An *Arabidopsis* flavonoid transporter is required for anther dehiscence and pollen development

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Supplementary information

Alignment of FFT and related sequences

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FFT             LLTHGGEVEEDYAPARSWTDVKRVLSTESAKLWMIAAPVGFNIICQYGVSSVTNIFVGHI 66
SlAnthPerm      ERNDLIGADGDYRPAKSTKDWWAIFCVETLKLWRIGGPIAFNIICQYGVNSLTNIFVGHL 73
At4g00350       TLLGETTGDADFPPIQSFRDAKLVCVVETSKLWEIAAPIAFNILCNYGVNSFTSIFVGHI 120
OsXP            ------------------------------------------------------MFIGHL 6
At3g26590       RSDLPFLSVDDIPPITTVGGFVREFNVETKKLWYLAGPAIFTSVNQYSLGAITQVFAGHI 77
At5g38030       PPALPFSSVEDIPPITTVGGFVKEFNVEVKKLWYLAGPAIFMSITQYSLGAATQVFAGHI 77
At1g47530       PELTGTKSASKV--------WAKEFGEESKRLWELAGPAIFTAISQYSLGALTQTFSGRL 67
Q96FL8          PAPVRGGPEATLEVRGSRCLRLSAFREELRALLVLAGPAFLVQLMVFLISFISSVFCGHL 66
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FFT             GEVELSAVSISLSVIGTFSFGFLLGMGSALETLCGQAYGAGQVNMLGVYMQRSWIILFVS 126
SlAnthPerm      GNVELSAISIAQTVISTFSFGFMMGMGSALETLCGQAYGAGQVHMLGVYMQRSIIILLAT 133
At4g00350       GDLELSAVAIALSVVSNFSFGFLLGMASALETLCGQAFGAGQMDMLGVYMQRSWLILLGT 180
OsXP            GNLPLAAASVGLSVFATFALGFLLGMGSALETLCGQAFGAGQVSMLGVYLQRSWIILLGA 66
At3g26590       STIALAAVSVENSVVAGFSFGIMLGMGSALETLCGQAFGAGKLSMLGVYLQRSWVILNVT 137
At5g38030       STIALAAVSVENSVIAGFSFGVMLGMGSALETLCGQAFGAGKLSMLGVYLQRSWVILNVT 137
At1g47530       GELELAAVSVENSVISGLAFGVMLGMGSALETLCGQAYGAGQIRMMGIYMQRSWVILFTT 127
Q96FL8          GKLELDAVTLAIAVINVTGVSVGFGLSSACDTLISQTYGSQNLKHVGVILQRSALVLLLC 126
                . : * * ::  :*.   .... :*:.** :** .*::*: ::  :*: :*** ::*

FFT             CFFLLPIYIFATPVLRLLGQAEEIAVPAGQFTLLTIPQLFSLAFNFPTSKFLQAQSKVVA 186
SlAnthPerm      CVFLLPIYLFTTPLLVLLGQETAIADLSGRYTMLLIPQLFSLAINFPTSKFLQAQSKVDV 193
At4g00350       SVCLLPLYIYATPLLILLGQEPEIAEISGKFTTQIIPQMFALAINFPTQKFLQSQSKVGI 240
OsXP            TVLMVPVYVLAEPLLLLVGQDPEVARAAGRFTLYILPGAFAFAVNFPSGKFLQAQSKVGV 126
At3g26590       ALILSLLYIFAAPILASIGQTAAISSAAGIFSIYMIPQIFAYAINFPTAKFLQSQSKIMV 197
At5g38030       AVILSLLYIFAAPILAFIGQTPAISSATGIFSIYMIPQIFAYAVNYPTAKFLQSQSKIMV 197
At1g47530       ALFLLPVYIWAPPILSFFGEAPHISKAAGKFALWMIPQLFAYAANFPIQKFLQSQRKVLV 187
Q96FL8          CFPCWALFLNTQHILLLFRQDPDVSRLTQTYVTIFIPALPATFLYMLQVKYLLNQGIVLP 186
                 .    ::: :  :*  . :   ::  :  :    :*   :        *:*  *  :

