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Supplemental information

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Figure S1.

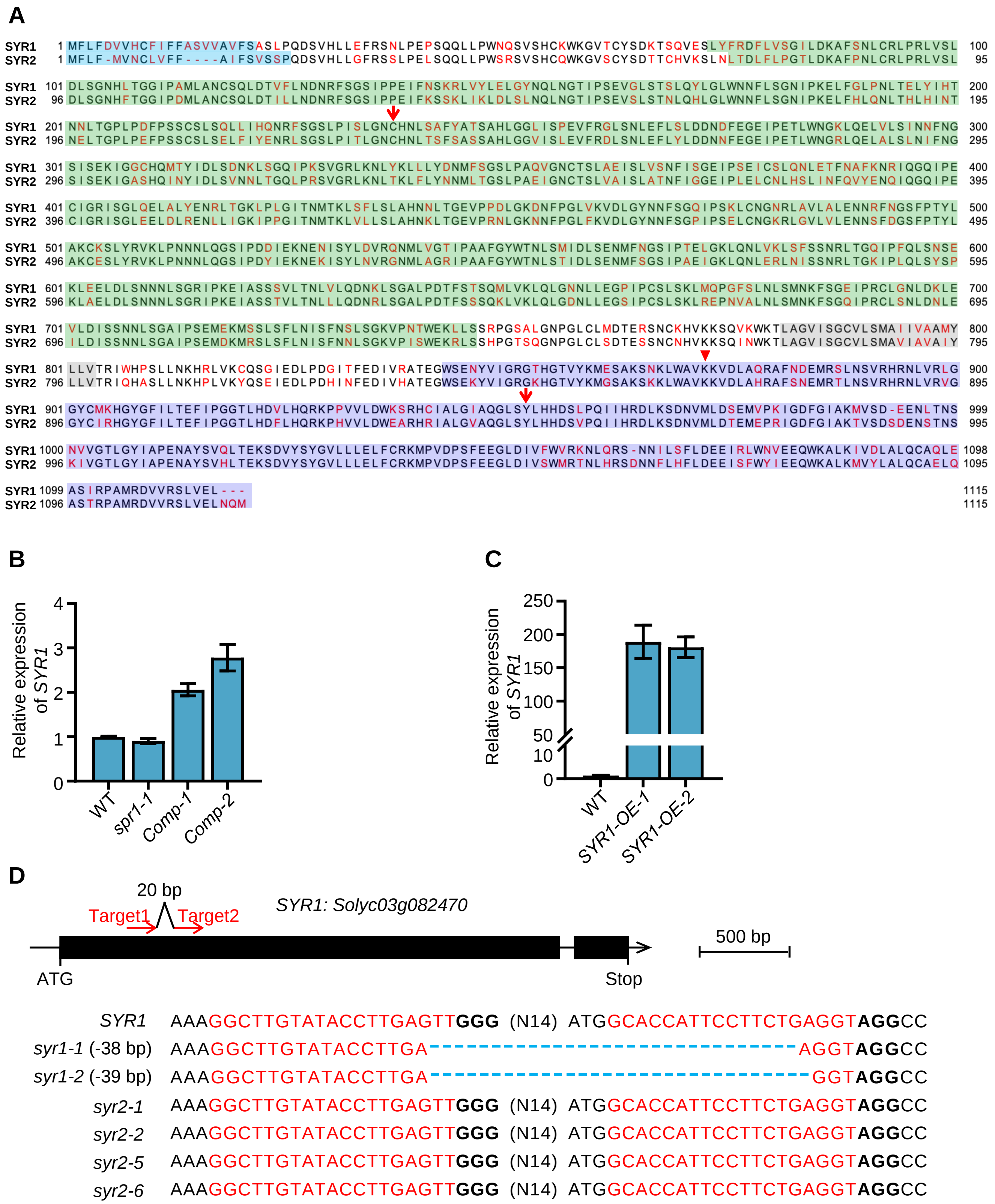

A
SYR1
SYR2
B
Relative expression of SYR1
WT
spr1-1
Comp-1
Comp-2
C
Relative expression of SYR1
WT
SYR1-OE-1
SYR1-OE-2
D
20 bp
Target1
Target2
SYR1: Solyc03g082470
500 bp
ATG
Stop
SYR1 AAAGGCTTGTATACCTTGAGTTGGG (N14) ATGGCACCATTCCTTCTGAGGTAGGCC
syr1-1 (-38 bp) AAAGGCTTGTATACCTTGA AGGTAGGCC
syr1-2 (-39 bp) AAAGGCTTGTATACCTTGA GGTAGGCC
syr2-1 AAAGGCTTGTATACCTTGAGTTGGG (N14) ATGGCACCATTCCTTCTGAGGTAGGCC
syr2-2 AAAGGCTTGTATACCTTGAGTTGGG (N14) ATGGCACCATTCCTTCTGAGGTAGGCC
syr2-5 AAAGGCTTGTATACCTTGAGTTGGG (N14) ATGGCACCATTCCTTCTGAGGTAGGCC
syr2-6 AAAGGCTTGTATACCTTGAGTTGGG (N14) ATGGCACCATTCCTTCTGAGGTAGGCC

