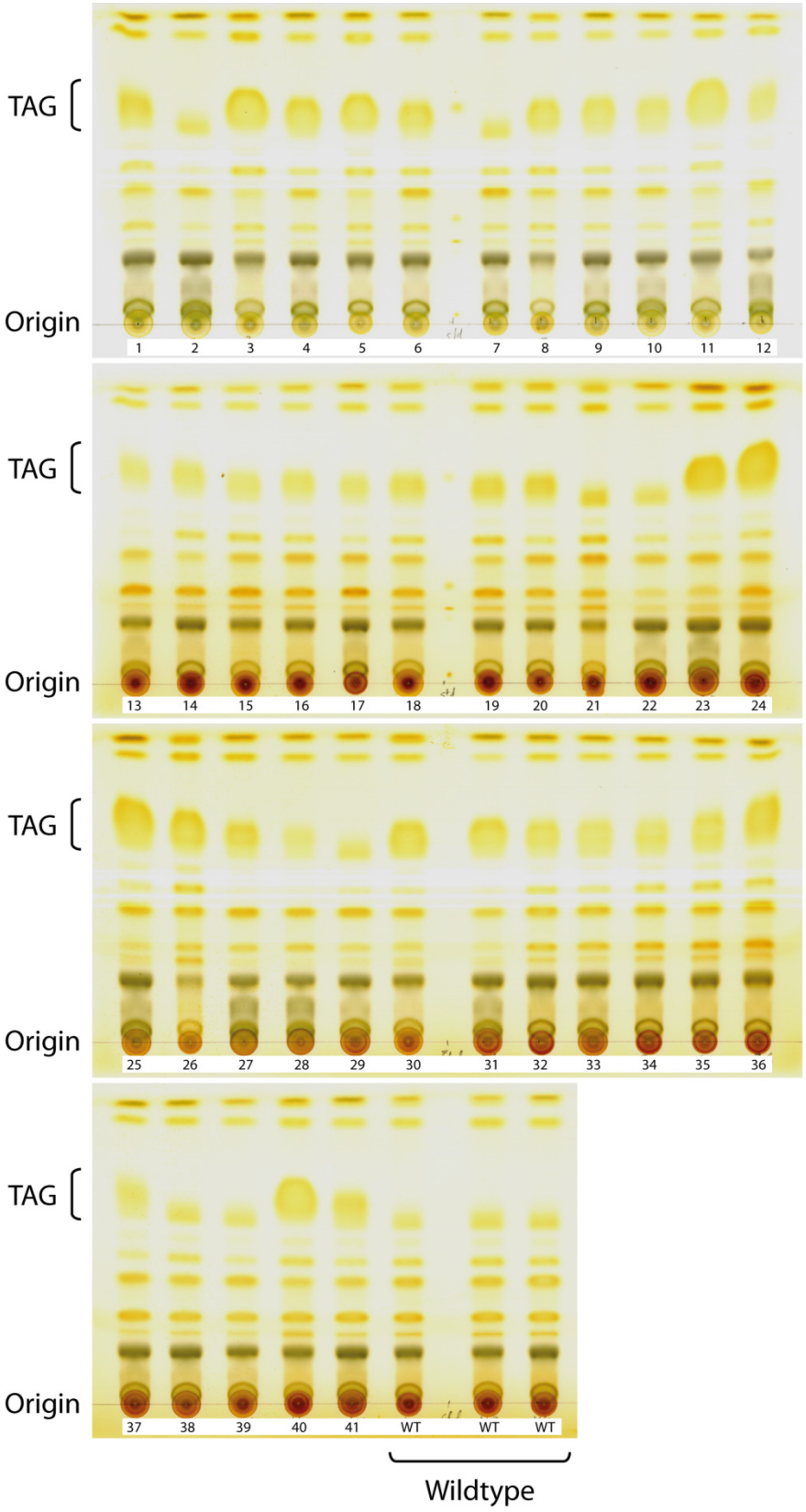
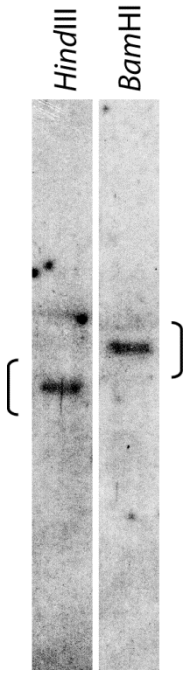
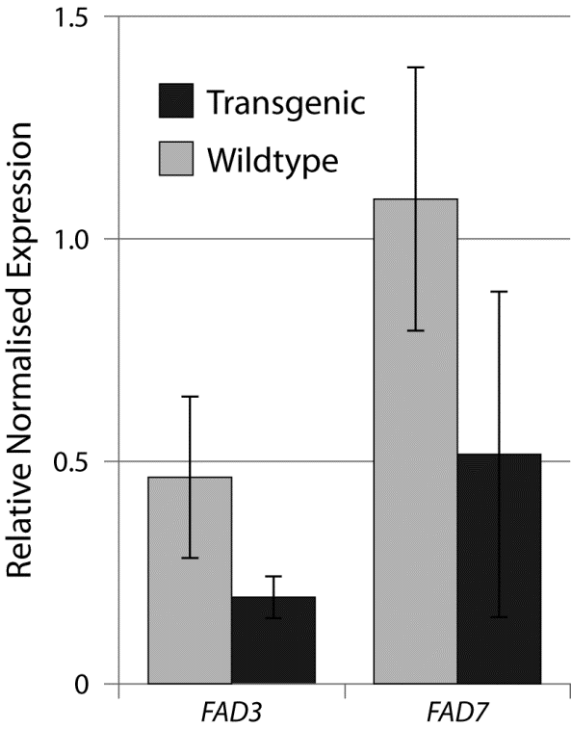
Supplementary



