SUPPLEMENTARY MATERIAL

Robust Cre recombinase activity in the biotrophic smut fungus *Ustilago maydis* enables efficient conditional null mutants *in planta.*

Antonio de la Torre, Matteo Jurca, Kai Hoffmann, Lara Schmitz, Kai Heimel, Jörg Kämper and José Pérez-Martín

Supplementary Figures

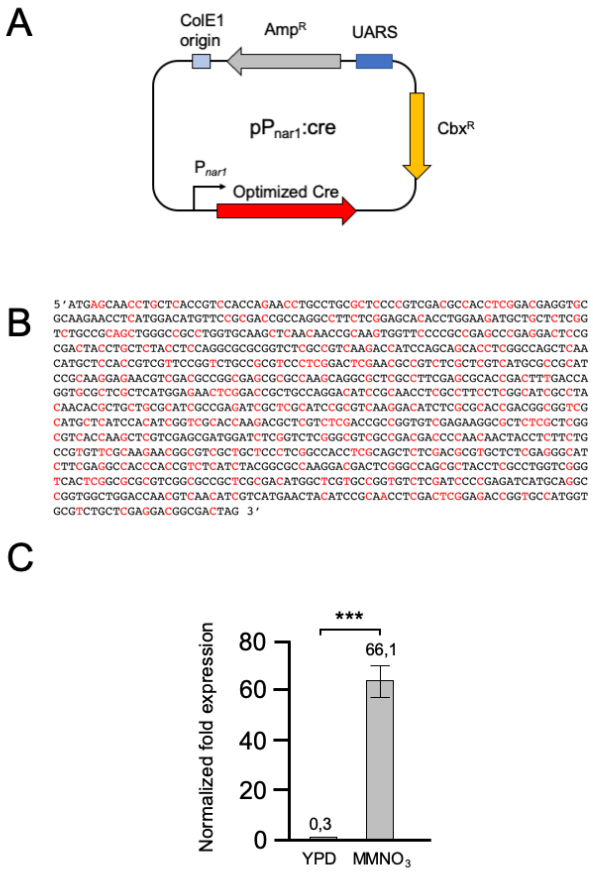


Figure S1. Expression of a *U. maydis* dicodon-optimized version of Cre. (A) Schematic representation of plasmid pP_{nar1}:cre (pKPH7) expressing the dicodon-optimized Cre recombinase under control of the promoter of the nitrate reductase gene *nar1* (UARS: *Ustilago* Autonomous Replication Sequence). (B) Nucleotide sequence of the dicodon-optimized *cre* gene. Nucleotide alterations with respect to the native *cre*-sequence from phage P1 are in red. (C) qRT-PCR analysis of *cre* gene expression when expressed from self-replicating plasmid pP_{nar1}:cre. Primers specifically detecting *cre* transcript were used (CreRT1 and CreRT2). RNA was isolated from a *U. maydis* strain carrying the pP_{nar1}:cre plasmid incubated for 6 hours in minimal nitrate medium ($MMNO_3$, permissive conditions

for *nar1* expression) and Yeast Peptone Dextrose medium (YPD, restrictive conditions for *nar1* expression) amended with carboxin (2μg/ml). The expression of *tub1* (encoding Tubulin α) was used for normalization. Expression values represent the mean of three biological replicates with two technical duplicates each. Error bars represent the SEM. Statistical significance was calculated using the Student's t-test. ***P < 0.001.

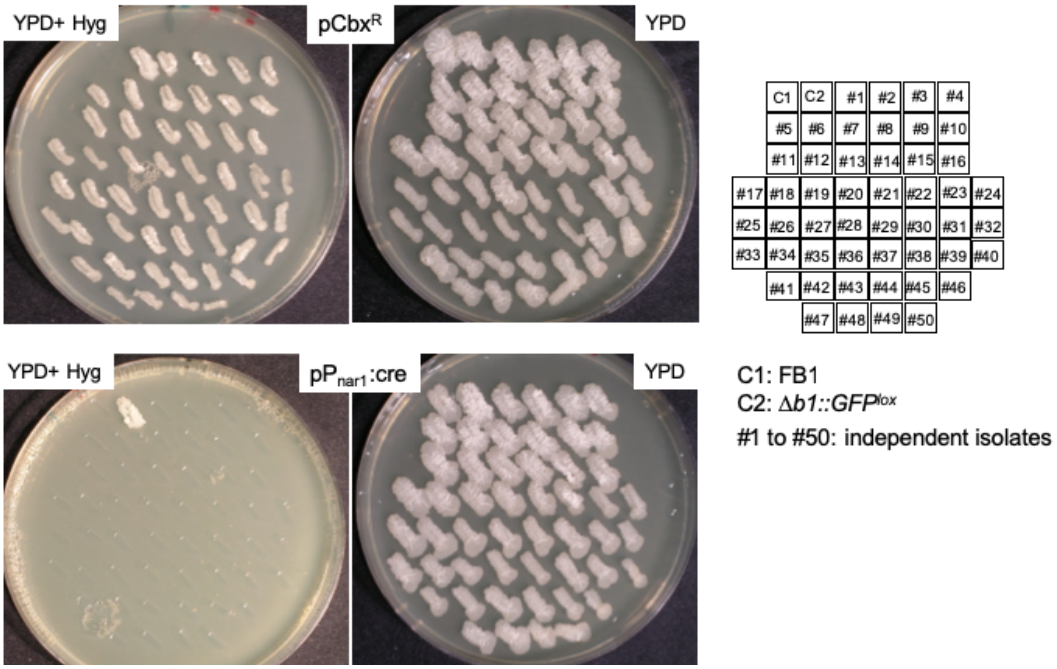


Figure S2. Phenotypical analysis of Cre-dependent recombination of a lox-flanked GFP-hygromycin cassette. Sensitivity to hygromycin (indicative for cre-mediated recombination of the lox-flanked GFP-hyg cassette) of colonies obtained upon transformation of strain Δ*b1*::*GFP*^{lox} with either the control plasmid pCM54 (pCbx^R) or plasmid pP$_{nar1}$:cre harbouring the *cre* gene under control of the *nar1* promoter. 50 colonies resistant to carboxin (used for plasmid selection) were isolated from transformation plates and streaked out on YPD plates with or without hygromycin. Control strains were also included (wild-type FB1 and Δ*b1*::*GFP*^{lox}). The experiment was repeated twice with same results. One of the experiments is shown.

