# Supporting Information

## Böhm et al. 10.1073/pnas.1217943110

### SI Materials and Methods

**Culture Conditions for *Penicillium chrysogenum*.** All strains investigated in this study were grown at 27 °C and 120 rpm in liquid complete culture medium (CCM); in case of the liquid-static cultures, shaking was avoided. For solid media, CCM, M322, or minimal medium (MM) was used as previously described (1, 2). For microarray analysis, strains were grown on sterile membranes (Pall Life Sciences) layered on solid M322 medium. All liquid media were inoculated with $5.0 \times 10^6$ spores from freshly prepared spore suspensions derived from cultures grown on M322 medium for 4–5 d. For quantitative real-time analysis, CCM was inoculated with $2 \times 10^8$ spores. Solid media were inoculated with $10^7$ spores. For quantifications of conidia, mycelia were grown on CCM for 168 h.

**Light Microscopy.** The microscopic observations of hyphal morphology at different growth phases were performed as described previously (3). For pellet quantification assays, strains were grown at 27 °C and 120 rpm in CCM, and samples were taken at different time points. The flasks were inoculated with $1 \times 10^7$ spores/mL freshly prepared spore suspensions. For each time point, three 2-mL samples were taken from the culture. Images were obtained with a stereomicroscope (Stemi 2000-C; Zeiss) equipped with a digital camera (AxioCamERc 5s) and digitally processed using Adobe Photoshop CS4. Pellet sizes were measured using the programs ImageJ (http://rsbweb.nih.gov/ij/) and Microsoft Excel 2010.

**Scanning Electron Microscopy.** Cleistothecia were removed with a sterile needle tip. The samples were fixed as described (4) for at least 24 h at room temperature, dehydrated in an ascending ethanol series, transferred to formaldehyde–dimethylacetal for 48 h, critical point dried, and mounted on metal stubs, sputter coated with gold for 180 s using an SEM Coating Unit E 5100 (Polaron Equipment Ltd), and examined on a Zeiss DSM 950 scanning electron microscope. Recorded images were processed with Adobe Photoshop CS4 software.

**Examination of Crosses and Ascospore Isolates.** The crosses were examined for cleistothecial production periodically with a Zeiss Stemi 2000 stereomicroscope. Cleistothecia were removed with a sterile needle tip, cleaned of adhering conidia by rolling on preparation agar [7% (wt/vol) agar], and then squashed with a needle tip in 200 μL of sterile water. Aliquots of 50 μL were plated on oatmeal agar medium or Köllnflocken agar supplemented with biotin (6.4 μg/L), incubated at 27 °C, and examined daily for germinating ascospores. Ascospore isolates were grown on CCM and characterized phenotypically with respect to spore color, chrysogenin production, and penicillin biosynthesis (3). Two different types of genetic markers were chosen for molecular characterization. First, mating type was determined by Southern hybridization. EcoRI-restricted genomic DNA from individual strains was blotted and hybridized with probes specific for the *MAT1-1–1* or *MAT1-2–1* gene. In a second approach, genome databases (www.ncbi.nlm.nih.gov) were searched with BLASTn to identify suitable molecular markers from the major contigs. Selected genes from the parental strains Q176 and IB 08/921 were amplified by PCR and sequenced for comparison. Finally, restriction fragment length polymorphisms (RFLPs) of 11 gene sequences were chosen and used to identify single point mutations by endonuclease restriction of amplified genomic DNA. As listed in Table S5, oligonucleotide pairs were used to amplify the corresponding gene fragments from the two parental strains. Sequencing of both amplicons identified RFLPs for differential restriction enzyme analysis. In a subsequent step, amplified DNA from each ascospore group was cut with the selected restriction enzymes (Fig. S1*A*) to detect which parental gene copy was present.

**Transformation.** DNA-mediated transformation of *P. chrysogenum* strains was performed as recently described (3, 5). The *ergA* gene was used as a selection marker as described (6) with some modifications: for regeneration of protoplasts, solid MM was used containing 5% (wt/vol) KCl and 2% (wt/vol) glucose as the sole carbon source. Twenty-four hours after transformation, the medium was overlaid with agar containing 0.7 μg/mL terbinafine.

**Construction of *MAT1-1–1* Deletion Strains.** The sequences of all plasmids and oligonucleotides used for construction of the gene deletion cassette are listed in Tables S4 and S5. For construction of the deletion vector, the strategy recently described by Hoff et al. (3) was used to generate the recombinant plasmid pKOMAT-1, containing the Tn5Phleo marker gene, flanked by sequences located 5′ and 3′ of the *MAT1-1–1* gene. This plasmid was used as a template to amplify the linear KO-MAT-1 cassette with primers 5′-Mat_sense and 3′-MAT_anti. The PCR fragment was used for transformation of ΔPcku70 (7), which facilitates homologous recombination (7). Resulting transformants were screened and analyzed as previously described (3). DNA from single spore isolates was used to verify the complete lack of the *MAT1-1–1* gene by Southern hybridization analysis (Fig. S2*A*).

**Rescue of *MAT1-1–1* Deletion Strains.** For complementation analysis, ΔMAT1-1–1 EK5 and EK6 were transformed with plasmid pKompMAT-1_ergA. This plasmid carries the *MAT1-1–1* gene with the native 5′ and 3′ regions using the *ergA* gene as a selectable marker, under the control of the strong P*acn*-promoter of *P. chrysogenum* (6). The successful rescue of the *MAT1-1–1* gene was verified by Southern hybridization, and the corresponding strains were designated ΔMAT1-1–1::MAT1-1–1 (Fig. S2*C*).

**Construction of *MAT1-1–1* Overexpression Strains.** For generating *MAT1-1–1* overexpression strains, plasmid pPgpd-MAT-1-ptrA was transformed into strain P2niaD18. Here, the *MAT1-1–1* gene is under the control of the strong constitutive *gpd* promoter of *Aspergillus nidulans*. Resulting transformants were selected using pyrithiamine-supplemented agar plates, as the constructs carry the *ptrA* resistance gene and were named P2::MAT1-1–1. Copy numbers of integrated plasmids were tested by Southern hybridization using a $^{32}$P-radiolabeled *MAT1-1–1* probe (Fig. S2*B*).

**Interaction Studies with Pheromones and Receptors from *P. chrysogenum*.** For the heterologous expression of the *P. chrysogenum* pheromone receptor gene *Pcpre2* in *Saccharomyces cerevisiae*, we used the yeast expression vector pPGK (8). The ORF of *Pcpre2* was amplified by PCR with the oligonucleotides Pre2-hom-f and Pre2-hom-r (Table S5) and inserted into the BamHI linearized vector pPGK by homologous recombination (9) to obtain the desired plasmid pPGK-PcPRE2. Plasmid pPGK-PcPRE2 was transformed into the yeast strain YDB103 (MATa *ste2*Δ *sst2::KanMX4*) (1). Shmoo formation (Fig. 4*A*) was assayed as described previously (10).

