Supporting Information for

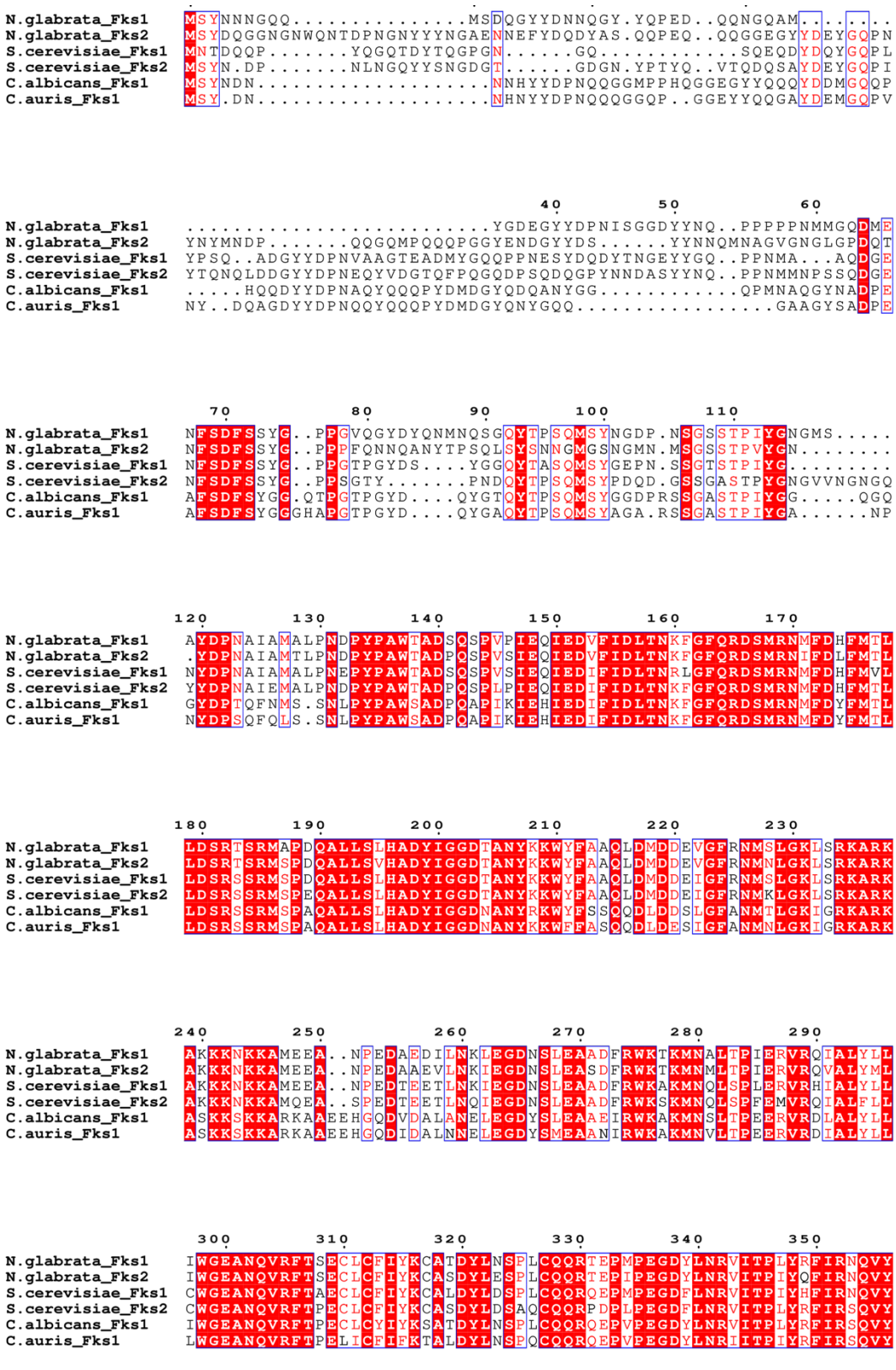
Structural basis of caspofungin inhibition in the post-catalytic state of *Nakaseomyces glabratus* Fks1

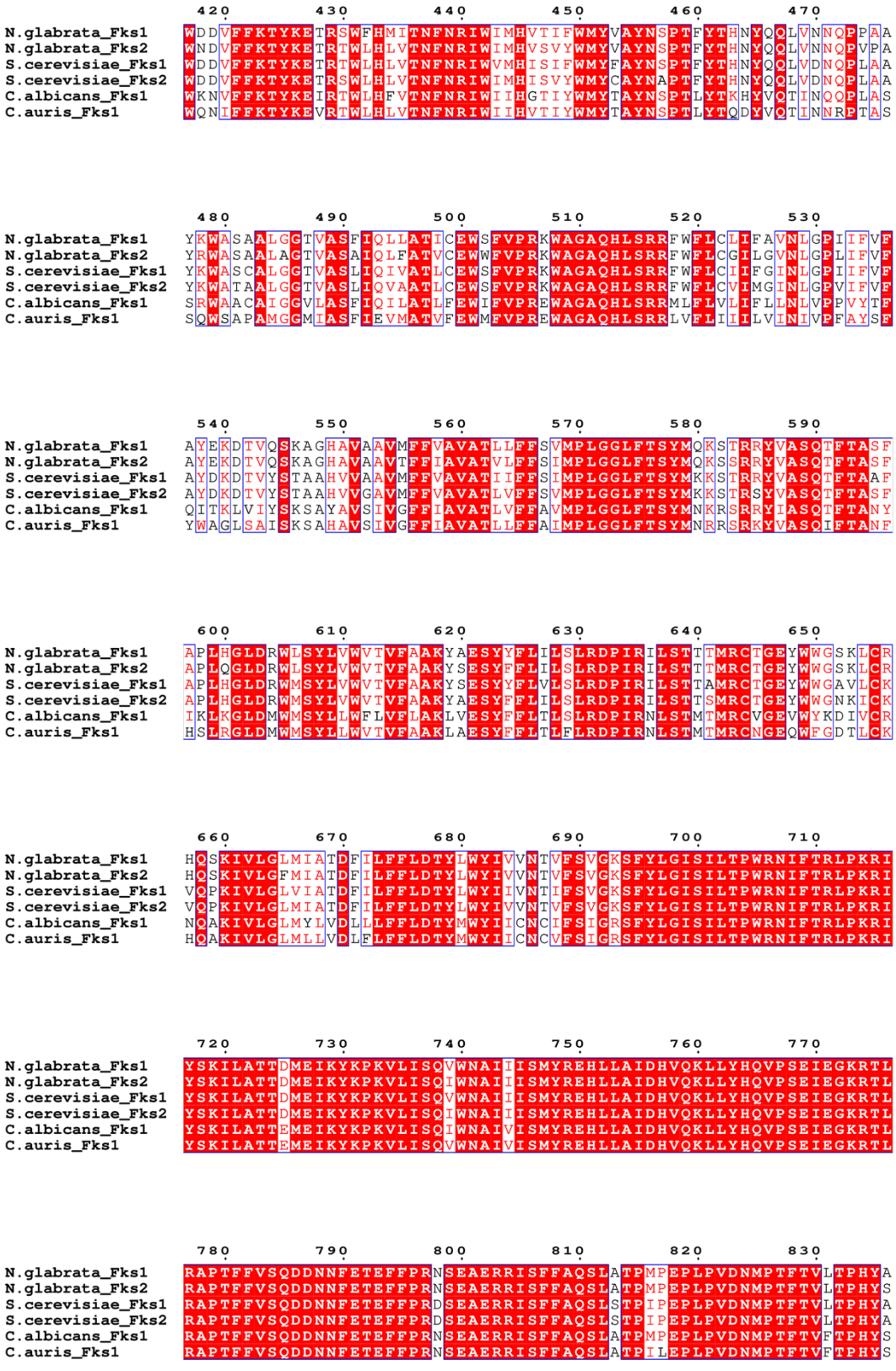
[1]Rasel, Md Shamiul Islam, [1]Raghav, D, [1]Suchana, Fahrin Jahan,[1] Souza Amado De Carvalho, Rodolpho, [1]Tomasiak TM.

