Table S1: SinR peaks.

Peak[a]	Gene[b]	Location[c]	MEME[d]	Reference[e]
2555393	*tapA/yqzG*	intergenic	ATCGTTCTCTTTAAAGAACTT TTTGTTCGTTTAAAAGAATGT ATCGTTGATTATACTCTATTT	1,2,3,4
3530041	*epsA/slrR*	intergenic	TTCGTTCATTATAAGGAATTT TTTGTTCTCTAAAGAGAACTT TTCCTCTAATTTACTGCACTT	3

[a]Peak indicates the nucleotide position of the center of the peak of ChIP-seq signal in *Bacillus subitilis* 3610 genome (CP020102). Peaks that were enriched in "*wt*" and abolished in a *sinR* mutant are indicated in this table. Peaks that were abolished in the absence of SinR are included in this table. Only peaks over 10 ChIP/input units were included in the table. Analysis was done with atleast 2 biological replicates. Complete data set is available in **Table S11**.
[b]Gene indicates the closes proximal gene to peak center. Two genes are indicated when the peak was between two genes and could regulate either.
[c]Location indicates whether the peak was in the non-coding region between two genes(intergenic) or in the coding region within a gene (intragenic).
[d]21bp putative binding site(s) obtained from MEME analysis of 200bp region surrounding the Peak[a].
[e]Reference for the respective targets.

Table S2: SlrR peaks

Peak[a]	Gene[b]	Location[c]	MEME[d]	Reference[e]
231712	*ybeC*	intragenic	AGAACTCATATATTGAACGGA	
383033	*srfAA*	intragenic	TGGCGCAACATATGAAACACC	6
413611	*ubiD*	intragenic	AGAGGCAGTATATCGAACAAA	
635197	*B4U62_03360*	Intragenic	GCGCGCCATATAAGATACCAC	
1153278	*yisL*	Intergenic	GGGGAACATATATGACACACC	
1273134	*manP*	intragenic	AGGAACCATAAATGAAACGCC	
1291225	*yjiA/yjiB*	intergenic	AGGGGGAATATATGATGCCTG	
1377262	*purU*	intergenic	TGAAATCATATATGACTCAGC	
1715599	*cheC*	intragenic	AAAGGCATTATAAAGAACATT	
1791694	*pksH*	intragenic	AAGCGCATTATATGACGCTTA	
1817424	*pksL*	intragenic	AAGAGACATATATGAAACATA	
1971030	*ppsD*	intragenic	GCACGCTTTATATGACGCTTC	
2175497	*yorM*	intragenic	AGGGCTCGTATATAGCGCTAA	
3109475	*bceB*	intragenic	GAGCGCAGTATATAACACCAT	
3465091	*spbB*	intragenic	ACAGGTAATATAAGAAAAGAA AAGGTTAATATAAAAATCATT	
3663106	*lytA*	Intragenic	AGAAGCAATATAAAGAACAGT	5,6
3669448	*B4U62_19215/ggaB*	intragenic	TGAGTTAATATATGATGCGGT	
3699576	*pgsB*	intragenic	CGACGCCATATATGACACGGT	
3993511	*yxjM*	intragenic	GGAGGCAACATATTACTCCTA AGGCGGCATATATCATTAAAA	
4174619	*yybL*	intragenic	CGGGACAATATAAAACAAGGT	

[a]Peak indicates the nucleotide position of the center of the peak of ChIP-seq signal in *Bacillus subitilis* 3610 genome (CP020102). Peaks less that 10 ChIP/input units were eliminated from analysis. Peaks that were enriched in a *sinR* mutant and abolished in a *sinR slrR* double mutant are indicated in this table. Complete data set is available in **Table S11**.

[b]Gene indicates the closes proximal gene to peak center. Two genes are indicated when the peak was between two genes and could regulate either.

[c]Location indicates whether the peak was in the non-coding region between two genes(intergenic) or in the coding region within a gene (intragenic).

[d]21bp putative binding site(s) obtained from MEME analysis of 200bp region surrounding the Peak[a].

[e]Reference for the respective targets.

