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# STAT3 inhibition reduces macrophage number and tumor growth in neurofibroma

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## Abstract

Plexiform neurofibroma, a benign peripheral nerve tumor, is associated with the biallelic loss of function of the *NF1* tumor suppressor in Schwann cells. Here, we show that FLLL32, a small molecule inhibitor of JAK/STAT3 signaling, reduces neurofibroma growth in mice with conditional, biallelic deletion of *Nf1* in the Schwann cell lineage. FLLL32 treatment or *Stat3* deletion in tumor cells reduced inflammatory cytokine expression and tumor macrophage numbers in neurofibroma. Although STAT3 inhibition down-regulated the chemokines CCL2 and CCL12, which can signal through CCR2 to recruit macrophages to peripheral nerves, deletion of *Ccr2* did not improve survival or reduce macrophage numbers in neurofibroma-bearing mice. Interestingly, macrophages accounted for ~20-40% of proliferating cells in untreated tumors. FLLL32 suppressed this proliferation, as well as Schwann cell proliferation, implicating STAT3-dependent, local proliferation in neurofibroma macrophage accumulation. The functions of STAT3 signaling in neurofibroma Schwann cells and macrophages, and its relevance as a therapeutic target in neurofibroma, merit further investigation.



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Competing Interests

The authors have no relevant competing interests to disclose.

Study Approval

The animal care and use committees of Cincinnati Children's Hospital Medical Center approved all animal procedures. Human paraffin-embedded tissues were collected under Cincinnati Children's Hospital Medical Center (CCHMC) IRB approval.

## Introduction

Plexiform neurofibromas occur in 20-50% of persons with Neurofibromatosis type 1 (NF1), an autosomal-dominant disorder affecting approximately 1 in 3000 individuals (1). These tumors can be disfiguring and may compress neighboring vital organs, causing significant morbidity (1–3). Therefore, current research efforts are focused on identifying molecular targets critical to plexiform neurofibroma development and growth. *NF1* encodes neurofibromin, a negative regulator of the Ras family of proto-oncogenes, implicating intracellular signaling downstream of Ras as potential therapeutic targets in NF1 (4–6).

Plexiform neurofibroma formation is associated with biallelic loss of *NF1* in the Schwann cell (SC) lineage (7–9). *Dhh*-Cre mediated, conditional deletion of *Nf1* in SC precursors is sufficient to induce the formation of multiple plexiform neurofibromas in a mouse model (10). In this model, deletion of *Stat3* in SC precursors reduces tumor number and size, and prolongs mouse survival (11). Furthermore, Stat3 activation via Egfr and Il-6 signaling is important for the growth and/or survival of *Nf1*-null SC precursors in vitro (12). These data are consistent with established roles STAT3 signaling in tumor cell survival and proliferation (13).

STAT3 signaling is also strongly implicated in establishing a pro-tumorigenic inflammatory microenvironment (13,14). STAT3 signaling in tumor cells can facilitate the expression of chemokines and cytokines, including the macrophage chemoattractant CCL2 (13,15). This is of interest in neurofibroma, as macrophages constitute a remarkable 30-40% of the cells in plexiform neurofibromas, and macrophage reduction correlates with reduced tumor growth in neurofibroma-bearing mice treated with a CSF1R inhibitor (16). Macrophages have context dependent pro and anti-tumorigenic effects (17–20). CCL2, IL-6 and other tumor cell factors can polarize macrophages towards a tumor supportive phenotype (19–23). STAT3 activation in macrophages can also enhance macrophage proliferation and survival, and potentiate pro-tumorigenic functions such as angiogenesis, immune tolerance, extracellular matrix remodeling, and efferocytosis, the removal of dying cells by phagocytosis (17–20). Small molecule inhibitors of STAT3 pathway signaling may have therapeutic potential (24). Here, we test the effects of FLLL32, a specific JAK2/STAT3 inhibitor, on the growth of plexiform neurofibroma in a mouse model (25). We show that FLLL32 affects both Schwann cells and macrophages in neurofibroma.

## Results

### Pharmacological inhibition of JAK2/STAT3 decreases neurofibroma growth and macrophage numbers in vivo

FLLL32 is a JAK2/STAT3 inhibitor with no significant antagonism of a panel of related tyrosine kinases and disease-relevant kinases (25). FLLL32 treatment at 200 mg/kg/day for 5 days inhibited STAT3-Y705 phosphorylation, a measure of STAT3 activation, in neurofibromas from *Dhh*-Cre;$Nf1^{fl/fl}$ mice (Figure 1A) relative to vehicle treated controls (n = 4/group, mice collected 1 hour after the final dose). To test the therapeutic effects of FLLL32 in neurofibroma, we first established baseline neurofibroma volume and growth rates in a mixed sex population of *Dhh*-Cre;$Nf1^{fl/fl}$ (n = 10 FLLL32, n = 5 vehicle) mice on

