## **Supplementary Figures and Tables**

# Effect of Mutations in GvpJ and GvpM on Gas Vesicle Formation of *Halobacterium salinarum*

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**Supplementary Figure S1**. Alignment of the as sequence of GvpA, GvpJ and GvpM derived from the p-vac region of *Halobacterium salinarum* PHH1. The highlighted sequence motifs of GvpJ and GvpM (GAV, RAAIA, EYGL) are discussed in the text. The consensus of the three sequences depicted by black beams is shown below. Predicted  $\alpha$ -helices of GvpJ are labelled  $\alpha$ 1 through  $\alpha$ 3.



**Supplementary Figure S2**. Colonies of  $\Delta J+J_{wt}$  and  $\Delta J+J_{mut}$  transformants on solid media (top), and cells analyzed by transmission electron microscopy (underneath). The respective GvpJ variant is labelled on top. Colonies of gas vesicle containing cells are turbid and pink (see  $\Delta J+J_{wt}$  bottom row, right). The red color is due to the caroteine bacterioruberin. In contrast, colonies of Vac<sup>-</sup> cells are transparent. The difference in the darkness of the red color depends on the oxygen supply during growth. Cells of several colonies were inspected by transmission electron microscopy for the possession of gas vesicles. In each case, a representative cell is shown underneath.



**Supplementary Figure S3**. Western analyses to determine the presence of GvpJ. Total proteins were isolated from  $\Delta J+J_{wt}$  or  $\Delta J+J_{mut}$  transformants, and 20 µg of proteins were separated by SDS-PAGE, followed by transfer on a PVDF membrane and treatment with an antiserum raised against GvpJ. The respective substitution- or deletion variant is indicated on top. Arrows on the right mark the position of GvpJ.



**Supplementary Figure S4**. Western analysis to determine the presence of GvpJ and GvpL in the interaction study. Total proteins were isolated from cells containing  $J_{mut}CGFP$  and NGFPL, and 20 µg of proteins were separated by SDS-PAGE, followed by transfer to a PVDF membrane and treatment with the respective antiserum to detect NGFPL (top) or JCGFP (bottom). Lysates of *Hfx. volcanii* transformants expressing *gvpJ*-pJAS35 or *gvpL*-pJAS35 were used as control. Green arrows mark the position of the expected Gvp-GFP fusion protein, grey arrows the original GvpJ or GvpL.



**Supplementary Figure S5.** Colonies of  $\Delta M+M_{wt}$  and  $\Delta M+M_{mut}$  transformants on solid media (top), and representative cells analyzed by transmission electron microscopy for the possession of gas vesicles. The respective GvpJ variants are labeled on top. (A) Transformants producing gas vesicles similar to wild type (wt) (Vac<sup>+</sup> phyenotype). (B) Transformants containing a few gas vesicles only (Vac<sup>±</sup>). (C) Transformants exhibiting a Vac<sup>-</sup> phenotype. Further explanations are given in the text.



**Supplementary Figure S6**. Western analyses to determine the presence of GvpM. Total proteins were isolated from  $\Delta M+M_{wt}$  or  $\Delta M+M_{mut}$  transformants, and 20 µg of proteins were separated by SDS-PAGE followed by transfer on a PVDF membrane and treatment with an antiserum raised against GvpM. The respective substitution of deletion variant is indicated on top. The arrow on the right marks the position of GvpJ.

		10	20	30	40	50	60 70	
4QSG	1	M <mark>G</mark> H <mark>H</mark>	HHHHM <mark>MT</mark> V <mark>GL</mark> Y	LYGIFPEPIF	<mark>2 D G L V L Q G I D</mark>	N – <mark>E P V H S</mark> E M I D	<mark>G F S F L Y S</mark> A A	HKEKYLA 60
pgvpl	1 MTDHR	<mark>P</mark> S	N E E R T <mark>V S</mark> N <mark>G</mark> R Y	<u>' L Y C V V D T </u> T S S	5	D – N <mark>P V Y V V E A D</mark>	<mark>G V G A V V H D</mark> C E T V <mark>Y</mark>	ETEDLEQ 77
pgvpf	1		– – – – – <mark>MT</mark> E N L Y	' <mark>TYG</mark> II <mark>EQ</mark>	$-\mathbf{E}\mathbf{D}\mathbf{L}\mathbf{E}\mathbf{L}\mathbf{D}\mathbf{V}\mathbf{E}\mathbf{G}\mathbf{V}\mathbf{A}$	GAEQVYPVDYK	T L <mark>S</mark> A V V <mark>S D</mark> I	DTTDPER 51
	80	90	100	110	120	130	140	150
4QSG	61 <mark>5 r r y</mark> l	ICHEKVLETV	M <mark>E</mark> A – <mark>G</mark> FT <mark>T</mark> LL <mark>F</mark>	L	VESVTEQLITPY	K T Q L K E L F A K L	S	<b>D</b> NQWE 134
pgvpl	78 <mark>v k r w</mark> l	V <mark>THQ</mark> QVV <mark>D</mark> AA	SDA – – FGTPLF	MRFDTVLEGC	DA <mark>SIE</mark> RWLEDH	Y E <mark>G F R D</mark> E L A S F .	A <mark>G VWEY</mark> R I <u>N</u> LLW-	- – <mark>D S A P</mark> F 150
pgvpf	52 <b>TDE</b> DV	E	L K H E E E R T V V F	MSFGMAF <mark>KS</mark> A	$\mathbf{R} - \mathbf{T} \mathbf{L} \mathbf{K} \mathbf{G} \mathbf{V} \mathbf{L} \mathbf{R} \mathbf{G} \mathbf{A}$	<b>R</b> R A <b>L R</b> S T <b>L</b> N D <b>I</b>	E <mark>G T V E L G V K</mark> I L <mark>G P</mark>	GDDTVP - 127
	160	170	180	190	200	210	220	230
4QSG	135 L <mark>Q</mark> AAL	ESNPKLKQER	DAMMGKN		QLIEATVLRRK		NHRAQEVIESDPN	<b>ITDD</b> 204
pgvpl	151 E <mark>E</mark> TIA	DRDD <mark>RLRE</mark> L <mark>R</mark>	Q	F	-	<mark>Q E L</mark> K R E R <mark>R</mark> T <mark>E</mark> L .	4 <mark>D</mark> Q L K <mark>E A I T</mark> – – <mark>P</mark> V	VNDLTEQ 216
pgvpf	128				<mark>R</mark>	EEIQENVTDQL.	ADLSINE <mark>TE</mark> NDLF	<b>TD</b> R 155
		240	250 2	60 2	70 280	290	300	
4QSG	205			A   DO - OF GDR	R	IRYNNITAPYT	FAOL - I	253
	205							
pgvpl	217 DTNT		EQIVRFAVLAC	EDDETALGOR	LDTIVEHE <mark>GV</mark> E	I <mark>R</mark> F <mark>T </mark> G P W P P Y T	F A <mark>P</mark> D – I <mark>G</mark> K – – – –	281

**Supplementary Figure S7**. Alignment of GvpF derived from the cyanobacterium *Microcystis aeruginosa* and the GvpF and GvpL proteins of the haloarchaeon *Halobacterium salinarum*.

