Formation of retinal direction-selective circuitry initiated by starburst amacrine cell homotypic contact

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Impact statement: Selective synapse formation in a retinal motion-sensitive circuit is orchestrated by starburst amacrine cells, which use homotypic interactions to initiate formation of a dendritic scaffold that recruits projections from circuit partners.
SUMMARY

A common strategy by which developing neurons locate their synaptic partners is through projections to circuit-specific neuropil sublayers. Once established, sublayers serve as a substrate for selective synapse formation, but how sublayers arise during neurodevelopment remains unknown. Here we identify the earliest events that initiate formation of the direction-selective circuit in the inner plexiform layer of mouse retina. We demonstrate that radially-migrating newborn starburst amacrine cells establish homotypic contacts on arrival at the inner retina. These contacts, mediated by the cell-surface protein MEGF10, trigger neuropil innervation resulting in generation of two sublayers comprising starburst-cell dendrites. This dendritic scaffold then recruits projections from circuit partners. Abolishing MEGF10-mediated contacts profoundly delays and ultimately disrupts sublayer formation, leading to broader direction tuning and weaker direction-selectivity in retinal ganglion cells. Our findings reveal a mechanism by which differentiating neurons transition from migratory to mature morphology, and highlight this mechanism’s importance in forming circuit-specific sublayers.
INTRODUCTION

In the developing nervous system, neurons form selective synapses to generate circuits comprised of cell-type-specific connections. This selectivity is important for circuit function because it ensures connectivity between neurons specialized for particular information-processing tasks. Despite its importance, basic questions about selective synapse formation remain unanswered. For example, we do not know how cell types fated to form synapses coordinate their growth to establish contact with each other. This is a significant cell biological challenge, because the neurons that comprise a single circuit are often born at disparate times and physical locations.

In many tissues, notably the insect and vertebrate visual systems, synaptic specificity is facilitated by laminar specificity, the phenomenon whereby circuit partners project their axons and dendrites to narrow strata within a laminated neuropil (Sanes and Zipursky, 2010). The inner plexiform layer (IPL) of the vertebrate retina comprises at least 10 distinct sublayers built from the axons and dendrites of different amacrine, bipolar, and retinal ganglion cell (RGC) types (Baier, 2013). By projecting to the same IPL sublayer, circuit partners can be assured of encountering each other. The developmental events that create sublayers and guide circuit partners to converge upon them are therefore essential for establishment of retinal circuitry. At later developmental stages, when rudimentary IPL sublayers have already formed, neurons rely on molecular cues localized to those sublayers for guidance to the appropriate IPL strata (Duan et al., 2014; Matsuoka et al., 2011; Sun et al., 2013; Yamagata and Sanes, 2008; Visser et al., 2015). However, a crucial question remains unresolved: How do sublayers form in the first place? Understanding the mechanisms that initiate creation of sublayers will provide significant insight into the earliest step in circuit formation.
To learn how members of a single circuit create layers and converge upon them to achieve synapse specificity, we studied the direction-selective (DS) circuit of mouse retina (Fig. 1A). This circuit reports the direction of image motion to the brain through the spiking activity of distinct DS ganglion cell (DSGC) types that are tuned to prefer stimuli moving in particular directions (Demb, 2007; Vaney et al., 2012). The DS circuit comprises a limited number of well-described cell types amenable to genetic marking and manipulation (Kay et al., 2011; Huberman et al., 2009; Duan et al., 2014): In addition to DSGCs, circuit members include GABAergic/cholinergic interneurons called starburst amacrine cells (SACs); and four subtypes of glutamatergic bipolar cells (Chen et al., 2014; Duan et al., 2014; Greene et al., 2016; Kim et al., 2014). The circuit occupies two IPL sublayers, ON and OFF, named for the light response profiles of the neurons that project to them. ON-OFF DSGCs (ooDSGCs) send dendrites to both sublayers, while SACs and bipolar cells project to one or the other, depending on their subtype (Fig. 1A). Several molecular perturbations have been described that influence ON vs. OFF laminar targeting in the mouse DS circuit (Sun et al., 2013; Duan et al., 2014), but in these cases IPL sublayers still form in the right place; errors are limited to choosing the wrong DS sublayer. Thus, neither the establishment of the DS circuit sublayers nor their positioning in the appropriate IPL region depends on molecules that have been studied to date.

Here we seek to understand the earliest events leading to formation of the DS circuit IPL sublayers. Two lines of evidence suggest that SACs may take the lead in assembling this circuit. First, SACs are among the first cells to stratify the IPL: Even though other neurons innervate it contemporaneously, SACs are precocious in restricting their arbors into sublayers (Stacy and Wong, 2003; Kay and Sanes, 2013). Second, in mutant mice that entirely lack RGCs or bipolar cells, SAC IPL projections are largely normal, indicating SACs can form sublayers in the
absence of their circuit partners (Moshiri et al., 2008; Green et al., 2003). Thus, we set out to test the hypothesis that SACs orchestrate assembly of the DS circuit sublayers. We find evidence supporting this hypothesis, and we identify a surprising cellular mechanism initiating SAC lamination: Rather than immediately innervating the IPL, newborn SACs first produce a transient homotypic arbor network outside the IPL. These early homotypic contacts serve as a cue promoting SAC dendrite development and circuit integration upon conclusion of their radial migration to the inner retina. When deprived of homotypic contacts, SAC IPL innervation – and consequent sublayer formation – is impaired. We identify the SAC cell-surface protein MEGF10 as the molecular mediator of IPL innervation upon homotypic contact. In the absence of MEGF10, SACs persist in growing arbors outside the IPL, delaying IPL innervation. This in turn delays formation of the DS circuit sublayers and leads to SAC sublaminar targeting errors that persist to adulthood. We further show that impaired SAC sublayer formation has consequences for laminar targeting of their circuit partners: While partnering remains intact, lamination is disrupted, leading to spatial inhomogeneity in the DS circuit network. Finally, we show that these MEGF10-dependent anatomical changes both broaden and weaken direction tuning across the population of ooDSGCs. These results demonstrate that SACs orchestrate DS circuit assembly, first by initiating sublayer formation via homotypic contact, and then by using their laminated dendrites as a scaffold that guides projections of their circuit partners.

RESULTS

Timing of DS circuit IPL sublayer formation

To explore how the DS circuit creates its IPL sublayers, we began by determining when the sublayers first emerge in mouse. This analysis focused on SACs and ooDSGCs because
bipolar cells develop later (Morgan et al., 2006). Previous estimates of layer emergence vary widely (Stacy and Wong, 2003; Sun et al., 2013) due to the lack of adequate markers to study dendrite development in neonatal SACs. We therefore assembled a suite of mouse lines and antibody markers for this purpose, enabling anatomical studies of the full SAC population as well as individual cells (Fig. 1B-C; Fig. 1-Supplement 1; Fig. 2-Supplement 1). These markers revealed that SAC dendrites form two continuous well-defined laminae by P1. Some dendrites were stratified already at P0, even though the P0 IPL neuropil is less than one cell diameter wide (Fig. 1B; Fig. 1-Supplement 1). Further supporting this timeline, individual P1 SACs made lamina-specific projections (Fig. 1C): 96% of OFF SACs in the inner nuclear layer (INL), and 99% of ON SACs in the ganglion cell layer (GCL), stratified within the expected IPL sublayer ($n = 49/51$ OFF; $78/79$ ON; 4 mice). By contrast, ooDSGC dendrites were rudimentary and unstratified at P1 ($n = 18$ cells, 3 mice; Fig. 1-Supplement 1; also see Peng et al., 2017). Even at P2, only 30% of ooDSGCs co-fasciculated with SAC arbors; the rest projected diffusely within the IPL ($n = 23$ cells, 2 mice; Fig. 1D; Fig. 1-Supplement 1). These results indicate that SACs form IPL sublayers at P0-P1, and are joined later by their synaptic partners.

**Early SAC projections target neighboring SAC somata**

To gain insight into how SACs form their sublayers, we next investigated the events immediately preceding SAC stratification. At P0-1, other IPL sublayers do not yet exist (Kay and Sanes, 2013; Stacy and Wong, 2003), so SACs are unlikely to innervate their sublayers by following pre-existing cues. Instead, we hypothesized that SACs create their sublayers by engaging in homotypic interactions. To test this idea, we examined embryonic retina to determine if and when SACs first establish homotypic contact. SACs exit the cell cycle at the apical retinal surface and migrate radially through the outer neuroblast layer (ONBL). They next
arrive at the inner neuroblast layer (INBL), where postmitotic neurons reside (Hinds and Hinds, 1978; Fig. 2A,B). Then they begin to innervate the nascent IPL, which begins to appear in some retinal regions at E16 (Fig. 2A). To reveal SAC morphology throughout these steps, we used mice expressing membrane-targeted GFP (mGFP) under control of the *Isl1* gene (Galli-Resta et al., 1997). In these *Isl1* mG mice, we found that migrating SACs in the E16 ONBL rarely contacted each other (Fig. 2A-C). However, upon arriving at the INBL, SAC arbors contacted the soma or primary dendrite of neighboring SACs (Fig. 2D,E). The majority of INBL SACs projected these soma-directed neurites, such that a GFP⁺ arbor network connected them (Fig. 2E,H).

In retinal regions where IPL neuropil had emerged by E16 (Fig. 2A,B), SACs also sent a distinct set of arbors to innervate the IPL (Fig. 2E), raising the question of whether the soma- or IPL-directed projection establishes the first homotypic contact. We concluded that soma-directed SAC contact precedes IPL innervation, for two reasons. First, soma contacts were found in retinal regions where the IPL had not yet emerged (Fig. 2-Supplement 2). Second, soma contacts were observed among cells that still showed migratory morphological features, such as apical and/or basal processes (Deans et al., 2011; Hinds and Hinds, 1978), and did not yet project into the IPL (Fig. 2D; Fig. 2-Supplement 2). Indeed, rather than projecting to the IPL, SACs oriented their primary dendrites towards their neighbors. This was shown by staining for the intermediate filament protein internexin (Knabe et al., 2007), a selective marker of SAC primary dendrites (Fig. 2–Supplement 1). Unlike P1-2 SACs, which exclusively directed primary dendrites towards the IPL, E16 SACs projected in a variety of directions, including within the INBL (Fig. 2F,G; Fig. 2-Supplement 1). In many cases, SACs projected directly towards each other (Fig. 2F). Thus, INBL SACs appear to transiently seek out soma contact before shifting to target the IPL.
Together, these data indicate that SACs first make homotypic contact by projecting arbors toward neighboring SAC somata, and that this contact occurs at, or perhaps just before, completion of their radial migration (Fig. 2N).

To determine how long these soma-directed SAC contacts persist, we examined SAC anatomy at early postnatal ages using Isl1mG and ChatmG (Fig. 1-Supplement 1) mice. At P0-1, SAC arbors within the soma layers remained remarkably prominent (Fig. 2H). Most OFF SACs assumed a bi-laminar morphology, with one set of arbors in the IPL and another set in the INL (Fig. 2I-K,M; Fig. 2-Supplement 2). As with E16 contacts, the P1 INL arbors selectively contacted somata or arbors of SAC neighbors (Fig. 2J; 89% of branches terminated on a homotypic neighbor; n = 122 arbor tips from 22 cells). By P2-3, however, this dense INL network was mostly gone (Fig. 2H,M; Fig. 2-Supplement 2). ON SACs also made soma layer projections between P0-P3 that selectively contacted neighboring SAC somata (Fig. 2L,M; Fig. 2-Supplement 2). While the GCL SAC network was not as prominent as the INL network, some ON SACs established soma contact without a separate GCL projection; instead, these cells sent fine soma-directed branches from their IPL arbors (Fig. 2-Supplement 2). Together, these observations demonstrate that both ON and OFF SACs make transient soma-directed homotypic contacts that arise prior to IPL dendrite elaboration, and are disassembled at P2-3 after SAC sublayers have formed (Fig. 2N).

**Homotypic contact is required for SAC IPL innervation and dendrite lamination**

SAC homotypic contacts arise at a time when they could serve as a cue for IPL innervation and sublayer formation. To test this idea, we developed a genetic strategy to prevent SACs from contacting each other in vivo. Ptf1a encodes a transcription factor required for progenitor cells to assume an amacrine fate (Fujitani et al., 2006; Nakhai et al., 2007; Fig. 3-
Supplement 1). We crossed conditional Ptf1a$^{\text{floxed}}$ mutant mice (Krah et al., 2015) to a Cre line (Six3:Cre; Furuta et al., 2000), that drives widespread recombination in central retina but spares some progenitors from Cre activity in peripheral retina (Fig. 3A; Fig. 3-Supplement 1). In Six3:Cre; Ptf1a$^{\text{floxed/floxed}}$ mice (abbreviated Ptf1acKO), only these spared Cre$^{-}$ progenitors were capable of giving rise to SACs, indicating that any SACs produced in these mutants are wild-type at the Ptf1a locus (Fig. 3C). Therefore, the Ptf1acKO mutant creates a situation where otherwise-normal SACs are present at significantly lower density than in wild-type retina (Fig. 3B,C). In P1-2 mutants, some SACs were effectively segregated from their neighbors – these were termed “solitary” SACs – while others had neighbors sufficiently nearby that they touched (Fig. 3B,E,F; Fig. 3-Supplement 1).

Comparing solitary to touching SACs in Ptf1acKO retinas revealed a role for homotypic contacts in promoting IPL innervation and sublayer formation. At P1-2, touching SACs projected normally to the IPL, similar to SACs from Ptf1a$^{+}$ littermates (Fig. 3D,E,G). This suggests that any changes in retinal cell type composition caused by loss of Ptf1a (Fig. 3-Supplement 1) are not by themselves sufficient to perturb SAC sublayer formation. By contrast, solitary SACs largely failed to innervate the IPL (Fig. 3F,G). This was not caused by abnormal migration: Solitary SACs were properly positioned at the IPL border, but sent only rudimentary arbors into it (Fig. 3F; Fig. 3-Supplement 1). Solitary SACs were also more likely to project processes into the soma layers (Fig. 3G), and when they did so, the projections were typically more elaborate than those observed in wild-type retina (Fig. 3D,F; Fig. 3-Supplement 1). Thus, solitary SACs overgrew arbors directed towards neighboring somata instead of growing IPL dendrites. Both types of projection errors were also seen at P15, indicating that early errors persist to retinal maturity (Fig. 3-Supplement 1). Misprojecting SACs were still in contact with numerous other
amacrine cells and their arbors, strongly suggesting that generic amacrine contacts are not sufficient to ensure normal dendrite targeting (Fig. 3-Supplement 1). Instead, homotypic interactions are specifically required for IPL innervation and sublayer formation.

**Requirement for MEGF10 in SAC IPL innervation and sublayer formation**

To understand how SACs initiate IPL innervation upon homotypic contact, we next sought to identify the molecular cues that SACs use to recognize that contact has occurred. The cell-surface protein MEGF10 (Fig. 4A) is a strong candidate to mediate homotypic recognition in this context, for four reasons. First, it is selectively expressed by SACs during the perinatal period (Fig. 1B; Fig. 1-Supplement 1). Second, the onset of its expression coincides with onset of SAC homotypic contact at the conclusion of radial migration (Fig. 4B; Kay et al., 2012). Third, MEGF10 protein is present on soma-directed SAC arbors, making it available to transduce signals arising on these arbors (Fig. 4C). Finally, MEGF10 mediates SAC-SAC interactions in a separate context – during formation of the orderly “mosaic” among SAC cell bodies across the retina (Kay et al., 2012). Thus, we tested whether MEGF10 also mediates SAC-SAC recognition to initiate IPL innervation. If so, SACs from mice lacking Megf10 gene function should have phenotypes similar to solitary Ptf1a<sup>k<sup>ko</sup></sup> SACs – i.e., reduced IPL innervation and increased arborization in cell body layers.

To test this prediction, we examined SAC anatomy in Megf10 null mutants (Kay et al., 2012) and littermate controls at P0-1, when sublayers are first forming. We found a striking deficit in sublayer formation – both ON and OFF strata were absent or severely disrupted in mutants (Fig. 5A). This phenotype was not due to aberrant SAC migration, because mutant SACs reached the inner retina in normal numbers (wild-type, 2600 ± 287 SACs/mm<sup>2</sup>; mutant, 3153 ± 145 SACs/mm<sup>2</sup>; p = 0.144, 2-tailed t-test; n = 3 each group) and assumed a normal
location adjacent to the IPL at P0 (Fig. 5A). Rather, the absence of SAC sublayers was due to
innervation of the soma layers instead of the IPL – a phenotype reminiscent of solitary Ptf1aKO
SACs. This phenotype was specific to SACs: Other amacrine cell types showed normal dendritic
morphology in Megf10 mutants (Fig. 5-Supplement 1). The severe disruption to SAC IPL
innervation was evident from pan-SAC labeling (Fig. 5A) as well as single cell analysis, which
revealed that most Megf10−/− SACs made rudimentary, unstratified IPL projections at P0-1 (Fig.
5C,E; n = 1/15 OFF SACs were stratified). Mutant IPL dendrites appeared undifferentiated, with
a lack of space-filling branches (Fig. 5C,E). As a result, not only did their arbors enclose a
significantly smaller IPL territory, but they also failed to sample as much of their enclosed
territory as control SACs (Fig. 5C; also compare to control cell in Fig. 2I). By P3 some ON SAC
IPL innervation was evident, but OFF SAC arbors remained largely confined to the soma layer;
those that did reach the IPL remained undifferentiated (Fig. 5B,E). These observations indicate
that deletion of MEGF10 causes a profound impairment of IPL-directed SAC dendrite growth,
preventing timely sublayer formation.

Instead of innervating the IPL, Megf10 mutant SACs ramified exuberantly in the soma
layers. Both ON and OFF SACs were affected (Fig. 5-Supplement 1), but the OFF SAC
phenotype was particularly striking: Between P0 and P1, the mutant INL network grew to
become much more elaborate than the control network of any age (Fig. 5A,B,E,F; Fig. 5-
Supplement 1). Individual P1 SACs had larger INL arbors than control SACs (Fig. 5-Supplement
1), even though their IPL arbors were smaller (Fig. 5C), suggesting that they preferentially
projected to the soma layer. Supporting this notion, P1 mutant SACs often projected primary
dendrites towards each other, reminiscent of E16 newborn SACs (Fig. 5D). These findings
suggest that mutant SACs continued to grow soma-directed arbors at an age when control SACs
exclusively targeted the IPL (Fig. 5D; Fig. 2-Supplement 1). In addition to being more elaborate, mutant soma-directed arbors were also more persistent: Control SACs mostly eliminated these arbors by P3 but mutants maintained them (Fig. 5B,F). Together, our data suggest that MEGF10 governs a developmental transition from soma-directed to IPL-directed arbor growth: Whereas control SACs have only a brief period of soma-directed growth, switching to IPL ramification by P0, Megf10 mutant SACs do not make this transition and instead persist in soma-layer innervation (Fig. 5G). This phenotype is consistent with a failure of homotypic recognition (Fig. 3). We conclude that, because MEGF10 regulates IPL innervation in this way, MEGF10 is required for initial formation of SAC IPL sublayers at P0-1.

