Supplementary material

Functional characterization of B class MADS-box transcription factors in *Gerbera hybrida*

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Running title: B class MADS-box transcription factors in *Gerbera*

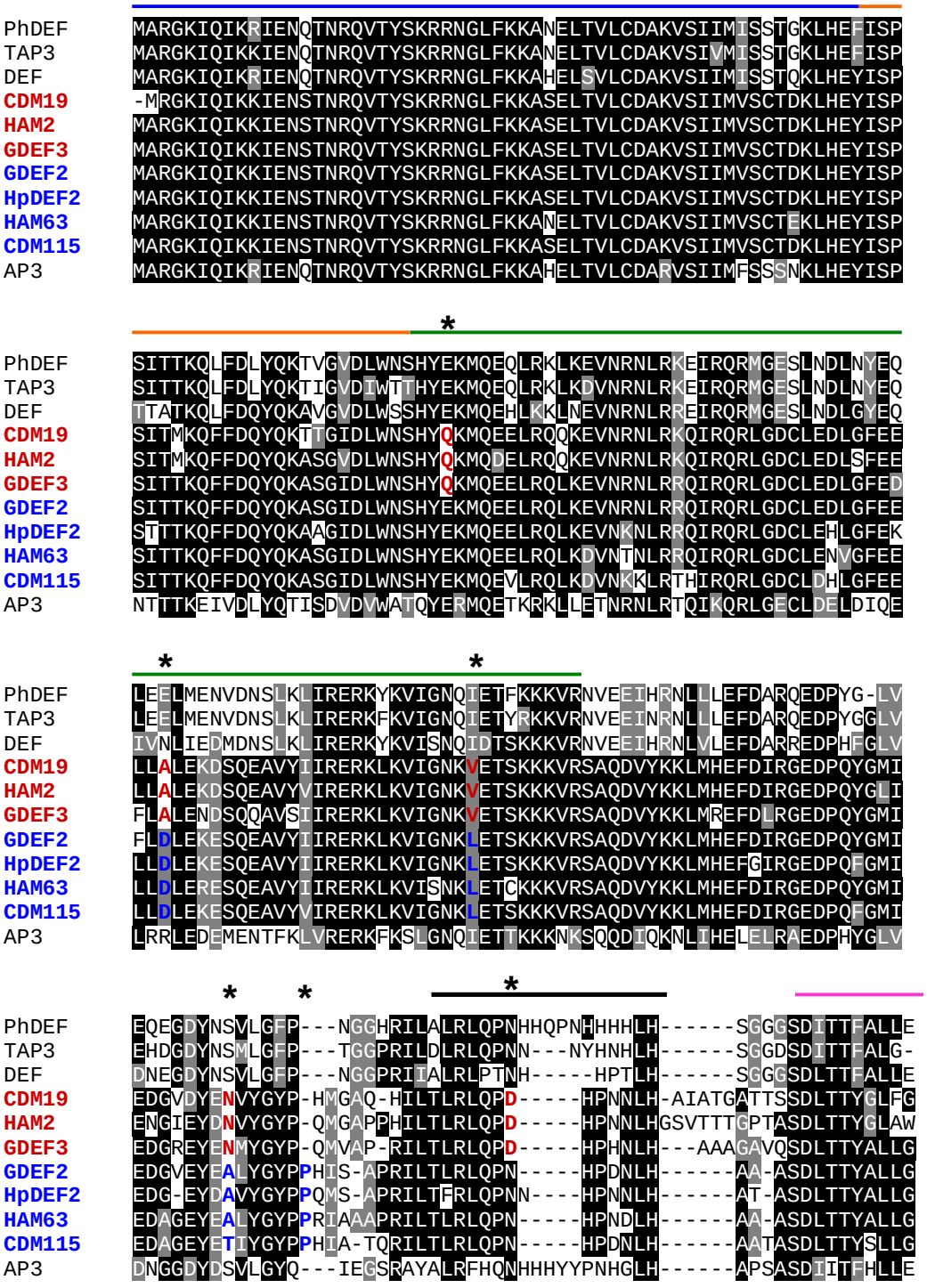

Figure S1

Amino acid alignment for a selected set of eu*AP3*-clade MADS-box genes. Amino acid changes specific to GDEF3/HAM2/CDM19 and/or GDEF2/HpDEF2/HAM63/CDM115 (coloured with red and blue, respectively), are marked with asterisk (*). Conserved domains are marked with coloured lines: MADS (blue), Intervening (orange), K (green), PI (black), and euAP3 (pink). Three diagnostic amino acid changes are found in the K domain and three in the C-terminal domain, of which one is specifically in the PI-derived domain.

