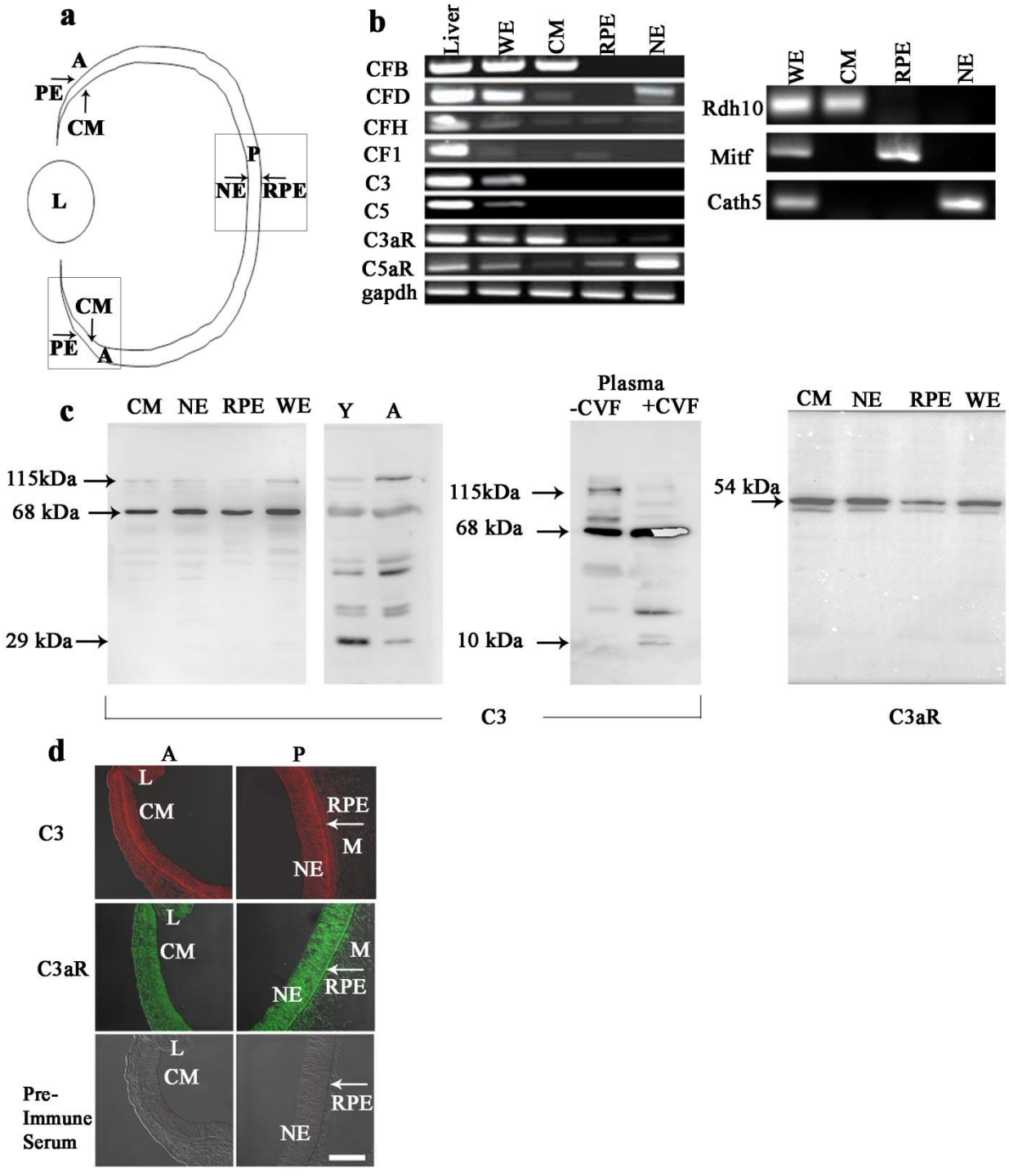
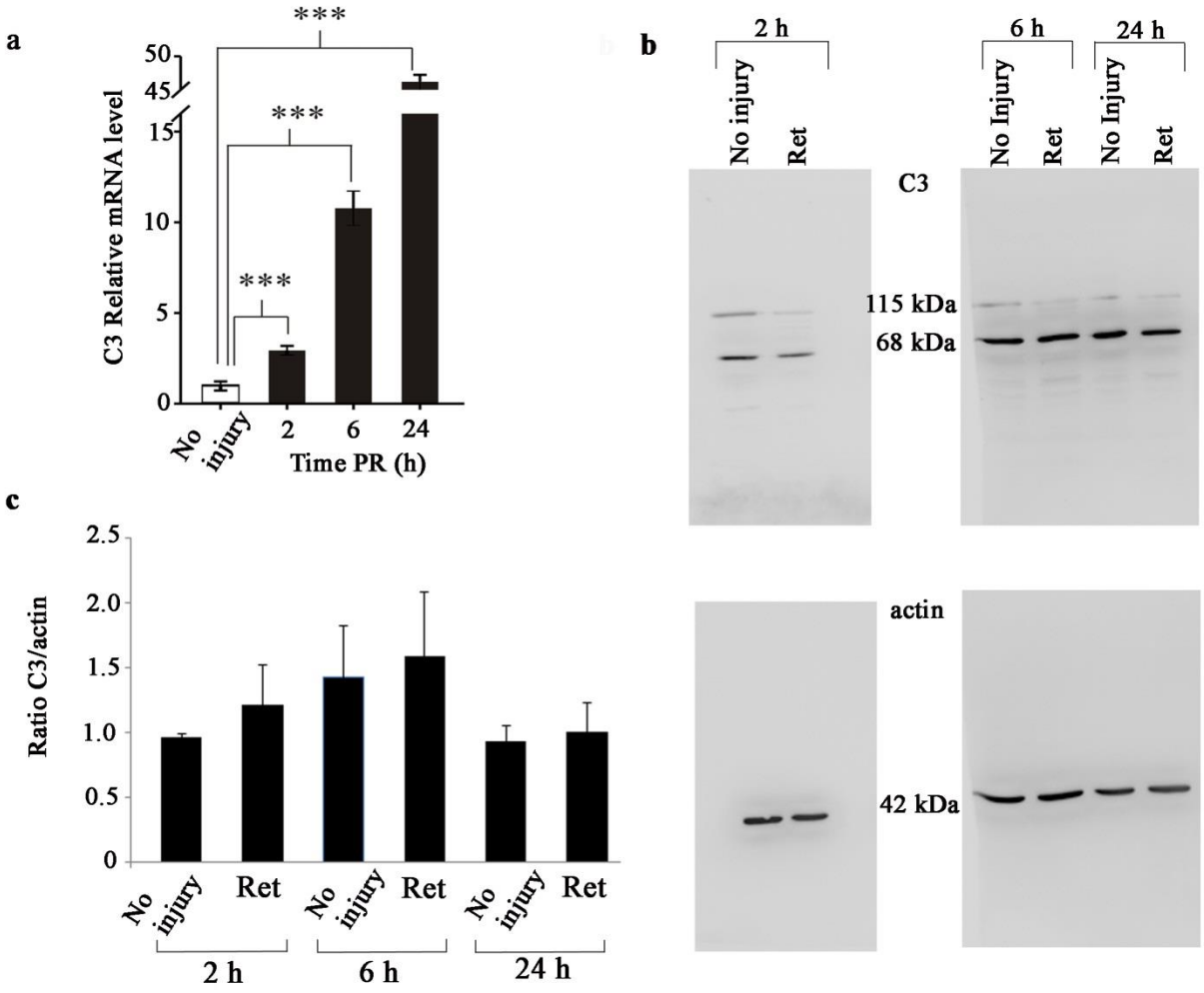


