

Supplemental Material

Journal

Applied Microbiology and Biotechnology

Title

Class VI G protein-coupled receptors in *Aspergillus oryzae* regulate sclerotia formation through GTPase-activating activity

Authors

Dong Min Kim¹, Itsuki Sakamoto¹, and Manabu Arioka^{1, 2, *}

Affiliation

^aDepartment of Biotechnology, The University of Tokyo, Japan

^bCollaborative Research Institute for Innovative Microbiology (CRIIM), The University of Tokyo, Japan

*Corresponding author

Department of Biotechnology, The University of Tokyo

1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Tel.: +81-3-5841-5163; Fax: +81-3-5841-8033

E-mail: arioka@mail.ecc.u-tokyo.ac.jp

Table S1. Overview of *A. oryzae* GPCRs and potential roles of their orthologues in *A. flavus*

Class	Gene	Amino acids	Predicted roles	Conserved domain	Observed roles in <i>A. flavus</i> (Affeldt et al. 2014)
I	<i>AogprA</i>	374	Mating	STE2 GPCR (<i>S. cerevisiae</i> pheromone receptor)	Germination; AF repression; carbon source sensing; oxylipin sensing
II	<i>AogprB</i>	465	Mating	STE3 GPCR (<i>S. cerevisiae</i> pheromone receptor)	Germination; quorum sensing; MeJA sensing
III	<i>AogprC</i>	444	Glucose sensing	Git3; Gi3C (<i>Schizosaccharomyces pombe</i> glucose receptor)	Germination; carbon and nitrogen sensing; 13(S)-HpODE sensing
	<i>AogprD</i>	415	Nitrogen sensing		Nitrogen and 13(S)-HpODE sensing; ROS, cell wall, acidic pH stress response
IV	<i>AogprF</i>	300	Nitrogen sensing	PQ loop repeat (<i>Schizosaccharomyces pombe</i> nitrogen sensor)	Light sensing; quorum sensing; acidic pH stress response; oxylipin sensing
	<i>AogprG</i>	426			ROS and acidic pH stress responses; oxylipin sensing
	<i>AogprJ</i>	322			Germination; carbon and 13(S)-HpODE sensing
	<i>AogprS</i>	266			Germination; cell wall stress response; MeJA sensing
V	<i>AogprH</i>	428	Methionine sensing	Secretin family (signal through cAMP pathways)	Germination; ROS stress response
VI	<i>AogprK-1</i>	560	Unknown	RGS domain (regulator of G protein signaling)	Germination; carbon, MeJA sensing; cell wall, osmotic, and acidic stress response
	<i>AogprK-2</i>	562			Germination; light; carbon and nitrogen sensing; osmotic and alkaline pH stress responses; lipid and oxylipin sensing
	<i>AogprR</i>	523			
VII	<i>AogprM</i>	490	Unknown	No conserved domains	Carbon, MeJA sensing, osmotic and pH stress responses
VIII	<i>AogprO</i>	282	Unknown	Hemolysin III related (broad range of ligands)	Oxylipin sensing
	<i>AogprP</i>	508			Germination; AF repression; carbon sensing; Oxylipin sensing
IX	<i>AonopA</i>	312	Light sensing	Bacteriorhodopsin-like (photoreactive)	Unknown

AF, aflatoxin; MeJA, methyl jasmonate; 13(S)-HpODE, 13S-hydroperoxy-9Z,11E-octadecadienoic acid;
ROS, reactive oxygen species

Affeldt KJ, Carrig J, Amare M, Keller NP (2014)

Global survey of canonical *Aspergillus flavus* G protein-coupled receptors. mBio 5: 1501. doi: 10.1128/mBio.01501-14

Table S2. Strain list

A. oryzae strain list

Strain	Host	Genotype	Reference
NSPID1 (Control)	NSID	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i>	Maruyama and Kitamoto (2008)
Δ AoGprK-1	NSPID1	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1	This study
Δ AoGprK-2	NSPID1	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -2	This study
Δ AoGprR	NSPID1	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprR</i>	This study
Δ AoGprK-1 <i>Δ</i> K-2	Δ AoGprK-1	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2	This study
Δ AoGprK-1 <i>Δ</i> R	Δ AoGprK-1	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprR</i>	This study
Δ AoGprK-2 <i>Δ</i> R	Δ AoGprR	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i>	This study
Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R	Δ AoGprK-1 <i>Δ</i> K-2	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i>	This study
Δ AoGprK-1+ <i>Δ</i> AoGprK-1	Δ AoGprK-1	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 <i>niaD</i> ⁻ ::(<i>AogprK</i> -1- <i>egfp</i>)	This study
Δ AoGprK-2+ <i>Δ</i> AoGprK-2	Δ AoGprK-2	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -2 <i>niaD</i> ⁻ ::(<i>AogprK</i> -2- <i>egfp</i>)	This study
Δ AoGprR+ <i>Δ</i> AoGprR	Δ AoGprR	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> - <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2+ <i>Δ</i> AoGprK-1	Δ AoGprK-1 <i>Δ</i> K-2	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 <i>niaD</i> ⁻ ::(<i>AogprK</i> -1- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2+ <i>Δ</i> AoGprK-2	Δ AoGprK-1 <i>Δ</i> K-2	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 <i>niaD</i> ⁻ ::(<i>AogprK</i> -2- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> R+ <i>Δ</i> AoGprK-1	Δ AoGprK-1 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprK</i> -1- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> R+ <i>Δ</i> AoGprR	Δ AoGprK-1 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> - <i>egfp</i>)	This study
Δ AoGprK-2 <i>Δ</i> R+ <i>Δ</i> AoGprK-2	Δ AoGprK-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprK</i> -2- <i>egfp</i>)	This study
Δ AoGprK-2 <i>Δ</i> R+ <i>Δ</i> AoGprR	Δ AoGprK-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> - <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R+ <i>Δ</i> AoGprK-1	Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprK</i> -1- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R+ <i>Δ</i> AoGprK-2	Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprK</i> -2- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R+ <i>Δ</i> AoGprR	Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> - <i>egfp</i>)	This study
Δ AoGprK-1+ <i>Δ</i> AoGprK-1 (ΔRGS)	Δ AoGprK-1	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 <i>niaD</i> ⁻ ::(<i>AogprK</i> -1 ΔRGS- <i>egfp</i>)	This study
Δ AoGprK-2+ <i>Δ</i> AoGprK-2 (ΔRGS)	Δ AoGprK-2	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -2 <i>niaD</i> ⁻ ::(<i>AogprK</i> -2 ΔRGS- <i>egfp</i>)	This study
Δ AoGprR+ <i>Δ</i> AoGprR (ΔRGS)	Δ AoGprR	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> ΔRGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2+ <i>Δ</i> AoGprK-1 (ΔRGS)	Δ AoGprK-1 <i>Δ</i> K-2	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 <i>niaD</i> ⁻ ::(<i>AogprK</i> -1 ΔRGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2+ <i>Δ</i> AoGprK-2 (ΔRGS)	Δ AoGprK-1 <i>Δ</i> K-2	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 <i>niaD</i> ⁻ ::(<i>AogprK</i> -2 ΔRGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> R+ <i>Δ</i> AoGprK-1 (ΔRGS)	Δ AoGprK-1 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprK</i> -1 ΔRGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> R+ <i>Δ</i> AoGprR (ΔRGS)	Δ AoGprK-1 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> ΔRGS- <i>egfp</i>)	This study
Δ AoGprK-2 <i>Δ</i> R+ <i>Δ</i> AoGprK-2 (ΔRGS)	Δ AoGprK-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprK</i> -2 ΔRGS- <i>egfp</i>)	This study
Δ AoGprK-2 <i>Δ</i> R+ <i>Δ</i> AoGprR (ΔRGS)	Δ AoGprK-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> ΔRGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R+ <i>Δ</i> AoGprK-1 (ΔRGS)	Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprK</i> -1 ΔRGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R+ <i>Δ</i> AoGprK-2 (ΔRGS)	Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprK</i> -2 ΔRGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R+ <i>Δ</i> AoGprR (ΔRGS)	Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> ΔRGS- <i>egfp</i>)	This study
Δ AoGprK-1+ <i>Δ</i> AoGprK-1-RGS	Δ AoGprK-1	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 <i>niaD</i> ⁻ ::(<i>AogprK</i> -1 RGS- <i>egfp</i>)	This study
Δ AoGprK-2+ <i>Δ</i> AoGprK-2-RGS	Δ AoGprK-2	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -2 <i>niaD</i> ⁻ ::(<i>AogprK</i> -2 RGS- <i>egfp</i>)	This study
Δ AoGprR+ <i>Δ</i> AoGprR-RGS	Δ AoGprR	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> RGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2+ <i>Δ</i> AoGprK-1-RGS	Δ AoGprK-1 <i>Δ</i> K-2	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 <i>niaD</i> ⁻ ::(<i>AogprK</i> -1 RGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2+ <i>Δ</i> AoGprK-2-RGS	Δ AoGprK-1 <i>Δ</i> K-2	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 <i>niaD</i> ⁻ ::(<i>AogprK</i> -2 RGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> R+ <i>Δ</i> AoGprK-1-RGS	Δ AoGprK-1 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprK</i> -1 RGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> R+ <i>Δ</i> AoGprR-RGS	Δ AoGprK-1 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> RGS- <i>egfp</i>)	This study
Δ AoGprK-2 <i>Δ</i> R+ <i>Δ</i> AoGprK-2-RGS	Δ AoGprK-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprK</i> -2 RGS- <i>egfp</i>)	This study
Δ AoGprK-2 <i>Δ</i> R+ <i>Δ</i> AoGprR-RGS	Δ AoGprK-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> RGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R+ <i>Δ</i> AoGprK-1-RGS	Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprK</i> -1 RGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R+ <i>Δ</i> AoGprK-2-RGS	Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprK</i> -2 RGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R+ <i>Δ</i> AoGprR-RGS	Δ AoGprK-1 <i>Δ</i> K-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> RGS- <i>egfp</i>)	This study
Δ AoGprK-1+ <i>Δ</i> EN/AA AoGprK-1	Δ AoGprK-1	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 <i>niaD</i> ⁻ ::(<i>AogprK</i> -1 EN/AA RGS- <i>egfp</i>)	This study
Δ AoGprK-2+ <i>Δ</i> EN/AA AoGprK-2	Δ AoGprK-2	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -2 <i>niaD</i> ⁻ ::(<i>AogprK</i> -2 EN/AA RGS- <i>egfp</i>)	This study
Δ AoGprR+ <i>Δ</i> EN/AA AoGprR	Δ AoGprR	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> EN/AA RGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2+ EN/AA AoGprK-1	Δ AoGprK-1 <i>Δ</i> K-2	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 <i>niaD</i> ⁻ ::(<i>AogprK</i> -1 EN/AA RGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> K-2+ <i>Δ</i> EN/AA AoGprK-2	Δ AoGprK-1 <i>Δ</i> K-2	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprK</i> -2 <i>niaD</i> ⁻ ::(<i>AogprK</i> -2 EN/AA RGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> R+ <i>Δ</i> EN/AA AoGprK-1	Δ AoGprK-1 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprK</i> -1 EN/AA RGS- <i>egfp</i>)	This study
Δ AoGprK-1 <i>Δ</i> R+ <i>Δ</i> EN/AA AoGprR	Δ AoGprK-1 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i> ⁻ Δ <i>ligD</i> :: <i>argB</i> Δ <i>pyrG</i> :: <i>adeA</i> Δ Ao <i>gprK</i> -1 Δ Ao <i>gprR</i> <i>niaD</i> ⁻ ::(<i>AogprR</i> EN/AA RGS- <i>egfp</i>)	This study
Δ AoGprK-2 <i>Δ</i> R+ <i>Δ</i> EN/AA AoGprK-2	Δ AoGprK-2 <i>Δ</i> R	<i>niaD</i> ⁻ <i>sC</i> ⁻ <i>adeA</i> ⁻ Δ <i>argB</i> :: <i>adeA</i>	