Supplementary Table 1. Oligonucleotides used in this study.

Name

### **Oligonucleotide sequence (5'-3')\***

### p-gvpM substitutions in $M^{Ex}$

f-pM/E02A	atgg <u>ca</u> ccaacaaaagacgag
f-pM/P03A	atggaggcaacaaaagacgag
f-pM/T04A	atggagccagcaaaagacgag
f-pM/K05A	atggagccaacagcagagag
f-pM/D06A	atggagccaacaaaag <u>c</u> cgag
f-pM/E07K	gac <u>a</u> agacacacgcgatcgtt
f-pM/T08A	gacgaggcacacgcgatcgtt
f-pM/H09A	gacgagaca <u>gc</u> cgcgatcgtt
f-pM/A10D	gacgagacacacg <u>ac</u> atcgtt
f-pM/I11A	gcg <u>gc</u> cgttgagttcgt
f-pM/I11D	gcg <u>ga</u> cgttgagttcgt
f-pM/V12Y	gcgatc <u>ta</u> tgagttcgt
f-pM/F14A	gcgatcgttgag <u>gc</u> cgt
f-pM/V15A	ttcgccgacgtgttactgc
f-pM/V17A	ttcgtcgacg <u>c</u> gttactgc
f-pM/L18A	ttcgtcgacgtggcactgc
f-pM/L19A	ttcgtcgacgtgtta <u>gc</u> gc
f-pM/G22A	gacg <u>c</u> agccgtgattcaag
f-pM/V24A	gacggagccg <u>c</u> gattcaag
f-pM/I25A	gacggagccgtg <u>gc</u> tcaag
r-pM/E02, P03, T04, K05, D06	ctgcagagttcggcttcc
r-pM/E07, T08, H09, A10	ttttgttggctccatctgcagag
r-pM/I11, V12, F14	gtgtgtctcgtcttttgttggctc
r-pM/V15, V17, L18, L19	ctcaacgatcgcgtgtgtctc
r-pM/G22, V24, I25	gcgcagtaacacgtcgac
f-pM/D28G	aagcgggcgtgatcgt
r-pM/D28G	gaatcacggctccgtc
f-pM/T32K	gtgatcgtga <u>a</u> ggtcgccg
r-pM/T32K	gtccgcttgaatcacggctc

#### p-gvpM substitutions in split-GFP vector pM(25N)<sub>N</sub>

pM_25N-E02A_fwd
pM_25N-E02A_rev
pM_25N-P03A_fwd
pM_25N-T04A_fwd
pM_25N-K05A_fwd
pM_25N-K05A_rev
pM_25N-D06A_fwd
pM_25N-E07A_fwd
pM_25N-T08A_fwd
pM_25N-H09A_fwd
pM_25N-H09A_rev
pM_25N-A10D_fwd
pM_25N_I11A_fwd
pM_25N_I11D_fwd
pM_25N_V12Y_fwd
pM_25N_E13A_fwd
pM_25N_I11A_rev

atgcccatgg<u>c</u>gccaacaaa cactgcagagttcggcttccga atgcccatggaggcaacaaaa atgcccatggagccagcaaaag gccaaca<u>gc</u>agacgagacac tccatgggcatcactgcagag gccaacaaaag<u>ca</u>gagacacacg gccaacaaaagacg<u>c</u>gacacac gccaacaaaagacgaggcacacg cgagacagccgcgatcg tcttttgttggctccatgggcatc cgagacacactgggatcgttgag acacgcggcagttgagttc acacgcggacgttgagttc acacgcgatc<u>*ta*</u>tgagttcgtcg acacgcgatcgttg<u>ca</u>ttcgtc gtctcgtcttttgttggctccatg

3'-BlpI-pM(25N)_D21A	tgttgtt gctcagc cc aatcacggctccggc
3'-BlpI-pM(25N)_G22A	tgttgtt gctcagc cc aatcacggctgcgtc
3'-BlpI-pM(25N)_A23D	tgttgtt gctcagc cc aatcacgtctccgtcgc
3'-BlpI-pM(25N)_V24A	tgttgtt gctcagc cc aatcgcggctccgtc
3'-BlpI-pM(25N)_I25A	tgttgtt gctcagc cc agccacggctccg
5'-NcoI-pM	tgttgtt ccatgg atggagccaacaaaag
3'-BlpI-pM(25N)∆stop	tgttgtt gctcagc cc aatcacggctccgtcg

## p-gvpM substitutions in split-GFP vector pM<sub>C</sub>

5'-NcoI-pM	tgttgtt ccatgg atggagccaacaaaag
3'-BamHI-pM∆stop	agttet ggatee c gteetetegeeg

## p-gvpJ substitutions in pBSK(II)+ and pJAS35

5'-PstI-pJ	attc ctgcag atg agtgaccccaaaccgac
3'-KpnI-pJ	atte ggtace tea tttggteteetegetg
f-pJ/L13A	gcgac <u>g<i>ca</i></u> gccgagatg
f-pJ/A14S	gcgacctc <u>t</u> ccgagatg
r-pJ/L13, A14	cctgcgagcgcgtc
f-pJ/L13I	<u>a</u> tcgccgagatgctggagatgc
f-pJ/A14V	ctcgtcgagatgctggagatgctc
f-pJ/A14D	ctcgacgagatgctggagatgctc
r-pJ/L13, A14	gtcgccctgcgagcgcg
f-pJ/E15A	ggcgacctcgccg <u>c</u> gatgctggagatg
r-pJ/E15A	catctccagcatcgcggcgaggtcgcc
f-pJ/E15R	ctcgcc <u>ag</u> gatgctggag
f-pJ/E15Q	ctcgcc <u>c</u> agatgctggag
f-pJ/E15D	ctcgccga <u>t</u> atgctggagatg
r-pJ/E15	gtcgccctgcgagc
f-pJ/M16A	gag <u>gc</u> gctggagatgc
f-pJ/L17A	gagatg <u>gcggagatgctcc</u>
f-pJ/E18R	gagatgctg <u>c</u> agatgctccta
f-pJ/E18Q	gagatgctg <u>ag</u> gatgctccta
f-pJ/E18D	gagatgctgga <u>t</u> atgctcctagac
r-pJ/M16, L17, E18	ggcgaggtcgccc
f-pJ/E18A	gccgagatgctgg <u>cg</u> atgctcctagac
r-pJ/E18A	gtctaggagcat <u>cg</u> ccagcatctcgg
f-pJ/E18M	atgatgctcctagacaaaggagtcgtcg
f-pJ/M19E	gaggagetectagacaaaggagtegte
f-pJ/E18, M19	cagcatctcggcgaggtcgc
f-pJ/M19A	gag <u>gc</u> gctcctagacaaagg
f-pJ/L20A	atgctggagatg <u>gcg</u> ctagacaaagga
r-pJ/L20A	tcctttgtctag <u>cgc</u> catctccagcat
f-pJ/L20I	gagatgatcctagacaaaggagtcgtc
f-pJ/L21I	gagatgctc <u>a</u> tagacaaaggagtcgtc
f-pJ/D22N	gagatgctccta <u>a</u> acaaaggagtcg
f-pJ/D22E	gagatgctcctaga <u>a</u> aaaggagtcgtc
r-pJ/M19, L20, L21, D22	cagcatctcggcgaggtc
f-pJ/L21A	gcagacaaaggagtcgtcgtcaac
f-pJ/D22K	cta <u>a</u> aaaggagtcgtcgtcaacgc
f-pJ/K23D	ctagacgatggagtcgtcgtcaacg
f-pJ/D22A	gagatgctcctag <u>cg</u> aaaggagtcgtc
r-pJ/D22A	gacgactcctttccgctaggagcatctc