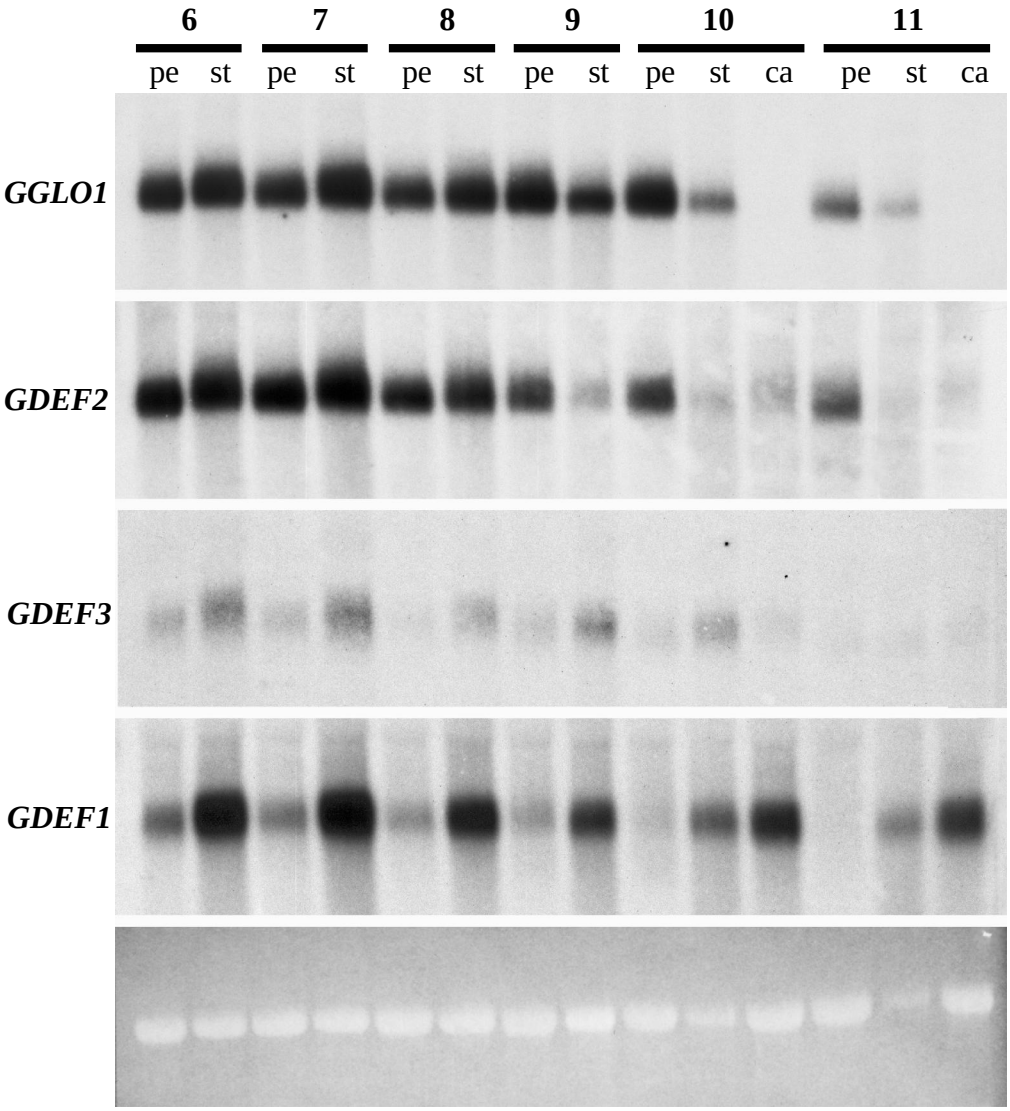

Figure S2

The expression of gerbera B class genes in disc flower petals and stamens (stages 6-11) and carpels (stages 10-11). *GGLO1* and *GDEF2* show strong and consistent expression in disc flower petals and stamens, whereas the expression of both *GDEF3* and *GDEF1* in petals diminishes earlier. At the late stage 11, *GGLO1* and *GDEF1* are the only genes detected in stamens. *GDEF1* is also the only gene showing strong expression in carpels at the stages studied (10 and 11). The lowest panel shows ethidium bromide-stained ribosomal RNA bands to control RNA loading.

