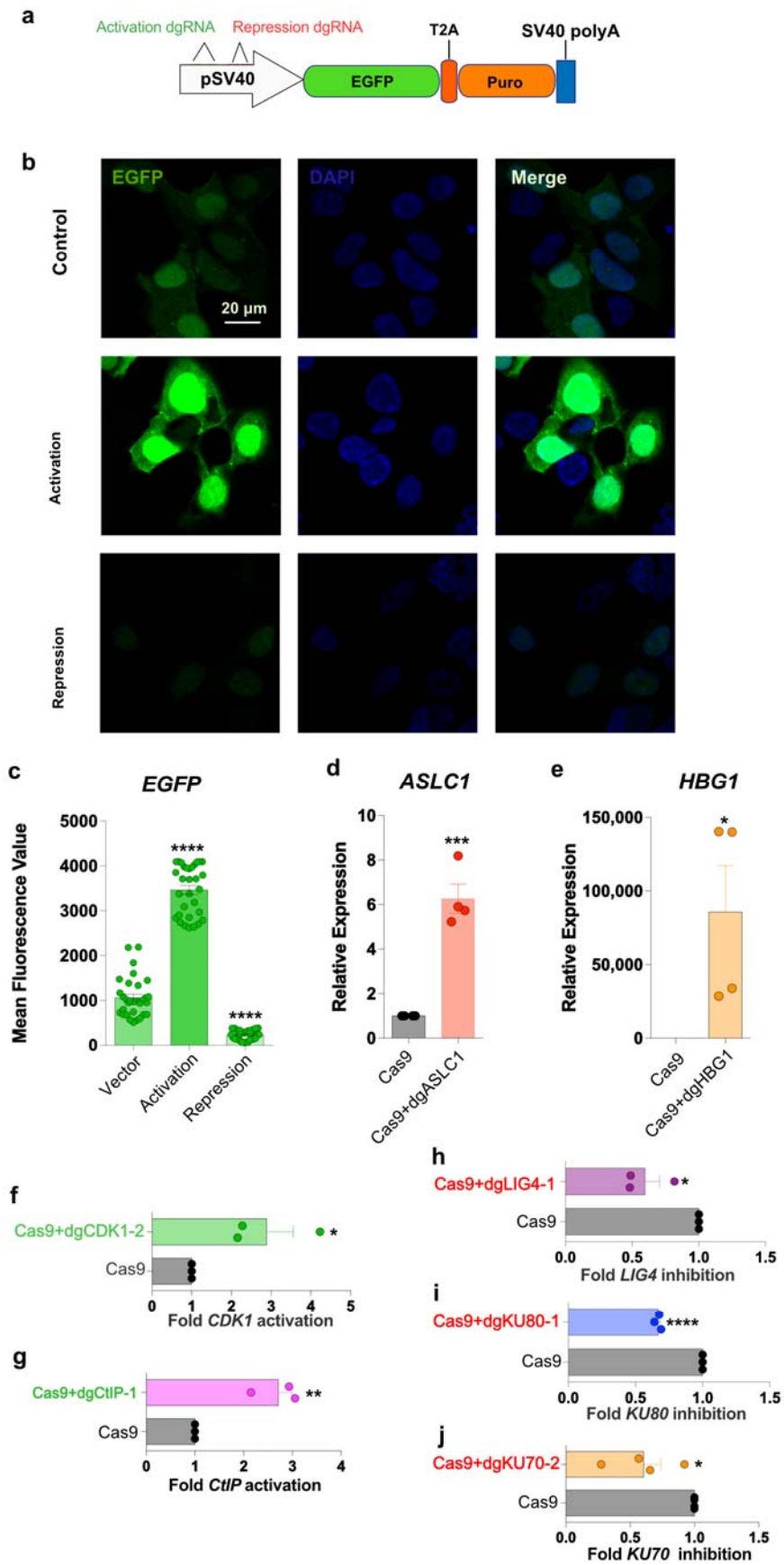
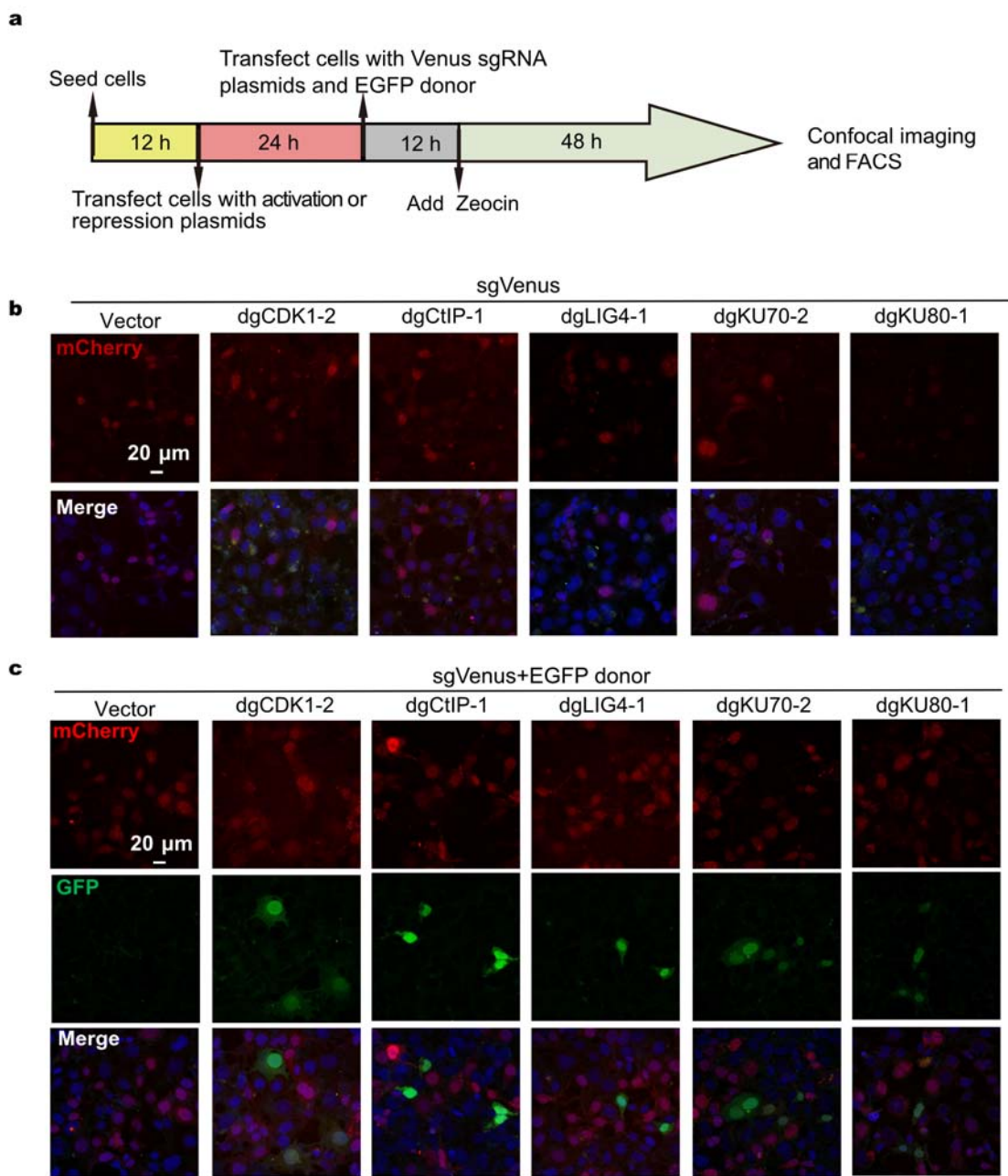
