Supplementary Information

Gene-in-gene coding generates a dual-isoform condensate that promotes the ecological resilience of *Vibrio cholerae*

Running title: Dual coding drives *Vibrio* success

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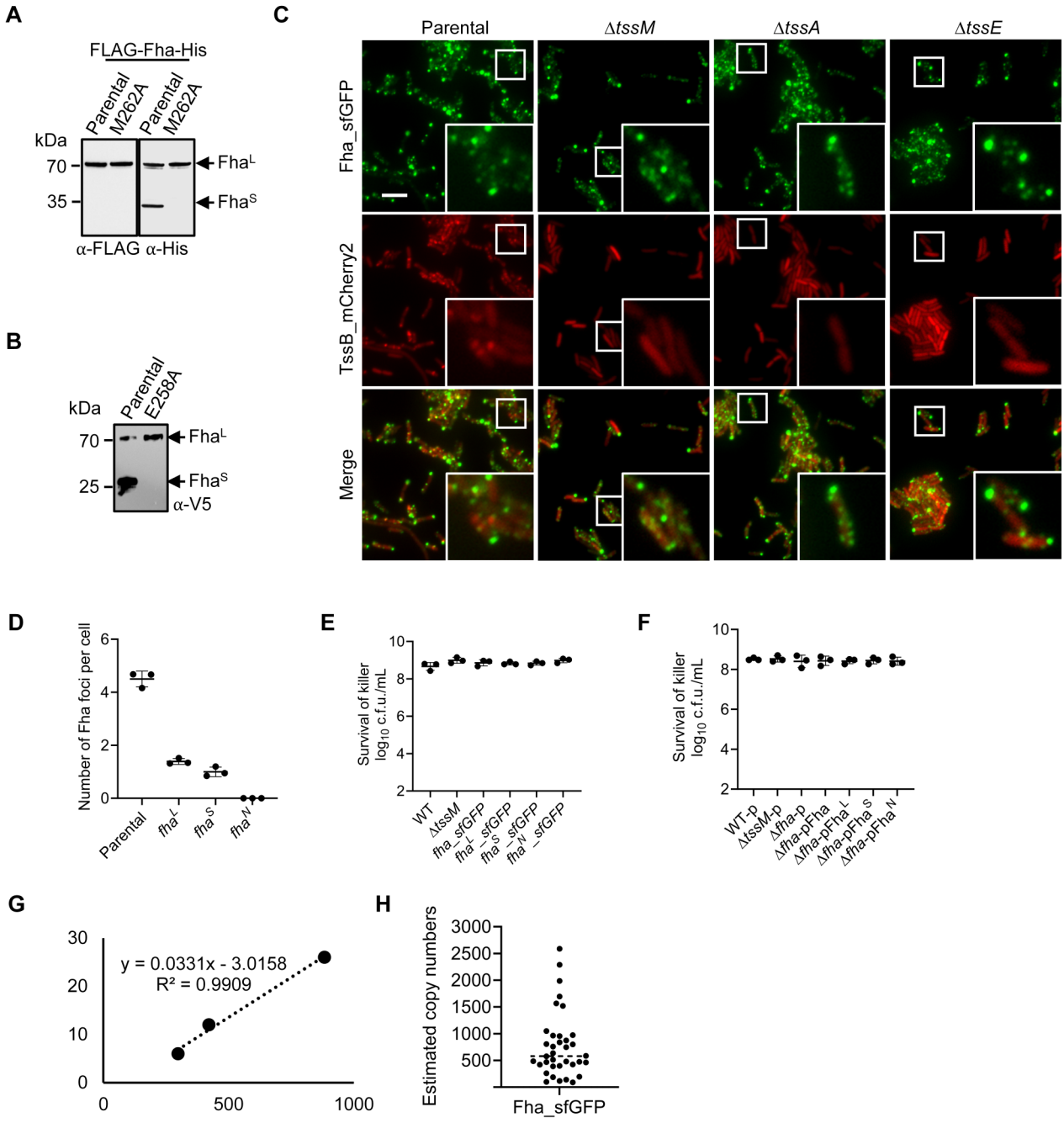
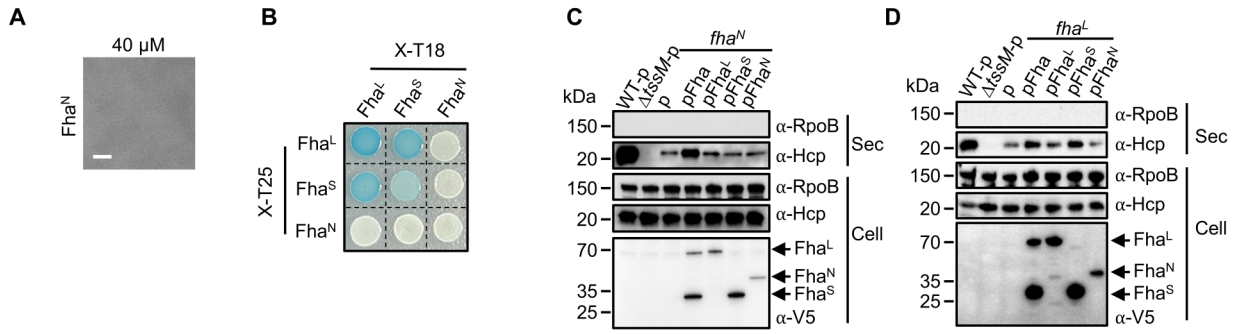
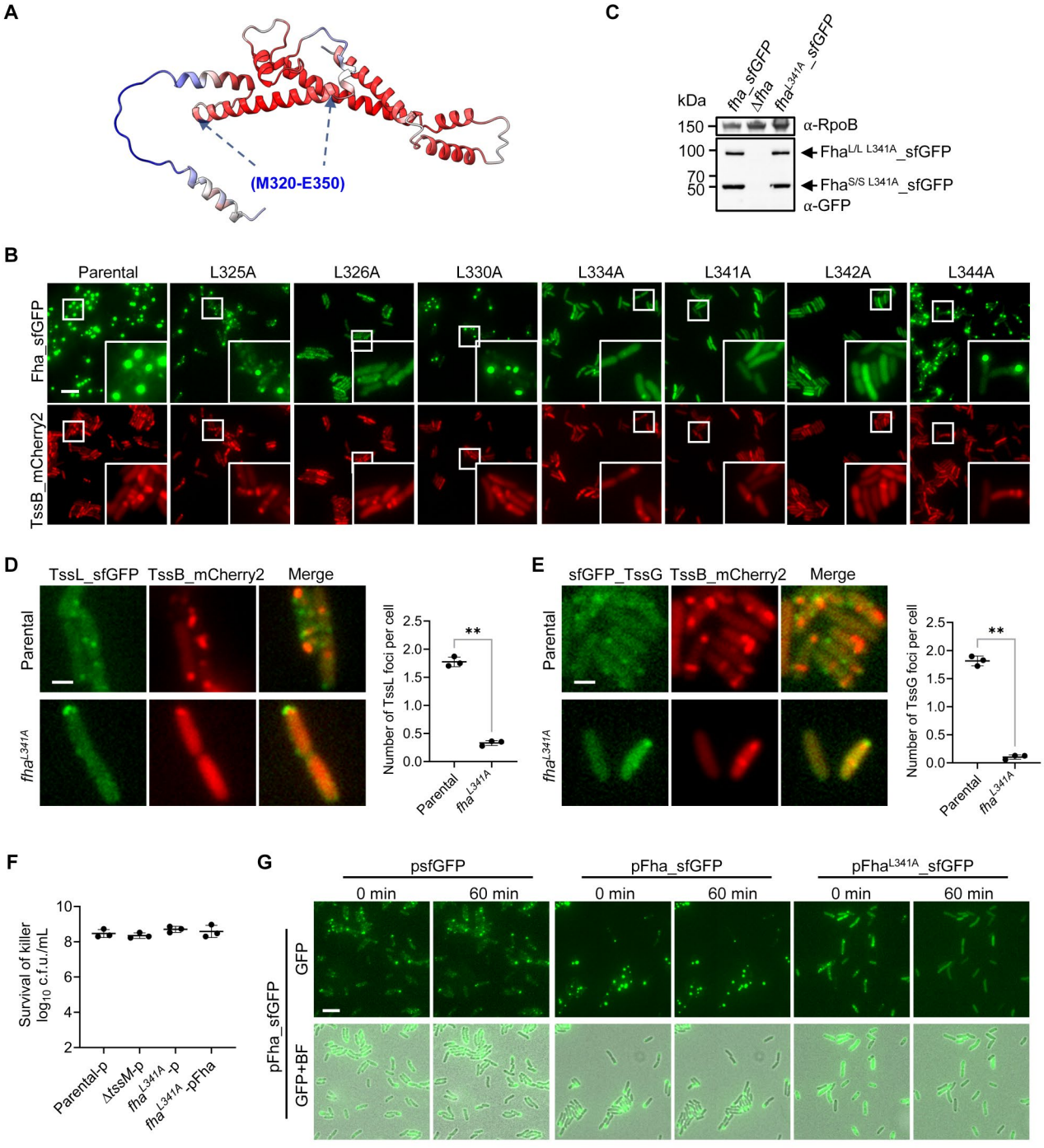


