Supplementary Figure 1 | Cell cycle effects of POLD depletion.

(*A*) Schematic of the ZITR assay. Top panel: *AAVS1* locus with zinc finger nuclease (ZFN) cut site downstream of the endogenous promoter; cassette of promoter-less *GFP* transgene downstream of an *AAVS1* recognition site integrated on a heterologous chromosome. Bottom panel: Chromosomal translocation juxtaposes the endogenous *AAVS1* promoter with *GFP* transgene leading to GFP expression. Scissors, *AAVS1* ZFNs. Dotted lines, targeted cutting at *AAVS1* recognition sequences. (*B*) Schematic of the CRITR assay. Top two panels: endogenous *CD71* and *CD4* loci showing the orientation of the *CD71* promoter and the *CD4* open reading frame (ORF) on human chromosomes 3 (chr3, green) and 12 (chr12, blue) respectively. DNA sequences targeted by the *CD71* and *CD4* CRISPR gRNAs are shown by orange lines, PAM sequence indicated by red letters, CRISPR/Cas9 cut site indicated by pink dotted lines. Bottom panel: Sequence of translocation junction without any end processing (referred to as 'exact'). Translocation juxtaposes the *CD71* promoter with the *CD4* ORF leading to the expression of the *CD4* gene, which is quantified by flow cytometry. **(***C***)** Cell cycle dynamics for 293T cells transfected with control non-targeting siRNA (siCTRL) or siPOLD2 #1 or #2. Data are presented as mean ± Standard Error (SE) of n=3. *P* values calculated using a one-way ANOVA with Tukey's correction. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* <0.0001.

Supplementary Figure 2 | Single antibody controls for PLA assays.

Proximity ligation assay (PLA) using antibodies against the indicated targets at 2 hours following 10 Gy ionizing radiation (IR) or mock treatment.

Supplementary Figure 3 | POLD-dependent CD4/CD71 translocation junctions.

(*A*) Alignment of insertion sequences ≥ 20 bp from CD4/CD71 translocation junctions in 293T cells. (*B*) Deletion lengths and (*C*) microhomology usage at CRISPR/Cas9-mediated CD4/CD71 translocation junctions in 293T cells. Deletion lengths include complex junctions (i.e. junctions with both sequence

loss and gain). Data are combined from three independent experiments. *P* values calculated using a Student's *t*-test (*B*) or two-way ANOVA with Tukey's correction (*C*). There were no significant differences.

Supplementary Figure 4 | Linear PCR for Hi-FIBR

Optimization of linear PCR to produce *CD4* and *ESR1* amplicons for deep sequencing and Hi-FIBR analysis.

Supplementary Figure 5 | POLD1 mutants in intrachromosomal repair

Hi-FIBR analysis of (*A*) DSB repair classes, (*B*) microhomology usage, (*C*) deletion and (*D*) insertion lengths for intrachromosomal repair of CRISPR/Cas9-induced DSB in the *ESR1* locus in 293T cells transduced with siRNA-resistant POLD1 WT, D316G, or S605del or empty vector (EV). Cells were transfected with siCTRL or siPOLD1 #2 and 72 hrs later transfected with the same siRNA and Cas9/gRNA targeting *ESR1*. DNA was harvested 48 hrs later. (*E*) Deletion and (*F*) insertion lengths for intrachromosomal repair of CRISPR/Cas9-induced DSB in the *CD4* locus in 293T cells, as in (*C,D*). Data are presented as mean ± SE of n = 3 independent experiments. *P* values calculated using a two-way ANOVA (*A,B*) or one-way ANOVA with Tukey's correction (*C-F*). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****p<0.001.

Supplementary Figure 6 | Alt-NHEJ factors in intrachromosomal repair

(*A*) Relative proliferation of 293T cells 5 days after transfection with the indicated siRNA. CTRL, non-targeting control siRNA; D2, siPOLD2 #1; Q, POLQ; LIG3, Ligase 3; B, POLB. (*B*) RT-qPCR for the indicated transcripts. B, POLB; D2, POLD2 #1. (*C*,*D*) DSB repair class and microhomology usage for intrachromosomal repair of CRISPR/Cas9-induced DSB in the *CD4* locus in 293T cells. Cells were transfected with the indicated siRNA and 72

hrs later transfected with the same siRNA and Cas9/gRNA targeting *CD4*. DNA was harvested 48 hrs later. Data are presented as mean ± SE of n = 3 independent experiments. *P* values calculated using a one-way (*A*,*B*) or two-way (*C*,*D*) ANOVA with Tukey's correction. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* <0.0001.

