Supplementary Materials & Methods

MTT assay

Cell viability was measured through the reduction of yellow 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase to an insoluble, dark purple formazan product.[1] Approximately $2.5x10^4$ HEK293T cells were seeded in 96-wells plates and transfected with 50-250 ng AH, AL, LH, or LL plasmid using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Two days after transfection, MTT was added to all wells (1 mg/mL). After 30 minutes incubation, medium was removed from cells and cells were solubilized with DMSO. The released formazan reagent was measured spectrophotometrically at 570 nm. Puromycin (Sigma, St. Louis, MO) was added at 0-1.8 µg/mL to separate wells to create a killing curve.

RNA isolation and quantitative real-time RT-PCR

To determine U1 snRNA expression *in vitro*, approximately $2x10^5$ HEK293T cells were seeded in 24-wells plates and transfected the next day with 1 µg shRNA/U1i construct using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Total RNA was isolated from cells two days post-infection using the Nucleospin kit (Clontech, Mountain View, CA). To determine U1 snRNA expression *in vivo*, total RNA was isolated from four snap-frozen adductor muscles per AAV-shRNA/U1i construct at eight weeks post-transduction using Trizol (Invitrogen, Carlsbad, CA). Genomic DNA was removed by DNase treatment using TURBO DNase (Ambion, Austin, TX). First strand cDNA was reverse transcribed using random hexamer primers with the Dynamo kit (Finnzymes, Espoo, Finland). qPCR amplification (Applied Biosystems, Foster City, CA) was performed using primers pr484 and pr486 (Supplementary Table 2) that specifically recognize exogenous U1i plasmids, due to a mutation in stem-loop three in the U1 snRNA.[2] U1 snRNA expression was normalized to the beta-actin housekeeping gene and expression in mock-transfected cells and PBS-injected muscles was set at one for *in vitro* and *in vivo* experiments, respectively. Human beta-actin (pr480 and pr481) was used for *in vitro* experiments, and murine beta-actin was used (pr530 and pr531) for *in vivo* experiments (Supplementary Table 2).

Reference List

1. Peters AK, Steemans M, Hansen E, Mesens N, Verheyen GR, Vanparys P. Evaluation of the embryotoxic potency of compounds in a newly revised high throughput embryonic stem cell test. *Toxicol Sci* 2008; 105: 342-350.

2. Fortes P, Cuevas Y, Guan F, Liu P, Pentlicky S, Jung SP *et al.* Inhibiting expression of specific genes in mammalian cells with 5' end-mutated U1 small nuclear RNAs targeted to terminal exons of pre-mRNA. *Proc Natl Acad Sci U S A* 2003; 100: 8264-8269.