Supplementary Figure 1. Fha^{L} and Fha^{S} contribute to T6SS activities in *V. cholerae* V52. A, Western blotting analysis of Fha and Fha^{M262A}. All constructs were cloned to pET22b vectors with an N-terminal FLAG tag and a C-terminal His tag. Proteins were induced in *E. coli* BL21(DE3) with 1 mM IPTG at 20°C for 2 h. **B**, Western blotting analysis of Fha and Fha^{E258A}. All constructs were cloned to pBAD24 vectors with a C-terminal 3V5 tag. Proteins were induced in *E. coli* with 0.1% arabinose at 30°C for 1 h. **C**, Fluorescence images showing co-localization between Fha_sfGFP and TssB_mCherry2 in V52 Parental (*fha_sfGFP tssB_mCherry2*), Δ*tssM*, Δ*tssA*, and Δ*tssE*. A representative 30- × 30-μm field of cells with a 3× magnified 5- × 5-μm inset (marked by box) is shown. Scale bar: 5 μm. **D**, Quantification of cells forming Fha_sfGFP foci in Parental (*fha_sfGFP tssB_mCherry2*), *fha^{L}*, *fha^{S}*, and *fha^{N}* strains. Each data point represents the number of foci per cell quantified from an individual 30- × 30- μm field of view. **E**, Survival of killer strains during competition assays for which the survival of

the prey is depicted in Figure 2G. **F**. Survival of killer strains during competition assays for which the survival of the prey is depicted in Figure 2I. For E and F, error bars indicate the mean +/- standard deviation of three biological replicates. **G**, Calibration of fluorescence intensity using $LacI^{mut}$_sfGFP-bound *lacO* arrays. The fluorescence intensities of sfGFP foci were quantified in *V. cholerae* V52 strains carrying *lacO3*, *lacO6*, and *lacO13* arrays. Assuming that each *lacO* site binds two copies of $LacI^{mut}$_sfGFP, the number of bound fluorophores was used to generate a standard curve correlating sfGFP copy number with measured fluorescence intensity. **H**, Estimated sfGFP-equivalent copy numbers of Fha foci. Raw fluorescence intensities of Fha foci in *V. cholerae* V52 were converted into estimated sfGFP copy numbers using the calibration established in G.

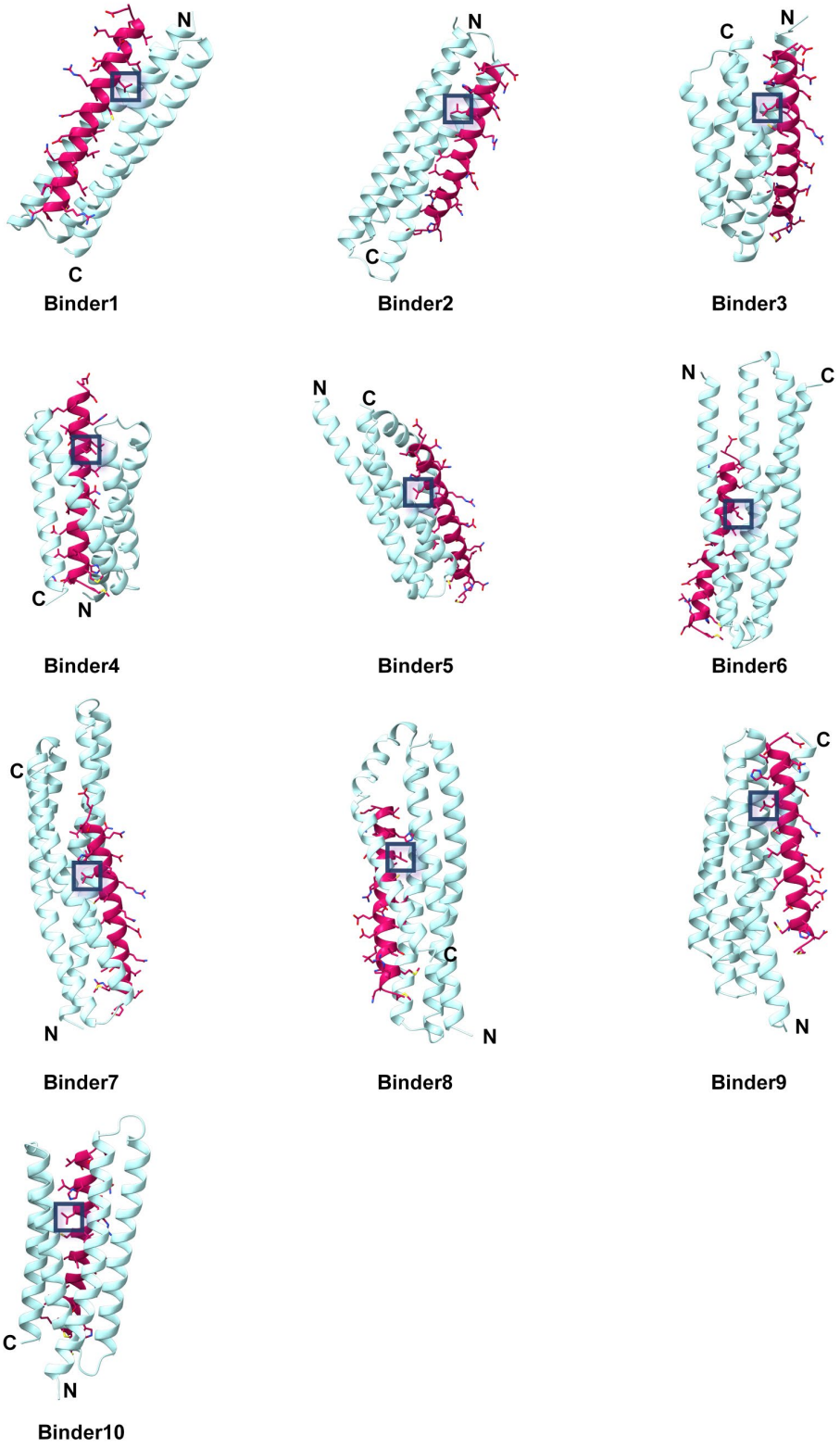


Supplementary Figure 2. Functional characterization of Fha^{L} and Fha^{S}. A, DIC images of Fha^{N} at the indicated concentration. Scale bar: 5 μm. **B**, Bacterial two-hybrid analysis of Fha^{L}, Fha^{S}, and Fha^{N}. Proteins fused with the adenylate cyclase T25 or T18 subunits were co-expressed in the BTH101 reporter strain as indicated. A positive interaction is indicated by color development on X-Gal plates. **C**, Secretion analysis of Hcp in V52 wild-type, T6SS-null mutant Δ*tssM*, *fha*^{N}, and *fha*^{N} strains expressing plasmid-borne Fha and its variants, as indicated. **D**, Secretion analysis of Hcp in V52 wild-type, T6SS-null mutant Δ*tssM*, *fha*^{L}, and *fha*^{L} strains expressing plasmid-borne Fha and its variants, as indicated. For C and D, RpoB serves as a control for equal loading and autolysis.