Yeast halo assays were conducted as described previously (10). YDB103 containing pPGK-PcPRE2 or strain Y06055 [MATa, his3Δ1, leu2Δ0, lys2Δ0, ura3Δ0, YLR452c::kanMX4 (sst2Δ); Euroscarf] were used. Five microliters of synthetic pheromone from either *Sordaria macrospora* (SmPPG1; GeneScript Corporation),

*P. chrysogenum* (PcPPG1; GeneScript Corporation) or *S. cerevisiae* (α-factor; Sigma-Aldrich) were applied to filter disks (6 mm; Sartorius Stedim) at a concentration of 3 nmol. No halo formation was seen when synthetic *S. cerevisiae* α-factor or DMSO was added in control experiments. As a positive control, we applied the synthetic *S. cerevisiae* α-factor to strain Y06055, which produces the *S. cerevisiae* α-factor receptor Ste2p.

**Identification of *P. chrysogenum* Genes and Sequence Analysis.** The sequences for all genes in this study were obtained from the public National Center for Biotechnology Information Entrez database (www.ncbi.nlm.nih.gov/entrez/). The genome sequence of *P. chrysogenum* ATCC28089 (Wisconsin 54–1255) served as the source. Sequence alignments were performed with the program MultAlign (http://multalin.toulouse.inra.fr/multalin/) and displayed using GeneDoc (www.nrbsc.org/gfx/genedoc/index.html).

**Penicillin Bioassay and HPLC Analysis.** For penicillin bioassays with *Staphylococcus aureus* as a sensitive indicator bacterium, 30 μL of supernatant from the culture broth was used in a halo test. For calibration of penicillin activity, the area of the inhibition zone was normalized to the dry weight of the mycelium. Media were buffered to pH 6 to obtain optimal conditions for penicillin biosynthesis. Penicillin titers were also determined using HPLC as described (11). The standard protocol involved measurement of penicillin production in liquid shaking cultures after 72 h growth, as this is the optimal time point for penicillin biosynthesis.

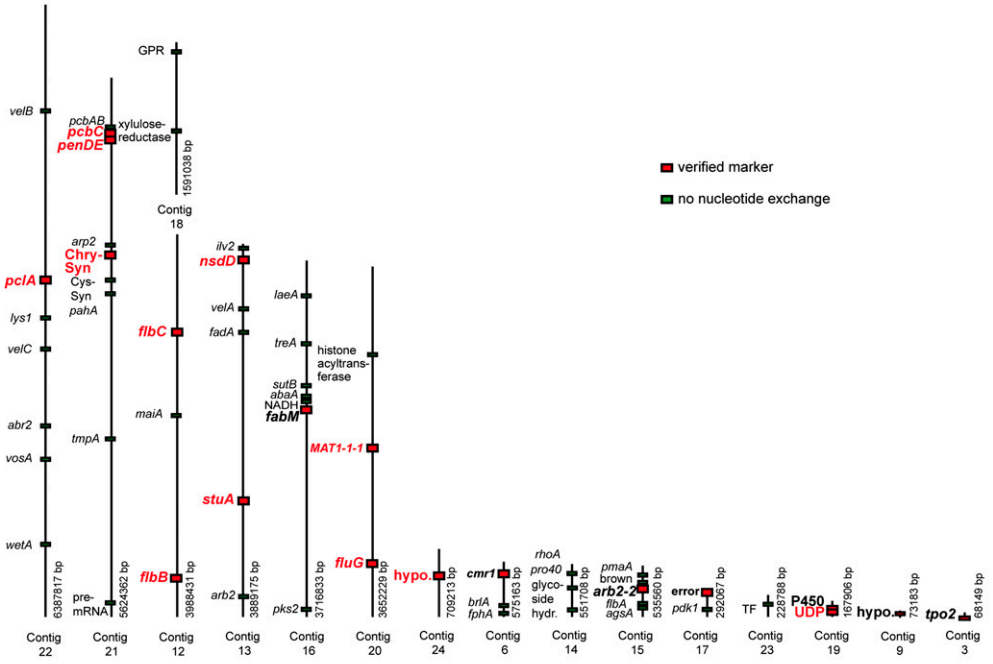
**Nucleic Acids Isolation, cDNA Synthesis, Microarray, and Quantitative Real-Time PCR.** Preparation of nucleic acids, hybridizations, and cDNA synthesis for microarray and quantitative real-time PCR (qRT-PCR) analysis were carried out as described recently (7). The time-course microarray analysis was performed using ΔPcku70 as the reference strain (7). qRT-PCR was carried out as described previously (7) with the following modifications: The Promega GoTaq qPCR Master Mix was used as recommended by the manufacturer, and incubation cycles were performed on a StepOnePlus Real-Time PCR System (Applied Biosystems) with the primers listed in Table S5.


1. Ballon DR, et al. (2006) DEP-domain-mediated regulation of GPCR signaling responses. *Cell* 126(6):1079–1093.
2. Haas H, Angermayr K, Zadra I, Stöffler G (1997) Overexpression of *nreB*, a new GATA factor-encoding gene of *Penicillium chrysogenum*, leads to repression of the nitrate assimilatory gene cluster. *J Biol Chem* 272(36):22576–22582.
3. Hoff B, et al. (2010) Two components of a velvet-like complex control hyphal morphogenesis, conidiophore development, and penicillin biosynthesis in *Penicillium chrysogenum*. *Eukaryot Cell* 9(8):1236–1250.
4. McDowell EM, Trump BF (1976) Histologic fixatives suitable for diagnostic light and electron microscopy. *Arch Pathol Lab Med* 100(8):405–414.
5. Kamerewerd J, Zadra I, Kürnsteiner H, Kück U (2011) PcchiB1, encoding a class V chitinase, is affected by PcVelA and PcLaeA, and is responsible for cell wall integrity in *Penicillium chrysogenum*. *Microbiology* 157(Pt 11):3036–3048.
6. Sigl C, Handler M, Sprenger G, Kürnsteiner H, Zadra I (2010) A novel homologous dominant selection marker for genetic transformation of *Penicillium chrysogenum*: overexpression of squalene epoxidase-encoding *ergA*. *J Biotechnol* 150(3):307–311.
7. Hoff B, Kamerewerd J, Sigl C, Zadra I, Kück U (2010) Homologous recombination in the antibiotic producer *Penicillium chrysogenum*: Strain DeltaPcku70 shows up-regulation of genes from the HOG pathway. *Appl Microbiol Biotechnol* 85(4):1081–1094.
8. Kang YS, Kane J, Kurjan J, Stadel JM, Tipper DJ (1990) Effects of expression of mammalian G alpha and hybrid mammalian-yeast G alpha proteins on the yeast pheromone response signal transduction pathway. *Mol Cell Biol* 10(6):2582–2590.
9. Colot HV, et al. (2006) A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. *Proc Natl Acad Sci USA* 103(27): 10352–10357.
10. Mayrhofer S, Pöggeler S (2005) Functional characterization of an alpha-factor-like *Sordaria macrospora* peptide pheromone and analysis of its interaction with its cognate receptor in *Saccharomyces cerevisiae*. *Eukaryot Cell* 4(4):661–672.
11. Schmitt EK, et al. (2004) Winged helix transcription factor CPCR1 is involved in regulation of beta-lactam biosynthesis in the fungus *Acremonium chrysogenum*. *Eukaryot Cell* 3(1):121–134.

