

Supporting Information

Rhoades *et al.* " Identification of *rfk-1*, a meiotic driver undergoing RNA editing in *Neurospora*".

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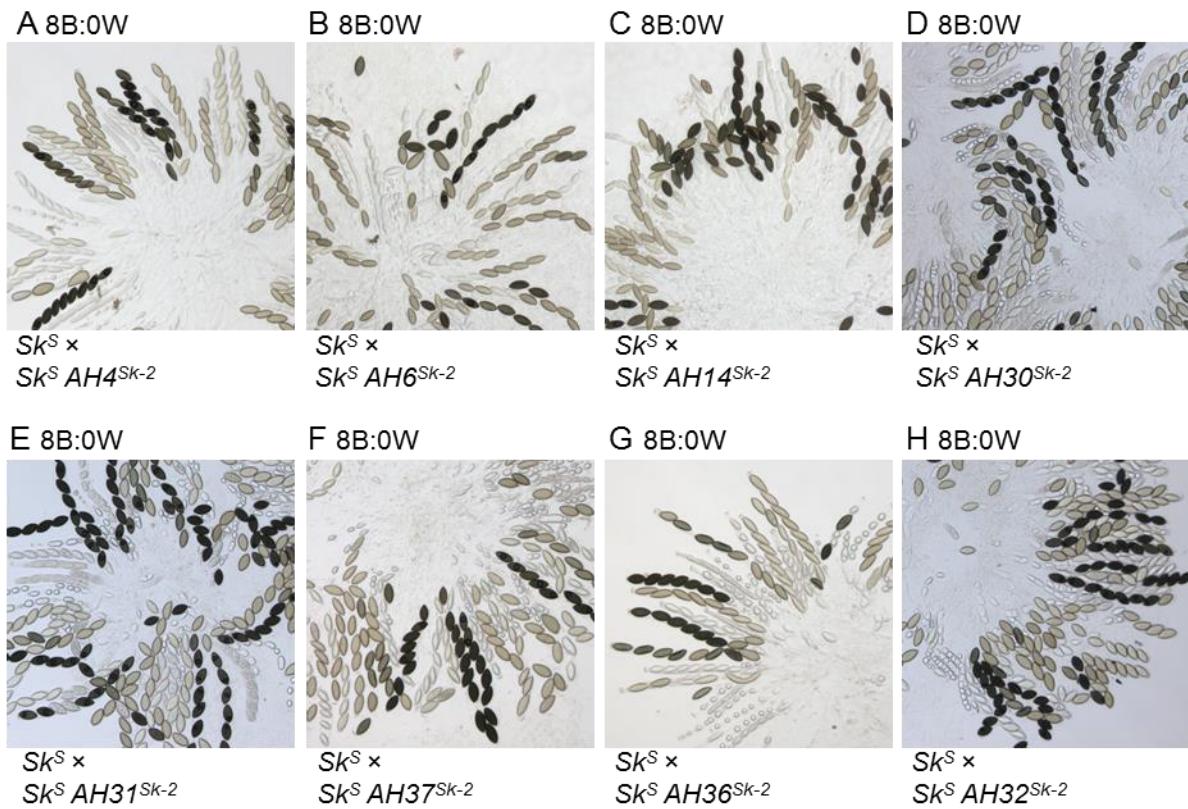
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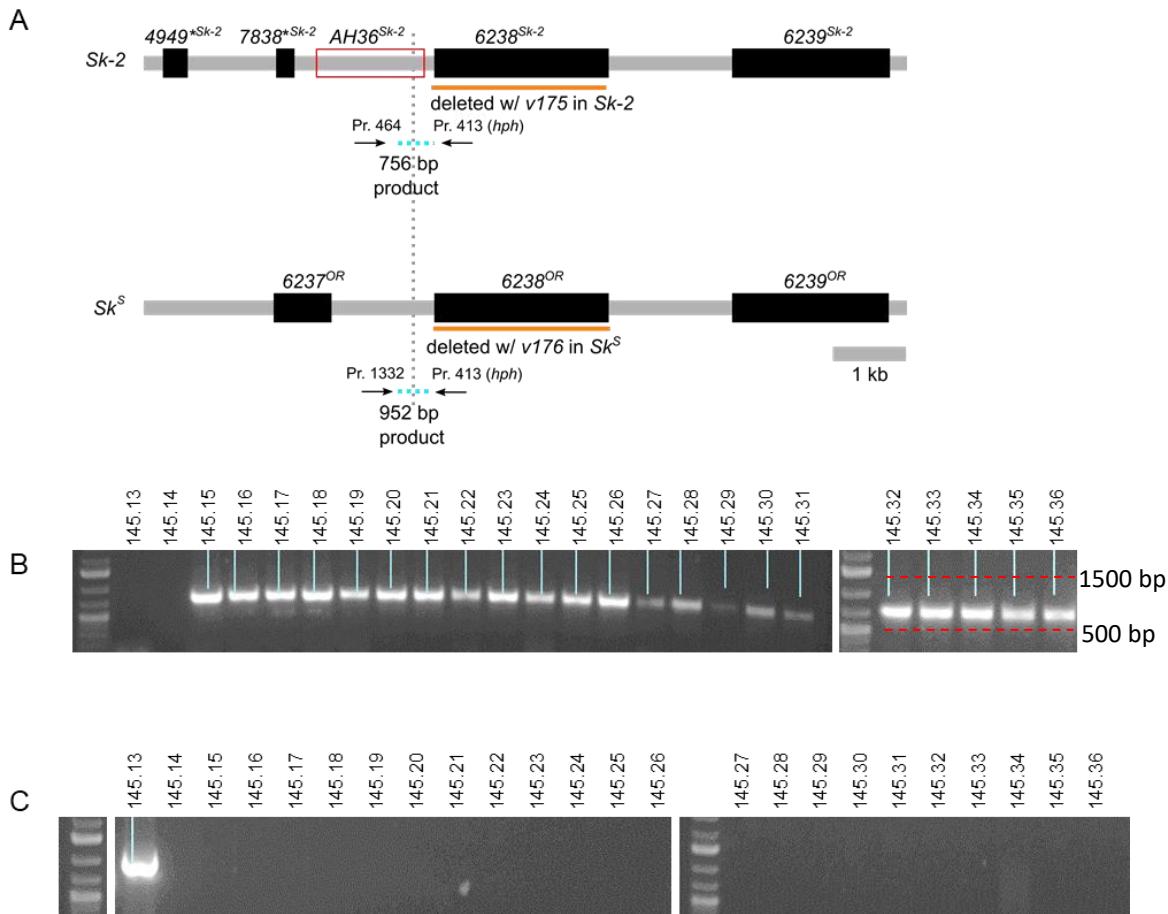
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Figure S1 *v5*-subintervals do not kill ascospores when unpaired in MSUD-proficient crosses



