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## Cellular cryo-tomography of nidovirus replication organelles

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# REFERENCES

1. Cavanagh, D. (1997) Nidovirales: a new order comprising Coronaviridae and Arteriviridae. *Arch Virol* 142(3):629-633.
2. den Boon, J. A., Snijder, E. J., Chirnside, E. D., de Vries, A. A., Horzinek, M. C., Spaan, W. J. (1991) eritis virus is not a togavirus but belongs to the coronaviruslike superfamily. *Journal of virology* 65(6):2910-2920.
3. Pringle, C. R. (1996) Virus Taxonomy 1996 — A Bulletin from the Xth International Congress of Virology in Jerusalem. *Archives of Virology* 141(11):2251-2256.
4. Zhou, Z., Qiu, Y., Ge, X. (2021) The taxonomy, host range and pathogenicity of coronaviruses and other viruses in the Nidovirales order. *Animal Diseases* 1(1):5.
5. Van Vliet, A., Smits, S., Rottier, P., De Groot, R. (2002) Discontinuous and non-discontinuous subgenomic RNA transcription in a nidovirus. *The EMBO journal* 21(23):6571-6580.
6. Saberi, A., Gulyaeva, A. A., Brubacher, J. L., Newmark, P. A., Gorbalenya, A. E. (2018) A planarian nidovirus expands the limits of RNA genome size. *PLoS pathogens* 14(11):e1007314.
7. Ksiazek, T. G. *et al.* (2003) A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 348(20):1953-1966.
8. van Boheemen, S., de Graaf, M., Lauber, C., Bestebroer, T. M., Raj, V. S., Zaki, A. M., Osterhaus, A. D., Haagmans, B. L., Gorbalenya, A. E., Snijder, E. J., Fouchier, R. A. (2012) Genomic characterization of a newly discovered coronavirus associated with acute respiratory distress syndrome in humans. *mBio* 3(6).
9. Zhou, P. *et al.* (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579(7798):270-273.
10. Farinholt, T., Doddapaneni, H., Qin, X., Menon, V., Meng, Q., Metcalf, G., Chao, H., Gingras, M.-C., Avadhanula, V., Farinholt, P. (2021) Transmission event of SARS-CoV-2 Delta variant reveals multiple vaccine breakthrough infections. *BMC medicine* 19(1):1-6.
11. Singanayagam, A., Hakki, S., Dunning, J., Madon, K. J., Crone, M. A., Koycheva, A., Derqui-Fernandez, N., Barnett, J. L., Whitfield, M. G., Varro, R. (2021) Community transmission and viral load kinetics of the SARS-CoV-2 delta (B. 1.617. 2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study. *The Lancet Infectious Diseases*.
12. Tian, D., Sun, Y., Xu, H., Ye, Q. (2022) The emergence and epidemic characteristics of the highly mutated SARS-CoV-2 Omicron variant. *J Med Virol*.
13. Dance, A. (2022) Omicron's lasting mysteries: four questions scientists are racing to answer. *Nature* 603(7899):22-24.
14. Veldhoen, M., Simas, J. P. (2021) Endemic SARS-CoV-2 will maintain post-pandemic immunity. *Nature Reviews Immunology* 21(3):131-132.
15. Creech, C. B., Walker, S. C., Samuels, R. J. (2021) SARS-CoV-2 vaccines. *Jama* 325(13):1318-1320.
16. Pan, H. *et al.* (2021) Repurposed Antiviral Drugs for Covid-19- Interim WHO Solidarity Trial Results. *N Engl J Med* 384(6):497-511.
17. Joshi, S., Parkar, J., Ansari, A., Vora, A., Talwar, D., Tiwaskar, M., Patil, S., Barkate, H. (2021) Role of favipiravir in the treatment of COVID-19. *Int J Infect Dis* 102:501-508.
18. Kozlov, M. (2022) Why scientists are racing to develop more COVID antivirals. *Nature* 601(7894):496.
19. Lednicky, J. A., Tagliamonte, M. S., White, S. K., Elbadry, M. A., Alam, M. M., Stephenson, C. J.,



Bonny, T. S., Loeb, J. C., Telisma, T., Chavannes, S., Ostrov, D. A., Mavian, C., Beau De Rochars, V. M., Salemi, M., Morris, J. G. (2021) Independent infections of porcine deltacoronavirus among Haitian children. *Nature* 600(7887):133-137.

20. Terpstra, C., Wensvoort, G., Pol, J. (1991) Experimental reproduction of porcine epidemic abortion and respiratory syndrome (mystery swine disease) by infection with Lelystad virus: Koch's postulates fulfilled. *Veterinary Quarterly* 13(3):131-136.
21. Holtkamp, D. J., Kliebenstein, J. B., Neumann, E., Zimmerman, J. J., Rotto, H., Yoder, T. K., Wang, C., Yeske, P., Mowrer, C. L., Haley, C. A. (2013) Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. *Journal of Swine Health and Production* 21(2):72.
22. Thomann, B., Rushton, J., Schuepbach-Regula, G., Nathues, H. (2020) Modeling Economic Effects of Vaccination Against Porcine Reproductive and Respiratory Syndrome: Impact of Vaccination Effectiveness, Vaccine Price, and Vaccination Coverage. *Frontiers in Veterinary Science* 7(500).
23. Doll, E. (1957) Isolation of a filterable agent causing arteritis of horses and abortion by mares: Its differentiations from equine abortion (influenza) virus. *Cornell Vet.* 47:3-42.
24. Balasuriya, U. B. R., Carossino, M., Timoney, P. J. (2018) Equine viral arteritis: A respiratory and reproductive disease of significant economic importance to the equine industry. *Equine Veterinary Education* 30(9):497-512.
25. Huang, Y., Yang, C., Xu, X.-f., Xu, W., Liu, S.-w. (2020) Structural and functional properties of SARS-CoV-2 spike protein: potential antiviral drug development for COVID-19. *Acta Pharmacologica Sinica* 41(9):1141-1149.
26. Li, F. (2016) Structure, Function, and Evolution of Coronavirus Spike Proteins. *Annu Rev Virol* 3(1):237-261.
27. Veit, M., Matczuk, A. K., Sinhadri, B. C., Krause, E., Thaa, B. (2014) Membrane proteins of arterivirus particles: Structure, topology, processing and function. *Virus Research* 194:16-36.
28. Nauwynck, H. J., Duan, X., Favoreel, H. W., Van Oostveldt, P., Pensaert, M. B. (1999) Entry of porcine reproductive and respiratory syndrome virus into porcine alveolar macrophages via receptor-mediated endocytosis. *The Journal of general virology* 80 ( Pt 2):297-305.
29. Sarkar, S., Chelvarajan, L., Go, Y. Y., Cook, F., Artiushin, S., Mondal, S., Anderson, K., Eberth, J., Timoney, P. J., Kalbfleisch, T. S., Bailey, E., Balasuriya, U. B. (2016) Equine Arteritis Virus Uses Equine CXCL16 as an Entry Receptor. *Journal of virology* 90(7):3366-3384.
30. Hofmann, H., Pöhlmann, S. (2004) Cellular entry of the SARS coronavirus. *Trends in Microbiology* 12(10):466-472.
31. Jackson, C. B., Farzan, M., Chen, B., Choe, H. (2022) Mechanisms of SARS-CoV-2 entry into cells. *Nature Reviews Molecular Cell Biology* 23(1):3-20.
32. Meng, B. *et al.* (2022) Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts tropism and fusogenicity. *Nature*.
33. Zhao, H., Lu, L., Peng, Z., Chen, L. L., Meng, X., Zhang, C., Ip, J. D., Chan, W. M., Chu, A. W., Chan, K. H., Jin, D. Y., Chen, H., Yuen, K. Y., To, K. K. (2022) SARS-CoV-2 Omicron variant shows less efficient replication and fusion activity when compared with Delta variant in TMPRSS2-expressed cells. *Emerg Microbes Infect* 11(1):277-283.
34. Firth, A. E., Brierley, I. (2012) Non-canonical translation in RNA viruses. *The Journal of general virology* 93(Pt 7):1385-1409.



35. Irigoyen, N., Firth, A. E., Jones, J. D., Chung, B. Y., Siddell, S. G., Brierley, I. (2016) High-Resolution Analysis of Coronavirus Gene Expression by RNA Sequencing and Ribosome Profiling. *PLoS pathogens* 12(2):e1005473.
36. Ziebuhr, J., Snijder, E. J., Gorbalenya, A. E. (2000) Virus-encoded proteinases and proteolytic processing in the Nidovirales. *The Journal of general virology* 81(Pt 4):853-879.
37. Gulyaeva, A. A., Gorbalenya, A. E. (2021) A nidovirus perspective on SARS-CoV-2. *Biochem Biophys Res Commun* 538:24-34.
38. Mielech, A. M., Chen, Y., Mesecar, A. D., Baker, S. C. (2014) Nidovirus papain-like proteases: multifunctional enzymes with protease, deubiquitinating and deISGylating activities. *Virus Res* 194:184-190.
39. Posthuma, C. C., Te Velhuis, A. J. W., Snijder, E. J. (2017) Nidovirus RNA polymerases: Complex enzymes handling exceptional RNA genomes. *Virus research* 234:58-73.
40. Malone, B., Urakova, N., Snijder, E. J., Campbell, E. A. (2022) Structures and functions of coronavirus replication–transcription complexes and their relevance for SARS-CoV-2 drug design. *Nature Reviews Molecular Cell Biology* 23(1):21-39.
41. Romero-Brey, I., Bartenschlager, R. (2014) Membranous replication factories induced by plus-strand RNA viruses. *Viruses* 6(7):2826-2857.
42. de Haan, C. A., Rottier, P. J. (2005) Molecular interactions in the assembly of coronaviruses. *Adv Virus Res* 64:165-230.
43. Ghosh, S., Dellibovi-Ragheb, T. A., Kerviel, A., Pak, E., Qiu, Q., Fisher, M., Takvorian, P. M., Bleck, C., Hsu, V. W., Fehr, A. R., Perlman, S., Achar, S. R., Straus, M. R., Whittaker, G. R., de Haan, C. A. M., Kehrl, J., Altan-Bonnet, G., Altan-Bonnet, N. (2020)  $\beta$ -Coronaviruses Use Lysosomes for Egress Instead of the Biosynthetic Secretory Pathway. *Cell* 183(6):1520-1535.e1514.
44. Angelini, M. M., Akhlaghpour, M., Neuman, B. W., Buchmeier, M. J. (2013) Severe acute respiratory syndrome coronavirus nonstructural proteins 3, 4, and 6 induce double-membrane vesicles. *mBio* 4(4).
45. Hagemeyer, M. C., Monastyrska, I., Griffith, J., van der Sluijs, P., Voortman, J., van Bergen en Henegouwen, P. M., Vonk, A. M., Rottier, P. J., Reggiori, F., de Haan, C. A. (2014) Membrane rearrangements mediated by coronavirus nonstructural proteins 3 and 4. *Virology* 458-459:125-135.
46. Doyle, N., Neuman, W. B., Simpson, J., Hawes, C. P., Mantell, J., Verkade, P., Alrashedi, H., Maier, J. H. (2018) Infectious Bronchitis Virus Nonstructural Protein 4 Alone Induces Membrane Pairing. *Viruses* 10(9).
47. Snijder, E. J., van Tol, H., Roos, N., Pedersen, K. W. (2001) Non-structural proteins 2 and 3 interact to modify host cell membranes during the formation of the arterivirus replication complex. *The Journal of general virology* 82(Pt 5):985-994.
48. van der Hoeven, B., Oudshoorn, D., Koster, A. J., Snijder, E. J., Kikkert, M., Barcena, M. (2016) Biogenesis and architecture of arterivirus replication organelles. *Virus Res* 220:70-90.
49. Stertz, S., Reichelt, M., Spiegel, M., Kuri, T., Martínez-Sobrido, L., García-Sastre, A., Weber, F., Kochs, G. (2007) The intracellular sites of early replication and budding of SARS-coronavirus. *Virology* 361(2):304-315.
50. Snijder, E. J., van der Meer, Y., Zevenhoven-Dobbe, J., Onderwater, J. J., van der Meulen, J., Koerten, H. K., Mommaas, A. M. (2006) Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex.