A

| Marker | Chry.syn. Pc21g 16000 | *flbB* Pc12g 01640 | *flbC* Pc12g 12190 | *fluG* Pc20g 02420 | hypo. Pc24g 01940 | *nsdD* Pc13g 15350 | *pcbC* Pc21g 21380 | *pclA* Pc22g 14900 | *penDE* Pc21g 21370 | *stuA* Pc13g 04920 | UDP Pc19g 00330 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Restriction enzyme | *Ssp*I | *HpyAV* | *Mnl*I | *Hin1*II | *Hinf*I | *Pdm*I | *Taq*I | *Mva*I | *Alu*I | *Nae*I | *Hpa*II |
| Q176 # of fragments | 2 | 4 | 13 | 6 | 3 | 2 | 7 | 5 | 3 | 3 | 3 |
| IB 08/921 # of fragments | 3 | 3 | 12 | 5 | 2 | 3 | 6 | 4 | 2 | 4 | 4 |

B




C

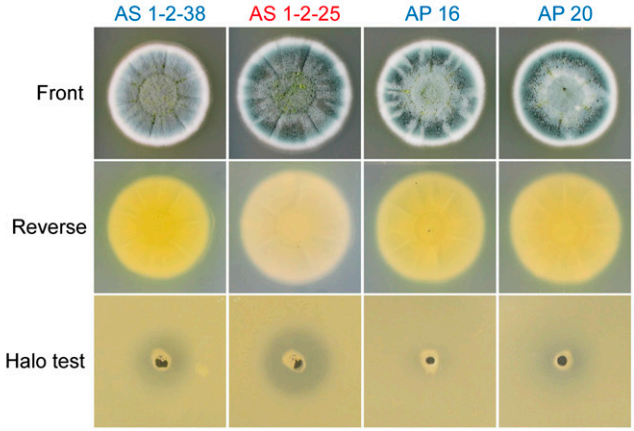



**Fig. S1.** Analysis of recombinant ascospore lineages. (*A*) RFLP analysis with different marker genes to detect genetic recombination. Sequence analysis identified point mutations in different genes derived from both parental strains Q176 and IB 08/921. In some cases, these point mutations generated new restriction sites, which were used to distinguish the two gene variants. Numbers indicate restriction fragments when PCR products were digested with the indicated endonucleases. (*B*) Location of marker genes used for RFLP analysis of recombinant ascospore groups displayed on contigs of the *P. chrysogenum* genome (www.ncbi.nlm.nih.gov/genbank/). All genes shown were tested for sequence polymorphisms between the two parental strains Q176 and IB 08/921. The genes with suitable polymorphisms are highlighted in bold. Only the genes highlighted in red were used in the RFLP analysis. (*C*) Phenotypes of parental

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strains (AS 38 and AS 25) and ascospore progeny (AP 16, AP 20) as evidence of meiotic recombination during a sexual cross. The two parental strains are F1 progeny from a cross between Q176 and IB 08/921 that differed in spore color (light green versus dark green; *Top*), chrysogenin production (bright yellow versus fawn reverse coloration; *Middle*), and penicillin production (differential size of halo; *Bottom*). Recombinant ascospore progeny AP 16 and AP 20 have novel phenotypes of dark green conidia, chrysogenin production, and a reduced halo size. Strain designation color indicates either *MAT1-1* (blue) or *MAT1-2* (red) genotype. Molecular analysis of progeny was not performed.

