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Supplemental Information

Gbx2 Identifies Two Amacrine Cell Subtypes with Distinct Molecular, Morphological, and Physiological Properties

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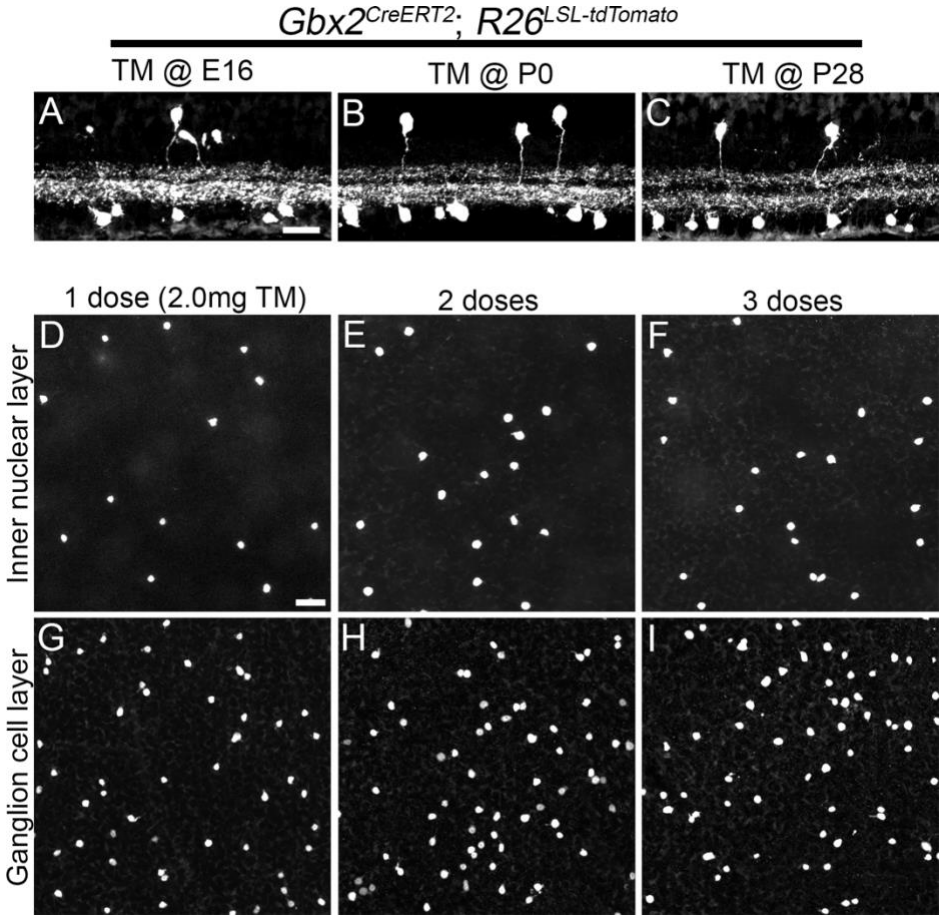

Figure S1. *Gbx2*$^{CreERT2\text{-}IRES\text{-}EGFP}$ expression is consistent throughout the mouse development and adulthood. Related to Figure 1.
(A-C) Adult (P35) Retinal cross-sections from *Gbx2*$^{CreERT2\text{-}IRES\text{-}EGFP}$*; R26*$^{LSL\text{-}tdTom}$ mice dosed with of Tamoxifen (60mg/kg) 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*$^{CreERT2\text{-}IRES\text{-}EGFP}$*; R26*$^{LSL\text{-}tdTom}$ 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**).

