

Supplementary Information

Impact of SARS-CoV-2 3CL Protease Mutations on Nirmatrelvir Inhibitory Efficiency. Computational Insights into Potential Resistance Mechanisms

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Table S1. Contribution of each residue to the MMGBSA binding free energy of nirmaltrelvir (g_I) and the natural substrate (g_S) in the active site of wild type 3CLpro. Side-chain only contributions are also provided ($g_{I,sc}$ and $g_{S,sc}$) Values are given in kcal·mol⁻¹. The standard deviation is given in parenthesis.

Residue	g_I	$g_{I,sc}$	g_S	$g_{S,sc}$
S1	3.47 (0.1443)	0.15 (0.0119)	2.56 (0.0962)	0.02 (0.0752)
G2	-0.11 (0.0063)	-0.01 (0.0010)	-0.08 (0.0142)	-0.00 (0.0018)
F3	-0.02 (0.0019)	-0.02 (0.0008)	-0.02 (0.0017)	-0.03 (0.0009)
R4	-0.50 (0.0108)	-0.43 (0.0084)	-0.86 (0.0358)	-0.55 (0.0411)
K5	-0.31 (0.0075)	-0.25 (0.0060)	-0.66 (0.0307)	-0.38 (0.0345)
M6	-0.01 (0.0013)	-0.01 (0.0009)	-0.01 (0.0011)	-0.02 (0.0013)
A7	0.01 (0.0004)	-0.02 (0.0002)	0.02 (0.0032)	-0.02 (0.0015)
F8	0.01 (0.0006)	-0.01 (0.0002)	0.02 (0.0018)	-0.01 (0.0008)
P9	-0.01 (0.0003)	-0.10 (0.0009)	-0.02 (0.0018)	-0.10 (0.0090)
S10	-0.00 (0.0001)	-0.02 (0.0003)	-0.00 (0.0004)	-0.01 (0.0011)
G11	-0.01 (0.0002)	0.01 (0.0001)	-0.01 (0.0016)	0.01 (0.0009)
K12	-0.43 (0.0037)	-0.32 (0.0028)	-0.41 (0.0318)	-0.22 (0.0252)
V13	-0.00 (0.0002)	-0.01 (0.0002)	-0.01 (0.0011)	-0.01 (0.0009)
E14	0.53 (0.0049)	0.45 (0.0044)	0.57 (0.0388)	0.38 (0.0328)
G15	-0.01 (0.0002)	0.01 (0.0001)	-0.01 (0.0007)	0.01 (0.0007)
C16	-0.01 (0.0003)	-0.01 (0.0001)	-0.03 (0.0010)	-0.01 (0.0007)
M17	-0.03 (0.0024)	-0.03 (0.0022)	-0.01 (0.0041)	-0.04 (0.0029)
V18	0.05 (0.0009)	-0.01 (0.0001)	0.06 (0.0072)	-0.00 (0.0004)
Q19	-0.06 (0.0032)	-0.04 (0.0035)	0.03 (0.0259)	0.04 (0.0245)
V20	0.06 (0.0015)	-0.02 (0.0003)	-0.10 (0.0102)	-0.02 (0.0020)
T21	-0.01 (0.0052)	-0.01 (0.0065)	-0.04 (0.0231)	-0.16 (0.0177)
C22	-0.02 (0.0018)	-0.01 (0.0009)	-0.03 (0.0169)	-0.02 (0.0040)
G23	0.03 (0.0013)	0.00 (0.0003)	-0.07 (0.0196)	-0.02 (0.0091)
T24	0.03 (0.0016)	-0.01 (0.0021)	-1.85 (0.2788)	-1.29 (0.2037)
T25	-0.14 (0.0126)	-0.07 (0.0133)	-3.53 (0.5208)	-2.81 (0.3626)
T26	0.21 (0.0075)	-0.04 (0.0063)	-5.84 (0.7846)	-2.21 (0.1831)
L27	-0.79 (0.0063)	-0.64 (0.0057)	-2.28 (0.1128)	-1.67 (0.0330)
N28	-0.29 (0.0061)	-0.26 (0.0060)	-0.54 (0.0425)	-0.32 (0.0249)
G29	0.04 (0.0006)	0.01 (0.0003)	0.04 (0.0030)	-0.01 (0.0014)
L30	-0.01 (0.0002)	-0.02 (0.0003)	-0.03 (0.0009)	-0.02 (0.0009)
W31	0.01 (0.0002)	0.00 (0.0000)	0.01 (0.0008)	-0.01 (0.0008)
L32	-0.01 (0.0001)	-0.01 (0.0001)	-0.01 (0.0005)	-0.00 (0.0006)
D33	0.23 (0.0034)	0.18 (0.0026)	0.08 (0.0177)	0.04 (0.0101)
D34	0.17 (0.0033)	0.13 (0.0026)	-0.03 (0.0132)	-0.02 (0.0089)
V35	0.01 (0.0002)	-0.00 (0.0001)	0.04 (0.0014)	-0.00 (0.0003)
V36	-0.02 (0.0007)	-0.01 (0.0001)	-0.01 (0.0012)	-0.00 (0.0004)
Y37	0.03 (0.0009)	-0.02 (0.0002)	0.04 (0.0025)	-0.02 (0.0009)

