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Supplemental information

CRISPR-MiX: A pooled single-stranded donor strategy to enhance HDR efficiency in human iPSCs

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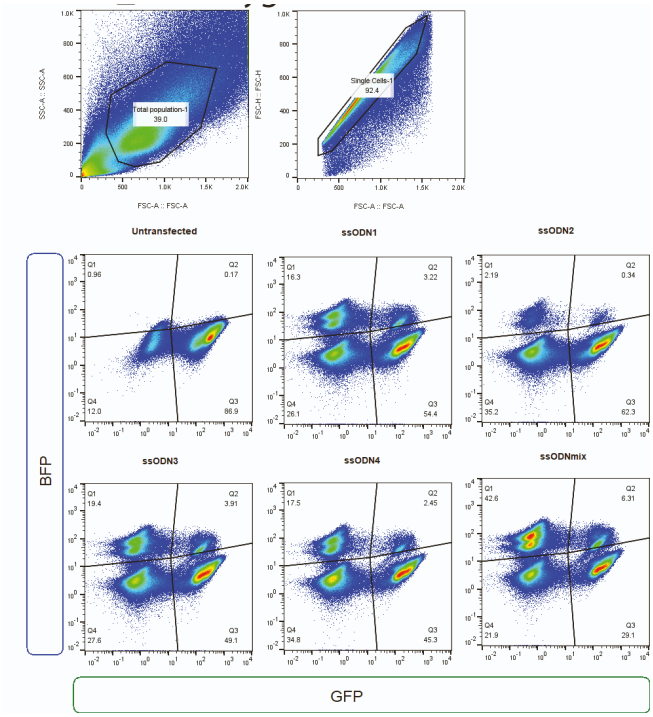
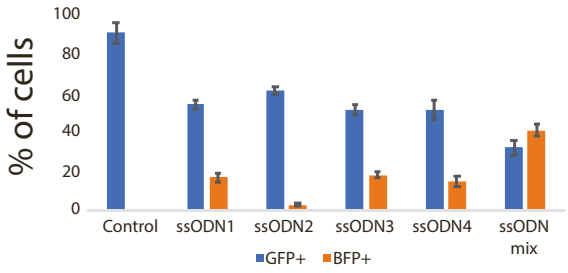
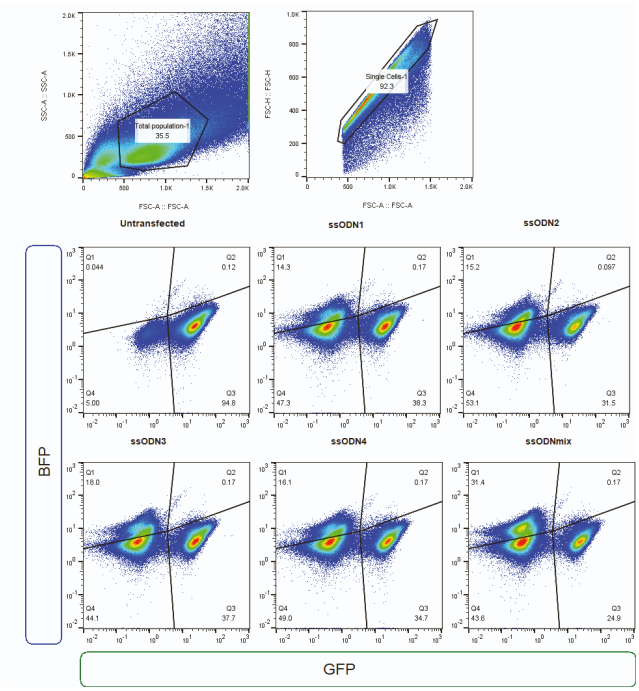
BIH001-A-2 (Homozygous AAVS1-GFP)





BIH001-A-11 (Heterozygous; POUF51-GFP)



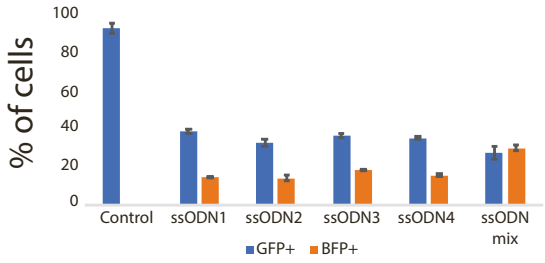


Figure S1. Representative FACS plots from GFP-to-BFP HDR reporter assays.

