

## 1 **SUPPLEMENTARY DATA**

### 2 **Methods**

#### 3 **Human T cell isolation and culture**

4 Fresh umbilical cord blood (UCB) units obtained from healthy volunteer donors  
5 who had provided informed consent from the Beijing Cord Blood Bank (Beijing, China).  
6 Peripheral blood mononuclear cells (PBMCs) were separated by density gradient  
7 centrifugation with human mononuclear cells separation medium 1.077  
8 (DongFangHuaHui, 25710). CD3<sup>+</sup> T cells were isolated using the EasySep human T cell  
9 enrichment kit (Stemcell Technologies, 19051), activated and expanded with CD3/CD28  
10 Dynabeads (Gibco, 11163D) at a bead to T cell ratio of 1:1 according to the  
11 manufacturer's instructions. UCB-derived CD3<sup>+</sup> T cells were cultured in X-vivo15  
12 medium (Lonza, 04-418Q) supplemented with 5% (v/v) heat-inactivated fetal bovine  
13 serum (Gibco, 10091-148) in the presence of 100 IU/mL recombinant human IL-2. All  
14 cells were cultured at 37 °C in 5 % CO<sub>2</sub> atmosphere.

#### 15 **Cell culture and electroporation**

16 K562 cells were maintained in RPMI 1640 (Gibco, C11875500BT) supplemented  
17 with 10 % FBS, 100 mg/ml streptomycin and 100 units/ml penicillin (Gibco, 15140-122).  
18 K562 cells were electroporated with the 4D-Nucleofector X and core Unit (Lonza) and  
19 SF cell line 4D-Nucleofector Kit (Lonza, V4XC-1024). For gene editing experiments in  
20 K562 cells: 10 µg unmodified or CTsgRNA and 10 µg Cas9 mRNA were electroporated

21 into 1 million cells using program FF120. For gene activation experiments in K562 cells:  
22 10 µg unmodified or CT modified sgRNA and 10 µg dCas9-P65 mRNA or 1 µg  
23 dCas9-P65 expressing plasmid were electroporated into 1 million cells using program  
24 FF120.

25 T cells were activated for three days with CD3/CD28 Dynabeads before  
26 electroporation. T cells were electroporated using the Lonza Nucleofector 4D (Lonza)  
27 and P3 Primary Cell 4D-Nucleofector Kit (Lonza, V4XP-3024). For gene editing  
28 experiments in T cells: 10 µg unmodified or CT modified sgRNA and 10 µg Cas9 mRNA  
29 were electroporated into 1 million cells using program EO115. For gene activation  
30 experiments in T cells: 10 µg unmodified or CT modified sgRNA and 10 µg dCas9-P65  
31 mRNA or as the dose in figure legend were electroporated into 3 million cells using  
32 program EO115.

### 33 ***In vitro* transcription**

34 Guide sequences of sgRNAs and oligo primers used in this study were listed in  
35 Table S1. The sequence of DNA templates for T7-sgRNA PCR were listed in Table S2.  
36 The T7-sgRNA PCR product was recovered and used as template for *in vitro*  
37 using MEGA shortscript T7 kit (Ambion, AM1354) and mMESSAGING mMACHINE®  
38 T7 Ultra Kit for CT modification (Ambion, AM1345). RNAs were purified with  
39 MEGAclean columns (Ambion, AM1908) and eluted with RNase-free water.

### 40 **Surveyor assay and Tracking of Indels by Decomposition (TIDE) sequencing**

41 The levels of genomic disruption of *AAVS1*, *VEGFA*, *EMX1*, and *HBB* in K562 cell

42 line or primary T cells was determined by Surveyor assay using Surveyor mutation  
43 detection kit (Integrated DNA Technologies, Inc). The percentage of target disruption was  
44 quantified by densitometry and calculated as described (Guschin et al., 2010). The PCR  
45 products are also sequenced for TIDE analysis using specially designed software that  
46 provided as a simple web tool (available at <http://tide.nki.nl>). The PCR primers used for  
47 the amplification of target locus and sequencing are listed in Table S1.

#### 48 **QuantStudio<sup>®</sup> 3D Digital PCR**

49 Digital PCR is a method of quantitative analysis of sample nucleic acids based on  
50 single-molecule template PCR amplification without the use of standard curves. Primers  
51 and probes were designed as previously described (Mock et al., 2016). Genomic DNA  
52 was extracted by using EasyPure Genomic DNA Kit (Transgene, EE101). Mixture  
53 consisted of 900nM PD1-dPCR-F, 900nM PD1-dPCR-R , 2x QuantStudio<sup>®</sup> 3D digital  
54 PCR master mix (Thermo, A26358), 200nM VIC-Insensitive probe-MGB, 200nM  
55 FAM-sensitive probe-MGB and sample gDNA was loaded onto chips (Thermo, A26316 )  
56 using the QuantStudio<sup>®</sup> 3D Digital PCR Chip Loader. The chips were sealed and loaded  
57 onto ProFlex<sup>™</sup> 2x Flat PCR System (Applied Biosystem) and cycled as following  
58 parameters: 96°C for 10 min, followed by 39 cycles of 52°C for 2 min and 98°C for 30  
59 sec and then extension at 60°C for 2 min. After cycling, the chips were measured by  
60 QuantStudio<sup>™</sup> 3D Digital PCR Instrument. The data was analyzed by AnalysisSuite<sup>™</sup> at  
61 <https://china.apps.thermofisher.com/quantstudio3d/>.

