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Supplemental Information

Transplanted human neural stem cells rescue phenotypes in zQ175 Huntington's disease mice and innervate the striatum

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Figure S1

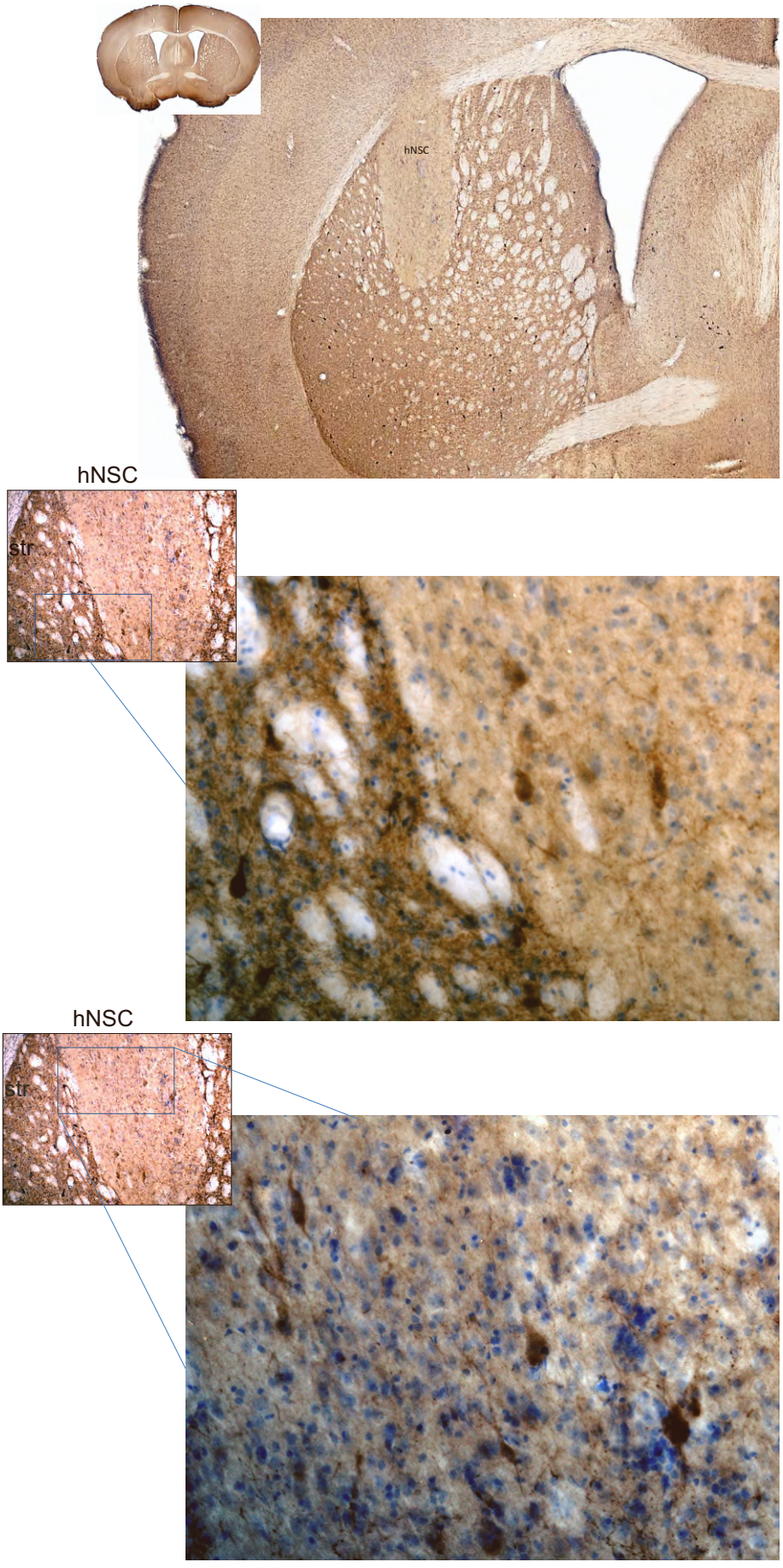


Figure S1. IHC for ChAT demonstrated that some hNSCs express the cholinergic marker. 5x mag. of hNSCs in zQ175 showing overall implant site. 10X mag. then box indicating 20x mag. showing area in and around implant site that has DAB positive (brown) ChAT expressing cells.