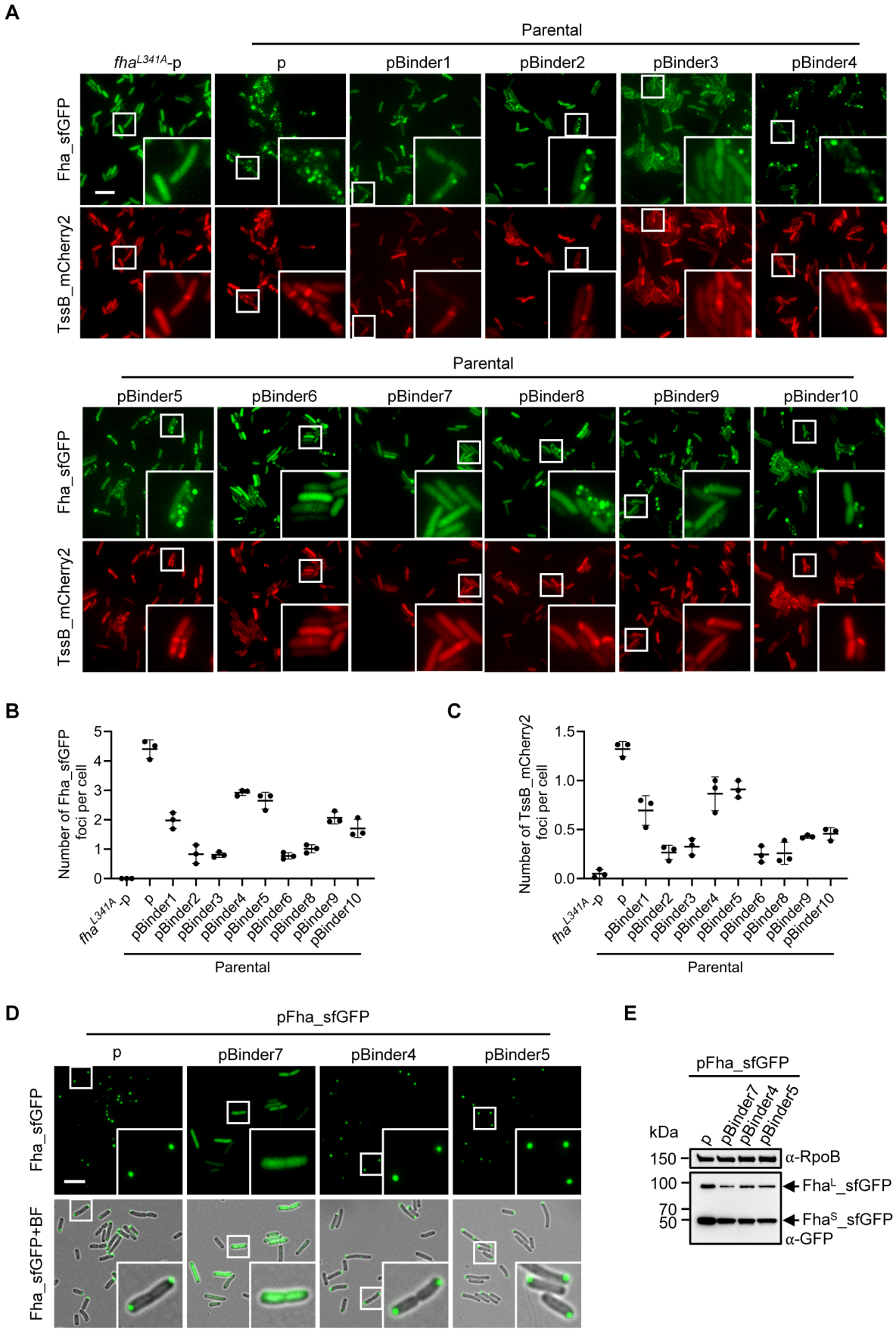


Supplementary Figure 3. Identification of LLPS-disrupting mutations in Fha. A, Predicted structures of Fha^{S} using AlphaFold2. The predicted structures are colored based on the reported confidence of the AlphaFold modeling, from blue (pLDDT < 50) to red (pLDDT > 70). **B**, Fluorescence images showing the subcellular localization of plasmid-expressed Fha_sfGFP and its mutants in the V52 Δ*fha tssB_mCherry2* strain. Proteins were induced with 0.01% arabinose for 40 min prior to imaging. A representative 30- × 30-μm field of cells with a 3× magnified 5- × 5-μm inset (marked by box) is shown. Scale bar: 5 μm. **C**, Western blotting analysis of chromosomally encoded Fha in V52 *fha_sfGFP*, Δ*fha*, and *fha*^{L341A}*_sfGFP* strains. RpoB serves as a control for equal loading. **D**,

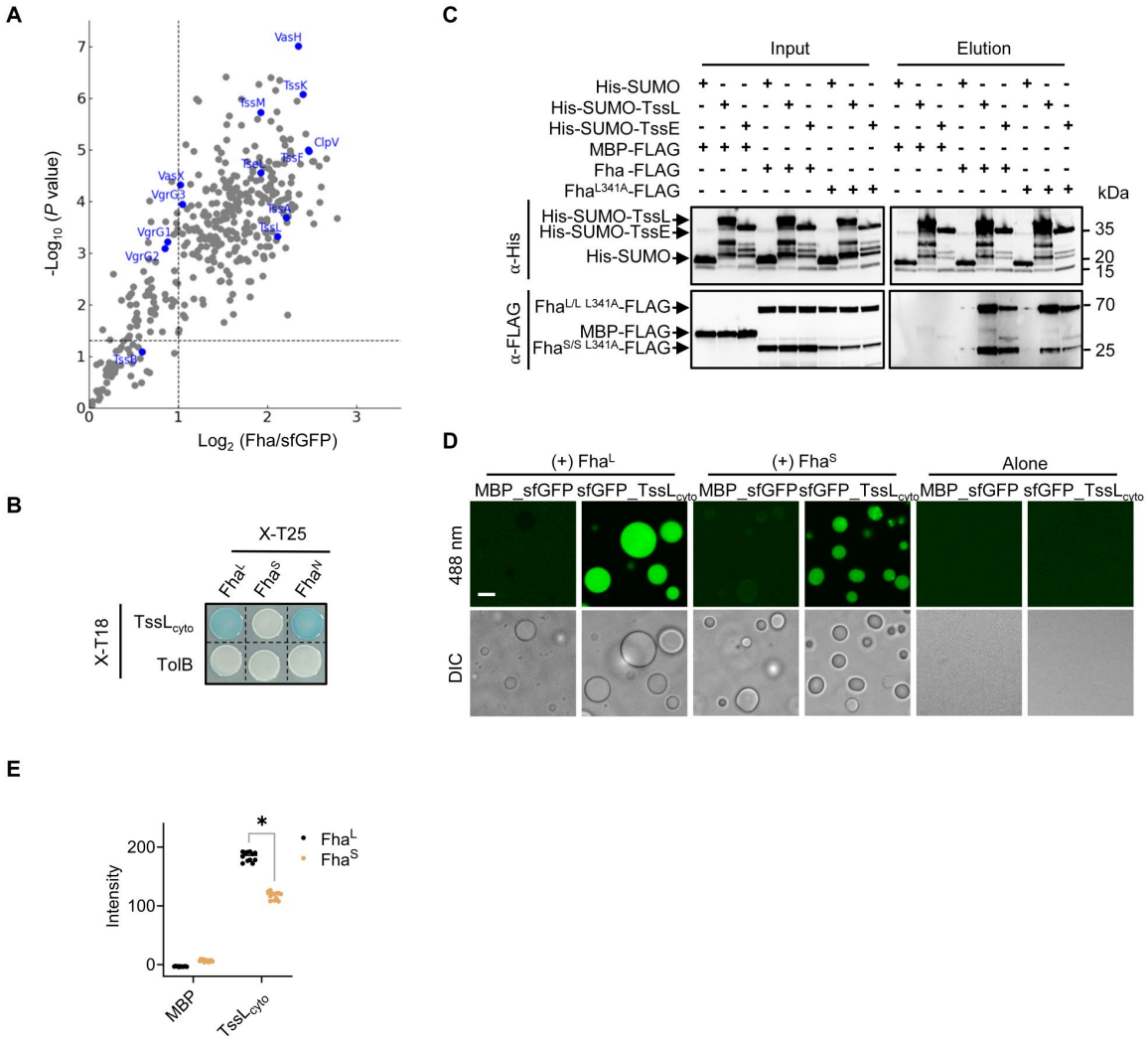
Fluorescence images showing co-localization between TssL_sfGFP and TssB_mCherry2 in V52 Parental (*tssL_sfGFP tssB_mCherry2*) and *fha*^{L341A}. A representative 5- × 5-μm field of cells is shown. Scale bar: 1 μm. Quantification of cells forming TssL_sfGFP foci in Parental (*tssL_sfGFP tssB_mCherry2*) and *fha*^{L341A} is shown on the right. **E**, Fluorescence images showing co-localization between sfGFP_TssG and TssB_mCherry2 in V52 Parental (*sfGFP_tssG tssB_mCherry2*) and *fha*^{L341A}. A representative 5- × 5-μm field of cells is shown. Scale bar: 1 μm. Quantification of cells forming sfGFP_TssG foci in Parental (*sfGFP_tssG tssB_mCherry2*) and *fha*^{L341A} is shown on the right. Each data point represents the number of foci per cell quantified from an individual 30- × 30- μm field of view. **F**, Survival of killer strains during competition assays for which the survival of the prey is depicted in Figure 3D. Error bars indicate the mean +/- standard deviation of three biological replicates. **G**, Time-lapse imaging of the V52 *fha_sfGFP* strain expressing plasmid-borne sfGFP, Fha_sfGFP, or Fha^{L341A}_sfGFP. Cells were imaged at 0 min and 60 min in the same field of view to monitor changes in pre-existing Fha_sfGFP foci. Proteins were induced for 1 h with 0.01% arabinose prior to imaging. A representative 30- × 30-μm field is shown. Scale bar: 5 μm.



