Comparative single-cell transcriptomic analysis reveals key differentiation drivers and the potential origin of the vertebrate retina

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Abstract

Background: The retina plays a vital role in capturing and processing visual information in vertebrates, allowing them to adapt to diverse environments. Single-cell expression profiles of the vertebrate retina have been described; however, a deeper understanding of the expression patterns in the context of development and evolution among homologous cell types is lacking. To identify such shared patterns, we examined and compared approximately 230,000 retinal cells from three species: mouse, chicken, and zebrafish.

Results: We found significant similarities among homologous cell types, indicating inherent regulatory patterns. To understand these shared patterns, we constructed gene regulatory networks for each developmental stage for the three species. We identified 690 regulons with 381 regulators across the three species. In addition, we identified 10 common cell type-specific regulators and 16 preserved regulons. Using RNA velocity analysis, we pinpointed conserved driver genes key to retinal cell differentiation in both mouse and zebrafish, and by intersecting regulators, we extracted the crucial regulators that facilitate these differentiation processes. Finally, investigation of the potential origins of photoreceptors and retinal ganglion cells by examining conserved expression patterns among the three vertebrate species and the invertebrate *Ciona intestinalis* revealed functional similarities in light transduction mechanisms between *Ciona* photoreceptor-related cells and vertebrate retinal cells.

Conclusions: Our findings offer insights into the conserved regulatory frameworks intrinsic, evolutionarily preserved differentiation programs, and ancestral origins of vertebrate retinal cells.

Keywords: Comparative cross-species transcriptomics, Gene regulatory network, RNA

velocity, Evolutionary origin, Retina, Single-cell transcriptomics

Background

The retina acts as the primary sensory interface between the visual system and the external world and is crucial for vertebrate adaptation to varied ecological niches. Because of its structural complexity and accessibility, the retina is a pivotal model for studying the intricate functions of the central nervous system (CNS) [1]. For example, the presence of β-amyloid in both Alzheimer's and age-related macular degeneration not only underscores the interconnected pathways of the retina and CNS, but also highlights the critical role of retina research in understanding and potentially treating CNS disorders [2]. The vertebrate retina comprises one embryonic retinal progenitor cell (RPC) and seven mature cell types that collaborate in visual information processing: rod and cone photoreceptors (PRs), bipolar cells (BCs), horizontal cell (HCs), amacrine cells (ACs), retinal ganglion cells (RGCs) and müller glia (MG) [3]. Notably, certain developmental phases in the zebrafish retina feature ciliary marginal zone (CMZ) progenitors or retinal stem cells (RSCs) akin to RPCs [4]. Unraveling the intricate regulatory mechanisms of the vertebrate retina has been challenging because of the high cellular heterogeneity and the various patterns of cell body distributions and connectivity [5].

The rapid advancement of single-cell RNA-seq (scRNA-seq) has enabled the detailed profiling of thousands of retinal cells, revealing molecular features of diverse cell (sub)types in model organisms such as *Mus musculus* (mouse) [6], *Gallus gallus* (chicken) [7], and *Danio rerio* (zebrafish) [8]. These studies have deepened our understanding of cellular identities within the vertebrate retina. Although the transcriptomes of retinal cells appear similar between mammals and birds [7], the limited scope of the study, particularly the exclusion of fish retinal cells, suggests a need

for a more comprehensive analysis across vertebrates. In addition, while the focus of many studies has been on identifying marker genes in these species, the analysis of their wider regulatory networks remains largely unexplored.

Recently, methods such as SCENIC [9], which predict gene regulatory networks based on co-expression and transcription factor binding information, have been developed to uncover the regulatory networks that provide more comprehensive insights than marker genes alone. While these approaches have been instrumental in exploring conserved regulatory programs across various species and tissues [10,11], a thorough understanding of the intricate and conserved regulatory networks in the vertebrate retinal cells is still an area awaiting further exploration. Furthermore, identifying genes driving retinal cell differentiation is crucial in understanding retinal development. While studies have been conducted to study retinal differentiation in mouse and zebrafish using methods based on trajectory inference [6,8], these methods often struggle to accurately capture the dynamic and complex nature of cellular changes over time [12], limiting the inference of cell type-specific driver genes. In recent developments, methods based on RNA velocity, which model the ratio of unspliced and spliced RNAs, have emerged as more comprehensive tools for capturing information driving differentiation [13,14]. Identifying conserved driver genes underlying vertebrate retinal differentiation using these methods is vital for advancing our understanding of broader mechanisms in retinal development.

In addition, considering the complexity of vertebrate retina, exploring corresponding cell types in simpler invertebrate organisms offers a feasible method to grasp the fundamental regulatory mechanisms of the vertebrate retina. *Ciona intestinalis* Type A (*Ciona*), one of the closest relatives of vertebrates, featuring a relatively simplistic nervous system and visual structures, is a fitting model to

investigate the evolutionary origins of the vertebrate retina [15–17]. Although a previous study suggested that dopamine-synthesizing cells that are located close to *Ciona* photoreceptors have some vertebrate AC characteristics [18], an in-depth exploration of shared expression patterns between vertebrate retina and *Ciona* PR-related cells is lacking.

To bridge these gaps, we systematically investigate the transcriptional profiles of vertebrate retinal cells from three representative species: mouse, chicken, and zebrafish [6–8], focusing on conserved gene regulatory networks (GRNs) and key driver genes for retinal cell differentiation. We also extend our analysis by comparing these profiles with the invertebrate *Ciona* [19] to trace the potential origins of retinal cells.

Results

Overview of the study strategy

We collected and annotated scRNA-seq data from GEO database (Fig. 1a). We initially focused on assessing the conservation of transcriptomes in vertebrate retinal cells across different species (Fig. 1b). Subsequently, we applied SCENIC [9] to discern cell-type-specific regulatory networks within each species (Fig. 1c). We thus identified conserved regulators and regulons across vertebrates, elucidating evolutionarily preserved regulatory frameworks. In addition, we utilized RNA velocity analysis to identify conserved driver genes that propel retinal cell differentiation in both mouse and zebrafish (Fig. 1d) and extracted the regulators that drive differentiation by intersecting regulatory networks (Fig. 1e). Finally, we collected scRNA-seq data from *Ciona* (Fig. 1f), identified shared differentially expressed genes (DEGs) between vertebrate retinal cells and *Ciona* photoreceptor-related cells (Fig. 1g), and inferred the potential origin of vertebrate retinal cells.

Comparison of vertebrate retinal cell landscapes

We downloaded scRNA-seq data of retinal cells for the three species from the GEO database (see Methods). Across the three species, we collected data obtained at 18 different time points (Fig. 2a-f). In total, 230,000 cells were retained for subsequent analysis, including 100,506 mouse cells (Fig. 2a), 86,056 chicken cells (Fig. 2b), and 49,311 zebrafish cells (Fig. 2c). The data from all three species comprised seven mature cell types distributed over stages. In the mouse data, RPCs were divided into three categories: early RPCs, late RPCs, and neurogenic cells, representing different states of RPCs. RGCs and HCs were predominantly represented in the data from the early stages of retinal development during Embryonic day 11 to 16 (E11–E16). ACs, cones, and rods were more represented in the intermediate stages during E18 to postnatal day 2 (E18–P2), and BCs and MG in the late stages (P5–P14, Fig. 2d). Chicken data at Embryonic day 18 (E18) included all mature cell types except RGCs, which were represented only in the E16 stage. Cells at E12 represent the early stages of cell development (Fig. 2e). Zebrafish data from at 24 hours post-fertilization (hpf) and 36 hpf comprised of RPCs and RGCs, and the data at the 72 hpf stage included all retinal cell types. Data at 48 hpf and 14 days post-fertilization (dpf) represented newly born retinal cells from different regions. It is worth noting that it has been reported that the transcriptome profiles and functions of RPCs and CMZ progenitors are largely congruent [8]. Therefore, we categorized CMZ progenitor cells as RPCs (Fig. 2f).

To comprehensively evaluate transcriptome conservation across species, we systematically compared cell similarities among the three species included in this study (see Methods). The heatmap (Fig. 2g) revealed that the overall gene expression patterns in vertebrate retinal

homologous cell types were conserved, indicating that individual cell types displayed less variation in their transcriptome profiles during evolution than different cell types within the same species. All MG shared similar expression patterns with RPCs, indicating their ability to differentiate into other retinal cell types [20]. Cones and rods shared more similar transcriptional profiles because of their common role in converting light signals to neural signals [21]. Additionally, ACs, HCs, and RGCs demonstrated closer relationships than other cell types, indicating they share similar expression patterns. Unsupervised clustering of integrated pseudo-cell data revealed six major clusters (Fig. 2h, see Methods). Each cell type was confined to a specific cluster, and almost all the homologous cell types were grouped into one cluster, indicating the similarity of expression profiles among homologous cell types (Fig. 2i).

Identification and reconstruction of gene regulatory networks (GRNs) in vertebrate retina cells across species

To construct the GRNs for cells from the three species, we first built the TF-motif ranking database for chicken and zebrafish (see Methods). Then, we estimated the GRNs for cell types from each developmental stage from each species using the pySCENIC pipeline [22] (see Methods). Approximately 2700 regulons were inferred from the 18 datasets. We calculated the regulon specificity scores for each regulon and extracted the top 20 cell type-specific regulons. We then removed low-quality regulons whose regulators were not specifically expressed in one cell type. Ultimately, 690 cell type-specific regulons were retained for further analysis (Fig. 3a, Additional file 2: Table S1-3). We identified numerous regulators in various cell types across different stages (Additional file 1: Fig. S1a–g, Fig. S2a, Fig. S3a–d). Furthermore, we reconstructed GRNs for

representative cell types in each stage (Additional file 1: Fig. S1h−q, Fig. S2b−g, Fig. S3e−m), providing detailed maps of the complex interactions among regulators that orchestrate retinal development and function. To provide a focused discussion, we have selected one representative stage from each species for an in-depth analysis.

In our study focused on E18 for mouse, we identified both well-established and novel cell type-specific regulons (Fig. 3b). Notably, Sox9 and Hmga2 were active in late RPCs [23,24]. In neurogenic cells, Atoh7 was verified to be active [25], whereas Tfdp1, Olig2, and Dlx1 appeared to have novel regulatory functions. Gbx2 was particularly noted in a subtype of gamma-aminobutyric acid (GABA)ergic ACs [26], and Barhl2 [27] was identified as an active regulator in ACs and RGCs. We identified several regulators (i.e., Irf5, Lhx5, Pitx1, Pou3f1, Dlx1, and Mafb) that were specifically active in glycine ACs. Dlx1, essential for RGCs development [28], was also active in glycine ACs. Established regulators Isl2 and Rxrg were observed in RGCs and PRs [29,30], with Zfp444 and Lcorl as potential key regulators in PRs.

At the E18 stage in chicken, we identified known and novel retinal cell regulators (Fig. 3c). PAX6 was identified in AC [31]. In BCs, SOX6, DMBX1, and LHX3 were identified as potential active regulators. HMX3 was found in HCs and MEOX2 in PRs. The known regulator THRB[32] was identified in cones, and VAX1 in rods. In MG, mouse homologs SOX9 and NR2E1 were verified [23,33], with SMAD5 and PAX2 as potential regulators.

Our analysis of retinal cell types at 72 hpf in zebrafish (Fig. 3d) validated known regulators and identified novel ones. Key findings include: tcf7l1a [34] and sox9b [35] were found in RPCs along with the novel regulator, thap12a; in MG, sox10 and jdp2b were identified as potential novel regulators. In RGCs, our data suggested activity for the novel regulators sox6 and tbr1b, and

corroborated klf7a [36]. We identified roraa as a potential active regulator across all cell types examined. In ACs and RGCs, pbx3a, nr4a3, sox4b, and pparg were identified. In HCs, the activity of lhx1a was confirmed [37] and hmx2, hmx3a were identified as novel regulators. In BCs, vsx1 [38], dmbx1a and rorab were active. Our analysis confirmed the activity of thrb and crx in cones and nrl in rods, wherein we also identified a novel regulator, mafba.

Based on regulon enrichment in specific cell types, we determined cell type-specific regulons to reconstruct a GRN. As expected, the regulators within these regulons exhibited pronounced interactions, implying a collaborative effort to preserve cell identity and foster cell differentiation. In glycine ACs in the mouse E18 stage, hub regulators such as Tfap2c, Pou3f1, and Lhx5 significantly influence the GRN (Fig. 3e). In cones, Crx, Thrb, Otx2, Zfp444, and Lcorl (Fig. 3f) have multiple regulatory ties. In MG in the chicken E18 stage, SMAD5 and PAX2 are interacting with SOX9 and JUN (Fig. 3g). In the zebrafish 72 hpf HC GRN (Fig. 3h), regulators such as onecut2, lhx1a, and rxraa, hmx3a, form a grouped pattern, suggesting that cooperate in certain biological functions.

