

Supplementary Materials for

Phase transition specified by a binary code patterns the vertebrate eye cup

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Published 10 November 2021, *Sci. Adv.* 7, eabj9846 (2021)

DOI: 10.1126/sciadv.abj9846

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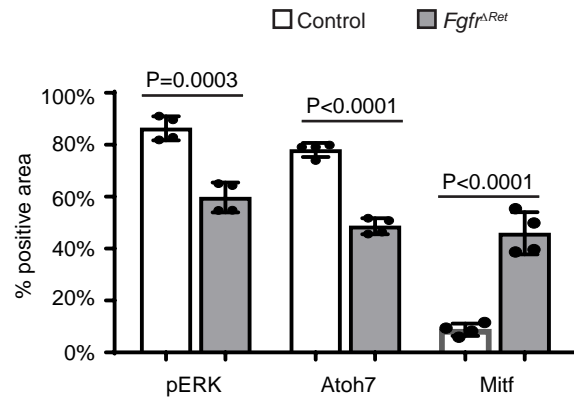


Figure S1. Quantification of FGF signaling mutant phenotype. The relative area expressing pERK, *Atoh7* or Mitf was normalized against the entire retina and the statistical significance was evaluated using Students' t test. *n*=4 for all markers.

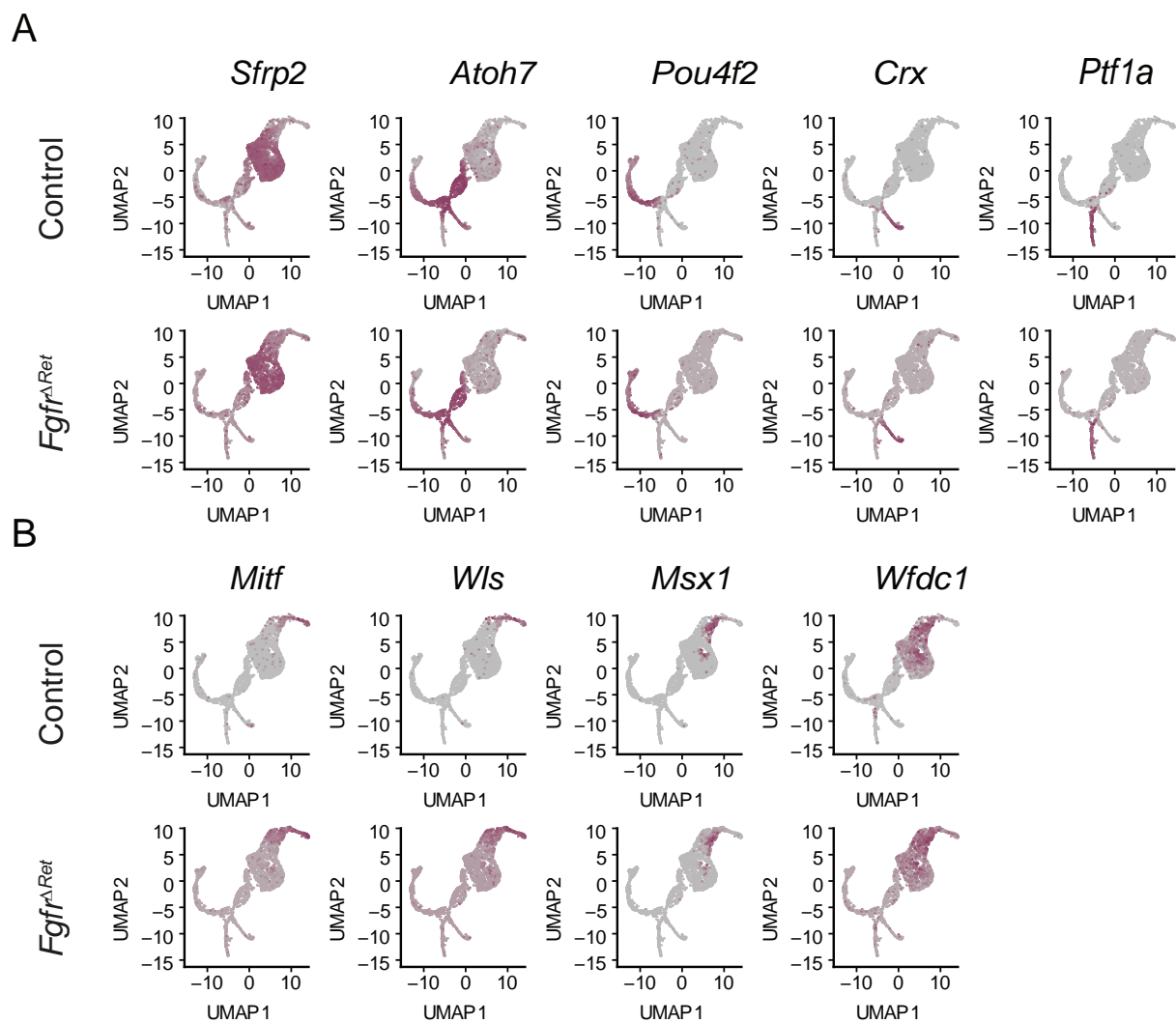


Figure S2. Identification of single-cell clusters by known cell type specific markers. (A) Feature plots identified RPC clusters expressing *Sfrp2*, neurogenic clusters expressing *Atoh7*, the RGC cluster expressing *Pou4f2*, the photoreceptor cluster expressing *Crx* and the AC/HC cluster expressing *Ptf1a*. **(B)** Feature plots of the CM specific genes. Notice the increased expression of *Mitf* and *Wls*, the reduced expression of *Wfdc1* and the loss of *Msx1* in *Fgfr^{ΔRet}* mutants.

