Figure S1. Related to Figure 1

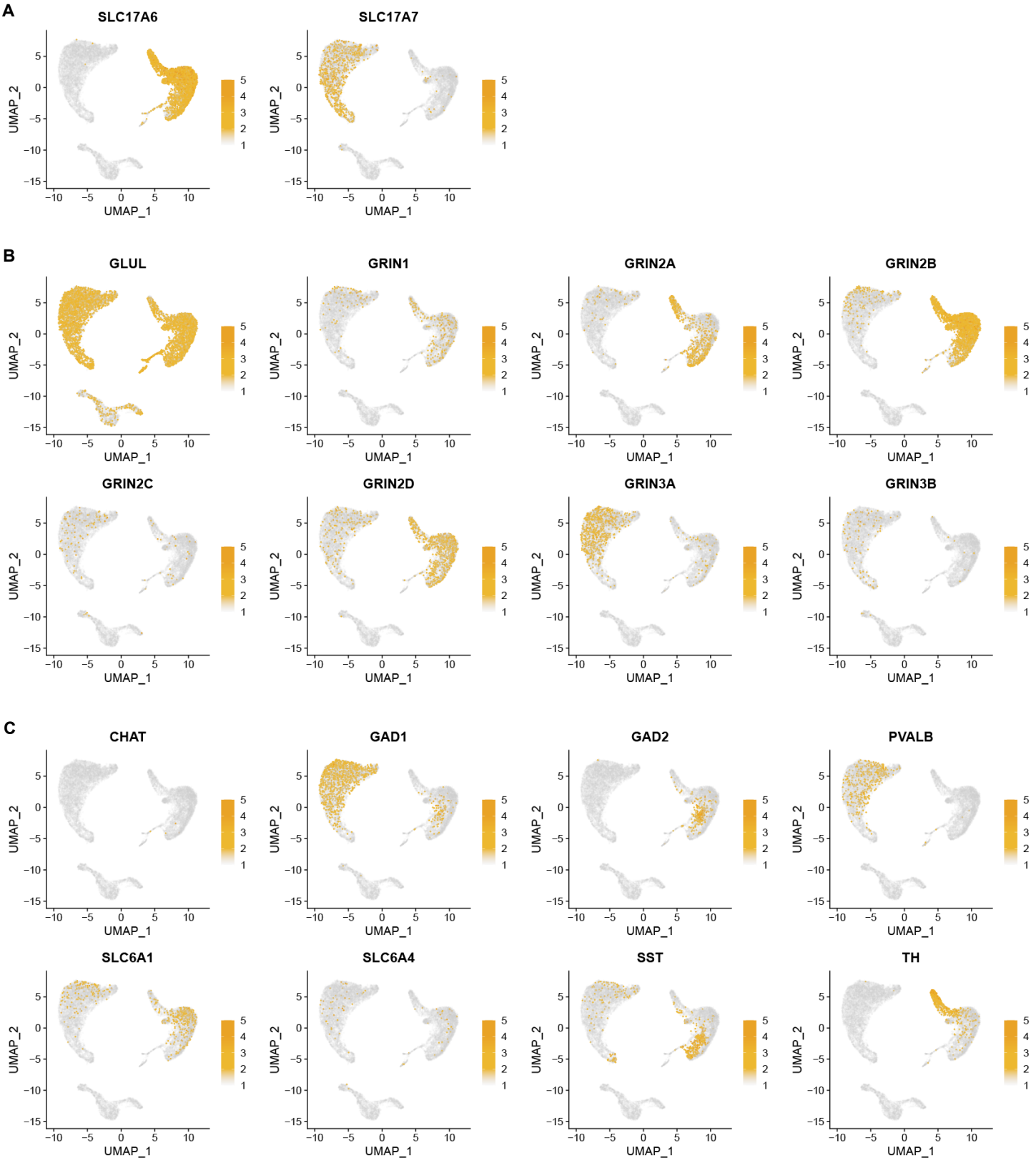

Figure S1. Single cell RNAseq subtyping, Related to Figure 1

(A) UMAP of the glutamatergic markers SLC17A6 (VGLUT2) and SLC17A7 (VGLUT1) across cell populations.

(B) Additional glutamatergic marker expression including GLUL, and the NMDA receptor subunits GRIN1, GRIN2A, GRIN2B, GRIN2C, GRIN2D, GRIN3A, GRIN3B.

(C) Expression patterns of non-glutamatergic neuronal lineages, including the cholinergic marker CHAT, inhibitory markers GAD1, GAD2, PVALB, SLC6A1, SST, serotonergic marker SLC6A4, and dopaminergic marker TH.

See also Figure 1.

