

Supplementary Information for

Mechanism of a rabbit monoclonal antibody broadly neutralizing SARS-CoV-2 variants

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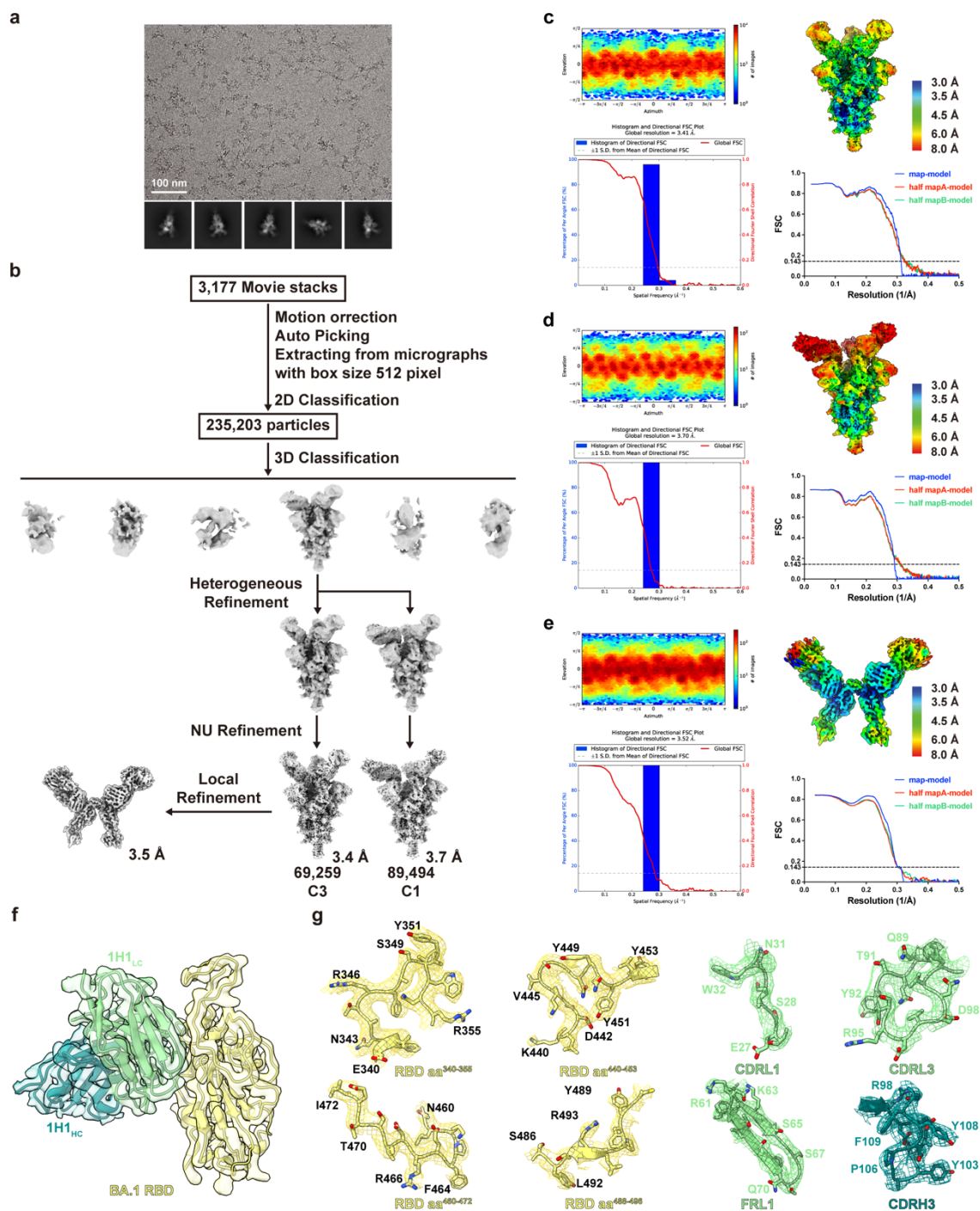
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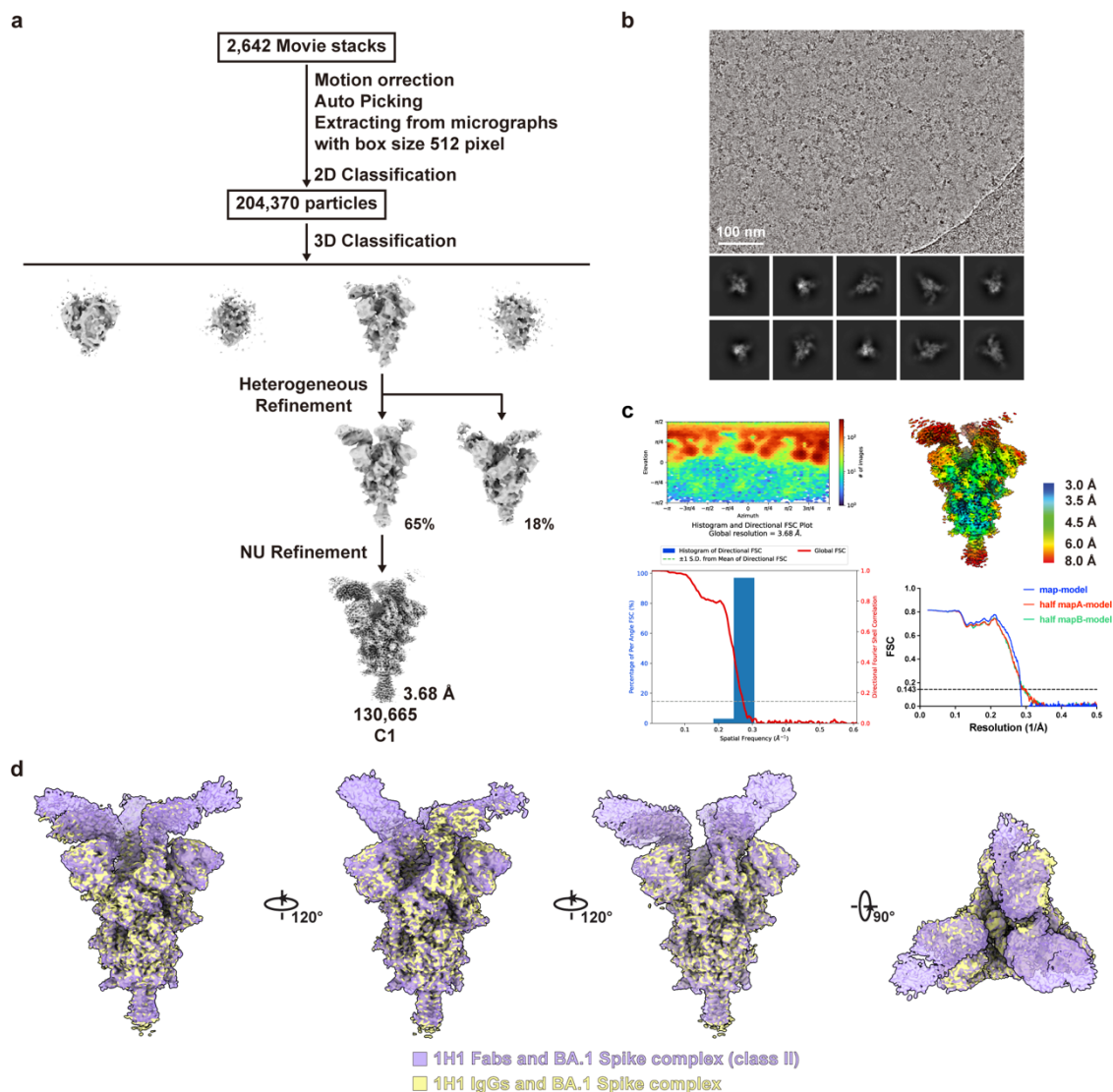
Supplementary Figures 1–5



