Strain	Mating type	Genotype	Source	Reference
FGSC#2489	A	*wildtype*	The Fungal Genetics Stock Center	ref. 39
FGSC#4200	a	*wildtype*	The Fungal Genetics Stock Center	ref. 39
FGSC#8594	a	*dim-2Δ, his-3*	The Fungal Genetics Stock Center	ref. 39
FGSC#9539	a	*mus-52Δ, his-3*	The Fungal Genetics Stock Center	ref. 39
FGSC#9720	A	*mus-52Δ, his-3*	The Fungal Genetics Stock Center	ref. 39
FGSC#11198	a	*ddb1Δ*	The Fungal Genetics Stock Center	ref. 39
FGSC#12374	a	*cul4Δ*	The Fungal Genetics Stock Center	ref. 39
FGSC#12353	a	*ridΔ*	The Fungal Genetics Stock Center	ref. 39
FGSC#12354	A	*ridΔ*	The Fungal Genetics Stock Center	ref. 39
FGSC#12440	A	*spo11Δ*	The Fungal Genetics Stock Center	ref. 39
FGSC#14522	a	*hpoΔ*	The Fungal Genetics Stock Center	ref. 39
FGSC#15885	a	*dim-5Δ*	The Fungal Genetics Stock Center	ref. 39
S-353	a	*dim-5Δ, set-7Δ*	Zachary Lewis, University of Georgia, USA	ref. 26
S-354	A	*dim-5Δ, set-7Δ*	Zachary Lewis, University of Georgia, USA	ref. 26
T103.3	A	***802-bp**, mus-52Δ, his-3*	FGSC#9720 transformed with pEAG115A	ref. 18
T208.3	A	***RANDOM**, mus-52Δ, his-3*	FGSC#9720 transformed with pEAG186B	ref. 18
T221.18	A	***4H-7N**, mus-52Δ, his-3*	FGSC#9720 transformed with pEAG186K	ref. 18
T223.4	A	***3H-8N**, mus-52Δ, his-3*	FGSC#9720 transformed with pEAG186L	ref. 18
T337.1h	a	***csr-1(Dp)::his-3+**, ridΔ, mus-52Δ*	C03.1 transformed with pEAG82B	this study
T364.6	A	***802-bp**, ridΔ*	FGSC#12354 transformed with pEAG115A	this study
T382.32	a	***802-bp**, dim-2Δ, his-3*	FGSC#8594 transformed with pEAG115A	this study
T402.1h	A	***csr-1(Dp)::his-3+**, mus-52Δ*	FGSC#9720 transformed with pEAG82B	this study
T451.6h	A	***4x::his-3+**, ridΔ, mus-52Δ*	C02.1 transformed with pEAG236A	this study
T452.1h	A	***4x::his-3+**, ridΔ, dim-2Δ, mus-52Δ*	C58.40 transformed with pEAG236A	this study
T475.6h	a	*ridΔ, dim-5Δ, set-7Δ, mus-52Δ, his-3::dim-5+*	C146.1 transformed with pEAG244B	this study
T482.3	A	***RANDOM**, ridΔ, mus-52Δ, his-3*	C02.1 transformed with pEAG186B	this study
T484.1	A	***3H-8N**, ridΔ, mus-52Δ, his-3*	C02.1 transformed with pEAG186L	this study
T485.4h	A	***4x::his-3+**, mus-52Δ*	FGSC#9720 transformed with pEAG236A	this study
