

LINE-1 produces cytosolic DNA products from self and non-self RNAs



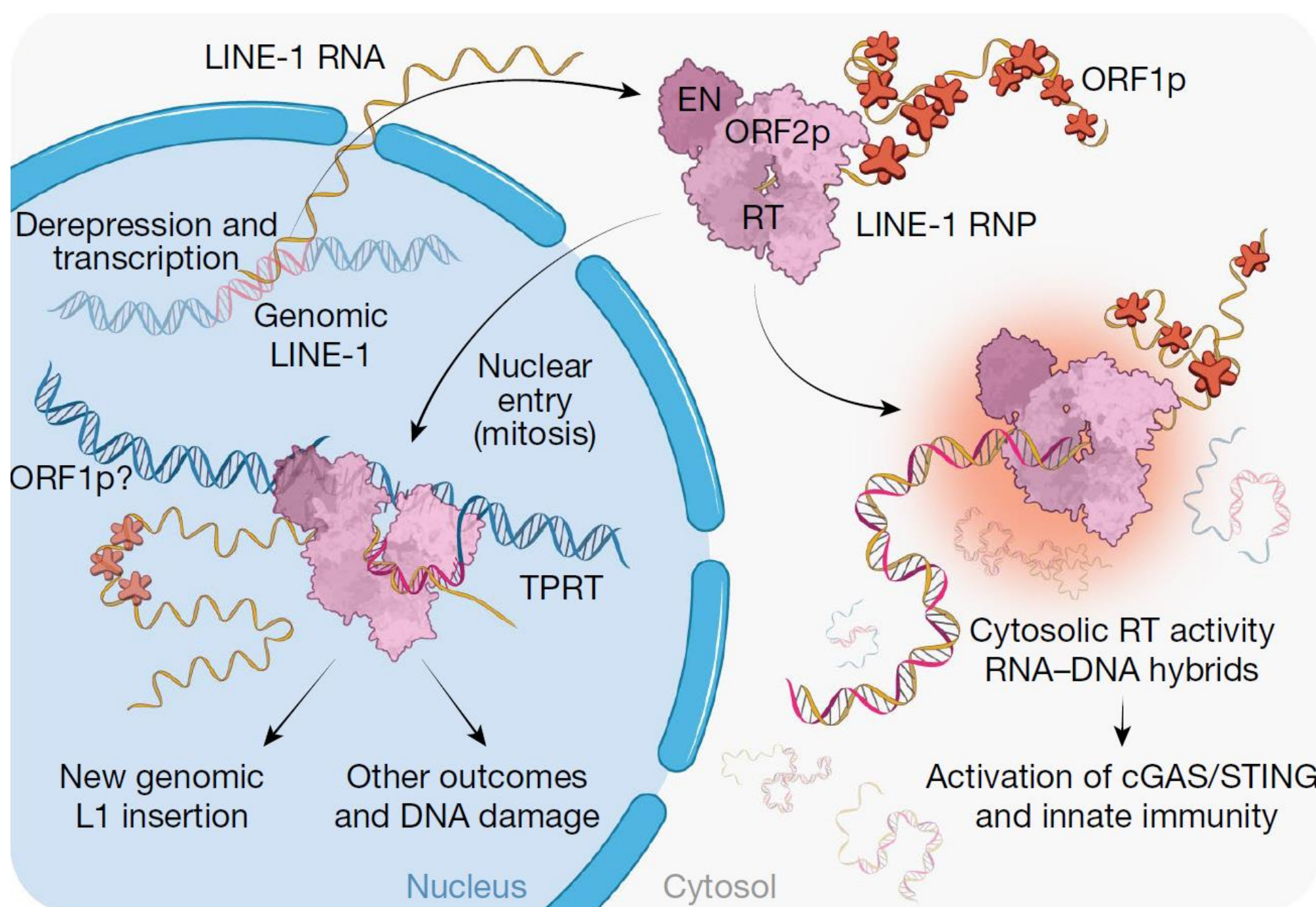
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Abstract

