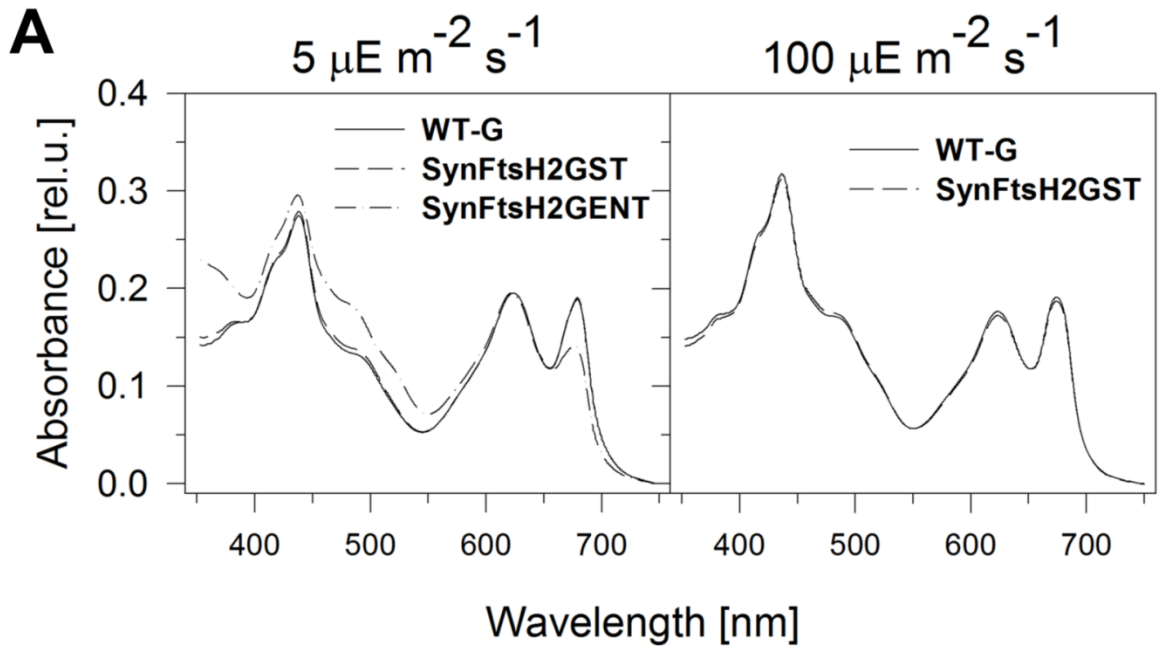
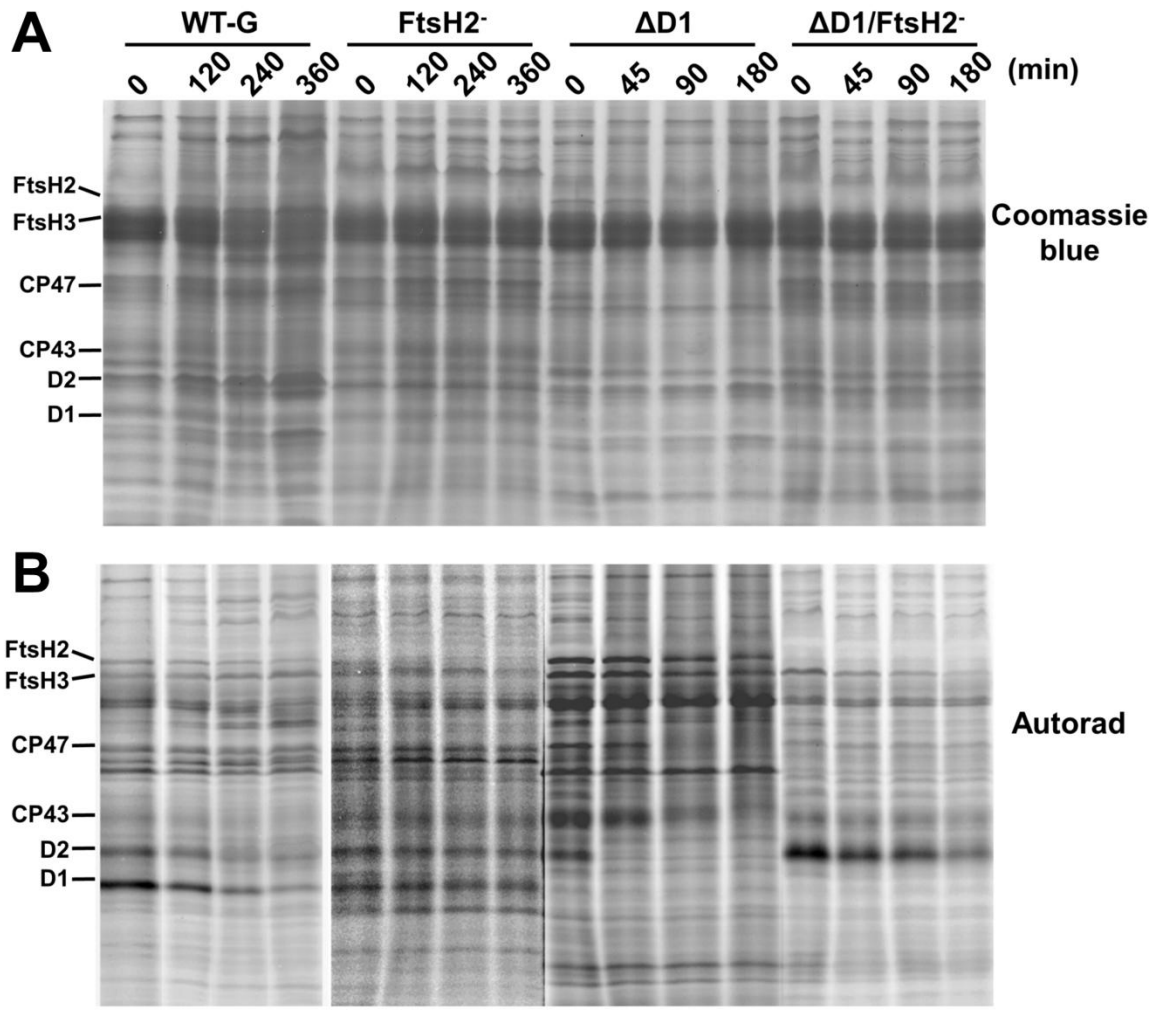


