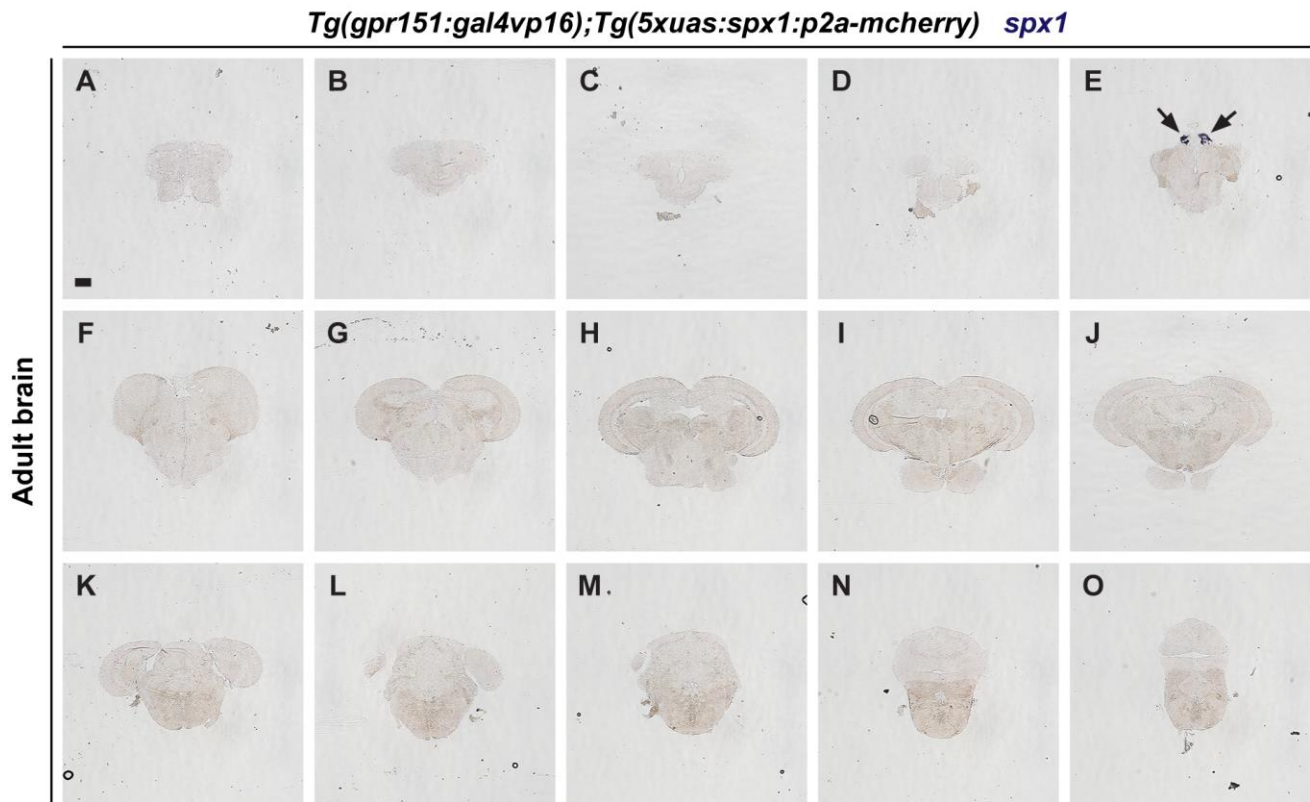


## Supplementary Material

### 1.1 Supplementary Table 1. Primer sequences for quantitative RT-PCR

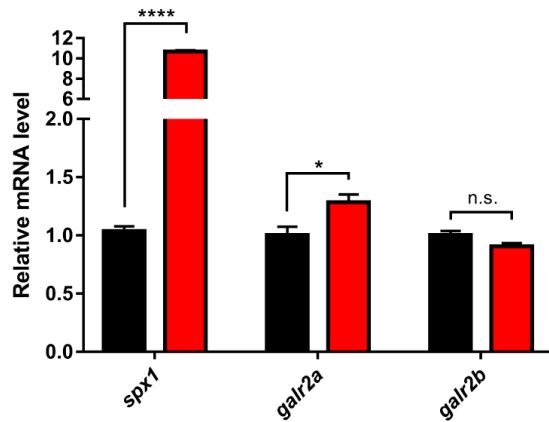
Primer names	Primer sequences
<i>β-actin</i> forward	5'-AGAGCTATGAGCTGCCTGACG-3'
<i>β-actin</i> reverse	5'-CCGCAAGATTCCATACCCA-3'
<i>spx1</i> forward	5'-CGCACTTGCGCTGTTACTTCTTG-3'
<i>spx1</i> reverse	5'-GCTGGAGGATGTTTCAGGAGAAACG-3'
<i>garl2a</i> forward	5'-GAAACGCGAACTCCACGTAA-3'
<i>garl2a</i> reverse	5'-GAAGGTGCAGATGTCCATGG-3'
<i>garl2b</i> forward	5'-ATGTCTGATCACGAGGACCT-3'
<i>garl2b</i> reverse	5'-CCCAACCTGCCCACTTCTAA-3'
<i>pet1</i> forward	5'-GCAACTTCGCTCCCATTCA-3'
<i>pet1</i> reverse	5'-TGGTAGAGGGTTGGAGGAGATC-3'
<i>tph2</i> forward	5'-TCAGACACTAAAGGAACAACAAGGA-3'
<i>tph2</i> reverse	5'-AAGCACAACATTAGGCACAAAGAG-3'
<i>slc6a4a</i> forward	5'-ACATCTCCTCAAAGCCCCAAA-3'
<i>slc6a4a</i> reverse	5'-CCACCAGAGTCCTAAATGTTCCA-3'

**Supplementary Figure 1.**

**Supplementary Figure 1. Specifically overexpressed *spx1* in the dorsal habenula of the whole adult brain**

(A-O) Serial transverse section views of the brain of *Tg(gpr151:gal4vp16);Tg(5xuas:spx1:p2a-mcherry)* zebrafish labeled by *spx1* RNA probe (E) Black arrows indicate overexpressed *spx1* in the dorsal habenula Scale bar: 200  $\mu$ m

## Supplementary Figure 2.



### Supplementary Figure 2. Upregulation of *spx1* and *galr2a* in the whole brain upon overexpression of *spx1* in the dHb

Quantitative RT-PCR in *Tg(gpr151:gal4vp16);Tg(5xuas:spx1:p2a-mcherry)* zebrafish (dHb-*spx1* OV) and wildtype siblings ( $n = 5$  per group).  $\beta$ -actin expression was used to normalize all samples, and data were acquired in triplicate. Data are represented as mean  $\pm$  standard error of the mean (SEM);  $n = 3$ . (A) Relative *spx1*, *galr2a*, and *galr2b* mRNA expression: *Spx1*: siblings,  $1.037 \pm 0.04177$ ; dHb-*spx1* OV,  $10.68 \pm 0.1323$ . *Galr2a*: siblings,  $1.003 \pm 0.07126$ ; dHb-*spx1* OV,  $1.283 \pm 0.06839$ . *Galr2b*: siblings,  $1.003 \pm 0.03528$ ; dHb-*spx1* OV,  $0.9067 \pm 0.02848$ . (\* $p < 0.05$ , \*\*\*\* $p < 0.0001$ . Unpaired  $t$ -test, n.s.: not significant.)