SUPPLEMENTARY DATA

Microbial Single-strand Annealing Proteins Enable CRISPR Gene-editing Tools with Improved Knock-in Efficiencies and Reduced Off-target Effects

Supplementary Notes and Sequences

Supplementary Figs. S1 to S12

Supplementary Tables S1 to S3

Supplementary Sequences

SUPPLEMENTARY NOTES AND SEQUENCES

SUPPLEMENTARY NOTE

Step-by-step gene-editing protocol using REDIT plasmids

A. Design of guideRNA sequences at target genomic loci

This step is the same as standard Cas9 experiments. Briefly, based on the Cas9 enzyme used, target sequence (usually 20-bp) near the knock-in or editing sites can be selected next to the protospacer adjacent motif (PAM). For SpCas9 use “NGG” and for SaCas9 use “NNGRRT”. We usually append extra “G” base to the beginning of the guide sequence to facilitate U6/Pol-III transcription initiation if the first base of the guide sequence is not “G”. Two DNA oligos could be ordered based on selected guides, with golden gate cloning overhangs, as shown below.

5′ –CACCGNNNNNNNNNNNNNNNNNNN **–3′**

3′ –CNNNNNNNNNNNNNNNNNNNCAAA **–5′**

N denotes the guide sequences. Standard desalting oligos are sufficient for this cloning. The two oligos above will be annealed to form the insert fragments in the next step.

B. Annealing of two DNA oligos for each guideRNA target. Perform phosphorylation and annealing of each pair of oligos via reaction setup below.

oligo1 Top (100uM)	1ul
oligo2 Bottom (100uM)	1ul
10X T4 ligation Buffer(NEB)	1ul
ddH2O	6.5ul
T4 PNK (NEB)	0.5ul
Total	10ul

Anneal in a thermocycler using the following parameters:

37C 30 min

95C 5 min and then ramp down to 25C at 5C/min

C1. Golden Gate Cloning of annealed oligos into sgRNA/Cas9 (REDIT) plasmid

For wild-type Cas9 REDIT, one guide RNA is needed and the backbone vectors for the cloning will bear BbsI cloning sites matching the annealed oligos from Step B. The REDIT plasmids for this step will be: **pREDIT_Cas9-MS2-BB_BbsI** (see list of plasmids at end of protocol)

Item	**Volume**	**Note**
Water	4.3 ul	
Cutsmart Buffer	0.8 ul	10x
T4 ligase	0.2 ul	
BbsI-HF	0.4 ul	
ATP (25mM)	0.3 ul	~ final 1mM
REDIT plasmid/vector	1 ul	~ 50ng total REDIT plasmid
Annealed Oligo (1:10 diluted)	1 ul	diluted 10ul into 100ul
Total	8 ul	

This protocol uses a minimal amount of enzyme and could be scaled up as needed. After set up the golden gate reaction (on ice), immediately move the reaction into Thermocycler and perform the golden gate reaction using the following parameters:

37C 5 min
16C 5 min
cycle for ~20 cycles, additional cycles up to 50 could be used to maximize efficiency
65C 5 min
4C hold

After the reaction, perform bacterial transformation as per standard protocol of the competent cells used in the lab.

C2. Golden Gate Cloning of annealed oligos into sgRNA/Cas9n (REDITn) plasmid

For Cas9 REDITn using Cas9n (nickase), one or two guide RNAs can be used with double guideRNAs providing slightly better efficiency of editing. The backbone vectors for the cloning will bear BbsI cloning sites matching the annealed oligos from Step B. The REDIT plasmids for this step will be: **pREDIT_Cas9n-MS2-BB_BbsI** (see list of plasmids at end of protocol)

Item	**Volume**	**Note**
Water	4.3 ul	Add first

Cutsmart Buffer	0.8 ul	10x
T4 ligase	0.2 ul	
BbsI-HF	0.4 ul	
ATP (25mM)	0.3 ul	~ final 1mM
REDITn plasmid/vector	1 ul	~ 50ng total REDIT plasmid
Annealed Oligo (1:10 diluted)	1 ul	diluted 10ul into 100ul
Total	8 ul	

Golden Gate reaction setup and transformation steps are similar as above.

D. Preparation of HDR templates

Please refer to Supplementary Sequences for template used in the study and examples of template designs are illustrated as in Fig. 1A. We recommend using a dsDNA template with at least 200bp of homology arms on each end of the insertion/replacement sequences (the edited portion of the template). We suggest cloning the template into simple plasmids such as pUC19, then, restriction digestion of plasmids or standard PCR (using primers such as listed in the Supplementary table S2) could be employed for generating large amounts of dsDNA templates.

E. Perform gene-editing via delivery of REDIT/REDITn plasmids and template DNA

With previous steps, the 3 components of REDIT editing method are ready for experiments: the guideRNA/Cas9 plasmid (cloned in step A-C), the template DNA (from step D), and the SSAP plasmid (pREDIT_MCP-RecT, can be obtained from Addgene). For delivery into cells in vitro, routine transfection or electroporation could be performed following the recommended conditions by the reagent or equipment manufacturer and selected based on the cell types. For HEK293T cells as an example, a typical transfection condition is described below:

1. One day before transfection, 2.5E4 HEK293T cells seeded on each well of 96-well plate, the cell density should be around 70% on the next day at the time of transfection.
2. For lipofectamine 3000 as the transfection reagent, use a total of 250 ng DNA + 0.4 ul Lip3000 reagents (ea.) and perform the reagent set up using 10 ul of Opti-MEM per well, as in the manufacturer's protocol.
3. Transfection material:
   REDIT guideRNA plasmids, 125ng (for double-nicking design, use equal amount of the

two guideRNA plasmids, i.e. 62.5ng each); pREDIT_MCP-RecT, 75ng; Template DNA, up to 50ng.

4. Mix plasmids with template DNA and perform transfection according to the manufacturer's protocol for HEK293T cells.
5. 12-24 hours after transfection, if applicable could switch to fresh media.
6. After at least 3 days post transfection, cells could be harvested or proceed to downstream experiments or analysis as needed.

List of REDIT and REDITn Plasmids (all will be available at Addgene via plasmid ID)

Plasmid ID	**Detailed Description**
SpCas9 REDIT Plasmids	
pREDIT_Cas9-MS2-BB_BbsI	pU6-MS2-gRNA-backbone(BbsI)-CBH-SpCas9-T2A-EBFP
pREDIT_MCP-RecT	pLenti-EF1A-MCP-EXTEN-RecT-NLS
SpCas9 REDITn/dn Plasmids	
pREDIT_Cas9n-MS2-BB_BbsI	pU6-MS2-gRNA-backbone(BbsI)-CBH-SpCas9n(D10A)-T2A-EBFP
pREDIT_MCP-RecT	Same as above
SaCas9 REDIT plasmids	
pREDIT_SaCas9-MS2-BB_BsaI	pU6-MS2-gRNA-backbone(BsaI)-CBH-SaCas9-T2A-EBFP
pREDIT_MCP-RecT	Same as above
SaCas9 REDITn/dn plasmids	
pREDIT_SaCas9n-MS2-BB_BsaI	pU6-MS2-gRNA-backbone(BsaI)-CBH-SaCas9(D10A)-T2A-EBFP
pREDIT_MCP-RecT	Same as above

Suntag RecT plasmids	
pCBH-Cas9-Suntag-BB_BbsI	pU6-gRNA-backbone(BbsI)-CBH-SpCas9-Suntag-T2A-EBFP
pEF1A-scFV-RecT	pLenti-EF1A-scFV-GS-NLS-RecT

SUPPLEMENTARY SEQUENCES

SV40 NLS amino acid sequence:

PKKKRKV

Ty1 NLS amino acid sequence:

NSKKRSLEDNETEIKVSRDTWNTKNMRSLEPPRSKKRIH

c-Myc NLS amino acid sequence:

PAAKRVKLD

biSV40 NLS amino acid sequence:

KRTADGSEFESPKKKRKV

GS linker sequence:

GGGGSGGGGSGGGGS

modified E-XTEN Linker amino acid sequence:

SGGSSGGSSGSETPGTSESATPESSGGSSGGS

Bacteriophage lambda, Recombination protein bet amino acid sequence:

MSTALATLAGKLAERVGMDSVDPQELITTLRQTAFKGDASDAQFIALLIVANQYGLNPWTKEIYA
FPDKQNGIVPVVGVDGWSRIINENQQFDGMDFEQDNESCTCRIYRKDRNHPICVTEWMDECR
REPFKTREGREITGPWQSHPKRMLRHKAMIQCARLAFGFAGIYDKDEAERIVENTAYTAERQP

ERDITPVNDETMQEINTLLIALDKTWDDDLLPLCSQIFRRDIRASSELTQAEAVKALGFLKQKAAE
QKVAA*

Bacteriophage T7, Single-stranded DNA-binding protein gp2.5 amino acid sequence:

MAKKIFTSALGTAEPYAYIAKPDYGNEERGFGNPRGVYKVDLTIPNKDPRCQRMVDEIVKCHEE
AYAAAVEEYEANPPAVARGKKPLKPYEGDMPFFDNGDGTTTFKFKCYASFQDKKTKETKHINL
VVVDSKGKKMEDVPIIGGGSKLKVKYSLVPYKWNTAVGASVKLQLESVMLVELATFGGGEDD
WADEVEENGYVASGSAKASKPRDEESWDEDDEESEEADEDGDF*

Rac prophage RecT (EcRecT) amino acid sequence:

MTKQPPIAKADLQKTQGNRAPAAIKNNDVISFINQPSMKEQLAAALPRHMTAERMIRIATTEIRK
VPALGNCDTMSFVSAIVQCSQLGLEPGSALGHAYLLPFGNKNEKSGKKNVQLIIGYRGMIDLAR
RSGQIASLSARVVREGDEFNFEFGLDEKLIHRPGENEDAPVTHVYAVARLKDGGTQFEVMTRK
QIELVRSQSKAGNNGPWVTHWEEMAKKTAIRRLFKYLPVSIEIQRAVSMDEKEPLTIDPADSSV
LTGEYSVIDNSEE*

MS2 coat protein

MASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISSNSRSQAYKVTCSVRQSSAQKRKYTIK
VEVPKVATQTVGGVELPVAAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANS
GIY*

10XGCN

MEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEV
ARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLS
KNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKG
SGSGEELLSKNYHLENEVARLKKGSGSGEELLSKNYHLENEVARLKKGSGSGTAVNIGGGTGP
MDLQRPLNGGGPKKKRKV*

scFV

MGPDIVMTQSPSSLSASVGDRVTITCRSSTGAVTTSNYASWVQEKPGKLFKGLIGGTNNRAPG
VPSRFSGSLIGDKATLTISSLQPEDFATYFCALWYSNHWVFGQGTKVELKRGGGGSGGGGSG
GGGSSGGGSEVKLLESGGGLVQPGGSLKLSCAVSGFSLTDYGVNWVRQAPGRGLEWIGVIW
GDGITDYNSALKDRFIISKDNGKNTVYLQMSKVRSDDTALYYCVTGLFDYWGQGTLVTVSS*

MS2 stem loop insertion sgRNA scaffold (N denotes guide RNA target site sequence)

NNNNNNNNNNNNNNNNNNNNGTTTAAGAGCTAGGCCAACATGAGGATCACCCATGTCTGC
AGGGCCTAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTC
GGTGCGCGCACATGAGGATCACCCATGTGC

Template DNA sequences

Annotations of the replaced or inserter editing sequences are detailed below with each of the templates. Unless otherwise noted, when different homology arms are used in the study, we used primers listed in **Table S2** to obtain templates with different homology arm lengths.