Figure S2. Related to Figure 2

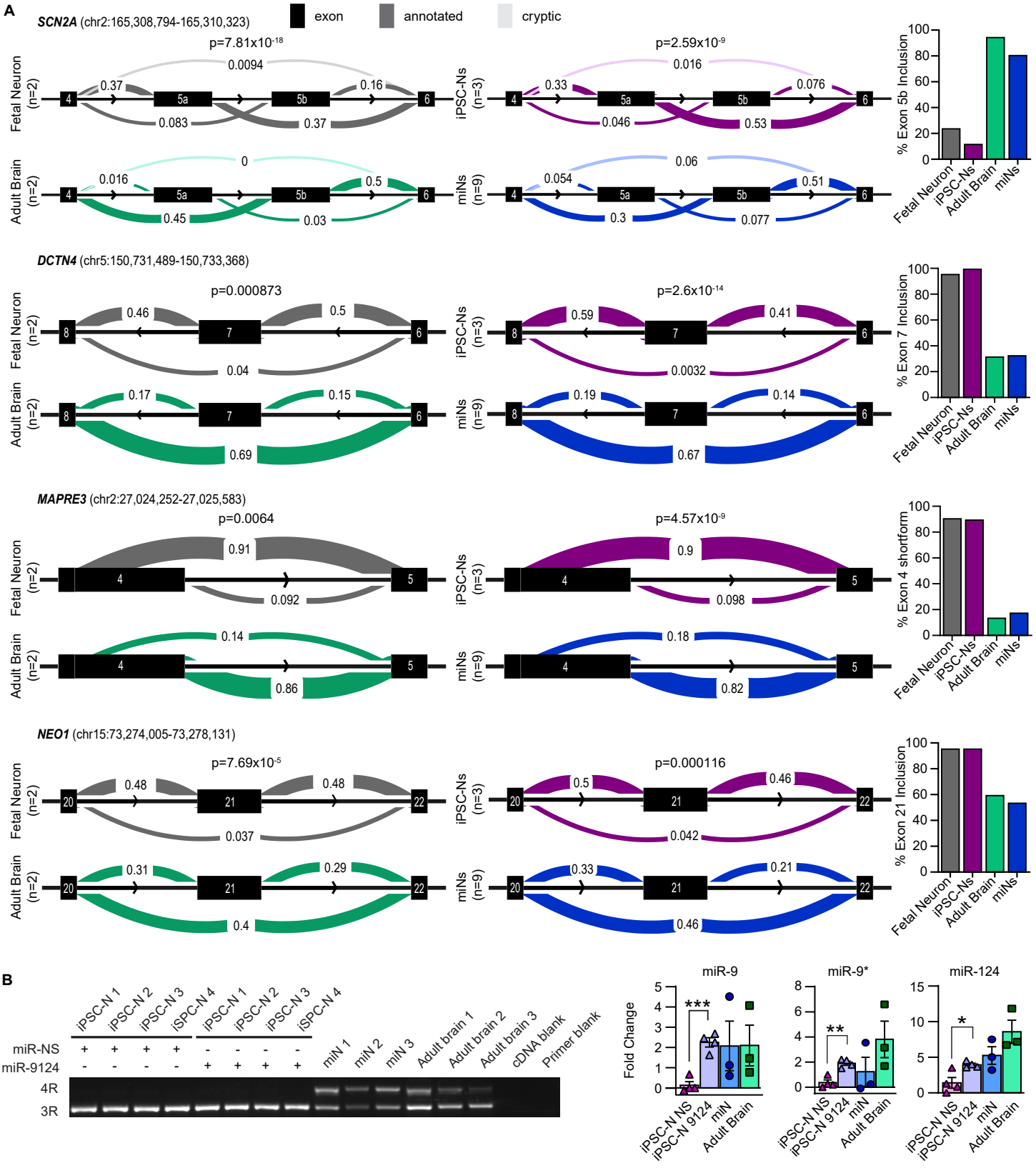

Figure S2. Age-associated splicing events in miNs and 4R expression independent of miRNA over-expression, Related to Figure 2

(A) Differential splicing events between fetal neurons and iPSC-Ns compared to adult brain and miNs. Examples include SCN2A, DCTN4, MAPRE3, and NEO1.

(B) Left, semi-quantitative PCR of 3R and 4R tau isoforms in differentiated iPSC-Ns with overexpression of either nonspecific miRNAs (miR-ns) or miRNAs-9/9*-124 (miR-9124) demonstrating no expression of 4R tau in either condition. Right, qPCR validating the overexpression of miRs-9, -9*, and -124.