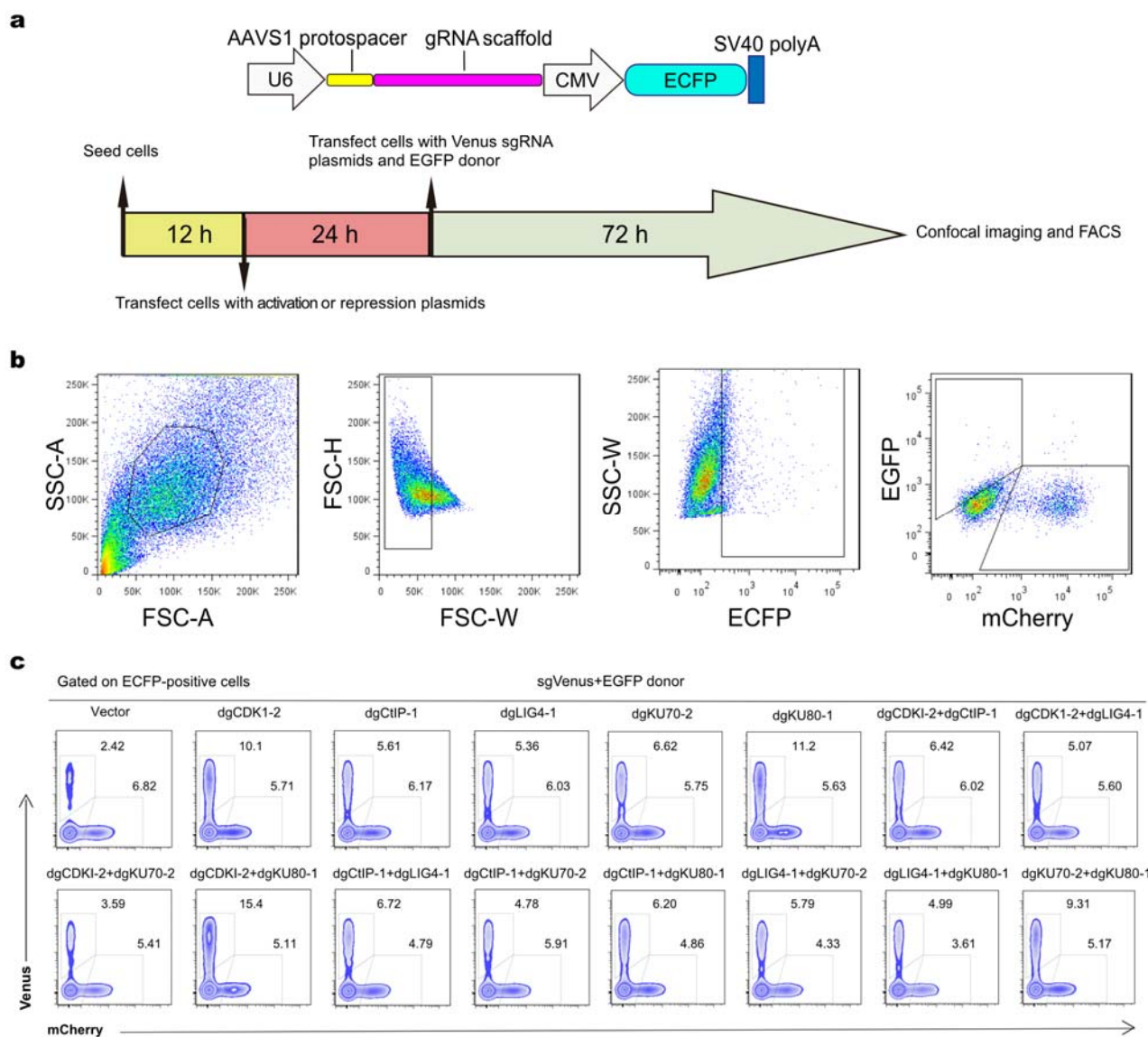




