Fruit-Specific RNAi-mediated suppression of *SlNCED1* increases both lycopene and β-carotene contents in tomato fruit

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Supplementary Fig. S1. Hairpin construct of the *SlNCED1* gene for double-stranded RNAi under the control of the tomato *E8* promoter. The conserved sequence of *SlNCED1* cDNA fragments was used for hairpin RNAi construction using the pCAMBIA1305.1 vector, from which a recombinant plasmid containing the *E8*::antisense *SlNCED1*::GUS::sense *SlNCED1*::Nos-terminator fusion gene driven by the fruit-specific *E8* promoter was constructed.

Supplementary Fig. S2. Genetic transformation of *SlNCED1*-RNAi tomato. A: Pre-culture of tomato cotyledons; B: removal of agrobacterium from cotyledons after transformation mediated by *Agrobacterium tumefaciens*; C: selection and differentiation culture; D: transgenic seedlings; E: rooting culture of the transgenic seedlings; and F: transgenic tomato plants.

Supplementary Fig. S3. Histochemical GUS assay for transgenic tomato

plants. A: Leaf of transgenic tomato after GUS assay. B: micrograph (40×) of transgenic tomato leaf after GUS assay. C: flower of transgenic tomato after GUS assay. D: young fruit of transgenic tomato after GUS assay. E: roots of transgenic tomato after GUS assay. F: micrograph (40×) of transgenic tomato root after GUS assay.

Supplementary Fig. S4. Detection for transformed tomato plants. A, PCR analysis of transformed plants with *Hygromycin* gene. M: DNA marker; lane 1: negative control (non-transgenic tomato genome DNA); lane 2: positive control (pCAMBIA1305.1-*SlNCED1*-RNAi); lanes 3 to 5: transgenic plant lines after GUS assay (positive plants). B, Detection of 25bp siRNA specific to the *SlNCED1* gene in the control and RNAi fruit.

Supplementary Fig. S5. Wavelength scanning of standards for lycopene and carotenoid.

Supplementary Fig. S6. HPLC chromatogram of standards for lutein and zeaxanthin.

Supplementary Fig. S7. HPLC chromatogram of the four carotenoid standards.

