TTI-101: A competitive inhibitor of STAT3 that spares oxidative phosphorylation and reverses mechanical allodynia in mouse models of neuropathic pain

Moses M. Kasembeli\textsuperscript{a}, Pooja Singhmar\textsuperscript{b}, Jiacheng Ma\textsuperscript{b}, Jules Edralin\textsuperscript{b}, Yongfu Tang\textsuperscript{b}, Clydell Adams III\textsuperscript{a}, Cobi J. Heijnen\textsuperscript{b}, Annemieke Kavelaars\textsuperscript{b}, David J. Tweardy\textsuperscript{a,}\textsuperscript{*}

\textsuperscript{a}The Department of Infectious Diseases, Infection Control & Employee Health, Division of Internal Medicine, University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030-4009, United States

\textsuperscript{b}The Department of Symptom Research, Division of Internal Medicine, University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030-4009, United States

Abstract

Signal Transducer and Activator of Transcription (STAT) 3 emerged rapidly as a high-value target for treatment of cancer. However, small-molecule STAT3 inhibitors have been slow to enter the clinic due, in part, to serious adverse events (SAE), including lactic acidosis and peripheral neuropathy, which have been attributed to inhibition of STAT3’s mitochondrial function. Our group developed TTI-101, a competitive inhibitor of STAT3 that targets the receptor pY705-peptide binding site within the Src homology 2 (SH2) domain to block its recruitment and activation. TTI-101 has shown target engagement, no toxicity, and evidence of clinical benefit in a Phase I study in patients with solid tumors. Here we report that TTI-101 did not affect mitochondrial function, nor did it cause STAT3 aggregation, chemically modify STAT3 or cause neuropathic pain. Instead, TTI-101 unexpectedly suppressed neuropathic pain induced by chemotherapy or in a spared nerve injury model. Thus, in addition to its direct anti-tumor effect, TTI-101 may be of benefit when administered to cancer patients at risk of developing chemotherapy-induced peripheral neuropathy (CIPN).

Keywords

STAT3; neuropathic pain; allodynia; CIPN; TTI-101; VEGF

\textsuperscript{*}Corresponding author. dtweardy@mdanderson.org (D.J. Tweardy).

Declaration of Competing Interest

Baylor College of Medicine, with David Tweardy as inventor, has filed 8 patent families covering the use of TTI-101, a small-molecule inhibitor of STAT3 used in this study. These patents are exclusively licensed to Tvardi Therapeutics, Inc., in which David Tweardy owns stock.
1. Introduction

STAT3 is known to play an essential role in biological processes important for development, including cell growth and survival, as well as in restoring homeostasis after injury. However, persistent STAT3 signaling has been linked to a number of pathological conditions including cancer, chronic inflammation and fibrosis. An extensive body of preclinical data indicates that inhibition of STAT3 signaling may be of substantial therapeutic benefit [1,2]. However, agents that target STAT3 have been slow to enter the clinic, in part, because of difficulties inherent in targeting transcription factors, a class of proteins deemed “undruggable” due to the large size of their protein–protein interaction interfaces [3]. In addition, serious adverse events (SAE), including lactic acidosis and peripheral neuropathy, have been observed with some small-molecule STAT3 inhibitors in clinical-stage development [4,5]. These have been attributed to targeting of STAT3’s non-canonical functions, most notably, its contribution to mitochondrial-mediated oxidative phosphorylation [6], which relies on phosphorylation of STAT3 on serine 727, in contrast to phosphorylation on tyrosine 705 required for its canonical function [7,8].

Many STAT3-directed drug development programs have focused on STAT3’s SH2 domain, in particular its phosphotyrosine (pY) peptide binding pocket. However, the finding that some inhibitors induce mitochondrial toxicity suggests they may target other regions of STAT3 and affect STAT3 structure and stability. In fact, Genini et al. demonstrated that OPB-51602, and other small-molecule STAT3 inhibitors designed to directly target STAT3, caused STAT3 aggregation and altered intracellular protein homeostasis [6]. They further argued that induction of cell death by these agents is mediated, in part, through a proteotoxic mechanism in metabolically stressed cancer cells and suggested that this may be a common mechanism underlying the anticancer activity of any inhibitor that directly targets the SH2 domain within STAT3.

Our group, working in collaboration with Tvardi Therapeutics, Inc., developed TTI-101 (formerly C188–9), a competitive inhibitor of STAT3 designed to target the pY-peptide binding site within STAT3’s SH2 domain and thereby directly block two key steps in its activation—recruitment to activated cytokine receptor complexes and homodimerization [9,10]. We previously performed good laboratory practice (GLP)-compliant, 28-day pharmacotoxicology studies of TTI-101 [10] that demonstrated no drug-related toxicity up to the maximum dose administered (200 mg/kg/day in rats and 100 mg/kg/day in dogs). Moreover, an ongoing Phase I clinical trial of TTI-101 that has enrolled 40 patients with advanced solid tumors [11] up to dose level 4 (25.6 mg/kg/day) for as long as 12 months, has not demonstrated any serious adverse events, including lactic acidosis.

The studies reported here were undertaken to determine in vitro if TTI-101 affects STAT3 mitochondrial function, causes STAT3 aggregation, chemically modifies STAT3, or induces peripheral neuropathy in mice. We tested TTI-101 and four other STAT3 inhibitors purported to target the STAT3 SH2 domain and demonstrated that two of these—WP-1066 and cryptotanshinone—induced STAT3 aggregation and caused mitochondrial toxicity in metabolically stressed cells. Importantly, our studies revealed that TTI-101 does not: 1) affect mitochondrial function, 2) chemically modify STAT3, 3) cause STAT3 aggregation.
in metabolically stressed cells, or 4) cause peripheral neuropathy. In fact, TTI-101 administration unexpectedly reversed mechanical allodynia in models of chemotherapy-induced peripheral neuropathy (CIPN) and spared nerve injury (SNI). These findings indicate that TTI-101 may be of special benefit when administered to patients receiving CIPN-inducing agents as part of their cancer therapy regimen.

2. **Material and methods**

2.1. **Materials**

STAT3 inhibitors - Stattic, cryptotanshinone, WP1066 and STA21 were obtained from Selleck Chemicals (Houston, TX, USA). TTI-101 was custom synthesized by Regis technologies Inc. (Morton Grove, IL, USA). Molecular grade dimethyl sulfoxide (DMSO), reduced glutathione (GSH), iodoacetamide, and N-ethylmaleimide were obtained from Sigma-Aldrich (St. Louis, MO, USA). Cisplatin was acquired from (TEVA Pharmaceuticals, North Wales, PA). All LC/MS reagents, including ammonium acetate, formic acid, acetonitrile, methanol and water, were obtained from Honeywell Fluka (Morris Plains, NJ, USA). STAT3 antibody was purchased from Cell Signaling Technology (Danvers, MA, USA). Antibodies to histone H2B (ab52484) and GAPDH (ab9485) were purchased from Abcam (Toronto, ON, Canada). Antibody to Vimentin (sc66002) was obtained from Santa Cruz Biotechnology (Dallas, TX, USA). DMEM XF base medium, FCCP, and rotenone/antimycin A were obtained from Agilent Technologies (Santa Clara, CA, USA). A C18 Synergi™ 4 μm Fusion-RP 80 Å LC column (50 × 2 mm) was purchased from Phenomenex, (Torrance, CA, USA); a Waters Symmetry C18 column (100 Å, 3.5 μm, 4.6 mm × 150 mm) was purchased from Waters (Milford, MA, USA).

2.2. **Cell line and culture**

The human prostate cancer cell line DU-145 was obtained from American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in RPMI 1640 medium (ATCC modification) containing 10% fetal bovine serum and Antibiotic-Antimycotic from Gibco, Invitrogen (Carlsbad, CA, USA). The cells were cultured at 37 °C with an atmosphere of 5% CO₂.

2.3. **Mitochondrial assays**

DU-145 cells (2.5 × 10⁴) were seeded per well in XF24 plates and incubated at 37 °C / 5% CO₂ in complete RPMI medium. After 12Hrs complete medium was replaced with nutrient depleted four-day culture media: conditioned media (CM) and incubated for 4 Hrs. Cells were then treated for another 2 Hrs with STAT3 inhibitors prior to analysis using a Seahorse XF24 Analyzer. The Oxygen Consumption Rate (OCR) was measured in DMEM XF base medium containing 10 mM glucose, 2 mM glutamine and 1 mM pyruvate, before and after the sequential injection of oligomycin, FCCP and rotenone/antimycin A, as indicated in (Fig. 1), to final concentrations of 1 uM, 1 uM and 0.5 uM, respectively.

2.4. **Cell fractionation**

Cells were treated for 16 Hrs with STAT3 inhibitors at a concentration of 10 uM and 1% DMSO in glucose depleted conditioned media (CM), as described [6]. Lysates

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were fractionated into cytosol, organelle, nuclei, and cytoskeleton subcellular fractions using ProteoExtract kit (Calbiochem, San Diego, California, USA) according to the manufacturer’s directions. Enrichment of each fraction was assessed by SDS-PAGE and immunoblotting using antibodies against GAPDH (cytosol and organelles, Fractions I and II), Histone H2B (nucleus, Fraction III), and Vimentin (cytoskeleton and insoluble proteins, Fraction IV).

2.5. Expression and purification of recombinant STAT3

STAT3 (127–722) cDNA was cloned into a pET15b vector and transformed in BL21 (DE) (Life Technologies, Inc. Woburn, MA, USA). Expression of the recombinant protein was induced by 0.5 mM IPTG, at 20 °C for 5 Hrs. The recombinant STAT3 protein was purified by ammonium sulfate precipitation followed by an ion exchange step with a HiTrap Q column (GE Healthcare Bio-Sciences, Uppsala Sweden) and size exclusion chromatography to achieve purity of over 98%.

2.6. Glutathione reaction studies

STAT3 inhibitors (10 ul of 10 mM stock in DMSO) were spiked into reaction buffer [50 mM HEPES pH7.5 containing 10 mM reduced glutathione (GSH)] the samples were mixed thoroughly and placed in an autosampler set at 20 °C. The reactions were monitored by high performance liquid chromatography (HPLC) using an Exion LC Sciex unit equipped with a UV detector. The stationary phase used was a C18 Synergi™ 4 μm Fusion-RP 80 Å LC column (50 × 2 mm), the mobile phase was water (A) and acetonitrile (B). The elution process consisted of a gradient starting at 20% mobile phase B to 80% for 2 min. The flow rate was maintained at 0.5 mL/min during the run. Measurements were conducted at intervals of 5 min for a period of 50 min total. The presence of the STAT3 inhibitors were quantified by calculating the area under the curves (AUCs) of the compound peaks at 260–295 nm.

2.7. STAT3 alkylation studies

Purified recombinant core fragment of STAT3β protein in ammonium bicarbonate buffer (10 uM) was mixed with each compound at a final concentration of 100 uM. The protein mixture was then incubated at 37 °C overnight. Samples were reduced with 5 mM DTT at 37 °C for one hour and further alkylated with iodoacetamide (15 mM) for 30 min at room temperature in the dark, followed by digestion with trypsin gold in a dry incubator at 37 °C overnight. Formic acid was added the next day to a final concentration of 5% and each protein sample was diluted in 5 mM ammonium acetate containing 0.5% formic acid immediately prior to analysis by targeted mass spectrometry Multiple Reaction Monitoring (MRM).

2.8. Lc–MS/MS

A QTRAP 5500 Sciex hybrid quadrupole-linear ion trap system with a turbo ion spray source coupled to a Sciex LC Exion liquid chromatography system (Redwood City, CA, USA) were used to analyze tryptic digests of STAT3 protein samples treated with STAT3 inhibitors. Fractionation of the samples was done using a Waters Symmetry C18 column
(100 Å, 3.5 μm, 4.6 mm × 150 mm) with a 30 min linear gradient of acetonitrile containing 0.1% formic acid at a flow rate of 300 uL/min. A transition list of cysteine-containing peptides with expected drug-cysteine adducts was generated in Skyline software and exported to the QTRAP mass spectrometer for the development of the acquisition method. Resultant raw data files were imported back into Skyline for analysis and additional processing.

2.9. High resolution LC/MS

Aliquots of the tryptic digests of STAT3 proteins treated with DMSO (control) and those treated with Stattic and TTI-101 were analyzed by LC-MS/MS on an Ultimate 3000 RSLC-Nano chromatograph interfaced to an Orbitrap Fusion high-resolution mass spectrometer (Thermo Scientific, Waltham MA). All MS/MS data were analyzed using Sequest-HT (Thermo Scientific). Proteins were identified by searching their fragment spectra against the Swiss-Prot protein database (EBI). The iodoacetamide derivative of cysteine, stactic adducts of cysteine, and the predicted TTI-101 adducts of cysteine were specified as variable modifications. To access for potential unknown modifications, the data was analyzed in MaxQuant using the dependent peptide search option [12]. An all-peptides output list was analyzed by comparing Stattic, TTI-101 and TTI-101ox with DMSO treated samples, as described in [13].

2.10. Animals

Male and female C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME) and housed at the University of Texas MD Anderson Cancer Center animal facility (Houston, TX) on a regular 12-hour light/dark cycle with free access to food and water. Mice were group-housed on the same rack in individually ventilated cages. Mice were 8 – 10 weeks of age at the start of the experiment and were randomly assigned to groups (cage) by animal care givers not involved in the experiment. Investigators were blinded to treatment until group data were analyzed and the code was broken by an investigator not involved in the study. All experimental procedures were consistent with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and the Ethical Issues of the International Association for the Study of Pain [14] and were approved by the Institution for Animal Care and Use Committee (IACUC) of M.D. Anderson Cancer Center. Experiments were performed and reported in compliance with the ARRIVE guidelines [15].

