SUPPLEMENTARY INFORMATION

Modulation of Histone H3 Lysine 56 Acetylation as an Antifungal Therapeutic Strategy

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Supplementary Methods

Strains and culture conditions

The *C. albicans* strains used in this study are listed in **Supplementary Table 2** and **Supplementary Fig. 4a**. *C. albicans* cells were grown routinely at 30 ^{o}C in YPD (1% yeast extract, 2% Bacto Peptone and 2% glucose) or SD medium (0.67% Difco nitrogen base, 2% glucose and a mixture of amino acids), including 2% agar for solid media. We used spider medium to induce hyphae formation (1% Difco nutrient broth, 2% glucose and 0.2% K_2HPO_4). For *Tet* promoter repression, an overnight YPD culture was diluted into fresh YPD medium containing 20-50 μg ml^{-1} of doxycycline (Sigma) at an optical density at 600 nm (OD_{600}) of 0.005 and grown for 24 h unless otherwise indicated[1]. *Escherichia coli* DH10B cells were used for DNA cloning procedures. *E. coli* cells were grown in Luria-Bertani (LB) medium to which chloramphenicol (34 μg ml^{-1}) was added when required. The *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* strains (**Fig. 5b** and **Supplementary Fig. 4a**) were grown as described above for *C. albicans*. The *Aspergillus* strains (**Fig. 5c** and **Supplementary Fig. 4a**) were grown on complete medium (CM) plates (0.1% yeast extract, 0.2% Bacto Peptone, 1% glucose, 0.15% casamino acids, 0.6% $NaNO_3$, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, 1% Vitamin stock solution (http://www.fgsc.net/), pH 6.8 and 2% agar).

Deletion of the *RTT109* or *HST3* genes in *C. albicans* strain SN152

The *RTT109*- or *HST3*-deletion strains were constructed using a PCR-based deletion approach[2] (see **Supplementary Table 3** for the list of primers). The deletion cassettes were constructed to carry the *HIS1* or *ARG4* markers flanked by 120-bp sequences from the upstream and downstream regions of the targeted gene (*RTT109* or *HST3*). The *HIS1*-containing amplicon was used to transform *C. albicans* strain SN152 using the standard lithium acetate protocol with minor modifications as previously described[1]. To disrupt the

Nature Medicine: doi:10.1038/nm.2175

second *RTT109* allele, two independently selected His^+ transformants carrying a properly deleted allele, as determined by Southern blotting, were transformed with the *ARG4*-containing amplicon to generate two independent gene deletion strains.

Construction of *RTT109* revertants

Plasmid pSFS2A containing the *SAT1-FLP* cassette was used to construct the *RTT109* revertant[3-4]. A PCR-amplified SN152 DNA fragment located downstream of *RTT109* (+1,204 to +1,740) was cloned into the *Xho*I-*Apa*I sites of plasmid pSFS2A, creating $pRTT109_{down}$. Next, a PCR-amplified fragment containing the *RTT109* open reading frame (ORF, +1 to +1,080 from ATG) as well as its upstream (-800 to -1 bp upstream of the ATG) and downstream (+1,081 to +1,740 from the ATG) regions was cloned into the *Sac*II-*Not*I sites of plasmid $pRTT109_{down}$, resulting in $pRTT109_{rev}$. The 7.3-kb *Apa*I-*Apa*I *RTT109-SAT1* fragment derived from $pRTT109_{rev}$ (**Supplementary Fig. 1b**) was used to transform the *rtt109*Δ/Δ#1 strain. Selection of the integrants was performed on YPD supplemented with 200 μg ml^{-1} of nourseothricin (Werner BioAgents, Jena, Germany).

Construction of a conditional *hst3* mutant

Conditional mutants of the *HST3* gene were constructed in the *C. albicans* CaSS1 strain (**Supplementary Table 2 and Supplementary Fig. 2c-e**) which expresses a tetracycline-dependent transactivation fusion protein (TetR-ScGal4AD) (GRACE technology) as described previously[1,6]. His^+ heterozygote mutants for *HST3* were constructed using a PCR-generated deletion cassette containing a *HIS3* selectable marker flanked by *HST3* upstream (positions -1 to -120 upstream of the ATG) and downstream (+1,465 to +1,584 downstream of the ATG) sequences. The resulting amplicon was transformed into *C. albicans* strain CaSS1[6] which was then plated on medium without histidine and incubated at 30 °C for 2 days. His^+ clones were

Nature Medicine: doi:10.1038/nm.2175

screened by colony PCR and analyzed by Southern blotting. The endogenous promoter (from -3 to -250 bp upstream of the ATG) of the remaining *HST3* allele was replaced in the *HST3/hst3*Δ heterozygote with a repressible tetracycline promoter[6]. The PCR-generated *Tet* promoter cassette containing the *SAT1* dominant selectable marker flanked by sequences homologous to *HST3* (-251 to -370 upstream of the ATG and -4 usptream of the ATG to +116 downstream of the ATG) was amplified from the template plasmid pSAT-Tet[1,6]. The amplicon was transformed as described above into the *HST3/hst3*Δ heterozygote. The resulting integrants were selected on YPD medium supplemented with 200 μg ml^{-1} of nourseothricin (Werner BioAgents, Jena, Germany) and tested by colony PCR and Southern blotting. To repress the *Tet* promoter of the *HST3*Δ*/pTET-HST3* strain, an overnight culture in YPD was diluted into fresh YPD medium containing 20 μg ml^{-1} of doxycycline (Sigma)[1,6] at an initial OD_{600} of 0.005 and the cells were grown overnight.

Deletion of the *HST3* gene in the *C. albicans rtt109*Δ/Δ#1 strain

The *rtt109*Δ/Δ *hst3*Δ/Δ strains were also constructed using a PCR-based deletion approach[2] (see **Supplementary Table 3** for the list of primers and **Supplementary Fig. 2f-h**). The deletion cassettes were constructed to carry the *LEU2* marker amplified from plasmid pSN40[5] or the *SAT1* marker from pSFS2A[3]. Both cassettes contained 120-bp sequences from the upstream and downstream regions of *HST3*. The *LEU2*-containing amplicon was used to transform *C. albicans* strain *rtt109*Δ/Δ#1 by electroporation as previously described[4]. A selected Leu^+ heterozygote shown by colony PCR to have the correct *rtt109*Δ/Δ *HST3*+/Δ genotype was electrotransformed with the *SAT1* cassette. The resulting transformants were selected on YPD medium supplemented with 200 μg ml^{-1} of nourseothricin (Werner BioAgents, Jena, Germany) and tested by colony PCR. Clones carrying the correctly disrupted alleles with *LEU2* and *SAT1* were then grown overnight in YP medium

Nature Medicine: doi:10.1038/nm.2175

supplemented with 2% maltose to loop out the *SAT1* cassette, leaving only the *FRT* sequence. As controls, the *C. albicans* strain SN152 was also transformed as described above by electroporation with the *LEU2* cassette or the *SAT1* cassette followed by a loop-out. The resulting double *rtt109*Δ/Δ *hst3*Δ/Δ strains and the SN152 derivatives used for their construction were tested by Southern analysis.

Southern and Northern blots

C. albicans genomic DNA (gDNA) was purified using the glass-bead method[7]. Total RNA was extracted using the hot phenol method[8]. Southern and Northern blots were performed as previously described[9]. Probes for Southern and Northern analyses were generated by PCR amplification (**Supplementary Table 3**).

Immunoblots

Whole-cell yeast extracts were prepared for SDS–polyacrylamide gel electrophoresis using an alkaline method[10] or a glass bead method[11]. Briefly, cells in exponential phase were harvested and resuspended in 100 µl of TE buffer (50 mM Tris-HCl pH 7.5 and 1.5 mM EDTA) containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride and 5 µg ml^{-1} each of leupeptin, pepstatin and aprotinin). Cells were disrupted by vortexing in the presence of glass beads (5 cycles of 1 min vortex followed by 1 min cooling on ice). Total protein extracts were harvested by centrifugation. Antibodies against H3K56ac (AV105), the H3 N-terminal domain (AV71/72) and recombinant *S. cerevisiae* histone H4 (AV 94) have been previously described[11-13].

