**Gene knock-in by CRISPR/Cas9 and cell sorting in macrophage and T cell lines**

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**Figure S1.**

To evaluate the cleavage activities of guide RNAs, electroporation of 4.0 × 105 cells was performed in the format one 10 μL nucleofection tip with 0.5 μg of each CRISPR expression vectors in the absence of a targeting vector. 48-72 h post-transfection, 1000 single cells express both DsRed2 and EBFP2 fluorescent reporters were sorted into a total 50 μL of lytic reaction with 25 μL 2 × lysis buffer (100 mM KCl, 20 mM Tris-HCl pH 9.0, 0.2% Triton X-100, and 0.8 mg/mL proteinase K) for isolating genomic DNA. Samples were incubated at 56 °C for 15 min and subsequently at 94 °C for 5 min. 1 μL of the crude extraction was used as template in a 10 μL of fPCR using 5’-fluorescein-amidite (FAM)-labeled forward primers and non-labeled reverse primers. 200~400 bp of PCR amplicons spanning the CRISPR target sites were designed. To test the activities of mR26-sg1 and mR26-sg2, Fm-FAM (5’ - 3’): TAAGGGAGCTGCAGTGGAGTA; Rm (5’ - 3’): CCCGACAAAACCGAAAATCTGT. To test the activities of hR26-sg1 and hR26-sg2, Fh-FAM (5’ - 3’): GGAGTGCCGCAATACCTTTATG; Rh (5’ - 3’): TGCATAAAATCAGCCCCAGGT. PCR products were subjected to capillary array electrophoresis (CAE) using an ABI 3730 DNA analyzer1. Perform data analysis by GeneMapper software v3.1, which enables determination of the positions and areas of the peaks, indicating the lengths and relative amounts of PCR products, respectively2.



**Figure S1. Analysis of on-target activity by fluorescence PCR and capillary array electrophoresis (fPCR-CAE).** CRISPR-editing efficiency is evaluated by the frequencies of small insertions and/or deletions (Indel) resulted from the nonhomologous end joining (NHEJ) pathways. Over 80% cells are edited by CRISPR/Cas9 system in both RAW264.7 and Jurkat cells. **(A)** For RAW264.7 macrophages transfected with pDsR-mR26-sg1 and pDsR-mR26-sg2,crude extracts containing genomic DNA of DsRed2+EBFP2+ cells were analysis by fPCR-CAE using Fm-FAM and Rm. Genomic DNA of the WT RAW264.7 cells was used as control with a predicted size of 308 bp. **(B)** For Jurkat T cells transfected with pDsR-hR26-sg1 and pDsR-hR26-sg2, genomic DNA of DsRed2+EBFP2+ cells were analysis by fPCR-CAE using Fh-FAM and Rh. The predicted PCR band testing the WT Jurkat control cells has size of 342 bp. The size and frequency of Indels are indicated near selected peaks.

**References:**

1. Velasco, E. et al. Heteroduplex analysis by capillary array electrophoresis for rapid mutation detection in large multiexon genes. *Nature Protocols.* **2** (1), 237-246 (2007).
2. Lonowski, L. A. et al. Genome editing using FACS enrichment of nuclease-expressing cells and indel detection by amplicon analysis. *Nature Protocols.* **12** (3), 581-603 (2017).

**Figure S2.**



**Figure S2. Three-primers PCR for simultaneous detection of the wild-type allele and the targeted allele.** **(A)** The position of PCR primers is depicted in the diagram. Three-primers PCR for detection of *mRosa26* and *hROSA26* knock-in are designed in the same manner. **(B)** Genomic DNA of two candidate knock-in RAW264.7 cells (#4 and #25) was analysis by PCR reaction with either mFwt/mRwt/mRki primers (left) or mFwt/mRwt/mFki primers (right). The 308 bp of PCR products correspond to wild-type (WT) and the 415 bp/439bp of PCR amplicons correspond to the targeting vector (TV) positive control which suggesting the occurrence of insertion of the targeting vector. **(C)** Genomic DNA of two candidate knock-in Jurkat (#1 and #2) are amplified by PCR with either hFwt/hRwt/hRki primers (left) or hFwt/hRwt/hFki primers (right). Both WT allele (342 bp) and the targeted allele (724 bp of region spanning the junction of 5’HA and CAG promoter and 571 bp region spanning the junction of EBFP2 and 3’HA) are present in the candidate knock-in cells. M, DNA ladder. The primers used were (5’ – 3’):

mFwt, TAAGGGAGCTGCAGTGGAGTA;

mRwt, CCCGACAAAACCGAAAATCTGT;

mFki, GAGCAAAGACCCCAACGAGA;

mRki, CCAAGTGGGCAGTTTACCGTA;

hFwt, GGAGTGCCGCAATACCTTTATG;

hRwt, TGCATAAAATCAGCCCCAGGT;

hFki, GACTTCAAGGAGGACGGCAA;

hRki, GGCTATGAACTAATGACCCCGT.

