Supplemental information for:

A xylose-inducible expression system and a CRISPRi-plasmid for targeted knock-down of gene expression in *Clostridioides difficile*

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Fig S1

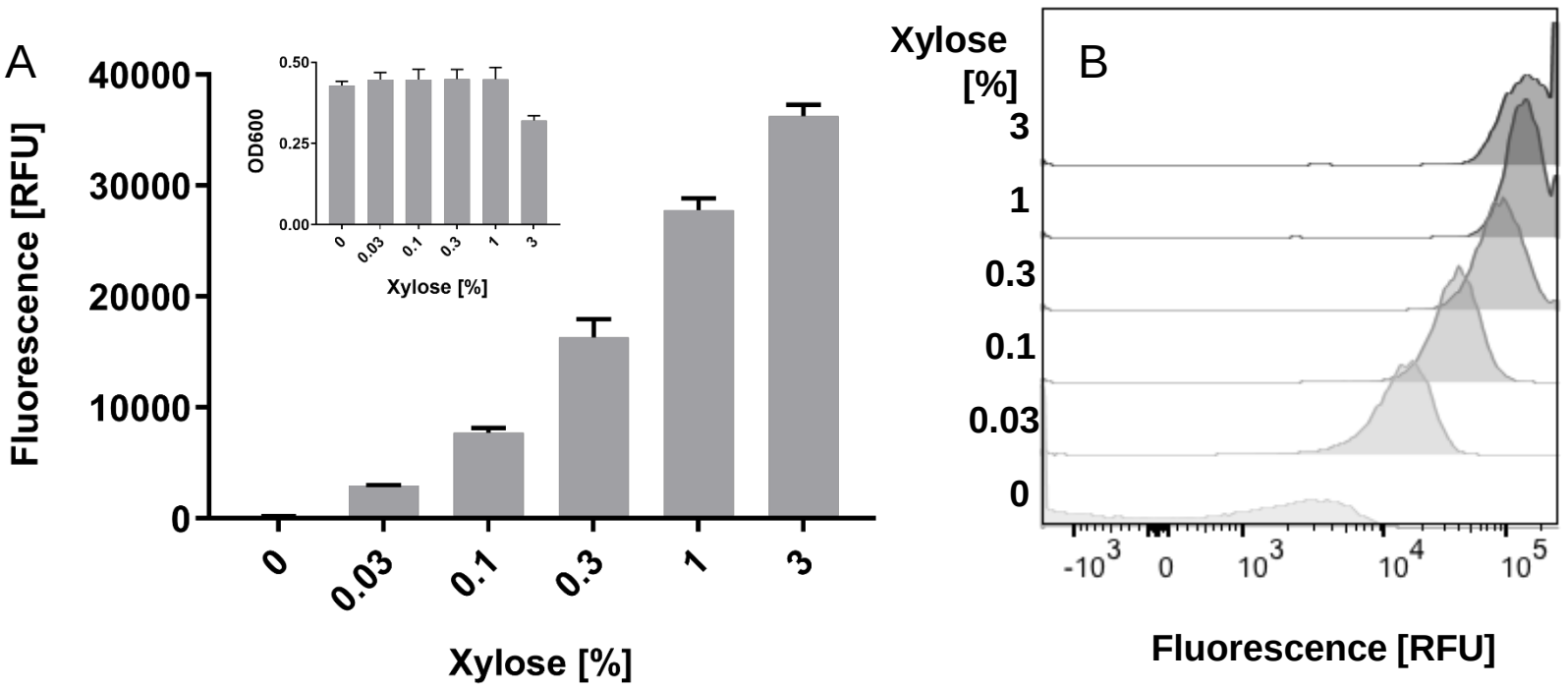

Fig S1. Tunable induction from P_{xyl} in 630Δ*erm*. An overnight culture of 630Δ*erm*/pAP114 was diluted to a starting OD_{600} = 0.05 into TY Thi_{10} with the indicated concentration of xylose. Once cells had reached an OD_{600} = 0.5 (~5h) they were fixed and processed to allow RFP development. (A) A plate reader was used to measure relative fluorescence and OD_{600} of bulk samples. (B) Flow cytometry was used to measure fluorescence of individual cells. RFU is relative fluorescence units normalized to OD_{600}. Data in (A) represent the mean and standard deviation of triplicate cultures. These results are representative of at least two independent experiments.

