Structural basis for transport and inhibition of nucleotide sugar transport in pathogenic fungi.

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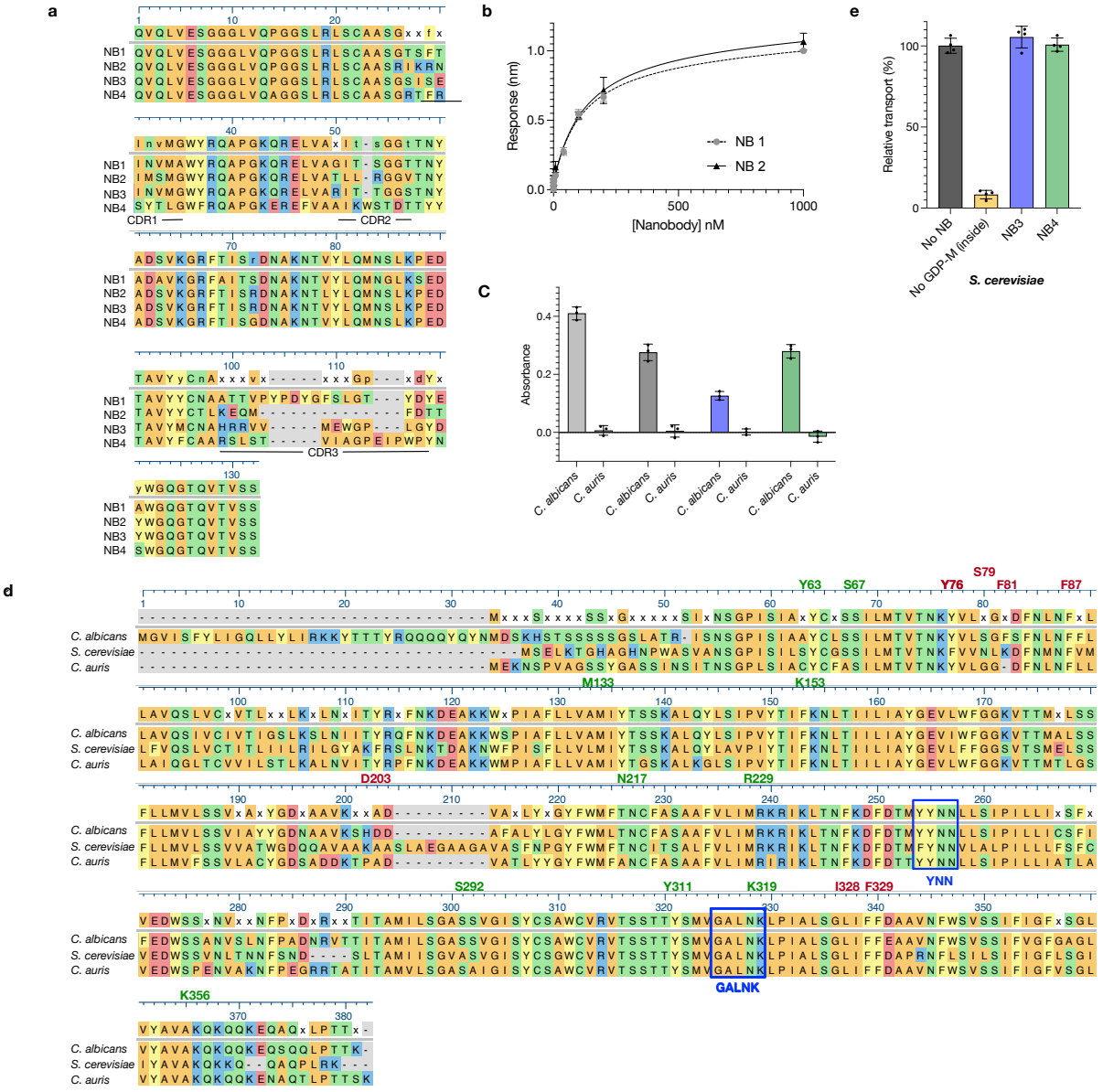
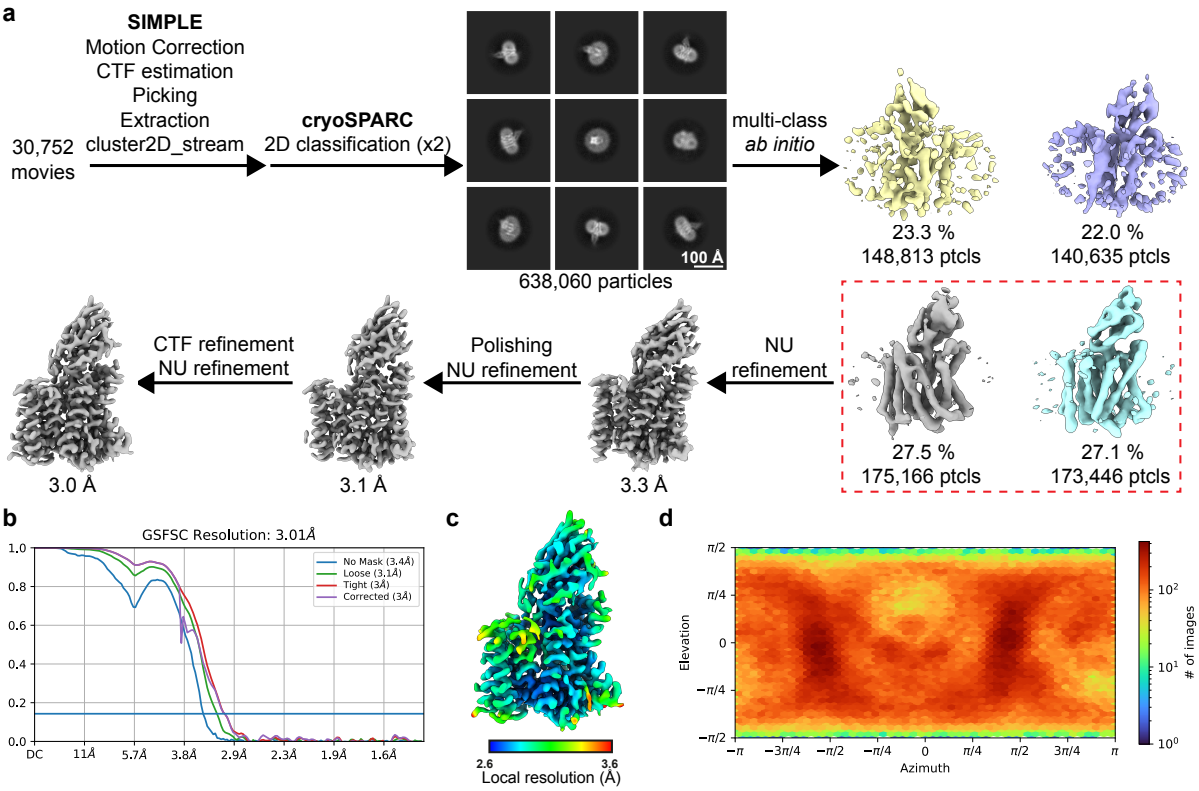
Extended Data Figures 1-5

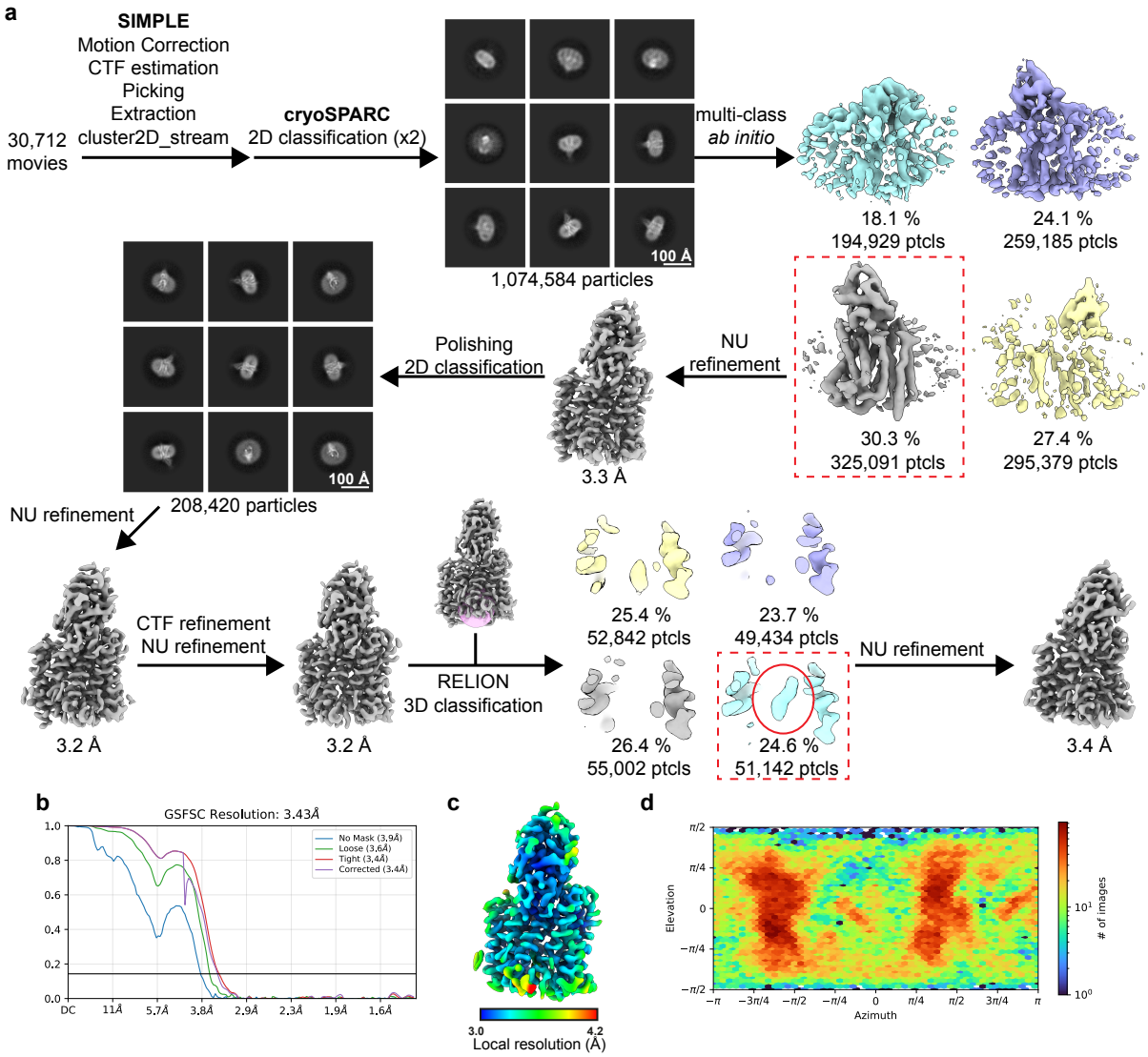


Extended Data Fig. 1. Characterisation of nanobodies to CaVrg4. ***a****, Sequence alignment of the four nanobodies used in this study, the position of the CDR1, 2, and 3 loops is indicated.* ***b,*** *ELISA data showing normalised absorbance (normalised to the highest absorbance for each NB) against NB concentration for NB1 and NB2. The calculated* K_D *for both NBs was ~ 110 nM (109 ± 5 for NB1 and 125 ± 12 for NB2) (n = 3 independent experiments performed on different days; the mean is shown, and errors indicate SD).* ***c****, ELISA data showing the specificity of the NBs towards C. albicans Vrg4 as compared to Vrg4 from C. auris. 