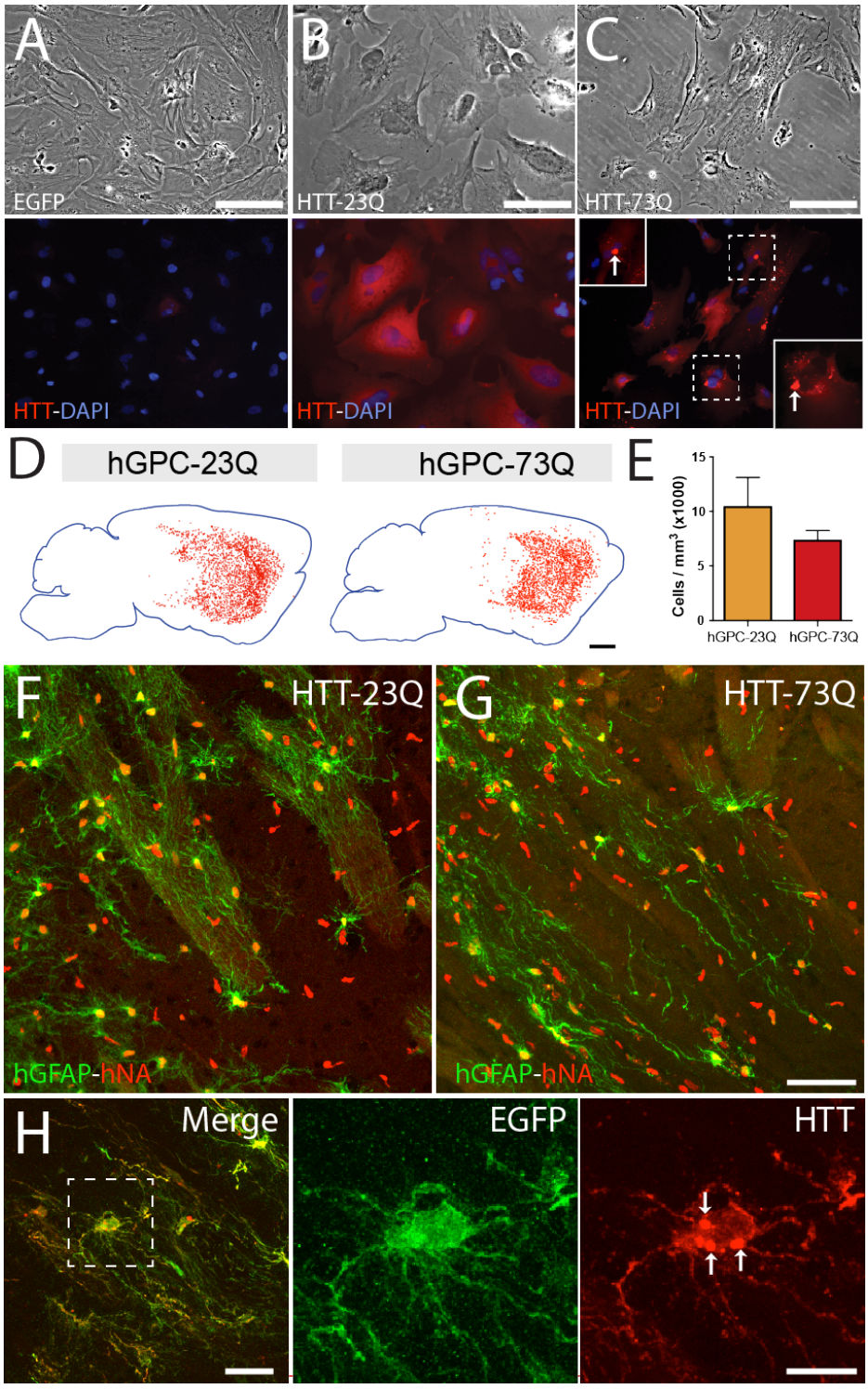
SUPPLEMENTARY FIGURES

Supplementary Figure 1 (Related to Figure 3)

Chimerization of normal striata by Q23 and Q73 HTT-transduced hGPCs



See caption on next page.

