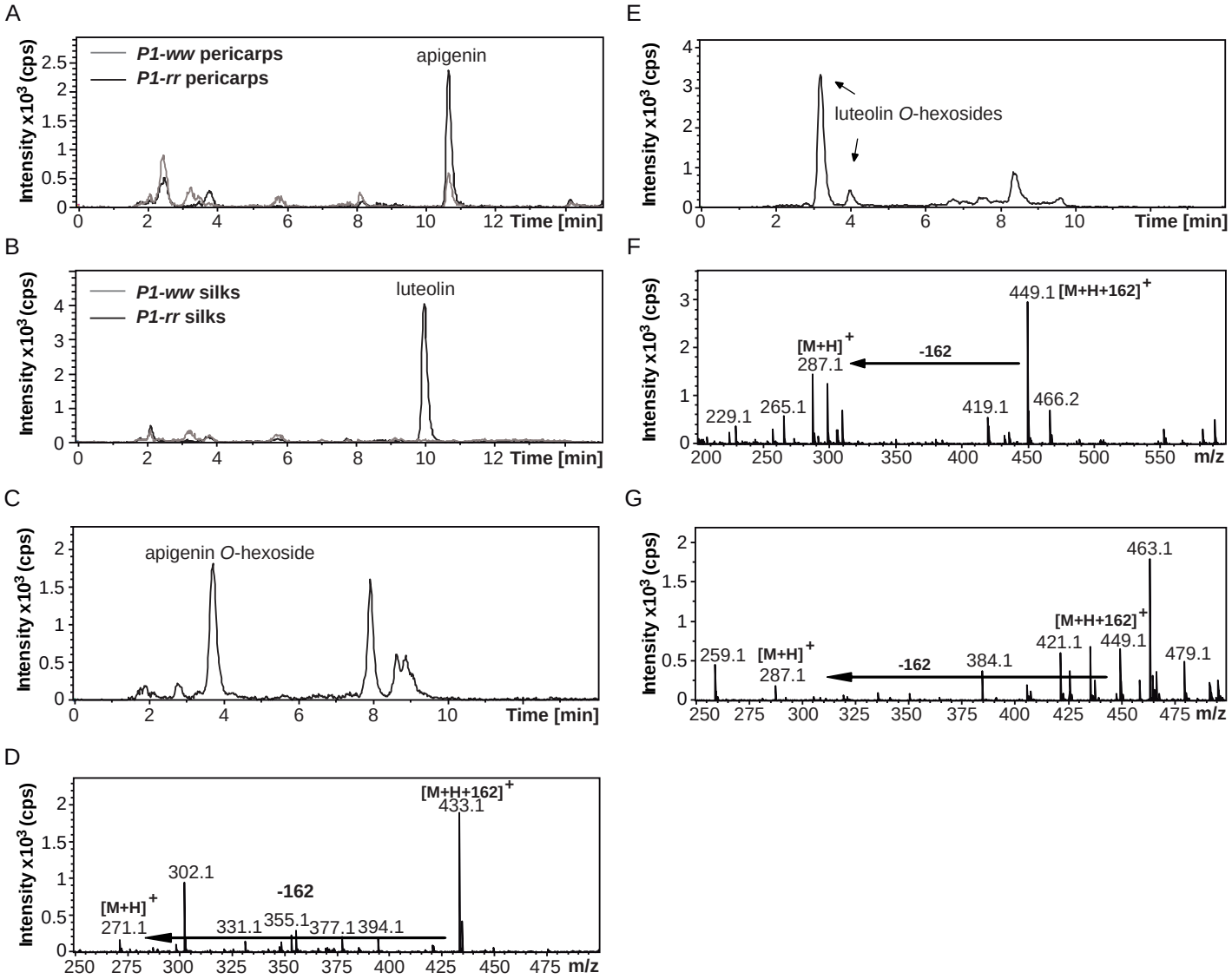
Supplemental Fig. S1



LC-MS/MS analysis of flavone-derived metabolites in maize pericarps (14 DAP) and silks expressing or not the *P1* gene (*P1-rr* and *P1-ww*). (A, B) Representative ion chromatograms for molecular ions of *m/z* = 271 and 287 corresponding to apigenin and luteolin, respectively; detected in pericarps (A) and silks (B) expressing or not the P1 gene. (C) Representative ion chromatograms for molecular ions of *m/z* = 433 corresponding to apigenin *O*-hexoside detected in silks. (D) MS/MS fragmentation profile of apigenin *O*-hexoside detected in (C). (E) Representative ion chromatograms for molecular ions of *m/z* = 449 corresponding to luteolin *O*-hexosides detected in pericarps expressing the *P1* gene. (F, G) MS/MS fragmentation profiles of luteolin *O*-hexosides detected in (D).

Supplemental Fig. S2

A

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ZmFNSI-1          ------MAEHLLST-AVHDTLPGSYVRPEPERPRLAEVVTGARIPVVDLGSPDRGAVVAA 53
Os10g39140        MAAEAEQQHQLLST-AVHDTMPGKYVRPESQRPRLDLVVSDARIPVVDLASPDRAAVVSA 59
Os03g03034        ------MADQLIST-ADHDTLPGNYVRPEAQRPRLADVLSDASIPVVDLANPDRAKLVSQ 53
AtDMR6            ------MAAKLISTGFRHTTLPENYVRPISDRPRLSEVSQLEDFPLIDLSSTDRSFLIQQ 54
                            :*:**   * *:* .**** .:****  *     :*::**...**. ::