Table S3: SinR•SlrR peaks

Peak[a]	Gene[b]	Location[c]	MEME[d]	Reference[e]
44865	*abrB*	Intergenic	AAAACGTTCTTGTTATGACAC	
231709	*ybeC*	intragenic	CCTCCGTTCAATATATGAGTT	
413578	*ubiD*	intragenic	AATTTGTTCGATATACTGCCT	
739925	*yefB*	intragenic	GACCCCTTCGTTATACAATCC	
743331	*yeeB*	intragenic	CTTTTGTTCGTTTTATGGAAT	
813897	*B4U62_04195*	Intergenic	TTTTTATTCGTTATAATGCAT	
1692292	*fliE*	intragenic	TATTAGTTCGTTAAATGAGTC	
1696778	*fliI*	intragenic	TCAGTGTTCGCTATTTTACCT CACCTGTTCGTTCTAGTAAAC	
1707609	*flhB*	intragenic	TGCCCGTGCGTTATATGATCA	
1715602	*cheC*	intragenic	TCAATGTTCTTTATAATGCCT CTTCCATTCTTAAAAAGATGC	
1842327	*pksN*	intragenic	TTCACGTTCGTTTTATTGACT	
2047545	*yoaW*	intergenic	AAACTGTTCTTATTAAGGCTC CCTCTATTCGAAATAAGGCTT TCTATATTCTTTTTAAAAGTT	
2068474	*yobI*	intragenic	GCACTGTTCTTTATAGTATAT	
2141427	*yodP*	intragenic	GCGCTGTGCGATATATGGCTT	
2166868	*yosJ*	intragenic	TTTCTGATCTTTATAAAACCC	
2346317	*asnC/dnaD*	Intragenic	CTCACGTTCGTGAAACGATTC	
2456372	*ansA*	intragenic	TTTTCGTTCTTAATTTGATCG	
2611221	*cdd*	intragenic	GCCGCGTTCTCAATATTGCAG	
2619631	*yqeZ*	intragenic	GTCCCGTTCGTTACAAGACGC	
2692910	*yqaO*	Intergenic	TCAGTGTTCTTGATATGAACT	
2735546	*yrdA*	intergenic	TTTTTGTTCTTTTTAACGAAA	
2859212	*minC*	Intragenic	TCTTTGTTCCTTTTATTGTTA TGCGTGTTCTTTTGATGAGCT	
3025807	*sspA/ytbJ*	Intragenic	TTCTTGTTCGCTATAAAGGGT	
3043216	*ytxE*	intragenic	CTTGTGTTCTTTATTTTACTC	6
3048025	*ytxG*	intergenic	TTATTATTCTTTATTTGAGCG	
3055447	*ytoP*	intragenic	AATTCGTTCTTGATATGGCTG CCGCTGTTCGAAATATGAACC	
3119744	*ytzC*	intragenic	TTCTTGATCGTTATAATGCTC	
3424044	*yvgJ*	intragenic	TAATCGTTCGTAATAAAGCTG	
3458169	*yvaQ*	intragenic	TAGGCGTTCTTAATATGCAGC	
3663100	*lytA*	Intragenic	TTACTGTTCTTTATATTGCTT	5,6
3728345	*ywqH/B4U62_19510*	intergenic	TTTTTGTTCGTTATACAGCTA	
3776591	*ywmC*	intergenic	TCGGCTTTCGTTATATGAGTC	
3943570	*sacX*	intragenic	ATTTTGTTCATTATATGATCG	
4011182	*citH*	intragenic	TCCCTGTTCTTTTTATGACTG	

[a]Peak indicates the nucleotide position of the center of the peak of ChIP-seq signal in *Bacillus subitilis* 3610 genome (CP020102). Peaks that were either enriched or enhanced in *slrA*$^+$ and

abolished in *slrR slrA*$^{+}$ are included in this table. Only peaks over 10 ChIP/input units were included in the table. Analysis was done with atleast 2 biological replicates. Complete data set is available in **Table S11**.

[b]Gene indicates the closes proximal gene to peak center. Two genes are indicated when the peak was between two genes and could regulate either.

[c] Location indicates whether the peak was in the non-coding region between two genes(intergenic) or in the coding region within a gene (intragenic).

[d]21bp putative binding site(s) obtained from MEME analysis of 200bp region surrounding the Peak[a].

[e]Reference for the respective targets.

Table S4- Expression of P_{lytA}-*lacZ* and P_{lytA}^{ext}-*lacZ* in Figure 4B (Miller units)[a]

Strains	P_{lytA}	P_{lytA}^{ext}
"wt"	24.34 ± 4.46	26.26 ± 1.13
sinR	26.88 ± 1.69	35.89 ± 10.04
sinR slrR	28.83 ± 5.08	31.22 ± 4.89
slrA$^+$	7.34 ± 0.66	3.99 ± 0.94
slrR slrA$^+$	28.20 ± 1.03	37.11 ± 2.56
site(12345) slrA*$^+$	32.07 ± 2.11	5.12 ± 0.39
site(12345) slrR slrA*$^+$	29.83 ± 2.16	32.75 ± 4.05

[a]± is the standard deviation of three replicates.

Table S5- Expression of P_{flache}-*insert'-lacZ* reporters in Figure 4D (Miller units)[a]

Reporters	*"wt"*	*slrA*$^+$	*slrR slrA*$^+$
site1	63.39 ± 14.49	26.62 ± 1.60	67.06 ± 7.61
site23	79.99 ± 19.32	38.14 ± 3.67	109.28 ± 53.43
site4	83.93 ± 26.15	85.07 ± 14.38	64.41 ± 5.52
site5	55.71 ± 11.24	34.05 ± 4.59	40.75 ± 11.08
fliF'	59.53 ± 14.18	75.79 ± 12.01	53.30 ± 7.90
*site1**	101.69 ± 8.52	99.38 ± 9.44	76.22 ± 15.36
*site1*2*	132.19 ± 16.45	120.46 ± 6.92	105.79 ± 31.58

[a]± is the standard deviation of three replicates.

Table S6: Expression of P_{ygzD}-*lacZ* in Figure S1 (Miller units)[a]

Strains	P_{ygzD}
"wt"	4.71 ± 0.39
sinR	4.81 ± 1.21
sinR slrR	5.19 ± 0.29
sinR slrR ygzD	133.36 ± 7.46

[a]± is the standard deviation of three replicates.

Table S7: Expression of P_{hag}-*lacZ* in Figure S5 (Miller units)[a]

Strains	P_{hag}
"wt"	132.40 ± 6.09
slrA$^+$	5.83 ± 1.51
slrA$^+$ *slrR*	129.02 ± 7.15
slrA$^+$*site1**	32.25 ± 3.47
slrA$^+$*site2**	24.26 ± 7.02
slrA$^+$*site3**	6.98 ± 0.80
slrA$^+$*site4**	5.89 ± 1.78
slrA$^+$*site5**	7.22 ± 1.55
slrA$^+$*site(12)**	111.34 ± 1.38
slrA$^+$*site(123)**	119.26 ± 8.67
slrA$^+$*site(1234)**	107.56 ± 5.22
slrA$^+$*site(12345)**	131.46 ± 15.84

[a]± is the standard deviation of three replicates.