(A–H) Images depict asci from crosses between Sk^S and an Sk^S mating partner. Each Sk^S mating partner carries a different subinterval of *v5*. Crosses are as follows: (A) F2-26 × ISU-3224, (B) F2-26 × ISU-3228, (C) F2-23 × ISU-3243, (D) F2-26 × ISU-3656, (E) F2-26 × ISU-3658, (F) F2-23 × ISU-4269, (G) F2-26 × ISU-4271, and (H) F2-26 × ISU-3660.

Figure S2 Meiotic drive is functional in an *Sk^S* *nclu06238^Δ* × *Sk-2* *nclu06238^Δ* cross



(A) Diagram of the *Sk-2* right border, relative to the corresponding region in *Sk^S*. Gene *nclu06238* was deleted from *Sk-2* and *Sk^S* with vectors *v175* and *v176*, respectively. (B) Genomic DNA was isolated from progeny of ISU-4559 (*Sk^S* *nclu06238^Δ*) and ISU-4561 (*Sk-2* *nclu06238^Δ*). The genomic DNA was then used as the template with primers 413 and 464. These primers amplify a 756 bp product from *Sk-2* genotypes only. (C) Similar to panel B, except that the genomic DNA was used as the template with primers 413 and 1332. These primers amplify a 952 bp product from *Sk^S* genotypes only. The results indicate that strain 145.13 is of the *Sk^S* genotype, strain 145.14 is of an unknown genotype (no products with both primer sets), and 145.15 through 145.36 are of the *Sk-2* genotype. In addition, twelve other progeny were examined by similar methods with similar results (all twelve progeny were *Sk-2*; data not shown). Overall, we found that 34 out of 35 progeny from ISU-4559 × ISU-4561 were of the *Sk-2* genotype, indicating that *nclu06238* is not required for meiotic drive by *Sk-2*.

Primers

- 413 5' CGCTACTGCTACAAGTGGGGCTGA 3'
- 464 5' GGCCCTCCAAGACAATCCCACT 3'
- 1332 5' CCGCGAATGGTTAACTGCACGGC 3'

Figure S3 Alignment of AH36 sequences from *N. crassa* F2-19 and *N. intermedia* 7426

F2-19 7426	CATTGATACCGAGTCTTCCGTTCTAAGGTGGAGTGAGGATATGATCCGGCACGTCGAAGGAGGAACT CATTGATACCGAGTCTTCCGTTCTAAGGTGGAGTGAGGATATGATCCGGCACGTCGAAGGAGGAACT	70
F2-19 7426	ATGGTAAAATAAGTCAGTCATCATCAAGGAAAAGAACAGTATTAGGTGCCTCAAGTTACCTACCTTCTCC ATGGTAAAATAAGTCAGTCATCATCAAGGAAAAGAACAGTATTAGGTGCCTCAAGTTACCTACCTTCTCC	140
F2-19 7426	GACGGGGGCCCTGCCTCCCTAGTTCTCTCATGAAGCTGGAAGTTATATCCTAACCCACTACTGACAAA GACGGGGGCCCTGCCTCCCTAGTTCTCTCATGAAGCTGGAAGTTATATCCTAACCCACTACTGACAAA	210
F2-19 7426	ACAAAGCAAGAGCTGCTGCCCAACTATAGACAGCACGCTTCCACCTCAGTTGGGCACCTAGAAAGC ACAAAGCAAGAGCTGCTGCCCAACTATAGACAGCACGCTTCCACCTCAGTTGGGCACCTAGAAAGC	280
F2-19 7426	TATAAGATCCCTTCCCCCGGCCAACCTCTCCTCAGAATTCTTTCTCCAACATTGTAAGAAC TATAAGATCCCTTCCCCCGGCCAACCTCTCCTCAGAATTCTTTCTCCAACATTGTAAGAAC	350
F2-19 7426	TTTGTTTTGGAAAATGGCTGCCAACAGGGTTTTT T ACCGCTTTGGCAAACCTCCTCACCATCCC TTTGTTTTGGAAAATGGCTGCCAACAGGGTTTTT C ACCGCTTTGGCAAACCTCCTCACCATCCC	420
F2-19 7426	CATTTGGGTGTTGGTGTATTCAATGCTCTGTTGTTCTCCCCGGTTTGGGTCTGGTAAGTCTCC CATTTGGGTGTTGGTGTATTCAATGCTCTGTTGTTCTCCCCGGTTTGGGTCTGGTAAGTCTCC	490
F2-19 7426	TTCATGTTCCAATTCAATTGTTCTCTCGTCTCCTCATGTTCCAGTTCAATTCAATTGTTGTT TTCATGTTCCAATTCAATTGTTCTCTCGTCTCCTCATGTTCCAGTTCAATTCAATTGTTGTT	560
F2-19 7426	TTTTTCCCTTCTCTCGTCTCCTCATGTTCCAGTTCAATTGTTGTTTCTCTCGTCTCCTCATGTTCCAGTTCAATTGTTGTT TTTTTCCCTTCTCTCGTCTCCTCATGTTCCAGTTCAATTGTTGTTTCTCTCGTCTCCTCATGTTCCAGTTCAATTGTTGTT	630
F2-19 7426	CCTTCATGTTCCAATTCAATTGTTGTTTCTCTCGTCTCCTCATGTTCCAGTTCAATTGTTGTTTCTCTCGTCTCCTCATGTTCCAGTTCAATTGTT CCTTCATGTTCCAATTCAATTGTTGTTTCTCTCGTCTCCTCATGTTCCAGTTCAATTGTTGTTTCTCTCGTCTCCTCATGTTCCAGTTCAATTGTT	700
F2-19 7426	TGTTTTTCCCTTCTCTCGTCTCCTCATGTTCCAATTCAATTGTTGTTTCTCTCGTCTCCTCATGTTCCAGTTCAATTGTTGTTTCTCTCGTCTCCTCATGTTCCAGTTCAATTGTT TGTTTTTCCCTTCTCTCGTCTCCTCATGTTCCAATTCAATTGTTGTTTCTCTCGTCTCCTCATGTTCCAGTTCAATTGTTGTTTCTCTCGTCTCCTCATGTTCCAGTTCAATTGTT	770
F2-19 7426	TCTCCTTCATGTTCCAATTCAATTGTTT TCTCCTTCATGTTCCAATTCAATTGTTT G TTTTTTCCCTTCTCTCGTCTCCTCATGTTCCAATTCAATTGTTTCTCTCGTCTCCTCATGTTCCAATTCAATTGTTT	796