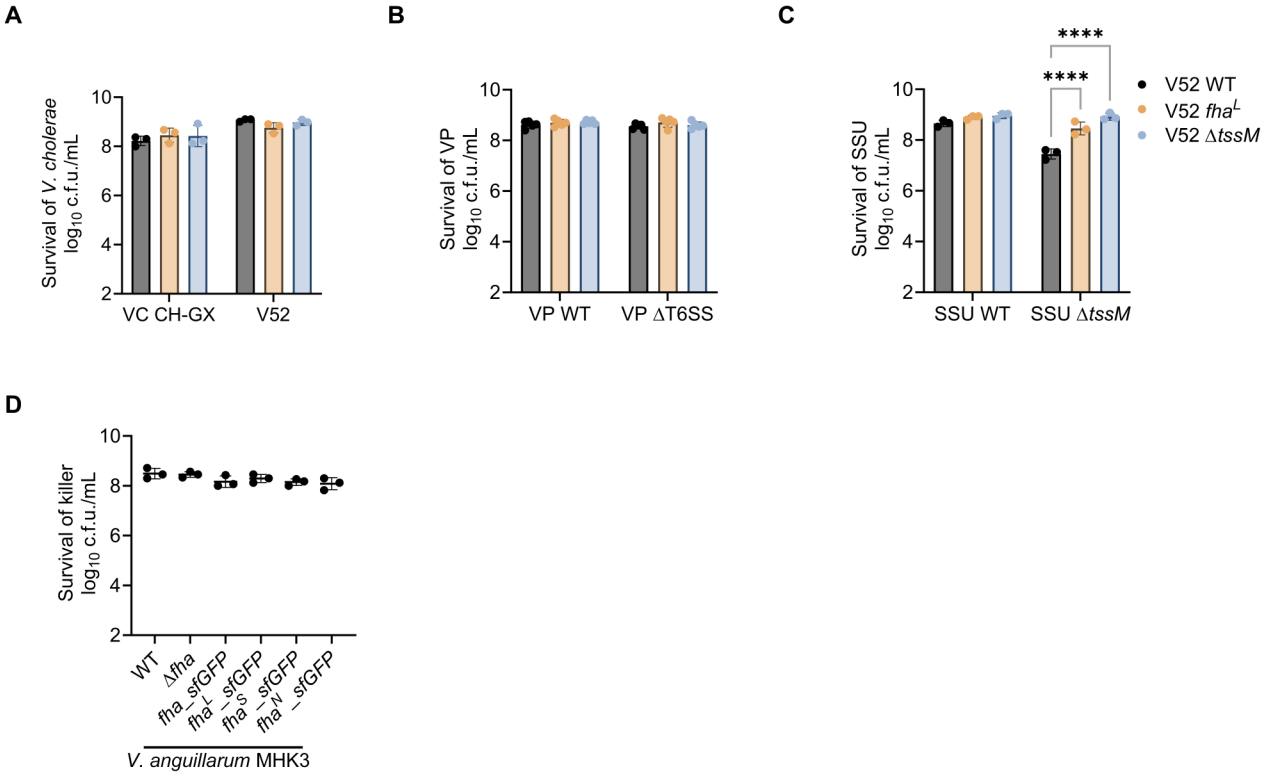
Supplementary Figure 4. AlphaFold2-predicted structures of the Fha leucine-rich helix in complex with designed binders. The Fha leucine-rich helix is shown in magenta, and the binders are shown in light blue, with their N- and C-termini indicated. Residue L341 of Fha is highlighted with a blue box.


A
Parental
fhaL341A-p
p
pBinder1
pBinder2
pBinder3
pBinder4
Fha_sfGFP
TssB_mCherry2
Parental
pBinder5
pBinder6
pBinder7
pBinder8
pBinder9
pBinder10
Fha_sfGFP
TssB_mCherry2
B
Number of Fha_sfGFP foci per cell
0
1
2
3
4
5
fhaL341A-p
p
pBinder1
pBinder2
pBinder3
pBinder4
pBinder5
pBinder6
pBinder8
pBinder9
pBinder10
Parental
C
Number of TssB_mCherry2 foci per cell
0.0
0.5
1.0
1.5
fhaL341A-p
p
pBinder1
pBinder2
pBinder3
pBinder4
pBinder5
pBinder6
pBinder8
pBinder9
pBinder10
Parental
D
pFha_sfGFP
p
pBinder7
pBinder4
pBinder5
Fha_sfGFP
Fha_sfGFP+BF
E
pFha_sfGFP
p
pBinder7
pBinder4
pBinder5
kDa
150
100
70
50
α-RpoB
FhaL_sfGFP
FhaS_sfGFP
α-GFP

Supplementary Figure 5. Designed binders impair Fha foci formation and T6SS assembly. A, Fluorescence images showing the localization of chromosomally encoded Fha_sfGFP and TssB_mCherry2 in the V52 Parental (*fha_sfGFP tssB_mCherry2*) strains expressing designed binders. Binders were induced with 0.1% arabinose. **B**, Quantification of cells forming Fha_sfGFP foci in V52 Parental (*fha_sfGFP tssB_mCherry2*) strains expressing designed binders. **C**, Quantification of cells forming TssB_mCherry2 foci in V52 *fha_sfGFP tssB_mCherry2* strains expressing designed binders. Each data point represents the number of foci per cell quantified from an individual 30- × 30- μm field of view. Data points for the Parental carrying the empty vector and the fha^{L341A} mutant carrying the empty vector are reused from Fig. 3g (Fha_sfGFP foci) in B and from Fig. 3h (TssB_mCherry2 foci) in C. **D**, Fluorescence microscopy of plasmid-borne Fha_sfGFP in *E. coli* strains expressing designed binders. Fha_sfGFP and Binders were induced with 0.01% arabinose for 1 h before imaging. For A and D, a representative 30- × 30-μm field of cells with a 3× magnified 5- × 5-μm inset (marked by box) is shown. Scale bar: 5 μm. **E**, Western blotting analysis of plasmid-borne Fha_sfGFP expression in the *E. coli* strains shown in D. RpoB serves as a control for equal loading.



