Science Advances
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Supplementary Materials for

Improving the efficiency of precise genome editing with site-specific Cas9-oligonucleotide conjugates

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Published 8 April 2020, *Sci. Adv.* **6**, eaaz0051 (2020)
DOI: 10.1126/sciadv.aaz0051

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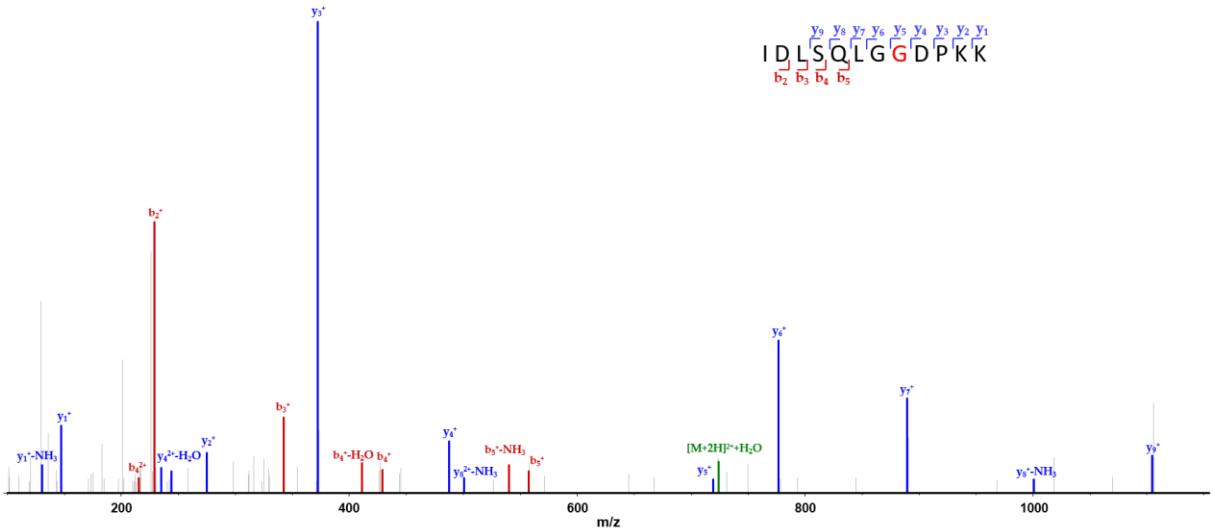

Fig. S1. Peptide mass spec of G1367-AeF.

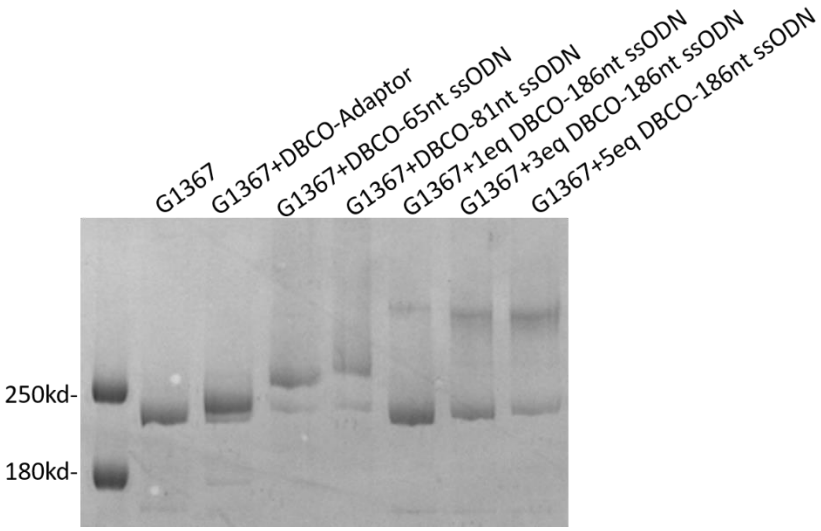

Fig. S2. SDS-PAGE analysis the G1367 conjugate with different length DBCO modified ssODN at 1:1 stoichiometry. DBCO-adaptor(25nt), DBCO-ssODN 65nt/81nt and DBCO-186nt were mixed with pre-assembled G1367AeF RNP (500ng) at the equivalent 3 hours at 4 degree and analysis by SDS-PAGE.

