

Expression of HSATII noncoding RNA in transfected fibroblast cells

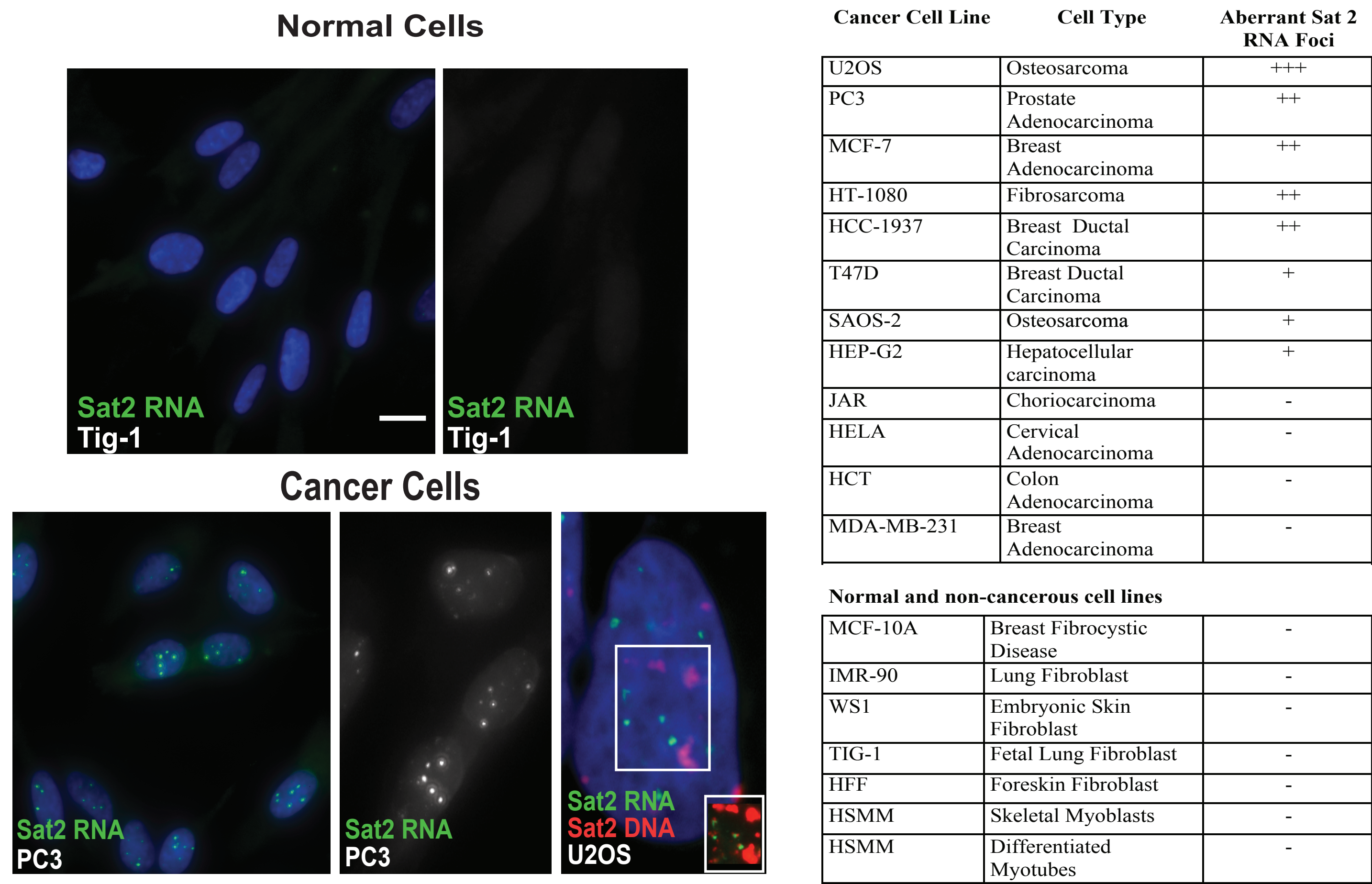
Lia R. D'Alessandro, Christina A. Rabeler, Emily Ferrari, Dawn M. Carone
Department of Biology, Swarthmore College, Swarthmore, PA
dcarone1@swarthmore.edu



