

Supplementary files. Terry et al., “The snapdragon LATE ELONGATED HYPOCOTYL plays a dual role in activating floral growth and scent emission”

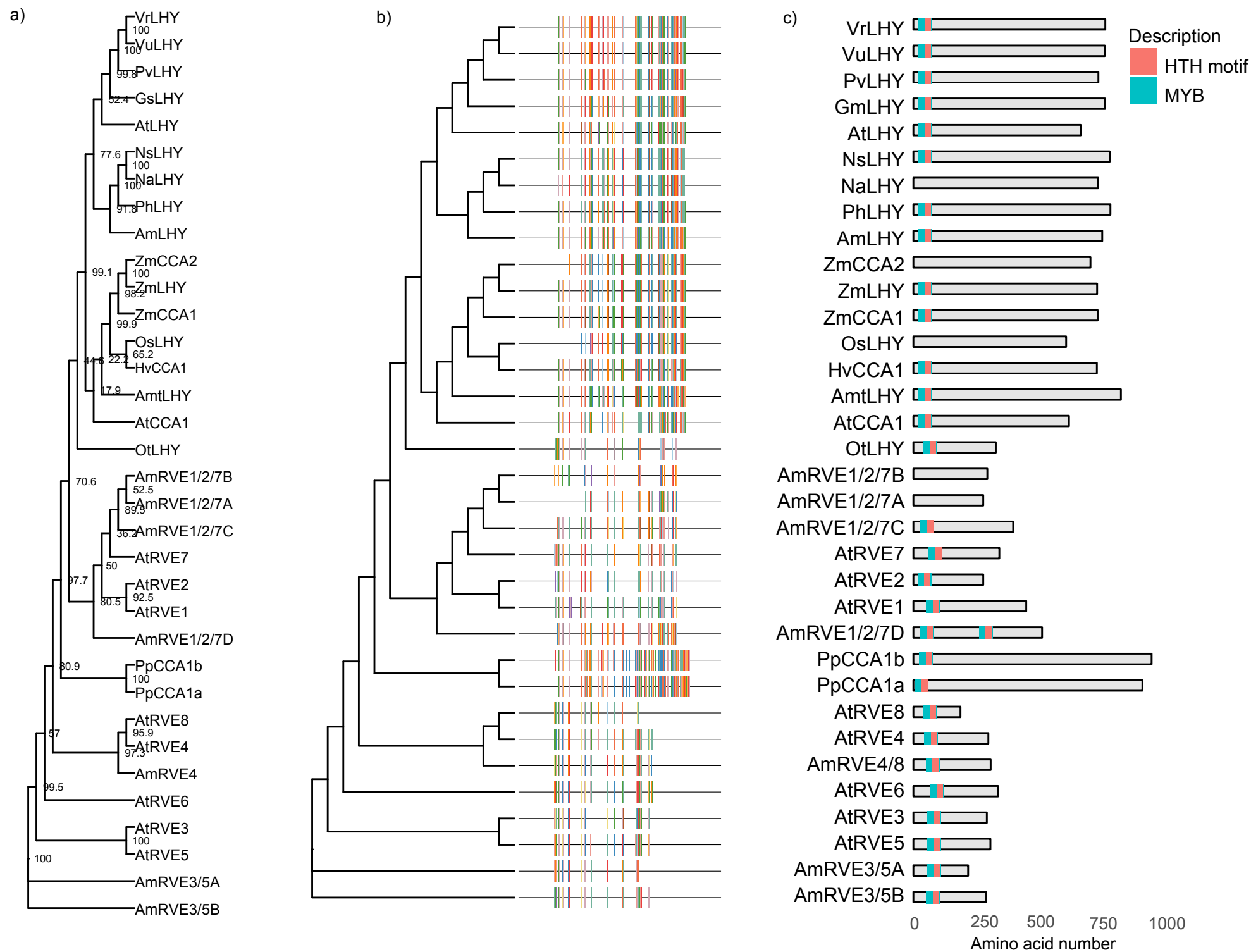
Supplementary Figure S1. Multiple sequence alignment of the predicted AmLHY and other proteins belonging to the LHY/CCA1 family. Vu (*Vigna unguiculata*), Vr (*Vigna radiata*), Pv (*Pisum vulgaris*), Gm (*Glycine max*), At (*Arabidopsis thaliana*), Na (*Nicotiana attenuata*), Ns (*Nicotiana sylvestris*), Ph (*Petunia hybrida*), Am (*Antirrhinum majus*), Amt (*Amborella trichopoda*), Zm (*Zea mays*), Os (*Oryza sativa*), Hv (*Hordeum vulgare*), Pp (*Physcomitrella patens*), Ot (*Ostreococcus tauri*).

