Supplemental Information

Transformation of the Radial Glia Scaffold Demarcates Two Stages of Human Cerebral Cortex Development

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Supplemental Figures and Legends

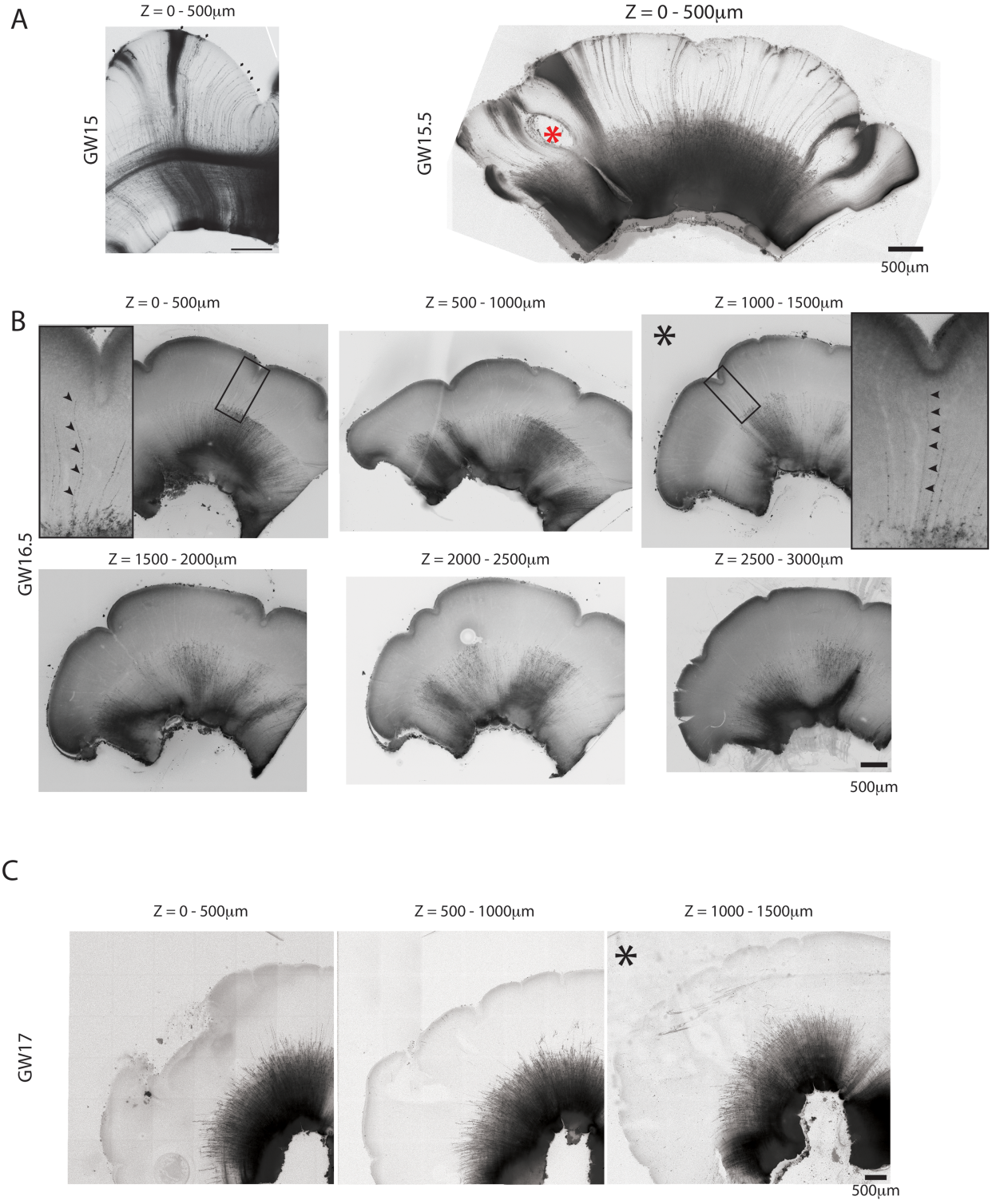

Figure S1: Truncation of the Ventricular Radial Glia Fibers, Related to Figure 1. Additional examples of DiI labeled primary tissue samples from a range of developmental stages. Z indicates tissue depth calculated based on imaging of consecutive 500μm tissue slices. **(A)** Ventricular labeling of one GW15 and two GW15.5 specimens reveals abundant fiber bundles extending to the pial surface. Red asterisk indicates mechanical tissue damage acquired during processing. **(B-C)** Slices through GW16.5 **(B)** and GW17 **(C)** specimens show that the majority of ventricular radial glia have truncated fibers,

but sparse examples of fibers extending to the pial surface can still be identified at GW16.5 (arrows). Multiple 500μm tissue slices are displayed to ensure no broad fiber bundles reaching the cortical plate can be identified through the depth of the tissue. Asterisks indicate slices that are also presented in Figure 1.

