Supplemental Information

Deciphering the essentiality and function of the anti-σ^M factors in *Bacillus subtilis*

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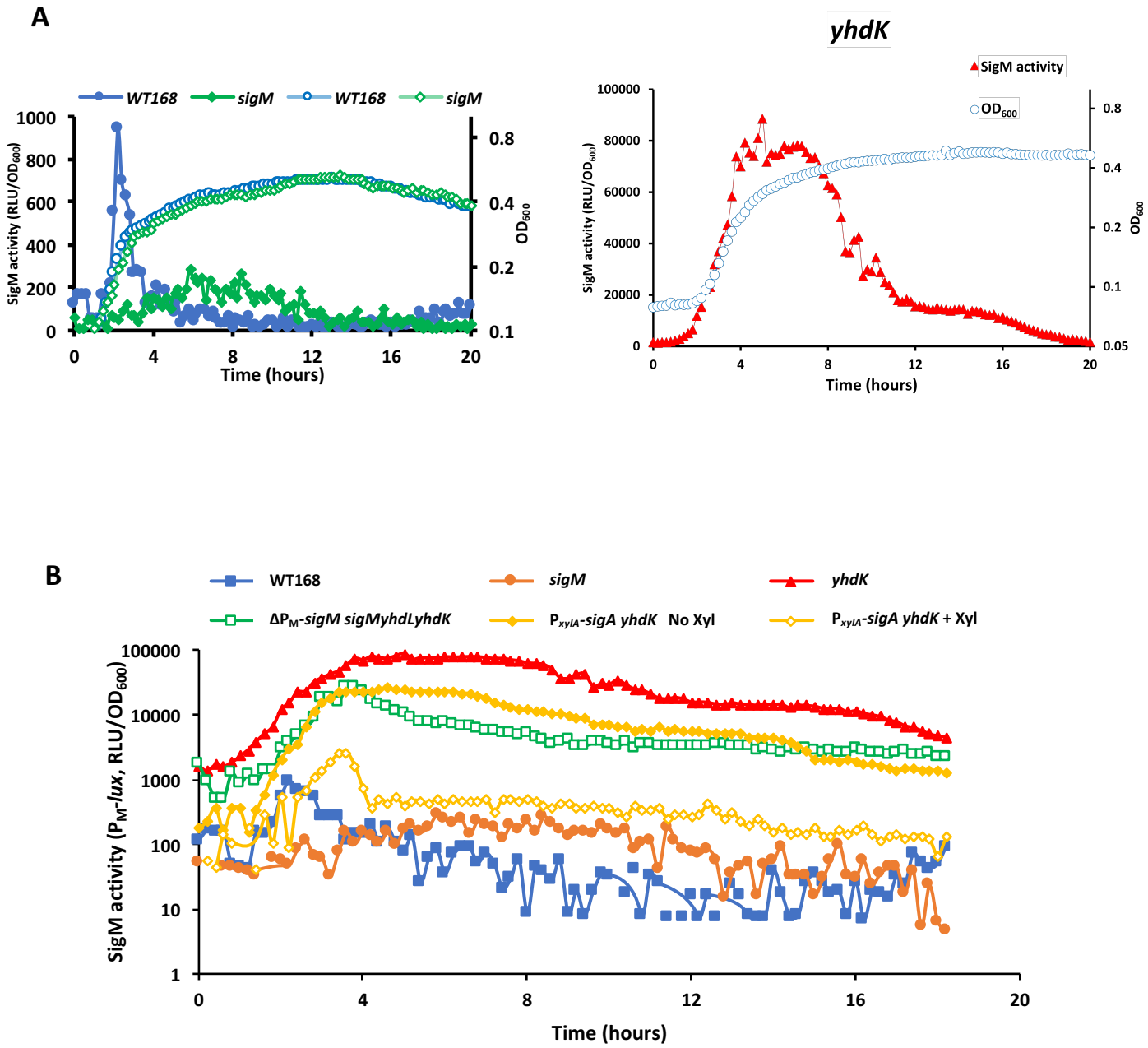
Table S1. Primers and gBlock used in this study