DYNLT1 P2A-mKate knock-in HDR template sequence

Left Homology Arm-Insertion Sequence-Right Homology Arm

(Underlined are the inserted **mKate** fluorescent protein sequence, the proceeding non-underlined part is the **P2A peptide** sequence)

AGTGACCTGTGTAATTATGCAGAAGAATGGAGCTGGATTACACACAGCAAGTTCCTGCTTC
TGGGACAGCTCTACTGACGGTATGATTTTCATTCATGTTTGTGAAGTTTTGTTGTGTGAAAT
ATATGACTGGAAGTTTCCTATCTTTGAATGCAATGCATGTTTATCACCTTTTAAAACATTTAA
TAATAGACTTGCCAAGGTTCTTTGTGTAGCATAGAGATGGGTACTTGAATGTTGGCCTTATT
GTGAGTAAAACGTCGTCCCCCAGCTTTCCCTGCCGTAAATGCTGCTCTCTTCCCTCCCGCA
GGGAGCTGCACTGTGCGATGGGAGAATAAGACCATGTACTGCATCGTCAGTGCCTTCGGA
CTGTCTATTGGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAG
GAGAACCCTGGACCTGCCACCGTGAGCGAGCTGATTAAGGAGAACATGCACATGAAGCTG
TACATGGAGGGCACCGTGAACAACCACCACTTCAAGTGCACATCCGAGGGCGAAGGCAAG
CCCTACGAGGGCACCCAGACCATGAGAATCAAGGCGGTCGAGGGCGGCCCTCTCCCCTT
CGCCTTCGACATCCTGGCTACCAGCTTCATGTACGGCAGCAAAACCTTCATCAACCACACC
CAGGGCATCCCCGACTTCTTTAAGCAGTCCTTCCCCGAGGGCTTCACATGGGAGAGAGTC
ACCACATACGAAGATGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCCAGGACGG
CTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCATCCAACGGCCCTGTGAT
GCAGAAGAAAACACTCGGCTGGGAGGCCTCCACCGAGACACTGTACCCCGCTGACGGCG
GCCTGGAAGGCAGAGCCGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACCTGATCTGC
AACCTTAAGACCACATACAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCCGGCGTCT
ACTATGTGGACAGGAGACTGGAAAGAATCAAGGAGGCCGACAAAGAGACATACGTCGAGC
AGCACGAGGTGGCTGTGGCCAGATACTGCGACCTCCCTAGCAAACTGGGGCACAAACTTA
ATTCCTAACCAGCTGTCCtGCCTATGGCCTTTCTCCTTTTGTCTCTAGTTCATCCTCTAACCA
CCAGCCATGAATTCAGTGAACTCTTTTCTCATTCTCTTTGTTTTGTGGCACTTTCACAATGTA
GAGGAAAAAACCAAATGACCGCACTGTGATGTGAATGGCACCGAAGTCAGATGAGTATCC
CTGTAGGTCACCTGCAGCCTGCGTTGCCACTTGTCTTAACTCTGAATATTTCATTTCAAAGG
TGCTAAAATCTGAAATCTGCTAGTGTGAAACTTGCTCTACTCTCTGAAATGATTCAAATACA
CTAATTTTCCATACTTTATACTTTTGTTAGAATAAATTATTCAAATCTAAAGTCTGTTGTGTTC
TTCATAGTCTGCATAGTATCATAAACG

HSP90AA1 P2A-mKate knock-in HDR template sequence

Left Homology Arm-Insertion Sequence-Right Homology Arm
(Underlined are the inserted **mKate** fluorescent protein sequence, the proceeding non-underlined part is the **P2A peptide** sequence)

GCAGCAAAGAAACACCTGGAGATAAACCCTGACCATTCCATTATTGAGACCTTAAGGCAAA
AGGCAGAGGCTGATAAGAACGACAAGTCTGTGAAGGATCTGGTCATCTTGCTTTATGAAAC
TGCGCTCCTGTCTTCTGGCTTCAGTCTGGAAGATCCCCAGACACATGCTAACAGGATCTAC
AGGATGATCAAACTTGGTCTGGGTAAGCCTTATACTATGTAATGTTAAAAAGAAAATAAACA
CACGTGACATTGAAGAAAATGGTGAACTTTCAGTTATCCAAACTTGGAGCACCTTGTCCTG
CTTGCTGCTTGGAGGTATTAAAGTATGTTTTTTTTAGGGATAAGTAAGGTCTTACAAGAGCA
AAGAAATGAAATTGAGACTCATATGTCCTGTAATACTGTCTTGAAAGCAGATAGAAACCAAG
AGTATTACCCTAATAGCTGGCTTTAAGAAATCTTTGTAATATGAGGATTTTATTTTGGAAACA
GGTATTGATGAAGATGACCCTACTGCTGATGATACCAGTGCTGCTGTAACTGAAGAAATGC
CACCCCTTGAAGGAGATGACGACACATCACGCATGGAAGAAGTAGACGGAAGCGGAGCTA
CTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTGTGAGC
GAGCTGATTAAGGAGAACATGCACATGAAGCTGTACATGGAGGGCACCGTGAACAACCAC
CACTTCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAG
AATCAAGGCGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTT
CATGTACGGCAGCAAAACCTTCATCAACCACACCCAGGGCATCCCCGACTTCTTTAAGCAG
TCCTTCCCCGAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGATGGGGGCGTGCTG
ACCGCTACCCAGGACACCAGCCTCCAGGACGGCTGCCTCATCTACAACGTCAAGATCAGA
GGGGTGAACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGC
CTCCACCGAGACACTGTACCCCGCTGACGGCGGCCTGGAAGGCAGAGCCGACATGGCCC
TGAAGCTCGTGGGCGGGGGCCACCTGATCTGCAACCTTAAGACCACATACAGATCCAAGA
AACCCGCTAAGAACCTCAAGATGCCCGGCGTCTACTATGTGGACAGGAGACTGGAAAGAA
TCAAGGAGGCCGACAAAGAGACATACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATAC
TGCGACCTCCCTAGCAAACTGGGGCACAAACTTAATTCCTAAATCTGTGGCTGAGGGATGA
CTTACCTGTTCAGTACTCTACAATTCCTCTGATAATATATTTTCAAGGATGTTTTTCTTTATTT
TTGTTAATATTAAAAAGTCTGTATGGCATGACAACTACTTTAAGGGGAAGATAAGATTTCTG
TCTACTAAGTGATGCTGTGATACCTTAGGCACTAAAGCAGAGCTAGTAATGCTTTTTGAGTT
TCATGTTGGTTTATTTTCACAGATTGGGGTAACGTGCACTGTAAGACGTATGTAACATGATG
TTAACTTTGTGGTCTAAAGTGTTTAGCTGTCAAGCCGGATGCCTAAGTAGACCAAATCTTGT
TATTGAAGTGTTCTGAGCTGTATCTTGATGTTTAGAAAAGTATTCGTTACATCTTGTAGGATC
TACTTTTTGAACTTTTCATTCCCTGTAGTTGACAATTCTGCATGTACTAGTCCTCTAGAAATA
GGTTAAACTGAAGCAACTTGATGGAAGGATCTCTCCACAGGGCTTGTTTTCCAAAGAAAAG
TATTGTTTGGAGGAGCAAAGTTAAAAGCCTACCTAAGCATATCGTAAAGCTGTTCAAAAATA
ACTCAGACCCAGTCTTGTGGA

AAVS1 P2A-mKate knock-in HDR template sequence

Left Homology Arm-Insertion Sequence-Right Homology Arm
(Underlined are the inserted **mKate** fluorescent protein sequence, the proceeding non-underlined part is the **P2A peptide** sequence)

GATGCTCTTTCCGGAGCACTTCCTTCTCGGCGCTGCACCACGTGATGTCCTCTGAGCGGA
TCCTCCCCGTGTCTGGGTCCTCTCCGGGCATCTCTCCTCCCTCACCCAACCCCATGCCGT
CTTCACTCGCTGGGTTCCCTTTTCCTTCTCCTTCTGGGGCCTGTGCCATCTCTCGTTTCTTA
GGATGGCCTTCTCCGACGGATGTCTCCCTTGCGTCCCGCCTCCCCTTCTTGTAGGCCTGC
ATCATCACCGTTTTTCTGGACAACCCCAAAGTACCCCGTCTCCCTGGCTTTAGCCACCTCT
CCATCCTCTTGCTTTCTTTGCCTGGACACCCCGTTCTCCTGTGGATTCGGGTCACCTCTCA
CTCCTTTCATTTGGGCAGCTCCCCTACCCCCCTTACCTCTCTAGTCTGTGCTAGCTCTTCCA
GCCCCCTGTCATGGCATCTTCCAGGGGTCCGAGAGCTCAGCTAGTCTTCTTCCTCCAACC
CGGGCCCCTATGTCCACTTCAGGACAGCATGTTTGCTGCCTCCAGGGATCCTGTGTCCCC
GAGCTGGGACCACCTTATATTCCCAGGGCCGGTTAATGTGGCTCTGGTTCTGGGTACTTTT
ATCTGTCCCCTCCACCCCACAGTGGGGCAAGCTTCTGACCTCTTCTCTTCCTCCCACAGGG
CCTCGAGAGATCTGGCAGCGGAGGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGG
CTGGAGACGTGGAGGAGAACCCTGGACCTGTGAGCGAGCTGATTAAGGAGAACATGCACA
TGAAGCTGTACATGGAGGGCACCGTGAACAACCACCACTTCAAGTGCACATCCGAGGGCG
AAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCAAGGCGGTCGAGGGCGGCCCT
CTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTTCATGTACGGCAGCAAAACCTTCATCA
ACCACACCCAGGGCATCCCCGACTTCTTTAAGCAGTCCTTCCCCGAGGGCTTCACATGGG
AGAGAGTCACCACATACGAAGATGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTC
CAGGACGGCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCATCCAACGGC
CCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCTCCACCGAGACACTGTACCCCGCT
GACGGCGGCCTGGAAGGCAGAGCCGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACC
TGATCTGCAACCTTAAGACCACATACAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCC
CGGCGTCTACTATGTGGACAGGAGACTGGAAAGAATCAAGGAGGCCGACAAAGAGACATA
CGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTGCGACCTCCCTAGCAAACTGGGGC
ACAAACTTAATTCCTAAACTAGGGACAGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCT
CCTCCTTCCTAGTCTCCTGATATTGGGTCTAACCCCCACCTCCTGTTAGGCAGATTCCTTAT
CTGGTGACACACCCCCATTTCCTGGAGCCATCTCTCTCCTTGCCAGAACCTCTAAGGTTTG
CTTACGATGGAGCCAGAGAGGATCCTGGGAGGGAGAGCTTGGCAGGGGGTGGGAGGGA
AGGGGGGGATGCGTGACCTGCCCGGTTCTCAGTGGCCACCCTGCGCTACCCTCTCCCAG
AACCTGAGCTGCTCTGACGCGGCTGTCTGGTGCGTTTCACTGATCCTGGTGCTGCAGCTT
CCTTACACTTCCCAAGAGGAGAAGCAGTTTGGAAAAACAAAATCAGAATAAGTTGGTCCTG
AGTTCTAACTTTGGCTCTTCACCTTTCTAGTCCCCAATTTATATTGTTCCTCCGTGCGTCAG
TTTTACCTGTGAGATAAGGCCAGTAGCCAGCCCCGTCCTGGCAGGGCTGTGGTGAGGAGG
GGGGTGTCCGTGTGGAAAACTCCCTTTGTGAGAATGGTGCGTCCTAGGTGTTCACCAGGT
CGTGGCCGCCTCTACTCCCTTTCTCTTTCTCCATCCTTCTTTCCTTAAAGAGTCCCCAGTGC
TATCTGGGACATATTCCTCCGCCCAGAGCAGGGTCCCGCTTCCCTAAGGCCCTGCTCTGG
GCTTCTGGGTTTGAGTCCTTGGC

