Supplementary Methods

Western Blotting

Supplementary Table 1. Primary Antibodies.

Antibody	Source	Host	Dilution	Application
TRA-1-60	Cell Signaling	Mouse	1:200	ICC
TRA-1-81	Cell Signaling	Mouse	1:200	ICC
SSEA-4	Cell Signaling	Mouse	1:200	ICC
OCT4	Cell Signaling	Rabbit	1:200	ICC
SOX2	Cell Signaling	Rabbit	1:200	ICC
NANOG	Cell Signaling	Rabbit	1:200	ICC
Phospho-DARPP-32 (Thr34)	Santa Cruz	Goat	1:100	WB
DARPP-32	Cell Signaling	Rabbit	1:500	WB
Neurofibromin (C)	Santa Cruz (sc-67)	Rabbit	1:250	WB, IHC
Neurofibromin (N)	Santa Cruz (sc-68)	Rabbit	1:250	WB
RAS (Clone RAS10)	Millipore	Mouse	1:1000	WB
SMI-312	Covance	Mouse	1:1000	ICC
α-Tubulin	Life Technologies	Mouse	1:10000	WB

WB, Western Blot; IHC, immunohistochemistry; ICC, immunocytochemistry

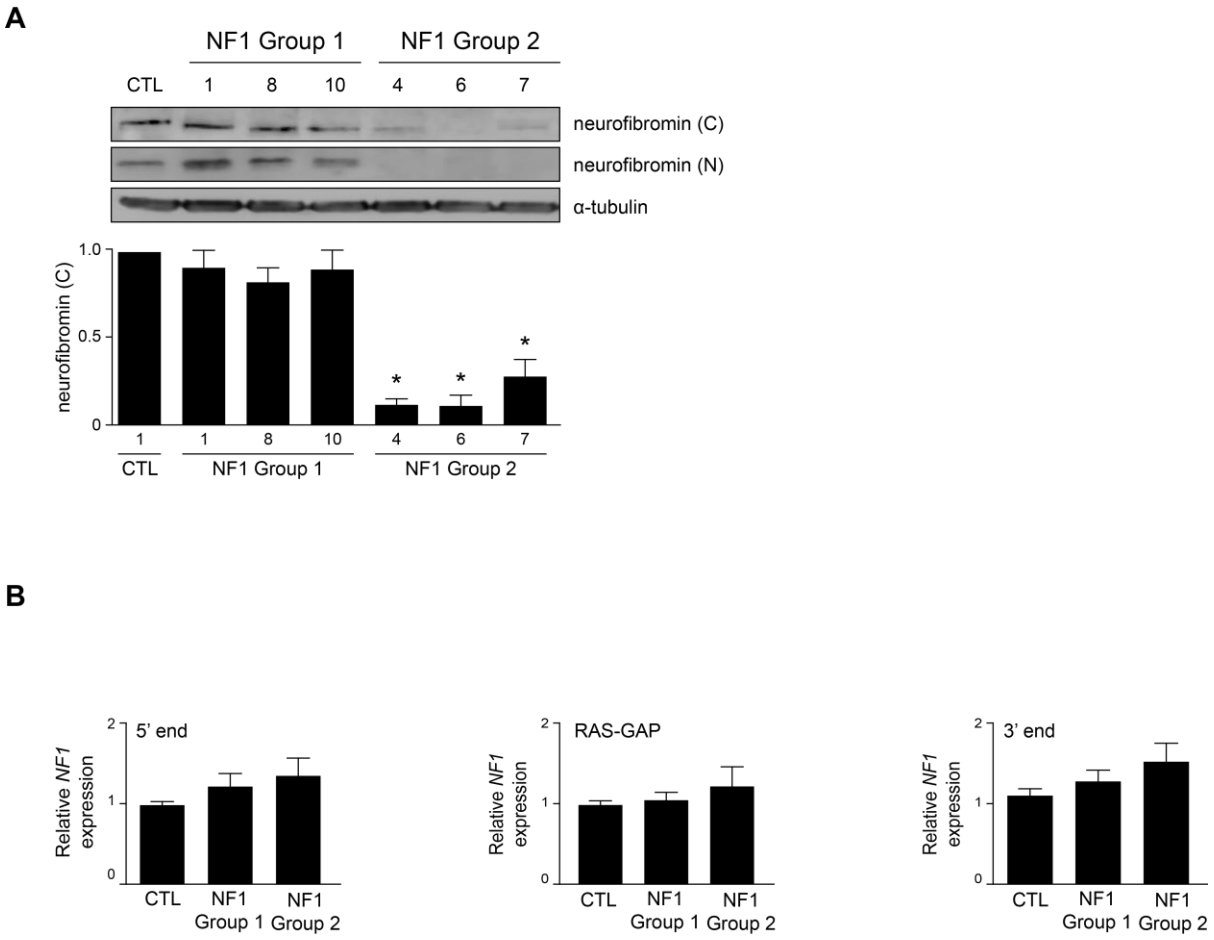
Real-time quantitative reverse transcription-PCR (qRT-PCR)

