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**).

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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.