536 **Supplementary Fig. S1 Functional test of the dgRNA-Com:CK and dgRNA-MS2:MPH expression vectors. (a)**
537 Schematics of plasmids used for testing dgRNA-Com:CK and dgRNA-MS2:MPH systems. **(b)** Confocal analysis of
538 dgRNA-Com:CK and dgRNA-MS2:MPH systems in HEK293 cells. HEK293 cells were transfected with pSV40-EGFP
539 plasmid. One day later, the dgRNA-Com:CK or dgRNA-MS2:MPH expression vector targeting SV40 promoter (pSV40)
540 was transfected. After 2 days, the fluorescence intensity was assessed using confocal microscopy. **(c)** Quantitative
541 fluorescence intensity of EGFP after activation and repression (Vector vs Activation, $p < 0.0001$; Vector vs Repression, p
542 < 0.0001). The activation efficiency of *ASLC1* (Cas9 vs Cas9 + dgASLC1, $p = 0.0002$) **(d)** and *HBG1* (Cas9 vs Cas9 +
543 dgHBG1, $p = 0.0344$) **(e)** in HEK293 cells using dgRNA-MS2:MPH expression vector targeting *ASLC1* or *HBG1*
544 promoter region. Three days later, total RNA was extracted and the gene transcriptional level was determined by RT-
545 qPCR. **(f-j)** The activation or suppression efficiency of essential genes related to DNA repair. Five dgRNAs were
546 designed for each gene to screen the best dgRNA for *CDK1* (Cas9 vs Cas9 + dgCDK1-2, $p = 0.0485$) and *CtIP* (Cas9 vs
547 Cas9 + dgCtIP-1, $p = 0.0038$) activation and *LIG4* (Cas9 vs Cas9 + dgLIG4-1, $p = 0.0216$), *KU80* (Cas9 vs Cas9 +
548 dgKU80-1, $p < 0.0001$) and *KU70* (Cas9 vs Cas9 + dgKU70-2, $p = 0.0254$) repression. Data was represented as the mean
549 $\pm$ SEM from three independent experiments. Significance was calculated using the Paired t test. * $P < 0.05$, ** $P < 0.01$,
550 *** $P < 0.001$, **** $P < 0.0001$.
551