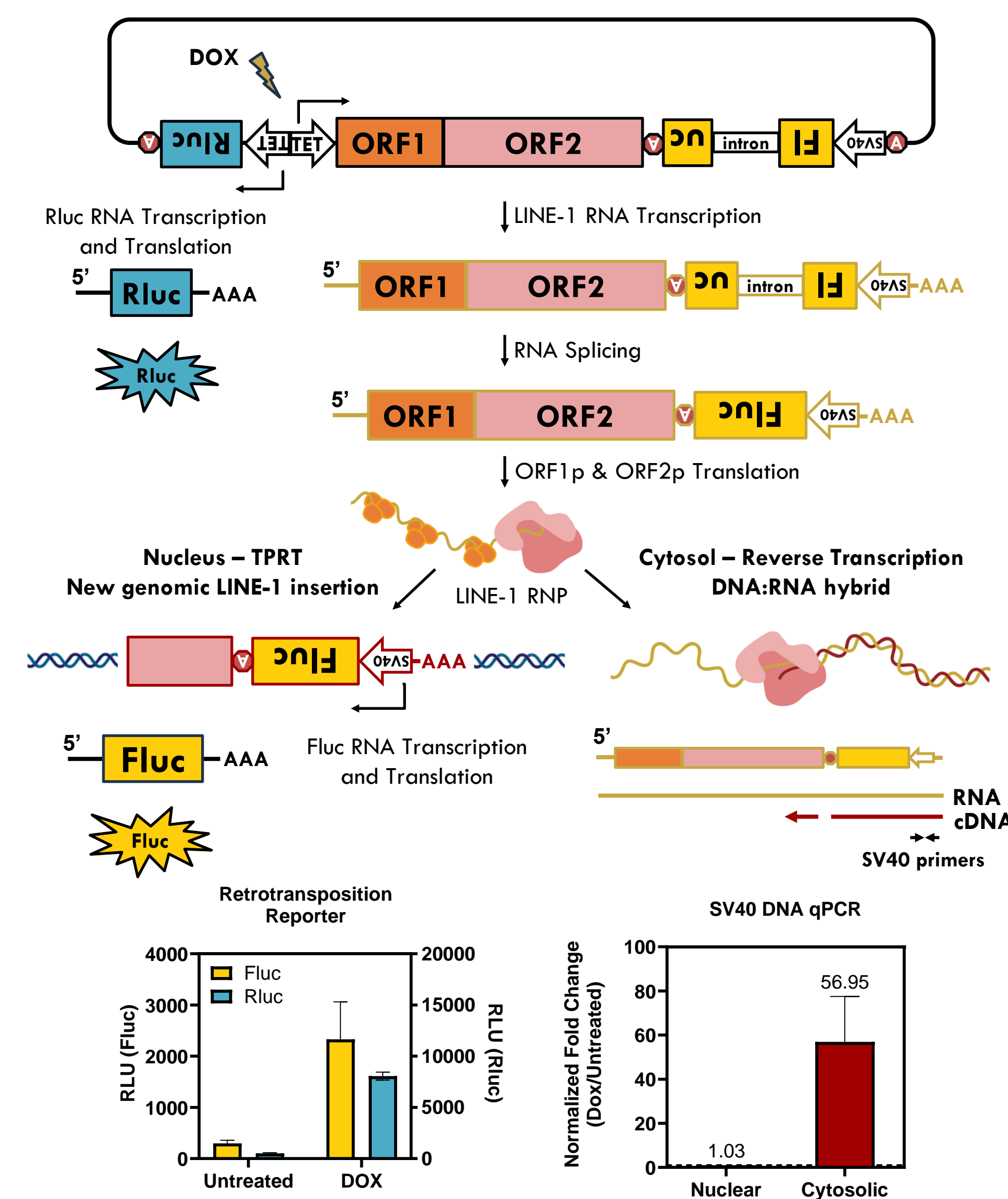
Long Interspersed Element-1 (LINE-1, L1) retrotransposons are genetic parasites that self-propagate through a 'copy and paste' mechanism driven by the LINE-1 encoded open reading frame 2 protein (ORF2p) that contains both endonuclease and reverse transcriptase (RT) domains. The RT activity of ORF2p has been linked to the development of cancer, autoimmune diseases, aging, and neurodegenerative disorders, making ORF2p a potential therapeutic target. ROME is currently developing novel LINE-1 reverse transcriptase inhibitors (RTIs) for therapeutic use. To date, efforts to explore LINE-1 as a therapeutic target have been hindered by a limited mechanistic understanding of LINE-1 RT activity in cells, particularly regarding the diversity of potential cytosolic reverse transcription products generated by LINE-1 RT using both its own RNA and other non-LINE-1 RNA templates. To address this, we have developed methods to identify and quantify LINE-1-mediated cytosolic DNAs, including DNA:RNA hybrids, from cells. These methods enabled us to demonstrate that LINE-1 generates cytosolic DNA products of varying lengths from its own RNA and non-LINE-1 RNAs, both of which depend on LINE-1 RT activity. Treatment with ROME RTIs reduced the abundance and size of these products. These findings provide key mechanistic insights into how LINE-1 reverse transcribes itself and non-LINE-1 RNAs, advancing the development of potential LINE-1 RT inhibitors as therapeutic candidates.

