Supporting Information

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SI Methods

Bacterial Strains and Growth Conditions. Synchronized cultures were grown in a 3-(*N*-morpholino)propanesulfonic acid (Mops)-buffered minimal medium (50 mM Mops, 1 mM $MgSO_4$, 0.25 mM $CaCl_2$, 19 mM glutamic acid, 0.004 mM biotin, pH 7.0) supplemented with 1% casamino acids at 30 °C for 40 min. Then 1 mL of culture was treated with NCR247, LL-37, melittin, or polymyxin B at the indicated concentrations. This treatment was designated time 0 for all experiments. DNA content per cell was measured as described (1).

RNA Isolation, Gene-Expression Profiling, and Heatmap Generation. Five 1-mL cultures treated or not treated with 4 μM NCR247 peptide were mixed in a ratio of 1:2 (vol/vol) with RNAprotect bacteria reagent (Qiagen). Fixed cells then were collected by filtration through a 0.22-μM Durapore filter (Millipore). Fixed cells on the filter were suspended and lysed in Tris-EDTA buffer (pH 8.0) containing 15 mg/mL of lysozyme and 15 μL of Proteinase K (Qiagen). RNA was isolated from lysate using the RNeasy Plus Mini kit (Qiagen).

For quantitative PCR (qPCR) experiments, 500 μg RNA was reverse transcribed using the IScriptcDNA synthesis kit (Bio-Rad). qPCR analysis of cDNA of indicated genes and the internal control gene, *smc00128*, (2) was performed with Power SYBR Green PCR Master Mix (Life Technologies) on a StepOnePlus real-time PCR machine (Life Technologies). The $2^{\Delta\Delta CT}$ method was used to determine the expression level of indicated genes relative to *smc00128* expression. For qPCR data in Figs. 2 and 4 *B* and *C* and in Fig. S4, relative gene-expression data from three biological replicates were normalized, log transformed, mean centered, and autoscaled as described (3). Oligonucleotide primers are available upon request.

Single-channel expression normalization of microarray data was done using the *limma* package in R (4, 5). Briefly, signals were background corrected by fitting a convolution model of normal and exponential distributions to the foreground intensities using the background intensities as a covariate. The expected signal given the observed foreground becomes the corrected intensity (6). Background corrected values then were quantile normalized for between-array accuracy. For each time point, biological replicate samples were averaged to generate $\log_2$ expression values for each gene.

Normalized microarray data of nontreated and NCR247-treated samples were compared directly using the *limma* package in R. A linear model was fitted to the normalized $\log_2$ values for each gene and used to generate estimated coefficients and SEs for each time point of the compared samples. The estimated coefficients and SEs then were used to compute moderated t-statistics, moderated F-statistics, and log-odds of differential expression by empirical Bayes shrinkage of the SEs toward a common value. Genes identified as differentially expressed had logFC values greater than or equal to 1.0 or less than or equal to −1.0 and an adjusted *P* value of ≤0.01.

To generate heatmaps, the replicate-average $\log_2$ expression values for cell-cycle–, CtrA-, ExoS/ChvI-, FeuP/FeuQ-, and RirA-regulated genes were row normalized $[X - \overline{X}/(\text{std } X)]$ across the time points. Normalized values then were clustered by using Gene Cluster 3.0 and the city block similarity metric with complete linkage clustering (7). For the heat map of all 902 genes significantly affected by NCR247-peptide treatment, the replicate-average $\log_2$ expression value for a gene at each time point was divided by the $\log_2$ expression value at time 0, before being row normalized across the time points. Differentially expressed genes also were subjected to hierarchical clustering.

Comparison with Other Microarray Studies. We compared our microarray data with microarray data on *Sinorhizobium meliloti* cells exposed to iron, acid, heat, osmotic, carbon, phosphate, or nitrogen stress (Table S1) by first applying the cutoffs (fold change, *P* value) from these studies to determine differentially expressed genes in our data for cultures treated for 90 min with a sublethal dose of NCR247. The resulting gene list was compared with the gene list from each study to test for significant overlap using a hypergeometric test. Genes designated as being specifically affected by NCR247-peptide treatment exhibited insignificant changes in *S. meliloti* expression under other stress conditions (Dataset S1).

