GW2T2 on Genome A AGGGSCTGTACGAGCACAGGGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCCCCTGCTACCCGGGGGCTGACGACGCCGCGAGGGCTTGTACGACGACGAGAAGAAGCTACGACAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCC-GGGGCTGACGACGCCGCGAGGGCTTGTACGACGACGAAGAAGCTACGAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCC-GGGCTGACGACGACGCCGCGAGGGCTTGTACGACGACGACGACGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCCCCTGCTACC-GGGGCTGACGACGACGCCGCGAGGGCTTGTACGACACAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCCCCTGCTAC-GGGGGCTGACGACGACGCCGCG CGGGGGCTGACGACGCCGCG GGGGCTGACGACGCCGCG GGGGGCTGACGACGCCGCG GW2T2 on Genome B tgtacgagcaca<mark>g</mark>ggatatcgaccagaagaagctacgcaagttgatcctcgaggccaa<mark>gc1</mark> CGGGGGCTGACGACGCCGCG TGTACGAGCACASGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCC-GGGGCTGACGACGCCGCG TGTACGAGCACASGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCTACCC--GGGCTGACGACGACGCCGCG TTTTACGAGCACASGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTTGCGCCCTGCTAC CTGTACGAGCACASGATATCGACCAGAAGAAGCTACGCATGATGCTCCTCGAGGCCAAGCTTGCGCCCTGCTAC CTGTACGAGCACASGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTTGCGCCCTGCT CTGTACGAGCACASGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCCTGCT CTGTACGAGCACASGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCCAGGCCCAAGCTCGCGCCC CTGTACGAGCACASGGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCC CTGTACGAGCACAGGGAGATATCGACCAGAAGAAGCTACGCAAGTTGATCCTCGAGGCCAAGCTCGCGCCC GGGGGCTGACGACGCCGCG AGGG -- CGGGGGCTGACGACGCCGCG GGGGCTGACGACGCCGCG AGGG GGGGGCTGACGACGCCGCG GCCGCG GW2T2 on Genome D LPX1T2 on Genome B CGCACCTACGTCGACACCACCCCCGGCGAGTTCGACTCCTTCCAGGACATCATCAACCTC CGCACCTACGTCGACACCA-CCCCGGCGAGTTCGACTCCTTCCAGGACATCAT CAACCTC CAACCTC CGCACCTACG-CGACACCACCCCGGCGAGTTCGACTCCTTCCAGGACATCAT CAACCTC CGCACCTACG--GACACCACCCCGGCGAGTTCGACTCCTTCCAGGACATCAT ACCCCGGCGAGTTCGACTCCTTCCAGGACATCATCAACCTC -CCCCGGCGAGTTCGACTCCTTCCAGGACATCATCAACCTC CGCACCTACG---ACACCACCCCGGCGAGTTCGACTCCTTCCAGGACATCAT CGCACCTACG-LPX1T2 on Genome D CGCACCTACGTCGACACCACCCCCGGGGAGTTCGACTCCTTCCAGGACATCATGAACCTC CGCACCTACGTCGACACCA-CCCCGGCGAGTTCGACTCCTTCCAGGACATCATGAACCTC CGCACCTACG--GACACCACCCCGGCGAGTTCGACTCCTTCCAGGACATCATGAACCTC CGCACCTACG: --cccccggcgagttcgactccttccaggacatcatgaacctc MLOT1 on Genome A TCTCGCTGCTGCTCGCCGTCACGCAGGACCCAATCTCCGGGGATATGCATCTCCCAGAAGG TCTCGCTGCTCGCCGTCA-GCAGGACCCAATCTCCGGGATATGCATCTCCCAGAAGG TCTCGCTGCTGCTCGCCGTCA GGACCCAATCTCCGGGATATGCATCTCCCAGAAGG

SUPPLEMENTARY FIG. S5. Comparison of the gene editing efficiency of single and multiplex gene editing CRISPR-Cas9 constructs using the protoplast transient expression assay. The mutations were detected by the NGS of the PCR amplicon libraries generated from the protoplast DNA. Representative reads with mutations in the target sites GW2T2, LPX1T1, and LPX1T2 induced by the MGE construct pBUN421-GLM are shown. The first rows correspond to the wild-type genomic sequences from the A, B, or D genomes. The targeted regions are shown within the red rectangles; the PAM sequences are underlined. The genome specific nucleotide bases are shown within the black rectangles.