Fig S2

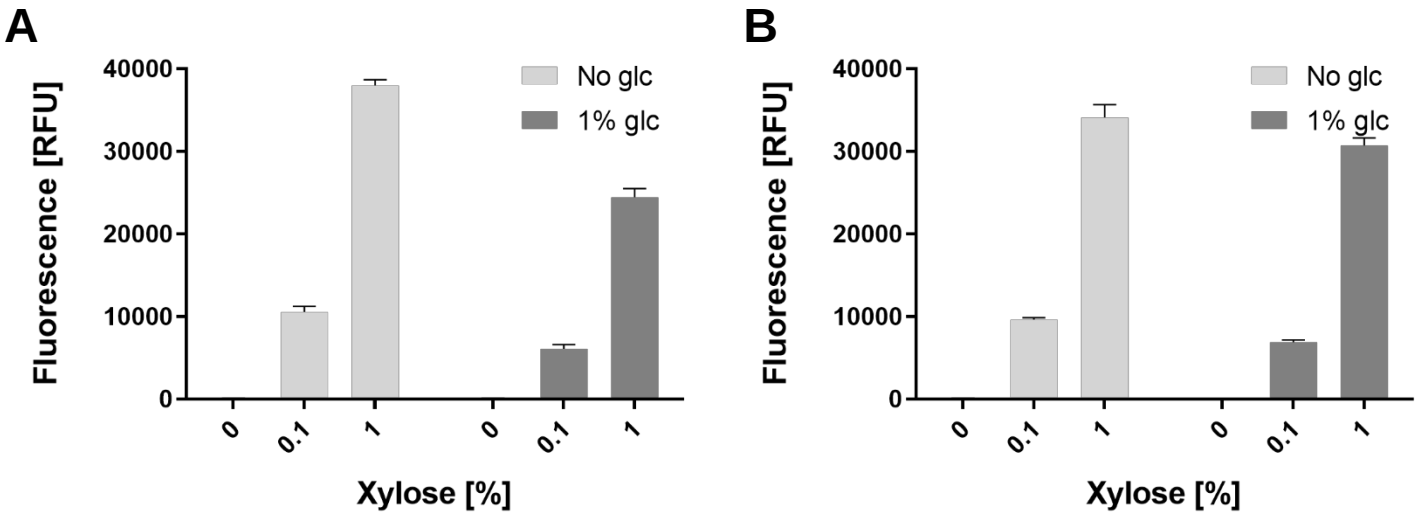

Fig S2. Glucose has a modest effect on xylose induction. (A) R20291/pAP114. (B) 630Δ*erm*/pAP114. Procedures as described in the legend to Fig. S1. Data are graphed as the mean and standard deviation of triplicate cultures. Results are representative of at least two experiments.