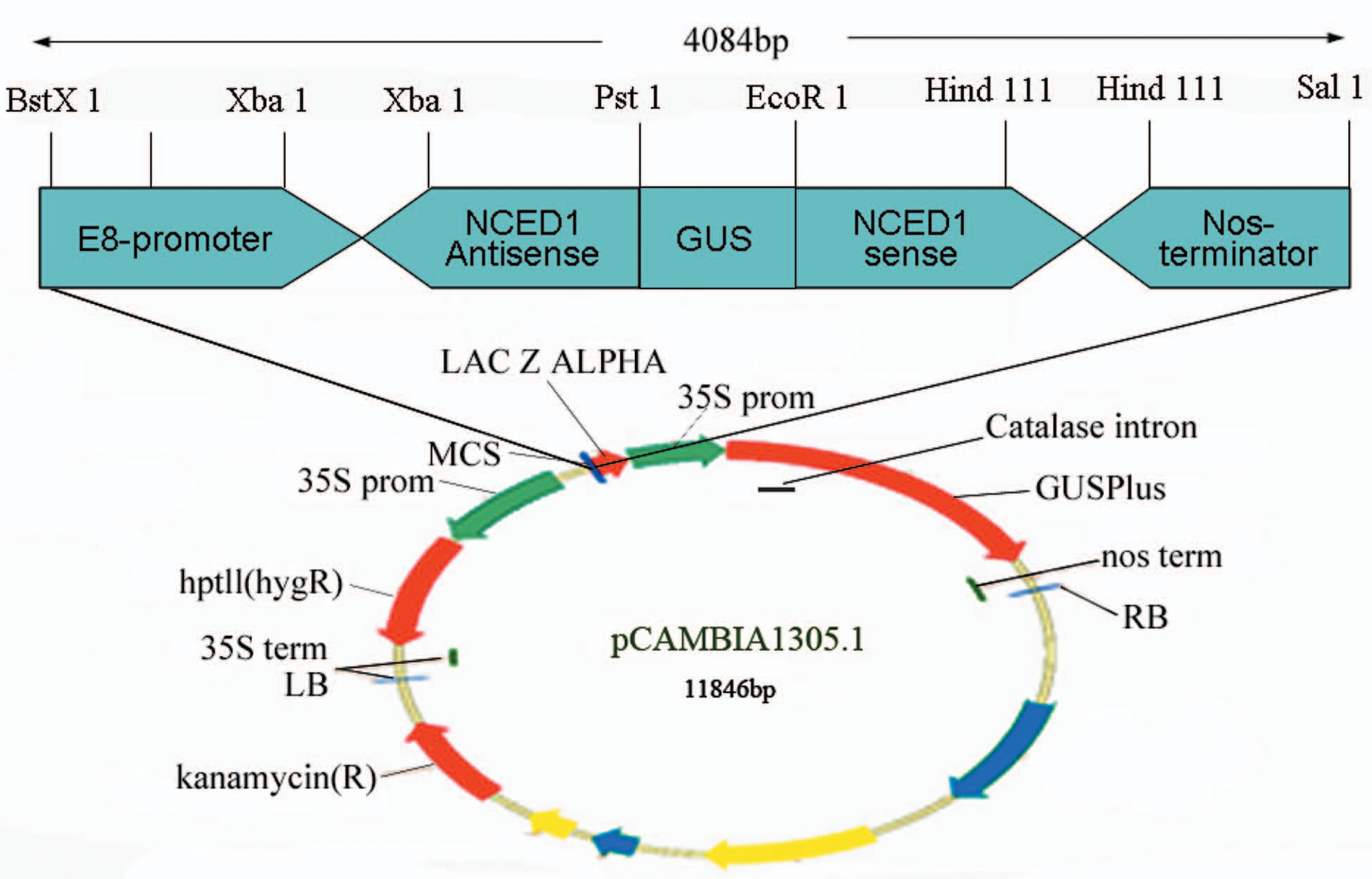
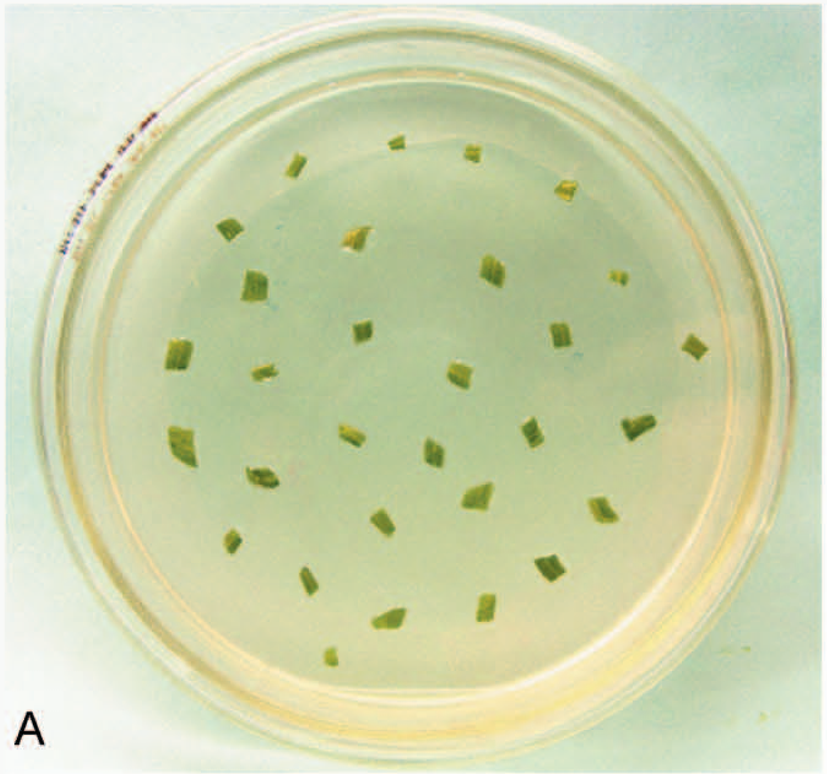
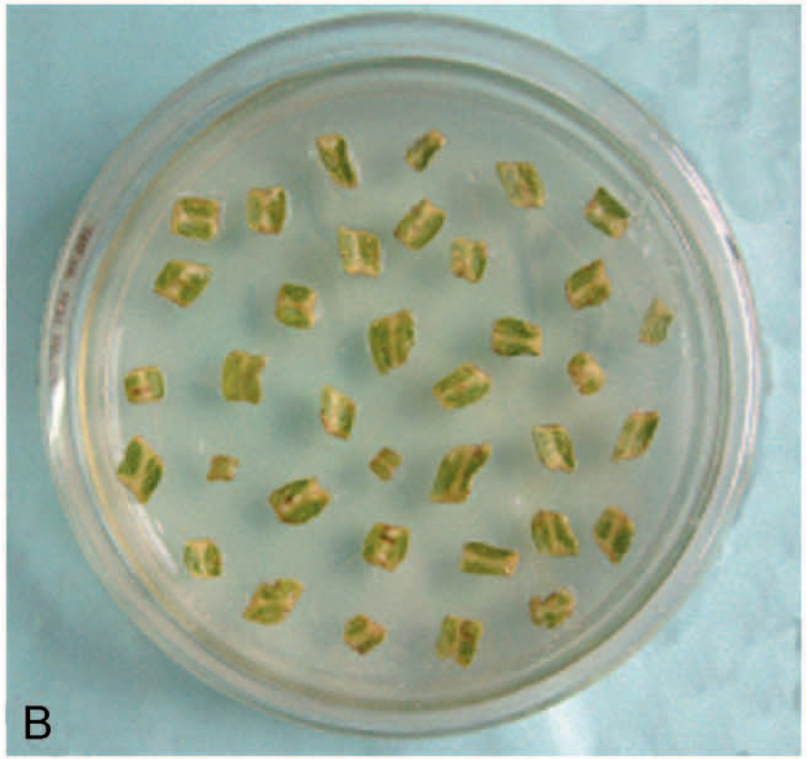
