Supporting Information

Kopera et al. 10.1073/pnas.1100275108

SI Materials and Methods

Cultured Cell Retrotransposition Assay. The cultured cell retrotransposition assay was previously described (1). Briefly, HeLa cells were plated at 2×10^3, 2×10^4, and 2×10^5 cells per well in a six-well dish (BD Falcon). The next day cells were transfected with 1.0 μg of plasmid DNA with or without 0.5 μg of hr-GFP plasmid, using Fugene6 (Roche) and OptiMEM (Invitrogen) according to the manufacturer's instructions. The next day, medium was replenished. Two days after transfection cells cotransfected with hr-GFP were analyzed by flow cytometry to determine transfection efficiency. Three days (G418) or 5 d (blasticidin) after transfection, retrotransposition events were selected for using the appropriate drug in cell maintenance media: 400 μg/mL G418 for cells transfected with pJM102/L1.3 and pAJ101/L1.3 plasmid constructs and 10 μg/mL blasticidin for cells transfected with pJJ101/L1.3 and pJBM101/L1.3 constructs. Drug-containing media were changed until 12 d after transfection; then cells were washed, fixed, and stained with crystal violet for 30 min at room temperature. Stained cells were rinsed, dried, counted, and scanned. Retrotransposition assays shown are representative of experiments repeated at least three times.

For retrotransposition assays in CHO cells, cells were plated at 2×10^4 and 2×10^5 cells per well in six-well plates or 2×10^5 and 2×10^6 in T75 flasks (BD Falcon). Cells were transfected 6–8 h after plating. Transfection efficiencies, drug selection, staining, and counting were performed as stated above.

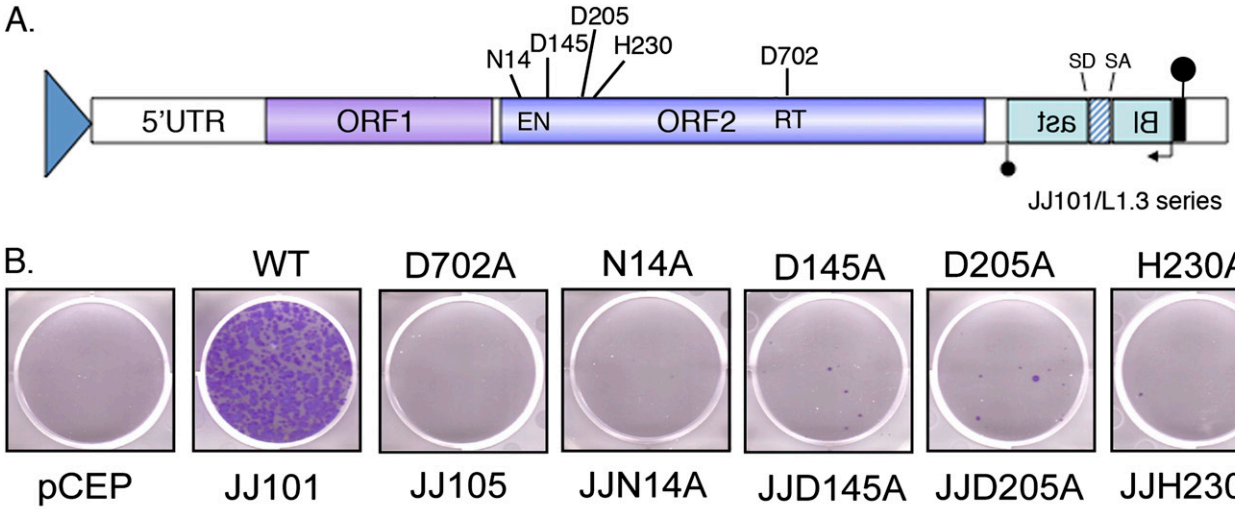
Telomere-L1 Southern Blot Analysis. CHO cells were transfected for the cultured cell retrotransposition assay as previously described (2). Twelve to fourteen days after transfection, drug-resistant colonies harboring retrotransposition events were isolated and expanded. Genomic DNA was isolated from each clonal cell line and subjected to PCR using a telomere oligonucleotide primer (Telo3T: 5′-GGTTAGGGTTAGGGTTAGGGTTT-3′; Telo2T: 5′-GGTTAGGGTTAGGGTTAGGGTT-3′; or Telo: 5′-GGTTAGGGTTAGGGTTAGGG-3′) and an engineered-L1 specific primer (J-L1-end186: 5′-AAGTATAATAAAGACGTCAGGGTTC-3′) (3). PCR products were run on a 2% agarose 1XTris-Acetate-EDTA gel and transferred onto 0.45-μm nylon membrane (GE-Amersham Hybond-XL) overnight. An oligonucleotide probe specific to the transfected long interspersed element-1 (L1) (J-L1-probe: 5′-TGTGATGCTATTGCTTTATTTGTA-3′; or CEP2as: 5′-CTGCATTCTAGTTGTGGTTTGTCC-3′) was phosphorylated with dATP-γP^{32} (MP Biomedicals) using T4-polynucleotide kinase (New England Biolabs) at 37 °C for 30 min. All blot incubations and washes were done at 42 °C. Blots were first incubated for 30 min in prehybridization buffer (6× SSC, 5× Denhardt's solution, and 0.1% SDS), then incubated for 30 min in hybridization buffer (6× SSC and 0.1% SDS) and finally incubated with the labeled probe overnight in hybridization buffer. Blots were washed three times for 30 min with hybridization buffer, were exposed to film, and positive bands were identified. PCR products that gave a positive Southern blot signal were ligated into pCR-Blunt (Invitrogen) or pGEM-T-easy (Promega) vector and sequenced at the University of Michigan DNA Sequencing Core.

