YMTHE, Volume *31*

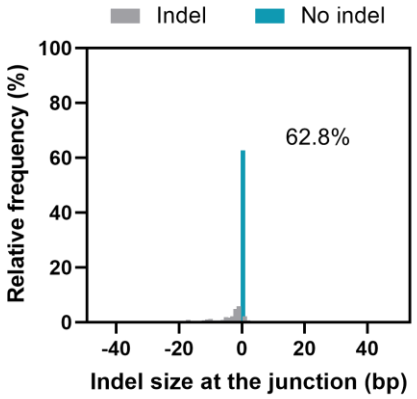
Supplemental Information

Targeted genomic translocations and inversions generated using a paired prime editing strategy

Jiyeon Kweon, Hye-Yeon Hwang, Haesun Ryu, An-Hee Jang, Daesik Kim, and Yongsub Kim

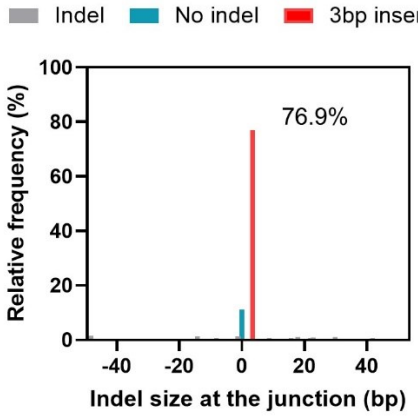
Figure S1. Analysis of Cas9-mediated DNA recombination in episomal reporter system.

Targeted sequencing results of amplicons generated using recombination-specific primers.




```
Direct junction
GATCCGGCACTGCGGCTGGAGGCGTGCTCAGTCTGGGCCAGCCAC Ref. (total: 5744)
GATCCGGCACTGCGGCTGGAGGCGTGCTCAGTCTGGGCCAGCCAC   3211 (55.9%)
GATCCGGCACTGCGGCTGGAGG-GTGCTCAGTCTGGGCCAGCCAC    223  (3.8%)
GATCCGGCACTGCGGCTGGAG--GTGCTCAGTCTGGGCCAGCCAC    213  (3.7%)
GATCCGGCACTGCGGCTGGAG----GCTCAGTCTGGGCCAGCCAC     67  (1.2%)
GATCCGGCACTGCGGCTGGA-GCGTGCTCAGTCTGGGCCAGCCAC     62  (1.1%)
GATCCGGCACTGCGGCTGGAGGACGTGCTCAGTCTGGGCCAGCCA     46  (0.8%)
GATCCGGCACTGCGGCTGGGGG---GCTCAGTCTGGGCCAGCCAC     45  (0.7%)D
```

Figure S2. Analysis of PETI-mediated genomic translocation in reporter integrated cells.
Targeted sequencing results of amplicons generated using translocation-specific primers.

