

Supplementary Information for:

Native-like SARS-CoV-2 spike glycoprotein expressed by ChAdOx1
nCoV-19/AZD1222 vaccine

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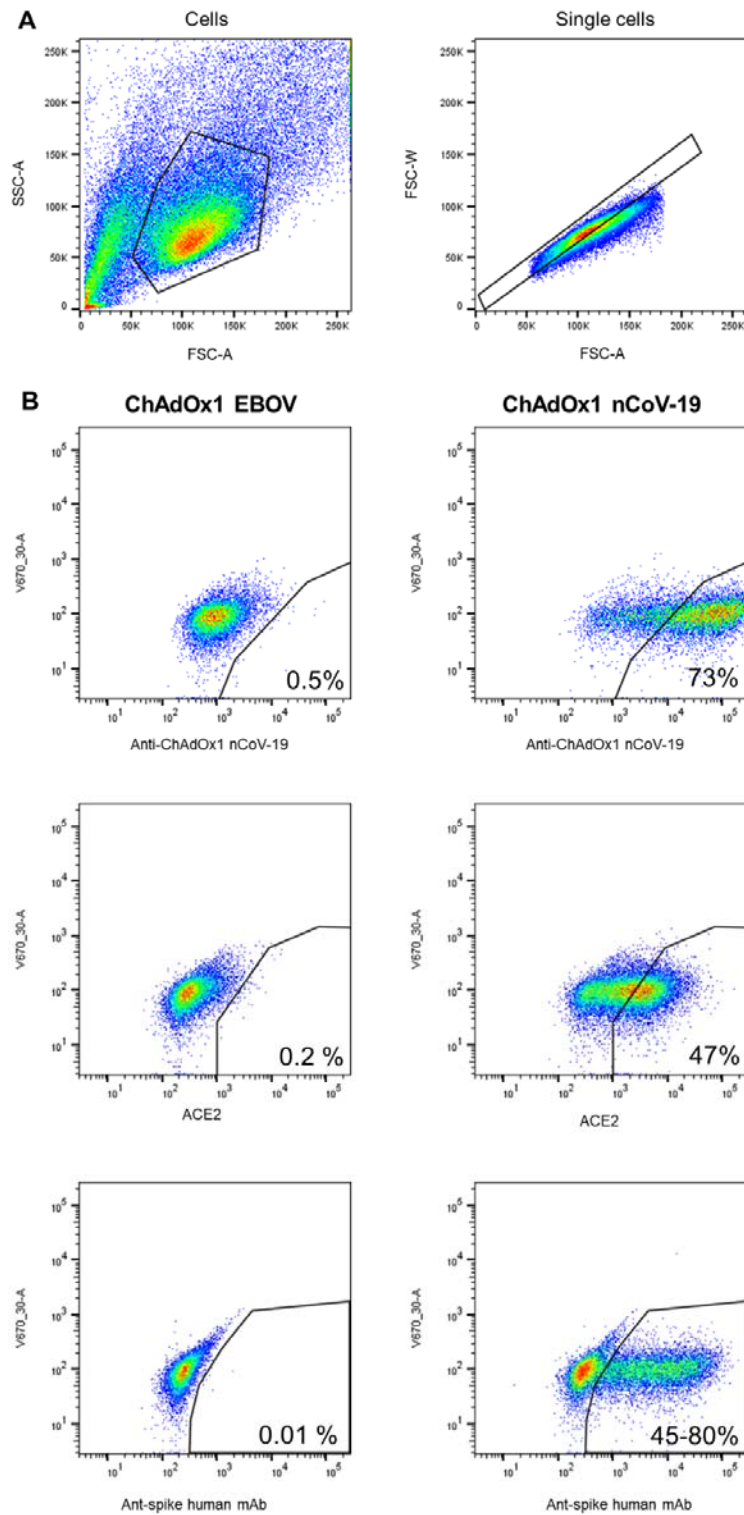
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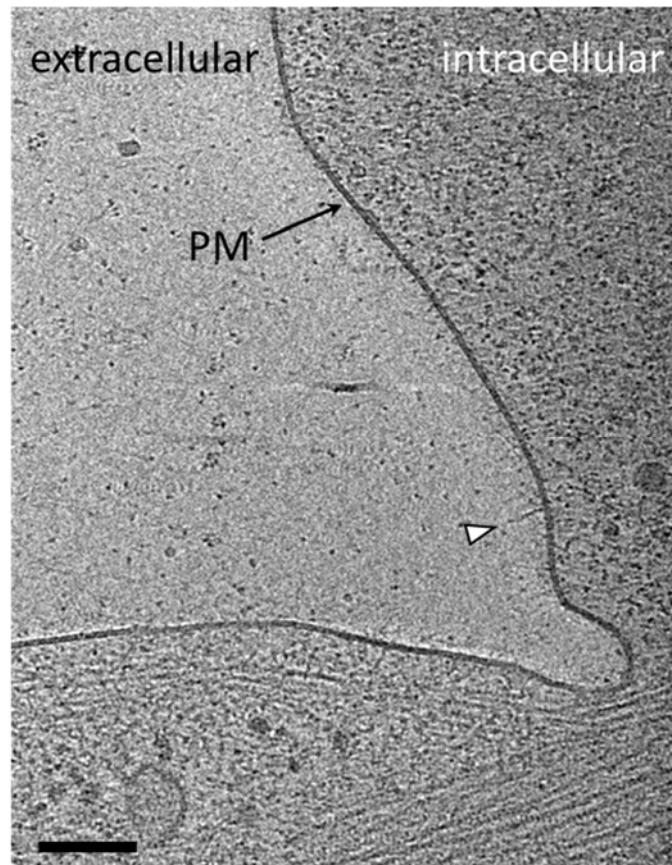
Supplementary Figures 1-4

