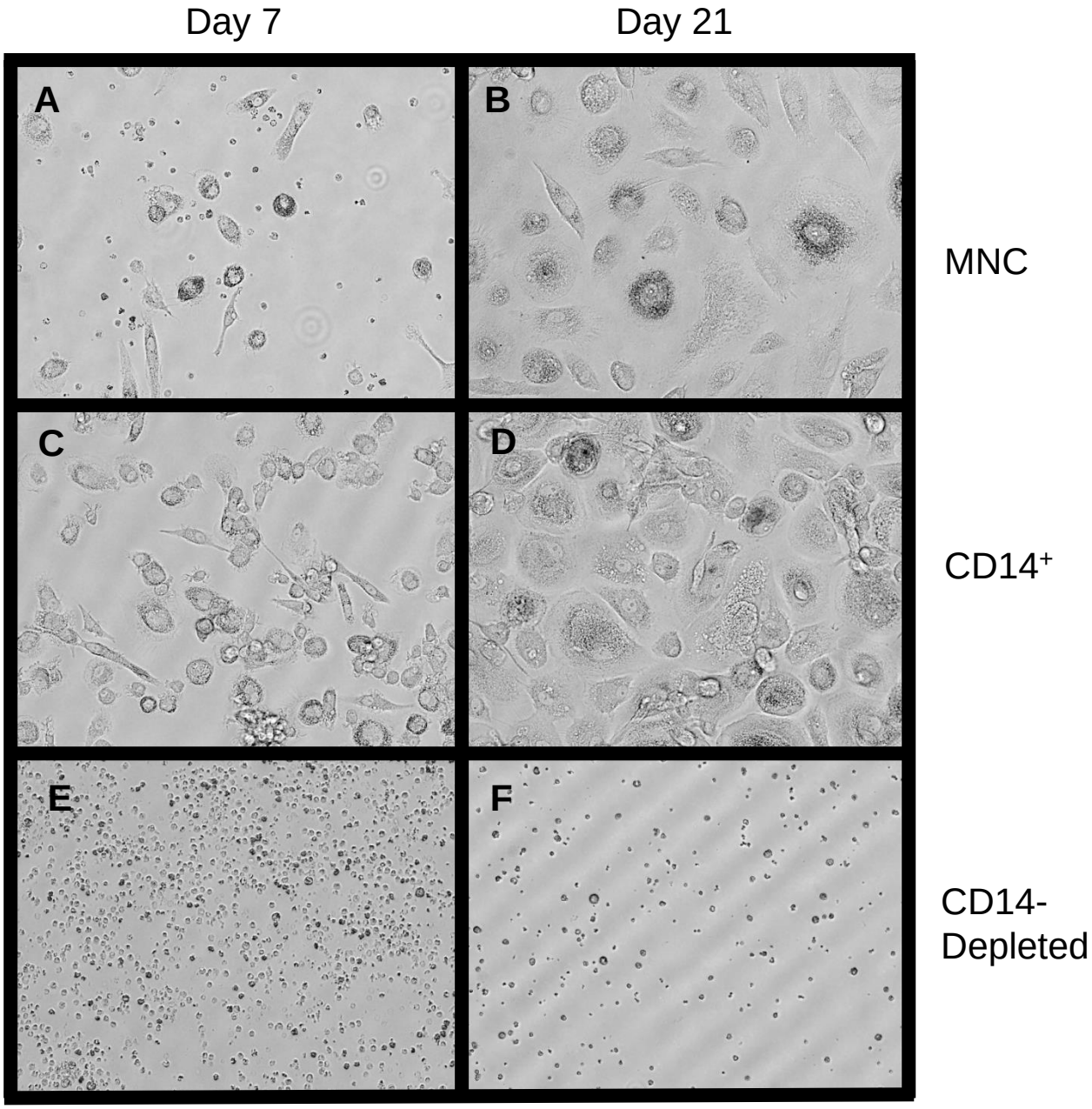
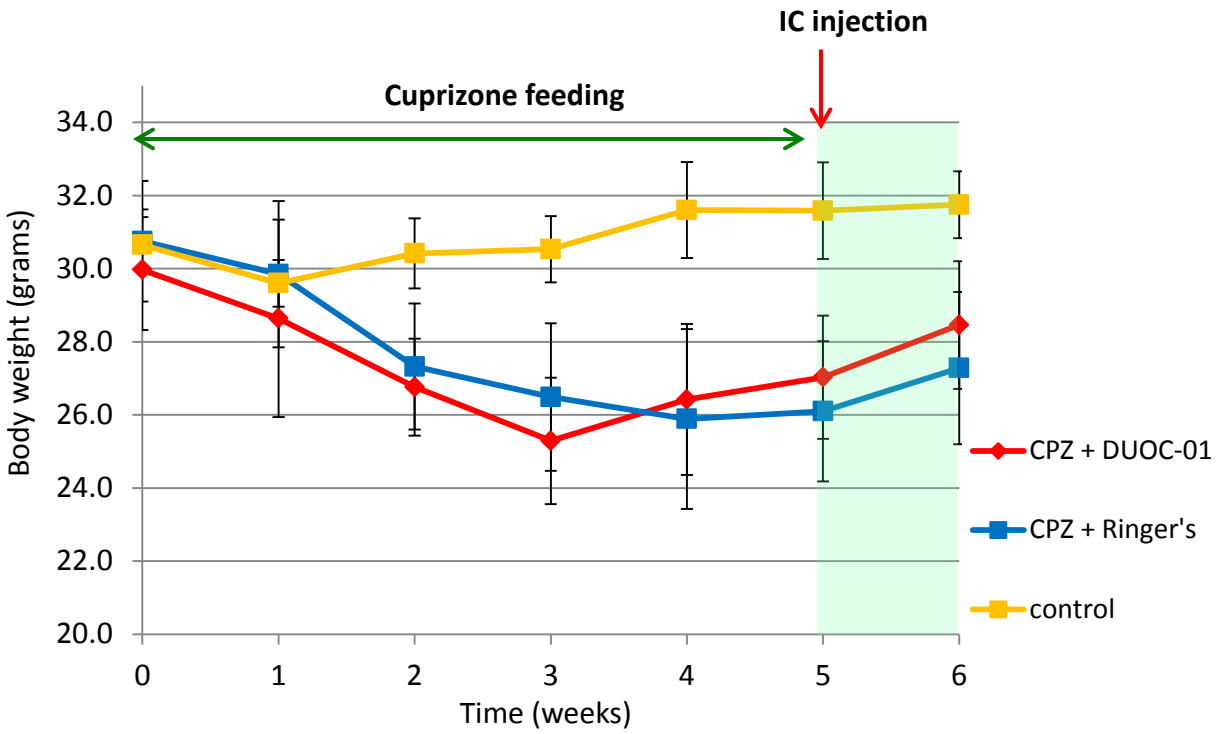
