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Supplementary Information for:

POLQ Suppresses Interhomolog Recombination and Loss of Heterozygosity at Targeted DNA Breaks

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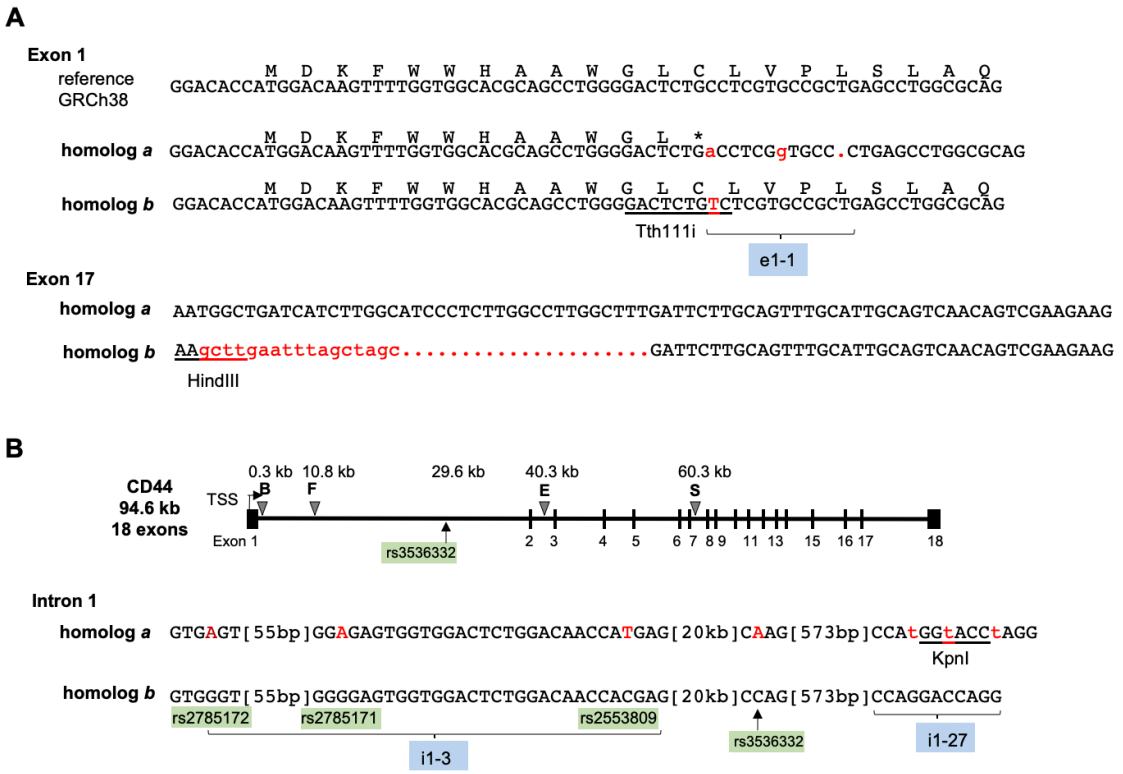

Fig. S1. Sequences of regions bearing natural or engineered polymorphisms

(A) Sequences of the mutations engineered in exons 1 and 17 of homologs ***a*** and ***b*** of the HT1080-K1 and HT1080-K2 cell lines, indicating diagnostic Tth111I and HindIII sites (underlined) used to assay heterozygosity by RFLP analysis and the polymorphism e1-1 in exon 1 that was used to assay heterozygosity by NGS. For exon 1, the GRCh38 reference sequence is shown, with encoded protein sequence above starting with the initiator methionine. For exon 17, the sequence of homolog ***a*** is identical to that of the reference sequence, while homolog ***b*** contains a 17 bp insertion that replaces 38 bp of sequence. Restriction enzyme sites, underlined; SNPs and engineered polymorphisms, red uppercase; insertions, red lower case; deletions, red dots.