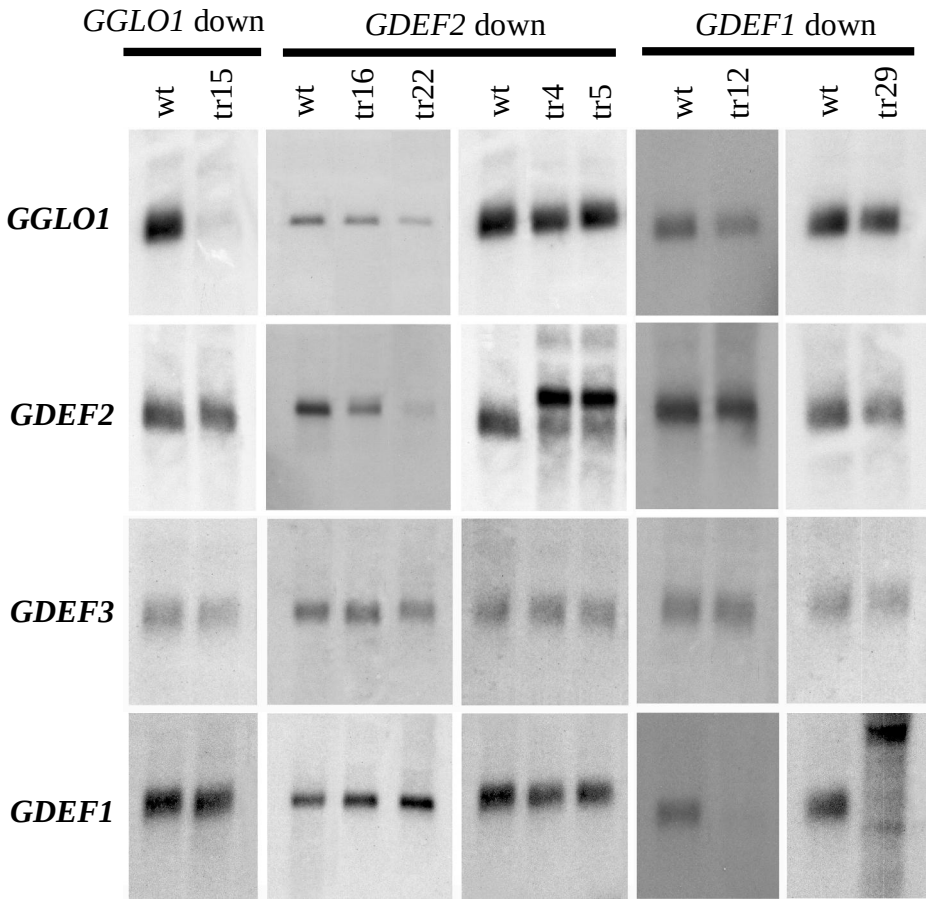

Figure S3

Expression of gerbera class B genes in young inflorescences of the transgenic lines described in this study. The *GGLO1* cosuppression line tr15 shows reduced *GGLO1* expression, whereas the level of *GDEF2*, *GDEF3* and *GDEF1* expression is similar to wild type (wt). For *GDEF2*, we obtained two cosuppression-lines (tr16 and tr22) and two antisense-lines (tr4 and tr5) with reduced *GDEF2* expression. In tr22, *GGLO1* expression is also reduced, but in the other lines the level of *GGLO1*, *GDEF3* and *GDEF1* expression is similar to wt. Two lines transformed with 35S::antisense-*GDEF1* construct show reduced *GDEF1* expression, whereas all the other gerbera B class genes are expressed like in wt.

