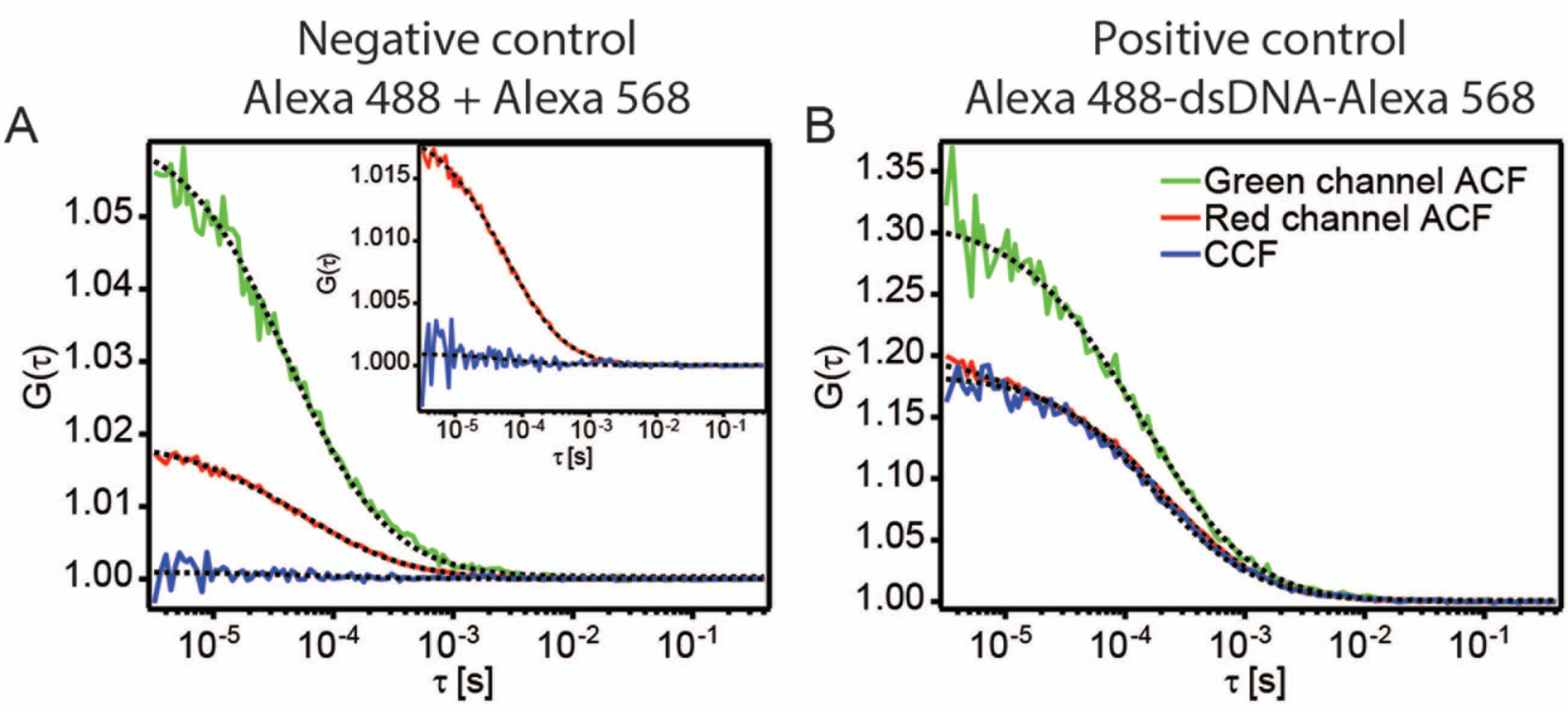
Supplementary Figure Legends

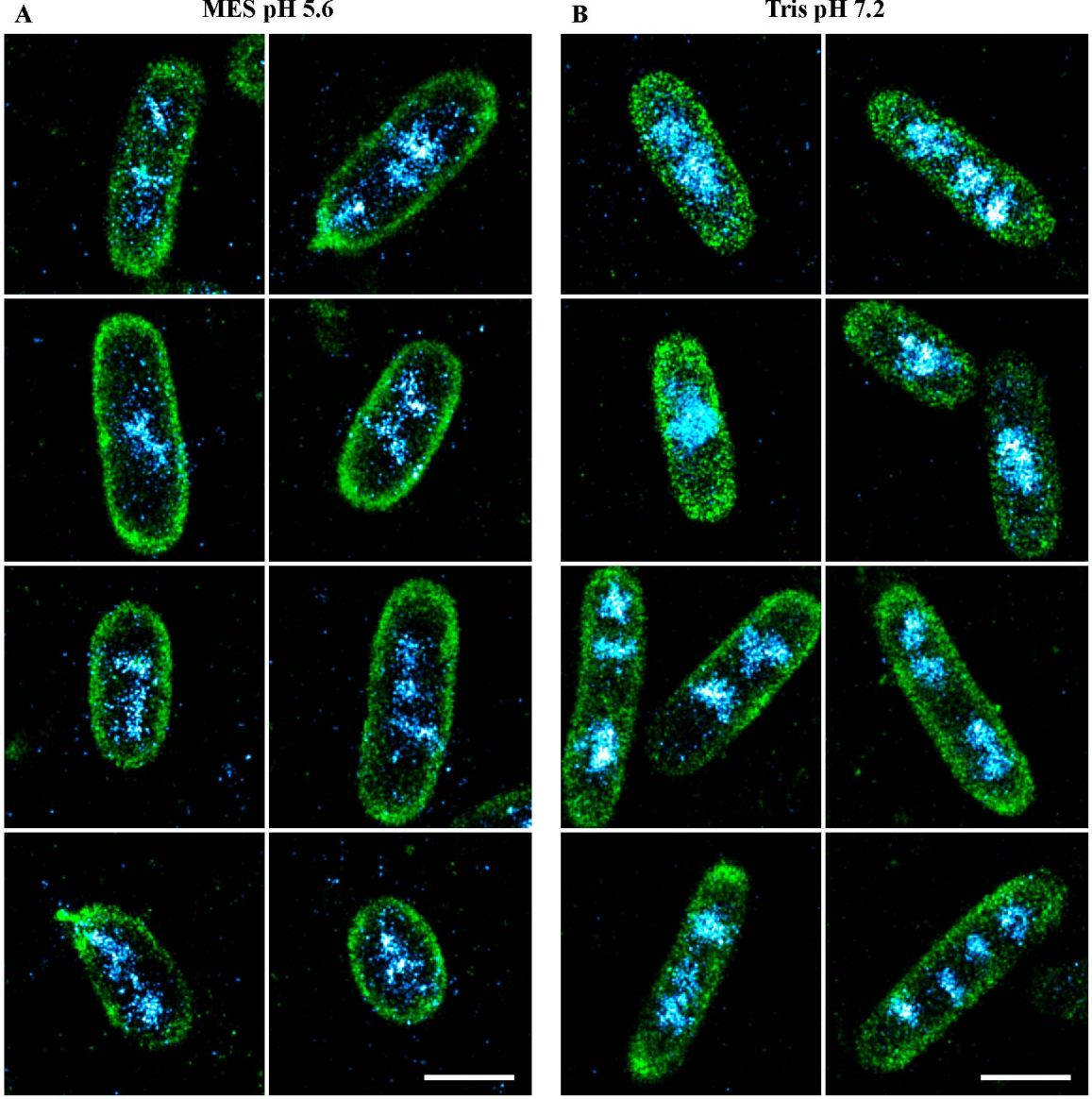
Supplementary Fig. 1 Fluorescence cross-correlation spectroscopy (FCCS) measurements of control samples. The green and red curves are the autocorrelation functions (ACFs) of the "green" molecules and "red" molecules, respectively. The blue curves are the cross-correlation functions (CCF). The ratio of the amplitude of the CCF to the red ACF (inset) indicates the amount of "green" in complex. (A) A negative control of Alexa 488 + Alexa 568. The cross-correlation ratio is ~0.02. (B) A positive control of double-stranded, gel purified DNA labeled with Alexa 488 and Alexa 568 on each end. The cross-correlation ratio is ~0.99. Both controls indicate that with these microscope settings, the cross-correlation ratio can range from a lower limit of 0.02 to an upper limit of 0.99.

