Supporting Information

Coexpression of methyltransferase gene *dmt50* and methylene tetrahydrofolate reductase gene increases *Arabidopsis thaliana* dicamba resistance

Le Chen[a], Shigang Yao[a], Tao Chen[a], Qin Tao[b], Xiangting Xie[b], Xiang Xiao[b], Derong Ding[b], Qin He[a *], Jian He[a]

[a] Key Laboratory of Agricultural Environmental Microbiology, Ministry of Agriculture, College of Life Sciences, Nanjing Agricultural University (210095), Nanjing, Jiangsu, China.

[b] Beijing DBN Biotech Co., Ltd. (100080), Beijing, China.

[*] Address correspondence to Qin He, qhe@njau.edu.cn;

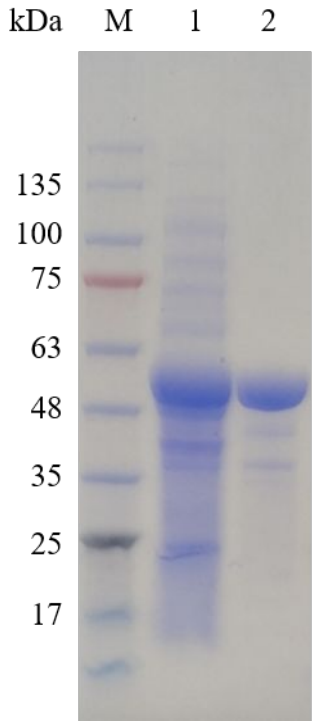
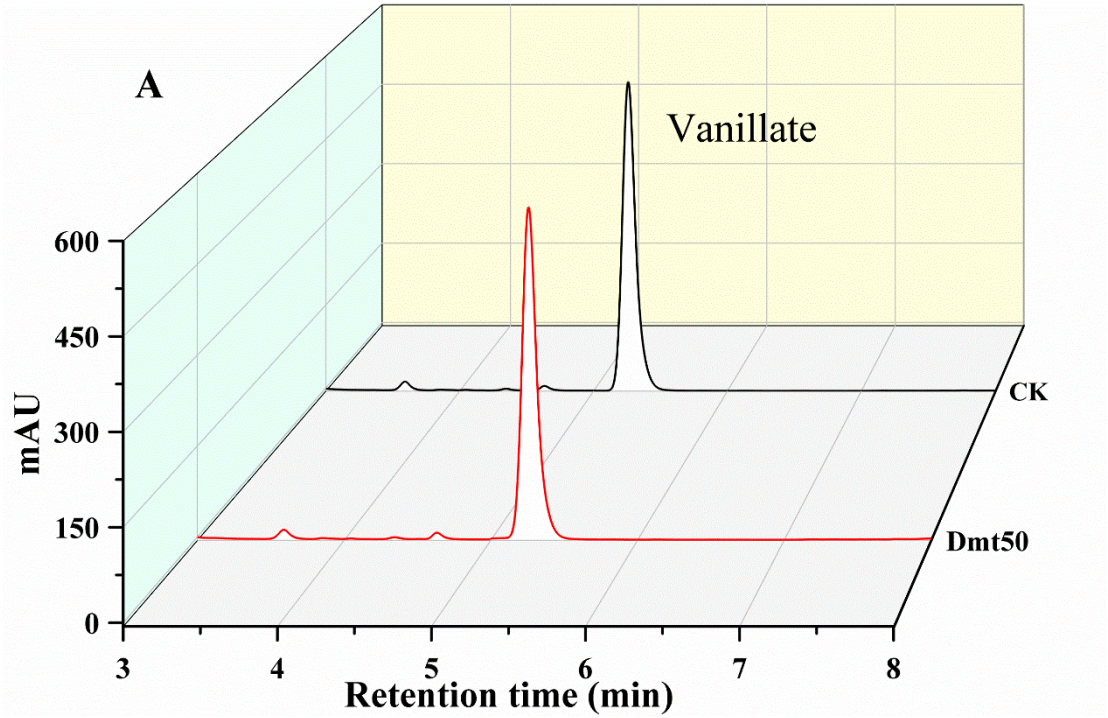
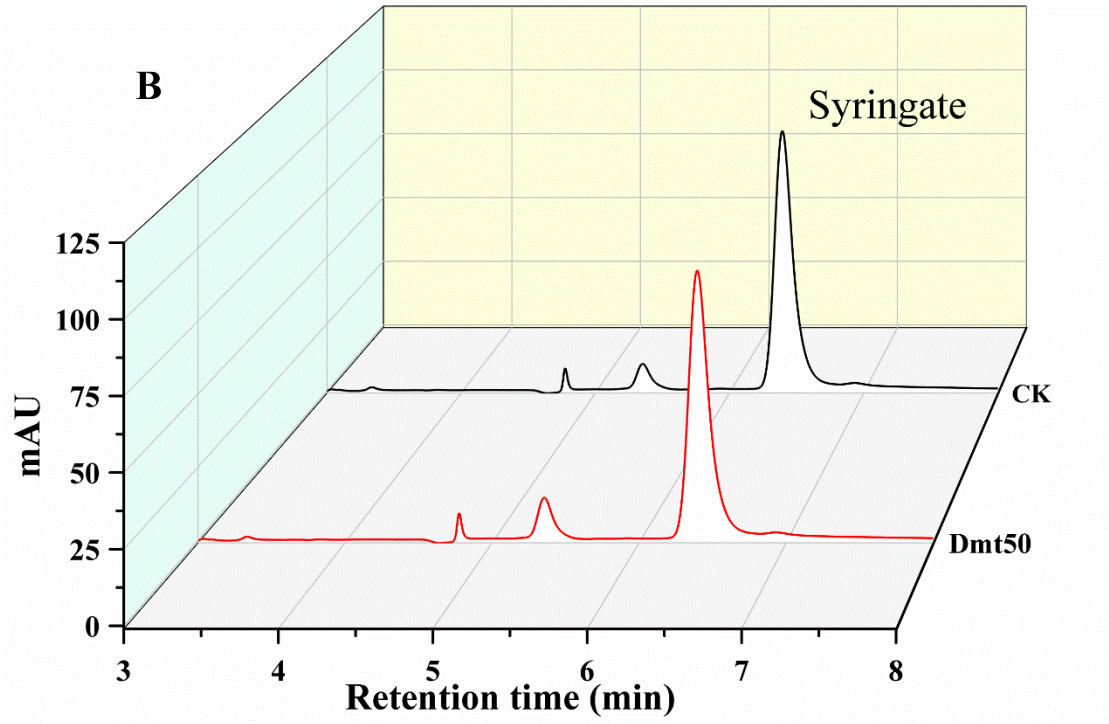
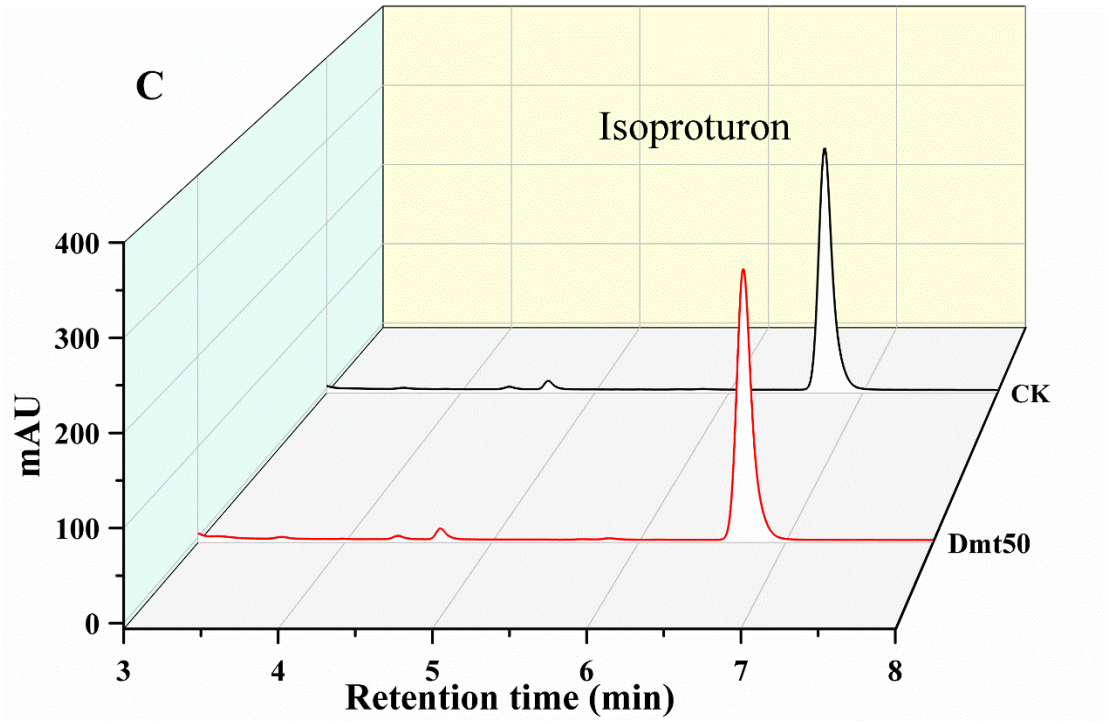