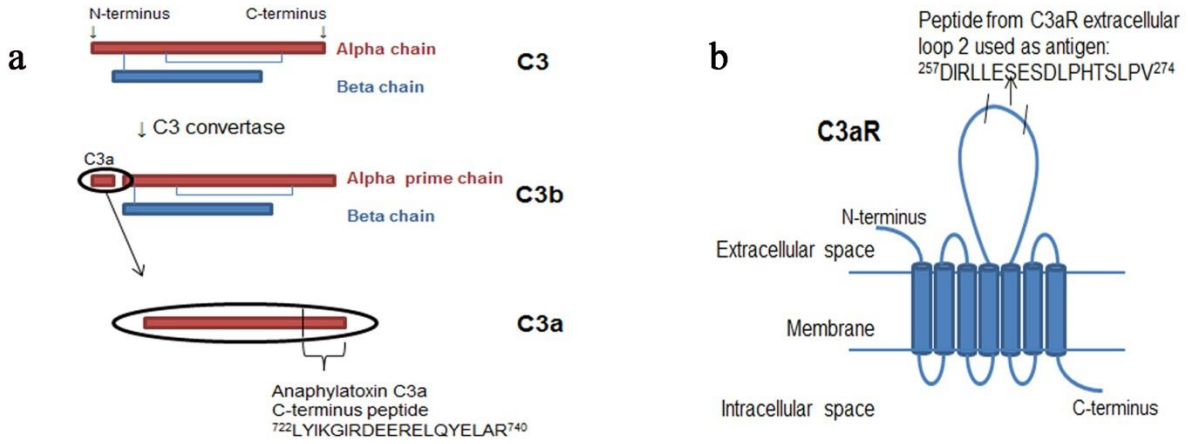
Supplementary Figure S1. Expression of complement components in E4 chick eyes. (**a)** A schematic of the eye showing the ciliary margin (CM) of the anterior region, and the neuroepithelium (NE) and retinal pigmented epithelium (RPE) of the posterior region labeled to

be used as reference for all figures. The pigmented epithelium (PE) in the anterior region connects with the RPE in the posterior region, while the CM joins the NE. The NE will develop into the retina as the eye develops. Also, pigmentation becomes more pronounced as the eye develops, in both the anterior and posterior regions. Boxed areas represent regions used for RT-PCRs and regions magnified in the immunohistochemical staining panel (**d)**. (**b)** Expression of *Complement factors B* (416 bp), *D* (444 bp), *H* (327 bp) and *I* (407 bp), and *C3* (231 bp), *C5* (228 bp), *C3aR* (230 bp), and *C5aR* (278 bp) mRNA in E4 whole eye (WE), CM, RPE, and NE. *Rdh10* (139 bp), *Mitf* (199 bp), and *Cath5(*149 bp*)* are specifically expressed in the CM, RPE and NE, respectively, and were used as positive controls for the different eye tissues, while *GAPDH* (432 bp) is ubiquitously expressed and therefore used as a standard control. **(c)** C3, C3 activation fragments, and C3aR were detected in eye tissues, yolk (Y) and albumin (A) by Western blotting using an antibody specific for the alpha-chain of chicken C3. The 115 kDa band denotes C3 alpha chain while the 68 kDa denotes iC3b and the 29 kDa denotes the N-terminus alpha chain fragment and suggests further degradation to C3c and c3dg. Low levels of C3a (10 kDa) can only be seen in chicken plasma that has been activated by cobra venom factor (CVF) using an anti C3a antibody. **(d)** Immuno-histochemical staining shows that C3 and C3aR are present in the anterior (A) and posterior (P) regions of the E4 chick eye, including the CM, NE, and RPE. Pre-immune serum was used as a negative control to show non-specific binding. The scale bar represents 20 μm and applies to all panels. L= lens; M = Mesenchyme.

