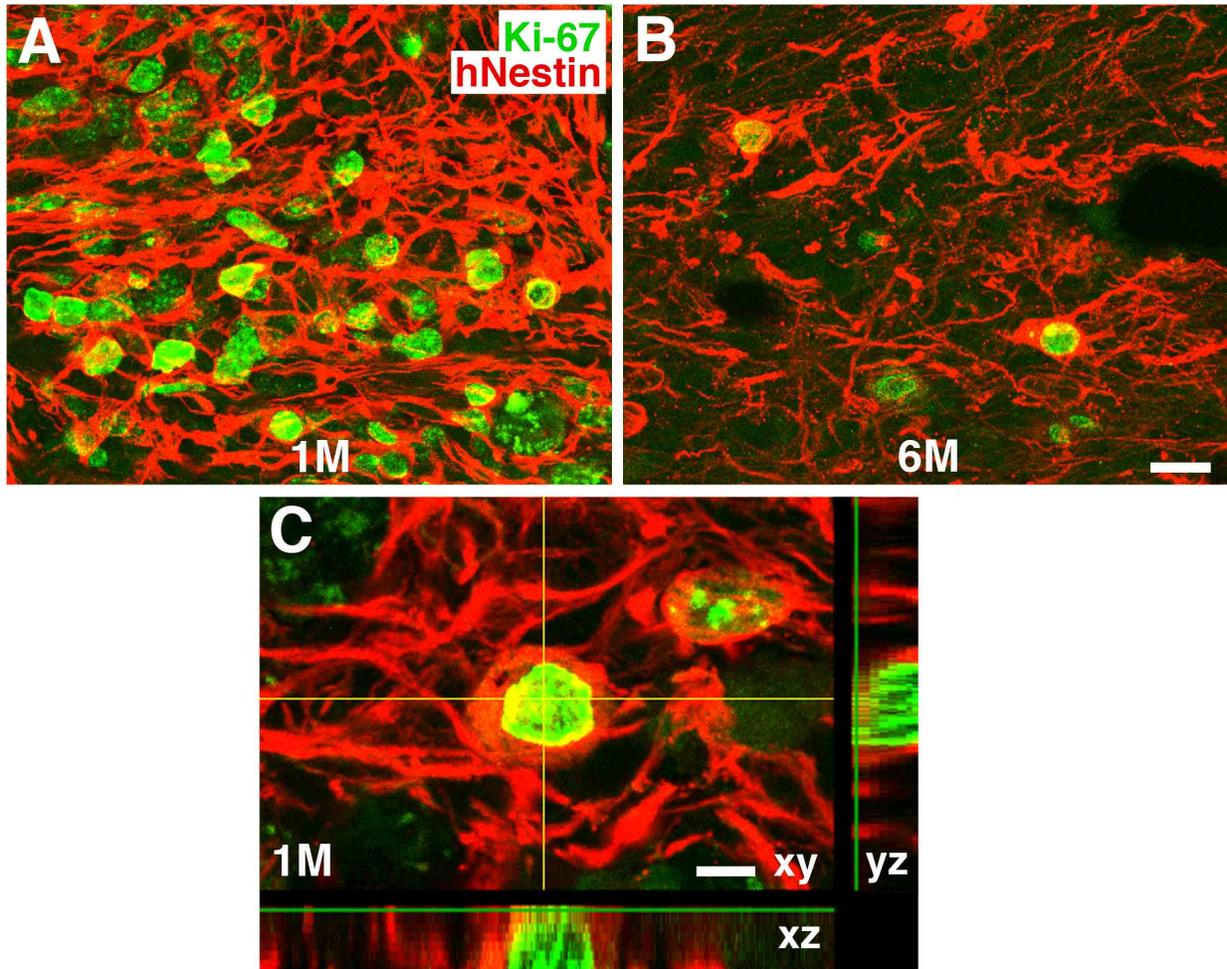
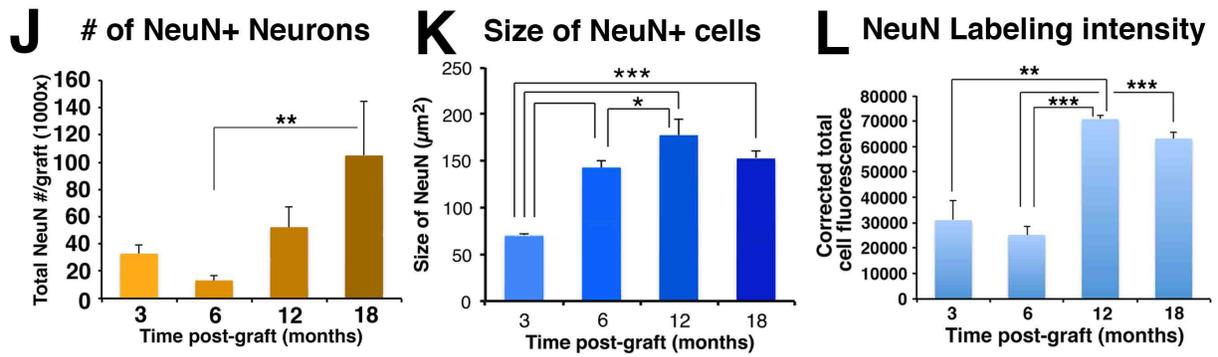
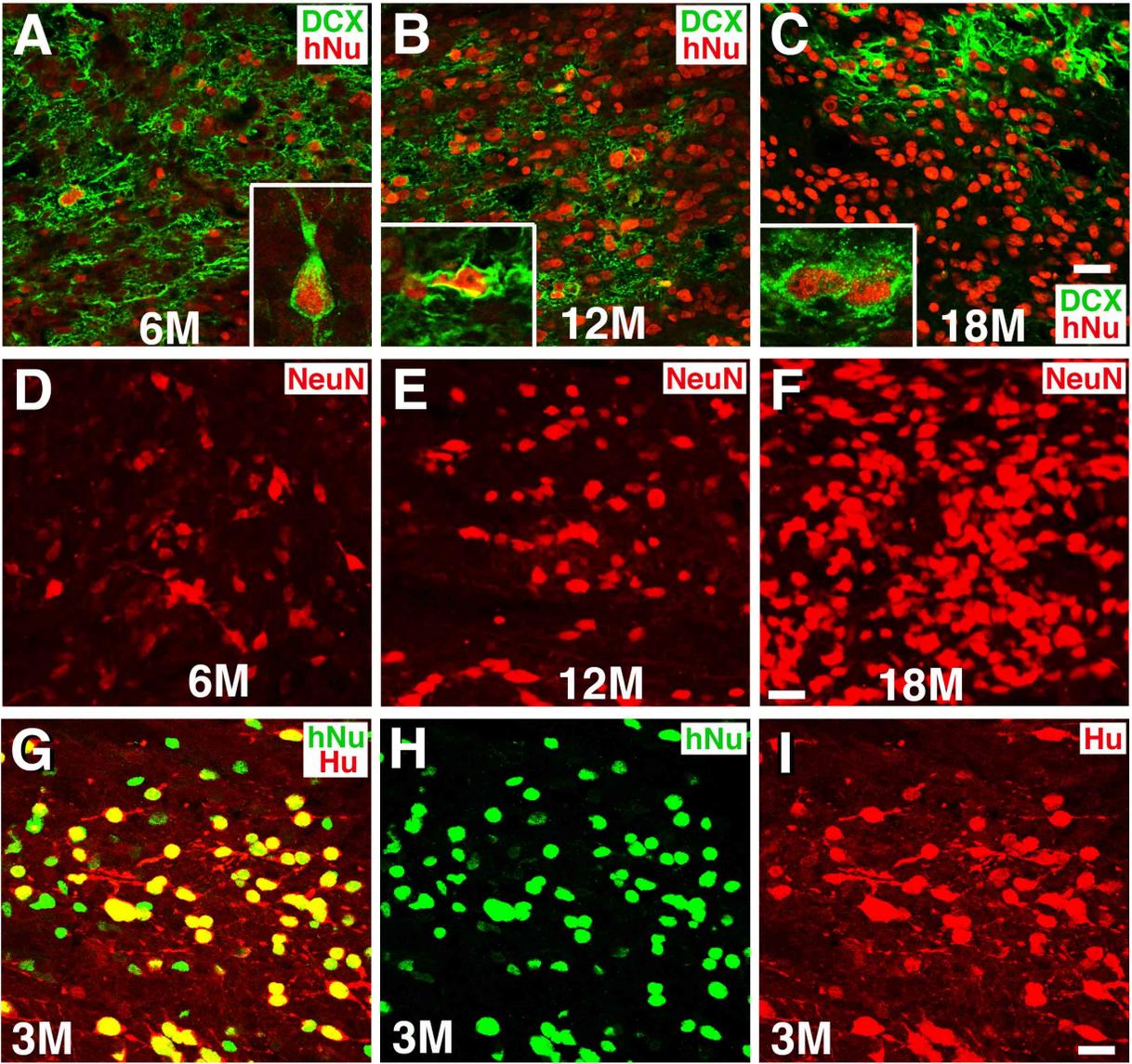


