Supplemental Materials

NXD30001 solutions and formulation

For *in vitro* exposure, NXD30001 was prepared in dimethyl sulfoxide (DMSO) at 20mM and diluted in culture media. For *in vivo* administration, NXD30001 was dissolved in DMSO at 400mg/mL, then formulated to the final concentration of 20mg/mL in 5% DMSO (with drug), 9.5% PEG400, 76% Plurol® Oleique CC497 (Gattefosse), 9.5% Lauroglycol™ FCC (Gattefosse), and 0.095% Tween20, and delivered in a volume of 2.5-7.5mL/kg by intraperitoneal (i.p.) injection.

NF2-deficient cell lines, mouse Schwann cells and patient-derived schwannoma primary cultures

ESC-FC1801 mouse embryonic Schwann cell line, in which exon 2 of NF2 was deleted *in vitro,* was cultured in serum-free N2 medium (DMEM/F12 with 1% N2 supplement (Invitrogen), 10ng/mL neuregulin-β1 (NRG1) (R&D Systems) and 2μM forskolin (Calbiochem), 50μg/mL gentamycin, and 1% fungizone (Invitrogen)) as previously described (1, 2). Mouse schwannoma cell line 08031-9 expressing the human Sch-Δ(39–121) dominant-negative mutant (3) was cultured in proliferation PM medium (DMEM with 10% FCS (Invitrogen), 1% N2 supplement, 10ng/mL NRG1 and 2μM forskolin, 50μg/mL gentamycin, and 1% fungizone). Mouse primary Schwann cells (mSC) were isolated from the sciatic nerves of 1-2 month-old adult mice and cultured in N2 or PM media. An immortalized human schwannoma cell line HEI193 (4) was cultured in DMEM supplemented with 10% FCS, 50U/mL penicillin, and 50μg/mL streptomycin. Human schwannoma and meningioma samples, immediately after the surgical removal, were dissociated overnight with 0.05% collagenase type I (Invitrogen) and 1.25U/mL dispase (Roche) in DMEM/10% FCS, and then plated at $10^5/cm^2$ in PM medium supplemented with 0.5mM 3-isobutyl-1-methylxanthine (IBMX). All cells were cultured on poly-L-lysine (Sigma) /laminin (Invitrogen) coated dishes at 37°C in 7.5% CO_2, except for HEI193 which was cultured in non-coated flask at 37°C in 5% CO_2.

MTS-based cell viability assay

The number of living cells was measured by using the MTS-based CellTiter 96 Aqueous One Cell Proliferation assay (Promega), which quantifies the bio-reduction of MTS tetrazolium into colored formazan by metabolically active cells. Cultured cells from established cell lines ESC-FC1801 and 08031-9 were plated at 10^3 cells/well in 96-well plates, and primary cultures from patient-derived

tumors at $5x10^3$ cells/well. NXD30001 in various concentrations was administered the day after plating and cells were cultured for another 72hrs.

Soft agar clonogenic assay

Clonogenic assay for NF2-deficient cell lines was performed in a 60-mm dish format according to the method introduced by Hamburger *et. al.* (5). Briefly, single cell suspensions ($2.5\sim5x10^3$ cells/60mm dish) in 4ml of RPMI medium containing 0.35% low-melting-temperature agarose (supplemented with 10% FCS and NRG1/folskolin as required), were plated on top of the 2mL base layer, then sealed by the 2mL top layer (both 0.7% agarose). After 24 hrs, the drug was added in an additional 4ml of appropriate media. Cultures were incubated at 37°C, 7.5% CO_2 for 2~3 weeks until the colonies became visible. The liquid media was changed every 3~4 days. Numbers of colonies were counted, and drug effects are expressed in percentage of survival (% of control).

Allograft models and antitumor efficacy assay

Female athymic nude (*nu/nu)* mice and FVB/N mice (Charles River Laboratory) were housed under optimized conditions with a 12-hour light cycle and fed standard food and water *ad libitum*. The mouse $Nf2^{-/-}$ Schwann cells, ESC-FC1801, and the transplantable mouse schwannoma homogenate expressing the human Sch-Δ(39–121) mutant, 08031-9, were resuspended in DMEM and mixed with Matrigel (1:1) (BD bioscience), then grafted subcutaneously (s.c.) in nude and in FVB/N syngenic mice, respectively. The tumor volume was estimated using the formula ($WxLxLx\pi/6$), where width (W), length (L) are the two largest diameters. When the average tumor volume reached approximately $100mm^3$, NXD30001 was administered intraperitoneally (i.p.), three days a week at 50-150 mg/kg/day. Mice in the control group were treated with vehicle under the same schedule. After the last injection, animals were anesthetized, the blood was collected by cardiac puncture, and the allograft tumors were dissected out of the subcutaneous cavity.