(B) *Above*, map of CD44 gene, showing the 18 exons; the sites targeted by gRNAs B, F, E and S with distance from TSS indicated above each site, and the natural polymorphism rs353632; 29.6 kb downstream of the CD44 TSS, that is targeted by gRNA M on homolog ***b***.

Below, sequences of intronic polymorphic region i1-3 (bearing natural SNPs rs2785172, rs2785171 and rs2553809); SNP rs353632; and engineered polymorphism i1-27 in intron 1 of homologs ***a*** and ***b*** in the HT1080-K2 cell line. Distances between sequences, bracketed; other notations as above.

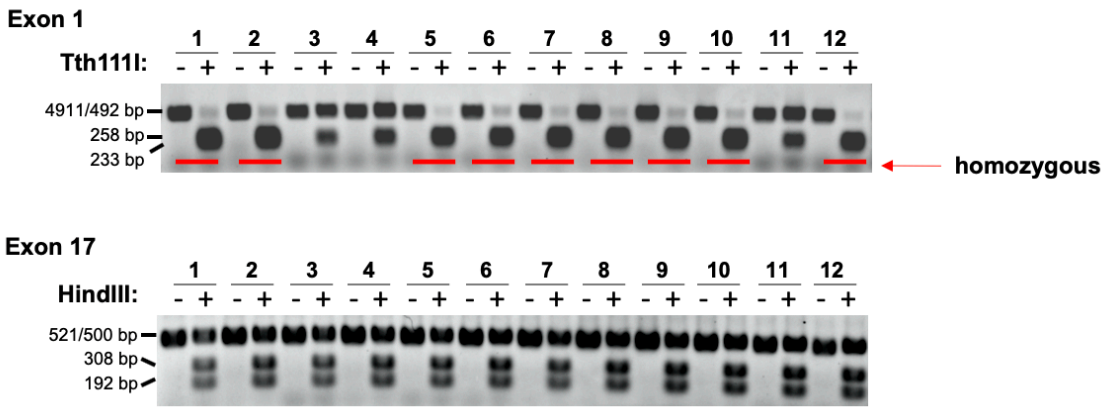

Fig. S2. RFLP assay of homozygosity of exons 1 and 17

Representative agarose gel electrophoresis of amplicons spanning regions in exons 1 or 17 of the CD44 gene analyzed for polymorphisms in cleavage sites for Tth111I (exon 1) or HindIII (exon 17). In this example, DNA was prepared from populations amplified from single HT1080 K1 cells in which nicks were targeted to intron 7 by transfection of plasmids expressing Cas9D10A and gRNAS. Of the 12 samples analyzed, 9 exhibited homozygosity at exon 1 (red) and none at exon 17.

