Supplementary information

Figure S1. Generation of PKA deletion and over-expression mutants. (A) Deletion of *pkaC2*. The *pkaC2* ORF was replaced with the phleomycin resistance cassette (PHLEO) via the spit marker method. Southern blot analysis of BamHI-digested genomic DNA revealed the loss of the WT 1.48 Kb band, consistent with *pkaC2* deletion. Several clones demonstrated addition ectopic integrations and were not used for further analyses. Black rectangle represents the probe. (B) Deletion of *pkaC1*. The *pkaC1* ORF was replaced with the hygromycin resistance cassette (HYG) in both the WT and Δ*pkaC2* strains. Southern Blot analysis of Pst1 digested genomic DNA identified the expected 4.14 Kb band in WT (left blot) or Δ*pkaC2* (right blot), which was truncated in Δ*pkaC1* or Δ*pkaC1*Δ*pkaC2* mutants. Black rectangle represents the probe. (C) Over-expression of *pkaC2*. The *pkaC1* mutant was transformed with a construct that placed *pkaC2* under control of the *gpdA* promoter. RT-PCR revealed a large increase in the steady-state mRNA levels of *pkaC2* in the Δ*pkaC1*::*PgpdA-pkaC2* transformants relative to either the WT or the Δ*pkaC1* parental strain. The upper band in the Δ*pkaC1*::*PgpdA-pkaC2* lane corresponds to unspliced transcript. qRT-PCR confirmed the over-expression (right).

Figure S2. Trehalose assay. Conidia were harvested from AMM plates cultured at 37^{o}C and then adjusted to equivalent densities with dH_20. Conidial suspensions were then incubated at 100 ^{o}C for twenty minutes and lysate supernatants were incubated with trehalase overnight at 37^{o}C. Following trehalase incubations, samples were assayed for the presence of glucose (Sigma, GAHK20). Data represent mean glucose concentrations in each sample, following subtraction of respective no-trehalase controls. Error bars represent the ± SD of a triplicate experiment. No statistical difference in glucose was detected between any of the strains.

Figure S3. Hypersensitivity of the PKA mutants to SDS. Conidia were point inoculated into wells that contained increasing concentrations of SDS in AMM agar. Plates were incubated at 37^{o}C for 3 d.

Figure S4. Growth and virulence phenotypes of additional Δ*pkaC1*Δ*pkaC2* isolates. (A) Radial growth rates of several Δ*pkaC1*Δ*pkaC2* isolates compared to the growth rate of the Δ*pkaC1* strain. The data represent changes in colony diameter between 24 and 48 h incubation on AMM medium at 37^{o}C. Error bars represent the ± SD of a triplicate experiment. (B) CF-1 mice were immunosuppressed with triamcinolone acetonide and inoculated intranasally with 10^5 conidia. Mortality of mice infected with two additional Δ*pkaC1*Δ*pkaC1* isolates was indistinguishable from saline controls.

Figure S5. Histopathology of infected mice at 48 h post-infection. Fungal lesions with associated neutrophilic infiltration are observed in all groups. Notably, sparse fungal growth could be seen in Δ*pkaC1*Δ*pkaC2* infected mice.