FFT             IAWIGFVALSLHVIMLWLFIIEFGWGTNGAALAFNITNWGTAIAQIVYVIG-WCNEG-WT 244
SlAnthPerm      LAGIGFAAVLVHALFLWLFIYTLEWGTNGAAIAFDLTNWLTAMAQLAYVVG-WCKDG-WK 251
At4g00350       MAWIGFFALTLHIFILYLFINVFKWGLNGAAAAFDVSAWGIAIAQVVYVVG-WCKDG-WK 298
OsXP            LAWIGVAGLAFHVGITYLAVSVLGWGLPGAAAAYDVSQWASSLAQVAYIMG-WCREG-WR 184
At3g26590       MAVISAVALVIHVPLTWFVIVKLQWGMPGLAVVLNASWCFIDMAQLVYIFSGTCGEA-WS 256
At5g38030       MAAISAVALVLHVLLTWFVIEGLQWGTAGLAVVLNASWWFIVVAQLVYIFSGTCGEA-WS 256
At1g47530       MAWISGVVLVIHAVFSWLFILYFKWGLVGAAITLNTSWWLIVIGQLLYILITKSDGA-WT 246
Q96FL8          QIVTGVAANLVNALANYLFLHQLHLGVIGSALANLISQYTLALLLFLYILGKKLHQATWG 246
                    .     .:    :: :  :  *  * * .   :     :  . *:.      . *

FFT             GLSWLAFKEIWAFVRLSIASAVMLCLEIWYMMSIIVLTGRLDNAVIAVDSLSICMNINGL 304
SlAnthPerm      GLSWSAFNEIWAFVRLSIASAVMLCLEIWYMMSIILLVGHLNNAVIAVGSISICMNINGW 311
At4g00350       GLSWLAFQDVWPFLKLSFASAVMLCLEIWYFMTIIVLTGHLEDPVIAVGSLSICMNINGW 358
OsXP            GWSMAAFHDLAAFLRLSIESAVMLCLEIWYLGLITVLTGDLDDAQMAVDSLGICMNINGY 244
At3g26590       GFSWEAFHNLWSFVRLSLASAVMLCLEVWYFMAIILFAGYLKNAEISVAALSICMNILGW 316
At5g38030       GFSWEAFHNLWSFVRLSLASAVMLCLEVWYLMAVILFAGYLKNAEISVAALSICMNILGW 316
At1g47530       GFSMLAFRDLYGFVKLSLASALMLCLEFWYLMVLVVVTGLLPNPLIPVDAISICMNIEGW 306
Q96FL8          GWSLECLQDWASFLRLAIPSMLMLCMEWWAYEVGSFLSGILG--MVELGAQSIVYELAII 304
                * *  .:.:   *::*:: * :***:* *       .. * *    : : : .*  ::

FFT             EAMLFIGINAAISVRVSNELGLGRPRAAKYSVYVTVFQSLLIGLVFMVAIIIARDHFAII 364
SlAnthPerm      ESMLFIGINAAISIRVSNELGQGHPRATKYSVYITVFQSLLIGILCMVIVLVARDHLAII 371
At4g00350       EGMLFIGINAAISVRVSNELGSGHPRAAKYSVIVTVIESLVIGVVCAIVILITRDDFAVI 418
OsXP            EGMIFIGLNAAISVRVSNELGSGRPRAAMHAVVVVVAESLLIGLLCMALVLAFSDKLALV 304
At3g26590       TAMIAIGMNTAVSVRVSNELGANHPRTAKFSLLVAVITSTLIGFIVSMILLIFRDQYPSL 376
At5g38030       TAMIAIGMNAAVSVRVSNELGAKHPRTAKFSLLVAVITSTVIGLAISIALLIFRDKYPSL 376
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At1g47530       TAMISIGFNAAISVRVSNELGAGNAALAKFSVIVVSITSTLIGIVCMIVVLATKDSFPYL 366
Q96FL8          VYMVPAGFSVAASVRVGNALGAGDMEQARKSSTVSLLITVLFAVAFSVLLLSCKDHVGYI 364
                  *:  *:..* *:**.* **      :  :  :    : ::..     ::   *    :