r-pJ/L21, K23 f-pJ/K23A f-pJ/K23R f-pJ/G24S r-pJ/K23, G24 f-pJ/G24A f-pJ/V25A f-pJ/V26A f-pJ/V27A r-pJ/G24, V25, V26, V27 f-pJ/A29D f-pJ/D30A r-pJ/A29, D30 f-pJ/V33A f-pJ/V33D r-pJ/V33 f-pJ/S34A f-pJ/V35A r-pJ/S34, V35 f-pJ/E39A f-pJ/L41A r-pJ/E39, L41 f-pJ/E44A f-pJ/L45A f-pJ/R46A r-pJ/E44, L45, R46 f-pJ/R46E f-pJ/A47D f-pJ/A48D r-pJ/R46, A47, A48 f-pJ/I49A f-pJ/A50D r-pJ/I49, A50 f-pJ/A50Y f-pJ/A50E f-pJ/S51A r-pJ/A50, S51 f-pJ/F52A f-pJ/E53A f-pJ/T54R r-pJ/F52, E53, T54 f-pJ/T54A r-pJ/T54A f-pJ/A55D f-pJ/A56D r-pJ/A55, A56 f-pJ/Y58A r-pJ/Y58A f-pJ/E57A f-pJ/Y58E f-pJ/G59A r-pJ/E57, Y58, G59 f-pJ/L60A r-pJ/L60A f-pJ/E61A r-pJ/E61A

gagcatetecagcatetegge gacgcaggagtcgtcgtc gac aga gga gtc gtc gtc aac gac aaa <u>agt</u> gtc gtc gtc aac g taggagcatctccagcatctcg gcagtcgtcgtcaacgcggatatcg ggag<u>ca</u>gtcgtcaacgcggatatcg ggagtcg<u>ca</u>gtcaacgcggatatcg ggagtcgtcgcaacgcggatatcg tttgtctaggagcatctccagcatctcgg gacgatatcgcagtcagcgtcgg gcggctatcgcagtcagcgtc gttgacgacgactcctttgtctaggagc g<u>c</u>cagcgtcggggacacgg g<u>a</u>cagcgtcggggacacggaac tgcgatatccgcgttgacgacg gcagtcggggacacggaactcctc gactgcgatatccgcgttgacgacg gcactcctcggtatcgagttacgggc gaactcgcaggtatcgagttacgggcc cgtgtccccgacgctgactgc gcattacgggccgcgattgcttc gag<u>gc</u>acgggccgcgattgc gagttagcagccgcgattgcttcgttc gataccgaggagttccgtgtccccgac g<u>ag</u>gccgcgattgcttcgttc cggg<u>a</u>cgcgattgcttcgttc cgggccgacattgcttcgttc taactcgataccgaggagttccgtgtc gctgcttcgttcgagacagcggc attg<u>a</u>ttcgttcgagacagcggcc cgcggcccgtaactcgataccg tattcgttcgagacagcggccgaatac g*aa*tcgttcgagacagcggccgaatac gctgcattcgagacagcggccg aatcgcggcccgtaactcgatacc <u>gc</u>cgagacagcggccgaatac ttcg<u>c</u>gacagcggccgaatac ttcgagagagcggccgaatacg cgaagcaatcgcggcccg gcagcggccgaatacgggctc ctcgaacgaagcaatcgcggcc g<u>acg</u>ccgaatacgggctcgag gcgg<u>a</u>cgaatacgggctcgag tgtctcgaacgaagcaatcgcg gcagggctcgagttcccaacgg ttcggccgctgtctcgaacgaag g<u>ct</u>tacgggctcgagttcccaac gaagaggggctcgagttcccaac gaatacgctctcgagttcccaacgg ggccgctgtctcgaacgaagc gctgagttcccaacggggacg cccgtattcggccgctgtctc g<u>ca</u>ttcccaacggggacggatatg gagcccgtattcggccgctgtc

f-pJ/E69A	g <u>ca</u> cgcgtcgagtccgccg
r-pJ/E69A	catatccgtccccgttgggaactcg
f-pJ/A74D	gacgcgaatatctcaccggaccag
r-pJ/A74D	ggactcgacgcgctccatatcc

#### p-gvpJ deletions in pBSK(II)+ and pJAS35

f-Start+PstI-pJxpBSK+ catctgcagcccggg r-∆3N-pJ cccaaaccgacgcgc r-∆4N-pJ aaaccgacgcgctcg r-∆5N-pJ ccgacgcgctcg r-∆6N-pJ acgcgctcgcagg r-∆9N-pJ cagggcgacctcgc r-∆12N-pJ ctcgccgagatgctgg f-∆5C-pJ tgacgtggatgccgtc f-Δ7C-pJ ggatgccgtcggcg f-∆9C-pJ cgtcggcgtcgaatcgtc f-Δ10C-pJ cggcgtcgaatcgtcc f-∆12C-pJ cgaatcgtccgacagtggg f-∆15C-pJ cgacagtgggttcgtcgac f-Δ20C-pJ cgactcggtctccgattgg f-Δ25C-pJ ttgggtttccgacgccg f-Δ30C-pJ cgggtcggactggtcc r-Stop+KpnI-pJxpBSK+ tgaggtacccaattcgccct 5'-PstI-pJ attc ctgcag atg agtgaccccaaaccgac 3'-KpnI-pJ attc ggtacc tca tttggtctcctccgctg 5'-PstI-pJ∆3N attc ctgcag atg cccaaaccgacgc 5'-PstI-pJ∆4N attc ctgcag atg aaa ccg acg cgc tcg 5'-PstI-pJ∆5N attc ctgcag atg ccg acg cgc tcg 5'-PstI-pJ∆6N attc ctgcag atg cccaaaccgacgc 5'-PstI-pJ∆9N attc ctgcag atg cagggcgacctc 5'-PstI-pJ∆12N attc ctgcag atg ctcgccgagatgc 3'-KpnI-pJ∆5C attc ggtacc tca tgacgtggatgccgtc 3'-KpnI-pJ∆7C attc ggtacc tca ggatgccgtcggcg 3'-KpnI-pJ∆9C attc ggtacc tca cgtcggcgtcgaatcgtc 3'-KpnI-pJ∆10C attc ggtacc tca cggcgtcgaatcgtc 3'-KpnI-pJ∆12C attc ggtacc tca cgaatcgtccgacagtggg 3'-KpnI-pJ∆15C attc ggtacc tca cgacagtgggttcgtc 3'-KpnI-pJ∆20C atte ggtace tea egaeteggteteeg 3'-KpnI-pJ∆25C attc ggtacc tca ttgggtttccgacgc 3'-KpnI-pJ∆30C attc ggtacc tca cgggtcggactgg

