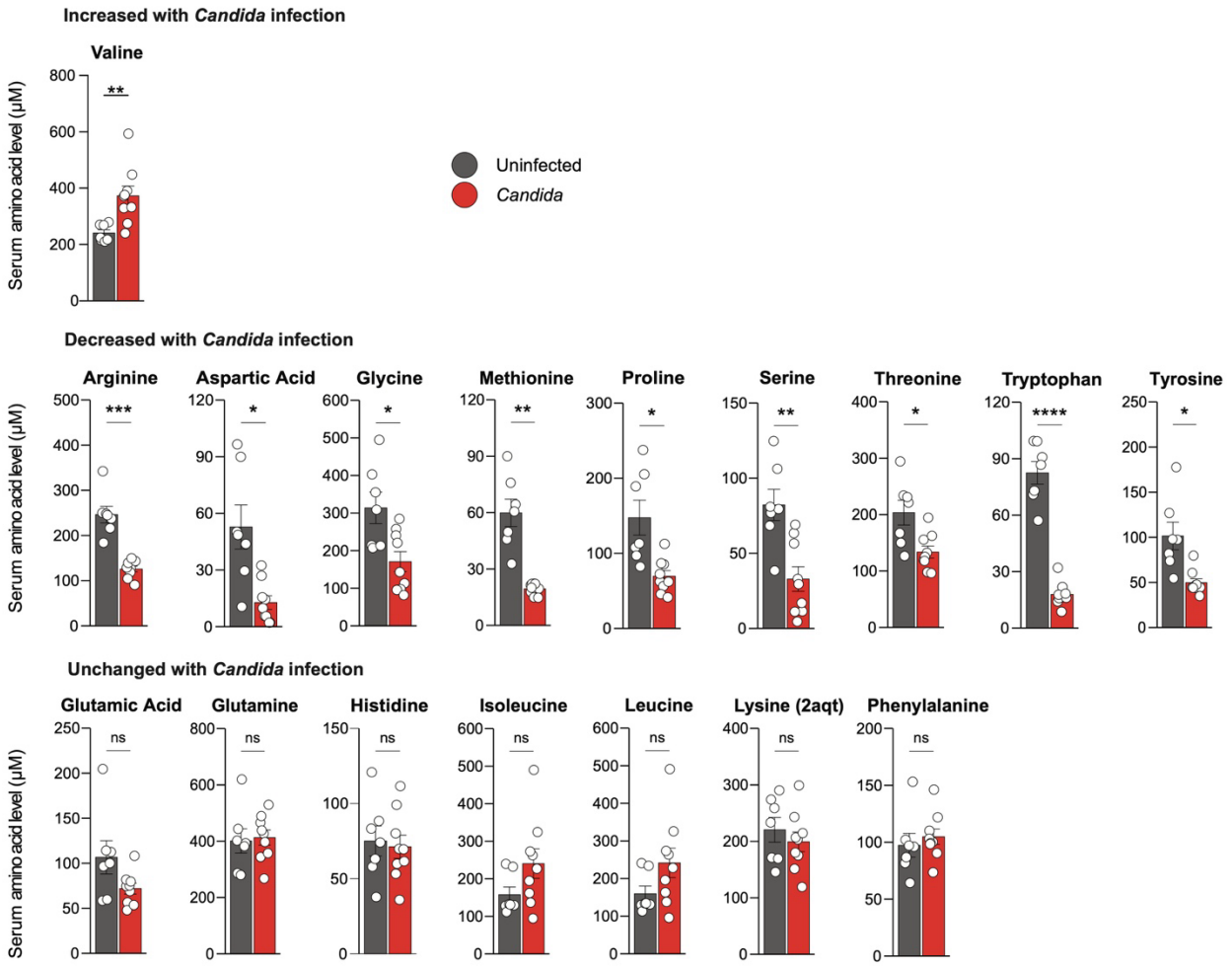

FIGURE S1. *C. albicans*-infected mice display disrupted amino acids metabolism
Serum amino acid levels from mice systemically infected with *C. albicans* SC5314, 24 h after infection. Uninfected mice were mock-infected with PBS. Shown are means and SEM of at least 6 individual animals. (students t-test with multiple comparison analysis, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$). ns-not significant. The alanine data is shown in **Fig. 1A**.

