



n=50

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AGGCTACGTCCAGGAGCGCACCATCTTC:TTCAAGGACGACGGCAACTACAAGACC (WT)
AGGCTACGTCCAGGAGCGCACCATCTTCtTTCAAGGACGACGGCAACTACAAGACC (+1bp, x4)
AGGCTACGTCCAGGAGCGCACCATCTTC:TT:::GGACGACGGCAACTACAAGACC (-3bp)
AGGCTACGTCCAGGAGCGCACCATCTT:::GGACGACGGCAACTACAAGACC (-6bp)
AGGCTACGTCCAGGAGCGCACCATCTTC:::GACGACGGCAACTACAAGACC (-6bp)
AGGCTACGTCCAGGAGCGCACCATCTTC:::AAGGACGACGGCAACTACAAGACC (-3bp)
AGGCTACGTCCAGGAGCGCACCATC:::AAGGACGACGGCAACTACAAGACC (-6bp, x3)

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**Supplementary information, Figure S9** Cas9/RNA site-specifically cut endogenous EGFP

of EGFP transgenic mouse. Transgenic founders were generated by co-injection of 20 ng/μl NLS-flag-linker-Cas9 mRNA and 20 ng/μl pre-annealed EGFP-A chimeric RNA into zygotes obtained by mating of CAG-EGFP transgenic males. T7EN1 cleavage assay (Upper) was performed as described in materials and methods by PCR amplification of the target site using genomic DNA extracted from tails of the transgenic founders as template. Arrowheads indicated that two cleavage bands (about 295 bp and 198 bp) were detected in #4 founder. Sequencing results of T-A clone for #4 founder showed 11 mutant colonies were detected out of 50 sequenced (Lower).