ZmFNSI-1          VGDACRSHGFFQVVNHGIHAALVAAVMAAGRGFFRLPPEEKAKLYSDDPARKIRLSTSFN 113
Os10g39140        VGDACRTHGFFQVVNHGIDAALIASVMEVGREFFRLPAEEKAKLYSDDPAKKIRLSTSFN 119
Os03g03034        VGAACRSHGFFQVLNHGVPVELTLSVLAVAHDFFRLPAEEKAKLYSDDPAKKIRLSTSFN 113
AtDMR6            IHQACARFGFFQVINHGVNKQIIDEMVSVAREFFSMSMEEKMKLYSDDPTKTTRLSTSFN 114
                  :  **  .*****:***:   :   :: ..: ** :. *** *******::. *******

ZmFNSI-1          VRKETVHNWRDYLRLHCHPLDEFLPDWPSNPPDFKETMGTYCKEVRELGFRLYAAISESL 173
Os10g39140        VRKETVHNWRDYLRLHCYPLHQFVPDWPSNPPSFKEIIGTYCTEVRELGFRLYEAISESL 179
Os03g03034        VRKETVHNWRDYLRLHCYPLHRYLPDWPSNPPSFREIISTYCKEVRELGFRLYGAISESL 173
AtDMR6            VKKEEVNNWRDYLRLHCYPIHKYVNEWPSNPPSFKEIVSKYSREVREVGFKIEELISESL 174
                  *:** *:**********:*:..:: :******.*:* :..*. ****:**::   *****

ZmFNSI-1          GLEASYMKEALGEQEQHMAVNFYPPCPEPELTYGLPAHTDPNALTILLMDPDVAGLQVLH 233
Os10g39140        GLEGGYMRETLGEQEQHMAVNYYPQCPEPELTYGLPAHTDPNALTILLMDDQVAGLQVLN 239
Os03g03034        GLEQDYIKKVLGEQEQHMAVNFYPKCPEPELTFGLPAHTDPNALTILLMDQQVAGLQVLK 233
AtDMR6            GLEKDYMKKVLGEQGQHMAVNYYPPCPEPELTYGLPAHTDPNALTILLQDTTVCGLQILI 234
                  *** .*:::.**** ******:** *******:*************** *  *.***:*

ZmFNS1            AGQWVAVNPQPGALIINIGDQLQALSNGQYRSVWHRAVVNSDREPRMSVASFLCPCNHVVL 293
Os10g39140        DGKWIAVNPQPGALVINIGDQLQALSNGKYRSVWHRAVVNSDREPRMSVASFLCPCNSVEL 299
Os03g03034        EGRWIAVNPQPNALVINIGDQLQALSNGRYKSVWHRAVVNSDKAPRMSVASFLCPCNDVLI 293
AtDMR6            DGQWFAVNPHPDAFVINIGDQLQALSNGVYKSVWHRAVTNTENPPRLSVASFLCPADCAVM 294
                   *:*.****:*.*::************** *:*******.*::. *:********.: . :

ZmFNSI-1          GPARKLVTED---TPAVYRNYTYDKYYAKFWSRNLDQEHCLELFRT------ 336
Os10g39140        GPAKKLITDD---SPAVYRNYTYDEYYKKFWSRNLDQEHCLELFRT------ 342
Os03g03034        GPAQKLITDG---SPAVYRNYTYDEYYKKFWSRNLDQEHCLELFRTTPTDTS 342
AtDMR6            SPAKPLWEAEDDETKPVYKDFTYAEYYKKFWSRNLDQEHCLENFLNN----- 341
                  .**: *       : .**:::** :** ************** * .
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B

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PcFNS             MAPTTITALAKEKTLNLDFVRDEDERPKVAYNQFSNEIPIISLAGLDDDSDGRRPEICRK 60
AgFNS             MAPSTITALSQEKTLNLDFVRDEDERPKVAYNQFSNEVPIISLAGLDDDSNGRRAEICRK 60
DcFNS             MAPTTITALAKEKTLNSDFVRDEDERPKVAYNQFSTEIPIISLAGIDDDSNGRRPEVCRK 60
CcFNS             MAPTTITALAQEKTLNSDFVRDEDERPKVAYNQFSTEIPIISLAGIDDDSKGRRPEVCRK 60
ZmFNSI-1          -MAEHLLSTAVHDTLPGSYVRPEPERPRLAEVVTGARIPVVDLGSPD------RGAVVAA 53
                    .  : : : ..**  .:** * ***::*    . .:*::.*.. *      *  :

PcFNS             IVKACEDWGIFQVVDHGIDSGLISEMTRLSREFFALPAEEKLEYDTTG-GKRGGFTISTV 119
AgFNS             IVEAFEEWGIFQVVDHGIDSGLISEMSRLSREFFALPAEEKLVYDTTG-EKKGGFTISTH 119
DcFNS             IVEAFEDWGIFQVVDHGIDSGLIAEMSRLSREFFALPAEEKLRYDTTG-GKRGGFTISTH 119
CcFNS             IVEAFEDWGIFQVVDHGVDSALISEMSRLSREFFALPAEEKLRYDTTG-GKRGGFTISTH 119
ZmFNSI-1          VGDACRSHGFFQVVNHGIHAALVAAVMAAGRGFFRLPPEEKAKLYSDDPARKIRLSTSFN 113
                  : .* .. *:****:**:.:.*:: :   .* ** **.***    : .  ::  :: *

PcFNS             LQGDDAMDWREFVTYFSYPINARDYSRWPKKPEGWRSTTEVYSEKLMVLGAKLLEVLSEA 179
AgFNS             LQGDDVRDWREFVTYFSYPISARDYSRWPKKPEGWRSTTEVYSEKLMVLGAKLLEVLSEA 179
DcFNS             LQGDDVKDWREFVVYFSYPVDARDYSRCPDKPEGWRSVTEVYSEKLMALGAKLLEVLSEA 179
CcFNS             QQGDDVRDWREFVTYFSYPVDARDYSRWPEKPEGWRSVTEVYSEKLMVLGAKLLEVLSEA 179
ZmFNSI-1          VRKETVHNWRDYLRLHCHPLDEFLP-DWPSNPPDFKETMGTYCKEVRELGFRLYAAISES 172
                   : : . :**:::  ..:*:.       *.:* .::..  .*.:::  ** :*  .:**:

PcFNS             MGLEKGDLTKACVDMEQKVLINYYPTCPQPDLTLGVRRHTDPGTITILLQD-MVGGLQAT 238
AgFNS             MGLEKEALTKACVEMEQKVLINYYPTCPEPDLTLGVRRHTDPGTITILLQD-MVGGLQAT 238
DcFNS             MGLEKEALTEACVNMEQKVLINYYPTCPQPDLTLGVRRHTDPGTITILLQD-MVGGLQAT 238
CcFNS             MGLDKGALTKACVNMEQKVLINYYPTCPEPDLTLGVRRHTDPGTITILLQD-MVGGLQAT 238
ZmFNSI-1          LGLEASYMKEALGEQEQHMAVNFYPPCPEPELTYGLPAHTDPNALTILLMDPDVAGLQVL 232
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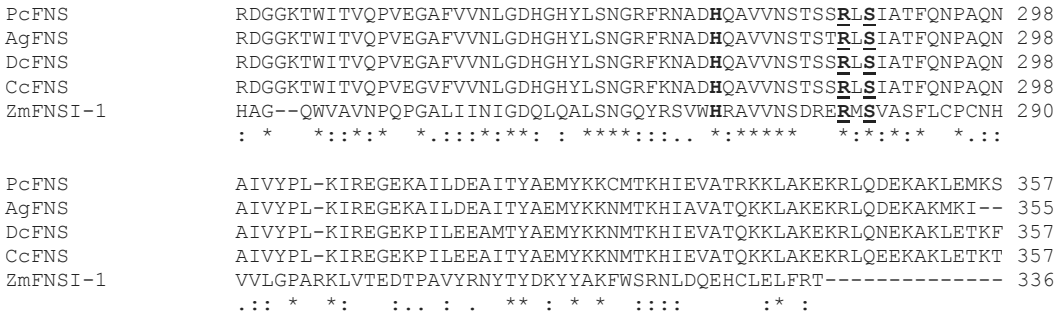
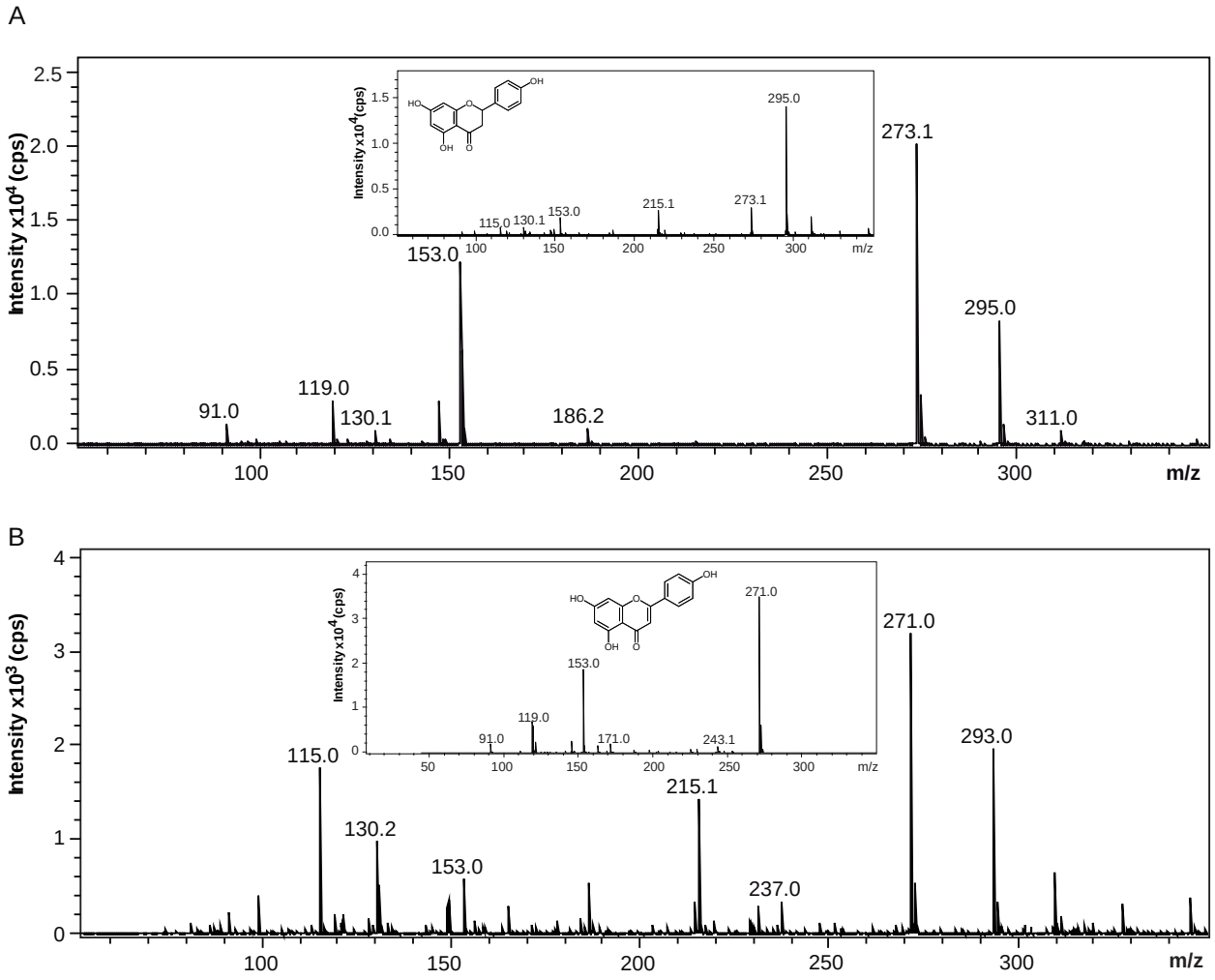


