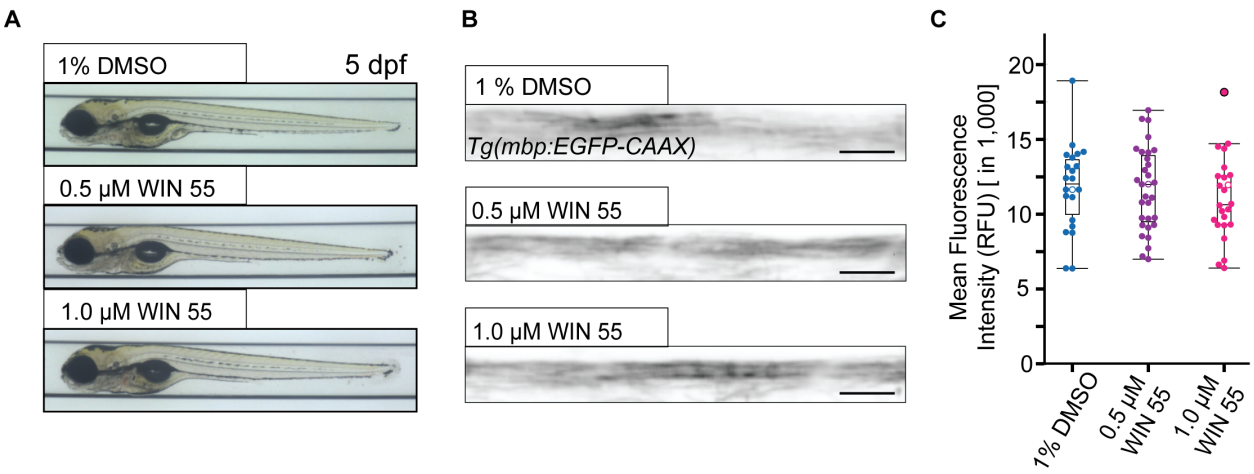
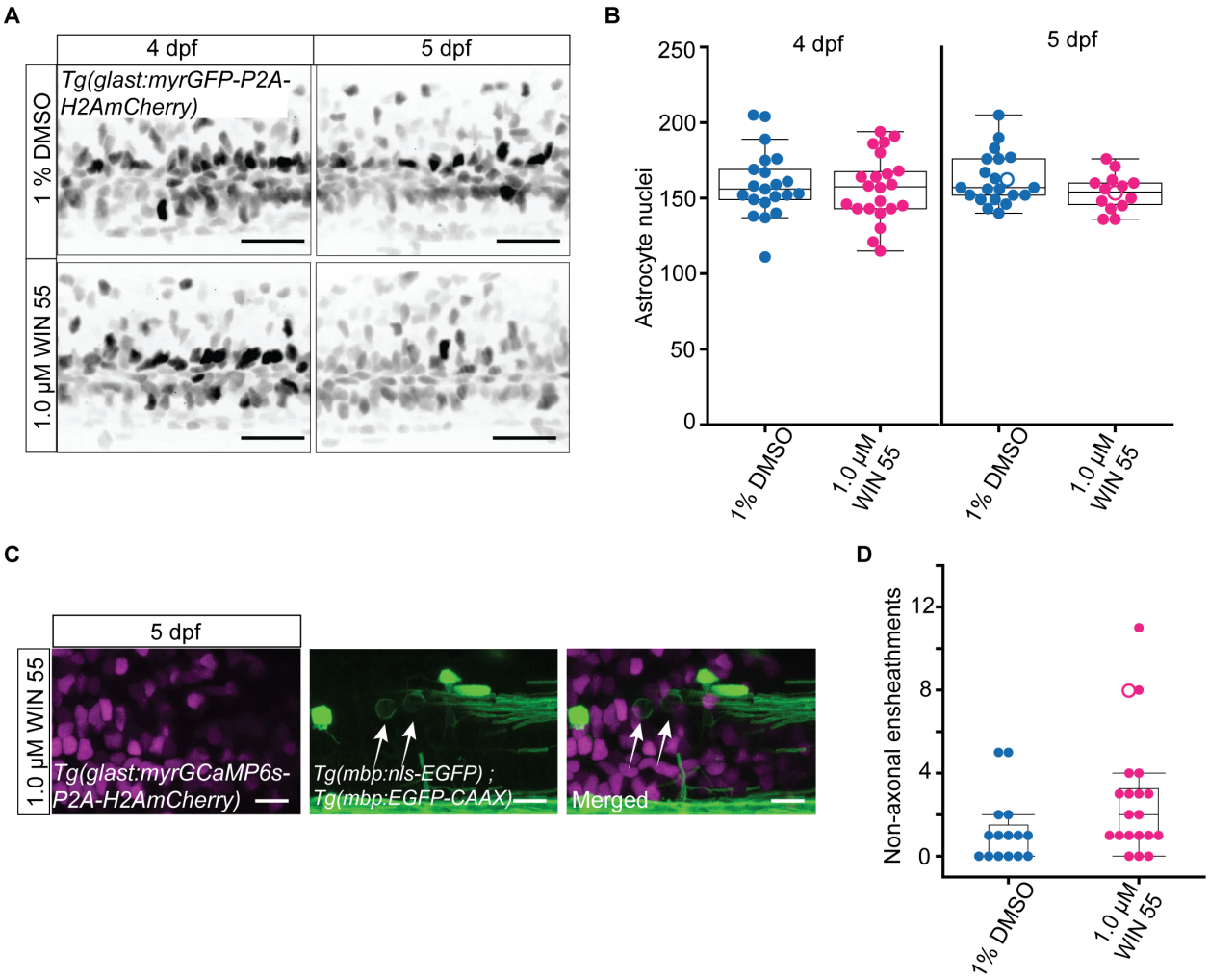
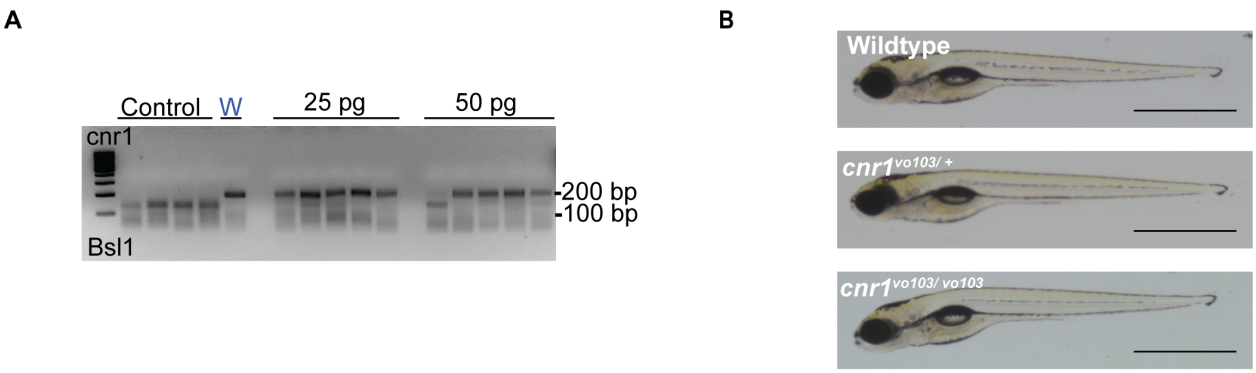


