Supplementary Figure S1

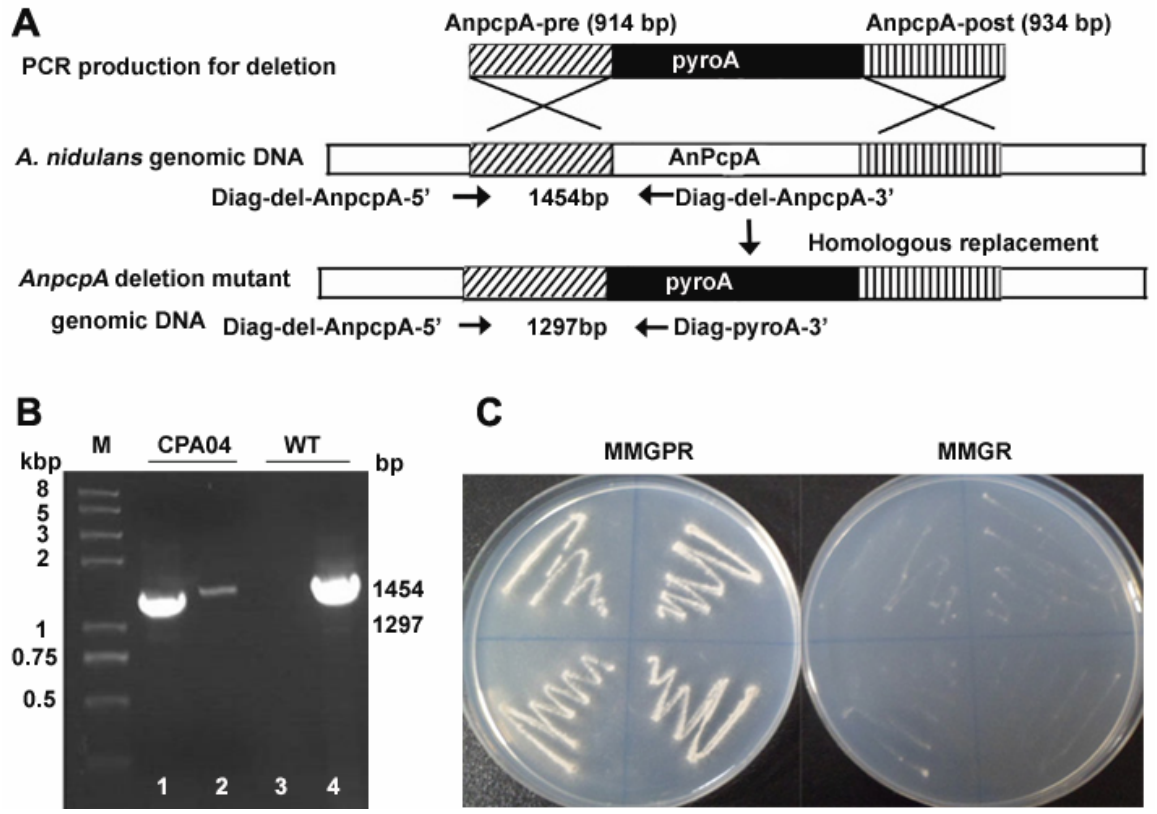

Fig S1 *AnpcpA* gene was deleted by homologues integration in the background of the SJW100 (GFP-MTs) strain. (A) Diagram showing the deletion strategy for *AnpcpA*. (B) PCR analysis showing the inegration of *AnpyroA* nutritional marker into the genome at the original *AnpcpA* locus in the CPA04 strain while *AnpcpA* gene still can be detected, suggesting transformants belonged to the heterokaryons. For lanes 2 and 4, primers were Diag-del-AnpcpA-5' and Diag-del-AnpcpA-3' to detect whether *AnpcpA* still exists in the genome, the expected size is 1454 bp. For lanes 1 and 3, primers were Diag-del-AnpcpA-5' and Diag-pyroA-3' to detect whether there was a homologous recombination to replace *AnpcpA* with nutritional marker gene *pyroA* in the genome, the expected size is 1297 bp. In lanes of 3 and 4, genomic DNA of TN02A7 was used for PCR template, 1 and 2 were using genomic DNA of transformants as PCR templates. (C) Replica transformant spores onto

MMGPR and MMGR. When the transformant spores were inoculated onto non-selective MMGPR media, they germinated and formed into colonies. However, the same mixed spores from the transformants streaked on selective MMGR media were unable to form colonies.