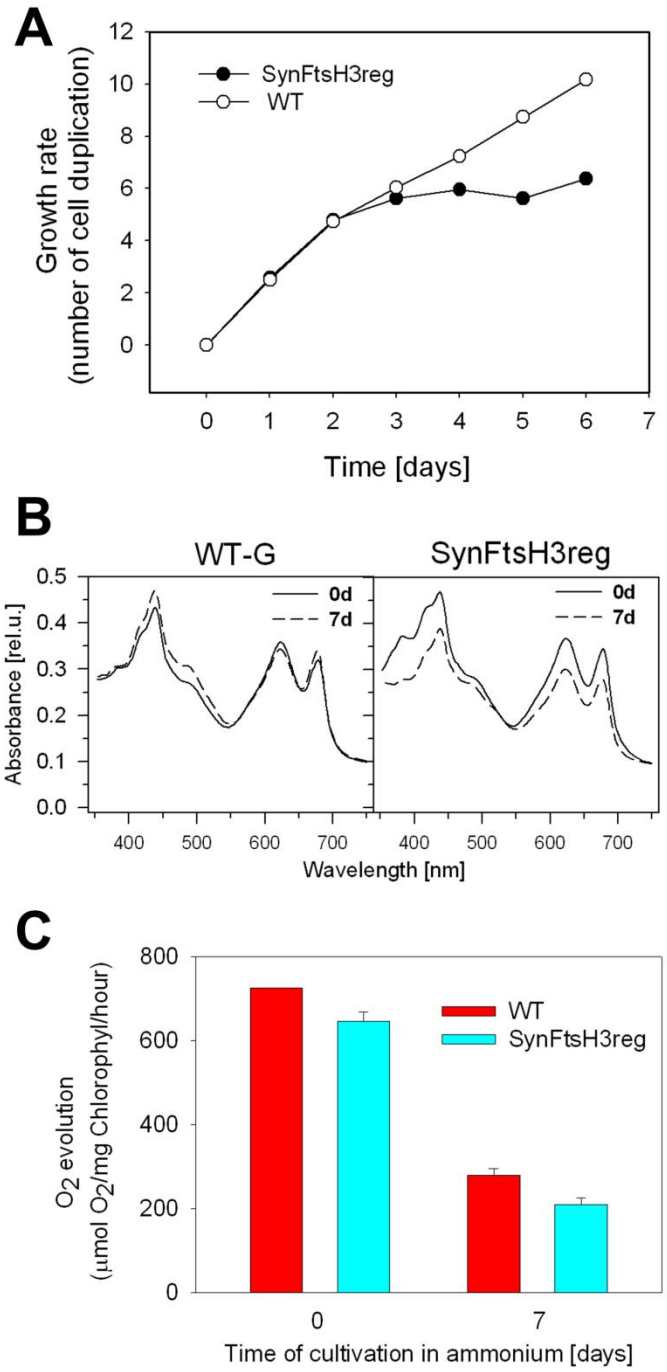
Supplemental Fig. 1. Absorption spectra of photoautotrophically grown cells.

Cells of WT-G, SynFtsH2GST and SynFtsH2GENT (FtsH2^{-}) were cultivated at an irradiance of 5 µE m^{-2} s^{-1} and then transferred to 100 µE m^{-2} s^{-1}. All spectra were measured by spectrophotometer Shimadzu UV3000 and have an identical OD_{750nm}.