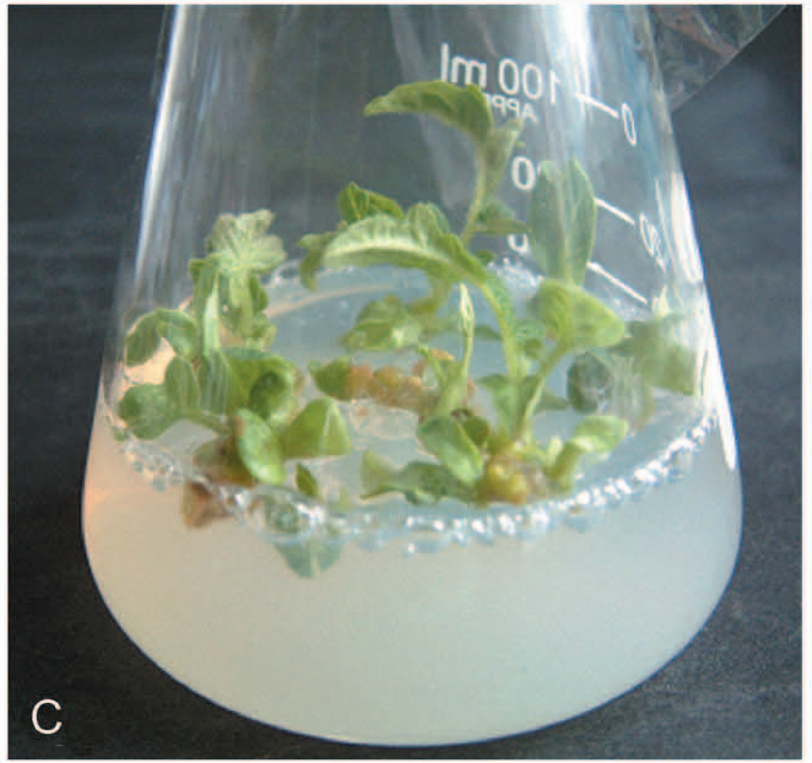
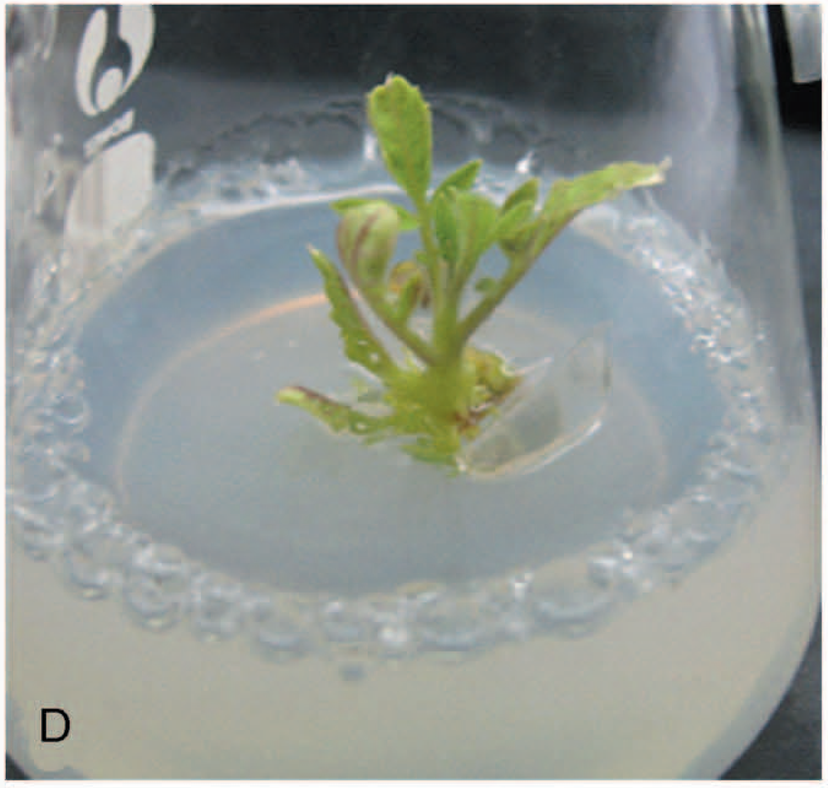

