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Supplemental Information

Genome editing in human hematopoietic stem and progenitor cells via CRISPR-Cas9-mediated homology-independent targeted integration

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Supplemental Figures

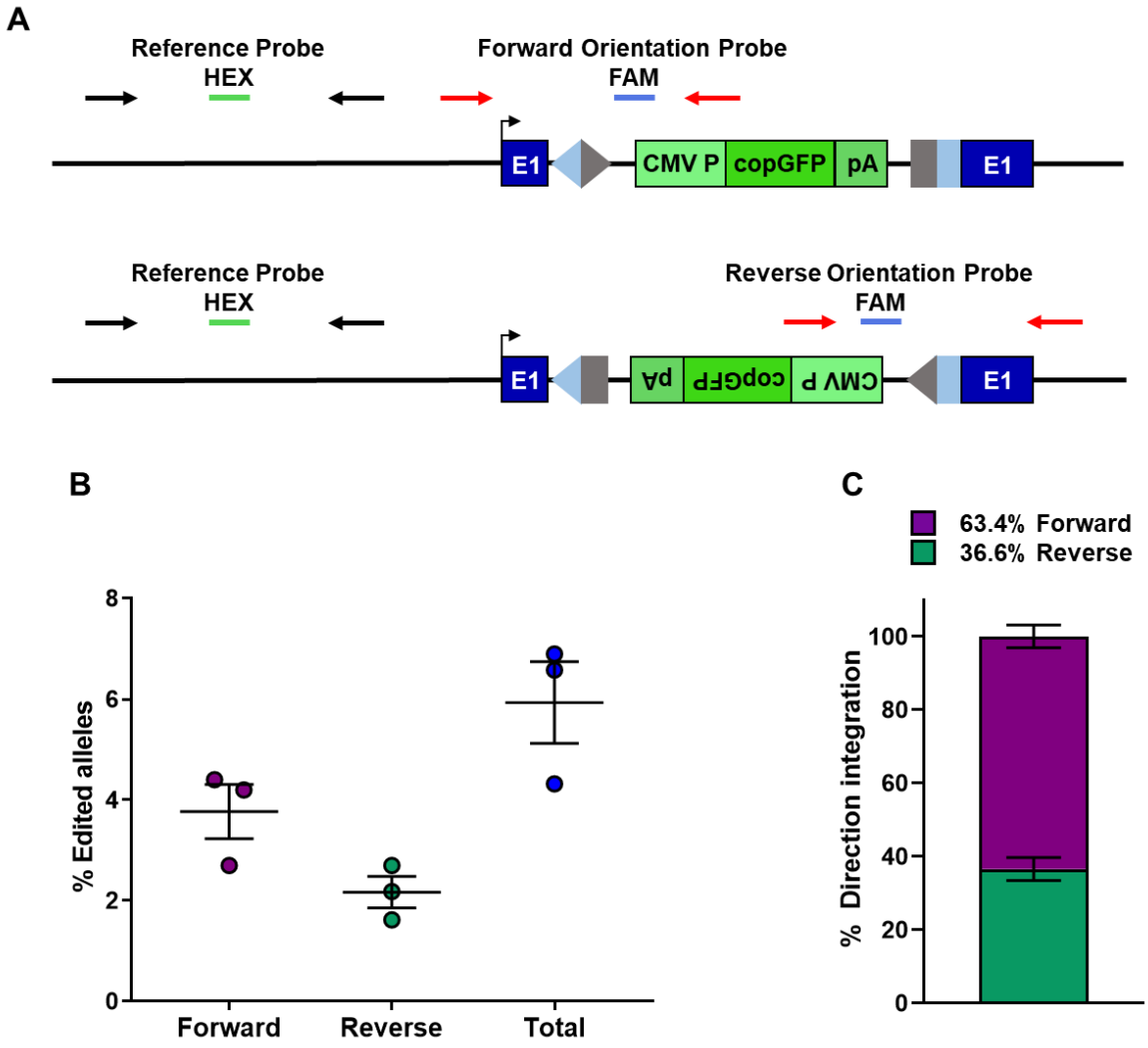

Figure S1 | Droplet Digital PCR (ddPCR) for quantification of allelic editing in the forward- and reverse-orientations (refers to Figure 3, C & D). A) Schematic of ddPCR primer-probe design for detection of forward- and reverse-orientation integrations. **B)** Frequency of alleles edited by HITI at day 4 post-electroporation as measured by ddPCR (n = 3 independent donors). **C)** Percent orientation of edited alleles (n = 3 independent donors). In panels (B) and (C), results are displayed as mean ± SEM.