siRNA	Vector	Replicate	Total Reads	Threshold Reads	Excluded Reads	% Excluded	Exact Reads	Deletion Reads	Complex Reads	Insertion Reads
None	Empty No Cas9	1	55982	55862	120	0.214354614	55294	0	568	0
None	Empty	2	85966	76735	9231	10.73796617	30537	36321	3398	6479
None	Empty	3	97867	86997	10870	11.1069104	36548	38978	3828	7643
None	POLD2	1	96149	86072	10077	10.48060822	31081	43077	3711	8203
None	POLD2	2	79881	70727	9154	11.45954607	28318	33268	2802	6339
None	POLD2	3	67300	58490	8810	13.09063893	23450	27509	2126	5405
siCTRL	Empty	1	86593	76674	9919	11.45473653	32296	35057	3383	5938
siCTRL	Empty	2	99019	85538	13481	13.61455882	31204	41680	4338	8316
siCTRL	Empty	3	109294	97263	12031	11.00792358	42345	42556	4593	7769
siCTRL	POLD2	1	36011	30451	5560	15.43972675	12092	14674	1006	2679
siCTRL	POLD2	2	38304	32226	6078	15.86779449	12879	15125	1154	3068
siCTRL	POLD2	3	98128	85023	13105	13.35500571	31212	41952	3802	8057
siPOLD2	Empty	1	65698	57721	7977	12.14192213	32299	18877	1892	4653
siPOLD2	Empty	2	51860	45148	6712	12.9425376	25544	14326	1357	3921
siPOLD2	Empty	3	66449	59022	7427	11.17699288	34810	17734	1832	4646
siPOLD2	POLD2	1	91830	80245	11585	12.61570293	29380	39873	3859	7133
siPOLD2	POLD2	2	81416	71013	10403	12.77758672	29965	32627	3011	5410
siPOLD2	POLD2	3	121372	107042	14330	11.80667699	43787	50141	4706	8408

Supplementary Table 1. NGS read counts from the *CD4* locus. Total Reads: The total number of reads in each file following Mapping and Hi-FiBR analysis. Threshold Reads: The number of reads remaining after excluding sequences represented by <10 reads. Excluded reads: The number of reads excluded from the dataset because the sequences were represented by <10 reads. % Excluded = 100*Excluded Reads/ Total Reads. Exact reads: The number of reads corresponding to perfect repair. Deletion Reads: The number of reads corresponding to simple deletions. Complex Reads: The number of reads corresponding to complex repair. Insertion Reads: The number of reads corresponding to simple insertions.

siRNA	Vector	Replicate	Total Reads	Threshold Reads	Excluded Reads	% Excluded	Exact Reads	Deletion Reads	Complex Reads	Insertion Reads
CTRL	Empty	1	43354	40245	3109	7.171195276	12757	22725	1179	3584
CTRL	Empty	2	54167	50596	3571	6.592574815	17064	27755	1655	4122
CTRL	Empty	3	78774	73969	4805	6.099728337	21768	42635	2744	6822
siPOLD2	Empty	1	84736	79748	4988	5.886518127	34193	34895	3020	7640
siPOLD2	Empty	2	33425	30928	2497	7.470456245	13542	13365	851	3170
siPOLD2	Empty	3	42101	39212	2889	6.862069785	16216	16824	1285	4887

Supplementary Table 2. NGS read counts from the *ESR1* locus. As in Supplementary table 1.

CD4 Proximal Junctions								
Left Alignment	Insertion	Right Alignment	Untransfected		siCTRL		siPOLD2	
TATACAGGATGCTACCGTA		CTAGGGTAAGACACCTCTG	Empty	POLD2	Empty	POLD2	Empty	POLD2
TATACAGGATGCTACCGTA		CTAGGGTAAGACACCTCTG	36.57	34.05	35.89	32.58	50.35	35.01
TATACAGGATGCTACCGTA	A	CTAGGGTAAGACACCTCTG	5.22	5.64	4.87	5.55	5.09	4.85
TATACAGGATGCTACCGTA		----------AGACACCTCTG	7.19	7.29	6.65	6.86	4.33	6.92
TATACAGGATGCTA----------		-------GGGTAAGACACCTCTG	6.56	7.43	6.24	7.22	3.62	6.53
TATACAGGATGCTACCGTA		------GGGTAAGACACCTCTG	4.71	4.23	5.03	4.84	3.42	5.01
TATACAGGATGCTACCGTA		--TAGGGTAAGACACCTCTG	1.37	1.30	1.38	1.26	1.29	1.22
TATACAGGATGCTACC-------		--TAGGGTAAGACACCTCTG	2.34	2.52	2.29	2.58	1.24	2.62
TATACAGGATGCTACCGT--		CTAGGGTAAGACACCTCTG	0.92	0.93	0.99	1.06	1.00	1.06
TATACAGGATGCTACCGTA		----------TAAGACACCTCTG	0.84	0.94	0.81	0.90	0.94	0.89
TATACAGGATGCTACCGTA		----------GACACCTCTG	0.80	0.79	0.72	0.82	0.70	0.76
TATACAGGATGCTAC---------		--TAGGGTAAGACACCTCTG	0.70	0.83	0.74	0.82	0.59	0.79
TATACAGGATGCTACCGTA		---AGGGTAAGACACCTCTG	0.55	0.46	0.56	0.47	0.51	0.46
TATACAGGATGCTACCG----		CTAGGGTAAGACACCTCTG	0.58	0.52	0.64	0.59	0.50	0.52
TATACAGGATGCTACCGTA	T	CTAGGGTAAGACACCTCTG	0.54	0.59	0.48	0.63	0.47	0.49
TATACAGGATGCTACCGTA		----------ACACCTCTG	0.50	0.58	0.50	0.57	0.43	0.56
TATACAGGATGCTACC-------		CTAGGGTAAGACACCTCTG	0.90	0.88	0.95	0.89	0.43	1.00
TATACAGGATGCTACCGTA	CTA	---------GGTAAGACACCTCTG	0.57	0.41	0.63	0.57	0.42	0.49
TATACAGGATGCTACCGTA	G	CTAGGGTAAGACACCTCTG	0.48	0.52	0.55	0.63	0.42	0.52
TATACAGGATGCTACC------		----------TCTG	0.71	0.83	0.69	0.70	0.41	0.70
TATACAGGATGCTACCGTA	TA	CTAGGGTAAGACACCTCTG	0.26	0.25	0.21	0.24	0.40	0.21
TATACAGGATGCTACCGTA		----------GTAAGACACCTCTG	0.40	0.38	0.38	0.38	0.36	0.38

