

Host skin lipids trigger MAT-dependent mating, pathogenic hyphal growth, and parasexual reproduction of *Malassezia furfur*

Giuseppe Ianiri

giuseppe.ianiri@unimol.it

Department of Agricultural, Environmental and Food Sciences, University of Molise

<https://orcid.org/0000-0002-3278-8678>

Erica Patriarca

Department of Agricultural, Environmental and Food Sciences, University of Molise

Bastien Tirtiaux

University of Molise, Department of Agricultural, Environmental and Food Sciences, Campobasso, Italy ;
NAMur Research Institute for Life Sciences, Faculty of Medicine, University of Namur, Namur

Márcia David-Palma

Department of Molecular Genetics and Microbiology, Duke University, Durham, North Carolina, USA

Anna Averette

Duke University

Eduardo Gushiken-Ibañez

University of Zurich <https://orcid.org/0000-0002-2510-9163>

Yves Poumay

NAMur Research Institute for Life Sciences (NARILIS), Faculty of Medicine, University of Namur, Namur, Belgium <https://orcid.org/0000-0001-5200-3367>

Salomé LeibundGut-Landmann

University of Zurich <https://orcid.org/0000-0002-5724-4837>

Joseph Heitman

Duke University School of Medicine <https://orcid.org/0000-0001-6369-5995>

Marco Coelho

Duke University Medical Center <https://orcid.org/0000-0002-5716-0561>

Article

Keywords: *Malassezia*, mating type, parasexual, hyphae, pathogenesis

Posted Date: July 27th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10372643/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: There is **NO** Competing Interest.

Host skin lipids trigger *MAT*-dependent mating, pathogenic hyphal growth, and parasexual reproduction of *Malassezia furfur*

Erica Patriarca^{1,6}, Bastien Tirtiaux^{1,2,6}, Márcia David-Palma³, Anna Floyd Averette³, Eduardo Gushiken-Ibañez⁴, Yves Poumay², Salomé Leibundgut-Landmann^{4,5}, Joseph Heitman³, Marco A. Coelho³, and Giuseppe Ianiri^{1*}.

¹University of Molise, Department of Agricultural, Environmental and Food Sciences, Campobasso, Italy

²NAMur Research Institute for Life Sciences (NARILIS), Faculty of Medicine, University of Namur, Namur, Belgium

³Department of Molecular Genetics and Microbiology, Duke University, Durham, North Carolina, USA

⁴Section of Immunology at Vetsuisse Faculty and Institute of Experimental Immunology, University of Zurich, Switzerland

⁵Medical Research Council Centre for Medical Mycology at the University of Exeter, Department of Biosciences, Faculty of Health and Life Sciences, Exeter, UK

⁶Contributed equally.

*Correspondence to giuseppe.ianiri@unimol.it

Abstract

The lipophilic yeast genus *Malassezia* dominates the human skin mycobiome and, while typically commensal, is associated with skin disorders in which hyphal cells are frequently observed. However, the mechanisms underlying hyphal differentiation and its contribution to pathogenesis remain poorly understood. Here, we show that hyphal growth in *Malassezia furfur* requires a complete mating-type system and is promoted by noncanonical reproductive interactions. Interspecific mating generates stable diploid hybrids with evidence of recombination, whereas intraspecific mating produces cell-fusion products that undergo recombination and haploidization, consistent with a parasexual cycle. Functional analyses reveal that the pheromone/receptor (*P/R*) locus governs cell recognition and fusion, while the homeodomain (*HD*) locus is required for hyphal development despite having lost canonical mating-type determination. Leveraging *in vitro* and *in vivo* skin models, we show that hyphal cells invade the epidermis more efficiently and elicit stronger inflammatory responses than yeast cells. Together, these findings link parasexual-like reproduction, mating-type loci function, and morphogenesis in *Malassezia* to pathogenic interactions with the host.

Keywords: *Malassezia*, mating type, parasexual, hyphae, pathogenesis

Introduction

The skin microbiome of warm-blooded vertebrates comprises diverse microbial communities that interact with host tissues and contribute to both homeostasis and disease. Within this ecosystem, the fungal component (the mycobiome) is frequently dominated by the lipophilic basidiomycetous yeast genus *Malassezia*. In humans, *Malassezia* spp. are by far the most abundant fungal colonizers of the skin and thrive in sebum-rich areas such as the scalp, back, or neck. This association reflects a strong reliance on external lipids, consistent with the loss of fatty acid synthase genes across the genus^{1,2}. Among the 21 species formally described, *M. globosa* and *M. restricta* are the most commonly found on humans, followed by *M. sympodialis* and *M. furfur*². In addition to their role as commensals, *Malassezia* species can protect their hosts from *Staphylococcus aureus*, a bacterium implicated in atopic dermatitis pathogenesis, through protease-mediated biofilm degradation by *M. globosa*³, and secretion of a hydroxy palmitic acid isomer by *M. sympodialis*⁴. Elevated *Malassezia* abundance on the skin also appears to limit colonization by the globally emerging multidrug-resistant fungal pathogen *C. auris*⁵. Last, *M. furfur* can convert tryptophan into indole metabolites that activate the aryl hydrocarbon receptor (AhR), improving barrier function and controlling inflammation in diseased skin⁶.

Despite these protective roles, *Malassezia* spp. are also implicated directly or indirectly in several skin pathologies, including atopic dermatitis, *Malassezia* folliculitis, seborrheic dermatitis (SD), and pityriasis versicolor (PV)^{7,8}. PV is characterized by *Malassezia* predominantly in the hyphal rather than the yeast form. While such morphology was thought to be PV-specific, hyphal structures have also been observed in SD skin lesions, indicating that hyphal growth is not restricted to PV⁹. The ability of specific *M. furfur* strains to produce hyphae has recently been demonstrated in a reconstructed human epidermis (RHE) infection model, where hyphae invaded the cornified layer and induced strong inflammatory keratinocyte responses, while neither response was observed for yeast-form strains^{10,11}. Despite establishing strain-level differences in hyphal growth potential, and a key role of hyphae in epidermal invasion and pathogenicity, the genetic mechanisms governing the yeast-to-hyphae transition remain elusive.

In most basidiomycetes, the transition from yeast to hyphal growth is linked to sexual reproduction between two compatible mating partners, and it is controlled by two mating type (*MAT*) loci¹². The first is *MAT A*, which is generally biallelic and encodes pheromones (*MFA*) and pheromone receptors (*PRA*) that govern cell recognition and fusion. The second is *MAT B*, which encodes adjacent, divergently transcribed homeodomain genes (*bE/bW* or *HD1/HD2*), often multiallelic, that typically form a functional heterodimer only when bE and bW are derived from different *MAT B* alleles. This heterodimer acts after syngamy to drive developmental programs, such as hyphal development and host invasion, as demonstrated in the plant pathogen *Ustilago maydis*^{13–15}. Compatibility at both loci is thus typically required for sexual development¹². The *MAT A* and *MAT B* loci can be unlinked on distinct chromosomes (tetrapolar system), or linked on the same chromosome with suppression of recombination between the two loci (bipolar system). An intermediate pseudobipolar system, identified in some *Malassezia* species, retains both loci on the same chromosome but sufficiently far apart that recombination occurs¹⁶.

Genome sequencing across *Malassezia* first revealed that some *M. furfur* strains have genomes approximately twice the size of other isolates, suggesting a non-haploid status and possible hybridization¹⁷. A subsequent study identified two parental haploid lineages within *M. furfur* (P1 and P2) hypothesized to represent two distinct species, and two derived hybrid lineages (H1 and H2,

diploid or aneuploid) resulting from hybridization of P1 and P2 strains¹⁸. Genome analysis further revealed a pseudobipolar mating system with two *MAT A* and four *MAT B* alleles; P1 and P2 isolates are either *MAT a1* or *a2*, while *MAT B* is fixed within each lineage (*b2* in P1, and *b1* in P2). H1 lineage strains carry *MAT a2b1b4* or *a2a2b1b4*, and are diploid or aneuploid, suggesting chromosome loss and/or loss of heterozygosity (LOH) during their evolution. Conversely, H2 lineage strains are diploid with a fully compatible set of *MAT* alleles, namely *MAT a1a2* and *b3b4*^{16,18}.

With the aim of linking *MAT* composition to hyphal formation in *M. furfur*, H2 lineage strains were grown in a number of conditions in earlier studies^{16,18}, but hyphae were never observed. The first evidence for *MAT* genes involvement in hyphal development came from a genetic engineering experiment in which insertion of compatible *MAT A* and *B* alleles (*a1b2*) or *MAT A* only (*a1*) into a haploid *M. furfur* strain (*MAT a2b1*) triggered robust hyphal formation under diverse growth conditions. The resulting strains were called “Aon Bon” (or “solo”; GI156) and “Aon Boff” (GI166)¹⁶ following the nomenclature of *U. maydis* solopathogenic strains¹⁹. Despite sharing the same *MAT* allelic composition as H2-lineage strains, only the engineered strains produced hyphae in culture. However, during a follow-up study assessing virulence in an RHE infection model, we noticed that, besides the “Aon Bon” and “Aon Boff” strains, some wild type *M. furfur* isolates also formed hyphae whereas others remained as yeast cells¹⁰. Further experiments revealed that the hyphal growth in RHE was restricted to H2 lineage strains (eg CBS7019 and CBS6000), which are diploid and harbor a fully compatible set of *MAT* alleles; in contrast, H1 lineage strains and P1 or P2 haploid strains carrying an incomplete set of *MAT* alleles, failed to form hyphae¹⁰. Together, these observations provided indirect evidence linking *MAT* composition to hyphal growth, but left unresolved whether sexual reproduction occurs in *Malassezia* and how it contributes to the yeast-to-hyphae transition.

Here, we define the nutritional conditions that induce hyphal growth in *M. furfur*, and show that they also promote cell-cell fusion. Mating between *MAT*-compatible strains from the two parental lineages generated diploid fusion products that acquired the ability to produce hyphae, whereas within-lineage crosses also yielded hypha-forming fusion products, although with variable ploidy that decreased to haploid or near haploid upon serial passaging, reminiscent of a parasexual-like cycle. Functional genetic analysis confirms a role for *MAT A* in cell recognition and fusion, as in other basidiomycetes, and for a single *MAT B* allele in hyphal development, without requiring a biparental HD protein combination. With *in vitro* and *in vivo* models, we demonstrate that *M. furfur* hyphae invade the skin more efficiently and elicit stronger host inflammatory responses than yeast cells, supporting a key role for hyphal growth in pathogenesis. Overall, our findings identify a noncanonical parasexual reproductive route in *Malassezia* that links cell fusion and genome plasticity with hyphal growth and pathogenic interactions with the host.

RESULTS

Olive oil promotes *Malassezia furfur* hyphal growth

Because some *M. furfur* strains undergo hyphal growth in the RHE infection model but not under laboratory conditions that instead readily induce hyphal growth in the engineered “Aon” strains, we reasoned that the RHE model contains additional morphogenetic cues. We therefore dissected this system component by component with the hybrid strain CBS7019 as a representative isolate (Supplementary Table 1). Across combinations of individual RHE components and culture parameters, we consistently identified olive oil as the only factor associated with hyphal growth of CBS7019; olive oil was specifically included as an external lipid source to support *Malassezia* growth

135 in the RHE model¹⁰. Remarkably, inoculating *M. furfur* CBS7019 into a drop of olive oil on a minimal
136 medium (MM) agar plate at 30°C was sufficient to induce hyphal growth without any other RHE
137 component (**Fig. 1a**). Hyphae were observed from the second day of incubation, and their abundance,
138 length, and branching, increased over time (**Fig. 1b**). Individual hyphae showed a clearly
139 distinguishable initial cell, septate hyphal compartments, and an apical growth cone (**Fig. 1c**). The
140 presence of both a carbon source (glucose) and a nitrogen source (ammonium sulphate, or ammonium
141 nitrate) was essential for hyphal development in the presence of olive oil, with growth being most
142 robust when both nutrients were present in MM agar, a condition we hereafter refer to as filamentation
143 medium (**Supplementary Table 2**).

144 Analysis of nuclear dynamics and hyphal architecture revealed that *M. furfur* CBS7019
145 diploid yeast cells are uninucleate, whereas hyphae are monokaryotic, with nuclei typically observed
146 near the center of each hyphal compartment, and frequently near the septa (**Supplementary Fig. 1a-**
147 **e**). Two nuclei were often observed near the terminal septum of growing hyphae (**Supplementary**
148 **Fig. 1f-g**), suggesting that nuclear division occurs within a hyphal compartment before nuclear
149 migration into the adjacent compartment. Consistent with a monokaryotic organization, true clamp
150 connections (hook-like structures typical of basidiomycetes that ensure nuclear distribution in
151 dikaryotic hyphae) were not observed in CBS7019 or, upon re-examination, in the self-filamenting
152 strains GI156 and GI166 (**Supplementary Fig. 2**), although hook-like septal protrusions were
153 occasionally noted in these strains¹⁶. Together, these observations establish olive oil as a cue for
154 monokaryotic, clamp-less hyphal growth in *M. furfur*.

156 **Unsaturated fatty acids promote *MAT* gene expression under hyphae-inducing conditions**

157 To define the lipid component(s) that triggers hyphal growth, we grew *M. furfur* CBS7019 on
158 filamentation medium supplemented with a panel of commercial oils. In addition to olive oil, other
159 oils induced hyphal formation, including jojoba, peanut, corn, sunflower, colza, and stearic oil (a
160 commercial preparation rich in unsaturated fatty acids) while others, such as coconut oil, did not
161 (**Supplementary Table 3**). A common feature between oils that promoted hyphal formation is their
162 abundance in unsaturated fatty acids. Supplementation with individual unsaturated fatty acids (oleic,
163 linoleic, linolenic acids) and the polyunsaturated hydrocarbon squalene, induced hyphal formation in
164 CBS7019, but only observed after prolonged incubation. The addition of Tween 60 (1%) to support
165 better *M. furfur* grow in the presence of individual unsaturated fatty acids or squalene, enhanced
166 hyphal formation, although less strongly than olive oil. By contrast, neither Tween alone nor the
167 saturated fatty acid palmitic acid induced hyphal formation (**Supplementary Table 3**).

168 The *M. furfur* H2-lineage strains CBS7019 and CBS6000, both diploid and capable of hyphal
169 growth, carry a fully compatible *MAT* configuration, specifically *MAT a1a2*, and *b3b4*^{16,18} (**Fig. 1d**),
170 which we hypothesized might contribute to hyphal growth. To test this, we first assessed *MAT* gene
171 expression by RT-qPCR in CBS7019 grown on filamentation medium supplemented with olive oil,
172 oleic acid, linoleic acid, linolenic acid, or squalene, or grown in mDixon. At 24 h, olive oil induced
173 the highest expression of *MAT A* genes, with significantly increased expression of *MFA1*, *PRAI*, and
174 *MFA2* relative to the MM reference and most other conditions. Oleic acid also induced high *MAT A*
175 gene expression, although responses were more variable and several pairwise comparisons were not
176 statistically significant (**Fig. 1e**). Conversely, linoleic acid, linolenic acid, and squalene showed
177 modest *MAT A* gene induction and were not statistically distinguishable from MM at 24 h (**Fig. 1e**).
178 After 48 h, olive oil and oleic acid still induced the highest *MAT A* gene expression, while linoleic
179 acid showed intermediate levels; linolenic acid, squalene, and mDixon, showed variable and generally

180 modest induction, frequently indistinguishable from MM or from each other (**Fig. 1e**). For the *MAT*
181 *B* locus, *bE3* and *bE4* alleles showed their strongest and most consistent upregulation with olive oil
182 at 24 h and 48 h, and with oleic acid 48 h, whereas *bW3* and *bW4* did not differ significantly across
183 conditions (**Fig. 1e**).

