Figure S1 Predicted truncated *dyt1* (**a**) and *gstaa* (**b**) protein sequences. Deduced protein sequences of dyt1 and gstaa in *dyt1 gstaa/*TB0993#1*, dyt1 gstaa/*TB0249#1 and their corresponding wild type were aligned by ClustalW.
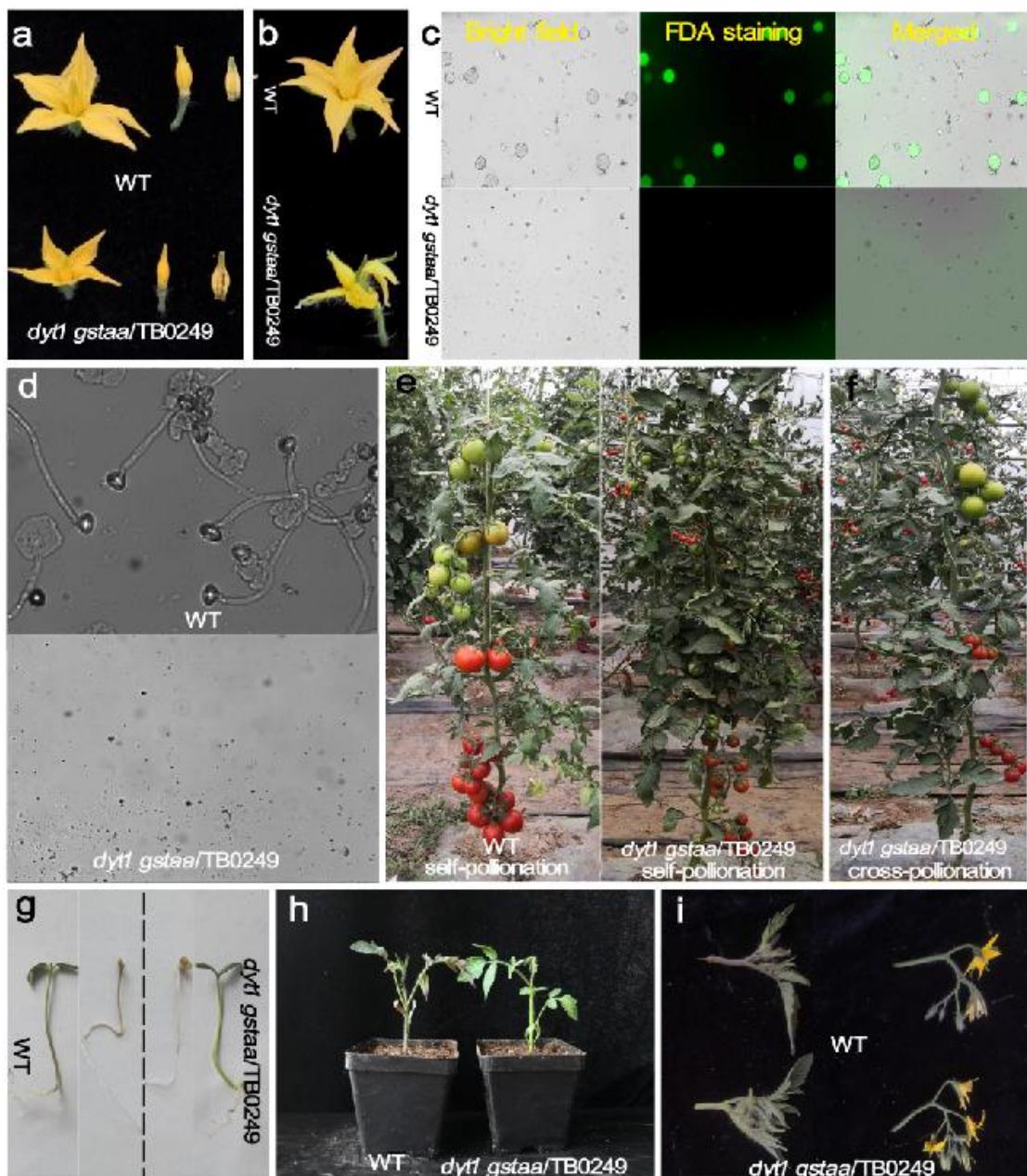
Figure S2 Phenotyping for male sterility and anthocyanin deficiency of CRISPR-Cas9 derived mutants. a *dyt1 