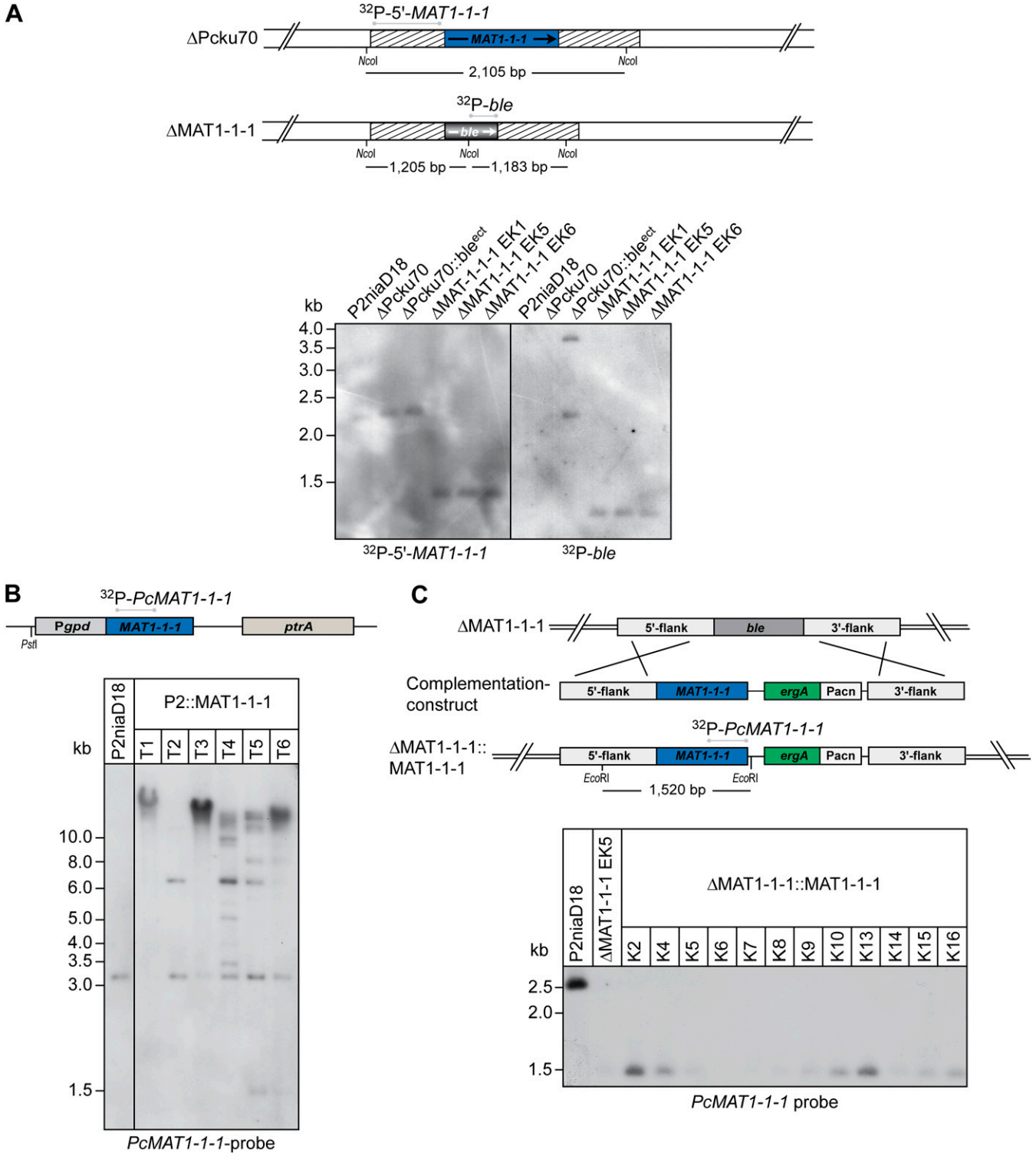


**Fig. S2.** Recombinant *P. chrysogenum MAT1-1–1* strains produced for functional analysis of the *MAT1-1–1* gene. Constructs to generate (*A*) deletion, (*B*) overexpression, and (*C*) complementing strains are shown, together with results of the corresponding Southern hybridizations used to characterize the recombinant fungal strains. Lane headings indicate results for specific control or recombinant *MAT1-1–1* strains. *ble*, phleomycin resistance gene; *ergA*, gene encoding squalene epoxidase (which confers resistance to terbinafine); P*acn*, promoter sequence of the *P. chrysogenum* actin gene; P*gpd*, promoter sequence of the *A. nidulans gpdA* gene; *ptrA*, pyrithiamine resistance gene.

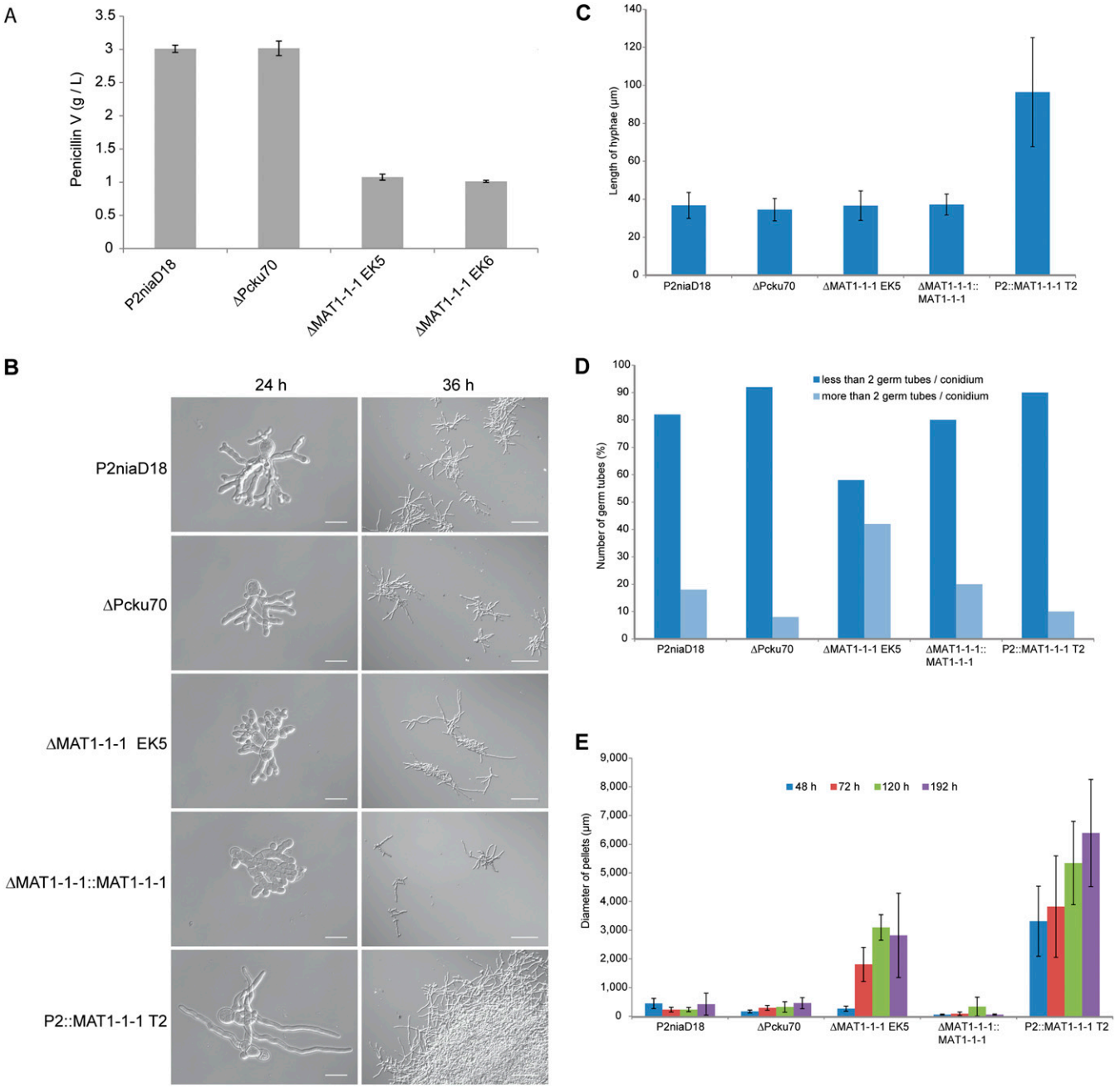


**Fig. S3.** Penicillin biosynthesis and hyphal morphology of recombinant *P. chrysogenum* strains. (*A*) HPLC analysis of penicillin V production in ΔMAT1-1–1 deletion strains and their corresponding reference strains. Measurements were taken after 96 h cultivation. Error bars represent mean ± SD ($n = 3$) from three independent experiments. (*B*) Representative examples of hyphal morphology and polarity of germinating conidia when parental and recombinant *MAT1-1–1* strains were grown in liquid shaking CCM for the times indicated. The morphology of the germinating conidia from the *MAT1-1–1* deletion strain is distinct from the reference strains as shown by the dichotomous branching of the hyphal tips and by the increased number of germ tubes. The P2::MAT1-1–1 overexpression strain shows elongated hyphae without branching, which is further evidenced by the quantitative analysis in *C*. These observations are identical to those seen on solid media, as shown in Fig. 2*B*. Scale bars: 20 µm (24 h) and 100 µm (36 h). (*C*) Quantification of hyphal length and (*D*) quantification of germ tubes from 50 germinating conidia. As an example, one out of two independent *MAT1-1–1* knockout and overexpression strains is shown. (*E*) Quantification of pellet diameter when parental and recombinant *MAT1-1–1* strains were grown in shaking liquid CCM cultures. Measurements were taken at four different time points as indicated. Error bars represent mean ± SD of 100 random pellets. One out of two independent *MAT1-1–1* knockout and overexpression strains is shown.

