Supplementary Information for:

Nucleoid Occlusion protein Noc recruits DNA to the Bacterial Cell Membrane

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**Supplementary Table S1** – Localisation and functionality of noc alleles

<table>
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<tr>
<th>Protein</th>
<th>Localisation</th>
<th>Complements Δnoc ΔminCD?a</th>
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<tr>
<td></td>
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<td>NA</td>
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<tr>
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<tr>
<td>K2E</td>
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<tr>
<td>S4A</td>
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<tr>
<td>S4L/G86S</td>
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* The ability of the indicated –YFP fusions to rescue the growth defect of a noc minCD mutant was assayed after growth for 18 h at 39 °C, either with no additions (NA), 0.05 % or 0.5 % w/v xylose. All proteins were produced at similar levels (Supplementary Figure S11).
**Supplementary Methods**

**Protein expression and purification**

*E. coli* strains BL21 (DE3), harbouring plasmids pDWA23, 31 or 32 were grown in LB at 37 °C to an 
OD$_{600}$ of 0.5, at which point IPTG was added to a final concentration of 1 mM to induce protein 
expression. After 3 h at 30 °C, cells were harvested by centrifugation (4000 g; 10 min; 4 °C), washed 
once with ice-cold PBS containing 1 mM PMSF and snap-frozen in liquid nitrogen. Cell pellets were 
stored at -80 °C overnight to aid cell wall breakage.

Cell pellets were re-suspended in ice-cold 50 mM HEPES-KOH pH 6.8; 150 mM NaCl (Buffer 
A) containing a complete EDTA-free protease inhibitor tablet (Roche) and 32 mg/ml lysozyme, and 
i incubated on ice for 1 h with gentle agitation. Cells were then lysed by sonication on ice (13 W; pulse 
3) and cell-debris removed by centrifugation (31,000 g; 45 min; 4 °C). The clarified lysate was applied 
to a 1 ml HiTrap Q HP column (GE Healthcare) pre-equilibrated with buffer A. The column was 
washed with 20 column volumes (CV) of buffer A and proteins eluted using a linear gradient of 150- 
1000 mM NaCl over 20 CV. Fractions containing Noc were identified by absorbance at 280 nm and 
were analysed for purity by SDS-PAGE. Appropriate fractions were diluted in 50 mM HEPES-KOH pH 
7.6; 100 mM NaCl; 1 mM EDTA (Buffer B) and applied to a 1 ml HiTrap Heparin HP Sepharose column 
(GE Healthcare) pre-equilibrated with the same buffer. The column was washed with 20 CV of buffer 
B and proteins eluted using a linear gradient of 100-1000 mM NaCl over 20 CV. Appropriate fractions 
were diluted in 50 mM HEPES-KOH pH 7.6; 100 mM NaCl (Buffer C) and applied to a 1 ml HiTrap Q 
HP column (GE Healthcare) pre-equilibrated with the same buffer. The column was washed with 20 
CV of buffer C and proteins eluted using a linear gradient of 100-1000 mM NaCl over 20 CV. 
Fractions containing Noc were identified by absorbance at 280 nm and were analysed for purity by 
SDS-PAGE. Appropriate fractions were pooled (≤ 3 ml) and loaded onto HiLoad 16/60 Superdex 75 pg 
gel filtration column (GE Healthcare) pre-equilibrated with 30 mM HEPES-KOH pH 7.6; 300 mM NaCl 
(Buffer D) and eluted in the same buffer according to its hydrodynamic volume. Fractions containing
pure Noc were identified by SDS-PAGE, pooled, mixed with glycerol (10 % v/v final), aliquoted and snap-frozen in liquid nitrogen before storage at -80 °C until needed.

**Analytical size-exclusion chromatography**

Approximately 250 μg of purified protein was applied to a Superdex 200 10/300 GL (GE Healthcare) gel filtration column in a sample volume of 100 μl. Samples were run in 30 mM HEPES-KOH pH 7.6; 300 mM NaCl at a flow rate of 0.5 ml/min. The column was calibrated using a Gel Filtration Calibration Kit (GE Healthcare) comprising Aprotinin (6,500 Mr), Ribonuclease A (13,700 Mr), Carbonic anhydrase (29,000 Mr), Ovalbumin (43,000 Mr), Conalbumin (75,000 Mr), Aldolase (158,000 Mr) and Ferritin (440,000 Mr). The void volume was determined using Blue Dextran 2000 (> 2,000,000 Mr). The partition coefficients ($K_{av}$) of the protein standards were calculated according to the manufacturer’s instructions and used to plot a standard curve (Supplementary Figure S3). The curve was then used to estimate the molecular weights of unknown samples by comparing the $K_{av}$ of the sample with those of the known protein standards.

**Reagents for Western blotting**

Antibodies used for Western blotting were as follows: anti-FtsZ (Lucet et al, 2000); anti-DnaA (Scholefield et al, 2012); anti-PBP2B (Daniel et al, 2000); anti-GFP (laboratory stock, controls for specificity are shown in Supplementary Fig. S6D and E); anti-Noc (laboratory stock, controls for specificity are shown in Supplementary Figures S6E and S7C).
Plasmid construction

The sequences of all plasmids were verified by DNA sequencing (DNA Sequencing & Services, University of Dundee, Scotland). Unless otherwise specified, PCR amplification used *B. subtilis* 168 chromosomal DNA as a template and was done using Phusion® High-Fidelity DNA Polymerase (New England Biolabs). Site-directed mutagenesis was done using PfuTurbo DNA Polymerase (Agilent Technologies).

