HmbC, a protein of the HMG family, participates in the regulation of carotenoid biosynthesis in *Fusarium fujikuroi*

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SUPPLEMENTARY MATERIAL

Table S1. Primers used for the generation of the constructs, probe and verification of DNA integration in the transformants.

Set	Primer	Sequence [5′ to 3′]	Use
PS1	HMGc_5_1F	GTAACGCCAGGGTTTTCCCAGTCACGACG ACCAGTCAGCGCTGCCCGCG	PCR *hmbC* deletion cassette
	HMGc_3_2R	GCGGATAACAAGTTCACACAGGAAACAG CCCACATCAAAAGCAAAGGCC	
PS2	HMGc_3_2F	CTCCTTCAATATCATCTTCTGTCTCCGACG CTTTCTTTACTCTCCGTTG	PCR 3′ fragment of *hmbC*
	HMGc_3_2R	GCGGATAACAAGTTCACACAGGAAACAG CCCACATCAAAAGCAAAGGCC	
PS3	HMGc_5_1F	GTAACGCCAGGGTTTTCCCAGTCACGACG ACCAGTCAGCGCTGCCCGCG	PCR 5′ fragment of *hmbC*
	HMGc_5_1R	ATCCACTTAACGTTACTGAAATCTCCAAC CGCTGACGACTTGATGGGACGCGT	
PS4	HPH-6F	GTCGGAGACAGAAGATGATATTGAAG GAGC	PCR Hyg^R cassette
	HPH-6R	GTTGGAGATTTCAGTAACGTTAAGTGGAT	
PS5	HMGc_5_1F	GTAACGCCAGGGTTTTCCCAGTCACGACG ACCAGTCAGCGCTGCCCGCG	PCR 5′ region of *hmbC*-Hyg^R cassette
	HPH-6R	GTTGGAGATTTCAGTAACGTTAAGTGGAT	
PS6	hmgbC_5_2F	GCAGCCAACACCTGTAGCAT	PCR test Δ*hmbC*
	Hph-11R	TGAGCTGATGCTTTGGGCCGA	
PS7	HMGc_5_1F	GTAACGCCAGGGTTTTCCCAGTCACGACG ACCAGTCAGCGCTGCCCGCG	PCR test Δ*hmbC*
	HMGc_5_1R	ATCCACTTAACGTTACTGAAATCTCCAAC CGCTGACGACTTGATGGGACGCGT	
PS8	hmgbC_3_2F	GCATGATACCTAAGGAGAGT	Southern probe
	hmgbC_3_2R	GAACTCCGTGAGCAATACAG	

Table S2. Primers used for quantitative RT-PCR

Gene	Primers	Sequence [5′ to 3′]
carS	RtFfcarS-1F	GATACCCGGCGGAAAGGTTA
	RtFfcarS-1R	CTGACAGTCCATTTCAGCGC
carB	RTcarB-1F	TCGGTGTCGAGTACCGTCTCT
	RTcarB-1R	TGCCTTGCCGGTTGCTT
carRA	RTcarRA-1F	CAGAAGCTGTTCCCGAAGACA
	RTcarRA 1R	TGCGATGCCCATTTCTTGA
β-tub	Tub-2F	CCGGTGCTGGAAACAACTG
	Tub-2R	CGAGGACCTGGTCGACAAGT
gpdA	RT-gpdh-1F	GTGACCTCAAGGGCGTTCTG
	RT-gpdh-1R	CGAAGATGGAGGAGTTGGTGTT

Table S3. Sequences of primers used for amplification of *car* promoters

Name	Target promoter	Sequence [5′ to 3′]	Use
PcarB_fw_biotin	P*carB*	**Biotin**-TGGGGTGAAGCGTG GAGGATG	Amplification of *carB* promoter
PcarB_bw	P*carB*	TTTGGCTGTAAAAAGTGAA GATGCTCAGTG	
PcarRAcarX_fw_biotin	P*carRA*/*carX*	**Biotin**-CATGATGATATGTG GACTATGATATTAC	Amplification of double *carRA*/*carX* promoter
PcarRAcarX_bw	P*carRA*/*carX*	CATTTTGACAAAGATTTAT TTCGTG	

