Supplemental Data

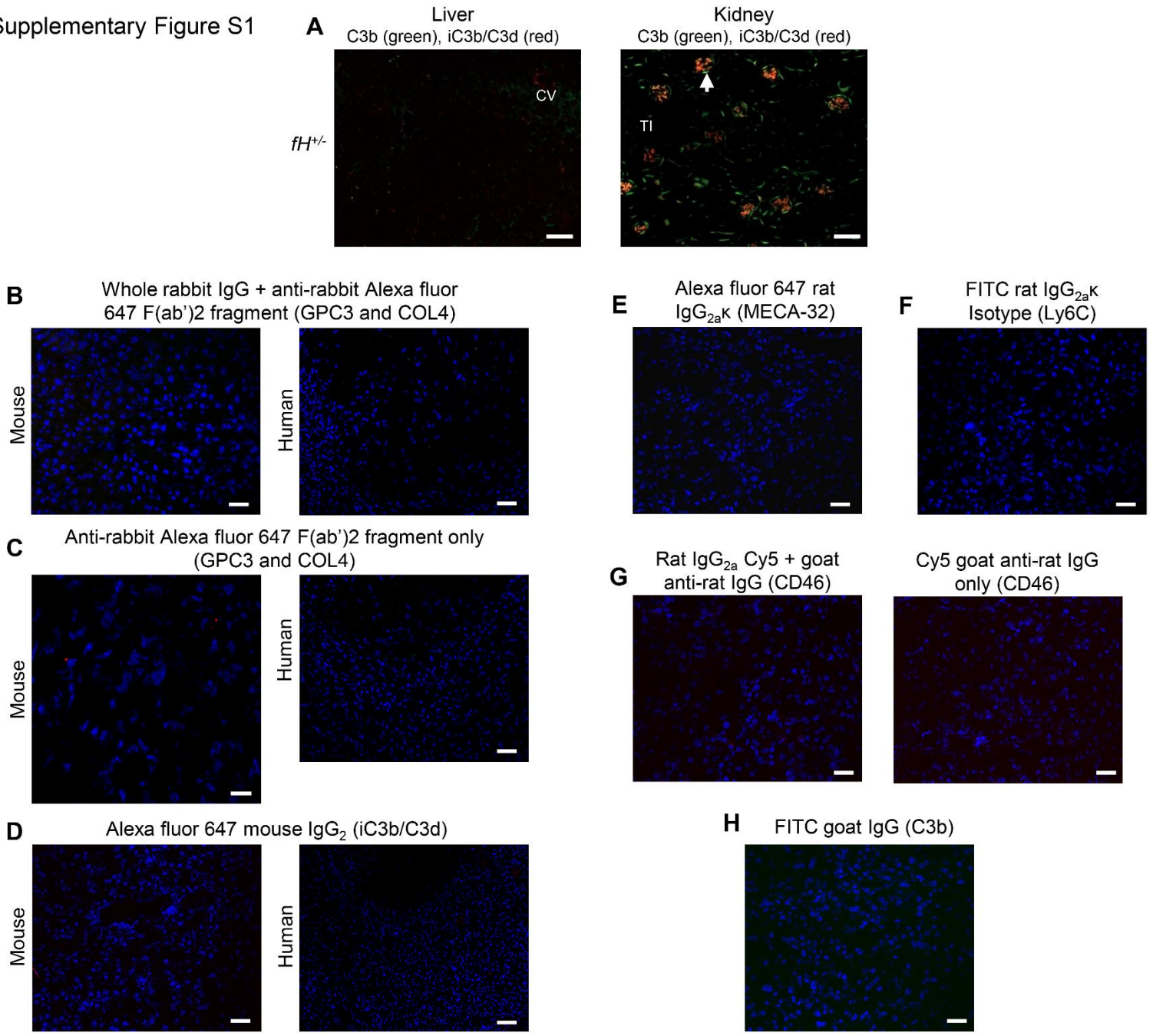
Complement factor H-deficient mice develop spontaneous hepatic tumors