Table S8: Plasmids

Plasmid	Genotype	Reference
pminiMAD3	*oriBsTs spec amp*	
pDG268	*amyE::lacZ cat amp*	7
pAP10	*thrC::*P_{hag}*-GFP mls amp*	
pAM58	*thrC::fliE-lacZ mls*	
pAM59	*thrC::fliI-lacZ mls*	
pAM60	*thrC::flhB-lacZ mls*	
pAM61	*thrC::cheC-lacZ mls*	
pAM62	*thrC::fliF-lacZ mls*	
pAM81	*thrC::*P_{ygzD}*-lacZ mls amp*	
pKM087	*ycgO::kan amp*	8
pDP96	*thrC::*P_{hag}*-lacZ mls amp*	
pDP99	*thrC::*P_{flache}*-lacZ mls*	
pDP100	*thrC::*P_{lytA}*-lacZ mls amp*	
pAM103	*ycgO::*P_{slrA}*-slrA kan amp*	
pAM109	*amyE::*P_{lytA}^{ext}*-lacZ cat amp*	
pAM112	*amyE::*P_{flache}*-lacZ cat amp*	
pAM113	*amyE::*P_{flache}*-fliE-lacZ cat amp*	
pAM114	*amyE::*P_{flache}*-fliI-lacZ cat amp*	
pAM115	*amyE::*P_{flache}*-flhB-lacZ cat amp*	
pAM116	*amyE::*P_{flache}*-cheC lacZ cat amp*	
pAM117	*amyE::*P_{flache}*-fliF lacZ cat amp*	
pAM118	*amyE::*P_{flache}*-fliE(site1) cat amp*	
pAM119	*amyE::*P_{flache}*-fliI(site2) cat amp*	
pDP620	*Ωsinslrsite1 oriBsTs spec amp*	

pDP621	*Ωsinslrsite2 oriBsTs spec amp*	
pDP622	*Ωsinslrsite3 oriBsTs spec amp*	
pDP623	*Ωsinslrsite4 oriBsTs spec amp*	
pDP624	*Ωsinslrsite5 oriBsTs spec amp*	
pDP638	*Ωsinslrsite23 oriBsTs spec amp*	
pDG1663	*thrC::lacZ mls amp*	9

Table S9: Primers

Number	Primer sequence
8423	CTCCTGGATCCTAGGCTAGCTCAGTTTTTTTCACCCTCAATATCCT
8424	AGGAGGCTAGCAACAAATCAAGTAAACAATAGCC
8425	AGGAGGGATCCCATGACCTCATCAAGATTTACA
8426	AGGAGGCTAGCAGCGAGAGATTGGACTGGCT
8427	AGGAGGGATCCCAATCGGCTCATTCATGTCAT
8428	AGGAGGCTAGCCACCTTGCGTTAAAAATCAGG
8429	AGGAGGGATCCACGTAAGCCAGAATTTCTGCT
8430	AGGAGGCTAGCGATGAGCTTGTTGATTTTTTCG
8431	AGGAGGGATCCGGTTTCCAATCAGCTCTCTG
8432	AGGAGGCTAGCGCAGCATCTGCATCAATCGT
8433	AGGAGGGATCCGCATCGCTGTCTGATTTGTC
8008	AGGAGGAATTCCGTTCTGTTATTGTGAACGCA
8139	AGGAGGAATTCAACAGCGTCAACCACTATCC
8140	AGGAGGGATCCACAATCTCGATTGATTATATTGT
8141	AGGAGGAATTCTCCATTGTGCCTTTTCCGATA
8142	AGGAGGGATCCTTAAATGTTACTGTCTGGCGATAC
7924	AGGAGGAATTCTGCCCGATCAAGCTTAATCTT
7925	AGGAGGGATCCCCAACCTGACTGAGCTCCAT
7926	AGGAGGGATCCCATTGTGGTGAGAAGCACGA
7927	AGGAGGAATTCCTGAAGGAGACCCGATTATC
7928	AGGAGGAATTCGCCTAATGTTGATCCATTAAAG
7929	AGGAGGGATCCAGAGTACGATTCATGTTAACC
7930	AGGAGGAATTCCGAGAGGTTCGGGAGTTTAT
7931	AGGAGGGATCCGTTCACGGAATTTATTTGCCG
8337	AGGAGGAATTCCGGCTCTGCTGATATCTTCT
8338	AGGAGGGATCCCAGATCAGGTGATAGAAAAGC
8273	CAGTGAATTCGAGCTCGGTACCCCGTCATGTTGATTTTTCTGATACTG
8274	GTCAGAAGCTACTTGGGACTCATTTAAACTCGAAATAGAGTTTTTTAAAAGCTCCGAAAAG
8275	CTTTTCGGAGCTTTTAAAAAACTCTATTTCGAGTTTAAATGAGTCCCAAGTAGCTTCTGAC
8276	GGTCGACTCTAGAGGATCCCCCGTTGATTTGGCTTTGATCAAGTG
8277	CAGTGAATTCGAGCTCGGTACCCGATGCAATTGAAAGATAAACTTCTG
8278	GTTCGTTCTAGTAAACGAGGTAAAATAGCAAAGACTGAAGGTGTATAGCCTTTTGTCG
8279	CGACAAAAGGCTATACACCTTCAGTCTTTGCTATTTTACCTCGTTTACTAGAACGAAC

