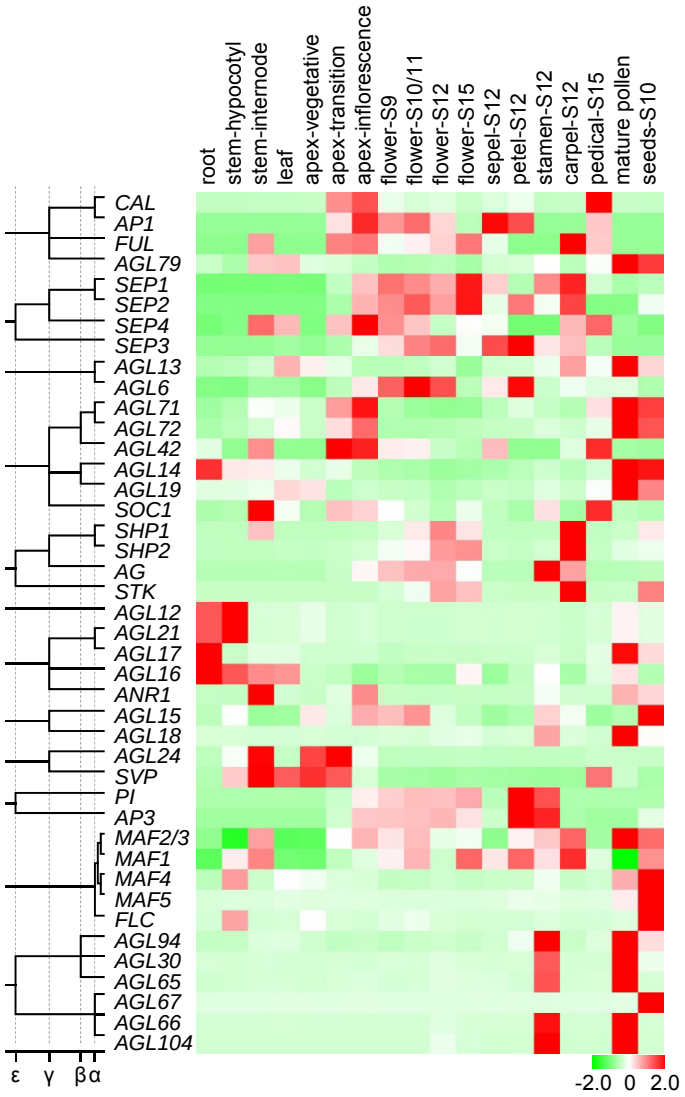
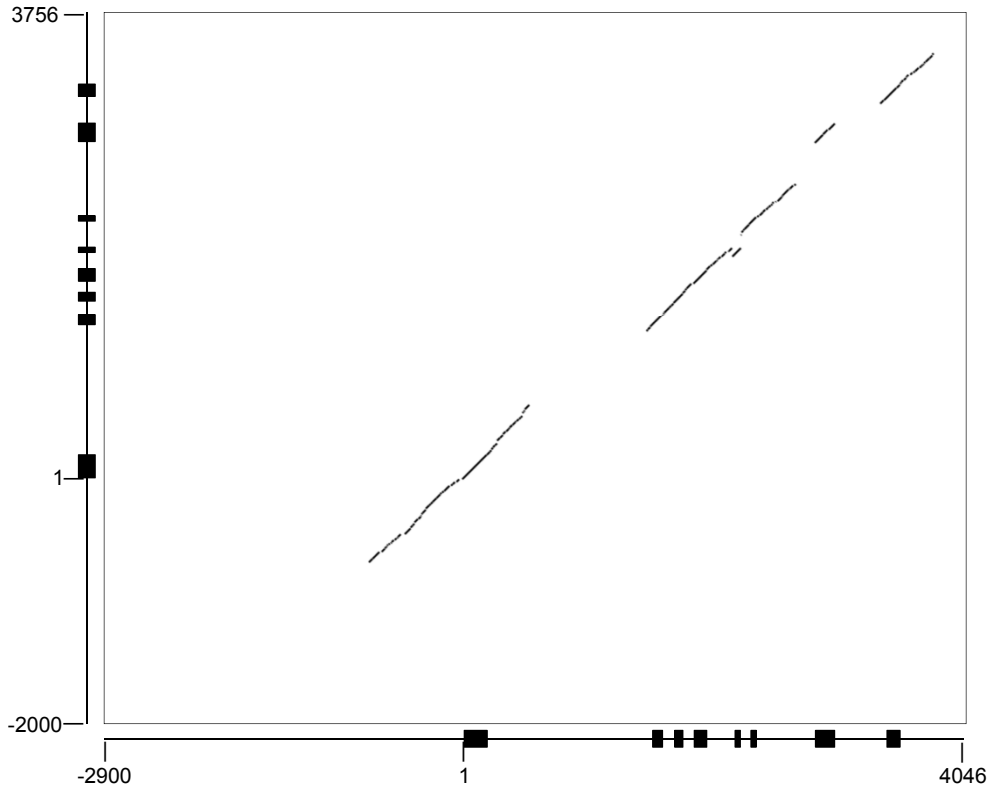
Supplemental Figure S1