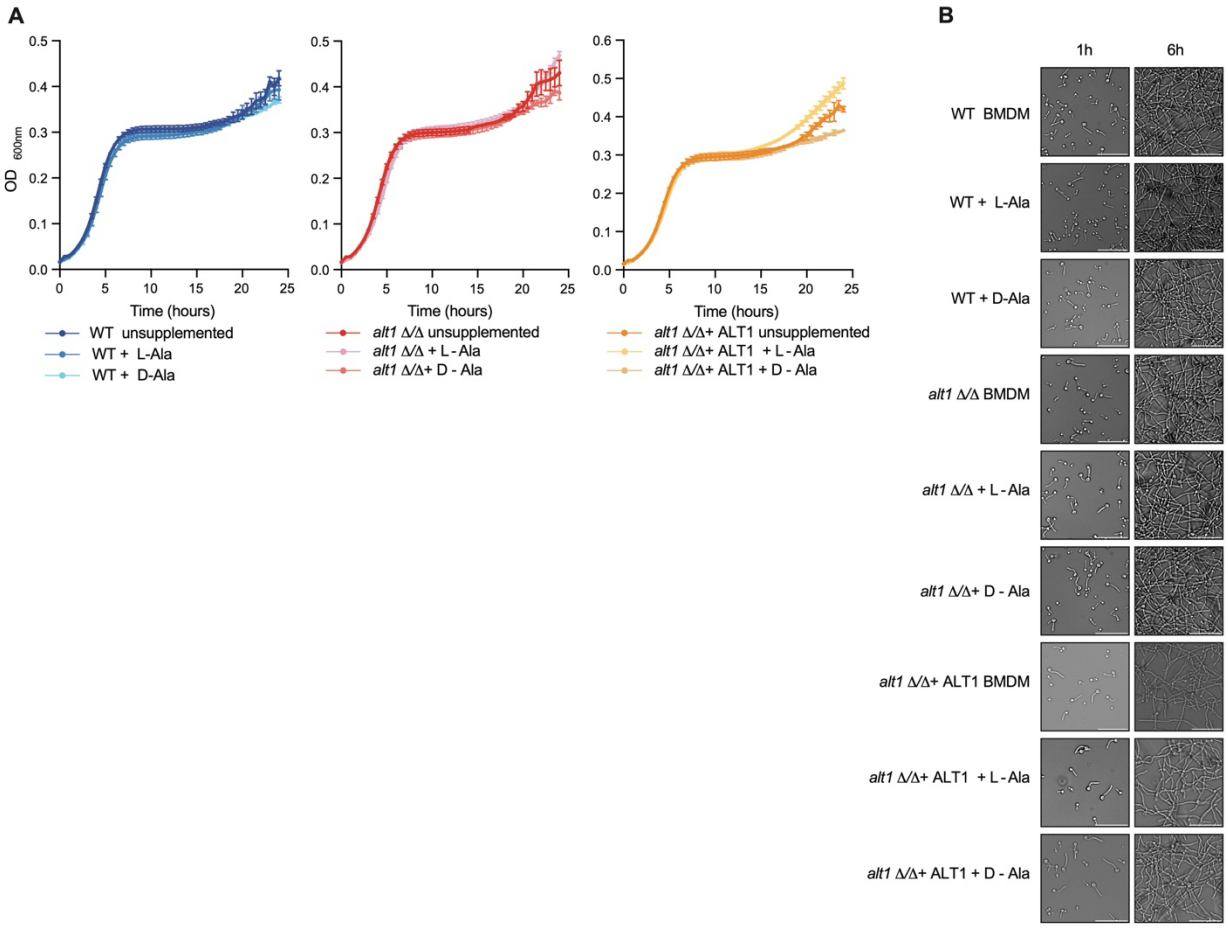

FIGURE S2. Impact of alanine on *C. albicans* growth and filamentation

A. The growth rate of wild type *C. albicans* (WT), *alt1*Δ/Δ, and *alt1*Δ/Δ+*ALT1* strains in BMDM medium with or without 10 mM L- or D-alanine. The optical density (OD) at 600 nm was measured every 30 min for 24 h at 37 °C. Shown are means and SEM for 3 independent replicates.