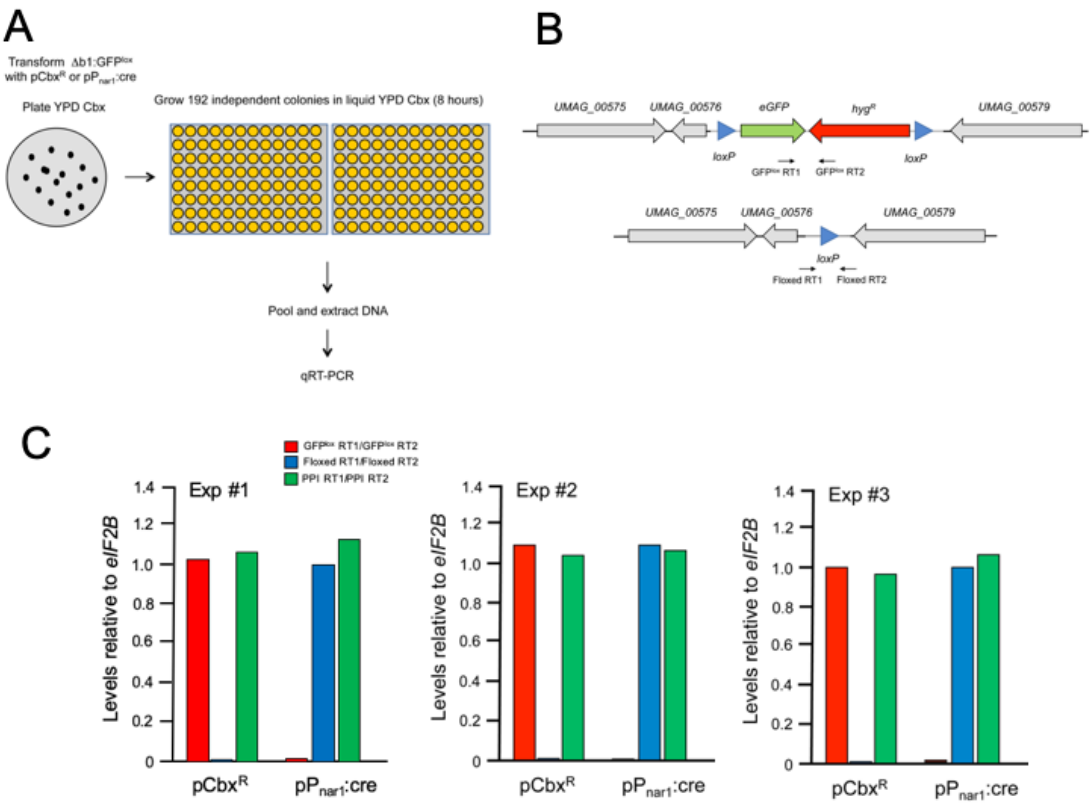


Figure S3. Genotypical analysis of Cre-dependent recombination of a lox-flanked GFP-hygromycin cassette. (A) Protoplasts from strain Δ*b1*::*GFP*^{lox}, harbouring a lox-flanked GFP-hygromycin cassette, were transformed with either the control plasmid pCM54 (pCbx^{R}) or plasmid pP$_{nar1}$:cre harbouring the *cre* gene under control of the *nar1* promoter. 192 colonies resistant to carboxin from each transformation were independently inoculated in liquid YPD amended with carboxin and grown for 8 hours. Cultures from these independent transformants were pooled, total DNA was extracted and submitted to qPCR analysis. (B) The scheme indicates the location of the two primer pairs designed to detect the excision of the *lox*-flanked cassette. GFPloxRT1/GFPloxRT2 primers amplify a 208 bp region encompassing the 5' ends from GFP- and Hyg^{R}-encoding genes. Floxed RT1/Floxed RT2 primers amplify a 202 bp region spanning the retaining *lox* site after recombination. (C) Results from 3 independent transformations of

each plasmid. DNA isolated from (A) was submitted to qPCR assay with the primer pairs indicated in (B). The gene encoding the *U. maydis* peptidyl-prolyl cis-trans isomerase (*ppi*, accession number EAK84904) was also analyzed as a control gene unaffected by the Cre-mediated recombination (RT_PPI_fw/RT_PPI_rv primers). RT_eIF2B_f/RT_eIF2B_r primers, amplifying the eIF2B-encoding gene, were used for normalization.

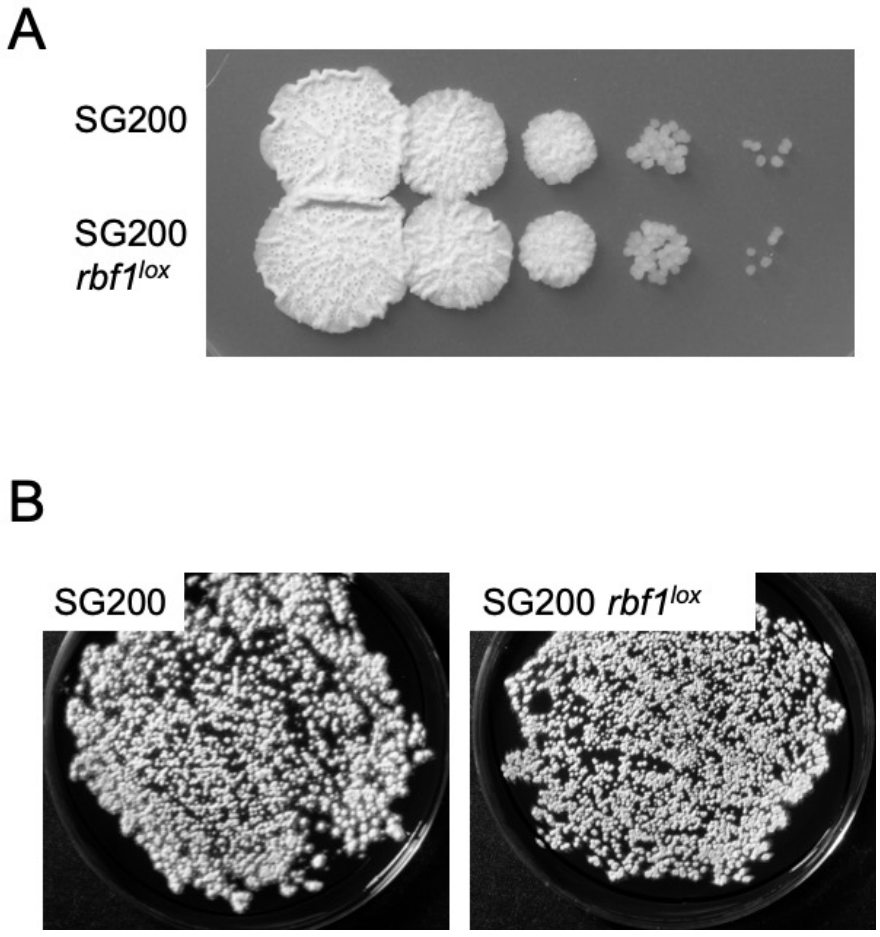


Figure S4. *U. maydis* strain SG200 harboring the *rbf1^{lox}* allele behaves similarly to wild-type SG200 with respect to growth and filament formation. (A) Growth of the indicated strains on CMD (Complete Medium Dextrose) plates. Serial ten-fold dilutions were spotted on plates and incubated for 2 days at 28ºC. (B) Development of *fuz*+ phenotype on charcoal PD (Potato Dextrose) plates. Plates were incubated for 2 days at RT.

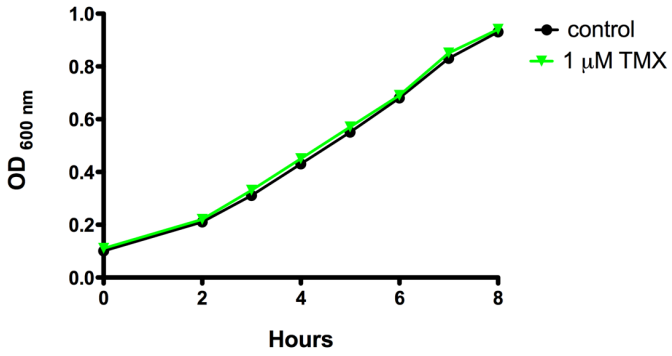


Figure S5. 1μM tamoxifen does not affect growth of *U. maydis* cells. Growth curves of *U. maydis* cultures incubated in YPD amended or not with 1μM Tamoxifen.