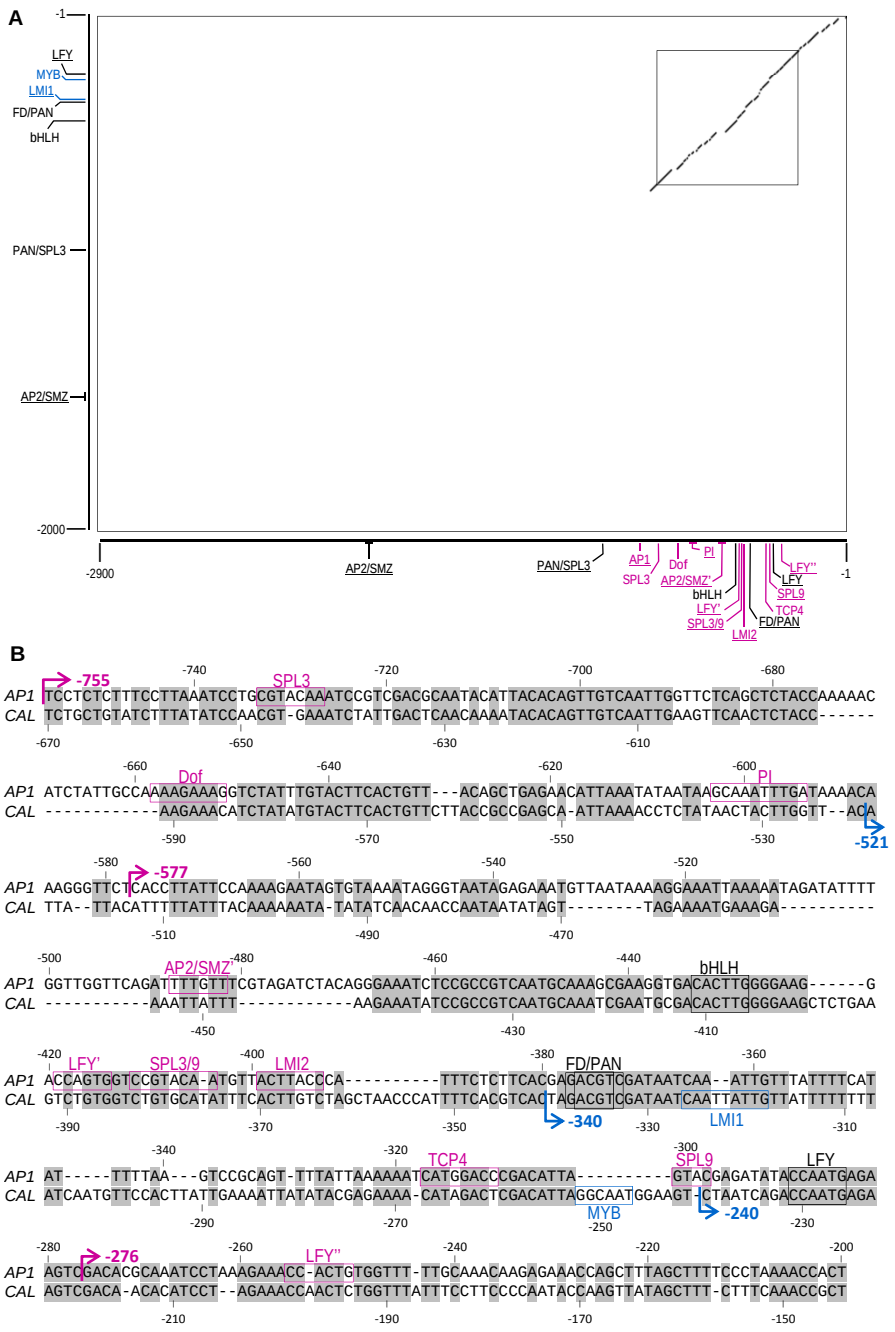
Supplemental Figure S1. Heatmap showing the prevalence of expression divergence between duplicated MIKC-type MADS-box genes in *Arabidopsis thaliana*. The expression values were retrieved from published microarray data (Schmid et al., 2005). Data from different samples were normalized by Z scores. Red, white and green indicate high, medium and low expression levels, respectively. Phylogenetic relationships of the MADS-box genes were based on (Kramer et al., 1998; Lawton-Rauh et al., 1999; Kramer et al., 2004; Zahn et al., 2005; Shan et al., 2007; Schauer et al., 2009; Cheng et al., 2013; Liu et al., 2013; Ruelens et al., 2013). The dashed lines on the trees refer to respective genome duplications that occurred before origins of Brassicaceae (α), Cleomaceae and Brassicaceae (β), core eudicots (γ), and extant angiosperms (ε) (Jiao et al., 2011).

