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

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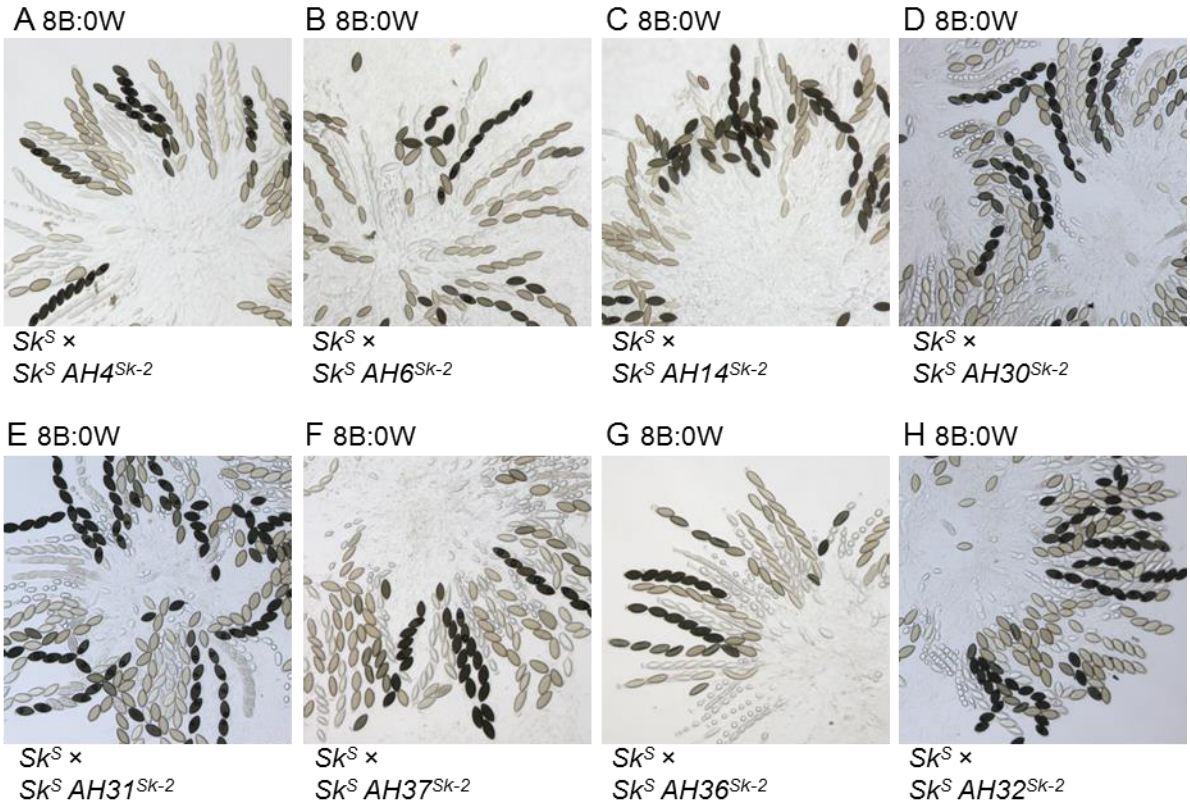
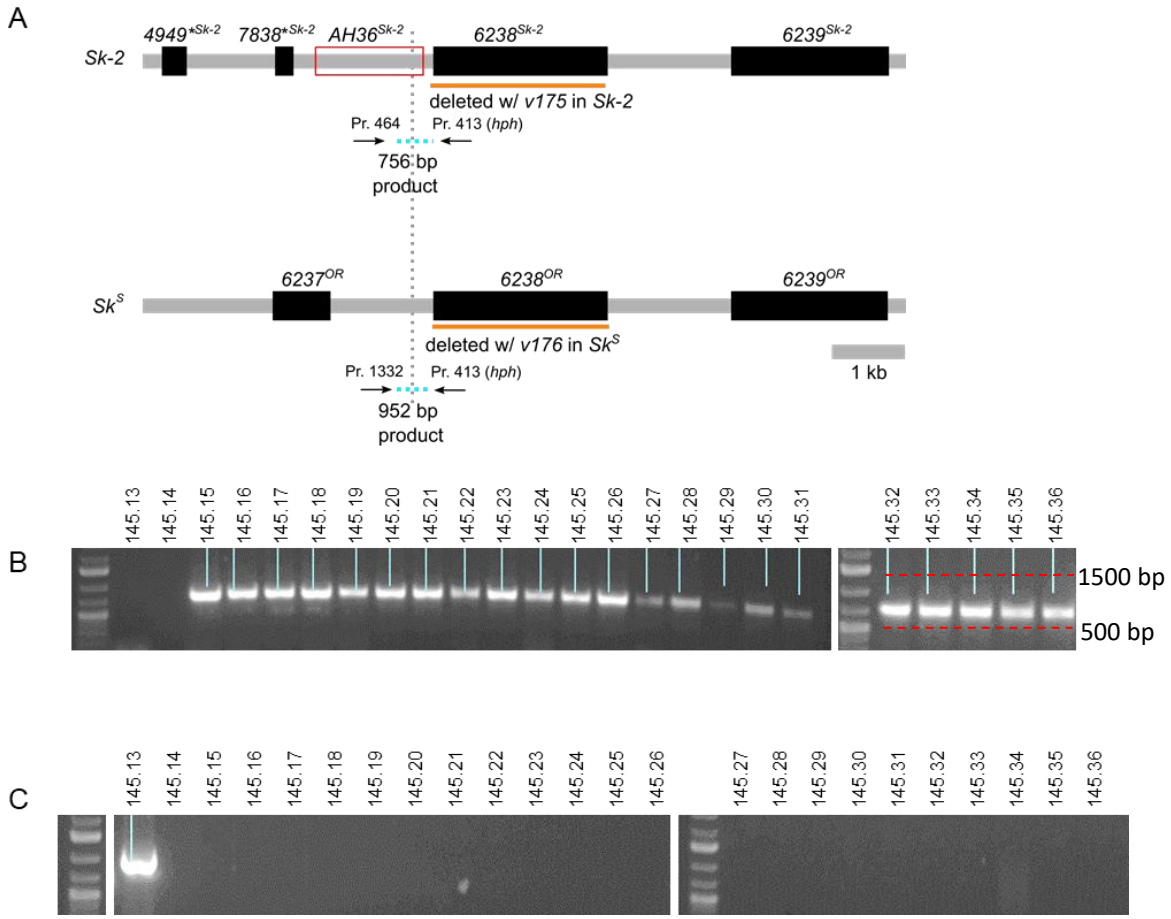
Figure S1 *v5*-subintervals do not kill ascospores when unpaired in MSUD-proficient crosses