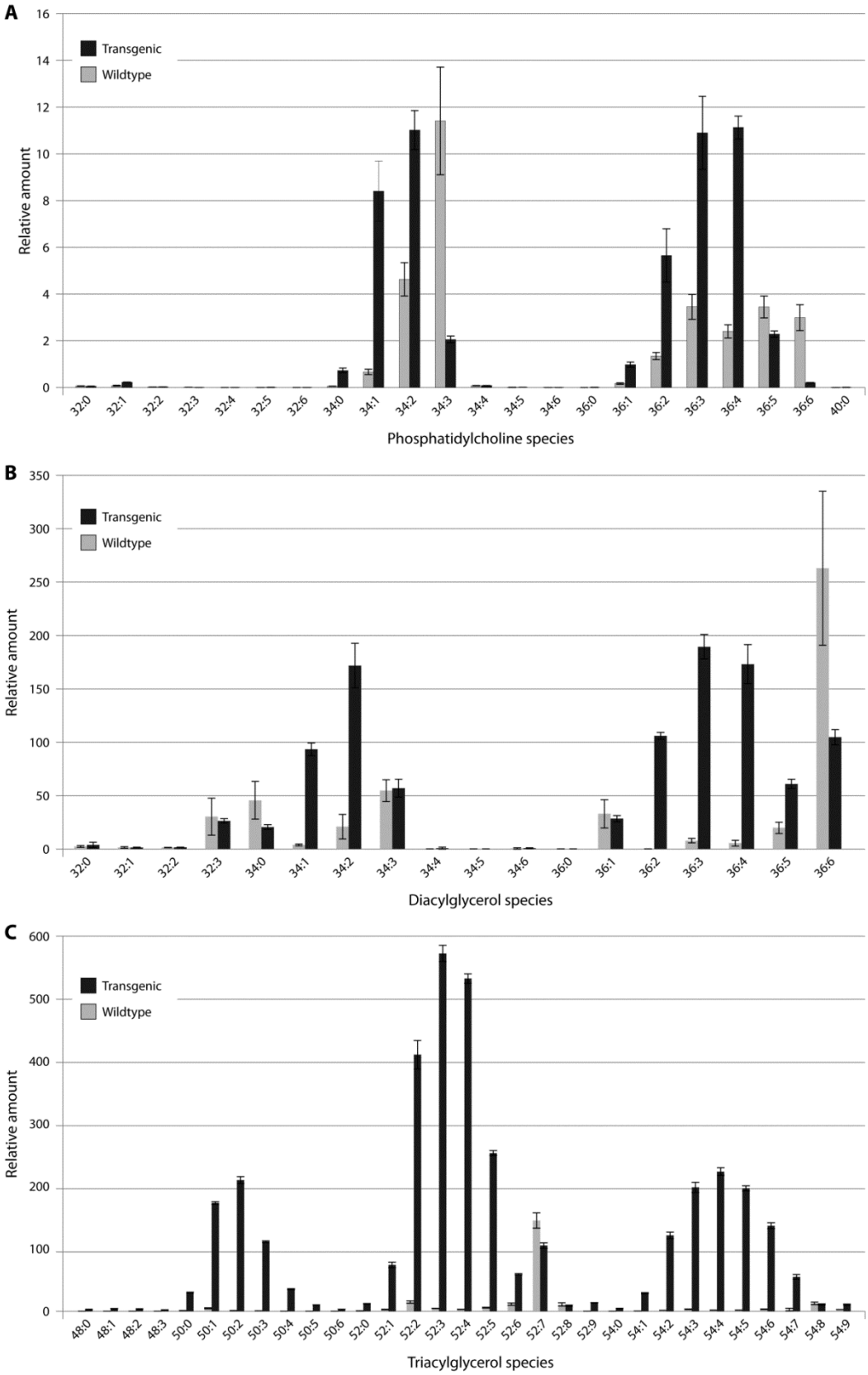
Supplementary Figure 1 TLC screening of leaf tissue of *Nicotiana tabacum* T_0 events at vegetative stage as described in materials and methods. Lanes 42-44 are wildtype controls which have relatively low TAG compared with the T_0 transgenic event in lane 3 (described in this study).



