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# SUPPLEMENTARY MATERIAL

**corresponding to:**

# Neural cells and their progenitors in regenerating zebrafish spinal cord

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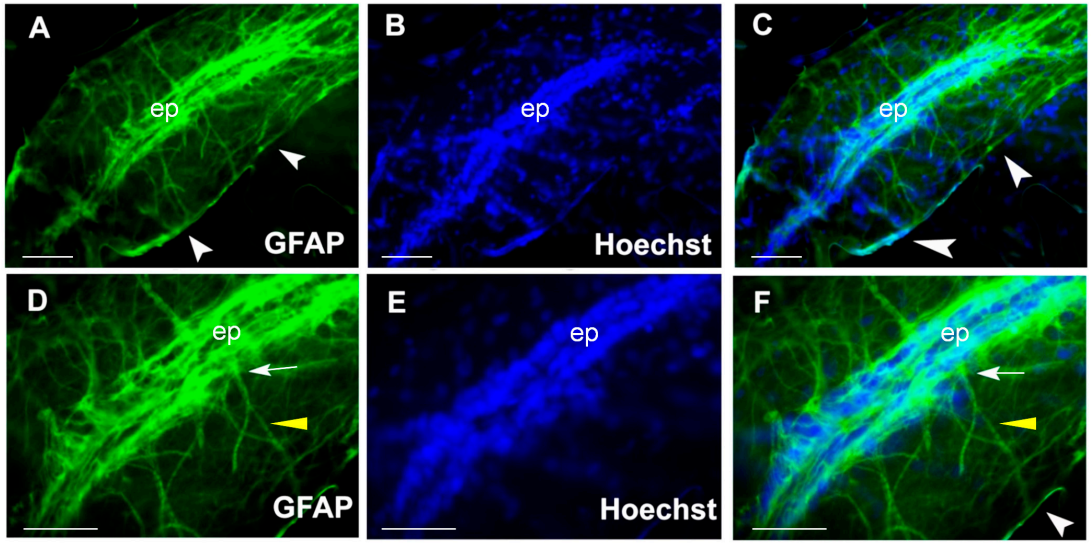
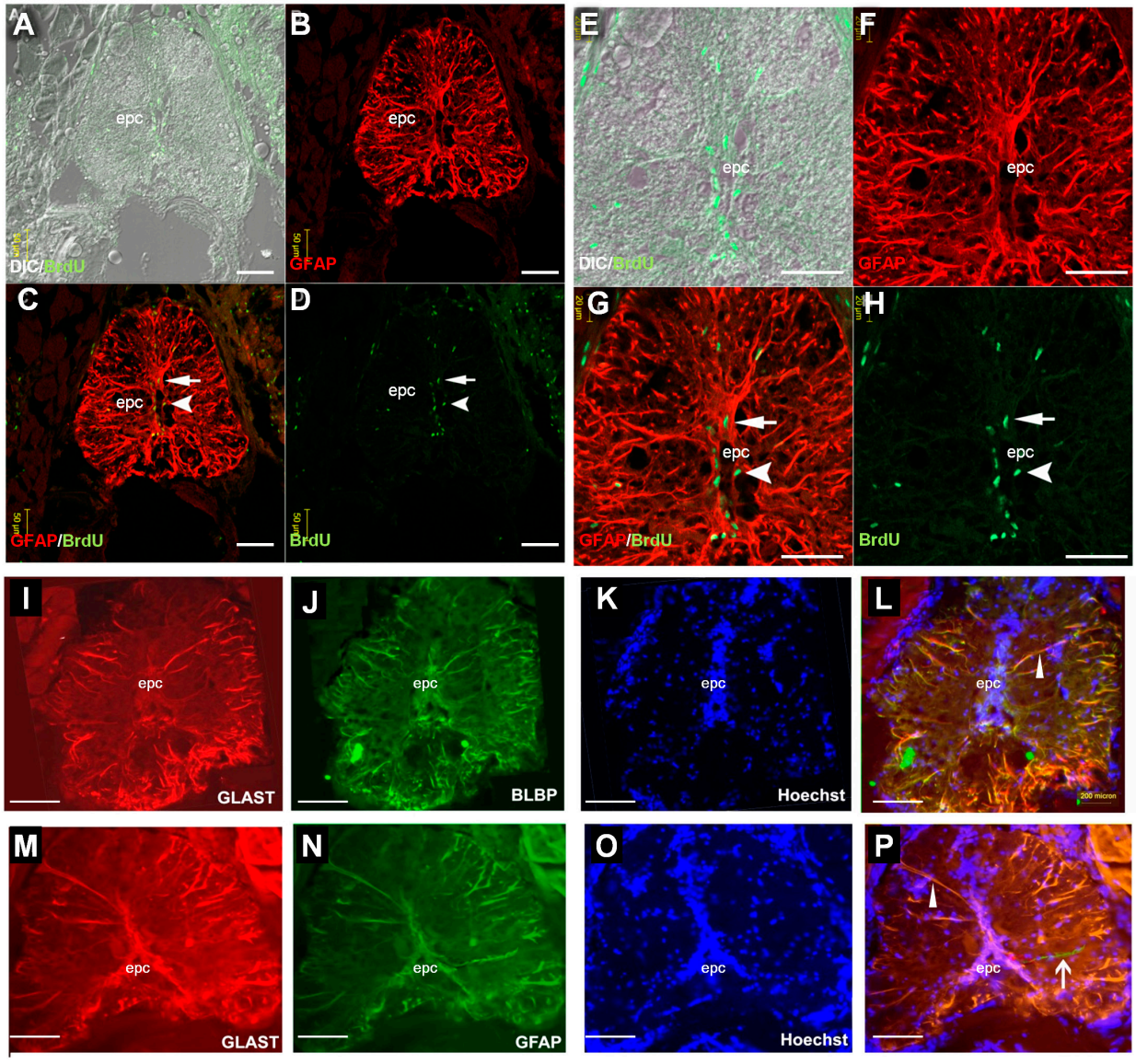
SUPPLEMENTARY TABLE1

