Supplemental Figure 1

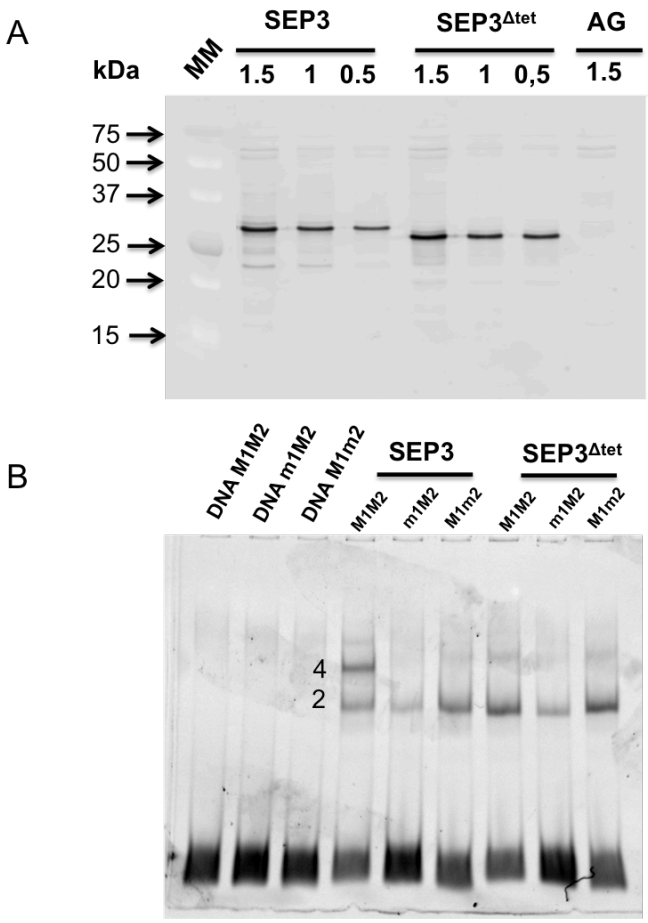

Figure S1. SEP3 and SEP3$^{\Delta tet}$ protein production and DNA binding**. (A)** Western blot analysis showing similar levels of accumulation of SEP3 and SEP$^{\Delta tet}$ after transcription/translation protein production. 1.5, 1 and 0.5 µl of protein were loaded with 1.5 µl of AGAMOUS protein as a control in the right lane. The SEP3-specific antibody recognizes a C-terminal peptide of the protein present in both SEP3 and SEP$^{\Delta tet}$ (1). AG was used as a negative control. **(B)** EMSA performed with a 103 bp fragment of the *SEP3* promoter with two CArG boxes (labeled M1 and M2) and one CArG box (labeled m1 and M2 when CArG 1 is mutated and M1 and m2 when CArG 2 is mutated, see Table S3 for sequences). 4 µl of transcription/translation protein product was used. The first 3 lanes correspond to DNA alone. Both SEP3 and SEP3$^{\Delta tet}$ dimers can bind to CArG box 1 or 2, however based on band intensity, CArG box 1 is preferred. The complex "4" appears only with SEP3 in the presence of 2 CArG boxes, highlighting the strong impairment of cooperative DNA binding and tetramer formation in SEP3$^{\Delta tet}$.

