New Phytologist Supporting Information

Article title: Discrete bHLH transcription factors play functionally overlapping roles in pigmentation patterning in flowers of Antirrhinum majus

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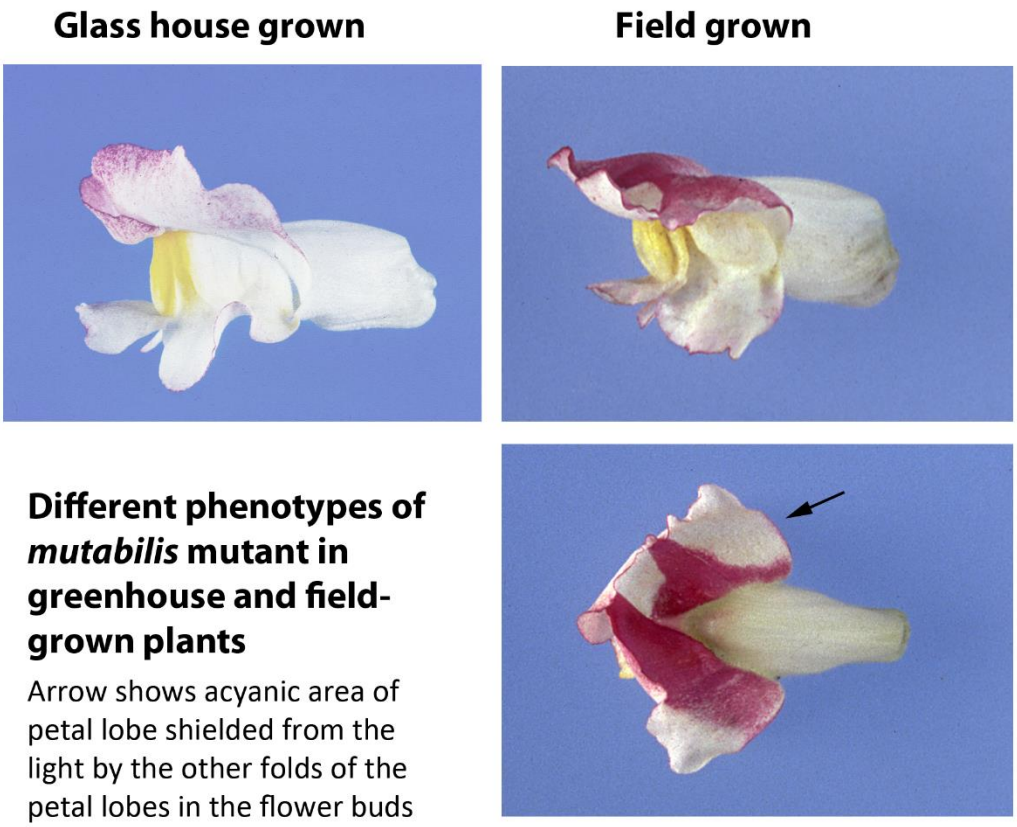
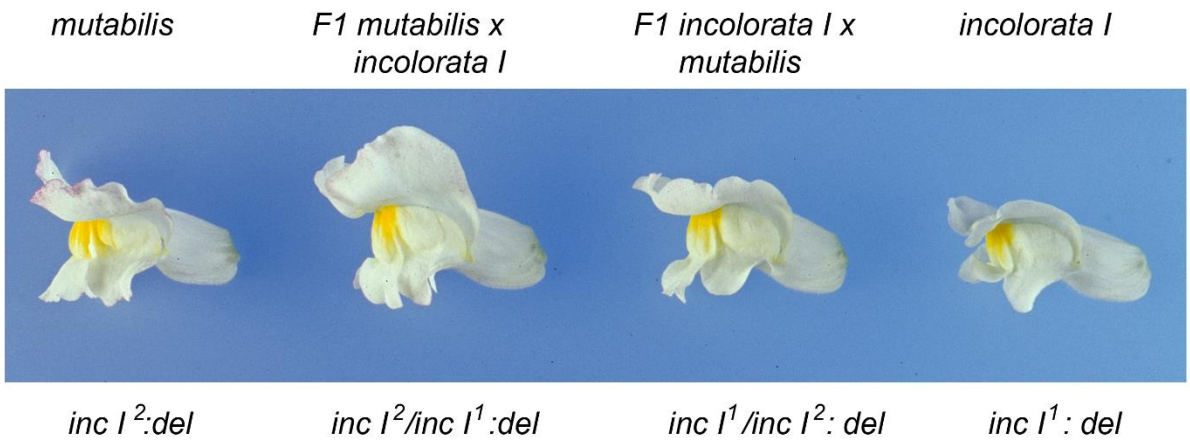
Supporting Figure S1: Differences in *mutabilis* phenotypes of *A. majus* between greenhouse and field-grown plants.