*Journal of virology* 80(12):5927-5940.

51. Pedersen, K. W., van der Meer, Y., Roos, N., Snijder, E. J. (1999) Open reading frame 1a-encoded subunits of the arterivirus replicase induce endoplasmic reticulum-derived double-membrane vesicles which carry the viral replication complex. *Journal of virology* 73(3):2016-2026.
52. Ulasli, M., Verheije, M. H., de Haan, C. A., Reggiori, F. (2010) Qualitative and quantitative ultrastructural analysis of the membrane rearrangements induced by coronavirus. *Cellular microbiology* 12(6):844-861.
53. Doyle, N., Hawes, P. C., Simpson, J., Adams, L. H., Maier, H. J. (2019) The Porcine Deltacoronavirus Replication Organelle Comprises Double-Membrane Vesicles and Zippered Endoplasmic Reticulum with Double-Membrane Spherules. *Viruses* 11(11).
54. Maier, H. J., Hawes, P. C., Cottam, E. M., Mantell, J., Verkade, P., Monaghan, P., Wileman, T., Britton, P. (2013) Infectious bronchitis virus generates spherules from zippered endoplasmic reticulum membranes. *mBio* 4(5):e00801-00813.
55. Knoops, K., Kikkert, M., Worm, S. H., Zevenhoven-Dobbe, J. C., van der Meer, Y., Koster, A. J., Mommaas, A. M., Snijder, E. J. (2008) SARS-coronavirus replication is supported by a reticulovesicular network of modified endoplasmic reticulum. *PLoS biology* 6(9):e226.
56. Knoops, K., Barcena, M., Limpens, R. W., Koster, A. J., Mommaas, A. M., Snijder, E. J. (2012) Ultrastructural characterization of arterivirus replication structures: reshaping the endoplasmic reticulum to accommodate viral RNA synthesis. *Journal of virology* 86(5):2474-2487.
57. Zhang, W., Chen, K., Zhang, X., Guo, C., Chen, Y., Liu, X. (2018) An integrated analysis of membrane remodeling during porcine reproductive and respiratory syndrome virus replication and assembly. *PLoS one* 13(7):e0200919.
58. Cortese, M. *et al.* (2020) Integrative Imaging Reveals SARS-CoV-2-Induced Reshaping of Subcellular Morphologies. *Cell host & microbe*.
59. Ávila-Pérez, G., Rejas, M. T., Chichón, F. J., Guerra, M., Fernández, J. J., Rodríguez, D. (2021) Architecture of torovirus replicative organelles. *Mol Microbiol*.
60. Gosert, R., Kanjanahaluethai, A., Egger, D., Bienz, K., Baker, S. C. (2002) RNA replication of mouse hepatitis virus takes place at double-membrane vesicles. *Journal of virology* 76(8):3697-3708.
61. Snijder, E. J., Limpens, R., de Wilde, A. H., de Jong, A. W. M., Zevenhoven-Dobbe, J. C., Maier, H. J., Faas, F., Koster, A. J., Barcena, M. (2020) A unifying structural and functional model of the coronavirus replication organelle: Tracking down RNA synthesis. *PLoS biology* 18(6):e3000715.
62. Avila-Perez, G., Rejas, M. T., Rodriguez, D. (2016) Ultrastructural characterization of membranous torovirus replication factories. *Cellular microbiology* 18(12):1691-1708.
63. Korogod, N., Petersen, C. C., Knott, G. W. (2015) Ultrastructural analysis of adult mouse neocortex comparing aldehyde perfusion with cryo fixation. *eLife* 4.
64. Tamada, H., Blanc, J., Korogod, N., Petersen, C. C., Knott, G. W. (2020) Ultrastructural comparison of dendritic spine morphology preserved with cryo and chemical fixation. *eLife* 9.
65. Dubochet, J., Adrian, M., Chang, J.-J., Homo, J.-C., Lepault, J., McDowell, A. W., Schultz, P. (1988) Cryo-electron microscopy of vitrified specimens. *Quarterly reviews of biophysics* 21(2):129-228.
66. Rigort, A., Bäuerlein Felix, J. B., Villa, E., Eibauer, M., Laugks, T., Baumeister, W., Plitzko Jürgen, M. (2012) Focused ion beam micromachining of eukaryotic cells for cryoelectron tomography.



*Proceedings of the National Academy of Sciences* 109(12):4449-4454.

67. Kruger, D. H., Schneck, P., Gelderblom, H. R. (2000) Helmut Ruska and the visualisation of viruses. *Lancet* 355(9216):1713-1717.
68. Callaway, E. (2020) Revolutionary cryo-EM is taking over structural biology. *Nature* 578(7794):201.
69. Cheng, Y. (2018) Single-particle cryo-EM-How did it get here and where will it go. *Science* 361(6405):876-880.
70. Koning, R. I., Koster, A. J., Sharp, T. H. (2018) Advances in cryo-electron tomography for biology and medicine. *Ann Anat* 217:82-96.
71. Castaño-Díez, D., Zanetti, G. (2019) In situ structure determination by subtomogram averaging. *Curr Opin Struct Biol* 58:68-75.
72. Pfeffer, S., Burbaum, L., Unverdorben, P., Pech, M., Chen, Y., Zimmermann, R., Beckmann, R., Förster, F. (2015) Structure of the native Sec61 protein-conducting channel. *Nature Communications* 6(1):8403.
73. Bharat, T. A. M., Kureisaite-Ciziene, D., Hardy, G. G., Yu, E. W., Devant, J. M., Hagen, W. J. H., Brun, Y. V., Briggs, J. A. G., Löwe, J. (2017) Structure of the hexagonal surface layer on *Caulobacter crescentus* cells. *Nat Microbiol* 2:17059.
74. Wan, W., Kolesnikova, L., Clarke, M., Koehler, A., Noda, T., Becker, S., Briggs, J. A. G. (2017) Structure and assembly of the Ebola virus nucleocapsid. *Nature* 551(7680):394-397.
75. Schur Florian, K. M., Obr, M., Hagen Wim, J. H., Wan, W., Jakobi Arjen, J., Kirkpatrick Joanna, M., Sachse, C., Kräusslich, H.-G., Briggs John, A. G. (2016) An atomic model of HIV-1 capsid-SP1 reveals structures regulating assembly and maturation. *Science* 353(6298):506-508.
76. Mendonça, L., Sun, D., Ning, J., Liu, J., Kotecha, A., Olek, M., Frosio, T., Fu, X., Himes, B. A., Kleinpeter, A. B., Freed, E. O., Zhou, J., Aiken, C., Zhang, P. (2021) CryoET structures of immature HIV Gag reveal six-helix bundle. *Communications Biology* 4(1):481.
77. Tegunov, D., Xue, L., Dienemann, C., Cramer, P., Mahamid, J. (2021) Multi-particle cryo-EM refinement with M visualizes ribosome-antibiotic complex at 3.5 Å in cells. *Nature Methods* 18(2):186-193.
78. Al-Amoudi, A., Chang, J.-J., Leforestier, A., McDowall, A., Salamin, L. M., Norlén, L. P. O., Richter, K., Blanc, N. S., Studer, D., Dubochet, J. (2004) Cryo-electron microscopy of vitreous sections. *The EMBO journal* 23(18):3583-3588.
79. McDowall, A. W., Chang, J. J., Freeman, R., Lepault, J., Walter, C. A., Dubochet, J. (1983) Electron microscopy of frozen hydrated sections of vitreous ice and vitrified biological samples. *J Microsc* 131(Pt 1):1-9.
80. Han, H. M., Zuber, B., Dubochet, J. (2008) Compression and crevasses in vitreous sections under different cutting conditions. *J Microsc* 230(Pt 2):167-171.
81. Marko, M., Hsieh, C., Schalek, R., Frank, J., Mannella, C. (2007) Focused-ion-beam thinning of frozen-hydrated biological specimens for cryo-electron microscopy. *Nat Methods* 4(3):215-217.
82. Wagner, J., Schaffer, M., Fernández-Busnadiego, R. (2017) Cryo-electron tomography-the cell biology that came in from the cold. *FEBS Lett* 591(17):2520-2533.
83. Buckley, G., Gervinskis, G., Taveneau, C., Venugopal, H., Whisstock, J. C., de Marco, A. (2020) Automated cryo-lamella preparation for high-throughput in-situ structural biology. *Journal of*



*structural biology* 210(2):107488.

84. Zachs, T., Schertel, A., Medeiros, J., Weiss, G. L., Hugener, J., Matos, J., Pilhofer, M. (2020) Fully automated, sequential focused ion beam milling for cryo-electron tomography. *eLife* 9.
85. Narayan, K., Subramaniam, S. (2015) Focused ion beams in biology. *Nat Methods* 12(11):1021-1031.
86. Mahamid, J., Pfeffer, S., Schaffer, M., Villa, E., Danev, R., Cuellar, L. K., Förster, F., Hyman, A. A., Plitzko, J. M., Baumeister, W. (2016) Visualizing the molecular sociology at the HeLa cell nuclear periphery. *Science* 351(6276):969-972.
87. Erdmann, P. S., Hou, Z., Klumpe, S., Khavnekar, S., Beck, F., Wilfling, F., Plitzko, J. M., Baumeister, W. (2021) In situ cryo-electron tomography reveals gradient organization of ribosome biogenesis in intact nucleoli. *Nat Commun* 12(1):5364.
88. Wang, Z., Grange, M., Wagner, T., Kho, A. L., Gautel, M., Raunser, S. (2021) The molecular basis for sarcomere organization in vertebrate skeletal muscle. *Cell* 184(8):2135-2150.e2113.
89. Bykov, Y. S., Schaffer, M., Dodonova, S. O., Albert, S., Plitzko, J. M., Baumeister, W., Engel, B. D., Briggs, J. A. G. (2017) The structure of the COPI coat determined within the cell. *eLife* 6:e32493.
90. Zimmerli, C. E., Allegretti, M., Rantos, V., Goetz, S. K., Obarska-Kosinska, A., Zagoriy, I., Halavatyi, A., Hummer, G., Mahamid, J., Kosinski, J., Beck, M. (2021) Nuclear pores dilate and constrict in cellulose. *Science* 374(6573):eabd9776.
91. Zila, V., Margiotta, E., Turoňová, B., Müller, T. G., Zimmerli, C. E., Mattei, S., Allegretti, M., Börner, K., Rada, J., Müller, B., Lusic, M., Kräusslich, H.-G., Beck, M. (2021) Cone-shaped HIV-1 capsids are transported through intact nuclear pores. *Cell* 184(4):1032-1046.e1018.
92. Scutigliani, E. M., Kikkert, M. (2017) Interaction of the innate immune system with positive-strand RNA virus replication organelles. *Cytokine & growth factor reviews* 37:17-27.
93. de Castro, I. F., Volonte, L., Risco, C. (2013) Virus factories: biogenesis and structural design. *Cellular microbiology* 15(1):24-34.
94. Liu, L., Cooper, T., Howley, P. M., Hayball, J. D. (2014) From crescent to mature virion: vaccinia virus assembly and maturation. *Viruses* 6(10):3787-3808.
95. Tenorio, R., Fernandez de Castro, I., Knowlton, J. J., Zamora, P. F., Sutherland, D. M., Risco, C., Dermody, T. S. (2019) Function, Architecture, and Biogenesis of Reovirus Replication Neorganelles. *Viruses* 11(3).
96. Harak, C., Lohmann, V. (2015) Ultrastructure of the replication sites of positive-strand RNA viruses. *Virology* 479-480:418-433.
97. Cortese, M., Goellner, S., Acosta, E. G., Neufeldt, C. J., Oleksiuk, O., Lampe, M., Haselmann, U., Funaya, C., Schieber, N., Ronchi, P., Schorb, M., Pruunsild, P., Schwab, Y., Chatel-Chaix, L., Ruggieri, A., Bartenschlager, R. (2017) Ultrastructural Characterization of Zika Virus Replication Factories. *Cell Rep* 18(9):2113-2123.
98. Fernandez de Castro, I., Fernandez, J. J., Barajas, D., Nagy, P. D., Risco, C. (2017) Three-dimensional imaging of the intracellular assembly of a functional viral RNA replicase complex. *Journal of cell science* 130(1):260-268.
99. Limpens, R. W., van der Schaar, H. M., Kumar, D., Koster, A. J., Snijder, E. J., van Kuppeveld, F. J., Barcena, M. (2011) The transformation of enterovirus replication structures: a three-dimensional study of single- and double-membrane compartments. *mBio* 2(5).





