

1 **Supplementary materials and methods**

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3 **Cel I assay**

4 PCR products amplified using the following primers were subjected to Cel I nuclease
5 reaction and analyzed by agarose gel electrophoresis. OsLig4-1F
6 5'-TGACAAGCTTGAGGAAAATGAGAAGGCTGA-3', OsLig4-1R
7 5'-ATGGCAACCTACTCCTCTCACAACACAACG-3'.

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9 **Western blot analysis**

10 Proteins extracted from calli were fractionated by SDS-PAGE on a 5–20% Tris-glycine
11 SDS gradient precast polyacrylamide gel (ATTO, Tokyo, JAPAN) and subjected to
12 immunoblotting with Cas9 antibody (Active motif, Carlsbad, CA, USA) using
13 immunoreaction enhancer solution Can Get Signal (TOYOBO, Osaka, Japan). After
14 rinsing with 1xTBST, blots were hybridized with second-antibody, stabilized goat
15 anti-mouse IgG HRP-conjugated (Thermo Fisher Scientific, Waltham, MA) and signals
16 were detected using Super Signal West Dura Maximum Sensitivity Substrate (PIERCE,
17 Rockford, IL, USA) and the ChemiDoc Touch Imaging system (Bio Rad, Hercules, CA,
18 USA).

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