SUPPLEMENTAL INFORMATION

Supplementary Figures and Tables

Figure S1: Analyses cyst cell formation in wild-type, *Δrc1_3783* and *Δrc1_3788* strains after 3 days growth on encystment inducing agar solidified CENS-8xN media. Strains were cultured overnight in liquid CENS media, concentrated 20x then spotted in 5 μl aliquots onto plates. Media supplemented with nucleotides (cAMP, AMP, ADP, ATP, cGMP, GMP, GDP, GTP) had final concentrations of 50 μM. Following macroscopic morphology observations, colonies were used in wet mounts observations of cellular morphologies. (**a**) In all conditions assayed, the wild-type colonies were dry and ridged, whereas the *Δrc1_3783* and *Δrc1_3788* strains consistently appeared shiny and vegetative, with the exception of the *Δrc1_3783* strain in the presence of cGMP. (**b)** In microscopic observations cyst cells were detected within wild-type colonies under all conditions assayed, and in the *Δrc1_3783* strains only when grown in the presence of cGMP. No cyst cells were detected in the *Δrc1_3788* strain under any condition assayed.

Figure S2: Thermal denaturation resistance of RC1_3788 in the presence of (**a**) cGMP and (**b**) cAMP. Both panels depict negative derivative curves of RC1_3788 (10 μM) thermal denaturation in response to increasing ligand concentration. Ligand concentrations (0, 10, 20, 30, 40 μM) used were equivalent in both assays, and are respectively represented by the following marker and line color schemes: green, closed circle (●); magenta, closed triangle (▲); blue, closed square (■); black, open circle (○); red, crossed lines (**x**). Panel (**c**) depicts the ΔT_m (^oC) of RC1_3788 in the presence of increasing cAMP (open circles, ○) or cGMP (closed circles, ●) relative to RC1_3788 without ligand.

Figure S3: Representative ITC titrations of cGMP (**a**) and cAMP (**b**) into WT RC1_3788 dimer. Lines of best-fit were generated based on one-site (cAMP) and two site sequential binding (cGMP) sites (See Table 2 for fitted parameters).

Figure S1

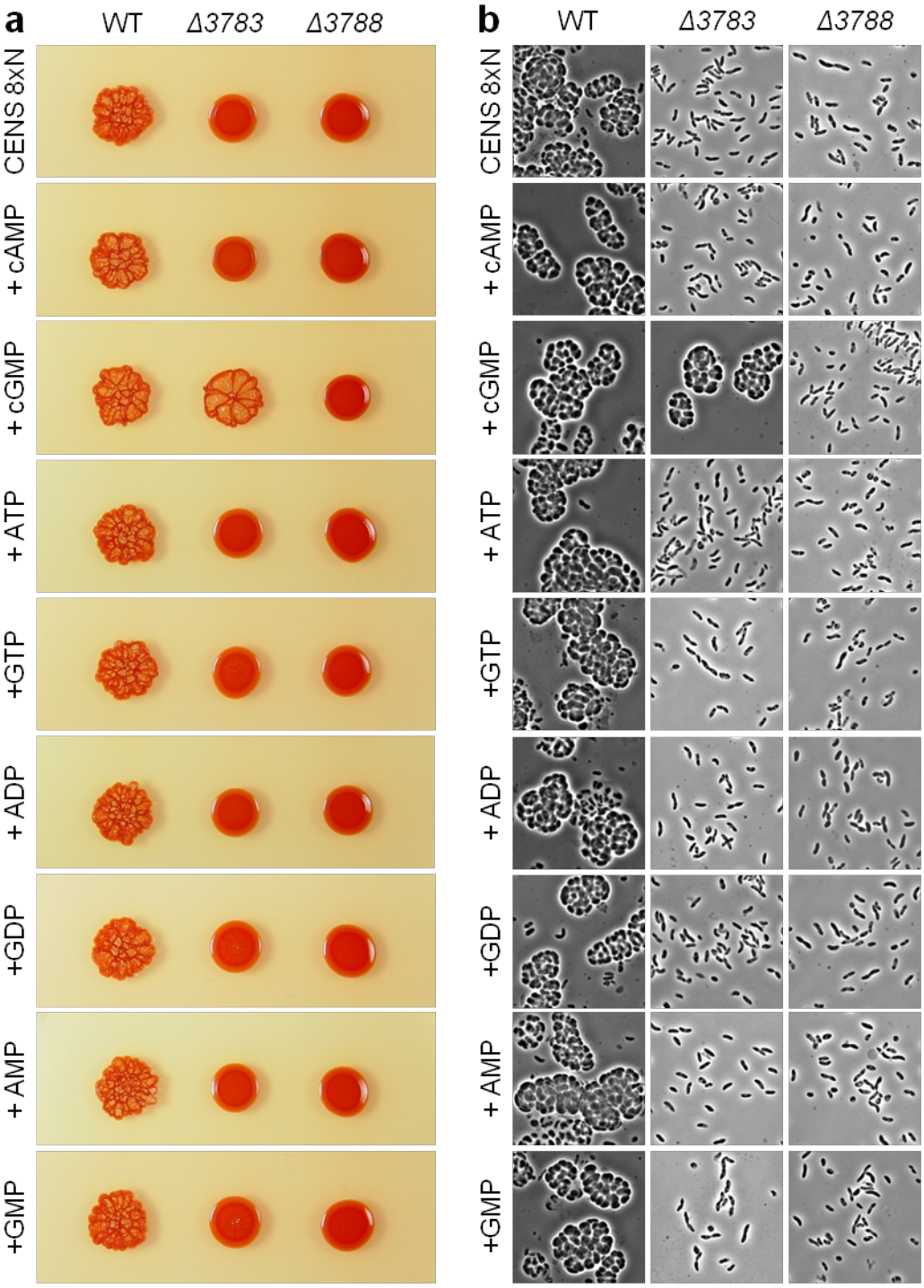
a
WT
Δ3783
Δ3788
CENS 8xN
+ cAMP
+ cGMP
+ ATP
+GTP
+ ADP
+GDP
+ AMP
+GMP
b
WT
Δ3783
Δ3788
CENS 8xN
+ cAMP
+ cGMP
+ ATP
+GTP
+ ADP
+GDP
+ AMP
+GMP