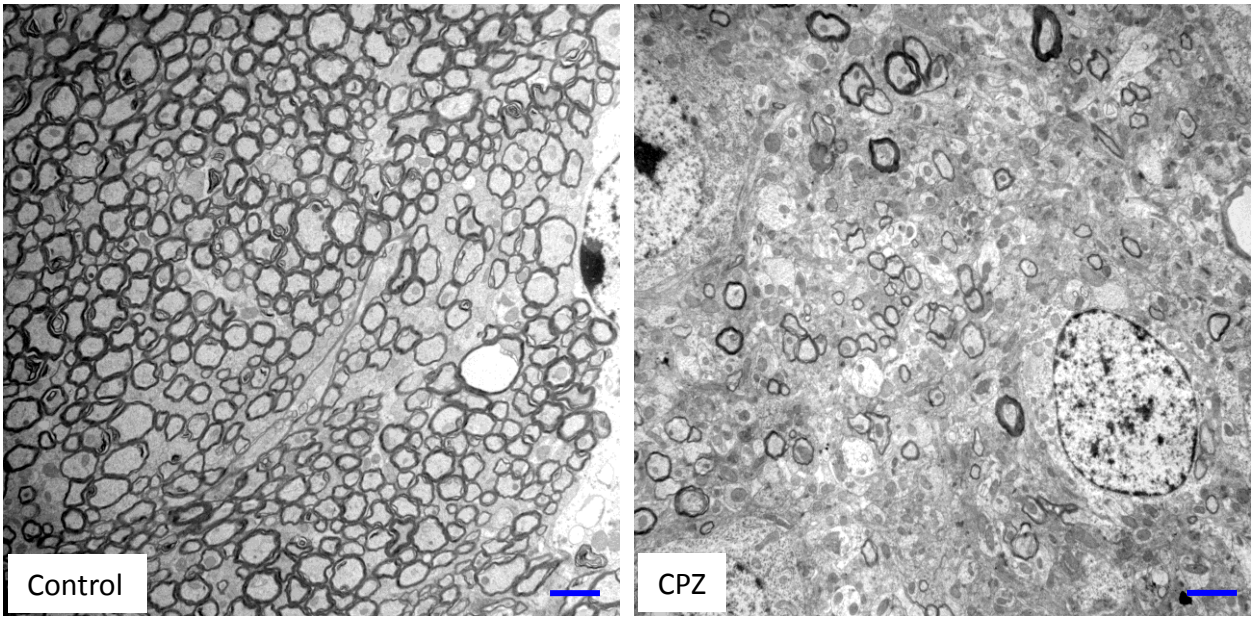


