Supplementary data

	(Cm^R colonies/10^8 cells; moi=1)		
oriC allele	AQ7664 λ recombinants	AQ10033 λ recombinants	MG1655 P1 transductants
oriC201	104	-	79
oriC227	129	-	105
oriC228	66	-	57
oriC229	78	-	41
oriC230	38	-	122
oriC231	9 (all Km^R)	681	84
oriC237	0	512	0
oriC238	0	825	3*
oriC233	137	-	92
oriC234	71	-	59
oriC235	0	418	0
oriC239	55	-	114
oriC243	0	1231	0

Table S1. Frequency of recombinants obtained during the in vivo replacement procedure as outlined in Figure S1. 7/12 mutant *oriC* alleles were successfully transfered onto the chromosome of *rnhA*$^+$ cells (AQ7664). The remaining 5 *oriC* alleles were transferred to the chromosome of an *rnhA224 strain* (AQ10033), and one allele, *oriC231*, exhibited cSDR-independent replication (Fig. 2). The 8 $OriC^+$ mutants were transferred into MG1655 by P1 transduction selecting for Cm^R and confirmed by Southern blot hybridization (Fig. S2) and direct sequencing (data not shown). None of the 4 remaining $OriC^-$ alleles were able to be transduced into MG1655. *The three Cm^R MG1655 *oriC238* transductants retained a wild-type copy of *oriC* by adjacent integration.