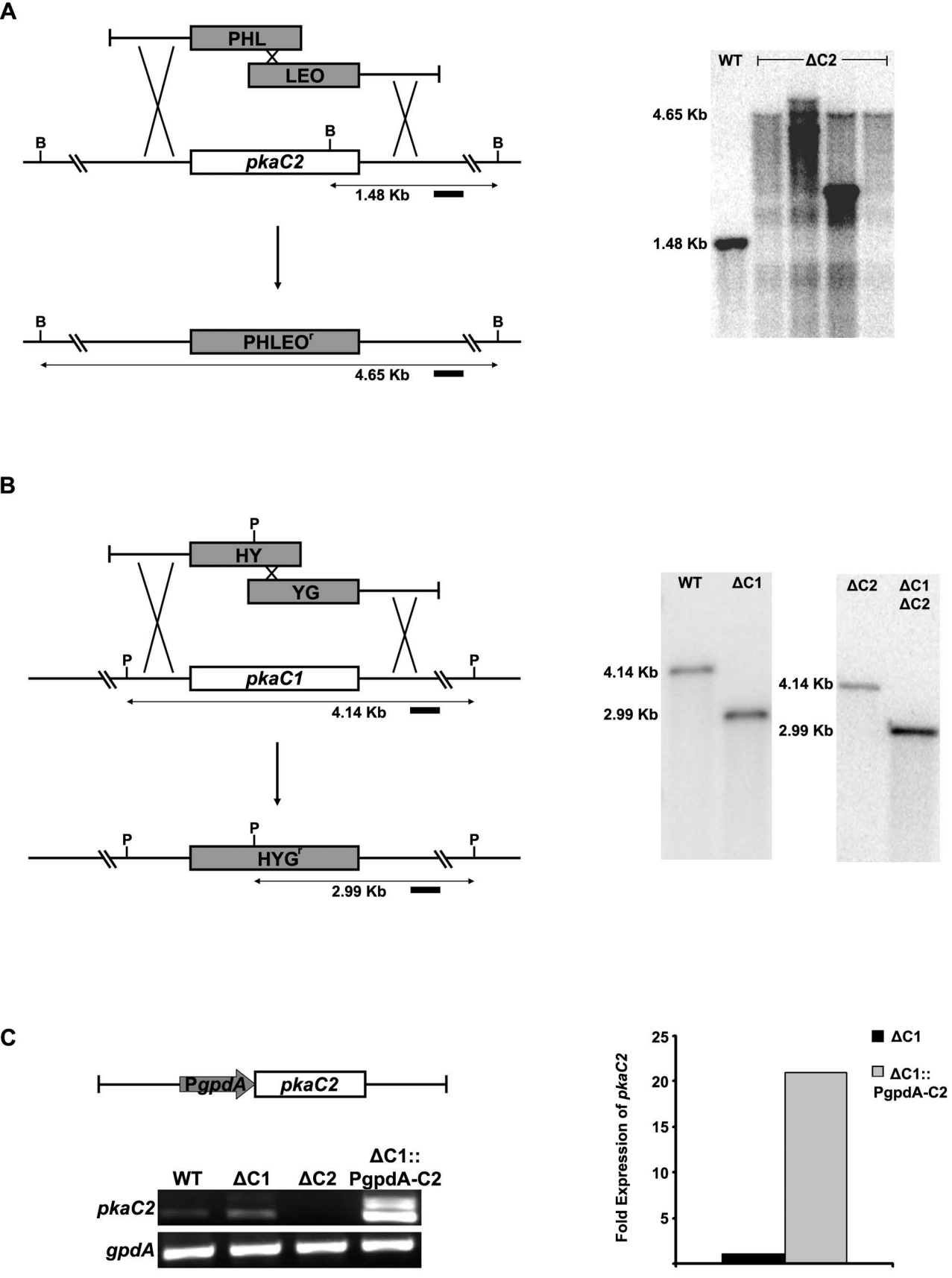
Figure S6. Expression of *ags3*. qRT-PCR was used to measure message levels of the transcript for *ags3* in the WT and the Δ*pkaC1* mutant. The primer is pair is that described by Ejzykowicz, et al., 2009, and is given in Table 1.

Figure S7. *In vitro* phosphorylation of the PKA substrate kemptide. Conidia of the indicated strains were shaken in YG at 37^{o}C overnight. Total protein was isolated by crushing the mycelium in liquid nitrogen and suspending the lysate in extraction buffer [25 mM Tris-HCl, 1 mMEDTA, 1mM DTT, 50 mM calyculin A phosphatase inihibitor (Invitrogen), protease inhibitor cocktail (Sigma, Cat.# P2714)]. Protein concentrations were determined by the BCA assay. Equivalent protein amounts were tested for the ability to phosphorylate kemptide

(Invitrogen Kit, Cat.#V5340). Phosphorylated substrate migrates towards the anode (bottom), while non-phosphorylated substrate migrates towards the cathode (top).

