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Supplemental information

The histone chaperone HIR maintains chromatin states to control nitrogen assimilation and fungal virulence

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Figure S1

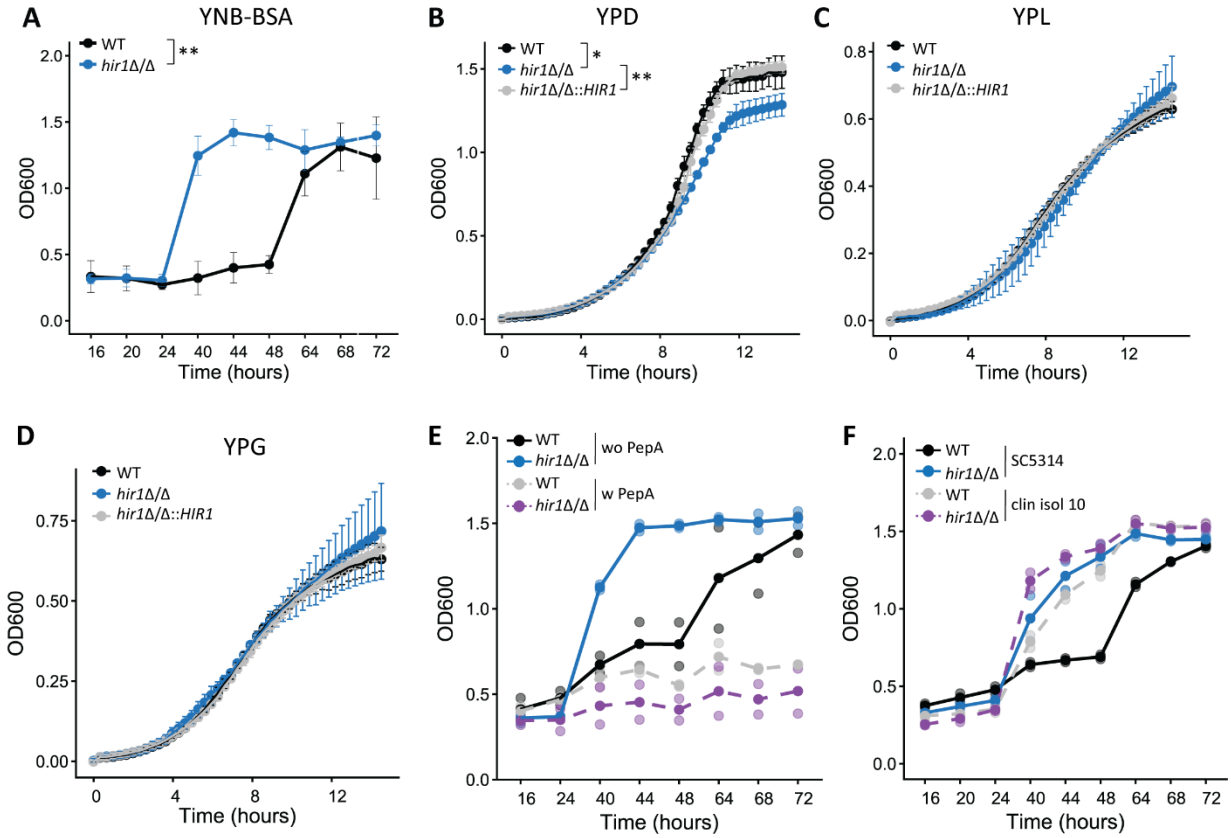

Figure S1: *HIR1*-deletion enhances growth on protein. *Related to Figure 1.*

A. Growth of *C. albicans* in YNB-BSA at 30 °C. Graphs show the mean +/- SD from 3 biological replicates. **p < 0.01 with Student's *t*-test after equal variance testing (F test) after 40 hours. **B-D**. Growth of the indicated *C. albicans* strain in YP medium supplemented with different carbon sources at 30 °C over a 12 hours period. OD_{600} values were measured every 20 minutes. Graphs show the mean +/- SD from 3 biological replicates. *p < 0.05, **p < 0.01 with one-way ANOVA followed by Tukey's multiple comparison test at the 12.2 hour time point after equal variance testing (Bartlett's test). **E-F.** Growth of the indicated *C. albicans* strains in YCB-BSA +/- Pepstatin A (PepA; E) and in YCB-BSA (F). Panel E is related to Figure 1C, showing the parallel treatment of PepA. Panel F depicts an additionally tested clinical isolate and is linked to Figure 1G. These additional conditions were split into two graphs to increase clarity. Graphs show the mean (solid dots) and single measurement values (opaque dots) from 2 biological replicates.

YP yeast peptone, YPD YP dextrose, YPG YP glycerol, YPL YP lactate, YPR YP raffinose, wo without, w with.