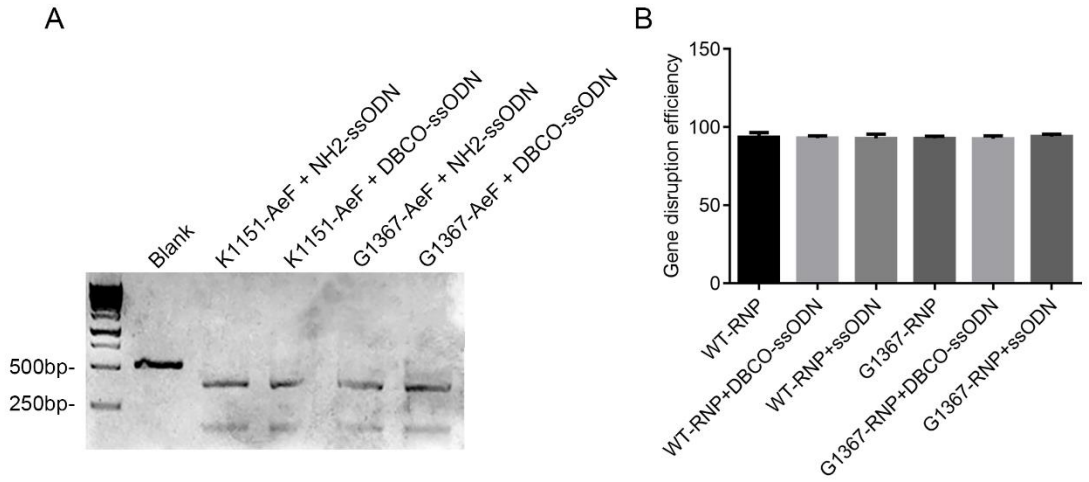

Fig. S3. Gene disruption activity of Cas9-ssODN conjugate *in vitro* and *in cell*. (a) Different Cas9 mutants were assembled into RNPs and incubated with DBCO or NH_2 modified ssODN. The resulting complex were measured for cleavage activity in vitro, and the cleavage products were measured on a 1% agarose. (b) Cas9 G1367-AeF mutant and wt Cas9 were assembled into RNP with DBCO or NH2 modified ssODN, the complex was delivered in HEK293 EGFP Teton cell through electroporation and analyzed by Flow cytometry 48h post-transduction.