Supplementary Table 1

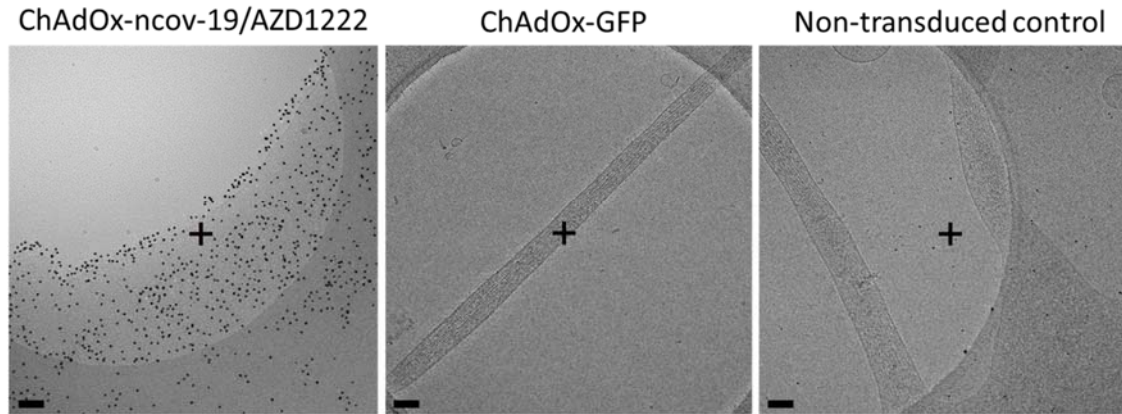


Supplementary Figure 1. Gating strategy for vaccine expression FACS

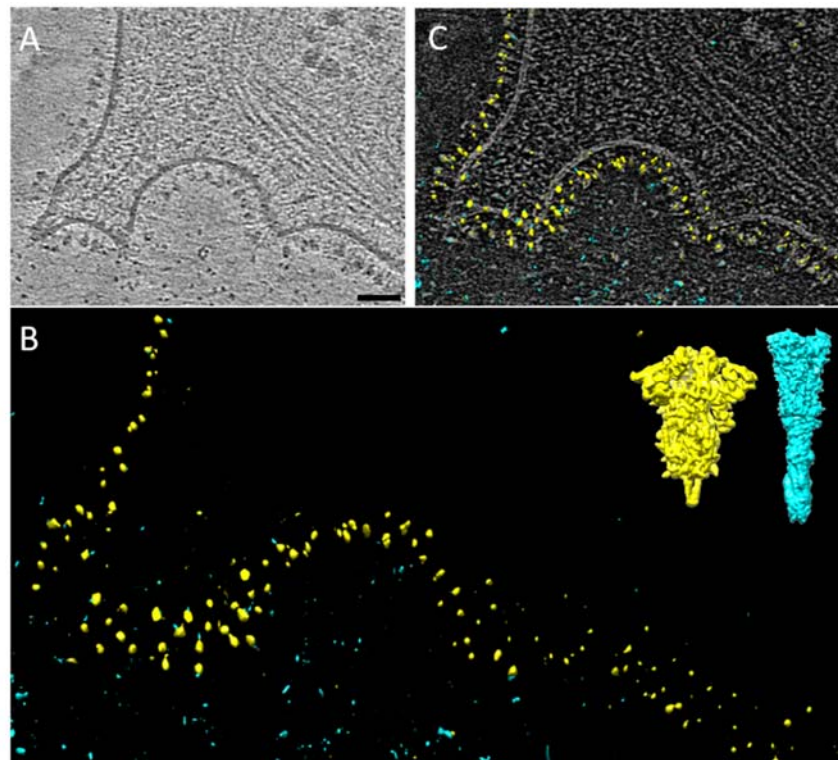
(A) Single cells were isolated for further analysis. **(B)** Percentages of positive cells were determined using the gating strategy determined in uninfected cells and displayed for ChAdOx1- EBOV (left panels) and ChAdOx1-nCoV19 (right panels) infected