SUPPORTING INFORMATION

Figure S1. Sequence alignment of the *Waxy* genomic sequence in *Nipponbare*, 9522 and XS134
The sequence of *Waxy* gene in 9522 is the same with that in XS134, which contain the typical Wx^b allele. Compared with *Nipponbare*, 9522 and XS134 contains 18 CT repeats in the CT-microsatellite regions, while *Nipponbare* contains 17 CT repeats. Two SNPs between 9522/XS134 and *Nipponbare* are indicated in red box. Start code and stop code are indicated in pink box.

Figure S2. Plant height, grain number per panicle, panicle number per plant and yield per plot in CRISPR-*waxy* mutants and their corresponding WT plants
(**A**) Plant height of *waxy*-9522, *waxy*-XS134 mutant and corresponding 9522, XS134 WT. (**B**) Grain number per panicle of *waxy*-9522, *waxy*-XS134 mutant and corresponding 9522, XS134 WT. (**C**) Panicle number per plant of *waxy*-9522, *waxy*-XS134 mutant and corresponding 9522, XS134 WT. (**D**) Yield per plot of *waxy*-9522, *waxy*-XS134 mutant and corresponding 9522, XS134 WT. Data are presented as means ±sd. n=20 in A-C and n=3 in D; two-tailed, two-sample Student t-test. NS, no significant

Figure S3. Grain width, length and 1,000 grains weight of CRISPR-*waxy* mutants and their corresponding WTs
(**A**) and (**C**) Grain width of *waxy*-9522 mutant and corresponding 9522 WT. (**B**) and (**D**) Grain length of *waxy*-9522 mutant and corresponding 9522 WT. (**E**) 1,000 grains weight of *waxy*-9522 mutant and corresponding 9522 WT. (**F**) and (**H**) Grain width of *waxy*-XS134 mutant and corresponding XS134 WT. (**G**) and (**I**) Grain length of *waxy*-XS134 mutant and corresponding XS134 WT. (**J**) 1,000 grains weight of *waxy*-XS134 mutant and corresponding XS134 WT. Data are presented as means ±sd. n= 50 in C, D, H and I; n=5 in E and J; two-tailed, two-sample Student t-test. NS: no significant

Figure S4. Detection of transgene DNA in CRISPR-*waxy* lines in 9522 and XS134 backgrounds
Red arrows indicate the transgene-free lines.

Table S1. Percentage of T0 plants with mutations in the target locus
*, the T0 homozygous mutations were further confirmed in the T1 generation.

Table S2. Mutation analysis of putative sgRNA off-target sites
Mismatching bases are shown in red color; the PAM motif (NGG) is shown in blue color.

Table S3. Amylose content (AC), gel consistency (GC) and gelatinization temperature (GT) in mature seeds of *waxy* mutants and corresponding WT lines

mm, millimetres; ASV, alkali spreading value. Data are presented as means ± sd. $^{**}P < 0.01$.

Table S4. Production of transgene-free homozygous lines in CRISPR-*waxy* mutants

Table S5. Primers used in this study