

Supplementary Information

A Genetically encoded BRET-based SARS-CoV-2 M^{pro} protease activity sensor

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Supplementary Text

BRET-based M^{pro} sensor construct details:

mNG-M^{pro}-Nter-auto-NLuc:

Protein Sequence:

MGSSHHHHHHSSGLVPRGSHMVSKGEEDNMASLPATHELHIFGSINGVDFDMVGQGTGNPNDGYEELNLKSTKGDLO
FSPWILVPHIGYGFHQYLPYPDGMSPFQAAMVDGSGYQVHRTMQFEDGASLTVNYRYTYEGSHIKGEAQVKGTGFPA
DGPVMTNSLTAADWCRSKKTYPNDKTIIISTFKWSYTTGNGKRYRSTARTTYTFAKPMAANYLKNQPMYVFRKTELKH
SKTELNFKEWQKAFTDVMGMDELYKEFGTENLYAVLQSGFRGSGGSMVFTLEDFVGDWRQTAGYNLDQVLEQGGVSS
LFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVPVDDHHFKVILHYGTLVIDGVTPNM
IDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLFRVTINGVTGWRLCERILA*

Key:

- Yellow: His-tag
- Green: mNeonGreen
- Grey: Linkers
- Red: cleavage sequence
- Cyan: NLuc

Predicted pI: 6.31

Predicted Mol. Wt.: 50048.54 (~50 kDa)

Predicted Mol. Wt. after cleavage (His-tagged fragment): Theoretical pI/Mw: 6.92 / 30178.78
(30 kDa)

Plasmid DNA Sequence:

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTACGGTAAA
TGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGT
CAATGGGTGGAGTATTTACGGTAACTGCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAAT
GGCCCGCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCG
GTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGGATTTCGAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTGGCAC
CAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCGCCCCATTGACGCAAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATTAAGCAGAG
CTCTCTGGCTAACTAGAGAACCCTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGCGTTTAACTTAAG
CTTGCCACCATGGGAAGTTCACATCATCATCATCACTCATCAGGACTGGTGCCACGGGGGTCTCATATGGTCTCCAAGGGGGAAGAGGACAACA
TGGCCTCTCTGCTGCCACACAGAGCTGCACATCTTCGGCAGCATCAATGGCGTGGACTTTGATATGGTGGGCCAGGGCACAGGCAACCCAAATGA
CGGCTACAGGAGCTGAACCTGAAGAGCACAGCCAGAACCACATATACCTTTGCCAAGCCAATGGCCGCAACTATCTGAAGAATCAGCCCATGTACGTGT
CTGCCCTACCCTGACGGCATGTCCCCCTTCAGGCCGCCATGGTGGATGGCTCTGGCTACCAGGTGCACAGGACCATGCAGTTTGAGGACGGCGCCT
CCCTGACAGTGAATTACCGGTATACCTACGAGGGCTCTCACATCAAGGGCGAGGCCAGGTGAAGGGCACAGGCTTCCAGCCGATGGCCCCGTGAT
GACAACTCTCTGACCGCCGCCGACTGGTGCCGGAGCAAGAAGACCTACCCTAATGATAAGACAATCATCTCCACCTTTAAGTGGTCTTATACCACA
GGCAACGGCAAGCGGTACAGAAGCACAGCCAGAACCACATATACCTTTGCCAAGCCAATGGCCGCAACTATCTGAAGAATCAGCCCATGTACGTGT
TCAGGAAGACAGAGCTGAAGCACTCCAAGACCGAGCTGAATTTCAAGGAGTGGCAGAAGGCCCTTACAGACGTGATGGGCATGGATGAGCTGTACAA
GGAGTTTGGCACCAGAGAACCTGTATGCAGTGTCCAAAGCGGATTTCCGCGGCTCTGGCGGCAGCATGGTGTTCACACTGGAGGACTTTGTGGGCGAT
TGGCGGCAGACCGCCGGCTATAATCTGGATCAGGTGCTGGAGCAGGGCGGCGTGAGCTCCCTGTTCCAGAACCTGGGCGTGAGCGTGACCCCTATCC