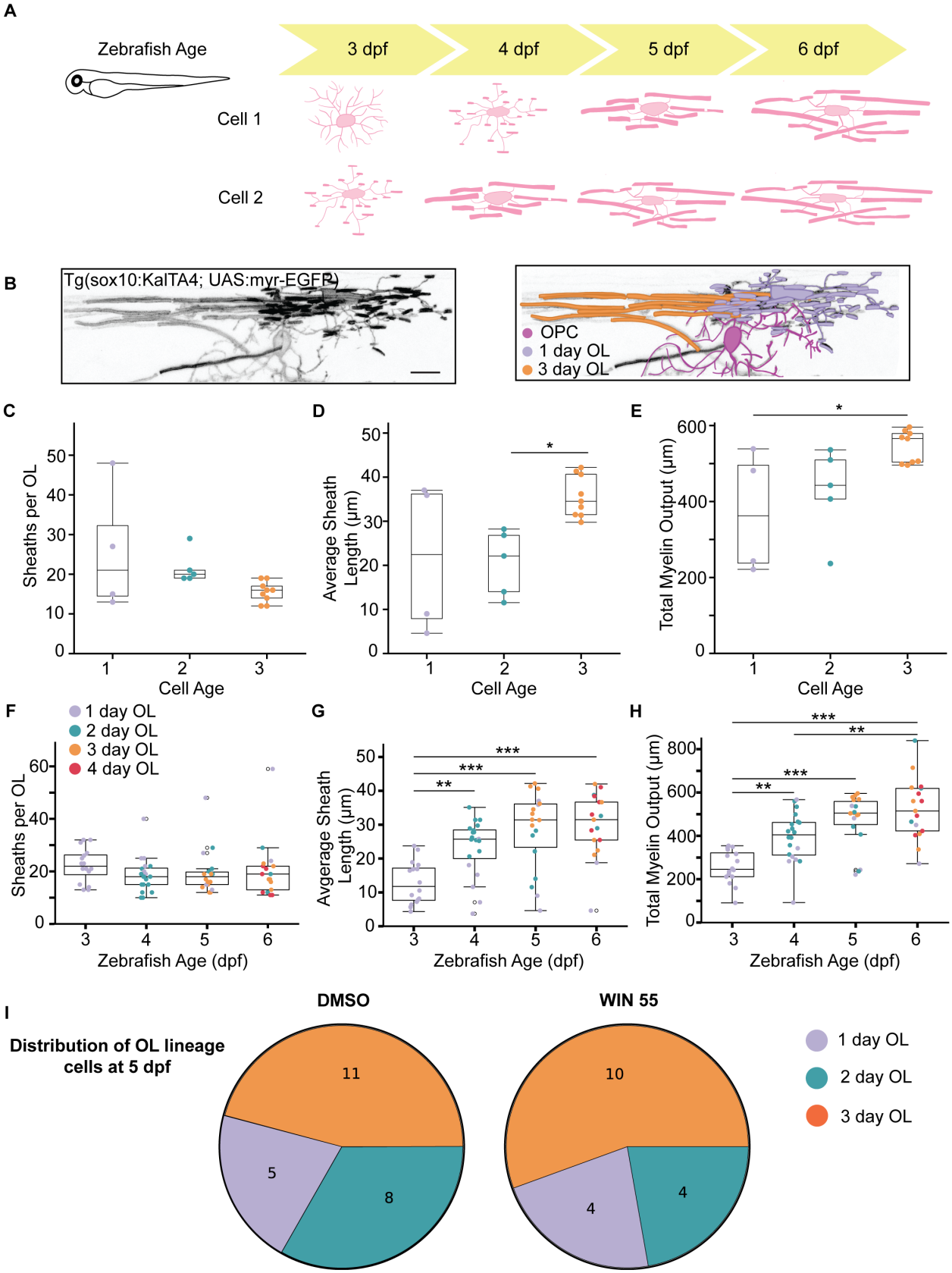
Supplemental Figure 1: **WIN 55 treatment does not change morphological development or overall mbp:EGFP fluorescence intensity.** (**A**) Representative images of fish at 5 dpf after vehicle or WIN 55 (0.5 or 1.0 µM) treatment. (**B**) Representative sum projection images of dorsal spinal cord sections used to calculate mean fluorescence intensity values at 5 dpf for vehicle and WIN 55 (0.5 or 1.0 µM) treatment. Scale bar = 25 µm. (**C**) Mean fluorescence intensity quantification of dorsal spinal cord sections at 5 dpf in relative fluorescence units (RFU). Each dot represents one fish, quantification from representative images in (**B**) shown as hollow dots. 4dpf: DMSO (n = 28), WIN 55 0.5 µM (n = 41), WIN 55 1.0 µM (n = 37), 5 dpf: DMSO (n = 22), WIN 55 0.5 µM (n = 31), WIN 55 1.0 µM (n = 24). Kruskal-Wallis Test.



