**Supplementary material**

**Table S1: Plant 2-ODDs with proven F3H, FNS I, FLS and ANS functionality, used in phylogenetic analysis.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Name** | **Species** | **GenBank ID** | **Reference** | **Function** |
| MtF3H | *Medicago truncatula* | FJ529406.1 | Shen et al. (2010) | F3H |
| OsF3H | *Oryza sativa* | NM\_001060692.1 | Kim et al. (2008) | F3H |
| PcF3H | *Petroselinum crispum* | AY230248.1 | Martens et al. (2001) | F3H |
| PhF3H | *Petunia hybrida* | AF022142.1 | Britsch et al. (1986) | F3H |
| ZmF3H | *Zea mays* | AAA91227.1 | Deboo et al. (1995) | F3H |
| AcFLS | *Allium cepa* | KY369209.1 | Park et al. (2017) | FLS |
| AtFLS | *Arabidopsis thaliana* | NP\_001190266.1 | Owens et al. (2008) | FLS |
| CuFLS | *Citrus unshiu* | Q9ZWQ9.1 | Wellmann et al. (2002) | FLS |
| FaFLS | *Fragaria x ananassa* | DQ087252 | Almeida et al. (2007) | FLS |
| MdFLS | *Malus domestica* | AY965343 | Halbwirth et al. (2006) | FLS |
| OsFLS | *Oryza sativa* | XP\_015624815.1 | Park et al. (2019) | FLS |
| PcFLS | *Petroselinum crispum* | Q7XZQ6.1 | Martens et al. (2003) | FLS |
| PhFLS | *Petunia hybrida* | Q07512.1 | Holton et al. (1993) | FLS |
| ZmFLS | *Zea mays* | BT039956 | Falcone Ferreyra et al. (2010) | FLS |
| AtDMR6 | *Arabidopsis thaliana* | OAO94377.1 | Falcone Ferreyra et al. (2015) | FNSI |
| CjFNSI | *Conocephalum japonicum* | QEP99662.1 | Li et al. (2020) | FNSI |
| CmFNSI | *Conium maculatum* | AY817677 | Gebhardt et al. (2005) | FNSI |
| MeFNSI | *Marchantia emarginata* | QEP99659.1 | Li et al. (2020) | FNSI |
| MpaFNSI | *Marchantia paleacea* | QEP99658.1 | Li et al. (2020) | FNSI |
| PaFNSI | *Plagiochasma appendiculatum* | QEP99657.1 | Li et al. (2020) | FNSI |
| PcFNSI | *Petroselinum crispum* | Q7XZQ8.1 | Martens et al. (2001) | FNSI |
| ZmFNSI | *Zea mays* | NP\_001151167.1 | Falcone Ferreyra et al. (2015) | FNSI |
| AcANS | *Allium cepa* | EF192598.1 | Kim et al. (2004) | ANS |
| AtANS | *Arabidopsis thaliana* | NP\_194019.1 | Wilmouth et al. (2002) | ANS |
| FaANS | *Fragaria x ananassa* | AY695817.1 | Almeida et al. (2007) | ANS |
| GbANS | *Ginkgo biloba* | ACC66092.1 | Xu et al. (2008) | ANS |
| OsANS | *Oryza sativa* | Y07955.1 | Reddy et al. (2007) | ANS |
| PaANS | *Phytolacca americana* | AB198870.1 | Shimada et al. (2005) | ANS |
| PfANS | *Perilla frutescens* | O04274.1 | Saito et al. (1999) | ANS |
| PhANS | *Petunia hybrida* | AB027454.1 | Nakajima et al. (2001) | ANS |
| SoANS | *Spinacia oleracea* | AB198869.1 | Shimada et al. (2005) | ANS |
| TfANS | *Torenia fournieri* | AB044091.1 | Nakajima et al. (2001) | ANS |
| ZmANS | *Zea mays* | NP\_001106074 | Nakajima et al. (2001) | ANS |
| AtGA3ox1 | *Arabidopsis thaliana* | NP\_173008.1 | Kawai et al. (2014) | outgroup |

**Table S2: Sequence Read Archive IDs for RNA-Seq raw data used for expression analysis.**

|  |  |
| --- | --- |
| **biolocial source** | **Sequence Read Archive IDs** |
| embryogenic | SRR8761343, SRR8761344, SRR8761345 |
| seedling | SRR7340404, SRR7340406 |
| root | SRR2984591, SRR2984594, SRR2984596, SRR2984598, SRR2984601, SRR4450942, SRR4450934, SRR4450941, SRR4450933, SRR4450944 |
| leaf | SRR6320573, SRR6320439, SRR6320438, SRR6320441, SRR6320440, SRR6320443, SRR5405140, SRR5405141 |
| young\_leaf | SRR6320573, SRR6320439 |
| adult\_leaf | SRR6320438, SRR6320441 |
| old\_leaf | SRR6320440,SRR6320443 |
| pulp | SRR5405133, SRR6320444, SRR5405132,SRR6320447, SRR5405131, SRR6320446, SRR5405130, SRR6320531 |
| pulp S1 | SRR5405133, SRR6320444 |
| pulp S2 | SRR5405132, SRR6320447 |
| pulp S3 | SRR5405131, SRR6320446 |
| pulp S4 | SRR5405130, SRR6320531 |
| peel | SRR5405139, SRR5405138, SRR5405137, SRR6320442, SRR5405136, SRR6320445, SRR5405135, SRR5405134 |
| peel S1 | SRR5405139, SRR5405138 |
| peel S2 | SRR5405137, SRR6320442 |
| peel S3 | SRR5405136, SRR6320445 |
| peel S4 | SRR5405135, SRR5405134 |

