MEGF10 and MEGF11 mediate homotypic interactions required for mosaic spacing of retinal neurons

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In many parts of the nervous system, neuronal somata display orderly spatial arrangements. In the retina, neurons of numerous individual subtypes form regular arrays called mosaics: they are less likely to be near neighbours of the same subtype than would occur by chance, resulting in ‘exclusion zones’ that separate them. Mosaic arrangements provide a mechanism to distribute them evenly across the retina, ensuring that all parts of the visual field have access to a full set of processing elements. Remarkably, mosaics are independent of each other: although a neuron of one subtype is unlikely to be adjacent to another of the same subtype, there is no restriction on its spatial relationship to neighbouring neurons of other subtypes. This independence has led to the hypothesis that molecular cues expressed by specific subtypes pattern mosaics by mediating homotypic (within-subtype) short-range repulsive interactions. So far, however, no molecules have been identified that show such activity, so this hypothesis remains untested. Here we demonstrate in mouse that two related transmembrane proteins, MEGF10 and MEGF11, have critical roles in the formation of mosaics by two retinal interneuron subtypes, starburst amacrine cells and horizontal cells. MEGF10 and 11 and their invertebrate relatives Caenorhabditis elegans CED-1 and Drosophila Draper have hitherto been studied primarily as receptors necessary for engulfment of debris following apoptosis or axonal injury. Our results demonstrate that members of this gene family can also serve as subtype-specific ligands that pattern neuronal arrays.

The retina contains over 70 neuronal subtypes, divided into broad categories of photoreceptors, interneurons and retinal ganglion cells. To seek molecules involved in cell–cell recognition events during retinal circuit assembly, we purified 13 subtypes of retinal neurons from transgenic mice and used microarrays to inventory the genes required for mosaic spacing of retinal neurons. Among bipolar, amacrine and ganglion cells, MEGF10 and 11 have been identified that show such activity, so this hypothesis remains untested. Here we demonstrate in mouse that two related transmembrane proteins, MEGF10 and MEGF11, have critical roles in the formation of mosaics by two retinal interneuron subtypes, starburst amacrine cells and horizontal cells. MEGF10 and 11 and their invertebrate relatives Caenorhabditis elegans CED-1 and Drosophila Draper have hitherto been studied primarily as receptors necessary for engulfment of debris following apoptosis or axonal injury. Our results demonstrate that members of this gene family can also serve as subtype-specific ligands that pattern neuronal arrays.

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The retina contains over 70 neuronal subtypes, divided into broad categories of photoreceptors, interneurons and retinal ganglion cells. To seek molecules involved in cell–cell recognition events during retinal circuit assembly, we purified 13 subtypes of retinal neurons from transgenic mice and used microarrays to inventory the genes they expressed. We collected cells at postnatal day (P)6, a time at which synapse formation and mosaic refinement are underway. From this data set we identified genes selectively expressed by specific subtypes, including starburst amacrine cells (SACs), an interneuronal subtype that has critical roles in motion detection. The approximately 100 genes that met our criteria for selective SAC expression included most known SAC markers as well as potential new markers (Fig. 1a and Supplementary Table 1). Thirty-one of the novel genes were strongly expressed in SACs (Fig. 1a–c). Both were also expressed in horizontal cells (HCs), which were not part of the data set. SACs are present in both the inner nuclear and ganglion cell layers, and form independent mosaics in each. These mosaics develop during late embryonic stages, as newborn SACs migrate from the site of their birth, the outer neuroblast layer, to their final laminar locations. SACs begin to exhibit mosaic spacing upon arrival at their destinations, presumably due to contacts with their homotypic neighbours. As SACs develop, the outer neuroblast layer, to their final laminar locations. SACs begin to exhibit mosaic spacing upon arrival at their destinations, presumably due to contacts with their homotypic neighbours. As SACs develop, the outer neuroblast layer, to their final laminar locations. SACs begin to exhibit mosaic spacing upon arrival at their destinations, presumably due to contacts with their homotypic neighbours.
new SACs are added to the array, local cellular rearrangements maintain mosaic spacing\textsuperscript{1,4,9}. Mosaic spacing is maintained even as SAC dendrites grow to overlap with those of their neighbours\textsuperscript{9,10}; thus, mosaicism is distinct from the phenomenon called tiling, which minimizes dendritic overlap\textsuperscript{15}. Co-staining at embryonic day (E)16 with the early SAC marker islet 1 (refs 9, 21) demonstrated that newborn SACs activated Megf10 expression as they finished their migration and became integrated into mosaics (Fig. 1d). Megf10 expression persisted in SACs from E16 to the first postnatal week, and began in HCs at P0 (Supplementary Fig. 1 and data not shown). In the second postnatal week, Megf10 was downregulated in neurons but appeared in Müller glia (Fig. 1e), consistent with previous reports that Megf10 is expressed by brain glia\textsuperscript{22}. To determine the subcellular localization of MEGF10 in neurons, we generated an antibody to recombinant protein (Supplementary Fig. 1). MEGF10 was present both on the somata of SACs and HCs and on their processes (Fig. 1f and Supplementary Fig. 1). As expected, immunoreactivity levels on SACs and HCs were highest during the first postnatal week, and then declined (data not shown). Thus, Megf10 is expressed by SACs and MEGF10 protein is present on SAC processes during the time that mosaic forms. Because Megf11 expression was not observed in retina until after SAC mosaics had formed (see later), we focused first on Megf10.

