Table S1. Primers used in this study

Primer name	Sequence (5'→3')	Source or reference
Cloning of *der* homolog genes		
DrDer-5-NdeI (P1)	ATCATATGCATAAAGTCGCCATCGTGGGC (*Nde*I)	This study
DrDer-3-BamHI (P4)	ATGGATCCTTAGTCCTCGTCGGCTTCGCC (*Bam*HI)	This study
KpDer-5-NdeI	AACATATGATACCTGTGGTCGCGCTTG (*Nde*I)	This study
KpDer-3-BamHI	GCGGATCCTTATTTGCTTTTCTTGATGTG (*Bam*HI)	This study
NgDer-5-NdeI (P1)	ACCATATGAAACCAACCATCGCGCTTA (*Nde*I)	This study
NgDer-3-EcoRI (P4)	GTGAATTCTTATTTGCTGAATTGTTTTTTCACA (*Eco*RI)	This study
SaDer-5-NdeI	CGCATATGACTAAACCTATAGTAGCT (*Nde*I)	This study
SaDer-3-BamHI	CAGGATCCTTAATTTCTCTTTCGAGC (*Bam*HI)	This study
StDer-5-NdeI	AACATATGGTACCTGTGGTCGCGCTTG (*Nde*I)	This study
StDer-3-BamHI	CGGGATCCTTACTTGCTTTTCTTGATATG (*Bam*HI)	This study
Construction of chimeric *der*		
DrGD1-EcLinkerN (P2)	TCAAATTGCGCCCAGTATTCAGCGTCTTCGTCGACTT CCTCTTGCGGTGCGAGATCCTCGGGCAGGTACTTCAT CA	This study
EcLinkerC-DrGD2 (P3)	AGCGGAAGAGAACGGCGAAGAAGAAGAGGAAGACG ACTTCGACCCGCAAAGTCTGATCCGCATTTCGCTCAT CGG	This study
DrC2-L-3 (P5)	CGGGTTTTCCCCTTCCTTCCACTTCAGGCG	This study
DrC2-R-5 (P6)	CGCCTGAAGTGGAAGGAAGGGGAAAACCCG	This study
NgGD1-EcLinkerN (P2)	TCAAATTGCGCCCAGTATTCAGCGTCTTCGTCGACTT CCTCTTGCGGTGCGAGATCCTCGGGGAAGTTTTCTAA AA	This study
EcLinkerC-NgGD2 (P3)	AGCGGAAGAGAACGGCGAAGAAGAAGAGGAAGACG ACTTCGACCCGCAAAGTCTGGTTTTTGCCGTTATCGG TCG	This study
NgC2-L-3 (P5)	CGGGTTTTCCCCTTCGTATTGAATCCGCAAC	This study
NgC2-R-5 (P6)	GTTGCGGATTCAATACGAAGGGGAAAACCCG	This study
T7terminator (P7)	GCTAGTTATTGCTCAGCGG	Novagen

[a]The solid underlines are the sites of listed restriction enzymes.

[b]The dotted underlines are linker region of EcDer.

[c] Primers (P#) indicate the primer sets used for construction of chimeric *der* and their positions are presented in in Fig. S8.

Table S2. Subclones constructed in this study

Plasmids	Description	Source or reference
pUC19	*E. coli* cloning vector	New England Biolabs
pUCDerK	*der*::kan, pUC19	(14)
pUC19DrDer	*der*$^+$ (*Deinococcus radiodurans*), pUC19	This study
pUC19KpDer	*der*$^+$ (*Klebsiella pneumoniae*), pUC19	This study
pUC19NgDer	*der*$^+$ (*Neisseria gonorrhoeae*), pUC19	This study
pUC19SaDer	*der*$^+$ (*Staphylococcus aureus*), pUC19	This study
pUC19StDer	*der*$^+$ (*Salmonella enterica* serovar Typhimurium), pUC19	This study
pCR2.1-Topo	*E. coli* cloning vector	Invitrogen
T;AtDer	N' truncated *der* (*Arabidopsis thaliana*), pCR2.1-Topo	(30)
T;NbDer	N' truncated *der* (*Nicotiana benthamiana*), pCR2.1-Topo	(30)
T;T1458	*der*$^+$ (*Nicotiana benthamiana*), pCR2.1-Topo	(30)