OCT4 P2A-mKate knock-in HDR template sequence

Left Homology Arm-Insertion Sequence-Right Homology Arm
(Underlined are the inserted **mKate** fluorescent protein sequence, the proceeding non-underlined part is the **P2A peptide** sequence)

GCGACTATGCACAACGAGAGGATTTTGAGGCTGCTGGGTCTCCTTTCTCAGGGGGACCAG
TGTCCTTTCCTCTGGCCCCAGGGCCCCATTTTGGTACCCCAGGCTATGGGAGCCCTCACT
TCACTGCACTGTACTCCTCGGTCCCTTTCCCTGAGGGGGAAGCCTTTCCCCCTGTCTCCGT
CACCACTCTGGGCTCTCCCATGCATTCAAATGGAAGCGGAGCTACTAACTTCAGCCTGCTG
AAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCTGCCACCATGGTGAGCGAGCTGAT
TAAGGAGAACATGCACATGAAGCTGTACATGGAGGGCACCGTGAACAACCACCACTTCAA
GTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCAAGG
CGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCTTCATGTACG
GCAGCAAAACCTTCATCAACCACACCCAGGGCATCCCCGACTTCTTTAAGCAGTCCTTCCC
CGAGGGCTTCACATGGGAGAGAGTCACCACATACGAAGATGGGGGCGTGCTGACCGCTA
CCCAGGACACCAGCCTCCAGGACGGCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGA
ACTTCCCATCCAACGGCCCTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCTCCACCG
AGACACTGTACCCCGCTGACGGCGGCCTGGAAGGCAGAGCCGACATGGCCCTGAAGCTC
GTGGGCGGGGGCCACCTGATCTGCAACCTTAAGACCACATACAGATCCAAGAAACCCGCT
AAGAACCTCAAGATGCCCGGCGTCTACTATGTGGACAGGAGACTGGAAAGAATCAAGGAG
GCCGACAAAGAGACATACGTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTGCGACCT
CCCTAGCAAACTGGGGCACAAACTTAATTCCTAATGACTAGGAATGGGGGACAGGGGGAG
GGGAGGAGCTAGGGAAAGAAAACCTGGAGTTTGTGCCAGGGTTTTTGGGATTAAGTTCTT
CATTCACTAAGGAAGGAATTGGGAACACAAAGGGTGGGGGCAGGGGAGTTTGGGGCAAC
TGGTTGGAGGGAAGGTGAAGTTCAATGATGCTCTTGATTTTAATCCCACATCATGTATCACT
TTTTTCTTAAATAAAGAAGCCTGGGACACAGTAGATAGACACACTT

ACTB P2A-mKate knock-in HDR template sequence

Left Homology Arm-Insertion Sequence-Right Homology Arm
(Underlined are the inserted **mKate** fluorescent protein sequence, the proceeding non-underlined part is the **P2A peptide** sequence)

TGTGGTGTGTGGGGAGCTGTCACATCCAGGGTCCTCACTGCCTGTCCCCTTCCCTCCTCA
GATCATTGCTCCTCCTGAGCGCAAGTACTCCGTGTGGATCGGCGGCTCCATCCTGGCCTC
GCTGTCCACCTTCCAGCAGATGTGGATCAGCAAGCAGGAGTATGACGAGTCCGGCCCCTC
CATCGTCCACCGCAAGTGTTTCGGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGC
TGGAGACGTGGAGGAGAACCCTGGACCTGTGAGCGAGCTGATTAAGGAGAACATGCACAT
GAAGCTGTACATGGAGGGCACCGTGAACAACCACCACTTCAAGTGCACATCCGAGGGCGA
AGGCAAGCCCTACGAGGGCACCCAGACCATGAGAATCAAGGCGGTCGAGGGCGGCCCTC
TCCCCTTCGCCTTCGACATCCTGGCTACCAGCTTCATGTACGGCAGCAAAACCTTCATCAA
CCACACCCAGGGCATCCCCGACTTCTTTAAGCAGTCCTTCCCCGAGGGCTTCACATGGGA
GAGAGTCACCACATACGAAGATGGGGGCGTGCTGACCGCTACCCAGGACACCAGCCTCC
AGGACGGCTGCCTCATCTACAACGTCAAGATCAGAGGGGTGAACTTCCCATCCAACGGCC
CTGTGATGCAGAAGAAAACACTCGGCTGGGAGGCCTCCACCGAGACACTGTACCCCGCTG
ACGGCGGCCTGGAAGGCAGAGCCGACATGGCCCTGAAGCTCGTGGGCGGGGGCCACCT
GATCTGCAACCTTAAGACCACATACAGATCCAAGAAACCCGCTAAGAACCTCAAGATGCCC
GGCGTCTACTATGTGGACAGGAGACTGGAAAGAATCAAGGAGGCCGACAAAGAGACATAC
GTCGAGCAGCACGAGGTGGCTGTGGCCAGATACTGCGACCTCCCTAGCAAACTGGGGCA
CAAACTTAATTCCTAATAGGCGGACTATGACTTAGTTGCGTTACACCCTTTCTTGACAAAAC

CTAACTTGCGCAGAAAACAAGATGAGATTGGCATGGCTTTATTTGTTTTTTTTGTTTTGTTTT
GGTTTTTTTTTTTTTTTTGGCTTGACTCAGGATTTAAAAACTGGAACGGTGAAGGTGACAGC
AGTCGGTTGGAGCGAGCATCCCCCAAAGTTCACAATGTGGCCGAGGACTTTGATTGCACA
TTGTTGTTTTTTTAATAGTCATTCCAAATATGAGATGCGTTGTTACAGGAAGTCCCTTGCCAT
CCTAAAAGCCACCCCACTTCTCTCTAAGGAGAATGGCCCAGTCCTCTCCCAAGTCCACACA
GGGGAGGTGATAGCATTGCTTTCGTGTAA

EMX1 HDR template sequence

Left Homology Arm-Insertion/Replacement Sequence-Right Homology Arm
(Underlined are the inserted **BsrGI** restriction site, i.e. TGTACA)

CATTCTGCCTCTCTGTATGGAAAAGAGCATGGGGCTGGCCCGTGGGGTGGTGTCCACTTT
AGGCCCTGTGGGAGATCATGGGAACCCACGCAGTGGGTCATAGGCTCTCTCATTTACTAC
TCACATCCACTCTGTGAAGAAGCGATTATGATCTCTCCTCTAGAAACTCGTAGAGTCCCAT
GTCTGCCGGCTTCCAGAGCCTGCACTCCTCCACCTTGGCTTGGCTTTGCTGGGGCTAGAG
GAGCTAGGATGCACAGCAGCTCTGTGACCCTTTGTTTGAGAGGAACAGGAAAACCACCCT
TCTCTCTGGCCCACTGTGTCCTCTTCCTGCCCTGCCATCCCCTTCTGTGAATGTTAGACCC
ATGGGAGCAGCTGGTCAGAGGGGACCCCGGCCTGGGGCCCCTAACCCTATGTAGCCTCA
GTCTTCCCATCAGGCTCTCAGCTCAGCCTGAGTGTTGAGGCCCCAGTGGCTGCTCTGGGG
GCCTCCTGAGTTTCTCATCTGTGCCCCTCCCTCCCTGGCCCAGGTGAAGGTGTGGTTCCA
GAACCGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGC
AGAAGAAGAAGGGCTCCCATCACATCAACCGGTGGCGCATTGCCACGAAGCAGGCCAATG
GGGAGGACATCGATGTCACCTCCAATGACTCGGATGTACACGGTCTGCAACCACAAACCC
ACGAGGGCAGAGTGCTGCTTGCTGCTGGCCAGGCCCCTGCGTGGGCCCAAGCTGGACTC
TGGCCACTCCCTGGCCAGGCTTTGGGGAGGCCTGGAGTCATGGCCCCACAGGGCTTGAA
GCCCGGGGCCGCCATTGACAGAGGGACAAGCAATGGGCTGGCTGAGGCCTGGGACCACT
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GGTGGGGTACTGGTGGAGGGGGTCAAGGGTAATTCATTAACTCCTCTCTTTTGTTGGGGG
ACCCTGGTCTCTACCTCCAGCTCCACAGCAGGAGAAACAGGCTAGACATAGGGAAGGGCC
ATCCTGTATCTTGAGGGAGGACAGGCCCAGGTCTTTCTTAACGTATTGAGAGGTGGGAATC
AGGCCCAGGTAGTTCAATGGG

VEGFA HDR template sequence

Left Homology Arm-Insertion/Replacement Sequence-Right Homology Arm
(Underlined are the inserted **XbaI** restriction site, i.e. TCTAGA)

AGGTTTGAATCATCACGCAGGCCCTGGCCTCCACCCGCCCCCACCAGCCCCCTGGCCTCA
GTTCCCTGGCAACATCTGGGGTTGGGGGGGCAGCAGGAACAAGGGCCTCTGTCTGCCCA
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CTCTTCCCTCCCAGTCACTGACTAACCCCGGAACCACACAGCTTCCCGTTCTCAGCTCCAC
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ACATGAAGCAACTCCAGTCCCAAATATGTAGCTGTTTGGGAGGTCAGAAATAGGGGGTCCA
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CCCCACTTGAAT

DYNLT1 mKate-T2A-EGFP HDR template

Left Homology Arm-mKate-T2A-EGFP-Right Homology Arm
(Underlined are the inserted **mKate/EGFP** fluorescent protein sequence, with the connecting non-underlined **T2A peptide** sequence)

TGCCGTAAATGCTGCTCTCTTCCCTCCCGCAGGGAGCTGCACTGTGCGATGGGAGAATAA
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CCACTTCAAGTGCACATCCGAGGGCGAAGGCAAGCCCTACGAGGGCACCCAGACCATGA
GAATCAAGGCGGTCGAGGGCGGCCCTCTCCCCTTCGCCTTCGACATCCTGGCTACCAGCT
TCATGTACGGCAGCAAAACCTTCATCAACCACACCCAGGGCATCCCCGACTTCTTTAAGCA
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GAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCA
GCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCA
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TCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTGACCAGCTGTCC
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AACTCTTTTCTCATTCTCTTTGTTTTGTGGCACTTTCACAATGTAGAGGAAAAAACCAAATGA
CCGCACTGTGATGTGAATGGCACCGAAGTCAGATGAGTATCCCTGTAGGTCACCTGCAGC
CTGCGTTGCCACTTGTCTT