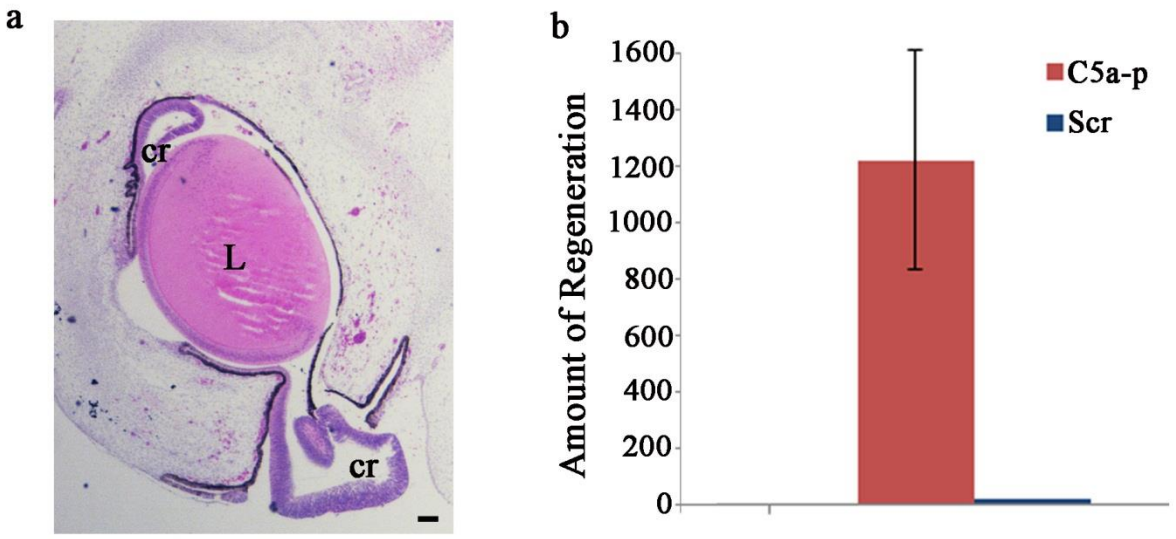


Supplementary Figure S2. *C3* mRNA and protein presence in response to injury. (a) Levels of *C3* mRNA detected by RT-qPCR in the CM at E4 before and after retinectomy. Expression is induced 2 h PR ($P = 0.0001$), and its expression is increased at 6 h ($P = 0.001$) and 24 h ($P = 0.000001$) PR. All *P* values were determined by comparing the level of *C3* in developing eyes to the induction after retinectomy. Statistical analysis was determined using the Student's t test. Error bars represent s.e.m. All analyses were performed in triplicate with at least three independent biological samples. The mean ratio of C3 expression, s.e.m., and range are given in

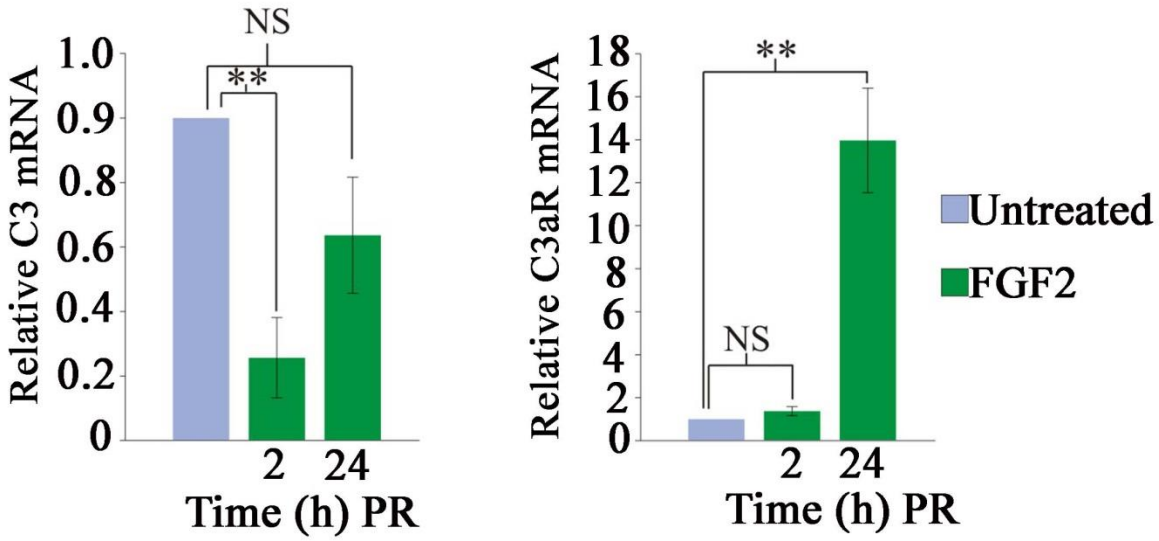
Supplementary Table S5. *** = *P* value < 0.005. **(b)** Western blotting using an antibody specific for the alpha-chain of chicken C3 shows that the level of activated C3 (iC3b= 68 kDa) does not change after retina removal in E4 eyes at 2 h, 6 h, or 24 h PR. Levels of actin (42 kDa) were used for normalization purposes. **(c)** Quantification of C3 in relation to actin levels. The exact binomial test was used for statistical analysis. *n* = 5 biological samples. The mean ratio of C3/actin, s.e.m., and range are given in **Supplementary Table S**3.