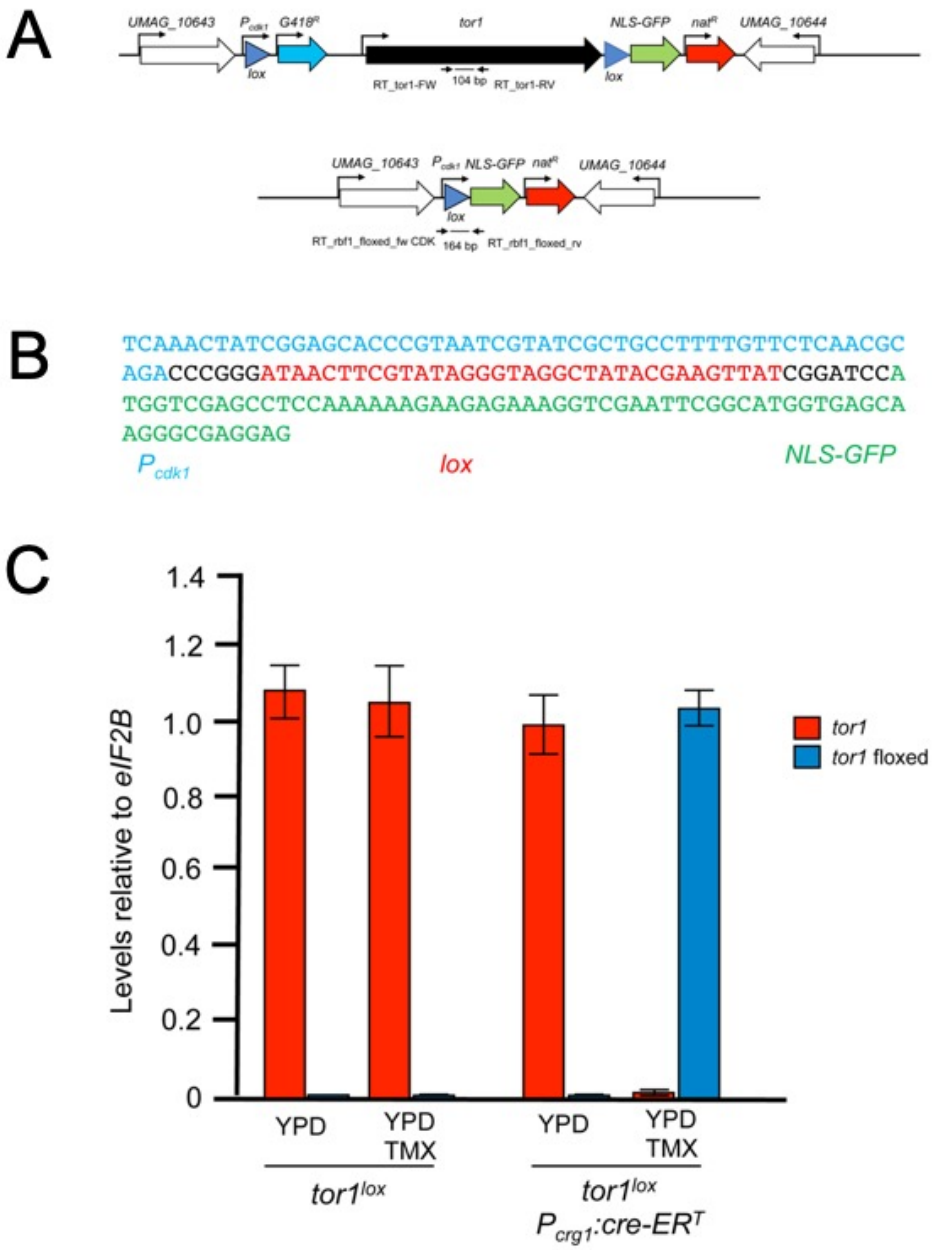


Figure S6. Cre-mediated excision of the *tor1*^{lox} allele. (A) Scheme of the *tor1*^{lox} allele before (top panel) and after (bottom panel) Cre-mediated recombination, indicating the primers designed for detection of the presence of *tor1* ORF (RT_tor1_FW/ RT_tor1_RV) and the recombination product (RT_rbf1_floxed_fw CDK/ RT_rbf1_floxed_rv). (B) Sequence from isolated PCR product resulting

from amplification of DNA obtained from *tor1*^{lox} *P*$_{crg1}$*:cre-ER*^{T} cells grown for 6 hours in liquid YPD amended with 1μm Tamoxifen. The primer pair used was RT_rbf1_floxed_fw CDK/ RT_rbf1_floxed_rv. (C) DNA isolated from cultures from the indicated strains grown for 6 hours in YPD medium supplemented or not with 1μm Tamoxifen (TMX) was submitted to qPCR assay with the indicated primer pairs (*tor1*: RT_tor1_FW/ RT_tor1_RV; *tor1* floxed: RT_rbf1_floxed_fw CDK/ RT_rbf1_floxed_rv). RT_eIF2B_f/RT_eIF2B_r primers, amplifying the eIF2B-encoding gene, were used for normalization. Level values represent the mean of two biological replicates. Error bars represent the SEM.

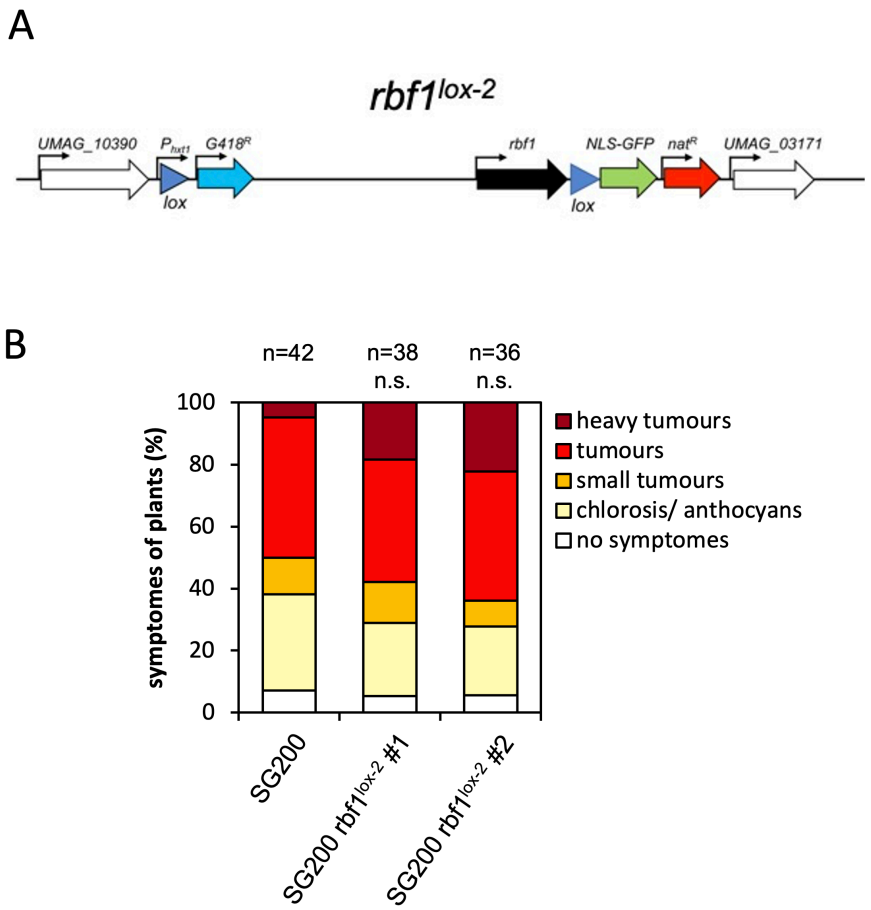


Figure S7. The *rbf1*$^{lox-2}$ allele is functional during plant infection. (A) Scheme of the *rbf1*$^{lox-2}$ allele. The *rbf1*$^{lox-2}$ allele is similar to *rbf1*^{lox1}, but the the *cdk1* promoter upstream of the 5' *lox* site in *rbf1*^{lox1} was replaced by the constitutive *hxt1* promoter, which allows high gene expression also when *U. maydis* is growing inside the plant tissue (SCHULER *et al.* 2015). (B) SG200 strains harboring the *rbf1*$^{lox-2}$ allele are not altered in virulence. 7 day-old maize plants were inoculated with SG200 and two independent SG200 transformants harboring the *rbf1*$^{lox-2}$ allele. Plants were scored for symptoms 14 days post-inoculation. n indicates the number of plants analyzed. n.s. indicate non-significant differences (Mann-Whitney U-test; SG200 *rbf1*$^{lox-2}$ #1: p= 0.197, SG200 *rbf1*$^{lox-2}$ #2: 0.083) in infection rates compared to SG200.