T486.3h	a	***4x::his-3+**, ridΔ, dim-5Δ, set-7Δ, mus-52Δ*	C146.1 transformed with pEAG236A	this study
T487.3	A	***4H-7N**, ridΔ, mus-52Δ, his-3*	C02.1 transformed with pEAG186K	this study
T488.1h	a	*ridΔ, dim-5Δ, set-7Δ, mus-52Δ, his-3::dim-5(Y283F)*	C146.1 transformed with pEAG244G	this study
T489.1	A	***NO-REPEAT**, mus-52Δ, his-3*	FGSC#9720 transformed with pEAG66	this study
C02.1	A	*ridΔ, mus-52Δ, his-3*	Cross progeny of FGSC#9539 and FGSC#12354	this study
C03.1	a	*ridΔ, mus-52Δ, his-3*	Cross progeny of FGSC#9720 and FGSC#12353	this study
C50.3	A	*ridΔ, dim-2Δ*	Cross progeny of FGSC#8594 and FGSC#12354	this study
C52.1	a	***csr-1(Dp)::his-3+**, ridΔ, dim-2Δ*	Cross progeny of C50.3 and T337.1h	this study
C57.8	A	***802-bp**, ridΔ, dim-2Δ*	Cross progeny of C52.1 and T364.6	this study
C58.7	a	*ridΔ, dim-2Δ, mus-52Δ, his-3*	Cross progeny of C50.3 and C03.1	this study
C58.40	A	*ridΔ, dim-2Δ, mus-52Δ, his-3*	Cross progeny of C50.3 and C03.1	this study
C96.1	A	*dim-2Δ*	Cross progeny of FGSC#8594 and FGSC#2489	this study
C97.2	A	***802-bp**, ridΔ, hpoΔ*	Cross progeny of FGSC#14522 and T364.6	this study
C97.6	a	***802-bp**, hpoΔ*	Cross progeny of FGSC#14522 and T364.6	this study
C98.18	A	***802-bp**, ridΔ, dim-5Δ*	Cross progeny of FGSC#15885 and T364.6	this study
C135.3	A	***802-bp**, set-7Δ, spo11Δ, hpoΔ*	Cross progeny of XG1.2 and FGSC#12440	this study
C139.10	a	*ridΔ, set-7Δ, mus-52Δ, his-3*	Cross progeny of C58.7 and S-354	this study
C146.1	a	*ridΔ, dim-5Δ, set-7Δ, mus-52Δ, his-3*	Cross progeny of XG3.4 and C139.10	this study
C146.3	a	*ridΔ, dim-5Δ, set-7Δ*	Cross progeny of XG3.4 and C139.10	this study
R88.6	A	***csr-1(Dp)::his-3+***	Cross progeny of C52.1 and FGSC#2489	this study
XG1.2	a	***802-bp**, set-7Δ, dim-5Δ, hpoΔ*	Cross progeny of C97.2 and S-353	this study
XG1B.17	a	*set-7Δ*	Cross progeny of C97.2 and S-353	this study
XG3.4	A	***802-bp**, dim-5Δ, set-7Δ*	Cross progeny of C97.6 and S-354	this study
XG3.7	A	***802-bp**, set-7Δ*	Cross progeny of C97.6 and S-354	this study
XG3B.11	a	*set-7Δ, hpoΔ*	Cross progeny of C97.6 and S-354	this study
XG26.8	A	***802-bp**, dim-5Δ, set-7Δ, ridΔ*	Cross progeny of C98.18 and C146.3	this study

