

## Supplementary Data

**Title:**

Activating glutamate decarboxylase activity by removing the autoinhibitory domain leads to hyper  $\gamma$ -aminobutyric acid (GABA) accumulation in tomato fruit

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**Authors:**

Mariko Takayama, Chiaki Matsukura, Tohru Ariizumi, Hiroshi Ezura

**Corresponding author:**

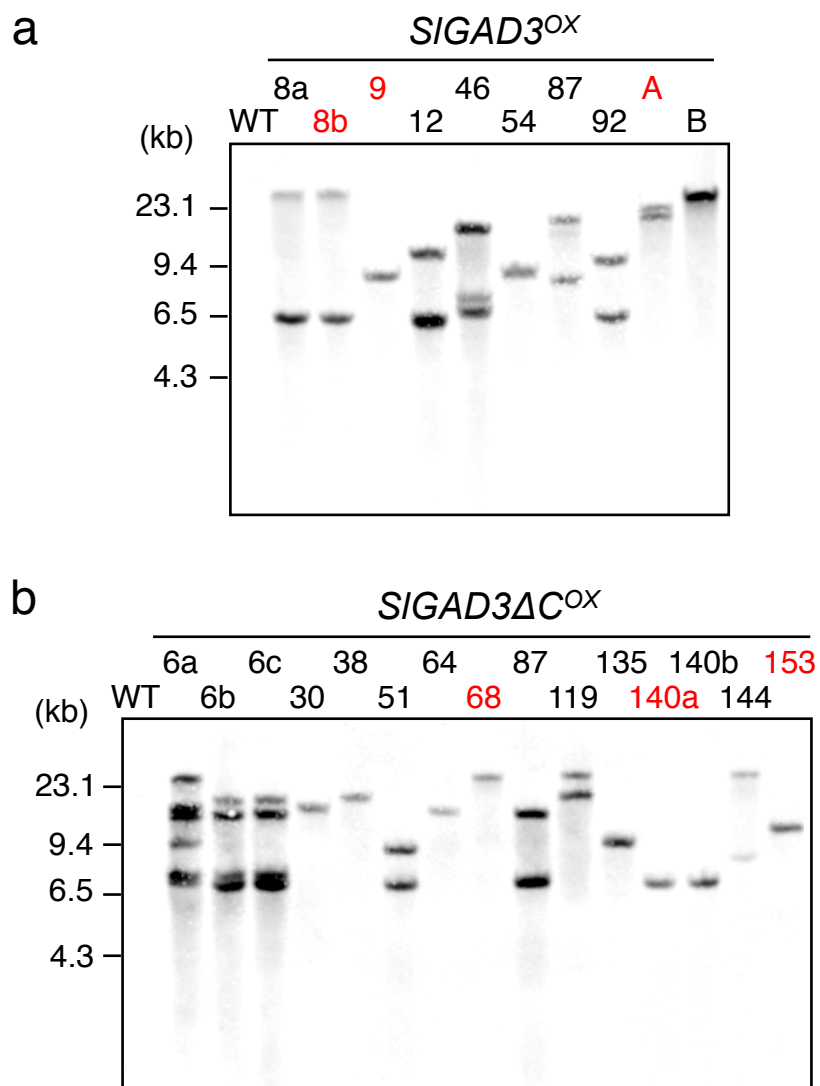
Hiroshi Ezura

Faculty of Life and Environmental Sciences, University of Tsukuba,  
Tennodai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan

[ezura@gene.tsukuba.ac.jp](mailto:ezura@gene.tsukuba.ac.jp)

