**SUPPLEMENTAL MATERIAL**

# Cas12a mediates efficient and precise endogenous gene tagging via MITI: microhomology-dependent targeted integrations

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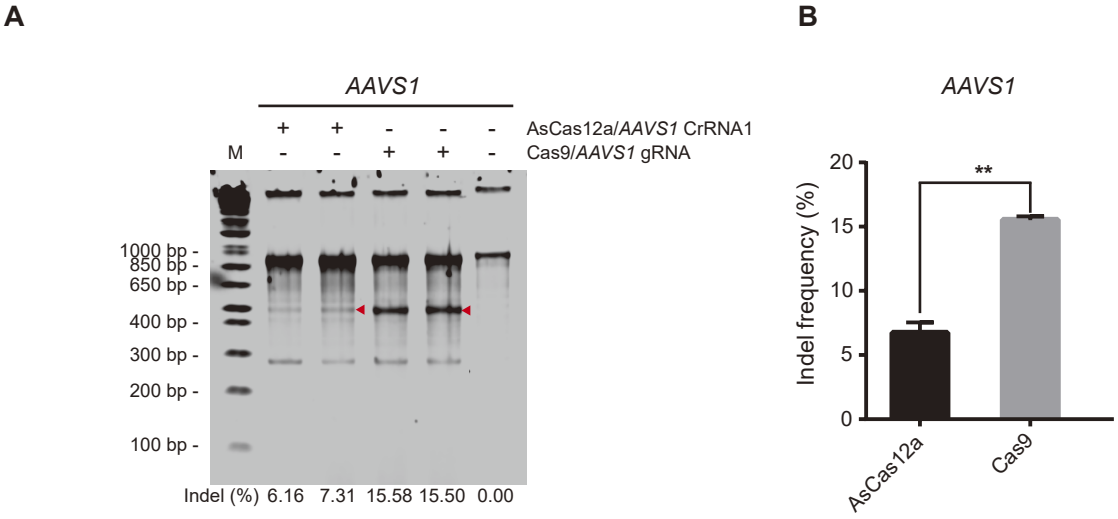
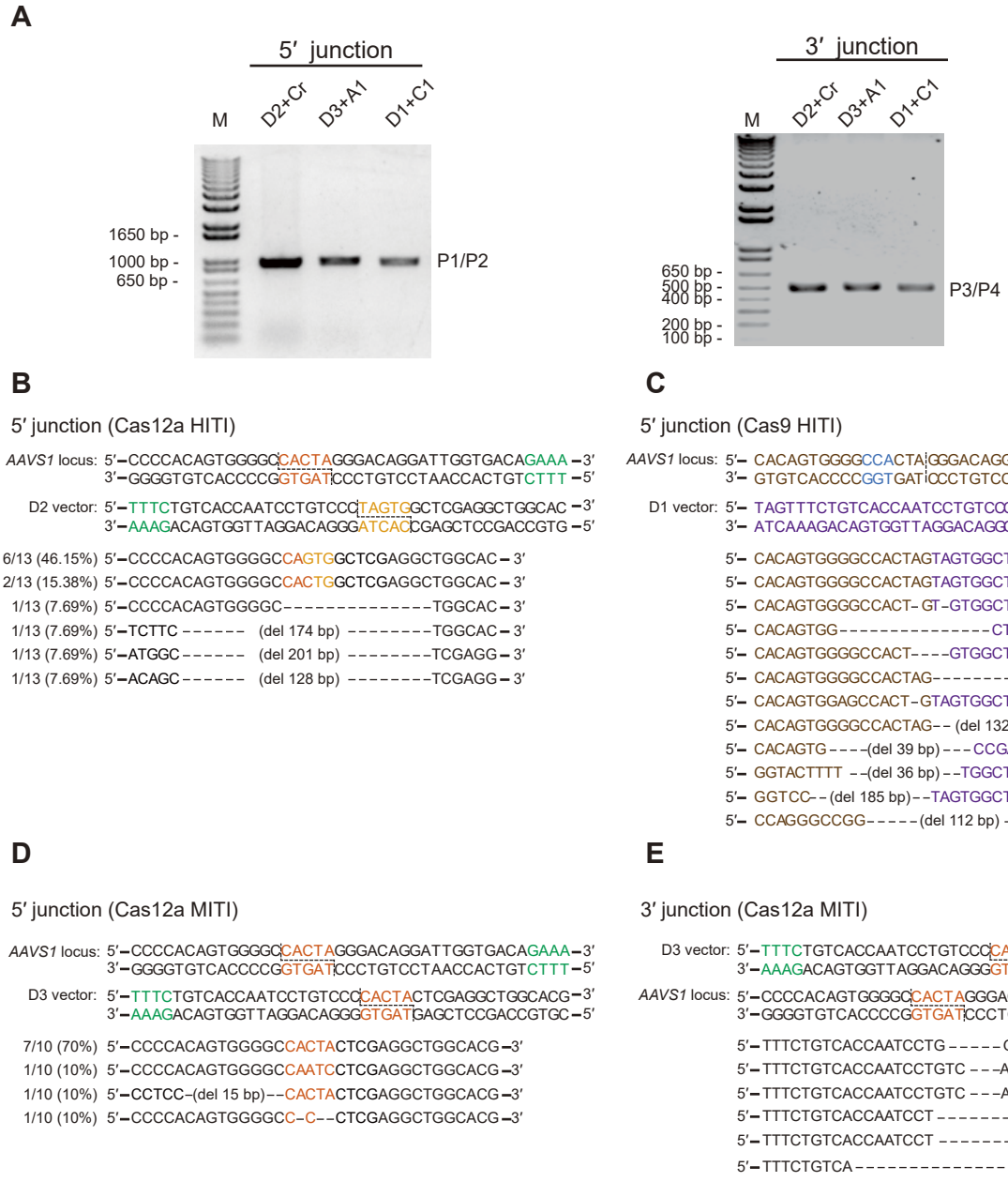
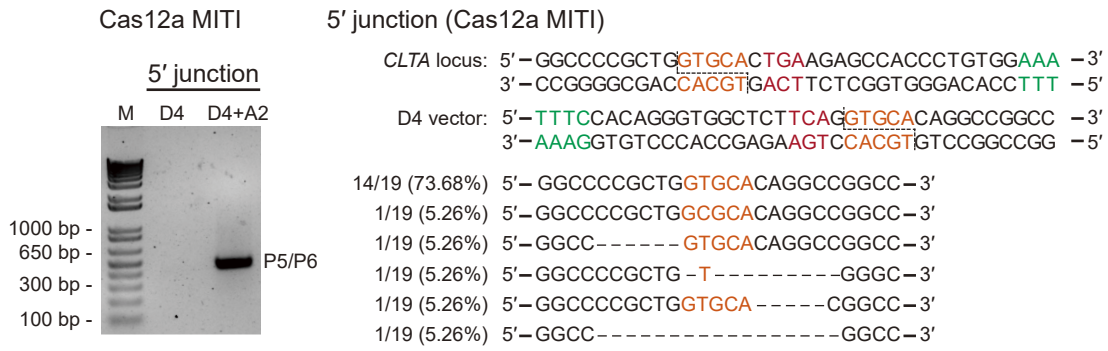
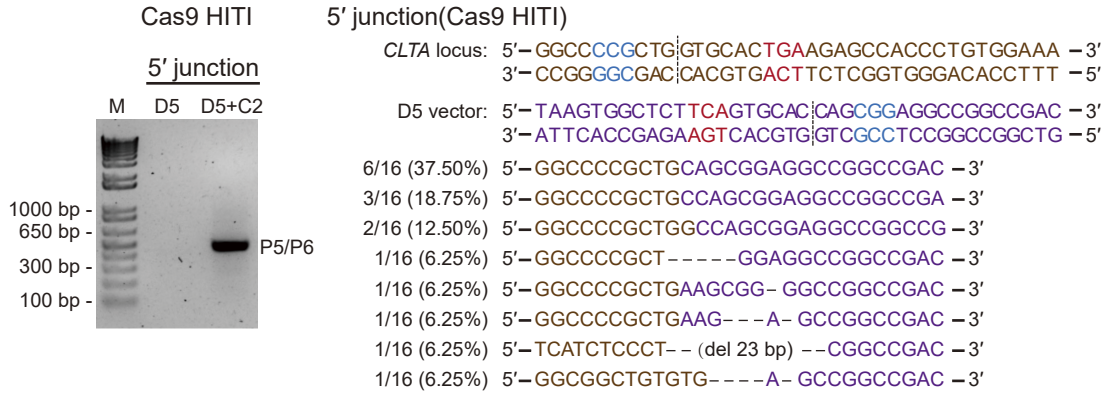