Histopathological analysis of NF2 transgenic mice

F1 mice of NF2 transgenic mice expressing human Sch-Δ(39–121) dominant-negative mutant are routinely generated for drug testing by crossing the strain TgP0-Sch-D(39–121)-27 in FVB/NCrl background to C3H/HeNCrl mice. 100% of the F1 transgenic mice were found to develop a panel of peripheral nerve lesions, from Schwannomal tumorlets to frank schwannomas, by 3 months of age. The

administration regime of 17-DMAG was adopted from the previous report (6). Briefly, the drug was dissolved in water and administered with feeding needles three days per week for eight weeks.
The lumber and sacral spines were dissected and processed for standard HE staining. About a hundred microscipic fields per each group were recorded and subjected for histopathological measurement. The number and the area size of tumorlets and the total nerve root were scored by investigators blinded to the treatments.

Quantitative RT-PCR

Total RNA was isolated using the RiboPure total RNA isolation kit (Ambion), reverse transcribed using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). The TaqMan probes used were: Egfr (Hs01076092, Mm01187858), ErbB2 (Hs00170433, Mm00658541), ErbB3 (Hs00176538, Mm01159999), ErbB4 (Hs00171783, Mm01256793), PdgfrA (Hs00998018, Mm00440701), PdgfrB (Hs00182163, Mm00435546), S100β (Mm00485897), $Ngfr^{p75}$ (Mm00446296), Pmp22 (Mm00476978), Mpz (Mm01290519), Mbp (Mm01266402), Hprt1 (Mm01545399, endogenous control) (Applied Biosystems).

Primary antibodies used for Western blotting and immunohistochemistry

Antibodies used in this study are: ErbB2 (A0485; DAKO), ErbB3 (sc-285; Santa Cruz Biotechnology), Axl (sc-1096; Santa Cruz Biotechnology), Met (sc-162; Santa Cruz Biotechnology), PI3K (#4257; Cell Signaling Technology), phosphor-PI3K (#4228; Cell Signaling Technology), Akt (#9272; Cell Signaling Technology), phospho-Akt(Ser473) (#4060; Cell Signaling Technology), Raf (610151, BD transduction laboratories), Erk (#4696; Cell Signaling Technology), phosphor-Erk (#4370; Cell Signaling Technology), S6 (#2317; Cell Signaling Technology), phosphor-S6 (#4858; Cell Signaling Technology), Cyclin D1 (sc-753; Santa Cruz Biotechnology), Cdk4 (sc-53636; Santa Cruz Biotechnology), HSP70 (#4876; Cell Signaling Technology), HSP90 (#4874; Cell Signaling Technology), β-tubulin (T-0198; Sigma-Aldrich), S100β (Z0311; DAKO), and PECAM (sc-1506; Santa Cruz Biotechnology).

TUNEL staining

DNA fragmentation of apoptotic cells was assessed using DeadEnd™ Fluorometric TUNEL System (Promega). In brief, ESC-FC1801 and 08031-9 cells on the coated cover glass and treated with

NXD30001 for 24 hrs. Fragmented DNA was detected by the catalytic incorporation of fluorescein-12-dUTP at the 3'-OH ends using the Terminal deoxynucleotidyl Transferase and visualized under the fluorescent microscope AxioImager.M1 (Carl Zeiss).

BrdU staining

The cells under DNA replication (S-phase) in the tumors were detected. Four hours prior to the dissection, mice were dosed with BrdU at 1mg/kg. The tumor sections were stained using a BrdU staining Kit (Invitrogen) and the mitotic index was determined by scoring the percentage of the BrdU positive nuclei. For cultured cells, the ESC-FC1801 cells were incubated for four hours with 10μg/mL BrdU and the immunocytochemical staining was performed using anti-BrdU primary antibody (#20-BS17, Fitzgerald) and Alexa488-conjugated anti-sheep IgG secondary antibody (Molecular probe). The fluorescein-labeled DNA was visualized using a fluorescent microscope (Zeiss).

Transcriptome analysis by high-throughput mRNA sequencing

Illumina Genome Analyzer IIx and HiSeq 2000 platforms were used for the RNA sequencing of in vivo and in vitro samples. Raw sequence data was aligned to the UCSC mouse genome database (build mm9) using TopHat (v. 1.2.0) (7), and the expression of 35,604 gene loci was assembled and quantified with Cufflinks (v 1.0.2) (8). Pathway analysis was conducted using the integrated knowledge database and analysis platform MetaCore (Thomson Reuters).

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