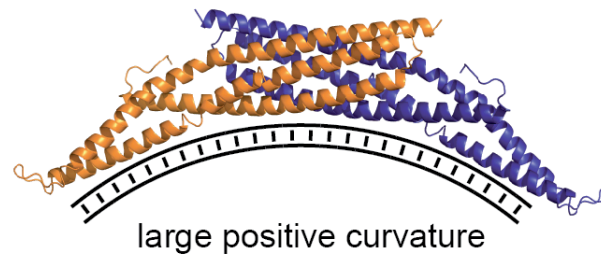


Supplementary data and figures

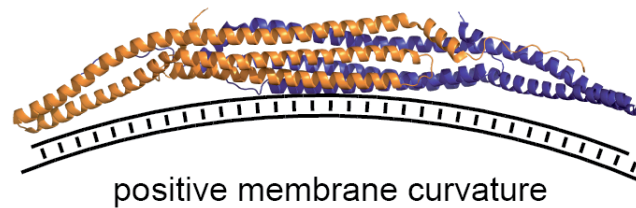
"Features critical for membrane binding revealed by DivIVA crystal structure", Oliva et al., 2010

Curvature sensing BAR domains

Amphiphysin BAR (PDB 1URU)



FCHo2 F-BAR (PDB 2V0O)



IRSp53 I-BAR (PDB 1WDZ)

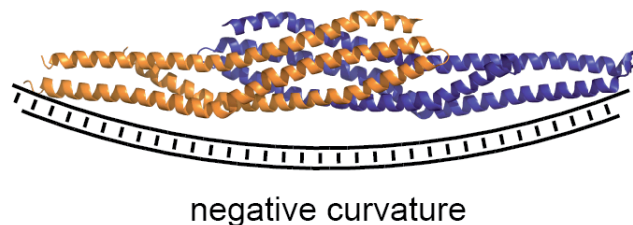


Figure S1: Is DivIVA related to eukaryotic BAR domains? Shown are three representative crystal structures of eukaryotic BAR-like domains. Amphiphysin bends membranes strongly and inserts an amphipathic helix into the membrane for doing so (Gallop et al., 2006; Peter et al., 2004). F-BAR domains form lipid tubes with larger diameters (Henne et al., 2007) and I-BAR domains bind to membranes with negative curvature (such as the inside of liposomes).

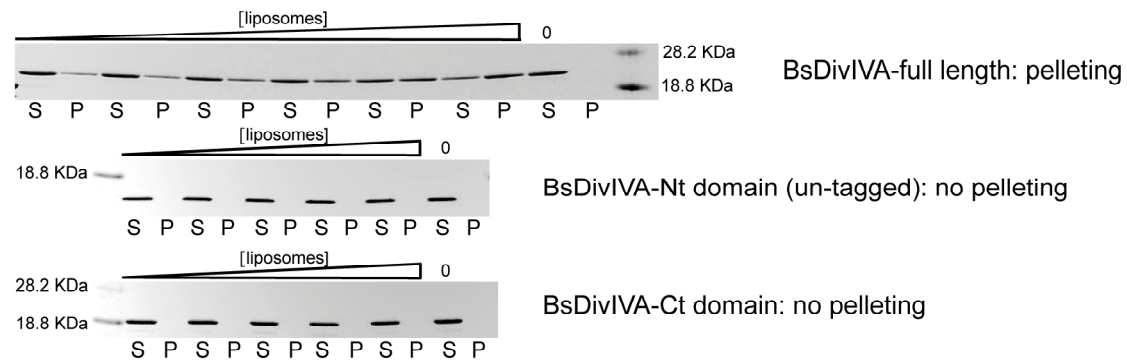


Figure S2: Spin assays showing membrane interaction of DivIVA to require full-length protein (top). Neither the N- (middle) nor the C-terminal domain (bottom) spin down with liposomes. This is in contrast to the sucrose-gradient assay shown in Figure 4E, bottom and the polar localisation of the N-terminal domain in *E. coli* (Figure 4E, top). The difference between those experiments is that here completely un-tagged protein was used that is only dimeric. The GFP-tag on the previous constructs might induce tetramerisation that may support localisation and lipid binding as demonstrated (GFP somehow mimicking the role of the C-terminal domain of DivIVA).