8280	GGTCGACTCTAGAGGATCCCCCTAATGGCCTTGAATCATAAACTGC
8281	CAGTGAATTCGAGCTCGGTACCCGCTGATGAAAAAGCTCAAAAAGGAAC
8282	GGTTCCATGCTCGTTTGCACCTGTGCGCTCTAGTAAACGAGGTAAAATAGCGAAC
8283	GTTCGCTATTTTACCTCGTTTACTAGAGCGCACAGGTGCAAACGAGCATGGAACC
8284	GGTCGACTCTAGAGGATCCCCGATACCAGCAGCCCAAAGAACAATC
8285	CAGTGAATTCGAGCTCGGTACCCGATCGCTGTCAGTTTCATTATGCTC
8286	CTGGTCAATTTCCACTTGATCATATAATGCCCGGGCAAGCGGTCTGTTTTCTACC
8287	GGTAGAAAACAGACCGCTTGCCCGGGCATTATATGATCAAGTGGAAATTGACCAG
8288	GGTCGACTCTAGAGGATCCCCCTTCCTCAAGAATCTCATCCACTTC
8289	CAGTGAATTCGAGCTCGGTACCCGTCAGTGTGAAATCAGCCGAGG
8290	GCTCCGCCTGAAAAAAAGGCATTATAAAGAAGATTGAACCCGTTAAATCGCCTTC
8291	GAAGGCGATTTAACGGGTTCAATCTTCTTTATAATGCCTTTTTTTCAGGCGGAGC
8292	GGTCGACTCTAGAGGATCCCCCAGCCAATCTTCTTTACGAAGCC
8397	GGTTCCATGCTCGTTTGCACCTGTGCGCTCTAGTAAACGAGGTAAAATAGCAAAG
8398	CTTTGCTATTTTACCTCGTTTACTAGAGCGCACAGGTGCAAACGAGCATGGAACC

SUPPLEMATAL FIGURE LEGENDS

Figure S1: YgzD represses expression of P_{ygzD}. A) ChIP-Seq performed using α-SinR that also cross reacts with SlrR. For each sample, sequencing reads were normalized to the total number of reads. Number of normalized sequencings reads from samples treated with α-SinR was divided by the normalized reads for the corresponding untreated samples to determine fold enrichment (ChIP/Input). ChIP/input (Y-axis) was plotted in 1kb bins against genome position in kilobases (X-axis). Each strain used in this figure had *epsH* deleted to avoid cell-clumping sure to absence of SinR. The genotype of the strain used to generate each panel is indicated in the top right corner. The following strains were used: top panel, *sinR slrR* (DK9314), bottom panel *sinR slrR ygzD* (DB225). **B)** Enlarged data from panel A. The ChIP/input (Y-axis) was plotted in 10bp bins over a 4kb range. Ticks on the X-axis represent 1kb intervals. An overlay of the enrichment profile of *sinR slrR* (red) and *sinR slrR ygzD* (blue) is displayed at the desired locations. The gene of interest (highlighted in orange) and the neighboring genome architecture is cartooned below each vertical panel. **C)** β-galactosidase activity of P_{ygzD}-*lacZ* in Miller units (MU) plotted on the Y-axis in linear

scale. Each experiment is the average of three replicates and error bars indicate standard deviation. The genetic background in which each reported was tested is indicated on the X-axis. Each strain used in this panel was mutated for *epsE* to avoid clumping of cells in the absence of SinR and thus an *epsE* mutant was considered as "*wt*" for this experiment. The following strains were used to generate this panel, "*wt*" (DB995), *sinR* (DB996), *sinR slrR* (DB997), and *sinR slrR ygzD* (DB998). Raw data are presented in **Table S6**.

Figure S2: Predicted structures of SinR, SlrR and YgzD homodimers and SinR•SlrR heterodimer. A) Structures predicted by Alphafold2. In each case, DNA binding domain are shaded in a darker color. B) Alignment of SinR, SlrR and YgzD amino acid sequence. Residues over 50% identity are shaded in purple.

FigS3: SinR•SlrR heteromer binding sites do not indicate internal promoters within the *fla/che* operon. Cells containing transcription reporters in which 500 bp surrounding the four SinR•SlrR peaks, *site1*, *site23*, *site4* and *site5* within the *fla/che* operon were transcriptionally fused to *lacZ* and integrated at an ectopic site were inoculated on LB plates supplemented with 80 µg/ml of the chromogenic substrate X-gal. As controls, cells containing the P_{flache} and P_{hag} promoters transcriptionally fused to *lacZ* were plated on the same media. The following strains were used to generate this figure, P_{flache}-*lacZ* (DB643), P_{hag}-*lacZ* (DB402), *site1-lacZ* (DB143), *site23-lacZ* (DB144), *site4-lacZ* (DB145) and *site5-lacZ* (DB146).

Figure S4: Mutation of multiple SinR•SlrR binding sites within the *fla/che* operon restores swarming motility to cells expressing multiple copies of *slrA*. A) The sequence of each of the five SinR•SlrR putative binding sites within the *fla/che* operon and the mutations generated at

each of the sites (site*, highlighted in pink). Pink lines on the top indicate the putative SinR-binding half-site (with consensus indicated). **B)** Quantitative swarm assay for strains mutated for single and multiple SinR•SlrR binding sites as indicated on each panel. Each strain used in this assay was mutated for EpsE to exclude the effect of the the EpsE bifunctional flagellar clutch/EPS glycosyltransferase in the absence of SinR, and an *epsE* mutant was considered "*wt*" for this experiment. Binding sites were mutated and tested in *"wt"* (gray circles) and *(slrA$^+$)* (black circles) genetic backgrounds respectively. The following strains were used to generate this panel. *"wt"* (DK9699, DB154), *site1** (DB1360, DB1438), *site2** (DB1419, DB1439), *site3** (DB1420, DB1440), *site4** (DB1357, DB1436), *site5** (DB1358, DB1437), *site(12)** (DB1515, DB1522), *site(123)** (DB1598, DB1609), *site(1234)** (DB1667, DB1669) and *site(12345)** (DB1702, DB1705). Each data point is the average of three replicas.