61 AGCGGATCGTGCTGTCCGGCGAGAACGGCCTGAAGATCGACATCCACGTGATCATCCCATACGAGGGCCTGTCTGGCGATCAGATGGGCCAGATCGA
62 GAAGATCTTCAAGGTGGTGTACCCCGTGGACGATCACCACCTTCAAAGTGATCCTGCATATGGCACCCCTGGTATCGACGGCGTGACCCCCAATATG
63 ATCGATTATTTCCGGCCGGCTTACGAGGCATCGCCGTTTGGACGGCAAGAAGATCACCGTGACAGGCACCCCTGTGGAAACGGCAATAAGATCATCG
64 ACGAGAGACTGATCAACCCCGATGGCTCCCTGCTGTTTCAAGGGTGACTATTAACGGAGTGACTGGCTGGCGGCTGTGCGAAAGGATTCTGGCTTGACT
65 CGAGTCTAGAGGGCCCGTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGGCAGCCATCTGTTGTTGGCCCTCCCGCGTCCCTTCCCTTG
66 ACCCTGGAAGGTGCCACTCCCACTGTCTTTTCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCTATCTATCTGGGGGGTGGGG
67 TGGGCGAGGACGAAGGGGAGGATTGGGAAGACAATAGCAGGCATGTGGGGATGCGGTGGGCTCTATGGGTTCTGAGGCGGAAAGAACAGCTG
68 GGGCTCTAGGGGGTATCCCCACCGCCCTGTAGCGGCCATTAAAGCGCGCGGGTGTGGTGGTTACGCGCAGCGTGACCCTACACTTGGCAGCGCC
69 CTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCTTTCGCGCCAGTTTCGCGGCTTTCCCGCTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCC
70 GATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGATGGTTACGTAAGTGGGCCATCGCCCTGATAGACGGTTTTTTCGCCCTTT
71 GACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACCTGGAACAACACTCAACCTATCTCGGTCTATTCTTTTGATTATAAGGGATT
72 TTGCCGATTTTCGCCCTATTTGGTTAAAAAATGAGTCTGATTTAAACAAAAATTAACGCGAATTAATTTCTGTGGAATGTCGGCTAGTTAGGCTGTGAAA
73 GTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAGTCCCCAGGCTCCCCAGCAGGCAGA
74 AGTATGCAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAAGTCCGCGCCATCCCGCCCTAAGTCCGCGCCAGTTCGCGCCATCTC
75 CGCCCATGGCTGACTAATTTTTTTTATTATGCAGAGGCCGAGGCCCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGAGG
76 CCTAGGCTTTTGCAAAAAGCTCCCGGAGCTTATGATATCTCCGCTCCCGATCAAGAGACAGGATGAGGATCTGTGCCTATTTGACGAAGAAT
77 GGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCC
78 GGTGTACAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAAGTGCAGGACGAGGCAGCGCGGCTATCGTGGCT
79 GGCCACGACGGGCGTTCCTTGCAGAGCTGTGCTCGACGTTGTCACTGAAGCGGAAGGACTGGTGCTATTGGGCGAAGTGCCGGGCGAGGATCTC
80 CTGCTATCTCATGTTCTGTATACCGTCGAAAGTATGATGCAATGCGCGGCTGCATACGCTTGATCGGGCTAGTGCCTGATTCGACCTGCGAAC
81 ACCAAGCGAAACATCGCATCGAGCGAGCAGTACTCGGATGGAAGCCGGTCTTGTGCTAGCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCC
82 AGCCGAAGTGTTCGCGCAGGCTCAAGGCGCGCATGCCCCGACGGCGAGGATCTCGTCTGACCCATGGCGATGCCTGCTTGCAGGAATATCATGGTGGAA
83 AATGGCCGCTTTTCTGGATTCACTGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGC
84 TTGGCGGCGTATGGGCTAGCCGTTTCTCGTGTTTACGGTATTCGGCTCCCGATCCGAGCGCATCGCCTTCTATGCGGCTTGTGACGATCTCTT
85 CTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCCCAACCTGCCATCAGAGATTTTCGATTCCACCGCCGCTTCTATGAAAGGTTGG
86 GCTTCGGAATCGTTTTTCGGGACGCGCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCACCCCACTTGTATTATTCGAGC
87 TTATAATGGTTACAAATAAAGCAATAGCATCACAATTTACAAATAAAGCATTTTTTTCACCTGCATTTCTAGTTGTGGTTTGTCCAACTCATCAAT
88 GTATCTTATCATGTTCTGTATACCGCTCGACCTTAGCTAGAGCTTGGCGTAATCATGGTGCATGCTATGCTTCTGTGGAATTTGTTATCCGCTCACA
89 TTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCATATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCCG
90 TTTCCAGTCGGGAAACCTGTCTGTCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGCGGTTTGCCTATTGGGCGCTCTTCCGCTTCTCTG
91 CTCCTGACTCGCTGCGCTCGGTCTGGCTCGCGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACG
92 CAGGAAAGCAATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCTGAAAGGCGCGGTTGCTGCGCTTTTTTCCATAGGCTCCGCCCCCTGACGA
93 GCATCACAATAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCGCTGGAAGCTCCCTCGTGCGCTCT
94 CCTGTTCCGACCCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTTCTCATAGCTCAGCTGTAGGTATCTCAGTT
95 CGGTGTAGGTCTGCTCCTCAAGCTGGGCTGTGTGCACGAACCCCGGTTACGCCCCAGCGCTGCGCCTTATCCGGTAAGTATCGTCTTGAGTCCAA
96 CCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGG
97 TGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCG
98 GCAACAAACACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTC
99 TACGGGGTCTGACGCTCAGTGGAACGAAACTCACGTTAAGGGATTTTGGTCTAGAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAA
100 AAATGAAGTTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTC
101 TATTTTCGTTTATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAAAGTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCG
102 AGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAAGTGGTCTGCAACTTTATCCGCTCCATC
103 CAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTTCGCCAGTTAATAGTTTGGCGAACGTTGTTGCCATTGCTACAGGCATCTGGGTGTCAC
104 GCTCGTCTGTTGGTATGGCTTCATTTCAGCTCCGCTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTT
105 CGGTCTCCGATCGTTGTGCAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCTATGCCATCCGTA
106 AGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGATGCGGCGACCGAGTTGCTCTTGGCCGGCGTCAATACGGGATA
107 ATACCGCGCCACATAGCAGAAGTTTAAAGTGCTCATCTATGGAAGCGTTCTTTCGGGGCGAAAACTCTCAAGGATCTTACCCTGTTGAGATCCAG
108 TTCGATGTAACCCACTCTGTCACCCCACTGATCTTCAGCATCTTTTACTTTTCCAGCGGTTTCTGGGTGAGCAAAAAAGGCAAGCAAAATGCCGCA
109 AAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTACATACTCTTCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCG
110 GATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCCGGCGACATTTCCCGAAAAGTCCACCTGACGTCGACGGATCGGGAGATCT
111 CCCGATCCCTATGGTGCATCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGT
112 AGTCCGAGCAAAATTAAGCTACAACAAGGAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTTTCGCTTCTTCGCA
113 TGTACGGGCGAGATATACGCGTT

115 *mNG-M^{pro}-Nter-auto-L-NLuc*:

116 Protein Sequence:

117 MGSSHHHHHHSSGLVPRGSHMVSKGEEDNMASLPATHELHIFGSINGVDFDMVGQGTGNPNDGYEELNLKSTKGDLO

118 FSPWILVPHIGYGFHQYLPYPDGMSPFQAAMVDGSGYQVHRTMQFEDGASLTVNYRYTYEGSHIKGEAQVKGTGFPA

119 DGPVMTNSLTAADWCRSKKTYPNDKTIISTFKWSYTTGNGKRYRSTARTTYTFAKPMAANYLKNQPMYVFRKTELKH

120 SKTELNFKEWQKAFTDVMGMDELYKEFGTENLYKTSAVLQSGFRKMEGSSGSMVFTLEDFVGDWRQTAGYNLDQVLE

121 OGGVSSSLFQNLGVSVTPIQIRIVLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVPVDDHHFKVILHYGTLVID

122 GVTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKIIDERLINPDGSLLFRTINGVTGWRLCERILA*

123

124 Key:

125 Yellow: His-tag

126 Green: mNeonGreen

127 Grey: Linkers

128 Red: cleavage sequence

129 Cyan: NLuc

130

131 Predicted pI: 6.41

132 Predicted Mol. Wt.: 50753.38 (~51 kDa)

133 Predicted Mol. Wt. after cleavage (His-tagged fragment): Theoretical pI/Mw: 7.26 / 30495.14

134

135 Plasmid DNA Sequence:

136 GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCGCGGTACATAACTTACGGTAAA
137 TGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGT
138 CAATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAAT
139 GGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATTGGTGATGCG
140 GTTTTGGCAGTACATCAATGGCGGTGGATAGCGGTTTACTCACGCGGATTTCCAAAGTCCACCCCATTTGACGTCAATGGGAGTTTGTTTTGGCAC
141 CAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCATTTGACGCAAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAG
142 CTCTCTGGCTAACTAGAGAACCCTGCTTACTGGCTTATCGAAATTAATACGACTCACTATAGGGAGACCCAAGCTGGCTAGCGTTTAAACTTAAG
143 CTTGCCACCATGGGAAGTTCACATCATCATCATCACTCATCAGGACTGGTGCCACGGGGGTCTCATATGGTCTCCAAGGGGGAAGAGGACAACA
144 TGGCCTCTCTGCCCGCCACACAGCAGCTGCACATCTTCGGCAGCATCAATGGCGTGGACTTTGATATGGTGGGCCAGGGCACAGGCAACCCAAATGA
145 CGGCTACGAGGAGCTGAACCTGAAGAGCACCAAGGGCGATCTGCAGTTCTCCCTTGGATCCTGGTGCCACACATCGGCTATGGCTTTTACCAGTAT
146 CTGCCCTACCCTGACGGCATGTCCCCCTTCCAGGCCGCCATGGTGGATGGCTCTGGCTACCAGGTGCACAGGACCATGCAGTTTGGAGACGGCGCCT
147 CCCTGACAGTGAATTACCGGTATACCTACGAGGGCTCTCACATCAAGGGCGAGGCCAGGTGAAGGGCACAGGCTTCCAGCCGATGGCCCCGTGAT
148 GACAAACTCTCTGACCCCGCCGACTGGTGCCGAGCAAGAAGACCTACCCTAATGATAAGACAATCATCTCCACCTTTAAGTGGTCTTATACCACA
149 GGCAACGGCAAGCGGTACAGAAGCACAGCCAGAACCATATACCTTTGCCAAGCCAATGGCCGCCAATATCTGAAGAATCAGCCCCATGTACGTGT
150 TCAGGAAGACAGAGCTGAGCACTCCAAGACCGAGCTGAATTTCAAGGAGTGGCAGAAGGCCCTTTACAGACGTGATGGGCATGGATGAGCTGTACAA
151 GGAGTTTGGCACCGAGAACCTGTATAAAACGAGTGCCGTATTGCGAGTGGGTTTCGGAAAATGGAAGGCTCTGGCGGCAGCATGGTGTTCACACTG
152 GAGGACTTGTGGCGGATTGGCGGCAGACCGCCGCTATAATCTGGATCAGGTGCTGGAGCAGGGCGGCGTGAGCTCCCTGTTCCAGAACCTGGCG
153 TGAGCGTGACCCCTATCCAGCGGATCGTGCTGTCCGGCGAGAAGCGCCTGAAGATCGACATCCACGTGATCATCCCATACGAGGGCCTGTCTGGCGA
154 TCAGATGGGCCAGATCGAGAAGATCTTCAAGGTGGTGACCCCGTGGACGATACCACTTCAAGTGTCTGCATCTGGCACCCCTGGTCATCGAC
155 GCGGTGACCCCAATATGATCGATTATTTCCGGCCGGCCTTACGAGGGCATCGCCGTGTTTACAGGCAAGAAGATCACCGTGACAGGACCCCTGTGGA
156 ACGGCAATAAGATCATCGACGAGAGACTGATCAACCCGATGGCTCCCTGCTTTCAGGGTGACTATTAACGGAGTGACTGGCTGGCGGCTGTGCGA
157 AAGGATTCTGGCTTGACTCGAGTCTAGAGGGCCGTTTAAACCCGCTGATCAGCCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTGCCCC
158 TCCCCCGTGCTTCTTACCCCTGGAAGGTGCCACTCCCACTGTCTTCTTAATAAAATGAGGAAATGCGATCGCATGTCTGAGTAGGTGTGATT
159 CTATTTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGA
160 GCGGAAAGAACCAGCTGCGGGCTTAGGGGGTATCCCCACGCCCTGTAGCGGCGCATTAAGCGCGCGGGTGTGGTGGTTACGCGCAGCAATCGGCTGACC
161 GCTACACTTGCCAGCGCCCTAGCGCCGCTCCTTTCTGCTTTCTTCCCTTCTTCTCGCCACGTTCCGCGGCTTTCCCGCTCAAGCTCAAATCGGG
162 GGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACCTTGATTAGGGTGATGGTTACGTAGTGGCCATCGCCCTGATA
163 GACGGTTTTTTCGCCCTTTGACGTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACCTGGAACAACACTCAACCCCTATCTCGGTCTATTCT
164 TTTGATTTATAAGGATTTTGGCGATTTCCGCCATTTGGGTTAAAAATGAGCTGATTTAACAAAAATTAACGCGAATTAATCTGTGGAATGTGTG
165 TCAGTTAGGGTGTGGAAGTCCCAAGGCTCCCGCAGCAGAGGATGTTGGTGGAGAGGCTATTCCGGCTATGACTGGGCAACAGACAACTCGGCTGCT
166 GGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGCCATCCCGCCCTAACTCCGC
167 CCAAGTTCCGCCCATTTCTCCGCCCATGGCTGACTAATTTTTTTTATTTATGACAGAGCCGAGGCCGCTCTGCCTCTGAGCTATTCAGAAAGTAGTG
168 AGGAGGCTTTTTTGGAGGCTTAGGCTTTTGCAAAAAGCTCCCGGGAGCTTGTATATCCATTTTTCGGATCTGATCAAGAGACAGGATGAGGATCGTTT
169 CGCATGATTGAACAAGATGGAATGACACGAGGTTCTCCGCCGCTTGGGTGGAGAGGCTATTCCGGCTATGACTGGGCAACAGACAACTCGGCTGCT
170 CTGATGCCGCGGTGTTCCGGCTGTGACGCGAGGGCGCCCGGTTCTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAAGTGCAGGACGAGGC
171 AGCGCGGTATCGTGGCTGGCCACGACGGCGCTTCTTGCAGAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAA
172 GTGCCGGGCGAGGATCTCTGTCTACCTTGCTCTGCCGAGAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGG
173 CTACCTGCCCATTCGACCAAGTGAAGTGCATCGCAGGAGTCTCGGATGGAAGCCGCTTGTGCTGAGGATGATCTGACGATCGGACGAAGA
174 GCATCAGGGGCTCGGCCAGCCGAAGTGTTCGCCAGGCTCAAGGGCGCATGCCCCAGCGGAGGATCTCGTGTGACCCATGGCGATGCCTGCTTG
175 CCGAATATCATGGTGAAAATGGCCGCTTTTCTGGATTTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCC

176 GTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCTCTGCTTTACGGTATCGCCGCTCCCGATTTCGAGCGCATCGCCTTCTATCG
177 CCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCAACCTGCCATCACGAGATTTTCGATTCCACCGCCG
178 CCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCGGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCACCC
179 CAACTTGTTTATTGTCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTCTACTGCATTCTAGTTGTGGT
180 TTGTCCAAACTCATCAATGTATCTTATCATGTCTGTATACCGTCGACCTCTAGCTAGAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAA
181 ATTGTTATCCGCTCACAATTTCCACACACATACGAGCCGGAAGCATAAAGTGTAAGCCTGGGGTGCCATATGAGTGAGCTAACTCACATTAATTGC
182 GTTGGCGTCACTCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGCGGTTTCGCTATTGGG
183 CGCTCTTCCGCTTCTCTGCTCACTGACTCGCTCGCTCGGTCGTTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCA
184 CAGAATCAGGGGATAACGCGAGGAAGAATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAGGCCGCGTTGCTGGCGTTTTTCCATAG
185 GCTCCGCCCCCTGACGAGCATCACAAAATCAGCGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGA
186 AGCTCCCTCGTGCGCTCTCTGTTCGACCCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAC
187 GCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTTCGCTCCAGCTGGGCTGTGTGCACGAACCCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAA
188 CTATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCT
189 ACAGAGTTCTTGAAGTGGTGGCTAACTACGGGTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAGAAAGAG
190 TTGGTAGCTCTTGATCCGGCAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGA
191 AGATCCTTTGATCTTTTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCAGCTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACC
192 TAGATCCTTTTAAATTAATAATGAAGTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCAC
193 CTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAG
194 TGCTGCAATGATACCGCGAGACCACGCTCACC GGCTCCAGATTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCA
195 ACTTTATCCGCCCTCCATCCAGTCTATTAATTGTTGCCGGAAGCTAGAGTAAGTAGTTGCGCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTA
196 CAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCATTGAGCTCCGGTTCCTCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAA
197 AAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTCAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTT
198 ACTGTCTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCC
199 CGCGCTCAATACGGGATAATACCGGCACATAGCAGAACTTTAAAAGTGCTCATCATTTGGAACAGTTCTTCGGGGCGAAAACCTCAAGGATCTT
200 ACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAAACA
201 GGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCTTTTCAATATATTGAAGCATTTATCAGG
202 GTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGT
203 CGACGGATCGGGAGATCTCCCGATCCCTATGGTGCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGTATCTGCTCCCTGCTGTG
204 TGTGGAGGTGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGGCAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCG
205 TTTTGCGTGTCTCGCGATGTACGGGCCAGATATACGCGTT

206

207 **M^{pro} protease constructs details:**

208 *WT M^{pro} Sequence:*

209 As expressed from the pLVX-EF1alpha-SARS-CoV-2-nsp5-2xStrep-IRES-Puro¹ plasmid

210 MSGFRKMAFPSGKVEGCMVQVTCGTTTTLNGLWLDDVVYCPRHVICTSEDMLNPNYEDLLIRKSN

211 HNFLVQAGNVQLRVIGHSMQNCVLKLKVD TANPKTPKYKFVRIQPGQTFSVLACYNGSPSGVYQ

212 CAMRPNFTIKGSFLNGSCGSVGFNIDYDCVSFCYMHMELPTGVHAGTDLEGNFYGPVDRQTA

213 QAAGTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNYEPLTQDHVDILGPLSA

214 QTGIAVLDMCASLKELLQNGMNGRTILGSALLEDEFTPFDVVRQCSGVTFQLEGGGGWSHPQFE

215 KGGSGGGSGGGWSHPQFEK

216

217 *C145A mutant M^{pro} Sequence:*

218 As expressed from the pLVX-EF1alpha-SARS-CoV-2-nsp5-C145A-2xStrep-IRES-Puro¹

219 plasmid

220 MSGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCPRHVICTSEDMLNPNYEDLLIRKSN
221 HNFLVQAGNVQLRVIGHSMQNCVLKLKVD TANPKTPKYKFVRIQPGQTF SVLACYNGSPSGVYQ
222 CAMRPNFTIKGSFLNGSAGSVGFNIDYDCVSFCYMHMELPTGVHAGTDLEGNFYGPFVDRQTA
223 QAAGTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNYEPLTQDHVDILGPLSA
224 QTGIAVLDMCASLKELLQNGMNGRTILGSALLEDEFTPFDVVRQCSGVTFQLEGGGGWSHPQFE
225 KGGGSGGGSGGGSGWSHPQFEK

226

227 Key:
228 Green: M^{pro}
229 Yellow: Strep-tag II
230 Red: C145A mutation

231

232

Supplementary Methods

Dynamic Light Scattering (DLS) measurements

DLS measurements of recombinantly purified proteins were performed using the Zetasizer Nano ZS (Malvern Panalytical, Malvern, United Kingdom). Proteins, either bovine serum albumin (BSA; Tocris Bioscience, Cat. No. 5217) or the short M^{pro} sensor, were prepared in 1× TBS at a final concentration of 1 μM and 400 nM, respectively and light scattering measurements were performed. The purified short M^{pro} biosensor protein was centrifuged prior to measurement at 14000 rpm for 1 hour at 4 °C and supernatant taken to remove any aggregates. Multiple size spectra ($N = 40$ for BSA and 61 for the short M^{pro} biosensor) obtained from triplicate measurements for 5 s were used for the determination of average molecular size of the proteins.

Script used for determining FlipGFP-based M^{pro} sensor activity in live cells

The following script was used for determining the percentage of GF positive (GFP⁺), number of transfected cells per image frame and total number of cells analyzed for each time points.

```
dir_img = getDirectory("Choose your directory of images");
list_img = getFileList(dir_img);
setBatchMode(true);
for (i=0; i<list_img.length; i++) {
    open(dir_img + list_img[i]);
    name=getTitle;
    run("Split Channels");
    selectWindow("C1-" + name);
    run("Duplicate...", "title=duplicate");
    selectWindow("duplicate");
    run("Subtract Background...", "rolling=5");
    run("Enhance Contrast...", "saturated=0.3");
```

```

267 run("Gaussian Blur...", "sigma=1");
268 run("Auto Local Threshold", "method=Bernsen radius=5 parameter_1=0
269 parameter_2=0 white");
270 run("Analyze Particles...", "size=5-Infinity circularity=0.10-1.00
271 add");
272
273 selectWindow("duplicate");
274 run("Close");
275
276 selectWindow("C1-" +name);
277 roiManager("Measure");
278 //print("C1-" +name);
279 selectWindow("C2-" +name);
280 roiManager("Measure");
281
282     selectWindow("Results");
283     saveAs("Text", dir_img+name+".txt");
284     run("Close");
285
286 roiManager("Delete");
287
288 selectWindow("C1-" +name);
289 run("Close");
290
291 selectWindow("C2-" +name);
292 run("Close");
293 }
294 run("Close All");
295
296

```

297 **Bacterial expression and purification of M^{pro} sensor**

298 The mNG-M^{pro}-Nter-auto-NLuc (short) sensor was expressed in *Escherichia coli* (*E. coli*) BL21-
 299 CodonPlus cells (Agilent Technologies) in 100 mL of LB medium, as described previously².
 300 Protein expression was induced by the addition of 0.5 mM isopropyl- β -D-thiogalactopyranoside
 301 (IPTG), followed by overnight incubation at 20°C. After harvesting the cells by centrifugation
 302 (10000 g, 10 min, 4°C), the pellet was resuspended in lysis buffer (10 mL per gram cell pellet; 10
 303 mM phosphate buffer, 2.7 mM KCl, 507 mM NaCl, 10% glycerol, 20 mM imidazole and 0.1 mM
 304 DTT), followed by sonication. The supernatant was collected after centrifugation (18000 g, 90

min, 45°C). The sensor construct was purified using Ni-NTA affinity chromatography. The concentration of the sensor was determined using Bradford assay³.