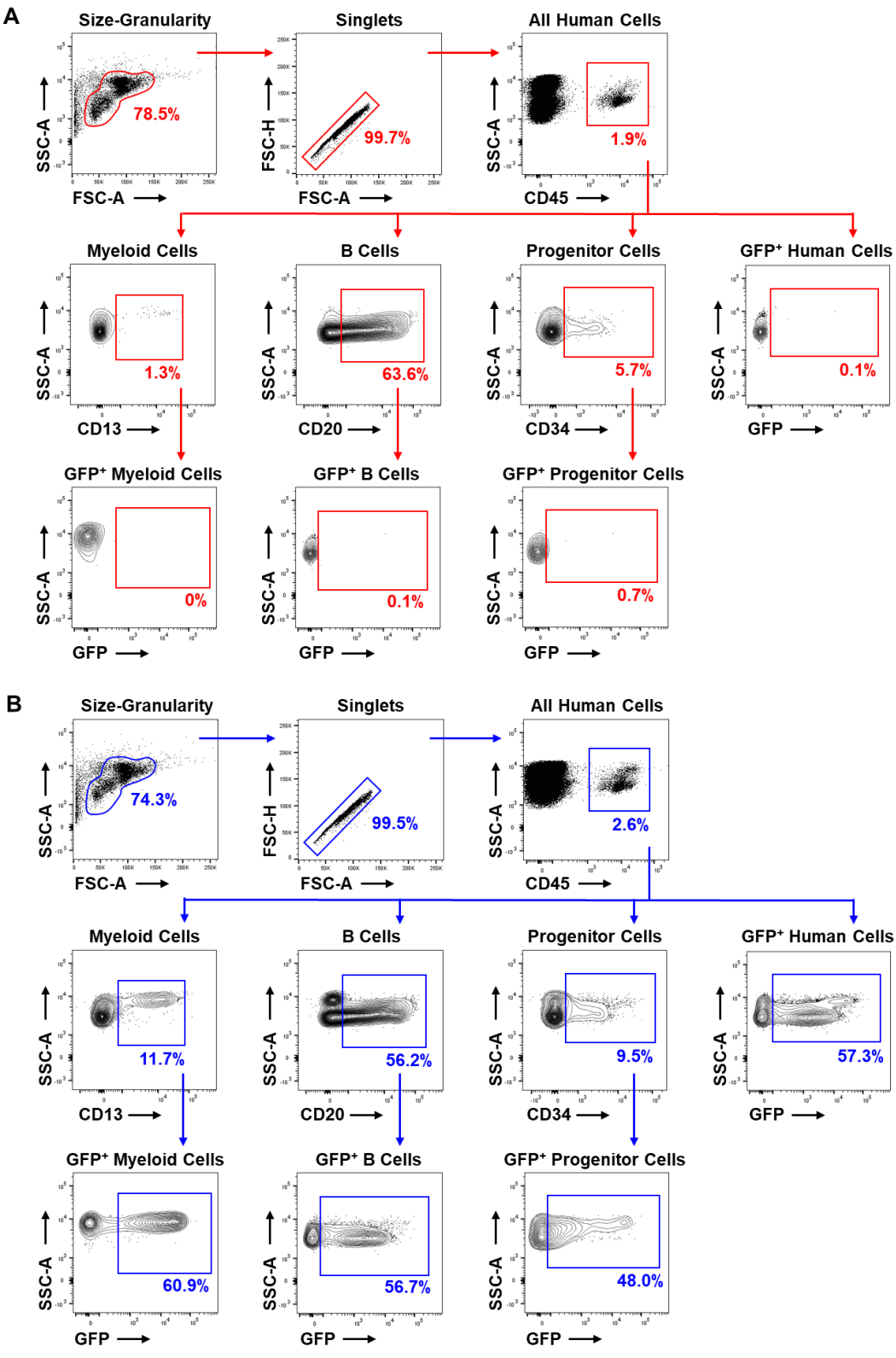

Figure S2 | Flow cytometry gating strategies for NSG mice analysis (refers to Figure 5, B to E). A) Representative flow cytometry plots for NSG mouse in rAAV6 group. **B)** Representative flow cytometry plots for NSG mouse in rAAV6+RNP group.