a C57/BL6 background by magnetic resonance imaging (MRI) at 5 and 7 months of age. Mice were then treated with 200 mg/kg/day FLLL32 for 60 days and re-imaged at 9 months to measure final tumor burdens. Tumor growth in vehicle-treated mice was consistent with historic controls. Overall, neurofibroma volumes were significantly decreased in FLLL32-treated mice (Figure 1B), however there was considerable heterogeneity in the response of individual animals. FLLL32 treatment did not cause weight loss, but 50% of FLLL32-treated mice developed intestinal adhesions, a reported effect of this compound (25). Interestingly, colorimetric (DAB) staining for STAT3-Y705 phosphorylation was abolished in FLLL32 treated mice with a quantitative response (reduction in neurofibroma volume) to treatment, but was weakly present in non-responders (Figure 1C). FLLL32 treatment strongly suppressed cell proliferation (Ki-67$^+$ cells, Figure 1D) in FLLL32 responding mice (responders) relative to vehicle treated mice, while non-responders displayed considerable variation in Ki67 positivity. Gross changes in neurofibroma morphology were not evident by hematoxylin and eosin staining (Supplemental Figure 1A). Given the known roles of STAT3 in modulating the inflammatory tumor microenviroment, and the importance of macrophages and mast cells in neurofibroma growth and development, we also examined the effects of FLLL32 on these cells types (16,26). FLLL32 significantly reduced tumor macrophage numbers in both FLLL32 responders and non-responders relative to vehicle controls (Iba-1$^+$ cells, Figure 1E). Mast cell numbers did not differ between vehicle and FLLL32 treated mice (Figure 1F). Tumor macrophage numbers were also reduced, to a lesser extent, in *Dhh*-Cre;*Nf1*$^{fl/fl}$;*Stat3*$^{fl/fl}$ mice (Supplemental Figure 1B), suggesting that macrophage accumulation is partially dependent on STAT3 regulated SC factors.

### STAT3 inhibition in neurofibroma suppresses macrophage-trophic chemokine expression.

To explore the role of STAT3 in regulating gene expression in mouse neurofibroma, we utilized RNA-Seq. In transcriptomes of neurofibromas isolated from *Dhh*-Cre;*Nf1*$^{fl/fl}$ mice treated with FLLL32 or vehicle for 60 days, KEGG mapping of differentially expressed genes identified a single pathway (#04060; cytokine-cytokine receptor interaction) satisfying a global pathway significance p-value filter (pNDE=6.850337e-05, pPERT=0.191, and pGFDR=0.017) (Figure 2A). Three chemokines associated with this pathway: *Ccl6*, *Ccl12*, *Ccl22* were significantly decreased in FLLL32 treated neurofibroma versus control, and in *Dhh*-Cre;*Nf1*$^{fl/fl}$;*Stat3*$^{fl/fl}$ neurofibromas. A trend towards reduced *Ccl2*, an important macrophage chemokine, was also observed (Figure 2B, Supplemental Figure 2A). These factors can contribute to the recruitment and functional polarization of tumor macrophages/myeloid cells (13,20,23,27).

Next, we examined whether these chemokines correlate with disease severity. As in human disease, considerable heterogeneity in rate of neurofibroma growth and response to therapy is present in the *Dhh*-Cre;*Nf1*$^{fl/fl}$ mouse model. Vehicle and FLLL32-treated samples were segregated by rate of growth and response to therapy, respectively. We defined those tumors that increase in volume >35mm$^3$ in a 60-day period, based on volumetric MRI scans performed at 7 and 9 months of age, as fast growing neurofibromas. We defined those tumors that increase in volume <5mm$^3$ in a 60-day period, based on volumetric MRI scans performed at 7 and 9 months of age, as slow growing neurofibromas. We defined those tumors that shrink in a 60-day period in response to FLLL32, based on volumetric MRI

scans performed at 7 and 9 months of age, as responders and those tumors that continue to increase in volume in a 60-day period even when exposed to drug, based on volumetric MRI scans performed at 7 and 9 months of age, as non-responders. Expression of the CCR2 ligands *Ccl2* and *Ccl12* were significantly increased in fast-growing neurofibromas and were reduced in FLLL32 responders and *Dhh*-Cre;*Nf1*$^{fl/fl}$;*Stat3*$^{fl/fl}$ relative to vehicles and FLLL32 non-responders (Figure 2C). *Ccl6* and *Ccl22* did not correlate with disease severity (Supplemental Figure 2B).

To determine if similar expression of these chemokines occurs in human neurofibromas, we examined their expression in a published gene expression microarray data set (28). *CCL2* expression was significantly increased in human plexiform neurofibromas and neurofibroma SCs relative to normal human SCs and nerves (Figure 2D). Expression of *CCL13*, the human ortholog of *Ccl12,* was similar in normal and neurofibroma Schwann cell populations, and increased in both human nerve and neurofibroma (Figure 2D). Thus, *Ccl2* correlates with disease severity and FLLL32 response in mice, and *CCL2* with disease state in human SCs and tissues. Together, these data demonstrate that STAT3 signaling regulates cytokine-chemokine signaling in neurofibroma.