ΔAoGprK-1ΔK-2ΔR+EN/AA AoGprK-2	ΔAoGprK-1ΔK-2ΔR	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ ΔligD::argB ΔpyrG::adeA ΔAogprK-1 ΔAogprK-2 ΔAogprR niaD⁻ ::(AogprK-2 EN/AA RGS-egfp)</i>	This study
ΔAoGprK-1ΔK-2ΔR+EN/AA AoGprR	ΔAoGprK-1ΔK-2ΔR	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ ΔligD::argB ΔpyrG::adeA ΔAogprK-1 ΔAogprK-2 ΔAogprR niaD⁻ ::(AogprR EN/AA RGS-egfp)</i>	This study
NSRku70-1-1A	NSRku70-1-1	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ Δku70::arg adeA</i>	Higuchi et al. (2009)
SK-1	NSRku70-1-1	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ Δku70::arg adeA niaD⁻ ::(PamyB niaD)</i>	
pUt-gpaA Q/L mut	NSRku70-1-1A	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ Δku70::arg adeA niaD⁻ ::(PamyB -AogpaB QL mut niaD)</i>	This study
pUt-gpaB Q/L mut	NSRku70-1-1A	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ Δku70::arg adeA niaD⁻ ::(PamyB -AogpaB QL mut niaD)</i>	This study
pUt-ganA Q/L mut	NSRku70-1-1A	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ Δku70::arg adeA niaD⁻ ::(PamyB -AoganA QL mut niaD)</i>	This study
Δ3 sC	ΔAoGprK-1ΔK-2ΔR	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ ΔligD::argB ΔpyrG::adeA ΔAogprK-1 ΔAogprK-2 ΔAogprR pisCA (sC)</i>	This study
Δ3 ΔgpaA	ΔAoGprK-1ΔK-2ΔR	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ ΔligD::argB ΔpyrG::adeA ΔAogprK-1 ΔAogprK-2 ΔAogprR AogpaA::sC</i>	This study
Δ3 ΔgpaB	ΔAoGprK-1ΔK-2ΔR	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ ΔligD::argB ΔpyrG::adeA ΔAogprK-1 ΔAogprK-2 ΔAogprR AogpaB::sC</i>	This study
Δ3 ΔganA	ΔAoGprK-1ΔK-2ΔR	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ ΔligD::argB ΔpyrG::adeA ΔAogprK-1 ΔAogprK-2 ΔAogprR AoganA::sC</i>	This study
NSPID1 velB OE	NSID	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ ΔligD::argB ΔpyrG::adeA niaD⁻ ::(PamyB-AovelB niaD)</i>	This study
NSPID1 ΔvelB	NSID	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ ΔligD::argB ΔpyrG::adeA AovelB:sC</i>	This study
Δ3 velB OE	ΔAoGprK-1ΔK-2ΔR	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ ΔligD::argB ΔpyrG::adeA ΔAogprK-1 ΔAogprK-2 ΔAogprR niaD⁻ ::(PamyB-AovelB niaD)</i>	This study
Δ3 ΔvelB	ΔAoGprK-1ΔK-2ΔR	<i>niaD⁻ sC⁻ adeA⁻ ΔargB::adeA⁻ ΔligD::argB ΔpyrG::adeA ΔAogprK-1 ΔAogprK-2 ΔAogprR AovelB::sC</i>	This study

S. cerevisiae strain list

Strain	Host	Genotype	Reference
BY4741		<i>MAT</i> a; <i>ura3</i> Δ0; <i>leu2</i> Δ0; <i>his3</i> Δ1; <i>met15</i> Δ0	Euroscarf (Frankfurt, Germany)
BY4741 (<i>URA3⁺</i> , <i>HIS3⁺</i>)	BY4741	<i>MAT</i> a; <i>ura3</i> Δ0; <i>leu2</i> Δ0; <i>his3</i> Δ1; <i>met15</i> Δ0; BYP5232 (P _{FUS1} - <i>lacZ</i> <i>URA3</i>); pYES2-His3 (<i>HIS3</i>)	This study
YO6055 (<i>Δsst2</i>)	BY4741	<i>MAT</i> a; <i>ura3</i> Δ0; <i>leu2</i> Δ0; <i>his3</i> Δ1; <i>met15</i> Δ0; <i>YLR452c::kanMX4</i>	Euroscarf
YO6055 Control (<i>Δsst2</i> , <i>URA3⁺</i> , <i>HIS3⁺</i>)	Y06055	<i>MAT</i> a; <i>ura3</i> Δ0; <i>leu2</i> Δ0; <i>his3</i> Δ1; <i>met15</i> Δ0; <i>YLR452c::kanMX4</i> ; BYP5232 (P _{FUS1} - <i>lacZ</i> <i>URA3</i>); pYES2-His3 (<i>HIS3</i>)	This study
YO6055 K-1 RGS (AoGprK-1 RGS)	Y06055	<i>MAT</i> a; <i>ura3</i> Δ0; <i>leu2</i> Δ0; <i>his3</i> Δ1; <i>met15</i> Δ0; <i>YLR452c::kanMX4</i> ; BYP5232 (P _{FUS1} - <i>lacZ</i> <i>URA3</i>); pYES2-His3 (P _{GAL1} - <i>AogprK-1</i> <i>rgs</i> <i>HIS3</i>)	This study
YO6055 K-1 RGS (AoGprK-2 RGS)	Y06055	<i>MAT</i> a; <i>ura3</i> Δ0; <i>leu2</i> Δ0; <i>his3</i> Δ1; <i>met15</i> Δ0; <i>YLR452c::kanMX4</i> ; BYP5232 (P _{FUS1} - <i>lacZ</i> <i>URA3</i>); pYES2-His3 (P _{GAL1} - <i>AogprK-2</i> <i>rgs</i> <i>HIS3</i>)	This study
YO6055 R RGS (AoGprR RGS)	Y06055	<i>MAT</i> a; <i>ura3</i> Δ0; <i>leu2</i> Δ0; <i>his3</i> Δ1; <i>met15</i> Δ0; <i>YLR452c::kanMX4</i> ; BYP5232 (P _{FUS1} - <i>lacZ</i> <i>URA3</i>); pYES2-His3 (P _{GAL1} - <i>AogprR</i> <i>rgs</i> <i>HIS3</i>)	This study

