iScience, Volume 27

Supplemental information

A reduced vernalization requirement is a key component of the early-bolting trait in globe artichoke (*Cynara cardunculus* var. *scolymus*) Rick Berentsen, Reyes Benlloch, Peter Visser, Francisco Madueño, and Vicente Balanzà 1 Supplementary Figures



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4 Supplementary Figure S1: Schematic representation and photographic illustration of experimental 5 conditions. Related to STAR METHODS (Experimental design and treatment). A. Schematic 6 representation of the experiment. 1 = access door in outer wall, 2 = cold compartment (vernalization / 7 control) (blue fill), 3 = double wall separating heated and non-heated sectors, 4 = heater, 5 = heated 8 compartment (red fill), 6 = border rows (grey), 7 = inner compartment wall, 8 = viewpoint for the 9 photograph below. B. Photograph of the heated compartment from the viewpoint indicated by marker 10 (8). The inner compartment wall facing the camera has been raised while the lateral inner wall and outer 11 wall are still lowered for comparison. In the back of the image is the double wall separating the sectors, 12 behind which is the cold compartment. Yellow bands along the outer wall serve as pest control. The table

13 and tools illustrate the scale of the image.



15 Supplementary Figure S2: Accumulation and distribution of hours < 10°C. Related to Figure 2. A

- 16 Accumulated numbers of hours <10°C for each season in the no-vernalization and vernalization
- 17 compartments. **B** Number of hours <10°C for each individual day. Data from three seasons.



- 19 Supplementary Figure S3: ML cladograms of Type I and Type II MADS box genes. Related to STAR
- 20 METHODS (Identification of MADS box genes in globe artichoke). A Type I MADS box genes from
- 21 globe artichoke and Arabidopsis. Red tip names indicate ungrouped genes. **B** Type II MADS box genes
- 22 from globe artichoke (Cc), Arabidopsis (At), tomato (SI) and safflower (Ct). Green strips annotate
- 23 subclades for both Type I and Type II cladograms, with the AP1 and SOC1 subclades highlighted in
- 24 blue.



26 Supplementary Figure S4: Protein sequence alignments for SOC1, AP1 and FUL subfamilies. Related

- to Figure 4. Genes used in the phylogenetic study. A SOC1 subfamily, TM3/SOC1 motifs marked with a
 cyan box. B AP1 subfamily. Red box denotes the euAP1 acidic domain and the blue box the farnesylation
- 29 motif. C FUL subfamily. The euFUL/paleoAP1 domain is marked by a yellow box.

30 Supplementary tables

- 31 Supplementary Table S1: Comparison between phenological stages around the time of floral transition
- 32 and the stages added to describe morphological changes. Related to Figure 1. Grey fill accentuates
- the positioning of the added bolting stages in relation to the existing scales.

phenological stages according to BBCH scale phenological stages according to (Foury, 1967) and (Archontoulis et al., 2009) (Pesce and Mauromicale, 2019) (modified)				phenological stages a	added to describe morphological changes at the microscopic level
stage	stage	description		developmental stage	description
rincipal growth stage 4: development of vegetative lant parts (Codes 41-49)	bolting stage 0	No signs of bolting on macroscopic scale (if no dissection of apex)		pre-bolting stage 0	Apex round, later flat at dissection.
	bolting stage R	caulinar apex in transition from the vegetative to reproductive phase		pre-bolting stage 1	Apex assuming a pointed shape at dissection.
incipal growth stage 5: inflorescence emergence ad development, code 501	bolting stage A	Primary inflorescence perceptable by palpation		pre-bolting stage 2	Primordial primary head visible to the naked eye at dissection of apex
rincipal growth stage 5: inflorescence emergence nd development, code 501	bolting stage B	Top of primary inflorescence visible in center of the rosette		pre-bolting stage 3	Primary head developing, height ±1 cm at dissection of apex.
rincipal growth stage 5: inflorescence emergence nd development, codes 501-503	bolting stage C	Primary inflorescence more than 10 cm above center of rosette.		pre-bolting stage 4	Primary head fully developed, seconday primoridia heads visible to naked eye at dissection. First inflorescence stem elongation.