C38	-0.08 (0.0026)	-0.02 (0.0007)	-0.00 (0.0061)	-0.05 (0.0038)
P39	-0.05 (0.0026)	-0.19 (0.0022)	-0.19 (0.0129)	-0.00 (0.0134)
R40	0.57 (0.0098)	0.42 (0.0099)	1.12 (0.0215)	0.62 (0.0143)
H41	-2.96 (0.1191)	-2.92 (0.1148)	-6.12 (0.0804)	-6.29 (0.1539)
V42	-0.10 (0.0036)	-0.06 (0.0011)	-0.42 (0.0169)	-0.25 (0.0162)
I43	0.00 (0.0029)	-0.01 (0.0006)	-0.00 (0.0032)	-0.04 (0.0038)
C44	-0.05 (0.0164)	-0.14 (0.0169)	-0.19 (0.0266)	-0.20 (0.0134)
T45	0.01 (0.0071)	0.00 (0.0021)	-0.08 (0.0219)	-0.46 (0.1465)
S46	0.03 (0.0051)	0.01 (0.0014)	-0.08 (0.0125)	-0.03 (0.0142)
E47	-0.38 (0.0283)	-0.29 (0.0236)	-0.48 (0.0433)	-0.23 (0.0674)
D48	-0.37 (0.0670)	-0.22 (0.0597)	-0.58 (0.1177)	-0.09 (0.1447)
M49	-3.13 (0.2395)	-2.68 (0.2007)	-3.83 (0.0821)	-3.05 (0.1482)
L50	-0.59 (0.1621)	-0.44 (0.1395)	-0.41 (0.0183)	-0.26 (0.0080)
N51	-0.04 (0.0208)	-0.00 (0.0056)	0.04 (0.0027)	-0.01 (0.0024)
P52	-0.08 (0.0066)	0.07 (0.0036)	-0.07 (0.0044)	0.12 (0.0075)
N53	0.01 (0.0047)	-0.01 (0.0054)	0.02 (0.0018)	0.00 (0.0025)
Y54	-0.29 (0.0296)	-0.31 (0.0304)	-0.25 (0.0122)	-0.32 (0.0255)
E55	-0.24 (0.0318)	-0.22 (0.0294)	-0.43 (0.0124)	-0.33 (0.0152)
D56	-0.15 (0.0114)	-0.13 (0.0091)	-0.36 (0.0157)	-0.23 (0.0285)
L57	0.03 (0.0009)	0.00 (0.0003)	0.04 (0.0010)	0.01 (0.0024)
L58	0.01 (0.0012)	-0.00 (0.0002)	0.01 (0.0009)	0.00 (0.0007)
I59	0.01 (0.0003)	0.00 (0.0002)	0.01 (0.0005)	0.00 (0.0008)
R60	0.14 (0.0066)	0.12 (0.0046)	0.48 (0.0245)	0.21 (0.0508)
K61	0.05 (0.0083)	0.05 (0.0067)	0.73 (0.0403)	0.25 (0.1220)
S62	0.00 (0.0004)	0.00 (0.0002)	-0.02 (0.0014)	0.00 (0.0021)
N63	0.01 (0.0003)	-0.00 (0.0003)	-0.00 (0.0013)	0.00 (0.0005)
H64	0.01 (0.0003)	0.01 (0.0002)	0.00 (0.0012)	-0.01 (0.0016)
N65	0.01 (0.0004)	-0.01 (0.0002)	0.01 (0.0019)	-0.00 (0.0057)
F66	-0.02 (0.0006)	-0.01 (0.0002)	0.01 (0.0030)	-0.04 (0.0026)
L67	0.02 (0.0005)	-0.01 (0.0002)	-0.04 (0.0026)	-0.02 (0.0040)
V68	-0.02 (0.0002)	-0.01 (0.0001)	-0.00 (0.0022)	-0.01 (0.0005)
Q69	0.02 (0.0005)	-0.01 (0.0004)	0.00 (0.0022)	0.01 (0.0059)
A70	-0.01 (0.0002)	-0.01 (0.0001)	-0.02 (0.0016)	-0.00 (0.0006)
G71	-0.00 (0.0003)	0.01 (0.0001)	-0.02 (0.0019)	0.00 (0.0006)
N72	-0.00 (0.0002)	0.00 (0.0001)	-0.00 (0.0008)	0.00 (0.0005)
V73	0.01 (0.0001)	-0.00 (0.0001)	0.01 (0.0016)	-0.00 (0.0002)
Q74	-0.01 (0.0003)	-0.01 (0.0002)	0.02 (0.0028)	0.01 (0.0013)
L75	0.00 (0.0002)	-0.01 (0.0001)	-0.01 (0.0013)	-0.00 (0.0005)
R76	-0.14 (0.0034)	-0.10 (0.0023)	0.12 (0.0119)	0.02 (0.0197)
V77	-0.00 (0.0002)	-0.00 (0.0001)	0.01 (0.0007)	0.00 (0.0011)
I78	-0.01 (0.0001)	-0.00 (0.0001)	-0.01 (0.0007)	0.00 (0.0004)
G79	0.01 (0.0001)	0.00 (0.0001)	0.01 (0.0007)	-0.00 (0.0007)
H80	0.01 (0.0008)	0.01 (0.0006)	0.03 (0.0008)	0.00 (0.0023)
S81	0.01 (0.0005)	-0.00 (0.0001)	-0.01 (0.0010)	0.00 (0.0008)
M82	-0.00 (0.0009)	-0.02 (0.0007)	0.00 (0.0016)	-0.03 (0.0011)