Figure S1. *SPR1* encodes the systemin receptor SYR1, related to Figure 2

(A) Protein sequence alignment between SYR1 and SYR2. Protein sequences were aligned and visualized by program Jalview (version, 2.1.1, https://www.jalview.org/). Protein domains were predicted by InterPro (http://www.ebi.ac.uk/interpro/). The leucine-rich-repeat (LRR) domain was predicted based on the consensus LRR sequences (xLxxLxLxxNxLS/TGxIPxxLGxLx). Blue box, signal peptide; Green box, LRR domain; Grey box, transmembrane domain; Purple box, kinase domain. Arrowheads indicate $SYR1^{C237}$ and $SYR1^{Y952}$. Triangles indicate $SYR1^{K872}$ and $SYR2^{K867}$.

(B) RT-qPCR showing *SYR1* expression in indicated *spr1-1* complementation plants (*Comp*). The error bars represent SD, data are mean $\pm$ SD, n = 3 repeats. CM was used as WT.

(C) RT-qPCR showing *SYR1* expression in indicated *SYR1-OE* lines. The error bars represent SD, data are mean $\pm$ SD, n = 3 repeats. AC was used as WT.

(D) CRISPR/Cas9-mediated editing of *SYR1*. *SYR1* exons are targeted by two sgRNAs. sgRNA target (red) and PAM (bold) sequences are indicated. Blue dashes indicate deletions, numbers indicate the number of nucleotides involved.

Figure S2.