#### 62 **Quantitative PCR**

63 Total RNA was extracted with the Trizol reagent (Life Technologies, 15596-026 ). 1  
64 µg RNA of each sample was used for reverse transcription with TransScript-Uni  
65 One-Step gDNA Removal and cDNA Synthesis Supermix Kit (TransGen Biotech,  
66 AU311). QPCR reactions were performed using qPCR Master Mix (Takara, QPK-212) in  
67 CFX96 real-time detection system (Bio-Rad). Housekeeping gene *GAPDH* was used as  
68 internal control. The qPCR primers are listed in Table S1.

#### 69 **Flow cytometry**

70 CytoFLEX (Beckman Coulter Inc) was used to perform fluorescent expression  
71 analysis. Cells were harvested 48 hours, 72 hours and 96 hours post electroporation and  
72 stained with mouse anti-human FOXP3 antibody (eBioscience, 11-4776-42) for 1 hour in  
73 the dark refrigerator.

#### 74 **Statistics**

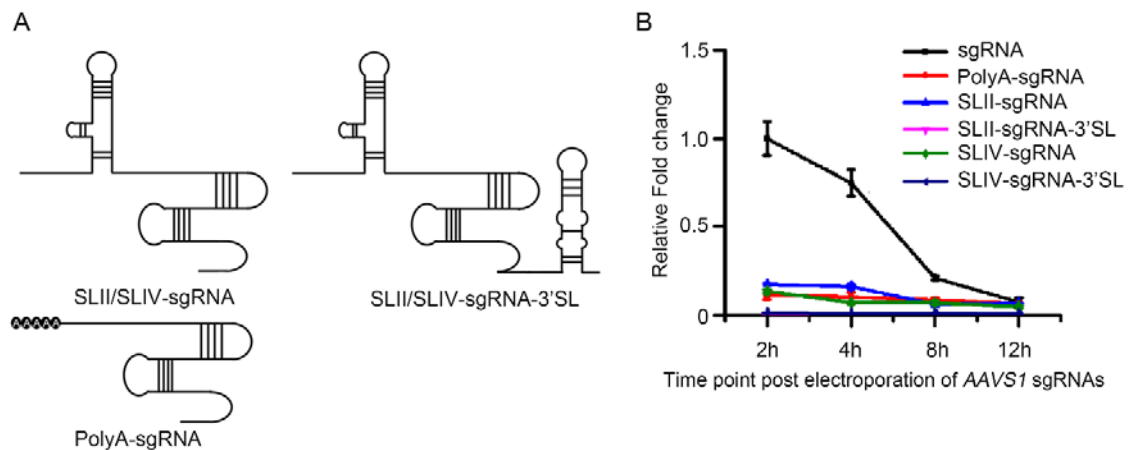
75 Statistical significance was analyzed by unpaired *t*-tests using GraphPad Prism 5 with  
76 P-value considered as \*\*\*P < 0.001; \*\*P < 0.01; \*P < 0.1.

#### 77 **REFERENCE**

78 Guschin, D.Y., Waite, A.J., Katibah, G.E., Miller, J.C., Holmes, M.C., and Rebar, E.J  
79 (2010) A Rapid and General Assay for Monitoring Endogenous Gene Modification. In  
80 Engineered Zinc Finger Proteins: Methods and Protocols, J.P. Mackay, and D.J. Segal,  
81 eds. (Totowa, NJ: Humana Press), pp. 247-256.

82 Mock, U., Hauber, I. and Fehse, B. (2016) Digital PCR to assess gene-editing frequencies  
83 (GEF-dPCR) mediated by designer nucleases. *Nat. Protoc.*, 11, 598

#### 84 **Supplementary Figures**



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86 **Figure S1** The stability of differently modified sgRNA structures in K562 cell line.

87 (A) Schematic structures of differently modified sgRNAs. SLII: stem loop II of Dengue

88 virus sfRNA, SLIV: stem loop IV of Dengue virus sfRNA, 3'SL: stem loop at 3' end of

