

**Table S1 : primers used for T-DNA construct validation**

Target	Name	Forward primer		Reverse primer	
		5'-3' sequence	Name	5'-3' sequence	
ELF3a	LG1051	ATTGCATCATTCACATGAAGCCG	LG1052	AAACCGGCTTCATGTGAATG/	
ELF3b	LG1055	ATTGTCCTTCTCAATACCACTCT	LG1056	AAACAGAGTGGTATTGAGAA	
LHP1a	LG1081	ATTGAGGATATGAAAGGGGCAAG	LG1082	AAACCTTGCCCCCTTTCATATC/	
LHP1b	LG1085	ATTGGGTTTGTTCAGTGACGACGG	LG1086	AAACCCGTCGTCACTGACAA/	
FLCa	LG1091	ATTGCTGTAGAGCTTGCCGGAGG	LG1092	AAACCCCTCCGGCAAGCTCTAC/	
FLCb	LG1095	ATTGGCTGATGATCTCAAAGCCT	LG1096	AAACAGGCTTTGAGATCATC/	
TFL1a	LG1101	ATTGAATGTAGCATCTGTTGTAC	LG1102	AAACGTACAACAGATGCTAC	
TFL1b	LG1105	ATTGAATTGCCAAGGCCAAGCAT	LG1106	AAACATGCTTGGCCTTGGCA/	
SVPa	LG1111	ATTGTTCTTTACTCATTTCGGGCG	LG1112	AAACCGCCCGAATGAGTAAA	
SVPb	LG1115	ATTGCGGGTCAAACCAGCTTCAA	LG1116	AAACTTGAAGCTGGTTTGACC	

**Table S2 : Line and plant naming and pedigree**

<b>T4 line names*</b>	<b>T4 line pedigree*</b>	<b>T5 plant names**</b>	<b>T5 plant pedigree**</b>
A40-24	6-1-104-40-24	A40-24-25	6-1-104-40-24-25
A4-12	6-1-111-4-12	A4-12-25	6-1-111-4-12-25 6-1-111-4-12 -26
A4-19	6-1-111-4-19	A4-19-25 A4-19-26	6-1-111-4-19-25 6-1-111-4-19-26
A1-14	6-1-112-1-14	A1-14-25	6-1-112-1-14-25
B1-2	6-2-109-1-2	B1-2-25	6-2-109-1-2-25
B23-3	6-2-3-23-3	B23-3-25	6-2-3-23-3-25
B28-12	6-2-7-28-12		
B33-8	6-2-7-33-8		
B1-11	6-2-12-1-11		
B20-2	6-2-109-20-2		
C2-23	6-3-9-2-23		
C16-4	6-3-12-16-4		
C16-9	6-3-12-16-9		
C27-4	6-3-14-27-4		
C2-19	6-3-120-2-19	C2-19-25	6-3-120-2-19-25
A1-4	6-1-112-1-4		
B33-8	6-2-7-33-8		

\* T4 lines are listed in Table1

\*\* T5 plants used for genotyping are listed in Table2

Principle of line/plant numbering : First number, construct number (Flo6); second number, T1 parent line; third number, T2 parent line; fourth number, T3 parent line; fifth number, T4 parent line; Sixth number, T5 plant

Table S3. Primers used for amplification and sequencing

Gene	SVP			FLC sgRNA-a			FLC sgRNA-b		
Subgenome	SG-1	SG-2	SG-3	SG-1	SG-2	SG-3	SG-1	SG-2	SG-3
Fwd primer	LG1720	SVP-F3	LG1720	LG1750	LG1750	LG1750	LG1776	LG1775	LG1773
Rev primer	LG2475	SVP-R2	LG2473	LG1755	LG1756	LG1753	LG1770	LG1770	LG1770
Seq primer	LG1728	TA Cloning	LG1728	LG 1758	LG 1758	LG 1758	LG1770	LG1770	LG1770

