

Supplementary Information for:

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

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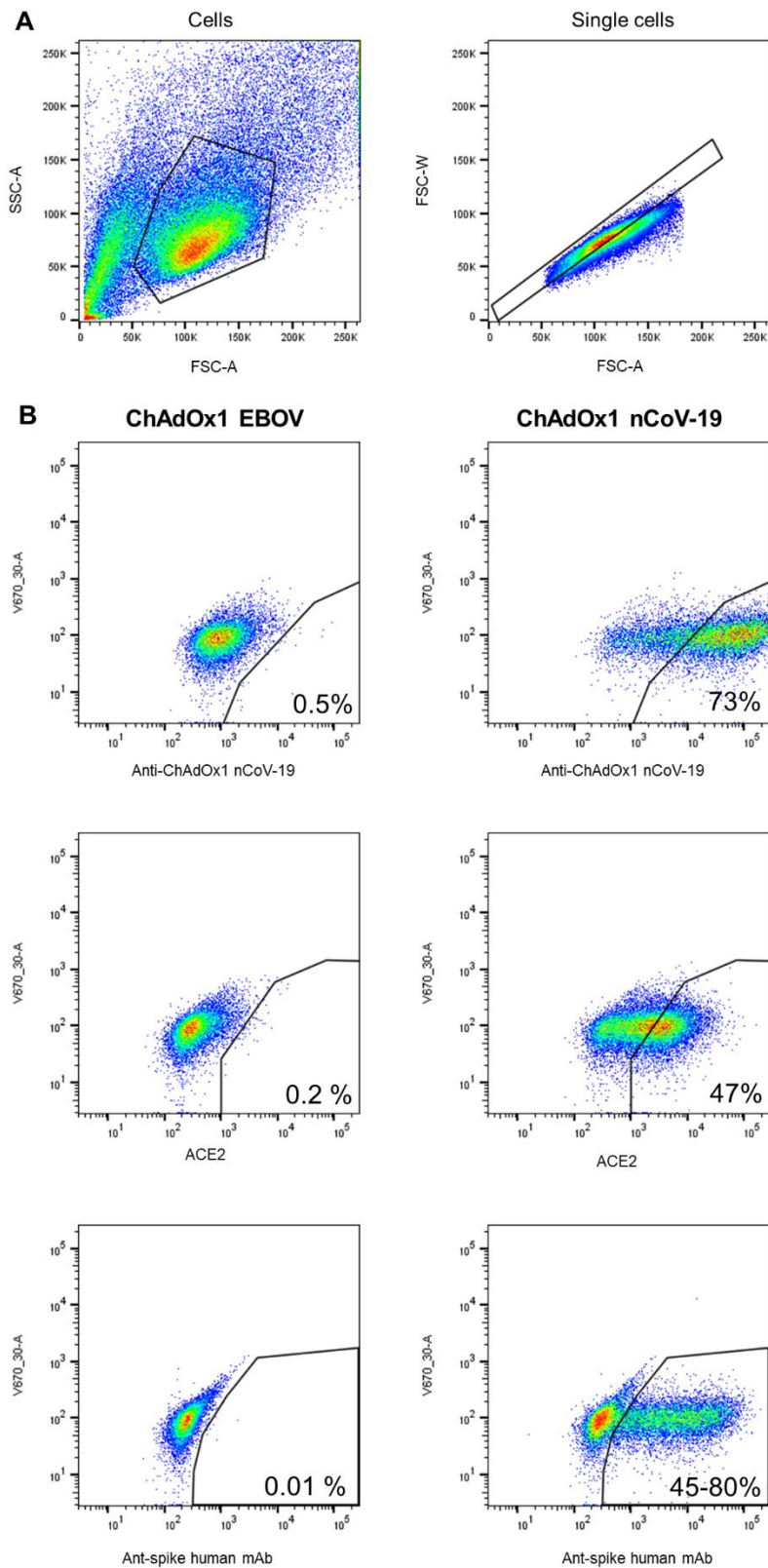
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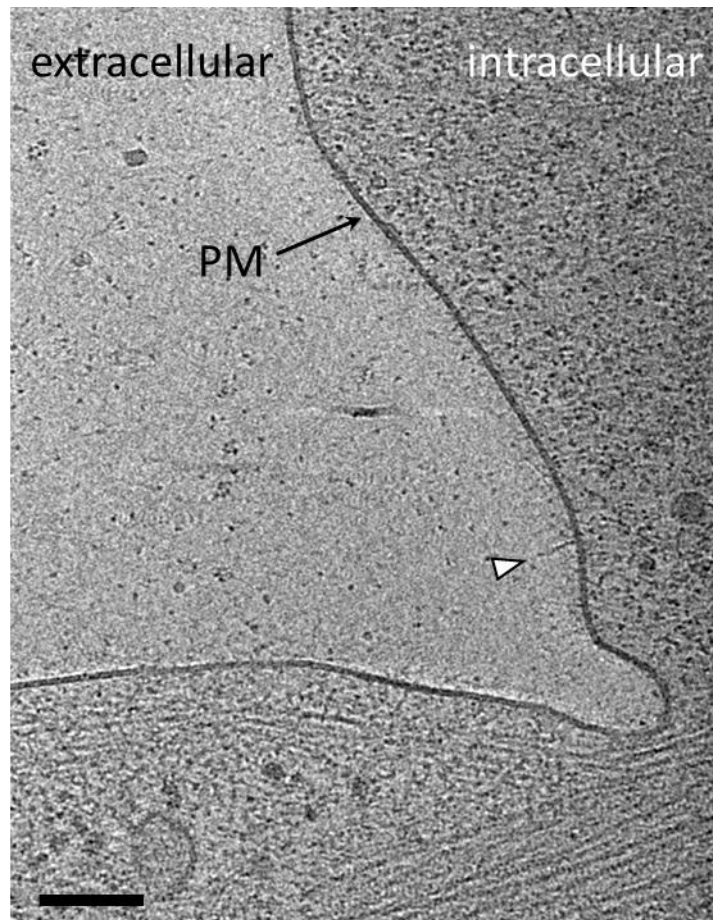
Supplementary Figures 1-4