Supplementary data

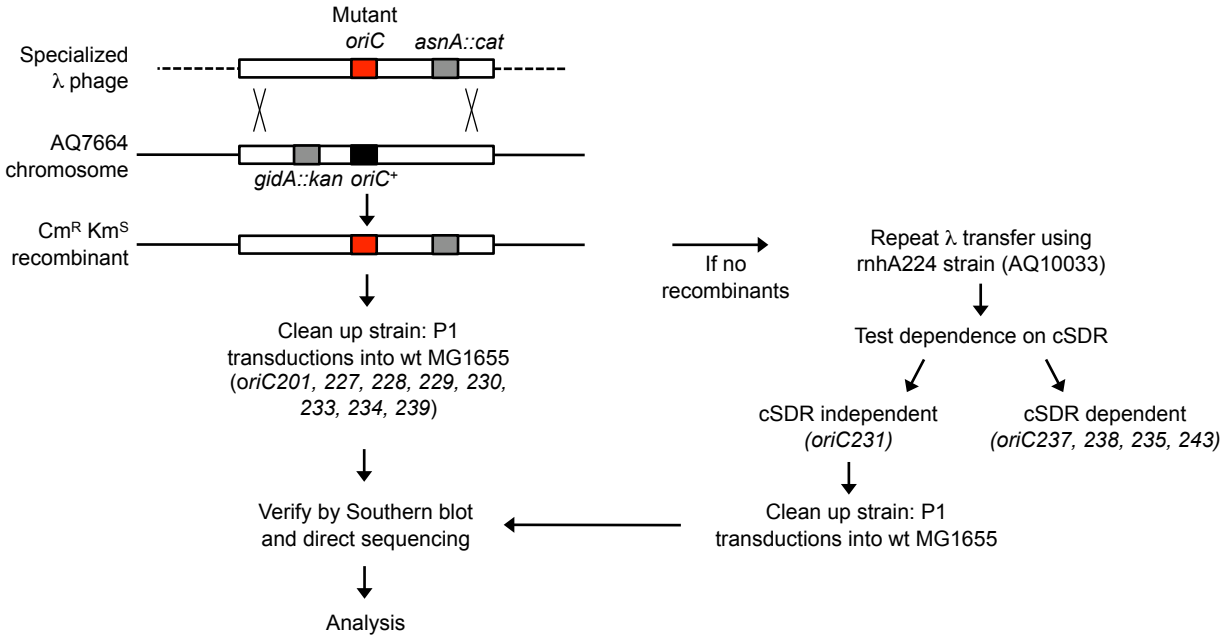

Figure S1

Supplementary data

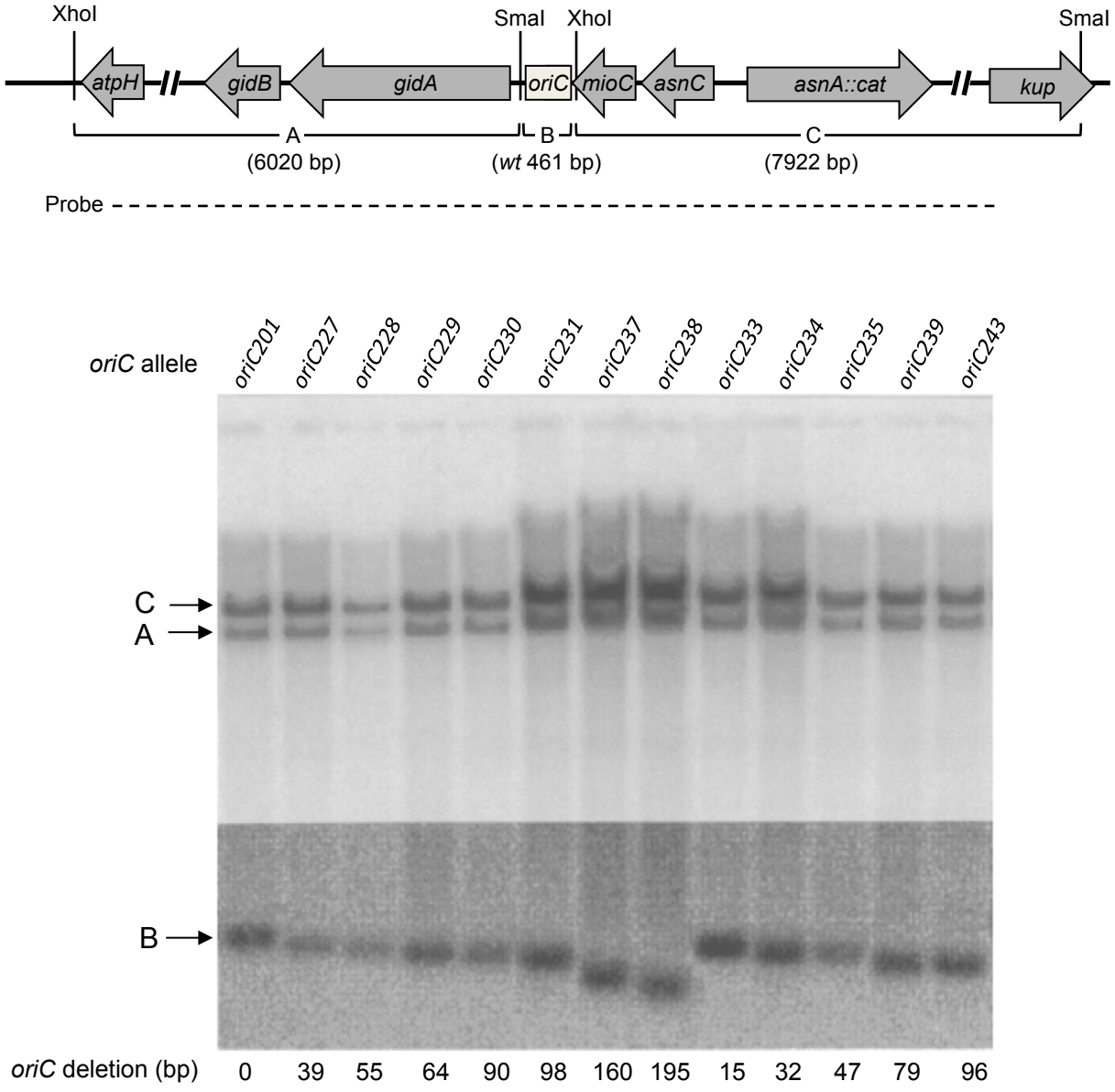

Figure S2

Supplementary data

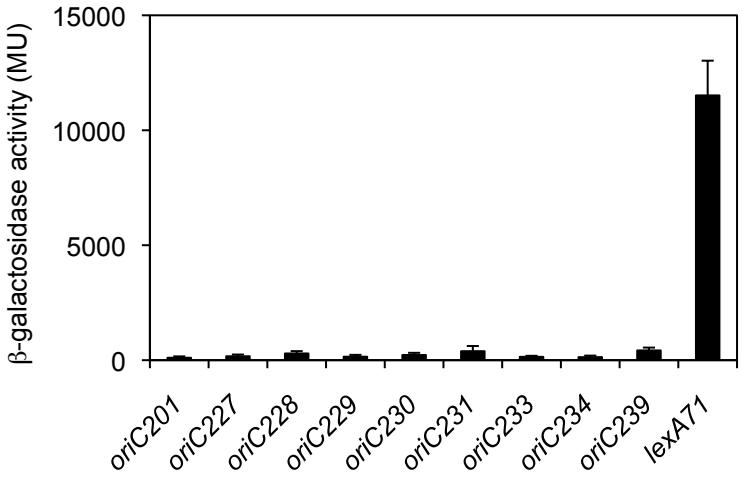

Figure S3

Supplementary data

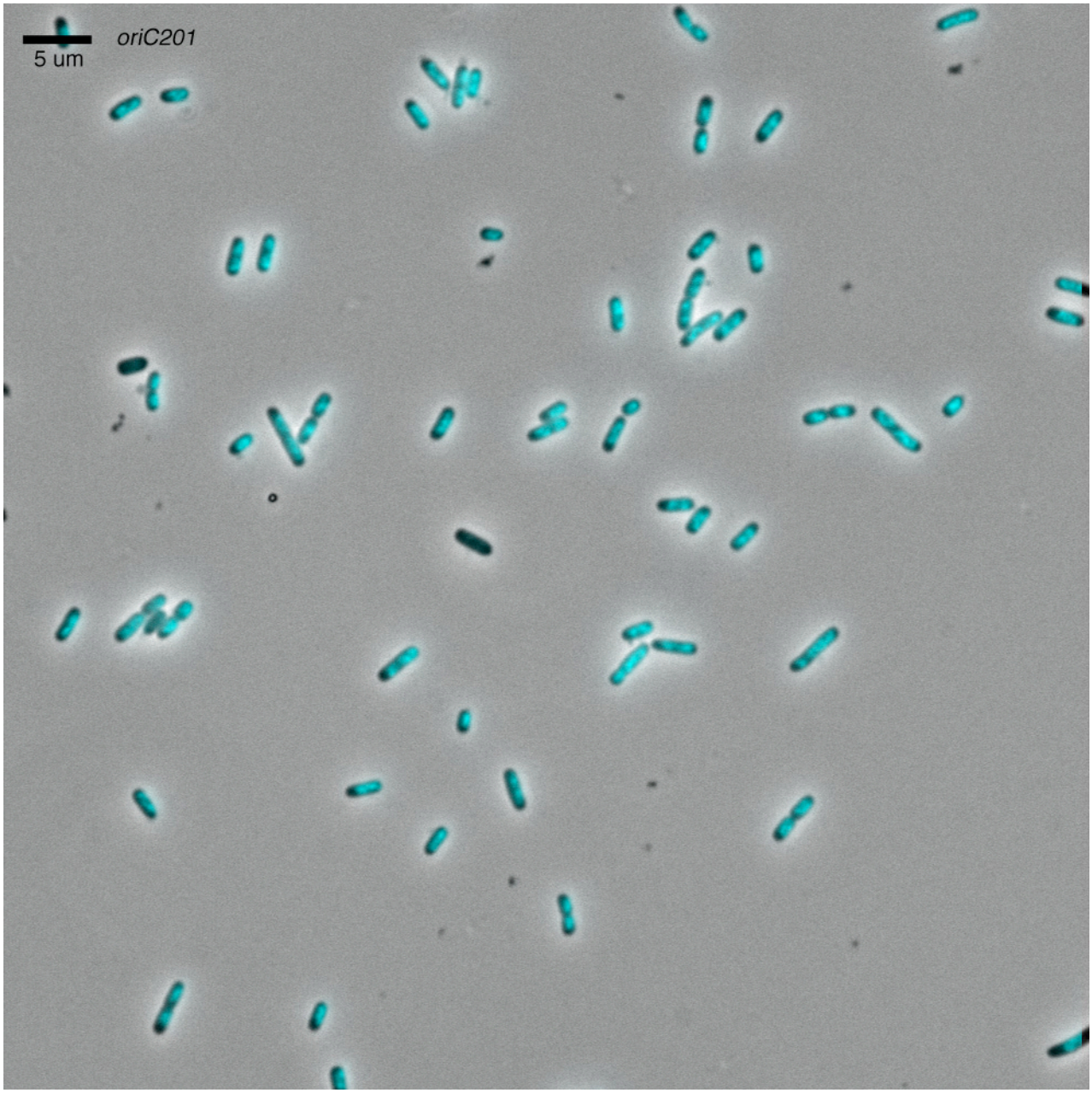

Figure S4

Supplementary data

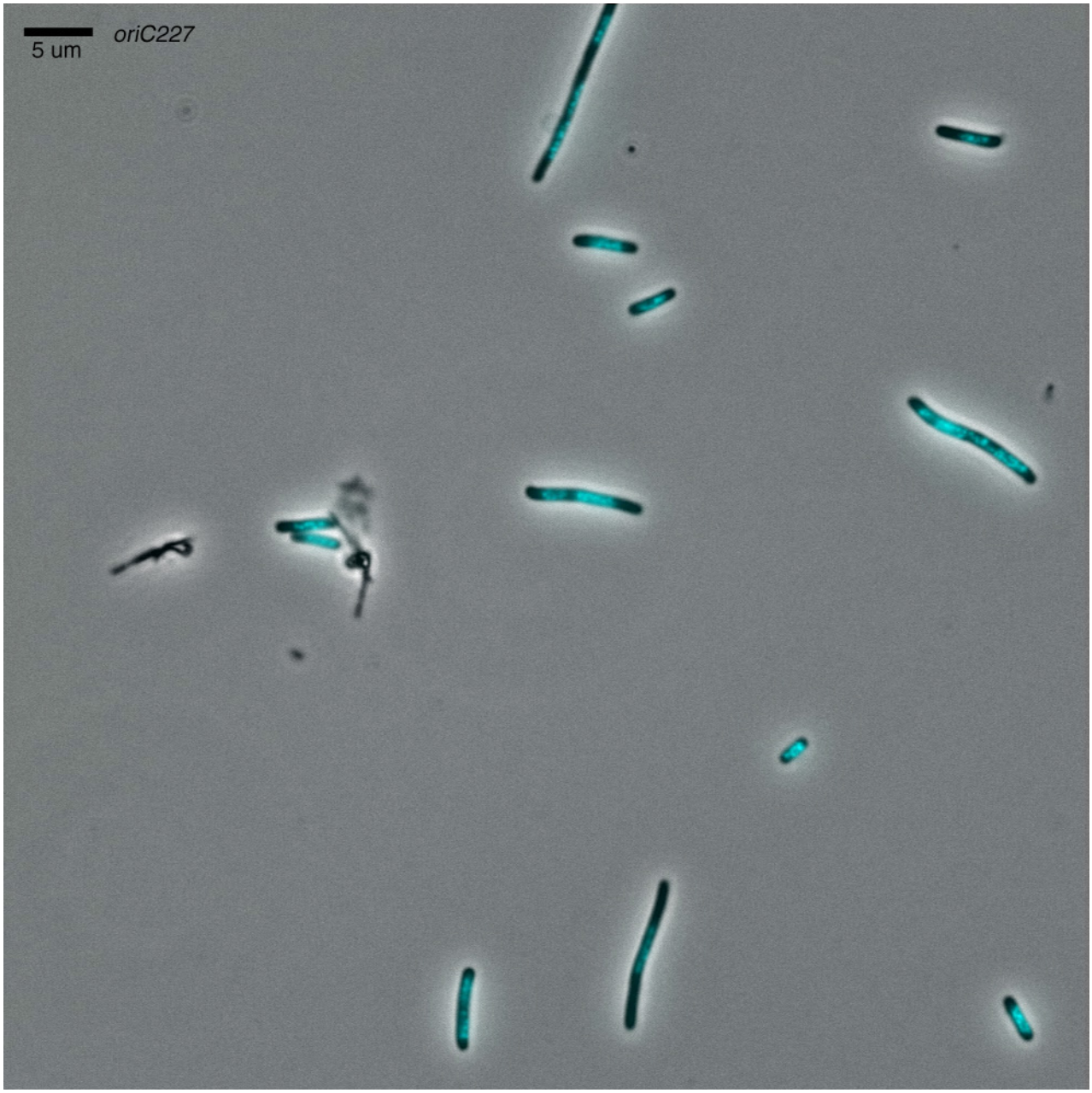

Figure S5

Supplementary data

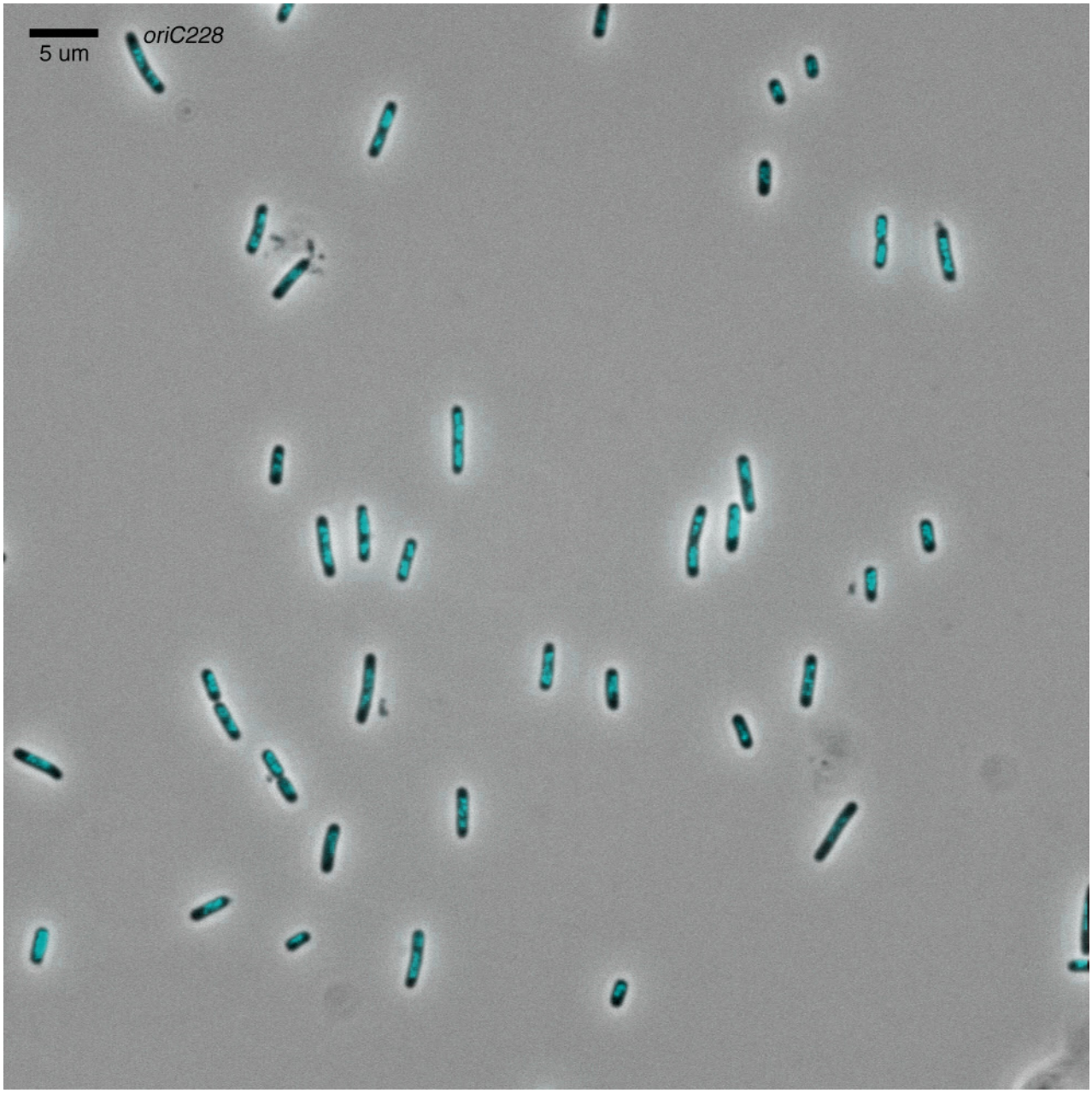

Figure S6

Supplementary data