HSP90AA1 mKate-T2A-EGFP HDR template

Left Homology Arm-mKate-T2A-EGFP-Right Homology Arm
(Underlined are the inserted **mKate/EGFP** fluorescent protein sequence, with the connecting non-underlined **T2A peptide** sequence)

TACTGTCTTGAAAGCAGATAGAAACCAAGAGTATTACCCTAATAGCTGGCTTTAAGAAATCT
TTGTAATATGAGGATTTTATTTTGGAAACAGGTATTGATGAAGATGACCCTACTGCTGATGA
TACCAGTGCTGCTGTAACTGAAGAAATGCCACCCCTTGAAGGAGATGACGACACATCACG
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Supplementary Figures

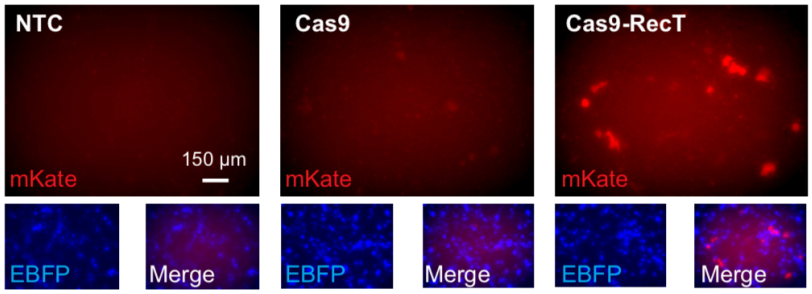

Fig. S1 Imaging of cells using fluorescent microscopy to confirm mKate knock-in

Imaging of the expression of knock-in mKate cassette in HEK293T cells under different conditions at *HSP90AA1* locus. EBFP fluorescence represents the transfection positive cells. (NTC, non-targeting control group. scale bar: 150 um).

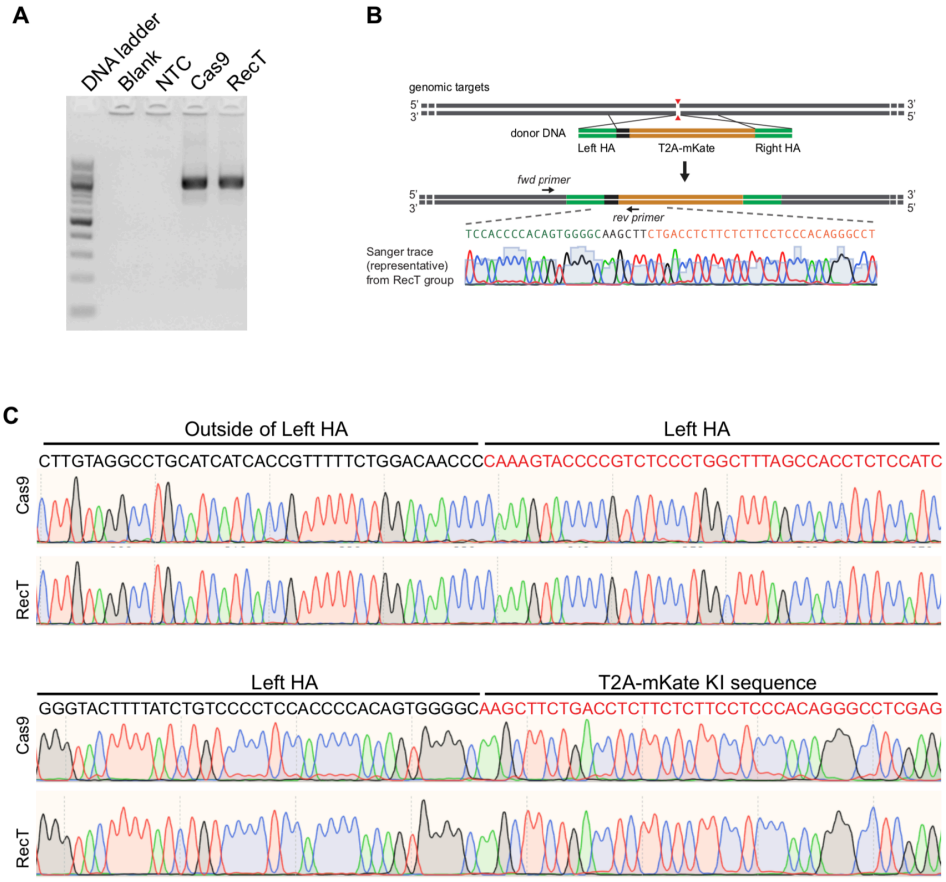

Fig. S2 Additional data and illustration for junction sequencing for Figure 1

(A) Full agarose gel image of junction PCR that validates mKate knock-in. (B) Detailed illustration of the junction PCR assay followed by Sanger sequencing (C) Sanger sequencing result of junction PCR product of mKate knock-in at *HSP90AA1* locus.

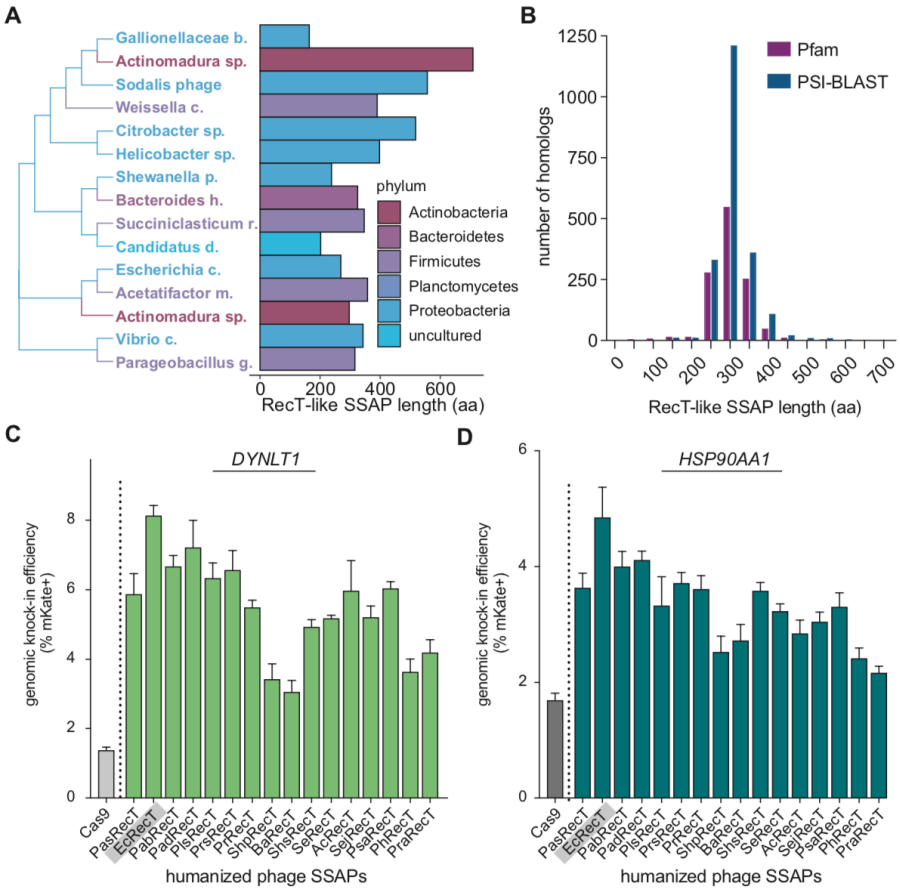

Fig. S3 Metagenomic screening of RecT homologs for mammalian gene-editing.

(A) Phylogenetic tree and lengths of representative RecT homologs spanning the diversity of metagenomic RecT-like SSAPs using PSI-BLAST mining, as detailed in the Methods section. (B) Histogram showing the length distribution of RecT-like SSAPs from two major sources for mining RecT homologs, Pfam database and PSI-BLAST results. (C-D) Measuring the gene-editing activities of RecT homologs using the 2A-mKate knock-in assay on two endogenous targets: (C) *DYNLT1* and (D) *HSP90AA1*. The groups corresponding to the top SSAP candidate, EcRecT (the original Rac prophage RecT), are highlighted.

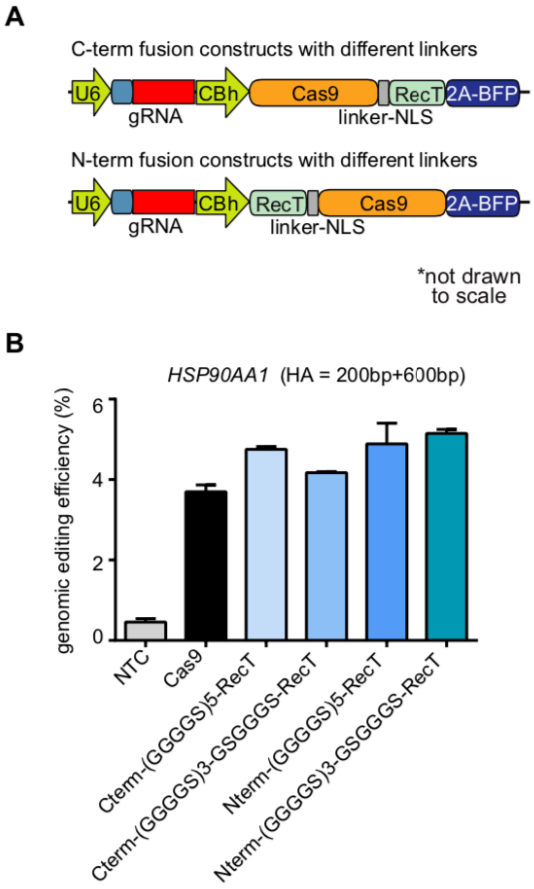

Fig. S4 Test direct fusion design of using SSAP for gene-editing in HEK293 cells.
(A) Schematic of direct fusion design. (B) Relative knock-in efficiency of constructs fused RecT to the N-term or C-term of Cas9 using different amino acid linkers at HSP90AA1 loci in HEK293T cells. Donor HA lengths are labeled on top.

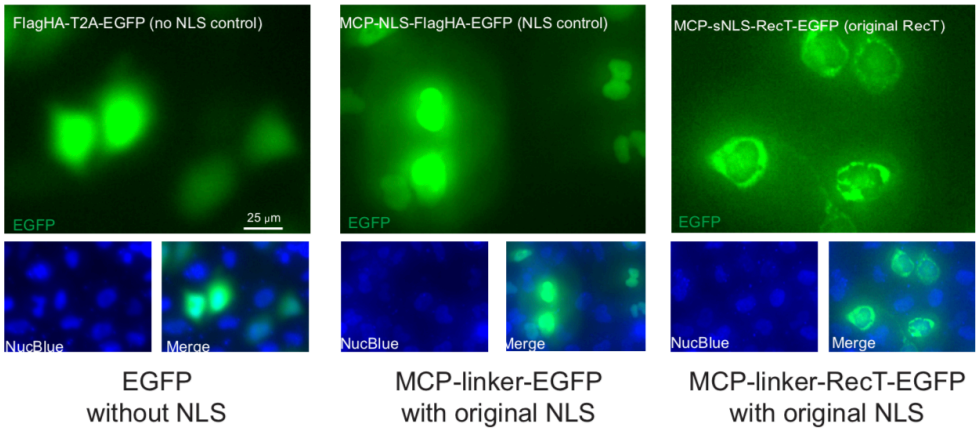

Fig. S5 RecT nuclear-targeting analysis using fusion GFP design

To image RecT protein and observe its nuclear-targeting using our originally-selected nuclear-localization signal design, we fused EGFP to the RecT original designed protein. After expression of these GFP-fusion RecT (along with control groups where no or validated NLS were used), we imaged the cells using fluorescent microscopy. RecT is shown here with the presence of fluorescent signals (green channel). Nuclei were stained with NucBlue Probes Reagent (Fisher Scientific) to visualize the cellular nucleus as reference (blue channel). Our original RecT design did not efficiently enter the nucleus, requiring further optimization.