cells, with percentage of positive cells indicated in gate. Representative data is shown, for human mAbs data for mAb 71 is shown.



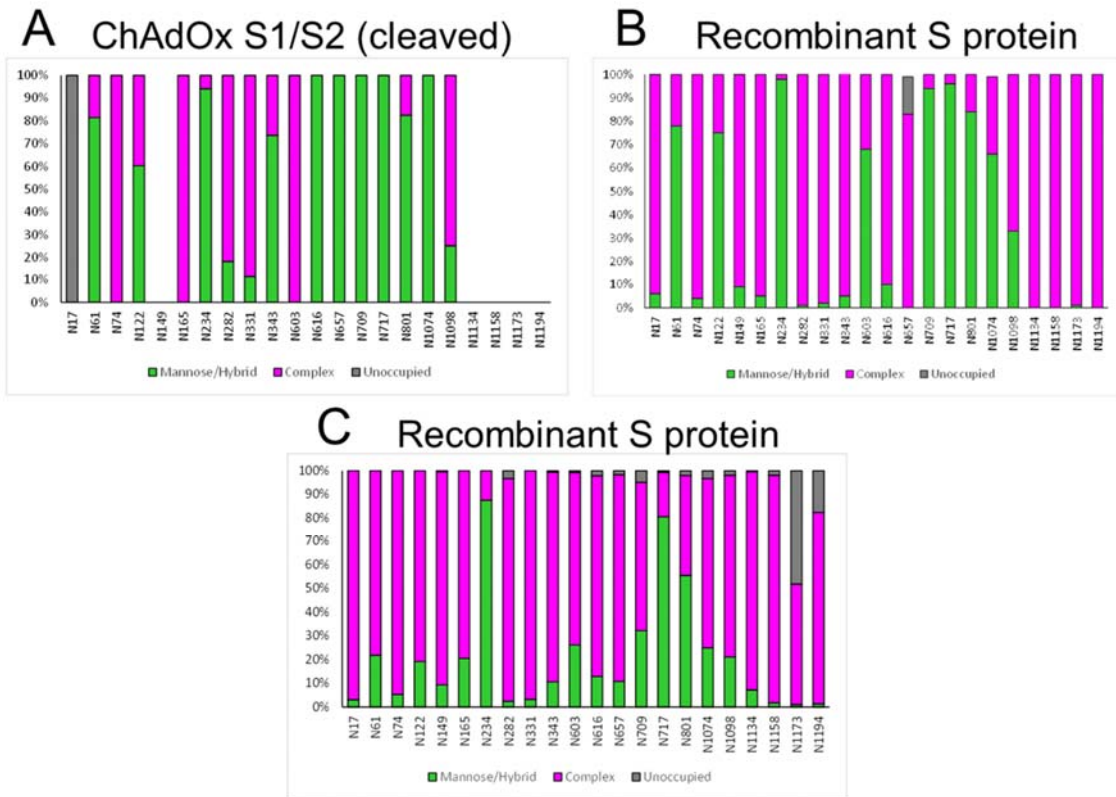
Supplementary Figure 2. Non-transduced cells lack prefusion-like surface receptors. Tomographic slice of U2OS control cell surface. PM – Plasma membrane. Slice is 2.13 Å thick. White arrowhead point to a thin and elongated surface receptor. Scale bar is 100 nm.