Plasmids for protein localisation in *B. subtilis*

Plasmids used for protein localisation were made using derivatives of pSG4924, which is an integrative vector (*amyE*) for creating C-terminal YFP fusions under the control of the xylose-inducible $P_{xyl}$ promoter. All –YFP fusions used the same linker (VEVDGIDKDIEFLQ) and Shine-Dalgarno sequence (AGGAGA). To create plasmids pDWA41 (mYFP) and pDWA42 (Noc-mYFP), the monomeric A206K mutation (Landgraf et al., 2012) was introduced into *yfp* by site-directed mutagenesis using pSG4924 and pSG4926, respectively, as templates. To create pDWA40, *noc bp 1-150* encoding the first 50 amino acids of Noc was amplified by PCR. The PCR product was digested with *Bln*I and *Sal*I and cloned into pDWA41 between the *Bln*I and *Xho*I sites. To create pDWA38 (HVCAH-NocNΔ10-YFP), the sequence encoding the HVCAH (MILSSLTVTQLLRRLHQWI) was included in the forward primer and was used to amplify nocNΔ10 by PCR. The PCR product was digested with *Bln*I and *Sal*I and cloned into pSG4924 between the *Bln*I and *Xho*I sites. The HVCAH is connected to NocNΔ10 by a GSGSGS linker sequence. To create pDWA45 (TM-NocNΔ10-mYFP), the sequence encoding the WALP23 (Nyholm et al., 2007) trans-membrane (TM) domain (MAWWLALALALALALALALALWWA) was included in the forward primer and was used to amplify nocNΔ10 by PCR. The PCR product was digested with *Bln*I and *Sal*I and cloned into pDWA41 between the *Bln*I and *Xho*I sites. The TM domain is joined to NocNΔ10 by a GSGSGS linker sequence. To create pDWA110 (NocNΔ10-mYFP), nocNΔ10 was amplified by PCR, digested with *Bln*I and *Sal*I and cloned
into pDWA41 between the BlmI and XhoI sites. All -YFP fusion derivatives encoding amino acid substitutions in Noc were made by site-directed mutagenesis using the templates pSG4926, pDWA42 or pDWA75, as appropriate.

**Plasmids for Noc overproduction in B. subtilis**

To construct pSG4922, *noc* was amplified by PCR, the product was digested with XbaI and ClaI and cloned into pPL82 cut with the same enzymes. pPL82 is an integrative vector (amyE) that carries *lacI* and the strong synthetic LacI-regulated $P_{spac(hy)}$ promoter. All constructions within pPL82 used the native *noc* Shine-Dalgarno sequence and spacer. To create pDWA79, *nocNΔ10* was amplified by PCR, digested with XbaI and ClaI and cloned into pPL82 cut with the same enzymes. Plasmid derivatives encoding deletions or substitutions were created by site-directed mutagenesis using pSG4922 as a template.

To create pSG4943, which integrates at the *noc* locus, a 3.5 kb DNA fragment containing $P_{spac(hy)}$-*noc lacI* was isolated from pSG4922 by digestion with EcoRI and BamHI, and then cloned into pUK19 (Wu et al., 2009) using the EcoRI and BamHI sites.

**Plasmids for Noc overproduction in E. coli**

To create pDWA37, *noc* was amplified by PCR, the product digested with EcoRI and SalI and cloned into pMG25 cut with EcoRI and SalI. pMG25 is a pUC-derived vector that allows tightly regulated, IPTG-inducible expression. The plasmid carries *lacI* and the strong synthetic LacI-regulated $P_{A1/O3/O4}$ promoter. All pMG25 constructs used the same optimised Shine-Dalgarno sequence (AGGAGG). To create pDWA61, *nocNΔ10* was amplified by PCR, the product digested with EcoRI and SalI and cloned into pMG25 cut with the same enzymes. pDWA62 (NocΔ50) was created by site-directed mutagenesis using pDWA37 as a template. To create pDWA66, *spo0J* was amplified by PCR, the
product digested with EcoRI and SalI and cloned into pMG25 cut with the same enzymes. pDWA68 was constructed in three stages. First, spo0J was amplified by PCR, the product digested with BlnI and EcoRI and cloned into pSG1728 digested with the same enzymes. Second, the sequence encoding the N-terminal 30 amino acids of Noc was cloned into this plasmid between BlnI (within vector) and StuI (within spo0J). Third, using this intermediate plasmid as a template, noc^{bp1-90-spo0J} was amplified by PCR, the product digested with EcoRI and SalI and cloned into pMG25 cut with the same enzymes. Note that in order to avoid any potential complications due to Spo0J or Noc30-Spo0J proteins binding to the parS site within the spo0J gene, plasmids pDWA66 and pDWA68 were assembled using a parS-minus allele of spo0J, which contains changes in 7 bp (of 16) (WT, TGTTCCACGTAAACA; parS-minus, CGTGCCCAGGGAGACC) in the parS site within spo0J without affecting the amino acid sequence of the gene product (Lin & Grossman, 1998). To create pDWA69, HCV_{AH}-nocNΔ10 was amplified by PCR using pDWA38 as a template. The PCR product was digested with EcoRI and SalI and cloned into pMG25 cut with the same enzymes.

**GFP-JunZ-MTS derivatives**

pDWA20 encodes an arabinose inducible fusion protein comprised of GFP, JunLZ and the first 30 amino acids of Noc, with a triglycine linker between JunLZ and Noc^{1-30}. JunLZ is a homodimerization-enhanced mutant of the leucine zipper motif from the c-Jun transcription factor (Szeto et al, 2003). To create pDWA20 the sequence encoding the E. coli MinD membrane targeting sequence (MTS) in pTS37 was replaced with the sequence encoding the first 30 amino acids of Noc by PCR.

**Plasmids for protein purification**

To create pDWA23, noc was amplified by PCR and cut with BspHI and XhoI. The digested PCR product was cloned between the Ncol and XhoI sites of pET16b such that the native protein (**i.e.**
without any tag) is produced under the control of the T7 promoter. To create pDWA31, nocNΔ10 was amplified by PCR, digested with Ncol and Xhol and cloned into pET16b cut with the same enzymes. pDWA32 (NocCΔ50) was created by site-directed mutagenesis of pDWA23.
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<th>Strain/Plasmid</th>
<th>Relevant genotype(^a)</th>
<th>Reference / Origin(^b)</th>
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<tr>
<td>168CA</td>
<td>trpC2</td>
<td>Laboratory stock</td>
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<td>ΔatpB::erm</td>
<td>(Strahl &amp; Hamoen, 2010)</td>
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<td>trpC2 ΔminCD::kan</td>
<td>(Wu &amp; Errington, 2004)</td>
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<td>DWA306</td>
<td>trpC2 ΔminCD::kan</td>
<td>3309 &gt; 168 (Km)</td>
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</table>
DWA346  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{cat laci } P_{\text{spac(hy)^-noc}}\Delta F5, S6) \)
\( \text{pSG4941 } > \text{DWA117 } (\text{Cm}) \)

