

SUPPLEMENTARY DATA

Supplementary Figures:

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AtCOI1  1 medpdikrcklscvatvddvieqvmtyitdpkdrdsaslvcrrwfkidsetrehvtmalcytatpdrslrrfpnlrskl
NtCOI1  -----
                                     F-Box
AtCOI1  81 kgkpraamfnlipenwgggyvtpwvteisnnlrqlksvvhfrmrivsdldldrlakaraddletlkldkscsgfttdgllsiv
NtCOI1  -----

AtCOI1  161 thcrkikttlmeessfsekdgkwlhelaqhntslevlnfymtefakispkdletiarncrslsvskvgdfeilelvgffk
NtCOI1  1  -----tdlvqvraedlelmarncskslvsmkiseceelanllgffr

AtCOI1  241 aaanleefcggslnedigmp-----ekymnlvfpkrlcrlglsymgpnempilfpfaaqirkldllyalletedh
NtCOI1  40 aavaleefgggsfn-dqpepvaengyneqlekyaaavvspprlcqlgltylgkyempilfpfiasrltkldllyalldtaah

AtCOI1  311 ctliqkcpnlevletrnvigdrglevlaqyckqlkrlriergadeqgmedeeglvsqrqlialaqqcqeley mavyvsvdi
NtCOI1  119 cflrqrcpnlviletrnvvgdrglevlqyckrlkrlriergaddqmedeeggavthrgltdlakgcleley mavyvsvdi

AtCOI1  391 tneslesigtlyknlcdfrlvlldreeritdpldngvrsliligckklrrfafylrqqgltldglsyigqyspnvrwml
NtCOI1  199 tneafenigtlyknlcdfrlvlldreeritdpldngvrallrgcyklrrfalyvrpggltadvglsvgyrpspnvrwml

AtCOI1  471 gyvgesdeglmefsrqcpnlqklemrgccfseraiaaavtklpslrylwvgyrasmtgqdlmqmarpywnielipsrv
NtCOI1  279 gyvgesdegllefskgcpslqklevrgccfse-----

AtCOI1  551 pevngqgeiremehpahilayyslagqrtdcptttrvlkepi
NtCOI1  -----

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Fig. S1. Schematic diagram of the NtCOI1 region used for RNAi-mediated gene silencing. The RNAi vector was constructed using the *NtCOI1* cDNA fragment corresponding to the black boxed region in the alignment of NtCOI1 (AB433899) with AtCOI1 (AF036340). The blue boxed region indicates the F-box of AtCOI1.

