SUPPLIMENTARY MATERIAL

MATERIAL AND METHODS

β-galactosidase assays to assess copper inducible *copA* promoter activity.

This assay was performed as previously described with few modification (1). Strains bearing plasmids with the *lacZ* gene controlled by copper inducible promoter *copAB* were inoculated from freshly grown colonies into 5 ml marine broth containing 5 µg/ml Kan and incubated at 30˚C overnight. Overnight cultures were diluted in the same culture medium to OD_{600} = 0.10 and incubated until an OD_{600} = 0.4 was reached, where copper sulfate dissolved in marine broth was added to a final concentration of 0 - 250 µM. The induced cultures and controls were incubated for 2 - 4 h at 30 ˚C. β-galactosidase activity was measured colorimetrically as described previously (2). Briefly, 200 µl of culture was mixed with 600 µl Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, 50 mM ß-mercaptoethanol). Cells were then permeabilized using 50 µl chloroform and 25 µl 0.1% SDS. 200 µl of substrate *o*-nitrophenyl- β-D-galactoside (4 mg/ml) was added to the permeabilized cells. Upon development of a yellow color, the reaction was stopped by raising the pH to 11 with addition of 400 µl of 1 M Na_2CO_3. Absorbance at 420 nm (A_{420}) was determined and the Miller Units of β-galactosidase activity were calculated as $(A_{420})(1000)/(OD_{600})(t)(v)$ where t is the time in minutes and v is the volume of culture used in the assay in mL.

Growth measurements.

Impact of $CuSO_4$ on *H. baltica* growth was measured using 24-well plates. 1 ml of cultures (Starting OD_{600} = 0.05) were incubated for 12 h at 30˚C, using marine Broth and various $CuSO_4$ concentrations. OD_{600} were recorded after overnight incubation to determine the growth yield for the different $CuSO_4$ concentrations. Growth curves using 0 or 500 µM $CuSO_4$ were recorded every 30 min for 20 h. All OD_{600} were recorded using a Biotek Synergy HT.