B. Images of WT, *alt1*Δ/Δ, and *alt1*Δ/Δ +*ALT1 C. albicans* filamentation in base BMDM medium with or without 10 mM L- or D-alanine. Images were taken at the indicated time points. Scale bar =50 µm.

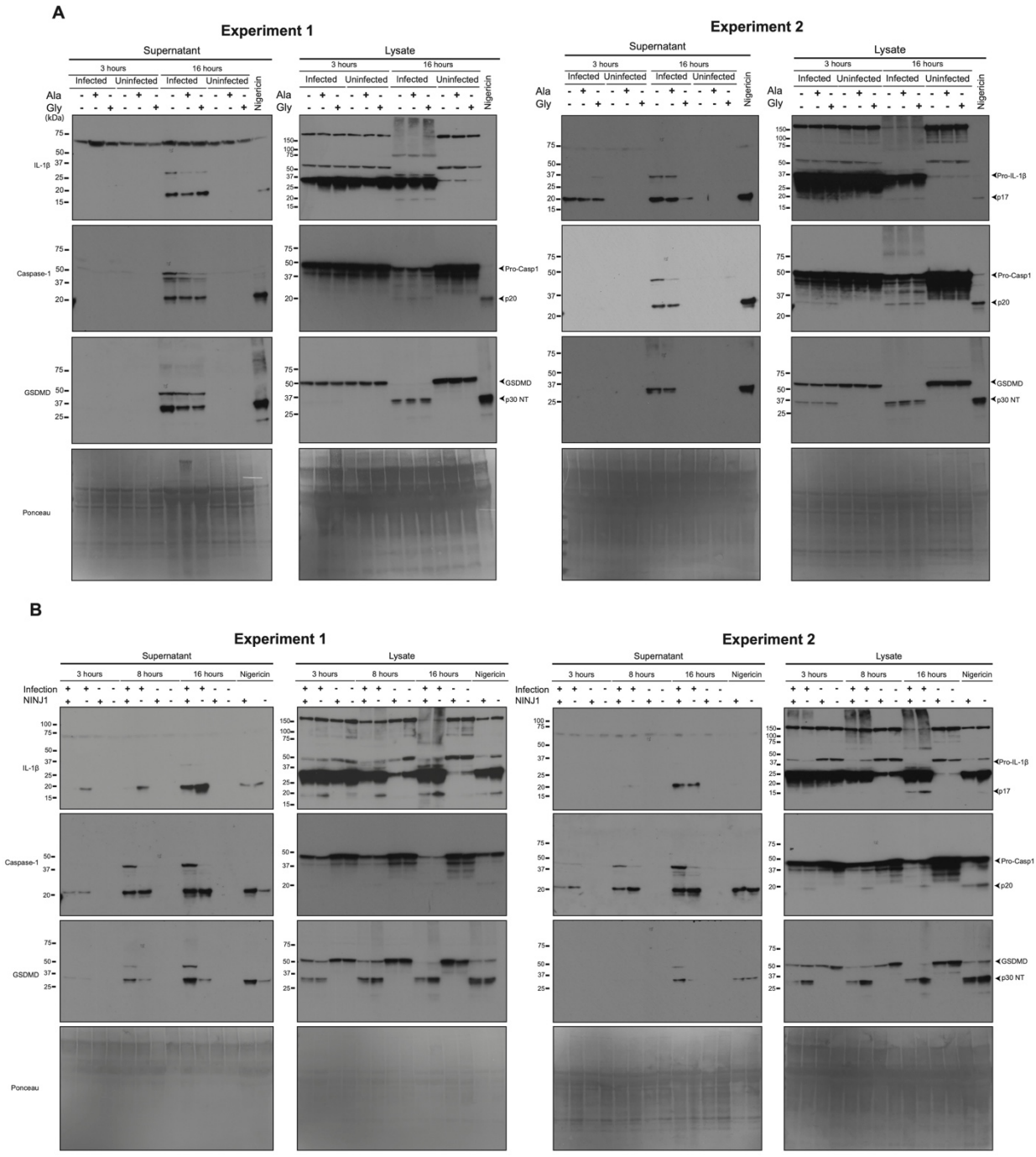

FIGURE S3. Uncropped Western blots

A. Uncropped westerns from **Fig. 4C**. Experiment 1 is represented in the figure.

B. Uncropped westerns from **Fig. 5J**. Experiment 1 is represented in the figure.

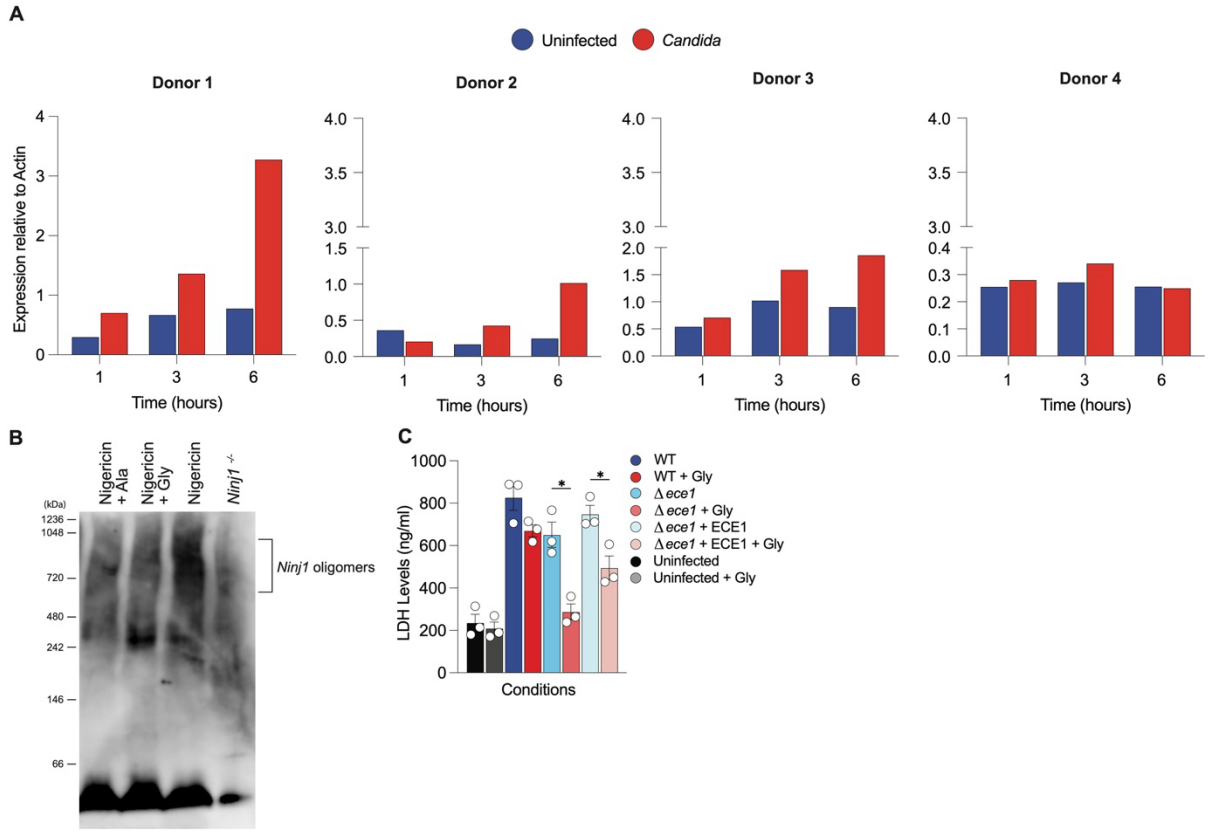

FIGURE S4. Further characterisation of the roles of NINJ1 following *C. albicans* infection and inhibition with alanine

- **A.** Quantitative PCR analysis of *Ninj1* gene expression in hMDMs after *C. albicans* challenge. Gene expression was normalized to actin (*Act1*). Shown are means from 4 independent donors, each analysed in two technical replicates.
- **B.** BN-PAGE of NINJ1 in mouse BMDMs following activation with nigericin, in the presence or absence of glycine and alanine. NINJ1 oligomers are indicated with a square bracket.
- **C.** LDH release from tdBLaER1 cells infected with *C. albicans* wild type (WT), *ece1Δ/Δ* mutant and *ece1Δ/Δ +ECE1* revertant. Uninfected cells are shown as control. Samples were taken 24 h post infection. Shown are means and SEM from 3 independent experiments. Measurements for all of WT cells (infected and uninfected groups) were performed and analyzed within the same experiments as those shown **Fig. 5H,** using a shared control group across the figures. (Welsh's t-test correction * $p \leq 0.05$).

