**Supplementary Data & Figure Legends**

**Materials and methods for**

**Western blot analysis for STAT3 and phosphorylated STAT3 expression**

The expression levels of T-STAT3 and phosphorylated STAT3 protein in NB cell lines was determined by Western blot analysis. Antibodies against phosphorylated STAT3 (Y705, S727), STAT3, and GAPDH were purchased from Santa Cruz (Santa Cruz, CA). For Nuclear/Cytosolic Protein Extraction: After culture in RPMI complete media (10% FBS, 1x Pen/Strep/L-Glutamine), IMR-32, NGP and AS cell lines were harvested and proteins from whole cells (W), or cytosolic (Cyto) or nuclear(Nuc) fractions were isolated according to manufacturer’s recommendations (NE-PER Nuclear and Cytoplasmic Extraction Reagents Catalog #78833**).** For IL-6 stimulation:One million NGP cells were plated in 10cm dish format in RPMI complete media (10% FBS, 1x Pen/Strep/L-Glutamine). The following day, cells were treated with 1µM ntASO or AZD9150. Treatment was continued for six days. Media was replaced with 10ng/ml IL6 (Miltenyi Biotec) for 10 minutes. Cells were washed three times with 1xPBS. Cells were lysed with RIPA buffer for Western blot analysis. Total protein(10ug) was loaded in each lane. For Western blot analysis, relative normalized density is reported under each lane for each target. Band density was first normalized to tubulin band density. Relative densities were then normalized to 1µM ASO.

**Immunocytofluorescence**

Cells were cultured in Lab-Tek II 8 chamber slides (Nalge Nunc International, Naperville, IL) for 72 hours. AS, NGP, IMR32 expressing Tet-inducible STAT3 shRNA were cultured with or without Tet (1 μg/ml). AS, NGP, IMR32 cells were cultured with ntASO 1μM or AZD9150 1μM. Cells were then fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton-X100 in 15% FBS in PBS for 10 minutes. The fixed cells were incubated for 1 hour in blocking solution (5% BSA with 0.1% Triton-X100 in PBS). Immunostaining was performed by incubating the slides with anti-STAT3, anti-pSTAT3 (Y705), or anti-pSTAT3 (S727) at 4℃ overnight, followed by Alexa fluor 488 antibody for 1 hour at room temperature.

**Wound-healing assays *in vitro***

Cells were plated into Essen ImageLock 24-well plates, incubated overnight. NB cells were treated with ntASO (1μM) or AZD9150 (1μM), and stable clones were treated with or without Tet (1μg/ml) for 6 days. The medium was changed to complete medium with mitomycin-C (10μg/ml) for 2 hours to inhibit cell proliferation. After washing with low serum medium (0.5% FBS) three times, the confluent monolayer of cells was scratched using the Essen 24-well Wound Maker (Ann Arbor, MI, USA) following the manufacture’s protocol. The NB cells were cultured in low serum medium (0.5% FBS) with ntASO (1μM) or AZD9150 (1μM), and stable clones were cultured with or without Tet (1 μg/ml). The wound confluence was obtained and analyzed using the IncuCyte phase-contrast imaging and scratch wound assay system (Essen Bioscience, MI).

**Primers used for qPCR**

|  |  |  |
| --- | --- | --- |
| **Genes** | **Forward** | **Reverse** |
| HPRT1 | TGCAGACTTTGCTTTCCTTGGTCAGG | CCAACACTTCGTGGGGTCCTTTTCA |
| Cyclin D1 | CTTCCTCTCCAAAATGCCAG | AGAGATGGAAGGGGGAAAGA |
| Cyclin D3 | GACCGACAGGCCTTGGTCAA | AGTGCCAGTGATCCCTGCCA |
| Bcl-2 | CTGCACCTGACGCCCTTCACC | CACATGACCCCACCGAACTCAAAGA |
| Survivin | AGAACTGGCCCTTCTTGGAGG | CTTTTTATGTTCCTCTATGGGGTC |
| c-myc | AAACACAAACTTGAACAGCTAC | ATTTGAGGCAGTTTACATTATGG |
| MYCN | GGACACCCTGAGCGATTCAGA | AGGAGGAACGCCGCTTCT |
| Beta-actin | GACCTCTATGCCAACACAGT | AGTACTTGCGCTCAGGAGGA |

**Revised Supplementary Figure Legends**

**Revised Supplementary figure S1.**

**Panel A. T-STAT3 and phosphorylated STAT3 protein expression in NB cell lines.**

Western blot analysis demonstrated the presence of T-STAT3 and phosphorylated STAT3 in 14 NB cell lines tested.

**Panel B. Expression of Activated nuclear STAT3 in NB cell lines.**

**Three micrograms of proteins** were analyzed by Western blot analysis for levels of P-STAT3Y705 and P-STAT3Y727, total STAT3. GAPDH was used to assess cytosolic protein levels and H3 was analyzed to assess nuclear proteins.