Figure S2

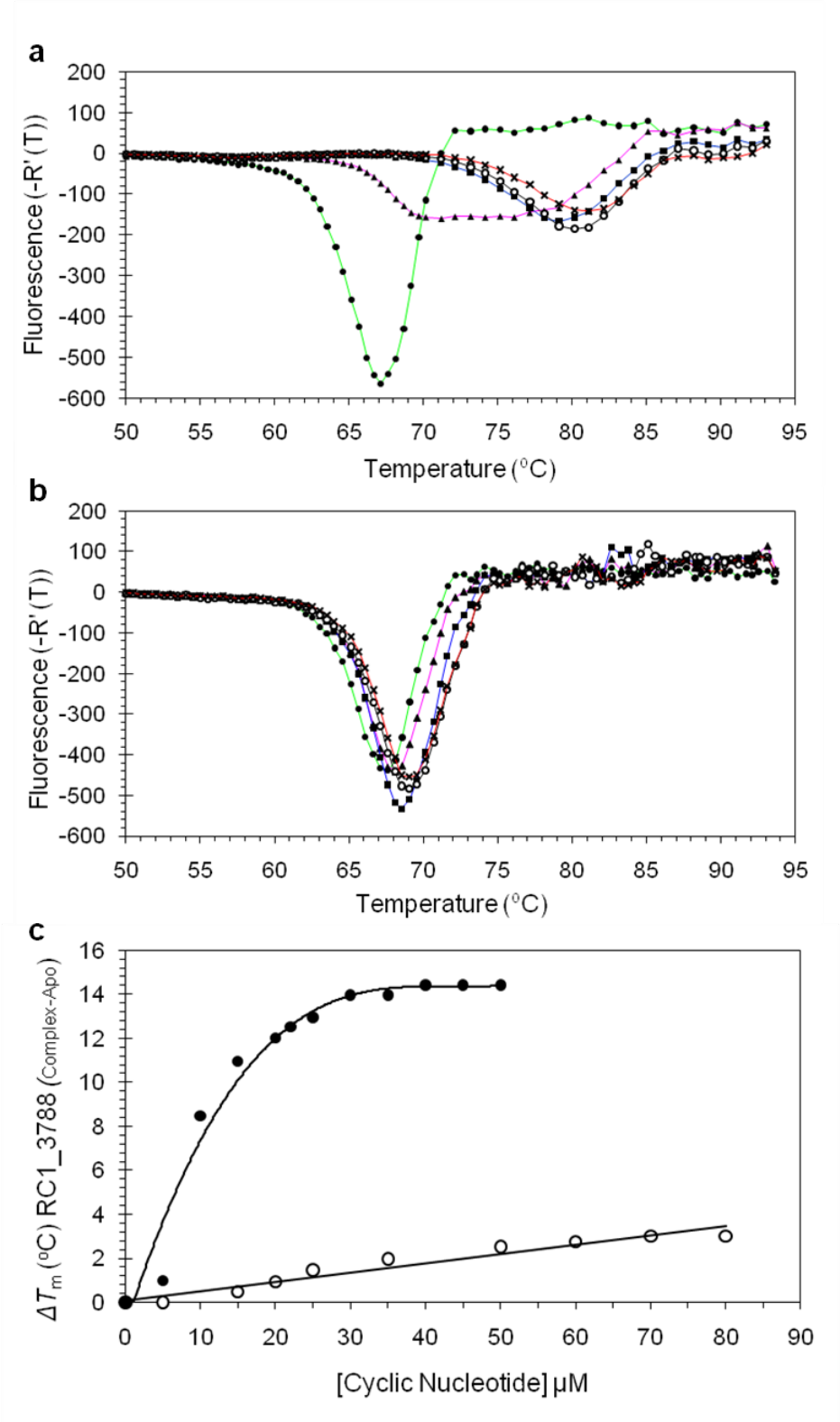
a
Fluorescence (-R' (T))
Temperature (°C)
b
Fluorescence (-R' (T))
Temperature (°C)
c
ΔTm (°C) RC1_3788 (Complex-Apo)
[Cyclic Nucleotide] µM