Supplemental Figure 2

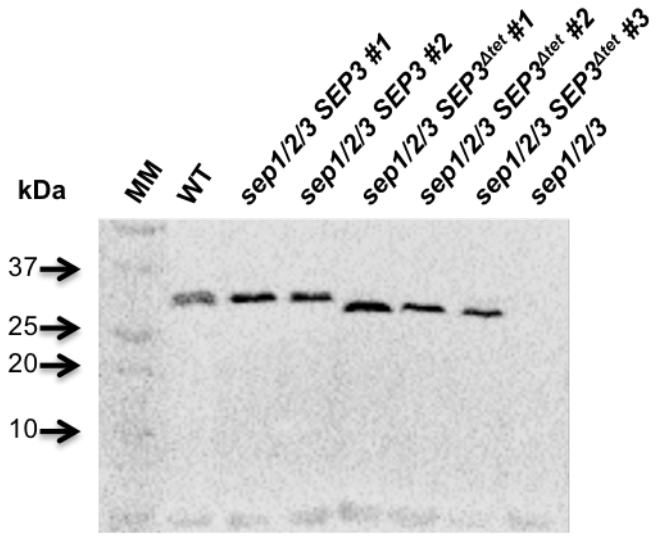

Figure S2. SEP3 and SEP3$^{\Delta tet}$ protein levels in *sep1/2/3* lines expressing either *SEP3* or *SEP3*$^{\Delta tet}$. Western blotting was performed on protein extracted from IM and young flower buds from WT, *sep1/2/3 SEP3* or *sep1/2/3 SEP3*$^{\Delta tet}$ (2 and 3 independent lines, respectively) and *sep1/2/3* triple mutant. The SEP3 antibody recognizes a region at the C-terminal part of the protein present in both isoforms, SEP3 and SEP3$^{\Delta tet}$ (1). MM: molecular markers.