Supplementary Table S2: Environmental data calculated and collected during the experiments.Related to Table 1.

			da	ily mean ('	°C)	accumulated hours
season	treatment	n_days	min	mean	max	< 10°C
2019-2020	no vernalization	201	19.1	14.4	28.3	5
2020-2021	no vernalization	201	18.1	13.4	27.4	62
2021-2022	no vernalization	195	17.6	13.6	26.2	69
2019-2020	vernalization	201	16.7	10.6	27.5	698
2020-2021	vernalization	201	16.1	9.9	26.8	837
2021-2022	vernalization	169	15.8	9.9	27.5	832

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39 Supplementary Table S3: Wald tests for non-zero predictors. Related to Figure 2. Wald tests for models

- 40 per genotype (genotypes c1, c70, c20, and c154) and per category (early-bolting genotypes c1 and c70
- 41 vs late-bolting genotypes c20 and c154).
- 42

model ^a	source	Df ^b	denDF ^c	F.inc ^d	Pr ^e
per genotype	(Intercept)	1	100.6	94810	1.85E-151
	genotype	6	107.3	615.1	1.16E-80
	treatment	1	99.8	271.8	3.01E-30
	season	2	51.6	1.537	2.25E-01
	genotype:treatment	6	106.8	6.383	9.09E-06
	season:treatment	2	52.5	7.253	1.66E-03
per category	(Intercept)	1	154.7	24030	1.35E-171
	earliness	1	147.7	718.6	1.29E-58
	treatment	1	154.6	48.21	9.95E-11
	season	2	74.2	0.3074	7.36E-01
	earliness:treatment	1	146.9	14.47	2.08E-04
	treatment:season	2	74.1	6.669	2.17E-03

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^a Model used: "per genotype", each genotype, "per category", early-bolting vs late-bolting genotypes

45 ^b Df: Degrees of freedom

46 ^c denDF: denominator degrees of freedom

47 ^d F.inc: F-statistic value

48 ^e Pr = p-value

50 Supplementary Table S4: MADS box genes from globe artichoke. Related to STAR METHODS

51 (Identification of MADS box genes in globe artichoke).