Table S3. Identity and similarity scores between Der homologs

Der homologs	Full sequence	GD1	Linker	GD2	KH domain	C-terminus
DrDer	35/55	43/59 (5-160)	- (161-171)	37/64 (172-339)	26/51 (345-425)	- (426-438)
KpDer	92/96	92/96 (12-165)	73/90 (166-209)	96/98 (210-380)	94/96 (386-466)	88/96 (467-498)
NgDer	49/67	52/67 (6-163)	- (164-177)	60/80 (178-345)	48/68 (351-430)	71/71 (431-485)
SaDer	42/61	49/66 (7-163)	- (164-174)	48/72 (175-346)	37/53 (353-433)	- (434-436)
StDer	97/99	99/100 (6-159)	88/95 (160-201)	99/99 (202-372)	95/98 (378-485)	97/100 (459-490)

* Scores are represented as identity/similarity.

The numbers in parentheses refer to amino acid positions.

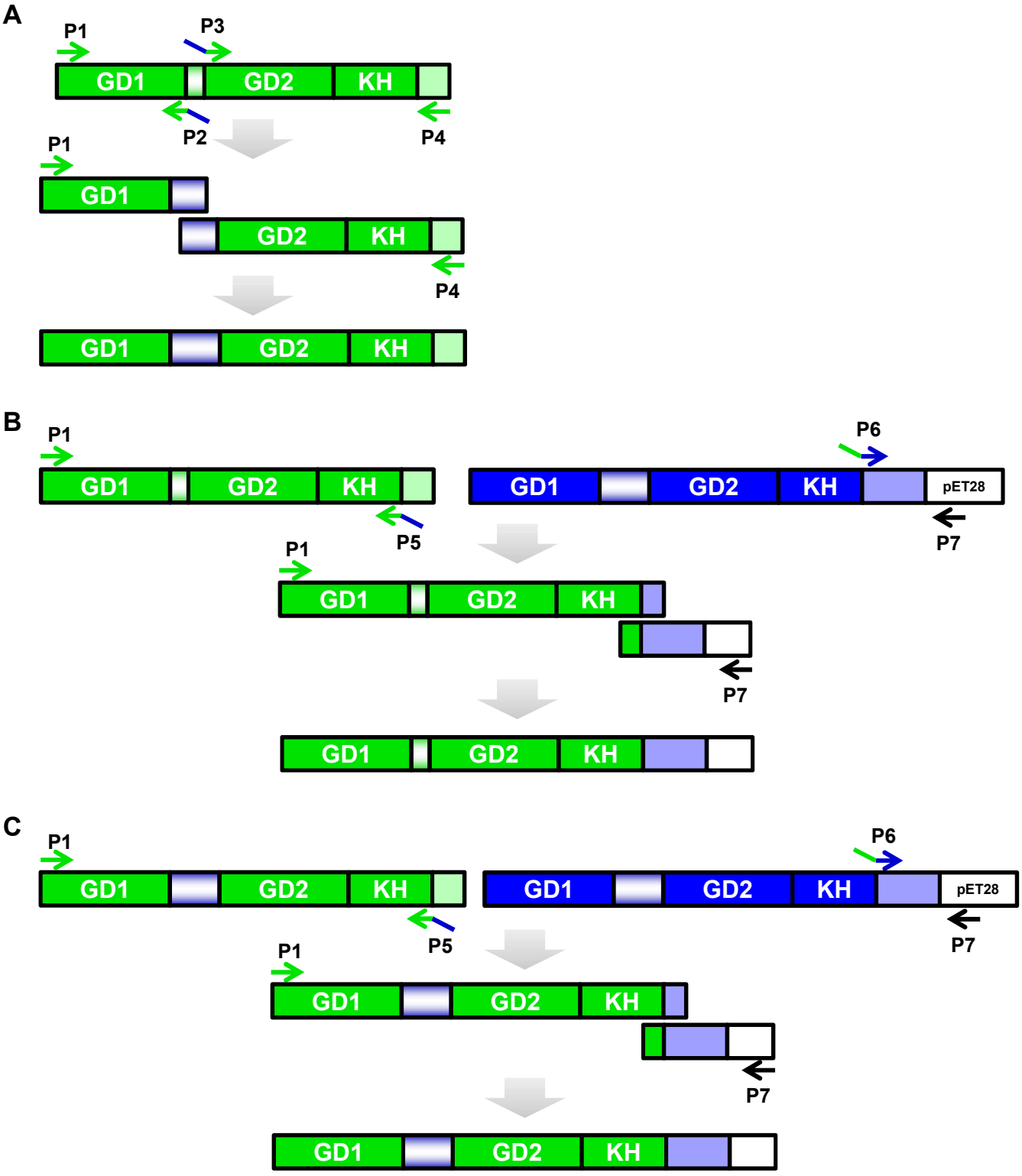

Figure S1. Schematic diagrams of the construction of chimeric *der* using overlapping PCR method. The domain arrangement of EcDer and Der homologs (DrDer and NgDer) are in blue and in green, respectively. Left or right fragment of each chimeric Der was amplified with corresponding primers, and the both fragments were fused together in the second PCR. **(A)** Because EcDer linker was too short to be amplified by PCR, we designed P2 and P3 containing *Ecder* linker sequence. By