RT-PCR

Nature Medicine: doi:10.1038/nm.2175

Total RNA was extracted from 2×10^7 cells using the hot phenol method[8]. One microgram of total RNA was reverse-transcribed to cDNA using the SuperscriptIII reverse transcriptase enzyme and random primers (Invitrogen) according to the manufacturer's protocol. Quantitative PCR was performed on 1/20 of the cDNA preparation in triplicate using a SYBR green master mix containing Jumpstart Taq DNA polymerase (Sigma) and SYBR green nucleic acid stain (Invitrogen). Quantitative PCR was performed on a Step One qPCR instrument (Applied Biosystems Inc.). Results were normalized to the *ACT1* gene signal. See **Supplementary Table 3** for primer sequences.

Identification of H3 K56 acetylation by mass spectrometry

Histones were isolated from *C. albicans* as previously described for *S. cerevisiae*[14]. Acid-extracted histones were further purified by reverse phase HPLC[15]. Fractions containing histone H3 were pooled, evaporated in a Speed-Vac and resuspended in 0.1 M ammonium bicarbonate. After derivatization with deuterated acetic anhydride, tryptic digests were loaded onto a 4 mm length, 360 μm i.d. trap column and separated using a 10 cm length, 150 μm i.d. analytical column packed in-house with 3 μm C_{18} particles (Jupiter 300 Å, Phenomenex, Torrance, CA)[15]. The mobile phases consisted of 0.2% formic acid in water (solvent A) and 0.2% formic acid in acetonitrile (solvent B). The pump flow rate was set to 0.6 μl min^{-1} and peptide elution was achieved using a linear gradient of 5 to 40% B for the first 65 min followed by a rapid increase to 80% B for the next 3 min. Nano LC-MS/MS analyses of H3 tryptic peptides using multiple reaction monitoring (MRM) were performed on an AB/MDS Analytical Technologies 4000 Q-Trap mass spectrometer (Thornhill, ON, Canada) equipped with a Nanospray II interface. MRM transition pairs were monitored to detect the *in vivo* K56-acetylated tryptic peptide (*m/z* 638.9 → 831.5, 638.9 → 1001.6) and the peptides K56-acetylated *in vitro* with deuterated acetic anhydride (*m/z* 640.3 → 831.5 and 640.3 → 1004.6)

Nature Medicine: doi:10.1038/nm.2175

or propionic anhydride (*m/z* 645.9 → 831.5 and 645.9 → 1015.6). Peptide sequences were confirmed by the MIDAS strategy with MRM triggering an enhanced product ion (EPI) scan in data dependant mode. Samples were injected in triplicate using MRM only for relative quantitation. A 10-ms dwell time was applied to each unique histone peptide ion MRM transition; tryptic peptides were eluted from the HPLC column using the conditions specified above.

Accurate determination of the stoichiometry of H3 acetylation at specific lysine residues

250 µl of protein A-Sepharose beads (GE healthcare) were incubated with 300 µg of affinity-purified antibody against the N-terminal domain of yeast H3 (AV71/72) for 1 h at 4 ºC in 10 mM Tris-HCl pH 8.0. The antibody beads were then washed three times with the same buffer. *C. albicans* cells (1 ml cell pellet) were resuspended in 1 ml of lysis buffer (100 mM Tris-HCl pH 8.0, 200 mM NaCl, 1X EDTA-free complete protease inhibitor cocktail (Roche), 100 mM sodium butyrate, 10 µM trichostatin A, 100 mM nicotinamide, 1 mM dithiothreitol) and lysed using a 6850 Freezer Mill (SPEX certiprep). The cell lysates were then incubated in the presence of 200 µg ml^{-1} of ethidium bromide for 30 min on ice to force histone dissociation from DNA. After two brief (5-10 seconds) pulses of sonication, the cell lysates were centrifuged at 13,000 rpm and 4ºC for 15 min in a tabletop centrifuge (Heraeus Multifuge 3S-R). The supernatant was mixed with 50 µl of H3 antibody beads and incubated overnight at 4 ºC. The beads were washed five times in wash buffer (100 mM Tris-HCl pH 8.0, 100 mM NaCl), resuspended in 1X SDS-PAGE sample buffer and boiled for 5 min to elute bound proteins. Protein samples were resolved in an SDS-15% polyacrylamide gel (29:1 acrylamide:N,N`-methylene bisacrylamide molar ratio), which was stained with Bio-Safe Coomassie G-250 stain (Bio-Rad) and destained overnight in water. The band corresponding to *C. albicans* H3 was then cut from the gel for mass spectrometry (MS).

Nature Medicine: doi:10.1038/nm.2175

We found that *in vitro* acetylation with deuterated acetic anhydride (see above) or propionylation of non-modified H3K56 did not appreciably influence the stoichiometry of H3K56 acetylation determined experimentally. Therefore, the *in vivo* stoichiometry of H3K56 acetylation can be determined accurately with both methods. Gels bands were destained using deionized (DI) water, 50:50 DI water: acetonitrile (ACN), and pure ACN. The bands were washed and resuspended in a 0.1 M ammonium bicarbonate (Ambic) solution (pH 8.0). A propionic anhydride reagent (2:1 PA:water) was added to the bands in a 1:1 volume ratio and incubated for 1 h at room temperature with shaking. The buffer and derivatization mixture was replaced with fresh reagents to perform a second propionylation reaction for 1 h. After removing excess reagent, the bands were washed with a 0.1 M Ambic solution, and evaporated to dryness in a Speed-vac. Rehydration with 0.1 M Ambic was followed by the addition of 1 μg of trypsin and overnight protein digestion. The digest supernatant was placed in a separate Eppendorf tube. Peptides were extracted twice with a 50:50 DI water:ACN solution containing 5 % TFA (v:v:v). The bands were incubated for 15 min at room temperature with shaking. The two solutions for peptide extraction were recovered, combined with the original tryptic digest supernatant, and evaporated to dryness in a Speed-Vac. Peptides were dissolved in the initial mobile phase (95:5 DI water:ACN, 0.2% formic acid (v:v:v) prior to injection onto the, 4000 Q-Trap mass spectrometer.

Precursor – product ion pairs corresponding to the following MRM transitions were monitored (Ac, acetylation; Pr, propionylation of ε-amino group):

A) H3 peptide 54-FQK(Ac)STELLIR-63: *m/z* 638.9 → 1001.6

B) H3 peptide 54-FQK(Pr)STELLIR-63: *m/z* 645.9 → 1015.6

C) H3 peptide 18-K(Ac)QLASK(Pr)AAR-26: *m/z* 535.8 → 772.5

D) H3 peptide 18-K(Pr)QLASK(Ac)AAR-26: *m/z* 535.8 → 758.5

Nature Medicine: doi:10.1038/nm.2175

E) H3 peptide 18-K(Ac)QLASK(Ac)AAR-26: *m/z* 528.8 → 758.5

F) H3 peptide 18-K(Pr)QLASK(Pr)AAR-26: *m/z* 542.8 → 772.5

H) H3 peptide YK(Pr)PGTVALR, a non-modified peptide used for normalization of H3 quantities in each gel band and for fluctuations in the MS response: *m/z* 530.8 → 713.4

First, the abundance for each peptide MRM transition (A to F) was normalized to the ion abundance of the non-modified peptide transition (H). These normalized values (An to Fn) were then used to calculate the stoichiometry of acetylation at each lysine residue as follows.