Supplementary Fig. 1 Cryo-EM data processing and validation for the SARS-CoV-2

Omicron BA.1 spike protein in complex with 1H1 Fab. a representative cryo-EM micrograph and 2D classes. **b** workflow for BA.1 spike-1H1 Fab 3D Reconstructions. **c-e**

the viewing direction distribution plot, global FSC and histogram, cryo-EM density colored according to the local resolution and model-map FSC for the class I (**c**), class II (**d**) and local refinement (**e**), respectively. **f** model-map fitting of the local-refined BA.1RBD-1H1 structures. **g** sharpened cryo-EM density maps (BA.1 RBD and 1H1 Fab interaction regions) are rendered as mesh with the corresponding models shown as sticks.



Supplementary Fig. 2 Cryo-EM data processing and validation for the SARS-CoV-2

Omicron BA.1 spike protein in complex with 1H1 IgG. a workflow for BA.1 spike-1H1

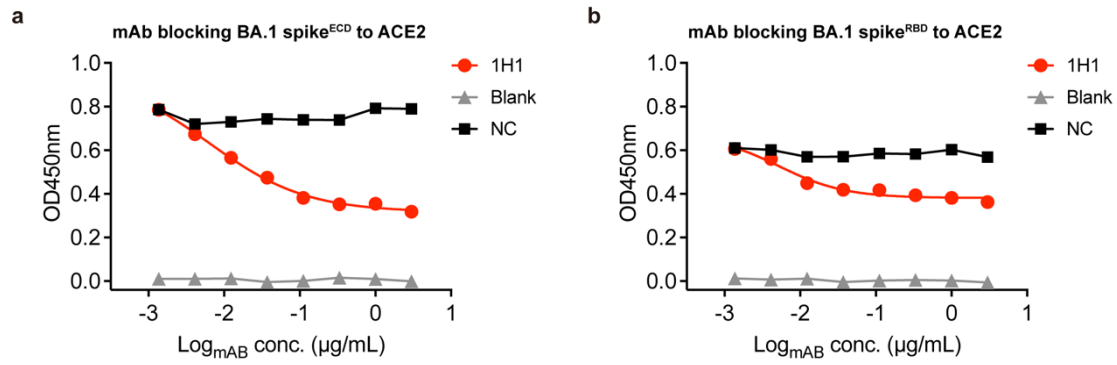
IgG 3D Reconstructions. **b** representative cryo-EM micrograph and 2D classes. **c** the

viewing direction distribution plot, global FSC and histogram, cryo-EM density colored

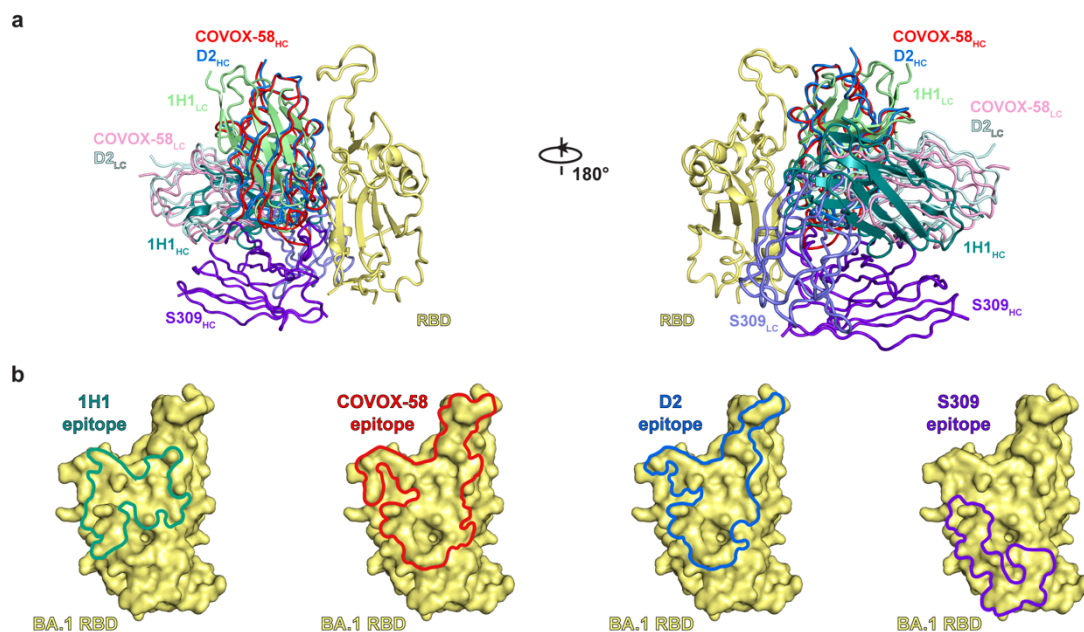
according to the local resolution and model-map FSC for the 1H1 IgG dataset. **d**

comparison of the electron density maps between BA.1 spike-1H1 IgGs complex (yellow)

and BA.1 spike-1H1 Fabs complex (class II, purple).



Supplementary Fig. 3 Competing ELISA assays. a, b the ability of 1H1 to compete with ACE2 for binding to Omicron BA.1 spike ECD (**a**) and RBD (**b**). The competition capacity of 1H1 was indicated by the level of reduction in the response unit of ACE2 compared with or without the addition of 1H1. Error bars indicate standard deviation (SD) of at least two biological replicates.



Supplementary Fig. 4 Comparison of 1H1 with COVOX-58, D2 and S309. **a** structural comparison of the RBD-1H1 and RBD-COVOX-58 (PDB ID: 7QNY), RBD-D2 (PDB ID: 7XMZ) and RBD-S309 (PDB ID: 7XCO) complexes. Superposition of 1H1, COVOX-58 and D2 shows a similar binding mode, where COVOX-58 and D2 share a nearly identical epitope and the epitope of 1H1 is slightly different from the other two. **b** the footprints of 1H1, COVOX-58, D2 and S309, respectively.