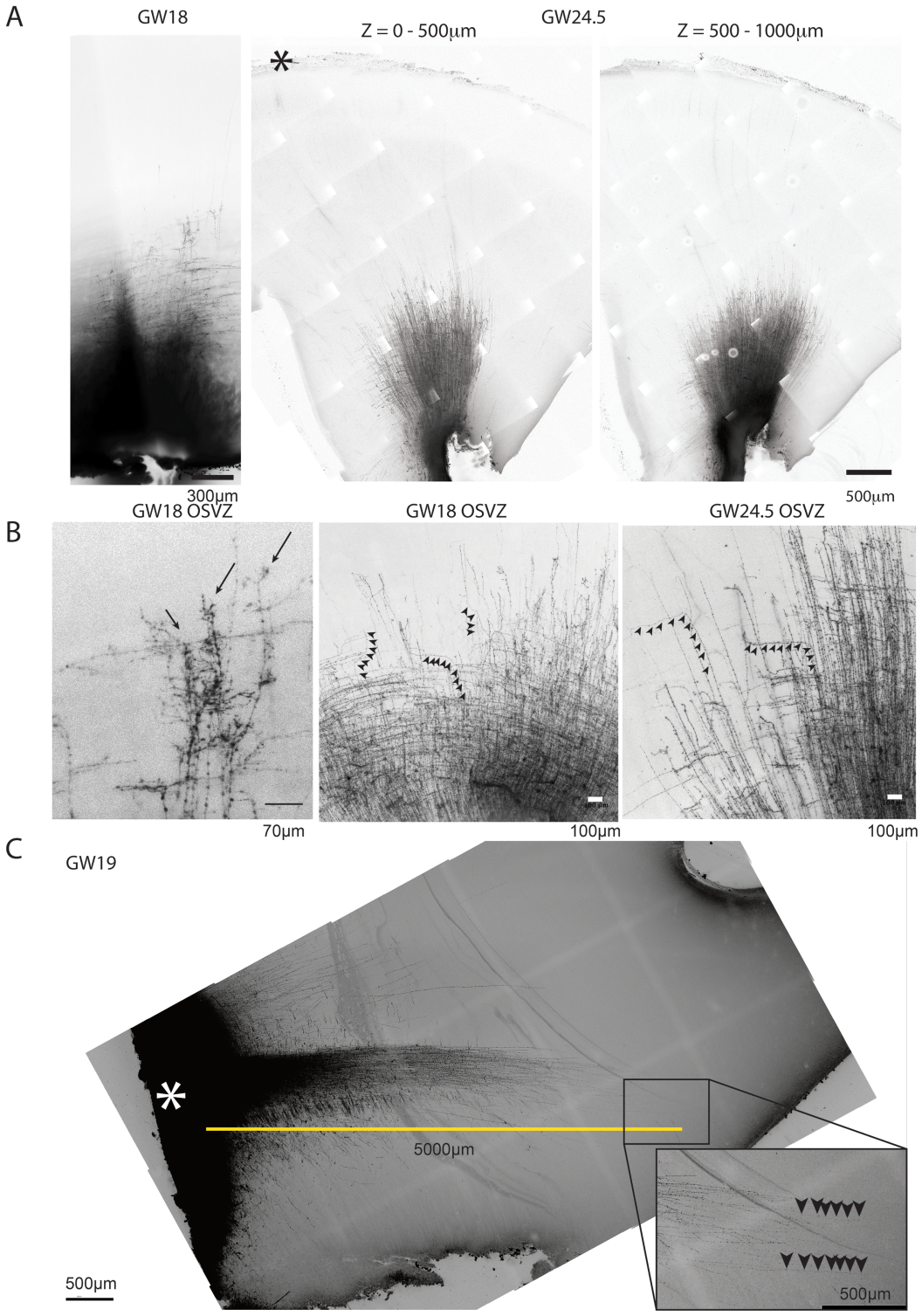

Figure S2: Truncated Radial Glia Cells Persist Throughout Neurogenesis, Related to Figure 1. (A) Ventricular labeling of the radial glia fibers after GW17 reveals radial fibers extending into the OSVZ persist despite the loss of pial connections through GW24. Asterisk indicates slice also shown in Figure 1. **(B)** Representative examples of OSVZ fiber endings of

truncated radial glia. Arrows indicate example of growth-cone like morphologies and arrowheads indicate segments of individual fibers with tangential orientation. **(C)** Axonal fiber tract labeling of a GW19 specimen – following tissue dissection, DiI crystals were placed against the OSVZ region and incubated for 6 days. Over that period, axonal fibers labeled by DiI extended as far as 5000μm away from the crystal. Inset image shows magnified view of the farthest-extending fibers indicated with arrows. Asterisk indicates site of DiI crystal placement.