%H3K56ac = 100 × An / (An + Bn)

%H3K18ac = 100 × (Cn + En) / (Cn + Dn + En + Fn)

%H3K23ac = 100 × (Dn + En) / (Cn + Dn + En + Fn)

Drug susceptibility assays

Spot assays were performed as described previously[4]. Cells were grown overnight in YPD at 30 ºC and diluted to an OD_{600} of 0.1. Ten-fold serial dilutions of each strain were spotted onto drug-containing YPD plates. For the *Aspergillus* plate assay (**Fig. 5c**), cells were thawed from -80 °C glycerol stocks onto CM plates and grown for 3-4 days at 30 °C. Spores were then transferred with a toothpick onto NAM-containing CM plates and incubated at 30 °C for 3 days. Genotoxic and antifungal drugs were purchased from Sigma unless otherwise stated. Caspofungin (CSF) was a gift from Merck Frosst Canada and micafungin (MCF) was purchased from Astellas Pharma Canada. Liquid microtiter plate assays were performed as described previously[4]. Sensitivity to nicotinamide in liquid YPD cultures was measured by monitoring cell growth spectrophotometrically at an OD_{620} after 48 h of incubation at 30 ˚C.

Morphological observations

Nature Medicine: doi:10.1038/nm.2175

Microscopy was performed by differential interference contrast (DIC) and epifluorescence microscopy with a Zeiss Axio-Imager Z1 microscope. Image analysis was carried out with the Zeiss AxioVision 4.8 software. DAPI staining of DNA, calcofluor white (CFW) staining of cell walls and septa, and propidium iodide staining of dead cells were performed as previously described[1].

In vivo disseminated candidiasis model

Eight to twelve week old A/J mice were purchased from The Jackson Laboratories (Bar Harbor, ME). Housing and all experimental procedures were approved by the Biotechnology Research Institute Animal Care Committee, which operates under the guidelines of the Canadian Council of Animal Care. For the *HST3* virulence study (**Fig. 6a**), wild-type strains SC5314, KTY3 and the mutant *hst3*Δ/*pTET-HST3* were used. To turn off the expression of the *HST3* gene (**Fig. 6a**), 5% sucrose and 2 mg ml^{-1} tetracycline were added to the drinking water of the mice three days prior to infection with *C. albicans*. Mice (6 per *C. albicans* strain) were injected with 200 µl of PBS containing 5×10^4 blastospores via the tail vein. When infected with this number of wild-type *C. albicans* cells, A/J mice become moribund approximately 48 h post-infection[16], thus allowing ample time for tetracycline to act. Mice were closely monitored and the kidney fungal loads were determined as described above. For the *in vivo* nicotinamide treatments (**Fig. 6b**), mice (6 per treatment) were injected intra-peritoneally with 500 µl of PBS containing nicotinamide (500 mg kg^{-1}), 30 min prior to the *C. albicans* infection, since it takes 20-30 min for the nicotinamide concentration to peak in the blood stream[17]. In addition, given that the half-life of nicotinamide *in vivo* is approximately 2 h[17], a second dose of nicotinamide was injected 8 h after *C. albicans* infection in order to maintain high nicotinamide concentrations in circulation. 3×10^5 rather than 5×10^4 blastospores were injected in an effort to shorten the period during which nicotinamide levels had to be

Nature Medicine: doi:10.1038/nm.2175

maintained *in vivo*. At this higher dose, A/J mice are moribund within 24 h[18]. Fungal load was determined as described above.

For the *RTT109* virulence study (**Fig. 6c-d**), inoculums of strains SC5314, *rtt109*Δ/Δ and revertant *rtt109*Δ/Δ+*RTT109* were grown overnight in YPD medium and the blastospores were washed twice and resuspended in phosphate buffered saline (PBS). Mice (6 per *C. albicans* strain) were injected with 200 µl of PBS containing 3×10^5 blastospores via the tail vein. Mice were closely monitored for clinical signs such as lethargy, hunched back and ruffled fur. Mice exhibiting extreme lethargy were deemed moribund and were euthanized. For determination of fungal load, kidneys and hearts were homogenized in 5 ml PBS and 50 µl of an appropriate dilution was plated on Sabouraud broth-agar plates containing 0.35 mg L^{-1} chloramphenicol (**Fig. 6a-c**). Blood of sacrificed mice was also collected for cytokines analysis (**Fig. 6d**).

To determine whether nicotinamide administration could activate elements of the innate immune system directly, levels of six cytokines/chemokines were tested in the serum of A/J mice that had received nicotinamide treatment. For the sake of consistency with the experiment described in **Fig. 6b**, two nicotinamide injections were given, 9 h apart in a 24 h period. Nicotinamide was administered for either 1 or 3 days and cytokine levels were measured at indicated times post nicotinamide injection. Control mice received PBS injections.

Multiple sequence alignments

The *C. albicans* Hst3p sequence was used to search the Broad Institute Fungal Database (http://www.broadinstitute.org/) and the alignments were compiled with Probcons (http://probcons.stanford.edu/) (**Supplementary Fig. 3**). Residues conserved across all the fungal proteins were colored either in red (identical or highly related) or brown (related).

Nature Medicine: doi:10.1038/nm.2175

Amino acid residues with highly related side chains were grouped as follows: aliphatic (G, A, V, I, L, M), aromatic (H, F, W, Y), nucleophilic non-charged (C, S, T), acidic (D, E), basic (H, K, R), and amides (N, Q). Histidine was grouped with both aromatic and positively charged residues. Although proline is hydrophobic, it was not considered equivalent to any other residue owing to its heterocyclic side chain that incorporates the main chain amino group. In some cases, this has led us to avoid coloring conserved hydrophobic residues when at least one of the fungal proteins had a proline at that position. Aliphatic and aromatic residues were considered related by their hydrophobic nature and therefore colored in brown. Residues with nucleophilic non-charged, acidic, and amide side chains were also regarded as related.

Nature Medicine: doi:10.1038/nm.2175

Supplementary Results

Deletion of *RTT109* in *C. albicans* SN152 and construction of revertants

RTT109 deletion cassettes containing the *HIS1* or *ARG4* markers flanked by *RTT109* upstream and downstream sequences were obtained by PCR amplification. The *HIS1* cassette was first used to transform SN152 and two independently selected His^+ transformants were characterized by Southern blotting (**Supplementary Fig. 1a** and **1d**). The expected 615-bp band corresponding to the wild-type *RTT109* allele and 1,377-bp band corresponding to the *rtt109*Δ*::HIS1* allele were observed. These strains were used to delete the second allele of *RTT109,* using the *ARG4* cassette. Selected His^+Arg^+ transformants were analyzed by Southern blotting with the *RTT109* probe 1 (**Supplementary Fig. 1a, 1d** and **Supplementary Table 3**). The 1,816-bp and 1,377-bp bands consistent with the replacement of the *RTT109* alleles by the *ARG4* and the *HIS1* cassettes were detected for the two independent *rtt109*Δ/Δ mutants. A wild-type *RTT109* allele was reintroduced at its original locus in strain *rtt109*Δ/Δ*#1*, using the *SAT1-FRT* cassette as previously described (**Supplementary Fig. 1b-c**)[3-4]. The loss of the 1,816-bp band and the appearance of the wild type 615-bp band confirmed the successful reintegration of the *RTT109* gene at its locus (**Supplementary Fig. 1d**). A 1.8-kb transcript was detected by the *RTT109* ORF probe 2 in all the strains tested, except for the *rtt109*Δ/Δ strains (**Supplementary Fig. 1e**).

We noticed that the gene neighboring *RTT109* is *SWC4* (orf19.7492), which encodes a subunit of the NuA4 histone acetyltransferase complex. These two genes share a common intergenic region, but are expressed in opposite orientations. To verify that the phenotypes of the *rtt109*Δ/Δ mutants were not due to modulation of *SWC4* expression, a Northern blot was performed with total RNA isolated from strains SN152, *rtt109*+/Δ #1, *rtt109*Δ/Δ #1 and *rtt109*Δ/Δ +*RTT109*#1. The blot was probed with an *SWC4* ORF fragment (**Supplementary**

Nature Medicine: doi:10.1038/nm.2175

Fig. 1f). *SWC4* transcripts levels were similar in the four strains tested. Therefore, the *rtt109*Δ/Δ mutant phenotypes do not result from changes in *SWC4* gene expression.

