**Materials and Methods**

**Cell Culture and Drug Treatment**

Cultured 293T cells were maintained in Dulbecco’s modified Eagle’s medium (Gibco®, Thermo Fisher Scientific) containing 10% heat-inactivated fetal bovine serum (Gibco®, Thermo Fisher Scientific) and 1% penicillin-streptomycin (Gibco®, Thermo Fisher Scientific)32. Human dermal fibroblasts (HDFs) were cultured in medium 106 (Gibco®, Thermo Fisher Scientific) with low serum growth supplement (LSGS) (Gibco®, Thermo Fisher Scientific) and 1% penicillin-streptomycin (Gibco®, Thermo Fisher Scientific) as previously described33. Cells were incubated at 37°C in a humidified 5% CO2 atmosphere. NSC34 cells were cultured and processed for differentiation as previously described. Transfections were performed using LipofectamineTM 2000 or LipofectamineTM 3000 (Thermo Fisher Scientific) according to the manufacturer’s protocol. Briefly, cells were seeded into the wells of a 96-well plate (7.5×103 cells for viability assays), a 12-well plate (1.5×105 for immunocytochemistry (ICC) studies) or a 6-well plate (5×105 for time-lapse studies) and cultured overnight. Cells transfected with mCherry-SMNΔ7 or cotransfected with mCherry-SMNΔ7 and EGFP, TDP-PLD, or TDP-NP expression plasmids were assayed for neurite outgrowth at 48 h posttransfection. Baicalein (50 μM) was added at 24 h posttransfection, and neurite outgrowth was determined 24 h after drug addition. To determine the effects of compounds on the LLPS of prion-like proteins, the cells were treated with baicalein and b-isox for 24 or 48 h at the indicated concentrations (5, 10, 25, or 50 μM).

**Subcellular Fractionation and Western blotting**

Cultured 293T cells were harvested using a subcellular protein fractionation kit (Thermo Fisher Scientific) according to the manufacturer’s recommendations. Cell lysates were separated into five different cellular compartments. The fractionated proteins were used for Western blotting, as previously described32. The SMN and mCherry antibodies were kind gifts from Hung-Li. The profilin1 antibody was purchased from Proteintech (11680-1-AP).

**B-isox Precipitation**

Briefly, 293T cells were harvested and lysed with RIPA buffer. The protein concentration was adjusted to 1 to 10 mg/mL, and 10 mM b-isox was added to the cell lysate at a final concentration of 100 to 200 μM. The mixtures were then incubated at 4°C for 60 min and centrifuged at 15000 rpm for 15 min at 4°C, and the supernatant was discarded. All of the mixtures were subjected to SDS-PAGE and Western blotting.

**Mice**

A mouse model of SMA was produced by deleting exon 7 of the *Smn* gene and knocking in the human *SMN2* gene (*Smn−/−SMN2+/−*)35. We were able to generate a variant that exhibited more severe disease symptomatology by backcrossing to obtain a more homogenous genetic background with two copies of the *SMN2* transgene (*Smn−/−SMN2+/−*) (severe SMA model). We also generated a model of mild SMA, which carried four copies of the *SMN2* transgene (*Smn−/−SMN2+/+*)33. Both severe and mild SMA mice received daily intraperitoneal injections of baicalein (13.6 mg/kg/d in DMSO) or DMSO alone (control) from birth until death (severe SMA mice) or from 7 to 11 months of age (mild SMA mice). The severe SMA mice were then subjected to motor function tests and survival analysis. Mild SMA mice were subjected to motor function tests, survival analysis, electrophysiological studies, and pathological studies. Another group of mild SMA mice received the same dose of baicalein or DMSO at 4 months old for 2 weeks for Western blot and quantitative PCR analyses. Housing and husbandry were maintained with standard procedures in the NTUCM Laboratory Animal Center. All animal procedures in this study were approved by the Institutional Animal Care and Use Committee (protocol #20190314).

**Motor Function Tests**

For mild SMA mice, both fixed-speed (20 rpm for 5 min) and accelerating (from 4-40 rpm in 5 min) rotarod test protocols were conducted to evaluate motor function every 2 weeks, as previously described36. Mice were placed in a neutral position on the stationary 3-cm diameter cylinder of the rotarod apparatus (Singa RT-01), and the time to fall was recorded when each mouse fell off the rod. For severe SMA mice, the turnover, tube and negative geotaxis tests were conducted at 6 and 8 days old, as previously described37. In the turnover test, the time required for a mouse to right itself and place all four paws on the ground from a prone position was recorded (the cutoff time was 60 sec). The tube test was used to determine hind limb strength according to the posture of the hind limbs and the tail; scores ranged from 0 (the worst) to 4 (the best). In the negative geotaxis test, mice were placed on a 45° incline with their head pointing downward. The responses (turning and climbing) were scored from 0 (worst) to 4 (best).

