

951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971

972 **Supplemental Figure 1. Anxiety phenotype in NF1 mutant miniswine.** 9-10 month old, male
973 *NF1^{+/-ex42del}* miniswine took significantly longer to touch a novel object compared to control animal,
974 though they spent a similar amount of time with that same object (A). When miniswine were
975 exposed to a second novel object, in addition to the first object, the next day, this interaction delay

976 was no longer present (B). Data presented as mean \pm SEM. Outliers removed with ROUT method
977 Q=1%. *p<0.05, Unpaired two-tailed Student's *t*-test. N=5-12.

978

979 **Supplemental Figure 2. Neurofibromas present on miniswine.** Neurofibromas (arrows) on the
980 neck of a 17 month old, male *NF1*^{+/*ex42del*} mutant miniswine showing appearance of the skin (A).
981 Heterogeneous contrast enhancement is seen in the T2-weighted MR of two of the lesions from
982 the same animal (B and D). The pre-contrast LAVA MR show hyperintensity in (C) but not in (E).

983

984 **Supplemental Figure 3. Neurofibroma tissues from an NF1 mutant miniswine.** Serial
985 sections of the cutaneous neurofibroma isolated from an *NF1*^{+/*ex42del*} animal are outlined by arrows
986 (A, B) allowing visualization of the neurofibroma (A) and increased vWF immunostaining (B) in a
987 serial section. Note the increased vWF immunostaining (brown staining) within the neurofibroma
988 (C, arrows). Trichrome stain (A) and vWF immunohistochemistry with hematoxylin counterstain.
989 Bar = 313 (A, B) and 63 (C) μ m, respectively.

990

991 **Supplemental Figure 4. Histopathological features of neurofibromas from an NF1 mutant**
992 **miniswine.** Sections of the cutaneous tumors under polarized light show the normal "white"
993 dermal collagen fibers that distinguished the dark (black) regions of neurofibromas (white arrows)
994 in the dermis (A, B). Within the neurofibromas, uncommon aggregates of eosinophils (C, inset)
995 and mast cells (D, black arrows and inset) were detected. Sirius red (A-C) and toluidine blue
996 stains (D). Bar = 627 (A), 125 (B), 62 (C) and 20 (D) μ m, respectively.

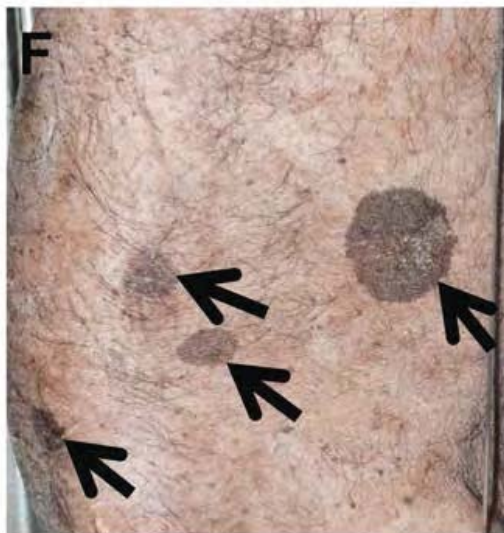
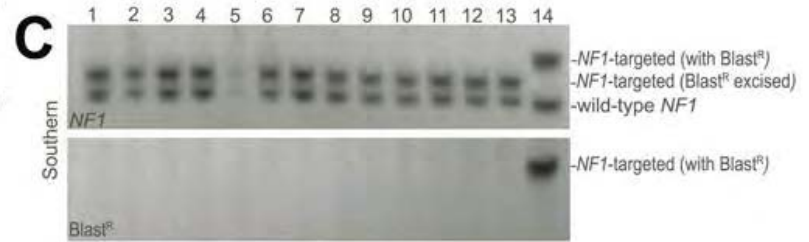
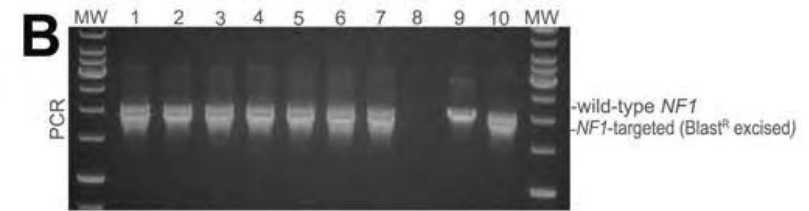
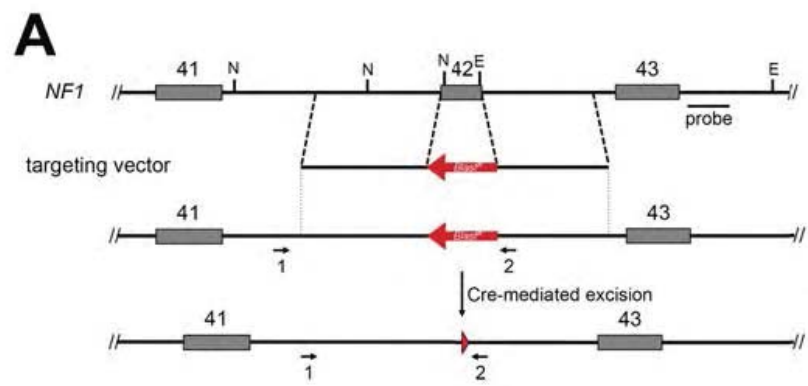
997

998 **Supplemental Table 1.** Primers used for sequencing, amplify NF1 genomic region, Targeting
999 Vector Construction, rAAV Production, and Cell Screening. Primers listed from 5' to 3'.