10 μM NB was used in the assay, and only in the presence of C. albicans, Vrg4 was binding (indicated by absorbance), detected with no binding to the C. auris protein.* ***d****, Sequence alignment of Vrg4 from C. albicans (Uniprot Q5A477), C. auris (A0A8F2VZF7) and*

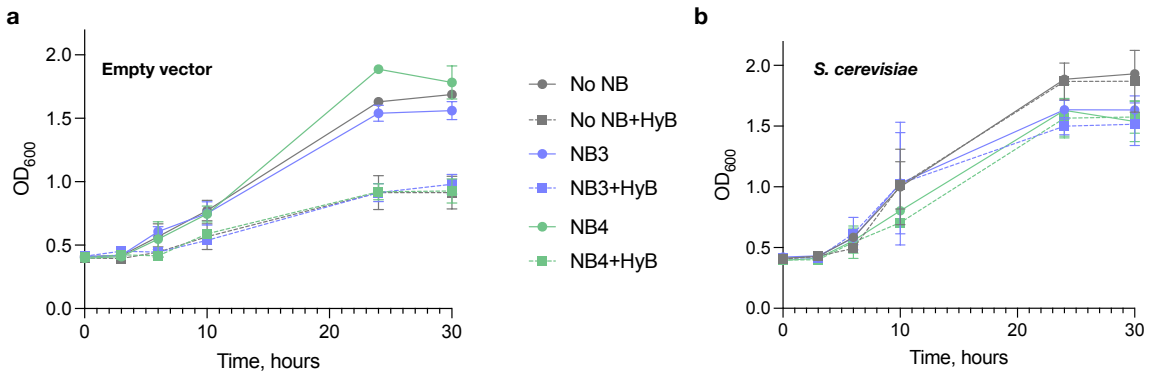
S. cerevisiae (P40107). Highlighted are the conserved motifs, GALNK (sugar specificity) and YNN (nucleotide specificity). Also indicated are residues mentioned in the manuscript where NB3 or NB4 contact the transporter (shown in red) or are important for function (shown in green). **e** *Transport assay data showing the transport of GMP via S. cerevisiae Vrg4 in the presence of a nanobody (NB) relative to a no NB control. Without GDP-mannose on the inside of the liposome (no GDP-M inside) this transporter cannot function. (n=4 independent experiments performed on different days, the mean is shown, and errors indicate SD).*



