

Supplemental Figure 1. FAMA Co-Expression Network Predicted by ATTED-II.

*FAMA* (At3g24140, a yellow shading octagon) was used as a query in the ATTED-II database (http://atted.jp). The output data show 20 genes were co-expressed with *FAMA*. *ESM1* (*EPITHIOSPECIFIER MODIFIER1*) encodes a protein in the myrosinase-glucosinolate system. Octagons indicate transcription factors and ovals indicate genes except transcription factors.



**Supplemental Figure 2.** *FAMA* Expression Pattern in Wild-Type Plants. GUS staining of Col-0 expressing *ProFAMA:GUS*.



**Supplemental Figure 3.** *ProFAMA:TagRFP-FAMAg* Expression Rescues *fama-1* Dwarfism. Col-0, *fama-1*, and two independent *fama-1* transgenic lines expressing *ProFAMA:TagRFP-FAMAg*.



**Supplemental Figure 4.** *ProFAMA:GUS* Expression Pattern in Early Developmental Stages of Leaves.

GUS staining of leaf primordia of Col-0 expressing ProFAMA:GUS.

(A) Typical images showing the first GUS-positive cell at the middle point in early leaf primordia. The first GUS-positive cell is detected in one (right leaf) of a pair of rosette leaves but not yet in the other (left leaf) in the upper panel.

**(B)** Leaf primordia at a later stage than those shown in **(A)**. In addition to the leaf inner tissue layers (focused in left panel), GUS signals appear in epidermal stomatal-lineage cells (focused in right panel). Arrows indicate stomatal-lineage cells.



**Supplemental Figure 5.** *ProTGG2:VENUS-2sc* Expression Pattern in a first true leaf primordium at 3.5 days after germination.

Merged overlays of the fluorescent images (green) and the bright-field images of leaf inner tissue primordia expressing *ProTGG2:VENUS-2sc*. VENUS-2sc localizes to the ER and vacuoles.



**Supplemental Figure 6.** The *fama*-Deficient Mutant Lacks Endogenous TGG1 Protein. Rosette leaves of Col-0 and *fama-1* were subjected to SDS-PAGE and electroblotted onto a PVDF membrane. The membrane was stained with CBB.





**Supplemental Figure 7.** Myrosin Cell Development in Leaf Primordia of *fama* Loss-of-Function Mutants.

(A) *GFP* Expression Pattern in Leaf Primordia of the E1728 Line. Confocal images of the epidermis (upper left panel) and the inner tissue (upper right and lower panels) in leaf primordia of E1728.

(B) Merged overlays of fluorescent images (green) and bright-field images of the rosette leaf inner tissue of Col-0 and *fama-1* plants expressing E1728. The boxed area in the left panel is enlarged in the middle panel.



**Supplemental Figure 8.** *FAMA* Expression Pattern in the *fama*-Deficient Mutant. GUS staining of rosette leaves of *fama-1* expressing *ProFAMA:GUS*.



Bars = 1 mm

Supplemental Figure 9. Development of Myrosin Cells in FAMA-sGFP Overexpression Lines.

Transgenic plants expressing *FAMA-sGFP* under the estrogen-inducible promoter (*ProEstro:FAMA-sGFP*) were generated. The 8-day-old plants were transplanted onto inductive medium containing no estrogen (-Estrogen) or 10  $\mu$ M estrogen (+Estrogen) and incubated for two weeks.

(A) GUS staining of *ProEstro:FAMA-sGFP* plants (independent lines #2 and #3) expressing *MYR001:GUS*.

(B) Enlarged images of GUS-stained leaves of ProEstro:FAMA-sGFP plants.