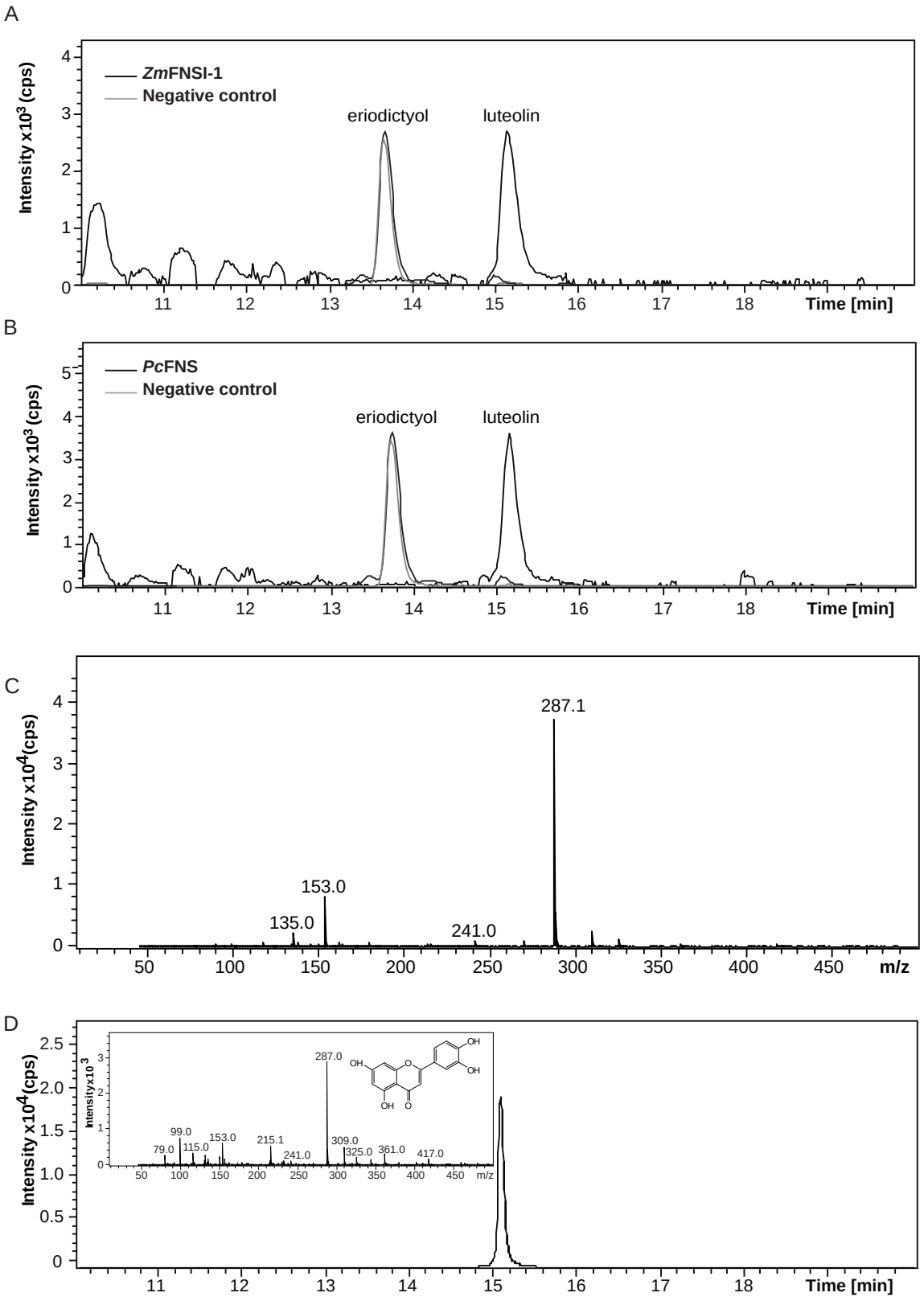
Amino acid sequences alignment of the predicted *Zm*FNSI-1 with FNS proteins from *O. sativa* and *A. thaliana* plants (A) and *Apiaceae* (B). The sequences were aligned using the Clustal W2 program. Dashes (-) indicate spaces introduced to promote optimal alignment, perfect matches are indicated by an asterisk (*), high amino-acid similarities by double dots (:), and weak similarities by a single dot (·). Amino acids coordinating the ferrous iron and residues participating in 2-oxoglutarate binding are in bold-underlined and bold letters, respectively.