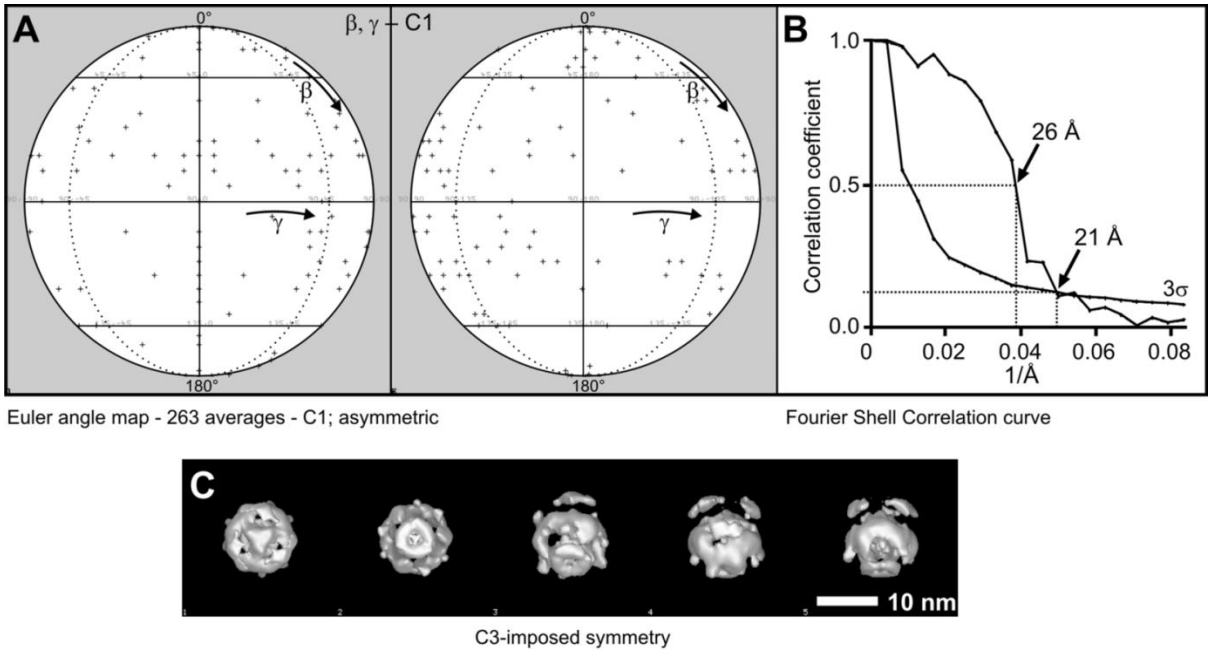
Supplemental Fig. 2. Enhanced degradation of FtsH3 in strains lacking FtsH2.

Cells of WT-G, *slr0228*:cm^R (FtsH2$^-$), the *psbA*-triple mutant ΔD1 and the *psbA/slr0228* quadruple mutant (ΔD1/FtsH2$^-$) (Komenda et al., 2006) were pulse-labeled using a mixture of [^{35}S]methionine and cysteine, at a light irradiance of 250 µE m^{-2} s^{-1} for 20 min at 29 °C. After washing and addition of unlabeled Met and Cys, the cells were exposed to 500 µE m^{-2} s^{-1} for the time indicated in min. Radiolabeled thylakoid membrane proteins were separated by SDS-PAGE, the gel stained (Coomassie stain), dried and exposed to a Phosphorimager plate (Autorad). Loaded thylakoids corresponded to 2 µg of Chl *a*.



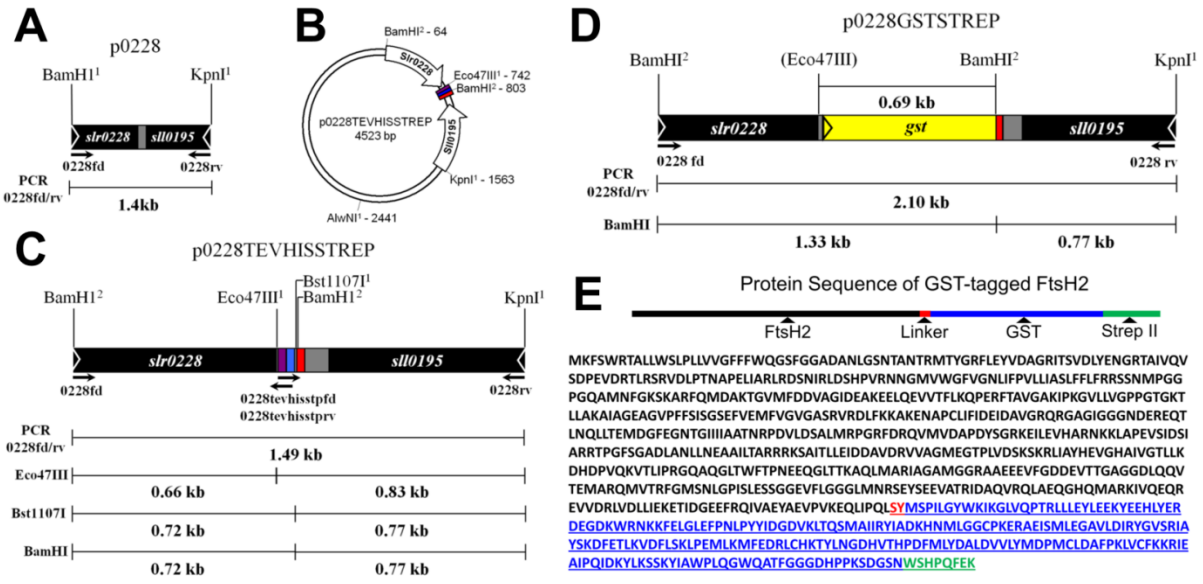
Supplemental Fig. 3. Phenotype of SynFtsH3reg.