**Supplemental Figure S1.** The verification and comparison of the target efficiency between the Cas9 and Cas12a at the *AAVS1* locus. T7E1 analyses showing that the gRNA of Cas9 is more efficient than that of Cas12a when the same *AAVS1* site was targeted.The result was presented as mean ± SD, n=2, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, unpaired Student's t-test.

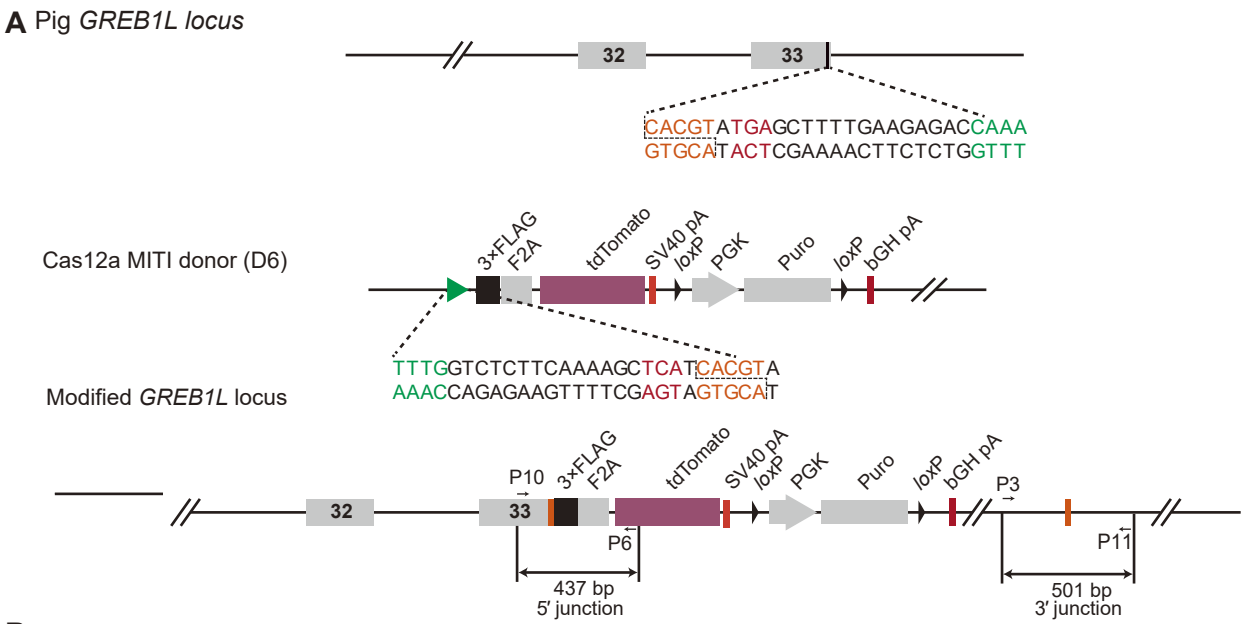


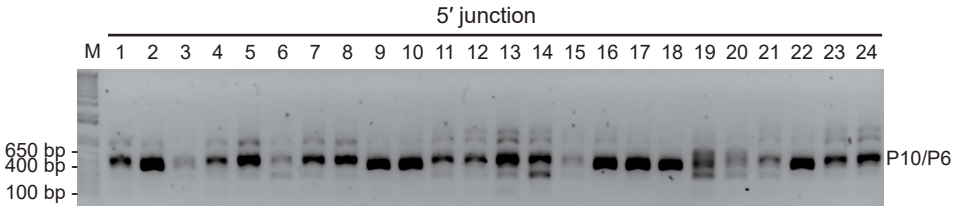

**Supplemental Figure S2.** Analyze the validity and accuracy of two junctions. **(A)** PCR analysis of 5' and 3' junctions of cells with Cas12a HITI, Cas12a MITI, and Cas9 HITI targeted integrations at the *AAVS1* locus in HeLa cells. Genomic PCR products amplified from pooled HeLa cells transfected with three groups of plasmids. The first group contained the Cas12a HITI donor (D2), *AAVS1* CrRNA1 and the Cas12a targeting vector (Cr); the second group included the Cas12a MITI donor (D3), *AAVS1* CrRNA1, *AAVS1* CrRNA1.1, and the Cas12a targeting vector (A1); and the third group had the Cas9 HITI donor (D1), *AAVS1* gRNA1 and Cas9 targeting vector (C1). M is the 1kb plus ladder maker. **(B)** The representative TA cloning sequence analysis at the 5' target junction of *AAVS1* targeted