To investigate whether MEGF10 is required for SAC development, we generated mutant mice (Supplementary Fig. 2). Megf10 mutants were viable and fertile and their retinas exhibited no gross abnormalities.

Mutant SACs migrated to the inner nuclear and ganglion cell layers as in controls, they were present in normal numbers, and their dendrites projected to appropriate sublaminae of the inner plexiform layer (Fig. 2a–c). Examination of whole mounts showed, however, a marked loss of regular spacing among SAC somata, suggesting that their mosaic arrangement had been disrupted (Fig. 2a and Supplementary Fig. 3).

To assess the degree to which SAC mosaics were disrupted, we measured the exclusion zone—the region surrounding each SAC in which other SACs are rarely found. This parameter is calculated from the density recovery profile, a plot of cell density as a function of distance from each SAC in the array\textsuperscript{23} (Fig. 2d). The SAC exclusion zone in mutants was smaller than in wild-type littermates, and was approximately equal to the diameter of a SAC soma (Fig. 2e). As the only limitation on proximity was soma size, SACs seem to be positioned randomly in Megf10 mutants. This conclusion was supported by two additional measurements of spatial order: the packing factor\textsuperscript{23}, another index of regularity calculated from the density recovery profile; and an independent measure, the Voronoi domain regularity index, which quantifies variations in the area of the territories nearest to each cell in an array\textsuperscript{20,24}. In each case the index calculated for SAC arrays in Megf10 mutants was similar to that measured for computer-generated random arrays, whereas SAC arrays from wild-type littermates were highly ordered (Fig. 2f, g); heterozygotes showed a mild disorganization (Supplementary Fig. 4). Together these results suggest that MEGF10 acts in SACs to impose a minimal intercellular spacing: in

