

SUPPLEMENTARY MATERIAL

Supplementary Table 1: strains and plasmids used in this study.

Strain Name (<i>Species</i>)	Genotype	Markers	Notes
Mach1 (<i>E. coli</i>)	F- $\Phi 80lacZ\Delta M15 \Delta lacX74 hsdR(rK-, mK+) \Delta recA1398 endA1 tonA$	T1 ^R	Cloning strain, purchased from Invitrogen.
DP10 (<i>E. coli</i>)	F-, <i>mcrA</i> , $\Delta(mrr-hsdRMS-mcrBC)$, $\Phi 80lacZ\Delta M15$, $\Delta lacX74$, <i>recA1</i> , <i>endA1</i> , <i>araD139</i> , $\Delta(ara, leu)7697$, <i>galU</i> , <i>galK</i> , λ -, <i>rpsL</i> (Str ^R), <i>nupG</i> D(<i>araFGH</i>) $\phi(DaraEp P_{cp8}-araE)$	Str ^R	Derived from DH10B. See reference ²⁵ for details.
CME03 (<i>E. coli</i>)	F-, <i>mcrA</i> , $\Delta(mrr-hsdRMS-mcrBC)$, $\Phi 80lacZ\Delta M15$, $\Delta lacX74$, <i>recA1</i> , <i>endA1</i> , <i>araD139</i> , $\Delta(ara, leu)7697$, <i>galU</i> , <i>galK</i> , λ -, <i>rpsL</i> (Str ^R), <i>nupG</i> D(<i>araFGH</i>) $\phi(DaraEp P_{cp8}-araE)$ <i>tnaA::kan</i>	Str ^R , Kan ^R	Derived from DP10, contains <i>tnaA::kan</i>
Plasmid name	Expression constructs	Markers	Notes
pJS33 ¹	<i>recA</i>	Amp ^R	Used to enable P1 transduction of <i>tnaA::kan</i> into DP10.
pWW307	P _{ara} empty vector, P _{tet} -mRFP1	Amp ^R	See reference ²⁶ .
pCDF-tet	<i>tetR</i> , P _{tet} empty vector	Spec ^R	Derived from pCDF-duet
pCAM12	P _{ara} =>p12/p9-mCitrine	Amp ^R	Derived from pWW307
pCAM15	P _{ara} =>p12/p9-mCerulean	Amp ^R	Derived from pWW307
pCAM17	P _{ara} =>p12/p9	Amp ^R	Derived from pWW307
pCAM33	P _{tet} =>p9-mCitrine	Spec ^R	Derived from pCDF-tet
pCAM35	P _{tet} =>p9-6His	Spec ^R	Derived from pCDF-tet
pCAM36	P _{tet} =>6His-p9	Spec ^R	Derived from pCDF-tet
pCAM53	P _{ara} =>p12/mCitrine-p9	Amp ^R	Derived from pWW307
pCAM66	P _{ara} =>p9-mCitrine	Amp ^R	Derived from pWW307
pCAM105	P _{tet} =>p9-FLAG-FMO	Spec ^R	Derived from pCAM35
pCAM106	P _{tet} =>p9*-FLAG-FMO	Spec ^R	Derived from pCAM105
pCAM108	P _{ara} =>p9*-mCitrine	Amp ^R	Derived from pCAM12
pCAM110	P _{ara} =>p12/p9-6His-tnaA	Amp ^R	Derived from pCAM15
pCAM111	P _{ara} =>p9-6His-tnaA	Amp ^R	Derived from pCAM66
pCAM112	P _{ara} =>p12/p9*-6His-tnaA	Amp ^R	Derived from pCAM110