gstaa*/TB0249 produced thinner and shorter anther cones when compared with WT plants. **b** Some flowers of *dyt1 gstaa*/TB0249 displayed a protruding stigma (right) in contrast to flush stigma 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
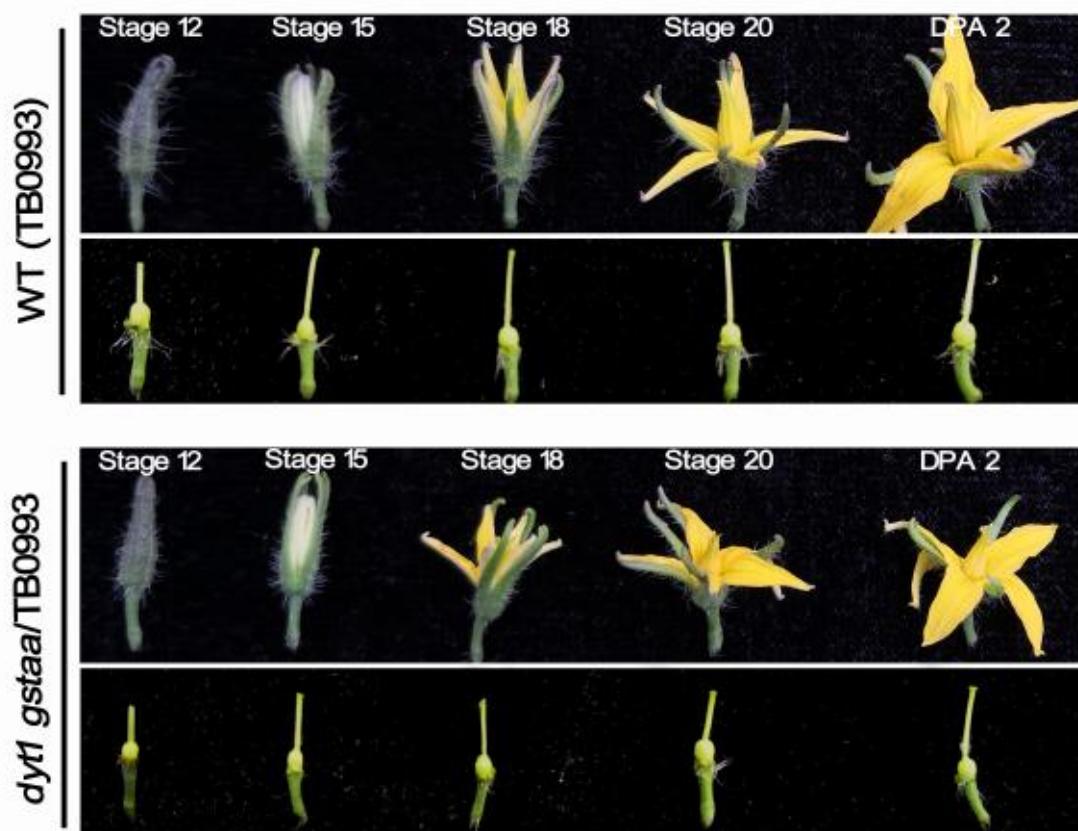
Figure S3 CRISPR-derived *dyt1* mutatnts developed smaller ovaries. DPA, days post anthesis.