**Supplemental Figure 10.** Accumulation Levels of TGG1 and TGG2 in *FAMA-sGFP* Overexpression Lines.

The 8-day-old *ProEstro:FAMA-sGFP* plants were transplanted onto inductive medium containing no estrogen (-Estrogen) or 10 µM estrogen (+Estrogen) and incubated for two weeks. Dilution series were prepared from three sets of rosette leaf extracts derived from independent *ProEstro:FAMA-sGFP* plants. Samples were subjected to immunoblotting with anti-TGG1 and anti-TGG2 antibodies. Signal intensities of the immunoblot band (×100 dilution) were quantified by densitometry. See also Figure 5B.



Supplemental Figure 11. Plant Morphology of FAMA-sGFP Overexpression Lines.

The 8-day-old *ProEstro:FAMA-sGFP* plants were transplanted onto inductive medium containing no estrogen (-Estrogen) or 10  $\mu$ M estrogen (+Estrogen) and incubated for two weeks.



**Supplemental Figure 12.** *FAMA* Expression in Myrosin Cells Is Independent of *MUTE*. GUS staining of rosette leaves of *mute-2* expressing *ProFAMA:GUS*. Representative two examples are shown.



Supplemental Figure 13. TGG1 and FAMA Transcript Levels in spch.

*TGG1* and *FAMA* mRNA levels in rosette leaves of Col-0, *fama-1*, and *spch-3* were assessed by RT-PCR. *ACTIN2* (*ACT2*) was used as a control.



**Supplemental Figure 14.** Accumulation Levels of TGG1 in ICE1 and SCRM2 Mutants. Stems and flowers of Col-0 and indicated mutants were subjected to immunoblotting with anti-TGG1 antibody.



Bars = 100 μm

**Supplemental Figure 15.** Distribution of Stomata in Wild-Type and *syp22-4*. (A) Stomatal density of a first pair of true leaves of wild-type and *syp22-4*. Images of abaxial epidermis of matured true leaves stained by FM4-64 were obtained. Error bars indicate standard deviation (n = 8). (B) Representative images of (A) in wild-type and *syp22-4*.

