

Supplemental Information

**The Aryl Hydrocarbon Receptor Pathway Defines
the Time Frame for Restorative Neurogenesis**

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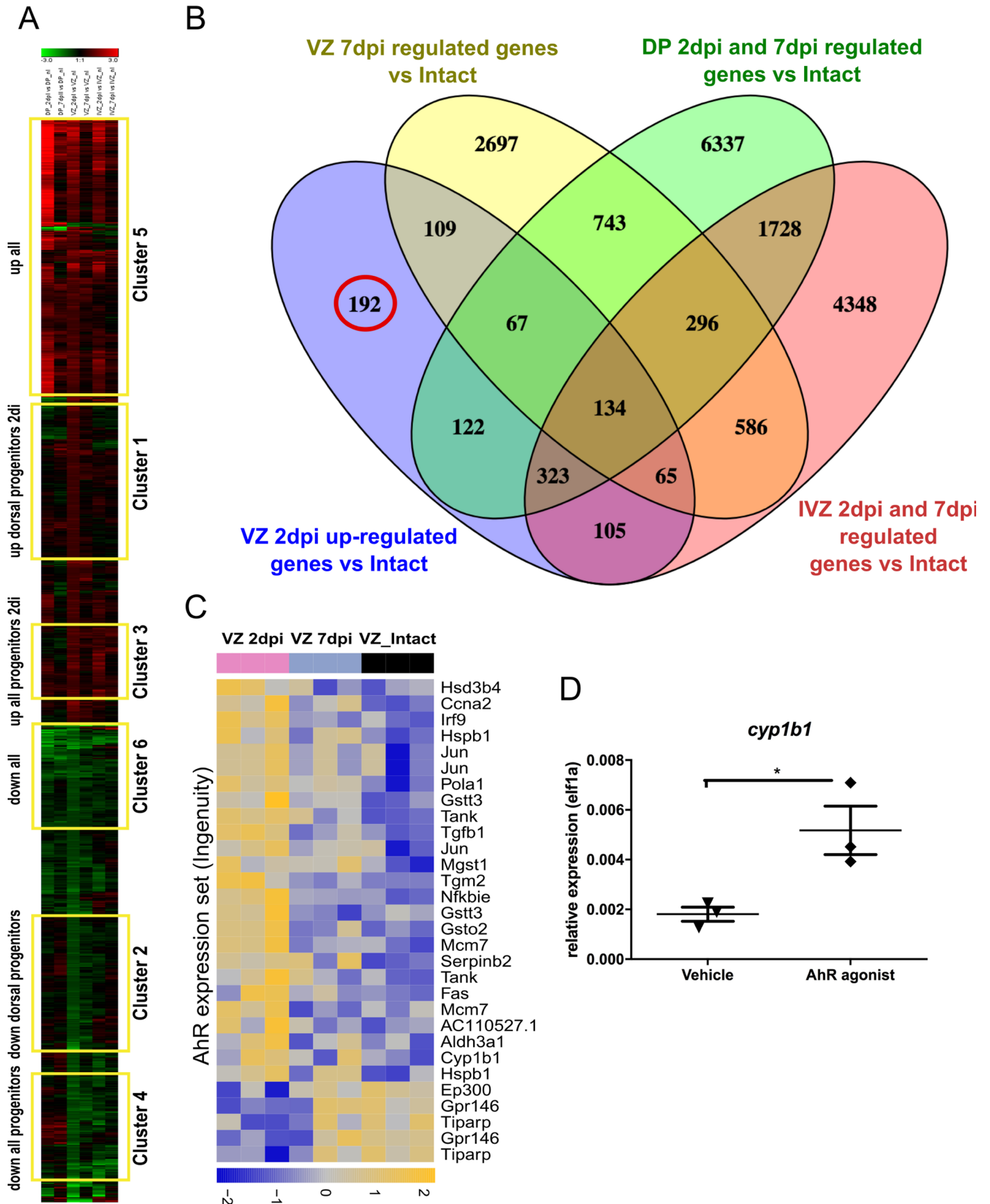


