**Supplemental Dataset S1.** Sanger sequencing results of the sgRNAs amplified from the 355 randomly selected *Agrobacterium* single colonies. Forward primers were used for the sequencing.

**Supplemental Dataset S2.** Sanger sequencing results of the sgRNAs amplified from the 82 randomly selected *Agrobacterium* single colonies. Reverse primers were used for the sequencing.

**Supplemental Dataset S3.** SgRNA sequences of the 28 selected positive transgenic plants obtained from the NGS were verified by Sanger sequencing. Forward or reverse primers were used for the sequencing.

**Supplemental Dataset S4.** Sangersequencing data for the editing interrogation of the target sites.

**Supplemental Dataset S5.** Sanger sequencing data for the off-target effect evaluation.

**Supplemental Dataset S6.** Sanger sequencing data for the editing interrogation of the target sites in T1 plants of lines 0369 and 0594.

**Supplemental Dataset S7.** Sanger sequencing data for the editing interrogation of the target sites in the plants used for the forward genetic screening.

**Supplemental Dataset S8.** SgRNA design data for selecting the target sites following the three principles.