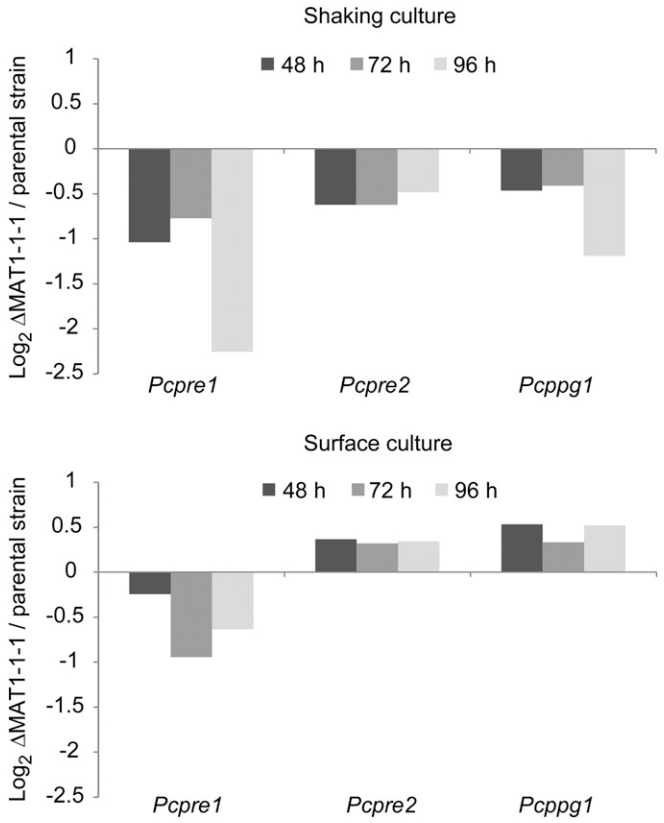


**Fig. S4.** qRT-PCR analysis to quantify transcriptional expression of genes for pheromone and pheromone receptor genes. Values are the $\log_2$-transformed average expression ratios of at least three biological replicates of two independently derived deletion strains (mean ΔMAT1-1–1 EK5/EK6, $n \geq 3$), relative to the ΔPcku70 parental strain. Strains were grown in liquid shaking (*Upper*) or surface (*Lower*) cultures.

**Table S1. *P. chrysogenum* isolates that were crossed in various combinations**

| | | *MAT1-2**,† | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | IB 08/921 | PC 0814C | PC 0819C | PC 0826A | DAOM 155628 | DAOM 59494C | IBT 30738 | Pc 131 | US 68 | Pc 105 | AS 25 |
| *MAT1-1* | P2niaD18 | 4, 5, 6, 8, 9 – (1, 2, 3, 12, 13) | – (3) | – (3) | | – (3) | | 10, 11 | | | | |
| | ATCC 10106 | 7 – (4) | | | | | | | | | | |
| | Q176 | 6,‡ 7, 8 – (1, 2, 3, 4) | – (3) | – (3) | | – (3) | | | | | | |
| | ΔMAT1 EK5 | 1, 12, 13 – (2, 3, 4, 5) | – (3) | | | | | | | | | |
| | P2::MAT1 T5 | 4, 5, 7 – (1, 2, 3, 12, 13) | | | | | | | | | | |
| | P2::MAT2 T2 | 3 | | | | | | | | | | |
| | P2::MAT2 T5 | 3 | | | | | | | | | | |
| | DAOM 193710 | 4 | | | | | | | | | | |
| | PC0820A | | 1, 2, 3 | 3 – (1, 2) | | 3 – (1, 2) | | | | | | |
| | PC088B | | 3 – (1, 2) | | | 3 – (1, 2) | 3 – (1, 2) | | | | | |
| | DAOM 155627 | – (4) | 3 | 1, 3 – (2) | | 3 – (1, 2) | 3 | | | | | |
| | PC08105C | | | | 3 – (1) | | | | | | | |
| | IBT 14508 | | | | | | | | – (10) | | – (10) | |
| | IBT 30427 | | | | | | | 10, 11 | – (10) | – (10) | – (10) | |
| | IBT 22703 | | | | | | | 10, 11 | | – (10) | | |
| | US 49 | | | | | | | | 10 | | – (10) | |
| | AS 38 | | | | | | | | | | | 6‡ |

Numbers represent different growth conditions where the formation of cleistothecia was observed. Numbers in parentheses indicate conditions where no fruiting bodies were detected (see *Materials and Methods, Mating and Analysis of Recombinant Ascospore Lines* for full details): 1, OA at 15 °C; 2, OA at 18 °C, 3, OA at 20 °C, 4, OA at 27 °C; 5, Kölln at 27 °C; 6, OA + biotin at 20 °C; 7, OA + Var at 27 °C; 8, OA + Sil at 27 °C; 9, OA + biotin at 27 °C; 10, OA (U.K.) at 20 °C; 11, OA (U.K.) + biotin at 20 °C; 12, Kölln at 15 °C; 13, Schmelz at 15 °C. OA, oatmeal agar medium (Pinhead Oatmeal; Odlums Group); OA (U.K.), Traditional Rolled Oats (Quaker Oats); Kölln, Köllnflocken medium; Schmelz, Schmelzflocken medium (Peter Kölln KGaA); Sil, sildenafil citrate; Var, vardenafil citrate.

*See Table S3 for isolate details.

†Shaded boxes indicate pairings that were not tested.

‡Cleistothecia contained viable ascospores under these conditions.

