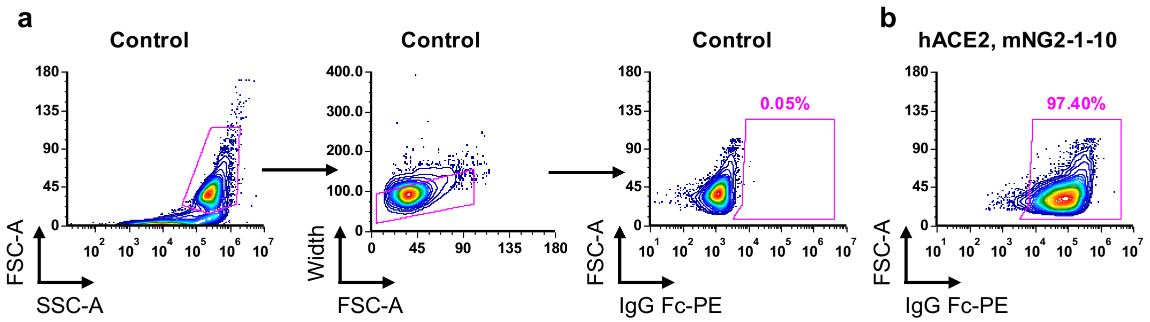
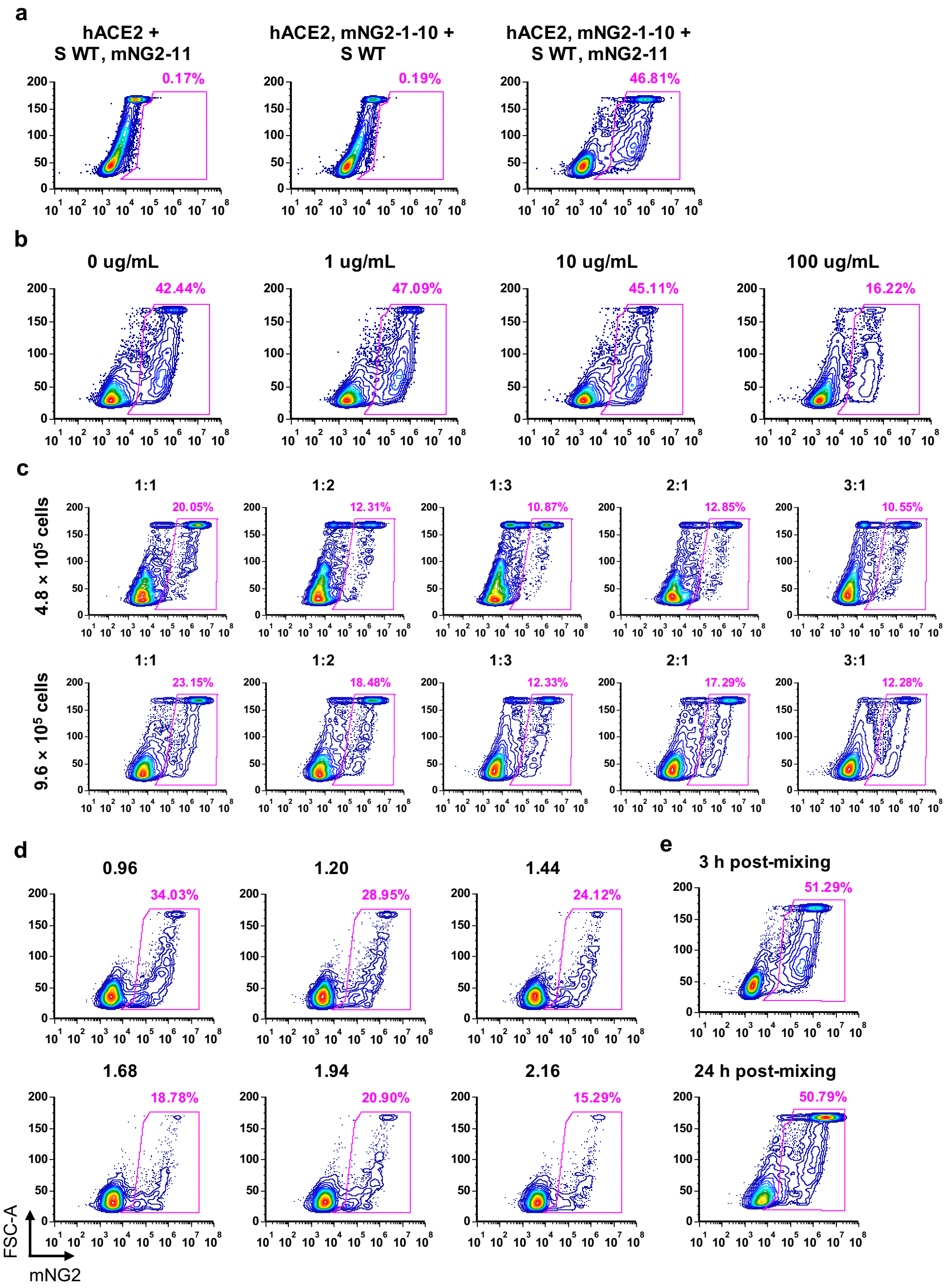
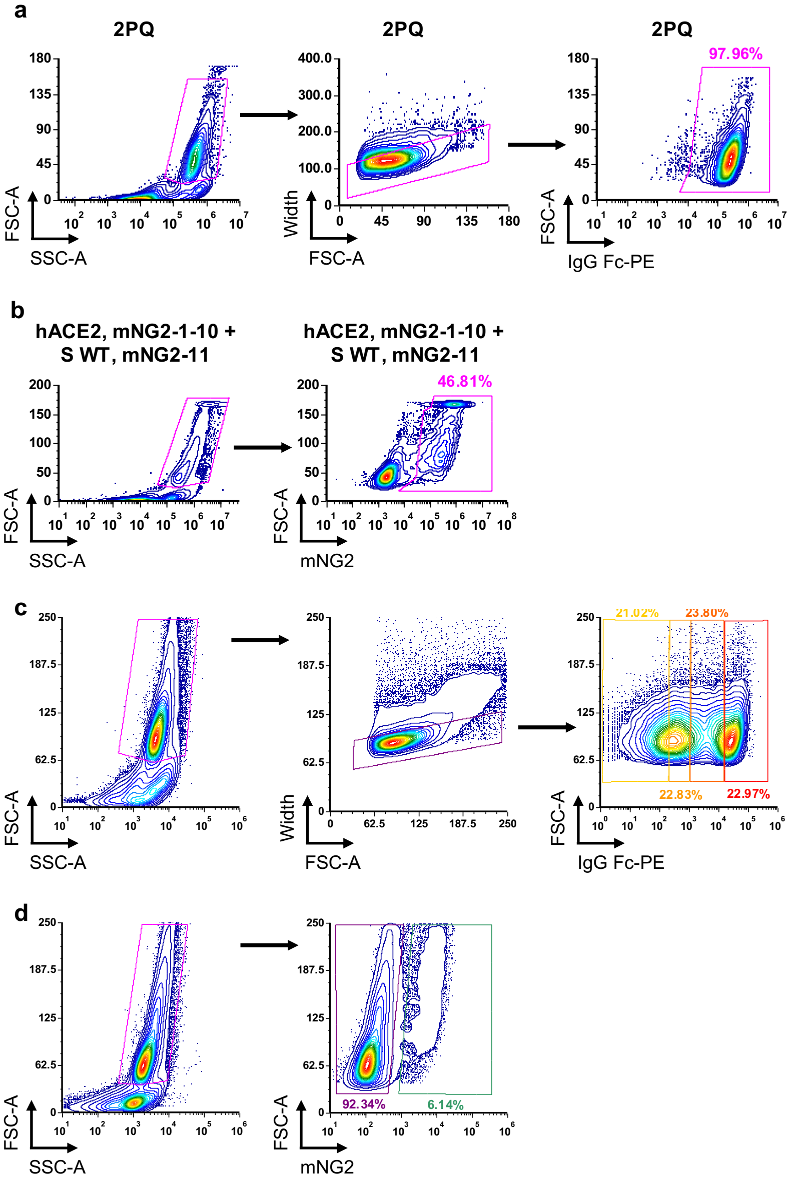