In vitro enzyme kinetics measurements

To determine the initial reaction velocity, a range of the short M^{pro} sensor concentrations were incubated with 200 and 500 nM of the recombinantly purified M^{pro} protein in a buffer containing 50 mM HEPES, 50 mM NaCl, 0.1% Triton X-100, 1 mM 1 mM Dithiothreitol (DTT) & 1 mM ethylenediamine tetraacetic acid (EDTA) in a total volume of 50 µL for 2.25 h at 37°C. Following incubation, each reaction was diluted to a final concentration of 20 nM of the M^{pro} sensor and BRET measured using a Tecan SPARK® multimode microplate reader after addition of NLuc substrate. For initial reaction velocity calculation at each sensor (substrate) concentration, a background BRET value of 0.25 (obtained using NLuc alone) was subtracted from the initial as well as final BRET values. Reaction rates were then calculated as:

$$Rate = [(BRET^{Initial} - BRET^{Final}) \times [M^{pro} \text{ sensor}]] / time$$

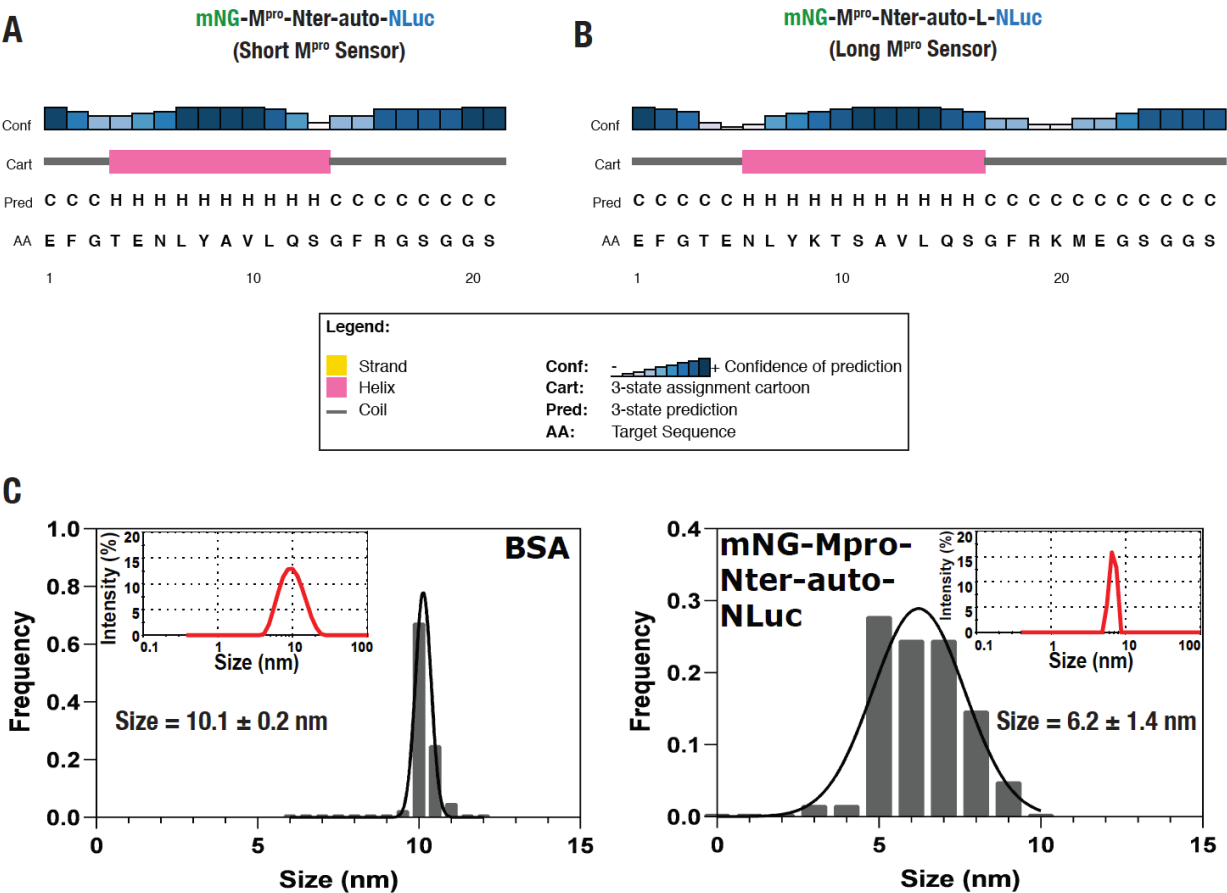
where Rate = reaction velocity at a M^{pro} sensor concentration

BRET^{initial} and BRET^{final} = initial and final BRET ratio, respectively,

[M^{pro} sensor] = concentration of M^{pro} sensor

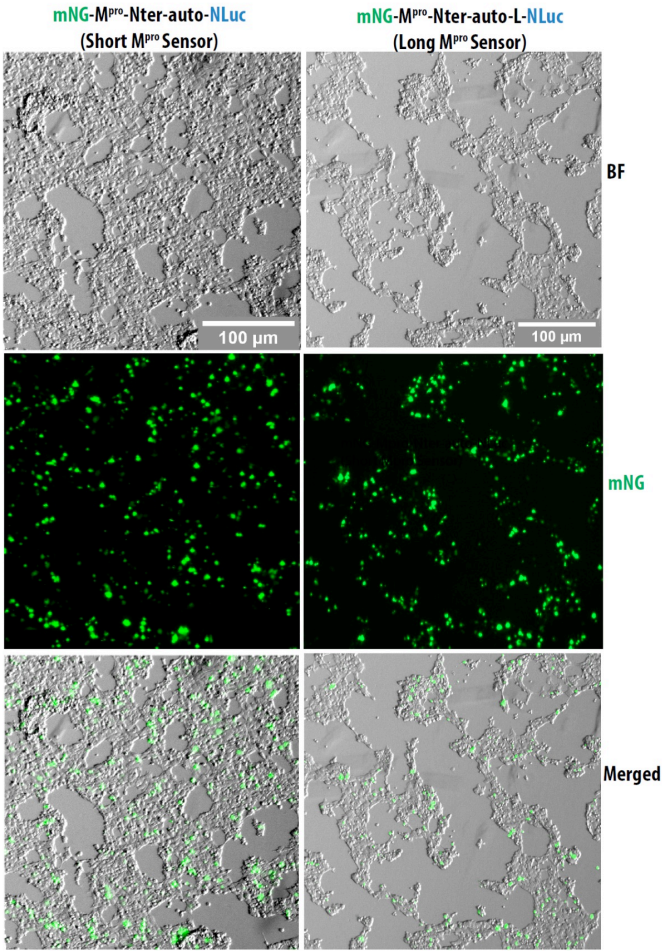
time = incubation time

Supplementary Figures



Supplementary Figure 1. Secondary structure prediction of the linkers and Dynamic Light Scattering (DLS) measurements of M^{pro} sensor. (A,B) Secondary structure prediction of the short (A) and long (B) M^{pro} BRET biosensor linkers containing M^{pro} cleavage sites. (C) Graph showing frequency distribution of size (diameter, nm) of BSA (left panel) and the short M^{pro} biosensor (right panel) determined from multiple ($N = 40$ for BSA and 61 from the short M^{pro} biosensor) DLS measurements. Insets in the respective graphs show a representative measurement.