Supplementary Figure 6. Fha^{L} and Fha^{S} recruit distinct T6SS components. A, Volcano plot showing differentially enriched proteins identified by mass spectrometry in the pull-down assay. Lysates from V52 strains overexpressing the Strep-tagged Fha or sfGFP were used. Proteins enriched in the Fha pull-down (experimental group) compared to the sfGFP control (control group) were identified via mass spectrometry. The x-axis represents the $\log_2$ fold change in abundance of the identified proteins between the Fha and sfGFP samples, while the y-axis represents the $-\log_{10}$ adjusted *P*-value. Predicted T6SS-related proteins are highlighted in blue, and non-T6SS proteins are shown in gray. The Fha protein itself was excluded from this plot due to its exceptionally high abundance. All proteins identified by mass spectrometry are listed in Supplementary Data 4, and only proteins with 10 or more peptide hits were used to generate this volcano plot. **B**, Bacterial two-hybrid analysis of TssL cytoplasmic domain (TssL$_{cyto}$) interactions with Fha^{L}, Fha^{S}, and Fha^{N}. Proteins fused with the adenylate cyclase T25 or T18 subunits were co-expressed in the BTH101 reporter strain as indicated. A positive interaction is indicated by color development on X-Gal plates. **C**, Interaction of Fha and Fha^{L341A} with TssL and TssE. Pull-down analysis was conducted using His-tagged SUMO (control), SUMO-TssL, or SUMO-TssE, and FLAG-tagged MBP (control), Fha, or Fha^{L341A}. **D**, Fluorescence images showing recruitment of the TssL cytoplasmic domain (TssL$_{cyto}$) into Fha^{L} and Fha^{S} droplets. Fha^{L} and Fha^{S} proteins were mixed with MBP_sfGFP or sfGFP_TssL$_{cyto}$ at a ratio of 10: 1 in a 10% dextran-70 solution before imaging, respectively. A representative 30- × 30-μm field is shown. Scale bar: 5 μm. **E**, Quantification of GFP fluorescence intensity within Fha^{L} and Fha^{S} droplets. Statistical significance was calculated using Student's *t*-test, $*P < 0.05$.



Supplementary Figure 7. *fha*^{L} mutants display impaired inter- and intra-bacterial killing activities. A-D. Survival of killer strains during competition assays for which the survival of the prey is depicted in Figure 4 A-C, and 5 E. Error bars indicate the mean +/- standard deviation of at least three biological replicates and statistical significance was calculated using One-way ANOVA test for each group, ****$P < 0.0001$.

Supplementary Table 1. Plasmids, strains, and primers

Plasmid	Description	Reference
pDS132	Suicidal conjugation vector for all chromosomal allelic changes	(1)
pDS132-tssM	Suicidal vector to construct the V52 in-frame deletion mutant of *tssM*	(2)
pDS132-tssA	Suicidal vector to construct the V52 in-frame deletion mutant of *tssA*	(3)
pDS132-tssE	Suicidal vector to construct the V52 in-frame deletion mutant of *tssE*	This study
pDS132-fha	Suicidal vector to construct the V52 in-frame deletion mutant of *fha*	This study
pDS132-fhaM262A	Suicidal vector to construct the V52 mutant of fha^{L}	This study
pDS132-fhaM1A	Suicidal vector to construct the V52 mutant of fha^{S}	This study
pDS132-fha1-261aa	Suicidal vector to construct the V52 mutant of fha^{N}	This study
pDS132-fhaL341A	Suicidal vector to construct the V52 mutant of fha^{L341A}	This study
pDS132-fha_sfGFP	Suicidal vector to construct the V52 mutant with chromosomal insertion Fha_sfGFP	This study
pDS132-tssB_mCherry2	Suicidal vector to construct the V52 mutant with chromosomal insertion TssB_mCherry2	Lab stock
pDS132-tssL_sfGFP	Suicidal vector to construct the V52 mutant with chromosomal insertion TssL_sfGFP	This study
pDS132-sfGFP_tssG	Suicidal vector to construct the V52 mutant with chromosomal insertion sfGFP_TssG	Lab stock
pDS132-MHK3 tssM	Suicidal vector to construct the MHK3 in-frame deletion mutant of *tssM*	This study
pDS132-MHK3 fha_sfGFP	Suicidal vector to construct the MHK3 mutant with chromosomal insertion Fha_sfGFP	This study
pDS132-MHK3 fhaM263A	Suicidal vector to construct the MHK3 mutant of fha^{L}	This study
pDS132-MHK3 fhaM1A	Suicidal vector to construct the MHK3 mutant of fha^{S}	This study
pDS132-MHK3 fha1-262aa	Suicidal vector to construct the MHK3 mutant of fha^{N}	This study
pPSV37	IPTG inducible expression vector, gentamicin resistance	Lab stock
pPSV37-sfGFP	IPTG inducible expression of sfGFP	Lab stock
pBAD18	Arabinose inducible expression vector, kanamycin resistance	Lab stock
pBAD18-sfGFP	Arabinose inducible expression of sfGFP	Lab stock
pBAD24	Arabinose inducible expression vector, kanamycin resistance	Lab stock
pBAD24-Fha-sfGFP	Arabinose inducible expression of Fha_sfGFP	This study
pBAD24-FhaL325A-sfGFP	Arabinose inducible expression of Fha^{L325A}_sfGFP	This study
pBAD24-FhaL326A-sfGFP	Arabinose inducible expression of Fha^{L326A}_sfGFP	This study
pBAD24-FhaL330A-sfGFP	Arabinose inducible expression of Fha^{L330A}_sfGFP	This study
pBAD24-FhaL334A-sfGFP	Arabinose inducible expression of Fha^{L334A}_sfGFP	This study
pBAD24-FhaL341A-sfGFP	Arabinose inducible expression of Fha^{L341A}_sfGFP	This study
pBAD24-FhaL342A-sfGFP	Arabinose inducible expression of Fha^{L342A}_sfGFP	This study
pBAD24-FhaL344A-sfGFP	Arabinose inducible expression of Fha^{L344A}_sfGFP	This study

pBAD24-Fha-3V5	Arabinose inducible expression of Fha with a C-terminal 3V5 tag	This study
pBAD24-Fha^{E258A}-3V5	Arabinose inducible expression of Fha^{E258A} with a C-terminal 3V5 tag	This study
pBAD24-Fha^{M262A}-3V5	Arabinose inducible expression of Fha^{L} with a C-terminal 3V5 tag	This study
pBAD24-Fha$^{\Delta 1\text{-}261aa}$ -3V5	Arabinose inducible expression of Fha^{S} with a C-terminal 3V5 tag	This study
pBAD24-Fha$^{1\text{-}261aa}$-3V5	Arabinose inducible expression of Fha^{N} with a C-terminal 3V5 tag	This study
pBAD24-Fha$^{\Delta 1\text{-}261aa}$-FLAG	Arabinose inducible expression of Fha^{S} with a C-terminal FLAG tag	This study
pBAD24-Fha$^{1\text{-}261aa}$-FLAG	Arabinose inducible expression of Fha^{N} with a C-terminal FLAG tag	This study
pBAD24-Binder1	Arabinose inducible expression of Binder1	This study
pBAD24-Binder2	Arabinose inducible expression of Binder2	This study
pBAD24-Binder3	Arabinose inducible expression of Binder3	This study
pBAD24-Binder4	Arabinose inducible expression of Binder4	This study
pBAD24-Binder5	Arabinose inducible expression of Binder5	This study
pBAD24-Binder6	Arabinose inducible expression of Binder6	This study
pBAD24-Binder7	Arabinose inducible expression of Binder7	This study
pBAD24-Binder8	Arabinose inducible expression of Binder8	This study
pBAD24-Binder9	Arabinose inducible expression of Binder9	This study
pBAD24-Binder10	Arabinose inducible expression of Binder10	This study
pBAD24-MBP-FLAG	Arabinose inducible expression of MBP with a C-terminal FLAG tag	Lab stock
pBAD24-TssL-FLAG	Arabinose inducible expression of TssL with a C-terminal FLAG tag	This study
pBAD24-TssE-FLAG	Arabinose inducible expression of TssE with a C-terminal FLAG tag	This study
pBAD24-LacI^{mut}-sfGFP	Arabinose inducible expression of LacI^{mut}_sfGFP	(4)
pBBR1MCS2-Fha-FLAG	Constitutive expression of Fha with a C-terminal FLAG tag	This study
pBBR1MCS2-Fha^{M262A}-FLAG	Constitutive expression of Fha^{L} with a C-terminal FLAG tag	This study
pBBR1MCS2-Fha^{L341A}-FLAG	Constitutive expression of Fha^{L341A} with a C-terminal FLAG tag	This study
pET-2Strep-Fha^{M262A}	IPTG inducible expression of Fha^{L} with a N-terminal 2Strep tag	This study
pET-2Strep-Fha$^{\Delta 1\text{-}261aa}$	IPTG inducible expression of Fha^{S} with a N-terminal 2Strep tag	This study
pET-2Strep-Fha$^{1\text{-}261aa}$	IPTG inducible expression of Fha^{N} with a N-terminal 2Strep tag	This study
pET22b-FLAG-Fha-His	IPTG inducible expression of Fha with a N-terminal FLAG tag and a C-terminal His tag	This study
pET22b-FLAG-Fha^{M262A}-His	IPTG inducible expression of Fha^{L} with a N-terminal FLAG tag and a C-terminal His tag	This study
pET22b-Fha-His	IPTG inducible expression of Fha with a C-terminal His tag	This study
pET22b-Fha^{M262A}-His	IPTG inducible expression of Fha^{L} with a C-terminal His tag	This study
pET22b-Fha^{M262A}-sfGFP-His	IPTG inducible expression of Fha^{L}_sfGFP with a C-terminal His tag	This study
pET22b-Fha$^{\Delta 1\text{-}261aa}$-sfGFP-His	IPTG inducible expression of Fha^{S}_sfGFP with a C-terminal His tag	This study