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\caption{Loss of SAC mosaic spacing in Megf10 mutant mice. a, SAC mosaic in inner nuclear layer (INL) of wild-type (left) and Megf10\textsuperscript{–/–} (centre) retina, revealed by whole-mount staining with anti-ChAT. Wild-type mice have evenly spaced SAC somata, whereas mutants exhibit clumps and gaps similar to those seen in a simulation of a random cellular array (right). See Supplementary Fig. 3 for similar results in ganglion cell layer (GCL) SACs. b, ChAT-stained retinal sections from wild-type and Megf10 mutant animals. Laminar positions of SAC somata and processes are normal in mutants, even in regions where soma are clumped. c, Density of SACs and VGLUT3 amacrine cells is similar in wild-type (+/+) and Megf10 mutant (–/–) retina. d, Density recovery profiles (DRPs) for the SAC (INL) and VGLUT3 amacrine arrays. Graphs show the density of cells in a ring of radius x, relative to the density of cells in the image as a whole. Dashed line, DRP of random point array. The exclusion zone characteristic of mosaic spacing is measured as a dip below this line. e, Exclusion zone radius measured from d. Dashed line, expected result for an array of cells distributed randomly; that is, the diameter of a single cell. Increases above this minimum indicate spatial order. The mutant SAC exclusion zone radius was similar in size to a SAC cell diameter, and was significantly smaller than wild type (*P < 0.0001). VGLUT3\textsuperscript{–/–} amacrine exclusion zones were unaffected. f, g, SAC packing factor (f) and Voronoi domain regularity index (g) were significantly lower in Megf10\textsuperscript{–/–} mice than in wild-type littermates (*P < 0.0001). Dashed line, mean for arrays of cells distributed randomly. Wild-type SACs and VGLUT3\textsuperscript{–/–} cells of both genotypes were non-randomly arrayed. The SAC array in mutants was not significantly different from random arrays (f, P = 0.16; g, P = 0.49). h, Morphology of single GCL SACs, labelled with adeno-associated virus driving membrane-targeted Cherry fluorescent protein, showed no gross abnormalities in Megf10 mutants (n ≥ 8 cells each genotype). Data from P15 (a–g) or P80 (h) mice. Scale bars, 50 µm. Error bars, s.e.m.}
\end{figure}
its absence SACs assume random positions relative to each other. By contrast, other amacrine cell types examined (VGLUT3- (also known as SLC17A8) and tyrosine hydroxylase-positive) as well as bistratified direction-selective retinal ganglion cells, which are prominent synaptic targets of SACs44, showed normal mosaic spacing (Fig. 2d–g and Supplementary Fig. 3).

In mice lacking the adhesion molecules DSCAM or DSCAML1, certain retinal cell mosaics form normally, then degrade secondary to hyperfasciculation of their neurites25,26. To investigate whether MEGF10 acts in a similar indirect fashion, we labelled individual SACs in mutants. Lack of MEGF10 had no obvious effect on SAC dendritic morphology (Fig. 2h). Moreover, soma disorganization was already evident by P0 (Supplementary Fig. 5), shortly after mosaics form, indicating that MEGF10 affected formation of SAC mosaics.

Next we asked whether MEGF10 is also essential for formation of the HC mosaic. Loss of MEGF10 had only a modest effect on HC regularity (Fig. 3). We therefore considered that MEGF10 might have a redundant role. Megf11 was not expressed in embryonic retina, but it appeared in HCs and SACs during the first postnatal week and persisted into adulthood (Fig. 3a–d). Importantly, SAC mosaics begin to form before P0, but HC mosaics are established postnatally4,6,9. We therefore generated Megf11 mutant mice (Supplementary Fig. 2), which, like Megf10 mutants, showed no gross retinal abnormalities (Supplementary Fig. 6). Examination of HC arrays in Megf11 mutants revealed a modest decrease in regularity similar to that in Megf10 mutants, whereas in Megf10−/−; Megf11−/− double-mutant animals, the HC mosaic was severely disrupted (Fig. 3e–m and Supplementary Fig. 7). By contrast, SACs were unaffected by loss of Megf11 function, and were no more affected in double mutants than in Megf10 single mutants (Supplementary Figs 6 and 8). Thus, Megf11 is dispensable for SAC arrangement, but acts together with Megf10 to shape the HC mosaic.

