

Supplemental Information

**Cell-Type-Specific Complement Expression
in the Healthy and Diseased Retina**

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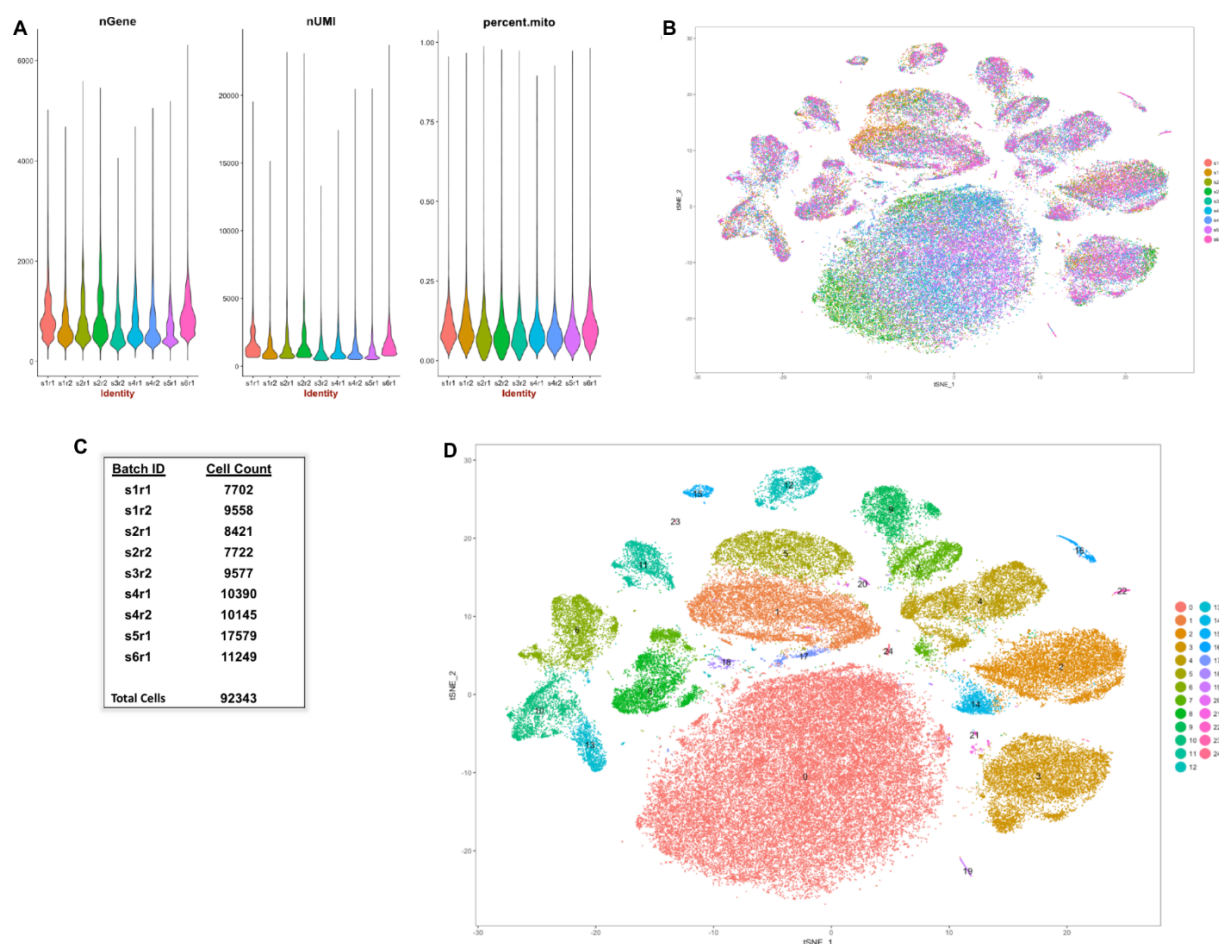


Figure S1. Quality control of scRNA sequencing data set. Related to Figure 1. Summary of the quality metrics and distribution of retinal cells from the six genetically identical C57BL/6J mice. We filtered out low-quality cells in which <90% of the reads did not map to the genome using the Cell Ranger pipeline from 10x genomics, and ultimately obtained 92,343 cells used in our subsequent analyses.