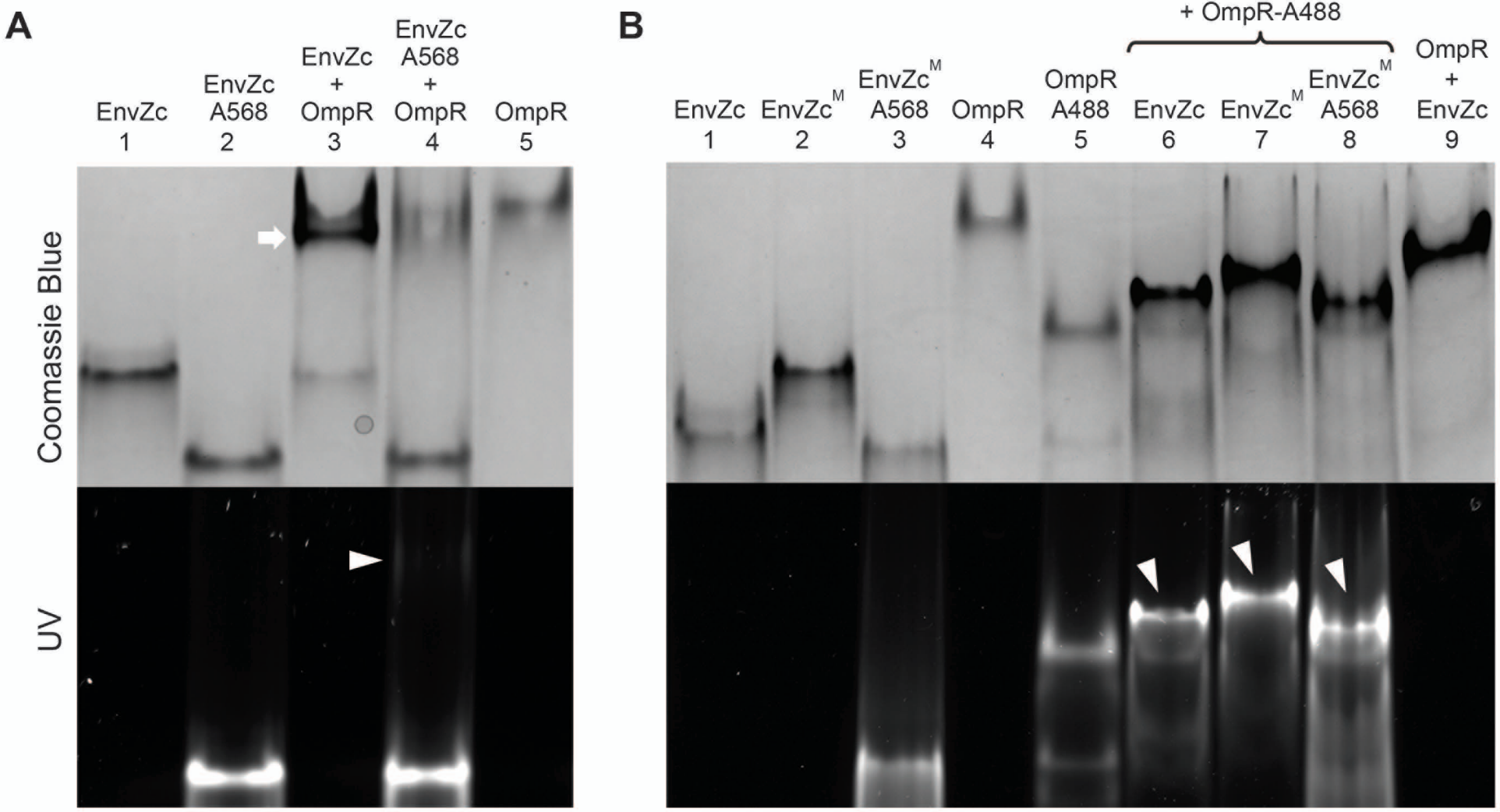
Supplementary Fig. 2. Comparison of nucleoids during acidic (MES pH 5.6) and neutral (Tris pH 7.2) growth. The nucleoid is shown in cyan (*d*STORM channel), while the respective cell boundaries are displayed green (PAINT channel). (A) Cells grown in an acidic environment show stronger nucleoid condensation and appear more rounded than cells grown in Tris (B) (scale bar 1μm).

Supplementary Fig. 3 The upper panel shows the Coomassie-stained native PAGE. Since most Alexa Fluor dyes can be excited around 260-280 nm, the labeled EnvZc-A568 can also be visualized using a UV gel imager prior to Coomassie staining (lower panel). The final concentration of the different EnvZc and OmpR variants used was 7 μM each. (A) Lane 1: EnvZc alone. Lane 2: EnvZc-A568. EnvZc-A568 runs faster, likely due to the charges on Alexa 568. Lane 3: EnvZc + OmpR (OmpR alone is shown in lane 5). The white arrow indicates the EnvZc/OmpR complex formed. Lane 4: EnvZc-A568 + OmpR. The bright bands in the UV panel in lanes 2 and 4 are Alexa-labeled EnvZc. The white triangle in the UV panel highlights a very faint EnvZc-A568/OmpR complex, indicating that the binding is very weak. (B) Lanes 1-5 are the individual proteins EnvZc, $EnvZc^{M}$ (C277M/M294C mutant), $EnvZc^{M}$-A568, OmpR and OmpR-A488, respectively. Lanes 6-8 are a mixture of OmpR-A488 and EnvZc, $EnvZc^{M}$ and $EnvZc^{M}$-A568, respectively. Lane 9 is the positive control of EnvZc + OmpR. Complexes in the UV panel are indicated by the white triangles.