Loss of *RTT109* affects morphogenesis

Under yeast growth conditions (YPD medium at 30 ^{o}C), overnight liquid cultures of the wild-type strain SN152 consisted only of yeast cells. While deleting one allele of *RTT109* did not result in any obvious phenotype (data not shown), deletion of both *RTT109* alleles affected cell morphology. A mixture of yeast, pseudohyphae and pseudohyphae-like filaments were observed in cultures of *rtt109*Δ/Δ mutants. In addition, anucleated and multinucleated cells were observed in a fraction of the pseudohyphal cells that spontaneously formed in the *rtt109*Δ/Δ mutants (**Supplementary Fig. 1g**). Interestingly, similar morphology defects have been observed previously in *C. albicans* mutants for dynein (*CaDYN1*) and dynactin (*CaNIP100*), two proteins involved in regulating nuclear migration and positioning[19].

Construction of *HST3* mutants in *C. albicans*

Deletion of *HST3* in strain SN152 was attempted, as described above for *RTT109*. We replaced one allele of the *HST3* with the *HIS1* cassette and the resulting *hst3*+/Δ heterozygous mutants were characterized by Southern blotting (**Supplementary Fig. 2a-b**). In strain SN152, two bands of 786 and 1,027 bp were detected using the *HST3* probe 1, due to an *Spe*I polymorphism in the promoter region of the two *HST3* alleles. Loss of the 786-bp band and appearance of a 6,813-bp band in both heterozygote strains confirmed proper deletion of the $HST3_{1934}$ allele. However, we were unable to construct the *hst3*Δ/Δ strain despite many attempts, suggesting that *HST3* is an essential gene.

Construction of a conditional *hst3* mutant strain

Nature Medicine: doi:10.1038/nm.2175

Since we were unable to generate an *hst3*Δ/Δ mutant, we constructed a conditional *HST3* mutant, using the GRACE (Gene Replacement and Conditional Expression) technology[1,6]. One allele of *HST3* (orf19.1934) was deleted and the endogenous promoter of the remaining *HST3* allele (orf19.9490) was replaced with a repressible tetracycline promoter in strain CaSS1 which expresses a chimeric tetracycline transactivator. We analyzed the resulting strains by Southern blotting (**Supplementary Fig. 2c-d**). The genomic DNA of strains CaSS1, *hst3*+/Δ and *hst3*Δ*/pTET-HST3* was isolated, digested with *Spe*I and *Cla*I and analyzed with the *HST3* probe 1. The presence of two bands of 786 bp and 1,027 bp in CaSS1 (**Supplementary Fig. 2d**) confirmed that this strain contains the same *Spe*I polymorphism that was detected in SN152 (**Supplementary Fig. 2b**). The disappearance of the 786-bp band and the appearance of a doublet around 1,000 bp in strain *hst3*+/Δ demonstrated the insertion of the *HIS3* cassette within the *HST3* (orf19.1934) allele. The doublet consists of a 995-bp *Cla*I-*Spe*I product derived from the $hst3_{1934}$Δ*::HIS3* allele and the 1,027-bp fragment corresponding to the remaining *HST3* (orf19.9490) allele. The 2,643-bp band corresponding to the replacement of the orf19.9490 promoter with the repressible tetracycline promoter and the 995-bp band confirmed the correct integration in the *HST3* mutant strain *hst3*Δ*/pTET-HST3*. Strains *hst3*+/Δ, *hst3*Δ*/pTET-HST3* as well as the control strain CaSS1 were analyzed by Northern blotting after growth for 24 h in the absence or the presence of doxycycline (DOXY), a tetracycline derivative (**Supplementary Fig. 2e**). In the absence of doxycycline, we detected a stronger signal for the *HST3* transcript in strain *hst3*Δ*/pTET-HST3* as compared to the signals derived from strains CaSS1 and *hst3*+/Δ (**Supplementary Fig. 2e**), suggesting that the tetracycline-repressible promoter confers stronger expression in the absence of doxycycline than the endogenous *HST3* promoter. Growth of the *hst3*Δ*/pTET-HST3* strain in the presence of 20 μg ml^{-1} of doxycycline decreased the expression of *HST3* mRNA to levels lower than those detected in strains CaSS1 and *hst3*+/Δ (**Supplementary Fig. 2e**). These

Nature Medicine: doi:10.1038/nm.2175

results were confirmed by RT-PCR analysis, using RNA from strains grown in 50 μg ml^{-1} of doxycycline (**Fig. 2a**).

Deletion of the *HST3* gene in the *rtt109*Δ/Δ mutant

The *LEU2* and *SAT1* cassettes flanked by *HST3* upstream and downstream sequences were obtained by PCR amplification. The *LEU2* cassette was first used to transform *C. albicans* strain *rtt109*Δ/Δ#1. One Leu^+ transformant, *rtt109*Δ/Δ *HST3*+/Δ, was characterized by Southern blotting (**Supplementary Fig. 2f-g**). The expected 3,961-bp band corresponding to the wild-type *HST3* allele and 1,411-bp band corresponding to the *hst3*Δ*::LEU2* allele were observed. This strain was used to delete the second *HST3* allele with the *SAT1* cassette. Two selected Leu^+ Nou^+ transformants, *rtt109*Δ/Δ *hst3*Δ/Δ#A and *rtt109*Δ/Δ *hst3*Δ/Δ#B, were grown in the presence of maltose to induce expression of the flippase and trigger genomic excision of the *SAT1* cassette, leaving only the *FRT* sequence. Two independent Leu^+ Nou^- strains, *rtt109*Δ/Δ *hst3*Δ/Δ#1 and #2, were analyzed by Southern blotting with the *HST3* probe 1 (**Supplementary Fig. 2g, Supplementary Table 3**). For all the *rtt109*Δ/Δ *hst3*Δ/Δ mutants analyzed, the detection of a 1,411-bp and 1,331-bp doublet was consistent with the replacement of the *HST3* alleles by the *LEU2* and the excised *SAT1* cassettes. Appearance of a 1,411-bp band in addition to the 3961-bp fragment in HST3+/Δ#3 and HST3+/Δ#4 corroborated the replacement of one *HST3* allele by the *LEU2* cassette. The observation of two bands of 3,961-and 1331-bp, for *HST3*+/Δ#5 and *HST3*+/Δ#6 confirmed the presence of the *FRT* sequence in addition to the wild-type *HST3* allele in these strains. Finally, only the *HST3* loci, corresponding to a band of 3,961 bp, were observed in SN152 and *rtt109*Δ/Δ#1 cells. Proper deletion of both *RTT109* and *HST3* was further confirmed by Southern analysis of the *rtt109*Δ/Δ *hst3*Δ/Δ mutants using both ORFs as probes (data not shown). Even following overexposure of the blots, no signal could be detected in the double mutant strains.

Nature Medicine: doi:10.1038/nm.2175

On YPD plates, the *rtt109*Δ/Δ *hst3*Δ/Δ and *rtt109*Δ/Δ *hst3*+/Δ mutants exhibited slower growth as compared to the *rtt109*Δ/Δ and *hst3*+/Δ mutants (**Supplementary Fig. 2h** and **Fig. 2g**). Nevertheless, the viability of the *rtt109*Δ/Δ *hst3*Δ/Δ mutant confirms that the lethality of the *hst3*Δ/Δ mutant is due to H3 K56 hyperacetylation.