Cross-species analysis of conserved regulators and regulons in mouse, chicken, and zebrafish retina

Given the conservation and comparability of the transcriptional profiles in retinal cells across the three species, we sought to determine if their GRNs were conserved. First, we identified 381 unique cell type-specific regulators for each species, yielding 162 regulators from mouse, 48 from chicken, and 171 from zebrafish. Then, we examined the overlap of cell type-specific regulators among these species (Fig. 4a). We identified 10 regulators that were consistently active in all three species, including Gbx2-homolog, Lhx9-homolog, and Pbx1-homolog in ACs and Sox9-homolog in MG

(Additional file 1: Fig. S4). Such overlap indicates their evolutionary conservation and pivotal roles in retinal biology. For instance, Gbx2 is expressed in a particular subtype of mouse ACs (Fig. 4b) [26], and its homologs are also specifically expressed in ACs. Similarly, the Maf-homolog demonstrated expression in ACs across all three species (Fig. 4b), but its role remains to be explored.

We also observed overlaps among two species in distinct retinal cell types. Hmx3 (hmx3a) was highly specific to HCs in chicken and zebrafish (Additional file1: Fig. S5a), likely because of the absence of Type 2 HC in mice and humans (Additional file1: Fig. S5b) [39]. Similarly, Dmbx1 (dmbx1a) was distinctively expressed in BCs of chicken and zebrafish but not in mouse (Additional file1: Fig. S5c). A set of regulators, including Tcf7l1(tcf7l1a) and Pax2 (pax2a), were predominantly linked to RPCs in mouse and zebrafish because of a lack of chicken RPC data (Additional file1: Fig. S6a-i). Regulators such as Ebf1 (ebf3a) and Lhx4 (lhx4), which were identified as active in mouse and zebrafish, were also expressed in the corresponding chicken cell types (Additional file1: Fig. S6j−p). regulators such as Tfap2c (TFAP2C) and Eomes (EOMES) were active in both mouse and chicken and were found in zebrafish as well (Additional file1: Fig. S7a, b). PAX6 (pax6a), SOX6 (sox6), and ONECUT3 (onecut3b), which were identified as active in chicken and zebrafish, were consistently expressed across all three species (Additional file1: Fig. S7c−e).

After having closely examined the conservation of regulators across the retinas of the three species, we expanded our analysis to the overarching regulatory networks they orchestrate. We employed AUCell [9] to measure the activity of a cell type-specific regulon from one species across all cell types in the other two species and computed the average scaled regulon activity for each cell type (see Methods). If a cell type-specific regulon presented a high average scaled activity in its homolog cell type, it was considered a conserved regulon. With an average scaled activity threshold

of 1.6, we found 16 conserved regulons: six from mouse (Fig. 4c), four from chicken (Fig. 4d), and six from zebrafish (Fig. 4e).

Having identified these 16 conserved regulons, our next aim was to delve deeper into the gene composition of these regulons to discern which genes show conserved expression patterns across species. Specifically, we examined the genes within these conserved regulons that were highly expressed in the homologous cell types across all three species (Additional file 1: Fig. S8−S11). Among the conserved regulons, we found that the Lhx4 regulon (Additional file 1: Fig. S8), which was highly active in mouse cones at E14 stage (Fig. 4c), contained several genes co-expressed with well-known marker genes such as *Thrb*-homolog and *Crx*-homolog, including *Gabrb3*-homolog, *Pcdh15*-homolog, *Pclo*-homolog, and *Prom1*-homolog. Mutation of *GABRB3* has been previously linked to human early infantile epileptic encephalopathy [40]. Similarly, we observed that the PBX1 regulon (Additional file 1: Fig. S9), active in chicken ACs at E12 stage (Fig. 4d), showed conserved expression of the *Auts2*-homolog across all three species. Mutations in *AUTS2* are strongly associated with autism spectrum disorders [41]. The mafba regulon was active in zebrafish rods at 72 hpf stage (Fig. 4e). Genes such as *Tulp1*-homolog, *Nr2e3*-homolog, and *Pde6g*-homolog showed cell type-specific expression in rods across all three species (Additional file 1: Fig. S10). The VAX1 regulon, which was active in chicken rods at E18 stage (Fig. 4d), includes gene ENSGALG00000051404, which was specifically expressed in chicken rods (Additional file 1: Fig. S11). Its zebrafish ortholog, si:ch211−113d22.2, shows similar specificity in rods. A mouse ortholog was not identified.

Identification of Conserved Cell-Type-Specific Regulators and Key Differentiation Drivers in

Mouse and Zebrafish Retinal Development

We identified an array of cell type-specific regulators, some of which were conserved across the three species. This led us to question their pivotal regulatory roles during developmental processes. To deduce differentiation trajectories for both mouse and zebrafish, we first calculated RNA velocities using scVelo [14] and visualized them through a two-dimensional uniform manifold approximation and projection (UMAP) (see Methods). The resulting embedding stream plot demonstrated a pronounced motion of RPCs in the direction of mature retinal cell types, suggesting that RNA velocities capture the critical information on key drivers of differentiation (Fig. 5a, b). Based on combined RNA velocities and transcriptional similarity, we determined the fate probability of each cell as well as the driver genes guiding their differentiation into specific cell types (see Methods). We visualized the top 50 correlated driver genes for each cell type in the two species (Additional file 1: Fig. S12, S13).

To assess the biological functions of the driver genes across mouse and zebrafish, we conducted Gene Ontology (GO) analysis focusing on the biological process (BP) category for the top 100 correlated driver genes in each species independently (see Methods, Additional file 3: Table S1, S2, Additional file 1: Fig. S14). The BPs of driver genes closely aligned with the specific functions of the corresponding cell types, and homologous cell types across species shared similar BPs, underscoring the existence of conserved driver genes.

We further identified two kinds of key differentiation drivers (Fig. 5c): 1) differentiation drivers shared between mouse and zebrafish and 2) regulators that drive differentiation in mouse and zebrafish.

We examined the intersection of top 100 driver genes in homologous cell types and observed an

overlap, particularly in ACs, RGCs, and cones (Fig. 5d). Specifically, 19 shared driver genes were identified in ACs in both species (Fig. 5e, f), followed by 16 in RGCs and 12 in cones (Additional file1: Fig. S15). Among the shared driver genes, *Pax6*-homolog, *Gng3*-homolog, *Stmn2*-homolog, *Rtn1*-homolog, *Syt4*-homolog, and *Rbfox2*-homolog drove both AC and RGC development (Fig. 5e, f, Additional file 1: Fig. S15a, b), suggesting that they may be modulated by similar signaling pathways in the early stages of retinal development. *Celf4*-homolog and *Gad1*-homolog were identified as specific drivers of AC differentiation (Fig. 5e, f), whereas *Elavl4*-homolog, *Pou6f2*-homolog, *Ebf1*-homolog, and *Mapt*-homolog were specific drivers of RGC differentiation (Additional file 1: Fig. S15a, b). *Guk1*-homolog and *Cuedc1*-homolog were important drivers in cones (Additional file 1: S15c, d). Finally, *Car14*-homolog and *Glul*-homolog were identified as important drivers of MG differentiation (Additional file 1: S15e, f).

To determine cell-type-specific regulators driving retinal cell development, we cross-referenced the top 100 correlated driver genes for each cell type with cell type-specific regulators identified using SCENIC. In mice, 37 regulators were identified, including widely known ones such as Nrl for rods and Rxrg in cones. We also identified regulators that have not been reported to drive differentiation (Fig. 5g). Sox4 expression displayed a consistent upward trend in all cell types as development progressed. Pou3f1 exhibited a similar increase in expression in all cell types during differentiation, with the most pronounced rise observed in the RGCs. Prdm1 expression also increased in all cell types, but the most marked increase was observed in rods. In contrast, Prox1 showed a differentiation-dependent increase in expression exclusively in BCs. Similarly, Irx2 expression increased only in the rod during the development. Using a similar approach for zebrafish, 44 regulators were found, including established ones such as klf7a in RGCs and pax6a in ACs. We also

discovered regulators not previously reported to regulate differentiation (Fig. 5h). The expression of sox6 increased in both RGCs and cones during development, whereas pbx1a exhibited a pronounced increase in ACs and RGCs. The expression of hmx2, hmx3a, and onecutl increased only in HCs, and rorab expression showed an increasing trend in ACs, BCs, HCs, and RGCs as development progressed.

Notably, some TFs had already been flagged as cell type-specific regulators in our preceding SCENIC analysis, validating their critical roles in cellular function and identity. For instance, in ACs, TFs such as Tfap2c-homolog, Tfap2a-homolog, and Barhl2-homolog (Fig. 5e, f) were not only identified as shared driver genes but also appeared in the SCENIC-identified list of regulators in both mouse and zebrafish. Some TFs, such as Tfap2b-homolog, Meis2-homolog, and Nhlh2-homolog were shared driver genes but not listed as regulators in the SCENIC analysis (Fig. 5e, f).

Exploring evolutionary origins: comparative analysis of retinal cells in vertebrates and invertebrate *Ciona*

To expand our evolutionary understanding beyond the conserved regulatory genes identified in the mouse, chicken, and zebrafish retinas, we included an invertebrate *Ciona* in our comparative study. We first identified a set of conserved DEGs in the retinal cells of vertebrates. The top 10 conserved DEGs are consistently expressed in the corresponding homologous cells (Figure 6a). Then, we collected scRNA-seq data from *C. intestinalis*, extracted eight PR-related cell types (Fig. 6b), and identified DEGs in these cell types (see Methods). We analyzed the intersection of DEGs between vertebrate retinal cell types and *Ciona* PR-related cells based on the 5894 orthogroups generated using Orthofinder (Fig. 6c, see Methods) [42]. Specifically, if DEGs in a certain type of *Ciona*

PR-related cells have orthologous DEGs in a certain type of vertebrate retinal cell, these two cell types may have a homologous relationship. Twenty-one orthologs of the 23 DEGs in vertebrate retinal cells also displayed differential expression in PR-related cells in *Ciona* (Fig. 6d, Additional file 4: Table S1).

Rx was reported to be specifically expressed in the *Ciona* developing PR [43]. Among the DEGs in Rx+ aSV cells of *Ciona*, four orthologous genes were specifically expressed in vertebrate PRs. In *Ciona* Rx+ aSV cells, the genes KH.C2.828 and KH.C1.346 were prominently expressed. These genes are orthologous to Phosducin (*PDC*) and *Prom1*, respectively, which were specifically expressed in PR in vertebrates (Fig. 6e, Additional file 1: Fig. S16a). *PDC* modulates G-protein-coupled receptor signaling by binding to βγ subunits [44], while mutations in *Prom1* have been associated with retinal degeneration [45]. These findings suggested that *Ciona* Rx+ aSV cells and vertebrate PRs share similar mechanisms for light transduction. Likewise, the gene KH.S215.4 was selectively expressed in Rx+ aSV cells and is an ortholog to Lhx4, a key regulator of retinal neural differentiation specifically expressed in cones and BCs [46,47] (Fig. 6f). KH.L152.2 was exclusively expressed in Rx+ aSV cells and corresponds to Isl2, predominantly expressed in RGCs and cones (Additional file 1: Fig. S16b). Together, these results suggest functional similarities between vertebrate PRs and Rx+ aSV cells of *Ciona*.

Among the DEGs of Ci-VP+ pSV cells of *Ciona*, six orthologous genes were specifically expressed in vertebrates RGCs. The gene KH.L71.1 was specifically expressed in Ci-VP+ pSV cells, and its ortholog *Rbpms2* was a DEG in RGCs (Fig. 6g). *Rbpms2*, which is an RNA binding protein with multiple splicing, has been used as an RGC marker [48]. Similarly, KH.C14.291 is specifically

expressed in Ci-VP+ pSV cells and is an ortholog to *Dach2* (Additional file 1: Fig. S16c), which was a DEG in vertebrate RGCs and HCs. Furthermore, KH.L24.10 and KH.C12.301 were both specifically expressed in Opsin1+ STUM+ aSV and Ci-VP+ pSV, and their orthologs *Ebf1* and *Fstl4* were specifically expressed in RGCs (Additional file 1: Fig. 16d, e). Ebf1 is a regulator driving the differentiation of RGCs [49] and Fstl4 is a marker for a subtype of RGCs [50]. Thus, the function of Ci-VP+ pSV may be related to that of RGCs.