Supplemental Figure S2



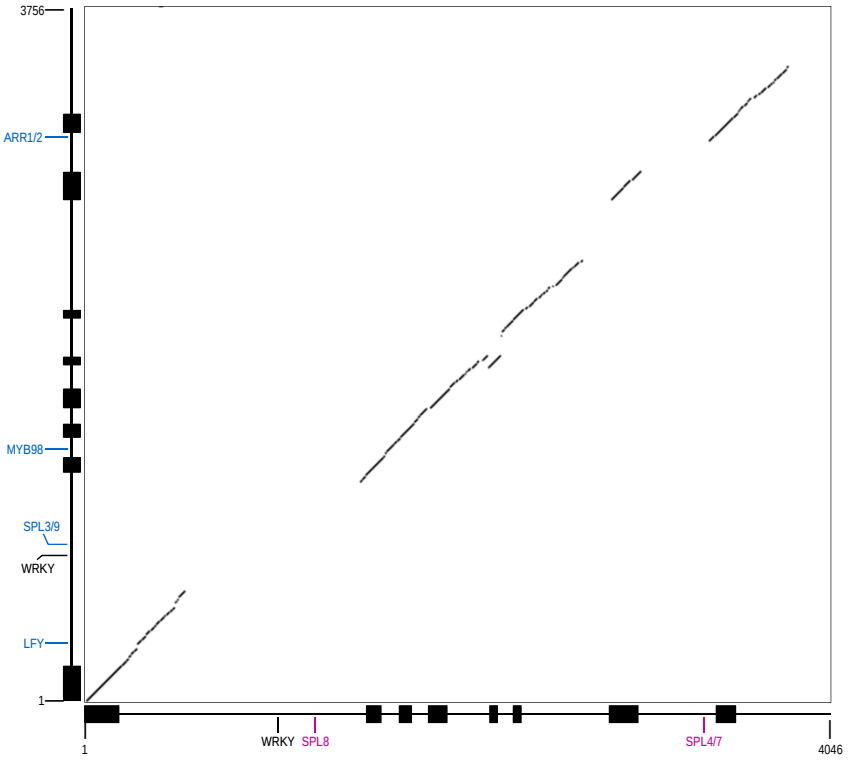
Supplemental Figure S2. The DotPlot result of *AP1* (6946 bps, horizontal axis) and *CAL* (5756 bps, vertical axis). It was performed using the PipMaker program (http://pipmaker.bx.psu.edu/pipmaker/). Black boxes represent exons.