Supplementary Figures



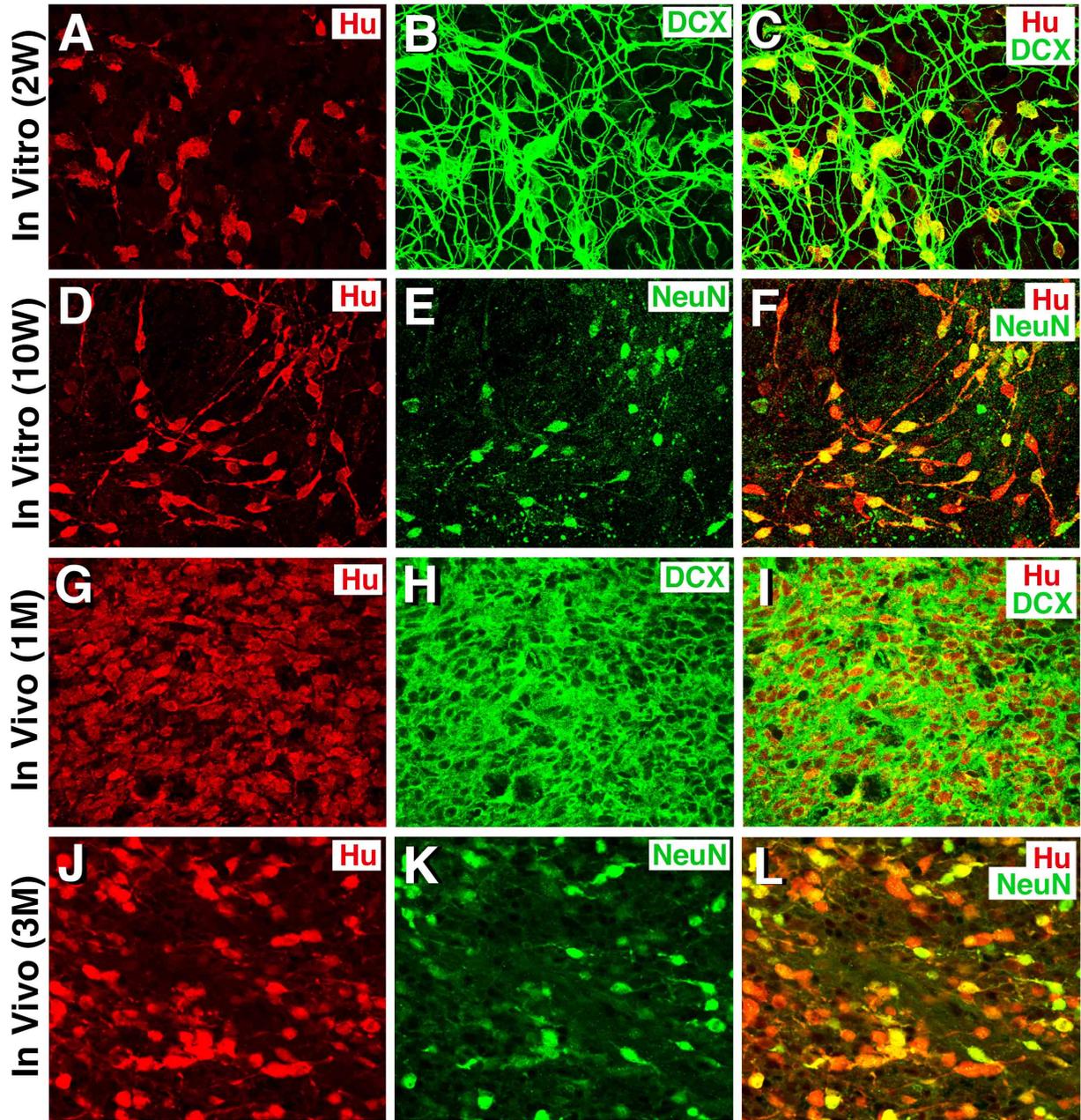
Suppl Fig. 1: Co-localization of Some Ki-67+ Cells with Human-specific Nestin in the Human Cell Graft