- A Violin plots showing the number of genes (*nGene*), UMIs (*nUMI*), and the percentage of mitochondrial genes (*percent.mito*) detected in each batch. The percentage of UMIs mapping to mitochondrial genes is a common scRNA-seq quality control metric.
- B tSNE plot showing the cell distribution from nine different batches. Note that cells from each batch contributed to every single cell cluster.
- C A table displaying the number of cells isolated from each of nine different retina dissection and scRNA-seq preparations from six healthy C57BL/6J mice.
- D Unsupervised clustering demonstrates 25 distinct cell clusters shown in a *t*-distributed stochastic neighbour embedding (tSNE) map, which we subsequently labelled into eleven major cell classes (N=92,343 cells).

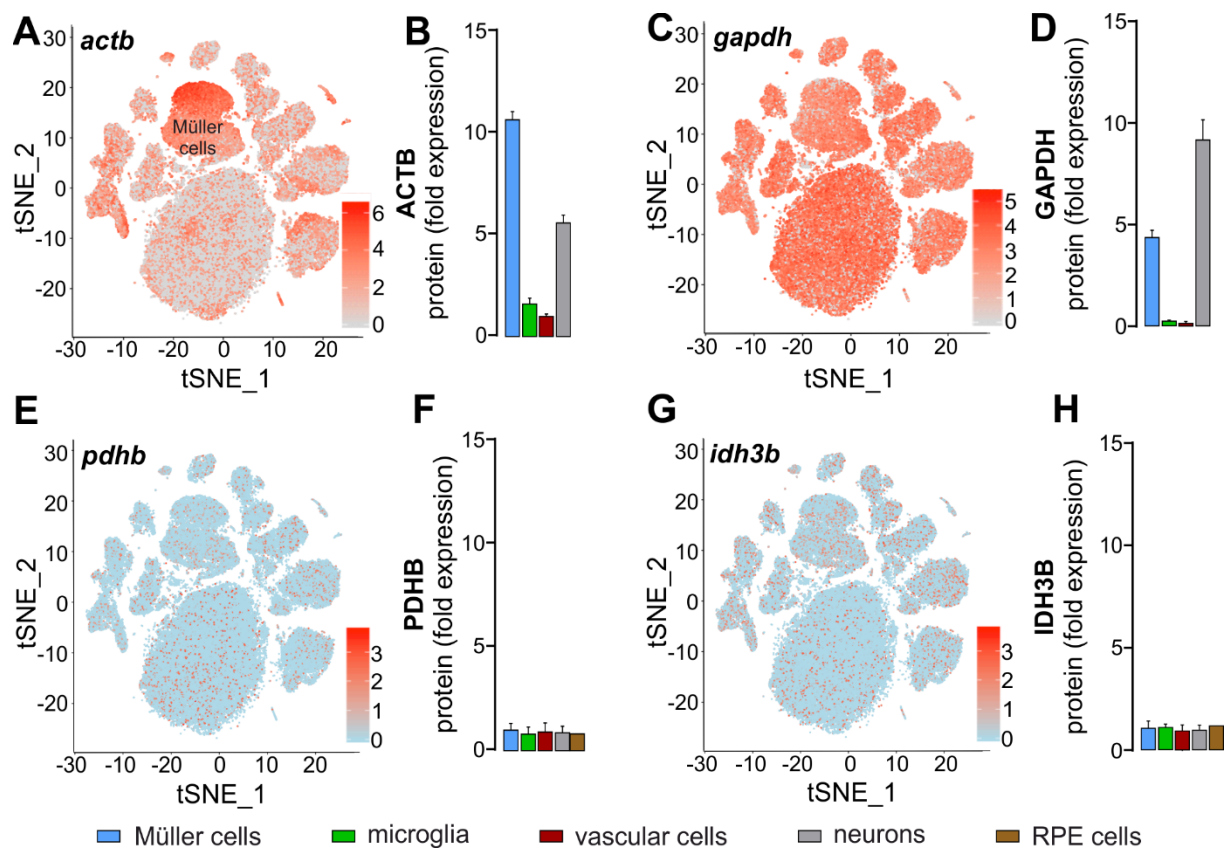


Figure S2: Putative housekeeping genes PDHB and IDH3b are expressed at comparable levels in all five investigated retinal cell populations. Relates to Figures 3-7.