Supplemental Figure 3

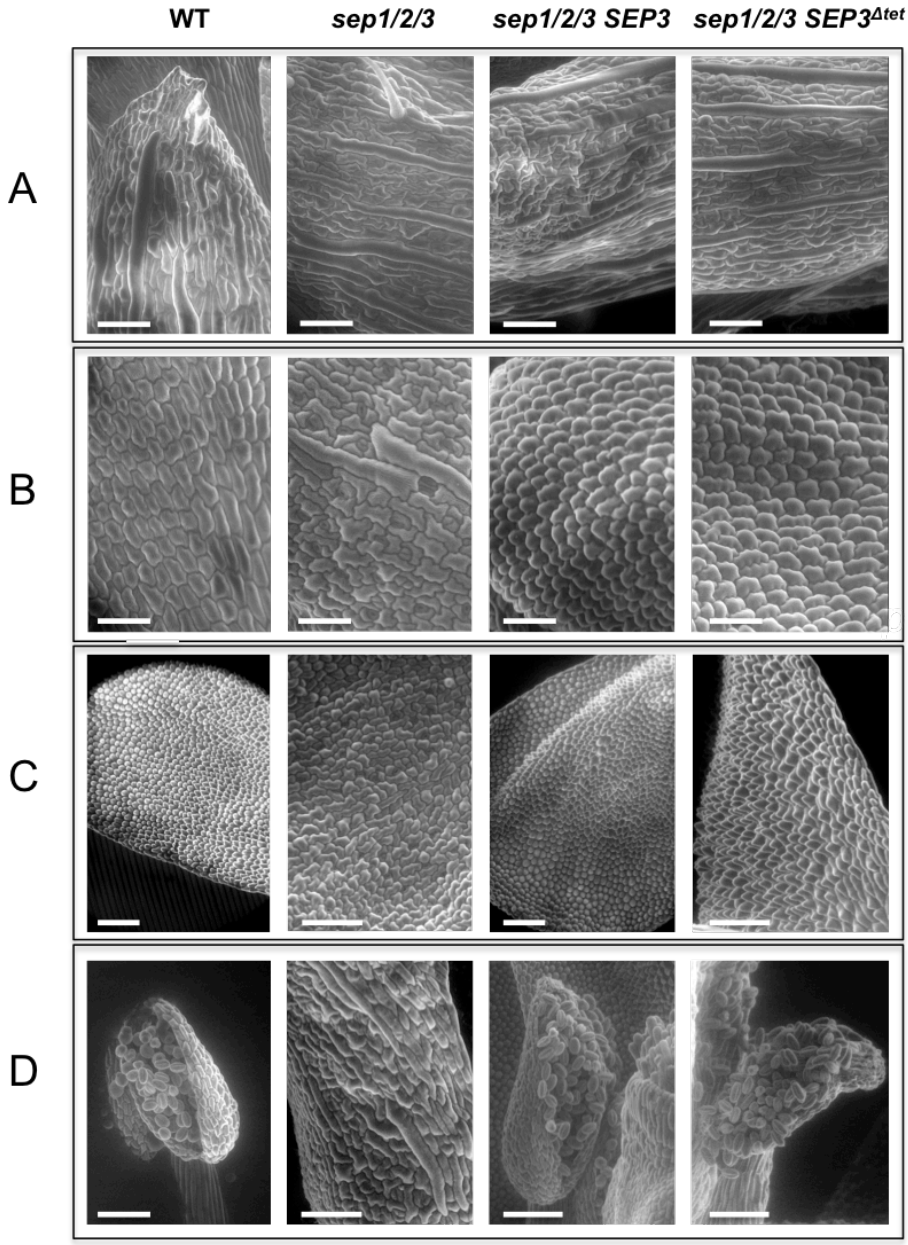

Figure S3. Scanning electron micrographs of Arabidopsis sepals, petals and stamens in WT, *sep1/2/3* and *sep1/2/3* lines expressing either *SEP3* or *SEP3*$^{\Delta tet}$. **(A)** WT sepal cell morphology with long epidermal cells and stomata is observed for all genotypes. **(B)** Arabidopsis petal abaxial surface in WT, *sep1/2/3 SEP3* or *sep1/2/3 SEP3*$^{\Delta tet}$ shows rounded cells with a typical « cobblestone » appearance. The second whorl organs in the triple mutant, *sep1/2/3,* shows sepaloid character. **(C)** Arabidopsis petal adaxial surface shows ovoid cells in WT, *sep1/2/3 SEP3* and *sep1/2/3 SEP3*$^{\Delta tet}$, but not in *sep1/2/3*. **(D)** Arabidopsis mature stamens after dehiscence show epidermal cells of uniform size with pollen grains in all genotypes except *sep1/2/3*. Scale bar indicates 100 µm in A, C, D and 50 µm in B.