F2-19 7426	-----TTTTGTCCTTCTCTTCTCGTCTCCTACAGTTACCTTACGGTCCCTCTGTCTTCGC TTTTGTCCTTCTCTTCTCGTCTCCTACAGTTACCTTACGGTCCCTCTGTCTTCGC	862 910
F2-19 7426	 TAACCAGGAACAGGCGCTTACCAACGGCTGCAACACGAGCAGCAGCAGGACCGAACGATGACGA TAACCAGGAACAGGCGCTTACCAACGGCTGCAACACGAGCAGCAGCAGGACCGAACGATGACGA	932 980
F2-19 7426	 ATGGCAGCGGGCAGCAACAGGACAGGGGGTTGTGGTTGGCACCCACCGCCCCCTCCAGACGTGGAGATG ATGGCAGCGGGCAGCAACAGGACAGGGGGTTGTGGTTGGCACCCACCGCCCCCTCCAGACGTGGAGATG	1002 1050
F2-19 7426	 GCCCTCCAAGACAATCCCACTGCCGCCCGCAGAGCCGGCTGACCTGACCACCCAGCGCCGTAGTGGC GCCCTCCAAGACAATCCCACTGCCGCCCGCAGAGCCGGCTGACCTGACCACCCAGCGCCGTAGTGGC	1072 1120
F2-19 7426	 GGCGCTGGTGGCGACGAGTAGTCAATGCTATTCCAGATTATGAAATGTATCGTGACAGTTGCACA GGCGCTGGTGGCGACGAGTAGTCAATGCTATTCCAGATTATGAAATGTATCGTGACAGTTGCACA	1142 1190
F2-19 7426	 CCAGTGCCTACCCGGCGTCCACTTCTCGTGACCGCAGCCAATGCGGTACGCAGGGTTGTAATTCCA CCAGTGCCTACCCGGCGTCCACTTCTCGTGACCGCAGCCAATGCGGTACGCAGGGTTGTAATTCCA	1212 1260
F2-19 7426	 CGTGAGCATTCCCCACCTTCTCGGGACCGACTTCCGTATCAACCCCAAATTATCGGACTGACCGTC CGTGAGCATTCCCCACCTTCTCGGGACCGACTTCCGTATCAACCCCAAATTATCGGACTGACCGTC	1282 1330
F2-19 7426	 CGAATCAAGGCGAACCGAGAGGACACAGACAAGGCCCACGTCCGCCATCAGCATTCCCAGCTGGCGACCC CGAATCAAGGCGAACCGAGAGGACACAGACAAGGCCCACGTCCGCCATCAGCATTCCCAGCTGGCGACCC	1352 1400
F2-19 7426	 GCACCGCCGCAACTCCCACCTTACCTCAACACCCAGAACGGGATCGGTACATCGACAGCAGCATCATCA GCACCGCCGCAACTCCCACCTTACCTCAACACCCAGAACGGGATCGGTACATCGACAGCAGCATCATCA	1422 1470
F2-19 7426	TCATCAATATCACCAACCTCCACTTGGCGCGCACTTGGGGAAAACGTCCCGCTACACCGT TCATCAATATCACCAACCTCCACTTGGCGCGCACTTGGGGAAAACGTCCCGCTACACCGT	1481 1529

The sequences of *AH36* intervals from strain F2-19 and FGSC 7426 are shown. The predicted location of 1) the *rfk-1* start site is marked with a bent arrow; 2) the four exonic sequences are marked with blue rectangles (■); and 3) the two stop codons are marked with red rectangles (■). The predicted transcription start and stop locations were estimated from RNA sequencing data (*i.e.*, they are the positions where coverage fell below 5% of the most covered *AH36* position).