L1 Element Amplification Protocol (LEAP) Assay. The LEAP assay was previously described (4). Briefly, HeLa cells were plated at 6×10^6 in T175 flasks (BD Falcon) and transfected with 30 μg L1.3 plasmid using Fugene6 (Roche) the next day. Selection with 225 μg/mL hygromycin B began 72 h posttransfection, changing medium daily. Nine days after transfection, cells were lysed [1.5 mM KCl, 2.5 mM $MgCl_2$, 5 mM Tris-Cl (pH 7.5), 1% deoxycholic acid, 1% Triton X-100, and 1× protease inhibitor EDTA-free mixture (Roche)] and subjected to sucrose cushion (8.5% and 17% sucrose) ultracentrifugation for 2 h at 178,000 × *g* for ribonucleoprotein particle (RNP) isolation. RNP pellets were resuspended in 1× protease inhibitor EDTA-free mixture (Roche), and protein concentration was determined by the Bradford reagent assay (BioRad). One microgram of L1-RNP was incubated with buffer [50 mM Tris-Cl (pH 7.5), 50 mM KCl, 5 mM $MgCl_2$, 10 mM DTT, 20 U RNasin (Promega), and 0.05% Tween-20], an HPLC-purified oligonucleotide adapter (0.4 μM), and dNTPs (0.2 mM) for the reverse transcription reaction at 37 °C for 1 h. The cDNAs were amplified by PCR, using primers specific for the transfected L1 (sense: 5′-GGGTTCGAAATCGATAAGCTTGGATCCAGA-3′) and the adapter (RACE: 5′-GCGAGCACAGAATTAATACGACT-3′). The PCR products were analyzed on a 2% agarose gel in 1× TAE. Bands were gel purified (Qiagen), ligated to the pCR-Blunt (Invitrogen) or pGEM-T-easy (Promega) vector, and sequenced. In LEAP assays in which bands are faint or difficult to visualize, gel slices were excised at the expected LEAP product size, and DNA was gel purified and pooled from independent reactions before cloning for sequencing. LEAP gels shown are representative data of experiments repeated a minimum of three times from at least two independent L1-RNP preparations. Sequences of adapters used are as follows:

T12: 5′-GCGAGCACAGAATTAATACGACTCACTATAGG-T_{12}-3′
T12+VN: 5′-GCGAGCACAGAATTAATACGACTCACTATAGG -T_{12}VN-3′
T4spacerP: 5′-GCGAGCACAGAATTAATACGACTCACTATAGG-T_4-(C_3H_6)(PO_4)-3′
T4AAC: 5′-GCGAGCACAGAATTAATACGACTCACTATAGG-TTTTAAC-3′
T4AAddC: 5′-GCGAGCACAGAATTAATACGACTCACTATAGG-TTTTAAddC-3′
Telo3: 5′-GCGAGCACAGAATTAATACGACTCACTATAGG -$(TTAGGG)_3$-3′
Telo3+TT: 5′-GCGAGCACAGAATTAATACGACTCACTATAGG -$(TTAGGG)_3$TT-3′
Telo2+TTAG: 5′-GCGAGCACAGAATTAATACGACTCACTATAGG-$(TTAGGG)_2$TTAG-3′
Telo2+TTAGG: 5′-GCGAGCACAGAATTAATACGACTCACTATAGG-$(TTAGGG)_2$TTAGG-3′
Telo3+T: 5′-GCGAGCACAGAATTAATACGACTCACTATAGG-$(TTAGGG)_3$T-3′
Telo3+TTA: 5′-GCGAGCACAGAATTAATACGACTCACTATAGG-$(TTAGGG)_3$TTA-3′

RNAs were isolated from RNP samples (RNeasy, Qiagen). L1 RNA and/or GAPDH RNA were reverse-transcribed by Moloney murine leukemia virus–RT (Promega) using the LEAP adapters stated. MMLV–RT-PCR products were visualized, cloned, and sequenced as stated above for LEAP.

1. Wei W, Morrish TA, Alisch RS, Moran JV (2000) A transient assay reveals that cultured human cells can accommodate multiple LINE-1 retrotransposition events. *Anal Biochem* 284:435–438.
2. Morrish TA, et al. (2002) DNA repair mediated by endonuclease-independent LINE-1 retrotransposition. *Nat Genet* 31:159–165.
3. Morrish TA, et al. (2007) Endonuclease-independent LINE-1 retrotransposition at mammalian telomeres. *Nature* 446:208–212.
4. Kulpa DA, Moran JV (2006) Cis-preferential LINE-1 reverse transcriptase activity in ribonucleoprotein particles. *Nat Struct Mol Biol* 13:655–660.



C.

plasmid	mutation	HeLa	AA8	V3
	WT	100%±10.3	100%±12	100%±20
full length	D702A	0.1%±0.2	0	0
RC-L1	N14A	0.5%±0.7	0	78.2%±23
	D145A	2.6%±0.5	2.2%±0.0	52.2%±3.8
	D205A	0.5%±0.3	1.2%±1.0	54.1%±8
	H230A	1.6%±0.6	1.1%±0.9	23.2%±17.5
AJ101	WT	0.1%±1.0	0.1%±0.0	1.5%±0.3
AJ105	D702A	0	0	0
AJN14A	N14A	0.04%±0.0	0.1%±0.1	1.6%±0.1
AJD145A	D145A	0.08%±0.0	0.1%±0.0	1.8%±0.1
AJD205A	D205A	0.02%±0.0	0	2.4%±0.2
AJH230A	H230A	0.07%±0.05	0.1%±0.0	1.7%±0.1
JBM101	WT	n.a.	100%±8.5	100%±7.2
JBM135	D702A	n.a.	0	0
JBM105	R261A/R262A	n.a.	0	3.4%±0.2
JBM205	R261A/R262A/H230A	n.a.	0	5.4%±0.4

Fig. S1. L1 endonuclease (L1-EN) is required for retrotransposition in a nonhomologous end-joining–intact cell line. (*A*) A retrotransposition-competent L1 is shown with conserved residues in the EN (N14, D145, D205, and H230) and RT (D702) domains shown. Blue triangle represents a CMV promoter in the pJJ101/L1.3 plasmid constructs. A blasticidin deaminase reporter cassette interrupts the 3′ UTR. The cassette contains its own transcription start site (upside-down arrow) and is interrupted by an intron with splice donor (SD) and splice acceptor (SA) sites noted. "Lollipops" mark polyadenylation signals in both transcriptional orientations. (*B*) Mutations in the EN or RT domain are unable to retrotranspose in HeLa cells. (*C*) Percentage of retrotransposition is shown in the table for all retrotransposition assays in Fig. 1*B* and Fig. S4 *A* and *B*. Wild type is set to 100% except for the pAJ101/L1.3 series retrotransposition assays, where pJM102/L1.3 in the respective cell line is set to 100%. Values are reported with SDs. Assays not performed are noted as n.a.