**Retrograde Tracing, Immunocytochemistry, and Immunohistochemistry**

For retrograde motor neuron tracing, fluorogold dye (Fluorochrome, LLC) was injected into the bilateral gastrocnemius and tibialis anterior muscles of mild SMA mice (2 μL for each muscle) 3 days before sacrifice. For immunocytochemistry, HDFs were fixed and blocked as previously described33. Gem number counts and SMN nuclear localization in fibroblasts were confirmed using DAPI counterstain. For immunohistochemistry, lumbar spinal cords and hamstring muscles from mild SMA mice were fixed with 4% paraformaldehyde (PFA) and dehydrated in a series of sucrose solutions. Lumbar spinal cord cryosections (10 μm) and hamstring muscle cryosections (15 μm) were blocked, permeabilized, and incubated with the appropriate antibodies as previously described38. Images were obtained using a Zeiss LSM880 microscope (Carl Zeiss) with a 10× or 20× objective lens (plan-apochromat 20×/0.8 M27 and plan-apochromat 40×/1.3, oil, DIC M27). The mean number of motor neurons per anterior horn, nuclear gem counts per motor neuron, and percentage of axonal innervation at the NMJ were calculated for each animal. The primary antibodies included anti-SMN, anti-ChAT (EMD Millipore, #AB144P), anti-neurofilament-H 200 kDa (EMD Millipore, #AB1989), anti-synaptophysin (Abcam, #ab32127), and α-bungarotoxin Alexa Fluor 555 conjugate (Thermo Fisher Scientific, #B35451).

**Electrophysiological Study**

The mice were anesthetized with Zoletil (20 mg/kg, ip). The skin in the right hind limb was shaved and adorned with surface nonpolarizable Ag/AgCl electrodes (Ambu® Blue Sensor NF, Ambu A/S, Denmark), which were trimmed to approximately 0.5cm ×0.5cm over the gastrocnemius muscle. An invasive needle electrode was placed near the sciatic notch for stimulation, and the ground was placed on the tail with an invasive needle electrode. The recording was performed with a Nicolet EDX System and Synergy interface (Natus Medical Incorporated, USA). The stimulation was increased gradually to obtain supramaximal stimulation and a stable CMAP amplitude.

**Time-lapse Study**

Briefly, 293T cells were first seeded on glass slides in 6-well culture plates and then transfected with mCherry-tagged plasmids. After 48 h, a glass slide was placed into a perfusion, open, and closed (POC) chamber (Pecon) and into an incubator containing a spinning disk confocal microscope (Axio-observer. Z1/7, Zesis). Images were acquired under a 63× objective lens (plan-apochromat 63×/1.40, oil, DIC, M27) using the mCherry (EX 572/35, EM 632/60) filter set for 2 min at appropriate time intervals. Analysis was conducted using the integrated ZEN (v.3.1) imaging program and Imaris software (v.9.6.0, Bitplane).

**Extended Data Figures**

**Extended Data Fig. 1｜RT-qPCR analysis of the mRNA levels of SMN-FL and SMNΔ7 in mice treated with or without baicalein.**

The data presented were normalized to the GAPDH level and are shown as the mean ± SD (*n* = 4) mice in each group, unpaired two-tailed Student’s *t*-test.

**Extended Data Fig. 2｜Effects of baicalein on SMA Type I Mice**

The therapeutic effects of baicalein on motor function in SMA mice**.** SMA mice and their heterozygous littermates were treated with daily intraperitoneal baicalein injections from birth and then subjected to motor functional analyses. The righting times (left panel), tube scores (middle panel), and tilting scores (right panel) of untreated (SMA, *n*=27; heterozygous, *n*=23) and baicalein-treated (SMA/baicalein, *n*=18; heterozygous/baicalein, *n*=27) SMA and heterozygous mice are shown. The motor function of SMA mice was significantly improved after baicalein treatment, particularly on postnatal day 6, and was partially improved on postnatal day 8 (*one-way* ANOVA with the LSD post hoc test). \* *p*<0.05, \*\* *p*<0.01, \*\*\* *p*<0.001. The lifespans of SMA mice were not significantly different after baicalein treatment (*p*=0.13, log-rank test). **b** & **c,** baicalein treatment did not improve body weight gains in SMA mice.

**Extended Data Fig. 3｜Images of gem in healthy HDFs treated with or without b-isox**

Reduction in gem body numbers in healthy HDFs treated with b-isox. The arrow indicates gems. Scale bars: 20 µm.

**Extended Data Fig. 4｜Analysis of the LLPS capability of Y272C and G279V**

**a**, Top panel, b-isox chemical binding analysis of the missense SMN mutants Y272C and G279V. Middle and bottom panels, solubility of the Y272C and G279V mutants. **b**, Cellular expression patterns and localization of the Y272C and G279V mutants.

**Supplementary Methods**

**Reverse Transcription and Real-time PCR**

Tissues from mice were dissected, and total RNA was extracted by TrizolTM reagent (Thermo Fisher Scientific) according to the manufacturer’s protocol. Reverse transcription was performed using an iScriptTM cDNA synthesis kit (Bio-Rad) and processed for quantitative PCR analysis on a CFX Connect Real-time system with built-in CFX Maestro software (Bio-Rad). The primer sequences for qPCR were as follows: SMNexon7 forward (F): 5’-GAAAAAGAAGGAAGGTGCTCACATT-3’; SMNexon 7 reverse (R): 5’-GTGTCATTTAGTGCTGCTCTATGC-3’; SMNΔ7 F: 5’-CATGGTACATGAGTGGCTATCATACTG-3’; SMNΔ7 R: 5’-TGGTGTCATTTA

GTGCTGCTCTATG-3’; GAPDH F: 5’-GACTTCAACAGCAACTCC-3’; and GAPDH R: 5’-TATTCATTGTCATACCAGGAA-3’.

**Supplementary References**

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