A

Step 1: Cl.25: Heterozygote (targeted/wildtype)

Step 1: Cl.26: Allele 1 targeted; Allele 2 indel (+T)

> Step 1: Cl.27: Messy read = indels

Step 1: Cl.28: Homozygote (targeted/targeted)

Step 1: Cl.29: untargeted (wildtype/wildtype)

Step 1: Cl.30: Homozygote (targeted/targeted)

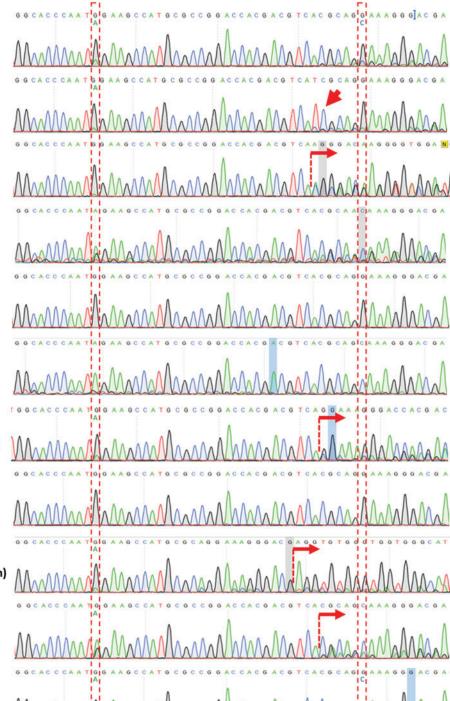
Step 1: Cl.31: Allele 1 targeted; Allele 2 indel (insert)

Step 1: Cl.32: untargeted (wildtype/wildtype)

Step 1: Cl.33: Allele 1 targeted; Allele 2 indel (deletion)

> Step 1: Cl.35: Allele 1 targeted; Allele 2 indel

Step 1: Cl.36: Heterozygote (targeted/wildtype)



**SUPPLEMENTARY FIG. S2.** (A, B) Sequencing data for *ADRB2* editing. Chromatograms derived from direct sequencing of PCR products are shown for the clones picked after step 1 (Fig. 2A; midpoint; after puromycin selection) and after step 2 (Fig. 2B; end; after ganciclovir selection and cassette excision). In (A) (read in forward direction), the polymorphic sites at nucleotide positions 46 and 79 are shown, but not the *TTAA* site since this is disrupted by cassette insertion. In (B) (read in reverse direction), the polymorphic sites at nucleotide positions 46 and 79, as well as the reconstituted *TTAA* site. Heterozygote (monoallelic) and homozygote (biallelic) targeting events are shown, with the former indicated by double peaks and both bases shown. *Arrows* indicate region where sequence becomes misaligned due to indels. PCR, polymerase chain reaction.

(continued)

## В

Step 2: Cl.m2: Homozygote (targeted/targeted)

Step 2: Cl.m6: untargeted (wildtype/wildtype)

Step 2: Cl.m7: Homozygote (targeted/targeted)

Step 2: Cl. m10: Heterozygote (targeted/wildtype)

Step 2: Cl. m11: Heterozygote (targeted/wildtype)

Step 2: Cl. m12: Heterozygote (targeted/indel)

Step 2: Cl. m13: Homozygote (targeted/targeted)

Step 2: Cl. m14: Homozygote (targeted/targeted)

Step 2: Cl. m21: Heterozygote (targeted/wildtype)

0 0 AC & C CCACCACCCACACCT C & T CCCTT G C C CA T CCO OCOCATO OCT T N 0 0 0 0 S 0.0 0 0 r 0]0 N S 0]0 N 6 6] AC ~ ..... M M

SUPPLEMENTARY FIG. S2. (Continued).