**Table S2. List of differentially expressed genes in the ΔMAT1-1–1 mutant according to a microarray analysis**

| Gene | ID | 36 h | 60 h | 96 h |
|---|---|---|---|---|
| Down-regulated at all time points | | | | |
| Hypothetical protein | Pc12g02560 | −2.28 | −1.87 | −1.46 |
| Hypothetical protein | Pc13g14700 | −2.06 | −1.01 | −1.03 |
| Tetratricopeptide repeat domain protein | Pc13g15900 | −1.63 | −2.21 | −1.42 |
| Hypothetical protein | Pc15g01560 | −1.50 | −1.38 | −2.42 |
| Hypothetical protein | Pc18g06580 | −3.15 | −2.74 | −2.39 |
| Hypothetical protein | Pc18g06600 | −3.38 | −4.06 | −3.47 |
| Hypothetical protein | Pc18g06610 | −4.30 | −5.13 | −5.51 |
| Hypothetical protein | Pc18g06620 | −1.48 | −1.36 | −1.67 |
| Hypothetical protein | Pc18g06660 | −3.13 | −1.37 | −3.08 |
| HMG box protein | Pc18g06680 | −2.81 | −2.20 | −1.53 |
| Vanillin dehydrogenase, putative | Pc20g02480 | −1.04 | −2.45 | −1.15 |
| Killer toxin sensitivity protein (Iki1) | Pc20g12630 | −2.28 | −2.13 | −2.11 |
| Small oligopeptide transporter, OPT family | Pc20g15530 | −1.00 | −1.69 | −1.20 |
| ABC multidrug transporter, putative | Pc21g10850 | −2.22 | −1.30 | −1.08 |
| Putative Transcription factor | Pc21g17180 | −1.15 | −1.92 | −1.99 |
| Glyoxalase family protein | Pc21g21290 | −6.09 | −6.88 | −6.41 |
| FAD dependent oxidoreductase, putative | Pc21g21350 | −1.06 | −1.23 | −1.03 |
| Hypothetical protein | Pc21g21440 | −7.04 | −1.44 | −1.45 |
| Phosphotransferase enzyme family protein | Pc21g21580 | −1.11 | −1.11 | −1.07 |
| Putative Transcription factor | Pc22g07530 | −3.21 | −2.27 | −1.20 |
| Integral membrane protein | Pc22g16710 | −1.82 | −1.09 | −1.27 |
| Hypothetical protein | Pc24g01440 | −1.31 | −2.59 | −1.51 |
| Hypothetical protein | Pc24g01600 | −4.43 | −2.99 | −4.91 |
| Up-regulated at all time points | | | | |
| MFS transporter, putative | Pc03g00020 | 2.52 | 3.16 | 1.35 |
| Arsenate reductase ArsC | Pc06g02220 | 1.03 | 2.43 | 1.69 |
| MFS monosaccharide transporter, putative | Pc12g02140 | 1.90 | 1.92 | 1.25 |
| Glycosyl hydrolase family protein | Pc12g04400 | 1.08 | 1.02 | 1.13 |
| Hypothetical protein | Pc13g09280 | 1.04 | 1.56 | 1.16 |
| MFS transporter, putative | Pc13g09900 | 3.72 | 3.14 | 3.17 |
| Flavin-binding monooxygenase | Pc13g14930 | 3.06 | 1.21 | 3.34 |
| MFS sugar transporter, putative | Pc15g00030 | 1.44 | 2.18 | 1.44 |
| Hypothetical protein | Pc16g14900 | 1.01 | 1.47 | 1.71 |
| Hypothetical protein | Pc17g00990 | 3.29 | 2.62 | 1.87 |
| Hypothetical protein | Pc17g01090 | 1.20 | 1.05 | 1.85 |
| Hypothetical protein | Pc18g06590 | 1.26 | 1.51 | 1.22 |
| Hypothetical protein | Pc19g00170 | 1.61 | 1.79 | 1.63 |
| MFS transporter, putative | Pc20g06200 | 1.76 | 1.70 | 3.91 |
| Alpha-ketoglutarate-dependent taurine dioxygenase | Pc20g06210 | 2.38 | 1.63 | 4.23 |
| High-affinity glucose transporter | Pc20g10820 | 2.05 | 1.35 | 1.48 |
| *sesA* | Pc20g13730 | 1.24 | 1.24 | 1.52 |
| Phytanoyl-CoA dioxygenase | Pc21g04130 | 1.40 | 1.97 | 1.41 |
| Cyclin | Pc21g20530 | 1.39 | 1.28 | 1.48 |
| Amino acid permease, putative | Pc21g20960 | 2.98 | 1.67 | 1.49 |
| FAD dependent oxidoreductase | Pc21g21750 | 1.86 | 1.89 | 3.29 |
| Hypothetical protein | Pc22g13240 | 1.31 | 2.49 | 2.44 |
| Hypothetical protein | Pc22g24550 | 1.82 | 2.23 | 2.63 |
| Hypothetical protein | Pc22g25960 | 1.54 | 2.70 | 1.49 |
| Hypothetical protein | Pc22g26500 | 1.32 | 1.67 | 1.54 |
| C2H2 finger domain protein | Pc22g27040 | 1.24 | 2.03 | 3.08 |
| Hypothetical protein | Pc24g00020 | 1.07 | 1.97 | 1.82 |
| Hypothetical protein | Pc24g00750 | 1.60 | 1.28 | 2.44 |
| Hypothetical protein | Pc24g00920 | 1.40 | 2.84 | 1.18 |
| Penicillin biosynthesis | | | | |
| *pcbAB* | Pc21g21390 | −0.87 | −2.84 | −2.27 |
| *pcbC* | Pc21g21380 | −0.82 | −1.73 | −1.28 |
| *penDE* | Pc21g21370 | −0.59 | −2.1 | −1.35 |
| Asexual development | | | | |
| *brlA* | Pc06g00470 | −1.22 | −0.23 | −0.45 |
| *dewA* | Pc16g06690 | −4.28 | 0.02 | 0.05 |
| *dewB* | Pc13g16010 | −1.14 | −0.09 | −0.02 |

**Table S2. Cont.**

| Gene | ID | 36 h | 60 h | 96 h |
|---|---|---|---|---|
| *flbA* | Pc15g00600 | 1.45 | −0.47 | 0.27 |
| *tmpA* | Pc21g07830 | −0.49 | 0.50 | 1.00 |
| *ppoA* | Pc22g06980 | 1.05 | −2.54 | −0.52 |
| Cell wall | | | | |
| *agsD* | Pc22g15390 | −1.81 | 0.45 | 0.27 |
| Fruit body lectin | Pc12g08560 | 0.20 | 2.19 | 2.62 |
| Transcription factors | | | | |
| *pacC* | Pc18g00420 | 0.47 | −1.24 | −0.46 |
| HMG box protein | Pc18g06680 | −2.81 | −2.2 | −1.53 |
| Hypothetical protein | Pc22g07530 | −3.21 | −2.27 | −1.2 |
| Hypothetical protein | Pc21g17180 | −1.15 | −1.92 | −1.99 |
| HLH transcription factor | Pc13g11100 | −2.86 | −1.18 | −0.8 |
| C6 zinc finger domain protein | Pc13g10310 | −0.07 | −2.8 | −3.18 |
| Phosphotransferase enzyme family protein | Pc18g03150 | −0.16 | −1.15 | −1.02 |
| C2H2 finger domain protein (Ezf) | Pc21g21760 | 0.65 | −2.16 | −1.14 |
| Hypothetical protein | Pc17g01190 | −4.16 | −0.1 | −1.13 |
| Hypothetical protein | Pc24g01630 | −3.47 | −0.29 | −1.3 |
| C2H2 finger domain protein | Pc22g27040 | 1.24 | 2.03 | 3.08 |
| bZIP transcription factor (Atf21) homolog | Pc22g26820 | 2.31 | 2.11 | 0.69 |
| bZIP transcription factor (Atf21) homolog | Pc19g00240 | 2.42 | 2.68 | 0.92 |
| Hypothetical protein | Pc04g00010 | 0.18 | 1.09 | 1.63 |
| Hypothetical protein | Pc13g01210 | −1.94 | 1.87 | 1.85 |
| zf-fungal binuclear cluster type transcription factor | Pc16g05270 | 0.00 | 1.1 | 1.32 |
| bZIP transcription factor JlbA | Pc20g15070 | −0.06 | 1.84 | 1.35 |
| C6 finger domain protein | Pc22g19540 | −1.32 | 1.44 | 1.39 |

Values are mean $\log_2$-transformed ratios relative to the ΔPcku70 parental strain. Colored values indicate genes with at least a twofold transcriptional up-regulation (red) or down-regulation (green). Listed are genes down-regulated or up-regulated at all time points, as well as those that control conidiation or morphology.