(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} *ncu06238*$^{\Delta}$ × *Sk-2 ncu06238*$^{\Delta}$ cross

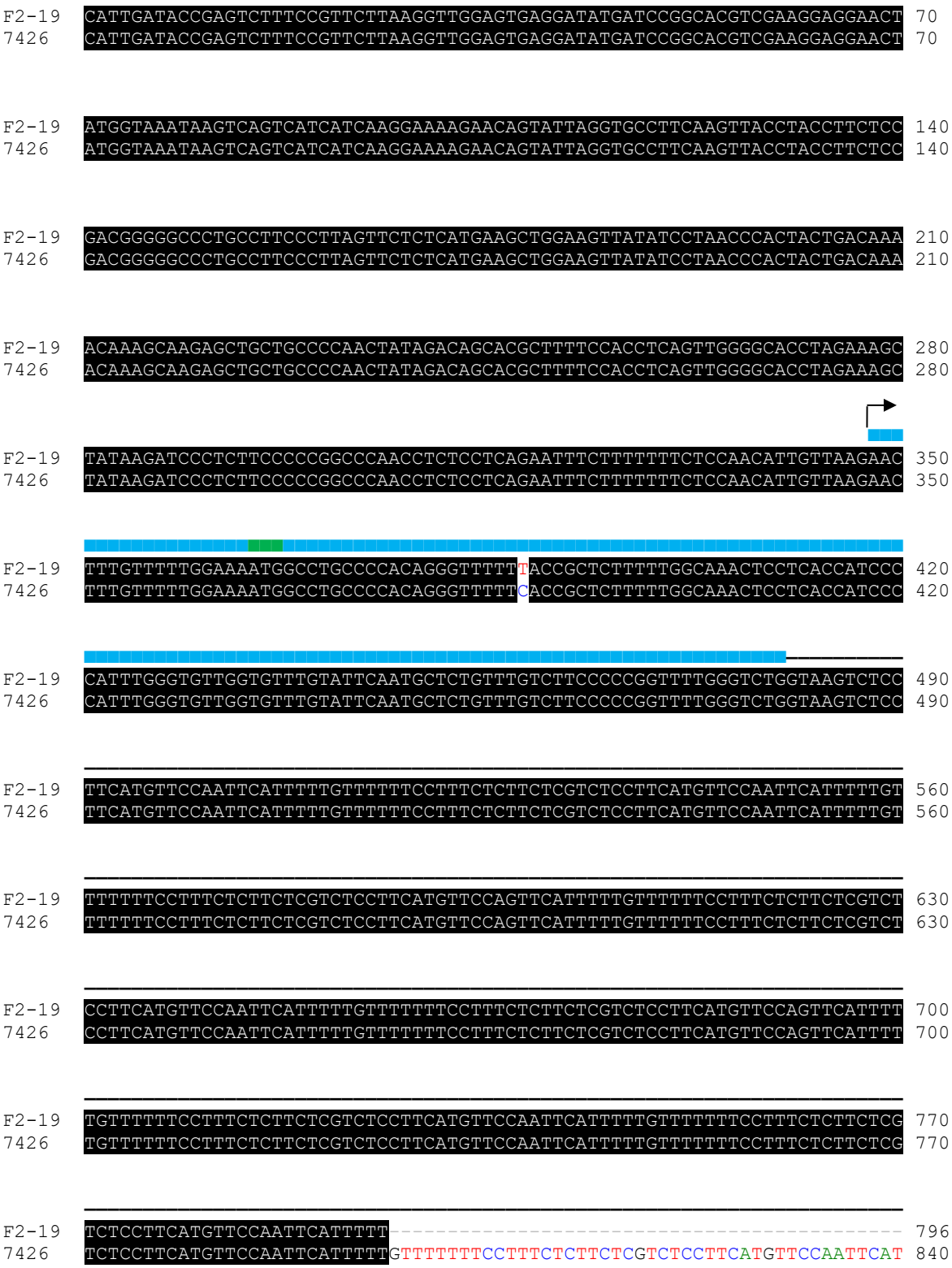


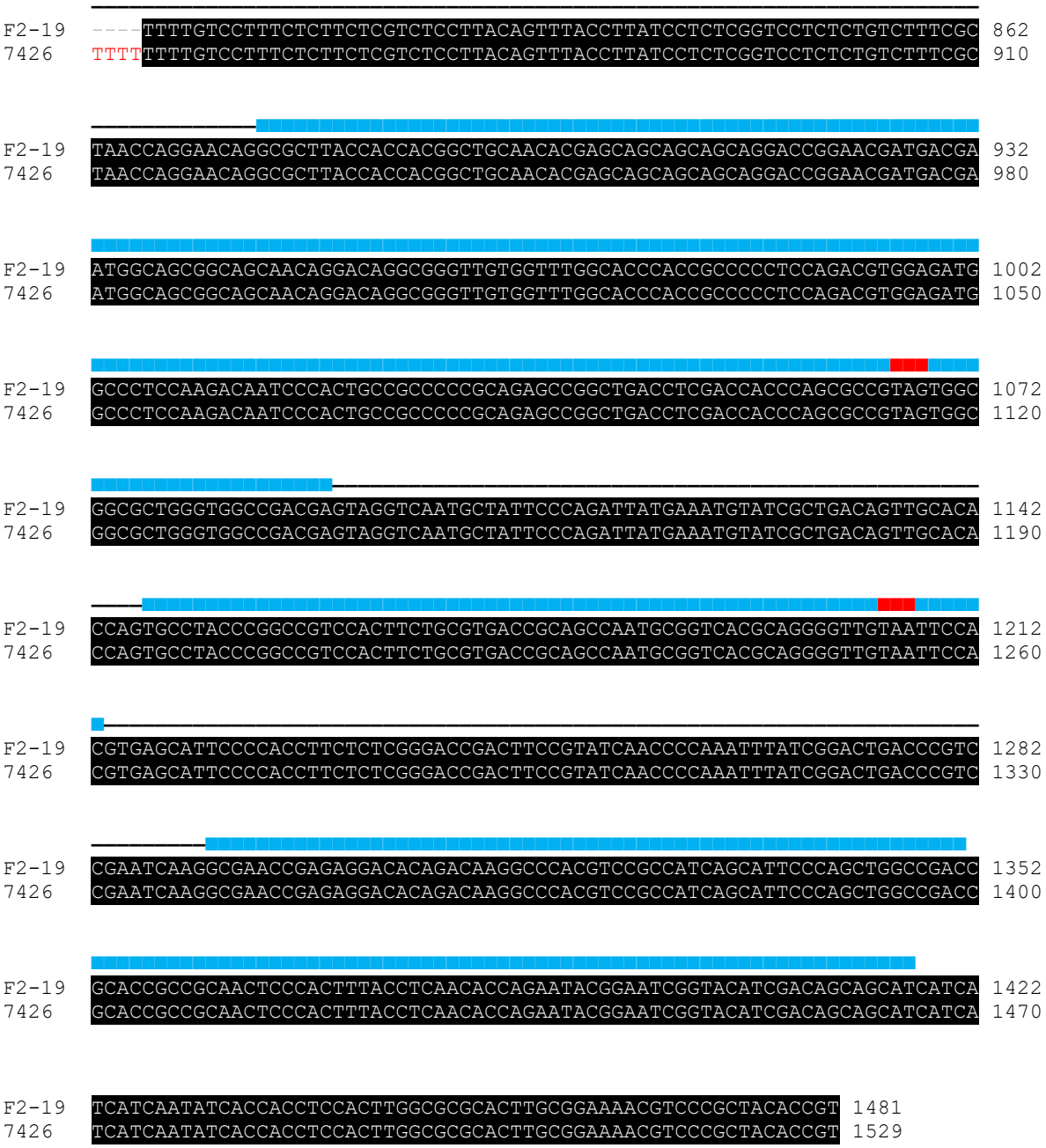
(A) Diagram of the *Sk-2* right border, relative to the corresponding region in *Sk*^{S}. Gene *ncu06238* was deleted from *Sk-2* and *Sk*^{S} with vectors *v175* and *v176*, respectively. (B) Genomic DNA was isolated from progeny of ISU-4559 (*Sk*^{S} *ncu06238*$^{\Delta}$) and ISU-4561 (*Sk-2 ncu06238*$^{\Delta}$). 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 *ncu06238* 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





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*$^{F2\text{-}19}$

CATTGATACCGAGTCTTTCCGTTCTTAAGGTTGGAGTGAGGATATGATCCGGCACGTCGAAGGAGGAACT
ATGGTAAATAAGTCAGTCATCATCAAGGAAAAGAACAGTATTAGGTGCCTTCAAGTTACCTACCTTCTCC
GACGGGGGCCCTGCCTTCCCTTAGTTCTCTCATGAAGCTGGAAGTTATATCCTAACCCACTACTGACAAA
ACAAAGCAAGAGCTGCTGCCCCAACTATAGACAGCACGCTTTTCCACCTCAGTTGGGGCACCTAGAAAGC
TATAAGATCCCTCTTCCCCCGGCCCAACCTCTCCTCAGAATTTCTTTTTTTCTCCAACATTGTTAAGAAC
TTTGTTTTTGGAAAATGGCCTGCCCCACAGGGTTTTTTACCGCTCTTTTTGGCAAACTCCTCACCATCCC