Figure S4 *N. crassa AH36* sequence and an *rfk-1* gene model

>*AH36*^{F2-19}

CATTGATACCGAGTCTTCCGTTCTTAAGGGTGGAGTGAGGATATGATCCGGCACGTCGAAGGAGGAAC
 ATGGTAAATAAGTCAGTCATCAAGGAAAAGAACAGTATTAGGTGCCTCAAGTTACCTACCTCTCC
 GACGGGGGCCCTGCCTCCCTAGTTCTCATGAAGCTGGAAGTTATATCCTAACCCACTACTGACAAA
 ACAAAAGCAAGAGCTGCTGCCCAACTATAGACAGCACGCTTTCCACCTCAGTTGGGGCACCTAGAAAGC
 TATAAGATCCCTCTTCCCCGGCCAACCTCTCCTCAGAATTCTTTCTCCAACATTGTTAAGAAC
TTTGTGTTGGAAA**ATG**GCCTGCCACAGGGTTTTACCGCTTTGGCAAACCTCCTCACCATCCC
 CATTGGGTGTTGGTATTCAATGCTCTGTTGTCTTCCGGTTGGGTCTGGTAAGTCTCC
TTCATGTTCAATTCTTGTCTTCTCTCGTCTCCTCATGTTCCAATTCTTGT
TTTTTCTCTCTCGTCTCCTCATGTTCCAGTCATTTGTTTCTCTCTCGTCTCCTCATGTTCCAGTC
CCTCATGTTCCAATTCTTGTCTTCTCTCGTCTCCTCATGTTCCAATTCTTGTCTTCTCTCGTCT
TGTTTCTCTCTCGTCTCCTCATGTTCCAATTCTTGTCTTCTCTCGTCTCCTTACAGTTACCTTACCT
CTCGGTCTCTGTCTCGTAACCAGGAACAGGCGCTTACCAACAGGCTGCAACACGAGCAGCAGC
AGCAGGACCGAACGATGACGAATGGCAGCGCAGCAACAGGACAGGGGGTGTGGTTGGCACCCACC
GCCCCCTCCAGACGTGGAGATGGCCCTCCAAGACAATCCCACTGCCGCCCCCGAGAGCCGGCTGACCTC
GACCACCCAGCGCCG**TAG**TGGCGCGCTGGGTGGCCACGAGTAGGTCAATGCTATTCCAGATTATGAA
ATGTATCGTGCACAGTTGCACACCAGTGCCTACCCGGCGCCACTTCTCGTGACCGCAGCCAATGCGG
TCACGCAGGGTTG**TAATTCCACGTGAGCATTCCCCACCTCTCGGGACCGACTCCGTATCAACCCC
AAATTATCGGACTGACCGTCCGAATCAAGGCGAACCGAGAGGACACAGACAAGGCCACGTCCGCCAT
CAGCATTCCCAGCTGGCGACCGCACGCCAACCTTACCTCAACACCAGAACATCGGAATCGG
TACATCGACAGCAGCATCATCATCAATATCACCACTCCACTGGCGCGACTTGCGGAAACGTCC
CGCTACACCGT**

>*rfk-1*^{F2-19} transcript

AACTTGTGTTGGAAA**ATG**GCCTGCCACAGGGTTTTACCGCTTTGGCAAACCTCCTCACCAT
 CCCCATTGGGTGTTGGTGTATTCAATGCTCTGTTGTCTTCCCGGTTGGGTCTGGCGCTTA
 CCACCACGGCTGCAACACGAGCAGCAGCAGGACCGAACGATGGCAGCAGCAACAGG
 ACAGGCGGGTTGTGGTTGGCACCCACCGCCCCCTCCAGACGTGGAGATGGCCCTCCAAGACAATCCCAC
 TGCCGCCCCCGAGAGCCGGCTGACCTCGACCACCGAGCGCG**TAG**TGGCGCGCTGGGTGGCCACGAT
 GCCTACCCGGCGTCCACTTCTCGTGACCGCAGCCAATCGGTACCGCAGGGTTG**TAATTCCACCGA**
 ACCGAGAGGACACAGACAAGGCCACGTCCGCCATCAGCATTCCCAGCTGGCGACCGCACCGCCGAAC
 TCCCACTTACCTAACACCAGAACCGGAATCGGTACATCGACAGCAGCAT

>*rfk-1*^{F2-19} protein (if the TAG stop codon is used)

MACPTGFFTALFGKLTIPIWVLVFVNALFVPRFWVRLPPRLQHEQQQQDRNDDEWQRQQQDRRVVV
 WHPPPPDVEMALQDNPTAAPAEPADLDHPAP*

>*rfk-1*^{F2-19} protein (if the TAG stop codon is edited to TIG)

MACPTGFFTALFGKLTIPIWVLVFVNALFVPRFWVRLPPRLQHEQQQQDRNDDEWQRQQQDRRVVV
 WHPPPPDVEMALQDNPTAAPAEPADLDHPAPWWRRWVADDAYPAVHFCVTAANAVTQGL*

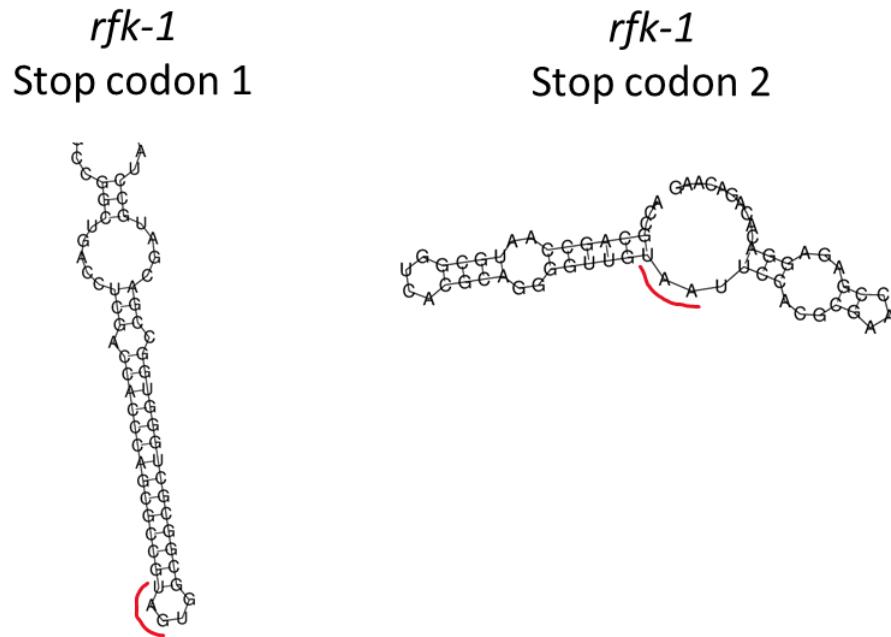
Figure S5 RNA sequencing suggests that *rfk-1* transcripts are subject to RNA editing