Supplementary Fig. 1 | Validation of hACE2, mNG2$_{1-10}$-expressing cell line. a,b, Flow cytometry plots showing (**a**) untransfected control and gating strategy in this verification experiment, and (**b**) surface expression of hACE2 in HEK293T landing pad cells.



Supplementary Fig. 2 | Validation and optimization of fusion assay. a, Flow cytometry plots of fusion assay between hACE2- and S-expressing cells showing detectable green fluorescence when both mNG2$_{1-10}$ and mNG2$_{11}$ were expressed, and negligible fluorescent background signal when either mNG2$_{1-10}$ or mNG2$_{11}$ was expressed. **b,** Addition of CC40.8, a neutralizing antibody targeting the stem helix, to S, mNG2$_{11}$-expressing cells one hour prior to mixing with hACE2,

$mNG2_{1\text{-}10}$-expressing cells decreased fusion events. Antibody concentration is indicated above each plot. **c,** Optimization of the ratio between hACE2, $mNG2_{1\text{-}10}$- and S, $mNG2_{11}$-expressing cells for fusion assay. Total cell number in co-culture is indicated, and the ratio of hACE2, $mNG2_{1\text{-}10}$- to S, $mNG2_{11}$-expressing cells is shown above each plot. **d,** Optimization of total cell numbers of hACE2, $mNG2_{1\text{-}10}$- and S, $mNG2_{11}$-expressing cells for fusion assay. Total cell number, in millions, of hACE2, $mNG2_{1\text{-}10}$- and S, $mNG2_{11}$-expressing cells in co-culture is shown above each plot. Equal numbers of hACE2, $mNG2_{1\text{-}10}$- and S, $mNG2_{11}$-expressing cells were co-cultured for 3 hours. **e,** Optimization of time point for fusion sorting. Equal numbers of hACE2, $mNG2_{1\text{-}10}$- and S, $mNG2_{11}$-expressing cells totaling 5.0×10^5 cells/mL were mixed and analyzed via flow cytometry at the indicated time point.



Supplementary Fig. 3 | Gating strategies for flow cytometry and fluorescence activated cell sorting (FACS). a, Gating strategy used for flow cytometry to assess surface expression of S via PE fluorescence. **b,** Gating strategy used for flow cytometry to determine fusion of hACE2- and S-expressing cells via mNG2 fluorescence. **c,** Gating strategy used for FACS to sort the DMS library of S-expressing cells based on levels of PE fluorescence. **d,** Gating strategy used for FACS to sort the co-culture of the DMS library of S-expressing cells and hACE2-expressing cells at 3 hours post-mixing, based on presence or absence of mNG2 fluorescence.