Table S1: Strains and Plasmids used in this study

Strain or Plasmid	Description and or genotype	Reference or source
E. coli		
α select	*deoR endA1 relA1 gyrA96 hsdR17($r_K^- m_K^+$) supE44 thi-1 Δ(lacZYA-argFV169) Φ80δlacZ*ΔM15 F^-	Bioline
YB8430	α select /pNPTS139Δ*hfsA*	This study
YB8431	α select /pNPTS139Δ*hfsL*	This study
YB8432	α select /pNPTS139Δ*hfaB*	This study
YB8439	α select /pNPTS139Δ*hfaD*	This study
YB8440	α select /pNPTS139Δ*hfsD*	This study
YB172	α select /pNPTS139Δ*hfsG*	This study
YB8441	α select/ pMR10:*hfsA*	This study
YB8442	α select/ pMR10:P*hfa-hfaB*	This study
YB8443	α select/ pMR10:P*hfsE-hfsL*	This study
YB8429	α select/ pMR10:P*cu-hfsL*	This study
YB8433	α select/pMR10:P*hfaA-hfaD*	This study
YB8436	α select/ pMR10:*hfsD*	This study
YB8437	α select/ pMR10:Pcu-*lacZ*	This study
YB173	α select/ pMR10:Pcu-*hfsG*	This study
C. Crescentus		
YB135	Wild-type strain CB15	(3)
YB4251	CB15 Δ*hfaB*	(4)
H. baltica		
YB5842	IFAM 1418^T Wild-type strain	(5)
YB8404	YB5842 Δ*hfsA*	This study
YB8405	YB5842 Δ*hfL*	This study
YB8406	YB5842 Δ*hfaB*	This study
YB210	YB5842 Δ*hfaB* Δ*hfaD*	This study
YB8409	YB5842 Δ*hfsA* /pMR10:*hfsA*	This study
YB8410	YB5842 Δ*hfaB* /pMR10:P*hfa-hfaB*	This study
YB8414	YB5842 Δ*hfsL* /pMR10:P*hfsE-hfsL*	This study
YB8417	YB5842 Δ*hfsG* Δ*hfaB* /pMR10:P*cu-hfsG*	This study
YB8418	YB5842 Δ*hfsL* Δ*hfaB* /pMR10:P*cu-hfsL*	This study
YB8424	YB5842 Δ*hfsL* /pMR10:P*cu-hfsL*	This study
YB8425	YB5842 Δ*hfaD*	This study
YB8426	YB5842 Δ*hfaD* /pMR10:P*hfaA-hfaD*	This study
YB8427	YB5842 ΔhfsD	This study
YB8434	YB5842 ΔhfsD /pMR10:*hfsD*	This study
YB8438	YB5842 pMR10:Pcu-*lacZ*	This study
YB173	YB5842 Δ*hfsG*	This study
YB174	YB5842 pMR10:Pcu-*hfsG*	This study
Plasmids		
pNPTS139	pLitmus 39 derivative, *ori*T, *sac*B, Kan^r	M.R.K Alley
pNPTS139Δ*hfsA*	pNPTS139 containing 500 bp fragments upstream and downstream of *hfsA*	This study
pNPTS139Δ*hfsL*	pNPTS139 containing 500 bp fragments upstream and downstream of *hfsL*	This study
pNPTS139Δ*hfaB*	pNPTS139 containing 500 bp fragments upstream and downstream of *hfaB*	This study
pNPTS139Δ*hfaD*	pNPTS139 containing 500 bp fragments upstream and downstream of *hfaD*	This study
pNPTS139Δ*hfsD*	pNPTS139 containing 500 bp fragments upstream and downstream of *hfsD*	This study
pNPTS139Δ*hfsG*	pNPTS139 containing 500 bp fragments upstream and downstream of *hfsG*	This study
pMR10	Mini-RK2 cloning vector; RK2 replication and stabilization functions	R. Roberts and C. Mohr
pMR10:*hfsA*	pMR10 containing *hfsA* gene with its native promoter	This study
pMR10:P*hfa-hfaB*	pMR10 containing native *hfaA* promoter and the *hfaB* gene	This study
pMR10:P*hfsE-hfsL*	pMR10 containing native *hfsE* promoter and the *hfsL* gene	This study
pMR10:P*cu-hfsL*	pMR10 containing copper inducible promoter of *CopA* and the *hfsL* gene	This study
pMR10:P*hfaA-hfaD*	pMR10 containing native *hfaA* promoter and the *hfaD* gene	This study
pMR10:*hfsD*	pMR10 containing *hfsD* gene with its native promoter	This study
pMR10:Pcu-*lacZ*	pMR10 containing copper inducible promoter of *CopA* and the *lacZ* gene	This study
pMR10:Pcu-*hfsG*	pMR10 containing copper inducible promoter of *CopA* and the *hfsG* gene	This study

