Title: Supplementary Data 1:

Description: Screening data of all gRNA-target pairs for three libraries (Lib T1-3) using dual-target system.

Title: Supplementary Data 2: Description: Screening data of all gRNA-target pairs for one library (Lib S1) using singletarget system.

Title: Supplementary Data 3: Description: Screening data of six libraries (Lib sg1-6) using single-target system.

Title: Supplementary Data 4:

Description: Screening data of all the 1-mismatch gRNAtarget pairs in three dual-target libraries, which were used for the decomposition of 1-mismatchdependent effects and guideintrinsic mismatch tolerance (GMT).

Title: Supplementary Data 5: **Description:** Screening data for allele-specific editing library (Lib Allele) using dual-target system.

Title: Supplementary Data 6: **Description:** Summary of the detailed components in each designed library.

Title: Supplementary Data 7: **Description:** Oligos and primers used for experiments.