**Table S3: Oligonucleotide primers, used in this work.**

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence (5’-3’)** | **Description** |
| RS1708 | attB1-ccATGGCTCCCGGCACCGCAGTCCTTCCC | fwd Primer with attB1 for *Musa*F3H1 (Ma02\_t04650) CDS |
| RS1709 | *attB2-*aAGCCAAGATCTCATCGAGGCCTGTAGCC | rev Primer with attB2 for *Musa*F3H1 (Ma02\_t04650) CDS |
| RS1710 | *attB1-*ccATGGCCCCCGGCACTGCATTGCTTCCC | fwd Primer with attB1 for *Musa*F3H2 (Ma07\_t17200) CDS |
| RS1712 | *attB2-*aAGCTAGGATCTCATTTAGGCATTTCGCC | rev Primer with attB2 for *Musa*F3H2 (Ma07\_t17200) CDS |
| RS1681 | *attB1-*ccATGGAGGTGGAGAGGGTGCAGGCC | fwd Primer with attB1 for *Musa*FLS1 (Ma03\_t06970) CDS |
| RS1682 | attB2-aCTGCGGAAGCTTGTTCAGCTTGCAG | rev Primer with attB2 for *Musa*FLS1 (Ma03\_t06970) CDS |
| RS1695 | attB1-ccATGGAGGTGGAGAGGGTTCAAGTCATCGCG | fwd Primer with attB1 for *Musa*FLS3 (Ma10\_t25110) CDS |
| RS1696 | attB2-aTTGCGGAAGCTTGTTGAGCTTGCAGTAGGCG | rev Primer with attB2 for *Musa*FLS3 (Ma10\_t25110) CDS |
| RS1704 | attB1-ccATGGCCACCAAGGTCGTGTCTG | fwd Primer with attB1 for *Musa*ANS (Ma05\_t03850) CDS |
| RS1705 | attB2-aGTTTGGGGTCTTGAAGTCCCCCCG | rev Primer with attB2 for *Musa*ANS (Ma05\_t03850) CDS |
| RS2101 | attB1-ccATGGCTCCTACAACAATAAC | fwd Primer with attB1 for *PcFNSI* CDS |
| RS2102 | attB2-AGCTAAATTTTCATCTGCAC | rev Primer with attB2 for *PcFNSI* CDS |

attB1: GGGGACAAGTTTGTACAAAAAAGCAGGCT, attB2: GGGGACCACTTTGTACAAGAAAGCTGGGT

**File S1: CDS of *Musa2-*ODDs from this work.**

Variant positions to the Pahang reference genome are given in bold-underlined.

*Musa*F3H1

ATGGCTCCCGGCACCGCAGTCCTTCCCACCAACGAGCAGACCTTGAGGGCGAGCTTCGTCCGGGACGAGGACGAGCGTCCCAAGGTGGCTTACAACCAGTTCAGCCTTGACATCCCCGTGATCTCGCTCTCCGGGATTGATGACGACGCCGCGGGCGGGAAGAGGGCGGAGATCCGCCGCAAGGTCGTGGAGGCCTGCGAGGACTGGGGTATATTCCAGGTGGTGGACCACGGGGT**G**GACGCCGGCCTTATCTCCGACATGACTAGGCTGGCCAAAGAGTTCTTCGCGCTGTCCCCGGA**T**GAGAAGCTCTTGTTCGACATGTCCGGTGGGAAGAAGGGAGGGTTCATCGTCTCCAGCCACCTCCAGGGCGAGGCAGTTCAGGACTGGAGAGAGATCGTGACGTACTTCTCATACCCAATTCGGGCTCGGGACTACTCGCG**G**TGGCCGGACAAGCCGGAAGGGTGGAGATCCGTGGTGGAGTCGTACAGCGAGAAGCT**G**ATGGGCTTGGCGTGCAAGCTCTTGGAGGTGTTGTCTGAGGCTATGGGGCTCGACAAGGAGGCCCTCACCGAGGCCTGCATCGACATGGATCAGAAGGT**G**GTCGTCAACTACTACCCCAAGTGCCCCCAGCCGGACCTCACTCTTGGCCTCAAACGACACACCGATCCCGGCACCATCACCCTCCTCCTCCAAGACCAAGTCGGCGGCCTCCAAGCCACCAAGGATGGCGGCAAGACATGGATCACGGTTCAGCCTGTGGAGGGAGCTTTTGTGGTCAACCTCGGGGACCATGGCCATTTTCTGAGCAATGGGAGGTTCAAGAATGC**A**GACCACCAGGCTGTGGTGAACTCAAACT**G**CAGCAGGCTCTCCATCGCCACGTTTCAGAACCCCGCGCCGGAGGCAATCGTGTATCCGCTGGCAGTAAGGGAGGGAGAGAAGCCGATTCTGGA**C**GAGCCCATCACGTTTGCCGAGATGTACCGCAGGAAGATGAGCCGAGACCTCGAGCTCGCCAAGCTCAAGAAACTGGCCAAGACGGAGCAG**C**AGCAG**A**AGCCGGAGCTACT**A**GAGAAGACTAAGGACATCAACTTGGCCAAGGCTACAGGCCTCGATGAGATCTTGGCT

*Musa*F3H2

ATGGCCCCCGGCACTGCATTGCTTCCCACGAACGAGGCGACCCTGAGGGCGAGCTTCGTGCGGGACGAGGACGAGCGACCCAAGGTGGCCTA**C**AACCA**G**TTCAGCAGCGACATCCC**C**GTGATCTCGCTCGCCGGGGTTGACGACGAGGACGGGGCCACCGGCGGGCGGAGGGCGGAGATCCGCCGCAAGATCGTGGA**A**GCCTGCGAGGACTGGGGCTTATTCCAGGTG**T**TGGACCATGGAGTGGACGCCGGCATGATCTCCGAGATGACCCGGCTCGCCCGGGATTTCTTCGCGCTACCCCCCGAGGAGAAGCT**A**CGGTTCGACATGTCCGGCGGAAAAAAGGGAGGCTTCATCGTGTCCAGCCATCTCCAGGGTGAGGCAGTGCAGGACTGGCGAGAGATCGTGACTTACTTCTCGTACCCGATCGGAGCTCGGGACTACTCGAGGTGGCCGGATAAGCCAGAAGGGTGGAGGTCGGTGGTGGAGTCATACAGCGAGAAGCTGATGGGGTTGGCGTGCAAGCTGCTGGAGGTGTTGTCGGAGGCAATGGGGCTCGACAAGGAAGCCCTCAC**T**GATGCCTGCGTAGACATGGATCAGAAGGTGGTGGTCAACTACTACCCCA**T**GTGCCCCCAGCCCGACCTCACCCTCGGCCTCAAACGCCACACCGATCCCGG**C**ACCATCACCCTCCTCCTCCAAGACCAAGTCGGCGGACTCCAAGCCACCAAGGACGGCGGCAAGACGTGGATCACAGTTCAGCCCGTGGAGGGAGCTTTCGT**T**GTCAACCTCGGGGACCATGGCCATTATCTGAGCAACGGGAGGTTCAAGAACGC**C**GATCATCAGGCGGTGGTGAACTCGAGCTGCAGCAGGCTGTCGATCGCCACGTTTCAGAACCCAGCGCCGGAGGCGATCGTGTATCCCCTGGCGATAAGGGAGGGAGA**G**AAGCCGATCCTGGACGAGCCCATCACGTTCAGCGAGATGTACCGTAGGAAGATGAGCCGAGATCTC**A**AGCTTGCCAACCT**T**AAGAA**G**CTGGCCA**A**GGCGGAGCATCAGCGGC