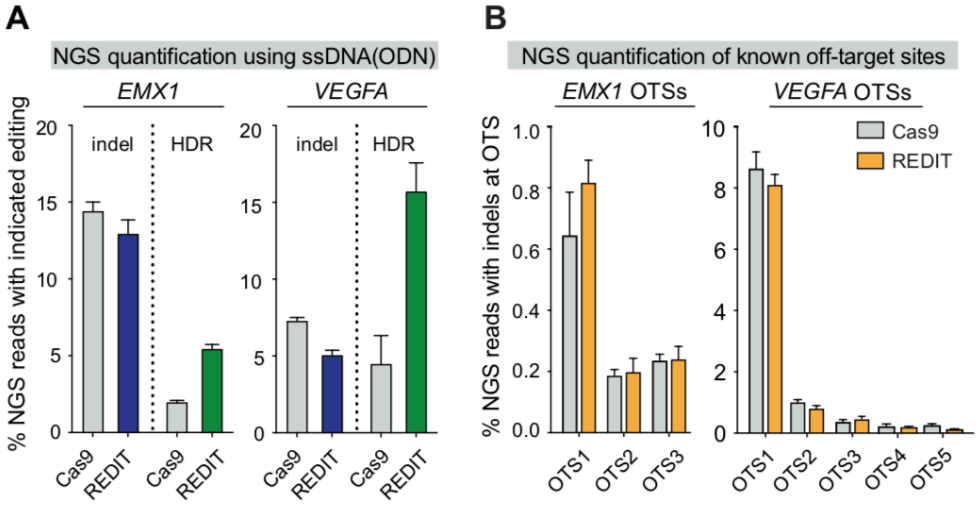

Fig. S6 Targeted NGS measurement of on/off-target editing events when using REDIT.

(A) NGS quantification of indel and HDR events at on-target sites at two genomic loci using ssODN donor in 293FT cells. (B) Targeted amplification and NGS quantification of known OTSs for the two guideRNAs being tested.

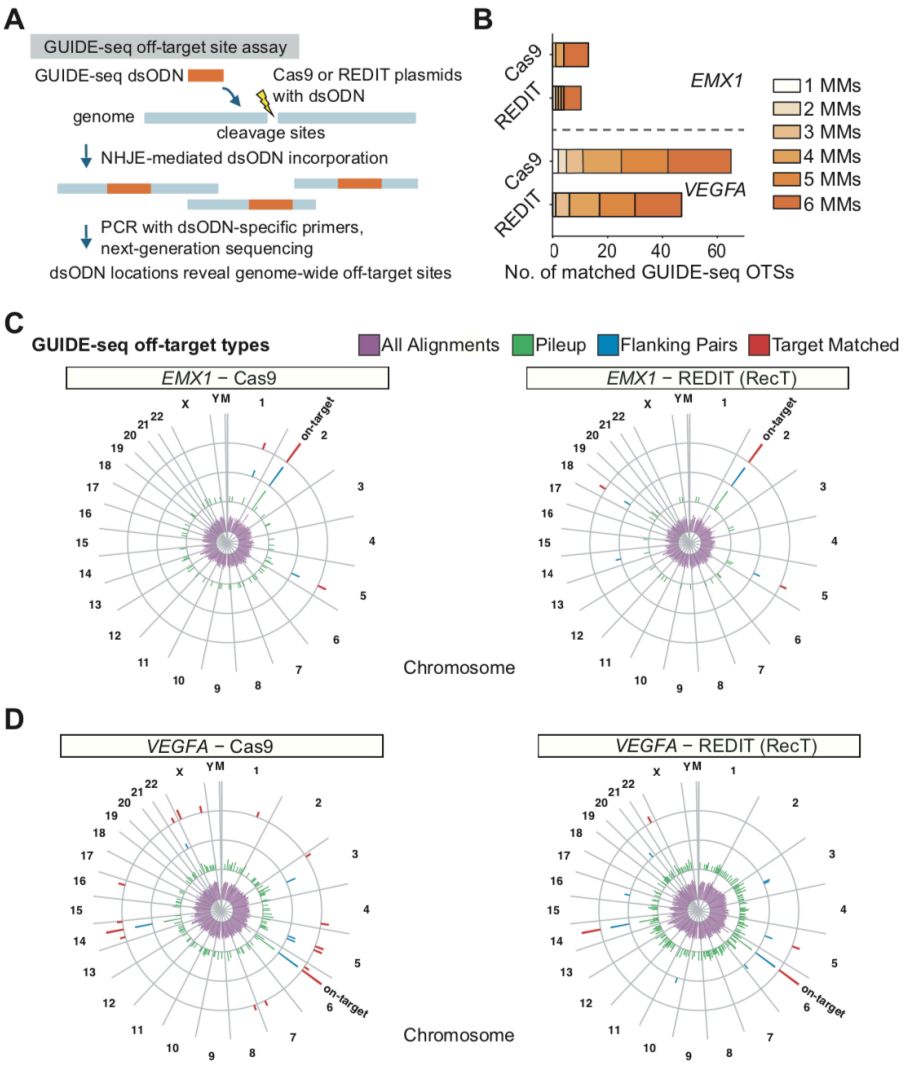

Fig. S7 GUIDE-seq off-target assay to characterize REDIT compared with Cas9.

(A) Schematic showing the steps for measuring genomic-wide off-target site (OTS) counts via GUIDE-seq in HEK293T cells (B) OTS sites summary from GUIDE-seq results using two gRNAs with REDIT and Cas9 reference. (C-D) OTS chromosomal distribution comparing Cas9 and REDIT groups, on-target and top OTSs are labeled for VEGF-targeting experiments. For all GUIDE-seq analysis, OTSs colored as: Pileup, alignments that have two or more reads overlapping with each other; flanking pairs, alignments that show up on opposite strands within 200bp upstream of each other; target matched, alignments that match to a treated target in the upstream sequence (up to 6 mismatches, including 1 mismatch in the PAM, are allowed in the target sequence).

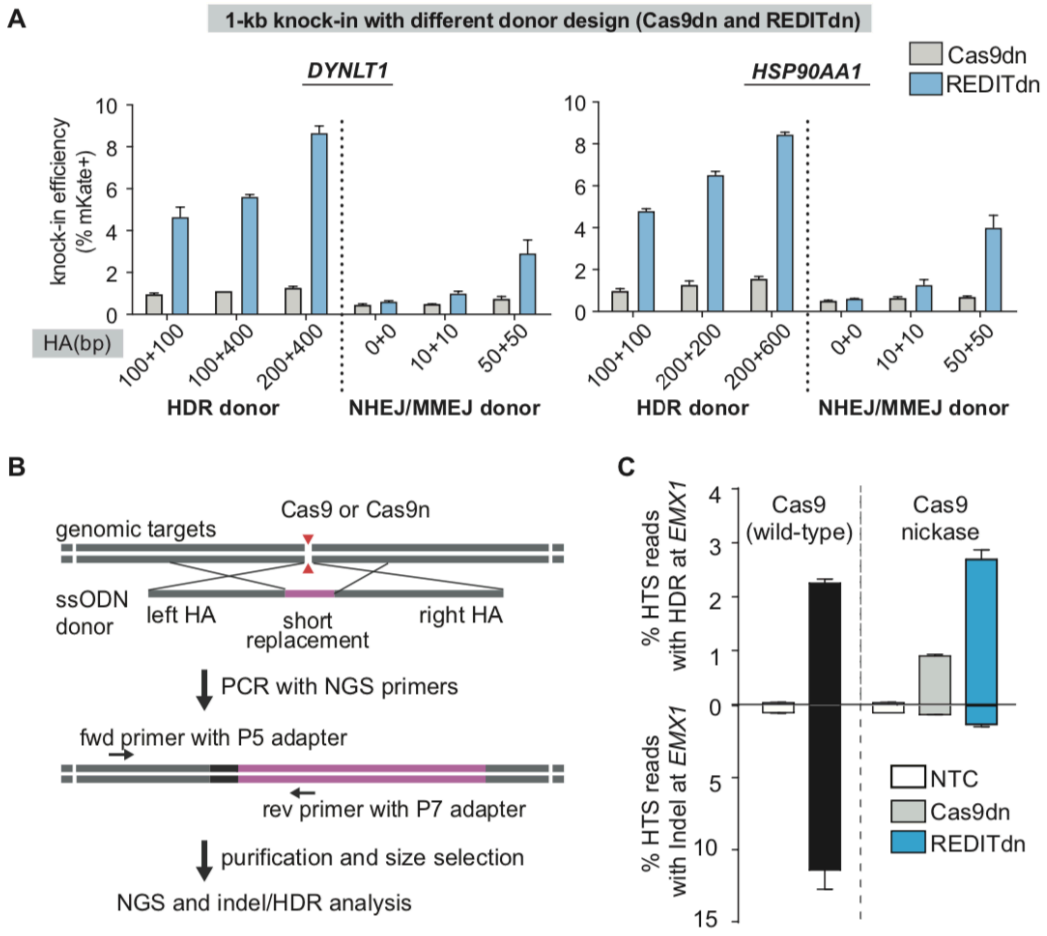

Fig. S8 Template design tests and NGS analysis of on-target editing for REDITdn

(A) HA length test comparing different template designs of HDR donors (longer HAs) or NHEJ/MMEJ donors (zero/shorter HAs) using REDITdn and Cas9dn references. (B) Schematic for NGS sample preparation. (C) NGS results for on-target HDR and indel for REDITdn showing the HDR reads and indel reads percentage at EMX1 loci.