Supplementary Figure S2

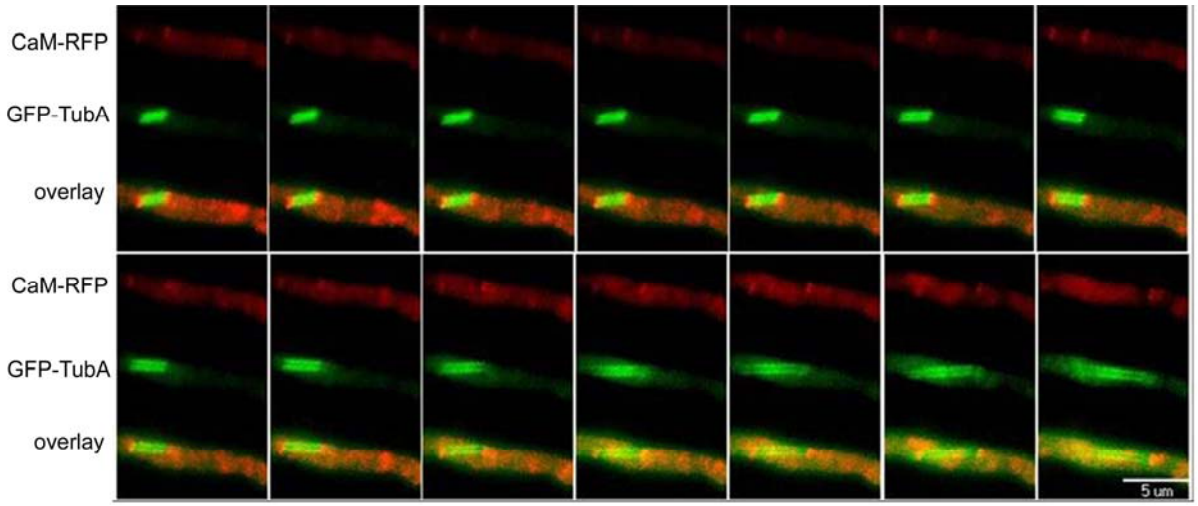

Fig S2 Localization relationship between GFP-tubA and CaM-RFP in SCA02 (generated from crossing CSA02 with SJW100). Time duration: 4 min, time interval: 5s. Bar: 5 μm.

