SUPPLEMENTARY MATERIALS

Title: CryoEM and AI reveal a structure of SARS-CoV-2 Nsp2, a multifunctional protein involved in key host processes.

Authors: Meghna Gupta[1*], Caleigh M. Azumaya[1*], Michelle Moritz[1*], Sergei Pourmal[1*], Amy Diallo[1*], Gregory E. Merz[1*], Gwendolyn Jang[2,3,4,5*], Mehdi Bouhaddou[2,3,4,5*], Andrea Fossati[2,3,4,5*], Axel F. Brilot[1], Devan Diwanji[1], Evelyn Hernandez[1], Nadia Herrera[1], Huong T. Kratochvil[1], Victor L. Lam[1], Fei Li[1], Yang Li[1], Henry C. Nguyen[1], Carlos Nowotny[1], Tristan W. Owens[1], Jessica K. Peters[1], Alexandrea N. Rizo[1], Ursula Schulze-Gahmen[1], Amber M. Smith[1], Iris D. Young[1], Zanlin Yu[1], Daniel Asarnow[1], Christian Billesbølle[1], Melody G. Campbell[1,6], Jen Chen[1], Kuei-Ho Chen[2,3,4,5], Un Seng Chio[1], Miles Sasha Dickinson[1], Loan Doan[1], Mingliang Jin[1], Kate Kim[1], Junrui Li[1], Yen-Li Li[1], Edmond Linossi[1], Yanxin Liu[1], Megan Lo[1], Jocelyne Lopez[1], Kyle E. Lopez[1], Adamo Mancino[1], Frank R. Moss III[1], Michael D. Paul[1], Komal Ishwar Pawar[1], Adrian Pelin[2,3,4,5], Thomas H. Pospiech Jr.[1], Cristina Puchades[1], Soumya Govinda Remesh[1], Maliheh Safari[1], Kaitlin Schaefer[1], Ming Sun[1,7], Mariano C Tabios[1], Aye C. Thwin[1], Erron W. Titus[1], Raphael Trenker[1], Eric Tse[1], Tsz Kin Martin Tsui[1], Feng Wang[1], Kaihua Zhang[1], Yang Zhang[1], Jianhua Zhao[1], Fengbo Zhou[1], Yuan Zhou[2,3,4,5], Lorena Zuliani-Alvarez[1,2,3,4,5], QCRG Structural Biology Consortium[1], David A Agard[1,2,3,8], Yifan Cheng[1,2,3,8,9], James S Fraser[1,2,3,8], Natalia Jura[1,2,3,11], Tanja Kortemme[1,2,3,10,12], Aashish Manglik[1,2,3,13], Daniel R. Southworth[1,2,3,8], Robert M Stroud[1,2,3,8], Danielle L Swaney[2,3,4,5], Nevan J Krogan[2,3,4,5,16,&], Adam Frost[1,2,3,8,14,&], Oren S Rosenberg[1,2,3,8,14,15,&], Kliment A Verba[1,2,3,13,&]

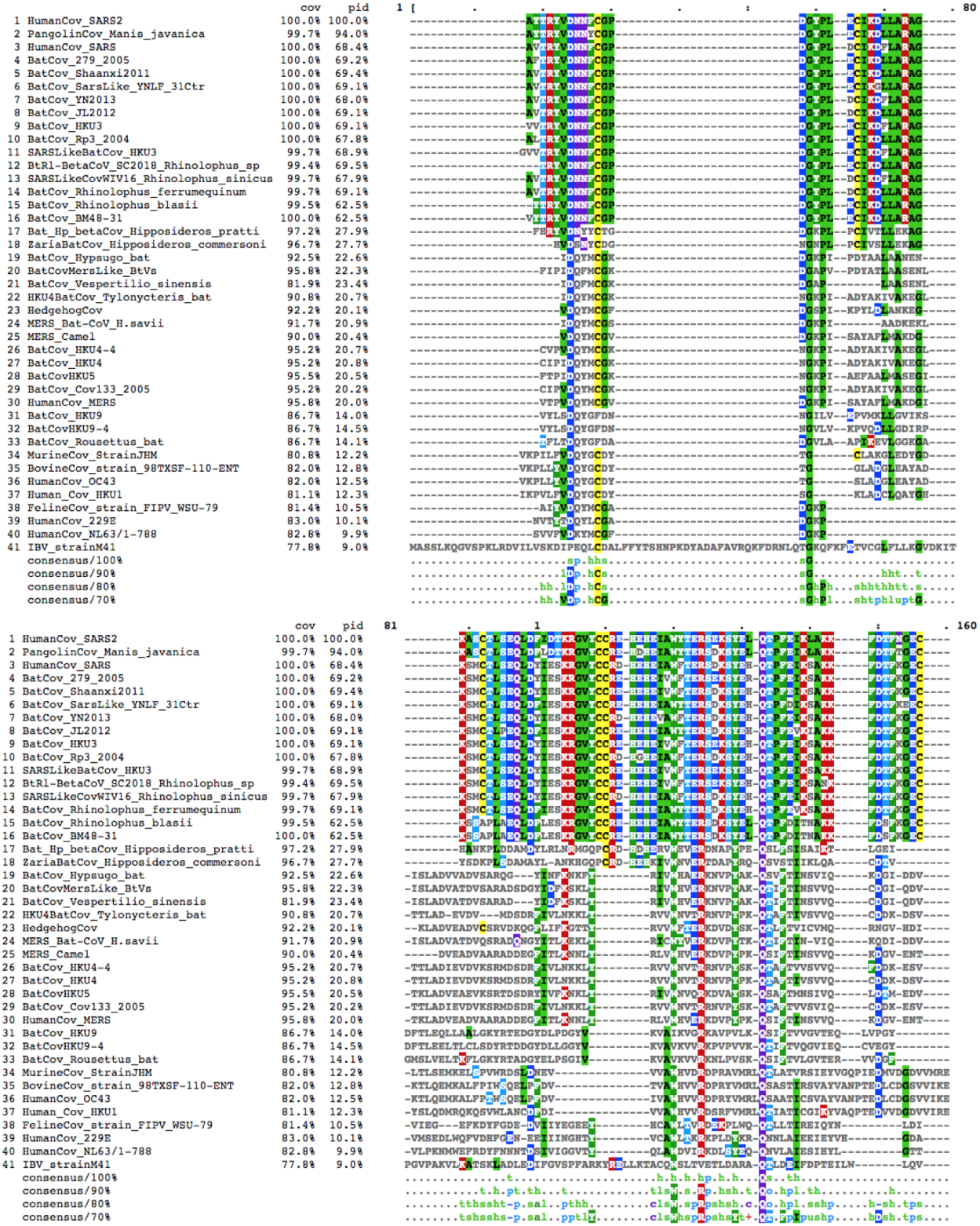
Affiliations

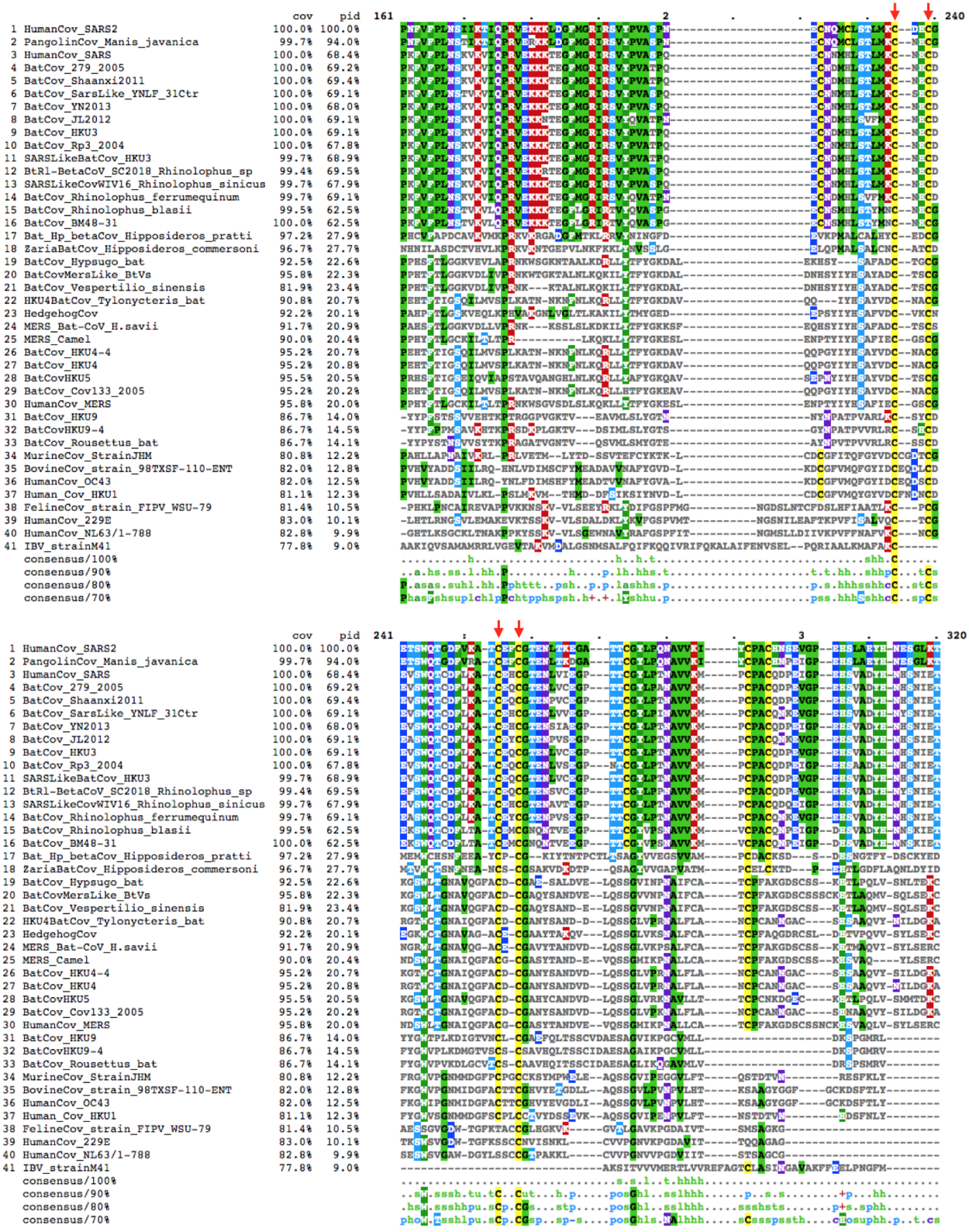
[1]QBI Coronavirus Research Group Structural Biology Consortium, University of California, San Francisco, CA 94158, USA.
[2]Quantitative Biosciences Institute (QBI) COVID-19 Research Group (QCRG), San Francisco, CA 94158, USA.
[3]QBI, University of California, San Francisco, CA 94158, USA.
[4]Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA 94158, USA.
[5]J. David Gladstone Institutes, San Francisco, CA 94158, USA.
[6]Current affiliation: Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA.
[7]Current affiliation: Beam Therapeutics, Cambridge, MA 02139, USA
[8]Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94158, USA.
[9]Howard Hughes Medical Institute, San Francisco, CA 94158, USA.
[10]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA 94158, USA.
[11]Cardiovascular Research Institute, University of California, San Francisco, CA 94158, USA.
[12]The University of California, Berkeley–University of California, San Francisco Graduate Program in Bioengineering, University of California, San Francisco, CA 94158, USA.
[13]Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94158, USA.
[14]Chan-Zuckerberg Biohub, San Francisco, CA 94158, USA.
[15]Department of Medicine, University of California, San Francisco, CA 94143, USA.
[16]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA.
* These authors contributed equally to this work.
[&] Corresponding authors. A.F., O.S.R. and K.A.V. are corresponding authors on behalf of QCRG Structural Biology Consortium