Three DEGs from the inhibitory neuron eminens had orthologs specifically expressed in various cell types from the vertebrate retina. KH.C2.526 and KH.L50.5 were predominantly expressed in eminens cells. Its orthologs, *Slc32a1*-homolog and *Slc24a2*-homolog, are specifically expressed in vertebrate ACs and cone, respectively (Fig. 6h, Additional file 1: Fig.16f). Slc32a1, also known as Vesicular GABA transporter (VGAT), is a transporter responsible for loading GABA into synapses [51]. Slc24a2, also known as NCKX2, is a sodium-calcium-potassium exchanger protein. It regulates calcium and sodium ion levels, enabling quick recovery and response to new light stimuli in cones [52]. KH.S761.6 was a DEG for eminens and Ci-VP+ pSV, its ortholog *Gad2*-homolog, was specifically expressed in vertebrate ACs and HCs (Additional file 1: Fig. 16g). Gad2 is a GABAergic neuron marker [53]. These findings suggest that vertebrate ACs and cones may share similar functions with *Ciona* eminens cells.

Finally, the KH.C3.298 was found to be specifically expressed in the GLGB+ pSV, its ortholog *Sept3*-homolog was the DEG for vertebrate AC and RGC (Additional file 1: Fig. 16h).

Discussion

In this study, we comprehensively explored the transcriptional landscape of vertebrate retinal cells across three representative species, unveiling transcriptomic conservation suggestive of shared regulatory mechanisms. Our detailed investigation into cell type-specific regulatory networks provided insights into the inherent regulatory dynamics of retinal cells, emphasizing the role of conserved regulators and regulons in evolutionary terms. Furthermore, comparative analysis with the invertebrate Ciona underscored potential evolutionary relations, hinting at shared ancestral origins of certain retinal cells. These discoveries not only expand our understanding of retinal cell biology but also pave the way for broader inquiries into the evolutionary trajectories of complex visual systems.

Our findings corroborate the idea that the transcriptomes of homologous cells are more similar across species than they are to other cell types within the same species. This not only affirms evolutionary conservation but also suggests the pivotal role of these cells in visual function across diverse vertebrates. While our study provided insights into general transcriptomic similarities among homologous cells in vertebrates, we did not delve into the finer subclasses of these cells. For

instance, 40 types of RGCs [50] and more than 60 types of ACs [54] have been identified. Because we used a broad classification approach, we may have overlooked nuanced differences or specializations within these subcategories. Future studies based on more refined cell-type classifications may unveil additional layers of transcriptomic and functional complexity, providing new avenues for the exploration of retinal biology and evolution.

Our application of SCENIC uncovered 690 high-quality regulons and their corresponding 381 regulators. These regulators showed a high overlap with regulators previously reported in the literature. For example, previous research has established the importance of Crx, Otx2, and Thrb in cone specification [55]. Our study extends this by demonstrating their high interaction with Zfp444 and Lcorl in cones at the mouse E18 stage, suggesting potential roles for these genes in cone specification (Fig. 3f). Additionally, while lhx1a is known as an HC marker gene[39], and onecut2 is a regulator of HC fate [56], we discovered their interaction with hmx3a and rxraa in zebrafish HCs at 72 hpf stage (Fig. 3h). The functions of hmx3a and rxraa may also be related to the identity and specification of zebrafish HCs. In summary, this extensive repository of regulons and regulators, many of which remain to be explored, represents a rich resource for future investigations into the intricate mechanisms of vertebrate retinal biology.

We pinpointed 10 conserved regulators and 16 conserved regulons in homologous cells across vertebrates. The conservation of these regulators suggests their fundamental importance to basic cellular functions, while also hinting at unexplored, potentially critical roles. For example, Gbx2, although proven to be expressed in a subtype of GABAergic ACs [26], has yet to have its function established in the AC of chicken and zebrafish. The role of Maf, a conserved regulator in ACs, emerges as an intriguing target for future functional verification. The conserved regulons we

identified not only enhance our understanding of the fundamental functions of the retina but offers insights into complex brain functions. For instance, we observed a conserved expression of Garbr3 and Auts2 in cones and ACs across the three species from the conserved regulons (Additional file 1: Fig. S8,9). While these genes has been linked to human brain diseases like human early infantile epileptic encephalopathy and autism spectrum disorders [40,41], their specific functions in these diseases remain to be further investigated. Studying the functions of these conserved genes in a simpler model, such as the zebrafish retina, provides a more feasible approach than exploring them in the complex environment of the human brain.

Our RNA velocity analysis in mouse and zebrafish significantly advanced our comprehension of genes driving retinal differentiation in vertebrates. For instance, we identified conserved driver genes between mouse and zebrafish, overlapping with previously identified marker genes, and demonstrated their intricate involvement in establishing cell identity. For example, Gad1, a known marker for ACs [54], was predominantly expressed in the terminal stages of differentiation (Fig. 5e, f); similar patterns were also observed in other driver genes such as Celf4. This not only corroborates the role of Gad1 as a marker gene but highlights its importance across species in establishing cell identity. In addition, we uncovered a set of key regulators that drive differentiation, which allowed us to pinpoint transcription factors and regulatory networks pivotal in guiding cellular differentiation pathways. Similarly, a recent study reported the regulatory role of Pou3f1 in RGC differentiation[57]. This finding closely aligns with our results (Fig. 5g), supporting the validity of our identified regulators.

Our comparative analysis with the invertebrate species *Ciona* allowed us to identify DEGs shared between vertebrate retinal cells and *Ciona* PR-related cells. These shared genes have been widely used as markers for vertebrate retinal cells though their functions are not yet fully understood.

For instance, *Rbpms2* is an RNA binding protein with multiple splicing. It has been used as a marker for vertebrate RGCs, but its specific role in these cells is still unknown [48]. Considering the compact genome of *Ciona* [58], exploring the regulatory programs of their *Ciona* orthologs could yield valuable insights into their functional conservation and evolutionary dynamics. Furthermore, through these shared DEGs, we identified corresponding cell types between vertebrate retina and Ciona PR-related cells, such as vertebrate PRs and *Ciona* Rx+ aSV cells, as well as vertebrate RGCs and *Ciona* Ci-VP+ cells. This correlation helps us better investigate the simple patterns of information transmission within retina, offering insights into more complex visual systems. However, it is important to note that our conclusions about ancestral relationships are drawn from a small number of conservative genes. Expanding this analysis to include a broader range of genes and other types of molecular data may provide a more comprehensive view of evolutionary relationships and might yield additional insights into the ancestral forms of these retinal cells. Future research should consider a more extensive comparative analysis for a deeper understanding of the evolutionary history of the vertebrate retina.

Conclusion

This study illustrated the intricated transcriptional landscape of vertebrate retinal cells, demonstrating the evolutionary ties that underpin their development and differentiation. Through a comprehensive analysis spanning multiple species, we revealed the depth of transcriptomic conservation, emphasizing the ubiquity of shared regulatory mechanisms. Our investigation of conserved regulatory networks and driver genes underscored the fundamental evolutionary principles that govern retinal cell biology. Furthermore, comparison with invertebrate species *C. intestinalis* yielded compelling evidence of the potential ancestral origins of specific retinal cells. These discoveries serve as a foundation for further inquiries into the evolutionary intricacies of retinal biology and beyond.

Methods

Single-cell RNA-seq data of retinal cells from mouse, chicken, and zebrafish

The FASTQ data from scRNA-seq retinal cells were retrieved from the GEO database for three species: mouse, chicken, and zebrafish (GSE118614, GSE159107, GSE122680). Specifically, the mouse dataset spans ten developmental stages: E11, E12, E14, E16, E18, P0, P2, P5, P8, and P14. Chicken data were collected on E12, E16, and E18. Zebrafish data were collected 24 h post-fertilization (hpf), 36 hpf, 48 hpf, 72 hpf, and 14 days post-fertilization (dpf).

CellRanger (Ver. 6.1.2) [59] was used to create original count matrices, using GRCm38, GRCg6a, and GRCz11 as the reference genomes. These raw count matrices were imported into R and analyzed using the Seurat 4 package [60]. Cells of low quality were identified and removed following specific criteria. For the data sets including cell type annotation (mouse and chicken cells at E16 and E18 stage), cells annotated as either non-retinal or low-quality were removed. In the

remaining cases (zebrafish and chicken cells at E12), cells expressing <200 genes and those with mitochondrial expression proportion >10% were excluded. For each stage without annotated information, filtered cells were normalized and scaled by *SCTransform* function, followed by principal component analysis (PCA) using top 3000 HVGs. The top 20 principal components (PCs) were used for clustering based on a shared nearest-neighbor modularity optimization-based clustering algorithm with default parameters and range of resolutions from 0.05 to 0.8. Cells were annotated based on existing marker genes (Additional file 5: Table S1). To integrate samples from the same species, the filtered cells were integrated, normalized, and scaled using *SCTransform* function, followed by PCA using top 3000 HVGs. Next, Canek [61] was used to eliminate the batch effect by the top 20 PCs. Non-linear dimensionality reduction was conducted on the corrected data using UMAP.

Transcriptional profile similarity analysis

Firstly, to reduce the noise from sequencing, we combined 10 cells within each cell type to construct pseudo-cells using all the retaining cells. Secondly, we collected 11,000 one-to-one orthologs for the three species from biomaRt [62] and homologene [63] package using *getLDS* and *homologene* function, respectively. Then, we employed the orthologs to integrate the pseudo-cell count matrices, which was used as input to MetaNeighbor. Specifically, we utilized *run_MetaNeighbor_US* function to identify the similarity between cell-type pairs among retinal cell types using the 391 highly variable genes (HVGs) generated by *get_variable_genes* function. In brief, MetaNeighbor uses highly variable genes (HVGs) to calculate Spearman correlations for gene expression across various cell types in different species, establishing a measure of similarity. The strength of these correlations is

then applied to compute the Area Under the Receiver Operating Characteristic (AUROC), which determines how well these correlations can distinguish between similar and dissimilar cell types across species, thus quantifying the reliability of cell type conservation.

For unsupervised clustering, the integrated pseudo-cell count matrix was used as input to Seurat 4 package. The data were log normalized and scaled, followed by PCA using HVGs generated above. Next, Harmony [64] was used to eliminate the batch effect by the top 20 PCs. The corrected data was used for clustering based on a shared nearest-neighbor modularity optimization-based clustering algorithm with default parameters and a resolution of 0.01. Non-linear dimensionality reduction was conducted using UMAP.

Ranking database development for chicken and zebrafish

We constructed the TF-motif ranking database for the two species according to the SCENIC tutorial instructions. First, TF lists for chicken and zebrafish were downloaded from the AnimalTFDB database [65]. Then, the transcription factor binding motifs in both species were obtained from JASPAR [66], CISBP [67], DBcorrDB [68], and HOCOMOCO [69]. Given that TF binding specificities are known to be conserved across species [70], the binding information from their human homologs was also used. Bind motif matrices for 80.6% (914 /1134) TFs in chicken and 44.3% (1128/2547) TFs in zebrafish were collected. The promoter sequences (upstream 5000bp and downstream 100bp from the transcription start site) used for ranking were downloaded from Ensembl (GRCg6a, GRCz11). Finally, promoter sequences were ranked.

GRN reconstruction

We used pySCENIC [22] for the reconstruction of the gene regulatory network. In brief, we generated co-expression modules based on the scRNA-seq data using GRNBoost2. A ranking database was used to filter out indirect targets by comparing the preliminary gene set with the transcription factor binding predictions, retaining only those genes with strong evidence of direct regulation. The minimum number of genes in a module was set to 8, with all other settings at their default values. The ranking database for mouse was obtained from cisTarget database. Finally, the activity of each filtered module in each cell was estimated using AUCell [9]. AUCell estimates the activity of specific gene sets in individual cells by calculating the area under the curve of the ranked gene expression distribution, assuming that if the genes in the set are highly active, they should appear earlier in the ranked list of gene expression. The inferred regulons were filtered by the regulon specificity scores and the expression specificity its regulator of a regulon.

Cross-species regulon comparison

We employed cell type-specific regulons for cross-species comparisons (Additional file 2: Table S1-3). First, gene names within these cell-specific regulons were transformed into their respective homologous gene names in other species, using the previously obtained set of 11,000 one-to-one homologous genes. Subsequently, the AUCell package was used to assess the activity of each regulon within the cell datasets corresponding to each species. For evaluation, we incorporated data from mouse cells extracted at four developmental stages: E14, E18, P8, and P14. In the case of chicken, data was derived from the E16 and E18 stages, while for zebrafish, we utilized data across all five stages (24, 36, 48, 72 hpf, and 14 dpf). Finally, we computed the mean z-score of the cell-specific regulons within their homologous cell types.