Figure S2

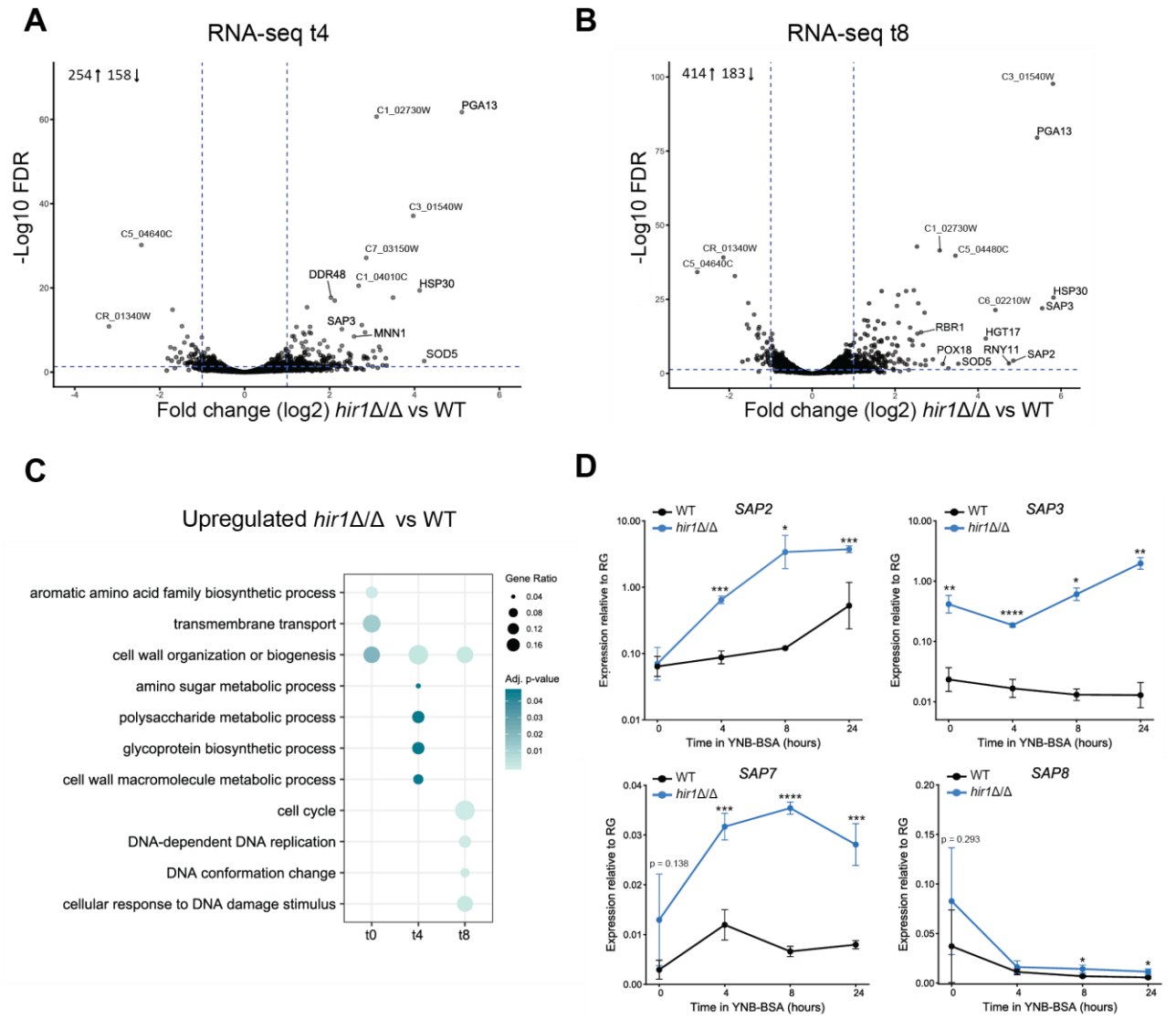

Figure S2: Hir1 functions in gene regulation during growth on protein. *Related to Figure 2.*

A-B. Volcano plots showing the global effect of deleting *HIR1* on gene expression after 4 hours (A; t4) and 8 hours (B; t8) in YCB-BSA. The x-axis represents the log2-fold change in mRNA expression in *hir1*Δ/Δ vs WT and the y-axis shows the negative log10 FDR values. Horizontal dashed blue lines indicate a FDR of 0.05, and vertical lines depict log2-fold change values of 0.58 and -0.58. The number insert in the top left corner indicates the number of up- and downregulated genes (FDR < 0.05 and log2-fold change < -0.58 or log2-fold change > 0.58 in the mutant relative to the WT). **C.** GO term analysis of upregulated genes (FDR < 0.05 and log2-fold change > 0.58) in *hir1*Δ/Δ cells at the indicated time (t0 YPD, t4 YCB-BSA 4 hours, t8 YCB-BSA 8 hours). The gene ratio shows the number of genes enriched in the relevant GO term relative to the total input gene number. **D.** RT-qPCR analysis of SAP gene expression (relative to the reference gene [RG] *PAT1*) in YNB-BSA medium. Graphs show the mean +/- SD from 3 biological replicates. * p< 0.05, ** p< 0.01, *** p< 0.001, **** p< 0.0001 with Student's *t*-test after equal variance testing (F test).

FDR false discovery rate.