Supplemental Figure S3



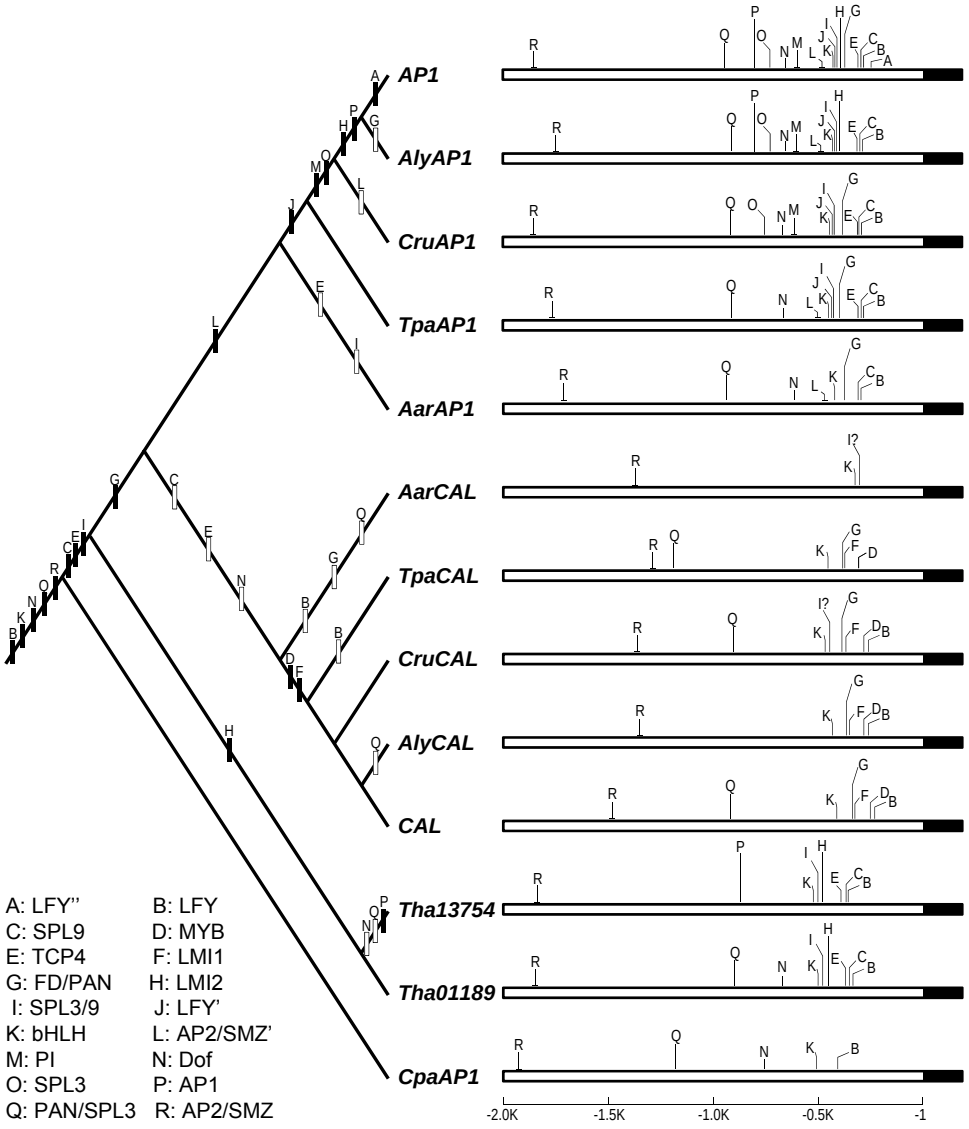
Supplemental Figure S3. Comparison of the promoter regions of *AP1* and *CAL*. (A)The DotPlot result of *AP1* (2900 bps, horizontal axis) and *CAL* (2000 bps, vertical axis), which was performed using the PipMaker program (http://pipmaker.bx.psu.edu/pipmaker/). The inset box shows the alignable region. TFBSs in black are those shared by *AP1* and *CAL*, whereas those in purple and blue are *AP1*- and *CAL*-specific, respectively. Experimentally confirmed TFBSs are underlined. (B) Sequence alignment of the alignable region, with TFBSs being boxed. Arrows indicate the positions of constructs in Figures 2 and 3.