**COMPARISONS BETWEEN UNINJURED AND INJURED CORD HIGHLIGHTING DISTINCT ULTRASTRUCTURAL FEATURES**

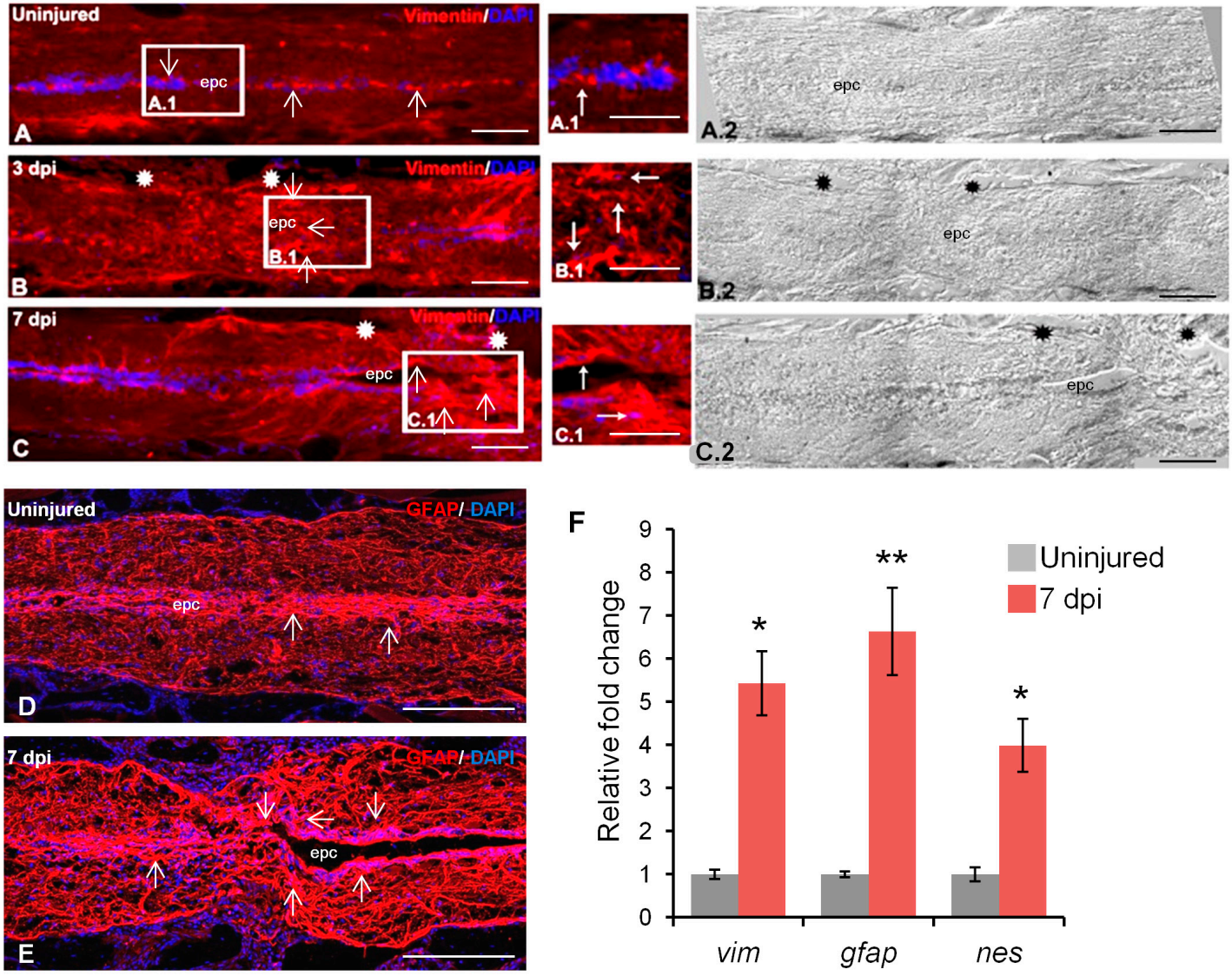
| | **Ultrastructural characters** | | **Antigenic markers used** | **References** |
|---|---|---|---|---|
| **Uninjured cord** | Resident microglia | Scanty cytoplasm, large nucleus with peripheral heterochromatin | - | Kalman 2004 |
| | Ependymal cell/ astroglia | Radial glia with extended microvilli towards lumen, present around the ependyma; desmosomes and intermediate filaments. | GFAP | Bernstein & Bernstein 1967<br>Yamada et al 1997 |
| | Neuron | Homogenously dispersed heterochromatin, large pale nucleus and thin rim of cytoplasm<br>Electron dense nucleus with scanty heterochromatin | HuC/D | Peters et al 1991<br>Hui et al 2010 |
| | Axon both myelinated and unmyelinated | Axoplasm showing mitochondria, neurofilament, compact myelination in myelinated axons | | Peters et al 1991 |
| **Injured cord**<br>*Early inflammatory stage* | Peripheral macrophage | Phagocytic macrophages with elongated nuclei, clumps of patchy chromatin near nuclear envelope, relatively dense cytoplasm with inclusions (myelin debris and lipid vacuoles and lysosomes) | N/D | Hui et al 2010<br>Van Ham et al 2014 |
| | Apoptotic neurons | Dark irregular mitochondria, retracted cytoplasm, fragmented nucleus | TUNEL Staining | Hui et al 2010 |
| | Degenerating axons | Hypertrophic appearance, loss of neurofilaments, void spaces within axon | | |