Q83	0.01 (0.0008)	0.00 (0.0005)	0.00 (0.0007)	0.02 (0.0015)
N84	-0.01 (0.0020)	-0.01 (0.0009)	-0.02 (0.0025)	-0.00 (0.0010)
C85	-0.06 (0.0030)	-0.03 (0.0013)	-0.09 (0.0023)	-0.01 (0.0005)
V86	-0.04 (0.0010)	-0.02 (0.0003)	-0.01 (0.0015)	-0.02 (0.0005)
L87	0.01 (0.0005)	-0.01 (0.0004)	0.01 (0.0028)	0.00 (0.0014)
K88	-0.12 (0.0059)	-0.08 (0.0040)	0.14 (0.0166)	0.08 (0.0097)
L89	0.00 (0.0003)	-0.01 (0.0002)	0.01 (0.0011)	-0.00 (0.0011)
K90	-0.14 (0.0032)	-0.10 (0.0023)	0.06 (0.0117)	0.03 (0.0090)
V91	-0.01 (0.0001)	-0.01 (0.0001)	0.00 (0.0007)	0.00 (0.0003)
D92	0.16 (0.0030)	0.14 (0.0025)	-0.06 (0.0120)	-0.01 (0.0157)
T93	0.00 (0.0002)	-0.00 (0.0002)	0.00 (0.0005)	0.00 (0.0005)
A94	-0.00 (0.0001)	-0.01 (0.0001)	0.00 (0.0004)	-0.00 (0.0003)
N95	-0.01 (0.0003)	-0.01 (0.0003)	-0.00 (0.0014)	0.00 (0.0011)
P96	-0.00 (0.0001)	-0.05 (0.0005)	-0.00 (0.0004)	-0.01 (0.0020)
K97	-0.34 (0.0031)	-0.28 (0.0025)	-0.21 (0.0224)	-0.14 (0.0151)
T98	-0.00 (0.0001)	-0.00 (0.0001)	-0.00 (0.0004)	-0.00 (0.0002)
P99	-0.01 (0.0001)	-0.06 (0.0007)	-0.02 (0.0011)	-0.04 (0.0042)
K100	-0.23 (0.0026)	-0.18 (0.0021)	-0.16 (0.0184)	-0.08 (0.0152)
Y101	-0.01 (0.0001)	-0.01 (0.0001)	-0.01 (0.0008)	-0.01 (0.0004)
K102	-0.21 (0.0034)	-0.17 (0.0026)	-0.20 (0.0194)	-0.11 (0.0183)
F103	-0.01 (0.0003)	-0.01 (0.0001)	-0.01 (0.0011)	-0.01 (0.0003)
V104	0.01 (0.0003)	-0.01 (0.0001)	0.01 (0.0010)	-0.01 (0.0005)
R105	-0.13 (0.0092)	-0.08 (0.0074)	-0.01 (0.0237)	0.10 (0.0206)
I106	-0.01 (0.0003)	-0.01 (0.0004)	-0.02 (0.0007)	-0.01 (0.0011)
Q107	0.02 (0.0004)	-0.00 (0.0002)	0.03 (0.0010)	-0.00 (0.0007)
P108	-0.01 (0.0005)	-0.04 (0.0014)	-0.01 (0.0008)	-0.03 (0.0048)
G109	-0.01 (0.0003)	0.01 (0.0002)	0.00 (0.0006)	0.01 (0.0008)
Q110	0.02 (0.0010)	-0.00 (0.0011)	0.02 (0.0016)	-0.01 (0.0014)
T111	-0.01 (0.0003)	-0.01 (0.0002)	-0.03 (0.0010)	-0.01 (0.0010)
F112	-0.01 (0.0002)	-0.03 (0.0003)	-0.00 (0.0005)	-0.04 (0.0005)
S113	-0.01 (0.0007)	-0.02 (0.0004)	-0.02 (0.0007)	-0.02 (0.0017)
V114	-0.04 (0.0010)	-0.03 (0.0004)	-0.02 (0.0016)	-0.03 (0.0013)
L115	0.04 (0.0014)	-0.02 (0.0001)	0.07 (0.0044)	-0.01 (0.0011)
A116	-0.10 (0.0007)	-0.05 (0.0004)	-0.15 (0.0083)	-0.06 (0.0029)
C117	0.09 (0.0008)	-0.06 (0.0008)	0.24 (0.0161)	-0.08 (0.0057)
Y118	-0.26 (0.0054)	-0.21 (0.0065)	-0.42 (0.0706)	-0.37 (0.0431)
N119	-0.07 (0.0197)	0.06 (0.0147)	-1.28 (0.3739)	-0.85 (0.2201)
G120	-0.06 (0.0012)	0.02 (0.0003)	-0.07 (0.0100)	-0.01 (0.0020)
S121	0.04 (0.0011)	-0.02 (0.0003)	0.08 (0.0065)	-0.00 (0.0035)
P122	-0.03 (0.0005)	-0.11 (0.0010)	-0.05 (0.0038)	-0.09 (0.0092)
S123	-0.03 (0.0036)	-0.01 (0.0033)	-0.03 (0.0032)	0.00 (0.0023)
G124	0.03 (0.0013)	0.02 (0.0002)	0.04 (0.0026)	0.02 (0.0016)
V125	-0.01 (0.0003)	-0.01 (0.0001)	0.00 (0.0006)	-0.01 (0.0007)
Y126	-0.03 (0.0006)	-0.07 (0.0005)	-0.08 (0.0025)	-0.10 (0.0044)
Q127	0.03 (0.0010)	0.01 (0.0012)	0.04 (0.0026)	0.00 (0.0019)

