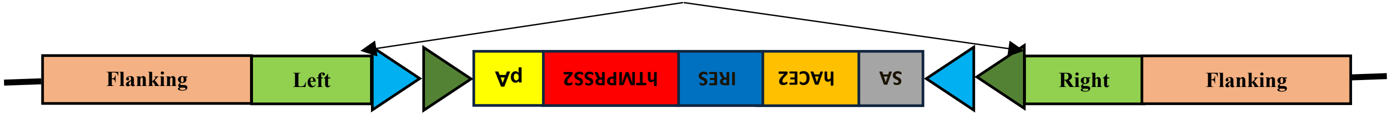
Models #28, #29 and #30

28) B6.mAce2^{cKO}-hACE2-IRES-hTMPRSS2^{cKI}
29) NSG.mAce2^{cKO}-hACE2-IRES-hTMPRSS2^{cKI}
30) BALB/c.mAce2^{cKO}-hACE2-IRES-hTMPRSS2^{cKI}

Notes: These are the same as models #25 to #27 except that IRES is used instead of P2A.

Sequence near the insertion site (the cleavage location expected for the chosen guide RNA is marked as '/').

aggctataagttccaatatgtgtcccatg/atttctaagtctagccctttctgttatagta



The gRNA sequence (in intron 1) is GGGCTAGACTTAGAAATCAT [*GGG*].
Sequence of the mAce2^{cKO}-hACE2-IRES-hTMPRSS2^{cKI} ssDNA:

AGCTCTAGCTGTCTTTGATTGGTAGACAAAGCTGTCCTCAAACTCAGAGATCTTCCTTCCTTTGTCTCCTGAGTGCTGGGATTAAAGGCATGGACCACCA
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