The expression of *NF1* (NM_001042492) was examined by real-time reverse transcription-PCR (RT-PCR) using SYBR Green detection. Forty nanograms of fibroblast cDNA were used as template for PCR amplification with primers listed in **Supplementary Table 2**. Each reaction was performed in triplicate and was repeated for at least three separate passages of each of the cultured fibroblasts. Bio-Rad CFX Manager 3.1 Software (Bio-Rad Laboratories, CA) was used to convert the fluorescent data into cycle threshold (CT) measurements, and the ΔΔCT method was used to calculate fold expression, using *GAPDH (*NM_002046) as an internal control.

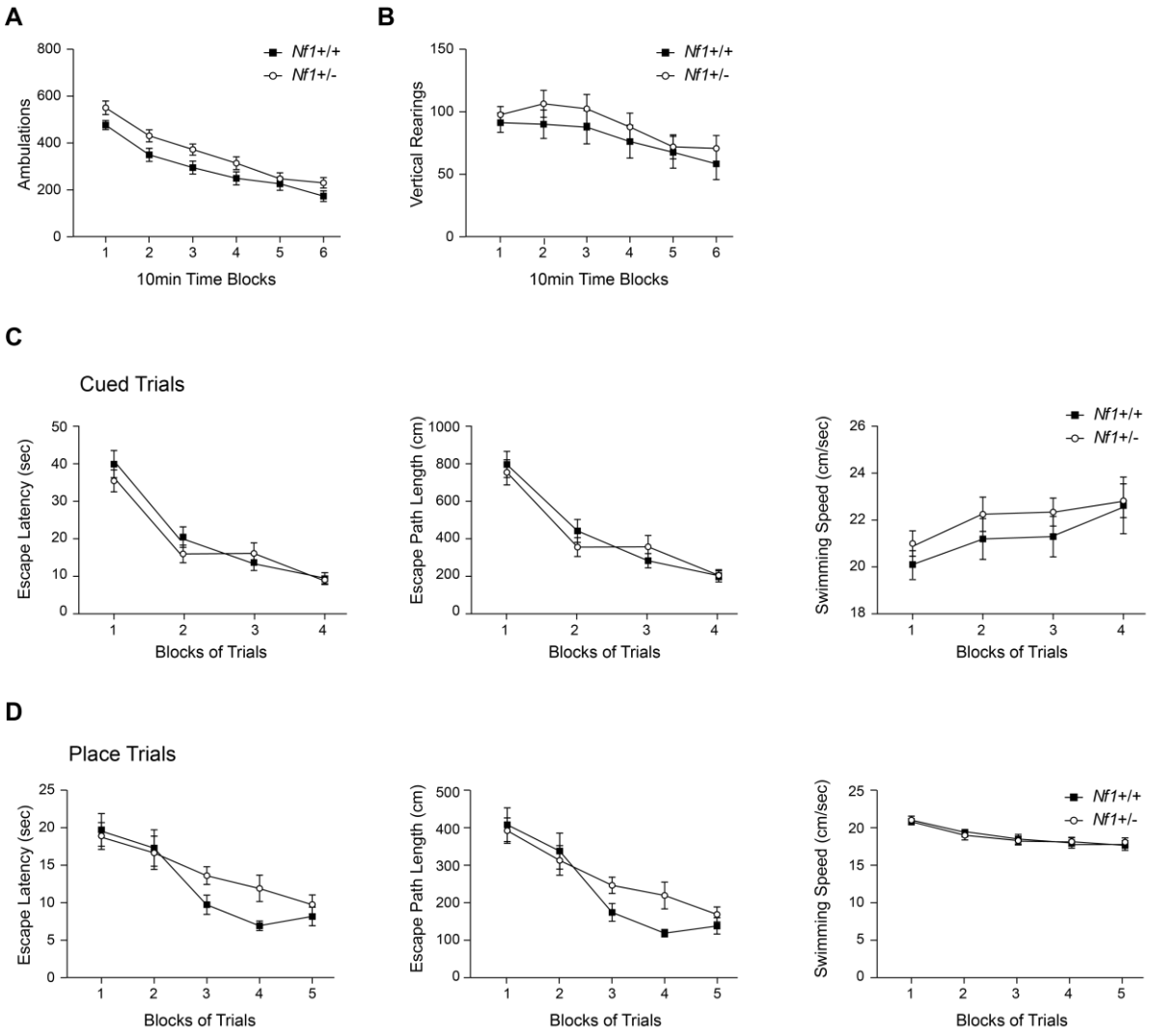
Supplementary Table 2. qRT-PCR Primers.