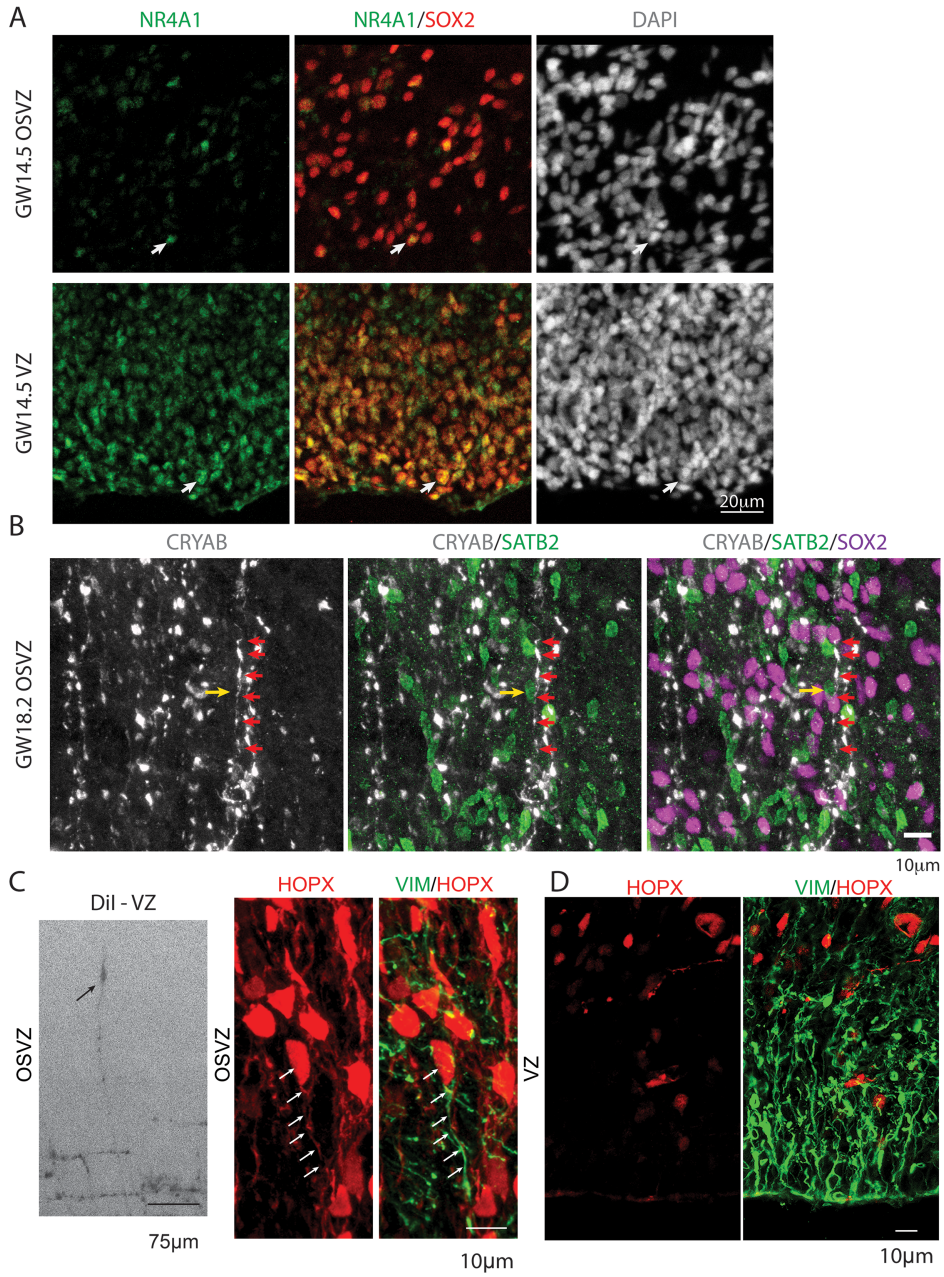

Figure S3: Diversity of Radial Glia Contacting the Ventricular Surface, Related to Figures 1, 2, and 3. (A) Higher magnification overview of NR4A1 staining of VZ (bottom row) and OSVZ (top row) radial glia. Arrows indicate examples of SOX2 and NR4A1 double-positive cells with nuclei counterstained with DAPI. **(B)** Additional example of CRYAB positive truncated radial glia fibers (example indicated with red arrows) in the OSVZ juxtaposed by SATB2 positive neuronal cell body (yellow arrow). **(C)** Left image shows an example of a rare OSVZ cell with direct contact with the ventricular surface in a GW18 sample (Hansen et al., 2010). Right panel shows an example of a HOPX positive oRG cell with the primary fiber directed towards the ventricle. **(D)** Representative staining for HOPX in the VZ, where in contrast to the cortical plate at GW18. VIM positive fibers do not double-label with HOPX.