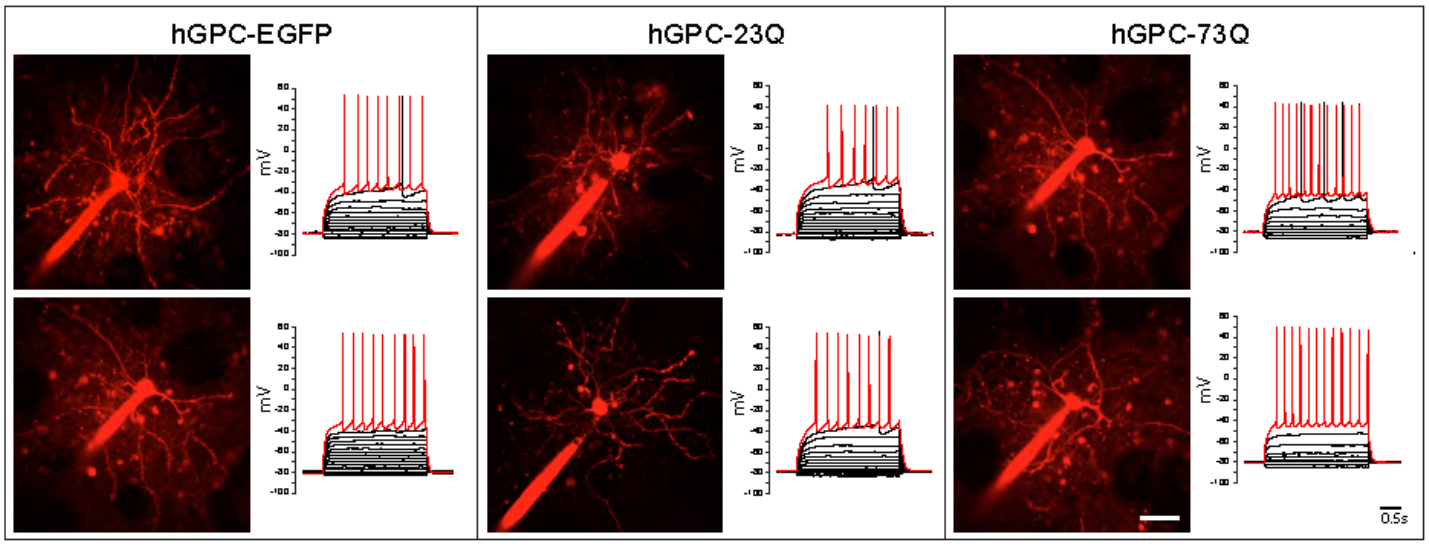
Supplementary Figure 1 *(see image on previous page)*

Chimerization of normal striata by Q23 and Q73 HTT-transduced hGPCs

A-C, Glia derived from Q73 mHtt-transduced hGPCs developed inclusions. **A**, Astroglia derived from hGPCs; no Htt immunostaining was detectable in untreated cells. **B**, Q23 HTT-transduced cells (23 CAG repeats in exon 1 of HTT) overexpress cytoplasmic Htt, but no aggregates or inclusions are noted. **C**, Q73 mHtt-expressing astroglia express both high levels of Htt, and discrete cytosolic inclusions of mutant Htt protein. **D-G,** Histological analysis revealed dense engraftment by human donor cells, in both the Q23 and Q73. mHtt glial-engrafted chimeric striata, whose distributions (**D**) and densities (**E**) of human nuclear antigen $(hNA)^+$ donor cells were similar at the 12 week time-point at which electrophysio-logical recordings were obtained. The distribution of donor-derived astroglia, revealed by anti-human GFAP, was also similar in Q23 (**F**) and Q73 (**G**) chimeric brains. **H**, Donor-derived glia transduced to express Q73 mHtt (which co-express EGFP, after lenti-mHtt-EGFP transduction) develop cytosolic inclusions (*arrows*) in vivo, 12 weeks after neonatal graft. Scale: **A**-**C**, 50 μm; **D**, 1 mm; **F**-**H**, 100 μm.