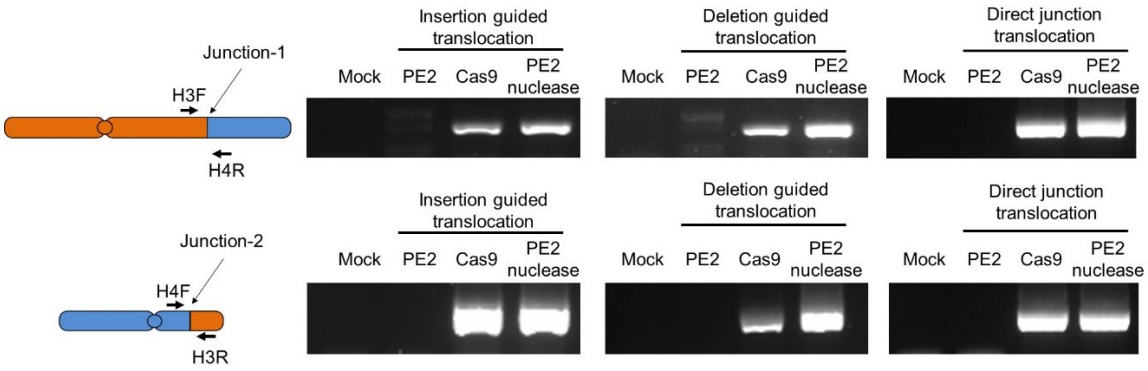


3bp insertion

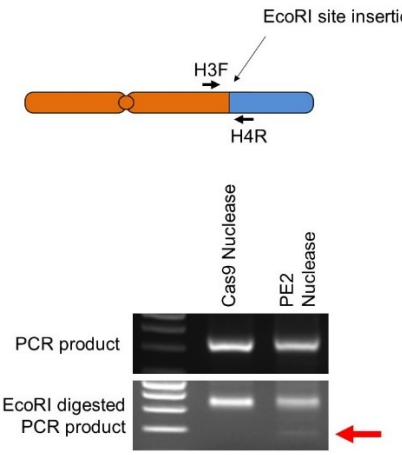
```
GATCCGGCACTGCGGCTGGAGG---CGTGCTCAGTCTGGGCCAGCCAC Ref. (total: 10680)
GATCCGGCACTGCGGCTGGAGGACGCGTGCTCAGTCTGGGCCAGCCAC   6916 (64.8%)
GATCCGGCACTGCGGCTGGAGG---CGTGCTCAGTCTGGGCCAGCCAC   1002  (9.4%)
GATCCGGCAC----------------TGCTCAGTCTGGGCCAGCCAC    135  (1.3%)
GATCCGGCACTGCGGCTGGAGG----GTGCTCAGTCTGGGCCAGCCAC    115  (1.1%)
GATCCGGCACTGCGGCTGGAGG----------GTCTGGGCCAGCCAC     67  (0.6%)
```

Figure S3. PCR assay for HEK3-HEK4 translocations in HEK293T cells. (a) PCR products were amplified by translocation-specific primers, either H3F (HEK3 forward) and H4R (HEK4 reverse) or H4F (HEK4 forward) and H3R (HEK3 reverse). (b,c) PE2-nuclease mediated insertion of a restriction enzyme site at translocation junctions.

a



b



c

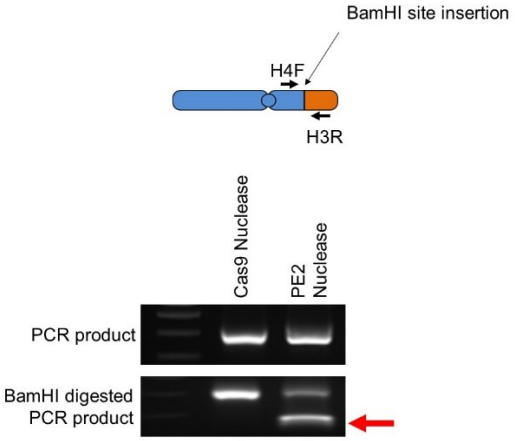


Figure S4. Estimation of HEK3-HEK4 translocation frequencies. The frequencies of translocations were estimated by digital PCR analysis using serially diluted samples. Genomic DNA samples isolated from Cas9 or PE2 nuclease treated cells were serially diluted in distilled water and diluted samples were then subjected to nested PCR using appropriate primers. Critical dilution points that support the amplification of breakpoint junctions were determined. The results were analyzed using the Extreme Limiting Dilution Analysis program (http://bioinf.wehi.edu.au/software/elda/).[1]

Dosage	Cas9				PE2 nuclesae											
					Insertion guided				Deletion guided				Direct junction guided			
	Junction-1		Junction-2		Junction-1		Junction-2		Junction-1		Junction-2		Junction-1		Junction-2	
pg	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response
66	12	1	12	1	12	0	12	0	12	0	12	0	12	0	12	0
660	24	5	24	9	24	4	24	12	24	2	24	11	24	2	24	4
3300	12	6	12	9	12	5	12	10	12	4	12	12	12	7	12	8

Figure S5. Types of translocations induced by DNA DSBs. Schematic of the chromosomal translocations induced by DNA DSBs. When two DSBs are induced simultaneously in different chromosomes, translocations can cause four different types of genomic rearrangements, two of reciprocal translocations, dicentric chromosome, acentric chromosome.