DWA347  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{cat laci } P_{\text{spac(hy)^-nocR7A}}) \)
\( \text{pSG4937 } > \text{DWA117 } (\text{Cm}) \)

DWA348  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{cat laci } P_{\text{spac(hy)^-nocK14A}}) \)
\( \text{pSG4938 } > \text{DWA117 } (\text{Cm}) \)

DWA349  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{spc } P_{\text{xyr-noc54L-G86S-myfp}}) \)
\( \text{pDWA103 } > \text{DWA117 } (\text{Sp}) \)

DWA350  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Delta \text{minCD}\cdot \text{kan, } \Omega \text{amyE}\cdot(\text{cat laci } P_{\text{spac(hy)^-noc}}) \)
\( \text{DWA306 } > \text{DWA117 } (\text{Km}) \)

DWA351  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Delta \text{minCD}\cdot \text{kan, } \Omega \text{amyE}\cdot(\text{cat laci } P_{\text{spac(hy)^-nocNA10}}) \)
\( \text{DWA306 } > \text{DWA282 } (\text{Km}) \)

DWA352  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Delta \text{minCD}\cdot \text{kan, } \Omega \text{amyE}\cdot(\text{cat laci } P_{\text{spac(hy)^-nocG86S}}) \)
\( \text{DWA306 } > \text{DWA282 } (\text{Km}) \)

DWA353  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Delta \text{minCD}\cdot \text{kan, } \Omega \text{amyE}\cdot(\text{cat laci } P_{\text{spac(hy)^-nocQ68R}}) \)
\( \text{DWA306 } > \text{DWA282 } (\text{Km}) \)

DWA354  \( \text{trpC2 } \Delta \text{minCD}\cdot \text{kan, } \Omega \text{amyE}\cdot(\text{cat laci } P_{\text{spac(hy)^-nocQ68S}}) \)
\( \text{DWA119 } > \text{DWA306 } (\text{Cm}) \)

DWA355  \( \text{trpC2 } \Delta \text{minCD}\cdot \text{kan, } \Omega \text{amyE}\cdot(\text{cat laci } P_{\text{spac(hy)^-nocNA10}}) \)
\( \text{DWA282 } > \text{DWA306 } (\text{Cm}) \)

DWA356  \( \text{trpC2 } \Delta \text{minCD}\cdot \text{kan, } \Omega \text{amyE}\cdot(\text{cat laci } P_{\text{spac(hy)^-nocNA10-yfp}}) \)
\( \text{DWA282 } > \text{DWA306 } (\text{Cm}) \)

DWA357  \( \text{trpC2 } \Omega \text{noc}\cdot(\text{spc } P_{\text{xyr-nocNA10-myfp}}) \)
\( \text{DWA146 } > \text{DWA127 } (\text{Sp}) \)

DWA358  \( \text{trpC2 } \Omega \text{noc}\cdot(\text{spc } P_{\text{xyr-nocNA10-myfp}}) \)
\( \text{DWA282 } > \text{DWA127 } (\text{Sp}) \)

DWA359  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{spc } P_{\text{xyr-nocNA10-myfp}}) \)
\( \text{pDWA110 } > \text{DWA117 } (\text{Sp}) \)

DWA360  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{spc } P_{\text{xyr-nocNA10-myfp}}) \)
\( 4705 > \text{DWA206 } (\text{Em}), \text{then } 4705 > \text{resulting strain } (\text{Cm}) \)

DWA361  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{spc } P_{\text{xyr-nocNA10-myfp}}) \)
\( 4705 > \text{DWA382 } (\text{Em}), \text{then } 4705 > \text{resulting strain } (\text{Cm}) \)

DWA362  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{spc } P_{\text{xyr-nocNA10-myfp}}) \)
\( 4705 > \text{DWA285 } (\text{Em}), \text{then } 4705 > \text{resulting strain } (\text{Cm}) \)

DWA363  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{spc } P_{\text{xyr-nocNA10-myfp}}) \)
\( 4712 > \text{HM773 } (\text{Km}) \)

DWA364  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{spc } P_{\text{xyr-tetR-MCherry}}) \)
\( \text{pDWA117 } > \text{DWA427 } (\text{Em}) \)

DWA365  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{spc } P_{\text{xyr-tetR-MCherry}}) \)
\( \text{pDWA117 } > \text{DWA427 } (\text{Em}) \)

DWA366  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{spc } P_{\text{xyr-tetR-MCherry}}) \)
\( \text{pDWA117 } > \text{DWA427 } (\text{Em}) \)

DWA367  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{spc } P_{\text{xyr-tetR-MCherry}}) \)
\( \text{pDWA117 } > \text{DWA427 } (\text{Em}) \)

DWA368  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{spc } P_{\text{xyr-tetR-MCherry}}) \)
\( \text{pDWA117 } > \text{DWA427 } (\text{Em}) \)

DWA369  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{spc } P_{\text{xyr-tetR-MCherry}}) \)
\( \text{pDWA117 } > \text{DWA427 } (\text{Em}) \)