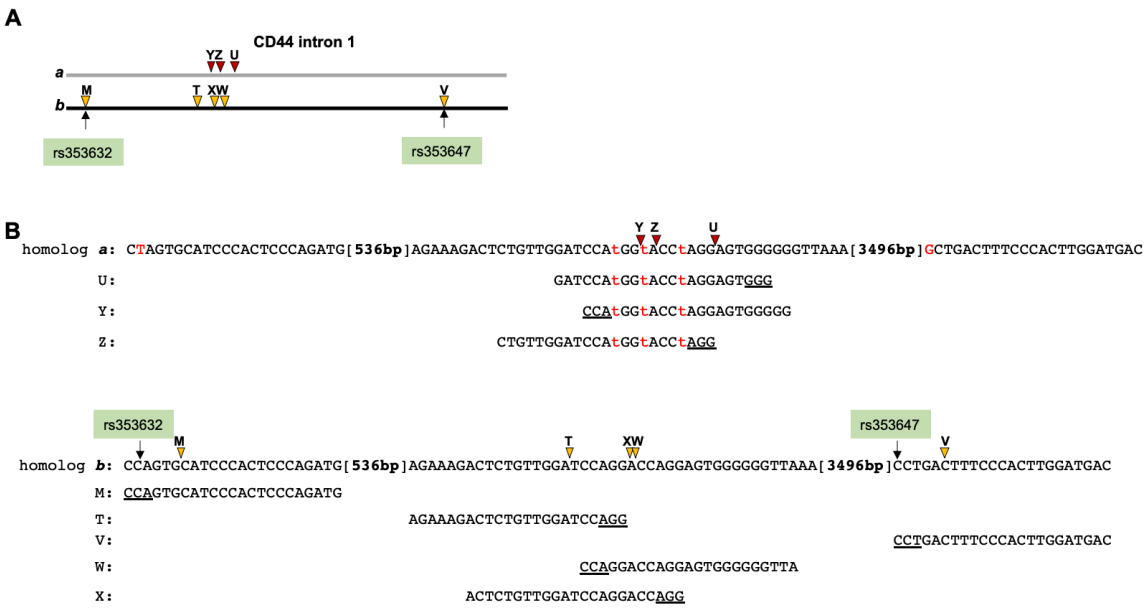

Fig. S3. Sites of gRNAs that target a single homolog

(A) Map of CD44 gene showing sites for gRNAs that target a single homolog of the engineered CD44 gene in HT1080-K2 cells. gRNAs Y, Z and U target homolog ***a***; and gRNAs M, T, X, W and V target homolog ***b***.

(B) Sequences within intron 1 targeted by gRNAs that cleave a single homolog. Engineering of homolog *a* generated 3 single base insertions of (red), enabling specific targeting by gRNAs U, Y and Z and conferring resistance to gRNAs T, V, W and X. Mutations and polymorphisms shown in red; insertions, red lower case; gRNA target sites, arrowheads; PAM sequences, underlined; and distances between sequences, bracketed.