1000

Primers used to amplify <i>NF1</i> genomic region:
pNF1-1F: AATTTACTGAGCAAGCCATTTCTTTTGCTG

pNF1-4R: GATCTTCAGGTATACTTTACCCAGCTTTAC
<u>Primers used for sequencing:</u>
pNF1seq5F: AAATGGGGAGAGCACTTTAATTAT
pNF1seq6F: GGTCAGCTGCCTATAATCTTCTGT
pNF1seq7F: GATTTTAGGGATATAGTCATTTCCG
pNF1seq8F: GCTATGAGGTTTGAGAATTAAGAA
pNF1seq9R: CCTCCTACGGTGAATATAAGGTCT
pNF1seq10F: AAGAGAAATGGAGTTCTGTCATGG
pNF1seq11F: AAGATAATATATATATATATCCACT
pNF1seq12F: CTGTTGCCATAGGTCTATTTTGCA
pNF1seq13F: GAGATCAGATGATACACCAATAGT
pNF1seq14R: GCTGCTGGCCAACGCTACAGCAGA
pNF1seq15F: TAGTACCTCCTTGATGCAGATATT
pNF1seq16F: ATGTGGGTCGGTGTGCTGCAGGC
pNF1seq17F: TGGACTGCTCCTTGAAAGTTTGTT
AAVF1: CTCTAGCTATAGTTCTAGTGG
AAVR2: GTGGTATGGCTGATTATGATC
pLDLR1: GGAGCATGCGCTTTAGCAG
p1338-puro B-F (short): CGCGCTGTTCTCCTCTTCC
p1338-puro B-R (short): GAAGGCACAGTCGAGGCTG
ScreenF (NeoR): AGACGTGCTACTTCCATTTGTCAC
<u>Primers used for Targeting Vector Construction:</u>
pNF1XhoI5armF28: AGTGATAGATGGGAACTCGAGGAA
pNF1SbfI5armR29: ATTCAATACCTCCTGCAGGAATACACATTA
pNF1SbfI3armF31: GGGAGCCTTGGGCAGGTCCTGCAGGCGCTG
pNF1Hind3armR33: TGTCTAGTAAAGCTTCGAGGAGGTCT
<u>Primers used for rAAV Production:</u>
pNF1Not46R: AGCATATGGAAGTTCCCAGGCGGCCGCTCTAATCGG
pNF1Not47F: TTCGAAATTGGCGGCCGCACAATTTCTCTAGAA
<u>Cell Screening Primers:</u>
Screen F (NeoR): AGACGTGCTACTTCCATTTGTCAC
LDLR-Exon5R1: AGCACTGGAACTCGTCAGG
<u>Primers used to detect excision of the Selectable Marker:</u>
pNF1seq26F: AGATCAACAGTAACTTGGGGCATG
pNF1Screen50R: TAGGTATCAGATAAATATCTGCATCA
<u>Primers used to amplify the Southern Blot Probes:</u>
pNF1probe52F: TACTAGACAGTTTCATCAAGACCA
pNF1probe53R: CTTTGCTAATGCCAAACAGTAGGT
BlastCD-F: ATGAAGACCTTCAACATCTCTCAG
BlastCD-R: GTTCCTGGTGTACTTGAGGGGGAT
<u>Primers used for Genotyping Offspring of a Heterozygous Cross:</u>
pNF1shorGen72F: CATATAATTTTTGTGACCAT
pNF1shorGen73R: TAAATATCTGCATCAAGGAGG

