

# Supplementary

Table S1. PCR primers used in this study.

NAME	SEQUENCE(5'-3')
FLY1-UP-F	TCTTGTGTCATCAGGCACTG
FLY1-UP-R	CAAAATAGGCATTGATGTGTTGACCTCCGTTGAGAACGAGTGAGG ATT
FLY1-DOWN-F	CTCGTCCGAGGGCAAAGGAATAGAGTAGATATGATACGATGGAAT GTT
FLY1-DOWN-R	AGAGTGGAGCGCTGCTCAGA
FLY1-ID-F	CCATGATCGCGTGCTTGAAC
FLY1-ID-R	CGCTGGTTATCAATCTCTGC
FLY1-NEST-F	GAAGCTCGCCCGGTAGCGTC
FLY1-NEST-R	CCAGTACAACGCTTTCCACC
NP-FLY1-GFP-F	ACTCACTATAGGGCGAATTGGGTAATAAATTGGTTAGACGAGAT ATAGGCTCTTG
NP-FLY1-GFP-R	CACCACCCCGGTGAACAGCTCCTCGCCCTTGCTCACGCAGCCAGA AGGCAGGGTGAA
ID-FLY1-GFP-F	TCCATGAAGCTCGTCATGGAC
ID-FLY1-GFP-R	GTCAGCTTGCCGTAGGTGGCA
CYFP-FLY1-F	CTAGTCGACTCTAGCCTCGAGATGGCACCCAACGCATCGCC
CYFP-FLY1-R	ATCGTATGGGTACATCCTAGGGCAGCCAGAAGGCAGGGTGAA
NYFP-CABT-F	TCTGAGGAGGATCTTCTAGGATGAATGTGATTCAATATTA
NYFP-CABT-R	GGGAGGCCTGGATCGACTAGTGCAGCAAATACATACACCAG
PPR3N-FLY1-F	GACGCGTGGCCATTACGGCCATGCGCTTCTCCGATAGTCT
PPR3N-FLY1-R	CTCGAGAGGCCGAGGCGGCCGCGCAGCCAGAAGGCAGGGTGAA
PBT3-FLY1-F	ATGTAATGGCCATTACGGCCATGCGCTTCTCCGATAGTCT
PBT3-FLY1-R	TGCAGATGGCCGAGGCGGCCCGCAGCCAGAAGGCAGGGTGAA
PBT3-ΔSPFLY1-F	ATGTAATGGCCATTACGGCCATGGCACCCAACGCATCGCC
PPR3-CABT-F	GACGCGTGGCCATTACGGCCATGAATGTGATTCAATATTA
PPR3-CABT-R	CTCGAGAGGCCGAGGCGGCCGCGCAGCAAATACATACACCAG
PBT3-CABT-F	ATGTAATGGCCATTACGGCCATGAATGTGATTCAATATTA
PBT3-CABT-R	TGCAGATGGCCGAGGCGGCCCGCAGCAAATACATACACCAG
NLUC-CABT-F	GAACACGGGGGACGAGCTCGGTACCATGAATGTGATTCAATATTA
NLUC-CABT-R	GCCTCAGTCGACGCGTTGTGGATCCGCAGCAAATACATACACCAG
CLUC-FLY1-F	CTCGTACGCGTCCCGGGGCGGTACCATGGCACCCAACGCATCGCC
CLUC-FLY1-R	GCCTCAGTCGACGCGTTGTGGATCCGCAGCCAGAAGGCAGGGTGA A
PSUC2-FLY1-F	TCCAAGCTCGGAATTTAATTAAGAATTCATGCGCTTCTCCGATAG TCT
PSUC2-FLY1-R	CGACTCACTATAGGGAGAACCTCGAGATGGGCACCGGCCAGGCCG G
PBIN-NSP-FLY1-F	CCCCGGGGTTCGACGGATCCATGGCACCCAACGCATCGCC
PBIN-FLY1-F	CCCCGGGGTTCGACGGATCCATGCGCTTCTCCGATAGTCT
PBIN-FLY1-R	CTCTAGTTCATCTAGAGGATCCGCAGCCAGAAGGCAGGGTGA
CLUC-JC-F	GAGTTGTGTTTGTGGACGAAGTACC
CLUC-JC-R	TTCCATTTACAGTTCGATAGCG
NLUC-JC-F	TTGGAGAGAACACGGGGGACG
NLUC-JC-R	TTGGAGAGAACACGGGGGACG
NLUC-JC-R	CAGGGCGTATCTCTTCATAGCC
RT-TRI1-F	GATGTTCTTCTCGACAGCGT
RT-TRI1-R	CACTGGTCGAAGATAGCTGG
RT-TRI3-F	TGTTACGATCAATGGCTTGG
RT-TRI3-R	TCCTCGTTGTAGTTTGCATCA