RNA velocity analysis

First, to generate spliced/unspliced count matrices for samples from mouse and zebrafish, the BAM files generated by CellRanger (Ver. 6.1.2) were processed using velocyto [13] with the repeat mask annotation file downloaded from UCSC genome browser (https://genome.ucsc.edu/). Samples from the same species were then integrated into one loom file. For the zebrafish dataset, cells with unspliced read proportions lower than 7% were removed. As a result, unspliced reads accounted for 26% of the total in mouse and 14% in zebrafish. Within individual cell types, these proportions ranged from 19% to 35% in mouse cells and from 9% to 17% in zebrafish cells (Additional file1: Fig. S17a, b).

The processed loom files were then used as input to Scanpy [71]. Initially, genes with larger than 10 shared counts were retained. The top 2000 HVGs were then used for PCA, and the top 30 PCs were used for the calculation of first-/second-order moments for each cell, considering the 30 nearest neighbors. The moments were used to determine RNA velocities for differentiation using dynamics model. The transition matrix of cells was estimated by combining gene expression similarity and RNA velocities using CellRank [72]. The weight for gene expression similarity was set to 0.2. Furthermore, the initial and terminal states were identified from the transition matrix. In addition, the lineage probabilities for each cell toward the terminal states of cell types were computed. For each cell type, genes whose expression levels are highly correlated with lineage probabilities were identified as driver genes. To visualize the trends for driver genes during differentiation, we estimated the Diffusion pseudotime (DPT pseudotime) for each cell (Additional file1: Fig. S17c, d). The estimated DPT pseudotime is consistent with stage information (Additional file1: Fig. S17e, f). Using this DPT

pseudotime to order the cells, we then fit Generalized Additive Models (GAMs) to the gene expression data, weighting the contribution of each cell according to fate probabilities.

GO analysis of top 100 cell type-specific driver genes

We employed the *compareCluster* function from the clusterProfiler [73] package for the analysis of drive genes identified in mouse and zebrafish. The analysis utilized the *enrichGO* function, targeting Biological processes (BP) with org.Mm.eg.db and org.Dr.eg.db as the gene ontology database for mouse and zebrafish. We applied the Benjamini-Hochberg method for p-value adjustment, setting the q-value cutoff at 0.05 for significance. The top 5 significant GO terms for each cell type were plotted.

Identification of DEGs in vertebrate retina and *Ciona* photoreceptors-related cells

We identified the vertebrate conserved DEGs for the vertebrate retina using the *FindAllMarkers* function by from Seurat in two datasets, applying criteria of an adjusted p-value <0.05 and setting '*only.positive* = *TRUE*' to focus exclusively on positive markers:

1. In the integrated pseudo-cells dataset (Fig. 1i), generating pseudo-cell-DEGs.
2. In datasets from each species separately (Fig. 1a-c). We took intersections by the 11,000 orthologs to generate ortholog-DEGs.

The final conserved DEGs were defined by retaining genes from pseudo-cell DEGs only if they were either in the ortholog-DEGs or HVGs from pseudo-cell-DEGs.

We collected scRNA-seq data of *Ciona* at late tailbud II and larval stage from the GEO database

(GSE131155). The single-cell raw count matrices were generated using CellRanger (Ver. 6.1.2) with KH2013 [58] as the reference genome. Annotations for cellular identities were kindly provided by the original authors. We focused on cells related to PR, which led to the extraction of eight PR-related cell types. The selected PR-related cells were normalized and scaled through *SCTransform* function. Subsequently, PCA was performed to identify the top 20 PCs, which were then used for batch correction by Canek. The corrected dimensions were further reduced in a non-linear fashion using UMAP. Marker genes were identified using the *FindAllMarkers* function, applying a threshold of an adjusted p-value < 0.01 and setting '*only.positive* = *TRUE*' to focus exclusively on positive markers

Identification of Orthologous gene among mouse, chicken, zebrafish, and *Ciona*

Orthologous gene identification among the four species was conducted using OrthoFinder 2.5.4 [42]. First, we downloaded the protein sequences of the mouse (GRCm38), chicken (GRCg6a), zebrafish (GRCz11), and Ciona (KH2013) from the Ensembl [74] and Ghost databases [58] (http://ghost.zool.kyoto-u.ac.jp/download_kh.html). To enhance accuracy and reduce computational complexity, only the longest transcript variant of a gene was used for multiple sequence alignments with MAFFT. Subsequently, orthogroups were estimated using the MCL graph clustering algorithm. 2,724 single-copy orthogroups and 6,374 orthogroups containing genes from mouse, chicken, zebrafish, and *Ciona* were identified. 5,894 orthogroups that contained genes from all species with less than five copies in each species were retained for further analysis.

Supplementary information

Additional file1: Figure S1-3. Reconstruction of GRN in mouse (**S1**), chicken (**S2**), zebrafish (**S3**) retinal cells at various stages. **Figure S4**. Expression of cell type specific regulators that are active in all three species. **Figure S5**. Expression of Hmx3-homolog and Dmbx1-homolog that are only active in chicken and zebrafish. **Figure S6**. Expression of regulators that are active in mouse and zebrafish. **Figure S7**. Expression of regulators that are only active in mouse and chicken, as well as zebrafish. and chicken. **Figure S8-11**. Top expressing genes in the E14_Lhx4 (**S8**), E12_PBX1 (**S9**), 72hpf_mafba (**S10**), E18_VAX1 (**S11**) regulon identified as active in mouse cones, chicken ACs, zebrafish rods, and chicken rods, respectively. Figure **S12-13**. Gene expression trends of top 50 driver genes along DPT pesudotime in mouse (**S12**) and zebrafish (**S13**) retina. **Figure S14**. GO analysis of top 100 cell type specific driver genes in mouse and zebrafish. **Figure S15.** Gene expression trends of shared driver genes along DPT pesudotime between mouse and zebrafish. **Figure S16** Shared DEGs between vertebrate retinal cells and *Ciona* PR-related cells. **Figure S17** RNA velocity preprocessing analysis in mouse and zebrafish retinal cells.

Additional file2: Table S1-3. Filtered cell type specific regulons in mouse (**S1**), chicken (**S2**), and zebrafish (**S3**)

Additional file3: Table S1-2. Top 100 cell type-specific driver genes identified in mouse (**S1**) and zebrafish (**S2**)

Additional file4: Table S1. Share DEGs between vertebrate retinal cells and *Ciona* PR-related cells

Additional file5: Table S1. Marker genes for annotating vertebrate retinal cells from chicken and zebrafish.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

FASTQ data from scRNA-seq retinal cells were retrieved from the GEO database for the four species: mouse, chicken, and zebrafish, *Ciona* (GSE118614, GSE159107, GSE122680, GSE131155). Code generated for this project is available on GitHub (https://github.com/xzengComBio/VertebrateRetina_scRNAseq),

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

XZ, FG, TGK, and KN conceived and designed the study; XZ performed the data analysis and visualization. YC, FG, and TGK contributed scientific expertise. XZ, ML, and KN wrote the

manuscript with input from all co-authors. The authors read and approved the final manuscript.

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References

1. Lucas-Ruiz F, Galindo-Romero C, Albaladejo-García V, Vidal-Sanz M, Agudo-Barriuso M. Mechanisms implicated in the contralateral effect in the central nervous system after unilateral injury: focus on the visual system. Neural Regen Res. 2021;16:2125.
2. Wang L, Mao X. Role of Retinal Amyloid-β in Neurodegenerative Diseases: Overlapping Mechanisms and Emerging Clinical Applications. IJMS. 2021;22:2360.
3. Stenkamp DL. Development of the Vertebrate Eye and Retina. Progress in Molecular Biology and Translational Science [Internet]. Elsevier; 2015 [cited 2023 Nov 2]. p. 397–414. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1877117315001088
4. Wan Y, Almeida AD, Rulands S, Chalour N, Muresan L, Wu Y, et al. The ciliary marginal zone of the zebrafish retina: clonal and time-lapse analysis of a continuously growing tissue. Development. 2016;dev.133314.
5. Hoon M, Okawa H, Della Santina L, Wong ROL. Functional architecture of the retina: Development and disease. Progress in Retinal and Eye Research. 2014;42:44–84.
6. Clark BS, Stein-O'Brien GL, Shiau F, Cannon GH, Davis-Marcisak E, Sherman T, et al. Single-Cell RNA-Seq Analysis of Retinal Development Identifies NFI Factors as Regulating Mitotic Exit and Late-Born Cell Specification. Neuron. 2019;102:1111-1126.e5.
7. Yamagata M, Yan W, Sanes JR. A cell atlas of the chick retina based on single-cell transcriptomics. eLife. 2021;10:e63907.

8. Xu B, Tang X, Jin M, Zhang H, Du L, Yu S, et al. Unifying Developmental Programs for Embryonic and Post-Embryonic Neurogenesis in the Zebrafish Retina. Development. 2020;dev.185660.
9. Aibar S, González-Blas CB, Moerman T, Huynh-Thu VA, Imrichova H, Hulselmans G, et al. SCENIC: single-cell regulatory network inference and clustering. Nat Methods. 2017;14:1083–6.
10. Han X, Zhou Z, Fei L, Sun H, Wang R, Chen Y, et al. Construction of a human cell landscape at single-cell level. Nature. 2020;581:303–9.
11. Li J, Wang J, Zhang P, Wang R, Mei Y, Sun Z, et al. Deep learning of cross-species single-cell landscapes identifies conserved regulatory programs underlying cell types. Nat Genet [Internet]. 2022 [cited 2022 Oct 30]; Available from: https://www.nature.com/articles/s41588-022-01197-7
12. Tritschler S, Büttner M, Fischer DS, Lange M, Bergen V, Lickert H, et al. Concepts and limitations for learning developmental trajectories from single cell genomics. Klein A, Treutlein B, editors. Development. 2019;146:dev170506.
13. La Manno G, Soldatov R, Zeisel A, Braun E, Hochgerner H, Petukhov V, et al. RNA velocity of single cells. Nature. 2018;560:494–8.
14. Bergen V, Lange M, Peidli S, Wolf FA, Theis FJ. Generalizing RNA velocity to transient cell states through dynamical modeling. Nat Biotechnol. 2020;38:1408–14.
15. Dufour HD, Chettouh Z, Deyts C, De Rosa R, Goridis C, Joly J-S, et al. Precraniate origin of cranial motoneurons. Proc Natl Acad Sci USA. 2006;103:8727–32.
16. Horie T, Sakurai D, Ohtsuki H, Terakita A, Shichida Y, Usukura J, et al. Pigmented and nonpigmented ocelli in the brain vesicle of the ascidian larva. J of Comparative Neurology. 2008;509:88–102.
17. Ryan K, Lu Z, Meinertzhagen IA. The CNS connectome of a tadpole larva of Ciona intestinalis (L.) highlights sidedness in the brain of a chordate sibling. Marder E, editor. eLife. 2016;5:e16962.
18. Razy-Krajka F, Brown ER, Horie T, Callebert J, Sasakura Y, Joly J-S, et al. Monoaminergic modulation of photoreception in ascidian: evidence for a proto-hypothalamo-retinal territory. BMC Biol. 2012;10:45.
19. Cao C, Lemaire LA, Wang W, Yoon PH, Choi YA, Parsons LR, et al. Comprehensive single-cell transcriptome lineages of a proto-vertebrate. Nature. 2019;571:349–54.
20. Goldman D. Müller glial cell reprogramming and retina regeneration. Nat Rev Neurosci. 2014;15:431–42.
21. Fain GL, Hardie R, Laughlin SB. Phototransduction and the Evolution of Photoreceptors. Current Biology. 2010;20:R114–24.
22. Van de Sande B, Flerin C, Davie K, De Waegeneer M, Hulselmans G, Aibar S, et al. A scalable SCENIC workflow for single-cell gene regulatory network analysis. Nat Protoc. 2020;15:2247–76.
23. Poché RA, Furuta Y, Chaboissier M-C, Schedl A, Behringer RR. Sox9 is expressed in mouse multipotent retinal progenitor cells and functions in Müller Glial cell development. J Comp Neurol. 2008;510:237–50.
24. Parameswaran S, Xia X, Hegde G, Ahmad I. *Hmga2* regulates self-renewal of retinal progenitors. Development. 2014;141:4087–97.
25. Ghiasvand NM, Rudolph DD, Mashayekhi M, Brzezinski JA, Goldman D, Glaser T. Deletion of a remote enhancer near ATOH7 disrupts retinal neurogenesis, causing NCRNA disease. Nat Neurosci. 2011;14:578–86.
26. Kerstein PC, Leffler J, Sivyer B, Taylor WR, Wright KM. Gbx2 Identifies Two Amacrine Cell Subtypes with Distinct Molecular, Morphological, and Physiological Properties. Cell Reports. 2020;33:108382.
27. Ding Q, Chen H, Xie X, Libby RT, Tian N, Gan L. BARHL2 Differentially Regulates the Development of Retinal Amacrine and Ganglion Neurons. J Neurosci. 2009;29:3992–4003.
28. Zhang Q, Zagozewski J, Cheng S, Dixit R, Zhang S, De Melo J, et al. Regulation of *Brn* 3b by *Dlx* 1 and *Dlx* 2 is required for retinal ganglion cell differentiation in the vertebrate retina. Development.