Figure S5: Mutation of SinR•SlrR sites 1 and 2 simultaneously restore *P$_{hag}$-lacZ* expression to *slrA*$^+$. β-galactosidase activity of *P$_{hag}$-lacZ* in Miller units (MU) plotted on the Y-axis in linear scale. Each experiment was performed in three replicates and error bars indicate standard deviation of the three replicates. The genetic background of strains used in this experiment is indicated on the X-axis. A line is drawn below all strains that contain an extra copy of *slrA* (*slrA*$^+$). Ø indicates that no additional mutation was added and each site mutation is indicated by a number, or sequence of numbers, followed by an asterisk. Each strain used in this panel was mutated for *epsE* to avoid clumping of cells in the absence of SinR. Therefore, an *epsE* mutant was considered as "*wt*" for this experiment. The following strains were used to generate this panel, "*wt*" (DB402), (*slrA$^+$)* (DB1662), *slrR* (*slrA$^+$)* (DB1661), *site1** (*slrA$^+$)* (DB1663), *site2** (*slrA$^+$)* (DB1787), *site3** (*slrA$^+$)* (DB1788), *site4** (*slrA$^+$)* (DB1785), *site5** (*slrA$^+$)* (DB1786), *site(12)** (*slrA$^+$)* (DB1652), *site(123)** (*slrA$^+$)* (DB1681), *site(1234)** (*slrA$^+$)* (DB1737) and *site(12345)** (*slrA$^+$)* (DB1709). Raw data are presented in **Table S7**.

Figure S6: Mutation of *sites1-4* abolished SinR•SlrR enrichment *in vivo* while mutation of *site5* did not. A) ChIP-Seq performed using α-SinR that also cross reacts with SlrR. For each sample, sequencing reads were normalized to the total number of reads. Number of normalized sequencings reads from samples treated with α-SinR was divided by the normalized reads for the corresponding untreated samples to determine fold enrichment (ChIP/Input). ChIP/input (Y-axis) was plotted in 1 kb bins against genome position in kilobases (X-axis). The genotype of the strain used to generate each panel is mentioned in the top right corner. The following strains were used to generate each panel. Top panel *slrA*$^+$ (DB154, red), bottom panel *site(12345)** (*slrA*$^+$*)* (DB1705, blue). **B)** Data are analyzed in the same way as in panel A. The ChIP/input (Y-axs) was plotted in 10 bp bins over a 4 kb range. Ticks on the X-axis represent 1 kb intervals. An overlay of the enrichment profile of (*slrA*$^+$*)* (red) and *site(12345)** (*slrA*$^+$*)* (blue) is displayed at the each of the SinR•SlrR sites within *fla/che* and a control SinR•SlrR site *lytA*. The genome architecture of the location depicted is cartooned below each vertical panel and the gene of interest is highlighted in orange.

Figure S7: Mutation of sites 1 and 2 in *(slrA*$^+$*)* restores transcript level of several genes within the SigD regulon, but not *lytA*. Data were analyzed in the same way as **Fig 7**. Percentage transcript per million (TPM) normalized to *sigA* TPM was calculated for each transcript in a variety of genetic background and the average of two replicates is plotted in the Y-axis in $\log_2$ scale. The dots in each panel represent percent TPM the values of each replicate. The name of the gene corresponding to each panel is mentioned on top on the panel. X-axis represents the different genotypes in which TPM was determined: **A)** "*wt*" (DK9699); **B)** (*slrA*$^+$*)* (DB154); **C)** *slrR* (*slrA*$^+$*)* (DB141); **D)** *site1**(*slrA*$^+$*)* (DB1438); and **E)** *site(12)** (*slrA*$^+$*)* (DB1522). Raw data are presented in **Table S10**.