pET22b-Fha$^{1\text{-}261aa}$-sfGFP-His	IPTG inducible expression of Fha^{N}_sfGFP with a C-terminal His tag	This study
pET22b-sfGFP-His	IPTG inducible expression of TssA with a C-terminal His tag	This study
pETSUMO	IPTG inducible expression of His-SUMO	Lab stock
pETSUMO-TssL	IPTG inducible expression of TssL with an N-terminal His-SUMO tag	This study
pETSUMO-TssE	IPTG inducible expression of TssE with an N-terminal His-SUMO tag	This study
pCH363-Fha^{M262A}	IPTG inducible expression of Fha^{L} for bacterial two-hybrid analysis	This study
pCH363-Fha$^{\Delta 1\text{-}261aa}$	IPTG inducible expression of Fha^{S} for bacterial two-hybrid analysis	This study
pCH363-Fha$^{1\text{-}261aa}$	IPTG inducible expression of Fha^{N} for bacterial two-hybrid analysis	This study
pCH363-TssL$_{cyto}$	IPTG inducible expression of TssL$_{cyto}$ for bacterial two-hybrid analysis	This study
pKNT25-Fha^{M262A}	IPTG inducible expression of Fha^{L} for bacterial two-hybrid analysis	This study
pKNT25-Fha$^{\Delta 1\text{-}261aa}$	IPTG inducible expression of Fha^{S} for bacterial two-hybrid analysis	This study
pKNT25-Fha$^{1\text{-}261aa}$	IPTG inducible expression of Fha^{N} for bacterial two-hybrid analysis	This study
pET22b-85003 Fha-His	IPTG inducible expression of 85003 Fha with a C-terminal His tag	This study
pET22b-85003 Fha^{M265A}-His	IPTG inducible expression of 85003 Fha^{M265A} with a C-terminal His tag	This study
pET22b-106-2A Fha-His	IPTG inducible expression of 106-2A Fha with a C-terminal His tag	This study
pET22b-106-2A Fha^{M260A}-His	IPTG inducible expression of 106-2A Fha^{M260A} with a C-terminal His tag	This study
pET22b-MHK3 Fha-His	IPTG inducible expression of MHK3 Fha with a C-terminal His tag	This study
pET22b-MHK3 Fha^{M263A}-His	IPTG inducible expression of MHK3 Fha^{M263A} with a C-terminal His tag	This study

Strain	**Genotype**	**Description**	**Reference**
Vibrio cholerae			
V52	Parental	Δ*hlyA* Δ*hapA* Δ*rtxA*, parental strain	(5)
	Δ*tssM*	In-frame deletion of *tssM*	(2)
	Δ*fha*	In-frame deletion of *fha*	This study
	fha^{L}	Chromosomal mutation of Fha^{M262A}	This study
	fha^{S}	Chromosomal mutation of Fha^{M1A}	This study
	fha_sfGFP	Chromosomal fusion of the Fha_sfGFP	This study
	fha^{L}_sfGFP	*fha_sfGFP* with chromosomal mutation of Fha^{M262A}	This study
	fha^{S}_sfGFP	*fha_sfGFP* with chromosomal mutation of Fha^{M1A}	This study
	fha^{N}_sfGFP	*fha_sfGFP* with chromosomal mutation of Fha$^{1\text{-}261aa}$	This study
	fha_sfGFP tssB_mCherry2	Chromosomal fusion of the Fha_sfGFP and TssB_mCherry2	This study
	fha^{L}_sfGFP tssB_mCherry2	*fha_sfGFP tssB_mCherry2* with chromosomal mutation of Fha^{M262A}	This study

	fha^{S}_sfGFP *tssB_mCherry2*	*fha_sfGFP tssB_mCherry2* with chromosomal mutation of Fha^{M1A}	This study
	fha^{N}_sfGFP *tssB_mCherry2*	*fha_sfGFP tssB_mCherry2* with chromosomal mutation of Fha$^{1\text{-}261aa}$	This study
	fha^{L341A}_sfGFP *tssB_mCherry2*	*fha_sfGFP tssB_mCherry2* with chromosomal mutation of Fha^{L341A}	This study
	fha_sfGFP tssB_mCherry2 Δ*tssM*	*fha_sfGFP tssB_mCherry2* with in-frame deletion of *tssM*	This study
	fha_sfGFP tssB_mCherry2 Δ*tssA*	*fha_sfGFP tssB_mCherry2* with in-frame deletion of *tssA*	This study
	fha_sfGFP tssB_mCherry2 Δ*tssE*	*fha_sfGFP tssB_mCherry2* with in-frame deletion of *tssE*	This study
	tssL_sfGFP tssB_mCherry2	Chromosomal fusion of the TssL_sfGFP and TssB_mCherry2	This study
	tssL_sfGFP tssB_mCherry2 Δ*fha*	*tssL_sfGFP tssB_mCherry2* with in-frame deletion of *fha*	This study
	tssL_sfGFP tssB_mCherry2 fha^{L341A}	*tssL_sfGFP tssB_mCherry2* with chromosomal mutation of Fha^{L341A}	This study
	sfGFP_tssG tssB_mCherry2	Chromosomal fusion of the sfGFP_TssG and TssB_mCherry2	This study
	sfGFP_tssG tssB_mCherry2 Δ*fha*	*sfGFP_tssG tssB_mCherry2* with in-frame deletion of *fha*	This study
	sfGFP_tssG tssB_mCherry2 fha^{L341A}	*sfGFP_tssG tssB_mCherry2* with chromosomal mutation of Fha^{L341A}	This study
	lacZ::lacO3	The chromosomal *lacZ* locus was replaced with three tandem *lacO* operator repeats (*lacO3*)	(4)
	lacZ::lacO6	The chromosomal *lacZ* locus was replaced with six tandem *lacO* operator repeats (*lacO6*)	(4)
	lacZ::lacO13	The chromosomal *lacZ* locus was replaced with thirteen tandem *lacO* operator repeats (*lacO13*)	(4)
C6706	Parental	Parental strain	Lab stock
	Δ*fha*	In-frame deletion of *fha*	This study
	fha^{L}	Chromosomal mutation of Fha^{M262A}	This study
	fha^{S}	Chromosomal mutation of Fha^{M1A}	This study
30167		Strain used for testing the expression of *fha*	Lab stock
523-955		Strain used for testing the expression of *fha*	Lab stock
3223		Strain used for testing the expression of *fha*	Lab stock
622-37		Strain used for testing the expression of *fha*	Lab stock
504-931		Strain used for testing the expression of *fha*	Lab stock
1742-79		Strain used for testing the expression of *fha*	Lab stock