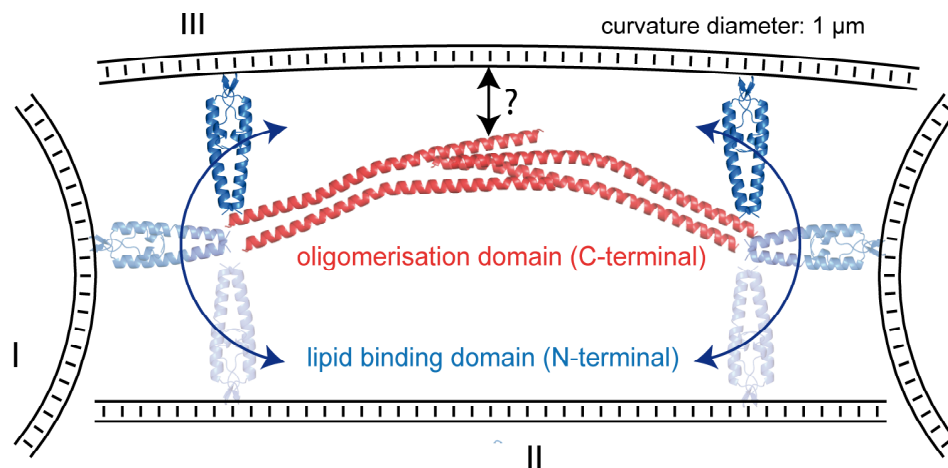


Figure S3: Some DivIVA membrane binding models. The molecule contains two membrane-interaction sites (each containing two hydrophobic residues inserting into the membrane and at least two positively charged residues for counteracting the negative charge of the membrane surface, as explained in Figures 6AB and hence can cross-link membranes (model I). If the N-terminal domain can swing around, the molecule would be able to bind to many different membrane shapes (II and III) and specificity for negative curvature, as reported previously (Lenarcic et al., 2009), would have to come from higher-order oligomers and their arrangement (this is the 'molecular bridging model' (Lenarcic et al., 2009)). If, like for BAR domains, the C-terminal domain also contains membrane-interacting residues (charged, binding to lipid head groups), specificity for certain membrane curvatures arises because of the shape of the molecule, as has been shown for BAR domains (Supplementary Figure S1). Oligomerisation of DivIVA would then strengthen this interaction (Lenarcic et al., 2009). The diameter of the negative curvature shown in III is 1 μm , representing a cell, obviously being much smaller than the curvature of DivIVA. Also shown to scale in Supplementary Figure S4.

