Supplementary Figures

Fig. S1. Developmental expression profile of the CrxGFP transgene

A-D, Analysis of CrxGFP expression and Rxrγ immunostaining during retinal histogenesis. **A**, GFP fluorescence is first detectable at E13.5 and localises to the outer aspects of the neuroblastic layer (NBL). **B**, At E15.5 the majority of the CrxGFP photoreceptor precursor cells co-label with Rxrγ (Rxrγ/GFP$^+$). **C**, At E17.5 the GFP layer of developing photoreceptors has increased and the proportion of Rxrγ/GFP$^+$ cells remains high. **D**, By P3 only a small percentage of CrxGFP photoreceptors are positive for Rxrγ. The percentage of Rxrγ$^+$ cells within the GFP$^+$ population was determined by averaging 10 fields of view per timepoint; these data indicate the relative ratios of cone to rod precursors as 3: 1, 2.2: 1 and 1: 15.6 at E15.5, E17.5 and P3 respectively. **E**, The mitotic marker pH3 labels retinal progenitor cells (open arrowheads) at the ventricular surface and does not co-label CrxGFP precursor cells. **A-E**, Hoechst nuclear stain (blue); **A'-E'**, CrxGFP labelling (green); **A''-E''**, Rxrγ and pH3 immunostaining (red); **A'''-E'''** three channel merge. NBL – neuroblastic layer, ONBL – outer neuroblastic layer, GCL – ganglion cell layer.

Fig. S2. CrxGFP expression in bipolar cells

A, Adult retinal section showing CrxGFP expression in the ONL and weak expression in the INL. **B-D,** CrxGFP cells in the subretinal space of recipient wildtype retina, 3 weeks after transplantation showing the presence of some Chx10 and PKCα positive cells (open

arrowheads). Cells that integrated into the ONL did not express these bipolar markers. Hoechst nuclear stain (blue). SRS – subretinal space, ONL – outer nuclear layer.

Fig. S3. Post mitotic CrxGFP cells integrate into the recipient ONL

A, B, CrxGFP cells in the subretinal space of recipient wildtype retina, 3 weeks after transplantation of P3 donor cells. **A**, Transplanted cells that did not integrate into the ONL, accumulated in the subretinal space and show presence of some BrdU positive cells (solid arrowheads). **B**, A micrograph displaying both non integrated BrdU positive cells in the subretinal space (**B'**, open arrowhead) and integrated CrxGFP BrdU negative cells in the ONL (**B,** closed arrowheads). No integrated cell in the ONL co-labelled for BrdU. SRS – subretinal space, ONL – outer nuclear layer.

Fig. S4. Expression of synaptic markers in integrated CrxGFP photoreceptor cells.

A-C, CrxGFP labelling (grey scale), **A'-C'** target protein immunostaining (red), **B''-D''** three channel merge with CrxGFP (green) and Hoechst nuclear stain (blue). **A**, **B**, Transplanted CrxGFP cells co-express the ribbon synapse marker Bassoon (arrowheads) and the synaptic marker Dystrophin. Insets show higher magnification and single optical sections of the small boxed areas in **A**, **B**. **C**, PKCα labels rod bipolar cells. Inset shows close association of the rod spherule (green) and the bipolar terminal (red). All retinae were examined 3 wks post transplantation. **A-C** are confocal z-projections. INL – inner nuclear layer, ONL – outer nuclear layer, OPL – outer plexiform layer.

Fig. S5. Transplanted CrxGFP cells remaining in the subretinal space express cone markers

A, B, View of transplanted CrxGFP cells that did not integrate into the ONL and accumulated in the subretinal space of a recipient eye. **A**, Subretinally located CrxGFP cells express the nuclear cone marker Rxrγ (solid arrowheads) and the cells organise in rosettes (dashed ellipse). **B**, CrxGFP cells in the sub retinal space also express the mature cone marker cone arrestin (Arr3) (solid arrowheads), as seen in cones in the recipient ONL (open arrowheads). **C,** CrxGFP cells in the sub retinal space express the mature rod marker phosducin (solid arrowheads), as seen in rods in the recipient ONL. **D,** CrxGFP/Rxrγ-positive cells observed after flow sorting. Arrowhead indicates a Rxrγ-negative cell. All retinae were examined 3 wks post transplantation. SRS – subretinal space, ONL – outer nuclear layer, RPE – retinal pigmented epithelium.