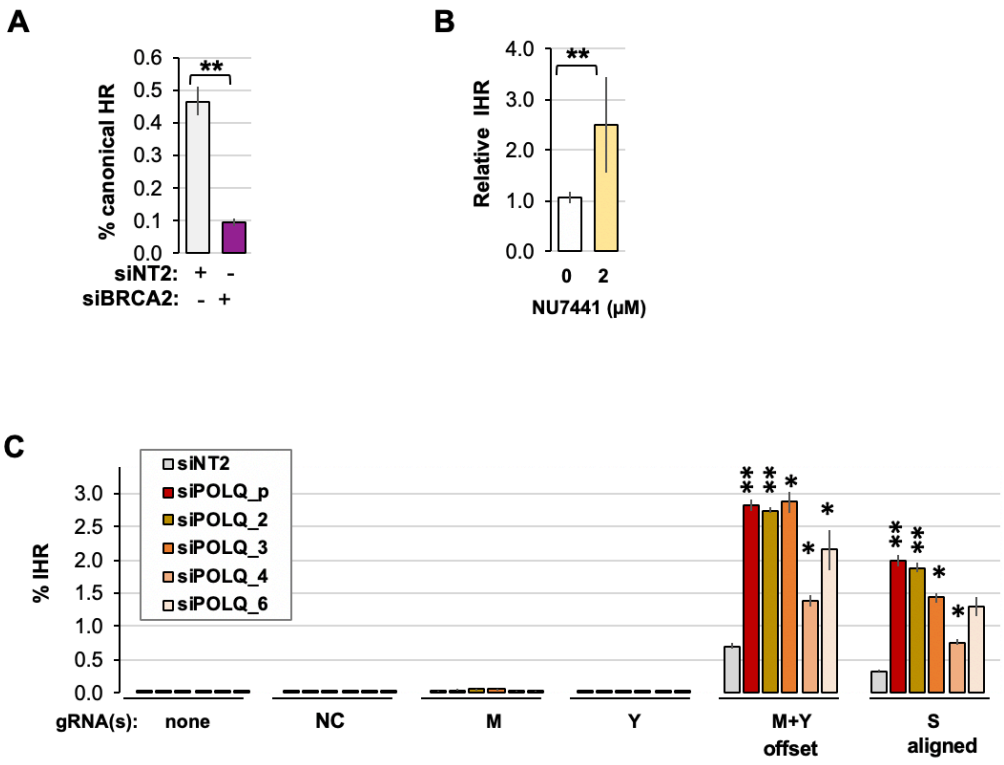

Fig. S4. Participation of BRCA2, DNA-PK and POLQ in IHR

(A) Canonical HR frequencies in HT1080 K1 cells treated with siNT2 (a non-specific control siRNA) or siBRCA2. Canonical HR was targeted to the mutant allele of CD44 exon 1 by cotransfection with a dsDNA donor plasmid and plasmids expressing Cas9 and gRNA K1D.

(B) Relative IHR frequencies from replicate assay of HT1080 K1 cells treated with DMSO or 2 μM NU7441, a selective DNA-PK inhibitor (Selleckchem). DSBs were targeted to CD44 intron 7 by transfection of RNPs containing Cas9 and gRNA S.

(C) IHR frequencies at DSBs targeted by transfection of RNPs containing Cas9 and the indicated gRNAs in HT1080-K2 cells pre-treated with non-targeting control siRNA (siNT2) or an equimolar pool of four siRNAs designed for POLQ depletion (siPOLQ_p, Qiagen catalog # SI02665215, SI00090083, SI00090076, SI00090069), or each of those four siRNAs individually (siPOLQ_6, #SI02665215; siPOLQ_4, #SI00090083; siPOLQ_3, #SI00090076; si-POLQ_2, #SI00090069). IHR frequencies are shown as mean and SD [n=1 for non-targeting gRNA NC, n=2 for gRNAs M and Y, and n=3 for gRNAs M+Y and S (except gRNA S/siPOLQ6 n=2)]. * and ** indicate significant differences from siNT2 ($p<0.05$ and $p<0.001$, respectively).