**SAC dendrite targeting requires transcellular MEGF10 signaling**

Given the similar phenotypes of Megf10 mutant and solitary Ptf1a\textsuperscript{cKO} SACs, we hypothesized that MEGF10 is the molecular cue that triggers IPL innervation upon SAC-SAC contact. A key prediction of this model is that SACs should require MEGF10 signals from their neighbors to target their dendrites properly. To test this prediction, we generated a conditional Megf10\textsuperscript{flox} allele and used it to create a situation where Megf10\textsuperscript{+} SACs were surrounded by Megf10\textsuperscript{−} mutant cells. This was accomplished via the same Six3:Cre strategy that we employed in our Ptf1a\textsuperscript{cKO} studies (Fig. 3A-C). In central retina of Six3:Cre; Megf10\textsuperscript{flox/lacZ} (Six3-Megf10\textsuperscript{cKO}) animals, the vast majority of cells expressed a Cre-dependent GFP reporter, indicating that they lacked Megf10 function (Fig. 6A). Accordingly, SACs projected exuberantly to the INL and sublayer formation was disrupted, as in null mutants (Fig. 6B; Fig. 6-Supplement 1). In peripheral retina, some SACs escaped Cre activity, leading to absence of the GFP reporter and continued MEGF10 protein expression (Fig. 6A,B; Fig. 6-Supplement 1). Our model predicts that these cells should have mutant dendrite phenotypes despite retaining
MEGF10. To test this prediction, we imaged βgal-stained OFF SACs from Six3-Megf10KO and littermate control mice at P2. This age was chosen because wild-type and null mutant mice showed a large difference in SAC INL projection frequency (Fig. 5F). In littermate controls, we found that βgal+ SACs rarely projected to the INL (Fig. 6C,D); therefore, they behaved like control SACs from earlier experiments (Fig. 5F). By contrast, Megf10+ SACs surrounded by mutant SACs in Six3-Megf10KO retina showed a high rate of INL projections, nearly identical to their Megf10− neighbors (Fig. 6B,D; Fig. 6-Supplement 1). Thus, when Megf10+ SACs are deprived of MEGF10 signal from adjacent SACs, they make exuberant soma-directed projections. This finding implicates MEGF10 as a transcellular signal that controls SAC dendrite targeting.

Next we investigated how SACs receive this MEGF10 signal from their neighbors. Given that MEGF10 can function as a receptor in other contexts (Chung et al., 2013; Kay et al., 2012), we speculated that MEGF10 might act as its own receptor. In support of this idea, co-immunoprecipitation experiments using intracellularly truncated Megf10 constructs showed that MEGF10 can interact with itself through its extracellular domain (Fig. 6I,J; Fig. 6-Supplement 2). Thus, MEGF10 appears biochemically capable of acting as both ligand and receptor.

If MEGF10 is indeed a receptor in this context, SACs should require it to detect contact with MEGF10-expressing homotypic neighbors. To test this prediction, we asked whether removal of Megf10 from a single SAC, during the period of soma-directed homotypic contact, would impair its IPL innervation despite normal MEGF10 expression by surrounding cells. We used ChatCre to achieve sparse recombination in SACs of neonatal mice, as in the anatomy experiments described above (Fig. 2I-L; Fig. 5E). In Chat-Megf10KO animals, MEGF10 immunostaining was used to identify SACs that lost MEGF10 protein prior to P3 – i.e., during
the period when soma-directed arbors are present (Fig. 6F,G; Fig. 6-Supplement 1). MEGF10− cells constituted a small minority of SACs at P3, meaning that they were generally surrounded by MEGF10+ neighbors (Fig. 6-Supplement 1). In this context, MEGF10− SACs produced more exuberant soma-directed arbors than neighboring MEGF10+ cells, while sending only minimal arbors into the IPL (Fig. 6E-H). Thus, single MEGF10− SACs had phenotypes similar to SACs from mice entirely lacking Megf10 (Fig. 6G,H; compare to Fig. 5E). By contrast, adjacent MEGF10+ cells in the same Chat-Megf10cKO retinas were indistinguishable from littermate control SACs (Fig. 6E,F,H). Therefore, when Megf10 is lost during dendro-somatic contact (but not after; see below), SACs make projection errors typical of neurons deprived of homotypic interactions, and they do so even if their neighbors express MEGF10 and are developing normally. Together, these experiments support the conclusion that MEGF10 is a receptor through which SACs detect each other to terminate soma-directed growth and initiate IPL innervation.

**SAC errors persist to adulthood in Megf10 mutants**

We next asked whether neonatal MEGF10-mediated interactions influence the anatomy of SAC IPL sublayers at maturity. We found that SAC sublayers eventually formed (by P5; Fig. 7I,J), and were present in the mature Megf10+/− retina, but they were marred by numerous errors. Sporadically, and at apparently arbitrary retinal locations, two kinds of local laminar disruptions were apparent. First, there were discontinuities in the ON and OFF strata, such that mutant SACs did not completely innervate their sublaminae (Fig. 7A-C). Innervation gaps were not observed for other amacrine cells, indicating that SACs were selectively affected (Fig. 7-Supplement 1). Examination of single SACs revealed that while dendritic patterning substantially recovered between P1 and adulthood, SAC arbor territories remained significantly smaller in mutants (Fig. 305).
These phenotypes suggest that mutant SACs never fully made up for their initial IPL innervation deficit, thereby contributing to gaps in the dendritic plexus.

The second type of error we observed in mature $\text{Megf10}^{−/−}$ IPL was ectopic SAC projections outside their typical IPL strata (Fig. 7A,B,E). We suspected that these adult ectopias arose due to persistence of exuberant P1-P3 projections, because both the morphology of the ectopic network at each age, and the number of SACs projecting into it, were quite similar (Fig. 7E-G). However, there were also two notable anatomical differences between P1-3 and adult. First, neonatal ectopically-projecting SACs sent fine arbors in many directions, while adult ectopic projections formed discrete aggregates (Fig. 7E,F). Second, these adult aggregates were located in the IPL, whereas neonatal arbors targeted the soma layers (Fig. 7A,B,E,F,H). These differences led us to consider the possibility that the two SAC arbor phenotypes might be unrelated.

To test the idea that diffuse neonatal exuberant arbors give rise to clumpy mature ones, we assessed changes in mutant SAC arbor anatomy across development. If our hypothesis is correct, we would expect this analysis to identify a time when ectopic SAC arbors transition between the two phenotypic states. Indeed, we found that this transition occurs at P5: Both arbor aggregation and IPL localization first arose at this time (Fig. 7F,H-J). These transitions occurred without a significant change in the number of mutant SACs projecting into the ectopic network (Fig. 7G; Fig. 7-Supplement 1), suggesting that the same cells continued to participate in the network but simply altered their anatomy between P3 and P5. Supporting this notion, we identified individual P5 $\text{Chat}^{mG}$-labeled SACs that projected both to ectopic IPL strata and to the soma layers, suggesting they were in the process of remodeling their arbors (Fig. 7H,J). Such anatomy was never observed at earlier or later stages (Fig. 7H). These observations support the
conclusion that early exuberant INL arbors are converted into IPL ectopias, starting between P3 and P5.

Together, these studies of adult SAC anatomy demonstrate that DS circuit sublayer formation is delayed and imperfect in the absence of MEGF10. While other mechanisms appear to partially compensate for MEGF10 in generating the sublayers, such mechanisms are not sufficient to prevent persistence of innervation gaps and laminar targeting errors. Thus, MEGF10 is essential for normal formation of the mature SAC IPL projection.

Next we sought to directly test the idea that MEGF10 is required early – at the time of initial SAC homotypic contact – to ensure normal SAC IPL lamination at maturity. To this end, we used \textit{Megf10}\textsuperscript{lox} mice to delete MEGF10 at different times. Deletion prior to the onset of homotypic contact, using the \textit{Six3:Cre} line, fully phenocopied \textit{Megf10}\textsuperscript{−/−} adult IPL errors (Fig. 8A), suggesting a requirement for MEGF10 at the time of contact. To remove MEGF10 from SACs that had already established homotypic contact, we used the \textit{ChatCre} line. In this line, the number of SACs expressing \textit{ChatCre} gradually increases over the first postnatal days to encompass the full SAC population (Xu et al., 2016). Therefore, \textit{Chat-Megf10cKO} mice can be used both for early, sparse MEGF10 deletion (Fig. 6F-H) and for later, broad MEGF10 deletion. MEGF10 immunostaining revealed that this late, broad deletion occurs between P3 and P5 (Fig. 6-Supplement 1), such that MEGF10 expression is largely preserved during the period when homotypic soma-layer contacts exist (Fig. 2M), but is eliminated shortly thereafter. In this \textit{ChatCre}-mediated deletion regime, SAC laminar targeting and gap errors were exceedingly rare (Fig. 8A). These experiments therefore define a time window for MEGF10 function: Adult IPL targeting phenotypes require absence of MEGF10 during the soma-directed projection phase of SAC development – i.e. prior to P3. Any additional activity of MEGF10 after P3 is dispensable
for the adult IPL phenotype. These findings strongly support a model whereby the functions of MEGF10 during early homotypic contact – i.e. promoting IPL innervation and terminating soma-directed arbor growth – are necessary for development of normal SAC IPL innervation at maturity.

**Mosaic spacing errors do not account for SAC IPL phenotype in Megf10 mutants**

In addition to these laminar targeting errors, Megf10 mutants also show disruptions in the mosaic spacing of SAC cell bodies across the retina: Instead of a regular, uniform distribution, mutant SAC positioning is random (Kay et al., 2012). We sought to determine whether loss of homogeneous SAC soma positioning in mutants contributes to their loss of homogeneous IPL innervation (Fig. 7A-E). If so, local variations in SAC soma and arbor density should be strongly correlated. We tested for soma-arbor correlations in two ways. First, we examined global correlations by determining the spatial cross-correlation between images of SAC cell bodies and of their underlying IPL arbors. This analysis revealed that soma and arbor positions were in fact more weakly correlated in mutants than in controls (Fig. 8-Supplement 1). Thus, even though soma-arbor correlations exist in mutants, they are not sufficient to explain mutant IPL arbor arrangements (Fig. 8-Supplement 1). Additional contributing factors likely include changes in dendritic arbor size (Fig. 7D), or other deficits arising from the early delay in IPL innervation.

Next we addressed soma-IPL correlations on a single-cell level. To do this, we first developed a way to score the severity of the mosaic phenotype on a cell-by-cell basis. The spatial arrangement of a SAC relative to all of its nearest neighbors was quantified by measuring its unique territory (i.e. Voronoi domain; see Methods). Because the mutant SAC distribution is random, there are many mutant cells that, by chance, are positioned quite normally relative to their neighbors; there are also many cells whose neighbors are abnormally near or far (Fig. 8C-E).
If soma position causes IPL projection errors, then SACs located in “normal” regions of mutant retina should make targeting errors less often than SACs located in perturbed regions. However, this was not the case: Across all territory sizes, the rate of ectopic IPL projection was quite uniform, and indistinguishable from the overall error rate for mutants (Fig. 8F). The only exception was cells with the very largest territories – larger than nearly all values ($n = 7/515$) in the control distribution. These cells made fewer ectopic projections than the typical mutant cell, but still made errors about 50% of the time (Fig. 8F). These results demonstrate that ectopic IPL lamination errors are largely independent of soma position, supporting the conclusion that ectopias arise due to persistence of early mistargeted SAC arbors.

These correlational anatomical studies of adult $\text{Megf10}^{-/-}$ retina suggested that disturbed SAC mosaics make only minimal contributions to the IPL projection phenotype. To test this idea experimentally, we used our $\text{Megf10}^{\text{lox}}$ conditional allele. We found that deletion of MEGF10 after P3 in $\text{Chat-Megf10}^{\text{cko}}$ mice dissociated the two phenotypes: Mosaic patterning was disturbed in these animals, but IPL projections were largely normal (Fig. 8A,B). This finding demonstrates that IPL laminar perturbations are not an inevitable consequence of altered soma positioning. Altogether, these experiments support the notion that delayed IPL innervation and exuberant soma-layer arborization are the major source of perturbed SAC projections at maturity.

**SAC IPL errors induce laminar targeting errors by their DS circuit partners**

We next tested the impact of SAC IPL stratification errors on laminar targeting by their circuit partners. First, we examined ooDSGC IPL projections using the $\text{Hb9:GFP}$ (Fig. 9A-C) and $\text{Drd4:GFP}$ (Fig. 9-Supplement 1) transgenic lines, which label ooDSGC subtypes with different preferred directions (Trenholm et al., 2011; Huberman et al., 2009). In littermate control mice ($n = 9$), ooDSGC dendrites were tightly and selectively associated with SAC arbors,
as shown previously (Vaney and Pow, 2000). This association was maintained in *Megf10* mutants: Both normal and ectopic SAC IPL arbors reliably recruited ectopic ooDSGC projections (Fig. 9A-B; Fig. 9-Supplement 1; \(n = 240\) ectopias from 5 mutants, >97% contained ooDSGC arbors). Further, when SAC gaps were present in the mutant IPL, ooDSGC dendrites typically grew around the gap edges and failed to enter them (Fig. 9C; Fig. 9-Supplement 1; \(n = 325\) gaps from 5 mutants, >95% devoid of ooDSGC arbors). Thus, SACs provide both permissive cues required for ooDSGC IPL innervation, and also attractive cues sufficient to recruit ooDSGCs to the wrong IPL sublayer.

Next we determined the impact of altered SAC lamination on the axons of bipolar cells that participate in the DS circuit – i.e., the four types (BC2, BC3a, BC5, and BC7) that make extensive monosynaptic connections with SACs and ooDSGCs (Duan et al., 2014; Ding et al., 2016; Greene et al., 2016; Kim et al., 2014; Chen et al., 2014). Bipolar cells were marked with type-specific antibodies and mouse lines reported previously (Wässle et al., 2009; Duan et al., 2014), as well as a novel transgenic marker of BC5 (*Gjd2::GFP*; Fig. 9-Supplement 1). In wild-type retina, DS-circuit bipolar cells arborized in close contact with SAC dendrites; however, unlike ooDSGCs, they remained adjacent to SACs rather than overlapping them (Fig. 9D,E,G; Fig. 9-Supplement 1). This arrangement was preserved in *Megf10* mutants: Axons of all four bipolar cell types were recruited to ectopic IPL locations by mistargeted SAC arbors, where they stratified adjacent to SACs (Fig. 9D-G; Fig. 9-Supplement 1). For example, BC5 and BC7 terminals always sandwiched SAC arbors, regardless of their IPL location – even when doing so required formation of a supernumerary BC axon field between the normal and ectopic SAC sublayers (Fig. 9D,E). To quantify the mistargeting effect we measured the position of BC5 and BC7 terminals adjacent to ON SAC ectopias. Their arbors were pushed farther apart by SAC
arbor clumps (Fig. 9E,F), which shifted BC7 terminals significantly towards the GCL by ~4 µm (69 ± 0.8% of IPL depth in control regions to 74 ± 1.9% in affected regions; mean ± S.E.M.; n = 21 control, 6 affected; 2-tailed t-test, p = 0.0024). No changes were seen in Syt2-labeled BC6 arbors, suggesting a specific effect on the bipolar cell types that make extensive contacts with SACs (data not shown). These observations indicate that DS-circuit bipolar cells, like ooDSGCs, respond to SAC attractive cues. However, in contrast to ooDSGCs, bipolar cell projections were minimally affected by SAC IPL gaps. While BC5 and BC7 terminals were slightly mispositioned in the absence of SAC arbors – they were closer together – innervation of gap regions was otherwise normal (Fig. 9D-F; Fig. 9-Supplement 1). Thus, DS-circuit bipolar axons either do not require SAC-derived signals for IPL innervation, or the relevant signals are capable of acting over larger distances than the typical SAC IPL gap size (35-45 µm maximum diameter). Altogether, these analyses of DS circuit anatomy in Megf10 mutants support the notion that early-stratifying SACs form a scaffold that directs IPL laminar targeting of their circuit partners using multiple guidance cues.

**Early SAC homotypic interactions impact DS circuit function**

Finally, we investigated the extent to which developmental events controlled by MEGF10 affect DS circuit function. We sought to determine whether the anatomical perturbations caused by loss of MEGF10 – SAC laminar targeting and mosaic spacing errors – alter direction coding by ooDSGCs. To do this we recorded from wild-type and Megf10–/– retinas on a large-scale multielectrode array (Field et al., 2007; Yu et al., 2017). ooDSGCs were identified based on their responses to drifting gratings and moving bars (see Methods), which unambiguously distinguished them from other recorded RGCs (Fig. 10A). Because MEGF10 is not expressed in
the adult DS circuit (Kay et al., 2012), we could be confident that any mutant physiological
phenotypes reflect anatomical changes that arose during development.

These experiments revealed that ooDSGCs with robust direction selectivity were present
in both wild-type and Megf10−/− retinas (Fig. 10A,B), and constituted a similar fraction of the
RGC population in both strains (wild-type: 80/609, 13.1%; mutant: 74/551, 13.4%).

Furthermore, loss of Megf10 did not alter the organization of ooDSGC preferred directions along
cardinal axes (Oyster and Barlow, 1967), or the fraction of ooDSGCs preferring each direction
(Fig. 10-Supplement 1). These results are consistent with the observation that mutant SACs
remain paired with ooDSGC dendrites and bipolar cell axons even when normal lamination and
arbor spacing are disrupted. They indicate that the qualitative functional properties of the circuit
are still present.

However, a more careful examination of DS tuning properties in Megf10−/− retinas
revealed clear quantitative differences in ooDSGC responses. Moving bars were used to measure
the width and strength of direction tuning for each identified ooDSGC across the populations
recorded on the electrode array (Fig. 10C). Tuning width was measured as the circular standard
deviation of the tuning curve, while tuning strength was measured as the normalized response
difference to motion in the preferred and null directions (see Methods). These experiments
revealed systematic shifts toward broader (Fig. 10D) and weaker (Fig. 10E) direction tuning
across the population of ooDSGCs in Megf10 mutant retinas. This was mainly due to higher null
direction spiking among ooDSGCs in mutants (Fig. 10B,C,E). Furthermore, these effects on
tuning width and strength persisted across a broad range of stimulus contrasts (Fig. 10-
Supplement 1). These results demonstrate that disruption of MEGF10-dependent developmental
patterning degrades the precision and strength of ooDSGC direction tuning. They further suggest
that perturbations to the anatomical regularly of the circuit across space (e.g. laminar uniformity and SAC spacing) may effectively introduce noise in the DS circuit that broadens and weakens direction tuning (see Discussion).