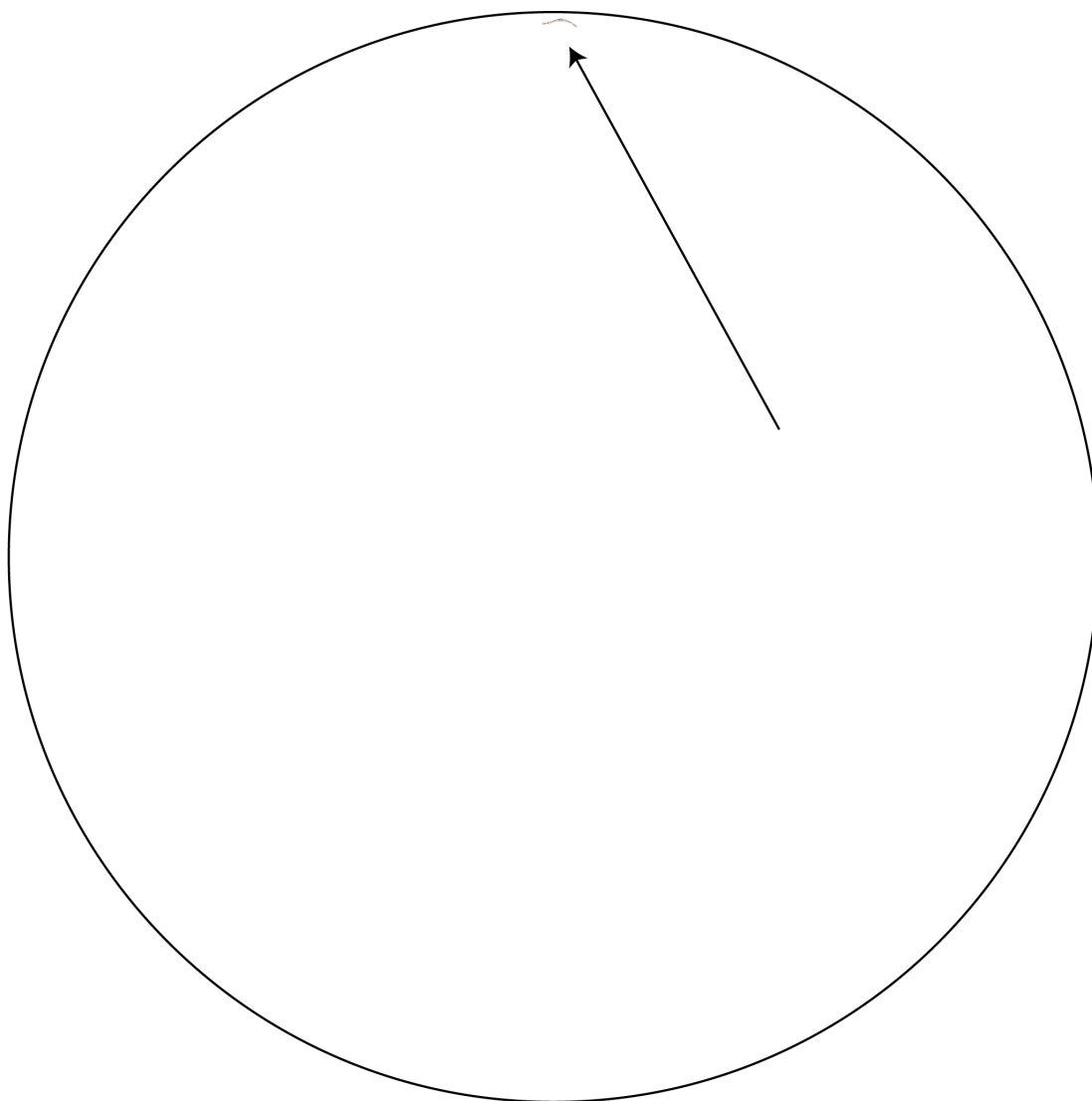


Figure S4: To-scale representation of the size of a bacterial cell and DivIVA. The cell is drawn with a diameter of 1000 nm and DivIVA has a length of 30 nm when maximally extended as determined in this study.

Table S5: Effects of amino acid exchanges in the N-terminal DivIVA domain on activity, localisation and dimerisation.

mutation	phenotype		localisation	N-terminal domain dimerisation*
	cell length	sporulation		
<i>tail</i>				
I8A	filamentous	-	inclusion bodies	-
K11A	short	+	spotty	n. d.
F13A	filamentous	-	inclusion bodies	-
S16G	short	+	wild type	n. d.
<i>crossed loops</i>				
F17G	filamentous	-	diffuse	wild type
F17A	filamentous	-	diffuse	wt, X-ray structure
F17V	filamentous	-	diffuse	n. d.
F17V E22K	short	+	wild type	n. d.
R18A	filamentous	-	regular spots	n. d.
G19A	filamentous	-	diffuse	n. d.
Y20A	intermediate	+	diffuse	n. d.
<i>coiled coil</i>				
D21A	short	+	wild type	n. d.
E22G	n. d.	n. d.	spotty	n. d.
E24A	short	+	spotty	n. d.
V25A	filamentous	-	inclusion bodies	-
F28A	filamentous	-	inclusion bodies	n. d.
L29A	filamentous	-	inclusion bodies	-
V32A	short	+	diffuse	n. d.
D35A	short	+	spotty	n. d.
E37A	short	+	wild type	n. d.
V39A	short	+	wild type	n. d.

* n. d. – not determined.