**Supplementary Table:**

**Primers used for plasmid construction and validation of knock-in allele in *mRosa26* locus.**

|  |  |  |
| --- | --- | --- |
| **Primer name** | **Sequence (5' - 3')** | **Description** |
| mR26-sg1f | CACC**G**CTCCAGTCTTTCTAGAAGAT | construction of pDsR-mR26-sg1 |
| mR26-sg1r | AAACATCTTCTAGAAAGACTGGAG**C** |
| mR26-sg2f | CACC**G**CGCCCATCTTCTAGAAAGAC | construction of pDsR-mR26-sg2 |
| mR26-sg2r | AAACGTCTTTCTAGAAGATGGGCG**C** |
| hR26-sg1f | CACCGGCGATGACGAGATCACGCG | construction of pDsR-hR26-sg1 |
| hR26-sg1r | AAACCGCGTGATCTCGTCATCGCC |
| hR26-sg2f | CACC**G**AATCGAGAAGCGACTCGACA | construction of pDsR-hR26-sg2 |
| hR26-sg2r | AAACTGTCGAGTCGCTTCTCGATT**C** |
| 5' external oligo | GTGGAGCCGTTCTGTGAGAC | primers spanning 5'HA for verifying precise HDR at the *mRosa26* locus |
| 5' internal oligo | CCAAGTGGGCAGTTTACCGT |
| 3' internal oligo | GAGCAAAGACCCCAACGAGA | primers spanning 3'HA for verifying precise HDR at the *mRosa26* locus |
| 3' external oligo | AGGTCCTGAAGAAGCTTGGC |

**Sequence information:**

**>expression cassette sequence**

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTCCGCCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCTCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGTCGGTCGGGCTGCAACCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTACGGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCATGCCTTCTTCTTTTTCCTACAGGGCGCGCCcccccccccctaacgttactggccgaagccgcttggaataaggccggtgtgcgtttgtctatatgttattttccaccatattgccgtcttttggcaatgtgagggcccggaaacctggccctgtcttcttgacgagcattcctaggggtctttcccctctcgccaaaggaatgcaaggtctgttgaatgtcgtgaaggaagcagttcctctggaagcttcttgaagacaaacaacgtctgtagcgaccctttgcaggcagcggaaccccccacctggcgacaggtgcctctgcggccaaaagccacgtgtataagatacacctgcaaaggcggcacaaccccagtgccacgttgtgagttggatagttgtggaaagagtcaaatggctctcctcaagcgtattcaacaaggggctgaaggatgcccagaaggtaccccattgtatgggatctgatctggggcctcggtgcacatgctttacatgtgtttagtcgaggttaaaaaaacgtctaggccccccgaaccacggggacgtggttttcctttgaaaaacacgatgataatatggccacaaccATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGAGGGGCGAGGGCGAGGGCGATGCCACCAACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGAGCCACGGCGTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCACCTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCGTCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTTCAACAGCCACAACATCTATATCATGGCCGTCAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACGTGGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAGCCACTACCTGAGCACCCAGTCCGTGCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCCGCACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAActgtgccttctagttgccagccatctgttgtttgcccctcccccgtgccttccttgaccctggaaggtgccactcccactgtcctttcctaataaaatgaggaaattgcatcgcattgtctgagtaggtgtcattctattctggggggtggggtggggcaggacagcaagggggaggattgggaagagaatagcaggcatgctgggga

NNN: CAG prometer

GGCGCGCC: AscI restriction site

nnn: internal ribosome entry site (IRES)