Figure S1. SDS-PAGE of Dmt50 heterogenous expressed in *E. coli* BL21 (DE3). M:

Marker; 1: The cell lysate supernatant of *E. coli* BL21 (DE3) carrying *dmt50* expression vector; 2: Purified Dmt50. The molecule weight of Dmt50 (His_6-tagged) was approximately 53 kDa.




B
Syringate
125
100
75
50
25
0
mAU
CK
Dmt50
3
4
5
6
7
8
Retention time (min)


C
Isoproturon
400
300
200
100
0
mAU
CK
Dmt50
3
4
5
6
7
8
Retention time (min)

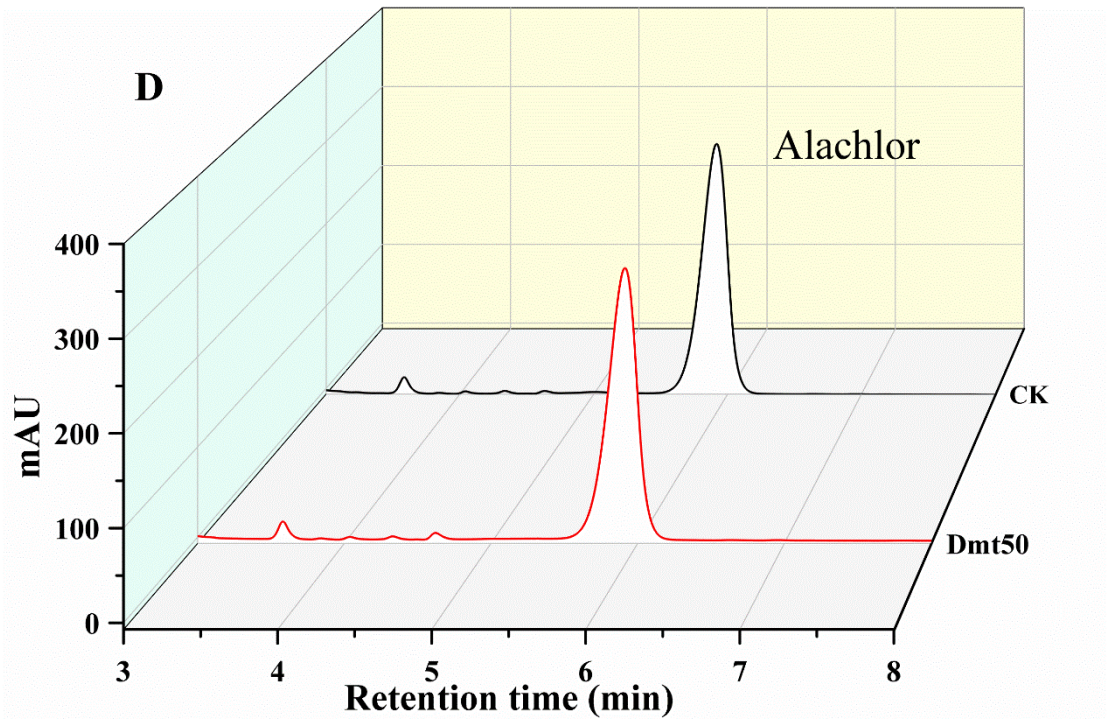


Figure S2. HPLC analysis of vanillate (A), syringate (B), isoproturon (C) and alachlor (D) degradation by Dmt50. "Dmt50": The mixture containing 0.1 mg of purified Dmt50, 2.0 mM THF and different subtract was added. The total volume was 300 μL and the reaction was incubated at pH 8.0, 45°C for 120 min. "CK": no enzyme was added, as control. Mobile phase and detection wavelengths: A, 10% acetonitrile, 90% water (contain 0.1% formic) 259 nm; B, 15% methanol, 85% water, 241 nm; C: 15% methanol, 85% water, 285 nm; D, 70% acetonitrile, 30% water, 210 nm. Other analysis conditions were as same as the dicamba detection.