This idea led us to consider additional functional properties of ooDSGCs that might depend on the spatial regularity of the DS circuit, and therefore might be perturbed in *Megf10* mutants. One such property is the generation of symmetric DS responses to stimuli that are darker or brighter than the background (Fig. 10F,G). This ON-OFF symmetry allows the DS response to be largely insensitive to contrast reversals (Amthor and Grzywacz, 1993); it arises because ooDSGCs receive highly symmetric SAC inputs in both ON and OFF sublayers (Fig. 1A). In *Megf10* mutants, ON-OFF anatomical symmetry is disturbed, because ON and OFF SAC errors are not spatially correlated (Fig. 7A-C). We hypothesized that this might lead to disparities in the direction tuning of individual cells’ ON and OFF responses. Indeed, *Megf10*−/− ooDSGCs exhibited greater separation (i.e. less coherence) between their ON and OFF preferred directions than wild-type ooDSGCs, across a broad range of contrasts (Fig. 10H; Fig. 10-Supplement 1).

These results support the idea that MEGF10 serves to establish a highly uniform and regular network of SAC dendrites (via controlling both the precise timing of INL lamination and through regularizing inter-SAC spacing), the net effect of which is to allow greater precision and coherence in the direction tuning of ooDSGCs.
DISCUSSION

Neural circuits typically consist of multiple cell types born at different places and times, raising the question of how circuit partners manage to converge at a common site for selective synapse formation. Here we describe a developmental strategy that the retinal DS circuit uses to solve this problem. We show that SACs coordinate amongst themselves to assemble a dendritic scaffold that subsequently recruits projections from their DS circuit partners. By identifying for the first time a genetic manipulation – loss of \textit{Megf10} – that causes SACs to misproject outside their two typical IPL layers, we uncover mechanisms by which SACs assemble this dendritic scaffold. Further, we use \textit{Megf10} mutants to examine the effects on DS circuit anatomy and function when SAC sublayer formation is disrupted. We find that MEGF10 establishes DS circuit spatial homogeneity across the retina, both by controlling IPL innervation patterns and by positioning SAC cell bodies. In \textit{Megf10} mutants, disruptions in circuit homogeneity occur with minimal effects on radial SAC dendrite anatomy or synaptic partnering, making the phenotype unique among DS circuit developmental mutants. Finally, we find that this abnormal spatial pattern degrades DS circuit function by broadening the range of directions to which ooDSGCs will respond, and by weakening overall direction selectivity. These results provide new insight into general strategies for circuit development, as well as the specific mechanisms that ensure functional assembly of the DS circuit.

Homotypic recognition as a mechanism regulating dendrite differentiation

During radial migration, newborn central nervous system neurons have a multipolar morphology, but on arrival at their final position within the tissue they become highly polarized (Nadarajah et al., 2001; Tabata and Nakajima, 2003; Cooper, 2014; Chow et al., 2015; Krol et al., 2016; Hinds and Hinds, 1978). This morphological change enables elaboration of dendrites and
integration into local circuitry. If dendrite differentiation begins early, migration is impaired (Hoshiba et al., 2016), suggesting that the transition from migratory to mature morphology must be highly regulated to ensure that neurons only differentiate once they arrive at their final position. The extracellular cues that signal arrival are poorly understood in most nervous system regions.

Here we show that SACs use homotypic recognition, mediated by MEGF10, to initiate IPL-directed dendrite morphogenesis. When deprived of homotypic neighbors or MEGF10, SACs at the IPL retain a multipolar morphology (compare Fig. 2C to Figs. 3F, 5B) instead of polarizing arbors towards the IPL. This indicates that the transition from migratory to mature morphology is impaired in the absence of SAC homotypic recognition. We show that migrating SACs first establish homotypic contact upon arrival at the inner retina. At this stage they are still multipolar (Fig. 2D,E), but they orient primary dendrites towards each other, projecting within the INBL to contact their SAC neighbors. These contacts occur prior to IPL innervation, and are required for it to occur in a timely manner. SACs lacking neighbors or the molecular means to detect them (i.e., MEGF10) appear to persist in this multipolar soma-targeting phase, causing over-innervation of the soma layers and delaying IPL innervation (Fig. 5G). Thus, establishment of homotypic contact is a key checkpoint for the progression of SAC dendrite differentiation and IPL sublayer morphogenesis.

We propose that the function of this checkpoint is to ensure that SACs elaborate dendrites only when they have arrived adjacent to the IPL. The presence of other SACs that have already completed their migration is a reliable indicator of arrival in the proper location. Because soma-directed SAC contacts appear earliest, and because MEGF10 selectively influences IPL innervation during the period when they exist, we favor the notion that the key homotypic
interactions occur through these arbors. However, we cannot exclude that IPL-based interactions also play a role. INL-directed arbors resembling those we describe can be discerned in many developing zebrafish amacrine cells (Godinho et al., 2005; Chow et al., 2015), raising the possibility that this mechanism applies across species and across other amacrine cell types.

Because most neurons require a way to control when and where they differentiate, we anticipate that this homotypic contact strategy, or variations upon it, may have important roles in the differentiation of other CNS neurons at the completion of their radial migration.

**MEGF10 as the signal mediating SAC homotypic recognition**

We conclude that MEGF10 is the molecule responsible for homotypic recognition during SAC IPL innervation, for four reasons. First, MEGF10 is expressed at the right time and place to assume this role: It is expressed selectively in SACs (Fig. 1), upon conclusion of their radial migration, and in the soma-layer arbors that we propose mediate recognition (Fig. 4). Second, Megf10 null mutant SACs phenocopy the dendrite polarization errors seen in solitary Ptf1a<sup>cKO</sup> SACs, suggesting that homotypic recognition requires Megf10. Third, co-immunoprecipitation experiments indicate that MEGF10 interacts with itself via its extracellular domain, suggesting it could act as both ligand and receptor. While this biochemical interaction may take place in the cis configuration, the fourth line of evidence indicates that MEGF10 interacts in trans as well: Using a conditional-null Megf10 allele in vivo, we show that MEGF10 is required on the cell that sends homotypic signals as well as the cell receiving those signals. Loss of MEGF10 on either side leads to dendritic phenotypes resembling solitary SACs and Megf10 null mutants. Together, these data are consistent with a model whereby SAC-SAC contact initiates a transcellular MEGF10 homophilic interaction, in which MEGF10 serves as both receptor and ligand to trigger the switch from migratory to mature morphology.
This homophilic model of MEGF10 function is consistent with its role during establishment of mosaic cell body patterning (Kay et al. 2012). In that context, MEGF10 acts as ligand and receptor to mediate cell-cell repulsion, thereby spacing SAC somata evenly across the retina. Here we discover a second MEGF10 function in SAC IPL innervation. Because the two SAC phenotypes have different underlying cell biology (soma movement vs. dendrite dynamics), and different temporal requirements for MEGF10 function (Fig. 8A,B), it seems unlikely that they reflect disruption of a single biological event. Instead, MEGF10 appears to act at distinct, albeit partially overlapping times, to control different aspects of SAC development, each of which are regulated by contact with homotypic neighbors.

Formation of SAC IPL sublayers

Our results shed light on the mechanisms controlling SAC dendrite lamination. While repulsion mediated by Sema6a and PlexinA2 prevents OFF SACs from straying to the ON sublayer (Sun et al., 2013), molecules required for formation of the SAC sublayers have not been identified. We show that SACs deprived of homotypic neighbors or MEGF10 initially fail to form IPL sublayers, and when they eventually do so, their strata are riddled with errors. Both the lack of sublayers at early stages and the dendritic mistargeting to inappropriate sublayers at maturity are novel SAC phenotypes; they implicate MEGF10 as a key player in forming SAC IPL sublayer-specific projections.

It is generally assumed that sublayer formation has two basic molecular requirements: 1) Attractive/adhesive molecules that mediate co-fasciculation of stratified arbors; and 2) repulsive cues that prevent straying of arbors into other sublayers (Lefebvre et al., 2015; Sanes and Yamagata, 2009). Our MEGF10 studies suggest an additional, earlier requirement for cell-cell interactions that occur prior to neuropil innervation. The purpose of this surprisingly early SAC-
SAC interaction, we propose, is to ensure that SACs grow dendrites at the right time and place to co-fasciculate with their SAC neighbors. When IPL arborization is delayed by loss of *Megf10*, two SAC errors ensue. First, SACs generate mistargeted dendritic material that persists as ectopic IPL sublayers. Second, SACs never completely innervate their sublayers, resulting in fragmented IPL strata. This failure is caused by delays rather than an ongoing requirement for MEGF10 during later stages of arbor growth, as shown by conditional mutant experiments. Thus, our findings support the idea that timing is critical to the sequential lamination of the IPL: When SAC dendrites arrive in the IPL too late, they encounter a different cellular and molecular milieu that may not support the proper development of their arbors. In this view, the normal role of MEGF10 in DS circuit assembly is to instigate SAC dendrite outgrowth at the crucial time when laminar self-assembly can occur.

SACs may face an additional obstacle to overcoming their delayed IPL innervation in *Megf10* mutants – abnormal soma positioning. While mosaic spacing errors do not account for much of the *Megf10* mutant IPL phenotype, the placement of IPL arbor gaps might be at least partly explained by soma position. This effect was only seen in mutant conditions that also produced IPL innervation delay – mosaic disturbance alone was not sufficient to produce gaps. If SACs are struggling to make up for their delayed IPL innervation, it is plausible that increasing the distance between SACs (as happens sporadically due to random positioning) might further hinder the development of complete retinal coverage.

**SACs as a scaffold for DS circuit assembly**

Because of their early stratification, SAC dendrites have been proposed to act as a scaffold that guides assembly of the DS circuit (Stacy and Wong, 2003). A key prediction of this model is that laminar targeting of later-stratifying cell types should depend on the existence of
this scaffold. We show using a SAC-specific manipulation – removal of Megf10 – that disruption of SAC stratification causes their bipolar and ooDSGC circuit partners to make corresponding projection errors. Based on the kinds of errors we observed, SACs appear to provide attractive, permissive, and even repulsive arbor sorting cues to influence the laminar positioning of their circuit partners. This work thus constitutes the first critical test of the scaffolding model, and provides strong support for it. We find that SACs use homotypic interactions to initiate formation of their circuit sublayers, and then heterotypic interactions to recruit circuit partners to join them. The scaffolding functions may be mediated in part by Cadherins 8 and 9, which regulate interactions between SAC dendrites and DS circuit bipolar cell axons (Duan et al., 2014). Molecular mediators of ooDSGC-SAC dendrite interactions remain to be identified.

Evidence that the SAC scaffold can be repulsive came from our observations of BC axon anatomy. In wild-type retina, we were surprised to note how completely the BC3a, BC5, and BC7 axon terminals were excluded from the SAC territory – they contacted it but did not enter (Fig. 9D-G; Fig. 9-Supplement 1). Moreover, in Megf10 mutants, the laminar distance between BC5 and BC7 terminals was reduced in the absence of SAC arbors, and increased in the presence of SAC ectopias, further suggesting the existence of local SAC-BC repulsion. The finding that SACs exclude bipolar circuit partners from their sublayers appears at first counterintuitive. But given that no bipolar cell type is exclusively devoted to the DS circuit (Wässle et al., 2009; Greene et al., 2016; Kim et al., 2014), a mechanism must exist to ensure that they can also contact non-DS partners. We speculate that SACs initially recruit their bipolar partners using long-range attractive cues, and then use contact-repulsion (or an equivalent arbor sorting mechanism) to displace bipolar arbors such that they remain in contact with the SAC layers but
also innervate adjacent layers. This model is consistent with bipolar arbor phenotypes in *Megf10* mutants, but will require further study.

**Role of MEGF10 in the functional assembly of DS circuitry**

We found that impairment of SAC interactions in the perinatal retina causes permanent functional DS circuit deficits. In *Megf10* mutants, direction tuning of ooDSGCs becomes broader and weaker, and their ON/OFF preferred directions are less aligned. Direction tuning is degraded in large part because mutant ooDSGCs have aberrant spiking responses to null-direction stimuli. This suggests that impaired null-direction inhibition – which arises from SACs – is a key contributor to the phenotype. Broader ooDSGC tuning curves have been shown, in modeling studies, to degrade population-level coding of directional information, and the ability of downstream neurons to extract such information (Fiscella et al., 2015). Thus, the physiological phenotypes we identified are likely sufficient to impair the ability of mutant retina to appropriately relay visual information.

Dysfunctional DS circuit physiology in *Megf10* mutants is almost certainly a consequence of its effects on development, because neurons do not express MEGF10 beyond the second postnatal week (Kay et al., 2012). Further, even though MEGF10 is expressed by Müller glia in adulthood, we have been unable to detect any changes in Müller glia anatomy or interactions with DS circuit synapses upon loss of *Megf10* function (Wang et al., 2017; J.W. and J.N.K., unpublished observations). We therefore conclude that anatomical changes to the DS circuit arising during development are responsible for circuit dysfunction.

The fundamental change to DS circuit anatomy in *Megf10* mutants is altered distribution of arbors and synapses, unlike other manipulations which simply serve to destroy SAC radial morphology or disrupt synaptic partnering among DS circuit cells (Sun et al., 2013; Duan et al.,
In *Megf10* mutants, the combined effect of mosaic spacing defects and IPL laminar targeting errors is to disturb the regularity of SAC IPL innervation. As a result, some parts of the visual map become over-innervated (e.g. Fig. 9A) while others are uninnervated (Fig. 9C). In turn, ooDSGCs are recruited to the over-innervated regions and excluded from uninnervated gaps, likely causing sporadic local inhomogeneity in synapse density across visual space. According to some models of DS, which posit that the total amount of SAC inhibition is the key factor underlying DS responsiveness, these relatively small-scale changes would be considered unlikely to change circuit function (Taylor and Vaney, 2002; Demb, 2007). A more recent alternate view is that the fine spatial arrangement of glutamatergic inputs to SACs, and the synaptic balance of SAC and bipolar input onto ooDSGC dendrites, are both important for DS responses (Ding et al., 2016; Vlasits et al., 2016; Poleg-Polsky and Diamond, 2016; Sethuramanujam et al., 2016, 2017). The finding that *Megf10* mutants have DS tuning phenotypes suggests that local synaptic arrangements are indeed important for the DS computation. More broadly, this finding shows that the developmental mechanisms we describe here are important for enabling circuit function, raising the possibility that other circuits throughout the retina and CNS may use similar developmental mechanisms to establish their functional connectivity.
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COMPETING INTERESTS

The authors have no competing interests to disclose.
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### MATERIALS AND METHODS

#### KEY REAGENTS TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Megf10: rabbit, 1:1000 | Kay et al., 2012 | ab97959 |
| Sox2: rabbit, 1:500  | Abcam |            |
| Sox2: goat, 1:500    | Santa Cruz | sc-17320 |
| ChAT: goat, 1:400    | EMD Millipore | AB144P |
| Beta Galactosidase: rabbit, 1:5000 | J. Sanes, Harvard | |
| GFP: chicken, 1:1000 | Life Technologies | A10262 |
| GFP (Co-IP): rabbit, 1:1000 | Thermo Fisher Scientific | A-6455 |
| AP-2a: mouse, 1:200 | Developmental Studies Hybridoma Bank | 3B5 |
| RBPMS: guinea pig, 1:2000 | N. Brecha, UCLA | |
| Chx10: sheep, 1:300 | Exalpha | X1180P |
| Chx10: goat, 1:500 | Santa Cruz | sc-21690 |
| GAD65: rabbit, 1:1000 | Millipore | AB1511 |
| FLAG: mouse, 1:500 | Sigma Aldrich | F-1804 |
| VGLUT3 guinea pig | synaptic systems | 135 204 |
| Synaptotagmin-2 (Syt2), mouse, 1:250 | Zebrafish International Resource Center | ZDB-ATB-081002-25 |
| Isl1: mouse, 1:25 | Developmental Studies Hybridoma Bank | 39.4D5 |
| Internexin: rabbit, 1:1000 | EMD Millipore | AB5354 |
| Normal Rabbit IgG | Cell signaling Technology | 2729S |
| Normal Mouse IgG | Cell Signaling Technology | 5415S |
| Alexa Fluor 488 AffiniPure Donkey | Jackson | 703-545-155 |
| Anti-Chicken: 1:1000 | ImmunoResearch | |
| Alexa Fluor 488 AffiniPure Donkey | Jackson | 711-545-152 |
Anti-rabbit: 1:1000 ImmunoResearch
Alexa Fluor 488 AffiniPure Donkey Anti-goat: 1:1000 Jackson ImmunoResearch
Alexa Fluor 488 AffiniPure Donkey Anti-mouse: 1:1000 Jackson ImmunoResearch
Alexa Fluor 647 AffiniPure Donkey Anti-rabbit: 1:1000 Jackson ImmunoResearch
Cy3-AffiniPure Donkey Anti-rabbit: 1:1000 Jackson ImmunoResearch
Cy3-AffiniPure Donkey Anti-Guinea Pig: 1:1000 Jackson ImmunoResearch
Cy3-AffiniPure Donkey Anti-Goat: 1:1000 Jackson ImmunoResearch
IRDye® 680RD Donkey anti-Mouse IgG (H + L): 1:1000 Li-Cor Biosciences 925-68072
IRDye® 800CW Donkey anti-Rabbit IgG (H + L): 1:1000 Li-Cor Biosciences 925-32213

Bacterial and Virus Strains
AAV9.hEF1a.lox.TagBFP.lox.eYFP.lox.WPRE.hGH-InvBYF(Harvard) Penn Vector Core AV-9-PV2453
AAV9.hEF1a.lox.mCherry.lox.mTFP1.lox.WPRE.hGH-InvCheTF(Harvard) Penn Vector Core AV-9-PV2454