Figure S2

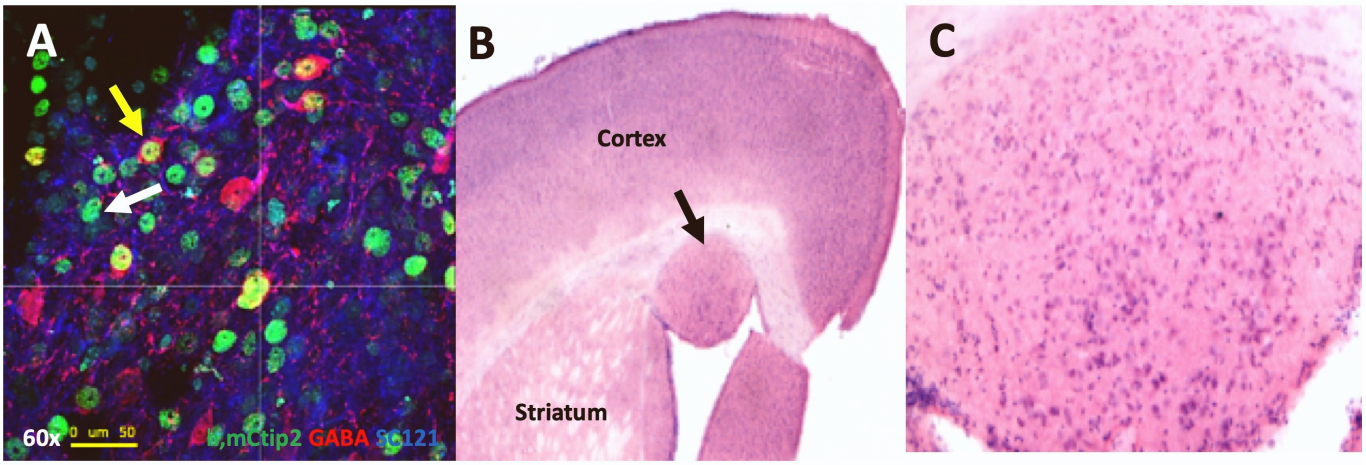


Figure S2. IHC for Ctip2 and GABA demonstrated that some hNSCs express the MSN and inhibitory markers. H&E on nodule. A. 60x image shows hNSCs (SC121, blue) expressing the MSN marker Ctip2 (green) and co-localization with the inhibitory neuronal marker GABA (red). **B.** 4x mag. and **C** (20x) showing H&E stains. A small nodule of cells that migrated away from the initial injection track are shown (at arrow, enlarged in L). Review from pathology included the comments that cytologically, the cells in the nodules are mostly well differentiated cells, with lots of large, mature-looking neurons.