89 Dengue virus sfRNA. Poly A: 55 consecutive A bases. (B) Stability of modified *AAVS1*

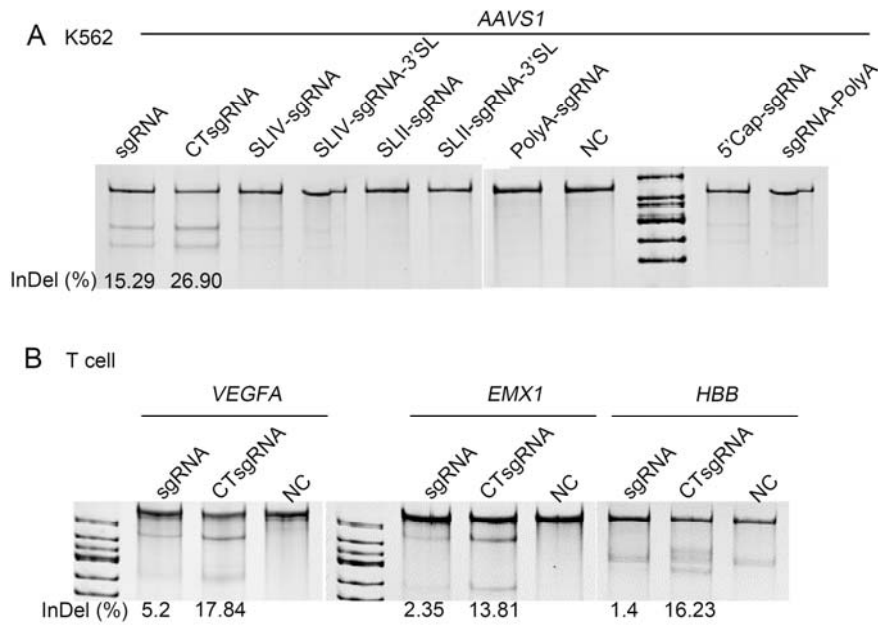
90 sgRNAs in K562 cells. 2 million K562 cells were electroporated with 10  $\mu$ g IVT *AAVS1*

91 sgRNA with indicated structures. The quantity of sgRNAs were measured by qPCR at

92 different time points, and Ru6B was used as an internal control. Each data point depicts

93 the relative abundance of sgRNA in electroporated cells at each time point (Mean  $\pm$  SD, n

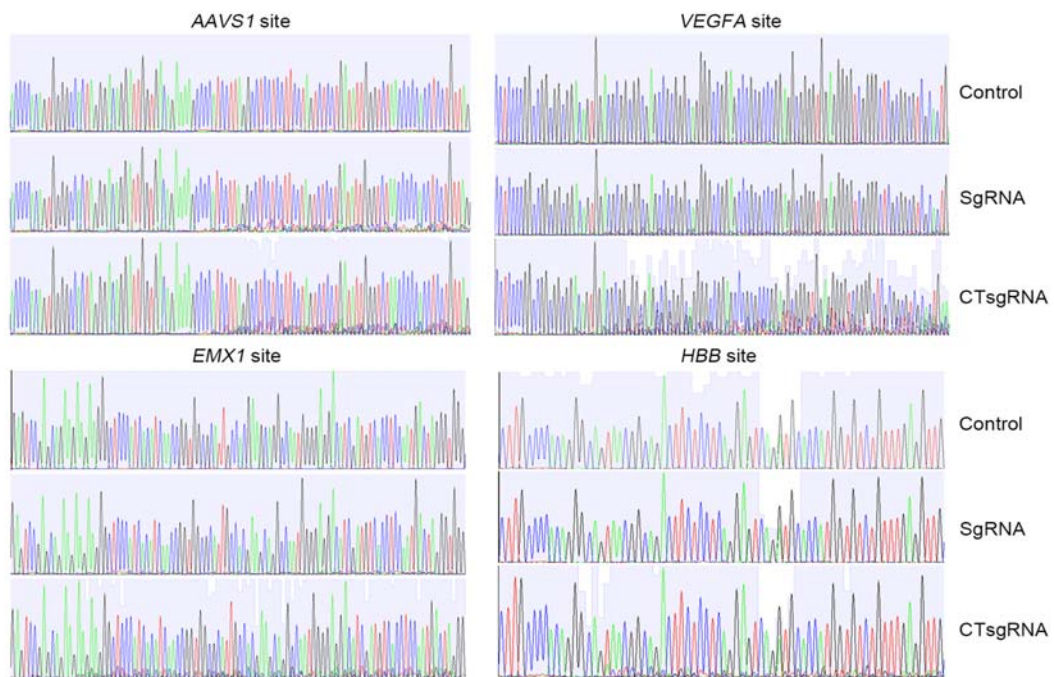
94 = 3).



95 **Figure S2** Indel frequencies of sgRNAs with different modifications in K562 and  
 96 primary CD3<sup>+</sup> T cells as measured by Surveyor assays. (A) Gene editing efficiency at  
 97 AAVS1 site mediate by different sgRNA structures in K562 cells. (B) CT modification  
 98 enhanced gene disruption at VEGFA, EMX1 and HBB sites in K562 cells. Percentage of  
 99 gene disruption is indicated under each panel.  
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104 **Figure S3** Sequencing profile of TIDE related to **Figure 1C**.