Table S4. PCR setups and programs used to amplify biotin-labelled promoters

P*carB*	**P*carRAcarX***	**µl**	**MM (x15)**	**Program**			
10x BioTaq		10	150	**P*carB***		**P*carRAcarX***	
$MgCl_2$		3	45	94°C-2′		94°C-2′	
PcarB_fw_biotin	PcarB_bw	4	60	94°C-30′′		94°C-30′′	
PcarRAcarX_fw_biotin	PcarRAcarX_bw	4	60	55°C-30′′	x35	52°C-30′′	x34
dNTPs		2	30	72°C-28′′		72°C-55′′	
template: *F. fujikuroi* WT genomic DNA		2	30	72°C-10′		72°C-10′	
BioTaq		1	15	4°C-hold		4°C-hold	
H_2O		74	1,110				
Total		100	1,500				

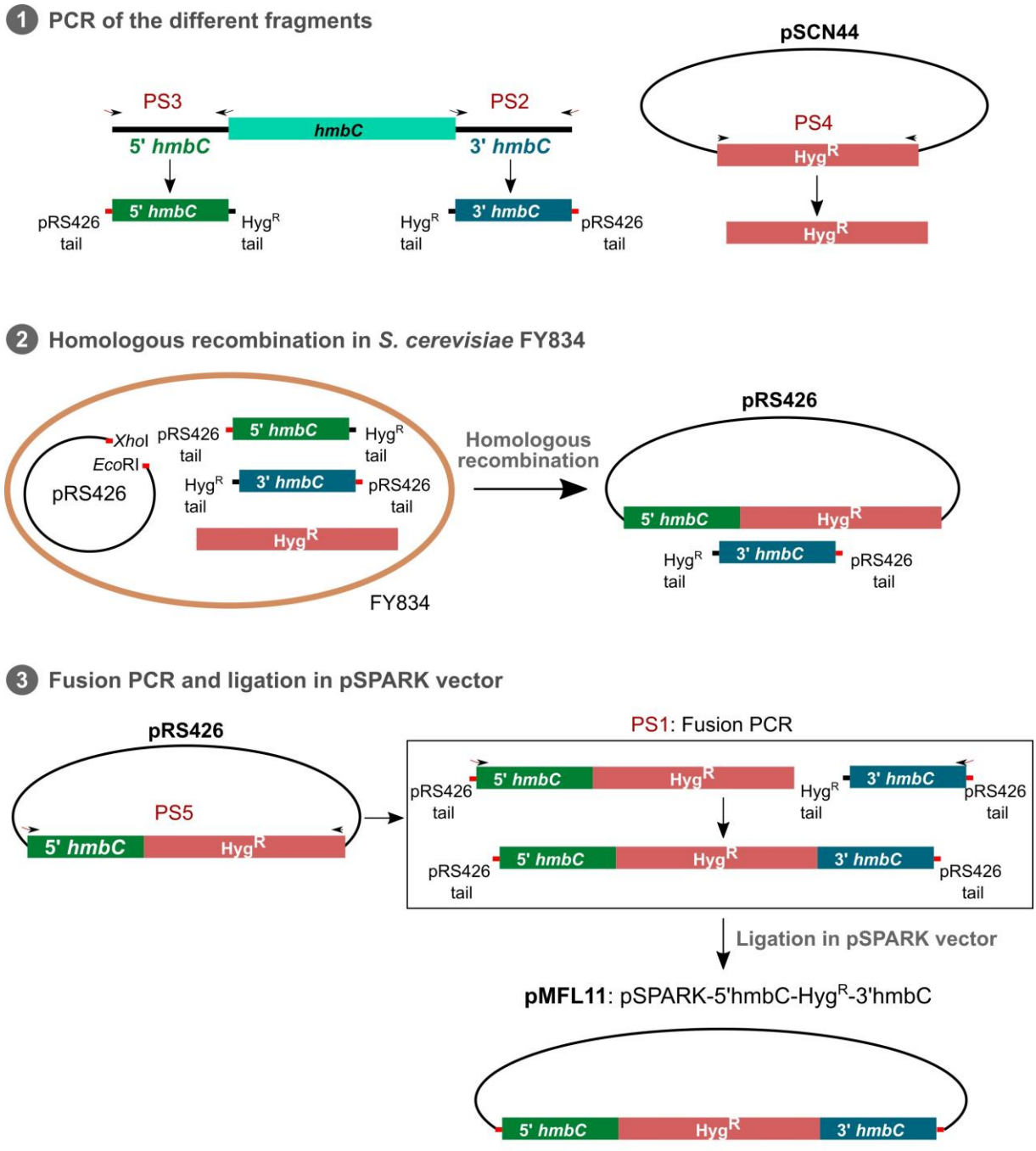

Figure S1. Construction of pMFL11 plasmid. Steps 1, 2 and 3 are described in the section of material and methods. Primer sets (PS#) are indicated in red. Primer locations are indicated by small arrows.

