

Table S1. Y2H screening results**A. Fsr1 N-terminal as bait**

Fv Locus	Gene name (domain)
FVEG_00233.3	Hypothetical protein
FVEG_00403.3	putative cyclophilin peptidyl-prolyl cis-trans isomerase
FVEG_00510.3	Hypothetical protein
FVEG_00562.3	40S ribosomal protein S6-B
FVEG_01143.3	putative alpha keto acid dehydrogenase
FVEG_01304.3	cystathionine gamma-lyase
FVEG_01449.3	conserved hypothetical protein (TF)
FVEG_01920.3	conserved hypothetical protein (Hex1)
FVEG_02319.3	putative mitochondrial carrier protein
FVEG_02948.3	C-1-tetrahydrofolate synthase
FVEG_03125.3	putative glyoxalase
FVEG_04097.3	SCP like extracellular protein
FVEG_05696.3	conserved hypothetical protein
FVEG_06063.3	conserved hypothetical protein
FVEG_07050.3	Heat shock protein HSP98
FVEG_07289.3	Eukaryotic translation initiation factor 5A-2
FVEG_07318.3	putative cystathionine beta-lyase
FVEG_07509.3	conserved hypothetical protein
FVEG_08088.3	putative transcription initiation factor
FVEG_08459.3	putative phospholipase B homolog
FVEG_08486.3	conserved hypothetical protein
FVEG_08639.3	conserved hypothetical protein
FVEG_08746.3	putative phospholipase B homolog
FVEG_08950.3	hypothetical protein
FVEG_09495.3	O-acetylhomoserine
FVEG_09511.3	putative glutamate synthase
FVEG_09578.3	putative choline sulfatase
FVEG_10001.3	conserved hypothetical protein
FVEG_10119.3	putative Antigen1 precursor (no domains)
FVEG_10127.3	conserved hypothetical protein
FVEG_10358.3	putative pyruvate dehydrogenase complex
FVEG_10441.3	putative short-chain dehydrogenase
FVEG_10833.3	hypothetical protein
FVEG_11066.3	putative glutamyl-tRNA synthetase
FVEG_11334.3	peroxisomal membrane anchor protein
FVEG_11642.3	putative 2-oxoglutarate dehydrogenase
FVEG_12134.3	Conserved hypothetical Sel1 repeat protein
FVEG_12379.3	conserved hypothetical protein
FVEG_12398.3	putative mannose-binding lectin
FVEG_12529.3	O-acetylhomoserine
FVEG_12531.3	putative FMN-dependent dehydrogease
FVEG_12848.3	conserved hypothetical protein
FVEG_12878.3	putative carboxypeptidase Y
FVEG_13501.3	conserved hypothetical protein
FVEG_13647.3	conserved hypothetical protein
No Fus homolog	hypothetical protein

Table S2. Additional Description of putative Fsr1-interacting proteins

Fv Locus	Gene Name	Key pfam motif	Proposed function
FVEG_00403	<i>FvCYP1</i>	cyclophilin-like domain (CLD) (PF04126)	Act as a virulence determinant during plant infection in <i>Magnaporthe grisea</i> and <i>Botrytis cinerea</i> (Viaud <i>et al.</i> , 2003); act as modulators of protein function in eukaryotes such as budding yeast cyclophilin Cpr1 (Arevalo-Rodriguez & Heitman, 2005)
FVEG_04097	<i>FvSCP1</i>	sperm-coating protein (SCP)-like extracellular protein (PF00188)	Act as a Ca ²⁺ -chelator in various signaling processes (Cantacessi <i>et al.</i> , 2009)
FVEG_12134	<i>FvSEL1</i>	Sel1-like repeat (SLR) (PF08238)	Involved in signal transduction pathways, involved in the ER-associated protein degradation under cellular stress (Kamauchi <i>et al.</i> , 2005) (Gardner <i>et al.</i> , 2000)
FVEG_01920	<i>FvHEX1</i>	Hex1 (cd04469)	Physiology and functioning of the Woronin body (Curach <i>et al.</i> , 2004), asexual production and pathogenicity in <i>F. graminearum</i> (Son <i>et al.</i> , 2013)
FVEG_11334	<i>FvPEX14</i>	Pex14_N (pfam04695)	Sustain the formation of fruiting bodies and the maturation and germination of sexual spores (Peraza-Reyes & Berteaux-Lecellier, 2013). Important for lipid metabolism (Wanders <i>et al.</i> , 2010) and are implicated in the homeostasis of reactive oxygen species (Fransen <i>et al.</i> , 2012).