2.11. Pain measurements and chemotherapy-induced peripheral neuropathy (CIPN)

The effect of TTI-101 and chemotherapy on mechanical sensitivity as a read out for pain were assessed over time using von Frey hairs (0.02, 0.07, 0.16, 0.4, 0.6, 1.0, and 1.4 g; Stoelting, (Wood Dale, Illinois, USA) and the up and down method as described previously [16,17]. Cisplatin was diluted in sterile PBS and administered i.p. at a dose of 2.3 mg/kg per day for 5 days followed by 5 days of rest and another 5 days of injections [18]. After 17 days of the last dose of cisplatin the mice were treated with TTI-101 (50 mg/kg i.p. every other day) for a total of seven doses.
2.12. Spared nerve injury (SNI)

SNI surgery was performed on male and female C57BL/6j mice (8 weeks old; Jackson Laboratories), as described [19]. The sural, common peroneal and tibial branches of the sciatic nerve of the left hind paw were exposed under isoflurane anesthesia. A silk suture was used to ligate the common peroneal and tibial branches and 2–4 mm of the distal ends were removed. The sural nerve was left intact. Mice received buprenorphine right before and 1 h after surgery. Mice were treated with 6 doses of TTI-101 (50 mg/kg in vehicle—60% Labrasol/40% PEG-400—or vehicle alone) administered by oral gavage every other day starting on day 10 after SNI. Mechanical sensitivity was monitored over time using von Frey hairs.

2.13. Data and analysis

Studies were designed to include groups of equal size, using randomization and blinded analysis. Statistical analysis was undertaken for studies where each group size was at least \( n = 5 \), with the exception of the CIPN study where 4 mice per group were used. Animal group size selection for mechanical allodynia was based on previously published data for similar experiments in which sample size calculations were established [19]. Pain behavior data were normally distributed and analyzed by Two-way repeated measures ANOVA followed by Tukey post tests using PRISM8 software; \( P < 0.05 \) was considered statistically significant. Data from all animals enrolled were included in the final analysis.

2.14. RNA-seq and transcriptome analysis of dorsal root ganglion (DRG)

Whole-genome RNA sequencing was used to identify transcriptional changes induced by cisplatin and TTI-101 in the DRG of 3 mice per group. Total RNA was isolated with the RNeasy MinElute Cleanup Kit (Qiagen, Hilden, Germany). Libraries were prepared with the Stranded mRNA-Seq kit (Kapa Biosystems, Wilmington, MA) following the manufacturer’s guidelines. Stranded-mRNA seq was performed with a HiSeq4000 Sequencer (Illumina, San Diego, CA) with 76nt PE format by the RNA Sequencing Core at MD Anderson Cancer Center.

Data analysis was performed as previously described [20,21]. Briefly, expression data of three samples per group were analyzed in R using bioconductor packages. STAR was used for alignment of paired-end reads to the mm10 version of the mouse reference genome; featureCounts was used to assign mapped sequence reads to genomic features, and DESeq2 was used to identify differentially expressed genes (padj < 0.05). Quality check of raw and aligned reads was performed with FastQC and Qualimap. Next, we used Ingenuity Pathway Analysis (IPA; Qiagen Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis/) for analysis of the canonical pathways implicated by cisplatin-induced transcriptome changes in DRG.

3. Results

3.1. TTI-101 does not affect mitochondrial function

Mitochondrial dysfunction has been demonstrated to contribute to drug-related SEA [22]. Examination of TTI-101 for safety in 28-day IND-enabling studies in rats and dogs [9], as
well as in a Phase I clinical trial of patients with advanced solid tumors through dose level 4 [11], did not demonstrate any serious toxicity including lactic acidosis, which is a clinical manifestation of mitochondrial dysfunction. However, to determine if TTI-101 caused subclinical abnormalities in mitochondrial function, we examined the effects of TTI-101 and four other direct STAT3 inhibitors on mitochondrial respiration using a Seahorse XF Cell Mito Stress Test kit that measured basal respiration, ATP production, maximal respiration, proton leak, and spare respiratory capacity. The OCR curves of cells incubated with TTI-101 at concentrations 10-fold higher than its IC_{50} for STAT3 inhibition [9] were similar to cells treated with DMSO control (Fig. 1A–C). Similarly, STA21 and Stattic did not consistently alter the OCR curves compared to DMSO at these concentrations (Fig. 1A–C).

In contrast, marked abnormalities were observed in the OCR curves of cells treated with cryptotanshinone and WP1066 (Fig. 1A, B). Overall, the effects of cryptotanshinone on the OCR curves mimicked a mitochondrial uncoupler, which creates a ‘shot-circuit’ in the oxidative process by inducing a proton leak (PL) such that the loss of proton motive force proceeds without ATP generation. Cells incubated with cryptotanshinone (30 uM) demonstrated a concentration-dependent increase in basal respiratory rate compared to control cells and decreased responses to oligomycin and to antimycin A/rotenone treatment. In addition, cryptotanshinone blocked cell responses to FCCP treatment and resulted in a 50% reduction in ATP level production. (Fig. 1A–C). To further examine the effects of cryptotanshinone on ATP production, we measured the fraction of basal mitochondrial oxygen consumption linked to ATP synthesis (coupling efficiency); coupling efficiency was significantly reduced (Fig. 1C), further indicative of mitochondrial dysfunction. In addition, the OCR after oligomycin treatment, which is a direct measure of the proton leak rate (Fig. 1C), showed a significant increase in proton leak in cells incubated with cryptotanshinone (30 uM) indicating that mitochondria are uncoupled and severely damaged [23,24]. Similar to cryptotanshinone, we observed marked abnormalities in the OCR curves of cells treated with WP1066. (Fig. 1A–C) indicative of mitochondrial dysfunction [24], including diminished basal OCR, ATP production rate, maximal respiration, spare respiratory capacity, and coupling efficiency.

### 3.2. TTI-101 does not induce STAT3 aggregation in cells

STAT3 inhibitors that were demonstrated to impair mitochondrial activity also were found to cause STAT3 to aggregate in cells under low glucose conditions [6]. Using similar experimental conditions, we assessed the effects of TTI-101 and other direct STAT3 inhibitors on the partitioning and oligomeric state of STAT3. Cells were incubated in medium containing each STAT3 inhibitor at 10 uM final concentration for 16 h. Cells were fractionated and fractions I through IV were separated by SDS-PAGE and immunoblotted using antibodies selective for each fraction (Fig. 2). TTI-101 had no effect on the intracellular localization of STAT3; similar results were obtained in cells incubated with STA21 and Stattic. In contrast, in cells treated with cryptotanshinone or WP1066, over half of STAT3 was found in the insoluble fraction (Fraction IV) indicating that each induced formation of STAT3 intracellular aggregates, which explains their adverse effects on mitochondrial function and confirms the findings of Genini et.al. [6].

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3.3. **TTI-101 does not react with GSH or covalently modify STAT3**

Surface Plasmon Resonance (SPR) studies that directly examined the ability of TTI-101 to inhibit STAT3 binding to its immobilized pY-peptide ligand were performed under reducing conditions [9, 25]. Furthermore, the shape of the binding inhibition curves was most consistent with competitive inhibition. However, intracellular protein depletion through aggregation has recently been described as a key mechanism of action of compounds, such as DUB Inhibitors b-AP15 and VLX1570 that possess α,β-unsaturated carbonyl moieties capable of covalently reacting with their target [26]. Soon after its discovery, Stattic was proposed to alkylate STAT3 via a Michael addition reaction at C687 located within the SH2 domain, but outside the pY-peptide binding pocket; this alkylation event was proposed to allosterically alter the structure of the pY-peptide binding pocket interfering with its ability to bind ligand [27]. More recently, SI3–201 and related compounds were shown to modify STAT3 in a manner consistent with thiolmediated O-tosyl substitution [28].

We performed two studies to determine directly if TTI-101 mediates its inhibitory effect on STAT3 through covalent modification. The first study was a UV-HPLC-based assay to determine the stability of TTI-101, as well as the other STAT3 inhibitors, in the presence of a natural nucleophile—reduced glutathione (GSH). TTI-101 and the other inhibitors were reconstituted at 100 uM in 50 mM HEPES buffer at pH 7.5 containing 10 mM GSH. Each reaction mixture was sampled at time 0 and every 5 min for 50 min; all samples were analyzed by HPLC. The amount of unreacted inhibitor was determined by measuring the area under the curve (AUC) and plotting this value as a percentage of the starting AUC as a function of time (Fig. 3). Consistent with early reports of it serving as a Michael’s acceptor, Stattic levels decreased rapidly within 5 min to < 10% of baseline in the presence of GSH while remaining constant in the absence of GSH (Fig. 3). In contrast, there was no loss of TTI-101 in the presence of GSH up to 50 min after exposure; similar results were observed for cryptotanshinone, WP1066 and STA21.

Review of the structure of TTI-101 did not reveal a potential mechanism for alkylation of STAT3 by a Michael addition or by thiolmediated O-tosyl substitution. However, enol-to-ketone oxidation within the first hydroxy-naphthalene group of TTI-101 would form TTI-101\textsubscript{OX} (Fig. 4), which potentially could undergo a Michael addition reaction. To examine this possibility, we generated recombinant STAT3 post-translationally unmodified in bacteria using a cDNA construct in which the domain containing the N-terminal oligomerization domain was deleted (STAT3\textsubscript{β tr}); this domain is not necessary for native folding of the core domains of STAT3 (CCD, DBD, linker domain, and SH2 domain) and its removal markedly improves recombinant STAT3 protein solubility. STAT3\textsubscript{β tr} contains 11 Cys residues. To determine how many of these Cys residues are available to be alkylated when the soluble protein is natively folded, we incubated STAT3\textsubscript{β tr} with two protein alkylating agents, iodoacetamide and N-ethylmaleimide (NEM) under conditions optimal for alkylation (Fig. 4A). Using data obtained on a quadrupole-linear ion trap MS (Sciex QTrap 5500), we detected adducts based on the presence of predicted MRM signal for peptides containing cysteine residues. The identity of the peptides were confirmed by performing full MS/MS spectra on the detected transitions. The LC-MS/MS of the tryptic digested protein...
revealed 6 peptides alkylated by iodoacetamide and NEM. Five of the peptides contained a single alkylated Cys, while one of the peptides contained two alkylated Cys residues.

We next performed targeted and untargeted LC-MS/MS analysis using a QTrap 5500 and an Orbitrap-Ellite mass spectrometer on tryptic digests of STAT3β tr incubated with TTI-101 or Stattic under optimal alkylating conditions. If TTI-101 or TTI-101Ox alkylated STAT3, we would expect a shift in the mass of peptides containing Cys residues by the equivalent of the exact mass of TTI-101ox as TTI-101 needs to undergo oxidation at the –OH located para to the sulfonamide group to form a Michael’s acceptor. We were unable to detect adducts of TTI-101 or TTI-101ox on Cys containing peptides by targeted LC-MS/MS (Fig. 4C). To ensure that the failure to detect alkylated protein incubated with TTI-101 was not due to insufficient generation of oxidized TTI-101 under the experimental conditions, we synthesized TTI-101Ox itself and incubated it with STAT3β tr. Similar to results obtained with TTI-101, no alkylated peptides were detected upon incubation with TTI-101Ox indicating that STAT3 is not alkylated by TTI-101 in either its reduced or oxidized form. In contrast to TTI-101, tryptic digests of STAT3β tr incubated with Stattic under similar conditions demonstrated that Stattic efficiently alkylated STAT3 at seven sites (Fig. 5A–C).

We then evaluated the possibility that TTI-101—reduced or oxidized—may covalently modify STAT3 and result in a mass shift on LC-MS/MS that is not detectable using the targeted detection approach described above. We performed high resolution LC-MS/MS analysis of protein digests after incubation of STAT3 with TTI-101 or TTI-101Ox using an Orbitrap-Ellite mass spectrometer and analyzed the data using an approach described by Antinori et al. that is tailored for the detection of unknown chemical adduct modifications on proteins [13]. Using this approach, we were able to detect Stattic adducts in protein digests of STAT3 incubated with Stattic. However, we did not identify adducts in digests of STAT3 incubated with either TTI-101 or TTI-101Ox, confirming that neither forms of TTI-101 covalently modify STAT3 (Fig. 5D).

### 3.4. TTI-101 suppresses chemotherapy-induced mechanical allodynia

Peripheral neuropathy has been observed with several small-molecule STAT3 inhibitors in clinical-stage development [4–6]. To assess whether TTI-101 causes peripheral neuropathy, male C57BL/6 mice were treated with 7 doses of TTI-101 (50 mg/kg i.p. every other day) and sensitivity to mechanical stimulation was followed over time using von Frey hairs. Administration of TTI-101 alone had no effect on mechanical sensitivity (Fig. 6A). To investigate whether TTI-101 aggravates existing neuropathic pain, we used the cisplatin model of chemotherapy-induced peripheral neuropathy (CIPN). This model was selected because we showed previously that it is mediated by mitochondrial damage in the peripheral nervous system [17,29]. Mice were treated with two cycles of cisplatin (5 daily doses of 2.3 mg/kg followed by 5 days rest), which induces mechanical allodynia (Fig. 6B) that lasts for at least 75 days [17]. TTI-101 administration (50 mg/kg i.p. every other day for a total of 7 doses) was started 17 days after the last dose of cisplatin, when mechanical allodynia had developed fully. TTI-101 administration markedly reduced cisplatin-induced mechanical allodynia (Fig. 6B). The beneficial effect of TTI-101 developed slowly over time—maximal
inhibition was obtained after the 4th dose of TTI-101 and was maintained while dosing continued. Mechanical alldynia returned to levels similar to those in mice treated with cisplatin alone 4 days after the last dose of TTI-101.