Supplementary Table 2. Primers used for PCR-based site directed mutagenesis (QuikChange) to generate mutations. The plasmid backbone used was attB-S-mNG2-11.

Primer	**Sequence (5′ to 3′)**
T961F-F	GCACTGAACTTCCTGGTCAAGCAGCTGTCC
T961F-R	CAGCTGCTTGACCAGGAAGTTCAGTGCCTGGGC
K986P-F	CTGAGCAGACTGGACCCGGTGGAAGCCGAGGTGCAG
K986P-R	CTGCACCTCGGCTTCCACCGGGTCCAGTCTGCTCAG
V987P-F	CTGAGCAGACTGGACAAGCCGGAAGCCGAGGTGCAG
V987P-R	CTGCACCTCGGCTTCCGGCTTGTCCAGTCTGCTCAG
D994E-F	CGAGGTGCAGATCGAGAGACTGATCACCGGAAG
D994E-R	CTTCCGGTGATCAGTCTCTCGATCTGCACCTCGGCT
D994Q-F	GCCGAGGTGCAGATCCAGAGACTGATCACCGGAAG
D994Q-R	CTTCCGGTGATCAGTCTCTGGATCTGCACCTCGGCT
Q1005R-F	GGCTGCAGTCCCTGCGGACCTACGTTACCCAG
Q1005R-R	CTGGGTAACGTAGGTCCGCAGGGACTGCAGCC
K986P/V987P-F	CTGAGCAGACTGGACCCGCCGGAAGCCGAGGTGCAG
K986P/V987P-R	CTGCACCTCGGCTTCCGGCGGGTCCAGTCTGCTCAG

Supplementary Table 3. Cassette primers to generate the DMS library.

Primer	**Sequence (5′ to 3′)**
Cassette1_1	GCCCTGCTGGCCGGCACAATCNNKAGTGGTTGGACATTTGGAGCTGGCGCCGCTCTGCAG
Cassette1_2	GCCCTGCTGGCCGGCACAATCACCNNKGGCTGGACCTTTGGAGCTGGCGCCGCTCTGCAG
Cassette1_3	GCCCTGCTGGCCGGCACAATCACCAGCNNKTGGACATTCGGAGCTGGCGCCGCTCTGCAG
Cassette1_4	GCCCTGCTGGCCGGCACAATCACAAGCGGTNNKACCTTTGGAGCTGGCGCCGCTCTGCAG
Cassette1_5	GCCCTGCTGGCCGGCACAATCACAAGTGGCTGGNNKTTCGGAGCTGGCGCCGCTCTGCAG
Cassette1_6	GCCCTGCTGGCCGGCACAATCACCAGCGGTTGGACCNNKGGTGCTGGCGCCGCTCTGCAG
Cassette1_7	GCCCTGCTGGCCGGCACAATCACCAGCGGTTGGACATTTNNKGCAGGCGCCGCTCTGCAG
Cassette1_8	GCCCTGCTGGCCGGCACAATCACCAGTGGTTGGACCTTCGGANNKGGCGCCGCTCTGCAG
Cassette2_1	AGCGGCTGGACATTTGGAGCTNNKGCAGCACTGCAGATCCCCTTTGCTATGCAGATGGCC
Cassette2_2	AGCGGCTGGACATTTGGAGCTGGTNNKGCTCTCCAGATCCCCTTTGCTATGCAGATGGCC
Cassette2_3	AGCGGCTGGACATTTGGAGCTGGTGCCNNKCTGCAAATCCCCTTTGCTATGCAGATGGCC
Cassette2_4	AGCGGCTGGACATTTGGAGCTGGCGCAGCTNNKCAAATCCCCTTTGCTATGCAGATGGCC
Cassette2_5	AGCGGCTGGACATTTGGAGCTGGCGCCGCACTCNNKATCCCCTTTGCTATGCAGATGGCC
Cassette2_6	AGCGGCTGGACATTTGGAGCTGGTGCAGCACTCCAANNKCCCTTTGCTATGCAGATGGCC
Cassette2_7	AGCGGCTGGACATTTGGAGCTGGTGCAGCTCTGCAAATANNKTTTGCTATGCAGATGGCC
Cassette2_8	AGCGGCTGGACATTTGGAGCTGGTGCCGCACTCCAGATACCCNNKGCTATGCAGATGGCC
Cassette3_1	GCCGCTCTGCAGATCCCCTTTNNKATGCAAATGGCATACCGGTTCAACGGCATCGGAGTG
Cassette3_2	GCCGCTCTGCAGATCCCCTTTGCANNKCAAATGGCCTATCGGTTCAACGGCATCGGAGTG
Cassette3_3	GCCGCTCTGCAGATCCCCTTTGCAATGNNKATGGCATATCGATTCAACGGCATCGGAGTG
Cassette3_4	GCCGCTCTGCAGATCCCCTTTGCTATGCAGNNKGCATATCGGTTCAACGGCATCGGAGTG
Cassette3_5	GCCGCTCTGCAGATCCCCTTTGCAATGCAAATGNNKTACCGATTTAACGGCATCGGAGTG
Cassette3_6	GCCGCTCTGCAGATCCCCTTTGCTATGCAAATGGCCNNKCGATTCAACGGCATCGGAGTG
Cassette3_7	GCCGCTCTGCAGATCCCCTTTGCAATGCAGATGGCCTATNNKTTTAACGGCATCGGAGTG