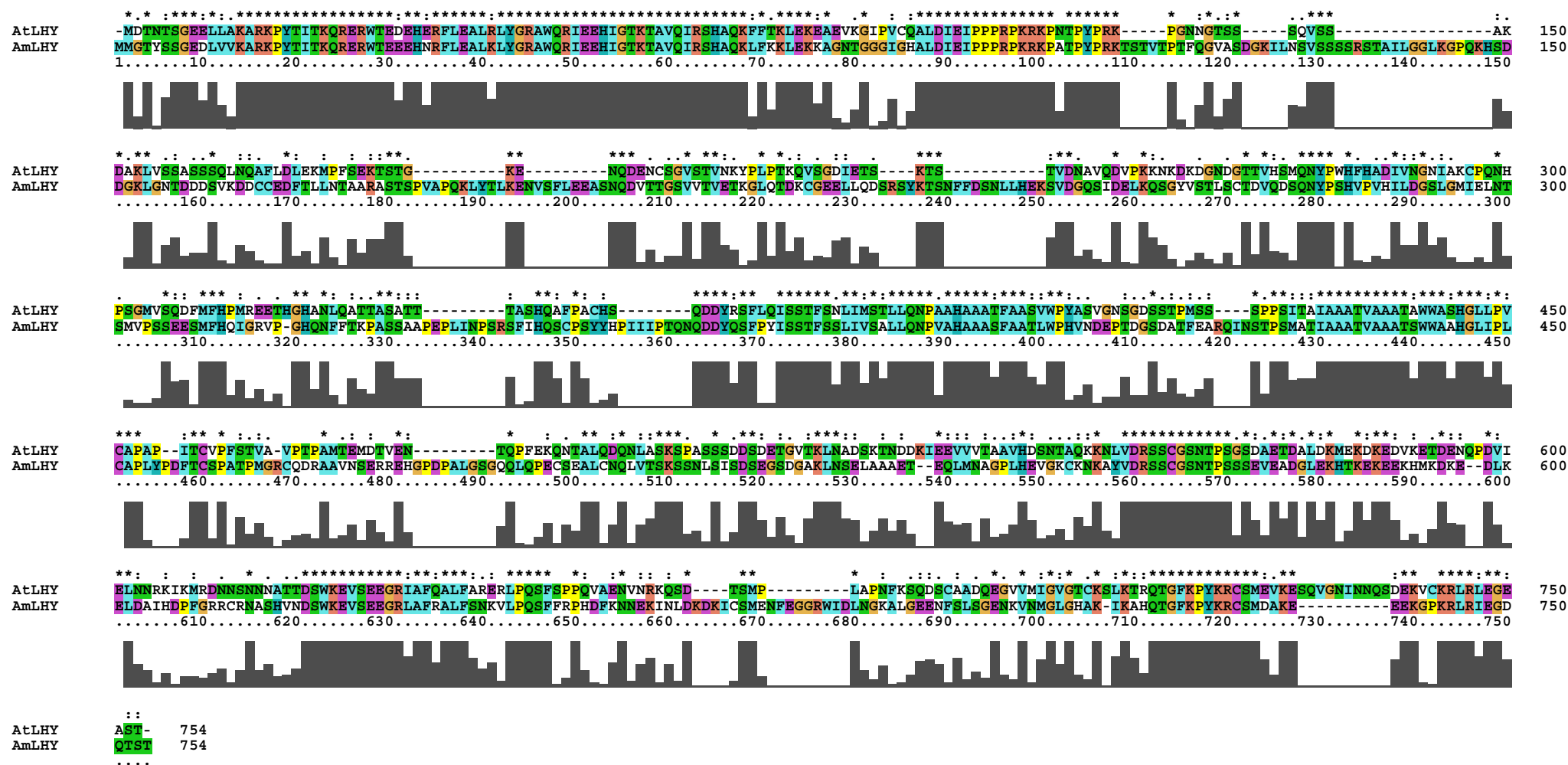
Supplementary Figure S2. AtLHY and AmLHY protein alignment.