Supplemental Figure 1. DUOC-01 cell product develops from CD14$^+$ monocytes in cord blood. Cultures were established with cord blood mononuclear cells (top row, MNC), with CD14$^+$ monocytes immunomagnetically selected from cord blood MNC (middle row, CD14$^+$), or with CD14-depleted MNC from the same cryopreserved cord blood unit (CD14-depleted, bottom row). The cultures were then maintained following the usual protocol for manufacturing DUOC-01 (1). Photomicrographs (200X) show the appearance of the cultures 7 (A, C and E) and 21 days (B, D and F). The DUOC-01 product is harvested on day 21. Cultures initiated with CD14-depleted cells contain only cellular debris. Cultures initiated with MNC and with CD14$^+$ monocytes are indistinguishable.

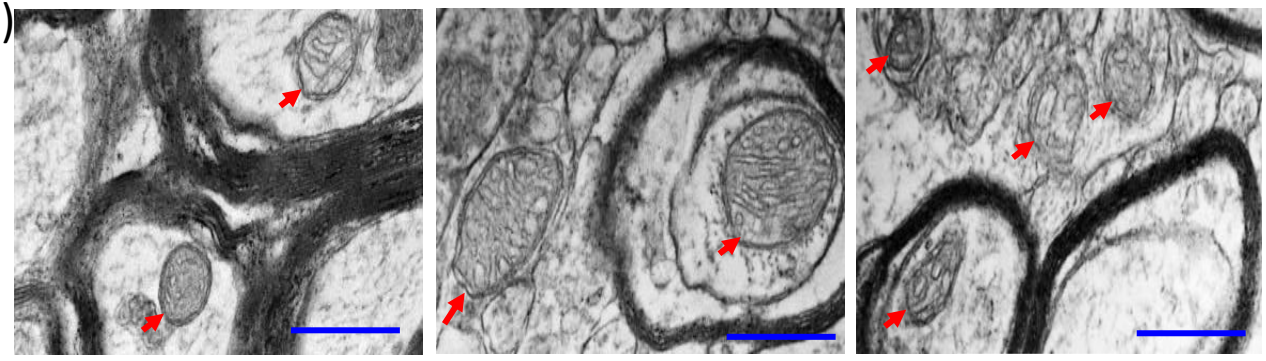


Supplemental Figure 2. Weight loss of NSG mice along the CPZ feeding schedule. Significant weight loss was observed in animals treated with 0.2% CPZ for 5-weeks [red (n=6) and blue [(n=6)] compared to the control mice fed milled chow without CPZ [yellow line (n=5)] for 5 weeks. Red arrow shows time CPZ feeding was stopped, leading to subsequent reversal of weight loss in CPZ fed groups. Mice were treated with DUOC-01 [red] or Ringer's solution [blue] one day after CPZ withdrawal. IC, intracranial.

A)