Figure S3 | Sanger sequencing of transgene junctions (refers to Figure 6A). A) Sequences of the 5' junction sites of forward-orientation alleles. Black arrowhead, Cas9 cleavage site. Yellow highlight, sgRNA target site. Blue, genomic sequences. Green, rAAV vector sequences. Red dots, continuation of rAAV vector sequences flanking HITI insert. Green dots, continuation of HITI insert. Red sequences, non-templated insertions. Dashes, deletions.

B

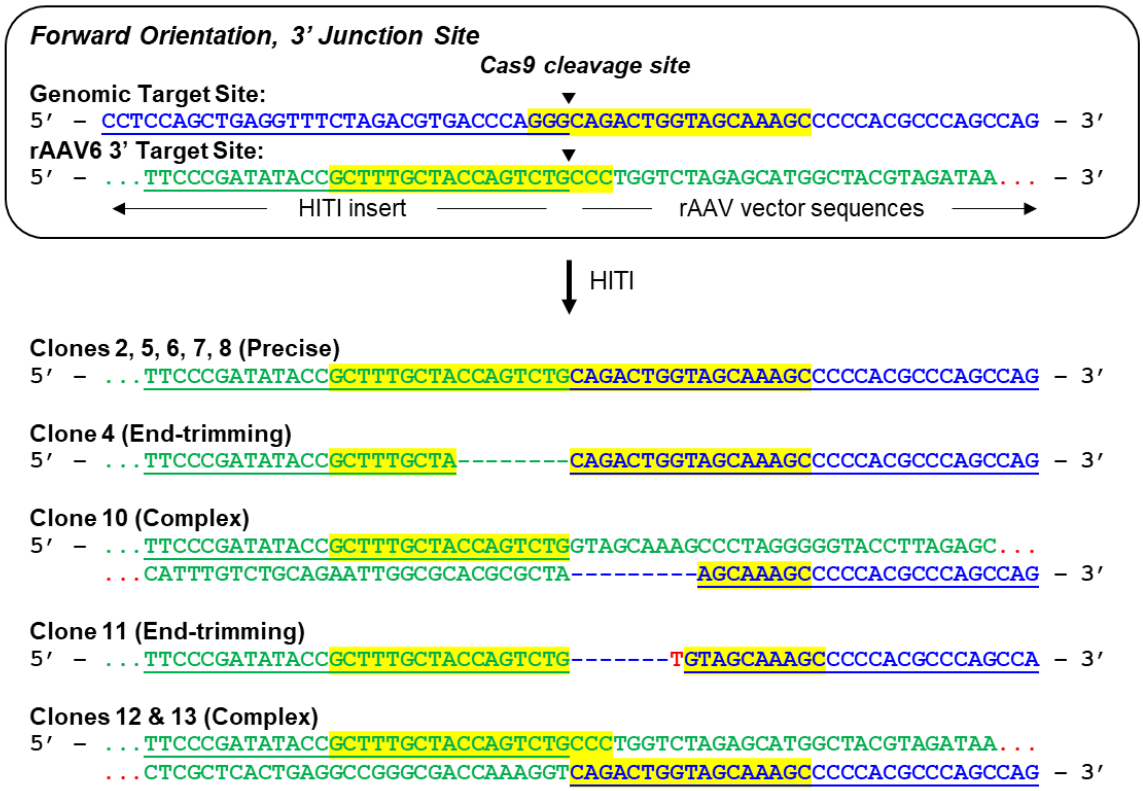

Figure S3 | Sanger sequencing of transgene junctions (refers to Figure 6A). B) Sequences of the 3' junction sites of forward-orientation alleles. Black arrowhead, Cas9 cleavage site. Yellow highlight, sgRNA target site. Blue, genomic sequences. Green, rAAV vector sequences. Red dots, continuation of rAAV vector sequences flanking HITI insert. Green dots, continuation of HITI insert. Red sequences, non-templated insertions. Dashes, deletions.