Figure S3

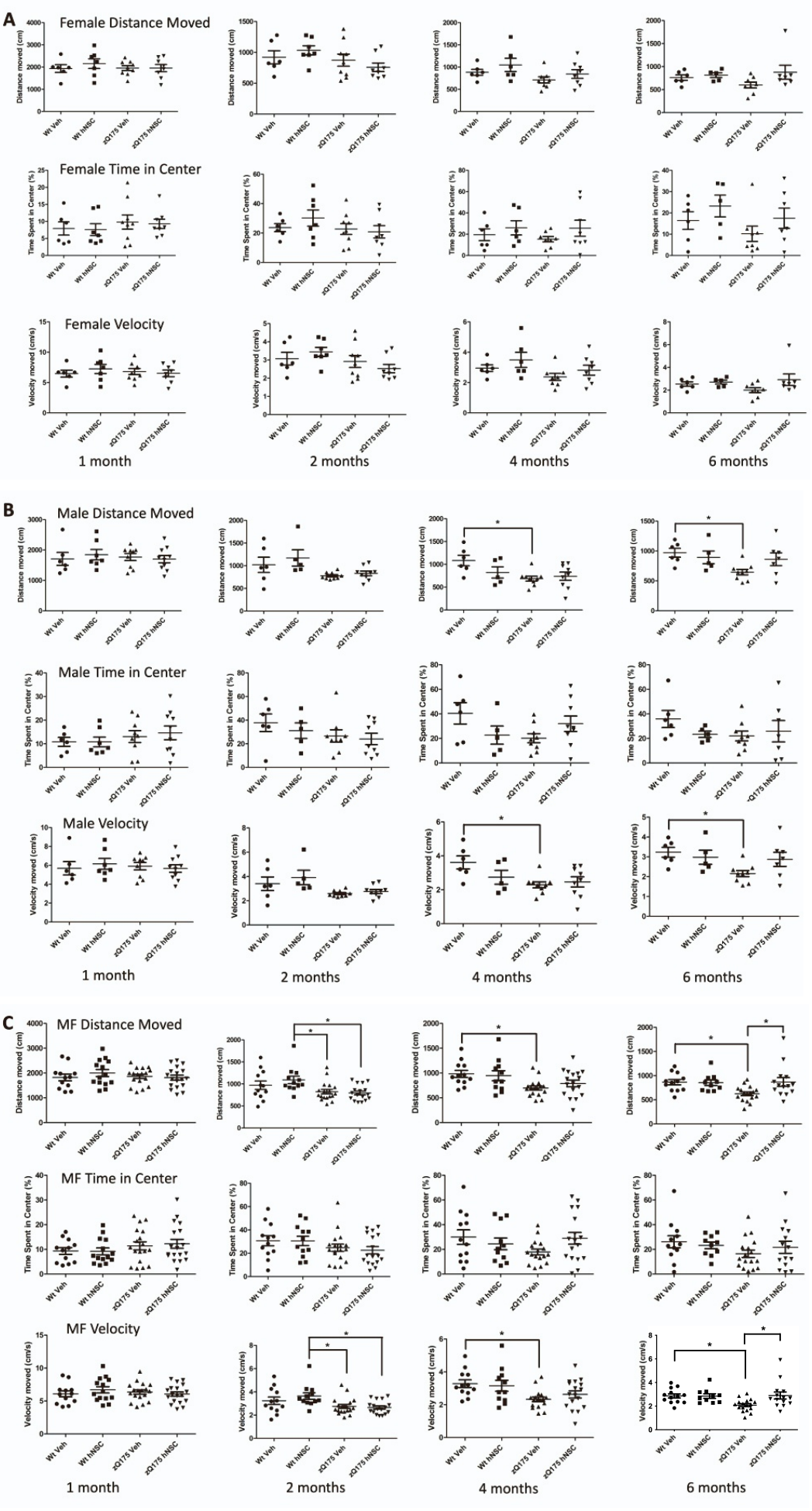


Figure S3. Open Field behavior. Total distance traveled in the open field 6 months post-implant. Mice were subjected to the open field and total distance in centimeters of their respective tracks were combined and statistically analyzed to visualize any differences in ambulation. Time spent in center was also measured. In addition, velocity traveled in centimeters/sec of their respective tracks were combined and statistically analyzed to visualize any differences in time of ambulation. **A.** Females **B.** Males and **C.** Males and Females combined. Groups for open field at 1 month included: 10 male zQ175 hNSC, 8 female hNSC, 9 male zQ175 Veh, 9 female zQ175 Veh, 7 male Wt hNSC, 7 female Wt hNSC, 6 male Wt Veh, 6 female Wt Veh. Groups for open field at 6 months included: 7 male zQ175 hNSC, 7 female hNSC, 9 male zQ175 Veh, 8 female zQ175 Veh, 5 male Wt hNSC, 5 female Wt hNSC, 6 male Wt Veh, 6 female Wt Veh. Results are expressed as the mean ± S.E.M with one-way ANOVA Bonferroni post hoc test: *In order of graphs p=0.03, p=0.04, p=0.03, p=0.04, p=0.01, p=0.01, p=0.01, p=0.01, p=0.01, p=0.01.

Figure S4

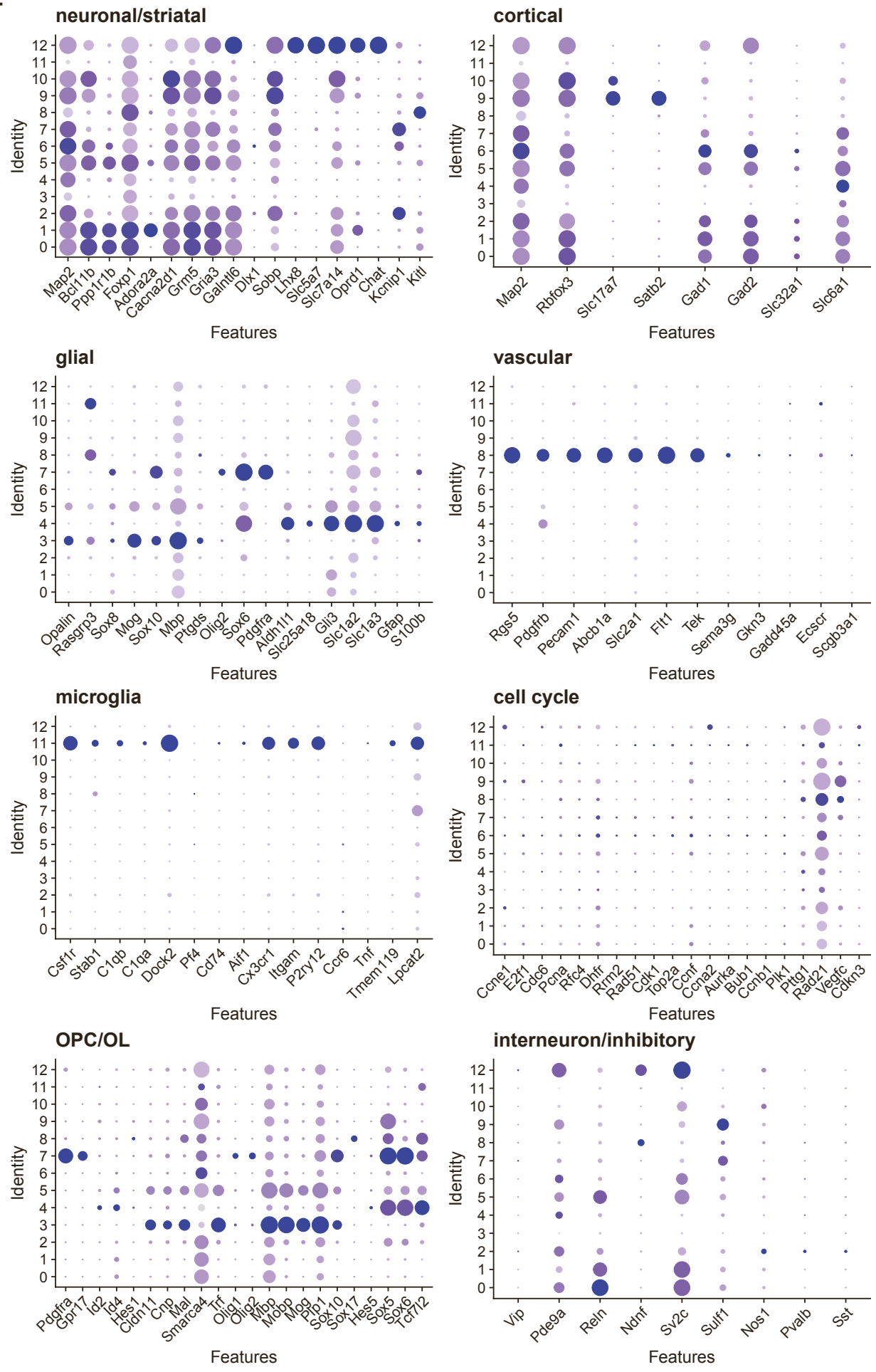
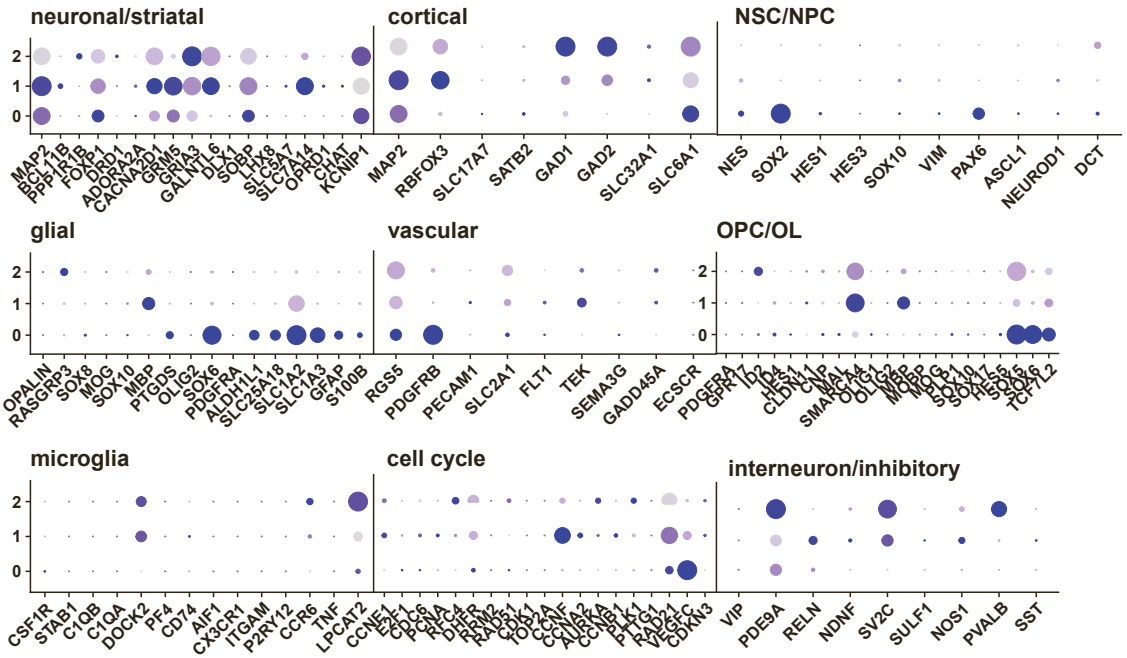