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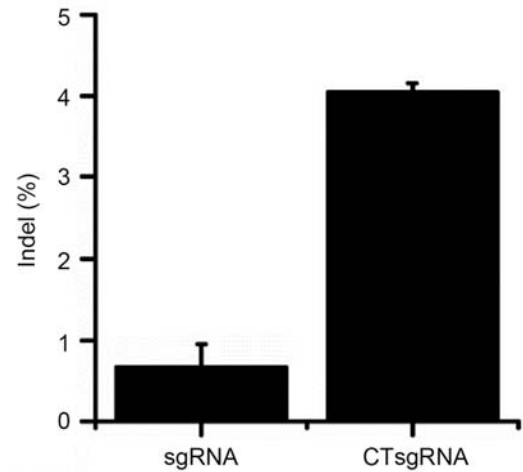
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Target	Sample	Copies/ $\mu$ l	CI Copies/ $\mu$ l	Precision
FAM	K562 -1	1541.2	1511.6 -- 1571.5	1.96%
VIC	K562 -1	1540.7	1511.1 -- 1571	1.96%
FAM	K562 -2	865.46	846.49 -- 884.86	2.24%
VIC	K562 -2	873.47	854.37 -- 892.99	2.23%
FAM	PD1 sgRNA-1	1370.2	1344.2 -- 1396.7	1.93%
VIC	PD1 sgRNA-1	1382.2	1356 -- 1408.8	1.93%
FAM	PD1 sgRNA-2	1398.8	1372.3 -- 1425.9	1.94%
VIC	PD1 sgRNA-2	1404.6	1378 -- 1431.8	1.93%
FAM	PD1 CTsgRNA-1	1461	1432.9 -- 1489.7	1.96%
VIC	PD1 CTsgRNA-1	1521.3	1492.2 -- 1551	1.95%
FAM	PD1 CTsgRNA-2	1467.1	1439.4 -- 1495.3	1.92%
VIC	PD1 CTsgRNA-2	1530.3	1501.6 -- 1559.5	1.91%

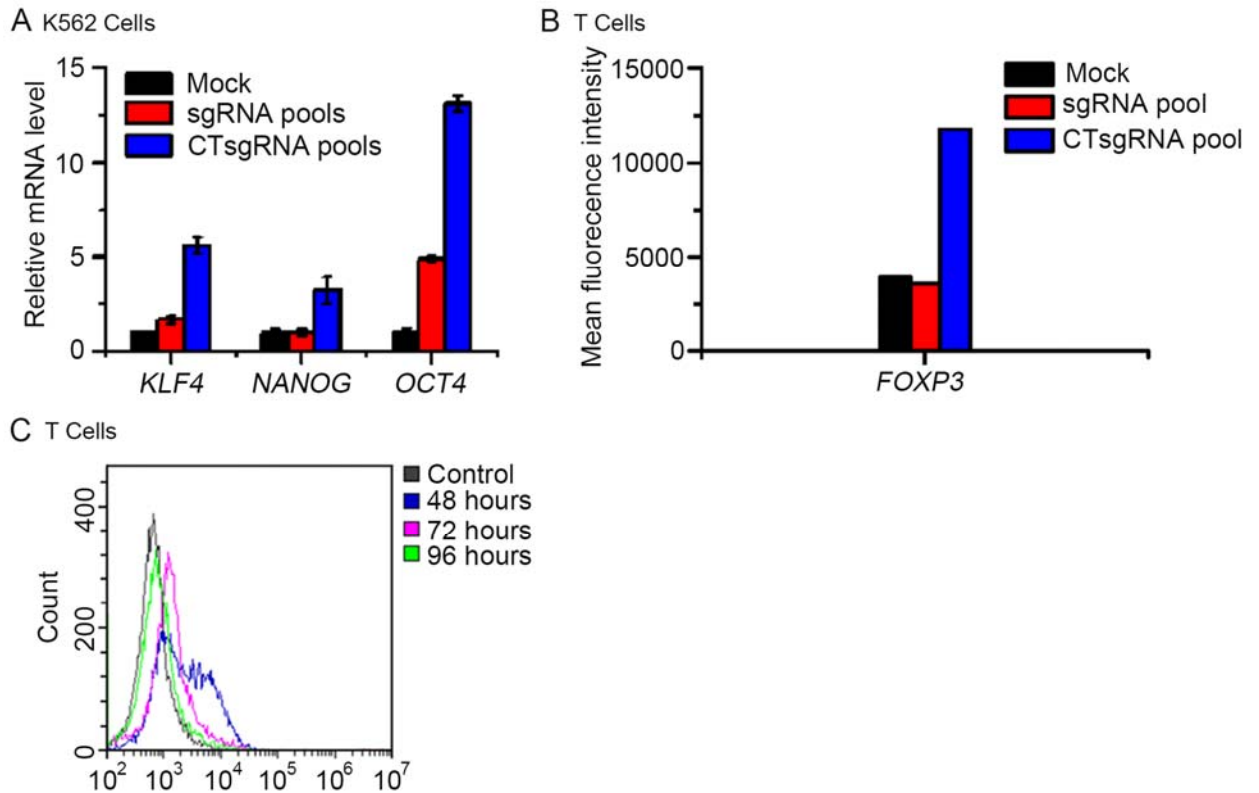


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115 **Figure S4.** CT modification enhanced gene editing efficiency at *PD1* loci in K562 cells  
 116 measured by QuantStudio<sup>®</sup> 3D Digital PCR. Left: copy numbers of FAM or VIC positive  
 117 signal in the gDNA of control and gene-edited K562 cells. Right: Gene editing efficiency  
 118 based on copy number analysis. Gene editing efficiency =  $(1 - \text{FAM}/\text{VIC})\%$ . Bars  
 119 represent average editing efficiency in two replicates  $\pm$  SD