4084bp
BstX 1
Xba 1
Xba 1
Pst 1
EcoR 1
Hind 111
Hind 111
Sal 1
E8-promoter
NCED1
Antisense
GUS
NCED1
sense
Nos-
terminator
LAC Z ALPHA
35S prom
Catalase intron
MCS
35S prom
GUSPlus
hptll(hygR)
nos term
RB
35S term
LB
pCAMBIA1305.1
11846bp
kanamycin(R)

Figure S1.









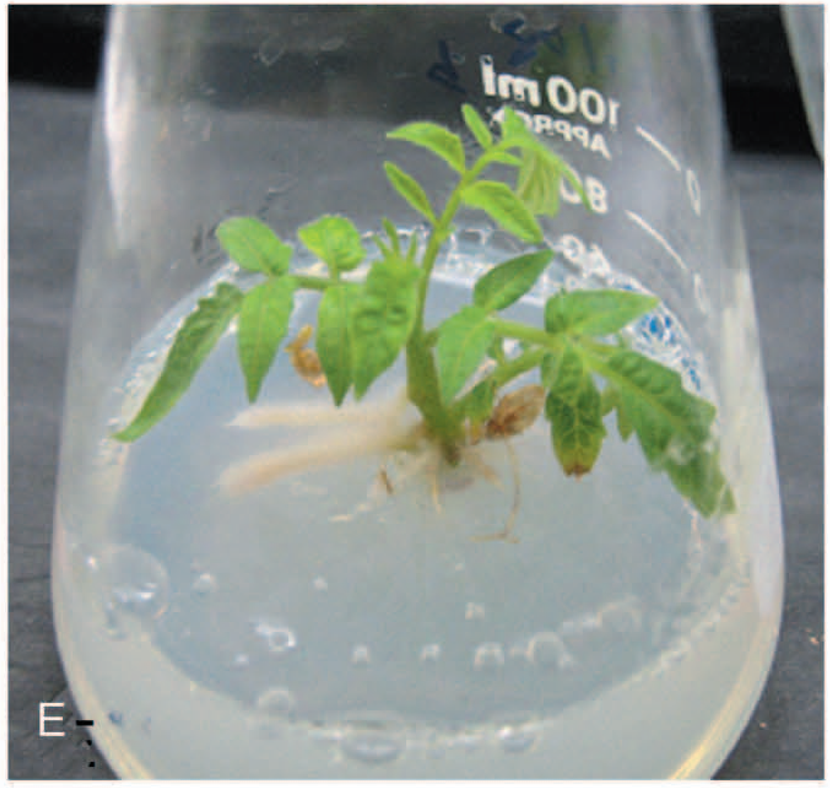
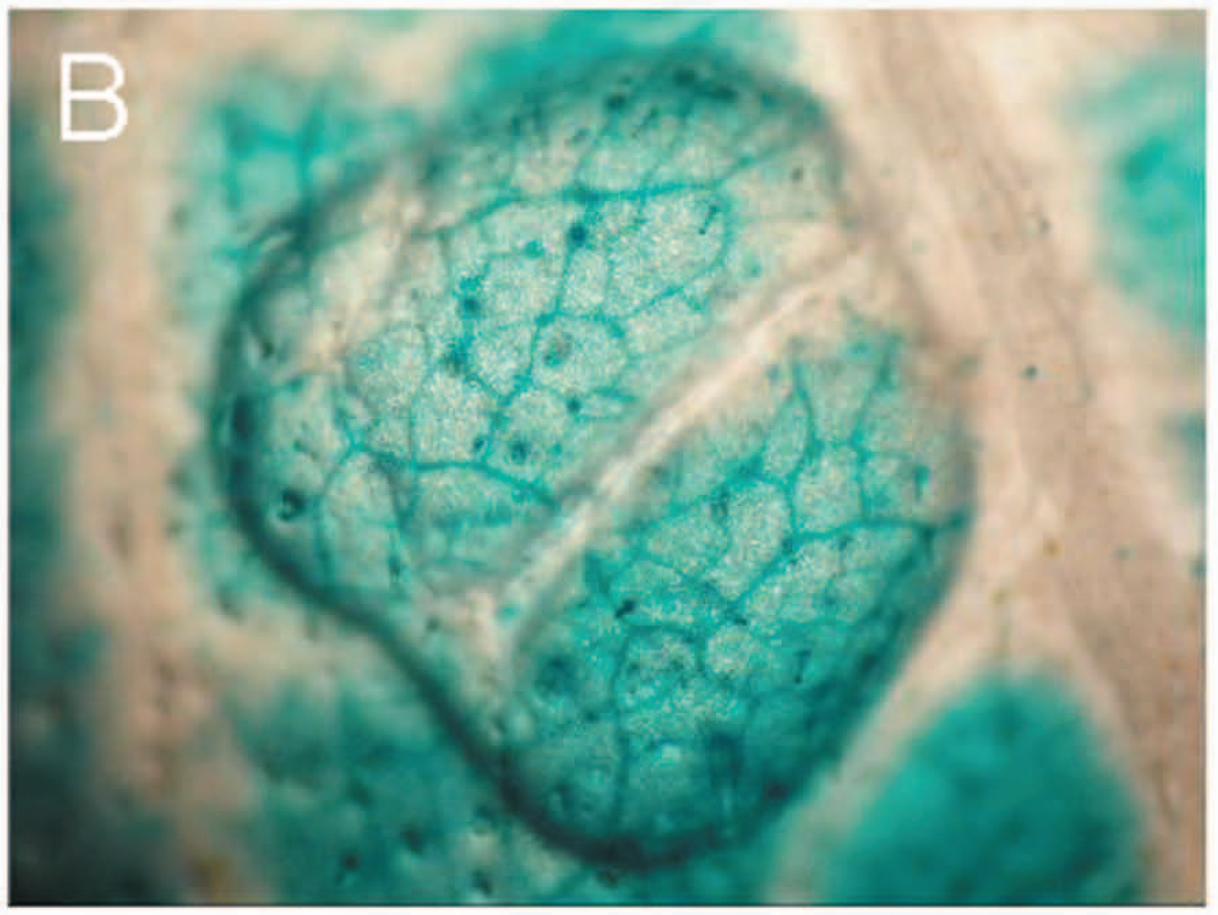
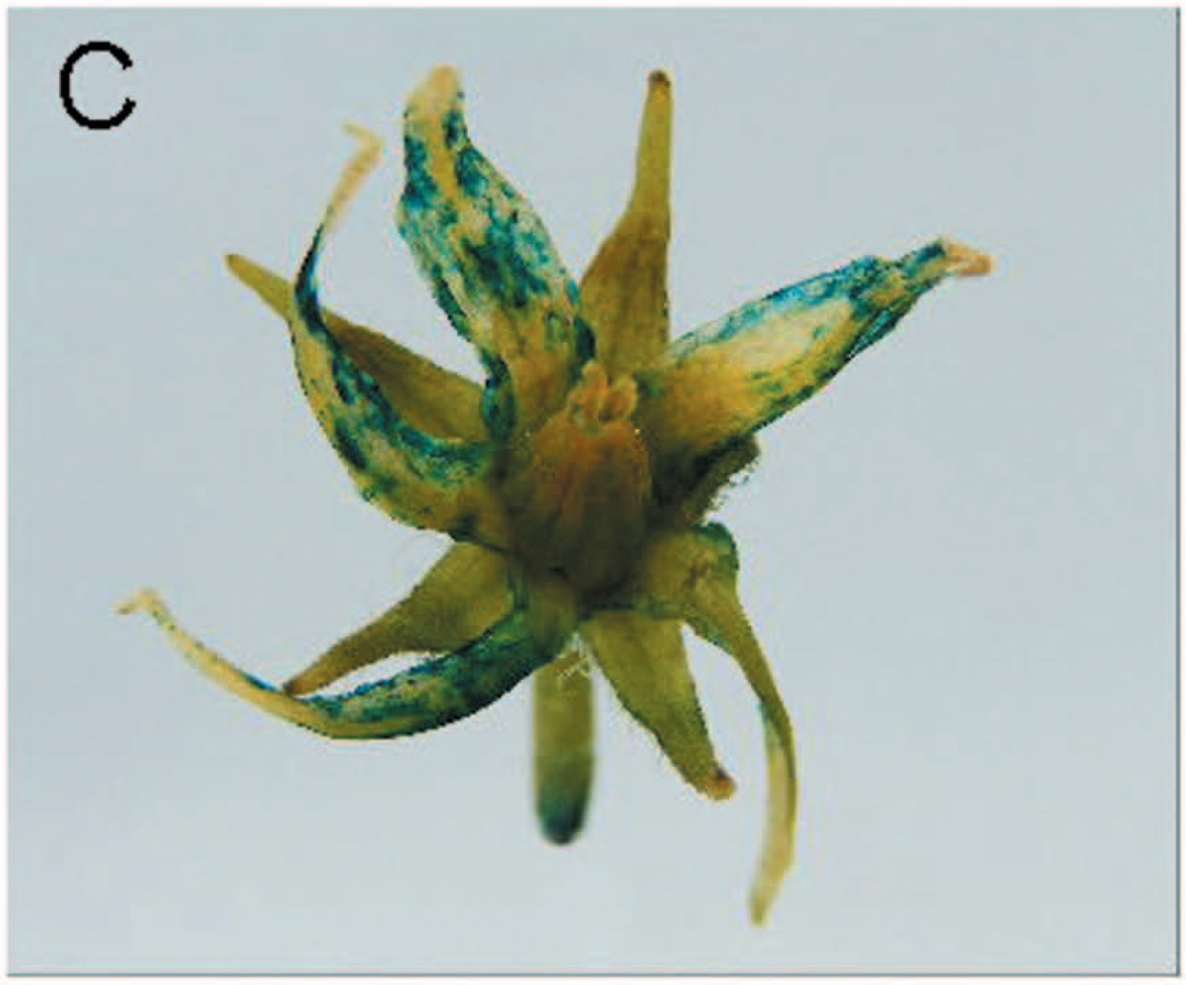


Figure S2.