To elucidate the cellular mechanisms by which Megf10 and Megf11 act, we used a gain-of-function approach in which we introduced MEGF10 into the retina by electroporation of plasmid DNA at P0. We first tested the hypothesis that MEGF10 can act as a signal that repels SACs, creating the exclusion zone that defines mosaic spacing. The electroporation method predominantly transfects dividing cells, leading to expression in neurons that exit the cell cycle postnatally, such as bipolar cells, Müller glia, photoreceptors and late-born amacrine cells27. Because SACs and HCs are born embryonically, they are rarely transduced25, allowing us to surround wild-type SACs and HCs with cells ectopically expressing excess MEGF10. Indeed, electroporation of plasmid encoding a fluorescent protein (FP) or a MEGF10–FP fusion produced retinal patches in which a large fraction of neurons, but no SACs or HCs, were FP+ (Fig. 4a and Supplementary Fig. 9). Expression of MEGF10–FP (but not FP alone) resulted in exclusion of SACs and HCs from a swath at the edge of the electroporated patch, whereas spacing of these cells was essentially normal in patch centres (Fig. 4a, b and Supplementary Fig. 10). A truncated MEGF10 lacking the cytoplasmic domain produced an identical phenotype (Supplementary Fig. 10), ruling out the possibility that MEGF10 acts indirectly by triggering production of a repellent factor by the transfected cells. Thus, MEGF10 can act as a ligand that signals to SACs and HCs.

Because MEGF10 relatives have been implicated in cell engulfment10–13, we asked whether its overexpression formed an exclusion zone by eliminating cells. We found no evidence for cell death at patch edges (no pyknotic nuclei or activated caspase-3 immunoreactivity; data not shown). Instead, cell density was increased at the outer edge of the cell-free swath, indicating that SACs had exited the patch to create the

Figure 3 | HC mosaic spacing requires Megf10 and Megf11. a–d, In situ hybridization for Megf11 at ages indicated. Megf11 (red) was not expressed at E16 (a). Calbindin immunostaining (green) labels SACs and HCs, which co-express Megf11 at P5 (b), P7 (c) and P14 (d). See Fig. 1 for abbreviations. e–i, Retinal whole mounts stained for calbindin to reveal the HC array. In wild-type mice (e), HCs are distributed evenly. Megf10/−/− mutants (f) and Megf11/−/− mutants (g) show subtle changes in the regular spacing of HCs, whereas double Megf10/−/−; Megf11/−/− mutants (h) show marked HC disorganization similar to a simulation of a random HC array (i). j–m, Quantification of HC spacing regularity in Megf10/−/− (red) or Megf11/−/− (green) single mutants; Megf10/−/−; Megf11/−/− double mutants (blue); and wild-type (WT) siblings (grey). In all genotypes, HCs were present at normal density (j) but were less regularly spaced relative to wild type based on exclusion zone radius (k), packing factor (l) and Voronoi domain regularity (m) measurements as in Fig. 2. Double mutants showed significantly less order than single Megf10/−/− or Megf11/−/− mutants and approach random arrangement, indicated by dashed lines (k, mean HC soma diameter; l, computed values for random arrays). P values: *P < 0.01, **P < 0.001, ***P < 0.0001. NS, not significant. Error bars give s.e.m. Data in j–m from P15 animals. Scale bars, 20 μm (a–d, b, c share scale) or 50 μm (e–i).
MEGF10 acts as both ligand and receptor to trigger SAC repulsion. a, A retinal patch transfected by electroporation with plasmid encoding MEGF10–FP fusion protein, viewed in flat mount. SAC somata in INL, stained with anti-ChAT, are excluded from a swath at the patch edge. SACs are evenly spaced elsewhere, except where the retina was pierced to inject DNA (Inj. site). b, Higher-magnification views of patch edges. FP misexpression (left) did not affect SAC spacing, but MEGF10–FP (right) produced a SAC-free zone just inside the transfected region and induced apparent crowding of SACs immediately outside it (arrows). Dashed line, patch edge. See Supplementary Fig. 11 for quantification of cell distribution at patch edges. c, Hypothesis for MEGF10 function based on a, b. In wild-type retina SAC-free zone (Fig. 4b and Supplementary Fig. 11). Our interpretation is that SACs and HCs are repelled by MEGF10, but that within MEGF10–FP patches the concentration of MEGF10 is similar in all directions, resulting in no net movement (Fig. 4c). Consistent with this view, SACs and HCs were entirely excluded from small patches (≤5–6 cell diameters; Supplementary Fig. 10). Together these results suggest that MEGF10 can act as a repellent ligand.