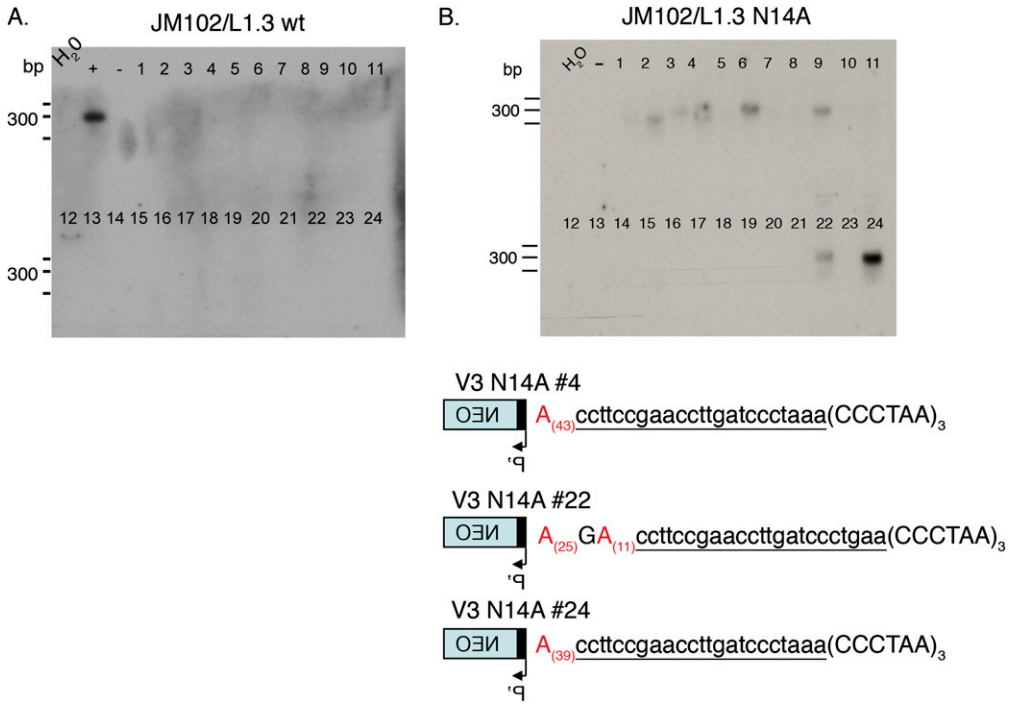
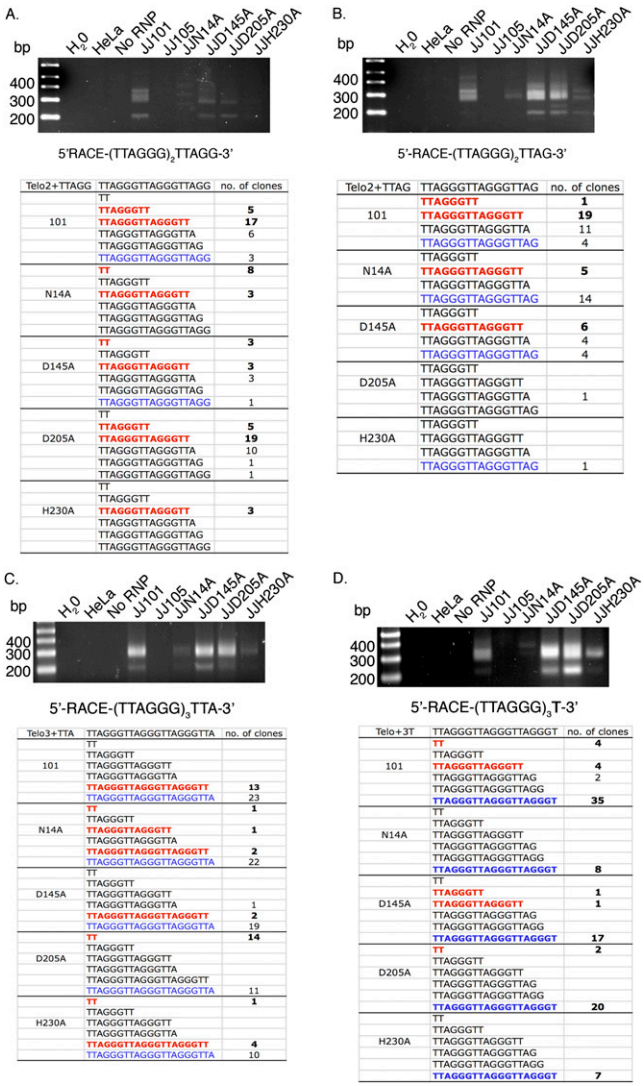