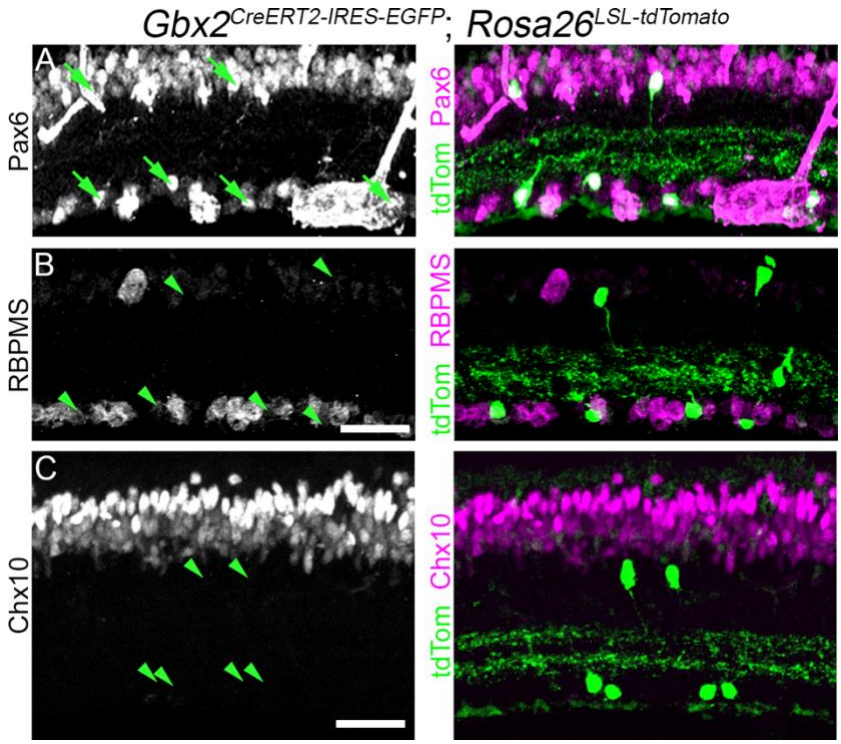

Figure S2. Gbx2+ retinal neurons are amacrine cells. Related to Figure 1.
Cross-sections of an adult retina from a *Gbx2*$^{CreERT2\text{-}IRES\text{-}EGFP}$; *Rosa26*$^{LSL\text{-}tdTom}$ mouse labeling the total Gbx2+ AC population. Left: Neurotransmitter markers, (**A**) Pax6, (**B**) RBPMS, and (**C**) 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.