3.5. TTI-101 suppresses SNI-induced mechanical alldynia

To determine whether the beneficial effect of TTI-101 is limited to CIPN or is more broadly applicable to other causes of neuropathic pain, we examined its effect on mechanical alldynia induced by SNI. SNI induces profound mechanical alldynia in male and female mice. Administration of TTI-101 in the SNI model reduced mechanical alldynia within 6 h of the first dose (Fig. 7A) and repeated dosing of TTI-101 over 14 days led to complete reversal of SNI-induced mechanical alldynia in male and female mice (Fig. 7B) that was sustained through day 52 of the experiment or 40 days after the last dose of TTI-101.

3.6. RNA-seq analysis of effect of TTI-101 on the DRG transcriptome in cisplatin-treated mice

To determine whether the beneficial effect of TTI-101 on CIPN is associated with changes in the transcriptome and, in particular, in expression of STAT3 target genes, we performed RNA-seq analysis on dorsal root ganglia (DRG). Mice were treated with cisplatin followed by TTI-101 as in Fig. 6 and lumbar DRG were collected at 4 Hrs after the fourth dose of TTI-101 or vehicle. Comparison of the transcriptome in DRG from mice treated with cisplatin vs. PBS showed that cisplatin changed the expression of 1,973 genes (675 down, 1,298 up; Fig. 8A). TTI-101 administration to cisplatin-treated mice changed expression of 1,713 genes (1,416 down, 297 up) vs. mice treated with cisplatin alone. Notably, the 443 genes that were altered in both groups (PBS vs. Cis and Cis vs. Cis + TTI-101) showed an overall opposite expression pattern between groups, indicating that TTI-101 administration normalized the expression of genes whose expression was altered in cisplatin-treated mice (Fig. 8B and Table 1).

Ingenuity pathway analysis (IPA) focused on canonical pathways (Fig. 8C) and showed that the 443 genes were mainly associated with neuronal health and survival pathways (namely synaptogenesis signaling pathways, TNFR signaling, axonal guidance signaling, Semaphorin Signaling in Neurons, Ephrin signaling). Upstream regulator analysis of the 1,713 differentially expressed genes (DEG) that changed with TTI-101 administration revealed huntingtin (HTT), amyloid beta precursor protein (APP), TP53, TGFβ1, and estrogen receptor as the top five upstream regulators driving downstream changes (Table 2). The mechanistic network of four regulators identified STAT3 as an intermediate regulator. Consistent with the activity of TTI-101 as a STAT3 inhibitor, STAT3 activity was reduced in all networks (Fig. 8D). For example, for the TP53 network, 14 regulators were part of the mechanistic network which together influence expression of 481 genes and out of these 481 genes, 64 target genes are regulated by STAT3.

To gain further insight into potential mechanisms underlying TTI-101’s reversal of CIPN, we used IPA to perform a comparison analysis (Fig. 9A). IPA identified VEGF as the top upstream regulator different between the groups—PBS vs. Cis and Cis vs. Cis + TTI-101. Specifically, IPA predicted activation of VEGF signaling in mice treated with cisplatin.
as compared to PBS. This pathway was inhibited when cisplatin-treated mice received TTI-101. The heat map in Fig. 9B shows the effect of TTI-101 administration on the target genes in VEGF signaling network.

4. Discussion

Small-molecule inhibitors that target the SH2 domain of STAT3 have been proposed recently as an example of synthetic lethality; upon STAT3 binding, they induce formation of proteotoxic aggregates that inhibit mitochondrial function leading to cell death, especially in cancer cells experiencing metabolic stress [6]. Perhaps, not surprisingly, cancer patients enrolled in Phase I/II clinical studies of some drugs within this class have experienced SAE, such as lactic acidosis and peripheral neuropathy, which are clinical manifestations of mitochondrial toxicity; these SAE have halted further patient enrollment. Our group developed TTI-101, a competitive inhibitor of STAT3 designed to target the pY-peptide binding site within STAT3’s SH2 domain and to directly block two key steps in its activation —recruitment to activated cytokine receptor complexes and homodimerization. The studies reported here were undertaken to determine if TTI-101 targets STAT3’s mitochondrial function, causes STAT3 aggregation, or induces peripheral neuropathy. We report that TTI-101 does not affect mitochondrial function, does not cause STAT3 aggregation through chemical modification or other means, and does not induce pain measured as mechanical allodynia. In fact, TTI-101 administration unexpectedly suppressed mechanical allodynia induced by the chemotherapeutic agent cisplatin, indicating it may be of special benefit when administered to cancer patients at risk of developing chemotherapy-induced peripheral neuropathy (CIPN).

While the Otsuka compounds were unavailable to us for study, we examined two compounds (WP1066 and cryptotanshinone) previously demonstrated to markedly affect mitochondrial function for evidence of covalent modification of STAT3, in particular, alkylation. LC-MS/MS of STAT3 protein incubated with WP1066 and cryptotanshinone revealed no alkylation at cysteine sites. In contrast, Stattic, which did not affect mitochondrial function, alkylated STAT3 at all detectable cysteines. STA21, which emerged from a virtual ligand screen for compounds that bind the STAT3 SH2 domain [30], behaved in manner identical to TTI-101; it neither alkylated STAT3 nor induced mitochondrial toxicity. Thus, the ability of STAT3 inhibitors to cause STAT3 aggregation and mitochondrial toxicity is not related to their ability to covalently modify STAT3 and their precise mechanism for inducing STAT3 aggregation remains uncertain.

We previously demonstrated that administration of TTI-101 to rats for 28 days (up to a dose of 200 mg/kg/day) and to dogs (up to a dose of 100 mg/kg/day) did not cause any metabolic abnormalities including lactic acidosis. Increasing evidence indicates that, in addition to lactic acidosis, mitochondrial dysfunction plays a key role in the development of chemotherapy-induced neuropathic pain [31–33]. Peripheral neuropathy is one of the SAE that have been observed with some small-molecule STAT3 inhibitors in clinical-stage development [5]. Our current findings show that treatment of naïve mice with TTI-101 does not induce neuropathic pain in the von Frey test of mechanical allodynia (Fig. 6A), which is commonly used to assess for peripheral neuropathic pain in rodent models.
It is conceivable that potential additional damage to mitochondria as a result of STAT3 inhibition would aggravate CIPN. However, our data demonstrated that TTI-101 treatment had the opposite effect; administration of TTI-101 suppressed established mechanical allodynia in mice treated with cisplatin. Preliminary data also indicated that TTI-101 suppressed existing mechanical allodynia in mice treated with paclitaxel or docetaxel, two chemotherapeutics of a different class. In addition, TTI-101 markedly reduced mechanical allodynia in the SNI model. SNI increases levels of pY-STAT3 in spinal cord astrocytes [34]. In addition, there is evidence for increased pY-STAT3 in microglia in the spinal cord in the spinal nerve injury model of neuropathic pain in rats [35]. While intrathecal administration of the Janus kinase 2 inhibitor, AG490, attenuated mechanical allodynia in both models [36], there are no published reports describing the effects of a STAT3 inhibitor on mechanical allodynia in response to SNI. Transgenic expression of constitutively active and dominant-negative forms of STAT3 in astrocytes located within the spinal dorsal horn (SDH) of mice and rats showed that activation of astrocytic STAT3 plays an important role in maintaining neuropathic pain [37]. Two previous studies reported the beneficial effect of STAT3 inhibitor treatment on chemotherapy-induced neuropathy; administration of the STAT3 inhibitor S3I-201 to rats during treatment with oxaliplatin, paclitaxel, or vincristine reduced mechanical allodynia [38,39]. However, the reduction in mechanical allodynia was only partial, and S3I-201 was administered intrathecally. Our data indicate that mechanical allodynia is reduced to baseline levels in response to oral administration of TTI-101, making this a more attractive compound for clinical application. It remains to be determined whether co-administration of TTI-101 with cisplatin will fully or partially prevent development of CIPN.

The beneficial effect of the STAT3 inhibitor S3I-201 in CIPN cited above was associated with normalization of the expression of CXCL12 a chemokine that was shown to be upregulated in the DRG of rats or mice treated with oxaliplatin, vincristine or paclitaxel. In our RNA-seq data set, we did not detect significant changes in CXCL12, although there was a trend towards increased levels (log2FC 0.176) in response to cisplatin and a trend towards reduced levels (log2FC − 0.474) in response to administration of TTI-101 in cisplatin-treated mice. In addition, our RNA-seq analysis of the DRG transcriptome indicated that TTI-101 administration to cisplatin-treated mice normalizes expression of 443 out of the 1,973 genes (22 %) that were changed in response to cisplatin treatment. These genes are mainly associated with neuronal health and survival pathways, indicating that TTI-101 also may help to restore the loss in intra-epidermal nerve fiber density that is associated with CIPN. We also saw enrichment in genes related to the TNFR1 pathway after TTI-101 administration. This may indicate inhibition of TNF signaling, which has been shown to reduce STAT3 activation. In oxaliplatin-mediated neuropathy, administration of a neutralizing antibody against TNF-α prevented STAT3 activation in DRG [38].

To get additional insight into the potential mechanism underlying the suppression of cisplatin-induced mechanical allodynia, we used the IPA comparison analysis tool to identify upstream regulators of the pathways that were altered in response to cisplatin and reversed by administration of TTI-101. The STAT3 target VEGF [40,41] was identified as the top upstream regulator. It has been shown that activation of retinal neuronal STAT3 increases neuronal VEGF production [42]. In addition, DRG expression of Flt1, the gene
encoding the VEGF receptor 1 (VEGF-R1), was upregulated in response to cisplatin (log2FC 0.295) and this was reversed by administration of TTI-101 (log2FC − 0.503). We also found the VEGF-associated downstream kinase, PI3K, was downregulated by TTI-101 (log2FC − 0.408). Interestingly, it has been shown that VEGF signaling to VEGFR1 on sensory neurons promotes pain in models of cancer pain [43]. Moreover, administration of an anti-VEGF antibody attenuated oxaliplatin-induced mechanical allodynia [44]. In addition, treatment with a PI3K inhibitor attenuated mechanical allodynia induced by VEGFA in mice. In contrast, however, there also is evidence for aggravation of chemotherapy-induced neuropathies by co-administration of anti-VEGF antibodies in patients and mice [45,46]. Further studies are needed to address the potential role of changes in local VEGF signaling in the DRG in the beneficial effect of TTI-101 on cisplatin-induced peripheral neuropathy.

We previously demonstrated that mitochondrial damage contributes to neuropathic pain [17,29]. STAT3 signaling is essential for mitochondrial function; consequently, reports of peripheral neuropathy as an SAE in clinical trials of STAT3 inhibitors that impair mitochondrial function were not totally unexpected. Here, we show that TTI-101, a competitive inhibitor STAT3, did not affect mitochondrial function and not only did not cause peripheral neuropathy, rather, it markedly reduced cisplatin-induced mechanical allodynia through modulating several signaling networks linked to CIPN. Thus, TTI-101 may be an attractive agent for cancer treatment, especially in combination with chemotherapy agents that cause CIPN.