CATTTGGGTGTTGGTGTTTGTATTCAATGCTCTGTTTGTCTTCCCCCGGTTTTGGGTCTGGTAAGTCTCC
TTCATGTTCCAATTCATTTTTGTTTTTTTCCTTTCTCTTCTCGTCTCCTTCATGTTCCAATTCATTTTTGT
TTTTTTCCTTTCTCTTCTCGTCTCCTTCATGTTCCAGTTCATTTTTGTTTTTTTCCTTTCTCTTCTCGTCT
CCTTCATGTTCCAATTCATTTTTGTTTTTTTCCTTTCTCTTCTCGTCTCCTTCATGTTCCAGTTCATTTT
TGTTTTTTCCTTTCTCTTCTCGTCTCCTTCATGTTCCAATTCATTTTTGTTTTTTTCCTTTCTCTTCTCG
TCTCCTTCATGTTCCAATTCATTTTTTTTTGTCCTTTCTCTTCTCGTCTCCTTACAGTTTACCTTATCCT
CTCGGTCCTCTCTGTCTTTCGCTAACCAGGAACAGGCGCTTACCACCACGGCTGCAACACGAGCAGCAGC
AGCAGGACCGGAACGATGACGAATGGCAGCGGCAGCAACAGGACAGGCGGGTTGTGGTTTGGCACCCACC
GCCCCCTCCAGACGTGGAGATGGCCCTCCAAGACAATCCCACTGCCGCCCCCGCAGAGCCGGCTGACCTC
GACCACCCAGCGCCGTAGTGGCGGCGCTGGGTGGCCGACGAGTAGGTCAATGCTATTCCCAGATTATGAA
ATGTATCGCTGACAGTTGCACACCAGTGCCTACCCGGCCGTCCACTTCTGCGTGACCGCAGCCAATGCGG
TCACGCAGGGGTTGTAATTCCACGTGAGCATTCCCCACCTTCTCTCGGGACCGACTTCCGTATCAACCCC
AAATTTATCGGACTGACCCGTCCGAATCAAGGCGAACCGAGAGGACACAGACAAGGCCCACGTCCGCCAT
CAGCATTCCCAGCTGGCCGACCGCACCGCCGCAACTCCCACTTTACCTCAACACCAGAATACGGAATCGG
TACATCGACAGCAGCATCATCATCATCAATATCACCACCTCCACTTGGCGCGCACTTGCGGAAAACGTCC
CGCTACACCGT

