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Supporting information

Dynamic visualization of mRNA splicing variants with a transactivating reporter

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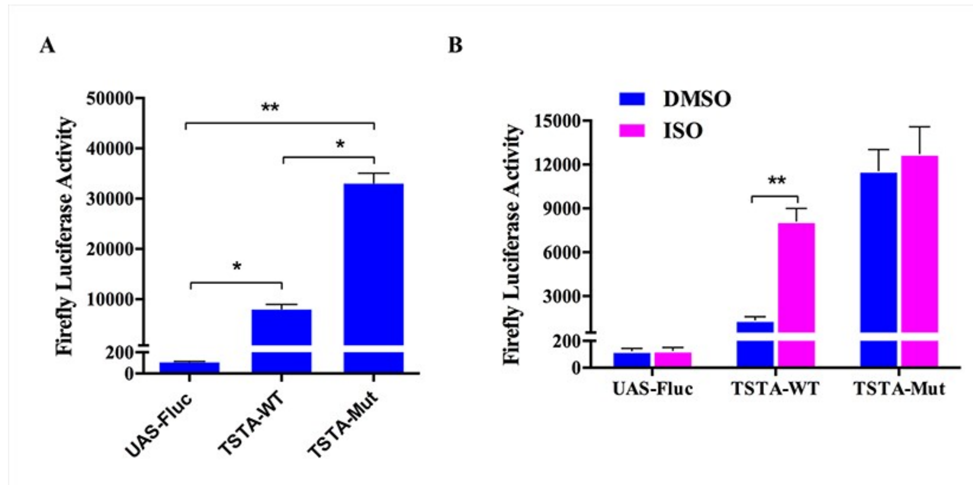

Supplementary Figure S1. Validation of the TSTA reporter in HepG2 cells. (A) Hepatocellular carcinoma HepG2 cells were transfected with UAS-Fluc, TSTA-WT or TSTA-Mut plasmids, respectively. The luciferase assay was carried out for luciferase activity from different transfected groups after 24 h. Data was shown as means ± SD. (B) The UAS-Fluc, TSTA-WT and TSTA-Mut reporter plasmids were transfected into HepG2 cells and treated or untreated with isoginkgetin (ISO) for 24 h. Then the firefly luciferase activity was measured in three transfected groups. Data was shown as means ± SD.

Materials and Methods

Plasmid construction

To construct a two-step transcriptional activation reporter, we developed two types of plasmids: an intron retained GAL4-VP16 vector and a UAS-Fluc vector. The intron retained GAL4-VP16 vector was generated by inserting a GAL4-VP16 fusion gene (synthesized by Shanghai Generay Biotech Co., Ltd, China) into the position 41 of intron 6 of human triose phosphate isomerase (TPI) minigene, which was described in our previous study (1). Then the TPI with GAL4-VP16 fusion gene was inserted into the KpnI/XhoI sites of pcDNA 3.1 (+) vector (Invitrogen). The pG5 luc vactor was purchased from Promega (Promega Corporation, US) to executive the function of UAS-Fluc construct. The mutant construct TSTA-Mut was designed by site-specific mutagenesis at both the 5 ' and 3' splice sites of TPI minigene but had no change in the UAS-Fluc vector. The pG5 luc plasmid with the function of UAS-Fluc vector serves as a control (U-Fluc-control).

Cell culture and transfection

Human lung adenocarcinoma A549 cells and hepatocellular carcinoma HepG2 cells were grown in DMEM medium (HyClone) supplemented with 10% fetal bovine serum (FBS, Gibco) and penicillin (100 U/ml; Gibco)/ streptomycin (100 g/ml; Gibco). The cells were maintained and incubated at 37°C with 5% CO_2. When the cells reached a density of about 80% confluence in plates, transfection of plasmids was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions.

Luciferase assay and in vitro bioluminescence imaging assay

The transfected cells were cultured for 12 hours and treated with Pladienolide B (CAS 445493-23-2, R&D Systems). Then the cell samples were collected and lysed separately for luciferase assay and for in vitro bioluminescence imaging. For luciferase assay, the luciferase activity was detected using Glomax-20/20 Luminometer (Promega). The luminescence signals for in vitro bioluminescence imaging were captured using a Xenogen Lumina Π system (Caliper Life Sciences).

RNA extraction and RT-PCR analysis

The transfected cells were harvested and the total RNA was isolated by Trizol reagents (Invitrogen). Then the RNA was reverse transcribed using a cDNA Synthesis Kit (ThermoFisher) following the manufacturer's directions. The region of spliced or unspliced mRNA was amplified by conventional PCR and the product of amplification was then applied to an agarose gel analysis. The expected size of PCR product is about 200 bp. The PCR were performed

using the following procedure: 95 °C for 5 min, 30 cycles of 94 °C (30 s), 56 °C (30 s), and 72 °C (30 s), and an extension step at 72 °C for 5 min. The primers are designed to locate at both ends of the exon of minigene. The PCR primer sequences were as follows: Forward 5'-TAC ACG AGA AGC TCC GAG GAT-3'; Reverse 5'-GCT GGC CAG CTC CTT GCA GGT-3'.

In vivo bioluminescence imaging

All the animal experiments were approved by Xi'an Jiaotong University Health Science Center following the Guidance for the Care and Use of Laboratory Animals. The TSTA reporter plasmids were transfected into A549 cells. 24 h later, the cells were harvested and 1x 10^7 cells were subcutaneously implanted into the right thigh region of nude mice (3 weeks, n=5, SPF Biotechnology Co. Ltd, Beijing). Twelve hours later, Pladienolide B (100 μg/kg) was injected intraperitoneally into the cell-inoculated mouse model. With the treatment of Pladienolide B at the indicated time, the mice were anaesthetized and the luciferase substrate D-luciferin (150 μg/kg, Shanghai YEASEN, China) was intraperitoneally injected into mice to acquire the in vivo bioluminescence image using a Xenogen Lumina Π system (Caliper Life Sciences). The regions of interest (ROI) of mice area was analyzed by using the Living Imaging Software 4.1 (Xenogen) to obtain the luminescence intensity, which was shown as p/sec/cm^2/sr.

Data and statistical analysis

All data are expressed as mean ± SD and analyzed by using GraphPad Prism version 6.0 software. The significant differences from three independent experiments were calculated using the Student's t-test. *P value <0.05, **P< 0.01 and ***P<0.001 was considered as significant.

Reference

1. Zheng, H., Chen, S., Wang, X., Xie, J., Tian, J. and Wang, F. (2019) Intron Retained Bioluminescence Reporter for Real-Time Imaging of Pre-mRNA Splicing in Living Subjects. *Analytical chemistry*, **91**, 12392-12398.