Supporting Information

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SI Materials and Methods

Reagents. Sodium fluoride, kanamycin, G418, sorbitol, glufosinate ammonium, yeast nitrogen base without amino acids, and yeast synthetic drop-out media supplement without histidine (or leucine) were purchased from Sigma-Aldrich. Bacto Yeast Extract, Bacto Peptone, Bacto Agar, and Difco yeast nitrogen base without amino acids were purchased from BD Biosciences. Glucose (dextrose) was purchased from J. T. Baker. Hygromycin B was purchased from EMD Millipore and Invitrogen. ^{18}F, in the form of $Na^{18}F$, was obtained from Cardinal Health.

Phylogenetic Tree for Eukaryotic *FEX* Genes. Eukaryotic FEX protein sequences were downloaded from UniProt (annotated there as CrcB homologs) (1). The program MUSCLE (2) was used to align the sequences and RAxML (3) was used to generate the tree file. A few sequences were excluded from the final alignment because they aligned poorly to the majority of sequences and/or were missing elements common to the rest of the collection (e.g., were not predicted to contain any transmembrane segments). Fewer than 10 sequences were excluded in the final alignment. Mega5 (4) was used to view and generate the figure of the tree.

Strains and Media. ***Neurospora crassa.*** The Fungal Genetics Stock Center was the source of *N. crassa* (FGSC#4200 and FGSC#9717) and the plasmid pCSN44 (5). The deletion of the *fex-1* gene (*NCU06262*) was accomplished following protocols developed by Hildur Colot (6). Briefly, a KO cassette was constructed that contains the upstream portion (about 1.5 kb) of the NCU0626 ORF, a hygromycin B resistance gene and the downstream fragment (about 1.5 kb) of the NCU06262 ORF. The 5′ flank fragment was amplified from the *N. crassa* (FGSC#9717) genome using primers Ncr-5F and Ncr-5R (Table S4) producing a 1,479-bp fragment before the start codon of *fex-1* (NCU06262). The 3′ flank fragment was then amplified using 3′F-ecoRV and 3′R-notI (Table S4). The hygromycin B KO (hph KO) cassette, including the TrpC promoter and terminator, was amplified from pCSN44 using hphF-pro and hphR-ter (Table S4). The 5′ flank fragment and the hph KO cassette were ligated by PCR and inserted into a topo-TA vector. This vector was shown to contain the insert in the arrangement of HindIII-5′ flank fragment-hph KO cassette-EcoRV. The plasmid was digested with EcoRV and NotI to insert the 3′ flank fragment. This plasmid now contained the entire *N. crassa fex-1* KO fragment that was then digested out of the plasmid using HindIII and NotI to use for electroporation into *N. crassa* strain FGSC#9717.

The macroconidia of the fungus were used for making the gene KO by homologous recombination following standard procedures as previously described (6). Briefly, the *N. crassa* mus KO strain FGSC#9717 (delta *mus-51*::*bar*+, *his-3* A) was cultured by incubating at 30 °C for 3 d and then 25 °C for 7–10 d. The conidia were harvested, and the spores were washed twice with water and once with 1 M sorbitol and resuspended in residual sorbitol. The macroconida were electroporated in the presence of the KO cassette described above. Successful transformants were selected on minimal media (28 °C) supplemented with hygromycin (300 μg/mL) and histidine. The presence of the hygromycin resistance gene was confirmed by PCR. This heterokaryotic KO mutant was crossed with the WT strain (*his*+ a) FGSC#4200. After the perithecia matured, we picked ascospores that grew in minimal media that contained hygromycin (200 μg/mL). We then tested for glufosinate ammonium (200 μg/mL) sensitivity to isolate transformants with WT mus-51. PCR was used to confirm that these transformants contained the hygromycin resistance gene, no *fex-1* ORF, and the whole KO cassette.

Rescue strains were constructed by integrating a WT *fex-1* copy (including original promoter and terminator) into the *his-3* locus in the deletion strain. The WT *NCU06262* fragment (3,164 bp) includes the promoter and terminator (the region between the *NCU06263* and the *NCU06261* genes) and was cloned from the *N. crassa* strain FGSC#9717 genome using primers EcoRI-ncuF and EcoRI-ncuR (Table S4). The fragment was inserted into the EcoRI site in plasmid pll07-2 (7), which contains a truncated *his-3* gene downstream of the insertion. The plasmid was electroporated into the *his-3 fex-1* KO strain, and transformants were selected on minimal media without histidine. PCR was used to confirm that the resulting rescue strain contained a WT copy of the *fex-1* gene.

Microconidia were prepared as previously described (8). Specifically, macrospore suspensions from rescue strains were inoculated to a test tube containing 2% (wt/vol) agar, 0.5% sucrose, 0.1 x synthetic crossing medium, and 1 mM iodoacetic acid (IAA). Cultures were grown at room temperature (25 °C) for 7–10 d. The microconidia were harvested with 2 mL water and were passed through 5-μm millex Durapore filter units. Diluted microspores were plated on Petri plates containing vogel's and sorbose agar.

Southern blotting was used to determine whether the rescue strain was homokaryotic or heterokaryotic. Briefly, 10 μg of genomic DNA from different strains of *N. crassa* including WT, KO, and rescue were digested with SacII and run on a 0.8% agarose gel. The DNA in the gel was depurinated, denatured, and transferred to a piece of positively charged nylon membrane. The probe was prepared by the random priming method and the 2.7-kb his-3 fragment was used as a template. The Southern blot indicates that the majority of rescue strains purified from microconidia are homokaryotic (Fig. S2). We confirmed that the strain used for growth rate measurements and IC_{50} determination is homokaryotic.

