**Supplementary Material**

# A Subset of Circulating Hemocytes Expresses Genes Indicating Neural Precursor Identity

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**Supplementary Table 1.** Custom branched probes for use with ViewRNA in situ hybridization assays (Invitrogen) were designed and provided by ThermoFisher Scientific according to the sequences below.

| **Custom branched probes for use with ViewRNA in situ hybridization assays:** | **Accession number** | **Fluorophore** |
|---|---|---|
| *pros* | XM_069310523.1 | Alexa Fluor 488 |
| *dcx* | GBYW01003677.1 | Alexa Fluor 594 |
| *brat* | GBYW01047025.1 | Alexa Fluor 647 |
| *numb* | GBYW01013236.1 | Alexa Fluor 647 |
| *numb* | GBYW01013236.1 | Alexa Fluor 750 |
| *pcna* | GBYW01027650.1 | Alexa Fluor 594 |
| *gcm* | MT407370 | Alexa Fluor 750 |
| *5htr1* | GBYW01015232.1 | Alexa Fluor 488 |
| *ast1* | AY787656.1 | Alexa Fluor 750 |
| *hml* | GBYW01036052 | Alexa Fluor 594 |
| *soxN* | MZ325488 | Alexa Fluor 594 |

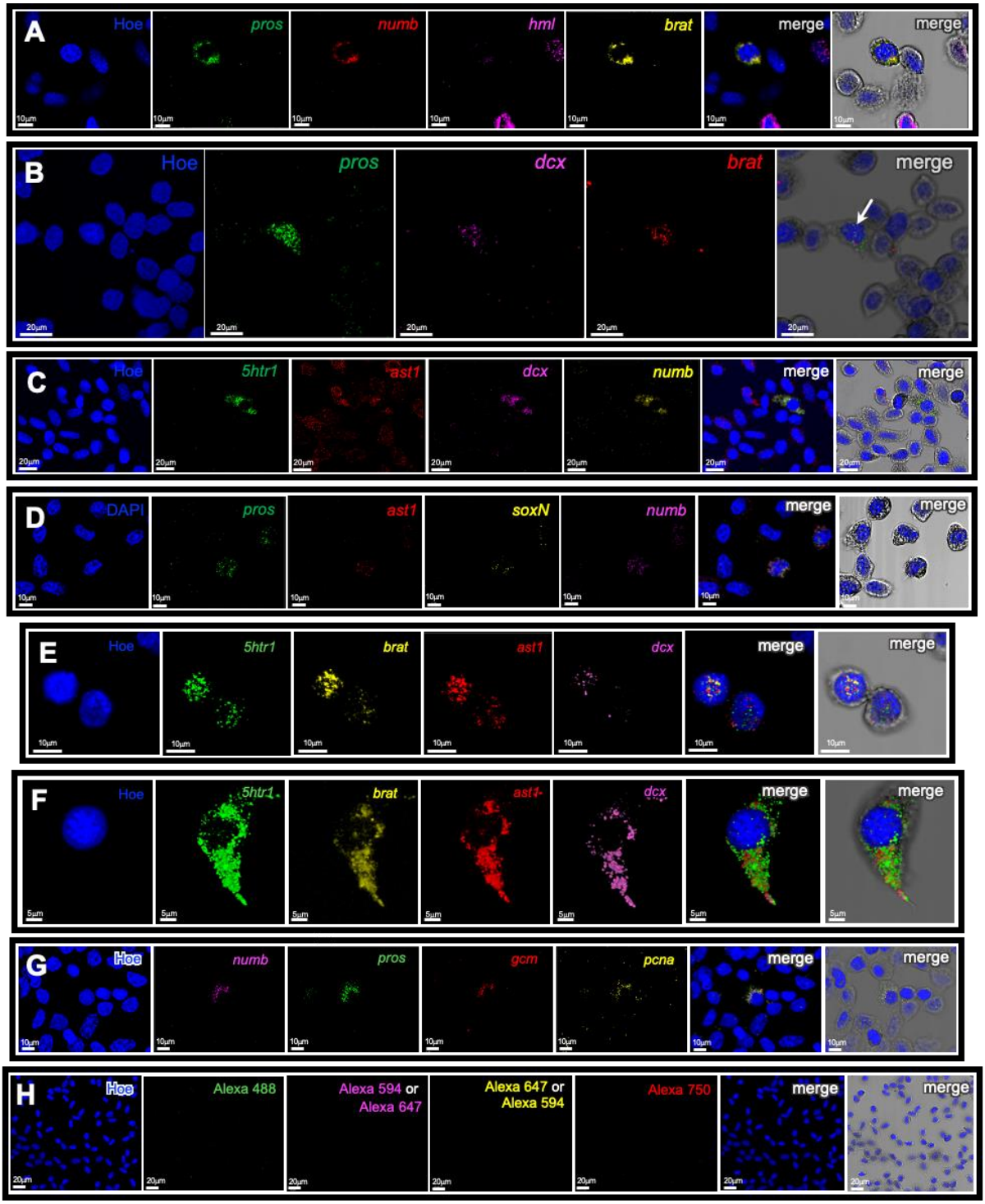
**Supplementary Table 2**. A list of specific probe combinations used in the experimental assays.