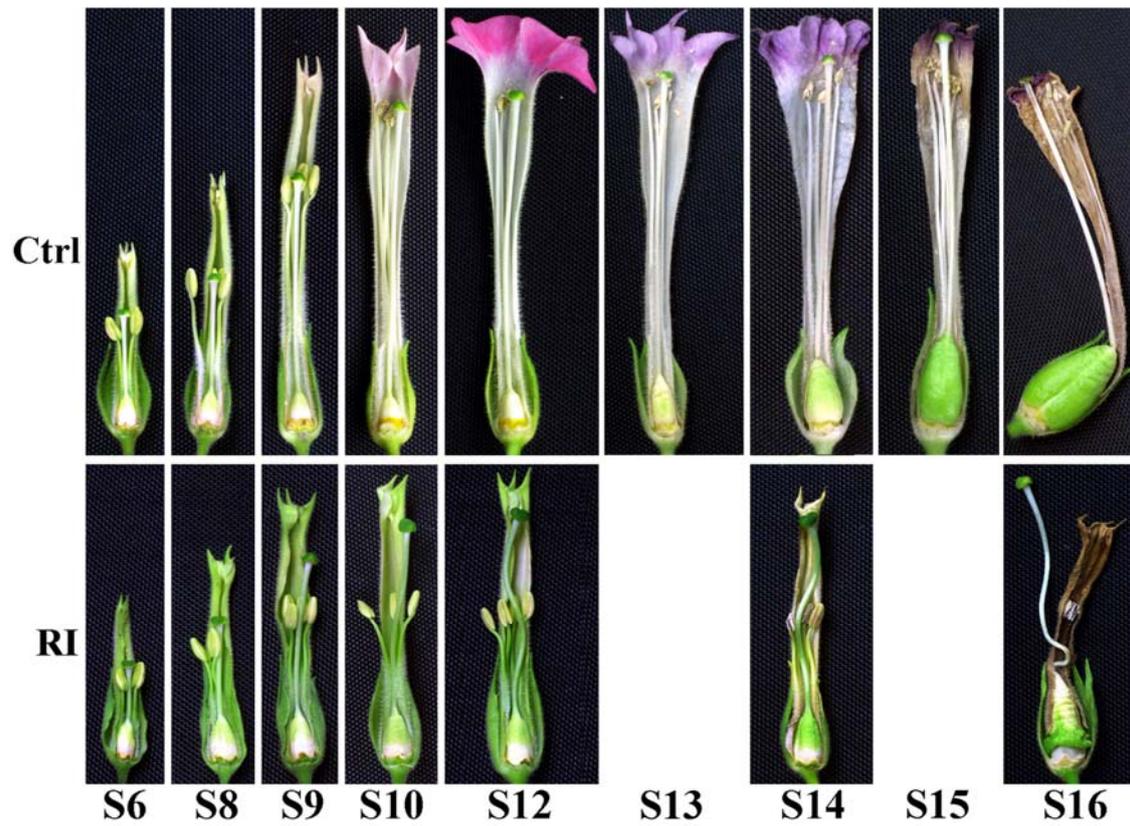


Fig. S2. Flower morphology of tobacco cv. TN90 (Ctrl) at different floral development stages. The *NtCOII*-silenced (RI) flowers at corresponding floral development stages are given for phenotype comparison.

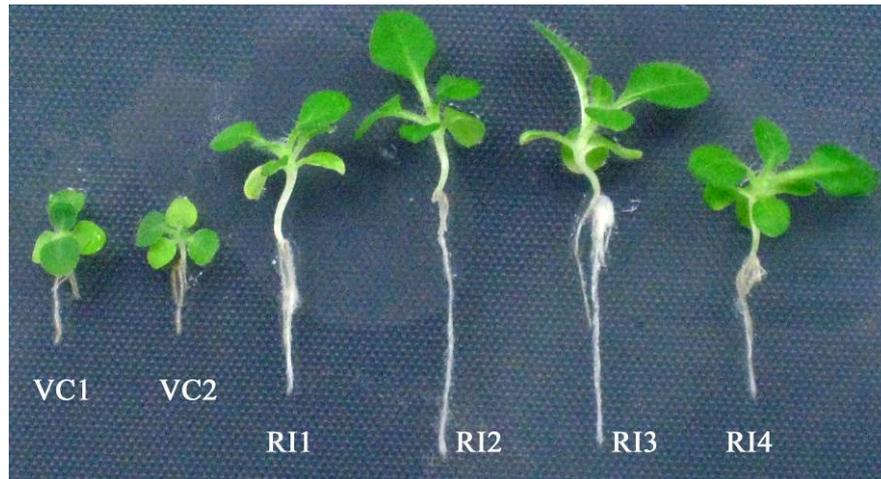


Fig. S3. JA-sensitivity assay for *NtCOII*-silenced tobacco seedlings.

Due to the male infertility of *NtCOII*-silenced (RI) plants, their T₁ seeds were generated by pollination using pollen grains from wild type plants. The prescreened hygromycin-resistant seedlings of RI and control vector transformed (VC) plants were used in this assay. Images of seedlings were taken after 20 days of growth on 1/2 MS agar plate containing 5 μ M MeJA. VC_N or RI_N indicate different independent transgenic lines.