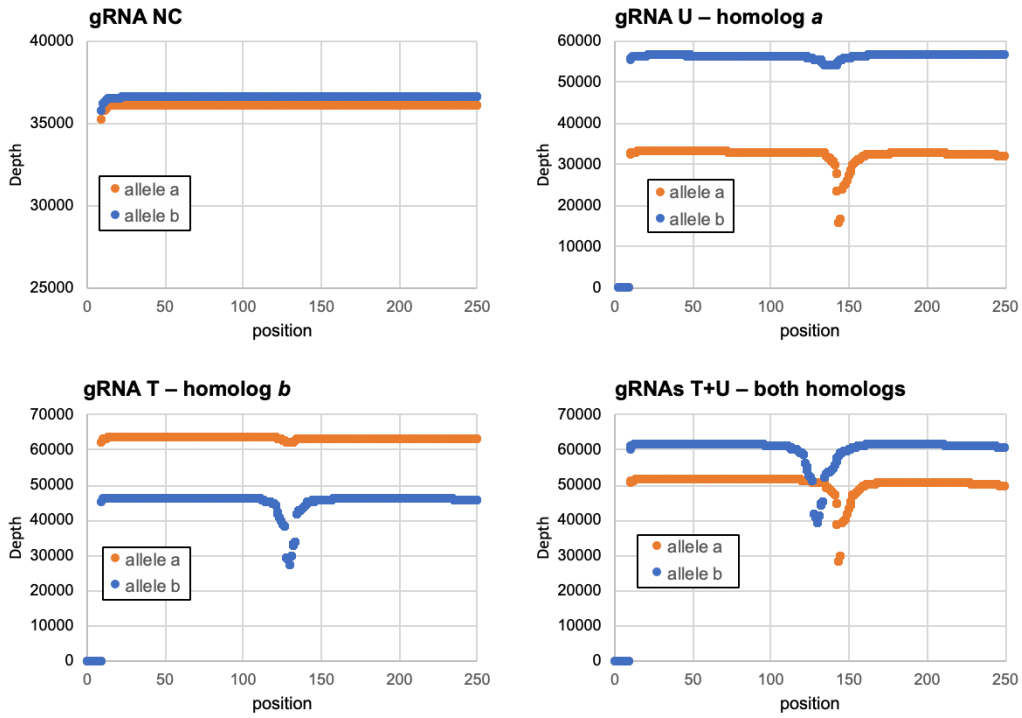

Fig. S5. Positional read depth across amplicon targeted by gRNAs T and U
Graphs of positional read depth, which are indicative of cumulative frequency of deletions, across the amplicon targeted by gRNA T, gRNA U, gRNAs T+U; or the non-targeting control gRNA, gNC. DSBs were targeted by transfection of siNT2 treated cells with RNPs containing Cas9 and the indicated gRNAs. The decrease in depth at the cut site for the targeted homolog, relative to the non-targeted homolog, is indicative of the specificity of the gRNAs for the targets.

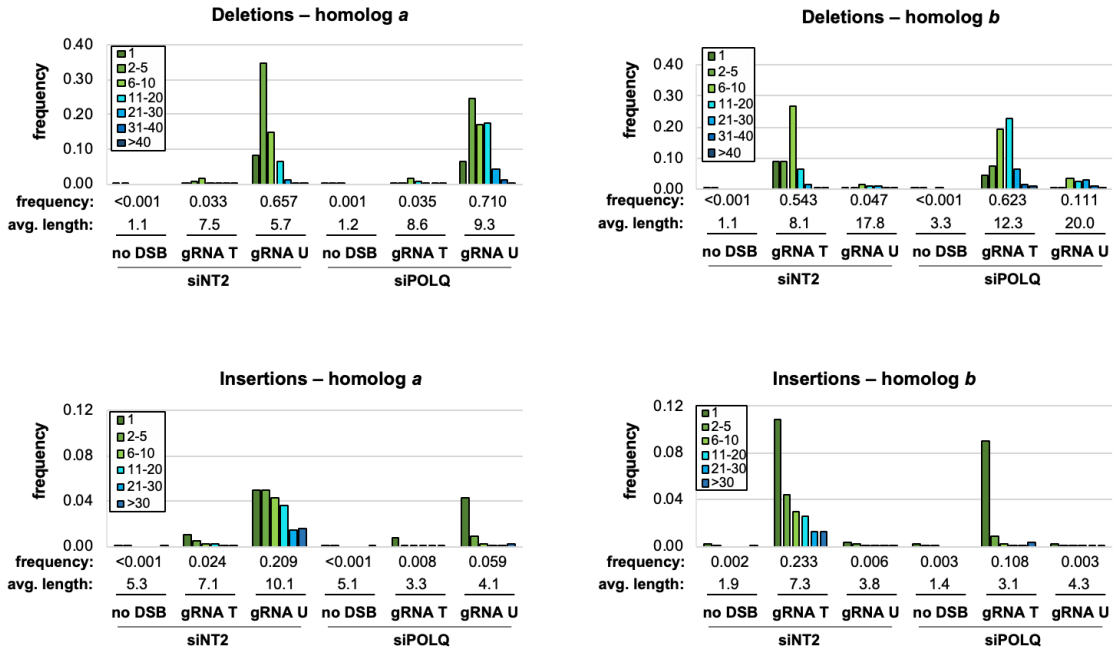

Fig. S6. Targeting of homolog-specific deletions and insertions by gRNA T or gRNA U

Frequencies of binned deletions (*above*) and insertions (*below*) surrounding the sites targeted by gRNA T and gRNA U on homologs ***a*** and ***b*** in HT1080 K2 cells. Cell populations were treated with siNT2 or siPOLQ; and transfected with gRNAs NC (non-targeting), T or U, as indicated. Frequencies were calculated among reads of homologs ***a*** or ***b***, as indicated above the graph. The specificity of cleavage is evidenced by the relatively low frequency of indels on the non-targeted homolog, although all non-targeted indel frequencies are significantly above background with the exception of insertions due to mistargeting by gRNA U on homolog ***b***. Frequencies and lengths of indels are shown below binned results for each sample; and tabulated in Fig. S7A.