using these primers, linker region of DrDer or NgDer was substituted by that of *E. coli*. **(B)** Similarly, in case of *E. coli* C-terminal region, P6 and P7 (T7 terminator primer) primers were utilized to amplify C-terminal region using pET28EcDer as template. Then, two fragments amplified in the first PCR were fused by the second PCR, resulting in DrC2 and NgC2. **(C)** To generate chimera harboring both linker and C-terminal region of EcDer, DrC2 or NgC2 were used as template for left fragment, and then C-terminal region was replaced in the same way described above.

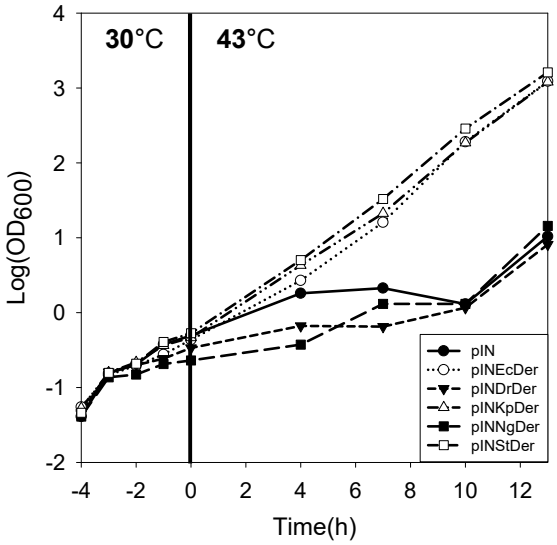

Figure S2. Growth curve of *der*-null strain expressing *E. coli* Der or Der homologs.
All transformants were grown at 30°C in LB medium containing ampicillin, kanamycin and chloramphenicol for 4 h. Portions of each culture were then centrifuged to remove antibiotics and resuspended in pre-warmed LB medium containing kanamycin and chloramphenicol with or without 1 mM IPTG. The cultures were then shifted to 43°C at the time indicated by the vertical line. During incubation at 43°C, the cultures were repeatedly diluted (1:3–1:5) into fresh pre-warmed medium.