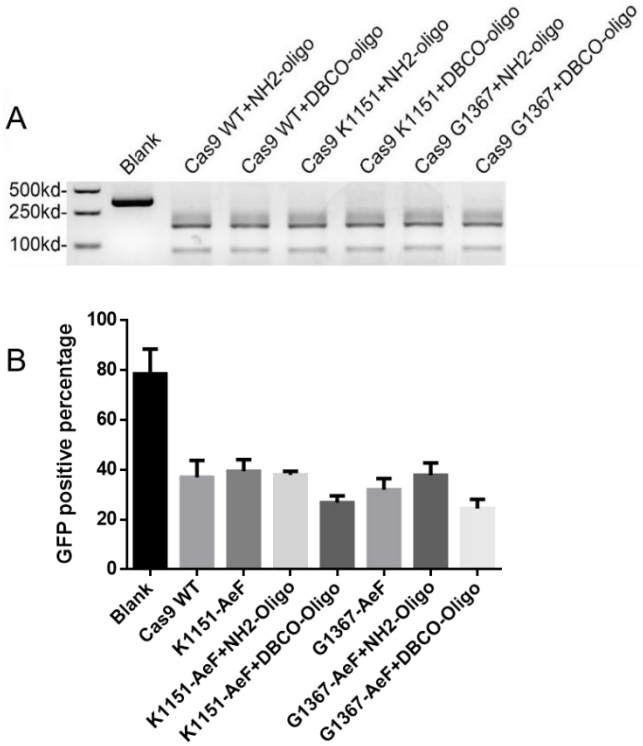

Fig. S4. Cleavage activity of Cas9 mutants reacted with modified oligos *in vitro* and *in cell*. (A) RNPs were pre-incubated with NH2 or DBCO modified oligo DNA, forming RNP-oligo DNA complex and analyzed for cleavage activity on agarose gel. (B) RNP with adaptor conjugated at different sites were transfected into HEK293-Teton-EGFP by RNAiMAX and

analyzed by flow cytometry 2 days post-transduction. Error bars represent the standard deviation of triplicates.

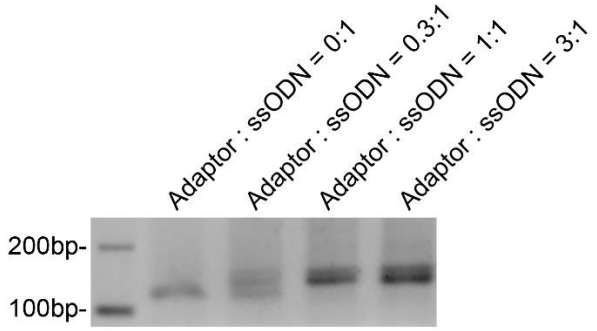

Fig. S5. Oligonucleotide-adaptor paired with ssODN. Oligonucleotide-adaptor was mixed with ssODN at different molar ratio in vitro, and analyzed on a 2% agarose gel.

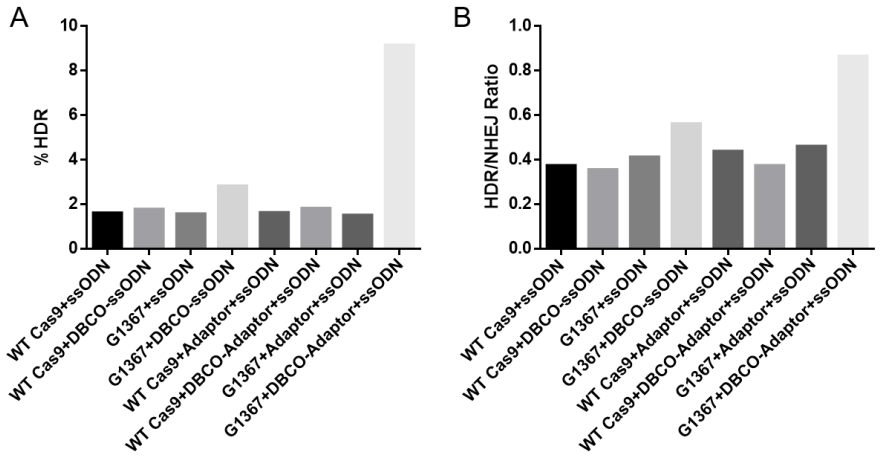

Fig. S6. Deep sequencing confirmation of HiBiT insertion and HDR enhancement.
(A)Absolute HDR efficiency was quantified by deep sequencing. (B) HDR/NHEJ ratio was calculated based on data from deep sequencing.