siRNA	gRNA	DEL frequency (av length, bp)		INS frequency (av length, bp)	
		homolog *a*	homolog b	homolog *a*	homolog b
siNT2	gRNA-NC	**0.0007** (1.1 bp)	**0.0005** (1.1 bp)	**0.0002** (5.3 bp)	**0.0024** (1.9 bp)
siNT2	gRNA-T	**0.0325** (7.5 bp)	**0.5427** (8.1 bp)	**0.0237** (7.1 bp)	**0.2326** (7.3 bp)
siNT2	gRNA-U	**0.6570** (5.7 bp)	**0.0473** (17.8 bp)	**0.2091** (10.1 bp)	**0.0063** (3.8 bp)
siPOLQ	gRNA-NC	**0.0010** (1.2 bp)	**0.0007** (3.3 bp)	**0.0006** (5.1 bp)	**0.0025** (1.4 bp)
siPOLQ	gRNA-T	**0.0345** (8.6 bp)	**0.6234** (12.3)	**0.0084** (3.3 bp)	**0.1077** (3.1 bp)
siPOLQ	gRNA-U	**0.7097** (9.3 bp)	**0.1110** (20.0 bp)	**0.0587** (4.1 bp)	**0.0028** (4.3 bp)

Fig. S7. The effect of POLQ depletion on deletion and insertion frequencies and lengths at DSBs targeted by gRNA T or U

Summary table of cumulative frequencies of deletions and insertions (DEL and INS) on the indicated homolog, as graphed in Fig. S6. Results for the homolog specifically targeted by the gRNA are highlighted (yellow). All frequencies are significantly different from the corresponding gRNA NC non-targeting control ($p<0.0001$: Fisher's exact test) except insertions on homolog b with gRNA U in siPOLQ-treated cells (lower right).

siRNA	gRNA		DEL frequency (av length, bp)	INS frequency (av length, bp)
siNT2	gRNA-NC	unsorted	**0.0006** (1.1)	**0.0015** (3.3)
siNT2	gRNAs T+U	unsorted	**0.5490** (7.7)	**0.2058** (8.4)
siNT2	gRNAs T+U	sCD44+	**0.4993** (13.2)	**0.5169** (11.0)
siPOLQ	gRNAs T+U	unsorted	**0.5984** (12.5)	**0.0656** (3.6)
siPOLQ	gRNAs T+U	sCD44+	**0.6705** (19.3)	**0.2867** (9.4)

Fig. S8. The effect of POLQ depletion on deletion and insertion frequencies and lengths at DSBs targeted by gRNAs T+U

Summary table of cumulative frequencies of deletions and insertions (DEL and INS) at DSBs targeted by gRNAs T+U to homologs ***a*** and ***b***, as graphed in Fig. 5C. Frequencies were calculated among total reads (both homologs). Results of analyses of sCD44+ cells are highlighted (green).

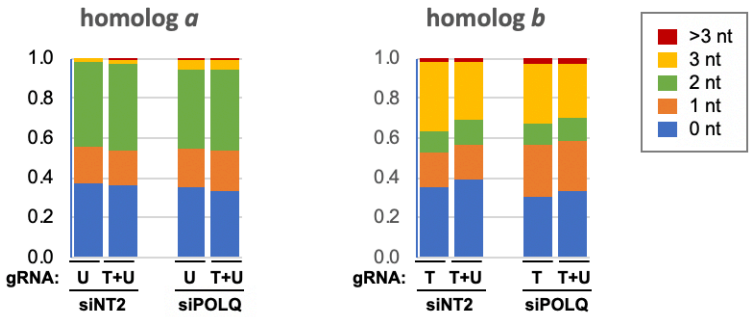

Fig. S9. Junctional microhomologies

Fractional representation of microhomologies of 0, 1, 2, 3 nt or >3 nt at deletions generated on homolog ***a*** or ***b*** at DSBs targeted by gRNA U (*homolog **a**)*, gRNA T (*homolog **b***), or gRNAs T+U (both homologs), in unsorted cells treated with siNT2 (non-targeting) or siPOLQ.

