Supplementary Materials: Functional Analysis of the Fusarielin Biosynthetic Gene Cluster

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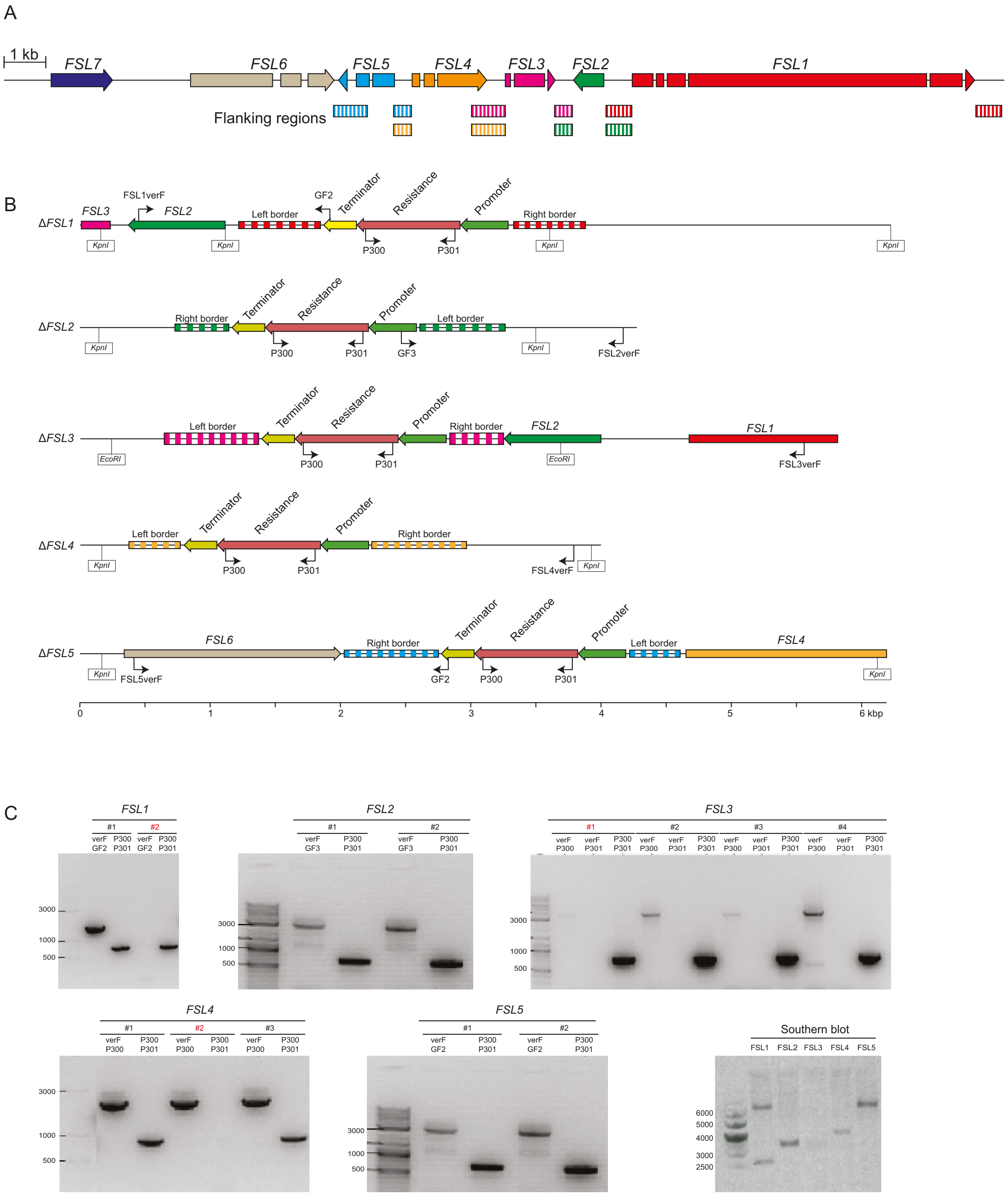

Figure S1. Generation and verification of deletion mutants. (**A**) Overview of the fusarielin gene cluster with highlighted border regions used for generation of knock-out vectors; (**B**) Illustration of the genomic DNA regions for knock-out mutants of *FSL1-5* showing the border regions, geneticin resistance cassette, primer sites used for verification and restriction sites (*KpnI* and *EcoRI*, respectively) used for Southern blot analyses; (**C**) Verification of mutants with PCR and Southern blotting. Incorrect mutants are labelled in red.