A, C, E, G Single cell RNA sequencing of 92,000 retinal cells revealed expression of *actb*, *gapdh*, *idh3b* and *pdhb* in all retinal cell populations. *Actb* expression was clearly higher in Müller cells compared to neuronal clusters or other cell types. *Gapdh*, *idh3b* and *pdhb* showed similar distribution. The expression level was lower for *idh3b* and *pdhb* than for *gapdh*.

B, D, F, H Quantitative mass spectrometric analysis revealed equal protein expression levels of PDHB and IDH3B relative to total protein input amounts in all investigated cell populations, while commonly used housekeepers like ACTB and GAPDH showed major differences regarding expression levels in the distinct cell populations (n=3 – 4, mean ± SEM).

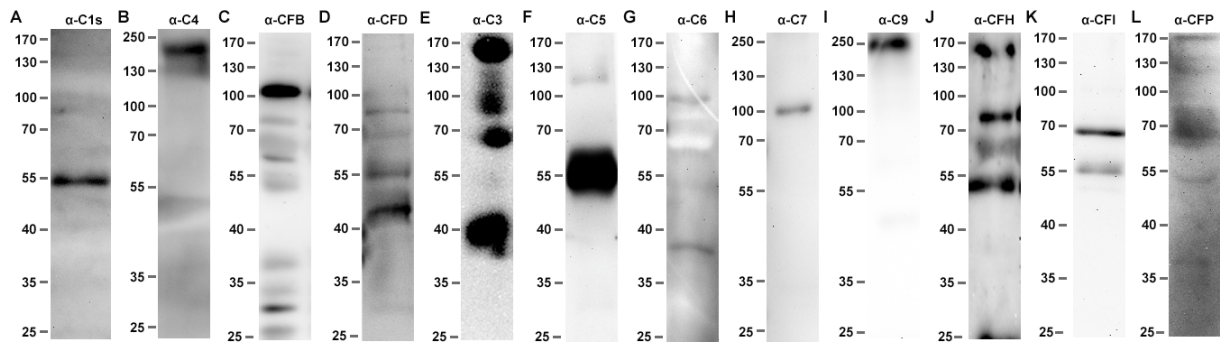


Figure S3: Validation of antibodies used for detection of complement components.

Related to Figure 4. Implemented antibodies for complement component detection in the retinal cell types were tested on mouse serum as positive controls, to assure that these antibodies do specifically detect the respective complement components.

- A C1s heavy chain (~50 kDa) was detected under reducing conditions.
- B C4 was detected in non-reduced serum at ~187 kDa.
- C Whole CFB (~100 kDa) and CFB cleavage products Bb (~57 kDa) and Ba (~33 kDa) were observed in reduced mouse serum.
- D CFD has a theoretical molecular weight of 25 kDa. We observed protein signals at ~85, 70, 55 and 45 kDa under reducing conditions in mouse serum.
- E C3 (~170 kDa), C3 alpha chain (~100 kDa) and C3 cleavage products (iC3b ~60 kDa, C3d/C3dg ~ 40 kDa) were detected under reducing conditions using a rabbit anti-C3 alpha chain antibody in mouse serum.
- F C5 heavy chain was detected at 112 kDa under reducing conditions together with the mouse Ig heavy chain at 55 kDa.
- G Goat anti- human C6 antibodies detected full-length C6 at 102 kDa and MACFP domains at 39 kDa under reducing conditions.
- H C7 has a theoretical size of 95 kDa and was detected at 100 kDa in non-reduced mouse serum.
- I Rabbit anti- human C9 antibodies showed the C9-complex in non-reduced mouse serum.
- J CFH (~170 kDa) and FHR-C (~90 kDa) were detected together with murine IgG heavy chain (~55 kDa) in reduced mouse serum.
- K CFI was detected at ~ 70 kDa and the heavy chain at ~50 kDa using a goat anti-human CFI antibody in murine reduced serum.
- L Murine properdin was detected at ~ 60 kDa using a rat anti-murine properdin serum.