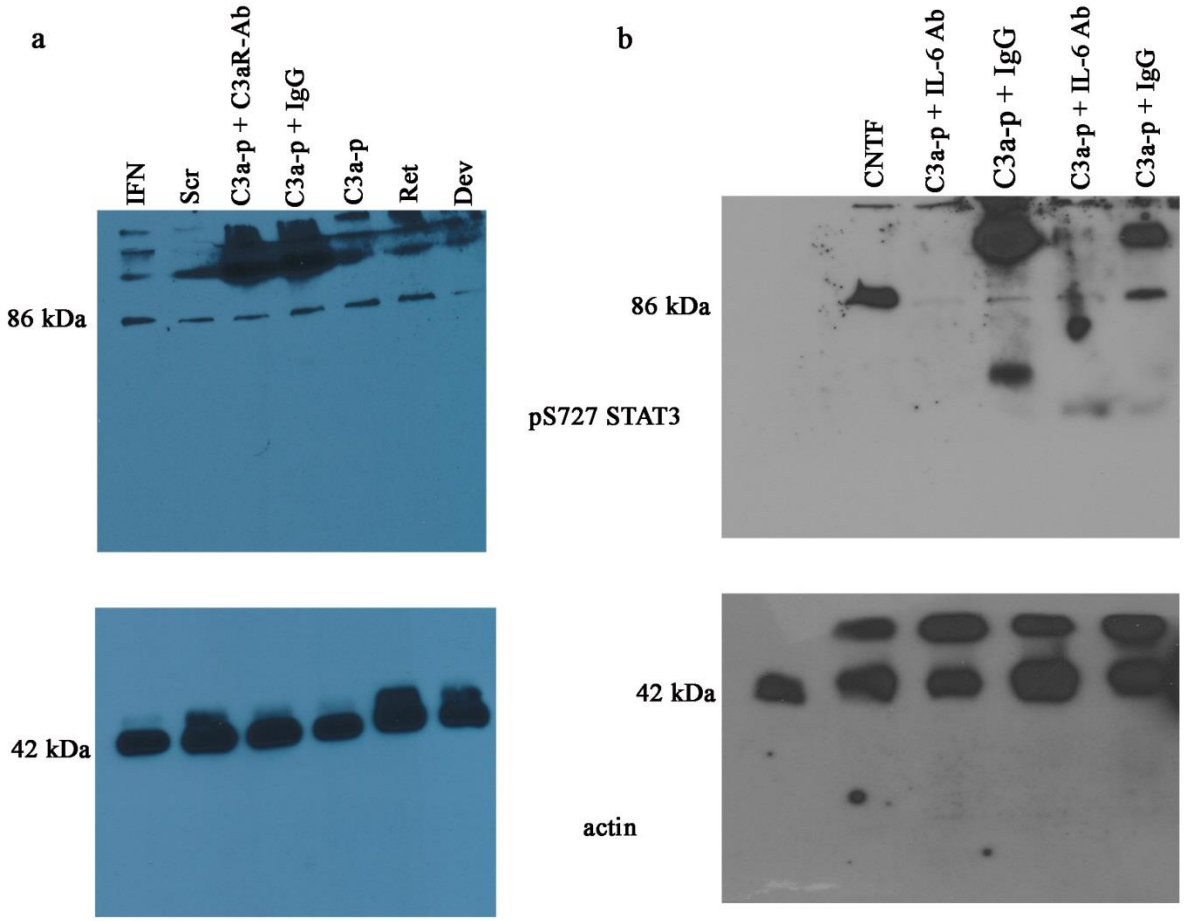
Supplementary Figure S3. **Schematic of C3a and C3aR**. (**a**) Schematic shows the cleavage of C3 alpha chain into C3a and C3b. The C-terminus sequence of C3a corresponds to the C3a peptide (C3a-p) used to induce retina regeneration. (**b)** Schematic of the C3a Receptor showing the region used to generate the C3aR-Ab.



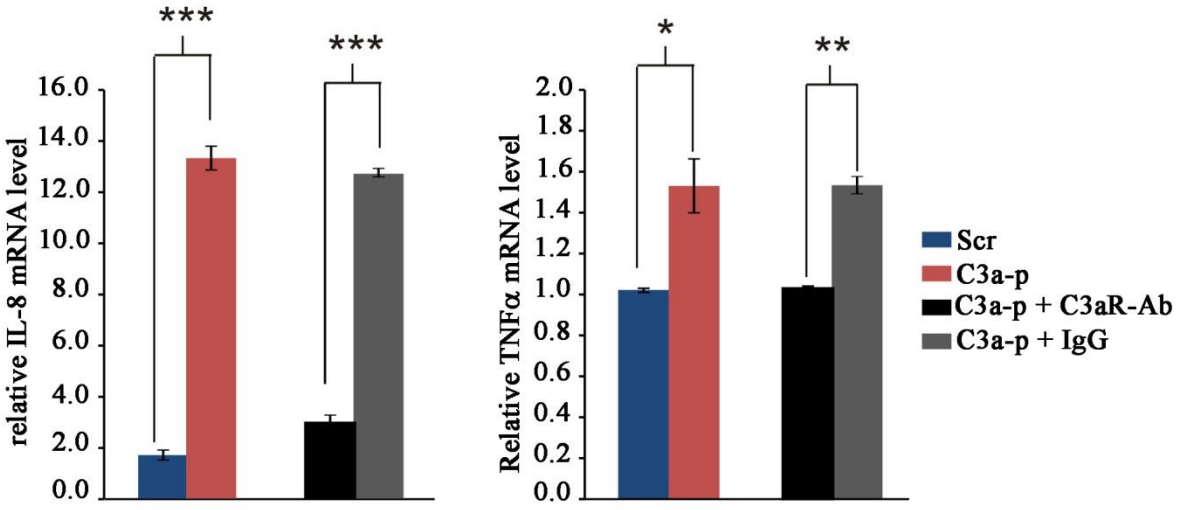
Supplementary Figure S4. C5a-p induces chick retina regeneration. (a) Histological analysis at 3d PR shows the amount of regeneration from the stem/progenitor cells of the CM (cr) after treatment with C5a-p. Scale bar represents 100 μm. **(b)** Graphical representation of the mean amount of regeneration induced by C5a-p (*n* = 8) compared to eyes treated by a peptide with a scrambled sequence (Scr) *(n* = 8). Error bars represent s.e.m. The mean, range and s.e.m. are provided in **Supplementary Table S7**. L= lens.