We next asked which cells are sensitive to MEGF10 ligand. In Megf10 mutants, SACs and HCs are selectively affected; this might be because they are uniquely sensitive to MEGF10 or because only these cells encounter endogenous MEGF10 at high concentration. Ectopic expression allowed us to distinguish between these possibilities. None of 13 other amacrine, bipolar or retinal ganglion cell subtypes assayed with cell-type-specific markers was detectably affected by MEGF10–FP (Fig. 4d and Supplementary Table 3). Thus, MEGF10 appears to act as a cell-surface ligand for a receptor specifically expressed by SACs and HCs.

This led us to ask whether MEGF10 might be the MEGF10 receptor. We used cultured epithelial cells (HEK293) to seek evidence for a MEGF10-dependent homotypic interaction. Whereas FP-expressing cells overlapped, MEGF10–FP–expressing cells formed sharp borders with narrow gaps (Supplementary Fig. 12a), consistent with previous results14. This ‘jigsaw’ pattern reflected suppression or elimination of filopodia at points of intercellular apposition (Supplementary Fig. 12b). Jigsaw formation and loss of filopodia required the MEGF10 cytoplasmic domain and did not occur when a MEGF10–FP-expressing cell contacted an untransfected or FP-transfected cell (Supplementary Fig. 12b, c). Although we have been unable to demonstrate MEGF10 homophilic binding using biochemical methods (data not shown), these results suggest that a MEGF10-containing signalling complex mediates a homotypic interaction resulting in intercellular repulsion.

Finally, we tested the idea that MEGF10 serves as both ligand and receptor in vivo. To this end, we electroporated MEGF10 into Megf10 mutant retina. Mutant SACs did not exit MEGF10–FP patches (Fig. 4e and Supplementary Fig. 11b), indicating that Megf10 gene function is required for SACs to respond to MEGF10 repulsive signals. Together these results suggest a model in which SACs and HCs use MEGF10 as part of a receptor complex that detects MEGF10 on their homotypic neighbours. This repulsive signal positions their somata so as to equalize MEGF10 signals on all sides, thereby creating exclusion zones (Fig. 4c).

The phenomenon of retinal mosaic implies a molecular system for cell-type-specific recognition. Several potential mechanisms have been proposed, based on imaging and computational studies, one being that short-range repulsive signals regulate tangential movements to establish each cell’s territory3,4,6–9. So far, however, direct mediators of this phenomenon have not been described. Here we provide evidence that, for SACs and HCs, mosaic spacing requires repellent homotypic interactions mediated by MEGF10 and 11. Signals initiated by MEGF10/11 in growing neurites could lead to repositioning of the soma within the cytoplasm, perhaps by mechanisms resembling those that direct soma translocation in response to signals at the leading process of migrating neurons28. The finding that MEGF10 and 11 facilitate formation of three independent mosaics (two for SACs and one for HCs) appears at first to contradict the idea that mosaics are independent of each other and must therefore be regulated by distinct molecules3,4,6–9. However, because the three mosaics occupy distinct cellular planes, they may be exempt from the requirement for molecular individuation. Different molecules are likely to mediate homotypic interactions in other retinal subtypes, some of which may lead to soma translocation and others to the death of cells that violate minimal spacing44.

We note two broader implications of our results. First, Draper/ced-1/MEGF10 homologues have until now been studied predominantly as receptors for cell engulfment10–14. Here we show that they also mediate cell–cell repulsion and can act as ligands as well as receptors, thereby expanding the roles for this gene family. Second, although mosaic arrangements have so far been studied formally only in retina, regularly arranged neuronal arrays are common features of central neural
organization. Mechanisms similar to those described here could be involved in promoting this regularity.