Supplemental Figure S4



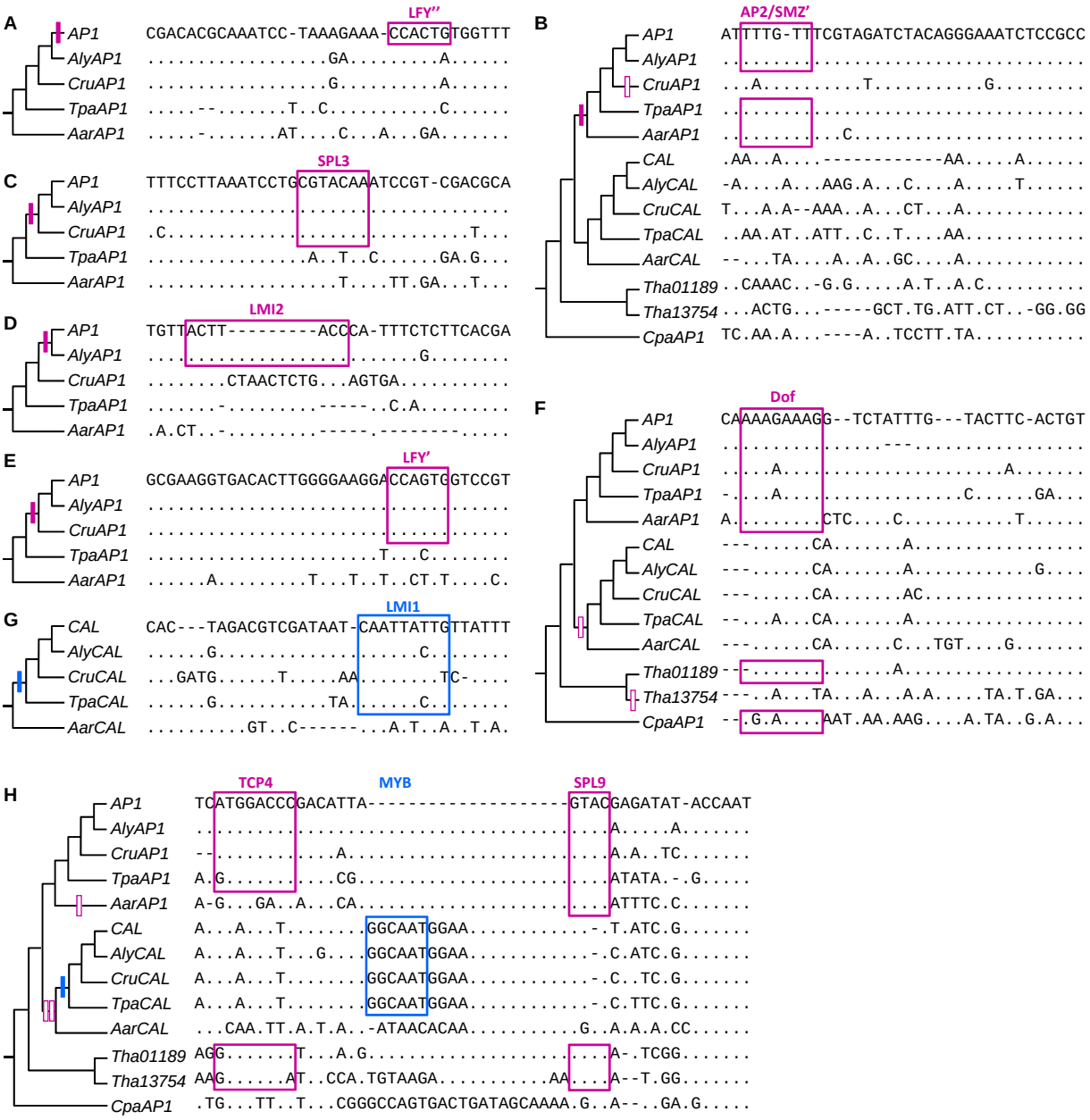
Supplemental Figure S4. The DotPlot result of genomic sequences of *AP1* (4046 bps, horizontal axis) and *CAL* (3756 bps, vertical axis). It was performed using the PipMaker program (http://pipmaker.bx.psu.edu/pipmaker/). TFBSs in black are those shared by *AP1* and *CAL*, whereas those in purple and blue are *AP1*- and *CAL*-specific, respectively. Black boxes represent exons.