#### Split-GFP plasmids *gvpJ* (substitutions, fragments, deletions)

5'-BspHI-pJ 3'-BamHI-pJ∆Stop 5'-BamHI-pJ 3'-BlpI-pJ\_1-56 +Stop 3'-BlpI-pJ\_1-56 3'-BamHI-pJ\_1-56 3'-KpnI-pJ +Stop 5'-NcoI-pJ\_47-114 5'-BamHI-pJ\_47-114 3'-BlpI-pJ actgac **tcatga** gtgaccccaaaccg tggatt **ggatce** c tttggtctcctccgc aggtcaa **ggatce** atgagtgaccccaaac atte **gctcage** tea ggecgetgtetegaac atte **ggtace** e ggecgetgtetegaac atte **ggtace** e ggecgetgtetegaac atte **ggtace** tea ggecgetgtetegaac atte **ggtace** tea ggecgetgtetegaac atte **ggtace** tea ggecgetgtetegaac atte **ggtace** atg geegegattgetteg atte **ggatce** atg geegegattgetteg gtaacet **geteage** teatttggteteeteg

3'-BlpI-pJ∆Stop	aagtaacct gctcagc cctttggtctcctccgc
3'-KpnI-pJ	atte ggtace teatttggteteeteegetg
5'-NcoI-pJ∆3N	attc ccatgg ca atg cccaaaccgacgc
5'-BspHI-pJ∆4N	attc tcatga aaccgacgcgctcg
5'-NcoI-pJ∆5N	attc ccatgg ca atg ccgacgcgctcg
5'-BspHI-pJ∆6N	attc tcatga cgcgctcgcag
5'-NcoI-pJ∆12N	attc ccatgg ca atg ctcgccgagatgc
3'-BamHI-pJ∆5C	atte ggatee e tgaegtggatgeegte
3'-BamHI-pJ∆7C	attc ggatcc c ggatgccgtcggcg
3'-BamHI-pJ∆9C	atte ggatee e egteggegtegaategte
3'-BamHI-pJ∆10C	atte ggatee e eggegtegaategte
3'-BamHI-pJ∆12C	attc ggatcc c cgaatcgtcgacagtggg
3'-BamHI-pJ∆15C	attc ggatcc c cgacagtgggttcgtc
3'-BamHI-pJ∆20C	atte ggatee e cgaeteggteteeg
3'-BamHI-pJ∆25C	attc ggatcc c ttgggtttccgacgc
3'-BamHI-pJ∆30C	attc ggatcc c cgggtcggactgg

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\*restriction sites are marked in bold; codons for substituted aa are marked in *italics* and are underlined; nucleotides added are separated by blanks

Substitution	position	Vac/GV*	J/L rf value*
L13A	α1	negative	
L13I	α1	few	
A14D	α1	negative	
A14V	α1	negative	
E15A/D/R	$\alpha$ 1	negative	
E150	$\alpha$ 1	negative	higher
M16A	$\alpha$ 1	negative	
L17A	$\alpha$ 1	negative	
E18A/M/D/R	$\alpha$ 1	negative	
E180	$\alpha 1$	wt	
M19A	$\alpha 1$	negative	
M19F		negative	lower
L20A	$\alpha$ 1	negative	lower
L2011		negative	
$I 21 \Delta / I$		negative	
$D^{22} \Delta$		negative	
D22A D22E		negative	 higher
D22E		negative	Inghoi
D22N		anindlo/oulindor	
$V_{23A}$	loon1	cylinder/unstable	
K23D	loop1	negative	
G24A	loop1	negative	lower
G24S	loop1	negative	lower
V25A	B1	spindle/unstable	
V26A	B1	negative	
V27A	B1	negative	
A29D	B1	negative	higher
D30A	B1	negative	
V33A/D	B1	negative	higher
\$34A	B1	wt	
V35A	B1	negative	
E39A	loop 2	few	
L41A	β2	negative	
E44A	β <b>-</b> β2	negative	
L45A	β <u>2</u>	spindle	
<b>R</b> 46A	β <u>2</u>	cylinder/ unstable	
<b>R</b> 46E	β <b>2</b>	negative	
A47D	β2 β2	negative	higher
A48D	β2 β2	negative	higher
149A	р <u>2</u> В2	negative	higher
A = 50  D/F	B2	negative	higher
A 50Y	B2	negative	
S51A	β2 β2	negative	higher
F52A	α2	negative	higher
F53A	a2	negative	
1.55Λ Τ5ΛΛ/D	α2 α2	negative	
1 J+A/IN A 55D	a2	negative	
A33D A56D	u2 a2	negative	
	u2 a2	negative	
EJ/A	0.2	negative	

# Supplementary Table S2. Effect of mutations in GvpJ on gas vesicle formation

Y58A	α2	negative	
Y58E	α2	negative	lower
G59A	α2	negative	
L60A	α2	negative	higher
E61A	α2	negative	
E69A	α2	cylinder	
A74D	loop3	negative	
$J_{WT}$		wt	

\*Vac phenotype: wt, wild type; few = few gas vesicles; Rf --- = similar to wild type GV, gas vesicle shape

**Supplementary Table S3.** Split-GFP analyses investigating the  $J_{mut}/L$  or  $M_{mut}/L$ . The fluorescence was measured in LAU/mm<sup>2</sup> and the relative fluorescence was calculated.