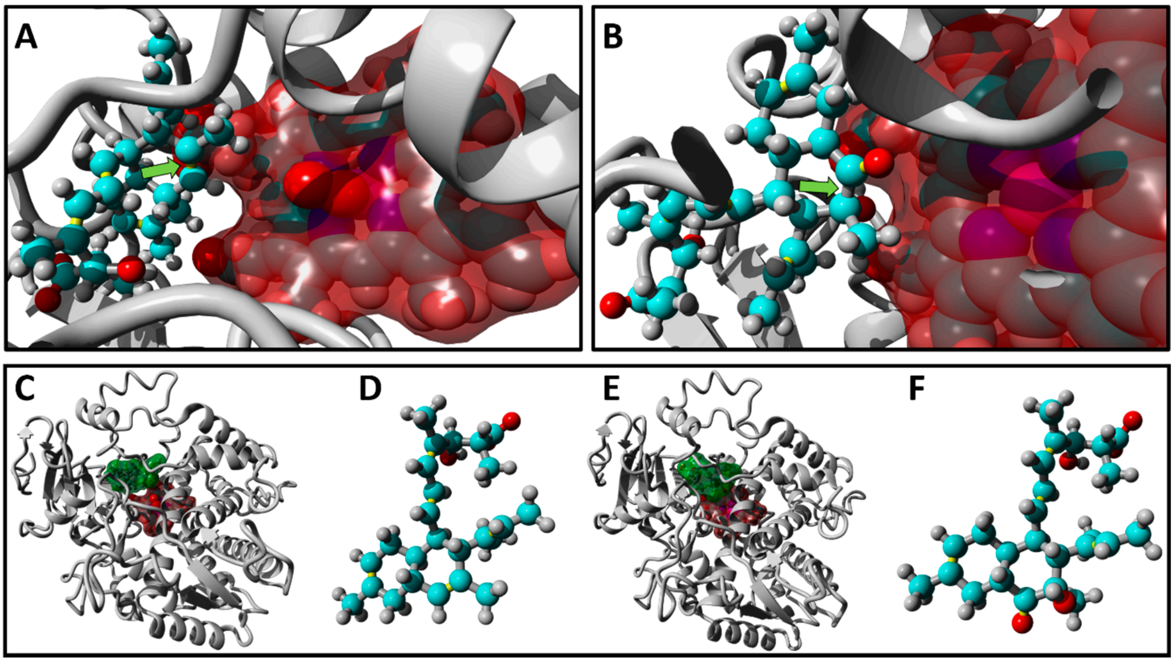

Figure S2. Docking of prefusarielin and fusarielin F into energy-minimized homology models of FSL4 (grey ribbon diagrams). (**A**) A prefusarielin docking pose near the oxy-heme group (red surface-rendering) bound within the oxy-heme FSL4 model. The green arrow indicates the position of the C15-C16 double-bond in prefusarielin; (**B**) A similar docking-pose of fusarielin F near the deoxy-heme group, with the green arrow indicating the position of the C15-C16 double-bond; (**C**) Global view of the prefusarielin docking pose (surface-rendered green) within the oxy-heme FSL4 homology model; (**D**) Energy-minimized prefusarielin ligand; (**E**) Global view of the fusarielin F docking-pose (surface-rendered green) within the deoxy-heme FSL4 homology model; (**F**) Energy-minimized fusarielin F ligand.

Table S1. Conservation of the fusarielin gene cluster in *Aspergillus clavatus*, *Penicillium nordicum* and *Colletotrichum simmondsii* and comparison to lovastatin gene cluster in *Aspergillus terreus* [6,7,10]. Numbers in parentheses are E values from BlastP analyses.

Gene Name	**Length (bp/aa)**	***Aspergillus clavatus***	***Penicillium nordicum***	***Colletotrichum simmondsii***	***Aspergillus terreus***
FSL1 (FGSG_10464)	8224/2642	ACLA_098920 (0.0)	ACN38_g5709 (0.0)	CSIM01_02879 (0.0)	LovB; ATEG_09961 (0.0)
FSL2 (FGSG_10463)	747/248	ACLA_098880 (1e-61)	ACN38_g5705 (8e-69)	CSIM01_02875 (5e-66)	LovG; ATEG_09962 (1e-08)
FSL3 (FGSG_17368)	1215/362	ACLA_098890 (1e-146)	ACN38_g5705 (1e-138)	CSIM01_02876 (5e-66)	
FSL4 (FGSG_10461)	1808/557	ACLA_098900 (2e-164)	ACN38_g5707 (2e-163)	CSIM01_02878 (7e-165)	LovA; Q9Y7C8.1 (no hit)
FSL5 (FGSG_17367)	1279/299	ACLA_098910 (5e-151)	ACN38_g5708 (8e-147)	CSIM01_02877 (5e-138)	LovC; ATEG_09963 (1e-82)
FSL6 (FGSG_10459)	3460/1045	-	-	-	
FSL7 (FGSG_10458)	1491/496	ACLA_098870 (0.02)	ACN38_g5704 (9e-09)	CSIM01_02875 (2e-14)	

Table S2. Primers used in the study to create knock-out vectors, verify *F. graminearum* mutants and for RT-PCR.