**Panel C. Figure B. AZD9150 treatment suppresses IL6-induced Y705 phosphorylation of STAT3 via downregulation of total STAT3 in NB.**

Western blot analysis indicates that AZD9150 inhibits endogenous and IL-6 induced STAT3 expression resulting in lower levels of P-STAT3Y705 and P-STAT3Y727.

**Supplementary figure S2.**

**AS, NGP, IMR32 cells expressed total and phosphorylated STAT3, and genetic or pharmacologic inhibition of STAT3 uniformly inhibited STAT3 expression.**

**A-D: AS NB cells.**  The presence of total and phosphorylated STAT3 in AS expressing Tet-inducible STAT3 shRNA without Tet **(A)**, AS expressing Tet-inducible STAT3 shRNA with Tet **(B)**, AS with ntASO 1μM treatment **(C)**, AS with AZD9150 1μM treatment **(D)**,

**E-H: NGP NB cells.** NGP expressing Tet-inducible STAT3 shRNA without Tet **(E)**, NGP expressing Tet-inducible STAT3 shRNA with Tet **(F)**, NGP with ntASO 1μM treatment **(G)**, NGP with AZD9150 1μM treatment **(H)**,

**I-L: IMR32 NB cells.**  IMR32 expressing Tet-inducible STAT3 shRNA without Tet **(I)**, IMR32 expressing Tet-inducible STAT3 shRNA with Tet **(J)**, IMR32 with ntASO 1μM treatment **(K)**, IMR32 with AZD9150 1μM treatment **(L)** by immunocytofluorescence.

**Supplementary Figure S3.**

**Genetic or pharmacologic inhibition of STAT3 inhibits NB cell migration *in vitro*.**

**A. Top**: Time course of wound closure for NB cells expressing Tet-inducible STAT3 shRNA expressed as relative wound density (%). Cells were grown to confluence and a wound was made using 24-well Wound Maker (Ann Arbor, USA). Migration was measured in presence of Tet (1 μg/ml) or solvent control by IncuCyte every 6h for 48h. AS-TetshSTAT3 NB cells (left panel); NGP-TetshSTAT3 NB cells(middle panel) IMR32-TetshSTAT3 NB cells (right panel). **Bottom**: images immediately after the start (0h) and after 48h.

**B. Top**: time course of wound closure for NB cells expressed as relative wound density (%). Cells were grown to confluence and a wound was made using 24-well Wound Maker (Ann Arbor, USA). Migration was measured in presence of AZD9150 (1 μM) or ntASO (1 μM) by IncuCyte every 6h for 48h. AS NB cells (left panel); NGP NB cells(middle panel) IMR32 NB cells (right panel). **Bottom:** images immediately after the start (0h) and after 48h.

**Supplementary Figure S4.**

**Genetic or pharmacologic inhibition of STAT3 increased chemosensitivity in NB cells *in vitro*.**

**A.** Relative cell numbers as assessed by MTS assay. NB cells expressing Tet-inducible STAT3 shRNA were treated with Tet or control for 3 days followed by cisplatin (0.15μg/ml) for another 3 days. Relative cell number was normalized to the value obtained for the Tet-, control solvent treated cells. The data is the mean of 3 replicate experiments ± SD. Statistical significance (\*, P<0.05) was indicated for the combination of Tet and cisplatin treated cells vs control cells (*t*-test). AS-Tet shSTAT3 NB cells (left panel); IMR32-Tet shSTAT3 NB cells (right panel).

**B.** Relative cell numbers as assessed by MTS assay. NGP were treated with AZD9150 (1μM) or ntASO (1μM) for 3 days followed by cisplatin (0.15 μg/ml) in combination for another 3 days. Cell viability was normalized to ntASO treated and cisplatin untreated cell. The data is the mean of 3 replicate experiments ± SD. Statistical significance (\*, P<0.05) was indicated for the combination of AZD9150 and cisplatin treated cells vs control cells (*t*-test). AS NB cells (left panel); IMR32 NB cells (right panel).

**C.** Relative cell numbers as assessed by MTS assay. NB cells expressing Tet-inducible STAT3 shRNA with Tet (1μg/ml) or solvent control were treated for 3 days followed by various concentrations of cisplatin in combination for another 3 days. Relative cell number was normalized to the value obtaining for the untreated cell. P-value was determined by a two-way ANOVA. AS-Tet shSTAT3 NB cells (left panel); IMR32-Tet shSTAT3 NB cells (right panel).

**D.** Relative cell numbers as assessed by MTS assay. NB cells treated with AZD9150 (1μM) or ntASO (1μM) were cultured for 3 days followed by incubation with various concentrations of cisplatin for an additional 3 days. Relative cell number was normalized to values obtained in untreated cell. P-value was determined by a two-way ANOVA. AS NB cells (left panel); IMR32 NB cells (right panel).