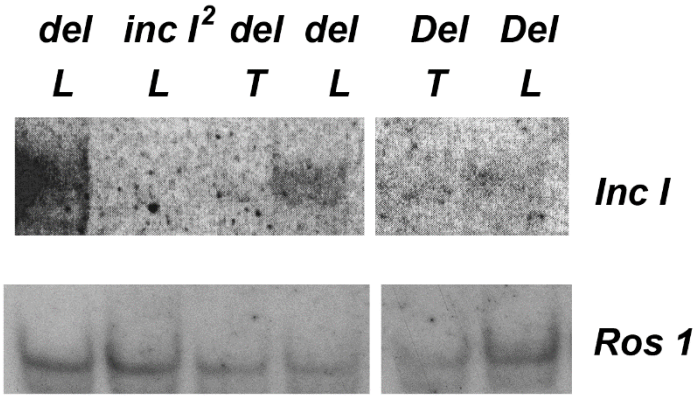


Supporting Figure S2:



Supporting Figure S2: Lack of complementation between *inc I*^{1} and *inc I*^{2} *in A. majus*
The phenotypes of the *mutabilis* and *incolorata I* mutants are shown with the phenotypes of two of their F_1 progeny**.**

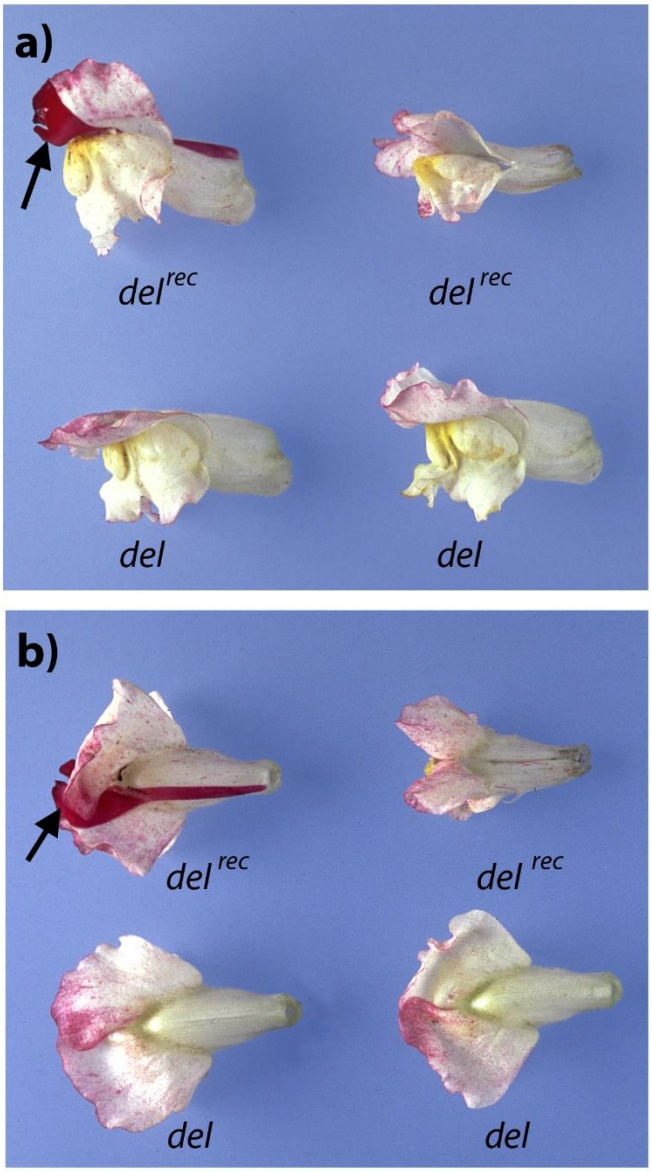
Supporting Figure S3:



Supporting Figure S3: RNA gel blot showing lack of *Inc I* transcript in flowers of the *mutabilis* mutant *of A. majus*