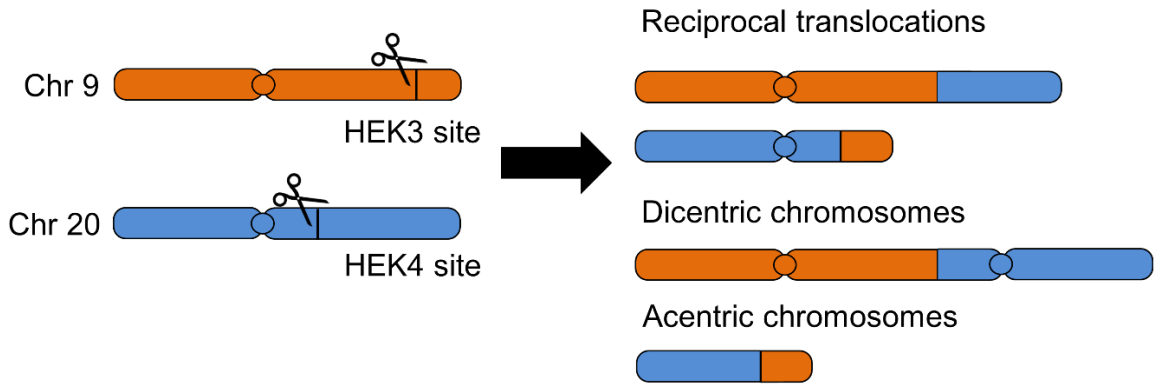
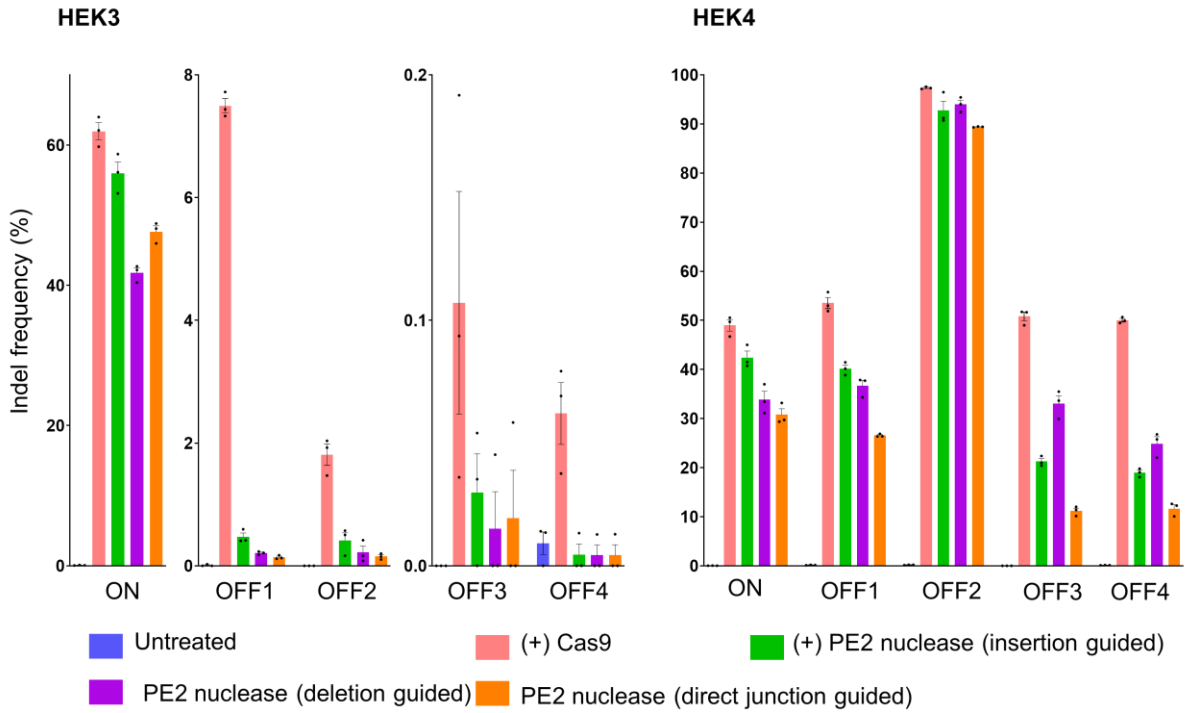


Figure S6. Off-target analysis of HEK3 and HEK4 target sites. Mutation frequencies induced by PE2, PE2 nuclease, or Cas9 nuclease at the HEK3 and HEK4 on- and off-target sites.