NNN: EBFP2

nnn: bGH poly(A) signal

**>5’HA sequence of plasmid pKR26-POI-iBFP**

CAGGAATGCGGTCCGCCCTGCAGCAACCGGAGGGGGAGGGAGAAGGGAGCGGAAAAGTCTCCACCGGACGCGGCCATGGCTCGGGGGGGGGGGGGCAGCGGAGGAGCGCTTCCGGCCGACGTCTCGTCGCTGATTGGCTTCTTTTCCTCCCGCCGTGTGTGAAAACACAAATGGCGTGTTTTGGTTGGCGTAAGGCGCCTGTCAGTTAACGGCAGCCGGAGTGCGCAGCCGCCGGCAGCCTCGCTCTGCCCACTGGGTGGGGCGGGAGGTAGGTGGGGTGAGGCGAGCTGGACGTGCGGGCGCGGTCGGCCTCTGGCGGGGCGGGGGAGGGGAGGGAGGGTCAGCGAAAGTAGCTCGCGCGCGAGCGGCCGCCCACCCTCCCCTTCCTCTGGGGGAGTCGTTTTACCCGCCGCCGGCCGGGCCTCGTCGTCTGATTGGCTCTCGGGGCCCAGAAAACTGGCCCTTGCCATTGGCTCGTGTTCGTGCAAGTTGAGTCCATCCGCCGGCCAGCGGGGGCGGCGAGGAGGCGCTCCCAGGTTCCGGCCCTCCCCTCGGCCCCGCGCCGCAGAGTCTGGCCGCGCGCCCCTGCGCAACGTGGCAGGAAGCGCGCGCTGGGGGCGGGGACGGGCAGTAGGGCTGAGCGGCTGCGGGGCGGGTGCAAGCACGTTTCCGACTTGAGTTGCCTCAAGAGGGGCGTGCTGAGCCAGACCTCCATCGCGCACTCCGGGGAGTGGAGGGAAGGAGCGAGGGCTCAGTTGGGCTGTTTTGGAGGCAGGAAGCACTTGCTCTCCCAAAGTCGCTCTGAGTTGTTATCAGTAAGGGAGCTGCAGTGGAGTAGGCGGGGAGAAGGCCGCACCCTTCTCCGGAGGGGGGAGGGGAGTGTTGCAATACCTTTCTGGGAGTTCTCTGCTGCCTCCTGGCTTCTGAGGACCGCCCTGGGCCTGGGAGAATCCCTTCCCCCTCTTCCCTCGTGATCTGCAACTCaAGTCTTTCTAGAAGA

**>3’HA sequence of plasmid pKR26-POI-iBFP**

GGCGGGAGTCTTCTaGGCAGGCTTAAAGGCTAACCTGGTGTGTGGGCGTTGTCCTGCAGGGGAATTGAACAGGTGTAAAATTGGAGGGACAAGACTTCCCACAGATTTTCGGTTTTGTCGGGAAGTTTTTTAATAGGGGCAAATAAGGAAAATGGGAGGATAGGTAGTCATCTGGGGTTTTATGCAGCAAAACTACAGGTTATTATTGCTTGTGATCCGCCTCGGAGTATTTTCCATCGAGGTAGATTAAAGACATGCTCACCCGAGTTTTATACTCTCCTGCTTGAGATCCTTACTACAGTATGAAATTACAGTGTCGCGAGTTAGACTATGTAAGCAGAATTTTAATCATTTTTAAAGAGCCCAGTACTTCATATCCATTTCTCCCGCTCCTTCTGCAGCCTTATCAAAAGGTATTTTAGAACACTCATTTTAGCCCCATTTTCATTTATTATACTGGCTTATCCAACCCCTAGACAGAGCATTGGCATTTTCCCTTTCCTGATCTTAGAAGTCTGATGACTCATGAAACCAGACAGATTAGTTACATACACCACAAATCGAGGCTGTAGCTGGGGCCTCAACACTGCAGTTCTTTTATAACTCCTTAGTACACTTTTTGTTGATCCTTTGCCTTGATCCTTAATTTTCAGTGTCTATCACCTCTCCCGTCAGGTGGTGTTCCACATTTGGGCCTATTCTCAGTCCAGGGAGTTTTACAACAATAGATGTATTGAGAATCCAACCTAAAGCTTAACTTTCCACTCCCATGAATGCCTCTCTCCTTTTTCTCCATTTATAAACTGAGCTATTAACCATTAATGGTTTCCAGGTGGATGTCTCCTCCCCCAATATTACCTGATGTATCTTACATATTGCCAGGCTGATATTTTAAGACATTAAAAGGTATATTTCATTATTGAGCCACATGGTATTGATTACTGCTTACTAAAATTTTGTCATTGTACACATCTGTAAAAGGTGGTTCCTTTTGGAATGC