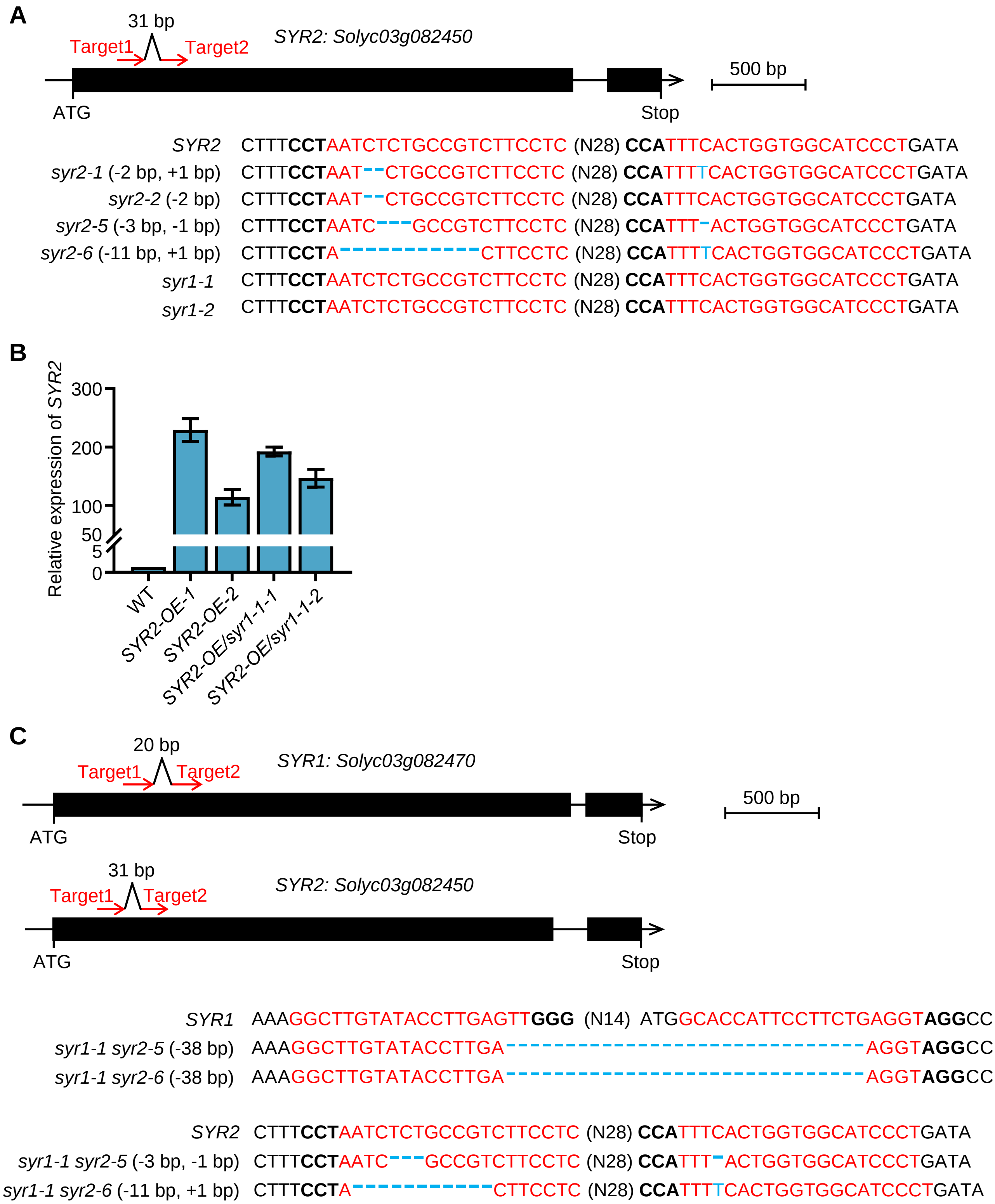

A
31 bp
Target1
Target2
SYR2: Solyc03g082450
500 bp
ATG
Stop
SYR2 CTTTCCTAATCTCTGCCGTCTTCCTC (N28) CCATTTCACTGGTGGCATCCCTGATA
syr2-1 (-2 bp, +1 bp) CTTTCCTAAT--CTGCCGTCTTCCTC (N28) CCATTTTCACTGGTGGCATCCCTGATA
syr2-2 (-2 bp) CTTTCCTAAT--CTGCCGTCTTCCTC (N28) CCATTTCACTGGTGGCATCCCTGATA
syr2-5 (-3 bp, -1 bp) CTTTCCTAATC---GCCGTCTTCCTC (N28) CCATTT-ACTGGTGGCATCCCTGATA
syr2-6 (-11 bp, +1 bp) CTTTCCTA-----------CTTCCTC (N28) CCATTTTCACTGGTGGCATCCCTGATA
syr1-1 CTTTCCTAATCTCTGCCGTCTTCCTC (N28) CCATTTCACTGGTGGCATCCCTGATA
syr1-2 CTTTCCTAATCTCTGCCGTCTTCCTC (N28) CCATTTCACTGGTGGCATCCCTGATA
B
Relative expression of SYR2
300
200
100
50
5
0
WT
SYR2-OE-1
SYR2-OE-2
SYR2-OE/syr1-1-1
SYR2-OE/syr1-1-2
C
20 bp
Target1
Target2
SYR1: Solyc03g082470
500 bp
ATG
Stop
31 bp
Target1
Target2
SYR2: Solyc03g082450
ATG
Stop
SYR1 AAAGGCTTGTATACCTTGAGTTGGG (N14) ATGGCACCATTCCTTCTGAGGTAGGCC
syr1-1 syr2-5 (-38 bp) AAAGGCTTGTATACCTTGA--------------------------------------AGGTAGGCC
syr1-1 syr2-6 (-38 bp) AAAGGCTTGTATACCTTGA--------------------------------------AGGTAGGCC
SYR2 CTTTCCTAATCTCTGCCGTCTTCCTC (N28) CCATTTCACTGGTGGCATCCCTGATA