1. De Nisco NJ, Abo RP, Wu CM, Penterman J, Walker GC (2014) Global analysis of cell cycle gene expression of the legume symbiont *Sinorhizobium meliloti*. *Proc Natl Acad Sci USA*, 10.1073/pnas.1400421111.
2. Krol E, Becker A (2004) Global transcriptional analysis of the phosphate starvation response in *Sinorhizobium meliloti* strains 1021 and 2011. *Mol Genet Genomics* 272(1): 1–17.
3. Willems E, Leyns L, Vandesompele J (2008) Standardization of real-time PCR gene expression data from independent biological replicates. *Anal Biochem* 379(1):127–129.
4. Smyth GK (2005) *Limma: Linear Models for Microarray Data* (Springer, New York).
5. Smyth GK (2004) Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 3:e3.
6. Ritchie ME, et al. (2007) A comparison of background correction methods for two-colour microarrays. *Bioinformatics* 23(20):2700–2707.
7. Eisen MB, Spellman PT, Brown PO, Botstein D (1998) Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA* 95(25):14863–14868.

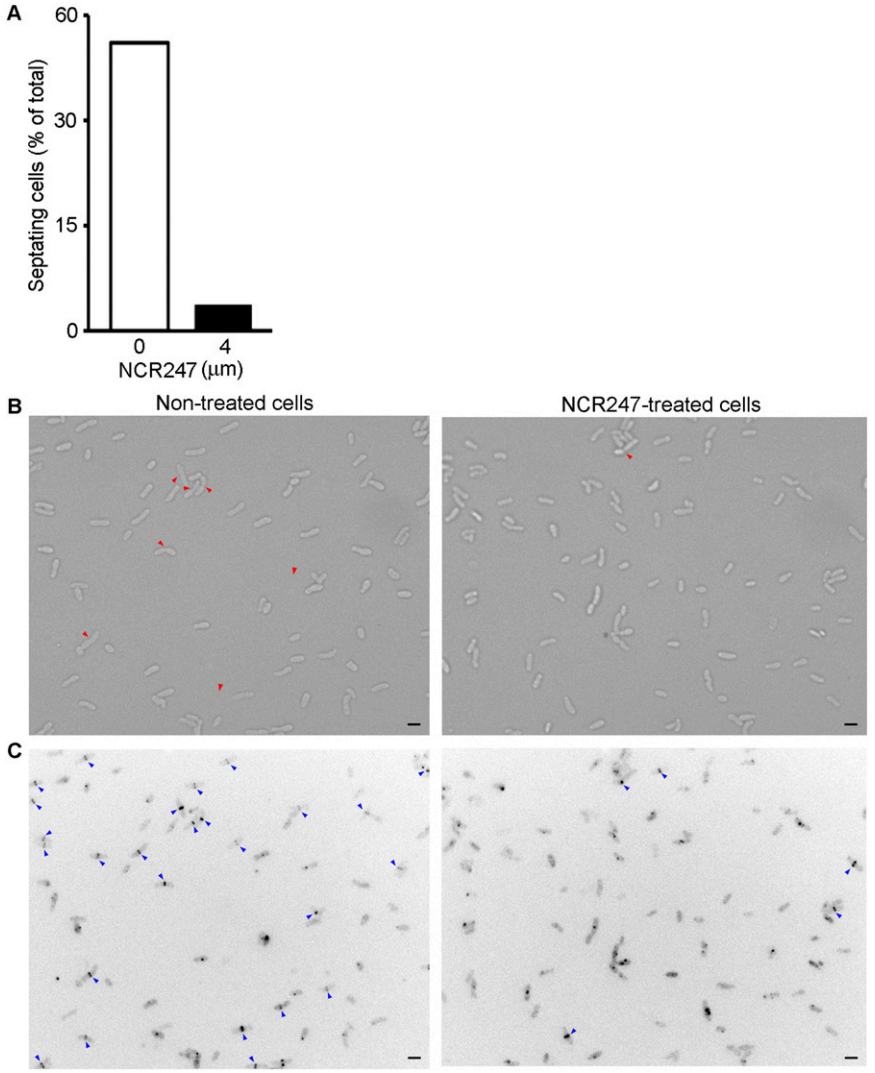

Fig. S1. Treatment with NCR247 peptide blocks cell division by antagonizing Z-ring formation or stability. (*A*) Frequency of septating cells in nontreated (white bar) and NCR247-treated (black bar) synchronized cultures at the G2 phase (155 min) of the cell cycle. Data represent >1,000 cells from one experiment. (*B* and *C*) Light (*B*) and fluorescence (*C*) micrographs of FtsZ-GFP–expressing cells in nontreated and NCR247-treated synchronized cultures at the G2 phase (155 min) of the cell cycle. Red arrowheads in *B* mark cells exhibiting morphological signs of septation. Blue arrowheads in *C* mark cells with a GFP-labeled Z-ring. (Scale bar, 1 μm.)

