Supplemental Information For

SMC translocation is unaffected by an excess of nucleoid associated proteins in vivo

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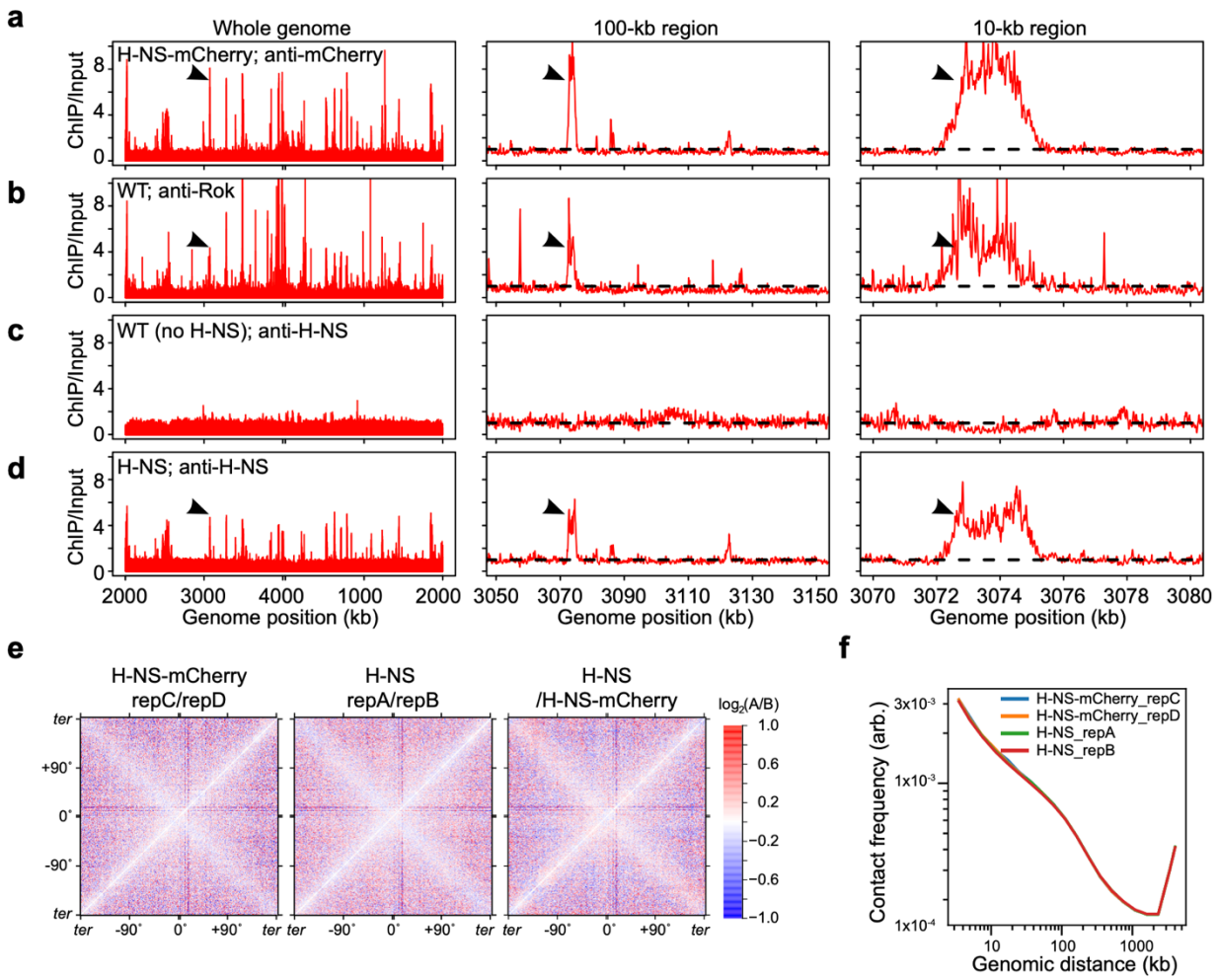


Figure S1. mCherry tag does not affect H-NS function.