Number	Name	Sequence
6578	xylR-F-BamHI	ATCGGGATCCCGGTGGATAAACAAAGGAGTGG
6579	xylR-R-NotI	ATCGGCGGCCGCGGAGCTCCTAACTTATAGGGGT
6580	sigM-F-NotI	ATCGGCGGCCGCGCACTATCTTTTGCGGCCAT
6581	sigM-R-HindIII	ATCGAAGCTTTGGTCGCTCATTTCCCCATT
6582	yhdL-cln-up-F	GCCGTTTTCGTTGCGAGAAT
6583	yhdL-cln-up-R	CGCCGACATTCGCTGATTTTTCCTGGTCGCTCATTTCCC
6584	yhdL-cln-mid-F	GGGAAATGAGCGACCAGGAAAAATCAGCGAATGTCGGCG
6585	yhdL-cln-mid-R	CCTATCACCTCAAATGGTTCGCTGTCCGAAAACCGGTATAACGAAA
6586	yhdL-cln-down-F	CGAGCGCCTACGAGGAATTTGTATCGAGATACGAATTTACAGTTTGGCT
6587	yhdL-cln-down-R	ACGAATCGGGCAATCATGTG
6588	chr-sigM-seq-F	CCATTGTGCCACTCCTTCAC
6589	chr-sigM-seq-R	TGCAGTCATTTCCTGGTCGC
6590	pAX01-check-F	GGGGGAAATGACAAATGGTCC
6591	pAX01-check-R	ACGAAAGGGCCTCGTGATAC
6599	Pxyl-yhdL-F-BamHI	ATCGGGATCCTAGAGGGGAGAAAAGGCAATGATGAATGAAGAATTTAAAAAGC
6600	Pxyl-yhdL-R-SacII	ATCGCCGCGGTCCAGCCGAATACATTGTG
6693	pAX01-erm-cm-up-F	GCCGCACTCTTCCTTTTTCAA
6694	pAX01-erm-cm-up-R	CTTGATAATAAGGGTAACTATTGCCTTTGGTTGAGTACTTTTTCACTCG
6695	pAX01-erm-cm-down-F	GGGTAACTAGCCTCGCCGGTCCACGCTGGGGGAGGAAATAATTCTATGAGTCGC
6696	pAX01-erm-cm-down-R	TCGGCATTTTTGCATGGAGC
6759	yhdL-check-F	ACGCTGGGAAGCTACCTCTA
6760	yhdL-check-R	TCTGCTTTTGCGGTCGTTTG
6808	PsigM-F-EcoRI	AGCTGAATTCGCCGTTTGCATGTAATGTG
6809	PsigM-R-PstI	AGCTCTGCAGCAGTAAGTCTTCAGCAAGATGC
6814	pBs1ClacZ(lux)-check-F	AAAGGATTTGAGCGTAGCGA
6815	pBs1ClacZ-check-R	TTGGGTAACGCCAGGGTTTT
6816	pBs3Clux-check-R	GAGAGTCCTCCTGTCGACCT
7249	yhdL-in-check-F	GAAAACACAGCACCGGCAAT
7250	yhdL-in-check-R	AATTCCACCTCGCCGACATT
7324	rpoB-gRNA-F	TACGGACTGTTAAGTCTGATGACG
7325	rpoB-gRNA-R	AAACCGTCATCAGACTTAACAGTC
7326	rpoB-repair-up-F	AAGGCCAACGAGGCCTGCGCTTCGCAATCTTGATG
7327	rpoB-repair-up-R	CTTCGTATGTTTTCACACGTCCAACAACATCATTAGACTTAACAGTCAGAAATTTCTTGA
7328	rpoB-repair-down-F	TCAAGAAATTCTGACTGTTAAGTCTAATGATGTTGTTGGACGTGTGAAAACATACGAAG
7329	rpoB-repair-down-R	AAGGCCTTATTGGCCTACGGCGTTGTCCTTGTGAA
7340	rpoC10034-gRNA-F	TACGGGTAAACGTGTCGATTACTC
7341	rpoC10034-gRNA-R	AAACGAGTAATCGACACGTTTACC
7344	rpoC1004-repair-up-R	CAACAACGATTACAGAACGTCCTGAATAGTCCACATGTTTACCAAGAAGGTTTTGACGGA
7345	rpoC1004-repair-down-F	TCCGTCAAAACCTTCTTGGTAAACATGTGGACTATTCAGGACGTTCTGTAATCGTTGTTG
7348	pJOE8999-check-F	CCTTTTTGCGTGTGATGCGA
7349	pJOE8999-check-R	GTCAGCTAGGAGGTGACTGA