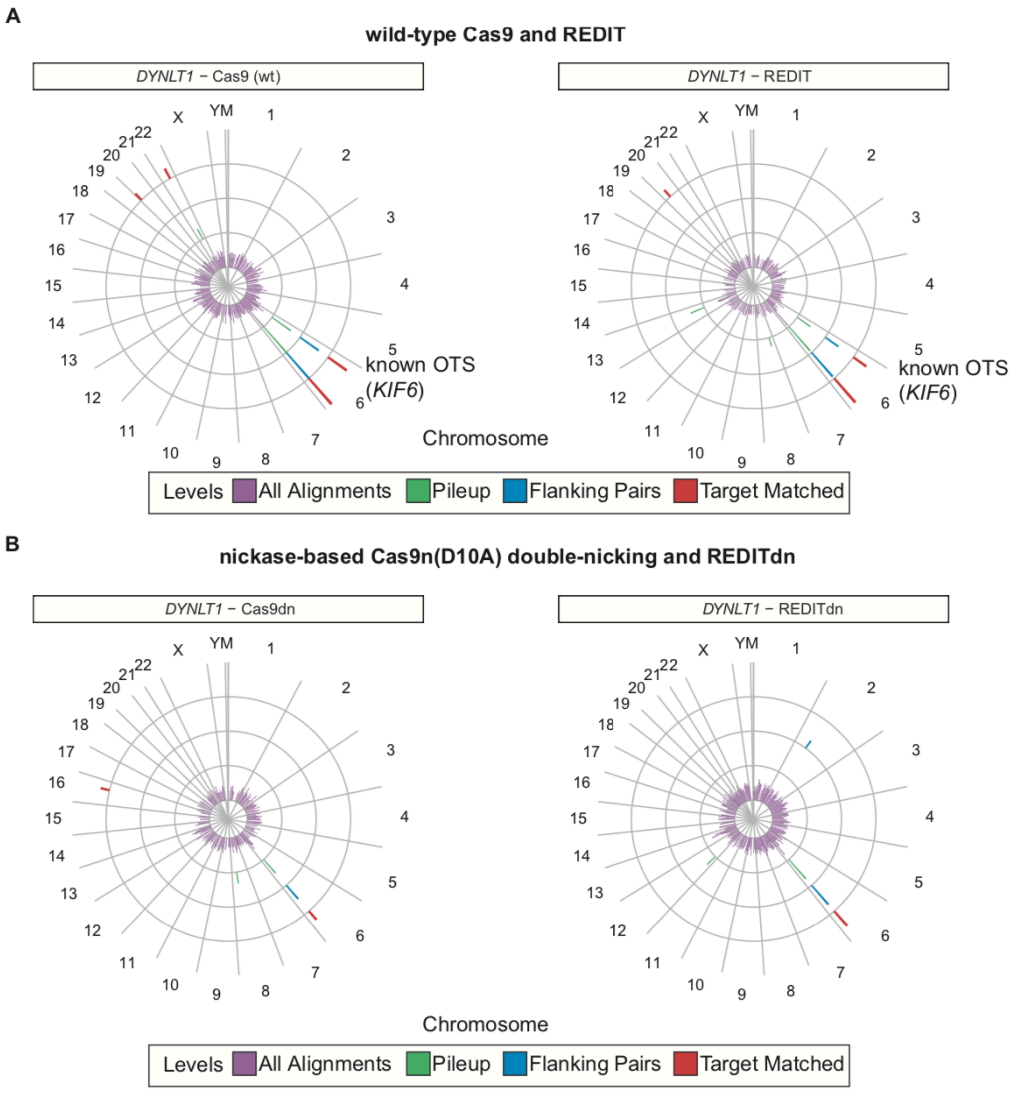

Fig. S9 GUIDE-seq analysis full data for REDITdn systems

Genomic distribution of detected off-target sites of REDIT (A) and REDITdn (B) versus Cas9 and Cas9dn benchmarks. Different off-target sites (OTSs) were colored as: Pileup, alignments that have two or more reads overlapping with each other; flanking pairs, alignments that show up on opposite strands within 200bp upstream of each other; target matched, alignments that match to a treated target in the upstream sequence (up to 6 mismatches, including 1 mismatch in the PAM, are allowed in the target sequence).

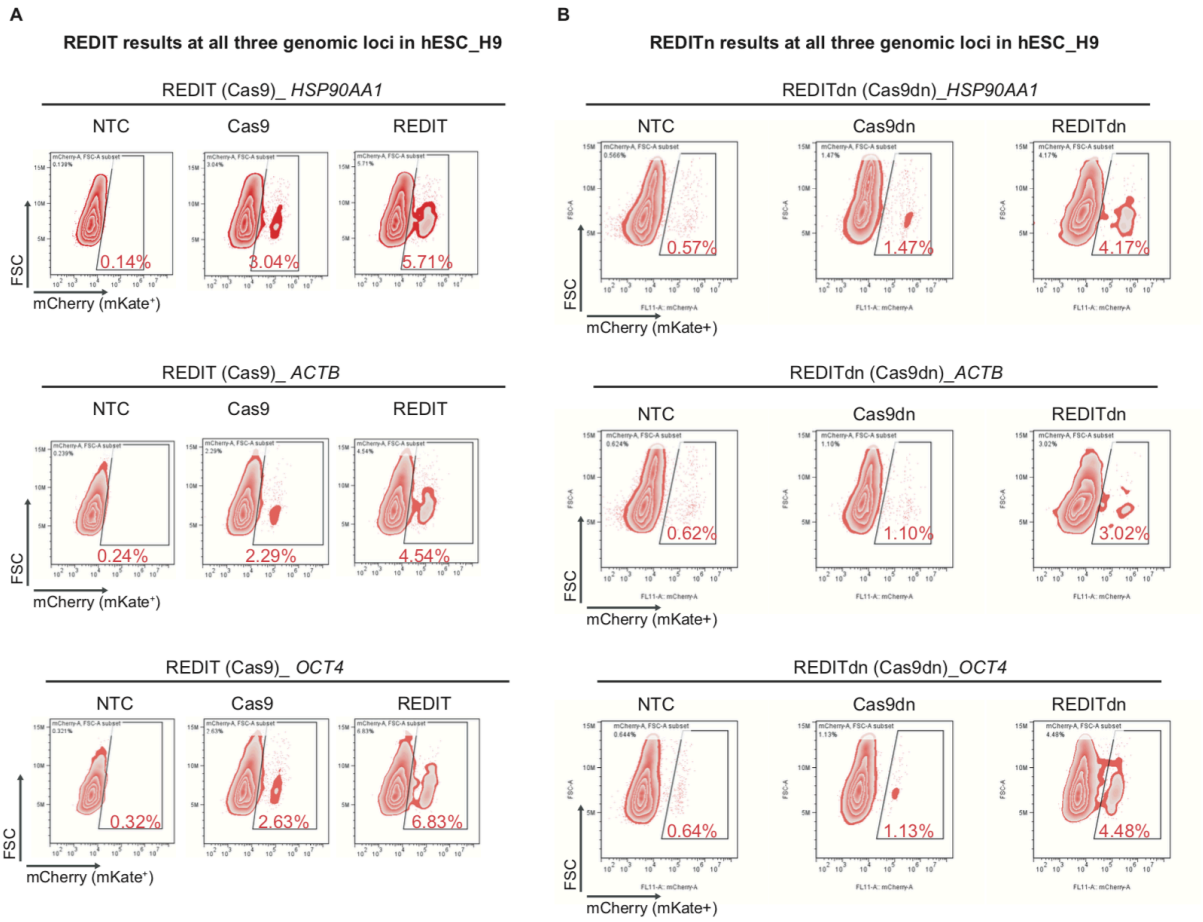

Fig. S10 Flow cytometry analysis of mKate knock-in experiments in human embryonic stem cells (hESC, H9)

(A-B) Original flow cytometry plots of mKate knock-in events at all three genomic loci: *HSP90AA1*, *ACTB*, *OCT4* (*POU5F1*), showing the stimulation of HDR efficiencies when using REDIT and REDITdn versus Cas9 and Cas9dn, respectively, along with negative controls.

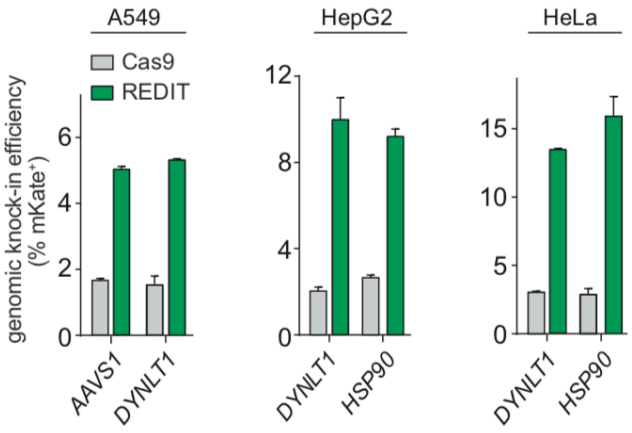

Fig. S11 Validation of the REDIT gene-editing method across different cell types

Measuring knock-in efficiencies using REDIT in A549, HepG2, HeLa, and compared with Cas9 reference using the same setup (donor DNA contains ~200bp of HA each side).

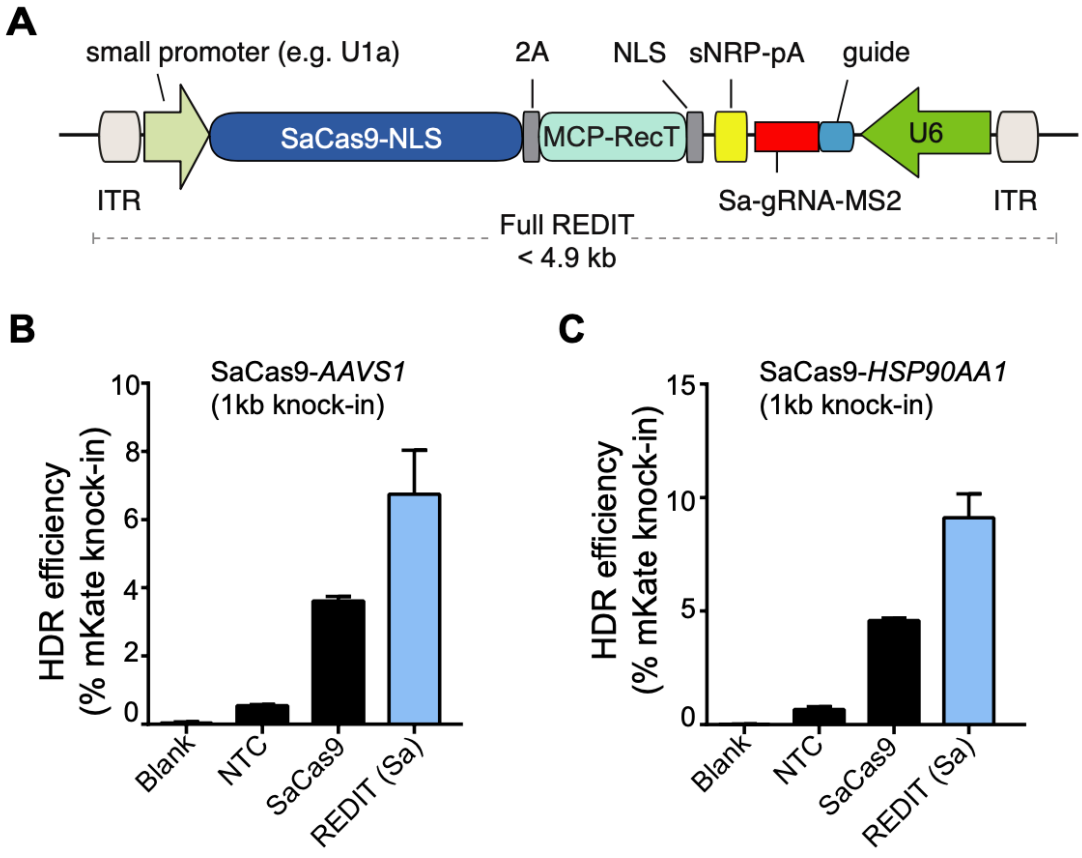

Fig. S12 REDIT (RecT) tool compatibility with the compact SaCas9 system

(A) Diagram of saCas9 express vector that has a small size that could potentially fit into a single AAV vector. Relative mKate knock-in efficiency at (B) *AAVS1* and (C) *HSP90AA1* locus using RecT protein in SaCas9 system. NTC, non-targeting negative control.