integration events mediated by Cas12a HITI strategy after PCR-based amplification. **(C)** The representative TA cloning sequence analysis at the 5' target junction of *AAVS1* targeted integration events mediated by Cas9 HITI strategy. **(D and E)** The representative TA cloning sequence analysis at the 5' and 3' target junction of *AAVS1* targeted integration events mediated by Cas12a MITI strategy.




**Supplemental Figure S3.** Detection of target integration at the 5' junction at the *CLTA* locus. Genomic PCR products amplified from pooled HEK293T cells transfected with *CLTA* donor and the corresponding Cas12a or Cas9 targeting vector and the TA cloning sequence analysis at the 5' junction of *CLTA* targeted integration events after PCR-based amplification. A2 represents the Cas12a co-expression plasmid with the *CLTA* CrRNA array. C2 represents the *CLTA* gRNA and Cas9 co-expression plasmid. P5 and P6 primers are utilized to amplify the 5' junction. The right panel is the representative TA cloning sequence results of the 5' target junction of *CLTA* integration using the Cas12a MITI or Cas9 HITI strategy.

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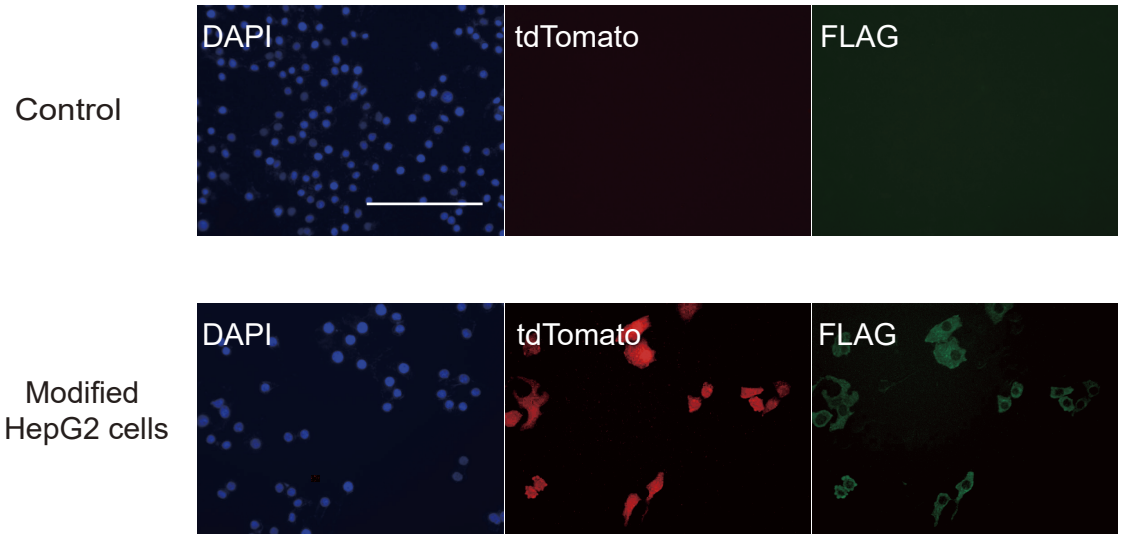
5′ junction (Cas12a MITI)