Fig. S6. Rxrγ labelsdevelopingcones

A-C, Confocal z-projections of CrxGFP retinae at E15.5, P0 and P21 that received a BrdU-pulse at E14. Photoreceptor cells born after the BrdU pulse colabel for BrdU and CrxGFP; developing cone photoreceptors are identified by Rxrγ immunostaining. **D,** Pulse chase time course. **E,** Graph of the percentage of Rxrγ-positive cells (+/- standard deviation) within the CrxGFP/BrdU positive population after each chase period at E15.5, P0 and P21 indicating that the majority of CrxGFP cells born early in retinogenesis develop as cone photoreceptors. N=number of retinae, n= number of cells. ONBL – outer neuroblastic layer, ONL – outer nuclear layer.

Fig. S7. Transplantation of embryonic CrxGFP photoreceptor precursors into P14 and adult wildtype recipient mice

A, Comparison of the cone integration efficiency of E15.5 CrxGFP donor cells transplanted into 6 weeks (n=7) (adult) and P14 wild-type (n=10) recipient retinae. No statistically significant difference was observed (Mann Whitney, p = 0.107).
B, CrxGFP Rxrγ-positive cone precursors within the inner and outer segment layer apparently migrating towards the recipient ONL at 4 days after transplantation of E15.5 CrxGFP donors into the adult.

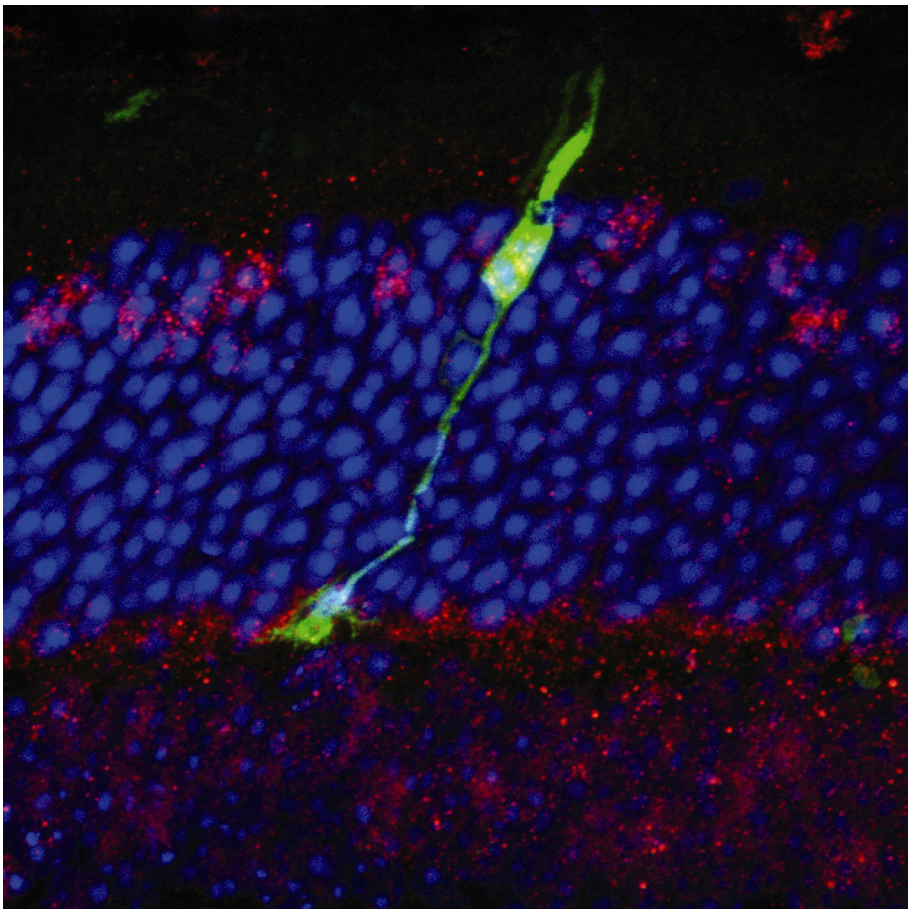
Fig. S8. The *Gucy2e*$^{-/-}$ retina shows reduced Rxrγ staining and is severely cone dystrophic by 2 months of age

