Supplemental Experimental Procedures

Mouse breeding

To generate insertional mutants, we used a T2/Onc transgenic line with the donor concatemer on chromosome 15 (Collier et al., 2005) (Supplemental Figure 1A). Transgenic animals heterozygous for *Rosa26*-lsl-SB11 and T2/Onc were interbred to obtain *Rosa26*-lsl-SB11; T2/Onc mice, which were then bred with $Nf1^{fl/fl}$ mice to generate triple transgenic *Rosa26*-lsl-SB11; T2/Onc;$Nf1^{fl/fl}$ mice. Then, doubly $Nf1^{fl/fl}$;*DhhCre* and *Rosa26*-lsl-SB11;T2/Onc;$Nf1^{fl/fl}$ mice were interbred to generate quadruple experimental (*Rosa26*-lsl-SB11;T2/Onc;$Nf1^{fl/fl}$;*DhhCre*) and control animals of various transgene combinations. The genetic backgrounds of these animals were mixed, allowing for diverse genetic population analyses. PCR genotyping was performed as described (Collier et al., 2005; Jaegle et al., 2003; Keng et al., 2009).

We bred the $Stat3^{fl/fl}$ mice (Raz et al., 1999) onto the $Nf1^{fl/fl}$ background to obtain F1 generation ($Stat3^{fl/+}$;$Nf1^{fl/+}$). We also bred the $Stat3^{fl/fl}$ mice with $Nf1^{fl/+}$;*DhhCre*+ to obtain $Stat3^{fl/+}$;$Nf1^{fl/+}$;*DhhCre* mice. We then bred $Stat3^{fl/+}$;$Nf1^{fl/+}$ with $Stat3^{fl/+}$;$Nf1^{fl/+}$;*DhhCre* mice to obtain $Stat3^{fl/fl}$;$Nf1^{fl/fl}$;*DhhCre* mice. $Nf1^{fl/fl}$;*DhhCre* mice or $Stat3^{fl/+}$;$Nf1^{fl/fl}$;*DhhCre* were used as controls.

Flow cytometry

We sorted 5×10^6 GEM-neurofibroma cells directly after enzymatic dissociation to identify EGFP high and negative cells. For human neurofibroma or normal human SCs, FACS was performed as previously described (Williams et al, 2008).

Cytospin

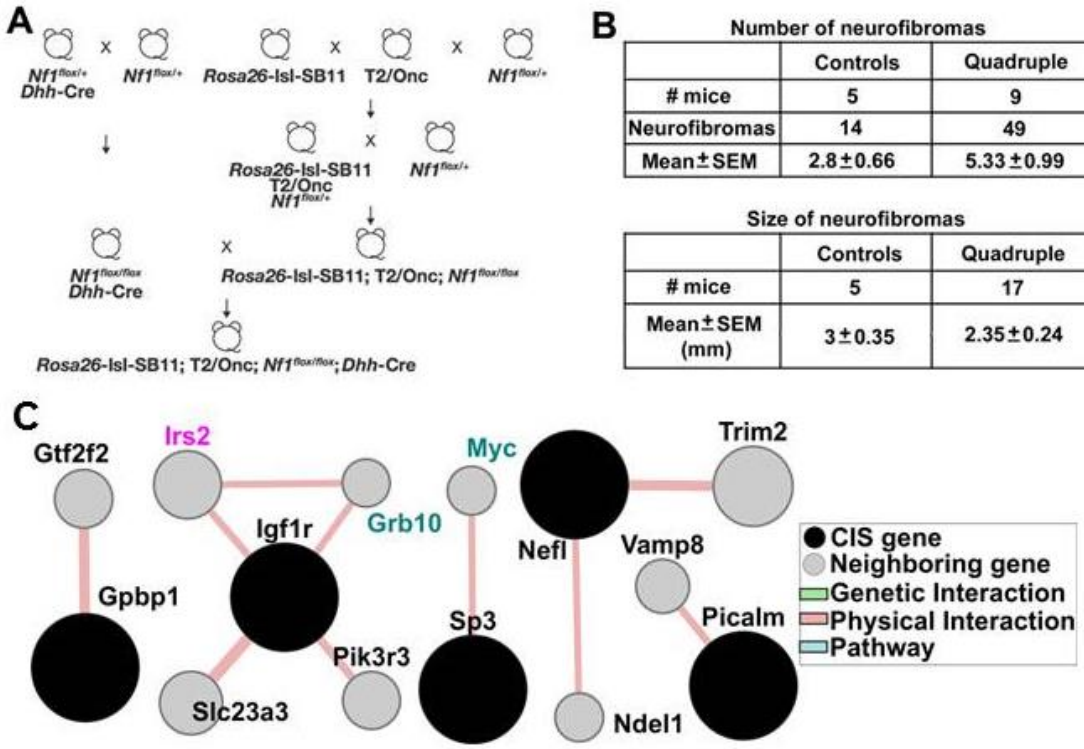
We cytospun 15,000 $EGFP^+$ or $EGFP^-$ cells onto 2% FBS pre-coated Super-plus slides. Slides were dried for 10 minutes at room temperature and then stored at -80°C.

