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Supplementary information

Structural and mechanistic insights into fungal β-1,3-glucan synthase FKS1

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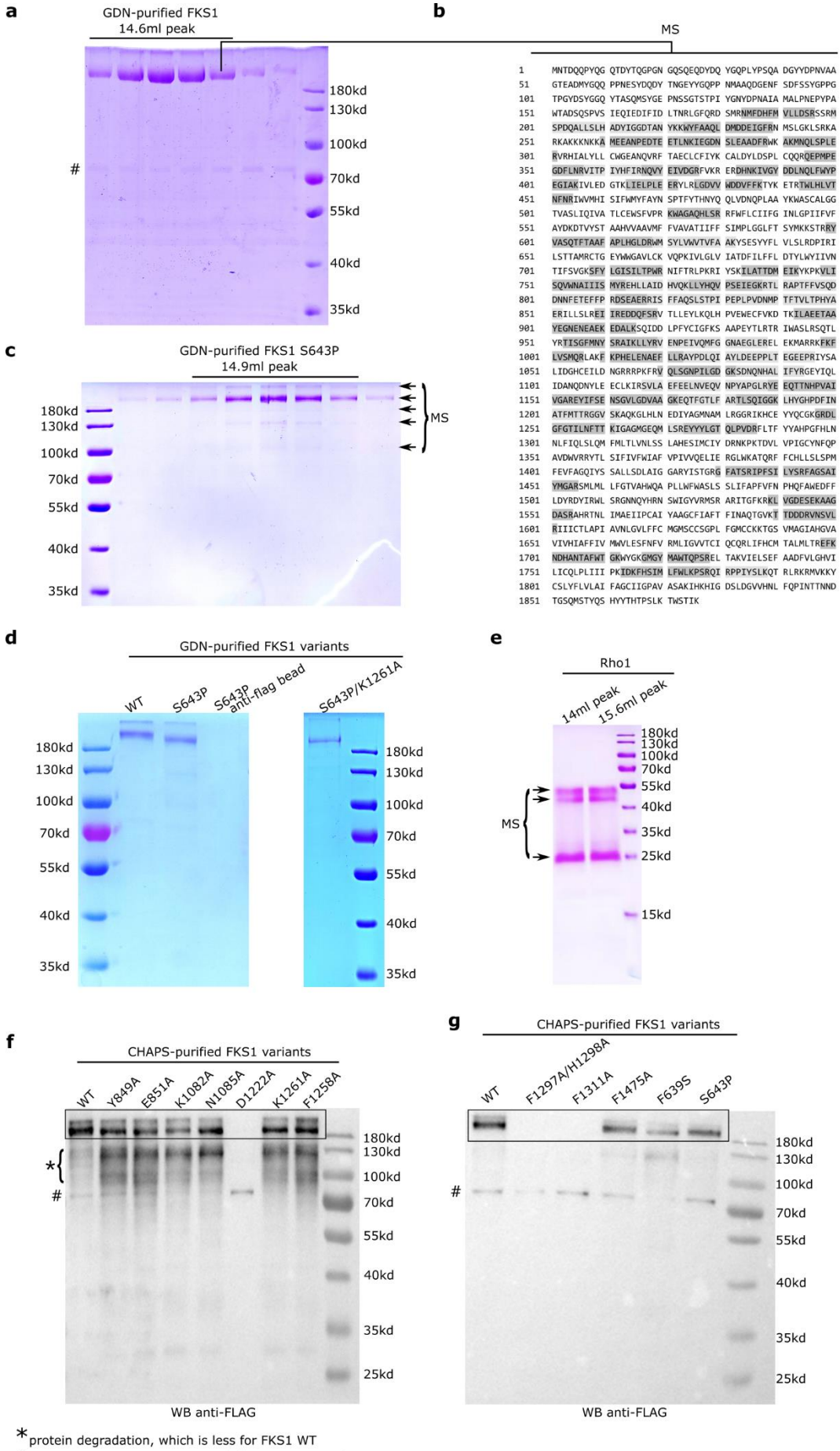
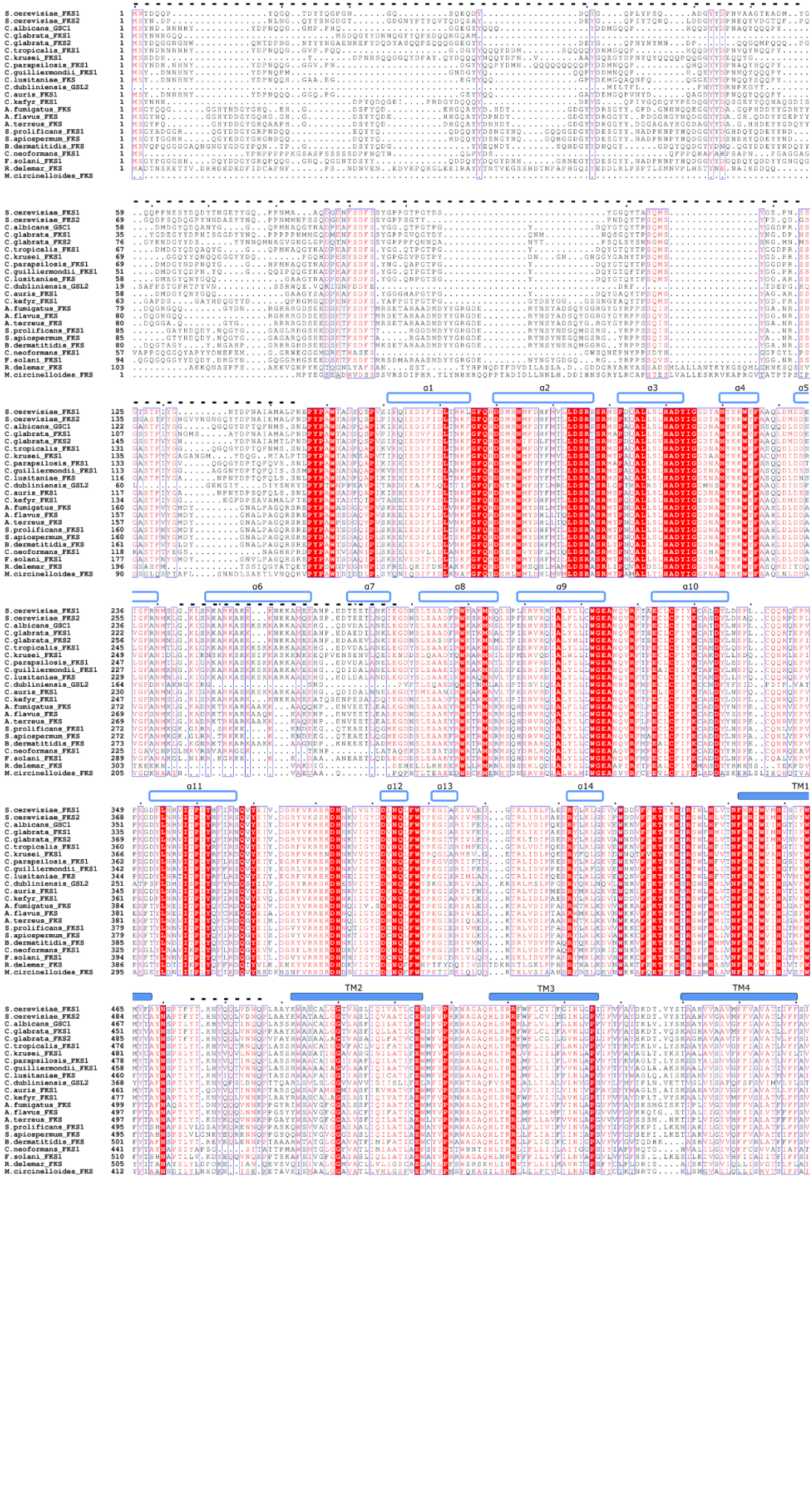
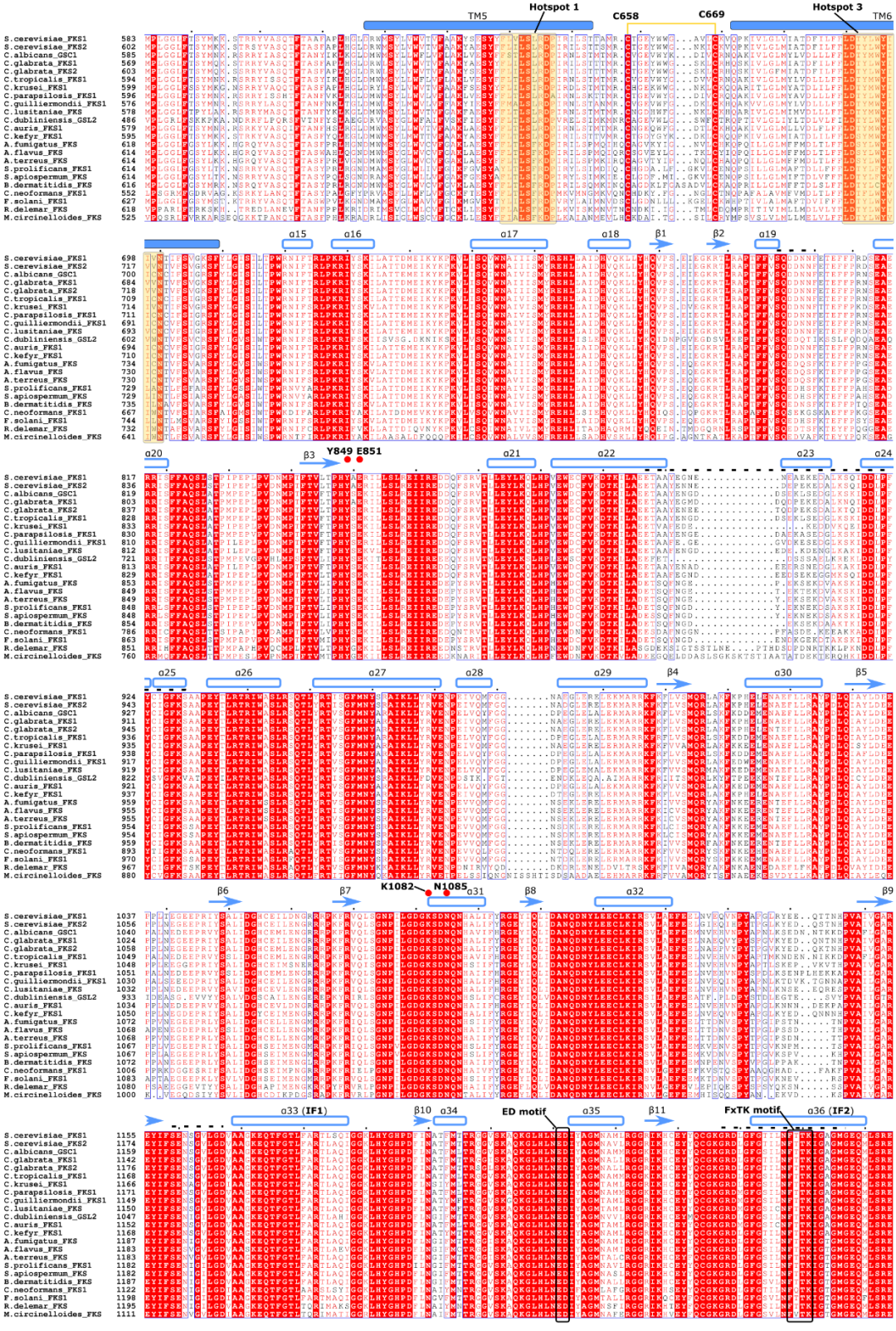