ON	GGCCCAGACTGAGCACGTGATGG
OFF1	caCCCAGACTGAGCACGTGcTGG
OFF2	aGCtCAGACTGAGCAaGTGAGGG
OFF3	GagCCAGAaTGAGCACGTGAGGG
OFF4	GGCCCAGACTGAGCAaaaGAAGG

ON	GGCACTGCGGCTGGAGGTGGGGG
OFF1	GGCAaTGCGGCTGGAGGcGGAGG
OFF2	GGCACgaCGGCTGGAGGTGGGGG
OFF3	GGCACTGCtGCTGGgGGTGGTGG
OFF4	aGCAgTGCGGCTaGAGGTGGTGG

Figure S7. Estimation of translocation frequencies between HEK3 on-target site and HEK4 off-target sites. The frequencies of translocations between HEK3 on-target site and HEK4 off-target sites were estimated by digital PCR analysis as mentioned in Supplementary Figure 4.

Dosage	HEK3 - HEK4 OFF1								HEK3 - HEK4 OFF2							
	Cas9				PE2 nuclease				Cas9				PE2 nuclease			
	Junction-1		Junction-2		Junction-1		Junction-2		Junction-1		Junction-2		Junction-1		Junction-2	
pg	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response
20000	1	1	1	1	5	4	1	1	-	-	-	-	4	3	4	4
10000	1	1	1	1	5	3	1	1	5	5	5	5	5	2	5	3
5000	5	4	1	1	5	1	5	4	5	4	5	5	5	1	5	2
2500	5	3	5	5	1	0	5	1	5	2	5	2	5	0	5	0
1250	5	3	5	2	1	0	5	0	5	1	5	2	1	0	1	0
625	1	0	5	0	1	0	1	0	1	1	1	0	1	0	1	0
313	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
156	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0
78	-	-	-	-	-	-	-	-	1	0	1	0	1	0	1	0

Figure S8. PCR assay for *NPM1-ALK* and *KIF5B-ALK* translocations in HEK293T cells. (a,b) Detection of PE2-, Cas9 nuclease-, or PE2 nuclease-mediated translocations using translocation-specific primers. (c) Schematic of the *NPM1-ALK* fusion transcript (left). Detection of the *NPM1-ALK* fusion transcript by RT-PCR using total RNA as template (right).