Figure S3

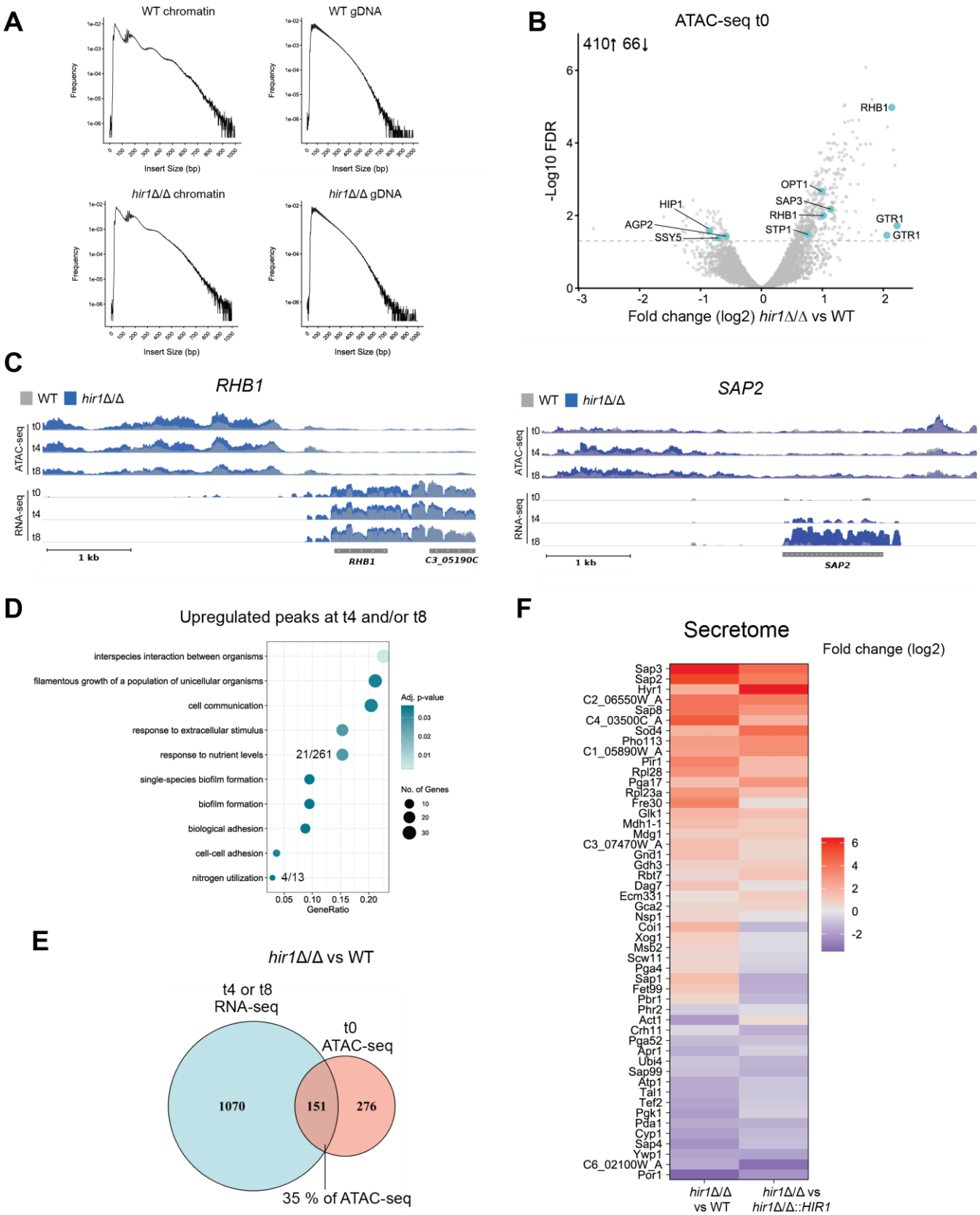

Figure S3: *HIR1* deletion alters chromatin accessibility upstream of genes related to nitrogen metabolism. *Related to Figure 3.*

Figure caption is on the next page.

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A. Fragment size distribution of ATAC-seq libraries prepared from intact chromatin and naked gDNA after paired-end sequencing. The x-axis shows the size in bp and the y-axis the frequency of the fragment length. **B.** Volcano plot representing differential ATAC-seq peak signals in *hir1*Δ/Δ cells vs WT during YPD growth (t0), with the log2-fold change plotted on the x-axis and the negative log10 FDR on the y-axis. Each dot corresponds to one ATAC-seq peak, which was annotated to the next adjacent gene. Turquoise colored dots represent selected genes involved in nitrogen metabolism, grey dashed line indicates a FDR of 0.05. The number insert in the top left corner depicts the number of significantly up- or downregulated peaks (FDR < 0.05 and log2-fold change > 0 or < 0, respectively). **C.** IGV tracks of normalized read coverage profiles from ATAC-seq and RNA-seq data for the *RHB1* and *SAP2* loci. Grey boxes represent ORFs, white arrows show direction of transcription. **D.** GO term analysis of genes with upregulated ATAC-seq peaks (located max. 2 kb upstream the TSS after 4 or 8 hours (t4 and t8) in YCB-BSA in *hir1*Δ/Δ cells. The GeneRatio represents the proportion of genes enriched in the depicted GO term relative to the total number of input genes. The number insert next to each dot shows the number of genes from the input dataset in comparison to the total number of genes associated with the depicted GO term. **E.** Venn diagram showing the overlap of genes with significantly altered ATAC-seq peak abundance during growth in YPD (t0) and differential RNA-seq signals (FDR < 0.05) after 4 and/or 8 hour culture in YCB-BSA (t4 or t8) in *hir1*Δ/Δ cells. **F.** Heatmap of proteins with significant altered abundance (adjusted p-value < 10^{-05}) in *hir1*Δ/Δ supernatants relative to WT and *HIR1* complemented cells. gDNA genomic DNA, FDR false discovery rate, ORF open reading frame, TSS transcription start site