## Ccr2 is not necessary for macrophage accumulation in neurofibroma

The Ccl2 receptor Ccr2 plays a critical role in the bone marrow egress and tissue recruitment of the monocyte population that develops into tumor macrophages (27,29–32). The loss of *Ccr2* reduces macrophage accumulation in the contexts of nerve injury and inflammation and in some tumor microenvironments (27,31–35). Given that both the expression of CCR2 ligands, *Ccl2* and *Ccl12*, and the number of neurofibroma macrophages were reduced by STAT3 inhibition, we hypothesized that STAT3-dependent SC production of these CCR2 ligands is important to tumor macrophage accumulation and neurofibroma growth (Figure 3A). We generated *Dhh*-Cre;*Nf1*$^{fl/fl}$;*Ccr2*-null mice to examine the importance of CCR2-mediated recruitment in macrophage accumulation in neurofibroma. *Dhh*-Cre;*Nf1*$^{fl/fl}$;*Ccr2*-null mice did not demonstrate increased survival relative to *Dhh*-Cre;*Nf1*$^{fl/fl}$;*Ccr2*-heterozygous and *Dhh*-Cre;*Nf1*$^{fl/fl}$;*Ccr2*-wild-type controls (Figure 3B). Surprisingly, loss of *Ccr2* also did not significantly reduce macrophage accumulation in neurofibromas isolated from these mice (Figure 3C). The lack of effect of *Ccr2* deletion in this setting could be due compensatory/redundant recruitment mechanisms. Alternatively, STAT3 signaling might facilitate macrophage accumulation by enhancing the proliferation and survival of resident macrophages.

## STAT3 inhibition suppresses Schwann cell and macrophage proliferation in neurofibroma.

Resident macrophage populations in dorsal root ganglia/peripheral nerves where neurofibromas form are reported to expand as much as ten times in response to inflammatory signals after injury (35). Both neurofibroma SCs and macrophages express activated (Y-705 phosphorylated) STAT3 (Figure 4A-B). To determine whether STAT3 signaling directly or indirectly regulates macrophage proliferation, we examined the impact of STAT3 inhibition on neurofibroma macrophage proliferation after a brief (10 day) FLLL32 treatment in 7-month old *Dhh*-Cre;*Nf1*$^{fl/fl}$ mice. Unlike 60-day treatment, 10-day FLLL32 treatment was insufficient to reduce tumor macrophage number (Iba-1$^{+}$ cells)

relative to vehicle controls (Supplemental Figure 4A). Ten-day treated mice were also administered EdU over the 24 hours prior to collection to facilitate the quantification of proliferating cells in subsequent co-localization experiments. Cell proliferation ($EdU^+$ cells) was suppressed in 10-day FLLL32-treated neurofibroma sections relative to vehicle controls (Supplemental Figure 4B). F4/80 was used to identify macrophages for co-localization experiments; F4/80, Iba-1, and CD11b identify the same neurofibroma macrophage population (>98% overlap). Macrophage markers did not co-localize with T cell (CD3 )or neutrophil (Gr-1) markers (Supplemental Figure 3). Schwann cells ($Sox10^+$ cells) and macrophages ($F4/80^+$ cells) represented the two largest populations of proliferating cells in vehicle treated neurofibroma, and FLLL32 treatment suppressed both Schwann cell and macrophage proliferation (Figure 4C-D, Supplemental Figure 4C). These data suggest that STAT3 regulated proliferation contributes to macrophage accumulation in neurofibroma.

We also examined whether STAT3 inhibition induces macrophage apoptosis in 10-day FLLL32 treatment. Apoptosis (cleaved caspase $3^+$) was modestly increased in F4/80-negative cells in FLLL32 treated neurofibromas relative to vehicle controls, and extensive contact suggestive of efferocytosis was observed between macrophage processes and apoptotic cells (Figure 4E). Co-localization of Sox10 and cleaved caspase 3 was ambiguous and so not quantified separately. These data suggest that macrophage reduction in FLLL32-treated neurofibromas results from a reduction in the chemokine-mediated recruitment of hematogenous macrophages and/or the reduction of tumor macrophage proliferation rather than an increase in macrophage cell death. Possible roles of FLLL32 and pSTAT3 in modulating macrophage accumulation and cell proliferation in neurofibroma are depicted in Figure 4F.

## Discussion

We found that inhibition of JAK/STAT3 signaling by FLLL32 reduces the growth of established neurofibromas in the *Dhh*-Cre;$Nf1^{fl/fl}$ mouse model, concomitant with a reduction in tumor macrophages. Consistent with the well-established roles of STAT3 in regulating inflammatory signaling (13,14,20), RNA-sequencing analysis of vehicle and FLLL32-treated neurofibromas demonstrated that STAT3 signaling also regulates cytokine expression in neurofibromas. Analysis of differential gene expression in FLLL32-treated neurofibromas implicated STAT3 signaling in the expression of macrophage-recruiting chemokines. These genes, including *Ccl2,* and Ccl12, have established roles in tumor macrophage recruitment and macrophage polarization towards pro-tumorigenic functions (13,19–23,32). *Ccl2* and *Ccl12* expression were positively associated with neurofibroma growth and resistance to Stat3 inhibition. *CCL2* expression was also associated with disease state in human Schwann cells and neurofibromas.