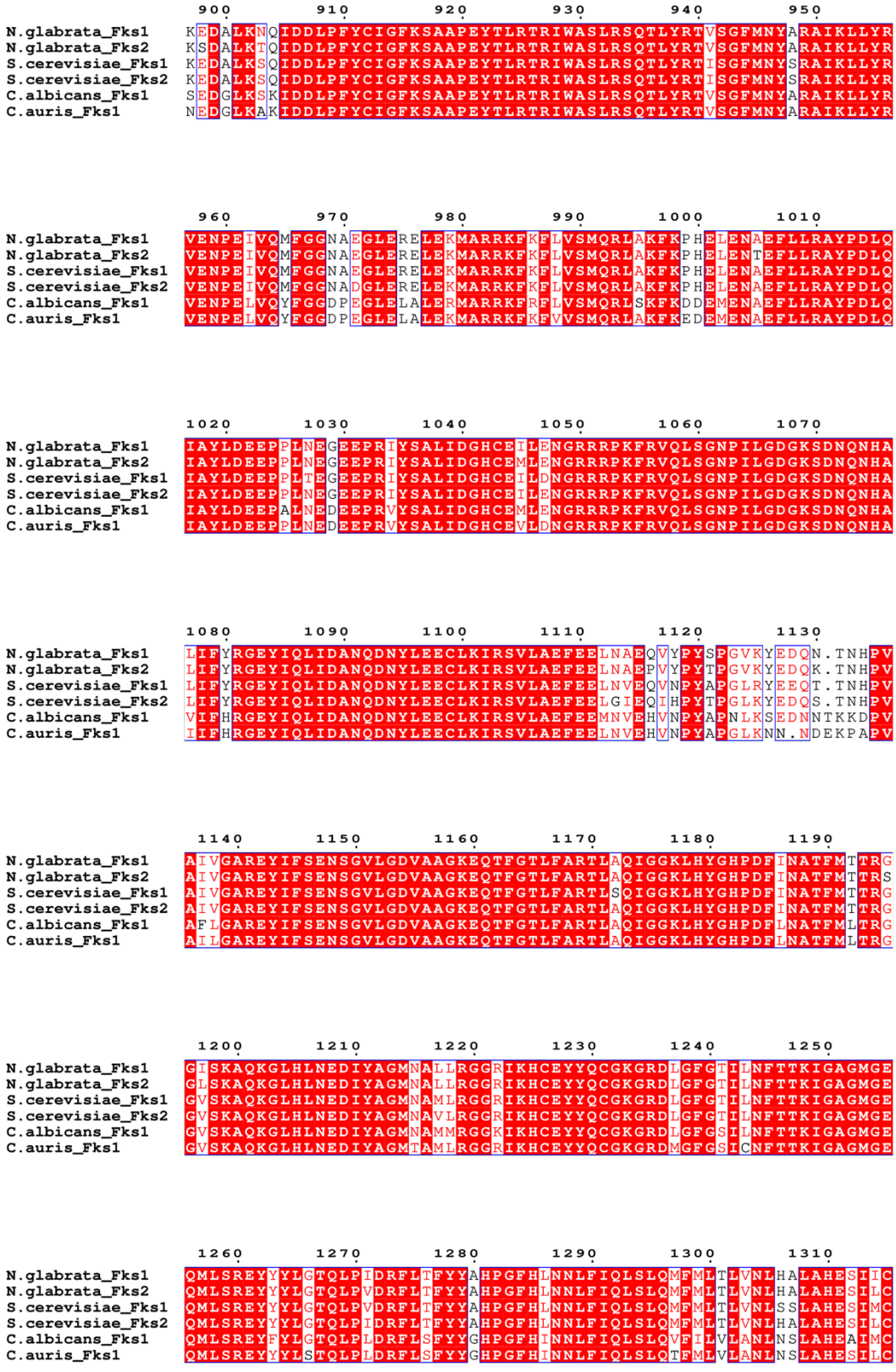
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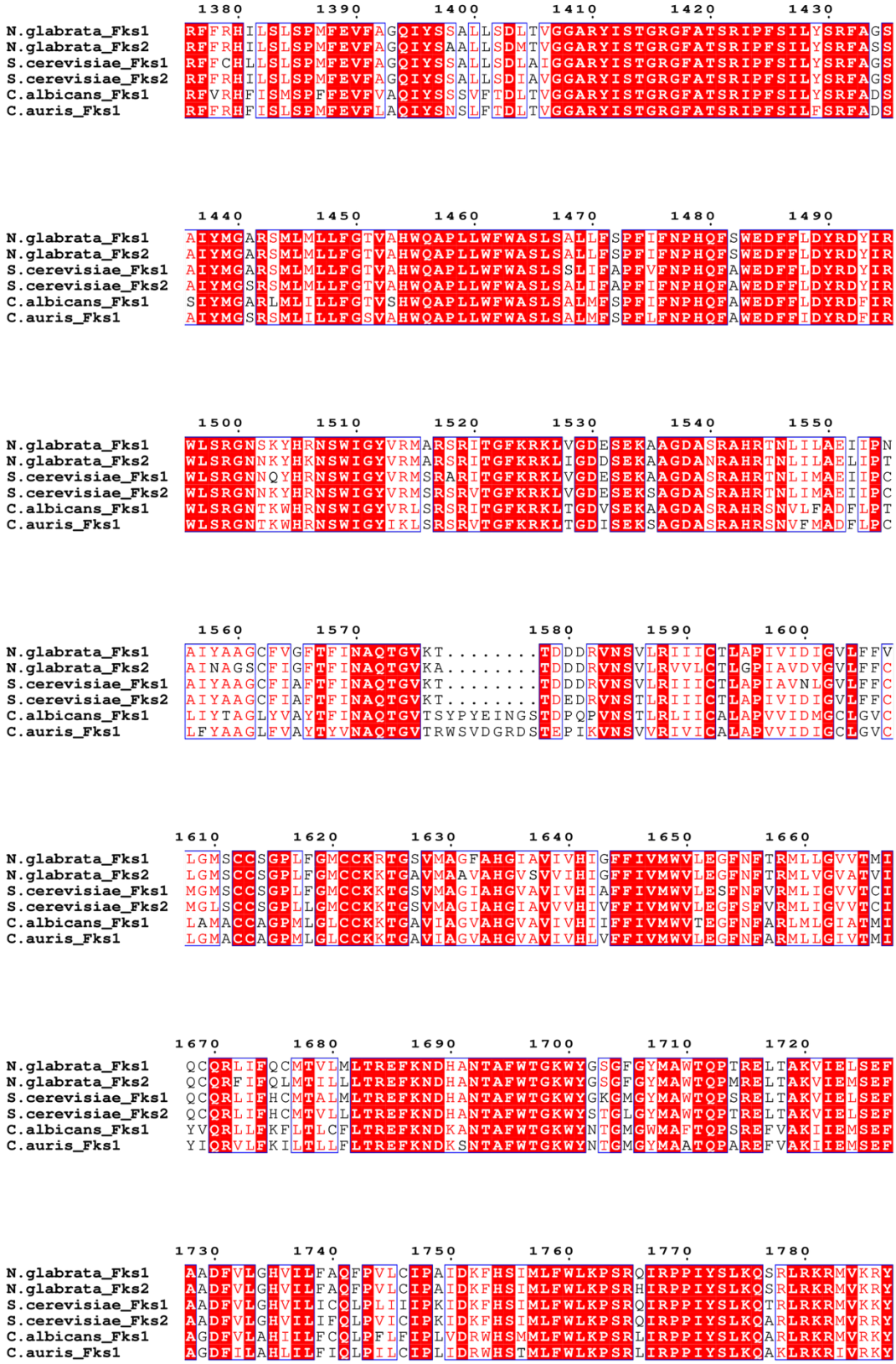
This PDF file includes:

Figures S1 to S10
Tables S1 to S2
SI References


N.glabrata_Fks1
N.glabrata_Fks2
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S.cerevisiae_Fks2
C.albicans_Fks1
C.auris_Fks1


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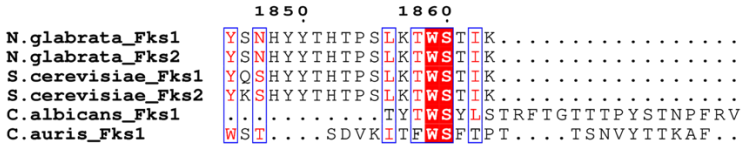


Fig. S1. Multiple sequence alignment of Fks. Fks1 and Fks2 sequences from N. glabrata (Uniprot: Q6FTN8, Q6FMZ6), S. cerevisiae (P38631, P40989), Fks1 C. auris (Uniprot: A0A3G2LW13), and the Fks1 sequence from C. Albicans (A0A1D8PCT0), are aligned using Clustal Omega (1)and then displayed using ESPript 3.2 (2).

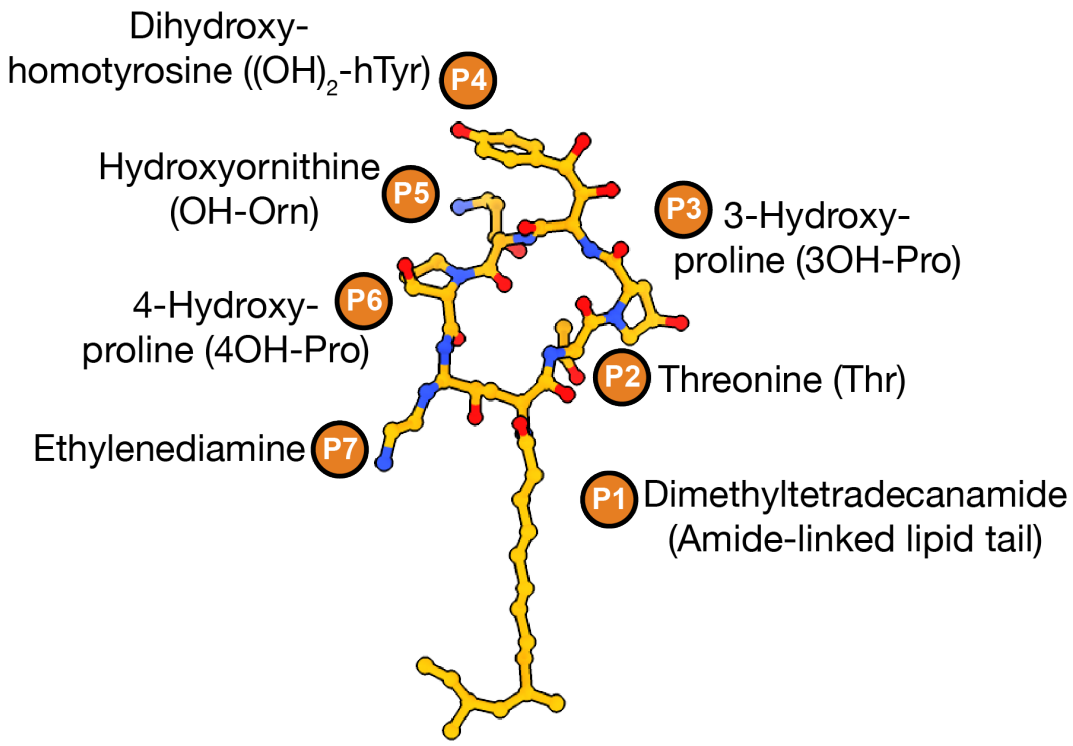


Fig. S2. Structural moieties of caspofungin. The lipidic tail is shown in a yellow shade containing the dimethyltetradecanamide (P1) group. The cyclic peptidic core is annotated in an orange shade, containing threonine (P2), 3-hydroxyproline (P3), dihydroxyhomotyrosine (P4), hydroxyornithine (P5), 4-hydroxyproline (P6), and the ethylenediamine (P7) group.