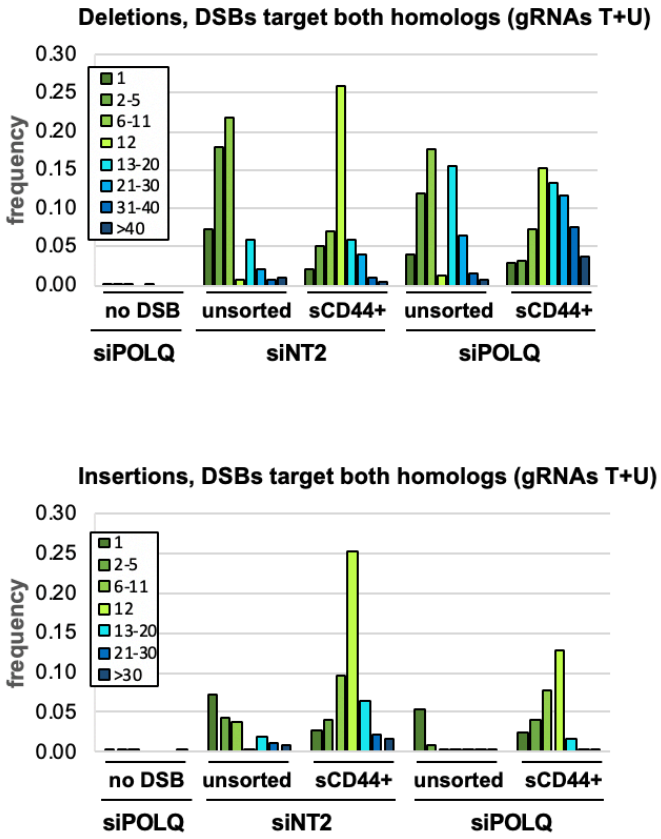

Fig. S10. Replicate analysis of deletions and insertions at DSBs targeted by gRNAs T+U

Frequencies of binned deletions *(above*) and insertions (*below*) in control parental cells (no DSB) or at DSBs targeted by gRNAs T+U to homologs ***a*** and ***b***. A separate bin is devoted to 12 bp deletions and insertions. Cells were unsorted or sorted for sCD44+; and treated with control siNT2 or siPOLQ, as indicated. Frequencies were calculated among total reads (both homologs). This is a replicate of the experiment presented in Fig. 5C, except in this case the "no DSB" sample was from cells treated with siPOLQ.

Table S1. Genomic sequence (+ strand) of gRNA targets

gRNA	location	sequence	plasmid or crRNA
CD44-4	exon 1	CCTcgtgccgctgagcctggcgc	plasmid
CD44-D1-2	exon 1	CCTcgtggccgctgagcctggcg	plasmid
CD44T-17-1	exon 17	accagaatggctgatcatctTGG	plasmid
CD44-K1B	exon 1	CCCtgagcctggcgcagatcggt	plasmid
CD44-K1D	exon 1	CCTggggactctgacctcggtgc	plasmid
CD44T-17-2	exon 17	CCAgaatggctgatcatcttggc	plasmid
Cleave both alleles			
B	intron 1	ggatgcgcacagtcgttgtcTGG	plasmid
E	intron 2	CCTgagctacatgagccgttgct	plasmid
F	intron 1	CCTatgcaactagggtagcctga	plasmid
S	intron 7	cccctccagagcttaatctaTGG	plasmid and crRNA
Allele-specific			
U	intron 1***a***	gatccatggtacctaggagtGGG	crRNA
Y	intron 1***a***	CCAtggtacctaggagtgggggg	plasmid and crRNA
Z	intron 1***a***	ctgttggatccatggtacctAGG	plasmid
M	intron 1***b***	CCAgtgcatcccactcccagat	plasmid and crRNA
T	intron 1***b***	agaaagactctgttggatccAGG	crRNA
V	intron 1***b***	CCTgactttcccacttggatgac	plasmid
W	intron 1***b***	CCAggaccaggagtgggggtta	plasmid
X	intron 1***b***	actctgttggatccaggaccAGG	plasmid
No cleavage			
NC	none	IDT catalog # 1072545	crRNA

UPPER CASE, PAM (protospacer adjacent motif); lower case, protospacer; red font, variants that distinguish intron 1 from intron 1-1. Also see maps and sequences in Fig. S3.