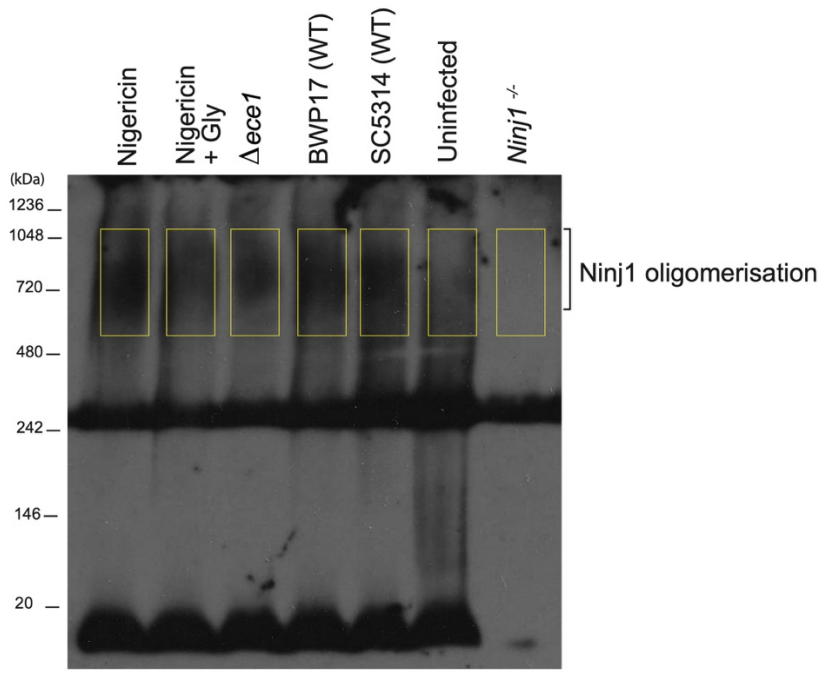

FIGURE S5. BN-PAGE of NINJ1 following *C. albicans* infection of macrophages.
BN-PAGE blot of NINJ1 oligomerization in BMDMs at 3 h post infection with WT and *ece1Δ/Δ C. albicans* strains. The quantified regions are demarcated by a yellow box, between 480 -1048 kDa as determined in [46]. Quantification was performed by ImageJ and is shown in **Fig. 6C**.