(a-d) ChIP-seq results of indicated strains. The x-axis shows genome positions, and the y-axis indicates ChIP enrichment (ChIP/input). Sequencing reads from ChIP and input samples were normalized to the total number of reads before plotting. Left panel: ChIP-seq profiles of the whole genome in 1-kb bins. Middle and right panels: High-resolution plots of a 100-kb region (3150-3150 kb) and a 10-kb region (3070-3080 kb), both of which were plotted in 100-bp bins. **(a)** Anti-mCherry ChIP-seq on H-NS-mCherry (BWX5256). H-NS-mCherry expression was induced using 0.5% xylose for 90 min. **(b)** Anti-Rok ChIP-seq on WT *B. subtilis* PY79. **(c)** Anti-H-NS ChIP-seq on WT *B. subtilis* PY79, which did not express the H-NS protein. **(d)** Anti-H-NS ChIP-seq on *B. subtilis* PY79 expressing H-NS (BWX5864). H-NS expression was induced using 0.5% xylose for 90 min. **(e)** Log_2 ratio plots comparing different Hi-C matrices. Log_2(matrix 1/matrix 2) was calculated and plotted in the heatmaps. Identities of matrix 1/ matrix 2 are shown at the top of each plot. The color scale is shown at the right of panel **(e). (f)** Contact probability decay Pc(s) curves of H-NS-mCherry (BWX5490) and H-NS (BWX5864). Two biological replicates of each strain were analyzed.

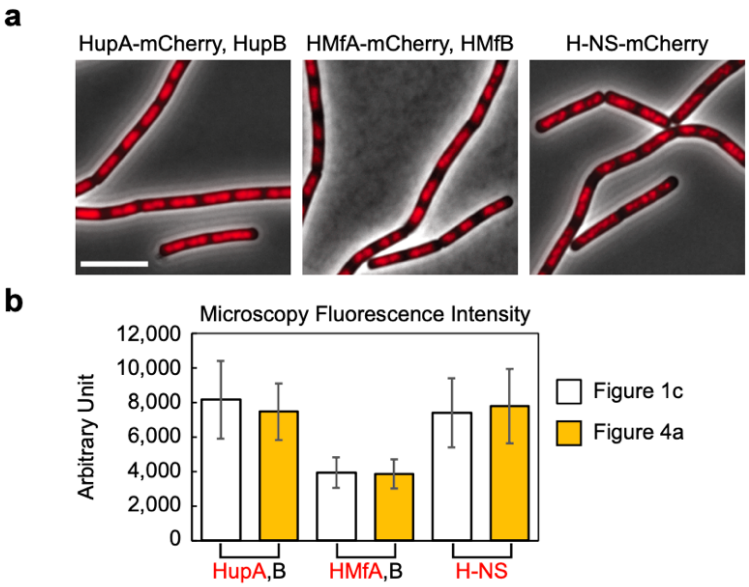


Figure S2. Exogenous DNA-binding proteins localize to the nucleoid.

(a) Fluorescence microscopy of *B. subtilis* expressing the indicated proteins in the strains that were used to measure SMC translocation rates in **Figure 4**. The HupA-mCherry/HupB heterodimer (BWX5602), the HMfA-mCherry/HMfB heterodimer (BWX5603), and H-NS-mCherry (BWX5663) were induced with 0.5% xylose for 90 min. The scale bar represents 4 μm. **(b)** Comparison of fluorescence intensity. Fluorescence intensities of the HupA-mCherry/HupB heterodimer (BWX5598 and BWX5602), the HMfA-mCherry/HMfB heterodimer (BWX5600 and BWX5603), and H-NS-mCherry (BWX5256 and BWX5663) were quantified. BWX5598, BWX5600 and BWX5256 were used in Figure 1; BWX5602, BWX5603 and BWX5663 were used in Figure 4. Red writing indicates the proteins that are tagged with mCherry. The mean pixel intensities were plotted as histograms. Error bars indicate the standard deviation of pixel intensity.