Table S2. Primer and donor oligonucleotide sequences.

oligo	region	sequence
CD44-F2	exon 1	5'-CTTGCTTGGGTGTGTCCTTC-3'
CD44-R2	exon 1	5'-CCAAATGGTGCTTCCACAGAC-3'
CD44-F4	exon 17	5'-GGCTGTTGGACAAATACCTTCA-3'
CD44-R4	exon 17	5'-CTGTCTCTAAAAACCGGGGC-3'
CD44-F16	intron 1	5'-CTGTCCCAGGTATGCACATCA-3'
CD44-R14	intron 1	5'-GCAGTCAACGCTGAACCAAC-3'
rs85074-F1	rs85074	5'-GTGACTACAGACCAGCTGATACA-3'
rs85074-R1	rs85074	5'-GGGCGATCCTTTCATCGTCA-3'
rs7950932-F1	rs7950932	5'-AGATGTAGGGCACTGGACTGT-3'
rs7950932-R1	rs7950932	5'-AGAGCCGTACGTGGCTTTTC-3'
Nxtra_CD44-i1-1-F1	intron 1	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGN NNNNNNNNNACGGGGTGGGTACTGTGGTTTC-3'
Nxtra_CD44-i1-1-R1	intron 1	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CTGTTACCCTGGCCCTTCAG-3'
Nxtra_CD44-3k-F1	intron 1	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNN NNNNNNNNACAGAGGAAGTACAATGAAGTGCCAG-3'
Nxtra_CD44-3k-R1	intron 1	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGT CTCCACTTTTTGCTTTCCCATAC-3'
NxtraCD44-V3-1-F	exon 1	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNN NNNNNNNNACTTCGGTCCGCCATCCTCGTC-3'
NxtraCD44-V3-1-R	exon 1	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GCAAATCCCAGCCCTGCTTTC-3'
CD44-1-TOP	exon 1 (silent mutations)	5'-CGGACACCATGGACAAGTTTTGGTGGCATGCCG CATGGGGACTCTGTCTCGTGCCGCTGAGCCTGGCGCAGAT CGGTGAGTGCCCGCCGCAGCCTGGGC-3'
CD44T-17eng1	exon 17 (insertion/ deletion)	5'-CTGAAGCTCACGCATGTCATTTAATTTACTCATACC AGAAgcttgaatttagctagcGATTCTTGCAGTTTGCATTGCAGTCAA CAGTCGAAGAAGg-3'
CD44-1-TOP3	exon 1 (wt)	5'-ACCATGGACAAGTTTTGGTGGCACGCAGCCTGGG GACTCTGCCTCGTGCCGCTGAGCCTGGCGCAGATCGGTGA GTGCCCGCCGCAGCCTGGGCAGCAA-3'
CD44-17-0	exon 17 (wt)	5'- CTGAAGCTCACGCATGTCATTTAATTTACTCATACC AGAATGGCTGATCATCTTGGCATCCCTCTTGGCCTTGGCTTT GATTCTTGCAGTTTGCATTGCAGTCAACAGTCGAAGAAGG-3'
CD44-intron1-1	intron 1	5'-GGGACATATACTTTCTTTTGCCAGAAAGACTCTGT TGGATCCAtGGtACCtAGGAGTGGGGGGTTAAACAGTTCTCC ATACCCTCACCTCCAAG-3'

red font, mutations; lower case, insertions