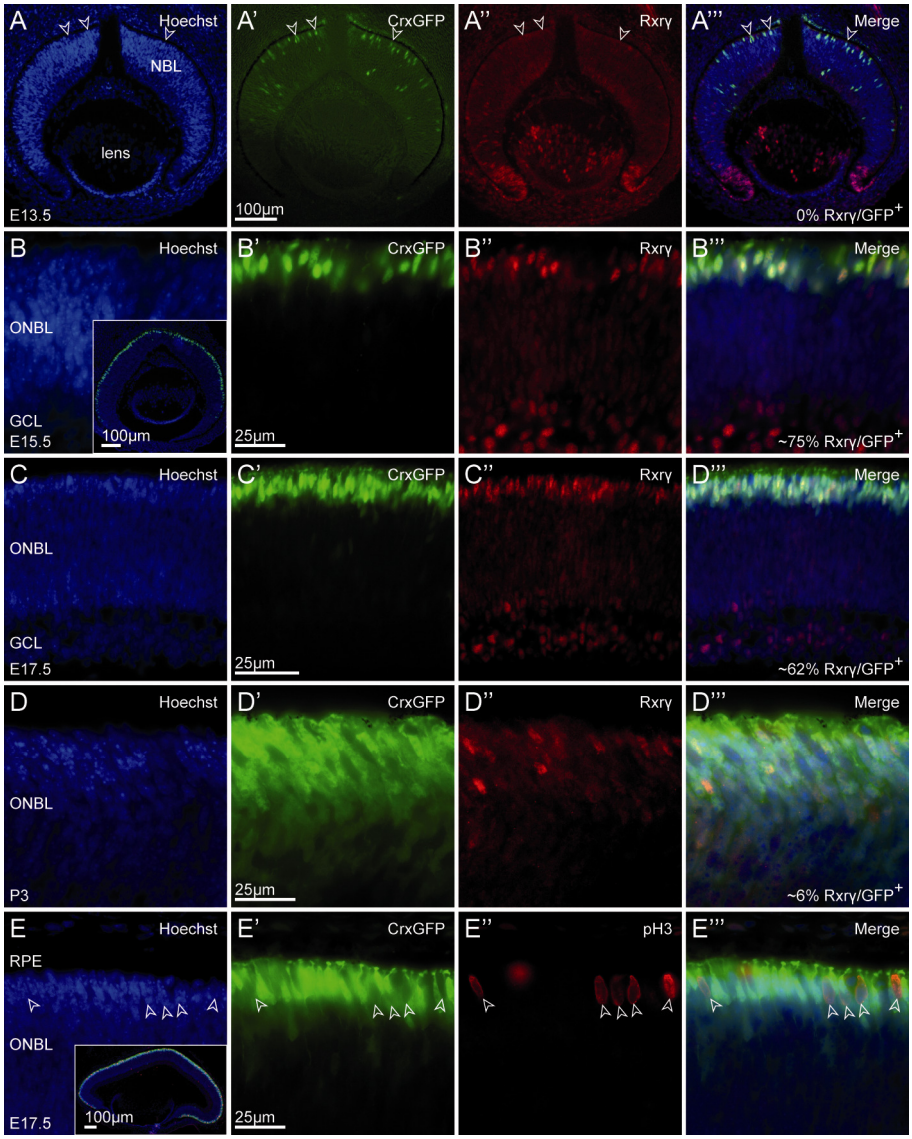
A, *Gucy2e*$^{-/-}$ central retina at 2 months of age. Cone nuclei are labelled by Rxrγ immunostaining at the outer aspect of the ONL (arrowheads). The number of cones is severely reduced (approximately four-fold) compared to wild-type, ONL-thinning is not apparent at this age. **B**, A control wild-type retina displaying a normal cone distribution visualised by Rxrγ immunostaining. **C**, Bar chart comparing the amount of Rxrγ^{+} nuclei per field of view between the mutant and wild-type retina. ONL – outer nuclear layer, INL – inner nuclear layer, GCL – ganglion cell layer.