| Treatment | Probe combination | Transcripts | Fluorophore (Alexa Fluor) |
|---|---|---|---|
| 5HT-injection<br>rAst1-injection<br>CFS-injection | Set 1 | *pros* | AF488 |
| | | *gcm* | AF750 |
| | | *pcna* | AF594 |
| | | *numb* | AF647 |
| | Set 2 | *5htr1* | AF488 |
| | | *numb* | AF750 |
| | | *soxN* | AF594 |
| | | *brat* | AF647 |
| LPS-injection | Set 1 | *dcx* | AF594 |
| | | *5htr1* | AF488 |
| | | *numb* | AF647 |
| | | *ast* | AF750 |
| | Set 2 | *hml* | AF594 |
| | | *pros* | AF488 |
| | | *brat* | AF647 |
| | | *numb* | AF750 |
| Brain injury | Set 1 | *dcx /soxN** | AF594 |
| | | *5htr1* | AF488 |
| | | *numb* | AF647 |
| | | *ast/gcm** | AF750 |
| | Set 2 | *hml/ pcna** | AF594 |
| | | *pros* | AF488 |
| | | *brat* | AF647 |
| | | *numb* | AF750 |

***** one of these probes were used in the combinations

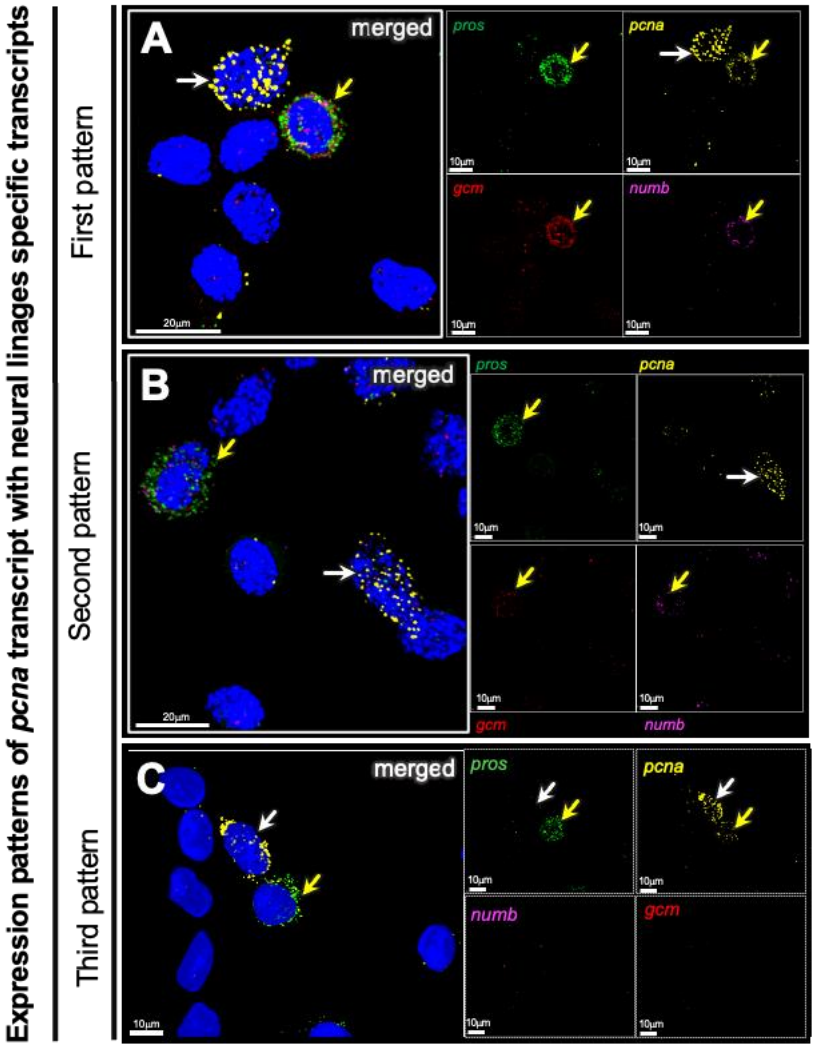
**Supplementary Figure 1. Separated fluorescent window-channels for the different probe combinations, as shown in Figure 2.**

(A-G) The separated fluorescent channels correspond to the pictures in Fig. 2, respectively. (H) Negative controls, performed without the application of probes as a standard for comparison.

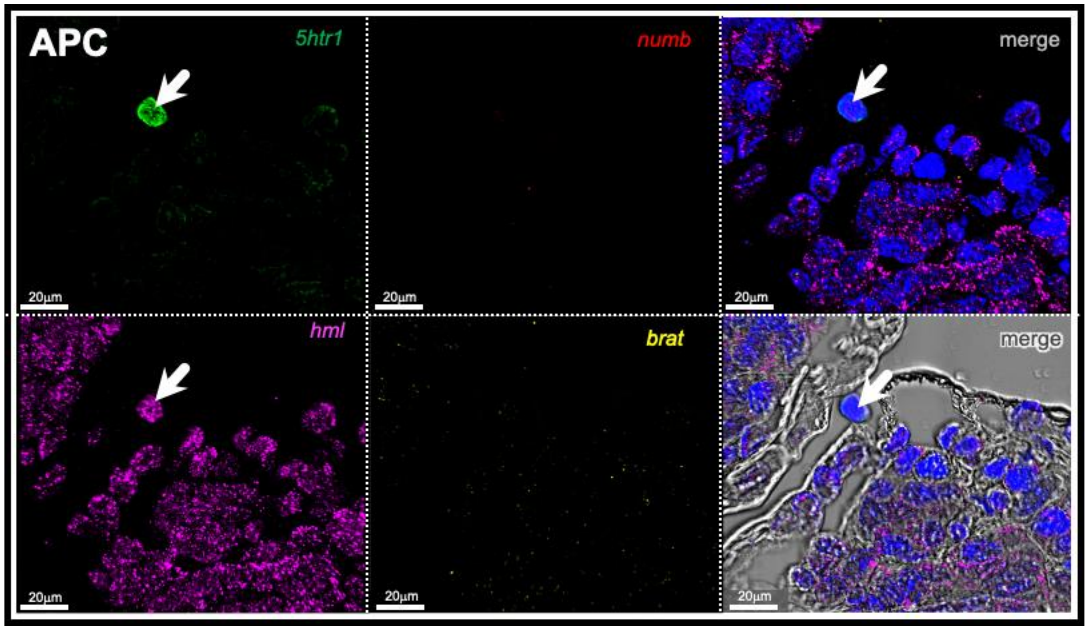


**Supplementary Figure 2. Spatial distribution of proliferating cell nuclear antigen (PCNA) transcript expressed in circulating hemocytes as demonstrated by RNA-FISH.**