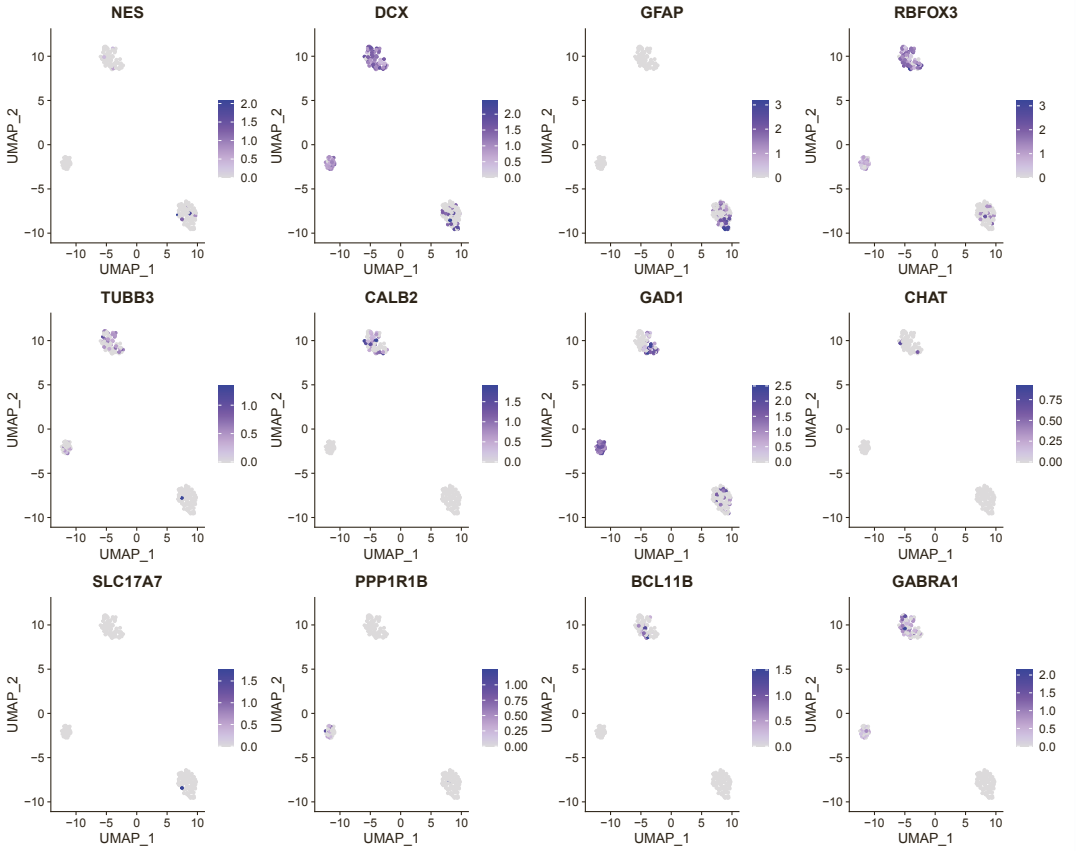
Figure S4. snRNAseq of hNSC Transplants marker expression for mouse cells. Dot plot visualization showing the expression levels of well-known representative cell-type[1]enriched marker genes in the 38,892 mouse nuclei from 13 clusters.