1. LINE-1 lifecycle diagram – DNA:RNA hybrids in cytosol

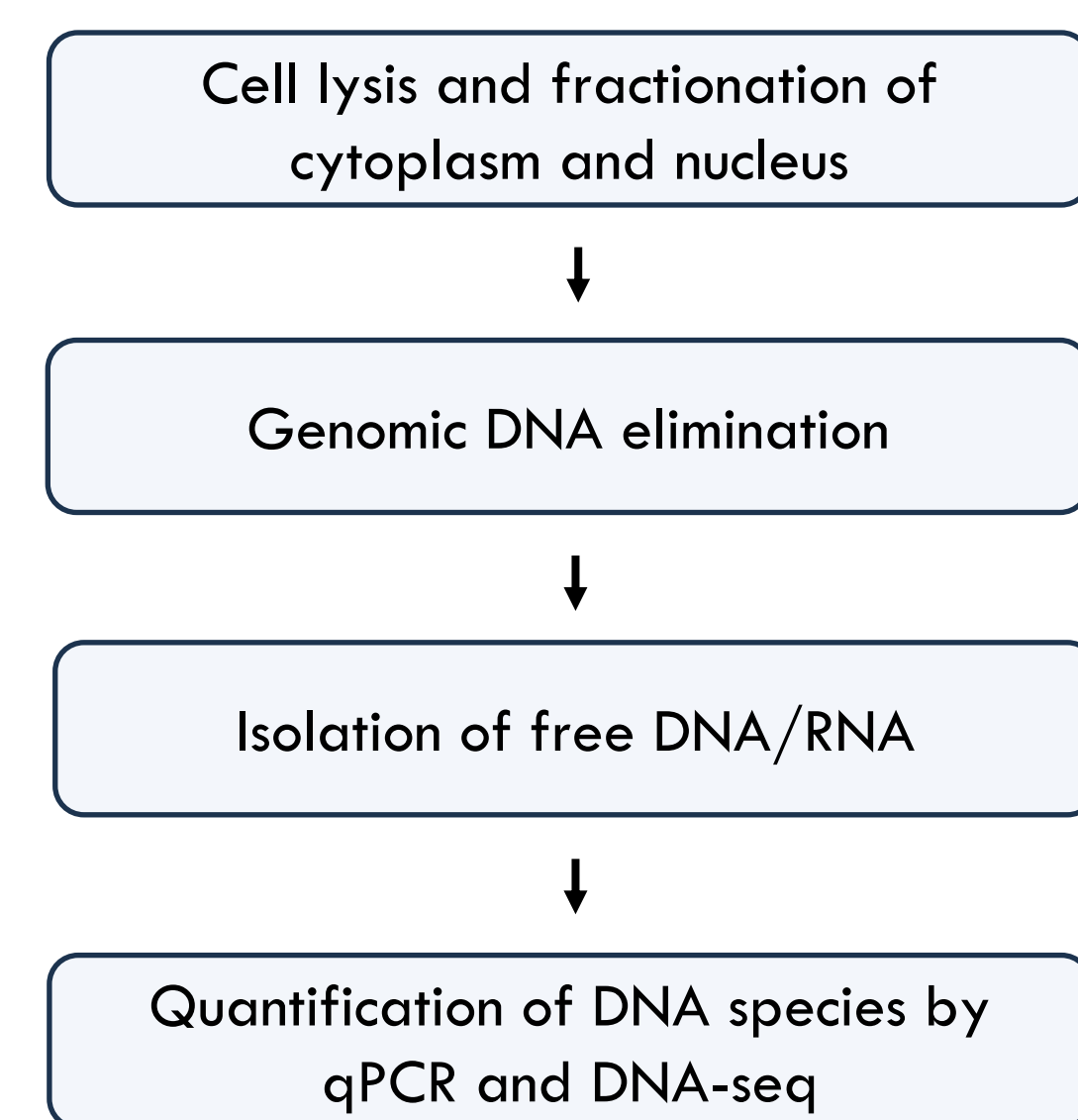


Baldwin et al Nature. 2024; 626(7997): 194–206