*GREB1L* locus: 5′–CTTTCTTACTGGACGTCACGTATGAGCTTTTGAAGAGACCAAAACTAG–3′
3′–GAAAGAATGACCTGCAGTGCATACTCGAAAACTTCTCTGGTTTTGATC–5′

D6 vector: 5′–TTAATTTGGTCTCTTCAAAAGCTCATCACGTAAGGCCGGCCGACTATA–3′
3′–AATTAAACCAGAGAAGTTTTCGAGTAGTGCATTCCGGCCGGCTGATAT–5′

#1 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#2 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#3 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#4 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#5 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#6 5′–CTTTCTTACTGGACGTCACGTACGGCCGGCCGACTATA–3′
#7 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#8 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#9 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#10 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#11 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#12 5′–CTTTCTTACTGGACGTCACACGTAAGGCCGGCCGACTATAA–3′
#13 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#14 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#15 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#16 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#17 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#18 5′–CTTTCTTACTGGACGTCACGTGGTAAGGCCGGCCGACTATAA–3′
#21 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#22 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#23 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′
#24 5′–CTTTCTTACTGGACGTCACGTAAGGCGGGCCGACTATA–3′

**Supplemental Figure S4.** Tagging the *GREB1L* gene in pig fetal fibroblasts (PFFs) cells using the MITI approach. **(A)** Strategy for targeting *GREB1L* locus in PFF cells. **(B)** PCR identification of positive PFF clones bearing predicted integration of 3×FLAG-F2A-tdTomato. **(C)** The 5' junction sequences of positive PFF clones.




**Supplemental Figure S5.** Immunostaining results of tdTomato positive HepG2 cells. The tdTomato positive HepG2 cells bearing 3×FLAG-2A-tdTomato integration in *CLTA* were fixed, stained with anti-FLAG antibody and examined by fluorescence microscopy. Scale bar, 200 μm

**Supplementary Tables**

**Supplementary Table S1**. The primers for donor construction. All sequences are in the 5' to 3' direction

| Primer name | Sequence |
|---|---|
| D1/D3-linker-F | CTAGTTTCTGTCACCAATCCTGTCCCTAGTGGC |
| D1/D3-linker-R | TCGAGCCACTAGGGACAGGATTGGTGACAGAAA |
| D2-linker-F | CTAGTTTCTGTCACCAATCCTGTCCCCACTAC |
| D2-linker-R | TCGAGTAGTGGGGACAGGATTGGTGACAGAAA |
| D4-linker-F | TATGttaattaaTTTCCACAGGGTGGCTCTTCAGGTGCAcAGGCCGG |
| D4-linker-R | CCTgTGCACCTGAAGAGCCACCCTGTGGAAAttaattaaCA |
| D5-linker-F | TATGttaattaaGTGGCTCTTCAGTGCACCAGCGGAGGCCGG |
| D5-linker-R | CCTCCGCTGGTGCACTGAAGAGCCACttaattaaCA |
| D6-linker-F | TATGttaattaaTTTGGTCTCTTCAAAAGCTCATCACGTaAGGCCGG |
| D6-linker-R | CCTtACGTGATGAGCTTTTGAAGAGACCAAAttaattaaCA |
| D7-linker-F | CGCGCCAGGATCTCTGGCTCCATCGTAAGCAAAACGCGTGTCGACA |
| D7-linker-R | GATCTGTCGACACGCGTTTTGCTTACGATGGAGCCAGAGATCCTGG |