Nature Medicine: doi:10.1038/nm.2175

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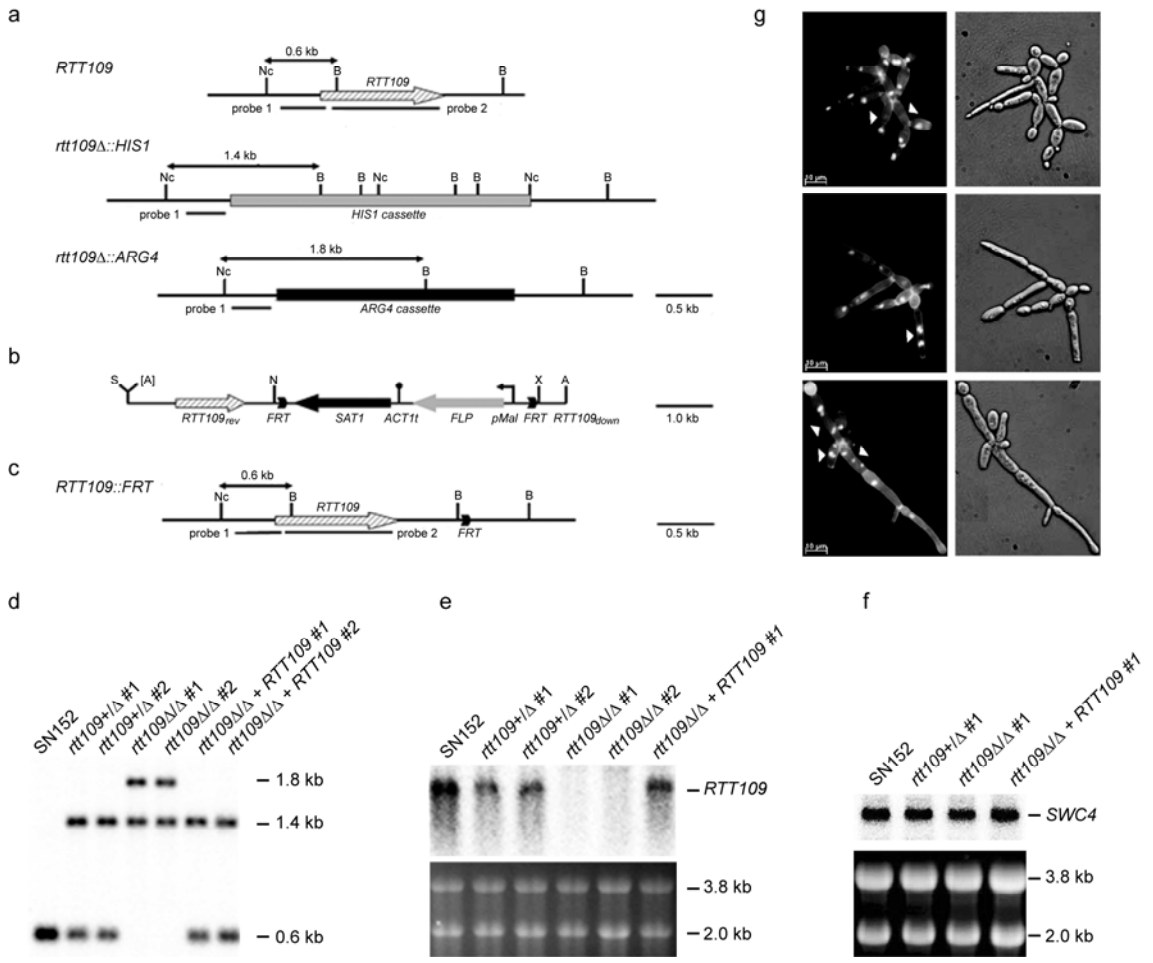
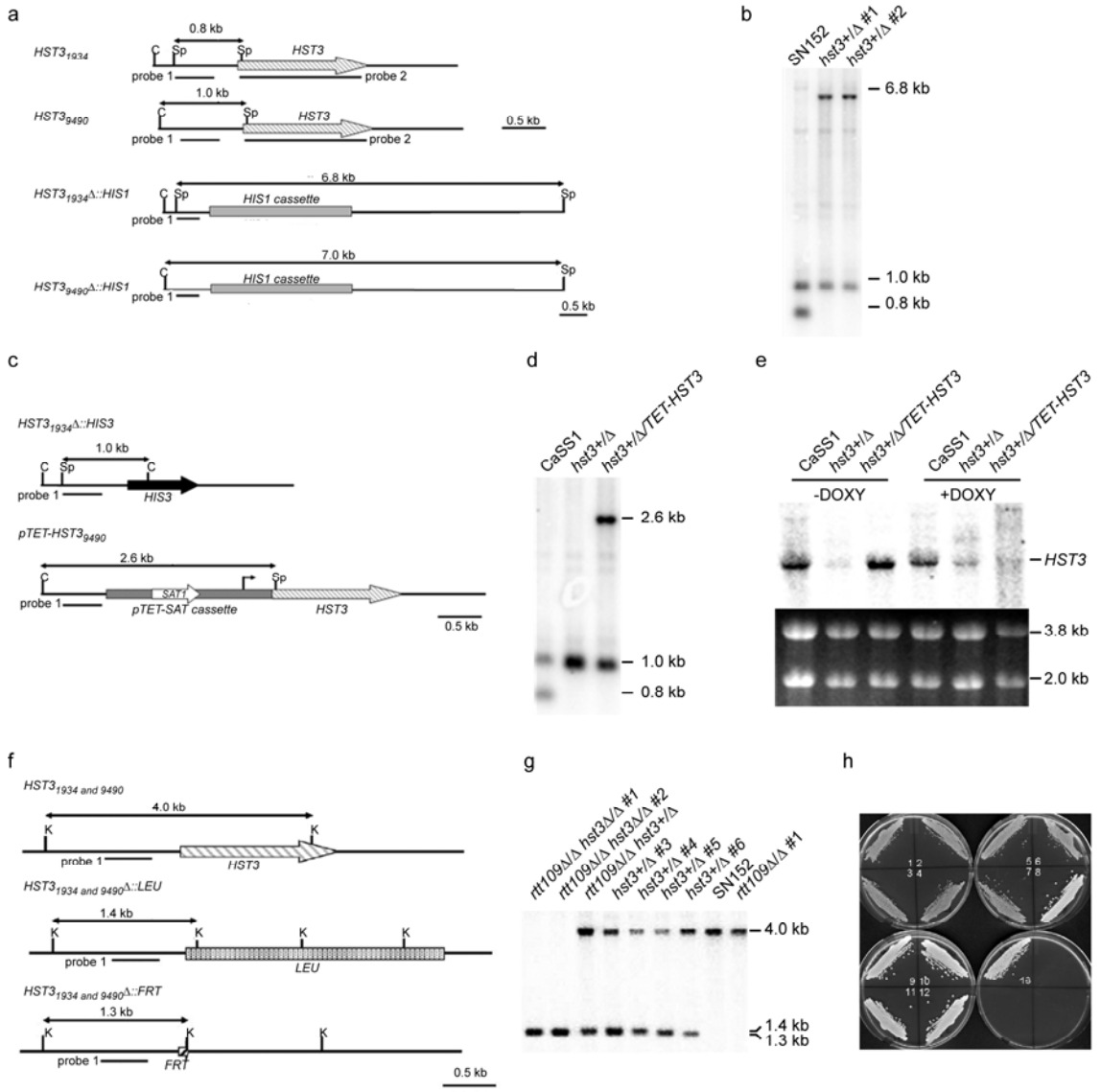
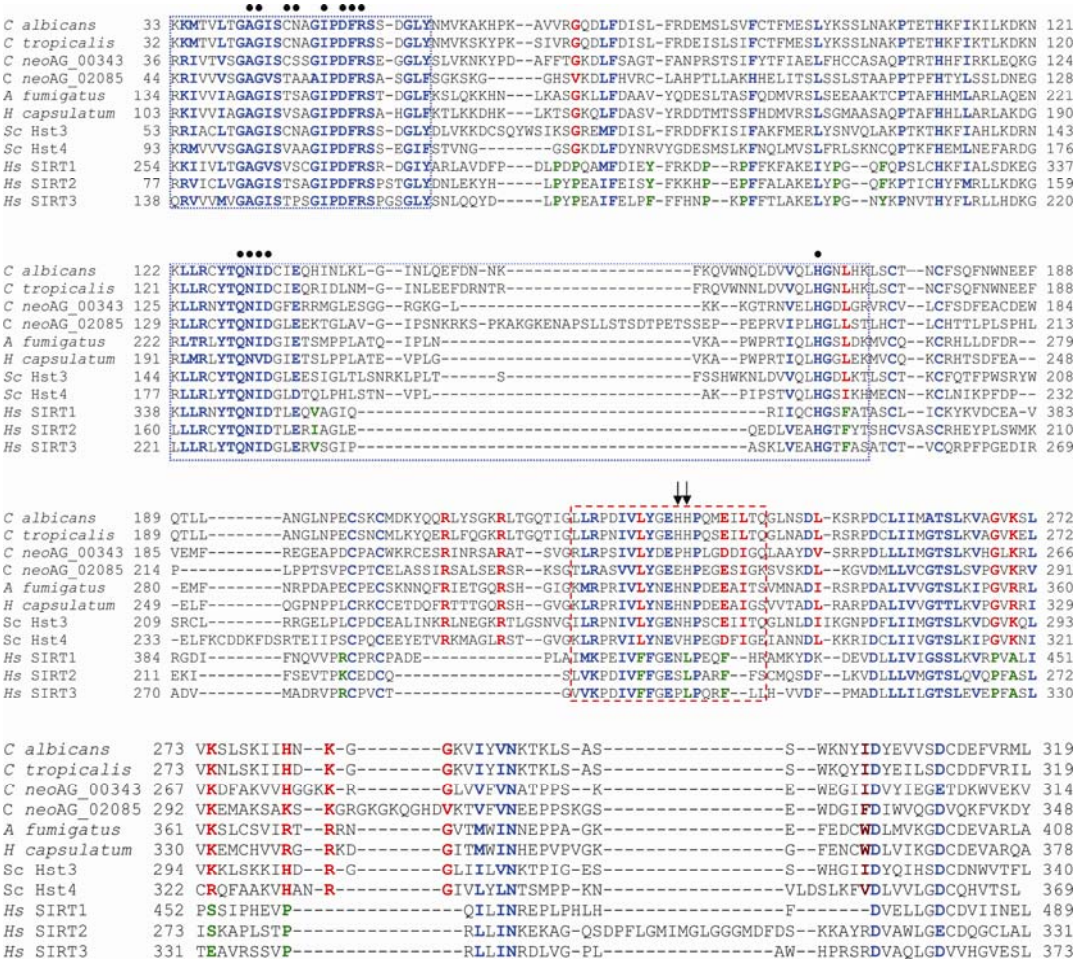