*Musa*FLS1

ATGGAGGTGGAGAGGGTGCAGGCCATCGCGTCCCTGAGCGTGGCCACCAACGACATACCGCCGGAGTTCGTGAGGTCGGAGCACGAGCAGCCGGGTATCACCACGTACCGCGGCC**T**GGTCCCGGAGATCCCGGTGATCGACCTCGAAGACGGGGACGAAGGCCGGGTGACGCGCGCCATCGCGGAGGCCAGCCAGGAGTGGGGCATCTTCCAGCTGGTGAACCACGGCATCCCCGGGGAGGTGATCCGGGCGCTGCAGCGCGTGGGCAGGGAGTTCTTCGAGCTGCCACCGGAGGAGAAGGAGAAGTACGCGGCGGCGCCGGGGAGCCTCCAGGGCTACGGAACCAAGCTGCAGAAGGACTTGGAAGGCAAGAAGGCGTGGGTGGACTTCCTCTTCCACAACATCTGGCCGCCGACGCACGTCGACCACCGCGCATGGCCGGAGAATCC**G**GTGGATTACAGGAAGGCAAATGAGGAGTACGCCAAACATTTGGTGGGATTGGTGGAGAAGATGTTGGTAAGCCTGTCCAAGGGACTGGGGCTGGAGGCCGACGTCCTCAAGCACGCAGTGGGAGGGGACGACTTGGAGTTCCTCCTCAAGATCAACTACTACCCGCCGTGCCCGAGACCCGACCTCGCCCTCGGCGTGGTGGCTCACACCGACATGTCCGCCATCACCATCCTGATCCCCAACGACGTCCCCGGCCTCCAGGTCTTCAAGGACGACCACTGGTTCGACGCCAAGTACGTCCCCGACGCCATCATCGTCCACATCGGGGACCAGATCGAGAAACTGAGCAACGGCAGGTACAAGAGCGTGCTGCACCGGACGACGGTGAACAAGGAGAAGGCGAGGATGTCGTGGCCTGTGTTCTGCTCCCCGCCGGGCGAGACGGTCATTGGGCCTCTGCCGCAGCTCGTCAGCGACGAACAGCCCGCTCAGTACAAGACGAAGAAGTACAAGGACTATGCTTTCTGCAAGCTGAACAAGCTTCCGCAG

*Musa*FLS3

ATGGAGGTGGAGAGGGTTCAAGTCATCGCTTCCGTCTGCGCCGCCGACGGTGTTATGCCTCCGGAGTTCATACGGTCGGAGCACGAGCAGCCGGGCATCACCACCTACCGCGGCCCCGCGCCGGAGATCCCCGTCATCGACCTCGCGGGCGCCGACCGGGA**C**CGGTTGACGATCGCCGTCGCCGAGGCCAGCCGGGAGTGGGGAATCTTCCAACTGCTGAACCACGGGATCCCGAGGGAGGTGATCCGTGAGCT**T**CAGCGCGTCGGCAAG**C**AATTCTTCGAGTTGCCGCAGGAGGAGAAGGAGATGTACGCGATGGAAT**G**CAAACCGGGGAGCTCGGAAGGATATGGGAC**C**AAGCTGCAGAGGGAGTTGGAGGGCAAAAAGGC**T**TGGGT**A**GACTTCTTCTTCCACTAC**A**T**T**TCGCCGCCGGCTCGCGTCAACCACGCCATCTGGCCCAAGAACCCT**G**CTGATTA**T**AGGAAAGCAAACGAGGAATATG**G**CAAACACCTGGTGGG**T**CTGGTGGACAAGATGCTGA**T**GAC**G**CTGTCGAGGGGACTGGGACTGGAGGAGCATGTCCTCAAGGGAGCACTCGGTGGAGATGGACTGGAGCTACG**T**CTAAAGATGAACTACTACCCACCGTGTCCTCGGCCTGACCTGGCTCTCGGCGTGGTGGCGCACACTGACATGTGTGCTATCACCTTCCTCGTCCCTAACCTTGTGCCGGGTTTGCAGGTCTTCAAAGATGAGCACTGGATTGACGTCAACTTCATTCCTAATGCTGTCATCGTCCACATCGGTGATCAGATCGAGATTTTGAGCAATGGAACATACAAGAGCGTGCTGCACAGAACGACCGTGAACAAAGAGAAGGTGAGGATGTCTTGGCCAGTGTTCTGCGCGCCTCCTGGTGAGATGGTAATTGGCCCTCTGCAACAGCTCGTCGGCGATGAGAGCCCAGCCAAGTATAAGCCGAAGAAGTACAAAGACTACGCCTACTGCAAGCTCAACAAGCTTCCGCAA

*Musa*ANS

ATGGCCACCAAGGTCGTGTCTGTGGCGCCCAGGGTGGAGATCCT**G**GC**G**AAGAGCGGCATCAACGAGATCCCGACCGAGTACGTCCGCCCCGAGTCGGAGCGGCTCGACCTCGGTGACGCGTTCGAGGAGGTGAAGAAGGCGGCGGAGGGGCCTCAGATTCCTGTGGTGGACCTCCAGGGTTTCGACTCGCCTGATGAAGAGGTGAGAAGGGCGTGCGTGGAGGAGGTGAGGAAGGCGGCGACGGAGTGGGGGGTGATGCACATCGTGAATCACGGCATCCCGTTGGAGCTCGTCGAGCAGCTGAGGAAGGTGGGGAAGGAGTTCTTCGACCTGCCCATAGAGCAGAAGGAGCAGTACGC**A**AACGACCAGTCATCAGGGAAGATCCAAGGGTACGGGAGCAAGCTGGCGAACAACGCGAGTGGGCAGCTCGAGTGGGAGGACTACTTCTTCCACCTTATATTCCCGGAGGAGAAGATCAACATCTCCATTTGGCCCAAGCAACCAACCGACTACATTGAGGTGACGAAGAAGTTCGGGAGGCAGCTGAGGGCGGTGGTCACCAAGATGCTGGAAGTTCTTTCCCTGGGTCTTGGACTGGAAGAGGGGAAGCTGGACAGGGAACTCGGAGGGATGGAGGACCTGCTGATGCAGTTGAAGATCAACTACTACCCTATCTGCCCACAGCCCGATCTCGCCCTCGGCGTCGAGGCGCACACCGACATCAGCGCGCTCTCCTTCATCCTCCACAACATGGTGCCGGGGCTGCAGATCTACTACGGCGGCAGGTGGGTCACCGCCAAATG**T**GTGCCGGACTCCATCATCATGCACGTCGGAGACTGCCTCGAGATCCTGAGCAATGGGCAGTACAAGAGCATCCTCCACCGCGGGCTCGTCAACAAGGAGAAGGTGCGCATCTCCTGGGCGGTCTTCTGCGAGCCTCCCAAAGACAAGATCGTGCTGAAGCCACTGGAGGAGCTCGTGGCCGACGGGACGCCGGCCAAGTTCCCCCCGCGCACCTTCGAGCAGCACATCCAGCACAAGCTCTTCAAGAAGACTCGGGGGGACTTCAAGACCCCAAAC