Gene	Domain	Forward primer	Reverse primer
NF1	5’ (exon 2)	5’-AAAAACAGGACAGCAGAACACAC- 3’	5’-TAGTGAGGCCGCTTATAACCAAA-3’
NF1	RAS-GAP (exon 27)	5’-ATCGGTTTGAGAGATTGGTGGAA-3’	5’-TCATCCCACTGAGAACAAGGAAC-3’
NF1	3’ (exon 52)	5’-AGGCAAGAAATGGAATCAGGGAT-3’	5’-GGTGCTGTTGTGATGAGGAAATC-3’
GAPDH	5’ (exon 1)	5’-ACAGTCAGCCGCATCTTCTTTTG-3’	5’-AATACGACCAAATCCGTTGACTC-3’

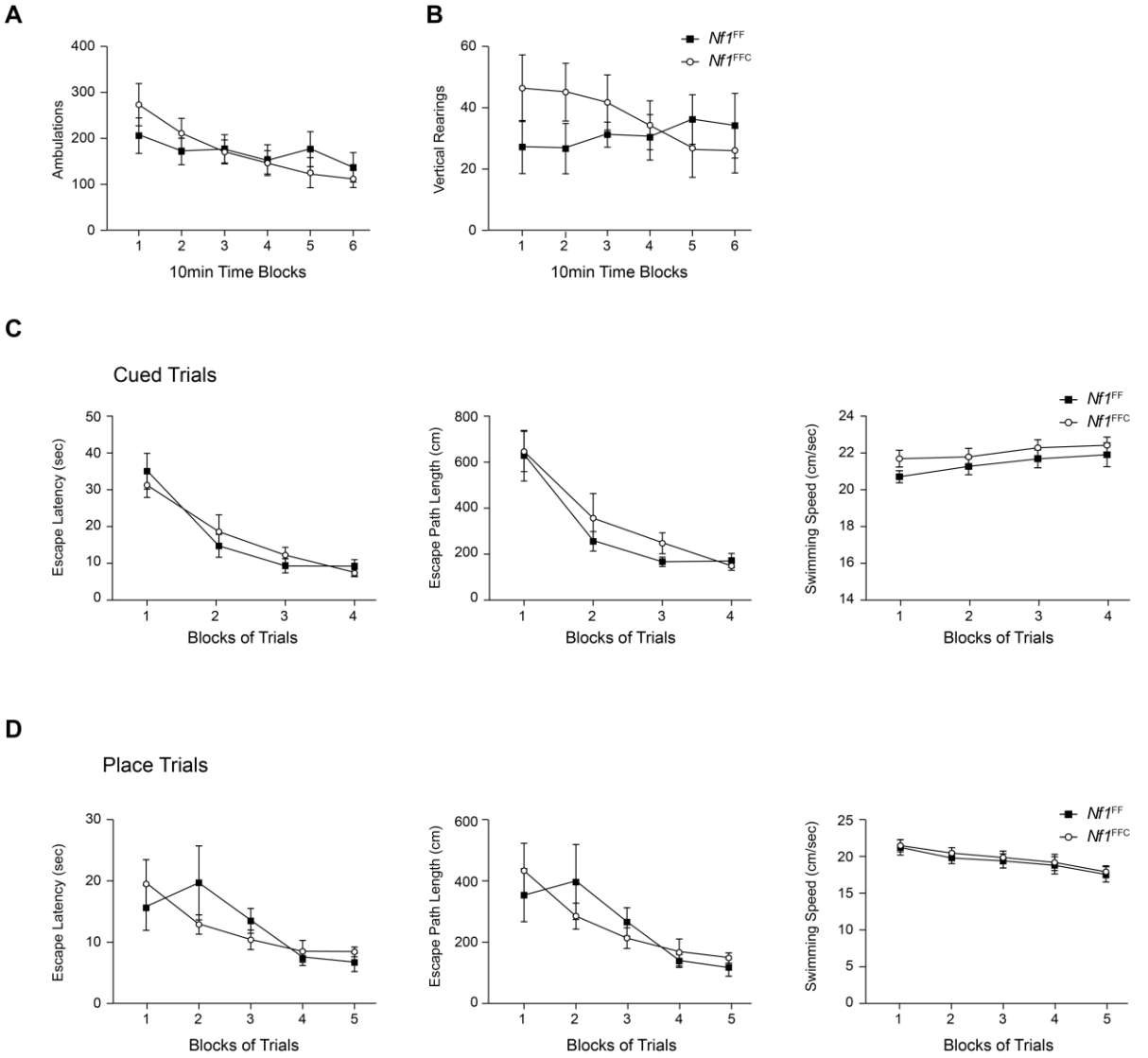
Supplementary Figures



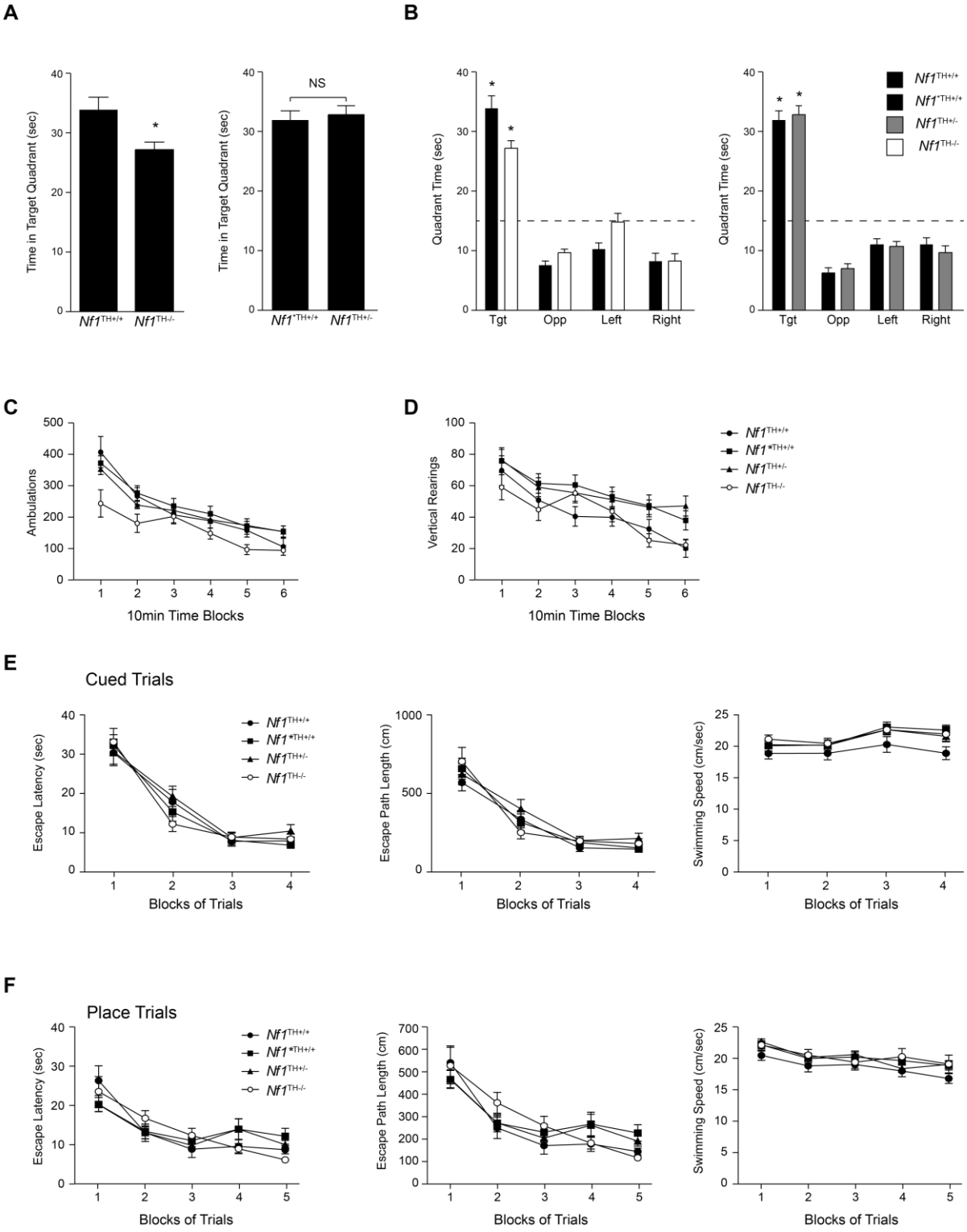
Supplementary Figure 1. Germline *NF1* gene mutations result in different levels of neurofibromin expression in NF1-patient fibroblasts. (**A**) Neurofibromin immunoblot with C- (C) and N- (N) terminal antibodies reveal significantly different protein expression levels only in Group 2 individuals with NF1 relative to controls. Tubulin was used as an internal protein loading control. (**B**) Combined *NF1* real-time quantitative PCR (RNA qPCR) analysis of NF1-patient fibroblasts with primers designed against the 5'end, the RAS-GAP domain and the 3'end of the transcript showed no significant difference relative to control fibroblasts. All quantitative data was normalized to GAPDH levels. All data are represented as means ± s.e.m. (*P<0.0001, one-way ANOVA with Bonferroni post-test).