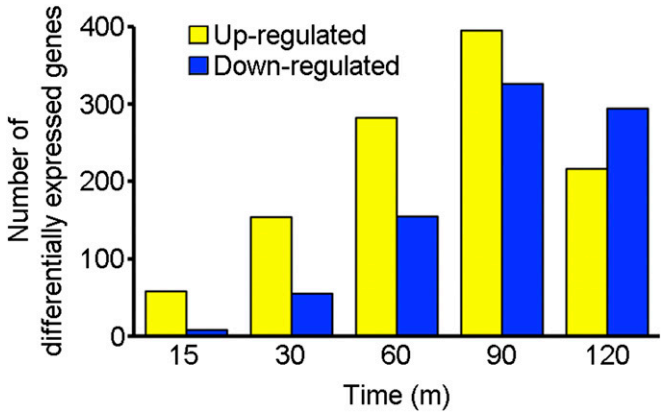

Fig. S2. The total number of differentially expressed genes increases progressively during the cell cycle of NCR247-treated cultures.

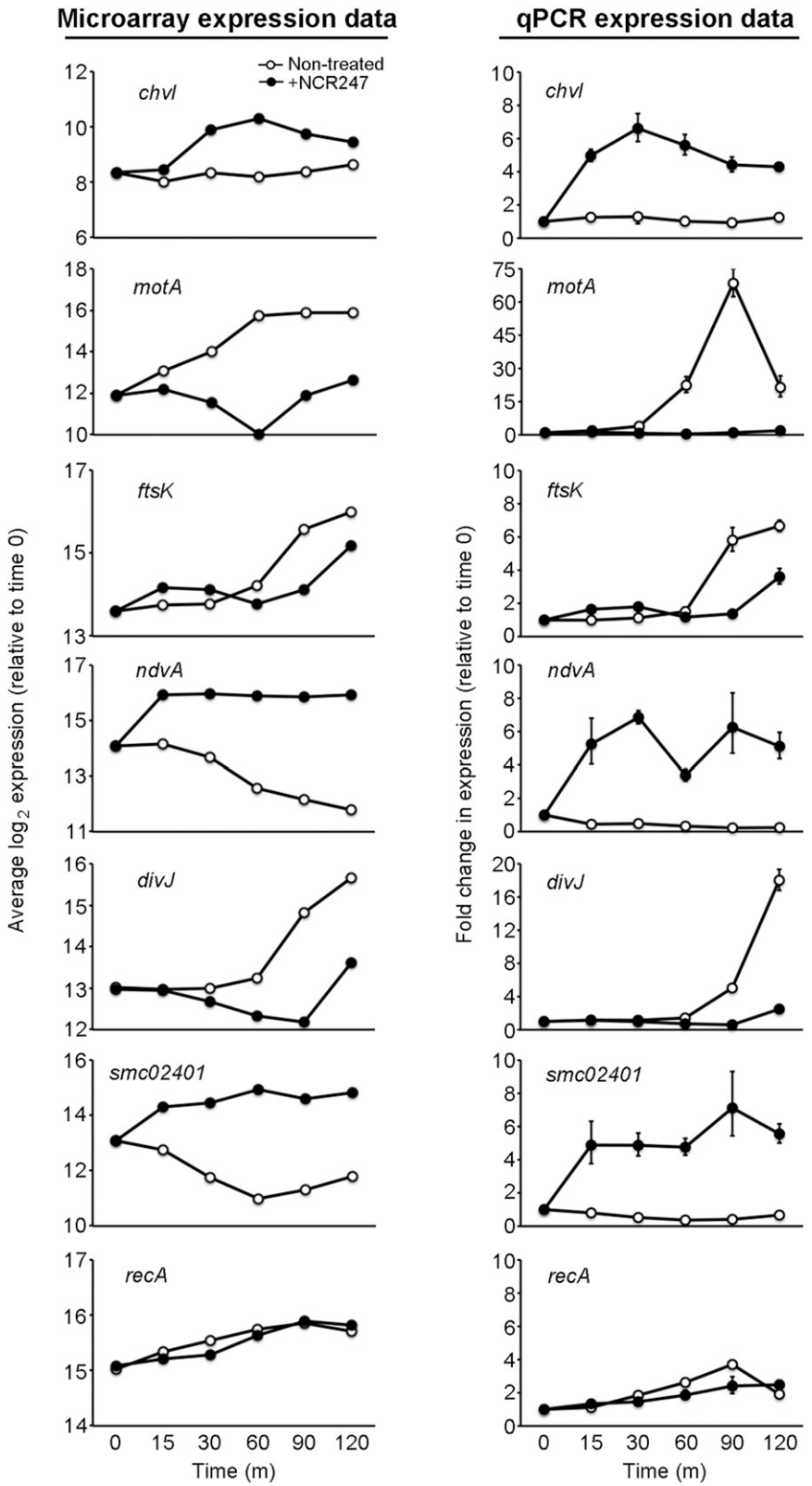

Fig. S3. Gene-expression levels and patterns during synchronized growth as detected by microarray and qPCR experiments. (*Left*) Average $\log_2$ hybridization values relative to time 0 from the microarray experiment. (*Right*) Fold change in the indicated genes in nontreated and NCR247-treated synchronized cultures relative to time 0. Quantitative PCR data from one biological replicate are shown. Error bars indicate SE.

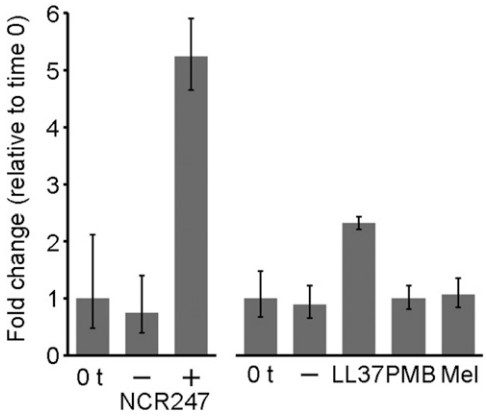

Fig. S4. Treatment of *S. meliloti* with a sublethal level of NCR247 or LL37 induces expression of the RirA-regulated *smc01747* gene. Shown is the fold change in *smc01747* expression relative to time 0 in cultures after 15 min of treatment with no peptide or with a sublethal inhibitory concentration of NCR247, LL-37 (1 μM), melittin (Mel) (0.5 μM), or polymyxin B (PMB) (1.25 μg/mL). The expression of the control gene *smc00128* was used to compare *smc01747* expression levels. *smc01747* is directly regulated by RirA. Error bars indicate the 95% confidence interval.

Table S1. Overlap of differentially expressed genes in *Sinorhizobium meliloti* exposed to NCR247 and other stresses

Description of stress	Reference	Differentially expressed genes that overlap	Differentially expressed genes in NCR247	Differentially expressed genes in stress condition	Total no. of genes in common between the arrays	*P* value
