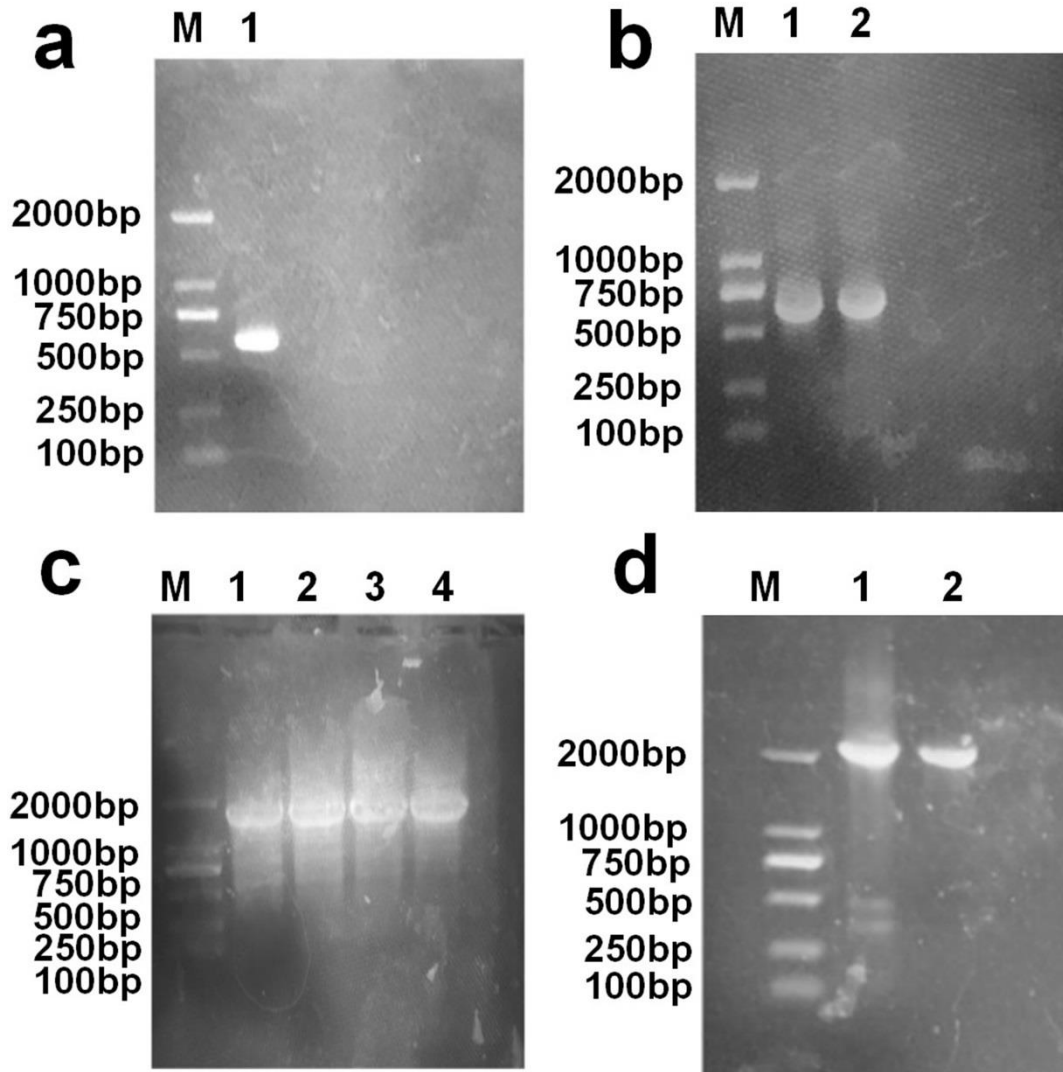
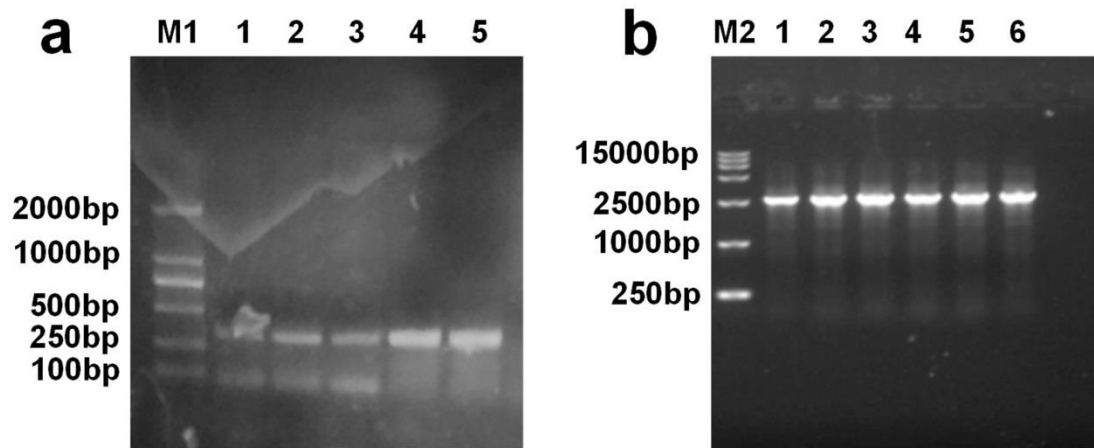