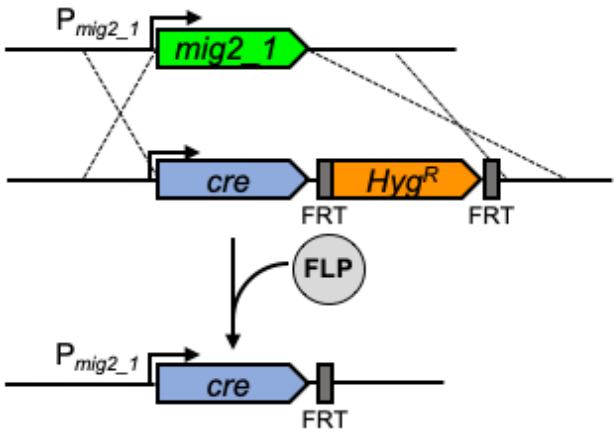


Figure S8. Scheme for construction of the *mig2_1* promoter-driven *cre*-recombinase gene. In a first step, the *mig2_1* coding sequence was replaced with the cre-recombinase to drive cre-expression by the *mig2-1* promoter. In a second step, the hygromycin-resistance cassette was removed via its flanking *FRT* sites by transformation with an autonomously replicating plasmid expressing the *flp* recombinase. Strains were cured of the *flp*-plasmid by passage on medium without selection pressure for maintaining the plasmid (KHRUNYK *et al.* 2010).

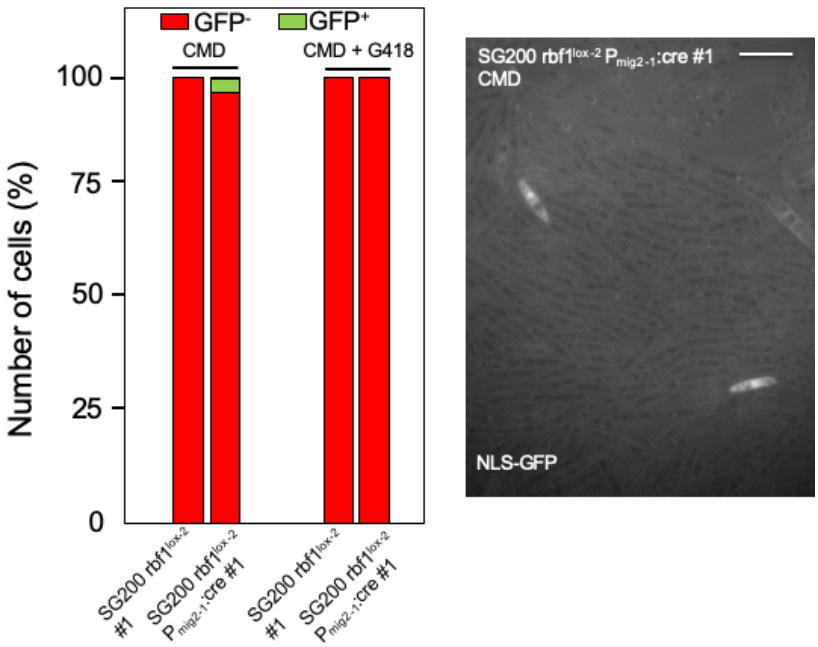


Figure S9. Basal expression of *P*$_{mig2\_1}$*:cre* in axenic cultures. Percentage of cells showing green nuclear fluorescence after growth of strains indicated for 8 hours in liquid CMD or CMD supplemented with G418 (2 independent experiments, 100 cells counted per sample in each). The image on the right shows a representative field of a dense culture of SG200 r*bf1*$^{lox-2}$ *P*$_{mig2-1}$*:cre#1* grown in CMD medium. Bar: 20 μm.

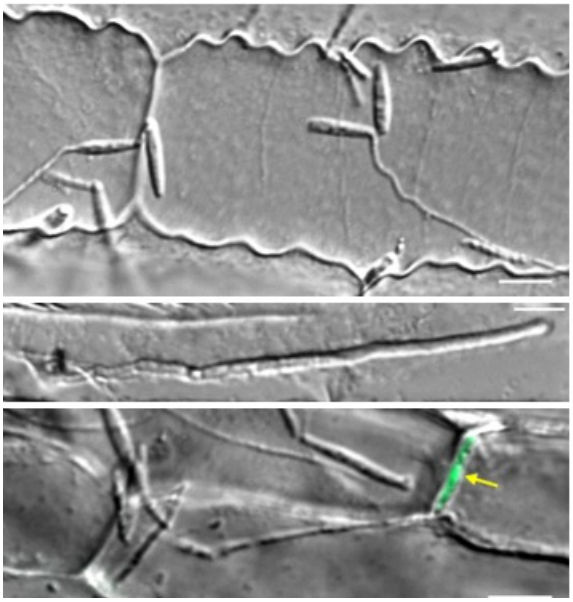


Figure S10. Basal expression of *P_{mig2_1}:cre* on plant surface. DIC and GFP channel merged images of plant surface from maize plants inoculated with SG200*rbf1*$^{lox-2}$ *P_{mig2_1}:cre* cells (1 dpi). Note the formation of infective filaments without associated GFP fluorescence. Also, note the presence of cells (bottom panel, yellow arrow) that have lost the *rbf1* gene already when growing on the plant surface, indicated by the green fluorescence due to the reconstituted *P_{hxt1}:NLS-GFP* expression cassette. Bar: 40 μm.