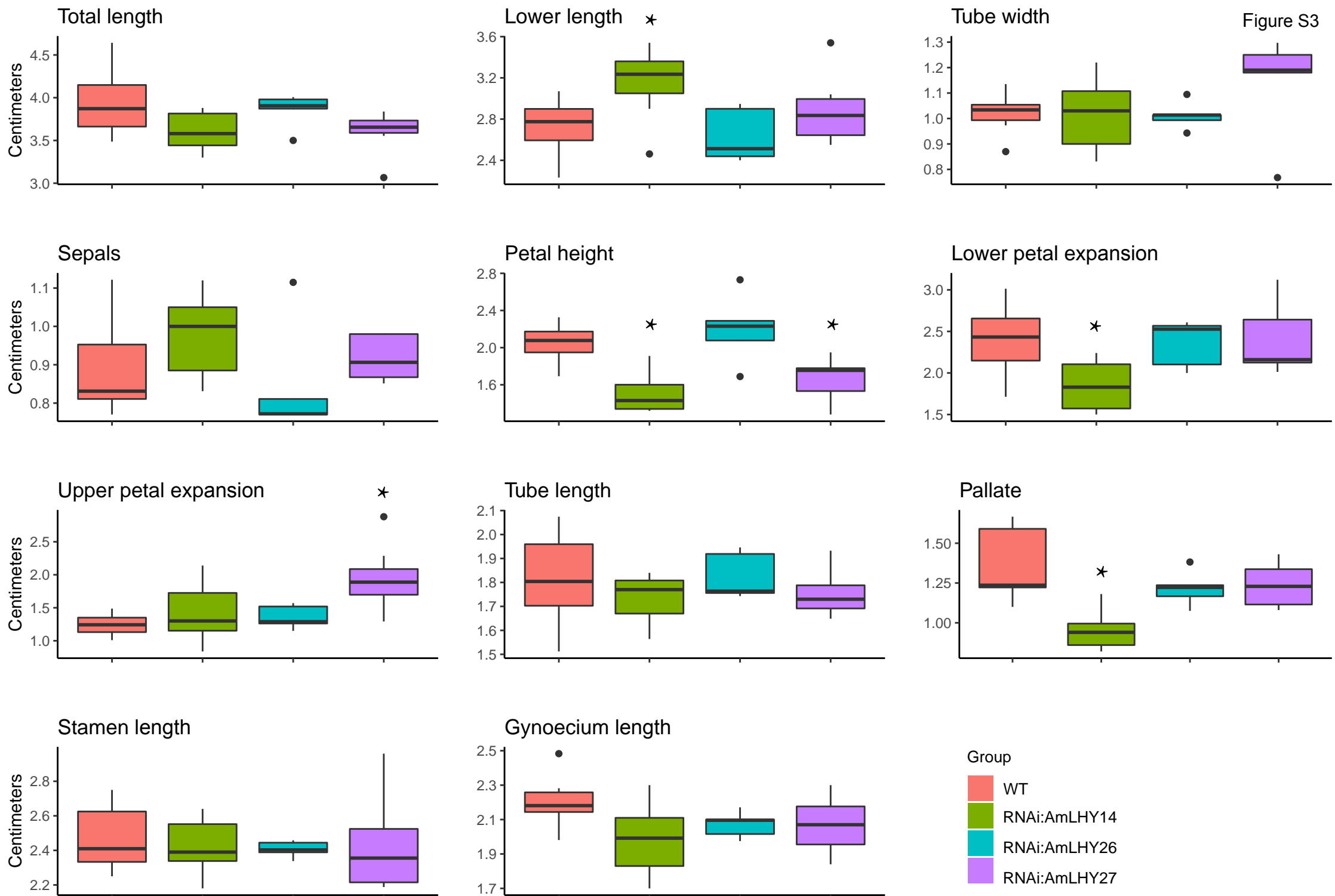
Supplementary Figure S3. Floral parameters in non-transgenic (WT) and transgenic lines (*RNAi:AmLHY*). Asterisk (*) denotes significant differences among wild type and transgenic flowers (see Supplementary Table S4).