Supplementary Figure 4

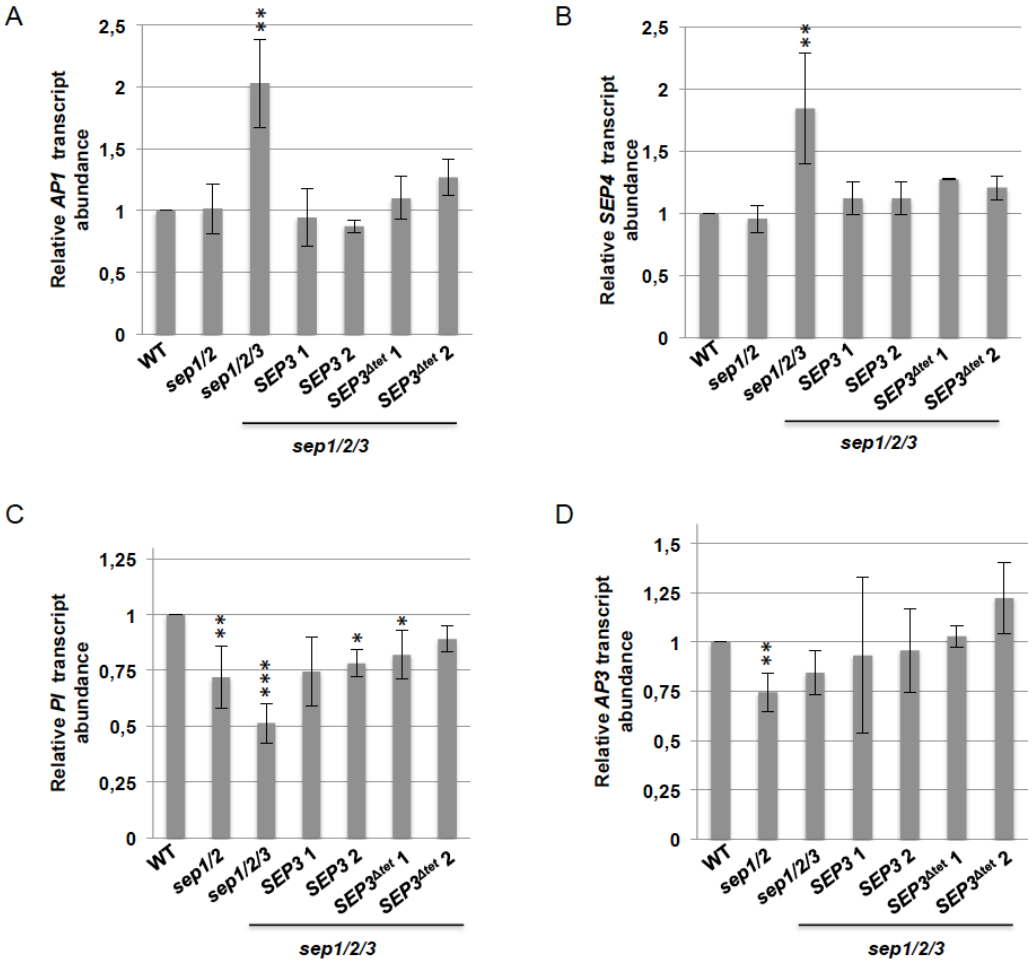

Figure S4. Expression of *AP1*, *SEP4*, *PI* and *AP3* in WT, *sep1/2* and *sep1/2/3* double and triple mutants, and *sep1/2/3* triple mutant expressing either *SEP3* or *SEP3*$^{\Delta tet}$. *AP1* **(A)**, *SEP4* **(B)**, *PI* **(C)** and *AP3* **(D)** expression levels were determined by qPCR on IMs and young flower buds up to stage 11 harvested from the different genotypes. Data correspond to the mean of 3 independent biological replicates for WT, *sep1/2* and *sep1/2/3* plants, and 2 biological replicates for *sep1/2/3 SEP3* and *sep1/2/3 SEP3*$^{\Delta tet}$ *lines*. Two independent lines were tested for *sep1/2/3 SEP3* and *sep1/2/3 SEP3*$^{\Delta tet}$. Error bars show standard deviation. WT expression was set to one. Expression was normalized to *ACTIN2* and *EF-1*α. Asterisks indicate significant differences from WT (* $P < 0.05$, ** $P < 0.01$). The *sep1/2/3* triple mutant shows a small but significant increase in *AP1* and *SEP4* expression, which correlates with the increased sepal number observed in *sep1/2/3* triple mutant plants.

Supplementary Figure 5

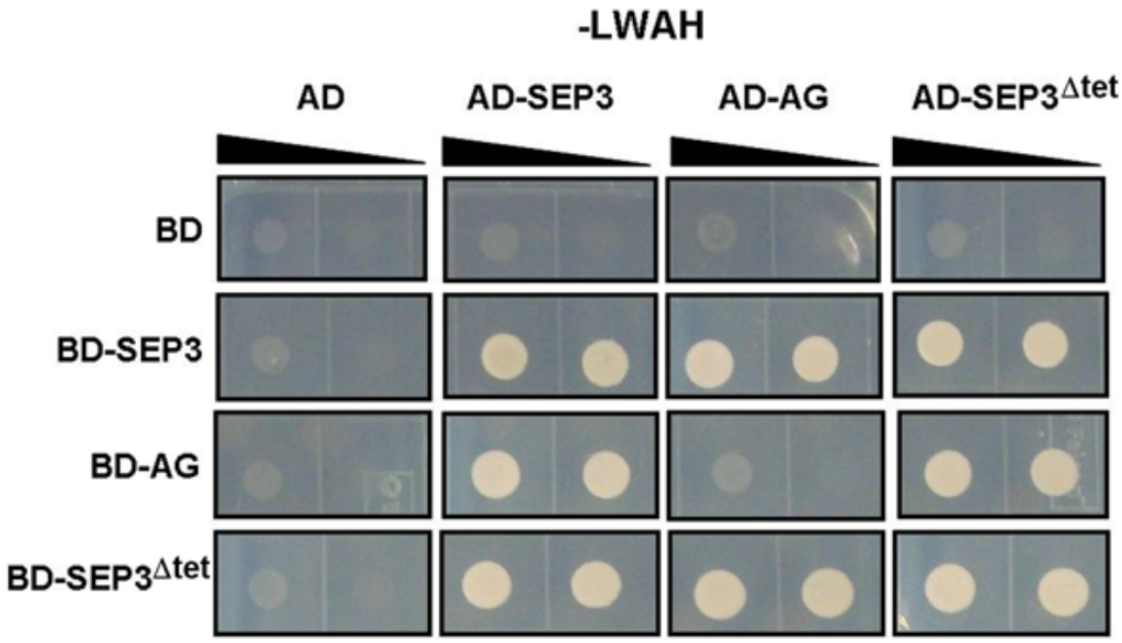

Figure S5. Yeast two hybrid interactions with AGAMOUS (AG), SEP3 and SEP3$^{\Delta tet}$. AD refers to activating domain and BD refers to DNA binding domain. Pictures shown were taken after 4 days of incubation at 30 ºC. The screening was performed in duplicate.