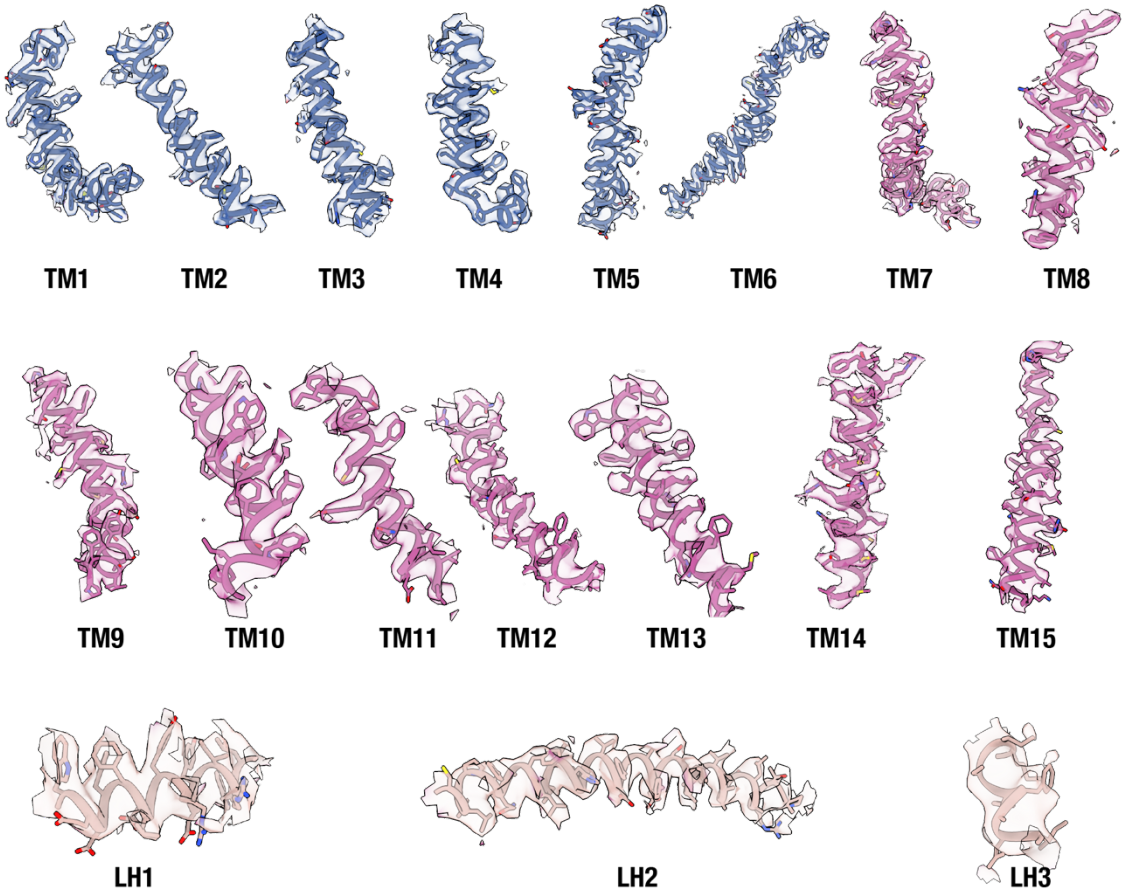


Fig. S3A. TM helices with Cryo-EM density for NgFks1 apo. Isolated TM helices and Lateral helices are shown along with their maps. TM1-6 is colored blue, TM7-15 is colored purple and Lateral helices are colored light tan. Maps shown are at a contour level of 0.075.

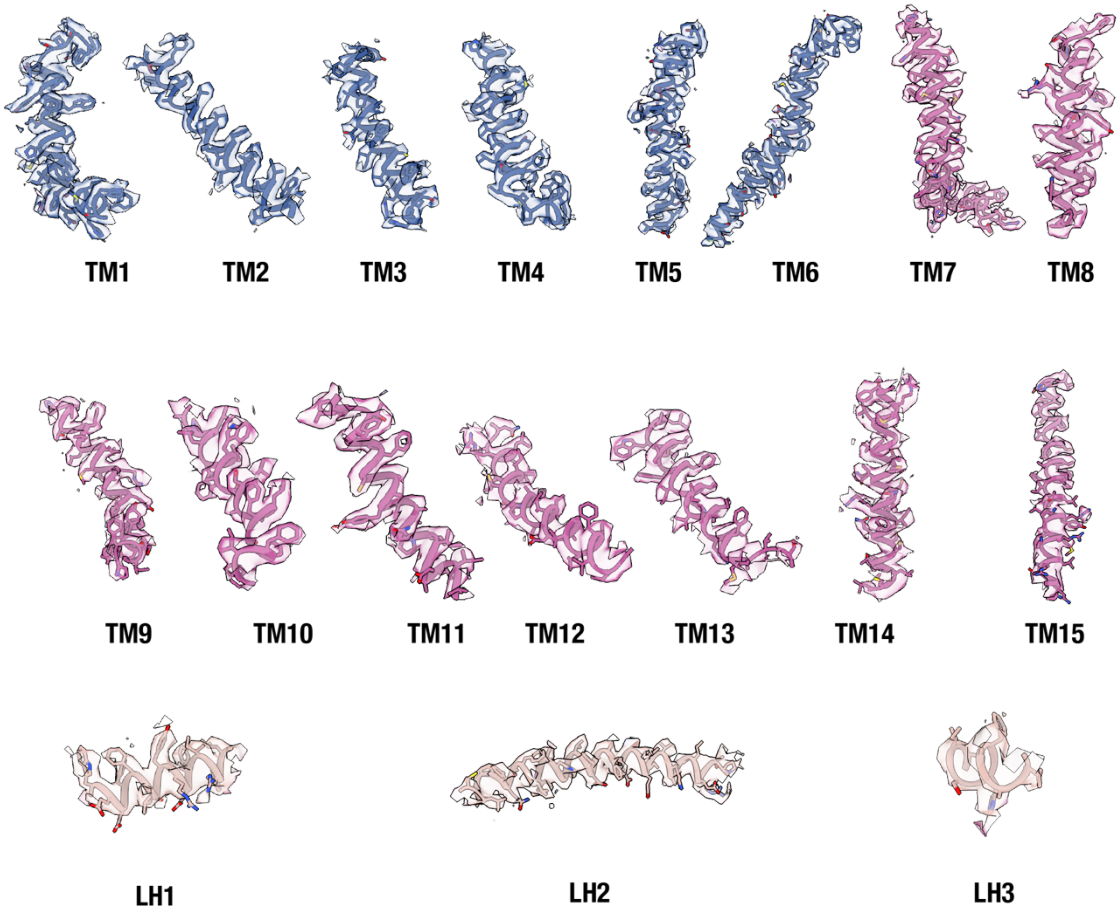


Fig. S3B. TM helices with Cryo-EM density for NgFks1 CFN. Isolated TM helices and Lateral helices are shown along with their maps. TM1-5 is colored blue, TM7-15 is colored purple and Lateral helices are colored light tan. Maps shown are at a contour level of 0.03.