Supplementary Fig. S2 Using the TLR reporter to evaluate HDR efficiency enhancement and confocal microscopy analysis. (a) The strategy used in this experiment. Firstly, cells were transfected with dgRNA-Com:CK or dgRNA-MS2:MPH vector to active or repressing targeted gene. After 1 day, these cells were co-transfected with EGFP HR donor and sgVenus vector. 2.5 days later, the samples were analyzed by confocal microscopy or flow cytometry. (b) HEK293-Cas9-TLR cells co-transfected with dgRNA-Com:CK or dgRNA-MS2:MPH plasmids and sgVenus vector. After 2 days, mCherry⁺ cells were analyzed by confocal microscopy. (c) HEK293-Cas9-TLR cells were co-transfected with intact EGFP PCR repair template and sgVenus plasmids after dgRNA-Com:CK or dgRNA-MS2:MPH plasmid transfection. 3 days later, samples were analyzed by confocal microscopy. The ratio of HDR-positive events was significantly increased after programming DNA repair pathways.



Supplementary Fig. S3 NHEJ and HDR efficiency evaluation by the TLR system using FACS. (a) The AAVS1 sgRNA plasmid schematics (upper) and the workflow of this experiment (lower). (b) Flow cytometry gating settings for TRL analysis of HDR and NHEJ. (c) HEK293-Cas9-TLR cell line was first transfected with dgRNA-MS2:MPH and/or dgRNA-Com:CK plasmids; 24 h later, these cells were co-transfected with intact EGFP PCR repair templates and sgVenus-ECFP plasmids. FACS analysis was performed after 72 h of transfection, where ECFP⁺ cells were positively gated for transfection, and the percentage of Venus⁺ (HDR) cells and mCherry⁺ (NHEJ) cells were determined.

a

	*	*	*	*						*	*	*	*	*	*							*	*	*	*	*			*	*	*	*	*	*															
Venus	C	T	G	C	A	G	G	G	A	G	C	A	G	C	G	T	C	T	T	C	G	A	G	A	G	T	G	A	G	G	A	C	A	C	T	A	G	T	G	T	G	A	A	C	C	C	T	G	A
EGFP	C	T	G	C	-	-	-	-	-	-	C	G	G	T	G	C	C	C	T	G	G	C	C	C	-	-	-	-	-	A	C	C	C	T	C	G	T	G	A	C	C	A	C	C	C	T	G	A	
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b

