#### **Supplementary Figure Legends**

Supplementary Figure 1. *Gamma radiation increases the L1 retrotransposition frequency in the absence of puromycin selection*. 143B cells were transfected with L1-EGFP and irradiated or mock treated two days later. EGFP was measured on day 8. Cotransfection with DsRed was used to normalize transfection efficiency. Percentage of EGFP+ cells on day 8 from two independent L1-EGFP transient transfections (squares, circles) following 0, 1, 2, or 4 Gys of gamma irradiation. Percentages of EGFP positive cells were adjusted for transfection efficiency by DsRed cotransfection. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, as compared to 0 Gy by 2-tailed Student's t-test.

Supplementary Figure 2. *L1 RNA is not increased in cells exposed to gamma radiation*. A. 143B cells were transfected with L1-EGFP and exposed to 4 Gy of gamma radiation or mock treated 2 days later. Strand specific quantitative RT-PCR was performed on RNA harvested at 3 and 24 hours post irradiation. See materials and methods for primers and conditions. Cycle counts were normalized to GAPDH expression. Data are expressed in log<sub>10</sub> units relative to average L1 expression in unirradiated cells at 3 hours. Black, white, and striped bars represent independent L1-EGFP transfections. RT-PCRs were performed in quadruplicate for each sample. Standard deviations are expressed as error bars. Lower levels of L1-EGFP RNA were noted at 72 hours post transfection as compared to 51 hours post transfection in both irradiated and unirradiated cells. Even when driven by a strong, plasmid-based promoter, host cells may negatively regulate L1 retrotransposons in the absence of antibiotic selection.

Supplementary Figure 3. Potential 5' flank integration intermediates during the insertion of L1-EGFP into chromosome 17p11. The insertion was notable for a target site duplication as well as an inversion of both the 5' genomic flank and the L1 5' truncated sequence. One potential mechanism for this insertion involves the formation of dual DNA hairpins. These hairpins are then cleaved, and the overhangs near a region of microhomology are used to repair the gaps.

# Supplementary Figure 1



## Supplementary Figure 2



### Supplementary Figure 3

1. Target site

#### ACTGCCAATTTAAAAAATTATATTTCATTTTT TGACGGTTAAATTTTTTAATATAAAGTAAAAA

2. cleavage and nibbling (loss of shaded bases)

ACTGCCAATTTAAAAAATTAT TGACGGTTA	TCATTTT TTTTTAATAAAGTAAAAA
3. RNA annealing	
ACTGCCAATTTAAAAAATTAT TGACGGTTA	TGGTGATTCCTCAAGGATCTAGGACTAGAAAAAAAAA TCATTTT TTTTTAATATAAAGTAAAAA
4. Reverse transcription from genomic p	rimer
ACTGCCAATTTAAAAAATTAT TGACGGTTA	TGGTGATTCCTCAAGGATCTAGGACTAGAAAAAAAAA TCATTTT ACCACTAAGGAGTTCCTAGATCCTGATCTTTTTTTTTAATATAAAGTAAAAA
5. filling in and hairpin formation	
A ACTGCCAATTTAAAAAATT T TGACGGTTAAATTTTTTAAA	TGGTGATTCCTCAAGGATCTAGAACTAGAAAAAAAAA TCATTTT ACCACTAAGGAGTTCCTAGATCTTGATCTTTTTTTTTAATATAAAGTAAAAA
6. RNA displacement and loop formation	
A ACTGCCAATTTAAAAAATT T TGACGGT <mark>TAAATTTTTTAAA</mark>	TGGTGATTCCTCAAGGATCTAG TCAC A A CA ACTAGAAAAAAAAA TCATTTT AGGAGTTCCTAGATCTTGATCTTTTTTTTAATATAAAGTAAAAA
7. Second strand synthesis from 1 <sup>st</sup> strand primer	
A ACTGCCAATTTAAAAAATT T TGACGGTTAAATTTTTTAAA	TCAC A CAAGGATCTAGAACTAGAAAAAAAAATTATATTTCATTTT AGGAGTTCCTAGATCTTGATCTTTTTTTTTAATATAAAGTAAAAA
8. Hairpin opening	
ACTGCCAATTTAAAAAATTAT <mark>AAATTTTTTAAAT</mark> TGACGGT	AAAAAAATTATATT <b>TCATTTT</b> AAGATCAAGATCTAGGA <mark>ACCACTAAGGAGTTCCTAGATCCTGATCTT TTTTTTTAATATAAAGTAAAAA</mark>
9. Microhomology annealing	
ACTGCCAATTTAAAAAATTAT <mark>AAATTTTTAAAT</mark> TGACGGT AAAGATCAAGATCAAGATCTAG	A AAAAAAATTATATTTCATTTTT GAACCACTAAGGAGTTCCTAGATCCTGAT TTTTTTTAATATAAAGTAAAAA

10. Repair (with junctional base modifications)

ACTGCCAATTTAAAAAATTATAAATTTTTTAAATAGATCCTTGGTGATTCCTCAAGGATCTAGGACTA...AAAAAAATTATATTTCATTTTT TGACGGTTAAATTTTTTAATATTTAAAAAATTTATCTAGGAACCACTAAGGAGTTCCTAGATCCTGAT...TTTTTTTAATATAAAGTAAAA