Biological Samples

Chemicals, Peptides, and Recombinant Proteins
Fetal Bovine Serum Life Technologies 16250-078
0.5% Trypsin-EDTA phenol red Life Technologies 25300-054
Dulbecco's Modified Eagle Medium (DMEM) Thermo Fisher Scientific 11995065
Penicillin Streptomycin Thermo Fisher Scientific 15070063
Opti-MEM® I Reduced Serum Medium Thermo Fisher Scientific 31985070
Polyethyleneimine (PEI), Linear (MW 25,000) VWR/Polysciences 23966-2
PBS Fisher Scientific BP3994
| Item                                                      | Supplier                | Code     |
|-----------------------------------------------------------|-------------------------|----------|
| 16% Paraformaldehyde                                       | Electron Microscopy     | 15710    |
| Normal Donkey Serum                                        | Jackson ImmunoResearch  | 017-000-121 |
| Immun-Blot Low Fluorescence PVDF membrane                  | Bio-Rad                 | 1620264  |
| Methanol                                                  | Sigma-Aldrich           | 322415   |
| Sodium Dodecyl Sulfate                                    | Thermo Scientific       | 28364    |
| Fluoromount G                                             | SouthernBiotech         | 0100-01  |
| Hoechst 33258                                             | Invitrogen              | H21491   |
| Isothesia: Isoflurane                                     | Henry Schein            | 11695-6776 |
| Tissue Freezing Medium                                    | VWR                     | 15148-031 |
| 2-methylbutane                                            | VWR                     | JtQ223-8 |
| Trizma(R) base                                            | Sigma-Aldrich           | T1503-250G |
| GLYCINE                                                   | Sigma-Aldrich           | G8898-1KG |
| Ammonium Persulfate (APS)                                 | Thermo Fisher Scientific | 17874   |
| TEMED                                                     | Bio-Rad                 | 161-0800 |
| Precision Plus Protein Dual Color Standards               | Bio-Rad                 | 1610374  |
| Acrylamide/Bis solution                                   | Bio-Rad                 | 161-0158 |
| 4x Laemmli Sample Buffer                                  | Bio-Rad                 | 1610747  |
| Immun-Blot® Low Fluorescence PVDF membrane                | Bio-Rad                 | 1620264  |
| 0.05% Trypsin-EDTA                                        | Thermo Fisher Scientific | 25300054 |
| Odyssey Blocking Buffer                                   | Li-Cor Biosciences      | 927-40000 |
| Dynabeads® Protein G for Immunoprecipitation             | Thermo Fisher Scientific | 10003D  |
| Sodium chloride,SigmaUltra, >=99.5%                      | Sigma-Aldrich           | S7653-1KG |
| 10% NP-40 solution                                        | Thermo Scientific       | 28324    |
| cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail Tablets | Roche             | 04693159001 |
| Whatman® gel blotting paper, Grade GB003                 | Thermo Fisher Scientific | 10426890 |
| Ames                                                      | Sigma-Aldrich           | A1420    |
| Sodium Bicarbonate                                        | Sigma-Aldrich           | S5761    |
Carbogen
Airgas
Z020x9512000000

Critical Commercial Assays
Bio-Rad DC Protein Assay Kit
Bio-Rad
5000112

Deposited Data

Experimental Models: Organisms/Strains

Mouse: Megf101lacZ
Kay et al., 2012

Mouse: Megf10flox
this study

Mouse: Ptf1acko
Krah et al., 2015

Mouse: Isl1Cre
Jax 024242

Mouse: Hb9;GFP
Jax 05029

Mouse: ChATCre
Jax 006410

Mouse: Six3;Cre
Jax 019755

Mouse: Kcng4Cre
Jax 029414

Mouse: Drd4;GFP
Huberman et al., 2009

Mouse: Gjd2;GFP
RRID:MMRRC_030611-UCD

Mouse: Rosa26mTmG
Jax 007676

Mouse: Rosa26EGFP
Rawlins et al., 2009

Mouse: Ai14
Jax 007914

Mouse: ACTB:FLPe B6;SJL
Jax 003800

Mouse: C57BL6/J
Jax 000664

Experimental Models: Cell Lines

Human: Hek293T
ATCC
293T (ATCC® CRL-3216™)

Oligonucleotides

M10flagNotI_Rev
ATAGCGGCCGCttaCTTGTCGTCATCGTCTTTGT
M10flag_Fwd AGTCttcactgctgctgctgctgctgctg
Cyto9_flag_Rev1 GGTACATGCCTGTGCGAAGCA
5’ATAGCGGCCGCttaCTTGTCATCGTCTTT
GTAGTC TTCCTTCCTTCTCTTGCTTGTGT

Recombinant DNA
CMV-M10-FLAG this paper
CMV-M10-GFP Kay et al., 2012
pCMV-MEGF10-ΔICD-GFP Kay et al., 2012
MEGF10-ΔICD-Flag this paper
pAAV-EF1a-Brainbow-tagBFP-EYFP-WPRE Addgene 45185
pAAV-EF1a-Brainbow-mTFP1-Cherry-WPRE Addgene 45816

Software and Algorithms
Fiji/ImageJ Schindelin et al., 2012
SnapGene SnapGene
NIS Elements Nikon Instruments
Custom JAVA scripts for spike sorting Oracle
Matlab Mathworks, Natick, MA
Image Studio™ LI-COR Biosciences
Photoshop Adobe

Other
Olympus FV 300 Confocal Microscope Olympus
Nikon A1 Confocal Microscope Nikon
Micro HM550 Cryostat Microtom Thermo Fisher Scientific
LI-COR Odyssey LI-COR Biosciences
Nikon Digital Sight Qi1Me Nikon Corporation
Automatic Temperature Controller Warner Instruments TC-324B Corporation
MEA 519 electrode Field et al., 2010
METHOD DETAILS

Animals

All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Duke University. The animals were maintained under a 12-hour light-dark cycle with ad lib access to food and water. Retinas from adult (4-8 weeks old) Megf10−/− mutant mice and wild-type control mice with same genetic background were used for experiments performed on the multielectrode array (MEA). Animals were dark-adapted overnight prior to the experiment.

For this study the following transgenic and mutant mouse lines were used: 1) Megf10tm1b(KOMP)Jrs (Kay et al., 2012), referred to as Megf10− or Megf10lacZ; 2) Ptf1a tm3Cvw (Krah et al., 2015), referred to as Ptf1a flox or (when crossed to Cre mice) Ptf1a cKO; 3) Isl1tm(cre)Sev (Yang et al., 2006), referred to as Isl1 Cre; 4) Hb9:GFP (Trenholm et al., 2011); 5) Chat tm2(cre)Lowl (Rossi et al., 2011), referred to as Chat Cre; 6) Tg(Six3-cre)69Frtv (Furuta et al., 2000) referred to as Six3:Cre; 7) Kcng4 tm1.1(cre)Jrs (Duan et al., 2014) referred to as Kcng4 Cre; 8) Tg(Drd4-EGFP)W18Gsat (Huberman et al., 2009), referred to as Drd4:GFP; 9) Tg(Gjd2-EGFP)JM16Gsat, referred to as Gjd2:GFP. Two Cre reporter strains were used that express membrane-targeted green fluorescent protein (mGFP) upon Cre recombination: 1) Gt(Rosa)26Sortm4(ACTB-tdTomato-EGFP)Luo, also known as mT/mG (Muzumdar et al., 2007); 2) Rosa26 GFP (Rawlins et al., 2009). An additional Cre reporter strain was used that expresses...
tdTomato fluorescent protein upon Cre recombination: $Gt(ROSA)26Sor^{tm14(CAG-tdTomato)Hze}$ (Madisen et al., 2010). See Key Reagents table for repository stock numbers where applicable.

To produce $Megf10^{flox}$ mice, $Megf10^{tm1a(KOMP)Jrs}$ mice (Kay et al., 2012) were crossed to germline Cre strain $B6;SJL-Tg(ACTFLPe)9205Dym/J$, thereby generating a functional allele (also known as $Megf10^{tm1c}$) in which exon 4 was flanked by loxP sites.

**Cell Culture**

HEK293T cells were obtained from ATCC. The cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% bovine growth serum, 4.5 g/L D-glucose, 2.0 mM L-glutamine, 1% Penicillin/Streptomycin in 10 cm cell culture dishes. Cells were passaged every 2-3 days to reach confluence. Before splitting, culture media were removed and Dulbecco’s phosphate-buffered saline (D-PBS) was used to rinse cell layers as well as removing residual serum. Cells were detached from dish with 4 ml of 0.05% Trypsin and incubated at 37ºC until cell layer is dispersed (about 5 minutes). Equal volume of complete culture media was added to the dish to inhibit protease activity. The suspension was centrifuged at 200 x g for 5 minutes. Supernatant was aspirated and the cells were suspended with appropriate amount of media and plated (1:4-1:8). Cells used for experiments were passaged no more than 10 times. Cell stocks were stored as 2 million cells per vial in complete culture media with 10% DMSO in liquid nitrogen.

**Identification of DS circuit cell types using antibody and transgenic markers**

*SAC markers in mature retina*

Antibodies to choline acetyltransferase (ChAT) were used as a SAC marker in mice older than P5. This antibody stains SAC somata and their dendrites in the IPL (e.g. Fig. 7A).
SAC markers in embryonic and neonatal retina

Antibodies to ChAT and vesicular acetylcholine transporter, typically used as SAC markers in the mature retina, do not stain reliably in the embryonic and neonatal (P0-P3) mouse retina, precluding their use as markers during one of the key time periods of this study. We therefore characterized several other SAC markers that we found to be suitable for definitive SAC identification and their anatomical characterization in the E16-P3 period:

The Megf10lacZ allele (Kay et al., 2012) drives strong, selective β-galactosidase (βgal) expression in all SACs starting at embryonic day (E)17 (Fig. 1B; Fig. 1-Supplement 1; data not shown). Horizontal cells are also labeled. Expression is strong enough to allow characterization of SAC dendrite anatomy at these early stages. Antibodies to Megf10 yield a similar staining pattern (Fig. 1-Supplement 1; Fig. 4B,C), but staining of fine dendritic arbors was brighter with anti-βgal staining of Megf10lacZ mice, so this approach was used for most of our anatomical experiments analyzing the full SAC population at or before P3. In some such experiments a Megf11lacZ allele (Kay et al., 2012) was also present; this allele drives βgal expression in essentially the same pattern as Megf10lacZ and therefore contributed to signal brightness. The presence of this allele had no apparent effect on SAC anatomy, in either wild-type or Megf10 mutant background.

Antibodies to Sox2 (Whitney et al., 2014) strongly label all SAC nuclei in the INL and GCL, starting at embryonic stages (Fig. 1-Supplement 1; Fig. 2D,E). Progenitor cells in the ONBL are also labeled. This marker was typically used in conjunction with Megf10lacZ to provide definitive identification of SACs as βgal⁺Sox2⁺ cells.

Antibodies to internexin label SAC intermediate filaments, which localize in a polarized manner to the primary dendrite(s) and the side of the cell body from which they emerge (Fig. 2-
Primary dendrites were defined as any first-order dendrite branch, i.e. those arising directly from the cell body. Internexin is a selective marker of SAC in perinatal mouse retina, as previously shown in tree shrew (Knabe et al., 2007). RGC axons are also labeled (Fig. 2-Supplement 1).

Antibodies to Isl1 (Fig. 2A) label all SAC nuclei, starting at cell cycle exit (Galli-Resta et al., 1997). A large subset of RGCs are also labeled. The Isl1Cre knock-in mouse (Yang et al., 2006) faithfully recapitulated this expression pattern (Fig. 2A,B) and was used to study SAC anatomy at embryonic stages (see below for further details).

**SAC single-cell labeling**

To assess the single-cell morphology of individual SACs during early postnatal development, the ChatCre line was used. In contrast to mature retina (e.g. Fig. 7B), in which all SACs were labeled, ChatCre expression was rare and sporadic in early postnatal retina (Fig. 1C; Fig. 1-Supplement 1), as reported previously (Xu et al., 2016). Therefore, when crossed with Cre reporter mice to make ChatmG animals, the full anatomy of individual SACs was clearly delineated (e.g. Fig. 2I-L). We did not typically observe Cre recombination in non-SAC cell types; nevertheless, we always co-stained with another SAC marker, either Sox2 or Megf10βgal, to confirm the SAC identity of the cells that were analyzed.

**ooDSGC markers**

Two mouse lines were used, each of which labels distinct types of ooDSGCs. Hb9:GFP labels the superior subtype of ooDSGC, while Drd4:GFP labels the posterior subtype of ooDSGC (Trenholm et al., 2011; Huberman et al., 2009).
**DS-circuit bipolar cell markers**

Four types of bipolar cells have been shown to make monosynaptic connections with SACs and/or ooDSGCs: Types BC2, BC3a, BC5, and BC7 (Duan et al., 2014; Ding et al., 2016; Greene et al., 2016; Kim et al., 2014; Chen et al., 2014). OFF bipolar cells BC2 and BC3a were labeled, respectively, by antibodies to Syt2 and HCN4 (Wässle et al., 2009). Syt2 also labeled the axon terminals of BC6 bipolar cells (Wässle et al., 2009). ON bipolar cells BC5 and BC7 were marked with Kcng4^Cre^ (Duan et al., 2014) crossed to mGFP Cre reporter mice (denoted Kcng4^mG^). Labeling of BC7 was more prominent with the Rosa26 locus mGFP Cre reporter line that we used, compared to the cytosolic GFP reporter driven by Thy1 that was used by Duan et al. (2014).

Gjd2-GFP was also used to label BC5 bipolar cells (Fig. 9-Supplement 1). In adult retina, GFP was strongly expressed by a bipolar cell type that ramified in a laminar location typical of BC5 (Sidney Kuo, University of Washington, personal communication). We confirmed this expression pattern; weak expression in amacrine cells was also noted (Fig. 9-Supplement 1). At earlier developmental stages the amacrine cell staining was much stronger and filled many amacrine processes throughout the IPL, precluding use of this line for developmental studies of bipolar axons (M. Stogsdill and J.N.K, unpublished observations).

**Immunohistochemistry**

**Retinal cross sections:**

Mice were anesthetized by isoflurane or cryoanesthesia (neonates only) followed by decapitation. Eyes were enucleated, washed in PBS, and fixed in PBS containing 4% formaldehyde (pH 7.5) for 1.5 hours at 4° C. After fixation, eyes were washed 3X with PBS and
stored in PBS containing 0.02% sodium azide at 4° C until further processing. Retinas were
dissected from the eyecup, cryoprotected by equilibration in PBS containing 30% sucrose, then
embedded in Tissue Freezing Medium and frozen by submersion in 2-methylbutane chilled by
dry ice. Tissue sections were cut on a cryostat to 20 µm and mounted on Superfrost Plus slides.
Slides were dried on a slide warmer for 1 h then stored at -80° C or used immediately.

For antibody labeling, slides were washed for 5 min with gentle agitation in PBS to
remove embedding medium and blocked for 1 h in PBS + 0.3% Triton X-100 (PBS-Tx)
containing 3-5% normal donkey serum. Primary antibodies were diluted in blocking buffer,
added to slides, then incubated overnight at 4° C. Slides were washed with PBS 3X for 10
minutes followed by incubation with secondary antibody diluted in PBS-Tx for 1-2 h at RT.
Slides were washed again with PBS 3X for 10 minutes then coverslipped using Fluoromount G.

Retinal whole-mounts:

Tissue was processed as above up to the point of dissection from the eyecup. After
dissection from eyecup, retinas were washed in PBS then blocked for 3 hours with agitation at 4°
C in blocking buffer (constituted as described above). Primary antibodies were diluted in
blocking buffer, added to retinas, and incubated for 5-7 days with gentle agitation at 4°C. Retinas
were washed 3X with PBS over the course of 2 hours with gentle agitation. Secondary antibody
was diluted in PBS containing 0.3% Triton X-100 and was added to retinas followed by
incubation overnight at 4° C with gentle agitation. Retinas were washed again 3X in PBS over
the course of 2 hours with gentle agitation. For mounting on slides, 4 radial incisions separated
by 90° were made centripetally, approximately 1/3 the radius of the retina. Retinas were flattened
on nitrocellulose paper photoreceptor side down and coverslipped with Fluoromount G.
Image acquisition and processing

Sections and whole-mounts were imaged on a Nikon A1 or an Olympus FV300 confocal microscope. Image z-stacks were imported to Fiji (Schindelin et al., 2012), de-noised by median-filtering (0.5 - 2.0 pixel radius), and projected to a single plane. Color channels were assembled, and minor adjustments to brightness and contrast were made, in Adobe Photoshop. When images were to be compared, equivalent adjustments were performed on all images in the experiment.

Analysis of SAC anatomy in embryonic retina

To study SAC anatomy during embryonic stages, Isl1\textsuperscript{Cre} was crossed to \textit{lox-stop-lox-}mGFP Cre reporter mice (\textit{mT/mG} or \textit{Rosa26\textsuperscript{GFPf}}; see Key Reagents) to generate Isl1\textit{mG} animals. Timed-pregnant dams were sacrificed at E16 and eyes collected from embryos (\textit{n = 11} mice from 3 litters). Tissue was processed as described for postnatal eyes, except fixation time was 60 min. Cross-sections were stained with anti-GFP to reveal the morphology of Isl1\textit{mG}-expressing neurons, as well as Sox2 to distinguish Isl1\textit{mG}-positive SACs from RGCs. (All cells shown in Fig. 2B-G were confirmed to be SACs by Sox2 co-labeling.) In combination with these markers, anti-internexin staining was used to assess orientation of primary dendrites. Location and/or presence of the IPL was determined using Hoechst nuclear staining, which revealed cell body-free neuropil regions, and/or by Isl1\textit{mG} labeling of neuronal processes, which filled these neuropil regions (Fig. 2-Supplement 2). We assessed anatomy of mGFP\textsuperscript{+} migrating SACs in the ONBL, as well as SACs in the INBL that were concluding their migration. Morphology of ON SACs in the GCL could not be discerned due to Isl1 expression by RGCs (Fig. 2A,B), but because displaced amacrine cells pause at the INL-IPL border before crossing to the GCL (Chow et al., 2015), the population of cells available to analyze might have included both ON and OFF SACs.
Characterization of SAC homotypic arbor network in soma layers

The homotypic nature of SAC soma-layer contacts was investigated by imaging single ChatmG-labeled OFF SACs in mice also carrying a single copy of the Megf10lacZ allele (Fig. 2I,J). Anti-βgal staining was used to reveal the full SAC population, including arbors. En-face images were captured in z-stacks spanning the INL and IPL; slices corresponding to each layer were separately z-projected. For the INL arbor of each ChatmG-labeled cell, we examined the termination site of each dendritic tip. The fraction of dendrites terminating on the βgal-positive soma or arbor of a neighboring SAC was quantified. Sample sizes are given in main text.

Generation and analysis of “solitary” SACs

Reduction of SAC density using Ptf1a^{flox} mice

Ptf1a^{flox} mutant mice (Krah et al., 2015) were crossed into the Six3:Cre background to generate Ptf1a^{cKO} mice. Six3:Cre is expressed by retinal progenitors starting at E9.5 in a high-central-to-low-peripheral gradient (Furuta et al., 2000; Fig. 3A). In central retina, where Cre is expressed in all progenitors, amacrine cells were completely absent but bipolar cells, RGCs, Müller glia, and photoreceptors remained (Fig. 3B; Fig. 3-Supplement 1; data not shown). In peripheral retina, where Cre recombination was incomplete, amacrine cells derived only from Cre-negative progenitors (Fig. 3C). Because the number of Cre-expressing progenitors in peripheral retina still vastly exceeded the number that escaped Cre, amacrine cell density in Ptf1a^{cKO} peripheral retina was markedly reduced compared to littermate controls (Fig. 3A,B; Fig. 3-Supplement 1).
Quantification of dendrite phenotypes in solitary and touching SACs

To visualize SACs and quantify their arbor targeting frequencies in $Ptf1a^{cKO}$ mice, we bred $Megf10^{lacZ}$ into the $Ptf1a^{flx}$ background. All $Ptf1a^{cKO}$ and littermate control mice in these experiments carried one copy of the $Megf10^{lacZ}$ allele. SAC morphology was revealed with anti-βgal. Sox2 was used to confirm the SAC identity of all cells included in the experiment. SACs were scored as “solitary” or “touching” based on whether their dendrites contacted neighboring SACs in the same or adjacent sections. If this could not be determined (e.g. because the adjacent section was missing or damaged), the cell was excluded from further analysis. Because SACs were only present in $Ptf1a^{cKO}$ peripheral retina, analysis of littermate control SACs was also limited to peripheral retina. In $Ptf1a^{cKO}$ mice, SACs were more frequently found in the INL than the GCL and it is possible that the INL SACs were a mixed population of ONs and OFFs. Therefore, we did not distinguish between SAC subtypes for the analyses.