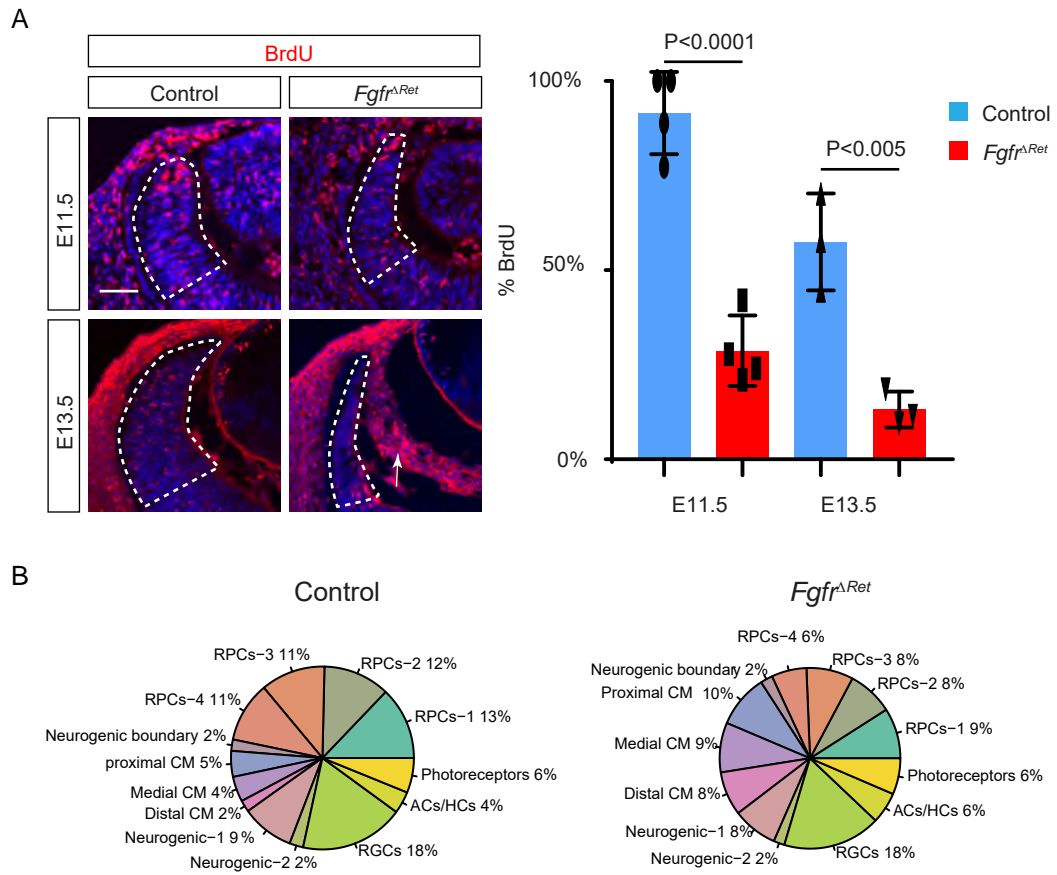


Figure S3. Loss of FGF signaling promoted differentiation of RPCs instead of proliferation. (A) The percentages of BrdU+ versus DAPI+ cells were significantly reduced in the peripheral retina (circled in dotted lines) in *Fgfr*^{ΔRet} mutants. Arrow points to hyaloid cells in the vitreous. Scale bar: 50 μm. (B) The proportions of CM clusters in *Fgfr*^{ΔRet} mutants were increased at the expense of RPCs.

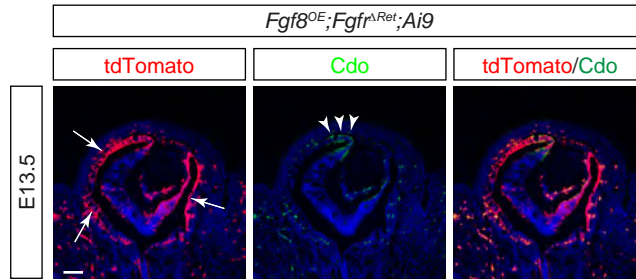


Figure S4. Characterization of the *Fgfr^{ΔRet};Fgf8^{OE};Ai9* mutant. Although Cre activities were induced throughout the presumptive RPE in the *Fgfr^{ΔRet};Fgf8^{OE};Ai9* mutant, the CM marker Cdo was still restricted to the distal eye cup.