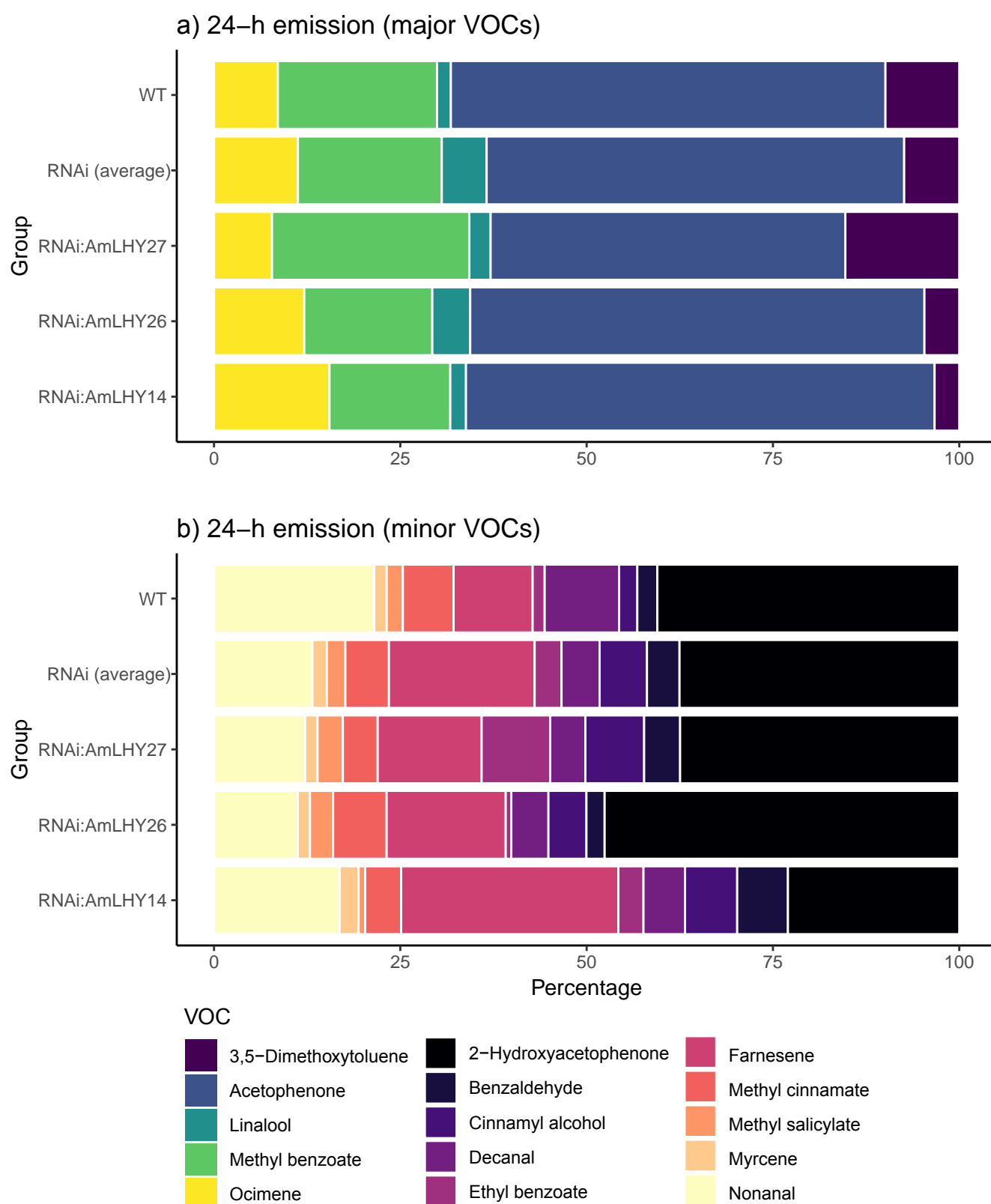
Supplementary Figure S4. 24-h scent profile. Major (A) and minor compounds (B) of wild-type, RNAi lines and its average are represented in bars.