IPL projections of βgal-stained cells were examined, and cells were assigned to one of three categories: 1) no arbors projecting to the IPL; 2) Arbors enter the IPL but fail to stratify; 3) Arbors enter the IPL and ramify in a laminar pattern. Examples of the first category of solitary SACs are shown in Fig. 3F, left, and Fig. 3-Supplement 1. Examples of the second category are shown in Fig. 3F, right, and Fig. 3-Supplement 1. The third category is exemplified by all touching SACs shown (Fig. 3E; Fig. 3-Supplement 1). Each cell in the dataset was also scored on an independent criterion: whether it projected to the soma layer (e.g. Fig. 3D,F, white arrows).

For each animal in the experiment, the following was calculated and plotted in Fig. 3G:

1) Percentage of SACs with projections to the soma layers; 2) percentage of SACs projecting to the IPL (i.e., the cells assigned to categories 2 and 3 above); 3) percentage of SACs with stratified IPL dendrites (i.e. the cells in category 3). Sample sizes: $n = 3$ wild-type littermates
(28, 62, 32 cells analyzed in each animal); \(n = 4\) Ptf1acKO animals (11, 35, 13, 12 solitary and 27, 44, 22, 23 touching SACs analyzed in each animal). Statistics: one-way ANOVA with Tukey’s post-hoc test.

**Quantification of SAC projection phenotypes in Chat\(^mG\) mice**

Single SACs labeled in Chat\(^mG\) and Chat\(^mG\);Megf10\(^{−/−}\) mice were morphologically assessed in cross-sections. GFP signal was amplified with anti-GFP antibody staining. All GFP\(^+\) SACs on any given slide were imaged and analyzed, to avoid cell selection bias, with the exceptions of: 1) cells severed by the sectioning process; 2) cells with arbors that could not clearly be distinguished from those of their neighbors; 3) cells in the far retinal periphery, where sections were oblique to retinal layers, obscuring IPL strata. In experiments analyzing Megf10 mutants, littermates were always used as controls to avoid complications arising from the fact that the precise state of retinal development at the time of birth might vary from litter to litter.

A cell was scored as innervating the IPL if it ramified branched dendrites within the neuropil. Dendrites that entered the neuropil but did not branch or stratify (e.g. Fig. 5E) were not sufficient. A cell was scored as projecting to the soma layer if arbors emanating from the cell soma or primary dendrite terminated or arborized in the INL (for OFF SACs) or GCL (for ON SACs). The arbor was required to be \(\geq 1\) cell diameter in length (i.e. small fine arbors were not counted). One other important exception that was not counted: We observed that many SACs at young ages had single unbranched arbors extending \(\sim 180^\circ\) away from the IPL (e.g. Fig. 2K,L – all four cells have such arbors, even the ones that do not project towards neighboring SAC somata). These processes were not counted for two reasons. First, their trajectory was such that they were unlikely to join the soma-layer dendrite network or contact neighboring somata. Second, these \(180^\circ\) arbors were sometimes still present in P5 SACs (Fig. 2-Supplement 2) and
therefore they did not appear to be subject to the same developmental regulation as soma-directed arbors (Fig. 2M). This observation suggests they are fundamentally different, and likely serve a different (as yet uncharacterized) purpose. No obvious difference in their frequency was observed between wild-type and Megf10 mutants.

To produce graphs in Figs. 2M, 5F, and 7G, the fraction of cells making ectopic projections – either to the soma layer or to inappropriate IPL sublayers – was calculated for each genotype and each time point. To determine whether a GFP+ IPL arbor was located in normal or abnormal IPL strata, Megf10:βgal was used as a counterstain. ChatCre was rarely expressed in OFF SACs at P0, making it difficult to obtain large sample sizes at this age. For this reason, and because soma-layer projection frequency did not appear to differ much between P0 and P1, the data from each time point was pooled for analysis of Megf10 litters.

Sample sizes for Fig. 2M: P0, n = 25 OFF, 63 ON; P1, n = 51 OFF, 79 ON; P2, n = 46 OFF, 55 ON; P3, n = 33 OFF, 49 ON; P5, n = 15 OFF, 26 ON; P7, n = 23 OFF, 34 ON. Data were from four litters of mice, each of which was assessed at no less than two of these time points.

Sample sizes for Megf10; ChatmG experiments (Figs. 5F, 7G): Megf10 heterozygous littermate controls: P0/1, n = 11 OFF, 25 ON; P2, n = 25 OFF, 23 ON; P3, n = 17 OFF, 22, ON; P5, n = 16 OFF, 16 ON. Megf10 mutants: P0/1, n = 6 OFF, 25 ON; P2, n = 14 OFF, 20 ON; P3, n = 34 OFF, 41 ON; P5, n = 48 OFF, 54 ON. Data were from two litters of mice.

For the adult data reported in Fig. 7G, a different procedure was used; see “Quantification of Mosaic Spacing Phenotypes” section below.
Analysis of *Chat-Megf10*\(^{KO}\) conditional mutants

**Characterization of timing of MEGF10 deletion**

For initial characterization of when MEGF10 protein is eliminated by the *Chat\(^{Cre}\)* driver line, the following experiment was performed: *Chat\(^{Cre}\); Megf10\(^{flox}\)* mice were intercrossed with *Chat\(^{Cre}\); Megf10\(^{lacZ}\)* carriers to generate *Chat\(^{Cre}\); Megf10\(^{flox/lacZ}\) (Chat-Megf10\(^{KO}\)) experimental animals and littermate controls (*Chat\(^{Cre}\); Megf10\(^{flox/+}\)*). These animals also carried a *Rosa26* mGFP Cre reporter allele. Animals were sacrificed at P1, P3, and P5; retinas were cross-sectioned and immunostained for anti-MEGF10 (Fig. 6-Supplement 1). Comparisons were made across animals from the same litter to assess how MEGF10 immunoreactivity changed over time. Two litters were analyzed in this way, each yielding the same conclusion: MEGF10 immunoreactivity was largely eliminated by P5 in *Chat-Megf10*\(^{KO}\) mice (Fig. 6-Supplement 1). At P3, overall MEGF10 levels were reduced, but most SACs still expressed detectable protein (Fig. 6-Supplement 1). The cells that lost MEGF10 immunoreactivity by P3 were not necessarily the same cells that recombined the mGFP reporter at the *Rosa26* locus (Fig. 6F,G). At P1, only a very small number of cells (< 5 per retina) could be identified that lacked MEGF10 immunoreactivity; most of these were ON SACs although a few recombined OFF SACs were identified (Fig. 6G). We conclude that a small fraction of SACs loses MEGF10 protein prior to P3, while the majority lose MEGF10 between P3 and P5. Further, ON SACs are somewhat more likely to lose MEGF10 before P3 than OFF SACs.

**Assessment of morphological and IPL projection phenotypes**

To ask if loss of MEGF10 prior to P3 affects dendritic targeting, *Chat\(^{mG}\)*-labeled single SACs were identified in retinal cross-sections from *Chat-Megf10*\(^{KO}\) and *Chat\(^{Cre}\); Megf10\(^{flox/+}\)*. However, the specific details and results of these analyses are not provided in the text.
control mice, as described above. Analysis was performed at P1 and P3; data in Fig. 6H is from P3 only. All mGFP+ SACs were first scored as to whether they expressed MEGF10 protein (see Fig. 6F,G). Subsequently, each cell was scored for soma-layer projection as described above for wild-type and Megf10+/− animals. This scoring was done blind to the cell’s MEGF10 expression status. The fraction of cells classified as either “soma-projecting” or “IPL-only” was calculated for MEGF10+ SACs, MEGF10− SACs, and littermate control SACs (Fig. 6H). Sample sizes: n = 26 OFF, 18 ON cells from controls; 24 OFF, 19 ON MEGF10+ cells from Chat-Megf10cKO; 9 OFF, 17 ON MEGF10− cells from Chat-Megf10cKO.

To assess SAC stratification at maturity, cross-sections from P17 Chat-Megf10cKO and littermate controls were stained for anti-ChAT. Four mutants and three littermate controls, from two litters, were examined.

**Analysis of Six3-Megf10cKO conditional mutants**

**Characterization of Cre recombination patterns**

Breeders carrying the relevant alleles were interbred to generate Six3:Cre; Megf10fl/flacZ (Six3-Megf10cKO) mice and littermate controls (Six3:Cre; Megf10+/+acZ or Cre− Megf10fl/flacZ). As noted above in Ptf1a section, Cre is expressed very early (~E9.5) in Six3:Cre retina, but expression is incomplete, with some parts of peripheral retina spared from Cre activity (Furuta et al., 2000). Therefore, all mice used for these experiments also carried the Rosa26GFP Cre reporter, to reveal retinal regions that either lacked MEGF10 (GFP+ cells) or were spared from MEGF10 deletion (GFP− cells). Anti-MEGF10 staining confirmed that the GFP Cre reporter is a reliable marker of MEGF10 expression status (Fig. 6-Supplement 1).
Assessment of morphological phenotypes

For quantification of INL projection frequency at P2, Six3-Megf10cKO and littermate control whole-mount retinas were stained for βgal, Sox2, and anti-GFP. This staining marked SACs (Sox2 and βgal), revealed their dendritic morphology (βgal), and defined their MEGF10 expression status (GFP). Confocal stacks were acquired through the INL, extending to the IPL (which was clearly discernable due to dense βgal and GFP expression). The INL was defined as the region above this in the image stack, containing Sox2+ neurons. Cells that projected soma-directed arbors into the INL were clearly discernable due to their multipolar morphology with numerous dendritic protrusions (e.g. Fig. 6B). Cells that did not project to the INL had a round morphology with only minor lateral branches less than one cell radius in length (Fig. 6C). Each βgal-labeled SAC was scored as to whether it expressed GFP, and whether it projected lateral arbors into the INL. If the cell had only INL branches directed towards the IPL through the stack Z-plane, it was not counted as INL-projecting. Scoring was done in separate sessions so that the scorer was blind to GFP expression status when determining INL projections. Sample sizes: \( n = \) 117 SACs from 2 control mice; \( n = 302 \) GFP+ SACs and 149 GFP– SACs from 2 Six3-Megf10cKO mice.

To assess SAC stratification in cross-sections, P2, P4, or P17 Six3-Megf10cKO and littermate control retinas were sectioned and stained for anti-βgal (P2) or anti-ChAT (P17). The number of animals examined was: P2, 4 mutants, 2 controls; P4, 2 mutants, 3 controls; P17, 2 mutants, 2 controls.
Quantification of area covered by SAC dendritic arbors

Embryonic SAC arbor territory

P0 Chat
tG retinas were imaged in whole-mount preparations stained with anti-Sox2 and anti-GFP antibodies to identify single GFP+ SACs. To avoid cell selection biases, all labeled SACs with arbors that were clearly distinguishable from their neighbors were imaged and analyzed, except for far-peripheral cells that may have been damaged during mounting. Z stacks were acquired through the GCL, IPL, and INL to encompass all arbors of a single cell. Images were imported into ImageJ, z-projected into a single plane, and polygons were drawn connecting the dendritic tips, nearest neighbor to nearest neighbor, until the dendritic field was captured. Area of this polygon was calculated using ImageJ. Sample sizes: OFF SACs, n = 16 wild-type and 16 Megf10−/−; ON SACs, n = 31 wild-type and 34 Megf10−/−. Statistics: two-tailed t-tests.

Adult SAC arbor territory

Individual SACs were labeled by injection of ChatCre mice with “Brainbow” Adeno-associated virus (AAV) driving fluorophore expression in a Cre-dependent manner (Cai et al., 2013). The two Brainbow AAV9 viruses, encoding farnesylated fluorescent proteins that are targeted to the plasma membrane (University of Pennsylvania Vector Core), were mixed to 1.5 x 10^12 genome copies per mL. Adult mice (P40-50) were anesthetized with ketamine-xylazine by intraperitoneal injection. Propracaine hydrochloride (0.5%) ophthalmic solution (Akorn, Lake Forest, IL) was applied to the eye to provide local anesthesia. A 30 1/2G needle was used to make a small opening near the ora serrata, and 1µl of virus was injected with a 33G blunt-ended Hamilton syringe intravitreally. Tissue was collected 3 weeks after the virus injection.
Retinas were stained in whole-mount with anti-GFP, anti-mCherry, and anti-mKate antibodies to reveal SACs. OFF SACs were not labeled in large numbers, so analysis was restricted to more abundantly labeled ON SACs. Imaging, image processing, and quantification were as for P0, except that only SACs in central and mid-peripheral retina were used to avoid confounding effects of eccentricity on arbor size. Sample sizes: \( n = 10 \) wild-type and \( 16 \) Megf10 mutant SACs.

**Hb9-GFP stratification**

P1-P2 retinas carrying \( \text{Megf10}^{\text{lacZ}} \) and \( \text{Hb9-GFP} \) were co-stained for \( \beta \text{gal} \) and GFP. RGCs with dendrites that co-fasciculated with \( \beta \text{gal} \)-positive IPL strata were counted. See Results for sample sizes.

**Quantitative assessment of IPL stratification level**

Images of retinal cross sections were processed in ImageJ. A vertical ROI (12.5 \( \mu \)m wide) was drawn to perpendicularly bisect the IPL strata, from the edge of the INL to the edge of the GCL. IPL stratification levels were reported as percentage of IPL width. Intensity was calculated for each pixel along the length of the ROI as an average across its width. Then all pixel intensity values were normalized to the maximum value of that ROI. Location of fluorescent peaks was calculated as the pixel with maximum intensity; if multiple pixels had the same intensity the peak was defined as the center of the plateau.

For BC5-BC7 arbor distance measurements (Fig. 9F), distances as percentage of total IPL width were compared by one-way ANOVA/Tukey’s post-hoc test. \( n = 14 \) measurements from 2 control mice; \( n = 7 \) normal IPLs, 11 SAC clumps, 11 SAC gaps from 3 \( \text{Megf10}^{-/-} \) mice.
Generation of Megf10-ΔICD Constructs

The MEGF10-ΔICD-GFP construct was reported previously (Kay et al., 2012), which was originally made from pUbC-MEGF10-GFP (Addgene #40207). It encodes a version of MEGF10 in which the cytoplasmic domain is truncated after the 9th amino acid and replaced by GFP. Inclusion of those 9 amino acids was necessary to achieve plasma membrane localization. For this study it was subcloned into the pEGFPN3 plasmid, containing the CMV promoter, to make pCMV-MEGF10-ΔICD-GFP.

To make the MEGF10-ΔICD-Flag construct, Megf10 (truncated after the 9th intracellular domain amino acid as above) was PCR amplified from pUbC-MEGF10-GFP vector using M10flag_Fwd forward primer and Cyto9_flag_Rev1 reverse primer. Resulting PCR products were digested with NotI and AscI restriction enzymes and ligation cloned into pEGFPN3 vector linearized with corresponding restriction enzymes.

Assay for interaction of MEGF10-ΔICD constructs

Co-Immunoprecipitation

HEK293T cells were grown to 80% confluency. Cells were then transfected using a linear polyethylenimine (PEI) transfection reagent: DNA, PEI, and Opti-MEM were mixed in a 1:3:30 ratio and incubated for 10 minutes at room temperature then applied to confluent cells. Cells were harvested 48-hour post transfection. Cells were lysed with NP-40 lysis buffer (1% NP-40, 150mM NaCl, 50mM Tris-Cl, and 1X proteinase inhibitor) by pipetting. Lysate was centrifuged at 14000 x g at 4°C for 15 min. to remove insoluble material. The soluble protein fraction was quantified with Bio-Rad DC assay. For immunoprecipitation, 500µl (1µg/µl) protein in NP-40 buffer lysis buffer was incubated overnight at 4°C with antibody (1µl of
chicken anti-GFP or 2µl of mouse anti-Flag). Protein G Dynabeads (10µl) were added to mixture
for 1 hour at 4°C while rotating. Beads were sequestered by magnet and flow-through was
removed. Beads were washed with 500µl lysis buffer (3x) on ice then eluted with 30µl 2X
Laemmli containing 5% β-mercaptoethanol.

**Western Blot**

Samples were prepared in 2X Laemmli sample buffer, heated at 95°C for 10 minutes, and
loaded onto SDS-acrylamide gel (running gel: 8% acrylamide/bis Tris-HCl with 0.1% SDS pH
8.8; stacking gel: 5% acrylamide pH 6.8; cross linked with TEMED and APS). Precision Plus
Protein Dual Color Standards (BioRad) were used as a molecular weight marker. The gel was
run on a BioRad mini gel running apparatus with SDS-PAGE running buffer (25 mM Tris, 192
mM glycine, 0.1% SDS). Electrophoresis was carried out at 50 V through the stacking gel then
adjusted to 120 V until the dye front reached the lower end of the gel. BioRad Immobilon-FL
PVDF membrane and Whatman filter paper were used with the BioRad mini cassette for transfer.
Samples were transferred in 25 mM Tris, 192 mM glycine, 20% methanol at 100 V for 90
minutes. Membranes were blocked with PBS/Odyssey blocking buffer and stained with chicken
anti-GFP 1:20000, mouse anti FLAG 1:20000 overnight at 4°C with shaking. After washing with
PBST for 4 times, membranes were stained with 1:20000 secondary antibodies for one hour at
room temperature. The membranes were washed with PBST four times and then rinsed with PBS
and water. Finally, the membranes were imaged with LI-COR Odyssey using the Image Studio
software.
Quantification of mosaic spacing phenotypes and their effects on SAC IPL projections

Regularity index

Regularity of SAC cell body distribution in Six3-Megf10<sup>KO</sup>, Chat-Megf10<sup>KO</sup>, and littermate control mice was calculated as previously described (Kay et al., 2012). The Voronoi domain regularity index (VDRI) was used as a measure of regularity. It is calculated by first assigning a Voronoi domain to each cell in an array (Fig. 8C), and then calculating the mean and standard deviation of the domain areas. The VDRI is defined as the mean area divided by the standard deviation. Arrays that are less regularly distributed will have a lower VDRI because their domain sizes are more variable (and hence have a higher standard deviation).