Table S3. Primer list

Primer name	Sequence (5' to 3')	Target gene	Comments
GW ΔgprK-1 Up-F	GGGGACAAC TTT GTATAGAAAAGTTGAGATGTACGGAAGGATTCTATTAAT	<i>AogprK-1</i>	Construct AoGprK-1 deletion strain (Gateway cloning system)
GW ΔgprK-1 Up-R	GGGGACTGCTTTTTTGTACAAACTTGGATGGCGGTGTTTCGCTGAA	<i>AogprK-1</i>	Construct AoGprK-1 deletion strain (Gateway cloning system)
GW ΔgprK-1 DR-F	GGGGACAGCTTTCTTGTACAAAGTGGAAGGGTGGGCCTTTGTGACTTC	<i>AogprK-1</i>	Construct AoGprK-1 deletion strain (Gateway cloning system)
GW ΔgprK-1 DR-R	GATGGCGGTGTTTCGCTGAAAGAAT	<i>AogprK-1</i>	Construct AoGprK-1 deletion strain (Gateway cloning system)
GW ΔgprK-1 Down-F	ATTCTTTCAGCGAAACACCCGCCATCTCGGCTTCTCGGGCTCGT	<i>AogprK-1</i>	Construct AoGprK-1 deletion strain (Gateway cloning system)
GW ΔgprK-1 Down-R	GGGGACAAC TTT GTATAATAAAAGTTGCCTCCAAACCCCTTCCCTACAA	<i>AogprK-1</i>	Construct AoGprK-1 deletion strain (Gateway cloning system)
GW ΔgprK-2 Up-F	GGGGACAAC TTT GTATAGAAAAGTTGAGCCCACTCGCGGTCGCTGTCTATA	<i>AogprK-2</i>	Construct AoGprK-2 deletion strain (Gateway cloning system)
GW ΔgprK-2 Up-R	GGGGACTGCTTTTTTGTACAAACTTGGATGTGCGATGTTTCTCGTACTACA	<i>AogprK-2</i>	Construct AoGprK-2 deletion strain (Gateway cloning system)
GW ΔgprK-2 DR-F	GGGGACAGCTTTCTTGTACAAAGTGGGAAAACCGAACGGGACTATTTGC	<i>AogprK-2</i>	Construct AoGprK-2 deletion strain (Gateway cloning system)
GW ΔgprK-2 DR-R	GATGTGCGATGTTTCTCGTACTACA	<i>AogprK-2</i>	Construct AoGprK-2 deletion strain (Gateway cloning system)
GW ΔgprK-2 Down-F	TGTAGTACGAGAAACATCGCACATCCTAGCCTGTAGACAGCACGG	<i>AogprK-2</i>	Construct AoGprK-2 deletion strain (Gateway cloning system)
GW ΔgprK-2 Down-R	GGGGACAAC TTT GTATAATAAAAGTTGGATCCATGCGATTAGGTTATGCAGG	<i>AogprK-2</i>	Construct AoGprK-2 deletion strain (Gateway cloning system)
GW ΔgprR Up-F	GGGGACAAC TTT GTATAGAAAAGTTGGCTTGTGAGCAATACGGAGTAACG	<i>AogprR</i>	Construct AoGprR deletion strain (Gateway cloning system)
GW ΔgprR Up-R	GGGGACTGCTTTTTTGTACAAACTTGACTTGATGGTCACTGGTCACGAA	<i>AogprR</i>	Construct AoGprR deletion strain (Gateway cloning system)
GW ΔgprR DR-F	GGGGACAGCTTTCTTGTACAAAGTGGCAGGTCAACCCATTGCACCAATT	<i>AogprR</i>	Construct AoGprR deletion strain (Gateway cloning system)
GW ΔgprR DR-R	ACTTGATGGTCACTGGTCACGAA	<i>AogprR</i>	Construct AoGprR deletion strain (Gateway cloning system)
GW ΔgprR Down-F	TCTTCGTGACCAGTGACCATCAAGTTTAAAGATAGAGAACTTCCGGGAG	<i>AogprR</i>	Construct AoGprR deletion strain (Gateway cloning system)
GW ΔgprR Down-R	GGGGACAAC TTT GTATAATAAAAGTTGACACCACGGGAGATTGGAAAC	<i>AogprR</i>	Construct AoGprR deletion strain (Gateway cloning system)
F-Native gprK-1	ACAACGAGCTGGGAACCCCACTGGTCTGTAGCCGAGAT	<i>AogprK-1</i>	Construct AoGprK-1 native promoter strain
R-gprK-1	CGCCCTTGCTCACCATCCCGACAGCCTCCTCCTTAATG	<i>AogprK-1</i>	Construct AoGprK-1 native promoter strain
F-Native gprK-2	ACAACGAGCTGGGAACCCCTCACTGGTGTAGATGGCTG	<i>AogprK-2</i>	Construct AoGprK-2 native promoter strain
R-gprK-2	GATTGACATGAAGACTGTCCCATGGTGAGCAAGGGCG	<i>AogprK-2</i>	Construct AoGprK-2 native promoter strain
F-Native gprR	ACAACGAGCTGGGAACCCCAACACATCAAGCAAGGGTT	<i>AogprR</i>	Construct AoGprR native promoter strain
R-gprR	ACAACGCCCTTGCTCACCATCCCGAATGTGATGGACAAGTCGTC	<i>AogprR</i>	Construct AoGprR native promoter strain
F-Inver K-1 RGS 7TMx	ATGTGGGAGGTGATGCGCCA	AoGprK-1 RGS domain	Construct AoGprK-1 RGS domian strain
R-Inver K-1 RGS 7TMx	GATGGCGGTGTTTCGCTGAA	AoGprK-1 RGS domain	Construct AoGprK-1 RGS domian strain
F-Inver K-2 RGS 7TMx	ATGTGGGAGGTGAGGCGCCG	AoGprK-2 RGS domain	Construct AoGprK-2 RGS domian strain
R-Inver K-2 RGS 7TMx	GATGTGCGATGTTTCTCGTA	AoGprK-2 RGS domain	Construct AoGprK-2 RGS domian strain
F-Inver R RGS 7TMx	ATGTCCAGAAAGCAGCGAGA	AoGprR RGS domain	Construct AoGprR RGS domian strain
R-Inver R RGS 7TMx	ACTTGATGGTCACTGGTCAC	AoGprR RGS domain	Construct AoGprR RGS domian strain
R-AoGprK-1 7TM RGSx	ACAACGCCCTTGCTCACCATCCCGCAGGGAAGAAAGACAGTG	AoGprK-1 7TM region	Construct AoGprK-1 7TM region strain
R-AoGprK-2 7TM RGSx	ACAACGCCCTTGCTCACCATCCCGCAGGGAATAATGATGGTG	AoGprK-2 7TM region	Construct AoGprK-2 7TM region strain
R-AoGprR 7TM RGSx	ACAACGCCCTTGCTCACCATCCCGACAATAAGCTCATAACAT	AoGprR 7TM region	Construct AoGprR 7TM region strain
Inv-E87,N88AMutK1-F	GTCGCCTTCCTCACCAGCGTCGCCG	AoGprK-1 RGS domain	Construct AoGprK-1 RGS EN/AA point mutation strain
Inv-E87,N88AMutK1-R	AGCAGCGCCGGAAAAGTCGTTGAGGG	AoGprK-1 RGS domain	Construct AoGprK-1 RGS EN/AA point mutation strain
Inv-E87,N88AMutK2-F	ATTGCCTTCCTGGTGAGCGTGTCTC	AoGprK-2 RGS domain	Construct AoGprK-2 RGS EN/AA point mutation strain
Inv-E87,N88AMutK2-R	AGCAGCACCAGAGAAGTCATGAAGAG	AoGprK-2 RGS domain	Construct AoGprK-2 RGS EN/AA point mutation strain
Inv-E87,N88AMutR-F	ATCATCTTCCTGAATTATGTGCGAG	AoGprR RGS domain	Construct AoGprR RGS EN/AA point mutation strain
Inv-E87,N88AMutR-R	AGCAGCGCCGCTGAATTCCTTCACTG	AoGprR RGS domain	Construct AoGprR RGS EN/AA point mutation strain
L-6myc-gprK-1,2&R-RGS-NotI-F	ATTTGCGGCCGCATGGAGCAGAAACTGATTT	AoGprK-1,2 R RGS domain	Insertion of class VI GPCR RGS domein for pheromon assay
L-6myc-gprK1-RGS-XbaI-R	GCTCTAGATCAGACAGCCTCCTCCTTAA	AoGprK-1 RGS domain	Insertion of AoGprK-1 RGS domein for pheromon assay
L-6myc-gprK2-RGS-XbaI-R	GCTCTAGATTAGACAGTCTTCATGTCAATCCCA	AoGprK-2 RGS domain	Insertion of AoGprK-2 RGS domein for pheromon assay
L-6myc-gprR-RGS-XbaI-R	GCTCTAGACAGAATGTGATGGACAAGTCGT	AoGprR RGS domain	Insertion of AoGprR RGS domein for pheromon assay
pUtNAN-gpaA-F	GAATTCGAGCTCGGTACCCATGGGTTTCGTGTGTAAGCA	<i>AogpaA</i>	Construct <i>AogpaA</i> in pUt-NAN plasmid
pUtNAN-gpaA-R	CCCTCTACTACAGATCCCCCTACAAGATACCTGAATCCTT	<i>AogpaA</i>	Construct <i>AogpaA</i> in pUt-NAN plasmid
pUtNAN-gpaB-F	GAATTCGAGCTCGGTACCCATGGGTTGTATGGGCTCAA	<i>AogpaB</i>	Construct <i>AogpaB</i> in pUt-NAN plasmid