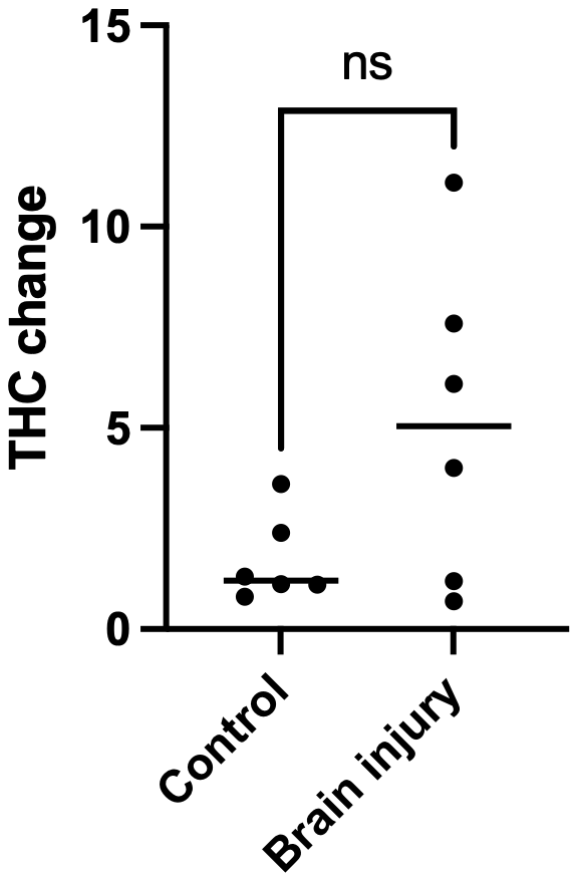
**(A-C)** *pcna*$^{+}$ cells can be divided into two major types: (1) *pcna*$^{+}$ cells without co-localization with neural lineage transcripts (white arrow), and (2) co-expression of the *pcna*$^{+}$ cells with neural lineage transcripts (yellow arrow). The latter can be subcategorized into three sub-patterns based on its co-localization with other neural lineage transcript markers (including *pros* and *numb*) and a glia-specific marker transcript (*gcm*), as well as by the intensity of positive signals, which is marked by yellow arrows in A-C.
**(A)** Yellow arrow showing moderate expression of *pcna,* with high expression of *pros*/*numb*, and high expression of *gcm* in the same cell.
**(B)** Yellow arrow shows very low *pcna* expression, high expression of *pros*$^{+}$, moderate expression of *numb*+, low expression of *gcm* in the same cell. White arrow shows a *pcna*$^{+}$/ *pros*$^{-}$/*numb*$^{-}$/*gcm*$^{-}$ cell
**(C)** Yellow arrow shows a cell which is characterized by low *pcna* expression, moderate to low expression of *pros*, and absence or very low expression of *numb/gcm*.



**Supplementary Figure 3. No putative neural precursor cells were detected in the APC**
Multiple RNA-FISH using probes for *5htr1*, *numb*, *brat* and *hml* in a section of the anterior proliferation center (APC) observed by confocal laser scanning microscopy. No neural progenitor marker transcript was detected, and only a *5htr1*-positive cell, that also expressed *hml* (white arrow), was observed in the tissue. No cells expressing *numb* or *brat* could be detected in the APC.