Supplementary Table 3. The top twenty mutated *CD4* alleles after CRISPR/Cas9 treatment and NGS. The values presented here are the mean percentages of each junction normalized to total read count. n=3 Blue = gRNA, Green = PAM, Red = inserted sequence.

ESR1 Proximal Junctions				
Left Alignment	Insertion	Right Alignment	siCTRL	siPOLD2
AGAGACGGGGAAAGCCAAAC		GACACAATGAGCGTTCTCA		
AGAGACGGGGAAAGCCAAAC		GACACAATGAGCGTTCTCA	29.26	39.90
AGAGACGGGGAAAGCCAAAC		GACACAATGAGCGTTCTCA	14.59	8.46
AGAGACGGGGAAAGCCAAAC	G	GACACAATGAGCGTTCTCA	6.22	4.23
AGAGACGGGGAAAGCCAA----		----------TGAGCGTTCTCA	5.44	6.46
AGAGACGGGGAAAGCCAAAC		----------AATGAGCGTTCTCA	5.38	3.54
AGAGACGGGGAAAGCCAA----		GACACAATGAGCGTTCTCA	1.69	2.56
AGAGACGGGGAAAGCCAAAC		--ACACAATGAGCGTTCTCA	1.48	1.35
AGAGACGGGGAA----------		GACACAATGAGCGTTCTCA	1.38	1.14
AGAGACGGGGA----------		GACACAATGAGCGTTCTCA	1.22	1.25
AGAGACGGGGAAAGCCAAAC	T	GACACAATGAGCGTTCTCA	1.15	1.17
AGAGACGGGGAAAG----------		--ACACAATGAGCGTTCTCA	1.04	0.65
AGAGACGGGGAAAGCCAAA--		GACACAATGAGCGTTCTCA	1.01	0.69
AGAGACGGGGAAAGCCAAAC	A	GACACAATGAGCGTTCTCA	1.00	0.70
AGAGACGGGG----------		GACACAATGAGCGTTCTCA	0.81	1.15
AGAGACGGGGAAAGCCAAAC		----------GAGCGTTCTCA	0.78	0.77
AGAGACGGGGAAAGCCAAA--		----------TGAGCGTTCTCA	0.74	0.74
AGAGACGGGGAAAGCCA------		--------CAATGAGCGTTCTCA	0.71	0.53
AGAGACGGGGAAAGCCA------		GACACAATGAGCGTTCTCA	0.69	0.94
AGAGACGGGGA----------		----CACAATGAGCGTTCTCA	0.63	0.68
AGAGACGGGGAAAGCCAAAC	GA	GACACAATGAGCGTTCTCA	0.62	0.47
AGAGACGGGGAAAGCCAAAC		----------29bp	0.59	0.26

Supplementary Table 4. The top twenty mutated *ESR1* alleles after CRISPR/Cas9 treatment and NGS. The values presented here are the mean of percentages of each junction normalized to total read count. n=3 Blue = gRNA, Green = PAM, Red = inserted sequence.

Gene product	Oligonucleotide primer sequences (5′–3′)	
	Forward	**Reverse**
CD4 break	GCTTGAGGGACCAGAGAGC	TGATTCCCATACTCAGCGCC
CD4 reference	AGAAGAGGCAAGGGGCATTC	GCTCCCTGTGTCTGTGTCTG
GAPDH	ACCCACTCCTCCACCTTTGA	CATACCAGGAAATGAGCTTGACAA
LIG3	GAACGATAAGCAGATTGTGAAGC	GGGAAAGACTTGCTCTGCTC
POLB	GTTTGCCAGCTTCCCAGTAA	AAACCCTTTTCTAGGGCATGA
POLD2	TCCAAATGAGACCCTTCCTG	CCACACAGCACTTCTCCTCA
POLQ	GGAGGTGGAGGTGATTCTGA	CTGCTGCTTCCCTTTCAGTT
CD4 gRNA	TACAGGATGCTACCGTACTA	
CD71 gRNA	ACTAGCATTGTGATCGATTC	
ESR1 gRNA	AACGACACAATGAGCGTTCT	

Supplementary Table 5. Primer sequences used for quantitative real-time PCR and gRNA for CRISPR/Cas9.