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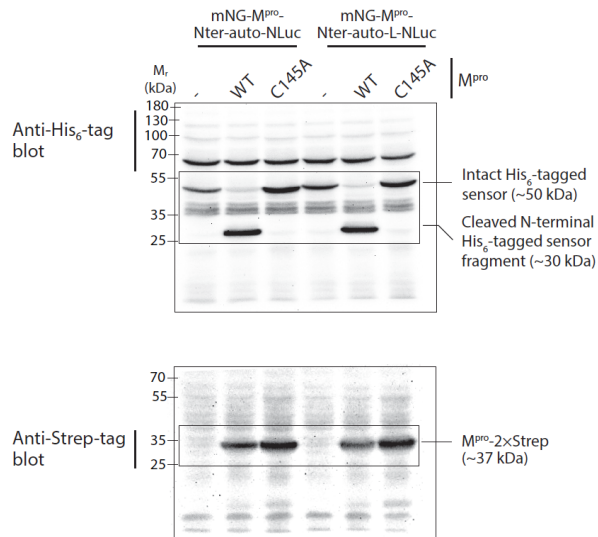
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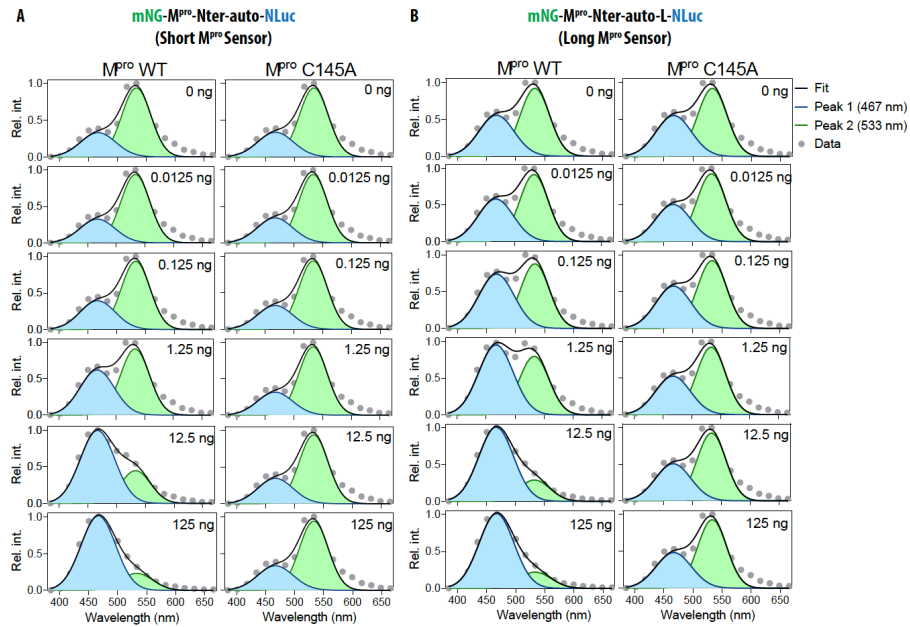
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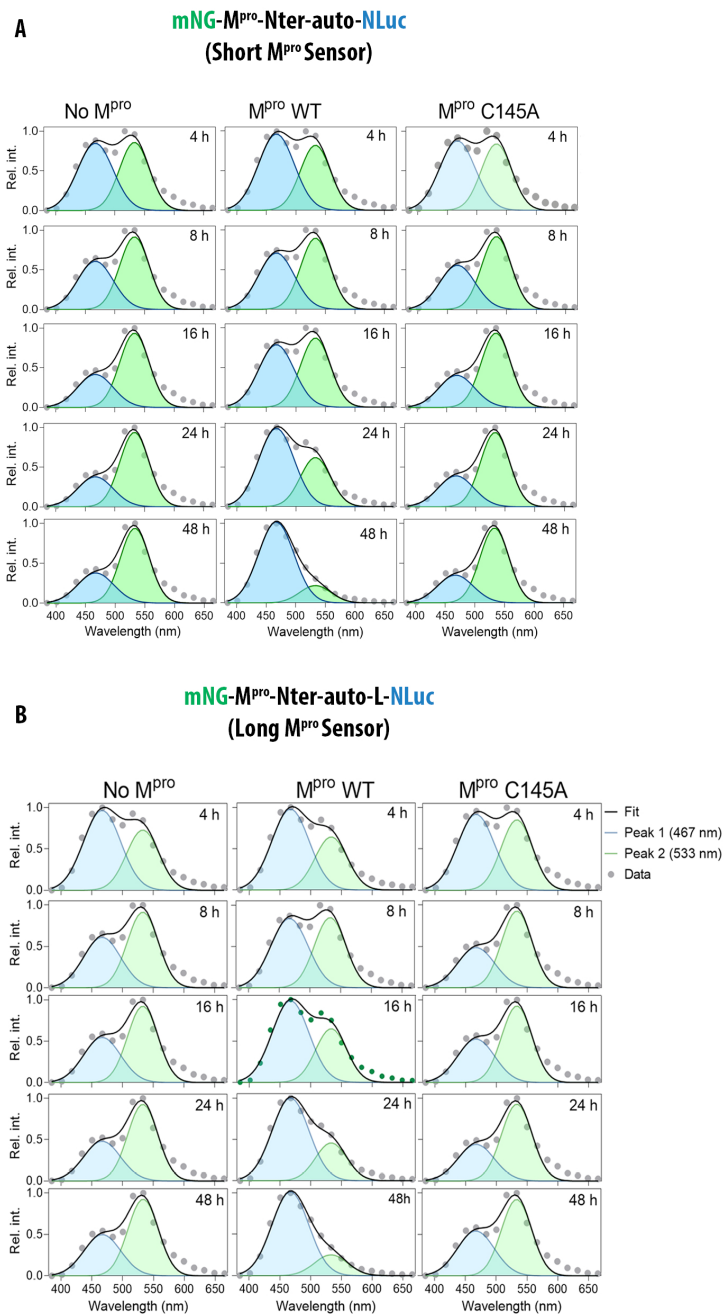
Supplementary Figure 2. Fluorescence image of live cells showing expression of the M^{pro} sensor. Epifluorescence images acquired using a 4× objective of HEK 293T cells transfected with either pmNG-M^{pro}-Nter-auto-NLuc (short; left panel) or pmNG-M^{pro}-Nter-auto-L-NLuc (long; right panel) plasmids showing robust expression of the sensor constructs in these cells.



Supplementary Figure 3. Western blot images of the M^{pro} sensor constructs and M^{pro} . Full western blot images used for preparing Fig. 3H top and bottom panels, respectively. Cropped regions used for preparing Fig. 3H are indicated with the rectangles in respective blots.