	*	*	*	*										*													*	*	*	*	*						*	*	*	*	*	*	*										
Venus	C	C	T	G	C	A	G	G	G	A	G	C	A	G	C	T	C	T	T	C	G	A	G	A	G	T	G	-	-	A	G	G	A	C	A	C	T	A	G	T	G	T	G	A	A	C	C	T	G	A	C	C	T
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C

Venus	*	*	*	*		*		*		*	*		*		*	*	*	*											*	*	*	*	*	*	*	*																				
#1	C	C	T	G	C	A	G	G	A	G	C	A	C	G	T	C	T	T	C	G	A	G	A	G	T	G	A	C	G	A	C	C	C	T	G	A	C	T	A	C																
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d

Donor	Inside of homology arm	SA-T2A-EGFP
Donor	C C A A C C C C A T G C C G T C T T C A C T C G C T G G G T T C C C T T T T C C	T T T C T C T T T C T G G G C C T G T G C C A T C T C T C G
#1	C C A A C C C C A T G C C G T C T T C A C T C G C T G G G T T C C C T T T T C C	T T T C T C T T T C T G G G C C T G T G C C A T C T C T C G
#2	C C A A C C C C A T G C C G T C T T C A C T C G C T G G G T T C C C T T T T C C	T T T C T C T T T C T G G G C C T G T G C C A T C T C T C G