Supplementary Figure 1. Characterization of *rtt109* mutants. (**a**) *Nco*I-*Bgl*II restriction maps of the *RTT109* locus and the *rtt109*Δ alleles disrupted with either *HIS1* or *ARG4* cassettes. *RTT109* probe 1 was used for Southern blot analysis. Probe 2, which detects *RTT109* ORF, was used for Northern blot analysis. (**b**) Schematic representation of the *RTT109-SAT1* complementation cassette. The cassette consists of, from left to right: $RTT109_{rev}$ including the upstream *RTT109* sequence, the entire *RTT109* ORF and the *RTT109* downstream sequences; *FRT*, FLP recombination target sites; *SAT1*, nourseothricin resistance marker; *ACT1t*, transcription termination sequence; *FLP*, flip-recombinase gene; *pMal*, maltose promoter and $RTT109_{down}$, *RTT109* downstream sequences. Restriction sites are: B, *Bgl*II; Nc, *Nco*I; S, *Sac*II; A, *Apa*I; N, *Not*I; X, *Xho*I; [A], *Apa*I site was introduced by PCR. (**c**) *Nco*I-*Bgl*II restriction map of the *RTT109* revertant allele. (**d**) Southern blot analysis of *Nco*I-*Bgl*II digested gDNA. (**e**) Northern blot analysis and corresponding denaturing RNA gel. (**f**) Testing the expression of the *SWC4* gene in *rtt109* mutants by Northern blot analysis. (**g**) Microscopy analysis of *rtt109*Δ/Δ cells with DAPI and calcofluor white staining; multinucleated or anucleated cells are indicated by arrowheads.



Supplementary Figure 2. Characterization of *hst3* mutants. (**a**) *Cla*I-*Spe*I restriction maps of the two *HST3* alleles (orf19.1934 and orf19.9490) and the corresponding *hst3*Δ::*HIS1* loci. The *HST3* probe 1 was used for Southern blot analysis. Probe 2, which detects the *HST3* ORF, was used for Northern blot analysis. (**b**) Southern blot analysis of *Cla*I-*Spe*I-digested gDNA. (**c**) *Cla*I-*Spe*I restriction maps of *hst3*Δ::*HIS3* (orf19.1934) locus (top panel) and the promoter of *HST3* (orf19.9490) after replacement by the *pTET-SAT1* cassette (bottom panel). (**d**) Southern blot analysis of *Cla*I-*Spe*I-digested gDNA. (**e**) Northern blot analysis of conditional *hst3* mutants using probe 2. DOXY (doxycycline, 20 μg ml^{-1}). (**f**) Construction of *rtt109*Δ/Δ *hst3*Δ/Δ double mutants. *Kpn*I restriction maps of the *HST3* loci (top panel), the *hst3*Δ::*LEU2* loci (middle panel) and the *hst3*Δ*::FRT* loci (bottom panel). (**g**) Southern blot analysis of *Kpn*I-digested genomic DNA. (**h**) Growth of *rtt109*Δ/Δ *hst3*Δ/Δ mutants. Strains tested are: 1, *rtt109*Δ/Δ *hst3*Δ/Δ#1; 2, *rtt109*Δ/Δ *hst3*Δ/Δ#2; 3, *rtt109*Δ/Δ *hst3*Δ/Δ#3; 4, *rtt109*Δ/Δ *hst3*Δ/Δ#4; 5, *rtt109*Δ/Δ *hst3*Δ/Δ#5; 6, *rtt109*Δ/Δ *hst3*Δ/Δ#6; 7, *rtt109*Δ/Δ *HST3*+/Δ; 8, *HST3*+/Δ #3; 9, *HST3*+/Δ #4; 10, *HST3*+/Δ #5; 11, *HST3*+/Δ #6; 12, SN152 and 13, *rtt109*Δ/Δ#1.

Nature Medicine: doi:10.1038/nm.2175



Supplementary Figure 3. Class Ic sirtuins homologous to Hst3p exist in different pathogenic fungi and contain potentially important sequence motifs that are different from human sirtuins. Only one putative Hst3p homologue was found in *C. tropicalis* (RT05386), *A. fumigatus* (1G1054) and *H. capsulatum* (EG02181). As is the case in *S. cerevisiae*, which possesses two related proteins (Hst3p, YOR025W and Hst4p, YDR191W), two potential Hst3p orthologs also exist in *C. neoformans*, (CneoHst3p, AG00343 and CneoHst4p, AG02085). The alignment is restricted to the catalytic cores and the human sirtuins (class Ia and Ib) that are most closely related to Hst3p are included for comparison (SIRT1: *NM_012238*; SIRT2: *NM_012237*; SIRT3: *NM_012239*). Residues colored in blue are common to all class I sirtuins. Residues colored in red and brown are conserved among Hst3p family members, but different in human sirtuins. In contrast, residues in green are conserved among human class I sirtuins, but are different in fungal Hst3p homologs. The red box includes residues that bind acetylated peptides in other sirtuins[23] and most likely plays a similar role in Hst3p homologues. The side chains of the residues highlighted with arrows have been implicated in peptide substrate selection[23]. The blue boxes bracket conserved regions that contain residues which directly interact with NAD^+ (black dots)[24-25].