7405	SigA-R-SacII	ATCGCCGCGGACAAAATTGAATAGAAACATGCCT
7406	SigA-F-SpeI	ATCGACTAGTTTGCGGAGGAGCAAATAGAT
7625	NFLAG-YhdK-Up-F	AAGGCCAACGAGGCCGGGAAATGAGCGACCAGGAA
7628	NFLAG-YhdK-Down-R	AAGGCCTTATTGGCCGACATCGAGCAAGCTGGAGA
7635	N-FLAG-YhdK-gRNA-F	TACGGCGAGGTGGAATTATGGAAC
7636	N-FLAG-YhdK-gRNA-R	AAACGTTCCATAATTCCACCTCGC
7656	PfosB-XbaI-F	CTAGACTGTATGAAACTTTCTTATGAAAAAAGTCGTATATGTGGATGATCAGCTTCTGCA
7657	PfosB-PstI-R	GAAGCTGATCATCCACATATACGACTTTTTTCATAAGAAAGTTTCATACAGT
7738	gBLOCK-CL-NK-up-R	TTGGCCCTGTAACGACCGAACCGTATACATTGACACCGTTTTTCTCTACA
7739	gBLOCK-CL-NK-down-F	TTCGCCATGGCTGCCGTTCCGTTCGGCGTTGTTGAACTCTT
7545	PsigW-XbaI-F	ATCGTCTAGAACTTTGACTCCGTCATGCGT
7827	sigM-int-F	CCGCTGTCTGGCACGAAATA
7828	sigM-int-R	TTTTCAAGTGGCGCGAAACG
8110	PspoIIM-XbaI-F	ATCGTCTAGACATACAGCAGTTGATGATAAGG
8111	PspoIIM-PstI-R	ATCGCTGCAGCGCTCTAGTGATTTGATTTAATA
8112	PspoVFA-XbaI-F	ATCGTCTAGAGCGGGCTCTAAAGAAAACAT
8113	PspoVFA-PstI-R	ATCGCTGCAGCTGGATCTCTAGTTGTTAAGC
8114	sigH-check-F	ATTTTCGGACAGGGGGCATT
8115	sigH-check-R	GGTTTCCGCATGTCTTGCAT
8116	sigE-check-F	CAGGGGAGTTGGTCAGCAAA
8117	sigE-check-R	ACCCCGCAGATTTCGACTTT
8118	spoIIIC-check-F	TGCCTGCAACTTGGACTGAT
8119	spoIIIC-check-R	AGCTTTTAGAACGTCCGGCT
8122	spoIVCB-check-F	ACAGGCCTGCCATCCATTTT
8123	spoIVCB-check-R	GGCCGCAAGCGTCTTTTAAC
8132	PspoVG42-up-F	ATCGTCTAGAATAAGAAAAGTGATTCTGGGAGA
8133	PspoVG42-up-R	TCCTGCTCGTTTTTAAAATATTTTTTAAAAAAATAGGATATAGTTACACAATTAGGT
8134	PspoVG42-down-F	ACTATATCCTATTTTTTtAAAAAATATTTTAAAAACGAGCAGGA
8135	PspoVG42-down-R	ATCGCTGCAGTCCCTATATAAAAGCATTAGTGTATCA
DR194	SigM operon Fwd	GGAAAAGGTGGTGAACTACTAGGCCGATTCTGGAGGCTTC
DR197	SigM auto rev	CTATGTTATACTTTCATAAGAAAGGTTTAAAGTTGCAC
DR196	sigM auto Fwd	TTTCTTATGAAAGTATAACATAGAGGG
DR198	sigM rev	CATGGCCTGCCCGGTTATTACTGGTCGCTCATTTCCCCA
	gBlock sequence for C-FLAG-yhdL N-FLAG-yhdK	TGTAGAGAAAAACGGTGTCAATGTATACGGTTCGGTCGTTACAGGGCCAACCAAGGAAATCCAGCGCATGCTGAAAAATAAATCTGTAAAATCAGCGA ATGTCGGAGAAGTGGAACTTTGGAAtTGGGGATCAGGCCAAGGACCGGGAAGCGGACAAGGCCCTGATTACAAAGATGACGATGACAAAGGCGAGGT GGAATTATGGAAtTAGTAAGAGATTATAAAGATGATGATGATAAAGGCTCAGGCCAAGGACCTGGATCAGGCCAAGGCCCTATGGAACTTGTTAGAATT TTTAAAGAACACAATGTATTCGGCTGGATCTCTGTCGGGACAGCGGTTCTGTCCCTGCTGTTGCTGAATTTGGCCATTATCAGCAACGTCACGTTTTATT CCTATCAAATGCTTCCGTTCGCCATGGCTGCCGTTCCGTTCGGCGTTGTTGAACTCTT