Figure S4

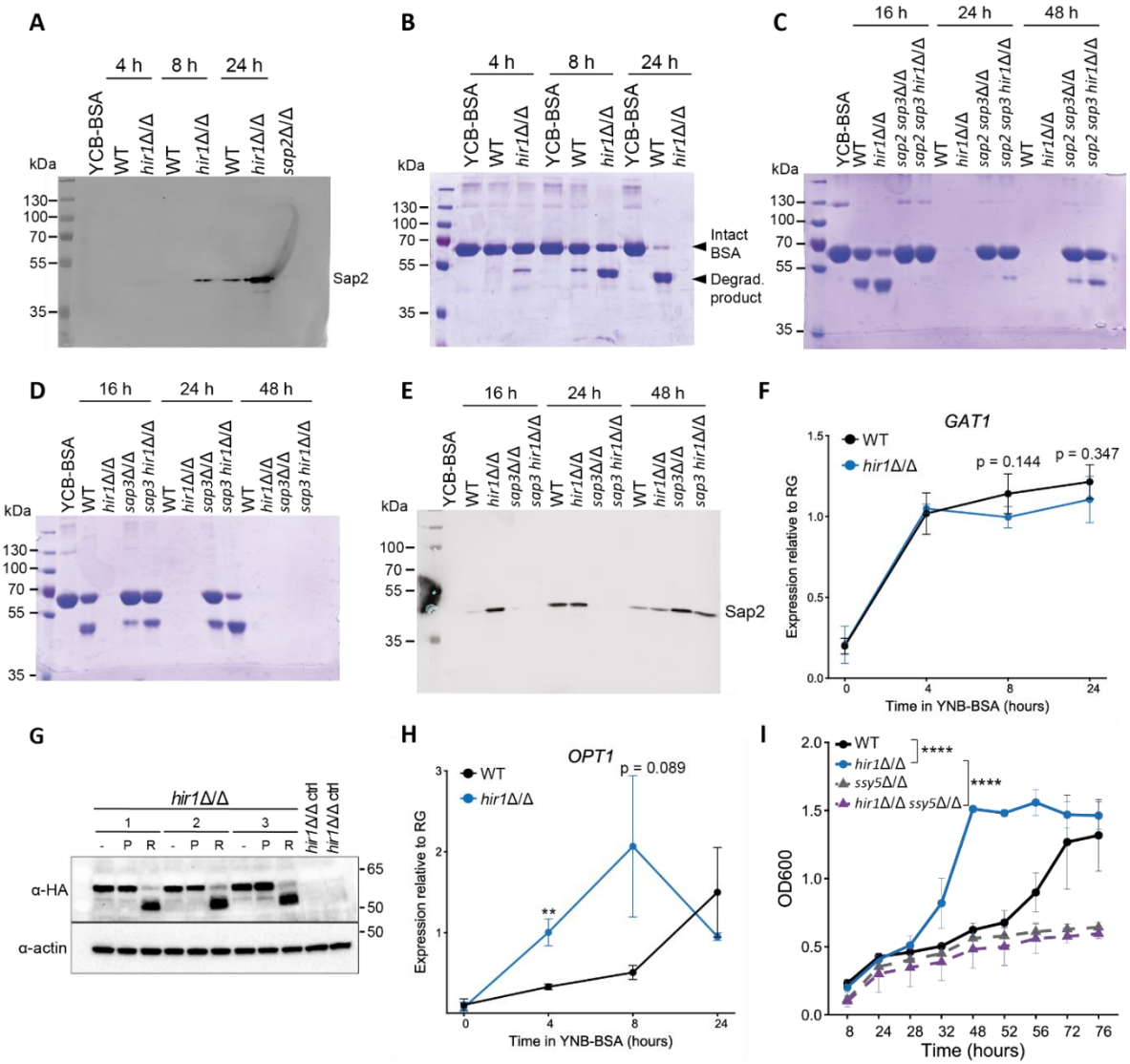

Figure S4: Proteolytic activity of *hir1*Δ/Δ requires Sap2, Sap3 and the SPS-sensor. *Related to Figure 4*. **A-B.** Immunoblot analysis for Sap2 (A) and Coomassie staining for BSA degradation (B) of WT and *hir1*Δ/Δ YCB-BSA culture supernatants used in parallel for both analyses. Results are from 3 independent experiments. **C-E.** Coomassie staining (C-D) and Sap2 immunoblot analysis (E) of YCB-BSA culture supernatants. Results are representative for 2 independent experiments. **F.** RT-qPCR analysis of *GAT1* expression (relative to the reference gene [RG] *PAT1*) in WT and *hir1*Δ/Δ cells in YNB-BSA. Graphs show the mean +/- SD from 3 biological replicates. P-values are derived from Student's *t*-test after equal variance testing (F test). **G.** Immunoblot analysis of 3xHA-tagged Stp1-3HA processing. Cultures of *hir1*Δ/Δ were grown for 2 hours in YNB-BSA at 30 °C, followed by treatment with arginine (R; SPS-inducer), proline (P; SPS non-inducer) or ddH_2O (-; solvent control). Protein extracts are from 3 biological replicates (1-3). Untagged strains grown for 2 hours in YNB-BSA served as control (ctrl). **H.** RT-qPCR analysis of *OPT1 g*ene expression (relative to the RG *PAT1*) in YNB-BSA. Graphs show the mean +/- SD from 3 biological replicates. **p < 0.01 with Student's *t*-test after equal variance testing (F test). **I.** Growth of the indicated strains in YCB-BSA at 30 °C. Graphs show the mean +/- SD from 3 biological replicates. ****p < 0.001 with one-way ANOVA followed by Tukey's multiple comparison test at 48 hours after testing for equal variances (Bartlett's test).