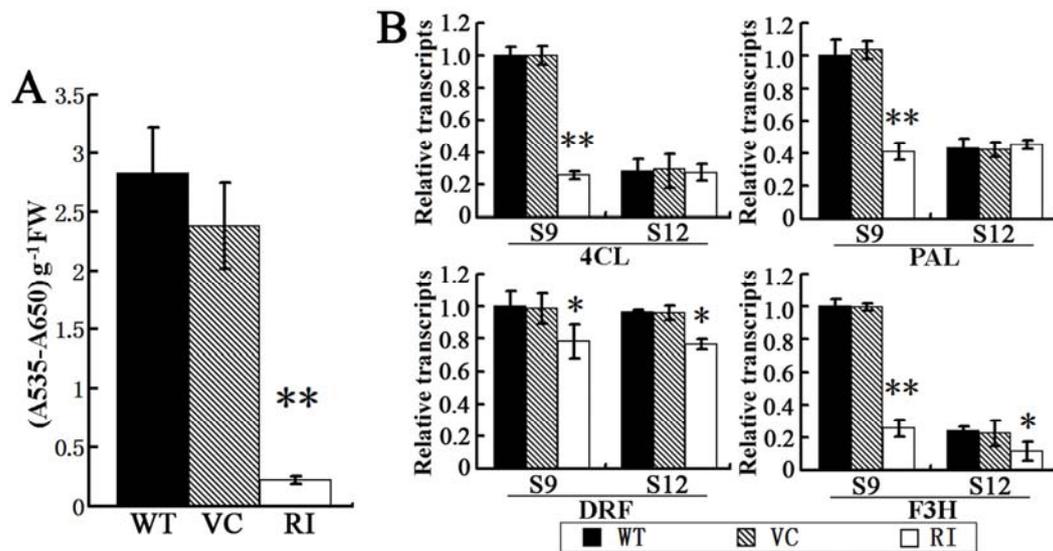


Fig. S4. Silencing of *NtCOII* down-regulated anthocyanin synthesis in tobacco corolla.

(A) Comparison of anthocyanin content in the corollas at S12. Anthocyanin in corolla was quantified as described in Supplementary Methods. WT, wild type; VC, control vector transformed; RI, *NtCOII*-silenced. (B) The relative transcript levels of anthocyanin synthetic genes in corollas at indicated floral development stages. Gene names and NCBI accessions: *4CL* (4-coumarate: coenzyme A ligase; U50845), *PAL* (phenylalanine ammonia-lyase; M84466.1), *F3H* (flavanone 3-hydroxylase; AF036093), *DRF* (dihydroflavonol 4-reductase; AB289448.1). The values for anthocyanin content and gene expression were determined based on three replicates, the value for VC is the average of three lines, and that for RI is the average of five lines. The transcription of each gene in S9 WT corollas is set as 1. Error bar, mean \pm SD. Asterisks indicate significant differences from the data in control plants ($*P < 0.05$, $**P < 0.005$, Student's *t* test).