Given the association between the expression of the Ccr2 ligands, *Ccl2* and *Ccl12*, with disease progression and resistance to FLLL32, and the established importance of Ccr2 signaling to hematogenous macrophage recruitment to other tumors and to injured peripheral nerve/dorsal root ganglia, we anticipated that loss of *Ccr2* would reduce macrophage accumulation and improve survival in neurofibroma-bearing mice (29–35). However, neither survival nor macrophage accumulation in tumors were affected by the loss of *Ccr2* in

neurofibroma-bearing mice. This led us to explore other roles for STAT3 signaling in driving macrophage accumulation in neurofibroma. Surprisingly, we found that significant macrophage proliferation occurs in neurofibroma, and that STAT3 inhibition suppresses this proliferation. Therefore, it seems likely that local proliferation is an important contributor to macrophage accumulation in neurofibroma. Although neurofibroma macrophage numbers were more strongly reduced by FLLL32-mediated global inhibition of STAT3 than by loss of *Stat3* in Schwann cells, it remains unclear whether this is a direct or indirect effect of STAT3 inhibition. Recruitment of hematogenous macrophages to these tumors may also involve redundant signaling through alternative chemokine receptors, such as Ccr5 or Cx3cr1 (36,37). Thus, further work is needed to delineate the relative importance of hematogenous recruitment and local proliferation in neurofibroma macrophage accumulation.

Macrophages are the predominant immune population in neurofibroma, accounting for ~25-35% of all human and mouse neurofibroma cells (16), and macrophage reduction correlates with tumor shrinkage in neurofibroma-bearing mice treated with an inhibitor of CSF1R signaling, a central regulator of macrophage survival and proliferation (16). CSF1, chemokines such as CCL2, and STAT3 are all important regulators of macrophage functional polarization (20). STAT3 inhibition significantly reduced tumor macrophages in both responder and non-responder neurofibroma-bearing mice. Therefore, changes in both macrophage number and function may be important for a therapeutic response. Gene expression data from neurofibroma macrophages suggests that these cells do not conform to simple M1/M2 paradigms (38). Additional work is needed to better characterize specific mechanisms by which macrophages contribute to neurofibroma development and growth.

While current evidence suggests that STAT3 signaling may not be relevant in human dermal neurofibroma, significant STAT3 activation is present in human plexiform neurofibroma and malignant peripheral nerve sheath tumor (11, 39). We demonstrate that inhibition of STAT3 signaling reduces tumor growth in a mouse model of plexiform neurofibroma, and that STAT3 plays a critical role in macrophage accumulation in these tumors. We show that STAT3 signaling is important for the production of macrophage-recruiting chemokines by neurofibroma SCs and for tumor macrophage proliferation. Together with prior work demonstrating the importance of neurofibroma SC intrinsic STAT3 signaling to tumor cell survival and proliferation, this work establishes STAT3 as a key regulator of both the neurofibroma SC and the neurofibroma inflammatory microenvironment.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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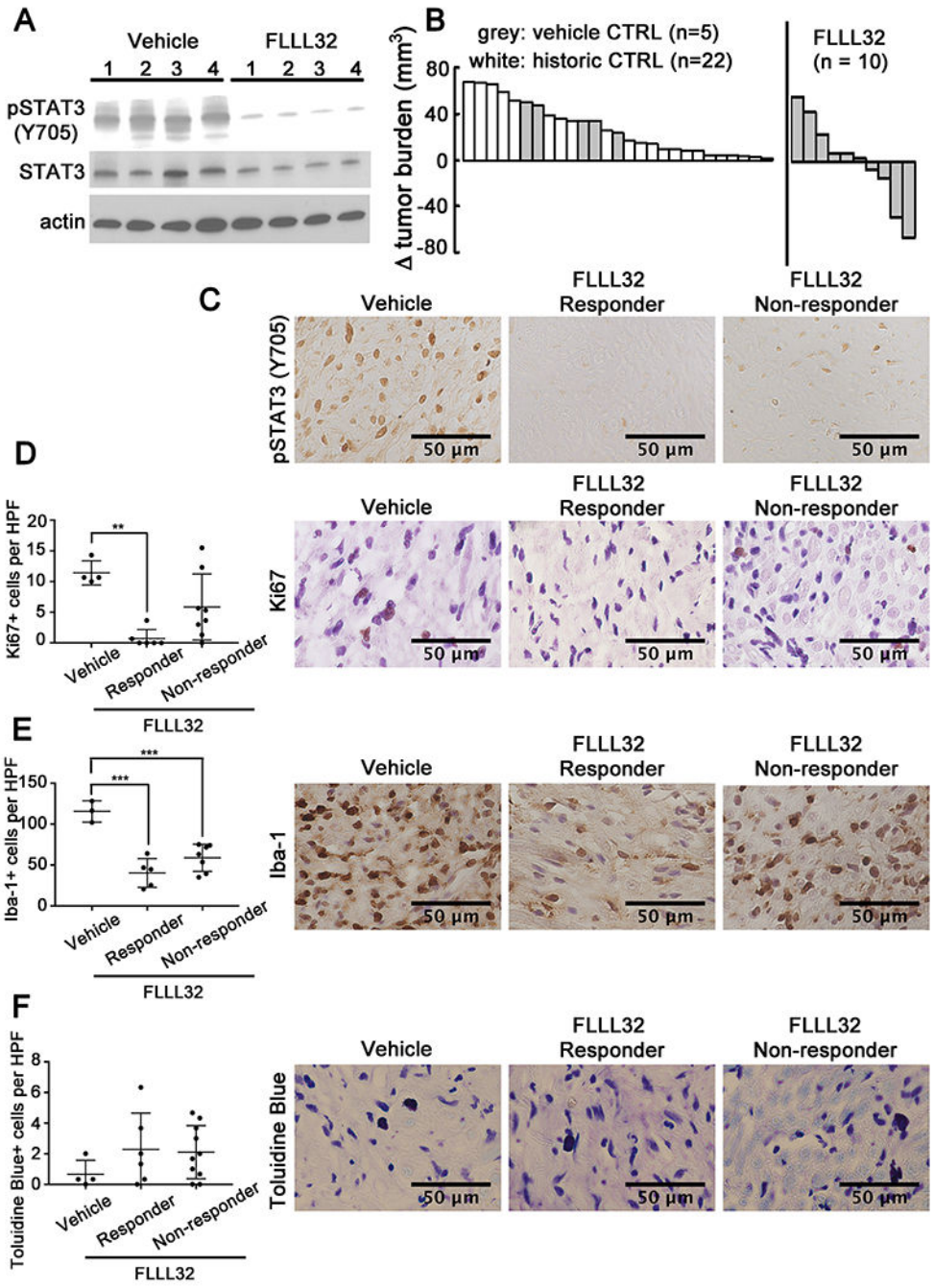