		_		Relative				_	Relative
transformant		LAU/mm <sup>2</sup>	σ	fluorescence	tra	nsformant	LAU/mm <sup>2</sup>	σ	fluorescence
			LAU/mm <sup>2</sup>	(rf)			-	LAU/mm <sup>2</sup>	(rf)
L/J subst	itution interactio	ns			L/J delet	ion interactions			
ы	la.	211 201	18 /03	12 1/		la la	2/1 252	0 866	15 72
NL	JC	207 722	16,495	13.14	NL		341,332 492.0EE	3,800	13.72
	J_LISAC	307,725	10,542	12.96			462,955	22,051	24.75
	J_LISIC	331,351	40,342	12.95			242,551	25,612	12.21
	J_A14Dc	290,015	9,074	11.19		J_ASCc	304,823	17,586	15.62
	J_A14Vc	283,532	7,068	10.92		$J_\Delta/C_c$	315,044	27,465	16.18
	J_E15A <sub>C</sub>	252,666	4,890	9.62		J_A9Cc	354,435	10,998	18.32
	J_E15Dc	278,911	25,654	11.80		J_∆12Cc	320,370	9,872	16.47
	J_E15Q <sub>C</sub>	372,085	15,175	16.08		$J_{\Delta 15C_{C}}$	262,504	7,931	13.31
	J_E15Rc	243,433	26,870	10.17		$J_{\Delta 20C_{C}}$	348,166	22,914	17.98
	J_M16A <sub>c</sub>	223,804	10,811	9.27		$J_{\Delta 25C_{C}}$	279,370	8,039	14.23
	J_L17Ac	365,522	16,107	14.37		J_Δ30Cc	268,576	6,970	12.16
	J E18A <sub>C</sub>	248,551	46,525	9.45					
	J E18Dc	337,776	32,110	14.50					
	J E18Mc	287,899	20,087	11.10	L/M sub	stitution interactio	ns		
	J_E180c	215,660	16.396	8.90					
	L F18Rc	219 553	12 849	9.08	Lc	Mc	199 613	34 656	7 77
	J_M10A	225,555	22,045	11 01	LC	M V15E	173 052	64 784	6.45
		102 425	10 140	7.00		M 110E	177,002	17 021	7.40
	J_10119EC	192,423	10,140	7.09		M_C22D	221.000	17,921	7.40
	J_LZUAc	217,108	8,807	8.13		IVI_GZZDc	231,066	27,107	9.58
	J_L20Ic	276,275	5,890	13.72		M_A23Dc	252,635	49,527	9.65
	J_L21A <sub>C</sub>	157,981	17,245	8.99		M_V24Dc	290,910	99,595	11.23
	J_L21I <sub>C</sub>	268,198	37,856	13.29		M_R44E <sub>c</sub>	162,403	22,786	7.54
	J_D22Ac	308,235	19,802	11.93		M_A45Ec	366,492	144,119	17.95
	J_D22Ec	315,506	17,616	15.81		M_A46Ec	155,180	21,823	5.57
	J_D22K <sub>C</sub>	325,261	19,633	12.64		M_I47A <sub>C</sub>	191,650	6,552	7.16
	J_D22Nc	289,244	14,942	14.41		M_A48Dc	177,806	19,685	7.45
	J K23Ac	349,753	32,151	13.67		_			
	L K23Dc	305.054	32,292	11.80					
	L K23Rc	282,251	12,297	14.04	I/M1-25	substitution intera	actions		
	L G24Ac	179 939	5 505	7.24	2,1112 23				
	J_024AL	1/2,555	4 024	7.24		M	1 692 960	5/1 910	65.25
	J_G2430	142,557	4,054	0.00	NL		1,002,009	341,810	67.15
	J_V25Ac	208,952	15,653	11.00		IVI_EUZAN	1,283,829	244,490	67.15
	J_V26Ac	311,333	54,863	13.25		IVI_PU3AN	1,594,677	50,768	84.10
	J_V27Ac	292,917	31,521	13.21		M_I04A <sub>N</sub>	1,414,952	273,203	69.90
	J_A29Dc	545,660	27,948	25.47		M_K05A <sub>N</sub>	1,526,740	209,660	77.64
	J_D30Ac	198,692	21,742	10.41		M_D06A <sub>N</sub>	1,247,417	111,424	65.57
	J_V33Ac	402,647	49,455	18.53		M_E07K <sub>N</sub>	124,587	106,469	80.46
	J_V33Dc	381,461	37,720	17.50		M_T08A <sub>N</sub>	2,141,202	159,651	113.26
	J_S34Ac	339,263	13,976	13.23		M_H09A <sub>N</sub>	1,220,235	133,869	62.05
	J V35Ac	307,214	18,694	11.89		M A10D <sub>N</sub>	1,184,781	261,837	58.23
	L E39Ac	266.255	7.059	9.50		M I11AN	1.641.347	189,102	84.36
	1   41Ac	369,689	11,923	13.57		 M_I11D <sub>N</sub>	2 073 193	209 138	106.83
	J_E41Ac	251 082	22,323	0.63		M_112VN	1 162 180	174 316	58 13
	J_L44AC	214 214	22,227	12 20		M E12A.	1 154 250	28 022	60.60
	J_L4JAC	242 575	10 200	12.50		NI_LIJAN	1,134,330	20,032	50.00
	J_R46AC	342,575	10,300	13.50		IVI_F14AN	1,137,529	213,254	50.98
	J_R46Ec	322,779	23,409	12.66		IVI_V15AN	2,104,044	441,164	101.78
	J_A47Dc	466,101	19,411	21.61		M_D16A <sub>N</sub>	877,442	101,732	45.82
	J_A48D <sub>c</sub>	353,028	30,056	16.13		M_V17A <sub>N</sub>	1,414,810	122,470	74.50
	J_I49Ac	392,753	27,279	18.05		M_L18A <sub>N</sub>	1,427,971	116,759	75.20
	J_A50Dc	376,845	15,255	17,28		M_L19A <sub>N</sub>	1,550,256	55,602	81.73
	J_A50Ec	353,769	16,645	16.16		M_R20A <sub>N</sub>	1,049,825	112,269	54.11
	J A50Y <sub>c</sub>	255,749	16,234	11.41		M D21A <sub>N</sub>	1,262,809	67,600	66.39
	J S51Ac	349,028	17,174	17.42		M G22A <sub>N</sub>	1,673,417	136,131	85.47
	L E52Ac	391,868	19.880	19.68		M A23D <sub>N</sub>	1.778.716	36.033	93.92
	L F53Ac	307 388	5 887	12.01		M_V24A <sub>N</sub>	1 423 728	59 154	74 97
	L T54Δ	316.080	16 789	12.32		M 125AN	1 228 149	88 782	64 54
	J_134AC	304 500	14 675	11 00		11_123AN	1,220,149	30,702	04.04
	J_134KC	304,308	14,0/0	11.09					
	J_ASSDC	233,848	4,217	12.43					
	J_A56D <sub>C</sub>	299,073	8,699	11.66					
	J_E57A <sub>C</sub>	284,417	5,288	11.04					
	J_Y58Ac	201,304	6,846	10.56					
	J_Y58Ec	209,186	20,640	7.86					
	J_G59A <sub>c</sub>	295,449	20,047	15.14					
	J_L60A <sub>c</sub>	307,556	6,128	15.80					
	J E61Ac	256,080	12,933	12.99					
	J E69Ac	275.470	11.256	14.05					
	J A74D	257 287	31,325	13.05					
		,,	- 1,010	20.00					

**Supplementary Table S4**. Split-GFP analyses investigating interactions of A-J-M fragments. The fluorescence was measured in LAU/mm<sup>2</sup> and the relative fluorescence calculated.