| At bHLH090  | MMMMRGGERVKEFLRPFVDSRTWDLCVIWKLGDDPSRFIEWVGCCCSGCYIDKNIKLENS  | 60  |
|-------------|---|-----|
| Al bHLH090  | MMMMRGGERVKEFLRPFVDSRTWDLCVIWKHGDDPSRFIEWVGCCCSGCYIDKNIKLENS  | 60  |
| Cr bHLH090  | -MMMRAGERVKEFLRPFVDSKTWDLCVIWKLGDDPSRFIEWVGCCCSGSYVDKNIKLENS  | 59  |
| Es bHLH090  | MMKSGERVKEFLRPFVDSRDWDLCVIWKLGDDPSRFIEWVGCCCSGSHIDKNIKHEKV  | 58  |
|             | *** ********  |     |
|             |   |     |
| At bHLH090  | EEGGTGRKKKASFCRDDHNKHRIRTLACEALSRFPLFMPLYPGIHGEVVMSKSPKWLVNS  | 120 |
| Al bHLH090  | EEE-TERRKKASFCRDEHNKHRTRTLACEALSHFPLFMPLYPGTHGEVVMSKSPKWLVNS  | 119 |
| Cr bHLH090  | EGTAREKKGSECRDEHNKHHMRTLACEALSHEPLEMPLYPGTHGEVVMSKSPKWLVNS  | 117 |
| Es bHLH090  | EEG-TERKOTGSTCRDEHNKHYTRSLACEALTHEPLEMPLYOCTHGEVVMSKSPKWLVNS  | 117 |
|             | *   |     |
|             |   |     |
| At bHLH090  | GSKMEMFSTRVI.VPVSDGI.VEI.FAFDMRPFDESMVHI.TMSRCTTFFEPFPEORI.OFRT   | 178 |
| Al bHT.H090 | CSKMDMFSTRVLVPVSDCLVELFSFDMKPFDESMVHLTMSRCTTFFEPLPEORLPFRT  | 177 |
| Cr bHLH090  | CDGLKKDMFNTRVLVPVSDGLTELFSFKMKPFDCPMVDMTTSRCNAFFEPFPEEKLOTRT  | 177 |
| Fe but H090 | CDCSKKDTFSTDUT TDUDDCI VEI FSFTMKDUDFSMUDMTTDDCNACFEDFDEOKI OFDT  | 177 |
|             |   | 1// |
|             | n * * * * * * * * * * * * * * * * * * *   |     |
| A+ 641.4090 | T D D A FFSMSSCUNT SUFCCCSSSUSNDSSFTONT FONVDNASCUFTI D FFOTDCLTMNKF                                      | 238 |
| AL_DILLIO90 |   | 230 |
|             | TPRAEESMODGVNLOFEGGGSSSVSNFSSETQNLFGSTSNARCVETLREEQAPCMVMNRE  | 237 |
| Cr_bhLh090  |   | 237 |
| ES_DHLH090  | ISKALLSMSSGVNLSFLGGGSSSVSNPLSLNQTLFGNQ-NARCGLHLPCLVVNRL   | 231 |
|             | ***************************************   |     |
| A+ bHT.H090 | KDWWONANDSKANKKTT.PTENEKSKNTHSERKRRERTNOAMVGT.RAWVDKTTKT.NKTGT  | 298 |
| AL DHLH090  | KDCLUONANDSKANKR_LDAENEKSKNLHSERKRRDDETNOAMVCLRAUWDKTTKLNKTCT   | 296 |
| Cr bHLH090  |   | 295 |
| Ec but H000 |   | 295 |
| LS_DHIH090  | + + + ++. + +++++++ ++++++++++++++  | 290 |
|             | · * * • * • • • • • • • • • • • • • • •   |     |
| A+ bHT.H090 | FSDAUDYTNELLVEKOKLEDELKOTNEMECKETAAFEOSATADDEAEDVSSKSNKDVKKN  | 358 |
| AL DILLIOSO | FODAVDI INELLVENQUEDBENGINEMECKETAAEEQSATADI EREKVOSKONAKVANN   | 256 |
| Cr but H000 | F SDAVDI INELLAENQALEDELIKGI DEMECKEIAAEEQSAIADF GAENVSSKINAKVAAN   | 255 |
| Cr_DHLH090  | FTDAVDIINELLVERARLEDELAGINERECREIAAEEESAIADPEAERAASRINAAMAAN  | 335 |
| ES_DHTH0A0  | FSDAVDIINELLVENQALEDELSGINEIECKEIAAEEESAIANPEAEAVSSAVNAAVN-D  | 349 |
|             | * <u>*********</u> **************************   |     |
| A+ 6414000  | FULT FULF TO THE TOTAL FULL OF FULL OF FULL FULL FULL FOR THE TOTAL FULL FULL FULL FULL FULL FULL FULL FU | 110 |
| AL DILINO90 | EVALEVISIONER CEDET TEMOREN CELETIDUNE TABLET NUV   | 416 |
| Cr but H000 | EVNLEVNETGERDET INVOGENOOGENET TENNOCCOLETIONNE INDUTVUTUUVA  | 410 |
| Ec but u000 | EVNLEVHEIGERDFLIRVVQEHKRDGFKLIEAVDSCGLEIIDVNFTRLDLIVKTVLNVK   | 415 |
| ES_DHTH0A0  | EVNEEVHELGERDELIRVAQEHIRDGERRLIEAVDSCGLEIIDVNETRLDLTVMTVLNVR  | 409 |
|             | nn;;nnnn nnndaaaa,aaaa;aaaaaaaaaaaa a aaaaaaaaaaaaaa  |     |
| At bHI.HOGO | ANKDGTACGTLEDILLEMMTTST   |     |
|             | ANKDOTTSCILEDILLEMITTSI   |     |
| Cr but u000 |   |     |
| Er PHIHODO  |   |     |
| TP_DUTUAAA  | WWVDGTOWGNTWUTUTUTUTUTUTUTUTUTUTUTUTUTUTUTUTUTUTU   |     |
|             |   |     |