Supplementary Figure 6

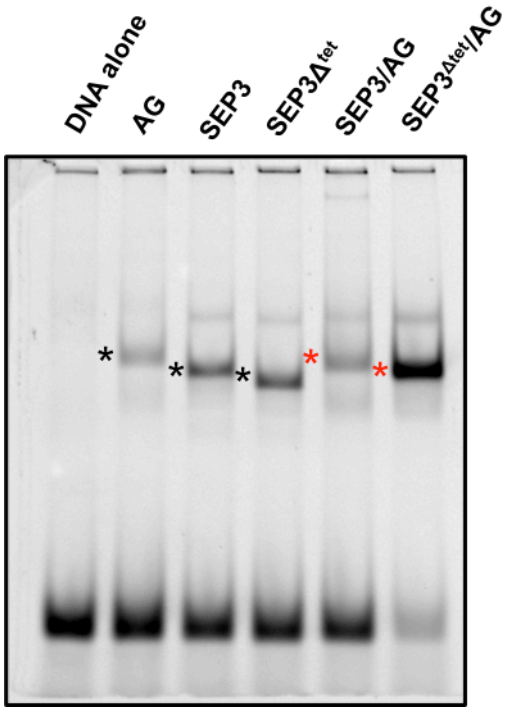

Figure S6. AG, SEP3 and SEP3$^{\Delta tet}$ bind DNA as homo- and heterodimers. EMSAs were performed using a 52 bp DNA fragment of the *SEP3* promoter with one CArG box (sequence given in Supplementary Table 3). Homodimers and heterodimers are labeled with black and red asterisks, respectively.