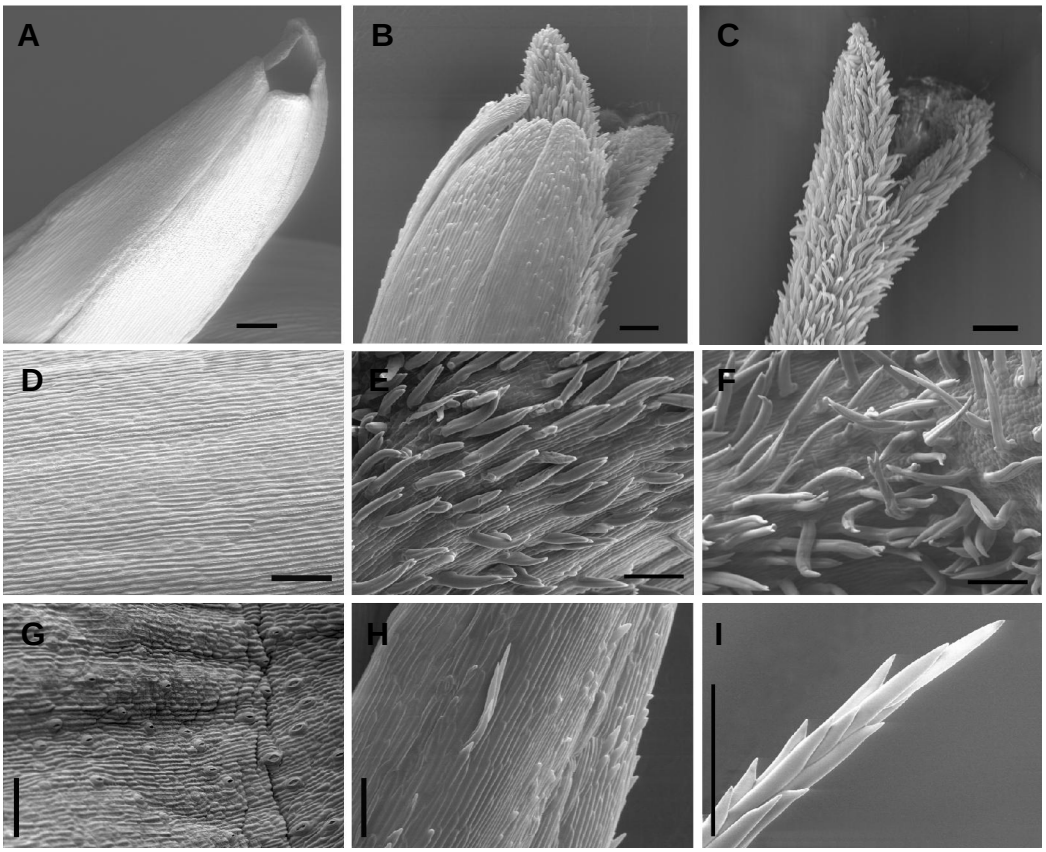

Figure S4
SEM analysis comparing the epidermal cell structure of gerbera floral organs between wild type (wt) and transgenic (tr) lines with cosuppressed *GDEF2* expression revealed similar homeotic changes in stamens and petals than in the transgenic line with reduced *GGLO1* expression. Wt stamen (A) and tr stamen (B), which resembles wt carpel (C). Wt petal tube (D) and tr petal tube (E) with trichomes similar to trichomes in the wt ovary wall (F). Wt petal ligule (G) and tr petal ligule (H) with trichomes that resemble wt whorl one pappus bristles (I). Scale bars 100 µm.