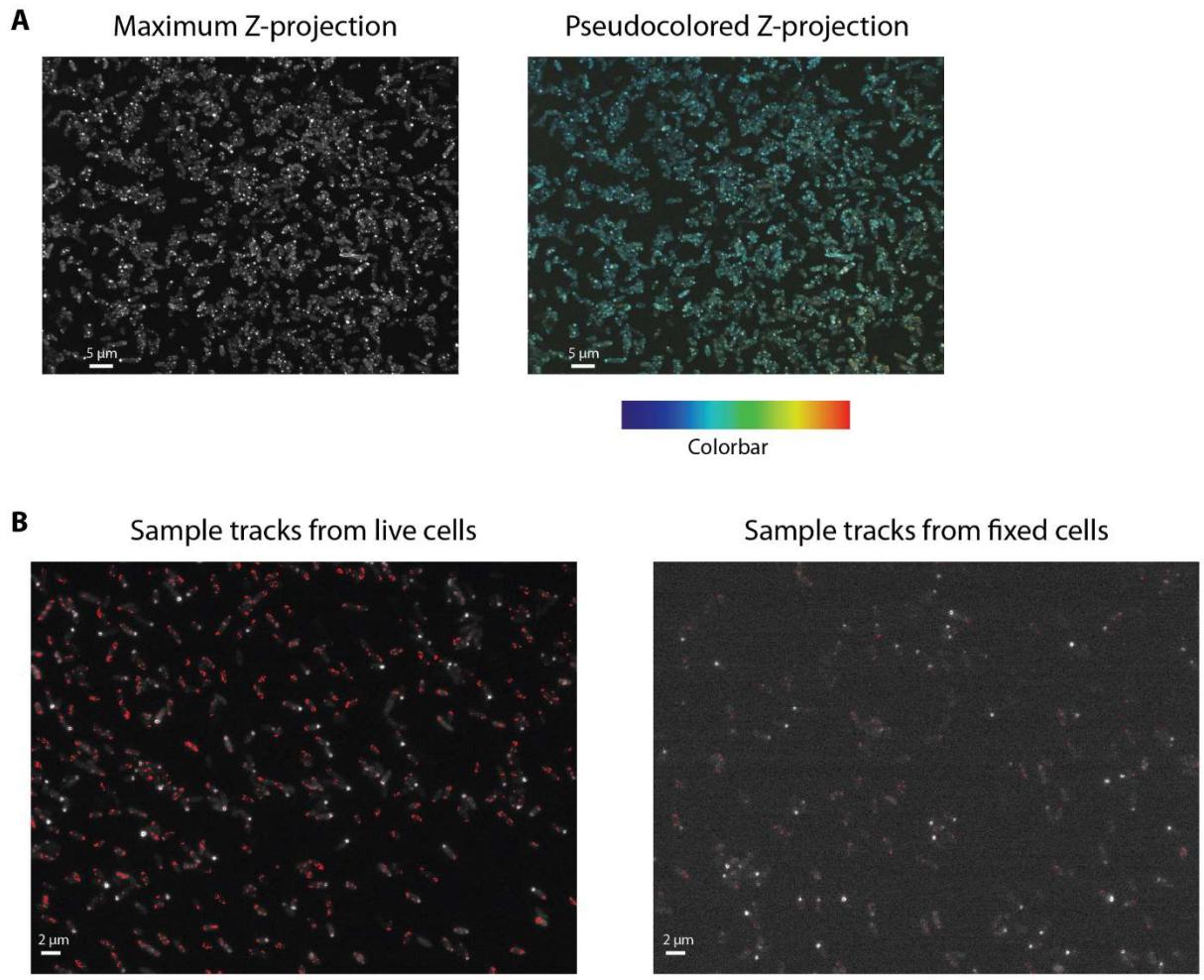
¹ pJS33 is described here²⁷.