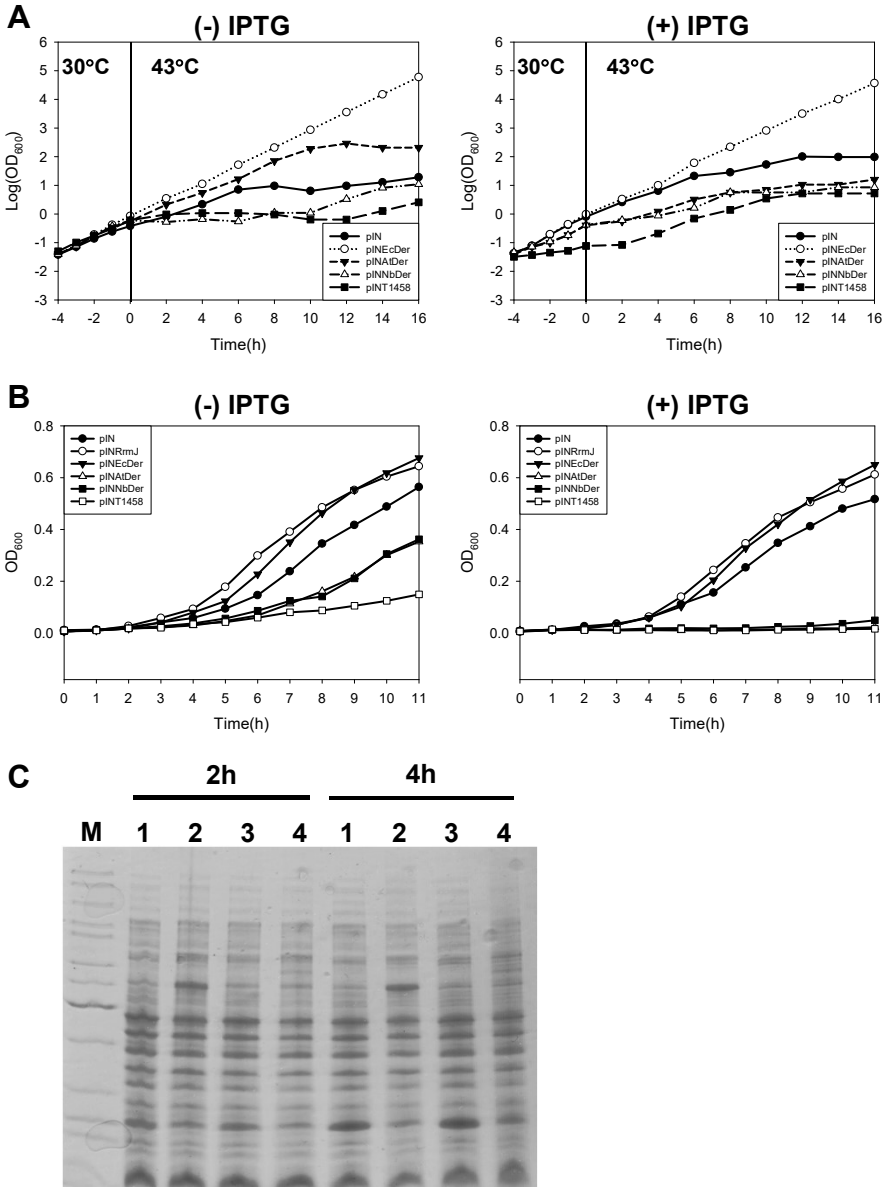

Figure S3. Growth curve of *der*-null strain **(A)** and *rrmJ*-null strain **(B)** expressing plant Der. **(A)** N-terminal truncated forms of *Arabidopsis thaliana der* (AtDer) and *Nicotiana benthamiana der* (NbDer) and full length *Nicotiana benthamiana der* (T1458) were cloned into pIN vector, yielding pINAtDer, pINNbDer and pINT1458, respectively. MK cells transformed with the indicated plasmids were then grown at 30°C in LB medium containing ampicillin, kanamycin and chloramphenicol for 4 h, during which time growth was monitored as described in Fig. 2. **(B)** HB23 cells transformed with each plasmid were cultured in LB medium containing chloramphenicol in the presence and absence of 1 mM IPTG at 30°C. Cell cultures were diluted five times before measurement of the optical density at 600 nm. **(C)** Coomassie blue staining of SDS-PAGE gel showing overexpression of plant Der homologs in MG1655. pIN (lane 1), pINAtDer (lane 2), pINNbDer (lane 3) or pINT1458 (lane 4) was transformed into MG1655. Each transformant was grown to an OD_{600} of 0.5–0.6, then induced with 0.5 mM IPTG for 2 h and 4 h at 37°C. Lane M, molecular marker. AtDer(56.64 kDa), NbDer(64.7kDa), and T1458(72.38 kDa).