Cassette3_8	GCCGCTCTGCAGATCCCCTTTGCTATGCAGATGGCATACCGANNKAACGGCATCGGAGTG
Cassette4_1	ATGCAGATGGCCTACCGGTTCNNKGGTATAGGAGTGACCCAGAATGTGCTGTACGAGAAC
Cassette4_2	ATGCAGATGGCCTACCGGTTCAATNNKATCGGTGTGACCCAGAATGTGCTGTACGAGAAC
Cassette4_3	ATGCAGATGGCCTACCGGTTCAACGGTNNKGGTGTAACCCAGAATGTGCTGTACGAGAAC
Cassette4_4	ATGCAGATGGCCTACCGGTTCAATGGCATANNKGTAACCCAGAATGTGCTGTACGAGAAC
Cassette4_5	ATGCAGATGGCCTACCGGTTCAACGGTATCGGANNKACACAGAATGTGCTGTACGAGAAC
Cassette4_6	ATGCAGATGGCCTACCGGTTCAACGGCATAGGTGTGNNKCAGAATGTGCTGTACGAGAAC
Cassette4_7	ATGCAGATGGCCTACCGGTTCAATGGTATCGGAGTAACCNNKAATGTGCTGTACGAGAAC
Cassette4_8	ATGCAGATGGCCTACCGGTTCAACGGCATCGGTGTAACACAGNNKGTGCTGTACGAGAAC
Cassette5_1	GGCATCGGAGTGACCCAGAATNNKCTCTATGAGAACCAGAAGCTGATCGCCAACCAGTTC
Cassette5_2	GGCATCGGAGTGACCCAGAATGTANNKTACGAAAACCAGAAGCTGATCGCCAACCAGTTC
Cassette5_3	GGCATCGGAGTGACCCAGAATGTACTGNNKGAGAATCAGAAGCTGATCGCCAACCAGTTC
Cassette5_4	GGCATCGGAGTGACCCAGAATGTGCTCTACNNKAATCAGAAGCTGATCGCCAACCAGTTC
Cassette5_5	GGCATCGGAGTGACCCAGAATGTGCTGTATGAANNKCAGAAGCTGATCGCCAACCAGTTC
Cassette5_6	GGCATCGGAGTGACCCAGAATGTACTCTATGAAAATNNKAAGCTGATCGCCAACCAGTTC
Cassette5_7	GGCATCGGAGTGACCCAGAATGTACTCTACGAGAACCAANNKCTGATCGCCAACCAGTTC
Cassette5_8	GGCATCGGAGTGACCCAGAATGTACTGTATGAGAACCAAAAGNNKATCGCCAACCAGTTC
Cassette6_1	CTGTACGAGAACCAGAAGCTGNNKGCAAATCAGTTCAACAGCGCCATCGGCAAGATCCAG
Cassette6_2	CTGTACGAGAACCAGAAGCTGATANNKAACCAATTCAACAGCGCCATCGGCAAGATCCAG
Cassette6_3	CTGTACGAGAACCAGAAGCTGATCGCANNKCAATTTAACAGCGCCATCGGCAAGATCCAG
Cassette6_4	CTGTACGAGAACCAGAAGCTGATAGCCAATNNKTTTAACAGCGCCATCGGCAAGATCCAG
Cassette6_5	CTGTACGAGAACCAGAAGCTGATCGCAAACCAGNNKAATAGCGCCATCGGCAAGATCCAG
Cassette6_6	CTGTACGAGAACCAGAAGCTGATCGCCAATCAATTCNNKAGCGCCATCGGCAAGATCCAG
Cassette6_7	CTGTACGAGAACCAGAAGCTGATAGCAAACCAGTTTAACNNKGCCATCGGCAAGATCCAG
Cassette6_8	CTGTACGAGAACCAGAAGCTGATCGCCAACCAATTTAATAGCNNKATCGGCAAGATCCAG