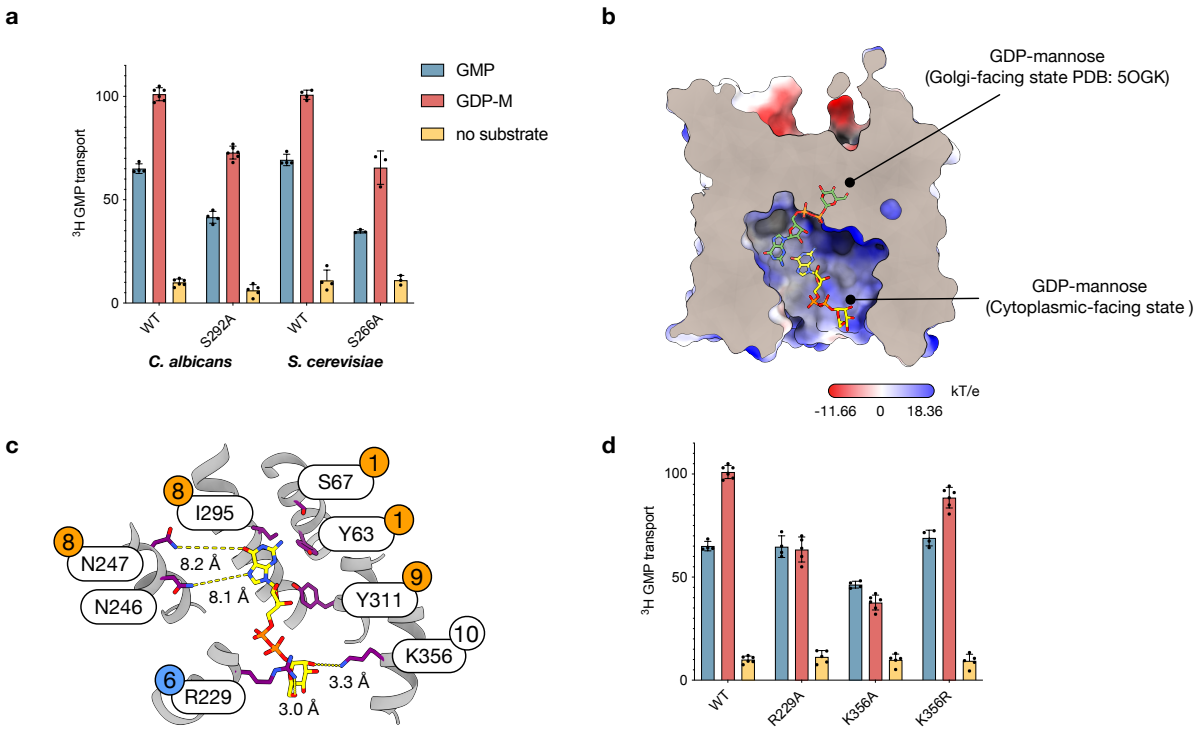
Extended Data Fig. 2 Cryo-EM processing workflow of Vrg4 with NB3. ***a,*** *Image processing workflow.* ***b,*** *Gold-standard Fourier Shell Correlation (FSC) curves for global resolution.* ***c,*** *Local resolution estimate of the volume.* ***d,*** *Orientation distribution plot.*



Extended Data Fig. 3 Cryo-EM processing workflow of Vrg4 with NB4. a, *Image processing workflow. Volume for the endogenous GDP-mannose bound to Vrg4 is shown in the red box.* ***b,*** *Gold-standard Fourier Shell Correlation (FSC) curves for global resolution.* ***c,*** *Local resolution estimate of the volume.* ***d,*** *Orientation distribution plot.*



Extended Data Fig. 4. Growth of NDY5 yeast strain. a*, Growth curves of the NDY5 yeast containing an empty yEP181 vector and either NB3, NB4 