**METHODS SUMMARY**

Retinal neurons expressing fluorescent proteins were purified and used to generate amplified RNA for hybridizing Affymetrix microarrays as described previously. SAC-specific genes were identified using dChip software. Megf10 and Megf11 mutant mice were produced from constructs generated by the Knockout Mouse Project and European Conditional Mouse Mutagenesis program. Histological methods and methods for electroporation of plasmid DNA in vivo were described previously. HEK293 cells (ATCC) were cultured and transfected using standard methods.

For analysis of spatial statistics, we sampled a 635.9 µm square at 3–4 locations per retina. X–Y cell coordinates, marked manually, were used to calculate Voronoi domain areas (Fiji) or DRP statistics such as the effective radius (that is, exclusion zone) and packing factor (WinDRP). We generated random arrays matched in density and soma size to real data; these were analysed in parallel with data from mutants. For measurement of SAC crowding in gain-of-function experiments, the Delaunay triangulation was used to define each cell’s nearest neighbours.

**Full Methods** and any associated references are available in the online version of the paper at www.nature.com/nature.

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**Supplementary Information** is linked to the online version of the paper at www.nature.com/nature.

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**Author Contributions** J.N.K. and J.R.S. designed experiments and wrote the paper. J.N.K. and M.W.C. performed experiments. J.R.S. performed data analysis. J.R.S. supervised the project.

**Author Information** Data have been deposited at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession code GSE35077. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to J.R.S. (sanesj@mcc.h.harvard.edu).
METHODS

Animals. CD1 and C57BL/6 mice were obtained from Charles River and Jackson Labs. All experiments were carried out in accordance with protocols approved by the Harvard University Standing Committee on the Use of Animals in Research and Teaching.

Megf10 mutant mice were produced from a construct provided by the Knockout Mouse Project (CHORI). Embryonic stem (ES) cell electroporation and chimera production were performed by the Harvard Genome Modification Facility. ES cell clones were screened for integration by PCR using primers listed in Supplementary Table 4 (also see Supplementary Fig. 2). Megf11 mutant mice were generated from gene-targeted ES cells provided by the European Conditional Mutagenesis program (EUCOMM). In both cases germline transmission was obtained from two chimaeric mice generated from independent ES cell clones; each had indistinguishable phenotypes. The primers used for genotyping Megf10 and Megf11 mice are listed in Supplementary Table 4. Dvl3-GFP mice were obtained from the Mutant Mouse Regional Resource Center.

Cell sorting and expression profiling. Retinal neurons expressing fluorescent proteins were purified as described33–37. Briefly, P6 retinas were dissociated, live-stained with antibodies recognizing cell surface antigens (if required for purification), and then passed through a flow cytometer (Mo Flo; Dako). Positive cells were then either: (1) plated, fixed, stained with cell-type-specific markers and counted after dye-purification; or (2) sorted directly into RNA lysis buffer (Picotorp Kit; MDS). Gene expression was profiled using Affymetrix Mouse 430 2.0 arrays, following two rounds of linear amplification (MessageAmp II; Applied Biosystems). Using these methods we generated a gene-expression database for 13 specific retinal neuron subtypes (five amacrine, two bipolar and six retinal ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2). SAC-specific genes (Supplementary Table 1) were identified using dChip software using fold-change over other ganglion cell types; see Supplementary Table 2) were then calculated as described above, and the means were plotted as dashed lines in Figs 2 and 3. The VDRI for these random simulations were similar to those calculated previously35.

For analysis of SAC cell position in MEGF10-misexpressing retina, we acquired images at the edge of FP+ or MEGF10–FP− misexpressing patches, and used the above method to obtain X–Y coordinates of all SACs in the field of view. SACs inside the patch, outside the patch, and at the edge of the patch were marked separately. 'Edge' cells were defined as those outside the FP region for which the shortest line drawn from that cell to the edge of the FP region did not pass the soma of another SAC. To ask whether SACs were present at higher density at patch edges, we calculated the distance from each SAC to its nearest neighbours. Neighbours were defined in an unbiased manner by computing the Delaunay triangulation for the X–Y location data set (Fiji), thereby defining line segments from each cell to its nearest neighbours.