184 These findings show that unsaturated fatty acids promote both hyphal formation and *MAT*
185 gene expression in *M. furfur*, with olive oil eliciting the strongest and most consistent response,
186 possibly reflecting a synergistic contribution of multiple lipid components. Based on these findings,
187 all of the subsequent experiments were carried out on filamentation medium with cells placed in a
188 drop of olive oil, unless stated otherwise.

189 190 **Compatibility at *MAT A* is required for *M. furfur* hyphal growth**

191 To test whether the *MAT* locus controls the yeast-to-hyphae transition, we first screened a
192 panel of *M. furfur* strains spanning different *MAT* genotypes and lineages (**Supplementary Table 1**)
193 for hyphal formation with the olive-oil droplet assay (as in **Fig. 1a**), scoring cell morphology by light
194 microscopy. The panel included haploid P1 and P2 lineage strains, H1-lineage hybrids (CBS1878 and
195 CBS4171) and H2-lineage hybrids (CBS7019 and CBS6000; positive controls), the self-filamenting
196 strains GI156 and GI166, and the newly engineered strain #216, generated by inserting the transgene
197 *MATa1-NEO-MATb4* into the P2 haploid strain PM315 (*MAT alb1*). Robust hyphae formation was
198 restricted to strains carrying compatible *MAT A* alleles (CBS7019, CBS6000, GI156, and GI166),
199 whereas those carrying an incomplete *MAT* system (haploid strains and H1-lineage hybrids)¹⁸
200 remained in the yeast form. Occasional pseudohyphal-like cells were observed in haploid strains
201 (**Supplementary Fig. 3**), but were readily distinguishable from true hyphae, which were
202 multicellular, with septa, and generally longer and wider. Together, these results are consistent with a
203 requirement for *MAT A* compatibility for hyphal growth.

204 Next, we performed mating assays under the same conditions with four *M. furfur* haploid
205 strains (PM315, CBS9574, CBS14141, and CD866; **Supplementary Table 1**), representing both P1
206 and P2 lineages and collectively covering both *MAT A* and *B* alleles, allowing interspecific P1 × P2
207 (CBS14141 × CBS9574 and CD866 × PM315) and intraspecific crosses (P1 × P1: CD866 ×
208 CBS9574; P2 × P2: CBS14141 × PM315). Although hyphae were observed in several conditions,
209 results were variable across replicates and timepoints; we therefore developed a selection-based assay
210 to directly recover and quantify cell fusion products.

211 212 ***MAT A*-compatible *M. furfur* strains undergo cell fusion generating fusion products that gain** 213 **the ability to produce hyphae**

214 Hyphal growth appeared to be the result of cell fusion events controlled by the *MAT* loci. To
215 directly test this, we utilized *Agrobacterium*-mediated transformation to generate haploid *M. furfur*
216 strains PM315, CBS9574, CBS14141, and CD866 carrying dominant selectable markers conferring
217 resistance to nourseothricin (NAT) or G418 (geneticin; NEO). One stable NAT- or NEO-resistant
218 transformant was selected for each strain, with the exception of PM315, for which two independent
219 NEO-resistant transformants were analyzed (**Supplementary Table 1**). Marked strain pairs were co-
220 inoculated in the olive-oil droplet assay (**Fig. 2a**) and after 3 days of incubation at 30°C, the co-
221 cultures were plated onto mDixon agar containing both NAT and NEO. Double drug-resistant
222 colonies were recovered only from crosses involving parental strains compatible at *MAT A* (with or
223 without *MAT B* compatibility), whereas crossing with compatibility only at *MAT B* did not yield
224 double drug-resistant colonies (**Fig. 2b**). Across five independent positive assays, double drug-

225 resistant colony counts were broadly consistent within each cross but differed among crosses, with
226 crosses CBS14141-NAT#1 $\times$ CBS9574-NEO#1 (P2 $\times$ P1) and CD866-NAT#1 $\times$ CBS9574-NEO#1
227 (P1 $\times$ P1) yielding significantly more putative fusion products (**Fig. 2c**; $P < 0,0001$). Further
228 experiments indicate that cell fusion can occur within 24 h (**Supplementary Fig. 4**). Cell fusion was
229 also tested on filamenting medium supplemented with the same individual lipids as in figure 1e-f;
230 besides olive oil (positive control), oleic acid yielded the highest recovery of double-drug resistant
231 colonies, while linoleic and linolenic acid yielded only 1 and 4 colonies, respectively, in a single cross
232 (**Supplementary Table 4**).

233 Because double selection confirms acquisition of *NAT* and *NEO* but does not resolve *MAT*
234 allele composition, we PCR-genotyped four randomly-selected double drug resistant colonies from
235 each cross. Colonies from crosses with strains compatible at both *MATA* and *MATB* loci were PCR
236 positive for the expected *MAT* alleles (*a1*, *a2*, *b1*, *b2*) as well as the selectable markers *NAT* and *NEO*
237 (**Fig. 2d**). Likewise, colonies from crosses with strains compatible at *MAT A* but not *MAT B* were
238 PCR-positive for *MAT a1*, *a2*, *NAT*, and *NEO*, and carried the same *MAT B* allele as the parental
239 strains (*b1* for CBS14141-NAT#1 $\times$ PM315-NEO#3; and *b2* for CD866-NAT#1 $\times$ CBS9574-NEO#1)
240 (**Fig. 2d**). Regardless of *MAT B* genotype, all double drug-resistant colonies PCR-positive for both
241 *MAT a1* and *a2* formed hyphae (**Fig. 2e, f**).

242 Quantification of hyphal formation for two independent fusion products from each cross
243 showed that fusion products #440 and #503 (P1 $\times$ P2) and #444 and #506 (P1 $\times$ P1) had the highest
244 levels ($\geq 10\%$), followed by #513 and #514 (P1 $\times$ P2). The natural hybrid CBS7019 displayed about
245 4% hyphal formation, whereas fusion products #564 and #565 (P2 $\times$ P2) showed about 2%, the lowest
246 levels recorded (**Fig 2e**). These values likely represent conservative estimates of hyphal formation,
247 as tangled hyphae could not be reliably counted and washing steps may have perturbed the samples.
248 Microscopic analysis of a representative fusion product from each cross revealed some variation in
249 hyphal morphology but no consistent traits distinguishing intra- from inter-specific fusion products
250 (**Fig 2f**; **Supplementary Fig. 5**).

251 Mitochondrial inheritance in *M. furfur* fusion products was assessed with previously reported
252 mitogenome polymorphic markers²⁰. Strains selected for this analysis were confirmed as cell fusion
253 products by PCR genotyping of *MAT* allele markers, and by their ability to form hyphae. In the
254 interspecific cross CBS14141-NAT#1 (P2, *a2b1*) $\times$ CBS9574-NEO#1 (P1, *a1b2*), primers targeting
255 the *cox3*–*nad3* intergenic region showed that 3/8 fusion products carried the CBS9574 mitogenome
256 and 5/8 carried the CBS14141 mitogenome (**Fig. 2g**). In the interspecific cross CD866-NAT#1 (P1,
257 *a2b2*) $\times$ PM315-NEO#2 (P2, *a1b1*), primers targeting the *cob* exon2–exon3 intronic region indicated
258 that 6/8 fusion products carried the PM315 mitogenome and 2/8 carried the CD866 mitogenome (**Fig.**
259 **2g**). In the intraspecific cross CBS14141-NAT#1 (P2, *a2b1*) $\times$ PM315-NEO#3 (P2, *a1b1*), 15/16
260 fusion products carried the CBS14141 mitogenome (**Fig. 2h**). For the intraspecific cross CD866-
261 NAT#1 (P1, *a2b2*) $\times$ CBS9574-NEO#1 (P1, *a1b2*), existing markers could not discriminate parental
262 mitochondria; we therefore designed parental-specific primers for *cox1* (**Supplementary Fig. 6**),
263 which showed that 10/16 fusion products carried the CD866 mitogenome, 4/16 carried the CBS9574
264 mitogenome, and 2/16 were positive for both, potentially reflecting residual heteroplasmy or mixed
265 template amplification (**Fig. 2h**). Overall, these results show that mitochondrial inheritance in P1 $\times$
266 P2 interspecific crosses was variable and consistent with biparental inheritance, whereas in
267 intraspecific crosses showed a bias toward inheritance from the *MATa2* parent, similar to uniparental
268 inheritance reported in taxonomically related fungi²¹.

To visualize cell fusion events directly, an mCherry-tagged derivative of the *M. furfur* strain CBS14141 was generated by *Agrobacterium*-mediated transformation and crossed with wild type strains CBS9574 (P2 × P1) or PM315 (P2 × P2). Microscopic examination of CBS14141-mCherry × CBS9574 mating cultures revealed conjugation tubes connecting mCherry-tagged and untagged cells (Supplementary Fig. 7a). In the intraspecific cross, we captured a conjugation tube emerging from a pseudohyphal cell of PM315 and connecting to a yeast cell of CBS14141-mCherry (Supplementary Fig. 7b). Cell-cell connections were frequently observed involving pseudohyphae (Supplementary Fig. 7c), but were also seen between yeast cells (Supplementary Fig. 7d). Conjugation tubes appeared morphologically distinct from pseudohyphae, suggesting they might represent a specialized mating structure. Finally, double drug resistant putative products generated by crossing CBS14141-mCherry with CBS9574-NEO#1 (strain #524) or PM315-NEO#3 (strain #644), displayed robust hyphal formation and cytoplasmic mCherry fluorescence (Supplementary Fig. 7e-f), consistent with m-Cherry-positive cell fusion products giving rise to hyphal growth.

The fusion products from interspecific crosses are diploid hybrids

To determine the ploidy and genome composition of fusion products from the two interspecific crosses, we analyzed the parental strains and two fusion products from each cross by flow cytometry (FACS) and Illumina sequencing (Fig. 3a). Mapping Illumina reads to a parental reference genome for each cross revealed chromosome-wide heterozygosity (Fig. 3b-c), except for small LOH tracts in fusion product #440 on chr. 2 (~9.2 kb) and in #503 on chr. 6 (~5.6 kb); a larger LOH region (~152 kb) was also detected at the left end of chr. 5 in #503. Consistent with their hybrid origin, FACS confirmed that all four fusion products were diploid whereas the parental strains were haploid, as expected (Fig. 3d). De novo genome assemblies further supported this, revealing genome sizes exceeding 16 Mb (Supplementary Table 5) in all four fusion products, approximately twice the size of the haploid parental genomes (~8 Mb). BLAST searches confirmed the presence of parental *MAT* allele markers (*a1*, *a2*, *b1*, and *b2*), and the *NAT* and *NEO* cassettes. The loci disrupted by random insertion of *NAT* and *NEO* in the parental strains are reported in Supplementary Fig. 8; although these insertions may influence cell fusion frequency or hyphal formation levels, they do not appear to impair either process. Together, these results confirm that the fusion products from interspecific crosses are diploid hybrids (allodiploids), comparable in genome size to naturally occurring *M. furfur* hybrids but with markedly lower levels of LOH¹⁸.

Intraspecific fusion products undergo ploidy reduction and recombination upon passaging

Unlike the diploid interspecific fusion products, the intraspecific fusion products #444 and #506 (P1 × P1) and #564 and #565 (P2 × P2) initially appeared haploid by FACS yet retained extensive genome-wide heterozygosity characteristic of diploid hybrid genomes (samples 0a; Supplementary Fig. 9). This discrepancy prompted us to investigate whether ploidy reduction had occurred during sample handling, specifically, during passaging in non-selective medium. To test this, we performed FACS and Illumina sequencing on two sample sets: (i) the same fusion products revived from frozen stocks on selective mDixon medium (Fig. 4; samples 0b), and (ii) three single colonies derived from each fusion product, isolated and passaged three times on non-selective mDixon medium (Figure 4a; samples 1-3). These analyses showed that the intraspecific fusion products were indeed diploid or of mixed 1n/2n ploidy at the time of freezing; retrospective comparison with the original screening samples (Supplementary Fig. 9, samples 0a) further

suggested that growth in non-selective conditions had already promoted LOH accumulation prior to the initial analysis (**Fig. 4; Supplementary Fig. 9**).

We then examined each cross in detail. For the P1 × P1 cross, fusion products #444 and #506 (samples 0b) showed extensive heterozygosity with a few LOH tracts, and mixed 1n/2n ploidy (**Fig. 4 b, c**). Passaged isolates derived from #444 were haploid and with recombinant genomes, having inherited markers from both parents across multiple chromosomes, with the exception of chromosome 4, which was inherited from a single parent. No differences were observed among the three #444-derived passaged isolates (**Fig. 4b-c; Supplementary Fig. 10**). In contrast, passaged isolates derived from fusion product #506 showed similar ploidy as the initial #506 fusion product, but accumulated additional LOH on chromosomes 1, 2, 3 and 7; notably, these LOH tracts overlapped with those observed in the initial #506 fusion product (sample 0a) growth in non-selective liquid medium, suggesting they may represent preferential sites of LOH accumulation (**Fig. 4b-c; Supplementary Fig. 9**).

The P2 × P2 fusion products showed a similar but not identical pattern. Fusion product #564 (sample 0b) and its passaged isolates were consistently heterozygous diploids, with the exception of isolate #564-3, which underwent LOH on a segment of chr. 2 encompassing the *NAT* marker, resulting in the loss of NAT resistance (**Fig. 4d**). For fusion product #565, the sample 0b was a heterozygous with mixed 1n/2n ploidy, while passaged isolates were haploid with recombinant genomes, showing recombination on chrs. 3, 4 and 6; chrs. 1 and 5 were inherited from one parent, chr. 2 from the other, and isolates #565-2 and #565-3 retained disomy of chr. 7 with persistent heterozygosity (**Fig. 4d-e**).

De novo genome assemblies of the passaged haploid isolates confirmed haploid genomes size (~8 Mb), with the exception of isolates #565-2 and #565-3, which had slightly larger (~8.34 Mb) and more fragmented assemblies, likely due to retention of chr. 7 from both parents (**Fig. 4d**). BLAST searches confirmed that all haploid isolates carried a single parental *MAT* configuration, rendering them unable to form hyphae, and had inherited both *NAT* and *NEO* resistance markers, consistent with independent segregation of the chromosomes carrying each marker during ploidy reduction (**Fig. 4f**); the only exception was isolate #564-3, which lost *NAT* resistance following LOH at the *NAT* locus as described above (**Fig. 4d**). Together, these findings demonstrate that intraspecific fusion products can undergo spontaneous ploidy reduction and recombination, generating recombinant haploid progeny, hallmarks of a parasexual cycle.

