Figure S1

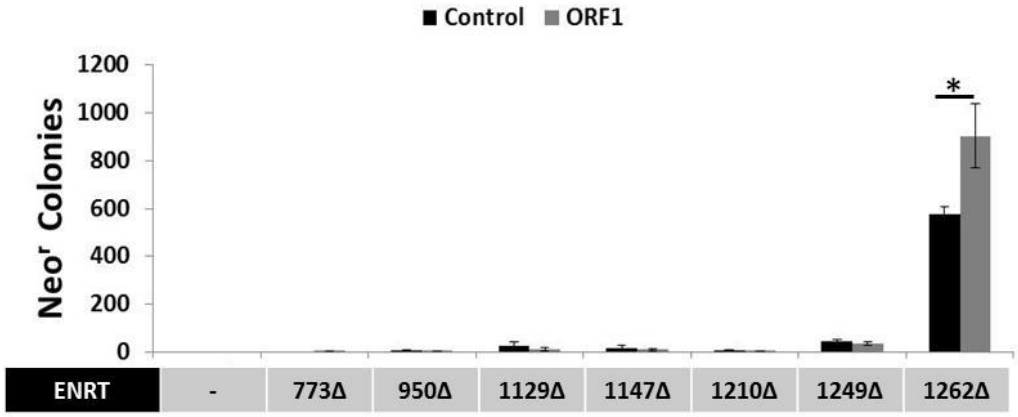

Figure S1: ORF1p-mediated increase in Alu retrotransposition driven by C-terminally truncated ORF2p fragments. Alu retrotransposition assay results. Neo^r colonies correspond to *de novo* retrotransposition events. Alu retrotransposition driven by Gal4-tagged ORF2 fragments alone (black) or with ORF1p supplemented (grey). Error bars denote standard deviation of n=3 experiments. Statistical significance assessed using Student's T-test (*p<0.05).

Figure S2

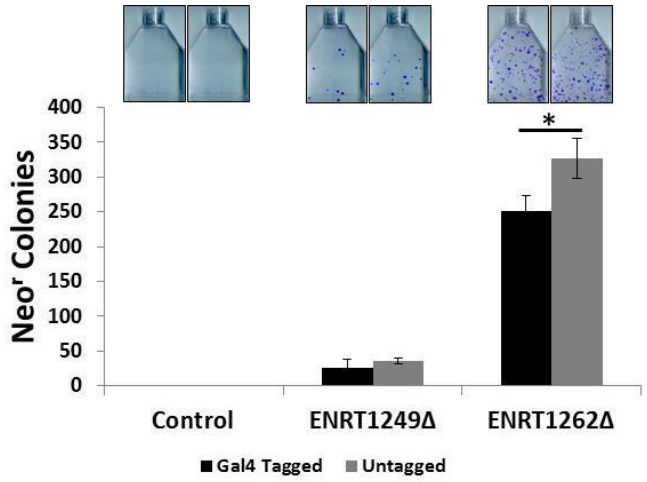

Figure S2: Gal4-tagged and Untagged ORF2 Driving Alu Retrotransposition. Alu retrotransposition assay results. Neo^r colonies correspond to *de novo* retrotransposition events. Alu retrotransposition driven by Gal4-tagged (black) or untagged (grey) ORF2 expression plasmids. Error bars denote standard deviation of n=3 experiments. Statistical significance assessed using Student's T-test (*p<0.05). Representative flasks are shown above corresponding graph bars.

Figure S3

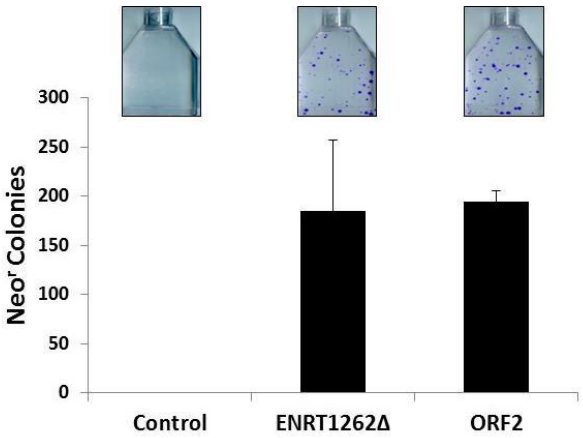

Figure S3: Untagged ORF2 fragment and ORF2 Driving Alu retrotransposition. Alu retrotransposition assay results. Neo^r colonies correspond to *de novo* retrotransposition events. Error bars denote standard deviation of n=3 experiments. Representative flasks are shown above corresponding graph bars.