FFT             FTSSKVLQRAVSKLAYLLGITMVLNSVQPVVSGVAVGGGWQGLVAYINLGCYYIFGLPFG 424
SlAnthPerm      FSNSKEMQEAVADLAYLLGITMVLNSVQPVISGVAVGGGWQALVAYINLGCYYVFGLPLG 431
At4g00350       FTESEEMRKAVADLAYLLGITMILNSLQPVISGVAVGGGWQAPVAYINLFCYYAFGLPLG 478
OsXP            YTSDAHLLRAVSRIAGLLGVTMVLNSVQPVLSGVAVGGGWQGLVAYINLACYYLFGLPVG 364
At3g26590       FVKDEKVIILVKELTPILALSIVINNVQPVLSGVAVGAGWQAVVAYVNIACYYVFGIPFG 436
At5g38030       FVGDEEVIIVVKDLTPILAVSIVINNVQPVLSGVAVGAGWQAVVAYVNIVCYYVFGIPFG 436
At1g47530       FTSSEAVAAETTRIAVLLGFTVLLNSLQPVLSGVAVGAGWQALVAYVNIACYYIIGLPAG 426
Q96FL8          FTTDRDIINLVAQVVPIYAVSHLFEALACTSGGVLRGSGNQKVGAIVNTIGYYVVGLPIG 424
                :  .  :   .  :. : ..: ::: :  . .**  *.* *   * :*   ** .*:* *

FFT             YLLGYIANFGVMGLWSGMIAGTALQTLLLLIVLYKTNWNKEVEETMERMKKWGG----SE 480
SlAnthPerm      YLLGYVAKLGTKGLWLGMIAGAALQTLLLLIILYKTNWNKEVNDTTERMRKWGGQDFETQ 491
At4g00350       FLLGYKTSLGVQGIWIGMICGTSLQTLILLYMIYITNWNKEVEQASERMKQWGAGYEKLE 538
OsXP            YLLGYYFNLGVGGVWGGMLCGVALQTLILLFVVWRTDWKAEAAQASARVHKWGGTDETKP 424
At3g26590       LLLGYKLNYGVMGIWCGMLTGTVVQTIVLTWMICKTNWDTEASMAEDRIREWGGEVSEIK 496
At5g38030       LLLGYKLNFGVMGIWCGMLTGTVVQTIVLTWMICRTNWDTEAAMAEGRIREWGGEVSD-- 494
At1g47530       LVLGFTLDLGVQGIWGGMVAGICLQTLILIGIIYFTNWNKEAEQAESRVQRWGGTAQE-- 484
Q96FL8          IALMFATTLGVMGLWSGIIICTVFQAVCFLGFIIQLNWKKACQQAQVHANLKVNNVPRSG 484
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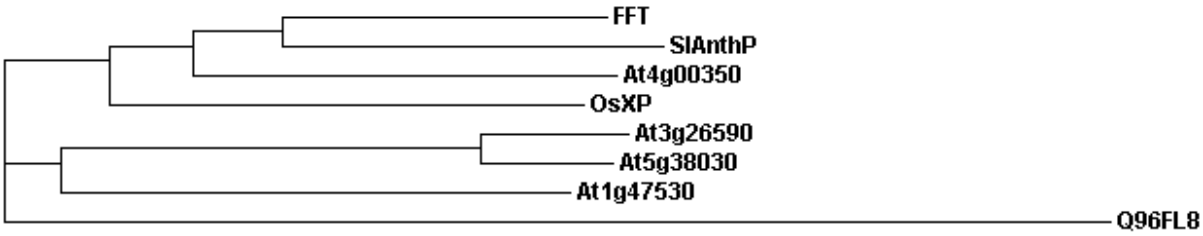
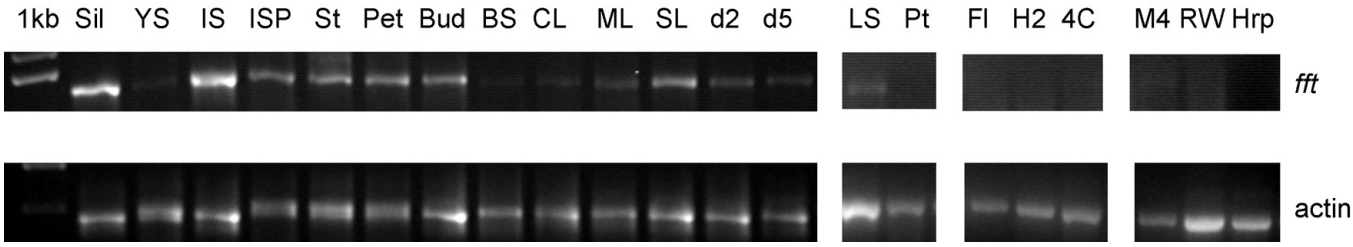