Fig S3

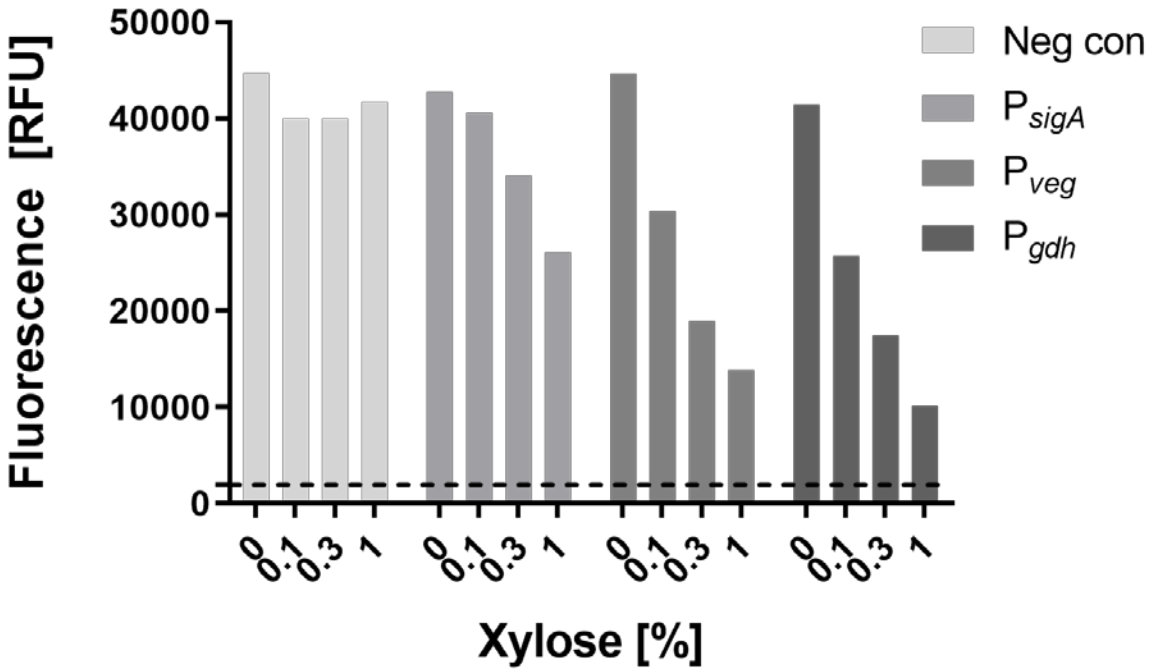

Fig S3. Test of three sgRNA promoters for CRISPRi. A set of CRISPRi plasmids was introduced into a *C. difficile* strain that expresses *rfp* constitutively. The CRISPRi plasmids express *dCas9* under P_{xyl} control and sgRNAs that target *rfp* under control of three constitutive promoters: P_{sigA} in pIA26, P_{veg} in pIA27, or P_{gdh} in pIA28. The negative control expressed neg^{sgRNA} from P_{gdh} (pIA25). Overnight cultures were used to inoculate TY Thi_{10} with xylose as indicated at OD_{600} = 0.05. After 5 hours, cells were fixed and processed to allow RFP development. The dashed line indicates fluorescence seen with strain 630Δ*erm*, which lacks *rfp*. RFU is relative fluorescence units normalized to OD_{600}. Results are representative of two experiments.

Fig S4

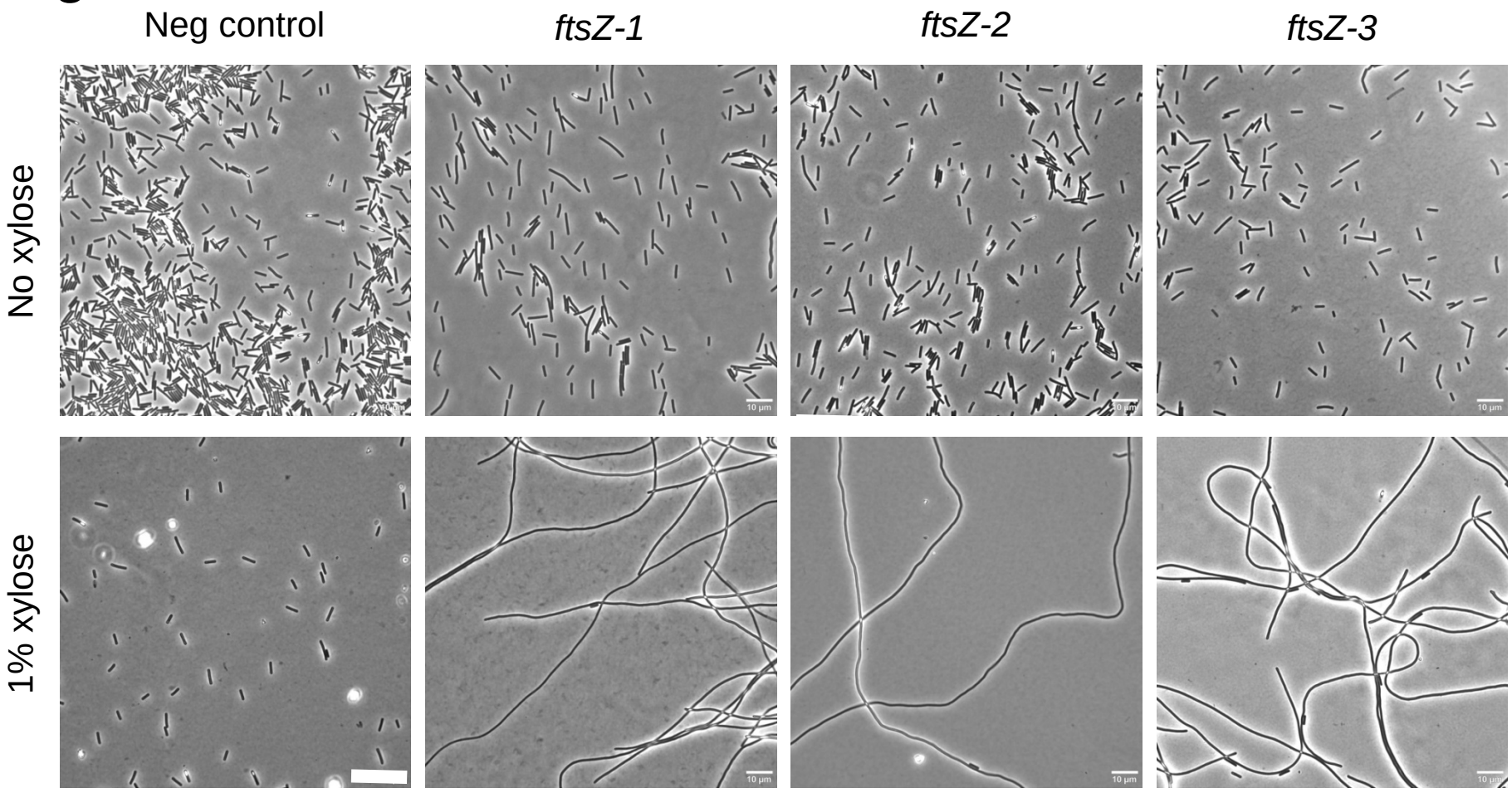

Fig S4. CRISPRi of *ftsZ* induces filamentation. Cells that grew from the undiluted samples spotted on the plates shown in Fig 4A were scraped from the plate and examined under phase contrast microscopy. Size bar on bottom left panel = 20 µm. Results are representative of two experiments.