Supplemental Figure S5



Supplemental Figure S5. TFBS evolution. TFBSs gained or lost during different evolutionary stages are indicated in the corresponding positions of the phylogenetic tree by filled and open boxes, respectively. The loss of the binding site of SPL3/9 (I) along the *CAL* lineage is not shown, however, because its exact position is still uncertain.

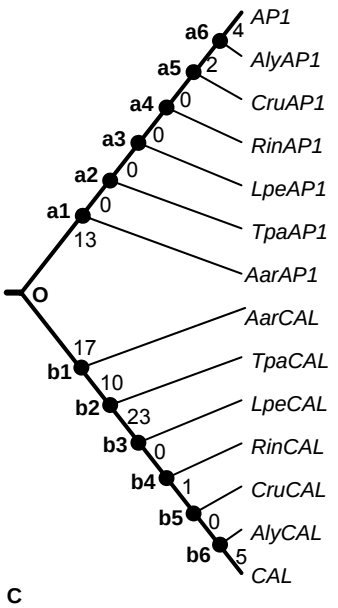
Supplemental Figure S6



Supplemental Figure S6. Alignments of representative TFBS-containing regions in a phylogenetic framework. Dots represent the same nucleotides as those in *AP1* whereas dashes indicate the alignment gaps. TFBSs gained or lost during evolution are indicated in the corresponding positions of the phylogenetic tree by filled and open boxes, respectively.