P17 whole-mount retinas were stained with an antibody to ChAT and imaged en face. One eye was processed from each animal used in the experiment. For each eye, 3 confocal image stacks were obtained using a 20x objective (636.5 µm<sup>2</sup> field of view). Images of INL SACs were analyzed using Fiji software. The location of each SAC in the field of view was marked; this information was used to count the number of SACs (Fig. 8-Supplement 1) as well as define Voronoi domains belonging to each cell, using Fiji functions. The area of each Voronoi domain (excluding edges) was calculated in Fiji.

For statistical analysis of regularity effects across genotypes, we first calculated the per-animal average cell density and VDRI from the 3 acquired images. Differences between genotypes were then evaluated using one-way ANOVA and Fisher’s PLSD. Previously published Megf10 null and simulation data was also included for comparison (Kay et al., 2012). The simulations define the VDRI that would be expected for a randomly-arranged array of cells matched in size and density to real SACs. Data collection and analysis was virtually the same as in the previous study, allowing us to include these data in our statistical comparisons.
Effects of soma position upon IPL errors: Single-cell analysis

To ask if soma position correlates with IPL errors, we first defined the ectopic projection status of each OFF SAC in a set of z-stacks acquired from ChAT-immunostained retinal whole-mounts. Sample sizes: \( n = 515 \) cells from 2 control (\( \text{Megf10}^{+/–} \)) mice; \( n = 584 \) cells from 2 \( \text{Megf10} \) mutant mice. The z-stacks encompassed, at different levels of the stack, SAC somata in the INL and their ramified arbors in the IPL. In \( \text{Megf10} \) mutants, the OFF ectopic IPL arbor network and the typical OFF DS circuit sublayer were identified at different stack levels (Fig. 7C,E). ChAT+ arbors arising from individual OFF SAC somata were traced through the stack to identify those that joined into the ectopic network. The fraction of SACs that did so was then calculated and plotted in Fig. 7G. For Fig. 7H, we further examined these stacks to look for SACs that made ectopic projections at the INL level.

Next, we defined the severity of mosaic spacing perturbations in the local neighborhood of each SAC. Because SAC position is random in \( \text{Megf10} \) mutants, SACs might be more crowded or more isolated from their neighbors than in controls; or, by chance, they might be located at a fairly normal distance from their neighbors. The size of a cell’s Voronoi domain is influenced by the distance of all nearest neighbors (Fig. 8C), and therefore serves as a convenient measure of local cell density. For simplicity we refer to Voronoi domains as “territory size” in Fig. 8. The effect of local cell density upon IPL projection errors was determined by plotting the ectopic error rate for each 100 \( \mu \text{m}^2 \) territory size bin (Fig. 8D,F). Sample size per bin, in order from smallest (<200 \( \mu \text{m}^2 \)) to largest (>1100 \( \mu \text{m}^2 \)): \( n = 32, 65, 89, 102, 91, 80, 39, 34, 24, 34 \).

Soma-arbor cross-correlation analysis

From the same z-stacks used for the above analysis, we made sub-stack z-projections capturing the OFF SAC soma array and the OFF SAC IPL sublayer. Prior to calculating the
correlations between these images, the following pre-processing steps were performed in Fiji: 1) Images were converted to 32-bit space. 2) To remove spurious correlations arising from vignetting, the images were flat-field corrected by low-pass filtering. 3) The pixel values in each image were normalized to an equivalent scale by subtracting the image mean value and dividing by the standard deviation. 4) Flipped images of the IPL arbors were generated by reflecting the image about both vertical and horizontal axes. On completion of these steps, cross-correlations between the soma image and the real or flipped arbor images were performed using the FD Math Fiji function. The Radial Profile Plot ImageJ plugin was used to quantify correlation intensities. To control for correlations unrelated to the specific locations of arbors and cell bodies, the intensity values at each radius were determined by subtracting the control (flipped image) value from the experimental (unflipped) value.

**Multielectrode array recordings**

*Isolation of retina, recording, and spike sorting*

Two wild-type and two *Megf10*−/− animals were used for multielectrode array (MEA) recordings. Immediately following euthanasia, retinas were isolated under infrared (IR, >900 nm) illumination with the assistance of IR-to-visual converters. This preserved the photosensitivity of the retina during the dissection. Dissections were performed in sodium bicarbonate-buffered Ames’ solution (Sigma, St. Louis, MO) equilibrated with 5% CO₂ + 95% O₂ to pH 7.4 and maintained at 32-34°C. Hemisection of the eye was performed along the ora serrata by first making a small incision, following which the vitreous was removed and the retina was isolated from the pigment epithelium and eye cup. A piece of dorsal retina (1-2 mm²) was dissected and placed RGC-side down on the planar MEA.
The MEA consisted of 519 electrodes with 30 µm inter-electrode spacing, covering a hexagonal region with 450 µm on a side (Field et al., 2010). The voltage on each electrode was digitized at 20 kHz and stored for post-hoc analysis. Details of recording methods and spike sorting have been described previously (Field et al., 2007). Spikes were identified using a threshold of four times the voltage standard deviation on each electrode. Principal component analysis applied to the ensemble of spike waveforms measured on each electrode provided a subspace for clustering spikes according to their shape. A Gaussian mixture model was used to cluster the spikes originating from individual RGCs. The clusters were manually inspected for each identified ooDSGC to ensure the spike waveforms were well isolated from other simultaneously recorded RGCs and all spikes were captured within each cluster. When a single cluster of spikes was captured by more than one Gaussian or when a single Gaussian included spikes from more than one cluster, the clustering was manually adjusted to generate a new set of initial conditions for re-fitting the mixture of Gaussians. Spike clusters with >10% estimated contamination based on refractory period violations, or spike rates <1 Hz, were excluded from further analysis.

Visual stimulation and RGC responses

Visual stimuli were focused on the photoreceptor outer segment, from an OLED display (Emagin, Inc.) with 60.35 Hz refresh rate. The mean intensity of the stimulus was 7000 photoisomerizations per rod per s, or 5000 photoisomerizations per cone per s for a cone containing all M-opsin. These estimates do not account for the effect of pigment self-screening. To measure the direction tuning of ooDSGCs as a function of contrast, a positive contrast bar (1200 µm wide) was presented on a gray background (Fig. 10B). On each presentation, the bar moved in one of twelve equally spaced directions at 400 µm/sec and was presented at one of the
following (Weber) contrasts: 5%, 10%, 20%, 40%, 80%, 150% and 300%. Responses to a total of 8 trials were collected for every condition; stimulus conditions were presented pseudo randomly. Spike times were binned at 1 ms resolution for all subsequent analyses.

To distinguish DSGCs from other RGCs recorded on the MEA, square-wave drifting gratings were used. These gratings drifted in one of twelve different and equally spaced directions and at two different speeds (225 µm/sec and 900 µm/sec; spatial period 400µm/cycle).

DSGCs were identified based on their direction selectivity index (DSI) defined as:

$$DSI = \frac{|\sum v_i|}{\sum n_i}$$

calculated from responses to drifting gratings and moving bars. Here, $n_i$ is the number of spikes elicited to stimulus movement along the direction $i$ defined by the vector $v_i$.

The distribution of DSIs across all recorded RGCs was bimodal, with DSGCs forming the high mode (Fig. 10A). Based on these distributions, a DSI of 0.25 reliably identified DSGCs in wild-type and $\text{Megf10}^{-/-}$ retinas. ooDSGCs were isolated from ON DSGCs by their distinct ON and OFF responses to a bar entering and exiting the receptive field (Fig. 10B). The total ooDSGC sample size obtained by this procedure was $n = 80$ from the two wild-type and $n = 74$ from the two $\text{Megf10}^{-/-}$ retinas. The paired Kolmogorov-Smirnov (KS) test was used to compare cumulative probability distributions from these two populations.

**Analysis of ooDSGC response**

**Measurement of direction tuning width**

First, the direction tuning curve for each ooDSGC was obtained by calculating the number of spikes elicited across all trials for each direction of bar movement. Due to the circular
nature of the data, the direction tuning curve was treated as circular normal distribution, also
called von Mises distribution (Oesch et al., 2005), and the tuning width was measured as the
circular standard deviation ($\sigma_{\text{circ}}$), defined by

$$\sigma_{\text{circ}} = \sqrt{-2\ln(R)}$$

where $R$ is the second moment of the von Mises distribution:

$$f(\theta, \mu) = \frac{1}{2\pi I_0(\kappa)} e^{\kappa \cos(\theta-\mu)}$$

This yielded a nonparametric estimate of the tuning curve width.

**Measurement of direction tuning strength**

To measure the strength of tuning, the difference between spike counts to motion in the
preferred and null directions was normalized by the sum of these responses. The tuning curves
were sampled at 30 degree intervals. To estimate the response in the preferred (null) direction,
which could fall between sampled directions, a cosine-weighted average of the two strongest
(weakest) responses was calculated. This yielded the following equation for measuring tuning
strength:

$$\text{Tuning strength} = \frac{\sum_{i=1}^2 r_i \cos(|\theta_{PD}-\theta_i|) - \sum_{j=1}^2 r_j \cos(|\theta_{ND}-\theta_j|)}{\sum_{i=1}^2 r_i \cos(|\theta_{PD}-\theta_i|) + \sum_{j=1}^2 r_j \cos(|\theta_{ND}-\theta_j|)}$$
where the summation $\sum_{i=1}^{2}$ is performed over the responses $r_i$ weighted by the cosine terms for the two nearest neighbor movement directions $\theta_i$ around the preferred direction $\theta_{PD}$ and the null direction $\theta_{ND}$. This resulting index for tuning strength varied between zero and unity.

**Measurement of direction tuning similarity between ON and OFF responses**

To separately analyze the ON and OFF responses of ooDSGCs, we first defined temporal windows for each ooDSGC that distinctly separate the ON and OFF responses. This was done by passing high-contrast moving bars (150% and 300% contrast) through the receptive field. In the resulting spike rasters, ON and OFF response phases were clearly discernible (Fig. 10B,F). The boundary for separating the ON and OFF responses was set halfway between the peak ON and OFF spike rate locations (Fig. 10F,G). Once the temporal boundary was defined, the preferred direction was calculated independently for the ON and OFF responses for each ooDSGC. The same ON-OFF temporal boundaries were used for all contrasts shown in Fig. 10-Supplement 1. The difference between the preferred directions, $\Delta \phi$, quantified the angular difference between the ON and OFF preferred directions (Fig. 10F).

**Analysis of ooDSGC subtypes**

ooDSGC subtype classification was performed using the K-means clustering algorithm. This was done by first assigning a set of four initial seed values corresponding to the four cardinal directions of ooDSGCs (Oyster and Barlow, 1967). Next, the angular difference between the seed values (for first iteration) or the cluster means (for later iterations), and the preferred directions of each ooDSGCs was calculated. The cluster for which the angular difference was minimum was the cluster to which an ooDSGC was assigned. This yielded the four subpopulations of ooDSGCs described in Fig. 10-Supplement 1.
Statistical analysis was performed using GraphPad Prism software (anatomy/development studies) or using custom JAVA based software and MATLAB software (physiology studies). Statistical tests used for each experiment are given in the METHOD DETAILS section above, and/or in the figure legends. Sample sizes for each experiment are given in the METHOD DETAILS section above or else in the Results. *P*-values (\( \alpha = 0.05 \)) are given in figure legends, or in the Results if no figure is shown. Error bars are defined in figure legends. Exact *p*-values are reported unless the value was less than 1.0 \( \times 10^{-7} \).
**Figure 1**: Initial formation of DS circuit IPL sublayers.

**A**: Schematic of mature direction-selective (DS) circuit and its cell types, depicted in cross-section. SACs (green) and bipolar cells (blue) project to one of two IPL sublayers (OFF, ON). OFF SACs reside in inner nuclear layer (INL); ON SACs reside in ganglion cell layer (GCL). ooDSGCs (purple) send dendrites to both DS circuit sublayers. ONL, outer nuclear layer.

**B**: SAC IPL sublayer formation assessed in *Megf10lacZ* mice. All SACs are double-positive for anti-Sox2 (purple) and anti-βgal (green). Progenitors in outer neuroblast layer (ONBL) also express Sox2. SAC IPL sublayers (arrowheads) begin to appear by P0, and are fully apparent by P1.

**C**: Sparse labeling of neonatal SACs in *ChatCre* mice. Individual SACs have laminar-specific projections by P1 (arrows). tdT, tdTomato.

**D**: ooDSGCs (labeled by *Hb9:GFP*) project diffusely in the IPL at P2 (arrow), whereas SAC arbors are stratified (right panel, arrowheads). Also see Fig. 1-Supplement 1.

Scale bars: 25 µm.
Figure 2: Newborn SACs contact each other via a network of soma-directed arbors.

A,B: Isl1 labels SACs and RGCs in embryonic retina. A, immunostaining; B, mGFP driven by Isl1Cre (Isl1mG). Arrows, newborn SACs migrating apico-basally through ONBL to inner retina. INBL SACs and RGCs predominantly reside in indicated regions. IPL neuropil (asterisks) exists in discontinuous patches at this age. NFL, nerve fiber layer containing RGC axons. Blue, nuclear counterstain.

B,C: Migrating SACs in ONBL (arrows) have multipolar morphology. They are far from other SACs and do not contact them.

D: Morphology of Sox2+Isl1+ SACs (large arrows) upon arrival at INBL. SACs contact each other outside the IPL (small arrow, connecting arbor). Their migratory morphology and distance from IPL (asterisks) indicate they have not yet innervated IPL (also see Fig. 2-Supplement 2).

E: A network of arbors connects somata of INBL SACs (small arrows). Arrowhead, IPL-directed projection.

F,G: Internexin immunostaining reveals polarization of SAC primary dendrites at E16 (F) and P2 (G). P2 SACs project exclusively towards the IPL. E16 INBL SACs often project towards neighboring SAC somata (F) as well as towards the ONBL (Fig. 2-Supplement 2).

H: Soma-directed SAC arbor network remains prominent in INL at P0 (arrows) but mostly gone by P2.

I,J: An individual P1 OFF SAC labeled by ChatmG (see Fig. 1-Supplement 1), imaged en face to show its arbor morphology at IPL and INL levels. J: INL arbors make selective contacts with SAC neighbors (purple; Megf10:βgal). GFP+ arbor tips terminate on SAC somata (orange arrow) or SAC arbors (white arrows). Right panel (J): Higher magnification view of touching arbors.
**K,L:** Individual P1 OFF (K) and ON (L) SACs labeled by *Chat<sup>mG</sup>* (green) in cross-section. Purple, full SAC population (F, *Megf10*:βgal; G, Sox2). Some SACs are bi-laminar with arbors that contact neighboring somata (arrows, left panels); others project only to IPL (right panels).

**M:** Frequency of soma layer projections across development, determined from single *Chat<sup>mG</sup>* cells as in K,L. Error bars, standard error. Sample sizes, see Methods.

**N:** Schematic of newborn SAC morphology based on B-L. Soma-directed homotypic contacts are established upon completion of migration, and are mostly eliminated by P3.

Scale bars: 25 µm (A,B,G,H); 10 µm (C-F, I-L)
Figure 3: SAC homotypic contact is required for IPL sublayer formation.

A: Top: Schematic illustrating Six3:Cre expression pattern in retinal cross-section. Bottom: En-face view of Six3:Cre recombination in peripheral retina, revealed using GFP Cre reporter. Asterisks, Cre– regions.

B. Reduced SAC density in Ptf1aαKO retina. SACs (labeled by Sox2 and Megf10lacZ) are completely eliminated from Ptf1aαKO central retina; some remain in peripheral retina (boxed regions, right panels). Top, littermate control (Ptf1a+/+).

C: En-face view of SACs in peripheral retina of Ptf1aαKO and littermate control. Green, GFP Cre reporter. Control SACs were either Cre+ or Cre–. Mutant SACs were Cre– (arrows), indicating that they derive only from cell lineages that maintain Ptf1a function.
D-F: SAC IPL laminar targeting in Ptf1a<sup>cKO</sup> (E,F) and littermate control (D). Ptf1a<sup>cKO</sup> SACs close enough to touch (E) form IPL strata (blue arrowheads), similar to control SACs (D). Solitary SACs (F) are not polarized towards IPL; they have extensive INL-directed arbors (white arrows) and rudimentary IPL-directed arbors (orange arrows). Some solitary SACs entirely fail to innervate IPL (F, left cell) and resemble migrating E16 SACs (Fig. 2C); others innervate IPL with minimally-branched, non-stratified arbors (F, right cell).

G: Quantification of SAC dendrite phenotypes at P1-2. Left, frequency of soma layer innervation. *<i>p</i> = 0.0350; **<i>p</i> = 0.0081; ns, <i>p</i> = 0.7516. Center, frequency of IPL innervation failure (e.g. F, left). ***<i>p</i> = 4.0 x 10<sup>-7</sup>; ns, <i>p</i> = 0.3723. Right, frequency of cells that send arbors into IPL but fail to stratify (e.g. F, right). * <i>p</i> = 0.0110; ***<i>p</i> < 1.0 x 10<sup>-7</sup>. Dots, individual animals. Error bars, S.E.M. P-values, Tukey’s post-hoc test. Sample sizes, see Methods.

Scale bars: 25 µm (A,C); 200 µm (B, left), 50 µm (B, right), 10 µm (D-F). Also see Fig. 3-Supplement 1.

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**Figure 4**

**A:** Schematic of MEGF10 protein. TM, transmembrane domain.

**B:** Left, MEGF10 immunostaining at E16 reveals onset of protein expression at conclusion of radial migration. INBL SACs express MEGF10, but SACs migrating through ONBL do not. Arrow, INBL SAC with migratory morphology suggesting it is newly-arrived. Right: Schematic illustrating timing of Megf10 expression onset in SACs (also see Kay et al., 2012).

**C:** Soma-directed SAC arbors in the INL (arrows) express MEGF10 protein. IPL dendrites are also labeled (arrowheads).

**Figure 4:** MEGF10 is expressed by SACs during early homotypic contact

**A:** Schematic of MEGF10 protein. TM, transmembrane domain.

**B:** Left, MEGF10 immunostaining at E16 reveals onset of protein expression at conclusion of radial migration. INBL SACs express MEGF10, but SACs migrating through ONBL do not. Arrow, INBL SAC with migratory morphology suggesting it is newly-arrived. Right: Schematic illustrating timing of Megf10 expression onset in SACs (also see Kay et al., 2012).

**C:** Soma-directed SAC arbors in the INL (arrows) express MEGF10 protein. IPL dendrites are also labeled (arrowheads).
Figure 5: Megf10 is required for initial formation of SAC IPL sublayers.

A: SAC sublayers are absent from P0-1 Megf10 mutant IPL. Antibodies to Sox2 and βgal reveal SACs in retinal cross-sections. Littermate control, Megf10lacZ/+ . Arrowheads, SAC IPL strata. Arrows, exuberant arbor growth in mutant INL and GCL. Note that mutant somata abut the IPL at P0, indicating their radial migration was normal. By P1 OFF somata have moved apically.