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Fig. 1.
Effects of STAT3 inhibitors on mitochondrial function. DU-145 cells were treated with the indicated STAT3 inhibitors at 30 uM for 2 Hrs. Seahorse experiments showing oxygen consumption rate (OCR) by DU-145 cells treated with DMSO alone or DMSO containing the indicated STAT3 inhibitor at 30uM prior to and following addition of oligomycin, FCCP, and antimycin A/rotenone, as indicated (n = 6). Data in (Fig. 1 A) combined into one graph, showing all the OCR curves relative to each other. Below, figure representing estimation of OCR parameters in(C) Mean ± SEM of basal respiration, maximal respiration, spare respiratory capacity, ATP production, coupling efficiency and proton leak (n = 6).
**Fig. 2.**  
Immunoblotting of fractions of DU-145 cells incubated with indicated drugs (10μM concentration for 16 Hrs). Fractions were separated using 4–20% SDS-PAGE and immunoblotted using antibodies against STAT3, Histone H2B, GAPDH and Vimentin. Data are representative of three independently performed experiments.
Fig. 3.
Stability of compounds incubated with GSH. The AUC of each compound measured at the times indicated by UV-HPLC and expressed as percent of the starting AUC of the peaks. Data are representative of four independently performed experiments.
Fig. 4.
Results of alkylation studies of STAT3 by iodoacetamide, NEM and TTI-101. Schematic depicting chemistry of possible alkylation of STAT3 by iodoacetamide the results of LC-MS chromatograms of STAT3 peptides demonstrating alkylated peptides, as predicted from the chemistry. Schematic depicting chemistry of possible alkylation of STAT3 by NEM the results of LC-MS chromatograms of STAT3 peptides demonstrating alkylated peptides, as predicted from the chemistry. Schematic depicting the chemistry of possible alkylation of STAT3 by TTI-101. The LC-MS chromatograms of STAT3 peptides, that, shows no MRM signal for predicted peptide adducts, indicating that no peptides were alkylated.
Fig. 5.
Alkylation of STAT3 by Stattic. Schematic depicting chemistry of possible alkylation of STAT3 by Stattic and results of LC-MS chromatograms of STAT3 peptides of alkylated peptides, as predicted from reaction chemistry. Results of LC-MS/MS demonstrating covalent modification of STAT3 by Stattic. Chromatograms show fragment ion analysis revealing alkylation of each cysteine-containing peptide, as indicated. Mass Spectra were annotated using IPSA [47]. Representative data of four independent experiments. Amino acid sequence of STAT3βtr indicating cysteine residues modified. Red residues indicates cysteine-containing tryptic fragments identified by LC-MS/MS, with some peptides containing more than one modified cysteine. Bolded residues are within tryptic fragments that can be identified by LC-MS/MS. Residues that are not bolded are within tryptic fragments that are either too large or too small to be detected. Z-score histograms comparing mass shifts of STAT3 peptides incubated with Stattic, TTI-101, or TTI-101ox vs. STAT3 incubated with DMSO. The dotted line indicates the cutoff for a significant Z-score. The peptide mass peak shifted by 211 Da represents the predicted addition of Stattic as a chemical adduct; no significant mass shifts were observed with TTI-101 or with TTI-101ox indicating that neither forms chemical adducts with STAT3.
Fig. 6.
TTI-101 does not cause mechanical allodynia; rather, it reverses mechanical allodynia caused by cisplatin. A. Male C57/Bl6 mice (n = 8 per group) received TTI-101 (50 mg/kg i.p. every other day) and mechanical allodynia was assessed using von Frey hairs and the up-and-down method. B. Male C57/Bl6 mice (n = 4 per group) were treated with cisplatin (two rounds of 5 daily doses of 2.3 mg/kg i.p. followed by 5 days of rest). Dosing with TTI-101 (50 mg/kg i.p. every other day) started 17 days after the last dose of cisplatin. Data were analyzed by two-way ANOVA repeated measures. Time: P < 0.001; Group: P < 0.001; Interaction P < 0.001. **P < 0.01 Tukey multiple comparison test.
Fig. 7.
STAT3 inhibitor reverses SNI-induced allodynia. Male and female mice underwent SNI surgery and were treated with TTI-101 (Males: n = 5; females: n = 6) or vehicle (Males: n = 4; Females: n = 6) by oral gavage for 6 doses every other day from day 10 after SNI. Mechanical allodynia in male and female mice. Data are shown as mean ± SEM and were analyzed using two-way ANOVA followed by Sidak’s post-hoc test. * P < 0.05. No signs of mechanical allodynia at 31 and 52 days after start of TTI-101 treatment (19 and 40 days after the last dose). Data are from 4 vehicle-treated and 5 TTI-101-treated male mice per group). Two-way ANOVA followed by Sidak’s post-hoc test: P < 0.05.
Fig. 8.
Effect of TTI-101 on the DRG transcriptome of cisplatin-treated mice. Genes differentially expressed among groups is shown in a Venn diagram. Expression of 1,973 genes was changed in response to cisplatin when compared to the PBS mice (PBS vs. Cis; n = 3 male mice per group). Expression of 1,713 genes was changed in response to TTI-101 administration vs. mice treated with cisplatin alone (Cis vs. Cis + TTI-101). A cutoff of ($-0.2 < \log_2\text{Fold Change} < 0.2$) and $p = 0.1$ was used for the analysis. Expression of 2,154 genes was changes in response to TTI-101 administration compared to PBS mice (PBS vs TTI-101). Subcellular clustering of 443 overlapping genes showing directionality of expression. Up-regulated and down-regulated genes are highlighted in red and green, respectively. Gray indicates effect cannot be predicted. Top IPA canonical pathways along with $-\log(p\text{-value})$ assigned to 443 common genes between PBS vs. Cis and Cis vs. Cis + TTI-101 mice. Mechanistic networks of four upstream regulators involving STAT3 as an intermediate regulator. Note that STAT3 (black outline) is an intermediate regulator in all networks and it is predicted to be inhibited. Color orange and blue indicate activation or inhibition respectively. Yellow arrow represents inconsistent relationship when the expected direction is different from direction observed.
Fig. 9.
Results of IPA comparison analyses. A. Top upstream regulators driving TTI-101-dependent changes in DRG identified using the IPA comparison analysis tool. IPA core analysis was performed between PBS vs. Cisplatin and Cisplatin vs. Cisplatin + TTI-101, followed by comparison of the two core analyses. B. Heat map showing details of target genes in VEGF network. Fold change data for target genes upregulated (blue) or downregulated (red) is shown.
Table 1
Details on the overlapping genes that were altered in both groups, PBS vs Cis, and Cis vs Cis + TTI-101.