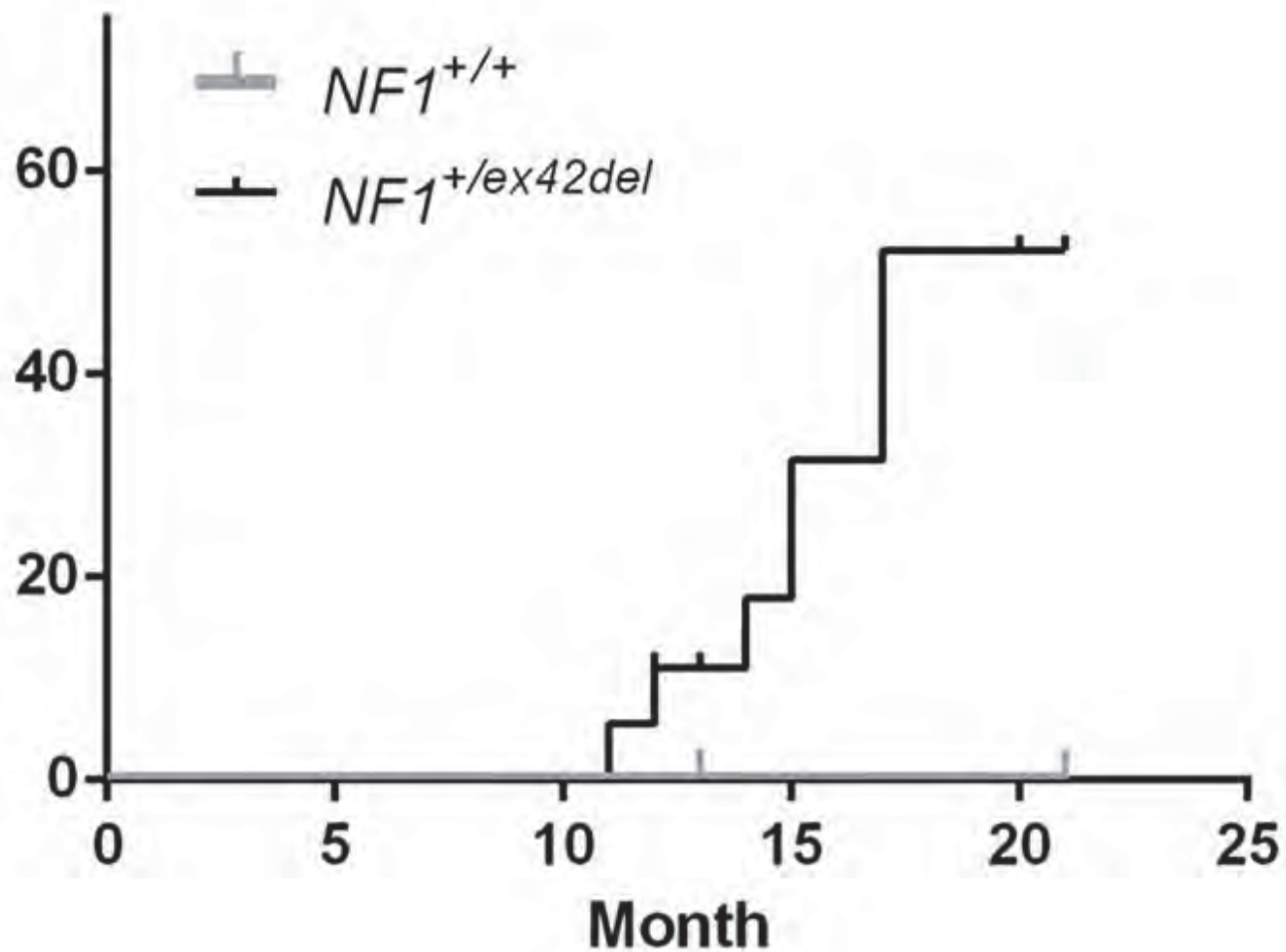


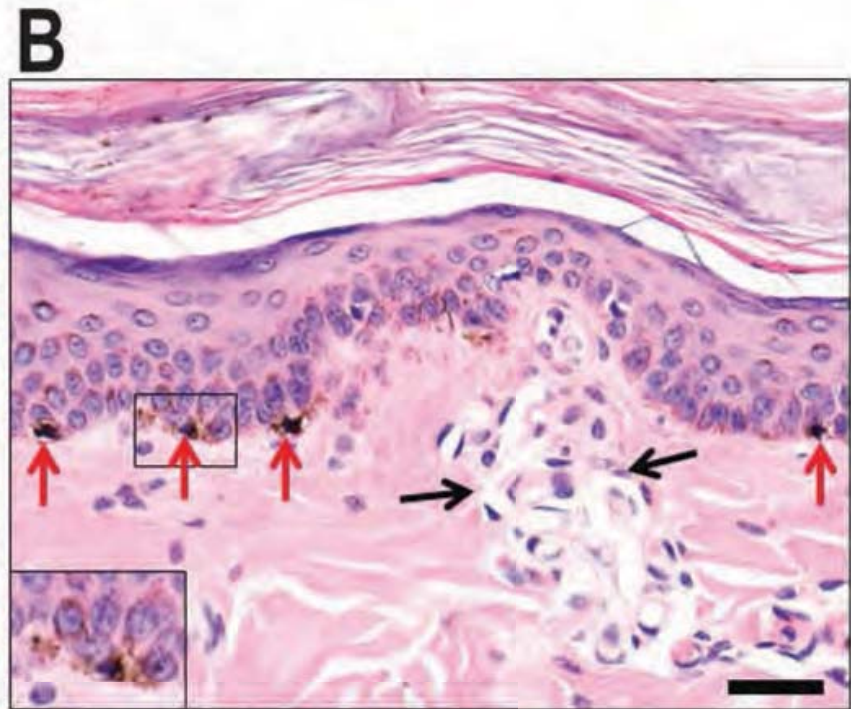
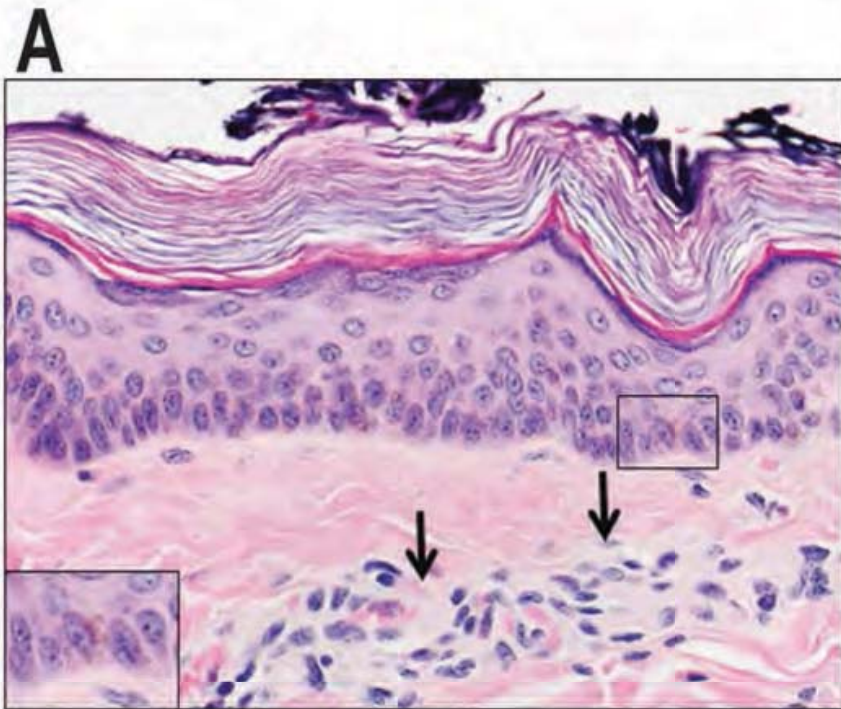
A

	Café au Lait Spots Present	Tumor Lesion Present	Initial T-maze Learning Deficit
<i>NF1^{+/+}</i>	0/6	0/6	0/5
<i>NF1^{+/Δex42del}</i>	18/18	8/18	3/7