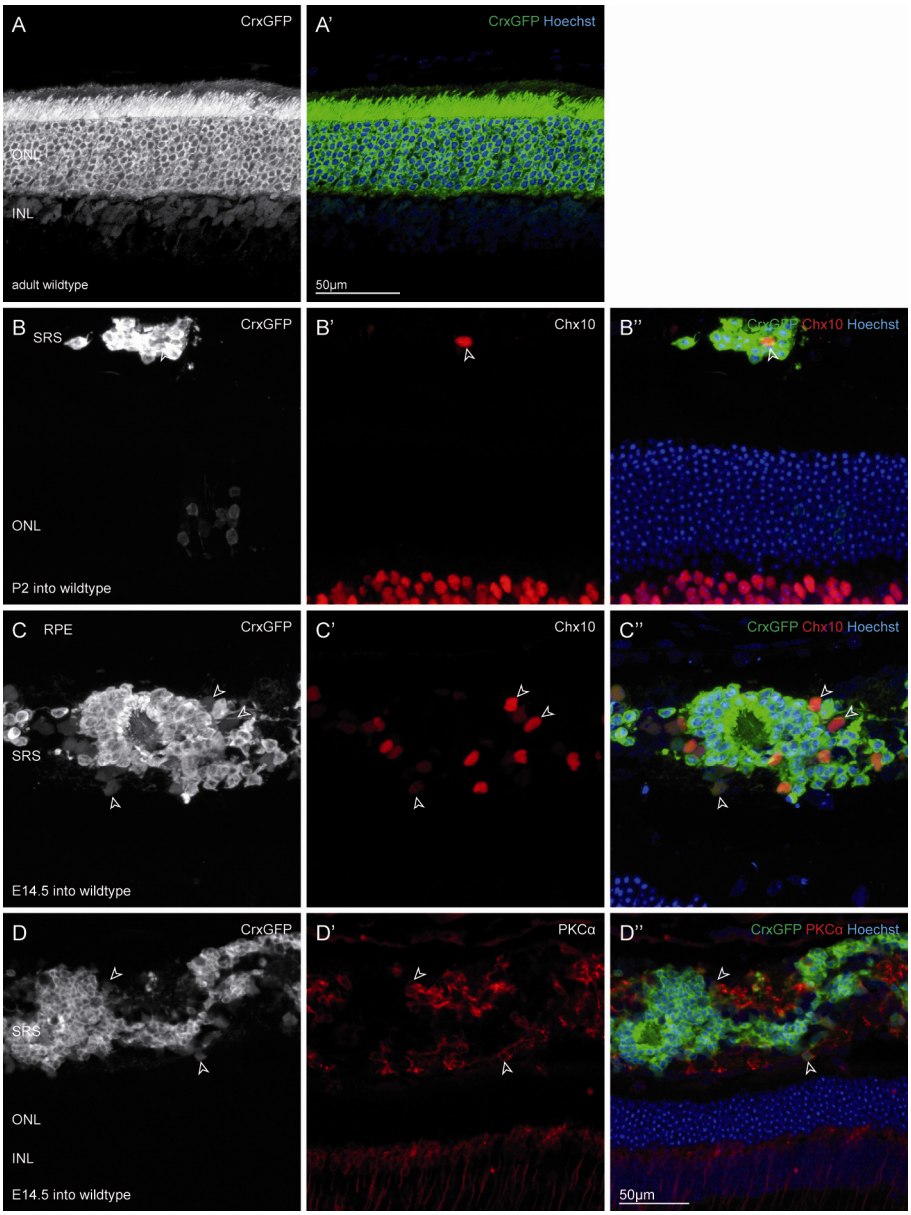
Supplementary Video 1. Transplants of CrxGFP precursor cells generate new cone photoreceptors

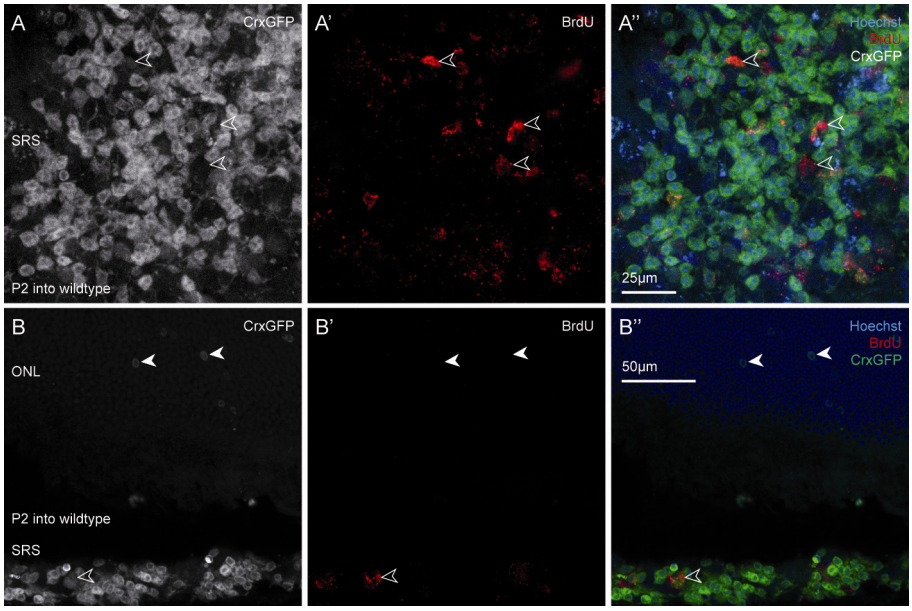
The video is a 360° rotation of a confocal stack, showing a cone photoreceptor generated from transplants of E15.5 CrxGFP cells into wildtype retina. The cone (green) co-labels with Rxrγ (red); displays inner and outer segment, as well as a cone pedicle including