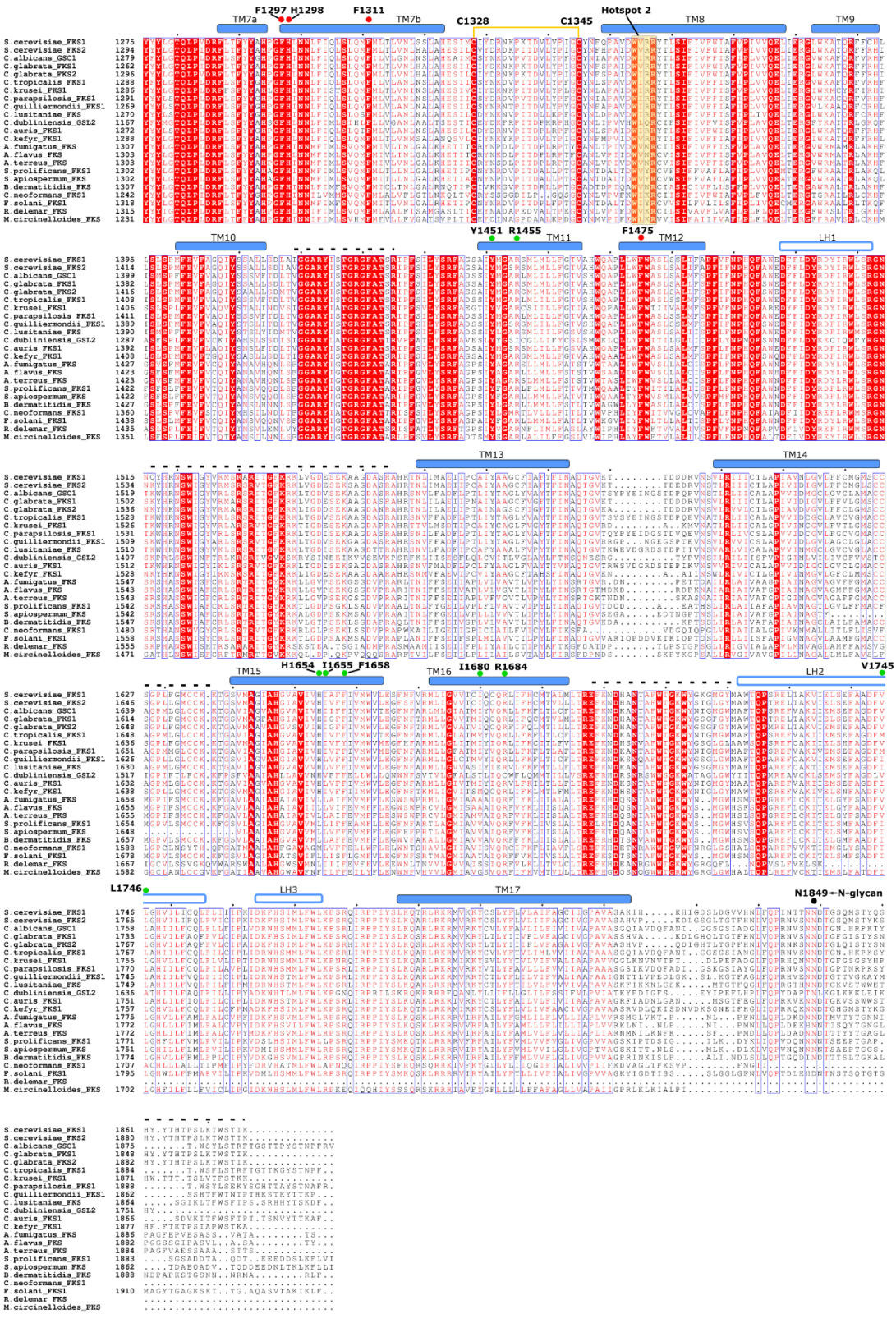
*protein degradation, which is less for FKS1 WT

