Supplementary information for:

Increasing Cas9-mediated homology-directed repair efficiency through covalent tethering of DNA repair template

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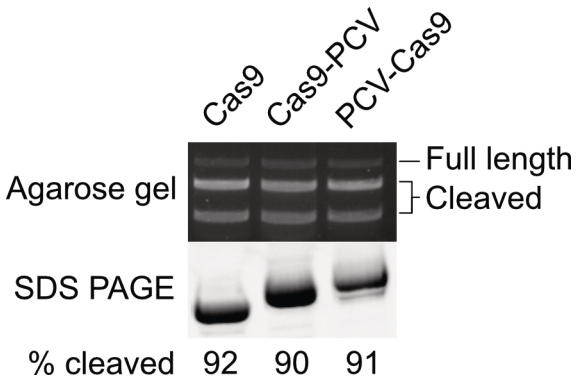
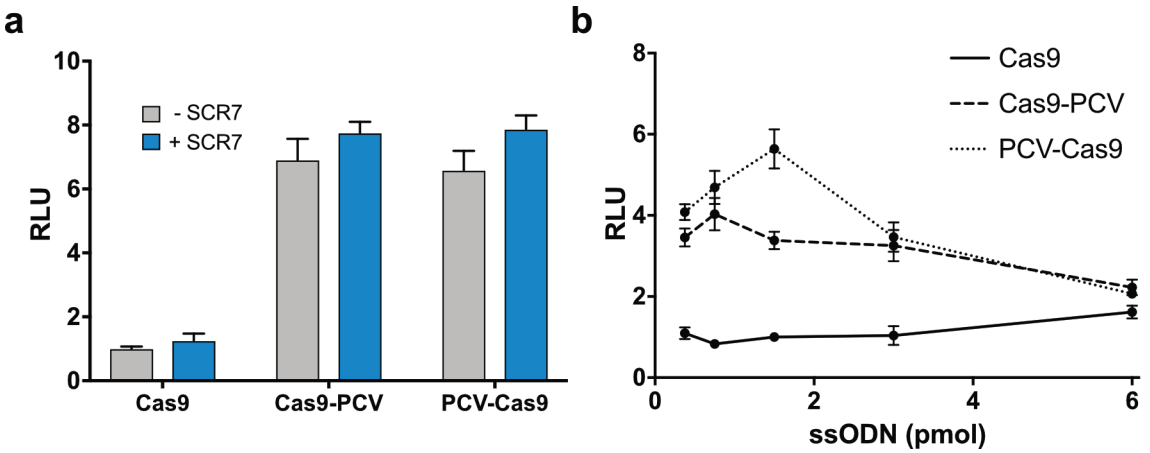
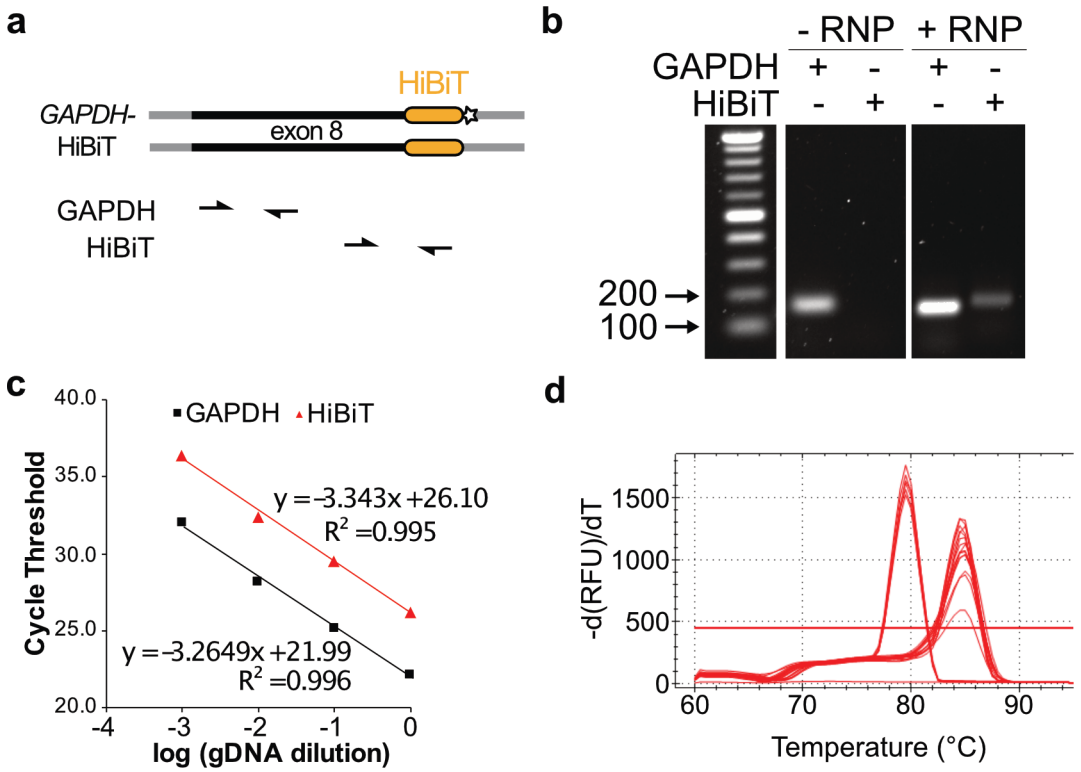


