

Supplementary Data

Physicochemical Properties and Antioxidant Activity of CRISPR/Cas9 Edited Tomato *SGR1* Knockout (KO) Line

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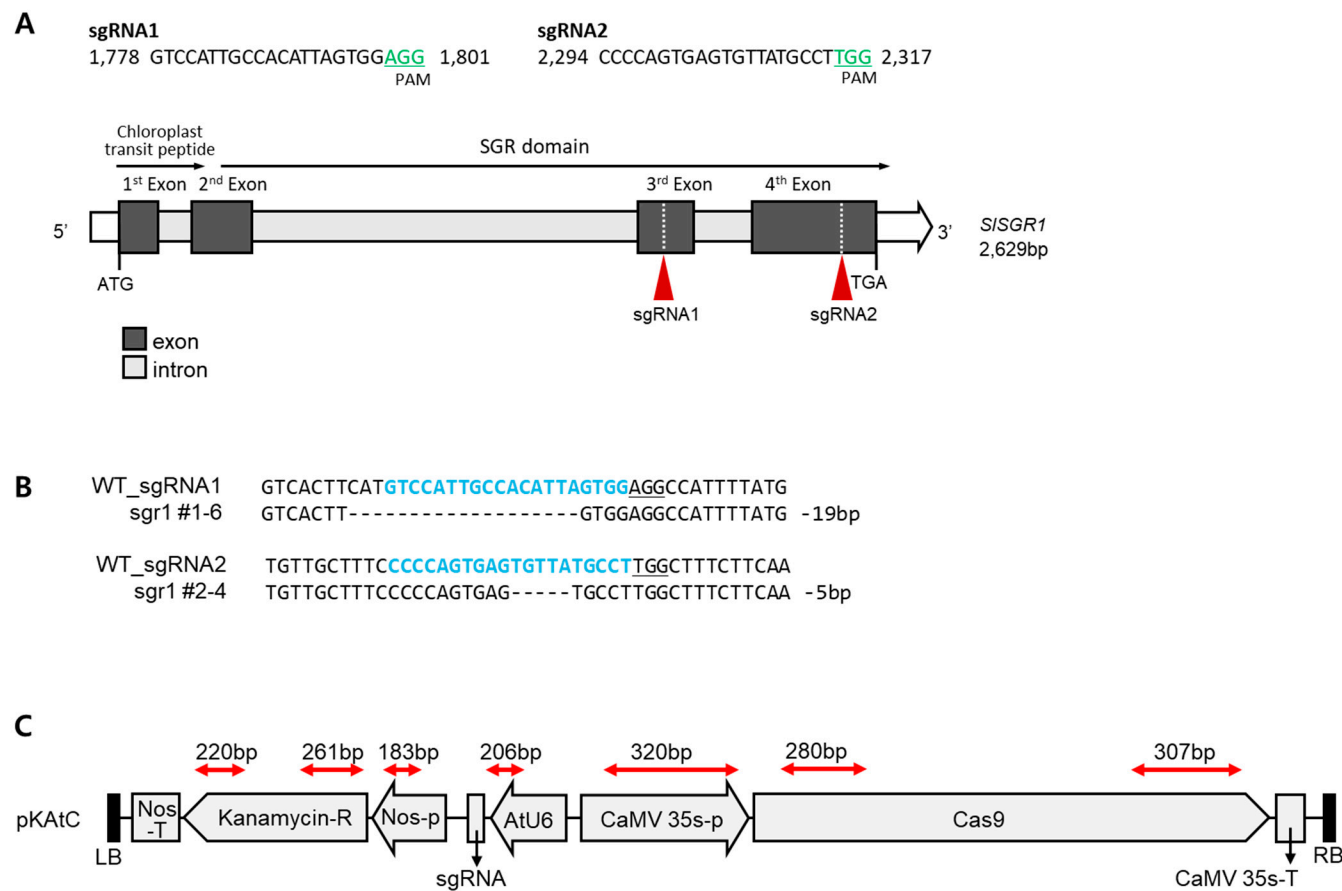
Figure S1. DNA sequence analysis of *sgr1* null lines. (A) Genetic map of *SlSGR1*. Red triangle indicates the position of sgRNA. (B) Sanger sequencing analysis. Blue letters indicate sgRNA, underline indicates PAM region, Asterisks indicates deleted base. (C) A schematic diagram of genes and primers in the T-DNA region of the pKAtC vector. Red arrows indicate PCR amplified DNA region and size. Published in reference from [9], International Journal of Molecular Sciences.