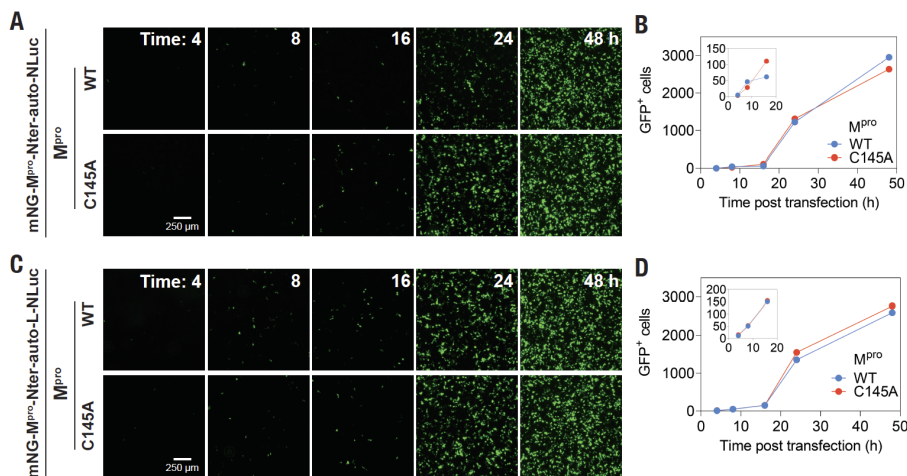
Supplementary Figure 4. M^{pro} plasmid DNA dose-dependent cleavage of the M^{pro} sensors in live cells. (A,B) Graphs showing bioluminescence spectra of the short (A) and long (B) M^{pro} sensor constructs in cells expressing either the WT or C145A mutant M^{pro} protease. Data were fit to a two Gaussian model reflecting mNG fluorescence and NLuc bioluminescence peaks. Note the dose-dependent cleavage of both the short (A) as well as the long (B) sensors specifically in the presence of the WT M^{pro} as reflected by the reduction in the mNG peak (533 nm) of the sensor constructs. Data shown are mean \pm S.D. from a representative of independent experiments, with each experiment performed in triplicates.



Supplementary Figure 5. Temporal dynamics of M^{pro} protease activity in live cells. (A, B)

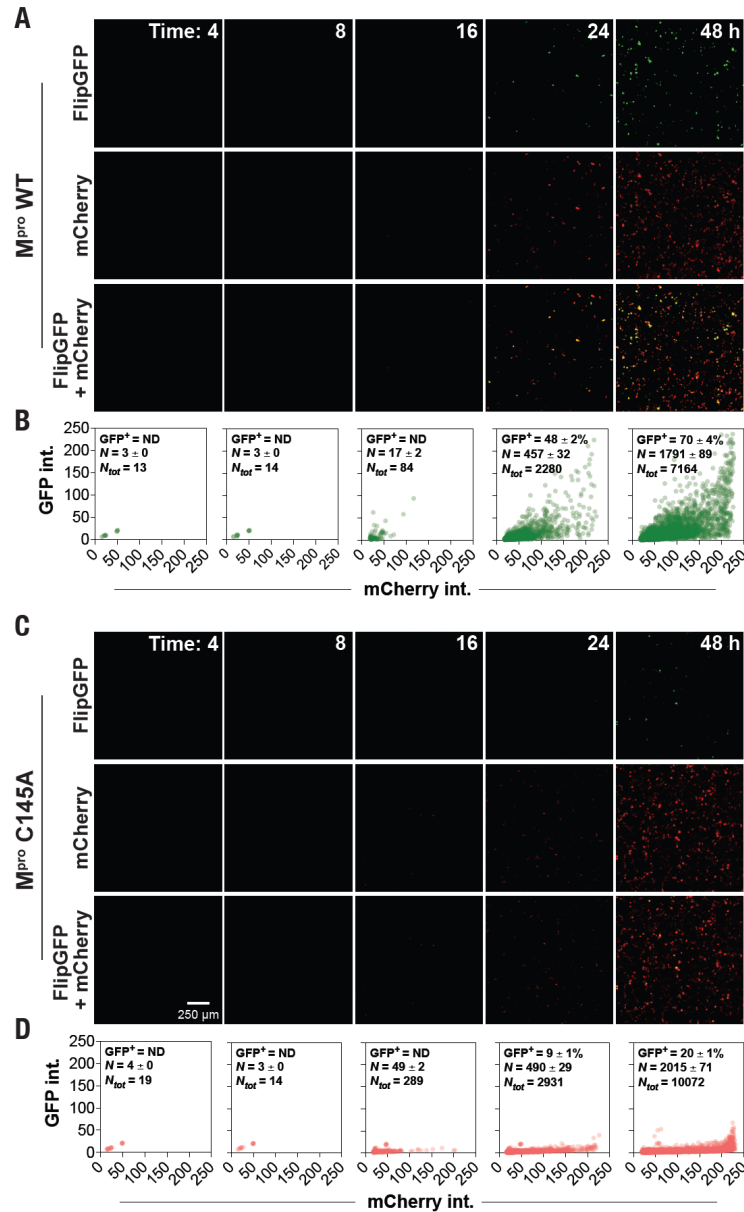
Graphs showing bioluminescence spectra of the short (A) and long (B) M^{pro} sensor constructs either in control cells or in cells expressing the WT or C145A mutant M^{pro} protease. Data were fit to a two Gaussian model reflecting mNG fluorescence and NLuc bioluminescence peaks. Note the time-dependent cleavage of short (A) and long (B) sensors specifically in the presence

of the WT M^{pro} as reflected by the reduction in the mNG peak (533 nm) of the sensor constructs. Data shown are mean \pm S.D. from a representative of independent experiments performed thrice.



Supplementary Figure 6. Time-dependent expression of the BRET-based M^{pro} sensors.

(A,C) Epifluorescence images acquired using a 4 \times objective of HEK 293T cells transfected with either pmNG- M^{pro} -Nter-auto-NLuc (short; A) or pmNG- M^{pro} -Nter-auto-L-NLuc (long; C) plasmids showing a time-dependent increase in the number of cells expressing the sensors. (B,D) Graph showing time-dependent increase in GFP+ cells after transfection with either pmNG- M^{pro} -Nter-auto-NLuc (short; B) or pmNG- M^{pro} -Nter-auto-L-NLuc (long; D) plasmids. Data shown are mean \pm S.D. from a representative of independent experiments, with each experiment performed in triplicates.