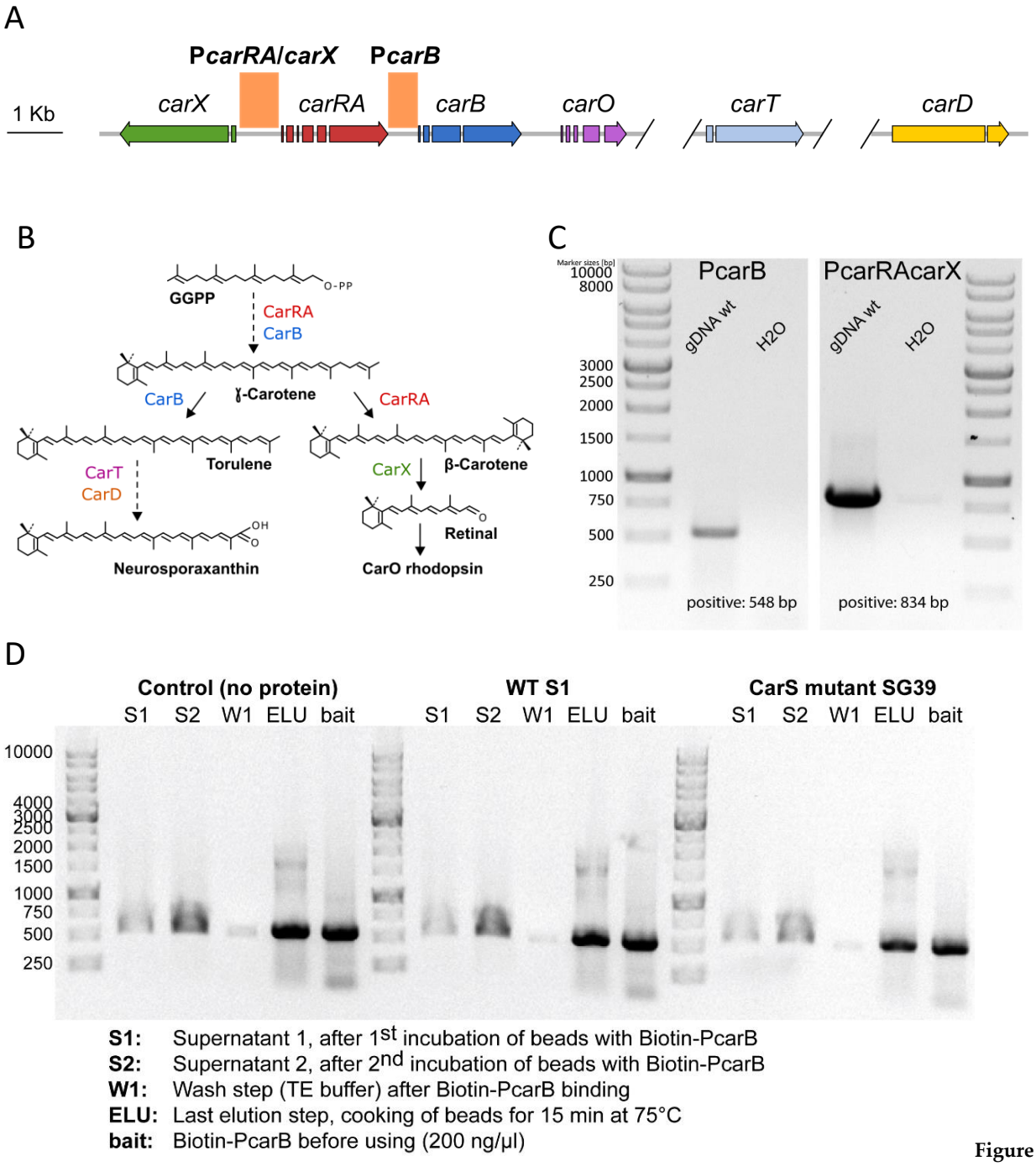

Figure S2. (**A**) Genomic organization of the genes of the carotenoid pathway in *F. fujikuroi*. The *carRA, carB,* and *carX* genes are clustered with the *carO* rhodopsin gene. The regulatory segments P*carRA/carX* and P*carB* used in the pulldown assay are indicated. Genes *carT* and *carD* are at other genomic locations. Gaps in the genes indicate introns. (**B**) Simplified scheme of the *F. fujikuroi* carotenoid pathway. The steps in which each enzyme is involved is indicated. Dashed lines represent several enzymatic steps. (**C**) Amplification of biotin-coupled promoter regions of P*carB* and P*carRA/carX*. gDNA wt: amplification from genomic DNA of the wild type. (**D**) Control steps of the streptavidin pulldown confirming the binding of biotinylated P*carB* (bait) to the streptavidin beads. Beads were incubated twice with biotinylated P*carB* and supernatant was analyzed (S1 and S2). Additionally, a washing step with TE buffer (W1) and the final eluate (ELU) were visualized.

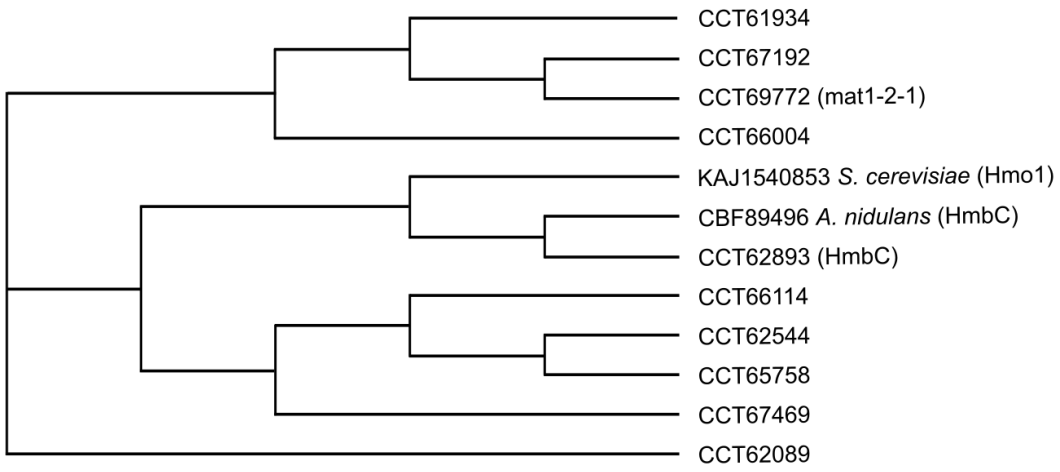

Figure S3. Cladogram representation of a neighbour-joining phylogenetic tree without distance corrections of the 10 proteins of the HMG-box family in *F. fujikuroi*. The sequences of the presumed HmbC orthologs in *S. cerevisiae* and *A. nidulans* are also included.