RT-TRI4-F	ACGTGTGGCTACTCAGGAGA
RT-TRI4-R	TGGAATTGCCTTGGGGTA
RT-TRI5-F	ATGGCGGATCTATCTATTAC
RT-TRI5-R	CCATTCATACGACGAAGGAAT
RT-TRI6-F	AAATGCCCATTCCTAGTTG
RT-TRI6-R	ATCTCGCATGTTATCCACCCT
RT-TRI7-F	TACCGTCGTCTTCAAAACCA
RT-TRI7-R	ACGCCAATGGTGTTCACAAA
RT-TRI8-F	ATATAACGGTACCCCCAGATG
RT-TRI8-R	TGTTTGTAGGACACTTCCGGT
RT-TRI9-F	CCGCTAAACTGATCGACTCAT
RT-TRI9-R	CCCATATGGTAGCGCATAAA
RT-TRI10-F	TCCCAACCTTTCAGAGGTTCA
RT-TRI10-R	TGATCCGTCAAGTCTTCCCAT
RT-TRI11-F	TGAGAACGACATGTGGGCAAT
RT-TRI11-R	AGGCTTGTCCATGCAAGAT
RT-TRI12-F	ACGAACAGCACTGCTACGGT
RT-TRI12-R	TTCCTGCTTGTGACTCCAAT
RT-TRI13-F	ACGCAGATCCTGGGATATCA
RT-TRI13-R	CAGCCCAGTATTTGCCAAA
RT-TRI14-F	AACTCCCGTTGTGATCAAGCA
RT-TRI14-R	AACAGTAATGTTGGCACCGT
RT-ACTIN-F	ATCCACGTCACCACTTCAA
RT-ACTIN-R	TGCCTTGAGATCCACATTTG

Table S2. Growth rate, average disease severity and plant infection between different types of strain

Strain	Growth rate (mm d <sup>-1</sup> ) <sup>1)</sup>	Average disease severity <sup>2)</sup>	Maize silk lesion length (cm) <sup>3)</sup>	Coleoptile length(cm) <sup>4)</sup>	Lesion
PH-1	11.5±0.3a	3.17a	10±0.2a	1.2±0.12a	
<i>ΔFgFly1</i>	9.2±0.2b	1.13b	4.2±0.1b	0.3±0.05b	
<i>ΔFgFly1-C</i>	11.4±0.3a	3.20a	9.8±0.3a	1.2±0.10a	

<sup>1)</sup> Daily extension in colony radius on potato dextrose agar (PDA) plates.

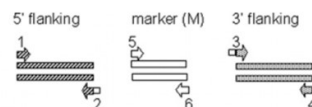
<sup>2)</sup> Average disease severity was rated by the disease severity of symptomatic spikelets per head at 14 days post-inoculation. (Total 30 spikes)

<sup>3)</sup> The average lesion length 7 days after infection of maize silk. (Total 10 spikes)

<sup>4)</sup> The average lesion length 7 days after infection of coleoptile. (Total 10 spikes)