Fig. S2. L1 wild type and N14A endonuclease-independent (ENi) retrotransposition events at telomere ends: PCR/Southern blot assay of genomic DNAs from (*A*) wild-type and (*B*) N14A ENi clonal retrotransposition events are shown. The sequences of the characterized insertions for N14A are also shown. The short sequences of unknown origin (see main text) between the L1 poly (A) tail and the telomeric repeats are underlined.



Telo2+TTAGG	TTAGGGTTAGGGTTAGG	no. of clones
	TT	
	TTAGGGTT	5
101	TTAGGGTTAGGGTT	17
	TTAGGGTTAGGGTTA	6
	TTAGGGTTAGGGTTAG	
	TTAGGGTTAGGGTTAGG	3
	TT	8
	TTAGGGTT	
N14A	TTAGGGTTAGGGTT	3
	TTAGGGTTAGGGTTA	
	TTAGGGTTAGGGTTAG	
	TTAGGGTTAGGGTTAGG	
	TT	3
	TTAGGGTT	
D145A	TTAGGGTTAGGGTT	3
	TTAGGGTTAGGGTTA	3
	TTAGGGTTAGGGTTAG	
	TTAGGGTTAGGGTTAGG	1
	TT	
	TTAGGGTT	5
D205A	TTAGGGTTAGGGTT	19
	TTAGGGTTAGGGTTA	10
	TTAGGGTTAGGGTTAG	1
	TTAGGGTTAGGGTTAGG	1
	TT	
	TTAGGGTT	
H230A	TTAGGGTTAGGGTT	3
	TTAGGGTTAGGGTTA	
	TTAGGGTTAGGGTTAG	
	TTAGGGTTAGGGTTAGG	

Telo2+TTAG	TTAGGGTTAGGGTTAG	no. of clones
	TTAGGGTT	1
101	TTAGGGTTAGGGTT	19
	TTAGGGTTAGGGTTA	11
	TTAGGGTTAGGGTTAG	4
	TTAGGGTT	
N14A	TTAGGGTTAGGGTT	5
	TTAGGGTTAGGGTTA	
	TTAGGGTTAGGGTTAG	14
	TTAGGGTT	
D145A	TTAGGGTTAGGGTT	6
	TTAGGGTTAGGGTTA	4
	TTAGGGTTAGGGTTAG	4
	TTAGGGTT	
D205A	TTAGGGTTAGGGTT	
	TTAGGGTTAGGGTTA	1
	TTAGGGTTAGGGTTAG	
	TTAGGGTT	
H230A	TTAGGGTTAGGGTT	
	TTAGGGTTAGGGTTA	
	TTAGGGTTAGGGTTAG	1