2017;dev.142042.
29. Triplett JW, Wei W, Gonzalez C, Sweeney NT, Huberman AD, Feller MB, et al. Dendritic and axonal targeting patterns of a genetically-specified class of retinal ganglion cells that participate in image-forming circuits. Neural Dev. 2014;9:2.
30. Roberts MR, Hendrickson A, McGuire CR, Reh TA. Retinoid X Receptor γ Is Necessary to Establish the S-opsin Gradient in Cone Photoreceptors of the Developing Mouse Retina. Invest Ophthalmol Vis Sci. 2005;46:2897.
31. Hsieh Y-W, Yang X-J. Dynamic Pax6 expression during the neurogenic cell cycle influences proliferation and cell fate choices of retinal progenitors. Neural Dev. 2009;4:32.
32. Enright JM, Lawrence KA, Hadzic T, Corbo JC. Transcriptome profiling of developing photoreceptor subtypes reveals candidate genes involved in avian photoreceptor diversification. J of Comparative Neurology. 2015;523:649–68.
33. Corso-Díaz X, Simpson EM. Nr2e1 regulates retinal lamination and the development of Müller glia, S-cones, and glycineric amacrine cells during retinogenesis. Mol Brain. 2015;8:37.
34. Young RM, Hawkins TA, Cavodeassi F, Stickney HL, Schwarz Q, Lawrence LM, et al. Compensatory growth renders Tcf7l1a dispensable for eye formation despite its requirement in eye field specification. eLife. 2019;8:e40093.
35. Yokoi H, Yan Y-L, Miller MR, BreMiller RA, Catchen JM, Johnson EA, et al. Expression profiling of zebrafish sox9 mutants reveals that Sox9 is required for retinal differentiation. Developmental Biology. 2009;329:1–15.
36. Veldman MB, Bemben MA, Thompson RC, Goldman D. Gene expression analysis of zebrafish retinal ganglion cells during optic nerve regeneration identifies KLF6a and KLF7a as important regulators of axon regeneration. Developmental Biology. 2007;312:596–612.
37. Rao MB, Didiano D, Patton JG. Neurotransmitter-Regulated Regeneration in the Zebrafish Retina. Stem Cell Reports. 2017;8:831–42.
38. Letelier J, Buono L, Almuedo-Castillo M, Zang J, Mounieres C, González-Díaz S, et al. Mutation of vsx genes in zebrafish highlights the robustness of the retinal specification network. eLife. 2023;12:e85594.
39. Boije H, Shirazi Fard S, Edqvist P-H, Hallböök F. Horizontal Cells, the Odd Ones Out in the Retina, Give Insights into Development and Disease. Front Neuroanat [Internet]. 2016 [cited 2023 Sep 23];10. Available from: http://journal.frontiersin.org/Article/10.3389/fnana.2016.00077/abstract
40. Papandreou A, McTague A, Trump N, Ambegaonkar G, Ngoh A, Meyer E, et al. *GABRB3* mutations: a new and emerging cause of early infantile epileptic encephalopathy. Dev Med Child Neurol. 2016;58:416–20.
41. Oksenberg N, Stevison L, Wall JD, Ahituv N. Function and Regulation of AUTS2, a Gene Implicated in Autism and Human Evolution. Noonan J, editor. PLoS Genet. 2013;9:e1003221.
42. Emms DM, Kelly S. OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol. 2019;20:238.
43. Oonuma K, Kusakabe TG. Spatio-temporal regulation of Rx and mitotic patterns shape the eye-cup of the photoreceptor cells in Ciona. Developmental Biology. 2019;445:245–55.
44. Lee BY, Thulin CD, Willardson BM. Site-specific Phosphorylation of Phosducin in Intact Retina. Journal of Biological Chemistry. 2004;279:54008–17.
45. Cehajic-Kapetanovic J, Birtel J, McClements ME, Shanks ME, Clouston P, Downes SM, et al. Clinical and Molecular Characterization of *PROM1* -Related Retinal Degeneration. JAMA Netw Open. 2019;2:e195752.
46. Buenaventura DF, Corseri A, Emerson MM. Identification of Genes With Enriched Expression in Early

Developing Mouse Cone Photoreceptors. Invest Ophthalmol Vis Sci. 2019;60:2787.
47. Dong X, Yang H, Zhou X, Xie X, Yu D, Guo L, et al. LIM-Homeodomain Transcription Factor LHX4 Is Required for the Differentiation of Retinal Rod Bipolar Cells and OFF-Cone Bipolar Subtypes. Cell Reports. 2020;32:108144.
48. Rodriguez AR, De Sevilla Müller LP, Brecha NC. The RNA binding protein RBPMS is a selective marker of ganglion cells in the mammalian retina: RBPMS identifies retinal ganglion cells. J Comp Neurol. 2014;522:1411–43.
49. Chuang J-H, Yarmishyn AA, Hwang D-K, Hsu C-C, Wang M-L, Yang Y-P, et al. Expression profiling of cell-intrinsic regulators in the process of differentiation of human iPSCs into retinal lineages. Stem Cell Res Ther. 2018;9:140.
50. Rheaume BA, Jereen A, Bolisetty M, Sajid MS, Yang Y, Renna K, et al. Single cell transcriptome profiling of retinal ganglion cells identifies cellular subtypes. Nat Commun. 2018;9:2759.
51. Wojcik SM, Katsurabayashi S, Guillemin I, Friauf E, Rosenmund C, Brose N, et al. A Shared Vesicular Carrier Allows Synaptic Corelease of GABA and Glycine. Neuron. 2006;50:575–87.
52. Sharon D, Yamamoto H, McGee TL, Rabe V, Szerencsei RT, Winkfein RJ, et al. Mutated Alleles of the Rod and Cone Na-Ca K- Exchanger Genes in Patients with Retinal Diseases. 2002;43.
53. Huang W, Xu Q, Liu F, Su J, Xiao D, Tang L, et al. Identification of TPBG-Expressing Amacrine Cells in DAT-tdTomato Mouse. Invest Ophthalmol Vis Sci. 2022;63:13.
54. Yan W, Laboulaye MA, Tran NM, Whitney IE, Benhar I, Sanes JR. Mouse Retinal Cell Atlas: Molecular Identification of over Sixty Amacrine Cell Types. J Neurosci. 2020;40:5177–95.
55. Musser JM, Arendt D. Loss and gain of cone types in vertebrate ciliary photoreceptor evolution. Developmental Biology. 2017;431:26–35.
56. Sapkota D, Chintala H, Wu F, Fliesler SJ, Hu Z, Mu X. Onecut1 and Onecut2 redundantly regulate early retinal cell fates during development. Proc Natl Acad Sci USA [Internet]. 2014 [cited 2023 Nov 26];111. Available from: https://pnas.org/doi/full/10.1073/pnas.1405354111
57. Fries M, Brown TW, Jolicoeur C, Boulan B, Boudreau-Pinsonneault C, Javed A, et al. Pou3f1 orchestrates a gene regulatory network controlling contralateral retinogeniculate projections. Cell Reports. 2023;42:112985.
58. Satou Y, Mineta K, Ogasawara M, Sasakura Y, Shoguchi E, Ueno K, et al. Improved genome assembly and evidence-based global gene model set for the chordate Ciona intestinalis: new insight into intron and operon populations. Genome Biol. 2008;9:R152.
59. Zheng GXY, Terry JM, Belgrader P, Ryvkin P, Bent ZW, Wilson R, et al. Massively parallel digital transcriptional profiling of single cells. Nat Commun. 2017;8:14049.
60. Hao Y, Hao S, Andersen-Nissen E, Mauck WM, Zheng S, Butler A, et al. Integrated analysis of multimodal single-cell data. Cell. 2021;184:3573-3587.e29.
61. Loza M, Teraguchi S, Standley DM, Diez D. Unbiased integration of single cell transcriptome replicates. NAR Genomics and Bioinformatics. 2022;4:lqac022.
62. Durinck S, Moreau Y, Kasprzyk A, Davis S, De Moor B, Brazma A, et al. BioMart and Bioconductor: a powerful link between biological databases and microarray data analysis. Bioinformatics. 2005;21:3439–40.
63. Sayers EW, Bolton EE, Brister JR, Canese K, Chan J, Comeau DC, et al. Database resources of the national center for biotechnology information. Nucleic Acids Research. 2022;50:D20–6.
64. Korsunsky I, Millard N, Fan J, Slowikowski K, Zhang F, Wei K, et al. Fast, sensitive and accurate integration of single-cell data with Harmony. Nat Methods. 2019;16:1289–96.
65. Shen W-K, Chen S-Y, Gan Z-Q, Zhang Y-Z, Yue T, Chen M-M, et al. AnimalTFDB 4.0: a comprehensive

animal transcription factor database updated with variation and expression annotations. Nucleic Acids Research. 2023;51:D39–45.
66. Castro-Mondragon JA, Riudavets-Puig R, Rauluseviciute I, Berhanu Lemma R, Turchi L, Blanc-Mathieu R, et al. JASPAR 2022: the 9th release of the open-access database of transcription factor binding profiles. Nucleic Acids Research. 2022;50:D165–73.
67. Weirauch MT, Yang A, Albu M, Cote AG, Montenegro-Montero A, Drewe P, et al. Determination and Inference of Eukaryotic Transcription Factor Sequence Specificity. Cell. 2014;158:1431–43.
68. Grau J, Grosse I, Posch S, Keilwagen J. Motif clustering with implications for transcription factor interactions. PeerJ PrePrints; 2015.
69. Kulakovskiy IV, Vorontsov IE, Yevshin IS, Sharipov RN, Fedorova AD, Rumynskiy EI, et al. HOCOMOCO: towards a complete collection of transcription factor binding models for human and mouse via large-scale ChIP-Seq analysis. Nucleic Acids Research. 2018;46:D252–9.
70. Nitta KR, Jolma A, Yin Y, Morgunova E, Kivioja T, Akhtar J, et al. Conservation of transcription factor binding specificities across 600 million years of bilateria evolution. eLife. 2015;4:e04837.
71. Wolf FA, Angerer P, Theis FJ. SCANPY: large-scale single-cell gene expression data analysis. Genome Biol. 2018;19:15.
72. Lange M, Bergen V, Klein M, Setty M, Reuter B, Bakhti M, et al. CellRank for directed single-cell fate mapping. Nat Methods. 2022;1–12.
73. Yu G, Wang L-G, Han Y, He Q-Y. clusterProfiler: an R Package for Comparing Biological Themes Among Gene Clusters. OMICS: A Journal of Integrative Biology. 2012;16:284–7.
74. Cunningham F, Allen JE, Allen J, Alvarez-Jarreta J, Amode MR, Armean IM, et al. Ensembl 2022. Nucleic Acids Research. 2022;50:D988–95.

Figure Legends

Fig. 1 Workflow illustrating the transcriptional analysis of vertebrate retinal cells and their comparison with the invertebrate species *Ciona*.

a scRNA-seq data from various stages of three representative species are collected and annotated. **b** Transcriptome similarity analysis showing transcriptome conservation among homologous retinal cells in vertebrates. **c** GRN analysis indicating cell type-specific regulatory networks and evolutionarily preserved regulatory frameworks within each vertebrate species. **d** RNA velocity-based analysis revealing conserved driver genes responsible for retinal cell differentiation in mouse and zebrafish. **e** Extraction of key differentiation-driving regulators through intersecting regulatory networks and RNA velocities. **f** scRNA-seq data from *Ciona* are collected. **g** Identification of genes with shared

expression between vertebrate retinal cells and PR-related cells in *Ciona*, implying a potential ancestral link in their origin.