B: At P3, SAC IPL sublayers remain disrupted in Megf10 mutants. Single SACs (ChatmG) and full population (Megf10:βgal) labeled in cross-sections. Mutant OFF SACs mostly project within INL (arrows). INL projections are absent from controls. Some ON SACs are stratified in mutants (arrowhead) but have not yet formed a continuous restricted sublayer as is seen in controls.
C: *En-face* view of single OFF SACs, imaged in whole-mount at IPL level. Mutant SAC dendrites appear undifferentiated, with less branching (arrow). Their arbors cover smaller arbor territories than SACs from wild-type (wt) littermate controls (quantified at right, mean ± s. e. m.). ***p(on) < 1.0 x 10^-7, p(off) = 9.38 x 10^-5; one-way ANOVA/Tukey’s post-hoc test. Sample size, see Methods.

D: Internexin immunoreactivity reveals orientation of SAC primary dendrites (arrows) at P1. Right: Example of mutant SACs projecting primary dendrites directly towards each other. Control primary dendrites were exclusively oriented towards IPL (left).

E: Single OFF SACs labeled by *Chat^mG* in cross sections (see Fig. 5-Supplement 1 for ON SACs). Arrows, arbors in INL. Mutant IPL projections (arrowheads) fail to arborize or stratify.

F: Frequency of soma layer projections across development in mutants (–/–) and littermate controls (+/–), determined from single *Chat^mG* cells as in E. Wild-type (WT) data replotted from Fig. 2M to show that +/– controls resemble WT. Error bars, standard error. Sample size, see Methods.

G: Summary of *Megf10–/–* phenotype. After initial contact at E16, mutant SACs do not immediately innervate the IPL, instead overgrowing arbors in cell body layers (P0). This leads to delayed sublayer formation and persistent soma-layer projections at P3.

Scale bars: 25 µm (A,B); 10 µm (C-E). Also see Fig.5-Supplement 1.
Figure 6: *Megf10* mediates transcellular SAC signals for dendrite development.

**A,B:** *En-face* images of INL in Six3-*Megf10*<sup>cko</sup> retinas stained for GFP Cre reporter (A) and βgal SAC marker (B). Reporter expression indicates loss of MEGF10 (see Fig. 6-Supplement 1). In central retina (top row), most SAC are mutant, and project extensive INL dendrites (B, arrows; compare to C). In peripheral retina (bottom row), some cells escape Cre (asterisks) and retain MEGF10 but still make ectopic INL projections. Purple, Cre reporter; green, βgal.

**C:** Littermate control retina imaged as in B; SACs rarely project INL dendrites.

**D:** Quantification of P2 INL projection phenotypes illustrated in A-C. Six3-*Megf10*<sup>cko</sup> (cko) SACs that escape Cre (M10<sup>+</sup>) make projection errors at similar rate as surrounding mutant cells from the same tissue (M10<sup>−</sup>).

**E-G:** *Chat-Megf10*<sup>cko</sup> phenotype. Morphology of single SACs, revealed by Chat<sup>mg</sup> in cross-sections. Anti-MEGF10 (M10) distinguished two classes of cKO SACs (orange arrows): Those that express MEGF10 (F) are anatomically similar to littermate control SACs (E). Those lacking MEGF10 (G) arborize extensively in INL (yellow arrowheads) but minimally in IPL (blue arrowheads). Vertical line, IPL.

**H:** Quantification of SAC soma-layer projection frequency at P3. Sparse M10 deletion (blue, −) phenocopied germline null (red). *Chat-Megf10*<sup>cko</sup> cells that retained M10 (blue, +) resembled controls (flox/+).

**I:** Schematic of MEGF10 proteins used for co-immunoprecipitation (IP). Intracellular domain was deleted (ΔICD) and replaced with epitope tags (Flag or GFP). Ex, extracellular; TM, transmembrane.

**J:** Co-IP from lysates of HEK 293T cells transfected with indicated constructs (I). Western blot with antibodies to GFP (green) and Flag (red). IP with anti-GFP, but not rabbit IgG control, pulled down both MEGF10-ΔICD constructs (2<sup>nd</sup> lane from right, orange text). IP with anti-Flag gave similar result (Fig. 6- Supplement 2). GFP alone did not co-IP with M10-Flag. Ladder molecular weights (kDa) at left. Full blots in Fig. 6-Supplement 2.

Error bars, 95% confidence interval. Sample sizes, see Methods. Scale bars: 25 µm (A), 10 µm (B-G).
Figure 7: SAC IPL errors persist to maturity in Megf10 mutants. 

A,B: SAC IPL phenotype in mature (two-week-old) retina, cross-section view. Blue, soma counterstain. Control IPL has two continuous SAC dendrite bands (blue arrowheads). Mutant IPL has sporadic SAC laminar gaps (white arrows) or ectopic arbors (yellow arrowheads).

C: En-face views of SAC dendrites, stained with anti-ChAT, in adult retinal whole-mounts. The same fields of view are shown at two different z-stack planes, corresponding to OFF and ON SAC sublayers. SAC dendrite plexus is uniform in littermate controls, but has holes (arrows) and large gaps (asterisks) in mutants. Note that errors are not spatially correlated between OFF and ON sublayers.

D: Single SAC labeling in adult (3 month old) mice, via ChatCre-dependent viral fluorescent protein expression. Megf10−/− SACs have relatively normal morphology but are significantly smaller than wild-type (WT) control cells (***p = 4.6 x 10⁻⁶, two-tailed t-test). Sample size, see Methods.

E: En-face images at INL-IPL border from same control and mutant z-stacks shown in C. A network of ectopic SAC dendrites (yellow arrows) is evident mutants but not controls.

F: Ontogeny of ectopic SAC network in Megf10 mutants, revealed by en-face images at INL level. Megf10:βgal labels SACs. At P1, INL-projecting cells send fine arbors in many directions. At P5, INL projections are
directed toward ectopic arbor aggregates, similar to adults (E). Arrows, cells making multipolar (left) or directed (right) INL projections. Littermate controls are shown in Fig. 7-Supplement 1.

**G**: Frequency of ectopic OFF SAC projections does not change over development, despite changes in arbor anatomy (F). P0-3 data replotted from Fig. 5F, with both control groups combined. Sample sizes, see Methods.

**H**: Classification of ectopic arbor location in *Megf10*−/− OFF SACs that made ectopic projections. Ectopic arbors localize to soma layer before P5, and to IPL in adults. P5 is a transitional stage when exuberant arbors can project to either or both ectopic targets. Sample sizes as in G (see Methods).

**I,J**: Transition of ectopic OFF SAC projections from INL to IPL at P5. Arbor fascicles (orange arrowhead) crossed the INL-IPL boundary at P5 (I), whereas they were confined to IPL in two-week-old mice (A). J: An individual P5 *Megf10*−/− SAC projects to three different locations: 1) correct IPL sublayer (blue arrowhead); 2) inappropriate IPL sublayer (yellow arrowhead); 3) ectopic INL arbor aggregate (orange arrowhead). ON SACs also make ectopic IPL projections (J, white arrowhead). Control cells are monostratified in IPL (left). Note that IPL sublayers have formed by P5 in mutants (I).

Error bars, 95% confidence intervals. Scale bars: 25 µm (A,B,F,I,J); 50 µm (C-E).
Figure 8

A: SAC IPL errors (yellow arrowheads) induced by early deletion of Megf10 in Six3-Megf10cKO mice, but not late deletion in Chat-Megf10cKO mice.

B: Mosaic spacing phenotype measured at P17 using Voronoi domain regularity index. Dashed line, index for simulated random SAC arrays. In both Six3 and Chat conditional mutants, SAC positioning is less regular than in controls (ChatCre; Megf10flox/+). Megf10−/− and simulation data from Kay et al. (2012). ns, *p = 0.6438; **p = 0.0023; ***p = 2.1 x 10^{-6}; ****p < 1.0 x 10^{-6} (one-way ANOVA/Tukey’s post-hoc test). Error bars, S.E.M.

C,D: Voronoi domain territory size as a single-cell measure of mosaic perturbation. Territory size images (C) and histograms (D, 100 µm² bins) for adult littermate controls and Megf10 mutants. Because mutant SAC positions are random, their locations are less constrained, leading to a wider range of territory sizes than in controls. Dashed lines (D), upper and lower 95% tolerance intervals of the control distribution. Mutant cells outside these lines experience crowding or isolation rarely seen in controls. Arrow denotes largest bin in F.

Sample sizes: n = 515 cells from 2 littermate control (Megf10+/−) mice; n = 584 cells from 2 Megf10−/− mice.

E: No obvious correlation between a mutant cell’s local neighborhood density and its projection to ectopic IPL sublayer. En-face view of SAC cell bodies and outer IPL, generated by z-projecting part of a confocal stack corresponding to these layers. Controls lack SAC dendrites at this IPL level. Arrows, examples of cells that are unusually far from their neighbors yet join the ectopic network (blue), or that are unusually crowded yet do not join (orange).

F: Frequency of ectopic IPL projections for mutant SACs in each 100 µm bin of histogram in D. Dark shading, bins outside dashed lines in D. Smallest and largest bins were pooled to ensure adequate sample size (n ≥ 24 SACs per bin; see Methods for bin sizes). Across all bins except the largest one (denoted in D by arrow), error rate was similar to the overall mutant error rate (red). X values denote bin center (aside from pooled bins <200 µm² and >1100 µm²). Error bars, 95% confidence intervals.

Scale bars (A,C,D), 25µm.
Figure 9: SACs guide IPL sublayer choice by their circuit partners. 
A: SACs (ChAT, purple) and ooDSGCs (Hb9:GFP, green) labeled in cross-sections (top, middle) and en-face view (bottom). In *Megf10* mutants, ectopic SAC arbors (arrowheads) are extensively innervated by ooDSGC dendrites.

B: Quantification of fluorescence intensity across IPL in cross-section images from A. ooDSGC dendrites (green) strictly co-localize with SAC arbors (purple) in ON and OFF sublayers, and in ectopic sublayer (arrowhead).

C: En-face view of ON (top) and OFF (bottom) SAC IPL sublayers. In *Megf10* mutants, ooDSGC dendrites (green) fail to enter IPL regions (asterisks) that are not innervated by SACs (purple).

D,E: BC5 and BC7 IPL projections (blue arrowheads), labeled in *Kcng4* mice. D, images; E, representative fluorescence plots of *Kcng4* (green) and ChAT (purple) across IPL. In littermate controls, or normal regions of mutant IPL (D, arrow), BC5 and BC7 arborize in sublayers immediately adjacent to ON SAC layer, but do not enter it. In *Megf10* mutants, ectopic SAC arbors displace BC5+7 terminals to new IPL locations, where they remain adjacent to SACs but non-overlapping (D, yellow arrowheads; E, center plots). Asterisk (E): ectopic BC arbors between normal and ectopic SAC strata. BC5/7 arbors that innervate SAC gaps are abnormally close together (D, white arrowhead; E, right plot). Vertical bars in E: distance between BC5/7 terminals.

F: Quantification of BC5-BC7 distance. *p = 0.0219; **p = 0.0012; ns, p = 0.3965 (Tukey’s post-hoc test). Sample sizes, see Methods. Error bars, S.E.M.

G: Mislocalized SAC arbors recruit BC3a bipolar axons (HCN4, green) to ectopic IPL locations.

All scale bars: 25 µm. Also see Fig. 9-Supplement 1.
Figure 10: Broader and weaker direction tuning of ooDSGCs in Megf10 mutants.

A: Histograms of RGC direction selectivity indices, measured on a multielectrode array, for wild-type (WT, black) and Megf10–/– (red) retinas. Bimodal histograms fit with two-Gaussian mixture model distinguished DSGCs (filled bars) from non-DSGCs (open bars).

B: Spike rasters from representative WT and Megf10–/– posterior-prefering ooDSGCs in response to a bright bar moving along 12 directions (arrows).

C: Direction tuning curves from cells in B normalized to the maximum response (line: von Mises fit). Non-zero values at tails of mutant curve reflect increase in null-direction spikes (B, left- and right-most bins).

D,E: Cumulative distribution of tuning widths (D) and tuning strengths (E) for all ooDSGCs recorded from two retinas of each genotype (WT n = 80 cells; Megf10–/– n = 74 cells). Mutant ooDSGC population is tuned more broadly (D, right shift of red curve) and more weakly (E, left shift of red curve) than WT. Mutant ooDSGCs also exhibit higher firing rate to null direction motion (E, inset). **p = 0.005 (D), p = 0.003 (E), paired KS-test.

F: Rasters and polar plot of a representative WT ooDSGC, highlighting preferred directions of ON (gray) and OFF (black) responses (arrows). Δϕ, angular difference between preferred directions of ON and OFF responses.

G: ON and OFF direction tuning curves for cell in F (line, von Mises fit). ON and OFF preferred directions (arrowheads) are well aligned in WT retina.

H: Cumulative distribution across all ooDSGCs of ON-OFF preferred direction difference (Δϕ). Same cells as in D,E. Rightward shift of mutant curve indicates larger ON-OFF misalignment. **p = 0.004, paired KS test.

For all panels, background light level was photopic (10^4 P*/M-cone/sec; contrast of moving bar was 60%). Error bars/bands, S.E.M. Also see Fig. 10-Supplement 1.
Formation of retinal direction-selective circuitry initiated by starburst amacrine cell homotypic contact

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SUPPLEMENTAL FIGURES & LEGENDS
Figure 1 - Supplement 1

Markers for SACs and ooDSGCs in neonatal retina. 

A,B: Characterization of Sox2, MEGF10, and \(\text{Chat}^{\text{Cre}}\) as markers that label SACs in the neonatal mouse. All images depict retinal cross-sections. 

A: Individual color channels of P0 image shown in Fig. 1B. Sox2 (A, left panel) is a pan-SAC nuclear marker. Antibodies to Sox2 strongly label all SACs in the inner nuclear layer (INL) and ganglion cell layer (GCL), as well as astrocytes in the nerve fiber layer (NFL). Progenitor cells in the outer neuroblast layer (ONBL) are weakly labeled. Antibodies to \(\beta\text{gal}\) (A, right panel) label the complete SAC population in \(\text{Megf10}^{\text{lacZ}}\) mice. Horizontal cells (HCs) in outer retina are also labeled. 

B: Antibodies to MEGF10 (purple) are selective for SACs and label the complete SAC population. \(\text{Chat}^{\text{mG}}\) mice (i.e. \(\text{Chat}^{\text{Cre}}\) crossed to membrane-targeted GFP Cre reporter) label a subset of SACs in the neonatal retina (green). Whereas \(\text{Chat}^{\text{Cre}}\) is a marker of the full SAC population at later stages, its expression in neonatal retina is more sporadic (Xu et al., 2016). We took advantage of this feature for two purposes: 1) Single-cell anatomy studies of SAC dendrite morphology, as shown here; 2) Sporadic early knock-out of genes in a sparse subset of SACs (See Fig. 6). 

C,D: Anatomy of neonatal ooDSGCs labeled with \(\text{Hb9:GFP}\). At P1 (C), ooDSGC dendrites are rudimentary with few branches. No IPL stratification is evident. At P2 most ooDSGCs remain unstratified as depicted in Fig 1D. However, a minority of P2 ooDSGCs have dendrites that co-stratify with SAC dendrites (\(\text{Megf10}^{\beta\text{gal}}\); blue arrowheads); an example is shown in (D). See main text for quantification of stratification frequency. 

Scale bars: 25 \(\mu\text{m}\).
Figure 2-Supplement 1:
Characterization of internexin as a primary dendrite marker of developing SACs.

A: Expression pattern of internexin in P2 mouse retina. Internexin (Intnx) immunoreactivity is detected in Sox2+ SACs, and in RGC axons within the nerve fiber layer (NFL). This pattern is typical of the entire first postnatal week. In RGCs, axons are selectively labeled; their cell bodies in the GCL are internexin-negative. In SACs, internexin selectively labels primary dendrites, as well as the portion of the soma from which the primary dendrites arise. Therefore, internexin+ intermediate filaments are trafficked to specific subcellular compartments of both SACs and RGCs. P2 SACs are strongly polarized towards the IPL (also at P1; see D). Note that this image is the same one depicted in Fig. 2G, but cropped differently to show NFL staining; also, colors have been reversed to match other panels of this figure.

B: An individual E16 INBL SAC (asterisk), surrounded by Isl1mG-positive RGCs (identified as RGCs because they lack Sox2 or internexin staining). At E16, internexin has the same subcellular localization within SACs as at P1-2 (A,D): It localizes to the primary dendrites (arrow) and the side of the cell body from which it emerges. However at E16, SAC primary dendrite orientation is more variable than at P2 (A). This SAC sends a primary dendrite towards the outer retina (ONBL) where it could potentially contact migrating SACs as they arrive at the INBL. Other SACs project within the INBL towards neighboring SAC cell bodies (Fig. 2F), or towards inner retina (not shown).

C: Antibodies to internexin strongly label SAC primary dendrites originating from the cell body (arrows), but fine dendritic branches within IPL are unlabeled. Occasionally, higher-order branches arising from the primary dendrites are weakly labeled. ChatmG was used to reveal the full dendritic arbor.

D: Internexin distinguishes IPL-directed primary dendrites from soma-directed arbors in neonatal SACs. Isl1mG labels full morphology of bi-laminar P1 OFF SACs (asterisks) that project to both INL and IPL. Only IPL-directed primary dendrites of these cells are internexin-positive (middle, right panels).

Scale bars: 25 µm (A), 10µm (B-D).
Figure 2-Supplement 2: Homotypic soma-directed SAC arbors across development.

A: E16 homotypic soma-directed contacts can be established prior to IPL formation. Right panel, same cells as Fig. 2F, showing INBL SACs projecting towards each other. Left panel, same field of view showing Sox2 SAC nuclear marker and Hoechst nuclear stain (blue). These SACs are surrounded by other INBL cell bodies, with no IPL neuropil evident in this retinal region.

B: E16 homotypic SAC soma contacts occur outside the IPL. Left panel, same cells as Fig. 2D. These cells have migratory morphology, as shown by their prominent apical and basal processes (arrowheads). They do not make obvious projections into the IPL, delineated by dense *Isl1*^mG^ staining. The contact between the two SACs (arrow) occurs outside of the IPL. Right panel, Hoechst nuclear stain confirms location of nascent IPL inferred from *Isl1*^mG^ labeling. The IPL is a narrow cell-free gap between cell bodies that corresponds to location of dense GFP+ arbors (left panel).

