Supplementary Figure S1: Integration and glial differentiation of intraretinally grafted PDGF-neurosphere cells.

Integrated EGFP-positive donor cells (some labeled with arrowheads in a, e) were detectable in the inner layers of host retinas as early as one week after transplantation of PDGF-neurospheres (a, e). Donor cells were either differentiated into astrocytes (arrowheads in b) or into oligodendrocytes that had myelinated small areas of the nerve fiber layer (asterisk in f). A fraction of grafted cells remained in the vitreous where they formed a cell layer that was attached to the vitread site of the retina (marked with asterisks in e). The number of EGFP-positive donor cells and donor-derived astrocytes (some labeled with arrowheads in c and d) in the inner retinal layers was significantly
increased four weeks after transplantation (c, d) when compared to the one week post-transplantation interval (a, b). At the sites of cell injections (arrows in b, d), expression of GFAP was elevated in endogenous astrocytes and Müller cells one (b) and four weeks after transplantation (d). PDGF-neurospheres were grafted into 5 (a, b) and 6 (c-f) days old mice. EGFP, enhanced green fluorescent protein; GFAP, glial fibrillary acidic protein; inl, inner nuclear layer; ipl, inner plexiform layer; MBP, myelin basic protein; nfl, nerve fiber layer; onl, outer nuclear layer; PDGF, platelet-derived growth factor. Bar in (f) for (a-f): 100µm.

Supplementary Figure S2: The distribution of myelin in the retina and optic nerve of control animals.

In control animals with intraretinal injections of PBS, myelin basic protein (MBP)-immunoreactivity was restricted to the distal region of the optic nerve, while the nerve fiber layer and intrabulbar (one asterisk) and proximal retrobulbar (two asterisks) regions of the nerve were MBP-negative. Analysis was performed four weeks after
intraretinal injection of PBS into a four days old mouse. nfl, nerve fiber layer; PBS, phosphate-buffered saline. Bar: 100µm.

Supplementary Figure S3: Ultrastructural analysis of myelinated ganglion cell axons in the retinal nerve fiber layer.

Electron microscopic analysis of a retina with grafted PDGF-neurospheres revealed the presence of numerous myelinated retinal ganglion cell axons (ax) in the nerve fiber layer (a; some non-myelinated axons are labeled with asterisks). Myelin sheaths display the normal ultrastructure of central nervous system myelin (a, b; b is a higher magnification of the boxed area in a). The retina was analyzed five weeks after intraretinal transplantation of PDGF-neurospheres into a five days old mouse. ax, axon; M, myelin; PDGF, platelet-derived growth factor. Bar (in a): 1µm; in (b): 0.5µm.
Supplementary information

Electron microscopy

Selected regions of retinas with a heavily myelinated nerve fiber layer were immersion-fixed in phosphate-buffered saline (pH 7.4) containing 4% paraformaldehyde and 2% glutaraldehyde (Serva, Heidelberg, Germany) overnight at 4°C. Tissue was then immersed in 2% osmiumtetroxide (Science Services, Munich, Germany) for 2 hours at room temperature, dehydrated in an ascending series of methanol, and embedded in Epon 812 (Serva). Ultrathin sections were counterstained with lead citrate (Sigma) and examined with a LEO 912 AB OMEGA electron microscope (Leo Elektronenmikroskopie, Oberkochen, Germany).