Table S1: Marker genes used to identify the major cell classes in the retina along with their cell type-specific expression parameters. Related to Figure 1.

Cell type	Marker genes*	% of cells with non-zero expression	mean expression (based on UMIs/cell) among cells with non-zero expression
rod photoreceptor	<i>rho</i>	99.5	21.6
	<i>gnat1</i>	94.5	5.1
	<i>rcvrn</i>	90.2	4.0
	<i>nr2e3</i>	68.9	2.2
	<i>nrl</i>	79.5	2.5
	<i>rbp3</i>	52.4	1.9
cone photoreceptor	<i>arr3</i>	98.0	8.3
	<i>gnat2</i>	93.7	4.6
	<i>opn1mw</i>	56.8	5.5
	<i>opn1sw</i>	82.1	20.0
rod bipolar cell (BC)	<i>prkca</i>	93.6	4.1
	<i>vstm2b</i>	55.7	1.7
	<i>prdm8</i>	26.8	1.2
	<i>pcp2</i>	99.5	20.1
cone BC	<i>trpm1</i>	30.2	1.5
	<i>vsx1</i>	16.0	1.6
	<i>isl1</i>	32.9	1.7
	<i>gsg1</i>	30.0	2.3
	<i>sox6</i>	6.6	1.2
microglia	<i>aif1</i>	64.9	2.6
	<i>tmem119</i>	58.3	3.3
	<i>cx3cr1</i>	65.8	4.2
	<i>trem2</i>	77.1	5.2
horizontal cell	<i>lhx1</i>	91.7	4.1
	<i>prox1</i>	94.2	5.4
	<i>pax6</i>	93.0	4.0
endothelial cell	<i>pecam1</i>	68.9	3.1
	<i>cspg4</i>	19.2	1.8
pericytes	<i>pdgfrb</i>	51.8	3.3
	<i>kcnj8</i>	45.2	4.2
Müller cells	<i>glul</i>	99.7	23.0
	<i>rlbp1</i>	93.3	5.7
retinal ganglion cells	<i>pou4f1</i>	91.6	5.3
	<i>sncg</i>	98.9	19.2
	<i>pou4f2</i>	29.8	1.5
	<i>tfap2d</i>	5.1	1.4
amacrine cells	<i>gad1</i>	35.9	2.3
	<i>gad2</i>	21.1	1.5
	<i>slc6a1</i>	40.5	2.4
	<i>slc6a9</i>	33.8	2.1

* Genes were selected based on the combination of an existing retina cell atlas from mouse single-cell transcriptomics data, additional immunohistochemical studies, and the *FindMarkers()* function in Seurat v2.

* Genes were selected based on an existing retina cell atlas from mouse single-cell transcriptomics data, and additional immunohistochemical studies (Shekhar et al. 2016, Macosko et al. 2015, Cheng et al. 2013b, Kim et al. 2008)

Table S2: Single-cell RNA sequencing identified all the major cell classes in mouse retina, with proportions comparable with past studies. Related to Figure 1.

% composition of cell types	Jeon et al. 1998	Macosko et al. 2015	Current single cell study %	# Cells
rod photoreceptors	79.9	65.6	40.6	37474
cone photoreceptors	2.1	4.2	8.1	7473
Müller cells	2.8	3.6	13.6	12597
RGCs	0.5	1.0	0.2	178
horizontal cells	0.5	0.6	0.6	516
amacrine cells	7.0	9.9	7.5	6941
bipolar cells	7.3	14.0	28.3	26153
endothelial cells	–	0.6	0.5	495
microglia	–	0.2	0.3	319
pericytes	–	0.2	0.2	197

Table S3: Cell type-specific expression of complement in the C57/BJ6 mouse retinae. Related to Figure 1.