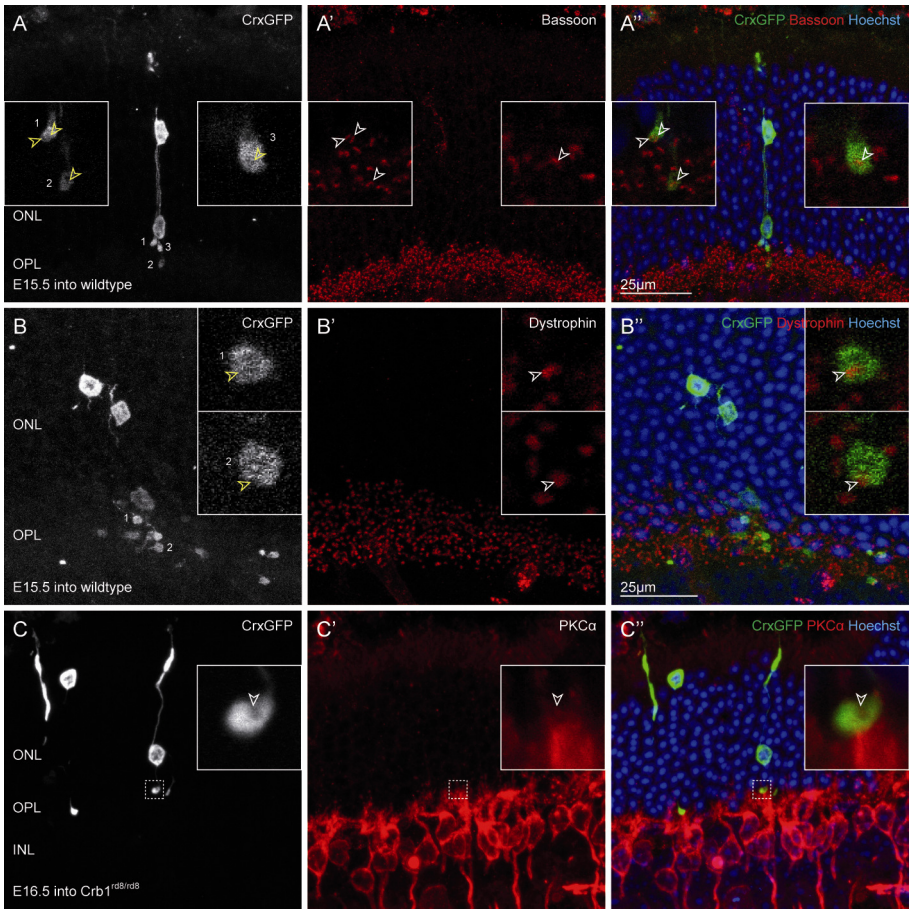
telodendria. The nucleus is differentiated from surrounding rods by multiple distinct heterochromatin foci (nuclear stain, blue).