Figure S5

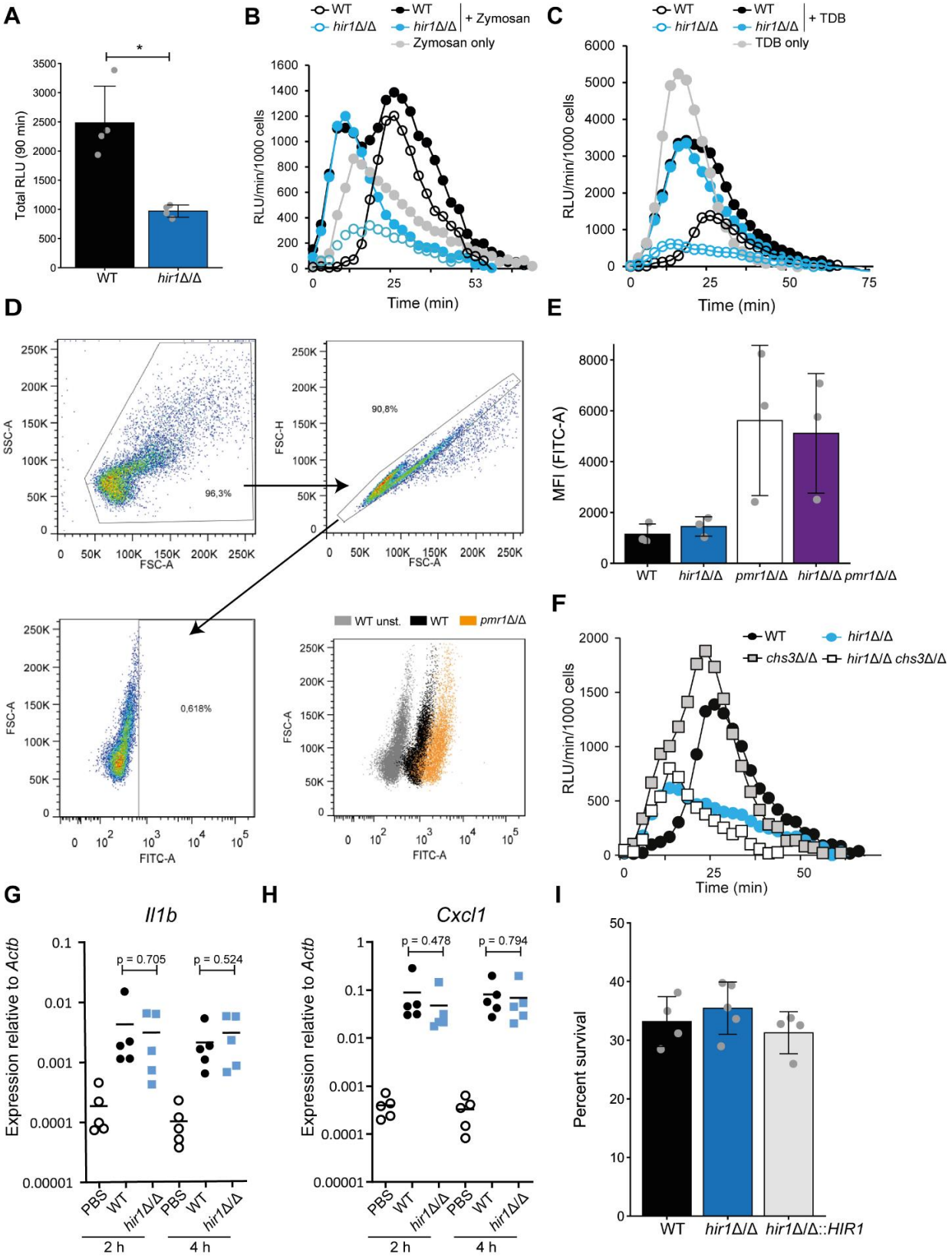

Figure S5: *In vitro* host response towards *hir1*Δ/Δ cells. *Related to Figure 5.*

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A-C. Isoluminol-dependent (A) or luminol-dependent (B-C) ROS assay with co-cultures of BMDMs and the indicated fungal strains alone or pre-mixed with Zymosan (B) or trehalose-6,6-dibehenate (TDB; C). Graphs show the mean +/- SD of the total RLU after 90 minutes from 4 independent replicates (shown as grey dots; A) or are representative for 3 replicates (B-C). *$p < 0.05$ with Student's *t*-test after equal variance testing (F-test). **D.** Representative FACS plot and gating strategy for fungal β-glucan exposure analysis shown in panel E. Example data from unstained (unst.) WT cells and one example showing the FITC-A signal from the indicated samples are depicted. **E.** Mean fluorescent intensity (MFI) values from FITC of flow-cytometry data. Graphs show the mean +/- SD from 3 independent replicates (grey dots). **F.** Luminol-dependent ROS assay of BMDMs challenged with the indicated fungal strains. RLU per minute per 1000 BMDMs over time are plotted. Data are representative for 3 independent experiments. **G-H.** RT-qPCR analysis of *Il1b* (G) and *Cxcl1* (H) expression in BMDMs stimulated with PBS, the WT or *hir1*Δ/Δ cells for the indicated times. Transcript levels are normalized to *Actb* (β-actin). Dots refer to single measurements. Horizontal lines indicate mean values from 5 replicates. Indicated p-values were calculated using Student's *t*-test of the indicated comparison. **I.** Fungal survival of the indicated genotypes co-cultured with BMDMs for 3 hours. Graphs show the mean +/- SD from 4-5 replicates (grey dots) pooled from two experiments using different BMDMs differentiation batches. BMDMs bone marrow-derived macrophages, RLU relative luciferase units.