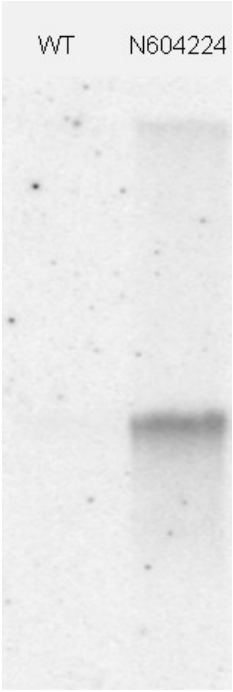
Supp.1. CLUSTALW alignment (N and C termini omitted to save space) and phylogram of FFT and three most similar *Arabidopsis* MATE proteins with tomato putative anthocyanin permease (SlAnthP) and rice ripening protein (OsXP). Human MATE1 sequence (Q96FL8) included to show invariant glutamate (boxed) in seventh transmembrane domain (Matsumoto *et al.*, 2008). Twelve putative transmembrane domains (TMPred) underlined.

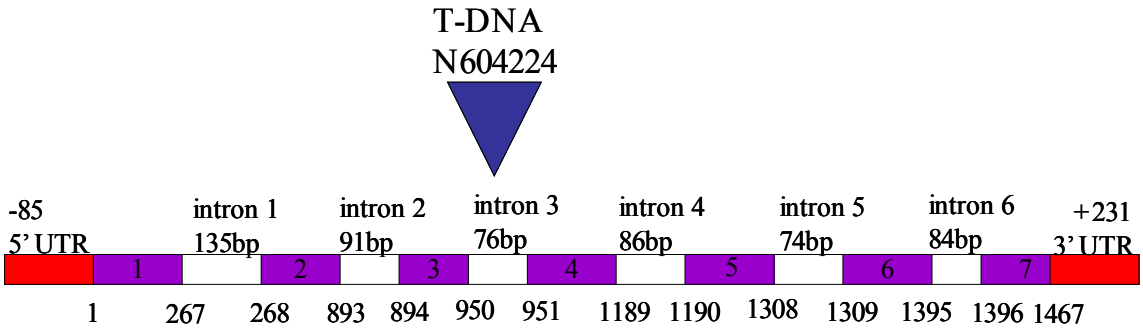
RTPCR



Supp.2. Tissue or stress-induced transcription of FFT-1. RTPCR conducted according to Materials and methods. Sil, Silique (green, >1cm); YS, young silique (green, <0.5cm); IS, immature silique (green, <1cm); ISP, IS with senescing petals attached; Pet, petals; Bud, unopened bud; BS, bolt stem; CL, cauline leaf; ML, mature rosette leaf; SL, senescent anthocyanin-pigmented leaf; d2/d5, day2 or d5 seedling; LS, rosette leaf of plant grown with 1% sucrose; Pt, petioles; Fl, plates flooded 25ml sterile water 36h; H2, 1mM H_2O_2 added to root tips 3h; 4C, plates incubated 4°C with light for 24h; *P. syringae* infiltrated into leaf as follows: M4, *P.s.* maculicola M4 (pathogenic), RW, *P.s.* phaseolica RW60 (nonpathogenic), Hrp, *P.s.* DC300 HrpA$^-$ (nonpathogenic).