Fig S5

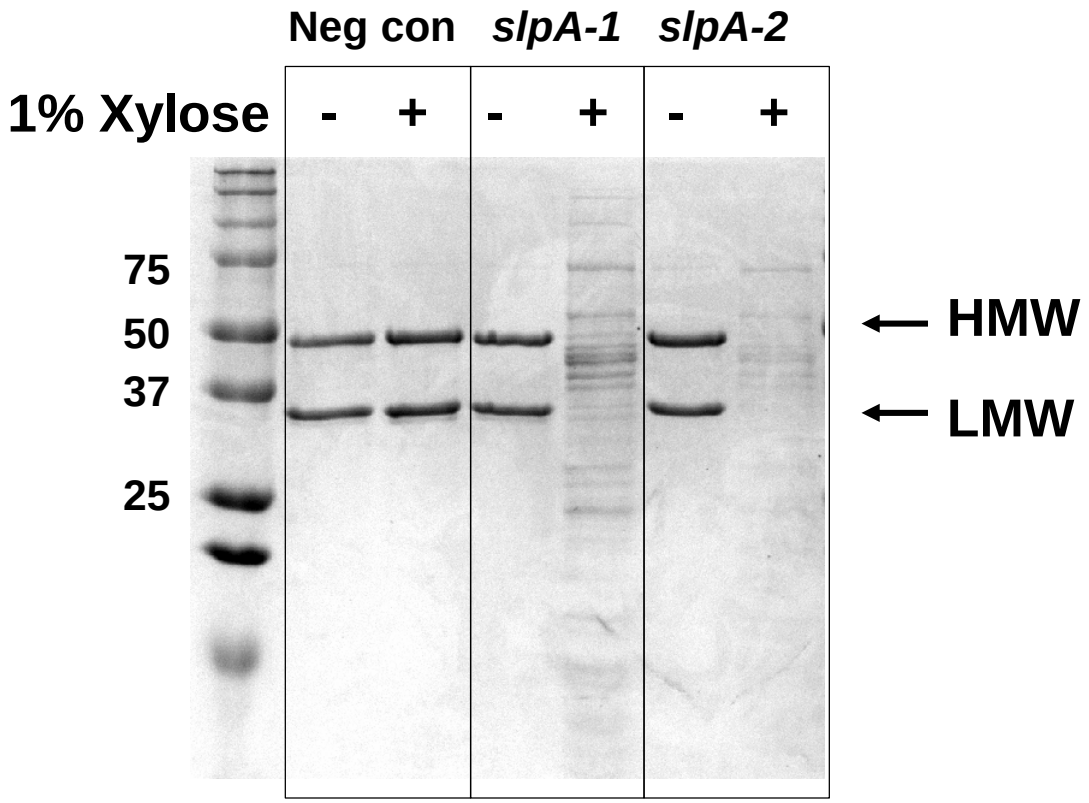

Fig S5. CRISPRi depletion of SlpA. Cultures of R20291 harboring pIA34 (negative control) or CRISPRi constructs with sgRNAs that target *slpA* (pIA38, pIA39) were grown in the presence or absence of 1% xylose as described in the legend to Fig. 5. Whole cell extracts were analyzed by SDS-PAGE followed by Coomassie staining. Molecular mass markers in kilodaltons are indicated to the left. The high molecular weight band (HMW) and the low molecular weight band (LMW) of SlpA are marked by arrows.