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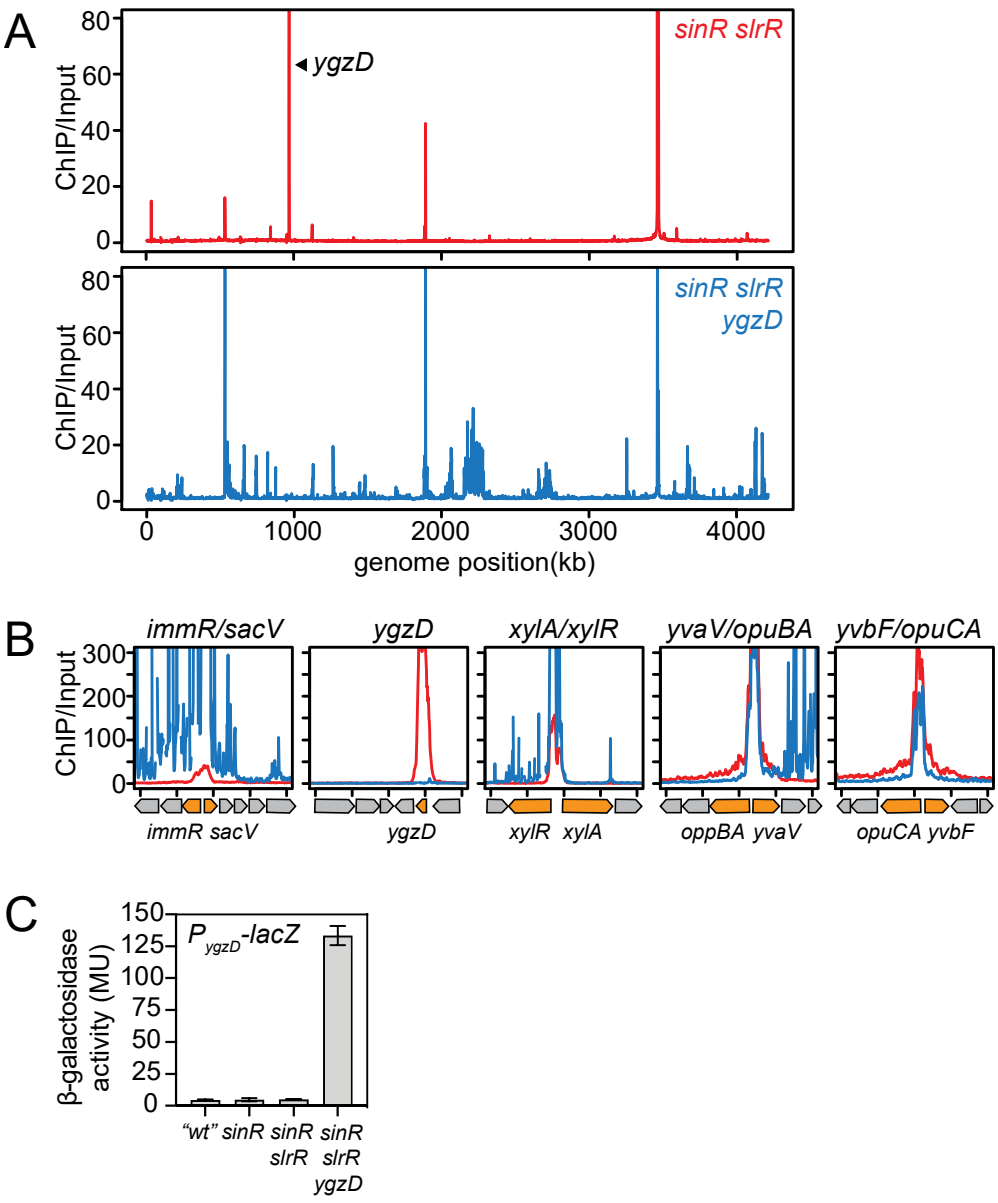
Figure S1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ygzD
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4000
genome position(kb)
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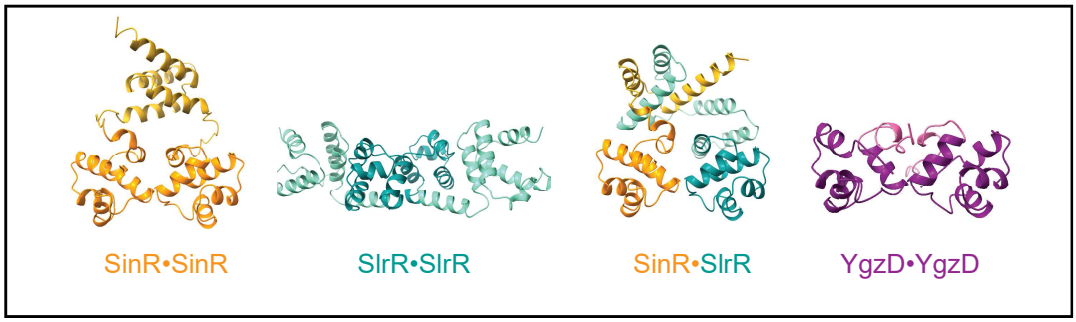
immR/sacV
ygzD
xylA/xylR
yvaV/opuBA
yvbF/opuCA
300
200
100
immR sacV
xylR xylA
oppBA yvaV
opuCA yvbF
C
β-galactosidase activity (MU)
150
125
100
75
50
25
"wt"
sinR
sinR slrR
sinR slrR ygzD

Figure S2

A



B

```
YgzD     1 -MKNKVKELRARFGYSQEKLGETVGVTRQTVAAIEKGDYV-PSLLLALKICKAFSMKMEDVFWLEEEN----------------------------
SinR     1 MIGQRIKQYRKEKGYSLSELAEKAGVAKSYLSSIERNLQTNPSIQFLEKVSAVLDVSVHTLLDEKHET-EYDGQLDSEWEKLVRDAMTSGVSKKQF
SlrR     1 MIGRIIRLYRKRKGYSINQLAVESGVSKSYLSKIERGVHTNPSVQFLKKVSATLEVELTELFDAETMMYEKISGGEEEWRVHLVQAVQAGMEKEEL

YgzD       ------------------------------------------------------------
SinR    96 REFLDYQK--------------------WRKSQKEE------------------
SlrR    97 FTFTNRLKKEQPETASYRNRKLTESNIEEWKALMAEAREIGLSVHEVKSFLKTKGR
```

