Supplemental Information

Gbtx2 Identifies Two Amacrine Cell Subtypes
with Distinct Molecular, Morphological,
and Physiological Properties

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Figure S1. *Gbx2<sup>CreERT2-IRE-EGFP</sup>* expression is consistent throughout the mouse development and adulthood. Related to Figure 1. 

(A-C) Adult (P35) Retinal cross-sections from *Gbx2<sup>CreERT2-IRE-EGFP</sup>; R26<sup>LSL-tdTom</sup>* mice dosed with of Tamoxifen (60mg/kg) at (A) E16, (B) P0, (C) P28. (D-I) Z-projections through the cell bodies of the inner nuclear layer (D-F) and ganglion cell layer (G-I) from retinal flatmounts of *Gbx2<sup>CreERT2-IRE-EGFP</sup>; R26<sup>LSL-tdTom</sup>* mice administered 2.0mg/day tamoxifen for (D, G) 1 day (dose), (E, H) 2 days, (F-I) 3 days. Scale bar, 25μm in (A) and (D).
Figure S2. Gbx2+ retinal neurons are amacrine cells. Related to Figure 1. Cross-sections of an adult retina from a Gbx2\textsuperscript{CreERT2-IRESGFP}; Rosa26\textsuperscript{LSL-tdTom} mouse labeling the total Gbx2+ AC population. Left: Neurotransmitter markers, (A) Pax6, (B) RBPMS, and (C) Chx10, label amacrine cells, retinal ganglion cells, and bipolar cells in the inner nuclear layer and ganglion cell layer, respectively. Right: Merged images showing both the select neurotransmitter marker (magenta) and Gbx2+ ACs (green). Arrows denote colocalization between the cell marker and Gbx2+ ACs, and arrowheads denote Gbx2+ ACs that do not colocalize with the specific cell marker. Scale bar, 25 µm.
Figure S3. Gbx2+ AC subpopulations have consistent density and spacing across the retina. Related to Figure 1. (A) TdTomato expressing Gbx2+ ACs in the ganglion cell layer (GCL) in a retina flatmount from a Gbx2<sup>CreER<sup>; R26<sup>LSL-tdTomato</sup> mouse. (B) Gbx2+ ACs (green) immunolabeled for calretinin (Calr, magenta) in the GCL. (C-D) Masked image of cell bodies of Gbx2+ neurons (C) Calretinin+ and (D) Calretinin- from the image in (B). (E) TdTomato expressing Gbx2+ ACs in the inner nuclear layer (INL). (F) Gbx2+ ACs (green) immunolabeled for calretinin (magenta) in the INL. (G-H) Masked image of cell bodies of Gbx2+ neurons (C) Calr+ and (D) Calr- from the image in (F). (I-K) Z-projection through the GCL and INL (pseudocolored green and magenta, respectively) for (I) all Gbx2+ ACs, (J) Calr+ Gbx2+ ACs, and (K) Calr- Gbx2+ ACs. (L) Quantification of the cell density of Calr+ and Calr- Gbx2+ ACs in the GCL and INL (n=24 measurements from 4 mice). (M-N) The density recovery profile (DRP) of Calr+ and Calr- Gbx2 ACs in the (M) GCL and (N) INL (n=32 measurements from 4 mice, respectively). (O-Q) The cell densities in the four quadrants of the retina of Gbx2+ AC in the (O) GCL and (P) INL (n=32 measurements from 4 mice, respectively). (Q) The cell density of Gbx2+ AC populations in the central and peripheral retina (n=32 measurements from 4 mice, respectively). Data represented as mean ± SEM. Scale bar, 50 μm in (A).
Figure S4. Flow cytometry plot of dissociated retinal neurons isolated from a Gbx2\textsuperscript{CreERT2-ires-EGFP}, Rosa26\textsuperscript{LSL-tdTomato} mouse. Related to Figure 2.
Using fluorescence-activated cell sorting, Gbx2+ ACs (tdTomato+) from P8 retina were isolated and separated into the S5-Gbx2+ ACs (tdTomato+, EGFP\textsuperscript{low}) and the S3-Gbx2+ ACs (tdTomato+, EGFP\textsuperscript{high}) groups for bulk RNA sequencing.
Figure S5. Gbx2+ ACs do not colocalize with many canonical neurotransmitter cell markers. Related to Figures 2 and 3.

Cross-sections of an adult retina from a Gbx2<sup>CreERT2-ires-EGFP</sup>; Rosa26<sup>LSL-tdTom</sup> mouse labeling the total Gbx2+ AC population (high-TM, 2.0mg tamoxifen). Left: Left, retinal sections from a Gbx2<sup>CreERT2-ires-EGFP</sup>; Rosa26<sup>LSL-tdTom</sup> mouse immunolabeled with (A) neuronal nitric oxide synthase (nNOS), (B) vesicular glutamate transporter 3 (Vglut3), (C) choline acetyl transferase transporter (ChAT), and (D) tyrosine hydroxylase (TH). Right: Merged images of Gbx2+ ACs (green) and the neurotransmitter marker (magenta). Arrows denote colocalization between the cell marker and Gbx2+ ACs, and arrowheads denote Gbx2+ ACs that do not colocalize with the specific cell marker. Scale bar, 25 µm.
Figure S6. Dendritic morphology and orientation of Gbx2+ ACs by retina location. Related to Figure 4.  
(A-C) S3- and S5-Gbx2+ AC morphology in (A) total dendrite length, (B) number of branches, (C) dendrite branch self-crossover. n=15, 7 cells for S3 and S5 respectively.  
(D, E) S3-stratifying Gbx2+ ACs show a similar dendrite asymmetry in (D) each retinal quadrant (n=3-5 retinas per quadrant) and (E) between central and peripheral retina (n=4, 9 retinas respectively).  
(F-I) A polar plot of dendrite orientation of S3-targeting Gbx2+ ACs in each retinal quadrant; black trace represents the mean and colored traces represent neurons quantified from a single retina (n >20 neurons per retina, n= 4 retinas). D, dorsal; V, ventral; N, nasal; T, temporal. Data represented as mean ± SEM. *p<0.05 by an unpaired t-test with a Welch’s correction.