<i>rfk-1</i> coding	
SRR7700963.16865053	ACCTCGACCACCCAGCGCCGTAGTGGCGCGCTGGGTGCCGACGA
SRR7700964.14211649	ACCTCGACCACCCAGCGCCGTAGTGGCGCGCTGGGTGCCGACGA
SRR7700963.10318102	ACCTCGACCACCCAGCGCCGTAGTGGCGCGCTGGGTGCCGACGA
SRR7700963.18013983	ACCTCGACCACCCAGCGCCGTAGTGGCGCGCTGGGTGCCGACGA
SRR7700964.18775380	ACCTCGACCACCCAGCGCCGTAGTGGCGCGCTGGGTGCCGACGA
SRR7700964.3833142	ACCTCGACCACCCAGCGCCGTAGTGGCGCGCTGGGTGCCGACGA
SRR7700964.3074722	ACCTCGACCACCCAGCGCCGTAGTGGCGCGCTGGGTGCCGACGA
SRR7700963.9874563	...CTCGACCACCCAGCGCCGTAGTGGCGCGCTGGGTGCCGACGA
SRR7700963.1181334CACCCAGCGCCGTAGTGGCGCGCTGGGTGCCGACGA
SRR7700964.16733332	ACCTCGACCACCCAGCGCCGTGGTGGCGGC
SRR7700964.5015165	ACCTCGACCACCCAGCGCCGTGGTGGCGCG
SRR7700963.4820833	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGG
SRR7700963.662554	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCG
SRR7700964.15753914	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700964.3596574	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.3780935	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.6730097	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.8314199	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.13888301	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700964.17623747	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.3795705	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.4692247	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.6925019	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700964.1825517	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700964.18301725	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.3939697	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.12197519	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.1654316	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700964.7818876	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.1482347	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.10644381	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.11324151	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.16396959	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700964.6407899	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.15097183	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.5818053	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.14783005	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.18225087	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700964.7654302	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700964.10425414	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.479699	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.1544396	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.2781496	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.5377885	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.7274183	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.9560480	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.13712567	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.14238711	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.14738056	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700964.8205190	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.379550	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.3457227	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.8941899	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.10653371	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.12683292	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.12902666	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700963.17269364	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700964.16240381	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA
SRR7700964.19352427	ACCTCGACCACCCAGCGCCGTGGTGGCGCGCTGGGTGGCCGACGA

SRR7700964.9294663	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.8052943	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.9008894	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.2076697	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.6630801	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.2933250	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.7782888	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.12120699	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.14405616	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.16319610	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.16797412	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.13250297	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.13409608	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.17528942	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.18066083	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.20492995	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.764481	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.13762049	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.11771506	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.11448950	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.18741171	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.3636521	ACCTCGAGCAGCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.1727758	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.15546968	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.1464720	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.13894582	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.6360242	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.10917553	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.11679577	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.12517142	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.17070534	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.18620507	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.1990623	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.5068722	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.4375738	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.19029627	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.6632902	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.9565909	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.10528016	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.21106932	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.3969824	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.16869670	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.19383062	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.5671306	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.15638118	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.6841324	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.8500708	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.17050453	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700963.6182260	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA
SRR7700964.2319191	ACCTCGACCACCCAGCGCCGTGGTGGCGGCCTGGGTGCCGACGA

Partial sequences of RNA reads from *N. intermedia* *Sk^S × Sk-2* perithecia that aligned to the “TAG” region of *rfk-1* are shown. Most reads have the sequence “TGG” instead of “TAG”, suggesting that the “TAG” stop codon of *rfk-1* is subject to A-to-I RNA editing.

Figure S6 Secondary structures surrounding first and second stop codons of *rfk-1*



Segments of sixty-three bases from the putative *rfk-1* mRNA sequence were analyzed on the RNAfold Server, a tool provided by the ViennaRNA Web Services Group at the University of Vienna (<http://rna.tbi.univie.ac.at/>). The location of the putative *rfk-1* stop codons are indicated with red curves. The potential secondary structure surrounding stop codon 1 places it within a hairpin loop (common for A-to-I editing sites in *N. crassa*). The potential secondary structure surrounding stop codon 2 is shown as a reference.

Table S1 Primers for DJ-PCR-based construction of deletion vectors

Nineteen deletion vectors were constructed by double joint (DJ)-PCR (Yu *et al.* 2004; Hammond *et al.* 2011). The table below lists the forward and reverse primer sequences (5' to 3') for the left recombination flank (L), the right recombination flank (R), and the nested amplification of each completed vector (N).

For each vector, the left and right DNA flanks were amplified from genomic DNA of the transformation host, which is also indicated in the table.

The center fragment for each vector is listed next to the name of each vector in the left-most column. Center fragments were either *hph*, *nat1*, or *hph-ccg-1(P)*. See Table S2 for more information on the center fragments.