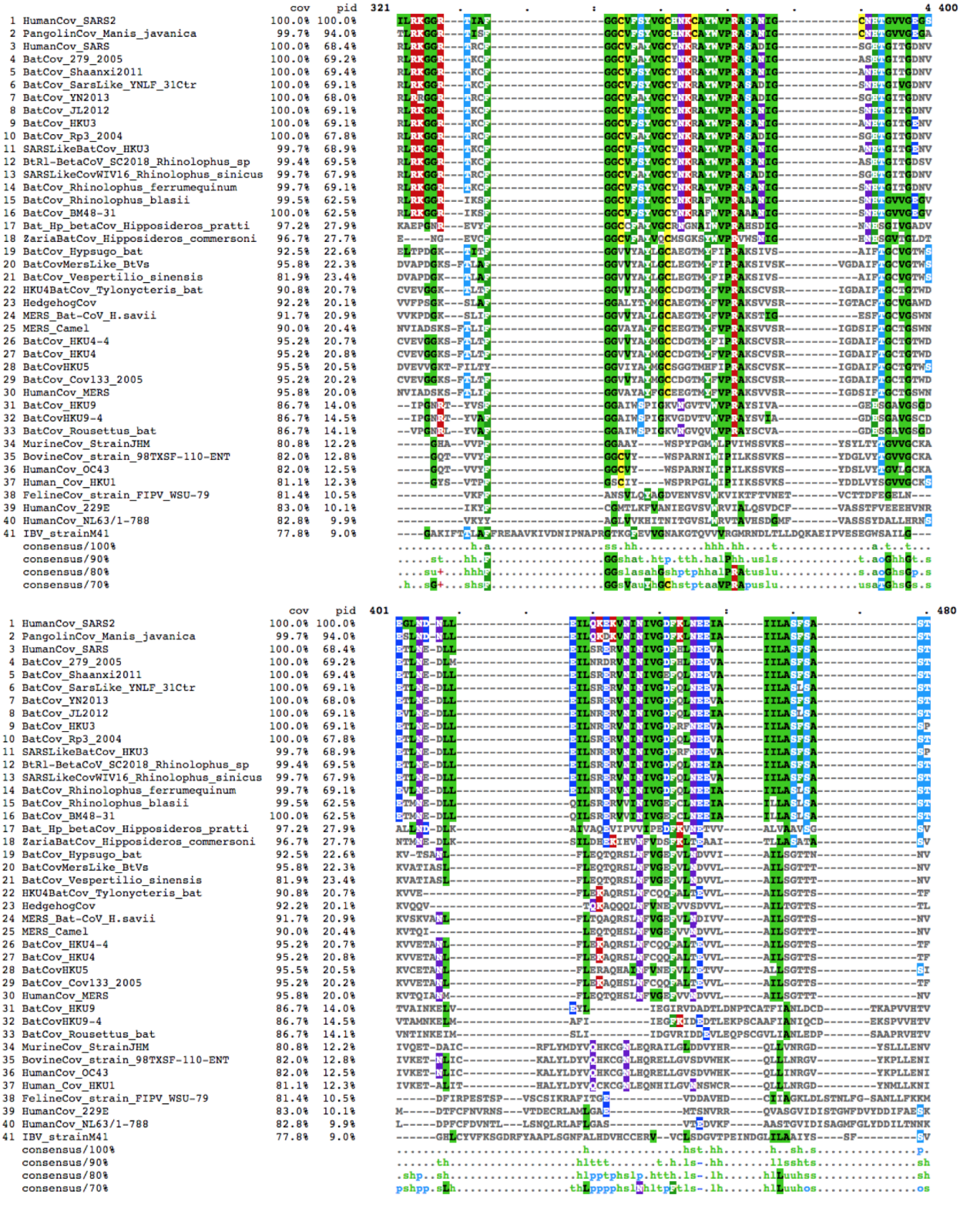
QCRG Structural Biology Consortium Author List

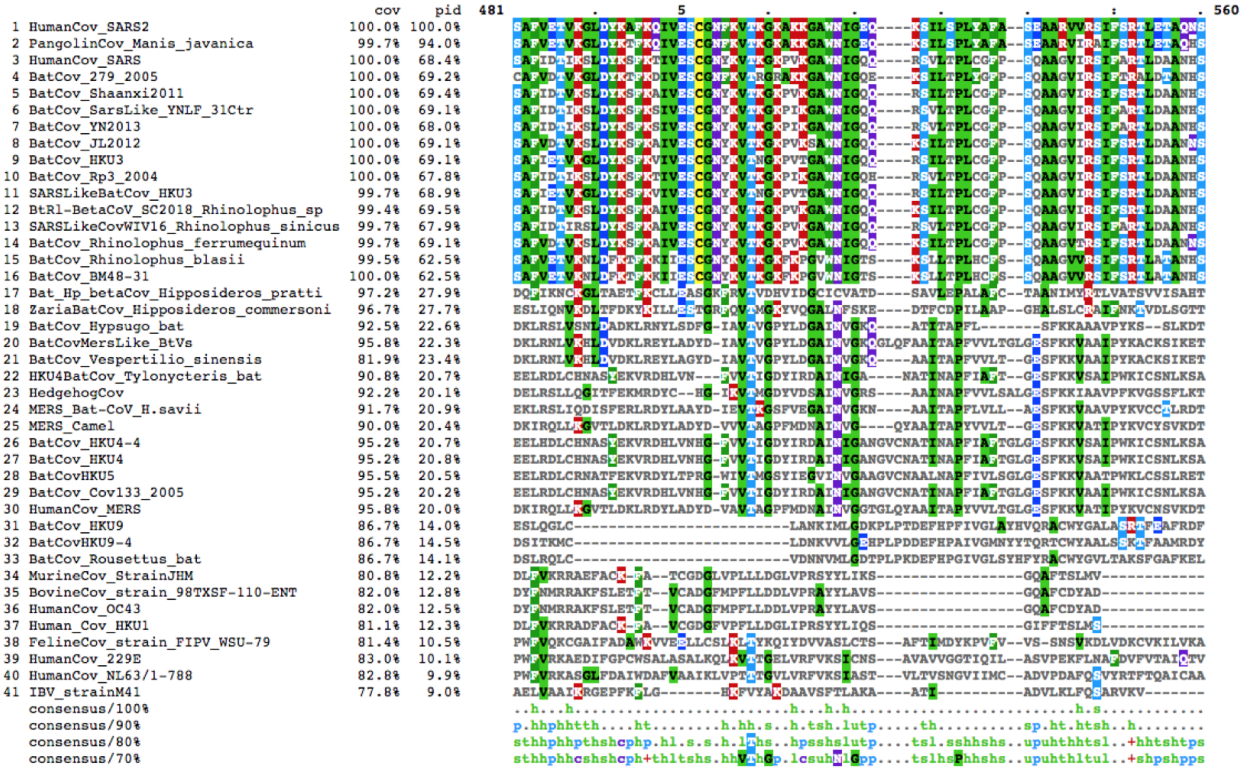
David A. Agard, Daniel Asarnow, Caleigh M. Azumaya, Christian Billesbølle, Axel F. Brilot, David Bulkley, Melody G. Campbell, Jen Chen, Yifan Cheng, Un Seng Chio, Amy Diallo, Miles Sasha Dickinson, Devan Diwanji, Loan Doan, James S. Fraser, Adam Frost, Meghna Gupta, Evelyn Hernandez, Nadia Herrera, Mingliang Jin, Arceli Joves, Almarie Joves, Natalia Jura, Kate Kim, Tanja Kortemme, Huong T. Kratochvil, Victor L. Lam, Fei Li, Yang Li, Junrui Li, Yen-Li Li, Edmond Linossi, Yanxin Liu, Megan Lo, Jocelyne Lopez, Kyle E. Lopez, Adamo Mancino, Aashish Manglik, Liam McKay, Melissa Mendez, Gregory E. Merz, Michelle Moritz, Frank R. Moss III, Henry C. Nguyen, Carlos Nowotny, Tristan W. Owens, Michael D. Paul, Joana Paulino, Komal Ishwar Pawar, Jessica K. Peters, Thomas H. Pospiech Jr., Sergei Pourmal, Cristina Puchades, Soumya Govinda Remesh, Alexandrea N. Rizo, Oren S. Rosenberg, Maliheh Safari, Kaitlin Schaefer, Ursula Schulze-Gahmen, Amber M. Smith, Daniel R. Southworth, Robert M. Stroud, Ming Sun, Mariano C. Tabios, Aye C. Thwin, Erron W. Titus, Raphael Trenker, Eric Tse, Tsz Kin Martin Tsui, Kliment A. Verba, Feng Wang, Iris D. Young, Zanlin Yu, Kaihua Zhang, Yang Zhang, Jianhua Zhao, Fengbo Zhou.