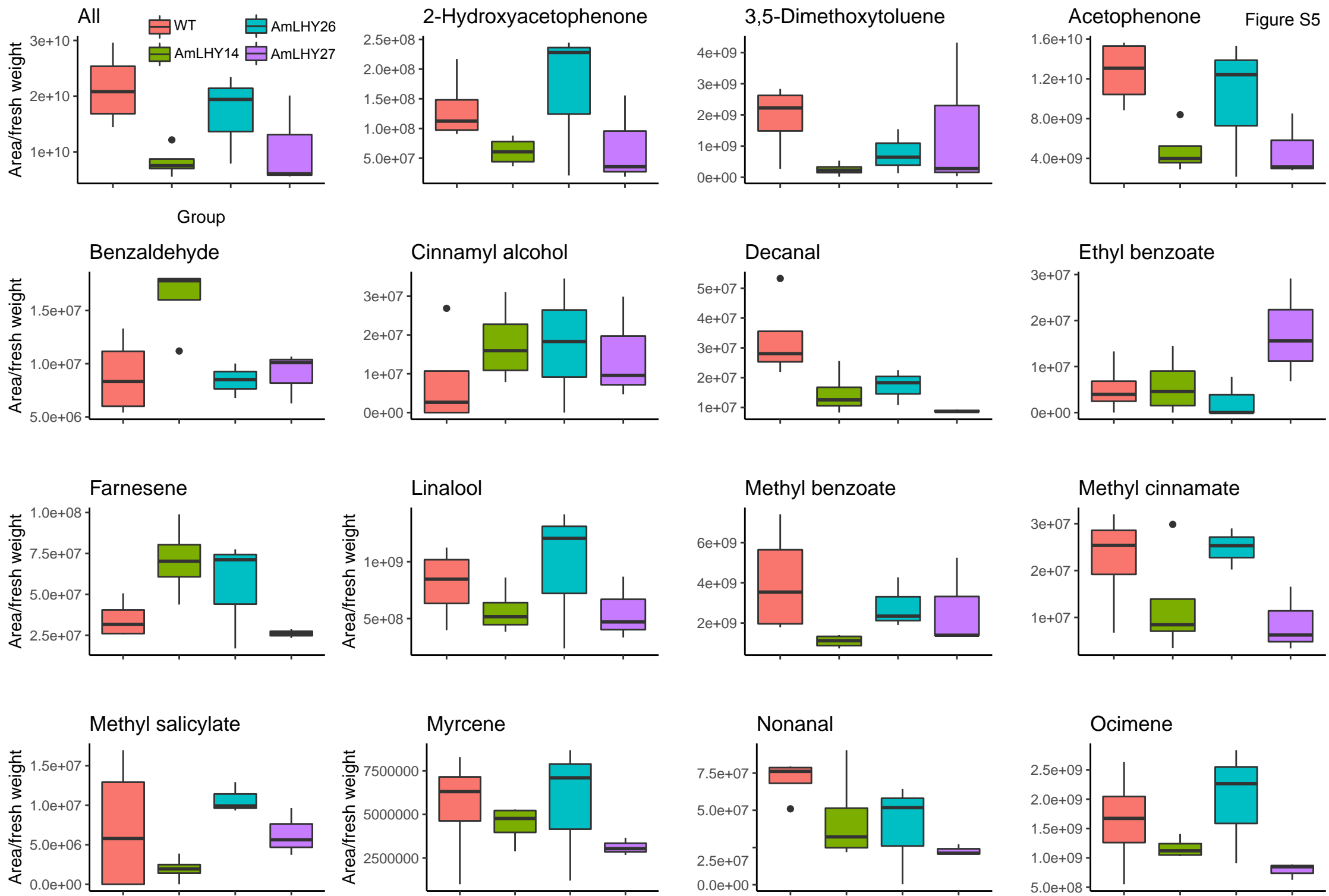
Supplementary Figure S5. Total volatile amounts, expressed as area divided by flower fresh weight, of wild type (WT) and transgenic lines (*RNAi:AmLHY*).











Supplementary Table S1. Accession numbers of proteins used in phylogenetic reconstruction.