Figure S1. Identification of transcriptome changes in the neurogenic niche after brain injury. Related to Figure 1. (A) Heatmap depicting clusters of differentially regulated genes in the dorsal ventricular zone (VZ), medial ventricular zone (MVZ) and parenchyma (DP) 2 and 7 days after brain injury compared to intact (nl) brains. Red (green) indicates up (down) regulation. Yellow boxes mark different clusters of co-regulated genes. (B) Venn diagram to identify genes specifically regulated at 2 dpi in the dorsal neurogenic zone (VZ). (C) Heatmap depicting the regulation of AhR target genes in the VZ after brain injury. Yellow (blue) indicates higher (lower) expression levels. (D) Dot plot depicting the expression of Cyp1b1 5 h after ventricular injection (CVMI) of AhR agonist (BNF) or vehicle. Single dots represent individual animals indicating biological replicates. Each biological replicate is the mean value of 4 technical replicates. Lines show mean \pm SEM. * ≤ 0.05 ; ** ≤ 0.01 (unpaired t test).

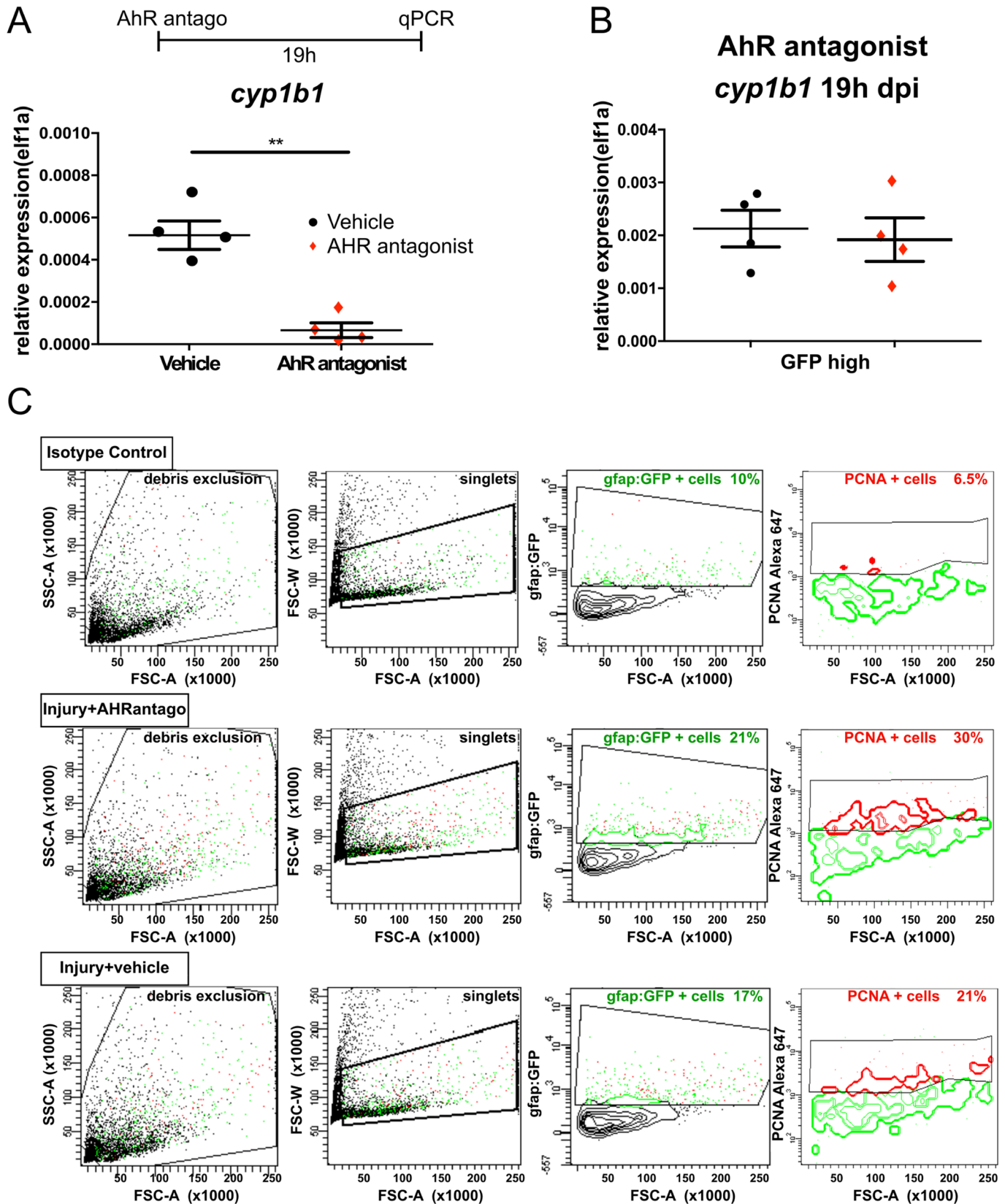


Figure S2. Decrease in AHR signalling promotes endymoglia proliferation. Related to Figure 2.

(A) Scheme depicting the experimental procedure to assess the efficiency of AhR antagonist and dot plot showing *cyp1b1* expression in endymoglia isolated from Tg(gfap:GFP) transgenic telencephalon 19 h after CVMI of vehicle or AhR antagonist. Single dots represent individual animals indicating biological replicates. Each biological replicate is the mean value of 4 technical replicates. Lines show mean±SEM. $**\leq 0.01$ (unpaired t test). (B) Dot plot showing real-time qPCR analysis of *cyp1b1* expression in GFP+ endymoglia sorted from the telencephalon of Tg(gfap:GFP) transgenic animals 19 h after CMVI of vehicle or AhR antagonist. Single dots represent individual animals indicating biological replicates. Each biological replicate is the mean value of 4 technical replicates. Lines show mean±SEM. (C) FACS plots depicting the definition of the sorting gates (isotype control) and sorting of PCNA+ and gfap:GFP+ endymoglia from injured telencephalons after AhR antagonist or vehicle treatment.