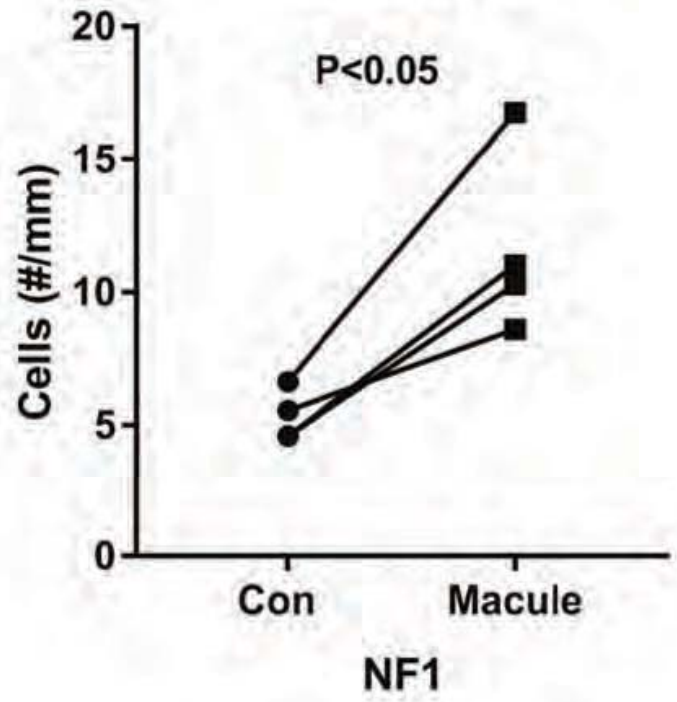
B

Observed Tumor Formation,
Percent of Animals

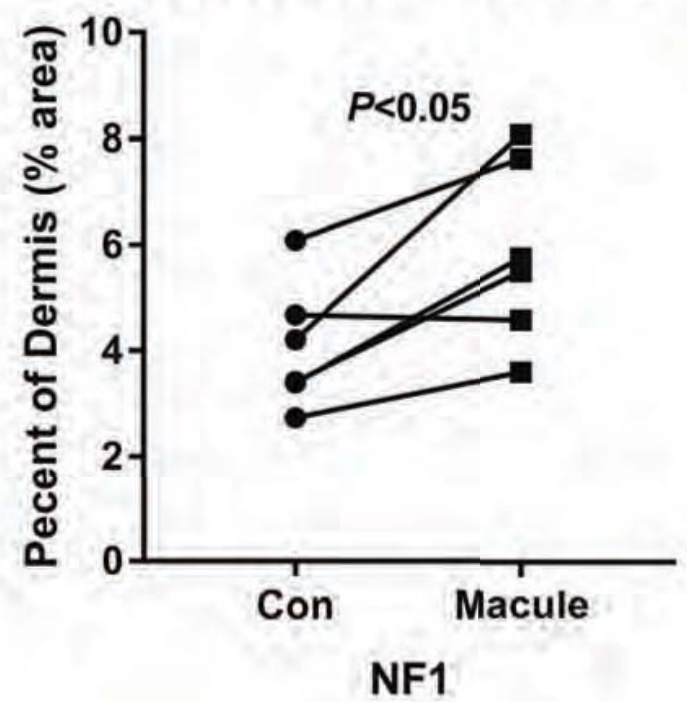


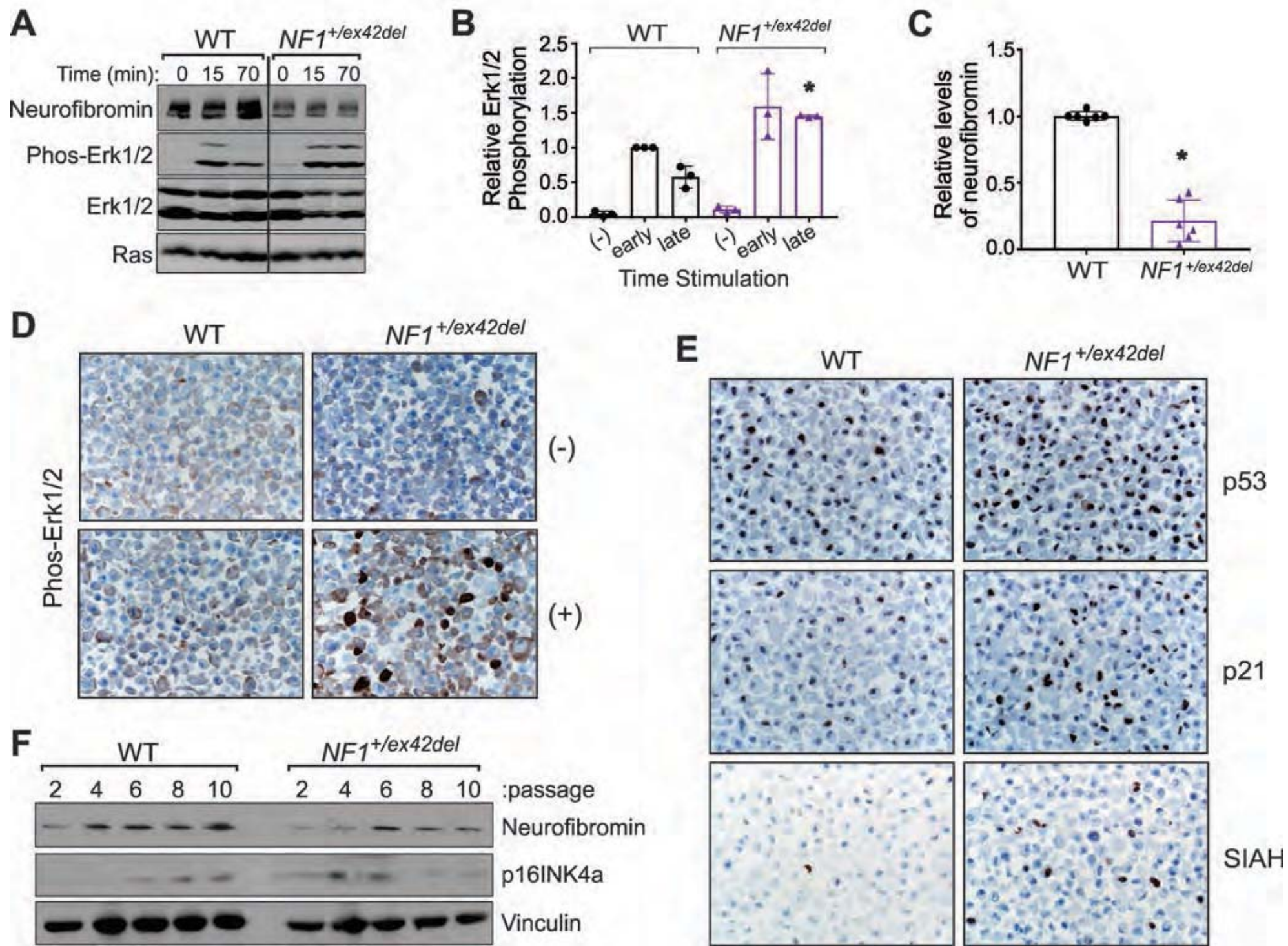