Functional dissection reveals distinct *MAT A* and *MAT B* roles in cell fusion and hyphal growth

To define the specific contributions of the *MAT A* and *MAT B* loci to cell fusion and hyphal growth in *M. furfur*, we generated targeted deletions of the *MAT a2* (*MFA2/PRA2*) and *MAT b1* (*bE1/bW1*) loci in the wild-type CBS14141 background, and validated mutant genotypes by diagnostic PCR (**Fig. 5 a-b**). We then performed unilateral crosses (i.e., crosses where only one parent carries the deletion allele) between the CBS14141 mutants and marked parental tester strains to test whether *MAT A* or *MAT B* is required for cell fusion. Unilateral interspecific and intraspecific crosses of CBS14141 *a2Δ* (*mfa2Δ/prA2Δ*) with CBS9574-NEO#1 (*a1b2*) and with PM315-NEO#3 (*a1b1*), respectively, did not yield colonies on double-drug selective medium, indicating that *MAT A* is required for cell fusion (**Fig. 5c**). Conversely, unilateral crosses of CBS14141 *b1Δ* (*bW1Δ/bE1Δ*) with CBS9574-NEO#1 and with PM315-NEO#3 yielded double drug-resistant colonies confirmed by PCR to carry the expected *MAT* alleles (*a1*, *a2* and *b2* for the CBS14141 *b1Δ* × CBS9574-NEO#1

357 fusion products, and *a1*, *a2* and *b1* for the CBS14141 *b1Δ* × PM315-NEO#3 fusion products) and the
358 *NAT* and *NEO* markers (**Fig. 5d**). Morphological analysis on filamentation medium revealed normal
359 hyphal growth in fusion products recovered from both interspecific (strain #592) and intraspecific
360 (strain #596) unilateral crosses (**Fig. 5e**), indicating that *MAT B* compatibility between mating
361 partners is dispensable for both cell fusion and hyphal growth.

362 Next, to assess the contribution of each *MAT* locus to hyphal growth independently of cell
363 fusion, we introduced the same *MAT a2::NAT* and *MAT b1::NAT* deletion cassettes into the self-
364 filamenting strains GI156 (“Aon Bon”) and GI166 (“Aon Boff”) ¹⁶ (**Fig. 5 f-g**). In GI156, deletion of
365 the native *MAT a2* locus abolished hyphal growth, whereas deletion of *MAT b1* (leaving *b4* intact)
366 did not affect hyphal formation compared to the parental strain (**Fig. 5h**), indicating that *MAT A*
367 compatibility is required for hyphal growth but *MAT B* allelic compatibility is dispensable. In GI166,
368 deletion of *MAT a2* similarly abolished hyphal growth, while deletion of *MAT b1* loci — which in
369 this background removes the only functional *MAT B* allele — also resulted in a yeast-only phenotype
370 (**Fig. 5h**), demonstrating that at least one functional *MAT B* allele is required for hyphal growth
371 independently of *MAT B* allelic compatibility. Gene expression analysis under hyphae-inducing
372 conditions confirmed successful deletion of the target loci, with *MAT A* gene expression remaining
373 strong and comparable across parental and mutant backgrounds; *MAT B* expression was overall low
374 in all backgrounds, suggesting that its role in hyphal development does not depend on strong
375 transcriptional induction under these conditions (**Supplementary Fig. 11**).

376 Given the yeast-only phenotype of the GI166 *b1Δ* mutant, we then tested whether *MAT B* is
377 also required for hyphal growth in fusion products from genetic crosses. To test this, a *b1Δ-NEO*
378 mutant was generated in PM315 (*a1b1*) and subjected to bilateral cross (i.e. in which both parents
379 carry the deletion allele) with the CBS14141 *b1Δ-NAT* mutant. RT-qPCR analysis of mating cultures
380 revealed high expression of *MFAl*, *PRA1*, *MFA2*, and *PRA2* with no significant differences relative
381 to the wild type control cross, and the expected absence of *bE1* and *bW1* expression, confirming
382 successful loss of *MAT b1* in both parents (**Fig. 5i**). PCR genotyping of recovered double drug-
383 resistant colonies confirmed they were genuine fusion products, carrying *MAT a1* and *a2* alleles from
384 each parent and lacking *MAT b1*, as expected from the bilateral deletion (**Fig. 5j**). Phenotypic analysis
385 of a representative *b1Δ* fusion product (#648) revealed a yeast-only phenotype (**Fig. 5k**),
386 demonstrating that *MAT B* is dispensable for cell fusion but required for hyphal formation. Taken
387 together with the unilateral *b1Δ* cross results — where fusion products carrying a single *MAT B* allele
388 (*b2* only in #592, *b1* only in #596) formed hyphae normally — these findings reveal that a single
389 functional *MAT B* allele is sufficient to drive hyphal growth, regardless of allelic identity.

390

391 **Fusion products exhibit enhanced virulence-associated phenotypes relative to parental strains**

392 To determine whether strains capable of hyphal growth differ from yeast-form counterparts in
393 their interaction with the host, one representative fusion product from each cross was tested *in vitro*
394 in the RHE infection model¹⁰, and *ex vivo* and *in vivo* with a murine model of *Malassezia* skin
395 colonization²²; the natural diploid hybrid CBS7019, and parental haploid strains were included as
396 controls. In the RHE model, fusion products formed hyphae and invaded the cornified layer similar
397 to CBS7019, whereas parental strains remained in the yeast form and were largely confined to the
398 epidermal surface (**Fig. 6a**). SEM imaging of the RHE surface further reinforced this observation,
399 revealing active tissue penetration and invasion by *M. furfur* hyphae (**Fig. 6b**). Consistent with
400 invasive growth, barrier integrity assessed by transepithelial electrical resistance (TEER) and lucifer

yellow (LY) permeability, was generally reduced in RHE infected with hypha-forming strains compared to the yeast-form parental strains, although significance was not reached for all strains tested in both assays (**Fig. 6c**). Hypha-forming strains also generally triggered higher expression of genes encoding proinflammatory cytokines IL-1 β , IL-8, and IL-17C in RHE, as well as of genes encoding the antimicrobial peptides β -defensin 2 (β D2), β -defensin 3 (β D3), and LL-37, compared to the parental strains, although significance and magnitude varied across strains and markers (**Fig. 6d-e**).

The ability of *M. furfur* fusion products to penetrate skin tissue was next assessed *ex vivo* by infecting mouse ear skin with hyphae induced prior to inoculation, and by microscopic examination of periodic acid-Schiff (PAS)-stained histological sections. Parental strains in their yeast form were restricted to the stratum corneum surface, whereas fusion products hyphae extended through the epidermis (**Fig. 6f**), indicating that hyphal growth is associated with enhanced capacity for tissue penetration. *In vivo*, epicutaneous infection of mice ear with the same strains and measurement of ear thickness as a proxy for skin inflammation showed that hypha-forming strains consistently caused significantly greater ear swelling compared to their yeast-form parental strains (**Fig. 6g**). Taken together, these results demonstrate that *M. furfur* hypha-forming fusion products invade skin tissue more efficiently and elicit stronger host inflammatory responses than yeast-form parental strains, supporting a link between hyphal morphogenesis and pathogenicity during skin colonization.

DISCUSSION

The skin fungus *M. furfur* has long been known to exist as both yeast and hyphal forms, with the latter associated with skin disease, yet the signals and genetic programs controlling this transition have remained poorly understood. Here, we show that skin-relevant lipids can trigger *MAT*-gene-dependent cell fusion and yeast-to-hyphae transition in *M. furfur*, and that intraspecific fusion products can undergo ploidy reduction and recombination reminiscent of a parasexual cycle, thereby linking host skin chemistry to fungal reproductive biology and pathogenesis.

Systematic dissection of individual RHE components identified olive oil as key trigger of hyphal formation in a natural diploid *M. furfur* strain. This effect is due to its abundance in unsaturated fatty acids, in particular oleic acid, which promotes *MAT* gene expression and the yeast-to-hyphae transition in strains with compatible *MAT* configurations within a single cell (**Fig. 1**). Other lipid sources, such as saturated fatty acids and Tween, commonly used to support *Malassezia* growth in axenic cultures, do not promote hyphal growth. Given that human sebum is rich in unsaturated fatty acids and squalene²³, our findings suggest that the host-associated cutaneous lipids likely provide the environmental cue that triggers mating and hyphal growth, providing a mechanistic basis for understanding how naturally-occurring *M. furfur* hybrids form.

Consistent with this, skin-relevant lipids also promote mating between haploid *M. furfur* cells of opposite mating type. *M. furfur* comprises two parental lineages (P1 and P2) hypothesized to represent two distinct species, each with strains carrying either *MAT a1* or *a2* alleles but sharing a single *MAT B* allele (*b2* in P1; *b1* in P2)¹⁸. Applying a dual drug-resistance selection strategy, we show that strains from both lineages can undergo both intraspecific and interspecific mating. In both cases, the initial step of cell recognition, conjugation, and cell fusion between haploid isolates requires compatibility only at the *MAT A* locus (**Figs. 2, 7**). Fused cells undergo karyogamy, generating a diploid monokaryon with a heterozygous genome, consistent with a chimera of homologous and homeologous parental genomes in intra- and interspecific fusion products, respectively. In fusion products that inherited compatible *MAT A* from both parents, a single *MAT B* allele, regardless of

identity, is both necessary and sufficient for the yeast-to-hyphae transition (**Fig. 2, 7**). The role of the *MAT A* pheromone/receptor (*P/R*) locus in cell recognition and fusion in *M. furfur* is hence consistent with its conserved role across basidiomycetes¹²; however, decoupling of *MAT B* from mating-type determination, while retaining its developmental role, represents a fundamental departure from canonical basidiomycete sexual development, and effectively renders *M. furfur* functionally bipolar.

In most basidiomycetes, hyphal development depends on heterodimerization of bE and bW homeodomain proteins derived from different *MAT B* alleles^{13,14}; the finding that a single allele suffices in *M. furfur* suggests that its HD regulatory mechanisms have been rewired to function independently of allelic complementation. While bE/bW self-recognition cannot be excluded, the consistently low expression of *bW* in different *M. furfur* strains under mating-inducing conditions (**Figs. 1, 5; Supplementary Fig. 11**) raises the intriguing possibility that a bE homodimer, rather than the canonical bE/bW heterodimer, drives hyphal development, as reported for bW/HD2 in the homothallic basidiomycete *Cystofilobasidium capitatum*²⁴. Notably, similar relaxation of *MAT B* allelic specificity has been observed in some *Microbotryum* anther-smut fungi²⁵, suggesting this may be a recurrent evolutionary strategy in basidiomycete fungi that have transitioned to non-canonical reproductive modes, or that have developed particular host-associations.

The genomic outcomes of cell fusion reveal a striking difference between interspecific and intraspecific crosses. Interspecific P1 × P2 fusion products are allodiploid hybrids with chromosome-wide heterozygosity and genome sizes of ~16 Mb, resembling the naturally occurring *M. furfur* H2 lineage hybrids isolated from human skin¹⁸, which suggests that H2 originated from interspecific mating events on the host. Interestingly, small LOH tracts were detected in fusion products from one of the two interspecific crosses (#440 and #503) but not the other, and affected different genomic regions in each strain, suggesting that LOH arises stochastically in *M. furfur* allodiploids, as previously observed in naturally occurring *M. furfur* hybrids¹⁸. These events recall the interstitial (I-LOH) and terminal (T-LOH) LOH described in *S. cerevisiae*, arising from mitotic double-strand break repair, the former through gene conversion involving short exchanges and the latter resulting from crossing over or break-induced replication spanning large regions²⁶. Notably, the markedly lower levels of LOH in experimentally generated hybrids compared to natural *M. furfur* H2-hybrids suggest that LOH accumulates progressively after hybridization. A recent study on the H1-hybrid strain CBS1878 reveal that post-hybridization genome stabilization involves DNA repair-mediated chromosome restructuring coupled with homeologous recombination and gene conversion, generating localized LOH events²⁷. Therefore, LOH events are a dynamic driver of post-hybridization genome evolution, linking genome stabilization with the generation of genetic diversity that may facilitate adaptation in hybrid *M. furfur*. The experimental system established here now provides a unique opportunity to investigate the mechanisms and dynamics underlying these processes during the earliest stages of hybridization.

In contrast, fusion products of intraspecific crosses follow a different genomic trajectory: upon passaging without selection, diploid fusion products can undergo spontaneous ploidy reduction and recombination, generating haploid progeny with recombinant genomes. This may explain why diploids derived from intraspecific crosses have never been isolated from the skin. This process, however, is variable in both rate and genomic outcome: (i) some fusion products remained diploid even after passaging, (ii) others were haploids and with recombinant genomes, and (iii) others displayed mixed ploidy with progressive LOH accumulation, likely representing intermediate steps toward genomic stabilization (**Fig. 4**). Compared with the hybrids, fusion products obtained in intraspecific crosses exhibited a higher number of I-LOH and terminal T-LOH events that involved

larger genomic regions, suggesting that their genomes are less stable and subjected to a different contribution of LOH-mediated stabilization. The similar LOH patterns observed across passaged isolates derived from the same fusion product are intriguing and could reflect several non-mutually exclusive processes. LOH events may have arisen very early after cell fusion, prior to single colony isolation, such that all passaged colonies inherited the same pre-existing LOH, although the largely heterozygous diploid genomes of the 0b samples suggest this would only have affected a minority of cells. Alternatively, the founder population (sample 0b) may already have been heterogeneous in ploidy and LOH status, with certain genotypes conferring a growth advantage on non-selective medium and being preferentially recovered as single colonies. Finally, specific genomic loci may represent hotspots for LOH, driven by selective pressure to eliminate deleterious alleles.

The generation of haploid progeny with recombinant genomes in intraspecific crosses is consistent with a parasexual cycle, in which cell fusion, nuclear fusion, and subsequent ploidy reduction generate genetically diverse haploid offspring without a canonical meiosis. Random segregation of *MAT* alleles in the haploid progeny likely ensures that both mating types are represented, enabling *MAT*-compatible offspring to re-enter the parasexual cycle and perpetuating genetic diversification (**Fig. 7**). Parasexuality in *M. furfur* shows notable analogies with the parasexual cycle of *Candida albicans*, in which, in the absence of canonical sexual structures, diploid cells fuse to form tetraploids that undergo ploidy reduction through concerted chromosome loss (CCL), a process in which chromosomes are lost in an apparently random and concerted fashion with recombination occurring between homologous parental chromosomes^{28,29}. Parasexual CCL in *C. albicans* is thought to involve chromosome non-disjunction during mitotic divisions, with the meiotic protein Spo11 generating DNA double-strand breaks that promote inter-homolog recombination in concert with Rec8 (a meiosis-specific cohesion subunit), together providing a mechanistic link between meiosis and parasexual chromosome loss³⁰. Interestingly, the *M. furfur* genome includes an ortholog of *SPO11* but lacks *REC8*, suggesting that Spo11 alone may drive parasexual ploidy reduction in *M. furfur*, potentially through a recombination mechanism that operates independently of meiotic cohesion. In *C. albicans*, parasex results in elevated recombination across all chromosomes, with very short recombination tracts³¹, while in *M. furfur* not all chromosomes showed evidence of recombination in individual progeny (**Fig. 4**). Recombination was nonetheless detected on all chromosomes across the combined progeny of both intraspecific crosses, indicating that no chromosome is refractory to recombination per se. The lower per-chromosome recombination frequency in *M. furfur* compared to *C. albicans* may reflect biological differences between the two parasexual systems; it is also possible that, unlike canonical meiosis where at least one crossover per chromosome is typically required for proper segregation³², parasexual ploidy reduction in *M. furfur* does impose such a per-chromosome constraint, though this remains to be demonstrated.