pUtNAN-gpaB-R	CCCTCTACTACAGATCCCCTCATAAGATAAGAGTGTGCAG	<i>AogpaB</i>	Construct <i>AogpaB</i> in pUt-NAN plasmid
pUtNAN-ganA-F	GAATTCGAGCTCGGTACCCATGGGTGCGGAATGAGTA	<i>AoganA</i>	Construct <i>AoganA</i> in pUt-NAN plasmid
pUtNAN-ganA-R	CCCTCTACTACAGATCCCCTTAAATCAGACCACAGAGTC	<i>AoganA</i>	Construct <i>AoganA</i> in pUt-NAN plasmid
AogpaA mutant F	GGACtcCGCAGCGAGCGGA	<i>AogpaA</i>	Insertion of mutation AG(3253 ~ 3254) → TC / Q → L in <i>AogpaA</i>
AogpaA mutant R	ACCCACATCAAACATGTGTATGCTCAGCTG	<i>AogpaA</i>	Insertion of mutation AG(3253 ~ 3255) → TC / Q → L in <i>AogpaA</i>
AogpaB mutant F	TGGCCtcCGGTCTGAGCGC	<i>AogpaB</i>	Insertion of mutation AA(3262 ~ 3263) → TC / Q → L in <i>AogpaB</i>
AogpaB mutant R	CCAACATCCATCATTTCGGAAGTTCA	<i>AogpaB</i>	Insertion of mutation AA(3262 ~ 3264) → TC / Q → L in <i>AogpaB</i>
AoganA mutant F	GGTGGTctCGTTCCGAACG	<i>AoganA</i>	Insertion of mutation AA(3244 ~ 3245) → TC / Q → L in <i>AoganA</i>
AoganA mutant R	GACGTCGAACATCCGGTATGTC	<i>AoganA</i>	Insertion of mutation AA(3244 ~ 3246) → TC / Q → L in <i>AoganA</i>
F- gpaA 1000 up	AGCTCGGTACCCGGGGATCGCTGCTCCAGTCATGTAC	<i>AogpaA</i>	Construct <i>gpaA</i> deletion strain
R- gpaA 1000 up	CATCTGCGAGATCGGATCGGTTGGCGGGTATTTAGTCA	<i>AogpaA</i>	Construct <i>gpaA</i> deletion strain
F- gpaA 1000 down	AATCTCAGAAAATCCGCGGTGATTAGGGTGCTTTGATTG	<i>AogpaA</i>	Construct <i>gpaA</i> deletion strain
R- gpaA 1000 down	AGGTCGACTCTAGAGGATCGTTGAGGGCCTGGACC	<i>AogpaA</i>	Construct <i>gpaA</i> deletion strain
F- gpaB 1000 up	AGCTCGGTACCCGGGGATCCCGGTCCACGTTTTAC	<i>AogpaB</i>	Construct <i>gpaB</i> deletion strain
R- gpaB 1000 up	CATCTGCGAGATCGGATCGCTCGGATTATATTCTAGACAGC	<i>AogpaB</i>	Construct <i>gpaB</i> deletion strain
F- gpaB 1000 down	AATCTCAGAAAATCCGCGGACTTGAATGCTCTGCGC	<i>AogpaB</i>	Construct <i>gpaB</i> deletion strain
R- gpaB 1000 down	AGGTCGACTCTAGAGGATCCCTACTGTTGAGAGACTACC	<i>AogpaB</i>	Construct <i>gpaB</i> deletion strain
F- ganA 1000 up	AGCTCGGTACCCGGGGATCCGATGTACCACTTGTGG	<i>AoganA</i>	Construct <i>ganA</i> deletion strain
R- ganA 1000 up	CATCTGCGAGATCGGATCGGTTGTCTATGATTTTCTCTCAC	<i>AoganA</i>	Construct <i>ganA</i> deletion strain
F- ganA 1000 down	AATCTCAGAAAATCCGCGGCAGAGACGATTGGCTTCC	<i>AoganA</i>	Construct <i>ganA</i> deletion strain
R- gnaA 1000 down	AGGTCGACTCTAGAGGATCCGGTTAGAAGTCGTCTGAG	<i>AoganA</i>	Construct <i>ganA</i> deletion strain
F-velB OE	GAATTCGAGCTCGGTACCCATGTATGCTATCGAAGAAA	<i>velB</i>	Construct <i>velB</i> overexpress strain
R-velB OE	ACAAGAAAGCTGGGTCCCCATCGTAATCGTCCCCATCG	<i>velB</i>	Construct <i>velB</i> overexpress strain
F- velB 1000 up	AGCTCGGTACCCGGGGATCCTGTACGTGATTTTAAAGA	<i>velB</i>	Construct <i>velB</i> deletion strain
R- velB 1000 up	ACATCTGCGAGATCGGATCGGGCGTGAGAGTGGAATAAA	<i>velB</i>	Construct <i>velB</i> deletion strain
F- velB 1000 down	AAATCTCAGAAAATCCGCGGGAAAGTCGTGTTGCTTTGC	<i>velB</i>	Construct <i>velB</i> deletion strain
R- velB 1000 down	AGGTCGACTCTAGAGGATCTGGATACTCTCCTGCTCTT	<i>velB</i>	Construct <i>velB</i> deletion strain
F- sC	CGATCCGATCTCGCAGATG	<i>sC</i>	Construct <i>velB</i> deletion strain (<i>sC</i> marker)
R- sC	AATCTCAGAAAATCCGCGG	<i>sC</i>	Construct <i>velB</i> deletion strain (<i>sC</i> marker)
qPCR velB-F	TCAACGTGGGTACTCAATCCAG	<i>velB</i>	Investigation of gene expression level using qRT-PCR
qPCR velB-R	GGGAAATTTCTTGGCGGAGAAG	<i>velB</i>	Investigation of gene expression level using qRT-PCR
qPCR laeA-F	TTCTTGGGACCTGATTCACCTG	<i>laeA</i>	Investigation of gene expression level using qRT-PCR
qPCR laeA-R	TCGATCTCCACCTGTTCAAACC	<i>laeA</i>	Investigation of gene expression level using qRT-PCR
qPCR veA-F	ACTTCCACCTGTACGAGGAAAC	<i>veA</i>	Investigation of gene expression level using qRT-PCR
qPCR veA-R	TCGAGACGAAACTCCAGGAATG	<i>veA</i>	Investigation of gene expression level using qRT-PCR
qPCR vosA-F	CGTCAATCGACTTTGGTAACCG	<i>vosA</i>	Investigation of gene expression level using qRT-PCR
qPCR vosA-R	ACCCTTGCA TGATAGGAGTTGG	<i>vosA</i>	Investigation of gene expression level using qRT-PCR
qPCR nsdD F2	TGTGCCCAATGAGCCTATGG	<i>nsdD</i>	Investigation of gene expression level using qRT-PCR
qPCR nsdD R2	CCGAAGTGATAGAACCGGCA	<i>nsdD</i>	Investigation of gene expression level using qRT-PCR
qPCR sclR F2	TAGGGACCATCTGCCAGCTC	<i>sclR</i>	Investigation of gene expression level using qRT-PCR
qPCR sclR R2	ACGACGTTGGGTATGAGCAC	<i>sclR</i>	Investigation of gene expression level using qRT-PCR