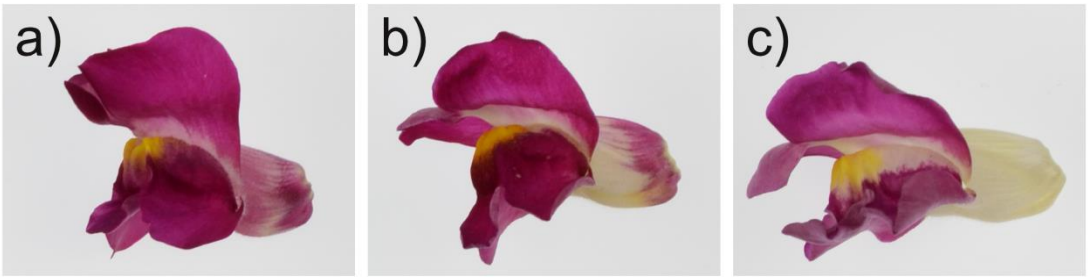
Total RNA from flower lobes (L) from the *inc I*^{2} mutant lack *Inc I* transcript compared to total RNA from lobes of *del* mutants. No *Inc I* transcript was detected in flower tubes (T) of the *del* mutant. *Inc I* transcript was detected in lobes of wild type (*Del*) flowers and also faintly in tube tissue from wildtype flowers. Transcript levels of *Ros 1* in the same RNA preparations are shown below as loading controls. The *Inc I* probe was pJAM1494, the *Ros 1* probe was pJAM1450 (Schwinn *et al.*, 2006).

Supporting Figure S4:



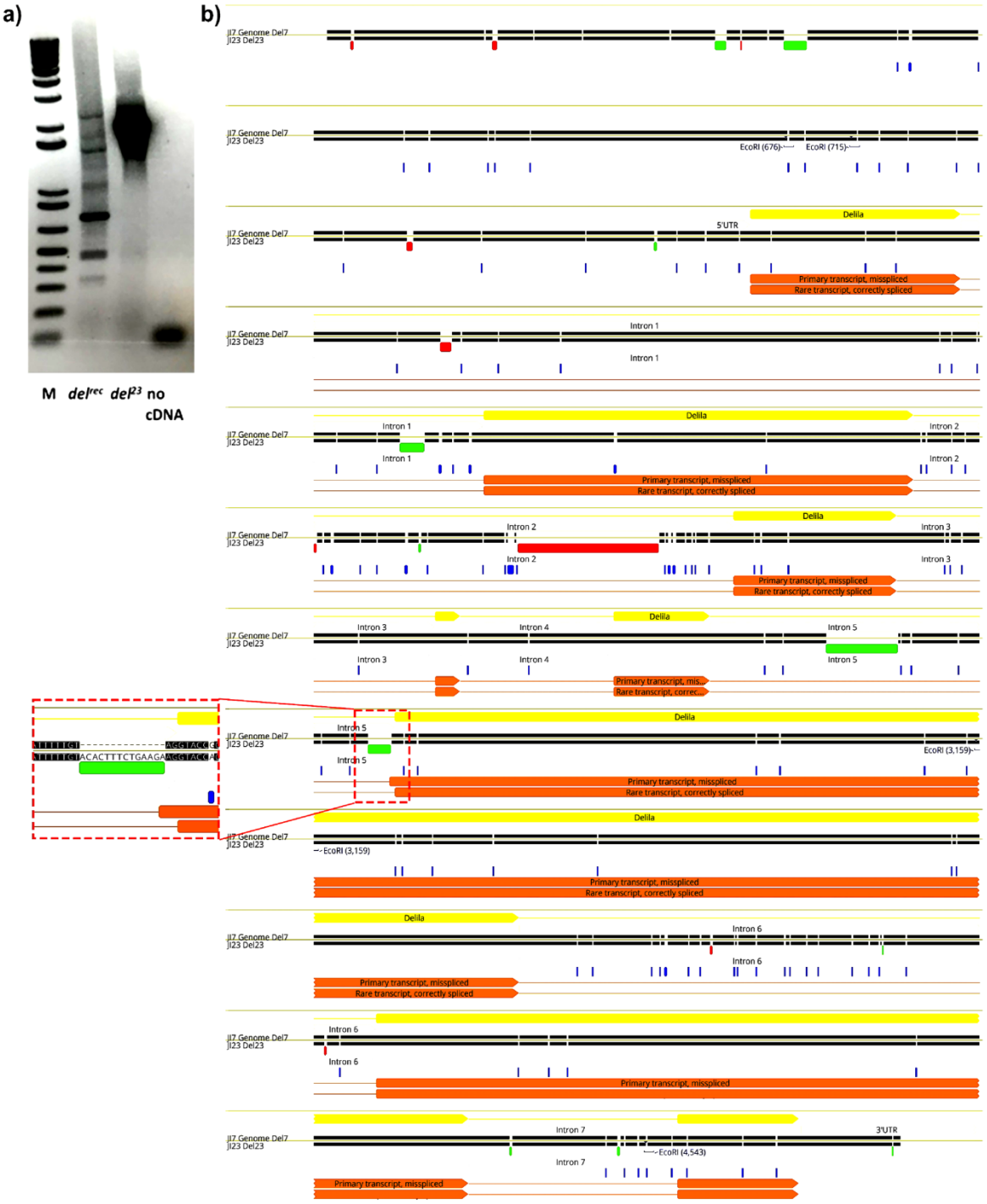
Supporting Figure S4: Sectors caused by excision of the transposable element (Tam 2) from the *Del* gene in *inc I^2: del^{rec}* *A. majus* plants showing that Del activity can complement Inc I activity in flower lobes. Arrows point to a *Del$^+$,* full red revertant sector that includes both lobe and tube tissue in an *inc I^2/inc I^2*: *del^{rec} /del^{rec}* mutant line **a)** shows side view of flowers **b)** shows dorsal view of same flowers.