syr1-1 syr2-5 (-3 bp, -1 bp) CTTTCCTAATC---GCCGTCTTCCTC (N28) CCATTT-ACTGGTGGCATCCCTGATA
syr1-1 syr2-6 (-11 bp, +1 bp) CTTTCCTA-----------CTTCCTC (N28) CCATTTTCACTGGTGGCATCCCTGATA

Figure S2. Generation of *syr2* mutants, *SYR2-OE* plants and *syr1 syr2* double mutants, related to Figure 3

(A) CRISPR/Cas9-mediated editing of *SYR2*. *SYR2* exons are targeted by two sgRNAs. sgRNA target (red) and PAM (bold) sequences are indicated. Blue dashes and letters indicate deletions and insertions, respectively, numbers indicate the number of nucleotides involved (+, insertion; -, deletion).

(B) RT-qPCR analysis showing *SYR2* expression levels of different *SYR2*-overexpression plants in the background of WT (*SYR2-OE*) or *syr1-1* (*SYR2-OE/syr1-1*). The error bars represent SD, data are mean ± SD, *n* = 3 repeats.

(C) CRISPR/Cas9-mediated editing of *SYR1* and *SYR2*. Exons of target genes are targeted by CRISPR/Cas9 using two sgRNAs. sgRNA target (red) and PAM (bold) sequences are indicated. Blue dashes and letters indicate deletions and insertions, respectively, and the numbers in parentheses indicate the number of nucleotides involved (+, insertion; -, deletion).