Saccharomyces cerevisiae. The *FEX* KO strains used in this study were made in the background of the BY4741 and BY4742 strains (obtained as a generous gift from M. Hochstrasser, Yale University, New Haven, CT) (9). To generate the two single deletion strains, the *FEX1* ORF (YOR390W) was replaced with the kanMX6 resistance cassette (10, 11) in BY4741 and the *FEX2* ORF (YPL279C) was replaced with the hphMX4 resistance cassette (12) in BY4742 using standard PCR-based deletion methods. Primers that contained 45 bp of homology to the region immediately upstream and downstream of the ORF were used to amplify each resistance cassette from the pFA6a-kanMX6 (kanMX6) and pAG32 (hphMX4) vectors (Table S3). Yeasts were transformed with these DNAs using the standard lithium acetate procedure (13). Deletion of an ORF was confirmed using PCR. Because *FEX1* and *FEX2* are nearly identical, both the upstream region of the deleted *FEX* gene as well as the coding region of the intact *FEX* gene were sequenced to determine which ORF had been deleted. The single *FEX1*Δ and *FEX2*Δ strains were mated to produce a diploid organism containing both deletions. This diploid strain was then allowed to sporulate and the resulting spores were subjected to tetrad analysis to generate the haploid *FEX1*Δ *FEX2*Δ double deletion strain.

To determine whether the double deletion strain could be rescued, the coding region, along with 700 bp upstream and 600 bp downstream, of *FEX1* was amplified from *FEX2*Δ genomic DNA. This sequence was ligated into pRS416 (obtained from the ATCC) between the NotI and XhoI restriction sites to create

pRS416-FEX1. The sequence in the vector was verified by sequencing. Yeast was transformed with both pRS416 and pRS416-FEX1 using the standard lithium acetate protocol and transformants were isolated on SC-ura media.

Unless otherwise noted, yeast was grown in YPD media that was not pH adjusted. We measured an average pH of YPD of 6.5. Selection for deletion strains was done using YPD media supplemented with 300 μg/mL G418 and/or hygromycin B. Selection for strains successfully transformed with vectors containing *URA3* was done using synthetic complete-ura media. For solid plate assays, NaF was added to YPD agar directly before the plates were poured.

Candida albicans. The *C. albicans* SN152 strain and plasmids pSN40, pSN52, and pSN69) were obtained as a gift from S. M. Noble (University of California, San Francisco). This strain of *C. albicans* contains one copy of a *FEX* gene (CaO19.7095) but because it is a diploid organism, both heterozygous KO and homozygous KO strains were made by deleting one or both alleles. These KO strains were made following protocols developed by Suzanne Noble (14). Briefly, fusion PCR was performed to create disruption fragments of both alleles of the *FEX1* gene. The two alleles of the *FEX1* gene were knocked out with two different nutrition markers. For each allele, a KO cassette containing either the *Candida maltosa LEU2* gene or the *Candida dubliniensis HIS1* gene and a flanking sequence of 500 bp upstream and downstream of the *C. albicans FEX1* gene (Cao19.7095) was constructed by amplifying partial sequences using PCR. These fragments were then ligated together to form the complete KO cassette. Two sequential rounds of electroporation were performed to produce first the heterozygous and then the homozygous KO. Electroporation was performed following the manufacturer's protocol for GenePulser (Bio-Rad). Specifically, a single colony of SN152 was cultured in 50 mL YPD in a 150 mL flask, incubating at 30 °C, and shaking at 200 rpm overnight (12 h) (VWR standard orbital shaker, VWR Signature Model DS-500; VWR International, West Chester, PA). The cells were chilled on ice and precipitated at 800 × *g* for 5 min. The pellet was washed twice with 30 mL ice cold water and once with 20 mL ice cold 1 M sorbitol. The washed cells were resuspended in 100 μL 1 M sorbitol. Sixty microliters of the cells and 5 μg of the KO cassette DNA were used for electroporation (1,500 V, 200 Ω, and 25 μF) in a 2-mm cuvette. Following transformation, the cells were immediately resuspended in 1 mL ice cold 1 M sorbitol, and 200 μL was plated on leu-dropout agar and incubated at 30 °C. PCR was used to confirm the insertion of the *C. maltosa LEU2* gene. This strain is referred to as the heterozygous KO strain. A subsequent round of electroporation to replace the second allele of the *FEX1* gene was accomplished using the *HIS1* gene KO cassette. For this step, all cultures were in leu-dropout and his-dropout media, and the 1 M sorbitol and water were all adjusted to pH 7.0 to prevent fluoride from entering and killing the cells when both alleles of *FEX1* were deleted. PCR was used to confirm the double allele KO.

The rescue strain was constructed by integrating a third nutrition marker and the WT copy of *FEX1* into the RPS10 locus of the homozygous KO *C. albicans* strain. This rescue method was similar to that used in a previous study (15). The rescue fragment contains 1-kb regions just upstream and downstream of the RPS10 gene, the *C. albicans FEX1* gene (including 1.2 kb upstream and 1.2 kb downstream of the ORF), and the *C. dubliniensis* ARG4. The ARG4 fragment was amplified from plasmid pSN69 (14), and the other three fragments were amplified from *C. albicans* genomic DNA. All four fragments were inserted one by one into the Topo-TA vector between the BamHI and ApaI sites. This complete rescue fragment was electroporated into the homozygous KO strain using the procedure described above. The transformants growing on arginine drop-out media were picked and tested by PCR to confirm the integration of WT *FEX1* into the genome.