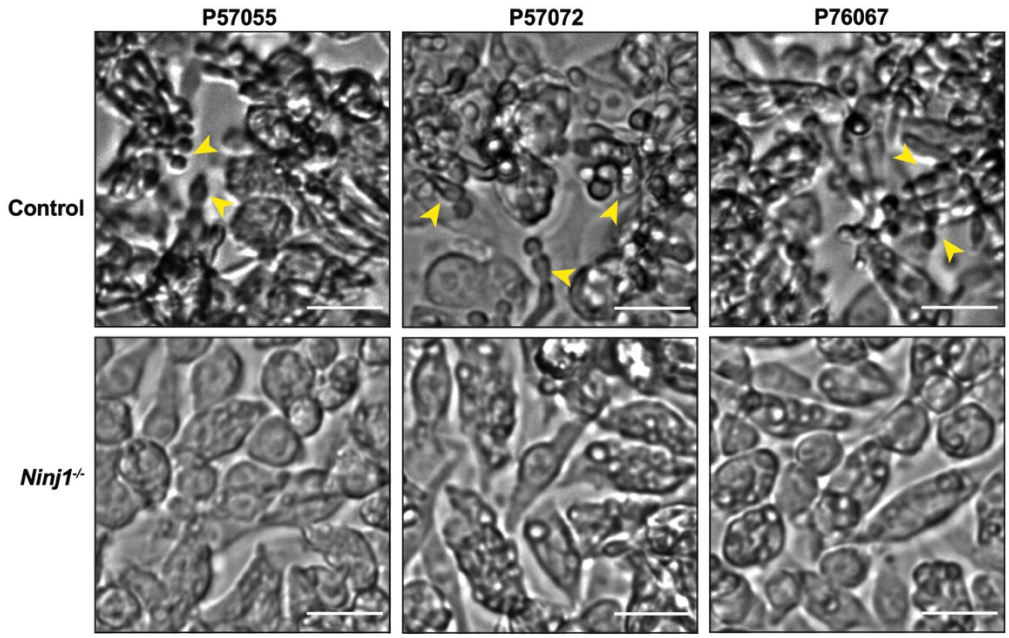

FIGURE S6. NINJ1 promotes macrophage escape of diverse *C. albicans* clinical isolates
Still images from live-cell imaging movies of control and *Ninj1*$^{-/-}$ iBMDMs infected with the indicated *C. albicans* clinical isolates. Yellow arrows indicate escaped hyphae. Images are from 9.5 h post challenge, and are taken from **Movie S2**. Scale bar =100 µm.

Table S1. Strains and cell lines used in this study

Experimental models: Cell lines		
Wild type iBMDMs: C57BL/6 Cre-J2 background	Gifted by Marco Herold, Walter and Eliza Hall Institute of Medical Research	43
Casp1$^{-/-}$*;Casp11*$^{-/-}$*;Casp12*$^{-/-}$*;Casp8*$^{-/-}$*;RIP3K*$^{-/-}$ iBMDMs: C57BL/6 Cre-J2 background	Gifted by Marco Herold, Walter and Eliza Hall Institute of Medical Research	43
Control iBMDMs: C57BL/6J Cas9+ background *Mlkl* $^{-/-}$	Walter and Eliza Hall Institute of Medical Research	69
Ninj1$^{-/-}$iBMDMs: *Mlkl* $^{-/-}$ *Ninj1*$^{-/-}$ C57BL/6J Cas9+ background	Walter and Eliza Hall Institute of Medical Research	69
Experimental models: Organisms/strains		
Mouse: wild type C57BL/6J	Monash Animal Research Platform (Melbourne, Australia)	RRID: IMSR_JAX:000664
Mouse: *Nlrp3*$^{-/-}$ C57BL/6J	Walter and Eliza Hall Institute of Medical Research	MGI ID:3721141
Mouse: wild type C57BL/6N	Monash Animal Research Platform (Melbourne, Australia)	RRID: IMSR_JAX:005304
Mouse: *Gsdmd*$^{-/-}$ C57BL/6J	Walter and Eliza Hall Institute of Medical Research	RRID: IMSR_JAX:032,410
Mouse: *Ninj* $^{+/+}$ C57BL/6J*	Contributed by James Vince, Walter and Eliza Hall Institute of Medical Research	MGI ID: 1196617
Mouse: *Ninj* $^{-/-}$ C57BL/6J*	Contributed by James Vince, Walter and Eliza Hall Institute of Medical Research	MGI ID: 1196617
C. albicans SC5314, Clade I	Clinical isolate	Traven lab
C. albicans P57055, Clade 3	Clinical isolate, BSI	BEI Resources, NIAID, NIH: *Candida albicans*, Strain P78042, NR-29443.
C. albicans P57072, Clade 2	Clinical isolate, BSI	BEI Resources, NIAID, NIH: *Candida albicans*, Strain P78042, NR-29443.
C. albicans P76067, Clade 2	Clinical isolate, BSI	BEI Resources, NIAID, NIH: *Candida albicans*, Strain P78042, NR-29443.
C. albicans P78042, Clade 3	Clinical isolate, BSI	BEI Resources, NIAID, NIH: *Candida albicans*, Strain P78042, NR-29443.
C. albicans SC5314: *ece1*Δ/Δ	*ece1*::Δ/*ece1*::Δ *NEUT5L*/*neut5l*::*FRT*	This study
C. albicans M2974: *ece1*Δ/Δ + *ECE1*	*ece1*::Δ/*ece1*::*ECE1*, *FRT NEUT5L*/*neut5l*::*FRT*	This study
C. albicans: SN152	*leu2Δ*/*leu2Δ*, *arg4Δ/arg4Δ, his1Δ/his1Δ, ura3Δ/URA3, iro1Δ/IRO1*	67
C. albicans: SN425,	*leu2Δ::C. dublinensis HIS1*/*leu2Δ::C. maltosa LEU2*, *arg4Δ/arg4Δ*::*C. dublinensis ARG4, his1Δ/his1Δ, ura3Δ/URA3, iro1Δ/IRO1*	67
C. albicans: alt1Δ/Δ	*alt1Δ*::*C. dublinensis HIS1*/*alt1Δ*::*C. maltosa LEU2*, *arg4Δ/arg4Δ, leu2Δ/leu2Δ::C. dublinensis ARG4, his1Δ/his1Δ, ura3Δ/URA3, iro1Δ/IRO1*	This study
C. albicans: alt1Δ/Δ+ALT1	*alt1Δ*::*C. dublinensis HIS1*/*alt1Δ*::*C. maltosa LEU2*, *arg4Δ/arg4Δ,leu2Δ/leu2Δ::ALT1::C. dublinensis ARG4, his1Δ/his1Δ, ura3Δ/URA3, iro1Δ/IRO1*	This study