Supplemental Fig. S3



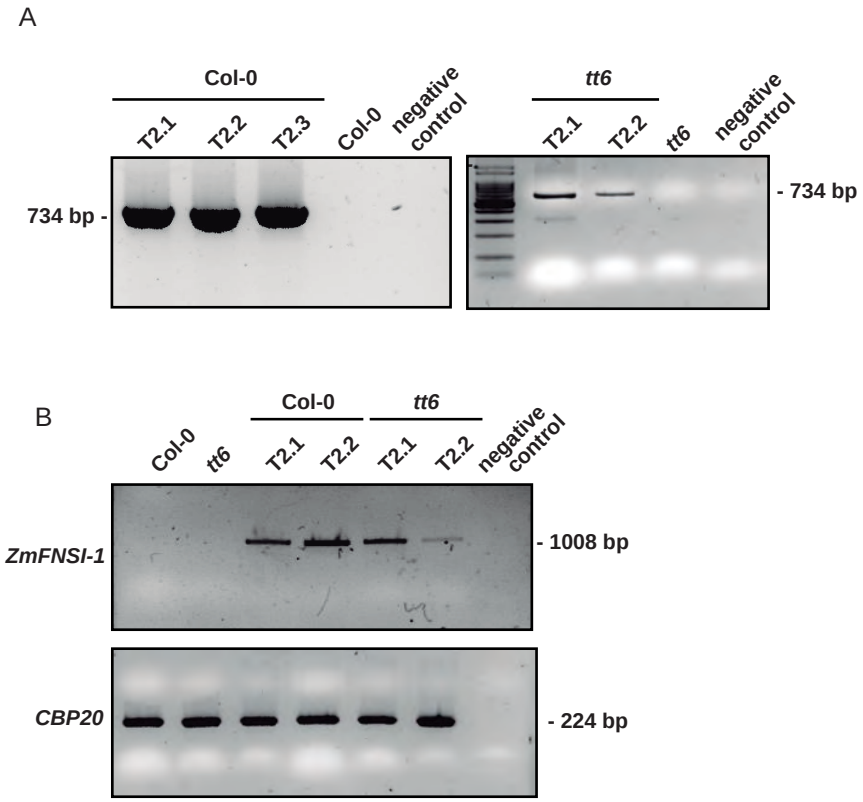
LC-MS analysis of *Zm*FNSI-1 activity products in *E. coli* bioconversion assays using naringenin as a substrate. Fragmentation of the naringenin substrate (A) and apigenin (B) produced by *Zm*FNS1-1. For comparison, the fragmentation patterns of the standards are shown on the middle top. Naringenin and apigenin produced molecular ions of *m/z* = 273 and 271, respectively.

Supplemental Fig. S4



LC-MS analysis of *Zm*FNSI-1 activity assayed with eriodictyol as a substrate. (A) Representative ion chromatogram for eriodictyol bioconversion in *E. coli* expressing *Zm*FNSI-1. The reaction products generated a molecular ion of *m/z* = 287 corresponding to luteolin, while *E. coli* cells transformed with the empty vector did not show the production of the product. (B) Representative ion chromatogram for eriodictyol bioconversion in *E. coli* expressing *Pc*FNS as a positive control. (C). MS/MS fragmentation profile of the product of the *Zm*FNSI-1 activity assay. (D) A luteolin standard was used as a control, its MS/MS fragmentation profile corresponds to that of the *Zm*FNSI-1 reaction product, which is shown inside the graph.

Supplemental Fig. S5



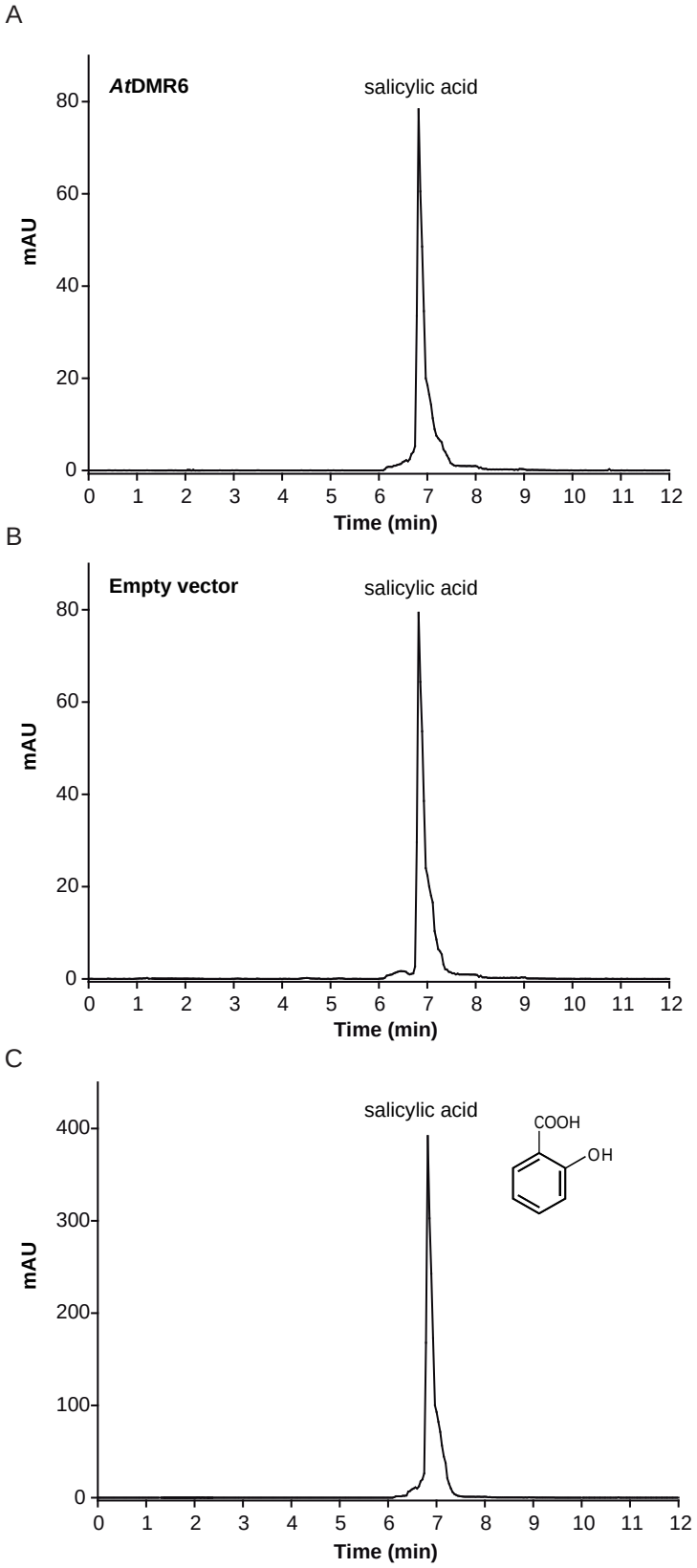
Presence and expression of the *ZmFNSI-1* transgene in transformed *A. thaliana* plants. (A) Amplification of the *ZmFNSI-1* transgene by PCR on genomic DNA from 15-days-old hygromycin-resistant plants transformed with the p35S::*Zm*FNSI-1 construct (3 lines in Col-0 background (T2.1, T2.2 and T2.3 lines) and 2 lines in *tt6* background (T2.1 and T2.2)). Positive PCR reaction amplified a 734 bp product (35 cycles). DNA from non-transformed Col- 0 and *tt6* mutant plants was used as negative controls for amplifications. Also, the PCR reaction was done without genomic DNA as a different negative control. (B) *ZmFNSI-1* mRNA levels in 15-days-old hygromycin-resistant Arabidopsis lines transformed with p35S::*Zm*FNSI-1 determined by RT-PCR, which amplifies a product of 1008 bp (35 cycles). CBP20 was used as an internal control. cDNA from non-transformed Col- 0 and *tt6* mutant plants was used as negative controls for amplifications. Also, the RT-PCR reaction was done without template as a different negative control.