Table S2: Primers used in this study

Primers	Sequence (5'→3')	Description
Hb*hfsA*UpF	GCGAATTCTGGATCCACGATGAAATACGCCCGGATTATTG	5' region for deletion of *hfsA*
Hb*hfsA*FR	ATACTTAGTCATTCTGATTCTGCTTTATCTAAAC	
Hb*hfsA*UpR	CAGAAAGCTTCCTGCAGGATTAATTTAGTATCCGCCACAC	3' region for deletion of *hfsA*
Hb*hfsA*RR	GAATCAGAATGACTAAGTATTTGTTATTTAATTAAAAAATATACTTTTC	
Hb*hfsGT*_pUpF	GCCAAGCTTCTCTGCAGGATCAGTATTGTTATTCCAACATTTCG	5' region for deletion of *hfsL*
Hb*hfsGT*_UpR	GTGAGTTTGGGTTGAATGCGATCCAAATC	
Hb*hfsGT*_DwF	CGCATTCAACCCAAACTCACAAACTGAG	3' region for deletion of *hfsL*
Hb*hfsGT*_pDwR	GCGAATTCGTGGATCCAGATTTGGTCCAGCTCATAACG	
Hb*hfaB*UpF	CGCGTTCGGCCGTGCTAGCGGATCATTGCTTATTCCCG	5' region for deletion of *hfaB*
Hb*hfaB*FRev	TCGCCAATTATTGCGAATTGGGCTAGTC	
Hb*hfaB*UpR	GCAGGATATCGTGGATCCAGGAAATATCGTTGACACTGG	3' region for deletion of *hfaB*
Hb*hfaB*RRev	CAATTCGCAATAATTGGCGATAAACTTCGC	
p*hfaD*upF	GTGCTAGCGAATTCTGGATCCACGATGTCTTGTCGAAACAGAATCTCTGGAAG	5' region for deletion of *hfaD*
*hfaD*upR	CTAAGTTTCTATATGTATATTGAGAACTTGGTGTCTGAGACCTTTTAGATAGGC	
*hfaD*dwF	GCCTATCTAAAAGGTCTCAGACACCAAGTTCTCAATATACATATAGAAACTTAG	3' region for deletion of *hfaD*
p*hfaD*dwR	GGCGCCAGAAAGCTTCCTGCAGGATATAGTGATGCAATGTTCGATGGTGG	
*hfsD*upF	GTGCTAGCGAATTCTGGATCCACGATTTTCTGCTATCTCTTGGGCAATTTTAG	5' region for deletion of *hfsD*
*hfsD*upR	CTAGTGTTTAGTTCAGCAATCTGAGGGTGCTTTCTTAATGCATCCGTTTTG	
*hfsD*dwF	CCAAAACGGATGCATTAAGAAAGCACCCTCAGATTGCTGAACTAAACACTAG	3' region for deletion of *hfsD*
*hfsD*dwR	GGCGCCAGAAAGCTTCCTGCAGGATACAGTAAAAGAAAATTCATGTACAAC	
*hfsA*_upF	ACGCCAAGCTTCCATGGGATGAAATACGCCCGGATTATTG	Complementation of *hfsA*
*hfsA*_DwpR	GCTCTGCAGGAGATCTCGATTAATTTAGTATCCGCCACAC	
*hfaB*_upF	ACGCCAAGCTTCCATGGGATAATTGCGCCATTGTG	Complementation of *hfaB*
*hfaB*_DwpR	GCTCTGCAGGAGATCTCGATGAAATATCGTTGACACTGGC	
P*hfsE_hfsL*upF	CCATGATTACGCCAAGCTTCCATGGGATGGCCATACAAATATAAGCGGTGCTC	Complementation of *hfsL*
P*hfsE_hfsL*upR	CAATACTGACTTTTACGGATTGGTTCATTCACGAAGAACACAGAGTGTCTCC	using *hfsE* promoter
P*hfsE_hfsL*dwF	GGAGACACTCTGTGTTCTTCGTGAATGAACCAATCCGTAAAAGTCAGTATTG	
P*hfsE_hfsL*dwR	CTAGAGCTCTGCAGGAGATCTCGATTTAAGTTGCGCTTTTGATAACTTTTTTG	
Pcu_*hfsL*upF	CTAGAGCTCTGCAGGAGATCTCGATTATACACGGATCGCACGCC	Complementation of *hfsL*
Pcu_*hfsL*upR	GGGTGTGTAATGCCAATCAATCATGATGTTCTCCTTCTTGCGTTGGAC	using copper promoter
Pcu_*hfsL*dwF	GTCCAACGCAAGAAGGAGAACATCATGATTGATTGGCATTACACACCC	
Pcu_*hfsL*dwR	CCATGATTACGCCAAGCTTCCATGGGATTTAAGTTGCGCTTTTGATAACTTTTTTG	
p*hfaABD*F	CCATGATTACGCCAAGCTTCCATGGGATCGAGACGAAAACATGAACAGTTTCAC	Complementation of *hfaB*
p*hfaABD*F	CTAGAGCTCTGCAGGAGATCTCGATCAGACAAACAGTTAGAAGAATTTAGAAATC	
comp*hfsD*upF	CCATGATTACGCCAAGCTTCCATGGGATTTTCTGCTATCTCTTGGGCAATTTTAG	Complementation of *hfsD*
comp*hfsD*dwR	CTAGAGCTCTGCAGGAGATCTCGATTTAGAAGGCGTTGTCTTTTAGGTTG	
Cu*lacZ*upF	CTAGAGCTCTGCAGGAGATCTCGATTATACACGGATCGCACGCC	Expression of *lacZ* under
Cu*lacZ*upR	CCCAGTCACGACGTTGTAAAACGACCATGATGTTCTCCTTCTTGCGTTGGACG	copper inducible promoter
Cu*lacZ*dwF	CGTCCAACGCAAGAAGGAGAACATCATGGTCGTTTTACAACGTCGTGACTGGG	
Cu*lacZ*dwR	GATTACGCCAAGCTTCCATGGGATCGGTGGCGGCCGCTCTAGAAC	
*hfsG*upF	GTGCTAGCGAATTCTGGATCCACGATGGTTTTAACAATCAGATTATTCGTGTC	5' region for deletion of *hfsG*
*hfsG*upR	CGTTTTAATTTGGCGGGAAGGGTACATTGGATGCCTAGCGCTGTGTTTTTG	
*hfsG*dwF	CAAAAACACAGCGCTAGGCATCCAATGTACCCTTCCCGCCAAATTAAAACG	3' region for deletion of *hfsG*
*hfsG*dwR	GGCGCCAGAAAGCTTCCTGCAGGATCAACATTAATTCCGGGAAGAATACC	
Pcu_*hfsG*upF	GATTACGCCAAGCTTCCATGGGATATCAATCATGAAGAGCCTCCGCATATATG	Complementation of *hfsG*
Pcu_*hfsG*upR	CGTCCAACGCAAGAAGGAGAACATCATGAACACAACGCCCCAACTTAGCG	using copper promoter
Pcu_*hfsG*dwF	CGCTAAGTTGGGGCGTTGTGTTCATGATGTTCTCCTTCTTGCGTTGGACG	
Pcu_*hfsG*dwR	CTAGAGCTCTGCAGGAGATCTCGATTATACACGGATCGCACGCCTGACAATG	