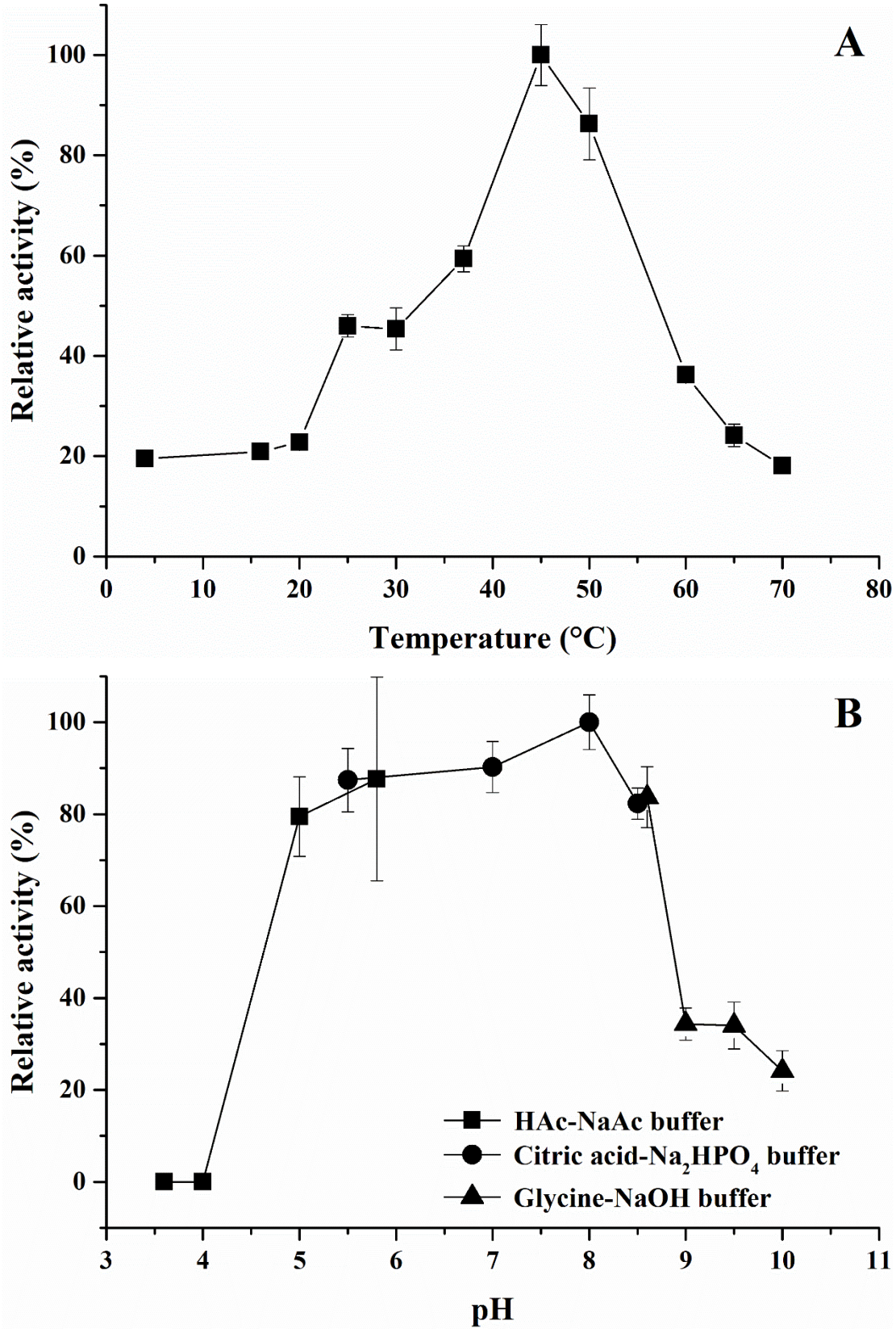


Figure S3. The relative demethylase activity of Dmt50 under a series of temperatures (A) and pH (B).

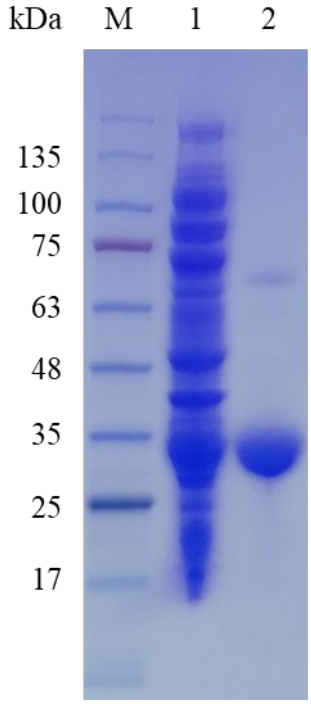


Figure S4. SDS-PAGE of Mthfr66 heterogenous expressed in *E. coli* BL21 (DE3). M: Marker; 1: The cell lysate supernatant of *E. coli* BL21 (DE3) carrying *mthfr66* expression vector; 2: Purified Mthfr66. The molecule weight of Mthfr66 (His_6-tagged) was approximately 30 kDa.