Fig. 2 Comparative single-cell transcriptomic landscapes of retinal cells in mouse, chicken, and zebrafish

a-c UMAP plots of retinal scRNA-seq data from mouse (**a**, N = 100,506), chicken (**b**, N = 86,056), and zebrafish (**c**, N = 49,311). **d-f** Proportions of cell types within each stage for mouse (**d**), chicken (**e**), and zebrafish (**f**). Red indicates a high proportion, blue indicates a low proportion. **g** Heatmap of AUROC scores computed using MetaNeighbor, indicating similarity among cell types based on orthologous gene expression from mouse, chicken, and zebrafish. In the heatmap, cell type prefixes denote their origin: 'M-' for mouse, 'C-' for chicken, and 'Z-' for zebrafish. Red indicates high similarity, blue indicates low similarity. **h** Unsupervised clustering of pseudo-cells into six clusters displayed in UMAP. **i** Heatmap displaying the proportion of each cell type within the 6 clusters identified in **h**. Red indicates a high proportion; blue indicates a low proportion.

Fig. 3 Gene regulatory network analysis in vertebrate retinal cells

a Number of identified and filtered regulons from scRNA-seq data across different stages in mouse, chicken, and zebrafish. Bar represents the number of regulons that have been filtered and identified from scRNA-seq data at distinct stages in the respective species. **b−d** Heatmaps of scaled regulon activity across different species and stages, including (**b**) mouse at E18, (**c**) chicken at E18, and (**d**) zebrafish at 72hpf. Each row represents a distinct regulon and each column denotes a specific cell type. Representative regulons for each cell type are highlighted in the right panel. Each regulon is

named following the format "TF (N)", where "TF" represents the regulator and "N" denotes the number of target genes in that regulon. Red indicates high activity, blue indicates low activity. **e-h** GRN derived from the regulons highlighted in **b-d**. (**e**) mouse E18 glycine ACs, (**f**) mouse E18 cones, (**g**) chicken E18 MG, and (**h**) Zebrafish 72hpf HC. The networks show the interplay among genes and regulators within the respective cell types. Purple nodes represent regulators, gray nodes represent target genes. Red edges indicate regulatory interactions between regulators, gray edges highlight regulatory relationships of regulators with other genes. The layout of the networks is based on centrality, emphasizing the significance of certain nodes within the GRN.

Fig. 4 Cross-species analysis of active regulators and regulons in homologous retinal cells

a Venn diagram illustrating the overlap of regulators in mouse, chicken, and zebrafish. **b** UMAP visualizations of Gbx2 (top) and Maf (bottom) among the shared regulators that are specifically actived in ACs in (**a**). Order from left to right: mouse, chicken, zebrafish. Blue indicates high expression, grey indicates low expression. **c-e** Average scaled activity of cell type-specific regulons generated from mouse (**c**), chicken (**d**) and zebrafish (**e**) in the homologous cell type from other two species. Dashed lines at 1.6 (both horizontal and vertical) indicate the thresholds for conserved regulons.

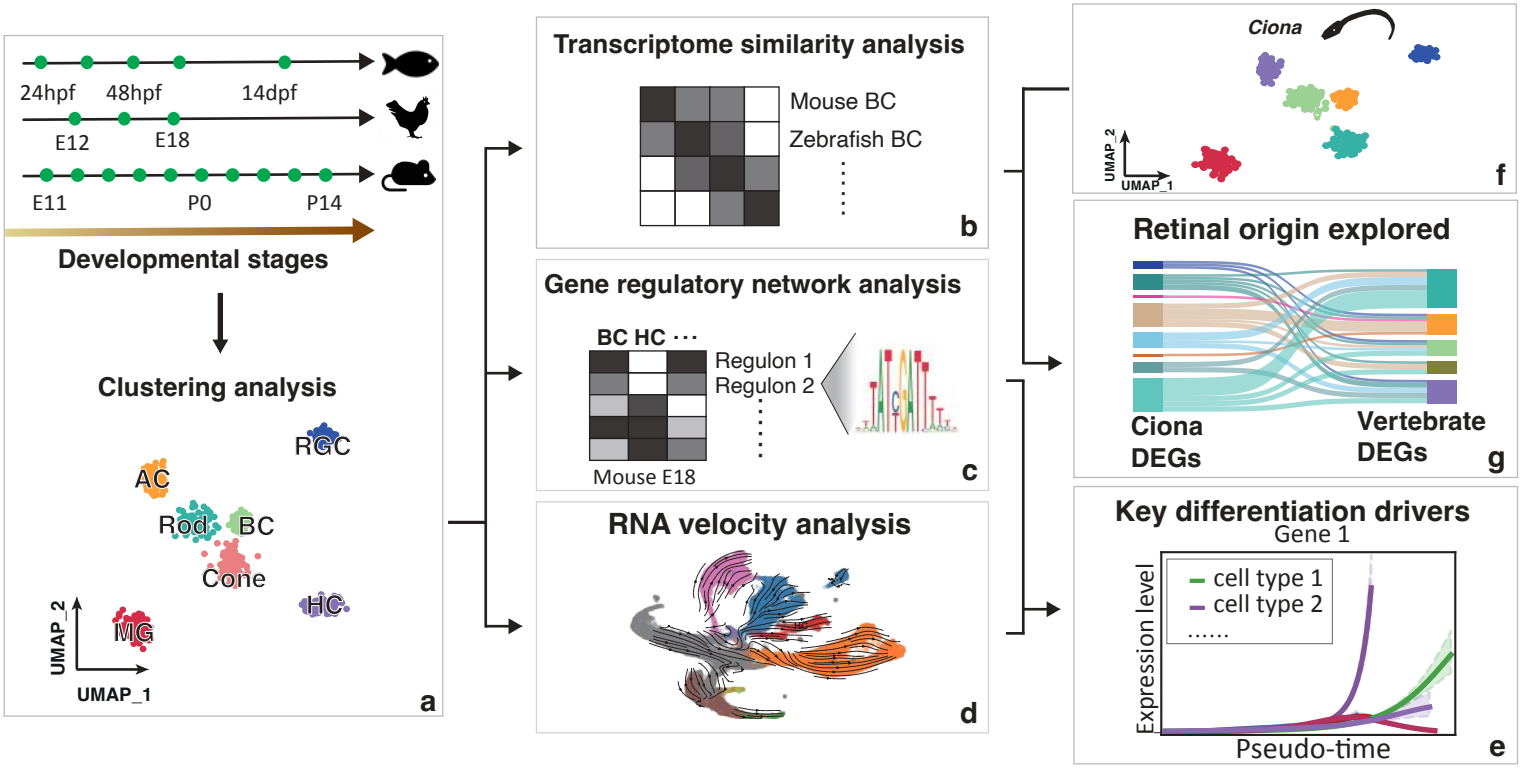
Fig. 5 Analysis of RNA velocities, shared driver genes, and regulators that drive differentiation in retinal cell development of mouse and zebrafish

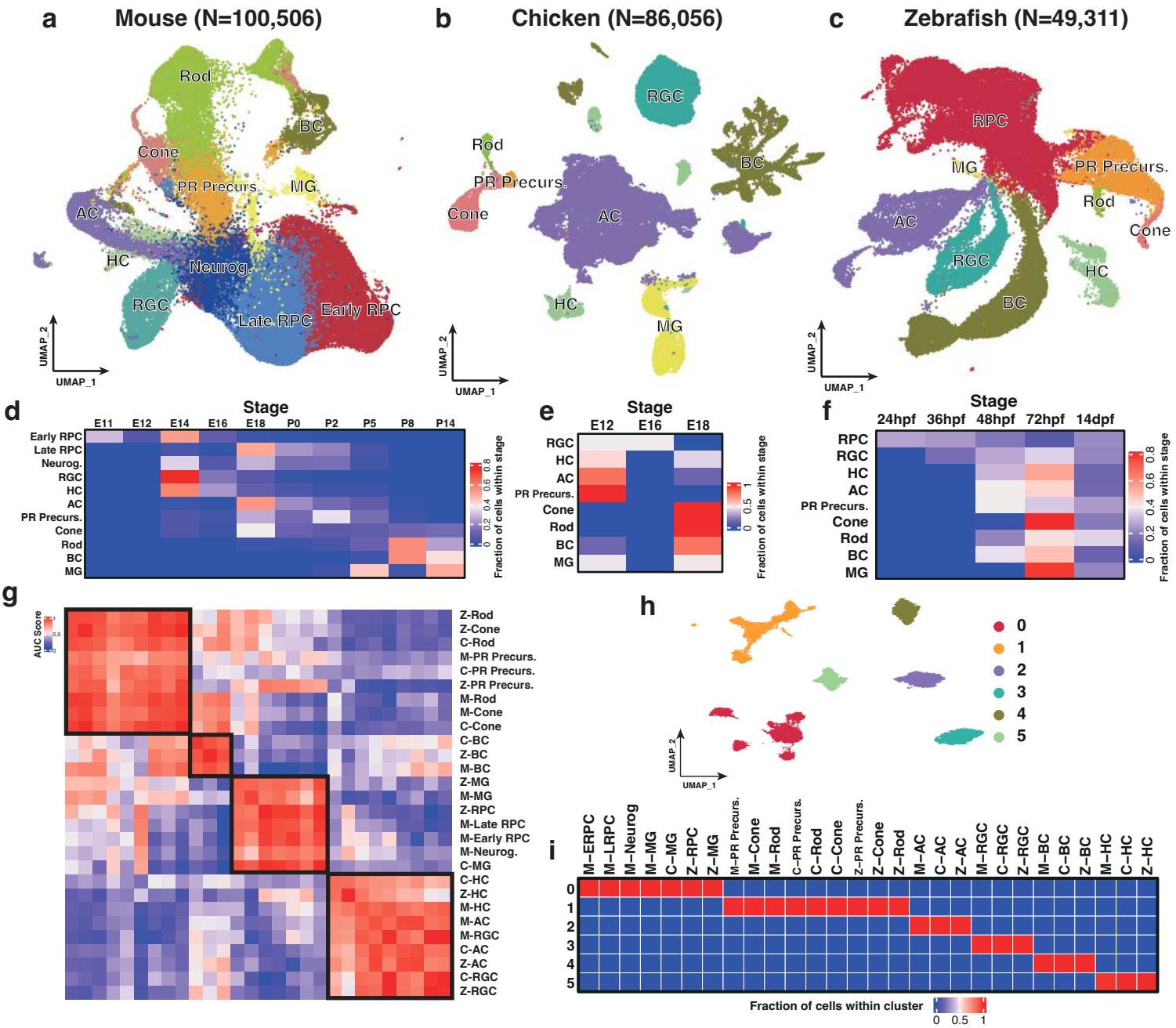
a, b UMAP-based projections of RNA velocities for retinal cell differentiation in mouse (**a**) and zebrafish (**b**). **c** Venn diagram illustrating the relationships among mouse driver genes, mouse