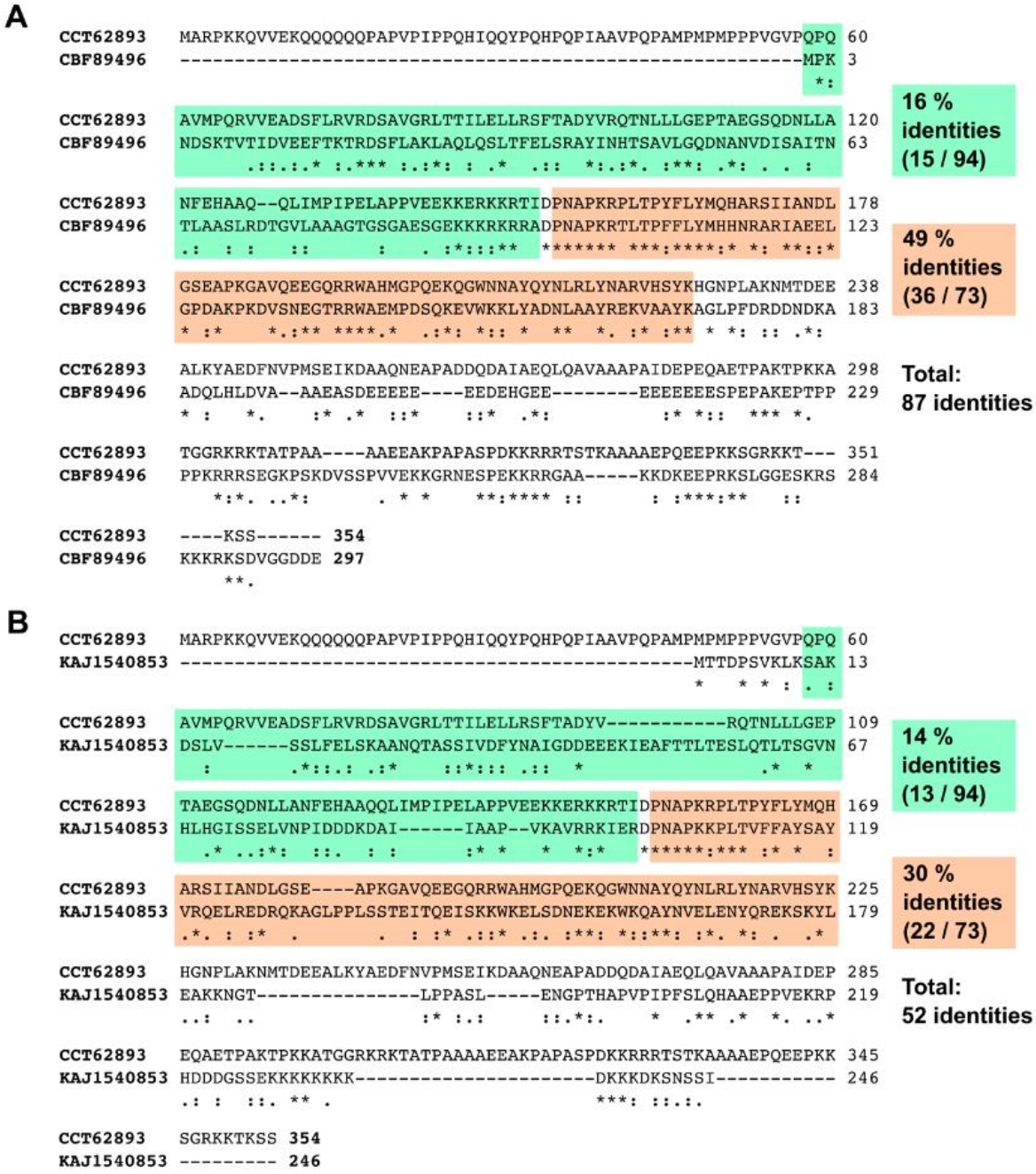

Figure S4. (A) Clustal alignment between the HmbC protein of *F. fujikuroi* (CCT62893) and **(A)** HmbC of *A. nidulans* (CBF89496) and **(B)** Hmo1 of *S. cerevisiae* (KAJ1540853). Partial identities in the A (orange) and B (green) domains as well as total found in the pairwise comparisons are indicated.