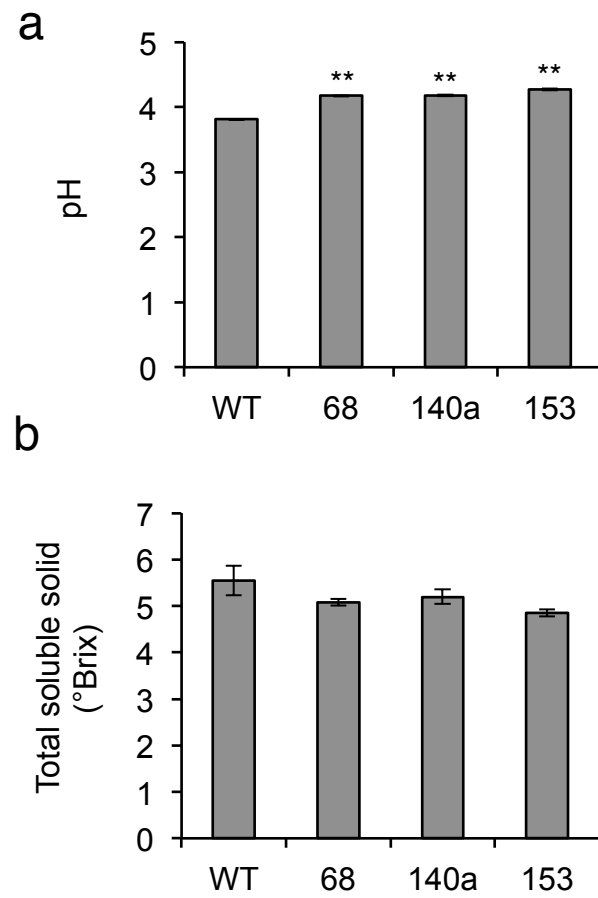
ATGGTTCTCTCTCAAAAACCTCCTTCTGATGATTCCTGTACACTCCACATTTGCTTCTCGCTATGTTCTGAACTT  
**SIGAD3-F**  
 CACTACCAAGGTTTGAGATGCTAGAGAACTCTATACCCAAAGAGGCAGCATACCAAATGATTAATGATGA  
 GTTAATGCTTGATGGGAATCCAAGGTTAAATTTGGCATCATTTGTAACCACATGGATGGAACCAGAATGT  
 GATAAGCTTATGATGGCTTCAATTAACAAGAATTATGTTGACATGGATGAATACCCTGTCACCACTGAGC  
 TTCAGAATCGATGTGTAAACATGATAGCGCGTTTATTCAATGCGCCTTTGAAAGAGGAAGAAATAGGAAT  
 TGGTGTGGGGACAGTGGGGTCATCAGAGGCCATAATGTTAGCAGGGCTGGCCTTCAAGAGGAACTGGCAA  
 AACAAACGCAAAGCTGAGGGAAAGCCTTATGATAAGCCCAACATTGTCACTGGTGCTAATGTTTCAGGTGT  
 GTTGGGAGAAATTTGCAAACCTACTTTGAAGTGAATTGAAACAAGTCAAGTTAAGTGAAGGGTACTATGT  
 GATGGACCCAATCAAAGCTGTGGAAATGGTAGATGACAACACTATTTGTGTTGCTGCTATTTTGGGTTCA  
 ACACTTAATGGAGAATTTGAAGATGTCAAACCTCTTGAATGATCTTTTGATTGAAAAGAATAAACAAACTG  
 GATGGGACACACCTATTCATGTGGATGCAGCAAGTGGTGGATTCAATGCACCATTTATCTATCCAGAGTT  
 GGAATGGGATTTTAGGCTTCCTTTAGTGAAAAGTATTAATGTGAGTGGACACAAATATGGGCTTGTTTAT  
 GCTGGTATTGGTTGGGTATTTTGAGAACTAAACAAGACTTGCCTCAACAACTCATTTTTTCATATCAATT  
 ATCTTGGTGCTGATCAGCCTACTTTTACTCTCAATTTCTCTAAAGGTTCAAGTCAAGTCATTGCTCAATA  
 TTATCAGCTTATCCGCTTGGGCTATGAGGGATATCGAAATGTAATGGAAAATTGTCGTGAAAATGCAATT  
 GTGCTAAGAAAAGGACTTGAAAAACAGGACGTTTCAATATAATCTCCAAAGATGAAGGTATACCCTTGG  
 TGGCATTTTCCCTCAAAGACAATAGCCTCCACAACGAATTCGAGGTCTCTGAGACCCTCCGTAGGTTTGG  
 GTGGATTGTCCCAGCCTACACTATGCCAGCTGACCTGCAACATGTTACAGTGTTGCGCGTTGTGATTAGA  
 GAGGACTTCTCCCGAACCTAGCAGATCGTCTTGCTCTTGACATCGTCAAGGTCCTCCACGAGCTCCCGA  
 ATGCCAAAAAAGTGAGGATAATTTGATGATCAAT**AATGAGAAGAAAACAGAAATTGAAGTTCAAAGGGC**  
**SIGAD3ΔC-R**  
**AATTGCTGAGTTTTGGAAGAAATATGTTTTAGCTAGGAAAGCATCTATTTGT** TAG  
**SIGAD3-R**

Full-length coding sequence (CDS) of *SIGAD3*. Arrows indicate the position of primers that were used for vector construction of *SIGAD3<sup>ox</sup>* and *SIGAD3ΔC<sup>ox</sup>*. The sequence deleted in *SIGAD3ΔC<sup>ox</sup>* construct is highlighted in yellow.