Gene	LHP1			TFL1			ELF3		
Subgenome	SG-1	SG-2	SG-3	SG-1	SG-2	SG-3	SG-1	SG-2	SG-3
Fwd primer	LG1740	LG1740	LG1740	LG1713	LG1716	LG1714	LG1680	LG1680	LG1680
Rev primer	LG1744	LG1747	LG1743	LG1711	LG1711	LG1711	LG2234	LG2236	LG2232
Seq primer	LG1748	LG1748	LG1748	LG1711	LG1711	LG1711	LG 1688	LG 1688	LG 1688

Table S4. Primer sequences

<i>C. Sativa</i> primers	
Name	5'-3' Sequence
SVP-LG1720	AGCCATCTCTTGAGTTACAGGT
SVP-LG1728	GCATTCTGTTATTGCCTACGATT
SVP-LG2473	CCCTAGGGAGTGGACTACCGT
SVP-LG2475	CGAGAATGTGCAAATTGAACGC
SVP-F3	GTAGAAGTGATGAATGGATGC
SVP-R2	TCTTTGAAGTTCGCTGATCTC
FLC-LG1750	CTCTCGGAGACTGAAGCCAT
FLC-LG1753	CGACATTGTTCTTCCATAGATTCA
FLC-LG1755	ACGGCTTGAAACTTCACTAAACGG
FLC-LG1756	GCAGAACGTGTCAAGCAAATCTTG
FLC-LG1758	GTCACCTTCTCCAAACGTCG
FLC-LG1770	CCTGGCCAAAGAAAAGGGAC
FLC-LG1773	TCCAGTGGTCTTTTCAAGGGTTGC
FLC-LG1775	CTCATAGTTTCCAGTGGTCTTTCG
FLC-LG1776	AGCCTGGTCAAGATCCTTGATCAT
LHP-LG1740	GGTCAGGTCTTCTTCCAGCA
LHP-LG1743	TCGAAATAGCCTTCATCAAGCTCT
LHP-LG1744	TACTCTTCACCATCCTCCGTACAT
LHP-LG1747	GCTTCGATTTTCGAAATACCCTTTG
LHP-LG1748	TGCTTCTCCTTCTTCTCTCCA
TFL1-LG1711	ACTCGACCGCAAATTTACGA
TFL1-LG1713	ACTTGGGGGCCCATATCCCATATAA
TFL1-LG1714	TTTCTTTACTTTCCTGTTTATTGT
TFL1-LG1716	AATTCATTTTTACCTTTTTTGCC
ELF3-LG1680	CGTAGTCGGTGGGTTAAGTT
ELF3-LG1688	GCTTGTTGTTGCTAAATTCGGT
ELF3-LG2232	ACAAAGCCACCTGACTTTTC
ELF3-LG2234	GCTCCCTACCCTCAAGGTGC
ELF3-LG2236	TTCCCTCTGTGACTGATAGG
EF1-AFor	CCACGAGTCTGTTCTTGAGGC
EF1-ARev	CATTTGGCACCCCTTCTTCAC

[illegible]

Table S5 : Compilation of transcriptomic data concerning the genes targeted for knockout

Gene	<i>A. thaliana</i> <sup>a</sup>	<i>C. sativa</i> <sup>b</sup>	genotype CO46 <sup>c</sup>	genotype DH55 <sup>d</sup>
<i>SVP</i>	At2g22540	Csa16g044290	12,192	7.28
		Csa09g086860	21,552	10.44
		Csa07g052630	21,103	10.87
<i>FLC</i>	At5g10140	Csa08g054450	43,364	15.38
		Csa20g015400	3,772	0.88
		Csa13g011890	0,987	0.00
<i>TFL1</i>	At5g03840	Csa20g005030	1,479	0.10
		Csa08g060650	0,184	0.00
		Csa13g003940	3,824	0.00
<i>LHP1</i>	At5g17690	Csa20g025860	3,082	1.15
		Csa13g020480	5,227	2.64
		Csa08g009910	3,973	1.43
<i>ELF3</i>	At2g25930	Csa16g047550	7,541	9.34
		Csa09g092140	13,567	15.60
		Csa07g056950	5,688	2.59

a: Position of gene in *A. thaliana* genome (TAIR)

b: Position of gene in *C. sativa* genome (Kagale *et al.* 2014)

c: Transcript abundance in young leaves (Anderson *et al.* 2019)