Chromatin immunoprecipitation assay

Primary $Nf1^{fl/fl}$;*DhhCre* mouse neurofibroma cells were used for the Chromatin immunoprecipitation (CHIP) assay according to Magna CHIP™ instruction (Millipore, Billerica, MA). For PCR, the primers were designed as described (Moh et al, 2008).

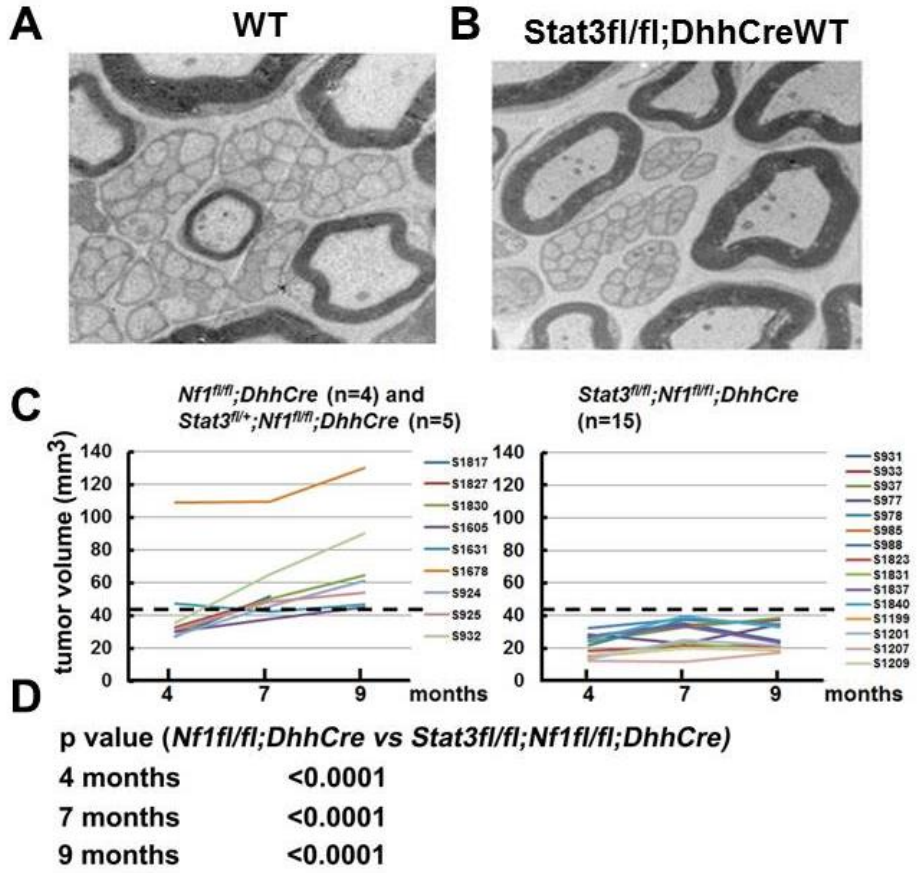
Supplemental Figures and Legends

Figure S1



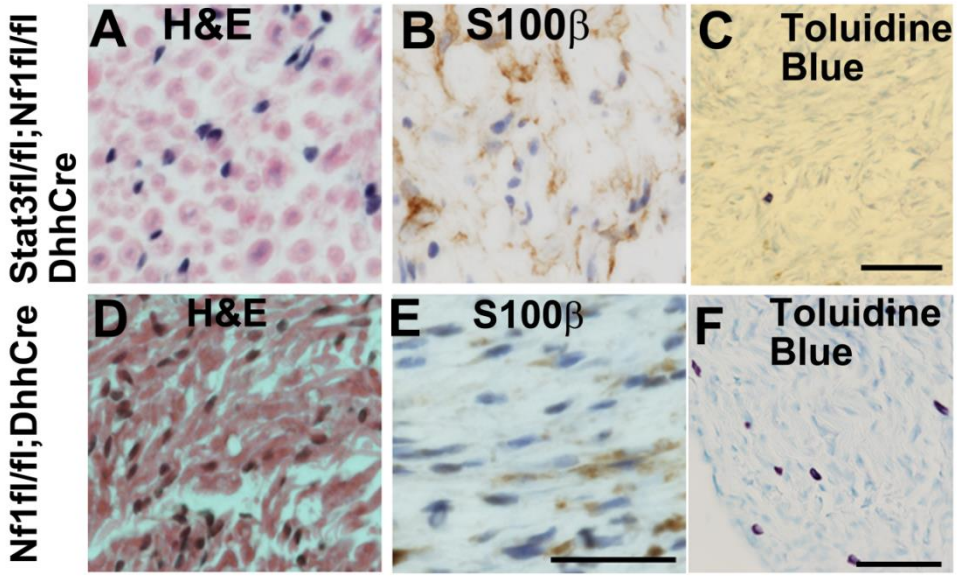
Supplemental Figure 1 related to Figure 1. Sleeping Beauty Transposon System identified common insertion sites and predict deregulated other pathway activation in neurofibroma (**A**) Strategy for generating experimental and control cohorts. Mice carrying both the desert hedgehog regulatory element driving Cre recombinase (*DhhCre)* and neurofibromin-fled allele ($Nf1^{fl/+}$) were bred with $Nf1^{fl/+}$ mice to generate doubly transgenic mice ($Nf1^{fl/fl}$*;DhhCre)*. Doubly transgenic mice carrying both the conditional Sleeping Beauty transposase gene knocked into the *Rosa26* locus (*Rosa26*-lsl-SB11) and the mutagenic transposon (T2/Onc) were bred with $Nf1^{fl/+}$ mice to generate triple transgenic mice (*Rosa26*-lsl-SB11; T2/Onc; $Nf1^{fl/fl}$). $Nf1^{fl/fl}$*;Dhh-Cre* mice were finally bred with *Rosa26*-lsl-SB11; T2/Onc; $Nf1^{fl/fl}$ mice to generate quadruple transgenic experimental mice (*Rosa26*-lsl-SB11; T2/Onc; $Nf1^{fl/fl}$*;DhhCre*) and control mice carrying various combinations of transgenes. (**B**) The number and size of neurofibromas isolated from experimental and control animals. Upper table shows the trend toward an increase in the number of neurofibromas isolated from experimental quadruple transgenic animals, compared to controls ($p = 0.10$). Data in the lower table shows no statistical difference in the average size of neurofibromas isolated from experimental and control animals ($p = 0.20$). (**C**) Genes identified by Sleeping Beauty Transposon System predict deregulated other pathway activation in neurofibroma. CIS candidate genes are shown in black. Circle size correlates to association probabilities to the network.