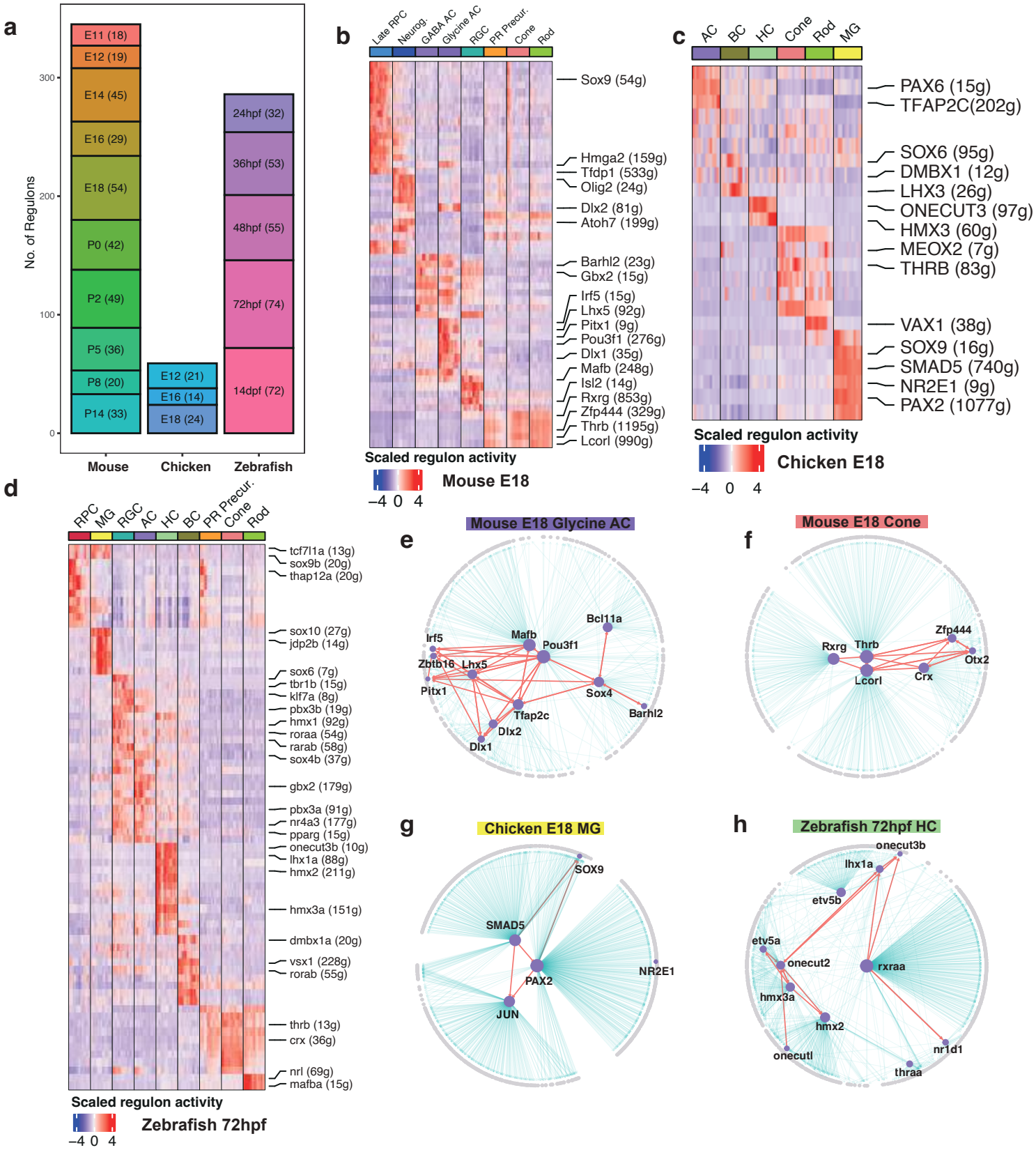
regulators, zebrafish regulators, and zebrafish driver genes. The intersections highlight (1) shared driver genes between mouse and zebrafish and (2) cell type-specific regulators that drive retinal cell development in mouse and zebrafish. **d** Bar chart representing the number of shared driver genes among the top 100 cell type-specific driver genes in both mouse and zebrafish. **e, f** Expression heatmaps of shared driver genes in ACs across diffusion pseudotime for mouse (**e**) and zebrafish (**f**). **g, h** Cell type-specific regulators that drive differentiation in mouse (**g**) and zebrafish (**h**)

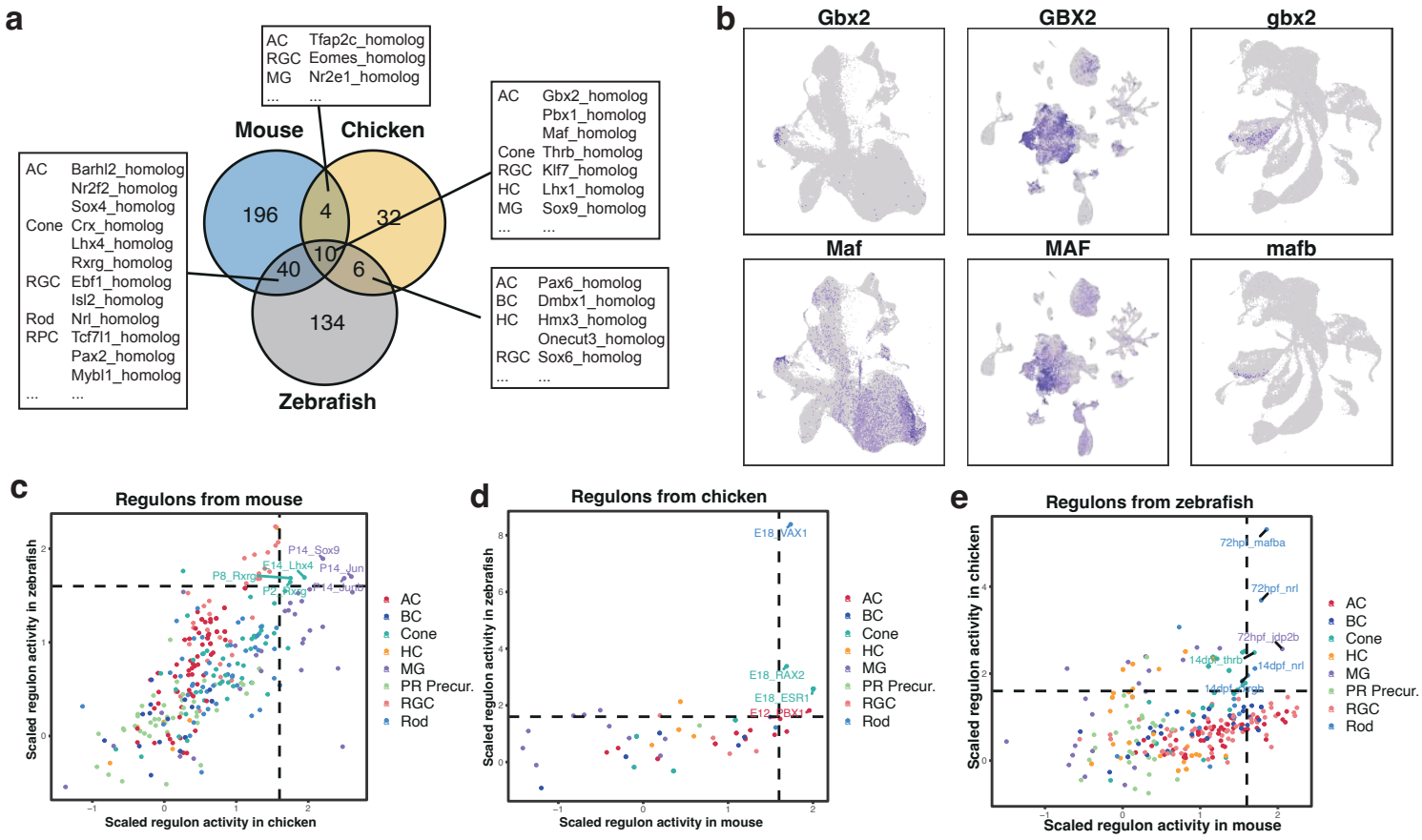
Fig. 6 Exploration of the origin of the vertebrate retina

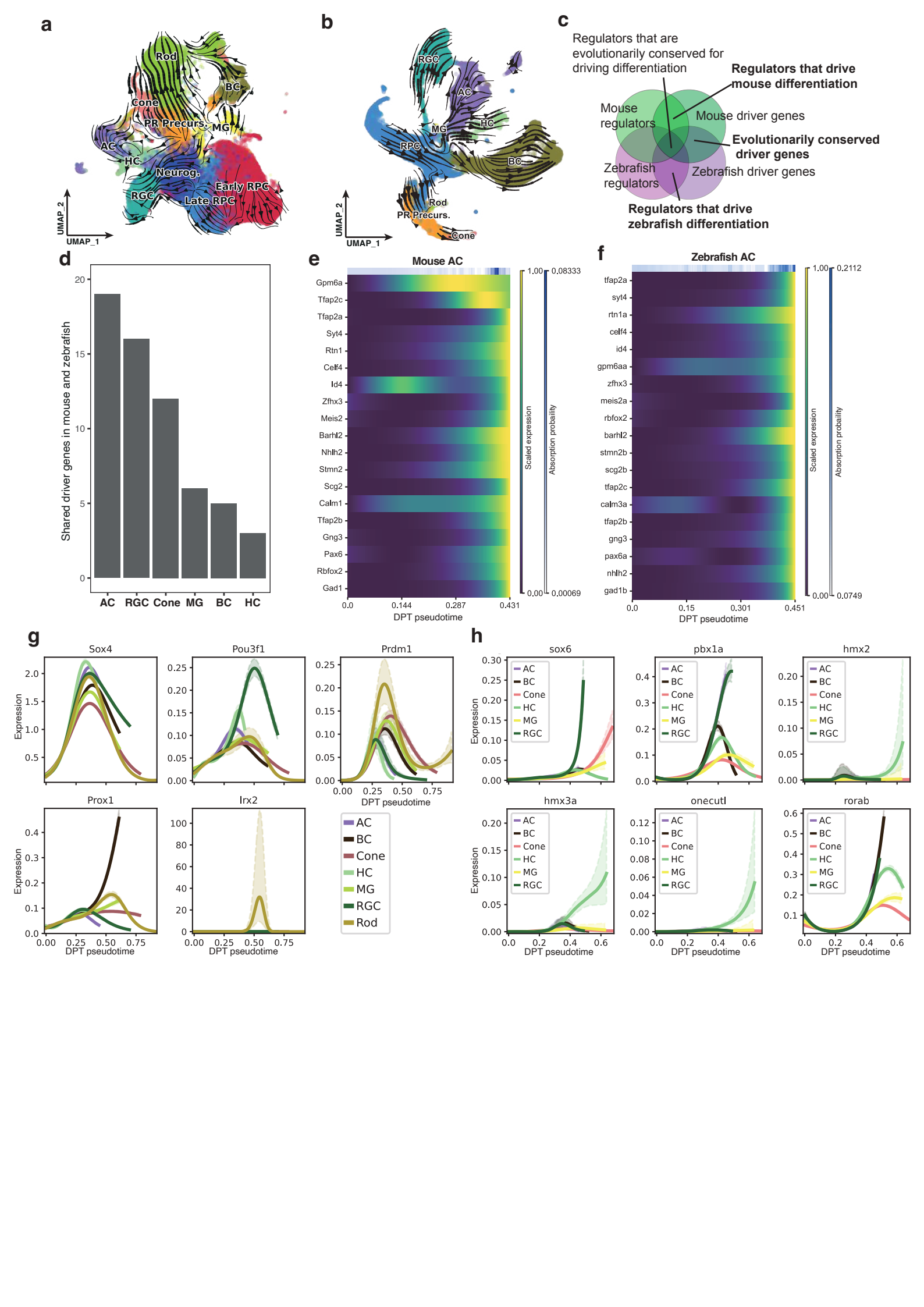
a Heatmap of the top 10 conserved DEGs in homologous cell types of the vertebrate retina. Red indicates high expression, blue indicates low expression. **b** UMAP visualization of scRNA-seq data from *Ciona* PR-related cells. **c** Venn diagram showing overlapping DEGs between vertebrate retina cells and Ciona PR-related cells, used to infer the origin of vertebrate retina. **d** Sankey diagram showing shared DEGs between *Ciona* PR-related cells and vertebrate retinal cells. **e-h** UMAP visualizations of gene expression levels across the four species. Each panel represents the expression of a specific gene: (**e**) *Pdc*-orth, (**f**) *Lhx4*-orth, (**g**) *Rbpms2*-orth, and (**h**) *Slc32a1*-orth. Order from left to right: mouse, chicken, zebrafish, and *Ciona*. Blue indicates high expression, gray indicates low expression.