Cassette7_1	GCCAACCAGTTCAACAGCGCCNNKGGTAAAATCCAGGACAGCCTGAGCAGCACAGCAAGC
Cassette7_2	GCCAACCAGTTCAACAGCGCCATANNKAAGATACAGGACAGCCTGAGCAGCACAGCAAGC
Cassette7_3	GCCAACCAGTTCAACAGCGCCATCGGTNNKATACAAGACAGCCTGAGCAGCACAGCAAGC
Cassette7_4	GCCAACCAGTTCAACAGCGCCATAGGCAAANNKCAAGACAGCCTGAGCAGCACAGCAAGC
Cassette7_5	GCCAACCAGTTCAACAGCGCCATCGGTAAGATCNNKGATAGCCTGAGCAGCACAGCAAGC
Cassette7_6	GCCAACCAGTTCAACAGCGCCATCGGCAAAATACAGNNKAGCCTGAGCAGCACAGCAAGC
Cassette7_7	GCCAACCAGTTCAACAGCGCCATAGGTAAGATCCAAGACNNKCTGAGCAGCACAGCAAGC
Cassette7_8	GCCAACCAGTTCAACAGCGCCATCGGCAAGATACAAGATAGCNNKAGCAGCACAGCAAGC
Cassette8_1	GGCAAGATCCAGGACAGCCTGNNKAGTACCGCAAGCGCCCTGGGAAAGCTGCAGGACGTG
Cassette8_2	GGCAAGATCCAGGACAGCCTGAGTNNKACAGCCAGCGCCCTGGGAAAGCTGCAGGACGTG
Cassette8_3	GGCAAGATCCAGGACAGCCTGAGCAGCNNKGCCAGTGCCCTGGGAAAGCTGCAGGACGTG
Cassette8_4	GGCAAGATCCAGGACAGCCTGAGCAGCACCNNKAGCGCACTGGGAAAGCTGCAGGACGTG
Cassette8_5	GGCAAGATCCAGGACAGCCTGAGCAGTACAGCANNKGCACTGGGAAAGCTGCAGGACGTG
Cassette8_6	GGCAAGATCCAGGACAGCCTGAGTAGCACCGCAAGTNNKCTGGGAAAGCTGCAGGACGTG
Cassette8_7	GGCAAGATCCAGGACAGCCTGAGTAGTACCGCCAGTGCCNNKGGAAAGCTGCAGGACGTG
Cassette8_8	GGCAAGATCCAGGACAGCCTGAGTAGTACAGCAAGTGCCCTGNNKAAGCTGCAGGACGTG
Cassette9_1	AGCACAGCAAGCGCCCTGGGANNKCTCCAAGACGTGGTCAACCAGAATGCCCAGGCACTG
Cassette9_2	AGCACAGCAAGCGCCCTGGGAAAANNKCAGGATGTGGTCAACCAGAATGCCCAGGCACTG
Cassette9_3	AGCACAGCAAGCGCCCTGGGAAAGCTCNNKGATGTAGTCAACCAGAATGCCCAGGCACTG
Cassette9_4	AGCACAGCAAGCGCCCTGGGAAAACTGCAANNKGTAGTCAACCAGAATGCCCAGGCACTG
Cassette9_5	AGCACAGCAAGCGCCCTGGGAAAGCTCCAGGACNNKGTAAACCAGAATGCCCAGGCACTG
Cassette9_6	AGCACAGCAAGCGCCCTGGGAAAGCTGCAAGATGTGNNKAACCAGAATGCCCAGGCACTG
Cassette9_7	AGCACAGCAAGCGCCCTGGGAAAACTCCAGGACGTAGTCNNKCAGAATGCCCAGGCACTG
Cassette9_8	AGCACAGCAAGCGCCCTGGGAAAGCTGCAAGACGTAGTAAACNNKAATGCCCAGGCACTG
Cassette10_1	CTGCAGGACGTGGTCAACCAGNNKGCACAAGCACTGAACACCCTGGTCAAGCAGCTGTCC

Cassette10_2	CTGCAGGACGTGGTCAACCAGAACNNKCAGGCCCTGAACACCCTGGTCAAGCAGCTGTCC
Cassette10_3	CTGCAGGACGTGGTCAACCAGAACGCCNNKGCACTCAACACCCTGGTCAAGCAGCTGTCC
Cassette10_4	CTGCAGGACGTGGTCAACCAGAATGCACAGNNKCTCAACACCCTGGTCAAGCAGCTGTCC
Cassette10_5	CTGCAGGACGTGGTCAACCAGAATGCCCAAGCCNNKAACACCCTGGTCAAGCAGCTGTCC
Cassette10_6	CTGCAGGACGTGGTCAACCAGAACGCACAAGCCCTCNNKACCCTGGTCAAGCAGCTGTCC
Cassette10_7	CTGCAGGACGTGGTCAACCAGAACGCACAGGCACTCAATNNKCTGGTCAAGCAGCTGTCC
Cassette10_8	CTGCAGGACGTGGTCAACCAGAACGCCCAAGCACTGAATACCNNKGTCAAGCAGCTGTCC
Cassette11_1	GCCCAGGCACTGAACACCCTGNNKAAACAACTGTCCTCCAACTTCGGCGCCATCAGCTCT
Cassette11_2	GCCCAGGCACTGAACACCCTGGTANNKCAGCTCTCCTCCAACTTCGGCGCCATCAGCTCT
Cassette11_3	GCCCAGGCACTGAACACCCTGGTAAAGNNKCTGTCTTCCAACTTCGGCGCCATCAGCTCT
Cassette11_4	GCCCAGGCACTGAACACCCTGGTCAAACAGNNKTCTTCCAACTTCGGCGCCATCAGCTCT
Cassette11_5	GCCCAGGCACTGAACACCCTGGTCAAGCAACTCNNKTCCAACTTCGGCGCCATCAGCTCT
Cassette11_6	GCCCAGGCACTGAACACCCTGGTAAAACAACTCTCTNNKAACTTCGGCGCCATCAGCTCT
Cassette11_7	GCCCAGGCACTGAACACCCTGGTAAAGCAACTCTCCTCTNNKTTCGGCGCCATCAGCTCT
Cassette11_8	GCCCAGGCACTGAACACCCTGGTAAAGCAGCTCTCTTCTAACNNKGGCGCCATCAGCTCT
Cassette12_1	AAGCAGCTGTCCTCCAACTTCNNKGCAATAAGCTCTGTGCTGAACGATATCCTGAGCAGA
Cassette12_2	AAGCAGCTGTCCTCCAACTTCGGTNNKATCAGTTCTGTGCTGAACGATATCCTGAGCAGA
Cassette12_3	AAGCAGCTGTCCTCCAACTTCGGTGCCNNKAGCTCGGTGCTGAACGATATCCTGAGCAGA
Cassette12_4	AAGCAGCTGTCCTCCAACTTCGGCGCAATCNNKTCGGTGCTGAACGATATCCTGAGCAGA
Cassette12_5	AAGCAGCTGTCCTCCAACTTCGGCGCCATAAGTNNKGTGCTGAACGATATCCTGAGCAGA
Cassette12_6	AAGCAGCTGTCCTCCAACTTCGGTGCAATAAGTTCGNNKCTGAACGATATCCTGAGCAGA
Cassette12_7	AAGCAGCTGTCCTCCAACTTCGGTGCAATCAGCTCTGTANNKAACGATATCCTGAGCAGA
Cassette12_8	AAGCAGCTGTCCTCCAACTTCGGTGCCATCAGTTCGGTACTGNNKGATATCCTGAGCAGA
Cassette13_1	GCCATCAGCTCTGTGCTGAACNNKATACTCAGCAGACTGGACAAGGTGGAAGCCGAGGTG
Cassette13_2	GCCATCAGCTCTGTGCTGAACGACNNKCTGAGTAGACTGGACAAGGTGGAAGCCGAGGTG