#3	C C A A C C C C A T G C C G T C T T C A C T C G C T G G G T T C C C T T T T C C	T T T C T C T T T C T G G G C C T G T G C C A T C T C T C G
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#5	C C A A C C C C A T G C C G T C T T C A C T C G C T G G G T T C C C T T T T C C	T T T C T C T T T C T G G G C C T G T G C C A T C T C T C G
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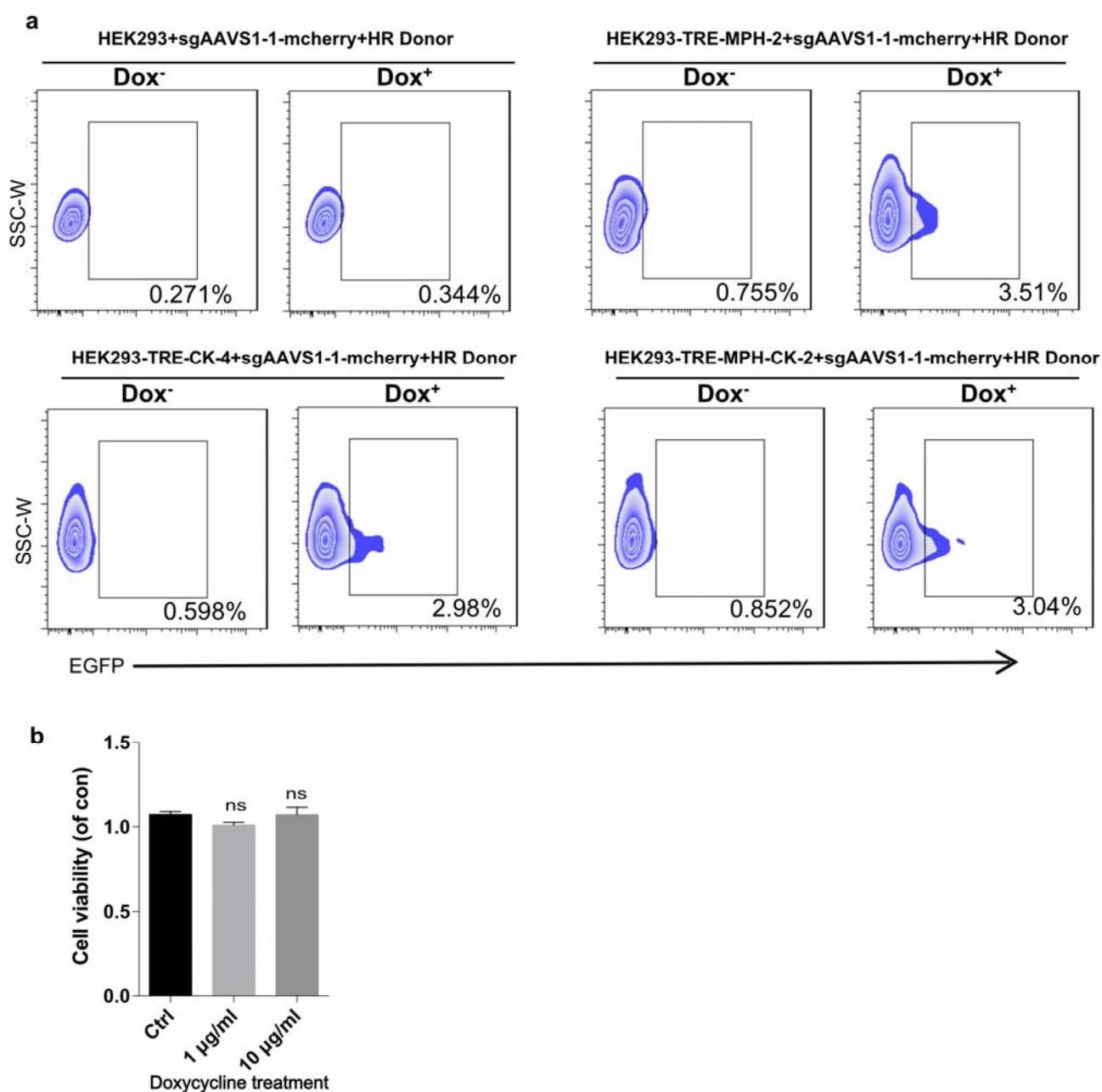
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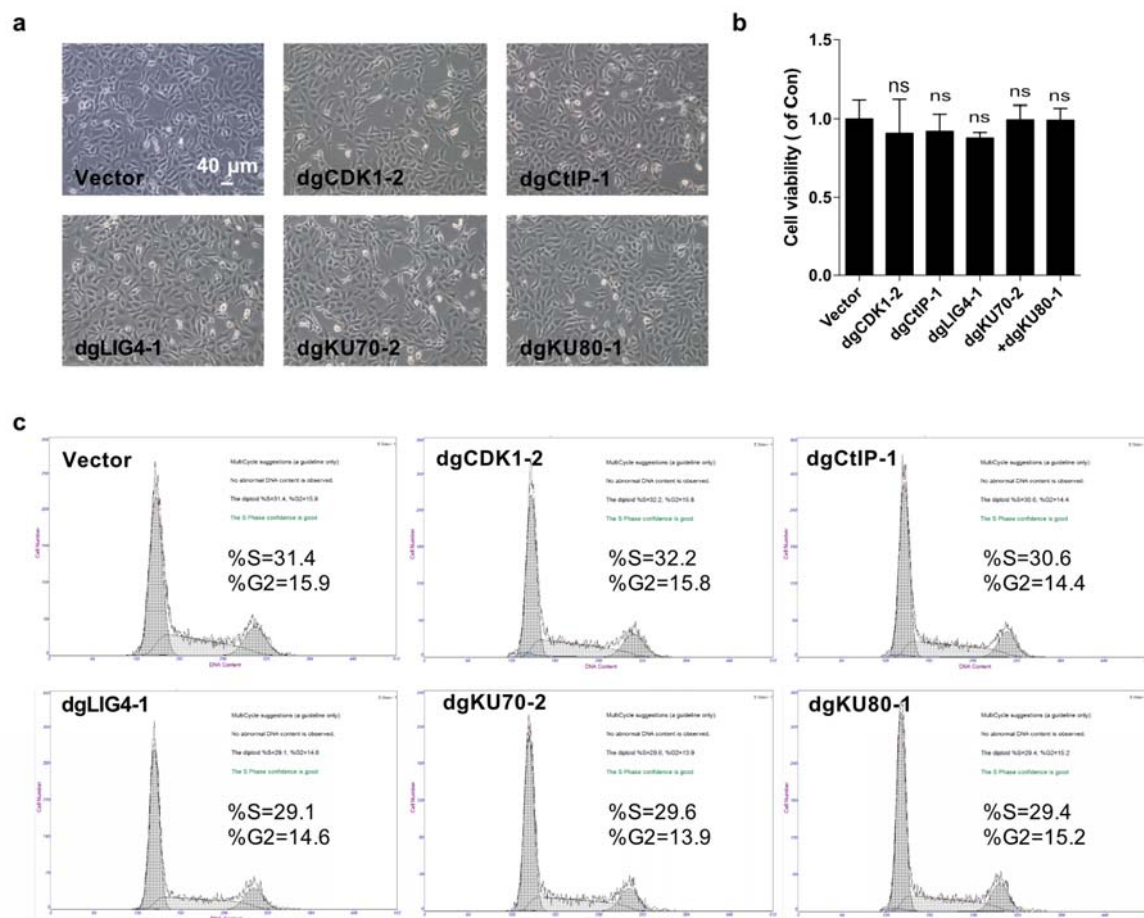
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Supplementary Fig S4 Sequencing confirmation of HDR- and NHEJ-positive events and exogenous gene into the endogenous AAVS1 locus. (a) GFP⁺/mCherry⁻, GFP⁻/mCherry⁺ and GFP⁻/mCherry⁻ individual clones were randomly picked, cultured, PCR and Sanger sequenced. Sequences from multiple clones were shown. (b) Sequencing confirmation of EGFP⁺ cell clones to make sure SA-T2A-EGFP was precisely integrated into AAVS1 locus.