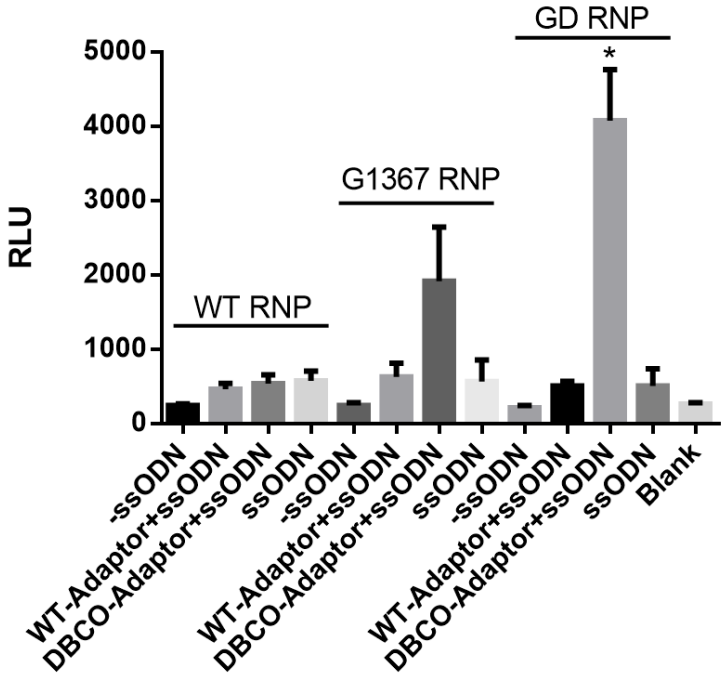

Fig. S7. The HDR efficiency of RNP-dual ssODN complexes. RNP with adaptor conjugated at different sites were transfected into HEK293 by RNAiMAX and analyzed by Hibit assay 2 days post-transduction. Error bars represent the standard deviation of triplicates. Significance was calculated by means of a 2-tailed Student's *t*-test; * $P < 0.05$.

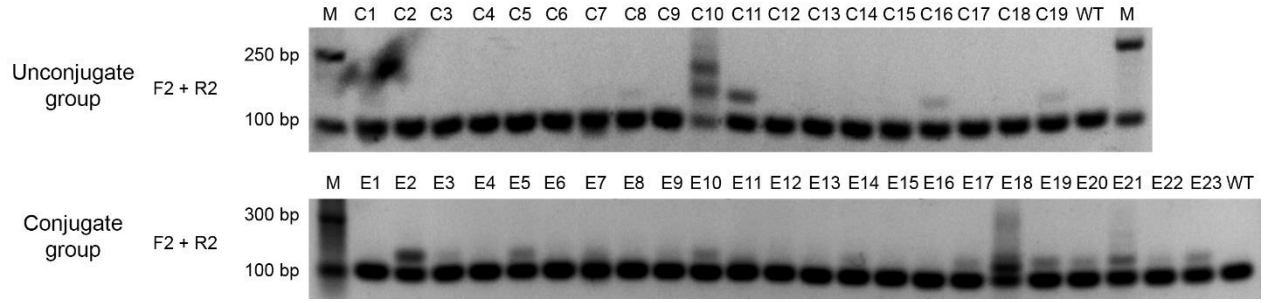

Fig. S8. Genotyping PCR of zygotes after RNP microinjection. A 141 bp fragment containing a 42 bp V5 tag can be specifically detected in edited zygotes using primer F2 and R2, and 99 bp band was amplified both in edited and unedited zygotes.

Table S1. Expression yield of Cas9 and its chemical mutants.

Mutation site	Expression yield	Mutation site	Expression yield
WT	3mg/L	K929	0.2mg/L
K3	Degradation seriously	L1004	2.7mg/L
D39	Inclusion body	K1151	2.8mg/L
H41	0.25mg/L	K1153	1.5mg/L
H754	0.17mg/L	G1361	0.8mg/L
L833	Low expression	G1367	3mg/L