Figure S5

A



B



C

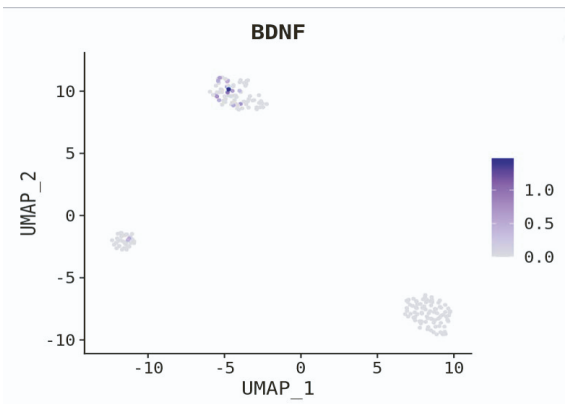
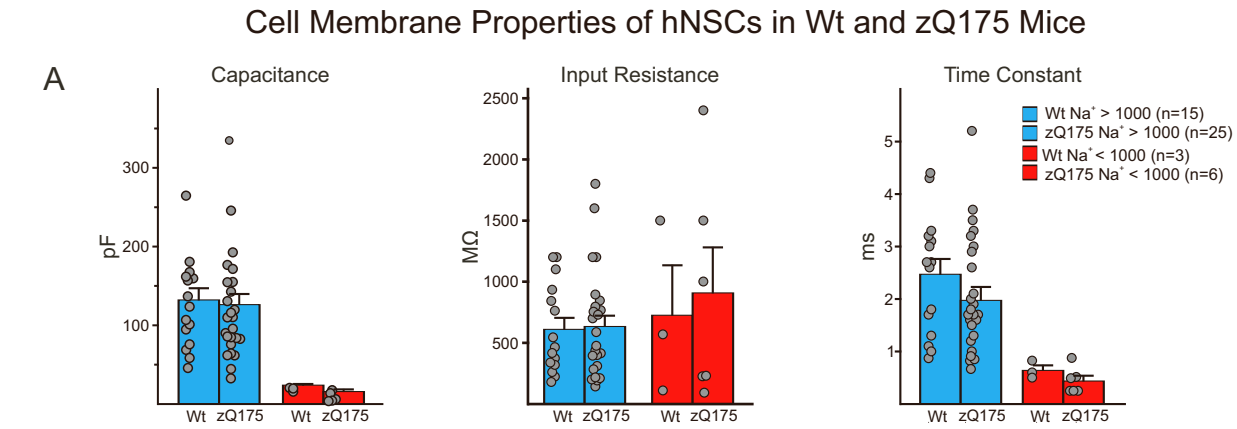
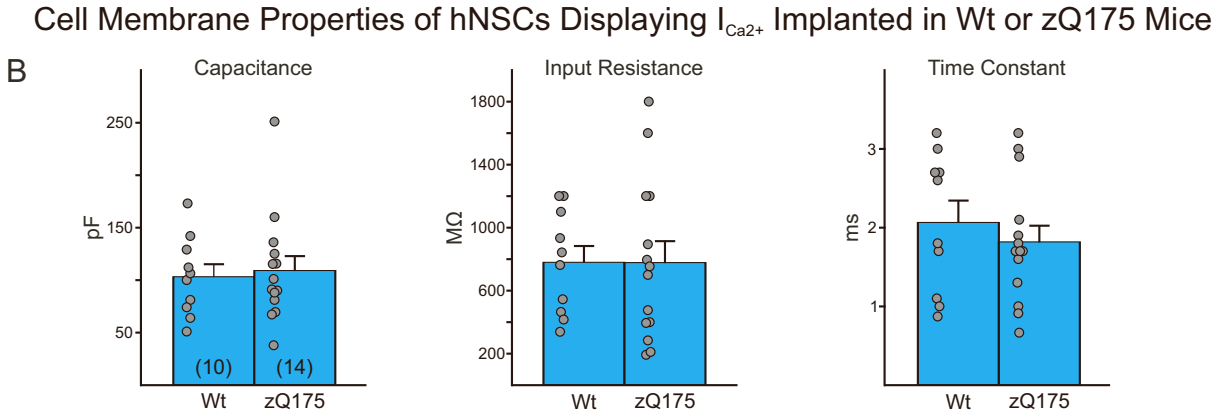


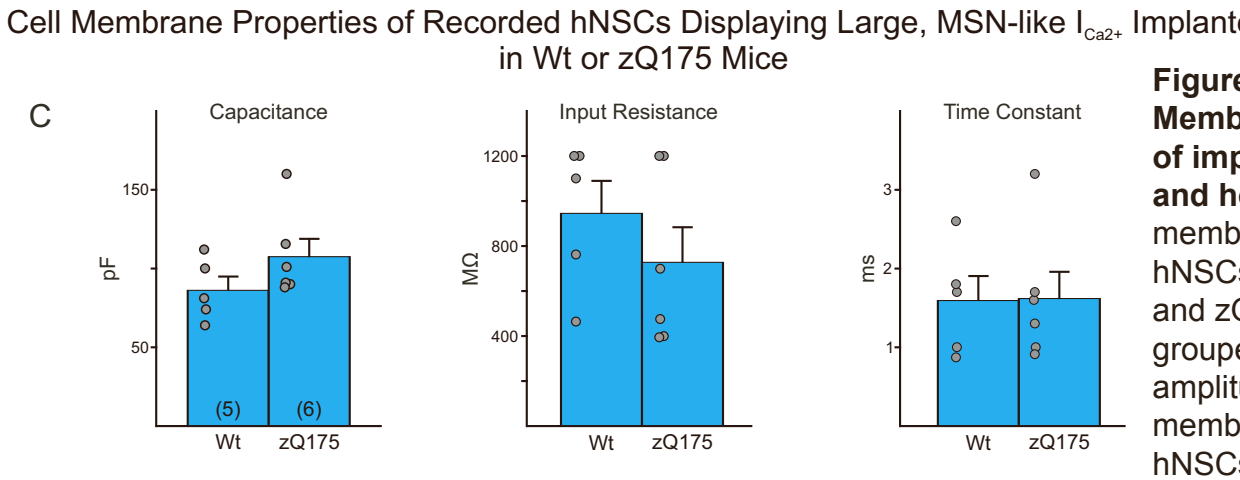
Figure S5. snRNAseq of hNSC Transplants marker expression for human cells.