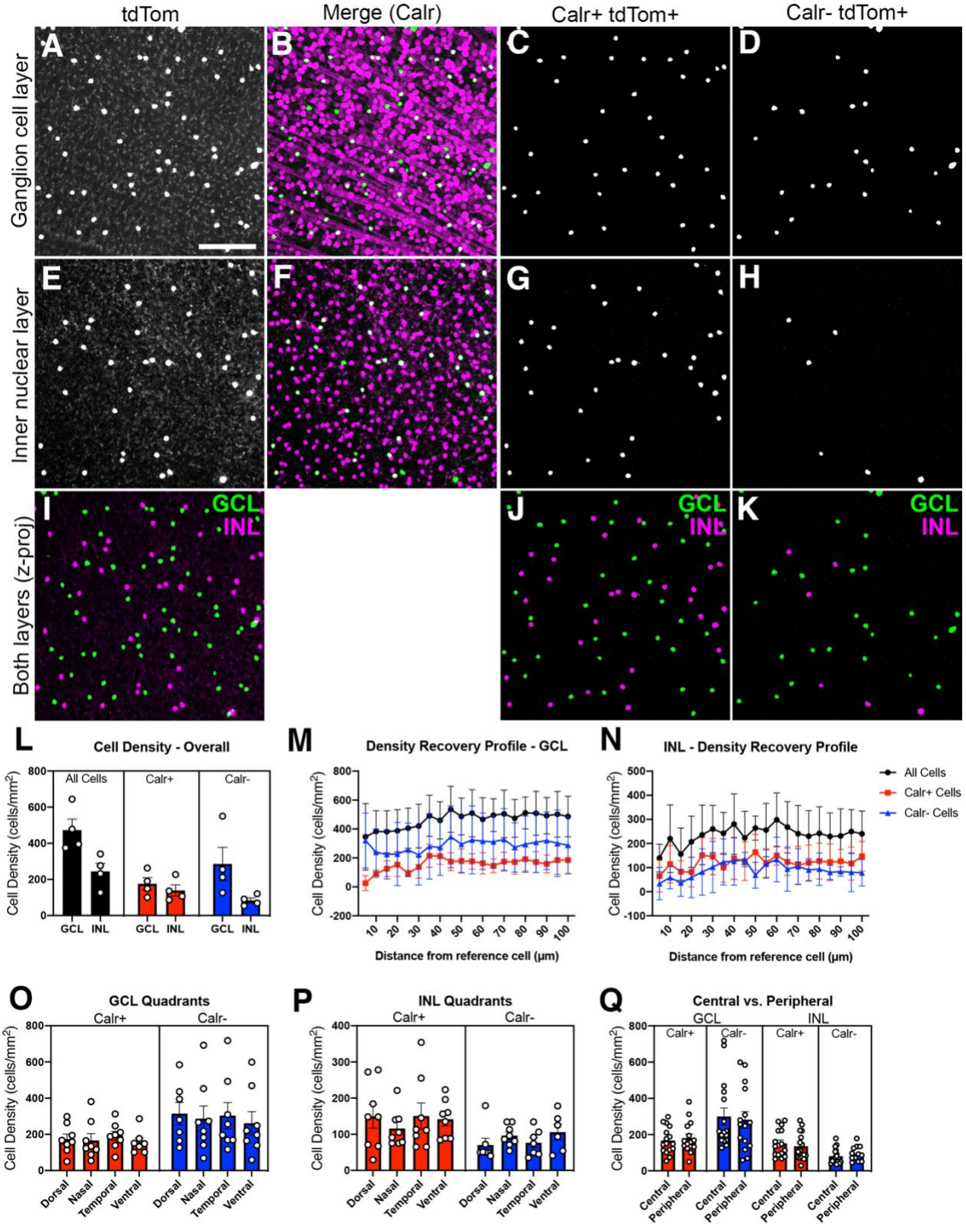
tdTom
Merge (Calr)
Calr+ tdTom+
Calr- tdTom+
Ganglion cell layer
Inner nuclear layer
Both layers (z-proj)
A
B
C
D
E
F
G
H
I
J
K
GCL
INL
L
Cell Density - Overall
All Cells
Calr+
Calr-
Cell Density (cells/mm²)
GCL
INL
M
Density Recovery Profile - GCL
Distance from reference cell (µm)
N
INL - Density Recovery Profile
All Cells
Calr+ Cells
Calr- Cells
O
GCL Quadrants
P
INL Quadrants
Q
Central vs. Peripheral
Dorsal
Nasal
Temporal
Ventral
Central
Peripheral

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*^{CreER}; *R26*$^{LSL\text{-}tdTomato}$ 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)**.

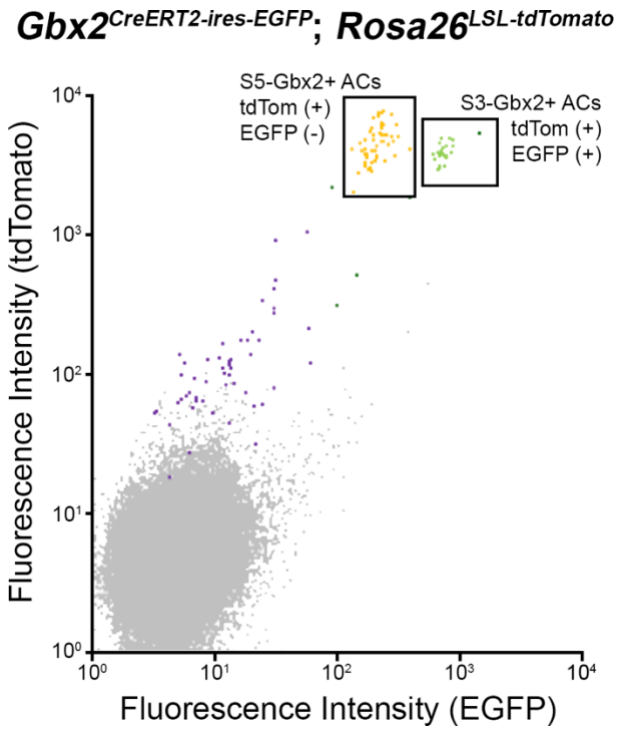

Figure S4. Flow cytometry plot of dissociated retinal neurons isolated from a *Gbx2*$^{CreERT2\text{-}IRES\text{-}EGFP}$; *Rosa26*$^{LSL\text{-}tdTomato}$ mouse. Related to Figure 2.