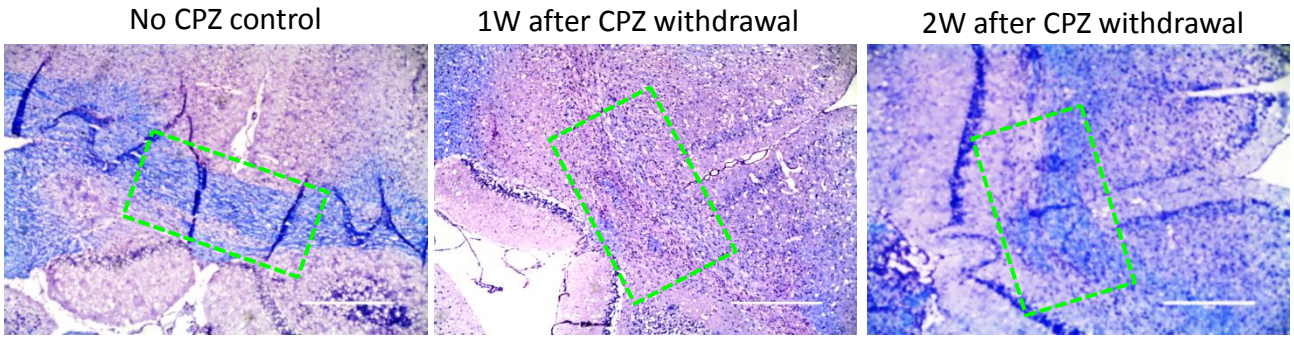


B)