**File S2: Multiple sequence alignment of flavonoid biosynthesis related 2-ODDs from banana and plant proteins with demonstrated 2-ODD activity.**

Substrate binding residues are highlighted in grey. Bold-underlined amino acids are involved in binding ferrous iron or 2-oxoglutarate (Chua et al., 2008). Asterisks mark conserved residues, colons indicate very similar amino acids and full stops indicate weakly similar properties.

AtF3H -------MAP-----GTLTELA---GESKLNSKFVRDEDERPKVAYNV----------FS 35

MusaF3H1 -------MAP-----GTAVLPT---NEQTLRASFVRDEDERPKVAYNQ----------FS 35

MusaF3H2 -------MAP-----GTALLPT---NEATLRASFVRDEDERPKVAYNQ----------FS 35

OsF3H -------MAPV----ATTFLPTAS-NEATLRPSFVRDEDERPRVAYNQ----------FS 38

ZmF3H -------MAPVSIS-AVPFLPTAAEGETNVRASFVREEDERPKVPHDR----------FS 42

AcFLS --------MEVE---RVQAIATLTANLGTIPSEFIRSDHERPDLTTYH---------GPV 40

MusaFLS1 --------MEVE---RVQAIASLSVATNDIPPEFVRSEHEQPGITTYR---------GLV 40

MusaFLS3 --------MEVE---RVQVIASVCAADGVMPPEFIRSEHEQPGITTYR---------GPA 40

MusaFLS4 --------MEVE---RVQVIASVCAADGVMPPEFIRSEHEQPGITTYR---------GPA 40

MusaFLS2 --------MAQV---KVQAIASMLHSKDTIPPEFVRVEDEQPGATTYR---------GPA 40

AtFLS1 --------MEVE---RVQDISSSSLLTEAIPLEFIRSEKEQPAITTFR---------GPT 40

ZmFLS --------MGGETHLSVQELAAS---LGALPPEFVRSEQDQPGATTYRG--------AAV 41

OsFLS -------MAEVQ---SVQALASS---LAALPPEFVRSEHERPGATTFRG--------GDA 39

AtANS -------MVAVE---RVESLAKS--GIISIPKEYIRPKEELESINDVFLEEKK----EDG 44

MusaANS MATK--VVSVAP---RVEILAKS--GINEIPTEYVRPESERLDLGDAFEEVKKA---AEG 50

ZmANS MESSPLLQLPAA---RVEALSLS—GLSAIPPEYVRPADERAGLGDAFDLARTHANDHTA 55

OsANS -------MTDAE--LRVEALSLS—GASAIPPEYVRPEEERADLGDALELARAASDDDAT 49

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AtF3H DEIPVISLAGI--DDVD----GKRGEICRQIVEACENWGIFQVVDHGVDTNLVADMTRLA 89

MusaF3H1 LDIPVISLSGI--DDD--AAGGKRAEIRRKVVEACEDWGIFQVVDHGVDAGLISDMTRLA 91

MusaF3H2 SDIPVISLAGV--DDEDGATGGRRAEIRRKIVEACEDWGLFQVLDHGVDAGMISEMTRLA 93

OsF3H DAVPVISLQGI--DE------AARAEIRARVAGACEEWGIFQVVDHGVDAGLVADMARLA 90

ZmF3H DEVPVVSLEGI--DGP-----RRRAEIRGRVAAACEDWGIFQVVDHGVDAALVADMARLA 95

AcFLS PELPVIDLANS-----------SQENVVKQISEAAREYGIFQLVNHGIPNEVINELQRVG 89

MusaFLS1 PEIPVIDLEDG-----------DEGRVTRAIAEASQEWGIFQLVNHGIPGEVIRALQRVG 89

MusaFLS3 PEIPVIDLAGA-----------DRDRLTIAVAEASREWGIFQLLNHGIPREVIRELQRVG 89

MusaFLS4 PEIPVIDLAGA-----------DRDRLTIAVAEASREWGIFQLLNHGIPREVIRELQRVG 89

MusaFLS2 PAVPVIDLADT-------------DRVVDAIADASREWGIFQLVNHGIPAAVIGELQRVG 87

AtFLS1 PAIPVVDLSDP-----------DEESVRRAVVKASEEWGLFQVVNHGIPTELIRRLQDVG 89

ZmFLS PDAPVIDISEP--------------GFGARMAAAAREWGLFQVVNHGVPSAAVAELQRVG 87

OsFLS PEIPVIDMAAP--------------ESAARVAEAAAEWGLFQVVNHGVPAAAVAELQRVG 85

AtANS PQVPTIDLKNI--ESDD---EKIRENCIEELKKASLDWGVMHLINHGIPADLMERVKKAG 99

MusaANS PQIPVVDLQGF--DSPD---EEVRRACVEEVRKAATEWGVMHIVNHGIPLELVEQLRKVG 105

ZmANS PRIPVVDISPFLDSSSQ---QQQRDECVEAVRAAAADWGVMHIAGHGIPAELMDRLRAAG 112

OsANS ARIPVVDISAF---DND---GDGRHACVEAVRAAAEEWGVMHIAGHGLPGDVLGRLRAAG 103

\*.:.: : \*. ::\*:::: .\*\*: : : .