Growth Assays. ***N. crassa.*** Liquid cultures of *N. crassa* were grown in test tubes as previously described (16). Briefly, *N. crassa* was recovered and one loop of spores was resuspended in 2 mL minimal medium (about 2.0 OD_{600}). Twenty microliters of this suspension was diluted into 2 mL minimal medium in an autoclaved 16 × 150-mm glass tube with cap. The fungus was allowed to grow for about 4 d at 25 °C.

Solid agar media cultures were grown in race tubes according to previously published methods (17) with small modifications. Specifically, 50-mL sterile disposable plastic pipettes were filled with minimal agar medium [Vogel's, 2% (wt/vol) glucose, and 1.5% (wt/vol) agar]. The media were supplemented with filter-sterilized sodium fluoride. The race tubes were capped with sterile test-tube caps and secured with micropore tape. Tubes were inoculated with 10 μL of spore suspension (OD 1.0) from the top of the tube with long, thin gel-loading tips (remove the filter with forceps before inoculation and reinsert the filter after inoculation). The tubes were kept at room temperature, and the growth front was marked every day for 4 d. Growth rates were measured for all strains. Growth curves were graphed, and IC_{50} values were calculated with GraphPad Prism using a nonlinear regression model with a sigmoidal dose–response (variable slope).

S. cerevisiae. Strains were grown overnight in YPD (SC-ura if they were transformed with a plasmid). For solid plate assays, these cultures were diluted to an OD_{600} of 0.2. Serial sixfold dilutions were made in sterile water. Cells were spotted onto YPD or YPD + fluoride plates and incubated at 30 °C. For liquid growth assays, cultures were diluted to a final OD_{600} of 0.1 in a Costar 24-well flat bottom plate in YPD and NaF. Each well contained a total volume of 1 mL. The assays were performed in a Biotek Synergy four-plate reader at 30 °C with continual shaking. OD_{600} measurements were recorded every 3 min. Curves were plotted and analyzed using GraphPad Prism. To determine IC_{50} values, the area under the curve was calculated for each growth curve (18). These values were normalized relative to the growth curve for each strain in YPD containing no fluoride. The normalized values were plotted vs. the log of the fluoride concentration and fit to a standard dose–response curve with GraphPad Prism. IC_{50} values were also calculated by plotting the OD_{600} at a single time point as a function of fluoride concentration. The time point was chosen to be in the linear region of the growth curve. Both of these methods gave very similar IC_{50} values. The values reported in Table S1 are from the area under the curve analysis.

C. albicans. Strains were grown overnight in YPD medium at 30 °C with shaking (200 rpm). For growth assays, each culture was diluted to an OD_{600} of 0.25, and 8 μL of this suspension was diluted in 400 μL of media in each well of a Honeycomb plate (Oy Growth Curves Ab Ltd.). NaF was supplemented if necessary. The assay was performed for 36 h at 30 °C, measuring OD_{600} every 15 min without shaking in a bioscreen C MBR instrument.

1. UniProt Consortium (2012) Reorganizing the protein space at the Universal Protein Resource (UniProt). *Nucleic Acids Res* 40(Database issue):D71–D75.
2. Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32(5):1792–1797.
3. Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Syst Biol* 57(5):758–771.
4. Tamura K, et al. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28(10):2731–2739.
5. McCluskey K, Wiest A, Plamann M (2010) The Fungal Genetics Stock Center: A repository for 50 years of fungal genetics research. *J Biosci* 35(1):119–126.
6. Colot HV, et al. (2006) A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. *Proc Natl Acad Sci USA* 103(27): 10352–10357.
7. Cheah MT, Wachter A, Sudarsan N, Breaker RR (2007) Control of alternative RNA splicing and gene expression by eukaryotic riboswitches. *Nature* 447(7143):497–500.
8. Ebbole D, Sachs MS (1990) A rapid and simple method for isolation of *Neurospora crassa* homokaryons using microconidia. *Fungal Genet Newsl* 37:17–18.

9. Brachmann CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14(2):115–132.
10. Wach A, Brachat A, Pöhlmann R, Philippsen P (1994) New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast* 10(13): 1793–1808.
11. Wach A, Brachat A, Alberti-Segui C, Rebischung C, Philippsen P (1997) Heterologous HIS3 marker and GFP reporter modules for PCR-targeting in *Saccharomyces cerevisiae*. *Yeast* 13(11):1065–1075.
12. Goldstein AL, McCusker JH (1999) Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast* 15(14):1541–1553.
13. Gietz RD, Schiestl RH, Willems AR, Woods RA (1995) Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. *Yeast* 11(4):355–360.
14. Noble SM, Johnson AD (2005) Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen *Candida albicans*. *Eukaryot Cell* 4(2):298–309.
15. Brand A, MacCallum DM, Brown AJP, Gow NAR, Odds FC (2004) Ectopic expression of URA3 can influence the virulence phenotypes and proteome of *Candida albicans* but can be overcome by targeted reintegration of URA3 at the RPS10 locus. *Eukaryot Cell* 3(4):900–909.
16. Li S, Breaker RR (2012) Fluoride enhances the activity of fungicides that destabilize cell membranes. *Bioorg Med Chem Lett* 22(9):3317–3322.
17. White B, Woodward D (1995) A simple method for making disposable race tubes. *Fungal Genet Newsl* 42:79.
18. North M, et al. (2011) Genome-wide functional profiling reveals genes required for tolerance to benzene metabolites in yeast. *PLoS ONE* 6(8):e24205.
19. Bähler J, et al. (1998) Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast* 14(10):943–951.