Figure S6

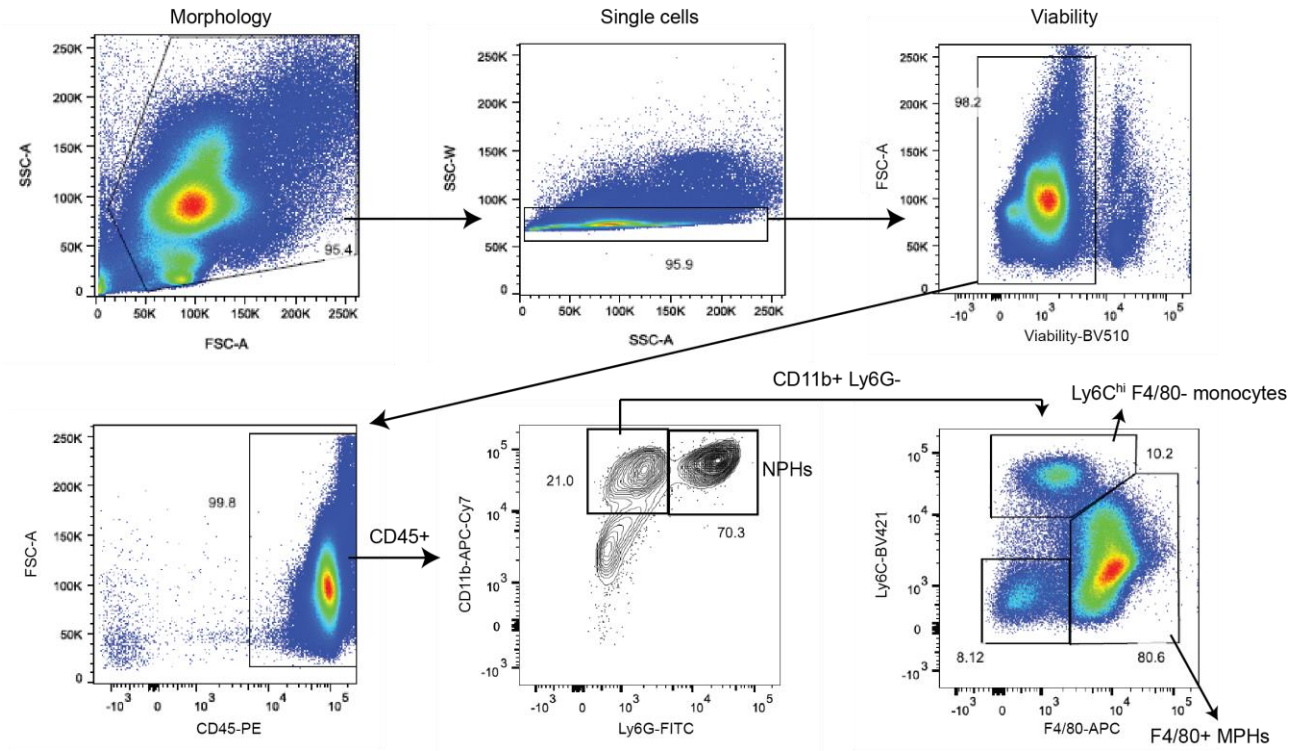

Figure S6: Gating strategy of flow cytometry data. *Related to Figure 6*.
Representative FACS plots and gating strategy of lavage samples from infected animals for assessing leukocyte recruitment to the peritoneum. Number inserts present percentage values of the gated population. NPHs neutrophils, MPHs macrophages.