Specie	Accession	Protein
<i>Arabidopsis thaliana</i> (At)	AT1G01060	LATE ELONGATED HYPOCOTYL (LHY)
	OAP07470.1	CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)
	OAO92169.1	REVEILLE 1 (RVE1)
	OAO90963.1	REVEILLE 2 (RVE2)
	AT1G01520	REVEILLE 3 (RVE3)
	gi 75324470	REVEILLE 4 (RVE4)
	gi 576017836	REVEILLE 5 (RVE5)
	AT5G52660	REVEILLE 6 (RVE6)
	gi 576017949	REVEILLE 7 (RVE7)
	OAP05994.1	REVEILLE 8 (RVE8)
<i>Amborella trichopoda</i> (Amt)	XP_006829218.1	LATE ELONGATED HYPOCOTYL (LHY)
<i>Antirrhinum majus</i> (Am)	Am03g44570	LATE ELONGATED HYPOCOTYL (LHY)
	LT978479.1	
	Am04g07010	REVEILLE 1A-like (RVE1A-like)
	Am06g21890	REVEILLE 1B-like (RVE1B-like)
	Am04g18800	REVEILLE 2A-like (RVE2A-like)
	Am06g21900	REVEILLE 2B-like (RVE2B-like)
	Am02g37040	REVEILLE 3/5A-like (RVE3/5A-like)
	Am03g44150	REVEILLE 3/5B-like (RVE3/5B-like)
	Am08g25850	REVEILLE 4/8-like (RVE4/8-like)
<i>Glycine soja</i> (Gs)	KHN11366.1	LATE ELONGATED HYPOCOTYL (LHY)
<i>Hordeum vulgare</i> (Hv)	AEW48245.1	CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)
<i>Nicotiana attenuata</i> (Na)	OIS99379.1	LATE ELONGATED HYPOCOTYL (LHY)
<i>Nicotiana glauca</i> (Ns)	XP_009794791.1	PREDICTED: protein LHY
<i>Oryza sativa</i> (Japonica Group) (Os)	XP_015649847.1	LHY isoform X4
<i>Ostreococcus tauri</i> (Ot)	AAU14271.1	LHY-like protein, partial
<i>Petunia hybrida</i> (Ph)	AKL88450.1	LATE ELONGATED HYPOCOTYL (LHY)
<i>Phaseolus vulgaris</i> (Pv)	CAD12767.2	LATE ELONGATED HYPOCOTYL (LHY)
<i>Physcomitrella patens</i> (Pp)	BAI39991.1	CIRCADIAN CLOCK ASSOCIATED 1A, partial
	BAI39992.1	CIRCADIAN CLOCK ASSOCIATED 1B, partial
<i>Vigna radiata</i> (Vr)	XP_014521593.1	LATE ELONGATED HYPOCOTYL (LHY)
<i>Vigna unguiculata</i> (Vu)	XP_027906072.1	LATE ELONGATED HYPOCOTYL-like (LHY)
<i>Zea mays</i> (Zm)	NP_001131529.2	LATE ELONGATED HYPOCOTYL (LHY)
	ADU60099.1	CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)
	AQK41466.1	CIRCADIAN CLOCK ASSOCIATED2 (CCA2)

Table S2 BLAST analysis. Arabidopsis accessions (Query ID) were used to identify REVEILLE (RVE, Protein column) sequences in the snapdragon genome database (<http://bioinfo.sibs.ac.cn/Am/index.php>). RVE names were assigned based on a tree generated by simple phylogeny (EMBL-EBI, <https://www.ebi.ac.uk/>).

Query ID	Protein	Snapdragon accession	P identity	e value	Assigned name
OAO92169.1	AtRVE1	Am04g07010	86.49	9e ⁻²³	AmRVE1/2/7A
		Am06g21890	47.15	5e ⁻²⁰	AmRVE1/2/7B
OAO90963.1	AtRVE2	Am04g18800	80.56	2e ⁻²³	AmRVE1/2/7C
		Am06g21900	51.00	2e ⁻²⁰	AmRVE1/2/7D
AT1G01520	AtRVE3	Am08g25850	76.79	1e ⁻¹⁸	AmRVE4/8
		Am02g37040	88.37	4e ⁻¹⁷	AmRVE3/5B
gi 75324470	AtRVE4	Am04g07010	72.97	8e ⁻¹⁴	AmRVE1/2/7A
		Am04g18800	70.27	1e ⁻¹³	AmRVE1/2/7C
gi 576017836	AtRVE5	Am03g44150	76.60	1e ⁻¹⁵	AmRVE3/5A
		Am02g37040	79.55	4e ⁻¹⁵	AmRVE3/5B
AT5G52660	AtRVE6	Am04g07010	72.97	3e ⁻¹⁴	AmRVE1/2/7A
		Am04g18800	70.27	5e ⁻¹⁴	AmRVE1/2/7C
gi 576017949	AtRVE7	Am04g18800	67.57	2e ⁻²¹	AmRVE1/2/7C
		Am06g21890	44.86	6e ⁻²⁰	AmRVE1/2/7B
OAP05994.1	AtRVE8	Am04g07010	78.38	2e ⁻¹⁵	AmRVE1/2/7A
		Am04g18800	72.97	5e ⁻¹⁵	AmRVE1/2/7C