Supplementary Table 1. *N. crassa* strains used in this study

All inserts were validated by PCR and sequencing; all gene deletions were validated by diagnostic PCR. Primer sequences are provided in Supplementary Table 3. Tester repeats are shown in bold.

Figure	Cross	Cross genotype		Parental strains			
		rid, dim-2	*other*	Female	Male	{N}	Reference
Fig. 1							
b	X1	*rid+/+, dim-2+/+*		FGSC#4200	**T103.3**	24	ref. 18
b	X2	*ridΔ/Δ, dim-2+/+*		T337.1h	**T364.6**	48	this study
b	X3	*ridΔ/Δ, dim-2Δ/Δ*		C52.1	**C57.8**	48	this study
b	X4	*rid+/+, dim-2Δ/Δ*		C96.1	**T382.32**	24	this study
b	X5	*rid+/Δ, dim-2+/+*		FGSC#4200	**T364.6**	24	this study
b	X6	*rid+/+, dim-2+/Δ*		FGSC#2489	**T382.32**	24	this study
c	X7	*rid+/+, dim-2+/+*		FGSC#4200	**T489.1**	48	this study
Fig. 2							
b	X8	*ridΔ/Δ, dim-2+/+*	*dim-5Δ/+, set-7Δ/+*	C146.3	**T364.6**	60	this study
b	X9	*ridΔ/Δ, dim-2+/+*	*dim-5Δ/Δ, set-7Δ/+*	C146.3	**C98.18**	60	this study
c	X10	*ridΔ/Δ, dim-2+/+*	*dim-5Δ/Δ, set-7Δ/Δ*	**XG26.8**	C146.1	60	this study
c	X11	*ridΔ/Δ, dim-2+/+*	*dim-5Δ/+, set-7Δ/Δ*	**XG26.8**	C139.10	60	this study
c	X12	*ridΔ/Δ, dim-2+/+*	*his-3::dim-5+, dim-5Δ/Δ, set-7Δ/Δ*	**XG26.8**	T475.6h	60	this study
c	X13	*ridΔ/Δ, dim-2+/+*	*his-3::dim-5 (Y283F), dim-5Δ/Δ, set-7Δ/Δ*	**XG26.8**	T488.1h	60	this study
d	X14	*rid+/Δ, dim-2+/+*	*dim-5+/+, set-7Δ/+*	XG1B.17	**T364.6**	24	this study
d	X15	*rid+/Δ, dim-2+/+*	*dim-5Δ/+, set-7Δ/+*	S-353	**T364.6**	24	this study
d	X16	*rid+/Δ, dim-2+/+*	*dim-5Δ/Δ, set-7Δ/+*	S-353	**C98.18**	48	this study
e	X17	*rid+/+, dim-2+/+*	*hpoΔ/Δ, set-7Δ/Δ, spo11Δ/+*	**C135.3**	XG3B.11	48	this study
f	X18	*rid+/+, dim-2+/+*	*set-7Δ/+*	**XG3.7**	FGSC#4200	24	this study
f	X19	*rid+/+, dim-2+/+*	*ddb1+/Δ, set-7Δ/+*	**XG3.7**	FGSC#11198	24	this study
f	X20	*rid+/+, dim-2+/+*	*cul4+/Δ, set-7Δ/+*	**XG3.7**	FGSC#12374	24	this study
Fig. 3							
c	X21	*ridΔ/Δ, dim-2+/+*		T337.1h	**T482.3**	60	this study
c	X22	*ridΔ/Δ, dim-2+/+*		T337.1h	**T484.1**	60	this study
c	X23	*ridΔ/Δ, dim-2+/+*		T337.1h	**T487.3**	60	this study
c	X24	*rid+/+, dim-2+/+*		FGSC#4200	**T208.3**	60	ref. 18
c	X25	*rid+/+, dim-2+/+*		FGSC#4200	**T223.4**	90	ref. 18
c	X26	*rid+/+, dim-2+/+*		FGSC#4200	**T221.18**	60	ref. 18
Fig. 4							
b	X27	*rid+/Δ, dim-2Δ/Δ*		FGSC#8594	**T452.1h**	14	this study
b	X28	*ridΔ/Δ, dim-2+/+*		C03.1	**T451.6h**	30	this study
Fig. 5							
d	X29	*rid+/+, dim-2+/+*		**R88.6**	FGSC#4200	10	this study
d	X30	*ridΔ/Δ, dim-2+/+*		**T337.1h**	C02.1	70	this study
d	X31	*ridΔ/Δ, dim-2Δ/Δ*		C50.3	**C52.1**	28	this study
SI Fig. 4							
b	X32	*rid+/+, dim-2+/+*	*set-7Δ/Δ*	XG1B.17	**XG3.7**	24	this study

Supplementary Table 2. Crosses analyzed in this study

Figure, Figure/panel with a corresponding RIP mutation profile; **Cross**, a unique cross identifier used in this study; **Cross genotype**, provided as pairs of maternal/paternal alleles; **Parental strains**, strains are provided in Supplementary Table 1. Strains with the assayed repeats are shown in bold; **{N}**, the total number of progeny spores analyzed (by sequencing) for a given cross/condition.