Supplemental Figure 2: **WIN 55 treatment does not affect the number of astrocytes or lead to ectopic wrapping of astrocyte cell bodies.** (**A**) Representative max projection images of *Tg(glast:myrGFP-P2A-H2AmCherry)* zebrafish mounted laterally. Zebrafish treated with DMSO control or WIN 55 (1.0 µM) at 4 and 5 dpf. Scale bar = 25 µm. (**B**) Quantification of the number of astrocytes nuclei in the spinal cord of zebrafish at 4 dpf (left) treated with DMSO control (n = 21) or WIN 55 (1.0 µM) (n = 22) and at 5 dpf (right), DMSO control (n = 21) and WIN 55 (1.0 µM) (n = 14). Quantification from representative images in (**A**) shown as hollow dots. Two-tailed unpaired t-test. (**C**) Representative images of laterally mounted transgenic zebrafish *Tg(glast:myrGCaMP6s-P2A-H2AmCherry*) and *Tg(mbp:EGFP-CAAX:mbp:nls-EGFP)* at 5 dpf treated with WIN 55 (1.0 µM). White arrows highlight non-axonal ensheathments. Scale bar = 10 µm. (**D**) Quantification of non-axonal ensheathments in zebrafish treated with DMSO control or WIN 55 (1.0 µM) at 5 dpf. Hollow dot highlights representative image in (**C**).