(A-B) Double labeling of **Ki67** and human-specific nestin (**hNestin**) reveals that some **Ki67**-labeled, dividing cells co-localize with **nestin**, 1 and 6 months post-grafting. (C) A high magnification confocal z-stack image shows a single **nestin**-labeled cells co-localized with **Ki67** at xy, xz, and yz views from 1-month grafted sample. Scale bar, 16 μm (A-B), 6 μm (C).



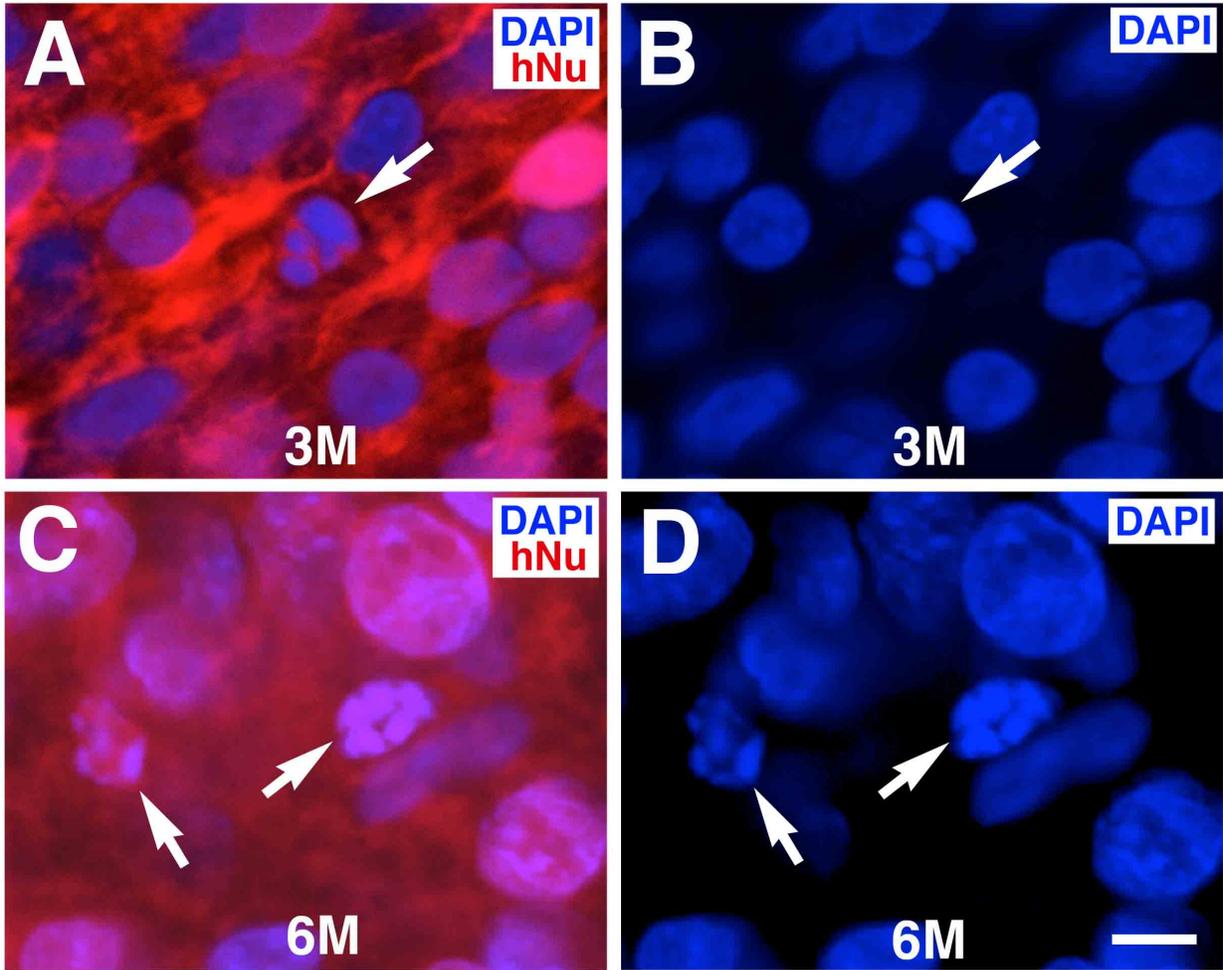
Suppl Fig. 2: Doublecortin (DCX), NeuN and Hu Labeling
 (A-C) Doublecortin (DCX) and human nuclear (hNu) immunolabeling reveal persistent expression of DCX at 6, 12, 18 months post-graft (insets show examples of DCX and hNu double labeling); this labeling is patchy and progressively rarer in longer term