Figure S3.

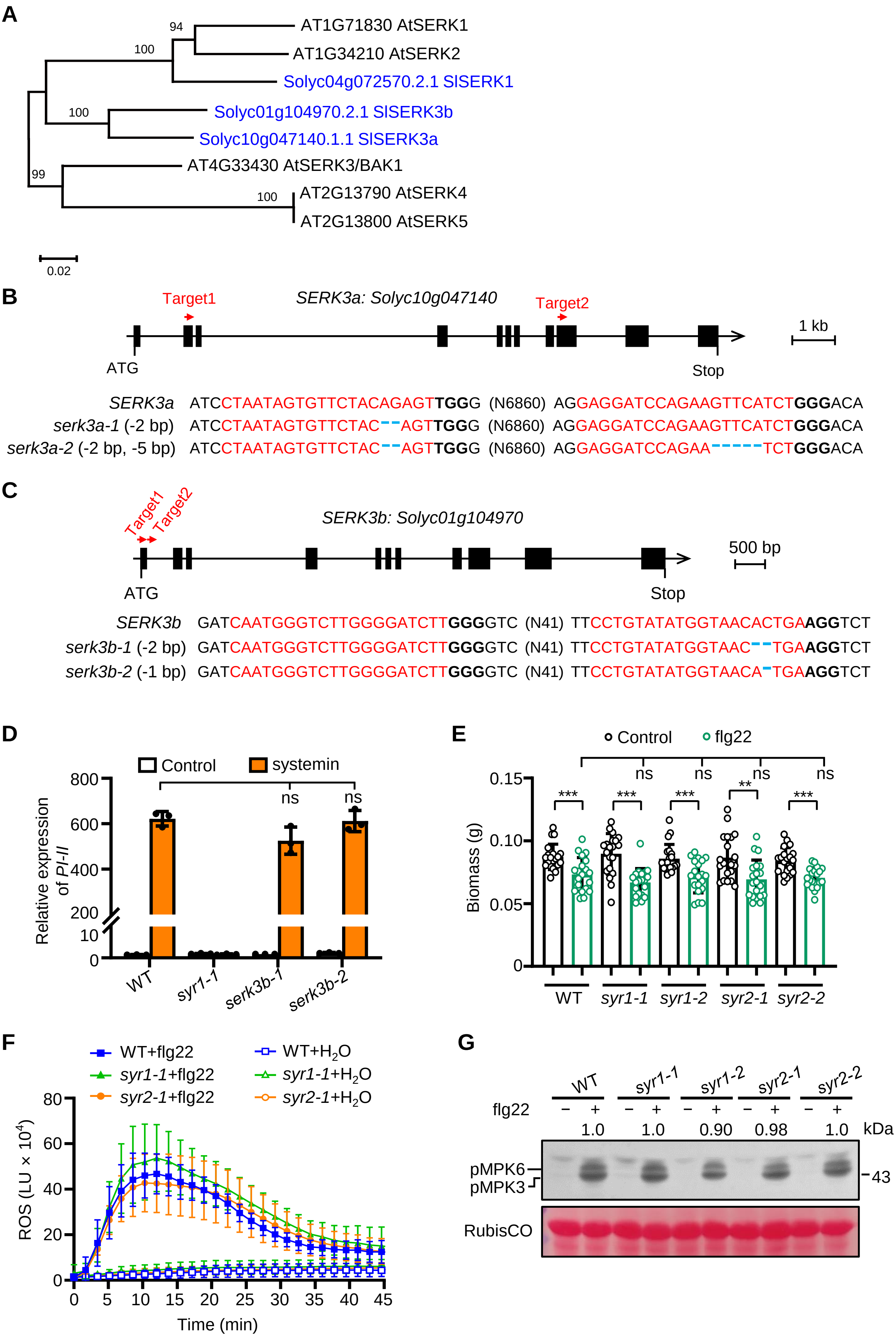

A
AT1G71830 AtSERK1
AT1G34210 AtSERK2
Solyc04g072570.2.1 SlSERK1
Solyc01g104970.2.1 SlSERK3b
Solyc10g047140.1.1 SlSERK3a
AT4G33430 AtSERK3/BAK1
AT2G13790 AtSERK4
AT2G13800 AtSERK5
0.02
B
Target1
SERK3a: Solyc10g047140
Target2
1 kb
ATG
Stop
SERK3a ATCCTAATAGTGTTCTACAGAGTTGGG (N6860) AGGAGGATCCAGAAGTTCATCTGGGACA
serk3a-1 (-2 bp) ATCCTAATAGTGTTCTAC--AGTTGGG (N6860) AGGAGGATCCAGAAGTTCATCTGGGACA
serk3a-2 (-2 bp, -5 bp) ATCCTAATAGTGTTCTAC--AGTTGGG (N6860) AGGAGGATCCAGAA-----TCTGGGACA
C
Target1
Target2
SERK3b: Solyc01g104970
500 bp
ATG
Stop
SERK3b GATCAATGGGTCTTGGGGATCTTGGGGTC (N41) TTCCTGTATATGGTAACACTGAAGGTCT
serk3b-1 (-2 bp) GATCAATGGGTCTTGGGGATCTTGGGGTC (N41) TTCCTGTATATGGTAAC--TGAAGGTCT
serk3b-2 (-1 bp) GATCAATGGGTCTTGGGGATCTTGGGGTC (N41) TTCCTGTATATGGTAACA-TGAAGGTCT
D
Control
systemin
Relative expression of PI-II
WT
syr1-1
serk3b-1
serk3b-2
E
Control
flg22
Biomass (g)
WT
syr1-1
syr1-2
syr2-1
syr2-2
F
WT+flg22
syr1-1+flg22
syr2-1+flg22
WT+H2O
syr1-1+H2O
syr2-1+H2O
ROS (LU × 10^4)
Time (min)
G
WT
syr1-1
syr1-2
syr2-1
syr2-2
flg22
1.0
1.0
0.90
0.98
1.0
kDa
pMPK6
pMPK3
43
RubisCO

Figure S3. SERK3a complexes with SYR1 and SYR2 in response to systemin, related to Figure 6

(A) Phylogenetic tree of *Arabidopsis thaliana* SERKs (in black) and their tomato orthologs (in blue) on the basis of protein sequences. The phylogenetic tree was constructed by Neighbor-joining Method. Bootstrap values for 1000 replicates are indicated at the forks. The scale bar indicates the average number of amino acid substitutions per site.

(B) CRISPR/Cas9-mediated editing of *SERK3a*. *SERK3a* exons are targeted by two sgRNAs. sgRNA target (red) and PAM (bold) sequences are indicated. Blue dashes indicate deletions, numbers indicate the number of nucleotides involved.

(C) CRISPR/Cas9-mediated editing of *SERK3b*. *SERK3b* exons are targeted by two sgRNAs. sgRNA target (red) and PAM (bold) sequences are indicated. Blue dashes indicate deletions, numbers indicate the number of nucleotides involved.

(D) Systemin-induced *PI-II* expression in indicated genotypes. Eighteen-d-old seedlings were treated with or without 100 nM systemin. *PI-II* levels were measured 12 hours after treatment. Data are mean ± SD, n = 3 repeats. ns, not significant (Student's *t* test).