Complement		Cell type-specific gene expression	% of cells within a given cell type with non-zero expression	Mean expression (based on UMIs) in cells with non-zero expression
Classical pathway components				
<i>c1q</i> complex (A, B and C subunit genes)		Microglia	86.2	4.1
<i>c1s1</i>		Pericytes,	10.7	2.2
		Endothelial cells	9.5	1.0
<i>c1s2</i>		<i>not detected</i>	—	—
<i>c1r</i>		Pericytes,	2.5	1.9
		Endothelial cells	2.2	0.65
<i>c1rl</i>		Müller cells,	0.8	1.6
		Microglia	2.8	1.4
Lectin pathway components				
<i>mb1-a</i>		<i>not detected</i>	—	—
<i>mb1-c</i>		<i>not detected</i>	—	—
<i>fcna</i>		<i>not detected</i>	—	—
<i>fcnb</i>		<i>not detected</i>	—	—
<i>colec11</i>		<i>not detected</i>	—	—
<i>masp1</i>		<i>not detected</i>	—	—
<i>masp2</i>		<i>not detected</i>	—	—
<i>masp3</i>		<i>not detected</i>	—	—
Classical and lectin pathway components				
<i>c2</i>		Cone BCs,	0.3	2.1
		RGCs	1.1	1.0
<i>c4</i>		Müller cells,	1.6	1.6
		Microglia,	0.6	1.8
		Pericytes,	1.0	2.0
		RGCs,	1.1	1.0
		Endothelial cells	0.4	1.2
Alternative pathway components				
<i>cfb</i>		<i>not detected</i>	—	—
<i>cfh</i>		<i>not detected</i>	—	—
Core complement components				
<i>c3</i>		Microglia	1.0	1.4
<i>c5</i>		<i>not detected</i>	—	—
<i>c6</i>		<i>not detected</i>	—	—
<i>c7</i>		<i>not detected</i>	—	—
<i>c8</i>		<i>not detected</i>	—	—
<i>c9</i>		<i>not detected</i>	—	—
Complement regulators				
<i>c1-inh (serping1)</i>	soluble	Müller cells,	3.8	1.6
		Cone BCs,	0.5	2.1
		Endothelial cells	10.7	0.9
<i>c4bp</i>	soluble	<i>not detected</i>	—	—
<i>cpn1</i>	soluble	<i>not detected</i>	—	—
<i>cfp</i>	soluble	Rod photoreceptors, Cone photoreceptors, Rod BCs,	0.4	2.5
		Cone BCs,	1.6	2.0
		Amacrine cells,	1.0	2.0
		Müller cells	1.0	2.1
			1.3	1.8
			1.2	1.5
<i>cfh</i>	soluble	Microglia,	29.8	1.7
		Endothelial cells, Pericytes,	28.3	1.3
		Müller cells	15.7	2.2
			2.2	1.6