grafts. **(D-F) NeuN** labeling is persistent at 6, 12 and 18 months post-grafting. The density and size of **NeuN+** cells increased over time (quantified in J, K), consistent with Hu labeling at Fig. 2. **(G-I)** Double labeling for the human cell marker **hNu** and the neuronal marker **Hu** reveals that the majority of hNu-labeled cells co-localized with Hu 3-months post-grafting. **(J)** Quantification of total **NeuN⁺** neurons 3, 6, 12 and 18 months post-grafting. **(K-L)** Quantification of **NeuN** size, and fluorescent label intensity. For J-L, $P < 0.001$, ANOVA and $***p < 0.001$; $**p < 0.01$; $*p < 0.05$, post-hoc Fischer's comparison, 1 (n=3), 3 (n=3), 6 (n=5), 12 (n=3), and 18 months (n=4). Scale bar 32 μ m, A-F; 24 μ m, G-I.



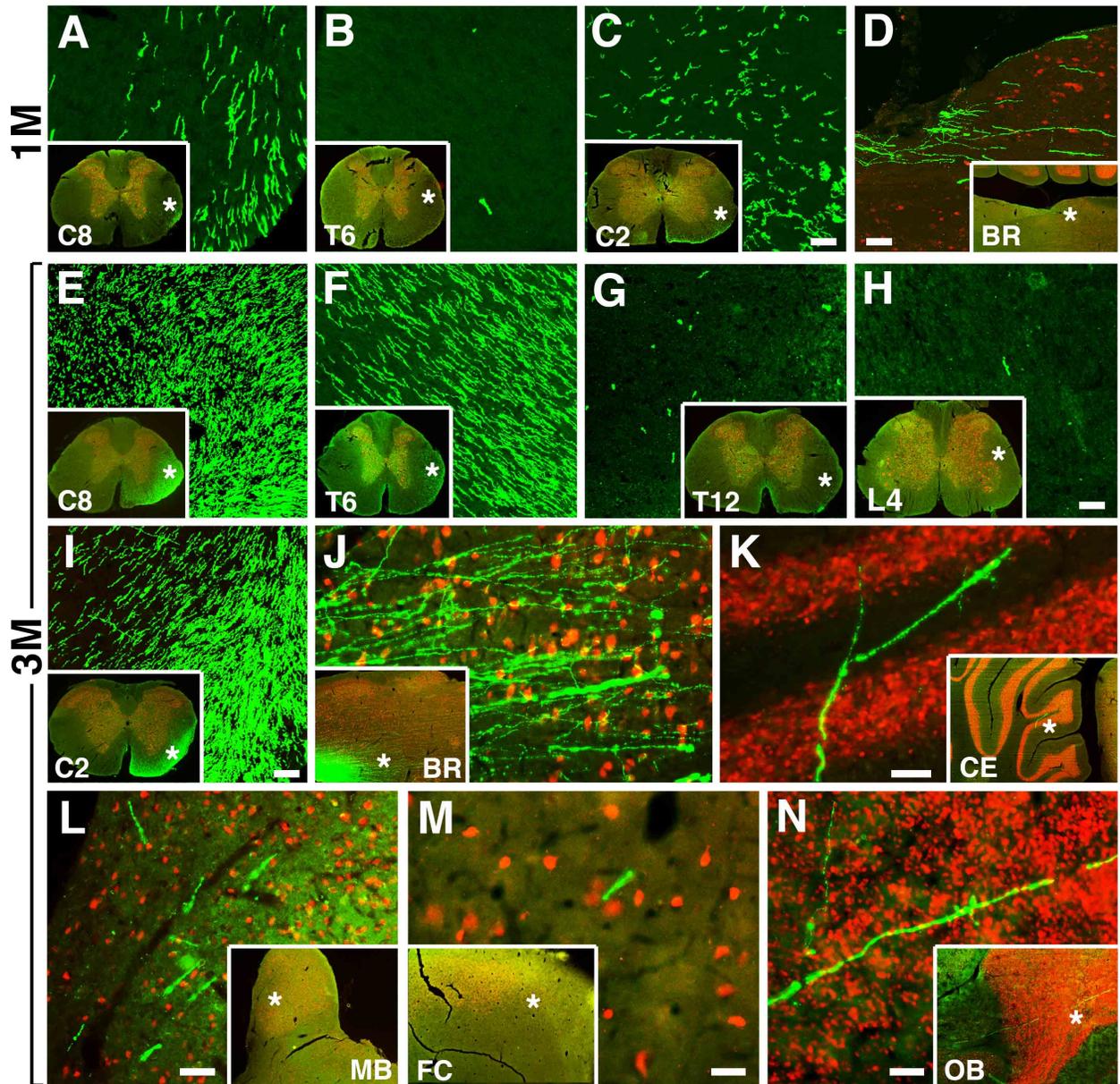
Suppl Fig. 3: Hu Labeling In Vitro and In Vivo

(A-C) The pan-neuronal label **Hu** and the early neuronal marker doublecortin (**DCX**) co-localize in H9-derived NSCs after 2 weeks in vitro, under a differentiation protocol. (D-F) After 10 weeks in vitro, NSCs mature and now predominantly co-localize with the mature neuronal marker **NeuN**. (G-I) After one month in vivo, grafted H9 NSCs co-localize for **Hu** and **DCX** in graft. (J-L) 3 months after in vivo grafting, NSCs predominantly co-localize for **Hu** and **NeuN**. Scale bar: 24 μ m.