Table 1. Oligonucleotides used in this study

Primer	Description	Sequence (5'-3')
101	*pkaC2* LA F	GTTTGCAGTTTTCACCCCGC
102	*pkaC2* LA R	GTCGTGACTGGGAAAACCCTGGCGTCGAGCACAGCGGGGAATG
103	*pkaC2* RA F	TCCTGTGTGAAATTGTTATCCGCTGCTCCTGCCACGACGTTACG
104	*pkaC2* RA R	AGTTTCAGTCCTGGTGTTGG
398	M13F-PHL F	CGCCAGGGTTTTCCCAGTCACGACAAGTGGAAAGGCTGGTGTGC
408	PHL R	TGCTCGCCGATCTCGGTCAT
410	LEO F	GACAAGGTCGTTGCGTCAGTC
409	LEO R	AGCGGATAACAATTTCACACAGGATTAAAGCCTTCGAGCGTCC
109	*pkaC2* probe F	CTTTGTGAACTTGCTTTGCG
110	*pkaC2* probe R	TTCCATTTCGGATGCGTGC
105	*pkaC1* LA F	ATGAAGTCACCAAGCTAGAGG
106	*pkaC1* LA R	GTCGTGACTGGGAAAACCCTGGCGGAGGAAACGAGAGTTAAAAG
107	*pkaC1* RA F	TCCTGTGTGAAATTGTTATCCGCTCGACATTTGATAGAGCAATG
108	*pkaC1* RA R	TCCTCCGCCGAACACACGTG
395	HY R	CTCCATACAAGCCAACCACGG
396	YG F	CGTTGCAAGACCTGCCTGAA
399	YG R	AGCGGATAACAATTTCACACAGGATCGCGTGGAGCCAAGAGCGG
201	*PgpdA-pkaC2* LA F	CTGTTTTCTTATCCCTTTCG
202	*PgpdA-pkaC2* LA R	GCACACCAGCCTTTCCACTTGCAAGCACATCATTGATTCG
203	*gpdA* promoter F	CGAATCAATGATGTGCTTGCAAGTGGAAAGGCTGGTGTGC
204	*gpdA* promoter R	CCTTTCCTGTAGCCATTGGGAACGGCACTGGTCAACTTGG
205	*PgpdA-pkaC2* RA F	CCAAGTTGACCAGTGCCGTTCCCAATGGCTACAGGAAAGG
206	*PgpdA-pkaC2* RA R	GAATCACTGCCTTAGAAATC
501	*gpdA* qPCR F	AGATCAAGCAGGCCATCAAG
502	*gpdA* qPCR R	GTAACCCCACTCGTTGTCGT
503	*pdbA* qPCR F	ATCCTGGGTGAAGAGGTTGC
504	*pdbA* qPCR R	GAAGGTCATAAACTCGCAGATAGG
505	*pkaC2* qPCR F	TGAGGTCATCCACAACAGCG
506	*pkaC2* qPCR R	CTCACTCGGATTGGTCTTGC
507	*gpdA* RT-PCR F	TCATCAACGACAAGTTCGGC
508	*gpdA* RT-PCR R	ACAACACGGCGAGAGTAACC
509	*pkaC2* RT-PCR F	AACAGAGCCTATATATGCTG
510	*pkaC2* RT-PCR R	TGGATGACCTCAGGAGCTAG
575	*ags3* RT-PCR F	CTTTGGAAGATGGTCCTGGT
576	*ags3* RT-PCR R	ACAAATCTATCGGCCTCCAC