(A) Growth, *(B)* absorption spectra and *(C)* oxygen-evolving activity of autotrophically grown cells of WT-G and SynFtsH3reg cultivated at an irradiance of 10 µE m^{-2} s^{-1} in the presence of 13 mM NH_4Cl. Growth was characterized by daily measurement of OD_{750nm} just before dilution and then the number of cell duplications was calculated; absorption spectra were measured by the same spectrophotometer at the identical OD_{750nm}; and oxygen evolution was measured from whole cells as described in METHODS.



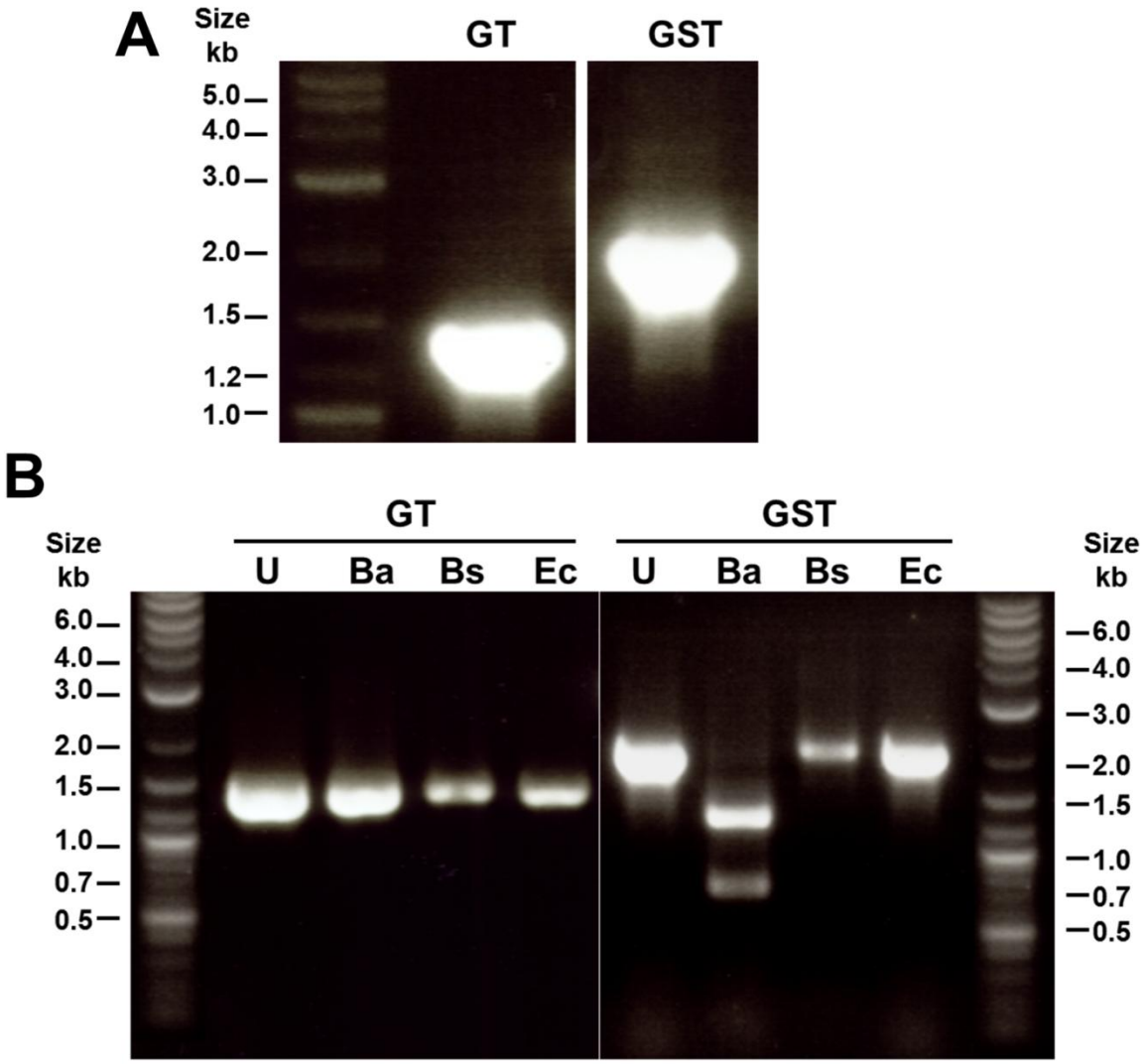
Supplemental Fig. 4. Image Processing Euler Map.