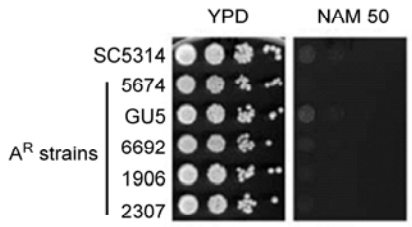
Nature Medicine: doi:10.1038/nm.2175

a

Species	Strain name	Reference or source
Candida albicans	5457	[9]
	5674	[9]
	GU5	[27]
	6692	[9]
	1906 (A2.1)	[28]
	2307 (A3.1)	[28]
	ATCC90028	[29]
	372.73374.1	[29]
	ATCC66027	[30]
	20464	[30]
Candida glabrata	ATCC90030	ATCC[a]
	MY067497	LSPQ[b]
Candida parapsilosis	ATCC90018	ATCC
	MY067743	LSPQ
Candida tropicalis	MY070362	LSPQ
	MY070589	LSPQ
Candida krusei	ATCC6258	ATCC
	MY069520	LSPQ
Aspergillus fumigatus	MY070954	LSPQ
	MY070968	LSPQ
Aspergillus nidulans	MY070627	LSPQ
	R153	C.F. Roberts

[a] American Type Cell Culture
[b] Laboratoire de Santé Publique du Québec

b



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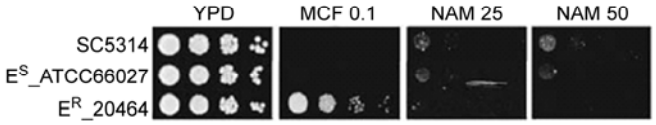

Supplementary Figure 4. Activity of nicotinamide against different pathogenic fungi. (**a**) Strain list of *Candida* species and *Aspergillus* species used in this study. (**b**) Nicotinamide spot assay of a collection of azole-resistant (A^R) strains. (**c**) Nicotinamide spot assay of echinocandin-susceptible (E^S) and echinocandin-resistant (E^R) strains. NAM, nicotinamide (mM), MCF, micafungin (μg ml^{-1}).

Nature Medicine: doi:10.1038/nm.2175

Supplementary Table 1. Stoichiometry of acetylation of H3 lysine residues determined by nano-LC MS/MS and MRM.

Strain	Condition	%K56Ac[2]	%K18Ac[2,3]	%K23Ac[2]
Wild-type (SN152)	ASY[1]	26.6 ± 0.3	2.52 ± 0.05	10.0 ± 0.5
*rtt109*Δ/Δ	ASY[1]	0.56 ± 0.07	3.11 ± 0.07	10.9 ± 0.3
HST3+/Δ #1[3]	ASY[1]	50.0 ± 0.6	3.49 ± 0.02	12.0 ± 0.05
HST3+/Δ #1[3]	ASY[1]	47.2 ± 0.5	2.68 ± 0.05	9.7 ± 0.1
*hst3*Δ/*pTET-HST3*	ASY (7.5h no doxy)[1]	47.8 ± 0.5	6.23 ± 0.05	20.2 ± 0.1
*hst3*Δ/*pTET-HST3*	ASY (7.5h doxy)[1]	86.0 ± 0.2	2.37 ± 0.08	9.5 ± 0.9
*hst3*Δ/*pTET-HST3*	ASY (7.5h doxy)[1]	88. 0 ± 0.1	2.41 ± 0.09	10.6 ± 0.1

[1] ASY = Asynchronously growing cells in exponential phase; doxy = doxycycline (40 μg ml^{-1}).

[2] %Ac of a given lysine residue was calculated as described in the Supplementary Materials and Methods section. The average values ± standard error of the mean were calculated from triplicate injections of the same tryptic digest.

[3] Independent replicates of the same strain.

Nature Medicine: doi:10.1038/nm.2175

Supplementary Table 2. List of *C. albicans* strains used in this study.

Strain Name	Parent	Genotype	Source or reference
SC5314		Wild type	[26]
SN152	RM1000#2	*arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434*	[5]
CaSS1	CAI4	*his3::hisG/his3::hisG leu2::tet*R-GAL4AD-*URA3/LEU2*	[6]
KTY3	CaSS1	*his3::hisG::HIS3/his3::hisG leu2::tet*R-GAL4AD-*URA3/LEU2*	[1]
rtt109+/Δ #1	SN152	*arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/ his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434 RTT109/ rtt109Δ::HIS1*	This study
rtt109+/Δ #2	SN152	*arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/ his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434 RTT109/ rtt109Δ::HIS1*	This study
*rtt109*Δ/Δ#1	*rtt109*+/Δ #1	*arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434 rtt109Δ::ARG4/rtt109Δ::HIS1*	This study
*rtt109*Δ/Δ#2	*rtt109*+/Δ #2	*arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434 rtt109Δ::ARG4/rtt109Δ::HIS1*	This study
*rtt109*Δ/Δ+*RTT109*#1	*rtt109*Δ/Δ#1	*arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/ his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434 RTT109-FRT/rtt109Δ::HIS1*	This study
*rtt109*Δ/Δ+*RTT109*#2	*rtt109*Δ/Δ#1	*arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/ his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434 RTT109-FRT/rtt109Δ::HIS1*	This study
HST3+/Δ #1	SN152	*arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/ his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434 HST3/hst3Δ::HIS1*	This study
HST3+/Δ #2	SN152	*arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/ his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434 HST3/hst3Δ::HIS1*	This study
HST3+/Δ #3	SN152	*arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/ his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434 HST3/hst3Δ::FRT*	This study
HST3+/Δ #4	SN152	*arg4Δ/arg4 leu2Δ/leu2Δ Δ his1Δ/ his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434 HST3/hst3Δ::LEU2*	This study
*rtt109*Δ/Δ *HST3*+/Δ	*rtt109*Δ/Δ #1	*arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434 rtt109Δ::ARG4/rtt109Δ::HIS1*	This study

Nature Medicine: doi:10.1038/nm.2175

*rtt109*Δ/Δ *hst3*Δ/Δ#1	*rtt109*Δ/Δ *HST3*+/Δ	*HST3/hst3Δ::LEU2 arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434 rtt109Δ::ARG4/rtt109Δ::HIS1 hst3Δ::FRT/hst3Δ::LEU2*	This study
*rtt109*Δ/Δ *hst3*Δ/Δ#2	*rtt109*Δ/Δ *HST3*+/Δ	*arg4Δ/arg4Δ leu2Δ/leu2Δ his1Δ/his1Δ URA3/ura3Δ::λimm434 IRO1/iro1Δ::λimm434 rtt109Δ::ARG4/rtt109Δ::HIS1 hst3Δ::FRT/hst3Δ::LEU2*	This study
HST3+/Δ	CaSS1	*his3::hisG/his3::hisG leu2::tet*R-GAL4AD-*URA3/LEU2 HST3/hst3Δ::HIS3*	This study
*HST3*Δ/*pTET-HST3*	*HST3*+/Δ	*his3::hisG/his3::hisG leu2::tet*R-GAL4AD-*URA3/LEU2* pTet-*HST3/hst3Δ::HIS3*	This study

Nature Medicine: doi:10.1038/nm.2175

Supplementary Table 3. List of primers used in this study.