Vector (<i>center</i>) Primer numbers	Name of transformation host Primer sequences	Pr.pur- pose
<i>v3 (hph)</i> 73 74 75 76 77 78	<i>Transformation host: ISU-3223</i> CAAGACCCAGAACACGCCAAC AAAAAATGCTCCTTCAATATCAGTTCTCGCTCCTTCCGCAAATT GAGTAGATGCCGACCGGGAAACCAGTTGGTGGATACTCGGTGCAGGTA CGACACCTCGAATAACGCCCTCTC CCGGAAACGTCAGCAAACACGTA GCGCCAGCTCCTCTACACTCTCC	L L R R N N
<i>v4 (hph)</i> 79 80 81 82 83 84	<i>Transformation host: ISU-3223</i> CCAAGCCAAACTCAAGGGAAATCG AAAAAATGCTCCTTCAATATCAGTTAATGGCGGTGATCTCGACTGCT GAGTAGATGCCGACCGGGAAACCAGTTGCCAGACTCAGCTTGACATTGAC TCACCTTGGCCCTGGAGTACCTG CAAACGGGACGCAACCTCTATGA CCAAGCGGGTTCCAGATAAGACG	L L R R N N
<i>v5 (hph)</i> 85 86 87 88 89 90	<i>Transformation host: ISU-3223</i> CACCATGTAGTCGGAGCGGAAGA AAAAAATGCTCCTTCAATATCAGTTCATCTTGACGGGCAGAACTGAA GAGTAGATGCCGACCGGGAAACCAGTTGCTAACCGAACAGGGCCTTAC CATCGAAAGGGAGAGGCACTTCG GCCTTCTTCTTCACACCGGAGGT ACAGGATCTGGTCATCCGCTT	L L R R N N
<i>v31 (hph)</i> 85 86 167 168 89 170	<i>Transformation host: ISU-3223</i> CACCATGTAGTCGGAGCGGAAGA AAAAAATGCTCCTTCAATATCAGTTCATCTTGACGGGCAGAACTGAA GAGTAGATGCCGACCGGGAAACCAGTTATTGAGGTGAGGACAAGCGATGA CATACGGCCCATTGTTACCGCACT GCCTTCTTCTTCACACCGGAGGT CAACGAAGCAGGCTCCACACAG	L L R R N N
<i>v32 (hph)</i> 85 86 173 174 89 176	<i>Transformation host: ISU-3223</i> CACCATGTAGTCGGAGCGGAAGA AAAAAATGCTCCTTCAATATCAGTTCATCTTGACGGGCAGAACTGAA GAGTAGATGCCGACCGGGAAACCAGTTGCTCGTGAATCGTGTACCTT AATTCCCGTGTACTTCGCTGTG GCCTTCTTCTTCACACCGGAGGT CGGTTGATCTGCCGGTTGAAGA	L L R R N N
<i>v33 (hph)</i> 85 86 3 4 89 6	<i>Transformation host: ISU-3223</i> CACCATGTAGTCGGAGCGGAAGA AAAAAATGCTCCTTCAATATCAGTTCATCTTGACGGGCAGAACTGAA GAGTAGATGCCGACCGGGAAACCAGTTGCTGGAGTGAAGTGGACAAGCTG GTGGTAAGCGCCTGTTCCGTTAG GCCTTCTTCTTCACACCGGAGGT TGCGGCCTGTTACGAAATCAA	L L R R N N