Cassette13_3	GCCATCAGCTCTGTGCTGAACGATATCNNKAGTAGGCTGGACAAGGTGGAAGCCGAGGTG
Cassette13_4	GCCATCAGCTCTGTGCTGAACGATATCCTCNNKAGACTCGACAAGGTGGAAGCCGAGGTG
Cassette13_5	GCCATCAGCTCTGTGCTGAACGATATACTGAGCNNKCTCGACAAGGTGGAAGCCGAGGTG
Cassette13_6	GCCATCAGCTCTGTGCTGAACGACATCCTCAGCAGGNNKGACAAGGTGGAAGCCGAGGTG
Cassette13_7	GCCATCAGCTCTGTGCTGAACGACATACTCAGTAGGCTGNNKAAGGTGGAAGCCGAGGTG
Cassette13_8	GCCATCAGCTCTGTGCTGAACGACATACTGAGCAGGCTGGACNNKGTGGAAGCCGAGGTG
Cassette14_1	ATCCTGAGCAGACTGGACAAGNNKGAGGCAGAGGTGCAGATCGACAGACTGATCACCGGA
Cassette14_2	ATCCTGAGCAGACTGGACAAGGTANNKGCCGAAGTGCAGATCGACAGACTGATCACCGGA
Cassette14_3	ATCCTGAGCAGACTGGACAAGGTAGAANNKGAGGTACAGATCGACAGACTGATCACCGGA
Cassette14_4	ATCCTGAGCAGACTGGACAAGGTGGAGGCCNNKGTACAGATCGACAGACTGATCACCGGA
Cassette14_5	ATCCTGAGCAGACTGGACAAGGTGGAAGCAGAANNKCAGATCGACAGACTGATCACCGGA
Cassette14_6	ATCCTGAGCAGACTGGACAAGGTAGAGGCAGAAGTANNKATCGACAGACTGATCACCGGA
Cassette14_7	ATCCTGAGCAGACTGGACAAGGTAGAGGCCGAGGTACAANNKGACAGACTGATCACCGGA
Cassette14_8	ATCCTGAGCAGACTGGACAAGGTAGAAGCAGAGGTGCAAATCNNKAGACTGATCACCGGA
Cassette15_1	GAAGCCGAGGTGCAGATCGACNNKCTCATAACCGGAAGGCTGCAGTCCCTGCAGACCTAC
Cassette15_2	GAAGCCGAGGTGCAGATCGACAGGNNKATCACAGGAAGGCTGCAGTCCCTGCAGACCTAC
Cassette15_3	GAAGCCGAGGTGCAGATCGACAGGCTGNNKACCGGTAGGCTGCAGTCCCTGCAGACCTAC
Cassette15_4	GAAGCCGAGGTGCAGATCGACAGACTCATCNNKGGTAGGCTGCAGTCCCTGCAGACCTAC
Cassette15_5	GAAGCCGAGGTGCAGATCGACAGACTGATAACANNKAGGCTGCAGTCCCTGCAGACCTAC
Cassette15_6	GAAGCCGAGGTGCAGATCGACAGGCTCATAACAGGTNNKCTGCAGTCCCTGCAGACCTAC
Cassette15_7	GAAGCCGAGGTGCAGATCGACAGGCTCATCACCGGTAGANNKCAGTCCCTGCAGACCTAC
Cassette15_8	GAAGCCGAGGTGCAGATCGACAGGCTGATCACCGGAAGACTGNNKTCCCTGCAGACCTAC
Cassette16_1	CTGATCACCGGAAGGCTGCAGNNKCTCCAAACCTACGTTACCCAGCAGCTGATCAGAGCC
Cassette16_2	CTGATCACCGGAAGGCTGCAGTCTNNKCAGACATACGTTACCCAGCAGCTGATCAGAGCC
Cassette16_3	CTGATCACCGGAAGGCTGCAGTCTCTGNNKACCTATGTTACCCAGCAGCTGATCAGAGCC