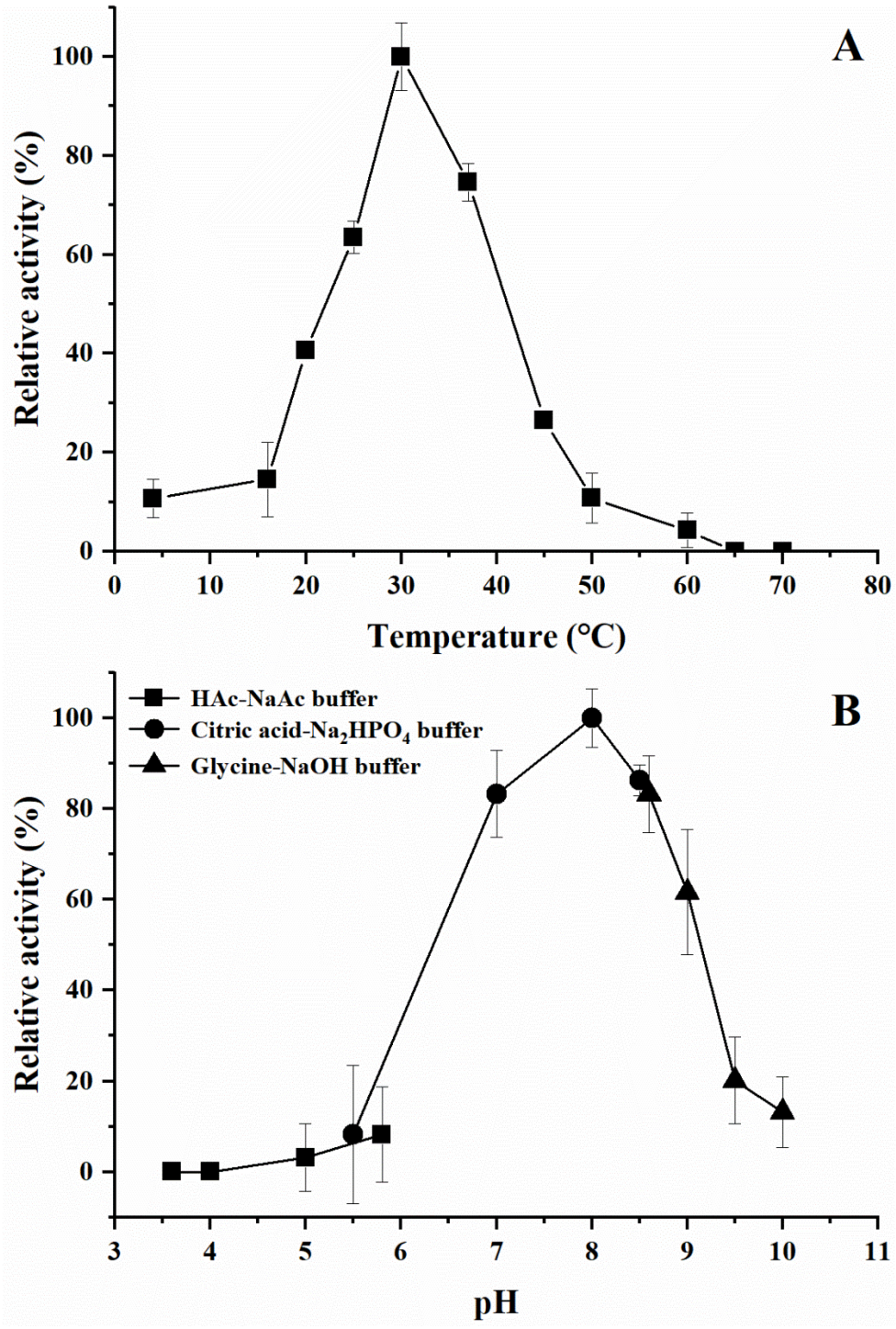
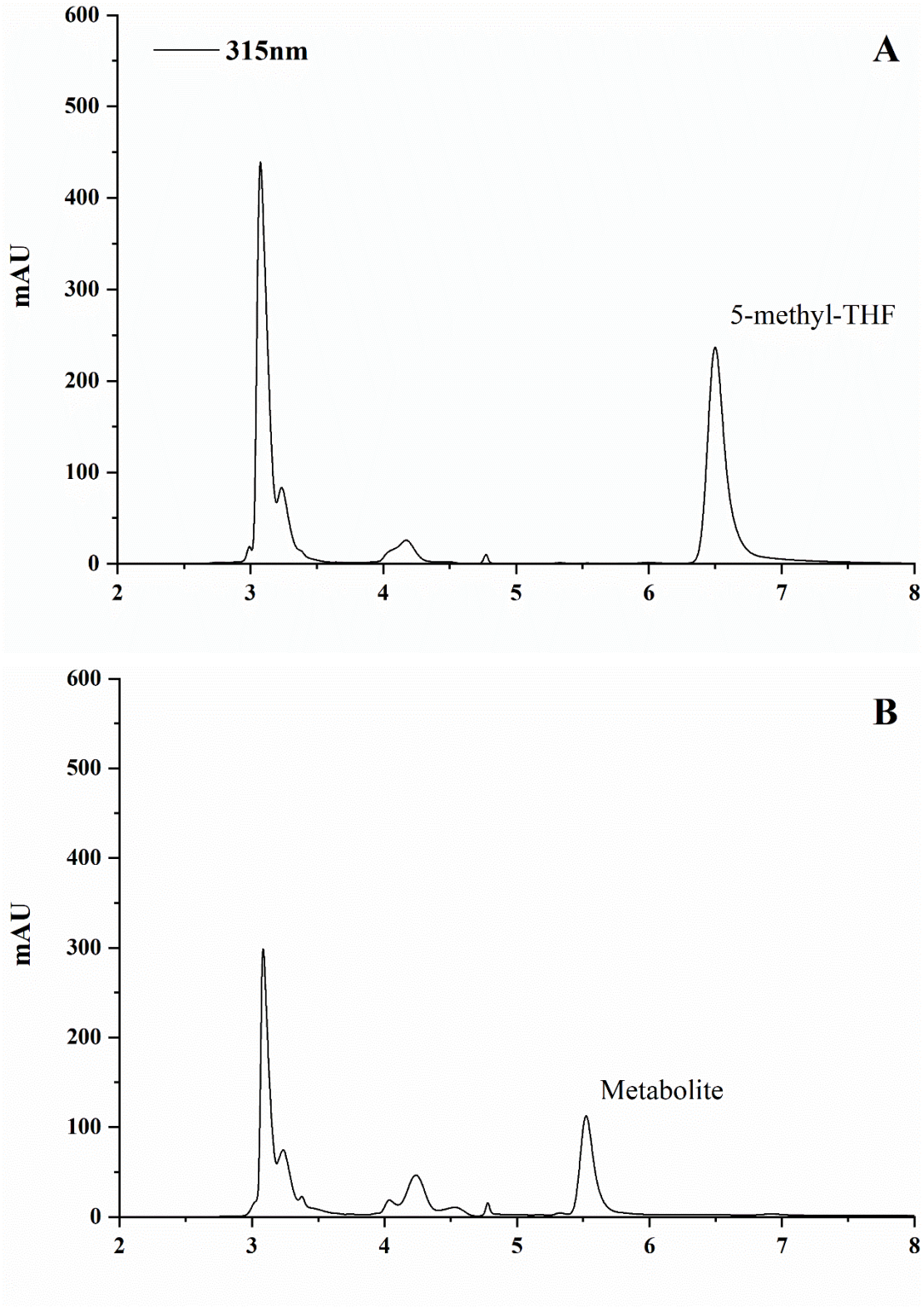


Figure S5. The Mthfr66 relative activity of 5-methyl-THF conversion under a series of temperatures (A) and pH (B).