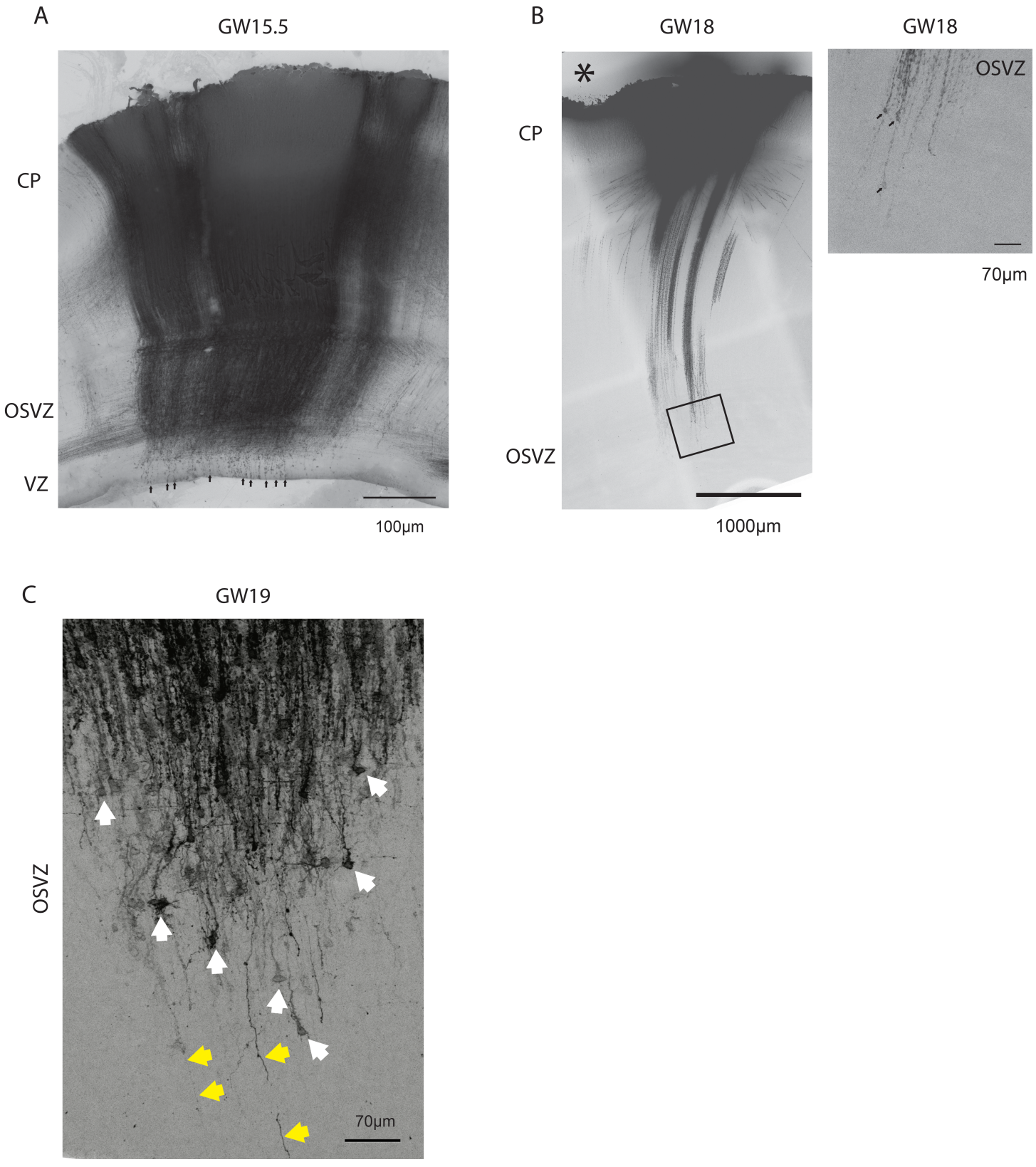

Figure S4: The Discontinuous Scaffold Originates from Cell Bodies in the OSVZ, Related to Figure 4. (A) DiI labeled at the pial surface in primary tissue samples. **(A)** Pial labeling at GW15.5 specimens reveals abundant fiber bundles and labeling of cell bodies in the VZ (arrows). **(B)** Pial labeling at GW18.5 shows that fibers terminate in the OSVZ with cell bodies

highlighted by arrows in the right panel. Asterisk indicates image that is also presented in Figure 1. (C) Labeling of a bundle of fibers labeled by DiI at the pial surface showing abundant termination in cell bodies (white arrows), and sporadic axonal projections extending past the oRG cell bodies (yellow arrow).

Table S1: Analysis of Single Radial Glia mRNA Sequencing Data, Related to Figure 2. Tab "Radial Glia" summarizes single cell mRNA sequencing datasets reanalyzed in the current manuscript. Cell identification numbers are preserved as distributed through dbGAP (Pollen et al., 2015) and NCBI Gene Expression Omnibus (Camp et al., 2015). Tab "WGCNA modules" lists gene membership for the modules depleted ("green") or enriched ("brown") in tRG cells (see Figure 2A and Experimental Procedures). Note that genes in the "green" module are further enriched in oRG cells and include transcripts that were previously shown enriched in oRG cells around GW16-18, such as HOPX, LIFR, FAM107A (Pollen et al., 2015; Thomsen et al., 2016). Genes enriched in radial glia in samples younger than GW16 are listed in the "magenta" module.

Supplemental References

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