Supplementary Table 1

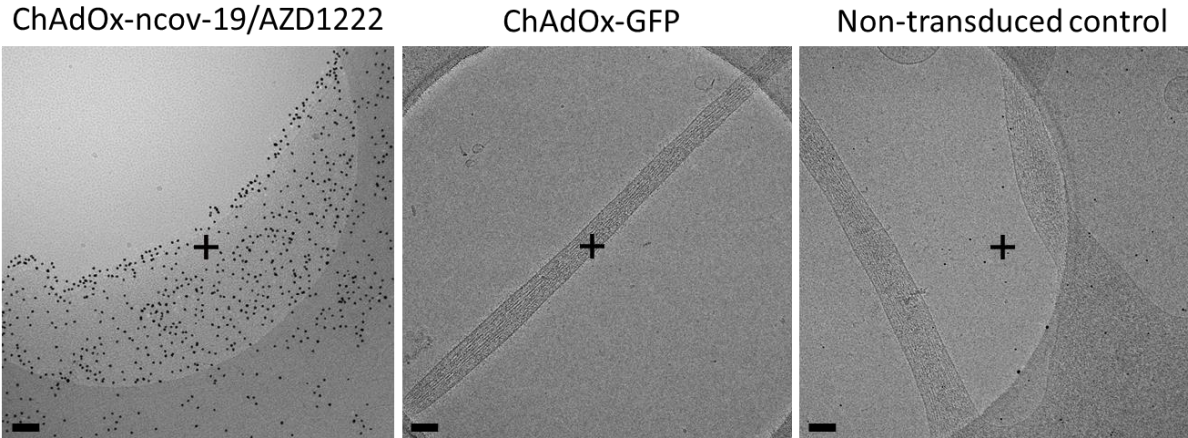


Supplementary Figure 1. Gating strategy for vaccine expression FACS

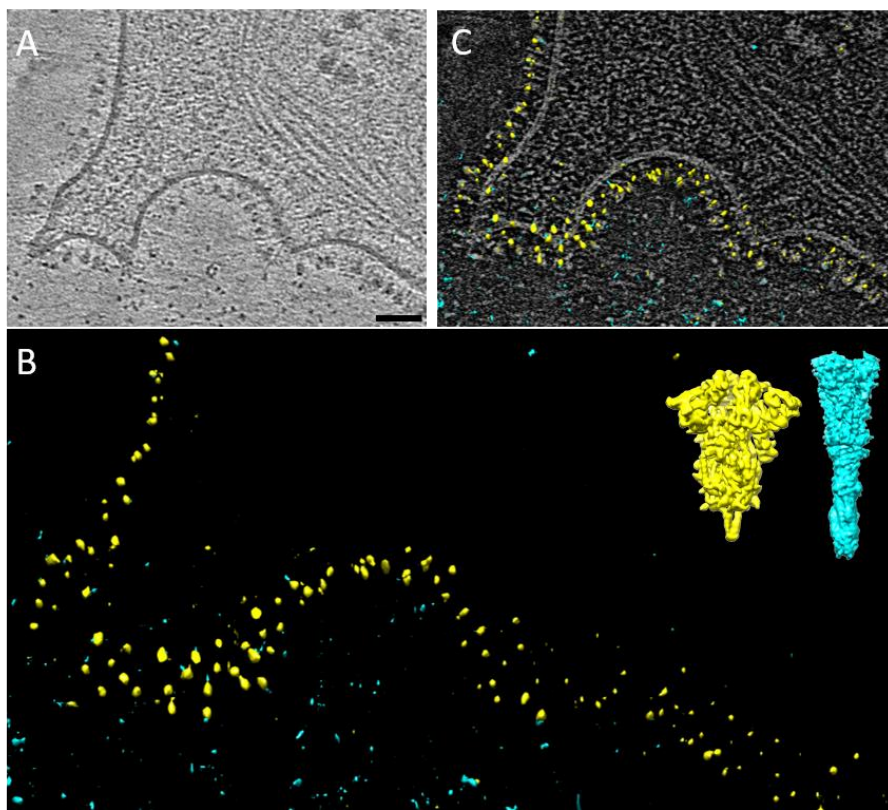
(A) Single cells were isolated for further analysis. (B) Percentages of positive cells were determined using the gating strategy determined in uninfected cells and displayed for ChAdOx1- EBOV (left panels) and ChAdOx1-nCoV19 (right panels) infected cells, with percentage of positive cells indicated in 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 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	N1156	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	N1156	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	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	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	0%	0%