Supplementary Fig. 4 Representative normalized autocorrelation functions (ACFs) obtained from FCS measurement of $EnvZc^{M}$ titration to OmpR-A488. From left to right are selected ACFs from the titration of $EnvZc^{M}$ with OmpR-A488. Dotted lines are the fits of the ACF. The shift of the ACF to a higher τ value is an indication of increased binding of the larger $EnvZc^{M}$ dimer to the smaller OmpR-A488.


Negative control
Alexa 488 + Alexa 568
A
G(τ)
τ [s]
Positive control
Alexa 488-dsDNA-Alexa 568
B
Green channel ACF
Red channel ACF
CCF


A
MES pH 5.6
B
Tris pH 7.2


A
EnvZc
1
EnvZc A568
2
EnvZc + OmpR
3
EnvZc A568 + OmpR
4
OmpR
5
Coomassie Blue
UV
B
+ OmpR-A488
EnvZc
1
EnvZc^M
2
EnvZc^M A568
3
OmpR
4
OmpR A488
5
EnvZc
6
EnvZc^M
7
EnvZc^M A568
8
OmpR + EnvZc
9

Supplementary Fig. 4

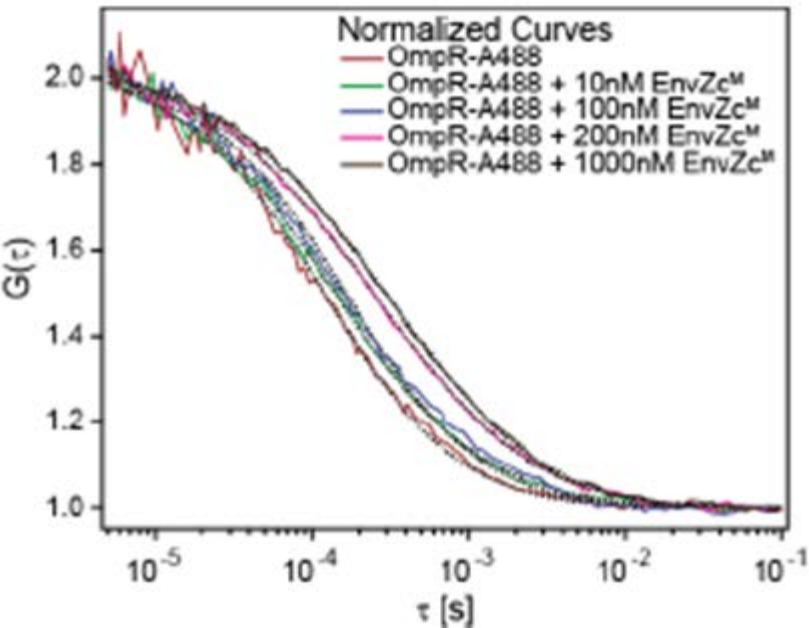


Table S1. Media composition and culture doubling times

Medium	reagent	Amount (per l)/ concentration	Osmolality [mOsm/Kg]	Doubling time [min]
LB	bacto-tryptone	10 g	460	24 ± 1
	yeast extract	5 g		
	NaCl	10 g		
Tris pH 7.2	Tris	100 mM	210	48 ± 2
	$(NH_4)_2SO_4$	7.5 mM		
	KCl	5 mM		
	K_2SO_4	0.5 mM		
	KH_2PO_4	1 mM		
	$MgCl_2$	10 µM		
	Glucose	2 mM		
	casamino acids	0.1 %		
MES pH 5.6	Change Tris for MES	100 mM	205	48 ± 3
0.5x M9*	Na_2HPO_4-$7H_2O$	32 g	115	52 ± 3
	KH_2PO_4	7.5 g		
	NaCl	1.25 g		
	NH_4Cl	2.5 g		
	Glucose	0.1%		
	$MgSO_4$	2 mM		
	$CaCl_2$	0.1 mM		
	casamino acids	0.05%		

* Cells were pre-cultured in LB to OD_{600} = 0.2 and transferred into 0.5x M9 medium