PCR-product or plasmid	Primer	Sequence (5'-3')
Gene deletion in SN152		
RTT109$_{short}$	MR1801	GATTCTTAAAATCGCGTCCTTTCATACTTTCCAATCGGTCCAAGTGAATTACTGGATTACTGTGGAATTGTGAGCGGATA[1]
	MR1803	CCCAGGTTTTAATCGAACTGGCCCCAATGCAATTACGTATCGTGTGAGGTTATCGTCGATGTTTTCCCAGTCACGACGTT[1]
RTT109$_{long}$	MR1802	TGTTGGGAATCGATTGAGGCAGAAAAAATTTCAAGCATATTGAGAATAACTAGAAGTGTTGATTCTTAAAATCGCGTCCT[2]
	MR1804	ACATCTATACTCTAATCACAATATATGATAAAATAATGACAGAGGTCAAGACCAATCGGTCCCAGGTTTTAATCGAACTG[2]
HST3$_{short}$	MR1808	ATTTGACAAGATTATCAGTACTGTCAAGAAAACTCACTATAAAACAACTACTTTTAGACGTGTGGAATTGTGAGCGGATA[1]
	MR1810	TCATTGCAATTGATTCATTAATATTACATAATGTTTACATAATTTCCAACTCTGAAACCAGTTTTCCCAGTCACGACGTT[1]
HST3$_{long}$	MR1809	AAATACAACCATCGCCAGTCTAGTTGAACAATACATAGGACATATAAAGTCCATACACCCATTTGACAAGATTATCAGTA[2]
	MR1811	TCAACAAAAGTATCTTCTACAGGTTATAATTGGTATATATGTGTGTGTCATAACATTCAGTCATTGCAATTGATTCATTA[2]
HST3$_{short\ SAT1}$	MR2344	TTTGACAAGATTATCAGTACTGTCAAGAAAACTCACTATAAAACAACTACTTTTAGACGGAGCTCCACCGCGGTGGCGGCCGCT[3]
	MR2345	TCATTGCAATTGATTCATTAATATTACATAATGTTTACATAATTTCCAACTCTGAAACCAGGTACCGGGCCCCCCCTCGAGGAA[3]
Gene reintroduction		
pRTT109$_{rev}$	MR1945	TCCCCGC**GGGCCC**TGATCTGGTTCTGTGGATTTTGTTGTG[4]
	MR1946	AAGGAAGCGGCCGCCTACCACAGTTGAACCACTACTACTAG[4]
pRTT109$_{down}$	MR1947	GCGCTCGAGCTGTATGTATGATTGTTGGAGGGTGAC[4]
	MR1948	GCGGGGCCCCTACCACAGTTGAACCACTACTACTAG[4]
GRACE strains construction		
HST3 HIS3$_{short}$	MR1970	TTTGACAAGATTATCAGTACTGTCAAGAAAACTCACTATAAAACAACTACTTTTAGACGGATCTTTCTGTGACTCAATTC[5]
	MR1972	TCATTGCAATTGATTCATTAATATTACATAATGTTTACATAATTTCCAACTCTGAAACCAATGGATTTTAGTCAGTAAC[5]
HST3 HIS3$_{long}$	MR1971	AAATACAACCATCGCCAGTCTAGTTGAACAATACATAGGACATATAAAGTCCATACACCCATTTGACAAGATTATCAGTA[2]
	MR1973	TCAACAAAAGTATCTTCTACAGGTTATAATTGGTATATATGTGTGTGTCATAACATTCAGTCATTGCAATTGATTCATTA[2]
HST3 P$_{short}$	MR1974	TAGAGTATTATACCAAGTATCTTTTGCACATATTCCTCATAGTCAATTACGTAAAAAAAGAGCGTCAAAACTAGAG[6]
	MR1976	GAGGTGTCAGCTGGCATACTAGTGCCTGAATTGTCAAGTAAGTCAATCAGTATCATCGTCTAATTTAGTGTGTGTATTTG[6]
HST3 P$_{long}$	MR1975	TGTTTTTTACTTGTTCAGTATTCTAATAATCATTACCTACACATTTTCATCCAATAGCTTTAGAGTATTATACCAAGTAT[2]
	MR1977	GTAAGAACAGTCATTTTCTTACTTTTAGATATAAATTTGATCACCTCATGTAGCTTTATCGAGGTGTCAGCTGGCATACT[2]
Probes		
RTT109 probe 1	MR2008	GTTTATAGGTGGTGTATTTGGACCTAC
	MR2009	TCCCAATTGTAGATTTGGGGATATGTATCG

Nature Medicine: doi:10.1038/nm.2175

RTT109 probe 2	MR2127	ATCACCGATACATATCCCCAAATCTAC
	MR2128	ATTGTCACAGTTGAAACCAGATCATCG
HST3 probe 1	MR2017	TCATTTTTACTATGGCTTTGGTGTTG
	MR2018	TGAGGAATATGTGCAAAAGATACTTG
HST3 probe 2	MR2058	GATACTGATTGACTTACTTGACAATTC
	MR2129	TCAAATAGCACTTTAGCCACAGGTTCATC
SWC4 probe	MR2056	ATGTCAGCAAATGATATTCTTGATG
	MR2057	CATCGTCCTTGATCTGGTTCTGTGG
RT-PCR		
ACT1	Act1_CA_F	TCCAGAAGCTTTGTTCAGACCAGC
	Act1_CA_R	TGCATACGTTCAGCAATACCTGGG
HST3	Hst3_CA_F	AAAGAAGAGCCAATTCCCACACCG
	Hst3_CA_R	TTGGCACTGATTTCATCAGGCAGC

[1] Each primer contains 60 bp complementary to the *RTT109* or the *HST3* locus and 20 bp complementary to pGEM-HIS1 or pRS-Arg4ΔSpeI (plasmid sequences are underlined).

[2] Each primer contains a 20-bp region complementary to the 5'-end of the first set of primers (underlined) and an extended 60-bp region of the *RTT109* or *HST3* locus.

[3] Each primer contains 60 bp complementary to the *HST3* locus and 24-25 bp complementary to the *SAT1* flipper cassette present on the plasmid pSFS2A (plasmid sequences are underlined).

[4] Each primer contains a region complementary to the *RTT109* locus. Restriction site(s) (underlined or bolded) were added for cloning of the amplicon into pSFS2A and for recovery of the *RTT109* integration cassette used to transform the rtt109Δ/Δ strain.

[5] Each primer contains a region complementary to the *HST3* locus and a region complementary to pHIS3 (plasmid sequence is underlined).

[6] Each primer contains a region complementary to the *HST3* locus and a region complementary to pTET (plasmid sequence is underlined.

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Supplementary Table 4. Quantification of proinflammatory cytokines in mice treated with NAM.

Experimental conditions[a]			Cytokines (pg mL^{-1})					
Mouse groups	Total # injections	Mice euthanized at	IL-6	MCP-1	KC	TNF-α	IL-10	IFN-γ
0 NAM-24 h	0	24 h	6.6 ± 0.8	21.7 ± 6.1	14.3 ±0.8	12.7 ± 0.8	7.3 ± 2.2	5.1 ± 1.1
2 NAM-24 h	2	24 h	8.7 ± 1.3	26.7 ± 9.4	16.5 ± 2.8	11.0 ±1.6	6.5 ± 2.8	6.0 ± 2.3
2 NAM-72 h#1	2	72 h	1.7 ± 1.1	N.D[c]	10.0 ± 0.9	10.0 ± 0.6	N.D[c]	N.D[c]
2 NAM-72 h#2[b]	6	72 h	3.8 ± 1.2	N.D[c]	11.0 ± 0.6	8.7 ± 0.9	N.D[c]	1.8 ± 1.2
2 NAM-120 h	2	120 h	8.0 ± 0.2	44.7 ± 10.5	14.2 ± 0.6	13.6 ± 0.5	13.3 ± 1.1	7.3 ± 0.3
0 NAM-120 h	0	120 h	7.7 ± 0.2	34.3 ± 1.7	15.7 ± 0.6	13.8 ± 0.8	14.2 ± 0.5	7.7 ± 0.2

[a] Mice were injected with NAM (500 mg kg^{-1} per injection) twice a day and were euthanized at the indicated time after the first injection.

[b] Two injections daily were performed for three consecutive days.

[c.] N.D. = not detected.