Supplementary Table 2: primer sequences used in this study

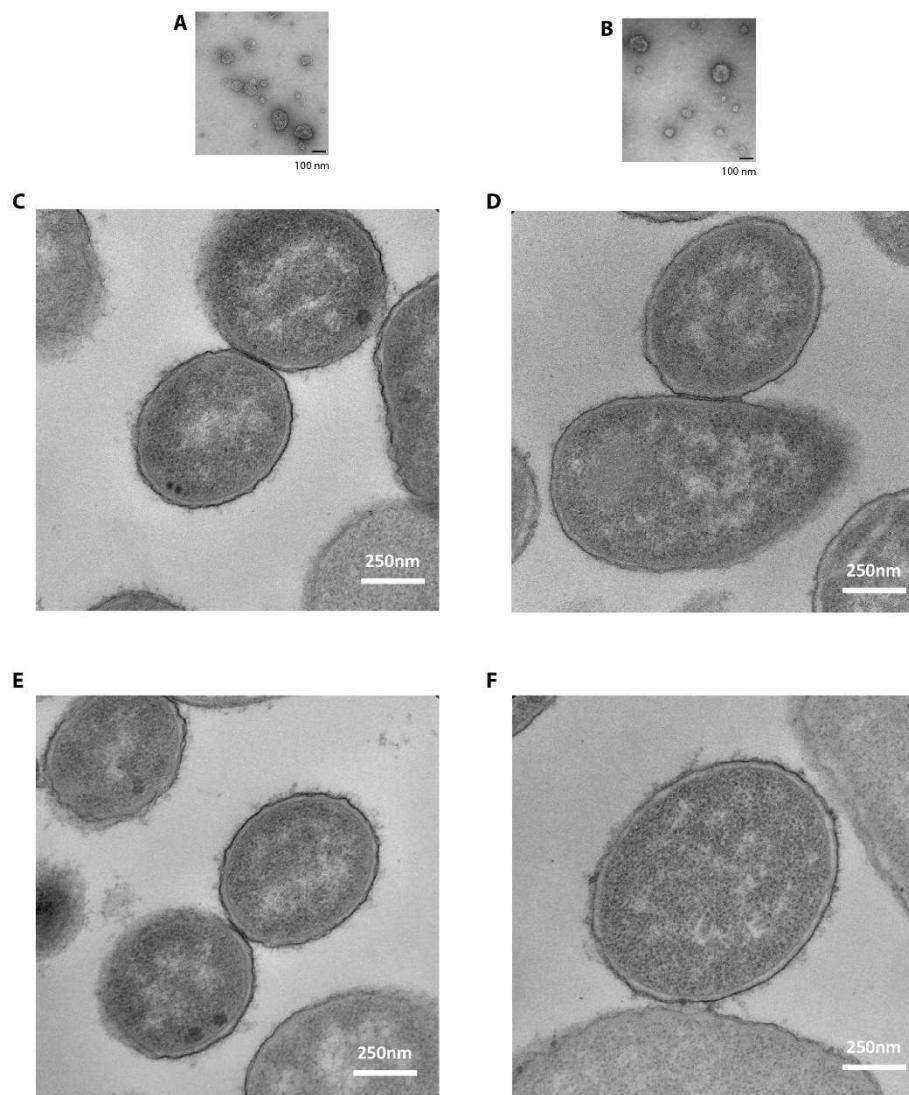
Name	Sequence	Used for
CP26	ACCCGCTAGCaaagaggagaaaAGAatggttatcggtccctgaagtatctcac	pCAM17
CP27	gtgagatacttcaggagaccgataaccatTCTtttctctttGCTAGCGGGT	pCAM17
CP32	Gccaacatcccttcgtggcgtagcaagggcgaggagc	pCAM12
CP33	Gctcctcgcccttgctcacgccaggaaaggatgttgc	pCAM12
CP34	ggcatggacgactgtacaagtagTCTccctatcagtatagagattgacatcc	pCAM12, 53, 66
CP35	ggatgtcaatcttatcactgtataggAAAGActacttgtacagctcgccatgcc	pCAM12, 53, 66
CP38	gccaacatcccttcgtggcGTGAGCAAGGGCGAGGAGCT	pCAM15
CP39	AGCTCCTGCCCTGCTCACggccaggaaaggatgttgc	pCAM15
CP40	GGCATGGACGAGCTGTACAAGtagTCTccctatcagtatagagattgacatcc	pCAM15
CP41	ggatgtcaatcttatcactgtataggAAAGActaCTTGATACAGCTCGCCATGCC	pCAM15
CP56	ccaacatcccttcgtggctaaTCtaaCTCGAatgagaagcttgg	pCAM17
CP57	ccaagttctattaCTCGAGttaGGAttaggccaggaaaggatgttgg	pCAM17
CP68	TACTGTTCTCCATAACCGCTAGCtaaggatgttccgtatgccatttct	pCAM66
CP69	agggaaatggcattacggacatcctaGCTAGCGGGTATGGAGAACAGTA	pCAM66
CP99	ccctctagaaataatttgttaacttaagaaggagatataccatgccatcccttggtaaagcaag	pCAM33, 35, 36
CP100	cttgcttaccagggaaatggcatggtatatctccctttaagttaaacaaaattttctagaggg	pCAM33, 35, 36
CP101	ggcatggacgactgtacaagtagatgtatggcagaattccatgtgcaga	pCAM33
CP102	tctgcagcatatggattctcactcatctactgtacagctcgccatgcc	pCAM33