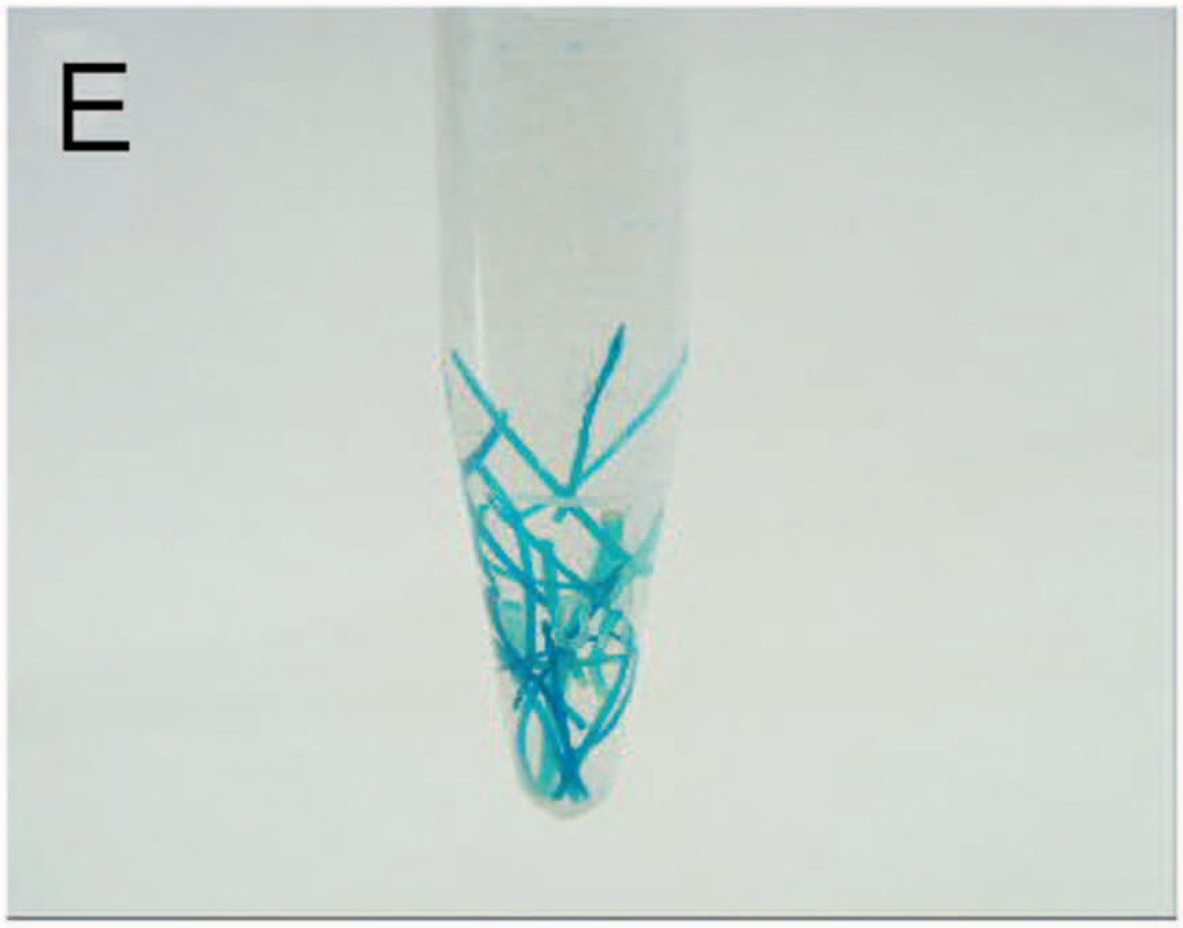


Figure S3.