Figure S1. Growth and SigM activity in the absence of each anti-sigM factor

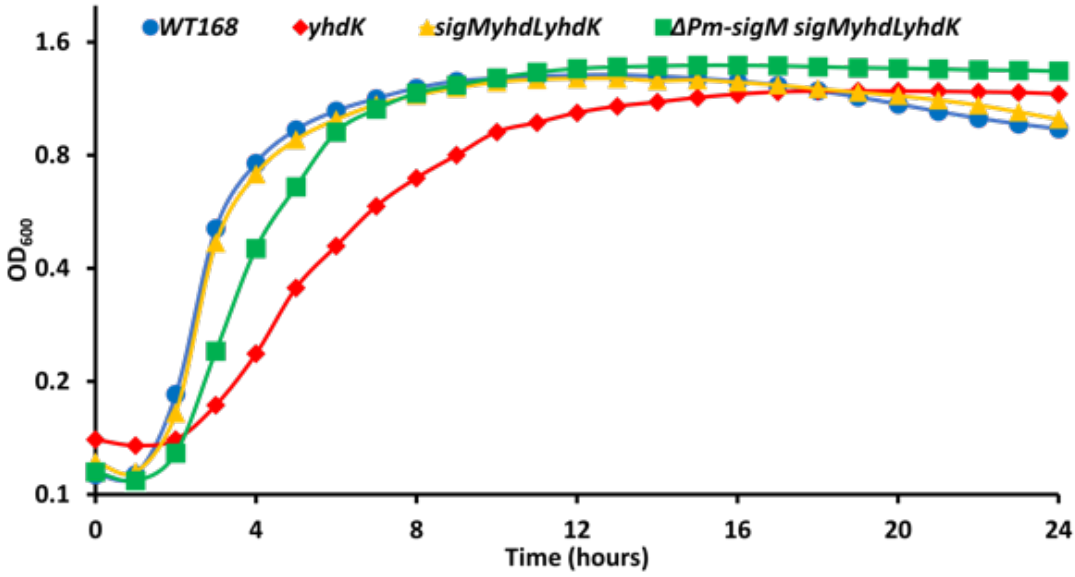


A. [Left panel] SigM activity of WT (168) strain (HB17325) and the *sigM* null mutant (HB17494) during growth in LB medium at 37°C as measured using a P_M-*lux* reporter (promoter activity is in closed symbols and was calculated by dividing relative light unit (RLU) by OD_{600}, in open symbols, with measurements every 12 min). [Right panel] SigM activity of the *yhdK* null mutant during growth in LB medium. Note that OD_{600} was measured with a culture volume of 100 µl per well in a 96-well plate.