Supplementary Fig. S5 FACS plot for AAVS1 targeting HDR enhancement using inducible CRISPRa/i system. (a) HEK293-TRE-MPH, HEK293-TRE-CK, and HEK293-TRE-MPH-CK cell lines were co-transfected with SA-T2A-EGFP donor and sgAAVS1-mCherry plasmid, 24 h later, 1 µg/ml doxycycline was provided. After 2 days' doxycycline treatment, the frequency of EGFP⁺ cells within the population of mCherry⁺ cells were analyzed by flow cytometry. **(b)** Cell viability was detected after Doxycycline treatment.



Supplementary Fig. S6 Cell viability and cell cycle confirmation after programming HDR and NHEJ pathways using CRISPRa/i system. (a-b) Cell viability was measured after doxycycline treatment. **(c)** Cell cycle was detected by Flow Cytometry after programming HDR and NHEJ pathways.

Programmable DNA Repair with CRISPRa/i

Enhanced Homology-Directed Repair Efficiency with a Single Cas9

Lupeng Ye^{1,2,3,*}, Chengkun Wang^{1,*}, Lingjuan Hong^{1,4}, Ninghe Sun¹, Danyang Chen¹, Sidi Chen^{2,3,#}, and Feng Han^{1,#}

Supplementary Table S1 Target sequences of dgRNAs

Target gene	Name	Sequence (5'>3')
<i>CDK1</i>	dgCDK1-1	GCGCTCTAGCCACC
	dgCDK1-2	ACGGGCTACCCGAT
	dgCDK1-3	GCGCTCGCACTCAGT
	dgCDK1-4	CTAGTCAGCGGAGC
	dgCDK1-5	GAACTGTGCCAATGC
<i>CtIP</i>	dgCtIP-1	GCGTGACGTCGCGC
	dgCtIP-2	GGGCAGCTGGAGGAA
	dgCtIP-3	ATCGCCCTCCGGGAT
	dgCtIP-4	GTCGCCAGACTCTTC
	dgCtIP-5	GCATCAAGCCCTTG
<i>Ligase IV</i>	dgLIG4-1	GGCCCTTAAAACTT
	dgLIG4-2	ACACTTCAGTGCAC
	dgLIG4-3	TACCTCGGCGGCGT
	dgLIG4-4	GAGCCCCCGCGACGG
	dgLIG4-5	GGGGCTCACTGGCAG

<i>KU70</i>	dgKU70-1	GGTAGAAGCTGGTTG
	dgKU70-2	GTTGGCTTTCGTCA
<i>KU80</i>	dgKU80-1	GCATGCTCAGAGTTC
	dgKU80-2	GCCTTTCAGGCCTAGC
	dgKU80-3	GTACTAGCGTTTCAGG
<i>ASCL1</i>	dgASCL1	GCTCGCTGCAGCAG
<i>HBG1</i>	dgHBG1	GAGGCCAGGGGCCGG
<i>EGFP</i>	dgGFP-A1	ATTAGTCAGCAACC
<i>EGFP</i>	dgGFP-A2	ACTGGGCGGAGTTAG
<i>EGFP</i>	dgGFP-R1	GGCCGAGGCCGCCT
<i>EGFP</i>	dgGFP-R2	CAGAAGTAGTGAGG