AtF3H RDFFALPPEDKLRF--DMSGGKKGGFIVSSHLQGEAVQDWREIVTYFSYPVRNRDYSRWP 147

MusaF3H1 KEFFALSPDEKLLF--DMSGGKKGGFIVSSHLQGEAVQDWREIVTYFSYPIRARDYSRWP 149

MusaF3H2 RDFFALPPEEKLRF--DMSGGKKGGFIVSSHLQGEAVQDWREIVTYFSYPIGARDYSRWP 151

OsF3H RDFFALPPEDKLRF--DMSGGKKGGFIVSSHLQGEAVKDWREIVTYFSYPVKSRDYSRWP 148

ZmF3H RDFFALPPEDKLRF--DMSGGKKGGFIVSSHLQGEAVQDWREIVTYFSYPVKARDYSRWP 153

AcFLS KEFFQLPQEEKEVYATVPDSGSFEGYGTKLQKDLEGKKAWVDYLFHNVWPKHKINYKFWP 149

MusaFLS1 REFFELPPEEKEKYAAAP--GSLQGYGTKLQKDLEGKKAWVDFLFHNIWPPTHVDHRAWP 147

MusaFLS3 KQFFELPQEEKEMYAMECKPGSSEGYGTKLQRELEGKKAWVDFFFHYISPPARVNHAIWP 149

MusaFLS4 KEFFELPQEEKEMYAMEFKPGSSEGYGTKLQRELEGKKAWVDFFFHYVSPPARVNHAIWP 149

MusaFLS2 REFFELPQEEKESYAADPRSGSIEGYGTQIQKDPNGKKAWGDYLFHNVWPPSRINHGMWP 147

AtFLS1 RKFFELPSSEKESVAKPEDSKDIEGYGTKLQKDPEGKKAWVDHLFHRIWPPSCVNYRFWP 149

ZmFLS RAFFALPTEEKERYAMDPASGKIEGYGTKLQRDLEGKKTWNDFFFHVVAPPEKVDHAVWP 147

OsFLS REFFALPQEEKARYAMDASSGKMEGYGSKLQKDLEGKKAWADFFFHNVAPPAMVNHDIWP 145

AtANS EEFFSLSVEEKEKYANDQATGKIQGYGSKLANNASGQLEWEDYFFHLAYPEEKRDLSIWP 159

MusaANS KEFFDLPIEQKEQYANDQSSGKIQGYGSKLANNASGQLEWEDYFFHLIFPEEKINISIWP 165

ZmANS TAFFALPVQDKEAYANDPAAGRLQGYGSRLATNTCGQREWEDYLFHLVHPDGLADHALWP 172

OsANS EAFFALPIAEKEAYANDPAAGRLQGYGSKLAANASGKREWEDYLFHLVHPDHLADHSLWP 163

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AtF3H DKPEGWVKVTEEYSERLMSLACKLLEVLSEAM-GLEK-ESLTNAC----------VDMDQ 195

MusaF3H1 DKPEGWRSVVESYSEKLMGLACKLLEVLSEAM-GLDK-EALTEAC----------IDMDQ 197

MusaF3H2 DKPEGWRSVVESYSEKLMGLACKLLEVLSEAM-GLDK-EALTDAC----------VDMDQ 199

OsF3H DKPAGWRAVVEQYSERLMGLACKLLGVLSEAM-GLDT-NALADAC----------VDMDQ 196

ZmF3H DKPAAWRAVVERYSEQLMALACRLLGVLSEAM-GLDT-EALARAC----------VDMDQ 201

AcFLS RNPPAYRKANEEYTKHLQVVVDKMHSYLSLGL-GLES-HVLKEAVGG--------DDLEY 199

MusaFLS1 ENPVDYRKANEEYAKHLVGLVEKMLVSLSKGL-GLEA-DVLKHAVGG--------DDLEF 197

MusaFLS3 KNPADYRKANEEYGKHLVGLVDKMLMTLSRGL-GLEE-HVLKGALGG--------DGLEL 199

MusaFLS4 KNPSDYRKANEEYAKHLVGLVDKMLTTLSRGL-GLEE-HVLKGALGG--------DGLEL 199

MusaFLS2 RQPSSYREANEEYTKYLLGILDKILDSLSLGL-GLEG-SALKKALGG--------EEMDM 197

AtFLS1 KNPPEYREVNEEYAVHVKKLSETLLGILSDGL-GLKR-DALKEGLGG--------EMAEY 199

ZmFLS RSLAGYREANEEYCRHMQRLTRELFEHLSLGL-GLHG-GAMAEAFGG--------DGLVF 197

OsFLS SHPAGYREANEEYCKHMQRLARKLFEHLSTAL-GLDG-GAMWEAFGG--------DELVF 195

AtANS KTPSDYIEATSEYAKCLRLLATKVFKALSVGL-GLEP-DRLEKEVGGL-------EELLL 210

MusaANS KQPTDYIEVTKKFGRQLRAVVTKMLEVLSLGL-GLEE-GKLDRELGGM-------EDLLM 216

ZmANS AYPPDYIAATRDFGRRTRDLASTLLAILSMGLLGTDRGDALEKALTTTTTRTAADDDLLL 232

OsANS ANPPEYVPVSRDFGGRVRTLASKLLAILSLGL-GLPE-ETLERRLRGHEL-AGVDDDLLL 220

: . : : : \*\* .: \* :