Table S6: *B. subtilis* strains and plasmids used in this study.

name	relevant characteristics	source/ reference
<i>Bacillus subtilis</i> strains		
168	<i>trpC2</i>	lab collection
4041	<i>trpC2</i> Δ divIVA::tet	L. Hamoen, unpublished
4067	<i>trpC2 amyE::</i> (Pxyl-divIVA-gfp <i>spc</i>) Δ divIVA::tet	L. Hamoen, unpublished
BSN5	<i>trpC2 aprE::</i> (Pspac-divIVA) Δ divIVA::tet	this work
BSN38	<i>trpC2 aprE::</i> (Pspac-divIVAF17V <i>spc</i>) Δ divIVA::tet	this work
BSN54	<i>trpC2 aprE::</i> (Pspac-divIVAF17V <i>spc::neo amyE::</i> (Pxyl-divIVAF17V-gfpA206K <i>spc</i>) Δ divIVA::tet	this work
BSN57	<i>trpC2 aprE::</i> (Pspac-divIVAF17V E22K <i>spc::neo amyE::</i> (Pxyl-divIVAF17V-gfpA206K <i>spc</i>) Δ divIVA::tet	this work
BSN76	<i>trpC2 aprE::</i> (Pspac-divIVAF17V E22K <i>spc</i>) Δ divIVA::tet	this work
BSN224	<i>trpC2 amyE::</i> (Pxyl-divIVAF17V-gfpA206K <i>spc</i>) Δ divIVA::tet	this work
BSN225	<i>trpC2 amyE::</i> (Pxyl-divIVAF17VE22K-gfpA206K <i>spc</i>) Δ divIVA::tet	this work
PD44	<i>trpC2 amyE::</i> (Pxyl-divIVAI8A-gfp <i>spc</i>) Δ divIVA::tet	this work
PD45	<i>trpC2 amyE::</i> (Pxyl-divIVAK11A-gfpA206K <i>spc</i>) Δ divIVA::tet	this work
PD46	<i>trpC2 amyE::</i> (Pxyl-divIVAF13A-gfpA206K <i>spc</i>) Δ divIVA::tet	this work
PD47	<i>trpC2 amyE::</i> (Pxyl-divIVAS16G-gfp <i>spc</i>) Δ divIVA::tet	this work
PD48	<i>trpC2 amyE::</i> (Pxyl-divIVAF17G-gfp <i>spc</i>) Δ divIVA::tet	this work
BSN235	<i>trpC2 amyE::</i> (Pxyl-divIVAF17A-gfp <i>spc</i>) Δ divIVA::tet	this work
BSN108	<i>trpC2 amyE::</i> (Pxyl-divIVAR18A-gfpA206K <i>spc</i>) Δ divIVA::tet	this work
BSN106	<i>trpC2 amyE::</i> (Pxyl-divIVAG19A-gfpA206K <i>spc</i>) Δ divIVA::tet	this work
PD49	<i>trpC2 amyE::</i> (Pxyl-divIVAY20A-gfpA206K <i>spc</i>) Δ divIVA::tet	this work
PD50	<i>trpC2 amyE::</i> (Pxyl-divIVAD21A-gfpA206K <i>spc</i>) Δ divIVA::tet	this work
PD51	<i>trpC2 amyE::</i> (Pxyl-divIVAE22G-gfp <i>spc</i>) Δ divIVA::tet	this work
PD52	<i>trpC2 amyE::</i> (Pxyl-divIVAE24A-gfp <i>spc</i>) Δ divIVA::tet	this work
PD53	<i>trpC2 amyE::</i> (Pxyl-divIVAV25A-gfp <i>spc</i>) Δ divIVA::tet	this work
PD54	<i>trpC2 amyE::</i> (Pxyl-divIVAF28A-gfp <i>spc</i>) Δ divIVA::tet	this work