CP107	ccACCACCACCACCATTaaatggatgtgagaattccatgtgcag	pCAM35
CP108	ctctgcagcatatggattctcactcatttaATGGTGGTGGTGGTGGTGG	pCAM35
CP109	ccctctagaaataatttgttaacttaagaaggagatataccATGCGTGGTTCTCACCAACAC	pCAM36
CP110	GTGGTGGTGAGAACACGCATGgttatatctccctttaagttaaacaaaattttctagaggg	pCAM36
CP161	gtccaccgttaaggatgttccgtacttaagaaggagatataccgtgagcaagg	pCAM53
CP162	ccttgctacggtatatctccctttaagttacggacatcctacggtgac	pCAM53
CP239	tcccttcgtggcCACCACCACCACCATGAAAACCTTAAACATCTCCCTGAACCGT	pCAM110, 111, 112
CP240	CAGGGAGATGTTAAAGTTTCATGGTGGTGGTGGTGGTGGTGGTGGTGG	pCAM110, 111, 112
CP241	CACTCACCGCAAACCTAAAGAAGTTtaTCTccctatcagtatagagattgacatc	pCAM110, 111, 112
CP242	gatgtcaatcttatcactgtatggAGAttaAACTCTTAAGTTGCGGTGAAGTG	pCAM110, 111, 112
CP243	ccACCACCACCACCATggccactaggattgtatcttagg	pCAM105, 106
CP244	cctaagatagcaatcttagtggcatATGGTGGTGGTGGTGGTGG	pCAM105, 106
CP245	ccaaggaggctggatcctgaatgtatggcagaattccatgtgcag	pCAM105, 106
CP246	ctgcagcatatggattctcactcattcaggatccagcccttgg	pCAM105, 106
CP280	CGTGGTGCCTGGTGG	pCAM106, 108, 112
CP281	TTGTTCTGACGGGTACGAAC	pCAM106, 108, 112
CP282	GATGATGATAAAATGGCCACTAGGATTGCTATC	pCAM105
CP283	ATCTTTATAATGGCCAGAAAAGGGATGTTG	pCAM105
CP286	GGATCTCCAGGCATCAAATAAAC	pCAM110, 111, 112
CP288	AGATTAAAACTCTTAAGTTGCG	pCAM110, 111, 112