DWA370  \( \text{trpC2 } \Delta \text{noc}\cdot \text{tet, } \Omega \text{amyE}\cdot(\text{spc } P_{\text{xyr-tetR-MCherry}}) \)
\( \text{pDWA117 } > \text{DWA427 } (\text{Em}) \)
DWA524  trpC2 Δnoc::tet, ΩamyE::(lacI P<sub>spac</sub>(hy)⁻nocNΔ10) + pSG4929  pSG4929 > DWA282 (Em)
DWA525  trpC2 Δnoc::tet, ΩamyE::(lacI P<sub>spac</sub>(hy)⁻nocQ68R) + pSG4929  pSG4929 > DWA283 (Em)
DWA526  trpC2 Δnoc::tet, ΩamyE::(lacI P<sub>spac</sub>(hy)⁻nocG86S) + pSG4929  pSG4929 > DWA284 (Em)
DWA545  trpC2 Δnoc::tet, ΩamyE::(spc P<sub>xyl</sub>-nocR88A-myfp)  pDWA140 > DWA117 (Sp)
DWA546  trpC2 Δnoc::tet, ΩamyE::(spc P<sub>xyl</sub>-nocR89A-myfp)  pDWA141 > DWA117 (Sp)
DWA547  trpC2 Δnoc::tet, ΩamyE::(spc P<sub>xyl</sub>-nocR91A-myfp)  pDWA142 > DWA117 (Sp)
DWA548  trpC2 Δnoc::tet, ΩamyE::(spc P<sub>xyl</sub>-TM-NΔ10-myfp)  pDWA45 > DWA117 (Sp)
DWA551  trpC2 Δnoc::tet, ΩamyE::(cat P<sub>spac</sub>(hy)⁻noc R88A)  pDWA144 > DWA117 (Cm)
DWA552  trpC2 Δnoc::tet, ΩamyE::(cat P<sub>spac</sub>(hy)⁻noc R98A)  pDWA145 > DWA117 (Cm)
DWA553  trpC2 Δnoc::tet, ΩamyE::(cat P<sub>spac</sub>(hy)⁻noc R91A)  pDWA146 > DWA117 (Cm)
DWA559  trpC2 ΔminCD::kan  DWA551 > DWA306 (Cm)
DWA560  trpC2 ΔminCD::kan  DWA552 > DWA306 (Cm)
DWA561  trpC2 ΔminCD::kan  DWA553 > DWA306 (Cm)
DWA562  trpC2 ΔminCD::kan  DWA350 > DWA548 (Km)
DWA564  trpC2 ΔminCD::kan  DWA350 > DWA206 (Km)
DWA566  trpC2 ΔminCD::kan  DWA350 > DWA382 (Km)
DWA568  trpC2 ΔminCD::kan  DWA350 > DWA286 (Km)
DWA570  trpC2 ΔminCD::kan  DWA350 > DWA316 (Km)
DWA574  trpC2 ΔminCD::kan  DWA350 > DWA328 (Km)
DWA576  trpC2 ΔminCD::kan  DWA350 > DWA329 (Km)
DWA578  trpC2 ΔminCD::kan  DWA350 > DWA211 (Km)
DWA580  trpC2 ΔminCD::kan  DWA350 > DWA212 (Km)
DWA582  trpC2 ΔminCD::kan  DWA350 > DWA318 (Km)
DWA584  trpC2 ΔminCD::kan  DWA350 > DWA325 (Km)
DWA588  trpC2 ΔminCD::kan  DWA350 > DWA349 (Km)
DWA590  trpC2 ΔminCD::kan  DWA350 > DWA285 (Km)
DWA598  trpC2 ΔminCD::kan  DWA350 > DWA545 (Km)
DWA600  trpC2 ΔminCD::kan  DWA350 > DWA546 (Km)
DWA602  trpC2 ΔminCD::kan  DWA350 > DWA547 (Km)
DWA606  trpC2 ΔminCD::kan  pDWA147 > DWA117 (Sp)
DWA611  trpC2 ΔminCD::kan  DWA350 > DWA322 (Km)
DWA613 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(} \text{spc} P_{\text{xyl}}^{-}\text{nocF8A-} \text{myfp}) \), \( \Delta \text{minCD::kan} \)

DWA615 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(} \text{spc} P_{\text{xyl}}^{-}\text{nocK164A-} \text{myfp}) \)

DWA623 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(cat} P_{\text{spac(hy)}}^{-}\text{noc} \text{R88A} \text{)} + \text{pSG4929} \)

DWA624 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(cat} P_{\text{spac(hy)}}^{-}\text{noc} \text{R89A} \text{)} + \text{pSG4929} \)

DWA625 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(cat} P_{\text{spac(hy)}}^{-}\text{noc} \text{R91A} \text{)} + \text{pSG4929} \)

DWA626 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(} \text{spc} P_{\text{xyl}}^{-}\text{nocR88A-} \text{myfp}) \)

DWA627 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(} \text{spc} P_{\text{xyl}}^{-}\text{nocR91A-} \text{myfp}) \)

DWA628 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(} \text{spc} P_{\text{xyl}}^{-}\text{HCVAH-} \text{gfp}) \), \( \Delta \text{minCD::kan} \)

DWA629 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(} \text{spc} P_{\text{yr}}^{-}\text{HCVAH-} \text{gfp}) \), \( \Delta \text{minCD::kan} \)

DWA630 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(} \text{spc} P_{\text{yr}}^{-}\text{HCVAH-} \text{gfp}) \), \( \Delta \text{minCD::kan} \)

DWA631 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(} \text{spc} P_{\text{yr}}^{-}\text{HCVAH-} \text{gfp}) \), \( \Delta \text{minCD::kan} \)

DWA632 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(} \text{spc} P_{\text{yr}}^{-}\text{HCVAH-} \text{gfp}) \), \( \Delta \text{minCD::kan} \)

DWA633 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(} \text{spc} P_{\text{yr}}^{-}\text{HCVAH-} \text{gfp}) \), \( \Delta \text{minCD::kan} \)

DWA634 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(} \text{spc} P_{\text{yr}}^{-}\text{HCVAH-} \text{gfp}) \), \( \Delta \text{minCD::kan} \)

DWA635 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(} \text{spc} P_{\text{yr}}^{-}\text{HCVAH-} \text{gfp}) \), \( \Delta \text{minCD::kan} \)

DWA636 \( \text{trpC2} \Delta \text{noc::tet}, \Omega \text{amyE::(} \text{spc} P_{\text{yr}}^{-}\text{HCVAH-} \text{gfp}) \), \( \Delta \text{minCD::kan} \)

E. coli

MC1000 \( \Delta(\text{ara-leu}) \Delta lac rpsL150 \)

MC1000\( \Delta \text{minCD} \)

MC1000\( \Delta slmA \)

MG1655 \( \text{Wild-type E. coli K-12} \)

DWA260 \( \text{MG1655: PMG25} \)

DWA261 \( \text{MG1655: pDWA37} \)

DWA266 \( \text{MG1655: pDWA61} \)

DWA267 \( \text{MG1655: pDWA62} \)

DWA270 \( \text{MG1655: pDWA69} \)

DWA271 \( \text{MG1655: pDWA66} \)