Table S3: Lectin binding assays for all the lectins used.

Lectin	Specificity	*H. baltica holdfast*	*C. crescentus holdfast*
Wheat Germ Agglutinin	GlcNAc, sialic acid	√	√
Succinylated Wheat Germ Agglutinin	GlcNAc	√	√
Lycopersicon Esculentum Tomato	GlcNAc 1-4	√	√*
Datura Stramonium Lectin	GlcNAc 1-4	√*	-
Solanum Tuberosum Potato Lectin	GlcNAc, prefers trimers and tetramers	√	√*
Ricinus Communis Agglutinin	Galactose	√	-
Griffonia Simplicifolia Lectin 1	α-GalNAc, α-galactose	√	-
Soybean Agglutinin	α-GalNAc	-	-
Concanavalin A	α-linked mannose	-	-
Dolichos Biflorus Agglutinin	α-linked acetylgalactosamine	-	-
Peanut Agglutinin	Galactosyl β-1,3 N-acetylgalactosamine	-	-
Soybean Agglutinin	α or β acetylgalactosamine	-	-
Ulex Europaeus Agglutinin 1	N- acetylgalactosamine, sialic acid or chitobiose	-	-
Len Culinaris Agglutinin	α-linked mannose	-	-
Pisum Sativum Agglutinin	α-linked mannose, fucose or N-acetylchitobiose	-	-
Erythrina Cristagalli Lectin	Galactose, prefers Galactosyl β-1,4 N- acetylgalactosamine	-	-
Jacalin	Galactosyl β-1,3 N-acetylgalactosamine	-	-
Griffinia Simplicifolia Lectin 2	α or β acetylgalactosamine	-	-
Vicia Villosa Lectin	α or β terminal N-acetylgalactosamine	-	-

√ Fluorescent signal detected

- No fluorescent signal detected

* Binding is enhanced on rosettes but weaker signals on single cells.

Figure S1: Design of a copper inducible promoter system in *H. baltica.*

A. Chromosomal arrangement of one of the copper sensitive operons in *H. baltica* genome, showing copper operon repressor gene *csoR* and copper binding protein genes *copA* and *copB* (top panel). The bottom diagram shows the fusion of the *copAB* promoter (P_{cu}) to the *lacZ* reporter gene. **B.** Effect of different concentration of $CuSO_4$ added into marine broth on *H. baltica* growth. Growth yield (OD_{600}) was measured on overnight cultures with different concentration of $CuSO_4$. Data represent mean of four independent replicates and the error bars represent standard error. **C**. Representative growth curves of *H. baltica* growing in marine broth without or with 500 µM $CuSO_4$. OD_{600} representing bacterial growth in a 24 well plate was measured every 30 min. **D.** β–galactosidase activity representing the P_{cu} activity when induced with different concentrations of $CuSO_4$. Exponential cultures were induced for 4h. Data shown is representative of three independent replicates and the error bars represent the standard error.