Figure S7

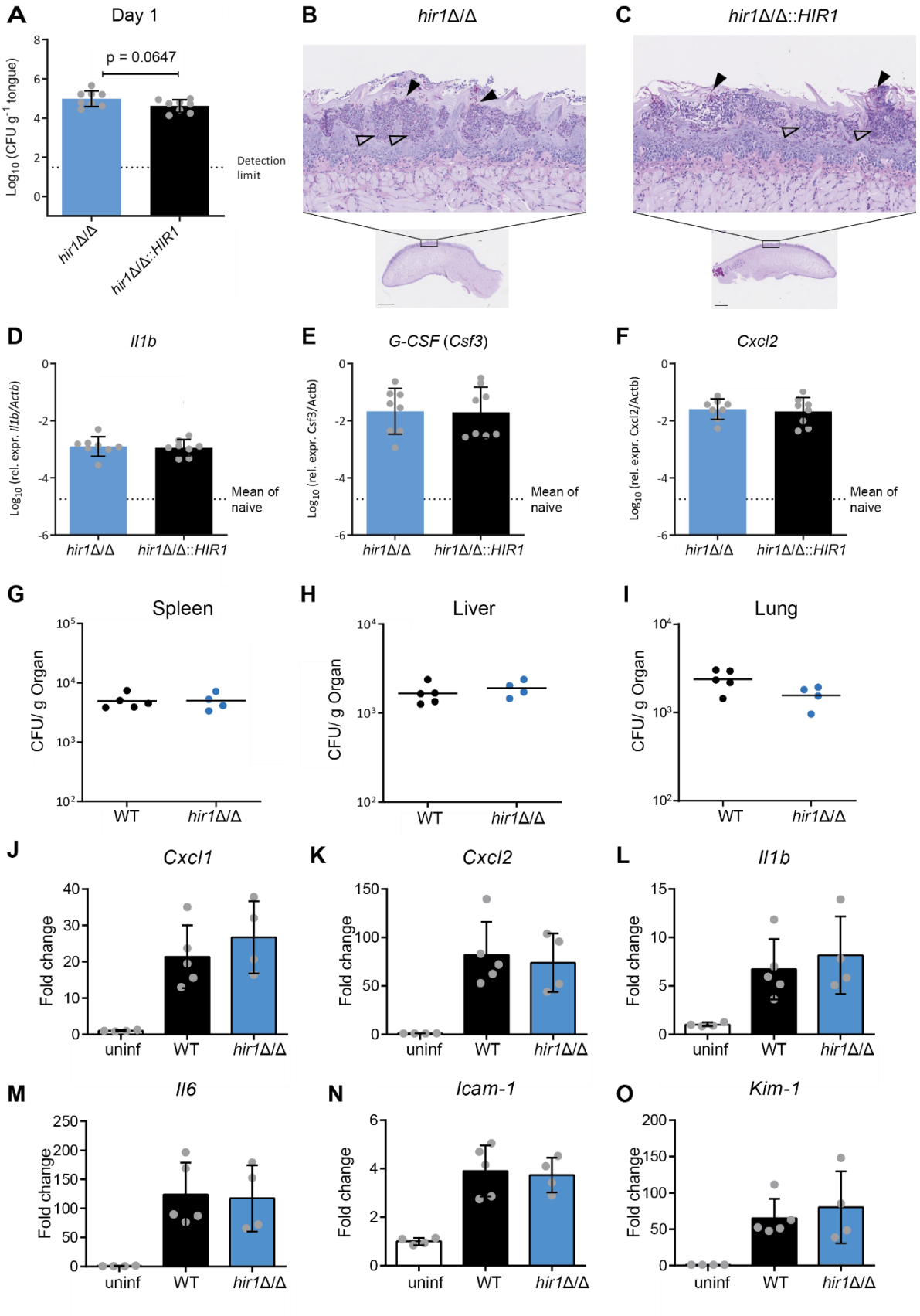

Figure S7: Host response to *hir1*Δ/Δ in oropharyngeal candidiasis (OPC) and systemic infection. *Related to Figure 7.*

Figure caption is on the next page.

Figure S7: Host response to *hir1*Δ/Δ in oropharyngeal candidiasis (OPC) and systemic infection. *Related to Figure 7*.

A. Fungal burden in murine tongues after 1 day of infection with the indicated genotypes. Graphs show the mean +/- SD from 8 animals per group that were pooled from two independent experiments (grey dots). The p-value from Student's *t*-test after equal variance testing (F test) is depicted. **B-C.** Histopathology of mouse tongues stained with periodic acid-Schiff (PAS) after 1 day of infection with *hir1*Δ/Δ cells (B) or the revertant strain (C). Representative images from whole tongue sections were taken with 2.15x (B) and 1.75x (C) magnification with 1 mm scale bar. Blow-ups represent 20x magnifications. Filled arrowheads indicate fungal filaments, empty arrowheads indicate leukocyte infiltrates. **D-F.** RT-qPCR analysis of whole tongue extracts after 1 day of infection with the indicated fungal strains. Gene expression is shown relative to β-*actin* (*Actb*). Graphs show the mean +/- SD from 8 animals per group that were pooled from two independent experiments (grey dots). **G-I.** Fungal burdens of mouse spleen (G), liver (H) and lungs (I) after 1 day of i.v. infection with the indicated fungal strains. The CFUs per g organ are shown. Each dot corresponds to one animal. Horizontal lines represent the mean value from 4-5 animals. **J-O.** RT-qPCR analysis of whole kidney lysates after 1 day of i.v. infection. Transcript levels are expressed as fold-change relative to the PBS (uninf) control after normalization to *Actb* (β-actin). Graphs show the mean +/- SD from 4-5 mice (grey dots).

CFU colony forming unit, uninf uninfected.

221 **Table S1. Oligos used in this study. *Related to STAR METHODS*.**

Strain construction		
Name	**Sequence (5'->3'; lower case letters denote overlaps used for Gibson assembly or *in vivo* cloning; underlined sequences represent restriction enzyme sites)**	**Purpose**
55IC_SAP3	ttctttcctgcgttatcccctgattctgtggataaccgtaccatggCCAAACCTTCAATGTACGTCC	Gene deletion
53IC_SAP3	gaggggggcccggtacccaattcgccctatagtgagtcgGTTGTTTTGATACACGGCACC	Gene deletion
35IC_SAP3	tagtgagggttaattgcgcgcttggcgtaatcatggtcatCCACAAACAATTACTCAAAGC	Gene deletion
33IC_SAP3	aacgcagaaaatgaaccggggatgcgacgtgcaagattaccatggCCAGTTGTTGTCAAATATGG	Gene deletion
5C_SAP3	GGATTGGATTATGCCGACTC	Integration check
3C_SAP3	GATAAAAACACTGCTGCTCAAG	Integration check
55_SAP3tag	GTGGGACATTGATTGCTTTACC	Loss of gene check
53_SAP3tag	ccactagcagcagaaccggaAGTAAGAGCAGCAATGTTAGAAG	Loss of gene check
55_IC_YEP_PMR1	ttctttcctgcgttatcccctgattctgtggataaccgtaccatggGATAGACGAAACAAACGAAGG	Gene deletion
53IC_PMR1	gaggggggcccggtacccaattcgccctatagtgagtcgGATTAGCACTCATTGAACTGTATAC	Gene deletion
35IC_PMR1	tagtgagggttaattgcgcgcttggcgtaatcatggtcatGACTGTATTTGGCTCCATGAAAG	Gene deletion
33_IC_YEP_PMR1	aacgcagaaaatgaaccggggatgcgacgtgcaagattaccatggGCACCTAGTAAACGAGCTATAG	Gene deletion
5C_PMR1	CTAGGCACTACAGGGAATGAG	Integration check
3C_2_PRM1	GCACAACCACGAAATGTACTAGTTG	Integration check
PMR1_LOG_fo	GTAACAACGCCAGATATTCTACTG	Loss of gene check
PMR1_LOG_re	CAATACAGCATGGTCTCCAAC	Loss of gene check
53_reintIC_PMR1	gaggggggcccggtacccaattcgccctatagtgagtcgTTCATGTTCACCTCCTCCCT	Gene complementation
55IC_STP1	ttctttcctgcgttatcccctgattctgtggataaccgtaccatggGTGTGAAATGAGATATCAACCG	Gene deletion
53IC_STP1	gaggggggcccggtacccaattcgccctatagtgagtcgCGTTGAAAGCTTATGACTGATGC	Gene deletion
35IC_STP1	tagtgagggttaattgcgcgcttggcgtaatcatggtcatCTTTATGGTATAGGGTTTTCGG	Gene deletion
33IC_STP1	aacgcagaaaatgaaccggggatgcgacgtgcaagattaccatggGACTGTTAGCATTGGTTCAG	Gene deletion