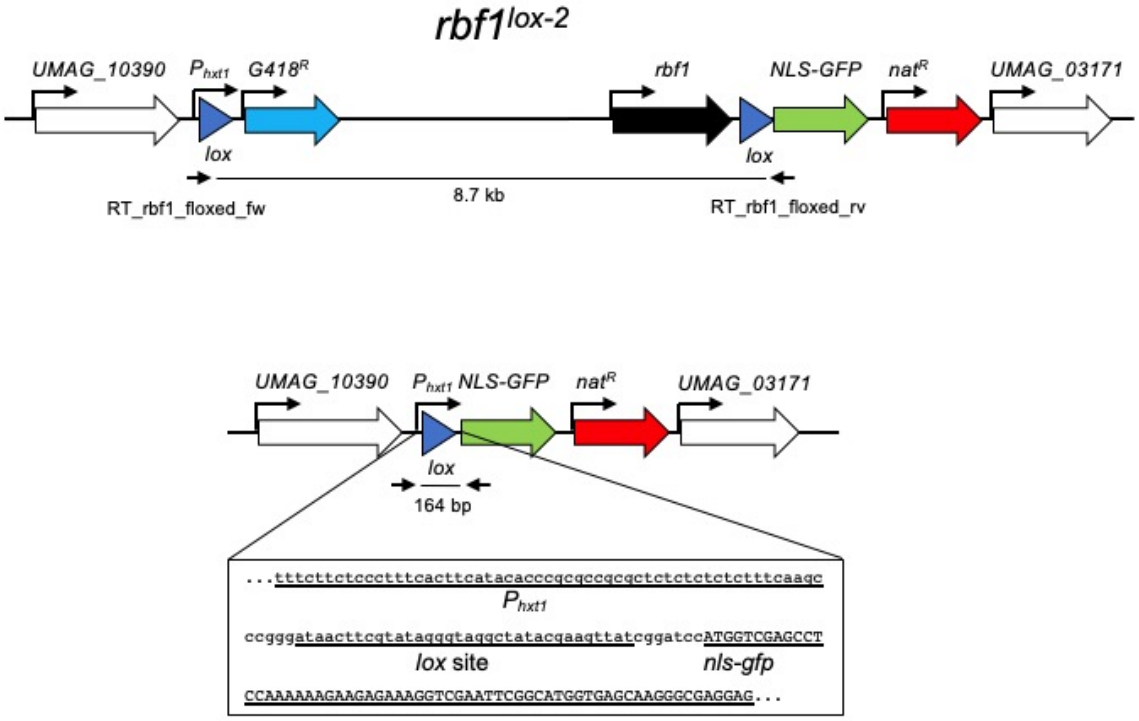


Figure S11. Cre-mediated excision of *rbf1*$^{lox-2}$ allele. (A) Scheme of the *rbf1*$^{lox-2}$ allele before (top panel) and after (bottom panel) Cre-mediated recombination. Position of PCR primers for detection of the recombination product (RT_rbf1_floxed_fw/ RT_rbf1_floxed_rv) are indicated. The boxed sequence was obtained by sequencing a PCR product obtained with the primers indicated; template DNA was isolated from plants infected with SG200*rbf1*$^{lox-2}$ P_{mig2_1}*:cre* #1cells 7 days post infection.

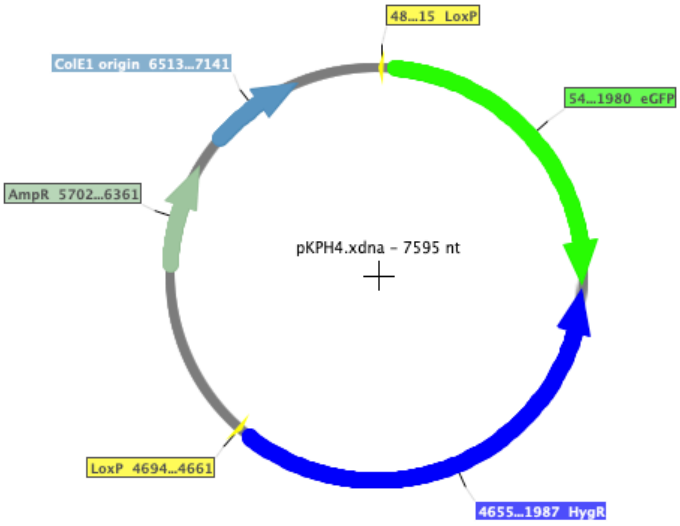
Supplementary Materials and Methods

Plasmid Constructions

pKPH4

The eGFP gene driven by the otef-promoter from plasmid p123 (BASSE *et al.* 2000) was PCR-amplified with primers p123_egfp_3 and lox_otef_5 to introduce a *loxP* site 5´ to the otef-promoter, cloned into PCR-TOPO-TA (Thermo Fischer) and sequenced.

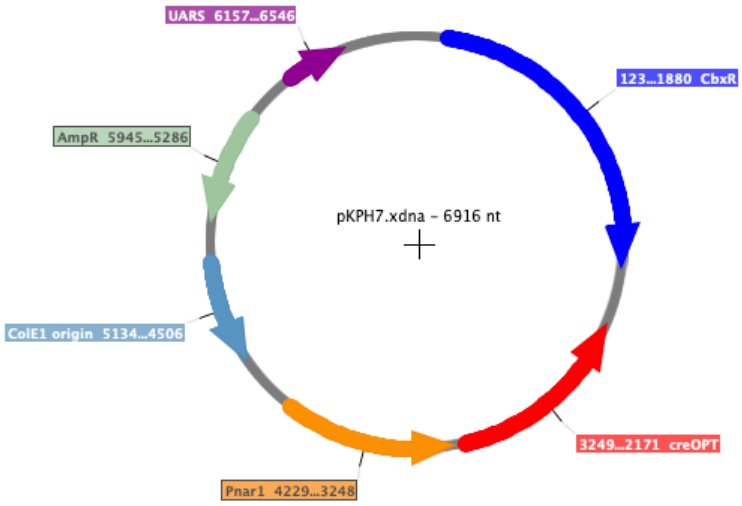
One of two *Not*I sites flanking the hygromycin-resistance cassette on plasmid pMF1-h (BRACHMANN *et al.* 2004) was deleted by partial digestion and a Klenow-fill in reaction; into the second *Not*I site (5´of the *hsp70*-promoter driving the hygromycin gene) a 49 bp fragment with second *lox*-site was integrated; the fragment was generated by annealing of oligonucleotides loxP_C and loxP_W. In the resulting plasmid, the above described eGFP cassette was integrated as *Mscl*/*Nsi*I into the related sites.



pP_{nar1}:cre (pKPH7)

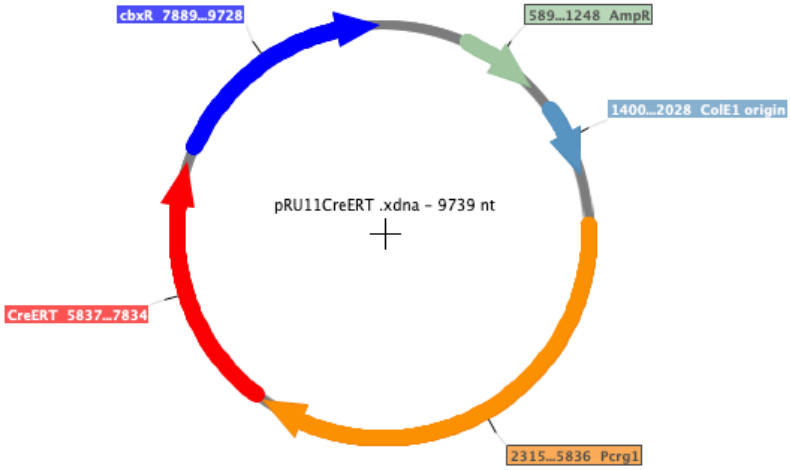
Plasmid pRU4 (BRACHMANN *et al.* 2001) harbors the *U. maydis ip*^{r} locus conferring carboxin resistance and the promoter region of the *U. maydis nar1* gene. To

confer replication autonomously, a 1.1 kb *Fsp*I/*Aat*II fragment from plasmid pCM54 (TSUKUDA *et al.* 1988) with a *U. maydis* UARS sequence was exchanged with the respective fragment in pRU4. Subsequently, the dicodon optimized cre gene was integrated as *Nde*I/*Not*I fragment into the vector's respective sites. The resulting plasmid pKPH7 replicates autonomously in *U. maydis*, confers carboxin resistance, and allows the nitrate-inducible expression of *cre*.



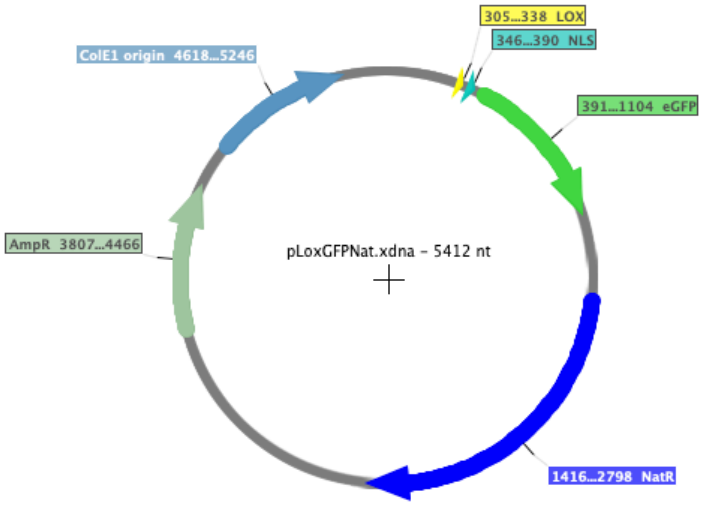
pP$_{crg1}$:cre-ER^{T} (pRU11CreER^{T})

A 2,02 kbp synthetic DNA encoding di-codon optimized CreER^{T} version was cloned as a *Nde*I-*EcoR*I fragment into the corresponding sites of pRU11 (BRACHMANN *et al.* 2001).