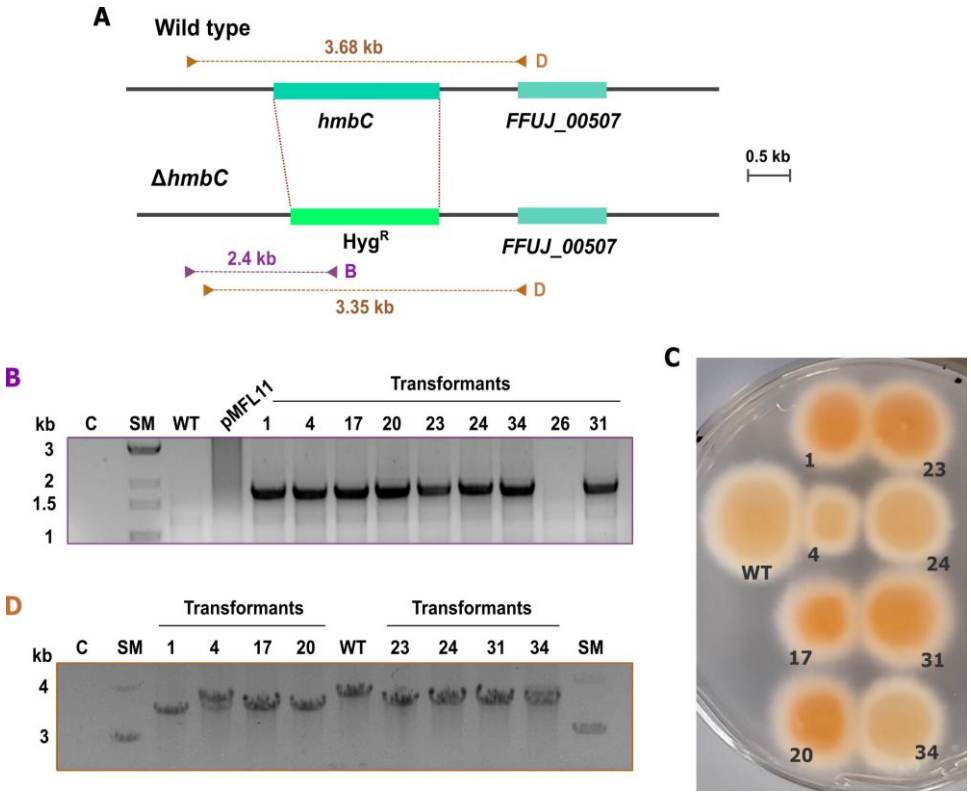

Figure S5. Screening of possible *hmbC* deletion transformants by PCR. **(A)** Map of the genomic *hmbC* region showing the expected homologous recombination generating the deletion. Expected amplicons of the PCR shown in panels B and D are indicated. **(B)** Electrophoresis of PCR carried out with genomic DNA of wild type and nine transformants using primer set PS6 to test the integration of the Hyg^R cassette in the *hmbC* locus by homologous recombination. The positive 2.4 kb band was observed in eight of the transformants. As expected, no amplification was obtained from the wild type and from pMFL11 plasmid. **(C)** Aspect of the pigmentation of 4-day old colonies of the wild type (WT) and the positive transformants in panel B. Five of them (1, 17, 20, 23 and 31) exhibit a deeper pigmentation. **(D)** Electrophoresis of PCR amplifications with genomic DNA of wild type and the eight transformants using PS7 primer set that bind to the surrounding of the *hmbC* locus, that are absent in the deletion cassette. The replacement should result in a 0.3 kb reduction in the size of the PCR product. Except transformant 4, the other transformants exhibit the expected shift in the product size. C: control without DNA. SM: size markers.