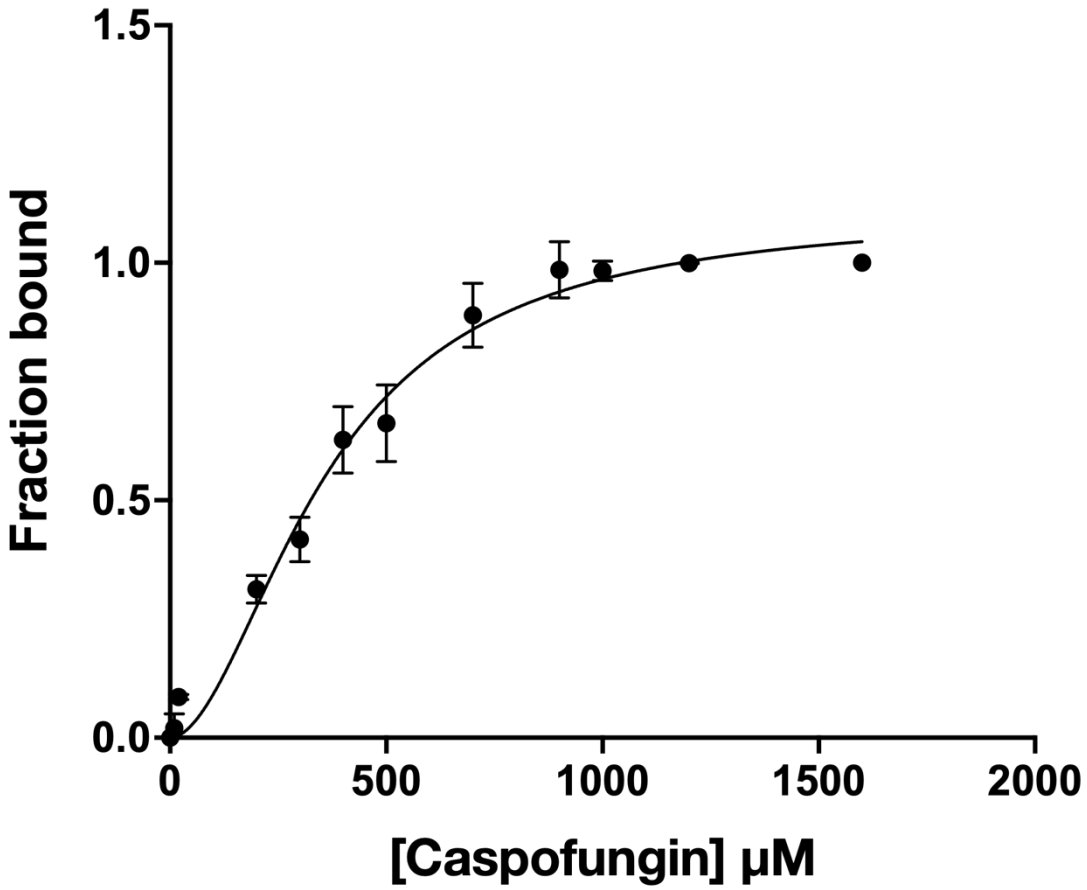


Fig. S4. Microscale Thermophoresis assay for caspofungin to Fks1. Fraction bound was plotted as a function of caspofungin concentration. Data were normalized from 0 (unbound) to 1 (fully bound). Error bars indicate variation among three replicate measurements where applicable.

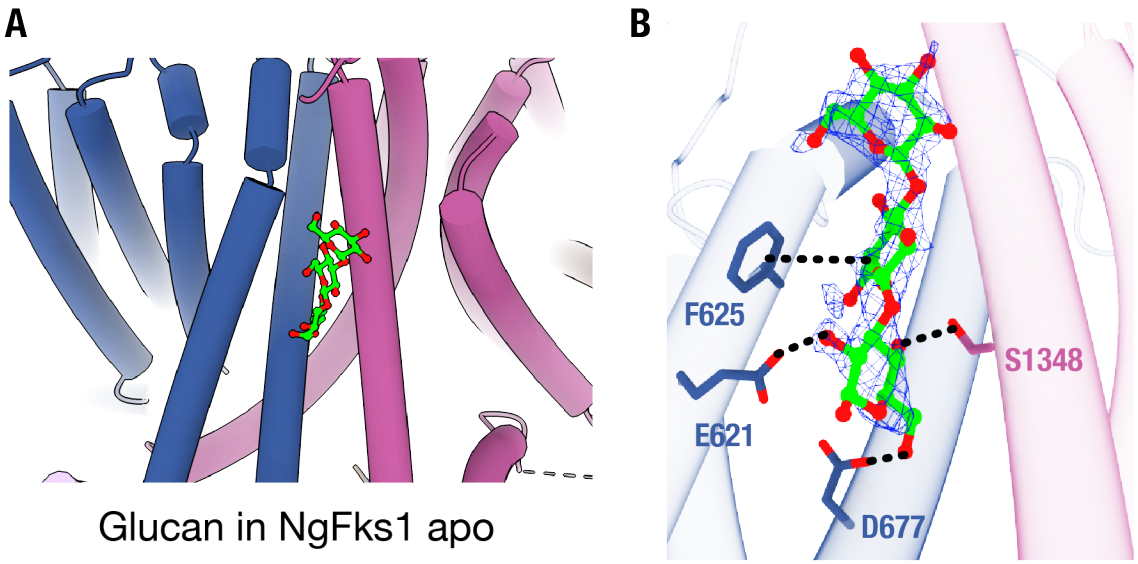


Fig. S5. Glucan-bound NgFks1 apo structure. (A) Overall view showing the position of the modeled glucan (lime) within the transmembrane helices of NgFks1. **(B)** Close-up view of the glucan model in the EM density shown in blue mesh with nearby stabilizing residues including E621, F625, D677 and S1348.