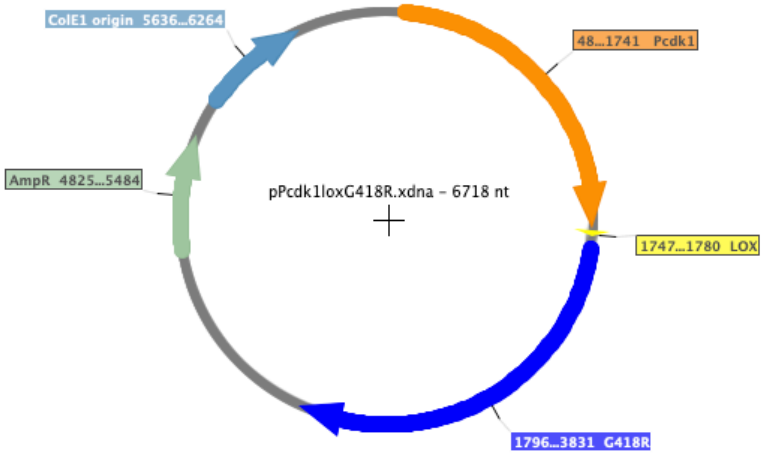
pLOXGFPNat (3' cassette)

A 0,79 kb fragment carrying the coding sequence of a GFP fused to an NLS was amplified from plasmid pnGFP (STRAUBE *et al.* 2005) with primers GFP-LOX-1/GFP-LOX-2, that introduced restriction sites for *Xho*I and *Bam*HI at the 5' end and *Asc*I at the 3´end. This fragment was cloned into the corresponding *Xho*I and *Asc*I sites into pMF5-1n (BRACHMANN *et al.* 2004). Finally, a 62 bp fragment with the *lox*-site, resulting from annealing oligonucleotides GFP-LOX-3 and GFP-LOX-4, was inserted between the *Xho*I and *Bam*HI sites of the previous plasmid.



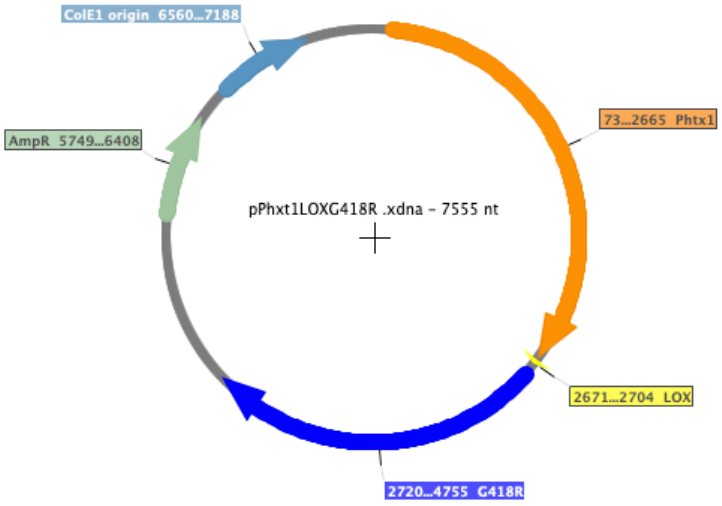
pPcdk1LOXG418R (5' cassette)

A 1,75 kb fragment carrying the promoter region of *cdk1* was amplified from *U. maydis* genomic DNA with primers pCDK1-1/pCDK1-2, flanking the fragment with restriction sites for *Sfi*I (5' end) and *Xma*I, *Eco*RI, and *Sfi*I (3' end). This fragment was cloned into pJET 1.2 (Thermo Fisher), and subsequently, a 48 bp fragment harboring the *lox* site (obtained by annealing of oligonucleotides LOX5 and LOX6) was cloned between the corresponding *Xma*I and *Eco*RI restriction sites. The resulting vector's *Eco*RI site was used to insert a *Mfe*I-*Eco*RI 2,05 kbp fragment carrying a gene encoding resistance to G418 from plasmid pCM150 (KOJIC AND HOLLOMAN 2000).



pPhxt1LOXG418R (5' cassette)

A 2,61 kbp fragment carrying the promoter region of *hxt1* was amplified from *U. maydis* genomic DNA with primers Phxt1-1/Phxt1-2, flanking the DNA fragment with *Kpn*I (5' end) and *Xma*I (3' end) restriction sites; the fragment was used to exchange the corresponding *Kpn*I/*Xma*I fragment in pPcdk1LOXG418R, resulting in the exchange of the *Pcdk1* promoter with the *Phxt1* promoter.



Procedure for *in planta* expression of Cre.

To express Cre recombinase *in planta*, the *mig2_1* open reading frame (ORF) was in a first step replaced via homologous recombination by the *cre* ORF associated with the *FRT* flanked hygromycin resistance cassette. The hygromycin resistance cassette was subsequently excised by FLP/*FRT* mediated recombination, according to (KHRUNYK *et al.* 2010).

The plasmid for replacement of *mig2_1* by *cre* (pJet1.2 mig2_1:cre) was constructed as follows: cre open reading frame (ORF) was PCR amplified from plasmid pKPH7 using primers cre_Sfi_f and cre_Sfi_r, adding *Sfi* I sites to the 5' and 3' end and integrated into *Sfi* I linearized plasmid pLS27 (pJET1.2 mig2_1-FL) (SCHMITZ *et al.* 2020). For transformation, the construct was PCR amplified using primers mig2_1_LB_f and mig2_1_RB_r, and integrated into strain SG200 via homologous recombination. The resulting strain was transformed with plasmid pFLPexpC to remove the hygromycin resistance marker by FLP/*FRT* mediated recombination according to (KHRUNYK *et al.* 2010), generating strain SG200mig2_1-cre. Correct genomic integration of the construct and excision of

the resistance marker cassette was confirmed at each step by Southern hybridization and phenotypic analysis (loss of hygromycin resistance).

Cassettes for *lox* alleles.

The integration cassettes for the distinct alleles described in this work were constructed following *Sfi*I-directed tripartite ligations (BRACHMANN *et al.* 2004). Briefly, a pair of DNA fragments (around 1kpb) flanking the region where the cassette will be inserted were amplified by PCR using primers that carried *Sfi*I (internal border) and *Pme*I (outer border) sites. The respective 5' and 3' fragments digested with *Sfi*I were ligated to the desired cassette, also previously digested with *Sfi*I. The ligation product was isolated and directly ligated to pJET1.2 (digested with *Eco*RV). The resulting plasmid, carrying the entire integration cassette, was digested with *Pme*I to release the integration cassette used to transform protoplasts of *U. maydis*.

Below, we describe the respective primers used for each construction and the used resistance cassette.

∆*b1::GFP^{lox}* (pVV17LC1). From pKPH4, the *lox*-flanked cassette harboring the HygR gene and eGFP was excised as *Sfi*I fragment and replaced with the *Sfi*I-fragment in plasmid pVV17 (KÄMPER 2004). In the resulting plasmid, the *lox*-cassette is flanked by sequences 5´and 3´to b-mating-type locus to delete the *b*-locus via homologous recombination.