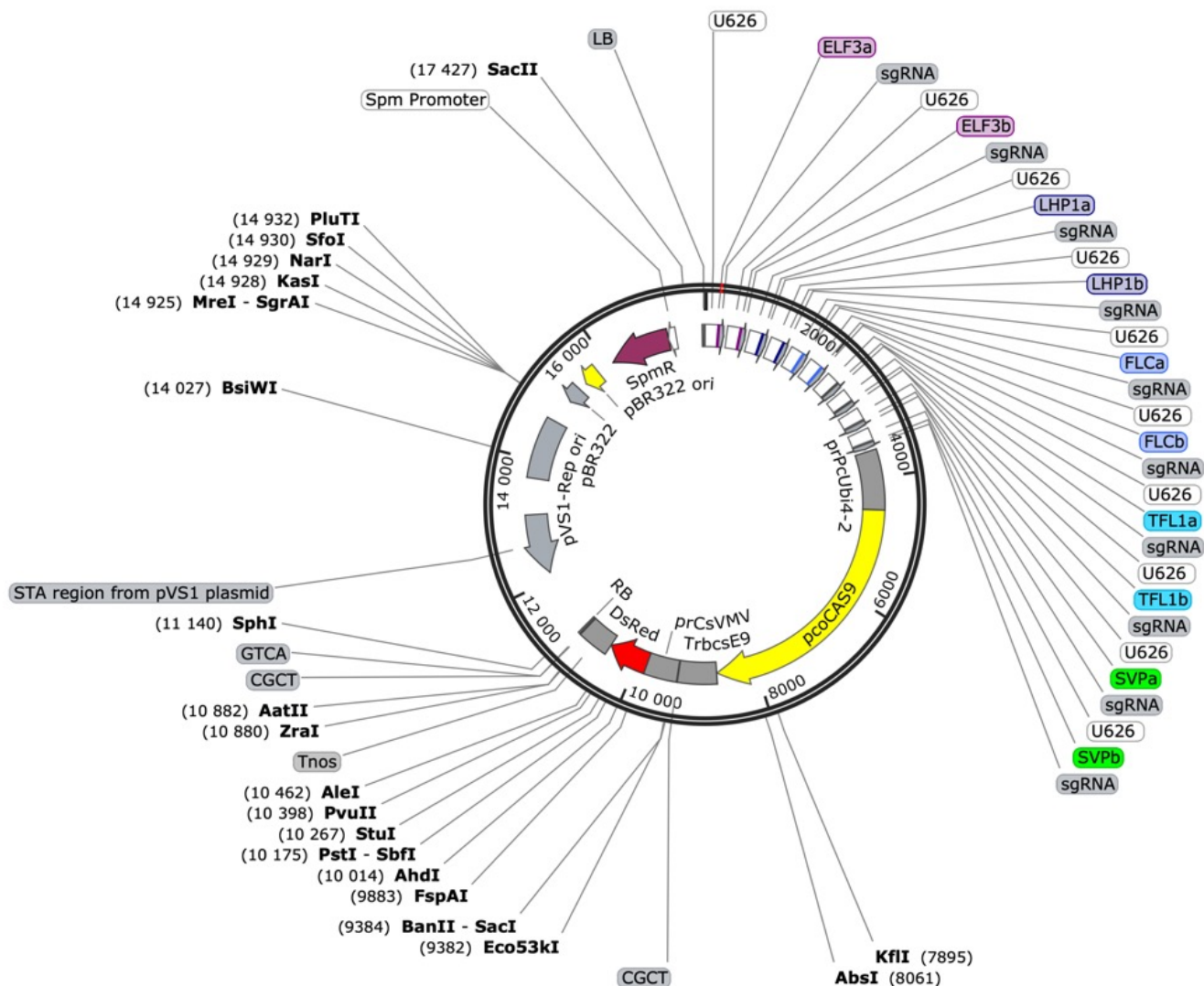
d: Transcript abundance in young leaves (Kagale *et al.* 2016)