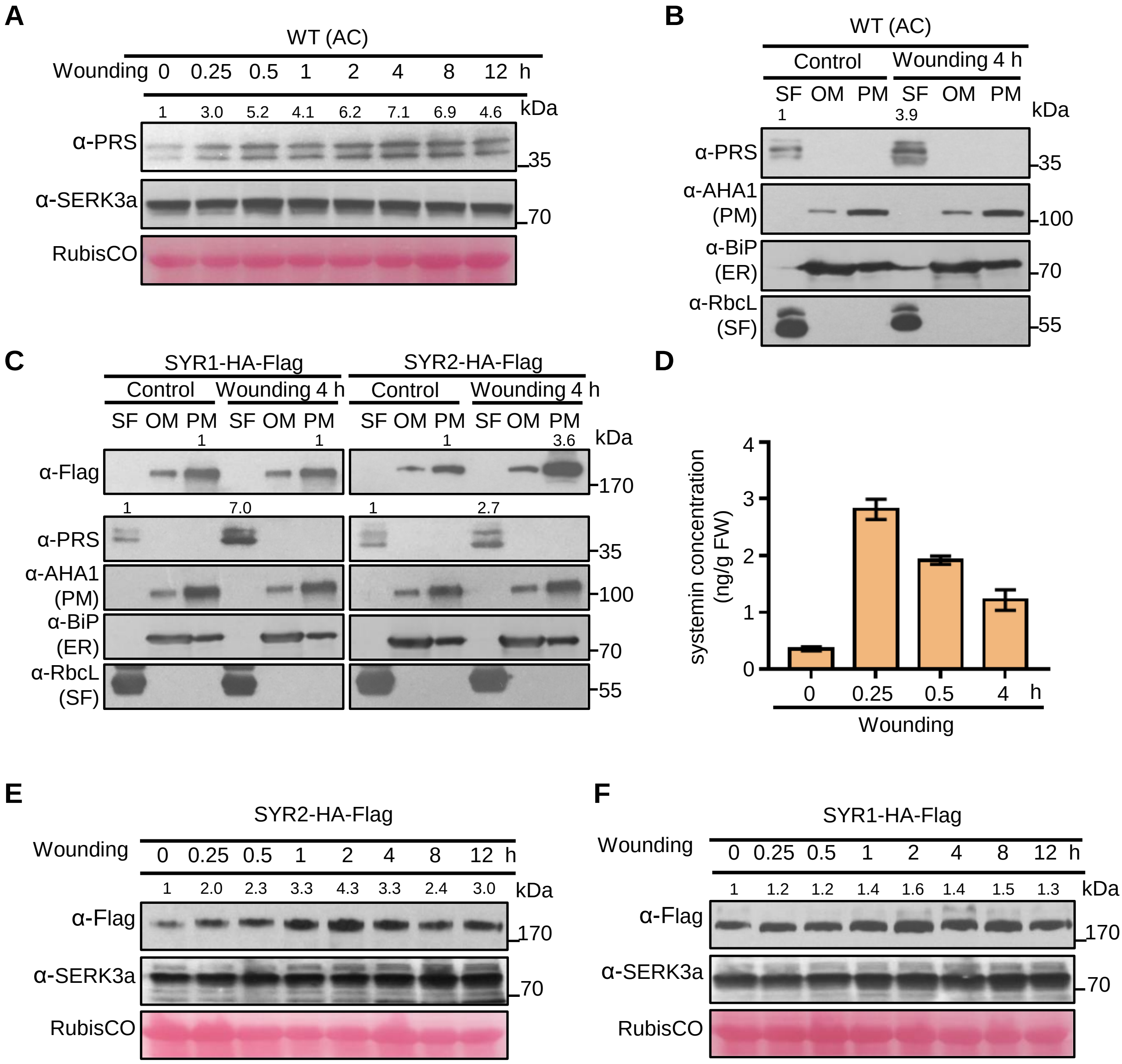
(E) Biomass of 6-d-old seedlings of indicated genotypes grown on medium with or without 100 nM flg22. Data are mean ± SD, n = 20 seedlings.

(F) Flg22-induced ROS burst in indicated genotypes. Leaves from 18-d-old seedlings were treated with or without 100 nM flg22. ROS production was detected by luminometer over 45 min. LU, light units. Data are mean ± SD, n = 6 leaf discs.

(G) Flg22-triggered MPK3/6 activation in indicated genotypes. Eighteen-d-old seedlings of indicated genotypes were treated with 100 nM flg22 for 30 min before immunoblot with anti-pERK antibody. Ponceau staining of RubisCO indicates equal loading. Numbers indicate arbitrary densitometry units of pMPK3/6 normalized to the WT.

In (D) and (E), the error bars represent SD. **p <0.01, ***p <0.001 (Student's *t* test); ns, not significant.

Figure S4.



A
WT (AC)
Wounding 0 0.25 0.5 1 2 4 8 12 h
1 3.0 5.2 4.1 6.2 7.1 6.9 4.6 kDa
α-PRS
35
α-SERK3a
70
RubisCO
B
WT (AC)
Control
Wounding 4 h
SF OM PM SF OM PM
1
3.9
kDa
α-PRS
35
α-AHA1 (PM)
100
α-BiP (ER)
70
α-RbcL (SF)
55
C
SYR1-HA-Flag
SYR2-HA-Flag
Control Wounding 4 h Control Wounding 4 h
SF OM PM SF OM PM SF OM PM SF OM PM
1 1 1 3.6
kDa
α-Flag
170
1 7.0 1 2.7
α-PRS
35
α-AHA1 (PM)
100
α-BiP (ER)
70
α-RbcL (SF)
55
D
systemin concentration (ng/g FW)
0 1 2 3 4
0 0.25 0.5 4 h
Wounding
E
SYR2-HA-Flag
Wounding 0 0.25 0.5 1 2 4 8 12 h
1 2.0 2.3 3.3 4.3 3.3 2.4 3.0 kDa
α-Flag
170
α-SERK3a
70
RubisCO
F
SYR1-HA-Flag
Wounding 0 0.25 0.5 1 2 4 8 12 h
1 1.2 1.2 1.4 1.6 1.4 1.5 1.3 kDa
α-Flag
170
α-SERK3a
70
RubisCO

Figure S4. Wounding differentially upregulates the protein abundance of SYR1, SYR2, SERK3a and PRS, related to Figure 7

(A) Wound-induced protein accumulation of PRS and SERK3a in WT plants. Eighteen-d-old WT seedlings were wounded and harvested at indicated time, prosystemin and SERK3a were detected by their antibodies. Numbers indicate band intensity normalized to untreated WT plants. Ponceau staining of RubisCO indicates equal loading. PRS, prosystemin.