Representative flow-cytometry plots showing GFP-to-BFP conversion following CRISPR-Mix–mediated editing in two iPSC lines: BIH001-A-2 (top; homozygous AAVS1-GFP) and BIH001-A-11 (bottom; heterozygous POU5F1-GFP). Quantification graphs display the percentage of BFP-positive cells for each donor design and pooled CRISPR-MiX condition. Data illustrate variable HDR efficiencies across individual ssODNs and the enhanced editing achieved with pooled donor templates.

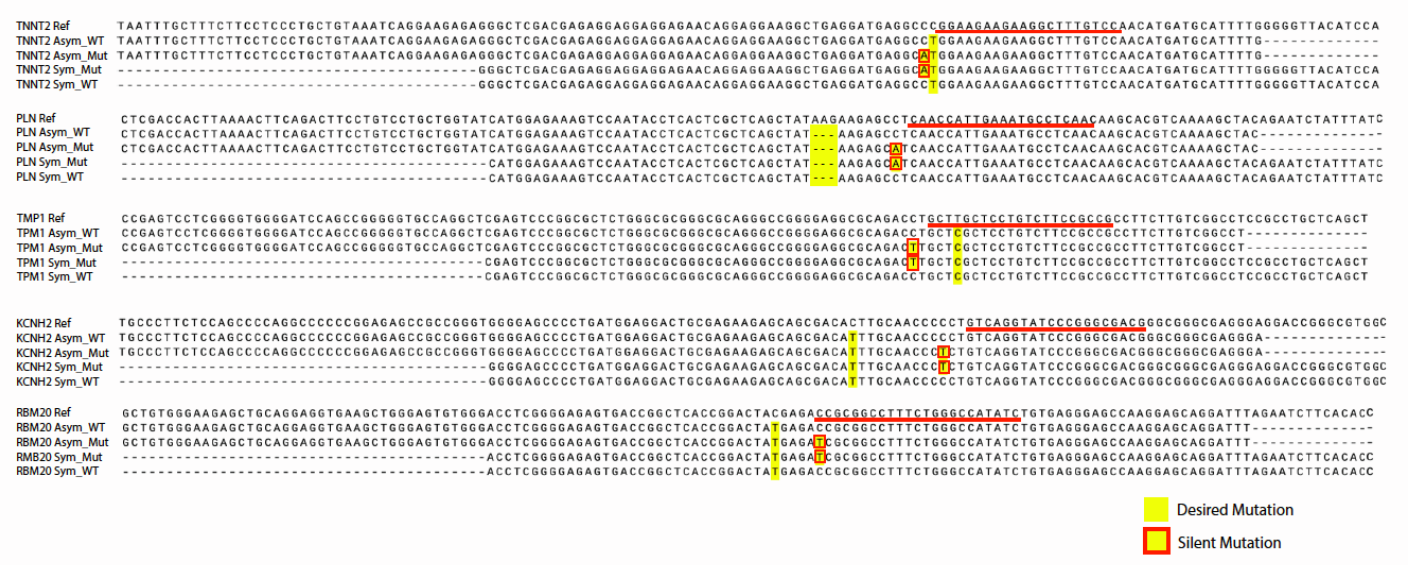


Figure S2: Expanded Sequences of the five loci. Sequences of each targeted cardiac gene, with the gRNA-targeted sequence underlined in red.

Table S1: ssODN sequences designed based on the non-targeted strand.