100. Belov, G. A., Nair, V., Hansen, B. T., Hoyt, F. H., Fischer, E. R., Ehrenfeld, E. (2012) Complex dynamic development of poliovirus membranous replication complexes. *Journal of virology* 86(1):302-312.
101. Melia, C. E., van der Schaar, H. M., de Jong, A. W. M., Lyoo, H. R., Snijder, E. J., Koster, A. J., van Kuppeveld, F. J. M., Barcena, M. (2018) The Origin, Dynamic Morphology, and PI4P-Independent Formation of Encephalomyocarditis Virus Replication Organelles. *mBio* 9(2).
102. Doerflinger, S. Y., Cortese, M., Romero-Brey, I., Menne, Z., Tubiana, T., Schenk, C., White, P. A., Bartenschlager, R., Bressanelli, S., Hansman, G. S., Lohmann, V. (2017) Membrane alterations induced by nonstructural proteins of human norovirus. *PLoS pathogens* 13(10):e1006705.
103. Romero-Brey, I., Merz, A., Chiramel, A., Lee, J. Y., Chlanda, P., Haselman, U., Santarella-Mellwig, R., Habermann, A., Hoppe, S., Kallis, S., Walther, P., Antony, C., Krijnse-Locker, J., Bartenschlager, R. (2012) Three-dimensional architecture and biogenesis of membrane structures associated with hepatitis C virus replication. *PLoS pathogens* 8(12):e1003056.
104. de Wilde, A. H., Raj, V. S., Oudshoorn, D., Bestebroer, T. M., van Nieuwkoop, S., Limpens, R. W., Posthuma, C. C., van der Meer, Y., Barcena, M., Haagmans, B. L., Snijder, E. J., van den Hoogen, B. G. (2013) MERS-coronavirus replication induces severe in vitro cytopathology and is strongly inhibited by cyclosporin A or interferon-alpha treatment. *The Journal of general virology* 94(Pt 8):1749-1760.
105. Gushchin, V. A., Solovyev, A. G., Erokhina, T. N., Morozov, S. Y., Agranovsky, A. A. (2013) Beet yellows virus replicase and replicative compartments: parallels with other RNA viruses. *Front Microbiol* 4:38.
106. Jin, X., Cao, X., Wang, X., Jiang, J., Wan, J., Laliberte, J. F., Zhang, Y. (2018) Three-Dimensional Architecture and Biogenesis of Membrane Structures Associated with Plant Virus Replication. *Frontiers in plant science* 9:57.
107. Wobus, C. E., Karst, S. M., Thackray, L. B., Chang, K. O., Sosnovtsev, S. V., Belliot, G., Krug, A., Mackenzie, J. M., Green, K. Y., Virgin, H. W. (2004) Replication of Norovirus in cell culture reveals a tropism for dendritic cells and macrophages. *PLoS biology* 2(12):e432.
108. Ferraris, P., Beaumont, E., Uzbekov, R., Brand, D., Gaillard, J., Blanchard, E., Roingear, P. (2013) Sequential biogenesis of host cell membrane rearrangements induced by hepatitis C virus infection. *Cellular and molecular life sciences : CMLS* 70(7):1297-1306.
109. Monaghan, P., Cook, H., Jackson, T., Ryan, M., Wileman, T. (2004) The ultrastructure of the developing replication site in foot-and-mouth disease virus-infected BHK-38 cells. *The Journal of general virology* 85(Pt 4):933-946.
110. Quinkert, D., Bartenschlager, R., Lohmann, V. (2005) Quantitative analysis of the hepatitis C virus replication complex. *Journal of virology* 79(21):13594-13605.
111. Paul, D., Hoppe, S., Saher, G., Krijnse-Locker, J., Bartenschlager, R. (2013) Morphological and biochemical characterization of the membranous hepatitis C virus replication compartment. *Journal of virology* 87(19):10612-10627.
112. Melia, C. E., van der Schaar, H. M., Lyoo, H., Limpens, R., Feng, Q., Wahedi, M., Overheul, G. J., van Rij, R. P., Snijder, E. J., Koster, A. J., Barcena, M., van Kuppeveld, F. J. M. (2017) Escaping Host Factor PI4KB Inhibition: Enterovirus Genomic RNA Replication in the Absence of Replication Organelles. *Cell Rep* 21(3):587-599.
113. Melia, C. E., Peddie, C. J., de Jong, A. W. M., Snijder, E. J., Collinson, L. M., Koster, A. J., van der Schaar, H. M., van Kuppeveld, F. J. M., Barcena, M. (2019) Origins of Enterovirus Replication



Organelles Established by Whole-Cell Electron Microscopy. 10(3):e00951-00919.

114. Paul, D., Madan, V., Ramirez, O., Bencun, M., Stoeck, I. K., Jirasko, V., Bartenschlager, R. (2018) Glycine Zipper Motifs in Hepatitis C Virus Nonstructural Protein 4B Are Required for the Establishment of Viral Replication Organelles. *Journal of virology* 92(4).
115. Oudshoorn, D., van der Hoeven, B., Limpens, R. W., Beugeling, C., Snijder, E. J., Barcena, M., Kikkert, M. (2016) Antiviral Innate Immune Response Interferes with the Formation of Replication-Associated Membrane Structures Induced by a Positive-Strand RNA Virus. *mBio* 7(6).
116. Oudshoorn, D., Rijs, K., Limpens, R., Groen, K., Koster, A. J., Snijder, E. J., Kikkert, M., Barcena, M. (2017) Expression and Cleavage of Middle East Respiratory Syndrome Coronavirus nsp3-4 Polyprotein Induce the Formation of Double-Membrane Vesicles That Mimic Those Associated with Coronaviral RNA Replication. *mBio* 8(6).
117. Kopek, B. G., Settles, E. W., Friesen, P. D., Ahlquist, P. (2010) Nodavirus-induced membrane rearrangement in replication complex assembly requires replicase protein a, RNA templates, and polymerase activity. *Journal of virology* 84(24):12492-12503.
118. Spuul, P., Balistreri, G., Hellstrom, K., Golubtsov, A. V., Jokitalo, E., Ahola, T. (2011) Assembly of alphavirus replication complexes from RNA and protein components in a novel trans-replication system in mammalian cells. *Journal of virology* 85(10):4739-4751.
119. Gorbalenya, A. E., Enjuanes, L., Ziebuhr, J., Snijder, E. J. (2006) Nidovirales: evolving the largest RNA virus genome. *Virus Res* 117(1):17-37.
120. Suh, D. A., Giddings, T. H., Jr., Kirkegaard, K. (2000) Remodeling the endoplasmic reticulum by poliovirus infection and by individual viral proteins: an autophagy-like origin for virus-induced vesicles. *Journal of virology* 74(19):8953-8965.
121. Wang, J., Ptacek, J. B., Kirkegaard, K., Bullitt, E. (2013) Double-membraned liposomes sculpted by poliovirus 3AB protein. *J Biol Chem* 288(38):27287-27298.
122. Romero-Brey, I., Berger, C., Kallis, S., Kolovou, A., Paul, D., Lohmann, V., Bartenschlager, R. (2015) NS5A Domain 1 and Polyprotein Cleavage Kinetics Are Critical for Induction of Double-Membrane Vesicles Associated with Hepatitis C Virus Replication. *mBio* 6(4):e00759.
123. Paul, D., Romero-Brey, I., Gouttenoire, J., Stoitsova, S., Krijnse-Locker, J., Moradpour, D., Bartenschlager, R. (2011) NS4B self-interaction through conserved C-terminal elements is required for the establishment of functional hepatitis C virus replication complexes. *Journal of virology* 85(14):6963-6976.
124. Gouttenoire, J., Montserret, R., Paul, D., Castillo, R., Meister, S., Bartenschlager, R., Penin, F., Moradpour, D. (2014) Aminoterminal amphipathic alpha-helix AH1 of hepatitis C virus nonstructural protein 4B possesses a dual role in RNA replication and virus production. *PLoS pathogens* 10(10):e1004501.
125. Posthuma, C. C., Pedersen, K. W., Lu, Z., Joosten, R. G., Roos, N., Zevenhoven-Dobbe, J. C., Snijder, E. J. (2008) Formation of the arterivirus replication/transcription complex: a key role for nonstructural protein 3 in the remodeling of intracellular membranes. *Journal of virology* 82(9):4480-4491.
126. Zhang, W., Chen, K., Guo, Y., Chen, Y., Liu, X. (2019) Involvement of PRRSV NSP3 and NSP5 in the autophagy process. *Virology Journal* 16(1):13.
127. Diaz, A., Ahlquist, P. (2012) Role of host reticulon proteins in rearranging membranes for positive-strand RNA virus replication. *Curr Opin Microbiol* 15(4):519-524.



128. Tang, W. F., Yang, S. Y., Wu, B. W., Jheng, J. R., Chen, Y. L., Shih, C. H., Lin, K. H., Lai, H. C., Tang, P., Horng, J. T. (2007) Reticulon 3 binds the 2C protein of enterovirus 71 and is required for viral replication. *J Biol Chem* 282(8):5888-5898.
129. Wu, M. J., Ke, P. Y., Hsu, J. T., Yeh, C. T., Horng, J. T. (2014) Reticulon 3 interacts with NS4B of the hepatitis C virus and negatively regulates viral replication by disrupting NS4B self-interaction. *Cellular microbiology* 16(11):1603-1618.
130. Lin, C. L., Chien, R. N., Liang, K. H., Ke, P. Y., Huang, Y. H., Yeh, C. T. (2017) Intrahepatic HCV RNA Level and Genotype 1 Independently Associate with Hepatic Reticulon 3 Expression. *Anticancer Res* 37(6):2885-2891.
131. Chao, T. C., Su, W. C., Huang, J. Y., Chen, Y. C., Jeng, K. S., Wang, H. D., Lai, M. M. (2012) Proline-serine-threonine phosphatase-interacting protein 2 (PSTPIP2), a host membrane-deforming protein, is critical for membranous web formation in hepatitis C virus replication. *Journal of virology* 86(3):1739-1749.
132. Jackson, W. T., Giddings, T. H., Jr., Taylor, M. P., Mulinyawe, S., Rabinovitch, M., Kopito, R. R., Kirkegaard, K. (2005) Subversion of cellular autophagosomal machinery by RNA viruses. *PLoS biology* 3(5):e156.
133. Reggiori, F., Monastyrska, I., Verheije, M. H., Cali, T., Ulasli, M., Bianchi, S., Bernasconi, R., de Haan, C. A., Molinari, M. (2010) Coronaviruses Hijack the LC3-I-positive EDEMosomes, ER-derived vesicles exporting short-lived ERAD regulators, for replication. *Cell host & microbe* 7(6):500-508.
134. Monastyrska, I., Ulasli, M., Rottier, P. J., Guan, J. L., Reggiori, F., de Haan, C. A. (2013) An autophagy-independent role for LC3 in equine arteritis virus replication. *Autophagy* 9(2):164-174.
135. Wang, L., Kim, J. Y., Liu, H. M., Lai, M. M. C., Ou, J. J. (2017) HCV-induced autophagosomes are generated via homotypic fusion of phagophores that mediate HCV RNA replication. *PLoS pathogens* 13(9):e1006609.
136. Fahmy, A. M., Labonté, P. (2017) The autophagy elongation complex (ATG5-12/16L1) positively regulates HCV replication and is required for wild-type membranous web formation. *Scientific Reports* 7(1):40351.
137. Mohl, B. P., Bartlett, C., Mankouri, J., Harris, M. (2016) Early events in the generation of autophagosomes are required for the formation of membrane structures involved in hepatitis C virus genome replication. *The Journal of general virology* 97(3):680-693.
138. Lee, J. Y., Cortese, M., Haselmann, U., Tabata, K., Romero-Brey, I., Funaya, C., Schieber, N. L., Qiang, Y., Bartenschlager, M., Kallis, S., Ritter, C., Rohr, K., Schwab, Y., Ruggieri, A., Bartenschlager, R. (2019) Spatiotemporal Coupling of the Hepatitis C Virus Replication Cycle by Creating a Lipid Droplet- Proximal Membranous Replication Compartment. *Cell Rep* 27(12):3602-3617 e3605.
139. Verheije, M. H., Raaben, M., Mari, M., Te Lintelo, E. G., Reggiori, F., van Kuppeveld, F. J., Rottier, P. J., de Haan, C. A. (2008) Mouse hepatitis coronavirus RNA replication depends on GBF1-mediated ARF1 activation. *PLoS pathogens* 4(6):e1000088.
140. Belov, G. A., Feng, Q., Nikovics, K., Jackson, C. L., Ehrenfeld, E. (2008) A critical role of a cellular membrane traffic protein in poliovirus RNA replication. *PLoS pathogens* 4(11):e1000216.
141. Goueslain, L., Alsaleh, K., Horellou, P., Roingard, P., Descamps, V., Duverlie, G., Ciczora, Y., Wychowski, C., Dubuisson, J., Rouille, Y. (2010) Identification of GBF1 as a cellular factor required for hepatitis C virus RNA replication. *Journal of virology* 84(2):773-787.