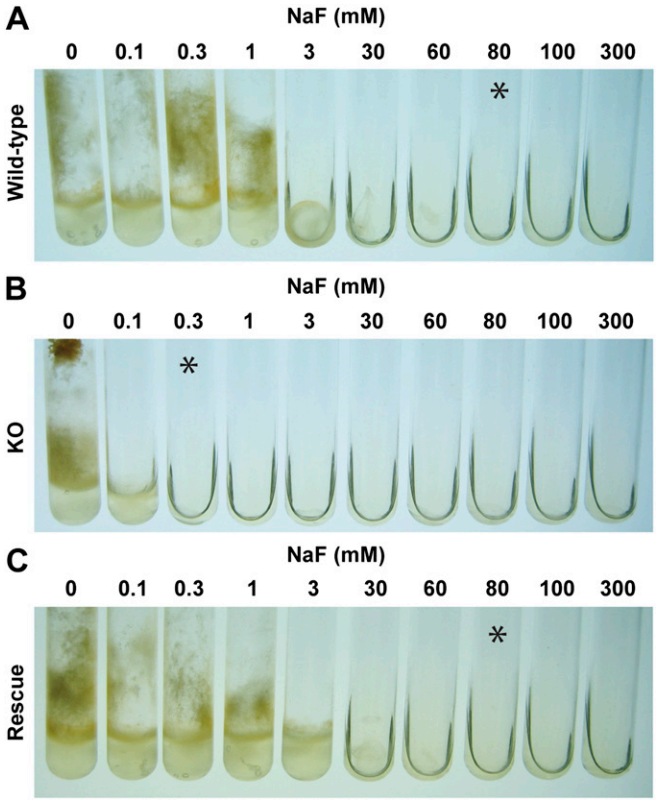

Fig. S1. Sensitivity of the *N. crassa fex-1* KO strain to NaF by liquid culture. The asterisks denote tubes with little or no cells visible. (*A*) NaF concentration required to inhibit mycelium growth in liquid culture of WT cells, (*B*) *fex-1* KO cells, and (*C*) rescue cells obtained by complementing *fex-1* KO with the WT *fex-1* gene.

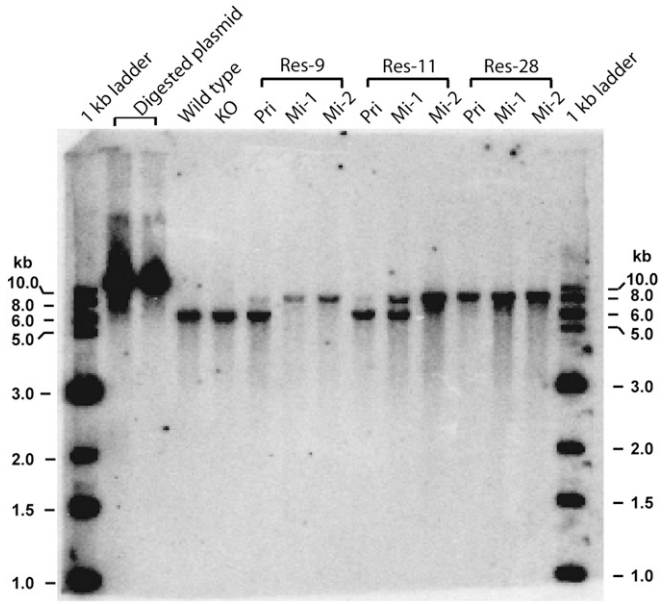

Fig. S2. Southern blot of *N. crassa* rescue strains. Genomic DNAs and the plasmid used for rescue were digested with SacII, separated on a 0.8% agarose gel, and visualized with a labeled DNA probe targeted to the *his3* gene. Res-9, Res-11, and Res-28 are three primary rescue strains from which microconidia strains Mi-1 and Mi-2 are isolated. WT and KO DNAs produced a band around 6 kb. Six rescue strains produced bands around 6 and/or 8 kb. Rescue strains that produce a single band are homokaryotic.