					Microhomology	
	Frequency of rearrangements	Proportion of deletions	Deletion length	Insertion length	0 bp	≥ 3 bp
POLD1 knockdown (% change relative to Wild-type)	Decreased $p = 0.0089$ (-26.4%)	Decreased $p = 0.0002$ (-2.37%)	ns	ns	Increased $p < 0.0001$ (2.05%)	Decreased $p < 0.0001$ (-2.44%)
		Complementation				
POLD1 Wild-type (% change relative to Wild-type)	Yes (0)	Yes (0.18%)	n/a	n/a	Yes (0.74%)	Partial (-1.08%)
POLD1 D316G. exonuclease mutant (% change relative to Wild-type)	No (-24.7%)	Yes (-0.95%)	n/a	n/a	No (1.70%)	No (-2.74%)
POLD1 del605S, synthesis mutant (% change relative to Wild-type)	No (-27.1%)	No (-2.14%)	n/a	n/a	No (1.27%)	No (-2.19%)

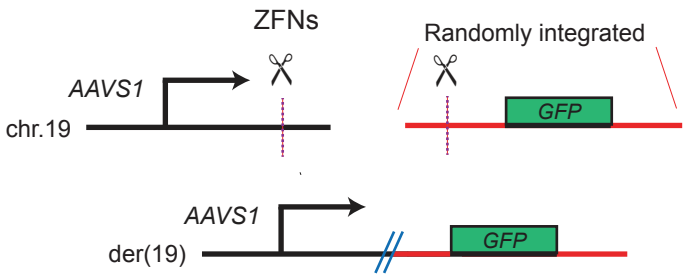
Supplementary Table 6. Statistics from Hi-FIBR analysis of intrachromosomal repair with siPOLD1 and complementation.

		Intrachromosomal				
					Microhomology	
	Frequency of rearrangements	Proportion of deletions	Deletion length	Insertion length	0 bp	≥ 3 bp
siPOLD2 (% change relative to Wild-type)	Decreased $p < 0.0001$ (-51.7%)	Decreased $p < 0.0001$ (-8.04%)	Decreased $p = 0.0017$ (-1.39 bp)	ns	Increased $p < 0.0001$ (5.96%)	Decreased $p < 0.0001$ (-3.38%)
siPOLQ (% change relative to Wild-type)	ns (-10.1%)	ns (-2.45%)	ns	ns	Increased $p = 0.0456$ (1.59%)	Decreased $p < 0.0001$ (-3.42%)
siLIG3 (% change relative to Wild-type)	Decreased $p = 0.0001$ (-26.9%)	Increased $p < 0.0001$ (4.91)	ns	ns	Decreased $p < 0.0001$ (-2.93%)	Increased $p = 0.0002$ (2.44%)
siLIG3 + siPOLD2 (relative to siLIG3) (% change relative to Wild-type)	Decreased $p = 0.0135$ (-31.9%)	Decreased $p = 0.0002$ (-4.00)	ns	ns	Increased $p < 0.0001$ (4.43%)	Decreased $p < 0.0001$ (-3.51%)

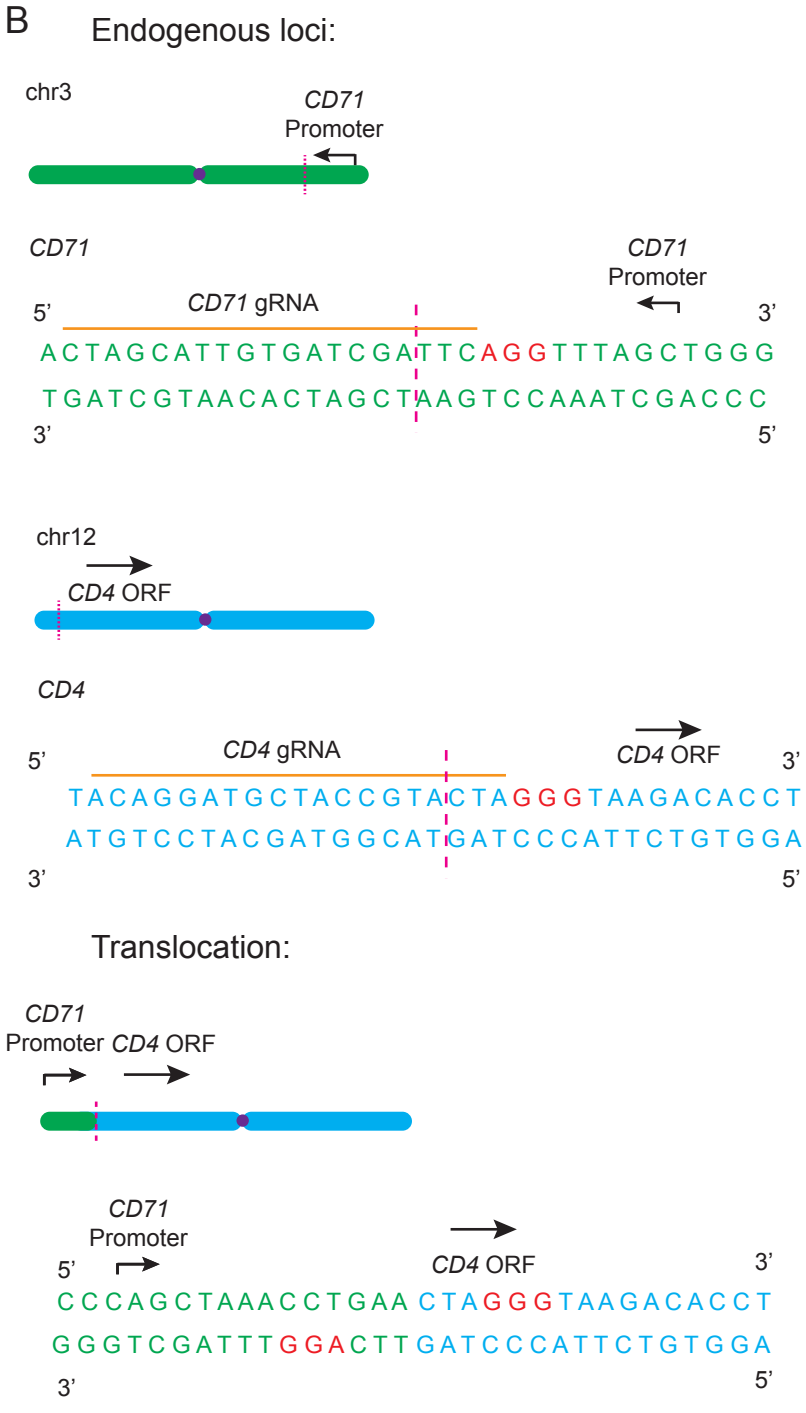
Supplementary Table 7. Statistics from Hi-FIBR analysis of intrachromosomal repair with siPOLD2, siPOLQ, and siLIG3.