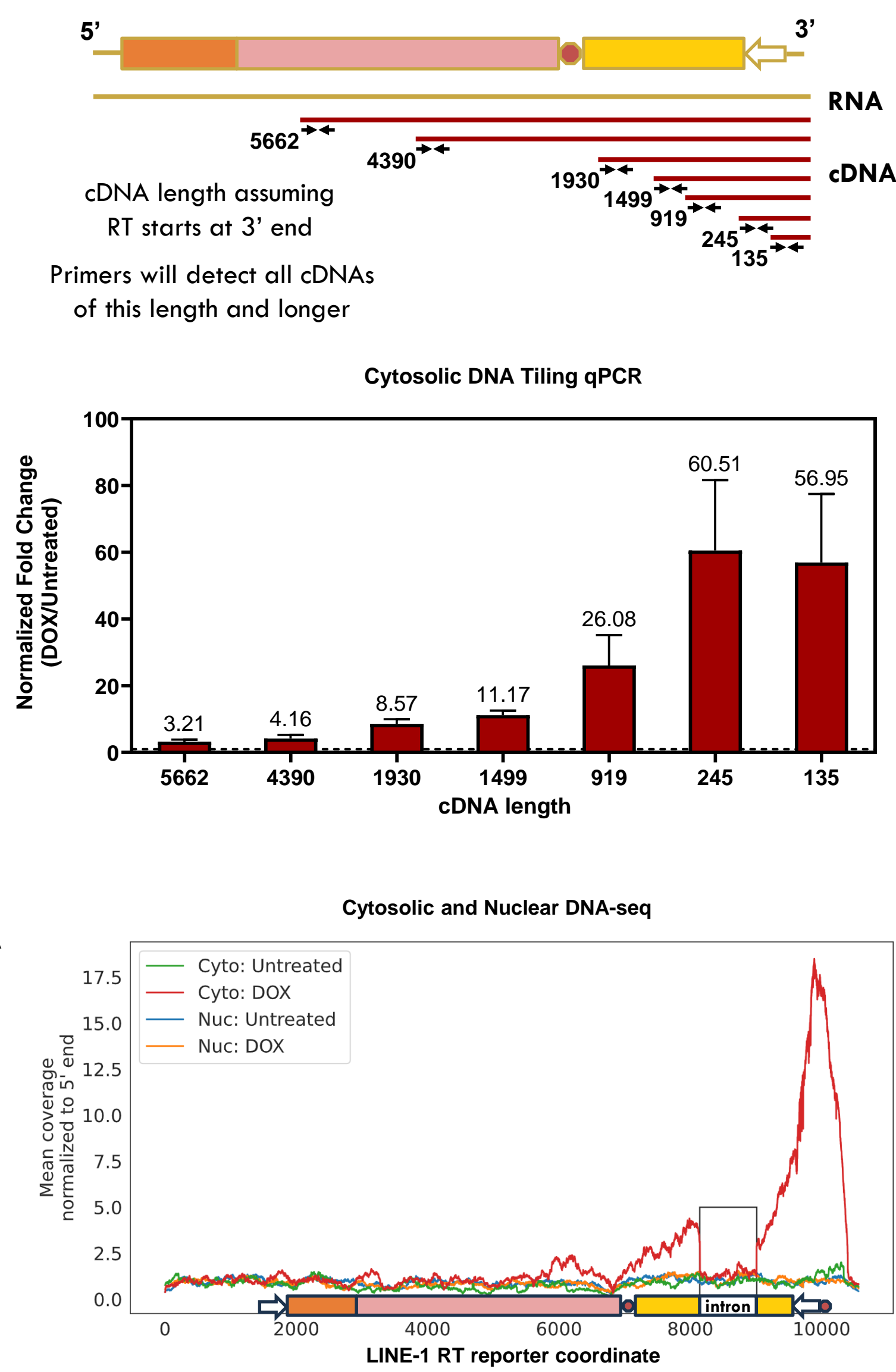
3. LINE-1 ORF2p RT activity can be measured by retrotransposition reporter and cytosolic DNA qPCR