C128	0.01 (0.0010)	0.01 (0.0005)	0.00 (0.0010)	0.00 (0.0009)
A129	0.03 (0.0005)	-0.01 (0.0002)	0.04 (0.0010)	-0.01 (0.0012)
M130	-0.02 (0.0007)	-0.02 (0.0005)	-0.02 (0.0019)	-0.03 (0.0017)
R131	-0.26 (0.0089)	-0.21 (0.0067)	-0.48 (0.0189)	-0.28 (0.0285)
P132	-0.00 (0.0003)	-0.04 (0.0015)	-0.02 (0.0005)	-0.04 (0.0053)
N133	0.05 (0.0018)	0.02 (0.0009)	-0.00 (0.0041)	-0.00 (0.0031)
F134	0.03 (0.0016)	-0.01 (0.0003)	-0.00 (0.0026)	-0.01 (0.0005)
T135	-0.04 (0.0031)	-0.00 (0.0013)	0.02 (0.0026)	0.04 (0.0012)
I136	0.02 (0.0013)	-0.07 (0.0012)	0.02 (0.0015)	-0.07 (0.0027)
K137	-0.30 (0.0104)	-0.12 (0.0077)	-0.62 (0.0213)	-0.25 (0.0295)
G138	-0.27 (0.0049)	0.02 (0.0004)	-0.26 (0.0047)	0.03 (0.0018)
S139	-0.15 (0.0215)	-0.00 (0.0102)	-0.05 (0.0144)	0.00 (0.0130)
F140	-4.02 (0.0423)	-1.36 (0.0158)	-4.89 (0.0913)	-1.24 (0.0094)
L141	-2.25 (0.0245)	-0.93 (0.0117)	-0.38 (0.0284)	-0.76 (0.0305)
N142	-3.09 (0.0784)	-2.17 (0.0215)	-10.01 (0.2917)	-6.00 (0.3014)
G143	-2.54 (0.0100)	-0.15 (0.0045)	-5.41 (0.1983)	-0.79 (0.0218)
S144	-1.15 (0.0471)	-0.64 (0.0437)	-2.52 (0.0882)	-0.84 (0.0856)
C145	-2.97 (0.0379)	-1.74 (0.0626)	-4.22 (0.1120)	-2.29 (0.0499)
G146	-0.06 (0.0037)	0.01 (0.0003)	-0.18 (0.0080)	-0.02 (0.0031)
S147	-0.21 (0.0012)	-0.17 (0.0014)	-0.34 (0.0155)	-0.24 (0.0090)
V148	0.05 (0.0010)	-0.01 (0.0001)	0.08 (0.0022)	-0.01 (0.0007)
G149	-0.01 (0.0002)	0.02 (0.0002)	-0.02 (0.0010)	0.01 (0.0012)
F150	0.01 (0.0003)	-0.02 (0.0002)	0.01 (0.0006)	-0.03 (0.0009)
N151	0.02 (0.0005)	0.01 (0.0005)	0.02 (0.0011)	0.02 (0.0015)
I152	-0.00 (0.0002)	-0.01 (0.0001)	0.01 (0.0009)	-0.01 (0.0013)
D153	0.25 (0.0033)	0.20 (0.0027)	0.29 (0.0241)	0.15 (0.0244)
Y154	0.01 (0.0003)	-0.01 (0.0002)	0.01 (0.0010)	-0.00 (0.0015)
D155	0.28 (0.0037)	0.22 (0.0029)	0.24 (0.0243)	0.11 (0.0205)
C156	0.01 (0.0001)	0.00 (0.0001)	0.01 (0.0007)	-0.00 (0.0004)
V157	-0.01 (0.0001)	-0.01 (0.0001)	-0.02 (0.0008)	-0.00 (0.0005)
S158	0.01 (0.0002)	-0.01 (0.0002)	0.02 (0.0006)	-0.01 (0.0014)
F159	-0.03 (0.0003)	-0.02 (0.0002)	-0.04 (0.0018)	-0.02 (0.0006)
C160	-0.01 (0.0004)	0.00 (0.0002)	-0.02 (0.0007)	0.00 (0.0006)
Y161	-0.16 (0.0037)	-0.19 (0.0045)	-0.19 (0.0045)	-0.23 (0.0059)
M162	-0.16 (0.0022)	-0.07 (0.0009)	-0.20 (0.0038)	-0.11 (0.0021)
H163	-6.65 (0.0665)	-6.51 (0.0668)	-7.37 (0.0540)	-7.16 (0.0614)
H164	-6.20 (0.1949)	-1.15 (0.0221)	-6.62 (0.1161)	-1.39 (0.0246)
M165	-7.66 (0.0549)	-4.69 (0.0235)	-7.83 (0.0591)	-4.78 (0.0448)
E166	-13.10 (0.0904)	-8.42 (0.1307)	-14.70 (0.1467)	-7.17 (0.1630)
L167	-1.75 (0.0185)	-1.14 (0.0089)	-2.21 (0.1074)	-1.50 (0.0515)
P168	-1.04 (0.0161)	-0.66 (0.0174)	-4.21 (0.0521)	-3.28 (0.0194)
T169	-0.04 (0.0008)	-0.04 (0.0024)	-0.12 (0.0086)	-0.11 (0.0016)
G170	0.08 (0.0029)	-0.04 (0.0013)	0.12 (0.0073)	-0.03 (0.0026)
V171	-0.26 (0.0095)	-0.05 (0.0004)	-0.31 (0.0153)	-0.05 (0.0013)
H172	-1.39 (0.0115)	-0.97 (0.0081)	-1.23 (0.0129)	-0.81 (0.0370)

A173	-0.22 (0.0091)	-0.12 (0.0024)	-0.19 (0.0165)	-0.09 (0.0058)
G174	-0.02 (0.0018)	-0.02 (0.0006)	-0.04 (0.0017)	-0.03 (0.0016)
T175	0.01 (0.0010)	-0.03 (0.0005)	0.02 (0.0055)	-0.04 (0.0019)
D176	0.10 (0.0086)	0.09 (0.0064)	-0.11 (0.0295)	-0.12 (0.0188)
L177	-0.02 (0.0003)	-0.02 (0.0001)	-0.04 (0.0008)	-0.02 (0.0007)
E178	0.11 (0.0055)	0.10 (0.0044)	-0.05 (0.0182)	-0.05 (0.0131)
G179	-0.04 (0.0008)	-0.00 (0.0002)	-0.05 (0.0011)	-0.01 (0.0006)
N180	0.02 (0.0003)	-0.02 (0.0003)	0.03 (0.0009)	-0.02 (0.0009)
F181	-0.21 (0.0041)	-0.23 (0.0038)	-0.25 (0.0083)	-0.27 (0.0088)
Y182	-0.02 (0.0016)	-0.05 (0.0008)	-0.06 (0.0018)	-0.04 (0.0019)
G183	-0.01 (0.0012)	0.00 (0.0004)	-0.02 (0.0013)	-0.01 (0.0007)
P184	-0.02 (0.0012)	-0.05 (0.0035)	-0.05 (0.0017)	0.01 (0.0054)
F185	-0.18 (0.0095)	-0.23 (0.0072)	-0.17 (0.0449)	-0.26 (0.0362)
V186	-0.44 (0.0174)	-0.13 (0.0021)	-0.47 (0.0101)	-0.10 (0.0038)
D187	-2.37 (0.0204)	-1.71 (0.0157)	-2.65 (0.0338)	-2.15 (0.0327)
R188	-0.76 (0.0362)	-0.82 (0.0248)	-0.63 (0.0867)	-0.68 (0.0314)
Q189	-4.13 (0.1323)	-2.84 (0.0751)	-7.31 (0.3966)	-6.56 (0.3784)
T190	-0.97 (0.0935)	-0.38 (0.0339)	-5.38 (0.4954)	-0.84 (0.0648)
A191	-0.50 (0.0396)	-0.24 (0.0212)	-2.23 (0.1079)	-1.33 (0.0601)
Q192	-0.76 (0.0412)	-0.73 (0.0332)	-1.94 (0.4387)	-0.47 (0.1101)
A193	-0.05 (0.0036)	-0.06 (0.0019)	-0.61 (0.1378)	-0.25 (0.0545)
A194	-0.04 (0.0015)	-0.03 (0.0013)	-0.07 (0.0233)	-0.04 (0.0064)
G195	0.01 (0.0011)	0.00 (0.0004)	0.03 (0.0037)	0.00 (0.0007)
T196	0.01 (0.0006)	-0.00 (0.0002)	0.00 (0.0009)	0.00 (0.0005)
D197	0.20 (0.0109)	0.18 (0.0092)	0.42 (0.0147)	0.25 (0.0297)
T198	0.02 (0.0007)	0.00 (0.0006)	0.01 (0.0006)	-0.00 (0.0005)
T199	-0.00 (0.0001)	0.00 (0.0001)	-0.01 (0.0005)	0.00 (0.0002)
I200	-0.00 (0.0002)	-0.01 (0.0002)	0.00 (0.0003)	-0.01 (0.0009)
T201	-0.00 (0.0001)	0.00 (0.0001)	0.01 (0.0005)	0.00 (0.0003)
V202	-0.01 (0.0002)	-0.00 (0.0001)	0.00 (0.0005)	-0.00 (0.0004)
N203	-0.02 (0.0005)	-0.01 (0.0003)	-0.01 (0.0010)	-0.02 (0.0013)
V204	-0.01 (0.0002)	-0.00 (0.0001)	0.01 (0.0007)	-0.00 (0.0004)
L205	-0.01 (0.0001)	-0.00 (0.0001)	0.00 (0.0006)	-0.00 (0.0005)
A206	-0.02 (0.0002)	-0.00 (0.0002)	-0.00 (0.0009)	-0.01 (0.0012)
W207	-0.03 (0.0004)	-0.02 (0.0003)	-0.02 (0.0010)	-0.02 (0.0006)
L208	-0.01 (0.0003)	-0.00 (0.0001)	0.00 (0.0007)	-0.00 (0.0005)
Y209	-0.02 (0.0003)	0.00 (0.0001)	-0.00 (0.0006)	-0.00 (0.0013)
A210	-0.04 (0.0005)	-0.00 (0.0002)	-0.01 (0.0016)	-0.01 (0.0015)
A211	-0.03 (0.0007)	0.00 (0.0002)	-0.01 (0.0014)	-0.01 (0.0012)
V212	-0.02 (0.0011)	-0.00 (0.0001)	0.00 (0.0011)	-0.00 (0.0005)
I213	-0.05 (0.0019)	-0.00 (0.0003)	-0.02 (0.0020)	-0.01 (0.0022)
N214	-0.09 (0.0055)	-0.08 (0.0051)	0.02 (0.0058)	-0.01 (0.0075)
G215	0.00 (0.0004)	-0.01 (0.0003)	-0.00 (0.0009)	-0.00 (0.0014)
D216	-0.10 (0.0089)	-0.07 (0.0074)	0.34 (0.0179)	0.16 (0.0340)
R217	0.22 (0.0082)	0.19 (0.0067)	-0.15 (0.0159)	0.01 (0.0299)