v34 (hph)		Transformation host: ISU-3223	
85	CACCATGTAGTCGGAGCGGAAGA	L	
86	AAAAAATGCTCCTCAATATCAGTTCATCTGACGGGCAGAACTGAA	L	
9	GAGTAGATGCCGACCGGGAACCCAGTCTGATTGCCGACACCTCTGT	R	
4	GTGGTAAGGCCCTTCTCACACGGAGGT	R	
89	GCCTCCTCTTCACACGGAGGT	N	
11	CGAAAGACAGAGAGGACCGAGAGGA	N	
v35 (hph)		Transformation host: ISU-3223	
1	TCGGAAGGATTGCTGACTTGTGTGT	L	
2	CCAAAAAATGCTCCTCAATATCAGTTAGTTGGTAGCTGGCGCGAAAG	L	
87	GAGTAGATGCCGACCGGGAACCCAGTTGCTAACCAAGAACAGGCCTTACC	R	
88	CATCGAAAGGGAGAGGCACCTCG	R	
5	GCGCAGACGAACATCAAGGAGAA	N	
90	ACAGGATCTGGTCATCCGCTTC	N	
v37 (hph)		Transformation host: P15-53	
7	GGCAGATAACAACCGACGACCAAA	L	
8	CCAAAAAATGCTCCTCAATATCAGTTCCGTTCGCTTATGATGTTAATGATG	L	
87	GAGTAGATGCCGACCGGGAACCCAGTTGCTAACCAAGAACAGGCCTTACC	R	
88	CATCGAAAGGGAGAGGCACCTCG	R	
10	CACGTAGGAAAGGAGAGGTGAAGGT	N	
90	ACAGGATCTGGTCATCCGCTTC	N	
v38 (hph)		Transformation host: P15-53	
309	ACGCCAAAAGGTGTAGGGGGATT	L	
310	CCAAAAAATGCTCCTCAATATCAGTTGACCGAACACCGGAATGACCT	L	
87	GAGTAGATGCCGACCGGGAACCCAGTTGCTAACCAAGAACAGGCCTTACC	R	
88	CATCGAAAGGGAGAGGCACCTCG	R	
311	AGGTCCGCAACTATTGTCCGTT	N	
90	ACAGGATCTGGTCATCCGCTTC	N	
v39 (hph)		Transformation host: P15-53	
309	ACGCCAAAAGGTGTAGGGGGATT	L	
312	CCAAAAAATGCTCCTCAATATCAGTTGACCGAACACCGGAATGACCT	L	
87	GAGTAGATGCCGACCGGGAACCCAGTTGCTAACCAAGAACAGGCCTTACC	R	
88	CATCGAAAGGGAGAGGCACCTCG	R	
311	AGGTCCGCAACTATTGTCCGTT	N	
90	ACAGGATCTGGTCATCCGCTTC	N	
v40 (hph)		Transformation host: P15-53	
309	ACGCCAAAAGGTGTAGGGGGATT	L	
310	CCAAAAAATGCTCCTCAATATCAGTTGACCGAACACCGGAATGACCT	L	
313	GAGTAGATGCCGACCGGGAACCCAGTTGCCAACCTCTCCTCAGAAT	R	
88	CATCGAAAGGGAGAGGCACCTCG	R	
311	AGGTCCGCAACTATTGTCCGTT	N	
90	ACAGGATCTGGTCATCCGCTTC	N	
v140 (hph)		Transformation host: P15-53	
1303	AACCAAGAACAGGCCCTTACAC	L	
1304	AAAAAATGCTCCTCAATATCAGTTACGGGTAGCGGGACGTTTC	L	
1305	GAGTAGATGCCGACCGGGAACCCAGTTCAACAAAGCGCGTGTATCTTCG	R	
871	GAACTCGAACCACCTCACGCAA	R	
1306	ACAGGACAGGCCGGTTGTGGTT	N	
872	CATGTCGGCTTGAGGTCGTTC	N	
v150 (hph)		Transformation host: P8-43	
1332	CCCGAATGGTTAACTGCACGGC	L	
1304	AAAAAATGCTCCTCAATATCAGTTACGGGTAGCGGGACGTTTC	L	
1305	GAGTAGATGCCGACCGGGAACCCAGTTCAACAAAGCGCGTGTATCTTCG	R	
1333	GTCGGCATAGGCTGTGGTGGTCG	R	
1334	ATGCCGCTTGATGCACTGGCTG	N	
1335	CGAGAGGGAGAGGCACCTCGCCA	N	
v160 (nat1)		Transformation host: ISU-3222	
10	CACGTAGGAAAGGAGGTTGAAGGT	L	
869	TGAATGCTAAAAGACACCATTCCCACACTCCCTCAGCAAGTAAGCCGGTCACGATCC	L	
870	GCTGGCTGCAATACAAGCGTCCCCACCTAACCAACTAACAAAGCGCGTGTATCTTCG	R	
871	GAACTCGAACCACCTCACGCAA	R	

		AGGTCCGCAACTATTGCCGTT CATGTCGGCTTGAGGTCGTTGC	N N
<i>v175 (hph)</i>		<i>Transformation host: ISU-3222</i> GGAACAGGCGCTTACCA AAAAAATGCTCCTCAATATCAGTTGAAATGTTGATGCCCTCCCTGGAT GAGTAGATGCCGACCGGGAACCGAGTTGGGTTAGGGAGGGCTGCAT TTCCTTCCCGCTCCGTTCG ACAGGACAGGCGGGTTGTGG CCGAATACCGACCCCCGATT	L L R R R N N
<i>v176 (hph)</i>		<i>Transformation host: P8-43</i> TGGCAGGTCAAGGTGATTGC AAAAAATGCTCCTCAATATCAGTTGAAATGTTGATGCCCTCCCTAGAT GAGTAGATGCCGACCGGGAACCGAGTTGGGTTAGGCAGGGCTGGAT TTCCTTCCCGCTCCGTTCG CGGCCGCGAATGGTTAATCTG CCGAATACCGACCCCCGATT	L L R R R N N
<i>v199 [hph-ccg-1(P)]</i>		<i>Transformation host: P15-53</i> CGAAGGACAAGAGGAACGGGAAA GCAGCCTGAATGGCGAATGGACGCGCGGGCAGCAGCTTGCTTGT TTCACAACCCCTCACATCAACCAAAATGGCTGCCACAGGGTT GTCACGGTGTAGCGGGACGTTT GGGGCGGAGAGGAGAAAGATGAGT GGAATTACAACCCCTGCGTGACC	L L R R R N N
<i>v214 [hph-ccg-1(P)-ATGT]</i>		<i>Transformation host: P15-53</i> CGAAGGACAAGAGGAACGGGAAA GCAGCCTGAATGGCGAATGGACGCGCGGGCAGCAGCTTGCTTGT TTCACAACCCCTCACATCAACCAAAATGTGCCTGCCACAGGGTT GTCACGGTGTAGCGGGACGTTT GGGGCGGAGAGGAGAAAGATGAGT GGAATTACAACCCCTGCGTGACC	L L R R R N N
<i>v221 [hph-ccg-1(P)-TAA]</i>		<i>Transformation host: P15-53</i> CGAAGGACAAGAGGAACGGGAAA GCAGCCTGAATGGCGAATGGACGCGCGGGCAGCAGCTTGCTTGT TTCACAACCCCTCACATCAACCAATAAGCTGCCACAGGGTT GTCACGGTGTAGCGGGACGTTT GGGGCGGAGAGGAGAAAGATGAGT GGAATTACAACCCCTGCGTGACC	L L R R R N N

Table S2 Primers for DJ-PCR center products

The forward and reverse primers used to amplify the center fragments for construction of DJ-PCR deletion vectors are described below.