Supplementary Table 3. Absolute indigo concentrations measured in Fig. 5. Units are mg indigo per 10^9 cells.

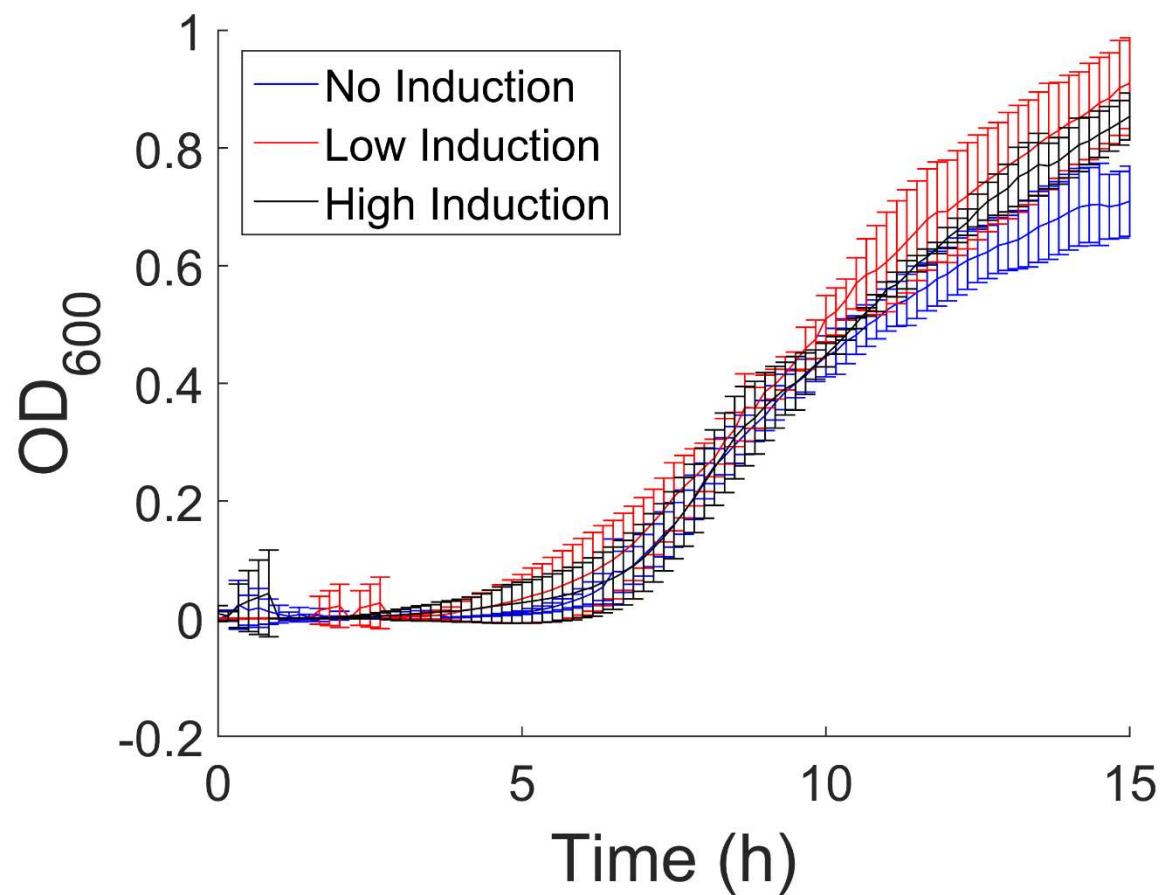
	P12 P9-TnaA P9-FMO	P9-TnaA P9-FMO only
Experiment 1	1.430801007	0.845100907
Experiment 2	0.725027449	0.268031054
Experiment 3	1.510661742	0.811617822
Experiment 4	0.831111025	0.178511727



Supplementary Figure 1. Microscopy images relevant to Figure 1. Large-view images of confocal microscopy (A), and particle tracking (B) are shown. The image on the right in (A) is pseudocolored from low (blue) to high (red), as indicated by the color bar.

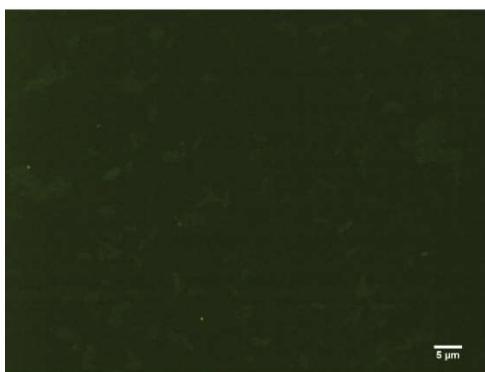


Supplementary Figure 2. Electron microscopy images relevant to Figure 3. Negative-stained TEM images from the red fractions in Figure 2B (A) and Figure 2D (B) are shown. High pressure freeze-substituted, Epon-embedded cells stained with uranyl formate and lead citrate. There is no detectable ultrastructural difference between cells not expressing SLSs (no plasmid, C and D) and cells expressing SLSs (pCAM12, E and F). Scale bars are indicated in each image.