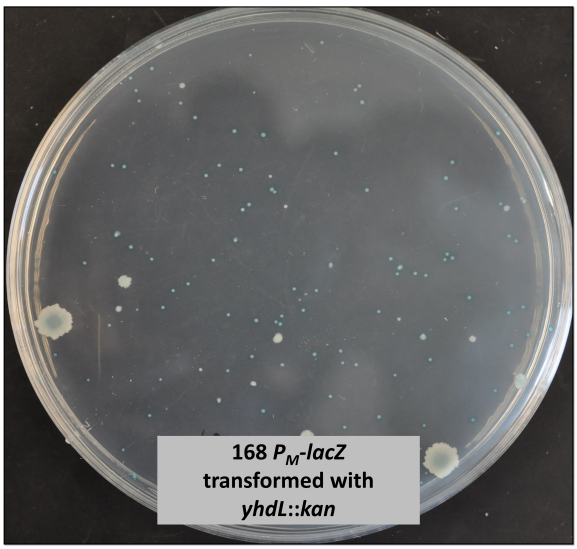
B. SigM activity of different strains as measured in panel A. A representative measurement is shown.

Figure S1. Growth and SigM activity in the absence of each anti-sigM factor

C



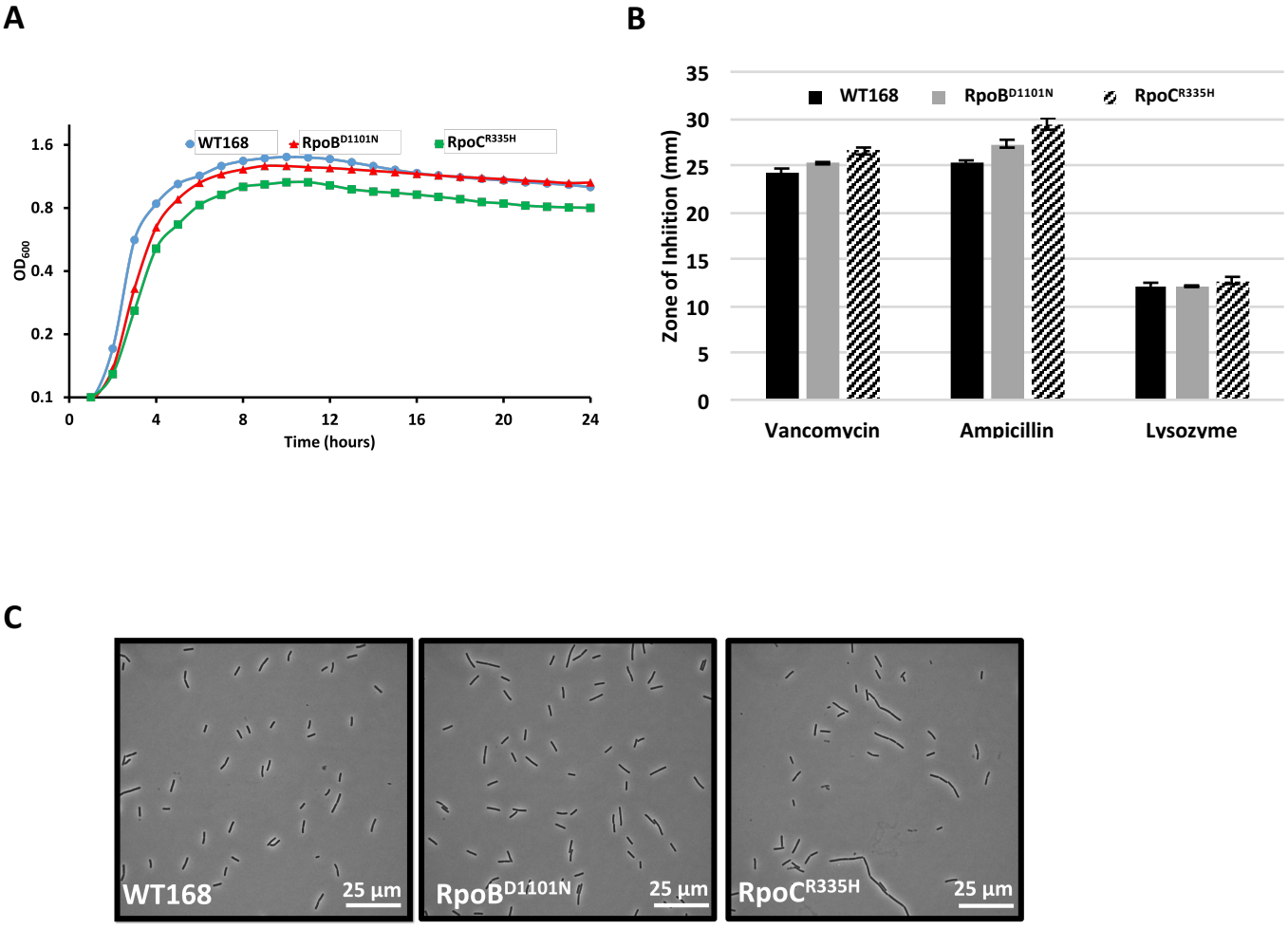
D



C. Representative growth curve of WT and mutants in liquid LB medium. OD_{600} was measured with a culture volume of 200 µl per well in a Bioscreen plate, so final OD_{600} values are twice those shown in Figure S1A and correspond roughly to those normally detected using a 1 cm path length in a spectrophotometer.

D. Transformation plate using WT 168 strain with a P_M-*lacZ* reporter as recipient, and chromosomal DNA of a *yhdL* deletion strain (HB17264) as donor. Transformed cells were plated on a LB plate containing kanamycin and X-gal and incubated at 37° C for 48 hours

Figure S2. Effect of *rpoB/C* mutations on cell growth, antibiotic sensitivity, morphology and alternative sigma factor activity.

