a

35S pro | Cas9+SV40 NLS | NOS Terminator

gRNA
GmU6 ─ GmU6 ─ gRNA2

b

                                            gRNA1   gRNA2
5'- GGAAGG**AAGAAGAGGCGCAATAGGTC**GGG**AACTG**TGG**CGCTTCGTGAG -3' CENH3(*WT*)   FS, frame shift
      G   R   K   K   R   R   N   R   S   **G**   T   V   A   L   R   E

C2
5'- GGAAGGAAGAAGAGGCGCAAT - - - - - - - - - - - - GTGGCGCTTCGTGAG -3' *cenh3-1* (12bp deletion)
      G   R   K   K   R   R   N   -   -   -   -   V   A   L   R   E

5'- GGAAGGAAGAAGAGGCGCAATAG-TCGGGAAgCTGTGGCGCTTCGTGAG -3' *cenh3-2* (1bp deletion+ 1bp insertion)
      G   R   K   K   R   R   N   S   R   E   A   V   A   L   R   E

C16
5'- GGAAGGAAGAAGAGGCGCAATAGGTCGG - - - CTGTGGCGCTTCGTGAG -3' *cenh3-3* (3bp deletion)
      G   R   K   K   R   R   N   R   S   A -   V   A   L   R   E

5'- GGAAGGAAGAAGAGG - - - - - - - GGTCGGGAACTGTGGCGCTTCGTGAG -3' *cenh3-4* (7bp deletion/FS, null)

C10
5'- GGAAGGAAGAAGAGGCGCA - - - GGTCGGGAACTGTGGCGCTTCGTGAG -3' *cenh3-5* (3bp deletion)
      G   R   K   K   R   R   -   R   S   **G**   T   V   A   L   R   E

5'- GGAAGGAAGAAGAGGCGCAAT - - - - - - - - - - - - - - - - - - TTCGTGAG -3' *cenh3-6* (19bp deletion/FS, null)

C12
5'- GGAAGGAAGAAGAGGCGC - - - - - GTCGGGA - CTGTGGCGCTTCGTGAG -3' *cenh3-7* (5-bp deletion+1-bp deletion)
      G   R   K   K   R   R   -   V   **G**   T   V   A   L   R   E

5'- GGAAGGAAGAAGAGGCGC - - - - - GTCGGGA - - - - - - GCGCTTCGTGAG -3' *cenh3-8* (5bp deletion+6bp deletion/FS, null)

C5
5'- GGAAGGAAGAAGAGGCGCAATAGGTCGGGAAaCTGTGGCGCTTCGTGAG -3' *cenh3-9* (1bp insertion/FS, null)
      G   R   K   K   R   R   N   R   S   **G**   N   C   G   A   S   .

5'- GGAAGGAAGAAGAGGCGCAATAGGTCGGGAACacTGTGGCGCTTCGTGAG -3' *cenh3-10* (2bp insertion/FS, null)
      G   R   K   K   R   R   N   R   S   **G**   T   L   W   R   F   V

Supplemental Figure 3. Edited *GmCENH3* using CRISPR/Cas9 technology.

(a) Scheme of CRISPR/Cas9 constructs for genome editing of *GmCENH3*. (b) The edited sequences of *GmCENH3* and the derived amino-acid changes.