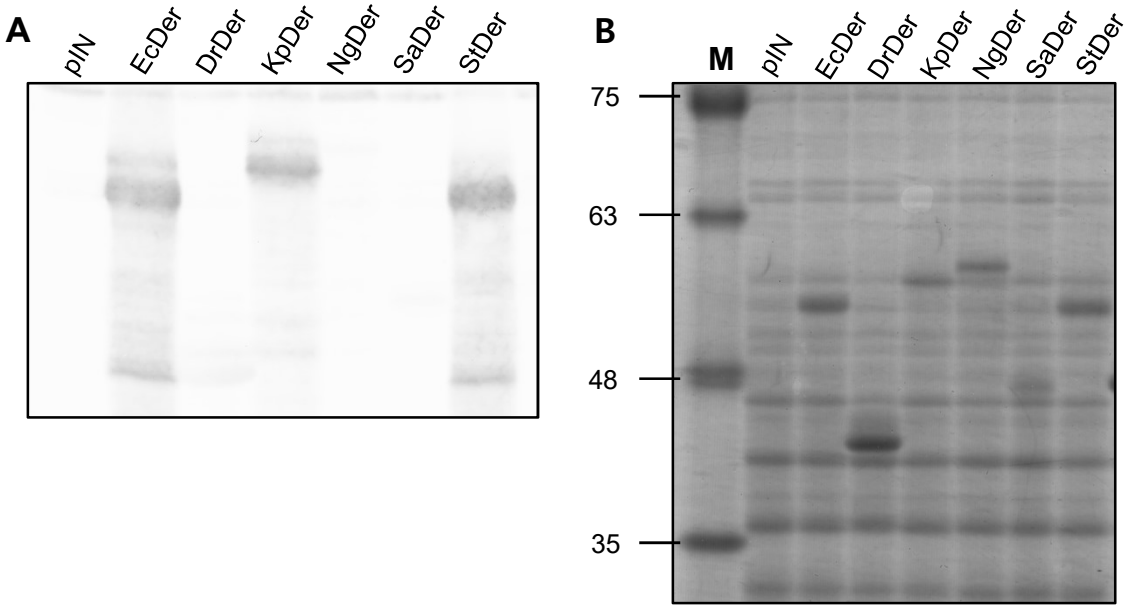

Figure S4. Cross-reactions of anti-EcDer antiserum with Der homologs. **(A)** Wild-type (MG1655) cells were transformed with plasmid, after which cell lysates were subjected to 12.5% SDS-PAGE followed by Western blot. **(B)** Coomassie blue staining of SDS-PAGE gel showing overexpression of Der homologs in MG1655 cells. pIN, pINEcDer, pINDrDer, pINKpDer, pINNgDer, pINSaDer or pINStDer was transformed into MG1655. Each transformant was grown to an OD_{600} of 0.5–0.6, then induced with 0.5 mM IPTG for 2 h at 37°C. GangNam-STAIN^{TM} Prestained Protein Ladder was used to identify the size (in kDa) of the recombinant protein (lane M). EcDer (55.04 kDa), DrDer (49.49 kDa), KpDer (55.99 kDa), NgDer (53.96 kDa), SaDer (48.99 kDa), and StDer (54.98 kDa).