**Table S3. List of bacterial and fungal strains used in this study**

| Strain | Characteristics and genotype | Mating type | Source |
|---|---|---|---|
| IB 08/921 | Wild type | *MAT1-2–1* | M. Kirchmair* |
| Pc105 | Wild type | *MAT1-2–1* | This study; indoor air |
| Pc131 | Wild type | *MAT1-2–1* | This study; indoor air |
| US 49 | Wild type | *MAT1-1–1* | BDUN Collection† |
| US 68 | Wild type | *MAT1-2–1* | BDUN Collection† |
| IBT 14508 | Wild type | *MAT1-1–1* | IBT Culture Collection of Fungi‡ |
| IBT 30738 | Wild type | *MAT1-2–1* | IBT Culture Collection of Fungi |
| IBT 30427 | Wild type | *MAT1-1–1* | IBT Culture Collection of Fungi |
| IBT 22703 | Wild type | *MAT1-1–1* | IBT Culture Collection of Fungi |
| PC0820A | Wild type | *MAT1-1–1* | (1) |
| PC088B | Wild type | *MAT1-1–1* | (1) |
| PC08105C | Wild type | *MAT1-1–1* | (1) |
| PC0814C | Wild type | *MAT1-2–1* | (1) |
| PC0819C | Wild type | *MAT1-2–1* | (1) |
| PC0826A | Wild type | *MAT1-2–1* | (1) |
| DAOM 193710 | Wild type | *MAT1-1–1* | (2) |
| DAOM 155627 | Wild type | *MAT1-1–1* | (2) |
| DAOM 155628 | Wild type | *MAT1-2–1* | (2) |
| DAOM 59494C | Wild type | *MAT1-2–1* | (2) |
| ATCC 10106 | Type strain; chrysogenin producer | *MAT1-1–1* | (3) |
| Q176 | chrysogenin producer | *MAT1-1–1* | (3) |
| P2niaD18 | *niaD*$^{-}$ | *MAT1-1–1* | (4) |
| ΔPcku70 | *Pcku70*Δ*::nat1*; *niaD*$^{-}$ | *MAT1-1–1* | (5) |
| ΔMAT1-1–1 EK5 | *MAT1-1–1*Δ*::ble*; *Pcku70*Δ*::nat1*; *niaD*$^{-}$ | | This study |
| ΔMAT1-1–1 EK6 | *MAT1-1–1*Δ*::ble*; *Pcku70*Δ*::nat1*; *niaD*$^{-}$ | | This study |
| ΔMAT1-1–1::MAT1-1–1 | *MAT1-1–1*Δ*::ble*; *Pcku70*Δ*::nat1*; P*MAT1-1–1::MAT1-1–1::TMAT1-1–1*; *ergA*; *niaD*$^{-}$ | *MAT1-1–1* | This study |
| P2::MAT1-1–1 T2 | P*gpd*::*MAT1-1–1*; *ptrA*; *niaD*$^{-}$ | *MAT1-1–1* | This study |
| P2::MAT1-1–1 T5 | P*gpd*::*MAT1-1–1*; *ptrA*; *niaD*$^{-}$ | *MAT1-1–1* | This study |
| P2::MAT1-2–1 T2 | P*gpd*::*MAT1-2–1*; *ptrA*; *niaD*$^{-}$ | *MAT1-1–1*<br>*MAT1-2–1* | This study |
| P2::MAT1-2–1 T5 | P*gpd*::*MAT1-2–1*; *ptrA*; *niaD*$^{-}$ | *MAT1-1–1*<br>*MAT1-2–1* | This study |
| AS 38 | F1 progeny from Q176 x IB 08/921 | *MAT1-1–1* | This study |
| AS 25 | F1 progeny from Q176 x IB 08/921 | *MAT1-2–1* | This study |
| YDB103 | *sst2*Δ::KanMX4 *ste2*Δ | *MAT*a | (6) |
| Y06055 | *his3*Δ*1, leu2*Δ*0, lys2*Δ*0, ura3*Δ*0*, YLR452c::KanMX4, *sst2*Δ | *MAT*a | Euroscarf |
| XL1-Blue K12 | *recA1, endA1, gyrA96, thi1, hsdR17, supE44, relA1, lac-*, [F′ *proAB, laclqZ*Δ*M15, Tn10*(tetr)] | | (7) |

*Institute for Microbiology, Innsbruck University, Innsbruck, Austria.
†BDUN, Department of Botany, University of Nottingham, Nottingham, UK.
‡IBT, Culture Collection of Fungi, Mycology Group, BioCentrum-DTU, Technical University of Denmark, Lyngby, Denmark.


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**Table S4. List of plasmids used in this study**

| Plasmid | Characteristics | Source |
|---|---|---|
| pDrive/PtrpC-Tn5Phleo | *trpC* promoter of *A. nidulans*, *ble* resistance gene of *Streptoalloteichus hindustanus* | (1) |
| pTn5Phleo-3′-FlankeMAT | *trpC* promoter of *A. nidulans*, *ble* resistance gene of *S. hindustanus*, 3′ flanking region of *MAT1-1–1* | This study |
| pKOMAT-1 | 5′ flanking region of *MAT1-1–1*, *trpC* promoter of *A. nidulans*, *ble* resistance gene of *S. hindustanus*, 3′ flanking region of *MAT1-1–1* | This study |
| pT3T7-gpd | *gpd* promoter of *A. nidulans* | This study |
| pT3T7Pgpd-MAT1 | *gpd* promoter of *A. nidulans*, *MAT1-1–1* gene of *P. chrysogenum* | This study |
| pDrive-ptrA | *ptrA* resistance gene of *Aspergillus oryzae* | This study |
| pPgpd-MAT-1-ptrA | *gpd* promoter of *A. nidulans*, *MAT1-1–1* gene of *P. chrysogenum*, *ptrA* resistance gene of *A. oryzae* | This study |
| pDrive-ergA | *acnP* promoter of *P. chrysogenum*, *ergA* gene of *P. chrysogenum* | (2) |
| ergA+3′FlankeMAT-1 | *acnP* promoter of *P. chrysogenum*, *ergA* gene of *P. chrysogenum*, 3′ flanking region of *MAT1-1–1* | This study |
| pKompMAT-1_ergA | 5′ flanking region of *MAT1-1–1*, *MAT1-1–1* gene of *P. chrysogenum*, *acnP* promoter of *P. chrysogenum*, *ergA* gene of *P. chrysogenum*, 3′ flanking region of *MAT1-1–1* | This study |
| pPGK-PcPRE2 | *Pcpre2* under control of the *S. cerevisiae PGK* promoter | This study |