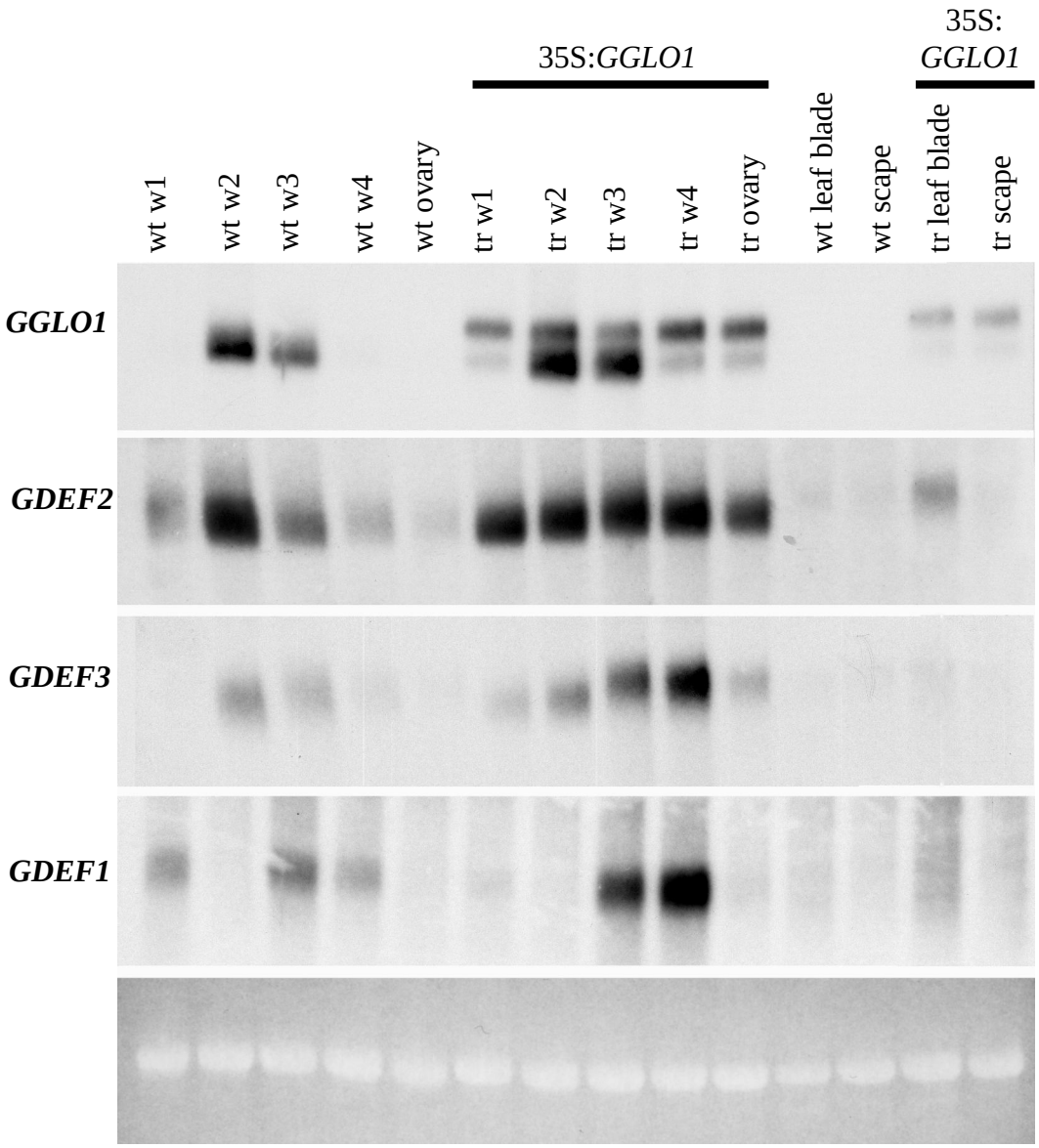

Figure S5

Expression of all the gerbera B class genes in 35S:*GGLO1* overexpression line that had petaloid organs in whorl 1 (w1) and stamenoid organs in whorl four (w4). The endogenous *GGLO1* transcripts (lower band) and the 35S promoter driven transcripts (upper band) can be distinguished. The expression of *GDEF2* is enhanced in all floral organs and in leaves. *GDEF3* expression is most strongly enhanced in w4. *GDEF1* expression is enhanced only in stamens (w3) and w4, whereas its expression is reduced in the petaloid w1 organs. The lowest panel shows ethidium bromide-stained ribosomal RNA bands to control RNA loading.