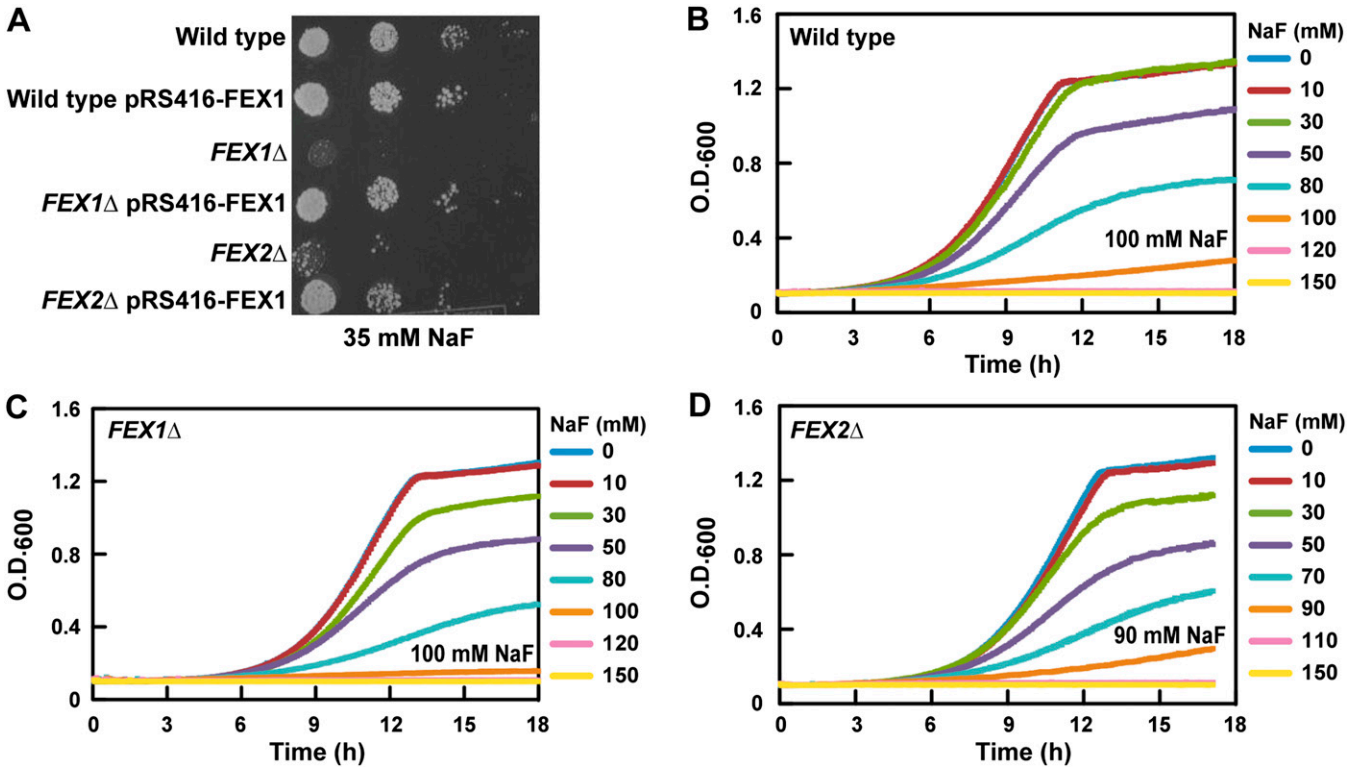

Fig. S3. Fluoride sensitivity of single *FEX* deletion mutants of *S. cerevisiae*. (*A*) Serial dilution assay. Media is YPD + 35 mM NaF. Image is after 6 d of growth at 30 °C. (*B*) Growth assay of WT *S. cerevisiae* in liquid YPD with added NaF. Growth was at 30 °C. (*C*) Growth assay of *FEX1*Δ *S. cerevisiae* in liquid YPD with added NaF. Growth was at 30 °C. (*D*) Growth assay of *FEX2*Δ *S. cerevisiae* in liquid YPD with added NaF. Growth was at 30 °C.

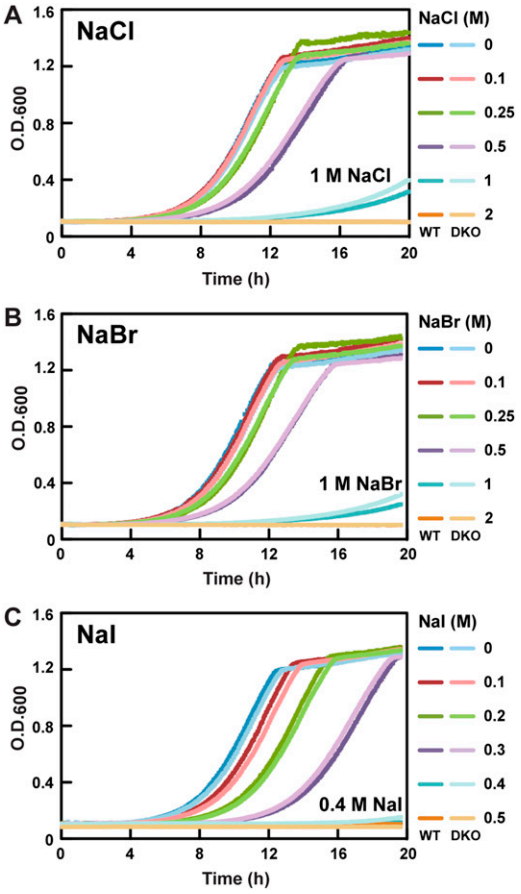

Fig. S4. Growth of WT and double KO *S. cerevisiae* with halide salts in liquid media. Growth of the WT is shown in dark colors; growth of the double KO (DKO) is shown in light colors. All assays were at 30 °C. (*A*) Growth in NaCl. (*B*) Growth in NaBr. (*C*) Growth in NaI.

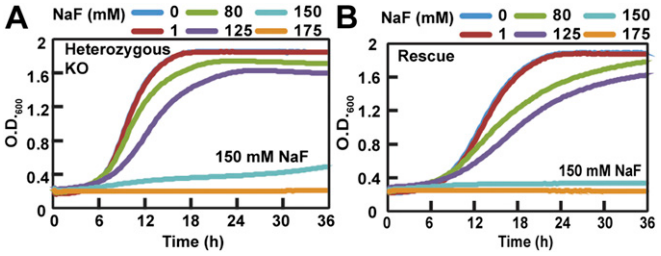

Fig. S5. Sensitivity of *C. albicans* strains to NaF. Growth curves of (*A*) heterozygous KO and (*B*) rescue strains grown in different concentrations of NaF.