Table S1. Strains used in this study

Strain	Genotype	Reference	Figures
BWX523	*sacA::hbsu-mcherry Kan*	This study	1bcd, 2b
BWX5269	*yhdG::Pxyl hupA-mcherry phleo*	This study	1bcd, 2c
BWX5271	*yhdG::Pxyl hupB-mcherry phleo*	This study	1bcd, 2d
BWX5598	*yhdG::Pxyl hupA-mcherry phleo, yuxG::Pxyl hupB erm*	This study	1bcd, 2e, S2b
BWX5256	*yhdG::Pxyl hns-mcherry phleo*	This study	1bcd, 2f, S1a, S2b
BWX5427	*yhdG::Pxyl hmfA-mcherry phleo*	This study	1bcd, 2g
BWX5429	*yhdG::Pxyl hmfB-mcherry phleo*	This study	1bcd, 2h
BWX5600	*yhdG::Pxyl hmfA-mcherry phleo, yuxG::Pxyl hmfB erm*	This study	1bcd, 2i, S2b
BWX5560	*yhdG::Pxyl mcherry phleo*	This study	1c
BWX5947	*yhdG::Pxyl nbhmfA-mcherry phleo*	This study	1c
BWX5949	*yhdG::Pxyl nbhmfB-mcherry phleo*	This study	1c
PY79	*wild-type*	[1]	2a, S1bc
BWX5492	*parSΔ9 no a.b., -1˚ parS no a.b., yhdG::Pxyl hupA-mcherry phleo*	This study	3a
BWX5494	*parSΔ9 no a.b., -1˚ parS no a.b., yhdG::Pxyl hupB-mcherry phleo*	This study	3a
BWX5496	*parSΔ9 no a.b., -1˚ parS no a.b., yhdG::Pxyl hmfA-mcherry phleo*	This study	3a
BWX5498	*parSΔ9 no a.b., -1˚ parS no a.b., yhdG::Pxyl hmfB-mcherry phleo*	This study	3a
BWX3370	*parSΔ9 no a.b., wt parS in spo0J no a.b.*	[2]	3a-c
BWX5556	*parSΔ9 no a.b., -1˚ parS no a.b., yhdG::Pxyl hupA-mcherry phleo, yuxG::Pxyl hupB erm*	This study	3a-c
BWX5558	*parSΔ9 no a.b., -1˚ parS no a.b., yhdG::Pxyl hmfA-mcherry, yuxG::Pxyl hmfB erm*	This study	3a-c
BWX5490	*parSΔ9 no a.b., -1˚ parS no a.b., yhdG::Pxyl hns-mcherry phleo*	This study	3a-c, S1ef
BWX4070	*parSΔ9 no a.b., Δspo0J spec (no loxP), wt parS in spo0J, yvbJ::Pspank (optRBS) spo0J ΔparS cat*	[3]	4ab
BWX5602	*parSΔ9 no a.b., Δspo0J spec (no loxP), wt parS in spo0J, yvbJ::Pspank (optRBS) spo0J ΔparS cat, yhdG::Pxyl hupA-mcherry phleo, yuxG::Pxyl hupB erm*	This study	4ab, S2ab
BWX5603	*parSΔ9 no a.b., Δspo0J spec (no loxP), wt parS in spo0J, yvbJ::Pspank (optRBS) spo0J ΔparS cat, yhdG::Pxyl hmfA-mcherry phleo, yuxG::Pxyl hmfB erm*	This study	4ab, S2ab
BWX5663	*parSΔ9 no a.b., Δspo0J spec (no loxP), wt parS in spo0J, yvbJ::Pspank (optRBS) spo0J ΔparS cat, yhdG::Pxyl hns-mcherry phleo*	This study	4ab, S2ab
BWX5864	*parSΔ9 no a.b., wt parS in spo0J no a.b., yhdG::Pxyl hns phleo*	This study	S1def
Strains used to build strains			
BWX5502	*parSΔ9* no a.b., *Δspo0J spec*, *wt parS in spo0J, yvbJ::Pspank (optRBS) spo0J ΔparS cat, yhdG::Pxyl hupA-mcherry phleo*	This study	
BWX5506	*parSΔ9* no a.b., *Δspo0J spec*, *wt parS in spo0J, yvbJ::Pspank (optRBS) spo0J ΔparS cat, yhdG::Pxyl hmfA-mcherry phleo*	This study	
BWX5549	*yuxG::Pxyl hupB erm*	This study	
BWX5551	*yuxG::Pxyl hmfB erm*	This study	
BWX5843	*yhdG::Pxyl hns phleo*	This study	