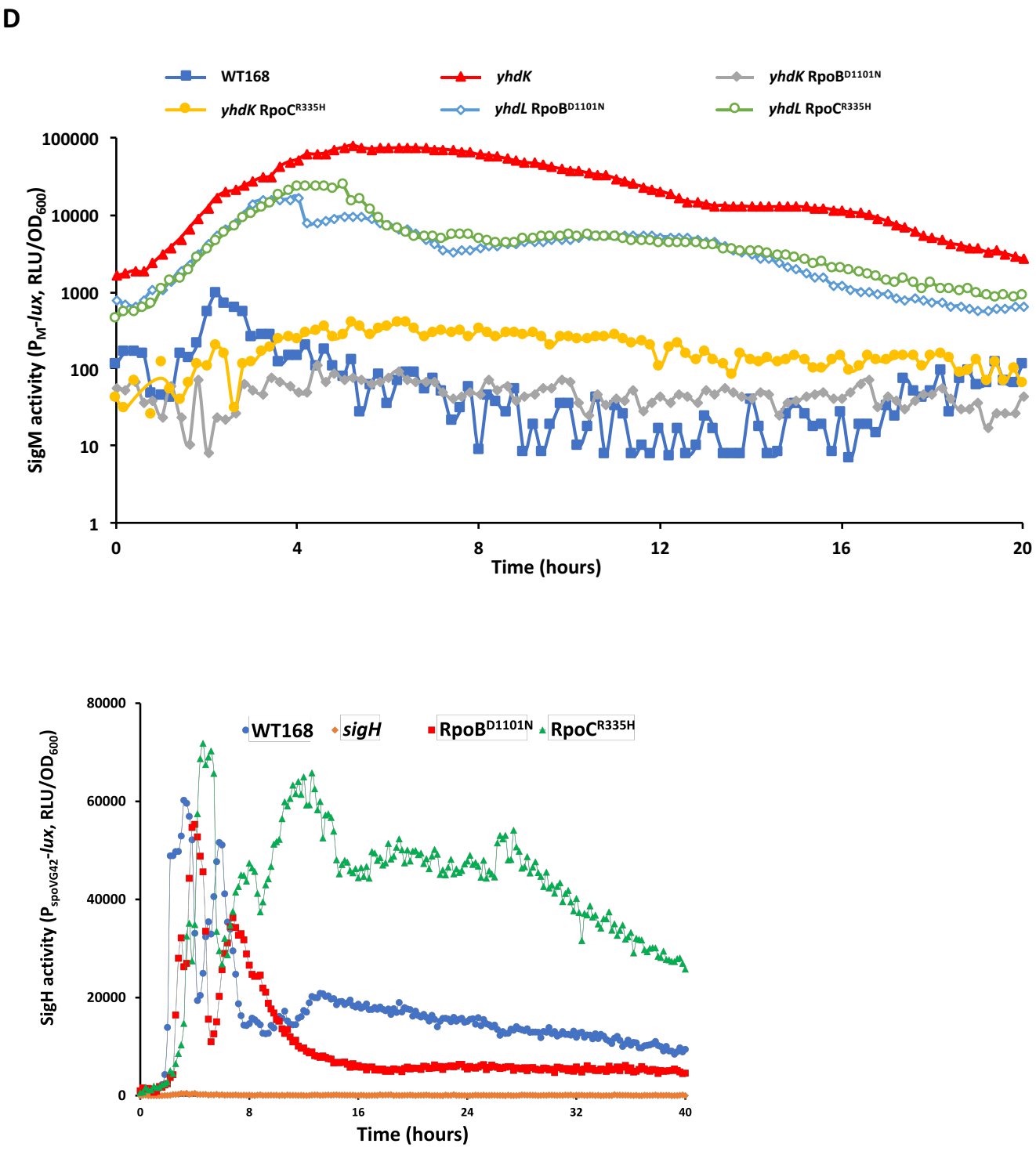


A. Growth curve of WT and *rpoB/C* mutants in LB medium at 37 °C in Bioscreen.

B. Zone of inhibition assay showing sensitivity of WT and *rpoB/C* mutants against vancomycin, ampicillin, and lysozyme. Data was presented as mean ± SEM, n=3.