Table S6 : Floraison-6 target genes, subgenomes, sgRNAs used to mutate genes

Gene	Accession	Position chromosome	Sub- genome	sgRNA a	sgRNA b
<i>CsELF3</i>	XM_010474320	Csa16g047550	g1		
	XM_010419071	Csa07g056950	g2	332rev GCATCATTACATGAAGCCG AGG	714rev TTCCTTCTCAATACCACTCT TGG
	XM_010431310	Csa09g092140	g3		
<i>CsFLC</i>	XM_010454736	Csa13g011890	g1		
	XM_010424583	Csa08g054450	g2	299rev GCTGTAGAGCTTGCCGAGG CGG	395for TGCTGATGATCTCAAAGCCT TGG
	XM_010493366	Csa20g015400	g3		
<i>CsLHP1</i>	XM_010455670	Csa13g020480	g1		
	XM_019228312	Csa08g009910	g2	245for GAGGATATGAAAGGGCAAG TGG	368for GGGTTTGTCACTGACGACGG CGG
	XM_010494428	Csa20g025860	g3		
<i>CsTFL1</i>	XM_010453977	Csa13g003940	g1		
	XM_010425355	Csa08g060650	g2	395rev AAATGTAGCATCTGTTGTAC CGG	459for GAATTGCCAAGGCCAAGCAT AGG
	XM_010492576	Csa20g005030	g3		
<i>CsSVP</i>	XM_010473941	Csa16g044290	g1		
	XM_010418712	Csa07g052630	g2	390rev TTTCTTTACTCATTGGGCG TGG	500rev GCGGGTCAAACCAGCTTCAA GGG
	XM_010430875	Csa09g086860	g3		

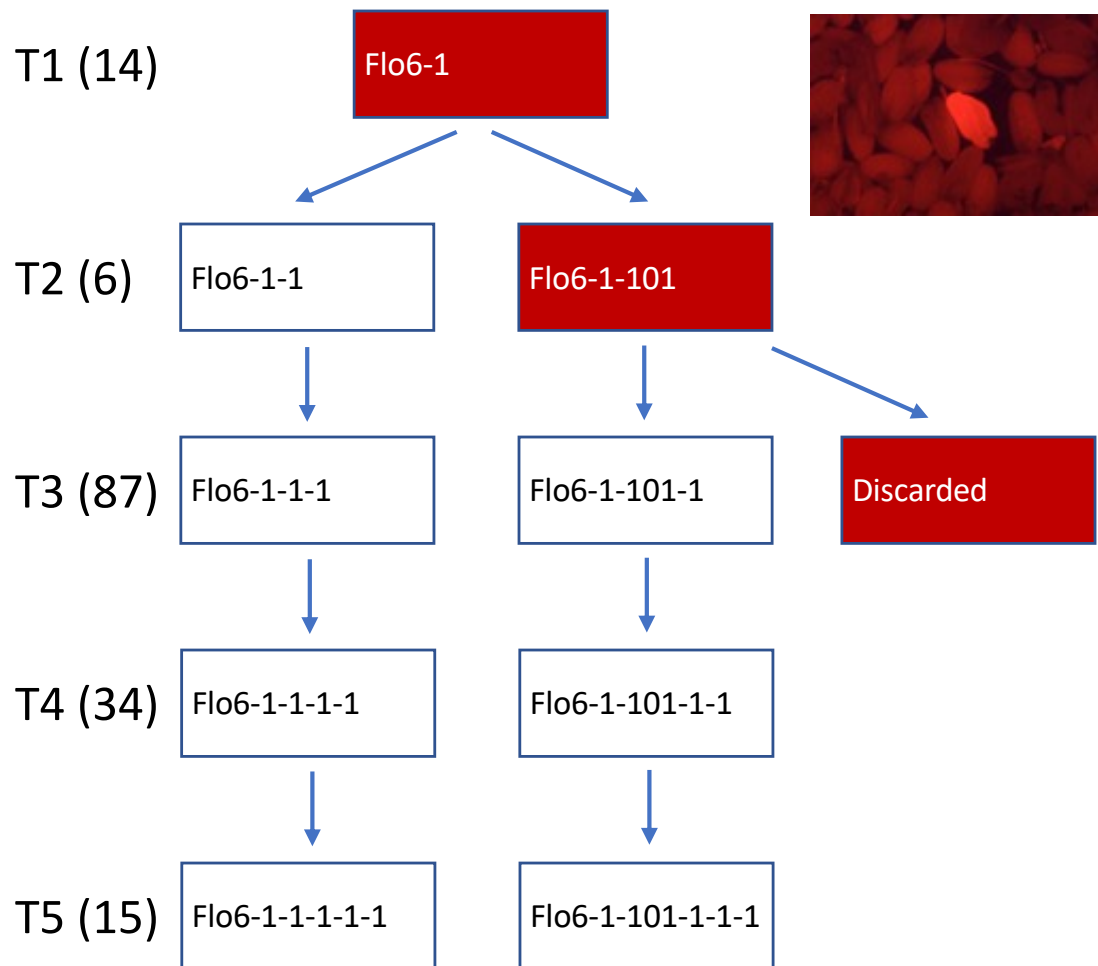
Table S7 : Progressive appearance of phenotypes