Oligo Name	Sequence
GFP to BFP SymWT	CTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACC CTCGTGACCACCCTGAGCCACGGGGTGCAGTGCTTCAGCCGCTACCCC GACCACATGAAGCAGCACGACTTCTTCAAGTCCGCC
GFP to BFP SymMut	CTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACC CTCGTGACCACACTGAGCCACGGGGTGCAGTGCTTCAGCCGCTACCCC GACCACATGAAGCAGCACGACTTCTTCAAGTCCGCC
GFP to BFP AsymWT	GAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCA CCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGAGCC ACGGGGTGCAGTGCTTCAGCCGCTACCCCG
GFP to BFP AsymMut	GAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCA CCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACACTGAGCC ACGGGGTGCAGTGCTTCAGCCGCTACCCCG
RBM20 SymWT	ACCTCGGGGAGAGTGACCGGCTCACCGGACTATGAGACCGCGGCCTTT CTGGGCCATATCTGTGAGGGAGCCAAGGAGCAGGATTTAGAATCTTCAC ACC
RBM20 SymMut	ACCTCGGGGAGAGTGACCGGCTCACCGGACTATGAGATCGCGGCCTTT CTGGGCCATATCTGTGAGGGAGCCAAGGAGCAGGATTTAGAATCTTCAC ACC
RBM20 AsymWT	GCTGTGGGAAGAGCTGCAGGAGGTGAAGCTGGGAGTGTGGGACCTCG GGGAGAGTGACCGGCTCACCGGACTATGAGACCGCGGCCTTTCTGGGC CATATCTGTGAGGGAGCCAAGGAGCAGGATTT
RBM20 AsymMut	GCTGTGGGAAGAGCTGCAGGAGGTGAAGCTGGGAGTGTGGGACCTCG GGGAGAGTGACCGGCTCACCGGACTATGAGATCGCGGCCTTTCTGGGC CATATCTGTGAGGGAGCCAAGGAGCAGGATTT
KCNH2 SymWT	GGGGAGCCCCTGATGGAGGACTGCGAGAAGAGCAGCGACATTTGCAAC CCCCTGTCAGGTATCCCGGGCGACGGGCGGGCGAGGGAGGACCGGGC GTGGC
KCNH2 SymMut	GGGGAGCCCCTGATGGAGGACTGCGAGAAGAGCAGCGACATTTGCAAC CCTCTGTCAGGTATCCCGGGCGACGGGCGGGCGAGGGAGGACCGGGC GTGGC
KCNH2 AsymWT	TGCCCTTCTCCAGCCCCAGGCCCCCCGGAGAGCCGCCGGGTGGGGAG CCCCTGATGGAGGACTGCGAGAAGAGCAGCGACATTTGCAACCCCCTG TCAGGTATCCCGGGCGACGGGCGGGCGAGGGA
KCNH2 AsymMut	TGCCCTTCTCCAGCCCCAGGCCCCCCGGAGAGCCGCCGGGTGGGGAG CCCCTGATGGAGGACTGCGAGAAGAGCAGCGACATTTGCAACCCTCTGT CAGGTATCCCGGGCGACGGGCGGGCGAGGGA
PLN SymWT	CATGGAGAAAGTCCAATACCTCACTCGCTCAGCTATAAGAGCCTCAACC ATTGAAATGCCTCAACAAGCACGTCAAAAGCTACAGAATCTATTTATC
PLN SymMut	CATGGAGAAAGTCCAATACCTCACTCGCTCAGCTATAAGAGCATCAACCA TTGAAATGCCTCAACAAGCACGTCAAAAGCTACAGAATCTATTTATC
PLN AsymWT	CTCGACCACTTAAAACTTCAGACTTCCTGTCCTGCTGGTATCATGGAGAA AGTCCAATACCTCACTCGCTCAGCTATAAGAGCCTCAACCATTGAAATGC CTCAACAAGCACGTCAAAAGCTAC
PLN AsymMut	CTCGACCACTTAAAACTTCAGACTTCCTGTCCTGCTGGTATCATGGAGAA AGTCCAATACCTCACTCGCTCAGCTATAAGAGCATCAACCATTGAAATGC CTCAACAAGCACGTCAAAAGCTAC
TNNT2 SymWT	GGGCTCGACGAGAGGAGGAGGAGAACAGGAGGAAGGCTGAGGATGAG GCCTGGAAGAAGAAGGCTTTGTCCAACATGATGCATTTTGGGGGTTACA TCCA