VO4175		Strain used for testing the expression of *fha*	Lab stock
SCE258		Strain used for testing the expression of *fha*	Lab stock
pv145		Strain used for testing the expression of *fha*	Lab stock
M4		Strain used for testing the expression of *fha*	Lab stock
Haitian		Strain used for testing the expression of *fha*	Lab stock
Bangladesh 2006		Strain used for testing the expression of *fha*	Lab stock
CH-GX-YL-YZ-T2-3-2021		Strain used for competition assay	Fu lab
Vibrio anguillarum			
MHK3	Parental	Parental strain	Mo lab
	Δ*fha*	In-frame deletion of *fha*	Mo lab
	Δ*tssM*	In-frame deletion of *tssM*	This study
	fha_sfGFP	Chromosomal fusion of the Fha_sfGFP	This study
	fha^{L}*_sfGFP*	*fha_sfGFP* with chromosomal mutation of Fha^{M263A}	This study
	fha^{S}*_sfGFP*	*fha_sfGFP* with chromosomal mutation of Fha^{M1A}	This study
	fha^{N}*_sfGFP*	*fha_sfGFP* with chromosomal mutation of $Fha^{1\text{-}262aa}$	This study
Vibrio parahaemolyticus			
RIMD2210633	Parental	Parental strain	Lab stock
	Δ*tssM1&2*	In-frame deletion of *tssM1* and *tssM2*	Lab stock
Aeromonas dhakensis			
SSU	Parental	Parental strain	Lab stock
	Δ*tssM*	In-frame deletion of *tssM*	Lab stock
Escherichia coli			
T-Fast		Strain used for cloning and gene expression	TIANGEN
WM6026		Strain used for conjugation	Lab stock
BL21(DE3)		Strain used for protein expression	Lab stock
MG1655		Strain used for competition assay	Lab stock

Primer	**Sequence (5'-3')**	**Description**
pDS132-hifi-f	cttctagaggtaccgcatgcgatatc	Forward primer to amplify pDS132 vector
pDS132-hifi-r	cgatccttttttaacccatcacatatacct	Reverse primer to amplify pDS132 vector
pDS132-f	tgttgcatgggcataaagttgc	Forward confirmation primer of pDS132 vector
pDS132-r	acggctgacatgggaattcc	Reverse confirmation primer of pDS132 vector

0112-KO1	gtgatgggttaaaaaggatcgcaagcctctccgttgagcttg	Forward primer to amplify the upstream of *vca0112* for constructing in-frame deletion of *vca0112*
0112-KO2	ttatagctccagttgtgtcactgagttcatggttattgccttac	Reverse primer to amplify the upstream of *vca0112* for constructing in-frame deletion of *vca0112*
0112-KO3	atgaactcagtgacacaactggagctataaccgtgaacaaagt	Forward primer to amplify the downstream of *vca0112* for constructing in-frame deletion of *vca0112*
0112-KO4	gcatgcggtacctctagaagcaactcatgcttgagcgagagg	Reverse primer to amplify the downstream of *vca0112* for constructing in-frame deletion of *vca0112*
0112-KO5	agtcgtggttaacatcagcaatcaag	Forward primer to confirm the in-frame deletion of *vca0112*
0112-KO6	catcccttgtcacttgaatgtagtgg	Reverse primer to confirm the in-frame deletion of *vca0112*
0112C-KO1	gtgatgggttaaaaaggatcgagtgctgtttggtaacgccatcg	Forward primer to amplify *vca0112* for constructing fha^N
0112C-KO2	gttcacggttatgtgaagccctctgatacgttgctttc	Reverse primer to amplify *vca0112* for constructing fha^N
0112C-KO3	gagggcttcacataaccgtgaacaaagtgctgtggatg	Forward primer to amplify the downstream of *vca0112* for constructing fha^N
0112C-KO4	gcatgcggtacctctagaagcattccagatcactcggttacgc	Reverse primer to amplify the downstream of *vca0112* for constructing fha^N
0112-M1A-mut1	gtgatgggttaaaaaggatcgagtgctgtttggtaacgccatcg	Forward primer to amplify the upstream of *vca0112* for constructing fha^N
0112-M1A-mut2	gttcacggttatgtgaagccctctgatacgttgctttc	Reverse primer to amplify the upstream of *vca0112* for constructing fha^N
0112-M1A-mut3	gagggcttcacataaccgtgaacaaagtgctgtggatg	Forward primer to amplify *vca0112* for constructing fha^S
0112-M1A-mut4	gcatgcggtacctctagaagcattccagatcactcggttacgc	Reverse primer to amplify *vca0112* for constructing fha^S
0112-M262A-mut1	gtgatgggttaaaaaggatcgccatgcactgtgaagtgatgatg	Forward primer to amplify *vca0112* for constructing fha^L
0112-M262A-mut2	tccagtactttctcgtcagctgtgaagccctctgatacgttgc	Reverse primer to amplify *vca0112* for constructing fha^L
0112-M262A-mut3	cacagctgacgagaaagtactggatttgttagaagaggaagtcg	Forward primer to amplify *vca0112* for constructing fha^L
0112-M262A-mut4	gcatgcggtacctctagaaggttggcggttagaggtcagctc	Reverse primer to amplify *vca0112* for constructing fha^L
0109-KO1	tgatgggttaaaaaggatcgatggcgcatgctcctttcat	Forward primer to amplify the upstream of *vca0109* for constructing in-frame deletion of *vca0109*
0109-KO2	caagcctgattatgacgtacatcgcatatcgagtgtttta	Reverse primer to amplify the upstream of *vca0109* for constructing in-frame deletion of *vca0109*
0109-KO3	catcgcatatcgagtgttttaacctctatgacgcaagac	Forward primer to amplify the downstream of *vca0109* for constructing in-frame deletion of *vca0109*
0109-KO4	gatttgactgggcgtcgttcacttctagaggtaccgcatg	Reverse primer to amplify the downstream of *vca0109* for constructing in-frame deletion of *vca0109*
0109-KO5	tttacccccttcaacgccagatatg	Forward primer to confirm the in-frame deletion of *vca0109*
0109-KO6	cgattcgatactctgaacgacgc	Reverse primer to confirm the in-frame deletion of *vca0109*
sfGFP-X-hifif	gcagctgcaggaggaggatctaaaggtgaag	Forward primer to amplify *sfGFP*
sfGFP-X-hifir	acggcatggatgagctctacaaataagagaagcaactggagctataaccg	Reverse primer to amplify *sfGFP*
Fha-sfGFP-KI1	gtgatgggttaaaaaggatcgcgaaggccacctttgctaagc	Forward primer to amplify *vca0112* for constructing chromosomal *fha_sfGFP*
Fha-sfGFP-KI2	gatcctcctcctgcagctgctagctccagttgcttctcgcgaattttgc	Reverse primer to amplify *vca0112* for constructing chromosomal *fha_sfGFP*