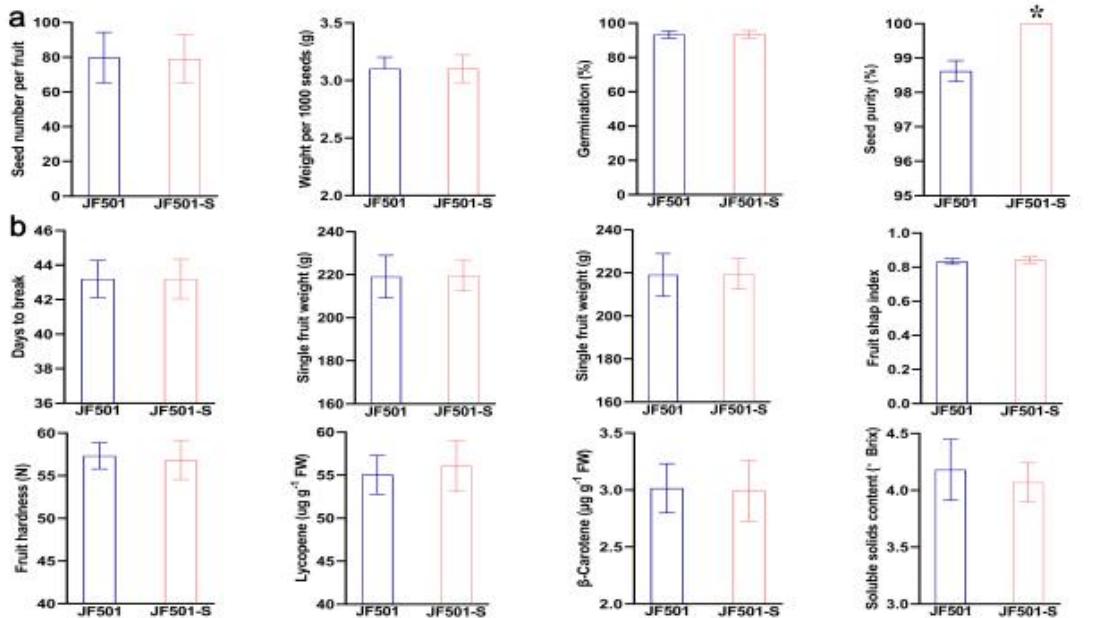
Figure S4 Performance of the *dyt1 gstaa*- and WT-derived elite F1 hybrids. a Seed yield and seed quality of *dyt1 gstaa*/TB0249-derived F1 JF501-S showed a comparable level to that of WT-derived F1 JF501. **b** Performance of important agronomic traits of *dyt1 gstaa*/TB0249-derived JF501-S were all equivalent to that of WT-derived JF501. **c** Typical pictures of *dyt1 gstaa*-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	TTGACAAGCTGTTCATCCAG
	PTX-zCas9-402R	CCTTCGTAATCTCGGTGTTC
For kocking-out mutant screening using PCR	DYT1-target1-419F	CAACTCTGAAGAAAGGGAAGTAGG
	DYT1-target1-419R	CTCGTCTGCAGTATCGATAATCTC
	GSTAA-target2-163F	CCTGGTGTACTAGGACATCACAAC
	GSTAA-target2-163R	CTGCTGGAGAGAATCAAGATCAAC
For *DYT1* genotyping of *gyt1 gstaa*/TB0993 #1 based on PCR method	CR-dyt-112F	TGAAGAAAGGGAAGTAGGAAGAAG
	CR-dyt-112R	AAGTTTTTGACGCCTATTTCTCTC
For *DYT1* genotyping *gyt1 gstaa*/TB0993 #1 based on KASP method	dyt-993-KF1	GAAGGTCGGAGTCAACGGATTTGAAGTTAAAGAATACAAATCCAAGA
	dyt-993-KF2	GAAGGTGACCAAGTTCATGCTAAATTGATGGTGAAGTTAAAGAATACT
	dyt-993-R	GACGCCTATTTCTCTCAGCCT
For *DYT1* genotyping of *gyt1 gstaa*/TB0249 #1 based on PCR method	ms-249-KF1	GAAGGTCGGAGTCAACGGATTTTAAAGAATACAAATCCAAGAACCTT
	ms-249-KF2	GAAGGTGACCAAGTTCATGCTAAGTTAAAGAATACAAATCCAAGAACA
For *DYT1* genotyping *gyt1 gstaa*/TB0249 #1 based on KASP method	ms-249-R	TGACGCCTATTTCTCTCAGCC
	CR-gstaa-98F	CCTGGTGTACTAGGACATCACAAC
	CR-gstaa-98R	CCATGACCCTTTGTGGACATGCAG
For *GSTAA* genotyping *gyt1 gstaa*/TB0993 #1 based on KASP method	aa-993-KF1	GAAGGTCGGAGTCAACGGATTATGGTAGTGAAAGTGTATGGTTCAG
	aa-993-KF2	GAAGGTGACCAAGTTCATGCTAGGAAAAATGGTAGTGAAAGTGTATC
	aa-993-R	ATTCTATAAGACAAACCATGACCCT
For *GSTAA* genotyping *gyt1 gstaa*/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	*Ty1*-F	CAACAGCAATGTACCTGGTCAG
	Ty1-R	CTGTGGCATACGTTGGTGACAC