Figure S4

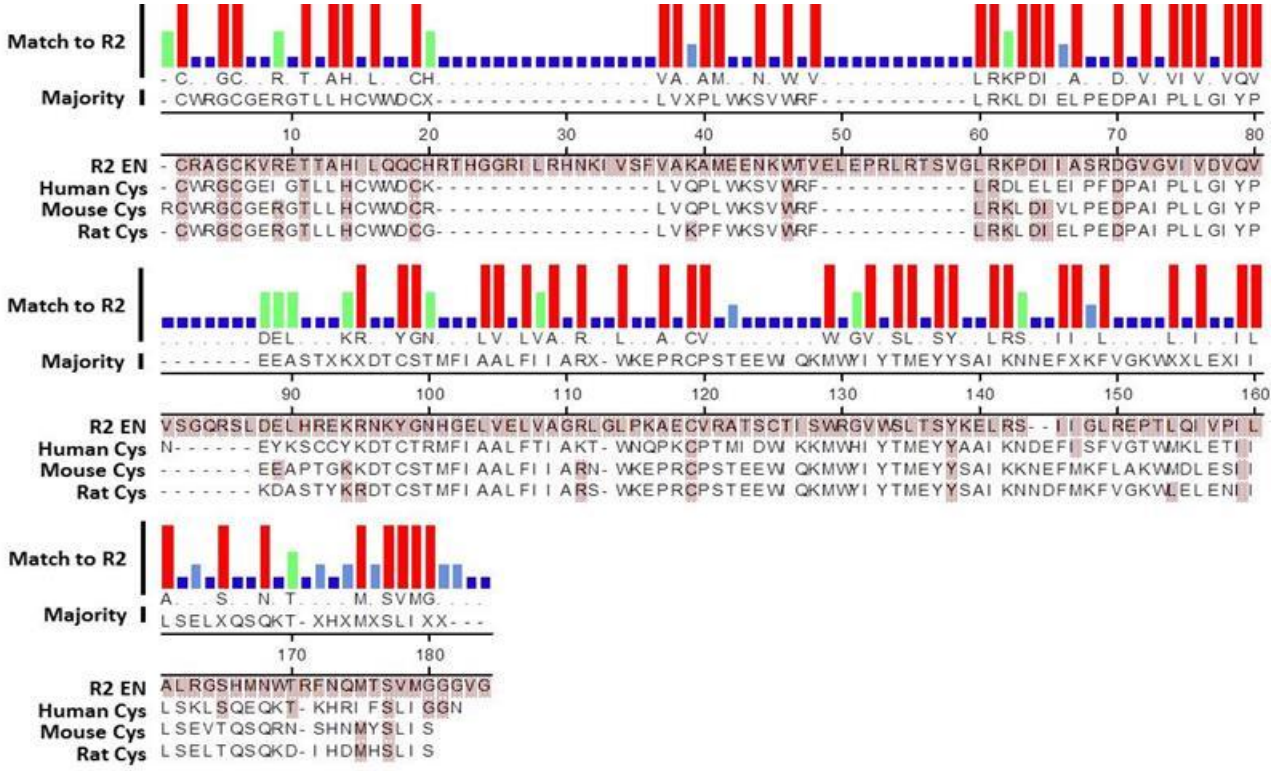

Figure S4: Full protein aligment between R2 C-terminal EN domain and C-termini of Human, Mouse, and Rat ORF2p. The origin of aligned ORF2p/ORFp is indicated at the left. Histogram plots strength of conservation of amino acid functional group relative to R2 amino acid sequence.

