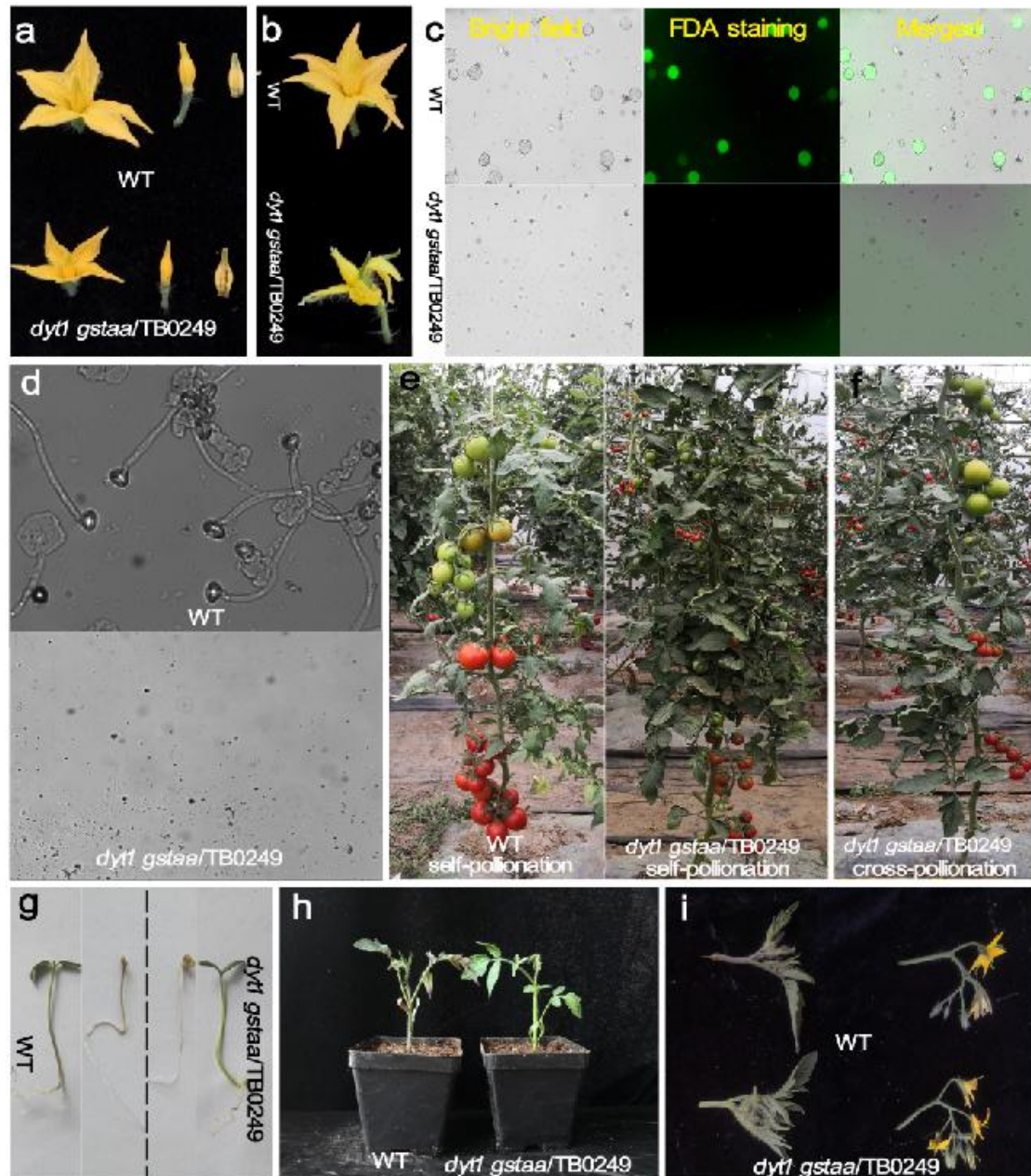
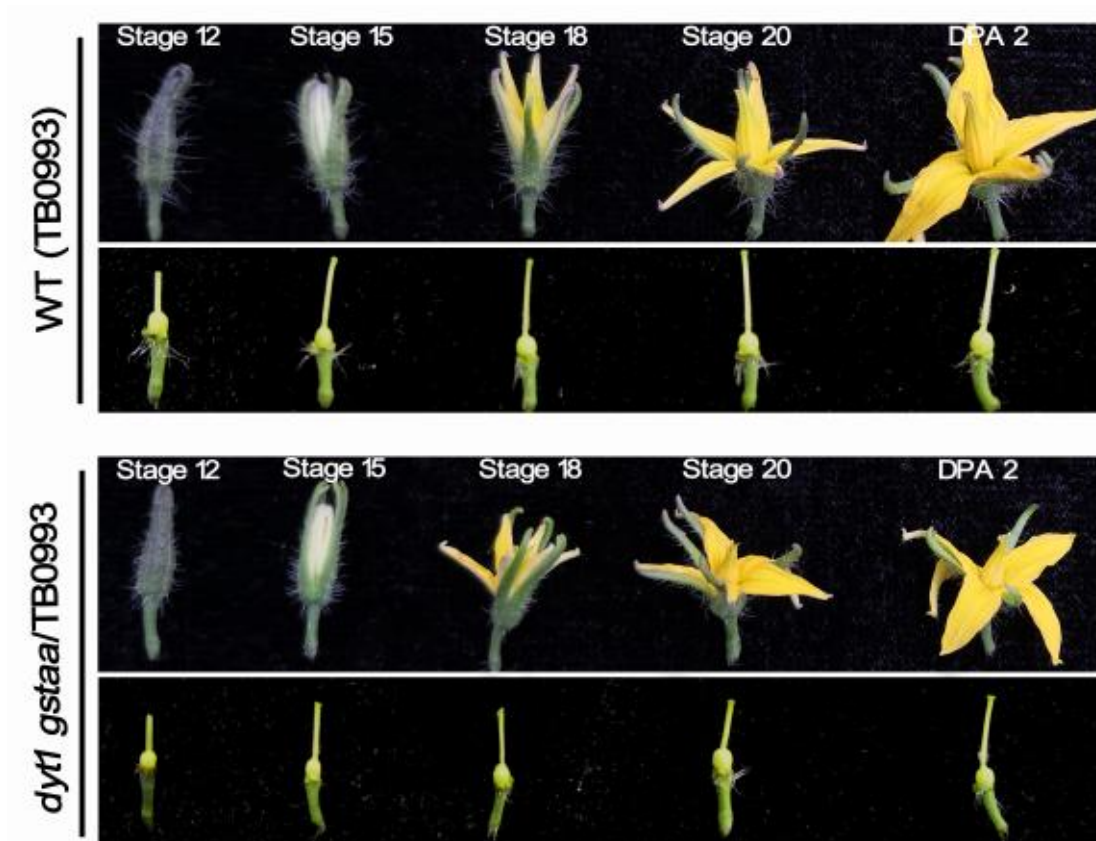