89

**Supplementary Table S2**. The primers for CrRNAs construction. All oligos are in the 5' to 3' direction.

| Primer name | Sequence |
|---|---|
| *AAVS1*-CrRNA1-F | agatTGTCACCAATCCTGTCCCTAGTG |
| *AAVS1*-CrRNA1-R | aaaaCACTAGGGACAGGATTGGTGACA |

| | |
|---|---|
| *AAVS1*-CrRNA1.1-F | agatTGTCACCAATCCTGTCCCCACTA |
| *AAVS1*-CrRNA1.1-R | aaaaTAGTGGGGACAGGATTGGTGACA |
| *AAVS1*-sgRNA1-F | CACCGTCACCAATCCTGTCCCTAG |
| *AAVS1*-sgRNA1-R | AAACCTAGGGACAGGATTGGTGAC |
| *CLTA*-CrRNA-array-F | agatCACAGGGTGGCTCTTCAGTGCACaatttctactcttgtagatCACA<br>GGGTGGCTCTTCAGGTGCA |
| *CLTA*-CrRNA-array-R | aaaaTGCACCTGAAGAGCCACCCTGTGatctacaagagtagaaattGT<br>GCACTGAAGAGCCACCCTGTG |
| *CLTA*-sgRNA-F | CACC GTGGCTCTTCAGTGCACCAG |
| *CLTA*-sgRNA-R | AAAC CTGGTGCACTGAAGAGCCAC |
| *GREB1L*-CrRNA-array-F | agatGTCTCTTCAAAAGCTCATACGTGaatttctactcttgtagatGTCT<br>CTTCAAAAGCTCATCACGT |
| *GREB1L*-CrRNA-array-R | aaaaACGTGATGAGCTTTTGAAGAGACatctacaagagtagaaattCA<br>CGTATGAGCTTTTGAAGAGAC |
| *AAVS1*-CrRNA-array-F1 | agatTGTCACCAATCCTGTCCCTAGTGAATTTCTACTCTTGT<br>AGATTGTCACCAATCCTGTCC |
| *AAVS1*-CrRNA-array-F2 | CCACTAaatttctactcttgtagatCTTACGATGGAGCCAGAGAGGAT<br>aatttctactcttgtagatCTTACGATGGAGCCAGAGATCCT |
| *AAVS1*-CrRNA-array-R1 | aaaaAGGATCTCTGGCTCCATCGTAAGatctacaagagtagaaattATC<br>CTCTCTGGCTCCAT |

| | |
|---|---|
| | CGTAAGatctacaagagtagaaattTAGTGGGGACAGGATTGGTGAC |
| *AAVS1*-CrRNA-array-R2 | AATCTACAAGAGTAGAAATTCACTAGGGACAGGATTGGT GACA |

90

**Supplementary Table S3**. Primers used for Knock-in detection

| Primer name | Sequence |
|---|---|
| P1 | TGCCATCTCTCGTTTCTTAGGATG |
| P2 | cagaTcgataaaacacatgcgtcaattt |
| P3 | GCGTTTCGGTGATGACGGTG |
| P4 | CTGCCAAGCTCTCCCTCCCAG |
| P5 | GGGACAAATAGGCAGTTGCT |
| P6 | tcctcgcccttgctcaccat |
| P7 | CTCTGAATGCCAGGGAGAAC |
| P8 | TCTGTTCCACATACACTTCATTC |
| P9 | CCCGGTGCCTGAGATaaacG |
| P10 | CGGCTGTCACATCTTGGTTT |
| P11 | TCCAAAGCATCTCCTCAGGC |
| P12 | CAGGACGGGGCTGGCTACTG |

91

**Supplementary Table S4**. Primers used for T7E1 assay

| Primer name | Sequence |
|---|---|
| *AAVS1*-T7E1-sur-F | TGCCATCTCTCGTTTCTTAGGATG |

| | |
|---|---|
| *AAVS1*-T7E1-sur-R | CTGCCAAGCTCTCCCTCCCAG |

92