Supplementary Figure S3

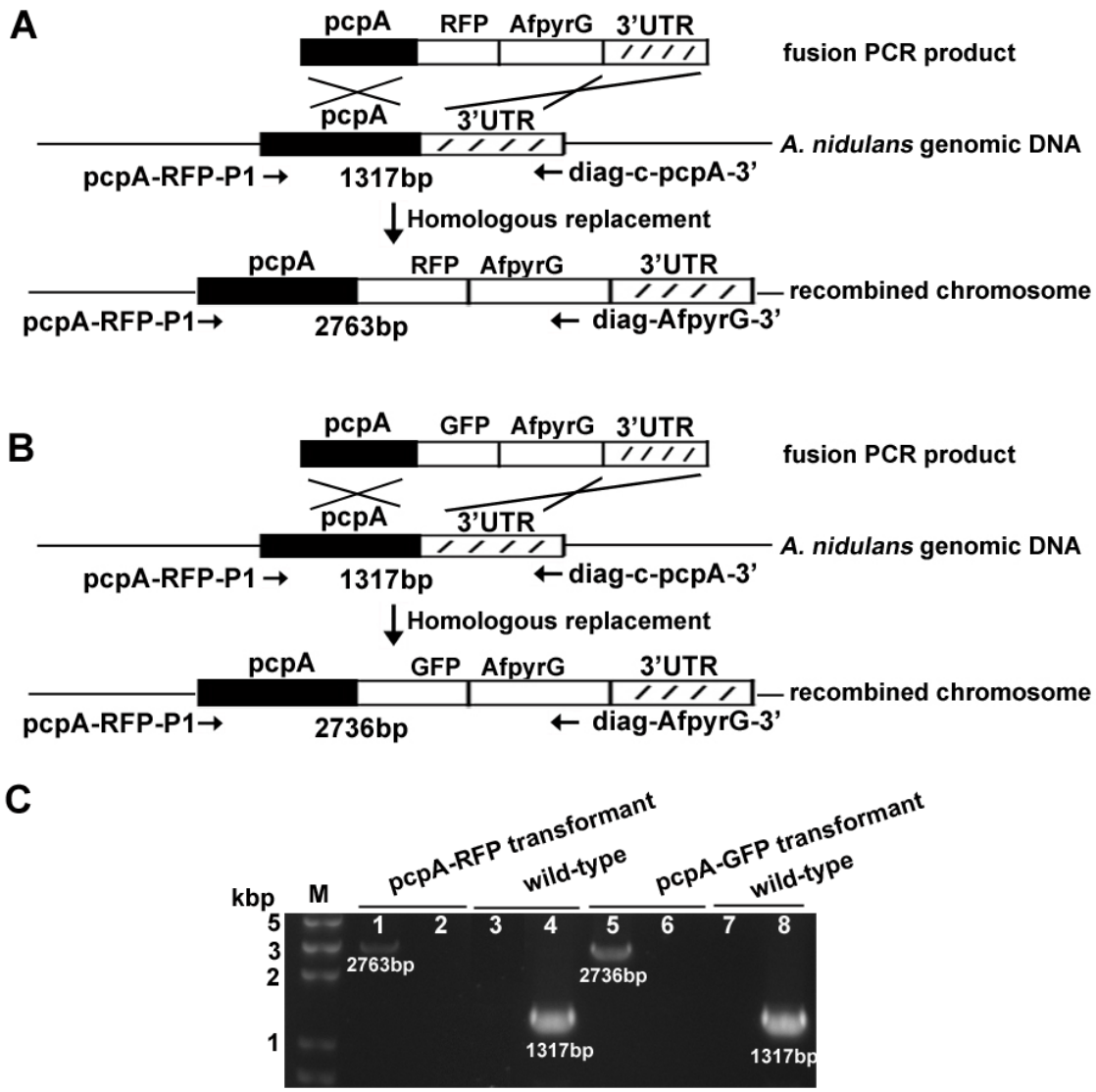

Fig S3 *pcpA* gene tagging strategy and identification. (A, B)Diagram showing the *pcpA* gene tagging strategy at C-terminal by highly efficient gene homologous integration strategy. (C) PCR analysis showing a cassette encoding the RFP or GFP and AfpyrG into the 3' end of the *pcpA* gene by highly efficient gene homologous integration strategy. In lanes of 3, 4, 7 and 8, genomic DNA of TN02A7 was used for PCR template, 1, 2, 5 and 6 were using genomic DNA of transformants as PCR templates.

Supplementary movie 1

Movie 1 Shape remodeling of cell from CPA02 after shifted from MMGPR to YAG for 4 h with the decreasing of GFP-AnPcpA expression.