(A) Map of the assigned Eulerian angles, assigned *a priori* for 263 class averages by angular reconstitution (van Heel, 1987), used in the asymmetric 3D reconstruction. Averages from each subpopulation were merged to ensure the broadest range of relative orientations. *(B)* Fourier-shell correlation at 3σ and a correlation co-efficient of 0.5 gave a resolution of 26 Å for the 3D model calculated without imposed symmetry. *(C)* Surface-rendered views of the 3D model with C3 (three-fold) symmetry imposed.



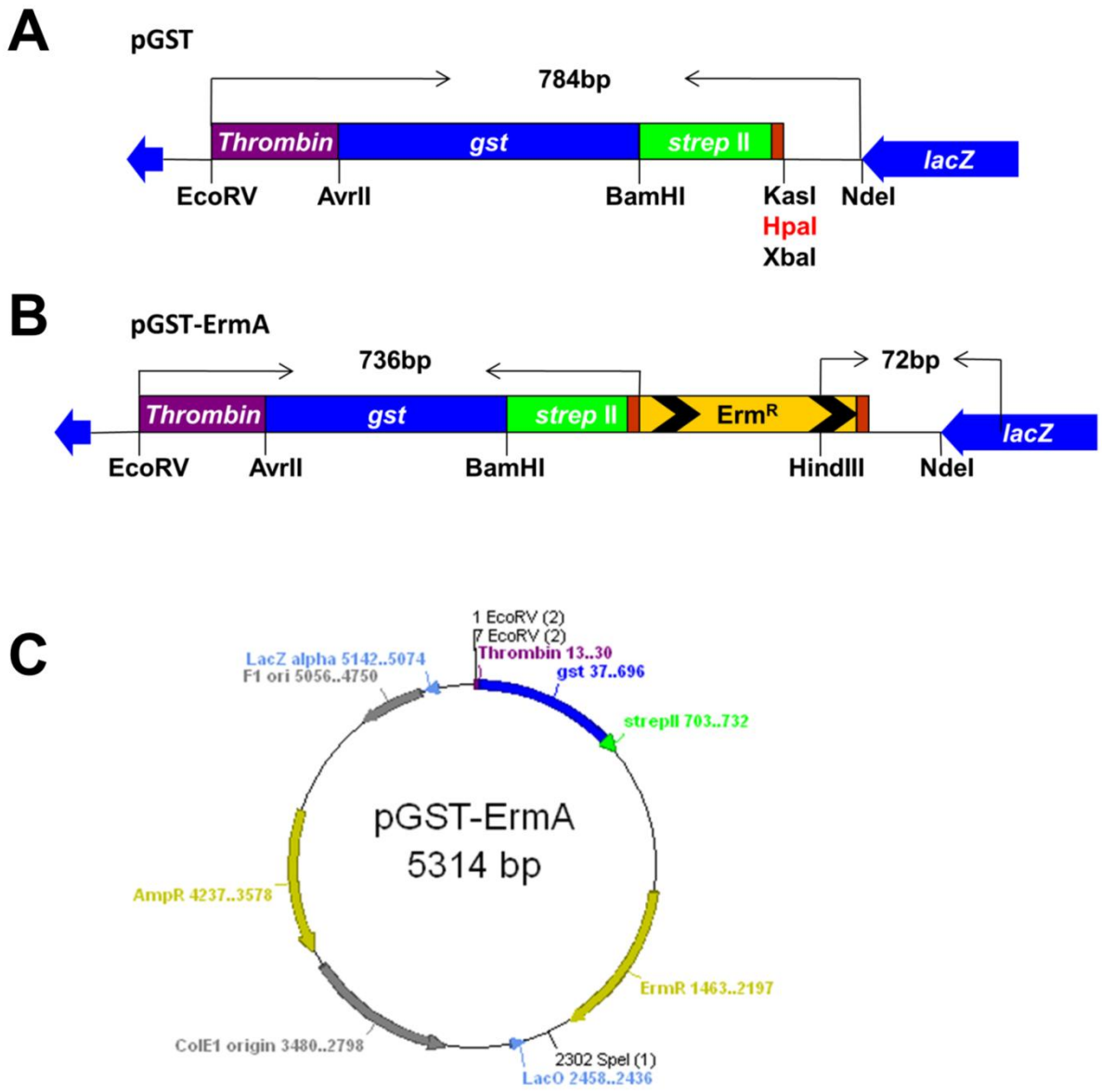
Supplemental Fig. 5. Plasmids used to construct SynFtsH2GST.