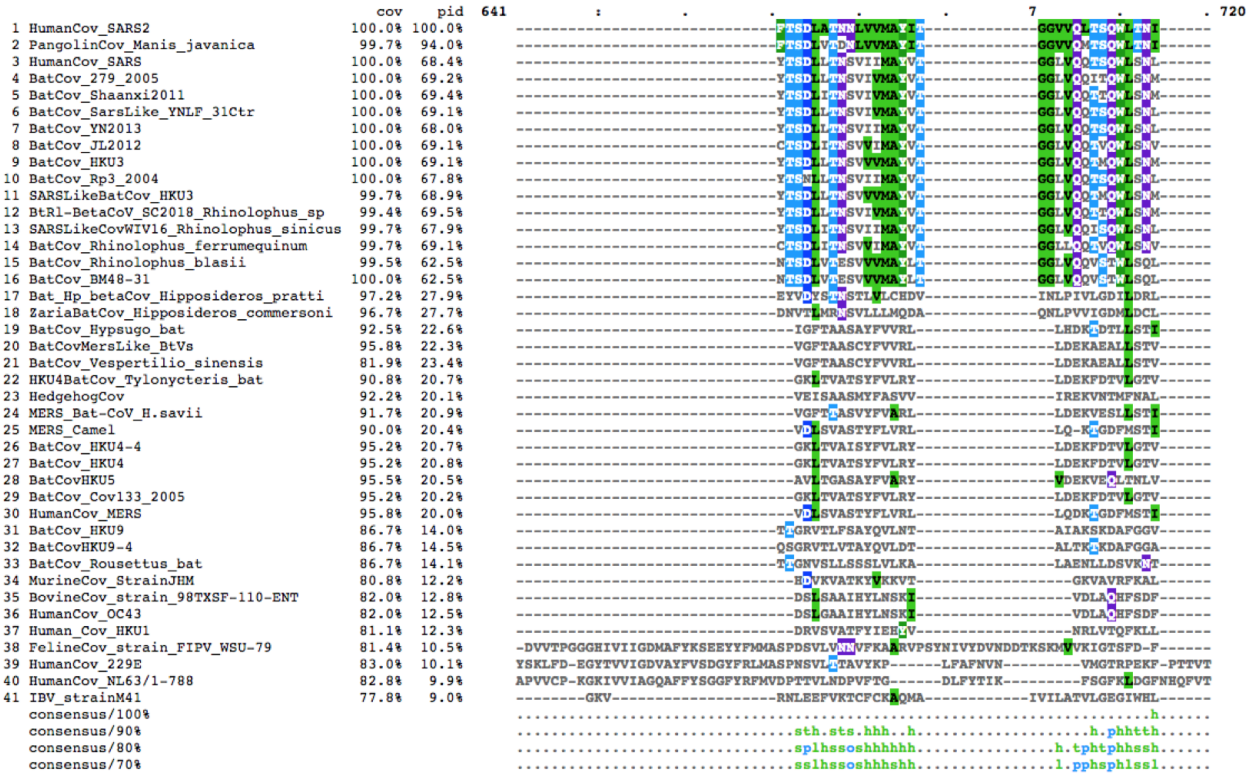
Supplementary figure 1. Multiple sequence alignment of Nsp2. Conserved cysteines are marked with red arrows (see positions 230-260 in the alignment).