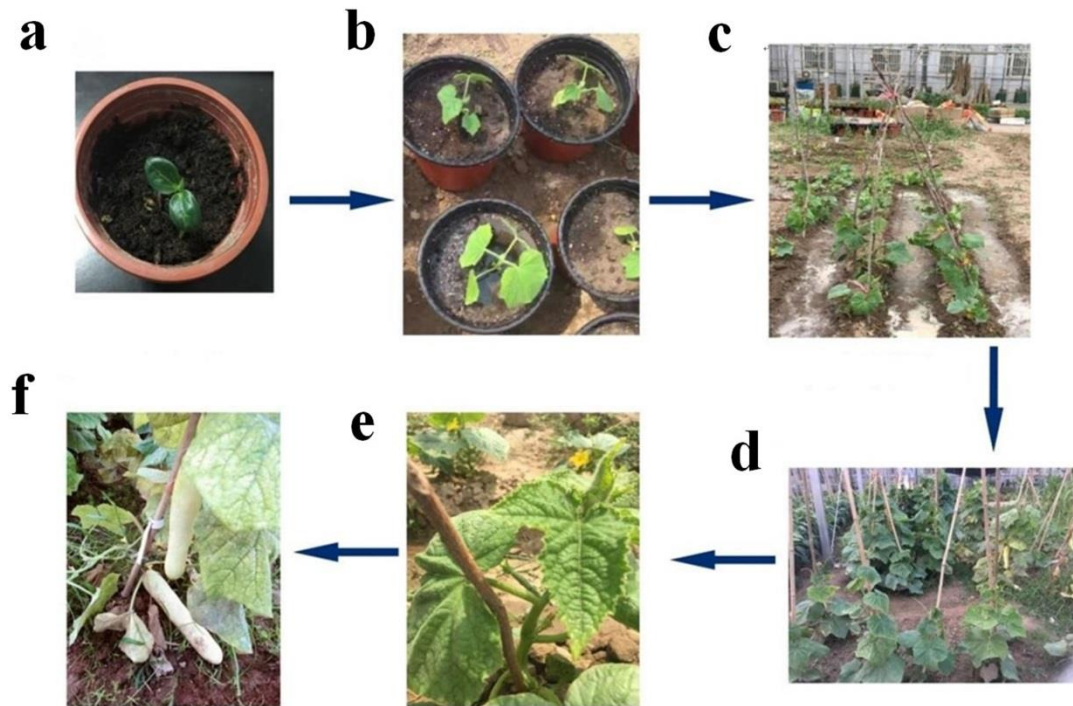
*Supplementary Fig. 1. Identification of NK cloning and point mutation. a. Lane M: DL15000 DNA marker. Lanes 1–8: Colony PCR results; b. Sequencing results for mutant NK.*



*Supplementary Fig. 2. Construction of LHA-U6-NK-T-RHA by overlap PCR. First, overlap PCR was used to amplify the left homologous arm (LHA) (311 bp, with the PCR primers LHA-U and LHA-D) and right homologous arm (RHA) (321 bp, with the PCR primers RHA-U and RHA-D) of the sgRNA targeting the Bt gene of cucumber. Second, the pZHY988 vector was used as a template to amplify the U6 promoter (with primers U6-U and U6-D) and terminator (with primers T-U and T-D). The fusion fragment LHA-U6 was obtained by overlap PCR using LHA and U6 as templates (primers LHA-U and U6-D), and T-RHA was amplified by overlap PCR using terminator and RHA as templates (primers T-U and RHA-D). Then, LA-U6-NK was amplified using LHA-U6 and NK as templates (primers LHA-U and NK-D). Finally, LHA-U6-NK-T-RHA was obtained by overlap PCR using LHA-U6-NK and T-RHA as templates (primers LHA-U and RHA-D). a. Fusion segments of *LHA-U6*; b. Fusion segments of *T-RHA*; c. Fusion segments of *LHA-U6-NK*; d. Fusion segments of *LHA-U6-NK-T-RHA*.*



*Supplementary Fig. 3.* PCR verification of pZHY988-Cas9-sgRNA and pX6-LHA-U6-NK-T-RHA vectors transformed into *A. tumefaciens* EHA105. a. PCR products using the pZHY988-Cas9-sgRNA vector as a template. Lane M1: DL2000 DNA marker, Lanes 1–5: PCR products. b. PCR products using the pX6-LHA-U6-NK-T-RHA vector as a template. Lane M2: DL15000bp DNA Marker; Lanes 1–6: PCR products.



*Supplementary Fig. 4.* The cultivation and transformation processes of cucumber. Seedlings were transferred outdoors for seedling training when they grew 4–5 true leaves. The experimental field was turned over, fertilised, and watered to increase the humidity. Then, the seedlings were transplanted into the experimental field and field management, such as watering, was performed in the transplantation stage. Seedling growth was in a stable state after one week of transplantation. a. grow seedlings; b. acclimatization; c. Transplant seedlings to the experimental field; d. Field management; e. Female flowers before flowering; f. Harvest fruits.