142. Knorr, R. L., Dimova, R., Lipowsky, R. (2012) Curvature of double-membrane organelles generated by changes in membrane size and composition. *PLoS one* 7(3):e32753.
143. Furse, S., Brooks, N. J., Seddon, A. M., Woscholski, R., Templer, R. H., Tate, E. W., Gaffney, P. R. J., Ces, O. (2012) Lipid membrane curvature induced by distearoyl phosphatidylinositol 4-phosphate. *Soft Matter* 8(11):3090-3093.
144. Yang, S. T., Kreutzberger, A. J. B., Lee, J., Kiessling, V., Tamm, L. K. (2016) The role of cholesterol in membrane fusion. *Chem Phys Lipids* 199:136-143.
145. Hsu, N.-Y., Ilnytska, O., Belov, G., Santiana, M., Chen, Y.-H., Takvorian, P. M., Pau, C., van der Schaar, H., Kaushik-Basu, N., Balla, T., Cameron, C. E., Ehrenfeld, E., van Kuppeveld, F. J. M., Altan-Bonnet, N. (2010) Viral Reorganization of the Secretory Pathway Generates Distinct Organelles for RNA Replication. *Cell* 141(5):799-811.
146. Reiss, S. *et al.* (2011) Recruitment and activation of a lipid kinase by hepatitis C virus NS5A is essential for integrity of the membranous replication compartment. *Cell host & microbe* 9(1):32-45.
147. Ilnytska, O., Santiana, M., Hsu, N. Y., Du, W. L., Chen, Y. H., Viktorova, E. G., Belov, G., Brinker, A., Storch, J., Moore, C., Dixon, J. L., Altan-Bonnet, N. (2013) Enteroviruses Harness the Cellular Endocytic Machinery to Remodel the Host Cell Cholesterol Landscape for Effective Viral Replication. *Cell host & microbe* 14(3):281-293.
148. Roulin, P. S., Lotzerich, M., Torta, F., Tanner, L. B., van Kuppeveld, F. J., Wenk, M. R., Greber, U. F. (2014) Rhinovirus uses a phosphatidylinositol 4-phosphate/cholesterol counter-current for the formation of replication compartments at the ER-Golgi interface. *Cell host & microbe* 16(5):677-690.
149. Wang, H. L., Perry, J. W., Lauring, A. S., Neddermann, P., De Francesco, R., Tai, A. W. (2014) Oxysterol-Binding Protein Is a Phosphatidylinositol 4-Kinase Effector Required for HCV Replication Membrane Integrity and Cholesterol Trafficking. *Gastroenterology* 146(5):1373-+.
150. Stoeck, I. K., Lee, J. Y., Tabata, K., Romero-Brey, I., Paul, D., Schult, P., Lohmann, V., Kaderali, L., Bartenschlager, R. (2018) Hepatitis C Virus Replication Depends on Endosomal Cholesterol Homeostasis. *Journal of virology* 92(1).
151. Muller, C., Hardt, M., Schwudke, D., Neuman, B. W., Pleschka, S., Ziebuhr, J. (2018) Inhibition of Cytosolic Phospholipase A2alpha Impairs an Early Step of Coronavirus Replication in Cell Culture. *Journal of virology* 92(4).
152. Plagemann, P. G., Cleveland, P. H., Shea, M. A. (1970) Effect of mengovirus replication on choline metabolism and membrane formation in novikoff hepatoma cells. *Journal of virology* 6(6):800-812.
153. Zhang, J., Zhang, Z., Chukkapalli, V., Nchoutmboube, J. A., Li, J., Randall, G., Belov, G. A., Wang, X. (2016) Positive-strand RNA viruses stimulate host phosphatidylcholine synthesis at viral replication sites. *Proc Natl Acad Sci U S A* 113(8):E1064-1073.
154. Viktorova, E. G., Nchoutmboube, J. A., Ford-Siltz, L. A., Iverson, E., Belov, G. A. (2018) Phospholipid synthesis fueled by lipid droplets drives the structural development of poliovirus replication organelles. *PLoS pathogens* 14(8):e1007280.
155. Laufman, O., Perrino, J., Andino, R. (2019) Viral Generated Inter-Organelle Contacts Redirect Lipid Flux for Genome Replication. *Cell* 178(2):275-289 e216.
156. Miyanari, Y., Atsuzawa, K., Usuda, N., Watashi, K., Hishiki, T., Zayas, M., Bartenschlager, R., Wakita, T., Hijikata, M., Shimotohno, K. (2007) The lipid droplet is an important organelle for



- hepatitis C virus production. *Nat Cell Biol* 9(9):1089-1097.
157. Schwartz, M., Chen, J., Lee, W. M., Janda, M., Ahlquist, P. (2004) Alternate, virus-induced membrane rearrangements support positive-strand RNA virus genome replication. *Proc Natl Acad Sci U S A* 101(31):11263-11268.
  158. Xu, K., Nagy, P. D. (2014) Expanding use of multi-origin subcellular membranes by positive-strand RNA viruses during replication. *Current opinion in virology* 9:119-126.
  159. Neufeldt, C. J., Joyce, M. A., Van Buuren, N., Levin, A., Kirkegaard, K., Gale, M., Jr., Tyrrell, D. L., Wozniak, R. W. (2016) The Hepatitis C Virus-Induced Membranous Web and Associated Nuclear Transport Machinery Limit Access of Pattern Recognition Receptors to Viral Replication Sites. *PLoS pathogens* 12(2):e1005428.
  160. Neufeldt, C. J., Joyce, M. A., Levin, A., Steenbergen, R. H., Pang, D., Shields, J., Tyrrell, D. L., Wozniak, R. W. (2013) Hepatitis C virus-induced cytoplasmic organelles use the nuclear transport machinery to establish an environment conducive to virus replication. *PLoS pathogens* 9(10):e1003744.
  161. Bienz, K., Egger, D., Pfister, T., Troxler, M. (1992) Structural and functional characterization of the poliovirus replication complex. *Journal of virology* 66(5):2740-2747.
  162. Du, X., Zhang, Y., Zou, J., Yuan, Z., Yi, Z. (2018) Replicase-mediated shielding of the poliovirus replicative double-stranded RNA to avoid recognition by MDA5. *The Journal of general virology* 99(9):1199-1209.
  163. van Hemert, M. J., van den Worm, S. H., Knoops, K., Mommaas, A. M., Gorbalenya, A. E., Snijder, E. J. (2008) SARS-coronavirus replication/transcription complexes are membrane-protected and need a host factor for activity in vitro. *PLoS pathogens* 4(5):e1000054.
  164. Chen, Y. H., Du, W., Hagemeyer, M. C., Takvorian, P. M., Pau, C., Cali, A., Brantner, C. A., Stempinski, E. S., Connelly, P. S., Ma, H. C., Jiang, P., Wimmer, E., Altan-Bonnet, G., Altan-Bonnet, N. (2015) Phosphatidylserine vesicles enable efficient en bloc transmission of enteroviruses. *Cell* 160(4):619-630.
  165. Guo, R., Katz, B. B., Tomich, J. M., Gallagher, T., Fang, Y. (2016) Porcine Reproductive and Respiratory Syndrome Virus Utilizes Nanotubes for Intercellular Spread. *Journal of virology* 90(10):5163-5175.
  166. Grunvogel, O. *et al.* (2018) Secretion of Hepatitis C Virus Replication Intermediates Reduces Activation of Toll-Like Receptor 3 in Hepatocytes. *Gastroenterology* 154(8):2237-2251 e2216.
  167. Berger, C., Romero-Brey, I., Radujkovic, D., Terreux, R., Zayas, M., Paul, D., Harak, C., Hoppe, S., Gao, M., Penin, F., Lohmann, V., Bartenschlager, R. (2014) Daclatasvir-like inhibitors of NS5A block early biogenesis of hepatitis C virus-induced membranous replication factories, independent of RNA replication. *Gastroenterology* 147(5):1094-1105 e1025.
  168. Garcia-Nicolas, O., V'Kovski, P., Vielle, N. J., Ebert, N., Zust, R., Portmann, J., Stalder, H., Gaschen, V., Vieyres, G., Stoffel, M., Schweizer, M., Summerfield, A., Engler, O., Pietschmann, T., Todt, D., Alves, M. P., Thiel, V., Pfaender, S. (2018) The Small-Compound Inhibitor K22 Displays Broad Antiviral Activity against Different Members of the Family Flaviviridae and Offers Potential as a Panviral Inhibitor. *Antimicrob Agents Chemother* 62(11).
  169. Lundin, A., Dijkman, R., Bergstrom, T., Kann, N., Adamiak, B., Hannoun, C., Kindler, E., Jonsdottir, H. R., Muth, D., Kint, J., Forlenza, M., Muller, M. A., Drosten, C., Thiel, V., Trybala, E. (2014) Targeting membrane-bound viral RNA synthesis reveals potent inhibition of diverse coronaviruses including the middle East respiratory syndrome virus. *PLoS pathogens*



10(5):e1004166.