or no NB in the absence or presence (+ HyB, dashed lines) of hygromycin B. This strain is sensitive to hygromycin B, resulting in slower and lower growth over time; however, this sensitivity is not altered in the presence of the NBs.* ***b****, Growth curves of the NDY5 yeast (similar to* ***a****) but containing the yEP181 vector overexpressing S. cerevisiae Vrg4 and either NB3, NB4 or no NB in the presence (+) and absence of hygromycin B (HyB). The presence of functional Vrg4 rescues the sensitivity to HyB of this strain, which is not altered in the presence of the NBs. (for a and b, n=2 independent experiments performed on different days, the mean is shown and errors indicate SD).*



Extended Data Fig 5. Recognition of GDP-mannose in CaVrg4. ***a****, Comparison of transport activity of WT and Ser292Ala in C. albicans Vrg4 and the equivalent serine (Ser266) in S. cerevisiae. Data is normalised to transport using GDP-mannose as the antiport substrate for the WT protein. Also shown is transport using GMP as the antiport substrate, as well as transport with no substrate, where only one turnover can occur. (n=4 independent experiments performed on different days, the mean is shown, and errors indicate SD).* ***b****, The position of GDP-mannose in CaVrg4 cytoplasmic facing state (shown as green sticks) with the GDP-mannose position from the Golgi facing state (PDB5:OGK, yellow sticks).* ***c,*** *Zoomed in view of the cytoplasmic facing binding site with bound GDP-mannose. Distances to key side chains are indicated.* ***d****, Comparison of transport activity for WT and mutant forms of CaVrg4 using either GMP, GDP mannose or no substrate as the counter substrate. Data is normalised to transport for WT with GDP mannose as the counter substrate (100%). (n=4, errors shown are SD).*