<b>a</b>		
WT	NVVKVYGSANAAACQQRVAVCIIL ELGVDEEII HVDLDSLQKKKPDFLLIQPFGQVPVI EEG	60
<i>dyl1 gstaa/TB0993 #1</i>	NVVKVYQVLHVHKGSVFVLNVEIINLIM..... I LI LSSRKNIISCFYSHLDRFL	52
<i>dyl1 gstaa/TB0249 #1</i>	NVVKVYGS EVLIVHKGSVFVL.....	21
WT	DFRLFEIRAIIRYYAAKYEDKGGKLTGTTLEEKALVDQVLEVESNNYNDLVYNVLQLLV	120
<i>dyl1 gstaa/TB0993 #1</i>	SLKRAISG.FS NLEQ.....	66
<i>dyl1 gstaa/TB0249 #1</i>	.....	21
WT	FPKMGHKSDLIVVQKCANNLEKVFDI YEQRLSKSKYLAGDFFSLADLSHPLSLRFLNNEG	180
<i>dyl1 gstaa/TB0993 #1</i>	.....	66
<i>dyl1 gstaa/TB0249 #1</i>	.....	21
WT	GFAHLVTQRKYLHDVYLDI SSRPSVSKVLDFNKKLEMLPGPPKEEVK	229
<i>dyl1 gstaa/TB0993 #1</i>	.....	66
<i>dyl1 gstaa/TB0249 #1</i>	.....	21
<b>b</b>		
WT	MEFPSTPPFDNSNNSEEREVGRRTDKRRQIDGEVKEYSKNLLKAERNRRQKLSERLQLRS	60
<i>dyl1 gstaa/TB0993 #1</i>	MEFPSTPPFDNSNNSEEREVGRRTDKRRQIDGEVKEY..... LRLRE	41
<i>dyl1 gstaa/TB0249 #1</i>	MEFPSTPPFDNSNNSEEREVGRRTDKRRQIDGEVKEYSKN..... RLRE	44
WT	LVPNTNNITKETIITDAITYI RELQMVDNLSHQLLEMEATQGEETKNEEI I DTADEM	120
<i>dyl1 gstaa/TB0993 #1</i>	IG..VKNLAKG..... FFNYAHVST.....	60
<i>dyl1 gstaa/TB0249 #1</i>	IG..VKNLAKG..... FFNYAHVST.....	63
WT	GKVGIEPEVQVANI GPTKLWI KIVCQKKRGGLTKLMEANALGFDI NDTSATASKGAI LI	180
<i>dyl1 gstaa/TB0993 #1</i>	.....	60
<i>dyl1 gstaa/TB0249 #1</i>	.....	63
WT	TSSVEVVRGGLTEANRI REI LLEI I HGI	208
<i>dyl1 gstaa/TB0993 #1</i>	.....	60
<i>dyl1 gstaa/TB0249 #1</i>	.....	63

**Figure S1** Predicted truncated *dyl1* (a) and *gstaa* (b) protein sequences. Deduced protein sequences of *dyl1* and *gstaa* in *dyl1 gstaa/TB0993#1*, *dyl1 gstaa/TB0249#1* and their corresponding wild type were aligned by ClustalW.