			с	Relative					
trans	formant	LAU/mm <sup>2</sup>	I ALI/mm <sup>2</sup>	fluorescence					
			2/0/1111	(rf)				6	Relative
					trans	formant	LAU/mm <sup>2</sup>	I ALI/mm <sup>2</sup>	fluorescence
control								LAO/IIIII	(rf)
	WR340	17,066	1,687	0.00					
					<sub>N</sub> J1-56	A44-76c	18,063	1,269	0.00
GvpJ intera	ictions					cA44-76	14,600	861	0.00
					J1-56 <sub>N</sub>	A44-76c	15,315	337	0.00
LΝ	A1-22c	15,515	1,185	0.06		cA44-76	13,922	1,686	0.00
	cA1-22	13,310	1.117	0.00	cJ1-56	A44-76 <sub>N</sub>	16.683	1,749	0.00
Ь	Δ1_22 σ	13 310	1 117	0.00	0	NΔ44-76	16 268	432	0.00
214	- 1 220	12,021	1,11/	0.00	11 56-	NA44 76.	10,200	F24	0.00
	(A1-22	12,931	1,974	0.01	11-200	A44-70N	10,303	534	0.00
cJ	A1-22 <sub>N</sub>	15,603	//9	0.05		<sub>N</sub> A44-76	18,091	508	0.00
	<sub>N</sub> A1-22	17,016	637	0.14					
Jc	A1-22 <sub>N</sub>	25,287	2,336	0.70					
	<sub>N</sub> A1-22	40,430	2,947	1.71	<sub>N</sub> J1-56	Mc1	105,270	3,549	0.34
						сM1	111,793	11,823	0.42
					J1-56 <sub>N</sub>	Mc1	84,466	7,502	0.08
мJ	A44-76c	11.772	569	0.10		cM <sup>1</sup>	118.065	18.329	0.35
110	cA44=76	10 993	803	0.05	cl1-56	M <sub>N</sub> <sup>1</sup>	111 356	7 800	0.00
	A 4 7 7 C	10,555	055	0.05	01 50	N/1	110,000	6 212	0.27
JN	A44-70C	10,641	007	0.04	14.50		119,462	0,515	0.57
	cA44-76	11,023	1,328	0.07	J1-56c	IVIN <sup>1</sup>	100,725	3,806	0.15
сJ	A44-76 <sub>N</sub>	11,040	1,271	0.06		NM¹	95,150	2,439	0.09
	<sub>N</sub> A44-76	9,293	2,029	0.01					
Jc	A44-76 <sub>N</sub>	21,842	1,550	1.03					
	NA44-76	16.121	1.185	0.50	₀J1-56	M1-25c	291.164	4.975	18.05
		- /	,			cM1-25	298,948	10,258	18.56
					11-56	M1_25-	276 530	22 476	17.00
	N41 25	10 005	1.000	0.00	31 30N	NA1 25	270,330	15 054	17.05
NJ	IVI1-25c	10,085	1,969	0.66	14 50	CIVI1-25	2/5,3/6	15,054	17.02
	cIVI1-25	11,355	1,839	0.14	cJ1-56	M1-25 <sub>N</sub>	273,638	10,757	16.90
JN	M1-25c	16,946	951	0.68		<sub>N</sub> M1-25	250,688	5,141	15.40
	cM1-25	12,195	1,030	0.21	J1-56c	M1-25 <sub>N</sub>	286,298	7,624	17.73
сJ	M1-25 <sub>N</sub>	11,296	1,514	0.15		⊾M1-25	284,322	11,170	17.60
	⊾M1-25	10.935	1.523	0.10					
lc.	M1-25	15 022	963	0 49					
30		15 780	1 /03	0.45		M60-84-	306 437	6 388	20.75
	NIVI1-25	15,785	1,495	0.57	N11-20	N100-04C	300,437	0,500	20.75
						cIVI60-84	280,733	7,194	18.93
					J1-56 <sub>N</sub>	M60-84c	289,429	4,594	19.55
LΝ	M60-84 <sub>c</sub>	16,396	458	0.01		<sub>c</sub> M60-84	301,170	8,442	20.38
	cM60-84	15,530	622	0.00	cJ1-56	M60-84 <sub>N</sub>	283,697	6,585	19.14
J <sub>N</sub>	M60-84 <sub>c</sub>	17,799	834	0.08		<sub>N</sub> M60-84	289,387	7,955	19.54
	cM60-84	16.423	1.561	0.03	J1-56c	M60-84 <sub>N</sub>	309.739	9.921	20.99
cl	M60-84N	15,259	887	0.00		M60-84	281,190	11,376	18.96
	M60-84	17 011	1 595	0.05			,	,	
	NIVIOU-04	20,220	1,555	0.05					
JC	IVI60-84N	20,220	2,125	0.23					
	<sub>N</sub> IVI60-84	47,920	3,298	1.91	<sub>N</sub> J47-114	Ac	34,030	3,212	0.14
						<sub>C</sub> A	17,794	886	0.00
					J47-114 <sub>N</sub>	Ac	112,572	93,078	2.93
GvpJ fragm	ents with GvpA	/GvpM				сA	18,683	205	0.00
					cJ47-114	AN	36,273	19,382	0.69
⊾I1-56	Ac	19.055	726	0.00		NА	19,039	709	0.00
102 00	cΔ	14 995	1 4 2 8	0.00	147-114	Δ.	17 438	353	0.00
11 50	•	17,555	1,420	0.00	J47 II40		10,70	042	0.00
JT-20N	Ac	15,844	369	0.00		NA	18,220	942	0.00
	<sub>c</sub> A	15,260	/50	0.00					
c <b>J1-56</b>	AN	15,867	1,088	0.00					
	NA	12,791	455	0.00	<sub>N</sub> J47-114	A1-22c	27,128	310	0.35
J1-56c	AN	14,375	1,302	0.00		c A1-22	12,503	669	0.00
	NА	14.825	463	0.00	J47-114 <sub>N</sub>	A1-22c	14.619	613	0.00
		,				c Δ1-22	12 024	1 194	0.00
					c 47-114	A1-22.	154 695	136 760	5 71
	A1 33	11 071	050	0.00	U-1 114		1090 1000	1 074	5.71
9C-T(N	AT-22C	14,821	859	0.00	147 444	N A1-22	20,322	1,074	0.00
	cA1-22	12,163	192	0.00	J4/-114 <sub>C</sub>	A1-22 <sub>N</sub>	11,812	812	0.00
J1-56 <sub>N</sub>	A1-22c	13,865	559	0.00		<sub>N</sub> A1-22	16,591	2,831	0.02
	cA1-22	12,505	252	0.00					
cJ1-56	A1-22 <sub>N</sub>	243,810	6,391	9.06					
	<sub>N</sub> A1-22	129.406	114.911	4.54					
11-56	A1-22M	243 821	9 884	9.06					
5- 500	MΔ1_77	10 00/	1 0/1	0.00					
	NAT-22	19,004	1,041	0.00					