Supporting Figure S5:



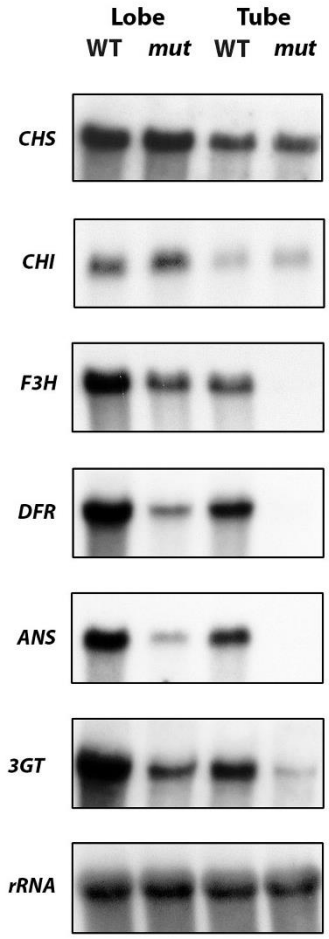
Supporting Figure S5: Phenotype of *del*^{23} allele of *A. majus*
a) shows wild type *Del* phenotype **b)** shows *del*^{23} phenotype **c)** shows *del* loss-of-function phenotype

Supporting Figure S6:



Supporting Figure S6: Molecular analysis of *del*^{23} of *A. majus*
a) qRT-PCR amplification of *Del* transcript in flowers of *del*^{rec} and *del*^{23} lines. **b)** Nucleotide sequence alignment of the reference genome WT JI:7 (upper sequence) and JI:23 (lower sequence). Annotations of sequence variants relative to the JI:7 reference genome are as follows: single nucleotide polymorphisms are indicated in blue, insertions in green, deletions in red below the aligned genomic sequences. The predominant transcript amplified from JI:23 flowers spliced into an insertion within intron five (see enlarged sequence in dashed-red box), inserting one additional amino acid (indicated by upper transcript shown scarlet bar). Rarely, the *del*^{23} transcript spliced correctly (indicated by lower transcript shown as scarlet bar).