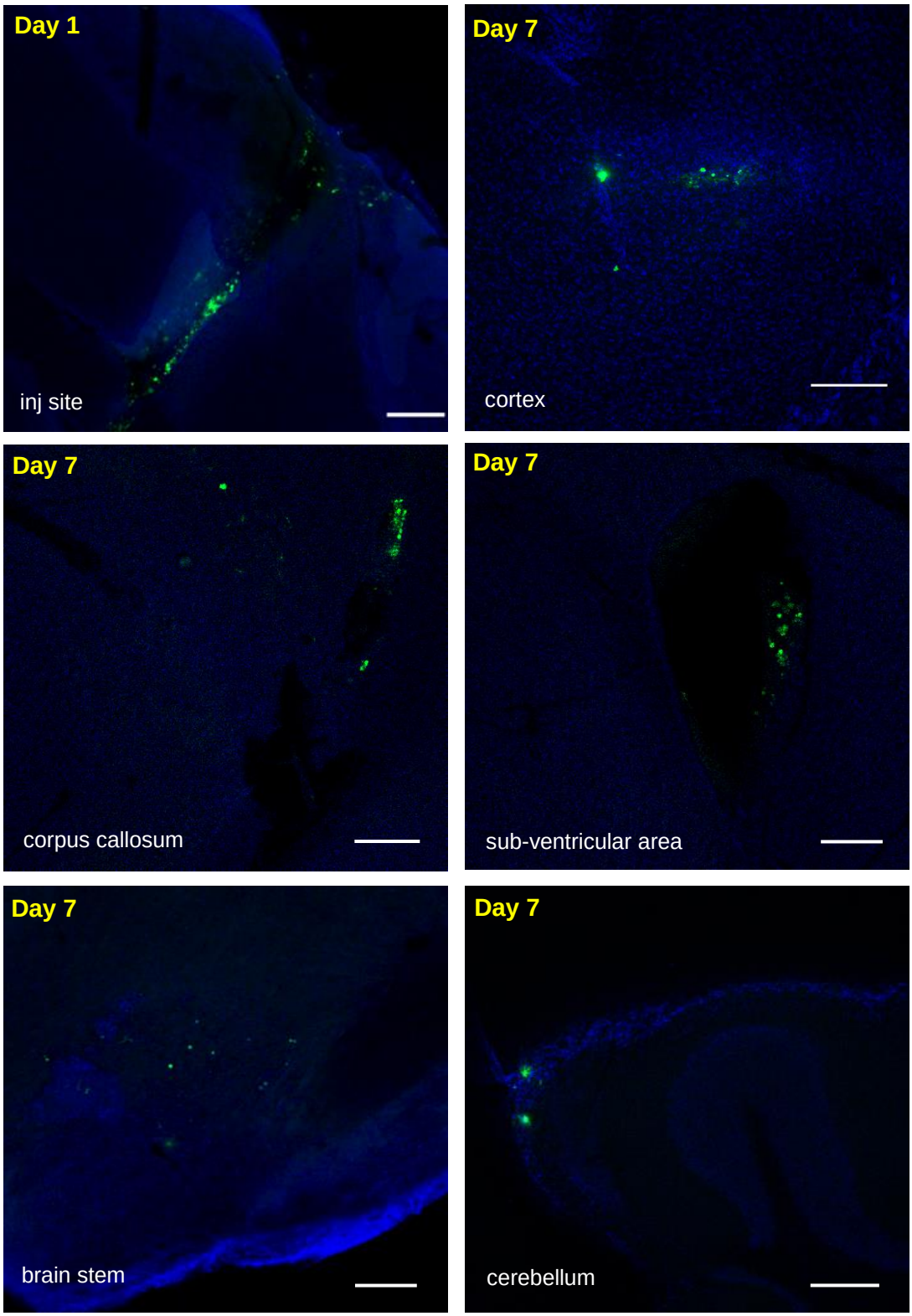


Control Ringer's DUOC-01

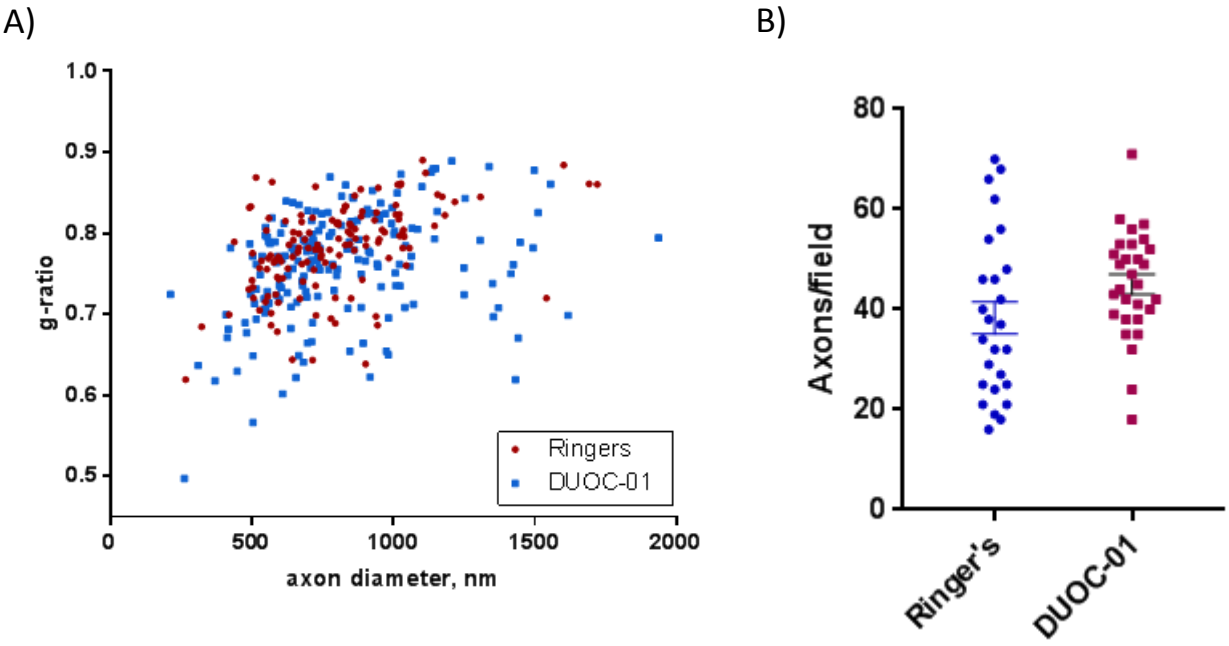
Supplemental Figure 3: Electron Microscopy: (A) Representative electron micrographs of CC of standard chow fed 'control' mice (left panel) and mice fed with CPZ for five weeks (right panel). Original magnification is 2650x. Scale bar is 2.0µm. (B) Representative transmission electron micrograph of CC region of no-CPZ fed control (left panel), CPZ-fed NSG mice one week after treatment with treated with Ringer's (middle panel) or DUOC-01 (right panel) showing differences in mitochondrial size (red arrows). Thicker myelin sheath in the normal control (non-demyelinated, left panel) are evidently visible compared to the re-myelinated brains (middle and right panels). Original magnification=31000x. Scale bar is 500nm.



Supplemental Figure 4: **Spontaneous remyelination following cessation of CPZ feeding**. LFB-PAS staining of mid-line CC regions (dotted green lines) of mice fed normal chow (left panels) or fed CPZ for five weeks and then switched to normal chow for 1 week (middle panels) or two weeks (right panels). Scale bar is 1000µm.