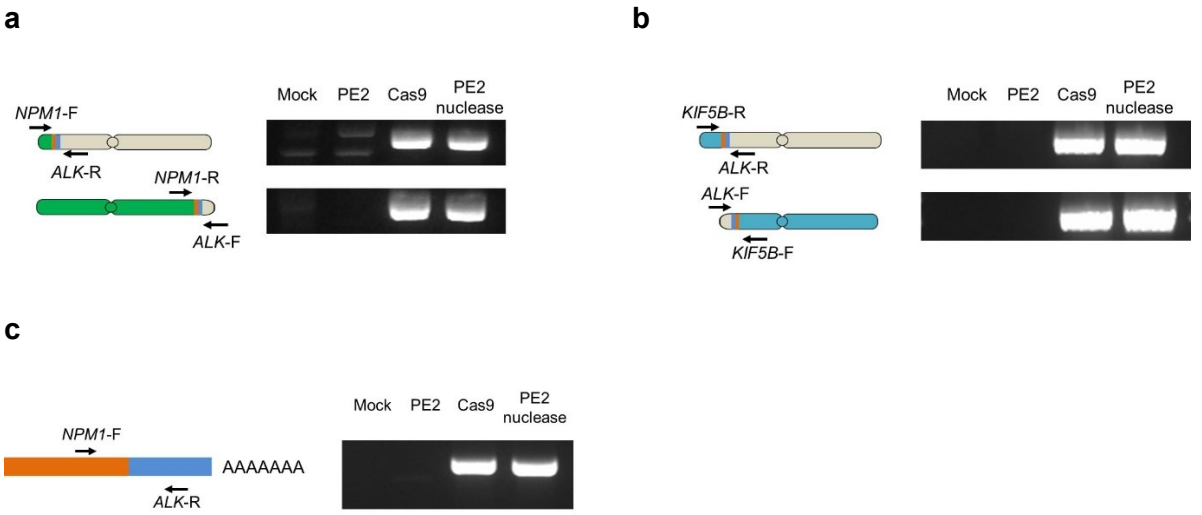


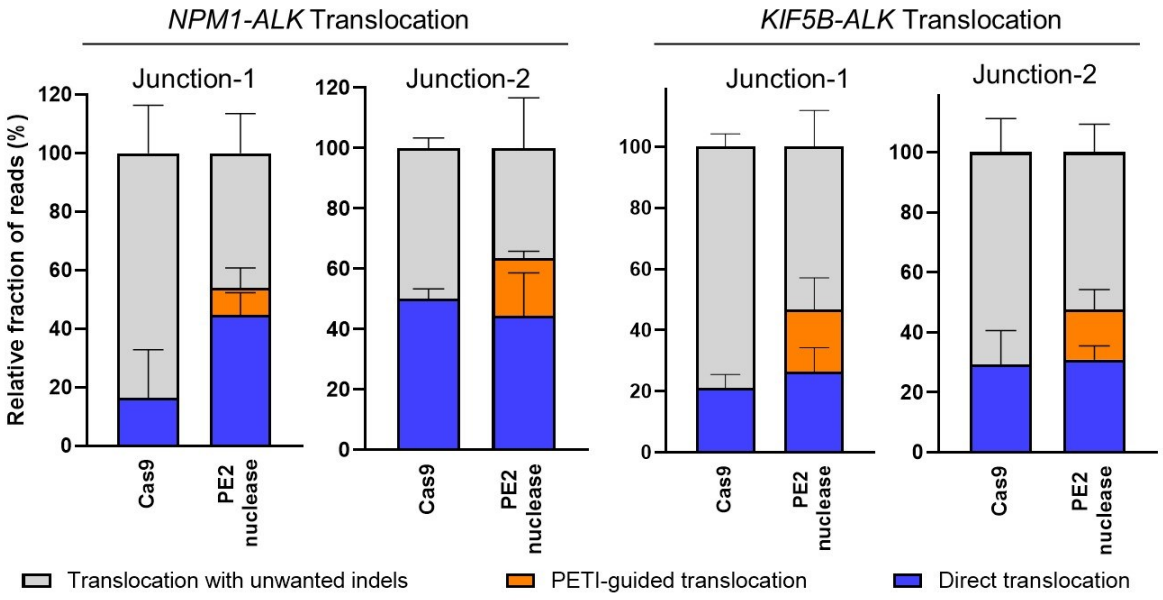
Figure S9. Estimation of *NPM1-ALK* and *KIF5B-ALK* translocation frequencies. The frequencies of *NPM1-ALK* or *KIF5B-ALK* translocations were estimated by digital PCR analysis as mentioned in Supplementary Figure 4.

Dosage	*NPM1-ALK*							
	Cas9				PE2 nuclease			
	Junction-1		Junction-2		Junction-1		Junction-2	
pg	Tested	Response	Tested	Response	Tested	Response	Tested	Response
20000	1	1	1	1	1	1	1	1
10000	5	4	1	1	1	1	1	1
5000	5	2	5	4	1	1	5	5
2500	5	1	5	2	1	1	5	4
1250	1	0	5	0	5	5	5	2
625	1	0	1	0	5	5	1	0
313	1	0	1	0	5	2	1	0
156	1	0	1	0	1	0	1	0
78	-	-	-	-	-	-	-	-

Dosage	*KIF5B-ALK*							
	Cas9				PE2 nuclease			
	Junction-1		Junction-2		Junction-1		Junction-2	
pg	Tested	Response	Tested	Response	Tested	Response	Tested	Response
30000	1	1	1	1	1	1	1	1
15000	5	5	1	1	1	1	1	1
7500	5	3	5	4	5	4	5	4
3750	5	0	5	1	5	3	5	1
1875	1	0	5	0	5	1	5	0
938	1	0	1	0	1	0	1	0
469	1	0	1	0	1	0	1	0
234	1	0	1	0	1	0	1	0
117	-	-	-	-	-	-	-	-

Figure S10. Chromosomal rearrangements in A549 cell lines. (a) Sequencing results of targeted amplicon amplified using translocation-specific primers in A549 cells. (b) Sequencing results of targeted amplicon amplified using inversion-specific primers in A549 cells.