**Figure 1.**

JAK2/STAT3 inhibition in *Dhh*-Cre;*Nf1*$^{fl/fl}$ mice treated with FLLL32. **(a)** Western blotting demonstrates reduced (P-Y705, Cell Signaling #9145) STAT3 activation in neurofibroma lysates from mice treated for 5 days with 200 mg/kg/day FLLL32 (n = 4 per group). **(b)** Global tumor burden as measured by volumetric MRI was reduced in neurofibroma-bearing mice after 60 day FLLL32 treatment relative to in-group (grey bars) and historic (white bars) vehicle controls ($p < 0.05$, random effects model analysis on log transformed tumor volume data). **(c)** Activated (P-Y705, Cell Signaling #9145) STAT3 signal was present in

neurofibroma tissue sections from *Dhh*-Cre;*Nf1*$^{fl/fl}$ vehicle control mice, but absent in FLLL32-responders and weak in FLLL32-non-responders treated for 60 days with FLLL32. **(d)** Proliferation, measured as Ki-67$^{+}$ (Cell Signaling, 12202) cells per high-powered field (HPF), was reduced (** $p < 0.01$, unpaired t-test, n = 4 control, n = 6 FLLL32 responders, n = 8 FLLL32 non-responders) in neurofibroma tissue from FLLL32 responders relative to vehicle controls. **(e)** Tumor macrophages, measured as Iba-1$^{+}$ (019-19741, Wako) cells per HPF, were reduced in both FLLL32 responder and non-responder neurofibroma tissue relative to vehicle controls (*** $p < 0.001$, unpaired t-test, n = 3 control, n = 5 FLLL32 responders, n = 7 FLLL32 non-responders). (f) Differences in the number of tumor mast cells, identified by toluidine blue staining, were not observed between vehicle and FLLL32-treated neurofibroma tissue (n = 4 control, n = 6 FLLL32 responders, n = 10 FLLL32 non-responders).