170. V'Kovski, P., Gerber, M., Kelly, J., Pfaender, S., Ebert, N., Braga Lagache, S., Simillion, C., Portmann, J., Stalder, H., Gaschen, V., Bruggmann, R., Stoffel, M. H., Heller, M., Dijkman, R., Thiel, V. (2019) Determination of host proteins composing the microenvironment of coronavirus replicase complexes by proximity-labeling. *eLife* 8.
171. Yuan, S. *et al.* (2019) SREBP-dependent lipidomic reprogramming as a broad-spectrum antiviral target. *Nat Commun* 10(1):120.
172. Ertel, K. J., Benefield, D., Castano-Diez, D., Pennington, J. G., Horswill, M., den Boon, J. A., Otegui, M. S., Ahlquist, P. (2017) Cryo-electron tomography reveals novel features of a viral RNA replication compartment. *eLife* 6:e25940.
173. Bárcena, M., Oostergetel, G. T., Bartelink, W., Faas, F. G., Verkleij, A., Rottier, P. J., Koster, A. J., Bosch, B. J. (2009) Cryo-electron tomography of mouse hepatitis virus: Insights into the structure of the coronavirus. *Proc Natl Acad Sci U S A* 106(2):582-587.
174. McDonald, K. L. (2014) Out with the old and in with the new: rapid specimen preparation procedures for electron microscopy of sectioned biological material. *Protoplasma* 251(2):429-448.
175. Bykov, Y. S., Cortese, M., Briggs, J. A., Bartenschlager, R. (2016) Correlative light and electron microscopy methods for the study of virus-cell interactions. *FEBS Lett* 590(13):1877-1895.
176. Engel, B. D., Schaffer, M., Albert, S., Asano, S., Plitzko, J. M., Baumeister, W. (2015) In situ structural analysis of Golgi intracisternal protein arrays. *Proceedings of the National Academy of Sciences* 112(36):11264.
177. Chaikerasitak, V., Nguyen, K., Khanna, K., Brilot, A. F., Erb, M. L., Coker, J. K., Vavilina, A., Newton, G. L., Buschauer, R., Pogliano, K. (2017) Assembly of a nucleus-like structure during viral replication in bacteria. *Science* 355(6321):194-197.
178. Böck, D., Medeiros, J. M., Tsao, H.-F., Penz, T., Weiss, G. L., Aistleitner, K., Horn, M., Pilhofer, M. (2017) In situ architecture, function, and evolution of a contractile injection system. *Science* 357(6352):713-717.
179. Guo, Q., Lehmer, C., Martínez-Sánchez, A., Rudack, T., Beck, F., Hartmann, H., Pérez-Berlanga, M., Frottin, F., Hipp, M. S., Hartl, F. U. (2018) In situ structure of neuronal C9orf72 poly-GA aggregates reveals proteasome recruitment. *Cell* 172(4):696-705. e612.
180. Al-Amoudi, A., Norlen, L. P. O., Dubochet, J. (2004) Cryo-electron microscopy of vitreous sections of native biological cells and tissues. *Journal of Structural Biology* 148(1):131-135.
181. Schaffer, M., Engel, B. D., Laugks, T., Mahamid, J., Plitzko, J. M., Baumeister, W. (2015) Cryo-focused ion beam sample preparation for imaging vitreous cells by cryo-electron tomography. *Bio Protoc.* 5:e1575.
182. Medeiros, J. M., Böck, D., Weiss, G. L., Kooger, R., Wepf, R. A., Pilhofer, M. (2018) Robust workflow and instrumentation for cryo-focused ion beam milling of samples for electron cryotomography. *Ultramicroscopy.*
183. Hayles, M. F., Stokes, D. J., Phifer, D., Findlay, K. C. (2007) A technique for improved focused ion beam milling of cryo-prepared life science specimens. *Journal of Microscopy* 226(3):263-269.
184. Schaffer, M., Mahamid, J., Engel, B. D., Laugks, T., Baumeister, W., Plitzko, J. M. (2017) Optimized cryo-focused ion beam sample preparation aimed at in situ structural studies of membrane proteins. *Journal of Structural Biology* 197(2):73-82.



185. Booy, F. P., Pawley, J. B. (1993) Cryo-crinkling: what happens to carbon films on copper grids at low temperature. *Ultramicroscopy* 48(3):273-280.
186. Toro-Nahuelpan, M., Zagoriy, I., Senger, F., Blanchoin, L., Théry, M., Mahamid, J. (2019) Tailoring cryo-electron microscopy grids by photo-micropatterning for in-cell structural studies. *bioRxiv*:676189.
187. Danev, R., Baumeister, W. (2017) Expanding the boundaries of cryo-EM with phase plates. *Current Opinion in Structural Biology* 46:87-94.
188. Zheng, S. Q., Palovcak, E., Armache, J. P., Verba, K. A., Cheng, Y., Agard, D. A. (2017) MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. *Nat Methods* 14(4):331-332.
189. Li, X., Mooney, P., Zheng, S., Booth, C. R., Braunfeld, M. B., Gubbens, S., Agard, D. A., Cheng, Y. (2013) Electron counting and beam-induced motion correction enable near-atomic-resolution single-particle cryo-EM. *Nature Methods* 10:584.
190. Davies, K. M., Daum, B., Gold, V. A., Muhleip, A. W., Brandt, T., Blum, T. B., Mills, D. J., Kuhlbrandt, W. (2014) Visualization of ATP synthase dimers in mitochondria by electron cryo-tomography. *J Vis Exp* (91):51228.
191. Zaki, A. M., van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D., Fouchier, R. A. (2012) Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* 367(19):1814-1820.
192. Zhu, N. *et al.* (2020) A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 382(8):727-733.
193. Abels, J. A., Moreno-Herrero, F., van der Heijden, T., Dekker, C., Dekker, N. H. (2005) Single-molecule measurements of the persistence length of double-stranded RNA. *Biophys J* 88(4):2737-2744.
194. Ding, K., Celma, C. C., Zhang, X., Chang, T., Shen, W., Atanasov, I., Roy, P., Zhou, Z. H. (2019) In situ structures of rotavirus polymerase in action and mechanism of mRNA transcription and release. *Nat Commun* 10(1):2216.
195. Snijder, E. J., Decroly, E., Ziebuhr, J. (2016) The nonstructural proteins directing coronavirus RNA synthesis and processing. *Adv Virus Res* 96:59-126.
196. Oostra, M., Hagemeijer, M. C., van Gent, M., Bekker, C. P., te Lintelo, E. G., Rottier, P. J., de Haan, C. A. (2008) Topology and membrane anchoring of the coronavirus replication complex: not all hydrophobic domains of nsp3 and nsp6 are membrane spanning. *Journal of virology* 82(24):12392-12405.
197. Oostra, M., te Lintelo, E. G., Deijs, M., Verheije, M. H., Rottier, P. J., de Haan, C. A. (2007) Localization and membrane topology of coronavirus nonstructural protein 4: involvement of the early secretory pathway in replication. *Journal of virology* 81(22):12323-12336.
198. Kanjanahaluethai, A., Chen, Z., Jukneliene, D., Baker, S. C. (2007) Membrane topology of murine coronavirus replicase nonstructural protein 3. *Virology* 361(2):391-401.
199. Neuman, B. W. (2016) Bioinformatics and functional analyses of coronavirus nonstructural proteins involved in the formation of replicative organelles. *Antiviral Research* 135:97-107.
200. Lei, J., Kusov, Y., Hilgenfeld, R. (2018) Nsp3 of coronaviruses: Structures and functions of a large multi-domain protein. *Antiviral Research* 149:58-74.
201. Serrano, P., Johnson, M. A., Almeida, M. S., Horst, R., Herrmann, T., Joseph, J. S., Neuman, B.



W., Subramanian, V., Saikatendu, K. S., Buchmeier, M. J., Stevens, R. C., Kuhn, P., Wuthrich, K. (2007) Nuclear magnetic resonance structure of the N-terminal domain of nonstructural protein 3 from the severe acute respiratory syndrome coronavirus. *Journal of virology* 81(21):12049-12060.

202. Hurst, K. R., Koetzner, C. A., Masters, P. S. (2013) Characterization of a critical interaction between the coronavirus nucleocapsid protein and nonstructural protein 3 of the viral replicase-transcriptase complex. *Journal of virology* 87(16):9159-9172.
203. Cong, Y., Ulasli, M., Schepers, H., Mauthe, M., V'Kovski, P., Kriegenburg, F., Thiel, V., de Haan, C. A. M., Reggiori, F. (2020) Nucleocapsid protein recruitment to replication-transcription complexes plays a crucial role in coronaviral life cycle. *Journal of virology* 94(4).
204. Freeman, M. C., Graham, R. L., Lu, X., Peek, C. T., Denison, M. R. (2014) Coronavirus replicase-reporter fusions provide quantitative analysis of replication and replication complex formation. *Journal of virology* 88(10):5319-5327.
205. Graham, R. L., Sims, A. C., Baric, R. S., Denison, M. R. (2006) The nsp2 proteins of mouse hepatitis virus and SARS coronavirus are dispensable for viral replication. *Adv Exp Med Biol* 581:67-72.
206. Gadlage, M. J., Sparks, J. S., Beachboard, D. C., Cox, R. G., Doyle, J. D., Stobart, C. C., Denison, M. R. (2010) Murine hepatitis virus nonstructural protein 4 regulates virus-induced membrane modifications and replication complex function. *Journal of virology* 84(1):280.
207. Kirchdoerfer, R. N., Ward, A. B. (2019) Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. *Nat Communications* 10(1):2342.
208. Sevajol, M., Subissi, L., Decroly, E., Canard, B., Imbert, I. (2014) Insights into RNA synthesis, capping, and proofreading mechanisms of SARS-coronavirus. *Virus Res* 194:90-99.
209. Subissi, L., Imbert, I., Ferron, F., Collet, A., Coutard, B., Decroly, E., Canard, B. (2014) SARS-CoV ORF1b-encoded nonstructural proteins 12-16: replicative enzymes as antiviral targets. *Antiviral Res* 101:122-130.
210. Gui, M., Liu, X., Guo, D., Zhang, Z., Yin, C. C., Chen, Y., Xiang, Y. (2017) Electron microscopy studies of the coronavirus ribonucleoprotein complex. *Protein & cell* 8(3):219-224.
211. Ogando, N. S., Dalebout, T. J., Zevenhoven-Dobbe, J. C., Limpens, R., van der Meer, Y., Caly, L., Druce, J., de Vries, J. J. C., Kikkert, M., Bárcena, M., Sidorov, I., Snijder, E. J. (2020) SARS-coronavirus-2 replication in Vero E6 cells: replication kinetics, rapid adaptation and cytopathology. *The Journal of general virology* 10.1099/jgv.0.001453.
212. Kuo, L., Koetzner, C. A., Masters, P. S. (2016) A key role for the carboxy-terminal tail of the murine coronavirus nucleocapsid protein in coordination of genome packaging. *Virology* 494:100-107.
213. Caly, L., Druce, J., Roberts, J., Bond, K., Tran, T., Kosteci, R., Yoga, Y., Naughton, W., Taiaroa, G., Seemann, T., Schultz, M. B., Howden, B. P., Korman, T. M., Lewin, S. R., Williamson, D. A., Catton, M. G. (2020) Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first patient diagnosed with COVID-19 in Australia. *Med J Aust* 212(10):459-462.
214. Wolff, G., Limpens, R., Zheng, S., Snijder, E. J., Agard, D. A., Koster, A. J., Barcena, M. (2019) Mind the gap: Micro-expansion joints drastically decrease the bending of FIB-milled cryo-lamellae. *Journal of structural biology* 208(3):107389.
215. Hagen, W. J. H., Wan, W., Briggs, J. A. G. (2017) Implementation of a cryo-electron tomography tilt-scheme optimized for high resolution subtomogram averaging. *Journal of structural biology*