All sequence alignments are provided in Supplementary Data Set 2. Data for crosses X1, X24, X25 and X26 were published previously (ref. 18) and are provided here for convenience.

Name	Sequence (5' → 3')
CSR1_SeqF	CGGATGTCTTTGGATAGCTCT
CSR1_SeqR	CGGACATGGACTTACGTTACC
CSR1_SeqR2	GGCATCTGGAACCGGACCAT
CSR1_SeqR3	GATGGCTTAGTAGAGCCTGTC
LNK_SeqR1	CAGATAGAGCTTGGAGCTTTGG
NcHis3_F4	GTACATGAACGGGGAAGCAC
NcHis3_F7	GTAGTGGGTTGATGTATATCTGG
NcHis3_R1	ATCCGGCCAAACCCTGACAT
NcHis3_R4	CCAACACCAAGCAGTAATCTCA
NcHis3_R6	GGATTGCACAGACGGTGGA
P236A_Seq1	GGTAGTGGAGGTTAAGCGTTA
P236A_Seq2	GGCCACCATGTTTTGGAAGTTA
P236A_Seq4	CGCCCTAATTAACCCTCACTA
P236A_Seq5	AATACGACTCACTATAGGGCGA
P236A_Seq8	CGGTACCAGCTTTTGTTCCC
P66_Seq1	CCACAACCGGTACGTTATCATT
P66_Seq3	GCTACCGCCATACGAAGTGTT
P66_Seq3	GCTACCGCCATACGAAGTGTT
P66_Seq4	ATGCGACCGAGCATCGTC
P66_Seq9	CCAGCCTTGATGTCGTAGAGT
P66_Seq10	CGCTTCCATGCATTTTGGATG
P66_Seq12	AGCGACAGTGATGACGATGC
P66_Seq17	AGGAAATCCACCGCGGCTT
P66_Seq18	GAGAATGGTGCCAGTGTGG
REG_A_F1	TTCAACCCTTTGGATGGCGATG
REG_A_R1	TTGGCCGGATCAGGGTATGTTT
REG_B_F1	TACCCAGACCAAGGGAAGTGTT
REG_B_R1	TTGCTCACAGGACACACCTAGA
REG_C_F1	ACACCAAGAAGCCAGTGGAAC
REG_C_R1	AAGACCTGAGACGAGAGGGA
RIP2_R1	TGCCCTGTCTATGATATGTGC
RIP2_R2	AGGAAGGCGGACCTGTCA
RIP2_R3	GACCAGTGCAACTCGGTTTG
CUL4_F1	GAGAGAGTACGTGAGGAGGTTT
CUL4_R1	TGTCTATCCAGCTCAGCATCTT
DDB1_F1	ATACCGCGTGATCTCGTCTT
DDB1_R1	AGGAAGCTTGTACCGACGAT
DIM2_F	CCGCCAAGACCAAAAGTAGC
DIM2_R	CTTTGGCTTGCCAAGATTCC
DIM5_F1	GTAAGTCATCCAACTGCATCCC
DIM5_R1	GAGGGCAAACAAGTAGACATCC
HP1_F1	CAAGTACACAATTCCAGAGCCC
HP1_R1	AATGTTATCAGCCGAGACTCCT
MUS52_F	CGAACACCACTTGGCGATAA
MUS52_R	GGCCTGTCTCTCCCATGTTC
RID_RTF	CCCATTTCCCTCACCAACTCAT
RID_RTR	GTGGGTGGAAATGCGGTTATTG
SET7_F1	GCTCACCTTTGTTCCTCATCTC
SET7_R1	CCGTTACTGTCTGATTGGTCAC
SPO11_F	GTGTCGGAAGTCCCATTACCA
SPO11_R	GTGACGCTGTGTAGTCGCTTG

Supplementary Table 3. Primers used in this study