Fig S6

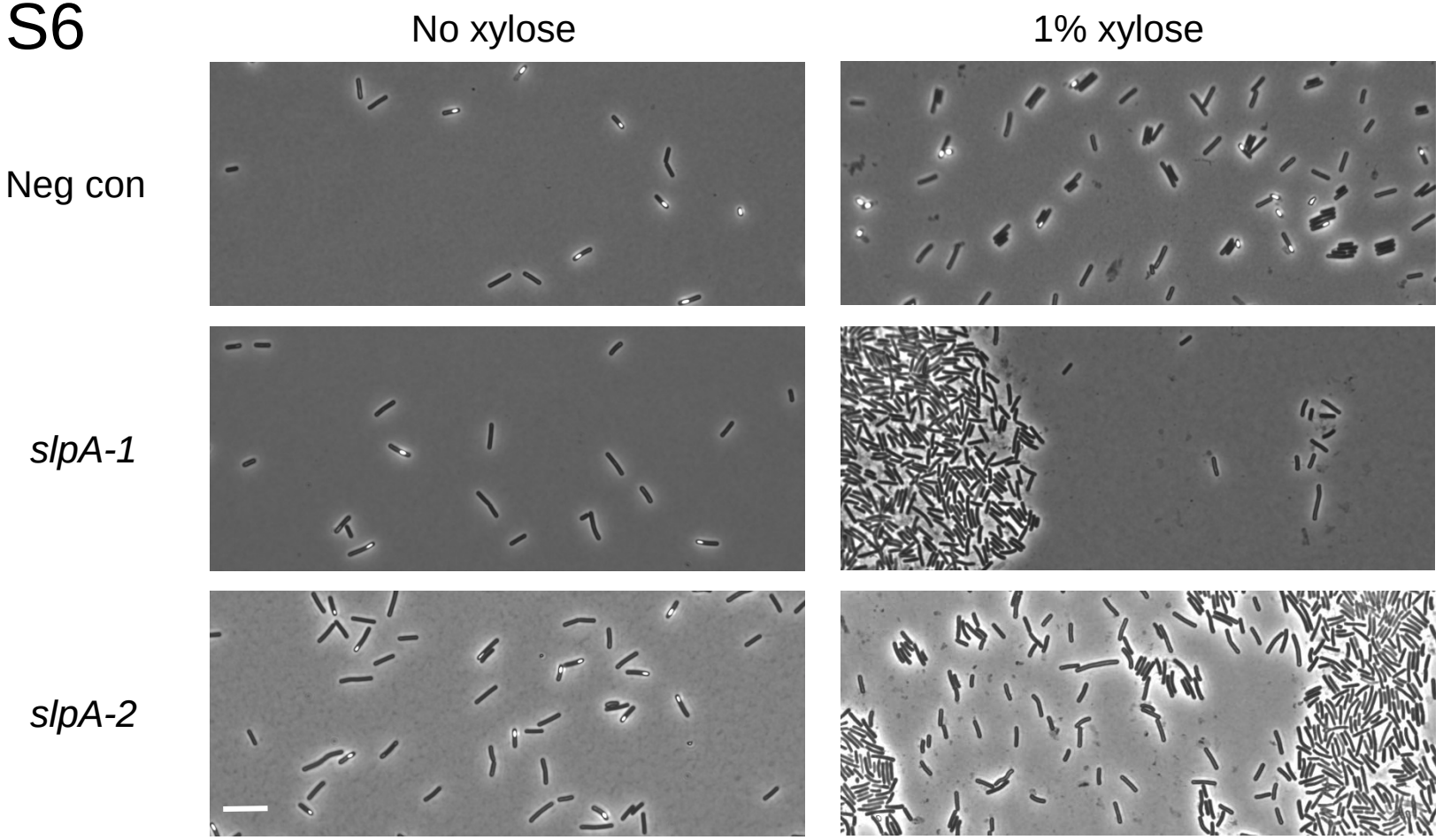

Fig S6. CRISPRi of *slpA* impairs sporulation but does not affect cell morphology. R20291 cells that grew from the undiluted samples spotted on the plates shown in Fig. 5A were scraped from the plate and examined under phase contrast microscopy. The phase-bright objects are spores. In the presence of 1% xylose, the numbers of spores were visibly reduced for the CRISPRi-*slpA*^{sgRNA} constructs but not the negative control. Size bar on bottom left panel = 10 µm. Results are representative of two experiments.

Fig S7

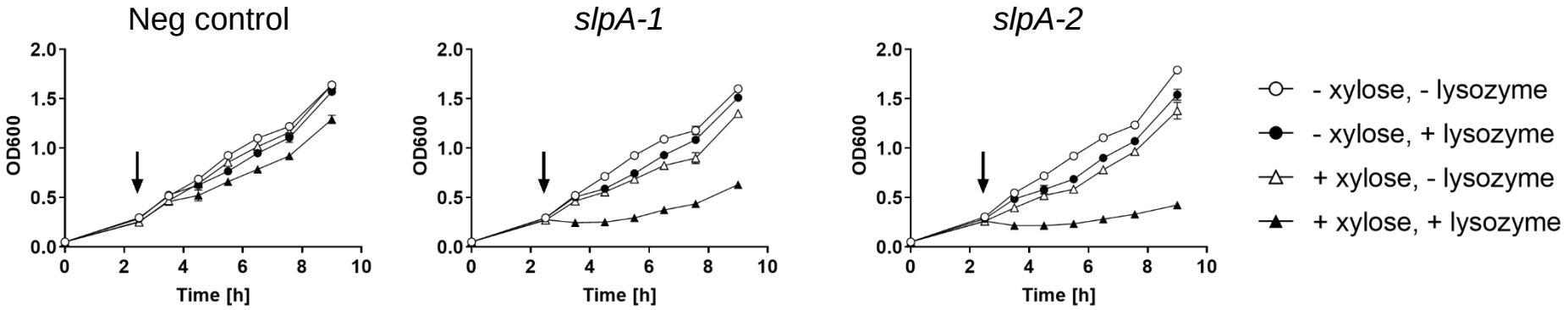

Fig S7. CRISPRi depletion of SlpA increases sensitivity to lysozyme. Duplicate cultures of R20291 harboring pIA34 (negative control) or CRISPRi constructs with sgRNAs that target *slpA* (pIA38, pIA39) were inoculated to OD_{600} = 0.05 in TY Thi_{10} with or without xylose. After 2.5h (**arrows**) lysozyme was added to 0.5 mg/mL and growth was monitored by OD_{600} for the next 6.5h. Results are representative of two experiments.