Using fluorescence-activated cell sorting, Gbx2+ ACs (tdTomato+) from P8 retina were isolated and separated into the S5-Gbx2+ ACs (tdTomato+, EGFP^{low}) and the S3-Gbx2+ ACs (tdTomato+, EGFP^{high}) groups for bulk RNA sequencing.

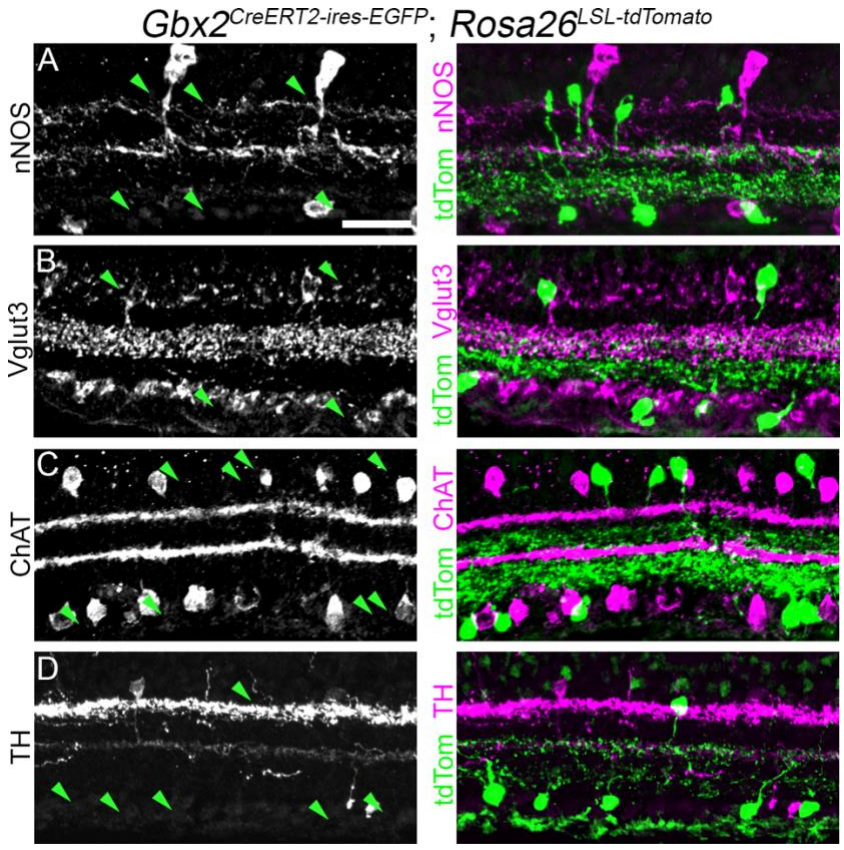

Figure S5. Gbx2+ ACs do not colocalize with many canonical neurotransmitter cell markers. Related to Figures 2 and 3.
Cross-sections of an adult retina from a *Gbx2*$^{CreERT2\text{-}IRES\text{-}EGFP}$; *Rosa26*$^{LSL\text{-}tdTom}$ mouse labeling the total Gbx2+ AC population (high-TM, 2.0mg tamoxifen). Left: Left, retinal sections from a *Gbx2*$^{CreERT2\text{-}IRES\text{-}EGFP}$; *Rosa26*$^{LSL\text{-}tdTom}$ 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.