C Heavily-Pigmented Melanocytes

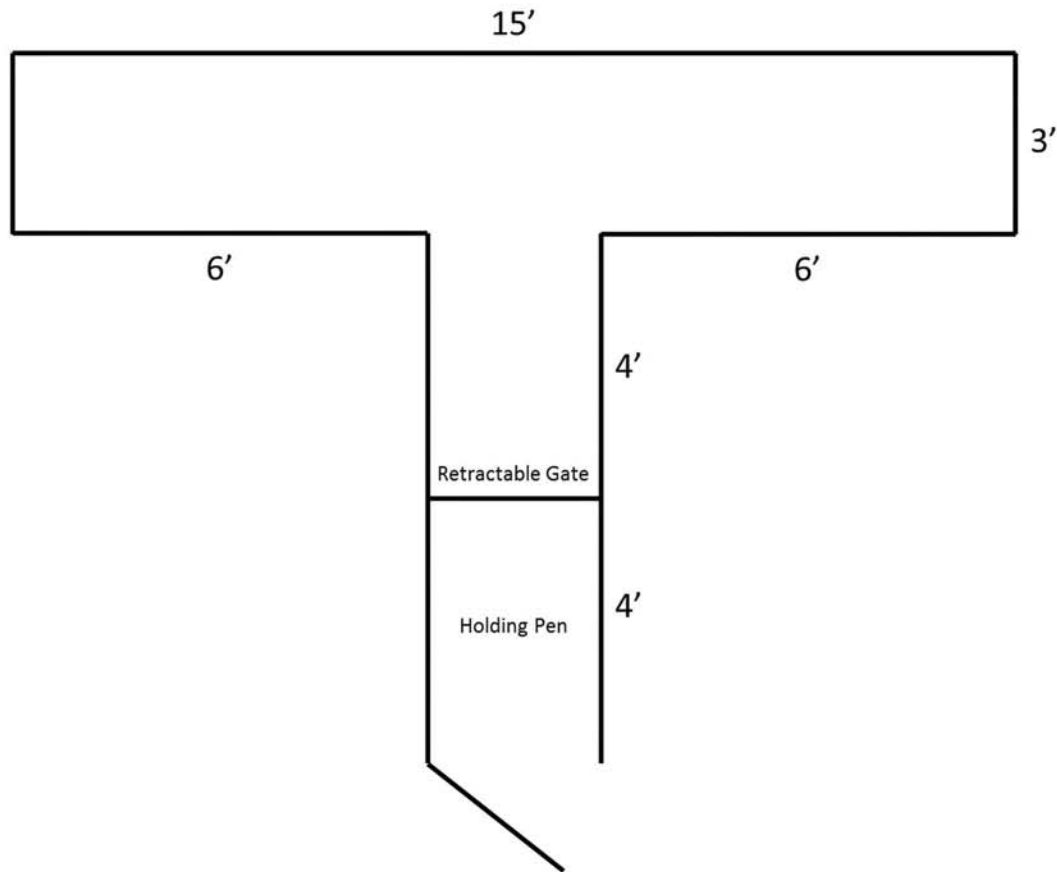


D Neurovascular Networks

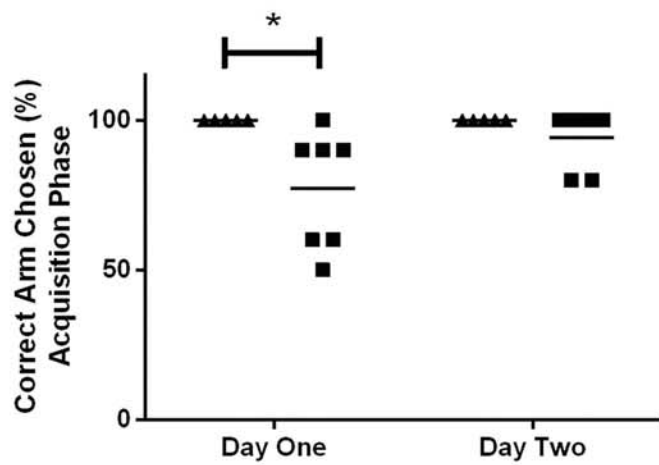




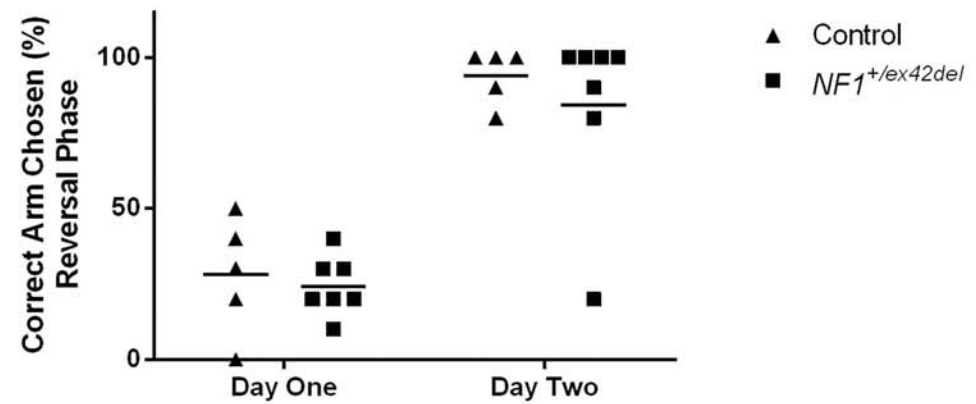