Lastly, the virulence data presented identify hyphal morphogenesis as a key determinant of *M. furfur* pathogenic interactions with the host, with hyphae-forming strains (regardless of whether they originate from intra or interspecific crosses) exhibiting enhanced tissue invasion, epidermal barrier disruption, and stronger immune activation compared to yeast-form counterparts in human and mouse skin models (**Fig. 6**). These findings are consistent with the known association of *M. furfur* hyphae with PV and seborrheic dermatitis^{7,9}, and with a well-established link between morphological transitions and pathogenicity in fungal pathogens, such as *U. maydis* and *C. albicans*^{19,28}. However, haploid parasexual progeny did not form hyphae and are thus unlikely to contribute directly to acute tissue invasion. We speculate that parasex may instead provide an adaptive route in host-associated niches by generating genetic diversity without requiring the production of potentially immunogenic

535 spores ³³. Parasexuality and hyphal morphogenesis may therefore act as complementary processes:
536 the former promoting population diversification over longer timescales, the latter driving tissue
537 invasion and inflammatory host responses when skin-associated lipids induce *MAT*-associated hyphal
538 development.

539 In conclusion, our findings point to a scenario in which the skin is not merely a passive
540 substrate for *Malassezia* colonization, but rather an active arena for (para)sexual reproduction,
541 hybridization, and genome diversification, initiated by the lipids that define this unique ecological
542 niche, and adding growing evidence that host-associated fungi may rely on reproductive strategies
543 distinct from canonical meiosis.

544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578

579 MATERIALS AND METHODS

580 Strains and culture conditions

581 *M. furfur* strains used in this study are listed in **Supplementary Table 1**. They were regularly
582 maintained on modified Dixon's media (mDixon) [mycological peptone (10 g/L), malt extract (36
583 g/L), glycerol (2 ml/L), tween 60 (10 ml/L), desiccated ox-bile (10 g/L), and agar (20 g/L) for solid
584 media] with incubation temperature of 30°C.

585 *A. tumefaciens* strain EHA105 was used for *Agrobacterium*-mediated transformation of *M.*
586 *furfur*. This strain was cultivated at 30°C in Luria Bertani [tryptone (10 g/L), yeast extract (5 g/L),
587 NaCl (10 g/L), and agar (20 g/liter) for solid media], or LB supplemented with 50 µg/mL of
588 kanamycin sulphate when transformed with binary vectors for *M. furfur* transformation.

589

590 *Agrobacterium*-mediated transformation of *M. furfur*

591 *Agrobacterium*-mediated transformation of *M. furfur* was carried out as previously reported
592 ³⁴. Haploid *M. furfur* strain PM315 was transformed with the binary vector containing the *MAT*
593 *a1* and *MAT b4* alleles together with a *NEO* marker ¹⁶. Haploid *M. furfur* strains CBS14141,
594 CBS9574, CD866 and PM315 were transformed with *A. tumefaciens* containing the binary vectors
595 pAIM2 and pAIM6 conferring resistance to nourseotricin (NAT) and neomycin G418 (NEO) ³⁵,
596 respectively. To obtain mCherry-tagged cells of *M. furfur* strain CBS14141, transformation was
597 carried out using EHA105 containing the bicistronic plasmid pJG201701 that confers resistance to
598 NAT ³⁶. In all cases *M. furfur* transformants were selected on mDixon supplemented with NAT [(100
599 µg/mL) clonNAT, AB-102L, Jena Bioscience] and NEO [(200 µg/mL) A6798, PanReac AppliChem].

600 *A. tumefaciens*-mediated targeted mutagenesis was carried out to mutate both the *MFA2* and
601 *PRA2* genes of the *MAT A2* locus, and both the *bE1* and *bW1* genes of the *MAT B1* locus of *M. furfur*
602 CBS14141, and of its derived self-filamenting strains GI156 and GI166; the *NAT* marker was used.
603 Mutation of the *MAT B1* locus was also carried out in *M. furfur* strain PM315 using the *NEO* marker.
604 Briefly, the 5' and 3' flanking regions for homologous recombination were amplified from the
605 genomic DNA of *M. furfur* CBS14141, using primer pairs listed in **Supplementary Table 6**.
606 The *NAT* and *NEO* cassettes were amplified, respectively, from plasmids pAIM2 and pAMI6 using
607 primers ALID2078-ALID2081. The PCR products and the double-digested (*KpnI* and *BamHI*) binary
608 vector pGI3 were transformed into *S. cerevisiae* FY834 using lithium acetate and PEG 3750, and
609 correctly recombined plasmids identified by PCR were transformed by electroporation in *A.*
610 *tumefaciens* EHA105 for transformation, as previously reported ³⁵. *M. furfur* transformants obtained
611 were colony purified on selective medium, subjected to phenol–chloroform–isoamyl alcohol
612 (25:24:1) DNA extraction, and the correct replacement of the target loci was assessed by diagnostic
613 PCR using primers reported in **Supplementary Table 6**.

614

615 Conditions promoting hyphal growth and mating in *M. furfur*

616 To induce filamentous growth, cellular pellet of *M. furfur* strain CBS7019 from a mDixon
617 agar plate was resuspended in sterile water, and 25 µL were spotted within a 50 µL-drop of olive oil
618 (O1514, Sigma-Aldrich) placed on filamentation medium agar (MM) [Glucose (15 g/L), Ammonium
619 sulphate (3.3 g/L), Magnesium sulfate (0.5 g/L), Potassium chloride (0.5 g/L), Potassium dihydrogen
620 phosphate (1g/L), agar (20g/L)] and incubated at 30°C; hyphal formation was monitored daily using
621 an optical microscope (Zeiss Axioscope 5 equipped with a Camera AxioCam 305 color, and with
622 Illumination System Colibri 3 for fluorescence) with a 100 X objective lens ("N-Achroplan"
623 100x/1.25Oil M27) and with immersion oil.

624 To define how nutrients availability affects filamentation, CBS7019 was spotted within a 50
625 μ L-drop of olive oil placed on 2% agarose- and 2% agar-based media in which all the MM
626 components were tested alone or in combination; hyphal formation was monitored as described above
627 after 3 days on incubation at 30°C.

628 To identify the lipid component(s) that triggers filamentation, CBS7019 cells were spotted on
629 filamenting medium within a drop of different commercial oils (jojoba, peanut, corn, sunflower,
630 colza, and coconut) bought in a local market, with the exception of stearic oil (Carl Roth).
631 Subsequently, CBS7019 cells were spotted on filamenting medium [(with and without 1% of Tween
632 60) P1629, Sigma-Aldrich] and then few drops of oleic acid (7213.1, Carl Roth), linoleic acid (62230,
633 Sigma-Aldrich), linolenic acid (6039.1, Carl Roth), or squalene (442785, Sigma-Aldrich) were
634 applied on the yeast cells. Olive oil was always used as a positive control while the negative control
635 was MM without olive oil; hyphal formation was monitored as described above after 3 days on
636 incubation at 30°C.

637 Mating assays were carried out using the same procedure reported to induce filamentous
638 growth. *M. furfur* strains resistant to NAT or NEO were resuspended in sterile water, their optical
639 density adjusted to OD₆₀₀ = 1 (about 1×10^9 CFU/mL), mixed in equal amount in water, and 25 μ L
640 of the mix spotted within a 50 μ L-drop of olive oil placed on filamenting medium agar as described
641 above. At the defined incubation time, the cellular mix was collected, washed with water, and plated
642 on mDixon supplemented with NAT (100 μ g/mL) and NEO (200 μ g/mL) to select fusion products.
643 The single strain represented the negative controls. Quantification of cell fusion rate was carried out
644 after 72 h of incubation, with five independent experiments (biological replicates) each consisting of
645 two or three technical replicates. Data of each biological replicates was obtained by the means of the
646 technical replicates, and subjected to ordinary one-way ANOVA with Tukey's multiple comparison
647 test.

648 Mating experiments were performed also on filamenting medium with few drops of oleic acid,
649 linoleic acid, linolenic acid, or squalene applied on the yeast cells inoculated with a loop; olive oil
650 was used as above (positive control). Three biological replicates were carried out. At the defined
651 incubation time, the cellular mix was plated on double drug medium as reported above.

652 The percentage of hyphal production of two characterized fusion products for each cross was
653 recoded after 3 days of incubation in the conditions reported above. Three or four independent
654 microscopic observations were carried out for each strain. The number of total cells and total hyphae
655 present in the observed sample were recorded and subjected to weighted one way ANOVA with
656 Tukey's multiple comparison test.

657 To observe a cell fusion event, the mCherry-tagged strain CBS14141 was crossed with *M.*
658 *furfur* strains CBS9574 and PM315 according to the conditions reported above and subjected to
659 microscopic observation after 24 – 72 h of incubation. Fluorescence microscopy was performed as
660 reported above using a filter compatible with mCherry excitation (550/25 nm) and emission (605/70
661 nm). DIC and mCherry images were super-imposed using Adobe Photoshop.

663 Scanning electron microscopy

664 Scanning electron microscopy was carried out as previously reported¹⁰. The *M. furfur* strains
665 were cultivated in filamenting medium with oil for 3 days or on RHE for 4 days (see below), and
666 fixed in 2.5% glutaraldehyde and 0.1 M cacodylate buffer (pH 7.4) at 4°C for 1 hour. Sample was
667 rinsed three time for 10 min each in 0.2 M cacodylate buffer, and maintained in 0.2 M cacodylate
668 buffer at 4°C for 48 h. Samples were gradually dehydrated in a graded ethanol series (30%, 50%,

669 70%, 80% and 100%), and dried overnight with hexamethyldisilazane. Gold-coated samples were
670 then analyzed using 6010LV scanning electron microscope (JEOL).

671

672 **Nuclear staining of *M. furfur* yeasts and hyphae**

673 Nuclear staining in *M. furfur* yeasts and hyphae was successfully achieved using a
674 combination of the protocols reported by Lachke et al. ³⁷ and Lin et al. ³⁸, with some modifications.
675 Briefly, cellular pellet of CBS7019 was placed on filamenting medium as reported above and
676 incubated at least for 3 days at 30°C. Cells were collected and washed with sterile water to remove
677 excess olive oil, then washed twice in HBSS balanced salt solution (HBSS: 10 mM HEPES, 150 mM
678 NaCl), and incubated for 10 min in HBSS with 40% ethanol. Cells were centrifuged, the supernatant
679 discarded, and the cellular pellet resuspended in 49 µL of 50% glycerol + 1 µL of a DAPI solution at
680 final concentration of 1 µg/mL. 10 µL of treated cells were placed on a sterile microscopic slide,
681 covered with a coverslip, and placed at 60°C for 40 min to aid penetration of DAPI solution into
682 fungal hyphae. Fluorescence microscopy was performed as reported above using a standard DAPI
683 filter. DIC and DAPI images were super-imposed using Adobe photoshop.

684

685 **PCR analyses**

686 Fusion products obtained in the crosses using NAT and NEO resistant haploid strains, in
687 unilateral crosses of CBS14141 *b1Δ* with CBS9574-NEO#2 and PM315-NEO#3, and in bilateral
688 crosses CBS14141 *b1Δ* × PM315 *b1Δ*, were subjected to PCR to characterize the markers inherited
689 following mating. Primers specific for the *MAT a1*, *a2*, *b1*, *b4* alleles were used according to the
690 condition reported by Theelen et al. ¹⁸, while the *NAT* and *NEO* marker as reported by Ianiri et al. ³⁵
691 (**Supplementary Table 6**). Primers specific for the *MAT* alleles were also used to further confirm the
692 mutation of the target *MAT a2Δ* and *b1Δ* in strains CBS14141, GI156 and GI166.

693 To determine which parental mitogenome was inherited by fusion products obtained from
694 interspecific and intraspecific crosses, primers targeting the *cox3–nad3* intergenic region and the *cob*
695 exon2–exon3 intronic region were used according to the condition reported by Theelen et al. ²⁰.
696 Specific primers for *cox1* were designed to discriminate the mitogenome of strain CD866 from that
697 of strain CBS9674 (**Supplementary Fig. 6**). PCRs were performed using EconoTaq PLUS 2x Master
698 Mix (Lucigen) according to manufacturer instruction, with initial denaturation at 94°C for 2 min,
699 denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min/Kb.

700

701 **RT-qPCR and statistical analyses**

702 Expression analysis of *MAT a1*, *a2*, *b1*, *b2* expression was carried out for several strains and
703 conditions. *M. furfur* CBS7019 was collected from mDixon agar, resuspended in water and spotted
704 on filamenting medium within a drop of olive oil, oleic acid, linoleic acid, linolenic acid, or squalene.
705 Cells were incubated for 24 h and 48 h and then subjected to RNA extraction. RT-qPCR analyses of
706 strains GI156 and GI166 and derived *MAT a2Δ* and *b1Δ* mutants, and of CBS14141 × PM315 and
707 CBS14141 *b1Δ* × PM315 *b1Δ*, was performed by inoculating the strains with a loop within a drop of
708 olive oil placed on filamenting medium with 24 h of incubation at 30°C. The comparative control
709 condition was represented by filamenting medium with no oil inoculated either with the parental
710 strains GI156 and GI166, or with the WT cross CBS14141 × PM315.

711 For RNA extraction, *M. furfur* cells were collected from plates and directly immersed in 1ml
712 of TRIzol reagent (Invitrogen) according to the manufacturer's instructions. To increase RNA yield,

200 µl of sterile glass beads were added to each tube and vortexed 2 times for 30 seconds before processing the samples. 500 µl of the aqueous phase was mixed with an equal volume of 70% ethanol. Total RNA was purified using the RNeasy Mini Kit (Qiagen). 500 ng of total RNA was retrotranscribed into cDNA using the SuperScript III Reverse Transcriptase kit (Invitrogen), and cDNA was 1:10 diluted in water. For keratinocytes, 1 µg of total RNA was retrotranscribed into cDNA using the SuperScript III Reverse Transcriptase kit, and cDNA was 1:20 diluted in water. Rt-qPCR were carried out using the Takyon No ROX SYBR Master Mix (Eurogentec), and with primers at a working concentration of 300 nM (**Supplementary Table 6**); 5 µl of cDNA were used for RT-qPCR. A “no-template control” and a water control were included for each target. Amplification protocol starts with 600s of pre-incubation at 95°C, followed by 45 cycles composed of 10 seconds at 95°C, 10 seconds at 60°C, and 10 seconds at 72°C. Technical and biological triplicates were performed for each sample. Gene expression levels were normalized using the reference genes *TUB2* for *M. furfur*, and *RPLP0* for keratinocytes, determined using the comparative $\Delta\Delta C_t$ method, and expressed as log₂ of the fold change.

For expression of *MAT a1*, *a2*, *b1*, *b2* in *M. furfur* CBS7019 grown with oil and individual fatty acids, statistical analysis was performed separately for each gene and time point on ΔC_t values using an ANOVA model with condition as the main factor and biological replicate as a blocking factor, followed by Tukey-adjusted pairwise comparisons.