W218	0.00 (0.0005)	-0.00 (0.0002)	-0.01 (0.0007)	-0.01 (0.0007)
F219	0.00 (0.0002)	-0.00 (0.0001)	-0.01 (0.0004)	-0.00 (0.0006)
L220	0.00 (0.0003)	0.00 (0.0001)	-0.00 (0.0004)	-0.00 (0.0007)
N221	-0.01 (0.0010)	-0.00 (0.0007)	-0.00 (0.0003)	-0.00 (0.0005)
R222	0.04 (0.0056)	0.03 (0.0048)	-0.13 (0.0073)	-0.04 (0.0133)
F223	-0.00 (0.0001)	-0.00 (0.0001)	0.00 (0.0003)	-0.00 (0.0002)
T224	0.00 (0.0001)	0.00 (0.0002)	-0.00 (0.0001)	-0.00 (0.0003)
T225	-0.00 (0.0001)	0.00 (0.0000)	0.00 (0.0001)	-0.00 (0.0003)
T226	0.00 (0.0001)	0.00 (0.0000)	0.00 (0.0001)	-0.00 (0.0002)
L227	0.00 (0.0001)	-0.00 (0.0001)	0.00 (0.0001)	-0.00 (0.0004)
N228	0.00 (0.0001)	0.00 (0.0001)	0.00 (0.0002)	0.00 (0.0001)
D229	0.01 (0.0024)	0.01 (0.0019)	0.13 (0.0064)	0.05 (0.0113)
F230	-0.00 (0.0001)	-0.00 (0.0001)	0.00 (0.0001)	-0.00 (0.0004)
N231	0.00 (0.0002)	0.00 (0.0001)	0.01 (0.0004)	0.00 (0.0004)
L232	0.00 (0.0001)	-0.00 (0.0000)	0.00 (0.0001)	-0.00 (0.0003)
V233	0.00 (0.0001)	-0.00 (0.0000)	0.00 (0.0001)	-0.00 (0.0003)
A234	0.00 (0.0001)	-0.00 (0.0001)	0.01 (0.0002)	-0.00 (0.0006)
M235	0.00 (0.0002)	-0.00 (0.0001)	0.00 (0.0002)	-0.00 (0.0004)
K236	-0.02 (0.0031)	-0.01 (0.0023)	-0.16 (0.0067)	-0.07 (0.0111)
Y237	0.00 (0.0001)	-0.00 (0.0001)	0.01 (0.0002)	-0.00 (0.0006)
N238	0.00 (0.0002)	-0.00 (0.0001)	0.01 (0.0003)	-0.00 (0.0006)
Y239	-0.00 (0.0001)	-0.00 (0.0001)	-0.01 (0.0003)	-0.00 (0.0005)
E240	0.12 (0.0055)	0.11 (0.0049)	0.24 (0.0120)	0.12 (0.0190)
P241	0.01 (0.0002)	-0.01 (0.0007)	0.01 (0.0005)	-0.02 (0.0033)
L242	-0.00 (0.0001)	-0.00 (0.0001)	-0.01 (0.0003)	-0.00 (0.0005)
T243	-0.00 (0.0001)	-0.00 (0.0001)	0.00 (0.0003)	-0.00 (0.0003)
Q244	-0.00 (0.0001)	-0.00 (0.0001)	0.00 (0.0003)	-0.00 (0.0005)
D245	0.07 (0.0029)	0.06 (0.0024)	0.15 (0.0108)	0.06 (0.0143)
H246	-0.01 (0.0002)	-0.01 (0.0001)	0.00 (0.0004)	-0.00 (0.0004)
V247	-0.00 (0.0001)	-0.00 (0.0000)	0.00 (0.0005)	-0.00 (0.0003)
D248	0.04 (0.0026)	0.04 (0.0021)	0.16 (0.0112)	0.06 (0.0164)
I249	-0.00 (0.0002)	-0.00 (0.0001)	0.01 (0.0008)	-0.00 (0.0008)
L250	-0.01 (0.0001)	-0.00 (0.0001)	0.00 (0.0006)	-0.00 (0.0005)
G251	-0.01 (0.0001)	0.00 (0.0001)	-0.00 (0.0005)	0.00 (0.0007)
P252	-0.01 (0.0002)	-0.01 (0.0007)	-0.00 (0.0007)	-0.02 (0.0061)
L253	-0.02 (0.0002)	-0.00 (0.0001)	-0.01 (0.0009)	-0.01 (0.0009)
S254	-0.01 (0.0001)	0.00 (0.0001)	-0.01 (0.0008)	-0.00 (0.0012)
A255	-0.01 (0.0002)	0.00 (0.0001)	-0.00 (0.0009)	-0.00 (0.0011)
Q256	0.02 (0.0005)	0.04 (0.0007)	0.02 (0.0020)	0.03 (0.0026)
T257	-0.00 (0.0004)	0.01 (0.0002)	-0.00 (0.0012)	0.00 (0.0015)
G258	-0.00 (0.0002)	-0.00 (0.0001)	-0.00 (0.0005)	0.00 (0.0008)
I259	0.00 (0.0002)	0.00 (0.0001)	0.00 (0.0004)	-0.00 (0.0009)
A260	-0.00 (0.0001)	0.00 (0.0001)	0.00 (0.0003)	-0.00 (0.0007)
V261	-0.00 (0.0001)	-0.00 (0.0001)	0.00 (0.0001)	-0.00 (0.0004)
L262	0.00 (0.0001)	-0.00 (0.0000)	0.00 (0.0002)	-0.00 (0.0003)