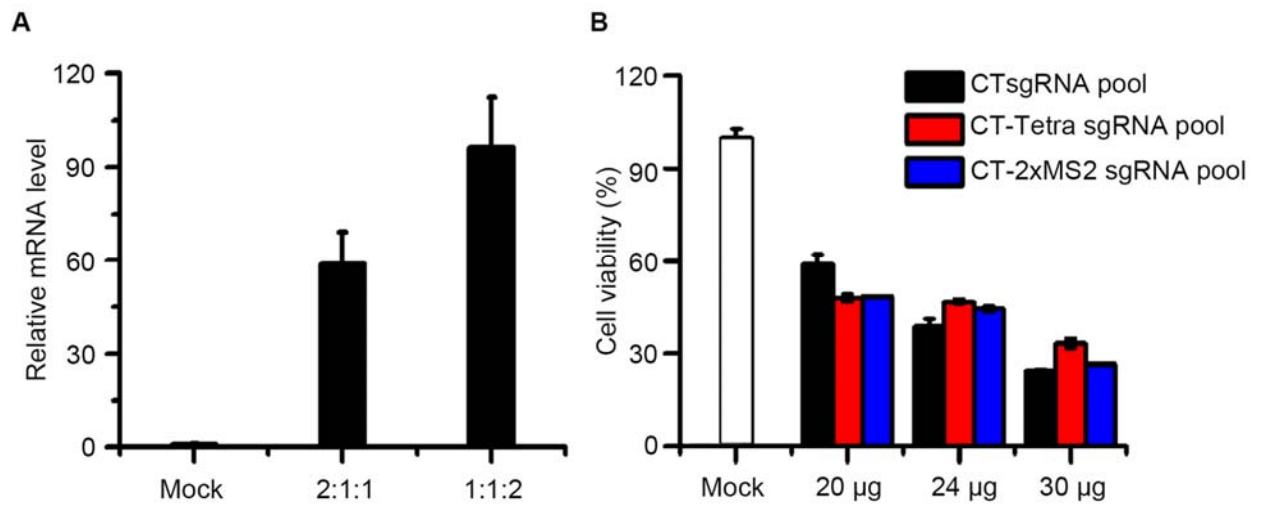
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122 **Figure S5** CT modification improved gene activation in K562 and primary T cells. (A)  
 123 CTsgRNA platform enabled simultaneous activation of three genes in K562 cells. The  
 124 expression of each gene was measured by qPCR with *GAPDH* as an internal control.  
 125 Bars represent average mRNA level of each gene in three replicates  $\pm$  SD. (B)  
 126 The protein level of endogenous *FOXP3* was represented by mean fluorescence intensity,  
 127 measured 48 hours post electroporation by flow cytometry. (C) Expression of *FOXP3*  
 128 protein in T cells at different time points post electroporation, analyzed by flow  
 129 cytometry.

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132 **Figure S6** Optimization of gene activation method in T cells.

133 (A) The optimal ratio of CT-Tetra platform components in activating endogenous *OCT4*

134 gene. 3 million stimulated human primary CD3<sup>+</sup> T cells were electroporated with 20  $\mu$ g

135 total amount of RNA. The ratio of dCas9-p65HSF1 mRNA, MS2-P65HSF1 mRNA and

136 CT modified tetra *OCT4* sgRNA pool is 2:1:1 or 1:1:2. The mRNA level of endogenous

137 *OCT4* was quantified by qPCR with *GAPDH* as an internal control. Bars represent

138 average mRNA level of *OCT4* in three replicates  $\pm$  SD. (B) Cell viability of three gene

139 activation platforms. Different quantity of RNAs of each gene activation platform were

140 delivered into 3 million stimulated human primary CD3<sup>+</sup> T cells and cell viability was

141 determined by cell counting 2 days after electroporation. The ratio of dCas9-p65HSF1

142 mRNA, MS2-P65HSF1 mRNA and CT modified tetra *OCT4* sgRNA pool is 1:1:2. Bars

143 represent average cell viability of electroporated cells in three replicates  $\pm$  SD.

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145 **Supplemental Tables**146 **Table S1. Sequences of sgRNA guide and DNA oligos used in this study**