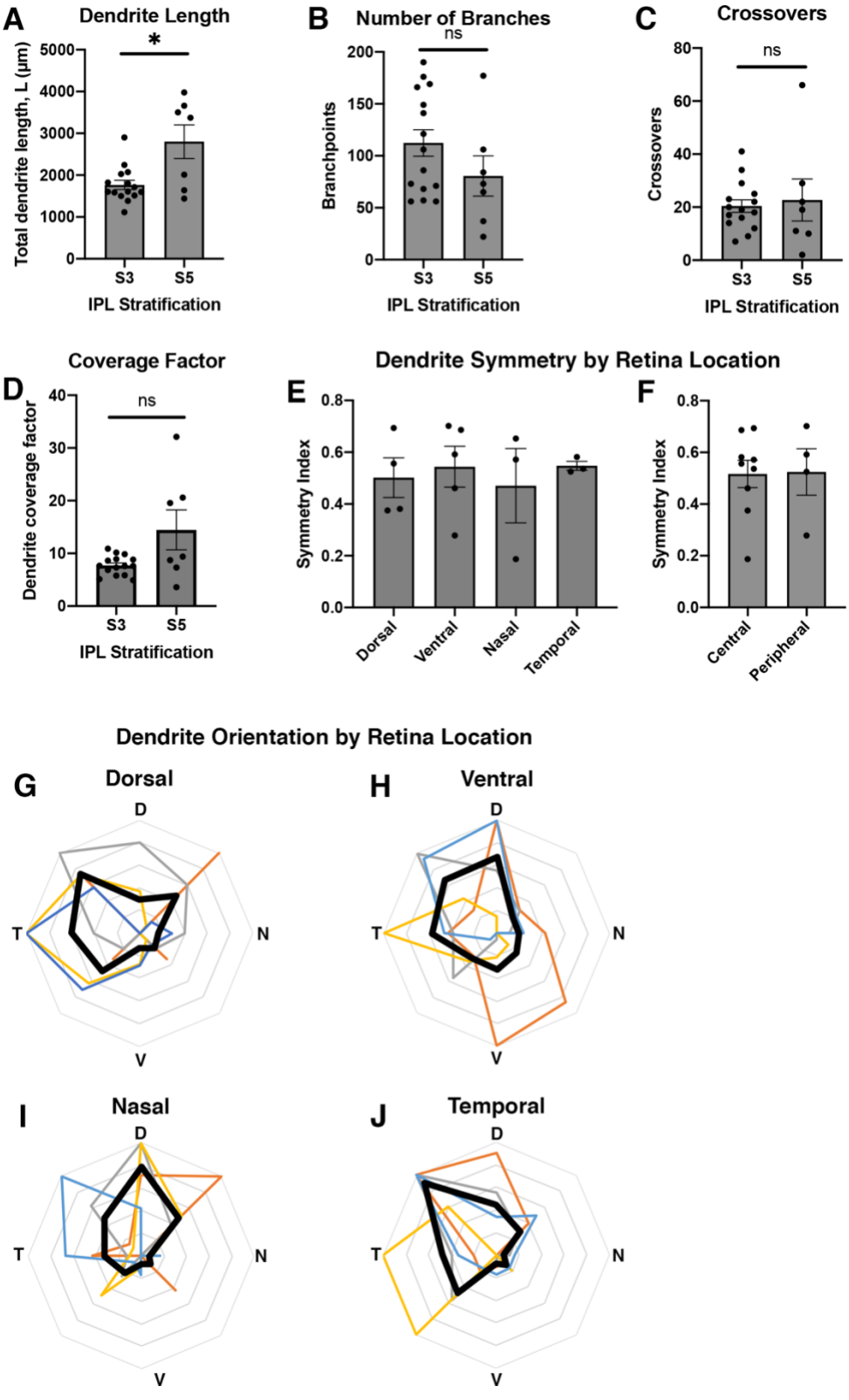
A
Dendrite Length
*
Total dendrite length, L (µm)
5000
4000
3000
2000
1000
0
S3
S5
IPL Stratification
B
Number of Branches
ns
Branchpoints
200
150
100
50
0
S3
S5
IPL Stratification
C
Crossovers
ns
Crossovers
80
60
40
20
0
S3
S5
IPL Stratification
D
Coverage Factor
ns
Dendrite coverage factor
40
30
20
10
0
S3
S5
IPL Stratification
E
Dendrite Symmetry by Retina Location
Symmetry Index
0.8
0.6
0.4
0.2
0.0
Dorsal
Ventral
Nasal
Temporal
F
Symmetry Index
0.8
0.6
0.4
0.2
0.0
Central
Peripheral
Dendrite Orientation by Retina Location
G
Dorsal
H
Ventral
I
Nasal
J
Temporal
D
T
N
V

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.