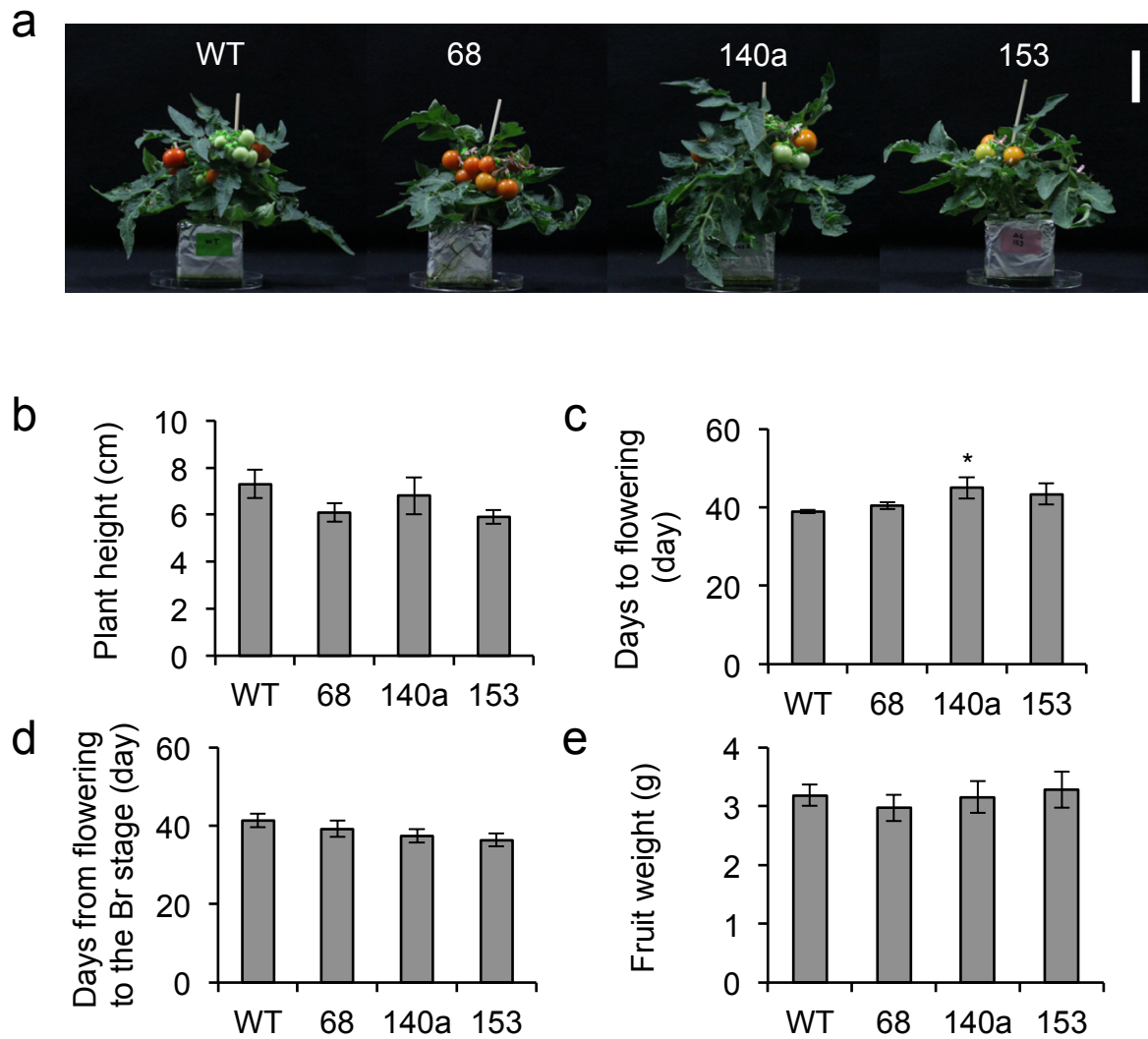
**Fig. S2**

Southern blot analysis of  $T_0$  transgenic plants. (a) *SIGAD3<sup>OX</sup>*. (b) *SIGAD3ΔC<sup>OX</sup>*. Genomic DNA (10  $\mu$ g) was digested with *Eco*RI and detected with the *NPTII* probe. WT was also tested as a negative control. The lines used for more detailed analyses are shown in red.