*Underlined letters in the sequence indicate the sequence of the plasmid

*Small letters in the sequence indicate the mutant sequence point

AoGpaA	MGC	GMS	----	---TEDK----	-----EGKARN	EEIENQLKRD	KMMQRNEIKM	LLL	GAGESGK	S	ILKQMKLI			
AoGpaB	MGS	CVST	EP	DN	-----EPKKRS	QAIDRRLEED	SRRLRRECKI	LLL	SSGESGK	S	IVKQMKII			
AoGanA	MGC	MGS	----	---KPVDTTDK	DALQRNARID	KVLKN----	KKVMDRTIKI	LLL	GAGESGK	S	TIKQMRII			
Gpa1	MGC	TVS	----	---TQTIGDES	DPFLQNKRRAN	DVIEQSLQLE	KQRDKNEIKL	LLL	GAGESGK	S	VLKQLKLL			
Gi	MGC	TL	S	----	AEDKAAVERS	KMIDRNLRD	GEKAAREVKL	LLL	GAGESGK	S	IVKQMKII			
Gs	MGC	LGN	----	---SKTEDQR	NEEKAQREAN	KKIEKQLQKD	KQVYRATHRL	LLL	GAGESGK	S	IVKQMRII			
Clustal Consensus	***								*****		***::***:::			
AoGpaA	HEGGYSRD	----	----	----	---ERESFKE	IIYSNTVQSM	RVILEAMESL	ELPLEDAR	----	----	----			
AoGpaB	HQNGYTV	----	----	----	---ELALYRL	TVCKNLLDCA	KSLVGAYHQF	SLEPSSQK	----	----	----			
AoGanA	HSGGFDD	----	----	----	---ERRQTRA	VIYSNVVIAF	KVLLDIMRTE	SIEFEQEK	----	----	----			
Gpa1	HQGGFSHQ	----	----	----	---ERLQYAQ	VIWADAIQSM	KILIIQARKL	GIQLDCDDPI	----	NNKDLFACKR	----			
Gi	HEAGYSEE	----	----	----	---ECKQYKA	VVYSNTIQSI	IAIIRAMGRL	KIDFGDSAR	----	----	----			
Gs	HVNGFNDEGG	----	----	----	EEDPQAARSN	SDGEKATKVQ	DIKNNLKEAI	ETIVAAMSNI	----	VPPVELANP	----			
Clustal Consensus	*	*	:		*									
AoGpaA	HEYHVQTI	FIM	QPAQIEG	DNL	PP	-----	----	----	----	----	----			
AoGpaB	VRDYVQYI	SD	YNIDDP	PHTT	LD	-----	----	----	----	----	----			
AoGanA	TKPLADY	MDT	LES	DVGS	DEA	FS	----	----	----	----	----			
Gpa1	ILLKAKAL	DY	INASV	AGGSD	FLNDY	VLYKYS	ERYETRRRVQ	STGRAKAAFD	EDGNISNVKS	DTDRDAETVT	----			
Gi	-ADDARQL	FV	LAGAA	EEGF	M	TA	----	----	----	----	----			
Gs	-ENQFRVDYI	LSVMNV	PDFD	FP	----	----	----	----	----	----	----			
Clustal Consensus														
AoGpaA	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----			
AoGpaB	-----	-----	-----	-----	-----	-----	-----	-----	A	KVGEAITYIW	N-DPCTSTVL			
AoGanA	-----	-----	-----	-----	-----	-----	-----	-----	DL	KVRDAMRDMW	N-DAGVQKAV			
Gpa1	QNEADADR	NNNS	SRINLQ	DICK	DLNQEG	DDQM	FVRKTSREIQ	GQNRRLNIHE	-----	DIAKAIKQLW	NNDKGIKQCF			
Gi	-----	-----	-----	-----	-----	-----	-----	-----	-----	ELAGVIKRLW	K-DSGVQACF			
Gs	-----	-----	-----	-----	-----	-----	-----	-----	P	EFYEHAKALW	E-DEGVRACY			
Clustal Consensus										*	*			
AoGpaA	KRSREYQL	ND	SAKYYF	DAIE	RIAQPDY	LPT	DQDVLRSRVK	TTGITETTFI	IGDLTYRMF	D	VGGQ	R	SERKK	
AoGpaB	EHQNEFY	LMD	SAPYFF	EEAK	RIASPDF	IPN	VNDVLRARTK	TTGIYETRFT	MGQLSIHMF	D	VGGQ	R	SERKK	
AoGanA	ARGHEFAL	HD	NLHYFF	DSLD	RIFAPGW	LPD	NQDMLQARLR	TTGITETLFE	LGMNFRMD	D	VGGQ	R	SERKK	
Gpa1	ARSNEFQ	LEG	SAAYYF	DNIE	KFASPNY	VCT	DEDILKGRIK	TTGITETEFN	IGSSKFKVL	D	AGGQ	R	SERKK	
Gi	NRSREYQL	ND	SAAYY	LDND	RIAQPNY	IPT	QQDVLRTVRK	TTGIYETHFT	FKDLHFKMF	D	VGGQ	R	SERKK	
Gs	ERSNEYQL	ID	CAQYFL	DKID	VIKQADY	VPS	DQDLLRCRVL	TSGIFETKFQ	VDKVNFHMF	D	VGGQ	R	DERRK	
Clustal Consensus	:	*	*	*	:	*	*	*	*	*	*	*	*	
AoGpaA	WIHCFENV	TT	ILFLVAI	SEY	DQLLFE	DET	V	NRMQEALT	TF	DSICNSRWF	V	KTSIILFL	LNK	IDRFKEKLPV
AoGpaB	WIHCFENV	TS	IIFCVAL	SEY	DQVLLE	ESNQ	NRMMESL	VLF	DSVVNSRWF	M	RTSIILFL	LNK	VDLFRQKLP	
AoGanA	WIHCFEGV	QC	LLFMVAL	SGY	DQCLVED	QNA	NQMHEAM	MLF	ESLVNGEW	F	RKPIILFL	LNK	IDLFKGKLSV	
Gpa1	WIHCFEG	ITA	VLFVLAM	SEY	DQMLFE	DERV	NRMHESI	MLF	DTLLNSK	WF	DTPFILFL	LNK	IDLFEEKVKS	
Gi	WIHCFEG	VTA	IIFCVAL	SDY	DLVLA	ED	DEEM	NRMHESM	KLF	DSICNNK	WFT	DTSIILFL	LNK	KDLFEKIKK
Gs	WIQCFND	VTA	IIFVVAS	SSY	NMVIRED	NQT	NRLQEA	LNLF	KSIWNNR	WLR	TISVILFL	LNK	QDLAEKVL	
Clustal Consensus	***	***	:	*	*	*	*	*	*	*	*	*	*	:
AoGpaA	--SPMKNY	FP	DYEGG	-ADYA	AACDYI	LN	----	----	----	RFVSLNQ	AEQKQIY	THF	TCATDTTQIR	
AoGpaB	--SPLSNY	FP	DYSGG	-NDVN	RAAKYLL	W	----	----	----	RFNQVNR	AH-LNLY	PHL	TQATDTTNIR	
AoGanA	--SPVSKH	FP	DYNGS	NTDFD	AAARYF	AD	----	----	----	RFGRGIR	IPDREI	YIHY	TNATDTTLLK	
Gpa1	--MPIRKY	FP	DYQGRV	GDAE	AGLKYF	EK	----	----	----	IFLSLNK	TN-KPIY	VKR	TCATDTQTMK	
Gi	--SPLTIC	YP	EYAGS	NTYEE	AAAYIQ	CQ	----	----	----	FEDLNKR	KDTKEI	YTHF	TCATDTKNVQ	
Gs	GKSKIEDY	FP	EFARYT	TPPED	ATPEP	GEDPR	VTRAKYF	IRD	EFLRIST	ASG	DGRHYCY	PHF	TCAVDTENIR	
Clustal Consensus	:	*	:	*	:	*	:	*	:	*	*	*	*	:
AoGpaA	FVMAAVN	DII	IQENL	RLCGL	I									
AoGpaB	LVFAAVK	ETI	LQNAL	KDSGI	L									
AoGanA	ATMDSV	QDMI	IQKNL	HTLIL	-									
Gpa1	FVLSAVT	DLI	IQQNL	KKIGI	I									
Gi	FVFDVAV	TDVI	IKNNL	KDCGL	F									
Gs	RVFNDCR	DII	QRMHL	RQYEL	L									
Clustal Consensus	:	*	:	*	:	*	:	*	:	*	*	*	*	:

Fig. S1

Sequence alignment of three *Gα* proteins in *A. oryzae*, AoGpaA, AoGpaB, and AoGanA, with yeast Gpa1, human Gαi, and human Gαs

Box in purple, the N-terminal myristoylation and palmitoylation sequences. Box in red, the guanine nucleotide binding motifs (GXGXXGKS). Box in blue, the GTPase domains (DXXGQ). To exogenously express GTPase-deficient mutants, the constructs in which Gln residue in the GTPase domain marked by the red asterisk was mutated to Leu were introduced to the control strain.

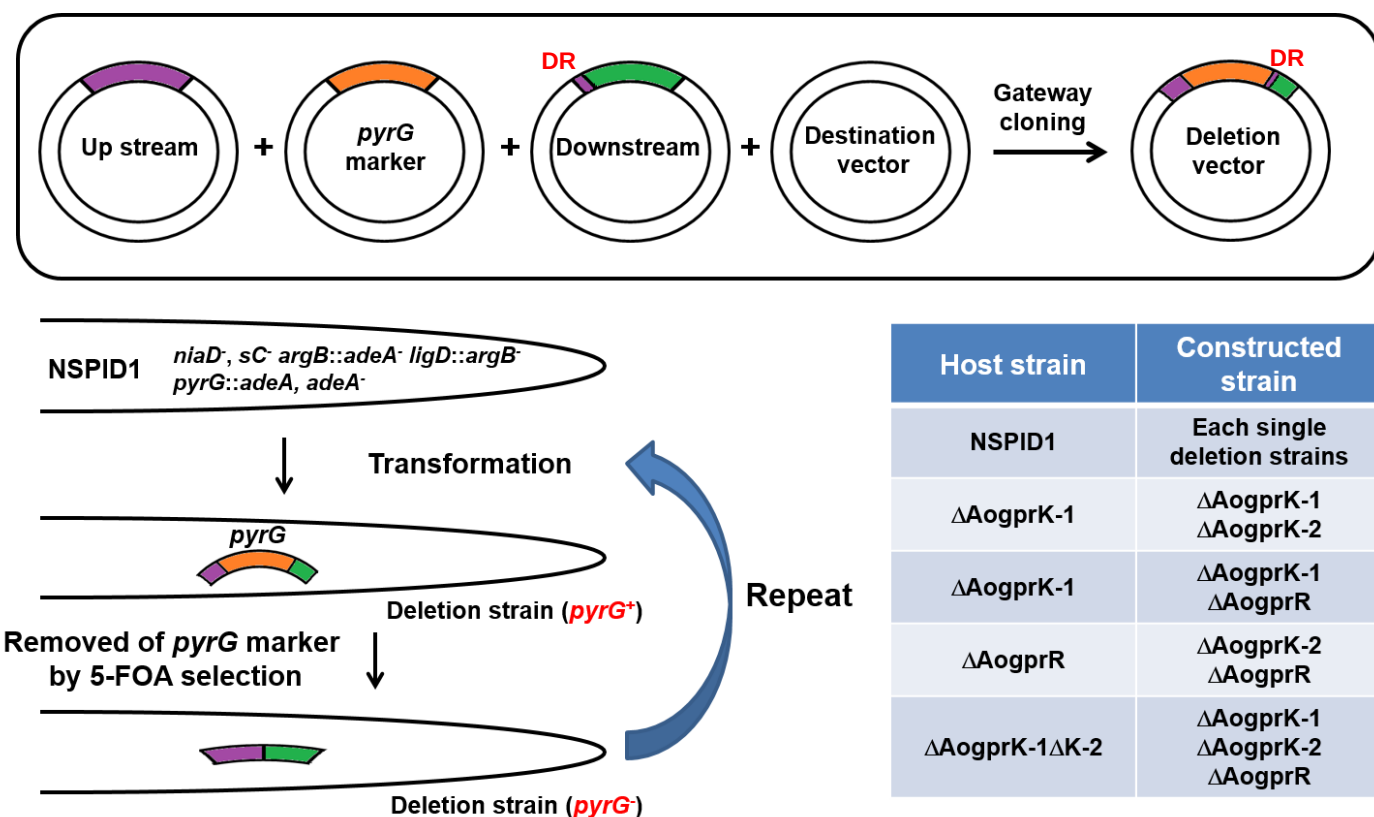


Figure S2

Construction of single and multiple gene deletion mutants using the marker recycling system

Strains with single and multiple gene deletion were constructed using the *pyrG* marker recycling system as described in the Materials and methods. The *pyrG*⁺ transformants with the deletion of target gene were grown on the medium containing 5-FOA to eliminate *pyrG* marker by homologous recombination. The resultant *pyrG*⁻ hosts were used for the subsequent gene deletion. The host and constructed strains are shown in the table in the right.