A. 192 human nuclei from 3 clusters. The size of the dot encodes the percentage of cells within a cluster that express the corresponding gene, while the color encodes the scaled average expression level across all cells within a cluster. B. Three distinct clusters of human cells annotated for cells expressing IHC staining markers. C. Three distinct clusters of human cells were indicated by cluster analysis and BDNF is in mainly in cluster 1.

Figure S6







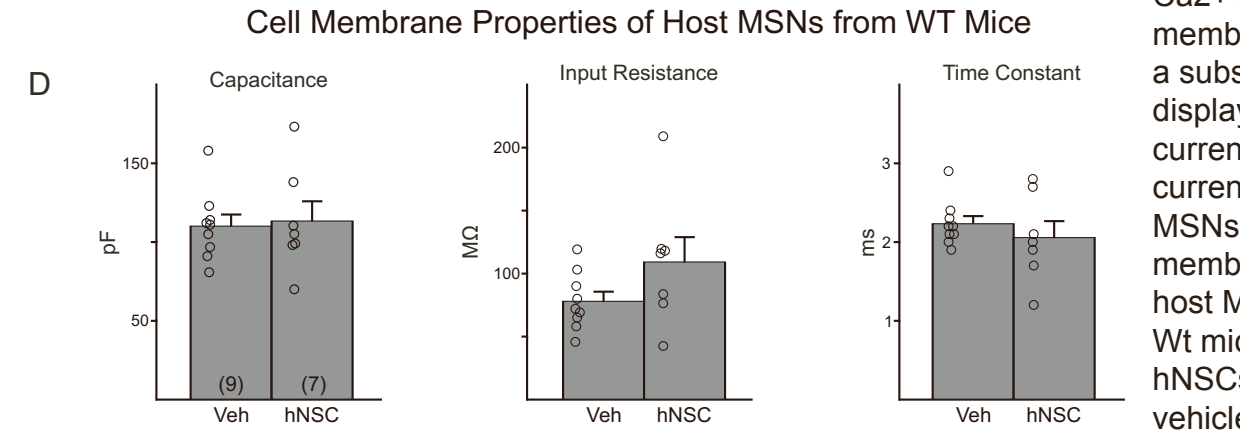


Figure S6. Cell Membrane Properties of implanted hNSCs and host MSNs. A. Cell membrane properties of hNSCs implanted in Wt and zQ175 mice grouped by Na+ current amplitudes. **B.** Cell membrane properties of hNSCs that displayed Ca2+ currents. **C.** Cell membrane properties of a subset of hNSCs that displayed large Ca2+ currents that resembled currents recorded in MSNs. **D.** Cell membrane properties of host MSNs recorded in Wt mice implanted with hNSCs or that received vehicle (Veh) only.