Table S2. Plasmids used in this study

Plasmid	Description	Reference
pWX722	*yvbJ::Pspank (optRBS) spo0J ΔparS cat*	[3]
pWX981	*ycgO::PftsW hns-mcherry phleo*	This study
pWX991	*yhdG::Pxyl hns-mcherry phleo*	This study
pWX993	*yhdG::Pxyl hupA-mcherry phleo*	This study
pWX994	*yhdG::Pxyl hupB-mcherry phleo*	This study
pWX1001	*ycgO::PftsW hupA-mcherry phleo*	This study
pWX1002	*ycgO::PftsW hupB-mcherry phleo*	This study
pWX1072	*yhdG::Pxyl hmfA-mcherry phleo*	This study
pWX1073	*yhdG::Pxyl hmfB-mcherry phleo*	This study
pWX1180	*yuxG Pxyl hupB erm*	This study
pWX1181	*yuxG Pxyl hmfB erm*	This study
pWX1182	*yhdG::Pxyl mcherry phleo*	This study
pWX1252	*yuxG::Pxyl hns erm*	This study
pWX1270	*yhdG::Pxyl nbhmfA-mcherry phleo*	This study
pWX1271	*yhdG::Pxyl nbhmfB-mcherry phleo*	This study

Table S3. Oligonucleotides used in this study

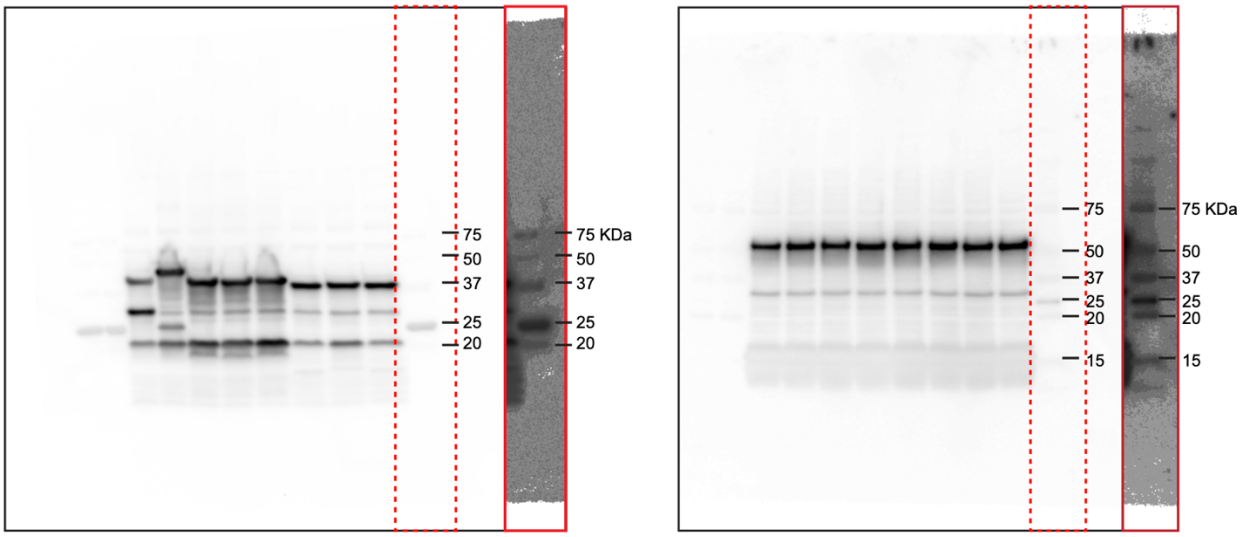
Oligos	Sequence	Use
odr198	GCCCTCGAGTTTTCCGGCAACTGCGTCTTTAAGCGC	BWX523
odr214	GCCGAATTCAAATCACCTTAAATCCTTGACGAGC	BWX523
odr829	CATCAAATCTTACAAATGTAG	sequencing
oML87	CCAGAAGTTTCTCAGAGTCGG	sequencing
oWX486	GCCGCTCTAGCTAAGCAGAAGGC	sequencing
oWX1894	ACATAGTACATAGCGAATCTTCCC	sequencing
oWX2227	CGCAAGCTTACATAAGGAGGAACTACTATGGGCGAGCTGCCAATTG CGCCG	pWX1072
oWX2229	CGCAAGCTTACATAAGGAGGAACTACTATGGAACTGCCAATTGCCCC TATC	pWX1073
oWX2231	GGTCATAACCTGAAGAATTGGATCCCTTATTGCTTGATCAGGAAATC GTCG	pWX1270
oWX2233	CGCAAGCTTACATAAGGAGGAACTACTATGGAACTGCCGATTGCGC CGATC	pWX1271
oWX2493	TGCGACATCGTATAACGTTACTGG	sequencing
oWX2555	CAGAAGCTTACATAAGGAGGAACTACTATGAGCGAAGCACTTAAAAT TCTG	pWX981, pWX1252
oWX2556	CATGAGCTCGAGGCCTGATCCGCCAGATCCTTGCTTGATCAGGAAA TCGTCG	pWX981
oWX2559	CAGAAGCTTACATAAGGAGGAACTACTATGAACAAGACTCAACTGAT TGATG	pWX1001
oWX2561	CAGAAGCTTACATAAGGAGGAACTACTGTGAATAAATCTCAATTGAT CGACAAG	pWX1002
oWX2587	CATGAGCTCGAGGCCTGATCCGCCAGATCCCTTAACTGCGTCTTTCA GTGCC	pWX1001
oWX2588	CATGAGCTCGAGGCCTGATCCGCCAGATCCGTTTACCGCGTCTTTC AGTGC	pWX1002
oWX2903	GAGCTCGAGGCCTGATCCGCCAGATCCCTTAAACATTTTGCGCGCC AGCTC	pWX1072
oWX2904	GAGCTCGAGGCCTGATCCGCCAGATCCCTTTTTGAAGCGACGAACT GCCAG	pWX1073
oWX3230	TAGCATCTCGAGACACTAGTTTAGTTTACCGCGTCTTTCAGTGC	pWX1180
oWX3231	TCCATTTTGACAGCCATTGAAGCTTTACATTGTAATCATGTCCAGAAAATG	pWX1180
oWX3232	TAGCATCTCGAGACACTAGTTTACTTTTTGAAGCGACGAACTGCCAG	pWX1181
oWX3233	TCCATTTTGACAGCCATTGAAGCTTTACATTGTAATCATGTCCAGAAA ATG	pWX1181
oWX3234	ATCTGTATTTGAATGAAGCTTACATAAGGAGGAACTACTATGG	pWX1182
oWX3235	TAACCTGAAGAATTGGATCCTTATTTGTATAATTCGTCCATTCCACC	pWX1182
oWX3573	GGTCATAACCTGAAGAATTGGATCCCTTATTGCTTGATCAGGAAATC GTCG	pWX1252
oWX3649	GGTCATAACCTGAAGAATTGGATCCCTTATTGCTTGATCAGGAAATC GTCG	pWX1270
oWX3650	GAGCTCGAGGCCTGATCCGCCAGATCCTTTCTTGAAACGGCGAACT GCCAAC	pWX1271