continued

<sup>1</sup> = LAU value of *Hfx. volcanii* WR340: 87,354

transformant		a ح LAU/mm² LAU/mr LAU/mr		σ Relative U/mm <sup>2</sup> fluorescen ce (rf)		transformant		σ LAU/mm²	Relative fluorescen ce (rf)
<sub>N</sub> J47-114	A44-76 <sub>c</sub>	26,216	1,771	0.31	<sub>N</sub> J47-114	Hc	272,516	6,881	12.36
	cA44-76	11,494	723	0.00		сH	20,393	775	0.02
J47-114 <sub>N</sub>	A44-76c	14,541	320	0.00	J47-114 <sub>N</sub>	Hc	29,273	1,266	0.44
	cA44-76	12,025	728	0.00		сH	14,794	827	0.00
<sub>c</sub> J47-114	A44-76 <sub>N</sub>	149,838	132,004	5.51	<sub>c</sub> J47-114	H <sub>N</sub>	15,861	844	0.00
	<sub>N</sub> A44-76	19,857	305	0.00		NH	15,230	975	0.00
J47-114 <sub>c</sub>	A44-76 <sub>N</sub>	12,264	1,280	0.00	J47-114 <sub>C</sub>	H <sub>N</sub>	14,412	850	0.00
	<sub>N</sub> A44-76	13,501	364	0.00		ΝН	14,023	1,437	0.00
⊾I47-114	Mc	35 279	3 507	0.70	NI47-114	lc.	13 982	1 228	0.00
M2+1 TT+	cM	14 559	168	0.00	M2+1 11+	d	24 422	457	0.00
147-114	Me	16 775	219	0.00	147-114.	La La	15 227	1 739	0.12
J+/ II+N	cΜ	13 815	1 401	0.00	347 II4N	ال حا	17 491	1,755	0.00
cl47-114	MN	12 745	1,005	0.00	cl47-114	lu lu	21 414	222	0.00
047 114	M	14 043	751	0.00	047 114	NI.	22,414	776	0.00
147-114c	Ma	13 073	751	0.00	147-114 <sub>c</sub>	NI IN	18 030	1 218	0.05
J47 1140	NМ	14,768	562	0.00	J47 1140	N	18,985	420	0.00
<sub>N</sub> J47-114	M1-25c	317,358	4,410	19.76	<sub>N</sub> J47-114	Jc	80,475	4,808	0.19
	<sub>c</sub> M1-25	325,525	8,722	20.30		cJ	18,586	691	0.00
J47-114 <sub>N</sub>	M1-25c	273,832	7,043	16.92	J47-114 <sub>N</sub>	Jc	19,376	877	0.00
	cM1-25	267,785	13,573	18.01		cJ	16,051	1,144	0.00
<sub>c</sub> J47-114	M1-25 <sub>N</sub>	291,/53	14,128	18.09	<sub>c</sub> J47-114	JN	15,770	699	0.00
	NM1-25	273,405	8,627	16.89	147 444	٩. ۲	15,141	535	0.00
J47-114 <sub>C</sub>	M1-25 <sub>N</sub>	306,459	7,336	19.05	J47-114 <sub>C</sub>	J <sub>N</sub>	15,060	1,128	0.00
	NIVI1-25	291,/15	5,997	18.09		ΓN	16,024	1,276	0.00
<sub>N</sub> J47-114	M60-84 <sub>c</sub>	303,601	20,553	20.55	<sub>N</sub> J47-114	Kc	101,494	3,641	3.67
	cM60-84	327,115	5,204	16.84		сK	20,158	881	0.00
J47-114 <sub>N</sub>	M60-84c	295,661	12,051	19.99	J47-114 <sub>N</sub>	Kc	19,932	661	0.00
	cM60-84	324,978	14,970	16.72		сK	16,395	702	0.00
<sub>c</sub> J47-114	M60-84 <sub>N</sub>	305,845	4,929	20.71	<sub>c</sub> J47-114	K <sub>N</sub>	12,912	694	0.00
	<sub>N</sub> M60-84	298,693	14,978	20.20		NК	15,697	686	0.00
J47-114 <sub>c</sub>	M60-84 <sub>N</sub>	289,178	29,570	19.53	J47-114 <sub>C</sub>	KN	15,915	483	0.00
	<sub>N</sub> M60-84	287,088	7,985	19.38		NК	16,293	441	0.00
GvpJ47-114 interactions with accessory Gvp				<sub>N</sub> J47-114	Lc	320,314	10,263	9.68	
						сL	24,013	565	0.00
<sub>N</sub> J47-114	Fc	16,562	860	0.00	J47-114 <sub>N</sub>	Lc	46,604	2,951	0.55
	сF	14,278	2,020	0.00		сL	20,888	361	0.00
J47-114 <sub>N</sub>	Fc	13,824	609	0.00	<sub>c</sub> J47-114	LN	112,470	92,340	3.87
	сF	13,240	510	0.00		NL	24,841	411	0.06
<sub>c</sub> J47-114	FN	13,716	294	0.00	J47-114 <sub>c</sub>	LN	20,225	481	0.00
	NF	13,166	442	0.00		NL	20,794	674	0.00
J47-114 <sub>c</sub>	FN	16,247	520	0.00					
	NF	15,313	716	0.00	GvpJ1-56 in	iteractions w	vith accessory Gvr	)	
	Ge	221 002	10 007	14 61		Ec1	01 070	24 226	0.15
NJ4/-114	-G	551,093 77 E10	40,997	14.01	0C-T(N	гс с <b>⊑</b> 1	01,078 100 777	24,200 1 100	0.12
147 114	G	22,318	0/0	0.00	11 56	CF -	100,007 66 710	4,420 21.006	0.53
34/-114N	cG	12 /55	3,303	0.08	JT-20N	cF1	76 7/1	21,330 8 601	0.05
c147-114	GN	16 /76	1 222	0.00	cl1-56	E <sub>N</sub> 1	0,244	8 022	0.02
J+/-114		12 751	1,223	0.00	(JT-20		55,550 101 754	0,033 Q //72	0.25
147-114 <sub>0</sub>	GN	14 915	601	0.00	11-56c	E <sub>N</sub> <sup>1</sup>	108 050	6 702	0.23
	NG	11 570	528	0.00	J1 300	NF <sup>1</sup>	95 692	16 835	0.55
		11,373	555	0.00		1.1.1	55,052	10,000	0.15

continued

transformant			-	Relative	transformant		LAU/mm <sup>2</sup>	σ LAU/mm²	Relative fluorescence (rf)
		LAU/mm <sup>2</sup>	LAU/mm <sup>2</sup>	fluorescence (rf)					
<sub>N</sub> J1-56	$G_{C^1}$	116,329	5,191	0.43	<sub>N</sub> J1-56	Jc	21,523	352	0.17
	cG1	100,934	8,438	0.24		сJ	12,922	1,854	0.00
J1-56 <sub>N</sub>	Gc1	95,945	2,425	0.18	J1-56 <sub>N</sub>	Jc	21,410	1,938	0.17
	cG1	81,555	21,073	0.13		сJ	14,624	1,355	0.00
<sub>c</sub> J1-56	$G_N^1$	110,939	8,529	0.37	cJ1-56	JN	18,658	542	0.02
	<sub>N</sub> G <sup>1</sup>	94,514	6,221	0.17		νJ	18,531	1,072	0.03
J1-56c	$G_N^1$	101,179	3,363	0.25	J1-56c	JN	16,474	629	0.00
	NG1	103,467	2,950	0.28		LΝ	17,288	1,587	0.01
<sub>N</sub> J1-56	Hc1	85,957	15,050	0.12	<sub>N</sub> J1-56	Kc1	107,987	1,078	0.37
	cH1	73,801	4,650	0.00		cK1	104,292	3,765	0.32
J1-56 <sub>N</sub>	Hc1	85,002	4,650	0.05	J1-56 <sub>N</sub>	Kc <sup>1</sup>	94,576	12,779	0.20
	cH1	69,432	14,878	0.02		cK1	83,818	4,811	0.07
<sub>c</sub> J1-56	$H_N^1$	109,585	3,404	0.35	<sub>c</sub> J1-56	K <sub>N</sub> <sup>1</sup>	115,008	8,435	0.46
	<sub>N</sub> H <sup>1</sup>	103,801	6,612	0.28		<sub>N</sub> K <sup>1</sup>	64,917	2,803	0.00
J1-56c	$H_N^1$	101,667	3,018	0.25	J1-56c	K <sub>N</sub> <sup>1</sup>	105,270	11,477	0.34
	$_{\rm N}{\rm H}^{1}$	126,969	18,753	0.57		NK1	72,108	29,437	0.14
<sub>N</sub> J1-56	lc1	97,928	7,984	0.21	<sub>N</sub> J1-56	Lc1	105,270	3,549	0.34
	cl1	97,230	4,010	0.20		cL1	111,793	11,823	0.42
J1-56 <sub>N</sub>	Ic <sup>1</sup>	79,291	28,865	0.17	J1-56 <sub>N</sub>	Lc <sup>1</sup>	84,466	7,502	0.08
	cl1	80,563	30,553	0.18		cL1	118,065	18,329	0.35
<sub>c</sub> J1-56	I <sub>N</sub> <sup>1</sup>	119,173	6,352	0.51	<sub>c</sub> J1-56	LN1	111,356	7,800	0.27
	NI1	91,665	7,211	0.16		NL <sup>1</sup>	119,482	6,313	0.37
J1-56c	I <sub>N</sub> <sup>1</sup>	114,719	5,457	0.46	J1-56c	LN1	100,725	3,806	0.15
	N <sup>1</sup>	106,568	15,370	0.35		NL <sup>1</sup>	95,150	2,439	0.09