***fft*-1 mutant: Southern and position of T-DNA insertion**

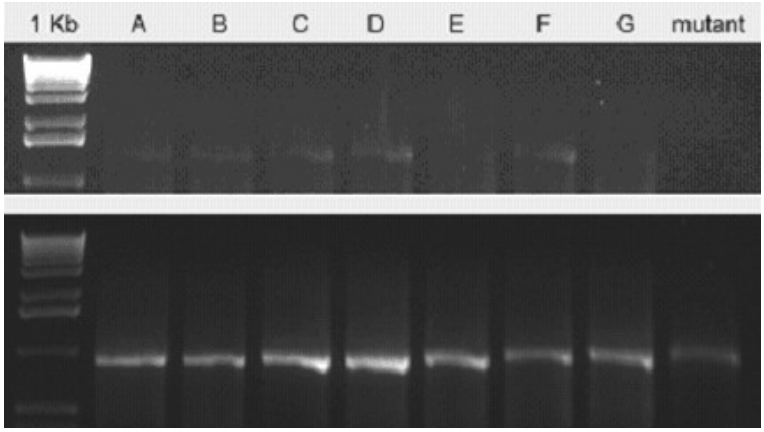




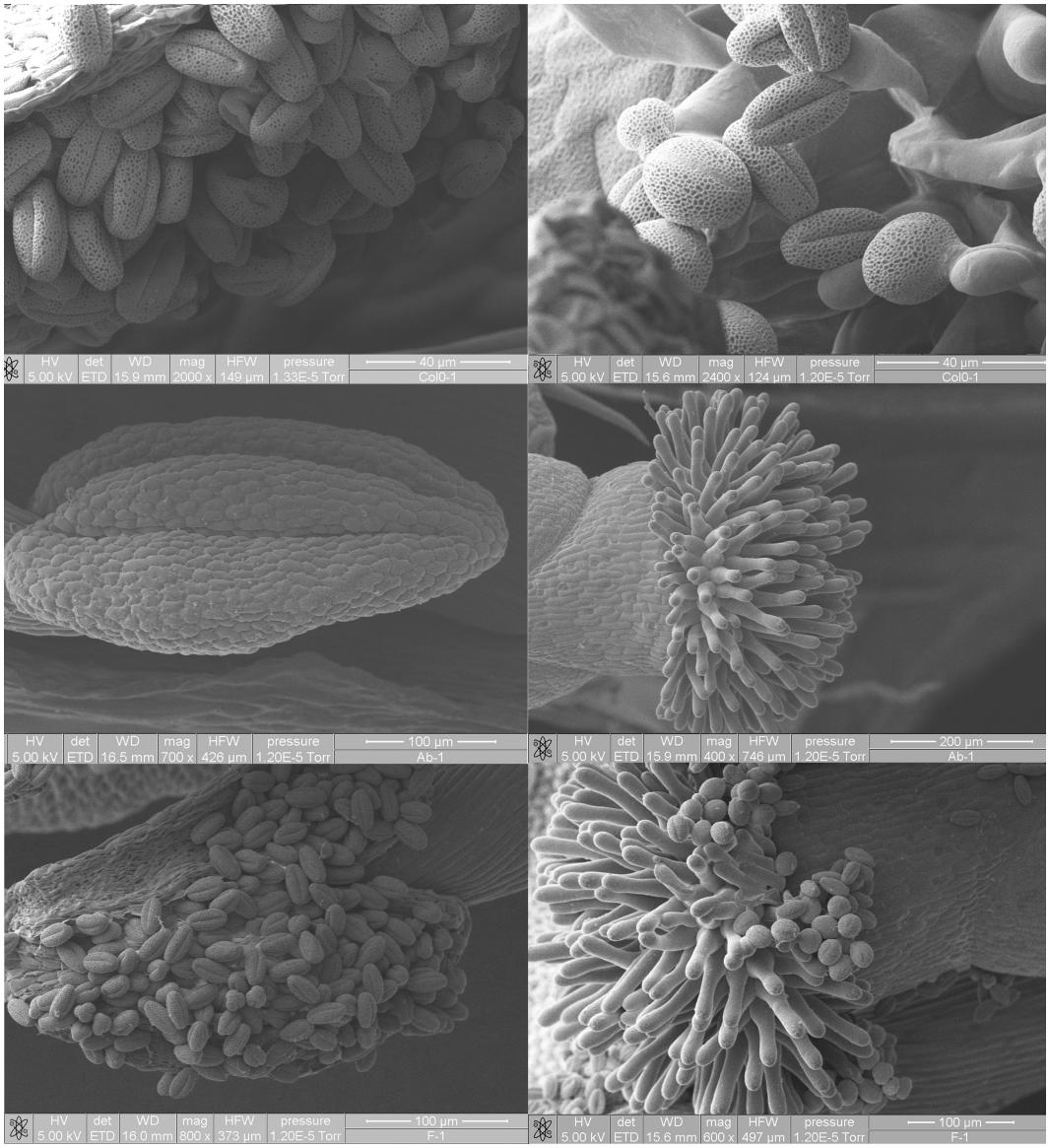
Supp.3. Southern blot with kanamycin probe showing one T-DNA band in genomic DNA from *fft*-1 (N604224) mutant and position of T-DNA insertion.