Supplemental Figure 3: **Global *cnr1* mutant zebrafish larvae show no abnormal development.** (**A**) Example of amplification of *cnr1*^{vo103} allele and restriction enzyme digestion with Bsl1 showing efficiency of Cas9 gene editing at 25 and 50 pg concentrations injected in zebrafish embryos on 3% agarose gel. CRISPR/Cas9 targeting disrupts a Bsl1 cut site resulting in 189 bp band uncut (mutant) and two bands 130 and 62 bp cut (WT). W denotes undigested control. (**B**) Representative images of wildtype, *cnr1*$^{vo103/+}$, and *cnr1*$^{vo103/vo103}$ zebrafish larvae at 5 dpf. Scale bar = 1 mm.



Supplemental Figure 4: **There is significant variability between individual OL cell development in age-matched control animals** (**A**) Schematic demonstrating how the previous method of analysis was matched to the zebrafish age. (**B**) Z at different developmental time points in a zebrafish imaged at 6 dpf. (Left) Max projection image (Right) Pseudo colored image highlighting the 3 different cells. The magenta represents an OPC, the lilac cell represents a cell defined as a 1-day-old OL, the orange represents a 3-day-old OL. Scale bar = 10 μm. (**C - E**) Quantification of the number of cells in each OL cell stage in DMSO treated fish at 5 dpf when quantifying (**C**) the number of sheaths per OL (**D**) Average Sheath Length (**E**) Total Myelin Output. One-way ANOVA, Tukeys HSD post hoc comparison, * = $p \leq 0.05$. (**F - H**) Quantification of (**F**) sheaths per OL (**G**) average sheath length (**H**) total myelin output across zebrafish age and the distribution of OL lineage cells of OLs in DMSO control zebrafish larvae at 5 dpf. ** = $p \leq 0.01$ *** = $p \leq 0.001$. (**I**) Distribution of OL lineage cells in DMSO and WIN 55 treated zebrafish at 5 dpf.

Supplemental Movies:

Supplemental Movie 1: Representative video through triple transgenic zebrafish spinal cord Tg(nbt:DsRed) and Tg(mbp:nls-EGFP;mbp:EGFP-CAAX) at 5 dpf, 1% DMSO treated larva on the left side and WIN 55 (1.0 μM) treated larva on the right side. White arrowhead denotes hollow non-axonal ensheathment and pink arrowhead highlights non-axonal ensheathment around a neuronal cell body. Scale bar = 10 μm.

Supplemental Movie 2: Representative video through transgenic zebrafish spinal cord treated with WIN 55 (1.0 μM) *Tg(glast:myrGCaMP6s-P2A-H2AmCherry)* and *Tg(mbp:EGFP-CAAX:mbp:nls-EGFP)* at 5 dpf. Scale bar = 10 μm.