Abstract

Human Satellite 2 (HSATII) is a tandemly repeated, ~26bp monomer sequence present in the pericentric heterochromatin on a subset of human chromosomes. Previously, it was demonstrated that HSATII RNA is expressed in many cancer types, producing a long non-coding RNA that accumulates in foci in cancer cells, while these transcripts are never observed in normal human cells. Further, this RNA accumulates in cancer cells in cis and binds key regulatory proteins, which are recruited to these nuclear foci. In an effort to understand the effect of HSATII RNA expression in cells that do not normally express HSATII, we created stably transfected primary human cells expressing HSATII RNA. In these stably expressing cells, we demonstrate that the HSATII expression construct is randomly integrated into the genome and the majority of cells contain a single site of integration. We observe focal accumulations of HSATII RNA in a subset of these transfected cells, demonstrating that ectopically-expressed HSATII RNA produces focal accumulations in the nucleus, with the capacity to recruit their protein binding partners and elicit changes in nuclear protein distribution.

HSATII (Sat2) expression in cancer cells

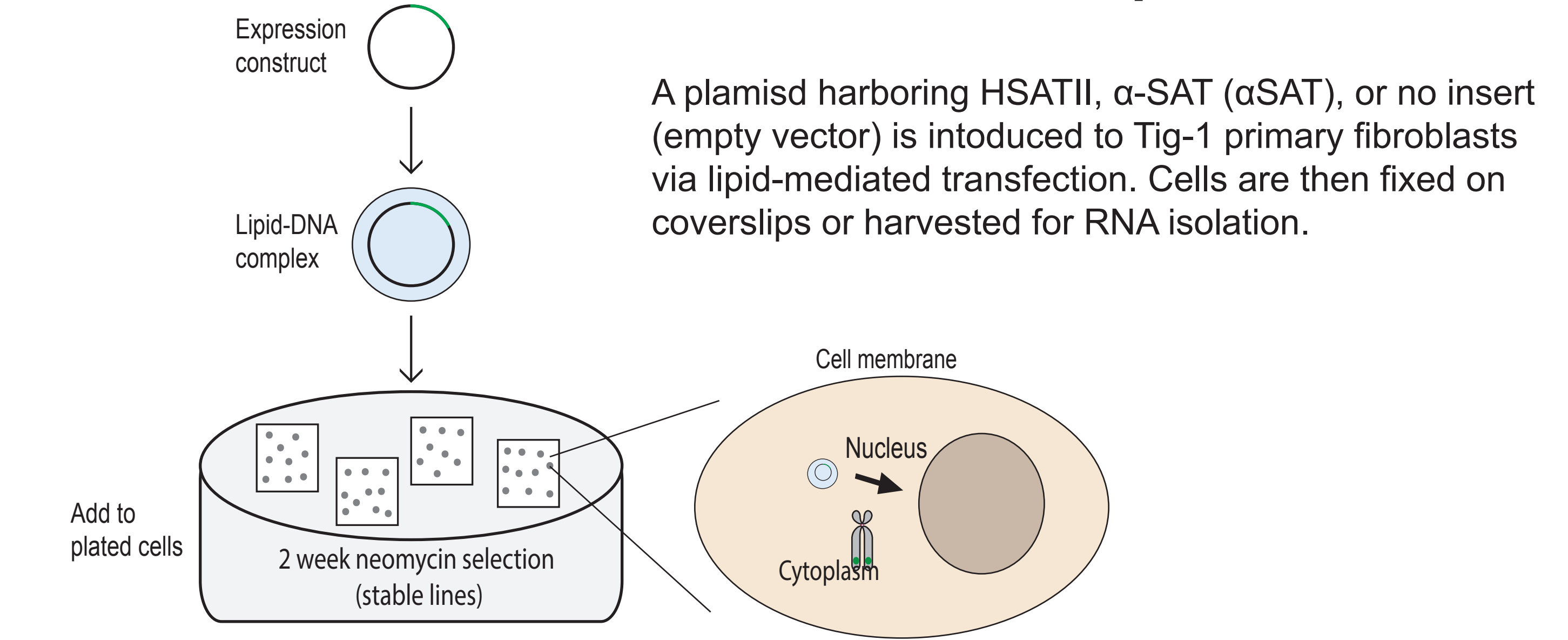


HSATII is aberrantly expressed and accumulates in cis in cancer cell lines. Fluorescence in situ hybridization (FISH) indicates HSATII aberrant focal accumulation is detected in a wide array of cancer cell lines. No expression is detected in a panel of normal (non-cancerous) cell lines. HSATII RNA foci accumulate adjacent to their pericentric DNA locations in the interphase nucleus (bottom, far right) (Hall et al., 2017).