Telo3+TTA	TTAGGGTTAGGGTTAGGGTTA	no. of clones
	TT	
	TTAGGGTT	
101	TTAGGGTTAGGGTT	
	TTAGGGTTAGGGTTA	
	TTAGGGTTAGGGTTAGGGTT	13
	TTAGGGTTAGGGTTAGGGTTA	23
	TT	1
	TTAGGGTT	
N14A	TTAGGGTTAGGGTT	1
	TTAGGGTTAGGGTTA	
	TTAGGGTTAGGGTTAGGGTT	2
	TTAGGGTTAGGGTTAGGGTTA	22
	TT	
	TTAGGGTT	
D145A	TTAGGGTTAGGGTT	
	TTAGGGTTAGGGTTA	1
	TTAGGGTTAGGGTTAGGGTT	2
	TTAGGGTTAGGGTTAGGGTTA	19
	TT	14
	TTAGGGTT	
D205A	TTAGGGTTAGGGTT	
	TTAGGGTTAGGGTTA	
	TTAGGGTTAGGGTTAGGGTT	
	TTAGGGTTAGGGTTAGGGTTA	11
	TT	1
	TTAGGGTT	
H230A	TTAGGGTTAGGGTT	
	TTAGGGTTAGGGTTA	
	TTAGGGTTAGGGTTAGGGTT	4
	TTAGGGTTAGGGTTAGGGTTA	10

Telo+3T	TTAGGGTTAGGGTTAGGGT	no. of clones
	TT	4
	TTAGGGTT	
101	TTAGGGTTAGGGTT	4
	TTAGGGTTAGGGTTAG	2
	TTAGGGTTAGGGTTAGG	
	TTAGGGTTAGGGTTAGGGT	35
	TT	
	TTAGGGTT	
N14A	TTAGGGTTAGGGTT	
	TTAGGGTTAGGGTTAG	
	TTAGGGTTAGGGTTAGG	
	TTAGGGTTAGGGTTAGGGT	8
	TT	
	TTAGGGTT	1
D145A	TTAGGGTTAGGGTT	1
	TTAGGGTTAGGGTTAG	
	TTAGGGTTAGGGTTAGG	
	TTAGGGTTAGGGTTAGGGT	17
	TT	2
	TTAGGGTT	
D205A	TTAGGGTTAGGGTT	
	TTAGGGTTAGGGTTAG	
	TTAGGGTTAGGGTTAGG	
	TTAGGGTTAGGGTTAGGGT	20
	TT	
	TTAGGGTT	
H230A	TTAGGGTTAGGGTT	
	TTAGGGTTAGGGTTAG	
	TTAGGGTTAGGGTTAGG	
	TTAGGGTTAGGGTTAGGGT	7

Fig. S3. LEAP activity on a cohort of telomeric ends. Gel figures show a representative LEAP assay with the sequence of the LEAP adapter shown below. As in Fig. 2*D* (main text), sequences of the processed adapters in the LEAP reactions are shown in the table. Bold red sequences represent LEAP products with perfect L1 poly (A)/telomere repeat junctions. Blue sequence represents LEAP products that were made with an unprocessed LEAP adapter and is in bold when the unprocessed adapter still gave rise to a perfect L1 poly (A) tail/telomere repeat junction. Black sequences represent LEAP products that have partial telomere sequences at the L1 poly (A) tail/telomere junction. (*A*) LEAP activity on the Telo2+TTAGG adapter [5′-RACE-$(TTAGGG)_2$TTAGG-3′]. (*B*) LEAP activity on the Telo2+TTAG adapter [5′-RACE-$(TTAGGG)_2$TTAG-3′]. (*C*) LEAP activity on the Telo3+TTA adapter [5′-RACE-$(TTAGGG)_3$TTA-3′]. (*D*) LEAP activity on the Telo3+T adapter [5′-RACE-$(TTAGGG)_3$T-3′].