Center Primer number	Name of template Primer sequences
<i>hph</i> 12 13	<i>pTH1256.1</i> (<i>GenBank MH550659</i>) AACTGATATTGAAGGAGCATTGG AACTGGTCCCGGTGGCAT
<i>nat1</i> 297 298	<i>pNR28.12</i> (<i>GenBank MH553564</i>) GAGGGAGTGTGGGAAATGGTGTC GTTGGTAGGTGGAACGCTTGT
<i>hph-ccg-1(P)</i> 550 1555	<i>pTH1117.12</i> (<i>GenBank JF749202</i>) GCGCGTCCATTGCCATTCA TTTGGTTGATGTGAGGGGTTGTGA

Table S3 Primers for cloning *Sk-2* intervals to pTH1256.1

Eight intervals of *Sk-2*^{INS1} were cloned to the *NotI* site of pTH1256.1 (GenBank MH550659), using the primers listed below. These cloning schemes created plasmids pAH4, pAH6, pAH14, pAH30, pAH31, pAH32, pAH36, and pAH37. Each plasmid was then used to transform strain P8-43.

Plasmid name Primer number	Name of transformation host Primer sequences
pAH4 248 249	<i>Transformation host: P8-43</i> AAAAGCGGCCGCAGGGTGGTGGGTGAGGATGT TTTGCGGCCGCGAGCGGAAGTGTGCTTGTGTGA
pAH6 252 253	<i>Transformation host: P8-43</i> AAAAGCGGCCGCATCGCCAACGGGCATTCAAG AAAAGCGGCCGCACCCGCCTACACATGCACCACATC
pAH14 302 314	<i>Transformation host: P8-43</i> AAAAGCGGCCGCTGCATGTGTAGGCGGGTATTGTG AAAAGCGGCCGCGGGCAGGGCAGCAAGTAAG
pAH30 304 251	<i>Transformation host: P8-43</i> AAAAGCGGCCGCGAGGACCAGCTGACGGTAGTAGG AAAAGCGGCCGCGAGGAATAGGACGTGAGGGTGTGG
pAH31 353 251	<i>Transformation host: P8-43</i> TTTGCGGCCGCATTGATACCGAGTCTTCCGTTC AAAAGCGGCCGCGAGGAATAGGACGTGAGGGTGTGG
pAH32 351 251	<i>Transformation host: P8-43</i> AAAAGCGGCCGCAACTCCTCACCCATCCCCATTG AAAAGCGGCCGCGAGGAATAGGACGTGAGGGTGTGG
pAH36 353 639	<i>Transformation host: P8-43</i> TTTGCGGCCGCATTGATACCGAGTCTTCCGTTC AAAAGCGGCCGCGACGGTAGCGGGACGTTC
pAH37 353 640	<i>Transformation host: P8-43</i> TTTGCGGCCGCATTGATACCGAGTCTTCCGTTC AAAAGCGGCCGCGCTCGTGAACCTCCGACCA

Table S4 Primers for amplification of $AH36^{Sk-2}::hph$

The $AH36^{Sk-2}::hph$ allele was amplified from ISU-4344 using the primers 10 and 871. These primers span the $v140^\Delta::hph$ allele in ISU-4344 and produce a PCR product containing $AH36^{Sk-2}$ and hph between recombination flanks suitable for replacing $AH36^\Delta::nat1$ in ISU-4562 with $AH36^{Sk-2}::hph$.

Target Primer number	Template; Name of transformation host Sequence
$AH36^{Sk-2}::hph$	Amplify from ISU-4344; Transformation host: ISU-4562
10	CACGTAGGGAAGGAGGTTGAAGGT
871	GAACTCGAACCACTCCACGCAA

Table S5 Primers for site-directed mutagenesis of *AH36^{Sk-2}*

Site-directed mutagenesis was performed essentially as described for the QuikChange II Site-Directed Mutagenesis Kit (Revision E.01, Agilent Technologies). The *AH36* interval from *Sk-2* was cloned to the *NotI* site of a standard 3 kb bacterial cloning vector with primers 353 and 639 (Table S3). Site-specific mutations were introduced into the resulting plasmid (pNR9.1) by PCR with the primer sets described below. PCR products were digested with *DpnI* and used to transform chemically-competent *E. coli* IgTM 5-alpha cells (Intact Genomics). The mutated-*AH36* intervals were transferred to the *NotI* site of pTH1256.1 and confirmed to be free of undesired mutations by Sanger sequencing. Mutated plasmids were used to transform P8-43.

Primer number	Primer sequence
Change G to A at position 27945 1138 1139	GTTGGAGTGAGGATATAATCCGGCACGTCGAAG CTTCGACGTGCCGGATTATATCCTCACTCCAAC
Change G to A at position 27972 1136 1137	GATGATGACTGACTTATTACTATAGTTCCCTCTCGACGT ACGTCGAAGGAGGAACATAGTAAATAAGTCAGTCATCATC
Change G to A at position 28052 1134 1135	CGACGGGGGCCCTACCTTCCCTTAGTT AACTAAGGGAAGGTAGGGCCCCGTCG
Change G to A at position 28104 1132 1133	CTAACCCACTACTAACAAAACAAGCAAGAGCTGCTGC GCAGCAGCTCTTGCTTTGTTAGTAGTGGTTAG
Change G to A at position 28300 1130 1131	GGGATGGTGAGGAGTTGCTAAAAAGAGCGGTAAAAAAC GTTTTTACCGCTTTAGCAAACCTCACCATCCC
Change G to A at position 28326 1128 1129	GAATACAAACACCAACACTCAAATGGGGATGGTGAG CTCACCATCCCCATTGAGTGTGGTGGTATTGATT

Table S6 Primers for *rfk-1* cDNA analysis

Primer number	Primer sequence
1741	GGCCTGCCCCACAGGGTTTT
1742	CCGATTCCGTATTCTGGTGGAG
1743	GAAAATGGCCTGCCCCACAGG
1744	CGTGGGCCTTGCTGTGTCCT
1745	CTTACCACCAACGGCTGCAA
1746	TGTCTTCGCTAACCAAGGAACA