Supplementary Figure S4

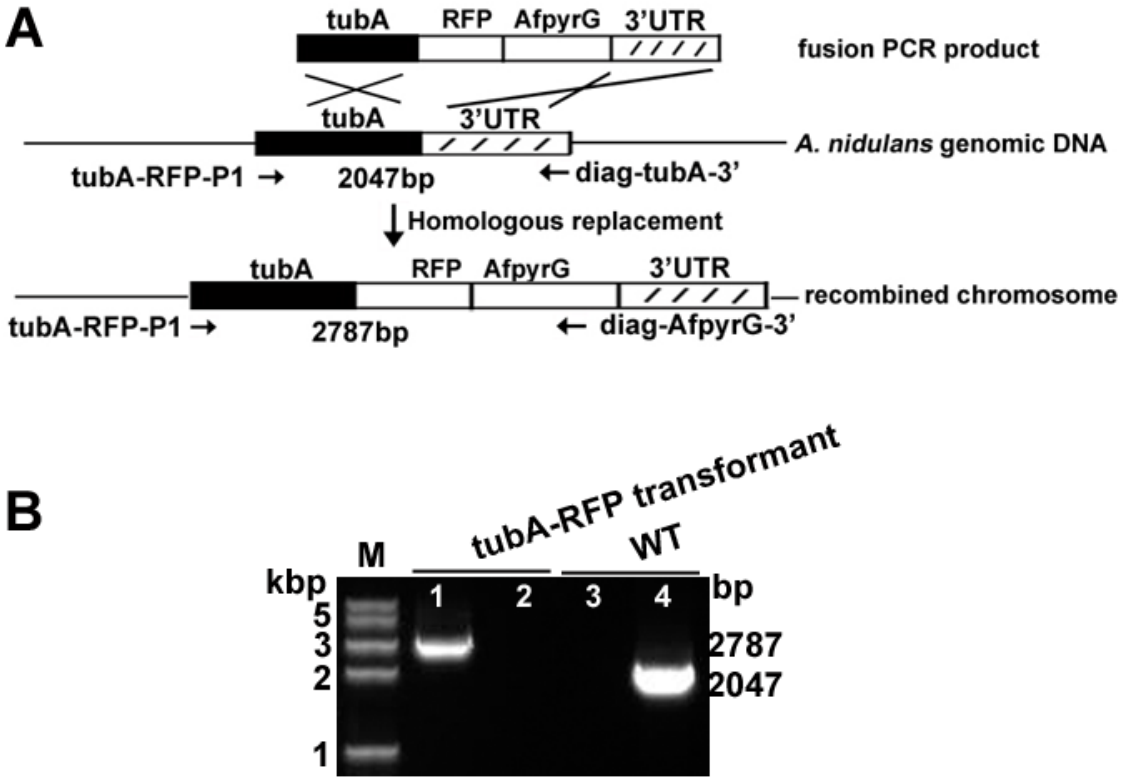

Fig S4 *tubA* gene tagging strategy and identification. (A)Diagram showing the *tubA* gene tagging strategy at C-terminal by highly efficient gene homologous integration strategy. (C) PCR analysis showing a cassette encoding the RFP and AfpyrG into the C-terminal of the *tubA* gene by highly efficient gene homologous integration strategy. In lanes of 3 and 4, genomic DNA of CPA02 was used for PCR template, 1 and 2 were using genomic DNA of transformants as PCR templates.

Supplementary Table S1. Primers used in this study

Primer name	DNA sequence 5'-3'
AnpcpA-5'	ATAAGAATGCGGCGGCAGATGGCCTACCCGTACATC
AnpcpA -3'	GCTCTAGAATCCTCGATATCGTCACGGAG
Diag-GFP-5'	GACACCCTCGTCAACAGGATCG

PyrG-5’	GCTCGAGCATGCATCTAGAG
PyrG-3’	CTGTCTGAGA GGAGGCACTG
Diag-AnpcpA -3’	GTTCATTTGTTGGAGTTTTAGACT
Diag-AnpcpA -5’	GCACGCTGGAGACTTTTGGACT
Diag-del-AnpcpA-5’	GCTTTTCGTTCTTCGGCTTTA
AnpcpA-pre-3’	CTCTAGATGCATGCTCGAGCCTTCGGAGGGAAAACGCGT
AnpcpA-post-5’	CAGTGCCTCCTCTCAGACAGAACACCTGCTGGAAGTCGG
AnpcpA-post-3’	GAAGATGTTCATGCTAGCTGTTGGC
Full5’	TAAATATCATACTGAGAACCTACT
Full3’	CGGTCACTGGCAGAGCAGAGCGAAG
Diag-AfpyrG-3’	TAGGGACCGAGACCTGTATC
Diag-del-AnpcpA-3’	CTTCATCAATCCCTGTCATTTCCAT
PyroA-5’	TTGGCGGGTAAGTCAGATAATAG
PyroA-3’	CTGACTTGACGCTTTCTCTTGG
P3-SJW100-de	CTATTATCTGACTTACCCGCCAACTTCGGAGGGAAAACGCGT
P4-SJW100-de	AAGAGAAAGCGTCAAGTCAGAACACCTGCTGGAAGTCGG
Diag-pyroA-3’	TTGACAACATCCATAATAACACCGC
RFP 5’	GGAGCTGGTGCAGGCGCTG
RFP 3’	CTGTCTGAGAGGAGGCACTGATG
pcpA-RFP-P1	ATCGGGAACTCGCACTCACC
diag-c-pcpA-3’	GTAAGCACCCATGAGCTCTCCTG
pcpA-RFP-P2	CAGCCGCCAGCAAATCAGGAG

pcpA-RFP-P5	TCCAACCCTATCAGCTCAGAAC
pcpA-RFP-P3	CAGCGCCTGCACCAGCTCCCTCCTTCGATA ACTCCCTTC
pcpA-RFP-P4	CAGTGCCTCCTCTCAGACAGCATAGACTGAGAAATTTGAAAC
pcpA-RFP-P6	GTCACTGGCAGAGCAGAGCGAAG
pcpA-GFP-P2	TCGAGAGACGTATCCATGAGCTG
pcpA-GFP-P5	GTCACTGGCAGAGCAGAGCGAAG
TubA-RFPuu-P1	AGGATGCCTCAAACAACTACGCTC
TubA-RFPuu-P2	GGAGATGATTGACCAGGTTCTTGAC
TubA-RFPuu-P3	TCCAGCGCCTGCACCAGCTCCGTACTCAACTTCCTCACCCTC
TubA-RFPuu-P4	CAGTGCCTCCTCTCAGACAGTCTATTGGGAGCCAGGGTAG
TubA-RFPuu-P5	CGACATACCAATCCGAGGGTTTCC
TubA-RFPuu-P6	CGCACTTTACGATGCTGGGATTTC