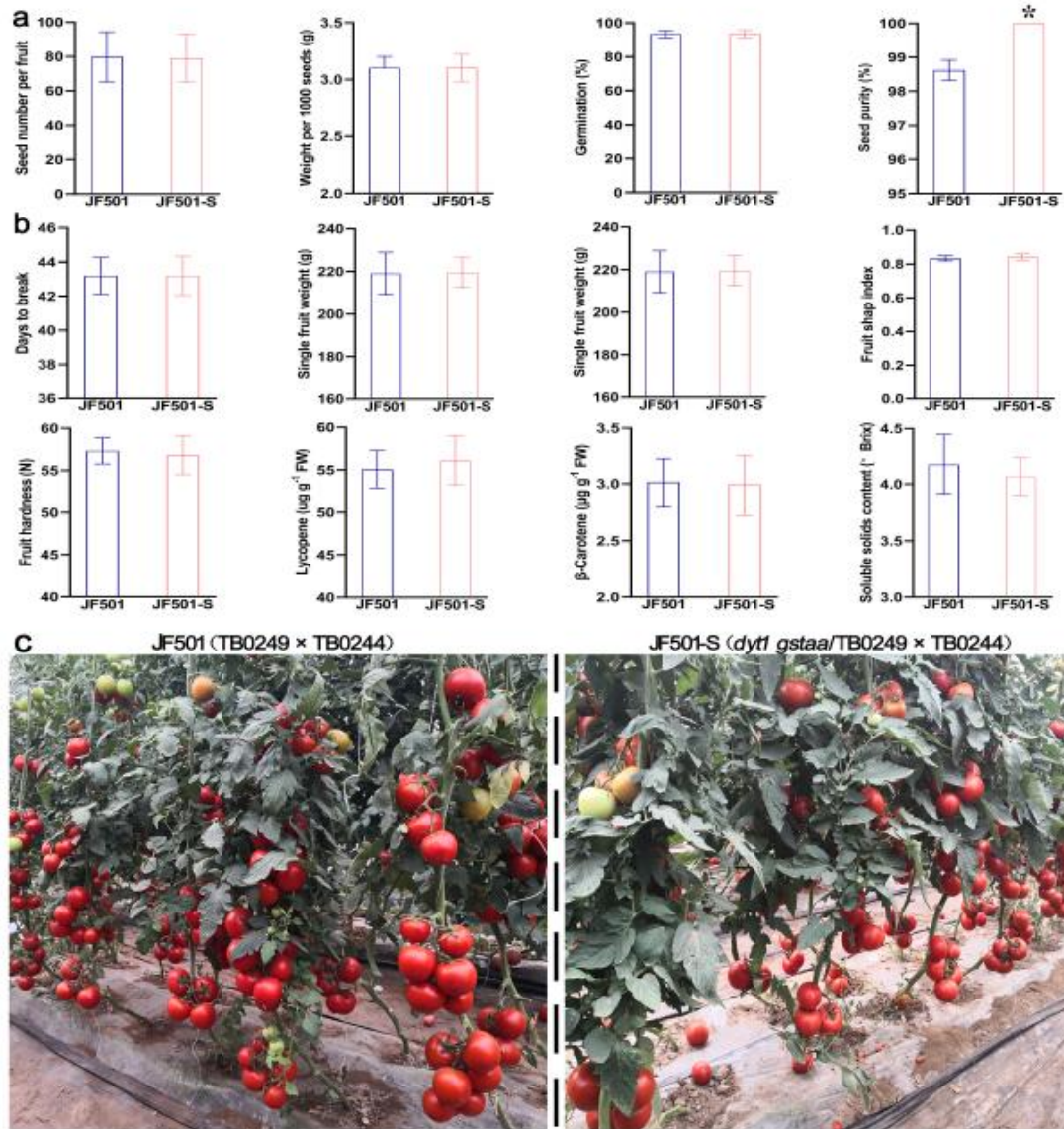


**Figure S2 Phenotyping for male sterility and anthocyanin deficiency of CRISPR-Cas9 derived mutants.** **a** *dyt1 gstaa/TB0249* produced thinner and shorter anther cones when compared with WT plants. **b** Some flowers of *dyt1 gstaa/TB0249* displayed a protruding stigma (right) in contrast to flush stigma of WT plants. **c and d** No visible pollen grains of *dyt1 gstaa/TB0249* were detected by the FDA assay and pollen germination assay. **e** *dyt1 gstaa/TB0249* failed to set seeded fruits in contrast to normal fruit set of WT plants. **f** *dyt1 gstaa/TB0249* gave rise to normal fruits as that of their corresponding WT plants when pollinated with WT pollens. **g** *dyt1 gstaa/TB0249* developed green hypocotyls and cotyledons owing to anthocyanin deficiency in contrast to purple tissues in WT plants. The position where the color difference is most obvious is circled. **h and i** Obvious color differences between *dyt1 gstaa/TB0249* and its corresponding WT plants were observed in hypocotyls, leaves, axillary bud and sepals



**Figure S3** CRISPR-derived *dyt1* mutants developed smaller ovaries. DPA, days post anthesis.





**Figure S4 Performance of the *dyt1 gsta*- and WT-derived elite F1 hybrids.** **a** Seed yield and seed quality of *dyt1 gsta*/TB0249-derived F1 JF501-S showed a comparable level to that of WT-derived F1 JF501. **b** Performance of important agronomic traits of *dyt1 gsta*/TB0249-derived JF501-S were all equivalent to that of WT-derived JF501. **c** Typical pictures of *dyt1 gsta*-derived JF501-S and WT-derived JF501 during the fruit ripening stage

**Table S1 Primers used in this study**

Usage	Name	Sequence (5'–3')