PD55	<i>trpC2 amyE::</i> (Pxyl-divIVAL29A-gfp <i>spc</i>) Δ divIVA::tet	this work
PD57	<i>trpC2 amyE::</i> (Pxyl-divIVAD35A-gfp <i>spc</i>) Δ divIVA::tet	this work
PD58	<i>trpC2 amyE::</i> (Pxyl-divIVAE37A-gfpA206K <i>spc</i>) Δ divIVA::tet	this work
PD59	<i>trpC2 amyE::</i> (Pxyl-divIVAV39A-gfp <i>spc</i>) Δ divIVA::tet	this work
PD74	<i>trpC2 aprE::</i> (Pspac-divIVAI8A <i>spc</i>) Δ divIVA::tet	this work
PD75	<i>trpC2 aprE::</i> (Pspac-divIVAK11A <i>spc</i>) Δ divIVA::tet	this work
PD76	<i>trpC2 aprE::</i> (Pspac-divIVAF13A <i>spc</i>) Δ divIVA::tet	this work
PD77	<i>trpC2 aprE::</i> (Pspac-divIVAS16G <i>spc</i>) Δ divIVA::tet	this work
PD78	<i>trpC2 aprE::</i> (Pspac-divIVAF17G <i>spc</i>) Δ divIVA::tet	this work
BSN234	<i>trpC2 aprE::</i> (Pspac-divIVAF17A <i>spc</i>) Δ divIVA::tet	this work
BSN97	<i>trpC2 aprE::</i> (Pspac-divIVAR18A <i>spc</i>) Δ divIVA::tet	this work
BSN95	<i>trpC2 aprE::</i> (Pspac-divIVAG19A <i>spc</i>) Δ divIVA::tet	this work
PD79	<i>trpC2 aprE::</i> (Pspac-divIVAY20A <i>spc</i>) Δ divIVA::tet	this work
PD80	<i>trpC2 aprE::</i> (Pspac-divIVAD21A <i>spc</i>) Δ divIVA::tet	this work
PD82	<i>trpC2 aprE::</i> (Pspac-divIVAE24A <i>spc</i>) Δ divIVA::tet	this work
PD83	<i>trpC2 aprE::</i> (Pspac-divIVAV25A <i>spc</i>) Δ divIVA::tet	this work
PD84	<i>trpC2 aprE::</i> (Pspac-divIVAF28A <i>spc</i>) Δ divIVA::tet	this work
PD85	<i>trpC2 aprE::</i> (Pspac-divIVAL29A <i>spc</i>) Δ divIVA::tet	this work
PD86	<i>trpC2 aprE::</i> (Pspac-divIVAV32A <i>spc</i>) Δ divIVA::tet	this work
PD87	<i>trpC2 aprE::</i> (Pspac-divIVAD35A <i>spc</i>) Δ divIVA::tet	this work
PD88	<i>trpC2 aprE::</i> (Pspac-divIVAE37A <i>spc</i>) Δ divIVA::tet	this work
PD89	<i>trpC2 aprE::</i> (Pspac-divIVAV39A <i>spc</i>) Δ divIVA::tet	this work
plasmids		
pAPNC213	<i>bla aprE5' spc lacI aprE3'</i>	Morimoto et al., 2002
pDG9	<i>bla amyE3' spc Pxyl-divIVA-gfp amyE5'</i>	this work
pDG15	<i>bla amyE3' spc Pxyl-divIVA¹⁻¹⁸⁰-gfp amyE5'</i>	Lenarcic et al.
pET11a	<i>bla lacI PT7</i>	Novagen
pSH3	<i>bla amyE3' spc Pxyl-divIVA-gfpA206K amyE5'</i>	this work
pSH105	<i>bla lacI PT7-gfp-strep</i>	this work