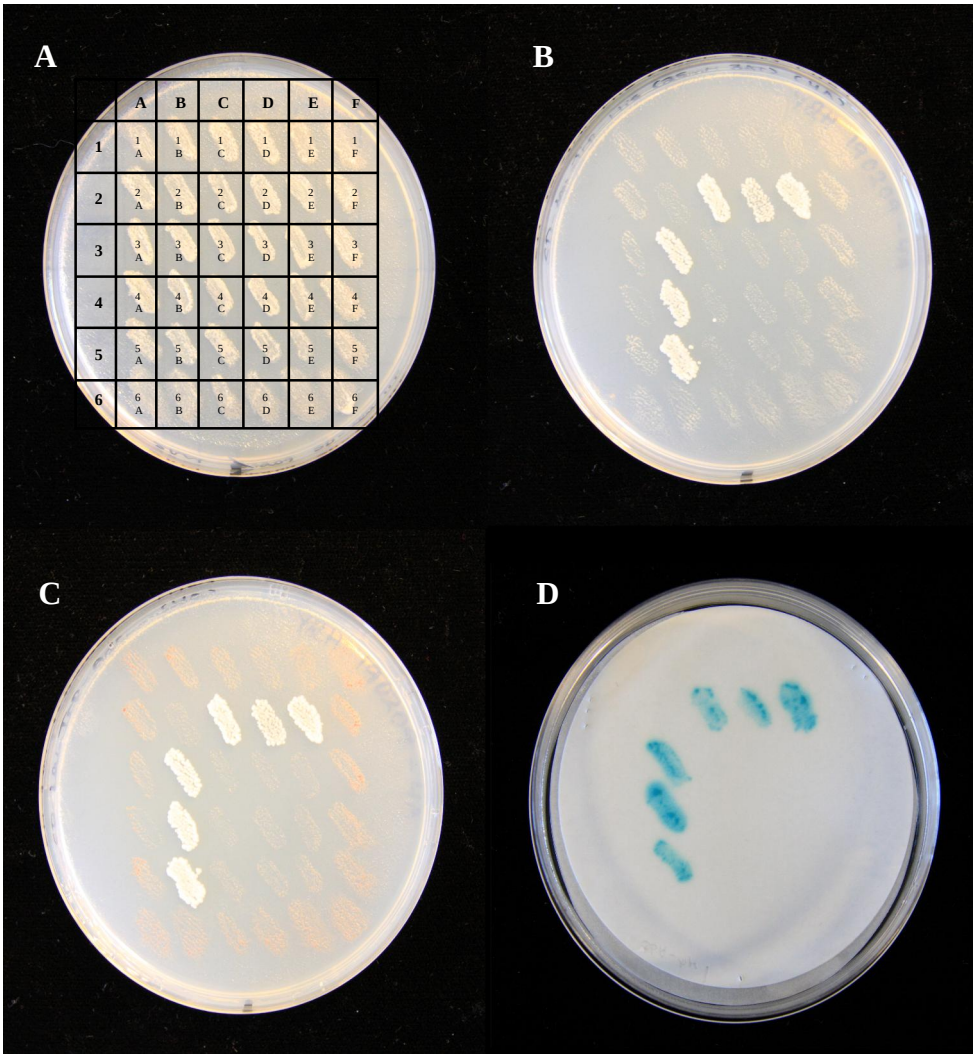

Figure S6

Yeast two-hybrid analysis of the gerbera B class MADS domain proteins. The constructs transformed in the yeast strains PJ694 α (1-6) and PJ694A (A-F) are shown in the table. Mating of these yeast strains resulted in growth on SD -Leu -Trp plates (A). Growth on selection plates SD -Leu -Trp -His +25 mM 3-AT (B) and SD -Leu -Trp -Ade (C) showed the interaction of GGLO1 protein with all the three DEF/AP3-like proteins GDEF1, GDEF2, and GDEF3. The same interactions were verified by the X-gal assay (D).

	A	B	C	D	E	F
1	1A	1B	1C	1D	1E	1F
2	2A	2B	2C	2D	2E	2F
3	3A	3B	3C	3D	3E	3F
4	4A	4B	4C	4D	4E	4F
5	5A	5B	5C	5D	5E	5F
6	6A	6B	6C	6D	6E	6F

1.	pBD32	A.	pAD22
2.	pBD32GGLO1	B.	pAD22GGLO1