Iron	(1)	108	772	319	6,142	5.0E-25
Acid	(2)	60	588	210	6,135	3.2E-16
Heat	(3)	394	812	2,023	6,135	1.8E-23
Osmotic	(4)	60	886	137	6,142	7.7E-18
Carbon starvation	(5)	19	507	199	6,135	0.208
Phosphate starvation	(5)	64	398	315	6,142	5.6E-18
Nitrogen	(6)	18	282	130	6,135	6.2E-06

1. Chao TC, Buhrmester J, Hansmeier N, Pühler A, Weidner S (2005) Role of the regulatory gene rirA in the transcriptional response of *Sinorhizobium meliloti* to iron limitation. *Appl Environ Microbiol* 71(10):5969–5982.
2. de Lucena DK, Pühler A, Weidner S (2010) The role of sigma factor RpoH1 in the pH stress response of *Sinorhizobium meliloti*. *BMC Microbiol* 10:265.
3. Barnett MJ, Bittner AN, Toman CJ, Oke V, Long SR (2012) Dual RpoH sigma factors and transcriptional plasticity in a symbiotic bacterium. *J Bacteriol* 194(18):4983–4994.
4. Rüberg S, et al. (2003) Construction and validation of a *Sinorhizobium meliloti* whole genome DNA microarray: Genome-wide profiling of osmoadaptive gene expression. *J Biotechnol* 106(2-3):255–268.
5. Krol E, Becker A (2004) Global transcriptional analysis of the phosphate starvation response in *Sinorhizobium meliloti* strains 1021 and 2011. *Mol Genet Genomics* 272(1):1–17.
6. Yurgel SN, Rice J, Kahn ML (2013) Transcriptome analysis of the role of GlnD/GlnBK in nitrogen stress adaptation by *Sinorhizobium meliloti* Rm1021. *PLoS ONE* 8(3):e58028.

Other Supporting Information Files

Dataset S1 (XLSX)