a



b

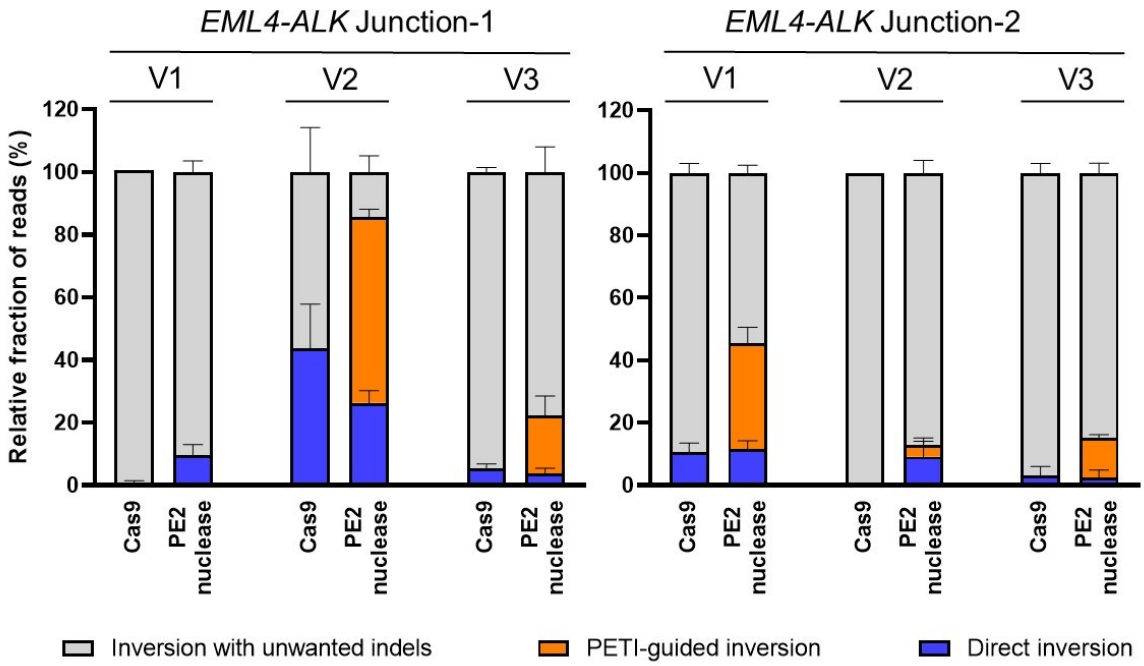
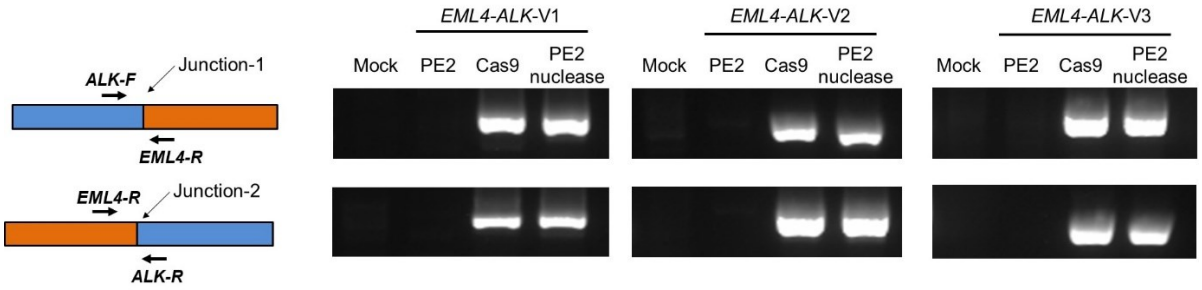


Figure S11. PCR assay for three types of *EML4-ALK* inversions in HEK293T cells. (a) PCR assay for inversions. Products were amplified by inversion-specific primers. (b) Detection of the *EML4-ALK* fusion transcript by RT-PCR using inversion-specific primers. Total RNA from HEK293T cells transfected with plasmids encoding PE2, Cas9 nuclease, or PE2 nuclease was used as template.