For expression of *MAT a1*, *a2*, *b1*, *b2* in GI156 and GI166, and in the bilateral crosses CBS14141 × PM315 and CBS14141 *b1*Δ × PM315 *b1*Δ, statistical analysis was performed separately for each gene on log₂FC values using an ANOVA model followed by Sidak-adjusted pairwise comparisons.

735

Reference genomes and construction of pseudo-references

To analyze the genotypes of *M. furfur* fusion products, genome assemblies from one of the parental strains served as references for read mapping and variant calling. High-quality chromosome-scale assemblies were available for two strains: CBS14141 and PM315¹⁸. These assemblies functioned as the references in comparisons where they corresponded to one of the parental strains. For the CD866 × CBS9574 fusion products, no high-quality genome assembly was initially available. To enable comparative analysis, a pseudo-reference genome for CD866 was generated by assembling Illumina reads de novo with SPAdes v3.15.3. The resulting scaffolds were then reordered using D-GENIES based on whole-genome alignment to CBS14141, ensuring a consistent chromosomal orientation for analysis. For reference or pseudo-reference assemblies other than *M. furfur* CBS14141, gene annotations were transferred from the CBS14141 reference genome using Liftoff v1.6.3³⁹, which performs coordinate lift-over via whole-genome alignment with minimap2 v2.17 to support downstream visualization and gene-aware plotting.

749

Mapping and Variant calling

Raw Illumina paired-end reads were trimmed with fastp v1.0.1⁴⁰ to remove adapter sequences and low-quality bases. Reads shorter than 50 bp after trimming were discarded. Adapter sequences were automatically detected in paired-end mode. Quality-filtered reads were aligned to a selected reference or pseudo-reference genome of *M. furfur* with BWA-MEM v0.7.17, and the resulting alignments were sorted and indexed with Samtools v1.17. Variants were called independently for each sample using FreeBayes v1.3.10⁴¹, assuming diploidy. Sites were retained only if they met the

757 following thresholds: minimum total depth ≥ 5 , alternate allele count ≥ 2 , base quality ≥ 20 , and
758 mapping quality ≥ 30 . Resulting VCFs were further filtered with bcftools v1.17 to exclude variants
759 with a quality score (QUAL) below 20.

760

761 **Parental marker identification and genotype classification**

762 For each fusion product analyzed, we identified a set of high-confidence parent-informative
763 SNPs by mapping sequencing reads from one parental strain (designated as parent 2) to the genome
764 assembly of the other parental strain, which served as the reference (designated as parent 1) in the
765 variant calling step. Variant calls for the parent 2 were filtered to retain only biallelic SNPs where the
766 genotype was homozygous for the alternate allele (GT = 1/1), with a minimum quality score (QUAL)
767 of 20, total read depth ≥ 5 , and at least 2 supporting reads for the alternate allele. These SNPs represent
768 positions where parent 2 differs from parent 1 and were used as informative markers for genotyping
769 the fusion products. This filtering step was implemented in a custom Python script using pysam
770 v0.22.0. Genotype calls at these parent-informative SNP positions were then extracted for each fusion
771 product and classified into three categories: homozygous for parent 1, homozygous for parent 2, or
772 heterozygous. For marker positions where no variant was detected in the fusion product, the genotype
773 was inferred to be homozygous for parent 1, as the absence of a variant indicates identity with the
774 reference allele.

775

776 **Genome-wide genotype visualization**

777 Genotype classifications across the genome were visualized using custom Python scripts built
778 with matplotlib v3.8.2, pandas v2.2.2, and Biopython v1.83. A matrix-style bar plot was generated to
779 depict genotype states (homozygous parent 1, homozygous parent 2, or heterozygous) across each
780 chromosome for all analyzed samples. To simplify the visualization of extended regions of consistent
781 genotype, adjacent variants of the same class were optionally merged into blocks if separated by
782 $\leq 5,000$ bp. In addition to the genome-wide view, zoomed-in plots were also generated and plotted
783 together with gene models from a GFF3 file to provide functional context.

784

785 **Coverage analysis and visualization**

786 To quantify genome-wide variation in read depth across fusion products, we computed
787 normalized read coverage in non-overlapping bins using bedtools v2.30.0. For each sample, read
788 alignments (BAM files) were processed to calculate coverage in fixed-size genomic windows of
789 5,000 bp. Genomic bins were defined with bedtools makewindows, and per-bin coverage was
790 computed with bedtools coverage. Coverage values were then normalized by dividing the number of
791 reads in each bin by the sample's genome-wide median read depth. This normalization step was
792 implemented in a custom Python script using pandas v2.2.2 and Biopython v1.83. The resulting
793 coverage profiles were visualized as heatmaps using matplotlib v3.8.2 and seaborn v0.13.2. Binned
794 coverage values were clipped to a range of 0 to 2x relative to the median to reduce the influence of
795 extreme values and enhance visual contrast. Final composite figures combining genotype
796 classification and coverage heatmaps were arranged manually in Adobe Illustrator for clarity of
797 presentation, using plots generated by the custom scripts described above.

798

799 **Fluorescence-activated cell sorting (FACS)**

800 Ploidy was assessed by fluorescence-activated cell sorting (FACS) analysis as described by
801 Theelen et al.¹⁸ with minor modifications. *M. furfur* strains were grown for 4 days on mDixon agar

at 30 °C; cells were collected in PBS, washed one time with PBS and fixed in 70 % ethanol at 4 °C overnight. Fixed cells were sonicated for 2 minutes and then washed with 1ml of NS buffer (10 mM Tris-HCl, pH 7.6, 250 mM sucrose, 1 mM EDTA, pH 8.0, 1 mM MgCl₂, 0.1 mM CaCl₂, 0.1 mM ZnCl₂, 0.55 mM PMSF, 7 mM 2-mercaptoethanol) and then stained with propidium iodide (30 µg/mL) in 0.2 ml of NS buffer containing RNase A (1 mg/ml) at 4 °C overnight in the dark. A volume of 50 µl of stained cells were added to 0.5 mL of Tris-PI mix [482 µl 1M Tris pH 7.5 + 18 µl Propidium Iodide (1 mg/µl)]. The final cell suspension was filtered through Miracloth (Calbiochem # 475855) prior to analysis. Flow cytometry was performed using a BD FACSCanto II analyzer as previously described ⁴².

811

812 ***M. furfur* infection on reconstructed human epidermis**

Reconstructed human epidermis (RHE) were obtained using human primary keratinocytes as previously described ⁴³. RHE on day 11 of reconstruction were pre-treated with sterile olive oil prior infection by 10⁴ yeasts per RHE according to a previously developed protocol ¹⁰. Infected RHE were collected four days after infection. Infected and control RHE were collected in a 4% formaldehyde solution for at least 24h before processing. Periodic acid-Schiff (PAS) staining was used to highlight *Malassezia* cell wall and RHE were counterstained with hemalum as previously described ⁴⁴. Barrier integrity of RHE during infection was assessed using trans-epithelial electrical resistance (TEER) and Lucifer Yellow (LY) assay as previously described ⁴⁵. Keratinocyte RNA was extracted using TRIzol reagent (Invitrogen). RHE were cut from the cell culture insert and dissolved in 50 µl of TRIzol using a grinder (Nippon Genetics). 950ul of TRIzol was then added to reach a total volume of 1 ml. Samples were processed according to the manufacturer's instructions. To ensure a pure and DNA-free RNA, 400 µl of the aqueous phase was collected and mixed with an equal volume of 70% ethanol. Total RNA was purified and subjected to DNase treatment according to manufacturer's instructions using the RNeasy Mini Kit (Qiagen). RNA concentration was determined by spectrophotometry using a Nanodrop device. RT-qPCR was carried out as described in the dedicated paragraph.

828

829 ***Ex vivo* and *in vivo* *M. furfur* infection on mice hear**

Animal experiments in this study were conducted in strict accordance with the guidelines of the Swiss Animals Protection Law and were performed under the protocols approved by the Veterinary office of the Canton Zurich, Switzerland (license number ZH200/2024). All efforts were made to minimize suffering and ensure the highest ethical and humane standards according to the 3R principles ⁴⁶. Female C57Bl/6 mice were mice purchased from Janvier Elevage and kept in individually ventilated cages under specific pathogen-free conditions at the Institute of Laboratory Animals Science (LASC, University of Zurich). *M. furfur* strains were grown on MM agar in 200 µl olive oil (2 × 10⁷ cells) at 30 °C for 7 days prior to infection experiments. The fungus was then collected from the agar and suspended homogenously. For *ex vivo* colonization assays, mouse ears were collected from euthanized mice, cut in half, and placed dorsal-side up in a 24-well plate. 50 µl fungal suspension was applied on top and the ears were incubated for 48 h at 30 °C under humid conditions. Samples were fixed overnight in 4% PBS-buffered paraformaldehyde, embedded in paraffin, sectioned sagittally (9 µm), and stained using periodic acid-Schiff (PAS). Slides were mounted using Pertex mounting medium (Biosystem) according to standard protocols. Histological images were acquired using a digital slide scanner (NanoZoomer 2.0-HT, Hamamatsu) and analyzed with NDP.view2 software. For *in vivo* infection experiments, epicutaneous colonization of mouse ear skin was performed as previously described ²². Mice were anesthetized by intraperitoneal injection of ketamine

846

847 (65 mg/kg) and xylazine (13 mg/kg), and a 100 µl fungal suspension (corresponding to 1×10^7 cells
848 in olive oil) was applied epicutaneously to the dorsal side of each ear. Ear thickness was measured
849 prior to colonization and then daily throughout the experiment using an Oditest S0247 0–5 mm
850 measurement device (Kroeplin).

851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891

References

1. Findley, K. *et al.* Topographic diversity of fungal and bacterial communities in human skin. *Nature* 498, (2013).
2. Ianiri, G., Leibundgut-Landmann, S. & Dawson, T. L. *Malassezia*: A Commensal, Pathogen, and Mutualist of Human and Animal Skin. *Annual Review of Microbiology* vol. 76 Preprint at <https://doi.org/10.1146/annurev-micro-040820-010114> (2022).
3. Li, H. *et al.* Skin Commensal *Malassezia globosa* Secreted Protease Attenuates *Staphylococcus aureus* Biofilm Formation. *Journal of Investigative Dermatology* 138, (2018).
4. Kowalski, C. H. *et al.* Skin mycobiota-mediated antagonism against *Staphylococcus aureus* through a modified fatty acid. *Current Biology* 35, (2025).
5. Proctor, D. M. *et al.* Integrated genomic, epidemiologic investigation of *Candida auris* skin colonization in a skilled nursing facility. *Nat. Med.* 27, (2021).
6. Gushiken-Ibañez, E. *et al.* Commensal yeast *Malassezia* produces tryptophan metabolites to promote tissue homeostasis via the aryl hydrocarbon receptor in mice. *Nat. Microbiol.* 11, 1934–1948 (2026).
7. Gaitanis, G., Magiatis, P., Hantschke, M., Bassukas, I. D. & Velegraki, A. The *Malassezia* genus in skin and systemic diseases. *Clin. Microbiol. Rev.* 25, (2012).
8. Abdillahi, A. & Ranque, S. Chronic diseases associated with *Malassezia* yeast. *Journal of Fungi* vol. 7 Preprint at <https://doi.org/10.3390/jof7100855> (2021).
9. Li, J. *et al.* Presence of *Malassezia* Hyphae Is Correlated with Pathogenesis of Seborrheic Dermatitis. *Microbiol. Spectr.* 10, (2022).
10. Tirtiaux, B. *et al.* Invasion of Human Epidermis by *Malassezia furfur* Is Strain Dependent. *Journal of Investigative Dermatology* 145, (2025).
11. Chua, W. *et al.* Aspartyl protease MfSAP2 is a key virulence factor in mycelial form of skin fungi *Malassezia furfur*. *Biochemical Journal* 483, (2026).
12. Coelho, M. A., Bakkeren, G., Sun, S., Hood, M. E. & Giraud, T. Fungal Sex: The Basidiomycota. *Microbiol. Spectr.* 5, (2017).
13. Schlesinger, R., Kahmann, R. & Kämper, J. The homeodomains of the heterodimeric bE and bW proteins of *Ustilago maydis* are both critical for function. *Molecular and General Genetics* 254, (1997).
14. Kämper, J., Reichmann, M., Romeis, T., Bölker, M. & Kahmann, R. Multiallelic recognition: Nonself-dependent dimerization of the bE and bW homeodomain proteins in *Ustilago maydis*. *Cell* 81, (1995).
15. Gillissen, B. *et al.* A two-component regulatory system for self/non-self recognition in *Ustilago maydis*. *Cell* 68, (1992).
16. Coelho, M. A. *et al.* Frequent transitions in mating-type locus chromosomal organization in *Malassezia* and early steps in sexual reproduction. *Proc. Natl. Acad. Sci. U. S. A.* 120, (2023).
17. Wu, G. *et al.* Genus-Wide Comparative Genomics of *Malassezia* Delineates Its Phylogeny, Physiology, and Niche Adaptation on Human Skin. *PLoS Genet.* 11, (2015).
18. Theelen, B. *et al.* Multiple Hybridization Events Punctuate the Evolutionary Trajectory of *Malassezia furfur*. *mBio* 13, (2022).
19. Kämper, J. *et al.* Insights from the genome of the biotrophic fungal plant pathogen *Ustilago maydis*. *Nature* 444, (2006).
20. Theelen, B., Christinaki, A. C., Dawson, T. L., Boekhout, T. & Kouvelis, V. N. Comparative analysis of *Malassezia furfur* mitogenomes and the development of a mitochondria-based typing approach. *FEMS Yeast Res.* 21, (2021).
21. Sun, S., Fu, C., Ianiri, G. & Heitman, J. The pheromone and pheromone receptor mating-type locus is involved in controlling uniparental mitochondrial inheritance in *Cryptococcus*. *Genetics* 214, (2020).