(B) Subcellular fractionation analysis of prosystemin in WT. Eighteen-d-old WT seedlings were wounded, and leaf tissues were harvested for fractionation analysis 4 hours after wounding. Plasma membrane (PM), organelle membrane (OM) and soluble fraction (SF) were separated, and each fraction was probed with anti-prosystemin, anti-AHA1 (plasma membrane marker), anti-BiP (endoplasmic reticulum membrane marker), and anti-RbcL (soluble fraction marker). PRS, prosystemin.

(C) Subcellular fractionation analysis of SYR1-HA-Flag and SYR2-HA-Flag in indicated genotypes. Eighteen-d-old SYR1-HA-Flag or SYR2-HA-Flag seedlings were wounded, and leaf tissues were harvested for fractionation analysis 4 hours after wounding. Plasma membrane (PM), organelle membrane (OM) and soluble fraction (SF) were separated, and each fraction was probed with anti-Flag, anti-PRS, anti-AHA1 (plasma membrane marker), anti-BiP (endoplasmic reticulum membrane marker), and anti-RbcL (soluble fraction marker). PRS, prosystemin.

(D) Wound-induced accumulation of systemin in WT plants at indicated time. FW, fresh weight. The error bars represent SD, data are mean $\pm$ SD, n = 3 repeats.

(E) Wound-induced accumulation of the SYR2-HA-Flag fusion and SERK3a in *SYR2-HA-Flag* plants. SYR2-HA-Flag was detected by anti-Flag antibody and SERK3a was detected by anti-SERK3a antibody. Numbers indicate band intensity normalized to untreated *SYR2-HA-Flag* plants. Ponceau staining of RubisCO indicates equal loading.

(F) Wound-induced accumulation of the SYR1-HA-Flag fusion and SERK3a in *SYR1-HA-Flag* plants. SYR1-HA-Flag was detected by anti-Flag antibody and SERK3a was detected by anti-SERK3a antibody. Numbers indicate band intensity normalized to untreated *SYR1-HA-Flag* plants. Ponceau staining of RubisCO indicates equal loading.

Figure S5.