Supplementary Figure 7

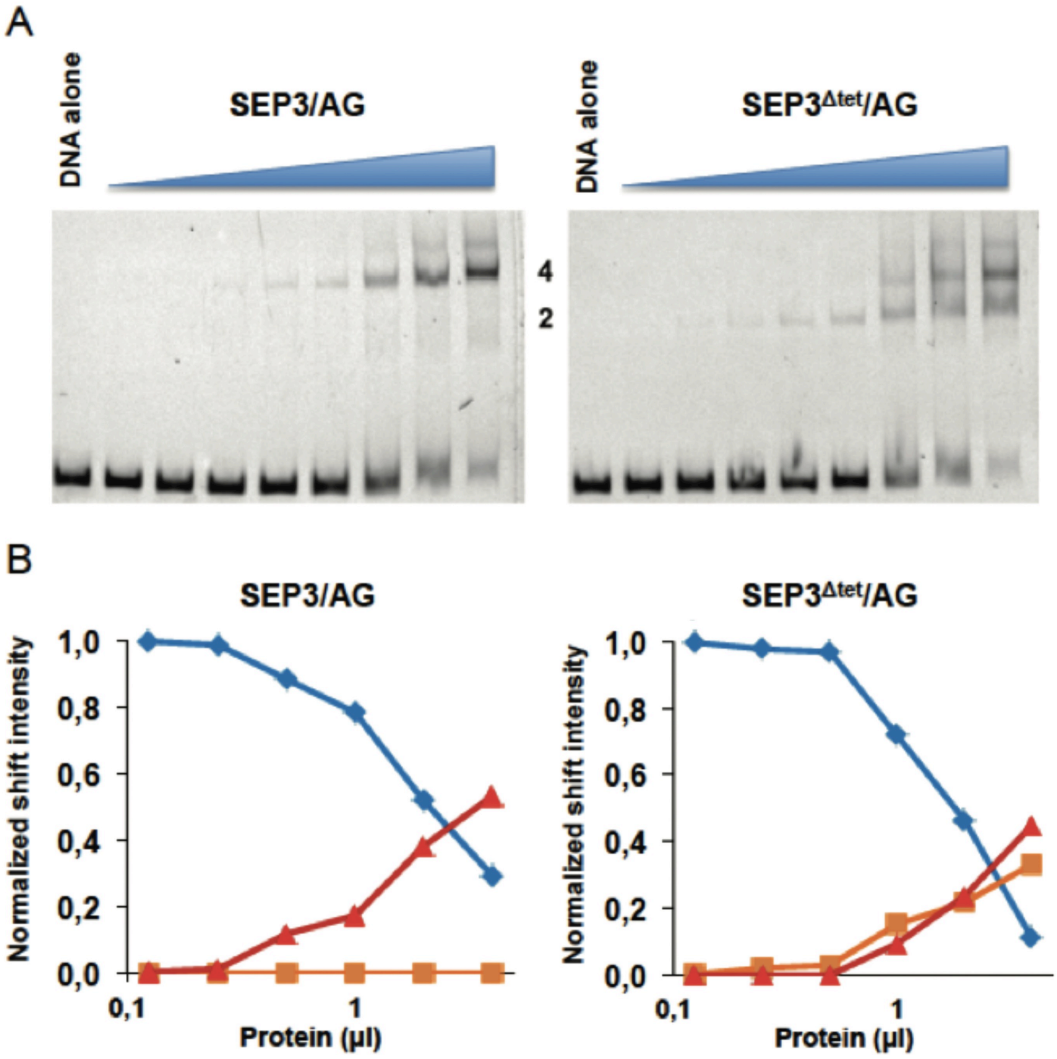

Figure S7. SEP3/AG and SEP3$^{\Delta tet}$/AG binding to DNA. **(A)** Binding of SEP3/AG and SEP3$^{\Delta tet}$/AG to 103 bp *SEP3* DNA probe containing two CArG boxes. Increasing amount of translated proteins from 0.03 µl of translation mix to 4 µl, were incubated with a constant concentration of DNA probe (7nm in the binding reaction mix). 2 and 4 indicate the number of protein monomers bound to the DNA probe. **(B)** Quantification of the signal in (A) is shown. Blue diamonds correspond to free DNA, orange squares to "2" (one dimer bound) and red triangles to "4" (two dimers bound). Bands were quantified using ImageJ. SEP3$^{\Delta tet}$/AG demonstrates reduced cooperative binding with respect to SEP3/AG as shown by the presence of a dimeric species under all protein concentrations tested.