- 941 22. Sparber, F. *et al.* The Skin Commensal Yeast *Malassezia* Triggers a Type 17 Response that
942 Coordinates Anti-fungal Immunity and Exacerbates Skin Inflammation. *Cell Host Microbe* 25,
943 (2019).
- 944 23. Knox, S. & O’Boyle, N. M. Skin lipids in health and disease: A review. *Chemistry and Physics*
945 *of Lipids* vol. 236 Preprint at <https://doi.org/10.1016/j.chemphyslip.2021.105055> (2021).
- 946 24. Cabrita, A. *et al.* Multiple pathways to homothallism in closely related yeast lineages in the
947 basidiomycota. *mBio* 12, (2021).
- 948 25. Lucotte, E. A. *et al.* Repeated loss of function at HD mating-type genes and of recombination
949 in anther-smut fungi. *Nature Communications* 16, (2025).
- 950 26. Dutta, A. & Schacherer, J. The dynamics of loss of heterozygosity events in genomes. *EMBO*
951 *Reports* vol. 26 Preprint at <https://doi.org/10.1038/s44319-024-00353-w> (2025).
- 952 27. Kukhta, T., NandyMazumdar, M. & Zhang, M. Telomere-to-telomere assembly reveals DNA
953 repair-driven genome restructuring in hybrid *Malassezia furfur*. *Genes Dis.* 102296 (2026)
954 doi:10.1016/j.gendis.2026.102296.
- 955 28. Bennett, R. J. The parasexual lifestyle of *Candida albicans*. *Curr. Opin. Microbiol.* 28, 10–17
956 (2015).
- 957 29. Forche, A. *et al.* The parasexual cycle in *Candida albicans* provides an alternative pathway to
958 meiosis for the formation of recombinant strains. *PLoS Biol.* 6, (2008).
- 959 30. Anderson, M. Z., Thomson, G. J., Hirakawa, M. P. & Bennett, R. J. A ‘parameiosis’ drives
960 depolyploidization and homologous recombination in *Candida albicans*. *Nature*
961 *Communications* 10, (2019).
- 962 31. Fillinger, R. J. *et al.* Parasex generates highly recombinant progeny in *Candida albicans* with
963 increased virulence. *Nat. Microbiol.* 11, (2026).
- 964 32. Payero, L. & Alani, E. Crossover recombination between homologous chromosomes in
965 meiosis: recent progress and remaining mysteries. *Trends in Genetics* vol. 41 Preprint at
966 <https://doi.org/10.1016/j.tig.2024.09.009> (2025).
- 967 33. Nielsen, K. & Heitman, J. Sex and Virulence of Human Pathogenic Fungi. *Advances in*
968 *Genetics* vol. 57 Preprint at [https://doi.org/10.1016/S0065-2660\(06\)57004-X](https://doi.org/10.1016/S0065-2660(06)57004-X) (2007).
- 969 34. Ianiri, G., Dagotto, G., Sun, S. & Heitman, J. Advancing functional genetics through
970 Agrobacterium-mediated insertional mutagenesis and CRISPR/Cas9 in the commensal and
971 pathogenic yeast *Malassezia*. *Genetics* 212, (2019).
- 972 35. Ianiri, G., Averette, A. F., Kingsbury, J. M., Heitman, J. & Idnurm, A. Gene function analysis
973 in the ubiquitous human commensal and pathogen *Malassezia* genus. *mBio* 7, (2016).
- 974 36. Goh, J. P. Z., Ianiri, G., Heitman, J. & Dawson, T. L. Expression of a *Malassezia* Codon
975 Optimized mCherry Fluorescent Protein in a Bicistronic Vector. *Front. Cell. Infect. Microbiol.*
976 10, (2020).
- 977 37. Lachke, S. A., Joly, S., Daniels, K. & Soll, D. R. Phenotypic switching and filamentation in
978 *Candida glabrata*. *Microbiology (N. Y.)* 148, (2002).
- 979 38. Lin, J. S., Happel, P. & Kahmann, R. Nuclear status and leaf tumor formation in the *Ustilago*
980 *maydis*–maize pathosystem. *New Phytologist* 231, (2021).
- 981 39. Shumate, A. & Salzberg, S. L. Liftoff: Accurate mapping of gene annotations. *Bioinformatics*
982 37, (2021).
- 983 40. Chen, S. Ultrafast one-pass FASTQ data preprocessing, quality control, and deduplication
984 using fastp. *iMeta* 2, (2023).
- 985 41. Garrison, E. & Marth, G. Haplotype-based variant detection from short-read sequencing --
986 Free bayes -- Variant Calling -- Longranger. *arXiv preprint arXiv:1207.3907* (2012).
- 987 42. Fu, C. *et al.* Dynamic genome plasticity during unisexual reproduction in the human fungal
988 pathogen *Cryptococcus deneoformans*. *PLoS Genet.* 17, (2021).
- 989 43. De Vuyst, E. *et al.* Reconstruction of normal and pathological human epidermis on
990 polycarbonate filter. *Methods in Molecular Biology* 1195, (2014).

991 44. Faway, É., Cambier, L., Mignon, B., Poumay, Y. & De Rouvroit, C. L. Modeling
992 dermatophytosis in reconstructed human epidermis: A new tool to study infection mechanisms
993 and to test antifungal agents. *Med. Mycol.* 55, (2017).

994 45. Faway, E. *et al.* Responses of Reconstructed Human Epidermis to *Trichophyton rubrum*
995 Infection and Impairment of Infection by the Inhibitor PD169316. *Journal of Investigative*
996 *Dermatology* 139, (2019).

997 46. Blache, D., Martin, G. B. & Maloney, S. K. Towards Ethically Improved Animal
998 Experimentation in the Study of Animal Reproduction. *Reproduction in Domestic Animals* 43,
999 (2008).

1000
1001
1002
1003
1004

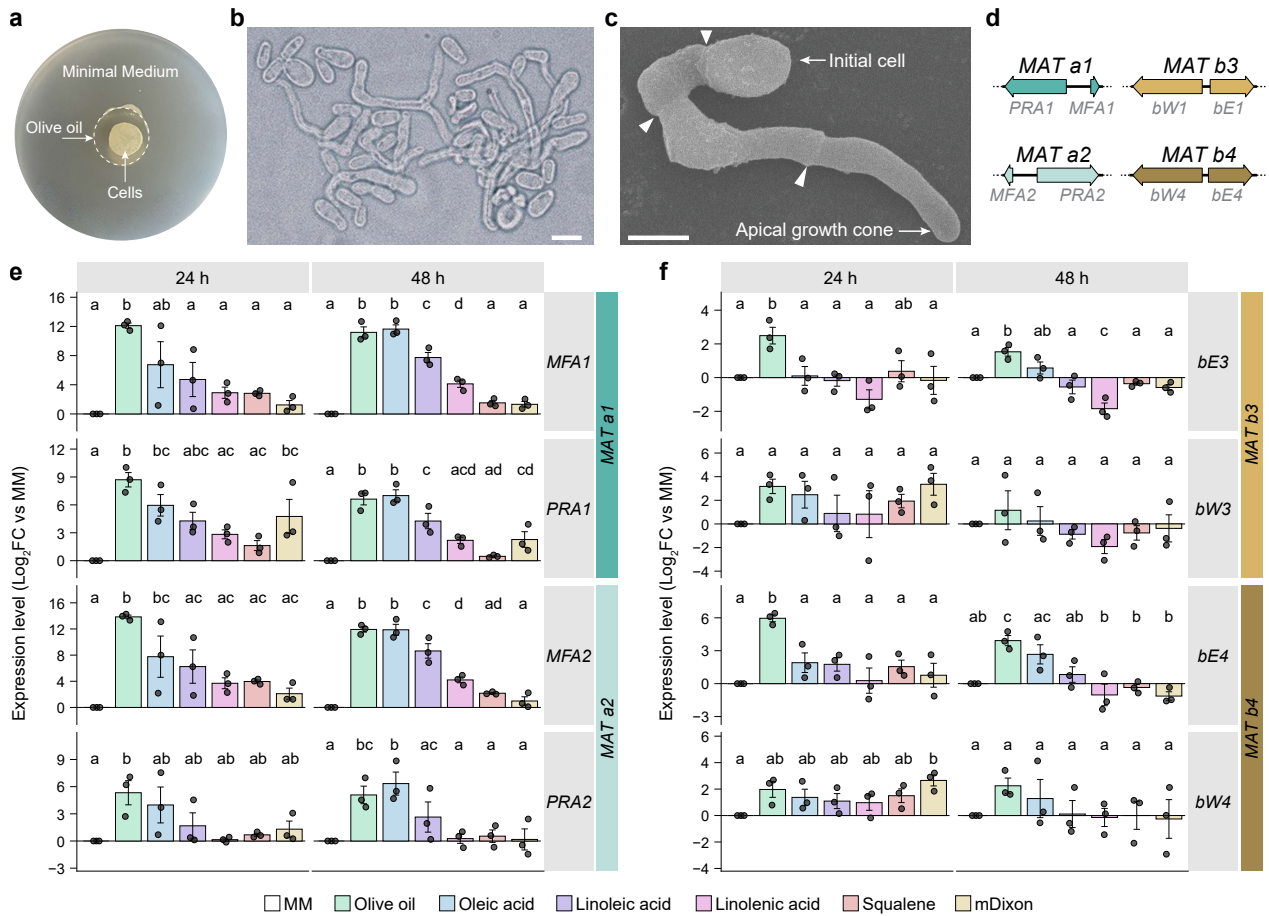


Figure 1. *M. furfur* hyphal formation and *MAT* gene expression under lipid-inducing conditions

(a) Experimental setup to induce hyphal growth in *M. furfur* CBS7019. Cells were resuspended in water and spotted within a drop of olive oil placed on filamentation medium (MM agar). **(b)** Hyphae of CBS7019 recovered after 3 days of incubation at 30°C (conditions as in panel a). **(c)** SEM image of a CBS7019 hypha; the initial cell, apical growth cone, and the septa (arrowheads) are indicated. **(d)** Schematic of the *MAT* locus organization in CBS7019, showing the two *MAT A* alleles (*a1* and *a2*) and two *MAT B* alleles (*b3* and *b4*) present in this diploid strain. Because the genome assembly of CBS7019 is not phased, the allelic combinations between *MAT A* and *MAT B* on each haplotype remain unknown. **(e, f)** RT-qPCR expression levels of **(e)** *MAT a1* and *a2* genes, and **(f)** *MAT b3* and *b4* genes in *M. furfur* CBS7019 after 24 h and 48 h on filamentation medium alone (MM; reference condition) or MM supplemented with olive oil, oleic acid, linoleic acid, linolenic acid, or squalene, or on mDixon. Expression levels are shown as log₂ fold change (FC) relative to MM, calculated using the $\Delta\Delta C_t$ method with *TUB2* as endogenous reference; MM log₂ FC is defined as 0. Bars show the mean log₂FC across biological replicates (n = 3); error bars indicate the standard error of the mean; dots indicate individual biological replicate values, each derived from the mean of three technical replicates. Statistical analysis was performed for each gene and time point on ΔC_t values using a one-way ANOVA with condition as the main factor and biological replicate as a blocking factor, followed by Tukey-adjusted pairwise comparisons. Letters above bars indicate Tukey groupings ($\alpha = 0.05$), with shared letters indicating no significant differences. Full pairwise comparison results are provided in Additional file 1.

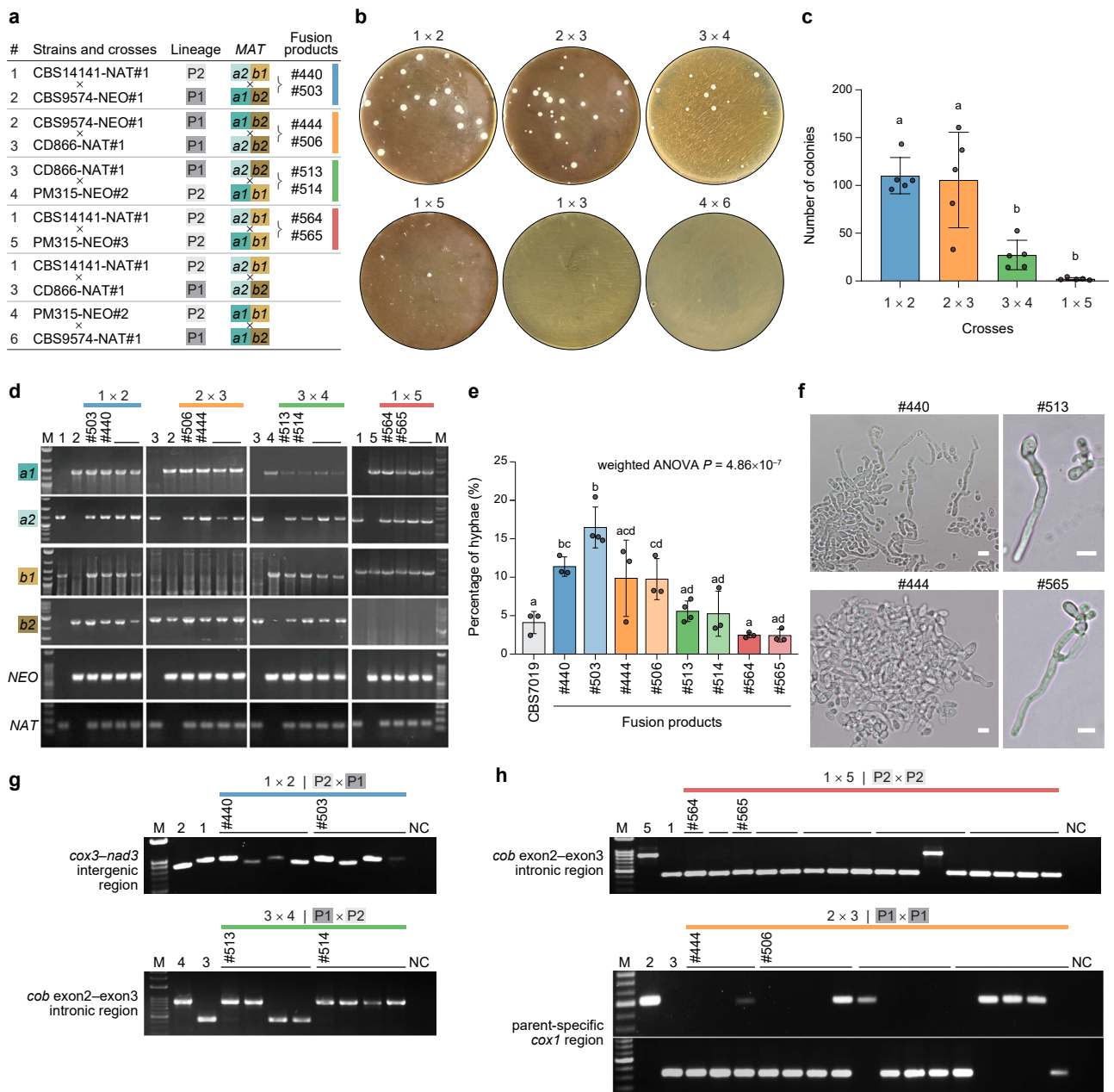


Fig. 2. MAT A-compatible *M. furfur* strains undergo cell fusion and generate fusion products that gain the ability to form hyphae. (a) Summary of the haploid parental strains, lineage assignment (P1 or P2), MAT allele genotypes, and the fusion products recovered from each cross (coded as 1×2, 2×3, 3×4, and 1×5, matching the parental numbering shown in the table). (b) Representative double-drug selection plates from the indicated inter-specific (P1 × P2) and intra-specific (P1 × P1 or P2 × P2) crosses showing recovery of fusion-product colonies. In cross 1×5, only one colony was recovered in the plate shown. (c) Quantification of colony formation under double-drug selection across independent crosses. Bars show the mean and error bars indicate SD; points represent individual plates from five independent mating assays per cross, each plated in duplicate or triplicate. Differences across crosses were determined by one-way ANOVA followed by Tukey's multiple-comparison test. Full pairwise comparison results are provided in Additional file 2. (d) PCR genotyping of the parental haploid strains and selected fusion products to assess the MAT genotype (*a1*, *a2*, *b1*, *b2*) and the NAT/NEO drug-resistance markers. Fusion products selected for downstream analyses are labeled (#). (e) Filamentation rate after 72 h of incubation on filamentation medium with

olive oil, reported as the percentage of hyphal cells, for the natural hybrid strain CBS7019 and selected fusion products. Bars show the mean across independent measurements and error bars indicate SD; points represent individual measurements ($n = 3$ per strain, except #503 and #513 where $n = 4$). Statistical differences across groups were assessed by weighted one-way ANOVA (weights = total cells scored per measurement) followed by Tukey's multiple-comparison test. Letters above bars indicate Tukey groupings ($\alpha = 0.05$), with shared letters indicating no significant differences. Full pairwise comparison results are provided in Additional file 3. **(f)** Representative microscopy images of filamentation phenotypes for fusion products from interspecific (#440 and #513) and intraspecific (#444 and #565) crosses; images of other fusion products are available in **Supplementary Fig. 5**. Scale bars, 5 μm . **(g–h)** Mitochondrial inheritance in representative fusion products assessed by PCR with markers distinguishing parental mitochondrial genotypes; amplicon regions are labeled to the left of each gel. Parental strains are included as reference; NC, no-template control; M, ladder.