>*rfk-1*$^{F2\text{-}19}$ transcript

AACTTTGTTTTTGGAAAATGGCCTGCCCCACAGGGTTTTTTACCGCTCTTTTTGGCAAACTCCTCACCAT
CCCCATTTGGGTGTTGGTGTTTGTATTCAATGCTCTGTTTGTCTTCCCCCGGTTTTGGGTCTGGCGCTTA
CCACCACGGCTGCAACACGAGCAGCAGCAGCAGGACCGGAACGATGACGAATGGCAGCGGCAGCAACAGG
ACAGGCGGGTTGTGGTTTGGCACCCACCGCCCCCTCCAGACGTGGAGATGGCCCTCCAAGACAATCCCAC
TGCCGCCCCCGCAGAGCCGGCTGACCTCGACCACCCAGCGCCGTAGTGGCGGCGCTGGGTGGCCGACGAT
GCCTACCCGGCCGTCCACTTCTGCGTGACCGCAGCCAATGCGGTCACGCAGGGGTTGTAATTCCACGCGA
ACCGAGAGGACACAGACAAGGCCCACGTCCGCCATCAGCATTCCCAGCTGGCCGACCGCACCGCCGCAAC
TCCCACTTTACCTCAACACCAGAATACGGAATCGGTACATCGACAGCAGCAT

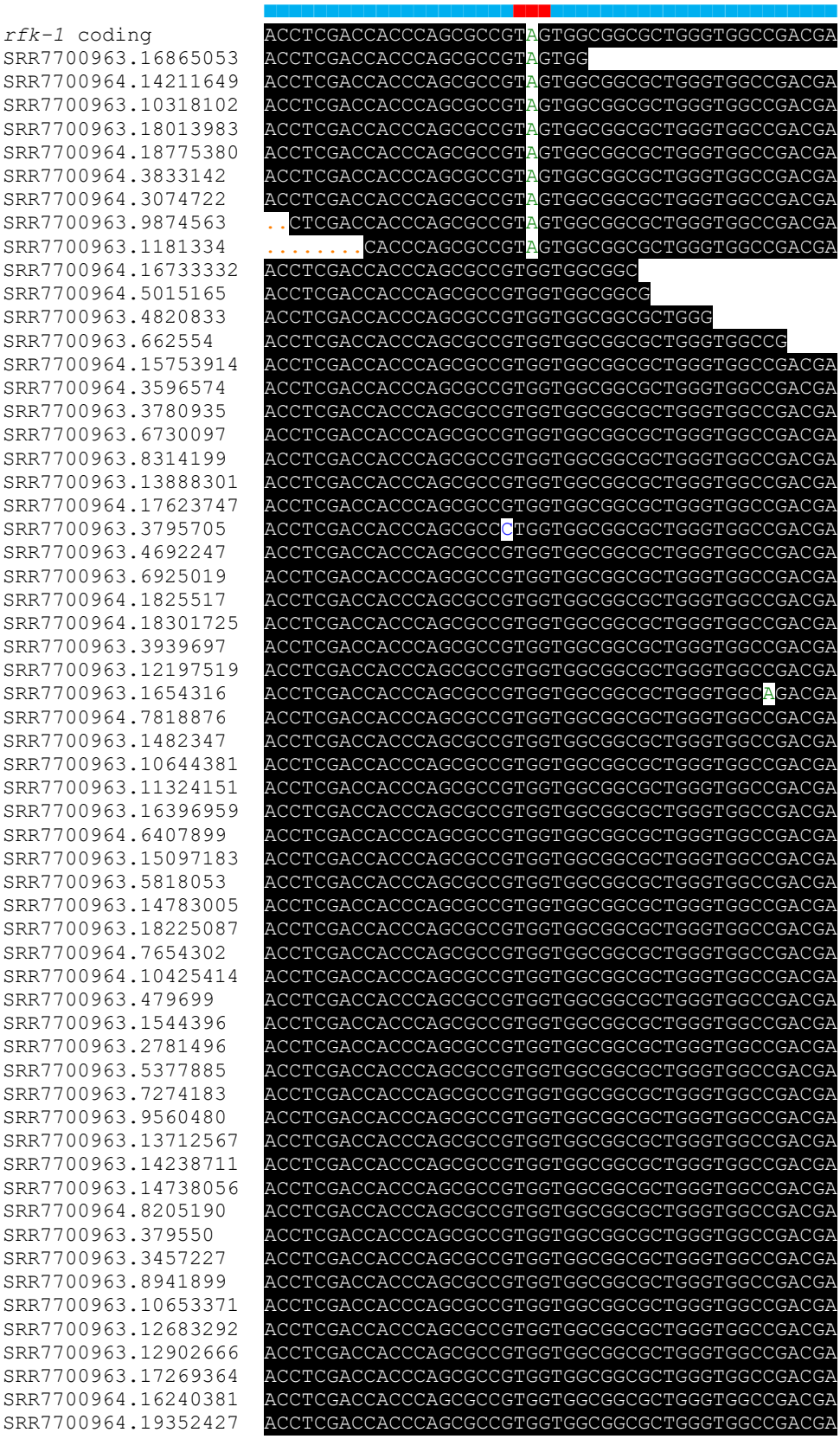
>*rfk-1*$^{F2\text{-}19}$ protein (if the TAG stop codon is used)