5' insertion of rbf1^{lox} (pPcdk1LOXG418-Rbf1). 5' fragment: Rbf1FG1-2/ Rbf1FG1-3 (1000 bp); 3' fragment: Rbf1FG2-1/ Rbf1FG2-2 (1040 bp). Ligated to the G418R cassette excised upon *Sfi*I digestion from pPcdk1LOXG418R.

5' insertion of rbf1^{lox} (pPhtx1LOXG418-Rbf1). 5' fragment: Rbf1FG1-2/ Rbf1FG1-3 (1000 bp); 3' fragment: Rbf1FG2-1/ Rbf1FG2-2 (1040 bp). Ligated to the G418R cassette excised upon *Sfi*I digestion from pPhtx1LOXG418R.

3' insertion of rbf1^{lox} (pRbf1-LOXGFPNAT). 5' fragment: Rbf1FG3-2/ Rbf1FG3-3 (1000 bp); 3' fragment: Rbf1FG4-1/ Rbf1FG4-2 (1042 bp). Ligated to the NatR cassette excised upon *Sfi*I digestion from pLOXGFPNat.

5' insertion of tor1^{lox} (pPcdk1LOXG418-Tor1). 5' fragment: Tor1FG1-2/ Tor1FG1-3 (1111 bp); 3' fragment: Tor1FG2-1/ Tor1FG2-2 (920 bp). Ligated to the G418R cassette excised upon *Sfi*I digestion from pPcdk1LOXG418R.

3' insertion of tor1^{lox} (pTor1-LOXGFPNAT). 5' fragment: Tor1FG3-2/ Tor1FG3-3 (1011 bp); 3' fragment: Tor1FG4-1/ Tor1FG4-2 (1025 bp). Ligated to the NatR cassette excised upon *Sfi*I digestion from pLOXGFPNat.

Table S1. *U. maydis* strains used in this study

Strain	Relevant genotype	Source
FB1	*a1 b1*	(BANUETT AND HERSKOWITZ 1989)
Δb1::GFP^{lox}	*a1 Δb1::lox-GFP-HygR-lox* (Hyg^R)	This work
Δb1::GFP^{lox}/ pCbxR#1	*a1 Δb1::lox-GFP-HygR-lox* (Hyg^R)/pCM54 (Cbx^R)	This work
Δb1::GFP^{lox}/ pCbxR#2	*a1 Δb1::lox-GFP-HygR-lox* (Hyg^R)/pCM54 (Cbx^R)	This work
Δb1::GFP^{lox}/ pPnar1:cre #1	*a1 Δb1::lox-GFP-HygR-lox* (Hyg^R)/pKPH7 (Cbx^R)	This work
Δb1::GFP^{lox}/ pPnar1:cre #2	*a1 Δb1::lox-GFP-HygR-lox* (Hyg^R)/pKPH7 (Cbx^R)	This work
SG200	*a1 mfa2 bW2 bE1* (Phleo^R)	(BÖLKER *et al.* 1995)
SG200 P_{crg1}*:creER^T*	*a1 mfa2 bW, bE1* (Phleo^R) *ip*[*Pcrg1:creER^T* (Cbx^R)]	This work
SG200 *rbf1^{lox}*	*a1 mfa2 bW2 bE1* (Phleo^R) *rbf1^{lox}* (G418^R, Nat^R)	This work
SG200 *rbf1^{lox}* P_{crg1}*:creER^T*	*a1 mfa2 bW2 bE1* (Phleo^R) *rbf1^{lox}* (G418^R, Nat^R), *ip*[*Pcrg1:creER^T* (Cbx^R)]	This work
FB1 *tor1^{lox}*	*a1 b1 tor1^{lox}*(G418^R, Nat^R)	This work
FB1 *tor1^{lox}* P_{crg1}*:creER^T*	*a1 b1 tor1^{lox}*(G418^R, Nat^R) *ip*[*Pcrg1:creER^T* (Cbx^R)]	This work
SG200 *rbf1^{lox-2}* #1	*a1 mfa2 bW2 bE1* (Phleo^R) *rbf1^{lox-2}* (G418^R, Nat^R)	This work
SG200 *rbf1^{lox-2}* #1	*a1 mfa2 bW2 bE1* (Phleo^R) *rbf1^{lox-2}* (G418^R, Nat^R)	This work
SG200 *rbf1^{lox-2}* P_{mig2_1}*:cre* #1	*a1 mfa2 bW2 bE1* (Phleo^R) *rbf1^{lox-2}* (G418^R, Nat^R) *mig2_1::cre-frt*	This work
SG200 *rbf1^{lox-2}* P_{mig2_1}*:cre* #2	*a1 mfa2 bW2 bE1* (Phleo^R) *rbf1^{lox-2}* (G418^R, Nat^R) *mig2_1::cre-frt*	This work

Table S2. Oligonucleotides used in this study

GFP-LOX-1	5’ ATCTCGAGATAGGATCCATGGTCGAGCCTCCAAAAAAGAAGAGA 3’
GFP-LOX-2	5’ ATGGCGCGCCTTACTTGTACAGCTCGTCCATGCCGAGAGT 3’
GFP-LOX-3	5’ TCGAGGCCAACGCGGCCATGATAACTTCGTATAGGGTAGGCTATA CGAAGTTATCG 3’
GFP-LOX-4	5’ GATCCGATAACTTCGTATAGCCTACCCTATACGAAGTTATCATGGCC GCGTTGGCC 3’
PCDK1-1	5’ GGCCTAGATGGCCACCATGGCGTGACAATTGCGGCCGCCGCGAAGC TTTCACGACTTGCACTCACGACTCGTGA 3’
PCDK1-2	5’ ACGGCCGGAGCGGCCGGAATTCTCCCGGGTCTGCGTTGAGAACAAA AGGCAGCGA 3’
LOX-5	5’ CCGGGATAACTTCGTATAGGGTAGGCTATACGAAGTTATCGG 3’
LOX-6	5’ AATTCCGATAACTTCGTATAGCCTACCCTATACGAAGTTATC 3’
PHXT1-1	5’ ATAGGTACCGGCCTAGATGGCCAAGATTCTTGGTCGATTCTACAG 3’
PHXT1-2	5’ TATCCCGGGCTTGAAAGAGAGAGAGAGCGCGGCGCG 3’
bLOCUS-2	5’ ATAGTTTAAACTGAAGGATTGTGTTCAGTTCGGTGAAC 3’
bLOCUS-3	5’ TATGGCCATCTAGGCCGATTTCAGTGATACGTTTAGTCCCTTT 3’
bLOCUS-4	5’ ATAGGCCTGAGTGGCCGACTTTGAAAGCGCCCACTGGATGGTG 3’
bLOCUS-5	5’ TATGTTTAAACGTTGAAGCTAGCGGAAAAGTGGGTGA 3’
b FWD	5’ CCCAAGCTCACTGTGCATGTGATTGCGAAC 3’
b REV	5’ ACAAGCTGAACGAAGCACATTGAGGAGCAC 3’
Rbf1FG1-2	5’ GACGTTTAAACGTGCCTCGTGTCGGCGTCGCCGTCTTTGTG 3’
Rbf1FG1-3	5’ GGTGGCCATCTAGGCCGACAATGACATTGCGTTTTCTTTGGTA 3’
Rbf1FG2-1	5’ CGCGGCCGGAGCGGCCACGAAGTGTAACCATTCCCTTGCAGTCC 3’
Rbf1FG2-2	5’ GTCGTTTAAACCACACGTTCCGTCGACGAGAGACATCAT 3’
Rbf1FG3-2	5’ GACGTTTAAACCACGCCACAGGAGGATCCACACGGGAAG 3’
Rbf1FG3-3	5’ CATGGCCGCGTTGGCCGTGTAGAGAAGGATGCGATTCCAGGAGC 3’
Rbf1FG4-1	5’ ATAGGCCTGAGTGGCCCTACCTTTATGCTTGTTGTGCTGGATAAC 3’
Rbf1FG4-2	5’ GTCGTTTAAACAGTGACGCGAGTGATGCTTTATGTGCTTG 3’