Supplementary Figure 3. Cryoimmunolabelling of ChAdOx-nCoV-19/AZD1222 derived spike. Cryo-EM image of U2OS cells transduced with ChAdOx-nCoV-19/AZD1222, ChAdOx-GFP and non-transduced controls. Cells were incubated with ChAdOx-nCoV-19/AZD1222 vaccinated mice sera and labelled with anti-mouse Fab conjugated with 10nm Au beads prior to plunge freezing. Scale bar is 100 nm.



Supplementary Figure 4. Template searching for pre- and post-fusion spike proteins. (A) Tomographic slice of a ChAdOx nCoV-19 infected U2OS cell. (B) Results of template matching by cross-correlation of tomographic volume with either a pre-fusion spike reference (EMD-21452, yellow) or a post-fusion spike reference (EMD-7040, cyan). The resulting cross-correlation maps are projected through 18.5 nm thick volume. Inset, the pre- (yellow) and post-fusion (cyan) spike references. (C) The template matching results shown in (B) overlaid with a 1.6 nm thick tomographic slice in (A). Scale bar is 50 nm.



Supplementary Figure 5. A comparison of site-specific glycosylation analysis between ChAdOx-derived S1/S2 protein (**A**), and stabilized recombinant S proteins expressed in HEK 293F cells (**B & C**). Panels B and C represent data reproduced from earlier published studies (1, 2).

Supplementary Table 1. Glycoform abundances observed across S0 and S1/S2 SARS-CoV-2 spike protein derived from ChAdOx1 nCoV-19.

Cleaved																							
	N17	N61	N74	N122	N149	N165	N234	N282	N331	N343	N603	N616	N657	N709	N717	N801	N1074	N1098	N1134	N1158	N1173	N1194	Total
Mannose/Hybrid	0%	81%	0%	60%	n/a	0%	94%	18%	11%	74%	0%	100%	100%	100%	100%	82%	100%	25%	n/a	n/a	n/a	n/a	56%
Complex	0%	19%	100%	40%	n/a	100%	6%	82%	89%	26%	100%	0%	0%	0%	0%	18%	0%	75%	n/a	n/a	n/a	n/a	38%
Unoccupied	100%	0%	0%	0%	n/a	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	n/a	n/a	n/a	n/a	6%

Uncleaved																								
	N17	N61	N74	N122	N149	N165	N234	N282	N331	N343	N603	N616	N657	N709	N717	N801	N1074	N1098	N1134	N1158	N1173	N1194	Total	
Mannose/Hybrid	n/a	100%	100%	81%	n/a	72%	98%	74%	78%	69%	69%	100%	100%	100%	100%	66%	86%	69%	100%	n/a	n/a	n/a	73%	85%
Complex	n/a	0%	0%	19%	n/a	28%	2%	26%	22%	31%	31%	0%	0%	0%	0%	34%	14%	31%	0%	n/a	n/a	n/a	27%	15%
Unoccupied	n/a	0%	0%	0%	n/a	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	n/a	n/a	n/a	0%	0%

Supplementary References

1. Y. Watanabe, J. D. Allen, D. Wrapp, J. S. McLellan, M. Crispin, Site-specific glycan analysis of the SARS-CoV-2 spike. *Science* (2020), doi:10.1126/science.abb9983.
2. P. Zhao, J. L. Praissman, O. C. Grant, B. Chen, I. Brief, Y. Cai, T. Xiao, K. E. Rosenbalm, K. Aoki, B. P. Kellman, R. Bridger, D. H. Barouch, M. A. Brindley, N. E. Lewis, M. Tiemeyer, R. J. Woods, L. Wells, Virus-Receptor Interactions of Glycosylated SARS-CoV-2 Spike and Human ACE2 Receptor. *Cell Host Microbe*. **28**, 1–16 (2020).