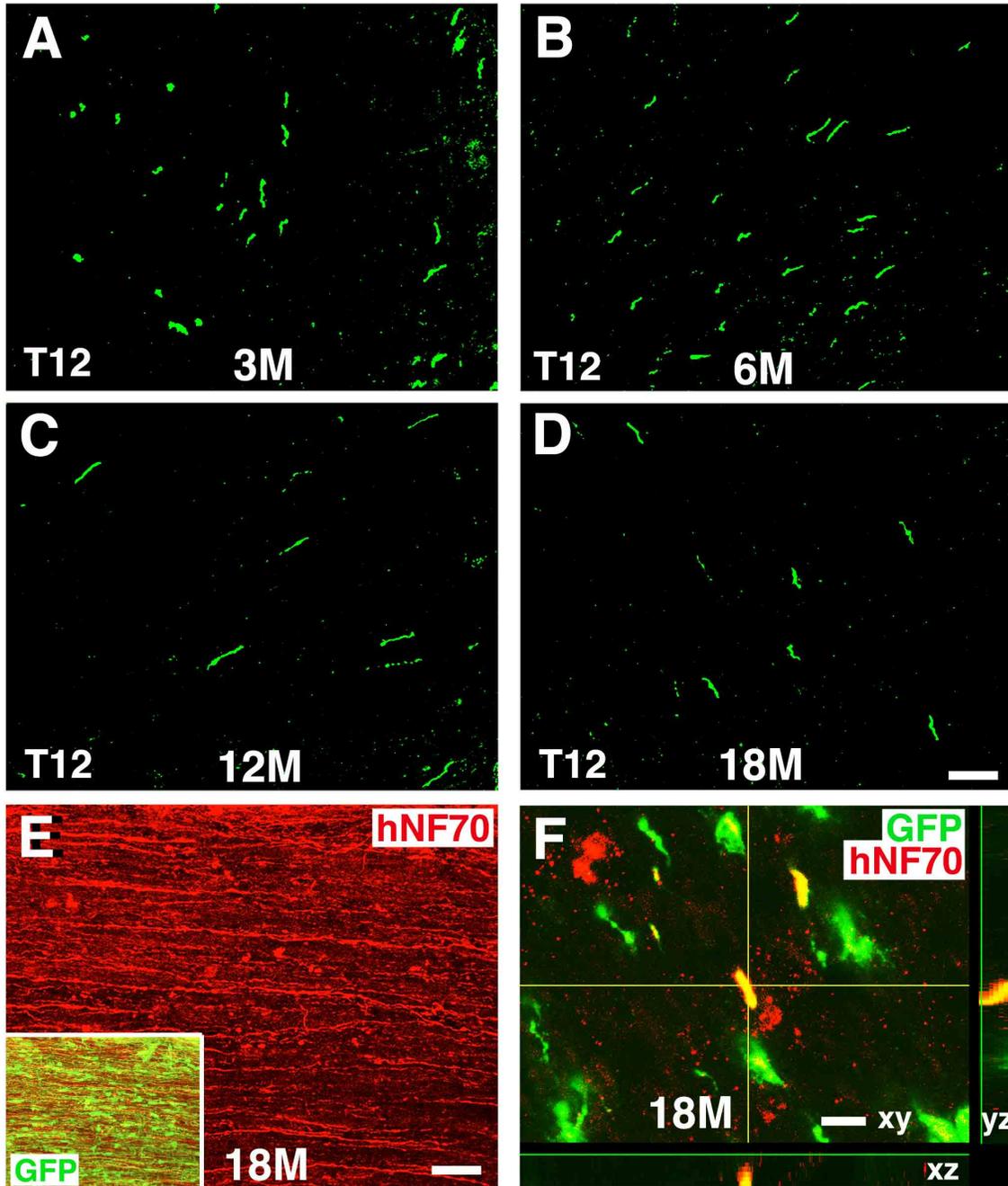
Suppl Fig. 4: DAPI Staining

DAPI immunolabeling reveals nuclear fragmentation (arrows) in human NSC graft identified by human cell specific marker **hNu** at (A-B) 3 and (C-D) 6 months post-grafting. Scale bar: 5 μ m.



Suppl Fig. 5: Long-Distance Axonal Growth from H9-Derived NSCs at 1 and 3 Months Post-Grafting