DWA272 \( \text{MG1655: pDWA68} \)

DWA334 \( \text{MC1000, pDWA37} \)

DWA335 \( \text{MC1000 \( \Delta slmA::cat, pDWA37 \) \( \Delta minCD::kan \)}} \)

DWA336 \( \text{MC1000 \( \Delta minCD::kan, pDWA37 \)}} \)

DWA337 \( \text{MC1000 \( \Delta slmA, pDWA37 \)}} \)

12
Plasmids

pET16b  
bla lacI P$_{77}$–10xhis  
Novagen  
(Wu et al., 2009)

pSG4924  
bla amyE’ spc P$_{xyr}$-yfp$^{mut1}$ ‘amyE  
(Wu et al., 2009)

pSG4926  
bla amyE’ spc P$_{xyr}$-noc-yfp$^{mut1}$ ‘amyE  
(Wu et al., 2009)

pSG4929  
P$_{spaO}$ 8xNBS(ydbO) erm  
(Quisel et al., 2001)

pPL82  
bla amyE’ cat lacI P$_{spac(ity)}$ ‘amyE  
(Christensen-Dalsgaard et al., 2008)

pMG25  
bla lacI $^{P_{A7}}$/403  
This work

pTS37  
cat P$_{aro}$-gfp-junLZ-EcMinD MTS$^{256–270}$  
tetO; Gm$^{R}$  
(Szeto et al., 2003)

pLAU44  
  
(Lau et al., 2003)

pDWA20  
cat P$_{aro}$-gfp-junLZ-BsNoc MTS$^{1–30}$  
This work

pDWA23  
bla lacI P$_{77}$-noc  
This work

pDWA31  
bla lacI P$_{77}$-noc NA10  
This work

pDWA32  
bla lacI P$_{77}$-noc CA50  
This work

pDWA37  
bla lacI $^{P_{A7}}$/403/noc  
This work

pDWA38  
bla amyE’ spc P$_{xyr}$-HCV AH-nocNA10-yfp ‘amyE  
This work

pDWA40  
bla amyE’ spc P$_{xyr}$-nocAA1–50-myfp ‘amyE  
This work

pDWA41  
bla amyE’ spc P$_{xyr}$-myfp ‘amyE  
This work

pDWA42  
bla amyE’ spc P$_{xyr}$-noc-myfp ‘amyE  
This work

pDWA43  
bla amyE’ spc P$_{xyr}$-nocF5E-yfp$^{mut1}$ ‘amyE  
This work

pDWA44  
bla amyE’ spc P$_{xyr}$-nocR7E-yfp$^{mut1}$ ‘amyE  
This work

pDWA45  
bla amyE’ spc P$_{xyr}$-TM-nocNA10-myfp ‘amyE  
This work

pDWA46  
bla amyE’ cat lacI P$_{spac(ity)}$-nocF5E ‘amyE  
This work

pDWA47  
bla amyE’ cat lacI P$_{spac(ity)}$-nocR7E ‘amyE  
This work

pDWA496  
bla lacI $^{P_{A7}}$/403/noc NA10  
This work

pDWA51  
bla lacI $^{P_{A7}}$/403/noc CA50  
This work

pDWA56  
bla lacI $^{P_{A7}}$/403/spo01(parS-)  
This work

pDWA68  
bla lacI $^{P_{A7}}$/403/noc30-spo01(parS-)  
This work

pDWA69  
bla lacI $^{P_{A7}}$/403/HCV AH-nocNA10  
This work

pDWA74  
bla amyE’ spc P$_{xyr}$-HCV AH-nocNA10 ‘amyE  
This work

pDWA75  
bla amyE’ spc P$_{xyr}$-nocG86S-myfp ‘amyE  
This work

pDWA78  
bla amyE’ cat lacI P$_{spac(ity)}$-nocQ68R ‘amyE  
This work

pDWA79  
bla amyE’ cat lacI P$_{spac(ity)}$-nocNA10 ‘amyE  
This work

pDWA81  
bla amyE’ spc P$_{xyr}$-nocQ68R-myfp ‘amyE  
This work

pDWA82  
bla amyE’ cat lacI P$_{spac(ity)}$-nocG86S ‘amyE  
This work

pDWA91  
bla amyE’ spc P$_{xyr}$-nocK2E-myfp ‘amyE  
This work

pDWA95  
bla amyE’ spc P$_{xyr}$-nocF9E-myfp ‘amyE  
This work

pDWA96  
bla amyE’ spc P$_{xyr}$-nocF5A-myfp ‘amyE  
This work

pDWA97  
bla amyE’ spc P$_{xyr}$-nocF8A-myfp ‘amyE  
This work

pDWA98  
bla amyE’ spc P$_{xyr}$-nocF9A-myfp ‘amyE  
This work

pDWA101  
bla amyE’ spc P$_{xyr}$-nocS4A-myfp ‘amyE  
This work

pDWA102  
bla amyE’ spc P$_{xyr}$-nocS4L-myfp ‘amyE  
This work

pDWA103  
bla amyE’ spc P$_{xyr}$-nocS4L,G86S-myfp ‘amyE  
This work

pDWA104  
bla amyE’ spc P$_{xyr}$-nocNA10-myfp ‘amyE  
This work

pDWA105  
P$_{spaO}$ 8xNBS(ydbO) erm + tetO array  
This work

pDWA140  
bla amyE’ spc P$_{xyr}$-nocR88A-myfp ‘amyE  
This work

pDWA141  
bla amyE’ spc P$_{xyr}$-nocR89A-myfp ‘amyE  
This work

pDWA142  
bla amyE’ spc P$_{xyr}$-nocR91A-myfp ‘amyE  
This work

pDWA144  
bla amyE’ cat lacI P$_{spac(ity)}$-nocR88A ‘amyE  
This work

pDWA145  
bla amyE’ cat lacI P$_{spac(ity)}$-nocR89A ‘amyE  
This work

pDWA146  
bla amyE’ cat lacI P$_{spac(ity)}$-nocR91A ‘amyE  
This work
pDWA147  
bla amyE' spc P<sub>xyl</sub>-nocK164A-myfp 'amyE'  
This work