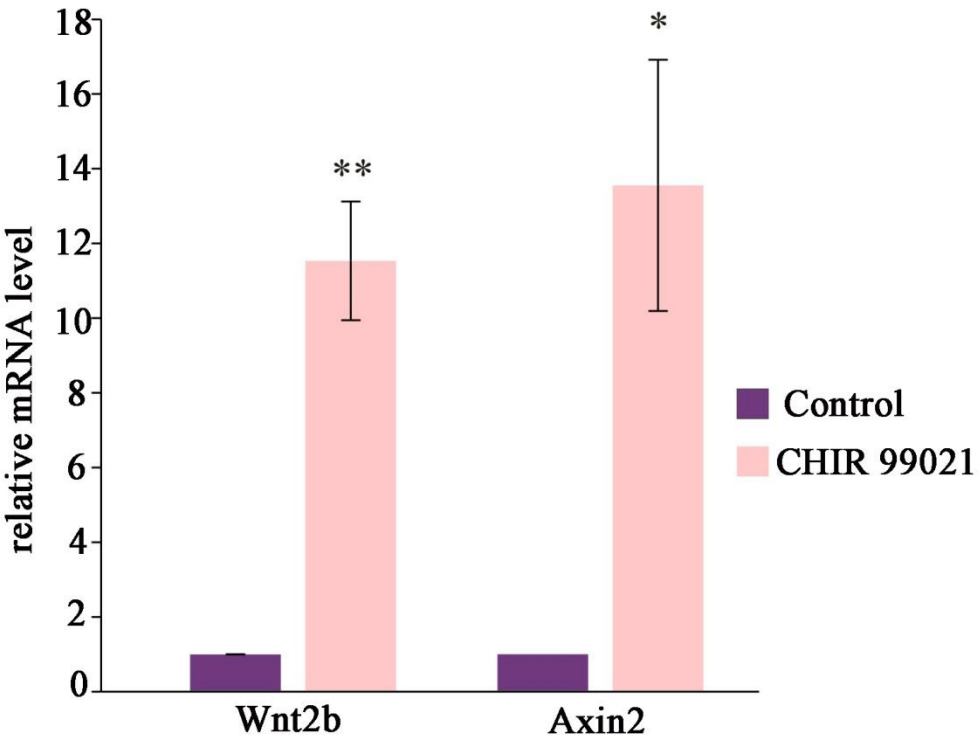
Supplementary Figure S5. FGF2 effects on *C3* and *C3aR* expression. The expression of *C3* and *C3aR* in eyes treated with FGF2 at 2 h and 24 h PR. Statistical analysis was determined by the Student's t- test. *P* value = 0.004 for *C3* expression at 2h PR in FGF2 treated eyes compared to eyes with retinectomy only. *P* value = 0.006 for *C3aR* expression at 24h PR in FGF2 treated eyes compared to eyes with retinectomy only. *n*= 3 biological samples done in triplicate. ** = $P < 0.01$. The level of *C3* and *C3aR* expression is normalized to the level expressed in the CM of developing eyes at E4. Error bars represent s.e.m. The mean ratio of *C3* and *C3aR* expression to *GADPH* expression as well as the s.e.m. and range are provided in **Supplementary Table S5.**



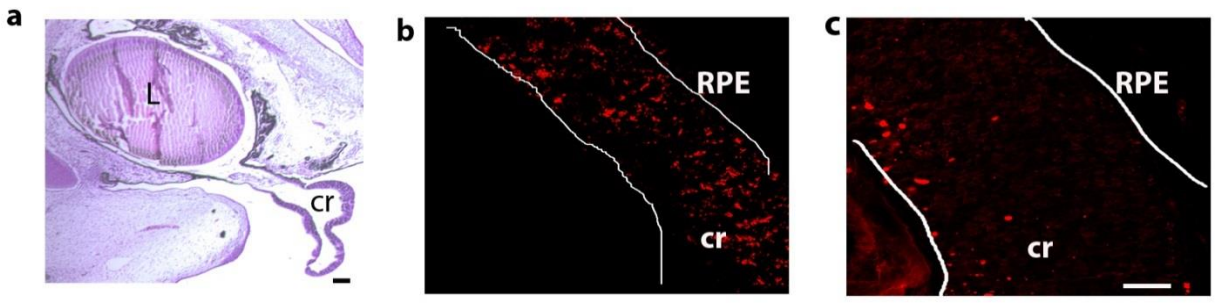
Supplementary Figure S6. Western blots used for the densitometry to measure the level of pS727 STAT3. (a) Representative Western blots used to calculate the mean ratio of pS727 STAT3 (86 kDa) and actin (42 kDa) shown in **Fig. 5a and b**. **(b)** A representative Western blot used to calculate the mean ratio of pS727 STAT3 and actin shown in **Fig. 6g**. Protein extracts from ciliary neurotrophic factor (CNTF) or interferon gamma (IFN)-treated DF-1 chick cells were used as a positive control for pS727 Stat3.