| Symbol | Entrez Gene Name | Location          | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|------------------|-------------------|------------|----------------------|
|        |                  |                   | log2 Fold  | Expr p-value         | log2 Fold  | Expr p-value |
| 1      | 6530402F18Rik    | RIKEN cDNA 6530402F18 gene | Other     | −0.27 0.07           | −0.44 0.02 |
| 2      | ABCA2            | ATP binding cassette subfamily A member 2 | Plasma Membrane | 0.23 0.01 | −0.42 0.04 |
| 3      | ABCA3            | ATP binding cassette subfamily A member 3 | Plasma Membrane | 0.28 0.00 | −0.32 0.06 |
| 4      | ABCA7            | ATP binding cassette subfamily A member 7 | Plasma Membrane | 0.24 0.01 | −0.36 0.03 |
| 5      | ABCG4            | ATP binding cassette subfamily G member 4 | Plasma Membrane | 0.24 0.00 | −0.32 0.04 |
| 6      | ABHD17A          | abhydrolase domain containing 17A | Plasma Membrane | −0.23 0.00 | −0.35 0.00 |
| 7      | ABHD8            | abhydrolase domain containing 8 | Cytoplasm | −0.27 0.00 | −0.21 0.06 |
| 8      | ACACB            | acetyl-CoA carboxylase beta | Cytoplasm | 0.30 0.02 | −0.30 0.04 |
| 9      | ADAMTS12         | ADAMTS like 2     | Extracellular Space | 0.37 0.02 | −0.31 0.08 |
| 10     | ADAP1            | ArfGAP with dual PH domains 1 | Nucleus | −0.22 0.00 | −0.23 0.05 |
| 11     | ADCK2            | Arf domain containing kinase 2 | Cytoplasm | 0.20 0.05 | −0.30 0.02 |
| 12     | AEBP1            | AE binding protein 1 | Nucleus | 0.60 0.00 | −0.23 0.08 |
| 13     | AKNA             | AT-hook transcription factor | Nucleus | 0.54 0.00 | −0.41 0.03 |
| 14     | ALAD             | aminolevulinate dehydratase | Cytoplasm | 0.30 0.01 | −0.43 0.01 |
| 15     | ALDH2            | aldehyde dehydrogenase 2 family member | Cytoplasm | 0.23 0.00 | −0.34 0.02 |
| 16     | ALDH4A1          | aldehyde dehydrogenase 4 family member A1 | Cytoplasm | 0.35 0.00 | −0.29 0.05 |
| 17     | ALOX5            | arachidonate 5-lipoxygenase | Cytoplasm | 0.76 0.00 | −0.38 0.07 |
| 18     | AMBRA1           | autophagy and beclin 1 regulator 1 | Cytoplasm | 0.22 0.01 | −0.32 0.02 |
| 19     | AMOTL2           | angiomotin like 2 | Plasma Membrane | 0.24 0.00 | −0.31 0.01 |
| 20     | AMPD2            | adenosine monophosphate deaminase 2 | Cytoplasm | 0.24 0.00 | −0.25 0.07 |
| 21     | AMPD3            | adenosine monophosphate deaminase 3 | Cytoplasm | 0.26 0.00 | −0.21 0.08 |
| 22     | ANKRD13B         | ankyrin repeat domain 13B | Plasma Membrane | −0.26 0.01 | −0.28 0.05 |
| 23     | ANO1             | anoctamin 1       | Plasma Membrane | 0.36 0.01 | −0.33 0.04 |
| 24     | APSZ1            | adaptor related protein complex 5 subunit zeta 1 | Nucleus | 0.24 0.01 | −0.27 0.04 |
| 25     | ARAP3            | ArfGAP with RhoGAP domain, ankyrin repeat and PH domain | Cytoplasm | 0.36 0.00 | −0.24 0.07 |
| 26     | ARHGEF10L        | Rho guanine nucleotide exchange factor 10 like | Cytoplasm | −0.34 0.00 | −0.28 0.05 |
| Symbol  | Entrez Gene Name | Location     | PBS vs Cis | Cis vs Cis + TTI-101 |
|---------|-----------------|--------------|------------|---------------------|
|         |                 |              | log₂ Fold  | Expr p-value        | log₂ Fold  | Expr p-value |
| 27      | ARHGEF2         | Cytoplasm    | 0.28       | 0.00               | −0.22      | 0.05        |
| 28      | ARID3A          | Nucleus      | 0.29       | 0.05               | −0.29      | 0.05        |
| 29      | ARMC7           | Cytoplasm    | 0.22       | 0.07               | −0.35      | 0.02        |
| 30      | ARMH3           | Other        | 0.25       | 0.00               | −0.28      | 0.09        |
| 31      | ARRDC1          | Cytoplasm    | 0.22       | 0.01               | −0.20      | 0.08        |
| 32      | ATP1B2          | Plasma Membrane | 0.25     | 0.00               | −0.42      | 0.01        |
| 33      | ATP2A3          | Cytoplasm    | 0.53       | 0.00               | −0.49      | 0.00        |
| 34      | ATP2B2          | Plasma Membrane | 0.39     | 0.00               | −0.45      | 0.02        |
| 35      | ATP6V1G2        | Cytoplasm    | 0.23       | 0.00               | −0.31      | 0.01        |
| 36      | B230206H07Rik   | Other        | 0.59       | 0.00               | −0.38      | 0.07        |
| 37      | BAG3            | Cytoplasm    | −0.27      | 0.00               | −0.38      | 0.01        |
| 38      | BCAR1           | BCL2 associated athanogene 3 | −0.23     | 0.03               | −0.31      | 0.05        |
| 39      | BCOR            | Plasma Membrane | 0.28     | 0.00               | −0.21      | 0.07        |
| 40      | BRD3            | Nucleus      | 0.34       | 0.00               | −0.26      | 0.06        |
| 41      | BRPF3           | Cytoplasm    | 0.26       | 0.00               | −0.22      | 0.05        |
| 42      | C11orf24        | Extracellular Space | 0.32    | 0.00               | −0.31      | 0.08        |
| 43      | C11orf98        | Other        | −0.24      | 0.03               | 0.25       | 0.04        |
| 44      | C15orf39        | Cytoplasm    | 0.37       | 0.05               | −0.50      | 0.03        |
| 45      | C1QC            | Extracellular Space | −0.38   | 0.01               | 0.52       | 0.01        |
| 46      | C3AR1           | Plasma Membrane | −0.44     | 0.06               | 0.63       | 0.02        |
| 47      | CACHD1          | Other        | 0.22       | 0.02               | −0.29      | 0.08        |
| 48      | CACNG5          | Plasma Membrane | 0.41     | 0.00               | −0.47      | 0.02        |
| 49      | CAD             | Cytoplasm    | 0.22       | 0.03               | −0.49      | 0.01        |
| 50      | CAMK2B          | Cytoplasm    | 0.23       | 0.00               | −0.28      | 0.03        |
| 51      | CARML2          | Plasma Membrane | −0.29     | 0.00               | −0.32      | 0.02        |
| 52      | CASP1           | Cytoplasm    | −0.36      | 0.06               | 0.33       | 0.06        |
| 53      | CASR            | Plasma Membrane | −0.36     | 0.09               | 0.33       | 0.07        |
| 54      | CBLL1           | Nucleus      | 0.24       | 0.02               | −0.30      | 0.07        |
| 55      | CCDC88B         | Nucleus      | 0.40       | 0.09               | −0.44      | 0.08        |
| Symbol | Entrez Gene Name | Location | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|----------------|----------|------------|---------------------|
|        |                |          | \( \log_{2} \) Fold | Expr p-value | \( \log_{2} \) Fold | Expr p-value |
| 56     | CCDC88C        | coiled-coil domain containing 88C | Cytoplasm | 0.88 | 0.00 | −0.44 | 0.04 |
| 57     | CCDC92B        | coiled-coil domain containing 92B | Other | −0.28 | 0.00 | −0.31 | 0.03 |
| 58     | CCN2           | cellular communication network factor 2 | Extracellular Space | 0.36 | 0.03 | −0.34 | 0.08 |
| 59     | CDC42EP3       | CDC42 effector protein 3 | Cytoplasm | 0.25 | 0.04 | −0.30 | 0.02 |
| 60     | CEP250         | centrosomal protein 250 | Nucleus | 0.22 | 0.00 | −0.21 | 0.07 |
| 61     | CHD4           | chromodomain helicase DNA binding protein 4 | Nucleus | 0.23 | 0.00 | −0.24 | 0.08 |
| 62     | CHD5           | chromodomain helicase DNA binding protein 5 | Nucleus | 0.21 | 0.00 | −0.35 | 0.01 |
| 63     | CHERP          | calcium homeostasis endoplasmic reticulum protein | Cytoplasm | 0.24 | 0.01 | −0.43 | 0.00 |
| 64     | CHIC2          | cysteine rich hydrophobic domain 2 | Plasma Membrane | −0.21 | 0.07 | 0.23 | 0.08 |
| 65     | CHPF           | chondroitin polymerizing factor | Cytoplasm | −0.28 | 0.00 | −0.24 | 0.05 |
| 66     | CHST2          | carbohydrate sulfotransferase 2 | Cytoplasm | −0.25 | 0.00 | −0.31 | 0.01 |
| 67     | CIART          | circadian associated repressor of transcription | Nucleus | 0.53 | 0.02 | 0.37 | 0.04 |
| 68     | CISD3          | CDGSH iron sulfur domain 3 | Cytoplasm | −0.23 | 0.00 | 0.21 | 0.04 |
| 69     | CITD2          | Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 2 | Nucleus | 0.24 | 0.03 | −0.27 | 0.05 |
| 70     | CLIP2          | CAP-Gly domain containing linker protein 2 | Cytoplasm | −0.21 | 0.01 | −0.33 | 0.03 |
| 71     | CLSTN2         | calsyntenin 2 | Plasma Membrane | 0.21 | 0.00 | −0.26 | 0.08 |
| 72     | CLSTN3         | calsyntenin 3 | Plasma Membrane | 0.22 | 0.01 | −0.29 | 0.07 |
| 73     | CLUH           | clustered mitochondria homolog | Cytoplasm | 0.40 | 0.00 | −0.41 | 0.03 |
| 74     | CNTN2          | contactin 2 | Plasma Membrane | 0.24 | 0.00 | −0.41 | 0.02 |
| 75     | CNTROB         | centrinin, centriole duplication and spindle assembly protein | Cytoplasm | 0.28 | 0.01 | −0.27 | 0.05 |
| 76     | COBL11         | cordon-bleu WH2 repeat protein like 1 | Extracellular Space | 0.25 | 0.03 | −0.27 | 0.02 |
| 77     | COL6A3         | collagen type VI alpha 3 chain | Extracellular Space | 0.43 | 0.00 | −0.25 | 0.08 |
| 78     | COMP           | cartilage oligomeric matrix protein | Extracellular Space | 0.39 | 0.00 | −0.31 | 0.06 |
| 79     | CORO6          | coronin 6 | Extracellular Space | 0.25 | 0.02 | −0.24 | 0.07 |
| 80     | CORO7/CORO7-PAM16 | coronin 7 | Cytoplasm | 0.32 | 0.00 | −0.29 | 0.07 |
| 81     | CPB1           | carboxypeptidase B1 | Extracellular Space | −0.26 | 0.04 | 0.26 | 0.06 |
| 82     | CPSF1          | cleavage and polyadenylation specific factor 1 | Nucleus | 0.54 | 0.00 | −0.30 | 0.07 |
| 83     | CRIP1          | cysteine rich protein 1 | Cytoplasm | −0.29 | 0.00 | 0.29 | 0.04 |
| 84     | CRMP1          | collapsin response mediator protein 1 | Cytoplasm | 0.27 | 0.00 | −0.27 | 0.07 |
| Symbol | Entrez Gene Name                                      | Location    | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|------------------------------------------------------|-------------|------------|----------------------|
|        |                                                      |             | log Fold   | Expr p-value         | log Fold | Expr p-value |
| 85     | CRTC2                                                | Nucleus     | 0.21       | 0.00                | -0.27    | 0.01         |
| 86     | CSDC2                                                | Cytoplasm   | 0.20       | 0.03                | -0.46    | 0.01         |
| 87     | CSF3R                                                | Plasma Membrane | 0.63    | 0.00                | -0.46    | 0.07         |
| 88     | CSMD2                                                | Other       | 0.25       | 0.03                | -0.33    | 0.01         |
| 89     | CSNK1G2                                              | Cytoplasm   | -0.23      | 0.00                | -0.24    | 0.04         |
| 90     | CSRNP1                                               | Nucleus     | 0.32       | 0.07                | -0.44    | 0.02         |
| 91     | CTSE                                                 | Cytoplasm   | 1.04       | 0.02                | -0.65    | 0.00         |
| 92     | Cux1                                                 | Nucleus     | 0.24       | 0.00                | -0.28    | 0.03         |
| 93     | CX3CL1                                               | Extracellular Space | 0.33    | 0.01                | -0.33    | 0.06         |
| 94     | DAG1                                                 | Plasma Membrane | 0.21    | 0.00                | -0.48    | 0.01         |
| 95     | DAP                                                  | Cytoplasm   | 0.21       | 0.02                | -0.23    | 0.03         |
| 96     | DDX19A                                               | Nucleus     | 0.23       | 0.02                | -0.24    | 0.06         |
| 97     | DGCR2                                                | Plasma Membrane | 0.20    | 0.00                | -0.27    | 0.08         |
| 98     | DGKD                                                 | Cytoplasm   | 0.23       | 0.01                | -0.21    | 0.07         |
| 99     | DHX34                                                | Other       | 0.24       | 0.02                | -0.33    | 0.06         |
| 100    | DIO2                                                 | Cytoplasm   | -0.47      | 0.04                | -0.59    | 0.01         |
| 101    | DISP1                                                | Plasma Membrane | 0.28    | 0.00                | -0.22    | 0.06         |
| 102    | DLC1                                                 | Cytoplasm   | 0.24       | 0.00                | -0.30    | 0.07         |
| 103    | DLGAP3                                               | Cytoplasm   | -0.28      | 0.01                | -0.36    | 0.05         |
| 104    | DNAJC15                                              | Cytoplasm   | -0.22      | 0.02                | 0.21     | 0.09         |
| 105    | DNMI                                                 | Cytoplasm   | -0.22      | 0.03                | -0.27    | 0.09         |
| 106    | DNMT1                                                | Nucleus     | 0.38       | 0.00                | -0.24    | 0.05         |
| 107    | DOCK1                                                | Cytoplasm   | 0.29       | 0.00                | -0.36    | 0.02         |
| 108    | DOCK6                                                | Cytoplasm   | 0.41       | 0.00                | -0.22    | 0.10         |
| Symbol | Entrez Gene Name                                      | Location      | PBS vs Cis log2 Fold | Expr p-value | Cis vs Cis + TTI-101 log2 Fold | Expr p-value |
|--------|------------------------------------------------------|---------------|----------------------|--------------|-------------------------------|--------------|
| 10     | DOCK8 dедакtor of cytokinesis 8                      | Cytoplasm     | 0.45                 | 0.00         | −0.30                         | 0.01         |
| 11     | DOK3 docking protein 3                               | Cytoplasm     | 0.48                 | 0.02         | −0.43                         | 0.07         |
| 11     | DOP1B DOP1 leucine zipper like protein B              | Cytoplasm     | 0.28                 | 0.01         | −0.41                         | 0.03         |
| 11     | DPYSL4 dihydroxynimidase like 4                     | Cytoplasm     | 0.34                 | 0.00         | −0.25                         | 0.02         |
| 11     | DUS3L dihydouridine synthase 3 like                  | Other         | 0.23                 | 0.00         | −0.20                         | 0.05         |
| 11     | DUSP15 dual specificity phosphatase 15               | Cytoplasm     | 0.28                 | 0.01         | −0.31                         | 0.05         |
| 11     | E2F2 E2F transcription factor 2                      | Nucleus       | 0.52                 | 0.00         | −0.33                         | 0.09         |
| 11     | EDC4 enhancer of mRNA decapping 4                   | Cytoplasm     | 0.43                 | 0.00         | −0.25                         | 0.08         |
| 11     | EFHC1 EF-hand domain containing 1                    | Cytoplasm     | −0.29                | 0.04         | 0.34                          | 0.03         |
| 11     | EGR2 early growth response 2                         | Nucleus       | 0.28                 | 0.04         | −0.52                         | 0.02         |
| 11     | ELANE elastase, neutrophil expressed                 | Extracellular Space | 0.86               | 0.00         | −0.72                         | 0.03         |
| 12     | ELMO1 engulfment and cell motility 1                 | Cytoplasm     | 0.26                 | 0.00         | −0.22                         | 0.08         |
| 12     | EMC1 ER membrane protein complex subunit 1           | Plasma Membrane | 0.27              | 0.00         | −0.22                         | 0.10         |
| 12     | EMCN endomucin                                       | Extracellular Space | −0.22           | 0.02         | 0.29                          | 0.03         |
| 12     | EPHA2 EPH receptor A2                                | Plasma Membrane | 0.30               | 0.09         | −0.43                         | 0.03         |
| 12     | ERBB2 erb-b2 receptor tyrosine kinase 2              | Plasma Membrane | 0.26               | 0.01         | −0.35                         | 0.01         |
| 12     | ERBB3 erb-b2 receptor tyrosine kinase 3              | Plasma Membrane | 0.26               | 0.00         | −0.27                         | 0.01         |
| 12     | ERMAP erythroblast membrane associated protein (Scianna blood group) | Cytoplasm | 1.31               | 0.00         | −0.54                         | 0.06         |
| Symbol | Entrez Gene Name                                | Location         | PBS vs Cis | Cis vs Cis + TTI-101 |
|-------|-----------------------------------------------|------------------|------------|---------------------|
|       |                                               |                  | log₂ Fold  | Expr p-value        |
|       |                                               |                  |            |                     |
| 12    | ESS2  | ess-2 splicing factor homolog             | Nucleus       | 0.28 0.00           | −0.22 0.09 |
| 12    | EXOSC9 | exosome component 9                      | Nucleus       | −0.20 0.03          | 0.20 0.09 |
| 12    | F2RL2  | coagulation factor II thrombin receptor like 2 | Plasma Membrane | −0.23 0.00        | 0.22 0.04 |
| 13    | F5    | coagulation factor V                      | Extracellular Space | 0.76 0.00     | −0.45 0.04 |
| 13    | F630028010Rik | RIKEN cDNA F630028010 gene              | Other         | 0.82 0.00          | −0.47 0.07 |
| 13    | FABP7 | fatty acid binding protein 7              | Cytoplasm     | −0.55 0.00         | −0.28 0.03 |
| 13    | FAM20A  | FAM20A golgi associated secretory pathway pseudokinase | Extracellular Space | 0.35 0.00 | −0.31 0.04 |
| 13    | FAM222B | family with sequence similarity 222 member B | Nucleus       | 0.41 0.00          | −0.36 0.05 |
| 13    | FAM234A | family with sequence similarity 234 member A | Plasma Membrane | 0.37 0.00        | −0.31 0.02 |
| 13    | FAM43B | family with sequence similarity 43 member B | Other         | −0.25 0.02         | −0.35 0.05 |
| 13    | FARSB | phenylalanyl-tRNA synthetase subunit beta | Cytoplasm     | −0.23 0.00         | 0.22 0.07 |
| 13    | FAT1  | FAT atypical cadherin 1                   | Plasma Membrane | 0.45 0.00        | −0.40 0.04 |
| 13    | FBLN1 | fibulin 1                                 | Extracellular Space | 0.40 0.00 | −0.34 0.02 |
| 13    | FBLN7 | fibulin 7                                 | Extracellular Space | 0.35 0.01 | −0.31 0.08 |
| 14    | FBXO42 | F-box protein 42                         | Other         | 0.21 0.00          | −0.28 0.07 |
| 14    | FERMT3 | fermitin family member 3                  | Cytoplasm     | 0.67 0.00          | −0.48 0.06 |
| 14    | FGGY  | FGGY carbohydrate kinase domain containing | Other         | −0.22 0.04         | 0.24 0.07 |
| 14    | FICD  | FIC domain containing                     | Nucleus       | 0.24 0.00          | −0.39 0.02 |
| Symbol | Entrez Gene Name | Location          | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|-----------------|-------------------|------------|---------------------|
|        |                 |                   | log₂ Fold | Expr p-value        | log₂ Fold | Expr p-value |
| 14     | FLOT1           | flotillin 1       | Plasma Membrane | 0.25 0.00 | −0.25 0.07 |
| 5      |                 |                   |            |                     |           |              |
| 14     | FLT1            | fms related tyrosine kinase 1 | Plasma Membrane | 0.29 0.00 | −0.50 0.00 |
| 6      | FMN2            | formin 2          | Cytoplasm | −0.24 0.00 | −0.26 0.04 |
| 14     | FMNL3           | formin like 3     | Cytoplasm | 0.26 0.05 | −0.25 0.09 |
| 8      | Folh1           | folate hydrolase 1 | Plasma Membrane | −0.28 0.00 | 0.28 0.01 |
| 15     | FRMPD1          | FERM and PDZ domain containing 1 | Cytoplasm | 0.27 0.00 | −0.37 0.04 |
| 0      | FRYL            | FRY like transcription coactivator | Other | 0.43 0.00 | −0.24 0.09 |
| 15     | FSCN1           | fascin actin-bundling protein 1 | Cytoplasm | −0.25 0.02 | −0.41 0.00 |
| 2      | GAA             | glucosidase alpha, acid | Cytoplasm | 0.35 0.00 | −0.40 0.03 |
| 15     | GAS2L1          | growth arrest specific 2 like 1 | Cytoplasm | −0.32 0.00 | −0.27 0.06 |