								Classific	ation
Name	Gene	GenelD	Length (a.a.)	Domains	Genomic position	CDS length	Exons	Group	Subfamily
	Gene	Genero	Lengui (a.a.)	Domains		CDO lengui	LAUIIS	Gloup	Sublaining
CCMADS1		V2_01g005930.1	682	SRF, SRF, SRF, SRF	Chr_01:6563154-6579934	2049	4	Μα	-
CcMADS2		V2_01g005940.1	755	SRF, SRF	Chr 01:6592245-6612897	2268	13	Μα	-
CcMADS3		V2_01a005950_1	470	SRE SRE	Cbr 01:6636827-6645096	1413	5	Μα	
CaMADC4		V2_01g0000000.1	404		Chr 01/6642226 6644702	FFF	4	Ma	
CCMAD54		vz_01g005960.1	104	SKF	CIII_01.0043230-0044793	555	4	IVIG	-
CcMADS5		V2_01g005970.1	196	SRF	Chr_01:6645121-6645711	591	1	Μα	-
CcMADS6		V2 01g005990.1	89	SRF	Chr 01:6659102-6659371	270	1	Μα	-
CcMADS7		V2_01c006010_1	210	SPE	Cbr 01:6672187-6672840	663	1	Ma	-
CONADO1		V2_01g000010.1	213	005	01-01-0072107-0072045	003		NG NG	
CCMAD58		V2_01g006020.1	267	SRF	Chr_01:6673382-6674418	804	2	Μα	-
CcMADS9		V2_01g009350.1	233	SRF	Chr_01:10447026-10447727	702	1	Μα	-
CcMADS10		V2_01q010600_1	126	SRF	Chr 01:11863297-11864466	381	2	MKCc	FLC
CoMADS11		V2_01g012420.1	100	SPE K box	Chr 01:12776559 12792794	572	5	MKCC	SED
COMADSTI		v2_01g012420.1	190	SKF, K-DUX	CIII_01.13770336-13782784	573	5	WIRC	3EF
CcMADS12		V2_01g012430.1	189	SRF, K-box	Chr_01:13817078-13825390	570	6	MIKC [©]	AP1
CcMADS13		V2 01g016650.1	171	SRF	Chr 01:19062272-19066258	516	5	MIKC ^C	SVP
CcMADS14		V2_01c026170_1	199	SRF	Cbr 01:49764431-49765030	600	1	Μα	
CaMADO14	CoSOC 11 like C	V2_01g020110.1	144	OPE	Chr 02:0005755 0004272	244		MIKOC	6001
CCMADS15	CCSOC ILIKE-C	V2_02g007180.1	111	SRF	Chr_02:8995755-9004272	344	2	MIKC	5001
CcMADS16		V2_02g007200.1	198	SRF, K-box	Chr_02:9035643-9049722	597	6	MIKC	AGL17
CcMADS17		V2_02q009710.1	127	SRF	Chr 02:14659672-14660193	384	2	Ma	-
CoMADS19		V2_02c000720_1	170	Khox	Chr 02:17606957 17702624	515	-	MIKC SPE incomplete	ACI 17
COMAD3 18		vz_03g009720.1	170	K-DUX	011_03.17090837-17702024	515	0	WIRC, SRF Incomplete	AGEIT
CcMADS19		V2_03g010800.1	239	K-box	Chr_03:32010378-32016725	720	6	MIKC,SRF incomplete	AG/STK
CcMADS20		V2 03g010810.1	78	SRF	Chr 03:32026348-32027307	237	2	MIKC ^C	AG/STK
CcMADS21		V2_03c011880_1	176	SPE	Cbr 03:46733084-46730542	531	6	MKCC	B-eistor
0.140.000		V2_00g011000.1	000	ODE Khan	01=00.50030404 50000300	001	7	MIKO	
CCMAD522		V2_03g015300.1	229	SRF, K-DOX	Chr_03:59976194-59980703	690	/	MIKC	AP3 (DEF/GLO)
CcMADS23	CcFULLike-B	V2_03g015980.1	205	K-box	Chr_03:61174353-61180637	816	9	MIKC,SRF incomplete	AP1
CcMADS24		V2 03g015990.1	75	SRF	Chr 03:61198079-61198306	228	1	MIKC	AP1
CcMADS25		V2_03d016560_1	252	SRE K-hov	Chr. 03:61999323.62016272	750	11	MKCC	SVP
O MAD 323		V2_03010300.1	202	K have	01=00.01000007.010072	139		MIKO ODE	THO
CcMADS26		V2_03g018790.1	115	K-box	Cnr_03:64996337-64997256	348	5	MIKC,SRF incomplete	IM8
CcMADS27		V2_04g002460.1	107	SRF	Chr_04:2743621-2750447	658	7	MIKC	SEP
CcMADS28		V2_04q006020_1	103	K-box	Chr 04:7740034-7740917	312	3	MIKC.SRF incomplete	AP1
Callado		V2_04g006020.1	74	CDE	Chr. 04:7759599 7759990	205	4	MICC ^C	AD1
CCMADS29		v2_04g006030.1	74	SRF	011_04.//50582-//58806	225	1	WIR C	
CcMADS30		V2_04g006040.1	248	SRF, K-box	Chr_04:7777716-7780938	765	8	MIKC	SEP
CcMADS31		V2 04q007630.1	193	SRF	Chr 04:10649060-10649641	582	1	Μα	-
CcMADS32		V2_04c007790_1	170	SPE K-box	Cbr 04:11004347-11005508	513	4	MKCC	
CONADOSZ		V2_049007730.1	170		01 04.11034347-11033500	515	-	WINCO .	TI(DEI/GEO)
CCMADS33		V2_04g008310.1	357	SRF	Chr_04:12663996-12673567	1321	6	Μα	-
CcMADS34		V2 04g008320.1	292	SRF	Chr 04:12664285-12671674	879	4	Μα	-
CcMADS35		V2_04q008330_1	607	SRF	Chr 04.12700422-12716552	1824	6	Μα	-
CoMADS26		V2_04g008240.1	102	CDE	Chr 04:12717400 12719071	5021	1	Ma	
CCMAD536		vz_04g006340.1	193	SKF	CIII_04.12/1/490-12/180/1	502		IVIG	-
CcMADS37		V2_04g008350.1	313	SRF	Chr_04:12736542-12748071	942	3	Μα	-
CcMADS38		V2 04q008360.1	120	SRF	Chr 04:12745559-12746192	634	1	Μα	-
CcMADS39		V2_05c000550_1	220	SPE K-box	Cbr 05:16157678-16162664	663	7	MKCC	SV/P
CONADO35		V2_00g0000000.1	220		01-05-50040400 50040040	400	6	MIKO	
CCMADS40		V2_05g014720.1	139	SRF	Chr_05:56612198-56616210	420	3	MIKC	AP3 (DEF/GLO)
CcMADS41		V2_05g016030.1	137	SRF, K-box	Chr_05:58719144-58728679	414	5	MIKC	AGL17