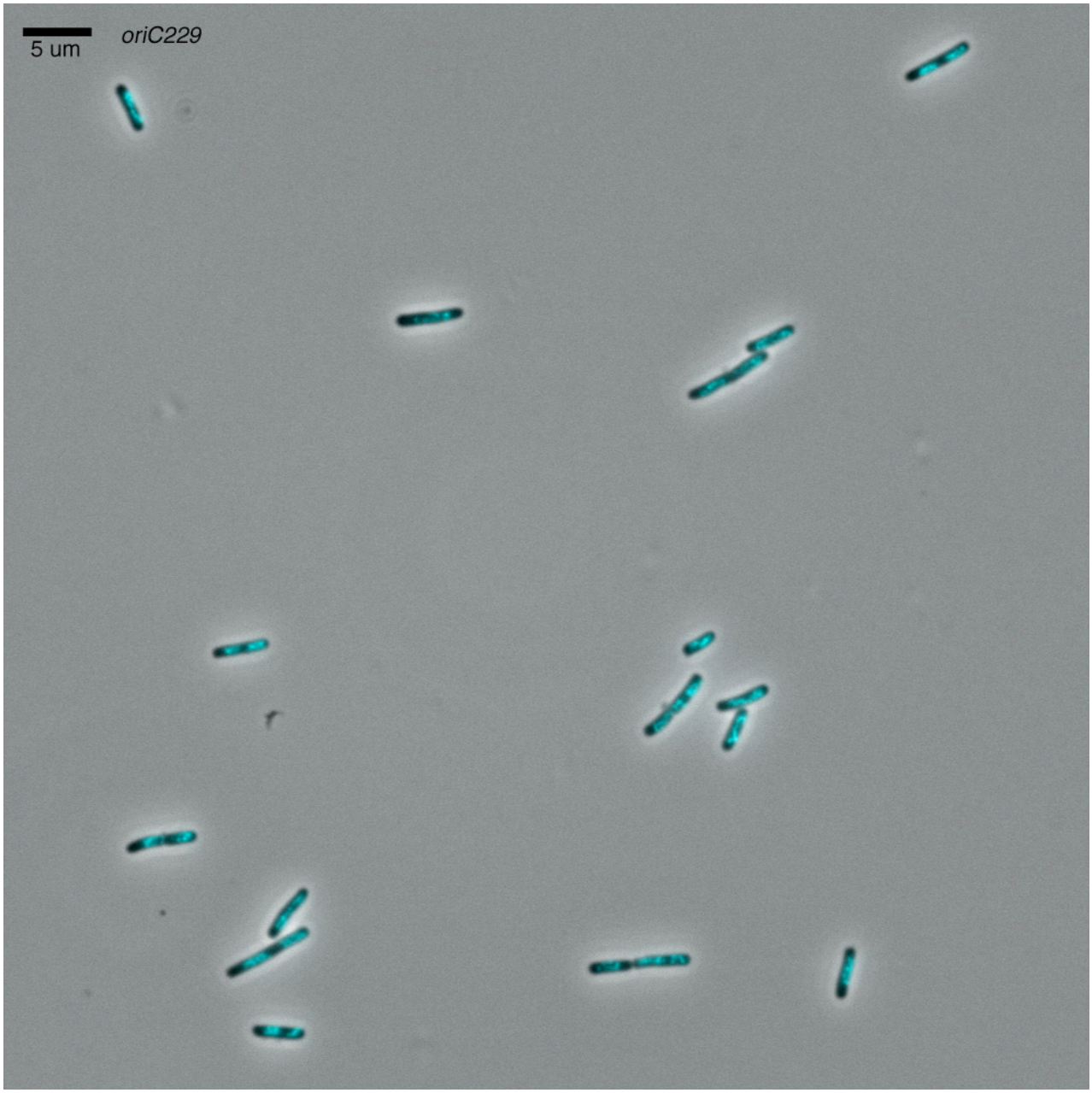

Figure S7

Supplementary data

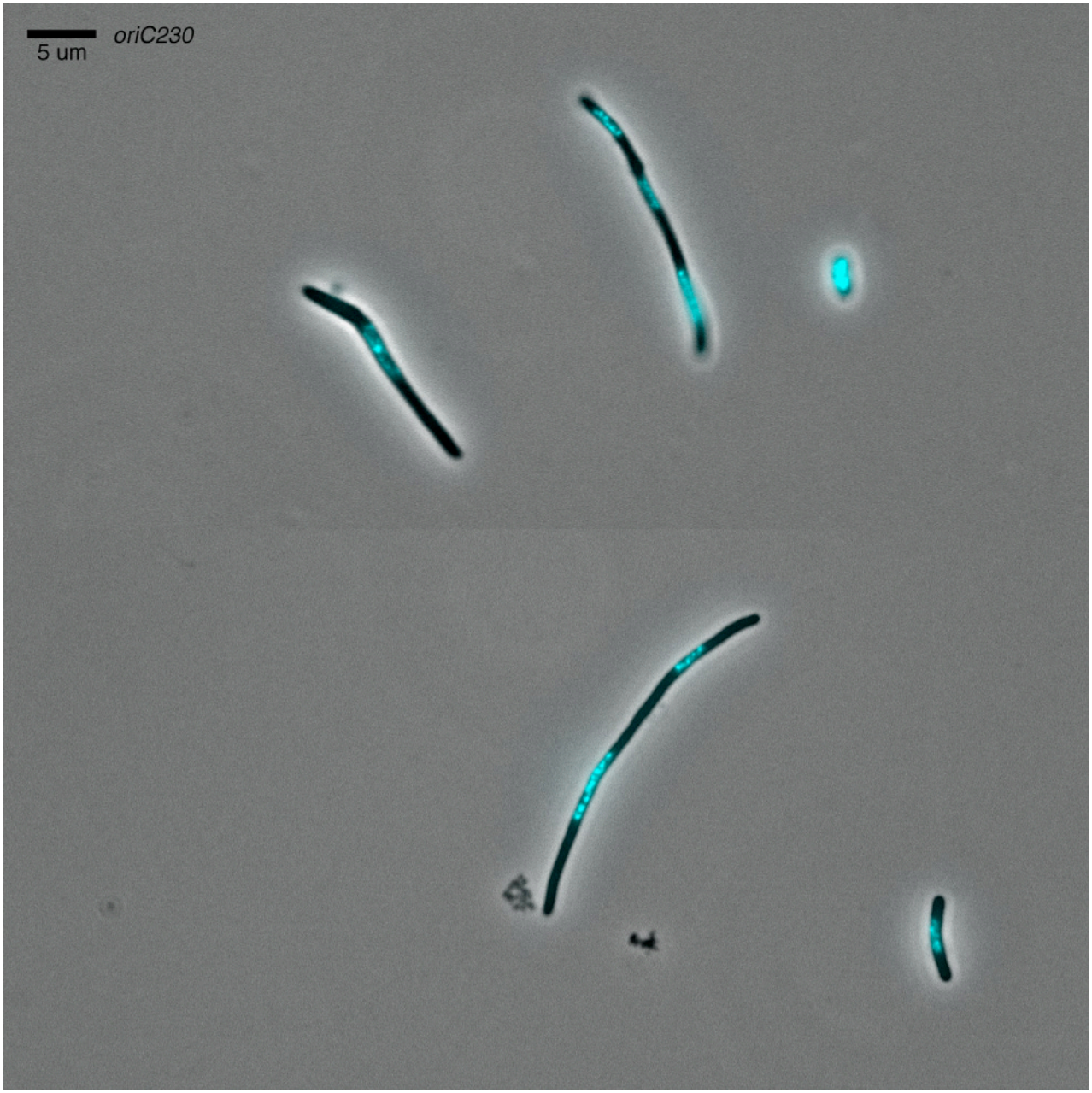

Figure S8

Supplementary data

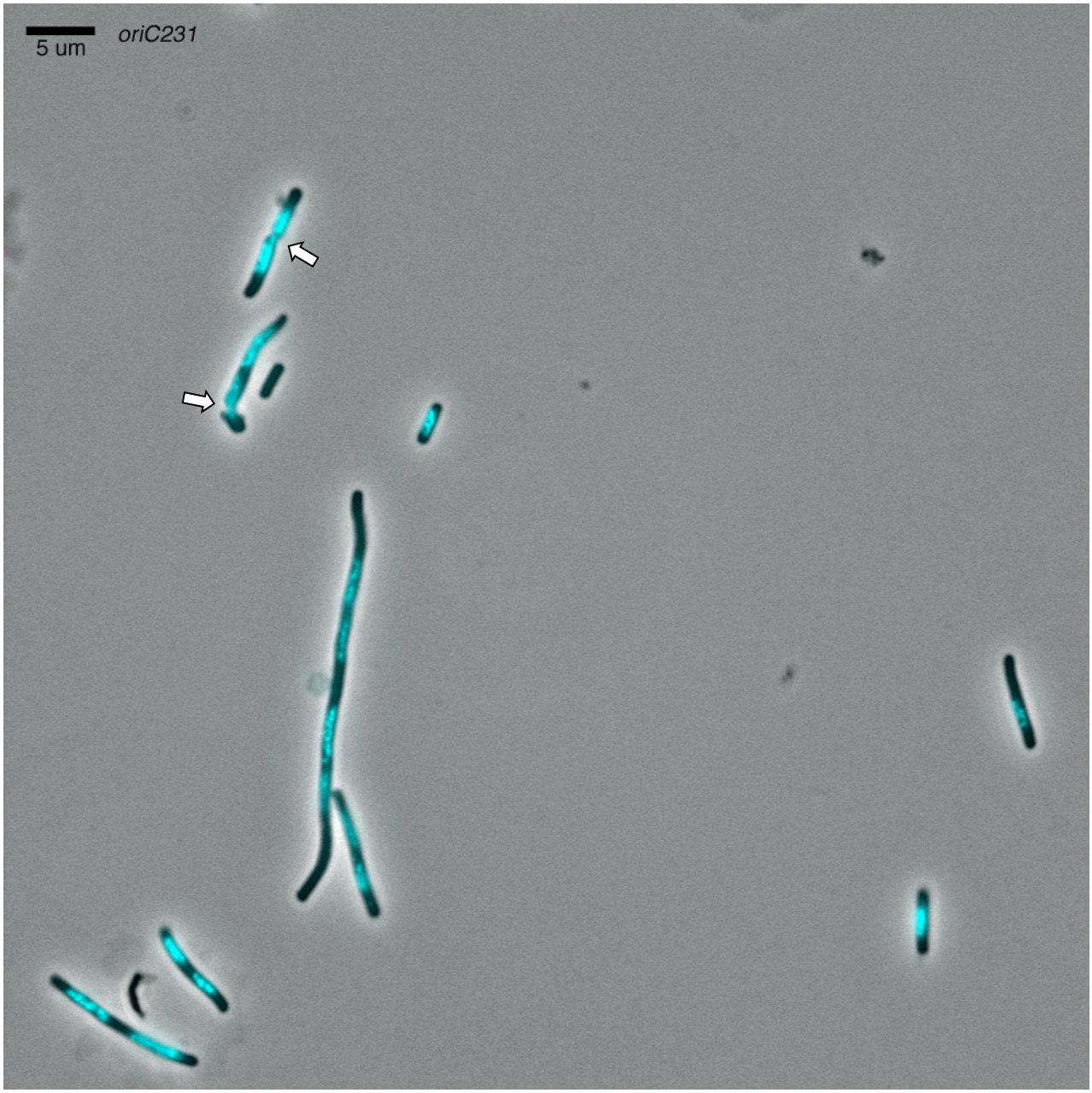

Figure S9

Supplementary data

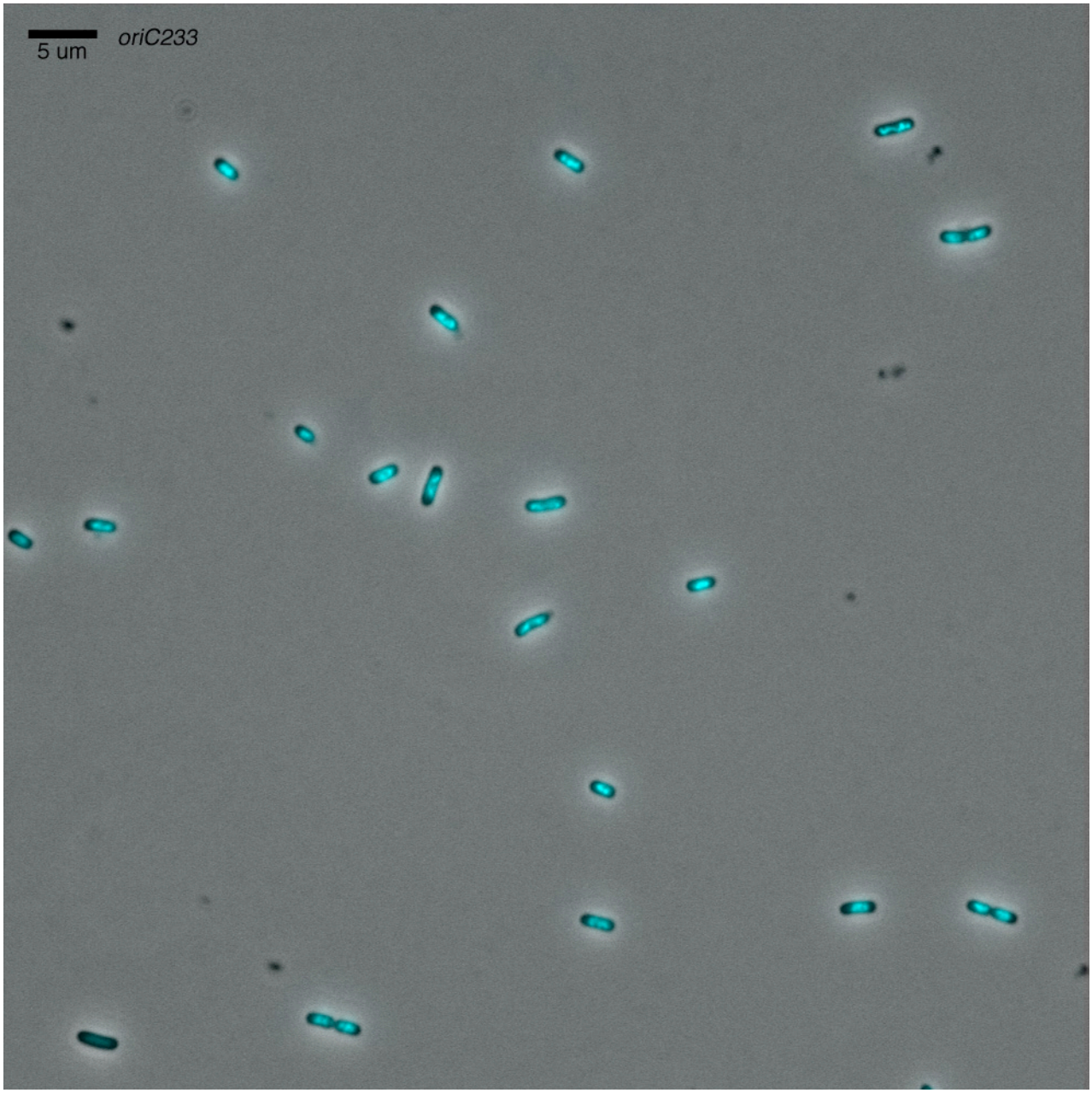

Figure S10

Supplementary data

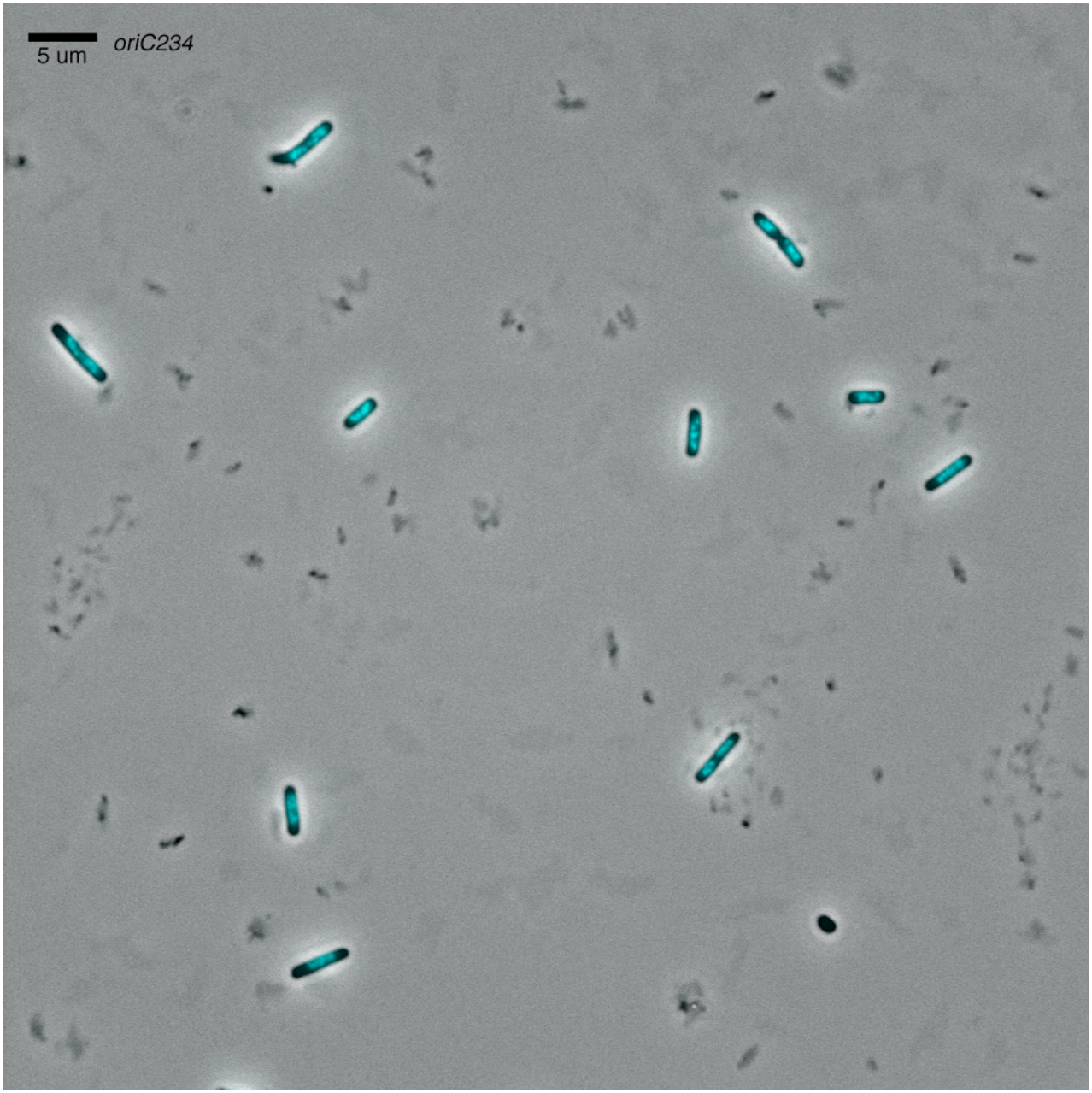

Figure S11

Supplementary data

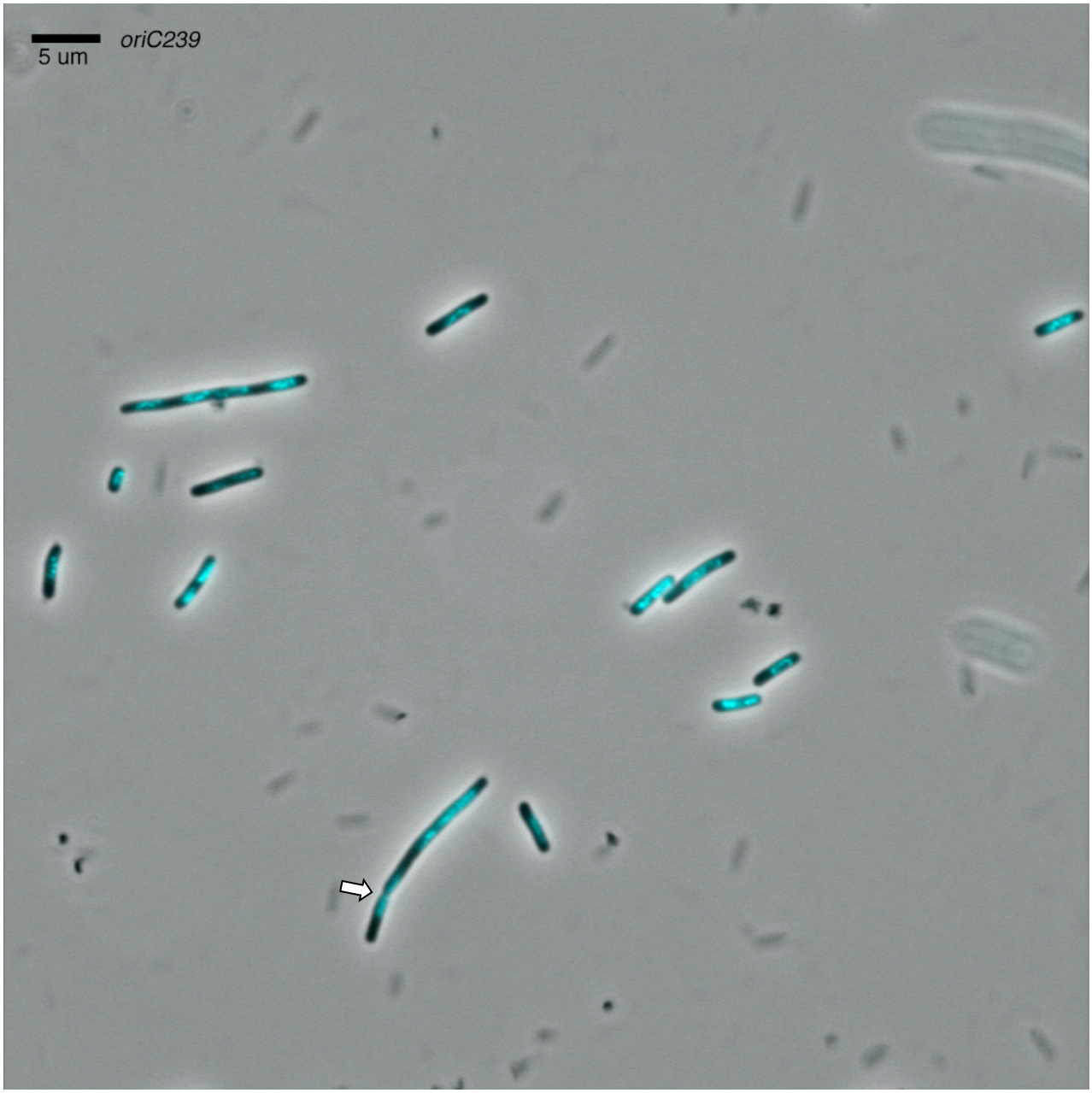

Figure S12

Figure S1. Outline of *in vivo* transfer proceedure and analysis