Supplemental Figure S7

A



B

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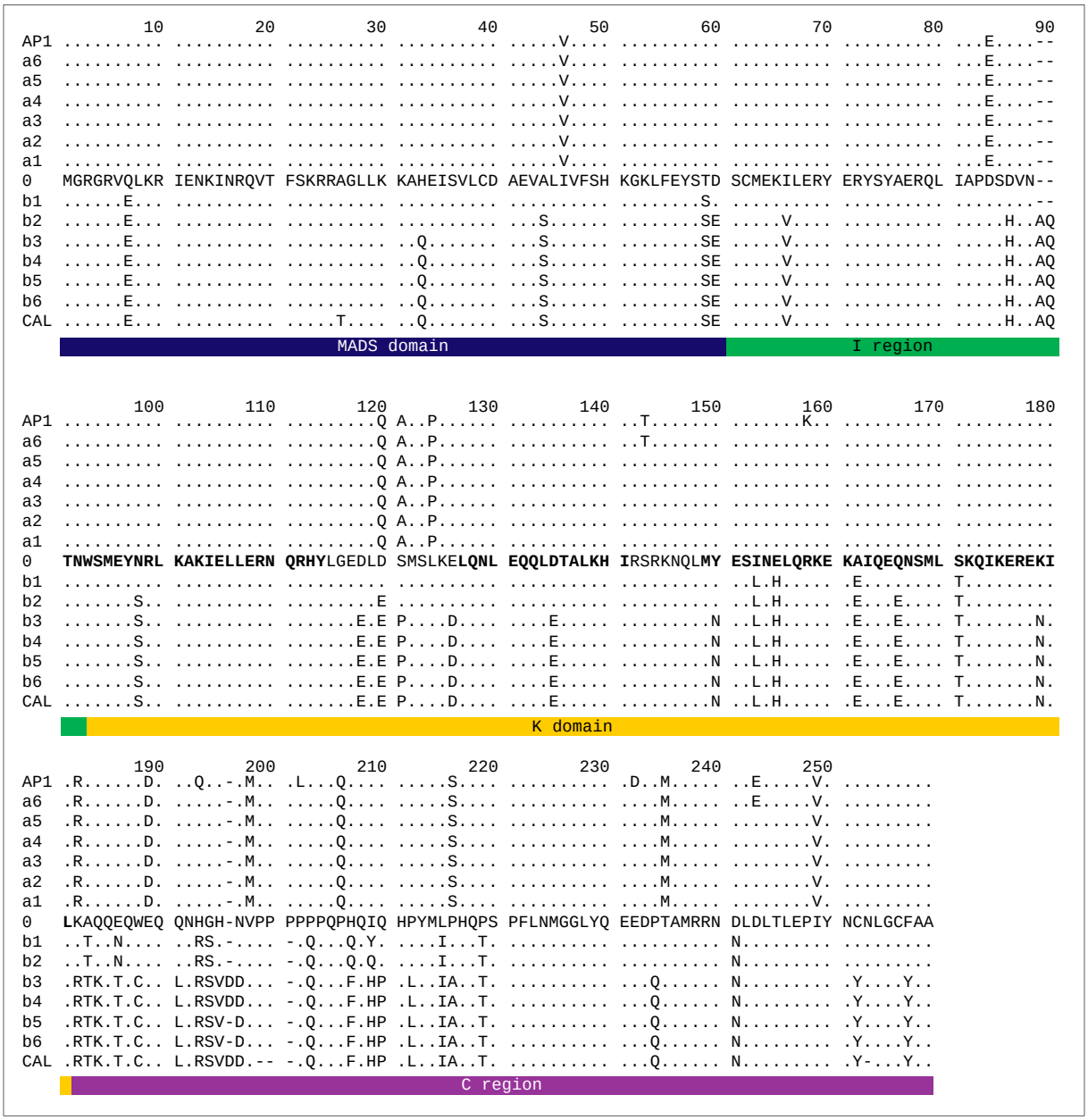
     0000000000 0001111111 1111111111 1111111111 1122222222 2222222222 222
     0234456688 8991222234 5555667788 8888999999 9900000001 1111333444 555
     7634690646 9088014653 0358261923 4689134567 8901236790 2569245139 237
AP1  ....V...E. --..QAP..T ...K....R. ...D.Q..-. M...L.Q... ..S.D.M.EV ...
a6   ....V...E. --..QAP..T ........R. ...D....-. M.....Q... ..S...M.EV ...
a5   ....V...E. --..QAP... ........R. ...D....-. M.....Q... ..S...M..V ...
a4   ....V...E. --..QAP... ........R. ...D....-. M.....Q... ..S...M..V ...
a3   ....V...E. --..QAP... ........R. ...D....-. M.....Q... ..S...M..V ...
a2   ....V...E. --..QAP... ........R. ...D....-. M.....Q... ..S...M..V ...
a1   ....V...E. --..QAP... ........R. ...D....-. M.....Q... ..S...M..V ...
O    QAHAITDIDD --NDDSLEDS YIERAQSKKA QEWEQHGH-N VPPPPPPHIQ PLPPEPTDDI CNF
b1   E....S.... .......... .LH.E.T..T .N...RS.-. ....-.Q.QY. .I.T...N.. ...
b2   E..S.SEV.H AQS.E..... .LH.EET..T .N...RS.-. ....-.Q.QQ. .I.T...N.. ...
b3   E.QS.SEV.H AQSEEP.DE. NLH.EETNRT KTC.LRSVDD ....-.Q.FHP LIAT.Q.N.. Y.Y
b4   E.QS.SEV.H AQSEEP.DE. NLH.EETNRT KTC.LRSVDD ....-.Q.FHP LIAT.Q.N.. Y.Y
b5   E.QS.SEV.H AQSEEP.DE. NLH.EETNRT KTC.LRSV-D ....-.Q.FHP LIAT.Q.N.. Y.Y
b6   E.QS.SEV.H AQSEEP.DE. NLH.EETNRT KTC.LRSV-D ....-.Q.FHP LIAT.Q.N.. Y.Y
CAL  ETQS.SEV.H AQSEEP.DE. NLH.EETNRT KTC.LRSVDD .---.Q.FHP LIAT.Q.N.. Y-Y
```

C

	a1 vs b1	a2 vs b2	a3 vs b3	a4 vs b4	a5 vs b5	a6 vs b6	AP1 vs CAL
Different sites	28	24	50	49	49	51	55
Gaps	1	3	4	4	3	3	7
Identity (%)	88.6	89.5	79.2	79.5	80.2	79.1	76.2