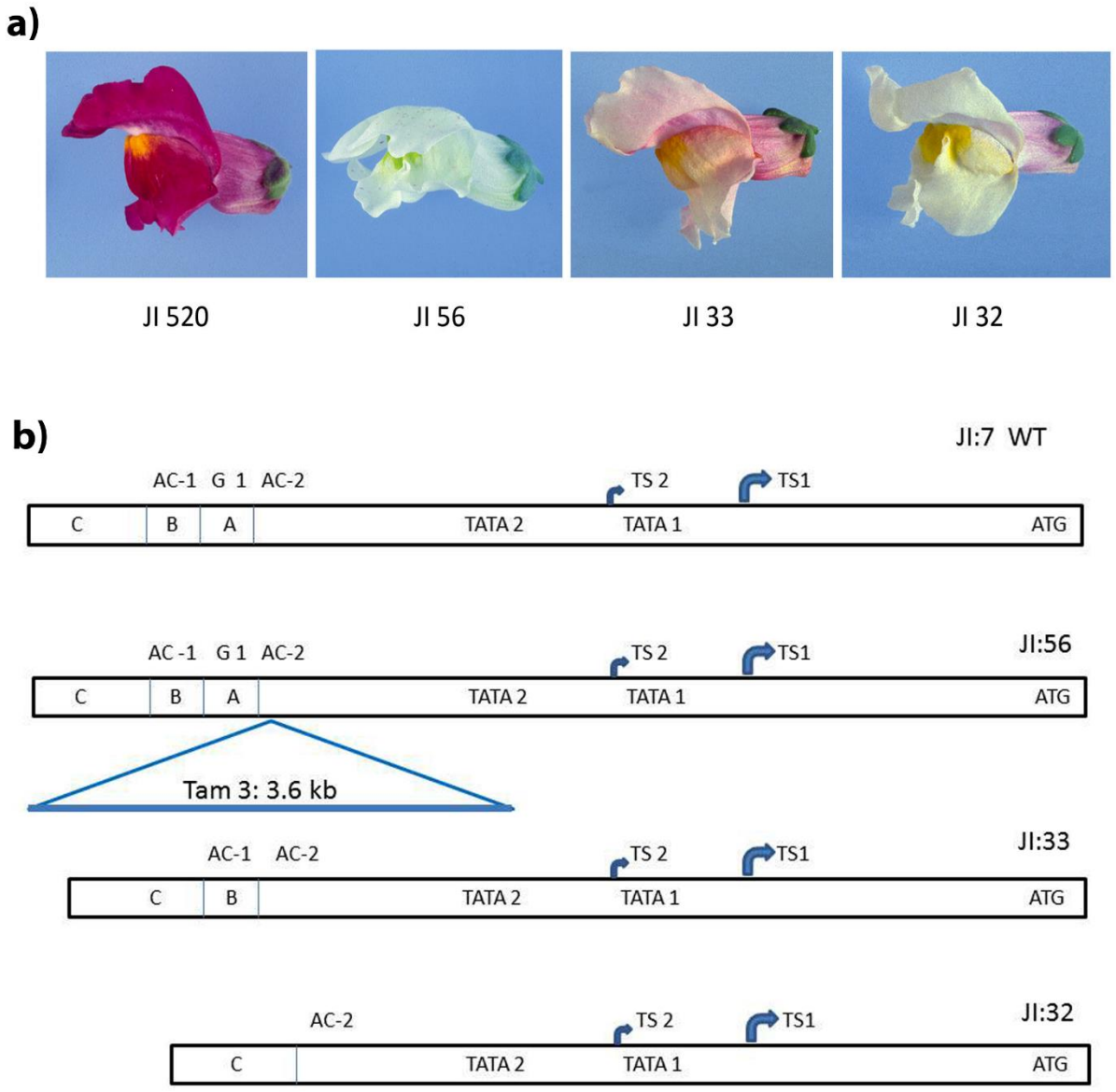
Supporting Figure S7:



Supporting Figure S7: **Impaired expression of anthocyanin biosynthetic genes in flowers of the *inc I²* mutant of *A. majus***

RNA gel blots of total RNA (25 µg) were hybridised with ^{32}P dCTP-labelled probes for the anthocyanin biosynthetic genes *CHS, CHI, F3H, DFR, ANS* and *3GT* or the 26S rRNA (loading control). RNA gel blots showed reduced transcript abundance for the anthocyanin biosynthetic genes *F3H, DFR, ANS* and *3GT* in the lobes of *inc I²* mutants compared with wild-type. This confirmed that the weak pigmentation in *inc I²* is because of impaired activation of the anthocyanin biosynthetic genes, and that *Inc I* encodes a regulator of anthocyanin biosynthesis.