Cassette16_4	CTGATCACCGGAAGGCTGCAGTCCCTCCAGNNKTATGTTACCCAGCAGCTGATCAGAGCC
Cassette16_5	CTGATCACCGGAAGGCTGCAGTCCCTGCAAACANNKGTTACCCAGCAGCTGATCAGAGCC
Cassette16_6	CTGATCACCGGAAGGCTGCAGTCTCTCCAAACATATNNKACCCAGCAGCTGATCAGAGCC
Cassette16_7	CTGATCACCGGAAGGCTGCAGTCTCTCCAGACCTATGTANNKCAGCAGCTGATCAGAGCC
Cassette16_8	CTGATCACCGGAAGGCTGCAGTCTCTGCAAACCTACGTAACCNNKCAGCTGATCAGAGCC
Cassette17_1	CTGCAGACCTACGTTACCCAGNNKCTCATAAGAGCCGCCGAGATTAGAGCCTCTGCCAAT
Cassette17_2	CTGCAGACCTACGTTACCCAGCAANNKATCAGGGCCGCCGAGATTAGAGCCTCTGCCAAT
Cassette17_3	CTGCAGACCTACGTTACCCAGCAACTGNNKAGAGCAGCCGAGATTAGAGCCTCTGCCAAT
Cassette17_4	CTGCAGACCTACGTTACCCAGCAGCTCATCNNKGCAGCCGAGATTAGAGCCTCTGCCAAT
Cassette17_5	CTGCAGACCTACGTTACCCAGCAGCTGATAAGGNNKGCCGAGATTAGAGCCTCTGCCAAT
Cassette17_6	CTGCAGACCTACGTTACCCAGCAACTCATAAGGGCANNKGAGATTAGAGCCTCTGCCAAT
Cassette17_7	CTGCAGACCTACGTTACCCAGCAACTCATCAGAGCAGCANNKATTAGAGCCTCTGCCAAT
Cassette17_8	CTGCAGACCTACGTTACCCAGCAACTGATCAGGGCAGCAGAGNNKAGAGCCTCTGCCAAT
Cassette18_1	CTGATCAGAGCCGCCGAGATTNNKGCATCGGCCAATCTGGCCGCCACCAAGATGTCTGAG
Cassette18_2	CTGATCAGAGCCGCCGAGATTAGGNNKTCTGCAAATCTGGCCGCCACCAAGATGTCTGAG
Cassette18_3	CTGATCAGAGCCGCCGAGATTAGGGCCNNKGCCAACCTGGCCGCCACCAAGATGTCTGAG
Cassette18_4	CTGATCAGAGCCGCCGAGATTAGAGCATCTNNKAACCTGGCCGCCACCAAGATGTCTGAG
Cassette18_5	CTGATCAGAGCCGCCGAGATTAGAGCCTCGGCANNKCTGGCCGCCACCAAGATGTCTGAG
Cassette18_6	CTGATCAGAGCCGCCGAGATTAGGGCATCGGCAAACNNKGCCGCCACCAAGATGTCTGAG
Cassette18_7	CTGATCAGAGCCGCCGAGATTAGGGCATCTGCCAACCTCNNKGCCACCAAGATGTCTGAG
Cassette18_8	CTGATCAGAGCCGCCGAGATTAGGGCCTCTGCAAACCTCGCCNNKACCAAGATGTCTGAG
Cassette19_1	GCCTCTGCCAATCTGGCCGCCNNKAAAATGTCGGAGTGTGTGCTGGGCCAGAGCAAGAGA
Cassette19_2	GCCTCTGCCAATCTGGCCGCCACANNKATGTCTGAATGTGTGCTGGGCCAGAGCAAGAGA
Cassette19_3	GCCTCTGCCAATCTGGCCGCCACCAAGNNKTCGGAATGTGTGCTGGGCCAGAGCAAGAGA
Cassette19_4	GCCTCTGCCAATCTGGCCGCCACCAAAATGNNKGAATGCGTGCTGGGCCAGAGCAAGAGA

Cassette19_5	GCCTCTGCCAATCTGGCCGCCACAAAGATGTCGNNKTGCGTGCTGGGCCAGAGCAAGAGA
Cassette19_6	GCCTCTGCCAATCTGGCCGCCACCAAGATGTCTGAANNKGTACTGGGCCAGAGCAAGAGA
Cassette19_7	GCCTCTGCCAATCTGGCCGCCACAAAAATGTCTGAGTGCNNKCTGGGCCAGAGCAAGAGA
Cassette19_8	GCCTCTGCCAATCTGGCCGCCACCAAGATGTCGGAGTGTGTANNKGGCCAGAGCAAGAGA