Figure S2



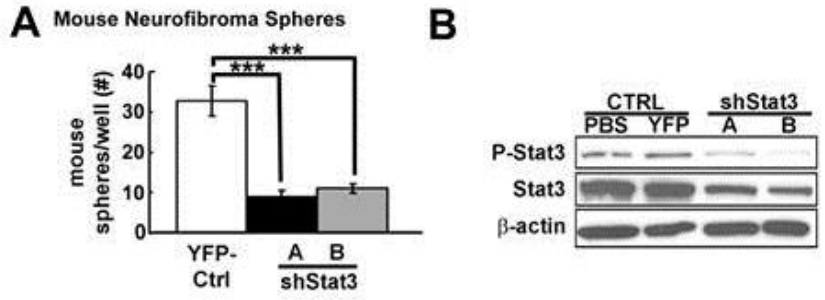
Supplemental Figure 2 related to Figure 3. Loss of Stat3 after E12.5 has no influence on Schwann cell development but delays neurofibroma formation *in vivo*. (**A-B**) Representative electron micrographs of saphenous nerves from 4-month-old WT (**A**) and Stat3 $^{fl/fl}$;DhhCre mice (**B**) show organized grouped axons and myeline-sheath. Small axons are considered grouped if three or more are wrapped by a single non-myelinating Schwann cell. We analyzed three mutant nerves in comparison to >5 controls at the same age. (**C-D**)Targeted genetic deletion of Stat3 in SCs and SCPs delays neurofibroma formation *in vivo* (**C**) Each mouse volume at 4, 7 and 9 months in controls (*Nf1*$^{fl/fl}$*;DhhCre* or *Stat3*$^{fl/+}$*;Nf1*$^{fl/fl}$*;DhhCre*) (left) and *Stat3*$^{fl/fl}$*;Nf1*$^{fl/fl}$*;DhhCre* mouse (right). Note that 2 control mice died between 7 and 9 months and therefor no volumes at 9 months were indicated. Dash lines indicate all the control mice volume >45 mm^3 at 9 months, while the double knock out mice <45 mm^3. (**D**) Mouse tumor volume statistical analysis: Mixed effects analysis of tumor volume showed p<0.001 between *Stat3*$^{fl/+}$*;Nf1*$^{fl/fl}$*;DhhCre* controls and *Stat3*$^{fl/fl}$*;Nf1*$^{fl/fl}$*;DhhCre* mice at 4, 7 and 9 months. Statistics: mixed model analysis.