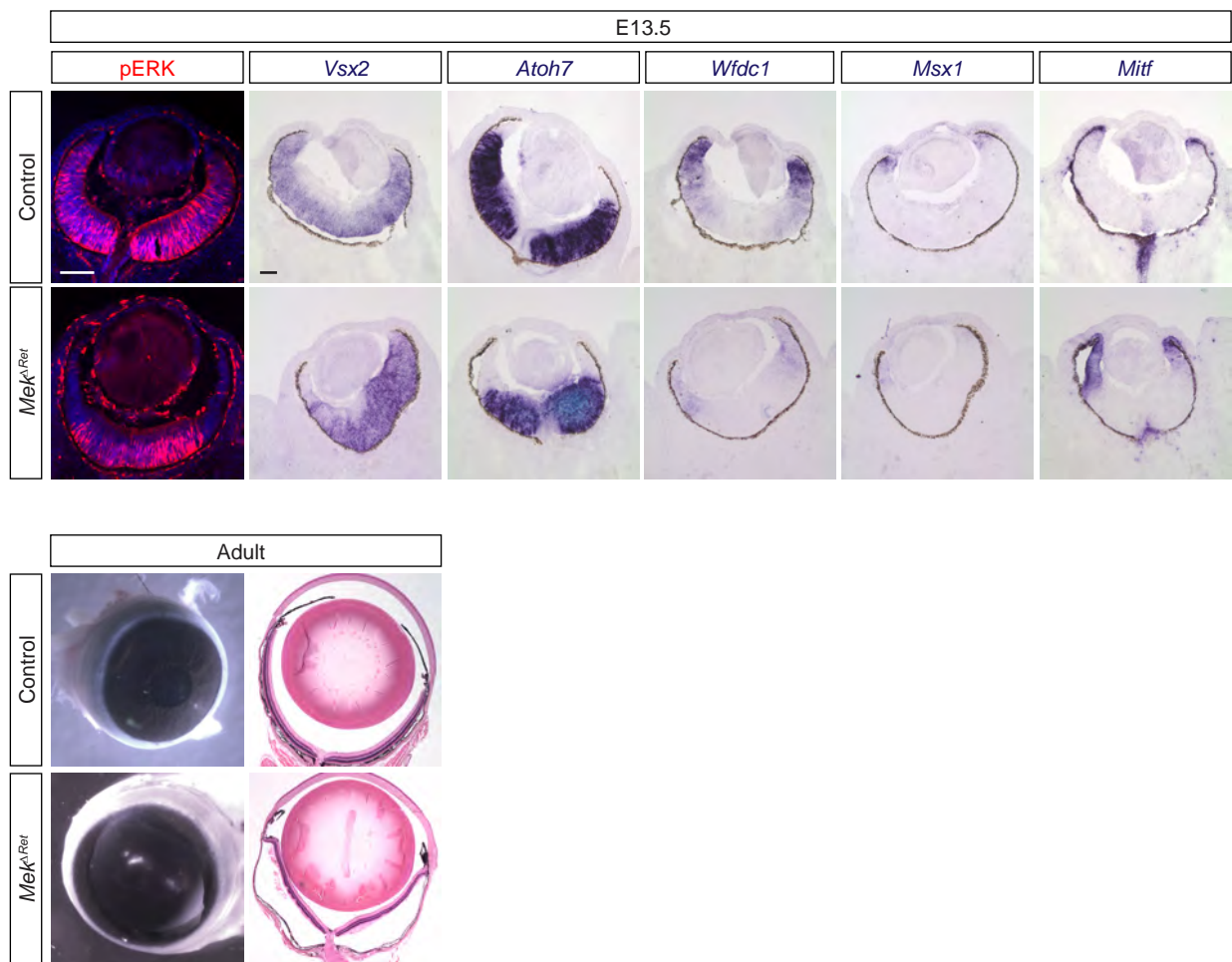


Figure S5: Inactivation of MAPK pathway phenocopied FGF signaling defects. Genetic ablation of *Mek1* and *2* in *Mek^{ARet}* mutants abolished pERK staining in the peripheral retina. This led to reduced expression of retinal markers *Vsx2* and *Atoh7*, loss of CM markers *Wfdc1* and *Msx1* and expansion of the RPE marker *Mitf*. The adult *Mek^{ARet}* animals also resemble *Fgf^{ARet}* mutants in the iris and ciliary body defects. Scale bars: 50 μ m.