Supporting Figure S8

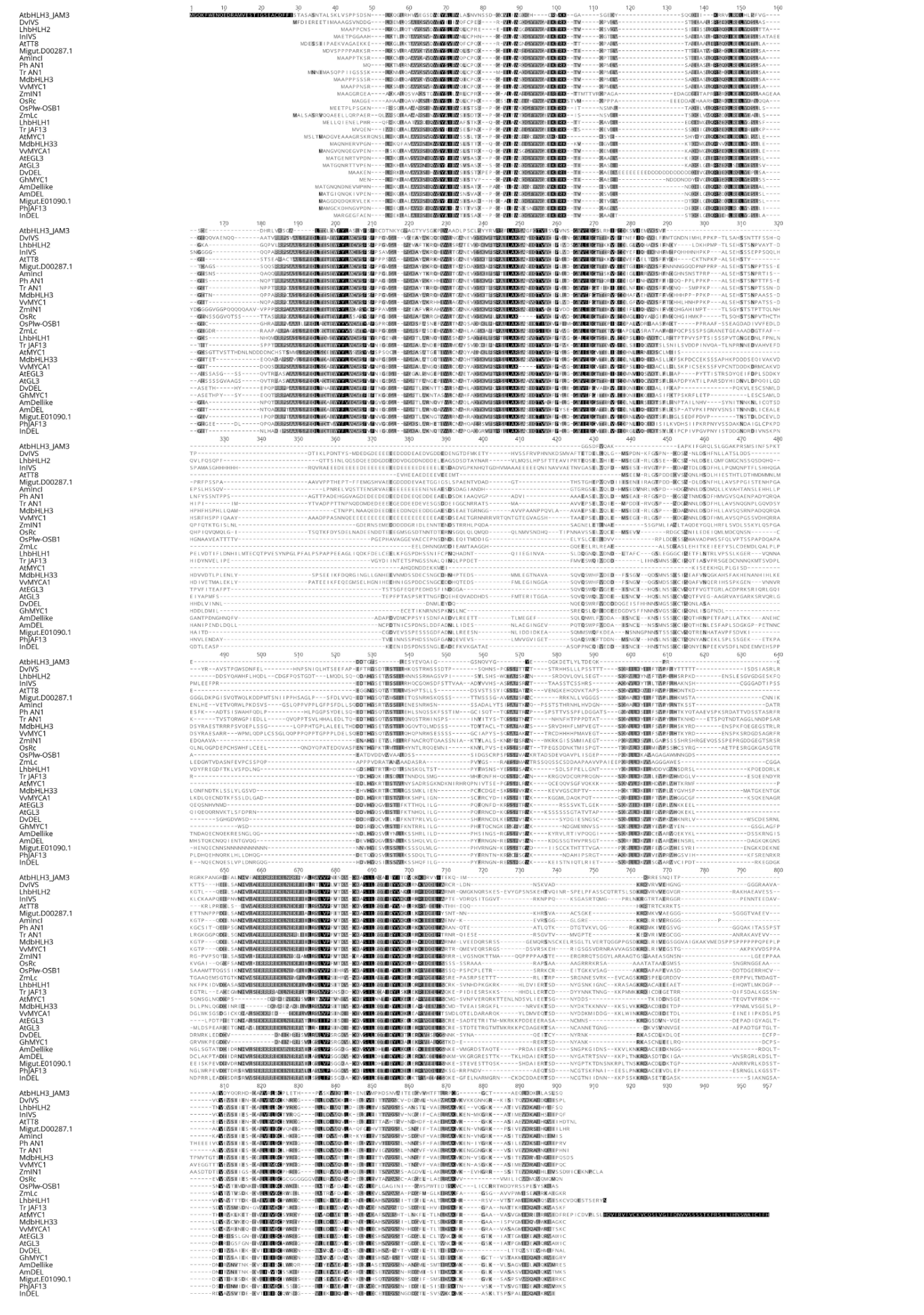


Supporting Figure S8: Phenotypes of *pallida* mutants of *A. majus* with deletions in their UAS controlling *DFR* expression caused by imprecise transposon excision and described by Almeida *et al* (1989)

a) Phenotypes of *pallida* alleles affected in the structure of the *DFR* promoter in *A. majus*.

b) Diagram of the UAS in *DFR* promoter in WT (JI:520) and mutant alleles of the *Pallida* locus encoding *DFR* in *A. majus*. The sequence of the UAS interacting with the MBW complex defined in this study (AC-box 1, G-box 1 and AC-box 2) are shown for each allele as well as the regions of the DFR promoter, boxes A, B and C, defined as important for regulation by Almeida *et al*., (1989). TS1 = transcriptional start site 1, TATA 1 = TATA box associated with TS1, TS2 = transcriptional start site 2 and TATA 2 = TATA box associated with TS2 in *DFR* promoter of *A. majus* (Coen *et al.*, 1986).

Supporting Dataset S1: Amino acid alignment of bHLH proteins. MUSCLE alignment of bHLH proteins used for Maximum likelihood phylogenetic tree in Figure 2c.