D263	-0.03 (0.0024)	-0.03 (0.0018)	0.14 (0.0087)	0.05 (0.0163)
M264	0.00 (0.0003)	0.00 (0.0002)	0.00 (0.0004)	-0.00 (0.0011)
C265	0.00 (0.0001)	0.00 (0.0001)	0.00 (0.0002)	0.00 (0.0001)
A266	0.00 (0.0001)	0.00 (0.0001)	0.00 (0.0001)	-0.00 (0.0006)
S267	-0.00 (0.0002)	-0.00 (0.0001)	-0.00 (0.0003)	-0.00 (0.0007)
L268	-0.00 (0.0001)	-0.00 (0.0001)	-0.00 (0.0002)	-0.00 (0.0006)
K269	-0.01 (0.0026)	-0.01 (0.0019)	-0.15 (0.0073)	-0.06 (0.0118)
E270	-0.01 (0.0027)	-0.01 (0.0022)	0.16 (0.0072)	0.07 (0.0142)
L271	0.00 (0.0001)	-0.00 (0.0001)	-0.00 (0.0003)	-0.00 (0.0006)
L272	-0.00 (0.0001)	-0.00 (0.0001)	0.00 (0.0002)	-0.00 (0.0005)
Q273	0.00 (0.0001)	0.00 (0.0002)	-0.00 (0.0002)	-0.00 (0.0005)
N274	-0.00 (0.0001)	0.00 (0.0001)	-0.00 (0.0004)	-0.00 (0.0006)
G275	-0.00 (0.0001)	0.00 (0.0001)	-0.01 (0.0003)	0.00 (0.0005)
M276	-0.00 (0.0001)	-0.00 (0.0002)	-0.00 (0.0002)	-0.00 (0.0007)
N277	-0.00 (0.0001)	0.00 (0.0001)	-0.00 (0.0002)	-0.00 (0.0003)
G278	0.00 (0.0002)	0.00 (0.0001)	0.00 (0.0002)	0.00 (0.0006)
R279	0.03 (0.0060)	0.03 (0.0046)	-0.22 (0.0094)	-0.09 (0.0178)
T280	-0.01 (0.0004)	0.00 (0.0002)	-0.01 (0.0009)	-0.00 (0.0008)
I281	0.02 (0.0004)	-0.00 (0.0001)	0.01 (0.0008)	-0.01 (0.0008)
L282	0.03 (0.0007)	-0.01 (0.0002)	0.02 (0.0017)	-0.01 (0.0009)
G283	0.01 (0.0004)	0.00 (0.0002)	0.00 (0.0008)	0.01 (0.0009)
S284	-0.01 (0.0003)	-0.00 (0.0002)	-0.01 (0.0007)	-0.01 (0.0011)
A285	-0.01 (0.0002)	-0.00 (0.0002)	-0.01 (0.0004)	-0.01 (0.0008)
L286	0.00 (0.0003)	-0.00 (0.0002)	0.00 (0.0008)	-0.01 (0.0008)
L287	0.01 (0.0002)	-0.00 (0.0001)	0.01 (0.0006)	-0.00 (0.0005)
E288	0.22 (0.0066)	0.21 (0.0059)	0.52 (0.0232)	0.34 (0.0323)
D289	0.21 (0.0068)	0.18 (0.0058)	0.44 (0.0191)	0.25 (0.0277)
E290	0.41 (0.0085)	0.35 (0.0075)	0.71 (0.0304)	0.46 (0.0386)
F291	-0.02 (0.0003)	-0.01 (0.0002)	-0.02 (0.0012)	-0.01 (0.0008)
T292	-0.00 (0.0006)	-0.01 (0.0002)	0.01 (0.0018)	-0.01 (0.0006)
P293	-0.01 (0.0005)	-0.02 (0.0008)	0.01 (0.0018)	-0.03 (0.0058)
F294	-0.00 (0.0006)	-0.01 (0.0002)	0.01 (0.0022)	-0.01 (0.0006)
D295	0.28 (0.0063)	0.25 (0.0046)	0.56 (0.0345)	0.31 (0.0335)
V296	-0.01 (0.0020)	0.00 (0.0001)	0.02 (0.0038)	-0.00 (0.0007)
V297	-0.02 (0.0017)	-0.00 (0.0001)	0.02 (0.0040)	-0.00 (0.0007)
R298	-0.34 (0.0118)	-0.32 (0.0118)	-0.55 (0.0338)	-0.35 (0.0348)
Q299	-0.02 (0.0102)	-0.01 (0.0035)	0.10 (0.0084)	0.01 (0.0020)
C300	-0.05 (0.0034)	-0.02 (0.0010)	0.00 (0.0047)	-0.01 (0.0008)
S301	0.03 (0.0009)	0.01 (0.0025)	0.03 (0.0041)	-0.00 (0.0027)
G302	0.03 (0.0034)	0.00 (0.0004)	-0.02 (0.0083)	0.00 (0.0016)
V303	0.04 (0.0026)	-0.01 (0.0034)	-0.09 (0.0451)	-0.07 (0.0298)
T304	-0.02 (0.0040)	-0.01 (0.0025)	-0.08 (0.0245)	-0.03 (0.0130)
F305	0.45 (0.0083)	0.07 (0.0014)	0.36 (0.0402)	0.00 (0.0183)