Tor1FG1-2	5’ CACGTTTAAACACGCCGGGGCAAGATCTGTACCGA 3’
Tor1FG1-3	5’ CATGGCCGCGTTGGCCCTCTCTTGTTTCCATCCTTTTGTTCAT 3’
Tor1FG2-1	5’ ATAGGCCTGAGTGGCCCGCTCAATGCTGAGTGGGAGGCTGCTG 3’
Tor1FG2-2	5’ GACGTTTAAACTGCTCAAGCGCGGCTTCCAGTTT 3’
Tor1FG3-2	5’ GATGTTTAAACGACGTCCGGCAAAGTGCTCGAGGCCA 3’
Tor1FG3-3	5’ GGTGGCCATCTAGGCCTATGATGACGGTGATGACAATGTGCGG 3’
Tor1FG4-1	5’ CGCGGCCGGAGCGGCCATGTCCGTCCTTGGCACCCATCAGAG 3’
Tor1FG4-2	5’ GATGTTTAAACGTCTGTGCTGACTCCTTGGGCGA 3’
CreRT-1	5’ CGTACTGACGGTGGGAGAAT 3’
CreRT-2	5’ CCCGGCAAAACAGGTAGTTA 3’
IN-Fwd	5’ CCCAAGCTCACTGTGCATGTGATTGCGAAC 3’
IN-Rev	5’ ACAAGCTGAACGAAGCACATTGAGGAGCAC 3’
cre_Sfi_f	5’ TATAGGCCTGAGTGGCCATGATGAGCAACCTGCTCACC 3’
cre_Sfi_r	5’ TATAGGCCATCTAGGCCCTAGTCGCCGTCCTCGAG 3’
mig2_1_LB_f	5’ CTGCACAGCGGATGGCAAAG 3’
mig2_1_RB_r	5’ CAGACTGAGAATGTCGATGTGG 3’
p123-egfp-3	5´CGCGCGTTGGCCGATTCATTAATG 3´
lox-otef-5	5´GCTGGCCAATAACTTCGTATAATGTATGCTATACGAAGTTATACCCG TACCGAGCTC GACTTTCAC 3´
loxP_C	5´ GGCCATAACTTCGTATAGCATACATTATACGAAGTTATGAATTCA 3´
loxP_W	5´ GGCCTGAATTCATAACTTCGTATAATGTATGCTATACGAAGTTAT 3´
GFPloxRT1	5´ GAAAACAAAATATAGCGCGC 3´
GFPloxRT2	5´ TTTGTCAACTTGACAGCGGG 3´
Floxed RT1	5´ GTGAAATTCCACGAGCAAAG 3´
Floxed RT2	5´ AGAGAAGAAGCAGGTATACG 3´
RT_PPI_fw	5´ ACATCGTCAAGGCTATCG 3´
RT_PPI_rv	5´ AAAGAACACCGGACTTGG 3´
RT_eIF2B_f	5´ ATCCCGAACAGCCCAAAC 3´
RT_eIF2B_r	5´ ATCGTCAACCGCAACCAC 3´
RT_tor1_FW	5´ TTGCTGGACTCAAGAGCAGA 3´

RT_tor1_RV	5´ GAACGAGGAGAGGCTGTCAC 3´
RT_rbf1_floxed_fw CDK	5´ TCAAACTATCGGAGCACCCG 3´
RT_rbf1_floxed_rv	5´ CTCCTCGCCCTTGCTCAC 3´
RT_rbf1_floxed_fw	5´ TTTCTTCTCCCTTTCACTTCATAC 3´
RT_rbf1-F	5´ AGTACGAGCTACGACGGATTC 3´
RT_rbf1-R	5´ GGGTAGGTGTTGGACACATTC 3´
Tub1-RT1	5´ CGAGATGACCTTCTCGTCGT 3´
Tub1-RT2	5´ AACATCACCACGGTACAGCA 3´
Rpl43B-RT1	5´ CGAACTGTCAAGGTCGGAAT 3´
Rpl43B-RT2	5´ ACTTGGAGTGCTGCGAGATT 3´
s12b-RT1	5´ ACCAACTTCCCCCTGTCTG 3´
s12b-RT2	5´ CTGGTAGCTCCAGACGTTGC 3´

Table S3. P-values of a two-sided Mann-Whitney U rank sum test with continuity correction for the plant infection experiments shown in Figure 7

		SG200 rbf1^{lox-2} P$_{mig2-1}$:cre #1		SG200 rbf1^{lox-2} P$_{mig2-1}$:cre #2	
		I	II	I	II
SG200 rbf1^{lox-2} #1	I	9.40E-05	-	1.82E-05	-
	II	-	4.16E-01	-	9.84E-01

REFERENCES

Banuett, F., and I. Herskowitz, 1989 Different a alleles of Ustilago maydis are necessary for maintenance of filamentous growth but not for meiosis. Proc Natl Acad Sci U S A 86**:** 5878-5882.

Basse, C. W., S. Stumpferl and R. Kahmann, 2000 Characterization of a Ustilago maydis gene specifically induced during the biotrophic phase: evidence for negative as well as positive regulation. Mol Cell Biol 20**:** 329-339.

Bölker, M., S. Genin, C. Lehmler and R. Kahmann, 1995 Genetic regulation of mating and dimorphism in Ustilago maydis. Can. J. Bot. 73**:** S320-S325.

Brachmann, A., J. Konig, C. Julius and M. Feldbrugge, 2004 A reverse genetic approach for generating gene replacement mutants in Ustilago maydis. Molecular genetics and genomics : MGG 272**:** 216-226.

Brachmann, A., G. Weinzierl, J. Kamper and R. Kahmann, 2001 Identification of genes in the bW/bE regulatory cascade in Ustilago maydis. Mol Microbiol 42**:** 1047-1063.

Kämper, J., 2004 A PCR-based system for highly efficient generation of gene replacement mutants in Ustilago maydis. Mol Genet Genomics 271**:** 103-110.

Khrunyk, Y., K. Munch, K. Schipper, A. N. Lupas and R. Kahmann, 2010 The use of FLP-mediated recombination for the functional analysis of an effector gene family in the biotrophic smut fungus Ustilago maydis. New Phytol 187**:** 957-968.

Kojic, M., and W. K. Holloman, 2000 Shuttle vectors for genetic manipulations in Ustilago maydis. Can J Microbiol 46**:** 333-338.

Schmitz, L., J. W. Kronstad and K. Heimel, 2020 Conditional gene expression reveals stage-specific functions of the unfolded protein response in the Ustilago maydis-maize pathosystem. Mol Plant Pathol 21**:** 258-271.

Schuler, D., R. Wahl, K. Wippel, M. Vranes, M. Münsterkötter *et al.*, 2015 Hxt1, a monosaccharide transporter and sensor required for virulence of the maize pathogen Ustilago maydis. New Phytol 206**:** 1086-1100.

Straube, A., I. Weber and G. Steinberg, 2005 A novel mechanism of nuclear envelope break-down in a fungus: nuclear migration strips off the envelope. Embo j 24**:** 1674-1685.

Tsukuda, T., S. Carleton, S. Fotheringham and W. K. Holloman, 1988 Isolation and characterization of an autonomously replicating sequence from Ustilago maydis. Mol Cell Biol 8**:** 3703-3709.