Figure S3

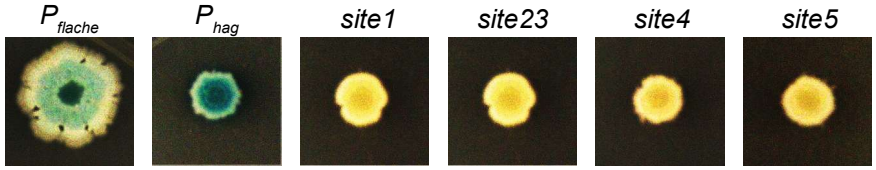


Figure S4

A

GTTCWTT

fliE (site1) TATTAGTTCGTTAAATGAGTC
fliE (site1)* TATT**TCGAGT**TTAAATGAGTC

fliI (site2) TCAGTGTTCGCTATTTTACCT
fliI (site2)* TCAGT**C**TT**T**GCTATTTTACCT

fliI (site3) CACCTGTTCGTTCTAGTAAAC
fliI (site3)* CACCTGT**G**CG**C**TCTAGTAAAC

flhB (site4) TGCCCGTGCGTTATATGATCA
flhB (site4)* TGCCCG**G**GC**A**TTATATGATCA

cheC (site5) TCAATGTTCTTTATAATGCCT
cheC (site5)* TCAAT**C**TTCTTTATAATGCCT

B

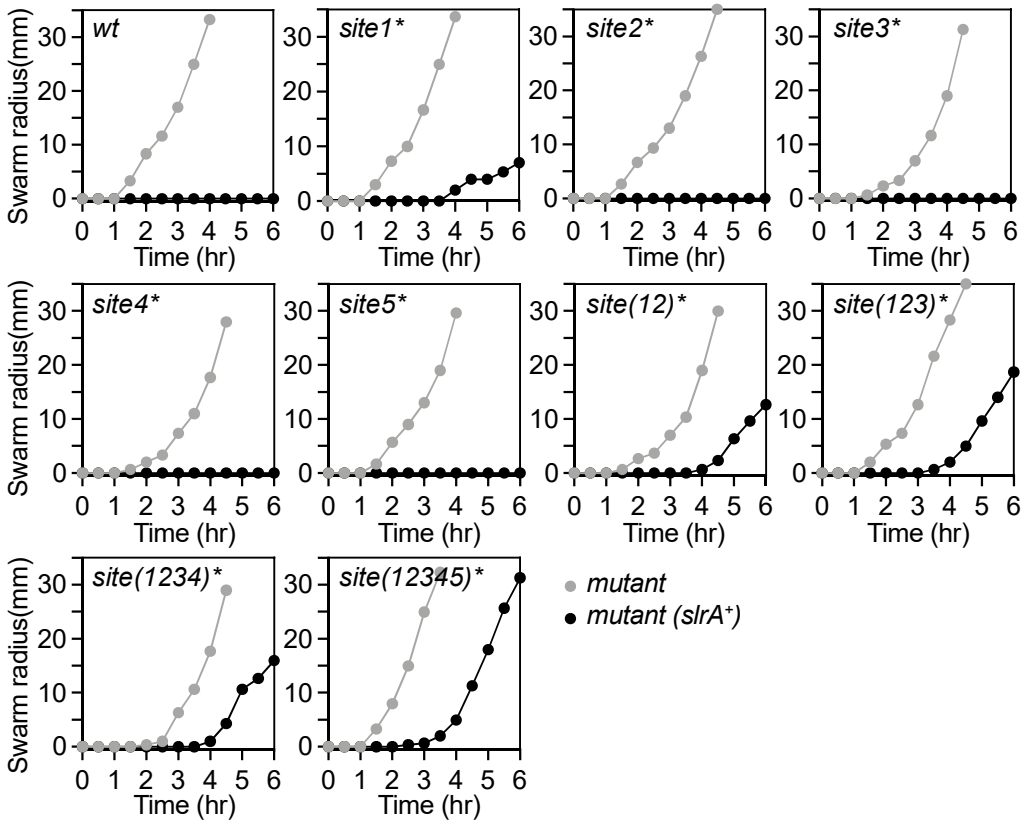


Figure S5

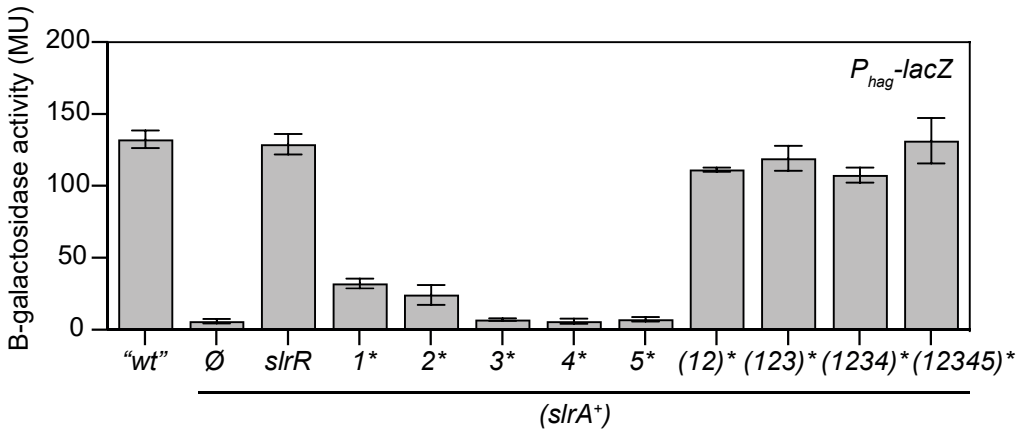

B-galactosidase activity (MU)
200
150
100
50
0
P_{hag}-lacZ
"wt"
Ø
slrR
1*
2*
3*
4*
5*
(12)*
(123)*
(1234)*
(12345)*
(slrA+)