Supplementary Figure 2 (Related to Figure 3)

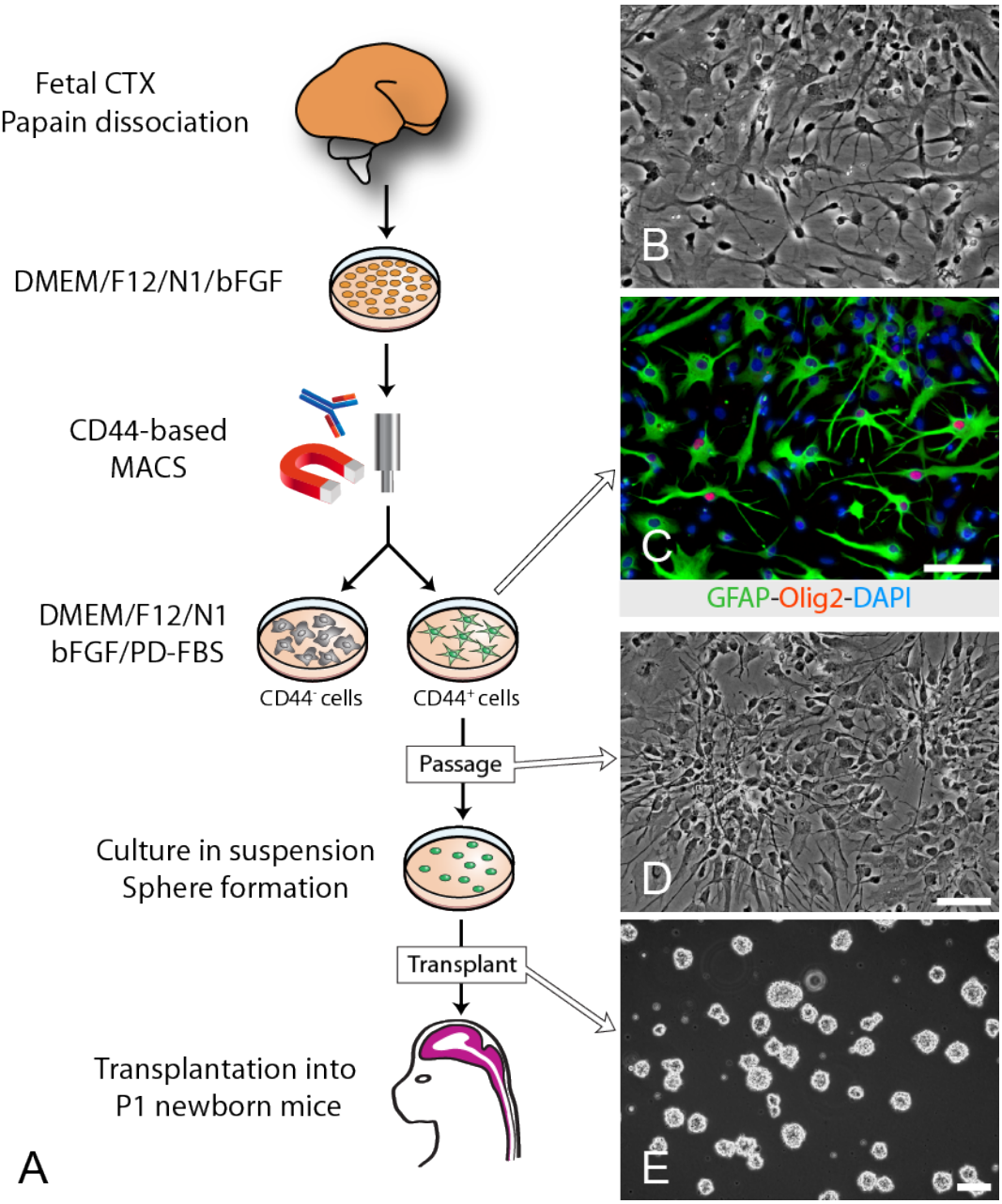
Representative recorded striatal neurons in Q23 and Q73 HTT hGPC-colonized striata



Examples of striatal neurons in hGPC-EGFP, hGPC-23Q and hGPC-73Q chimeric striata. Neurons, sampled and identified randomly throughout the striatum, were subjected to whole cell patch clamp in current-clamped configuration, and their responses to current injection recorded. The neurons displayed typical medium spiny neuron morphologies when filled with Alexa-594. Scale: 50 µm.