pSG4922  
bla amyE' cat lacI P<sub>spac(phy)</sub>-noc 'amyE'  
This work

pSG4937  
bla amyE' cat lacI P<sub>spac(phy)</sub>-nocR7A 'amyE'  
This work

pSG4938  
bla amyE' cat lacI P<sub>spac(phy)</sub>-nocK14A 'amyE'  
This work

pSG4939  
bla amyE' cat lacI P<sub>spac(phy)</sub>-nocΔK2 'amyE'  
This work

pSG4940  
bla amyE' cat lacI P<sub>spac(phy)</sub>-nocK2A 'amyE'  
This work

pSG4941  
bla amyE' cat lacI P<sub>spac(phy)</sub>-nocΔF5,S6 'amyE'  
This work

pSG4943  
bla noc::(P<sub>spac(phy)</sub>-noc lacI kan)  
This work

pSG4944  
bla amyE' spc P<sub>xyl</sub>-nocK14A-yfp 'amyE'  
This work

Resistance gene abbreviations: bla, ampicillin; cat, chloramphenicol; erm, erythromycin; kan, kanamycin; spc, spectinomycin; tet, tetracycline.

For strains constructed by transformation, the source of the DNA used in the transformation is given first. The recipient strain is indicated after the arrow, with the selected marker in parentheses: Amp, ampicillin; Cm, chloramphenicol; Em, erythromycin; Km, kanamycin; Sp, spectinomycin; Te, tetracycline.
Supplementary Figures

Supplementary Figure S1

(A-D) CCCP-induced Noc delocalisation is due to the loss of ΔΨ. Cells of strain 4702 expressing Noc-YFP were examined either without additions (A) or 5 min after treatment with Nigericin (5 μM) (B).

(C-D) Effect of CCCP on Noc-YFP localisation in a F1F0 ATP synthase-deficient background. To rule out the possibility that the altered Noc localisation resulted from the drop in cellular ATP levels that occurs upon CCCP treatment (Strahl & Hamoen, 2010) due to the ability of the F1F0 ATP-synthase to function as an ATP-driven proton pump (Hicks et al, 1994), the experiment was repeated in an F1F0 ATP-synthase deficient strain that is able to maintain normal ATP levels when grown in rich medium (Santana et al, 1994; Strahl & Hamoen, 2010). Cells of strain DWA103 (atpB::erm) expressing Noc-YFP were grown in LB + 0.4 % (w/v) glucose and examined either after no additions (C) or 5 min after treatment with CCCP (100 μM) (D). The corresponding phase contrast images are shown below each panel. Scale bar = 5 μm.
Figure S1

Noc-YFP

A

B

Nigericin

C

D

Phase

NA

Nigericin

NA

CCCP

atpB::erm
Supplementary Figure S2

(A) Multiple sequence alignment of Noc homologues. The *B. subtilis* Noc N-terminus was used as a query sequence for BLAST-P against a non-redundant protein database. The top 100 sequences returned were aligned using Clustal Omega. The figure was prepared using Jalview and is coloured according to percentage identity, as depicted in the key. The N-terminus is highlighted in red.

(B) Multiple sequence alignment of Noc and Spo0J homologues. Reference sequences were aligned using Clustal Omega. The figure was prepared using Jalview and is coloured according to percentage identity, as depicted in the key. Species abbreviations: *B. subtilis*, **Bs**; *B. anthracis*, **Ba**; *B. cereus*, **Bc**; *B. amyloliquefaciens*, **Bam**; *B. licheniformis*, **Bl**.
Figure S2

A  N-terminus

B

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<th>Noc</th>
<th>Bs</th>
<th>MKHSFSRFGLGKEQEEP</th>
<th>AEHDTNKREELETPVNAIVPNRFQPR</th>
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<td>Ba</td>
<td>MKNTFSRLFGDKESEFELQDSEHEIDKVVYEEEQEIPIVNITPNRYQPR</td>
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<tr>
<td>Bc</td>
<td>MKNTFSRLFGDKESEFELQDSEHEIDKVVYEEIQEIEPVNITPNRYQPR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bam</td>
<td>MKHSFSRFGLGKEEPEI</td>
<td>AEHDTNKREEILEIPVHAIVPNRFQPR</td>
<td></td>
</tr>
<tr>
<td>Bl</td>
<td>MKHSFSRLFGDKEEAEI</td>
<td>AEHDTNKREEIQEIEPVGDIIPNRFQPR</td>
<td></td>
</tr>
<tr>
<td>Bs</td>
<td>MAK---GGLKGINA-----LFNQVDLSEETVEEIKAIDLRPNPYQPR</td>
<td></td>
<td></td>
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<tr>
<td>Ba</td>
<td>MAK---GLGRINV------FPDLDVKEEEETVEIEVITELRPNPYQPR</td>
<td></td>
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<tr>
<td>Bc</td>
<td>MAK---GLGRINV------FPDLDVKEEEITQIEILIETERPNPYQPR</td>
<td></td>
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</tr>
<tr>
<td>Bam</td>
<td>MAKG---GLKGINA------LFNQVDLSEETVEEIKISDLRPNPYQPR</td>
<td></td>
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<tr>
<td>Bl</td>
<td>MPK---GLKGINA------LFSNVDLSEETVEDEKLQDLRPNPYQPR</td>
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> 80%
> 60%
> 40%
< 40%
Supplementary Figure S3

Size-exclusion chromatography of purified Noc, and NocNΔ10 and NocCΔ50 truncated proteins. Proteins were run on a calibrated Superdex 200 10/300 GL gel filtration column (GE Healthcare) and approximate molecular weights were estimated by comparison to a set of known protein standards (See Supplementary Methods). The apparent molecular weights were as follows with the theoretical values in brackets: Noc 162.3 kDa (monomer, 32.8 kDa; dimer, 65.6 kDa; tetramer, 131.2 kDa), NocNΔ10 172.6 kDa (monomer, 31.6 kDa; dimer, 63.2 kDa; tetramer, 126.4 kDa) and NocCΔ50 38.9 kDa (monomer, 26.9 kDa; dimer, 53.8 kDa). The standard curve used to calibrate the column is shown below the elution profile.
Figure S3

Retention volume (ml) vs. Normalised Intensity (mAU)