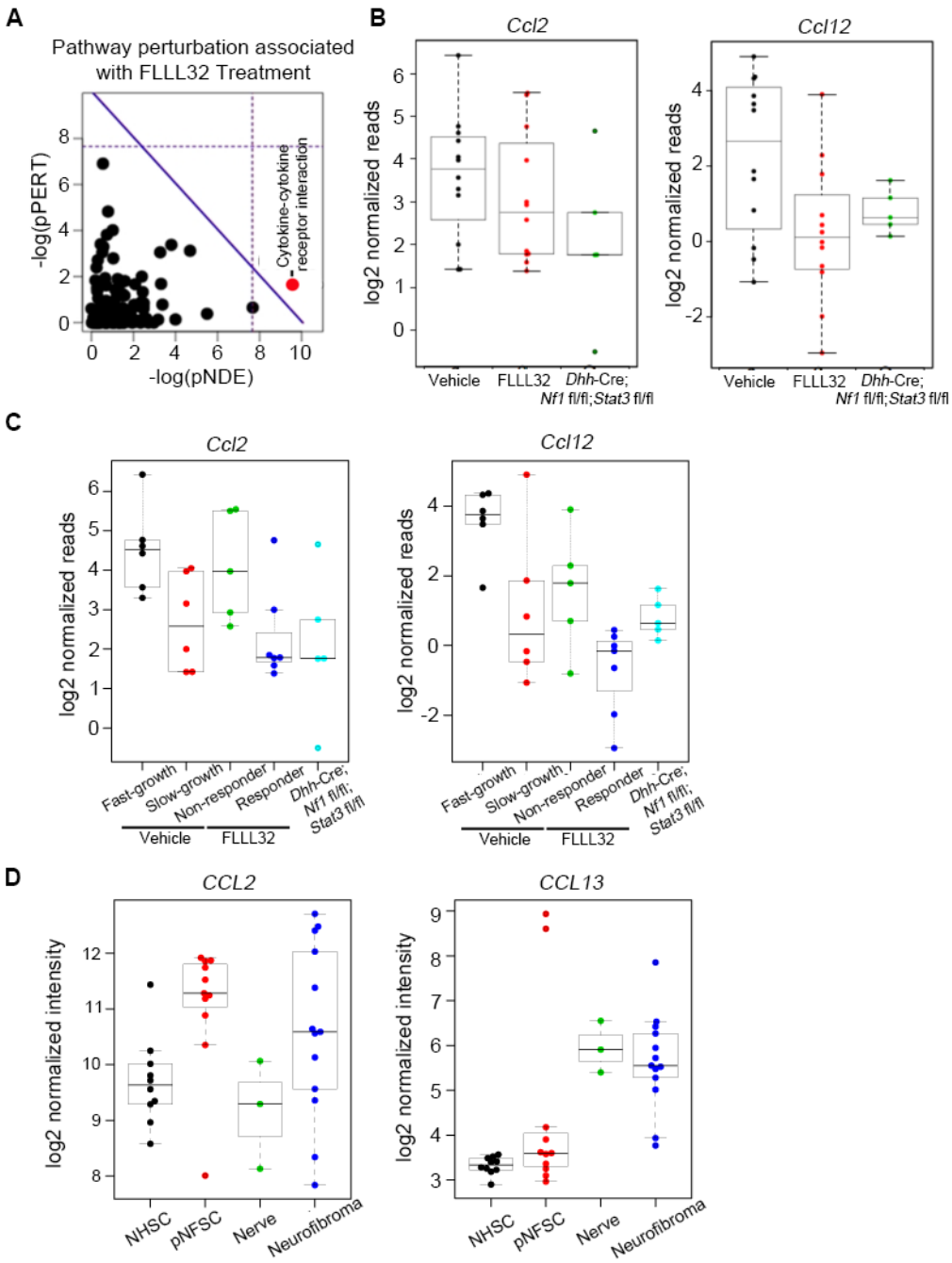


**Figure 2.**

STAT3 signaling stimulates the production of macrophage attractants by neurofibroma Schwann cells. **(a)** Single-end RNA-sequencing was performed on vehicle (n = 11) and FLLL32-treated (n = 10) neurofibromas from *Dhh*-Cre;*Nf1*$^{fl/fl}$ mice and *Dhh*-Cre;*Nf1*$^{fl/fl}$;*Stat3*$^{fl/fl}$ (n = 4) neurofibromas. Reads were trimmed and aligned to UCSC mm10 genome using TopHat. Gene-level read counts were computed using FeatureCounts. Gene expression was normalized by the trimmed mean of M-values method implemented in edgeR. **(a)** Signaling pathway impact analysis identified a single pathway (#04060;

cytokine-cytokine receptor interaction) satisfying a global pathway significance p-value filter (pNDE=6.850337e-05, pPERT=0.191, and pGFDR=0.017) decreased by FLLL32 treatment. **(b)** Expression of the CCR2 ligand *Ccl12* was decreased ($p < 0.05$, ANOVA with Tukey's HSD) in FLLL32-treated and *Dhh*-Cre;*Nf1*$^{fl/fl}$;*Stat3*$^{fl/fl}$ neurofibromas relative to vehicle, a trend towards decreased *Ccl2* was also observed. **(c)** Vehicle samples were subgrouped by their rate-of-growth (Fast-growth:>35mm$^3$ over 60 days, Slow-growth:<5mm$^3$ over 60 days). FLLL32 treated samples were segregated by response to therapy (responders: tumor shrinkage during 60 day FLLL32 treatment, non-responders: continued growth during the treatment period). Both *Ccl2* and *Ccl12* were increased in fast-growing neurofibromas relative to slow-growing, and were decreased in responders and *Dhh*-Cre;*Nf1*$^{fl/fl}$;*Stat3*$^{fl/fl}$ relative to vehicle treatment and non-responders ($p < 0.05$, ANOVA with Tukey's HSD). **(d)** Analysis of *CCL2* and *CCL13* (Ccl12 human orthology) in a previously published human neurofibroma microarray dataset. *CCL2* expression is significantly increased in human plexiform neurofibroma (pNF) and plexiform neurofibroma SCs (pNFSCs) relative to normal human SCs (NHSCs) and nerve ($p < 0.05$, ANOVA with Tukey's HSD). Relative *CCL13* expression was low in NHSCs and pNFSCs, and similar in pNF and nerve ($p < 0.05$, ANOVA with Tukey's HSD).