Table S4. Next Generation Sequencing samples used in this study

Sample name	Figure	Reference	Identifier
Wang_ChIP_antiHBsu_BWX523	2a	This study	GSM8326547
Wang_ChIP_antimCherry_BWX523	2b, S1a	This study	GSM8326548
Wang_ChIP_antimCherry_BWX5269	2c	This study	GSM8326550
Wang_ChIP_antimCherry_BWX5271	2d	This study	GSM8326551
Wang_ChIP_antimCherry_BWX5598	2e	This study	GSM8326552
Wang_ChIP_antimCherry_BWX5256	2f, S1a	This study	GSM8326549
Wang_ChIP_antimCherry_BWX5427	2g	This study	GSM8326553
Wang_ChIP_antimCherry_BWX5429	3h	This study	GSM8326554
Wang_ChIP_antimCherry_BWX5600	3i	This study	GSM8326555
Wang_WGS_BWX523	2ab, S1a	This study	GSM8326556
Wang_WGS_BWX5269	2c	This study	GSM8326558
Wang_WGS_BWX5271	2d	This study	GSM8326559
Wang_WGS_BWX5598	2e	This study	GSM8326560
Wang_WGS_BWX5256	2f, S1a	This study	GSM8326557
Wang_WGS_BWX5427	2g	This study	GSM8326561
Wang_WGS_BWX5429	3h	This study	GSM8326562
Wang_WGS_BWX5600	3i	This study	GSM8326563
Wang_HiC_BWX3370_repA	3abc	This study	GSM8326564
Wang_HiC_BWX3370_repB	3abc	This study	GSM8326565
Wang_HiC_BWX5492_repA	3a	This study	GSM8326566
Wang_HiC_BWX5494_repA	3a	This study	GSM8326567
Wang_HiC_BWX5556_repA	3abc	This study	GSM8326568
Wang_HiC_BWX5556_repB	3abc	This study	GSM8326569
Wang_HiC_BWX5490_repA	3abc	This study	GSM8326570
Wang_HiC_BWX5490_repB	3abc	This study	GSM8326571
Wang_HiC_BWX5496_repA	3a	This study	GSM8326572
Wang_HiC_BWX5498_repA	3a	This study	GSM8326573
Wang_HiC_BWX5558_repA	3abc	This study	GSM8326574
Wang_HiC_BWX5558_repB	3abc	This study	GSM8326575
Wang_HiC_BWX4470_IPTG0min	4ab	This study	GSM8326576
Wang_HiC_BWX4470_IPTG15min	4ab	This study	GSM8326577
Wang_HiC_BWX4470_IPTG25min	4ab	This study	GSM8326578
Wang_HiC_BWX4470_IPTG35min	4ab	This study	GSM8326579
Wang_HiC_BWX5602_IPTG0min	4ab	This study	GSM8326580
Wang_HiC_BWX5602_IPTG15min	4ab	This study	GSM8326581
Wang_HiC_BWX5602_IPTG25min	4ab	This study	GSM8326582
Wang_HiC_BWX5602_IPTG35min	4ab	This study	GSM8326583
Wang_HiC_BWX5603_IPTG0min	4ab	This study	GSM8326584
Wang_HiC_BWX5603_IPTG15min	4ab	This study	GSM8326585