TNNT2 SymMut	GGGCTCGACGAGAGGAGGAGGAGAACAGGAGGAAGGCTGAGGATGAG GCATGGAAGAAGAAGGCTTTGTCCAACATGATGCATTTTGGGGGTTACA TCCA
TNNT2 AsymWT	TAATTTGCTTTCTTCCTCCCTGCTGTAAATCAGGAAGAGAGGGCTCGACG AGAGGAGGAGGAGAACAGGAGGAAGGCTGAGGATGAGGCCTGGAAGA AGAAGGCTTTGTCCAACATGATGCATTTTG
TNNT2 AsymMut	TAATTTGCTTTCTTCCTCCCTGCTGTAAATCAGGAAGAGAGGGCTCGACG AGAGGAGGAGGAGAACAGGAGGAAGGCTGAGGATGAGGCATGGAAGAA GAAGGCTTTGTCCAACATGATGCATTTTG
TPM1 SymWT	CGAGTCCCGGCGCTCTGGGCGCGGGCGCAGGGCCGGGGAGGCGCAG ACCTGCTCGCTCCTGTCTTCCGCCGCCTTCTTGTCGGCCTCCGCCTGCT CAGCT
TPM1 SymMut	CGAGTCCCGGCGCTCTGGGCGCGGGCGCAGGGCCGGGGAGGCGCAG ACTTGCTCGCTCCTGTCTTCCGCCGCCTTCTTGTCGGCCTCCGCCTGCT CAGCT
TPM1 AsymWT	CCGAGTCCTCGGGGTGGGGATCCAGCCGGGGGTGCCAGGCTCGAGTC CCGGCGCTCTGGGCGCGGGCGCAGGGCCGGGGAGGCGCAGACCTGCT CGCTCCTGTCTTCCGCCGCCTTCTTGTCGGCCT
TPM1 AsymMut	CCGAGTCCTCGGGGTGGGGATCCAGCCGGGGGTGCCAGGCTCGAGTC CCGGCGCTCTGGGCGCGGGCGCAGGGCCGGGGAGGCGCAGACTTGCT CGCTCCTGTCTTCCGCCGCCTTCTTGTCGGCCT

Table S2: ssODN sequences designed based on the targeted strand.

Oligo Name	Sequence
RBM20 SymWT comp	GGTGTGAAGATTCTAAATCCTGCTCCTTGGCTCCCTCACAGA-TATGGCCCAGAAAGGCCGCGGTCTCATAGTCCGGTGAGCCGGTCAC TCTCCCCGAGGT
RBM20 SymMut comp	GGTGTGAAGATTCTAAATCCTGCTCCTTGGCTCCCTCACAGA-TATGGCCCAGAAAGGCCGCGATCTCATAGTCCGGTGAGCCGGTCAC TCTCCCCGAGGT
RBM20 AsymWT comp	ACAGAGACTGTGTGTCTGTGTGTGGGTGGGGTGGGATGG-GAGGTGTGAAGAT-TCTAAATCCTGCTCCTTGGCTCCCTCACAGATATGGCCCAGAAAGG CCGCGGTCTCATAGTCCGGTGAGCCGGTC
RBM20 Asym-Mut comp	ACAGAGACTGTGTGTCTGTGTGTGGGTGGGGTGGGATGG-GAGGTGTGAAGAT-TCTAAATCCTGCTCCTTGGCTCCCTCACAGATATGGCCCAGAAAGG CCGCGATCTCATAGTCCGGTGAGCCGGTC
KCNH2 SymWT comp	GCCACGCCCGGTCCTCCCTCGCCCGCCCGTCGCCCGGGATAC-CTGACAGGGGGTT-GCAAATGTCGCTGCTCTTCTCGCAGTCCTCCATCAGGGGCTCCCC
KCNH2 SymMut comp	GCCACGCCCGGTCCTCCCTCGCCCGCCCGTCGCCCGGGATAC-CTGACAGAGGGTT-GCAAATGTCGCTGCTCTTCTCGCAGTCCTCCATCAGGGGCTCCCC
KCNH2 AsymWT comp	AGAGAGCAGAGCTGGGTGAGCGGGGTAGACGCACCAC-CGCTGCCAC-GCCCGGTCCTCCCTCGCCCGCCCGTCGCCCGGGATACCTGACAG GGGGTTGCAAATGTCGCTGCTCTTCTCGCAGTCCTCC
KCNH2 Asym-Mut comp	AGAGAGCAGAGCTGGGTGAGCGGGGTAGACGCACCAC-CGCTGCCAC-GCCCGGTCCTCCCTCGCCCGCCCGTCGCCCGGGATACCTGACAG AGGGTTGCAAATGTCGCTGCTCTTCTCGCAGTCCTCC
PLN SymWT comp	GATAAATAGATTCTGTAGCTTTTGACGTGCTTGTT-GAGGCATTTCAATGGTT-GAGGCTCTTATAGCTGAGCGAGTGAGGTATTGGACTTTCTCCATG
PLN SymMut comp	GATAAATAGATTCTGTAGCTTTTGACGTGCTTGTT-GAGGCATTTCAATGGTT-GATGCTCTTATAGCTGAGCGAGTGAGGTATTGGACTTTCTCCATG
PLN AsymWT comp	ATACAGATCAGCAAGAGACATATTAAGATGAGACAGAAATTGA-TAAATAGATTCTG-TAGCTTTTGACGTGCTTGTTGAGGCATTTCAATGGTTGAGGCTCTTA TAGCTGAGCGAGTGAGGTATT
PLN AsymMut comp	ATACAGATCAGCAAGAGACATATTAAGATGAGACAGAAATTGA-TAAATAGATTCTG-TAGCTTTTGACGTGCTTGTTGAGGCATTTCAATGGTTGATGCTCTTA TAGCTGAGCGAGTGAGGTATT
TNNT2 SymWT comp	TGGATGTAACCCCCAAAATGCATCATGTTGGACAAA-GCCTTCTTCTTCCAGGCCTCATCCTCAGCCTTCCTCCTGTTCTCCT CCTCCTCTCGTCGAGCCC
TNNT2 SymMut comp	TGGATGTAACCCCCAAAATGCATCATGTTGGACAAA-GCCTTCTTCTTCCATGCCTCATCCTCAGCCTTCCTCCTGTTCTCCTC CTCCTCTCGTCGAGCCC