Figure S1

A



B



C

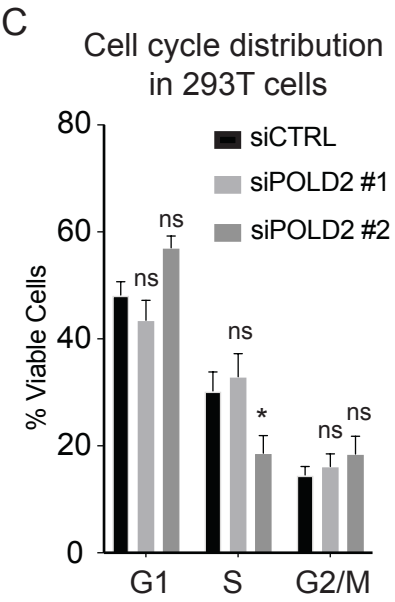


Figure S2

Single antibody PLA controls:

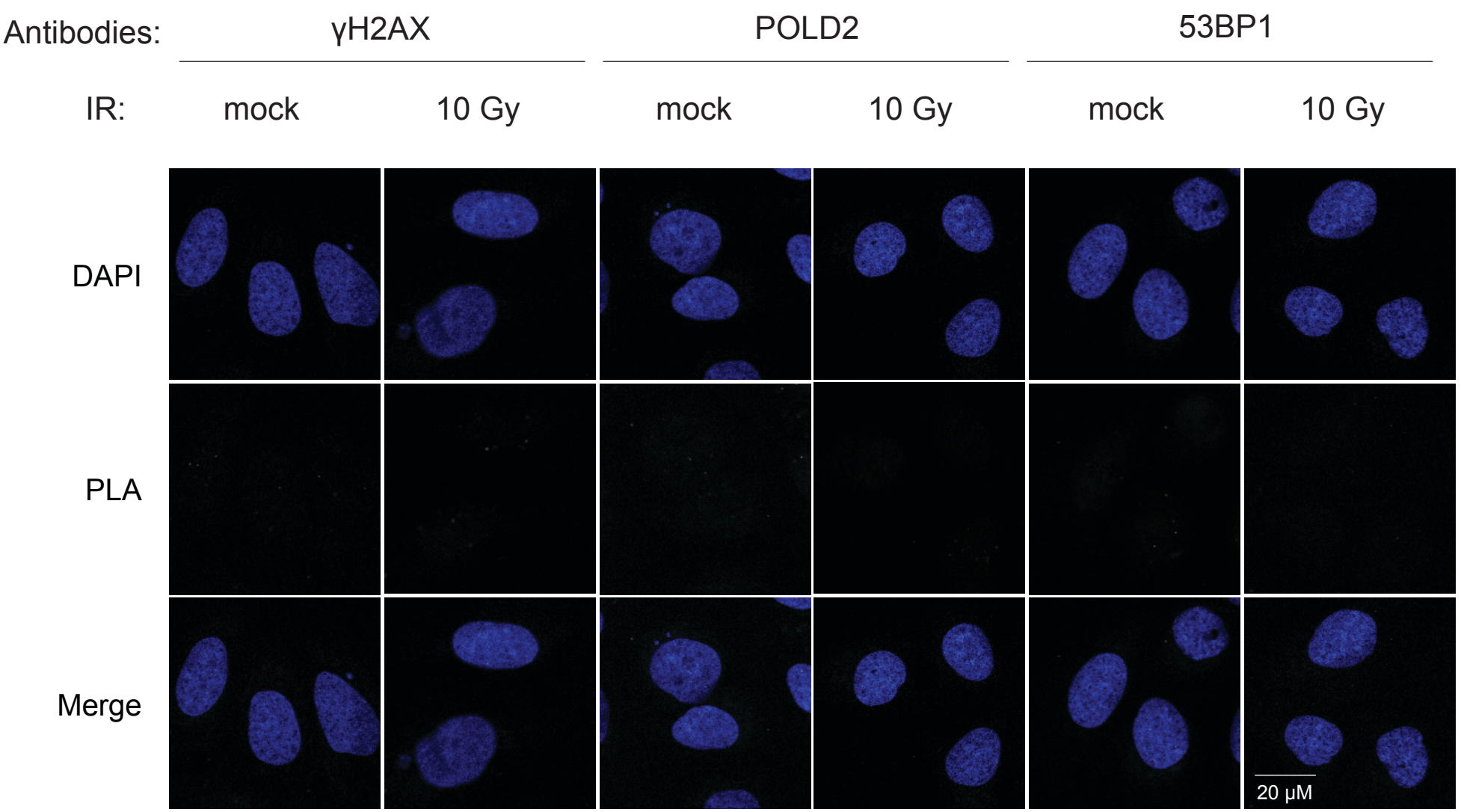


Figure S3

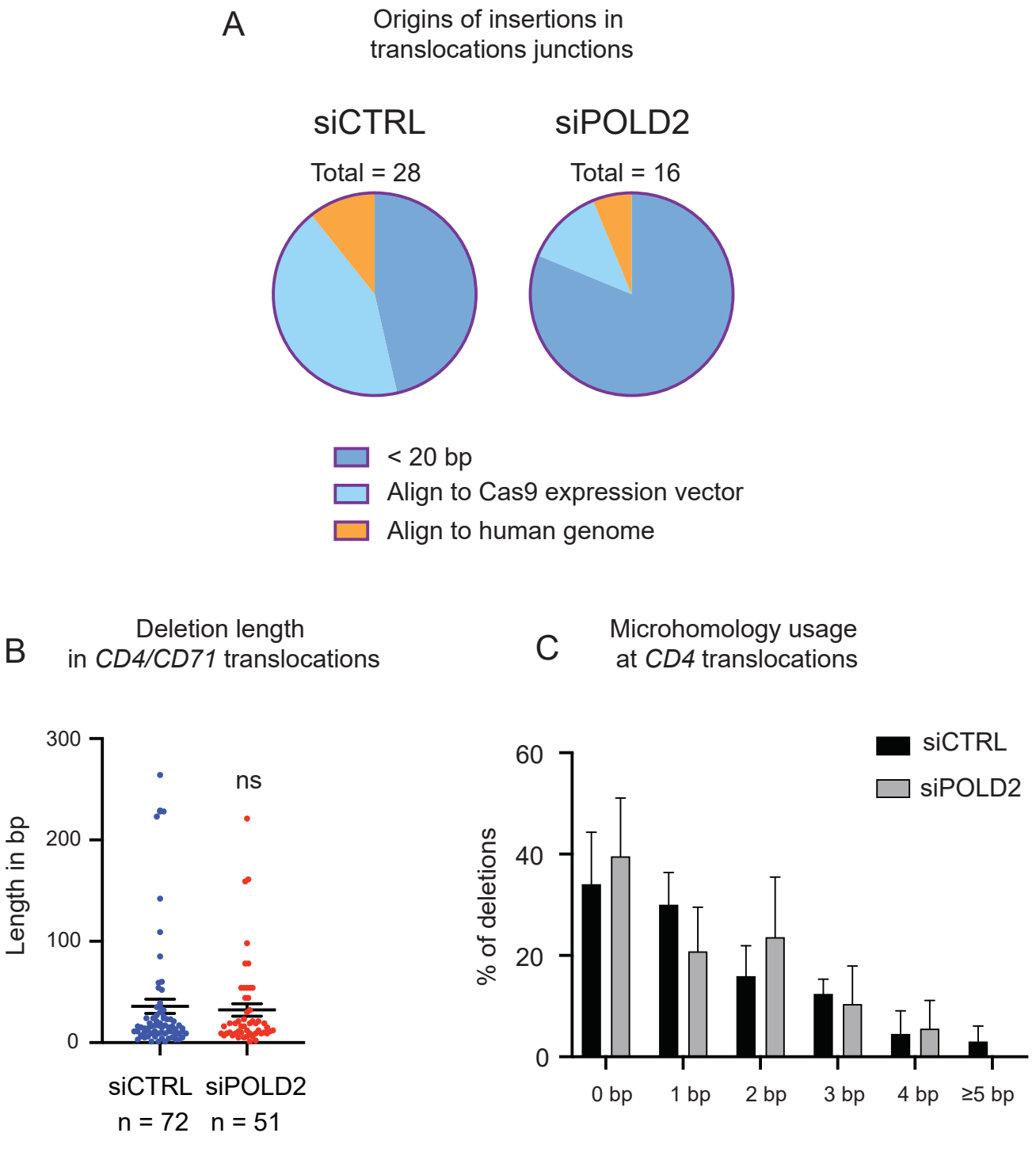

A
Origins of insertions in translocations junctions
siCTRL
Total = 28
siPOLD2
Total = 16
< 20 bp
Align to Cas9 expression vector
Align to human genome
B
Deletion length in CD4/CD71 translocations
300
200
100
0
Length in bp
ns
siCTRL
siPOLD2
n = 72
n = 51
C
Microhomology usage at CD4 translocations
60
40
20
0
% of deletions
siCTRL
siPOLD2
0 bp
1 bp
2 bp
3 bp
4 bp
≥5 bp