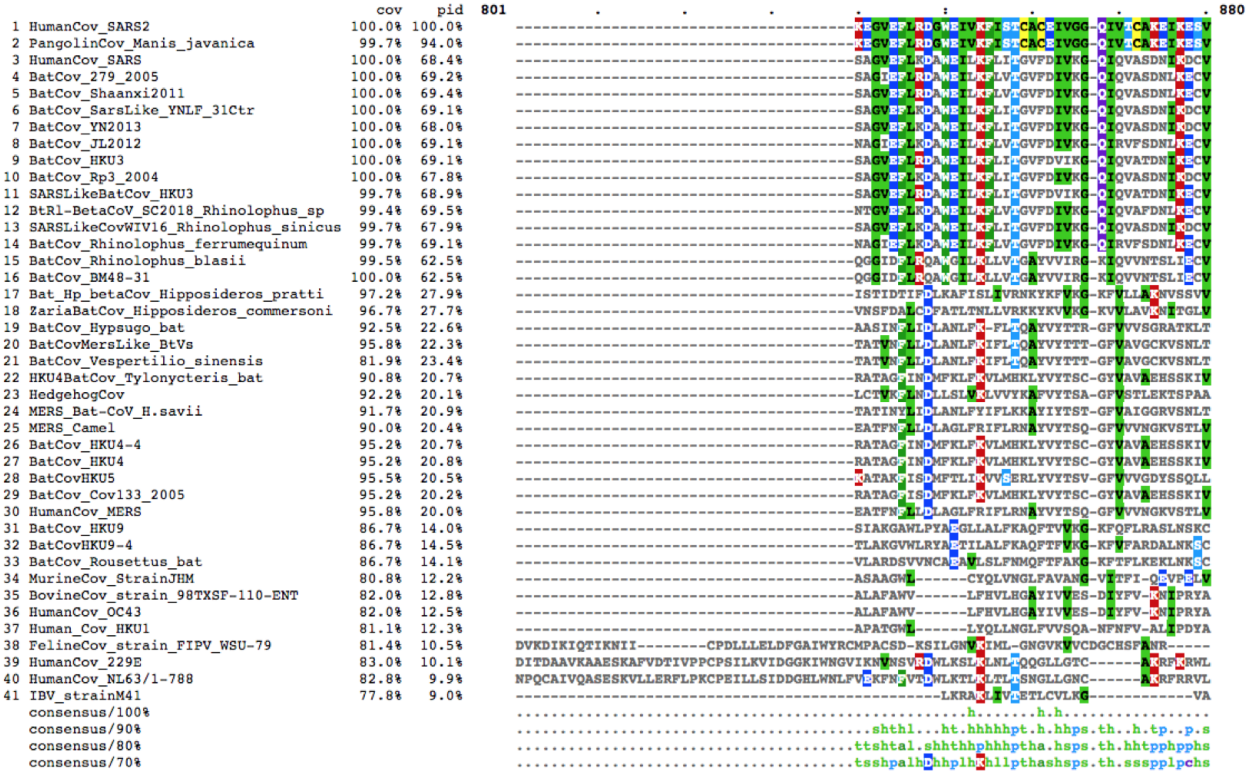


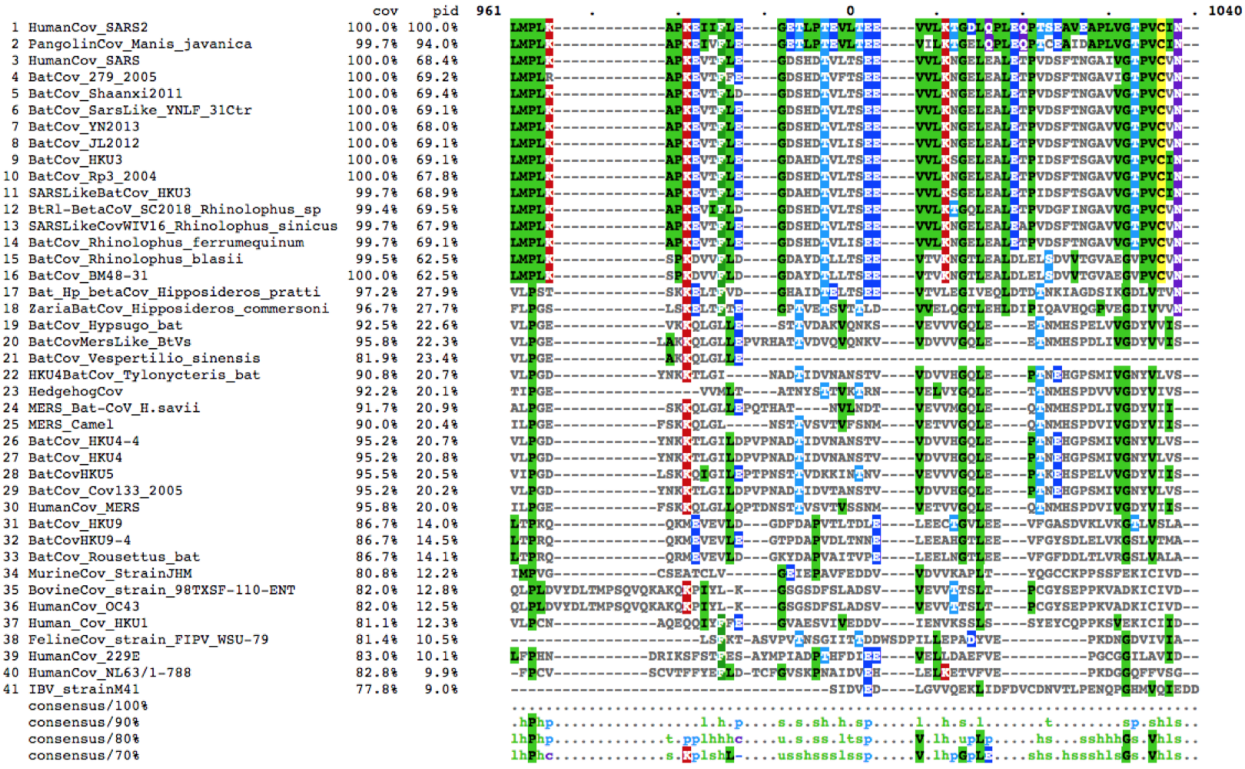

cov
pid
1 HumanCov_SARS2 100.0% 100.0%
2 PangolinCov_Manis_javanica 99.7% 94.0%
3 HumanCov_SARS 100.0% 68.4%
4 BatCov_279_2005 100.0% 69.2%
5 BatCov_Shaanxi2011 100.0% 69.4%
6 BatCov_SarsLike_YNLF_31Ctr 100.0% 69.1%
7 BatCov_YN2013 100.0% 68.0%
8 BatCov_JL2012 100.0% 69.1%
9 BatCov_HKU3 100.0% 69.1%
10 BatCov_Rp3_2004 100.0% 67.8%
11 SARSLikeBatCov_HKU3 99.7% 68.9%
12 BtRl-BetaCoV_SC2018_Rhinolophus_sp 99.4% 69.5%
13 SARSLikeCovWIV16_Rhinolophus_sinicus 99.7% 67.9%
14 BatCov_Rhinolophus_ferrumequinum 99.7% 69.1%
15 BatCov_Rhinolophus_blasii 99.5% 62.5%
16 BatCov_BM48-31 100.0% 62.5%
17 Bat_Hp_betaCov_Hipposideros_pratti 97.2% 27.9%
18 ZariaBatCov_Hipposideros_commersoni 96.7% 27.7%
19 BatCov_Hypsugo_bat 92.5% 22.6%
20 BatCovMersLike_BtVs 95.8% 22.3%
21 BatCov_Vespertilio_sinensis 81.9% 23.4%
22 HKU4BatCov_Tylonycteris_bat 90.8% 20.7%
23 HedgehogCov 92.2% 20.1%
24 MERS_Bat-CoV_H.savii 91.7% 20.9%
25 MERS_Camel 90.0% 20.4%
26 BatCov_HKU4-4 95.2% 20.7%
27 BatCov_HKU4 95.2% 20.8%
28 BatCovHKU5 95.5% 20.5%
29 BatCov_Cov133_2005 95.2% 20.2%
30 HumanCov_MERS 95.8% 20.0%
31 BatCov_HKU9 86.7% 14.0%
32 BatCovHKU9-4 86.7% 14.5%
33 BatCov_Rousettus_bat 86.7% 14.1%
34 MurineCov_StrainJHM 80.8% 12.2%
35 BovineCov_strain_98TXSF-110-ENT 82.0% 12.8%
36 HumanCov_OC43 82.0% 12.5%
37 Human_Cov_HKU1 81.1% 12.3%
38 FelineCov_strain_FIPV_WSU-79 81.4% 10.5%
39 HumanCov_229E 83.0% 10.1%
40 HumanCov_NL63/1-788 82.8% 9.9%
41 IBV_strainM41 77.8% 9.0%
consensus/100%
consensus/90%
consensus/80%
consensus/70%
161
240
241
320


cov pid 321 400
1 HumanCov_SARS2 100.0% 100.0%
2 PangolinCov_Manis_javanica 99.7% 94.0%
3 HumanCov_SARS 100.0% 68.4%
4 BatCov_279_2005 100.0% 69.2%
5 BatCov_Shaanxi2011 100.0% 69.4%
6 BatCov_SarsLike_YNLF_31Ctr 100.0% 69.1%
7 BatCov_YN2013 100.0% 68.0%
8 BatCov_JL2012 100.0% 69.1%
9 BatCov_HKU3 100.0% 69.1%
10 BatCov_Rp3_2004 100.0% 67.8%
11 SARSLikeBatCov_HKU3 99.7% 68.9%
12 BtRl-BetaCoV_SC2018_Rhinolophus_sp 99.4% 69.5%
13 SARSLikeCovWIV16_Rhinolophus_sinicus 99.7% 67.9%
14 BatCov_Rhinolophus_ferrumequinum 99.7% 69.1%
15 BatCov_Rhinolophus_blasii 99.5% 62.5%
16 BatCov_BM48-31 100.0% 62.5%
17 Bat_Hp_betaCov_Hipposideros_pratti 97.2% 27.9%
18 ZariaBatCov_Hipposideros_commersoni 96.7% 27.7%
19 BatCov_Hypsugo_bat 92.5% 22.6%
20 BatCovMersLike_BtVs 95.8% 22.3%
21 BatCov_Vespertilio_sinensis 81.9% 23.4%
22 HKU4BatCov_Tylonycteris_bat 90.8% 20.7%
23 HedgehogCov 92.2% 20.1%
24 MERS_Bat-CoV_H.savii 91.7% 20.9%
25 MERS_Camel 90.0% 20.4%
26 BatCov_HKU4-4 95.2% 20.7%
27 BatCov_HKU4 95.2% 20.8%
28 BatCovHKU5 95.5% 20.5%
29 BatCov_Cov133_2005 95.2% 20.2%
30 HumanCov_MERS 95.8% 20.0%
31 BatCov_HKU9 86.7% 14.0%
32 BatCovHKU9-4 86.7% 14.5%
33 BatCov_Rousettus_bat 86.7% 14.1%
34 MurineCov_StrainJHM 80.8% 12.2%
35 BovineCov_strain_98TXSF-110-ENT 82.0% 12.8%
36 HumanCov_OC43 82.0% 12.5%
37 Human_Cov_HKU1 81.1% 12.3%
38 FelineCov_strain_FIPV_WSU-79 81.4% 10.5%
39 HumanCov_229E 83.0% 10.1%
40 HumanCov_NL63/1-788 82.8% 9.9%
41 IBV_strainM41 77.8% 9.0%
consensus/100%
consensus/90%
consensus/80%
consensus/70%
cov pid 401 480
1 HumanCov_SARS2 100.0% 100.0%
2 PangolinCov_Manis_javanica 99.7% 94.0%
3 HumanCov_SARS 100.0% 68.4%
4 BatCov_279_2005 100.0% 69.2%
5 BatCov_Shaanxi2011 100.0% 69.4%
6 BatCov_SarsLike_YNLF_31Ctr 100.0% 69.1%
7 BatCov_YN2013 100.0% 68.0%
8 BatCov_JL2012 100.0% 69.1%
9 BatCov_HKU3 100.0% 69.1%
10 BatCov_Rp3_2004 100.0% 67.8%
11 SARSLikeBatCov_HKU3 99.7% 68.9%
12 BtRl-BetaCoV_SC2018_Rhinolophus_sp 99.4% 69.5%
13 SARSLikeCovWIV16_Rhinolophus_sinicus 99.7% 67.9%
14 BatCov_Rhinolophus_ferrumequinum 99.7% 69.1%
15 BatCov_Rhinolophus_blasii 99.5% 62.5%
16 BatCov_BM48-31 100.0% 62.5%
17 Bat_Hp_betaCov_Hipposideros_pratti 97.2% 27.9%
18 ZariaBatCov_Hipposideros_commersoni 96.7% 27.7%
19 BatCov_Hypsugo_bat 92.5% 22.6%
20 BatCovMersLike_BtVs 95.8% 22.3%
21 BatCov_Vespertilio_sinensis 81.9% 23.4%
22 HKU4BatCov_Tylonycteris_bat 90.8% 20.7%
23 HedgehogCov 92.2% 20.1%
24 MERS_Bat-CoV_H.savii 91.7% 20.9%
25 MERS_Camel 90.0% 20.4%
26 BatCov_HKU4-4 95.2% 20.7%
27 BatCov_HKU4 95.2% 20.8%
28 BatCovHKU5 95.5% 20.5%
29 BatCov_Cov133_2005 95.2% 20.2%
30 HumanCov_MERS 95.8% 20.0%
31 BatCov_HKU9 86.7% 14.0%
32 BatCovHKU9-4 86.7% 14.5%
33 BatCov_Rousettus_bat 86.7% 14.1%
34 MurineCov_StrainJHM 80.8% 12.2%
35 BovineCov_strain_98TXSF-110-ENT 82.0% 12.8%
36 HumanCov_OC43 82.0% 12.5%
37 Human_Cov_HKU1 81.1% 12.3%
38 FelineCov_strain_FIPV_WSU-79 81.4% 10.5%
39 HumanCov_229E 83.0% 10.1%
40 HumanCov_NL63/1-788 82.8% 9.9%
41 IBV_strainM41 77.8% 9.0%
consensus/100%
consensus/90%
consensus/80%
consensus/70%