TNNT2 AsymWT comp	CTAGGATCTCCTGGCAACCCCTGCTGCTCCCTACCTACCTTCTG-GATGTAACCCCCAAAATGCATCATGTTGGACAAAGCCTTCTTCTTC CAGGCCTCATCCTCAGCCTTCCTCCTGTTCTCCTCCT
TNNT2 AsymMut comp	CTAGGATCTCCTGGCAACCCCTGCTGCTCCCTACCTACCTTCTG-GATGTAACCCCCAAAATGCATCATGTTGGACAAAGCCTTCTTCTTC CATGCCTCATCCTCAGCCTTCCTCCTGTTCTCCTCCT
TPM1 SymWT comp	AGCTGAGCAGGCGGAGGCCGACAAGAAGGCGGCGGAAGACAG-GAGCGAG-CAGGTCTGCGCCTCCCCGGCCCTGCGCCCGCGCCCAGAGCGCCG GGACTCG
TPM1 SymMut comp	AGCTGAGCAGGCGGAGGCCGACAAGAAGGCGGCGGAAGACAG-GAGCGAG-CAAGTCTGCGCCTCCCCGGCCCTGCGCCCGCGCCCAGAGCGCCG GGACTCG
TPM1 AsymWT comp	ATGCAGATGCTGAAGCTCGACAAGGAGAACGCCTT-GGATCGAGCTGAGCAGGCG-GAGGCCGACAAGAAGGCGGCGGAAGACAGGAGCGAGCAGGTCTG CGCCTCCCCGGCCCTGCGCCCGCGCCCAG
TPM1 AsymMut comp	ATGCAGATGCTGAAGCTCGACAAGGAGAACGCCTT-GGATCGAGCTGAGCAGGCG-GAGGCCGACAAGAAGGCGGCGGAAGACAGGAGCGAGCAAGTCTG CGCCTCCCCGGCCCTGCGCCCGCGCCCAG

Table S3. gRNA sequences.

gRNA Name	gRNA Sequence
PLN	TTGAGGCATTTCAATGGTTG
TPM1	GGCGGAAGACAGGAGCAAGC
TNNT2	GGACAAAGCCTTCTTCTTCC
KCNH2	CGTCGCCCGGGATACCTGAC
RBM20	ATATGGCCCAGAAAGGCCG
GFP	GCACTGCACGCCGTAGGTCA

Table S4. Primer sequences used in amplicon sequencing.

Primer Name	Primer Sequence
PLN_F	TTTACATTCCAGGCTACCTAAA
PLN_R	TTAAGCTGATGTGGCAAGCTGC
TNNT2_F	CCACAAGCTTCAGCCCAGAATC
TNNT2_R	ATCTCTTCCTGGGACCTGACCT
TPM1_F	GGACGCCATCAAGAAGAAGAT
TPM1_R	GCTCTTGCGTTTCCCTTCCTTC
KCNH2_F	CCCTGAGAGCAGTGAGGATGAG
KCNH2_R	TGTTGGACACTCCTGAGAAGGC
RBM20_F	CTCTGTGTGGTTCTGTAGAGTTG
RBM20_R	TGGCATAGGGAGAGTGCT