Wang_HiC_BWX5603_IPTG25min	4ab	This study	GSM8326586
Wang_HiC_BWX5603_IPTG35min	4ab	This study	GSM8326587
Wang_HiC_BWX5663_IPTG0min	4ab	This study	GSM8326588
Wang_HiC_BWX5663_IPTG15min	4ab	This study	GSM8326589
Wang_HiC_BWX5663_IPTG25min	4ab	This study	GSM8326590
Wang_HiC_BWX5663_IPTG35min	4ab	This study	GSM8326591
Wang_ChIP_antiRok_BWX001	S1b	This study	GSM8326592
Wang_ChIP_antiHNS_BWX001	S1c	This study	GSM8326593
Wang_ChIP_antiHNS_BWX5864	S1d	This study	GSM8326594
Wang_WGS_BWX001	S1bc	This study	GSM8326595
Wang_WGS_BWX5864	S1d	This study	GSM8326596
Wang_HiC_BWX5490_repC	S1e	This study	GSM8326597
Wang_HiC_BWX5490_repD	S1e	This study	GSM8326598
Wang_HiC_BWX5864_repA	S1e	This study	GSM8326599
Wang_HiC_BWX5864_repB	S1e	This study	GSM8326600

Supplementary Uncropped Gels



Left gel: Western Blotting using anti-mCherry antibodies

Right gel: Western Blotting using anti-SigA antibodies

Lanes:

- ladder
- ladder
- BWX523
- BWX5256
- BWX5269
- BWX5271
- BWX5598
- BWX5427
- BWX5429
- BWX5600
- ladder

To label the molecular weight of the bands, we adjusted the image contrast of the right-most lane containing the protein ladder (regions in the red-dotted boxes) to generate the images in the red solid boxes on the right. The protein ladder was Precision Plus Protein Dual Color Standards (Bio-Rad 161-0347).

SI References

1. Youngman, P.J., Perkins, J.B., and Losick, R. (1983). Genetic transposition and insertional mutagenesis in Bacillus subtilis with Streptococcus faecalis transposon Tn917. Proc Natl Acad Sci U S A *80*, 2305-2309. 10.1073/pnas.80.8.2305.
2. Wang, X., Brandao, H.B., Le, T.B., Laub, M.T., and Rudner, D.Z. (2017). Bacillus subtilis SMC complexes juxtapose chromosome arms as they travel from origin to terminus. Science *355*, 524-527. 10.1126/science.aai8982.
3. Wang, X., Hughes, A.C., Brandao, H.B., Walker, B., Lierz, C., Cochran, J.C., Oakley, M.G., Kruse, A.C., and Rudner, D.Z. (2018). In Vivo Evidence for ATPase-Dependent DNA Translocation by the Bacillus subtilis SMC Condensin Complex. Mol Cell *71*, 841-847 e845. 10.1016/j.molcel.2018.07.006.