Figure S2. Antioxidant contents of WT and *sgr1* null lines at the ripening development stage. (A) Lycopene and β -carotene contents, (B) Chlorophyll *a* and *b* contents. The bars show a significant difference ($*0.01 < p < 0.05$, $**0.001 < p < 0.01$, $***p < 0.001$) between WT and *sgr1* null lines (Bonferroni's test). Vertical bars show the standard error of the mean for three replicates.

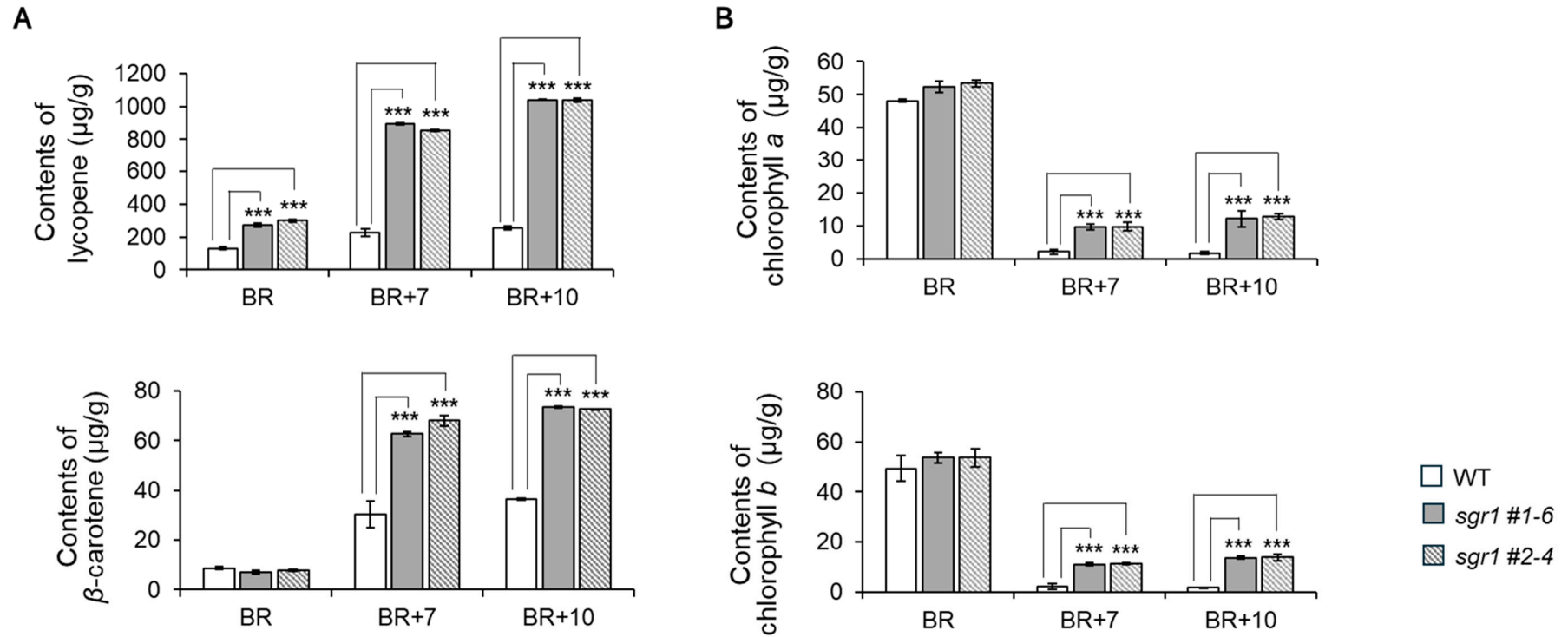
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Table S2. Antioxidant content during fruit development stages (Br, Br+7, Br+10) of WT and *sgr1* null lines.

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Supplementary Figure S2. Antioxidant contents of WT and *sgr1* null lines at the ripening development stage. (A) Lycopene and β-carotene contents, (B) Chlorophyll *a* and *b* contents. The bars show a significant difference (* $0.01 < p < 0.05$, ** $0.001 < p < 0.01$, *** $p < 0.001$) between WT and *sgr1* null lines (Bonferroni's test). Vertical bars show the standard error of the mean for three replicates.

Supplementary Table S1. The primers list used in this study.