A

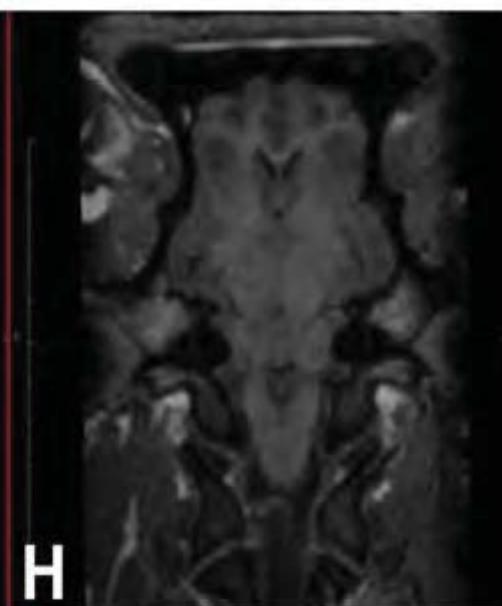
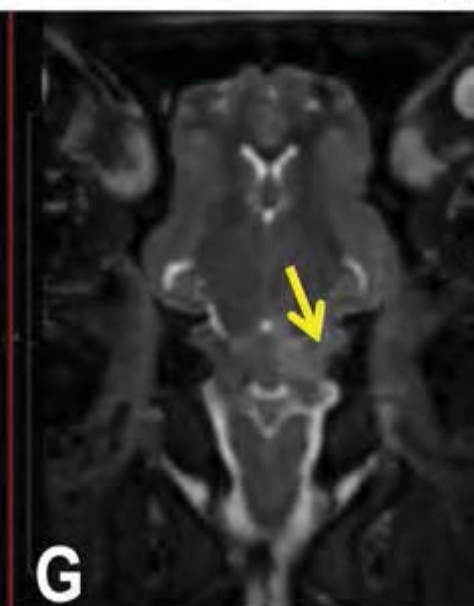
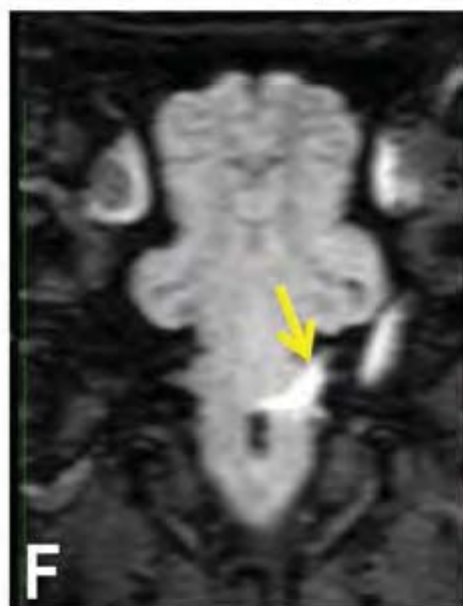
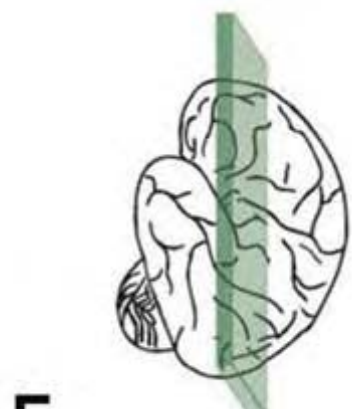