For Cas9-free screening using PCR	PTX-zCas9-402F	TTGACAAGCTGTTTCATCCAG
	PTX-zCas9-402R	CCTTCGTAATCTCGGTGTTC
For knocking-out mutant screening using PCR	DYT1-target1-419F	CAACTCTGAAGAAAGGGAAGTAGG
	DYT1-target1-419R	CTCGTCTGCAGTATCGATAATCTC
	GSTAA-target2-163F	CCTGGTGTACTAGGACATCACAAC
	GSTAA-target2-163R	CTGCTGGAGAGAATCAAGATCAAC
For <i>DYT1</i> genotyping of <i>gyt1 gsta</i> /TB0993 #1 based on PCR method	CR-dyt-112F	TGAAGAAAGGGAAGTAGGAAGAAG
	CR-dyt-112R	AAGTTTTTGACGCCTATTTCTCTC
For <i>DYT1</i> genotyping <i>gyt1 gsta</i> /TB0993 #1 based on KASP method	dyt-993-KF1	GAAGGTCGGAGTCAACGGATTGGAAGTTAAAGAATACAAATCCAAGA
	dyt-993-KF2	GAAGGTGACCAAGTTCATGCTAAATTGATGGTGAAGTTAAAGAATACT
	dyt-993-R	GACGCCTATTTCTCTCAGCCT
For <i>DYT1</i> genotyping of <i>gyt1 gsta</i> /TB0249 #1 based on PCR method	ms-249-KF1	GAAGGTCGGAGTCAACGGATTTTAAAGAATACAAATCCAAGAACCTT
	ms-249-KF2	GAAGGTGACCAAGTTCATGCTAAGTTAAAGAATACAAATCCAAGAACA
For <i>DYT1</i> genotyping <i>gyt1 gsta</i> /TB0249 #1 based on KASP method	ms-249-R	TGACGCCTATTTCTCTCAGCC
	CR-gsta-98F	CCTGGTGTACTAGGACATCACAAC
	CR-gsta-98R	CCATGACCCTTTGTGGACATGCAG
For <i>GSTAA</i> genotyping <i>gyt1 gsta</i> /TB0993 #1 based on KASP method	aa-993-KF1	GAAGGTCGGAGTCAACGGATTATGGTAGTGAAAGTGTATGGTTCAG
	aa-993-KF2	GAAGGTGACCAAGTTCATGCTAGGAAAAATGGTAGTGAAAGTGTATC
	aa-993-R	ATTCTATAAGACAAACCATGACCCT
For <i>GSTAA</i> genotyping <i>gyt1 gsta</i> /TB0993 #1 based on KASP method	aa-249-KF1	GAAGGTCGGAGTCAACGGATTGGTAGTGAAAGTGTATGGTTCAGC
	aa-249-KF2	GAAGGTGACCAAGTTCATGCTTGGTAGTGAAAGTGTATGGTTCAGA
	aa-249-R	ATTCTATAAGACAAACCATGACCCT
For seed purity determination through PCR based genotyping	<i>Ty1</i> -F	CAACAGCAATGTACCTGGTCAG
	<i>Ty1</i> -R	CTGTGGCATAACGTTGGTGACAC