Figure S3

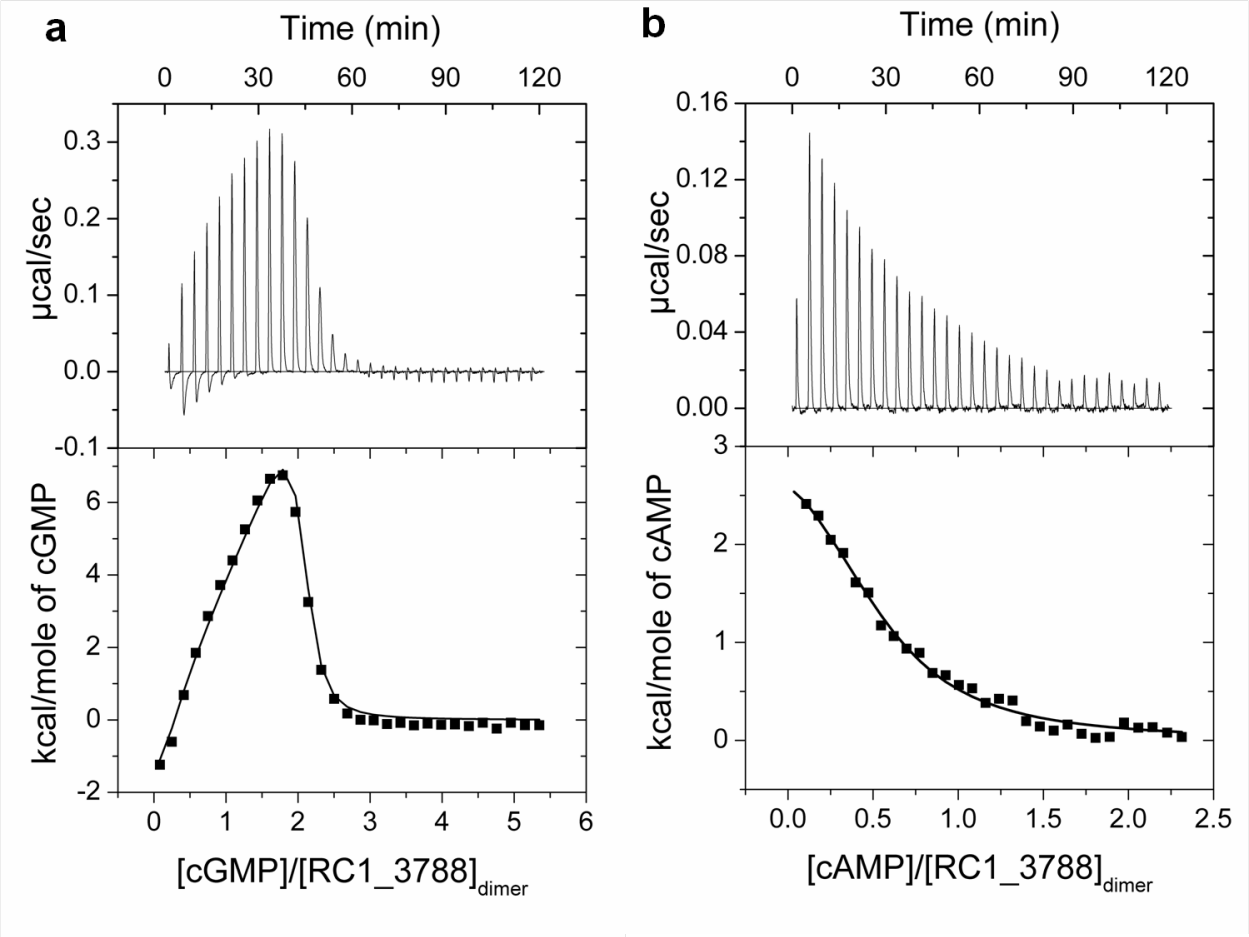
a
Time (min)
0
30
60
90
120
µcal/sec
0.3
0.2
0.1
0.0
-0.1
kcal/mole of cGMP
6
4
2
0
-2
0
1
2
3
4
5
6
[cGMP]/[RC1_3788]dimer
b
Time (min)
0
30
60
90
120
µcal/sec
0.16
0.12
0.08
0.04
0.00
kcal/mole of cAMP
3
2
1
0
0.0
0.5
1.0
1.5
2.0
2.5
[cAMP]/[RC1_3788]dimer