Schematic representations of the inserts found in the *(A)* p0228, *(C)* p0228TEVHISSTREP and *(D)* p0228GSTSTREP plasmids and *(B)* a vector map of the p0228TEVHISSTREP plasmid. A detailed description of how the individual plasmids were constructed can be found in the METHODS section. *(A-D)* Black and yellow boxes indicate genes, grey boxes indicate non-coding sequence and triangular arrows depict the direction of transcription of the labeled genes. The purple box indicates the TEV protease site, the blue box the His_9 and the red box the *Strep* tag-II encoding sequences. Restriction sites used for cloning steps or construct identification are labeled including the number of sites in superscript type. Restriction sites in brackets indicate that the respective site has been destroyed. Primers used for gene amplification, construct confirmation and sequencing are marked. Gene sizes, PCR products and restriction fragments are labeled in kb and annotated with the fragment name or restriction enzyme used to digest the fragment. *(E)* Predicted primary structure of the FtsH2-GST fusion protein including sequence of the linker.



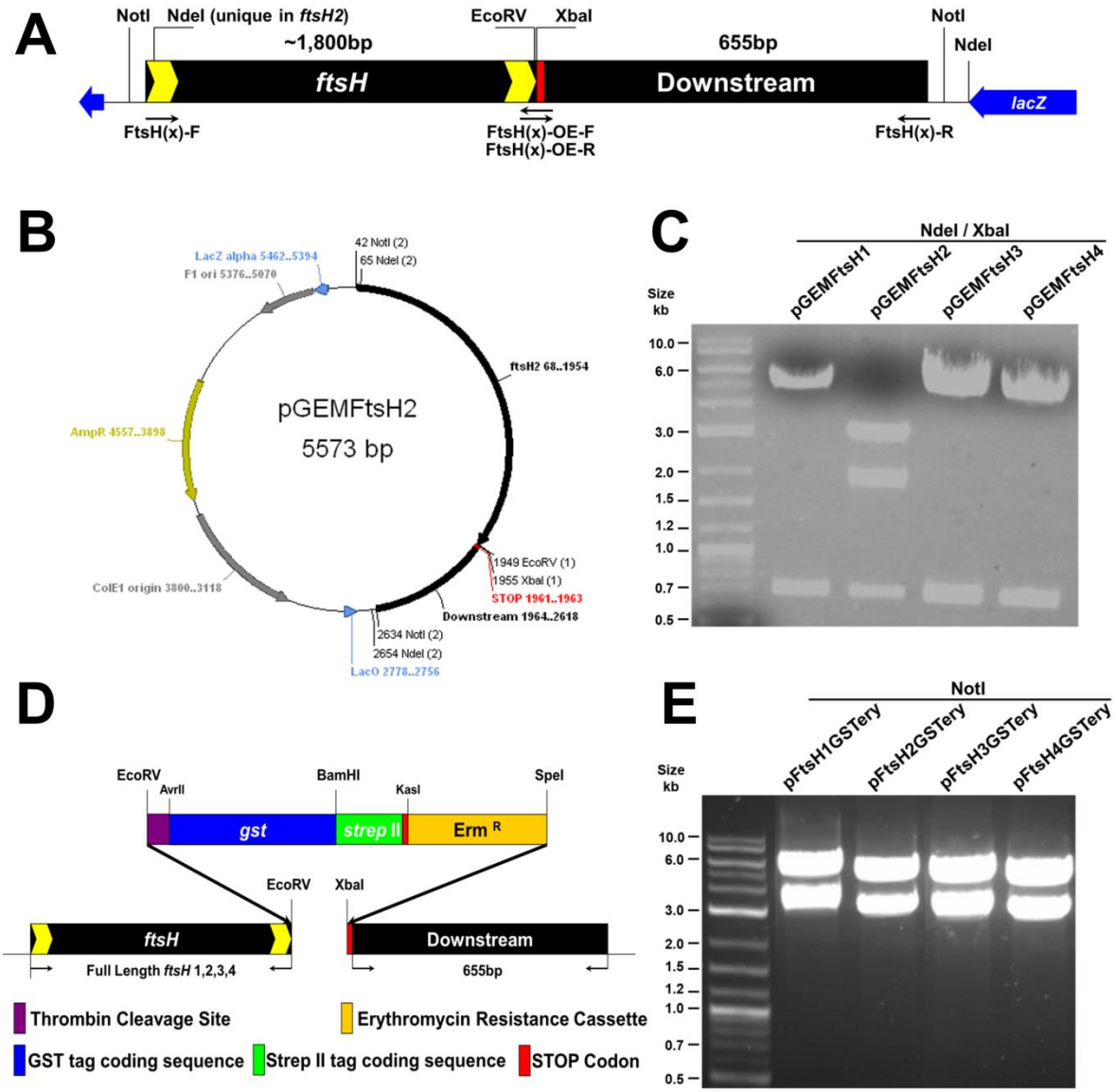
Supplemental Fig. 6. Genotype analysis of the FtsH2 mutants.

