



SUPPLEMENTARY FIG. S4. Guide RNA target sites and cutting activity for mouse *Rosa26* gene. **(A)** The locations of the MAD7 gRNAs tested for the *Rosa 26* in red, oligonucleotide PCR primers in green, and the oligo donor HA (solid black line; R, right; L, left). The bottom sequence contains the T7-promoter and *Bam*HI restriction enzyme integration sequences. **(B)** DNA mismatch detection assay using *Cel*-I enzyme of the above *Rosa26* gRNAs tested in Neuro-2A cells. The right arrow represents the expected size of the PCR amplification product (302 bp), and the asterisk marks the predicted fragments for gRNA #1 (201 bp, 101 bp), #2 (197 bp, 105 bp), #3 (157 bp, 145 bp), and #4 (187 bp, 115 bp). The numbers to the left represent the location of the DNA size markers in base pairs.