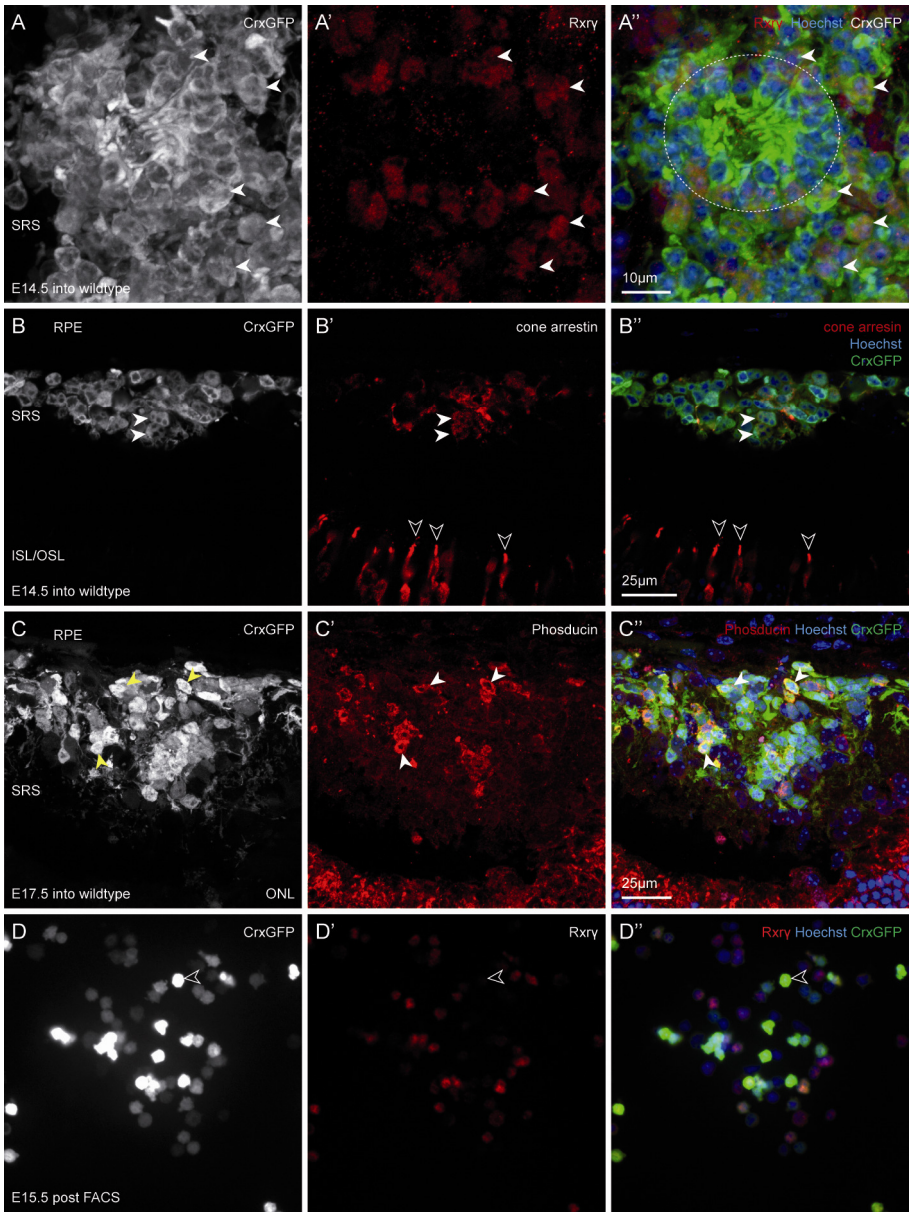


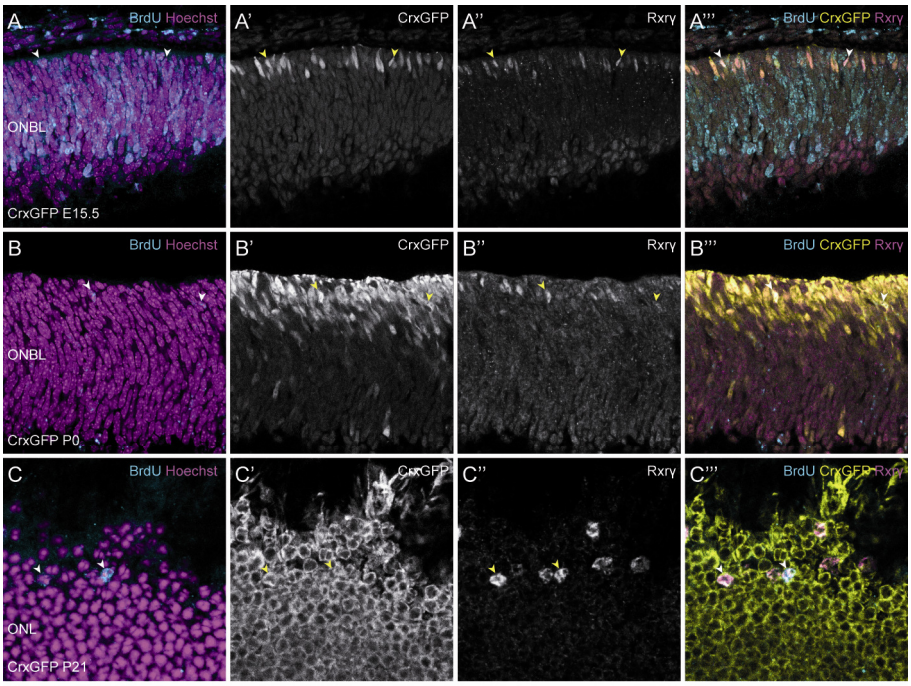

A
Hoechst
NBL
lens
E13.5
A'
CrxGFP
100μm
A''
Rxrγ
A'''
Merge
0% Rxrγ/GFP+
B
Hoechst
ONBL
GCL
E15.5
100μm
B'
CrxGFP
25μm
B''
Rxrγ
B'''
Merge
~75% Rxrγ/GFP+
C
Hoechst
ONBL
GCL
E17.5
C'
CrxGFP
25μm
C''
Rxrγ
D'''
Merge
~62% Rxrγ/GFP+
D
Hoechst
ONBL
P3
D'
CrxGFP
25μm
D''
Rxrγ
D'''
Merge
~6% Rxrγ/GFP+