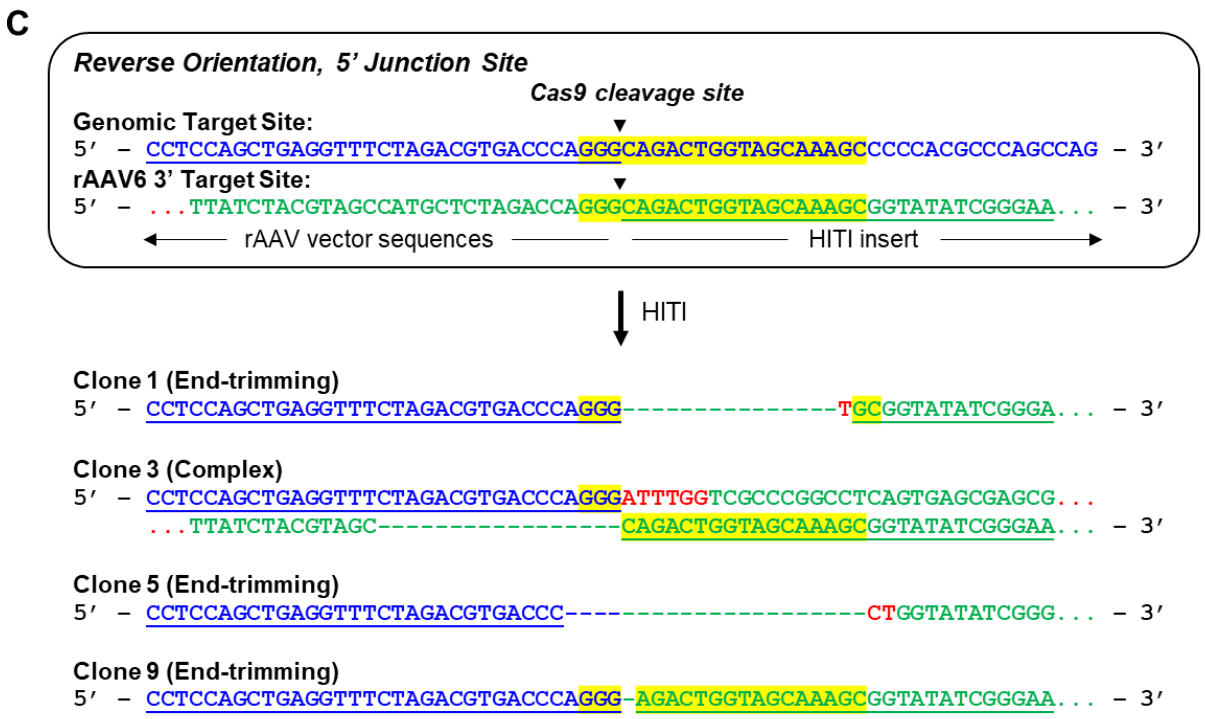

Figure S3 | Sanger sequencing of transgene junctions (refers to Figure 6A). C) Sequences of the 5' junction sites of reverse orientation alleles. Black arrowhead, Cas9 cleavage site. Yellow highlight, sgRNA target site. Blue, genomic sequences. Green, rAAV vector sequences. Red dots, continuation of rAAV vector sequences flanking HITI insert. Green dots, continuation of HITI insert. Red sequences, non-templated insertions. Dashes, deletions.