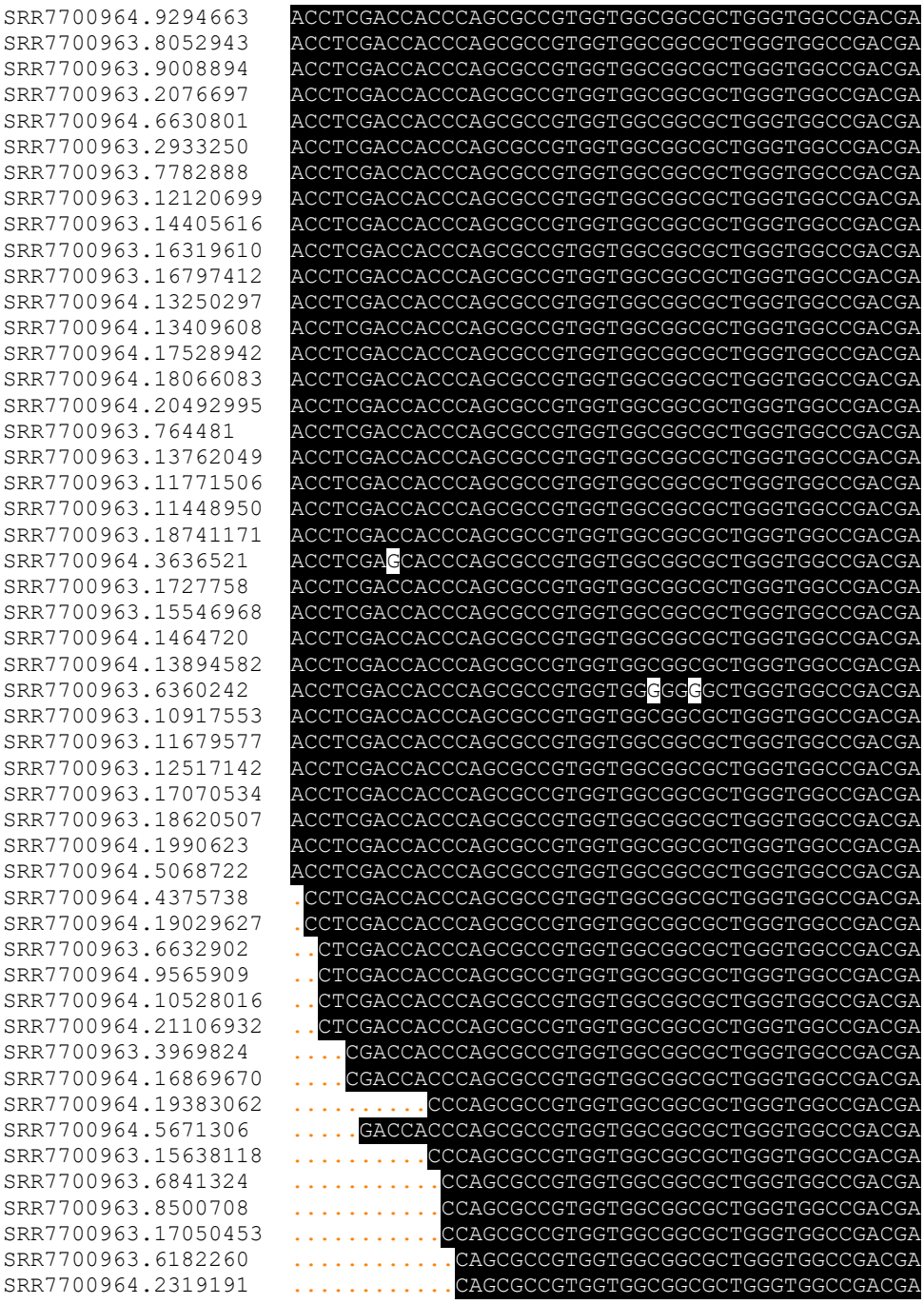
MACPTGFFTALFGKLLTIPIWVLVFVFNALFVFPRFWVWRLPPRLQHEQQQQDRNDDEWQRQQQDRRVVV
WHPPPPPDVEMALQDNPTAAPAEPADLDHPAP*

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

MACPTGFFTALFGKLLTIPIWVLVFVFNALFVFPRFWVWRLPPRLQHEQQQQDRNDDEWQRQQQDRRVVV
WHPPPPPDVEMALQDNPTAAPAEPADLDHPAPWWRRWVADDAYPAVHFCVTAANAVTQGL*