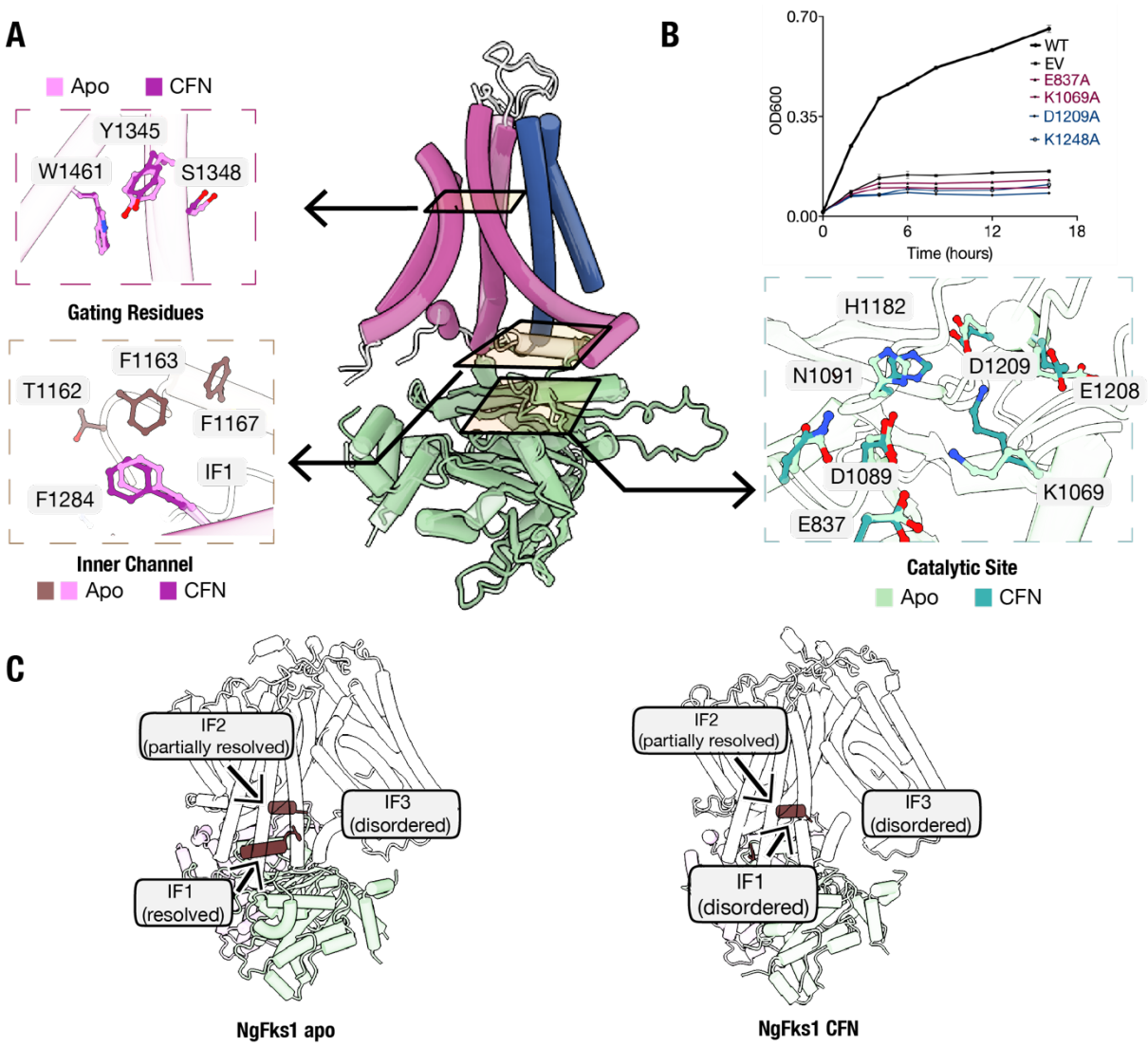


Fig. S6. Conserved architecture of the glucan translocation path. (A) The translocation path of NgFks1 based on the structure from pdb: 9pe1. The central channel is shown in the main view- the solid color represents the CFN bound state with the superimposition of the apo in transparent. The boxed inset shows a close-up comparison at key regions of the pathway: the upper exit gate (top left; CFN-bound in purple, apo in pink), the inner channel near IF1 (bottom left; CFN-bound in purple, apo in pink, with IF1 highlighted in dark brown), and the catalytic site (bottom right; CFN-bound in dark cyan, apo in light cyan). **(B)** Liquid growth assay of the catalytic site mutants. Each data point is a representation of 3 biological replicates. **(C)** Comparison of interfacial helices (IF1-IF3) organization in the apo and caspofungin-bound state. IF1 is resolved in apo but disordered in the CFN-bound state. IF2 is partially resolved and IF3 is disordered in both states.

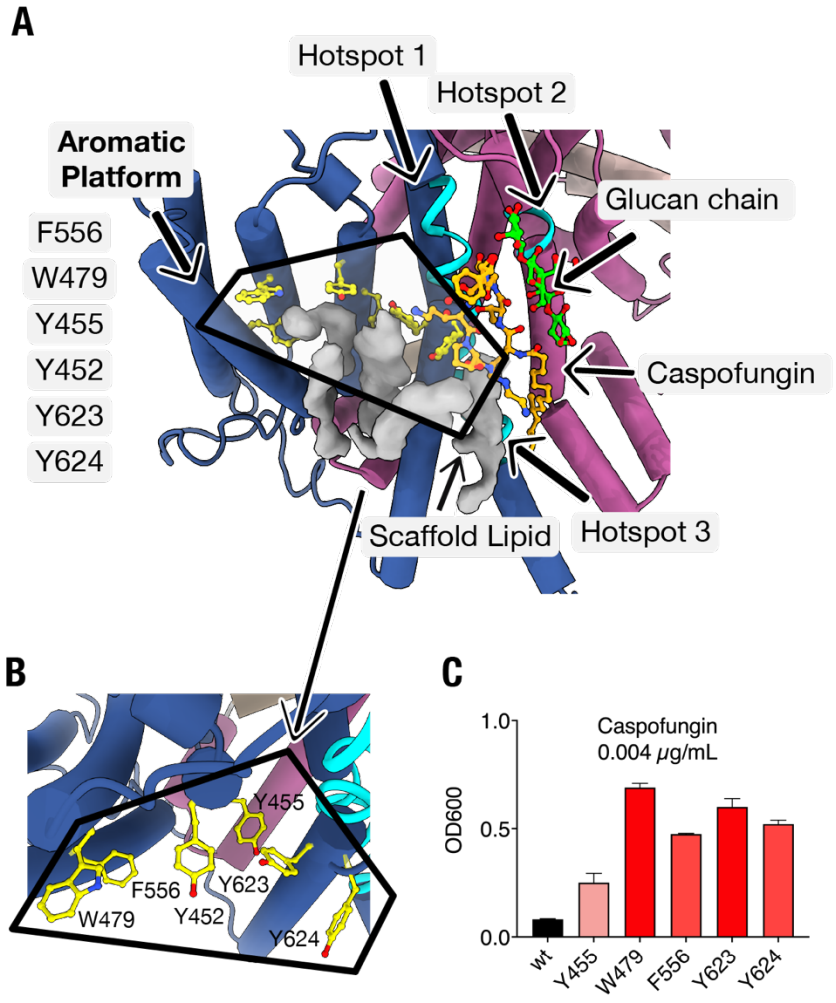


Fig. S7. Hotspot 1 adjacent aromatic lipid pocket. (A) Overview of the lipid densities (grey) relative to the glucan chain (lime), hotspot 1-3 (cyan), CFN (gold), and aromatic residues (yellow) (Y452, Y455, W479, F556, Y623, and Y624) surrounding the pocket like a platform. The lipid interacting with the caspofungin has been shown as a scaffold lipid **(B)** Close-up view of the aromatic platform residues. **(C)** Growth assay for aromatic platform alanine mutants in the presence of 0.004 μg/ml caspofungin showing a trend of reduction in caspofungin inhibition.