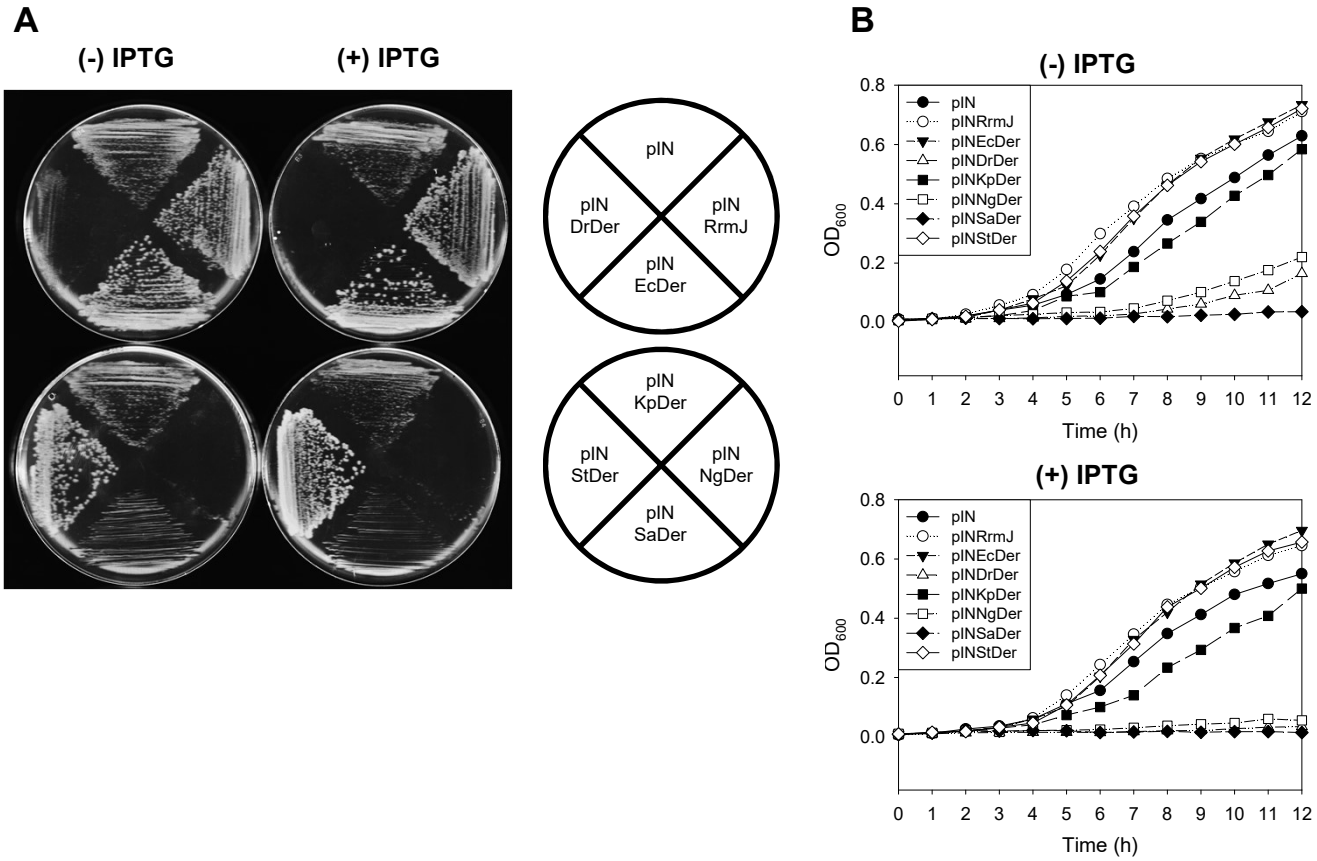

Figure S5. (A) Suppression of the slow growth of *rrmJ*-null by *E. coli* Der or Der homologs. HB23 cells were transformed with pIN, pINRrmJ, pINEcDer, pINDrDer, pINKpDer, pINNgDer, pINSaDer or pINStDer plasmid. The transformants were grown at 30°C overnight in LB medium containing chloramphenicol, and cultures were diluted 10^2 or 10^3-fold in LB medium. Next, 3 µl of diluted cultures were streaked onto LB plates containing chloramphenicol with or without 1 mM IPTG. **(B)** Growth curve of strain HB23 harboring *E. coli der* or other Der homolog expression vectors. Cells were cultured in LB medium containing chloramphenicol or chloramphenicol and 1 mM IPTG at 30°C, and cell cultures were diluted five times before measurement of the optical density at 600 nm (OD_{600}).