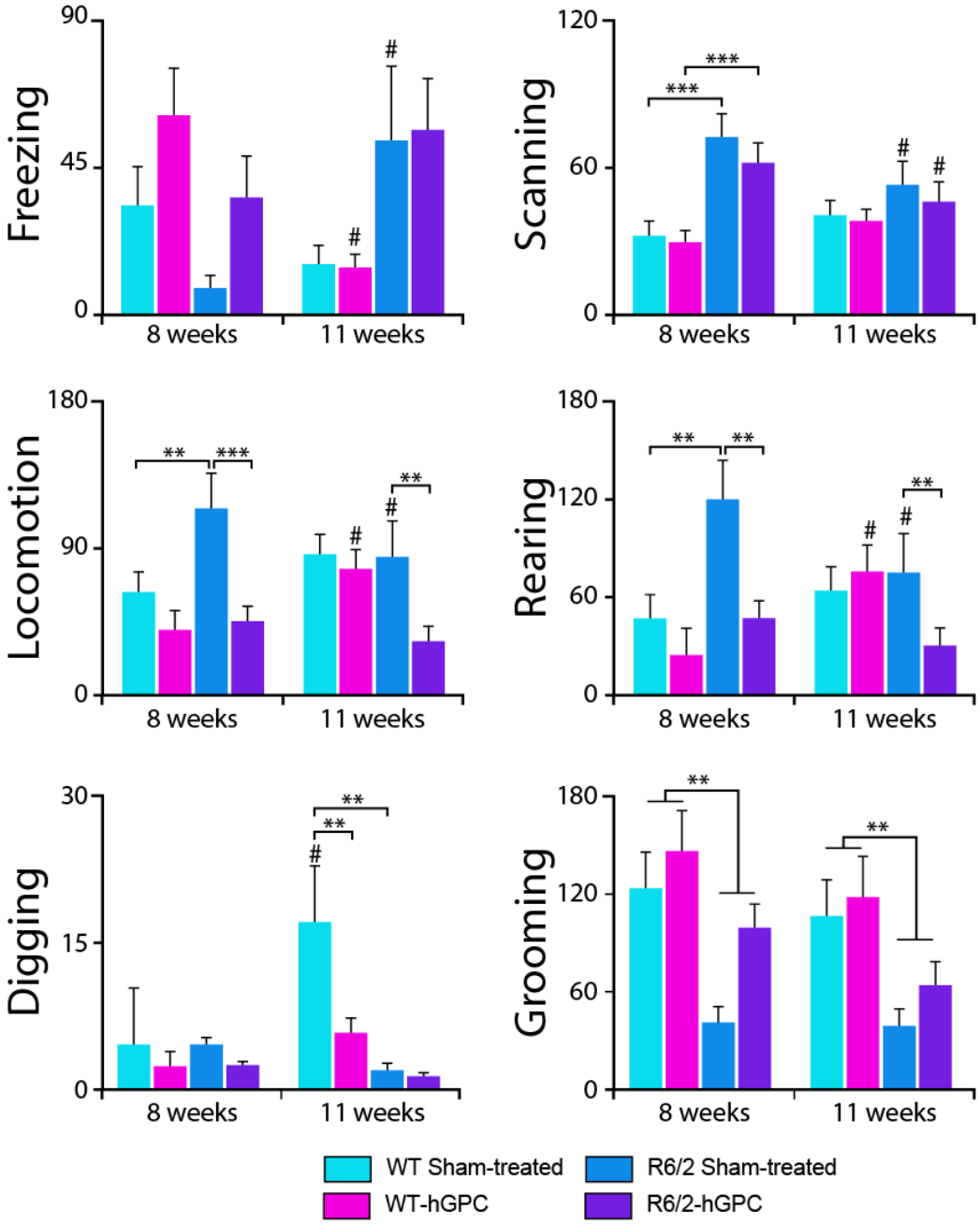
Supplementary Figure 3 (Related to Figure 4)

Fetal human CD44^{+}GPCs were used to colonize the R6/2 mouse striatum



This figure exhibits the morphology and antigenicity of hGPCs derived from 18-22 wk gestational age human fetal forebrain. **A**, The schematic illustrates the major steps involved in isolating and culturing CD44-defined astrocyte-biased hGPCs, prior to their transplantation into neonatal mice. **B** shows the appearance of the CD44-sorted cells after isolation. **C**, The cells include a mix of GFAP^{+}/olig2^{-} astrocytes and GFAP^{+}/olig2^{+} hGPCs. **D**-**E**, The cells were grown to confluency and passaged (**D**), then cultured on ultralow attachment dishes for 2-5 days, in which they formed clusters of 50-100 µm diameter; these were spun and collected for transplant (**E**). Scale: 100 µm.