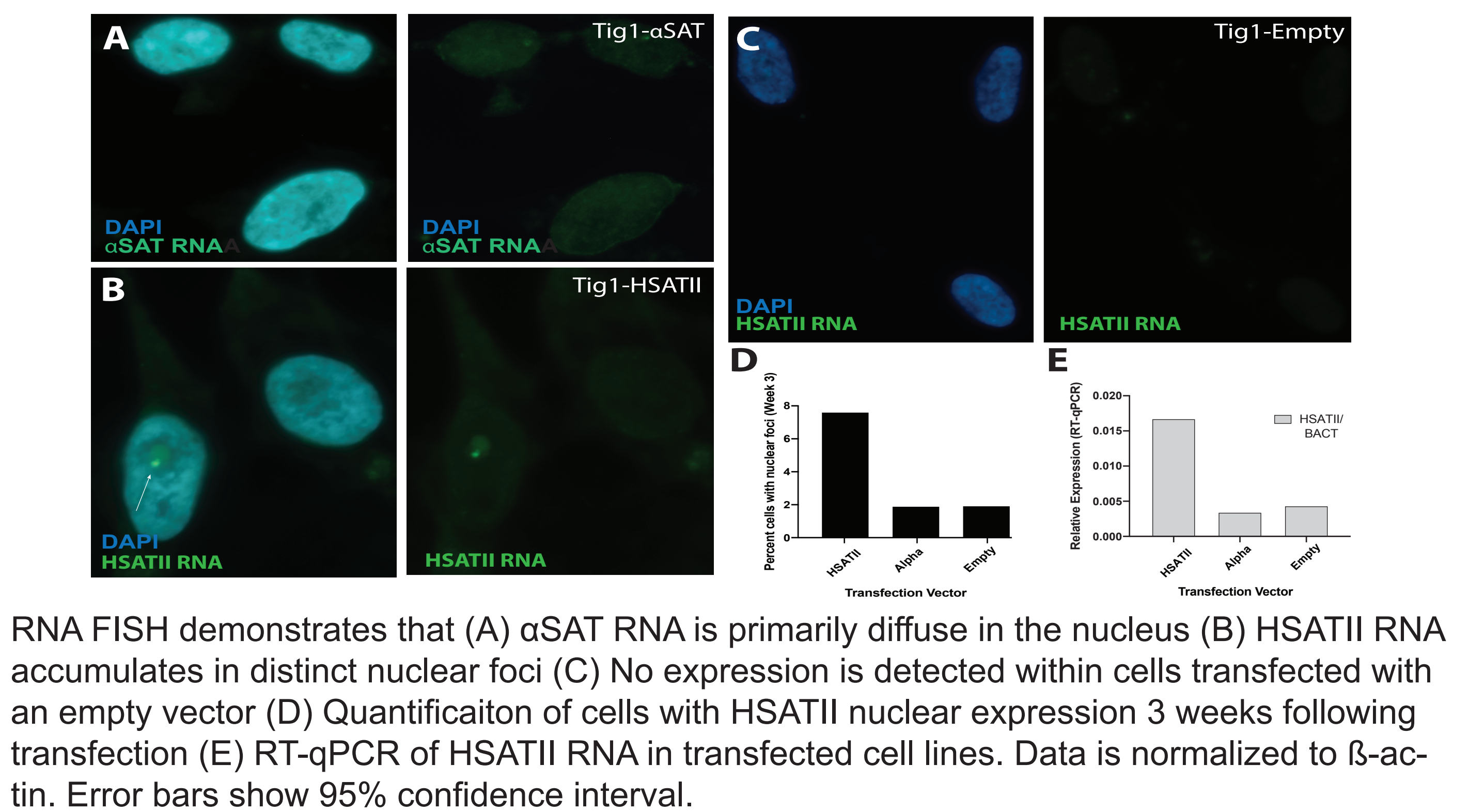
Overall Question

What are the consequences of HSATII expression?