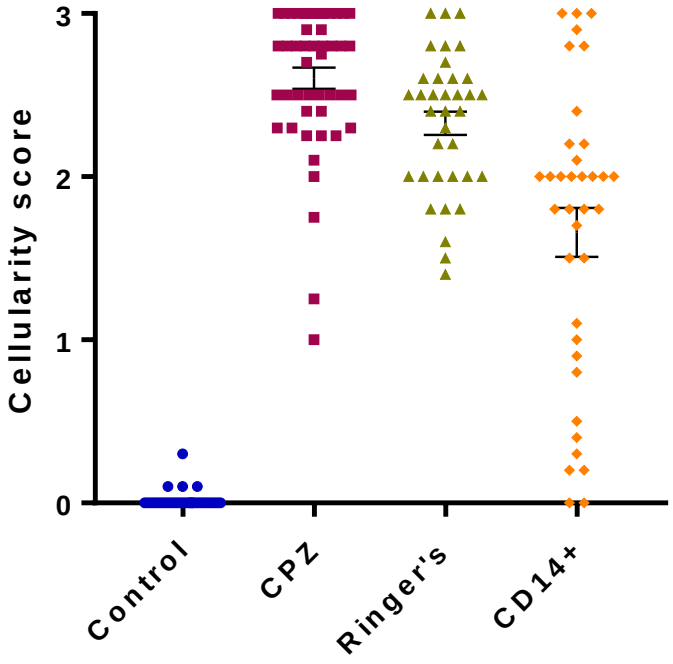


Supplemental Figure 5: CD14⁺ cells disseminated from the injection site and persisted in the brain for up to one week after IC injection: CPZ fed mice were stereotactically injected with CFSE-labelled CD14⁺ cells. All cell nuclei were stained with DAPI (blue). CFSE-labelled (green) CD14⁺ cells were found in numerous parts of the brain including the injection site. Scale bar is 200µm.



Supplemental Figure 6: Morphometric analysis of electron micrographs of CC regions of DUOC-01 and Ringer's-treated mice.

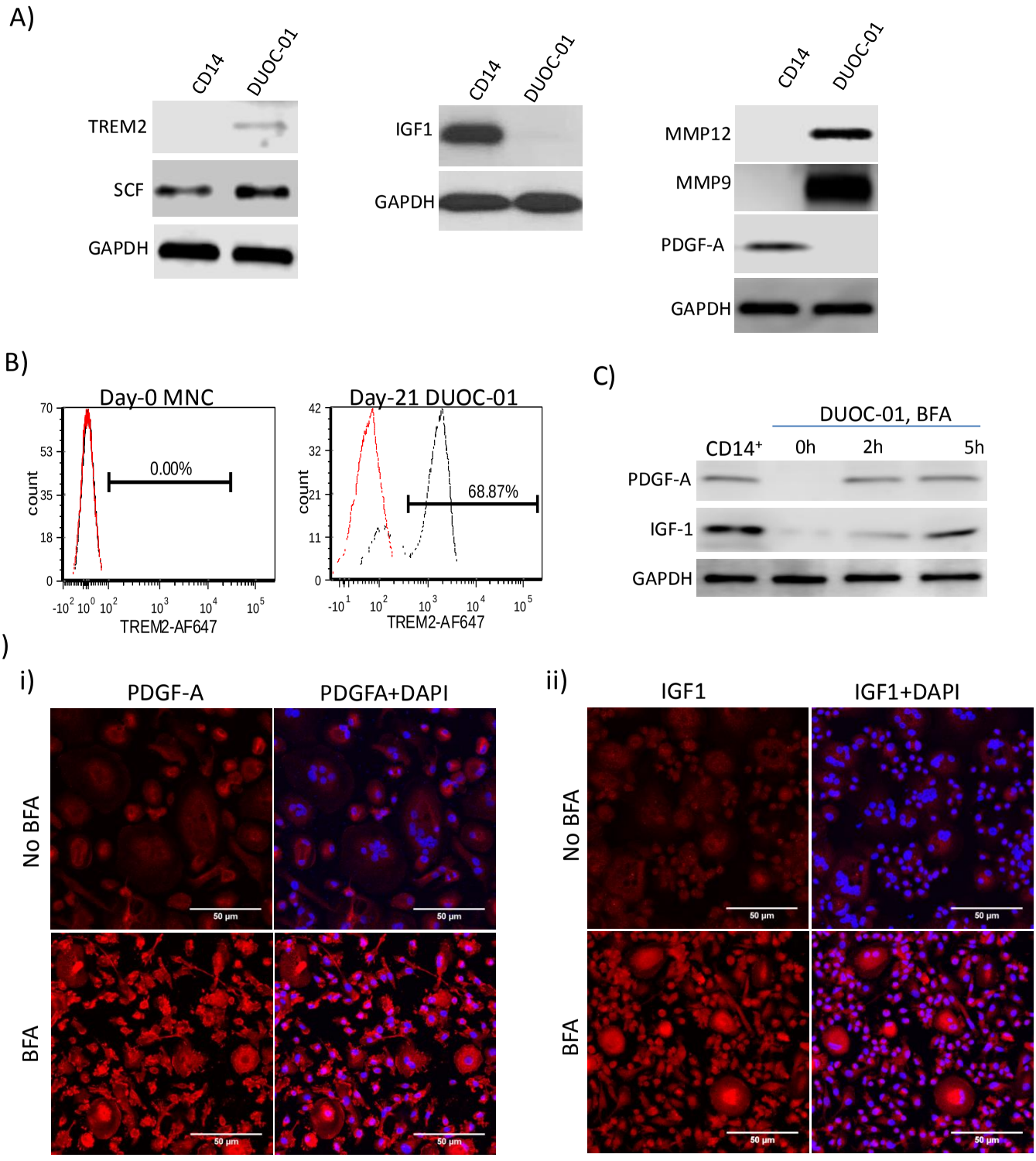
A) G-ratio plotted versus axonal diameters (of myelinated axons) displayed a similar distribution of higher and lower g-ratios across various axon diameter both in Ringer's and DUOC-01 treated groups. **B**) Axonal density as measured by number of axons present per microscopic field (8800x magnification). Although we see a higher axon density in the DUOC-01 treated group compared to the Ringer's control, but the difference was not statistically significant (p-value<0.075).