name	relevant characteristics	source/ reference
pVK73	<i>bla</i> <i>spc::neo</i>	Chary et al., 1997
pUT18	<i>bla</i> <i>Plac mcs-cyaA(T18)</i>	Karimova et al., 1998
pUT18C	<i>bla</i> <i>Plac cyaA(T18)-mcs</i>	Karimova et al., 1998
pKT25	<i>kan</i> <i>Plac cyaA(T25)-mcs</i>	Karimova et al., 1998
p25N	<i>kan</i> <i>Plac mcs-cyaA(T25)</i>	Claessen et al., 2008
pUT18- <i>divIVA</i>	<i>bla</i> <i>Plac divIVA-cyaA(T18)</i>	Lenarcic et al., 2009
pUT18C- <i>divIVA</i>	<i>bla</i> <i>Plac cyaA(T18)-divIVA</i>	Bramkamp et al., 2008
pKT25- <i>divIVA</i>	<i>kan</i> <i>Plac cyaA(T25)-divIVA</i>	Bramkamp et al., 2008
p25N- <i>divIVA</i>	<i>kan</i> <i>Plac divIVA-cyaA(T25)</i>	Lenarcic et al., 2009
pPD4	<i>bla</i> <i>amyE3' spc Pxyl-divIVAI8A-gfp amyE5'</i>	this work
pPD5	<i>bla</i> <i>amyE3' spc Pxyl-divIVAK11A-gfpA206K amyE5'</i>	this work
pPD6	<i>bla</i> <i>amyE3' spc Pxyl-divIVAF13A-gfpA206K amyE5'</i>	this work
pPD7	<i>bla</i> <i>amyE3' spc Pxyl-divIVAS16G-gfp amyE5'</i>	this work
pPD8	<i>bla</i> <i>amyE3' spc Pxyl-divIVAF17G-gfp amyE5'</i>	this work
pSH203	<i>bla</i> <i>amyE3' spc Pxyl-divIVAF17A-gfp amyE5'</i>	this work
pSH17	<i>bla</i> <i>amyE3' spc Pxyl-divIVAF17V-gfpA206K amyE5'</i>	this work
pSH152	<i>bla</i> <i>amyE3' spc Pxyl-divIVAF17VE22K-gfpA206K amyE5'</i>	this work
pSH52	<i>bla</i> <i>amyE3' spc Pxyl-divIVAR18A-gfpA206K amyE5'</i>	this work
pSH50	<i>bla</i> <i>amyE3' spc Pxyl-divIVAG19A-gfpA206K amyE5'</i>	this work
pPD9	<i>bla</i> <i>amyE3' spc Pxyl-divIVAY20A-gfpA206K amyE5'</i>	this work
pPD10	<i>bla</i> <i>amyE3' spc Pxyl-divIVAD21A-gfpA206K amyE5'</i>	this work
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pPD13	<i>bla</i> <i>amyE3' spc Pxyl-divIVAV25A-gfp amyE5'</i>	this work
pPD14	<i>bla</i> <i>amyE3' spc Pxyl-divIVAF28A-gfp amyE5'</i>	this work
pPD15	<i>bla</i> <i>amyE3' spc Pxyl-divIVAL29A-gfp amyE5'</i>	this work
pPD17	<i>bla</i> <i>amyE3' spc Pxyl-divIVAD35A-gfp amyE5'</i>	this work
pPD18	<i>bla</i> <i>amyE3' spc Pxyl-divIVAE37A-gfpA206K amyE5'</i>	this work
pPD19	<i>bla</i> <i>amyE3' spc Pxyl-divIVAV39A-gfp amyE5'</i>	this work
pSH2	<i>bla</i> <i>aprE5' Pspac-divIVA spc lacI aprE3'</i>	this work
pPD24	<i>bla</i> <i>aprE5' Pspac-divIVAI8A spc lacI aprE3'</i>	this work
pPD25	<i>bla</i> <i>aprE5' Pspac-divIVAK11A spc lacI aprE3'</i>	this work
pPD26	<i>bla</i> <i>aprE5' Pspac-divIVAF13A spc lacI aprE3'</i>	this work
pPD27	<i>bla</i> <i>aprE5' Pspac-divIVAS16G spc lacI aprE3'</i>	this work
pPD28	<i>bla</i> <i>aprE5' Pspac-divIVAF17G spc lacI aprE3'</i>	this work
pSH202	<i>bla</i> <i>aprE5' Pspac-divIVAF17A spc lacI aprE3'</i>	this work
pSH9	<i>bla</i> <i>aprE5' Pspac-divIVAF17V spc lacI aprE3'</i>	this work
pSH35	<i>bla</i> <i>aprE5' Pspac-divIVAF17V E22K spc lacI aprE3'</i>	this work
pSH47	<i>bla</i> <i>aprE5' Pspac-divIVAR18A spc lacI aprE3'</i>	this work
pSH45	<i>bla</i> <i>aprE5' Pspac-divIVAG19A spc lacI aprE3'</i>	this work
pPD29	<i>bla</i> <i>aprE5' Pspac-divIVAY20A spc lacI aprE3'</i>	this work
pPD30	<i>bla</i> <i>aprE5' Pspac-divIVAD21A spc lacI aprE3'</i>	this work
pPD32	<i>bla</i> <i>aprE5' Pspac-divIVAE24A spc lacI aprE3'</i>	this work
pPD33	<i>bla</i> <i>aprE5' Pspac-divIVAV25A spc lacI aprE3'</i>	this work
pPD34	<i>bla</i> <i>aprE5' Pspac-divIVAF28A spc lacI aprE3'</i>	this work
pPD35	<i>bla</i> <i>aprE5' Pspac-divIVAL29A spc lacI aprE3'</i>	this work
pPD36	<i>bla</i> <i>aprE5' Pspac-divIVAV32A spc lacI aprE3'</i>	this work
pPD37	<i>bla</i> <i>aprE5' Pspac-divIVAD35A spc lacI aprE3'</i>	this work
pPD38	<i>bla</i> <i>aprE5' Pspac-divIVAE37A spc lacI aprE3'</i>	this work
pPD39	<i>bla</i> <i>aprE5' Pspac-divIVAV39A spc lacI aprE3'</i>	this work
pSH92	<i>bla</i> <i>Plac divIVA¹⁻¹⁸³-cyaA(T18)</i>	this work
pSH93	<i>bla</i> <i>Plac cyaA(T18)-divIVA¹⁻¹⁸³</i>	this work
pSH94	<i>kan</i> <i>Plac cyaA(T25)-divIVA¹⁻¹⁸³</i>	this work
pSH95	<i>kan</i> <i>Plac divIVA¹⁻¹⁸³-cyaA(T25)</i>	this work
pSH139	<i>bla</i> <i>lacI PT7-divIVA(1-60)-gfp-strep</i>	this work
pSH144	<i>bla</i> <i>amyE3' spc Pxyl-divIVA¹⁻¹⁸⁰F17V-gfp amyE5'</i>	this work
pSH150	<i>bla</i> <i>amyE3' spc Pxyl-divIVA¹⁻¹⁸⁰E22K-gfp amyE5'</i>	this work
pSH151	<i>bla</i> <i>amyE3' spc Pxyl-divIVA¹⁻¹⁸⁰F17V E22K-gfp amyE5'</i>	this work
pSH153	<i>bla</i> <i>Plac divIVA¹⁻¹⁸³V25A-cyaA(T18)</i>	this work