- 197(2):191-198.
216. Rohou, A., Grigorieff, N. (2015) CTFIND4: Fast and accurate defocus estimation from electron micrographs. *Journal of structural biology* 192(2):216-221.
  217. Xiong, Q., Morphew, M. K., Schwartz, C. L., Hoenger, A. H., Mastronarde, D. N. (2009) CTF determination and correction for low dose tomographic tilt series. *Journal of structural biology* 168(3):378-387.
  218. Frangakis, A. S., Hegerl, R. (2001) Noise reduction in electron tomographic reconstructions using nonlinear anisotropic diffusion. *Journal of structural biology* 135(3):239-250.
  219. Kremer, J. R., Mastronarde, D. N., McIntosh, J. R. (1996) Computer visualization of three-dimensional image data using IMOD. *Journal of structural biology* 116(1):71-76.
  220. Castano-Diez, D., Kudryashev, M., Arheit, M., Stahlberg, H. (2012) Dynamo: a flexible, user-friendly development tool for subtomogram averaging of cryo-EM data in high-performance computing environments. *Journal of structural biology* 178(2):139-151.
  221. Kucukelbir, A., Sigworth, F. J., Tagare, H. D. (2014) Quantifying the local resolution of cryo-EM density maps. *Nat Methods* 11(1):63-65.
  222. Anger, A. M., Armache, J. P., Berninghausen, O., Habeck, M., Subklewe, M., Wilson, D. N., Beckmann, R. (2013) Structures of the human and Drosophila 80S ribosome. *Nature* 497(7447):80-85.
  223. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., Ferrin, T. E. (2004) UCSF Chimera--a visualization system for exploratory research and analysis. *Journal of computational chemistry* 25(13):1605-1612.
  224. Harpaz, Y., Gerstein, M., Chothia, C. (1994) Volume changes on protein folding. *Structure* 2(7):641-649.
  225. Scheres, S. H. (2012) RELION: implementation of a Bayesian approach to cryo-EM structure determination. *Journal of structural biology* 180(3):519-530.
  226. Lucic, V., Rigort, A., Baumeister, W. (2013) Cryo-electron tomography: the challenge of doing structural biology in situ. *The Journal of cell biology* 202(3):407-419.
  227. Neuman, B. W., Joseph, J. S., Saikatendu, K. S., Serrano, P., Chatterjee, A., Johnson, M. A., Liao, L., Klaus, J. P., Yates, J. R., 3rd, Wuthrich, K., Stevens, R. C., Buchmeier, M. J., Kuhn, P. (2008) Proteomics analysis unravels the functional repertoire of coronavirus nonstructural protein 3. *Journal of virology* 82(11):5279-5294.
  228. Snijder, E. J., Kikkert, M., Fang, Y. (2013) Arterivirus molecular biology and pathogenesis. *The Journal of general virology* 94(Pt 10):2141-2163.
  229. Spilman, M. S., Welbon, C., Nelson, E., Dokland, T. (2009) Cryo-electron tomography of porcine reproductive and respiratory syndrome virus: organization of the nucleocapsid. *The Journal of general virology* 90(Pt 3):527-535.
  230. Nagy, P. D., Strating, J. R. P. M., van Kuppeveld, F. J. M. (2016) Building Viral Replication Organelles: Close Encounters of the Membrane Types. *PLoS pathogens* 12(10):e1005912-e1005912.
  231. van Hemert, M. J., de Wilde, A. H., Gorbalenya, A. E., Snijder, E. J. (2008) The in vitro RNA synthesizing activity of the isolated arterivirus replication/transcription complex is dependent on a host factor. *J Biol Chem* 283(24):16525-16536.
  232. Tabata, K. *et al.* (2021) Convergent use of phosphatidic acid for hepatitis C virus and SARS-CoV-2 replication organelle formation. *Nature Communications* 12(1):7276.





233. Liu, G., Gack, M. U. (2020) Distinct and Orchestrated Functions of RNA Sensors in Innate Immunity. *Immunity* 53(1):26-42.
234. Wolff, G., Limpens, R., Zevenhoven-Dobbe, J. C., Laugks, U., Zheng, S., de Jong, A. W. M., Koning, R. I., Agard, D. A., Grunewald, K., Koster, A. J., Snijder, E. J., Barcena, M. (2020) A molecular pore spans the double membrane of the coronavirus replication organelle. *Science* 369(6509):1395-1398.
235. Klein, S., Cortese, M., Winter, S. L., Wachsmuth-Melm, M., Neufeldt, C. J., Cerikan, B., Stanifer, M. L., Boulant, S., Bartenschlager, R., Chlanda, P. (2020) SARS-CoV-2 structure and replication characterized by in situ cryo-electron tomography. *Nat Commun* 11(1):5885.
236. Luther, P. K., in *Electron Tomography: Methods for Three-Dimensional Visualization of Structures in the Cell*, J. Frank, Ed. (Springer New York, New York, NY, 2006), pp. 17-48.
237. Wolff, G., Melia, C. E., Snijder, E. J., Barcena, M. (2020) Double-Membrane Vesicles as Platforms for Viral Replication. *Trends Microbiol* 28(12):1022-1033.
238. Wieringa, R., de Vries, A. A., van der Meulen, J., Godeke, G. J., Onderwater, J. J., van Tol, H., Koerten, H. K., Mommaas, A. M., Snijder, E. J., Rottier, P. J. (2004) Structural protein requirements in equine arteritis virus assembly. *Journal of virology* 78(23):13019-13027.
239. Wissink, E. H., Kroese, M. V., van Wijk, H. A., Rijsewijk, F. A., Meulenberg, J. J., Rottier, P. J. (2005) Envelope protein requirements for the assembly of infectious virions of porcine reproductive and respiratory syndrome virus. *Journal of virology* 79(19):12495-12506.
240. Zevenhoven-Dobbe, J. C., Greve, S., van Tol, H., Spaan, W. J. M., Snijder, E. J. (2004) Rescue of disabled infectious single-cycle (DISC) equine arteritis virus by using complementing cell lines that express minor structural glycoproteins. *The Journal of general virology* 85(Pt 12):3709-3714.
241. Stueckemann, J. A., Ritzi, D. M., Holth, M., Smith, M. S., Swart, W. J., Cafruny, W. A., Plagemann, G. W. (1982) Replication of lactate dehydrogenase-elevating virus in macrophages. 1. Evidence for cytocidal replication. *The Journal of general virology* 59(Pt 2):245-262.
242. Wada, R., Fukunaga, Y., Kondo, T., Kanemaru, T. (1995) Ultrastructure and immunocytochemistry of BHK-21 cells infected with a modified Bucyrus strain of equine arteritis virus. *Arch Virol* 140(7):1173-1180.
243. Wassenaar, A. L., Spaan, W. J., Gorbalenya, A. E., Snijder, E. J. (1997) Alternative proteolytic processing of the arterivirus replicase ORF1a polyprotein: evidence that NSP2 acts as a cofactor for the NSP4 serine protease. *Journal of virology* 71(12):9313-9322.
244. Unchwaniwala, N., Zhan, H., Pennington, J., Horswill, M., den Boon, J. A., Ahlquist, P. (2020) Subdomain cryo-EM structure of nodaviral replication protein A crown complex provides mechanistic insights into RNA genome replication. *Proc Natl Acad Sci U S A* 117(31):18680-18691.
245. Jones, R., Bragagnolo, G., Arranz, R., Reguera, J. (2021) Capping pores of alphavirus nsP1 gate membranous viral replication factories. *Nature* 589(7843):615-619.
246. Zhang, K., Law, Y.-S., Law, M. C. Y., Tan, Y. B., Wirawan, M., Luo, D. (2021) Structural insights into viral RNA capping and plasma membrane targeting by Chikungunya virus nonstructural protein 1. *Cell host & microbe* 29(5):757-764.e753.
247. Bukhari, K., Mulley, G., Gulyaeva, A. A., Zhao, L., Shu, G., Jiang, J., Neuman, B. W. (2018) Description and initial characterization of metatranscriptomic nidovirus-like genomes from the proposed new family Abyssoviridae, and from a sister group to the Coronavirinae, the

- proposed genus Alphaletovirus. *Virology* 524:160-171.
248. Fang, Y., Snijder, E. J. (2010) The PRRSV replicase: exploring the multifunctionality of an intriguing set of nonstructural proteins. *Virus Res* 154(1-2):61-76.
249. Yan, L., Ge, J., Zheng, L., Zhang, Y., Gao, Y., Wang, T., Huang, Y., Yang, Y., Gao, S., Li, M., Liu, Z., Wang, H., Li, Y., Chen, Y., Guddat, L. W., Wang, Q., Rao, Z., Lou, Z. (2021) Cryo-EM Structure of an Extended SARS-CoV-2 Replication and Transcription Complex Reveals an Intermediate State in Cap Synthesis. *Cell* 184(1):184-193.e110.
250. Bäuerlein, F. J. B., Baumeister, W. (2021) Towards Visual Proteomics at High Resolution. *J Mol Biol* 433(20):167187.
251. Hillen, H. S., Kovic, G., Farnung, L., Dienemann, C., Tegunov, D., Cramer, P. (2020) Structure of replicating SARS-CoV-2 polymerase. *Nature* 584(7819):154-156.
252. Malone, B., Chen, J., Wang, Q., Llewellyn, E., Choi Young, J., Olinares Paul Dominic, B., Cao, X., Hernandez, C., Eng Edward, T., Chait Brian, T., Shaw David, E., Landick, R., Darst Seth, A., Campbell Elizabeth, A. (2021) Structural basis for backtracking by the SARS-CoV-2 replication–transcription complex. *Proceedings of the National Academy of Sciences* 118(19):e2102516118.
253. Cottam, E. M., Whelband, M. C., Wileman, T. (2014) Coronavirus NSP6 restricts autophagosome expansion. *Autophagy* 10(8):1426-1441.
254. Song, T., Fang, L., Wang, D., Zhang, R., Zeng, S., An, K., Chen, H., Xiao, S. (2016) Quantitative interactome reveals that porcine reproductive and respiratory syndrome virus nonstructural protein 2 forms a complex with viral nucleocapsid protein and cellular vimentin. *J Proteomics* 142:70-81.
255. Fang, Y., Rowland, R. R., Roof, M., Lunney, J. K., Christopher-Hennings, J., Nelson, E. A. (2006) A full-length cDNA infectious clone of North American type 1 porcine reproductive and respiratory syndrome virus: expression of green fluorescent protein in the Nsp2 region. *Journal of virology* 80(23):11447-11455.
256. Mastronarde, D. N. (2005) Automated electron microscope tomography using robust prediction of specimen movements. *Journal of structural biology* 152(1):36-51.
257. Zheng, S., Wolff, G., Greenan, G., Chen, Z., Faas, F. G. A., Bárcena, M., Koster, A. J., Cheng, Y., Agard, D. A. (2022) AreTomo: An integrated software package for automated marker-free, motion-corrected cryo-electron tomographic alignment and reconstruction. *Journal of Structural Biology*: X:100068.
258. Masters, B. R. (2009) History of the electron microscope in cell biology. *eLS*.
259. Kaelber, J. T., Hryc, C. F., Chiu, W. (2017) Electron Cryomicroscopy of Viruses at Near-Atomic Resolutions. *Annu Rev Virol* 4(1):287-308.
260. Bozzola, J. J., Russell, L. D., *Electron microscopy: principles and techniques for biologists*. (Jones & Bartlett Learning, 1999).
261. Dales, S., Franklin, R. M. (1962) A comparison of the changes in fine structure of L cells during single cycles of viral multiplication, following their infection with the viruses of Mengo and encephalomyocarditis. *The Journal of cell biology* 14:281-302.
262. Godman, G. C., Rifkind, R. A., Howe, C., Rose, H. M. (1964) A Description of Echo-9 Virus Infection in Cultured Cells. I. The Cytopathic Effect. *Am J Pathol* 44:1-27.
263. Dales, S., Eggers, H. J., Tamm, I., Palade, G. E. (1965) Electron Microscopic Study of the Formation of Poliovirus. *Virology* 26:379-389.