Figure S7

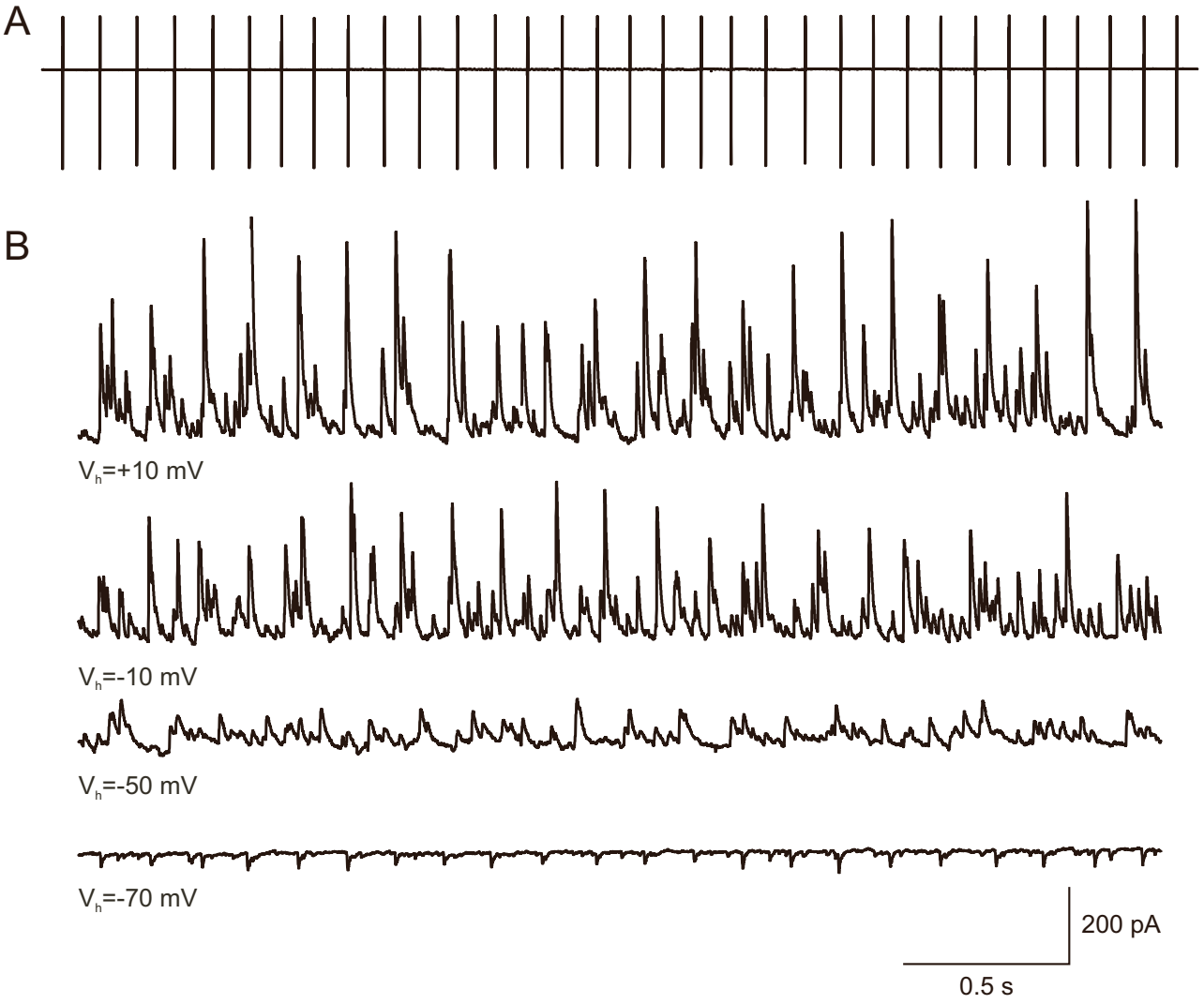


Figure S7. hNSCs display rhythmic activities. A. In cell-attached mode this cell from a zQ175 mouse displayed autonomous, rhythmic firing activity. **B.** In voltage clamp mode, spontaneous, rhythmic synaptic events can be observed at different holding potentials (bottom 4 traces). Spontaneous synaptic events were tentatively assumed to be GABAergic since the reversal potential for GABA occurred at ~-60 mV. This type of activity is not observed in normal conditions in striatal MSNs and this cell was assumed to be interneuron-like.