Supplementary Figure S7. ***IL-8* and *TNFα* expression is stimulated by C3a-p.** RT-qPCR shows the expression of *IL-8* and *TNFα* in the CM of eyes in response to Scr, C3a-p, C3a-p + C3aR-Ab, and C3a-p + IgG at 2 h PR. *P*= 0.00001 for *IL-8* and 0.01 for *TNFα* comparing C3a-p treated eyes to Scr treated eyes. *P* = 0.00001 for *IL-8* and 0.001for *TNFα* comparing C3a-p + IgG to C3a-p + C3aR-Ab. All expression values are normalized to the expression in eyes receiving retinectomy only. The Student's t-test was used for statistical analysis. n= 3 biological samples done in triplicate. Error bars represent s.e.m.The mean ratio of mRNA for *IL-8* and *TNFα* relative to mRNA for GADPH, as well as s.e.m. and range are given in **Supplementary Table S6**. * = *P* value < 0.05; **= *P* value < 0.01; *** = *P* value < 0.001.



Supplementary Figure S8. CHIR up-regulates *wnt2b* and *axin2* mRNA. RT-qPCR shows the expression of *wnt2b* and *axin2* in the CM at 24 h PR in response to treatment with the pharmacological agent CHIR 99021. All expression values are compared to the expression in the CM of eyes treated with DMSO only (control). *P* value = 0.002 for *wnt2b* expression and 0.02 for *axin2* expression. The Student's t-test was used for statistical analysis. n= 3 biological samples done in triplicate. Error bars represent s.e.m. The mean ratio of mRNA for *wnt2b* and *axin2* relative to mRNA for GADPH, as well as s.e.m. and range are given in **Supplementary Table S6**. * = *P* value < 0.05; **= *P* value < 0.01.



Supplementary Figure S9. Increased concentration of C3a-p induces retina degeneration and apoptosis. (a) Histological analysis at 7d PR shows the amount of regeneration induced by increased concentration of C3a-p (100 μg). **(b and c)** TUNEL analysis shows apoptotic cells at 7d PR in eyes treated with 100 μg of C3a-p **(b)** and 50 μg of C3a-p **(c).** Scale bar in **(c)** represents 100 μm and applies to **(b)** as well. L= lens; cr= regeneration from the ciliary margin; RPE = retinal pigmented epithelium.

Gene	Ensembl or GenBank ID	Sequence 5'-3'
Factor B	BI066262.1	F, GCTGAAGGGGCAGGAGAC
		R, GTGTGGGGGCTCTGTCTGT
Factor D	XM_003642860	F, GCAGCAACATCCACAACAAC
		R, CCATCACACCATCAATCCAC
Factor H	ENSGALG00000002431	F, GGGCCTCTGGAAAATGGTTA
		R, TGGAGACCAGCCATTCTTTT
Factor I	ENSGALT00000019810	F, AGGCAAACCAAAACATGAGG
		R, GCAGAGCGATGTCATTTTCA
C3	ENSGALT00000018763	F, TCCGTGAGGCTCATCAAGC
		R, TCGAGCCAGCTCGTATTGC
C5	ENSGALG00000001565	F, TCTGACTTTGAGGAGCAAG
		R, TCTTGCCAATATTAGAAGC
C3aR	ENSFM00500000273608	F, ACACAGGACCGATGGTTCTT
		R, GATGATGTTCACAGACCTCT
C5aR	ENSFM00670001235886	F, ATTTTCCTTCTGGGCATCCC
		R, GTGACCATAAGGCACCGATC
Gapdh	ENSGALG00000014442	F, TCCAAACTCATTGTCATACCAGGAA
		R, ACCACTGTCCATGCCATCACAGCC
Rdh10	ENSGALG00000015640	F, AGGAATGTTCAGAGGGTGCAGGAT
		R, ATACATGAGGCGAGGTGTGCAGAT
Mitf	ENSGALG00000007679	F, AAAGCATGCCTCCTCCAGGACTTA
		R, TTGGGTATCAAGGTGCCCAGTTCT
Cath5	ENSGALT00000006264	F, TGCAGAAGTGGATCAGGCTGTGTT
		R, TGTGGAACCACCTTCCTCAAACGA

Supplementary Table S1. Primers Sequences for RT-PCR (5'-3').

Name	Sequence
C3a	MRGSHHHHHHGSSVRLIKHKGTKMAEYSDKNLRKCCEDGMKENL MGYSCEKRATYVLDGKACTEAFLSCCLYIKGIRDEERELQYELAR
$C3a^{(722-740)}$-p	H-LYIKGIRDEERELQYELAR-COOH
$C5a^{(724-742)}$-p	H-EFANRLREEEPSKLLILAR-COOH
Scr	H-EAYKQRYEDRLELRIELIG-COOH
$C3a^{(721-740)}$ antigen	H-CLYIKGIRDEERELQYELAR-COOH
$C3^{(741-761)}$ antigen	H-SEVDDAFLSDEDITSRSLFPE-$CONH_2$
$C3aR^{(257-274)}$ antigen	H-DIRLLESESDLPHTSLPV-$CONH_2$

Supplementary Table S2. The sequences of the peptides and antigens used to make antibodies.