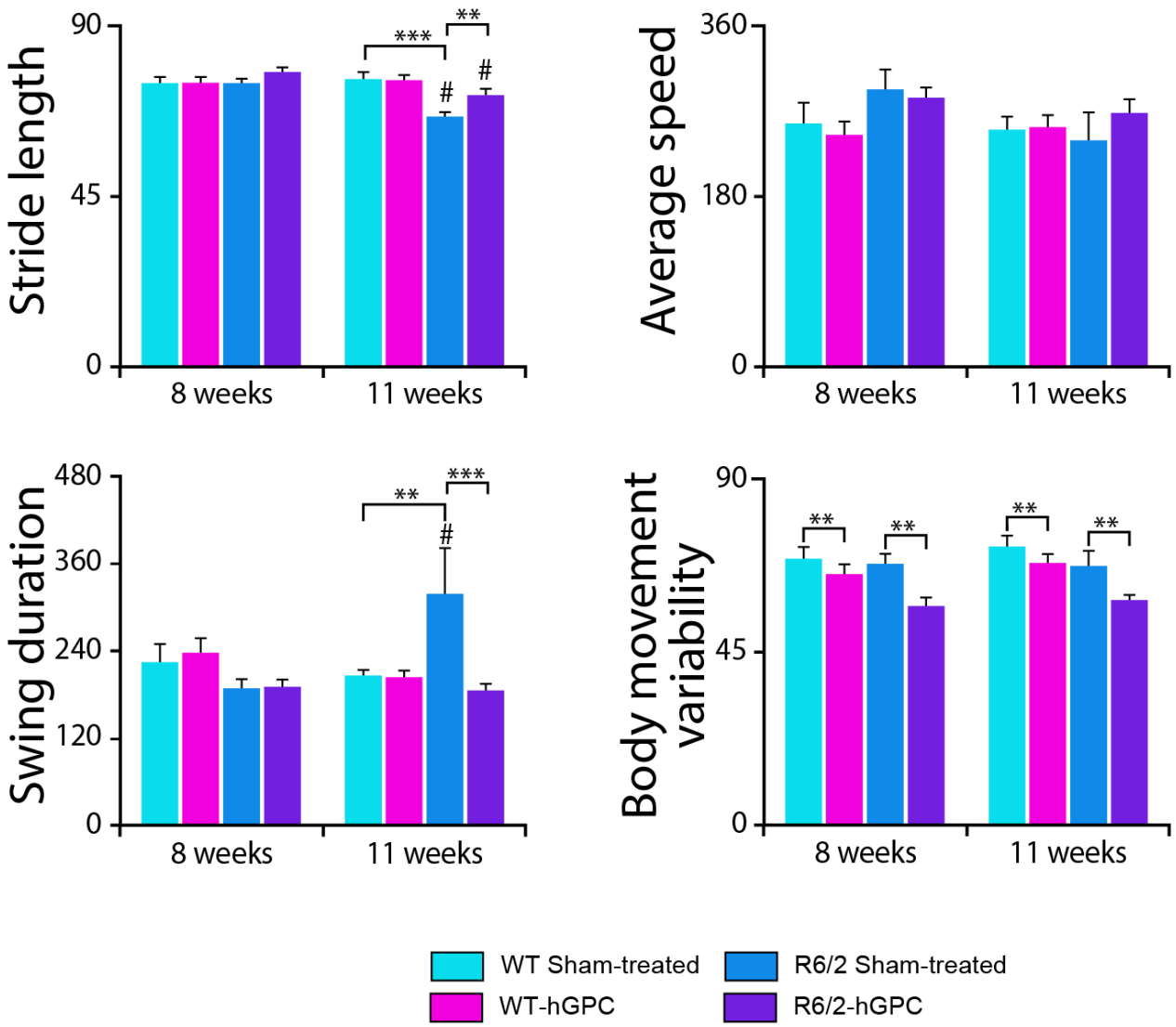
Supplementary Figure 4 (Related to Figure 6)



Chimerization with normal glia partially rescued behavioral phenotype in SmartCube

Using the SmartCube battery of behavioral tests (Psychogenics, Inc.), by 8 weeks of age sham-treated R6/2 mice showed more scanning, locomotion and rearing than wild-type controls; these disease-associated features were either attenuated or completely normalized by hGPC treatment. A reduction in grooming at both ages was marginally normalized by hGPC treatment. At 11 weeks the hyperactivity was no longer evident, freezing was increased, and digging decreased. hGPC treatment in the R6/2 mice reduced locomotion and rearing, but did not affect digging. Asterisks denote significant main effects or post hoc comparisons (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.)