AtF3H KIVVNYYPKCPQPDLTLGLKR**H**T**D**PGTITLLLQDQVGGLQATRDNGKTWITVQPVEGAFV 255

MusaF3H1 KVVVNYYPKCPQPDLTLGLKR**H**T**D**PGTITLLLQDQVGGLQATKDGGKTWITVQPVEGAFV 257

MusaF3H2 KVVVNYYPMCPQPDLTLGLKR**H**T**D**PGTITLLLQDQVGGLQATKDGGKTWITVQPVEGAFV 259

OsF3H KVVVNFYPKCPQPDLTLGLKR**H**T**D**PGTITLLLQDLVGGLQATRDAGKTWITVQPIPGSFV 256

ZmF3H KVVVNFYPRCPQPDLTLGLKR**H**T**D**PGTITLLLQDLVGGLQATRDGGRTWITVQPVEGAFV 261

AcFLS LLKINYYPPCPRPNLALGVVA**H**T**D**MSSLTILVPNEVPGLQVFKDD--HWFDAKYIPNALI 257

MusaFLS1 LLKINYYPPCPRPDLALGVVA**H**T**D**MSAITILIPNDVPGLQVFKDD--HWFDAKYVPDAII 255

MusaFLS3 RLKMNYYPPCPRPDLALGVVA**H**T**D**MCAITFLVPNLVPGLQVFKDE--HWIDVNFIPNAVI 257

MusaFLS4 RLKMNYYPPCPRPDLALGVVA**H**T**D**MCAITFLVPNLVPGLQVFKDE--HWIDVNFIPNAVI 257

MusaFLS2 LLKINYYPPCPRPDLALGVVA**H**S**D**LSAVTILVPSDVPGLQISKDD--RWIDIDYVPGALI 255

AtFLS1 MMKINYYPPCPRPDLALGVPA**H**T**D**LSGITLLVPNEVPGLQVFKDD--HWFDAEYIPSAVI 257

ZmFLS LQKINFYPPCPQPELTLGVAP**H**T**D**MSTLTVLVPNEVQGLQVFKDG--QWYEAKYVPDALI 255

OsFLS LHKINFYPPCPEPELTLGVAP**H**T**D**MSTFTVLVPNDVQGLQVFKDG--HWYDVKYVPDALI 253

AtANS QMKINYYPKCPQPELALGVEA**H**T**D**VSALTFILHNMVPGLQLFYEG--KWVTAKCVPDSIV 286

MusaANS QLKINYYPICPQPDLALGVEA**H**T**D**ISALSFILHNMVPGLQIYYGG--RWVTAKCVPDSII 274

ZmANS QLKINYYPRCPQPELAVGVEA**H**T**D**VSALSFILHNGVPGLQVLHGA--RWVTARHEPGTII 290

OsANS QLKINYYPRCPRPDLAVGVEA**H**T**D**VSALSFILHNGVPGLQVHHAG--SWVTARPEPGTIV 278

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AtF3H VNLGDHGH-----------FLSNGRFKNAD**H**QAVVNSNSS**R**L**S**IATFQNPAPDAT-VYPL 303

MusaF3H1 VNLGDHGH-----------FLSNGRFKNAD**H**QAVVNSNCS**R**L**S**IATFQNPAPEAI-VYPL 305

MusaF3H2 VNLGDHGH-----------YLSNGRFKNAD**H**QAVVNSSCS**R**L**S**IATFQNPAPEAI-VYPL 307

OsF3H VNLGDHAHIMHLLGNVNLQYLSNGRFKNAD**H**QAVVNSDCC**R**L**S**IATFQNPAPDAM-VYPL 315

ZmF3H VNLGDHGH-----------LLSNGRFKNAD**H**QAVVNSECS**R**L**S**IATFQNPAPDAT-VYPL 309

AcFLS CHIGDQLE-----------ILSNGKYKSVL**H**RTTVNKEKS**R**M**S**WPVFCSPPGDTM-IGPL 305

MusaFLS1 VHIGDQIE-----------KLSNGRYKSVL**H**RTTVNKEKA**R**M**S**WPVFCSPPGETV-IGPL 303

MusaFLS3 VHIGDQIE-----------ILSNGTYKSVL**H**RTTVNKEKV**R**M**S**WPVFCAPPGEMV-IGPL 305

MusaFLS4 VHIGDQIE-----------ILSNGTYKSVL**H**RTTVNKEKV**R**M**S**WPVFCAPPGEMV-IGPL 305

MusaFLS2 IHIGDQIE-----------ILSNGKYKSVL**H**RATVNKEKA**R**I**S**WPVFCSPPPEMT-VGPL 303

AtFLS1 VHIGDQIL-----------RLSNGRYKNVL**H**RTTVDKEKT**R**M**S**WPVFLEPPREKI-VGPL 305

ZmFLS VHIGDQIE-----------IFSNGAYKAVL**H**RTTVNKEKT**R**M**S**WPMFVEPPGELV-VGPH 303

OsFLS IHIGDQIE-----------ILSNGRYKAVL**H**RTTVDKDRT**R**M**S**WPVFVEPPPEHV-VGPH 301

AtANS MHIGDTLE-----------ILSNGKYKSIL**H**RGLVNKEKV**R**I**S**WAVFCEPPKDKIVLKPL 317

MusaANS MHVGDCLE-----------ILSNGQYKSIL**H**RGLVNKEKV**R**I**S**WAVFCEPPKDKIVLKPL 323

ZmANS VHVGDALE-----------ILSNGRYTSVL**H**RGLVNREAV**R**I**S**WVVFCEPPPDSVLLHPL 339

OsANS VHVGDALE-----------ILTNGRYTSVL**H**RGLVSRDAV**R**L**S**WVVFCEPPPESVLLQPV 327

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AtF3H K--VREGEKAILEEPITFAEMYKRKMGRDLELARLKKLAKEERDHK-EVDKP-------- 352