Primer name	Sequence (primer direction 5'-3')
<i>SlActin</i> Fw	GGGATGGAGAAGTTTGGTGGTGG
<i>SlActin</i> Rv	CTTCGACCAAGGGATGGTGTAGC
<i>SISGR1</i> Fw	TTCTTCTGGTGGGGTAGGTG
<i>SISGR1</i> Rv	AGGCATAAACTCACTGGGG
Deep sequencing primers	
sgRNA1 1st Fw	AAATCCCACACATCACATGC
sgRNA1 1st Rv	TCAAGGCTTTTGTTTCATGGA
sgRNA1 2nd Fw	ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCAGTTGCAAGGTTGGTA
sgRNA1 2nd Rv	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTGGGAAGTTCGAACGACAT
sgRNA2 1st Fw	GGAATTATCCAGAGTTACAAGAAGC
sgRNA2 1st Rv	GTTGGGTTGTGCCTAAATCAA
sgRNA2 2nd Fw	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGGTAGGTGGGGTGAAGAGT
sgRNA2 2nd Rv	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATCAAATCACATAATAAACCAAACA
DNA fragment analysis primers	
Cas9 (280bp) Fw	CGTGATCACCGACGAGTACA
Cas9 (280bp) Rv	CTTCTTGTCTCTCTCCACCA
Cas9 (307bp) Fw	AGAAGCAGCTGTTTCGTGGAG
Cas9 (307bp) Rv	TACAGACCGGTGATGCTCTG

CaMV 35s-p Fw	GAAACCTCCTCGGATTCCAT
CaMV 35s-p Rv	CGGAGTCCTCTCCAAATGAA
AtU6/sgRNA Fw	GGCCTGCTTCTCTTCTTTCA
AtU6/sgRNA Rv	TCGGTGGTGAACATGGTATG
Nos-p Fw	GGAACGTCAGTGGAGCATTT
Nos-p Rv	ACAAGCCGTTTTACGTTTGG
KanR (261bp) Fw	ATACTTTCTCGGCAGGAGCA
KanR (261bp) Rv	AGACAATCGGCTGCTCTGAT
KanR (220bp) Fw	AATATCACGGGTAGCCAACG
KanR (220bp) Rv	GGATGATCTGGACGAAGAGC

Supplementary Table S2. Antioxidant content during fruit development stages (Br, Br+7, Br+10) of WT and *sgr1* null lines.

Stage	BR			BR+7			BR+10		
Line	WT	<i>sgr1</i> #1-6	<i>sgr1</i> #2-4	WT	<i>sgr1</i> #1-6	<i>sgr1</i> #2-4	WT	<i>sgr1</i> #1-6	<i>sgr1</i> #2-4
<i>L</i> *	56.7±1.3	56.2±0.8	57.2±3.2	52.5±0.9	44.8±1.9	42.9±3.3	50.2±1.7	41.3±3.9	41.7±2.8
<i>a</i> *	-11.3±0.6	-12.3±1.2	-11.8±0.3	2.5±1.1	14.2±3.8	14.1±2.9	32.0±1.3	22.3±4.6	23.8±3.8
<i>b</i> *	24.2±2.2	25.1±2.3	24.1±1.1	25.8±1.9	26.1±4.1	25.9±1.9	27.2±1.5	25.7±1.3	22.7±3.2
Lutein (μg/g)	9.2±0.8	11.1±0.8	10.9±0.4	9.3±0.1	7.1±1.9	7.9±2.0	9.2±0.1	5.9±0.0	6.4±0.1
β-Carotene (μg/g)	8.7±0.4	7.0±0.8	7.9±0.4	30.3±5.4	62.7±0.9	68.0±2.2	36.5±0.4	73.5±0.3	72.6±0.1
Lycopene (μg/g)	130.4±7.1	272.1±9.4	298.4±8.7	226.7±21.5	894.0±4.9	851.5±4.9	254.6±12.0	1039.2±2.7	1038.2±11.7
Other carotenoids (μg/g)	40.5±5.7	103.4±7.4	108.5±14.2	201.4±14.8	286.4±13.8	276.8±10.3	233.5±5.1	334.4±6.3	394.2±14.6
Total carotenoids (μg/g)	205.4±17.6	397.9±13.4	412.6±14.8	462.7±17.2	1408.8±14.2	1427.4±20.4	531.4±10.7	1524.2±13.7	1527.5±18.4
Chlorophyll <i>a</i> (μg/g)	48.2±4.1	52.4±1.6	53.4±0.9	2.1±0.7	9.7±0.9	9.7±1.3	1.7±0.4	12.1±2.4	12.8±0.9
Chlorophyll <i>b</i> (μg/g)	49.4±5.2	53.7±2.1	53.7±3.7	2.2±1.1	11.1±0.7	11.3±0.5	1.8±0.2	13.7±0.6	13.9±1.3
δ-Tocopherol (μg/g)	0.54±0.07	0.97±0.09	0.96±0.11	1.08±0.10	2.85±0.65	1.29±0.12	2.08±0.08	3.85±0.08	3.29±0.12
γ-Tocopherol (μg/g)	5.16±1.27	9.44±1.12	9.46±1.11	9.29±0.52	21.46±3.57	23.45±3.14	9.75±0.47	29.78±10.54	27.28±9.03