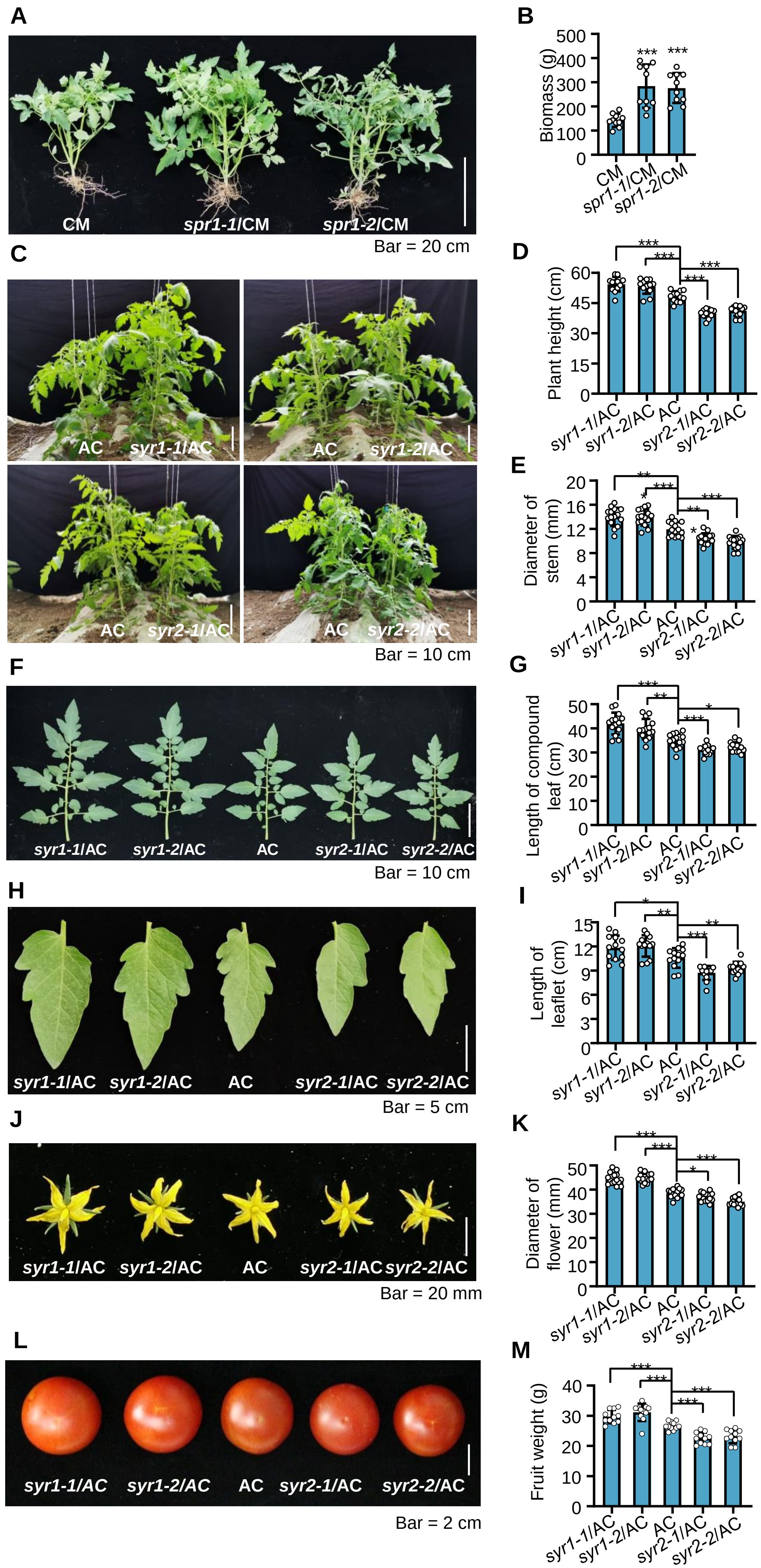

A
CM
spr1-1/CM
spr1-2/CM
Bar = 20 cm
B
Biomass (g)
500
400
300
200
100
0
***
***
CM
spr1-1/CM
spr1-2/CM
C
AC
syr1-1/AC
AC
syr1-2/AC
AC
syr2-1/AC
AC
syr2-2/AC
Bar = 10 cm
D
Plant height (cm)
60
45
30
15
0
syr1-1/AC
syr1-2/AC
AC
syr2-1/AC
syr2-2/AC
E
Diameter of stem (mm)
20
16
12
8
4
0
F
syr1-1/AC
syr1-2/AC
AC
syr2-1/AC
syr2-2/AC
Bar = 10 cm
G
Length of compound leaf (cm)
50
40
30
20
10
0
H
syr1-1/AC
syr1-2/AC
AC
syr2-1/AC
syr2-2/AC
Bar = 5 cm
I
Length of leaflet (cm)
15
12
9
6
3
0
J
syr1-1/AC
syr1-2/AC
AC
syr2-1/AC
syr2-2/AC
Bar = 20 mm
K
Diameter of flower (mm)
50
40
30
20
10
0
L
syr1-1/AC
syr1-2/AC
AC
syr2-1/AC
syr2-2/AC
Bar = 2 cm
M
Fruit weight (g)
40
30
20
10
0

Figure S5. *SYR* mutations have distinct effects on plant growth and organ size, related to Figure 7

(A and B) Representative image (A) and biomass (B) of 48-d-old plants of indicated genotypes. Data are mean ± SD, *n* = 10 plants. Tomato cv. Castlemart (CM) was the WT of *spr1* mutants.

(C) Representative image of 58-d-old plants of the indicated genotypes. Scale bar, 10 cm.

(D) Height of 58-d-old plants of indicated genotypes. Data are mean ± SD, *n* = 12.

(E) Main stem diameter of 58-d-old plants of indicated genotypes. Data are mean ± SD, *n* = 15.

(F) Representative compound leaf of 58-d-old plants of indicated genotypes. Scale bar, 10 cm.

(G) Compound leaf length of 58-d-old plants of indicated genotypes. Data are mean ± SD, *n* = 14.

(H) Representative leaflet of 58-d-old plants of indicated genotypes. Scale bar, 5 cm.

(I) Leaflet length of 58-d-old plants of indicated genotypes. Data are mean ± SD, *n* = 13.

(J) Representative flower of 80-d-old plants of indicated genotypes. Scale bar, 20 mm.

(K) Flower diameter of 80-d-old plants of indicated genotypes. Data are mean ± SD, *n* = 15.

(L) Representative fruit of plants of indicated genotypes at the red ripe stage. Scale bar, 2 cm.

(M) Fruit weight of plants of indicated genotypes at the red ripe stage. Data are mean ± SD, n = 10.

In (C) to (M): tomato cv. Ailsa Craig (AC) was the WT of *syr* mutants. In (B), (D), (E), (G), (I), (K), and (M): the error bars represent SD. *p <0.05, **p <0.01, ***p <0.001 (Student's *t* test).