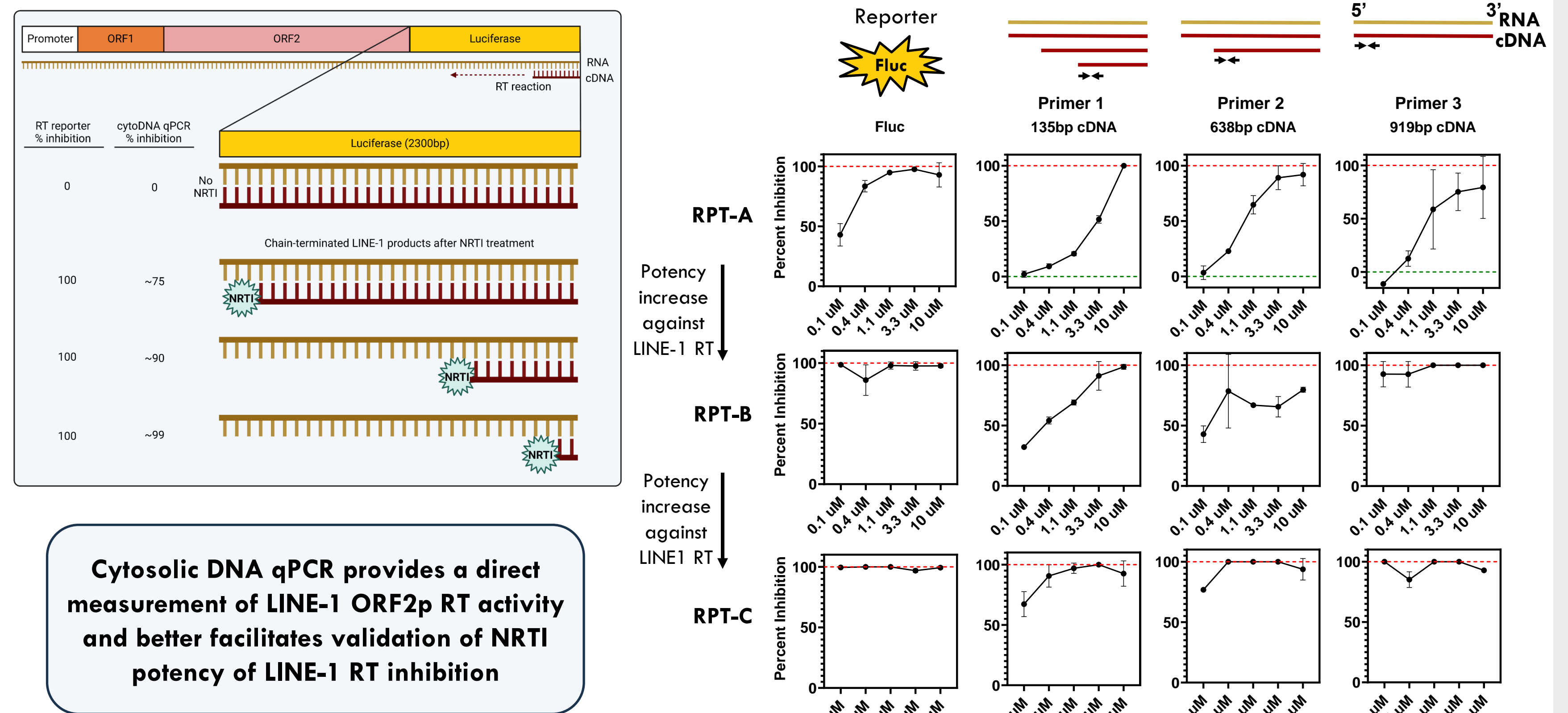
2. Isolation of cytosolic DNAs generated by LINE-1 reverse transcription