Supplementary Figure 7. Monitoring M^{pro} proteolytic activity using the FlipGFP-based

M^{pro} sensor in live cells. (A) Epifluorescence images of cells showing time-dependent

expression of GFP, which is converted from the non-fluorescent FlipGFP upon proteolytic

cleavage by M^{pro} (top panel), mCherry (middle panel) and merge (bottom panel) in cells

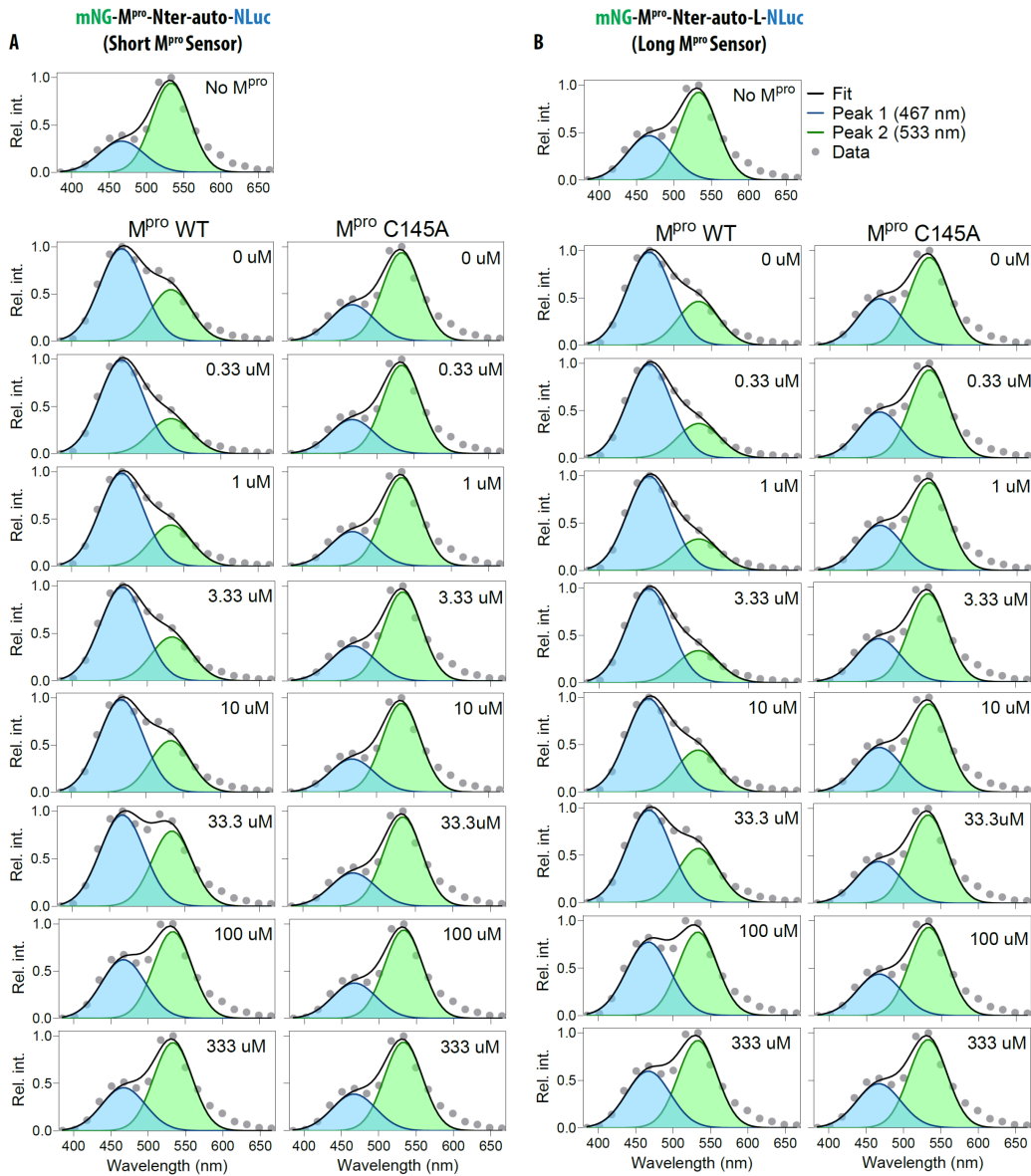
transfected with the WT M^{pro}. (B) Graphs showing GFP and mCherry fluorescence in individual

cells transfected with the WT M^{pro} at the indicated time points. (C) Epifluorescence images of

389 cells showing time-dependent expression of GFP (top panel), mCherry (middle panel) and merge
390 (bottom panel) in cells transfected with the C145A mutant M^{pro}. (D) Graphs showing GFP and
391 mCherry fluorescence in individual cells transfected with the C145A mutant M^{pro} at the indicated
392 time points. (E) Graph showing % GFP⁺ cells at the indicated time post transfection with either
393 the wild type or the C145A mutant M^{pro}.

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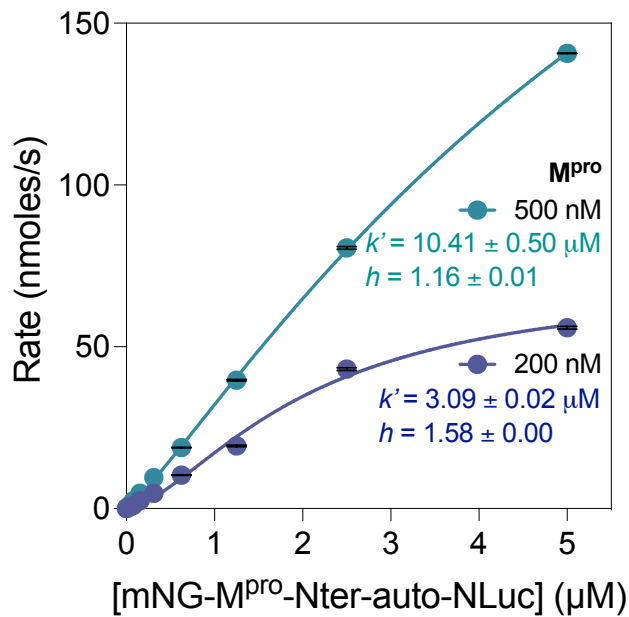
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Supplementary Figure 8. GC376-mediated M^{pro} inhibition monitored in live cells. (A,B)

Graphs showing bioluminescence spectra of the short (A) and long (B) M^{pro} sensor constructs in cells treated with the indicated concentrations of GC376 inhibitor in cells co-expressing either the WT or the C145A mutant M^{pro} protease. The bioluminescence spectra of the No M^{pro} control is also shown. Data were fit to a two Gaussian model reflecting mNG fluorescence and NLuc

bioluminescence peaks. Note the dose-dependent inhibition of protease activity of WT M^{pro} in cleaving both the short (A) as well as the long (B) sensors which is evident from the increased intensity of mNG peak (533 nm) of the sensor constructs. Data shown are mean \pm S.D. from a representative of independent experiments, with each experiment performed in triplicates.



Supplementary Figure 9. In vitro enzyme kinetics assay using the short M^{pro} sensor. Graphs showing kinetic measurements of the short M^{pro} sensor cleavage in reactions containing the indicated concentrations of M^{pro}. Data plotted are average of four measurements \pm SD and fit to the allosteric sigmoidal equation in GraphPad Prism. Note the decrease in the Hill coefficient (h) at 500 nM of M^{pro}.

417 **Supplementary References**

- 418 1. Gordon, D.E. et al. A SARS-CoV-2 protein interaction map reveals targets for drug
419 repurposing. *Nature* (2020).
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421 Intracellular Caspase Activity. *ACS Sens* **2**, 729-734 (2017).
- 422 3. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram
423 quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248-
424 54 (1976).
- 425