Supporting Table S1: Primers used in this study			
Number	**Primer**	**Sequence**	**Application**
NA587	*Incolorata l* Am02g53780	CTAAGAATTCAAGTTGCATCAGGC	Sequencing
NA588	*Incolorata l* Am02g53780	AGCCTTAGTTTGGTTCCCAGG	Sequencing
NA589	*Incolorata l* Am02g53780	GTGAGATTCACAATAGCTTTGGC	Sequencing
NA590	*Incolorata l* Am02g53780	CAAGTCAGAAAATTAGGTGGCG	Sequencing
NA591	*Incolorata l* Am02g53780	TGAATGTTCTCTGCACATGCC	Sequencing
NA592	*Incolorata l* Am02g53780	GCGCGAAACCACATGAAGG	Sequencing
SM119	*Incolorata l* Am02g53780	GAGAAGTACTAAAATGGCTGCAC	Sequencing
SM120	*Incolorata l* Am02g53780	CTTGGCTCCGCACCTAGTG	Sequencing
SM121	*Incolorata l* Am02g53780	CTAGTGTGGCGTGATGGATATTAC	Sequencing
SM122	*Incolorata l* Am02g53780	GCTGTTTGATTCTCCGGCAG	Sequencing
SM123	*Incolorata l* Am02g53780	GTTCGCATAACACTAGTAACCG	Sequencing
SM124	*Incolorata l* Am02g53780	CCTTAGCAAGAATTGTCCTGGA	Sequencing
SM125	*Incolorata l* Am02g53780	CATCCGAGTCCGACTCAGC	Sequencing
SM126	*Incolorata l* Am02g53780	GTGCTTCCGAATCGTGAACT	Sequencing
SM127	*Incolorata l* Am02g53780	GAGAATAGGTGATTATACGGGACA	Sequencing
SM128	*Incolorata l* Am02g53780	CCATGGACAAGTCAGTAGGCC	Sequencing
SM129	*Incolorata l* Am02g53780	CCCGAATTTTGACTAGCCAAATAC	Sequencing
SM130	*Incolorata l* Am02g53780	CTCTCTCTCAGCCCTGAAAGC	Sequencing
SM131	*Incolorata l* Am02g53780	CACTGAACAGCTTGAACCGC	Sequencing
SM132	*Incolorata l* Am02g53780	AGGCTGTAAGTCCACTGAACAG	Sequencing
SM133	*Incolorata l* Am02g53780	GTTAATCACTCGGTTCATTTCAATTAG	Sequencing
SM134	*Incolorata l* Am02g53780	TGGCATGGTTGAAGCAAGAC	Sequencing
SM135	*Incolorata l* Am02g53780	CTTACTTGTATTCCAGCACTCTTG	Sequencing
SM136	*Incolorata l* Am02g53780	CACACAAAAGGGGTGGAGGAG	Sequencing
SM137	*Incolorata l* Am02g53780	GGTGAAGGAGAATGTGAATGGTAG	Sequencing
SM138	*Incolorata l* Am02g53780	TTAGACTGAGAAGCCGGAGC	Sequencing
NA560	*Delila (del^{23} allele)*	CACCaaaATGGCTACTGGTGTCCAAAAC	Cloning/ Sequencing
NA561	*Delila (del^{23} allele)*	TCAAGACTTCATAATAACTTTCTGAAGAG	Cloning/ Sequencing
SM112	*Delila* Am02g33340	GGATTCAAGAATGGCTACTGGT	Sequencing
SM113	*Delila* Am02g33340	AGCCCTGCAAATTACTTAGCAA	Sequencing
SM114	*Delila* Am02g33340	GGTGATGGGTTCTACAATGGAG	Sequencing
SM115	*Delila* Am02g33340	GATTCAATTCGACAGATTGTACAGT	Sequencing
SM116	*Delila* Am02g33340	GTGCGGACACCAAAGTTTTC	Sequencing
SM117	*Delila* Am02g33340	GCTTTTGAATTGTCCAGACACG	Sequencing
SM118	*Delila* Am02g33340	GAAACCACGTCTTGTCAGAGAG	Sequencing
NA556	*Del-like* Am02g28470	CACCAAAATGGCAACTGGAAACCAAAATGATA ATG	Cloning/ Sequencing
NA559	*Del-like* Am02g28470	TCAAGACTCCCTCATAACCTTCTGC	Cloning/ Sequencing
NA557	*Del-like* Am02g28470	CTACCGCAATTCTCAACCATGTC	Sequencing