TABLE S1. Plasmids used in this study

Plasmid	Relevant features	Parent vector	Restriction enzymes to digest parent vector	PCR primers	PCR template	Comments	Reference
pJK02	P_{tet}::*Cas9-opt* P_{gdh}::*sgRNA colE1 CD6 ori RP4oriT-traJ catP*						McAllister et al 2017
pJMP2	P_{veg}::*sgRNA colE1 catP amp spc*						Peters et al 2016
pJMP4	P_{veg}::*rfp colE1 catP amp spc*						Peters et al 2016
pRFP185	*E. coli-C. difficile* shuttle vector with tetracycline-inducible promoter; P_{tet}::*gusA tetR CD6ori RP4oriT-traJ pMB1ori catP*						Fagan and Fairweather 2011
pdCas9_bacteria	P_{tet}::*dCas9 tetR ori15A catP*					Addgene 44249	Qi et al 2013
pMTL-YN1C	*E. coli-C. difficile* shuttle vector for inserting genes into *C. difficile* chromosome while restoring *pyrE*; c*olE1 RP4oriT-TraJ CB102ori-repH' catP*						Ng et al 2013
pDSW1728	P_{tet}::*mCherryOpt catP*						Ransom et al 2015
pDSW1963	P_{veg}::*rfp catP*	pDSW1728	BamHI, NheI	P2281+P2282	pJMP4	P_{veg}::*rfp* amplified from pJMP4 replaces P_{tet}::*mCherryOpt* in pDSW1728	This study
pIA17	P_{veg}::*rfp* in pMTL-YN1C *catP*	pMTL-YN1C	NotI, XhoI	4057+4058	pDSW1963	P_{veg}::*rfp* amplified from pDSW1963 is cloned into allelic exchange vector pMTL-YN1C	This study
pCE531	P_{tet}::*dCas9 catP*	pRFP185	SacI, BamHI	3083+3084	pdCas9_bacteria	*dCas9* amplified from pdCas9_bacteria cloned under P_{tet} control in pRFP185; primer 3084 includes P_{sigA}, and MscI and NotI sites for next cloning steps	This study
pIA18	P_{tet}::*dCas9* P_{sigA}::*sgRNA-neg catP*	pCE531	MscI, NotI	4083+4084	pJK02	*sgRNA* handle amplified from pJK02 and inserted into pCE531; bp-region for sgRNA-*neg* included	This study
pIA19	$P_{xylB'}$::*mCherryOpt catP*	pDSW1728	SacI, KpnI	4102+4103	gDNA R20291	*xylR* through first 63 nt of *xylB* ($P_{xylB'}$) replaces P_{tet} in pDSW1728 to control *mCherryOpt*	This study
pAP114	P_{xyl}::*mCherryOpt catP*	pDSW1728	SacI, KpnI	4102+4162	gDNA R20291	*xylR* and region immediately upstream of *xylB* (P_{xyl}) replaces P_{tet} in pDSW1728 to control *mCherryOpt*	This study
pIA20	$P_{xylB'}$::*dCas9* P_{sigA}::*sgRNA-neg catP*	pIA19	PstI (6271 bp)	4135+4136	pIA18	*dCas9* P_{sigA}::*sgRNA-neg traJ* amplified from pIA18 replaces *mCherryOpt traJ* in pIA19	This study
pIA21	P_{xyl}::*dCas9* P_{sigA}::*sgRNA-neg catP*	pIA20	PacI, BstZ171	4168+4169 & 4170+4171	pIA20	Delete 63 nt of *xylB* from promoter region driving *dCas9* expression (i.e. convert $P_{xylB'}$ to P_{xyl})	This study
pIA24	P_{xyl}::*dCas9* P_{veg}::*sgRNA-neg catP*	pIA21	AvrII, MscI	4143+4144	pJMP2	P_{veg} amplified from pJMP2 replaces P_{sigA} in pIA21	This study
pIA25	P_{xyl}::*dCas9* P_{gdh}::*sgRNA-neg catP*	pIA24	XhoI, MscI	4172+4173	pJK02	P_{gdh} amplified from pJK02 replaces P_{veg} in pIA24	This study
pIA26	P_{xyl}::*dCas9* P_{sigA}::*sgRNA-rfp catP*	pIA21	MscI, NotI	4184+4084	pIA21	Replace *sgRNA-neg* with *sgRNA-rfp*	This study
pIA27	P_{xyl}::*dCas9* P_{veg}::*sgRNA-rfp catP*	pIA24	MscI, NotI	4185+4084	pIA24	Replace *sgRNA-neg* with *sgRNA-rfp*	This study
pIA28	P_{xyl}::*dCas9* P_{gdh}::*sgRNA-rfp catP*	pIA25	MscI, NotI	4186+4084	pIA25	Replace *sgRNA-neg* with *sgRNA-rfp*	This study