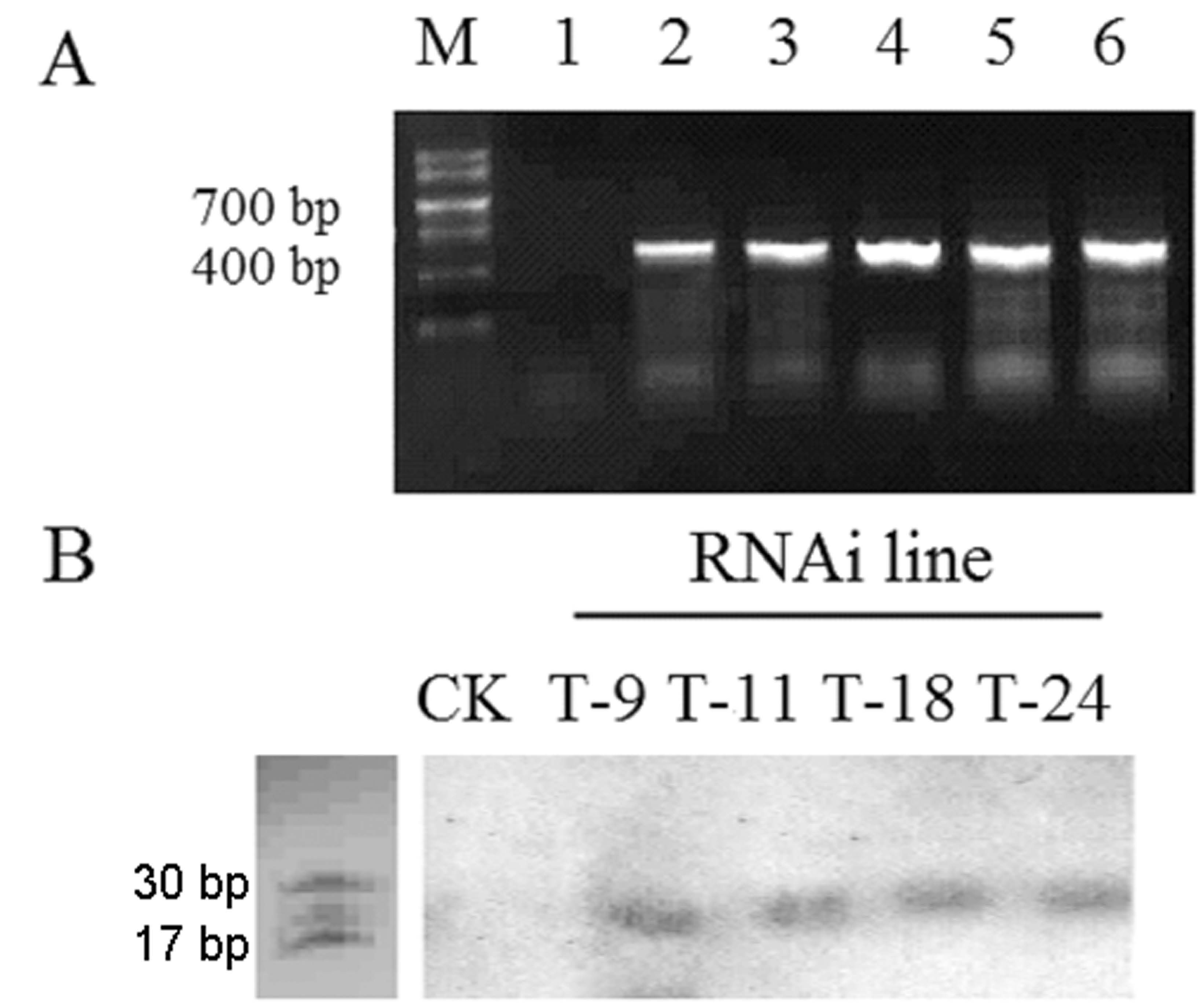
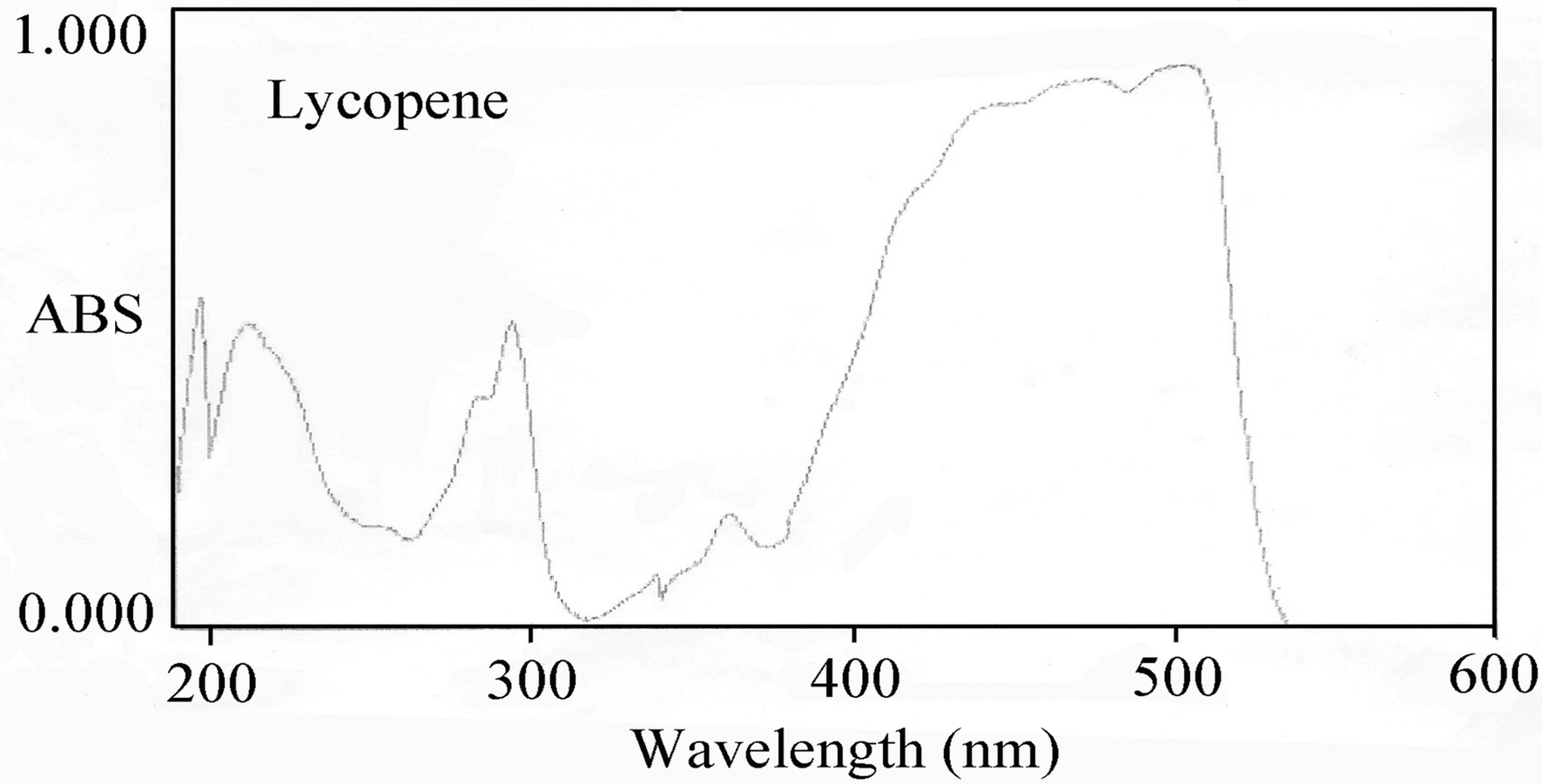