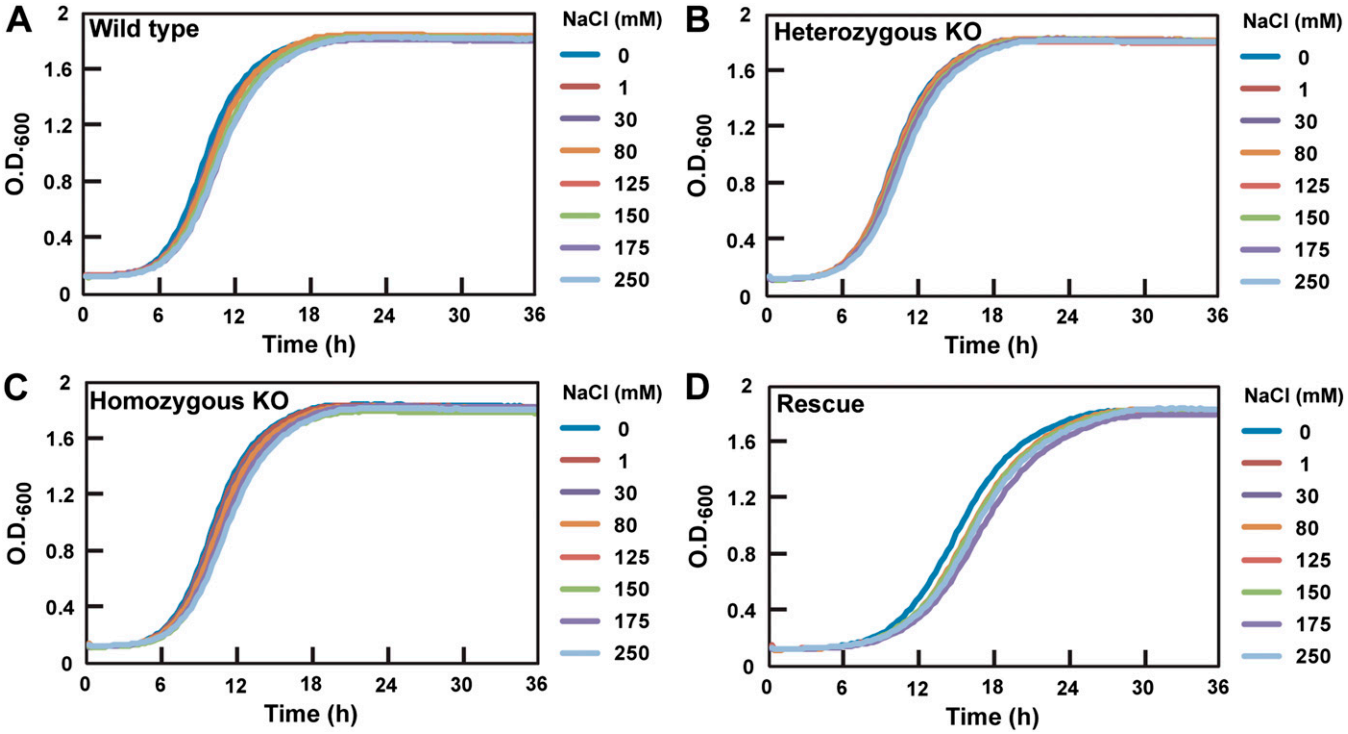

Fig. S6. Growth of *C. albicans* with NaCl in liquid media. (*A*) WT strain. (*B*) Heterozygous KO strain. (*C*) Homozygous KO strain. (*D*) Rescue strain.