(A) PCR analysis of the *slr0228* locus in the WT-G (GT) and SynFtsH2GST (GST) strains using the 0228fd/rv primer pair. *(B)* Restriction digests of the PCR reactions shown in panel A using restriction enzymes: BamHI (Ba), Bst1107I (Bs) and Eco47III (Ec). Uncut PCR product (U). DNA molecular mass marker band sizes are indicated in kb.



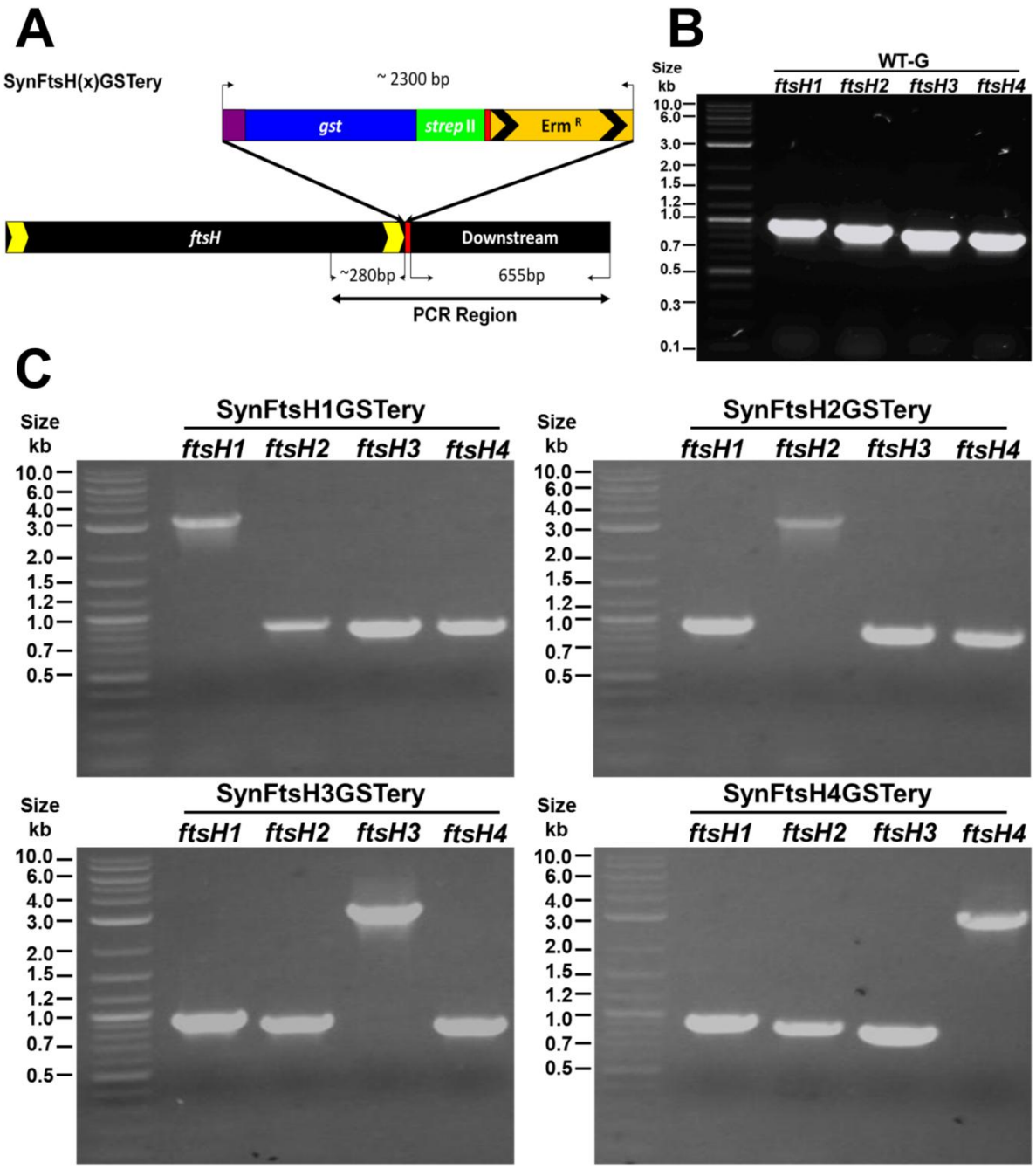
Supplemental Figure 7. Construction of *gst* tagging cassette.