Supplementary Figure 8

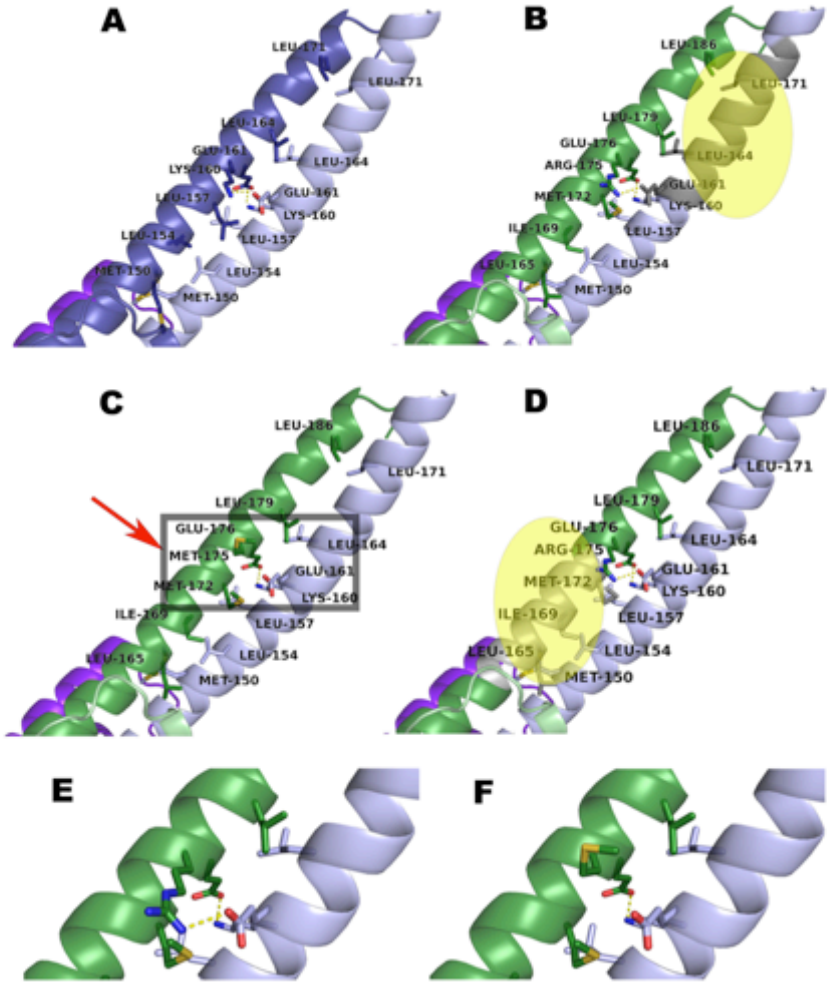

Figure S8. Homology models of SEP3/AG and SEP3$^{\Delta tet}$/AG interactions. **(A)** Close-up of the SEP3 homotetramerisation interface with one SEP3 in light blue and one in dark blue. Residues at the interface are labeled with numbering as per SEP3. **(B)** Homology model of SEP3 (light blue) and AG (green) with residues 161-174 circled in yellow. These residues are missing in SEP3$^{\Delta tet}$. **(C)** SEP3 and AG as per **B** with the region containing the AG-MET205 (R175M - SEP3 numbering) mutation boxed. **(D)** SEP3 and AG as per **B** with the region containing the *ag-4* mutation circled in yellow. **(E)** Close-up of the boxed region in **C** with R205 from AG forming a salt bridge with E161 from SEP3. **(F)** Close-up of the boxed region in **C** with the arginine to methionine mutation resulting in the loss of a salt bridge.

Table S1. Y2H interactions data between AP3, PI, SEP3, SEP3$^{\Delta tet}$, and AG.

AD / BD	SEP3	SEP$^{\Delta tet}$	AG	AP3	PI
SEP3	+	+	+	-	-
SEP$^{\Delta tet}$	+	+	+	n.t.	n.t.
AG	+	+	-	n.t.	n.t.
AP3	-	n.t.	n.t.	-	-
PI	-	n.t.	n.t.	-	-

n.t. – non-tested

+ indicates interaction, - indicates no interaction

Table S2. List of primers

Experiment	Template
I- Vector for complementation analyses	
Amplification of *SEP3* (At1g24260) 5' PROMOTER region F : CGC GGA TCC GCG GTAATTCAACATGTAGCATAGAG (BahmI site on 5') R : GTAGGTGATGGACACAAAGGTATG	Genomic DNA
Amplication of *SEP3* 3' PROMOTER and EXON1-INTRON1-EXON2 F : CATACCTTTGTGTCCATCACCTAC R : C TCT CTT GAA GGC ACA TTG GGT TCT	Genomic DNA
Amplication of *SEP3* EXON2-EXON8 F : AGA ACC CAA TGT GCC TTC AAG AGA R : CA CTT GGT CCT GCT CCC ATT CCA	*SEP3* cDNA
Amplication of *SEP3* EXON2-5,7,8 F : AGA ACC CAA TGT GCC TTC AAG AGA R : CA CTT GGT CCT GCT CCC ATT CCA	*SEP3*$^{\Delta tet}$ cDNA
Amplication of *SEP3*TERMINATER region F : TGG AAT GGG AGC AGG ACC AAG TG R : CGCGGATCCGCCATTACTATACATCAAGAGGCA (BamHI site on 5')	Genomic DNA
II- Gene expression analysis by qPCR	
***AG* (At4g18960)** F : CAACCGTTTGATTCACGGAA R : GGCGGATGAGTAATGGTGATTG	cDNA from IM and flowers buds
***AP3* (At3g54340)** F : CCAAGAAAAAGAACAAAAGTCAA R : GGTGGAAACGAAGAGCGTAA	"
***PI (*At5g20240)** F : GAGCACGCCATTGAACAT R : CTCCTCCGCCATCATCTT	"
***AP1* (At1g69120)** F : CAGCACCAAATCCAGCATC R : CGGGTTCAAGAGTCAGTTCG	"
***SEP4* (At2g03710)** F : TCCGAGATGGATGTGAATGA R : AAGCATAGACCGAGCCTTG	"
***KNU* (At5g14010)** F : TTCTTCGTCCTTACCCTTGC R : GTAGCCATCCATCGTCATCA	"
***CRC* (At1g69180)** F : CGCATCAAAAGTGCCAATCC R : CTTCTCACCGAATCCCAAGC	"
***ACTIN2* (At3g18780)** F : TGAGCAAAGAAATCACAGCACT R : CCTGGACCTGCCTCATCATAC	"
***EF-1α* (At1g07940)** F : GACAGGCGTTCTGGTAAGGAG R : GCGGAAAGAGTTTTGATGTTCA	"

III- Cloning for *in vitro* transcription translation

Primers include restriction site, and Kozak sequence for the F primer.