**Supplemental Figure 16.** The bHLH Domain and Alignment of bHLH090 Homologues. ClustalW alignment of four bHLH090 homologues. Identical residues among all three marked with \*, conservative substitutions with :, and semi-conservative changes with . . The bHLH domain is underlined. At=*Arabidopsis thaliana*, Al=*Arabidopsis lyrata*, Cr=*Capsella rubella*, Es=*Eutrema salsugineum*.

## Supplemental Table 1. Primer Sets Used in This Study.

| ID | purpose                                  | name       | sequence                                  |
|----|--|------------|---|
| 1  | genotyping of SALK T-DNA tagline         | LBa1       | 5'-TGGTTCACGTAGTGGGCCATCG-3'              |
| 2  | genotyping of SAIL T-DNA tagline         | LB1        | 5'-GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC-3'  |
| 3  | genotyping of FLAG_FST T-DNA tagline     | LB4        | 5'-CGTGTGCCAGGTGCCCACGGAATAGT-3'          |
| 4  | genotyping of fama-1, -3                 | FAMA LP    | 5'-TTTTCGTAACATTCTTGTTCTCCCTATC-3'        |
| 5  | genotyping of fama-1, -3                 | FAMA RP    | 5'-GTCTTCTTCTACTATCTTGCATGTCTTG-3'        |
| 6  | genotyping of spch-3                     | SPCH LP    | 5'-ATCTAAAACATAAATGAGACACGAAAAAC-3'       |
| 7  | genotyping of spch-3                     | SPCH RP    | 5'-AGAACTTGTTGTAACTCGCTTATGTACTC-3'       |
| 8  | genotyping of mute-2                     | MUTE LP    | 5'-TGAAAACTGTAAAACATCTCAATACAAAC-3'       |
| 9  | genotyping of mute-2                     | MUTE RP    | 5'-TATGCATAACATATACAAACAAGGAAGTC-3'       |
| 10 | genotyping of ice1-2                     | ICE1 LP    | 5'-GTTTTTCTTCACTCTTACATCATCAAAG-3'        |
| 11 | genotyping of ice1-2                     | ICE1 RP    | 5'-AGCTTGTTTACTTGTTGCTGTTATAGAG-3'        |
| 12 | genotyping of ice2-1                     | ICE2 LP    | 5'-TGAAATGTTTATTCAATATACTTTGTTGC-3'       |
| 13 | genotyping of ice2-1                     | ICE2 RP    | 5'-GAAGAGAAACTATACAAGCTTTCGTAGC-3'        |
| 14 | RT-PCR of FAMA, cloning of FAMA CDS      | FAMA-F     | 5'-CACCATGGATAAAGATTACTCGGCACCAAA-3'      |
| 15 | RT-PCR of FAMA, cloning of FAMA CDS      | FAMA-R1    | 5'-TCAAGTAAACACAATATTTCCCAGGTTAGA-3'      |
| 16 | cloning of FAMA CDS (not containing stop | codon) FAM | A-R2 5'-AGTAAACACAATATTTCCCAGGTTAGAGCT-3' |
| 17 | cloning of FAMA-SRDX FAMA-SRDX           | -R         |   |