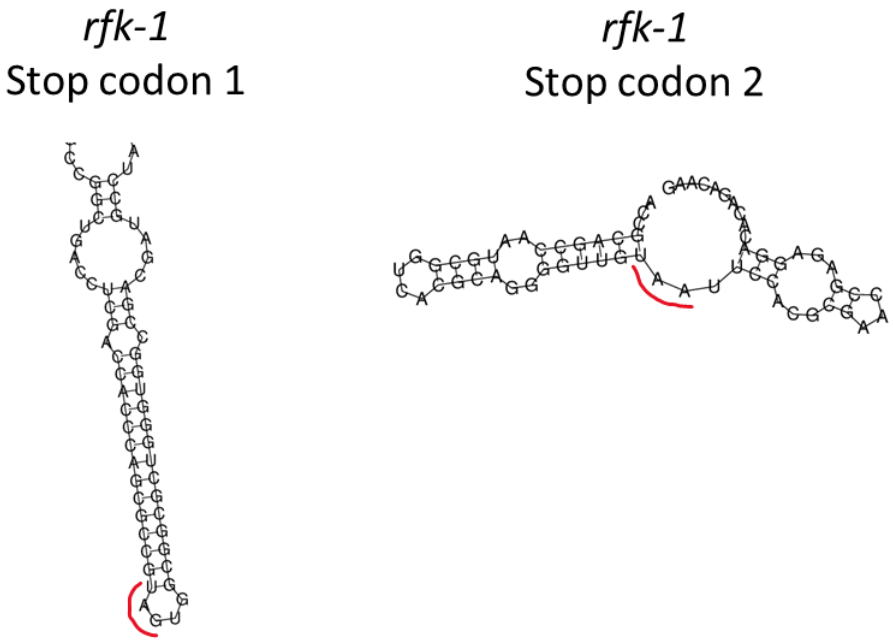
Figure S5 RNA sequencing suggests that *rfk-1* transcripts are subject to RNA editing





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 (*center*) Primer numbers	*Name of transformation host* Primer sequences	Pr.pur-pose
v3 (*hph*)	*Transformation host: ISU-3223*	
73	CAAGACCCAGAACAACGCCAACA	L
74	AAAAAATGCTCCTTCAATATCAGTTCCTCGCTCCTCTTCCGCAAATTA	L
75	GAGTAGATGCCGACCGGGAACCAGTTTGGTGGGATACTCGGTGCAGGTA	R
76	CGACACCTCGAATACGCCCTCTC	R
77	CCGGAAACGTCAGCAAACACGTA	N
78	GCGCCAGCTCCTCTACACTCTCC	N
v4 (*hph*)	*Transformation host: ISU-3223*	
79	CCAAGCCAAACTCAAGGGAATCG	L
80	AAAAAATGCTCCTTCAATATCAGTTAATGGCGGTGATCTTCGACTGCT	L
81	GAGTAGATGCCGACCGGGAACCAGTTGCCCAGACTCAGCTTGCATTGAC	R
82	TCACCTTGGCCCTGGAGTACCTG	R
83	CAAACGGGACGCAACCTCTATGA	N
84	CCAAGCGGGTTCCAGATAAGACG	N
v5 (*hph*)	*Transformation host: ISU-3223*	
85	CACCATGTAGTCGGAGCGGAAGA	L
86	AAAAAATGCTCCTTCAATATCAGTTTCATCTTGACGGGCAGAACTGAA	L
87	GAGTAGATGCCGACCGGGAACCAGTTGCTAACCAGGAACAGGCGCTTACC	R
88	CATCGAAAGGGAGAGGCACTTCG	R
89	GCCTTCCTTCTTCACACGGAGGT	N
90	ACAGGATCTGGTCATCCCGCTTC	N
v31 (*hph*)	*Transformation host: ISU-3223*	
85	CACCATGTAGTCGGAGCGGAAGA	L
86	AAAAAATGCTCCTTCAATATCAGTTTCATCTTGACGGGCAGAACTGAA	L
167	GAGTAGATGCCGACCGGGAACCAGTTATTGAGGTGAGGACAAGCGATGA	R
168	CATACGGCCCATGTTACCGCACT	R
89	GCCTTCCTTCTTCACACGGAGGT	N
170	CAACGAAGCAGGCTCCCATACAG	N
v32 (*hph*)	*Transformation host: ISU-3223*	
85	CACCATGTAGTCGGAGCGGAAGA	L
86	AAAAAATGCTCCTTCAATATCAGTTTCATCTTGACGGGCAGAACTGAA	L
173	GAGTAGATGCCGACCGGGAACCAGTTGTCGTCCGTGAATCGTGATCCTT	R
174	AATTCGCCGTGTACTTCGCTGTG	R
89	GCCTTCCTTCTTCACACGGAGGT	N
176	CGGTTGTATCTGCCGGTTTGAAGA	N
v33 (*hph*)	*Transformation host: ISU-3223*	
85	CACCATGTAGTCGGAGCGGAAGA	L
86	AAAAAATGCTCCTTCAATATCAGTTTCATCTTGACGGGCAGAACTGAA	L
3	GAGTAGATGCCGACCGGGAACCAGTTCATGGCAGTGAAGTGGACAAGCTG	R
4	GTGGTAAGCGCCTGTTCCTGGTTAG	R
89	GCCTTCCTTCTTCACACGGAGGT	N
6	TGCGGCCTGTTTACGAAATCCAA	N