Supplementary Table S2 Primers used for qRT-PCR

Target gene	Name	Sequence (5'>3')
<i>GFP</i>	GFP-qF	TGACCTACGGCGTGCAGTGCTT
	GFP-qR	CCTCGAACTTCACCTCGGCGC
<i>GADPH</i>	GADPH-qF	TTTGGTCGTATTGGGCGCCTGG
	GADPH-qR	CTCAGCCTTGACGGTGCCATGG
<i>ASCL1</i>	ASCL1-qF	GAGGAGCAGGAGCTTCTCGACT
	ASCL1-qR	AACGCCACTGACAAGAAAGCAC
<i>HBG1</i>	HBG1-qF	GGCTACTATCACAAGCCTGTGG
	HBG1-qR	TTGCCCATGATGGCAGAGGCA

<i>CDK1</i>	CDK1-qF	CTACAGGTCAAGTGGTAGCCATG
	CDK1-qR	CTGGAATCCTGCATAAGCACATCC
<i>CtIP</i>	CtIP-qF	CAACAGCTGAGGGAACAGCAG
	CtIP-qR	AGTTTAAGATCCTGCTGCCGG
<i>Ligase IV</i>	LIG4-qF	GGTAAAGGATCACGGGGTGG
	LIG4-qR	GCTGCTTGGTGGAGCTTTTC
<i>KU70</i>	KU70-qF	GGCTGTGGTGTCTATGGTACCG
	KU70-qR	CCGTGGCCCATCATGTCTTGGA
<i>KU80</i>	KU80-qF	GTTGTGCTGTGTATGGACGTGG
	KU80-qR	GTGCCATCAGTACCAAACAGGAC

Supplementary Table S3 Primers for PCR amplification of sgRNA target region

Target gene	Name	Sequence (5'>3')
<i>EGFP</i>	F	AGATCTATGGTGAGCAAGGGCGAGGA
	R	GAATTCTTACTTGTACAGCTCGTCCATG
<i>AAVS1</i>	Primer-F	GGGTCACCTCTACGGCTGG
	Primer-R	CGAATTCTTACTTGTACAGCTCGTCCA

Supplementary Table S4 Target sequences of sgRNAs

Target gene	Name	Sequence (5'>3')
<i>Venus</i>	sgVenus	GAGCAGCGTCTTCGAGAGTG

<i>AAVS1</i>	sgAAVS1-1	CACCCCACAGTGGGGCCACT
<i>AAVS1</i>	sgAAVS1-2	TGTCCCTAGTGGCCCCACTG
<i>ACTB</i>	sgACTB	CCACCGCAAATGCTTCTAGG

Supplementary sequence 1. The TLR DNA sequence

Venus

CRISPR targeting site

T2A

mCherry

atggtgagcaagggcgaggagctgttcaccggggtggtgccatcctggtcgagctggacggcgacg
taaacggccacaagttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctgacct
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aacagtacgaacgcgccgagggccgcccactccaccggcgcatggacgagctgtacaagtga

Supplementary sequence 2. The AAVS1 HDR donor DNA sequence

Left homology arm of *AAVS1*

SA-T2A-EGFP-ShortPA

Right homology arm of *AAVS1*

5'-ttctccttctggggcctgtgccatctctcgtttcttaggatggccttctccgacggatgtctcccttgcgt
cccgctcccccttctttaggcctgcatcatcaccgtttttctggacaaccccaaagtaccccgctctccctg
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Supplementary sequence 3. The ACTB HDR donor DNA sequence

Left homology arm of *ACTB*

T2A-EGFP

Right homology arm of *ACTB*

5'-CGGCTCTGCCTGACATGAGGGTTACCCCTCGGGGCTGTGCTG
TGGAAGCTAAGTCCTGCCCTCATTTCCTCTCAGGCATGGAGTC
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