Figure S4

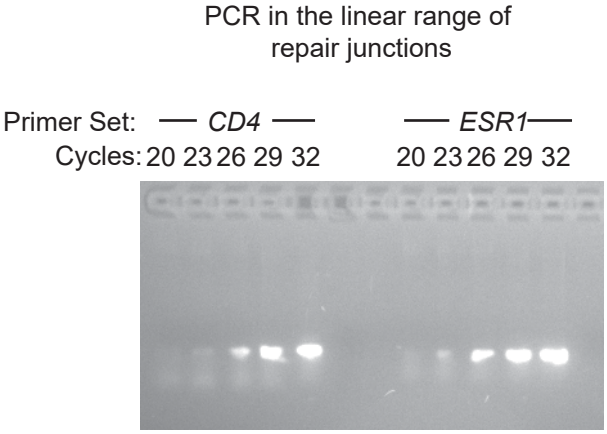

PCR in the linear range of
repair junctions
Primer Set: CD4 ESR1
Cycles: 20 23 26 29 32 20 23 26 29 32

Figure S5

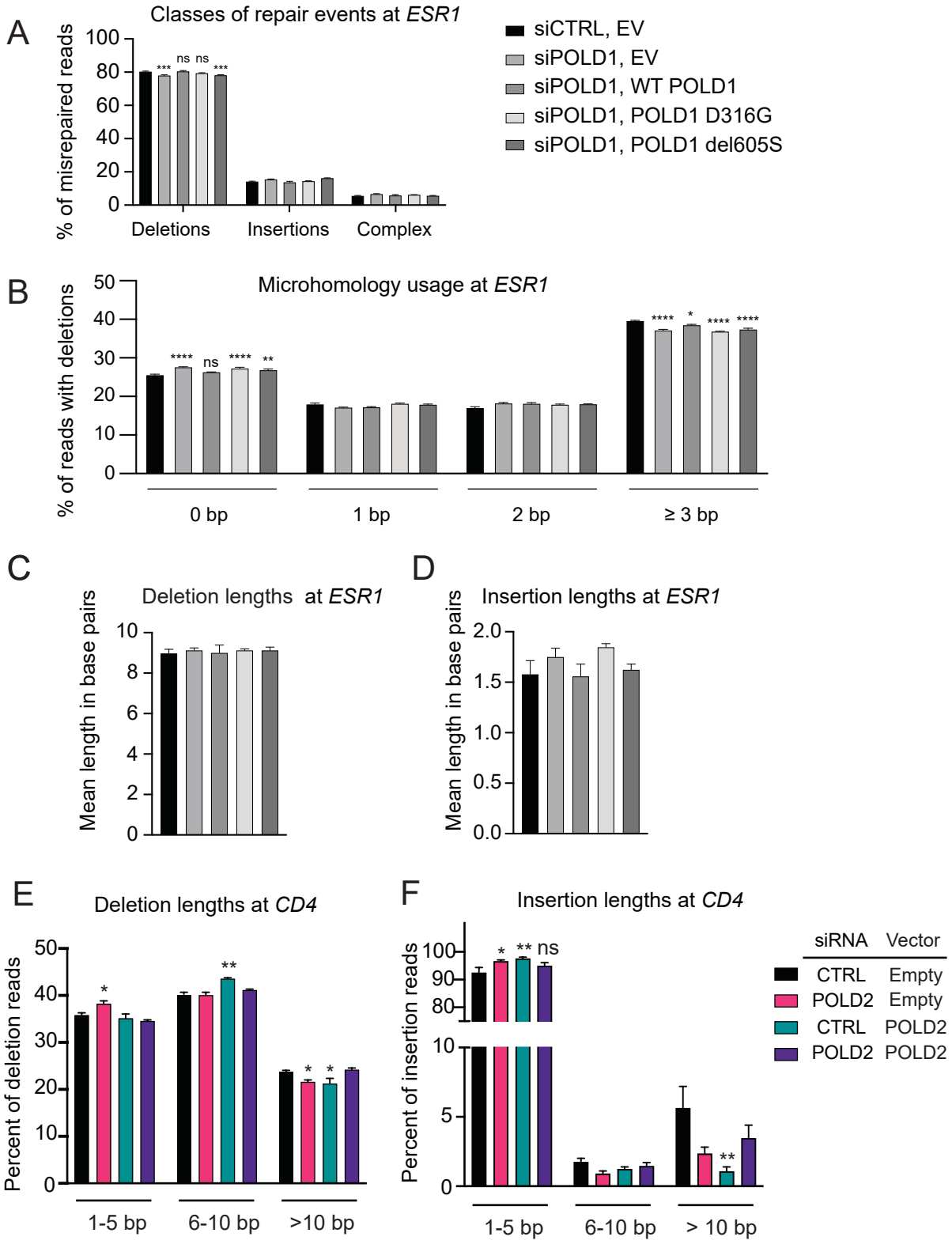
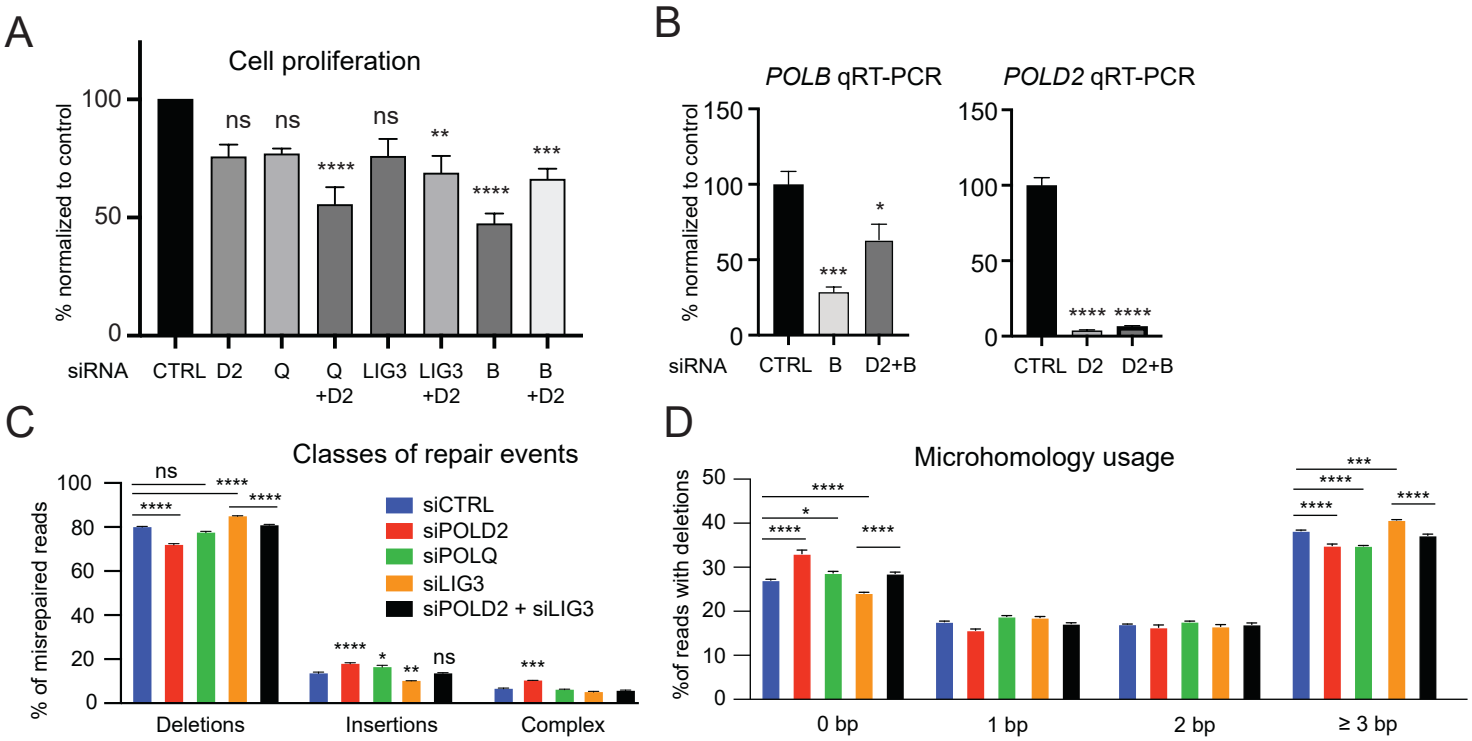