| *Proliferative response* | | | | |
| Neuronal progenitor | | High N/C ratio, less cytoplasm and few organelles in early stage. Proliferating progenitors arealong with astroglial filaments.<br>Appearance of organelles mitochondria, ribosome and Golgi bodies in newborn but specified neuron. | HuC/D, Sox2 & Brdu positive | Rakic 1971 |
| Glial Progenitor of | | | | |
| a) Astrocytic lineage | | Dark nucleus, scanty cytoplasm with intermediate filaments and glycogen granules. | | Yamada et al 1997<br>Kalman 2004 |
| b) Oligodendrocytic lineage | | N/C ratio high, less cytoplasm with clumps of heterochromatin,short RER, no intermediate filaments | NG2/BrdU | Peters et al 1991 |
| c) Schwann cell progenitor | Presence of Schwann cell progenitors(SCP) | Presence of SCP with basal membrane, remyelinating denuded axons | PNS like Collagen and Krox 20) N/D | Nona et al 1992<br>Hui et al 2010 |
| Astrogliosis after injury | | Hypertrophy of astroglial cells, bundles of intermediate filaments, increased organelles (mitochondria , Golgi and RER and glycogen granules) and junctional complexes | Increased synthesis of GFAP, vimetin, nestin | Bignami and Dahl 1976<br>Dimou and Gotz 2014 |
| **Late injury response**<br>*Remyelination and synapse formation* | Presence of denuded axons, remyelinating axons and various synapses | Axon terminal with synaptic vesicle and glycogen granules and mitochondria. | N/D | Peters et al 1991 |