Figure S3



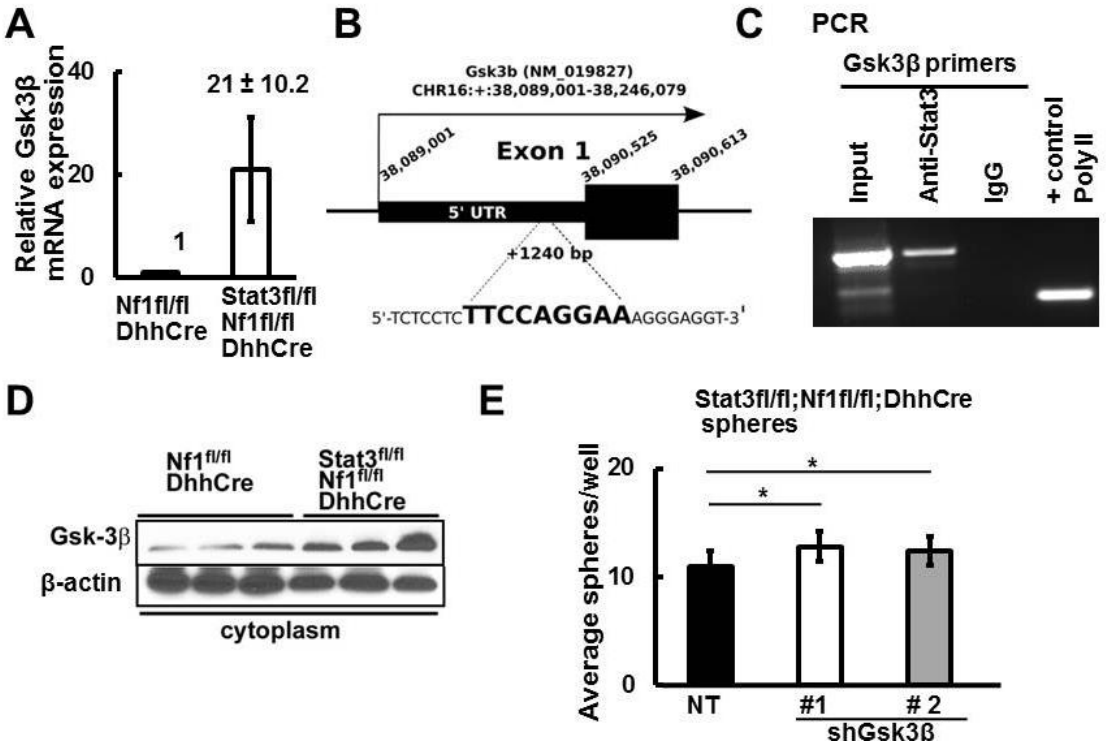
Supplemental Figure 3 related to Figure 3. Histology of *Stat3$^{fl/fl}$;Nf1$^{fl/fl}$;DhhCre* and *Nf1$^{fl/fl}$;DhhCre* mouse neurofibromas. (**A, D**) Representative H&E in *Stat3$^{fl/fl}$;Nf1$^{fl/fl}$;DhhCre* (**A**) and *Nf1$^{fl/fl}$;DhhCre* (**D**) of paraffin embedded mouse plexiform-GEM-neurofibroma sections. (**B, E**) Representative S100β-immunostaining in *Stat3$^{fl/fl}$;Nf1$^{fl/fl}$;DhhCre* (**B**) and *Nf1$^{fl/fl}$;DhhCre* (**E**) of paraffin embedded mouse plexiform-GEM-neurofibroma sections. (**C, F**). Toluidine-blue staining illustrating infiltration of metachromatic mast cells in *Stat3$^{fl/fl}$;Nf1$^{fl/fl}$;DhhCre* (**C**) and *Nf1$^{fl/fl}$;DhhCre* mouse (**F**). Bar=50µm.

Figure S4



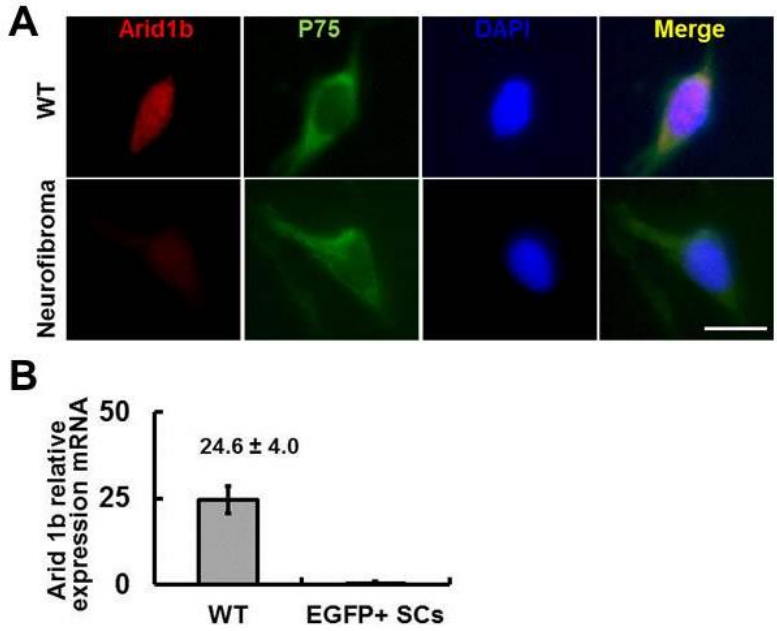
Supplemental Figure 4 related to Figures 3 and 4 . Genetic inhibition of Stat3 prevents neurofibroma sphere formation in vitro. (**A**) Two shStat3 shRNAs (A and B) each significantly decrease mouse neurofibroma sphere formation, compared to non-target lentivirus YFP control. (**B**) Western blot confirmation of shStat3-mediated knock down of P-Stat3-Y705 (P-Stat3) and total Stat3 (Stat3), compared to controls in mouse neurofibroma spheres. Anti-β-actin served as loading control. Statistics: Ordinary one way ANOVA. Three independent experiments were performed, data represented as mean ± SEM.