***AG* (At4g18960)** — cDNA in plasmid
F : ACGC CTCTAGA CC ACC ATG GCGTACCAATCGGAGCTAGGAGG
R : ACGC GG ATCC TTACACTAACTGGAGAGCGGTTTG

***SEP3* WT (At1g24260.2)** — "
F : ACGC TCTAGACC ACC ATGGGAAGAGGGAGAGTAGA
R : ACGC GGATCC TCAAATAGAGTTGGTGTCATAAGGT

***SEP3*$^{\Delta tet}$ (At1g24260.3)** — "
F : ACGC TCTAGACC ACC ATGGGAAGAGGGAGAGTAGA
R : ACGC GGATCC TCAAATAGAGTTGGTGTCATAAGGT

IV -Cloning of *SEP3*, *KNU* and *CRC* promoter regions

103 bp *SEP3* (At1g24260) promoter region (cloned in PCR blunt) — Genomic DNA
F : GACGATAACTCCATCTTTCTATTTTG
R : GAACCGTTGGATTAATCTGC

656 bp *KNU* (At5g14010) promoter region (cloned in PCR blunt) — "
F : GAGAGACAAGATTGAGAGACAAC
R : GAATCAAGTGGTGAGAGAAACTG

500 bp *CRC* (At1g69180) promoter region
(cloned in pAXE2 by Eurofins)

V -Amplification of DNA fragment for EMSA

Reverse primers were 5'Cy5 labelled (Eurofins)

103 bp *SEP3* (At1g24260) promoter region — Plasmid described in IV
F : GACGATAACTCCATCTTTCTATTTTG
R : GAACCGTTGGATTAATCTGC

52 bp *SEP3* promoter region — "
F : GACGATAACTCCATCTTTCTATTTTG
R : CGTAATGGGAAGGGGACCTCGTTAC

87 bp *KNU* (At5g14010) promoter region — "
F : GAGAAACATAGAAACCTTCCATG
R : TGTTCTTGTGATGCACAAAGAAGAC

136 bp *CRC* (At1g69180) promoter region — "
F : ATTTCTTATTAGCTCTCTTCCC
R : GACGATATCTATATCTGCATCTT

Table S3. Nucleotide sequences of the *SEPALLATA 3*, *KNUCKLES,* and *CRABS CLAW* DNA fragment used for EMSA experiments. CArG boxes are in bold.

***SEPALLATA* 3 2 CarG boxes 103 bp**

gacgataactccat**ctttctatttttgggtaac**gaggtccccttcccattacgtcttgacgtggaccctgtccg**tctatttttag**
cagattaatccaacggttc

***SEPALLATA* 3 1 CarG boxe m1M2 103 bp**

gacgataactaagtatgtatagggtttgtaacgaggtccccttcccattacgtcttgacgtggaccctgtccgt**ctatttttag**
cagattaatccaacggttc

***SEPALLATA* 3 1 CarG boxe M1m2 103 bp**

gacgataactccat**ctttctatttttgggtaac**gaggtccccttcccattacgtcttgacgtggaccctgtctctatgggtttatt
agattaatccaacggttc

***SEPALLATA* 3 1 CarG boxes 52 bp**

gacgataactccat**ctttctatttttgggtaac**gaggtccccttcccattacg

***KNUCKLES* 2 CarG boxes 87 bp**

gagaaacatagaaac**cttccatgtttggcaat**ttcatcttggaacttgattcactctc**tcttgtcttctttgtg**catcacaaaca

***CRABS CLAW* 2 CarG boxes 136 bp**

atttcttattagctct**cttccctttttttggcaat**cgtcccatctctcacagtcatagttgatagtccccaacctttttttaaaataaac
ataatttaatttcggtc**acctttttaag**atgcagatatagatatcgtc

1. Smaczniak C*, et al.* (2012) Characterization of MADS-domain transcription factor complexes in Arabidopsis flower development. *Proc Natl Acad Sci U S A* 109(5):1560-1565.