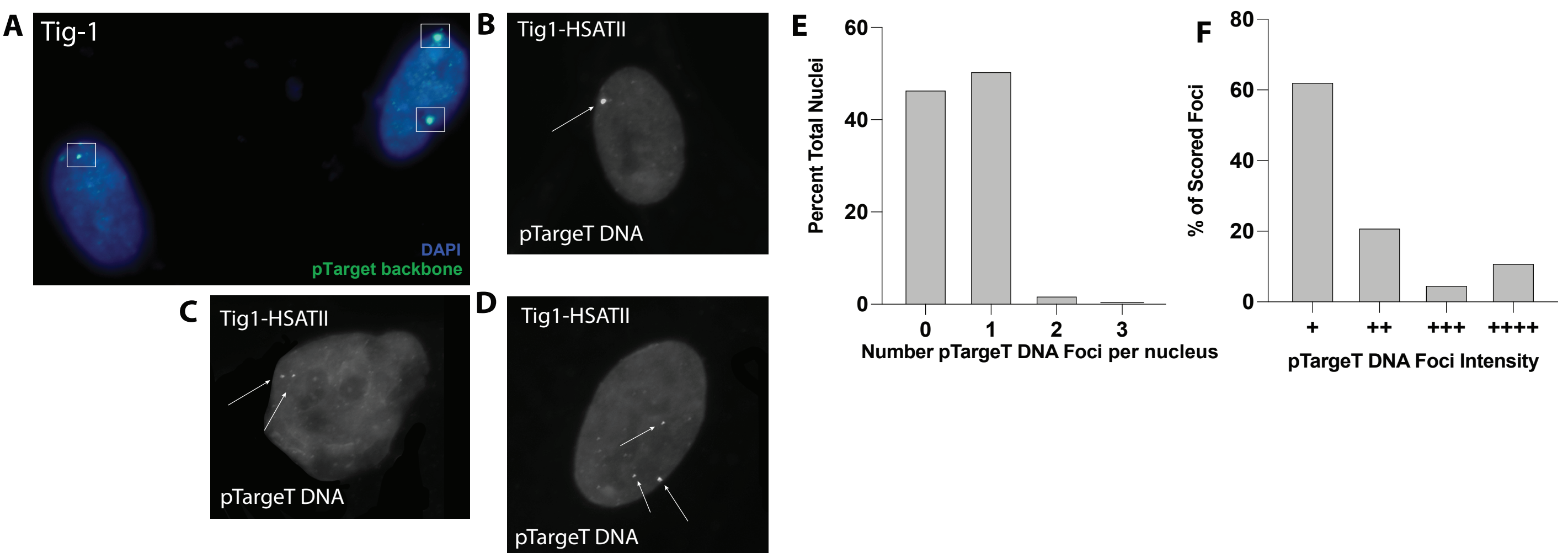
Stable transfection of satellite expression