C, D: At P0, ON SACs can contact neighboring SAC somata (arrows) without being bi-laminar. Cross-sections of P0 retina, co-stained for individual SACs (*Chat*^mG^) and for markers of the complete SAC population (C, *Megf10*:βgal; D, internexin). The existence of such cells may help explain why the frequency of soma layer-projecting ON SACs is lower than for OFF SACs (Fig. 2M). D: Internexin staining shows that these ON SACs (orange arrowhead) are polarized along the INL-GCL border towards their neighbors, adopting a horizontal
morphology distinct from surrounding OFF and ON SACs (white arrowheads). This morphology is typical of a displaced amacrine cell in the process of crossing from the INL to the GCL (Chow et al., 2015).

**E:** Examples of soma layer-projecting $Chat^{mG}$-labeled SACs in mice that are wild-type at the $Megf10$ locus ($Megf10^{+/+}$), demonstrating that the soma-contacting arbors shown in Fig. 2K,L are not a consequence of $Megf10$ heterozygosity. Arrows, arbors in INL. Arrowhead, arbors arising from a neighboring ON SAC with cell body located in adjacent section.

**F-H:** Examples of P3 (F,G) and P5 (H) cells used to generate graph in Fig. 2M. SAC single-cell morphology was revealed using $Chat^{mG}$ labeling. At P3, most SACs project only to the IPL (G), but some SACs still make soma-directed projections (F). Representative INL-projecting OFF cell (F, left) and GCL-projecting ON cell (F, center, right) are depicted. The ON cell makes contact with the neighboring internexin-positive SAC soma (arrow in right panel of F). Arrows, soma-layer projecting arbors. Arrowhead, arbor of a neighboring ON SAC only partially present in the section. At P5 (H), all SACs project exclusively to IPL (vertical bar).

All scale bars: 10 µm.
Figure 3-Supplement 1: Retinal phenotype of Ptf1a^cKO mutants.
A: Immunostaining with pan-amacrine marker AP2α (green) and pan-bipolar marker Chx10 (red), in littermate control and Ptf1a^cKO retinal cross sections. Blue, Hoechst nuclear counterstain. Top panels: low-power view illustrating center-peripheral differences in amacrine number that arise due to Cre expression pattern (see Fig.
3A-C). Bottom panels: Higher magnification views of mid-peripheral retina. AP2α+ cells are completely eliminated from Ptf1a<sup>cko</sup> central retina. Some amacines that have escaped Cre recombination (see Fig. 3C) are produced in the periphery, albeit at lower density than controls. Arrow marks central-most amacrine cells. Bipolar cell number is not obviously different between genotypes. Asterisks, non-specific staining, due to anti-mouse secondary antibody, in blood vessels and sclera. Note that sclera became detached from control section prior to imaging.

**B**: Cross-sections through central retina of littermate control and Ptf1a<sup>cko</sup> mutant, stained for pan-RGC marker RBPMS (red) and Sox2 (green) to mark SACs. Blue, Hoechst nuclear counterstain (nuc). Optic nerve head (ohn) marks center of retina. In Ptf1a<sup>cko</sup> mice, SACs are entirely absent from central retina, but Sox2+ astrocytes (a) in nerve fiber layer are present in normal numbers. RGC cell number appears to be increased, consistent with previous observations in embryonic retina of Ptf1a null mice (Fujitani et al., 2006; Nakhai et al., 2007).

**C-E**: Additional examples of SACs in Ptf1a<sup>cko</sup> retinal cross-sections, from dataset used to compile graph in Fig. 3G. Neurons were validated as SACs by co-expression of Megf10:βgal and Sox2. Touching SACs (C) stratify their arbors normally (arrowhead). Note that the right-hand cell appears to be polarized towards the left-hand cell, suggesting asymmetric growth towards the side with homotypic contact and away from the side lacking it. Representative solitary SACs are shown in D,E. One cell (D) is an example of the class that failed to project to the IPL. The other cell (E) exemplifies the class that sends only abnormal unstratified arbors into the IPL. In this case (E) the cell innervated the IPL with a single minimally-branched dendrite that fails to ramify in a laminar fashion (compare to C). This cell also has particularly exuberant arbors in the INL that were much larger than those seen in any cells that touched their neighbors (e.g. C; also see Fig. 3D,E). White arrows, soma-directed arbors. Orange arrows, IPL-directed arbors.

**F-G**: SAC errors in Ptf1a<sup>cko</sup> mutants persist to maturity. P15 littermate control (F) and mutant (G) retinal cross-sections stained for anti-ChAT to label SACs (red) and anti-GAD65 to label a broad non-SAC amacrine population (green). Control SACs no longer have soma-directed arbors at this age; the only processes not directed toward the IPL were very short and minimal (F, arrowhead). In mutant retina, SACs from low-density regions often innervated the INL (G, white arrowheads), or failed to innervate gaps in the SAC IPL network (G, orange arrowhead). SACs that made errors had extensive interactions with GAD65+ amacrine cells (G, arrows) and their arbors (green) suggesting that generic amacrine contacts are not sufficient to prevent SAC errors. Instead, because these SACs had few homotypic neighbors, the errors were likely due to paucity of SAC-SAC interactions.

Scale bars: 200 µm (A top); 100 µm (A bottom, B); 10 µm (C-E); 25 µm (F,G).
**Figure 5-Supplement 1**: IPL innervation and sublayer formation phenotypes in *Megf10* mutants.

**A**: Retinal cross-sections from P1 *Megf10* mutants and littermate (*Megf10*+/−) controls carrying the *Gad1:GFP* transgene. A broad subset of non-SAC amacrine cells is labeled by GFP in these mice. Unlike SACs (Fig. 5A), *Gad1:GFP*+ amacrine cells innervated the INL normally in *Megf10* mutants, and did not make exuberant projections within the INL.

**B-C**: Examples of P1 (B) and P3 (C) *ChatmG*-labeled ON SACs that were part of the dataset used to generate graphs in Fig. 5F. At P1 (B) many mutant ON cells are bi-laminar, with projections in both IPL and GCL (arrow, GCL arbor). IPL projections were underdeveloped relative to controls, and not sufficient to generate a clear sublayer (Fig. 5A). C: P3 control and mutant ON SACs, both of which project to the GCL. The control cell sends a single arbor to the GCL (left, arrow), typical of those few SACs that still project to the soma layers at this age. The mutant cell (right) makes a dense dendritic arborization in the GCL (right, arrow), which was never seen in P3 controls.

**D**: *En-face* view of OFF SACs at INL level shows extensive soma-layer arbor network in P1 mutants. Single SACs (*ChatmG*, purple) have larger and more elaborate INL-directed arbors in mutants than in littermate controls. A single-color version of the left panel, showing only the βgal channel, appears in Fig. 7F.

Scale bars: 25 µm (A,D); 10 µm (B,C).
Figure 6-Supplement 1: Megf10 cell autonomy: Characterization of conditional mutant mice

A: Six3-Megf10KO mice phenocopy SAC sublayer formation errors seen in null mutants. Cross-sections through central retina of P2 Six3-Megf10KO and littermate control mice. Immunostaining for Megf10: βgal and Sox2 revealed SAC morphology. Control SACs (left) have formed IPL sublayers by P2 and they rarely project to soma layers. In Six3-Megf10KO mice (right), sublayers are absent and SACs project exuberantly to soma layers (arrows).

B: The same Six3-Megf10KO Cre-negative SAC from Fig. 6B (arrow). βgal and GFP (Cre reporter) channels are shown separately (middle, bottom) to demonstrate lack of GFP expression in this cell.

C: Another example of a Cre reporter-negative Six3-Megf10KO SAC (arrow) surrounded by mutant Cre-positive cells (asterisks). All 5 cells, including the unrecombined one, participate in an aberrant INL dendritic network (bottom).

D: GFP Cre reporter is a reliable proxy for MEGF10 protein expression status in Six3-Megf10KO mice. Cre+ SACs (left, center panels) express the GFP reporter and lack MEGF10 immunoreactivity. Cre− SACs lack GFP reporter expression and retain MEGF10 immunoreactivity. Arrows denote position of Sox2+ SACs in each panel.
**E,F:** Timing of MEGF10 protein loss in *Chat-Megf10*KO mice. At P3 (E), MEGF10 immunoreactivity is much lower in mutants (right) than in littermate controls (left), but most SACs still express some protein (arrows indicate examples of MEGF10-positive cells). At P5 (F), MEGF10 immunoreactivity is virtually absent in mutants but readily detectable in controls. Arrowheads, SAC IPL strata. Vertical bar, IPL.

Scale bars: 10 µm (A-D); 25 µm (E,F). Scale bar in C applies to B, and bar in E applies to F.

**Figure 6 - Supplement 2**

**A**

|        | Inputs | IP: anti-GFP | IP: IgG-R |
|--------|--------|--------------|-----------|
| M10-gfp | −      | +            | +         |
| M10-flag | +     | −            | +         |
| GFP     | +      | −            | −         |

**B**

|        | Inputs | IP: anti-Flag | IP: IgG-M |
|--------|--------|---------------|-----------|
| M10-gfp | −      | +            | +         |
| M10-flag | +     | −            | +         |
| GFP     | +      | −            | −         |

**Figure 6-Supplement 2:** MEGF10 co-immunoprecipitation experiments

**A:** Uncropped blot image for co-IP experiment depicted in Fig. 6J. Anti-GFP was used for pull-down. Blot was stained for anti-GFP (green) and anti-Flag (red). Orange box indicates the condition in which cells were transfected with both MEGF10-ΔICD constructs. In this condition, pull-down with anti-GFP precipitated both MEGF10-ΔICD-GFP and MEGF10-ΔICD-Flag constructs, demonstrating that they interact. Ladder markings in kDa. Expected sizes for MEGF10-ΔICD constructs, GFP, and IgG are indicated (arrows). R, rabbit IgG control. See Fig. 6I for illustration of in MEGF10-ΔICD construct design.

**B:** Independent replicate of MEGF10-ΔICD co-IP experiment, using anti-Flag for pull-down. Labels as in F. IP with anti-Flag co-precipitated both GFP and Flag-tagged MEGF10-ΔICD constructs (orange box). Mouse (M) IgG control did not precipitate MEGF10 constructs, and MEGF10-ΔICD-Flag did not co-precipitate with GFP alone.
Figure 7-Supplement 1: SAC phenotypes in *Megf10* mutants at P5 and at maturity.

A: Specificity of *Megf10*−/− SAC IPL innervation phenotype. The same cross-sections from Fig. 7A are shown here, overlaid with anti-Vglut3 staining (green) to label amacrine cells that project to an IPL sublayer between the SAC strata (arrowheads). Regions of mutant IPL not innervated by SACs (arrow) are still innervated by Vglut3+ amacrine cells, demonstrating that absence of ChAT+ arbors is not due to tissue damage and that failure to innervate the IPL is a SAC-specific phenotype.

B: Littermate control images matching the P1 and P5 en-face mutant images shown in Fig. 7F. Images were acquired at the INL level, at a z-stack position comparable to the Fig. 7F mutant images. SACs are labeled by *Megf10*:βgal. At P1 (left), control SACs still project arbors within INL (arrows), but their network is not as extensive as in mutants (compare to Fig. 7F, left). At P5 (right), control SACs do not project to INL. By contrast, mutant SAC arbor aggregates are observed in INL (Fig. 7F, right).

C: Additional characterization of *Meg10* mutant phenotype at P5, using *Chat*mutG to label single cells and *Megf10*:βgal to label the full SAC population. Control OFF and ON SACs (left panels) are monostratified within the DS circuit IPL sublayers (blue arrowheads). Center: Example of a mutant ON SAC that makes an ectopic projection to inappropriate IPL sublayer (yellow arrowhead) while also projecting to the expected DS circuit sublayer (blue arrowheads). OFF SACs in this same field of view make ectopic projections within the INL (orange arrowhead), illustrating the simultaneous soma-layer and IPL ectopias observed only at P5. Right: Many mutant SACs still show perturbed IPL innervation at P5. Even though this SAC has innervated the IPL, and begun to ramify arbors that stratify in the appropriate sublayer, its arbors are far less extensive than controls.
(left), and it covers a smaller IPL territory. Thus, even though SAC sublayers have formed, individual SACs still demonstrate severe errors in IPL innervation that likely lead to persistence of IPL gaps.

D,E: ON SAC ectopic projections transition to the IPL at P5 in Megf10 mutants, similar to OFF SAC projections (Fig. 7G,H). Frequency of mutant ectopic ON SAC projections does not change over development (D), even though arbor anatomy changes by P5 (C). P0-3 data in D replotted from Fig. 5F, with both control groups combined. E: As with mutant OFF SACs, ON SACs can make ectopic projection errors either within the GCL or the IPL at P5.

Scale bars: 25 µm.
**Figure 8-Supplement 1**: Correlations between SAC soma and arbor position.

**A**: En-face views at different levels of individual confocal z-stacks, depicting OFF SAC cell bodies (left) and their underlying arbors in the IPL (right). Images are from adult (P46) ChAT-stained whole-mount retinal preparations. *Megf10* mutants have less orderly soma positions, and less uniform arbor distributions, than controls. Qualitatively, it is possible that gaps in the mutant arbor plexus line up, at least in some cases, with gaps in the soma array.

**B**: Spatial cross-correlation map generated by correlating soma and arbor images like those in A. Left, soma vs. underlying arbors. Right, soma vs. flipped image of underlying arbors, which controls for correlations in the image data unrelated to soma and arbor position. Bright pixels indicate positive correlations; dark pixels indicate negative correlations. Dashed red line indicates size of average SAC cell body. The bright region at the center of the “cells vs. arbors” map shows that when the two images are perfectly aligned, or offset by about 1
cell radius, correlations are high. Such correlations are absent from the control “flipped arbors” map, indicating that they arise due to the specific locations of somata and arbors.

C: Quantification of soma-arbor cross-correlations, from maps like those shown in B. Correlation intensities were measured radially out from the center. Values obtained from correlating real data were normalized by subtracting the equivalent-radius values from the flipped-arbor images. In control animals (gray), there is a strong positive correlation on a spatial scale approximating the size of a SAC cell body (dashed vertical line). There is also a weaker negative correlation at the 10 - 20 µm spatial scale. Beyond ~25 µm, soma and arbor positions are uncorrelated. In mutants (−/−, red), both correlations are attenuated. This finding suggests that soma-arbor correlations still exist in mutants to some extent; however, there are also additional factors influencing arbor position in mutants that reduce the influence of soma position. Sample size: n = 9 sets of soma & arbor images from 2 animals of each genotype (P46 adults). Error bars, S.E.M.

D: SAC cell density did not differ among Megf10 germline-nulls, conditional-nulls, or littermate controls (ChatCre; Megf10flx/+) . Therefore cell density differences cannot explain arbor patterning or mosaic spacing phenotypes (e.g. Fig. 8B). Megf10−/− data from Kay et al. (2012). One-way ANOVA, F(3, 15) = 0.6063; p = 0.6210. Error bars, S.E.M.

Scale bar = 25 µm. Bar applies to both A and B.
Figure 9-Supplement 1: IPL innervation by DS circuit neurons in Megf10 mutants.

A: Drd4:GFP mouse line was used to label a subset of ooDSGCs that is mutually exclusive with Hb9:GFP. IPL laminar targeting by Drd4:GFP ooDSGCs was assessed in cross-sections of Megf10−/− and littermate control retinas, co-stained for ChAT to reveal SAC dendrites. In mutants, Drd4:GFP+ cells made the same laminar targeting errors observed in the Hb9:GFP line (Fig. 9A-C): When SACs projected to inappropriate laminar locations, ooDSGC dendrites were recruited to join them (center panel, large arrows). GFP+ dendrites also failed to enter IPL regions not innervated by SACs (bottom panel, white arrow).

B: Laminar targeting errors by BC2 bipolar cells in Megf10 mutants. In control retina, BC2 axon terminals (stained with anti-Syt2, green) fill the entire IPL region between the INL border and the OFF SAC layer. In mutants, OFF SAC misprojection errors typically occur in the region that is normally innervated by BC2 (e.g. Fig. 9A,G), precluding a quantitative analysis of BC2 error rate. However, we did find a small number of cases, such as the one shown here, in which OFF SACs project inappropriately to central IPL regions where BC2 terminals are not normally found (yellow arrowhead). In these cases BC2 arborizations are recruited to join SAC arbors in their abnormal laminar location. Thus, BC2 IPL projections are likely guided by similar SAC-derived cues as the other DS circuit-projecting bipolar cell types.

C: Gjd2:GFP mouse line was used as an independent marker of BC5 bipolar cells. In cross-sections of adult retinas stained for anti-GFP (green) and anti-ChAT (red), GFP was found to label BC5 neurons arborizing in their characteristic position adjacent to the ON SAC sublayer (blue arrowhead). Thus, unlike the Kcng4mG line in which both BC5 and BC7 were labeled, this line could be used to specifically assess BC5 phenotypes. In Megf10 mutants, an ectopic SAC projection near the GCL border (yellow arrowhead) recruited BC5 terminals to an inappropriate IPL location. BC5 terminals are also seen innervating a gap in the SAC sublayer (white arrowhead). BC5 neurons therefore appear to respond similarly to SAC-derived cues as the other DS circuit bipolar cell types.

D: Fluorescence intensity plot across IPL obtained from a wild-type image similar to Fig. 9G – i.e. tissue stained with anti-ChAT (purple) and the BC3a marker HCN4 (green). BC3a arbors are excluded from the OFF SAC sublayer and arborize adjacent to it, similar to the behavior of BC5 and BC7 (Fig. 9E).

Scale bars: 25 µm (A,C,D); 50 µm (B).
Figure 10-Supplement 1: Contrast-dependence of direction-tuning phenotypes in $\text{Megf10}^{-/-}$ ooDSGCs.

A: Tuning curves from representative wild-type (WT) and $\text{Megf10}^{-/-}$ mutant ooDSGCs measured at 3 contrasts (10, 60 & 150% Weber contrast). Circles show responses, solid lines show von Mises fits. Mutant tuning curves are broader than controls at all three contrasts.
B: Preferred directions of WT ooDSGCs (left) align to the four cardinal ocular axes: superior, inferior, anterior and posterior (Oyster and Barlow, 1967). K-means clustering was used to separate the recorded ooDSGC population into these four subtypes (see Methods). Population mean (solid line) and standard deviation (shaded region) of preferred directions for each subtype is plotted; circles denote preferred direction of individual ooDSGCs. Preferred directions of Megf10−/− ooDSGCs (right) were also aligned to the cardinal axes, and there was no appreciable change in the fraction of ooDSGCs populating each subtype.

C-E: Cumulative distributions of tuning width (quantified by circular standard deviation; C), tuning strength (D), and ON-OFF preferred direction difference (E), measured at different bar contrasts (identified at the top of each plot) for WT and Megf10−/− ooDSGC populations. Insets (D) show responses to null direction stimuli. The analyzed RGC populations were the same as for data shown in Fig. 10 (n = 80 WT and 74 mutant ooDSGCs, two retinas each genotype). The width and speed of the moving bar was 1200 µm and 550 µm/sec, respectively. Error bars/bands, S.E.M.