315nm
A
mAU
600
500
400
300
200
100
0
2
3
4
5
6
7
8
5-methyl-THF
B
mAU
600
500
400
300
200
100
0
2
3
4
5
6
7
8
Metabolite

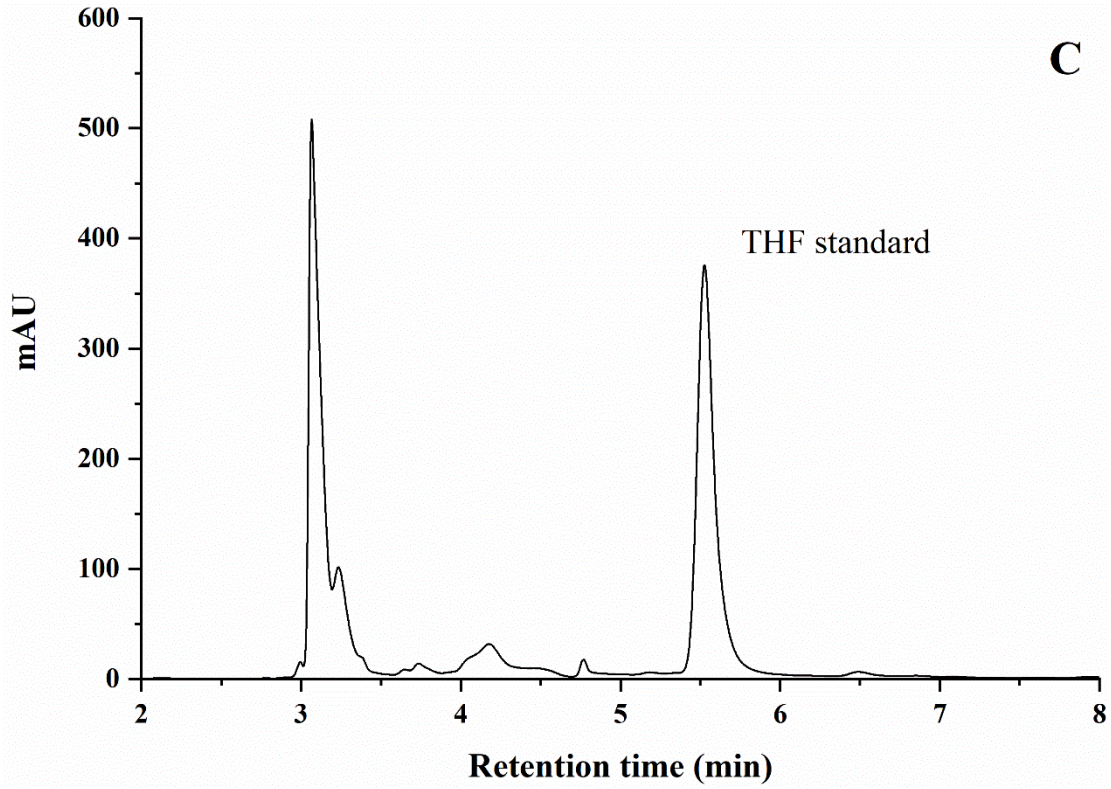


Figure S6. HPLC analysis of the products generated during 5-methyl-THF conversion by Mthfr66. The mixture containing 1.0 mM NAD^+, 1.0 mM menadione and 100 mM Tris-HCl buffer (pH 8.0) in a 300 μL volume and the reaction was incubated at 30 °C for 120 min. A, contains 1.0 mM 5-methyl-THF and no enzyme was added; B, contains 1.0 mM 5-methyl-THF and 0.1 mg of purified Mthfr66 was added; C, contains 3.5 mM THF standard and no enzyme was added, shows a peak with the same retention time as the metabolite peak. The reactions were terminated by boiling at 100 °C for 1 min, then the mixture was filtered through a 0.22 μm Millipore membrane to remove protein precipitate produced during boiling. The filter liquor was diluted 5 times with 0.1 M KH_2PO_4 (pH 6.8, contain 1% ascorbic acid and 0.1% β-mercaptoethanol). Detection wavelengths 315 nm was used for 5-methyl-THF, THF and potential metabolic detection. The mobile phase was a mixture of 0.05 M KH_2PO_4 (pH 3.0, 90%) and acetonitrile (10%).