3.	pBD32GDEF1	C.	pAD22GDEF1
4.	pBD32GDEF2	D.	pAD22GDEF2
5.	pBD32GDEF3	E.	pAD22GDEF3
6.	pBD32GAGA1	F.	pAD22GAGA1

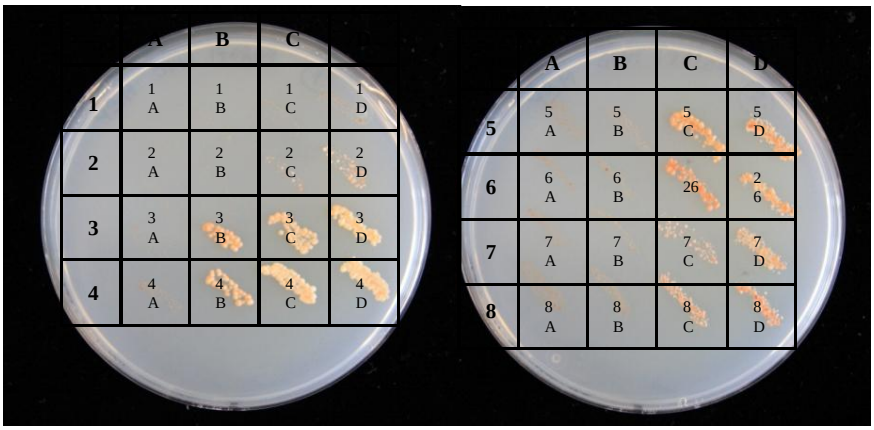

Figure S7

Yeast three hybrid (Y3H) analysis of the gerbera B class MADS domain proteins. The constructs transformed in the yeast strains PJ694A (combinations 1-8) and PJ694α (A-D) are listed below. Growth on high stringency selection plates (SD -Leu -Trp -Ura -Ade) showed that GGLO1-GDEF2 and GGLO1-GDEF3 (and GGLO1-GDEF1) heterodimers were capable to form ternary complexes with the gerbera class C proteins (GAGA1-2). Both GGLO1-GDEF2 and GGLO1-GDEF3 also formed ternary complexes with the gerbera AP1/FUL-like proteins (GSQUA1, 2, 3, 5), whereas the GGLO1-GDEF1 heterodimer did not.