| 4      | GATB            | glutamyl-tRNA amidotransferase subunit B | Cytoplasm | −0.22 0.02 | 0.22 0.09 |
| 5      | GBF1            | golgi brefeldin A resistant guanine nucleotide exchange factor 1 | Cytoplasm | 0.33 0.00 | −0.31 0.10 |
| 15     | GCN1            | GCN1 activator of EIF2AK4 | Cytoplasm | 0.25 0.01 | −0.34 0.06 |
| 7      | GDF11           | growth differentiation factor 11 | Extracellular Space | −0.25 0.00 | −0.21 0.05 |
| 15     | GDPD5           | glycerolphosphodiester phosphodiesterase domain containing 5 | Plasma Membrane | −0.25 0.00 | −0.22 0.03 |
| 9      | GLRX            | glutaredoxin      | Cytoplasm | −0.20 0.01 | 0.22 0.06 |
| 16     | Gm12696         | predicted gene 12,696 | Other | −0.39 0.00 | 0.27 0.08 |
| 1      | Gm16907         | predicted gene, 16,907 | Other | 0.59 0.01 | 0.45 0.02 |
| Symbol | Entrez Gene Name                                      | Location          | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|------------------------------------------------------|-------------------|------------|---------------------|
|        |                                                      |                   | log<sub>2</sub> Fold | Expr p-value | log<sub>2</sub> Fold | Expr p-value |
| 16     | GNG7                                                 | Plasma Membrane   | 0.23       | 0.01                | -0.38           | 0.01        |
| 16     | GPC1                                                 | Plasma Membrane   | -0.20      | 0.00                | -0.23           | 0.05        |
| 16     | GPD1                                                 | Cytoplasm         | -0.20      | 0.03                | -0.25           | 0.02        |
| 16     | GPR153                                               | Plasma Membrane   | -0.25      | 0.01                | -0.26           | 0.06        |
| 16     | GRINA                                                | Other             | 0.37       | 0.00                | -0.30           | 0.07        |
| 16     | GTF3A                                                | Nucleus           | 0.29       | 0.04                | -0.29           | 0.07        |
| 16     | GUCY1A1                                              | Cytoplasm         | 0.33       | 0.01                | -0.27           | 0.05        |
| 17     | GYPC                                                 | Plasma Membrane   | 0.37       | 0.00                | -0.39           | 0.01        |
| 17     | HCFC1                                                | Nucleus           | 0.32       | 0.00                | -0.39           | 0.04        |
| 17     | HCN2                                                 | Plasma Membrane   | -0.35      | 0.06                | -0.33           | 0.09        |
| 17     | HCN4                                                 | Plasma Membrane   | -0.20      | 0.06                | -0.36           | 0.02        |
| 17     | HDCC2                                                | Cytoplasm         | -0.27      | 0.00                | 0.22            | 0.08        |
| 17     | HELZ                                                 | Nucleus           | 0.24       | 0.01                | -0.21           | 0.09        |
| 17     | HEMGN                                                | Nucleus           | 1.31       | 0.00                | -0.69           | 0.03        |
| 17     | HGF                                                  | Extracellular Space| -0.32      | 0.00                | 0.28            | 0.04        |
| 17     | HHATL                                                | Cytoplasm         | 0.20       | 0.04                | -0.24           | 0.09        |
| 17     | HIVEP1                                               | Nucleus           | 0.21       | 0.01                | -0.34           | 0.05        |
| 18     | HOXA7                                                | Nucleus           | 0.25       | 0.01                | -0.35           | 0.02        |
| Symbol | Entrez Gene Name | Location  | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|-----------------|-----------|------------|---------------------|
|        |                 |           | log₂ Fold  | Expr p-value        | log₂ Fold  | Expr p-value |
| 18     | HOXC10          | Nucleus   | 0.29       | 0.01               | −0.28      | 0.04         |
| 1      | homeobox C10    |           |            |                     |            |              |
| 18     | HPS4            | Cytoplasm | 0.30       | 0.00               | −0.28      | 0.04         |
| 2      | HPS4 biogenesis of lysosomal organelles complex 3 subunit 2 | Cytoplasm | 0.30       | 0.00               | −0.28      | 0.04         |
| 18     | HSP90AA1        | Cytoplasm | −0.26      | 0.00              | 0.21       | 0.07         |
| 3      | heat shock protein 90 alpha family class A member 1 | Cytoplasm | −0.26      | 0.00              | 0.21       | 0.07         |
| 18     | Ifi27           | Cytoplasm | −0.23      | 0.00              | 0.22       | 0.03         |
| 4      | interferon, alpha-inducible protein 27 | Cytoplasm | −0.23      | 0.00              | 0.22       | 0.03         |
| 18     | Ifi27l2a/Ifi27l2b | Cytoplasm | −0.38      | 0.02              | 0.70       | 0.09         |
| 5      | interferon, alpha-inducible protein 27 like 2A | Cytoplasm | −0.38      | 0.02              | 0.70       | 0.09         |
| 18     | IFRD2           | Nucleus   | 0.64       | 0.00              | −0.53      | 0.01         |
| 6      | interferon related developmental regulator 2 | Nucleus | 0.64       | 0.00              | −0.53      | 0.01         |
| 18     | IGLON5          | Other     | −0.23      | 0.01              | −0.32      | 0.02         |
| 7      | IgLON family member 5 | Other | −0.23      | 0.01              | −0.32      | 0.02         |
| 18     | INCENP          | Nucleus   | 0.61       | 0.00              | −0.38      | 0.03         |
| 8      | inner centromere protein | Nucleus | 0.61       | 0.00              | −0.38      | 0.03         |
| 18     | INP5E           | Cytoplasm | 0.21       | 0.01              | −0.20      | 0.08         |
| 9      | inositol polyphosphate-5-phosphatase E | Cytoplasm | 0.21       | 0.01              | −0.20      | 0.08         |
| 19     | INSRR           | Plasma Membrane | 0.62      | 0.01              | −0.43      | 0.04         |
| 0      | insulin receptor related receptor | Plasma Membrane | 0.62      | 0.01              | −0.43      | 0.04         |
| 19     | INSYN1          | Plasma Membrane | −0.32     | 0.00              | −0.31      | 0.05         |
| 1      | inhibitory synaptic factor 1 | Plasma Membrane | −0.32     | 0.00              | −0.31      | 0.05         |
| 19     | IPO4            | Nucleus   | 0.26       | 0.00              | −0.26      | 0.09         |
| 2      | importin 4      | Nucleus   | 0.26       | 0.00              | −0.26      | 0.09         |
| 19     | IRF2BP1         | Nucleus   | −0.26      | 0.01              | −0.37      | 0.03         |
| 3      | interferon regulatory factor 2 binding protein 1 | Nucleus | −0.26      | 0.01              | −0.37      | 0.03         |
| 19     | IRF2BP1         | Nucleus   | −0.25      | 0.01              | −0.30      | 0.05         |
| 4      | interferon regulatory factor 2 binding protein like | Nucleus | −0.25      | 0.01              | −0.30      | 0.05         |
| 19     | ITGA2B          | Plasma Membrane | 0.47      | 0.01              | −0.61      | 0.01         |
| 5      | integrin subunit alpha 2b | Plasma Membrane | 0.47      | 0.01              | −0.61      | 0.01         |
| 19     | ITPR3           | Cytoplasm | 0.33       | 0.00              | −0.43      | 0.02         |
| 6      | inositol 1,4,5-trisphosphate receptor type 3 | Cytoplasm | 0.33       | 0.00              | −0.43      | 0.02         |
| 19     | KCNA1           | Plasma Membrane | 0.22      | 0.01              | −0.31      | 0.07         |
| 7      | potassium voltage-gated channel subfamily A member 1 | Plasma Membrane | 0.22      | 0.01              | −0.31      | 0.07         |
| 19     | KIAA1522        | Other     | −0.21      | 0.01              | −0.30      | 0.06         |
| 8      | KIAA1522        | Other     | −0.21      | 0.01              | −0.30      | 0.06         |
| Symbol | Entrez Gene Name                                           | Location                        | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|-----------------------------------------------------------|---------------------------------|------------|----------------------|
|        |                                                           |                                 | log₂ Fold | Expr p-value         | log₂ Fold | Expr p-value         |
| 19     | KIAA1549L                                                 | Cytoplasm                       | 0.20       | 0.01                 | -0.53     | 0.00                 |
| 20     | KMT2D                                                     | Nucleus                         | 0.20       | 0.08                 | -0.24     | 0.06                 |
| 20     | KNDC1                                                     | Plasma Membrane                 | 0.26       | 0.01                 | -0.44     | 0.03                 |
| 20     | LAMA5                                                     | Extracellular Space             | 0.37       | 0.00                 | -0.32     | 0.04                 |
| 20     | LAMC3                                                     | Extracellular Space             | 0.49       | 0.00                 | -0.50     | 0.06                 |
| 20     | LDLR                                                      | Plasma Membrane                 | 0.39       | 0.00                 | -0.33     | 0.08                 |
| 20     | LGALS1                                                    | Extracellular Space             | -0.31      | 0.00                 | 0.26      | 0.06                 |
| 20     | LIMK1                                                     | Cytoplasm                       | -0.29      | 0.00                 | -0.28     | 0.03                 |
| 20     | LITAF                                                     | Nucleus                         | 0.24       | 0.00                 | -0.20     | 0.05                 |
| 20     | LOC                                                       | Cytoplasm                       | 0.23       | 0.04                 | 0.49      | 0.01                 |
| 20     | LRP1                                                      | Plasma Membrane                 | 0.41       | 0.00                 | -0.36     | 0.01                 |
| 21     | LRRC32                                                    | Plasma Membrane                 | 0.42       | 0.01                 | -0.37     | 0.10                 |
| 21     | LRRK1                                                     | Cytoplasm                       | 0.27       | 0.01                 | -0.22     | 0.05                 |
| 21     | LRRN3                                                     | Extracellular Space             | -0.22      | 0.02                 | 0.21      | 0.07                 |
| 21     | LSM7 homolog, U6 small nuclear RNA and mRNA degradation associated | Nucleus                          | -0.25      | 0.02                 | 0.25      | 0.07                 |
| 21     | MADD                                                      | Cytoplasm                       | 0.20       | 0.01                 | -0.23     | 0.10                 |
| 21     | MAP3K14                                                   | Cytoplasm                       | 0.26       | 0.09                 | -0.25     | 0.09                 |
| 21     | MAP4K2                                                    | Cytoplasm                       | 0.31       | 0.00                 | -0.27     | 0.01                 |
| Symbol | Entrez Gene Name | Location  | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|-----------------|-----------|------------|---------------------|
|        |                 |           | log$_2$ Fold | Expr p-value | log$_2$ Fold | Expr p-value |
| 21 7   | MAP7D1          | Cytoplasm | −0.22      | 0.01            | −0.25       | 0.05        |
| 21 8   | MAPK7           | Cytoplasm | 0.23       | 0.01            | −0.32       | 0.01        |
| 21 9   | MARK4           | Cytoplasm | −0.23      | 0.06            | −0.33       | 0.08        |
| 22 0   | MAST4           | Other     | 0.31       | 0.01            | −0.42       | 0.03        |
| 22 1   | MCC             | Cytoplasm | 0.29       | 0.00            | −0.21       | 0.06        |
| 22 2   | MDC1            | Nucleus   | 0.31       | 0.00            | −0.30       | 0.04        |
| 22 3   | MED15           | Nucleus   | 0.36       | 0.00            | −0.37       | 0.05        |
| 22 4   | MEX3D           | Nucleus   | −0.28      | 0.01            | −0.29       | 0.07        |
| 22 5   | MFS2B           | Plasma Membrane | 0.76 | 0.00 | −0.32 | 0.08 |
| 22 6   | MMP15           | Extracellular Space | −0.28 | 0.00 | −0.44 | 0.01 |
| 22 7   | MMP9            | Extracellular Space | 0.86 | 0.00 | −0.80 | 0.00 |
| 22 8   | MPO             | Cytoplasm | 1.26       | 0.00            | −0.96       | 0.03        |
| 22 9   | MRC2            | Plasma Membrane | 0.23 | 0.00 | −0.23 | 0.04 |
| 23 0   | MRGRX4          | Plasma Membrane | −0.27 | 0.01 | 0.26 | 0.04 |
| 23 1   | MRV11           | Cytoplasm | 0.36       | 0.00            | −0.35       | 0.04        |
| 23 2   | MTSS2           | Plasma Membrane | −0.31 | 0.00 | −0.38 | 0.01 |
| 23 3   | MXD1            | Nucleus   | 0.40       | 0.01            | −0.35       | 0.07        |
| 23 4   | MYO1D           | Cytoplasm | 0.33       | 0.00            | −0.32       | 0.07        |
| Symbol | Entrez Gene Name | Location  | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|-----------------|-----------|------------|----------------------|
|        |                 |           | log2 Fold  | Expr p-value         | log2 Fold  | Expr p-value |
| 23     | MYO1F myosin IF | Cytoplasm | 0.63       | 0.00                 | −0.39      | 0.06         |
| 23     | MYO7A myosin VIIA | Cytoplasm | 0.29       | 0.00                 | −0.24      | 0.08         |
| 23     | MYPOP Myb related transcription factor, partner of profilin | Nucleus | −0.25 | 0.00 | −0.29 | 0.02 |
| 23     | NAA80 N(alpha)-acetyltransferase 80, NatH catalytic subunit | Cytoplasm | 0.25 | 0.00 | −0.38 | 0.01 |
| 23     | Naip1 (includes others) NLR family, apoptosis inhibitory protein 1 | Cytoplasm | −0.23 | 0.00 | −0.36 | 0.02 |
| 24     | NAT8L N-acetyltransferase 8 like | Cytoplasm | 0.30 | 0.00 | −0.25 | 0.07 |
| 24     | NCSTN nicastrin | Plasma Membrane | 0.00 | 0.00 | 0.22 | 0.06 |
| 24     | NDUFA3 NADH:ubiquinone oxidoreductase subunit A3 | Cytoplasm | −0.21 | 0.00 | 0.20 | 0.04 |
| 24     | NDUFAF4 NADH:ubiquinone oxidoreductase complex assembly factor 4 | Cytoplasm | −0.21 | 0.01 | 0.30 | 0.01 |
| 24     | NECTIN1 nectin cell adhesion molecule 1 | Plasma Membrane | −0.42 | 0.00 | −0.36 | 0.07 |
| 24     | NFAM1 NFAT activating protein with ITAM motif 1 | Plasma Membrane | 0.81 | 0.00 | −0.43 | 0.09 |
| 24     | NFATC1 nuclear factor of activated T cells 1 | Nucleus | 0.32 | 0.01 | −0.30 | 0.05 |
| 24     | NFE2 nuclear factor, erythroid 2 | Nucleus | 1.09 | 0.00 | −0.61 | 0.03 |
| 24     | NLGN2 neurologin 2 | Plasma Membrane | −0.25 | 0.01 | −0.32 | 0.03 |
| 24     | NLGN3 neurologin 3 | Plasma Membrane | 0.33 | 0.00 | −0.34 | 0.00 |
| 25     | NMD3 NMD3 ribosome export adaptor | Nucleus | −0.21 | 0.01 | 0.26 | 0.01 |
| 25     | NOL6 nucleolar protein 6 | Nucleus | 0.25 | 0.00 | −0.39 | 0.05 |
| 25     | NOTCH2 notch receptor 2 | Plasma Membrane | 0.31 | 0.00 | −0.27 | 0.01 |
| Symbol | Entrez Gene Name                     | Location       | PBS vs Cis log₂ Fold | PBS vs Cis Expr p-value | Cis vs Cis + TTI-101 log₂ Fold | Cis vs Cis + TTI-101 Expr p-value |
|--------|-------------------------------------|----------------|----------------------|-------------------------|-------------------------------|----------------------------------|
| 25     | Nppb natriuretic peptide type B     | Other          | −0.40                | 0.02                    | 0.34                          | 0.05                             |
| 25     | NPTXR neuronal pentraxin receptor   | Plasma Membrane| −0.22                | 0.01                    | −0.29                         | 0.08                             |
| 25     | NR1D1 nuclear receptor subfamily 1 group D member 1 | Nucleus | 0.36                | 0.01                    | −0.29                         | 0.02                             |
| 25     | NR4A2 nuclear receptor subfamily 4 group A member 2 | Nucleus | 0.27                | 0.03                    | −0.29                         | 0.09                             |
| 25     | NRSN2 neurexin 2                    | Plasma Membrane| 0.58                | 0.00                    | −0.38                         | 0.07                             |
| 25     | NRXN2 neurexin 2                    | Plasma Membrane| −0.26               | 0.01                    | −0.32                         | 0.06                             |
| 25     | NUDCD1 NadC domain containing 1     | Nucleus        | −0.20                | 0.02                    | 0.20                          | 0.07                             |
| 26     | NUMA1 nuclear mitotic apparatus protein 1 | Nucleus | 0.34                | 0.00                    | −0.26                         | 0.08                             |
| 26     | NUP188 nucleoporin 188              | Nucleus        | 0.29                | 0.00                    | −0.21                         | 0.08                             |
| 26     | OGDH oxoglutarate dehydrogenase    | Cytoplasm      | 0.36                | 0.00                    | −0.34                         | 0.05                             |
| 26     | OGDHL oxoglutarate dehydrogenase like | Other   | 0.25                | 0.01                    | −0.27                         | 0.08                             |
| 26     | OLFM2 olfactomedin 2                | Cytoplasm      | 0.31                | 0.00                    | −0.25                         | 0.04                             |
| 26     | OPRM1 opioid receptor mu 1          | Plasma Membrane| −0.28               | 0.00                    | 0.23                          | 0.01                             |
| 26     | OSBPL7 oxysterol binding protein like 7 | Cytoplasm | 0.27                | 0.00                    | −0.26                         | 0.03                             |
| 26     | P2RY2 purinergic receptor P2Y2      | Plasma Membrane| 0.31                | 0.00                    | −0.30                         | 0.07                             |
| 26     | PALM paralemmin                     | Plasma Membrane| −0.30               | 0.00                    | −0.27                         | 0.05                             |
| 26     | PAPLN papilin, proteoglycan like sulfated glycoprotein | Extracellular Space | 0.20                | 0.09                    | −0.34                         | 0.08                             |
| 27     | PC pyruvate carboxylase              | Cytoplasm      | 0.21                | 0.00                    | −0.30                         | 0.04                             |
| Symbol | Entrez Gene Name | Location               | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|-----------------|------------------------|------------|---------------------|
|        |                 |                        | \(\text{log}_2\) Fold | Expr p-value        | \(\text{log}_2\) Fold | Expr p-value |
|        |                 |                        | 0.25       | 0.01                | -0.34                | 0.04        |
|        |                 |                        | 0.24       | 0.01                | -0.37                | 0.04        |
|        |                 |                        | -0.32      | 0.10                | -0.36                | 0.05        |
|        |                 |                        | 0.32       | 0.01                | -0.39                | 0.02        |
|        |                 |                        | 0.54       | 0.00                | -0.27                | 0.09        |
|        |                 |                        | 0.37       | 0.00                | -0.31                | 0.10        |
|        |                 |                        | 0.52       | 0.01                | -0.37                | 0.08        |