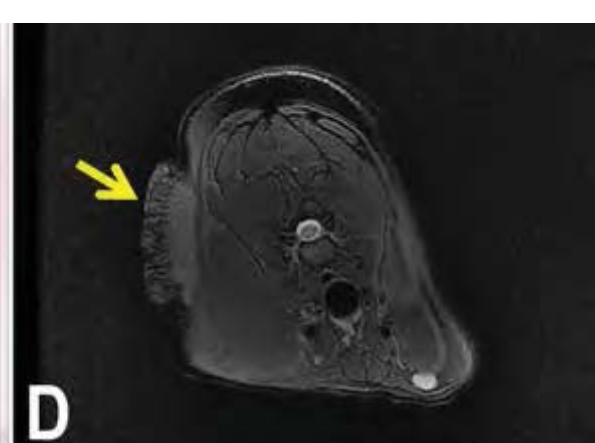
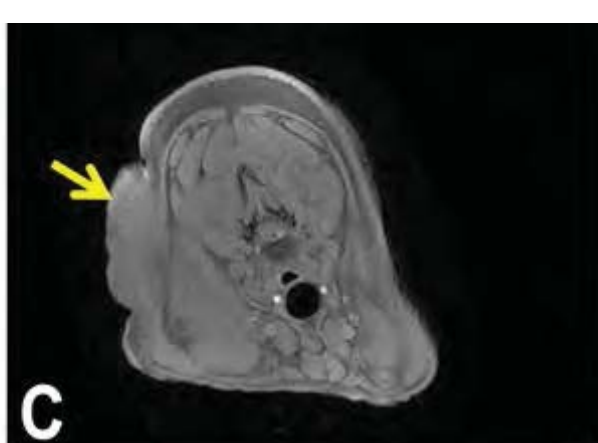
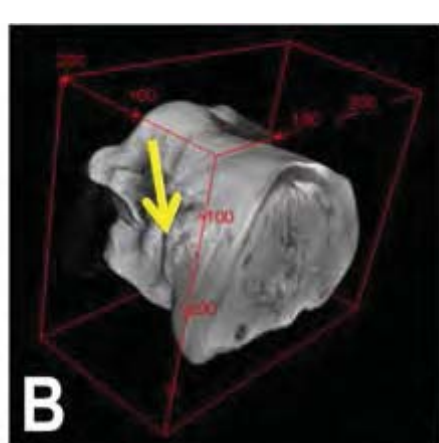
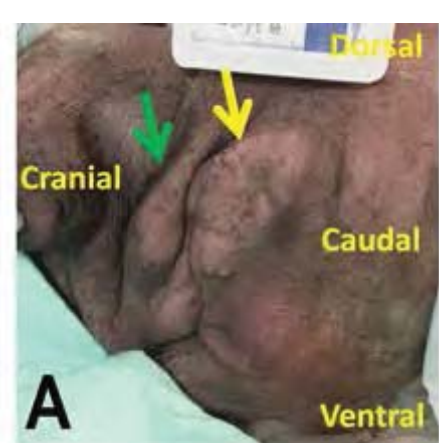