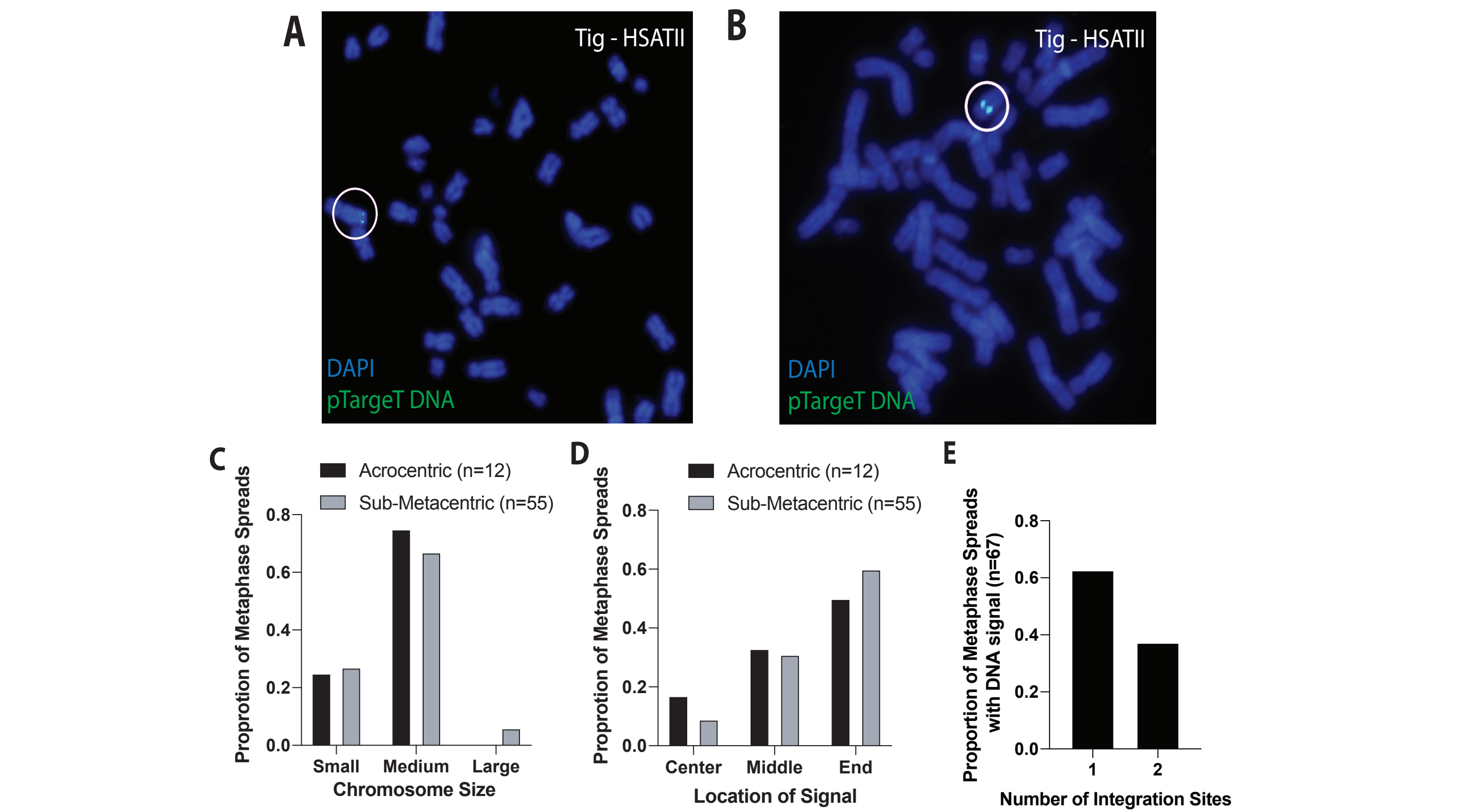
Expression of satellite RNA results in focal nuclear accumulation of HSATII RNA