A
PHL
LEO
B
B
B
pkaC2
1.48 Kb
B
B
PHLEO^r
4.65 Kb
WT
ΔC2
4.65 Kb
1.48 Kb
B
P
HY
YG
P
P
pkaC1
4.14 Kb
P
P
P
HYG^r
2.99 Kb
WT
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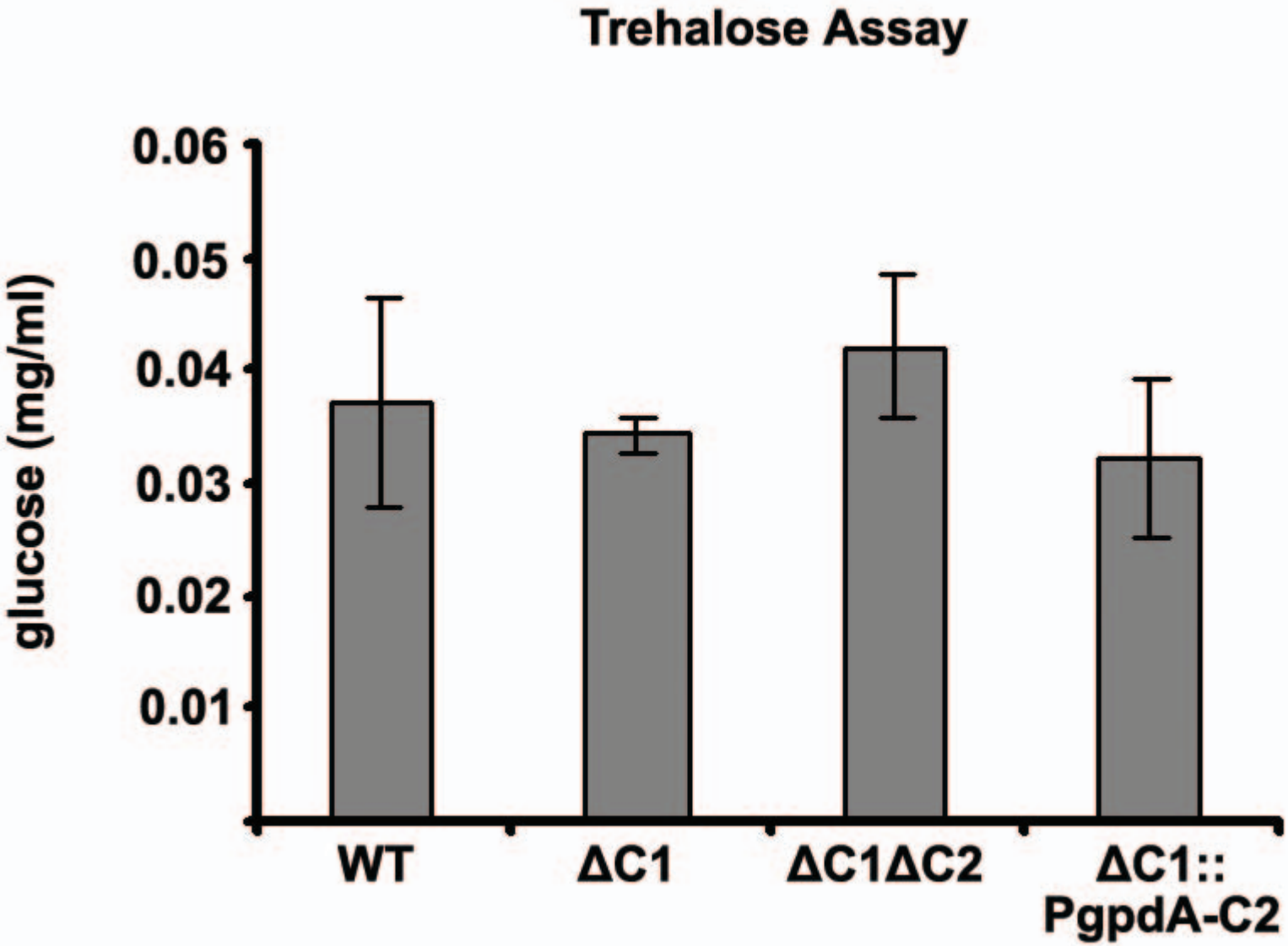
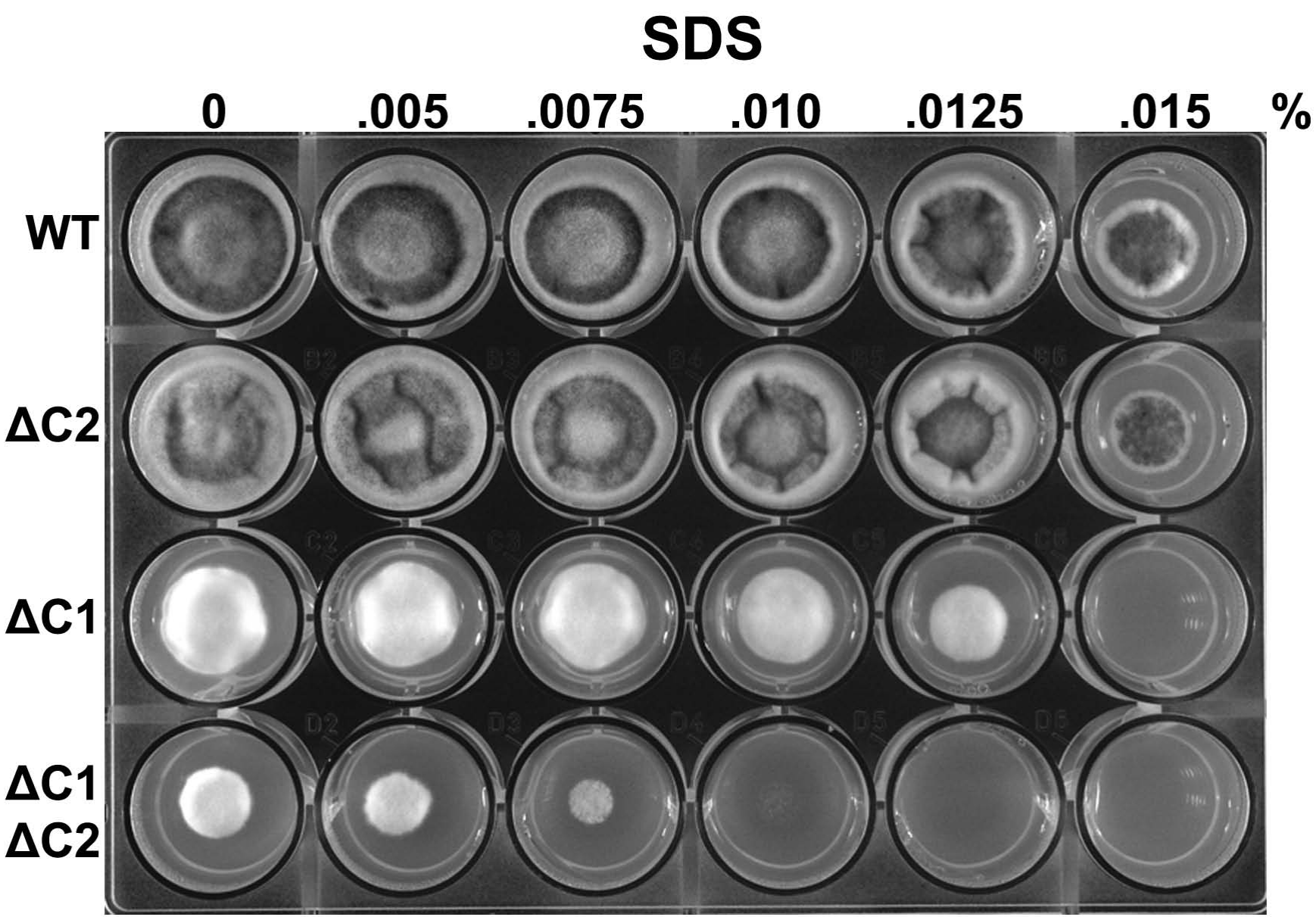