Fha-sfGFP-KI3	gagaagcaactggagctataaccgtgaacaaagtgctgtggatg	Forward primer to amplify the downstream of *vca0112* for constructing chromosomal *fha_sfGFP*
Fha-sfGFP-KI4	gcatgcggtacctctagaagagcaattgaggcatacgcactg	Reverse primer to amplify downstream of *vca0112* for constructing chromosomal *fha_sfGFP*
Fha-sfGFP-KI5	ggcgtatgacctcgagctgg	Forward primer to confirm the chromosomal *fha_sfGFP*
Fha-sfGFP-KI6	catcccttgtcacttgaatgtagtgg	Reverse primer to confirm the chromosomal *fha_sfGFP*
TssL-sfGFP-KI1	gtgatgggttaaaaaggatcgcaagactactggttccaattgcg	Forward primer to amplify *vca0115* for constructing chromosomal *tssL_sfGFP*
TssL-sfGFP-KI2	gatcctcctcctgcagcggcaagtatttgattgagttgattgagcacatc	Reverse primer to amplify *vca0115* for constructing chromosomal *tssL_sfGFP*
TssL-sfGFP-KI3	gagctctacaaataaacataattaggctaggtggtacg	Forward primer to amplify the downstream of *vca0115* for constructing chromosomal *tssL_sfGFP*
TssL-sfGFP-KI4	gcatgcggtacctctagaagcaacacctgcttcacccac	Reverse primer to amplify downstream of *vca0115* for constructing chromosomal *tssL_sfGFP*
TssL-sfGFP-KI5	cgatgttgactcattccagcg	Forward primer to confirm the chromosomal *tssL_sfGFP*
TssL-sfGFP-KI6	gccaaaccttcaatcatggcac	Reverse primer to confirm the chromosomal *tssL_sfGFP*
MHK3 tssM-KO1	gtgatgggttaaaaaggatcgcctcattacctgagcacgacgtc	Forward primer to amplify the upstream of MHK3 *tssM* for constructing in-frame deletion of *tssM*
MHK3 tssM-KO2	cgagagtttgaatgacccaacaataaatttccacatggg	Reverse primer to amplify the upstream of MHK3 *tssM* for constructing in-frame deletion of *tssM*
MHK3 tssM-KO3	tgttgggtcattcaaactctcgaaaacccttac	Forward primer to amplify the downstream of MHK3 *tssM* for constructing in-frame deletion of *tssM*
MHK3 tssM-KO4	gcatgcggtacctctagaaggcacctgctcaacctgttcc	Reverse primer to amplify the downstream of MHK3 *tssM* for constructing in-frame deletion of *tssM*
MHK3 tssM-KO5	ccttggcaatacgagtcaggc	Forward primer to confirm the in-frame deletion of MHK3 *tssM*
MHK3 tssM-KO6	ggccacatgcgtaacacgtg	Reverse primer to confirm the in-frame deletion of MHK3 *tssM*
MHK3 fhaC-KO1	gtgatgggttaaaaaggatcgaagtgatgcgcattggcagc	Forward primer to amplify MHK3 *fha* for constructing fha^N
MHK3 fhaC-KO2	agctgccgctaagccctcaggtacgttgtaaatg	Reverse primer to amplify MHK3 *fha* for constructing fha^N
MHK3 fhaC-KO3	tgagggcttagcggcagctgcaggaggaggatc	Forward primer to amplify *sfGFP* for constructing fha^N_*sfGFP*
MHK3 fhaC-KO4	gcatgcggtacctctagaaggcttttcgttcgggtctttgg	Reverse primer to amplify *sfGFP* for constructing fha^N_*sfGFP*
MHK3 fha-M1A-mut1	gtgatgggttaaaaaggatcgccttgtatatcgcgtatggcgaaag	Forward primer to amplify the upstream of MHK3 *fha* for constructing fha^N
MHK3 fha-M1A-mut2	agggtctgtttcttttgtgcatcgctattgccttacttgtataagcacg	Reverse primer to amplify the upstream of MHK3 *fha* for constructing fha^N
MHK3 fha-M1A-mut3	cgatgcacaaaagaaacagaccctatcaatggtagtgacgaatgtgcaac	Forward primer to amplify MHK3 *fha* for constructing fha^S
MHK3 fha-M1A-mut4	gcatgcggtacctctagaaggtgctcaacagccggaatagg	Reverse primer to amplify MHK3 *fha* for constructing fha^S
MHK3 fha-M263A-mut1	gtgatgggttaaaaaggatcgatgcagaagaagcaaacgctttc	Forward primer to amplify MHK3 *fha* for constructing fha^L
MHK3 fha-M263A-mut2	ctagcacctttcatctgccgctaagccctcaggtacgttg	Reverse primer to amplify MHK3 *fha* for constructing fha^L

MHK3 fha-M263A-mut3	cggcagatgaaaaggtgctagatctacttgaggaagaagtggcgagaag	Forward primer to amplify MHK3 *fha* for constructing *fha*^{L}
MHK3 fha-M263A-mut4	gcatgcggtacctctagaaggcatcagcaggacaaaagaccg	Reverse primer to amplify MHK3 *fha* for constructing *fha*^{L}
MHK3 Fha-sfGFP-KI1	gtgatgggttaaaaaggatcggagactgcgcaaccaccgac	Forward primer to amplify MHK3 *fha* for constructing chromosomal *fha_sfGFP*
MHK3 Fha-sfGFP-KI2	cctgcagctgctagctccagctgtttttcacgaattttcttg	Reverse primer to amplify MHK3 *fha* for constructing chromosomal *fha_sfGFP*
MHK3 Fha-sfGFP-KI3	taaacagctggagcttaaacggtgaac	Forward primer to amplify the downstream of MHK3 *fha* for constructing chromosomal *fha_sfGFP*
MHK3 Fha-sfGFP-KI4	gcatgcggtacctctagaagccactgactgatgagatgcgttc	Reverse primer to amplify downstream of MHK3 *fha* for constructing chromosomal *fha_sfGFP*
MHK3 Fha-sfGFP-KI5	ccacctattccggctgttgag	Forward primer to confirm the chromosomal MHK3 *fha_sfGFP*
pBAD-hifiF	gcatgcggtaaacctattcctaatcctc	Forward primer to amplify pBAD vector
pBAD-hifiR	catggtacctcctgctagcccaa	Reverse primer to amplify pBAD vector
pBAD-f	agtccacattgattatttgcacgg	Forward confirmation primer of pBAD vector
pBAD-r	ttcacttctgagttcggcatgg	Reverse confirmation primer of pBAD vector
VC Fha-pBAD-hifif	gggctagcaggaggtaccatgaactcagtgacattaccttcgttgac	Forward primer to amplify *vca0112*
VC Fha-pBAD-hifir	ggaataggtttaccgcatgctagctccagttgcttctcgcgaa	Reverse primer to amplify *vca0112*
pBBR2-hifiF	agctgtttcctgtgtgaaattg	Forward primer to amplify pBBRMCS2 vector
pBBR2-hifiR	gattacaaggacgacgatgacaagtgagcgttaatattttgttaaaattcgcgtt	Reverse primer to amplify pBBRMCS2 vector
pBBR2-f	agcgcaacgcaattaatgtgag	Forward confirmation primer of pBBRMCS2 vector
pBBR2-r	catcgcagtacggcctattgg	Reverse confirmation primer of pBBRMCS2 vector
VC Fha-pbbr-hifif	atttcacacaggaaacagctatgaactcagtgacattaccttcgttgac	Forward primer to amplify *vca0112*
VC Fha-pbbr-hifir	cacttgtcatcgtcgtccttgtaatcagaacctagctccagttgcttctcgcga	Reverse primer to amplify *vca0112*
pETDuet-f	cacgatgcgtccggcgtagagg	Forward confirmation primer of pET vector
pETDuet-r	ggttatgctagttattgctcagcggt	Reverse confirmation primer of pET vector
pET22b-hifi-f	aagcttgcggccgcactcga	Forward primer to amplify pET22b vector
pET22b-hifi-r	catatgtatatctccttcttaaagttaaacaaaattatttctagagg	Reverse primer to amplify pET22b vector
22b-Fha-f	ctttaagaaggagatatacatatgaactcagtgacattaccttcgttgac	Forward primer to amplify *vca0112*
22b-Fha-r	gccgcaagctttagctccagttgcttctcgcgaa	Reverse primer to amplify *vca0112*
petstrep-hifi-f	tgataacactgagatccggctgctaacaaag	Forward primer to amplify pET vector
petstrep-hifir	accgctgccggatttttcgaactg	Reverse primer to amplify pET vector
Strep-Fha-f	tcgaaaaatccggcagcggtaactcagtgacattaccttcgttgacc	Forward primer to amplify *vca0112*
Strep-Fha-r	agccggatctcagtgttatcatagctccagttgcttctcgcgaa	Reverse primer to amplify *vca0112*

Note for references

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