Supplemental Figure S6

GAGAGTTTGTGCTTGTCACTCTTGTTGATCGACATCACCTAAACGACTTGGTAGCGACCGAGAGTTTAGTG
ATCACCTGTTGAAGATTGTGGATGGTCTAACTAATGCTTAAACGACTACGGGGTAATTCATCGTGCTGGAG
TGACGAAGAATGAATCTATACTTGATCCTTACGCAGATCAAGAAAGAGTCTCACCCTTGTGTGGATGCTCC
AACGAGGACTACTAATTTAGTCTCTAAATTGCCAAATACGAAAATTAAAACTCTATTTTATTTTCTGTATT
TGGCAACTCAGTGACTAAAATGAAATAAAATAGATGGACTAAAAATTAGTCCCTAGAAACCAAACAACTCC
TTAGTCTATTTTTCCATCAAACGTAGATGGCCTTTAAAAAAGATGCAACCTTCTTTAGCCTTATAGAAATA
TTCCCTCTATCCTAATATATAAAGCGTAACCACGATCTGATTCAAATATAAAAAAATAAATTTAATTCTCT
CTATCAACACTAATACTACTGCATTGATTTACGTATGTCTAGATATAGCATGCTTTATAATATGATACTAG
AATAAAATATGATAGGACTGGCGGAGGGCAGTAACTTATAGTAATGTAAAGTAAGGAGCAAATTGTGCGGG
GTTCGACACATGGAATTGCTTCGTGGTCCCAATTAGAAAACATGAAAAATATCCTGGTGTCCCTCCAAAAG
CAGAAGGGAAACTCGAGTGTTTGAACAAGCATGGGATGGAATCGATTGGCACACGGCGACTTGGCGAGGAA
ACGTTAGCCATCCATTGTGCGAAAAGCTCGCAATCGCAATGCGCCAGATTCTCGAGTAACGAAATCGCGGA
TCCACATTCGCCGTGGACCACCGCATACAATGGTTGGTTCCCGTGTACGCCGTCGATACAGCAGAGCACGT
GGACGTGGATCTGATTGGACTTGGTTGGTGGTGCACCCGACCGATGTTGCGTCGACGAGACATCCTCGCAC
GACGGGGAATCCGCCGCTCTGGCAAGCCACTCTGTCATGTGCCTCTTGGATCCTGCGATCCGCCGCTGGAG
CGTAACCACCGCGTCCACATGCTGCTTCATTGGTGCCGGCGCGGAGGCTGCTTAGCTCGCCGCCGAGGGAC
TACTTTGCCCGCCACGGTTACGGCGTGATGCGGAGGGGTGGTGACGTCACTCACCGCCACGGCGATGACGC
GAGGGGATATCAGTCATCAGTCCACCCAACGACCGCGGGGGGTCAACCTCTTCTCGGTCGTCGTCGCGTCG
TCACGGCTGACGCCACGGGCCCGCTGCCTGCCGGAACGCCCACGCCGAGCCACGGCGCAGTCACCACCAGA
CATCAAGTTACGCGTCCGGCTTCCCGGCCGCTATAAAGAGCGCGCGACGGCGGCCCCTGGGAGAGCCATGC
GAGACTGGAGGCGGAACCGCGCACGACACCAAGCTGCCGCGCCGGACTGCTGCACGCAAGCGCAGCGCAGG
ACCGACCGACCTCCGTAGGCACGCACGGCGCCGGCGGC