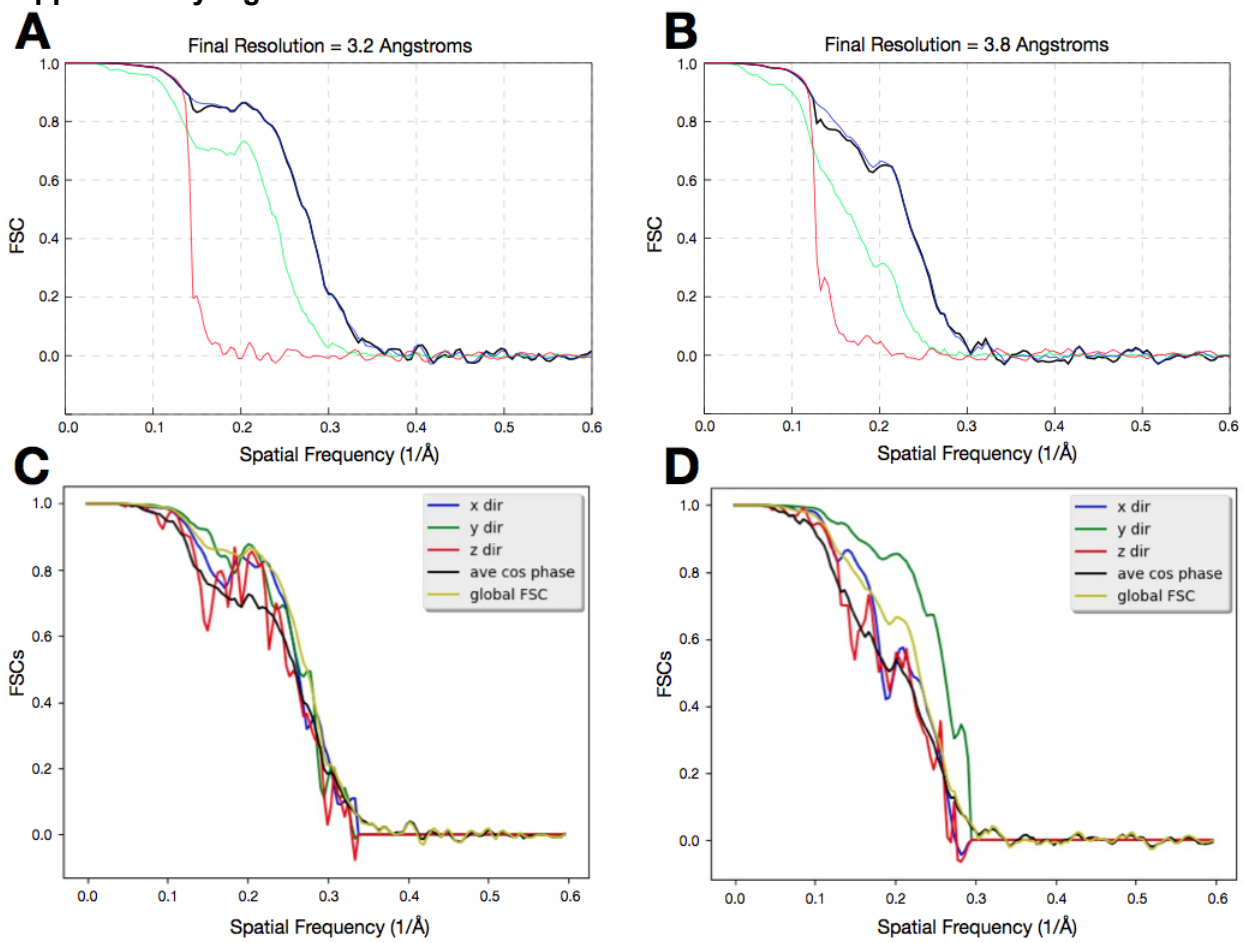

cov pid 481 5 560
1 HumanCov_SARS2 100.0% 100.0%
2 PangolinCov_Manis_javanica 99.7% 94.0%
3 HumanCov_SARS 100.0% 68.4%
4 BatCov_279_2005 100.0% 69.2%
5 BatCov_Shaanxi2011 100.0% 69.4%
6 BatCov_SarsLike_YNLF_31Ctr 100.0% 69.1%
7 BatCov_YN2013 100.0% 68.0%
8 BatCov_JL2012 100.0% 69.1%
9 BatCov_HKU3 100.0% 69.1%
10 BatCov_Rp3_2004 100.0% 67.8%
11 SARSLikeBatCov_HKU3 99.7% 68.9%
12 BtRl-BetaCoV_SC2018_Rhinolophus_sp 99.4% 69.5%
13 SARSLikeCovWIV16_Rhinolophus_sinicus 99.7% 67.9%
14 BatCov_Rhinolophus_ferrumequinum 99.7% 69.1%
15 BatCov_Rhinolophus_blasii 99.5% 62.5%
16 BatCov_BM48-31 100.0% 62.5%
17 Bat_Hp_betaCov_Hipposideros_pratti 97.2% 27.9%
18 ZariaBatCov_Hipposideros_commersoni 96.7% 27.7%
19 BatCov_Hypsugo_bat 92.5% 22.6%
20 BatCovMersLike_BtVs 95.8% 22.3%
21 BatCov_Vespertilio_sinensis 81.9% 23.4%
22 HKU4BatCov_Tylonycteris_bat 90.8% 20.7%
23 HedgehogCov 92.2% 20.1%
24 MERS_Bat-CoV_H.savii 91.7% 20.9%
25 MERS_Camel 90.0% 20.4%
26 BatCov_HKU4-4 95.2% 20.7%
27 BatCov_HKU4 95.2% 20.8%
28 BatCovHKU5 95.5% 20.5%
29 BatCov_Cov133_2005 95.2% 20.2%
30 HumanCov_MERS 95.8% 20.0%
31 BatCov_HKU9 86.7% 14.0%
32 BatCovHKU9-4 86.7% 14.5%
33 BatCov_Rousettus_bat 86.7% 14.1%
34 MurineCov_StrainJHM 80.8% 12.2%
35 BovineCov_strain_98TXSF-110-ENT 82.0% 12.8%
36 HumanCov_OC43 82.0% 12.5%
37 Human_Cov_HKU1 81.1% 12.3%
38 FelineCov_strain_FIPV_WSU-79 81.4% 10.5%
39 HumanCov_229E 83.0% 10.1%
40 HumanCov_NL63/1-788 82.8% 9.9%
41 IBV_strainM41 77.8% 9.0%
consensus/100%
consensus/90%
consensus/80%
consensus/70%
cov pid 561 6 640
1 HumanCov_SARS2 100.0% 100.0%
2 PangolinCov_Manis_javanica 99.7% 94.0%
3 HumanCov_SARS 100.0% 68.4%
4 BatCov_279_2005 100.0% 69.2%
5 BatCov_Shaanxi2011 100.0% 69.4%
6 BatCov_SarsLike_YNLF_31Ctr 100.0% 69.1%
7 BatCov_YN2013 100.0% 68.0%
8 BatCov_JL2012 100.0% 69.1%
9 BatCov_HKU3 100.0% 69.1%
10 BatCov_Rp3_2004 100.0% 67.8%
11 SARSLikeBatCov_HKU3 99.7% 68.9%
12 BtRl-BetaCoV_SC2018_Rhinolophus_sp 99.4% 69.5%
13 SARSLikeCovWIV16_Rhinolophus_sinicus 99.7% 67.9%
14 BatCov_Rhinolophus_ferrumequinum 99.7% 69.1%
15 BatCov_Rhinolophus_blasii 99.5% 62.5%
16 BatCov_BM48-31 100.0% 62.5%
17 Bat_Hp_betaCov_Hipposideros_pratti 97.2% 27.9%
18 ZariaBatCov_Hipposideros_commersoni 96.7% 27.7%
19 BatCov_Hypsugo_bat 92.5% 22.6%
20 BatCovMersLike_BtVs 95.8% 22.3%
21 BatCov_Vespertilio_sinensis 81.9% 23.4%
22 HKU4BatCov_Tylonycteris_bat 90.8% 20.7%
23 HedgehogCov 92.2% 20.1%
24 MERS_Bat-CoV_H.savii 91.7% 20.9%
25 MERS_Camel 90.0% 20.4%
26 BatCov_HKU4-4 95.2% 20.7%
27 BatCov_HKU4 95.2% 20.8%
28 BatCovHKU5 95.5% 20.5%
29 BatCov_Cov133_2005 95.2% 20.2%
30 HumanCov_MERS 95.8% 20.0%
31 BatCov_HKU9 86.7% 14.0%
32 BatCovHKU9-4 86.7% 14.5%
33 BatCov_Rousettus_bat 86.7% 14.1%
34 MurineCov_StrainJHM 80.8% 12.2%
35 BovineCov_strain_98TXSF-110-ENT 82.0% 12.8%
36 HumanCov_OC43 82.0% 12.5%
37 Human_Cov_HKU1 81.1% 12.3%
38 FelineCov_strain_FIPV_WSU-79 81.4% 10.5%
39 HumanCov_229E 83.0% 10.1%
40 HumanCov_NL63/1-788 82.8% 9.9%
41 IBV_strainM41 77.8% 9.0%
consensus/100%
consensus/90%
consensus/80%
consensus/70%