Treatment	Ratio	Mean	s.e.m.	Range
E4 No Treatment	C3/actin	0.97	0.02	0.93-1.00
E4 Retinectomy 2 h PR	C3/actin	1.22	0.30	0.77-1.79
E4 No Treatment	C3/actin	1.43	0.46	0.94-2.35
E4 Retinectomy 6 h PR	C3/actin	1.59	0.51	0.84-2.55
E5 No Treatment	C3/actin	0.93	0.12	0.81-1.05
E4 Retinectomy 24 h PR	C3/actin	1.01	0.22	0.79-1.23
E4 Development	pS727 STAT3/actin	0.11	0.06	0.05-.17
E4 Retinectomy	pS727 STAT3/actin	0.16	0.10	0.05-0.23
C3a-p	pS727 STAT3/actin	0.35	0.17	0.1-0.80
Scr	pS727 STAT3/actin	0.22	0.14	0.06-0.50
C3a-p + IgG (1)	pS727 STAT3/actin	0.25	0.07	0.06-0.50
C3a-p + C3aR-Ab	pS727 STAT3/actin	0.17	0.06	0.05-0.40
C3a-p + IgG (2)	pS727 STAT3/actin	0.40	0.13	0.1-0.86
C3a-p + IL-6 Ab	pS727 STAT3/actin	0.18	0.07	0.04-0.38

Supplementary Table S3. Mean ratio of C3 to actin or pS727 STAT3 to actin with s.e.m. and range. Each set of biological samples were run on separate gels but all comparisons were done within the same gel. C3a-p + IgG (1) was compared to C3a-p + C3aR-Ab and C3a-p + IgG (2) was compared to C3a + IL-6 Ab.

Gene	Ensembl or GenBank ID	Sequence 5'-3'
C3	ENSGALT00000018763	F, TCATCAAGCACAAGGGCACCAAGA R, TTTCCTTCATGCCGTCCTCACAG
C3aR	ENSFM00500000273608	F, GGCATAATCACAGCAGCTCA R, GATGTCAGGATAGGCAGACGA
Gapdh	ENSGALG00000014442	F, CCATGTTTGTGATGGGTGTC R, CTCCACAATGCCAAAGTTGT
FGF2	ENSGALG00000011835	F, TGCAGCTTCAAGCAGAAGAA R, CATGCACTGGCTGTGAGTTC
IL6	ENSGALG00000010915	F, TCGCCTTTCAGACCTACCTG R, TGACCACTTCATCGGGATTT
Sox2	ENSGALG00000008848	F, TGAACGGATCGCCTACCTAC R, CTGGATTCCGTCTTGACCAC
Wnt2b	ENSGALG00000001532	F, GATCCGTGAGTGCCAGTACC R, GAGGAGATGGCGTAGACGAA
Six3	NM_204364.1	F, CCCCACGAAGAGTTGTCAAT R, TATGTCTCCGGTCTCCTCCA
Axin2	ENSGALG00000004021	F, ACCCACCTCTCCCTCCACT R, GCGATGCTCTCCACTCCTC

Supplementary Table S4. Primers Sequences for RT-qPCR (5'-3').

Treatment	Gene	Mean	s.e.m.	Range
C3a-p (2h)	*FGF2*	1.02	0.02	1-1.05
Scr (2h)	*FGF2*	1.01	0.01	1-1.03
C3a-p (24 h)	*FGF2*	2.16	0.08	1.99-2.25
Scr (24 h)	*FGF2*	0.91	0.06	0.80-1.02
2 h PR	*C3*	2.90	0.14	2.7-3.20
6 h PR	*C3*	10.70	1.2	8.35-11.87
24 h PR	*C3*	46.20	1.05	44.08-47.25
FGF2 (2 h PR)	*C3*	0.26	0.12	0.09-0.5
FGF2 24 h PR)	*C3*	0.64	0.18	0.4-0.99
FGF2 (2 h PR)	*C3aR*	1.40	0.21	1.03-1.75
FGF2 (24 h PR)	*C3aR*	14.0	2.42	10.16-18.48

Supplementary Table S5. Mean ratio of fold change mRNA levels for the gene of interest to the level of mRNA for GADPH with s.e.m. and range.

Treatment	Gene	Mean	s.e.m.	Range
Scr	*IL-6*	1.06	0.02	1.01-1.09
C3a-p	*IL-6*	1.52	0.04	1.45-1.59
C3a-p + DMSO	*IL-6*	1.5	0.09	1.35-1.67
C3a-p + PD98059	*IL-6*	0.96	0.08	0.80-1.07
C3a-p+ Stattic	*IL-6*	0.10	0.01	0.09-0.13
C3a-p + IgG	*IL-6*	1.50	0.05	1.49-165
C3a-p + C3aR-Ab	*IL-6*	1.11	0.05	1.02-1.21
Scr	*Wnt2b*	0.90	0.01	0.87-0.92
C3a-p	*Wnt2b*	0.05	0.01	0.03-0.076
C3a-p + DMSO	*Wnt2b*	0.32	0.05	0.25-0.42
C3a-p + PD98059	*Wnt2b*	1.11	0.07	0.98-1.20
C3a-p + Stattic	*Wnt2b*	0.81	0.02	0.77-0.85
C3a-p + IgG	*Wnt2b*	0.38	.092	0.20-0.50
C3-pa + C3aR-Ab	*Wnt2b*	1.05	.04	0.98-1.10
Scr	*Six3*	0.94	0.08	0.84-1.10
C3a-p	*Six3*	1.82	0.04	1.78-1.90
C3a-p + DMSO	*Six3*	0.98	0.006	0.97-.99
C3a-p + PD98059	*Six3*	0.65	0.09	0.50-0.82
C3a-p + Stattic	*Six3*	0.04	0.007	0.03-0.06
C3a-p + IgG	*Six3*	1.60	0.06	1.50-1.70
C3a-p + C3aR-Ab	*Six3*	0.83	0.08	0.72-0.98
Scr	*Sox2*	0.86	0.03	0.8-0.90
C3a-p	*Sox2*	17.80	1.30	15.22-19.7
C3a-p + DMSO	*Sox2*	11.20	0.71	10.1-12.5
C3a-p +PD98059	*Sox2*	0.36	0.15	0.18-0.65
C3a-p + Stattic	*Sox2*	0.025	0.004	0.021-0.033
C3a-p + IgG	*Sox2*	15.80	0.93	14.3-17.5
C3a-p + C3aR-Ab	*Sox2*	1.29	0.02	1.26-1.33
Scr	*IL-8*	1.72	2.0	1.42-2.10
C3a-p	*IL-8*	13.30	0.43	12.9-14.2
C3a-p +IgG	*IL-8*	12.70	0.19	12.50-13.10