<b>Generation</b>	<b>Number of Populations screened</b>	<b>Number of Plants per population</b>	<b>Phenotypes observed</b>
T1	14	1	- Early flowering
T2	6	24	- Early flowering
T3	87	48	- Early flowering - late flowering - Short stature - Determinate growth - Increased basal branching
T4	34	24	- Early flowering - late flowering - Short stature - Determinate growth - Increased basal branching - extreme dwarfism
T5	15	24	- No new phenotypes

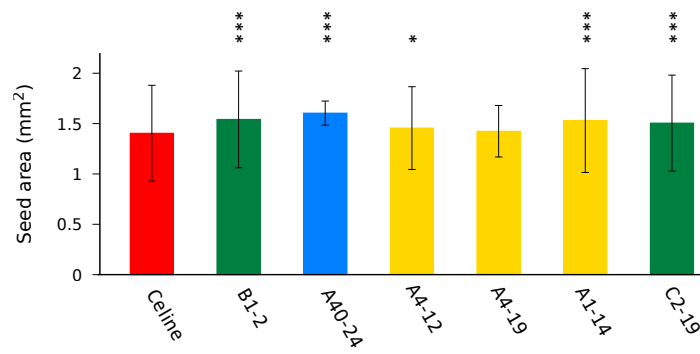


**Figure S1. Structure of binary plasmid carrying TDNA with sgRNA, Cas and DsRED marker.**

GoldenBraid II technology was used to assemble the vector pFlo6, in which, between the left and right T-DNA border sequences are: ten sgRNA sequences transcribed from the U6-26 promoter, the pcoCas9 coding sequence between the parsley ubiquitin 4 (PcUbi4\_2) promoter and the pea RbcsE9 terminator, and the DsRed coding sequence between the Cassava vein mosaic virus (CaVMV) promoter and the *A. tumefaciens* Nos terminator.



**Figure S2. Geneology of edited camelina populations.** The different generation (T) are indicated with the number of family analyzed in bracket. T1 corresponds to the initial transformed seeds borne by the plants treated by floral dip (as illustrated in the photo). Transgenic (DsRed-positive) plants are indicated by red boxes, and non-transgenic (DsRed-negative) segregants are indicated in white boxes. Successive generations of progeny through the T5 generation were produced by single-seed descent, with each plant designated by an additional number incremented at each generation. 24 plants were phenotyped at the T2, T4, and T5 generations, and 48 plants at the T3.



**Figure S3. Phenotype of T5 plants.** Seed size, expressed as surface area, was determined on samples of seeds borne by T5 plants. Values shown are means  $\pm$  SEM.  $n = 430, 480, 685, 506, 788, 634,$  and  $482,$  from left to right, respectively.  
Student test \*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ ; \*  $P < 0.05$ .