Cassette1_Rprimer	GATTGTGCCGGCCAGCAGGGC
Cassette2_Rprimer	AGCTCCAAATGTCCAGCCGCT
Cassette3_Rprimer	AAAGGGGATCTGCAGAGCGGC
Cassette4_Rprimer	GAACCGGTAGGCCATCTGCAT
Cassette5_Rprimer	ATTCTGGGTCACTCCGATGCC
Cassette6_Rprimer	CAGCTTCTGGTTCTCGTACAG
Cassette7_Rprimer	GGCGCTGTTGAACTGGTTGGC
Cassette8_Rprimer	CAGGCTGTCCTGGATCTTGCC
Cassette9_Rprimer	TCCCAGGGCGCTTGCTGTGCT
Cassette10_Rprimer	CTGGTTGACCACGTCCTGCAG
Cassette11_Rprimer	CAGGGTGTTCAGTGCCTGGGC
Cassette12_Rprimer	GAAGTTGGAGGACAGCTGCTT
Cassette13_Rprimer	GTTCAGCACAGAGCTGATGGC
Cassette14_Rprimer	CTTGTCCAGTCTGCTCAGGAT
Cassette15_Rprimer	GTCGATCTGCACCTCGGCTTC
Cassette16_Rprimer	CTGCAGCCTTCCGGTGATCAG
Cassette17_Rprimer	CTGGGTAACGTAGGTCTGCAG
Cassette18_Rprimer	AATCTCGGCGGCTCTGATCAG
Cassette19_Rprimer	GGCGGCCAGATTGGCAGAGGC

Supplementary Table 4. Numbers of cells collected per bin in expression sorting.

Bin	Replicate 1	Replicate 2	Replicate 3
Bin 0	8.03×10^5	1.30×10^6	1.70×10^6
Bin 1	7.51×10^5	1.30×10^6	1.70×10^6
Bin 2	7.70×10^5	1.30×10^6	1.70×10^6
Bin 3	8.20×10^5	1.30×10^6	1.70×10^6

Supplementary Table 5. Numbers of cells collected per bin in fusion sorting.

Bin	Replicate 1	Replicate 2
mNG2$^-$	3.53×10^6	5.51×10^6
mNG2$^+$	1.84×10^5	4.87×10^5

Supplementary Table 6. *p*-values from Student's *t* test of expression and fusion scores between mutation types. Data are related to **Extended Data Fig. 2c,d**.

Expression			
	Missense	Nonsense	Silent
Missense		6.46×10^{-60}	2.93×10^{-2}
Nonsense	6.46×10^{-60}		3.80×10^{-44}
Silent	2.93×10^{-2}	3.80×10^{-44}	
Fusion			
	Missense	Nonsense	Silent
Missense		3.10×10^{-34}	7.08×10^{-4}
Nonsense	3.10×10^{-34}		1.44×10^{-31}
Silent	7.08×10^{-4}	1.44×10^{-31}	

Supplementary Table 7. Betacoronaviruses used for sequence conservation analysis. Data are related to **Extended Data Fig. 3c,d**.

Accession ID	**Database**	**Name**
gb_MN908947.3_	GenBank	Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome
gb_MN996532.2_	GenBank	Bat coronavirus RaTG13, complete genome
gb_MZ937000.1_	GenBank	Bat coronavirus isolate BANAL-20-52/Laos/2020, complete genome
EPI_ISL_410543	GISAID	hCoV-19/pangolin/Guangxi/P3B/2017
EPI_ISL_471465	GISAID	hCoV-19/pangolin/Guangdong/cDNA20-S/2019
gb_MZ937003.1_	GenBank	Bat coronavirus isolate BANAL-20-236/Laos/2020, complete genome
gb_MZ937001.1_	GenBank	Bat coronavirus isolate BANAL-20-103/Laos/2020, complete genome
gb_MG772933.1_	GenBank	Bat SARS-like coronavirus isolate bat-SL-CoVZC45, complete genome
gb_MG772934.1_	GenBank	Bat SARS-like coronavirus isolate bat-SL-CoVZXC21, complete genome
gb_KT444582.1_	GenBank	SARS-like coronavirus WIV16, complete genome
gb_DQ497008.1_	GenBank	SARS coronavirus strain MA-15, complete genome
gb_KC881007.1_	GenBank	Bat SARS-like coronavirus WIV1 spike protein (S) gene, complete cds
gb_KY417144.1_	GenBank	Bat SARS-like coronavirus isolate Rs4084, complete genome
gb_DQ412042.1_	GenBank	Bat SARS coronavirus Rf1, complete genome
gb_KJ473813.1_	GenBank	BtRf-BetaCoV/SX2013, complete genome
gb_KJ473815.1_	GenBank	BtRs-BetaCoV/GX2013, complete genome
gb_KF294457.1_	GenBank	Bat SARS-like coronavirus isolate Longquan-140 orf1ab polyprotein, spike glycoprotein, envelope protein, membrane protein, and nucleocapsid protein genes, complete cds
gb_DQ022305.2_	GenBank	Bat SARS coronavirus HKU3-1, complete genome
gb_DQ071615.1_	GenBank	Bat SARS coronavirus Rp3, complete genome
gb_FJ588686.1_	GenBank	Bat SARS CoV Rs672/2006, complete genome
gb_KJ473814.1_	GenBank	BtRs-BetaCoV/HuB2013, complete genome
gb_KF569996.1_	GenBank	*Rhinolophus affinis* coronavirus isolate LYRa11, complete genome
gb_KY352407.1_	GenBank	Severe acute respiratory syndrome-related coronavirus strain BtKY72, complete genome
ref_NC_014470.1_	GenBank	Bat coronavirus BM48-31/BGR/2008, complete genome
gb_MZ937004.1_	GenBank	Bat coronavirus isolate BANAL-20-247/Laos/2020, complete genome
gb_MZ937002.1_	GenBank	Bat coronavirus isolate BANAL-20-116/Laos/2020, complete genome
EPI_ISL_412977	GISAID	hCoV-19/bat/Yunnan/RmYN02/2019