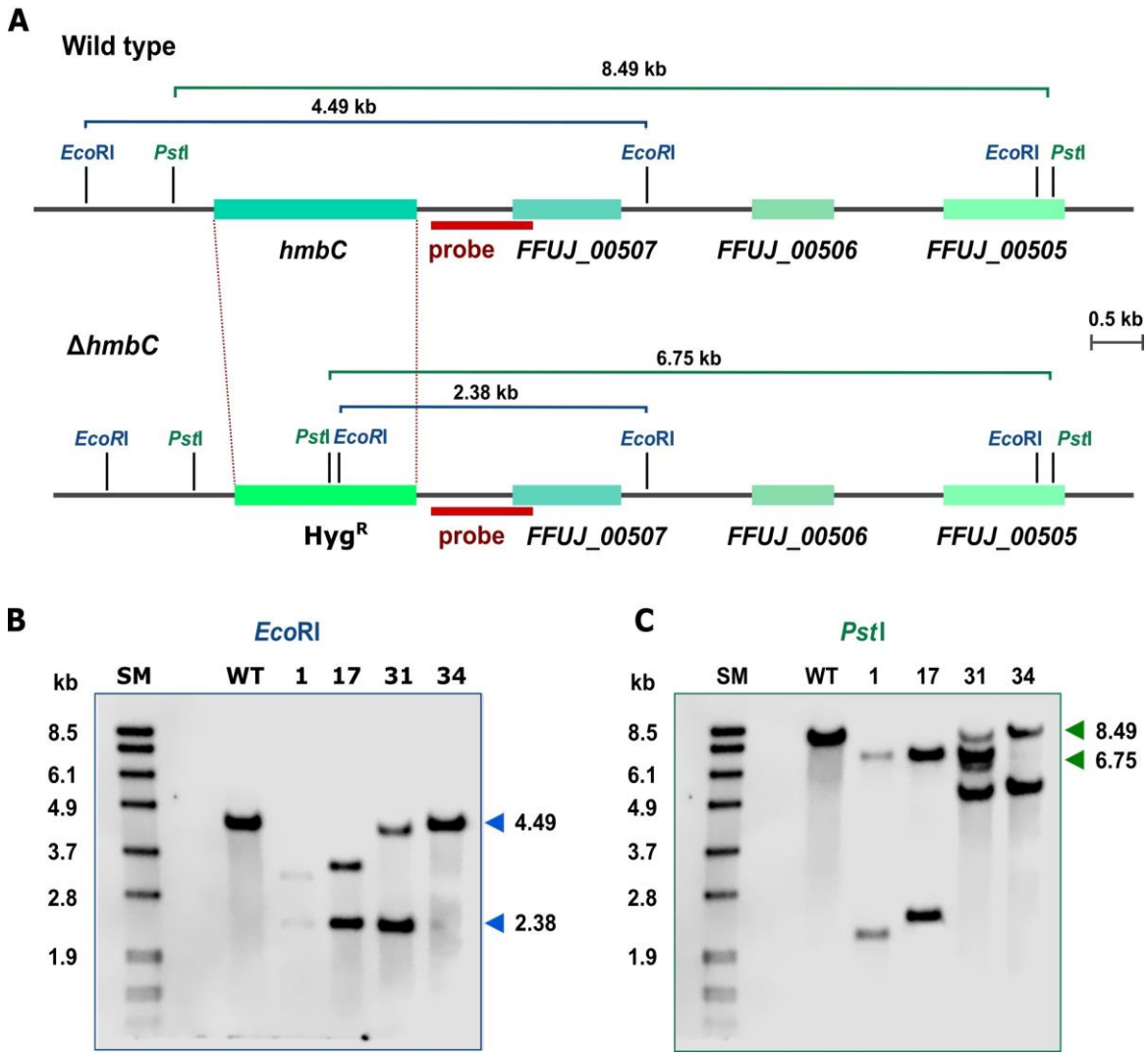

Figure S6. Identification by Southern blot of transformants with *hmbC* deletion. **(A)** Restriction map of the genomic *hmbC* region showing the expected homologous recombination generating the deletion. A probe obtained by PCR using PS8 primer set is shown as well as the expected hybridizing bands upon *Eco*RI or *Pst*I digestions. (**B)** and **(C),** Southern blot analysis of genomic DNA from the wild type and transformants 1, 17, 31, and 34 digested with *Eco*RI or *Pst*I. SM: Size markers. Sizes of relevant bands are indicated on the right.

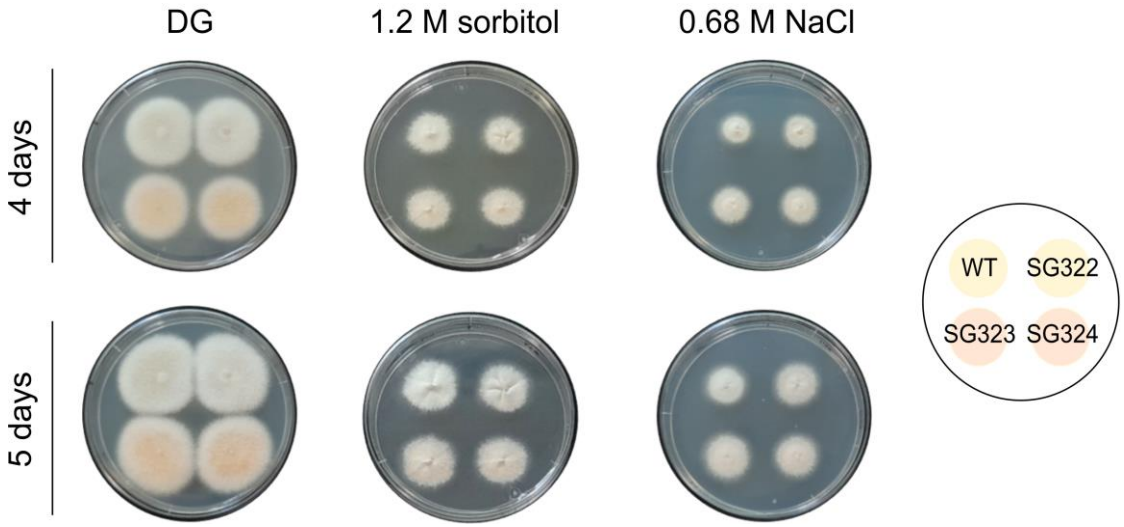

Figure S7. Effect of osmotic stress in the *hmbC* mutants and control strains. A small sample of mycelium from the wild type and *hmbC* deletions strain were inoculated on different osmotic stress media with tweezers from a fresh colony. These strains were grown for 4 and 5 days at 30 °C under dark conditions in DGasn minimal medium, without or with 1.2 M sorbitol or 0.68 M NaCl.