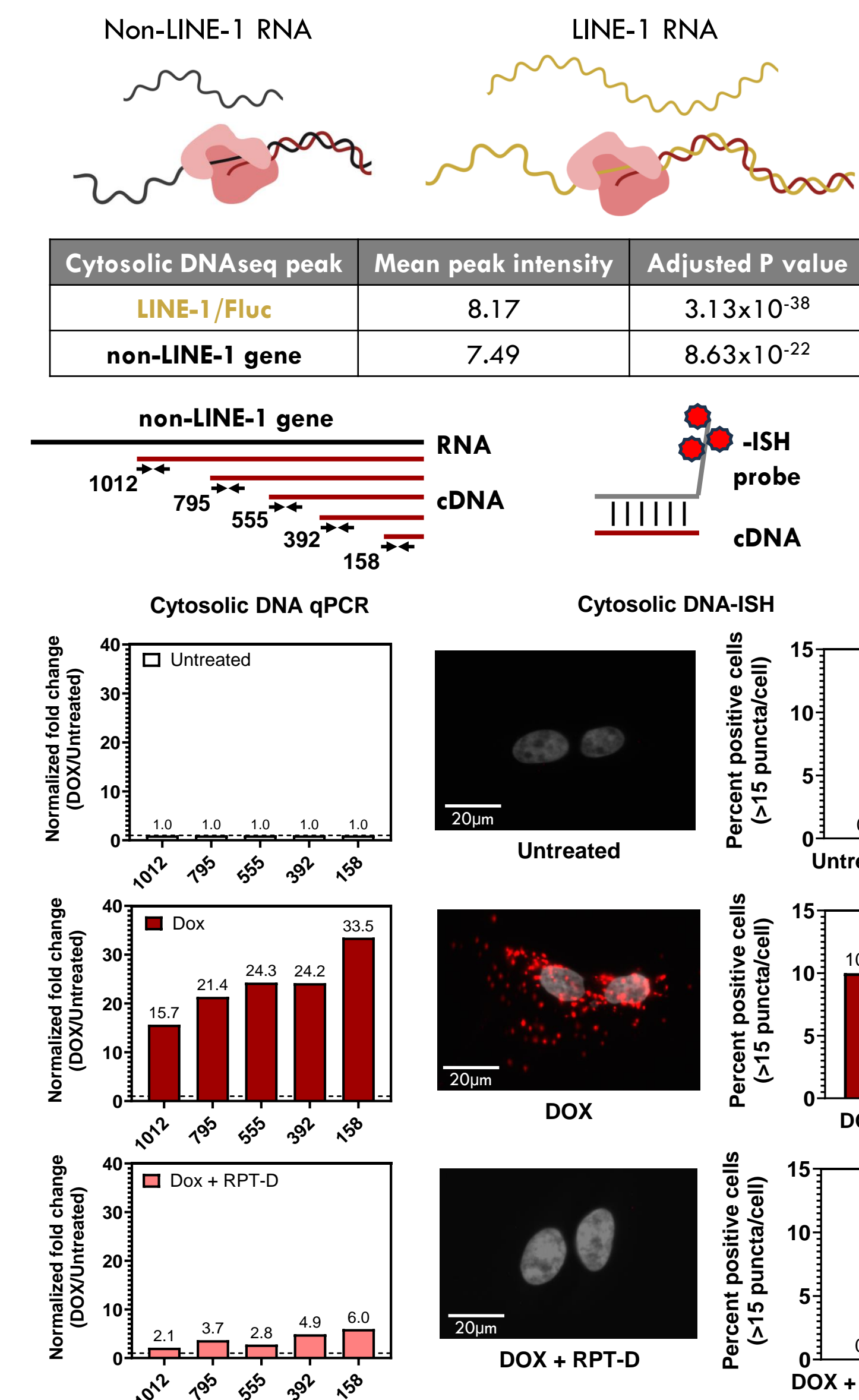
4. LINE-1 produces DNAs of varying sizes originating at the 3' end of LINE-1 mRNA