E
Hoechst
RPE
ONBL
E17.5
100μm
E'
CrxGFP
25μm
E''
pH3
E'''
Merge

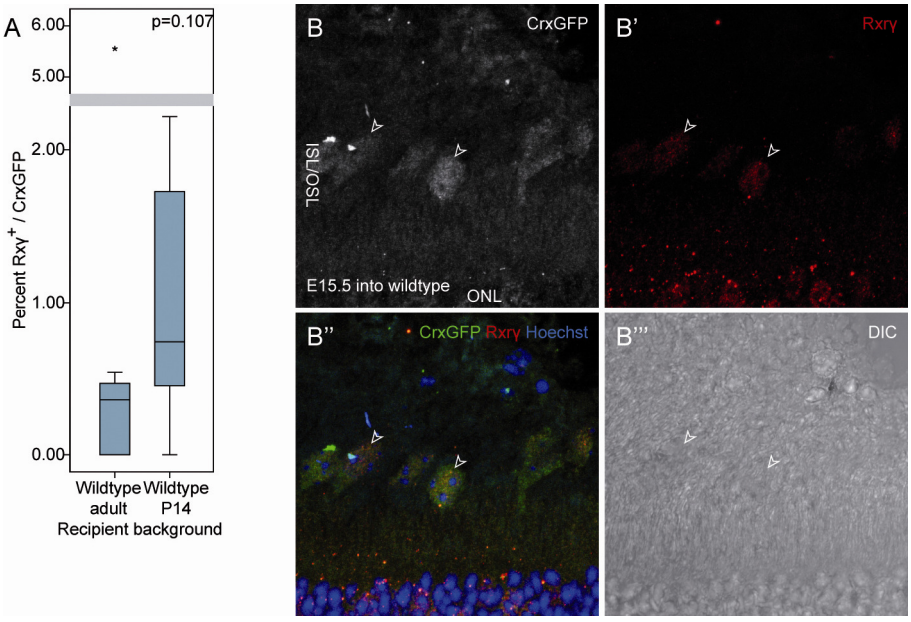

A
CrxGFP
ONL
INL
adult wildtype
A'
CrxGFP Hoechst
50µm
B
SRS
CrxGFP
ONL
P2 into wildtype
B'
Chx10
B''
CrxGFP Chx10 Hoechst
C
RPE
CrxGFP
SRS
E14.5 into wildtype
C'
Chx10
C''
CrxGFP Chx10 Hoechst
D
CrxGFP
SRS
ONL
INL
E14.5 into wildtype
D'
PKCα
D''
CrxGFP PKCα Hoechst
50µm