MusaF3H1 A--VREGEKPILDEPITFAEMYRRKMSRDLELAKLKKLAKTEQQQKPELLEK-------T 356

MusaF3H2 A--IREGEKPILDEPITFSEMYRRKMSRDLKLANLKKLAKAEHQRL-ELPEN-------A 357

OsF3H A--VRDGEEPILEEPITFAEMYRRKMARDLELAKLKKKAKEQRQLQ-QAALPPPPPTQVA 372

ZmF3H A--VRDGEAPILDHPITFAEMYRRKMARDIELARLKKQAKADKKQQ-QQSAN-------- 358

AcFLS PQLVNDEN-PPKFKTKKYKDYAYCKINK---------LPQ-------------------- 335

MusaFLS1 PQLVSDEQ-PAQYKTKKYKDYAFCKLNK---------LPQ-------------------- 333

MusaFLS3 QQLVGDES-PAKYKPKKYKDYAYCKLNK---------LPQ-------------------- 335

MusaFLS4 QQLVGDES-PAKYKPKKYKDYAYCKLNK---------LPQ-------------------- 335

MusaFLS2 PQFVSDQN-PAKYKTKKYKDYQYCKLNK---------LPQ-------------------- 333

AtFLS1 PELTGDDN-PPKFKPFAFKDYSYRKLNK---------LPLD------------------- 336

ZmFLS PKLVTEES-PAKYKAKKYKDYQHCKINK---------LPM-------------------- 333

OsFLS PQLVTDGS-PAKYKAKKFKDYRHCKINK---------LPM-------------------- 331

AtANS PEMVSVES-PAKFPPRTFAQHIEHKLFG---------KEQEELVSE-KN----------- 355

MusaANS EELVADGT-PAKFPPRTFEQHIQHKLFK---------KTRGDFKTP-------------- 359

ZmANS PELVTEGH-PARFTPRTFKQHLDRKLFK---------KKQQHKAKA-EEEDGG------N 382

OsANS QELLADGAGKPLFAPRTFKQHVQRKLFKKL-------KDQQDNNAA-AASNG-------- 371

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AtF3H ----------VDQIFA 358

MusaF3H1 KDINLAKATGLDEILA 372

MusaF3H2 NDINVAKAKCLNEILA 373

OsF3H AELAAQKPKSLDEILA 388

ZmF3H KEFADSKP--LDAIFA 372

AcFLS ---------------- 335

MusaFLS1 ---------------- 333

MusaFLS3 ---------------- 335

MusaFLS4 ---------------- 335

MusaFLS2 ---------------- 333

AtFLS1 ---------------- 336

ZmFLS ---------------- 333

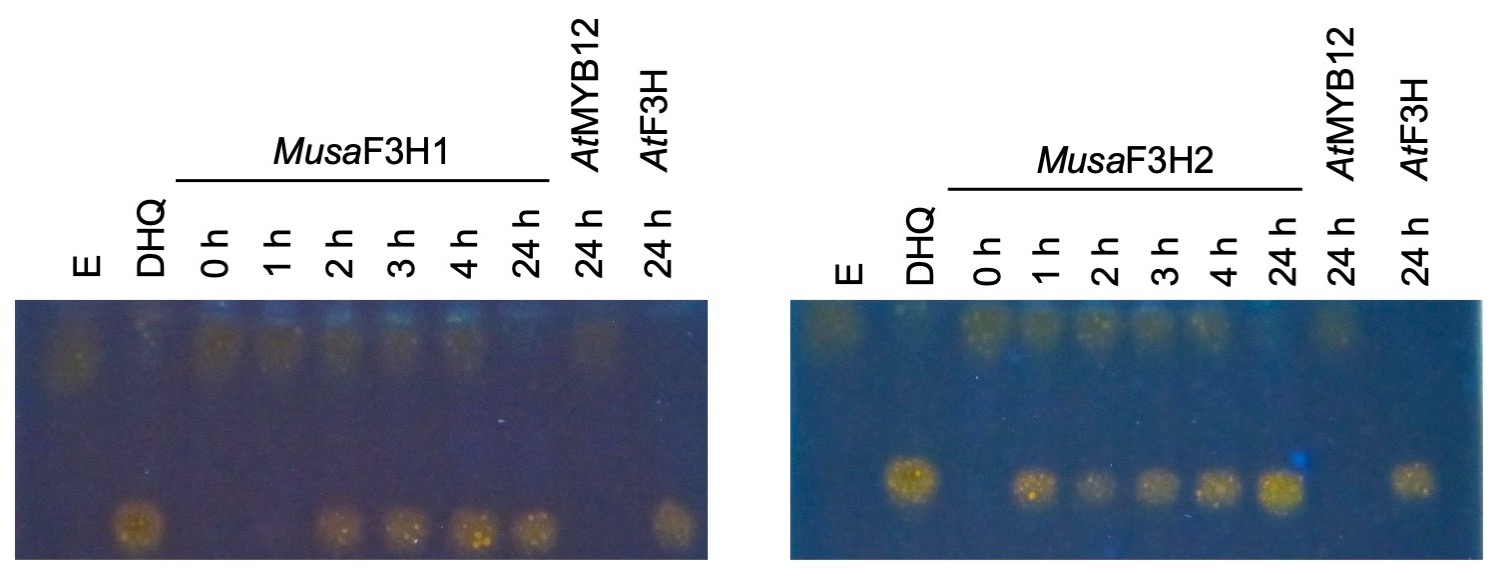
OsFLS ---------------- 331

AtANS ---------------D 356

MusaANS ---------------N 360

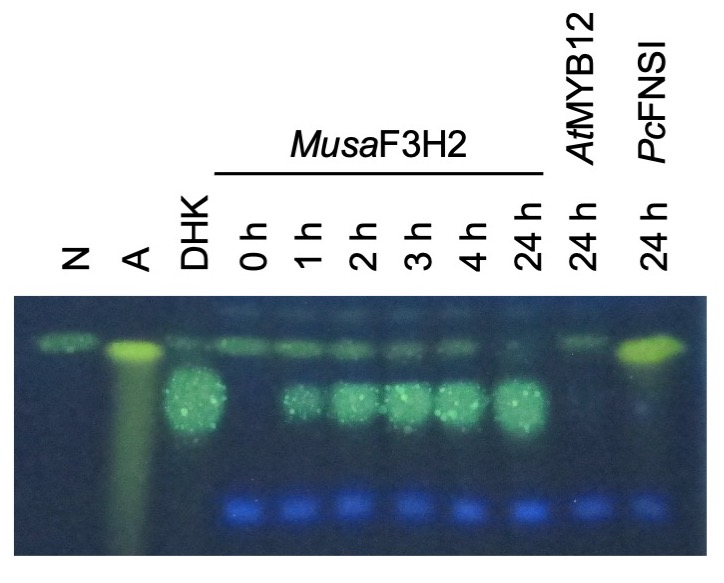
ZmANS GDHHRHEP---PPQTN 395

OsANS ------------MITK 375

****

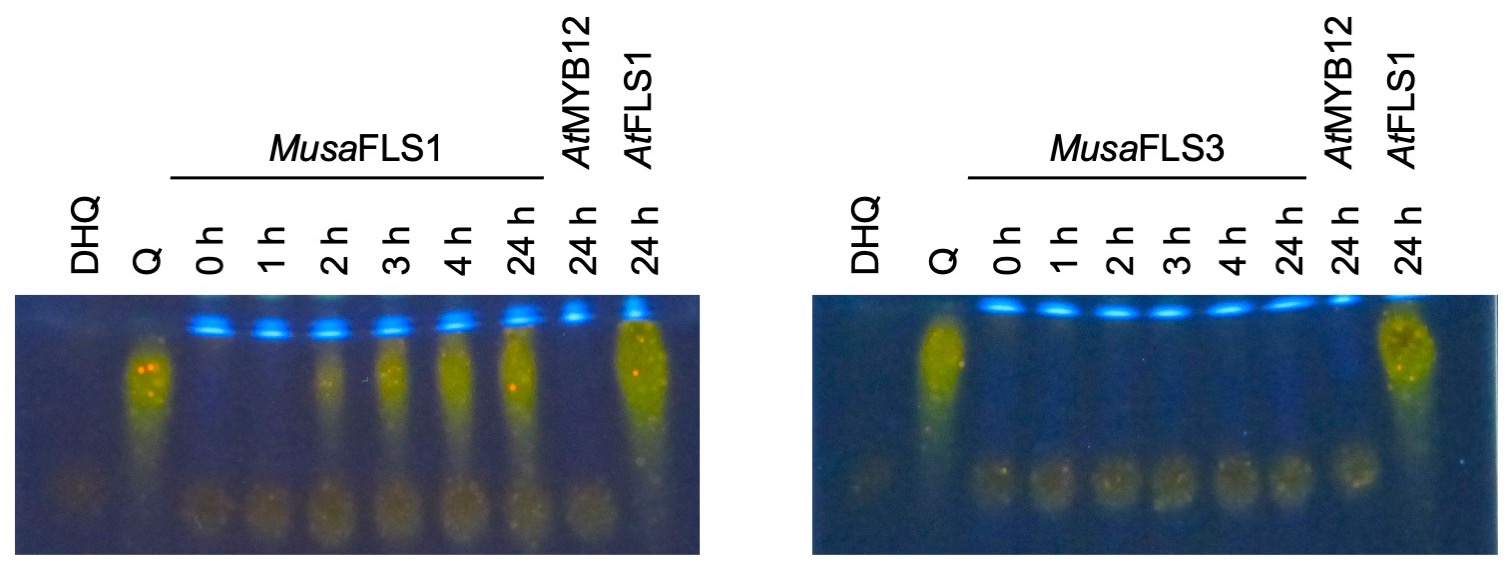
**File S3: *Musa*F3H1 and *Musa*F3H2 convert eriodictyol to dihydroquercetin.**