A
Classes of repair events at ESR1
% of misrepaired reads
siCTRL, EV
siPOLD1, EV
siPOLD1, WT POLD1
siPOLD1, POLD1 D316G
siPOLD1, POLD1 del605S
Deletions
Insertions
Complex
B
Microhomology usage at ESR1
% of reads with deletions
0 bp
1 bp
2 bp
≥ 3 bp
C
Deletion lengths at ESR1
Mean length in base pairs
D
Insertion lengths at ESR1
Mean length in base pairs
E
Deletion lengths at CD4
Percent of deletion reads
1-5 bp
6-10 bp
>10 bp
F
Insertion lengths at CD4
Percent of insertion reads
1-5 bp
6-10 bp
> 10 bp
siRNA Vector
CTRL Empty
POLD2 Empty
CTRL POLD2
POLD2 POLD2

Figure S6


A
Cell proliferation
% normalized to control
100
50
0
ns
ns
****
ns
**
****
***
siRNA
CTRL
D2
Q
Q +D2
LIG3
LIG3 +D2
B
B +D2
B
POLB qRT-PCR
POLD2 qRT-PCR
% normalized to control
150
100
50
0
***
*
****
****
siRNA
CTRL
B
D2+B
CTRL
D2
D2+B
C
Classes of repair events
% of misrepaired reads
100
80
60
40
20
0
ns
****
****
****
****
*
**
ns
***
siCTRL
siPOLD2
siPOLQ
siLIG3
siPOLD2 + siLIG3
Deletions
Insertions
Complex
D
Microhomology usage
%of reads with deletions
50
40
30
20
10
0
****
*
****
****
***
****
****
****
0 bp
1 bp
2 bp
≥ 3 bp