|        |                 |                        | -0.35      | 0.00                | -0.29                | 0.02        |
|        |                 |                        | 0.24       | 0.05                | -0.26                | 0.07        |
|        |                 |                        | -0.28      | 0.05                | -0.32                | 0.05        |
|        |                 |                        | 0.28       | 0.00                | -0.32                | 0.06        |
|        |                 |                        | 0.34       | 0.03                | -0.45                | 0.08        |
|        |                 |                        | 0.25       | 0.00                | -0.20                | 0.04        |
|        |                 |                        | 0.24       | 0.00                | -0.41                | 0.01        |
|        |                 |                        | -0.21      | 0.04                | 0.24                 | 0.09        |
|        |                 |                        | 0.43       | 0.00                | -0.31                | 0.06        |
|        |                 |                        | 0.29       | 0.00                | -0.26                | 0.06        |
|        |                 |                        | 0.26       | 0.00                | -0.29                | 0.05        |
| Symbol | Entrez Gene Name                                           | Location          | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|-----------------------------------------------------------|-------------------|------------|----------------------|
|        |                                                           |                   | log2 Fold  | Expr p-value         | log2 Fold  | Expr p-value         |
| 28     | PLRG1           | pleiotropic regulator 1 | Nucleus | −0.21  | 0.01 | 0.21  | 0.07 |
| 29     | PLTP            | phospholipid transfer protein | Extracellular Space | 0.49  | 0.00 | −0.30 | 0.04 |
| 29     | PLXNA2          | plexin A2          | Plasma Membrane | 0.25  | 0.00 | −0.28 | 0.08 |
| 29     | PLXNB1          | plexin B1          | Plasma Membrane | 0.28  | 0.02 | −0.36 | 0.02 |
| 29     | PLXNB3          | plexin B3          | Plasma Membrane | 0.38  | 0.00 | −0.30 | 0.05 |
| 29     | PMEPA1          | prostate transmembrane protein, androgen induced 1 | Plasma Membrane | −0.27 | 0.00 | −0.33 | 0.01 |
| 29     | POLR2A          | RNA polymerase II subunit A | Nucleus | 0.21  | 0.06 | −0.44 | 0.02 |
| 29     | POR             | cytochrome p450 oxidoreductase | Cytoplasm | 0.41  | 0.00 | −0.33 | 0.04 |
| 29     | PPEF1           | protein phosphatase with EF-hand domain 1 | Extracellular Space | −0.22 | 0.00 | 0.23  | 0.02 |
| 29     | PPP1R10         | protein phosphatase 1 regulatory subunit 10 | Nucleus | 0.26  | 0.00 | −0.58 | 0.01 |
| 29     | PPP1R3E         | protein phosphatase 1 regulatory subunit 3E | Cytoplasm | −0.28 | 0.04 | −0.30 | 0.09 |
| 30     | PPP1R9B         | protein phosphatase 1 regulatory subunit 9B | Cytoplasm | −0.34 | 0.00 | −0.26 | 0.04 |
| 30     | PRELP           | proline and arginine rich end leucine rich repeat protein | Extracellular Space | 0.35  | 0.00 | −0.25 | 0.07 |
| 30     | Prx1l           | paired related homeobox protein-like 1 | Other | 0.24  | 0.00 | −0.29 | 0.06 |
| 30     | PRTN3           | proteinase 3       | Extracellular Space | 0.58  | 0.01 | −0.45 | 0.09 |
| 30     | PSMA5           | proteasome subunit alpha 5 | Cytoplasm | −0.22 | 0.00 | 0.21  | 0.05 |
| 30     | PSMB10          | proteasome subunit beta 10 | Cytoplasm | −0.22 | 0.08 | 0.29  | 0.10 |
| 30     | PSMB3           | proteasome subunit beta 3 | Cytoplasm | −0.22 | 0.00 | 0.22  | 0.03 |
| Symbol | Entrez Gene Name | Location     | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|-----------------|--------------|------------|---------------------|
| 30 7   | PSMB7           | Cytoplasm    | −0.21      | 0.00                |
| 30 8   | PTPRU           | Plasma Membrane | 0.37      | 0.00                |
| 30 9   | PXN             | Cytoplasm    | 0.25       | 0.01                |
| 31 0   | RAB4A           | Cytoplasm    | 0.24       | 0.00                |
| 31 1   | RASL10B         | Other        | −0.21      | 0.00                |
| 31 2   | RAVER1          | Nucleus      | 0.22       | 0.03                |
| 31 3   | RBP38           | Nucleus      | 0.85       | 0.00                |
| 31 4   | RCC2            | Nucleus      | −0.21      | 0.00                |
| 31 5   | RCSD1           | Other        | 0.22       | 0.02                |
| 31 6   | REEP4           | Cytoplasm    | 0.33       | 0.01                |
| 31 7   | REEP6           | Plasma Membrane | 0.38      | 0.04                |
| 31 8   | RELN            | Extracellular Space | 0.28  | 0.00                |
| 31 9   | Retnlg          | Extracellular Space | 0.49  | 0.07                |
| 32 0   | RFXANK          | Nucleus      | 0.20       | 0.09                |
| 32 1   | RHCE/RHD        | Plasma Membrane | 1.07      | 0.02                |
| 32 2   | RHOT2           | Cytoplasm    | 0.20       | 0.00                |
| 32 3   | Rn18s-rs5       | Other        | −0.98      | 0.00                |
| 32 4   | RNF123          | Cytoplasm    | 0.32       | 0.00                |
| Symbol | Entrez Gene Name                      | Location    | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|--------------------------------------|-------------|------------|---------------------|
| 32     | RNF208 ring finger protein 208       | Other       | −0.22 0.02 | −0.35 0.02          |
| 32     | RNF216 ring finger protein 216       | Cytoplasm   | 0.33 0.00  | −0.36 0.08          |
| 32     | RPAP1 RNA polymerase II associated protein 1 | Other    | 0.33 0.00  | −0.34 0.03          |
| 32     | RPH3A rabphilin 3A                   | Plasma Membrane | 0.21 0.01  | −0.50 0.01          |
| 32     | Rpl22l1 ribosomal protein L22 like 1 | Other       | −0.27 0.00 | 0.20 0.06           |
| 33     | RPL26 ribosomal protein L26          | Cytoplasm   | −0.21 0.00 | 0.22 0.04           |
| 33     | RPL37A ribosomal protein L37a        | Cytoplasm   | −0.21 0.00 | 0.22 0.04           |
| 33     | RPS6KA1 ribosomal protein S6 kinase A1 | Cytoplasm   | 0.32 0.00  | −0.42 0.01          |
| 33     | RRM2 ribonucleotide reductase regulatory subunit M2 | Nucleus | 0.91 0.00  | −0.44 0.03          |
| 33     | RTN4R reticulin 4 receptor           | Plasma Membrane | −0.22 0.08 | −0.26 0.07          |
| 33     | RXRG retinoid X receptor gamma       | Nucleus     | 0.28 0.00  | −0.21 0.08          |
| 33     | SAMD14 sterile alpha motif domain containing 14 | Other | −0.21 0.01  | −0.28 0.03          |
| 33     | SAP130 Sim3A associated protein 130  | Nucleus     | 0.42 0.00  | −0.24 0.09          |
| 33     | SCG2 secretogranin II               | Extracellular Space | −0.22 0.01  | 0.20 0.08          |
| 33     | SCN5A sodium voltage-gated channel alpha subunit 5 | Plasma Membrane | 0.23 0.05  | −0.31 0.05          |
| 34     | SCRT1 scratch family transcriptional repressor 1 | Nucleus | −0.23 0.04  | −0.41 0.02          |
| 34     | SCUBE1 signal peptide, CUB domain and EGF like domain containing 1 | Plasma Membrane | 0.26 0.01  | −0.48 0.02          |
| 34     | SDC3 syndecan 3                     | Plasma Membrane | −0.22 0.00  | −0.25 0.07          |
| Symbol | Entrez Gene Name | Location          | PBS vs Cis          | Cis vs Cis + TTI-101          |
|--------|-----------------|-------------------|---------------------|-----------------------------|
| 34     | SEC24C           | SEC24 homolog C, COPII coat complex component | Cytoplasm | 0.20 0.00 | −0.29 0.03 |
| 34     | SEMA4B           | semaphorin 4B     | Plasma Membrane     | 0.28 0.00 | −0.23 0.07 |
| 34     | SEMA4G           | semaphorin 4G     | Plasma Membrane     | 0.32 0.01 | −0.47 0.00 |
| 34     | SEPTIN8          | septin 8          | Extracellular Space | 0.21 0.00 | −0.23 0.05 |
| 34     | SERPINA3         | serpin family A member 3 | Extracellular Space | −0.30 0.00 | 0.23 0.01 |
| 34     | Serpina3g (includes others) | serine (or cysteine) peptidase inhibitor, clade A, member 3G | Cytoplasm | −0.49 0.01 | 0.22 0.03 |
| 34     | SERPINB1         | serpin family B member 1 | Cytoplasm | −0.36 0.00 | 0.21 0.07 |
| 35     | Serpinb1b        | serine (or cysteine) peptidase inhibitor, clade B, member 1b | Other | −0.23 0.01 | 0.22 0.08 |
| 35     | SEZ6L            | seizure related 6 homolog like | Plasma Membrane | 0.41 0.00 | −0.59 0.01 |
| 35     | SFRP5            | secreted frizzled related protein 5 | Plasma Membrane | −0.21 0.00 | −0.30 0.00 |
| 35     | SH2D3C           | SH2 domain containing 3C | Cytoplasm | 0.23 0.00 | −0.24 0.10 |
| 35     | SHMT2            | serine hydroxymethyltransferase 2 | Cytoplasm | 0.23 0.02 | −0.27 0.05 |
| 35     | SIX5             | SIX homeobox 5    | Nucleus             | 0.32 0.09 | −0.34 0.09 |
| 35     | SKIV2L           | Ski2 like RNA helicase | Nucleus | 0.21 0.00 | −0.32 0.03 |
| 35     | SLC16A10         | solute carrier family 16 member 10 | Plasma Membrane | 0.51 0.03 | −0.45 0.05 |
| 35     | SLC25A37         | solute carrier family 25 member 37 | Cytoplasm | 0.64 0.00 | −0.24 0.08 |
| 35     | SLC40A1          | solute carrier family 40 member 1 | Plasma Membrane | 0.56 0.00 | −0.33 0.06 |
| 36     | SLC4A11          | solute carrier family 4 member 11 | Plasma Membrane | 0.29 0.00 | −0.28 0.04 |
| Symbol | Entrez Gene Name | Location                      | PBS vs Cis | Cis vs Cis + TTI-101  |
|--------|-----------------|-------------------------------|------------|-----------------------|
|        |                 |                               | log₂ Fold | Expr p-value          |
| 36     | SLC7A8          | solute carrier family 7 member 8 | Plasma Membrane | 0.48 | 0.00 | -0.36 | 0.07 |
| 36     | SLC9A1          | solute carrier family 9 member A1 | Plasma Membrane | 0.23 | 0.01 | -0.42 | 0.04 |
| 36     | SLIT1           | slit guidance ligand 1        | Extracellular Space | 0.24 | 0.01 | -0.36 | 0.06 |
| 36     | SLX4            | SLX4 structure-specific endonuclease subunit | Nucleus | 0.39 | 0.00 | -0.24 | 0.06 |
| 36     | SMTN            | smoothelin                    | Extracellular Space | 0.49 | 0.00 | -0.37 | 0.03 |
| 36     | SNCB            | synuclein beta                | Cytoplasm  | -0.32 | 0.00 | -0.25 | 0.06 |
| 36     | SNPH            | syntaphilin                   | Plasma Membrane | 0.22 | 0.00 | -0.31 | 0.02 |
| 36     | SOBP            | sine oculis binding protein homolog | Nucleus | -0.23 | 0.01 | -0.34 | 0.02 |
| 36     | SOD3            | superoxide dismutase 3        | Extracellular Space | 0.41 | 0.00 | -0.49 | 0.02 |
| 37     | SOX6            | SRY-box transcription factor 6 | Nucleus | 0.24 | 0.04 | -0.30 | 0.04 |
| 37     | Spaca6          | sperm acrosome associated 6   | Other | 0.42 | 0.02 | 0.33 | 0.04 |
| 37     | SPECC1          | sperm antigen with calponin homology and coiled-coil domains 1 | Nucleus | 0.22 | 0.05 | -0.26 | 0.07 |
| 37     | SPRE2           | spire type actin nucleation factor 2 | Cytoplasm | -0.31 | 0.00 | -0.27 | 0.02 |
| 37     | SPSB3           | splA/ryanodine receptor domain and SOCS box containing 3 | Cytoplasm | 0.23 | 0.06 | -0.34 | 0.03 |
| 37     | SPTBN5          | spectrin beta, non-erythrocytic 5 | Plasma Membrane | 0.32 | 0.01 | -0.36 | 0.05 |
| 37     | SRCAP           | Snf2 related CREBBP activator protein | Cytoplasm | 0.20 | 0.10 | -0.31 | 0.02 |
| 37     | ST5             | suppression of tumorigenicity 5 | Cytoplasm | 0.26 | 0.00 | -0.25 | 0.09 |
| 37     | STK10           | serine/threonine kinase 10    | Cytoplasm | 0.21 | 0.03 | -0.36 | 0.00 |
| Symbol | Entrez Gene Name                                      | Location        | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|------------------------------------------------------|-----------------|------------|----------------------|
| 37     | STUB1                                                | Cytoplasm       | 0.24       | 0.00                | -0.23 0.05 |
| 38     | SUGP1                                                | Nucleus         | 0.32       | 0.00                | -0.21 0.06 |
| 38     | SYT2                                                 | Cytoplasm       | 0.24       | 0.01                | -0.43 0.03 |
| 38     | TAF5                                                 | Extracellular Space | -0.25   0.00 | -0.24 0.05 |
| 38     | TBC1D17                                              | Cytoplasm       | 0.24       | 0.00                | -0.29 0.08 |
| 38     | TCOF1                                                | Nucleus         | 0.23       | 0.07                | -0.43 0.05 |
| 38     | TECPR2                                               | Other           | 0.20       | 0.01                | -0.25 0.08 |
| 38     | TENM2                                                | Plasma Membrane | 0.42       | 0.00                | -0.28 0.08 |
| 38     | TENM3                                                | Plasma Membrane | 0.26       | 0.00                | -0.34 0.05 |
| 38     | TENM4                                                | Plasma Membrane | 0.20       | 0.06                | -0.46 0.02 |
| 38     | TGFB1I1                                               | Nucleus         | 0.35       | 0.00                | -0.36 0.01 |
| 39     | THBS1                                                | Extracellular Space | 0.68    0.00 | -0.24 0.02 |
| 39     | THEM6                                                | Other           | -0.23      | 0.00                | -0.32 0.02 |
| 39     | TIAM1                                                | Cytoplasm       | 0.30       | 0.00                | -0.44 0.01 |
| 39     | TINAGL1                                               | Extracellular Space | 0.28    0.01 | -0.35 0.03 |
| 39     | TLN1                                                 | Plasma Membrane | 0.39       | 0.00                | -0.39 0.05 |
| 39     | TMEM151B                                              | Other           | -0.30      | 0.00                | -0.27 0.07 |
| 39     | TMEM160                                               | Cytoplasm       | -0.25      | 0.02                | 0.24 0.07 |
| Symbol | Entrez Gene Name                               | Location          | PBS vs Cis |     | Cis vs Cis + TTI-101 |     |
|--------|-----------------------------------------------|-------------------|------------|-----|---------------------|-----|
| 39     | TMEM205 transmembrane protein 205             | Cytoplasm         | −0.26      | 0.00| 0.23                | 0.08|
| 39     | TNFAIP2 TNF alpha induced protein 2           | Extracellular Space| 0.53       | 0.00| −0.42               | 0.06|
| 39     | TNFRSF1A TNF receptor superfamily member 1A  | Plasma Membrane   | 0.23       | 0.01| −0.29               | 0.08|
| 40     | TNIP1 TNFAIP3 interacting protein 1           | Nucleus           | 0.21       | 0.02| −0.38               | 0.01|
| 40     | TNXB tenascin XB                              | Extracellular Space| 0.26       | 0.01| −0.27               | 0.02|
| 40     | TOM1L2 target of myb1 like 2 membrane trafficking protein | Cytoplasm | 0.21 | 0.00 | −0.29 | 0.05 |
| 40     | TONSL tonsoku like, DNA repair protein       | Cytoplasm         | 0.43       | 0.00| −0.38               | 0.05|
| 40     | TPRA1 transmembrane protein adipocyte associated 1 | Plasma Membrane | 0.28 | 0.00 | −0.30 | 0.00 |
| 40     | TPRN taperin                                  | Extracellular Space| −0.28      | 0.02| −0.26               | 0.08|
| 40     | TRPA1 transient receptor potential cation channel subfamily A member 1 | Plasma Membrane | −0.31 | 0.00 | 0.29 | 0.02 |
| 40     | TRPC6 transient receptor potential cation channel subfamily C member 6 | Plasma Membrane | −0.20 | 0.04 | 0.29 | 0.01 |
| 40     | TRPM2 transient receptor potential cation channel subfamily M member 2 | Plasma Membrane | 0.26 | 0.00 | −0.23 | 0.05 |
| 40     | TRRAP transformation/transcription domain associated protein | Nucleus | 0.37 | 0.00 | −0.25 | 0.05 |
| 41     | TSPAN18 tetraspanin 18                        | Other              | 0.25       | 0.00| −0.27               | 0.08|
| 41     | TSPAN33 tetraspanin 33                        | Plasma Membrane   | 0.46       | 0.02| −0.35               | 0.07|
| 41     | TTBK1 tau tubulin kinase 1                    | Other              | −0.26      | 0.00| −0.40               | 0.02|
| 41     | TTC9B tetratricopeptide repeat domain 9B      | Other              | −0.38      | 0.00| −0.24               | 0.09|
| 41     | TTLL3 tubulin tyrosine ligase like 3          | Extracellular Space| 0.61       | 0.00| −0.36               | 0.04|
| Symbol | Entrez Gene Name                        | Location         | PBS vs Cis       | Cis vs Cis + TTI-101 |
|--------|----------------------------------------|------------------|------------------|----------------------|
|        |                                        |                  | log₂ Fold | Expr p-value | log₂ Fold | Expr p-value |
| 41     | TTYH3                                  | Plasma Membrane  | −0.21  | 0.02        | −0.23  | 0.09         |
| 41     | UBC                                    | Cytoplasm        | 0.58   | 0.00        | −0.40  | 0.05         |
| 41     | UBE3B                                  | Extracellular Space | 0.31  | 0.00        | −0.38  | 0.05         |
| 41     | UBL7                                   | Other            | 0.22   | 0.02        | −0.25  | 0.10         |
| 41     | UFSP2                                  | Other            | −0.22  | 0.00        | 0.20   | 0.06         |
| 42     | ULK3                                   | Cytoplasm        | 0.27   | 0.00        | −0.22  | 0.05         |
| 42     | UNC5A                                  | Plasma Membrane  | −0.27  | 0.05        | −0.33  | 0.07         |
| 42     | USP10                                  | Cytoplasm        | 0.31   | 0.00        | −0.30  | 0.08         |
| 42     | USP19                                  | Cytoplasm        | 0.20   | 0.01        | −0.24  | 0.06         |
| 42     | VGF                                    | Extracellular Space | −0.73 | 0.00        | −0.35  | 0.07         |
| 42     | VPS18                                  | Cytoplasm        | 0.22   | 0.02        | −0.35  | 0.05         |
| 42     | VPS9D1                                 | Other            | 0.22   | 0.01        | −0.29  | 0.05         |
| 42     | VWA5A                                  | Nucleus          | −0.28  | 0.00        | 0.24   | 0.02         |
| 42     | WASF1                                  | Nucleus          | −0.27  | 0.02        | −0.29  | 0.06         |
| 42     | WBP2                                   | Cytoplasm        | 0.28   | 0.00        | −0.36  | 0.04         |
| 43     | WDFY3                                  | Cytoplasm        | 0.22   | 0.01        | −0.37  | 0.03         |
| 43     | YLPM1                                  | Nucleus          | 0.25   | 0.00        | −0.24  | 0.02         |
| 43     | ZDHHC18                                | Cytoplasm        | −0.23  | 0.01        | −0.29  | 0.05         |
| Symbol | Entrez Gene Name | Location | PBS vs Cis | Cis vs Cis + TTI-101 |
|--------|-----------------|----------|------------|---------------------|
|        |                 |          | log₂ Fold | Expr p-value | log₂ Fold | Expr p-value |
| 43     | ZFP36L1         | Nucleus  | 0.23       | 0.03            | −0.25     | 0.10         |
| 43     | Zip651          | Other    | −0.23      | 0.00           | −0.21     | 0.03         |
| 43     | ZFYVE26         | Cytoplasm| 0.44       | 0.00           | −0.28     | 0.05         |
| 43     | ZNF142          | Nucleus  | 0.30       | 0.00           | −0.23     | 0.09         |
| 43     | ZNF219          | Nucleus  | −0.21      | 0.01           | −0.28     | 0.02         |
| 43     | ZNF423          | Nucleus  | 0.26       | 0.01           | −0.46     | 0.01         |
| 43     | ZNF442          | Nucleus  | −0.22      | 0.05           | 0.35      | 0.09         |
| 44     | ZNF592          | Nucleus  | 0.27       | 0.01           | −0.29     | 0.05         |
| 44     | ZNF646          | Nucleus  | 0.26       | 0.00           | −0.26     | 0.04         |
| 44     | ZNF703          | Nucleus  | −0.30      | 0.00           | −0.27     | 0.07         |
| 44     | ZSWIM1          | Nucleus  | 0.35       | 0.00           | −0.40     | 0.05         |
Table 2