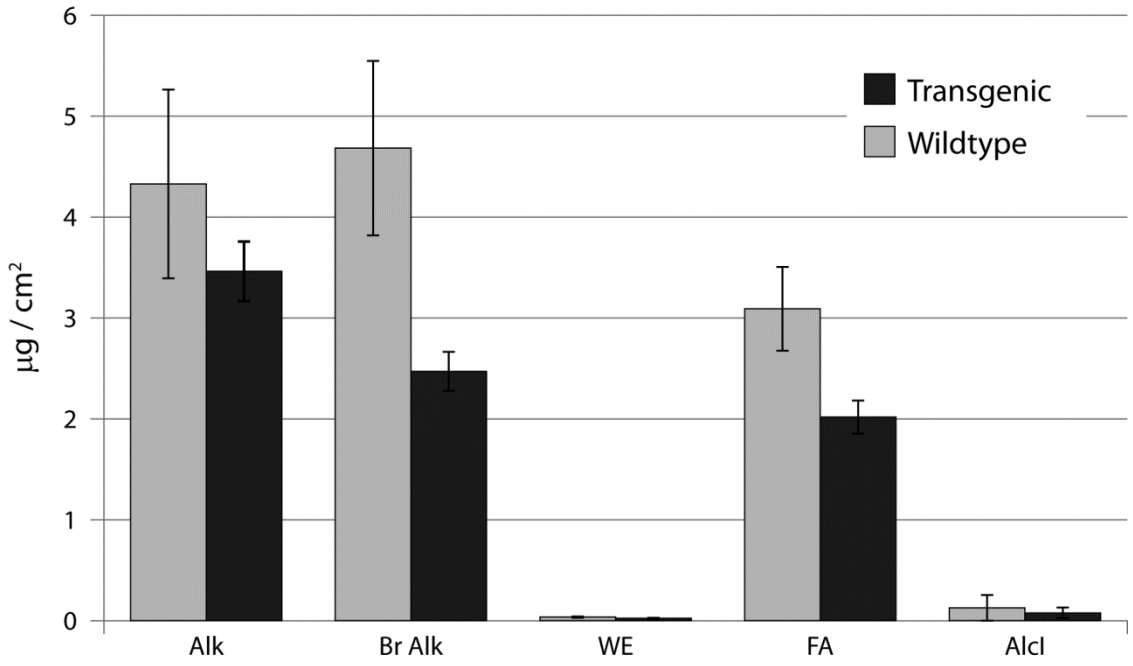
Supplementary Figure 2 Southern blot of transgenic *Nicotiana tabacum* leaf DNA with *NPTII* probe demonstrating that the T_0 line displaying maximum TAG levels was a single-copy event. This was corroborated by the segregation pattern of subsequent generations.