Table S2. Primer and donor sequence used in the study

a. Primers used for mutagenesis PCR

Name	Sequence
K3TAG-FW	CCAAGAAGAAGAGGAAGGTGATGGATtAGAAATACTCAATAGGCTTAGATATCGGCAC
K3TAG-RV	GTGCCGATATCTAAGCCTATTGAGTATTTCTaATCCATCACCTTCCTCTTCTTCTTGG
D39TAG-FW	GTTCAAGGTTCTGGGAAATACATAGCGCCACAGTATCAAAAAAAATC
D39TAG-RV	GATTTTTTTTGATACTGTGGCGCTATGTATTTCCCAGAACCTTGAAC
H41TAG-FW	GTTCTGGGAAATACAGACCGCTAGAGTATCAAAAAAAATCTTATAGGGGCTC
H41TAG-RV	GAGCCCCTATAAGATTTTTTTTGATACTCTAGCGGTCTGTATTTCCCAGAAC
H754TAG-FW	GAATTGGTCAAAGTAATGGGGCGGTAGAAGCCAGAAAATATCGTTATTGAAATGGC
H754TAG-RV	GCCATTTCAATAACGATATTTTCTGGCTTCTACCGCCCCATTACTTTGACCAATTC
L833TAG-F	AGAATTAGATATTAATCGTTAGAGTGATTATGATGTCGATC
L833TAG-R	GATCGACATCATAATCACTCTAACGATTAATATCTAATTCT
K929TAG-F	GTTGAAACTCGCCAAATCACTTAGCATGTGGCACAAATTTTG
K929TAG-R	CAAAATTTGTGCCACATGCTAAGTGATTTGGCGAGTTTCAAC
L1004TAG-F	CTGCTTTGATTAAGAAATATCCAAAATAGGAATCGGAGTTTGTCTATGGTG
L1004TAG-R	CACCATAGACAAACTCCGATTCCTATTTTGGATATTTCTTAATCAAAGCAG
K1151TAG-F	CTAGTGGTTGCTAAGGTGGAATAGGGGAAATCGAAGAAGTTAAA
K1151TAG-R	TTTAACTTCTTCGATTTCCCCTATTCCACCTTAGCAACCACTAG
K1153TAG-FW	GTTGCTAAGGTGGAAAAAGGGTAGTCGAAGAAGTTAAAATCCGTT

K1153TAG-R	AACGGATTTTAACTTCTTCGACTACCCTTTTTCCACCTTAGCAAC
D1361TAG-F	GTCTTTATGAAACACGCATTTAGTTGAGTCAGCTAGGAGGTGA
D1361TAG-R	TCACCTCCTAGCTGACTCAACTAAATGCGTGTTTCATAAAGAC
G1367TAG-FW	CGCATTGATTTGAGTCAGCTAGGATAGGACCCCAAGAAGAAGAGGAAGGTG
G1367TAG-RV	CACCTTCCTCTTCTTCTTGGGGTCCTATCCTAGCTGACTCAAATCAATGCG

b. Primers used for V5 genotype verification

Name	Sequence
F1	ACATGGGCAAGCCCATCC
R1	GCGTTAATTTGGATGGGATTGGTGG
F2	GGCCATTAACGGCACACTG
R2	CCTCCCAATTCCCTTGTATCTC

c. Primers used for deep sequencing

Name	Sequence
CX-FW	GAATTTGGCTACAGCAACAGGG
CX-RV	GGTCTCTCTCTTCCTCTTGTGC

d. ssODN sequences and fragments used for ligation

Name	Sequence
GAPDH-186nt ssODN	TCTTCTAGGTATGACAACGAATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGGCCTCCAAGGAGGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCTAAGACCCCTGGACCACCAGCCCCAGCAAGAGCACAAGAGGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTGCCAC
Sox2-162nt ssODN	TACCAGAGCGGCCCGGTGCCCGGCACGGCCATTAACGGCACACTGCCCCTGTCGCACATGGGCAAGCCCATCCCCAACCCCCTGCTGGGCCTGGACAGCACCTGAGGGCTGGACTGCGAACTGGAGAAGGGGAGAGATTTTCAAAGAGATACAAGGGAATTG

e. Sequences of gRNA

Name	Sequence
EGFP	GGGCGAGGAGCTGTTCACCG
SOX2	TGCCCCTGTCGCACATGTGA