Table S1: PCR Primers used for the construction of chromosomal deletion suicide plasmids, protein overexpression and sequencing. Restriction sequences are boldfaced.

Primer Use	Primer Name	Primer Sequence (5'-3')
RC1_3783 Overexpression	*rc1_3783*NdeI-f	**CATATG**GCGACGAGCGGAAGCACA
	*rc1_3783*SbfI-r	CTTCTT**CCTGCAGG**AATGCGT
	*rc1_3783*SbfI-f	ACGCATT**CCTGCAGG**AAGAAG
	*rc1_3783*XhoI-r	**CTCGAG**GACCCTGGACTAGCGGCC
RC1_3788 Overexpression	*rc1_3788*NdeI-f	**CATATG**ACGCCCGTGCCCAGCGA
	*rc1_3788*XhoI-r	**CTCGAG**TCAGCCGGCGAGCTTCTCCA
rc1_3783 Clean Deletion	*rc1_3783*SacI-f	GGA**GAGCTC**AGAACGACTACGAGCGCAT
	*rc1_3783*EcoRV-r	GGA**GATATC**TGTGCTTCCGCTCGTCGCC
	*rc1_3783*EcoRV-f	GG**AGATAT**CTTCGCCACGCTCCCGGCTG
	*rc1_3783*XbaI-r	GGA**TCTAGA**GTGTGAACTGCTTCACACG
rc1_3786 Clean Deletion	*rc1_3786*SacI-f	GGA**GAGCTC**GCTGATGAGCTGGCCGTCCAC
	*rc1_3786*SpeI-r	GGA**ACTAGT**TCGGCGGGGAGTCCGGTCAT
	*rc1_3786*SpeI-f	GGA**ACTAGT**GGGTACCGCCCACACTGATCC
	*rc1_3786*XbaI-r	GAG**TCTAGA**TGCCCGTGCCGATGATGCCGC
rc1_3787 Clean Deletion	*rc1_3787*SacI-f	GGA**GAGCTC**ATCCGGCCGGCGATCGGCTGC
	*rc1_3787*SpeI-r	GGA**ACTAGT**CGTGGGGCCGAGGAAGACGAT
	*rc1_3787*SpeI-f	GGA**ACTAGT**ATCCTGGGTGCAGCCGCATGA
	*rc1_3787*XbaI-r	GAG**TCTAGA**ACCGTGTCCTCGATGATGGCG
rc1_3788 Clean Deletion	*rc1_3788*SacI-f	GGA**GAGCTC**TGGAGGACGATGACGAGGTC
	*rc1_3788*EcoRV-r	GGA**GATATC**CTTTGTGTCGCTGGGCACGG
	*rc1_3788*EcoRV-f	GGA**GATATC**TACCTGATCGTCCACGACACG
	*rc1_3788*XbaI-r	GCA**TCTAGA**CGGTGGGCCGATTCCTGGCTG
Sequencing of Mini-Tn5 Disrupted Loci	Mini-Tn5-f	CTGTTCTTCTACGGCAAG
	Mini-Tn5-r	CACAGCCAAACTATCAGG