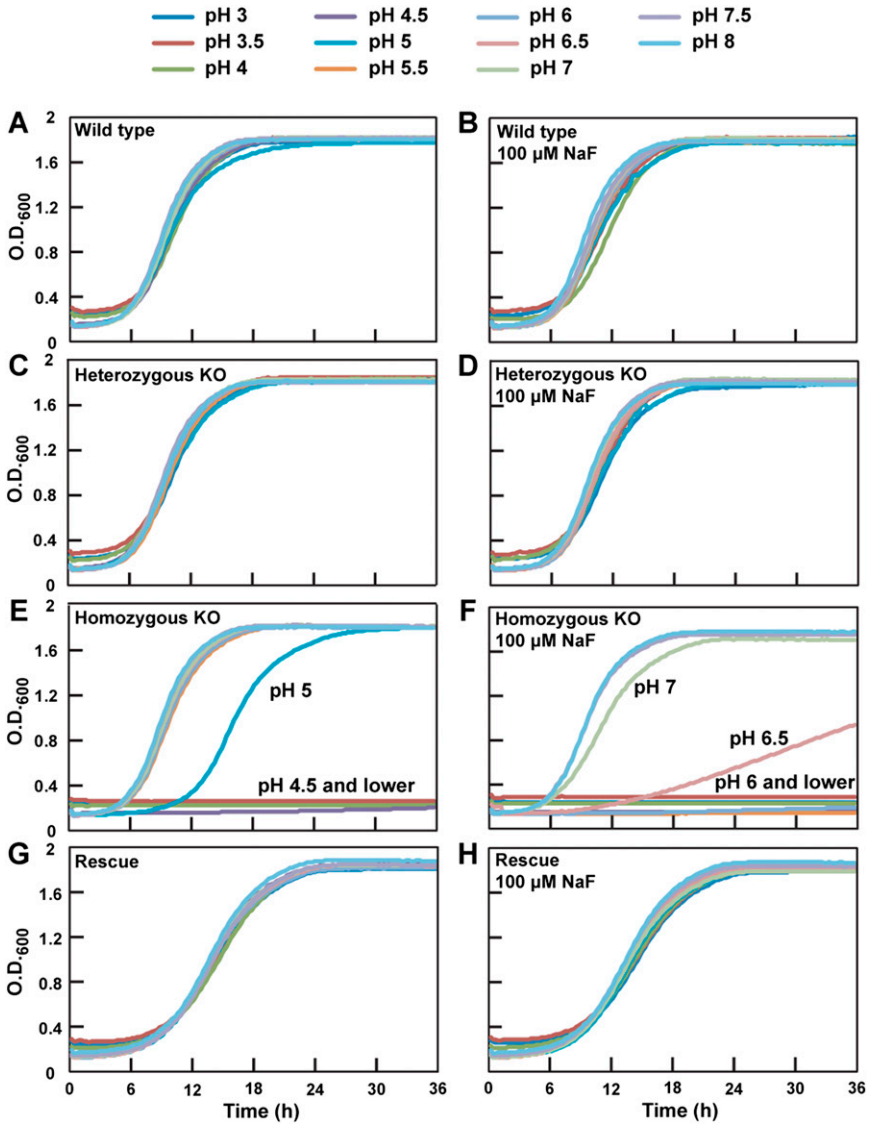

Fig. S7. Effect of pH on the growth of *C. albicans*. (*A*) WT strain. (*B*) WT with 100 μM NaF. (*C*) Heterozygous KO strain. (*D*) Heterozygous KO strain with 100 μM NaF. (*E*) Homozygous KO strain. (*F*) Homozygous KO strain with 100 μM NaF. (*G*) Rescue strain. (*H*) Rescue strain with 100 μM NaF.

Table S1. MIC and IC_{50} values for WT and *FEX* deletion strains

Organism	*S. cerevisiae*					*C. albicans*				*N. crassa*					
										WT		KO		Rescue	
Strain	WT	*FEX1*Δ	*FEX2*Δ	Double KO	Rescue	WT	Heterozygous KO	Homozygous KO	Rescue	Liquid	Solid	Liquid	Solid	Liquid	Solid
MIC (mM)	120	110	110	0.120	90	175	150	0.5	150	80	ND	0.3	ND	80	ND
IC_{50} (mM)	70 ± 1	59 ± 2	54 ± 5	0.060 ± 0.005	56 ± 7	142 ± 8	130 ± 7	0.098 ± 0.027	137 ± 11	ND	26.3 ± 0.4	ND	0.15 ± 0.01	ND	21.7 ± 0.2
Growth rates ($mm \cdot h^{-1}$)										ND	3.4 ± 0.2	ND	3.3 ± 0.2	ND	3.1 ± 0.1

IC_{50} represents the concentration of fluoride that inhibits cell growth by 50%. IC_{50} values are the average of at least three independent measurements ± SD. MIC, minimal inhibitory concentration of fluoride; ND, not determined.

Table S2. Strains used in this study

Name	Genotype	Reference
S. cerevisiae		
BY4741	*MATa his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0*	(1)
BY4742	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	(1)
SSY1	*MATa his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 FEX1Δ*	This study
SSY2	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 FEX2Δ*	This study
SSY3	*MATa his3Δ1 leu2Δ0 ura3Δ0 FEX1Δ FEX2Δ*	This study
SSY4	*MATα his3Δ1 leu2Δ0 ura3Δ0 FEX1Δ FEX2Δ*	This study
SSY5	*[FEX1] MATa his3Δ1 leu2Δ0 ura3Δ0 FEX1Δ FEX2Δ*	This study
C. albicans		
SN152	Arg^{-} Leu^{-} His^{-}	(2)
Single KO	*FEX1* first allele deletion (Arg^{-} His^{-})	This study
Double KO	*FEX1* both alleles deletion (Arg^{-})	This study
Rescue	*FEX1* both alleles deletion/FEX1	This study
N. crassa		
FGSC4200	Wild-type (*ORS-SL6a*)	From FGSC
FGSC9717	delta *mus-51*::*bar*+; *his-3 mat A*	
fex-1 KO	*his-3 fex-1Δ*	This study
Rescue	*fex-1Δ*+fex-1	This study

1. Brachmann CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14(2):115–132.
2. Noble SM, Johnson AD (2005) Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen *Candida albicans*. *Eukaryot Cell* 4(2):298–309.

Table S3. Plasmids used in this study

Name	Description	Reference
For *C. albicans*		
pSN40	To amplify *C. maltosa LEU2*	(1)
pSN52	To amplify *C. dubliniensis HIS* gene	
pSN69	To amplify *C. dubliniensis ARG4* gene	
For *N.crassa*		
pCSN44	To amplify hygromycin resistance gene	(2)
For *S.cerevisiae*		
pFA6a-kanMX6	To amplify the kanMX6 cassette	(3)
pAG32	To amplify the hphMX4 cassette	(4)
pRS416	Empty shuttle vector	(5)
pRS416-FEX1	pRS416 with the *FEX1* ORF inserted between the NotI and XhoI restriction sites	This study

1. Noble SM, Johnson AD (2005) Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen *Candida albicans*. *Eukaryot Cell* 4(2):298–309.
2. Colot HV, et al. (2006) A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. *Proc Natl Acad Sci USA* 103(27):10352–10357.
3. Bähler J, et al. (1998) Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*. *Yeast* 14(10):943–951.
4. Goldstein AL, McCusker JH (1999) Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast* 15(14):1541–1553.
5. Brachmann CB, et al. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14(2):115–132.