β -tocopherol ($\mu\text{g/g}$)	0.010 \pm 0.001	0.011 \pm 0.001	0.010 \pm 0.002	0.019 \pm 0.001	0.013 \pm 0.003	0.013 \pm 0.002	0.010 \pm 0.002	0.014 \pm 0.001	0.014 \pm 0.001
α -Tocopherol ($\mu\text{g/g}$)	53.2 \pm 6.7	61.5 \pm 18.5	62.4 \pm 4.2	63.7 \pm 12.1	75.8 \pm 4.6	85.9 \pm 20.3	68.4 \pm 6.4	99.4 \pm 5.7	99.2 \pm 4.1
Total tocopherols ($\mu\text{g/g}$)	109.1 \pm 11.1	110.7 \pm 8.4	117.4 \pm 9.1	133.7 \pm 4.5	155.4 \pm 21.1	157.4 \pm 14.1	140.1 \pm 11.1	179.4 \pm 12.4	179.5 \pm 9.5
GABA (mg/g)	9.26 \pm 0.24	10.42 \pm 0.18	10.79 \pm 0.68	6.16 \pm 0.06	6.62 \pm 0.07	6.89 \pm 0.11	5.39 \pm 0.20	5.79 \pm 0.15	5.94 \pm 0.53
Total amino acids (mg/g)	45.14 \pm 1.05	42.55 \pm 2.25	42.86 \pm 1.06	42.74 \pm 1.46	41.42 \pm 1.54	40.68 \pm 2.05	45.20 \pm 0.30	43.17 \pm 0.87	43.84 \pm 0.19
Vitamin C (mg AAE/g)	0.43 \pm 0.07	1.64 \pm 0.08	1.49 \pm 0.06	2.12 \pm 0.16	5.07 \pm 0.44	5.11 \pm 0.84	2.35 \pm 0.13	6.81 \pm 0.06	6.45 \pm 0.15
Antioxidant Activity of Lipophilic Extracts									
Total phenolics ($\mu\text{mol GAE/g}$)	1.61 \pm 0.05	1.82 \pm 0.08	1.83 \pm 0.17	2.04 \pm 0.02	2.14 \pm 0.10	2.13 \pm 0.04	2.10 \pm 0.08	2.67 \pm 0.08	2.39 \pm 0.03
Total flavonoids ($\mu\text{mol QE/g}$)	0.38 \pm 0.05	0.36 \pm 0.17	0.31 \pm 0.09	0.19 \pm 0.01	0.13 \pm 0.08	0.15 \pm 0.02	0.12 \pm 0.07	0.07 \pm 0.04	0.08 \pm 0.09
DPPH ($\mu\text{mol TE/g}$)	11.3 \pm 0.9	17.4 \pm 1.3	16.7 \pm 2.3	26.6 \pm 3.5	29.2 \pm 1.9	30.1 \pm 3.2	31.3 \pm 4.4	34.3 \pm 2.7	32.3 \pm 3.8
Antioxidant Activity of Hydrophilic Extracts									
Total phenolics ($\mu\text{mol GAE/g}$)	14.4 \pm 0.1	16.9 \pm 0.1	17.3 \pm 0.3	20.6 \pm 0.1	20.9 \pm 0.2	21.5 \pm 0.1	21.0 \pm 0.1	26.8 \pm 0.2	28.0 \pm 0.7
Total flavonoids ($\mu\text{mol QE/g}$)	1.96 \pm 0.23	1.82 \pm 0.27	1.86 \pm 0.18	0.27 \pm 0.05	0.18 \pm 0.03	0.25 \pm 0.10	0.19 \pm 0.01	0.12 \pm 0.07	0.18 \pm 0.06
DPPH ($\mu\text{mol TE/g}$)	30.9 \pm 0.9	34.9 \pm 2.4	34.3 \pm 1.4	39.9 \pm 1.0	44.5 \pm 2.4	45.4 \pm 1.4	45.9 \pm 1.1	52.1 \pm 1.0	52.5 \pm 1.0

Supplementary Table S3. Growth characteristics of WT and *sgr1* null lines.

	height of plant (cm)	width of stem (mm)	height of fruit (cm)	width of fruit (cm)	harvest/weight of fruit (g)
WT	147±4	3.4±0.5	4.4±0.3	5.6±0.2	178±0.6
<i>sgr1</i> #1-6	151±5	3.3±0.4	4.3±0.4	5.4±0.3	177±0.7
<i>sgr1</i> #2-4	150±4	3.5±0.4	4.4±0.2	5.7±0.3	180±0.4