Figure S2: DNA inhibition of holdfast binding and biofilm formation.

A. *C. crescentus* (upper panel) and *H. baltica* (lower panel) cells bound to a glass surface in presence of eDNA from each strain. Holdfasts labeled with WGA-AF488 lectins after exponentially grown cells were bound to a glass slide for 45 min. **B.** Biofilm quantification after 24 h for *C. crescentus and H. baltica* in presence of eDNA. Data are expressed as an average of 4 independent replicates and the error bars represent the standard error.

Figure S3: Fluorescence intensity of WGA-labeled holdfast

A. Box and whisker plots of WGA fluorescence intensity distribution from holdfast images collected in Figure 4 A-B. More than 500 holdfasts were measured in 10 independent images. The variance between *H. baltica* and *C. crescentus* holdfast fluorescent intensity was analyzed using a *t*-test. ns, not statistically significant ($p<0.38$).

Figure S1

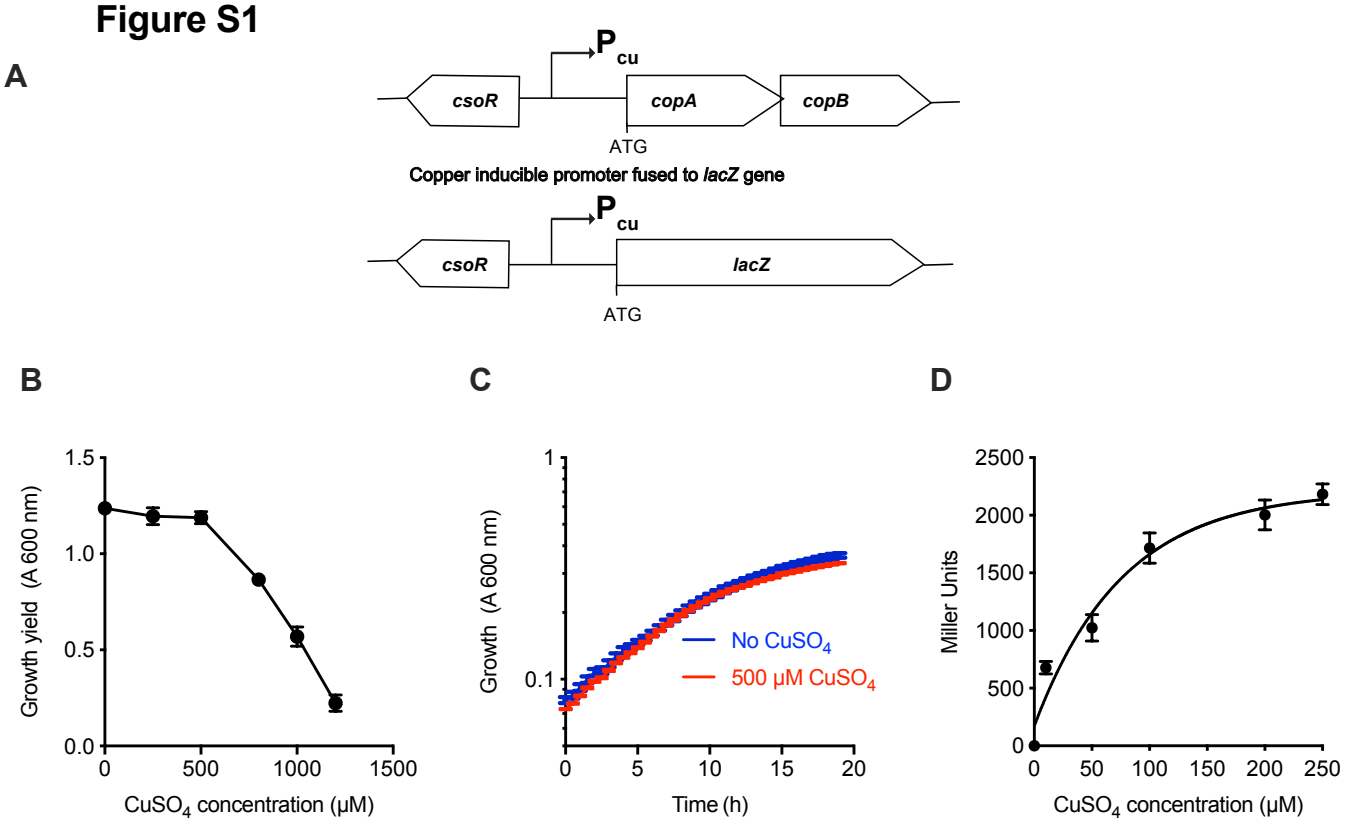

A
P cu
csoR
copA
copB
ATG
Copper inducible promoter fused to lacZ gene
P cu
csoR
lacZ
ATG
B
Growth yield (A 600 nm)
1.5
1.0
0.5
0.0
0
500
1000
1500
CuSO4 concentration (μM)
C
Growth (A 600 nm)
1
0.1
0
5
10
15
20
Time (h)
No CuSO4
500 μM CuSO4
D
Miller Units
2500
2000
1500
1000
500
0
0
50
100
150
200
250
CuSO4 concentration (μM)

Figure S2

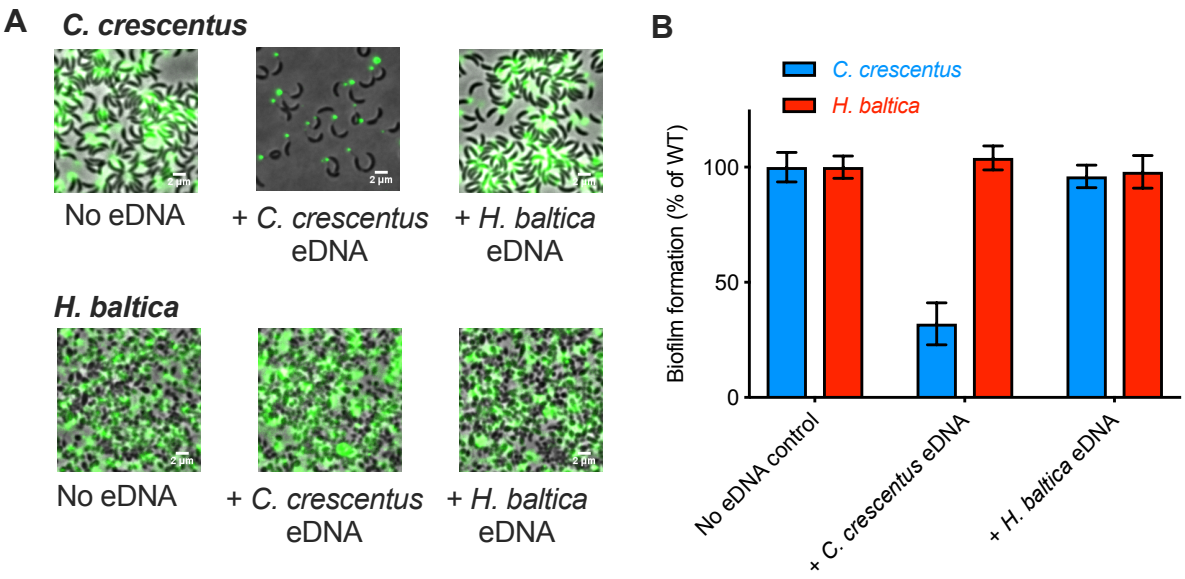
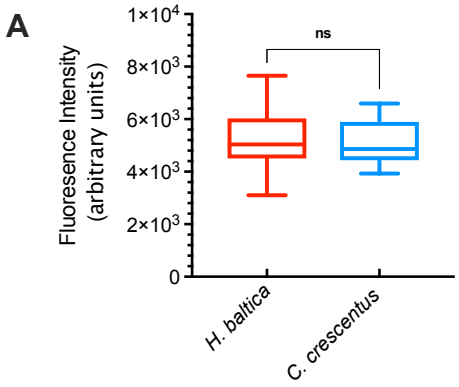

A
C. crescentus
No eDNA
+ C. crescentus eDNA
+ H. baltica eDNA
H. baltica
No eDNA
+ C. crescentus eDNA
+ H. baltica eDNA
B
C. crescentus
H. baltica
Biofilm formation (% of WT)
100
50
0
No eDNA control
+ C. crescentus eDNA
+ H. baltica eDNA

Figure S3


A
1×10^4
8×10^3
6×10^3
4×10^3
2×10^3
0
Fluoresence Intensity
(arbitrary units)
ns
H. baltica
C. crescentus

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