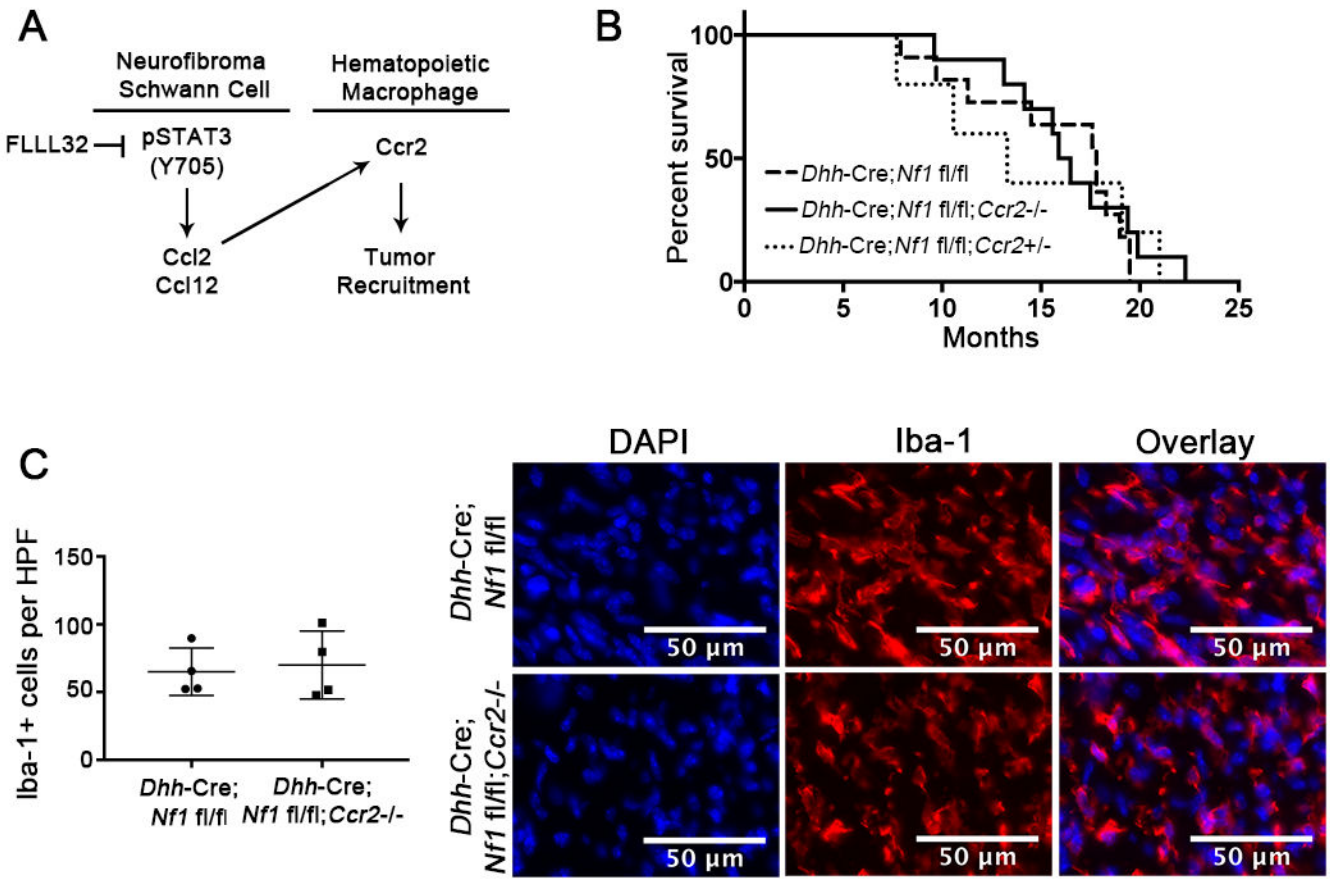


**Figure 3.**

Effects of *Ccr2* deletion on the survival of *Dhh*-Cre;$Nf1^{fl/fl}$ neurofibroma-bearing mice and the accumulation of neurofibroma macrophages. **(a)** Proposed interaction between Schwann cells and hematopoietic monocytes/macrophages. FLLL32 reduces tumor macrophages by decreasing the pSTAT3 dependent expression of macrophage recruiting chemokines, especially *Ccl2*. **(b)** Loss of *Ccr2* did not alter survival in neurofibroma-bearing mice (log-rank test, n= 11 *Dhh*-Cre;$Nf1^{fl/fl}$, n = 10 *Dhh*-Cre;$Nf1^{fl/fl}$;$Ccr2^{-/-}$, n = 5 *Dhh*-Cre;$Nf1^{fl/fl}$;$Ccr2^{+/-}$). **(c)** Iba-$1^{+}$ macrophages were quantified in frozen neurofibroma sections from *Dhh*-Cre;$Nf1^{fl/fl}$ and *Dhh*-Cre;$Nf1^{flox/flox}$;$Ccr2^{-/-}$ survival mice,. Nuclei were counterstained with DAPI. Neurofibroma macrophage accumulation was not affected by loss of *Ccr2* (t-test, n = 4 each group).