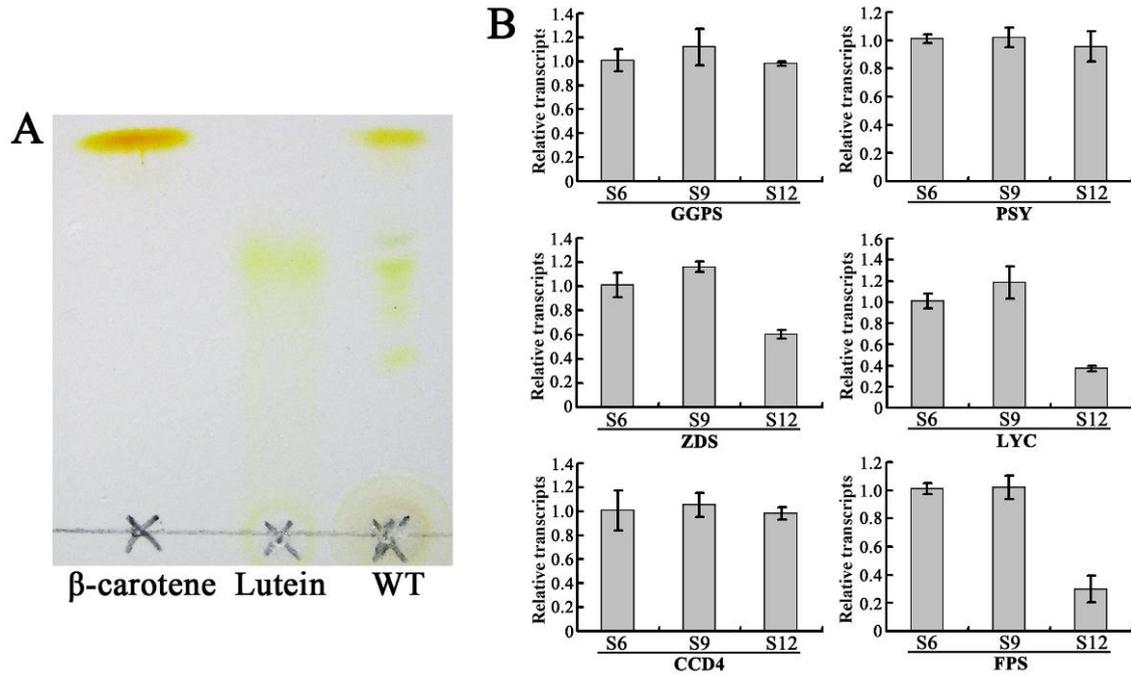


Fig. S5. Carotenoid assay and transcripts of carotenoid synthetic genes in floral nectary.

(A) TLC separation of β -carotene standard, lutein standard and, pigment extract from wild type (WT) nectary. (B) The relative transcript levels of carotenoid synthetic genes in WT nectaries at indicated floral development stages. Gene names and NCBI accessions: *FPS* (farnesyl pyrophosphate synthase; GQ410573.1), *GGPS* (geranylgeranyl diphosphate synthase; GQ911584.1), *PSY* (phytoene synthase; JF461341), *CCD4* (carotenoid cleavage dioxygenase; JF947192.1), *ZDS* (zeta-carotene desaturase; JF975566.1), *LYC* (lycopene cyclase; X81787.1). The values for gene expression levels were determined based on three replicates. The transcription of each gene in S6 nectaries is set as 1. Error bar, mean \pm SD.

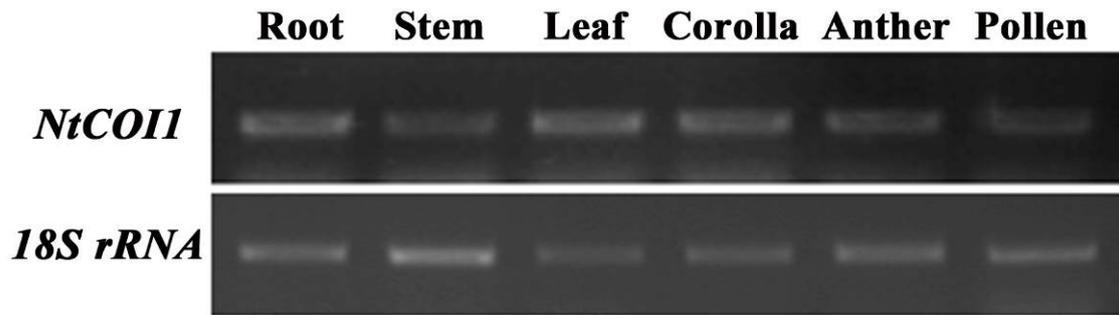


Fig. S8. Spatial expression patterns of *NtCOII*. *NtCOII* and *18S rRNA* (as an internal control) genes in indicated tobacco tissues were amplified for 25 cycles and 15 cycles respectively using primers same as those for qRT-PCR. The PCR products were separated on a 1.5% agarose gel with ethidium bromide, and visualized under UV light.

Supplementary Tables:

Table S1. Screening of hygromycin resistant progenies from *NtCOII*-silenced tobacco produced by pollination with wild type pollen grains.

Transgenic line	RI1	RI2	RI3	RI4	RI5
Number of hygromycin resistant seedlings	44	53	37	55	38
Number of screened seeds	89	91	84	112	74

Table S2. Oligonucleotide primers used for qRT-PCR.