Supplemental Figure 7: A quantitative cellularity scoring (by counting the nucleus in cc region of LFB stained brain slices) on a scale of 0 to 3, by blinded readers. 'Control' is no cuprizone fed, 'CPZ' is cuprizone fed, 'Ringers' is 1week after Ringer's injection, 'CD14+' is one week after $CD14^{+}$ cell injection. * indicates significance ($n \geq 5$, p-value≤ 0.01697). Statistical comparisons were performed using the Wilcoxon rank sum test for clustered data using the *clusrank* package in R.

Variable	CB1	CB2	CB3	CB4	OC1	OC2	OC3
CB1		0.986	0.985	0.984	0.899	0.905	0.897
CB2	0.986		0.989	0.990	0.879	0.891	0.883
CB3	0.985	0.989		0.990	0.867	0.879	0.869
CB4	0.984	0.990	0.990		0.870	0.883	0.874
OC1	0.899	0.879	0.867	0.870		0.974	0.972
OC2	0.905	0.891	0.879	0.883	0.974		0.984
OC3	0.897	0.883	0.869	0.874	0.972	0.984	

Supplemental Figure 8: Tabular representation of Pearson's co-relation coefficients between the samples, CD14 group (n=4, CB1-CB4) and DUOC-01 group(n=3, OC1-OC3).



Supplemental Figure 9: Protein expression of pro-myelinogenic factors. (A) Western blot analysis of TREM2, SCF, IGF1, MMP12, MMP9 and PDGF-A in DUOC-01 and CB CD14+ cells. GAPDH was used as loading control. (B) Expression of TREM2 in MNCs from cord blood (left panel) and DUOC-01 cells (right panel) as percent of all cells. Analyzed by flow cytometry and compared to isotype controls (red line). (C) Western blot analysis of IGF1 and PDGF-A after brefeldin-A (BFA) treatment for 2 hrs and 5 hrs in DUOC-01. CB CD14+ cells were used as control. GAPDH was used as loading control. Increased abundance of these proteins clearly indicates that both IGF-1 and PDGF-A highly secretory in DUOC-01 cells. (D) Immunocytochemistry of cultured DUOC-01cells. Five hours BFA treated and control cells not treated with BFA, were labeled with specific polyclonal antibodies directed against PDGF-A (i) and IGF1(ii). Nuclei were stained with DAPI. Scale Bars, 50 µm.