Supplementary Figure 5 (Related to Figure 6)



Treatment with hGPC partially rescued disease signatures in NeuroCube

At 8 weeks of age there were no differences in gait or related features. At 11 weeks of age deficits in gait (reduced stride length and increased swing duration) were rescued by hGPC treatment. Similarly, GPC treatment decreased body movement variability at both ages and in both the WT and R6/2 mice. Other features (e.g. speed) were not affected by genotype or treatment. Asterisks denote significant main effects or post hocs and numerals indicate age effects for a particular group; arrows indicate the effect of treatment independently of genotype and age (one symbol: $p < 0.05$; two symbols: $p < 0.01$; three symbols: $p < 0.001$.)

SUPPLEMENTARY TABLES

Supplementary Table 1 (Related to Methods)
Mouse allocations to each experiment

Mouse strain	Treatment	Experimental endpoint	n
R6/2 - Rag$^{-/-}$	P1 mice transplanted with fetal-derived glia	Histology	57
		Rotarod	36
		Survival	57
		Electrophysiology including interstitial K^+ measurement	49
		Cognitive assessment	75
Rag$^{-/-}$	Neonatal graft of fetal-derived hGPCs expressing Q23/Q73 Htt	Histology	16
		Electrophysiology	36
Rag$^{-/-}$	Neonatal graft of ESC-derived glia	Histology	16
		Rotarod	91

Supplementary Table 2 (Related to Fig. 2)

Rotarod performance of HD ESC-derived glial chimerized mice
Statistical table: p values with Tukey's multiple comparisons tests

Comparisons of treatments	p Values							
	8 weeks	12 weeks	16 Weeks	20 Weeks	24 Weeks	28 Weeks	32 Weeks	36 Weeks
Genea-19 (n=28) vs. Genea-20 (n=31)	0.7966	0.0299	< 0.0001	< 0.0001	0.0002	< 0.0001	0.0746	0.0565
Genea-19 (n=28) vs. Untreated (n=21)	0.9419	0.0772	0.2439	0.9988	0.9969	0.9999	0.2669	0.8725
Genea-19 (n=28) vs. Sham treated (n=11)	0.9552	0.9838	0.8064	0.3474	0.9793	0.8399	0.9849	0.7005
Genea-20 (n=31) vs. Untreated (n=21)	0.9935	< 0.0001	< 0.0001	0.0001	< 0.0001	< 0.0001	< 0.0001	0.0035
Genea-20 (n=31) vs. Sham treated (n=11)	0.9986	0.0704	< 0.0001	0.2308	0.0098	0.0107	0.0504	0.0034
Untreated (n=21) vs. Sham treated (n=11)	0.9999	0.4188	0.9255	0.4181	0.9393	0.8267	0.5768	0.976

Genea-19: GENEA19 (18Q)-derived hGPC chimeric Rag1$^{-/-}$.
Genea-20: mutant Htt GENEA20(48Q)-derived hGPC chimeric Rag1$^{-/-}$

Supplementary Table 3 (Related to Fig. 3)

Mice chimeric for human mHTT-expressing glia exhibit alterations in MSN physiology
Statistical table: p values with Tukey's multiple comparisons tests

Supplementary Table 3A
Mutant HTT glial chimeric mouse MSNS have higher absolute resting membrane potentials
Statistical table: p values, Tukey's multiple comparisons tests

Comparisons of treatments	p Values
Untreated vs. hGPC-EGFP	ns
Untreated vs. hGPC-23Q	ns
Untreated vs. hGPC-73Q	< 0.0001
hGPC-EGFP vs. hGPC-23Q	ns
hGPC-EGFP vs. hGPC-73Q	< 0.0001
hGPC-23Q vs. hGPC-73Q	< 0.01

Supplementary Table 3B
Mutant HTT glial chimeric mice have abnormally high MSN input resistance
Statistical table: p values, Tukey's multiple comparisons tests

Comparisons of treatments	p Values	
	R_{Neg}	R_{Pos}
Untreated vs. hGPC-EGFP	0.5718	0.6339
Untreated vs. hGPC-23Q	0.9142	0.0178
Untreated vs. hGPC-73Q	< 0.0001	< 0.0001
hGPC-EGFP vs. hGPC-23Q	0.2028	0.0007
hGPC-EGFP vs. hGPC-73Q	< 0.0001	< 0.0001
hGPC-23Q vs. hGPC-73Q	< 0.0001	< 0.0001