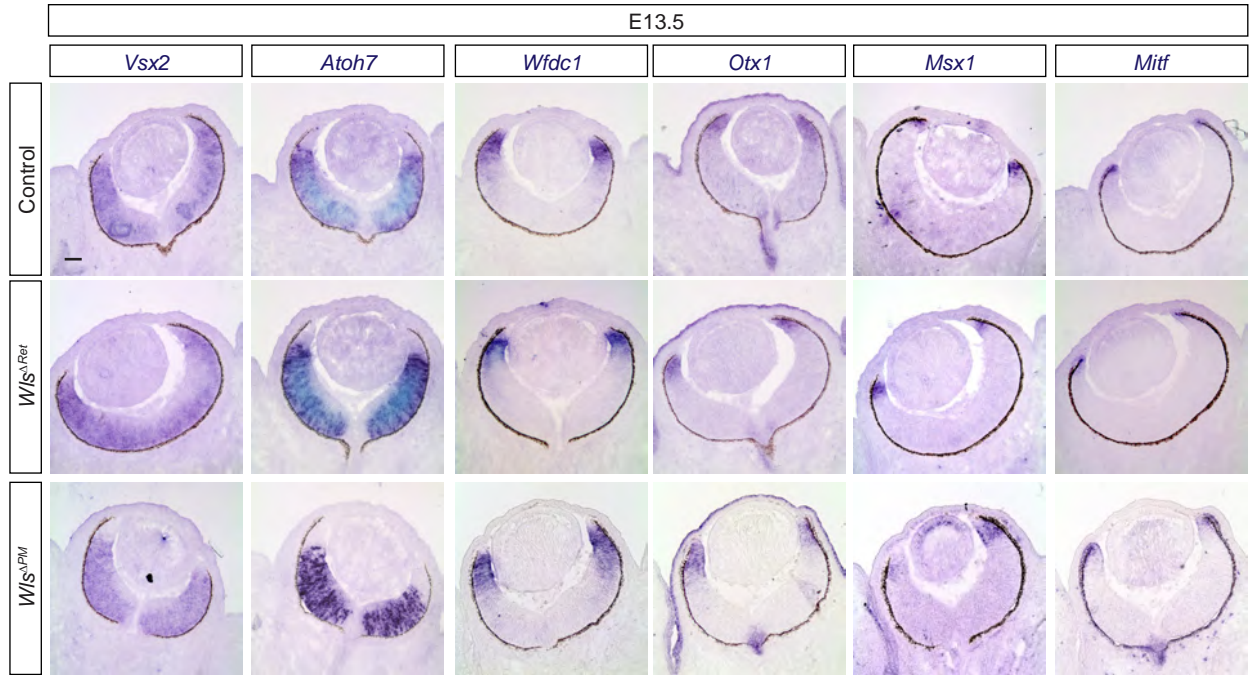


Figure S6: *Wls* is dispensable in the peripheral retina and the periocular mesenchyme. No retinal phenotype was observed in *Wls^{ΔRet}* where *Wls* was deleted in the peripheral retina and in *Wls^{ΔPM}* where *Wls* was deleted in the periocular mesenchyme. Scale bar: 50 μ m.

Supplementary Table 1: List of Mice Used in the Study

Reagent type or Resource	Designation	Reference	Identifiers	Source
Genetic reagent (<i>M.Musculus</i>)	<i>Ai9</i>	PMID: 20023653	RRID: MGI:3817869	Jackson Laboratory Stock #: 007908
Genetic reagent (<i>M.Musculus</i>)	β -catenin ^{fl3}	PMID: 10545105	RRID: MGI:1858008	Dr. Stavroula Kousteni (Columbia University Medical Center)
Genetic reagent (<i>M.Musculus</i>)	β -Catenin ^{fllox}	PMID: 11262227	RRID: MGI:2148567	Jackson Laboratory Stock #: 004152
Genetic reagent (<i>M.Musculus</i>)	<i>Fgf3</i> ^{fllox}	PMID: 20171206	RRID: MGI:4456396	Dr. Suzanne L Mansour (University of Utah)
Genetic reagent (<i>M.Musculus</i>)	<i>Fgf9</i> ^{fllox}	PMID: 16496342	RRID: MGI:3621452	
Genetic reagent (<i>M.Musculus</i>)	<i>Fgfr1</i> ^{fllox}	PMID:16421190	RRID: MGI:3713779	Jackson Laboratory Stock #: 007671
Genetic reagent (<i>M.Musculus</i>)	<i>Fgfr2</i> ^{fllox}	PMID: 12756187	RRID: MGI:3044690	Dr. David Ornitz (Washington University Medical School)