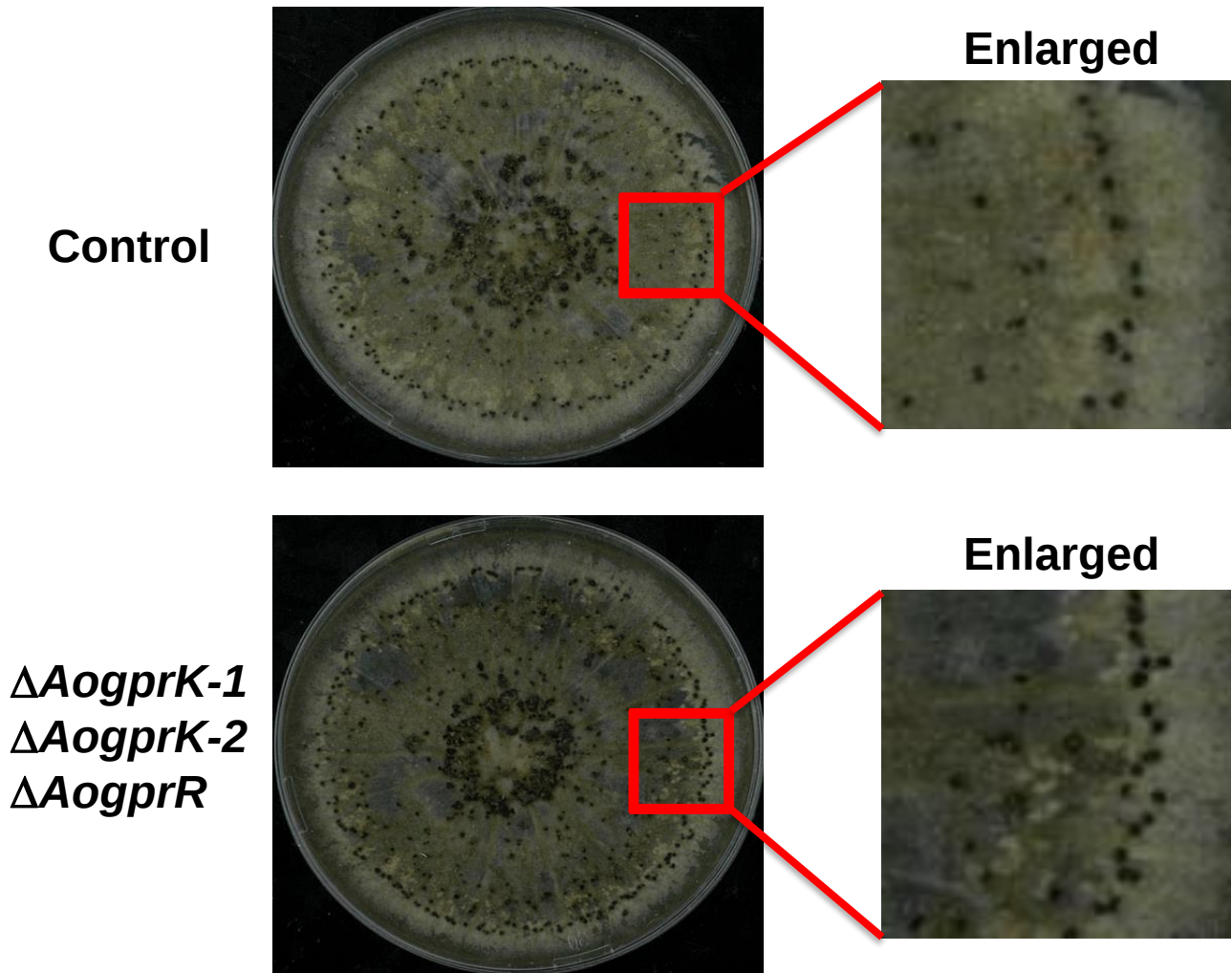


Fig. S3

Images of plates of the control and the triple gene deletion mutant

Control and the triple gene deletion mutant were grown on the DPY agar plates containing uridine and uracil for 20 days at 30°C. The plates were washed with 70% ethanol and observed from the bottom.

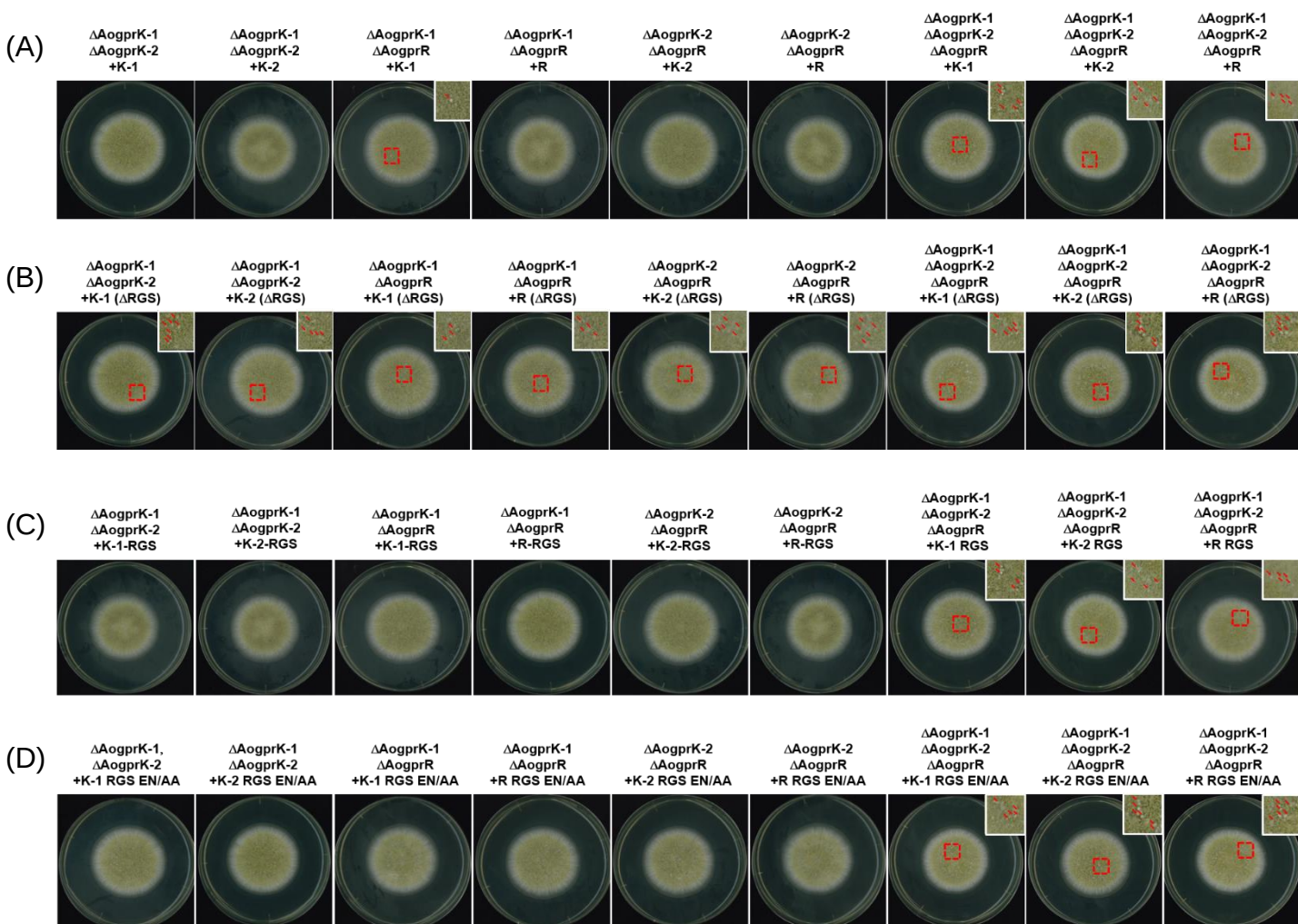


Fig. S4

Sclerotia formation in the complemented strains

Conidial suspensions ($10^3/5 \mu\text{L}$) were spotted on the DPY agar plates containing uridine and uracil, and incubated for 4 days at 30°C . Sclerotia formation in the strains complemented by the full-length constructs (A), the constructs containing only the 7-TM domain (B), the constructs containing only the RGS domain (C), and the full-length constructs containing EN/AA mutation in the RGS domains (D), respectively, was observed. The genotypes of the host strains are shown at the top, and the complemented gene is indicated by '+' (e.g. '+AogprK-1' indicates that the AogprK-1 was complemented, while ' Δ RGS' indicates that only the 7-TM domain was complemented).