NA558	*Del-like* Am02g28470	CGGCCACAAACTGATTATGACC	Sequencing
NA568	*Del-like* Am02g28470	GGTTGTGTAGCTGAGATGGAC	Sequencing
NA569	*Del-like* Am02g28470	GACCTAGTTGAGACACTCGAC	Sequencing
NA570	*Del-like* Am02g28470	TGGGCAGATCAGGGAGAC	Sequencing
NA571	*Del-like* Am02g28470	ATCGATTTGTGTGTGTGGAAC	Sequencing
K112	*WDR1*	ATHGAYACIACITGYACIATHTGGGA	3'RACE
K113	*WDR1*	TGGAAYAARCARGAYYTIMGITAYATGGC	3'RACE
(dT)17-adaptor	cDNA synthesis for 3'RACE Frohman et al., 1988	GACTCGAGTCGACATCGATTTTTTTTTTTTTTTT	3'RACE
adaptor	Adapter primer for 3'RACE Frohman et al., 1988	GACTCGAGCGACATCGAT	3'RACE
K127	*WDR1*	GCACCAAACATCGACATGGGAACAATC	Gene walking
K128	*WDR1*	AGCAACCGTCGGCAGTTCCCACAGCAA	Gene walking
K133	*WDR1*	tgcaGGTACCAATGGACATTTCAACCCAC	Cloning cDNA
K134	*WDR1*	tcgaTCTAGAGAGAGCTAAAGCCGTCGAC	Cloning cDNA
K564	*Am EF1a* Am01g17900	GACTGCCACACCTCCCACATTG	qPCR
K565	*Am EF1a* Am01g17900	TCACCATACCAGCGTCACCATTC	qPCR
K566	*Am Cyclophilin* Am05g43400	CCAGGGCGGCGATTTCACC	qPCR
K567	*Am Cyclophilin* Am05g43400	GCGTTCGCCATGGACAGGATTC	qPCR
K488	*Nivea*/CHS Am04g40840	TGCTGCGTATGGCGAAGGACT	qPCR
K489	*Nivea*/CHS Am04g40840	CCGCGGTAACGATCTGGAAAA	qPCR
K461	*Pallida*/DFR Am06g29640	CACATCAATGGACAAGAGAATGC	qPCR
K462	*Pallida*/DFR Am06g29640	GCCATCAGTATGATCGTTTGC	qPCR
NA621	*Candi*/ANS Am02g33790	GGTTGAGGAGAAGGAGAAGC	qPCR
NA622	*Candi*/ANS Am02g33790	CCACTCCAGTTGACCACTAG	qPCR
NA623	3-*O*-UFGT Am02g24600	TGAGAGTGTTATGGTCGTGG	qPCR
NA624	3-*O*-UFGT Am02g24600	CTCCTGAACTCCCTTATCACC	qPCR
NA625	CHI Am02g23710	CTGCGATCACCCAAATTCAG	qPCR
NA626	CHI Am02g23710	ACTTGATGAACTTCCCCTGG	qPCR
NA631	*Inc II*/F3H Am05g03330	TACTTTGCTGCTCCAGGATC	qPCR
NA632	*Inc II*/F3H Am05g03330	GAGTTCACAACTGCTTGGTG	qPCR
K562	*Rosea1* Am06g36450	AGGATGGGGAATTAGGAAACCTA	qPCR
K563	*Rosea1* Am06g36450	CTCTCCATAACATCAGTAATCTC	qPCR
K297	*WDR1* Am01g20780	TGGGCGCCGCACAGTTG	qPCR
K299	*WDR1* Am01g20780	GCTAAAGCCGTCGACTACCTCACA	qPCR
SM13	*Incolorata I* Am02g53780	CCTCGTGCCGTTTGTTACTA	qPCR
SM14	*Incolorata I* Am02g53780	CTCGAGCTCCTCAATCTTCTTC	qPCR
SM15	*Delila* Am02g33340	ATTCTTGCATCCCTAGTCCCATCC	qPCR
SM16	D*elila* Am02g33340	CCCCCGGCCCTTTACCATTTT	qPCR
NA562	*Del-like* Am02g28470	TGTGGAGGCAAAGAGTGATTTA	qPCR
NA563	*Del-like* Am02g28470	CCCTTCCACTTGGCTCTTATAG	qPCR
EB-665	*Pallida*/DFR promoter Am06g29640	GTTATCACGTGCCTCGCGA	Deletion for p2551 and

			p2552
EB-666	*Pallida*/DFR promoter Am06g29640	TTTAGGAAGGTGATAGTATTACGTACT	Deletion for p2551 and p2565
EB-668	*Pallida*/DFR promoter Am06g29640	GTATAAACTATGCCACTTCTATGACA	Deletion for p2552
EB-672	*Pallida*/DFR promoter Am06g29640	TTTATATAGTACGTAATACTATCACCTTC	Deletion for p2557
EB-674	*Pallida*/DFR promoter Am06g29640	AAGCTTATTCAAAAATCGTATAGAAATTCA	Deletion for p2557
EB-697	*Pallida*/DFR promoter Am06g29640	GTCTTGGAAAGTTAGGAGAATTTCGT	Deletion for p2565 and p2571
EB-719	*Pallida*/DFR promoter	AGGCACGTGATAACCCTACCA	Deletion for p2571

Supplementary references:

Almeida J, Carpenter R, Robbins TP, Martin C, Coen ES. 1989. Genetic interactions underlying flower color patterns in *Antirrhinum majus*. *Genes & Development* **3**(11): 1758-1767.

Coen ES, Carpenter R, Martin C. 1986. Transposable elements generate novel spatial patterns of gene-expression in *Antirrhinum majus*. *Cell* **47**(2): 285-296.

Schwinn K, Venail J, Shang Y, Mackay S, Alm V, Butelli E, Oyama R, Bailey P, Davies K, Martin C. 2006. A small family of MYB-regulatory genes controls floral pigmentation intensity and patterning in the genus *Antirrhinum*. *Plant Cell* **18**(4): 831-851.