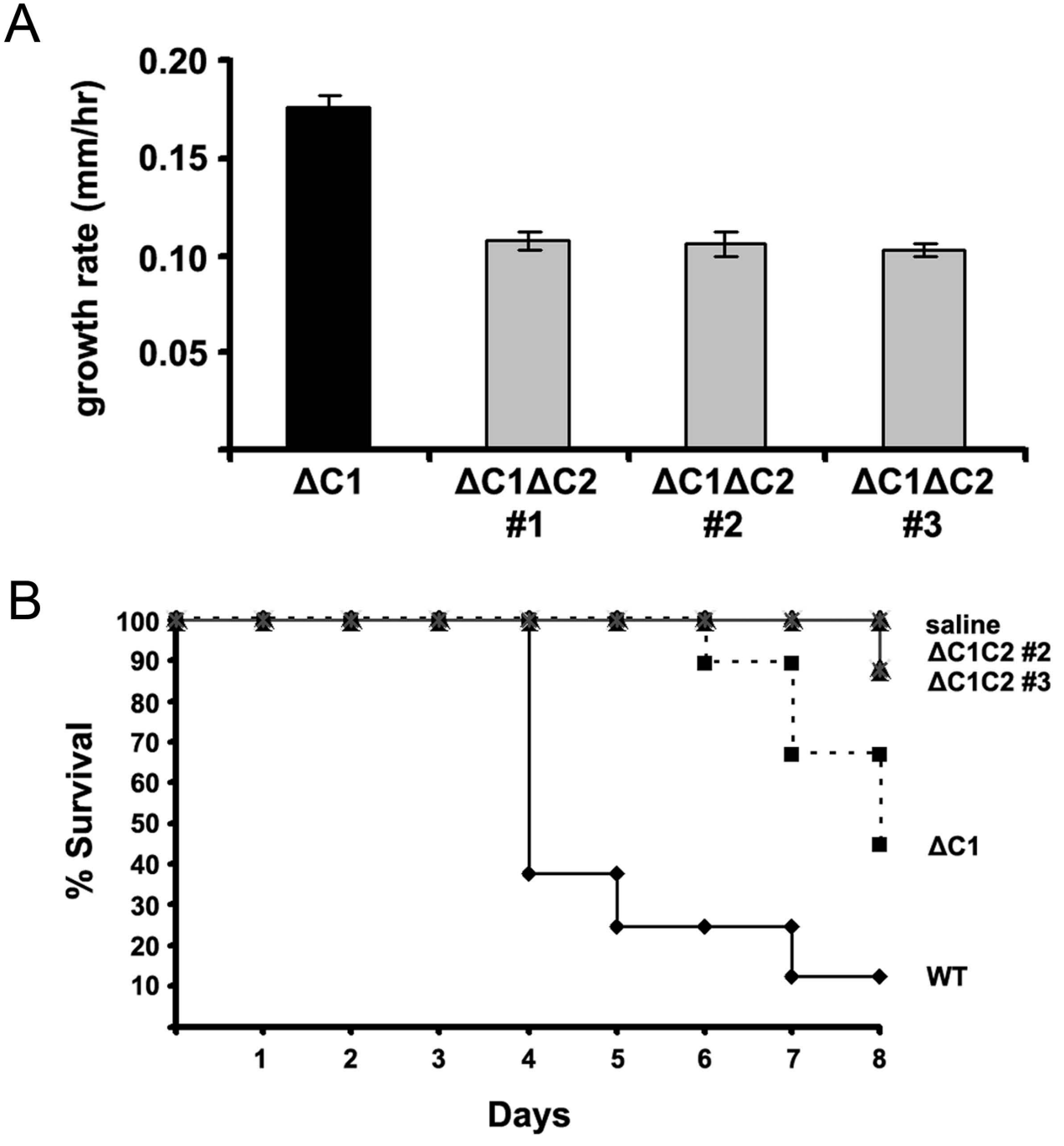
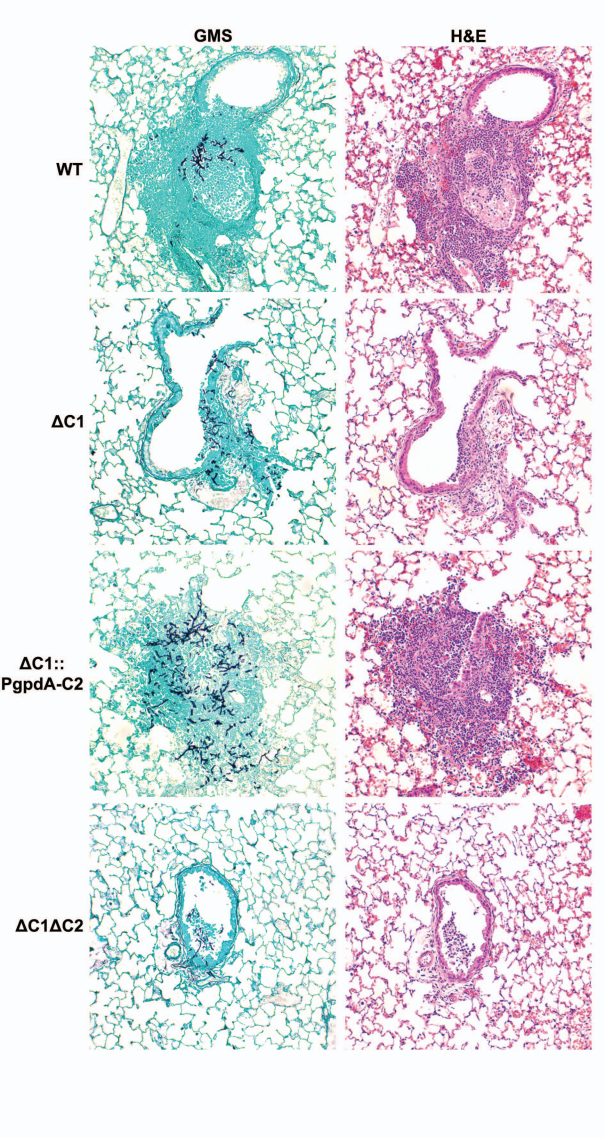
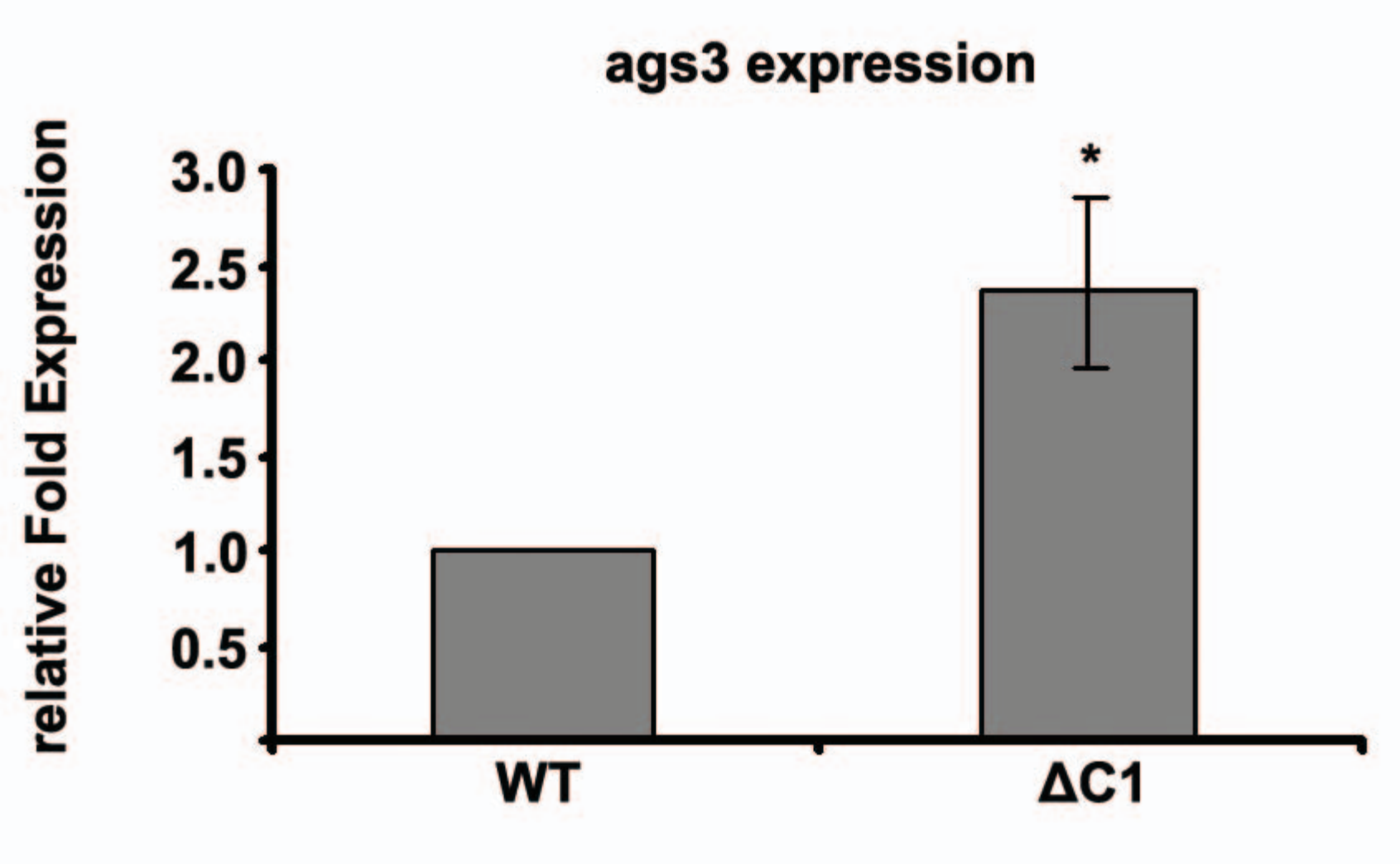
4.14 Kb
2.99 Kb
ΔC2
ΔC1
ΔC2
4.14 Kb
2.99 Kb
C
PgpdA
pkaC2
WT
ΔC1
ΔC2
ΔC1::
PgpdA-C2
pkaC2
gpdA
Fold Expression of pkaC2
25
20
15
10
5
ΔC1
ΔC1::
PgpdA-C2