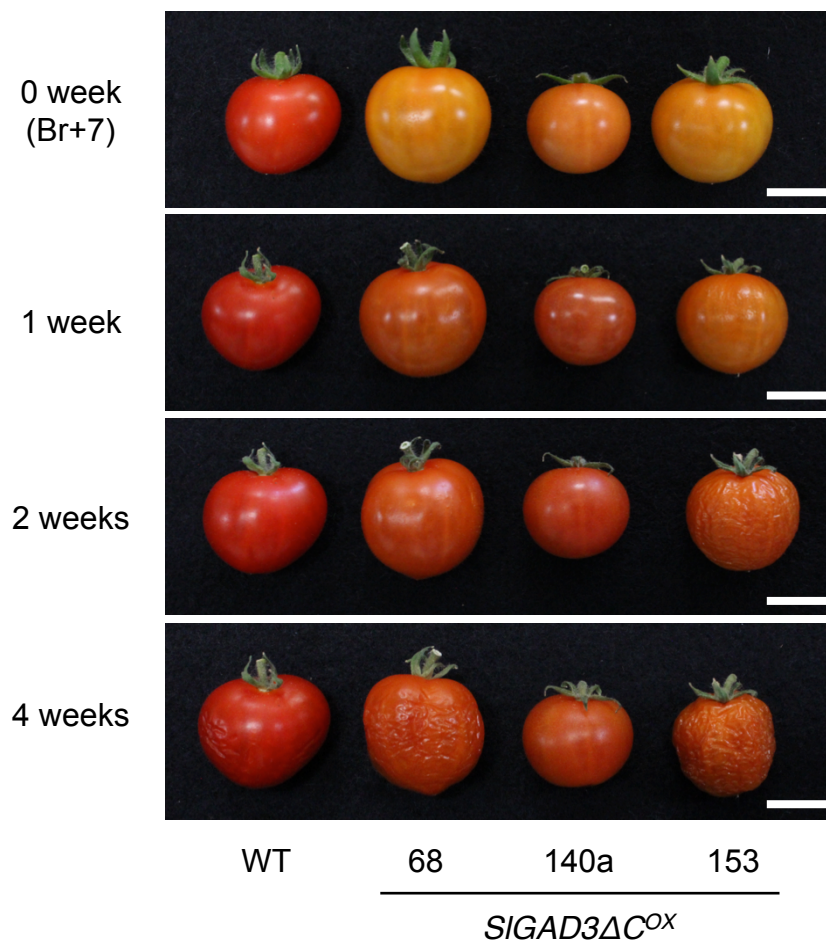
**Fig. S3**

Effects of *SIGAD3ΔC* over expression on the fruit pH and total soluble solid in  $T_1$  *SIGAD3ΔC<sup>OX</sup>* lines. pH (a) and total soluble solids (b) were determined using Br+10 fruits of WT and in  $T_1$  *SIGAD3ΔC<sup>OX</sup>* lines. The mean  $\pm$  SE of three biological replicates are shown. Asterisks indicate a significant difference between WT and transgenic lines according to Student's *t*-test (\* $P$  < 0.05 and \*\* $P$  < 0.01).



**Fig. S4**

Plant growth and fruit development of WT and  $T_1$  *SlGAD3ΔC<sup>OX</sup>* lines. (a) Appearance of 3-month-old plants. Bar = 5 cm. (b) Plant height when the first flower opened. Values are the mean  $\pm$  SE ( $n = 5$ ). (c) Days to flowering. (d) Days from flowering to the Br stage. (e) Fruit weight. (c–e) Values are the mean  $\pm$  SE ( $n = 13$ ). Asterisks indicate a significant difference between WT and transgenic lines according to Student's *t*-test (\* $P < 0.05$  and \*\* $P < 0.01$ ). Br, breaker



**Fig. S5**

Fruit shelf life of WT and T<sub>1</sub> *SIGAD3ΔC<sup>ox</sup>* lines. Fruits were harvested at the Br+7 stage and stored at 25°C for 4 weeks. Bar = 1 cm.

