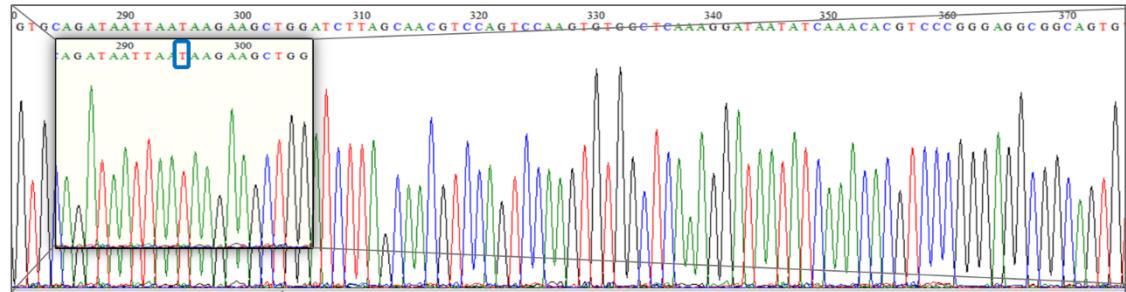
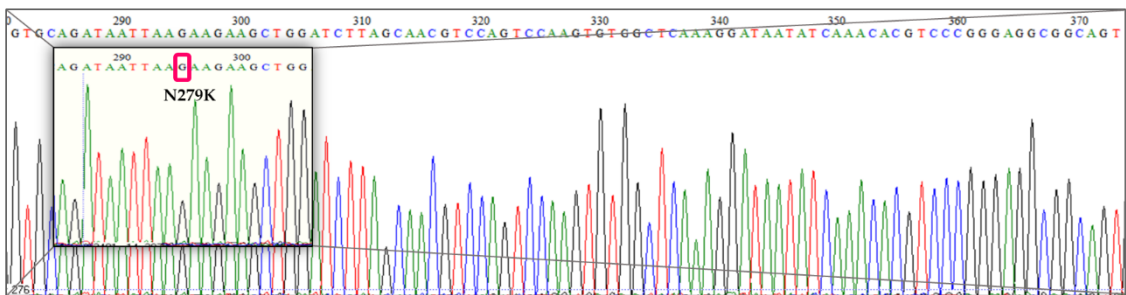