Figure S5



Supplemental Figure 5 related to Figures 5, 6 and 7. Stat3 alters Gsk3β in neurofibroma. (**A**) qRT-PCR shows increased relative *Gsk3β* mRNA expression in *Stat3*$^{fl/fl}$;*Nf1*$^{fl/fl}$;*DhhCre* mouse sciatic nerves (white bar, n=8) as normalized to *Nf1*$^{fl/fl}$;*DhhCre* mouse sciatic nerve (black bar, n=8) mRNA expression. (**B**) Schematic of exon 1 of the *Gsk3β* gene. Mouse *Gsk3β* gene is located on chromosome 16 (forward strand, ENSMUSG00000022812, chr16:38,089,001-38,246,084). One putative Stat3 binding motif (shown in bold) is present within the 5' UTR (38,089,001-38,090,525) within exon 1. (**C**) Chromatin immunoprecipitation (ChIP) of Stat3 on the *Gsk3β* promoter. PCR amplified a 300-bp *Gsk3β* DNA fragment from immunoprecipitated DNA after ChIP using a Stat3 antibody. IgG was used as a negative control. Anti-polymerase II antibody was used as internal positive control (165-bp PCR product). (**D**) Loss of Stat3 decreases P-Gsk3β in neurofibroma lysates. Western blots show a 290% increase in cytoplasmic total active Gsk3β in *Stat3* $^{fl/fl}$;*Nf1*$^{fl/fl}$;*DhhCre* compared to *Nf1*$^{fl/fl}$;*DhhCre* neurofibromas, after fractionation into nuclear and cytoplasmic fractions. n=3 for each group. (**E**) Knock-down *Gsk3β by shRNA* does not fully rescue sphere numbers in *Stat3*$^{fl/fl}$;*Nf1*$^{fl/fl}$;*DhhCre* mouse neurofibroma/DRG spheres. Statistics: Unparied student *t* –test. Three independent experiments were performed.

Figure S6



Supplemental Figure 6 related to Figures 6 and 7. Expression of Arid1b. (A) Representative immunofluorescence staining of Arid1b (red) and P75 (green) in wild type mouse Schwann cells (WT, top) and *Nf1*$^{fl/fl}$*;DhhCre* mouse neurofibroma Schwann cells (low). (**B**) Arid1b mRNA expression in FACS sorted wild type mouse Schwann cells (WT) and mouse neurofibroma Schwann cells (EGFP+ SCs). QRT-PCR results in triplicate were normalized to mRNA expression in EGFP+ SCs.

Figure S7

A. DNA sequence from mouse Chromosome 17(MMCHR17: 5003400-5007000) shows the potential Stat3 binding site (yellow highlight):

ATCGAATCATTATACAGAATGGTCTCTTCTTTTTCTCAGCTTTCTGCCTCACGCAGACTTTTGGAAACA
GGGAACAAGTCTATTGGGGAAGCGCCTTAGCTGGCTGGGTATTTCTGGGAACTGTTTAGATAATGCAC
GCTGGCTGAGCAGTACGGCAGCCACTGCTCCAGGTCCTGAAATAAACCTCAGAAAGCCAGGTGCAGT
GATTAGCGGAGACAGTTCCATATTCTCAATGTGGGTTTCTGGCTTTCTGTTGTGGGTGTCTCAGAAGTT
CATTTACTCTTCAGAAATGATTGGGAA

B. Primers:

Binding motif: TTCTGGGAA
mArid1b ChIP-F: TGGAAACAGGGAACAAGTCTATT
mArid1b ChIP-R: CACCTGGCTTTCTGAGGTTTA
Expected PCR size:139bp

Supplemental Figure 7 related to Figure 6: Potential Stat3 binding site and CHIP primers for Arid1b.
(**A**) mouse chromosome 17DNA sequence from 5003400 to 5007000. (**B**) Stat3 binding motif (yellow highlight) and CHIP primers. red: Forward; green: reverse.