Supplementary Figure 2. Water maze analysis of *Nf1*+/- mice. (**A**) *Nf1*+/- mice exhibited similar numbers of ambulations to WT controls. (**B**) *Nf1*+/- mice exhibited similar vertical rearing frequency compared to their WT controls (rmANOVA). Escape latencies, path lengths and swimming speeds on the (**C**) cued and (**D**) place trials in the Morris water maze were not significantly different from the WT controls (rmANOVA).



Supplementary Figure 3. Water maze analysis of *Nf1*^{FFC} mice. *Nf1*^{FFC} mice exhibited similar (**A**) ambulations and (**B**) vertical rearings as control mice (rmANOVA). *Nf1*^{FFC} had similar escape latency, path length and swimming speeds on the (**C**) cued and (**D**) place trials in the Morris water maze (rmANOVA).



Supplementary Figure 4. Behavioral analysis of *Nf1*$^{TH+/-}$ and *Nf1*$^{TH-/-}$ mice. (**A**) Time spent in target quadrant is significantly reduced in *Nf1*$^{TH-/-}$ compared to *Nf1*$^{TH+/+}$ mice, but not in *Nf1*$^{TH+/-}$ relative to *Nf1**$^{TH+/+}$ mice (*P=0.014; Student's t-test). The tests were performed separately with no significant differences between the two control groups. As such, all four groups were subsequently

analyzed collectively. (**B**) All *Nf1*TH mice showed spatial bias, spending significantly more time in the target quadrant than the other quadrants during probe trial 2 of the Morris water maze test (*P<0.0001; two-way ANOVA with Bonferroni post-test). *Nf1*$^{TH+/-}$ and *Nf1*$^{TH-/-}$ mice exhibited similar (**C**) ambulations and (**D**) rearings as control mice (rmANOVA). *Nf1*$^{TH+/-}$ and *Nf1*$^{TH-/-}$ mice exhibited similar escape latencies, path lengths, and swimming speeds on the (**E**) cued and (**F**) place trials in the Morris water maze.