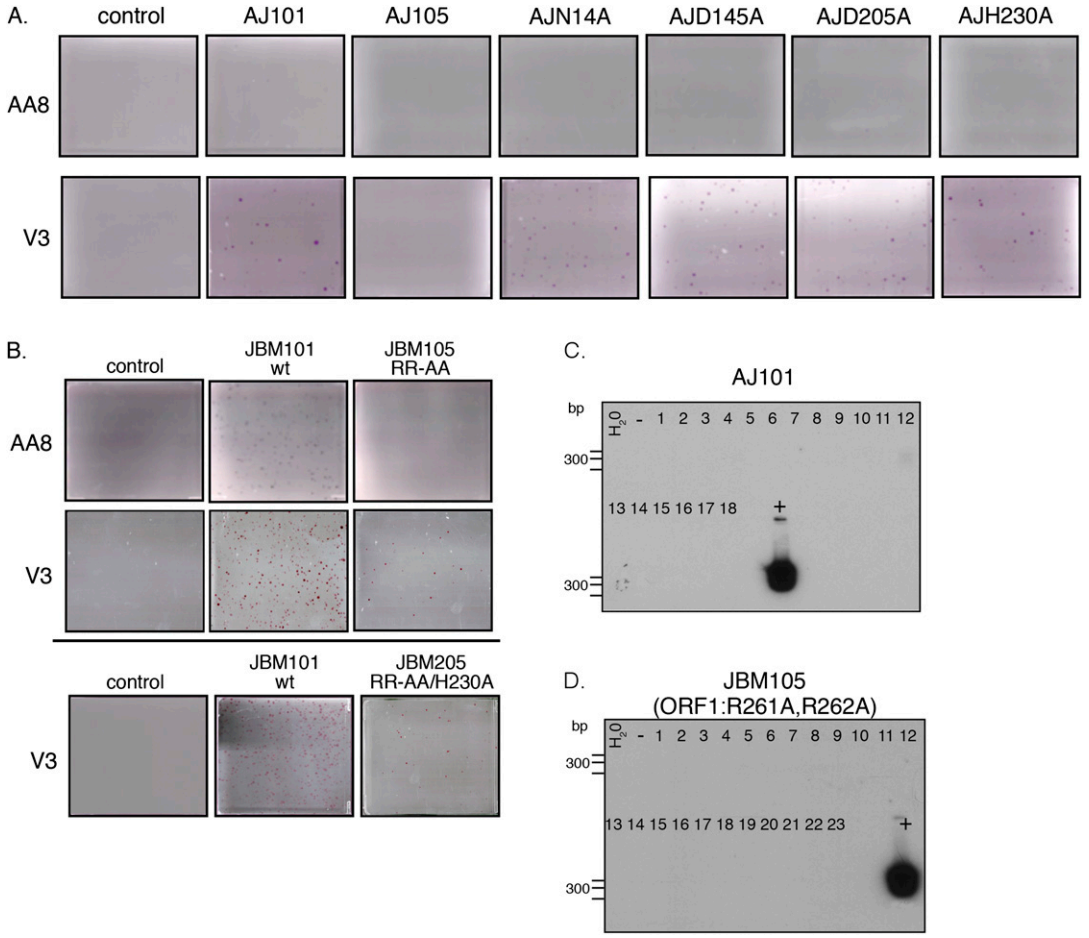


Fig. S4. L1-ORF2 only and L1-ORF1 mutant do not retrotranspose to telomere ends. (*A*) ORF2p-only wild type and EN mutants were able to retrotranspose in V3 CHO cells (*Lower*), but not in AA8 CHO cells (*Upper*). Retrotransposition assays were performed in T75 flasks. (*B*) The ORF1p double mutant (pJBM105: R261A/R262A) and the ORF1p/ORF2p triple mutant (pJBM205: R261A/R262A/H230A) can retrotranspose in V3 CHO cells (*Middle* and *Bottom*), but cannot retrotranspose in AA8 CHO cells (*Top*). PCR/Southern blot assay of genomic DNAs from (*C*) ORF2-only (AJ101) and (*D*) ORF1 mutant (JBM105) clonal retrotransposition events are shown.

Table S1. Endonuclease-independent retrotransposition events at dysfunctional telomeres

Table S1

Sequences of PCR products from genomic DNAs isolated from V3 clonal cell lines from Fig. 1*C* and Fig. 4*C* (main text). PCR primers are in blue, poly (A) tails are in red, and sequences corresponding to the probe used in the Southern blot assay are in green. L1/telomere junctions are underlined. Of note, one clone (V3 JBM205 #9) lacked a poly (A) tail and contained a 9-bp deletion upstream of the L1 mRNA poly adenylation site.