CcMADS42	CcFULLike-A	V2_05q017670_1	128	SRF	Chr 05:61226372-61236507	387	4	MKCc	AP1
CoMADS42		V2_05g010120_1	427	CDE	Chr. 05:62006209 62007521	1214	4	MP	
CCIVIAD343		v2_05g019120.1	437	SKF	CIII_03.03000208-03007321	1314		NID	
CcMADS44		V2_06g000670.1	200	SRF	Chr_06:2542888-2546558	603	6	MIKC*/Mð	MIKC*
CcMADS45		V2 06g002800.1	232	SRF, K-box	Chr 06:10366962-10371671	699	7	MIKC	AP3 (DEF/GLO)
CcMADS46		V2_06q003260 1	241	SRF	Cbr 06:11217724-11221447	726	2	Μα	
0.144.0047		V2_009000200.1	400	005	01-00-44004457 44004770	120	-	Ma	
CCMADS47		V2_06g003270.1	106	SRF	Chr_06:11221157-11221776	620	1	Μα	-
CcMADS48		V2_06g003320.1	127	K-box	Chr_06:11289297-11291933	384	5	MIKC,SRF incomplete	AP1
CcMADS49		V2_06q003330.1	78	SRF	Chr 06:11303009-11303245	237	1	MIKC	AP1
CcMADS50		V2_06q007930_1	365	SDE	Cbr 06:17105260-17109803	1008	10	MKC*/MA	MKC*
COMADOSO		V2_00g007330.1	505		01 00.17103200-17103003	1030	10		NIICO
CCMADS51		V2_06g008360.1	325	SRF, K-box	Chr_06:18606119-18608510	1036	1	MIKC	B-sister
CcMADS52		V2_06g010180.1	453	SRF, K-box	Chr 06:23081934-23106721	1370	14	MIKC	AGL17
CcMADS53		V2 06q010380.1	174	SRF, K-box	Chr 06:23474053-23479848	525	3	MIKC ^C	SVP
CcMADS54		1/2 070002680 1	1/2	SPE K-box	Cbr 07:2665138-2660960	120	4	MKCC	SED
CUNAD334		V2_079002000.1	142	0111,11-003	011_07.2003130-2003009	423	4	NIIKO NIIKO	
CcMADS55		V2_07g004870.1	123	SRF	Cnr_07:5959657-5962387	372	3	MIKC	AGL17
CcMADS56		V2_08g005070.1	93	SRF	Chr_08:6179130-6180671	282	2	MIKC	AGL17
CcMADS57		V2_08q005080_1	191	K-box	Chr 08:6193002-6199425	576	7	MIKC ^C	AGL17
Callabore		1/2 08/00/0000 4	200	CDE	Chr. 09:9157222 0150120	1000		My	
COMADS58		v∠_uoguubbuu.1	333	ORF IV	011_00.0107232-8158233	1002	1	IVIY	-
CcMADS59		V2_09g000690.1	234	SKF, K-DOX	Cnr_09:1966147-1970271	705	6	MIKC ⁻	AGL15
CcMADS60		V2_10g005330.1	236	SRF	Chr_10:5839834-5840544	711	1	Μα	-
CcMADS61		V2 10g008150.1	241	SRF, SRF	Chr 10:9337223-9338186	792	2	Μα	-
CcMADS62		V/2 10d000200 1	232	SRE	Chr 10:11534672-11535270	600	1	Ma	
COMADOUZ		V2_100000200.1	202	ODE	Ohr 10:11:00+072-11:00:07/0	474	-	MILCOU	Disister
CCMADS63		v2_10g010/10.1	156	SKF	CIII_10:14465025-14466948	4/1	5	WIRC	D-SISTER
CcMADS64		V2_10g010730.1	198	SRF, K-box	Chr_10:14543135-14547905	597	6	MIKC	AGL15
CcMADS65		V2 12g000460 1	318	SRF	Chr 12:433937-449367	957	10	MIKC	FLC
CoMADSee	CcSOC1h	1/2 12007100 1	210	SPE K-boy	Chr 12:0071115 0075040	657	7	MKCC	SOC1
CUMADSOD	0000010	V2_12007190.1	210	ONF, R-DUX	011 12.3071113-3073349	037	1	NIII CO	0001
CcMADS67		V2_12g009260.1	211	SRF, K-box	Cnr_12:12648980-12655523	692	6	MIKC	AG/STK
CcMADS68	CcSOC1Like-B	V2_13g009180.1	192	SRF, K-box	Chr 13:28823008-28829911	579	6	MIKC	SOC1
CcMADS69		V2 13q015600 1	137	SRE	Chr 13:38196053-38199798	414	3	MIKCC	SEP
Collabora	(CoAD1)	1/2 12:0015000.1	170	Khov	Chr 12:20260064 20272474	E10	7	MIKC SDE incomplete	AD1
CCMADS/0	(CCAPT)	V2_13g015610.1	172	N-DOX	CIII_13:38268961-382/31/4	519	1	WINC, SKF Incomplete	API
CcMADS70_corrected	CcAP1	-	211	K-box	Chr_13:38268961-38273724	636	8	MIKC,SRF incomplete	AP1
CcMADS71		V2 13q020630.1	215	SRF, K-box	Chr 13:44604397-44610355	648	7	MIKC ^C	SEP
CcMADS72	CcSOC1a	V2 14d001070 1	216	SRE K-boy	Chr 14:2757551_2762802	666	7	MKCC	SOC1
COMADS/2	000001a	V2_149001970.1	210	OINF, IN-DUX	011 14.2/0/001-2/02002	000		NIII CO	0001
CcMADS73		V2_14g006800.1	192	SRF, K-box	Cnr_14:23531833-23539409	579	4	MIKC	AG/STK
CcMADS74		V2_15g006880.1	227	SRF, K-box	Chr_15:7887245-7897105	704	6	MIKC	SEP
CcMADS75		V2 150009280 1	249	SRE	Chr 15:10879232-10879981	750	1	Mv	-
Callabore		V2_109000200.1	240	CDE	Chr 16:00050005 00056757	640	6	MIC*/ME	MIKC*
CCMADS/6		v2_16g008420.1	203	ort	CIII_10:22853005-22856757	012	6		WIKC"
CcMADS77		V2_16g010760.1	156	K-box	Chr_16:28395469-28404407	471	7	MIKC	AGL6
CcMADS78	CcSOC1Like-A	V2 16g010770 1	123	SRF	Chr 16:28415914-28421188	372	4	MIKC	SOC1
CoMAD070	JUSSOULINGA	1/2 17/0400404	254	K hov	Chr 17:21071252 24070404	760	-	MIKCC	
CCWAD579		v2_1/g010010.1	204	IV-DUX	011 1/ 319/ 1233-319/8191	/02	3	WING	-
CcMADS80		V2_ScYrq3g_1936g000100.1	190	SRF	ScYrq3g_1936:2832-3401	570	1	Μα	-
CcMADS81		V2 ScYrq3g 379a000100.1	232	SRF	ScYrg3g 379:2561-3259	699	1	Μα	-