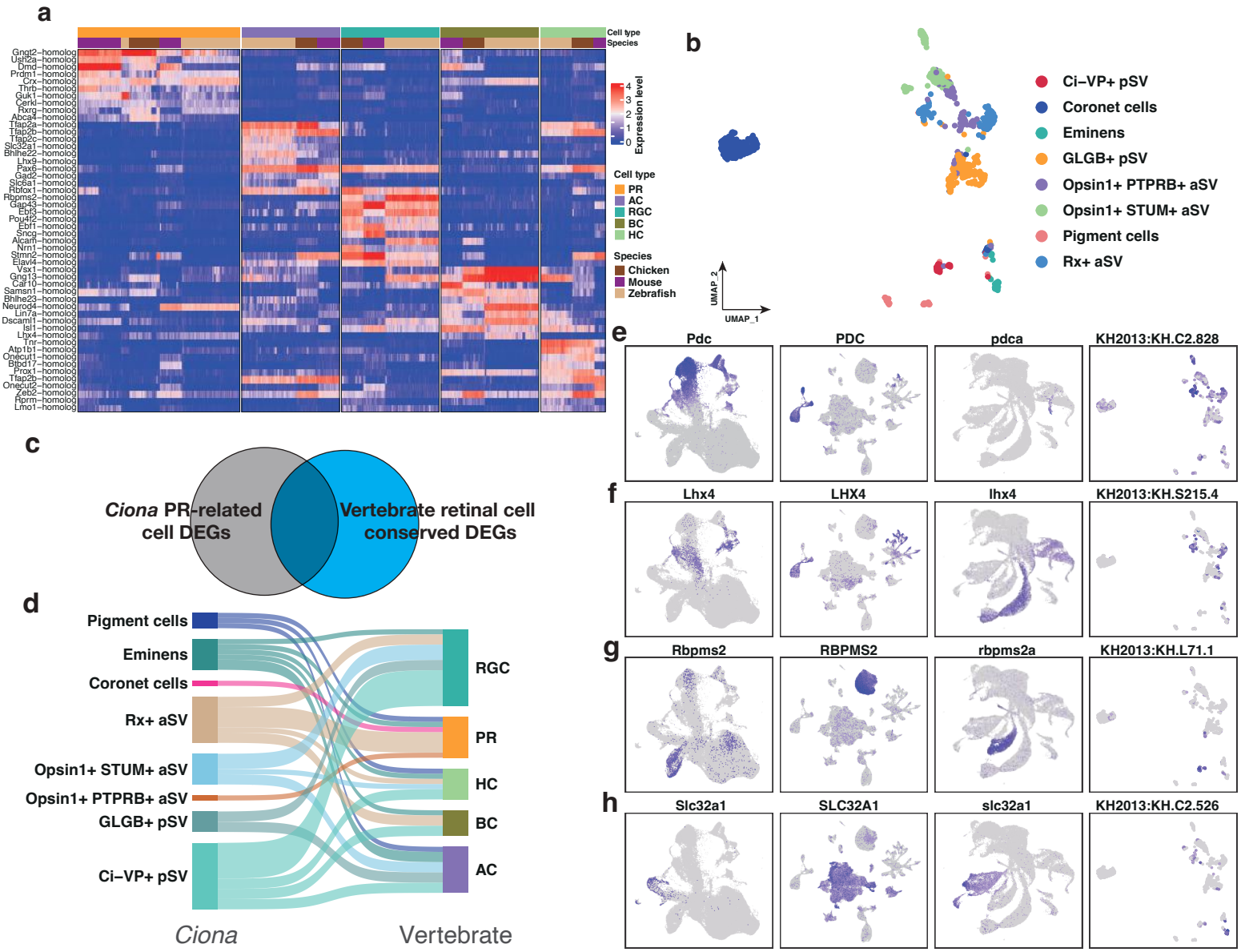

24hpf
48hpf
14dpf
E12
E18
E11
P0
P14
Developmental stages
Clustering analysis
RGC
AC
Rod
BC
Cone
HC
MG
UMAP_2
UMAP_1
a
Transcriptome similarity analysis
Mouse BC
Zebrafish BC
b
Gene regulatory network analysis
BC HC ···
Regulon 1
Regulon 2
Mouse E18
c
RNA velocity analysis
d
Ciona
UMAP_2
UMAP_1
f
Retinal origin explored
Ciona DEGs
Vertebrate DEGs
g
Key differentiation drivers
Gene 1
cell type 1
cell type 2
.....
Expression level
Pseudo-time
e


a
Mouse (N=100,506)
Rod
BC
Cone
PR Precurs.
MG
AC
HC
Neurog.
RGC
Late RPC
Early RPC
UMAP_2
UMAP_1
b
Chicken (N=86,056)
RGC
Rod
BC
PR Precurs.
Cone
AC
HC
MG
UMAP_2
UMAP_1
c
Zebrafish (N=49,311)
RPC
MG
PR Precurs.
Rod
Cone
AC
RGC
HC
BC
UMAP_2
UMAP_1
d
Stage
E11 E12 E14 E16 E18 P0 P2 P5 P8 P14
Early RPC
Late RPC
Neurog.
RGC
HC
AC
PR Precurs.
Cone
Rod
BC
MG
Fraction of cells within stage
e
Stage
E12 E16 E18
RGC
HC
AC
PR Precurs.
Cone
Rod
BC
MG
Fraction of cells within stage
f
Stage
24hpf 36hpf 48hpf 72hpf 14dpf
RPC
RGC
HC
AC
PR Precurs.
Cone
Rod
BC
MG
Fraction of cells within stage
g
AUC Score
Z-Rod
Z-Cone
C-Rod
M-PR Precurs.
C-PR Precurs.
Z-PR Precurs.
M-Rod
M-Cone
C-Cone
C-BC
Z-BC
M-BC
Z-MG
M-MG
Z-RPC
M-Late RPC
M-Early RPC
M-Neurog.
C-MG
C-HC
Z-HC
M-HC
M-AC
M-RGC
C-AC
Z-AC
C-RGC
Z-RGC
h
0
1
2
3
4
5
UMAP_2
UMAP_1
i
M-ERPC M-LRPC M-Neurog M-MG C-MG Z-RPC Z-MG M-PR Precurs. M-Cone M-Rod C-PR Precurs. C-Rod C-Cone Z-PR Precurs. Z-Cone Z-Rod M-AC C-AC Z-AC M-RGC C-RGC Z-RGC M-BC C-BC Z-BC M-HC C-HC Z-HC
0
1
2
3
4
5
Fraction of cells within cluster


a
No. of Regulons
300
200
100
0
E11 (18)
E12 (19)
E14 (45)
E16 (29)
E18 (54)
P0 (42)
P2 (49)
P5 (36)
P8 (20)
P14 (33)
E12 (21)
E16 (14)
E18 (24)
24hpf (32)
36hpf (53)
48hpf (55)
72hpf (74)
14dpf (72)
Mouse
Chicken
Zebrafish
b
Late RPC
Neurog.
GABA AC
Glycine AC
RGC
PR Precur.
Cone
Rod
Sox9 (54g)
Hmga2 (159g)
Tfdp1 (533g)
Olig2 (24g)
Dlx2 (81g)
Atoh7 (199g)
Barhl2 (23g)
Gbx2 (15g)
Irf5 (15g)
Lhx5 (92g)
Pitx1 (9g)
Pou3f1 (276g)
Dlx1 (35g)
Mafb (248g)
Isl2 (14g)
Rxrg (853g)
Zfp444 (329g)
Thrb (1195g)
Lcorl (990g)
Scaled regulon activity
−4 0 4
Mouse E18
c
AC
BC
HC
Cone
Rod
MG
PAX6 (15g)
TFAP2C(202g)
SOX6 (95g)
DMBX1 (12g)
LHX3 (26g)
ONECUT3 (97g)
HMX3 (60g)
MEOX2 (7g)
THRB (83g)
VAX1 (38g)
SOX9 (16g)
SMAD5 (740g)
NR2E1 (9g)
PAX2 (1077g)
Scaled regulon activity
−4 0 4
Chicken E18
d
RPC
MG
RGC
AC
HC
BC
PR Precur.
Cone
Rod
tcf7l1a (13g)
sox9b (20g)
thap12a (20g)
sox10 (27g)
jdp2b (14g)
sox6 (7g)
tbr1b (15g)
klf7a (8g)
pbx3b (19g)
hmx1 (92g)
roraa (54g)
rarab (58g)
sox4b (37g)
gbx2 (179g)
pbx3a (91g)
nr4a3 (177g)
pparg (15g)
onecut3b (10g)
lhx1a (88g)
hmx2 (211g)
hmx3a (151g)
dmbx1a (20g)
vsx1 (228g)
rorab (55g)
thrb (13g)
crx (36g)
nrl (69g)
mafba (15g)
Scaled regulon activity
−4 0 4
Zebrafish 72hpf
e
Mouse E18 Glycine AC
Bcl11a
Irf5
Mafb
Pou3f1
Zbtb16
Lhx5
Pitx1
Sox4
Tfap2c
Barhl2
Dlx2
Dlx1
f
Mouse E18 Cone
Zfp444
Rxrg
Thrb
Otx2
Lcorl
Crx
g
Chicken E18 MG
SOX9
SMAD5
PAX2
NR2E1
JUN
h
Zebrafish 72hpf HC
onecut3b
lhx1a
etv5b
etv5a
onecut2
rxraa
hmx3a
hmx2
nr1d1
onecutl
thraa


a
Mouse
Chicken
Zebrafish
196
4
32
10
40
6
134
AC Tfap2c_homolog
RGC Eomes_homolog
MG Nr2e1_homolog
... ...
AC Barhl2_homolog
Nr2f2_homolog
Sox4_homolog
Cone Crx_homolog
Lhx4_homolog
Rxrg_homolog
RGC Ebf1_homolog
Isl2_homolog
Rod Nrl_homolog
RPC Tcf7l1_homolog
Pax2_homolog
Mybl1_homolog
... ...
AC Gbx2_homolog
Pbx1_homolog
Maf_homolog
Cone Thrb_homolog
RGC Klf7_homolog
HC Lhx1_homolog
MG Sox9_homolog
... ...
AC Pax6_homolog
BC Dmbx1_homolog
HC Hmx3_homolog
Onecut3_homolog
RGC Sox6_homolog
... ...
b
Gbx2
GBX2
gbx2
Maf
MAF
mafb
c
Regulons from mouse
P14_Sox9
E14_Lhx4
P14_Jun
P8_Rxrg
Scaled regulon activity in zebrafish
Scaled regulon activity in chicken
d
Regulons from chicken
E18_VAX1
E18_HAX2
E18_ESR1
E12_PBX1
Scaled regulon activity in zebrafish
Scaled regulon activity in mouse
e
Regulons from zebrafish
72hpf_mafba
72hpf_nrl
72hpf_idp2b
14dpf_thrb
14dpf_nrl
Scaled regulon activity in chicken
Scaled regulon activity in mouse
AC
BC
Cone
HC
MG
PR Precur.
RGC
Rod


a
Rod
BC
Cone
PR Precurs.
MG
AC
HC
Neurog.
Early RPC
RGC
Late RPC
UMAP_2
UMAP_1
b
RGC
AC
HC
MG
RPC
BC
Rod
PR Precurs.
Cone
UMAP_2
UMAP_1
c
Regulators that are evolutionarily conserved for driving differentiation
Regulators that drive mouse differentiation
Mouse regulators
Mouse driver genes
Evolutionarily conserved driver genes
Zebrafish regulators
Zebrafish driver genes
Regulators that drive zebrafish differentiation
d
Shared driver genes in mouse and zebrafish
AC
RGC
Cone
MG
BC
HC
e
Mouse AC
Gpm6a
Tfap2c
Tfap2a
Syt4
Rtn1
Celf4
Id4
Zfhx3
Meis2
Barhl2
Nhlh2
Stmn2
Scg2
Calm1
Tfap2b
Gng3
Pax6
Rbfox2
Gad1
Scaled expression
Absorption probability
DPT pseudotime
f
Zebrafish AC
tfap2a
syt4
rtn1a
celf4
id4
gpm6aa
zfhx3
meis2a
rbfox2
barhl2
stmn2b
scg2b
tfap2c
calm3a
tfap2b
gng3
pax6a
nhlh2
gad1b
Scaled expression
Absorption probability
DPT pseudotime
g
Sox4
Pou3f1
Prdm1
Prox1
Irx2
Expression
DPT pseudotime
AC
BC
Cone
HC
MG
RGC
Rod
h
sox6
pbx1a
hmx2
hmx3a
onecutl
rorab
Expression
DPT pseudotime
AC
BC
Cone
HC
MG
RGC


a
Cell type
Species
Expression level
Cell type
PR
AC
RGC
BC
HC
Species
Chicken
Mouse
Zebrafish
b
Ci–VP+ pSV
Coronet cells
Eminens
GLGB+ pSV
Opsin1+ PTPRB+ aSV
Opsin1+ STUM+ aSV
Pigment cells
Rx+ aSV
UMAP_1
UMAP_2
c
Ciona PR-related cell DEGs
Vertebrate retinal cell conserved DEGs
d
Pigment cells
Eminens
Coronet cells
Rx+ aSV
Opsin1+ STUM+ aSV
Opsin1+ PTPRB+ aSV
GLGB+ pSV
Ci–VP+ pSV
RGC
PR
HC
BC
AC
Ciona
Vertebrate
e
Pdc
PDC
pdca
KH2013:KH.C2.828
f
Lhx4
LHX4
lhx4
KH2013:KH.S215.4
g
Rbpms2
RBPMS2
rbpms2a
KH2013:KH.L71.1
h
Slc32a1
SLC32A1
slc32a1
KH2013:KH.C2.526