<b>Guide sgRNA sequence</b>	
<i>AAVS1</i> sgRNA	ACCCACAGTGGGGCCACTA
<i>VEGFA</i> sgRNA	GACCCCTCCACCCCGCCTC
<i>EMX1</i> sgRNA	GAGTCCGAGCAGAAGAAGAA
<i>HBB</i> sgRNA	CTTGCCCCACAGGGCAGTAA
<i>OCT4</i> sgRNA1	GGCCCCGCCCCCTGGATGGG
<i>OCT4</i> sgRNA2	GGGGGAGAACTGAGGCGA
<i>OCT4</i> sgRNA3	GGTGGTGGCAATGGTGTCTG
<i>OCT4</i> sgRNA4	GACACA ACTGGCGCCCCTCC
<i>KLF4</i> sgRNA1	GCGCGCTCCACACA ACTCAC
<i>KLF4</i> sgRNA2	AAGGAACGCGCGCCGGCGGC
<i>KLF4</i> sgRNA3	ATGGGAGAAGGCGGAGGAAA
<i>KLF4</i> sgRNA4	GCAACGATGGAAGGGAGCCT
<i>NANOG</i> sgRNA1	GATTA ACTGAGAATTCACAA
<i>NANOG</i> sgRNA2	TCTAGTCCCCACCTAGTCT
<i>NANOG</i> sgRNA3	TGTCTTCAGGTTCTGTTGCT
<i>NANOG</i> sgRNA4	TGATT TAAAAGTTGGAAACG
<b><i>In vitro</i> transcription primers</b>	

SgRNA Forward primers	TAATACGACTCACTATAGNNNNNNNNNNNNNNNNNNNN NNN(20bp target sequence)GTTTAAGAGCTATGCTGGAAAC
SgRNA Reverse primers	AAAAGCACCGACTCGGTGCC
SL2S-sgRNA Forward primers	TAATACGACTCACTATAGGGCTAAAAGTCAGGTC GGATC
SL2S-sgRNA Reverse primers	AAAAGCACCGACTCGGTGCC
SL2S-sgRNA-3' SL Forward primers	TAATACGACTCACTATAGGGCTAAAAGTCAGGTC GGATC
SL2S-sgRNA-3' SL Reverse primers	GCAGAATTGGCGCACGCGCTA
SL4L-sgRNA Forward primers	TAATACGACTCACTATAGTAAAAGAAGTCAGGCC ATCAC
SL4L-sgRNA Reverse primers	AAAAGCACCGACTCGGTGCC
SL4L-sgRNA-3' SL Forward primers	TAATACGACTCACTATAGTAAAAGAAGTCAGGCC ATCAC
SL4L-sgRNA-3' SL Reverse primers	GCAGAATTGGCGCACGCGCTA

PolyA-sgRNA Forward primer	TAATACGACTCACTATAGGCAATTGGGCCGGCCA AA
PolyA-sgRNA Reverse primer	AAAAGCACCGACTCGGTGCC
sgRNA-polyA Forward primer	TAATACGACTCACTATAGNNNNNNNNNNNNNNNNNNNN NNGTTTAAGAGCTATGCTGGAAAC
SgRNA-polyA Reverse primer	GCAGAATTGGCGCACGCGCTA
Tetra sgRNA Forward primer	TAATACGACTCACTATAGNNNNNNNNNNNNNNNNNNNN NNNN GTTTAAGAGCTATGCTGG
Tetra sgRNA Reverse primer	AAAAGCACCGACTCGGTGCC
2xMBS sgRNA Forward primer	TAATACGACTCACTATAGNNNNNNNNNNNNNNNNNNNN NNNN GTTTAAGAGCTATGCTGG
2xMBS sgRNA Reverse primer	AAAAGCACCGACTCGGTGCC
DCas9-P65HSF1 Forward primer	TAATACGACTCACTATAGGGAGACCACCATGTACC CATACGATGTTCCAG
DCas9-P65HSF1 Reverse primer	TCAATCGATGGAGACAGTGGGGTC

MS2-P65HSF1 Forward primer	TAATACGACTCACTATAGGGAGACCACCATGGCTT CAAACCTTTACTCAG
MS2-P65HSF1 Reverse primer	TCAGGAGACAGTGGGGTCCTTG
SpCas9 Forward primer	TAATACGACTCACTATAGGGAGACCACCATGGACT ATAAGGACCACGAC
SpCas9 Reverse primer	GCGAGCTCTAGGAATTCTTAC
<b>Genotyping primers for surveyor assays</b>	
<i>AAVS1</i> -Forward primer	CTTACCTCTCTAGTCTGTGCTAGC
<i>AAVS1</i> -Reverse primers	GGATCCTCTCTGGCTCCATCG
<i>VEGFA</i> -Forward primer	AGAGAAGTCGAGGAAGAGAGAG
<i>VEGFA</i> -Reverse primer	CAGCAGAAAGTTCATGGTTTCG
<i>EMX1</i> -Forward primer	GGAGCAGCTGGTCAGAGGGG
<i>EMX1</i> -Reverse primer	CAGGGCAGAGCCATCTATT
<i>HBB</i> -Forward primer	TCTGTCTCCACATGCCAGT
<i>HBB</i> -Reverse primer	CAGGGCAGAGCCATCTATT
<i>VEGFA</i> -OT1-Forward primer	CCAGGTGGTGTCAGCGGAGG
<i>VEGFA</i> -OT1-Reverse primer	TGCCTGGCCCTCTCTGAGTCT