Table S1. Sequence for gRNAs

Annotations of the guideRNA names are: guides starting with sp indicate SpCas9 guide RNA targets, and guides starting with **nsp** indicate SpCas9 nickase guide RNA targets.

guideRNA Name	Genomic target	Guide sequence
sp-EMX1	*EMX1*	GTCACCTCCAATGACTAGGG
sp-VEGFA	*VEGFA*	GGTGAGTGAGTGTGTGCGTG
sp-DYNLT1	*DYNLT1*	AAGGCCATAGGCTGGACTGC
sp-HSP90AA1	*HSP90AA1*	GTAGACTAATCTCTGGCTGA
sp-OCT4	*OCT4*	TCTCCCATGCATTCAAACTG
sp-AAVS1	*AAVS1*	ACCCCACAGTGGGGCCACTA
sp-ACTB	*ACTB*	CCACCGCAAATGCTTCTAGG
nsp-DYNLT1-guide1	*DYNLT1*	AAGGCCATAGGCTGGACTGC
nsp-DYNLT1-guide2	*DYNLT1*	GGCACTGACGATGCAGTACA
nsp-HSP90AA1-guide1	*HSP90AA1*	GTAGACTAATCTCTGGCTGA

nsp-HSP90AA1-guide2	*HSP90AA1*	TCGTCATCTCCTTCAAGGGG
nsp-OCT4-guide1	*OCT4*	ATGCATGGGAGAGCCCAGAG
nsp-OCT4-guide2	*OCT4*	GCCTGCCCTTCTAGGAATGG
nsp-ACTB-guide1	*ACTB*	CCACCGCAAATGCTTCTAGG
nsp-ACTB-guide2	*ACTB*	GCTTGCTGATCCACATCTGC

Table S2. Primer Sequences.
Sequences for primers used for DNA template generation, targeted sequencing, and NGS assays are listed below. All NGS adapter sequences are shown in red color.

Primer name	Usage	Genomic Target	Primer sequence
EMX1-PCR-F	PCR template	*EMX1*	CATTCTGCCTCTCTGTATGGAAAAGAGC
EMX1-PCR-R	PCR template	*EMX1*	CCCATTGAACTACCTGGGCCTGATTC
VEGFA-PCR-F	PCR template	*VEGFA*	AGGTTTGAATCATCACGCAGGC
VEGFA-PCR-R	PCR template	*VEGFA*	ATTCAAGTGGGGAATGGCAAGC
DYNLT1-PCR-100bp-F	PCR template	*DYNLT1*	TGCCGTAAATGCTGCTCTCT
DYNLT1-PCR-200bp-F	PCR template	*DYNLT1*	AGACTTGCCAAGGTTCTTTGTG
DYNLT1-PCR-400bp-F	PCR template	*DYNLT1*	AGTGACCTGTGTAATTATGCAGAAG

DYNLT1-PCR-100bp-R	PCR template	*DYNLT1*	TGAAAGTGCCACAAAACAAAGAGA
DYNLT1-PCR-200bp-R	PCR template	*DYNLT1*	AAGACAAGTGGCAACGCAG
DYNLT1-PCR-400bp-R	PCR template	*DYNLT1*	CGTTTATGATACTATGCAGACTATGAAGAAC
DYNLT1-PCR-50-F	PCR template	*DYNLT1*	GGAGAATAAGACCATGTACTGC
DYNLT1-PCR-50-R	PCR template	*DYNLT1*	GAGGATGAACTAGAGACAAAAGG
DYNLT1-PCR-25-F	PCR template	*DYNLT1*	TCAGTGCCTTCGGACTG
DYNLT1-PCR-25-R	PCR template	*DYNLT1*	AAAGGCCATAGGCaGGAC
DYNLT1-PCR-10-F	PCR template	*DYNLT1*	ACTGTCTATTGGAAGCGGA
DYNLT1-PCR-10-R	PCR template	*DYNLT1*	GGACAGCTGGTTAGGAATTAAG
mKate-PCR-0-F	PCR template	*DYNLT1*	GGAAGCGGAGCTACTAACTT

mKate-PCR-0-R	PCR template	*DYNLT1*	TTAGGAATTAAGTTTGTGCCCC
HSP90AA1-PCR-100bp-F	PCR template	*HSP90AA1*	ATGAAGATGACCCTACTGCTGAT
HSP90AA1-PCR-200bp-F	PCR template	*HSP90AA1*	TACTGTCTTGAAAGCAGATAGAAACC
HSP90AA1-PCR-600bp-F	PCR template	*HSP90AA1*	GCAGCAAAGAAACACCTGGA
HSP90AA1-PCR-100bp-R	PCR template	*HSP90AA1*	GTTGTCATGCCATACAGACTTTTT
HSP90AA1-PCR-200bp-R	PCR template	*HSP90AA1*	AGCATTACTAGCTCTGCTTTAGTG
HSP90AA1-PCR-600bp-R	PCR template	*HSP90AA1*	TCCACAAGACTGGGTCTGAG
HSP90AA1-PCR-50bp-F	PCR template	*HSP90AA1*	AAATGCCACCCCTTGAAGG
HSP90AA1-PCR-50bp-R	PCR template	*HSP90AA1*	ATCAGAGGAATTGTAGAGTACTGA
HSP90AA1-PCR-25bp-F	PCR template	*HSP90AA1*	ACACATCACGCATGGAAGA

HSP90AA1-PCR-25bp-R	PCR template	*HSP90AA1*	GGTAAGTCATCCCTCAGCC
HSP90AA1-PCR-10bp-F	PCR template	*HSP90AA1*	AGAAGTAGACGGAAGCGG
HSP90AA1-PCR-10bp-F	PCR template	*HSP90AA1*	AGCCACAGATTTAGGAATTAAGTTT
OCT4-PCR-F	PCR template	*OCT4*	GCGACTATGCACAACGAGAGG
OCT4-PCR-R	PCR template	*OCT4*	AAGTGTGTCTATCTACTGTGTCCCAG
AAVS1-PCR-F	PCR template	*AAVS1*	GATGCTCTTTCCGGAGCACT
AAVS1-PCR-R	PCR template	*AAVS1*	GCCAAGGACTCAAACCCAGAA
ACTB-PCR-F	PCR template	*ACTB*	TGTGGTGTGTGGGGAGCT
ACTB-PCR-R	PCR template	*ACTB*	TTACACGAAAGCAATGCTATCACCTC
DYNLT1 KI PCR-F	Junction PCR	*DYNLT1*	AGGAGGTCCCATCAGATGCT

HSP90AA1 KI PCR-F	Junction PCR	*HSP90AA1*	GGCTGGACAGCAAACATGGA
AAVS1 KI PCR-F	Junction PCR	*AAVS1*	GATGCTCTTTCCGGAGCACT
Junction PCR universial-R	Junction PCR	*mKate*	TTGCTGCCGTACATGAAGCTG
EMX1-NGS-F	NGS	*EMX1*	CCATCTCATCCCTGCGTGTCTCCAGAAGAA GGGCTCCCATCAC
EMX1-NGS-R	NGS	*EMX1*	CCTCTCTATGGGCAGTCGGTGATGAGCAGC AAGCAGCACTCTG
VEGFA-NGS-F	NGS	*VEGFA*	CCATCTCATCCCTGCGTGTCTCCCAGCGTCT TCGAGAGTGAGG
VEGFA-NGS-R	NGS	*VEGFA*	CCTCTCTATGGGCAGTCGGTGATGTTGGAA TCCTGGAGTGACCC
EMX-OT1-F	Off Target	*EMX1 OT-1*	CCATCTCATCCCTGCGTGTCTCCACAAAAGC TCCACATGCTAGGA
EMX-OT1-R	Off Target	*EMX1 OT-1*	CCTCTCTATGGGCAGTCGGTGATGGCTGAC TTTGGGCTCCTTCT
EMX-OT2-F	Off Target	*EMX1 OT-2*	CCATCTCATCCCTGCGTGTCTCCACACACTC CCCAGGATCTCA

EMX-OT2-R	Off Target	*EMX1 OT-2*	CCTCTCTATGGGCAGTCGGTGATGAATGTC AGCTGAAGCAGGCT
EMX-OT3-F	Off Target	*EMX1 OT-3*	CCATCTCATCCCTGCGTGTCTCCGGCTACC CTGACAACTGCTT
EMX-OT3-R	Off Target	*EMX1 OT-3*	CCTCTCTATGGGCAGTCGGTGATGAGGACA GACATGACAAGGCA
VEGFA-OT1-F	Off Target	*VEGFA OT-1*	CCATCTCATCCCTGCGTGTCTCCGCAGGCA AGCTGTCAAGGGT
VEGFA-OT1-R	Off Target	*VEGFA OT-1*	CCTCTCTATGGGCAGTCGGTGATGCCCTCA CACCCACACCCTCA
VEGFA-OT2-F	Off Target	*VEGFA OT-2*	CCATCTCATCCCTGCGTGTCTCCGGAGGGG TGTCATCGTTCTG
VEGFA-OT2-R	Off Target	*VEGFA OT-2*	CCTCTCTATGGGCAGTCGGTGATGCAAATT GCGCCATAGCTGGG
VEGFA-OT3-F	Off Target	*VEGFA OT-3*	CCATCTCATCCCTGCGTGTCTCCTGAGCGC TCTTCGTCTTTCC
VEGFA-OT3-R	Off Target	*VEGFA OT-3*	CCTCTCTATGGGCAGTCGGTGATGGCCAGG AACACAGGAATGCTA
Junction NGS-5’ common-R	Junction NGS	*mKate*	CCTCTCTATGGGCAGTCGGTGATGGTACAG CTTCATGTGCATGT

Junction NGS-3’ common-F	Junction NGS	*mKate*	CCATCTCATCCCTGCGTGTCTCCGAGGCCGACAAAGAGACA
DYNLT1-Junction NGS-5’-F	Junction NGS	*DYNLT1-mKate*	CCATCTCATCCCTGCGTGTCTCCGTAAATGCTGCTCTCTTCCC
DYNLT1-Junction NGS-3’-R	Junction NGS	*DYNLT1-mKate*	CCTCTCTATGGGCAGTCGGTGATGTTGTGAAAGTGCCACAAAACA
HSP90AA1-Junction NGS-5’-F	Junction NGS	*HSP90AA1-mKate*	CCATCTCATCCCTGCGTGTCTCCCTACTGCTGATGATACCAGTG
HSP90AA1-Junction NGS-3’-R	Junction NGS	*HSP90AA1-mKate*	CCTCTCTATGGGCAGTCGGTGATGGTTGTCATGCCATACAGACT
DYNLT1-TA-F	TA colony	*DYNLT1*	AGTGGACAGAATGACATTTGTG
DYNLT1-TA-R	TA colony	*DYNLT1*	CGCCTGGTCTGGTTGTATA
HSP90AA1-TA-F	TA colony	*HSP90AA1*	AGACACATGCTAACAGGATCTA
HSP90AA1-TA-R	TA colony	*HSP90AA1*	ATGCAGAATTGTCAACTACAGG

Table S3. Sequence for all SSAP tested in this study.