See also Figure 2 and Tables S1 and S2

Figure S3. Related to Figure 4

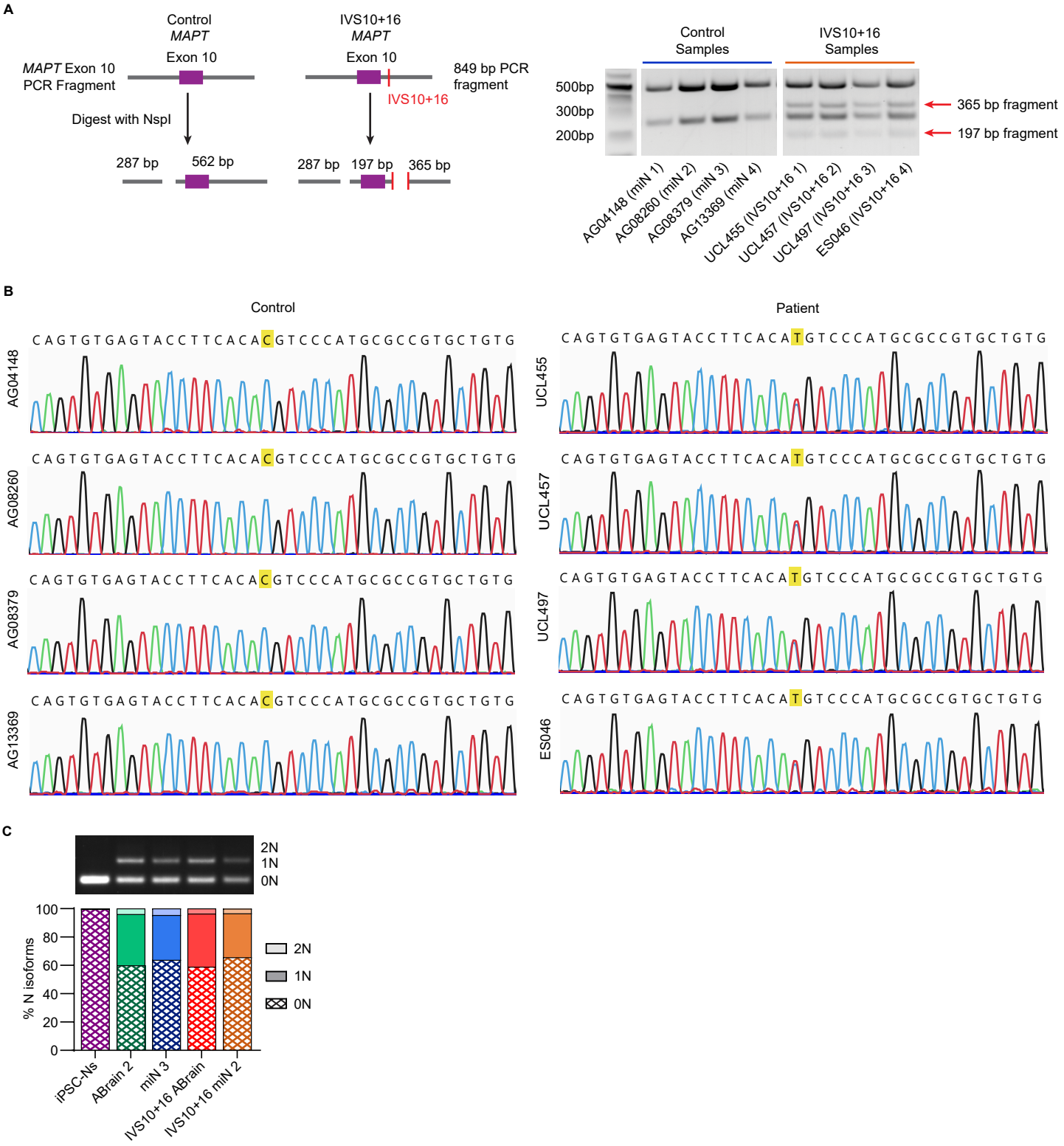

Figure S3. Confirmation of mutational status in control and IVS10+16 fibroblasts and comparison of N isoform expression, Related to Figure 4

(A) Diagram explaining restriction digest genotyping for IVS10+16 C>T mutation, which creates a novel NspI site, as previously published (Hutton et al., 1998). PCR fragment is generated from genomic DNA and digested with NspI. Control samples produced two bands, whereas patient samples had four, corresponding to heterozygous status.

(B) Sanger sequencing of IVS10+16 and surrounding region in MAPT for all lines confirms the presence of IVS10+16 C>T mutation in patient samples, where two peaks were present at the 10+16 location representing C and T. This mutation and no other MAPT mutations were present with exon 10 or in the known intronic mutation sites.

(C) Semi-quantitative PCR of N isoforms between fetal, healthy, and IVS10+16 samples. No significant change was detected.