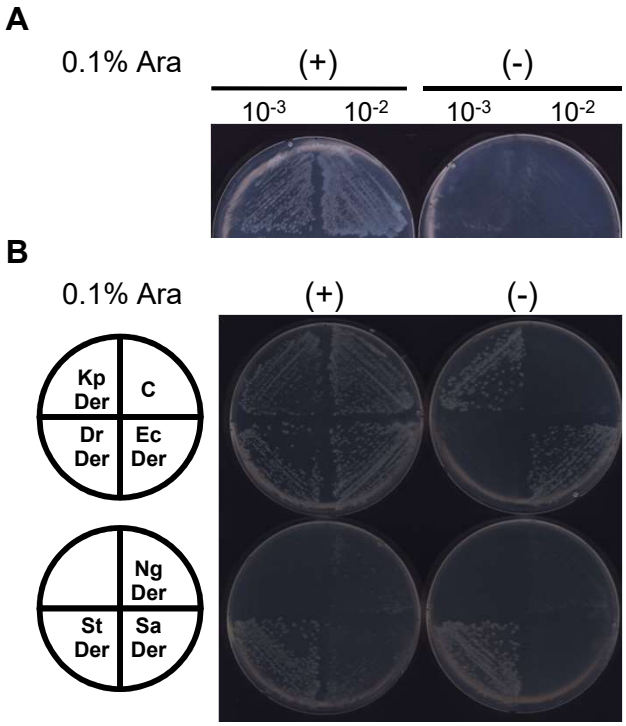

Figure S6. Arabinose-dependent growth of ESC08 and complementation of ESC08 cells by MBP-Der homologs. **(A)** To determine if MBP-Der homologs function in vivo, *der*-null cells were transformed with plasmids expressing MBP-Der homologs. The strain MK used in this paper had helper plasmid containing an ampicillin resistance cassette and pMAL-c5x vector also has ampicillin resistance cassette. Therefore, we constructed a new *der*-null strain, ESC08, that contains an arabinose-inducible helper plasmid, pBAD33EcDer. Overnight culture of ESC08 was diluted 10^2 or 10^3-fold in LB medium. Next, 2.5 µl aliquots of diluted cultures were streaked onto LB+KC plates with or without 0.1% arabinose and incubated 37°C overnight. **(B)** Complementation of strain ESC08 by expression of MBP-Der homologs. Overnight cultures were diluted 10^{-3}. Next, 2.5 µl aliquots of diluted cultures were streaked on LB+AKC plates with or without 0.1% arabinose and incubated at 37°C overnight. The vector pMAL-c5x harbors leaky Tac-promoter, so without IPTG Der homologs were expressed. C indicates ESC08 harboring pMAL-c5x as a negative control.

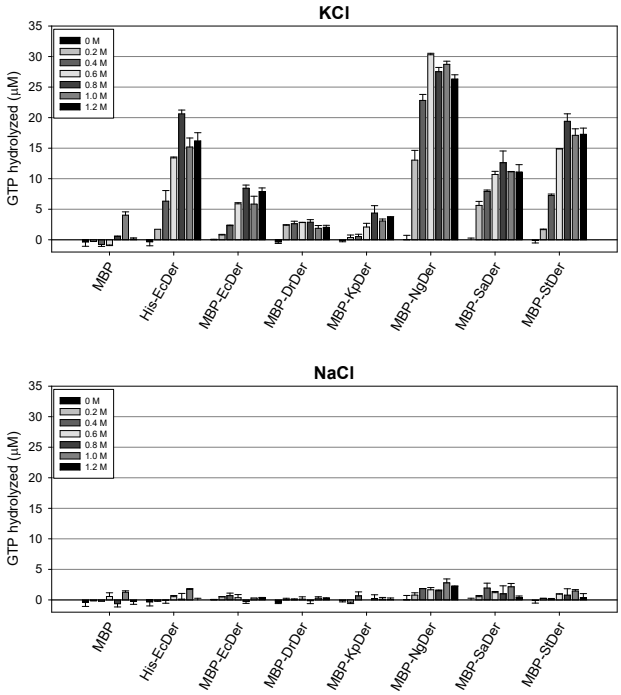

Figure S7. Effects of KCl or NaCl on the GTPase activities of Der homologs. GTPase assay was carried out in reaction mixture as described in Figure 6 but with different concentrations (0-1.2 M) of KCl or NaCl for 20 min at 37°C.

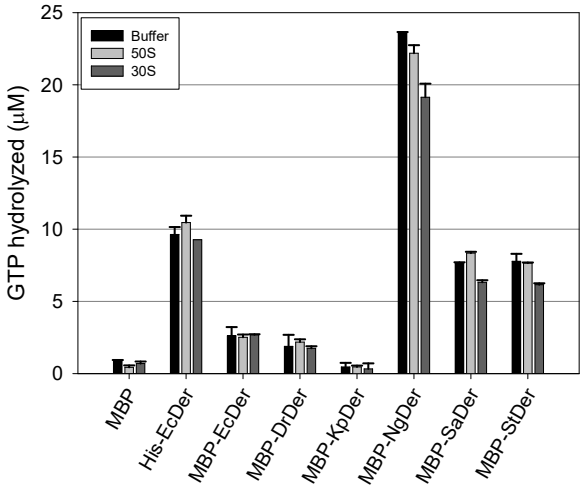

Figure S8. Effects of ribosomal subunit on the GTPase activities of Der homologs.
GTPase assay was carried out in a 100 μl reaction mixture containing 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 128 μM GTP, 0.2 μM protein, 200 mM KCl and 0.1 μM ribosomal subunit for 20 min at 37°C. Each measurement was repeated twice.