v34 (*hph*)	*Transformation host: ISU-3223*	
85	CACCATGTAGTCGGAGCGGAAGA	L
86	AAAAAATGCTCCTTCAATATCAGTTTCATCTTGACGGGCAGAACTGAA	L
9	GAGTAGATGCCGACCGGGAACCAGTTCTCGATTGCCCGACACCTTCTGT	R
4	GTGGTAAGCGCCTGTTCCTGGTTAG	R
89	GCCTTCCTTCTTCACACGGAGGT	N
11	CGAAAGACAGAGAGGACCGAGAGGA	N
v35 (*hph*)	*Transformation host: ISU-3223*	
1	TCGGAAGGATTGCTGACTTGTGTGT	L
2	CCAAAAAATGCTCCTTCAATATCAGTTAGTTGGTAGCTGGCGCGGAAAG	L
87	GAGTAGATGCCGACCGGGAACCAGTTGCTAACCAGGAACAGGCGCTTACC	R
88	CATCGAAAGGGAGAGGCACTTCG	R
5	GCGCAGACGAACATCAAGGAGAA	N
90	ACAGGATCTGGTCATCCCGCTTC	N
v37 (*hph*)	*Transformation host: P15-53*	
7	GGCAGATACAACCGACGACCAAA	L
8	CCAAAAAATGCTCCTTCAATATCAGTTTCCGTTTCGCTTATGATGTTAATGATG	L
87	GAGTAGATGCCGACCGGGAACCAGTTGCTAACCAGGAACAGGCGCTTACC	R
88	CATCGAAAGGGAGAGGCACTTCG	R
10	CACGTAGGGAAGGAGGTTGAAGGT	N
90	ACAGGATCTGGTCATCCCGCTTC	N
v38 (*hph*)	*Transformation host: P15-53*	
309	ACGCCAAAAGGTGTAGGGGGATT	L
310	CCAAAAAATGCTCCTTCAATATCAGTTGACCGAACAACCGGAATGACCT	L
87	GAGTAGATGCCGACCGGGAACCAGTTGCTAACCAGGAACAGGCGCTTACC	R
88	CATCGAAAGGGAGAGGCACTTCG	R
311	AGGTCCGCAACTATTGTCCGTTT	N
90	ACAGGATCTGGTCATCCCGCTTC	N
v39 (*hph*)	*Transformation host: P15-53*	
309	ACGCCAAAAGGTGTAGGGGGATT	L
312	CCAAAAAATGCTCCTTCAATATCAGTTGCAGCTCTTGCTTTGTTTTGTCAGT	L
87	GAGTAGATGCCGACCGGGAACCAGTTGCTAACCAGGAACAGGCGCTTACC	R
88	CATCGAAAGGGAGAGGCACTTCG	R
311	AGGTCCGCAACTATTGTCCGTTT	N
90	ACAGGATCTGGTCATCCCGCTTC	N
v40 (*hph*)	*Transformation host: P15-53*	
309	ACGCCAAAAGGTGTAGGGGGATT	L
310	CCAAAAAATGCTCCTTCAATATCAGTTGACCGAACAACCGGAATGACCT	L
313	GAGTAGATGCCGACCGGGAACCAGTTCGGCCCAACCTCTCCTCAGAAT	R
88	CATCGAAAGGGAGAGGCACTTCG	R
311	AGGTCCGCAACTATTGTCCGTTT	N
90	ACAGGATCTGGTCATCCCGCTTC	N
v140 (*hph*)	*Transformation host: P15-53*	
1303	AACCAGGAACAGGCGCTTACCAC	L
1304	AAAAAATGCTCCTTCAATATCAGTTACGGTGTAGCGGGACGTTTTC	L
1305	GAGTAGATGCCGACCGGGAACCAGTTTCAACAAAGCGCGTGATCTTTCG	R
871	GAACTCGAACCACTCCACGCAAA	R
1306	ACAGGACAGGCGGGTTGTGGTTT	N
872	CATGTCGGTCTTGAGGTCGTTGC	N
v150 (*hph*)	*Transformation host: P8-43*	
1332	CCGCGAATGGTTAACTGCACGGC	L
1304	AAAAAATGCTCCTTCAATATCAGTTACGGTGTAGCGGGACGTTTTC	L
1305	GAGTAGATGCCGACCGGGAACCAGTTTCAACAAAGCGCGTGATCTTTCG	R
1333	GTCGGCATAGGCTGTGGTGGTCG	R
1334	ATGCGGCCTTGATGCACTGGCTG	N
1335	CGAGAGGGAGAGGCACTTCGCCA	N
v160 (*nat1*)	*Transformation host: ISU-3222*	
10	CACGTAGGGAAGGAGGTTGAAGGT	L
869	TGAATGCTAAAAGACACCATTTCCCACACTCCCTCAGCAAGTAAGCCGGTCACGATCC	L
870	GCTGGCTGCAATACAAGCGTTCCCACCTAACCAACTCAACAAAGCGCGTGATCTTTCG	R
871	GAACTCGAACCACTCCACGCAAA	R

311	AGGTCCGCAACTATTGTCCGTTT	N
872	CATGTCGGTCTTGAGGTCGTTGC	N
v175 (*hph*)	*Transformation host: ISU-3222*	
1433	GGAACAGGCGCTTACCACCA	L
1434	AAAAAATGCTCCTTCAATATCAGTTTGAAATGTTGATGCCTCCCTGGAT	L
1435	GAGTAGATGCCGACCGGGAACCAGTTGGGGTTTAGGGAGGGCTGCAT	R
1436	TTCCTTTCCCGCTCCGTTCG	R
1437	ACAGGACAGGCGGGTTGTGG	N
1438	CCGAATACCGACCCCCGATT	N
v176 (*hph*)	*Transformation host: P8-43*	
1439	TGGCAGGTCAAGGTCGATTGC	L
1440	AAAAAATGCTCCTTCAATATCAGTTTGAAATGTTGATGCCTCCCTAGAT	L
1441	GAGTAGATGCCGACCGGGAACCAGTTGGGGTTTAGGCAGGGCTGGAT	R
1442	TTCCTTTCCCGCTCCTTTCG	R
1443	CGGCCGCGAATGGTTAACTG	N
1438	CCGAATACCGACCCCCGATT	N
v199 [*hph-ccg-1*(P)]	*Transformation host: P15-53*	
1538	CGAAGGACAAGAGGAACGGGAAA	L
1539	GCAGCCTGAATGGCGAATGGACGCGCGGGCAGCAGCTCTTGCTTTGTTT	L
1544	TTCACAACCCCTCACATCAACCAAAATGGCCTGCCCCACAGGGTTT	R
1541	GTCACGGTGTAGCGGGACGTTTT	R
1542	GGGGCGGAGAGGAGAAGATGAGT	N
1543	GGAATTACAACCCCTGCGTGACC	N
v214 [*hph-ccg-1*(P)-ATGT]	*Transformation host: P15-53*	
1538	CGAAGGACAAGAGGAACGGGAAA	L
1539	GCAGCCTGAATGGCGAATGGACGCGCGGGCAGCAGCTCTTGCTTTGTTT	L
1636	TTCACAACCCCTCACATCAACCAAAATGTGCCTGCCCCACAGGGTTT	R
1541	GTCACGGTGTAGCGGGACGTTTT	R
1542	GGGGCGGAGAGGAGAAGATGAGT	N
1543	GGAATTACAACCCCTGCGTGACC	N
v221 [*hph-ccg-1*(P)-TAA]	*Transformation host: P15-53*	
1538	CGAAGGACAAGAGGAACGGGAAA	L
1539	GCAGCCTGAATGGCGAATGGACGCGCGGGCAGCAGCTCTTGCTTTGTTT	L
1656	TTCACAACCCCTCACATCAACCAAATAAGCCTGCCCCACAGGGTTT	R
1541	GTCACGGTGTAGCGGGACGTTTT	R
1542	GGGGCGGAGAGGAGAAGATGAGT	N
1543	GGAATTACAACCCCTGCGTGACC	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
hph	*pTH1256.1(GenBank MH550659)*
12	AACTGATATTGAAGGAGCATTTTTTGG
13	AACTGGTTCCCGGTCGGCAT
nat1	*pNR28.12 (GenBank MH553564)*
297	GAGGGAGTGTGGGAAATGGTGTC
298	GTTGGTTAGGTGGGAACGCTTGT
hph-ccg-1(P)	*pTH1117.12 (GenBank JF749202)*
550	GCGCGTCCATTCGCCATTCA
1555	TTTGGTTGATGTGAGGGGTTGTGA