	TM1										TM2									
AoGprK-1	MGSELGITPE	TKPQAVYTPV	SIWWACWAGV	WTTAVALGMI	YLIANRNMP	LIRIRIGMSL	SAIVLLHLYW													
AoGprK-2	MGSELGVNPN	SKPRVSYTPV	SIWWCWGCT	WTTVIALGMA	YLIARNTPA	LRVRGLALS	SAIVVLIHYW													
AoGprR	-----MAS	TYPDGI FDNL	GKEYASVAIV	WMVALVGSV	FLVLRHEQC															
Clustal Consensus	..	: *	: :	: :	: *	: :	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *
	TM3										TM4									
AoGprK-1	ASVQFGVMIG	PIMPQDAQYW	IMGTYLGGI	ALFHASNTRF	LHVAKHQKRF	AHHNSRISES	VPDEKPKG--													
AoGprK-2	LSVQFGTMIG	ALMPGDVEYW	IMGTYLPCGI	ALFHLSNSQF	LYVAKLQRKY	VNYDSRCIRP	ATSLRPKA--													
AoGprR	ILCLMAYTMA	GAYPCGVEYW	IMSYLPLGI	ALFQANSML	LSVSGIQEKM	LHTAHPQRA	SYSTGSKGPN													
Clustal Consensus	..	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *
	TM5										TM6									
AoGprK-1	IAFFQFFWAW	IVAPFVLWKA	RHIHDTQGWR	VQTIGCAIAN	LHATPMWLIA	LY--VPAMQV	VNQYWIPPOW													
AoGprK-2	SIFWQSEFSW	IFAPIVLWKS	RRYDTQGWR	VQTIGCAIAN	LHATPMWLVA	LY--VPAMES	VNQYWLPPQW													
AoGprR	SILWQLFWSW	IFAPCILWKI	RKIREIHYWR	LQITICVIAA	LPGSPLWFIA	LNSTAEPWIT	INRYWVPALW													
Clustal Consensus	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *
	TM7										RGS domain region									
AoGprK-1	ICLSIWIMEI	FTVFLPCWEV	MRHHALRQET	FNAIEQWESK	MKKSQSEARS	LNSTPTLVDS	MMSGWKSNG													
AoGprK-2	ICLSILVIEI	FTTILPCWEV	RRRGASAERM	SSLITQKKLQ	HKKAISRFS	LSPTSTIAN	VTLDLETDNN													
AoGprR	FAPGIIAMEG	VTIFFPCYEL	IVSRKQRDRI	LGEIRAWNEK	KGGD-----	-----	--SESDSGTS													
Clustal Consensus	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *
	RGS domain region										RGS domain region									
AoGprK-1	SVDTTG-SRD	SILTMGALEH	VLERNPAPLQ	KFSALNDFSG	ENVAELTSTA	EWKNSLPKAL	RENTDPMN													
AoGprK-2	SVDMISDARN	NTLTINSLDY	ILEQDPAPLQ	TFAALHDFSG	ENIAFLVSVS	HWKSSLQQA	RNSTTPGGDC													
AoGprR	RSHAGSSRTN	ELYTIKALEK	CLSEDSHALL	RFAAVKEFGS	ENIIFLNIVR	DWKATWARIN	AKNPEYDWHR													
Clustal Consensus	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *
	RGS domain region										RGS domain region									
AoGprK-1	MKEILHEREN	-RALHIYVKE	ISVSQAEEFPV	NISSQDIRKI	ENIEEGPARS	LYGEKRAAVD	PVTPFDTPSF													
AoGprK-2	ETGLIREHEN	-RALRIYVDF	ISVYHAEFPV	NISSKDLKRL	EAVEEGPTRA	LYG-DMRDVD	PATPFEASDN													
AoGprR	DPQYHRLYFF	KIAVEIYSAC	VNLKTAEFPI	NVESRIYSGI	TTMFG--EAV	QCSGRRVSRG	AATHMEEDTR													
Clustal Consensus	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *
	RGS domain region										RGS domain region									
AoGprK-1	PMKSLSSPSF	GNGSQVELHP	VSSDDRQVFW	GEVPEAFGPT	VFENDAEKSIK	YIVLTNTWPK	FVKSRSSSDP													
AoGprK-2	SSKARLSPAS	LEG----AEQ	VSQVTNIFYT	GDVPETFTT	IFDDAQDSIK	YIVLTNTWPK	EVRTIQSLAD													
AoGprR	ALCLDENPYV	SQS-----I	MQIKSRVPDS	VVVPSEFIS	VEDEAEKSIL	ALVETNTWPK	FIDSSDDLS													
Clustal Consensus	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *
	RGS domain region										RGS domain region									
AoGprK-1	IKEEAV----	--																		
AoGprK-2	SSDIVGIDMK	TV																		
AoGprR	ITE-----	--																		
Clustal Consensus	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *	: *

Fig. S5

Sequence alignment of *A. oryzae* class VI GPCRs

The amino acid sequences of *A. oryzae* class VI GPCRs are aligned using Clustal W (<https://www.genome.jp/tools-bin/clustalw>). The underlines in red and blue indicate the transmembrane and RGS domains, respectively. For the complementation analysis, residues 1-360 in AoGprK-1, 1-361 in AoGprK-2, and 1-333 in AoGprR were used as the 7-TM domains; residues 361-559 in AoGprK-1, 362-561 in AoGprK-2, and 334-522 in AoGprR were used as the RGS domains. Amino acid residues (EN) shared by human RGS4 and mutated to AA in this study are marked by red asterisks.

(A)

HsRGS4	-SFDKLMHS-	PAGRSVFRAF	LRTEYSEENM	LFWLACEELK	AEANQHVVDE	KAR-----	-----
AoGprK-2 RGS	NSLDYILEQD	PAPLQTFAAL	H--DFSGENI	AFLVSVSHWK	SSLQQAIRNS	TTPGGDCFTG	LIREHFNRL
Clustal Consensus	** * : : . .	** . . * * :	: : * * :	* : : . . *	: . : * : : . .	: :	
HsRGS4	LIYEDYVSIL	S-----	-----	-----	-----PK	EVSLDSR---	----VREGIN
AoGprK-2 RGS	RIYVDFISVY	HAEFPVNISS	KDLKRLEAVF	EGPTRALYGD	MRDVPDPTPF	EASDNSSKAR	LSPASLEGAE
Clustal Consensus	** * : : *				* * * : *		** :
HsRGS4	KKMQEP----	-----SA	HTFDDAQLQI	YTLMHRDSYP	RFLSSPTYRA	LL---	
AoGprK-2 RGS	QVSQVTNIFY	TGDVPETFST	TIFDDAQDSI	KYLVLNTNWP	KFVRTLQSLA	DSSDI	
Clustal Consensus	: * .	* :	***** . *	* : : : *	: * : : *		

(B)

AoGprK-1 RGS	GALEHVLERN	PAPLQKFSAI	NDFSGENVAF	LTSVAEWKNS	LPKALRENTD	PMDNNMKELL	HERFN-RALH
AoGprK-2 RGS	NSLDYILEQD	PAPLQTFAAL	HDFSGENIAF	LVSVSHWKSS	LQQAIRNSTT	PGGDCFTGLI	REHFN-RALR
AoGprR RGS	-ALEKCLSED	SHALLRFAAV	KEFSGENIIF	LNYVRDWKAT	WARINAKNPE	YDWHRDPQYH	RLYFFKIAVE
Clustal Consensus	** : * . . :	. . * * : *	: : * * * : *	* * * . * * :	: : . . .		: * * : .
AoGprK-1 RGS	IYVKFISVSQ	AEFPVNISSQ	DLRKLENIFE	GPARSLYGEK	RAAVDPVTPF	DTPSFPMKSL	SSPSFGNGSQ
AoGprK-2 RGS	IYVDFISVYH	AEFPVNISSK	DLKRLEAVFE	GPTRALYGD	MRDVPDPTPF	EASDNSSKAR	LSPASLEG--
AoGprR RGS	IYSACVNLKT	AEFPINVESR	IYSGLTMMFG	--EAVQCSGR	RVSRGAATHM	EEDTRALCLD	ENPYVSQS--
Clustal Consensus	** : : . .	***** : * :	* : *	--EAVQCSGR	RVSRGAATHM	EEDTRALCLD	ENPYVSQS--
AoGprK-1 RGS	VELHPVSSDD	RVQFWGEVPE	AFGPTVFNDA	EKSIKYLVL	NTWPKFVKSR	RSSDPIKE-	
AoGprK-2 RGS	--AEQVSQVT	NIFYTGDVPE	TFSTTIFDDA	QDSIKYLVLT	NTWPKFVRTL	QSLADSSDI	
AoGprR RGS	----IMQIKS	RVPDSVVVPS	NFGISVFDEA	EKSILALVFT	NTWPKFIDSS	SDDL SITF-	
Clustal Consensus	: .	: .	** .	* : : * : *	: . * * * * :		

Fig. S6

Sequence alignment of RGS domains of class VI GPCRs and human RGS4

(A) Sequence alignment of RGS domain of AoGprK-2 and human RGS4 (accession: P49798). Conserved EN residues are marked by red asterisks. (B) Sequence alignment of RGS domains of AoGprK-1, AoGprK-2, and AoGprR. Conserved EN residues that were mutated to AA in this study are marked by red asterisks.