name	relevant characteristics	source/ reference
pSH154	<i>bla Plac cyaA(T18)-divIVA¹⁻¹⁸³V25A</i>	this work
pSH155	<i>kan Plac cyaA(T25)-divIVA¹⁻¹⁸³V25A</i>	this work
pSH156	<i>kan Plac divIVA¹⁻¹⁸³V25A-cyaA(T25)</i>	this work
pSH157	<i>bla Plac divIVA¹⁻¹⁸³L29A-cyaA(T18)</i>	this work
pSH158	<i>bla Plac cyaA(T18)-divIVA¹⁻¹⁸³L29A</i>	this work
pSH159	<i>kan Plac cyaA(T25)-divIVA¹⁻¹⁸³L29A</i>	this work
pSH160	<i>kan Plac divIVA¹⁻¹⁸³L29A-cyaA(T25)</i>	this work
pSH161	<i>bla lacI PT7-divIVA(1-60)F17V-gfp-strep</i>	this work
pSH162	<i>bla lacI PT7-divIVA(1-60)F17V E22K-gfp-strep</i>	this work
pSH168	<i>bla Plac divIVA¹⁻¹⁸³I8A-cyaA(T18)</i>	this work
pSH169	<i>bla Plac cyaA(T18)-divIVA¹⁻¹⁸³I8A</i>	this work
pSH170	<i>kan Plac cyaA(T25)-divIVA¹⁻¹⁸³I8A</i>	this work
pSH171	<i>kan Plac divIVA¹⁻¹⁸³I8A-cyaA(T25)</i>	this work
pSH172	<i>bla Plac divIVA¹⁻¹⁸³F13A-cyaA(T18)</i>	this work
pSH173	<i>bla Plac cyaA(T18)-divIVA¹⁻¹⁸³F13A</i>	this work
pSH174	<i>kan Plac cyaA(T25)-divIVA¹⁻¹⁸³F13A</i>	this work
pSH175	<i>kan Plac divIVA¹⁻¹⁸³F13A-cyaA(T25)</i>	this work
pSH176	<i>bla Plac divIVA¹⁻¹⁸³F17G-cyaA(T18)</i>	this work
pSH177	<i>bla Plac cyaA(T18)-divIVA¹⁻¹⁸³F17G</i>	this work
pSH178	<i>kan Plac cyaA(T25)-divIVA¹⁻¹⁸³F17G</i>	this work
pSH179	<i>kan Plac divIVA¹⁻¹⁸³F17G-cyaA(T25)</i>	this work

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