SUPPLEMENTAL METHOD:

Manufacture of DUOC-01: Cell products were manufactured from volume-reduced, red cell depleted, and MNC enriched cryopreserved cord blood units (from Carolinas Cord Blood Bank) through the use of validated standard operating procedures. All reagents were qualified for clinical use on the basis of manufacturing history files, certificates of analysis and testing for viruses, endotoxin and sterility. Red cell depletion was carried out with the use of immunomagnetic beads coated with anti-glycophorin A antibody. Isolated mononuclear cells from each CBU unit were plated in 75-mm^2 flasks (Corning Inc., Corning, NY, USA) at a concentration of 5×10^6 (for cryopreserved UCB) in 15 mL growth medium containing alpha-MEM (Gibco, USA), 10% fetal calf serum (HyClone, USA), insulin–transferrin–selenium supplementation, penicillin/streptomycin (Gibco), 2 mM L-glutamine (Gibco), 0.5 ng/mL platelet-derived growth factor (PDGF; PeproTech, USA), 0.16 ng/mL neurotrophin-3 (NT-3; PeproTech), 0.4 ng/mL vascular endothelial growth factor (VEGF; PeproTech) and 60 ng/mL triiodothyronine (T3 ; Sigma, USA). At day 7, half the medium was exchanged for fresh growth medium. At day 14, half the volume of medium in each flask was exchanged for an equal volume of medium containing neurocult NS-A basal medium (Stem Cell Technologies), neurocult NS-A proliferation supplement (Stem Cell Technologies, Canada), PDGF, VEGF and NT-3 in concentrations as above. At day 17, half the volume of medium in each flask was by an equal volume of the growth medium (used in the very beginning) was added.

Immunoblot analysis and antibodies: Cell lysates were prepared in 1x RIPA buffer (R0278, Sigma, USA) in the presence of the protease inhibitor cocktail(P8340, Sigma, USA). 50 µg of protein lysates were electrophoretically separated on 4–20% Mini-PROTEAN® TGX™ polyacrylamide gels (4561096, Bio-Rad, USA) and transferred to polyvinylidene fluoride (PVDF) membranes (162-0177, Bio-Rad, USA). Membranes were blocked for 1 hr. at room temperature in 5% dry milk in TBS buffer containing 0.2% Tween-20, followed by overnight incubation with primary antibodies; anti-TREM2 (1:500, ab175262, Abcam, USA), anti-SCF (1:1000) (ab83866, Abcam), anti-IGF1 (1:1000, ab9572, Abcam), anti-MMP12 (ab52897, 1:1000, Abcam, USA), anti-MMP9 (ab76003, 1:10000, Abcam, USA) and anti- PDGFα (1ug/ml, AB-221-NA, R&D systems). Anti-GAPDH (1:1,000, 5174, Cell Signaling Technology) was used as the loading control. Primary antibodies were detected using HRP-conjugated anti-rabbit (1:5,000, ab6721, Abcam), or anti-goat (1:5000, ab6741, Abcam) secondary antibodies. HRP activity was visualized using the Clarity Western ECL Kit (catalog#1705060, Bio-Rad, USA).

BFA treatment and immuno-staining: Day-21 DUOC-01 cells were re-plated at the density of $1.7x10^5$/well in the Lab-Tek II 8-well Chamber slides (cat#154534, Nalgen Nunc International) overnight. The cells were treated with or without 5µg/ml of Brefeldin A (B7651, Sigma, USA) for five hours. The cells were washed with DPBS, fixed with 4% PFA, then blocked for one hour in blocking buffer (2% BSA, 0.2% triton-X100 in DPBS). The cells were incubated with primary antibodies at RT for two hours: PDGF-α (1:200, AB-221-NA, R&D systems), and IGF-1 (1:200, ab9572, Abcam), followed by anti-goat or anti-rabbit secondary antibody (AlexaFluor-568, 1:500, Life Technologies) for one hour. Slides were mounted in Vectashield mounting medium with DAPI (Vector Laboratories) and analyzed using Leica SP8 fluorescence confocal microscope.

Flow cytometry: MNCs or DUOC-01 cells ($1.0x10^5$ cells in each case) in 50 µl of flow buffer (PBS+1%HSA) were incubated with anti-human TREM-2 monoclonal rat antibody conjugated to Alexa Fluor 647 (R&D Systems #FAB17291R) or with rat IgG2b-AF647 (R&D Systems) as isotype control, incubated on ice for 15 min, washed with 1 ml flow buffer and acquired on a BD FACSCanto II flow cytometer. Cells were gated first on SSC/FSC and then on viability using either SytoxBlue (Invitrogen) or 7AAD (Invitrogen). FCS files were analyzed using FCS-Express Software.