C3a-p + C3aR-Ab	***IL-8***	**3.00**	**0.34**	**2.30-3.40**
Scr	***TNFα***	**1.02**	**0.007**	**1.02-1.04**
C3a-p	***TNF α***	**1.53**	**0.12**	**1.32-1.72**
C3a-p + IgG	***TNF α***	**1.53**	**0.06**	**1.42-1.62**
C3a-p + C3aR-Ab	***TNF α***	**1.03**	**0.01**	**1.03-1.04**
CHIR 9021	***Wnt2b***	**11.50**	**1.59**	**8.39-13.51**
CHIR 9021	***Axin2***	**13.55**	**3.36**	**7.75-19.40**

Supplementary Table S6. Mean ratio of fold change mRNA levels for the gene of interest to the level of mRNA for GADPH with s.e.m. and range for each treatment.

Stage/Treatment	Mean Measurement	s.e.m.	Range (eyes with regeneration/n)
3d PR Scrambled C3a (Scr)	0	0	0 (0/8)
3d PR FGF2	1054	144	231-1418 (10/10)
3d PR C3a	307	93	245-620 (5/7)
3d PR C3a-p	754	170	407-1904 (9/11)
3d PR C5a-p	1028	313	137-2213 (7/8)
7d PR FGF2	1929	372	1089-3793 (9/10)
7d PR C3a-p	1417	329	1155-3237 (8/12)
3d PR FGF2 Transdifferentiation	258	51.6	231-804 (9/10)
3d PR C3a-p Transdifferentiation	13	8.2	68 and 73 (2/11)
7d PR FGF2 Transdifferentiation	938	242.6	354-2423 (9/10)
7d PR C3a-p Transdifferentiation	51	39.4	305 (1/12)
3d PR C3a-p + DMSO	746	187	369-1691 (7/9)
3d PR C3a-p + PD173074	790	332	543-1534 (6/8)
3d PR FGF2 + PD173074	0	0	0 (0/5)
3d PR FGF2 + C3aR-Ab	954	170	428-2045 (12/14)
3d PR C3a-p + IgG	974	212	755-1671 (5/7)
3d PR C3a-p + C3aR- Ab	49	16	439 (1/9)
3d PR C3a-p + PD98059	94	94	852 (1/9)
3d PR FGF2 + PD98059	56	56	279 (1/5)
3d PR C3a-p + Stattic	140	80	278-688 (4/9)
3d PR IL-6	508	109	143-1006 (8/10)

3d PR DPBS	**0**	**0**	**0 (0/6)**
3d PR C3a-p + IL-6 Ab	**215**	**146**	**651 and 1070 (2/8)**
3d PR C3a-p + CHIR 99021	**98**	**36**	**148-420 (6/16)**
3d PR C3a-p + DMSO (CHIR 99021 equivalent)	**547**	**163**	**167-1966 (8/11)**

Supplementary Table S7. Mean measurement of regeneration with s.e.m. and range for each treatment.

Treatment	Area Counted	Antibody	Mean	s.e.m.	Range
3d PR FGF2	Anterior	BrdU	21.0	2.7	21-30
3d PR C3a-p	Anterior	BrdU	24.7	6.2	8-42
3d PR FGF2	Posterior	BrdU	75.0	8.6	54-105
3d PR C3a-p	Posterior	BrdU	129.5	6.2	111-137
7d PR FGF2	Anterior	BrdU	12.3	5.2	12-21
7d C3a-p	Anterior	BrdU	16.0	2.6	4-22
7d PR FGF2	Posterior	BrdU	36.0	8.6	20-59
7d PR C3a-p	Posterior	BrdU	137.0	6.4	136-158
3d PR FGF2	Anterior	Chx10/Pax6	69.5	2.1	64-77
7d PR C3a-p	Anterior	Chx10/Pax6	95.4	4.8	87-114
7d PR FGF2	Anterior	Chx10/Pax6	246.6	3.4	16-35
7d PR C3a-p	Anterior	Chx10/Pax6	909.2	5.8	84-115
E11	Posterior	Brn3a	32.3	5.0	27-37
7d PR FGF2	Posterior	Brn3a	24.4	4.0	16-29
7d PR C3a-p	Posterior	Brn3a	16.5	4.3	11-29
E11	Posterior	AP-2	51.0	8.0	40-59
7d PR FGF2	Posterior	AP-2	41.0	7.4	33-51
7d PR C3a-p	Posterior	AP-2	209.8	110	94-363
E11	Posterior	Chx-10	62.0	7.0	57-70
7d PR FGF2	Posterior	Chx-10	78.0	4.6	78-82
7d PR C3a-p	Posterior	Chx-10	108.0	14.0	94-122
E11	Posterior	Pax-6	7.3	2.1	5-9
7d PR FGF2	Posterior	Pax-6	9.0	3.5	7-13
7d PR C3a-p	Posterior	Pax-6	13.7	7.7	5-29
E11	Posterior	Visinin	43.7	1.1	43-45
7d PR FGF2	Posterior	Visinin	61.3	8.6	49-78
7d PR C3a-p	Posterior	Visinin	60.3	8.4	50-77

Supplementary Table S8. Mean number of immunopositive cells with s.e.m. and range comparing the number of proliferating cells, progenitors, and/or differentiated cells during C3a-p and FGF2 induced regeneration as well as in developing retina at E11.