cov pid 641 : . . . . 7 . . 720
1 HumanCov_SARS2 100.0% 100.0%
2 PangolinCov_Manis_javanica 99.7% 94.0%
3 HumanCov_SARS 100.0% 68.4%
4 BatCov_279_2005 100.0% 69.2%
5 BatCov_Shaanxi2011 100.0% 69.4%
6 BatCov_SarsLike_YNLF_31Ctr 100.0% 69.1%
7 BatCov_YN2013 100.0% 68.0%
8 BatCov_JL2012 100.0% 69.1%
9 BatCov_HKU3 100.0% 69.1%
10 BatCov_Rp3_2004 100.0% 67.8%
11 SARSLikeBatCov_HKU3 99.7% 68.9%
12 BtRl-BetaCoV_SC2018_Rhinolophus_sp 99.4% 69.5%
13 SARSLikeCovWIV16_Rhinolophus_sinicus 99.7% 67.9%
14 BatCov_Rhinolophus_ferrumequinum 99.7% 69.1%
15 BatCov_Rhinolophus_blasii 99.5% 62.5%
16 BatCov_BM48-31 100.0% 62.5%
17 Bat_Hp_betaCoV_Hipposideros_pratti 97.2% 27.9%
18 ZariaBatCov_Hipposideros_commersoni 96.7% 27.7%
19 BatCov_Hypsugo_bat 92.5% 22.6%
20 BatCovMersLike_BtVs 95.8% 22.3%
21 BatCov_Vespertilio_sinensis 81.9% 23.4%
22 HKU4BatCov_Tylonycteris_bat 90.8% 20.7%
23 HedgehogCov 92.2% 20.1%
24 MERS_Bat-CoV_H.savii 91.7% 20.9%
25 MERS_Camel 90.0% 20.4%
26 BatCov_HKU4-4 95.2% 20.7%
27 BatCov_HKU4 95.2% 20.8%
28 BatCovHKU5 95.5% 20.5%
29 BatCov_Cov133_2005 95.2% 20.2%
30 HumanCov_MERS 95.8% 20.0%
31 BatCov_HKU9 86.7% 14.0%
32 BatCovHKU9-4 86.7% 14.5%
33 BatCov_Rousettus_bat 86.7% 14.1%
34 MurineCov_StrainJHM 80.8% 12.2%
35 BovineCov_strain_98TXSF-110-ENT 82.0% 12.8%
36 HumanCov_OC43 82.0% 12.5%
37 Human_Cov_HKU1 81.1% 12.3%
38 FelineCov_strain_FIPV_WSU-79 81.4% 10.5%
39 HumanCov_229E 83.0% 10.1%
40 HumanCov_NL63/1-788 82.8% 9.9%
41 IBV_strainM41 77.8% 9.0%
consensus/100%
consensus/90%
consensus/80%
consensus/70%
cov pid 721 . . : 8 800
1 HumanCov_SARS2 100.0% 100.0%
2 PangolinCov_Manis_javanica 99.7% 94.0%
3 HumanCov_SARS 100.0% 68.4%
4 BatCov_279_2005 100.0% 69.2%
5 BatCov_Shaanxi2011 100.0% 69.4%
6 BatCov_SarsLike_YNLF_31Ctr 100.0% 69.1%
7 BatCov_YN2013 100.0% 68.0%
8 BatCov_JL2012 100.0% 69.1%
9 BatCov_HKU3 100.0% 69.1%
10 BatCov_Rp3_2004 100.0% 67.8%
11 SARSLikeBatCov_HKU3 99.7% 68.9%
12 BtRl-BetaCoV_SC2018_Rhinolophus_sp 99.4% 69.5%
13 SARSLikeCovWIV16_Rhinolophus_sinicus 99.7% 67.9%
14 BatCov_Rhinolophus_ferrumequinum 99.7% 69.1%
15 BatCov_Rhinolophus_blasii 99.5% 62.5%
16 BatCov_BM48-31 100.0% 62.5%
17 Bat_Hp_betaCoV_Hipposideros_pratti 97.2% 27.9%
18 ZariaBatCov_Hipposideros_commersoni 96.7% 27.7%
19 BatCov_Hypsugo_bat 92.5% 22.6%
20 BatCovMersLike_BtVs 95.8% 22.3%
21 BatCov_Vespertilio_sinensis 81.9% 23.4%
22 HKU4BatCov_Tylonycteris_bat 90.8% 20.7%
23 HedgehogCov 92.2% 20.1%
24 MERS_Bat-CoV_H.savii 91.7% 20.9%
25 MERS_Camel 90.0% 20.4%
26 BatCov_HKU4-4 95.2% 20.7%
27 BatCov_HKU4 95.2% 20.8%
28 BatCovHKU5 95.5% 20.5%
29 BatCov_Cov133_2005 95.2% 20.2%
30 HumanCov_MERS 95.8% 20.0%
31 BatCov_HKU9 86.7% 14.0%
32 BatCovHKU9-4 86.7% 14.5%
33 BatCov_Rousettus_bat 86.7% 14.1%
34 MurineCov_StrainJHM 80.8% 12.2%
35 BovineCov_strain_98TXSF-110-ENT 82.0% 12.8%
36 HumanCov_OC43 82.0% 12.5%
37 Human_Cov_HKU1 81.1% 12.3%
38 FelineCov_strain_FIPV_WSU-79 81.4% 10.5%
39 HumanCov_229E 83.0% 10.1%
40 HumanCov_NL63/1-788 82.8% 9.9%
41 IBV_strainM41 77.8% 9.0%
consensus/100%
consensus/90%
consensus/80%
consensus/70%


cov pid 801 880
1 HumanCov_SARS2 100.0% 100.0%
2 PangolinCov_Manis_javanica 99.7% 94.0%
3 HumanCov_SARS 100.0% 68.4%
4 BatCov_279_2005 100.0% 69.2%
5 BatCov_Shaanxi2011 100.0% 69.4%
6 BatCov_SarsLike_YNLF_31Ctr 100.0% 69.1%
7 BatCov_YN2013 100.0% 68.0%
8 BatCov_JL2012 100.0% 69.1%
9 BatCov_HKU3 100.0% 69.1%
10 BatCov_Rp3_2004 100.0% 67.8%
11 SARSLikeBatCov_HKU3 99.7% 68.9%
12 BtRl-BetaCoV_SC2018_Rhinolophus_sp 99.4% 69.5%
13 SARSLikeCovWIV16_Rhinolophus_sinicus 99.7% 67.9%
14 BatCov_Rhinolophus_ferrumequinum 99.7% 69.1%
15 BatCov_Rhinolophus_blasii 99.5% 62.5%
16 BatCov_BM48-31 100.0% 62.5%
17 Bat_Hp_betaCoV_Hipposideros_pratti 97.2% 27.9%
18 ZariaBatCov_Hipposideros_commersoni 96.7% 27.7%
19 BatCov_Hypsugo_bat 92.5% 22.6%
20 BatCovMersLike_BtVs 95.8% 22.3%
21 BatCov_Vespertilio_sinensis 81.9% 23.4%
22 HKU4BatCov_Tylonycteris_bat 90.8% 20.7%
23 HedgehogCov 92.2% 20.1%
24 MERS_Bat-CoV_H.savii 91.7% 20.9%
25 MERS_Camel 90.0% 20.4%
26 BatCov_HKU4-4 95.2% 20.7%
27 BatCov_HKU4 95.2% 20.8%
28 BatCovHKU5 95.5% 20.5%
29 BatCov_Cov133_2005 95.2% 20.2%
30 HumanCov_MERS 95.8% 20.0%
31 BatCov_HKU9 86.7% 14.0%
32 BatCovHKU9-4 86.7% 14.5%
33 BatCov_Rousettus_bat 86.7% 14.1%
34 MurineCov_StrainJHM 80.8% 12.2%
35 BovineCov_strain_98TXSF-110-ENT 82.0% 12.8%
36 HumanCov_OC43 82.0% 12.5%
37 Human_Cov_HKU1 81.1% 12.3%
38 FelineCov_strain_FIPV_WSU-79 81.4% 10.5%
39 HumanCov_229E 83.0% 10.1%
40 HumanCov_NL63/1-788 82.8% 9.9%
41 IBV_strainM41 77.8% 9.0%
consensus/100%
consensus/90%
consensus/80%
consensus/70%
cov pid 881 960
1 HumanCov_SARS2 100.0% 100.0%
2 PangolinCov_Manis_javanica 99.7% 94.0%
3 HumanCov_SARS 100.0% 68.4%
4 BatCov_279_2005 100.0% 69.2%
5 BatCov_Shaanxi2011 100.0% 69.4%
6 BatCov_SarsLike_YNLF_31Ctr 100.0% 69.1%
7 BatCov_YN2013 100.0% 68.0%
8 BatCov_JL2012 100.0% 69.1%
9 BatCov_HKU3 100.0% 69.1%
10 BatCov_Rp3_2004 100.0% 67.8%
11 SARSLikeBatCov_HKU3 99.7% 68.9%
12 BtRl-BetaCoV_SC2018_Rhinolophus_sp 99.4% 69.5%
13 SARSLikeCovWIV16_Rhinolophus_sinicus 99.7% 67.9%
14 BatCov_Rhinolophus_ferrumequinum 99.7% 69.1%
15 BatCov_Rhinolophus_blasii 99.5% 62.5%
16 BatCov_BM48-31 100.0% 62.5%
17 Bat_Hp_betaCoV_Hipposideros_pratti 97.2% 27.9%
18 ZariaBatCov_Hipposideros_commersoni 96.7% 27.7%
19 BatCov_Hypsugo_bat 92.5% 22.6%
20 BatCovMersLike_BtVs 95.8% 22.3%
21 BatCov_Vespertilio_sinensis 81.9% 23.4%
22 HKU4BatCov_Tylonycteris_bat 90.8% 20.7%
23 HedgehogCov 92.2% 20.1%
24 MERS_Bat-CoV_H.savii 91.7% 20.9%
25 MERS_Camel 90.0% 20.4%
26 BatCov_HKU4-4 95.2% 20.7%
27 BatCov_HKU4 95.2% 20.8%
28 BatCovHKU5 95.5% 20.5%
29 BatCov_Cov133_2005 95.2% 20.2%
30 HumanCov_MERS 95.8% 20.0%
31 BatCov_HKU9 86.7% 14.0%
32 BatCovHKU9-4 86.7% 14.5%
33 BatCov_Rousettus_bat 86.7% 14.1%
34 MurineCov_StrainJHM 80.8% 12.2%
35 BovineCov_strain_98TXSF-110-ENT 82.0% 12.8%
36 HumanCov_OC43 82.0% 12.5%
37 Human_Cov_HKU1 81.1% 12.3%
38 FelineCov_strain_FIPV_WSU-79 81.4% 10.5%
39 HumanCov_229E 83.0% 10.1%
40 HumanCov_NL63/1-788 82.8% 9.9%
41 IBV_strainM41 77.8% 9.0%
consensus/100%
consensus/90%
consensus/80%
consensus/70%