Supplementary Table S4 (Related to Figure 5)

Weights of R6/2 mice as function of treatment and time

Age	R6/2-hGPC	R6/2-Untreated
8 weeks	22.32 (n=12)	20.33(n=11)
16 weeks	17.40 (n=8)	18.58(n=9)

Supplementary Table 5 (Related to Figure 5)

Rotarod scores of glial chimeric R6/2 mice as function treatment and age
Statistical table: p values with Tukey's multiple comparisons tests

Comparisons of treatments	p values			
	4 weeks	8 weeks	12 Weeks	16 Weeks
R6/2-hGPC vs. R6/2-untreated	0.919	0.937	0.044*	0.558
R6/2-hGPC vs. R6/2-Saline	0.706	0.421	0.012*	0.178
R6/2-untreated vs. R6/2-Saline	0.512	0.735	0.9	0.979

Of note, while **Supplementary Table 5** shows the data comparing treated and control mice through the 16-week time point. However, no similar analysis was possible for the 20 week time point, as all of the control animals had either died or were unable to balance on the rod by then.

Supplementary Table 6 (Related to Figure 5)

Analysis of survival of hGPC-engrafted R6/2 mice by gender

	Male survival (d)	Female survival (d)	Statistics p value (t test)
R6/2-hGPC	18.78 (n=16)	19.57 (n=13)	0.936
R6/2-untreated	18 (n=13)	16.85 (n=15)	0.249

Supplementary Table 7 (Related to Figure 5)

Striatal volumes of glial chimeric R6/2 mice as a dual function of treatment and age
Statistical table: p values with Tukey's multiple comparisons tests

Comparisons of treatments	p values		
	12 weeks	16 weeks	20 weeks
WT-untreated vs. R6/2-untreated	0.9985	0.0041	< 0.0001
WT-untreated vs. R6/2-hGPC	0.8368	0.3447	0.0585
R6/2-untreated vs. R6/2-hGPC	0.8436	0.1224	0.0066

WT: $Rag1^{-/-}$ immunodeficient mice; R6/2: $Rag1^{-/-}$ x R6/2(120CAG).

Supplementary Table 8 (Related to Fig. 7)

Human glial chimerized R6/2 mice exhibit restoration of MSN physiological parameters
Statistical table: p values with Tukey's multiple comparisons tests. All in WT: Rag1$^{-/-}$ immunodeficient mice.

A. Input resistance (Related to Fig. 7)

Comparisons of treatments	p Values	
	R_{Neg}	R_{Pos}
WT-untreated vs. R6/2-untreated	2.87439E-8	2.02057E-20
WT-untreated vs. R6/2-hGPC	6.67309E-5	1.01578E-12
R6/2-untreated vs. R6/2-hGPC	0.8760	0.0073
WT-untreated vs. WT-hGPC	0.5048	0.9899

B. EPSC Frequencies (Related to Fig. 7E)

Comparisons of treatments	p Values	
	sEPSC	mEPSC
WT-untreated vs. R6/2-untreated	6.88787E-7	0.1879
WT-untreated vs. R6/2-hGPC	0.0126	0.4888
R6/2-untreated vs. R6/2-hGPC	0.0077	0.1333
WT-untreated vs. WT-hGPC	0.0339	0.4814

C. EPSC Amplitudes (Related to Fig. 7F)

Comparisons of treatments	p Values	
	sEPSC	mEPSC
WT-untreated vs. R6/2-untreated	0.1178	0.5478
WT-untreated vs. R6/2-hGPC	0.7011	0.6753
R6/2-untreated vs. R6/2-hGPC	0.2054	0.7990
WT-untreated vs. WT-hGPC	0.0443	0.9728

Supplementary Table 9 (Related to Fig. 8)

Striatal interstitial potassium levels
Statistical table: p values with Tukey's multiple comparisons tests

Comparisons of treatments	p Values
WT-untreated vs. WT–hGPC	0.3701
WT-untreated vs. R6/2-untreated	< 0.0001
WT-untreated vs. R6/2-hGPC	0.3163
WT-hGPC vs. R6/2-untreated	0.0023
WT hGPC vs. R6/2-hGPC	0.9999
R6/2-untreated vs. R6/2-hGPC	0.0007

WT: Rag1$^{-/-}$ immunodeficient mice; R6/2: Rag1$^{-/-}$ x R6/2(120CAG).