Supplementary Figure 3 Expression analysis of *fad3* and *fad7* fatty acid desaturase genes in wildtype and transgenic T_1 *Nicotiana tabacum* leaf tissue as quantified by qRT-PCR. Error bars denote standard deviations (n=3).



Supplementary Figure 4 LC-MS analysis of different phosphatidylcholine (a), diacylglycerol (b) and triacylglycerol (c) molecular species in wildtype and transgenic T_1 *Nicotiana tabacum* leaf tissue. Error bars denote standard deviations (n=3).



Supplementary Figure 5 GC analysis of cuticular wax composition on wildtype and T_1 transgenic leaves. Alk, alkanes; Br Alk, branched alkanes; WE, wax esters; FA, fatty acids; Alc, fatty alcohols. Error bars denote standard deviations (n=3).

Supplementary Table 1 Representative triacylglycerol (TAG) and total fatty acid (TFA) levels (% dry weight) and fatty acid profiles in leaves of *Nicotiana tabacum* primary transformant #3 at the onset of senescence. A TLC chromatogram of this line is depicted in lane 3 of Supplementary Fig. 1.

	C14:0	C16:0	IsoC16:1	C16:1Δ3	C16:Δ9	C16:3	C18:0	C18:1	C18:1Δ11	C18:2	C18:3	C20:0	C20:1	C20:2	C20:3	C22:0	C24:0	%
TAG	0.1	28.6	0.4	0.0	1.6	0.2	4.1	25.8	1.0	24.3	9.8	2.0	0.0	0.1	0.0	1.1	1.0	17.3
TFA	0.1	26.6	0.0	0.6	1.6	0.3	3.8	25.6	1.1	25.2	11.0	1.8	0.3	0.1	0.0	1.0	0.8	23.4

Supplementary Table 2 Triacylglycerol content (% seed weight) and fatty acid profile in wildtype (WT) and pooled seed from a representative T_1 transgenic *Nicotiana tabacum* event.

	C14:0	C16:0	C16:Δ9	C18:0	C18:1	C18:1Δ11	C18:2	C18:3	C20:0	C20:1	C20:2	C22:0	C24:0	%
WT	0.0	9.8	0.1	3.3	11.1	0.7	74.2	0.4	0.2	0.1	0.0	0.1	0.0	35.5
Transgenic	0.0	9.9	0.1	3.5	16.4	0.7	68.2	0.4	0.4	0.1	0.0	0.2	0.1	37.2

Supplementary Table 3 Total fatty acid levels (% dry weight) and fatty acid profile in wildtype (WT) and T_1 transgenic *Nicotiana tabacum* senescing leaf tissue at the onset of senescence. Error bars represent standard deviations (n ≥ 3).

	C14:0	C16:0	C16:Δ9	C16:3	C18:0	C18:1	C18:1Δ11	C18:2	C18:3	C20:0	C20:1	C22:0	C24:0	%
WT	0.8 ± 0.2	18.2 ± 0.7	3.6 ± 0.5	1.8 ± 0.2	6.1 ± 0.5	3.3 ± 0.5	0.6 ± 0.1	14.8 ± 1.0	46.4 ± 3.8	1.2 ± 0.3	1.5 ± 0.4	1.1 ± 0.2	0.5 ± 0.1	0.8 ± 0.0
Transgenic	0.1 ± 0.0	25.3 ± 1.4	2.3 ± 0.1	0.2 ± 0.0	3.8 ± 0.2	29.6 ± 3.8	1.3 ± 0.1	25.4 ± 1.7	8.1 ± 1.0	1.7 ± 0.1	0.3 ± 0.2	1.1 ± 0.1	0.8 ± 0.1	17.7 ± 0.7

Supplementary Table 4 MS/MS (Q-TOF) identification of major triacylglycerols (TAG) and diacylglycerol (DAG) molecular species in wildtype and T_1 transgenic *Nicotiana tabacum* leaf tissue.