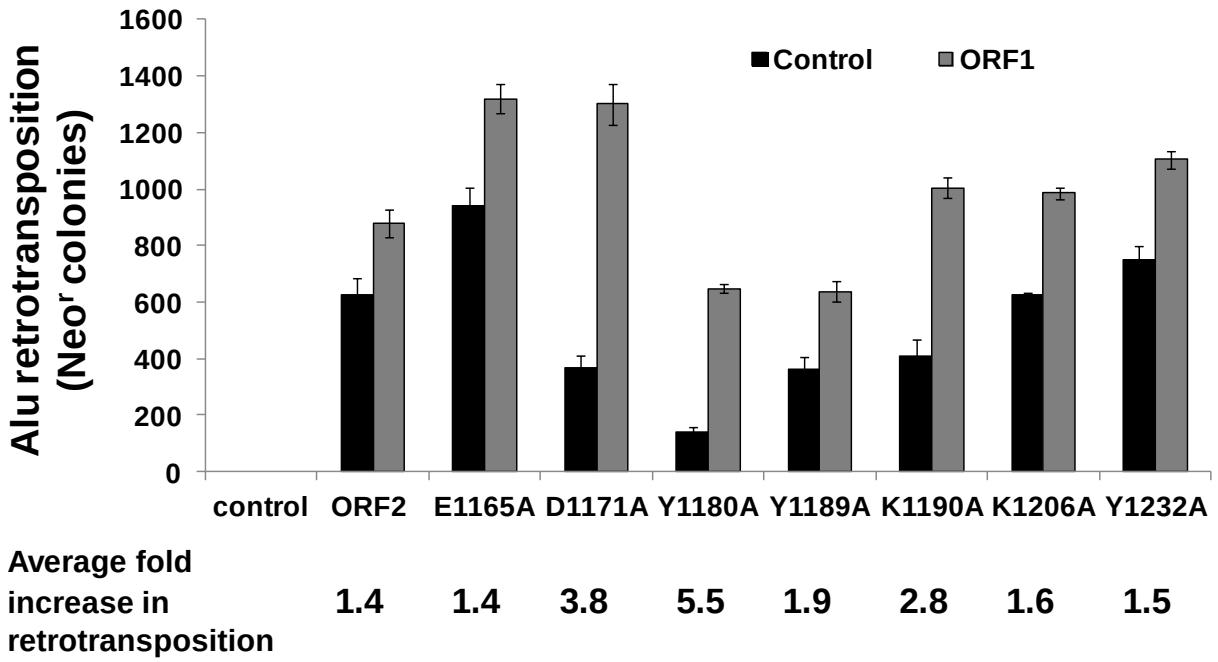

Figure S5: ORF1p-mediated increase in Alu retrotransposition driven by ORF2p.

Supplemental Materials and Methods

Alu Retrotransposition (Figures S2, S3)

HeLa cells were maintained in MEM supplemented with 1% sodium pyruvate, L-glutamate, NEAA, and 10% FBS as previously described. 500 thousand cells were seeded 16-18 hours prior to transfection. 0.2 µg indicated ORF2 expression construct plasmid was cotransfected with 0.1 µg of the previously described Alu retrotransposition reporter construct using 4 µL Plus reagent (Life Technologies) in 200 µL DMEM with 8 µL Lipofectamine reagent (Life technologies) in 92 µL DMEM . Cell culture media was supplemented with 0.45 mg/mL neomycin ~24 hours post transfection. Colonies were stained after 2 weeks with Neomycin selection with crystal violet solution (0.2% crystal violet, 5% acetic acid, 2.5% isopropanol) and counted with Oxford Optronics ColCount. Statistical significance assessed using Student's *t*-test for paired samples (*n*=3), with error bars denoting standard deviation.

ORF1-augmented ORF2p-driven Alu Retrotransposition (Figure S1, S5)

HeLa cells were maintained as previously described. 500 thousand cells were seeded 16-18 hours prior to transfection. 0.4 µg indicated ORF2 expression construct plasmid was cotransfected with either 0.4 µg of expression plasmid containing human codon-optimized ORF1 sequence or empty vector, in addition to 0.4 µg of the previously described Alu retrotransposition reporter construct using 6 µL Plus reagent (Life Technologies) in 200 µL DMEM with 8 µL Lipofectamine reagent (Life technologies) in 92 µL DMEM . Cells were selected, maintained, stained, counted, and analyzed as described above.