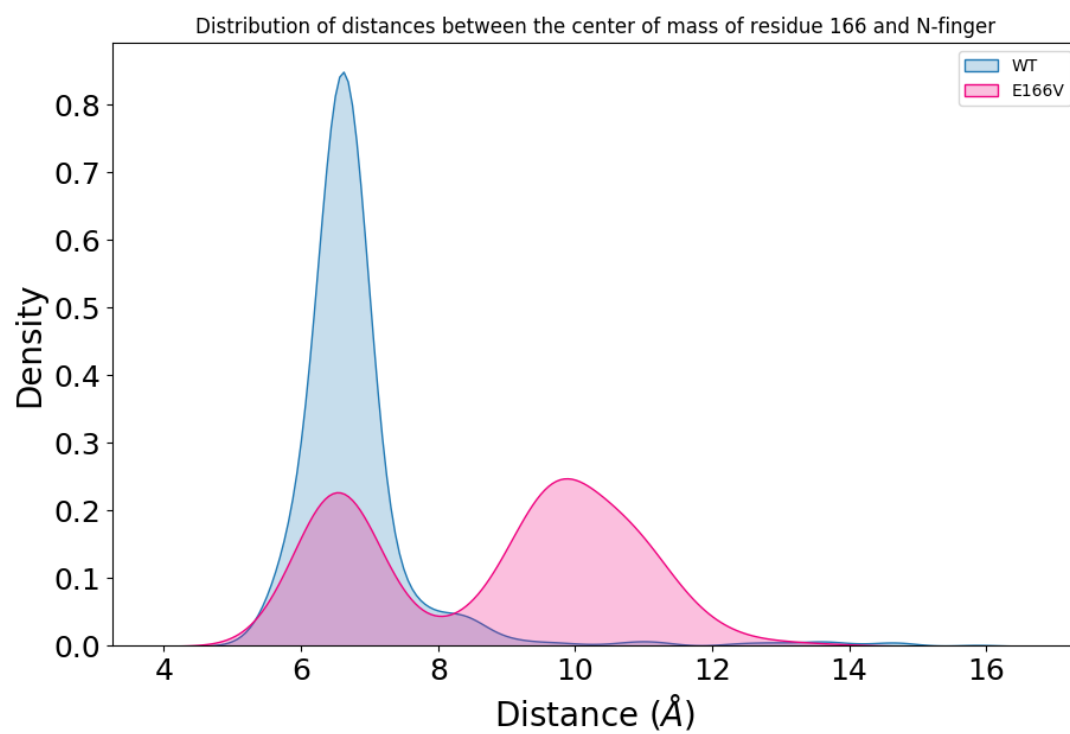


Figure S1. Distribution of distances between the center of masses of residue 166 and the N-terminal residue of the other protomer for the wild type enzyme (blue) and E66V variant (pink).

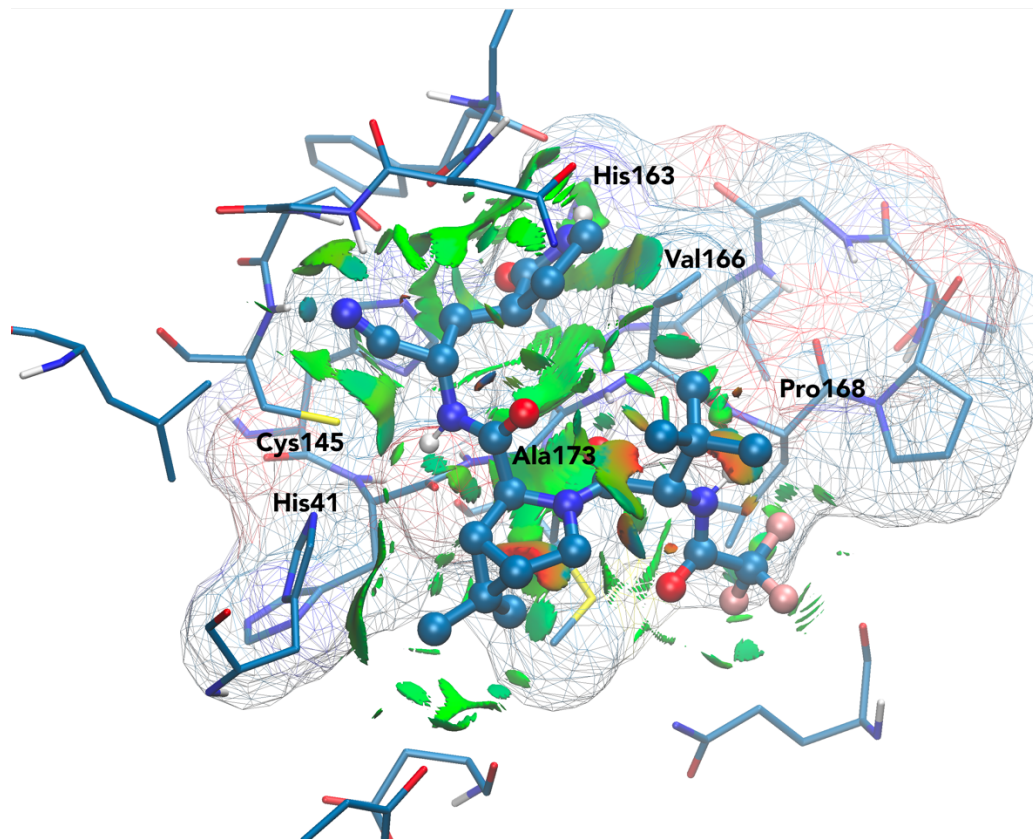


Figure S2. NCIPlot^{1,2} of the interactions established by nirmatrelvir (ball and stick representation, with carbon atoms in blue) in the active site of SARS-CoV-2 3CL^{pro}. Following the standard convention, blue color is used for strong attractive interactions, green for van der Waals and red for repulsive interactions.