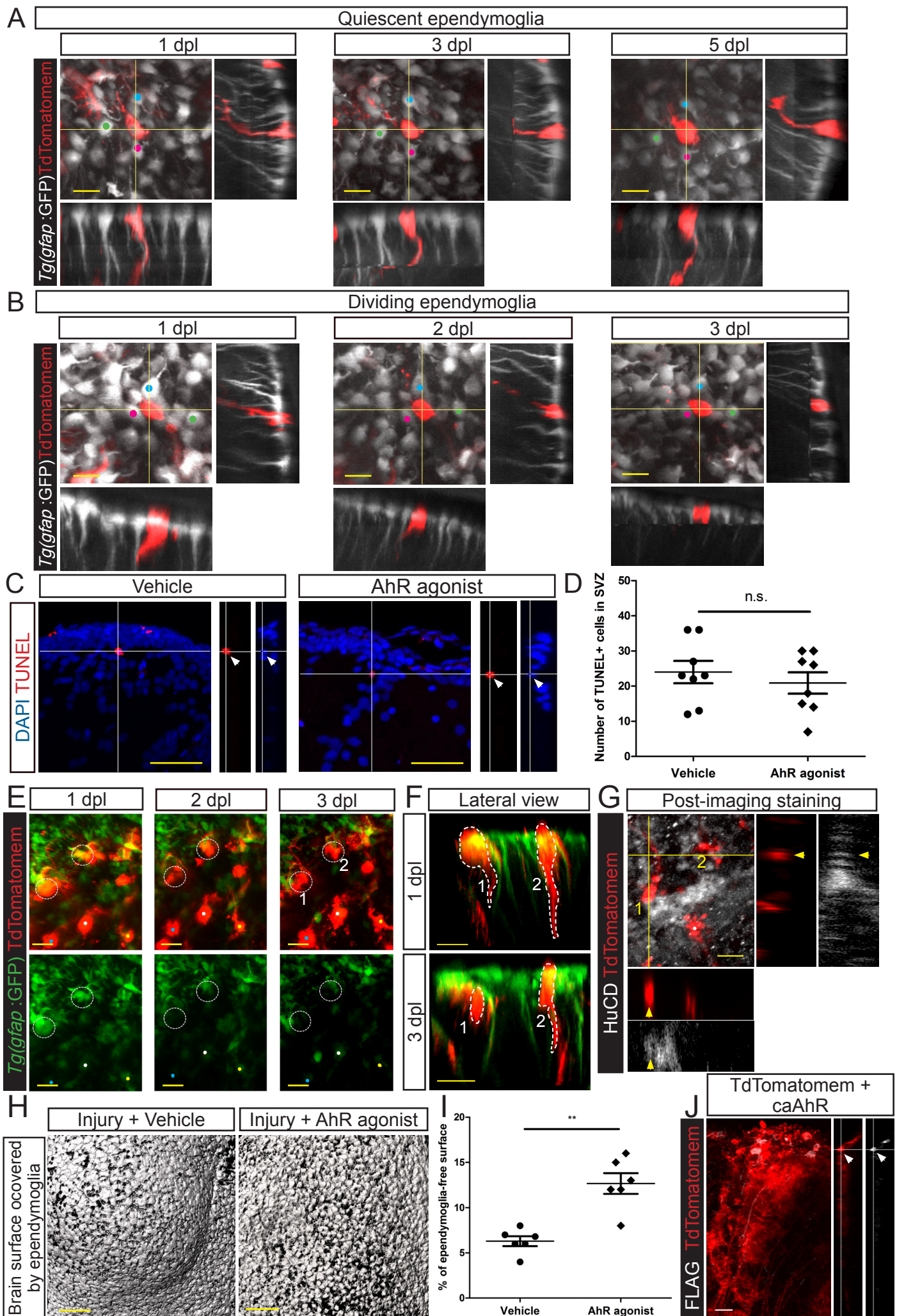


Figure S3. Live imaging of ependymoglia cells. Related to Figure 3.

(A, B) Micrograph depicting in vivo 2-photon images with orthogonal projections of the same TdTomatomem-labelled quiescent (A) and dividing (B) ependymoglia in the Tg(gfap:GFP) line followed for 5 days. (C) Confocal images with orthogonal projections depicting the TUNEL staining in vehicle- or AhR agonist-treated brains at 3 dpi. (D) Dot plot showing the total number of TUNEL-positive cells in the dorsal ventricular zone (SVZ) in the entire vehicle- or AhR agonist-treated brain. Single dots represent individual animals indicating biological replicates. Lines show mean \pm SEM (Mann-Whitney test). (E) In vivo 2-photon images following the evolution of TdTomatomem-labelled ependymoglia cells (labelled 1 and 2) in the Tg(gfap:GFP) line throughout 3 days. Lower panels show the downregulation of the gfap marker during imaging time. Dots label the individual cells used as references for the re-identification of tracked cells during imaging time. (F) 3D lateral views of the imaged cells (labelled 1 and 2) in order to assess the loss of radial processes and cell migration towards the parenchyma during direct conversion. Dashed lines define the shape of the cells. (G) Post-imaging immunostainings at 4 dpi for HuC/D and TdTomatomem in whole-brain samples with orthogonal projections confirming the neuronal (HuC/D+) identity of the imaged cells in E and F (yellow arrows). (H) Micrographs depicting the dorsal view on the central part of the hemisphere of the injured zebrafish brain covered with ependymoglia cells 5 days after injury in the Tg(gfap:GFP)mi2001 transgenic line after vehicle and AhR agonist treatment. (I) Dot plot showing the ependymoglia-free surface after treatment with AhR agonist or vehicle. Single dots represent individual animals indicating biological replicates. Lines show mean \pm SEM. $^{**}\leq 0.01$ (Mann-Whitney test). (J) Confocal image with orthogonal projections showing the co-electroporation of TdTomatomem and caAhR constructs confirming the colocalization of both plasmids in the same cells and allowing us to use TdTomatomem as a long-term tracer. Scale bars: 20 μ m in A and B, 30 μ m in C, 20 μ m in E, 30 μ m in F, 20 μ m in G, 20 μ m in H, 50 μ m in J.