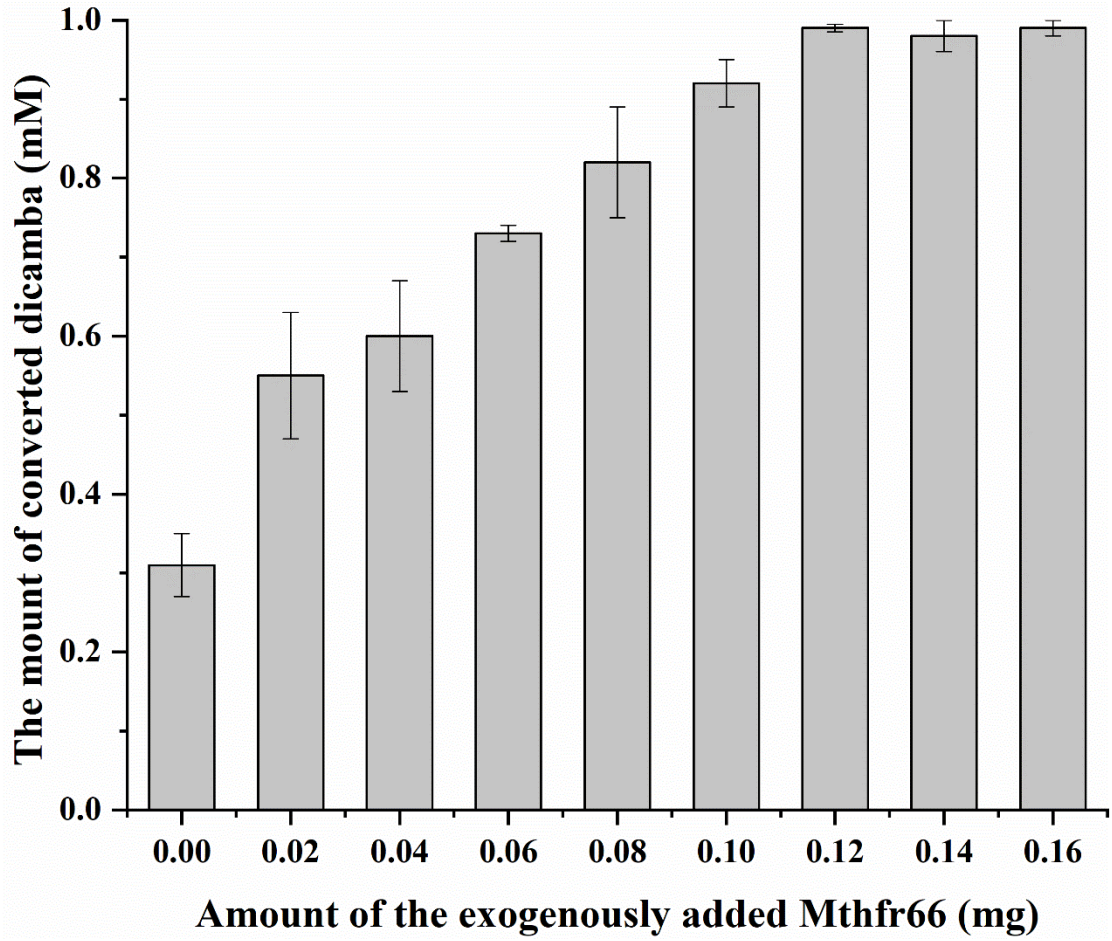


Figure S7. Effect of exogenously added Mthfr66 on the conversion of dicamba. The mixture containing 0.1 mg of purified Dmt50, 2.0 mM THF, 1.0 mM dicamba and different amount of Mthfr66. The total volume was 300 μL and the reaction was incubated at pH 8.0, 30°C for 120 min.

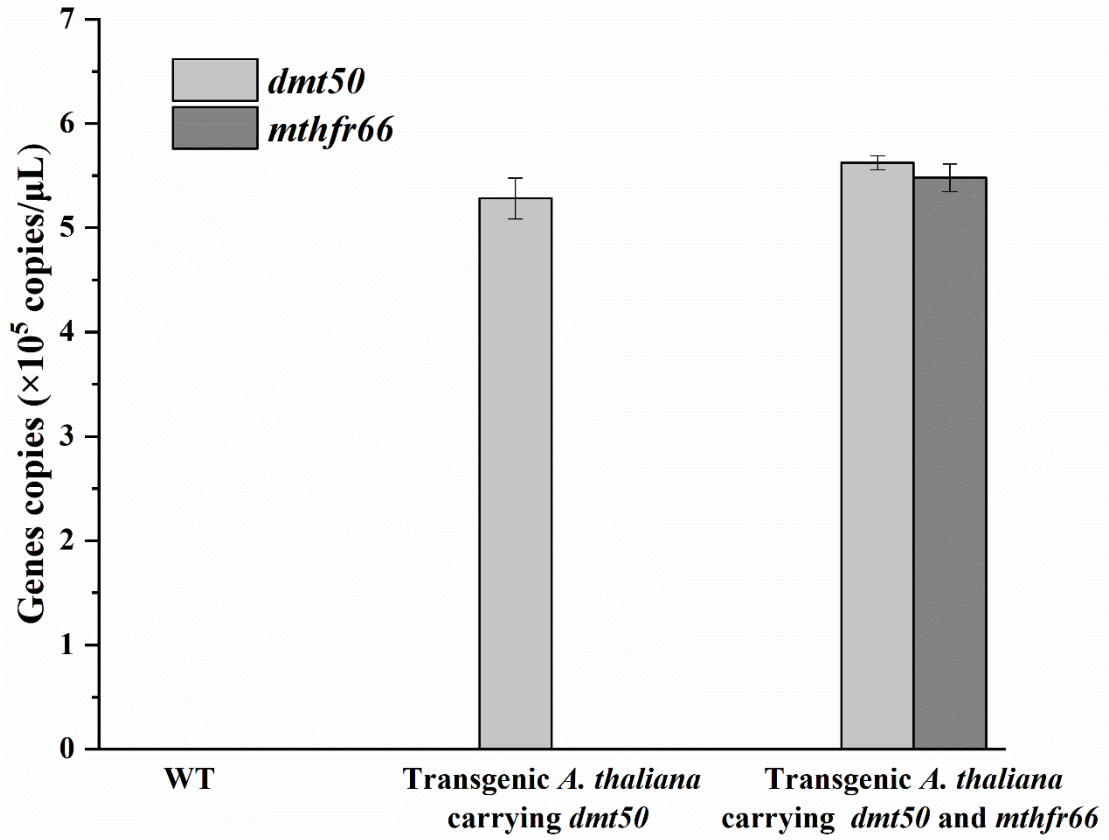


Figure S8. Real time-qPCR analysis of *dmt50* and *mthfr66* transcription in transgenic *A. thaliana*. "WT": wild type; Data are presented as the means ± SE of 2 independent determinations.