cov pid 961 0 1040
1 HumanCov_SARS2 100.0% 100.0%
2 PangolinCov_Manis_javanica 99.7% 94.0%
3 HumanCov_SARS 100.0% 68.4%
4 BatCov_279_2005 100.0% 69.2%
5 BatCov_Shaanxi2011 100.0% 69.4%
6 BatCov_SarsLike_YNLF_31Ctr 100.0% 69.1%
7 BatCov_YN2013 100.0% 68.0%
8 BatCov_JL2012 100.0% 69.1%
9 BatCov_HKU3 100.0% 69.1%
10 BatCov_Rp3_2004 100.0% 67.8%
11 SARSLikeBatCov_HKU3 99.7% 68.9%
12 BtRl-BetaCoV_SC2018_Rhinolophus_sp 99.4% 69.5%
13 SARSLikeCovWIV16_Rhinolophus_sinicus 99.7% 67.9%
14 BatCov_Rhinolophus_ferrumequinum 99.7% 69.1%
15 BatCov_Rhinolophus_blasii 99.5% 62.5%
16 BatCov_BM48-31 100.0% 62.5%
17 Bat_Hp_betaCov_Hipposideros_pratti 97.2% 27.9%
18 ZariaBatCov_Hipposideros_commersoni 96.7% 27.7%
19 BatCov_Hypsugo_bat 92.5% 22.6%
20 BatCovMersLike_BtVs 95.8% 22.3%
21 BatCov_Vespertilio_sinensis 81.9% 23.4%
22 HKU4BatCov_Tylonycteris_bat 90.8% 20.7%
23 HedgehogCov 92.2% 20.1%
24 MERS_Bat-CoV_H.savii 91.7% 20.9%
25 MERS_Camel 90.0% 20.4%
26 BatCov_HKU4-4 95.2% 20.7%
27 BatCov_HKU4 95.2% 20.8%
28 BatCovHKU5 95.5% 20.5%
29 BatCov_Cov133_2005 95.2% 20.2%
30 HumanCov_MERS 95.8% 20.0%
31 BatCov_HKU9 86.7% 14.0%
32 BatCovHKU9-4 86.7% 14.5%
33 BatCov_Rousettus_bat 86.7% 14.1%
34 MurineCov_StrainJHM 80.8% 12.2%
35 BovineCov_strain_98TXSF-110-ENT 82.0% 12.8%
36 HumanCov_OC43 82.0% 12.5%
37 Human_Cov_HKU1 81.1% 12.3%
38 FelineCov_strain_FIPV_WSU-79 81.4% 10.5%
39 HumanCov_229E 83.0% 10.1%
40 HumanCov_NL63/1-788 82.8% 9.9%
41 IBV_strainM41 77.8% 9.0%
consensus/100%
consensus/90%
consensus/80%
consensus/70%
cov pid 1041] 1082
1 HumanCov_SARS2 100.0% 100.0%
2 PangolinCov_Manis_javanica 99.7% 94.0%
3 HumanCov_SARS 100.0% 68.4%
4 BatCov_279_2005 100.0% 69.2%
5 BatCov_Shaanxi2011 100.0% 69.4%
6 BatCov_SarsLike_YNLF_31Ctr 100.0% 69.1%
7 BatCov_YN2013 100.0% 68.0%
8 BatCov_JL2012 100.0% 69.1%
9 BatCov_HKU3 100.0% 69.1%
10 BatCov_Rp3_2004 100.0% 67.8%
11 SARSLikeBatCov_HKU3 99.7% 68.9%
12 BtRl-BetaCoV_SC2018_Rhinolophus_sp 99.4% 69.5%
13 SARSLikeCovWIV16_Rhinolophus_sinicus 99.7% 67.9%
14 BatCov_Rhinolophus_ferrumequinum 99.7% 69.1%
15 BatCov_Rhinolophus_blasii 99.5% 62.5%
16 BatCov_BM48-31 100.0% 62.5%
17 Bat_Hp_betaCov_Hipposideros_pratti 97.2% 27.9%
18 ZariaBatCov_Hipposideros_commersoni 96.7% 27.7%
19 BatCov_Hypsugo_bat 92.5% 22.6%
20 BatCovMersLike_BtVs 95.8% 22.3%
21 BatCov_Vespertilio_sinensis 81.9% 23.4%
22 HKU4BatCov_Tylonycteris_bat 90.8% 20.7%
23 HedgehogCov 92.2% 20.1%
24 MERS_Bat-CoV_H.savii 91.7% 20.9%
25 MERS_Camel 90.0% 20.4%
26 BatCov_HKU4-4 95.2% 20.7%
27 BatCov_HKU4 95.2% 20.8%
28 BatCovHKU5 95.5% 20.5%
29 BatCov_Cov133_2005 95.2% 20.2%
30 HumanCov_MERS 95.8% 20.0%
31 BatCov_HKU9 86.7% 14.0%
32 BatCovHKU9-4 86.7% 14.5%
33 BatCov_Rousettus_bat 86.7% 14.1%
34 MurineCov_StrainJHM 80.8% 12.2%
35 BovineCov_strain_98TXSF-110-ENT 82.0% 12.8%
36 HumanCov_OC43 82.0% 12.5%
37 Human_Cov_HKU1 81.1% 12.3%
38 FelineCov_strain_FIPV_WSU-79 81.4% 10.5%
39 HumanCov_229E 83.0% 10.1%
40 HumanCov_NL63/1-788 82.8% 9.9%
41 IBV_strainM41 77.8% 9.0%
consensus/100%
consensus/90%
consensus/80%
consensus/70%

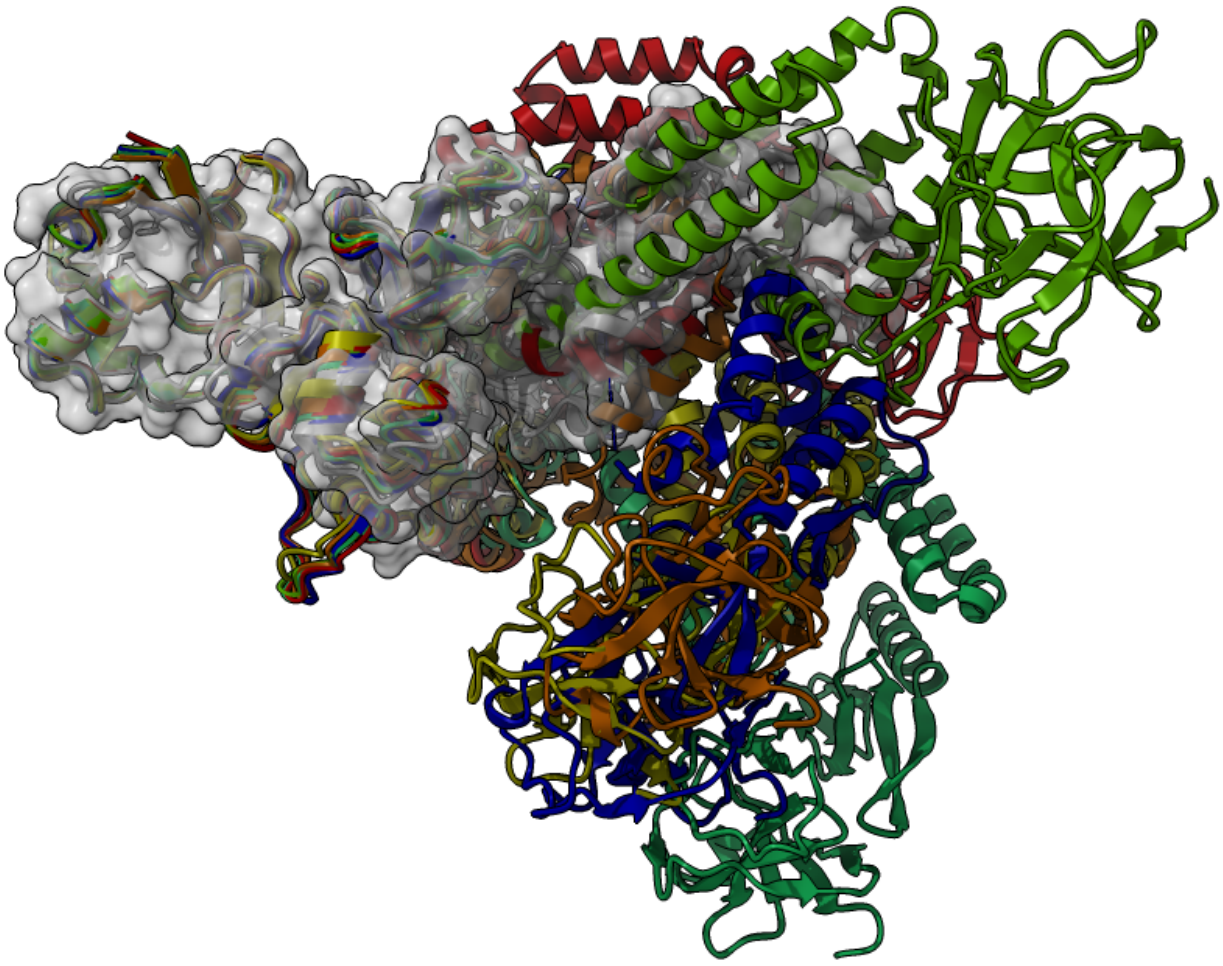
Supplementary Figure 2.



Supplementary Figure 2. Global and directional FSC curves for the Nsp2 cryo-EM reconstructions.