Gene	Forward Primer	Reverse Primer
<i>NtCOII</i>	5'-ACAGCAGCCATTGTTTCTT-3'	5'-GGACATAGAGGGCAAACCTT-3'
<i>4CL</i>	5'-GAAGACGCTGTGAGAACCAA-3'	5'-TCAGTGATGGCAGATCCATT-3'
<i>PAL</i>	5'-TGGTAGGCCTAATCCAAGG-3'	5'-GAAGTTGCCACCATGTAACG-3'
<i>F3H</i>	5'-GACGAGGCTAGCGAGAGAGT-3'	5'-GGTGGTCCGCATTCTTAAAT-3'
<i>DRF</i>	5'-ATTCCTCACGCCTACATTCC-3'	5'-CTGCTGTGCTTTGGGTAGAA-3'
<i>FPS</i>	5'-ATACACGTCGAGGTCAACCA-3'	5'-CCAACCAAGAGCACTTGAAA-3'
<i>GGPS</i>	5'-AAAGAGTGGTTCAAGCCGTT-3'	5'-TGGCAGCATCAAAGTAGCTC-3'
<i>PSY</i>	5'-TGCTTTGTTGTGGGTGTTT-3'	5'-ACTCAACAAGCCCAAATTCC-3'
<i>CCD4</i>	5'-TTATTACCCTCGGCCGTTAC-3'	5'-TCACCATCCCCTCTTCAAA-3'
<i>ZDS</i>	5'-AACATTATCGAGGGCCAAAG-3'	5'-AAGGGCAAGAGCTACAGCAT-3'
<i>LYC</i>	5'-GTTCTCCACTTCTGCCAAT-3'	5'-GAATTTGAGGCCATGGATTT-3'
<i>AGPs</i>	5'-GCTTTCTACAATGCCAATTTGG-3'	5'-ACTGTCTGTGACATCGGCAT-3'
<i>SS2</i>	5'-GGCATACTGCTTTATTGCCA-3'	5'-TGGTGGAAGATCTACGTATGAAA-3'
<i>BAM1</i>	5'-AGCGTGTC AAGCACTAATGG-3'	5'-CTGTCCAACGGCATCATAAC-3'
<i>Nec1</i>	5'-GATGAGGACATGCTCCAAGA-3'	5'-CACCACTGTTTCATGCACAA-3'
<i>INV1</i>	5'-CATCAAGGGTTTATCCGACA-3'	5'-TTGCAACTAGAGCAGTTCGG-3'
<i>INV2</i>	5'-CGGATATCAGCATACCAAG-3'	5'-GTAGTACTCAAACGCGCAA-3'
<i>INV3</i>	5'-AATGGATCAAGCCCGATAAC-3'	5'-GGACATTCCCAATTTCTGT-3'
<i>INV4</i>	5'-GAGTGCTCATTGGAAGCAA-3'	5'-AGTCGTTTCATGAAACCCACA-3'
<i>INV5</i>	5'-ATGCACAATCCGAAACATA-3'	5'-CCCAAGCAGTAGTTGGGTCT-3'
<i>NtMYB305</i>	5'-CATGGTGAAGGTGTTTGGAA-3'	5'-CATCAGGCCGGAGATAATTT-3'
<i>18S</i>	5'-GAAAGACGAACAACCTGCGAA-3'	5'-GAAGGATACCTCCGCATAG-3'

Supplementary Methods:

Anthocyanin quantification

Anthocyanin was quantified as described by Deikman and Hammer (1995). Pre-weighed fresh tobacco corollas were boiled in 2 ml of extraction buffer (18% 1-propanol, 1% HCl, and 81% water) for 3 minutes, and then incubated in darkness overnight at room temperature.

The absorbances (A_{535} and A_{650}) of the extracts were spectrophotometrically measured. The anthocyanin content was expressed as $(A_{535} - A_{650}) \text{ g}^{-1}$ fresh weight (FW). Three replicates were measured for each plant line.

Supplementary References:

Deikman J, Hammer PE. 1995. Induction of anthocyanin accumulation by cytokinins in *Arabidopsis thaliana*. *Plant Physiol* **108**, 47-57.