Figure S8

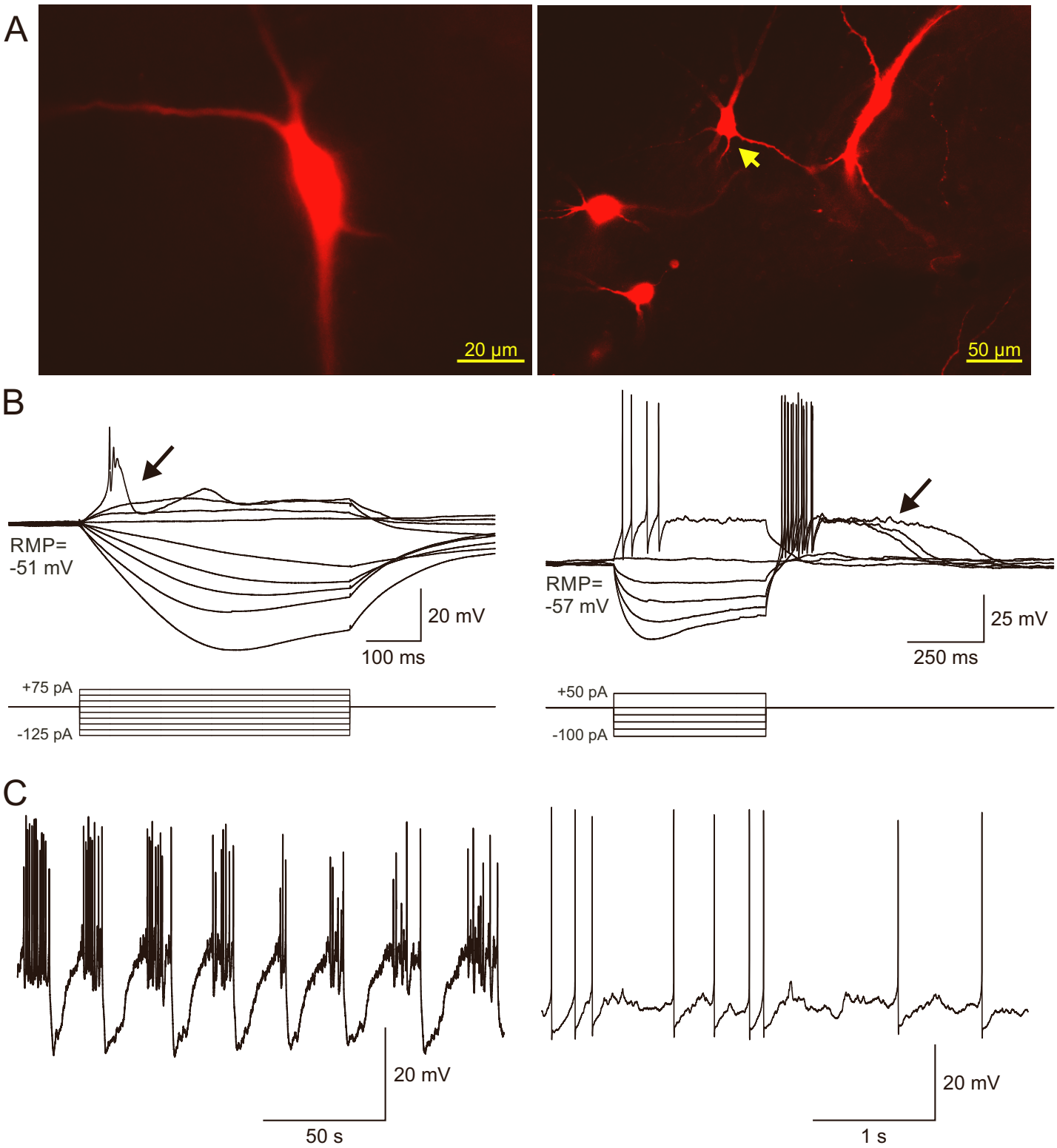


Figure S8. Some large hNSCs resemble striatal LTS interneurons. A. Large hNSCs from two zQ175 implanted mice were recorded and filled with biocytin. hNSC on the left and cell with yellow arrow on the right displayed LTS-like interneuron properties. **B.** Traces are electrophysiological recordings in current clamp mode. Both cells displayed prominent inward rectification and low-threshold Ca2+ spikes (arrows). **C.** Upon hyperpolarization by negative current injection cell on the left displayed rhythmic membrane oscillations and bursts of action potentials. The interneuron-like hNSC on right displayed low-threshold Ca2+ spikes and fired spontaneously at resting membrane potential (-57 mV). Both cells shared similarities with striatal LTS (somatostatin-expressing) interneurons.

Figure S9

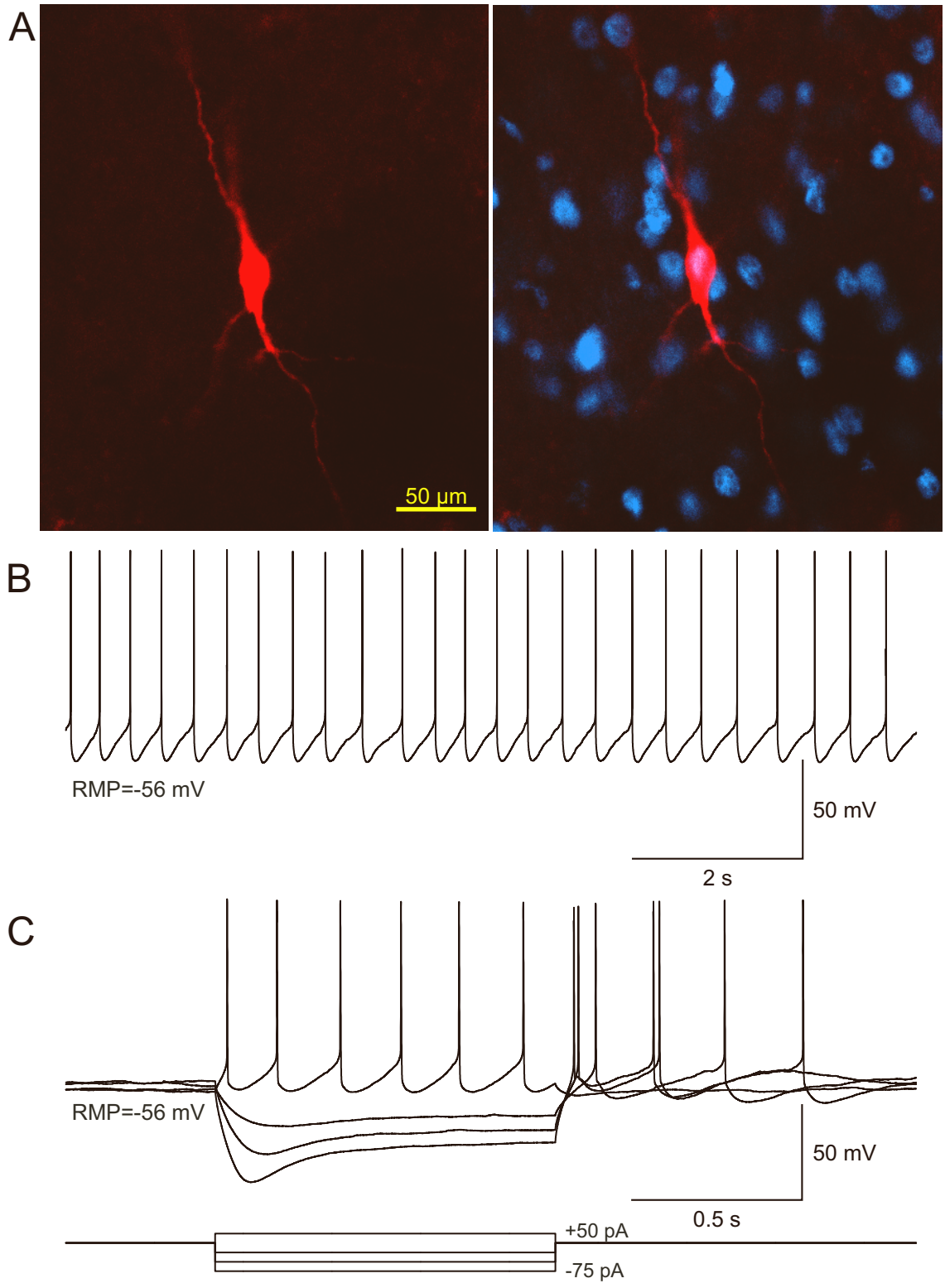


Figure S9. Some large hNSCs resemble cholinergic interneurons. A. Left panel shows a biocytin-filled, large hNSC. The right panel illustrates co-localization of the human marker Ku80 (blue) and biocytin (red). **B.** In current clamp mode, this cell fired spontaneous, rhythmic action potentials (2-3 Hz), typical of striatal cholinergic interneurons. **C.** Hyperpolarizing the cell also demonstrated delayed inward rectification, another signature of striatal cholinergic interneurons.

Tables included as excel files:

Table S1: Genotype DEGs comparing zQ175 vehicle treated cells against Wt vehicle treated cells.

Table S2: DEGs for the effect of treatment on gene expression in the zQ175 het mice (zQ175 hNSC-treated versus zQ175 vehicle).

Table S3: Treatment changes that overlapped with genotype DEGs.