<i>cfi</i>	soluble	Rod BCs	2.2	2.0
<i>fhr-a</i>	soluble	<i>Not detected</i>	—	—
<i>fhr-b</i>	soluble	<i>Not detected</i>	—	—
<i>fhr-c</i>	soluble	<i>Not detected</i>	—	—
<i>vtn</i>	soluble	Rod photoreceptors, Cone photoreceptors, Rod BCs, Cone BCs, Horizontal cells, Amacrine cells, Microglia, Pericytes, Endothelial cells, Müller cells, RGCs	63.2 54.5 11.0 11.8 19.2 14.7 15.0 59.9 57.4 14.5 22.5	3.0 2.4 2.1 2.2 1.0 1.9 1.9 3.8 2.4 1.7 1.0
<i>clu</i>	soluble	Rod photoreceptors, Cone Photoreceptors, Rod BCs, Cone BCs, RGCs, Müller cells, Microglia, Amacrine cells, Horizontal cells	2.7 16.4 4.4 8.4 28.1 86.8 6.6 10.2 37.6	2.5 2.1 2.0 2.1 1.0 2.8 1.8 1.8 1.1
<i>crry</i>	membrane	Rod photoreceptors, Cone photoreceptors, Cone BCs, Horizontal cells, Microglia, Müller cells, Amacrine cells, Endothelial cells, RGCs	0.4 2.1 1.1 6.0 7.2 2.2 2.2 27.3 5.1 2.2	2.5 2.0 2.0 0.9 1.5 1.5 1.8 1.3 1.0 2.1
<i>cd55 (daf-1/ daf-2)</i>	membrane	Microglia	2.2	2.1
<i>cd59a</i>	membrane	Rod photoreceptors, Cone photoreceptors, Rod BCs, RGCs, Müller cells, Endothelial cells, Amacrine cells, Cone BCs, Horizontal cells	6.2 31.3 1.9 19.1 6.2 40.1 5.9 2.9 57.8	2.5 2.7 1.9 1.0 1.6 1.4 1.7 2.1 1.3
<i>cd59b</i>	membrane	Müller cells, RGCs, Horizontal cells, Amacrine cells	1.5 6.2 3.7 0.8	1.6 0.9 1.0 1.6
Complement receptors				
<i>cr1</i>		<i>Not detected</i>	—	—
<i>cr2</i>		Horizontal cells, Müller cells	4.7 0.5	0.8 1.6
<i>cr3 (itgam and itgb2)</i>		Microglia	33.2	1.7
<i>cr4 (itgax and itgb2)</i>		Microglia	1.0	1.2
<i>c3ar1</i>		Microglia	27.9	1.8
<i>c5ar1</i>		Microglia	23.5	1.8
<i>c5ar2</i>		Microglia	11.9	1.7
<i>c1qr (cd93)</i>		Endothelial cells, Pericytes	50.9 3.6	1.7 1.7
<i>gc1qr (c1qbp)</i>		Rod photoreceptors, Cone photoreceptors, Rod BCs,	5.6 8.3 9.6	2.5 2.0 2.0

<i>cc1qr (calr)</i>	Cone BCs,	9.6	2.1
	Horizontal cells, Amacrine	32.6	1.0
	cells,	15.0	1.8
	Microglia,	15.0	1.5
	Pericytes,	14.2	2.0
	Endothelial cells,	22.4	1.2
	Müller cells,	10.1	1.6
	RGCs	39.9	1.1
	Rod photoreceptors, Cone	13.1	2.5
	photoreceptors,	18.4	2.1
	Rod BCs,	16.6	2.0
	Cone BCs,	16.8	2.1
	Horizontal cells,	48.6	1.2
	Amacrine cells,	20.9	1.9
	Microglia,	36.4	1.8
	Pericytes,	15.7	1.9
	Endothelial cells,	43.8	1.5
	Müller cells,	22.2	1.7
	RGCs	37.6	1.0
<i>vsig4 (Crlg)</i>	<i>Not detected</i>	—	—

Table S4: Percentage of murine, albino, aging retinal cell type-specific complement expression profiles. Related to Figure 6A.

Complement transcript	Müller cells			microglia			vascular cell			neuron			RPE		
	8 w	16 w	24 w	8 w	16 w	24 w	8 w	16 w	24 w	8 w	16 w	24 w	8 w	16 w	24 w
<i>c1s</i>	9%	5%	11%	2%	3%	2%	4%	14%	11%	8%	7%	2%	9%	4%	4%
<i>c3</i>	12%	8%	16%	3%	3%	4%	1%	3%	6%	6%	5%	7%	3%	1%	1%
<i>cfb</i>	7%	8%	13%	3%	4%	12%	4%	11%	13%	5%	7%	4%	25%	3%	4%
<i>cfp</i>	38%	64%	49%	11%	13%	9%	16%	21%	28%	46%	56%	47%	3%	2%	2%
<i>cfh</i>	32%	14%	10%	71%	73%	72%	72%	49%	38%	14%	7%	9%	60%	91%	89%
<i>cfi</i>	1%	0.8%	2%	9%	5%	2%	3%	3%	4%	22%	17%	31%	0.4%	0.3%	0.3%

Table S5: Percentage of murine, pigmented control and postischemic retinal cell type-specific complement expression profiles. Related to Figure 7A.