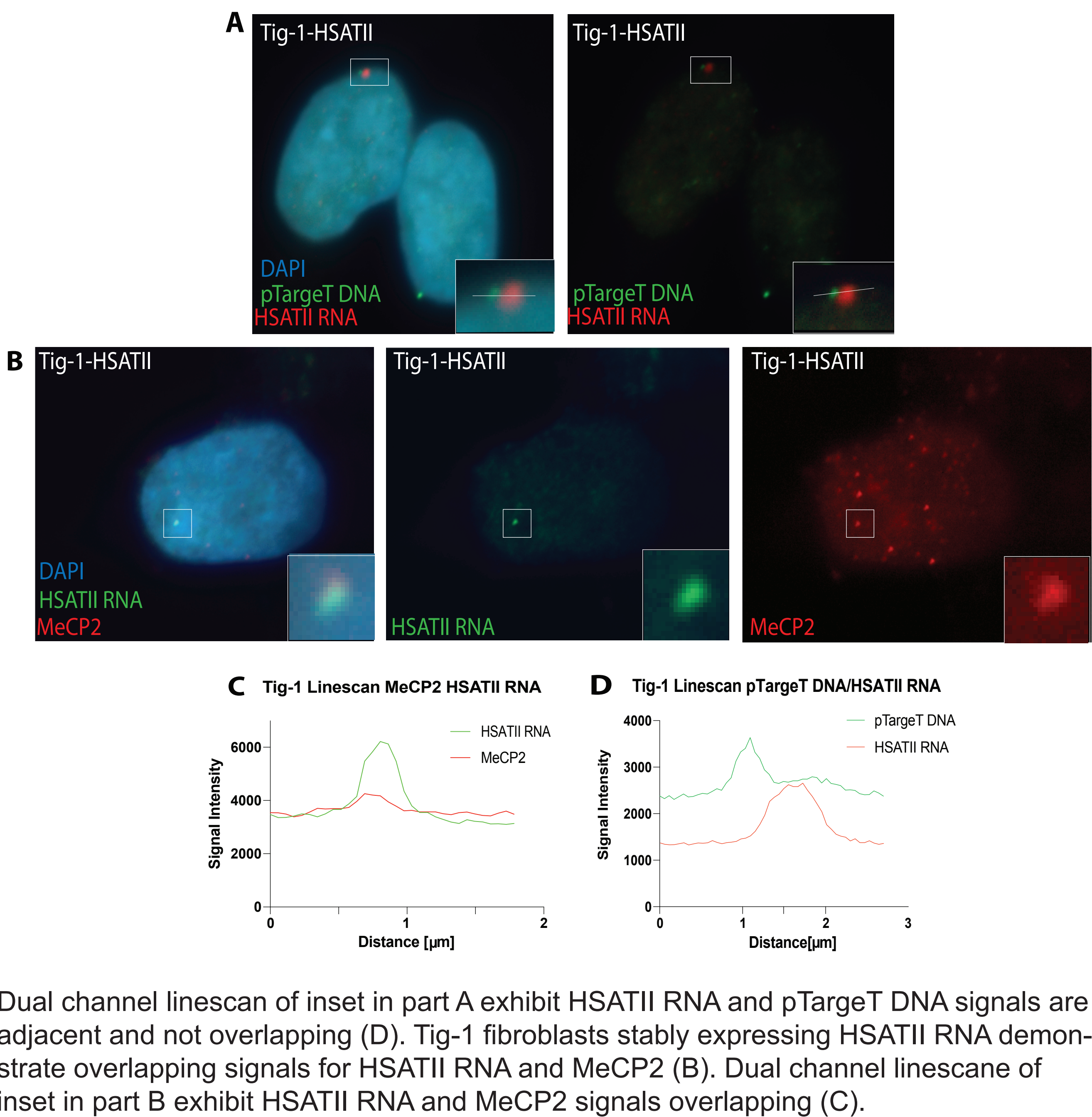
High efficiency integration of pTargetT vector DNA in HSATII transfected Tig-1 cells



pTargetT vector randomly integrates into Tig-1 chromosomes



HSATII RNA accumulates adjacent to site of integration and recruits MeCP2



Summary

We generated stable human cell lines exogenously expressing satellite sequences. We report that in a stably expressing HSATII cell line, HSATII RNA formed into distinct nuclear bodies, similar to the pattern of endogenous HSATII expression in cancer cells. HSATII expression leads to the accumulation of key regulatory proteins, such as MeCP2.

Timeline of ectopic HSATII expression