Mutant *oriC* alleles are transferred onto the chromosome of *wt* AQ7776 carrying a *gidA::kan* counter selection marker and moved into MG1655 by P1 transduction as described in Experimental procedures. If no lambda recombinants are obtained, the transfer procedure is repeated using an rnhA224 strain, clones are tested for cSDR dependence (Fig. 2), and cSDR-independent clones are moved into *wt* MG1655 by P1 transduction. All clones are verified by Southern blot hybridization (Fig. S3) and direct sequencing (data not shown). Data for efficiency of transfer is shown in Table S1.

Figure S2. Southern blot confirmation of chromosomal *oriC* deletions

Genomic DNA was prepared from *oriC* mutant strains after *in vivo* transfer and P1 transduction (Materials and methods). DNA was digested with SmaI and XhoI, electrophoresed in a 0.8% agarose gel, blotted onto a nylon filter, and hybridized with a ^{32}P-dCTP-labeled probe spanning a 13.1 kb *oriC* region (dashed line). Radioactive bands were visualized by a Phosphorimager (Molecular Dynamics). Three bands were present in all lanes, with the smallest band (B) corresponding to the *oriC*-containing fragment. Migration of B bands was proportional to the corresponding *oriC* deletion size shown below autoradiograph). The bottom half of the gel is shown after additional exposure to enhance visualization of the B fragment.

Figure S3. *oriC* mutants are not SOS-induced

Viable chromosomal *oriC* mutants were assayed for SOS induction by liquid β-galactosidase assay. Cells carrying a *sulA::lacZ* fusion were grown to exponential phase in M9 glucose with casamino acids and were assayed as described (Miller, 1972). Miller units (MU) were corrected for cell density (3 independent measurements +/- SD). A *lexA71* strain that is constitutively SOS-induced is shown for comparison. We conclude that cell elongation in several of the *oriC* mutants (Fig. 3), is independent of the DNA damage response cell division inhibitor SulA. Supporting this conclusion, cell elongation was not inhibited in any of the *oriC* mutants after introducing a *sulA11* mutation (data not shown). Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Figures S4-S12. Micrographs of viable *oriC* mutants

Combined phase / DAPI images were obtained for each strain as described in Experimental Procedures. White arrows indicate apparently guillotined nucleoids.