Supplementary Table S3. PCR primers used in the current study

Gene	Forward (5' – 3')	Reverse (5' – 3')
RNAi:AmLHY	TCTGGATGAAACAAGTGTCC	TAAAAAGTTTCTGGGCATGA
NPTII	CCTGCTTGCCGAATATCATGGTGG	CGAAATCTCGTGATGGCAGGTTGG
AmUBI	CCGATGGAAGTATATGTTTG	CCAGTCTTACAAGACCTAGAAGCTC
AmLHY	TTACTTGCTCTCCGGCAACT	TTGAAGTCACAAGCTGATTGC

Supplementary Table S4. Analysis of floral parameters in non-transgenic and transgenic lines. Parameters were analyzed using a Student's *t* test. *P* value < 0.05 indicates a significant difference between non-transgenic and transgenic snapdragon flowers, significance levels are indicated with one asterisks (* for *p* < 0.05), two asterisks (** for *p* < 0.001) and three asterisks (***) for *p* < 0.0001).

Parameter	RNAi:AmLHY14	RNAi:AmLHY26	RNAi:AmLHY27
Total length	0.037*	0.858	0.069
Lower length	0.014*	0.486	0.210
Tube width	0.927	0.927	0.001*
Sepal length	0.337	0.646	0.762
Petal height	0.000***	0.060	0.004*
Lower petal expansion	0.005*	0.885	0.885
Upper petal expansion	0.137	0.419	0.000***
Tube length	0.404	0.881	0.404
Pallate	0.000***	0.258	0.160
Stamen length	0.974	0.974	0.974
Gynoecium length	0.048*	0.278	0.278

Supplementary Table S5. Retention time in minutes (RT), CAS number of detected volatiles.

RT	CAS	Volatile
4.611	100-52-7	Benzaldehyde
5.235	123-35-3	Myrcene
6.296	3779-61-1	Ocimene
7.701	124-19-6	Nonanal
7.844	98-86-2	Acetophenone
8.411	93-58-3	Methyl benzoate
8.511	78-70-6	Linalool
8.778	118-93-4	2-Hydroxyacetophenone
9.387	119-36-8	Methyl salicylate
9.549	112-31-2	Decanal
9.839	93-89-0	Ethyl benzoate
10.554	4179-19-5	3,5-Dimethoxytoluene
11.192	104-54-1	Cinnamyl alcohol
12.349	19713-73-6, 103-26-4	Methyl cinnamate
14.006	502-61-4	Farnesene

Supplementary Table S6. Analysis of daily emitted volatiles with the algorithm JTK_CYCLE in wild type (WT) and RNAi lines. Phase indicates the time point (ZT hours) with maximum emission. Shift indicates if the maximum emission of a VOC was advanced (A), delayed (D) or did not changed (NC) compared to the wild type. No data indicates that a parameter could not be determined.

	WT	RNAi:AmLHY14		RNAi:AmLHY26		RNAi:AmLHY27	
Volatile	Phase	Phase	Shift	Phase	Shift	Phase	Shift
Myrcene	0	6	D	6	D	6	D
Decanal	3	3	NC	3	NC	3	NC
Ocimene	3	6	D	6	D	6	D
2-Hydroxyacetophenone	6	6	NC	6	NC	6	NC
3,5-Dimethoxytoluene	6	6	NC	6	NC	6	NC
Linalool	6	6	NC	6	NC	6	NC
Nonanal	6	6	NC	6	NC	6	NC
Methyl cinnamate	6	3	A	6	NC	3	A
Cinnamyl alcohol	9	9	NC	9	NC	9	NC
Farnesene	9	6	A	6	A	6	A
Methyl benzoate	15	18	D	18	D	18	D
Acetophenone	15	12	A	12	A	12	A
Methyl salicylate	15	21	D	21	D	21	D
Benzaldehyde	18	12	A	12	A	12	A
Ethyl benzoate	No data	No data		No data		No data	