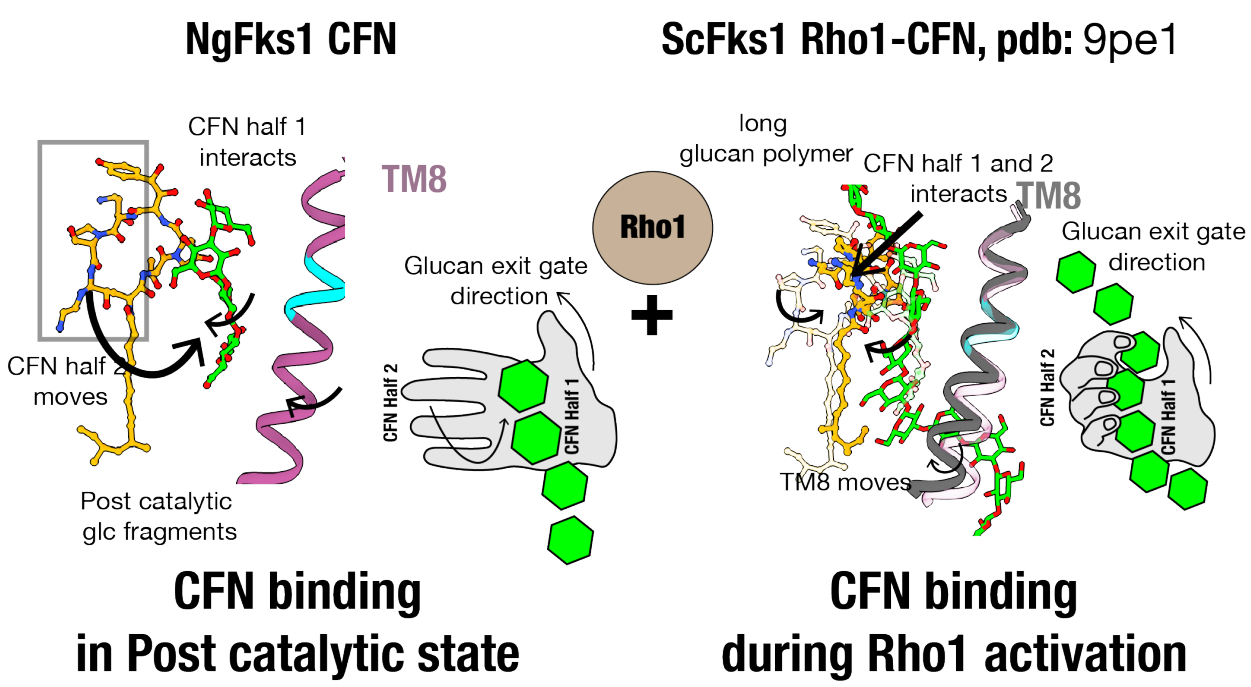


Fig. S8. Proposed model for caspofungin engagement and glucan-dependent stabilization. Comparison of our structure of the CFN-bound-post catalytic state of NgFks1 with the published structure of long glucan-CFN-bound ScFks1 in the rho1 activated state (pdb: 9pe1) (3). In our structure, CFN binds to Glc fragments on one side of the molecule (half1), while in the 9pe1 structure, both ends bind and capture the growing glucan chain. The cartoon representation by the side shows the mechanism, where the palm and figures represent half 1 and half 2 of the caspofungin molecule, respectively and the direction of the thumb represents the glucan exit gate. In this model, our structure is comparable to an open hand where the glucan fragment binds with the palm during the post-catalytic resting state. In the 9pe1 structure, the growing glucan chain is captured through a claw engaging both fingers and the palm.