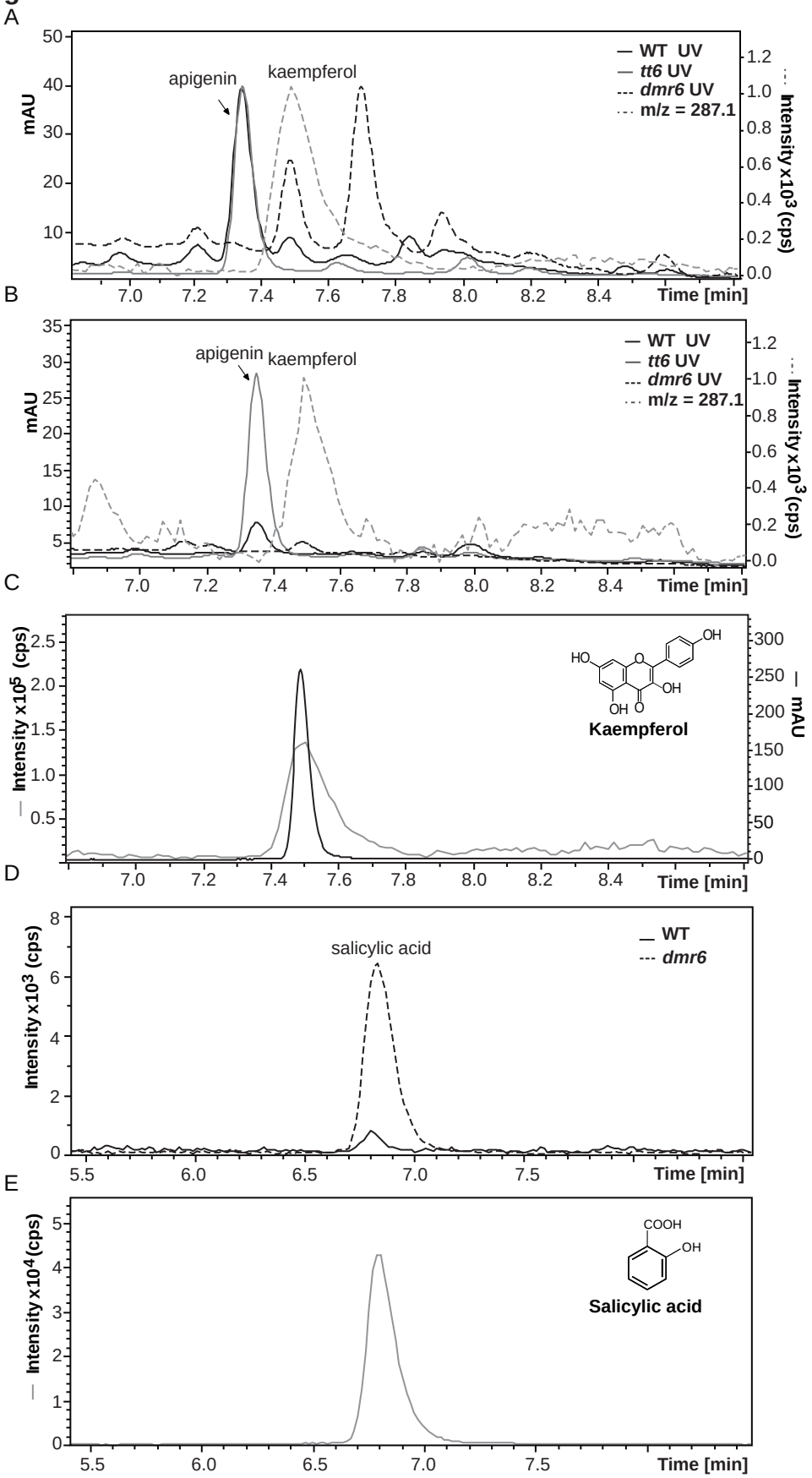
ZmFNSI-1 promoter sequence from the B73 maize line. The promoter region shown ranges from -1426 to +103 bp. C1/P1 binding sites and E-box are highlighted in red and grey, respectively. The 5'UTR region is indicated in red color. Primers used for PCR are highlighted in yellow.

Supplemental Fig. S7



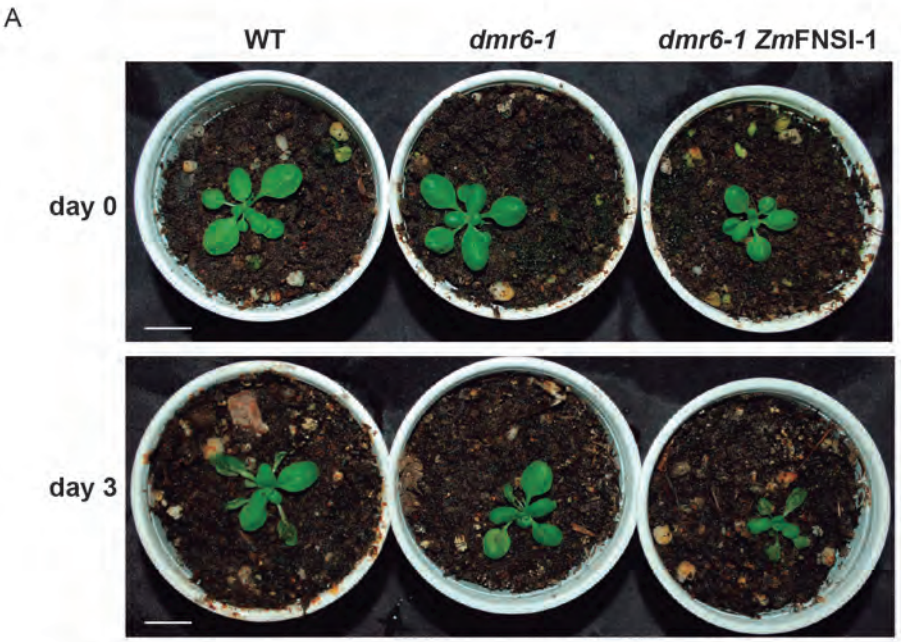
*In vitro At*DMR6 activity assayed with salicylic acid as a substrate. (A) HPLC profile of purified *His6-At*DMR6 activity assay using salicylic acid as a substrate, the reaction did not generate any hydroxylated product. (B) A negative control using an *E.coli* extract transformed with an empty vector did not show any product. (C) HPLC profile of a salicylic acid standard.