Supplementary Figure 1. Assessing Cas9 fusion *in vitro* cleavage activity. GFP-pcDNA3 DNA was linearized and incubated with RNP targeting GFP for 24 hours and electrophoresed on an agarose gel. The equivalent amount of RNP was also analyzed on SDS-PAGE. The percent of DNA cleaved was calculated using densitometry comparing the top band to the middle band.



Supplementary Figure 2. Further evidence for robustness of Cas9-PCV fusion. (**a**) RNP + ssODN containing PCV recognition sequence were transfected with and without the addition of SCR7. 24 hours post-transfection, cells were washed and replenished with fresh media, and luminescence was assayed another 24 hours later. Values normalized to Cas9 minus SCR7. (**b**) Titration of ssODN while keeping RNP concentration constant at 1.5 pmol. Values in relative luminescence units (RLU). Data are shown as mean +/- SD (n=3).



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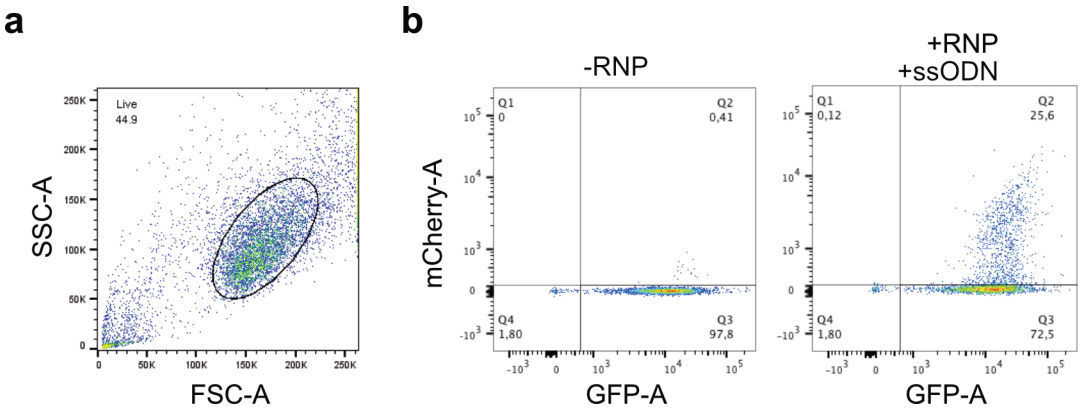
DNA source	Target	C_T	Average C_T
Cas9	GAPDH	21.89	
		21.93	21.94±0.06
		22.00	
	HiBiT	25.77	
		25.54	25.32±0.60
		24.64	
Cas9-PCV	GAPDH	22.17	
		22.00	22.07±0.09
		22.05	
	HiBiT	24.52	
		24.52	24.46±0.10
		24.34	
Change in HDR with Cas9-PCV: 1.98 fold*			

* Ratio calculated using Pfaffl Method where *GAPDH* is the reference

Supplementary Figure 3. Quantitative PCR data corroborates luminescence readout. (**a**) Diagram of PCR primer binding sites at the GAPDH locus. (**b**) End-point PCR using both primer pairs on unedited (-RNP) and edited (+RNP) cells. Expected amplicon sizes are 130 bp for GAPDH pair and 187 bp for HiBiT pair. (**c**) Serial dilution

of purified genomic DNA (gDNA) from a pool of edited cells using both primer pairs. Limit of detection is set at 40 cycles. Dynamic range represented by range between first and last values. Best fit line equation shown on graph with corresponding R^2 values. (**d**) Melt curve analysis comparing temperature to change in fluorescence. The first peak corresponds to GAPDH amplicon. (**e**) Calculated cycle threshold values. Relative change in HiBiT amplification is calculated using the Pfaffl method. Primer efficiencies used in calculation determined from (c) were 102% and 99% for GAPDH and HiBiT, respectively.