<i>VEGFA</i> -OT2-Forward primer	CCAGGTGGTGTTCAGCGGAGG
<i>VEGFA</i> -OT2-Reverse primer	TGCCTGGCCCTCTCTGAGTCT
<i>EMXI</i> -OT1- Forward primer	CTCTCCTTCAACTCATGACCAGC
<i>EMXI</i> -OT1- Reverse primer	CTGCACATGTATGTACAGGAGTC
<i>HBB</i> -OT1- Forward primer	TCCCGTTCTCCACCCAATAG
<i>HBB</i> -OT1- Reverse primer	GATTTCCAGGCTATGCTTCCA
<b>Primers for TIDE sequence</b>	
<i>AAVSI</i> Forward primer	GAGAGCTCAGCTAGTCTTCT
<i>AAVSI</i> Reverse primer	TTAGAGGTTCTGGCAAGGAG
<i>VEGFA</i> Forward primer	GTGCGAGCAGCGAAAGCGAC
<i>VEGFA</i> Reverse primer	CGCCTCGGCGAGCTACTCTT
<i>EMXI</i> Forward primer	GGAGCAGCTGGTCAGAGGGG
<i>EMXI</i> Reverse primer	GGGAAGGGGACACTGGGGA
<i>HBB</i> Forward primer	TCTGTCTCCACATGCCAGT
<i>HBB</i> Reverse primer	CAGGGCAGAGCCATCTATT
<b>Primers for qPCR</b>	
<i>GAPDH</i> -Forward primer	ATGACATCAAGAAGGTGGTG
<i>GAPDH</i> -Reverse primer	CATACCAGGAAATGAGCTTG

<i>OCT4</i> -Forward primer	GCTCGAGAAGGATGTGGTCC
<i>OCT4</i> -Reverse primer	CGTTGTGCATAGTCGCTGCT
<i>KLF4</i> -Forward primer	CAGTGCCAAAAATGCGACCGAGC
<i>KLF4</i> -Reverse primer	GACCATGATTGTAGTGCTTTCTGGC
<i>NANOG</i> -Forward primer	GCAGAAGGCCTCAGCACCTA
<i>NANOG</i> -Reverse primer	AGGTTCCCAGTCGGGTTC
<i>SgRNA</i> - Forward primer	GCTGGAAACAGCATAGCAAG
<i>SgRNA</i> -Reverse primer	GCAGGGTCCGAGGTATTC
<i>RNU6B</i> - Forward primer	CTCGCTTCGGCAGCACA
<i>RNU6B</i> -Reverse primer	AACGCTTCACGAATTTGCGT
sgRNA Reverse transcription primer	GTCGTATCCAGTGCAGGGTCCGAGGTAT TCGCACTGGATACGACAAAAAAGCACC G
Probes and primers for Quantstudio 3D digital PCR	
PD1-dPCR-F	ACCTGACCTGGGACAGTTTCC
PD1-dPCR-R	GCCAGCCCAGTTGTAGCA
FAM-Sensitive probe	AGATCCCACAGGCGCCCTGGC
VIC-Insensitive probe	CCGCTCACCTCCGCCTGAGC

148 **Table S2. Sequences of the DNA templates for different sgRNA expression cassettes.**

Name and Description	DNA Sequence
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U6 promoter-sgRNA	GGCGCGCCGAGGGCCTATTTCCCATGATTCCTTCATA TTTGCATATACGATACAAGGCTGTTAGAGAGATAATT GGAATTAATTTGACTGTAAACACAAAGATATTAGTAC AAAATACGTGACGTAGAAAGTAATAATTTCTTGGGT AGTTTGCAGTTTTTAAAATTATGTTTTTAAAATGGACTA TCATATGCTTACCGTAACTTGAAAGTATTTTCGATTTCT TGGCTTTATATATCTTGTGGAAAGGACGAAACACCG CAATTGGGCCGGCCCCCGTCTCGAAAGATCTAGAT CTCACNNNNNNNNNNNNNNNNNNNNNNNGTTTAAAGAG CTATGCTGGAAACAGCATAGCAAGTTTAAATAAGGC TAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC GGTGCTTTTTTTGTTTTAGAGCTAGAAATAGCAAGTTA AAATAAGGCTAGTCCGTAGCGCGTGCGCCAATTCTG CAGACAAATGGC
U6 promoter-PolyA-sgRNA	GGCGCGCCGAGGGCCTATTTCCCATGATTCCTTCATA TTTGCATATACGATACAAGGCTGTTAGAGAGATAATT GGAATTAATTTGACTGTAAACACAAAGATATTAGTAC AAAATACGTGACGTAGAAAGTAATAATTTCTTGGGT AGTTTGCAGTTTTTAAAATTATGTTTTTAAAATGGACTA TCATATGCTTACCGTAACTTGAAAGTATTTTCGATTTCT TGGCTTTATATATCTTGTGGAAAGGACGAAACACCG CAATTGGGCCGGCCAAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA ACCCCGTCTCGAAAGATCTAGATCTCACNNNNNNNN NNNNNNNNNNNNNGTTTAAAGAGCTATGCTGGAAAC AGCATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAA CTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGTT TTAGAGCTAGAAATAGCAAGTTAAATAAGGCTAGT CCGTAGCGCGTGCGCCAATTCTGCAGACAAATGGC
U6 promoter -SLII-sgRNA	GGCGCGCCGAGGGCCTATTTCCCATGATTCCTTCATA TTTGCATATACGATACAAGGCTGTTAGAGAGATAATT GGAATTAATTTGACTGTAAACACAAAGATATTAGTAC AAAATACGTGACGTAGAAAGTAATAATTTCTTGGGT AGTTTGCAGTTTTTAAAATTATGTTTTTAAAATGGACTA TCATATGCTTACCGTAACTTGAAAGTATTTTCGATTTCT TGGCTTTATATATCTTGTGGAAAGGACGAAACACCG CAATTGGGCTAAAAGTCAGGTCGGATCAAGCCATAG TACGGAAAAAACTATGCTACCTGTGAGCCCCGTCCA AGGACGTTAAAAAGATCTCACNNNNNNNNNNNNNN NNNNNNNNNGTTTAAAGAGCTATGCTGGAAACAGCATAG CAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAA

	AAAGTGGCACCGAGTCGGTGCTTTTTTGTTTTAGAG CTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTAG CGCGTGCGCCAATTCTGCAGACAAATGGC
U6 promoter -SLII-sgRNA-3'SL	GGCGCGCCGAGGGCCTATTTCCCATGATTCCTTCATA TTTGCATATACGATACAAGGCTGTTAGAGAGATAATT GGAATTAATTTGACTGTAAACACAAAGATATTAGTAC AAAATACGTGACGTAGAAAGTAATAATTTCTTGGGT AGTTTGCAGTTTTTAAAATTATGTTTTTAAAATGGACTA TCATATGCTTACCGTAACTTGAAAGTATTTTCGATTTCT TGGCTTTATATATCTTGTGGAAAGGACGAAACACCG CAATTGGGCTAAAAGTCAGGTCGGATCAAGCCATAG TACGGAAAAAACTATGCTACCTGTGAGCCCCGTCCA AGGACGTAAAAAGATCTCACCNNNNNNNNNNNNN NNNNNNNGTTTAAAGAGCTATGCTGGAAACAGCATAG CAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAA AAAGTGGCACCGAGTCGGTGCCAATTGCATATTGAC GCTGGGAAAGACCAGAGATCCTGCTGTCTCCTCAGC ATCATTCCAGGCACAGAACGCCAGAAAATGGAATGG TGCTGTTGAATCAACAGGTTCTAGATCTTTTTTTGTT TTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGT CCGTAGCGCGTGCGCCAATTCTGCAGACAAATGGC

<p>U6 promoter -SLIV-sgRNA</p>	<p>GGCGCGCCGAGGGCCTATTTCCCATGATTCCTTCATA  TTTGCATATACGATACAAGGCTGTTAGAGAGATAATT  GGAATTAATTTGACTGTAAACACAAAGATATTAGTAC  AAAATACGTGACGTAGAAAGTAATAATTTCTTGGGT  AGTTTGCAGTTTTTAAAATTATGTTTTTAAAATGGACTA  TCATATGCTTACCGTAACTTGAAAGTATTTTCGATTTCT  TGGCTTTATATATCTTGTGGAAAGGACGAAACACCG  CAATTGTAAAAGAAGTCAGGCCATCACAAATGCCAC  AGCTTGAGTAACTGTGCAGCCTGTAGCTCCACCTG  AGAAGGTGTAAAAAAGATCCCACCNNNNNNNNNNNN  NNNNNNNNNGTTTAAAGAGCTATGCTGGAAACAGCAT  AGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTG  AAAAAGTGGCACCGAGTCGGTGCCAATTGAGATCTT  TTTTTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAA  GGCTAGTCCGTAGCGCGTGCGCCAATTCTGCAGACA  AATGGC</p>
<p>U6 promoter -SLIV-sgRNA-3'SL</p>	<p>GGCGCGCCGAGGGCCTATTTCCCATGATTCCTTCATA  TTTGCATATACGATACAAGGCTGTTAGAGAGATAATT  GGAATTAATTTGACTGTAAACACAAAGATATTAGTAC  AAAATACGTGACGTAGAAAGTAATAATTTCTTGGGT</p>

	AGTTTGCAGTTTTTAAAATTATGTTTTAAAATGGACTA TCATATGCTTACCGTAACTTGAAAGTATTTTCGATTTCT TGGCTTTATATATCTTGTGGAAAGGACGAAACACCG CAATTGTAAAAGAAGTCAGGCCATCACAAATGCCAC AGCTTGAGTAACTGTGCAGCCTGTAGCTCCACCTG AGAAGGTGTAAAAAAGATCCCACCNNNNNNNNNN NNNNNNNNNGTTTAAAGAGCTATGCTGGAAACAGCAT AGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCCAATTGCATATTG ACGCTGGGAAAGACCAGAGATCCTGCTGTCTCCTCA GCATCATTCCAGGCACAGAACGCCAGAAAATGGAAT GGTGCTGTTGAATCAACAGGTTCTAGATCTTTTTTTG TTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTA GTCCGTAGCGCGTGCGCCAATTCTGCAGACAAATGG C
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