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**Table S5. List of oligonucleotides used in this study**

| Oligonucleotide | Sequence (5′–3′) | Specificity |
|---|---|---|
| 5′-Mat_sense | ACGCGTCTGTCAACAAGCAACGC | 5′ flanking region of *MAT1-1–1* |
| 5′-Mat_anti | GGATCCGGTAAACAAATATAGGACC | 5′ flanking region of *MAT1-1–1* |
| 3′-Mat_sense | AAGCTTCCCATTCACAATTTCGAC | 3′ flanking region of *MAT1-1–1* |
| 3′-Mat_anti | GCGGCCGCCCAACCTCCGTGGTTAG | 3′ flanking region of *MAT1-1–1* |
| ApaI-MAT1 | GGGCCCATGTCTACCTCTCTTGATGC | *MAT1-1–1* gene |
| MluI-MAT1 | ACGCGTCTAGTTGTGCCCAAAGATCC | *MAT1-1–1* gene |
| koMAT 5′-sense | GCACGGAGAGCCACGGATGTG | 5′ flanking region of *MAT1-1–1* |
| koMAT 3′-anti | CTTGGCTGTCTGTATATTCCGCG | 3′ flanking region of *MAT1-1–1* |
| PtrpC__anti1 | GGCATTCATTGTTGACCTCCACTAG | *trpC* promoter |
| Tn5-phleo | GCGCCTGATACAGAACGAATTGC | *ble* resistance gene |
| 3′MAT-1_inf_fw | ATTCGTCGACAAGCTTCCCATTCACAATTTCGACTCGGCT | 3′ flanking region of *MAT1-1–1* |
| 3′MAT-1_inf_rv | TAGAATACAGCGGCCGCCGAGGAGTTTGATATTGTCCGTG | 3′ flanking region of *MAT1-1–1* |
| 5′MAT-1_inf_fw | CATGCTGCAGACGCGTGAAATACATGTTCCAGGAAGGGGA | 5′ flanking region of *MAT1-1–1* and *MAT1-1–1* gene |
| 5′MAT-1_inf_rv | GATCCGATACTGTACGTACTAGTTGTGCCCAAAGATCCGG | 5′ flanking region of *MAT1-1–1* and *MAT1-1–1* gene |
| PcMAT1 -s | GGCTATTGCTGTCGACCAAGTT | *MAT1-1–1* gene |
| PcMAT1-a | GTCTGATCGTTGAGTCGTCCACTT | *MAT1-1–1* gene |
| Pc_HMG_sense | CGATGGCGTTCTTGACCTGG | *MAT1-2–1* gene |
| Pc_HMG_anti | GCCTGAACAAAAGGCAGG | *MAT1-2–1* gene |
| MAT1-1–1_s | CGCTTCGTCTACGCAAATGGTGTGCTGGAG | *MAT1-1–1* gene |
| MAT1-1–1_a | GAGAATGTGCTTGTCCCACTCTTCGTTGCG | *MAT1-1–1* gene |
| PclA_sense | TGTGGTATTACCGGGAAGTC | *pclA* (*Pc22g14900*) gene |
| PclA_anti | ACAATTCGTGCCTCGACTCC | *pclA* (*Pc22g14900*) gene |
| Chry1 | GAGTTTGACTCGGGTCTTCG | *Pc21g16000* gene |
| Chry4 | AGCCAATTCCATCTGCTCTG | *Pc21g16000* gene |
| PcpenDE_s | CTGCCACCAAAGAGATGATCC | *PcpenDE*gene |
| PcpenDE_a | CCTGGCGTTGAGCGCAGACCT | *PcpenDE*gene |
| pcbC_s | CACCCATGGCTTCCACCCCCAAGGCCAATG | *PcpcbC* gene |
| pcbC_a | GTGCCATGGCTGTCTGGCCGTTCTTGTTGATTAGAC | *PcpcbC* gene |
| PcFluG-s | CCACCATGCCCATAACCTATTGAA | *PcfluG* gene |
| PcFluG-a | GCAAATTTCCGATACAAAACCAAC | *PcfluG* gene |
| flbB_for | GCCAATGGCATGGACCACTC | *PcflbB* gene |
| flbB_rev | TGACCAAGTGCTGTCAAGAG | *PcflbB* gene |
| nsdD_for | TAGCGTGGCTTCGCCTAATG | *PcnsdD* gene |
| nsdD_rev | TAGAGCACCGAGTAGGGAAG | *PcnsdD* gene |
| Pc23g00420_for | TAGAGACCACGGTGCCGAAC | *Pc2300420* gene |
| Pc23g00420_rev | CATTCAAGCGTGCTAGATCC | *Pc2300420* gene |
| Pc24g01940_for | GTCAGCACGCCTATAGACAC | *Pc24g01940* gene |
| Pc24g01940_rev | CTCAGCAACCGGGATATTTC | *Pc24g01940* gene |
| Pc12g12190_for | GCCTTCCAGCTATGCCTACG | *PcflbC* gene |
| Pc12g12190_rev | ACGCCCAGGTCTAGCGAAAG | *PcflbC* gene |
| Pc13g04920_for | TGAGCAACCAGCGCTCAATG | *PcstuA* gene |
| Pc13g04920_for | CTTGCGCCTAGTTCTCCTCC | *PcstuA* gene |
| PcbAB-RT-s | GCCGTCAACGAGATATTGGA | *pcbAB* gene |
| PcbAB-RT-a | TGGTGAACGGAGAACCAGAC | *pcbAB* gene |
| PcbC-RT-s | CCCTCCCGTTCTTCGTCAATC | *pcbC* gene |
| PcbC-RT-a | CTGCAGATAGTCGCCGTACGA | *pcbC* gene |
| penDE-RT-s | GAATCATCGGGAAGGTTGGA | *penDE* gene |
| penDE-RT-a | TCATAGGCCTGGGAAGGAGA | *penDE* gene |
| Pcppg1-RT-s | GCTTGCCCCTTGTCCTTCAGA | *Pcppg1* gene |
| Pcppg1-RT-a | CGCTGGTACGCTTGACCTCA | *Pcppg1* gene |
| Pcpre1-RT-s | TGGGACACTGCTGGATGATCT | *Pcpre1* gene |
| Pcpre1-RT-a | GCTAATAACCTGCCGCACATG | *Pcpre1* gene |
| Pcpre2-RT-s | CATGGTGTGGTCCGAGTAGCA | *Pcpre2* gene |
| Pcpre2-RT-a | CGGCGGTGCTGAAAGTCTACT | *Pcpre2* gene |
| NcSSU1 | ATCCAAGGAAGGCAGCAGGC | *small subunit ribosomal* RNA |
| NcSSU2 | TGGAGCTGGAATTACCGCG | *small subunit ribosomal* RNA |
| Pre2-hom-f | GTAATTATCTACTTTTTACAACAAATATATGGCGACATCATCTCCAATTC | *Pcpre2* |
| Pre2-hom-r | ATTGATCTATCGATTTCAATTCAATTCATCACACGATTGAATTGTTCCTT | *Pcpre2* |