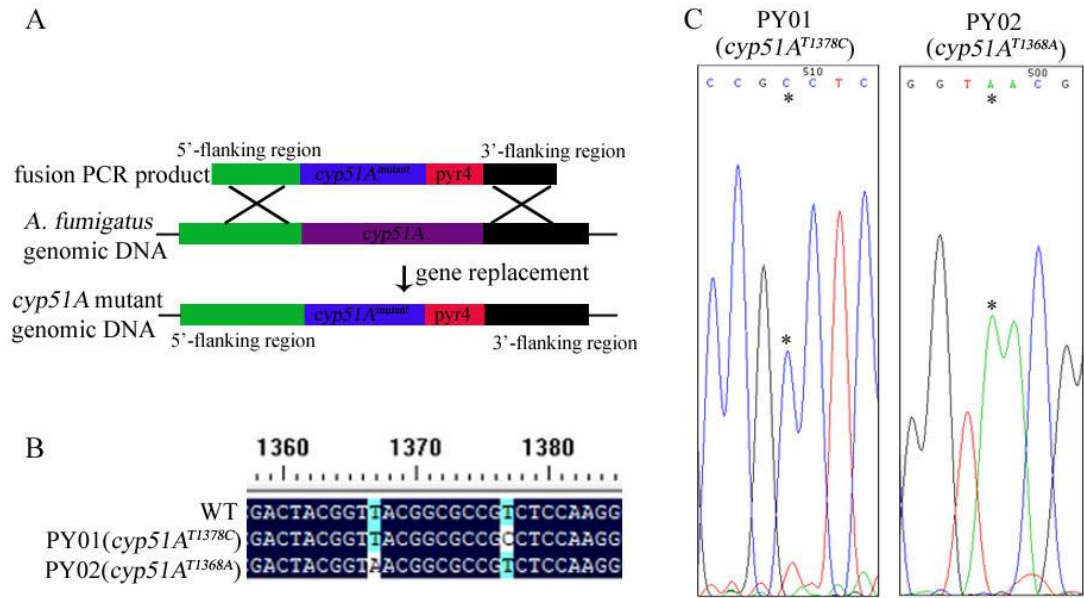


**Figure S1 PCR analysis showing the integration of the *pyrG::cyp51A<sup>point mutation</sup>* fragment into the genome at the original *cyp51A* locus in the PY01 or PY02 strains.**

For lanes 2 and 5, the primers *cyp51A*-F1 and *Diag-Ncpyr4*-3' were used to determine whether there was a homologous recombination to replace *cyp51A* with *pyrG::cyp51A<sup>point mutation</sup>* fragment in the genome of PY01 or PY02, and the expected size is 3686 bp; For lanes 3 and 6, primers *Diag-Ncpyr4*-5' and P6 were used to determine whether there was a homologous recombination to replace *cyp51A* with *pyrG::cyp51A<sup>point mutation</sup>* fragment in the genome of PY01 or PY02, and the expected size is 1133 bp; For lanes 4 and 7, primers *cyp51A*-F and *cyp51A*-R2 were used to determine whether wild-type gene *cyp51A* still exists in the genome of PY01 or PY02, and the expected size of wild-type gene *cyp51A* is 2113 bp, and the expected size of *pyrG::cyp51A<sup>point mutation</sup>* is 4321 bp. In lanes 2-4, genomic DNA of PY01 was used as a PCR template; for lanes 5-7, genomic DNA of PY02 was used as a PCR template.



**Figure S2 The construction of point mutations PY01 (*cyp51A*<sup>N248K/V436A</sup>) and PY02 (*cyp51A*<sup>Y433N</sup>)**

**and sequencing analysis for *cyp51A* gene.** (A) Diagram showing the gene replacement strategy for mutations. (B, C) The *cyp51A* gene sequencing analysis showing expected mutation sites in the PY01 and PY02 strains. Asterisk (C) show mutation sites.

TABLE S1 Primers used in this study

Primer name	DNA sequence 5'-3'
cyp51A-F1	CTCAGGACTTTCTTCTAACCACCAT
cyp51A-R1	TACAGTCATTTATTAGGCCCTCGAG
cyp51A-F2	GCAGCACCACTTCAGAGTTGTCTAG
cyp51A-R2	AGTATAGGCAACAACACTTCAGGGC
P2	AAGACTTGGCTTAGCTCCCTTACCG
P3	GTAACGCCAGGGTTTTCCAGTCTCACTTGGATGTGTTTTTCG
P4	TGCTCTGATGCCGCATAGTTGTAACCATCGAGGACTTCAAAGG
P5	CCGTCCACGTATGACATGCATATG
P6	TCTCGTGTGACTATGACCCCGT
Pyr4-up	GGAAAACCTGGCGTTAC
Pyr4-down	CTATGCGGCATCAGAGCA
Diag-Ncpyr4-5'	GTTGCATTTCGATAAGTAGCCAGTTC
Diag-Ncpyr4-3'	GTCTTGAGGACGACAATCGA

TABLE S2 *A. fumigatus* strains used in this study

Strain	Genotype	Reference or source
ATCC MYA-3626	NA	ATCC
NO.3	NA	This study
NO.7	NA	This study
A1160 <sup>e</sup>	$\Delta ku80$ ; A1160::pyrG; veA1	(Jiang et al., 2014)
A1160	$\Delta ku80$ ; pyrG; veA1	FGSC
PY01	$\Delta ku80$ ; pyrG; cyp51A <sup>N248K/V436A</sup> ::pyr4; veA1	This study
PY02	$\Delta ku80$ ; pyrG; cyp51A <sup>Y433N</sup> ::pyr4; veA1	This study

FGSC: Fungal Genetics Stock Center (<http://www.fgsc.net/>); NA: not applicable; ATCC: American Type Culture Collection.