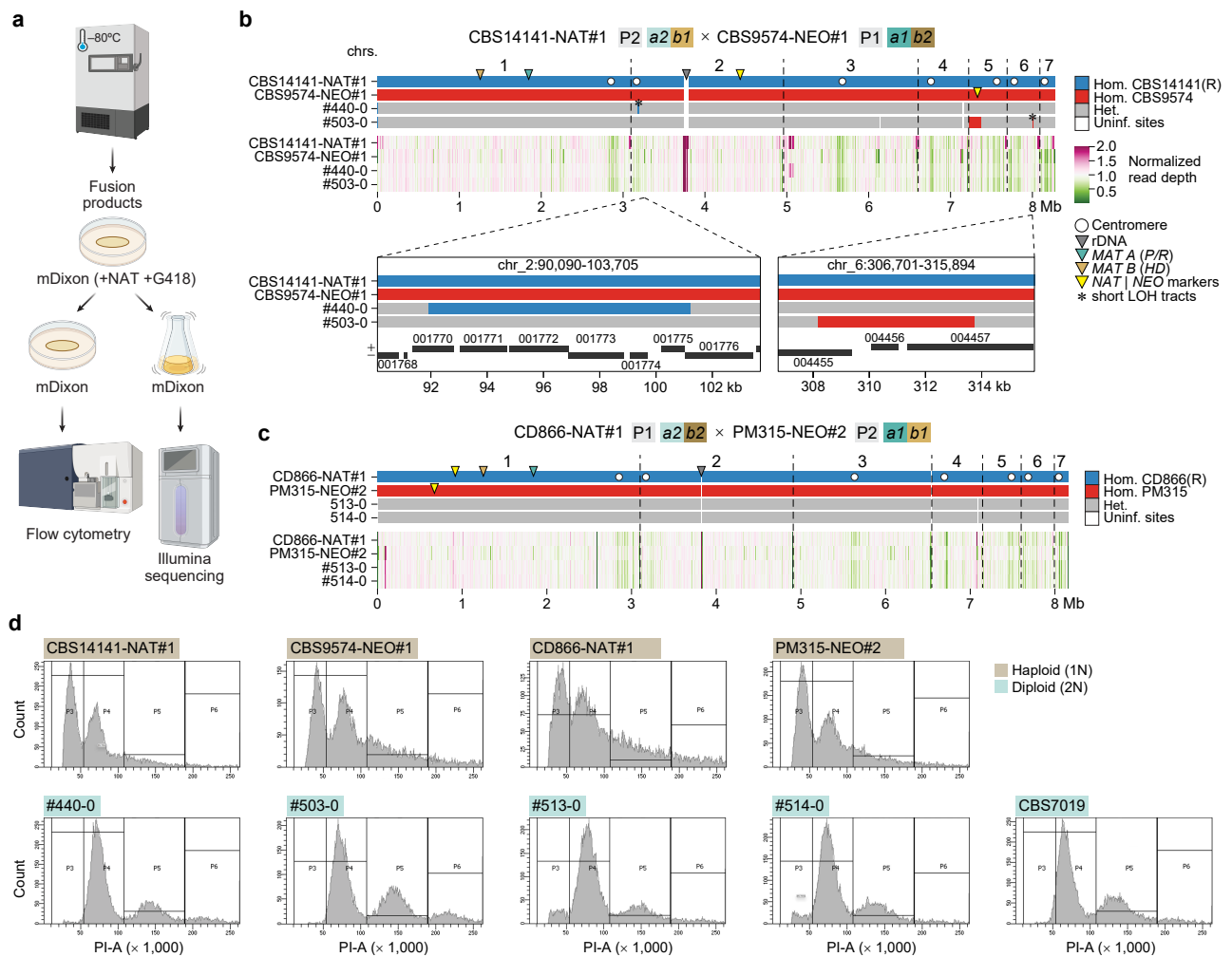


Fig. 3. Genome composition and ploidy of *M. furfur* fusion products from interspecific P1 × P2 crosses between marked, *MAT*-compatible strains. (a) Workflow for characterization of fusion products by flow cytometry and Illumina sequencing. Fusion products were revived from frozen stocks on mDixon agar supplemented with NAT and G418, then grown in liquid mDixon for DNA extraction, or on solid mDixon for flow cytometry. **(b, c)** Genotype (top tracks) and normalized read-depth (bottom tracks) profiles for two independent fusion products from each interspecific cross: **(b)** CBS14141-NAT#1 × CBS9574-NEO#1 (#440 and #503) and **(c)** CD866-NAT#1 × PM315-NEO#2 (#513 and #514). Genotypes were inferred from parent-informative SNPs mapped to the indicated parental reference genome (R) and classified as homozygous reference (blue), homozygous alternate parent (red), heterozygous (gray), or uninformative (i.e. regions identical between both parental); adjacent variants of the same genotype class separated by ≤ 5 kb were merged. Read depth was calculated in non-overlapping 5-kb windows and normalized to the genome-wide median. Arrows indicate the reference-coordinate positions of the rDNA locus, T-DNA insertions, and *MAT A* and *MAT B* loci, as indicated in the key; T-DNA insertion sites were inferred from strain-specific *de novo* assemblies and projected onto the reference genome. White circles depict centromeres, and dashed lines indicate chromosome boundaries. Small regions of read-depth variation may reflect copy-number differences or parental divergence affecting read mapping. Inserts in **b** show localized LOH tracts with gene models lifted from the *M. furfur* CBS14141 annotation (+ and – indicate strand orientation). **(d)** Flow cytometry profiles of parental strains, fusion products, and the diploid control CBS7019. Haploid and diploid profiles are labeled in brown and in light green, respectively.

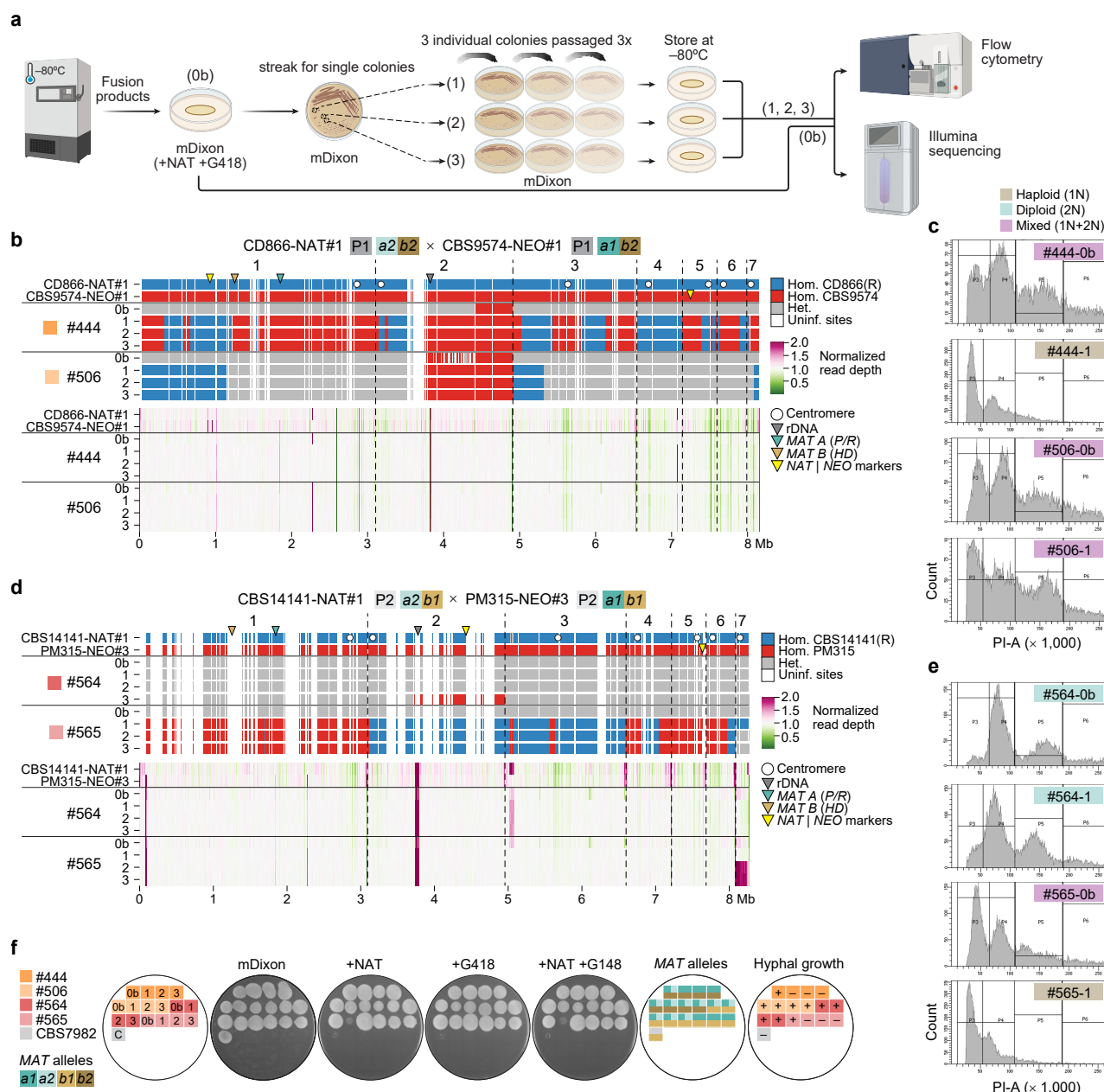


Fig. 4. Ploidy reduction and recombination in *M. furfur* fusion products from intraspecific crosses between marked strains carrying different *MAT A* alleles but matching *MAT B* alleles. (a) Workflow for characterization of intraspecific fusion products by flow cytometry and Illumina sequencing. Revived fusion products (labeled as 0b) on double-drug (NAT+G418) mDixon plates were sampled directly for DNA extraction and flow cytometry. A portion of the same growth patch was streaked on non-selective mDixon, and three single-colony derivatives (labeled 1, 2, and 3) were passaged three times without selection before analysis by flow cytometry and Illumina sequencing. (b–e) Genome composition, normalized read-depth and flow cytometry profiles for the indicated intraspecific P1 × P1 and P2 × P2 fusion products and their passaged derivatives; genome tracks are shown in b and d, and representative flow cytometry profiles in c and e. Flow cytometry profiles of all strains are in **Supplementary Fig. 10**. Genotype classes and read-depth normalization are as described in Fig. 3; additional annotations are indicated in panel keys. (f) Drug resistance, *MAT* allele content, and hyphal growth of revived fusion products (0b) and passaged derivatives (1–3), spotted on mDixon with no drug, NAT, G418 or both drugs, and incubated at 30°C for 5 days. Hyphal growth

was assessed on filamentation medium with olive oil and scored as present (+) or absent (–). *MAT* alleles and *NAT/NEO* markers were identified from *de novo* genome assemblies.

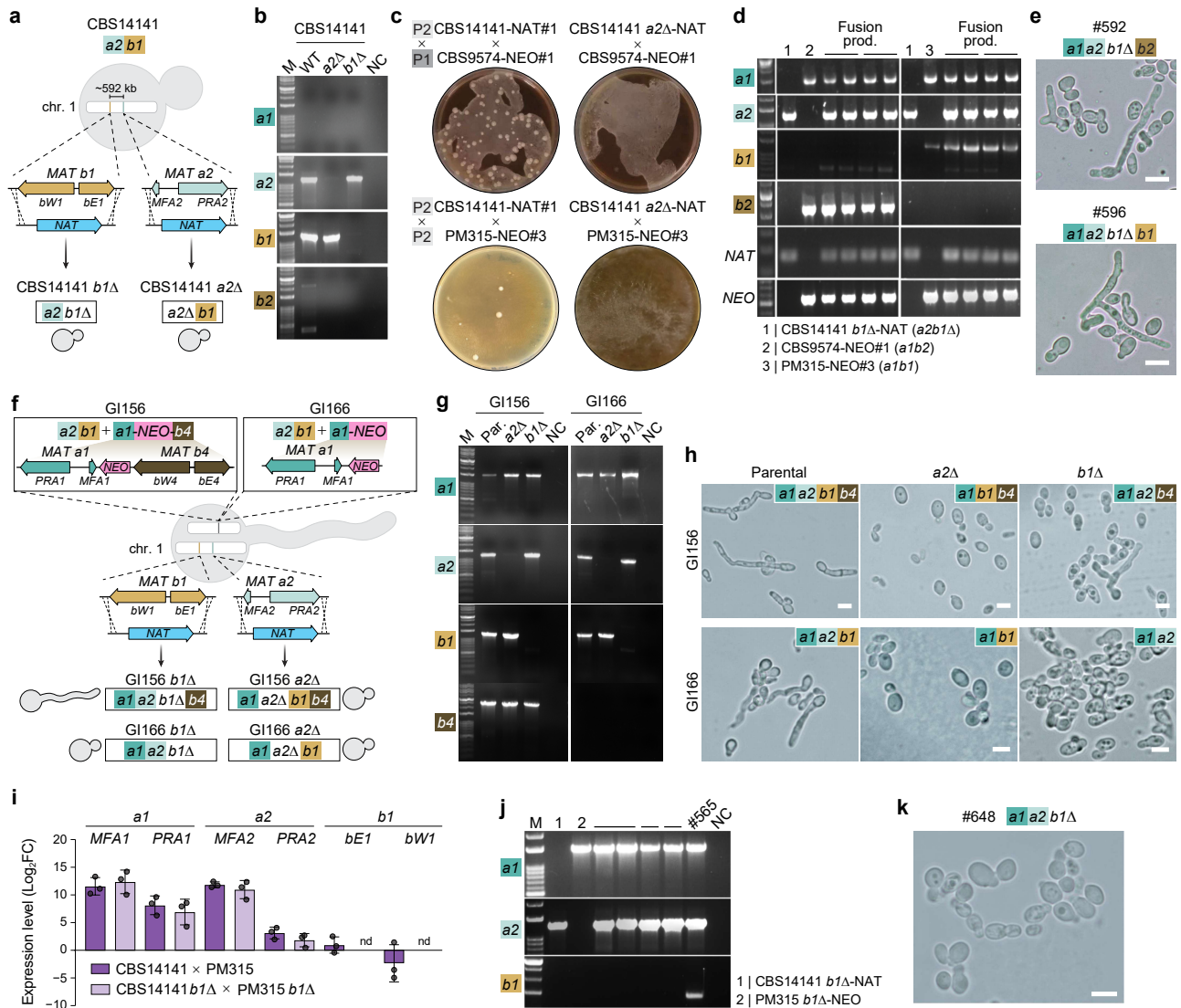


Fig. 5. Functional role of the *MAT A* and *MAT B* loci in cell fusion and hyphal formation. (a) Schematic representation of *M. furfur* CBS14141 *mat a2Δ* and *mat b1Δ* mutants, generated by NAT-marker disruption of *MFA2/PRA2* (*MAT a2*) or *bW1/bE1* (*MAT b1*) on chr. 1; the resulting genotypes and yeast-form morphology, as assessed on filamentation medium, are shown. (b) PCR validation of *mat a2Δ* and *mat b1Δ* mutants to assess presence/absence of *MAT a1*, *a2*, *b1* and *b2* alleles; NC, no-template control; M, ladder. (c) Representative double-drug selection plates showing recovery of fusion products in the control crosses CBS14141-NAT#1 x CBS9574-NEO#1 (P2 × P1) and CBS14141-NAT#1 × PM315-NEO#3 (P2 × P2), and their absence in the corresponding crosses carried out with the CBS14141 *mat a2Δ* mutant. (d) PCR genotyping of fusion products from the unilateral crosses CBS14141 *mat b1Δ* × CBS9574-NEO#1 (P1 × P2), and CBS14141 *mat b1Δ* × PM315-NEO#3 (P2 × P2). Parental strains are numbered 1 - 3, with genotypes reported below the gels; black lines above the gels indicates fusion products from independent crosses. The weak band in the *MAT b1* panel is a non-specific amplicon. (e) Fusion products #592 and #596, obtained from unilateral crosses in **d**, from hyphae on filamentation medium with olive oil; their *MAT* configurations are indicated. (f) Schematic representation of the self-filamenting *M. furfur* strains GI156 and GI166 and their derived *mat a2Δ* and *mat b1Δ* mutants. Top: original constructs (*MAT a1-NEO-b4*, and *MAT a1-NEO*) inserted into strain CBS14141 to generate strains GI156 and GI166, respectively (Coelho et al., 2023); bottom: native *MAT a2* and *MAT b1* alleles on chr. 1, and their mutation achieved through