Complemented mutant

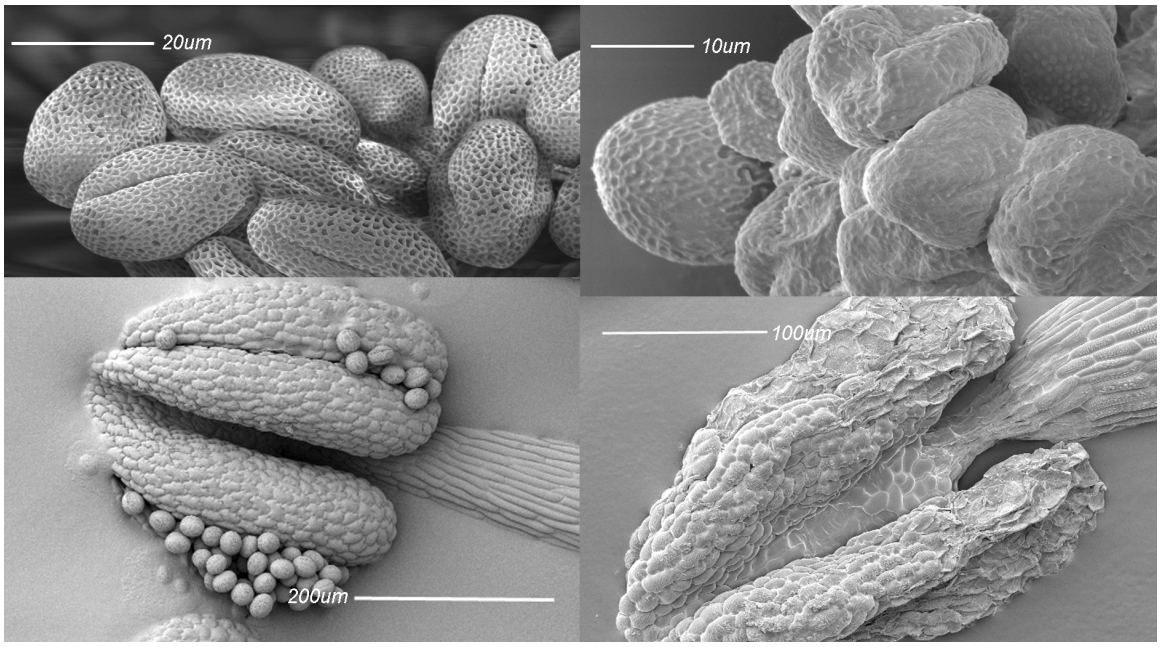
RTPCR for null mutant



Supp.4. RT-PCR in inflorescence tissues (top panel, FFT transcript; lower panel, actin8). From seven transformed mutants (lines A–G), lines D, F and G were investigated fully.