1. pARC351 + pDEST22
2. pY3HGGLO1 + pDEST22
3. pY3HGGLO1 + pADGAGA1
4. pY3HGGLO1 + pADGAGA2
5. pY3HGGLO1 + pADGSQUA1
6. pY3HGGLO1 + pADGSQUA2
7. pY3HGGLO1 + pADGSQUA3
8. pY3HGGLO1 + pADGSQUA5

A. pDEST32
B. pBDGDEF1
C. pBDGDEF2
D. pBDGDEF3

The Y3H method:

The plasmid pARC351 (Gateway compatible pRED-NLSa plasmid derivative, P. Ouwerkerk; Gateway modifications by R. Immink) was used to express GGLO1 in yeast cells. The expression clones were made by recombining the previously made entry clones (as described in materials and methods) with the destination vectors according to Invitrogen's instructions. The pARC351, pDEST22 and their derivatives carrying *GGLO1* and the gerbera *AG*- and *SQUA*-like MADS box genes, respectively, were transformed into yeast strain PJ69-4A (combinations 1-8). Instead, the pDEST32 and its derivatives were transformed into yeast strain PJ69-4α (constructs A-D). To obtain yeast triple transformants, the A and α types of yeast strains were mated by pipetting them on top of each other on SD Complete plates containing all the essential amino acids. Yeast triple transformants were further plated on selection plates SD -Leu -Trp-Ura, and these colonies were replica plated on SD -Leu -Trp -Ura -Ade, essentially as described in ProQuest Two-Hybrid System Manual (Invitrogen PQ10001-01). The plates were incubated at +22 °C for 5 days.

Table S1 The B class genes used in the phylogenetic analysis (Fig. 1)

Clade	Species	Gene Name	GeneBank Accession No.
Asterids			
Asteraceae	*Gerbera hybrida*	GDEF1	AJ009724
		GDEF2	AJ009725
		GDEF3	FJ817421
		GGLO1	AJ009726
	Helianthus annuus	HAM2	EF612597
		HAM31	AY173069
		HAM63	EF612598
		HAM91	AY173070
		HaPI	AY157725
	Dendranthema grandiflorum	CDM19	AY173064
		CDM86	AY173061
		CDM115	AY173060
	Hieracium piloselloides	HpDEF2	AF180365
Plantaginaceae	*Antirrhinum majus*	DEF	X52023
		GLO	X68831
Solanaceae	*Petunia hybrida*	PhDEF	AY205603
		PhTM6	AY532264
		PhGLO1	AAS46018
		PhGLO2	CAA49568
	Solanum lycopersicum	TAP3	DQ674532
		TM6	X60759
		LePI	SGN-U324841
		TPI	DQ674531
Rosids			
Brassicaceae	*Arabidopsis thaliana*	AP3	AF115814
		PI	AF115815
Rosaceae	*Rosa rugosa*	MASAKOeuB3	AB099875
		MASAKOB3	AB055966
		MASAKOBP	AB038462
	Malus domestica	MdTM6	AB081093
		MdMADS13	AJ251116
Vitaceae	*Vitis vinifera*	VvAP3	EF418603
		VvTM6	DQ979341
		VvPI	DQ988043

Table S2 Primer sequences of gerbera B class MADS-box genes used for Gateway (Invitrogen) conversion. The Gateway derived part of primer was added to the 5' end of gene specific primer sequence.

Gene	Forward primer 5'	Reverse primer 3'
GGLO1	TCATGGGGAGAGGAAAGATA	TTACATCCTCTCATGCAA
GDEF1	TCATGGGGAGGGGGAAGATA	TCAATTAGTGTGTTGATGATCATGGAG
GDEF2	TCATGGCGAGAGGAAAGATC	CTAGCCAAGCAAAGCATA
GDEF3	CAATGGCGAGAGGGAAGATC	GTTAGCCAAGCAAAGCATAT
GAGA1	TCATGGAAAATTCTGATGTGCTTGAGC	TTACACTAACTGGAGCGG
Gateway sequence	GGGGACAAGTTTGTACAAAAAAGCAGGCT	GGGGACCACTTTGTACAAGAAAGCTGGGT