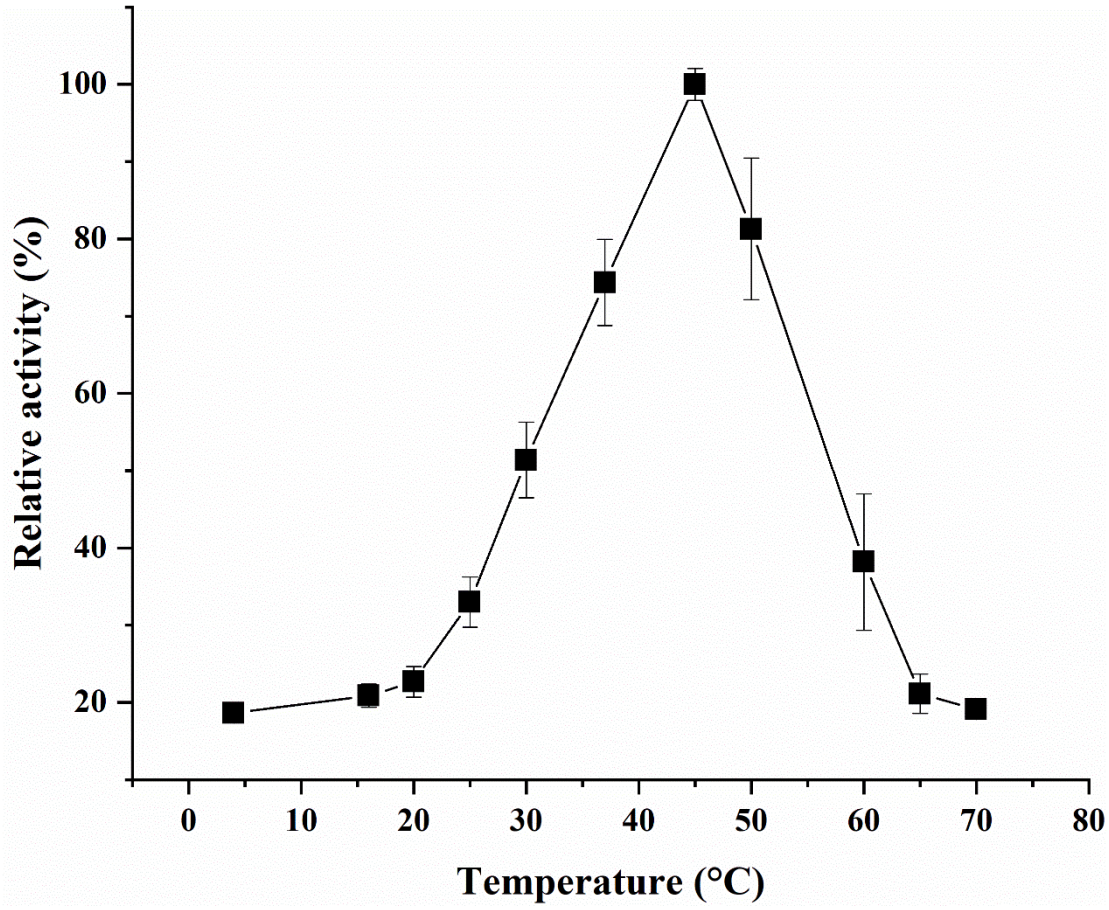


Figure S9. Effects of temperature on the dicamba demethylase activities of the crude lysates from *dmt50* transgenic *A. thaliana*. The relative activity was calculated by assuming that the activity observed at 45°C was 100%.

Table S1. Deduced function of each ORF within the scaffold 50 of the strain Ndbn-20

ORF in *scaffold 50*	Product size (amino acids)	Homologous protein (UniProtKB/Swiss-Prot/GenBank accession no.) and source	Identity (%)
orf0801	298	Methylenetetrahydrofolate dehydrogenase (A5V4U1.1), *Sphingomonas wittichii* RW1	75
orf0802	763	TonB-dependent receptor (WP_066970334.1), *Sphingomonadaceae*	58
orf0803	279	5,10-methylenetetrahydrofolate reductase (WP_084032862.1), *Chelativorans* sp. J32	52
orf0804	287	Formyltetrahydrofolate deformylase (WP_056451703.1), *Sphingomonas* sp. Leaf10	68
orf0805	475	Syringate *O*-demethylase (BAK67175), *Sphingobium paucimobilis* SYK-6	46
orf0806	494	MFS transporter (WP_068082783.1), *Novosphingobium rosa*	53