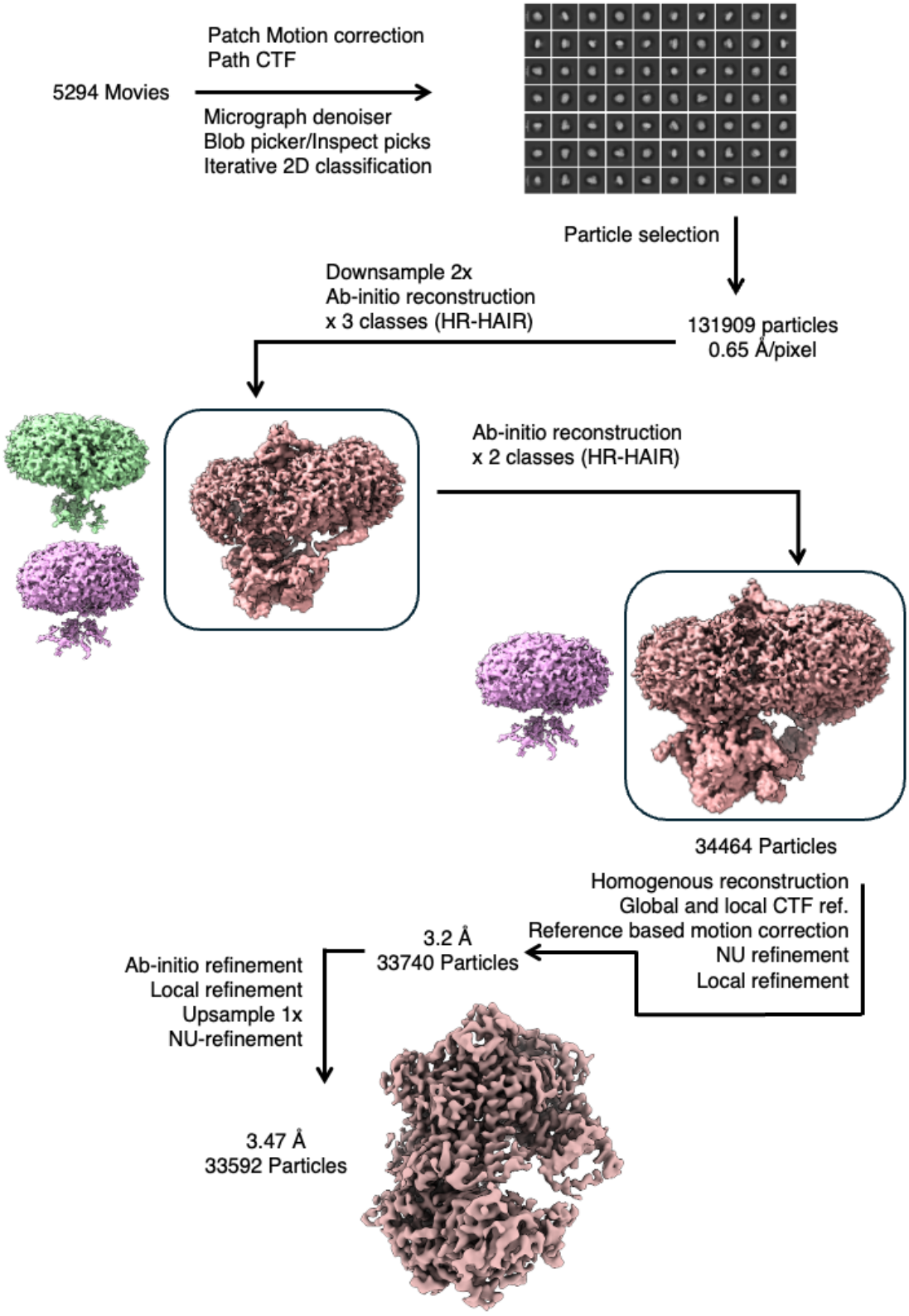


Fig. S9. CryoEM workflow for post-catalytic NgFks1 apo

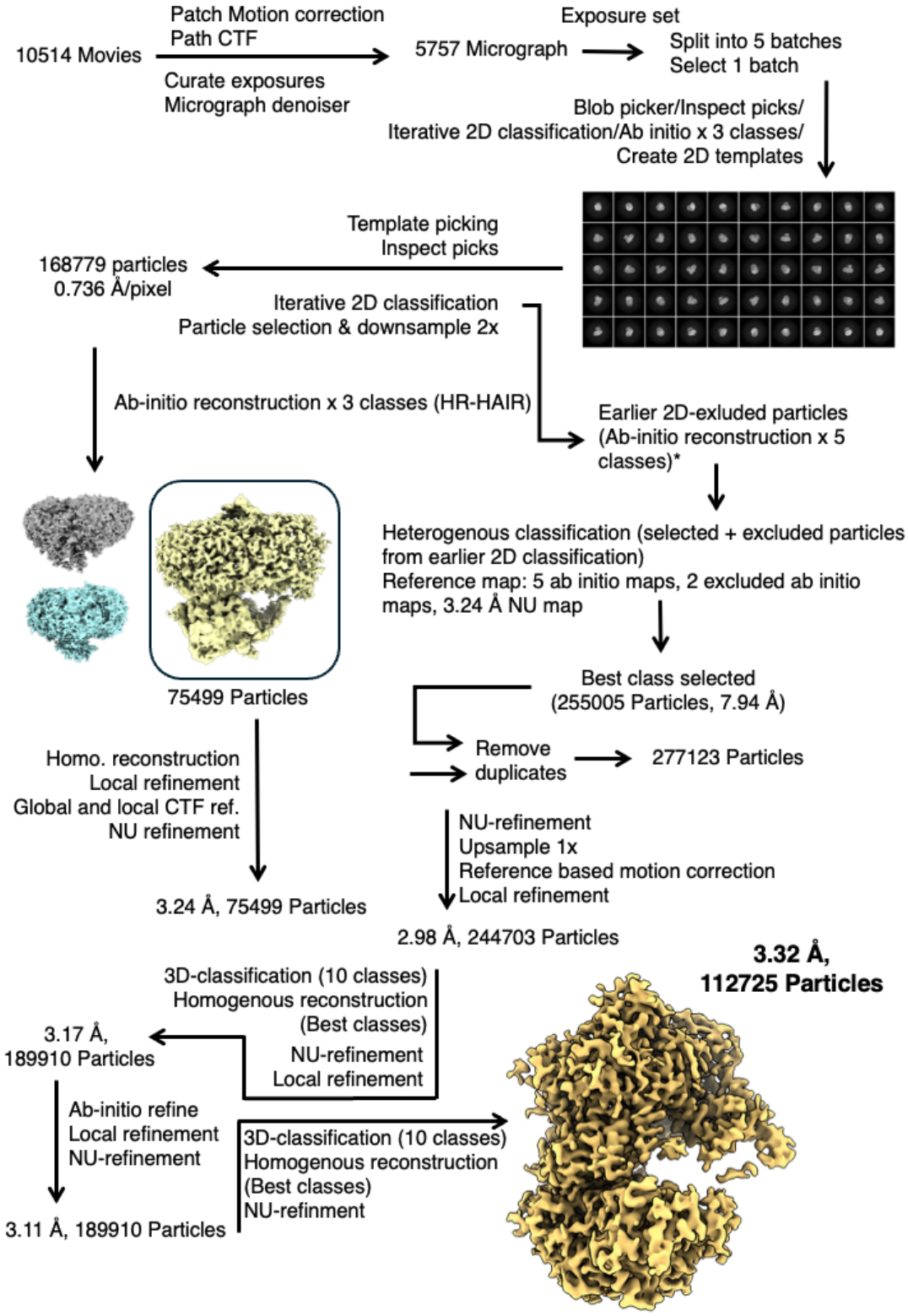


Fig. S10. Cryo-EM workflow for the post-catalytic NgFks1 CFN

Table S1. Cryo-EM data collection and refinement statistics for **Post-catalytic NgFks1 apo**	
Data Collection	
Microscope	TFS Krios
Detector	Gatan K3 BioContinuum (6k x 4k)
Image pixel size	0.65 Å
Defocus range	0.8-2.2 μm
Electron exposure	41 e-/Å^2
Number of micrographs	5294
Image Processing	
Number of particles used	33,592
Symmetry	C1
Final box size (pixels)	512
Reported resolution	3.47 Å
FSC threshold	0.143
Refinement	
Atoms	24,703 total; 12,315 non-H
Residues	1,487 total; 1,465 protein
Water	0
Supplied resolution	3.47 Å
B-factor (Å^2)	
Iso/Aniso (#)	12315/0
Protein (min/max/mean)	25.01/170.73/80.11
Length (Å)	0.005
Angles (°)	0.900
Validation	
MolProbity score	0.91
Clash score	0.41
Ramachandran plot (%)	
Outliners	0
Allowed	3.90
Favored	96.10
Map-model fit	
CC (mask)	0.79
CC (box)	0.60
CC (peaks)	0.49
CC (volume)	0.77
PDB ID	11KD (Held for publication)
EMDB ID	EMD-75764 (Held for publication)

Table S2. Cryo-EM data collection and refinement statistics for Post-catalytic NgFks1 CFN	
Data Collection	
Microscope	TFS Krios
Detector	TFS Falcon 4i (4k x 4k)
Image pixel size	0.734 Å
Defocus range	0.6-2.4 μm
Electron exposure	32 e-/Å^2
Number of micrographs	10514
Image Processing	
Number of particles used	112,725
Symmetry	C1
Final box size (pixels)	512
Reported resolution	3.32 Å
FSC threshold	0.143
Refinement	
Atoms	24,799 total; 12,309 non-H
Residues	1,474 total; 1,443 protein
Water	0
Supplied resolution	3.32 Å
B-factor (Å^2)	
Iso/Aniso (#)	12309/0
Protein (min/max/mean)	4.37/127.79/49.02
Length (Å)	0.007
Angles (°)	0.970
Validation	
MolProbity score	0.94
Clash score	0.73
Ramachandran plot (%)	
Outliners	0
Allowed	3.33
Favored	96.67
Map-model fit	
CC (mask)	0.79
CC (box)	0.64
CC (peaks)	0.56
CC (volume)	0.53
PDB ID	35UT (Held for publication)
EMDB ID	EMD-77198 (Held for publication)

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3. Z. Ren, *et al.*, Structural basis of fungal β-1,3-glucan synthase inhibition by caspofungin. *Nature* 1–9 (2026). https://doi.org/10.1038/s41586-026-10409-7.