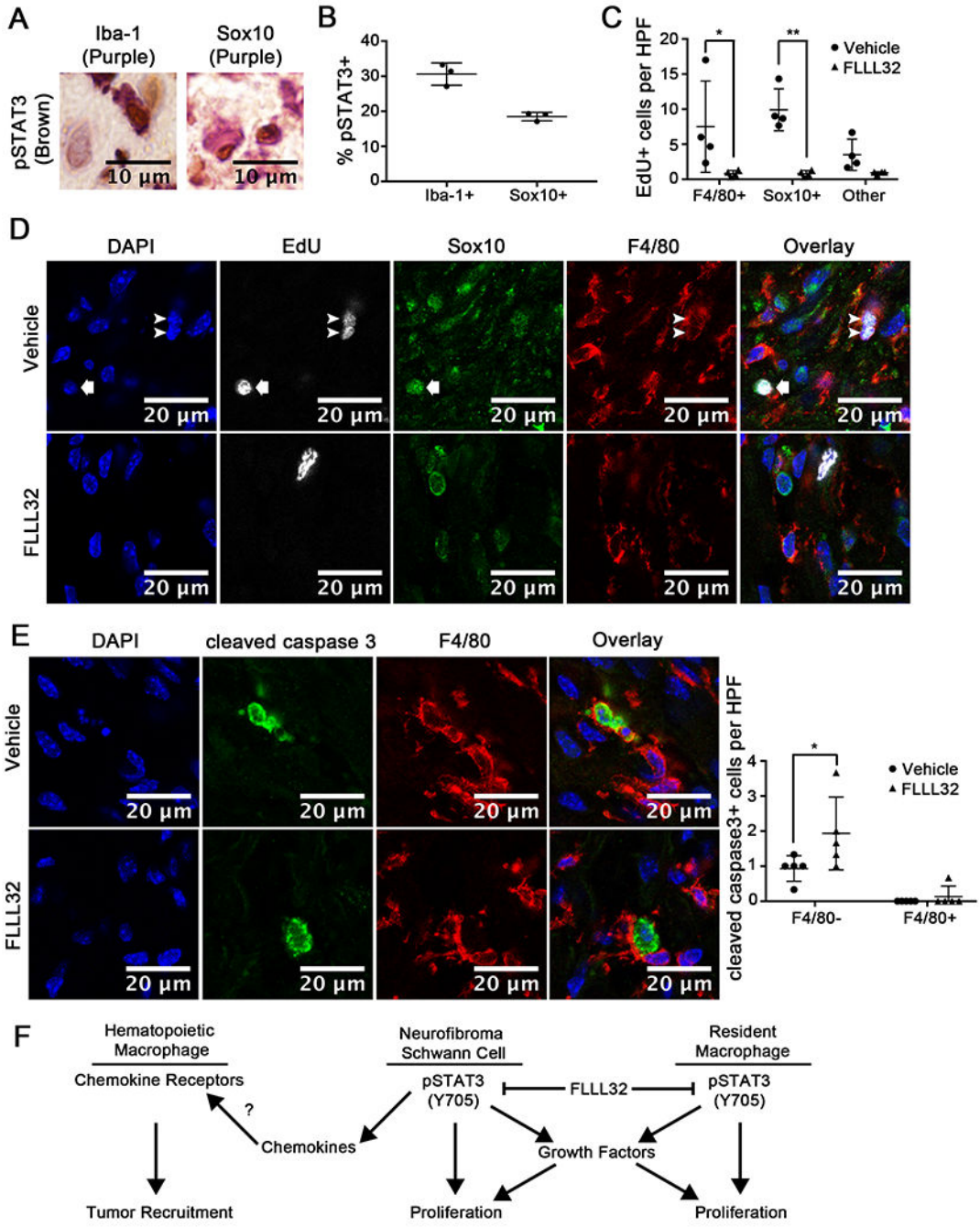


**Figure 4.**

**(a)** Colorimetric double labeling for pSTAT3 (DAB, brown) demonstrates pSTAT3 expression in (VIP, purple) macrophages (Iba-1+) and Schwann cells (Sox10+). **(b)** Quantification of pSTAT3 expression in macrophages and Schwann cells. **(c)** Co-staining of EdU stained neurofibroma sections for macrophages (F4/80, BioRad, MCA497) and Schwann cells (Sox10, Santa Cruz, sc-17342) demonstrates that these cell populations proliferate in neurofibroma. FLLL32 decreased both macrophage and non-macrophage proliferation in neurofibroma relative to vehicle (**p <0.01, two-way ANOVA with post-hoc

Sidak's multiple comparisons test, n = 5 each group). **(d)** Representative images of Sox10, F4/80, EdU triple staining with DAPI counterstain in FLLL32 and vehicle-treated neurofibromas. The rightward pointing white arrowheads indicate a pair of proliferating macrophages and the leftward pointing arrowhead a proliferating Schwann cell in a vehicle-treated neurofibroma. A rare $F4/80^{-}$;$Sox10^{-}$ proliferating is shown in an FLLL32-treated neurofibroma. **(e)** Frozen sections from 10-day FLLL32-treated and vehicle neurofibroma were stained for macrophage (F4/80) and apoptotic cell (cleaved caspase 3, Abcam, ab49822) markers and counterstained with DAPI. Cleaved caspase $3^{+}$ cells were rare in both vehicle and FLLL32 treated neurofibromas. These cells frequently had extensive contact with neighboring macrophages. FLLL32 treatment modestly increased apoptosis in non-macrophage cells in neurofibroma (*$p < 0.05$, two-way ANOVA with post-hoc Sidak's MCT, n = 5 each group). **(f)** Diagram representing possible mechanisms by which STAT3 signaling regulates proliferation and macrophage accumulation in neurofibromas: by regulating the SC expression of chemokines that recruit hematopoietic macrophages, and by directly or indirectly (through autocrine and paracrine secreted factors) that drive proliferation in neurofibroma SCs and resident macrophages.