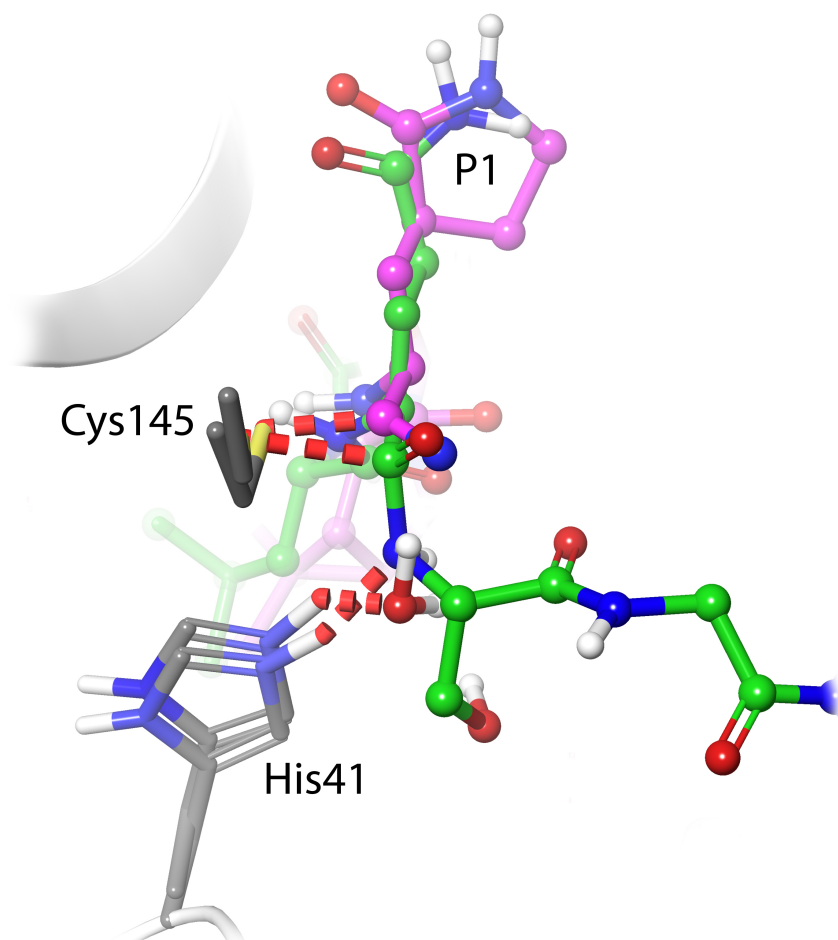


Figure S3. Overlap between the TS structures for the acylation reaction of a peptidic substrate (green carbon atoms) and nirmatrelvir (pink carbon atoms) in the active site of wild type SARS-CoV-2 3CLpro. Note that the reactive water molecule of nirmatrelvir acylation occupies the position of the amide group of the target peptide bind during the acylation of the peptidic substrate.

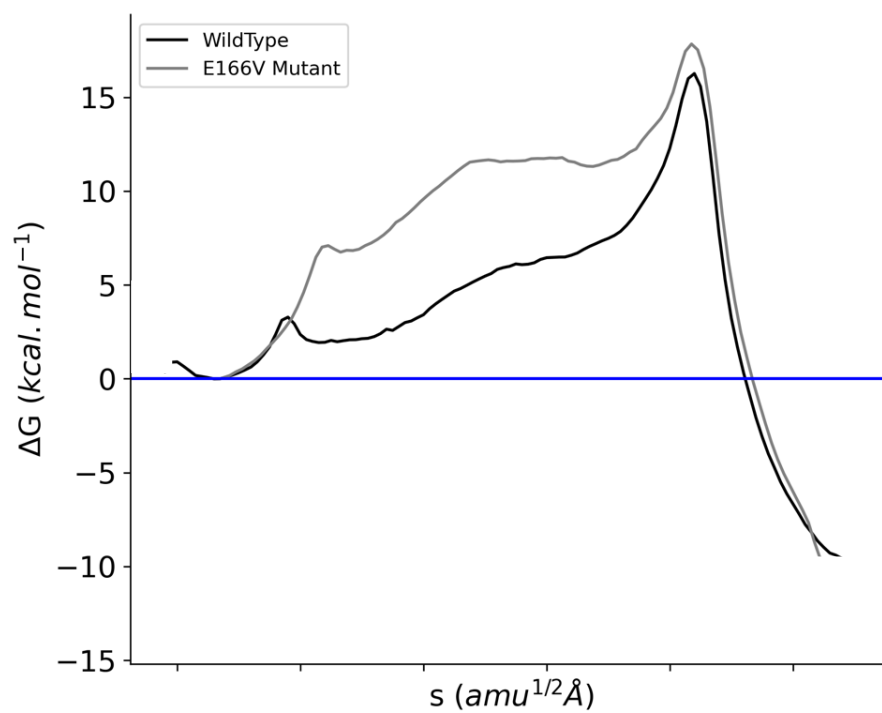


Figure S4. B3LYPD3/MM free energy profiles for the reaction of nirmatrelvir to form the covalent complex in the active site of the wild type 3CL protease of SARS-CoV-2 (black line)³ and in the E166V variant (grey line).

Data availability

Snapshots of the trajectory of the TS in the E166V variant can be downloaded from:

<https://zenodo.org/record/7501128#.Y7SHx-zMlfB>

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