**>5’HA sequence of plasmid pKhR26-POI-iBFP**

TTTGTTACGTTGGGAGGGAAAGGGGTGGCTGGATGCAGGCGGGAGGGAGGCCCGCCCTGCGGCAACCGGAGGGGGAGGGAGAAGGGAGCGGAAAATGCTCGAAACCGGACGGAGCCATTGCTCTCGCAGAGGGAGGAGCGCTTCCGGCTAGCCTCTTGTCGCCGATTGGCCGTTTCTCCTCCCGCCGTGTGTGAAAACACAAATGGCGTATTCTGGTTGGAGTAAAGCTCCTGTCAGTTACGCCGTCGGGAGTACGCAGCCGCTTAGCGACTCTCGCGTTGCCCCCTGGGTGGGGCGGGTAGGTAGGTGGGGTGTAGAGATGCTGGGTGTGCGGGCGCGGCCGGCCTCCTGCGGCGGGAGGGGAGGGTCAGTGAAATCGGCTCTGGCGCGGGCGTCCTCCCACCCTCCCCTTCCTTCGGGGGAGTCGGTTTACCCGCCGCCTGCTTGTCTTCGACACCTGATTGGCTGTCGAAGCTGTGGGACCGGGCCCTTGCTACTGGCTCGAGTCTCACATGAGCGAAACCACTGCGCGGGGCGCGGGGGTGGCGGGGAGGCGGGCGTTGGTACGGTCCTCCCCGAGGCCGAGCGCCGCAGTGTCTGGCCCCGCGCCCCTGCGCAACGTGGCAGGAAGCGCGCGCTGGAGGCGGGGGCGGGCTGCCGGCCGAGACTTCTGGATGGCGGCGGCCGCGGCTCCGCCCCGGGTTCCCACCGCCTGAAGGGCGAGACAAGCCCGACCTGCTACAGGCACTCGTGGGGGTGGGGGAGGAGCGGGGGTCGGTCCGGCTGGTTTGTGGGTGGGAGGCGCTTGTTCTCCAAAAACCGGCGCGAGCTGCAATCCTGAGGGAGCTGCGGTGGAGGAGGTGGAGAGAAGGCCGCACCCTTCTGGGCAGGGGGAGGGGAGTGCCGCAATACCTTTATGGGAGTTCTCTGCTGCCTCCCGTCTTGTAAGGACCGCCCTGGGCCTGGAAGAAGCCCTCCCTCCTTTCCTCaTCGCGTGATCT

**>3’HA sequence of plasmid pKhR26-POI-iBFP**

CGTCATCGCCTCtATGTCGAGTCGCTTCTCGATTATGGGCGGGATTCTTTTGCCTAGGCTTAAGGGGCTAACTTGGTCCCTGGGCGTTGCCCTGCAGGGGAGTGAGCAGCTGTAAGATTTGAGGGGCGACTCCGATTAGTTTATCTTCCCACGGACTAGAGTTGGTGTCGAGGTTATTGTAATAAGGGTGGGGTAGGGAAATGGAGCTTAGTCATTCACCTGGGGCTGATTTTATGCAACGAGACTGCGGATTATCACTACTTATCATTTTTGGAGCATTTTTCTAGAGACAGACATAAAGCATGATCACCTGAGTTTTATACCATTTGAGACCCTTGCTGCACCACCAAAGTGTAGCATCAGGTTAAATCTTAATAGAAAAATTTTAGCTTTTGCTTGAGAAACCAGTGCTTCCCTCCCTCACCCTCTCTCCCCAGGCTCTCTACCCCTTTGCATCCCTACCAGGCATCTTAGCAACTCTCACTCATACTTGATCCCATTTTCCATTTGTTGTACTTGCTCCTCTAGTATTCAGACATAGCACTAGCTTTCTCCCTCTCTTGATCTTGGGTAGCCTGGTGTCTCGCGAAACCAGACAGATTGGTTCCACCACAAATTAAGGCTTGAGCTGGGGCTTGACTCTTACCCAGCAGTGCTTTTATTCCTCCCTAGTTCACGTTCTTAAATGTTTATCTTGATTTTCATTTTATCCTTTTTCCTTAGCTGGGATTCTGTCCCTGACCGTCTTCACAGTCCAGGTGATCTTGACTACTGCTTTACAGAGAATTGGATCTGAGGTTAGGCAACATCTCCCTTTTTCTTCCTCTAAATACCTCTCATTTCTGTTCTTACCAGTTAGTAACTGATCTCAGATGCCTGTGTGATAGCTTCCAAATTGCTGTCTCTGTCTTTAGTGTTATCTTTTGATCCATCTTACATCTTGTTAGGATGATTGTCCTAAAGGAAGATAGAGCATGAAAATGACAGGTGAAACTCCATT

Note: Nucleotide changes (underlined lowercase) in the homology arms are introduced to destroy the PAM sequence for avoiding re-cut the targeted allele by CRISPR/Cas9.

**>OST tag with linker sequence**

ATGTGGAGCCACCCGCAGTTCGAGAAAGGTGGAGGTTCAGGAGGTGGATCGGGAGGTGGATCGTGGAGCCACCCGCAGTTCGAAAAAGGTTCCGGG

NNN: OST tag

NNN: GSG linker