HPTLC analysis of F3H bioconversion assays using extracts from *E. coli* expressing recombinant *Musa*F3H1 or *Musa*F3H2. The substrate eriodictyol (E) and the product dihydroquercetin (DHQ) were used as standards. *At*F3H was used as a positive control. *At*MYB12 was used as a negative control.



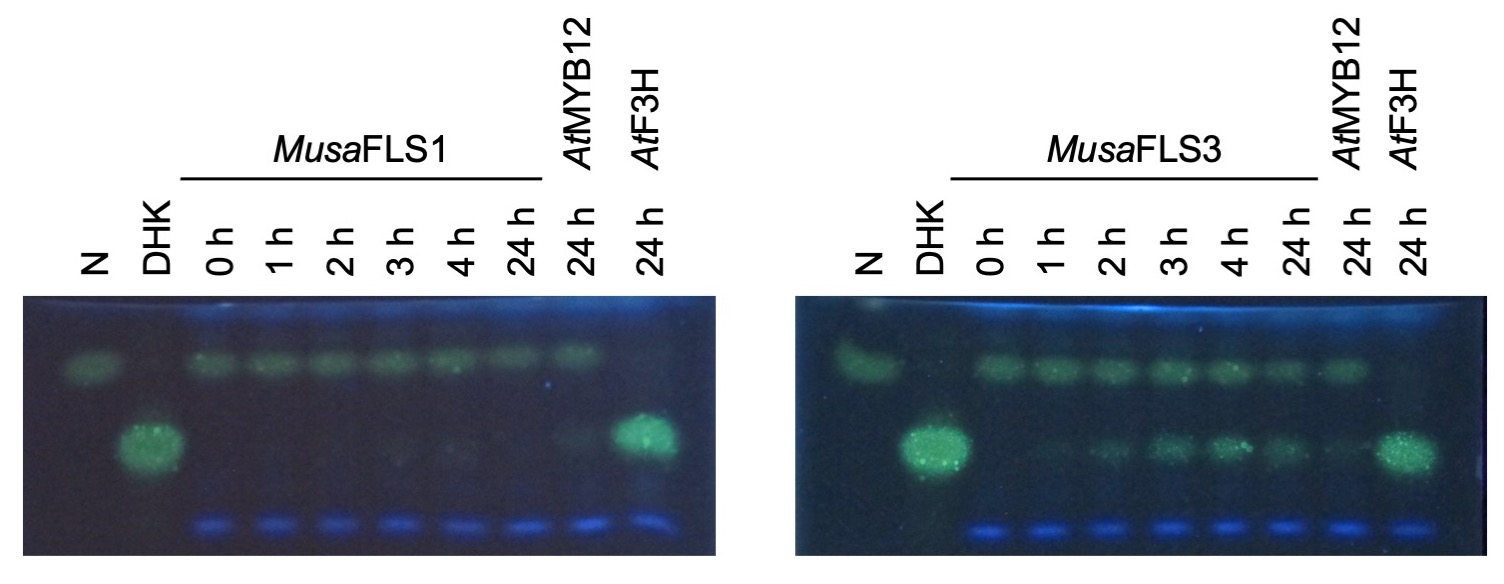
**File S4: Analysis of FNSI side activity of *Musa*F3H2.**

HPTLC analysis of FNSI bioconversion assay using extracts from *E. coli* expressing recombinant *Musa*F3H2. The substrate naringenin (N) and the product apigenin (A), as well as the F3H product dihydrokaempferol (DHK) were used as standards. *Pc*FNSI was used as a positive control. *At*MYB12 was used as a negative control.

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**File S5: MusaFLS1 converts dihydroquercetin to quercetin.**

HPTLC analysis of a FLS bioconversion assays using extracts from *E. coli* expressing recombinant *Musa*FLS1 or *Musa*FLS3. The substrate dihydroquercetin (DHQ) and the product quercetin (Q) were used as standards. *At*FLS1 served as a positive control and *At*MYB12 was used as a negative control.

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**File S6: Analysis of F3H side activity of *Musa*FLS1 and *Musa*FLS3.**

HPTLC analysis of F3H bioconversion assay using extracts from *E. coli* expressing recombinant *Musa*FLS1 or *Musa*FLS3. The substrate naringenin (N) and the product dihydrokaempferol (DHK) were used as standards. *At*F3H was used as a positive control. *At*MYB12 was used as a negative control.



**File S7: Analysis of recombinant *Musa*FLS1 and *Musa*FLS3 in *E. coli* by SDS-PAGE.**

Sample separation was performed by SDS-PAGE (Laemmli, 1970) using a 4 % stacking gel and 12 % resolving gel. Protein staining was performed using colloidal Coomassie Brilliant Blue G-250 (Dyballa and Metzger, 2012). The first lanes for each sample show the supernatant of *E. coli* cultures without addition of inducing arabinose, the second ones show the supernatant of induced *E. coli* cultures and the third lanes show the sediment of induced *E. coli* cultures. Including the polyhistidine-tag, *Musa*FLS1 and *Musa*FLS3 have a mass of about 41 kDa. The *Color Prestained Protein Standard, Broad Range* (NEB) was used as a standard.

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