**Supplementary Table S5**. Split-GFP analyses investigating interactions of GvpF and GvpL fragments. The fluorescence was measured in LAU/mm<sup>2</sup> and the relative fluorescence calculated.

transformant		o 1 (LAU/mm² (LAU/mm²)		Relative fluorescence (rf)	transfo	transformant		σ (LAU/mm²)	Relative fluorescence (rf)
GvpL fragme	nts				NL133-281	Kc	100,620	13,627	6.15
						сK	19,790	1,166	0.41
<sub>N</sub> L1-134	Gc	50,142	3,124	2.63	L133-281 <sub>N</sub>	Kc	64,662	2,487	3.60
	сG	14,096	266	0.02		сK	19,408	1,092	0.38
L1-134 <sub>N</sub>	Gc	16,343	1,129	0.18	<sub>c</sub> L133-281	K <sub>N</sub>	10,856	2,638	0.00
	сG	15,053	1,148	0.09		NК	14,461	886	0.08
cL1-134	GN	13,412	387	0.00	L133-281c	K <sub>N</sub>	18,570	2,518	0.32
	NG	12,496	620	0.00		NК	29,739	571	1.11
L1-134c	GN	12,312	317	0.00					
	NG	15,944	2,165	0.16					
					<sub>N</sub> L133-281	Mc	36,895	900	1.62
						сM	10,323	949	0.00
<sub>N</sub> L1-134	Jc	34,069	573	0.97	L133-281 <sub>N</sub>	Mc	16,662	560	0.18
	сJ	16,779	1,566	0.03		сM	10,619	811	0.00
L1-134 <sub>N</sub>	J <sub>C</sub>	15,699	1,027	0.00	<sub>c</sub> L133-281	M <sub>N</sub>	14,825	892	0.00
	сJ	15,669	653	0.00		NМ	10,023	871	0.00
cL1-134	J <sub>N</sub>	11,821	1,168	0.00	L133-281c	MN	14,410	711	0.00
	ΝJ	15,406	2,638	0.14		NМ	13,218	2,063	0.00
L1-134c	J <sub>N</sub>	10,553	1,233	0.00					
	ΝJ	12,335	825	0.00					
					GvpF fragme	ents			
NI 1-134	Kc	53 021	3,032	2.84	NF1-110	Ac	21,277	1,773	0.39
1122 201	cK	14 979	405	0.08	11.1.110	cΔ	17 132	1 592	0.12
I 1-134 <sub>N</sub>	Kc	15.034	392	0.09	F1-110 <sub>N</sub>	Ac	20,238	1,677	0.32
	cK	13 236	397	0.00	II IION	ς <b>Δ</b>	18 346	1 568	0.32
d 1-13/	K.	12,230	1 053	0.00	-E1-110	0A A.:	20 836	206	0.20
(L1-134		12,305	201	0.04	(1-110		20,830	200	0.30
11 124	NK	13,249	501	0.00	F1 110	NA A	20,129	912	0.32
L1-134C	NN K	14,500	048	0.05	F1-110C	AN	16,209	1,457	0.19
	ΝK	13,432	1,679	0.05		NA	20,154	1,399	0.32
NI 1-134	Mc	23 506	4,905	0.36	⊾F1-110	A1-22c	21 403	931	0.40
101	cM	12.265	1,518	0.00		c A1-22	20.632	636	0.35
I 1-134 <sub>N</sub>	Mc	12,682	581	0.00	F1-110 <sub>N</sub>	A1-22	21,243	576	0.39
	cM	11 926	968	0.00	N	c Δ1-22	20 265	2 809	0.32
d 1-134	M	11,520	585	0.00	cF1-110	Δ1_22N	21,205	483	0.32
(11 104	M	11 598	318	0.00	01110	Δ1-22	17 377	1 284	0.55
11-134-	N/M	11,550	1 077	0.00	E1_110_	NA1_22	20 320	1,204	0.33
LI-134(	NM	12,740	1,077	0.00	11-1100	N A1-22N	20,320	831	0.58
NL133-281	Gc	48.561	3 402	1.82	NF109-221	Ac	13 664	1 480	0.06
NE133 201	G	13,734	378	0.00	NI 105 221	cA	10,712	575	0.00
1133-281	Ge	27 798	1 1 7 7	0.56	F109-221	Δ.	12 824	704	0.01
L155 201N	-G	1/ 3/2	380	0.00	1105 221N	AL .	12,024	632	0.01
J 133-281	Gu	14,342	1 064	0.00	-F109-221	Δ	9 57/	372	0.00
(L133-201	GN	12 724	1,004	0.00	01109-221	AN A	10.002	372	0.00
1122 201-	NG Gu	14 200	1,213	0.00	E100 221-	NA A.:	14,090	2 2 2 1 0	0.00
L133-2010	GN	14,209	2 1 0 5	0.00	F109-2210	AN	14,000	3,210	0.15
	NG	16,994	2,105	0.04		NA	15,133	2,250	0.15
<sub>N</sub> L133-281	Jc	30.374	1.789	0.71	<sub>N</sub> F109-221	A1-22c	17.370	773	0.30
	d	13.146	735	0.00		cA1-22	9.356	726	0.00
1133-281	Lc.	20,040	1 263	0.13	F109-221	A1-22	12 880	244	0.00
2133 201N	d	12 166	£12	0.15	1 103 ZZIN	cΔ1-77	11 514	2 <del>74</del> Λ71	0.00
d 133-281	Lu Lu	12,100	013 AA7	0.00	-F109-221	Δ1_22.	10 052	471 1721	0.00
(2100-201	5 N	12,221	447 602	0.00	U 103-221		14 710	1,701	0.00
1132-291-	LN LN	11,00	610 610	0.00	F100_221 -	<u>N/1</u> _22	11 202	413	0.10
LT33-2010	JN ]	19,000	210	0.00	1 103-2210	<u>Ω1-</u> ∠∠Ν	11,203 E2 0E2	010	0.00
	NЛ	13,079	52/	0.00		NM1-22	22,923	0,750	5.03