C. Representative phase contrast microscopic images of WT and mutants grown in LB medium at exponential phase.

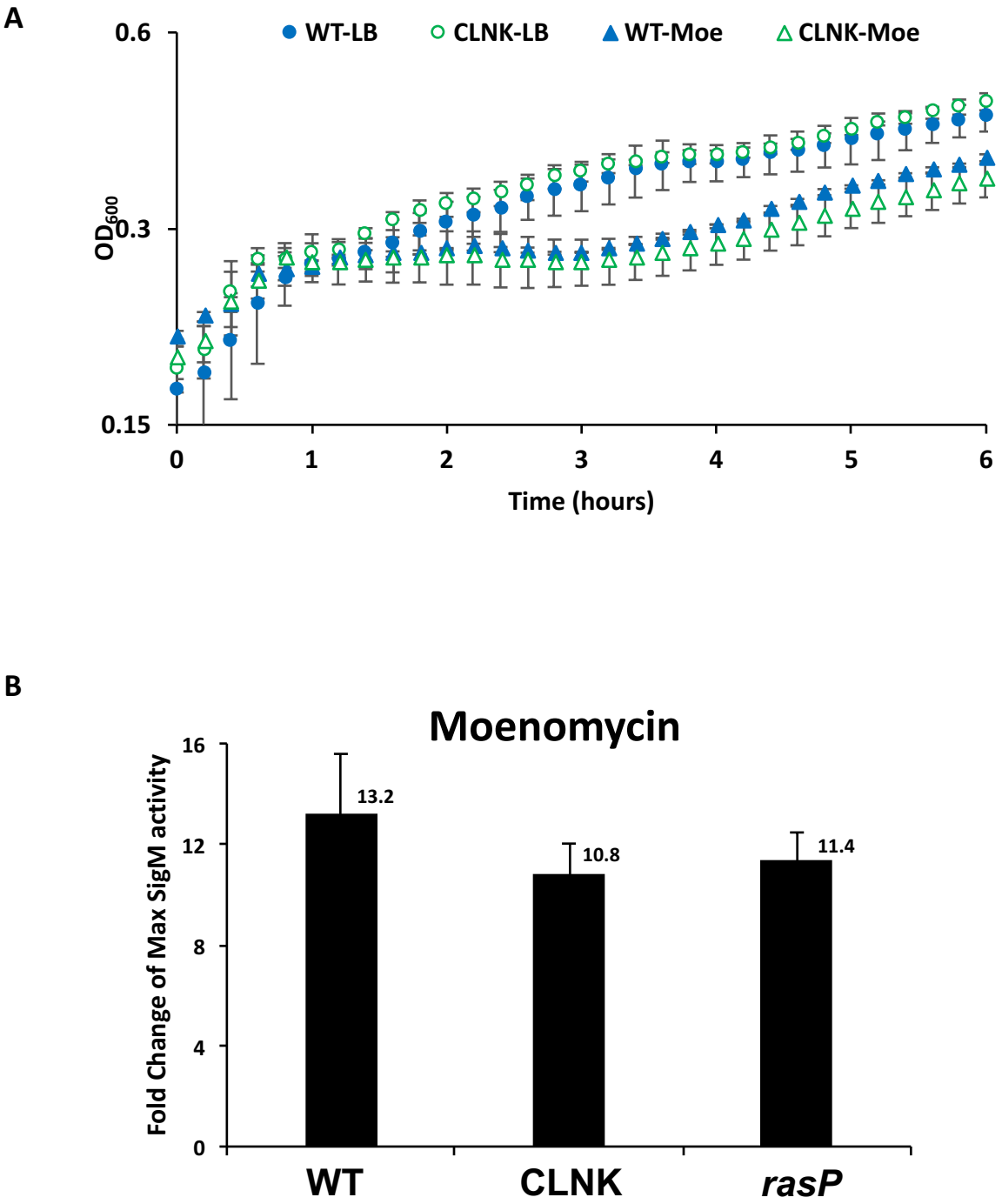
Figure S2. Effect of *rpoB/C* mutations on cell growth, antibiotic sensitivity, morphology and alternative sigma factor activity.



D. SigM activity of *yhdK* and *yhdL* mutants with or without single amino acid substitution in RpoB or RpoC.

E. SigH activity of WT168 strain and mutants during growth in LB medium. SigH activity was measured using a $P_{spoVG42}$-*lux* reporter, with relative light unit (RLU) and OD_{600} measured every 12 minutes. The promoter activity is calculated by dividing RLU with OD_{600}.

Figure S3. C-FLAG-YhdL and N-FLAG-YhdK exhibit function similar to WT



A. Curves of OD_{600} of WT and epitope tag labelled C-FLAG-YhdL N-FLAG-YhdK (CLNK) in LB or LB supplemented with 10 μg ml^{-1} moenomycin. Measurement was performed every 6 minutes and every other measurement was shown for simplicity. Data was presented as mean ± SD, n=3.

B. Fold change of maximum SigM activity of different strains after treatment of final concentration of 10 μg ml^{-1} moenomycin. SigM activity was measured every 6 minutes after treatment using the P_M-*lux* reporter and the maximum value was used to calculate fold changes between samples with or without treatment.