Name	Sequence	Amplicon
FSL5-A1	5′-GGTCTTAAXCTTGAAGCTTAGAGGCT	418 bp
FSL5-A2	5′-GGCATTAAXCTTGAGGGAGCTGAG	
FSL5-A3	5′-GGACTTAAXCACACGCAACTCACTCA	736 bp
FSL5-A4	5′-GGGTTTAAXGGAGGATGAGGTCTTG	
FSL4-A1	5′-GGTCTTAAXCTTGAGGGAGCTGAGTA	402 bp
FSL4-A2	5′-GGCATTAAXCTTGAAGCTTAGAGGCTG	
FSL4-A3	5′-GGACTTAAXCACCCAGCGCCG	729 bp
FSL4-A4	5′-GGGTTTAAXGGTATGACGACGGGTA	
FSL3-A1	5′-GGTCTTAAXCACCCAGCGCCG	745 bp
FSL3-A2	5′-GGCATTAAXGGTATGACGACGGGTA	
FSL3-A3	5′-GGACTTAAXGCTGGATCGCCATAAG	435 bp
FSL3-A4	5′-GGGTTTAAXAGATCACTGGTTTCAAGCA	
FSL2-A1	5′-GGTCTTAAXGATGAATTATAATGTATTTCTGAATGAAT	660 bp
FSL2-A2	5′-GGCATTAAXTTCTGTGCTTGTGATTGTTTC	
FSL2-A3	5′-GGACTTAAXAGATCACTGGTTTCAAGCA	419 bp
FSL2-A4	5′-GGGTTTAAXGCTGGATCGCCATAAG	
FSL1-A1	5′-GGTCTTAAXTTCTGTGCTTGTGATTGTTTC	660 bp
FSL1-A2	5′-GGCATTAAXGATGAATTATAATGTATTTCTGAATGAATG	
FSL1-A3	5′-GGACTTAAXGGAAAGACAAGAGCAGG	587 bp
FSL1-A4	5′-GGGTTTAAXGTTGATAATTGTAAAAGCGATATATG	
FSL5-verF	5′-TTCTTTGTACACAGGCCGACAGGACGCAATGA	2418 bp (in combination with GF2)
FSL4-verF	5′-GATGCAACTTGTGGTCGCGTACCGTTTTATCACT	2691 bp (in combination with P300)
FSL3-verF	5′-TCCTCAATAGCTGCACTGAGTGGCTTCA	3848 bp (in combination with P300)
FSL2-verF	5′-TCCTCAATAGCTGCACTGAGTGGCTTCA	1693 bp (in combination with GF3)
FSL1-verF	5′-CATTATAGCAGACCAGGCATCTTCTA	1472 bp (in combination with GF2)
GF2-verR	5′-GTTGTTGACCCCTATACTCTT	
GF3-verR	5′-AGACCCAATTACACCCTT	
P300	5′-GTAAAGCACGAGGAAGCG	690 bp (verification of geneticin)
P301	5′-TTGGGTGGAGAGGCTATT	
FSL7-RT292	5′-ACAGCGACGCTAAGCTTCGAGCCGCCTGCGATA	717 bp
FSL7-RT299	5′-TCGCATCAACAGTTGAATATAAACACCGTA	
FSL6-RT270	5′-CCAACACTATTTTACTCACTGGCGCCA	503 bp
FSL6-RT272	5′-TACCGCCGTTCCGCGATATTGCCCTT	
FSL5-RT296	5′-ATAGACGGCAAAATGGTTGGCAATCTGGCTA	409 bp
FSL5-RT297	5′-AACATGTGGTGATGGGGATCAACCCGGAGAGT	
FSL4-RT273	5′-GCCTTTTCGCCATCTTCCAACACCCGCGAGCCGTTCA	191 bp
FSL4-RT275	5′-GATTGGATTTCCCAAAGTCATGAGAATGGTTA	
FSL3-RT294	5′-AATCCTTACCTGAACGGTGCTATTGGTCGATA	422 bp
FSL3-RT295	5′-ATGCAACTTGTGGTCGCGTACCGTTTTATCACT	
FSL2-RT276	5′-TTCAAAGTACTGCGAATCATCAAGACGGATA	701 bp
FSL2-RT277	5′-TGCGCAGAGTCCCAACATGAAAGCACAG	
FSL1-RT278	5′-CAATTGGCATGGTCCCTCAATGACCATTGA	282 bp
FSL1-RT280	5′-TCCTCAATAGCTGCACTGAGTGGCTTCA	
TEF1-RT152	5′-GGAGGAGAAGACTCACCTTAACGTCG	260 bp
TEF1-RT153	5′-CGGTGACATAGTAGCGAGGAGTCTC	
TUB-RT150	5′-TGACTGGTCGCAACATGAAGACTTTCC	301 bp
TUB-RT151	5′-GCGGTGGAGTTGCCAACGAAA	