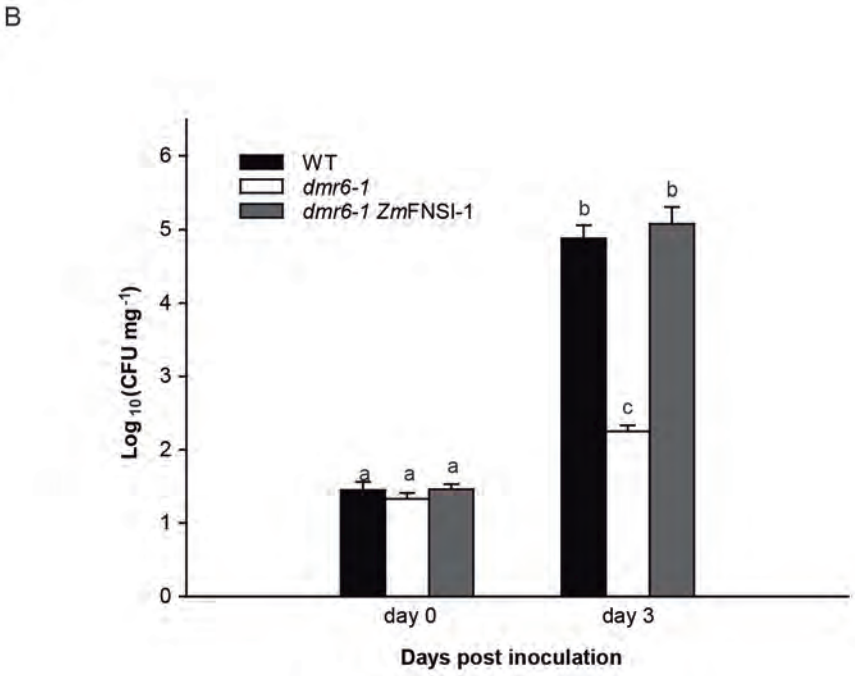
Supplemental Fig. S8



Accumulation of kaempferol, apigenin and salicylic acid in Arabidopsis. (A) UV and ion chromatogram profiles of hydrolyzed flavonoids of cauline leaves from WT, *tt6* and *dmr6* Arabidopsis mutant plants analyzed by LC-MS/MS showing the molecular ion of *m/z* = 287 in WT and *dmr6* mutant plants that corresponds to kaempferol. The peak at 7.35 min that corresponds to apigenin is also indicated. (B) UV and ion chromatogram profiles of hydrolyzed flavonoids of senescing leaves from WT, *tt6* and *dmr6* Arabidopsis mutant plants analyzed by LC-MS/MS showing the molecular ion of *m/z* = 287 in *dmr6* mutant plants that corresponds to kaempferol. The peak at 7.35 min that corresponds to apigenin is also indicated. (C) UV and ion chromatogram of kaempferol standard. (D) Ion chromatogram profiles of hydrolyzed flavonoids of cauline leaves from WT, and *dmr6* Arabidopsis mutant plants LC-MS/MS showing the molecular ion *of m/z* = 137 that corresponds to salicylic acid (negative ion chromatogram). (E) Negative ion chromatogram of salicylic acid standard.

Supplemental Fig. S9





*Zm*FNSI-1 complements susceptibility of Arabidopsis *dmr6-1* mutant plants towards *P. syringae* pv. *tomato* DC3000 (*Pst*). (A) Disease symptoms of WT plants, *dmr6-1* mutants and *dmr6-1* transformants expressing *Zm*FNSI-1, 0 and 3 days after *Pst* infection. Scale bar: 1 cm. (B) Leaf bacterial count at 0 and 3 days post infection (colony-forming units per mg of leaf tissue). A bacterial suspension with OD 0.05 was sprayed on the plants. Data show mean values ± S.E.M of three biological repeats. Different letters over the bars indicate statically significant differences with $P < 0.01$ (ANOVA test).

Supplemental Table S1. Substrates tested in bioconversion assays in *E. coli* expressing *Zm*FNSI-1 and *At*DMR6 and *in vitro* assays with the recombinant proteins. Product formation is indicated for each compound tested.

Compound	Type	Activity	Product formation
dihydroquercetin	dihydroflavonol	flavonol synthase	not detected
dihydrokaempferol	dihydroflavonol	flavonol synthase	not detected
leucocyanidin	leucoanthocyanidin	anthocyanidin synthase	not detected
salicylic acid	phenolic acid	salicylic acid 3-hydroxylase	not detected
naringenin	flavanone	flavanone 3-hydroxylase	not detected
eriodictyol	flavanone	flavanone 3-hydroxylase	not detected
naringenin	flavanone	flavone synthase	apigenin
eriodictyol	flavanone	flavone synthase	luteolin

Supplemental Table S2. Primers used for cloning*, RT-qPCR and screening.

Name	Sequence
ZmFNSI-F	5’ CACCATGGCGGAGCACCTCCTG3’
ZmFNSI-R1	5’ GGTTCTGAAGAGCTCGAGGCA3’
ZmFNSI-F-RT	5’AGGAGAAGGCCAAGCTCTACT3’
ZmFNSI-R-RT	5’CCCATGGTCTCCTTGAAATC3’
ZmFNSI-NdeI-F	5’ACAGCATATGGCGGAGCACCTCCTGTCGAC3’
ZmFNSI-BamH-R	5’TGTCAGGATCCTCAGGTTCTGAAGAGCTCG3’
*AtDMR6-Nde*I-F	5’ACAGCATATGGCGGCAAAGCTGATATCCACCGGT3’
AtDMR6-BamH-R	5’TGTCAGGATCCTTAGTTGTTTAGAAAATTCTCGAGGC3’
ZmFNS1-Kpn-prom-R2	5’CATAGGTACCGCCGCCGGCGCCGTGCGTG3’
*ZmFNS1-Not*I-prom-F2	5’CACCGCGGCCGCGAGAGTTTGTGCTTGTCACTC3’
ZmActin1-F	5’CTTCGAATGCCCAGCAAT3’
ZmActin1-R	5’CGGAGAATAGCATGAGGAAG3’
AtCBP20-F	5'CCGGCCTATTCGTGTGGATTTTGA3’
AtCBP20-R	5'CATAATTCGTTGGCGCAGCTTGAG3'
AtUBQ10-F	5'AAGCAGCTTGAGGATGGAC3'
AtUBQ10-R	5'AGATAACAGGAACGGAAACATAGT3'
AtDMR6-F-RT	5'ATCTTTGGCCTTTGTGTTTG3'
AtDMR6-R-RT	5'CAAACACAAAGGCCAAAGAT3'

*The restriction enzyme recognition sequences are underlined in primer sequences