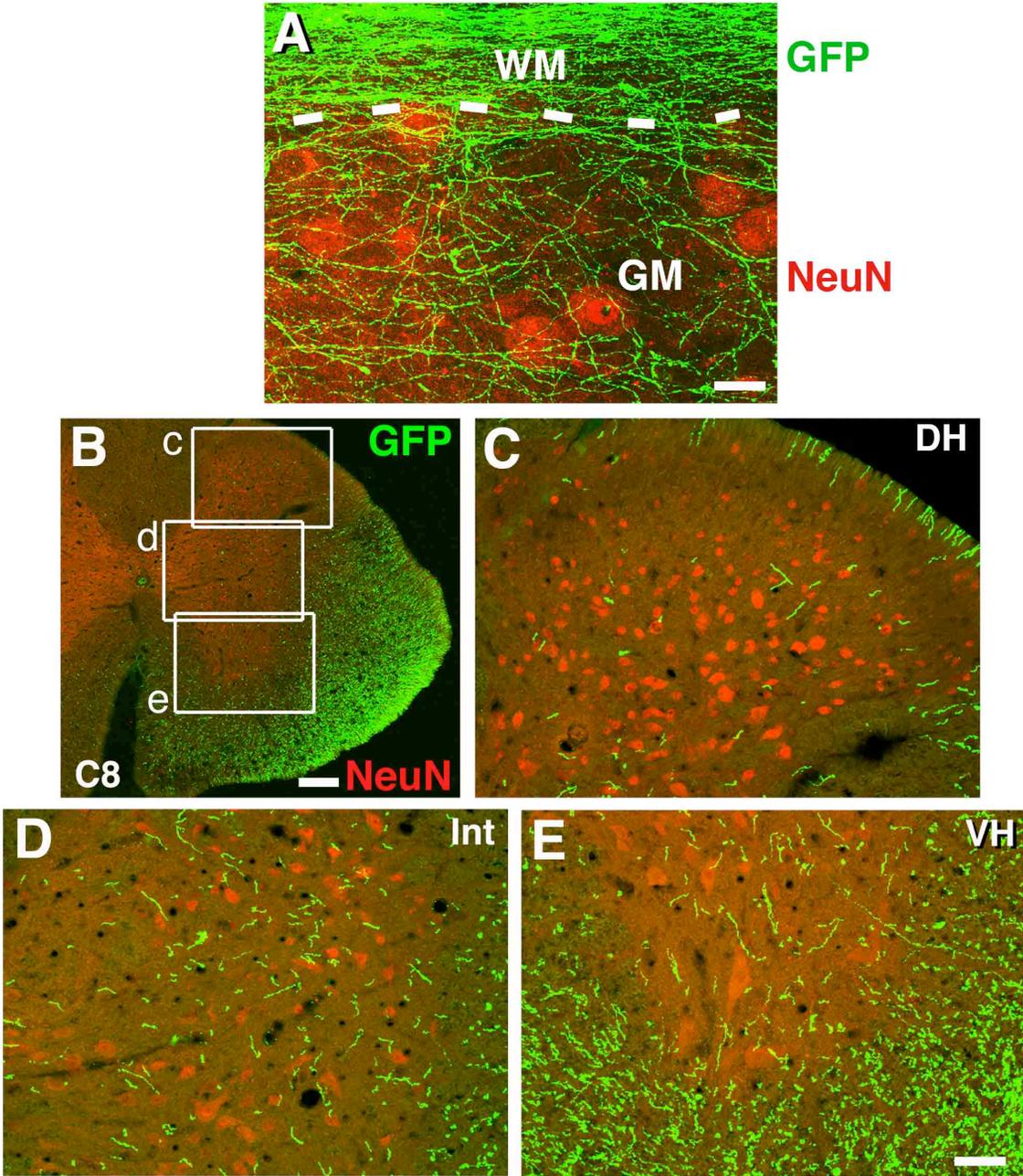
(A-D) **GFP** and **NeuN** immunolabeling reveals **GFP** positive axons from human H9-NSCs graft at C5 hemisection site at 1 month extended caudally into (A) C8 and as far as into (B) T6 spinal cord (coronal view), and rostrally into (C) C2 and (D) brainstem (BS). * at inset indicates region of sampling. (E-N) **GFP** labeled human axons at 3 months post-graft extended caudally into (E) C8, (F) T6, (G) T12 and as far as into (H) L4, and rostrally into (I) C2 and (J) brainstem (BR), (K) cerebellum (CE), (L) midbrain (MB), (M) front cortex (FC) and (N) olfactory bulb (OB). Scale bar: A-C, 30 μ m; D, 50 μ m; E-I, 30 μ m; J, 55 μ m; K-M, 50 μ m; N, 45 μ m.



Suppl Suppl Fig. 6: Long-Term Persistence and Maturation of Graft-Derived Human Axons

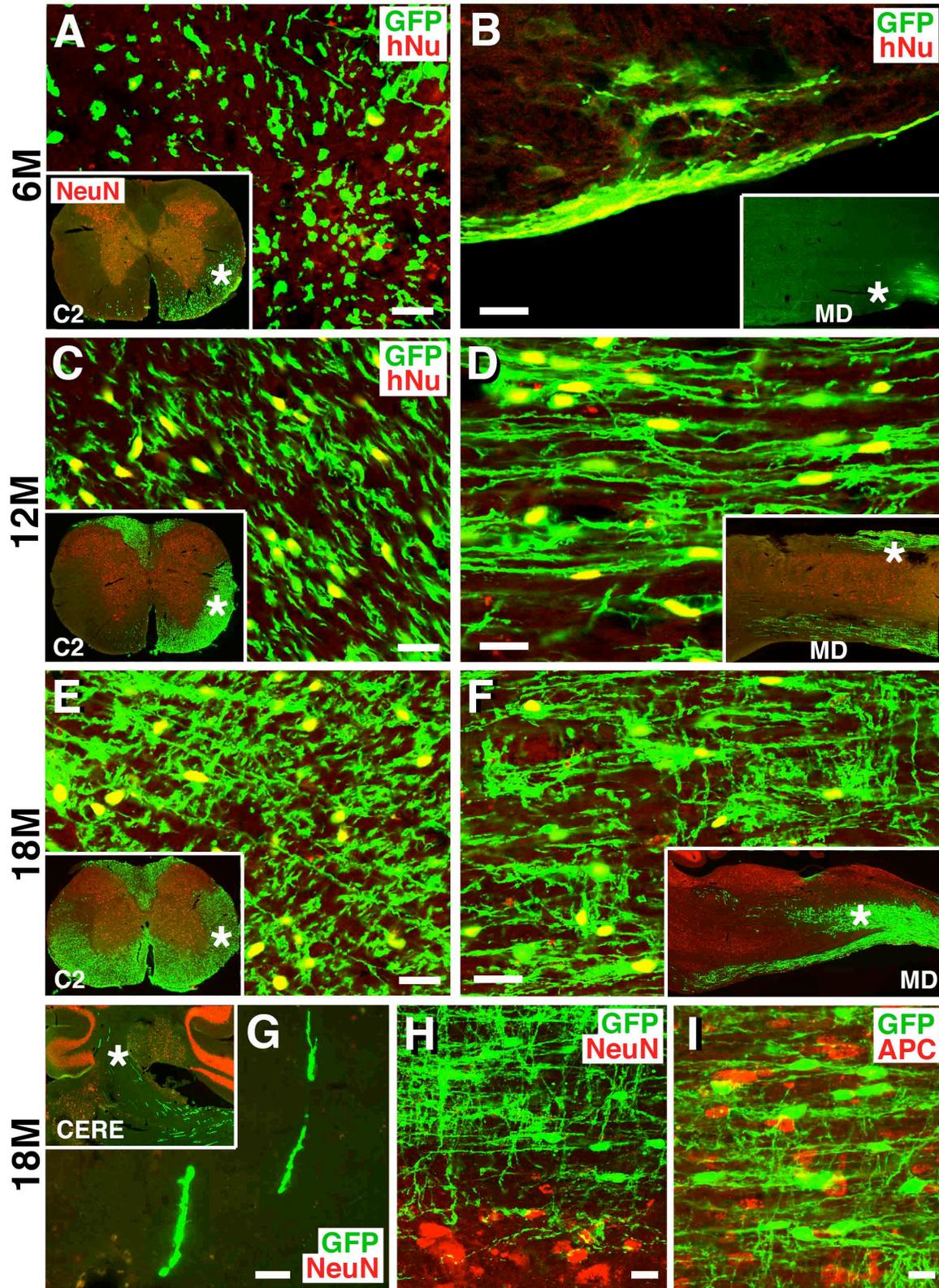
(A-D) GFP-immunoreactive human axons in coronal sections of host white matter at T12 (16 spinal segments caudal to the lesion site) 3, 6, 12 and 18 months post-grafting. Axons were not found in this level at 1 month post-grafting. Migrating glia did not reach this level 18 months post-grafting, allowing quantification of GFP-labeled axon numbers as a function of time (except in one 18-month subject that was excluded from axon counts, due to glial migration). (E) Human-specific NF70 (hNF70) labeling reveals persistence of human axons in host white matter 18 months post-grafting, 2mm caudal to the lesion site. Inset shows double labeling of hNF70 and GFP that detects both large