TABLE S1. Plasmids used in this study

Plasmid	Relevant features	Parent vector	Restriction enzymes to digest parent vector	PCR primers	PCR template	Comments	Reference
pIA33	P_{xyl}::*dCas9-opt* P_{gdh}::*sgRNA-rfp catP*	pIA28	SalI, XhoI	4197+4198 & 4199+4200	pJK02	*cas9-opt* amplified from pJK02, mutated to *dCas9-opt* and inserted into pIA28 to replace *dCas9*	This study
pIA34	P_{xyl}::*dCas9-opt* P_{gdh}::*sgRNA-neg catP*	pIA33	MscI, NotI	4238+4084	pIA33	Replace *sgRNA-rfp* with *sgRNA-neg*	This study
pIA35	P_{xyl}::*dCas9-opt* P_{gdh}::*sgRNA-ftsZ-1 catP*	pIA33	MscI, NotI	4234+4084	pIA33	Replace *sgRNA-rfp* with *sgRNA-ftsZ-1*	This study
pIA36	P_{xyl}::*dCas9-opt* P_{gdh}::*sgRNA-ftsZ-2 catP*	pIA33	MscI, NotI	4235+4084	pIA33	Replace *sgRNA-rfp* with *sgRNA-ftsZ-2*	This study
pIA37	P_{xyl}::*dCas9-opt* P_{gdh}::*sgRNA-ftsZ-3 catP*	pIA33	MscI, NotI	4236+4084	pIA33	Replace *sgRNA-rfp* with *sgRNA-ftsZ-3*	This study
pIA38	P_{xyl}::*dCas9-opt* P_{gdh}::*sgRNA-slpA-1 catP*	pIA33	MscI, NotI	4277+4084	pIA33	Replace *sgRNA-rfp* with *sgRNA-slpA-1*	This study
pIA39	P_{xyl}::*dCas9-opt* P_{gdh}::*sgRNA-slpA-2 catP*	pIA33	MscI, NotI	4278+4084	pIA33	Replace *sgRNA-rfp* with *sgRNA-slpA-2*	This study
pIA40	P_{xyl}::*dCas9-opt* P_{gdh}::*sgRNA-pbp-1 catP*	pIA33	MscI, NotI	4279+4084	pIA33	Replace *sgRNA-rfp* with *sgRNA-pbp-1*	This study
pIA41	P_{xyl}::*dCas9-opt* P_{gdh}::*sgRNA-pbp-2 catP*	pIA33	MscI, NotI	4280+4084	pIA33	Replace *sgRNA-rfp* with *sgRNA-pbp-2*	This study