264. David-Ferreira, J. F., Manaker, R. A. (1965) An Electron Microscope Study of the Development of a Mouse Hepatitis Virus in Tissue Culture Cells. *The Journal of cell biology* 24:57-78.
265. Moor, H., Riehle, U., in *Proceedings of the Fourth European Regional Conference of Electron Microscopy*. (1968), vol. 2, pp. 33-34.
266. McDonald, K. (2007) Cryopreparation methods for electron microscopy of selected model systems. *Methods Cell Biol* 79:23-56.
267. Sosinsky, G. E., Crum, J., Jones, Y. Z., Lanman, J., Smarr, B., Terada, M., Martone, M. E., Deerinck, T. J., Johnson, J. E., Ellisman, M. H. (2008) The combination of chemical fixation procedures with high pressure freezing and freeze substitution preserves highly labile tissue ultrastructure for electron tomography applications. *Journal of structural biology* 161(3):359-371.
268. Murk, J. L., Posthuma, G., Koster, A. J., Geuze, H. J., Verkleij, A. J., Kleijmeer, M. J., Humbel, B. M. (2003) Influence of aldehyde fixation on the morphology of endosomes and lysosomes: quantitative analysis and electron tomography. *J Microsc* 212(Pt 1):81-90.
269. Kopek, B. G., Perkins, G., Miller, D. J., Ellisman, M. H., Ahlquist, P. (2007) Three-dimensional analysis of a viral RNA replication complex reveals a virus-induced mini-organelle. *PLoS biology* 5(9):e220.
270. Welsch, S., Miller, S., Romero-Brey, I., Merz, A., Bleck, C. K., Walther, P., Fuller, S. D., Antony, C., Krijnse-Locker, J., Bartenschlager, R. (2009) Composition and three-dimensional architecture of the dengue virus replication and assembly sites. *Cell host & microbe* 5(4):365-375.
271. Gillespie, L. K., Hoenen, A., Morgan, G., Mackenzie, J. M. (2010) The endoplasmic reticulum provides the membrane platform for biogenesis of the flavivirus replication complex. *Journal of virology* 84(20):10438-10447.
272. Offerdahl, D. K., Dorward, D. W., Hansen, B. T., Bloom, M. E. (2012) A three-dimensional comparison of tick-borne flavivirus infection in mammalian and tick cell lines. *PLoS one* 7(10):e47912.
273. Miorin, L., Romero-Brey, I., Maiuri, P., Hoppe, S., Krijnse-Locker, J., Bartenschlager, R., Marcello, A. (2013) Three-dimensional architecture of tick-borne encephalitis virus replication sites and trafficking of the replicated RNA. *Journal of virology* 87(11):6469-6481.
274. Schwartz, M., Chen, J., Janda, M., Sullivan, M., den Boon, J., Ahlquist, P. (2002) A positive-strand RNA virus replication complex parallels form and function of retrovirus capsids. *Molecular cell* 9(3):505-514.
275. Cao, X., Jin, X., Zhang, X., Li, Y., Wang, C., Wang, X., Hong, J., Wang, X., Li, D., Zhang, Y. (2015) Morphogenesis of Endoplasmic Reticulum Membrane-Invaginated Vesicles during Beet Black Scorch Virus Infection: Role of Auxiliary Replication Protein and New Implications of Three-Dimensional Architecture. *Journal of virology* 89(12):6184-6195.
276. Grimley, P. M., Berezsky, I. K., Friedman, R. M. (1968) Cytoplasmic structures associated with an arbovirus infection: loci of viral ribonucleic acid synthesis. *Journal of virology* 2(11):1326-1338.
277. Froshauer, S., Kartenbeck, J., Helenius, A. (1988) Alphavirus RNA replicase is located on the cytoplasmic surface of endosomes and lysosomes. *The Journal of cell biology* 107(6 Pt 1):2075-2086.
278. Kujala, P., Ikaheimonen, A., Ehsani, N., Vihinen, H., Auvinen, P., Kaariainen, L. (2001) Biogenesis of the Semliki Forest virus RNA replication complex. *Journal of virology* 75(8):3873-3884.



279. Fontana, J., Lopez-Iglesias, C., Tzeng, W. P., Frey, T. K., Fernandez, J. J., Risco, C. (2010) Three-dimensional structure of Rubella virus factories. *Virology* 405(2):579-591.
280. Frolova, E. I., Gorchakov, R., Pereboeva, L., Atasheva, S., Frolov, I. (2010) Functional Sindbis virus replicative complexes are formed at the plasma membrane. *Journal of virology* 84(22):11679-11695.
281. Schlegel, A., Giddings, T. H., Jr., Ladinsky, M. S., Kirkegaard, K. (1996) Cellular origin and ultrastructure of membranes induced during poliovirus infection. *Journal of virology* 70(10):6576-6588.
282. Hyde, J. L., Sosnovtsev, S. V., Green, K. Y., Wobus, C., Virgin, H. W., Mackenzie, J. M. (2009) Mouse norovirus replication is associated with virus-induced vesicle clusters originating from membranes derived from the secretory pathway. *Journal of virology* 83(19):9709-9719.
283. Winey, M., Meehl, J. B., O'Toole, E. T., Giddings, T. H., Jr. (2014) Conventional transmission electron microscopy. *Mol Biol Cell* 25(3):319-323.
284. Möbius, W., Posthuma, G. (2019) Sugar and ice: Immunoelectron microscopy using cryosections according to the Tokuyasu method. *Tissue and Cell* 57:90-102.
285. Bienz, K., Egger, D., Pasamontes, L. (1987) Association of polioviral proteins of the P2 genomic region with the viral replication complex and virus-induced membrane synthesis as visualized by electron microscopic immunocytochemistry and autoradiography. *Virology* 160(1):220-226.
286. Bienz, K., Egger, D., Rasser, Y., Bossart, W. (1980) Kinetics and location of poliovirus macromolecular synthesis in correlation to virus-induced cytopathology. *Virology* 100(2):390-399.
287. Bienz, K. A. (1977) Techniques and applications of autoradiography in the light and electron microscope. *Microsc Acta* 79(1):1-22.
288. Zhou, X., Cong, Y., Veenendaal, T., Klumperman, J., Shi, D., Mari, M., Reggiori, F. (2017) Ultrastructural Characterization of Membrane Rearrangements Induced by Porcine Epidemic Diarrhea Virus Infection. *Viruses* 9(9).
289. Marsh, B. J. (2007) Reconstructing mammalian membrane architecture by large area cellular tomography. *Methods in cell biology* 79:193-220.
290. Peddie, C. J., Collinson, L. M. (2014) Exploring the third dimension: volume electron microscopy comes of age. *Micron* 61:9-19.
291. Dubochet, J., McDowell, A. (1981) Vitrification of pure water for electron microscopy. *Journal of Microscopy* 124(3):3-4.
292. Ke, Z., Oton, J., Qu, K., Cortese, M., Zila, V., McKeane, L., Nakane, T., Zivanov, J., Neufeldt, C. J., Cerikan, B., Lu, J. M., Peukes, J., Xiong, X., Kräusslich, H. G., Scheres, S. H. W., Bartenschlager, R., Briggs, J. A. G. (2020) Structures and distributions of SARS-CoV-2 spike proteins on intact virions. *Nature* 588(7838):498-502.
293. Turoňová, B. *et al.* (2020) In situ structural analysis of SARS-CoV-2 spike reveals flexibility mediated by three hinges. *Science* 370(6513):203-208.
294. Yao, H., Song, Y., Chen, Y., Wu, N., Xu, J., Sun, C., Zhang, J., Weng, T., Zhang, Z., Wu, Z., Cheng, L., Shi, D., Lu, X., Lei, J., Crispin, M., Shi, Y., Li, L., Li, S. (2020) Molecular Architecture of the SARS-CoV-2 Virus. *Cell* 183(3):730-738.e713.
295. Peukes, J., Xiong, X., Erlendsson, S., Qu, K., Wan, W., Calder, L. J., Schraidt, O., Kummer, S.,



Freund, S., Kräusslich, H.-G. (2020) The native structure of the assembled matrix protein 1 of influenza A virus. *Nature* 587(7834):495-498.

296. Al-Amoudi, A., Studer, D., Dubochet, J. (2005) Cutting artefacts and cutting process in vitreous sections for cryo-electron microscopy. *Journal of structural biology* 150(1):109-121.
297. Marko, M., Hsieh, C., Moberlychan, W., Mannella, C., Frank, J. (2006) Focused ion beam milling of vitreous water: prospects for an alternative to cryo-ultramicrotomy of frozen-hydrated biological samples. *Journal of microscopy* 222(1):42-47.
298. Unchwaniwala, N., Ahlquist, P. (2020) Coronavirus dons a new crown. *Science* 369(6509):1306-1307.
299. Jones, R., Bragagnolo, G., Arranz, R., Reguera, J. (2020) Capping pores of alphavirus nsP1 gate membranous viral replication factories. *Nature*.
300. Felts, R. L., Narayan, K., Estes, J. D., Shi, D., Trubey, C. M., Fu, J., Hartnell, L. M., Ruthel, G. T., Schneider, D. K., Nagashima, K., Bess, J. W., Jr., Bavari, S., Lowekamp, B. C., Bliss, D., Lifson, J. D., Subramaniam, S. (2010) 3D visualization of HIV transfer at the virological synapse between dendritic cells and T cells. *Proc Natl Acad Sci U S A* 107(30):13336-13341.
301. Do, T., Murphy, G., Earl, L. A., Del Prete, G. Q., Grandinetti, G., Li, G. H., Estes, J. D., Rao, P., Trubey, C. M., Thomas, J., Spector, J., Bliss, D., Nath, A., Lifson, J. D., Subramaniam, S. (2014) Three-dimensional imaging of HIV-1 virological synapses reveals membrane architectures involved in virus transmission. *Journal of virology* 88(18):10327-10339.
302. Dahm, T., Adams, O., Boettcher, S., Diedrich, S., Morozov, V., Hansman, G., Fallier-Becker, P., Schadler, S., Burkhardt, C. J., Weiss, C., Stump-Guthier, C., Ishikawa, H., Schrotten, H., Schwerk, C., Tenenbaum, T., Rudolph, H. (2018) Strain-dependent effects of clinical echovirus 30 outbreak isolates at the blood-CSF barrier. *J Neuroinflammation* 15(1):50.
303. Kantor, A. M., Grant, D. G., Balaraman, V., White, T. A., Franz, A. W. (2018) Ultrastructural analysis of chikungunya virus dissemination from the midgut of the yellow fever mosquito, *Aedes aegypti*. *Viruses* 10(10):571.
304. Schertel, A., Snaidero, N., Han, H.-M., Ruhwedel, T., Laue, M., Grabenbauer, M., Möbius, W. (2013) Cryo FIB-SEM: volume imaging of cellular ultrastructure in native frozen specimens. *Journal of structural biology* 184(2):355-360.
305. Vidavsky, N., Akiva, A., Kaplan-Ashiri, I., Rechav, K., Addadi, L., Weiner, S., Schertel, A. (2016) Cryo-FIB-SEM serial milling and block face imaging: Large volume structural analysis of biological tissues preserved close to their native state. *Journal of Structural Biology* 196(3):487-495.
306. Wu, G.-H., Mitchell, P. G., Galaz-Montoya, J. G., Hecksel, C. W., Sontag, E. M., Gangadharan, V., Marshman, J., Mankus, D., Bisher, M. E., Lytton-Jean, A. K. (2020) Multi-scale 3D cryo-correlative microscopy for vitrified cells. *Structure* 28(11):1231-1237. e1233.
307. Schaffer, M., Pfeffer, S., Mahamid, J., Kleindiek, S., Laugks, T., Albert, S., Engel, B. D., Rummel, A., Smith, A. J., Baumeister, W. (2019) A cryo-FIB lift-out technique enables molecular-resolution cryo-ET within native *Caenorhabditis elegans* tissue. *Nature methods* 16(8):757-762.
308. Yang, H., Rao, Z. (2021) Structural biology of SARS-CoV-2 and implications for therapeutic development. *Nat Rev Microbiol* 19(11):685-700.
309. Wrapp, D., Wang, N., Corbett Kizzmekia, S., Goldsmith Jory, A., Hsieh, C.-L., Abiona, O., Graham Barney, S., McLellan Jason, S. (2020) Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 367(6483):1260-1263.