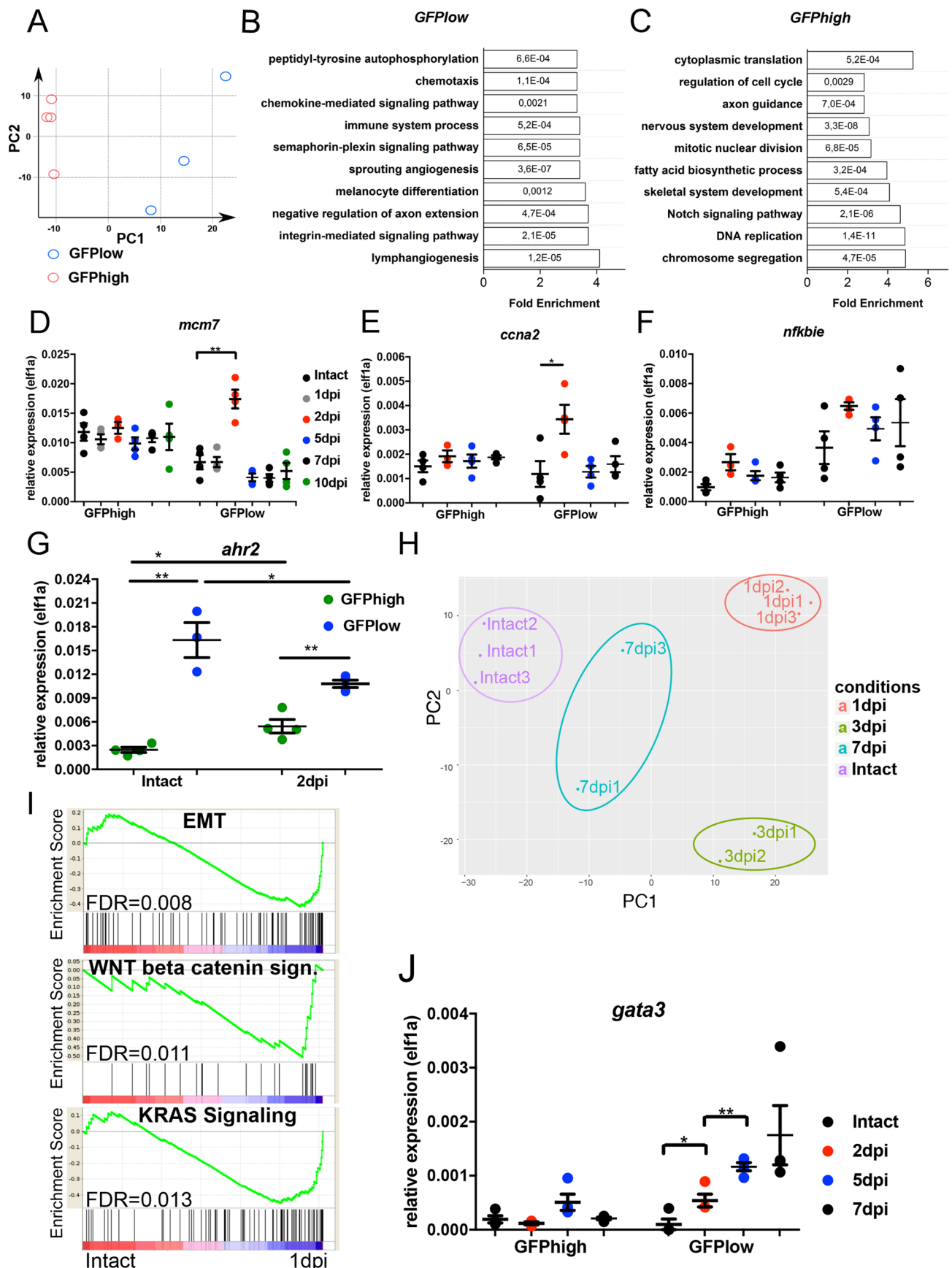


Figure S4. GFPlow ependymoglia population isolated from the Tg(gfap:GFP) transgenic line regulates AhR signalling after injury. Related to Figure 4.

(A) Dot plot depicting principle component analysis based on the total transcriptome of GFPhigh (red circles) and GFPlow (blue circles) ependymoglia population. (B, C) Histograms showing 10 biological processes enriched in GFPlow (B) and GFPhigh (C) transcriptomes based on the Gene Ontology (GO) analysis. (D, E, F, G) Dot plots depicting the expression of AhR-related genes in GFPhigh and GFPlow ependymoglia sorted from intact and injured brains. Single dots represent individual animals indicating biological replicates. Each biological replicate is the mean value of 4 technical replicates. Lines show mean±SEM. * ≤ 0.05; ** ≤ 0.01 (Unpaired t test). (H) Principal components analysis (PCA) of injury-induced transcriptome changes revealing overall transcriptional changes in the GFPlow ependymoglia in response to injury. (I) Graphs showing overrepresented gene sets in the GFPlow ependymoglia at 1 dpi using GSEA. (J) Dot plots depicting