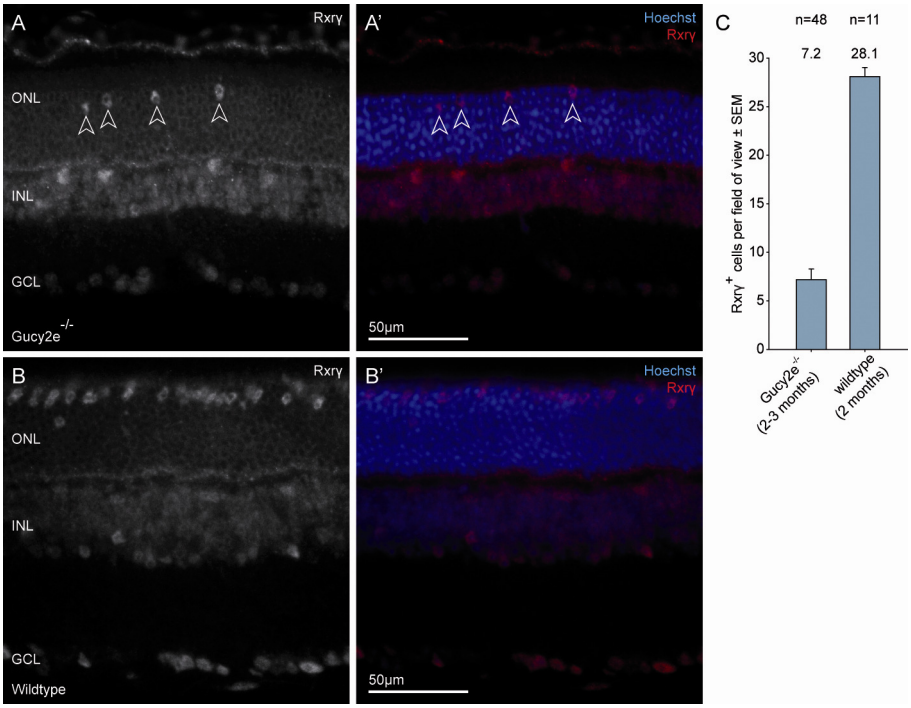

A
CrxGFP
SRS
P2 into wildtype
A'
BrdU
A''
Hoechst
BrdU
CrxGFP
25µm
B
CrxGFP
ONL
P2 into wildtype
SRS
B'
BrdU
B''
Hoechst
BrdU
CrxGFP
50µm


A
CrxGFP
ONL
OPL
E15.5 into wildtype
A'
Bassoon
A''
CrxGFP Bassoon Hoechst
25µm
B
CrxGFP
ONL
OPL
E15.5 into wildtype
B'
Dystrophin
B''
CrxGFP Dystrophin Hoechst
25µm
C
CrxGFP
ONL
OPL
INL
E16.5 into Crb1rd8/rd8
C'
PKCα
C''
CrxGFP PKCα Hoechst


A
CrxGFP
SRS
E14.5 into wildtype
A'
Rxrγ
A''
Rxrγ Hoechst CrxGFP
10μm
B
RPE
CrxGFP
SRS
ISL/OSL
E14.5 into wildtype
B'
cone arrestin
B''
cone arresin
Hoechst
CrxGFP
25μm
C
RPE
CrxGFP
SRS
E17.5 into wildtype
ONL
C'
Phosducin
C''
Phosducin Hoechst CrxGFP
25μm
D
CrxGFP
E15.5 post FACS
D'
Rxrγ
D''
Rxrγ Hoechst CrxGFP


A
BrdU Hoechst
ONBL
CrxGFP E15.5
A'
CrxGFP
A''
Rxry
A'''
BrdU CrxGFP Rxry
B
BrdU Hoechst
ONBL
CrxGFP P0
B'
CrxGFP
B''
Rxry
B'''
BrdU CrxGFP Rxry
C
BrdU Hoechst
ONL
CrxGFP P21
C'
CrxGFP
C''
Rxry
C'''
BrdU CrxGFP Rxry
D
BrdU injection
Time point 1
Time point 2
Time point 3
E14
E15.5
P0
P21
E
% Rxry+ within the BrdU/GFP+ population
91.9 ±7.2%
98.4 ±4.8%
91.8 ±2.5%
N=246 n=10
N=85 n=9
N=508 n=6
E15.5
P0
P21


A
p=0.107
Percent Rxy+ / CrxGFP
Wildtype adult
Wildtype P14
Recipient background
B
CrxGFP
ISL/OSL
E15.5 into wildtype
ONL
B'
Rxry
B''
CrxGFP Rxry Hoechst
B'''
DIC


A
Rxrγ
ONL
INL
GCL
Gucy2e-/-
A'
Hoechst
Rxrγ
50μm
B
Rxrγ
ONL
INL
GCL
Wildtype
B'
Hoechst
Rxrγ
50μm
C
n=48
n=11
7.2
28.1
Rxrγ+ cells per field of view ± SEM
30
25
20
15
10
5
0
Gucy2e-/- (2-3 months)
wildtype (2 months)