**Table S1** List of primers used in this study

Primer name	Primer sequence (5'→3')	Note
SIGAD3-F (SIGAD3ΔC-F)	<u>GCAAAAGAAG</u> <b>GGATCC</b> ATGGTTCTCTCAA AAACTCCTTCTG	Used for vector construction. The last 10 bases of the E8 promoter sequence (Underlined) and the <i>Bam</i> HI site (Bold letters) were added.
SIGAD3-R	<u>CTTCATCTTCATAT</u> <b>GAGCTC</b> CTAACAAATAG ATGCTTT	Used for vector construction. The first 14 bases of the HSP terminator sequence (Underlined) and the <i>Sac</i> I site (Bold letters) were added.
SIGAD3ΔC-R	<u>CTTCATCTTCATAT</u> <b>GAGCTC</b> CTAATTGATCA TCAAATTATCCTCCAC	Used for vector construction. The first 14 bases of the HSP terminator sequence (Underlined) and the <i>Sac</i> I site (Bold letters) were added.
SIUbiquitin-F SIUbiquitin-R	CACCAAGCCAAAGAAGATCA TCAGCATTAGGGCACTCCTT	Used for qRT-PCR of <i>SIUbiquitin</i> (Takayama et al. 2015)
SIGAD3-F SIGAD3-R	CAGGACGTTTCAATATAATC CCTACGGAGGGTCTCAGAG	Used for qRT-PCR of <i>SIGAD3</i> (Takayama et al. 2015)
ACS2-F ACS2-R	GGAGGTTCTGAGGTGTTGAG TAATGGTGAGGGAGGAATAGGT	Used for qRT-PCR of <i>ACS2</i> (Mantelin et al. 2013)
ACS4-F ACS4-R	AACAAGCACAAATGGAAGAGGA CGCACTACGAGCAAGGAAT	Used for qRT-PCR of <i>ACS4</i> (Mantelin et al. 2013)
ACO1-F ACO1-R	ACCATGTCCTAAGCCCGATT ATTCTGTGCCGTCTGTTTGT	Used for qRT-PCR of <i>ACO1</i> (Shinozaki et al. 2015)
PSY1-F PSY1-R	AACTTGTTGATGGCCCAAAC CTGTATCGGACAAAGCACCA	Used for qRT-PCR of <i>PSY1</i> (Ariizumi et al. 2014)
PDS-F PDS-R	AGCCGGTGACTACACGAAAC GCTTGCTTCCGACAACCTCT	Used for qRT-PCR of <i>PDS</i> (Ariizumi et al. 2014)
ZDS-F ZDS-R	CATGTCAAAGGCCACTCAGA ACGGTAACAACAGGCACTCC	Used for qRT-PCR of <i>ZDS</i> (Ariizumi et al. 2014)
CRTISO-F CRTISO-R	CCTGGGAATGCCTTTCAATA AACTCAGCTGCAACACGATG	Used for qRT-PCR of <i>CRTISO</i> (Ariizumi et al. 2014)
LCYB-F LCYB-R	GGACCCCATTTGAAGTTTTC AACCATGATGTGGGTTCAGA	Used for qRT-PCR of <i>LCY-B</i> (Guo et al. 2013)
CYCB-F CYCB-R	CTTTTCGGACATGGCTCAAAC GCTAGATTGCCAATCAGTCTAACCA	Used for qRT-PCR of <i>CYC-B</i> (Guo et al. 2013)
RIN-F RIN-R	ATGGCATTGTGGTGAGCAAAG GTTGATGGTGCTGCATTTTCG	Used for qRT-PCR of <i>RIN</i> (Shima et al. 2013)
TAGL1-F TAGL1-R	TGCCTGTAAATCTCCTGGAACC AGAATACCTGCTCCATGATTATCAGA	Used for qRT-PCR of <i>TAGL1</i> (Itkin et al. 2009)
FUL1-F FUL1-R	CAACAACCTGGACTCTCCTCACCTT TCCTTCCACTTCCCCATTATCTATT	Used for qRT-PCR of <i>FUL1</i> (Shima et al. 2013)
FUL2-F FUL2-R	CACACCCCTTTAACAATCTTCACA GCGATGATCCTTCTACTTCTCCAT	Used for qRT-PCR of <i>FUL2</i> (Shima et al. 2013)
SIERF6-F SIERF6-R	CCATGGAAATGCCCATAGTT TCAGTAGAACTGATGATGAGTTG	Used for qRT-PCR of <i>SIERF6</i> (Lee et al. 2012)