B



C



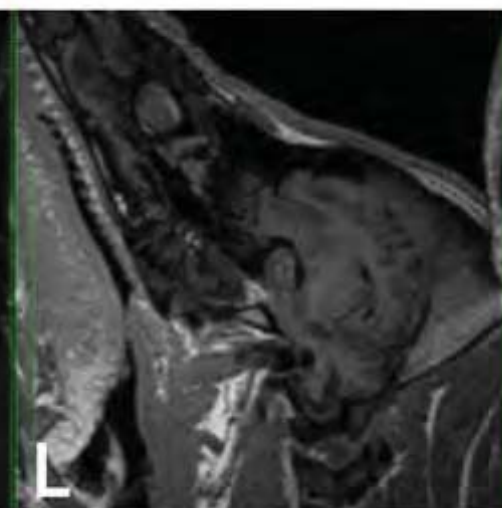
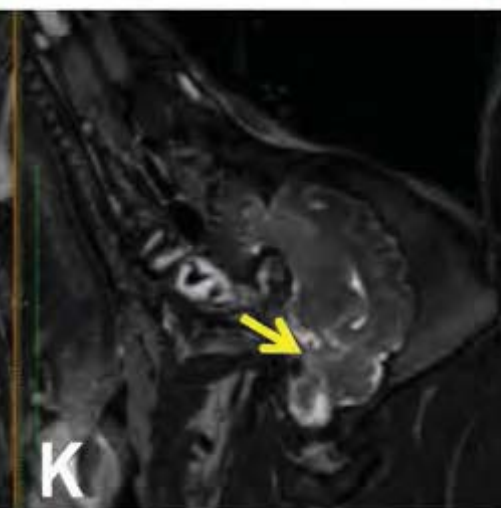
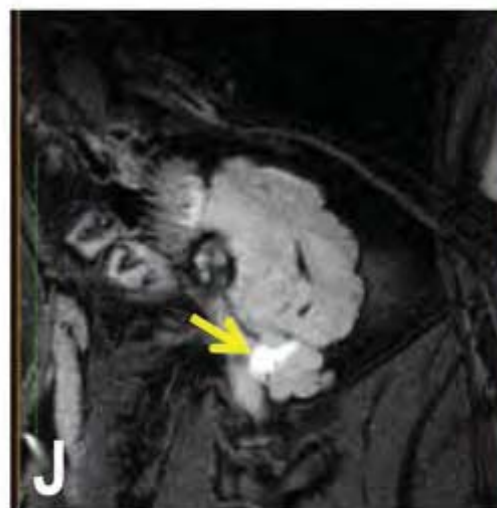


E

F

G

H



I

J

K

L