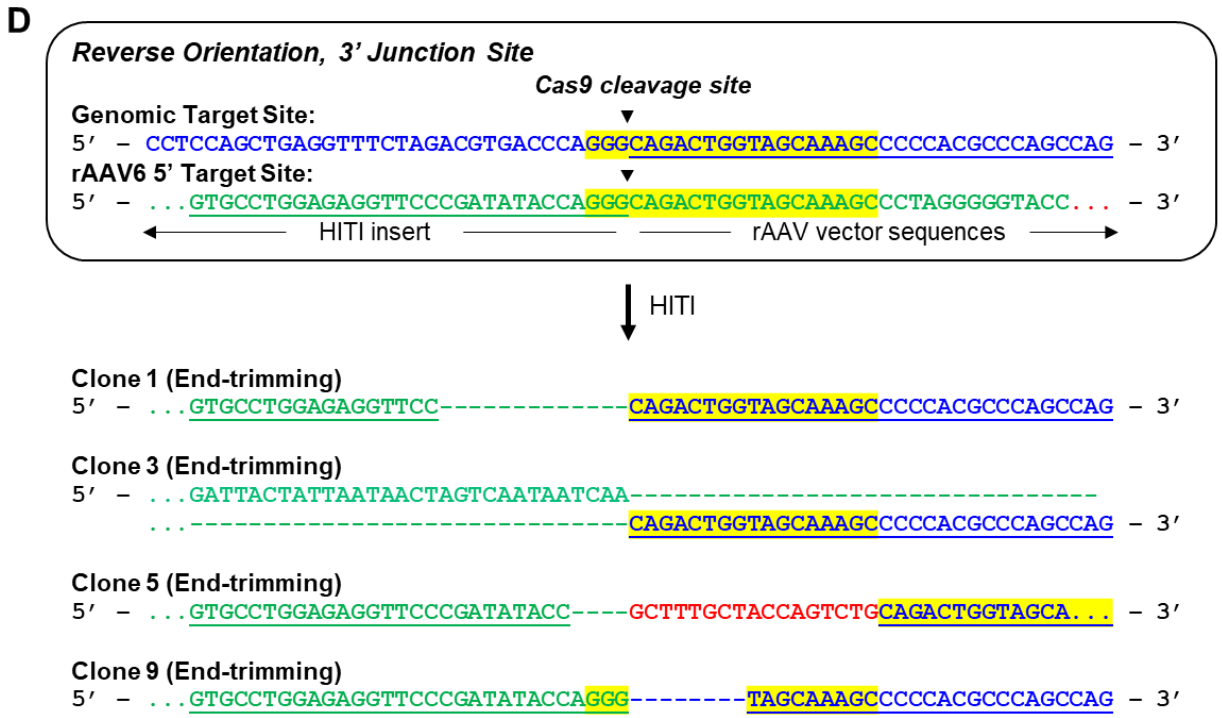

Figure S3 | Sanger sequencing of transgene junctions (refers to Figure 6A). D) Sequences of the 3' junction sites of reverse-orientation alleles. Black arrowhead, Cas9 cleavage site. Yellow highlight, sgRNA target site. Blue, genomic sequences. Green, rAAV vector sequences. Red dots, continuation of rAAV vector sequences flanking HITI insert. Green dots, continuation of HITI insert. Red sequences, non-templated insertions. Dashes, deletions.

Supplemental Tables

Table S1 | Summary of end-trimming at transgene junctions

Clone	Genomic DNA Trimming				Vector Trimming			
	Fw-5'	Fw-3'	Rv-5'	Rv-3'	Fw-5'	Fw-3'	Rv-5'	Rv-3'
1			0	0			15	13
2	0	0			0	0		
3			0	0			0	242
4	4	0			1	8		
5	6	0	4	0	0	0	17	4
6	0	0			4	0		
7	6	0			0	0		
8	0	0			0	0		
9			0	8			1	0
10	2	9			2	0		
11	6	7			377	0		
12	0	0			0	0		
13	0	0			0	0		
Range of trimming	2-6	7-9	4	8	1-4 (377)	8	1-17	4-13 (242)
# trimmed junctions	9 of 28				11 of 28			

Numbers of bases deleted within the targeted genomic locus or vector at each genomic DNA-transgene junction are indicated. Fw-5': Forward orientation, 5' junction site; Fw-3": Forward orientation, 3' junction site; Rv-5': Reverse orientation, 5' junction site; Rv-3': Reverse orientation, 3' junction site.