Molecular species	Wildtype	Transgenic	
	Major component	Major component	Minor component
TAG			
50:1		C16:0/C16:0/C18:1	
50:2		C16:0/C16:0/C18:2	C16:0/C16:1/C18:1
50:3		C16:0/C16:0/C18:3	C16:0/C16:1/C18:2
52:1		C16:0/C18:0/C18:1	
52:2	C16:0/C18:1/C18:1	C16:0/C18:1/C18:1	
52:3		C16:0/C18:1/C18:2	
52:4		C16:0/C18:2/C18:2	C16:0/C18:1/C18:3
52:5		C16:0/C18:2/C18:3	
52:7	C16:3/C18:1/C18:3	C16:3/C18:1/C18:3	C16:1/C18:3/C18:3
54:2		C18:0/C18:1/C18:1	
54:3		C18:0/C18:1/C18:2	C18:1/C18:1/C18:1
54:4		C18:1/C18:1/C18:2	C18:0/C18:1/C18:3
			C18:0/C18:2/C18:2
54:5		C18:1/C18:1/C18:3	C18:0/C18:2/C18:3
		C18:1/C18:2/C18:2	
54:6		C18:2/C18:2/C18:2	C18:1/C18:2/C18:3
54:7		C18:2/C18:2/C18:3	C18:1/C18:3/C18:3
DAG			
34:1		C16:0/C18:1	
34:2		C16:0/C18:2	
36:2		C18:1/C18:1	
36:3		C18:1/C18:2	
36:4	C18:2/C18:2	C18:2/C18:2	
		C18:1/C18:3	
36:5	C18:2/C18:3	C18:2/C18:3	
36:6		C18:3/C18:3	

Supplementary Table 5 Triacylglycerol (TAG) levels (% dry weight) and TAG fatty acid profile in stem and root tissues of wildtype (WT) and T_1 transgenic *Nicotiana tabacum* line. Error bars represent standard deviations (n=2, wildtype; N=3, transgenic).

Plant	Tissue	C14:0	C16:0	C16:1	C18:0	C18:1	C18:1Δ11	C18:2	C18:3	C20:0	C20:1	C20:2	C22:1	C24:0	C24:1	%
WT	Young stem	0.0	15.8 ± 3.6	0.4 ± 0.6	5.0 ± 0.3	11.3 ± 9.2	0.4 ± 0.5	34.8 ± 4.7	31.3 ± 10.4	0.6 ± 0.9	0.0	0.0	0.0	0.4 ± 0.6	0.0	0.0
Transgenic	Young stem	0.2 ± 0.0	26.5 ± 1.2	1.7 ± 0.2	3.7 ± 0.1	19.6 ± 1.1	1.5 ± 0.0	36.1 ± 0.5	5.8 ± 0.1	1.7 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	1.1 ± 0.0	0.0	1.3 ± 0.1	0.8 ± 0.1
WT	Mature stem	0.0	8.0 ± 0.7	0.0	3.2 ± 0.1	2.3 ± 0.3	0.0	44.0 ± 1.0	42.4 ± 2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1 ± 0.0
Transgenic	Mature stem	0.1 ± 0.0	28.7 ± 0.2	2.4 ± 0.0	3.4 ± 0.0	21.1 ± 0.8	1.7 ± 0.0	33.9 ± 0.4	4.6 ± 0.1	1.5 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	1.0 ± 0.0	0.0	1.1 ± 0.0	1.1 ± 0.1
WT	Root	0.3 ± 0.3	8.2 ± 0.7	1.4 ± 0.2	2.3 ± 0.5	3.8 ± 0.5	0.3 ± 0.3	62.2 ± 1.5	21.3 ± 0.9	0.0	0.2 ± 0.4	0.0	0.0	0.0	0.0	0.2 ± 0.1
Transgenic	Root	0.1 ± 0.0	21.5 ± 1.0	0.5 ± 0.0	4.4 ± 0.0	13.9 ± 0.8	0.5 ± 0.0	47.3 ± 1.1	5.3 ± 0.4	2.2 ± 0.1	0.3 ± 0.0	0.7 ± 0.1	1.8 ± 0.1	0.0	1.5 ± 0.1	1.4 ± 0.2

Supplementary Table 6 Cuticular wax content (μg/cm^2) and composition (% each molecular class) on the surface of wildtype (WT) and T_1 transgenic *Nicotiana tabacum* leaves. Error bars represent standard deviations (n=3).

	Alkanes	**Branched alkanes**	**Fatty acids**	**Fatty alcohols**	**Wax esters**	**Wax content**
WT	35.3	38.2	25.2	1.1	0.3	12.3 ± 2.3
Transgenic	43.0	30.7	25.1	0.9	0.3	8.1 ± 0.7