**A**

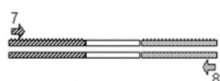
**A First Round PCR:**



**B Second Round PCR:**



**C Third Round PCR:**



**B**

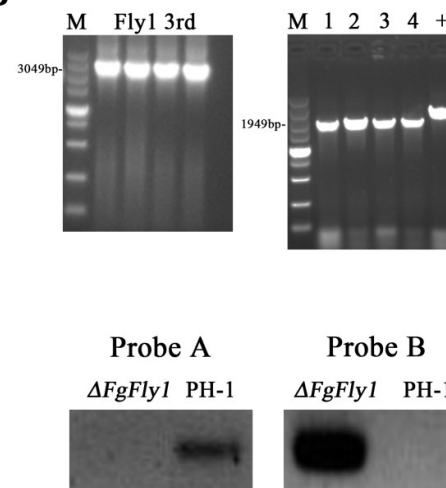


Figure S1.A. Schematic diagram of Double-joint PCR. B. Fly1 mutants were screened by PCR analysis and Southern blot.

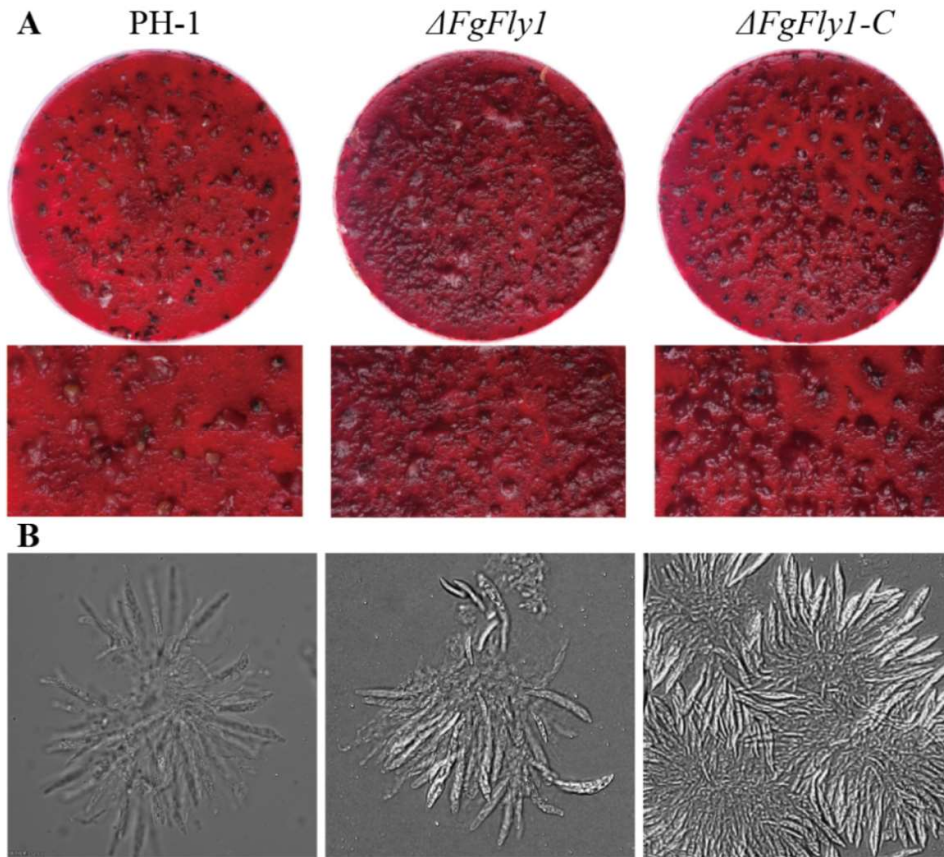


Figure S2. Analysis of ascospores discharge from PH-1,  $\Delta FgFly1$  and  $\Delta FgFly1-C$  on carrot plate for 15 d. A. Carrot agar cultures of the PH-1,  $\Delta FgFly1$  and  $\Delta FgFly1-C$  were examined 15 d post-fertilization (dpf). B. Ascospores of the PH-1,  $\Delta FgFly1$  and  $\Delta FgFly1-C$  were examined by differential interference contrast (DIC). Bar= 10  $\mu m$ .