migrating glia and fine axons. **(F)** A confocal z-stack image shows co-localization of fine **GFP+** human axons with **hNF70** in a C8 coronal section at 18 months post-graft. Migrating glial cells and processes are also present, labeled only for **GFP**. Scale bar = 30 μm (A-D); 48 μm (E); 4 μm (F).



Suppl Fig. 7: Human Axons Terminate in Host Gray Matter Regions

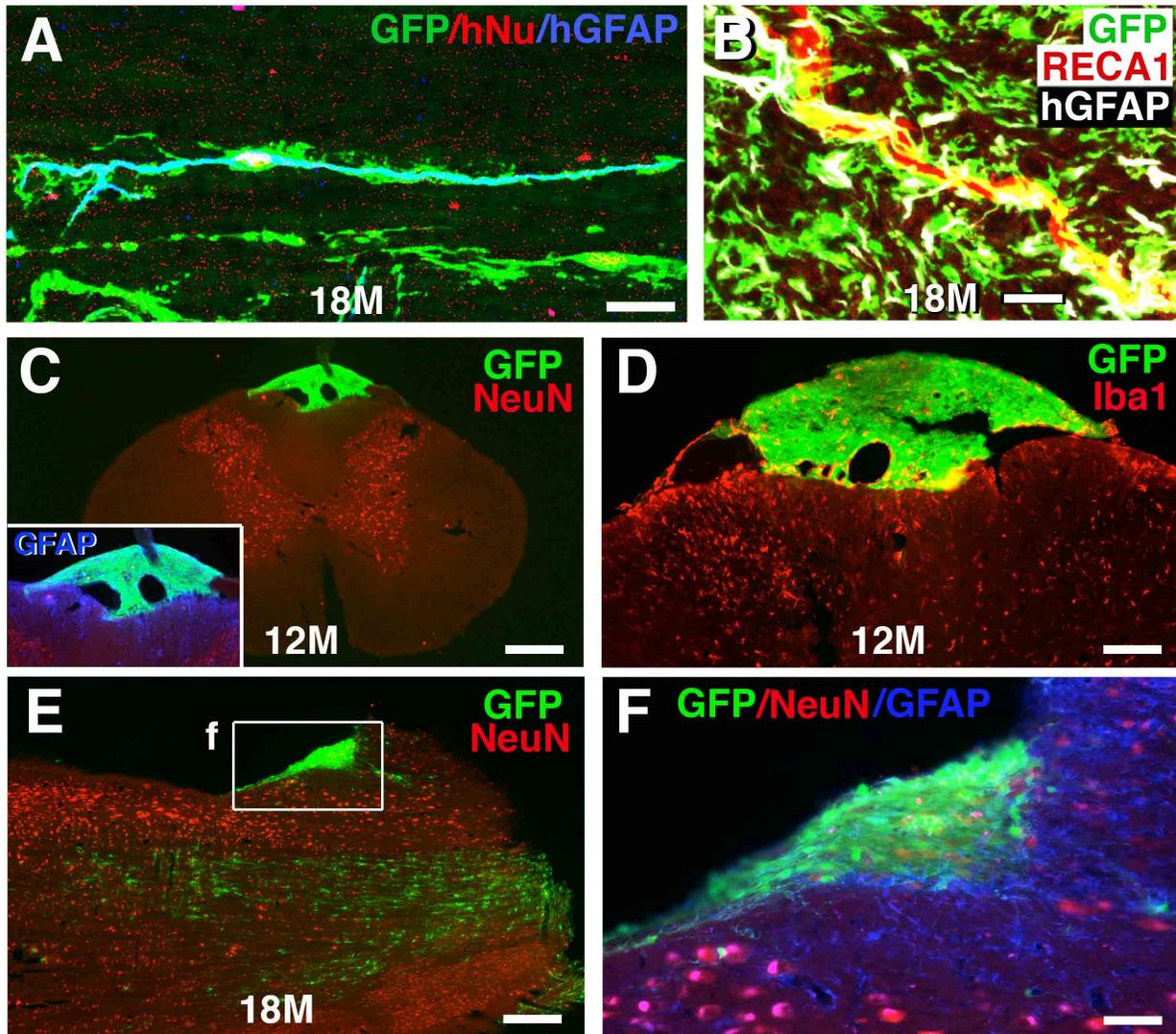
(A) GFP-labeled human axons in host white matter (WM) branch and enter host gray matter (GM, indicated by regions of NeuN labeling), 3 months post-grafting. Axons branch and arborize extensively. Horizontal section, 2mm caudal to graft; dashed lines indicate interface of white and gray matter. (B) GFP+ human axons terminate in different zones of host gray matter, 3 months post-grafting in a coronal section view at C8, 3 segments caudal to the graft. (C-E) High magnification views from boxed area in panel B showing human axons in host (C) dorsal horn (DH) gray matter, (D) intermediate zone (Int) gray matter, and (E) ventral horn (VH) gray matter. Scale bar = 48 μ m (A); 128 μ m (B); 32 μ m (C-D).