Figure S6

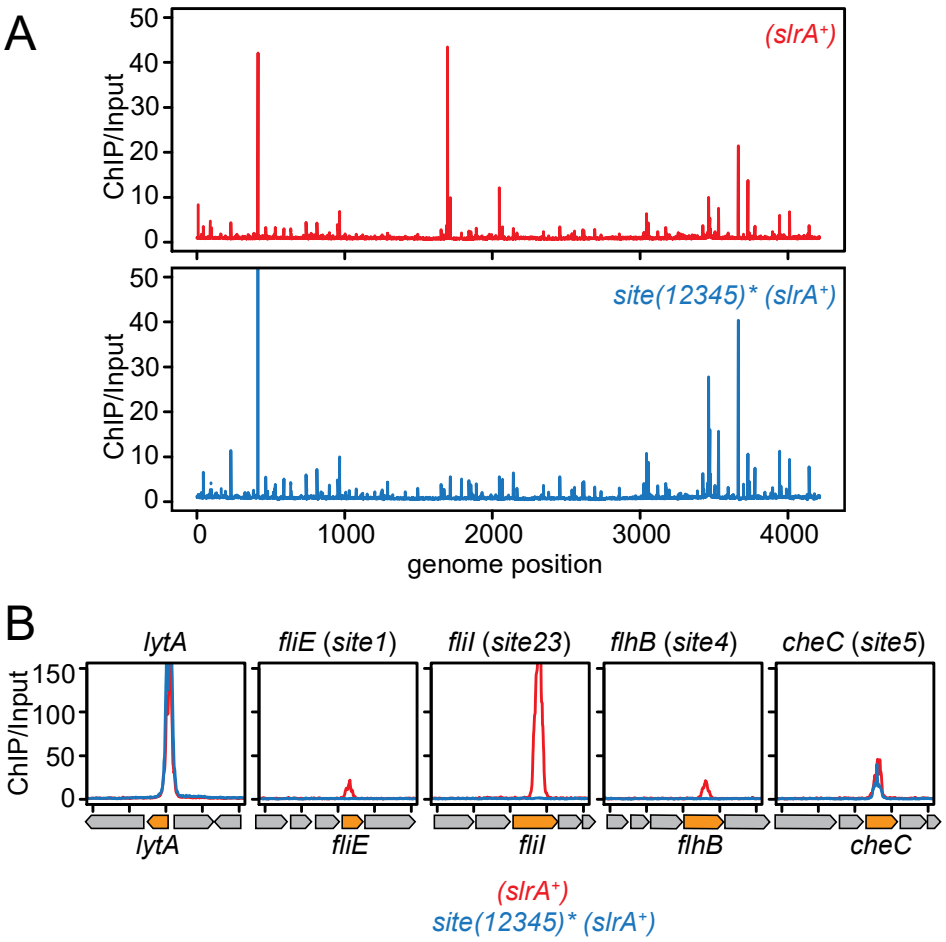
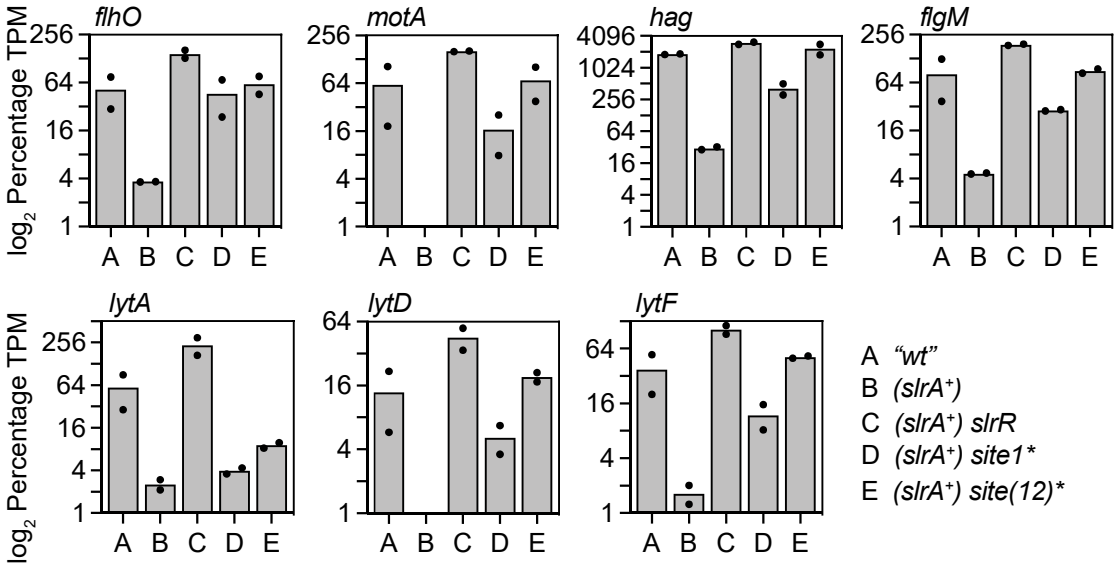

A
(slrA+)
site(12345)* (slrA+)
ChIP/Input
genome position
B
lytA
fliE (site1)
fliI (site23)
flhB (site4)
cheC (site5)
(slrA+)
site(12345)* (slrA+)

Figure S7


flhO
motA
hag
flgM
lytA
lytD
lytF
log2 Percentage TPM
A B C D E
A "wt"
B (slrA+)
C (slrA+) slrR
D (slrA+) site1*
E (slrA+) site(12)*