**Supplementary Fig. 1. Immunohistochemical localization of GFAP in radial glia in uninjured zebrafish spinal cord. (A-C)** *Longitudinal section immunostained with GFAP, Hoechst and merged image, respectively.* **(D-E)** *Higher magnification image of A to C. Note that soma of the radial glial cell (arrow) present around the ependyma (ep), yellow arrowhead indicates glial fibre and white arrowheads indicate end feet of glial cell near the pial membrane. Scale bar, 50 μm.*




**Supplementary Fig. 2. Immunohistochemical analysis of GFAP & and BrdU in injured and radial glial sub-type markers in uninjured spinal cord. (A-D)** *Transverse sections of 7 dpi spinal cord showing GFAP positive cells colocalized with BrdU positive nucleus (arrow), some cells are also not colocalized with BrdU (arrowhead) around ependymal canal (epc).* **(E-F)** *Higher magnification view of the same section shown in A and B.* **(G-H)** *Higher magnification view of C and D.* **(I-J)** *A transverse section of uninjured cord immunostained with GLAST and BLBP antibody respectively, showing radial glia around ependymal canal (epc).* **(K)** *Hoechst nuclear staining of same section shown in I and J.* **(L)** *Merged image of I and J shows presence of a both GLAST and BLBP positive radial glia (arrowhead).* **(M-N)** *A transverse section of a cord showing GLAST and GFAP immunostained radial glia around ependymal canal respectively.* **(O)** *Hoechst nuclear staining of same section of M and N.* **(P)** *Merged image of M and N, shows existence of a both GLAST and GFAP positive radial glia (arrowhead) and a GFAP positive but GLAST negative radial glia (arrow). Figures I-P have been adapted from Hui* et al.*, 2015. Scale bar, 50 μm.*




**Supplementary Fig. 3. Expression of intermediate filament markers in progenitors. (A-C)** *Longitudinal section of uninjured, 3 dpi and 7 dpi spinal cord immunostained with vimentin and nucleus stained with DAPI, respectively.* **(A.1-C.1)** *Higher magnification view of boxed areas of A to C, respectively showing vimentin immunoreactive radial glia.* **(A.2-C.2)** *Respective DIC image of A to C.* **(D-E)** *Longitudinal sections of uninjured and 7 dpi spinal cord immunostained with GFAP and nucleus stained with DAPI, respectively.* **(F)** *The relative expression of intermediate filament genes* vim, gfap *and* nes *in 7 dpi spinal cord compared to uninjured spinal cord by qRT-PCR represented as fold change. (mean ( SEM, n = 5)* *P *< 0.05,* **P *< 0.01, Mann–Whitney* U *test. Scale bar, 100 μm (A-E, A.1-C.1, A.2-C.2).*