Suppl. Fig. 8: Rostral Migration of Human Cells

(A) GFP+ and human nuclear+ (hNu+) cells were observed at C2, 6 mo post-grafting. Low magnification inset indicates section location; NeuN labels host gray matter

neurons. *indicates region of higher magnification view. **(B)** **GFP+** human cells labeled with **hNu+** in ventral medulla (MD), 6 mo post-grafting. **(C-D)** **GFP+** and **hNu+** human cells migrated rostrally toward **(C)** C2 and **(D)** dorsal medulla (MD), 12 months post-grafting. **(E-F)** **GFP+** and **hNu+** human cells migrated into **(E)** C2 and **(F)** medulla (MD) 18 mo post-grafting. **(G)** Thick **GFP+** human cell processes in cerebellum (CERE), 18 mo post-grafting. **(H-I)** **GFP+** human cells do not co-localize with **(H)** the neuronal marker **NeuN**, or **(I)** the oligodendrocyte marker **APC** (horizontal sections, 3mm from graft). Scale bar: A, C, E, 22 μ m; B, 25 μ m; D, F, 32 μ m; G, 40 μ m; H, 25 μ m; I, 18 μ m.



Suppl. Fig. 9: Morphology of Human Migrating Glia

(A) Morphology of a migrating **GFP+**, **hNu+** human cell that co-localizes with the human-specific astrocyte marker GFAP (**hGFAP**) in white matter, 2mm caudal to an 18-month graft site. (B) A migrated **GFP+** and **hGFAP+** (white) human glial cell associated with a host **RECA1+** endothelial cell in host white matter (coronal section at C8, 18 months post-graft). (C) **GFP+** human cells occasionally form a remote cell collection on the surface of host cord (coronal section at T12, 12 months post-graft). Inset shows differentiation of GFAP positive glia. (D) Graft does not express macrophage specific marker **Iba1**, and host cord under ectopic cell deposit does not show upregulation of **Iba1** immunoreactivity. (E) **GFP**-labeled sparse human cell deposit on dorsal surface of brainstem 18 months post-grafting. **NeuN** labels gray matter neurons. (F) A high magnification view from boxed area in E. **GFAP** labels astrocytes. Scale bars: 24µm (A); 13µm (B); 300 µm (C); 120 µm (D); 300 µm (E); 60 µm (F).

Table 1. Distance of Axonal Growth and Glial Migration

Time	Rat ID	Caudal								Rostral													
		C8		T6		T12		L4		C2		BR		CE		MB		HT /ST		FC		OB	
		Axons	Glial	Axons	Glial	Axons	Glial	Axons	Glial	Axons	Glial	Axons	Glial	Axons	Glial	Axons	Glial	Axons	Glial	Axons	Glial	Axons	Glial
1M	1	✓		✓						✓		✓											
	2	✓								✓													
	3	✓		✓						✓													
3M	4	✓		✓		✓		✓		✓		✓		✓		✓		✓		✓		✓	
	5	✓	✓	✓		✓		✓		✓		✓		✓		✓		✓		✓			
	6	✓		✓		✓				✓		✓		✓		✓		✓					
6M	7	✓	✓	✓		✓		✓		✓	✓	✓	✓	✓		✓		✓		✓			
	8	✓	✓	✓		✓		✓		✓	✓	✓	✓	✓		✓		✓					
	9	✓	✓	✓		✓		✓		✓		✓		✓		✓		✓		✓			
	10	✓	✓	✓		✓				✓	✓	✓	✓	✓		✓		✓		✓			
	11	✓		✓		✓				✓	✓	✓	✓	✓		✓		✓		✓			
12M	12	✓	✓	✓	✓	✓				✓	✓	✓	✓	✓		✓		✓		✓			
	13	✓	✓	✓		✓				✓	✓	✓	✓	✓		✓		✓		✓			
	14	✓	✓	✓	✓	✓				✓	✓	✓	✓	✓		✓							
18M	15	✓	✓	✓		✓		✓		✓	✓	✓	✓	✓	✓	✓		✓		✓		✓	
	16	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓		✓		✓					
	17	✓	✓	✓	✓	✓		✓		✓	✓	✓	✓	✓	✓	✓		✓		✓		✓	
	18	✓	✓	✓		✓		✓		✓	✓	✓	✓	✓		✓		✓					

BR, brainstem; CE, cerebellum; MB, midbrain; HT, hypothalamus; ST, stratum; FC, front cortex; OB, olfactory bulb.