See also Figure 4.

Figure S4. Related to Figure 6

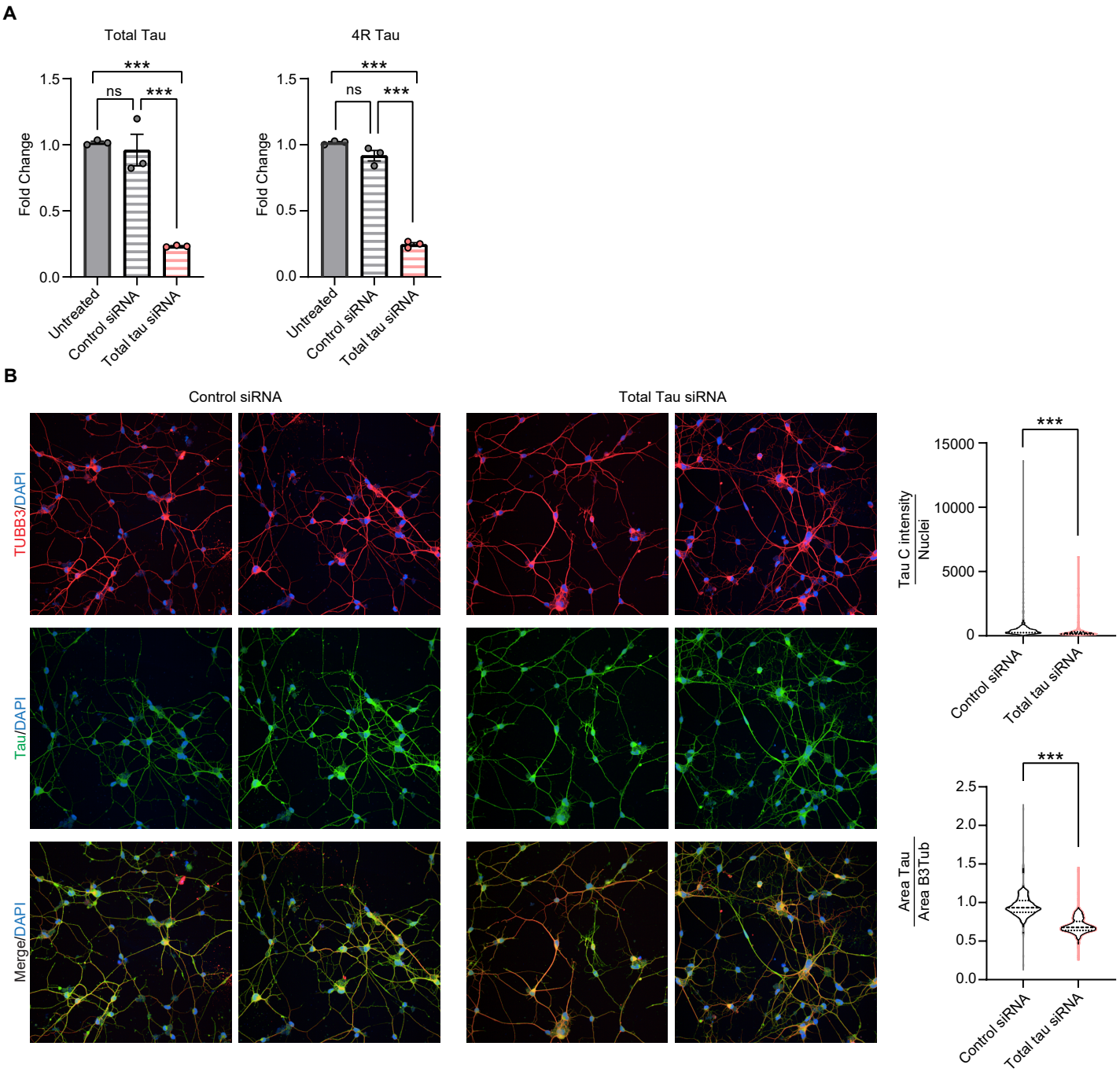

Figure S4. siRNA against total tau shows reduction in both mRNA and protein levels, Related to Figure 6

(A) qPCR of control miNs either untreated (solid grey), treated with control siRNA (grey stripe), or total tau siRNA(pink stripe) for their total tau and 4R tau expression relative to untreated. ***p < 0.001.

(B) Immunocytochemistry of PFA-fixed control miNs treated with control siRNA (grey) or total tau siRNA (pink) show a reduction in total tau fluorescent intensity compared to both total nuclie and total TUBB3 area. N=380-395 images per condition. Nuclei count: Control siRNA=9982; Total tau siRNA=12362. ***p < 0.001.

See also Figure 6.