a



b

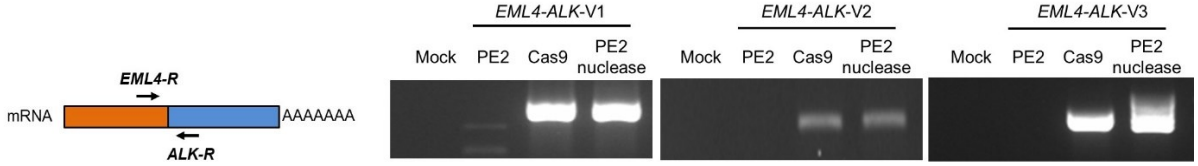
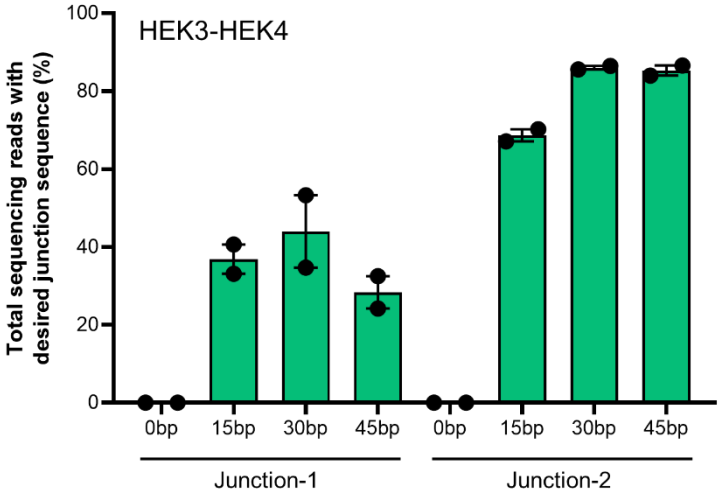


Figure S12. Estimation of three types of *EML4-ALK* inversions frequencies. The frequencies of inversions were estimated by digital PCR analysis as mentioned in Supplementary Figure 4.

Dosage	*EML4-ALK*-V1								*EML4-ALK*-V2								*EML4-ALK*-V3							
	Cas9				PE2 nuclease				Cas9				PE2 nuclease				Cas9				PE2 nuclease			
	Junction-1		Junction-2		Junction-1		Junction-2		Junction-1		Junction-2		Junction-1		Junction-2		Junction-1		Junction-2		Junction-1		Junction-2	
pg	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response	Tested	Response
20000	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-
10000	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5000	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2500	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1250	5	4	5	4	5	5	1	1	1	1	1	1	1	1	1	1	5	5	5	3	1	1	5	4
625	5	1	5	1	5	4	1	1	1	1	1	1	1	1	1	1	5	2	5	2	1	1	5	3
313	5	0	5	1	5	1	5	4	5	4	1	1	1	1	1	1	5	0	5	0	5	4	5	0
156	1	0	1	0	1	0	5	1	5	1	5	4	5	5	1	1	1	0	1	0	5	1	1	0
78	1	0	1	0	1	0	5	0	5	0	5	2	5	4	5	5	1	0	1	0	5	0	1	0
39	-	-	-	-	-	-	-	-	1	0	5	1	5	1	5	2	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	1	0	1	0	1	0	5	0	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	1	0	1	0	1	0	1	0	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	1	0	1	0	-	-	-	-	-	-	-	-	-	-	-	-

Figure S13. Analysis of PETI-mediated chromosomal translocation and inversion with various length of RTT in each pegRNAs. (a) Targeted deep sequencing results of amplicons generated using HEK3-HEK4 translocation-specific primers. (b) Targeted sequencing results of amplicons generated using *EML4-ALK* translocation-specific primers.

a



b

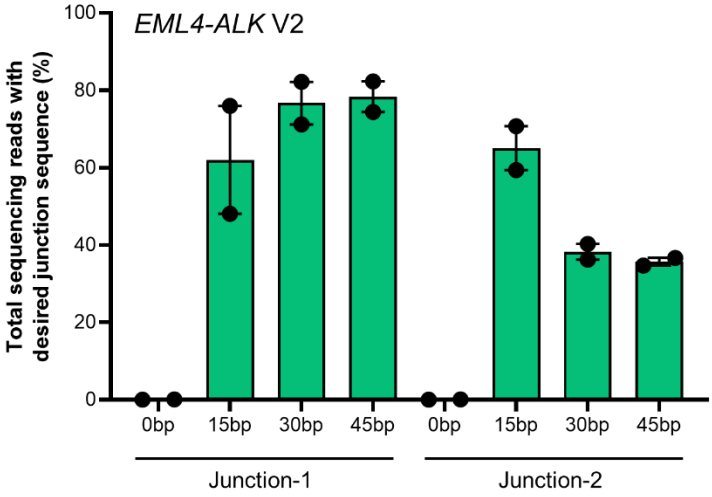
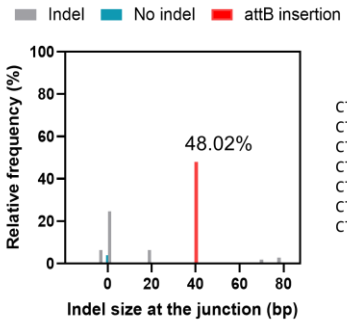