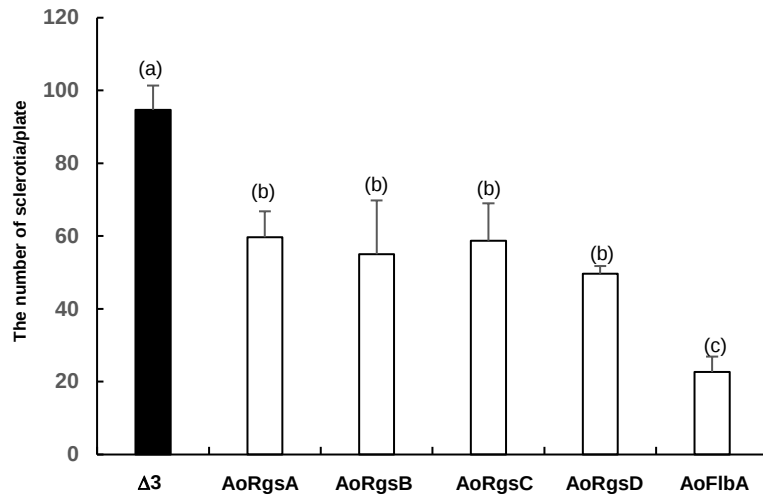


Fig. S7

Sclerotia formation in the strains overexpressing RGS domain-containing proteins in the triple gene deletion background ($\Delta 3$)

The triple gene deletion mutant ($\Delta 3$) and the strains overexpressing RGS domain-containing proteins (RgsA, RgsB, RgsC, RgsD, and FlbA) in the $\Delta 3$ background were grown on DPY+UU agar plate for 4 d at 30°C and the sclerotia formation was examined. Different letters indicate that they show significant difference (n=3).

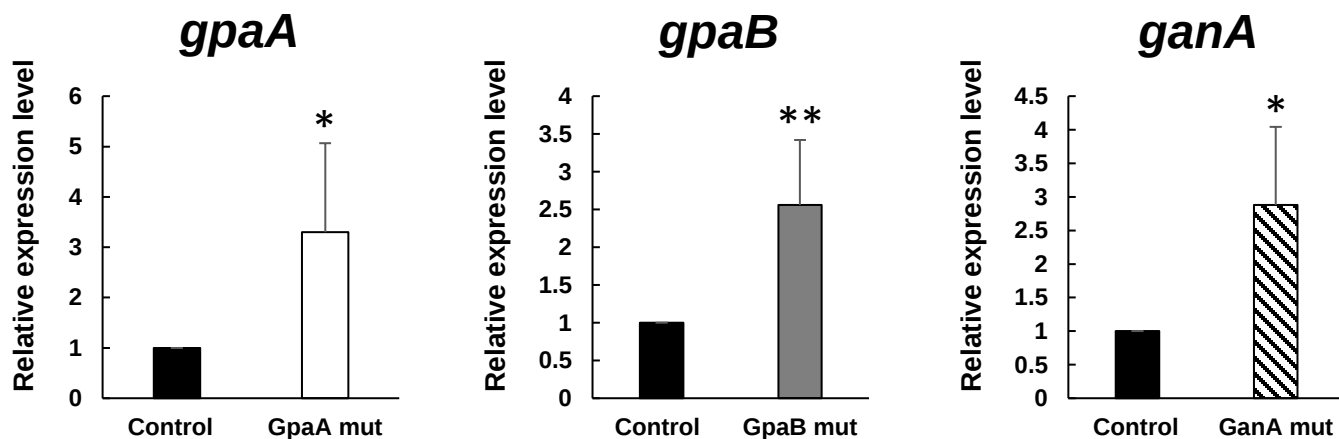


Fig. S8

Relative expression levels of the genes encoding G α subunits in the control and the mutants overexpressing GTPase-deficient form of G α (G α mut strains)

Control and G α mut strains were grown for 6 d at 30°C on the CD+Met agar plates containing dextrin instead of glucose as a carbon source. After incubation, the relative expression levels of each G α were analyzed by qPCR. Error bars represent S.D. (* and **, $p < 0.05$ and $p < 0.01$ by Student's t -test, respectively; $n=4$).

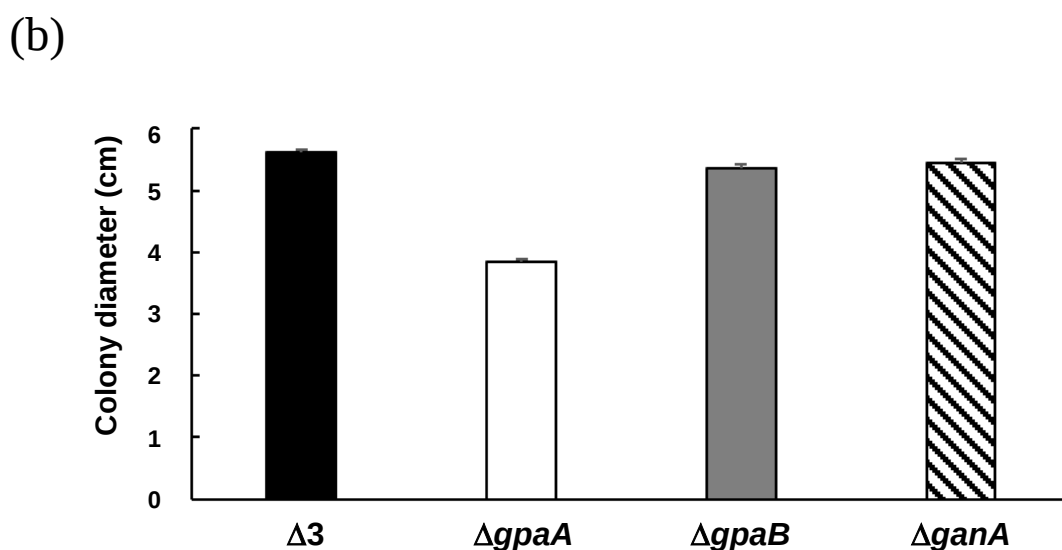
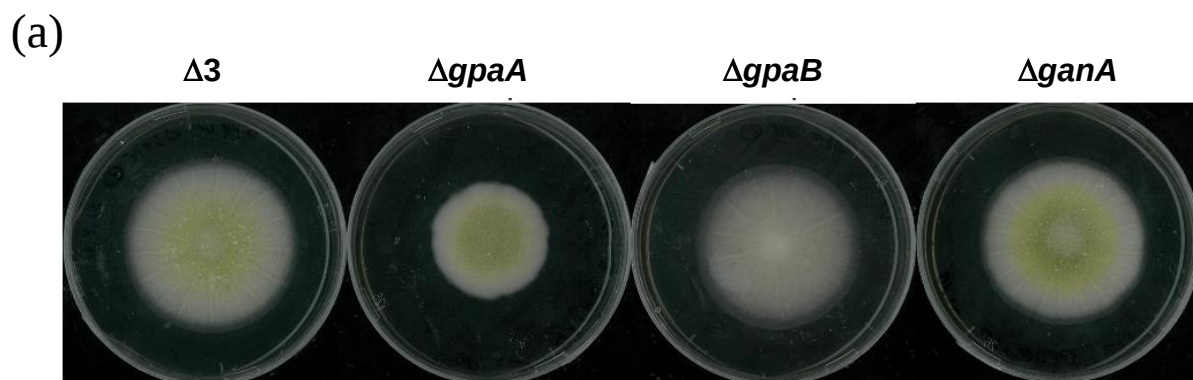


Fig. S9

Growth of the strains deleted for the genes encoding Gα subunits in the triple gene deletion background

The triple gene deletion mutant (Δ3) and the strains deleted for the genes encoding Gα subunits in the Δ3 background were grown on DPY+UU agar plate for 4 d at 30°C. Colony photograph (a) and colony diameter (b) (n=3) are shown.

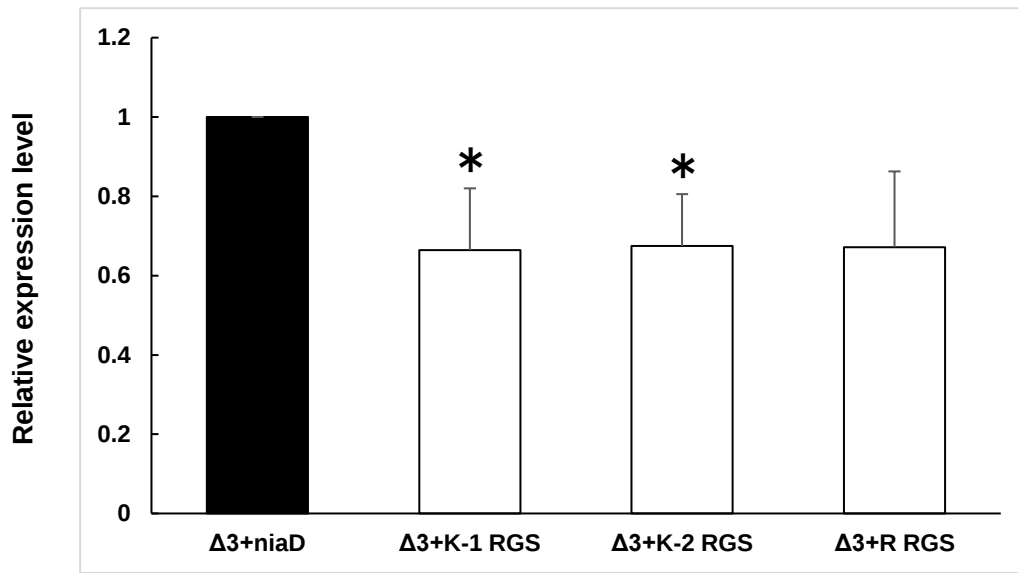


Fig. S10

Expression of *velB* in the triple gene deletion mutant complemented by one of the constructs carrying only the RGS domain of either AoGprK-1, AoGprK-2, or AoGprR

The triple gene deletion mutant ($\Delta 3$) transformed by the vector ($\Delta 3 + \text{niaD}$) or one of the constructs carrying only the RGS domain of either AoGprK-1, AoGprK-2, or AoGprR were examined for the expression of *velB*. Error bars represent S.D. (*, $p < 0.05$ by Student's *t*-test; $n=3$).