Supplemental Figures and Legend

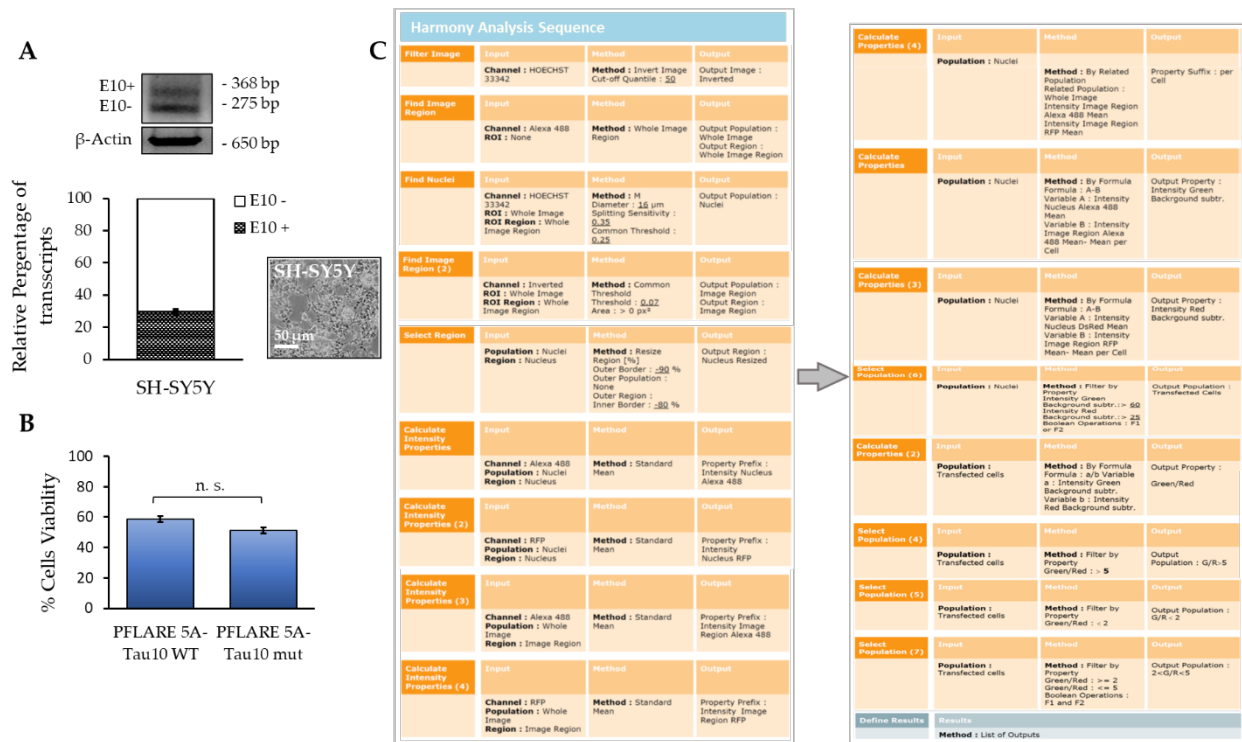
A Minigene PFlare5A-Tau10 WT



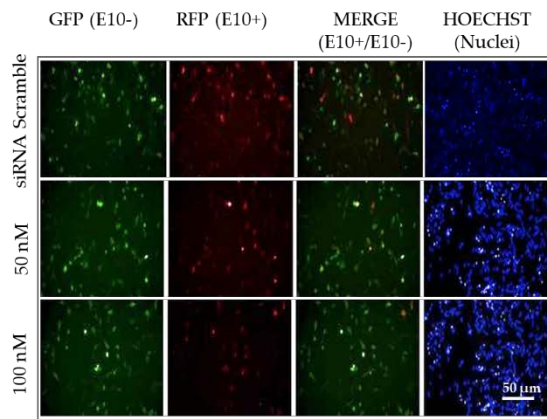
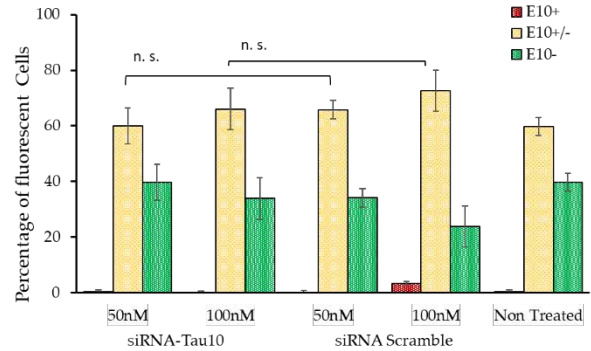
B Minigene PFlare5A-Tau10 mut



**Figure S1. Chromatogram sequence.** (a) of PFlare5A-Tau10 WT reporter plasmid (Minigene PFlare5A-Tau10 WT) and (b) of PFlare5A-Tau10 mut reporter plasmid (Minigene PFlare5A-Tau10 mut). Chromatogram shows the correct position of N279K point mutation in the PFlare5A-Tau10 mut reporter plasmid (BMR genomics, Padova, Italy).



**Figure S2. SH-SY5Y cell lines analysis and overall HCS-splice protocol procedure. (a)** Semiquantitative RT-PCR of Exon 10 endogenous expression levels in the SH-SY5Y cell line was performed as described in the material and methods section.  $\beta$  actin was used as a housekeeping gene (650 bp), and values were represented as mean  $\pm$  SD (n=3). **(b)** Percentage of cell viability in SH-SY5Y cells after transfection (48 hours) was measured by the Trypan Blue assay. Data represent mean  $\pm$  SEM obtained from triplicates. **(c)** Representative steps of the Harmony analysis protocol workflow used for the HCS-splice methods.

**A****B**

**Figure S3. Exon 10 expression of PFlare5A-Tau10 WT report plasmid after treatment with siRNA-Tau10.** (a) Representative images of SH-SY5Y cell line cotransfected with Minigene PFlare5A-Tau10 WT and treated with siRNA-Tau10 at concentrations of 50 and 100 nM (Top to bottom). The cells were acquired by a High-Content screening system (Operetta, PerkinElmer) after 48 hours of treatments, as described in Figure 2. The scale bar represents 50 μm. (b) The histogram represents the relative percentages of the three sub-populations of cells containing the reporter treated respectively with siRNA-Tau10 and Scramble siRNA control at a concentration of 50 nM and 100 nM. The values represent mean ± SD (n=3). Asterisks (\*) indicate significant differences (t-test, n.s.  $P > 0.05$ , \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ , \*\*\*\* $P \leq 0.0001$ ).