Table S4. Sequences of DNA primers used in this study

Name	DNA sequence (5′-3′)	Purpose
For *C. albicans* KO		
5′flankF	CTGTGGACGTTCTGATAGTATGTC	For amplifying 5′ flank of *C. albicans FEX1* KO (overlapping histidine selection marker)
5′flankR-his	GTATGCTTGCCATGGTATCAAACGTATATTGGACT AAAGAATGATAGTAT	
CdhisF	GTTTGATACCATGGCAAGCATAC	For amplifying histidine selection marker
CdhisR	CTAGAAAACCGTACCAGGTGAAC	
3′flankF-his	GTTCACCTGGTACGGTTTTCTAGAAATATGTGC TTCGACATACCCAG	For amplifying 3′ flank of *C. albicans FEX1* KO (overlapping histidine selection marker)
3′flankR	GGATTATAGTAATTCCAAGTGTGCAG	
5′flankR-leu	CCACCAGTGATGATTGGATCCGTATATTGGACT AAAGAATGATAGTAT	For amplifying 5′ flank of *C. albicans FEX1* KO (overlapping leusine selection marker)
CmleuF	GGATCCAATCATCACTGGTGG	For amplifying leucine selection marker
CmleuR	ACCTACCCATGTCTAGAAAG	
3′flankF-leu	CTTTCTAGACATGGGTAGGTAAATATG TGCTTCGACATACCCAG	For amplifying 3′ flank of *C. albicans FEX1* KO (overlapping leucine selection marker)
CrcupcheckF	CATTCCTTGTCGCTGTAGAAGTC	For confirming gene replacement
CrcdownchechR	GGATGGGCAGCAAGTTTCTC	
For *C. albicans FEX1* KO rescue		
RPS10-5F-BamHI	GATCGGATCCCCACGTTAACAATTTCATCAAG	For amplifying 5′ flank of RPS10 for rescue
RPS10-5R-notI	TAATGCGGCCGCGCCATGTTGTACTTGAGTTGGTG	
Cal-crcBres-F-notI	TAATGCGGCCGCCTGCCACTTTATCAACTTCCACATG	For amplifying *C. albicans FEX1*
Cal-crcBres-R-xhoI	TAATCTCGAGGTTGGCTCATAACATAAGGCTGG	
CdArgF-xhoI	TAATCTCGAGAAGAACATTTCTGTACCGCAAATG	For amplifying arginine selection marker
CdArgR-xbaI	CATGTCTAGAACAAAAGCTATTTGCATCGTTTG	
RPS10-3F-xbaI	CATGTCTAGAGGATAATGATAACTGAAGAGAAG	For amplifying 3′ flank of RPS10 for rescue
RPS10-3R-apaI	TAATGGGCCCTCGACTATTACTCATTGATAAAGAC	
Calcrc-res-check1F	GCGTATTATGTCTAGTTATGTAATAAATAGC	Rescue strains checking primers
Calcrc-res-check2R	GGTATGCAACTACCACAGACG	
Calcrc-res-check3F	CGAAGGTCACACTGACTTATGTC	
Calcrc-res-check4R	GTTCTTCTTTTGTTCCTCAGACTATG	
For *S. cerevisiae* KO		
Kan-F	GAATCTGCAGTTATTTAATTATTTAATCGAGCGTGT AATGCTCTG-CGGATCCCCGGGTTAATTAA	For amplifying the KanMX6 cassette
Kan-R	AAAAAAGTGGGATGATTATGCAGGG AAAAGTATAAAGAAAAGATC-GAA TTCGAGCTCGTTTAAAC	
Hph-F	GAATCTGCAGTTATTTAATTATTTAA TCGAGCGTGTAATGCTCTG-GCATA GGCCACTAGTGGATCTG	For amplifying the HphMX4 cassette
Hph-R	AAAAAGGTGGGATGATTGTGCAGGAAAAGGTA TGAAGAAAAGATC-CAGCTGAAGCTTCGTACGC	
For *S. cerevisiae* rescue		
416-F	TCCGTAATCCT-GCGGCCGC-TATATACA TATCAGATATCTGTTGAG	For amplifying the YOR390W (*FEX1*) ORF for insertion into pRS416
416-R	ATCGTTTACGATCTCGAGGAAATAAATA TGAATTATATTAAATGAAGTAAAAG	
For *N. crassa* KO		
Ncr-5F	GTAACGCCAGGGTTTTCCCAGTCACGACG CAGGAGTCGGTAGCAGGAAG	For amplifying 5′ flank of *N. crassa fex-1* KO
Ncr-5R	GCTCCTTCAATATCATCTTCTGTCTCCGACC ACTAATCCTAACAATACCGATATGG	
HphF-pro	GTCGGAGACAGAAGATGATATTGAAGGAGC	For amplifying hygromycin resistance gene including TrpC promoter and terminator
HphR-ter	GTTGGAGATCCTCTAGAAAGAAGGATTAC	
Ncr-3F-ecoRV	ATCGGATATCGTGGCAGGTGCAATGGTAG	For amplifying 3 flank of *N. crassa fex-1* KO
Ncr-3R-notI	GACTGCGGCCGCCTCTCACAGTATCTCGACTC	
For *N. crassa* rescue		
EcoRI-ncuF	GATCGAATTCGATGAGCAGTCTTGTGTTGG	To amplify *N. crassa fex-1* gene for rescuing
EcoRI-ncuR	CATGGAATTCGAAAGGCTTGAAAAGAGAGAGAG	