SSAP name	amino acid sequence
CspRecT	MNQIVKFTDDSGLAVQVTPDDVRRYICENATEKEVGLFLQLCQTQRLNPFVKD AYLVKYGGAPASMITSYQVFNRRACRDANYDGIKSGVVVLRDGDVVHKRGAA CYKKAGEELIGGWAEVRFKDGRETAYAEVALDDYSTGKSNWAKMPGVMIEK CAKAAAWRLAFPDTFQGMYAAEEMDQAQQPEQVRAQAEQPVDLQPIRELFK PYCEHFGITPAEGMTAVCGAVGAEGMHSMTEQQARRARAWMEEEMAAPAV EAEYEVVDEGEVF
PapRecT	MGTALTPLLTKFATRYEMGTTPEEVANTLKQTCFKGQVNDSQMVALLIVADQY KLNPFTKELYAFPDKNNGIVPVVGVDGWARIINENPQFDGMEFSMDQQGTEC TCKIYRKDRSHAISATEYMAECKRNTQPWQSHPRRMLRHKAMIQCARLAFGF AGIYDQDEAERIVERDVTPAEQYEDVSEAICLIKDSPTMEDLQAAFSNAWKAY KTKGARDQLTAAKDQRKKELLDAPIDVEFEETGDDRAA
EcRecT	MTKQPPIAKADLQKTQGNRAPAAIKNNDVISFINQPSMKEQLAAALPRHMTAE RMIRIATTEIRKVPALGNCDTMSFVSAIVQCSQLGLEPGSALGHAYLLPFGNKN EKSGKKNVQLIIGYRGMIDLARRSGQIASLSARVVREGDEFNFEFGLDEKLIHR PGENEDAPVTHVYAVARLKDGGTQFEVMTRKQIELVRSQSKAGNNGPWVTH WEEMAKKTAIRRLFKYLPVSIEIQRAVSMDEKEPLTIDPADSSVLTGEYSVIDNS EE
PasRecT	MSNQPPIASADLQKANTGKQVANKTPEQTLVGFMNQPAMKSQLAAALPRHM TADRMIRIVTTEIRKTPALATCDQSSFIGAVVQCSQLGLEPGSALGHAYLLPFG NGRSKSGQSNVQLIIGYRGMIDLARRSGQIVSLSARVVRADDEFSFEYGLDEN LIHRPGENEDAPITHVYAVARLKDGGTQFEVMTVKQIEKVKAQSKASSNGPWV THWEEMAKKTVIRRLFKYLPVSIEMQKAVILDEKAESDVDQDNASVLSAEYSVL DGSSEE
SeRecT	MTKQPPIAKADLQKTQENRAPAAIKNNDVISFINQPSMKEQLAAALPRHMTAE RMIRIATTEIRKVPALGNCDTMSFVSAIVQCSQLGLEPGSALGHAYLLPFGNKN EKSGKKNVQLIIGYRGMIDLARRSGQIASLSARVVREGDEFNFEFGLDEKLIHR PGENEDAPVTHVYAVARLKDGGTQFEVMTRRQIELVRSQSKAGNNGPWVTH WEEMAKKTAIRRLFKYLPVSIEIQRAVSMDEKEPLTIDPADSSVLTGEYSVIDNS EE

AcRecT	MTKQPPIAKADLQKTQGNRAPAAVNDKDVLCVINSPAMKAQLAAALPRHMTA ERMIRIATTEIRKVPELRNCDSTSFIGAIVQCSQLGLEPGSALGHAYLLPFGNGK AKNGKKNVQLIIGYRGMIDLARRSGQIISLSARVVRECDEFSYELGLDEKLVHR PGENEDAPITHVYAVAKLKDGGVQFEVMTKKQVEKVRDTHSKAAKNAASKGA SSIWDEHFEDMAKKTVIRKLFKYLPVSIEIQRAVSMDGKEVETINPDDISVIAGE YSVIDNPEE
SejRecT	MNAPQKQNTRAAVKKISPQEFAEQFAAIIPQVKSVLPAHVTFEKFERVVRLAVR KNPDLLTCSPASLFMACIQAASDGLLPDGREGAIVSRWSSKKSCNEASWMPM VAGLMKLARNSGDIASISSQVVFEGEHFRVVLGDEERIEHERDLGKTGGKIVA AYAVARLKDGSDPIREIMSWGQIEKIRNTNKKWEWGPWKAWEDEMARKTVIR RLAKRLPMSTDKEGERLRSAIERIDSLVDISANVDAPQIAADDEFAAAAHGVEP QQIAAPDLIGRLAQMQSLEQVQDIEPQVSHAIQEADKRGDSDTANALDAALQS ALSRTSTAKEEVPA
PsaRecT	MPKQPPIAKADLQKTQGARTPTAVKNNNDVISFINQPSMKEQLAAALPRHMTA ERMIRIATTEIRKVPALGDCDTMSFVSAIVQCSQLGLEPGGALGHAYLLPFGNR NEKSGKKNVQLIIGYRGMIDLARRSGQIASLSARVVREGDDFSFEFGLEEKLVH RPGENEDAPVTHVYAVARLKDGGTQFEVMTRKQIELVRAQSKAGNNGPWVT HWEEMAKKTAIRRLFKYLPVSIEIQRAVSMDEKETLTIDPADASVITGEYSVVEN AGVEENVTA
PhRecT	MGHLVSKTEQDYIKQHYAKGATDQEFEHFIGVCRARGLNPAANQIYFVKYRSK DGPAKPAFILSIDSLRLIAHRTGDYAGCSEPIFTDGGKACTVTVRRNLKSGETG NFSGMAFYDEQVQQKNGRPTSFWQSKPRTMLEKCAEAKALRKAFPQDLGQF YIREEMPPQYDEPIQVHKPKALEEPRFSKSDLSRRKGLNRKLSALGVDPSRFD EVATFLDGTPDRELGQKLKLWLKEAGYGVNQ
PraRecT	MNTDMIAMPPSPAISMLDTSKLDVMVRAAELMSQAVVMVPDHFKGKPADCLA VVMQADQWGMNPFTVAQKTHLVSGTLGYESQLVNAVISSSKAIKGRFHYEWS DGWERLAGKVQYVKESRQRKGQQGSYQVTVAKPTWKPEDEQGLWVRCGA VLAGEKDITWGPKLYLASVLVRNSELWTTKPYQQAAYTALKDWSRLYTPAVM QGSMTGKSWSLTGRLISPR
PabRecT	MSNQPPIASADLQKTQQSKQVANKTPEQTLVGFMNQPAMKSQLAAALPRHM TADRMIRIVTTEIRKTPQLAQCDQSSFIGAVVQCSQLGLEPGSALGHAYLLPFG NGRSKSGQSNVQLIIGYRGMIDLARRSGQIVSLSARVVRADDEFSFEYGLDEN

	LVHRPGENEDAPITHVYAVARLKDGGTQFEVMTVKQVEKVKAQSKASSNGP WVTHWEEMAKKTVIRRLFKYLPVSIEMQKAVVLDEKAESDVDQDNASVLSAE YSVLESGDEATN
PadRecT	MSNQPPLATADLQKTQQSNQVAKTPEQTLVGFMNQPAMKSQLAAALPRHMT ADRMIRIVTTEIRKTPALAQCDQSSFIGAVVQCSQLGLEPGSALGHAYLLPFGN GRSKSGQSNVQLIIGYRGMIDLARRSGQIVSLSARVVRADDEFSFEYGLDENLI HRPGDNESAPITHVYAVARLKDGGTQFEVMTAKQVEKVKAQSKASSNGPWV THWEEMAKKTVIRRLFKYLPVSIEMQKAVVLDEKAESDVDQDNASVLSAEYSV LESGTGE
PlsRecT	MSNQPPIASADLQKTQQSKQVANKTPEQTLVGFMNQPAMKSQLAAALPRHM TADRMIRIVTTEIRKTPALATCDQSSFIGAVVQCSQLGLEPGSALGHAYLLPFG NGRSKSGQSNVQLIIGYRGMIDLARRSGQIVSLSARVVRADDEFSFEYGLDEN LIHRPGDNEDAPITHVYAVARLKDGGTQFEVMTAKQVEKVKAQSKASSNGPW VTHWEEMAKKTVIRRLFKYLPVSIEMQKAVVLDEKAESDVDQDNASVLSAEYS VLEGDGGE
PrsRecT	MSNPPLAQADLQKTQGTEVKEKTKDQMLVELINKPSMKAQLAAALPRHMTPD RMIRIVTTEIRKTPALATCDMQSFVGAVVQCSQLGLEPGNALGHAYLLPFGNG KSKSGQSNVQLIIGYRGMIDLARRSGQIVSISARTVRQGDNFHFEYGLNENLTH VPGENEDSPITHVYAVARLKDGGVQFEVMTYNQIEKVRASSKAGQNGPWVS HWEEMAKKTVIRRLFKYLPVSIEMQKAVILDEKAEANIDQENATIFEGEYEEVG TDGK
PrRecT	MSNPPLAQSDLQKTQGTEVKVKTKDQQLIQFINQPSMKAQLAAALPRHMTPD RMIRIVTTEIRKTPALATCDMQSFVGAVVQCSQLGLEPGNALGHAYLLPFGNG KAKSGQSNVQLIIGYRGMIDLARRSNQIISISARTVRQGDNFHFEYGLNEDLTH TPSENEDSPITHVYAVARLKDGGVQFEVMTYNQVEKVRASSKAGQNGPWVS HWEEMAKKTVIRRLFKYLPVSIEMQKAVVLDEKAEANVDQENATIFEGEYEEV GTDGN
ShpRecT	MKAQLAAALPKHITSDRMIRIVSTEIRKTPSLANCDIQSFIGAVVQCSQLGLEPG NALGHAYLLPFGNGKSDNGKSNVQLIIGYRGMIDLARRSGQIISISARTVRQGD NFHFEYGLNENLTHIPEGNEDSPITHVYAVARLKDEGVQFEVMTYNQIEKVRD SSKAGKNGPWVTHWEEMAKKTVIRRLFKYLPVSIEMQKAVILDEKAEANIEQD HSAIFEAEFEEVDSNGN

BaRecT	MQTAQVKLSVPHQQVYQDNFNYLSSQVVGHLVDLNEEIGYLNQIVFNSLSTAS PLDVAAPWSVYGLLLNVCRLGLSLNPEKKLAYVMPSWSETGEIIMKLYPGYRG EIAIASNFNVIKNANAVLVYENDHFRIQAATGEIEHFVTSLSIDPRVRGACSGGY CRSVLMDNTIQISYLSIEEMNAIAQNQIEANMGNTPWNSIWRTEMNRVALYRR AAKDWRQLIKATPEIQSALSDTEY
ShsRecT	MSKQLTTVNTQAVVGTFSQAELDTLKQTIAKGTTNEQFALFVQTCANSRLNPF LNHIHCIVYNGKEGATMSLQIAVEGILYLARKTDGYKGIECQLIHENDEFKFDAK SKEVDHQIGFPRGNVIGGYAIAKREGFDDVVVLMESNEVDHMLKGRNGHMW RDWFNDMFKKHIMKRAAKLQYGIEIAEDETVSSGPSVDNIPEYKPQPRKDITP NQDVIDAPPQQPKQDDEAAKLKAARSEVSKKFKKLGIVKEDQTEYVEKHVPGF KGTLSDFIGLSQLLDLNIEAQEAQSADGDLLD