- Noc
- NocNΔ10
- NocCΔ50

$R^2 = 0.9983$

Log$_{10}$ Mr vs. $K_{av}$

- Aprotinin
- RNAse A
- Carbonic anhydrase
- Ovalbumin
- Conalbumin
- Aldolase
- Ferritin

$R^2 = 0.9983$
Supplementary Figure S4

(A) Histogram showing the effects of overproducing Noc mutants on cell length. Exponentially growing cells of strains 168 (WT), DWA117 (Δnoc), 119 (Δnoc, P_{spac(hy)}^{+}noc), 282 (Δnoc, P_{spac(hy)}^{+}nocNΔ10), 226 (Δnoc, P_{spac(hy)}^{+}nocF5E) and 227 (Δnoc, P_{spac(hy)}^{+}nocR7E) were examined after growth for 90 min in the presence of 1 mM IPTG. Cell membranes were stained with FM5-95 and used to measure cell length (WT, n =322; Δnoc, n =508; Noc, n =395; NΔ10, n =551; F5E, n =505; R7E, n =484).

(B) Effects of Noc mutants on sporulation. Strains DWA119 (Δnoc, P_{spac(hy)}^{+}noc), 343 (Δnoc, P_{spac(hy)}^{+}nocΔK2), 344 (Δnoc, P_{spac(hy)}^{+}nocK2A), 226 (Δnoc, P_{spac(hy)}^{+}nocF5E), 346 (Δnoc, P_{spac(hy)}^{+}nocΔF5, S6), 347 (Δnoc, P_{spac(hy)}^{+}nocR7A), 227 (Δnoc, P_{spac(hy)}^{+}nocR7E) and 348 (Δnoc, P_{spac(hy)}^{+}nocK14A) were grown on nutrient agar plates in the absence and presence of 1 mM IPTG, as indicated. Plates were photographed after growth for 48 h at 37 °C.
Supplementary Figure S5

(A) HCV AH-GFP is unable to rescue the growth defect of Δnoc ΔminCD at 39 °C. Strains DWA564 (Noc-mYFP) and DWA629 (HCV AH-GFP) were streaked on plates with either no additions (NA) or 0.5 % w/v xylose, as indicated, and photographed after incubation for 18 h at 39 °C.

(B) HCV AH-NocNΔ10-YFP rescues the growth defect of Δnoc ΔminCD at 39 °C. Strains DWA564 (Noc-mYFP) and DWA636 (HCV AH-NocNΔ10-YFP) were streaked on plates with either no additions (NA) or 0.5 % w/v xylose, as indicated, and photographed after incubation for 18 h at 39 °C.

(C) TM-NocNΔ10 is unable to rescue the growth defect of Δnoc ΔminCD at 39 °C. Strains DWA564 (Noc-mYFP) and DWA626 (TM-NocNΔ10-mYFP) were streaked on plates with either no additions (NA) or 0.5 % w/v xylose, as indicated, and photographed after incubation for 18 h at 39 °C.

(D) Production of TM-NocNΔ10 often leads to multiple defects in chromosome segregation. Exponentially growing cells of strain DWA548 (Δnoc, P_xyl-TM-nocNΔ10-mYFP) were cultured in the presence of 1 % w/v xylose and observed 2 h post-induction. Arrow-heads indicate some of the DNA-damage events. Cell membranes and DNA were stained with FM5-95 and DAPI, respectively. The insets show the corresponding phase contrast images. Scale bar = 5 μm.
Figure S5

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Scale bar: 10 μm
**Supplementary Figure S6**

(A-B) Localisation of GFP with a JunLZ dimerisation domain fused to either the amphipathic helix of *E. coli* MinD (pTS37) (Positive-control) (A), or the N-terminal 30 amino acids of Noc (pDWA20) (B). Constructs were expressed in *E. coli* DH5α by the inclusion of 0.2 % w/v arabinose in the media.

(C) Localisation in *B. subtilis* cells of a fusion between the N-terminal 50 amino acids of Noc and mYFP (Strain DWA225). The cartoons below each panel show a schematic of the relevant product.

(D) Western blots showing that the GFP-JunLZ-MinD (35 kDa) and GFP-JunLZ-Noc30 (36 kDa) fusions are intact and produced at comparable levels in *E. coli*. Exponentially growing cells were grown with (+) and without (-) induction (0.2 % w/v arabinose), as indicated, for 60 min at 37 °C, before being recovered and processed. Primary antibodies were used at a dilution of 1:20,000 (αNoc) and 1:10,000 (αGFP). The * denotes a non-specific band. The ** denotes limited degradation of the GFP-JunLZ-Noc30 protein.

(E) Western blots showing that the intact Noc50-mYFP (35.4 kDa) fusion is produced in *B. subtilis* (DWA215). Total protein from strains 168 (WT) and DWA117 (Δnoc) were included on the blot to facilitate size comparisons; Noc, 32.8 kDa. Exponentially growing cells were grown with induction (0.5 % w/v xylose) for 60 min at 37 °C, before being recovered and processed. Primary antibodies were used at a dilution of 1:20,000 (αNoc) and 1:10,000 (αGFP).
Figure S6

E. coli

A. MinD

B. Noc30

C. Noc50-mYFP

B. subtilis

D. αGFP

E. αNoc

Δnoc 168

Δnoc 168
Supplementary Figure S7

(A) Multiple sequence alignment of Noc and Spo0J homologues showing the conserved ParB boxes. Reference sequences were aligned using Clustal Omega. The figure was prepared using Jalview and is coloured according to percentage identity, the colour scheme is the same as in Supplementary FigS2. Species abbreviations: B. subtilis, Bs; B. anthracis, Ba; B. cereus, Bc; B. amyloliquefaciens, Bam; B. licheniformis, Bl. The Noc residues examined in this study are highlighted in red and labelled, with the equivalent B. subtilis Spo0J residue number shown below in brackets.

(B) Non-functional ParB-box mutants are dominant-negative. Overproduction of Noc Q68R, G86S, R88A or R89A in a noc ΔminCD background prevents growth at 42 °C but not at 30 °C. Strains encoding the indicated Pspac(hy)- driven noc alleles were streaked on plates containing 1 mM IPTG and incubated at the indicated temperature for 18 h before being photographed. Strains used: DWA362 (WT Noc), DWA363 (NΔ10), DWA364 (Q68R), DWA365 (G86S), DWA559 (R88A), DWA560 (R89A), and DWA561 (R91A).