TABLE S2. Primers used in this study

Oligo	Sequence	Relevant features
3083	GTTAACAGATCT**GAGCTC**GATCTAAAGAGGAGAAAGGATC	Bold: SacI
3084	GTTTTATTAAAACTTATA**GGATCC***GCGGCCGC* **TGGCCA**ATTATACCTAGGACTGAGCTAGCTGTCAAAGCGTTCACCGACAAACAAC	Bold & underlined: BamHI; Italic: NotI; Bold: MscI; Underlined: PsigA
4083	AGTCCTAGGTATAAT**TGGCCA**GACCGCTAAACTGAAAGTT GTTTTAGAGCTAGAAATAGC	Bold: MscI; Underlined: sgRNA-neg
4084	AACTTATAGGATCC**GCGGCCGC**TAGTCAGACATCATGCTGATCTAGA	Bold: NotI
4102	GCTTCTTATTTTTATGGTACGTATAATTTAAAGGGTTATGGCTGT	
4103	TCTCCTTTACTGCAGGAGCTAAATAAAACTGTTTTAGTACCTGATG	
4135	AGTTTTATTTAGCTCCTGCAAGATCTGAGCTCGATCTAAAG	
4136	AGAGACCGGTCAGATCTGCA**CTGCAG**AATTCGCCCTTCCT	Bold: PstI
4143	GACAGCTAGCTCAGT**CCTAGG**CCGCGATTTCCAATGAGGT	Bold: AvrII
4144	CTTTCAGTTTAGCGGTCT**TGGCCA**ATTGTACAACACGAGCCCAT	Bold: MscI
4162	TCTCCTTTACTGCAG**GAGCTC**ACTTCATAACTATCGTTTTCCTATC	Bold: SacI
4168	AGTCATAACCCTTATATTTCA**TTAATTAA**AGTTAAATTTTGTATATTCAAAAAGCTCT	Bold: PacI
4169	TGCGTCGACTCTCCTCTTTACCCTCCTTGAATGCCACTTC	
4170	GTAAAGAGGAGAGTCGACGCATGGATAAGAAATACTCAATAGGC	
4171	TACGATTCTTCCGACGT**GTATAC**CTTCTACGAGCTGTCCG	Bold: BstZ171
4172	CTAGGAGGTGACTAA**CTCGAG**CAAAAACATCGTAGAAATACG	Bold: XhoI
4173	TTTCAGTTTAGCGGTCT**TGGCCA**TTTACAGTTTAATTATAGCA	Bold: MscI
4184	AGTCCTAGGTATAATTGGCC AACTTTCAGTTTAGCGGTCT GTTTTAGAGCTAGAAATAGC	
4185	TCGTGTTGTACAATTGGCCA AACTTTCAGTTTAGCGGTCT GTTTTAGAGCTAGAAATAGC	
4186	AATTAAACTGTAAATGGCCA AACTTTCAGTTTAGCGGTCT GTTTTAGAGCTAGAAATAGC	
4197	AGGGTAAAGAGGAGA**GTCGAC**GCATGGATAAAAAATATAGTATAGGATTAGCAATAGGAA	Bold: SalI; Underlined: D10A
4198	ATCATCTTTAAGAAAACTTTGTGGTACTATTGCATCTACATCATAATCACTTAATCT	Underlined: H840A
4199	TGTAGATGCAATAGTACCACAAAGTTTTCTTAAAGATGATTCTATAGATAA	Underlined: H840A
4200	TCTACGATGTTTTTG**CTCGAG**TTAATCACCACCTAATTGAGATAAA	Bold: XhoI
4234	AATTAAACTGTAAA**TGGCCA** GCAGCTCTTTTCCCTACTTC GTTTTAGAGCTAGAAATAGC	Bold: MscI; Underlined: sgRNA-ftsZ-1
4235	AATTAAACTGTAAA**TGGCCA** TTTGCCAAGCCTGCAACCAC GTTTTAGAGCTAGAAATAGC	Bold: MscI; Underlined: sgRNA-ftsZ-2
4236	AATTAAACTGTAAA**TGGCCA** ATCTTACCTTCAAATGCAAA GTTTTAGAGCTAGAAATAGC	Bold: MscI; Underlined: sgRNA-ftsZ-3
4238	AATTAAACTGTAAA**TGGCCA** AGACCGCTAAACTGAAAGTT GTTTTAGAGCTAGAAATAGC	Bold: MscI; Underlined: sgRNA-neg
4277	AATTAAACTGTAAA**TGGCCA** ATATCTTCTGCTGCAAACAC GTTTTAGAGCTAGAAATAGC	Bold: MscI; Underlined: sgRNA-slpA-1
4278	AATTAAACTGTAAA**TGGCCA** GTTACTTTATCTCCCTCTGC GTTTTAGAGCTAGAAATAGC	Bold: MscI; Underlined: sgRNA-slpA-2
4279	AATTAAACTGTAAA**TGGCCA** GGAAATCTTATCTAATGGAA GTTTTAGAGCTAGAAATAGC	Bold: MscI; Underlined: sgRNA-pbp-1
4280	AATTAAACTGTAAA**TGGCCA** AGCATTTTGGATACCTGCAT GTTTTAGAGCTAGAAATAGC	Bold: MscI; Underlined: sgRNA-pbp-2
P2281	GTG**GCTAGC**CCTTATTAACGTTGATATAATTTAAATTTTATTTGAC	Bold: NheI
P2282	GTG**GGATCC**TTAAGCACCGGTGGAGTG	Bold: BamHI

All sequences are 5' to 3'

TABLE S3. Guide RNA base-pairing regions

sgRNA-	**Sequence**
neg	AGACCGCTAAACTGAAAGTT
rfp	AACTTTCAGTTTAGCGGTCT
ftsZ-1	GCAGCTCTTTTCCCTACTTC
ftsZ-2	TTTGCCAAGCCTGCAACCAC
ftsZ-3	ATCTTACCTTCAAATGCAAA
slpA-1	ATATCTTCTGCTGCAAACAC
slpA-2	GTTACTTTATCTCCCTCTGC
pbp-0712-1	GGAAATCTTATCTAATGGAA
pbp-0712-2	AGCATTTTGGATACCTGCAT

All sequences are 5' to 3'

References

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