Figure S14. PCR assay for insertion of attP site at translocation junctions in HEK293T cells.




```
CTTGCTATATCCTCGA----------------------------------------CGATGGTGAAAGCTCG Ref. (total: 16986)
CTTGCTATATCCTCGACGGCTTGTCGACGACGGCGGTCTCCGTCGTCAGGATCATCCGATGGTGAAAGCTCG  8214 (48.36%)
CTTGCTATATCCTCGAA---------------------------------------CGATGGTGAAAGCTC  4264 (25.10%)
CTTGCTATATCCT-------------------------------------------CGATGGTGAAAGCTCG  1094  (6.44%)
CTTGCTATAGGAAGAGATGAATCCTGCACCAGCCCCCAGCTTTCTTC---------CGATGGTGAAAGCTCG  1041  (6.13%)
CTTGCTATATCCTCGA----------------------------------------CGATGGTGAAAGCTCG   693  (4.08%)
CTTGCTATATCCTCGAAAG-------------------------------------CGATGGTGAAAGCTCG   663  (3.90%)
```

Table S1. Sequences of pegRNAs used in the experiments.

pegRNA name	Target gene	RT-name	Spacer sequence		PBS (5' to 3', 13 nt)	RTT sequence (5' to 3')
HEK3-E1	HEK3	E-HEK4-Perfect	ggcccagactgagcacgtga	TGG	cagactgagcacg	tgggggttaaagcggagactctggtgctgt
HEK3-E2	HEK3	E-HEK4-EcoRI-ins	ggcccagactgagcacgtga	TGG	cagactgagcacg	AATTCtgggggttaaagcggagactctggtgctgt
HEK3-E3	HEK3	E-HEK4-3bp-del	ggcccagactgagcacgtga	TGG	cagactgagcacg	gggttaaagcggagactctggtgctgtgtg
HEK4-E1	HEK4	E-HEK3-Perfect	ggcactgcggctggaggtgg	GGG	ctgcggctggagg	tgatggcagaggaaaggaaggaagccctgcttcc
HEK4-E2	HEK4	E-HEK3-BamHI-ins	ggcactgcggctggaggtgg	GGG	ctgcggctggagg	ATCCtgatggcagaggaaaggaaggaagccctgcttcc
HEK4-E3	HEK4	E-HEK3-3bp-del	ggcactgcggctggaggtgg	GGG	ctgcggctggagg	tggcagaggaaaggaagccctgcttcctcc
ALK-V1	ALK	ALK-1-BamHI-EXT	gcgagctttcaccatcgtga	TGG	gctttcaccatcg	GGATCCtgatggctaaataacagcccagttttcttg
EML4-V1	EML4	EML4-2-EcoRI-EXT	attcagctgtaccaatgtga	TGG	agctgtaccaatg	GAATTCtgatggacactgaaggagctccccaccccc
ALK-V2	ALK	ALK-EML4-V2(EcoRI)-EXT	tccttcagtgtccatcacga	TGG	tcagtgtccatca	GAATTctaaggtaatgagaatctcaaatgtgattc
ELM4-V2	EML4	EML4-V2(BamHI)-EXT	gcatatgttatgctgagcta	AGG	atgttatgctgag	GGATCCcgatggtgaaagctcgcccccaccctaga
ALK-NPM1	ALK	ALK-NPM1(BamHI)-EXT	gcgagctttcaccatcgtga	TGG	gctttcaccatcg	GATCCtcgaggatatagcaagctatgatcacatca
NPM1	NPM1	NPM1(EcoRI)-EXT	gtgaacccagtagcagttcg	AGG	acccagtagcagt	GAATTCtgatggacactgaaggagctccccaccccc
ALK-V3	ALK	ALK-EML4-V3(BamHI)-EXT	gcgagctttcaccatcgtga	TGG	gctttcaccatcg	GATCCtcctggctacagctaatcacaaattttagc
EML4-V3	EML4	EML4-V3(EcoRI)-EXT	tgatcaaccgcaactcttcc	TGG	caaccgcaactct	GAATTCtgatggacactgaaggagctccccaccccc

References

1. Hu, Y, and Smyth, GK (2009). ELDA: extreme limiting dilution analysis for comparing depleted and enriched populations in stem cell and other assays. *J Immunol Methods* **347**: 70-78.