Top five upstream regulators and their mechanistic network for differentially expressed.

| Upstream Regulator | Activation z-score | Overlap p-value | Mechanistic Network | Other Regulators in mechanistic network |
|-------------------|--------------------|-----------------|---------------------|----------------------------------------|
| 1 HTT             | -0.152             | 1.3E-10         |                     |                                        |
| 2 APP             | 2.394              | 1.67E-10        | 431 (8)             | TP53, NOTCH1, SP1, TP73, HIF1A, MYC, STAT3 |
| 3 TP53            | -2.078             | 1.77E-10        | 481 (14)            | STAT3, ERBB2, TP73, HIF1A, SP1, ESTROGEN RECEPTOR, NOTCH1, CTNNB1, CREBBP, SMAD4, SMAD3, FOS, STAT1 |
| 4 TGFBI           | -5.111             | 2.84E-09        | 436 (13)            | EGFP, ERBB2, NOTCH1, SP1, STAT3, EGR1, SMAD4, CREBBP, SMAD3, ESTROGEN RECEPTOR, CTNNB1, AR |
| 5 estrogen receptor | -0.614             | 6.22E-09        | 558 (15)            | TP53, BETA-ESTRADIOL, AR, CREBBP, ESR2, ESR1, SP1, TP73, HIF1A, STAT3, NOTCH1, DIETHYLSILBIBESTEROL,4-HYDROXYTAMOXIFEN, ESTROGEN |

genes in cisplatin dataset (cisplatin vs. cisplatin + TTI-101). Note, the number in the mechanistic network column denotes the number of target genes influenced by that particular regulator. The number in parenthesis denotes the total number of regulators in that mechanistic network.