*Indicates littermate mouse strains

Table S2. Primers used in this study

F = Forward primer
R = Reverse primer

Name	***Purpose***	***5' to 3' sequence***	***Source***
qPCR			
ACT1 (F)	For qPCR of *ActinA*	CGCCGCCAGCTCACC	This study
ACT1 (R)		ACGCAGCTCATTGTAGAAGGT	This study
NINJ1 (F)	For qPCR of *Ninj1*	AGTAGGACACCCAGGACCC	This study
NINJ1 (R)		CTGCAGCTGTCCAGTTCTGA	This study
Strain construction			
sgRNA-ECE1-F	For CRISPR deletion of *ece1*	ATTTGTAAAGCAAAAACAGTAGCACG	This study
sgRNA-ECE1-R		AAAACGTGCTACTGTTTTTGCTTTAC	This study
SM-ECE1-F	For CRISPR deletion of *ece1*	AACAAACAACTTTCCTTTATTTTACTACCAACTATTTTCCATTCGTTAAAATGCTCAGCA	This study
SM-ECE1-R		TGGAATAAAAGATTAAGCTTGTGGAAAACAAATTTTTATCTGCTGAGCATTGGTAGTAAA	This study
ECE1-3'-F-XhoI	For complementation of *ece1*	TGCTCTCGAGATAAAAATTTGTTTTCCACAAGC	This study
ECE1-3'-R-ApaI		AGTAGGGCCCTTAATTAAATGAATAACAAGAATC	This study
ECE1-5'-F-SacI	For complementation of *ece1*	TTTTGAGCTCTCATAACCTTAAGAAATTGTTTTC	This study
ECE1-3'-comp-R-SacII		AGTACCGCGGTTAATTAAATGAATAACAAGAATC	This study
ALT1 deletion (F)	For deletion of *ALT1*	CAATAATAATCACAGGCATTTGAAACAAACAAAGAAACTAGATACTAATACATATATACAAACAAGAAAATAAATAAAAAAAAAAATTAGACAATCAATATTTCCCAGTCACGACGTT	This study
ALT1 deletion (R)		TGTCTTAACTTTGATTTTTTTTTTTTCTTTTTTTGGTTATCATATTTTGTATTTTATAGACAAGACATATATGTACATATATAAATATATATAGGGTATGTGGAATTGTGAGCGGATA	This study
ALT1 complementation (F)	For complementation of *ALT1*	CCAGAAATACTCCCCTTTTCTTATTCCTTTCTTTTAAAACCGGGCCCCCCCTGACTCACCCACTCAACTC	This study
ALT1 complementation (R)		ACACCATCGAAAAAGTCGATACATTTGCGGTACAGAAATGTTCTTATCGAAACTTCCCGTTTCCACTGAA	This study

Movie S1. Ninj1 is the effector of glucose starvation-induced macrophage cell lysis during *C. albicans* infection

Movie S2. Ninj1 is a macrophage escape factor for *C. albicans* clinical isolates

Data S1. Numerical data used to construct the graphs shown in the figures.
Data for all graphs in figures is shown here.