Statistical analysis. For analysis of exclusion zone radius, packing factor, VDRI and cell density, the significance of measured differences between genotypes was evaluated by the Mann–Whitney U test. Sample sizes were ≥3 animals for ≥3 retinas per genotype (SAC and HC analysis) or ≥6 images for ≥2 retinas per genotype (VGLUT3 analysis). In double-mutant experiments on HCs, the Holm–Bonferroni procedure for multiple comparisons (four different genotypes) was applied when determining significance level. For measurement of soma diameter, sample size was ≥150 cells for each genotype.

Gain-of-function experiments. A Megf10 expression construct was generated by PCR amplifying the open reading frame (ORF), with stop codon deleted, from the wild-type sequence. Programitica software was used to generate the simulations (n = 10 for each cell type). Packing factor and VDRI were then calculated as described above, and the means were plotted as dashed lines in Figs 2 and 3. The VDRIs for these random simulations were similar to those calculated previously35.

For analysis of SAC cell position in MEGF10-misexpressing retina, we acquired images at the edge of FP− or MEGF10–FP+ misexpressing patches, and used the above method to obtain X–Y coordinates of all SACs in the field of view. SACs inside the patch, outside the patch, and at the edge of the patch were marked separately. 'Edge' cells were defined as those outside the FP region for which the shortest line drawn from that cell to the edge of the FP region did not pass the soma of another SAC. To ask whether SACs were present at higher density at patch edges, we calculated the distance from each SAC to its nearest neighbours. Neighbours were defined in an unbiased manner by computing the Delaunay triangulation for the X–Y location data set (Fiji), thereby defining line segments from each cell to its nearest neighbours.

Statistical analysis. For analysis of exclusion zone radius, packing factor, VDRI and cell density, the significance of measured differences between genotypes was evaluated by the Mann–Whitney U test. Sample sizes were ≥3 animals for ≥3 retinas per genotype (SAC and HC analysis) or ≥6 images for ≥2 retinas per genotype (VGLUT3 analysis). In double-mutant experiments on HCs, the Holm–Bonferroni procedure for multiple comparisons (four different genotypes) was applied when determining significance level. For measurement of soma diameter, sample size was ≥150 cells for each genotype.

Gain-of-function experiments. A Megf10 expression construct was generated by PCR amplifying the open reading frame (ORF), with stop codon deleted, from the IMAGE clone described earlier. The open reading frame, which was predicted to encode a protein equivalent to the Megf10 ReSeq sequence (NP_115822.1), was then cloned into the Gateway entry vector pCR8Gw-Topo (Invitrogen). Primers used for cloning were CAGTTGTTCTCTGACAGAATGCCG and TGTATGACTCTGCTGCTC. A cytoplasmic domain-deleting construct was made by replacing the TGTATGACTCTGCTGCTC to generate a truncated protein carrying only the first nine amino acids of the intracellular domain. For expression, these constructs were transferred to a Gateway destination vector bearing the ubiquitin-C promoter and an in-frame C-terminal GFP or monomeric Cherry tag. Mouse Megf11 was also cloned, but pilot experiments in HEK cells suggested a lack of surface expression, so we did not attempt in vivo experiments with mouse Megf11.

In vivo electroporation was performed as described12–17. Briefly, plasmid DNA (at least 1.5 mg ml−1) was injected into the subretinal space of neonatal mice (4–36 h postpartum), and current pulses (80 V) were applied across the head using paddle electrodes. We obtained identical results in MEGF10–GFP and MEGF10–Cherry misexpression experiments. Sample sizes were ≥20 animals each for MEGF10 misexpression and FP controls.

HEK 293 cells were cultured in DMEM with 10% fetal calf serum and transfected using TransIT reagent (Mirus). Cells were counterstained using Alexa Fluor.
dye-labelled cholera toxin B subunit (10 μg ml⁻¹; Invitrogen), added to media 30 min before fixation (4% paraformaldehyde/1× PBS for 20 min on ice).

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