LOG-STP1_fo	CATTAGATGACGAGTTTGTACC	Loss of gene check
LOG-STP1_re	CTCTGACTTGCCTTTACACC	Loss of gene check
5C_STP1	GAAACTTGAAAGCTCTCAACG	Integration check
3C_STP1	CATCCAATCGCAATATCTCCTAC	Integration check
STP1_HA_fo	ccatattgaagctggcaagtgtacgggaactgtgcaatataaacagaatgtaagcaatctattactagatAACATCTTTTACCCATACGATG	Amplification of 3xHA-NAT tagging cassette for Stp1
C-HATagSTP1R	tattaaaactaaaagaatataagaaaagaaaagaagagaaataataaccgaaaaccctataccataaagaagtaaaactaatatcacacaaactaagacGCAGGTTAACCTGGCTTATCG	Amplification of 3xHA-NAT tagging cassette for Stp1
55ic_CHS3	ttctttcctgcgttatcccctgattctgtggataaccgtaAGGTTATAACACCAACCAAG	Gene deletion
53ic_CHS3	cgaggggggcccggtacccaattcgccctatagtgagtcgGTAGAAGAAGAAGATTAAGCG	Gene deletion
35ic_CHS3	ttagtgagggttaattgcgcgcttggcgtaatcatggtcatCTTCTTCAGGGTCCAGTTG	Gene deletion
33ic_CHS3	aacgcagaaaatgaaccggggatgcgacgtgcaagattacCTTTTGACATAATGGAATATGG	Gene deletion
5C_CHS3	AAGATAATTGTAAGTTGAATGAGG	Integration check
3C_CHS3	CCCTTGAGTATTAGCATTTCAC	Integration check
LOGfwd_CHS3	TTCCACTGATTTATTGAACCGTCC	Loss of gene check
LOGrev_CHS3	GAATCACGCTTACCTCTATTACCT	Loss of gene check
hk3	CATCATCTGCCCAGATGCGAAG	Universal 3' integration check
SATflipp_5C	TTTGGAACTAACGATGCATACGAC	Universal 5' integration check
YEp_ic fwd	gtaatcttgcacgtcgcatcc	Amplification of YEp352 backbone
YEP_ic rev	tacggttatccacagaatcaggg	Amplification of YEp352 backbone
SATflipp_fwd	CGACTCACTATAGGGCGAATTGG	Amplification of the NAT-flipper cassette
SATflipp_rev	ATGACCATGATTACGCCAAGC	Amplification of the NAT-flipper cassette

qPCR

Name	**Sequence (5'->3')**
RT5_PAT1	CAGCAACTGATTTATCGGAATGG
RT3_PAT1	ACATCTTCAGGGTTAGGTGG
SAP2_fwd	CCCAGTTACTAATGGTCAAGAAGG

SAP2_rev	GCAGTTTGATCACTATAAGTGACTTG
SAP3_fwd	TCAAGTTTCATGTCAAGCTGGT
SAP3_rev	ATGTCCCTTGTGAAGTAGTTCC
SAP7_fwd	TCTCAATGTAAAGTCAATGGAGGG
SAP7_rev	TGACCCATAGTACCTGATGCC
SAP8_fwd	GGTGATGAAAGTAGTCCAACCT
SAP8_rev	GTACCAGATGAAGCAGAAGCAG
RT5_OPT1	CTGGAACCAAATTGCAGGGT
RT3_OPT1	TTGGGAGTACCAAGTGTTGGA
RT5_STP1	ACACGATTCAATTTCACCACCA
RT3_STP1	CCGTTTGATGTAACTGAAGAACTG
RT5_Gat1	TACGATCAATGTCGCAAACTCC
RT3_Gat1	TGTGGTGACGGTTGACTAGG
Actb_fwd_KK	AGTGTGACGTTGACATCCGT
Actb_rev_KK	TGCTAGGAGCCAGAGCAGTA
ICAM1_fwd	TGGATACCTGAGCATCACCA
ICAM1_rev	CTGCTACCTGCACTTTGCC
P-Selectin_fwd	GAACAATCCAGGTTGCCTTG
P-Selectin_rev	CAGTTCATGTGCGATGAAGG
Il1b_fwd	CCAACAAGTGATATTCTCCATGAG
Il1b_rev	TCTTTCATTACACAGGACAGGT
KIM1_fwd	ACATATCGTGGAATCACAACGAC
KIM1_rev	ACAAGCAGAAGATGGGCATTG
Csf3_fwd_KK	TCTCCGTTACTTGGGGACAC
Csf3_rev_KK	CCACACTCAAGAATGGTCGC
Cxcl2_fwd_KK	TCCAGGTCAGTTAGCCTTGC
Cxcl2_rev_KK	CGGTCAAAAAGTTTGCCTTG
Il6_fwd	GAGGATACCACTCCCAACAGACC
Il6_rev	AAGTGCATCATCGTTGTTCATACA
Actb_fwd_SLG	CCCTGAAGTACCCCATTGAAC
Actb_rev_SLG	CTTTTCACGGTTGGCCTTAG
Cxcl2_fwd_SLG	AGTGAACTGCGCTGTCAATGC
Cxcl2_rev_SLG	GCAAACTTTTTGACCGCCCT
Csf3_fwd_SLG	CTTAAGTCCCTGGAGCAAGTG
Csf3_rev_SLG	GTGGCCCAGCAACACCAG
Il1b_fwd_SLG	TACAGGCTCCGAGATGAACA
Il1b_rev_SLG	AGGCCACAGGTATTTTGTCG