(A) Global FSC curve for the 3.2 Å Nsp2 reconstruction as output from Relion. In black is the final corrected masked FSC. In blue is an uncorrected masked FSC. In green is FSC of unmasked volumes and in red is FSC of the volumes which were phase randomized beyond 7.3 Å. **(B)** Global FSC curve for the 3.8 Å Nsp2 reconstruction as output from Relion. In black is the final corrected masked FSC. In blue is an uncorrected masked FSC. In green is unmasked FSC and in red is FSC of the reconstructions which were phase randomized beyond 8 Å. **(C)** Directional FSC for the 3.2 Å reconstruction of Nsp2 as output by the 3DFSC server, estimated sphericity is 0.86. **(D)** Directional FSC for the 3.8 Å reconstruction of Nsp2 as output by the 3DFSC server, estimated sphericity is 0.81.

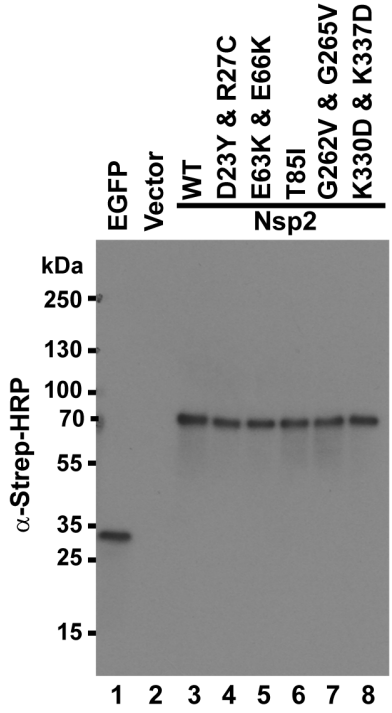
Supplementary Figure 3.



Supplementary Figure 3. AlphaFold2 predictions for Nsp2 have low accuracy in predicting the overall shape of the protein.

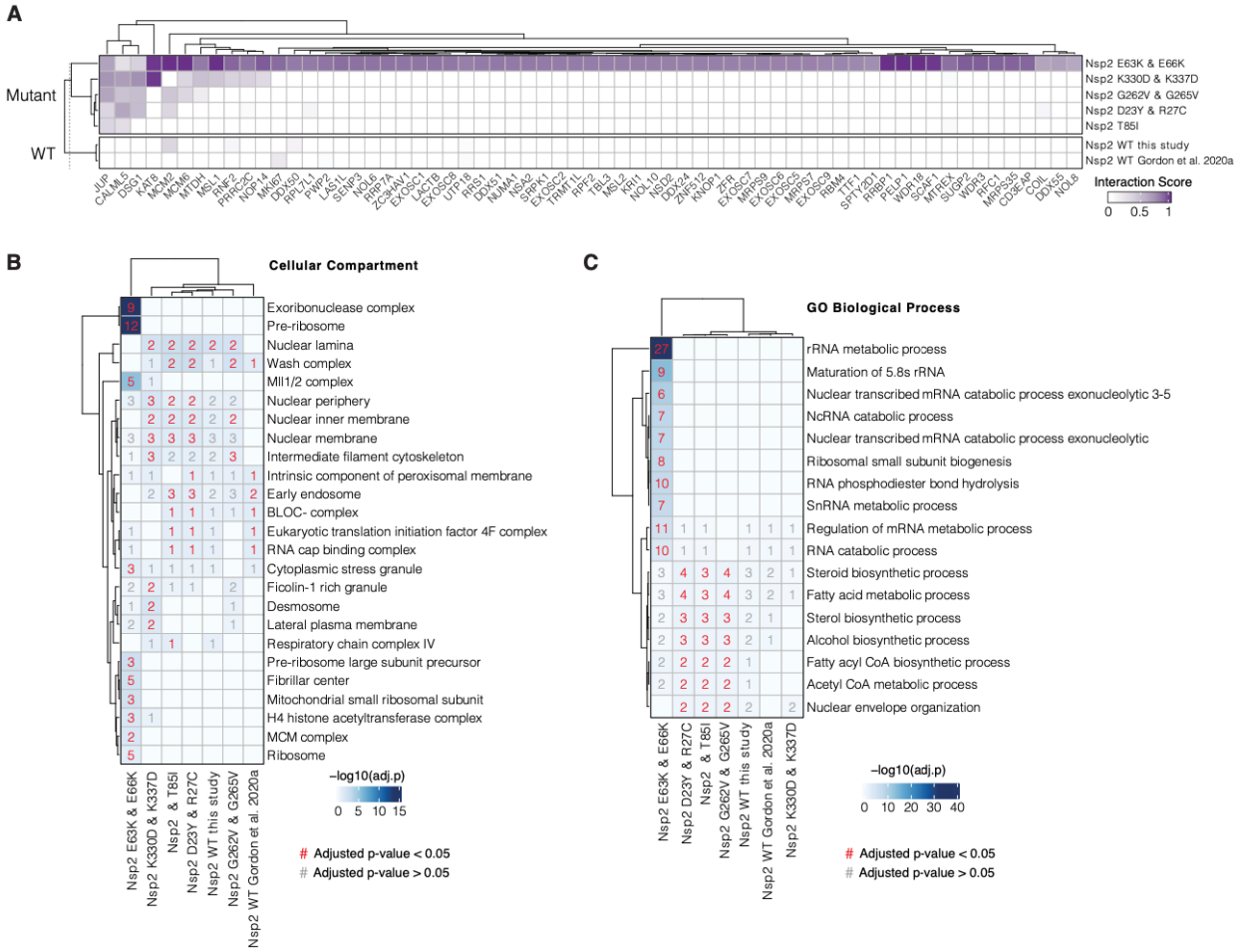
All publicly available predictions from AlphaFold2 team for Nsp2 (multicolored) were aligned to the experimental model of Nsp2 (gray surface). Although individual domains align well locally, globally there is a considerable deviation from the experimental model.

Supplementary figure 4.



Supplementary figure 4. Expression of wild type and mutant SARS-CoV-2 Nsp2 proteins in HEK293T cells. Reserved lysates (50 ul) were incubated at 95°C for 5 minutes with an equal volume of 2x sample buffer (Morganville Scientific). After further diluting 1:10 in 2x sample buffer, 5 ul was resolved on a 4-20% Criterion TGX Precast Midi Protein Gel (BioRad) and transferred to a 0.2 mm PVDF membrane using the Trans-Blot Turbo Transfer System (2.5 A, 25 V, 7 minutes). Membranes were blocked with 3% BSA in 1x Phosphate Buffered Saline (PBS) supplemented with 0.2% Tween 20 (0.2% T-PBS) at 4°C and incubated with Strep-Tag II Antibody HRP Conjugate (Millipore) at room temperature for 1 hour (1:10,000 in 1% BSA, 0.2% T-PBS). Membrane was washed in 0.2% T-PBS after blocking and antibody incubation steps and developed with Pierce ECL Western Blotting Substrate (ThermoFisher Scientific).

Supplementary figure 5.



Supplementary figure 5. Nsp2 E63K/E66K Mutation Gains Interactions with Exoribonuclease and Pre-ribosome Components.

(A) Interaction scores (average between MiST and Saint Scores) for human proteins (“preys”) deemed high-confidence interactions in at least one affinity purification (“bait”) mass spectrometry assay and detected to not interact with neither the wild-type Nsp2 in this study or in Gordon et al (2020a). Interaction scores range from zero to one, one being the most high-confidence. **(B)** Gene Ontology Cellular Compartment enrichment analysis (MSigDB) for preys passing the master scoring criteria (see Methods) for each bait (i.e. affinity purification experiment). The top 10 most significant enrichments for each bait are displayed as well as corresponding enrichments for other baits, if applicable. Color denotes the -$\log_{10}$(adjusted p-value). The number of preys enriched in each bait for each term are shown. Red numbers

indicated adjusted p-values < 0.05 whereas grey numbers indicate adjusted p-values > 0.05. **(C)** Same as in B using Gene Ontology Biological Process terms (MSigDB).

Supplementary Table 1. CryoEM collection, refinement and resulting model statistics.

	Nsp2 without Zn (EMDB-xxxx) (PDB xxxx)	Nsp2 with Zn (EMDB-xxxx) (PDB xxxx)
Data collection and processing		
Magnification	105,000x	105,000x
Voltage (kV)	300	300
Electron exposure (e–/Å^2)	66	67
Dose rate (e-/physical pixel/sec)	8	8
Exposure per frame (sec)	0.05	0.05
Defocus range (μm)	-0.8 to -2.4	-0.8 to -2.4
Pixel size (Å)	0.834 (physical)	0.834 (physical)
Symmetry imposed	C1	C1
Initial particle images (no.)	363145+577518	1515264
Final particle images (no.)	42579	81817
Map resolution (Å) FSC threshold	3.76	3.15
Map resolution range (Å)	3.5-6.6	3.0-4.3
Refinement		
Initial model used (PDB code)	ab-initio	ab-initio
Model resolution (Å) FSC threshold 0.143 (Unmasked) FSC threshold 0.5 (Unmasked)	 3.6 3.9	 3.12 3.46
Model resolution range (Å)	3.6-4.1	3.1-3.5
Map sharpening *B* factor (Å^2)	-122	-135
Model composition Non-hydrogen atoms Protein residues Ligands	 4922 635 Zn: 3	 3706 473 Zn: 3
B factors (Å^2) Protein Ligand	 194.4 79.8	 52.83 50.97

R.M.S. deviations		
Bond lengths (Å)	0.009	0.008
Bond angles (°)	0.982	0.946
Validation		
MolProbity score	0.86	0.75
Clashscore	0.71	0.81
Poor rotamers (%)	0.00	0.00
Ramachandran plot		
Favored (%)	97.31	98.07
Allowed (%)	2.53	1.93
Disallowed (%)	0.16	0.00