Complement transcript	Müller cells		microglia		vascular cell		neuron		RPE	
	C	I/R	C	I/R	C	I/R	C	I/R	C	I/R
<i>c1s</i>	17%	5%	1%	0.3%	1%	1%	1%	1%	17%	12%
<i>c3</i>	26%	38%	0.2%	40%	0.0%	6%	2%	6%	4%	28%
<i>cfb</i>	12%	47%	52%	38%	72%	70%	8%	19%	13%	39%
<i>cfp</i>	14%	5%	11%	19%	15.1%	14%	76%	58%	13%	6%
<i>cfh</i>	31%	5%	35%	3%	10%	7%	1%	1%	54%	14%
<i>cfi</i>	0.3%	0.3%	1%	0.3%	2%	2%	12%	16%	0.2%	0.3%

Table S6: Primer and TaqMan probe combinations for detection of complement components via qRT-PCR. Relates to Figures 3-7.

Gene ID	Primer sequences <i>forward</i> <i>reverse</i>	TaqMan® probe from Roche
<i>idh3b</i>	5' gctgcgcatctcaatct 3' 5' ccatgtctcgagtcctgacc 3'	# 67 95
<i>pdhb</i>	5' ttaaatcggccattcgatg 3' 5' caggaaatctttgactgagctt 3'	N# 4
<i>c1s</i>	5' ggtggatacttctgctcctgtc 3' 5' agggcagtgaaacacatctcc 3'	# 69
<i>c3</i>	5' accttacctcggcaagttct 3' 5' ttgtagagctgctggtcagg 3'	# 76
<i>c4</i>	5' ctagagacgcaggccaagtt 3' 5' ccaggtctctgacccaata 3'	# 64
<i>c5</i>	5' aaagccccataaacctgtc 3' 5' tcggatatctgccttcacac 3'	# 48
<i>c6</i>	5' cagaaaacgcatttacctgga 3' 5' gctgtgaatccagtaagacatgaa 3'	# 69
<i>c7</i>	5' tgctgatgaagacaaatgtgaa 3' 5' ttaccctggccagtaactacg 3'	# 4
<i>c8</i>	5' gacaggatttcagtgtagagagac 3' 5' ccctgacatcttcacagtcg 3'	# 21
<i>c9</i>	5' tgaccgagtagcggaagaat 3' 5' tcattgtcaaaagggtgtctcag 3'	# 58
<i>cfb</i>	5' ctgaacctgcagatccac 3' 5' tcaaagtcctgcggtcgt 3'	# 112
<i>cfd</i>	5' ctgggagcggctgtatgt 3' 5' cacggaagccatgtaggg 3'	# 79
<i>cfp</i>	5' tcttgagtggcagctacagg 3' 5' cagaccagccacccatct 3'	# 56
<i>cfh</i>	5' aaaaaccaaagtgccgagac 3' 5' ggaggtgatgtctccattgtc 3'	# 25
<i>cfi</i>	5' tttcttggctctccacttg 3' 5' tgcagtaagcatttctgatcg 3'	# 63

Table S7: Used dilutions for primary and secondary antibodies. Related to the STAR methods Key Resource Table.

Primary antibody	Dilution / Concentration
anti-PDHB	WB 1:1000
anti-C1s	WB 1:1000/ IS 1:100
anti-C4	WB 1:50
anti-C3-HRP	WB 1:1000
anti-C3	IS 1:100
anti-CFB	WB 1:1000
anti-CFD	WB 1:200
anti-C5	WB 1:1000
anti-C6	WB 1:100
anti-C7	WB 1:250
anti-C9	WB 1:500
anti-CFP	WB 1:250
anti-CFH	WB 1:500
anti-CFI	WB 1:500
anti-IBA1	WB 1:400
anti-glutamine sythetase	WB 1:1000
Secondary antibody	
anti-rat Ig-HRP	1:5000
anti-rabbit Ig-HRP	1:5000
anti-goat Ig-HRP	1:5000
Anti-rabbit-IG-Cy3	1:200

WB – Western blot, IS – immunostainings