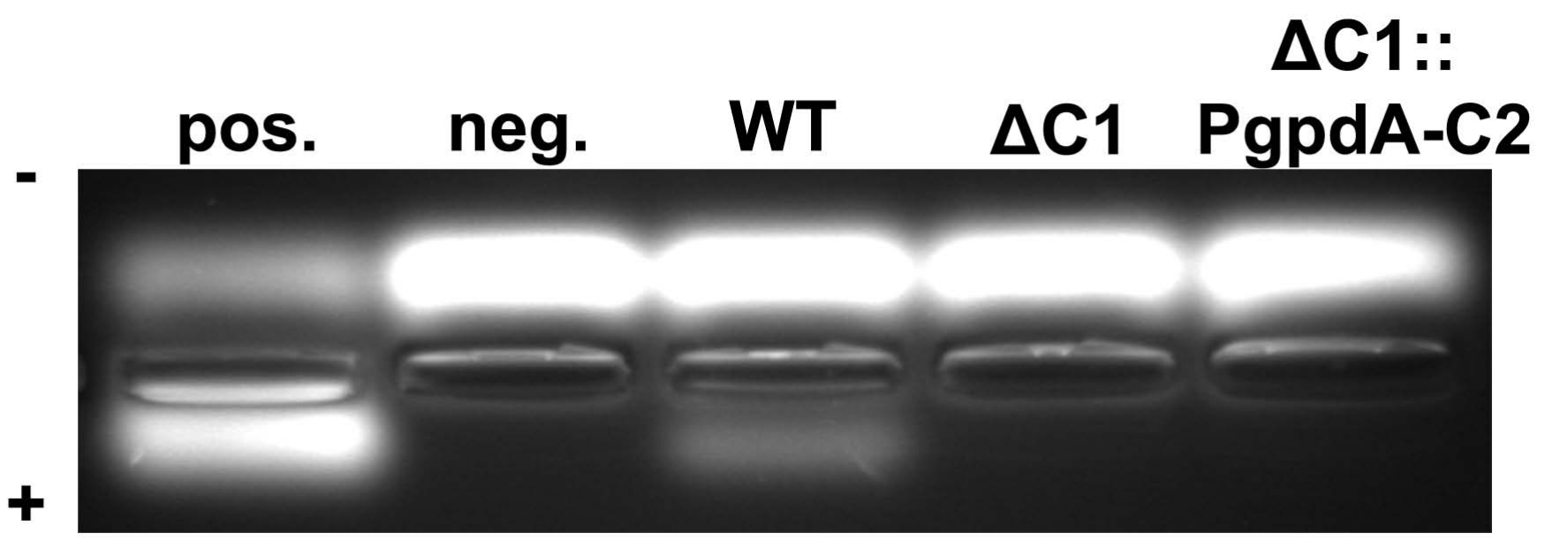

Trehalose Assay
glucose (mg/ml)
0.06
0.05
0.04
0.03
0.02
0.01
WT
ΔC1
ΔC1ΔC2
ΔC1::
PgpdA-C2


SDS
0
.005
.0075
.010
.0125
.015
%
WT
ΔC2
ΔC1
ΔC1
ΔC2


A
0.20
0.15
0.10
0.05
growth rate (mm/hr)
ΔC1
ΔC1ΔC2 #1
ΔC1ΔC2 #2
ΔC1ΔC2 #3
B
100
90
80
70
60
50
40
30
20
10
% Survival
1
2
3
4
5
6
7
8
Days
saline
ΔC1C2 #2
ΔC1C2 #3
ΔC1
WT


GMS
H&E
WT
ΔC1
ΔC1::
PgpdA-C2
ΔC1ΔC2


ags3 expression
relative Fold Expression
3.0
2.5
2.0
1.5
1.0
0.5
*
WT
ΔC1


pos.
neg.
WT
ΔC1
ΔC1::
PgpdA-C2
-
+