Supplementary Figure 4. Flow cytometry gating methodology. (**a**) Flow cytometry plot of gating used to select live cells based on the forward scatter (FSC) and side scatter (SSC). 10,000 events are measured. (**b**) Flow cytometry plots of gated live cells comparing GFP intensity to mCherry intensity in transfected (+RNP +ssODN) versus untransfected cells (-RNP). Gating is based on untransfected cells.

Supplementary Table 1. Sequences of primers, sgRNA, ssODNs, and proteins.

Primer	DNA sequence (5'-to-3')
GAPDH_F	CTCCCACCTTTCTCATCCAAG
GAPDH_R	ACATCACCCCTCTACCTCC
HiBiT_F	GAGACTGGCTCTTAAAAAGTGC
HiBiT_R	GCTAATCTTCTTGAACAGCCG
Cas9-PCV-Y96A_F	CGATCAGCAGAACAAAGAATTTTGTAGCAAAGAAGGCAAC
Cas9-PCV-Y96A_R	GTTGCCTTCTTTGCTACAAAATTCTTTGTTCTGCTGATCG
sgRNA target	**RNA sequence (5'-to-3')**
GAPDH	CCTCCAAGGAGTAAGACCCC
vinculin	TGAAGAGTTCCCTGAGCAGA
mCherry	TGGCCCCTCACCCTTCGCCT
GFP	CTCGTGACCACCCTGACCTA
ssODN	**DNA sequence (5'-to-3')**
PCV+ GAPDH	AAGTATTACCAGCCTCTTCTAGGTATGACAACGAATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGG CCTCCAAGGAGGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCTAAGACCCCTGGACCACCAGCCCCAGCAAGAG CACAAGAGGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTGCCAC
PCV- GAPDH	CTATTGTACTAATCTCTTCTAGGTATGACAACGAATTTGGCTACAGCAACAGGGTGGTGGACCTCATGGCCCACATGG CCTCCAAGGAGGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCTAAGACCCCTGGACCACCAGCCCCAGCAAGAG CACAAGAGGAAGAGAGAGACCCTCACTGCTGGGGAGTCCCTGCCAC
PCV vinculin	AAGTATTACCAGCCGCTCCTCCCAAACCACCTCTGCCTGAAGGTGAGGTCCCTCCACCTAGGCCTCCACCACCAGAG GAAAAGGATGAAGTGAGCGGCTGGCGGCTGTTCAAGAAGATTAGCGAGTTCCCTGAGCAGAAGGCCGGGGAGGTGA TTAACCAGCCAATGATGATGGCTGCCAGACAGCTCCATGATGAA
PCV mCherry	AAGTATTACCAGCCGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGC CCCTTACCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACC
Protein	**Amino acid sequence (N-to-C)**
Cas9-PCV	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQ EIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGH FLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNF KSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLK ALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNL PNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGR HKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSD YDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFI KRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKY PKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRK VLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITI MERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPED NEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRY TSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSGTRLPKKKRKVGGGSGSPSKKNGRSGPQPHKRWVFTLNNPSEDE RKKIRDLPISLFDYFIVGEEGNEEGRTPHLQGFANFVKKQTFNKVKWYLGARCHIEKAKGTDQQNKEYCSKEGNLLMECGA PRSQGQR*
PCV-Cas9	PSKKNGRSGPQPHKRWVFTLNNPSEDERKKIRDLPISLFDYFIVGEEGNEEGRTPHLQGFANFVKKQTFNKVKWYLGARC HIEKAKGTDQQNKEYCSKEGNLLMECGAPRSQGQRELAEAAAKEAAAKEAAAKEAAAKAAEAAAKEAAAKEAAAKEAAAK APKKKRKVGSSMDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRY TRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYL ALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGN LIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDN GSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQS FIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIE CFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYT GWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQT VKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRD MYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKA ERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHD AYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGE IVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKG KSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFL YLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAP AAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGGSGTRLPKKKRKVGGGSHHHHHH*