Schematic representations of the inserts found in *(A)* pGST, *(B)* pGST-ErmA and *(C)* the plasmid map of pGST-ErmA. A detailed description of the cloning strategy can be found in METHODS. In panels *(A)* and *(B),* blue arrows represent *lacZ'* on the pGEM-T Easy vector backbone. Purple boxes represent the coding sequence of the thrombin cleavage site. Blue and green boxes indicate the *gst:strep II* tagging sequence. The yellow box indicates the erythromycin-resistance cassette; the black arrows show the direction of transcription.



Supplemental Figure 8. Construction of transformation vectors to make SynFtsHxGSTery mutants.

(A) Schematic representation of the flanking sequence and how the restriction sites were introduced via overlap extension PCR. *(B)* Plasmid map of pGEMFtsH2 vector, which carries an additional NdeI site at the 5' end of the *ftsH2* ORF. *(C)* Restriction digests of each of the pGEMFtsHx vectors using NdeI and XbaI, where FtsHx represent the particular homologue. *(D)* Schematic representation of how the *gst* tagging cassette was introduced into each of the pGEMFtsHx vectors to create the final transformation vectors. *(E)* Restriction digest of the final transformation vectors (pFtsHxGSTery) using NotI to release the whole insert from each vector.



Supplemental Figure 9. Genotype analysis of the four SynFtsHxGSTery mutants.

(A) Schematic representation of the PCR region amplified using each FtsH-Seq3/FtsH-R primer pair. *(B)* PCR analysis of WT *ftsH1-4*. *(C)* PCR analysis of each SynFtsHxGSTery mutant.

#	Name	Primers
1	TGS-F	GAT ATC GAT ATC CTC GTT CCC CGC GGG TCC C CTAGG ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCT
2	TGS-R	T CTAGA T CTAGA GTT AAC G GCGCC TTATTTTTCAAATTGGGGATGGGACCA ATT G GATCC ATCCGATTTTGGAGGATGGTCGCCACCA
3	FtsH1-F	TTGGGGATGGGTTTACTGGTAGCTGGCA
4	FtsH1-OE-R	TCGGACATTGCACAGATAGGGGGCTA T CTAGA GAT ATCCTTACCGGCTAGAGCAGGCTGTT
5	FtsH1-OE-F	AACAGCCTGCTCTAGCCGGTAAG GAT ATC T CTAGA TAG CCCCCTATCTGTGCAATGTCCGA
6	FtsH1-R	TCACTTACCATTGATTAAATTCCATGCAACCTATGGAAAAGTCCT
7	FtsH1-Seq 3	GTCTAGTGGCCTTGGAAGAGGAAGGCGA
8	FtsH2-F	TAACATATGAAATTTTCCTGGAGAACTGCCCTACTTTGGT
9	FtsH2-OE-R	TGACCATACTAAATTGGTTGGAGAAGGGAATTTTTA T CTAGA GAT ATCTAGTTGGGGAATTAACTGTTCCTTGACGGGA
10	FtsH2-OE-F	TCCCGTCAAGGAACAGTTAATTCCCCAACTA GAT ATC T CTAGA TAAAAATTCCCTTCTCCAACCAATTTAGTATGGTCA
11	FtsH2-R	TGGGCGACCCCTGCCCTTGCACATTTTCGAGT
12	FtsH2-Seq 3	AACCGTTCTGAATACTCCGAAGAAGTAGCCA
13	FtsH3-F	TCTAGCGTGAGCAAAAATAATAAAAAATGGCGTAACGCGGGCCT
14	FtsH3-OE-R	ACTCTAGGAATTCCAATGCTTGGATTCTTA T CTAGA GAT ATC AACTAGAAGTGCCAATTTGGCATTGTTGTTAGCCA
15	FtsH3-OE-F	TGGCTAACAACAATGCCAAATTGGCACTTCTAGTT GAT ATC T CTAGA TAAGAATCCAAGCATTGGAATTCCTAGAGT
16	FtsH3-R	GAGCAAACCAAGTACCGAGTAGAGTAAGACCT
17	FtsH3-Seq 3	GATGAAACCGCTGCGGCGATCGATGAGGA
18	FtsH4-F	CAACCCATGGCCATCAAACCCCAACCCCAA
19	FtsH4-OE-R	TTTTTAAAGGGAGCAAAAAAGCAAGTTCCTTA T CTAGA GAT ATC TACCACTAGGGTGCCAGGAGCTTGA
20	FtsH4-OE-F	TCAAGCTCCTGGCACCCTAGTGGTAGAT ATC T CTAGA TAAGGAACTTGCTTTTTTGCTCCCTTTAAAAA
21	FtsH4-R	TTTTACCCCCAAACGATGCAACGCCTCAGCGGCCA
22	FtsH4-Seq 3	CTGCCAAAGAAATTGACCTAGAGGTCAAAGAAATCGT

Supplemental Table 1. Oligonucleotide primers used to generate and analyze mutants.