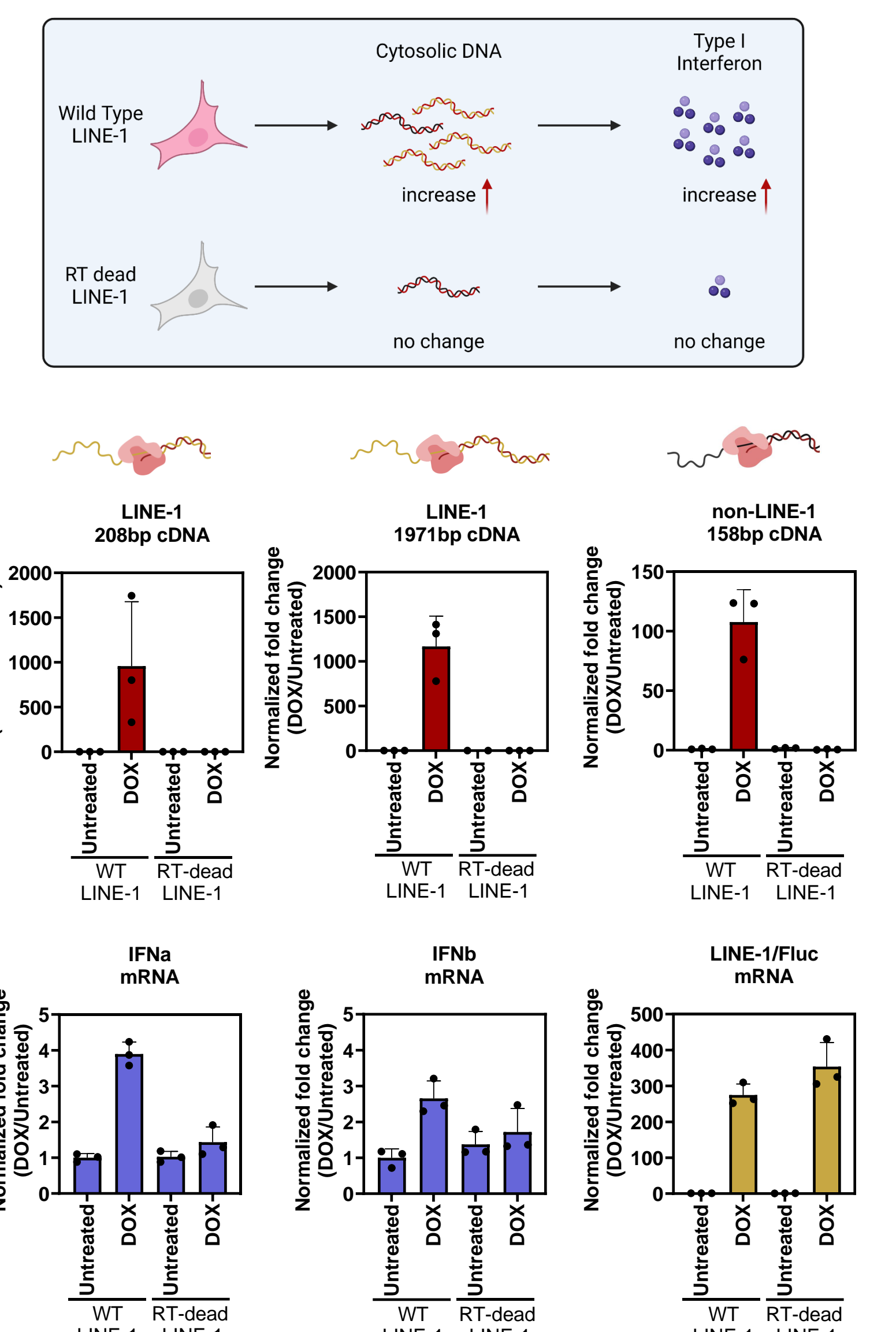
5. Nucleoside reverse transcriptase inhibitors (NRTIs) block formation of cytosolic LINE-1 DNA



6. LINE-1 can generate cytosolic DNA by reverse transcribing other cellular RNAs



7. Cytosolic DNA and interferon production are dependent on an active LINE-1 RT domain



Conclusions

- LINE-1 RT generates variable length DNA products from its own RNA and other cellular RNAs that are present in the cytosol
- Cytosolic DNA can be isolated and measured by qPCR and sequencing
- ROME NRTIs inhibit formation of cytosolic LINE-1 RT products
- Cytosolic DNA and interferon production are dependent on an intact LINE-1 RT domain
- ROME NRTIs are promising therapeutic candidates for diseases linked to aberrant interferon signaling and LINE-1