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

Eight intervals of *Sk-2*^{INS1} were cloned to the *Not*I 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	*Transformation host: P8-43*
248	AAAAGCGGCCGCAGGGTGGTGTGGGTGAGGATGT
249	TTTTGCGGCCGCGAGCGGAAGTGTTTGCTTGTGTGA
pAH6	*Transformation host: P8-43*
252	AAAAGCGGCCGCATCGCCAACGGGCATTCAAG
253	AAAAGCGGCCGCACCCGCCTACACATGCACCATC
pAH14	*Transformation host: P8-43*
302	AAAAGCGGCCGCTGCATGTGTAGGCGGGTATTGTG
314	AAAAGCGGCCGCGGGGCAGGGCAGCAAGTAAG
pAH30	*Transformation host: P8-43*
304	AAAAGCGGCCGCGAGGACCAGCTCGACGGTAGTAGG
251	AAAAGCGGCCGCGAGGAATAGGACGTGAGGGTGTGG
pAH31	*Transformation host: P8-43*
353	TTTTGCGGCCGCCATTGATACCGAGTCTTTCCGTTC
251	AAAAGCGGCCGCGAGGAATAGGACGTGAGGGTGTGG
pAH32	*Transformation host: P8-43*
351	AAAAGCGGCCGCAACTCCTCACCCATCCCCATTTG
251	AAAAGCGGCCGCGAGGAATAGGACGTGAGGGTGTGG
pAH36	*Transformation host: P8-43*
353	TTTTGCGGCCGCCATTGATACCGAGTCTTTCCGTTC
639	AAAAGCGGCCGCGACGGTGTAGCGGGACGTTTTCC
pAH37	*Transformation host: P8-43*
353	TTTTGCGGCCGCCATTGATACCGAGTCTTTCCGTTC
640	AAAAGCGGCCGCGTTCGCTGACTTTCCCGACCA

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

The *AH36$^{Sk\text{-}2}$::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$^{Sk\text{-}2}$* and *hph* between recombination flanks suitable for replacing *AH36$^{\Delta}$::nat1* in ISU-4562 with *AH36$^{Sk\text{-}2}$::hph*.

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

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 *Not*I 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 *Dpn*I and used to transform chemically-competent *E. coli* Ig^{TM} 5-alpha cells (Intact Genomics). The mutated-*AH36* intervals were transferred to the *Not*I 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	GTTGGAGTGAGGATATAATCCGGCACGTCGAAG
1139	CTTCGACGTGCCGGATTATATCCTCACTCCAAC
Change G to A at position 27972	
1136	GATGATGACTGACTTATTTACTATAGTTCCTCCTTCGACGT
1137	ACGTCGAAGGAGGAACTATAGTAAATAAGTCAGTCATCATC
Change G to A at position 28052	
1134	CGACGGGGGCCCTACCTTCCCTTAGTT
1135	AACTAAGGGAAGGTAGGGCCCCCGTCG
Change G to A at position 28104	
1132	CTAACCCACTACTAACAAAACAAAGCAAGAGCTGCTGC
1133	GCAGCAGCTCTTGCTTTGTTTTGTTAGTAGTGGGTTAG
Change G to A at position 28300	
1130	GGGATGGTGAGGAGTTTGCTAAAAAGAGCGGTAAAAAAC
1131	GTTTTTTACCGCTCTTTTTAGCAAACTCCTCACCATCCC
Change G to A at position 28326	
1128	GAATACAAACACCAACACTCAAATGGGGATGGTGAG
1129	CTCACCATCCCCATTTGAGTGTTGGTGTTTGTATTC

Table S6 Primers for *rfk-1* cDNA analysis

Primer number	Primer sequence
1741	GGCCTGCCCCACAGGGTTTTT
1742	CCGATTCCGTATTCTGGTGTTGAG
1743	GAAAATGGCCTGCCCCACAGG
1744	CGTGGGCCTTGTCTGTGTCCT
1745	CTTACCACCACGGCTGCAA
1746	TGTCTTTCGCTAACCAGGAACA