(C) Western blots showing the relative levels of Noc proteins (αNoc) produced under the control of the Pspac(hy) promoter. All proteins are intact and are expressed at comparable levels. FtsZ levels are shown to control for sample loading. Exponentially growing cells were grown with inducer (1 mM IPTG) for 60 min at 37 °C, before being recovered and processed. Primary antibodies were used at a dilution of 1:20,000 (αNoc) and 1:10,000 (αFtsZ). Strains used: 168 (WT parent), DWA117 (Δnoc), DWA119 (Noc), DWA282 (NΔ10), DWA283 (Q68R), DWA284 (G86S), DWA551 (R88A), DWA552 (R89A) and DWA553 (R91A).

(D-G) Effect of Noc overproduction (Pspac(hy)-noc) on the localisation of NocNΔ10-YFP and NocG86S-mYFP. Cells of strains DWA370 (NocNΔ10-YFP) and DWA371 (NocG86S-mYFP) were examined after growth for 1 h in the absence (D and E) and presence (F and G) of 1 mM IPTG. Xylose (0.5 % w/v) was included to induce the expression of the YFP fusions. Insets show the corresponding phase contrast images. Scale bar = 5 μm.
Figure S7

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Noc: NocB

Spo0J: Spo0J

B

- Noc
- Spo0J

C

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D, F

Xylose only

E, G

Xylose & IPTG

$P_{xyl}$'mutant'-YFP, $P_{spac(hy)}$-WT

Bar scale
Supplementary Figure S8

Strains DWA78 ($P_{spac\cdot noc} + pSG4929$) (A and B) and DWA429 ($P_{spac\cdot noc} + pDWA117$) (C and D) were grown in LB at 37 °C and examined after growth for 90 min either without further additions (A and C) or after induction with 0.1 mM IPTG (B and D). Cell membranes and DNA were stained with FM5-95 and DAPI, respectively. Insets show the corresponding phase contrast images. Scale bar = 5 μm.

(E) Toxicity of Noc variants in the presence of the NBS plasmid pSG4929. Strains carrying pSG4929 and encoding the indicated $P_{spac(hy)}$-driven noc alleles were streaked on plates with either no additions (NA) or containing 1 mM IPTG, as indicated. Strains used: DWA523 (WT), DWA524 (NΔ10), DWA525 (Q68R), DWA526 (G86S), DWA623 (R88A), DWA624 (R89A), and DWA625 (R91A). Plates were incubated at 37 °C for 18 h before being photographed.
Figure S8

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DWA429

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Supplementary Figure S9

Western blots using αNoc (A) or αSpo0J (B) showing the relative levels of the indicated proteins produced in *E. coli* MG1655. Exponentially growing cells were grown with induction (0.5 mM IPTG) for 30 min at 37 °C, before being recovered and processed. Primary antibodies were used at a dilution of 1:20,000. Strains used: DWA260 (Empty vector), DWA261 (Noc), DWA266 (NocΔ10), DWA267 (NocΔ50), DWA271 (Spo0J) and DWA272 (Noc-Spo0J). Lanes labelled with an asterisk contain proteins that were not used in this study.
Figure S9

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αNoc

B

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αSpo0J
Supplementary Figure S10

Cells of strains DWA334 (MC1000 parent), DWA337 (ΔsulA), DWA335 (ΔslmA) and DWA336 (ΔminCD) carrying plasmid pDWA37 (P_{34/04/03}-noc) were examined after growth for 1 h in the absence (A, C, E, and G) and presence (B, D, F, and H) of 1 mM IPTG. Cell membranes and DNA were stained with FM5-95 and DAPI, respectively. Insets show the corresponding phase contrast images. Scale bar = 5 μm.
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Supplementary Figure S11

Western blots showing the relative levels of Noc-YFP fusions (αNoc). All fusion proteins are intact and are produced at comparable levels (except S4L/G86S). FtsZ levels are shown to control for sample loading. Exponentially growing cells were grown with inducer (0.5 % w/v xylose) for 120 min at 37 °C, before being recovered and processed. Primary antibodies were used at a dilution of 1:20,000 (αNoc) and 1:10,000 (αFtsZ). Strains used: DWA206 (WT Noc-mYFP), DWA211 (F5E-YFP), DWA212 (R7E-YFP), DWA285 (Q68R-mYFP), DWA286 (G86S-mYFP), DWA316 (K2E-mYFP), DWA318 (F9E-mYFP), DWA322 (F5A-mYFP), DWA323 (F8A-mYFP), DWA325 (F9A-mYFP), DWA328 (S4A-mYFP), DWA329 (S4L-mYFP), DWA349 (S4L/G86S-mYFP), DWA382 (NΔ10-mYFP), DWA545 (R88A-mYFP), DWA546 (R89A-mYFP), DWA547 (R91A-mYFP), DWA606 (K164A-mYFP) and DWA634 (K14A-YFP).
Movie legends

Movies 1-3

Time-lapses showing localisation of Noc-mYFP (Movies 1 and 2) and NocNΔ10-mYFP (Movie 3) in strains DWA206 and 382 using TIRF microscopy. Cells were grown in CH + 0.5 % (w/v) xylose and examined either without further additions (Movies 1 and 3) or after treatment for 5 min with 100 μM CCCP (Movie 2). TIRF images were acquired automatically every 500 ms for c.a. 30 s. Images were scaled identically and the corresponding ‘zero-angle’ images are shown above each panel. Note the absence of NocNΔ10-mYFP signal from the cell surface in Movie 3.

Movies 4 and 5

Time-lapses showing localisation of plasmid DNA (TetR-mCherry) in strain DWA429 (Pxyt-tetR-mCherry, Pspac-noc + pDWA117). Cells were grown in competence medium + 0.5 % (w/v) xylose and examined either without further additions (Movie 4) or after induction for 2 hours with 1 mM IPTG (Movie 5). Images were acquired automatically every 10 s for 60 s. The corresponding phase-contrast image is shown above each panel. Scale bars = 5 μm.
Supplementary References