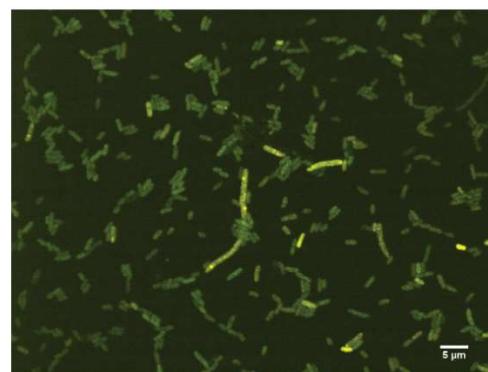


Supplementary Figure 3. SLS production does not impede growth. We compare the growth of DP10/pCAM15 cells experiencing no induction (blue), low induction (100 μ M arabinose, red), and high induction (1000 μ M arabinose, black) by monitoring the OD₆₀₀ over time. Error bars indicate one standard deviation based on 3 biological replicates.

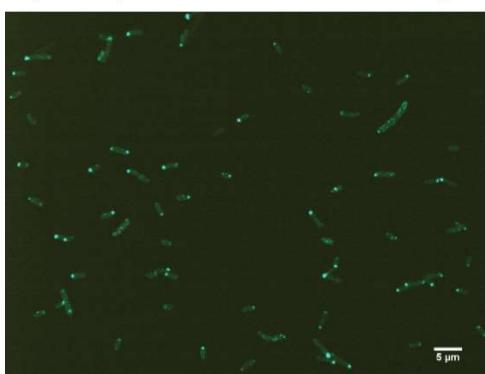
No fluorescent fusions



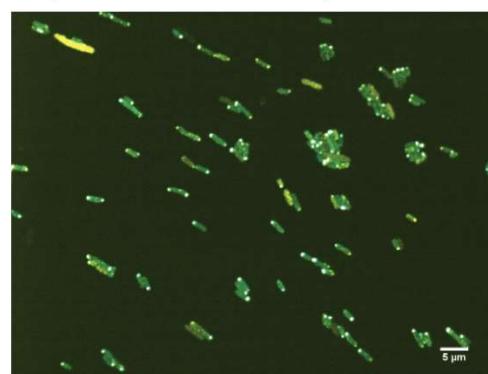
p9-mCitrine only



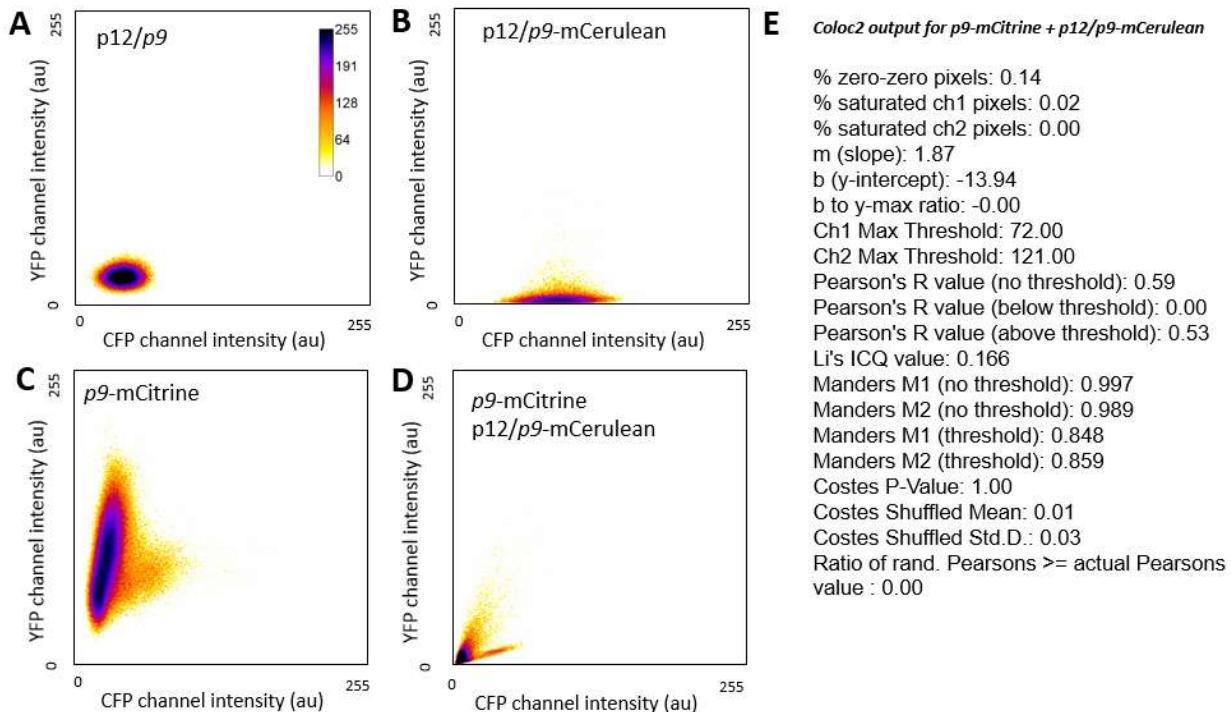
p12/p9-mCerulean only



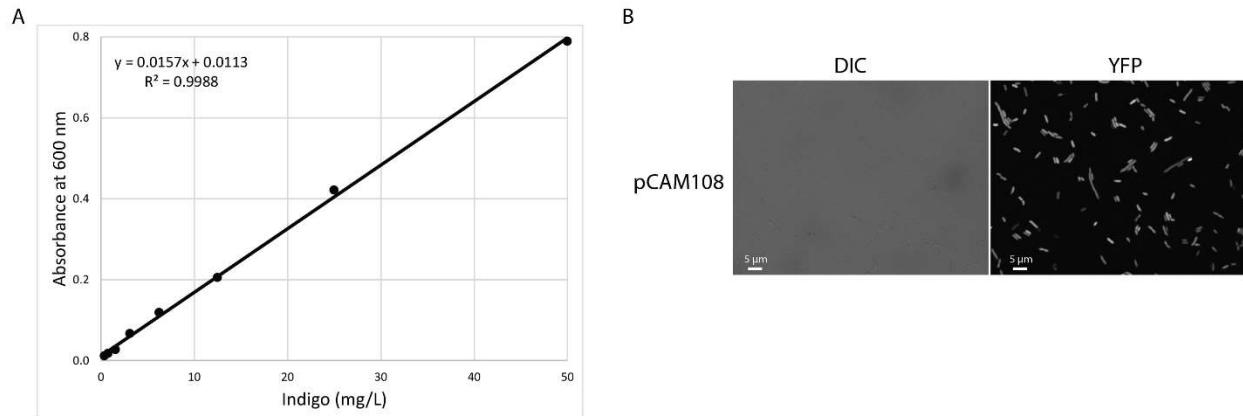
p12/p9-mCerulean, p9-mCitrine



Supplementary Figure 4. Large-scale view of co-localization of mCerulean and mCitrine to SLSs. We show large images for each of the conditions shown in Fig. 4A-D. Each image is a merge of the CFP channel (blue) and the YFP channel (yellow).



Supplementary Figure 5. Co-localization analysis of mCerulean and mCitrine to SLSs. (A-D) 2D histograms of intensities in both CFP and YFP channels for each of the four strains depicted in Supplemental Figure 4. (E) Full list of outputs provided by the Coloc2 FIJI plugin for the condition in (D).



Supplementary Figure 6. A sample standard curve for indigo quantification is shown (A). We used a linear fit (line) to the data (dots) to determine indigo concentrations. The equation of the fit and the R^2 value of the fit are shown. Fluorescence microscopy images of a strain expressing P12/P9*-mCitrine (pCAM108) is shown (B).