References

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Table S3. *F. verticillioides* strains used in this study.

Strain	Source
<i>F. verticillioides</i> 7600	FGSC
Δ fsr1	Shim et al., 2006
Δ Fvstp1	This study
Δ Fvcyp1	This study
Δ Fvscp1	This study
Δ Fvsel1	This study
Fvstp1c	This study
Fvstp1nc	This study
Fvcyp1c	This study
Fvscp1c	This study
Fvsel1c	This study
Fvcyp1-gfp	This study
Fvscp1-gfp	This study
Fvsel1-gfp	This study
Fsr1-gfp	This study
Fvcyp1-Fsr1-gfp	This study
Fvscp1-Fsr1-gfp	This study
Fvscp1-Fsr1-gfp	This study

Table S4. Primers used in this study. The underlined sequences were for fusion purpose. The recognition site of the restriction enzyme is shaded in select primer sequences.

Primer Name	5'-3' sequence
FsrExon1for	CATCTCTCCCAAACCGACTC
FsrExon1revt	CACTGGCATATCCTCCAAGGTAGATCTAAGCTTCTCTTGTATCTTTGC
FsrExon2fort	GCAAAGATACAAGAGAAGCTTAGATCTACCTTGGAGGATATGCCAGTG
FsrExon2rev	CTTCGTGGCCAGAAATGATAG
FNcos	GCCGCCATGGGCCCTA ATGCT
FBrev	CAGCCATGGGATCCGTCCACC
T7seq	TAATACGACTCACTATAGGGC
jRv	CTCCCGAGCCTGAAGCTGAGCCGTCGAGTTCCTATCGCGCTCATG
iFw	CATGAGCGCGATAGGAACTCGACGGCTCAGCTTCAGGCTCGGGAG
3'BDseq	TTTTCGTTTTAAACCTAAGAGTC
5'BDnfor	CATCATGGAGGAGCAGAAG
3'BDnrev	CATAAATCATAAGAAATTCGCCCGG
5'LD Amplimer	CTATTGATGATGAAGATACCCACCAAACCC
3'LD Amplimer	GTGAACTTGCGGGGTTTTTCAGTATCTACGAT
FCC1n LUC-F	<u>AAGCTCGAGTAGTCGAC</u> ATGTCAGCCAATTATTGGCAC
FCC1nLUC-R	<u>CGTACGAGATCTGGTCGAC</u> CTTATCTAAGCCTCTTGCCCTT
FCK1cLUC-F	<u>CGTCCCGGGGCGGTACC</u> CCTCTCCGTCCTCACATCG
FCK1cLUC-R	<u>TTGGATCCCCGGGTACC</u> CTATTCTTCTGCCTCTTGGC
STR1nLUC-F	<u>AAGCTCGAGTAGTCGAC</u> ATGGGCCCTAATGCTGGCAA
STR1nLUC-R	<u>CGTACGAGATCTGGTCGACT</u> CGTGCAAACACCTTGACCAC
STR2cLUC-F	<u>CGTCCCGGGGCGGTACC</u> CCTCATTCTTCAACAGATACTTCTAC
STR2cLUC-R	<u>TTGGATCCCCGGGTACC</u> TTACCAGACCTCACCCGG
STR3cLUC-F	<u>CGTCCCGGGGCGGTACC</u> AGACCCTCCCTCTTCAAGCC
STR3cLUC-R	<u>TTGGATCCCCGGGTACC</u> TTACAGCTCACCAGAGTCGACA
STR4cLUC-F	<u>CGTCCCGGGGCGGTACC</u> CCTTTTGTACCGTCTGTAGTG
STR4cLUC-R	<u>TTGGATCCCCGGGTACC</u> TTAGACAGTAACGCGCTTCTCG
STR5cLUC-F	<u>CGTCCCGGGGCGGTACC</u> GCCAGCTATCAGCAATATGC
STR5cLUC-R	<u>TTGGATCCCCGGGTACC</u> TTACATGAGGATCTGTCAATGGT
1F	CGTTACCAATTCCCAAGGCTG
2R	<u>TAGATGCCGACCGGGAACG</u> TTTGGGGCATGGCATTTTG
3F	<u>CCACTAGCTCCAGCCAAGG</u> CCTCCTCACTGTTACACAAAGG