Figure S4.



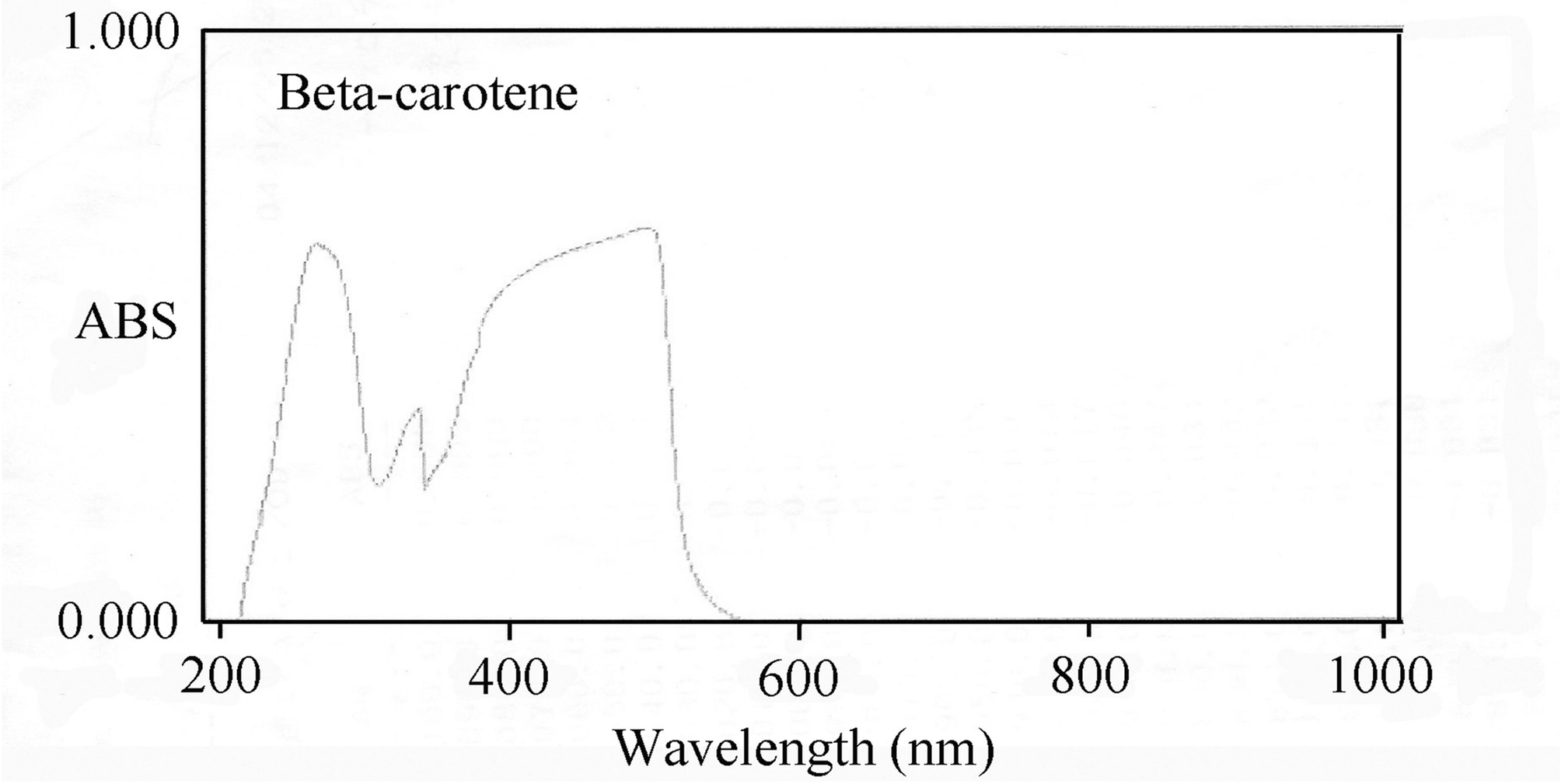
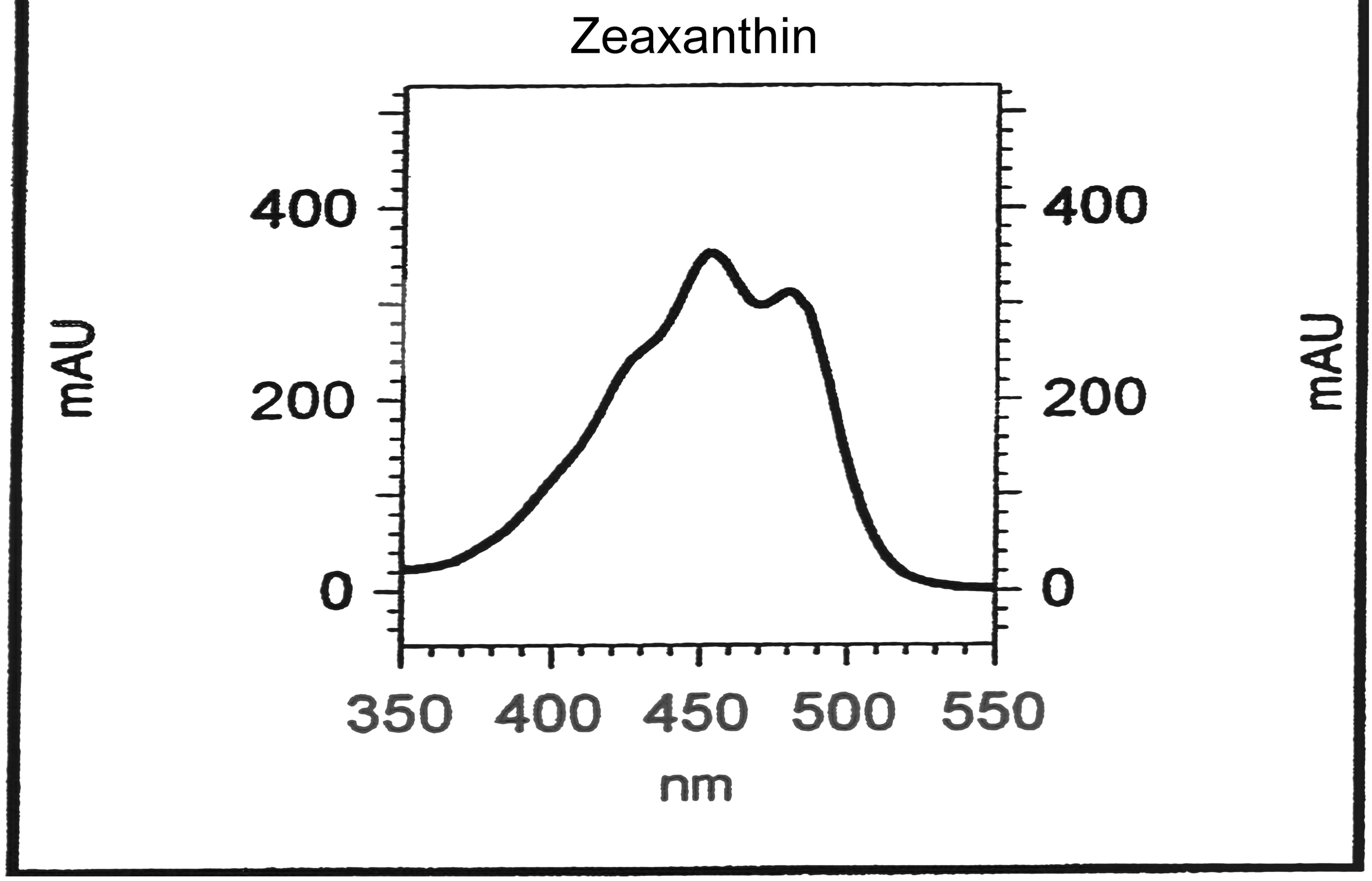


Figure S5. Wavelength scanning of standards for lycopene and beta-carotenoid.