Genetic reagent (<i>M.Musculus</i>)	<i>Mek1</i> ^{fllox}	PMID: 16887817	RRID: MGI:3714918	Dr. Jean Charron (Universite' Laval)
Genetic reagent (<i>M.Musculus</i>)	<i>Mek2</i> ^{-/-}	PMID: 12832465	RRID: MGI:2668345	Dr. Jean Charron (Universite' Laval)
Genetic reagent (<i>M.Musculus</i>)	<i>Msx1-Cre</i> ^{ERT2}	PMID: 21693521	RRID: MGI:5049923	Jackson Laboratory

				Stock #:027850
Genetic reagent (<i>M.Musculus</i>)	<i>Pax6^α-Cre</i> (<i>α-Cre</i>)	PMID: 11301001	RRID: MGI:3052661	Dr. Nadean Brown (Children's Hospital Research Foundation)
Genetic reagent (<i>M.Musculus</i>)	<i>Pax6^{Le}-Cre</i> (<i>Le-Cre</i>)	PMID: 11069887	RRID: MGI:3045795	Dr. Richard Lang (Children's Hospital Research Foundation)
Genetic reagent (<i>M.Musculus</i>)	<i>R26^{LSL-Fgf8}</i>	PMID: 23358455	RRID: MGI:5694148	Dr. Yiping Chen (Tulane University)
Genetic reagent (<i>M.Musculus</i>)	<i>R26^{LSL-Wnt1}</i>	PMID: 16054034	RRID: MGI:3589804	Dr. Thomas Carroll (UT Southwestern)
Genetic reagent (<i>M.Musculus</i>)	<i>Wls^{fllox}</i>	PMID: 20614471	RRID: MGI:4452362	Jackson Laboratory Stock #: 012888
Genetic reagent (<i>M.Musculus</i>)	<i>Wnt1-Cre</i>	PMID: 9843687	RRID: MGI:2386570	Jackson Laboratory Stock #: 003829