310. Juraszek, J., Rutten, L., Blokland, S., Bouchier, P., Voorzaat, R., Ritschel, T., Bakkers, M. J. G., Renault, L. L. R., Langedijk, J. P. M. (2021) Stabilizing the closed SARS-CoV-2 spike trimer. *Nature Communications* 12(1):244.
311. Cerutti, G., Guo, Y., Liu, L., Liu, L., Zhang, Z., Luo, Y., Huang, Y., Wang, H. H., Ho, D. D., Sheng, Z., Shapiro, L. (2022) Cryo-EM structure of the SARS-CoV-2 Omicron spike. *Cell Reports*:110428.
312. Cai, Y., Zhang, J., Xiao, T., Peng, H., Sterling, S. M., Walsh, R. M., Jr., Rawson, S., Rits-Volloch, S., Chen, B. (2020) Distinct conformational states of SARS-CoV-2 spike protein. *Science* 369(6511):1586-1592.
313. Wang, Y., Liu, C., Zhang, C., Wang, Y., Hong, Q., Xu, S., Li, Z., Yang, Y., Huang, Z., Cong, Y. (2022) Structural basis for SARS-CoV-2 Delta variant recognition of ACE2 receptor and broadly neutralizing antibodies. *Nature Communications* 13(1):871.
314. Yin, W. *et al.* (2020) Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. *Science* 368(6498):1499-1504.
315. Wang, Q. *et al.* (2020) Structural Basis for RNA Replication by the SARS-CoV-2 Polymerase. *Cell* 182(2):417-428.e413.
316. Ni, T., Frosio, T., Mendonça, L., Sheng, Y., Clare, D., Himes, B. A., Zhang, P. (2022) High-resolution in situ structure determination by cryo-electron tomography and subtomogram averaging using emClarity. *Nat Protoc* 17(2):421-444.
317. Tacke, S., Erdmann, P., Wang, Z., Klumpe, S., Grange, M., Plietzko, J., Raunser, S. (2021) A streamlined workflow for automated cryo focused ion beam milling. *Journal of structural biology* 213(3):107743.
318. Klumpe, S., Fung, H. K., Goetz, S. K., Zagoriy, I., Hampoelz, B., Zhang, X., Erdmann, P. S., Baumbach, J., Müller, C. W., Beck, M., Plietzko, J. M., Mahamid, J. (2021) A modular platform for automated cryo-FIB workflows. *eLife* 10.
319. Breese, S., McCollum, W., in *Int Conf Equine Infec Dis Proc.* (1970).
320. Bell, P. J. (2009) The viral eukaryogenesis hypothesis: a key role for viruses in the emergence of eukaryotes from a prokaryotic world environment. *Ann N Y Acad Sci* 1178:91-105.
321. Bell, P. J. L. (2020) Evidence supporting a viral origin of the eukaryotic nucleus. *Virus Research* 289:198168.
322. Takemura, M. (2020) Medusavirus Ancestor in a Proto-Eukaryotic Cell: Updating the Hypothesis for the Viral Origin of the Nucleus. *Frontiers in Microbiology* 11.
323. Hur, S. (2019) Double-Stranded RNA Sensors and Modulators in Innate Immunity. *Annu Rev Immunol* 37:349-375.
324. Zhang, X., Walker, S. B., Chipman, P. R., Nibert, M. L., Baker, T. S. (2003) Reovirus polymerase  $\lambda$ 3 localized by cryo-electron microscopy of virions at a resolution of 7.6 Å. *Nature structural & molecular biology* 10(12):1011-1018.
325. Diaz, A., Zhang, J., Ollwerther, A., Wang, X., Ahlquist, P. (2015) Host ESCRT proteins are required for bromovirus RNA replication compartment assembly and function. *PLoS pathogens* 11(3):e1004742.
326. Kovalev, N., de Castro Martín, I. F., Pogany, J., Barajas, D., Pathak, K., Risco, C., Nagy, P. D. (2016) Role of Viral RNA and Co-opted Cellular ESCRT-I and ESCRT-III Factors in Formation of Tombusvirus Spherules Harboring the Tombusvirus Replicase. *Journal of virology* 90(7):3611-3626.



327. Dahmane, S., Kerviel, A., Morado, D. R., Shankar, K., Ahlman, B., Lazarou, M., Altan-Bonnet, N., Carlson, L.-A. (2022) Membrane-assisted assembly and selective autophagy of enteroviruses. *bioRxiv:2021.2010.2006.463375*.
328. Unchwaniwala, N., Zhan, H., den Boon, J. A., Ahlquist, P. (2021) Cryo-electron microscopy of nodavirus RNA replication organelles illuminates positive-strand RNA virus genome replication. *Current opinion in virology* 51:74-79.
329. Doyle, N., Simpson, J., Hawes, P. C., Maier, H. J. (2021) Coronavirus RNA Synthesis Takes Place within Membrane-Bound Sites. *Viruses* 13(12):2540.
330. Perry, J. K., Appleby, T. C., Bilello, J. P., Feng, J. Y., Schmitz, U., Campbell, E. A. (2021) An atomistic model of the coronavirus replication-transcription complex as a hexamer assembled around nsp15. *Journal of Biological Chemistry* 297(4):101218.
331. Keane, S. C., Giedroc, D. P. (2013) Solution structure of mouse hepatitis virus (MHV) nsp3a and determinants of the interaction with MHV nucleocapsid (N) protein. *Journal of virology* 87(6):3502-3515.
332. Carlson, C. R., Asfaha, J. B., Ghent, C. M., Howard, C. J., Hartooni, N., Safari, M., Frankel, A. D., Morgan, D. O. (2020) Phosphoregulation of Phase Separation by the SARS-CoV-2 N Protein Suggests a Biophysical Basis for its Dual Functions. *Molecular cell* 80(6):1092-1103.e1094.
333. Bessa, L. M., Guseva, S., Camacho-Zarco, A. R., Salvi, N., Maurin, D., Perez, L. M., Botova, M., Malki, A., Nanao, M., Jensen, M. R., Ruigrok, R. W. H., Blackledge, M. (2022) The intrinsically disordered SARS-CoV-2 nucleoprotein in dynamic complex with its viral partner nsp3a. *Sci Adv* 8(3):eabm4034.
334. Guseva, S., Milles, S., Jensen, M. R., Schoehn, G., Ruigrok, R. W. H., Blackledge, M. (2020) Structure, dynamics and phase separation of measles virus RNA replication machinery. *Current opinion in virology* 41:59-67.
335. Liu, Y., Wang, C., Mueller, S., Paul, A. V., Wimmer, E., Jiang, P. (2010) Direct interaction between two viral proteins, the nonstructural protein 2C and the capsid protein VP3, is required for enterovirus morphogenesis. *PLoS pathogens* 6(8):e1001066.
336. Briegel, A., Chen, S., Koster, A. J., Plitzko, J. M., Schwartz, C. L., Jensen, G. J., in *Methods in enzymology*. (Elsevier, 2010), vol. 481, pp. 317-341.
337. Kaufmann, R., Hagen, C., Grünewald, K. (2014) Fluorescence cryo-microscopy: current challenges and prospects. *Curr Opin Chem Biol* 20(100):86-91.
338. Dobbie, I. M. (2019) Bridging the resolution gap: correlative super-resolution imaging. *Nature Reviews Microbiology* 17(6):337-337.
339. Schmidt, R., Weihs, T., Wurm, C. A., Jansen, I., Rehman, J., Sahl, S. J., Hell, S. W. (2021) MINFLUX nanometer-scale 3D imaging and microsecond-range tracking on a common fluorescence microscope. *Nature Communications* 12(1):1478.
340. Wolff, G., Hagen, C., Grünewald, K., Kaufmann, R. (2016) Towards correlative super-resolution fluorescence and electron cryo-microscopy. *Biol Cell* 108(9):245-258.
341. Moser, F., Pražák, V., Mordhorst, V., Andrade, D. M., Baker, L. A., Hagen, C., Grünewald, K., Kaufmann, R. (2019) Cryo-SOFI enabling low-dose super-resolution correlative light and electron cryo-microscopy. *Proc Natl Acad Sci U S A* 116(11):4804-4809.
342. Tuijtel, M. W., Koster, A. J., Jakobs, S., Faas, F. G. A., Sharp, T. H. (2019) Correlative cryo super-resolution light and electron microscopy on mammalian cells using fluorescent proteins. *Sci*





Rep 9(1):1369.

343. Noble, A. J., Stagg, S. M. (2015) Automated batch fiducial-less tilt-series alignment in Appion using Protomo. *Journal of structural biology* 192(2):270-278.
344. Burt, A., Gaifas, L., Dendooven, T., Gutsche, I. (2021) A flexible framework for multi-particle refinement in cryo-electron tomography. *PLoS biology* 19(8):e3001319.
345. Bepler, T., Kelley, K., Noble, A. J., Berger, B. (2020) Topaz-Denoise: general deep denoising models for cryoEM and cryoET. *Nature Communications* 11(1):5208.
346. Buchholz, T. O., Krull, A., Shahidi, R., Pigino, G., Jékely, G., Jug, F. (2019) Content-aware image restoration for electron microscopy. *Methods Cell Biol* 152:277-289.
347. Böhm, J., Frangakis, A. S., Hegerl, R., Nickell, S., Typke, D., Baumeister, W. (2000) Toward detecting and identifying macromolecules in a cellular context: Template matching applied to electron tomograms. *Proceedings of the National Academy of Sciences* 97(26):14245-14250.
348. Lucas, B. A., Himes, B. A., Xue, L., Grant, T., Mahamid, J., Grigorieff, N. (2021) Locating macromolecular assemblies in cells by 2D template matching with cisTEM. *eLife* 10.
349. Zeng, X., Kahng, A., Xue, L., Mahamid, J., Chang, Y.-W., Xu, M. (2021) DISCA: high-throughput cryo-ET structural pattern mining by deep unsupervised clustering. *bioRxiv*:2021.2005.2016.444381.
350. Moebel, E., Martinez-Sanchez, A., Lamm, L., Righetto, R. D., Wietrzynski, W., Albert, S., Larivière, D., Fourmentin, E., Pfeffer, S., Ortiz, J., Baumeister, W., Peng, T., Engel, B. D., Kervann, C. (2021) Deep learning improves macromolecule identification in 3D cellular cryo-electron tomograms. *Nature Methods* 18(11):1386-1394.
351. Brügger, B. (2014) Lipidomics: analysis of the lipid composition of cells and subcellular organelles by electrospray ionization mass spectrometry. *Annu Rev Biochem* 83:79-98.
352. Dupree, E. J., Jayathirtha, M., Yorkey, H., Mihasan, M., Petre, B. A., Darie, C. C. (2020) A Critical Review of Bottom-Up Proteomics: The Good, the Bad, and the Future of this Field. *Proteomes* 8(3).
353. Risco, C., Sanmartín-Conesa, E., Tzeng, W. P., Frey, T. K., Seybold, V., de Groot, R. J. (2012) Specific, sensitive, high-resolution detection of protein molecules in eukaryotic cells using metal-tagging transmission electron microscopy. *Structure* 20(5):759-766.
354. Fernandez, J.-J., Laugks, U., Schaffer, M., Bäuerlein, F. J., Khoshouei, M., Baumeister, W., Lucic, V. (2016) Removing contamination-induced reconstruction artifacts from cryo-electron tomograms. *Biophysical journal* 110(4):850-859.
355. Silvester, E., Vollmer, B., Pražák, V., Vasishtan, D., Machala, E. A., Whittle, C., Black, S., Bath, J., Turberfield, A. J., Grünewald, K., Baker, L. A. (2021) DNA origami signposts for identifying proteins on cell membranes by electron cryotomography. *Cell* 184(4):1110-1121.e1116.
356. Chan Zuckerberg Initiative (2020) <https://chanzuckerberg.com/science/programs-resources/imaging/frontiers/clonable-in-situ-label-for-high-resolution-cellular-imaging/> accessed at: March 4, 2022