4R	CAGCTGCACTGGCTATCTAG
5F	AAGAGCGCGAGCGATTTTC
6R	GAAATCGCTCGCGCTCTT
7F	GGCAAAGCAAAGGACAGAA
8F	TCTACCACAGAGAGCTCTGG
9R	<u>TAGATGCCGACCGGGAACCTC</u> ATTTTGAGGAAGCTGGAGG
10F	<u>CCACTAGCTCCAGCCAAGGACAAAGGGATGGGATTTGGGA</u>
11R	AGAAGCGACTGAACATGCACG
12F	TACTCAACTGAGAACAGCCA
13R	CACAGAGATGGACTTACCCT
14F	CCGCTCTCAACTCCGATAGTAG
15R	<u>TAGATGCCGACCGGGAACAAAGGCATGGCTCTGCTTC</u>
16F	<u>CCACTAGCTCCAGCCAAGGGCACGCTTCTACATCGATC</u>
17R	TGCCTCGCAAGTATCTCTACC
18F	GGCTTCTTTTGTACTCCCTG
19R	AGCACTCCGTCAATAAACAC
20F	AATTCGAGAAGCAGGCGAG
21R	<u>TAGATGCCGACCGGGAACGCCAAGCTGTGCATATTGCT</u>
22F	<u>CCACTAGCTCCAGCCAAGGGACTAAACCAAGTGGGACATG</u>
23R	TTGCACTTCCACATGCTGC
24F	GGCTTCTTTTGTACTCCCTG
25R	AGCACTCCGTCAATAAACAC
26F	GGAGAAGACCCAGAAGAAGG
27R	CGGGAGCAGAACTCACTACA
HYG-F	CTTGGCTGGAGCTAGTGGAGGTCAA
HY-R	GTATTGACCGATTCTTGCGGTCCGAA
YG-F	GATGTAGGAGGGCGTGGATATGTCCT
HYG-R	GTTCCCGGTCTGGCATCTACTCTAT
GFP-R2	<u>GGCACCGGCTCCAGCGCCTGCACCAGCTCCCTTGTACAGCTCGTCCATGC</u>
RP27-F	TCT TCG CTA TTA CGC CAG C
RP27-R	TTT GAA GAT TGG GTT CCT ACG
CYP-F	<u>GGA GCT GGT GCA GGC GCT GGA GCC GGT GCC</u> ATG AGA CCC TCC CTC TTC AAG
CYP-R	TTA CAG CTC ACC AGA GTC GAC A
SCP-F	<u>AGG AAC CCA ATC TTC AAA</u> ATG CCT TTT GTC ACC GTC TG

SCP-R	GGCACCGGCTCCAGCGCCTGCACCAGCTCCGAC AGT AAC GCG CTT CTC G
SEL-F	AGG AAC CCA ATC TTC AAA ATG GCT CCC CCA CAG TTA G
SEL-R	GGCACCGGCTCCAGCGCCTGCACCAGCTCCCAT GAG GAT CTG TCA ATG GTG TT
FSR1-F	AGG AAC CCA ATC TTC AAA ATG GGC CCT AAT GCT GGC AA
FSR1-R	GCTTGGCGGACCATGGTATTGT
GFP-SF	ACA TGA AGC AGC ACG ACT TC
GFP-SR	GAA GTC GTG CTG CTT CAT GT