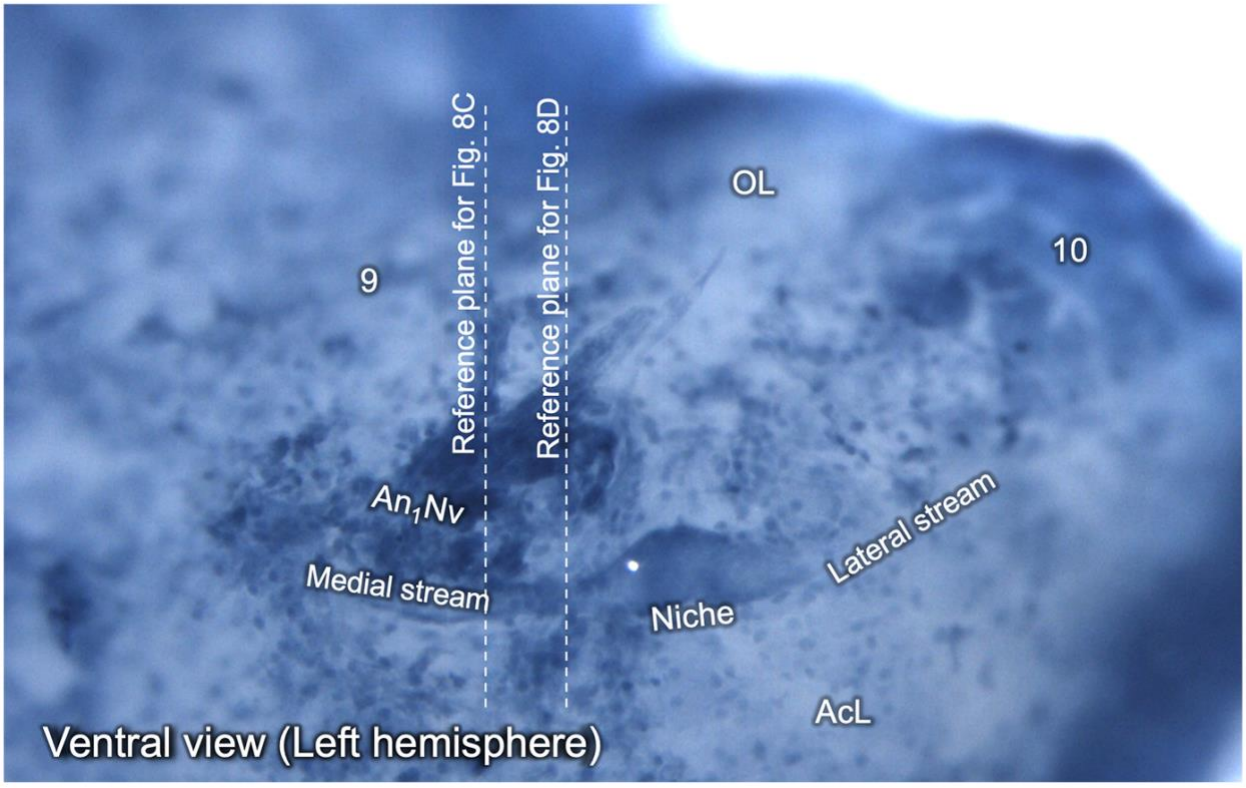


**Supplementary Figure 4. Total hemocyte count after brain injury compared to control.** Hemolymph samples were taken from six crayfish for each treatment 24 h before and 48 h after brain injury. The samples were mixed 1:1 with 4% PFA in PBS and counted in a Fuchs-Rosenthal cell counter. The number of hemocytes per mL hemolymph was compared as: THC after injure/THC before injury, and compared to untreated control crayfish n=6.



**Supplementary Figure 5. Whole mount preparation of the brain showing section planes for Fig. 8C-D.**

**(D)** The ventral side of the whole brain, treated with the autofluorescence quenching reagent (staining in blue), demonstrated the organization and interrelationship of the $An_1Nv$ bundle located nearby (medially) to the neurogenic niche and the stream. Abbreviations: AcL; accessory lobe, OL; olfactory lobe, the numbers 9 and 10 mark Cluster 9 and 10, respectively.



**Supplementary Figure 6.** Brain tissue sections (parasagittal sections) omitted probe application in the RNA-FISH system as a negative control. No autofluorescence and no positive signals were present in all fluorescent channels.