GATA3 expression in two ependymoglia states in intact and injured brains. Single dots represent individual animals indicating biological replicates. Each biological replicate is the mean value of 4 technical replicates. Lines show mean±SEM. * ≤ 0.05; ** ≤ 0.01 (unpaired t test).

Suppl. Table 2. Primers used for qPCR. Related to STAR Methods.

Gene Name	Primer forward	Primer reverse	Reference
Cyp1b1	AGATATTTTCGGGGCCAGTC	CACTACCCTGTCCACGTCCT	
Elf1a	CTTCTCAGGCTGACTGTGC	CCGCTAGCATTACCCTCC	McCurley and Callard, 2008
GFAP	TTGTGCGAACTGTTGAGACC	AGCAGGGAAAGTTGGTGAAG	
Nestin	GGTCTTTGGAGAGGAGTGGAG	CCCCTCATCAGCAGAATCAT	
Aldh1a2	CGTGAACTCGGAGAGATCGG	CCCACCAAAGGATAACGGCT	
Olig2	TTGCACCTGCTACCGGCAAT	CTTGACGGCGGACAGAAAG	
S100b	TAGAGAACTGCCTGGGAACC	CGGTGTCCAACTTTCCATC	
Aromat b	ACAGTCGGTTCCTCTGGATG	TATGCATTGCAGACCTTTGG	
SOX9b	GCCCAGACGGAGGAAATCAG	TGAGACTGACCGGAGGTGTTT	
Ahr2	CCCCATGGCTTGTCAACTAC	TCCTTAAGTGGACGGTTTTGC	
Mcm7	GAGATTTACGGCCATGAGGA	GGTGTACTGACTGCGTGGAG	
nfkbie	GCGCAGAACTGGAGAGGTAT	TATGTAACGCCGTCTTCCCG	
Ccna2	TGCGGGAAATGGAGGTC AAG	CTCCCACTTCCACCAACCAG	
Gata3	AACCTGCAAGGTGGAATGAC	AGCTGGAAGTCTGCAAGACAG	Kyritsis et al., 2012