5'TTAAGCGAAACCCAAACGGAGTTCTAGATCCAGATCCAGAGTAAACACAATATTTCC-3'

| ID | purpose                        | name            | sequence                                       |
|----|--------------------------------|-----------------|--|
| 18 | cloning of FAMA promoter       | FAMApro-F       | 5'-CACCCCAAACTTCATGTATGATTCCAAACT-3'           |
| 19 | cloning of FAMA promoter       | FAMApro-R       | 5'-TGCTATTCGTGGTAGTTGATTATAAACTGC-3'           |
| 20 | cloning of tagRFP-FAMA genomic | tRFP-FAMAg-1    | 5'-CAAAGGGTGGGCGCGATGGTGTCTAAGGGCGAA-3'        |
| 21 | cloning of tagRFP-FAMA genomic | tRFP-FAMAg-2    | 5'-AGCTGGGTCGGCGCGGTGTAAAAGCGTCACTTT-3'        |
| 22 | cloning of tagRFP-FAMA genomic | FAMAg-F         | 5'-CACCATGGATAAAGATTACTCGGTACGTAC-3'           |
| 23 | cloning of tagRFP-FAMA genomic | FAMAg-R         | 5'-GTGTAAAAGCGTCACTTTGTTTAACCGAATGGTCAA-3'     |
| 24 | cloning of ICE1 promoter       | ICE1pro-F       | 5'-CACCAAGACAACCGGACCACCGTCAATAAC-3'           |
| 25 | cloning of ICE1 promoter       | ICE1pro-R       | 5'-CGCCAAAGTTGACACCTTTACCCCCAAAGTC-3'          |
| 26 | cloning of amiRNA              | amiRNA-F        | 5'-CACCCTGCAAGGCGATTAAGTTGGGTAAC-3'            |
| 27 | cloning of amiRNA              | amiRNA-R        | 5'-GCGGATAACAATTTCACACAGGAAACAG-3'             |
| 28 | cloning of ICE1/ICE2 amiRNA    | ICE1/2_amiRNA_1 | 5'-GATTAAAGCCTATCATTAGGCTTTCTCTCTTTTGTATTCC-3' |
| 29 | cloning of ICE1/ICE2 amiRNA    | ICE1/2_amiRNA_2 | 5'-GAAAGCCTAATGATAGGCTTTAATCAAAGAGAATCAATGA-3' |
| 30 | cloning of ICE1/ICE2 amiRNA    | ICE1/2_amiRNA_3 | 5'-GAAAACCTAATGATACGCTTTATTCACAGGTCGTGATATG-3' |
| 31 | cloning of ICE1/ICE2 amiRNA    | ICE1/2_amiRNA_4 | 5'-GAATAAAGCGTATCATTAGGTTTTCTACATATATATTCCT-3' |
| 32 | RT-PCR of TGG1                 | TGG1-F          | 5'-CACCATGAAGCTTCTTATGCTCGCCTTTGT-3'           |
| 33 | RT-PCR of TGG1                 | TGG1-R          | 5'-TCATGCATCTGCAAGACTCTTCCGATCACG-3'           |
| 34 | RT-PCR of ACT2                 | ACT2-F          | 5'-GGCGATGAAGCTCAATCCAAACG-3'                  |
| 35 | RT-PCR of ACT2                 | ACT2-R          | 5'-GGTCACGACCAGCAAGATCAAGACG-3'                |
| 36 | cloning of bHLH090 promoter    | bHLH090pro-F    | 5'-CACCCTAAGCAAGAATCAACTTCTAATTCT-3'           |
| 37 | cloning of bHLH090 promoter    | bHLH090pro-R    | 5'-CTCACTCTCTCTCTCTCTCTCTCTCTCT-3'             |

| ID | purpose                  | name               | sequence                     |
|----|--------------------------|--------------------|------------------------------|
| 38 | Real-Time PCR of bHLH090 | bHLH090_RealTime_  | F 5'-TGGTCTCGTTGAGCTGTTCG-3' |
| 39 | Real-Time PCR of bHLH090 | bHLH090_RealTime_l | R 5'-ACTGCAGCCTTTGTTCAGGG-3' |
| 40 | Real-Time PCR of ACT2    | ACT2_RealTime_F    | 5'-TATGTCGCCATCCAAGCTGT-3'   |
| 41 | Real-Time PCR of ACT2    | ACT2_RealTime_R    | 5'-AAGGTCACGTCCAGCAAGGT-3'   |