Table S2 | Nested primers flanking *ITGB2* sgRNA target site

Primer Name	Primer Sequence	Annealing Temperature (°C)	Amplicon Size (bp)
ITGB2-Outer-F	5' CCAGCCTGGTCAACATAGTG 3'	58	1743
ITGB2-Outer-R	5' AGACTCCCCACATCACATGC 3'		
ITGB2-F	5' ATGTCCCACCTGTCTCAAGG 3'	65	633
ITGB2-R	5' GCAGCAGGTTCACAGAGGA 3'		

Table S3 | Primers for amplification of potential off-target sites and on-target control

Primer Name	Primer Sequence	Annealing Temperature (°C)	Amplicon Size (bp)
OT1-F	5' AAGGACTTAGCCCGAAACCT 3'	57	420
OT1-R	5' TCTCCCAACCACCCTTGAAG 3'		
OT2-F	5' TGGCCTCAGTTTTGCTTCTG 3'	57	402
OT2-R	5' GTGAAACTTCCTGGCTCGGA 3'		
OT3-F	5' CAGGGCCCTCTGTATGTAGG 3'	57	402
OT3-R	5' TTTCTGGCAAAGGGGTTTCC 3'		
OT4-F	5' TCGCTCTCTCTCTCTCTCAC 3'	57	414
OT4-R	5' GTGGTTGTGGGGTCAAAGTG 3'		
OT5-F	5' TCTCTGATACCCTGGGCAAC 3'	57	449
OT5-R	5' ACCAGCACATAGAAAGGCAT 3'		
ITGB2on-F	5' CTTCCTGCCAGACACCCC 3'	62.1	434
ITGB2on-R	5' TCCCAAGTGTGAATCTGATGGA 3'		

Table S4 | Nested primers for detecting integration in forward and reverse orientation

Primer Name	Primer Sequence	Annealing Temperature (°C)	Amplicon Size (bp)
5'-Forward-Integration-Outer-F	5' AGCTGCTGTAGAGCGGAGAG 3'	67	792
5'-Forward-Integration-Outer-R	5' CATTGGTGTACTGCCAAAACC 3'		
5'-Forward-Integration-Inner-F	5' CAAGGAGGAGCTGAGAGGAA 3'	60	665
5'-Forward-Integration-Inner-R	5' GCCAAGTAGGAAAGTCCCGTA 3'		
3'-Forward-Integration-Outer-F	5' GCACTTCAAGAGCGCCATC 3'	72	803
3'-Forward-Integration-Outer-R	5' CTCACAGCCCCTTGTCCTC 3'		
3'-Forward-Integration-Inner-F	5' TACCAGCACGCCTTCAAGA 3'	72	632
3'-Forward-Integration-Inner-R	5' ATGTGGCTCTGCTCTTGGT 3'		
5'-Reverse-Integration-Outer-F	5' AGCTGCTGTAGAGCGGAGAG 3'	67	978
5'-Reverse-Integration-Outer-R	5' GTGATGGGCTACGGCTTCTA 3'		
5'-Reverse-Integration-Inner-F	5' CCAAGGAGGAGCTGAGAGG 3'	60	872
5'-Reverse-Integration-Inner-R	5' GCTACGAGAACCCCTTCCTG 3'		
3'-Reverse-Integration-Outer-F	5' CGTAAGGTCATGTACTGGGC 3'	68	869
3'-Reverse-Integration-Outer-R	5' CTCACAGCCCCTTGTCCTC 3'		
3'-Reverse-Integration-Inner-F	5' GGCGGACTTGGCATATGATAC 3'	72	771
3'-Reverse-Integration-Inner-R	5' ATGTGGCTCTGCTCTTGGT 3'		

Table S5 | Primers and probes for ddPCR assay

Primer/Probe Name	Primer/Probe Sequence	Amplicon Size (bp)
ddPCR-Reference-F	5' TCCACAAAGAAAAACGTGCACAG 3'	191
ddPCR-Reference-R	5' ATAAAGGCTGGTGGAGGGAG 3'	
ddPCR-Reference-HEX-Probe	5' GCCCCACGGTCCCTAGCCCCT 3'	
ddPCR-Forward-Integration-F	5' CAAGGAGGAGCTGAGAGGAA 3'	186
ddPCR-Forward-Integration-R	5' TGACATGCATTGGTGGAGAT 3'	
ddPCR-Forward-Integration-FAM-Probe	5' ACCCCTCACTCGGCGCGCCA 3'	
ddPCR-Reverse-Integration-F	5' GCCAATATTGACATGCATTGGT 3'	185
ddPCR-Reverse-Integration-R	5' CACACTCACCCTCGGTGT 3'	
ddPCR-Reverse-Integration-FAM-Probe	5' TCCCGGTAGCGGGCGACGCA 3'	