Zeaxanthin
400
200
0
mAU
400
200
0
mAU
350 400 450 500 550
nm

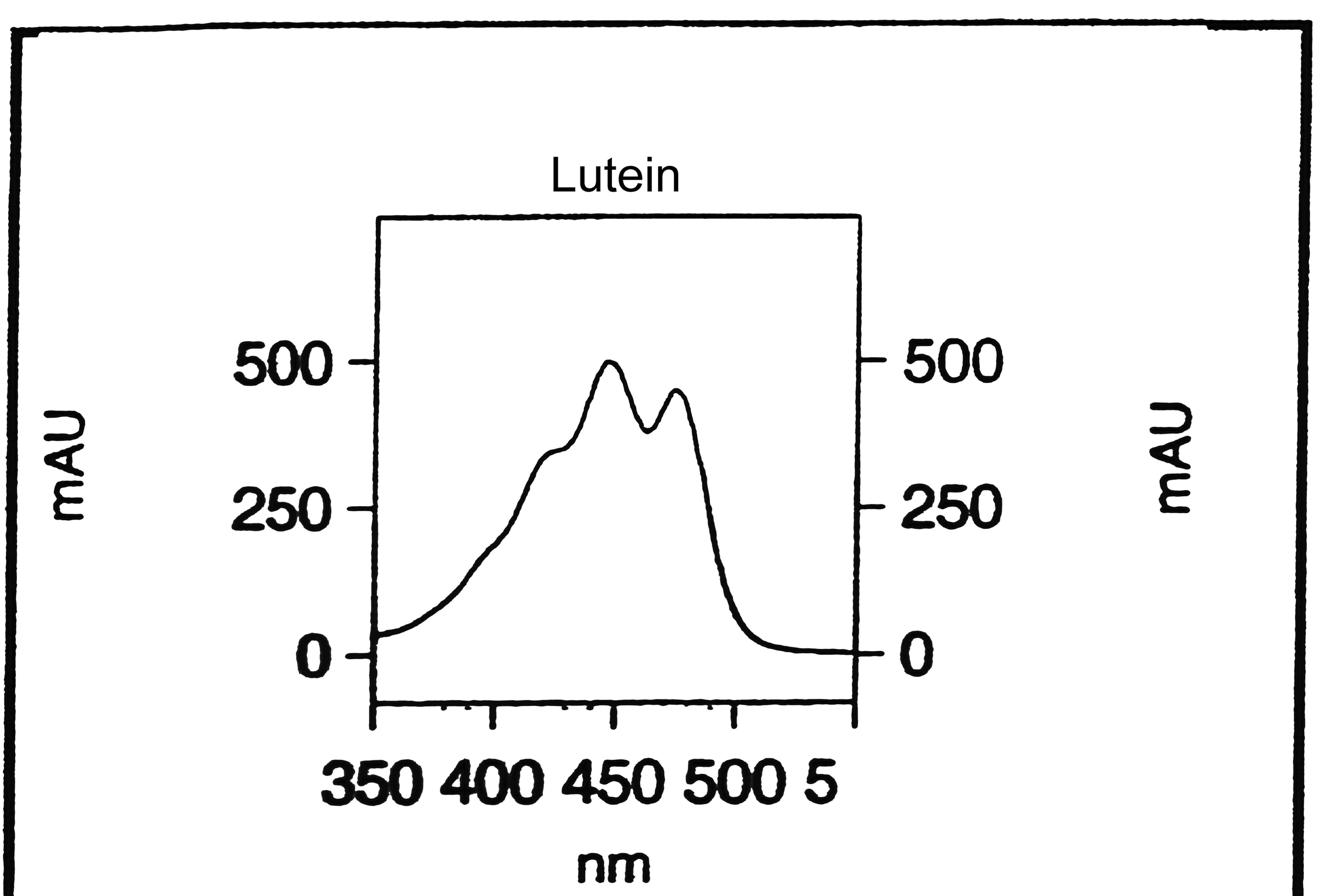

Lutein
500
250
0
mAU
500
250
0
mAU
350 400 450 500 5
nm

Figure S6

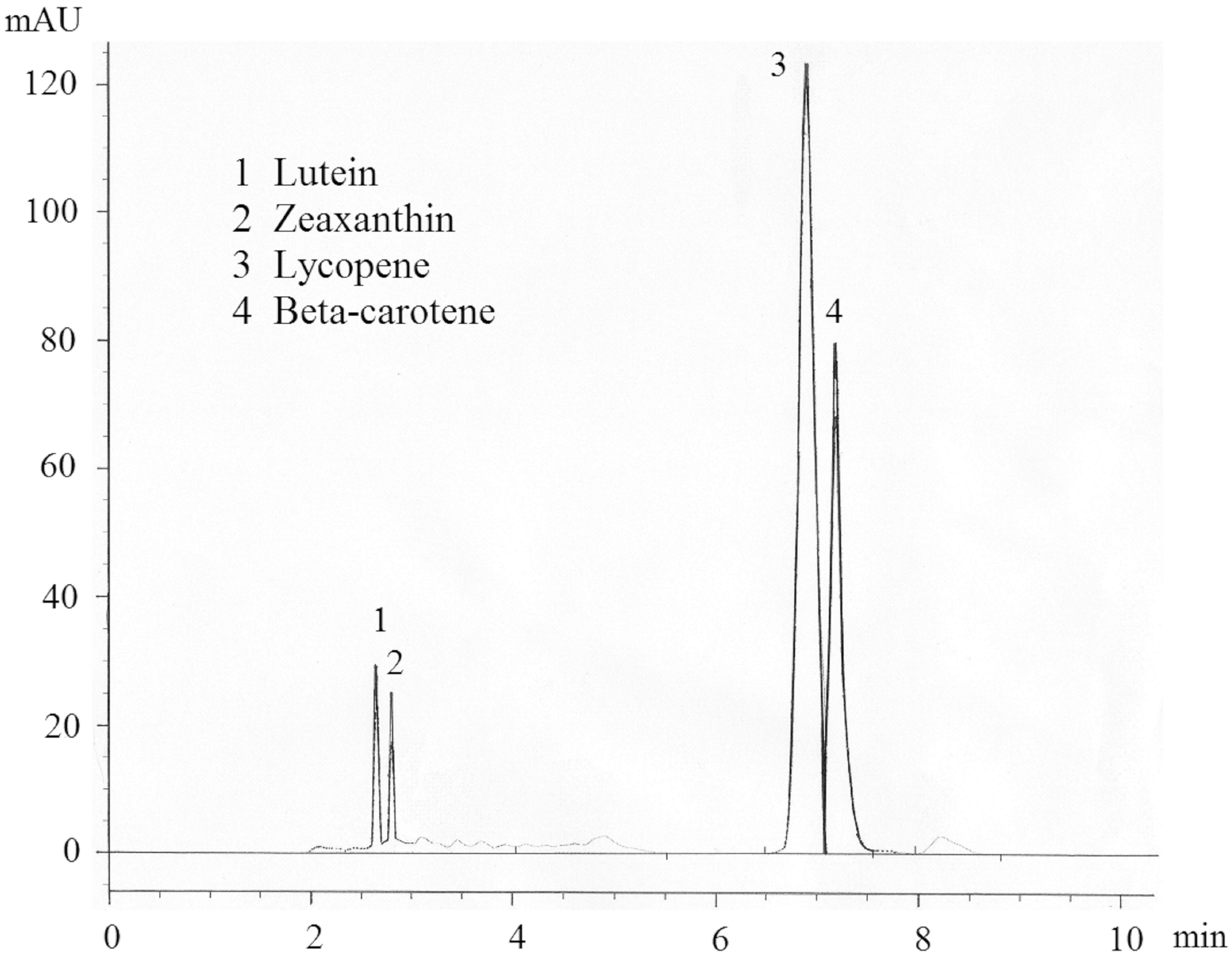


Figure S7. HPLC chromatogram of the four carotenoid standards.