#minor contaminating protein with flag-immunopurification

Supplementary Figure 1: Full scan of western-blot and SDS-PAGE gel. **a**, Full SDS-PAGE gel of Extended Data Fig. 1b. **b**, Mass spectrometry analysis identified the corresponding band in (**a**) as FKS1. The detected peptides of FKS1 are highlighted in grey. **c**, Full SDS-PAGE gel of Extended Data Fig. 1d. One major band and four minor bands (marked by arrows) are identified by Mass Spec as FKS1. **d**, Full SDS-PAGE gel of Extended Data Fig. 1h. **e**, Full SDS-PAGE gel of Extended Data Fig. 1f. The three bands (marked by arrows) were identified as Rho1 by Mass Spec analysis. **f-g**, Full scan of western-blot of Extended Data Fig. 1g. For (**f-g**), the bands marked by the star matches the proteolytic bands in (**c**).


α1
α2
α3
α4
α5
α6
α7
α8
α9
α10
α11
α12
α13
α14
TM1
TM2
TM3
TM4


TM5
Hotspot 1
C658
C669
Hotspot 3
TM6
α15
α16
α17
α18
β1
β2
α19
α20
β3
Y849
E851
α21
α22
α23
α24
α25
α26
α27
α28
α29
β4
α30
β5
β6
β7
K1082
N1085
α31
β8
α32
β9
α33 (IF1)
β10
α34
ED motif
α35
β11
FxTK motif
α36 (IF2)
S.cerevisiae_FKS1
S.cerevisiae_FKS2
C.albicans_GSC1
C.glabrata_FKS1
C.glabrata_FKS2
C.tropicalis_FKS1
C.krusei_FKS1
C.parapsilosis_FKS1
C.guilliermondii_FKS1
C.lusitaniae_FKS
C.dubliniensis_GSL2
C.auris_FKS1
C.kefyr_FKS1
A.fumigatus_FKS
A.flavus_FKS
A.terreus_FKS
S.prolificans_FKS1
S.apiospermum_FKS
B.dermatitidis_FKS
C.neoformans_FKS1
F.solani_FKS1
R.delemar_FKS
M.circinelloides_FKS



Supplementary Figure 2: Multiple sequence alignment of FKS orthologs from various pathogenic fungi and *Saccharomyces cerevisiae*.

Primary sequence alignment was generated using Clustal Omega. The secondary structural element, key motifs and residues are annotated and labeled above the alignment. The dashed lines marked the unstructured regions flexible in the cryoEM

map. Three hot spot regions of echinocandin-resistant mutations are shaded in orange. Protein sequence sources are FKS from *Saccharomyces cerevisiae* and multiple fungi pathogens, including: Candida (*C. albicans*; *C. glabrata*; *C. glabrata*; *C. tropicalis*; *C. krusei*; *C. parapsilosis*; *C. guilliermondii*; *C. lusitaniae*; *C. dubliniensis*; *C. auris*; *C. kefyr*); Aspergillus (*A. fumigatus*; *A. flavus*; *A. terreus*); *Scedosporium prolificans*; *Scedosporium apiospermum*; *Blastomyces dermatitidis*; *Cryptococcus neoformans*; *Fusarium solani; Rhizopus delemar*; *Mucor circinelloides*.