homologous recombination using the *NAT* marker. The resulting mutants and their morphology, as assessed on filamentation medium with olive oil, are shown. **(g)** PCR validation of GI156 and GI166 and derived *mat a2Δ* and *mat b1Δ* mutants to assess presence/absence of the *MAT a1*, *a2*, *b1* and *b4* alleles; NC, no-template control; M, ladder. **(h)** Morphology of GI156 and GI166 and derived *mat a2Δ* and *mat b1Δ* mutants on filamenting medium with olive oil. **(i)** RT-qPCR expression levels of the genes *MFA1*, *PRA1*, *MFA2*, *PRA2*, *bE1* and *bW1* during the WT cross (CBS14141-NAT#1 × PM315-NEO#3) and the bilateral cross of derived *mat-b1Δ* mutants, both after 24 h on filamentation medium with olive oil, relative to the WT crosses on filamenting medium without olive oil (MM), used as the comparative condition. Ct values were normalized to the endogenous reference *TUB2* and converted to log₂ fold change (log₂FC) relative to MM using the $\Delta\Delta C_t$ method; as MM is the calibrator, its log₂ FC is defined as 0. Bars show mean log₂FC across biological replicates (n = 3), with error bars indicating SEM; dots indicate individual biological replicate values, each the mean of three technical replicates. Statistical analysis was performed separately for each gene using an ANOVA model (condition as the main factor and biological replicate as a blocking factor), followed by Sidak pairwise comparisons. Nd, ‘not detected’. **(j)** PCR genotyping of fusion products from the bilateral cross CBS14141 *mat b1Δ* × PM315 *mat-b1Δ*; parental strains are indicated with numbers 1-2, with their genotypes shown next to the image; strain #565 served as positive control for *MAT B1*. Black line above the gel group fusion products from independent crosses. NC, no-template control; M, ladder. **(k)** Representative image of one fusion product (strain #648) from the bilateral cross in **j** on filamentation medium with olive oil. In all microscopic panels, the scale bar indicates 5 μ m.

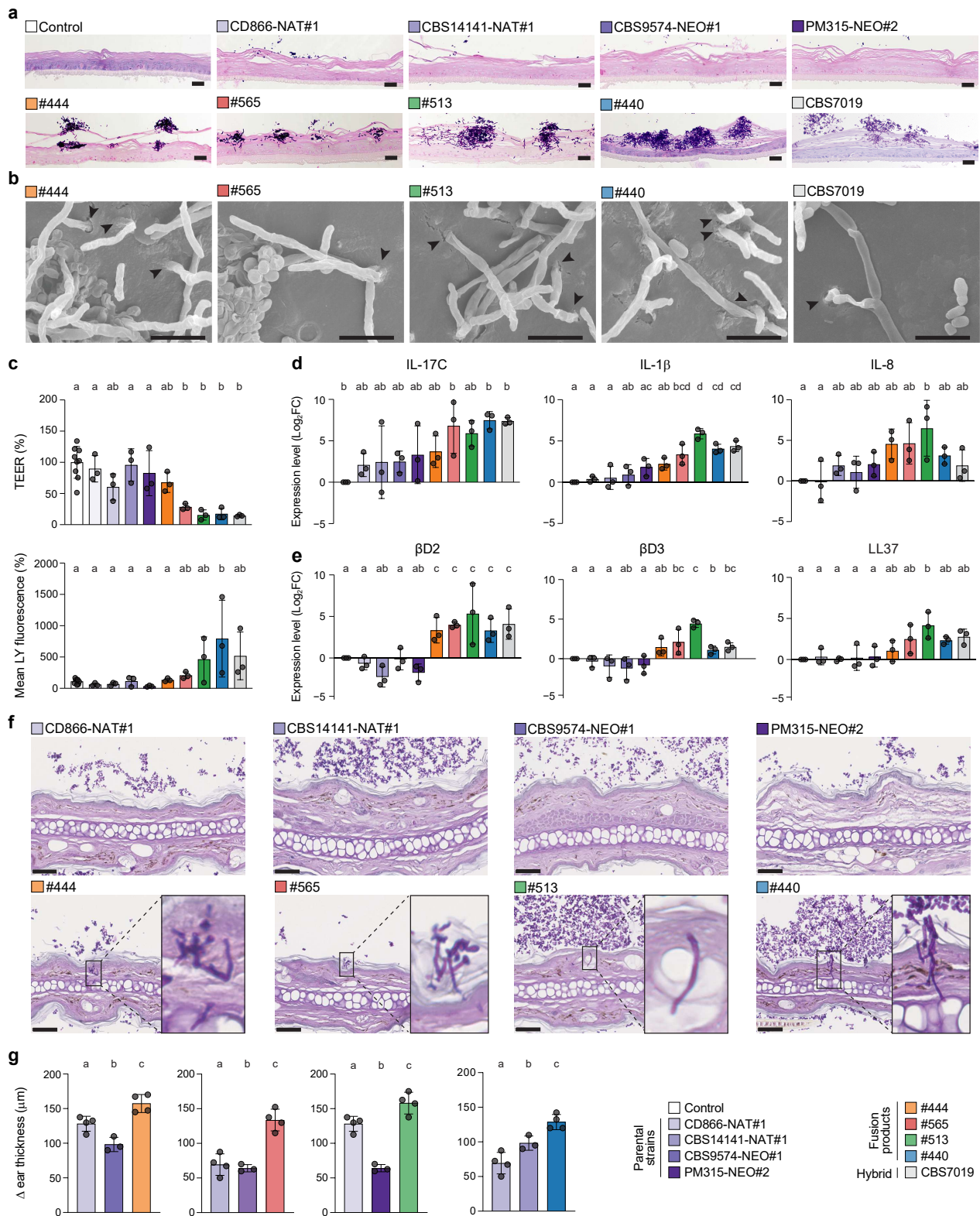


Fig. 6. *M. furfur* hyphae-forming strains are more pathogenic than yeast-forming strains on human and mouse models. (a) RHE topically inoculated with *M. furfur* strains and stained with PAS staining on day 4 of infection. Scale bars, 50 μ m. (b) Representative SEM images of hyphal strains during RHE infection; arrowheads indicate hyphae invading the cornified layer. (c) Barrier integrity assessed by transepithelial electrical resistance (TEER) and Lucifer Yellow (LY) permeability. Dots represents an individual measurement, and bars indicate mean $\pm$ SD. (d, e) Expression of genes encoding (d) proinflammatory cytokines (IL-1 β , IL-17C, and IL-8) and (e) antimicrobial peptide

(β D2, β D3 and LL37) in RHE infected with strains in the same order as in (a) and as given in the key. Expression levels ($\log_2$ FC) were derived from Ct values relative to the endogenous reference *RPLP0* ($\Delta\Delta$ ct method); bars show mean $\log_2$ FC $\pm$ SEM, with dots indicating individual values from three biological replicates ($n = 3$). In panels c and d, statistical analysis was carried out using one-way Anova, with condition as the main factor and biological replicate as a blocking factor, followed by Tukey-adjusted pairwise comparisons; letters above bars indicate Tukey groupings ($\alpha = 0.05$), with shared letters indicating no significant differences. Full pairwise comparison results are provided in Additional file 4. **(f)** PAS-stained histology sections of mouse ear skin colonized *ex vivo* with parental strains (top) or hyphae-induced fusion products (bottom). **(g)** Increase in ear thickness (Δ ear thickness, μ m) of mice ear colonized *in vivo* with the same set of strains. Each graph pairs a fusion product with its parental strains. Dots represents an individual mouse; bars indicate mean $\pm$ SD. Statistical significance of the differences between groups was determined by ordinary one-way Anova followed by Tukey-adjusted pairwise comparisons; letters above bars indicate Tukey groupings ($\alpha = 0.05$), with shared letters indicating no significant differences. Full pairwise comparison results are provided in Additional file 5.

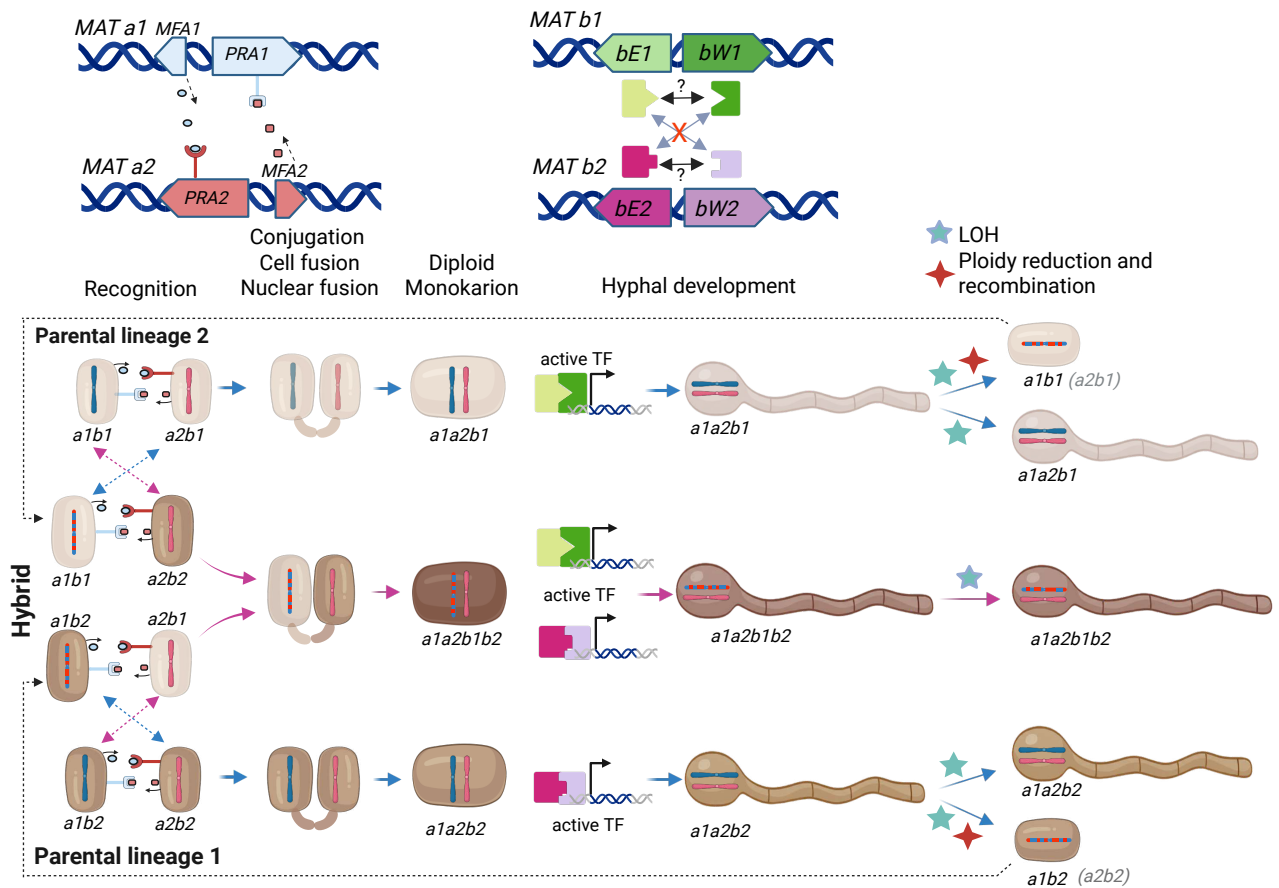


Figure 7. Model of *MAT*-dependent parasexual development in *Malassezia furfur*. The upper part of the figure summarizes the molecular machineries involved in *M. furfur* hybridization or parasexual reproduction and hyphal development, while the lower part shows the corresponding morphological changes and the *MAT* genotypes. Cell recognition, conjugation, and cell fusion are controlled by the *MAT A* locus through interactions between pheromone (encoded by *MFA* genes) and pheromone receptor (encoded by *PRA* genes) in *MAT-A* compatible partners. Following cell fusion and karyogamy, a diploid monokaryon is formed. These steps are in common in both inter and intraspecific crosses. Hyphal production is controlled by a single *MAT B* locus and does not require compatibility between *MAT B* alleles, hence excluding the *bE/bW* heterodimerization. Within each *M. furfur* parental lineages/species, intraspecific mating (blue arrows) between haploid cells of opposite *MAT A* alleles generates diploid monokaryotic cells carrying *MAT ala2* genotype and the *MAT B* of the parentals (*MAT b1* for the parental lineage 2 and *MAT b2* for the parental lineage 1). These diploids undergo hyphal development and can subsequently experience loss of heterozygosity (LOH) while remaining heterozygous filamenting strains, or undergo LOH, ploidy reduction, and recombination, producing haploid parasexual progeny with recombinant genomes and a haploid *MAT* configuration. The parasexual products are mating compatible and can mate with *MAT A* compatible strains (dashed blue arrows), thereby completing the *M. furfur* parasexual cycle. Interspecific mating (indicated by fuchsia arrows) between strains belonging to the two parental lineages generates a hybrid diploid monokaryon. The model shows a mating between a parasexual product derived from parental lineage 2 (*MAT a1b1*) with a strain of the parental lineage 1 (*MAT a2b2*), generating a hybrid with a *MAT ala2 b1b2* configuration. Hybrid cells undergo filamentation and accumulate LOH events during genome stabilization.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [AddFile3.xlsx](#)
- [AddFile4.xlsx](#)
- [AddFile1.xlsx](#)
- [AddFile2.xlsx](#)
- [SupplementalFiguresandlegend.pdf](#)
- [AddFile5.xlsx](#)