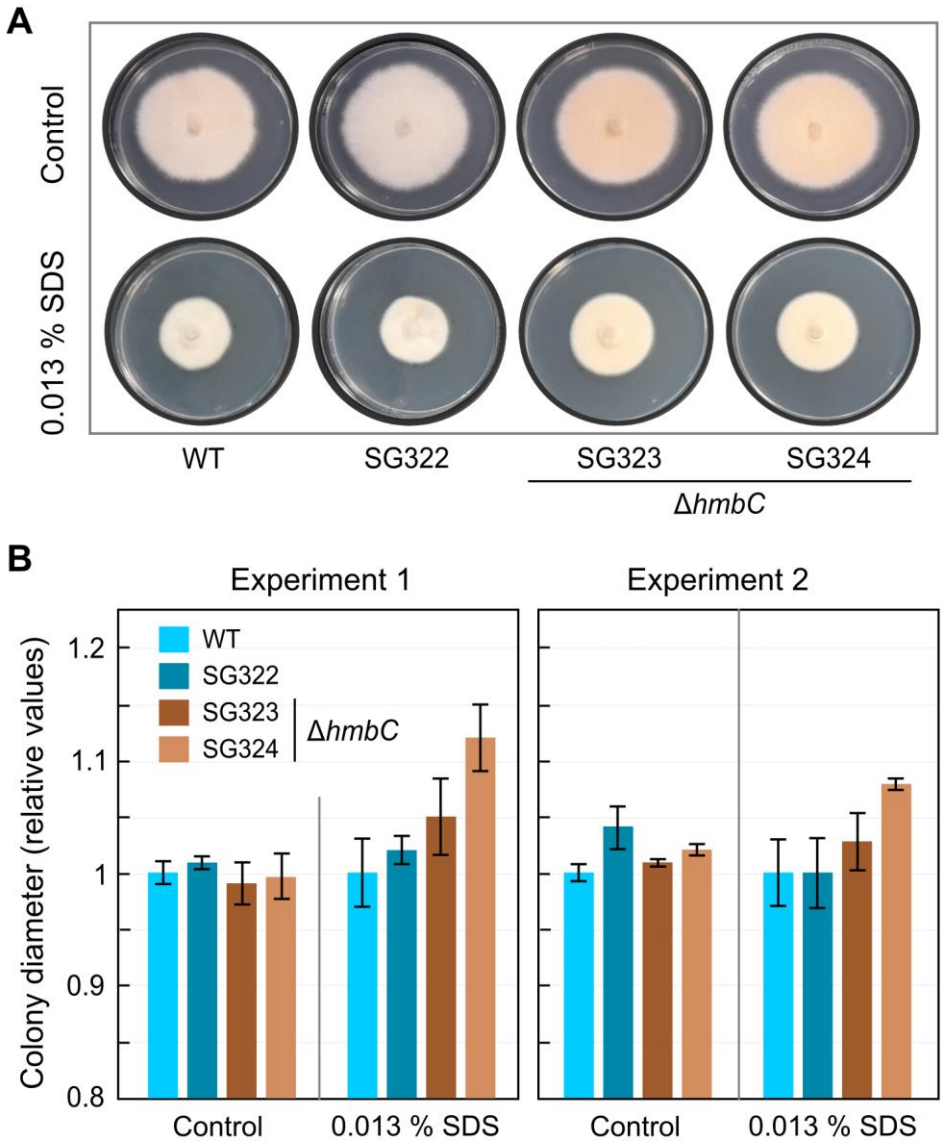

Figure S8. Effect of SDS on growth of Δ*hmbC* mutants and control strains. **(A)** Agar cultures of the indicated strains for 7 days on DG minimal medium and 0.013% SDS stress medium at 30 °C under dark conditions. **(B)** Size of the colonies after 7 days on DG minimal medium and 0.013% SDS medium at 30 °C. Data show the average and standard error from two indep*e*ndent experiments with four parallel cultures in Petri dishes. The statistic Student's *t* test gave values of $p > 0.05$, indicating no significant differences among the strains in the presence of SDS.