Supplementary Figure 1. Variable distances of Olig2+ cell migration into the retina. (a) DAPI and Olig2-stained retina from an E11 embryo to show extent of Olig2+ cell migration (DAPI only shown). Boxed regions are shown at higher resolution in "b," "c," "d," and "e". Red lines indicate distance between distal-most boxed regions, the boxes c and e show the distal-most area occupied by cells and the optic nerve head. Scale = 500um (b) Olig2 stain of region close to the optic nerve head (c) Olig2 stain of region furthest from the optic nerve head, which has Oligo2+ cells. (d) Olig2 stain of region close to optic nerve head on opposite side of optic nerve head. (e) Olig2 stain of region slightly further from optic nerve head showing no GCL cells positive for Olig2, reflecting the dramatic difference in glial distribution that can occur even within a single retina (compare to c). (f) Retina from an E15 embryo stained for Olig2 and DAPI (shown in blue), boxed region represents image in "g". (g) Olig2 staining showing extensive Olig2+ cells in GCL. (h) Olig2 staining of E9 retina showing Olig2+ cells in the ONL. Scale "a" and "f" = 500um, Scale other panels = 50um Green line = optic nerve head. Panels c-e and g-h show an orthogonal cross-section of the confocal stack on the bottom and side of the panel with an arrow indicating which nuclear layer is shown in main body of the panel.

Supplementary Figure 2. Development of glial cells. (a-b) Glial cells infected with pQ-mGFP virus were examined for cell morphology at different ages. Cell morphology at E14 showing shorter and thicker bipolar processes. (c-d) Cell morphology at E16 showing bipolar cells extending extra processes (c) and cells with many processes

resembling mature morphology (d). (e-f) Cell morphology at E18 with more cells resembling mature morphology (e) and a few branched bipolar cells (f).

Supplementary Figure 3. Morphological parameters quantified for the three cell types. (a'-a''') Image analysis for process asymmetry parameter as diagramed in Figure 5a. (a'), diacytes (a''), and astrocytes (a''') of its processes in the IPL and FL was measured. (b'-b''') A software-assisted filament tracing software (Bitplane's Imaris FilamentTracer) was used to get the 3-D process length of 10 of the longest IPL or FL processes for each cell (diagramed in Figure 5b), as exemplified in (b') for oligodendrocytes, (b'') for diacytes, and (b''') for astrocytes. (c'-c''') 20 processes for each cell had their diameter manually measured in Imaris (diagramed in Figure 5c) as shown in (c') for oligodendrocytes, (c'') for diacytes, and (c''') for astrocytes. FL and IPL processes were quantified separately. (d) Nucleus quantification for the three cell types (as diagramed in Figure 5d), with nucleus distance being the % of depth the nucleus is within the IPL (distance 1/distance1+distance2). (d'-d''') Shows how nuclear volume was quantified using the isosurface feature in Imaris that created a 3-D "cast" of the nucleus and could output the precise volume of the cast (d''''). (e) quantification of each morphological parameter across the three cell types. Characteristic measured is labeled on the top of the graph, numbers on each bar are the number of cells assayed for that characteristic. Blue = diacyte, maroon = astrocyte, green = oligodendrocyte.

Supplementary Figure 4. Examples of glial clone shapes and sizes. (a) A short glial clone, tapering off as it extends from the central (left side of image) to peripheral retina

(right side of image). (b) A glial clone, composed of three discrete clusters of cells. (c) A long, relatively uniform, wide glial clone with many cells (red arrow), and a smaller, somewhat shorter glial clone (yellow arrow). These two “clones” are an example of what may be a splitting error, i.e. they be subclones of the same clone. As discussed in the text, even if they are subclones, both subclones comprise all three glial cell types. Scale in "a" and "b" = 200um. Scale in "c" = 500um.

Supplementary Figure 5. Distribution of presumptive infected macrophages in the chick retina. (a) Low magnification view of presumptive macrophages in the posthatch chick retina after E3 injection into the heart. Scale = 500um (b) Presumptive macrophages show irregular cellular morphology. Scale = 50um

Supplementary Figure 6. Quantification of glial clone coverage and Ganglion cell axon density. The fraction of glial cells contributed by glial clones of different densities was estimated. Here the GFP+ cells within a clone are shown in green and the Olig2+ cells within the area of the clone are shown in red. Software-assisted quantification was employed to quantify the number of red and green cells. (a,b) and (c,d) are a matched pair of images for two different clones. Tick marks = 10um. (a, b) Sparse glial clone with only 2.5% of the Olig2 positive cells being GFP+. (c, d) Dense glial clone with 10% of the Olig2 positive cells being GFP+. (e,f) Quantification of ganglion cell axon fascicle distribution across the surface of the posthatch retina. (e) Raw image of neurofilament staining for peripheral retina (f) Raw image of neurofilament staining for central retina.

Supplementary Table 1. Estimation of clone size. 1=small (<50 cells), 2=medium (50-200 cells), 3 = large (>200 cells). G = GFP+ clones, R = tdTomato+ clones. If there are tdTomato as well as GFP+ clones in the retina, tdTomato-labeled clones are indicated by "R=" while GFP-labeled clones are indicated by "G=", if there is no letter indicated, only GFP-labeled clones were in that retina. The notation used if there are multiple clones in a retina of a given size is given by the size in parenthesis, for instance, a retina with 5 clones with more than 200 cells (size "3") is labeled 5(3).