54 Supplementary Table S5: Primers developed. Related to Figure 5.

55

Primer name	Sequence	F/R	Target	Fragment length (bp) ^a
ORB_7	TCCCAGGCTGATTGTGCTGTCCTTATTAT	F	CcElf1α	90
ORB_8	ATGCTCACGGGTCTGACCATCCTTA	R	CcElf1α	90
ORB_57	TGGAAGAAGGTCGAGCAAGT	F	CcSOC1A	98
ORB_58	CCTGGTCCGTTTTTCGGGTA	R	CcSOC1A	98
ORB_59	TGTTCGAGCCCGAAAGATGC	F	CcSOC1B	120
ORB_60	TGGTCCGTTTGGACTAGGCA	R	CcSOC1B	120
ORB_105	CTTGAACTTGCCTCGGGT	F	CcFULLike-B	145
ORB_106	GAGAAAGAAGTAGGGCAGCA	R	CcFULLike-B	145
ORB_113	CTGCTTACTGCACCTGAA	F	CcFULLike-A	74
ORB_114	TCATCCCTCATCACTGCT	R	CcFULLike-A	74
ORB_119	TCCCTTCAGCTGCTTCTCC	F	CcAP1 (CcMADS70)	95
ORB_120	AACCTTCCTCATGCCACC	R	CcAP1 (CcMADS70)	95

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57 ^a Fragment when amplified from cDNA