Supplementary Table 2: List of Antibodies used in the Study

Reagent type or Resource	Designation	Source	Identifiers	Additional Information
Antibody	Goat anti- Cdo	R&D Systems	RRID:AB_2078891	IHC 1:1000
Antibody	Rabbit anti-Cx43	Cell Signaling Technology	RRID: AB_2294590	IHC 1:400
Antibody	Rabbit anti- β catenin (β^{CTD})	Sigma-Aldrich	RRID: AB_476831	IHC 1:200
Antibody	Rabbit anti-Non-phospho β -catenin (β^{Ser45})	Cell Signaling Technology	RRID: AB_2650576	IHC 1:200
Antibody	Mouse anti-Brdu	Developmental Studies Hybridoma Bank	RRID:AB_1157913	IHC 1:200
Antibody	Rabbit anti-GFP	Thermo Fisher Scientific	RRID:AB_221569	IHC 1:1000
Antibody	Chick anti-GFP	Aves Labs	RRID:AB_10000240	IHC 1:1000
Antibody	Rabbit anti-Laminin	Sigma-Aldrich	RRID: AB_477163	IHC 1:1000
Antibody	Rabbit anti-Lef1	Cell Signaling Technology	RRID:AB_659971	IHC 1:200
Antibody	Rabbit anti-Mitf	Abcam	RRID:AB_10902226	IHC 1:100
Antibody	Goat anti-Msx1	R&D Systems	RRID:AB_2148804	IHC 1:100
Antibody	Rabbit anti-NICD	Cell Signaling Technology	RRID: AB_2153348	IHC 1:100
Antibody	Goat anti-Otx2	Santa Cruz Biotechnology	RRID:AB_2157183	IHC 1:500
Antibody	Rabbit anti-RFP	Rockland Immunochemicals	RRID: AB_828390	IHC 1:1000
Antibody	Rabbit anti-Pax6	Covance Research Products Inc.	RRID:AB_291612	IHC 1:200
Antibody	Goat anti-Pcad	R&D Systems	RRID: AB_355581	IHC 1:200
Antibody	Rabbit anti-pERK	Cell Signaling Technology	RRID:AB_2095853	IHC 1:200
Antibody	Rat anti-Sox2	Thermo Fisher Scientific	RRID:AB_11219471	IHC 1:500

Antibody	Rabbit anti- Wls	Seven Hills Bioreagents	Catalogue number: RLAB-177	IHC 1:200
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Supplementary Table 3: List of ISH probes used in the study

Reagent type or Resource	Designation	Source	Identifiers
ISH probe	Atoh7 (Math5)	Dr. Tom Glaser (University of California Davis)	PMID: 16038893
ISH probe	Etv5	Dr. Bridget Hogan (Duke University Medical Center)	PMID: 15809037
ISH probe	Gja1	Dr. Carol Mason (Columbia University)	PMID: 30254141
ISH probe	Gli1	Dr. Alexandra Joyner (New York University Medical Center)	
ISH probe	Mitf	Dr. Hans Arnheiter (NIH)	PMID: 15576400
ISH probe	Msx1	Dr. Valerie Wallace (University of Toronto)	PMID: 17574231
ISH probe	Otx1	Dr. Valerie Wallace (University of Toronto)	PMID: 17574231
ISH probe	Spry2	Dr. Gail Martin (University of California San Francisco)	PMID: 15809037
ISH probe	Ttr	Dr. Carol Mason (Columbia University)	PMID: 30254141
ISH probe	Vsx2 (Chx10)	Dr. Roderick McInnes (McGill University)	PMID: 7914735
ISH probe	Wfdc1	Dr. Jean Hebert (Albert Einstein College of Medicine)	PMID: 22102609
ISH probe	Wnt2b	Dr. Tom Jessell (Columbia University)	PMID: 30254141