Supp.5. Cryo-SEM of inflorescence tissue from WT (top panel, Col0-1), *fft-1* mutant (middle panel; Ab-1) and complemented mutant (lower panel, F-1). Mutant has non-dehisced anthers but receptive papillae on the stigma, whereas WT and complemented mutant have dehisced anthers and pollen germinating on stigma papillae.

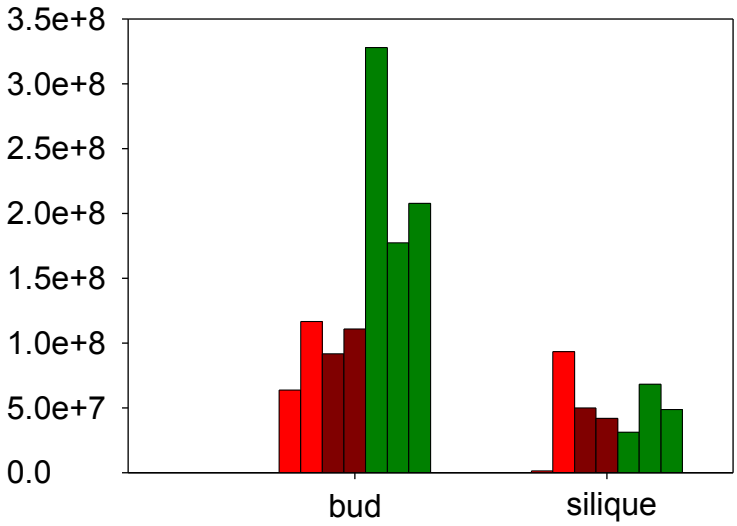


Col0 *fft-1*

Supp.6. SEM of WT (Col0) and *fft-1* mutant pollen and anthers, showing how a proportion of pollen and anthers are defective in the mutant.

Supp.7. Photosynthetic parameters. Since flavonoids may help to protect plants from u.v. light, it seemed prudent to ensure that the mutant plants were not suffering from a generalised inability to thrive, unlikely though this seemed from their appearance and increased rates of root growth. Basic photosynthetic parameters were checked, namely, Fv/Fm and chlorophyll content (*a*, *b* and *a*:*b*), to ensure no difference in photosynthetic efficiency between WT and mutant, and we also determined that there was no effect of different light intensities (10–250 μmol/m^2/s) on the pollen phenotypes.

(a)



(b)

Compound and sample	Col0 mean ± SEM	*fft-1* mutant mean ± SEM	*P**
kGG bud	$273 \times 10^3 \pm 67 \times 10^3$	$141 \times 10^3 \pm 29 \times 10^3$	0.018
RkG bud	$869 \times 10^3 \pm 225 \times 10^3$	$535 \times 10^3 \pm 85 \times 10^3$	0.18
RkG IS	$1070 \times 10^3 \pm 564 \times 10^3$	$796 \times 10^3 \pm 389 \times 10^3$	0.48
qRGR bud	$217 \times 10^3 \pm 104 \times 10^3$	$339 \times 10^3 \pm 102 \times 10^3$	0.19
qRG bud	$690 \times 10^3 \pm 285 \times 10^3$	$428 \times 10^3 \pm 113 \times 10^3$	0.13
qRG IS	$515 \times 10^3 \pm 303 \times 10^3$	$189 \times 10^3 \pm 67 \times 10^3$	0.23

*5 d.f.

Supp.8. (a) Example of LCMS runs for kGG in buds and immature siliques. Mutant, left (red); wild-type, right (cross-hatched, green). (b) Mean values from which Table 1 proportions are derived. k, Kaempferol; R, rhamnoside; G, glucoside; IS, immature silique; q, quercitin.