Supplemental Figure S7. *CAL* evolved under the less stringent constraint than *AP1*. (A) The phylogenetic tree showing the relationships of *AP1* and *CAL* genes. The gene duplication event leading to *AP1* and *CAL* lineages occurred at Node O. a1 to a6, and b1 to b6 indicate ancestral nodes. The total number of amino acid replacements and gaps between ancestral proteins are denoted beside each branch. Except for genes used for the analysis of regulatory evolution, genes from *Rorippa indica* and *Lepidium perfoliatum* are also included. (B) Amino acid residues showing differences in the alignment of ancestral and present-day AP1- and CAL-like proteins. The residue positions in the alignment are shown at the top. The amino acids are represented by single-letter codes. Dots represent the same amino acids as those of the sequences at Node o whereas dashes indicate the alignment gaps. The triangles refer to respective changes that occurred between hydrophobic and hydrophilic amino acids (pink), uncharged and charged amino acids (blue), and negatively and positively charged amino acids (orange). The nucleotides in the 5' end of exon 3 of *CAL* genes excluding *AarCAL*, which encode residues A and Q at positions 89 and 90, experienced a 6-bp exonization event. (C) Comparisons of protein sequences of ancestral paralogs with those of AP1 and CAL. Amino acid sequences at all interior nodes were inferred by using the distance-based and likelihood-based Bayesian methods, which were performed using the ANCESTOR software (Zhang and Nei, 1997) and the CODEML program in PAML 4.3 (Yang, 2007) respectively.

Supplemental Figure S8



Supplemental Figure S8. Sequence alignment of ancestral and present-day AP1- and CAL-like proteins.The MADS domain, I region, K domain, and C region are shown in blue, green, yellow and pink, respectively. The K1, K2, and K3 subdomains in the K domain, defined according to (Yang and Jack, 2004), are highlighted in bold.