Jennifer Laskowski, Brandon Renner, Matthew C. Pickering, Natalie J. Serkova, Peter M. Smith-Jones, Eric T. Clambey, Raphael A. Nemenoff, and Joshua M. Thurman

Supplementary Figures

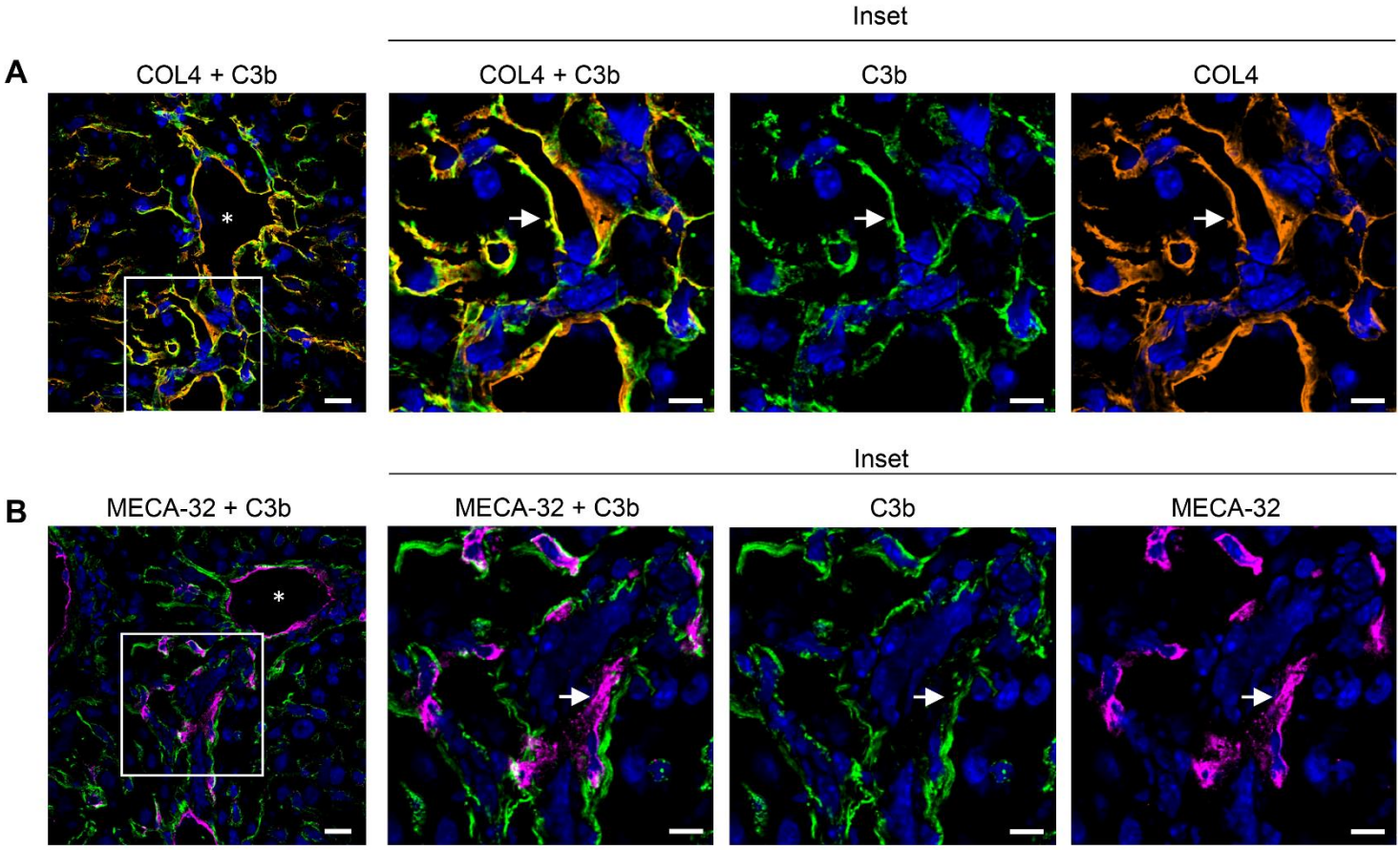
Supplementary Tables

Supplementary Figure S1



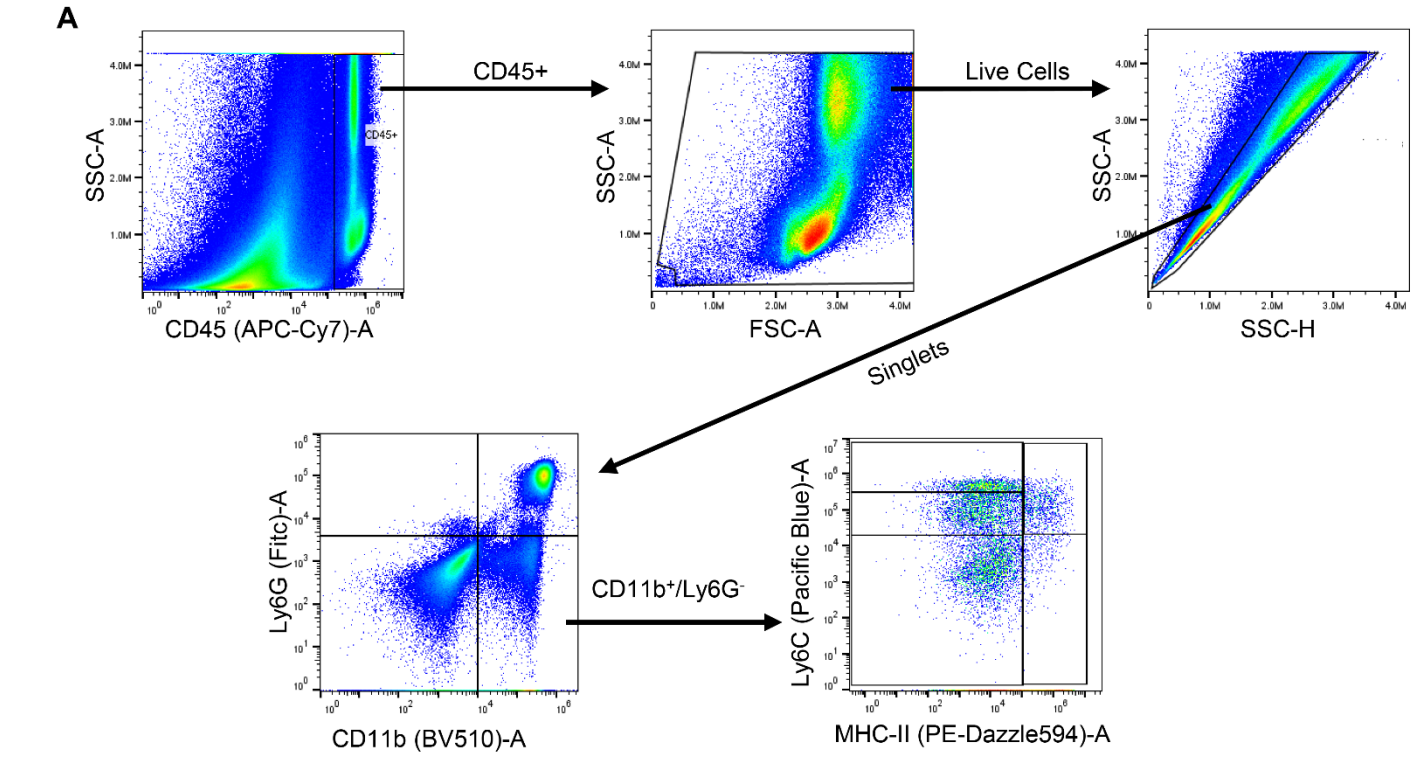
Supplementary Figure S1. (**A**) Representative C3b and iC3b/C3d staining in liver and kidneys (glomerulus indicated by arrow) from *fH*$^{+/-}$ mice. Scale = 50 µm (liver) and 100 µm (kidney); n=10 (liver) and 5 (kidney) males (3-5 months old). (**B**-**H**) Immunofluorescence isotype staining (target antigen indicated in parentheses). Scale = 50 µm and 100 µm (**B**, mouse and human, respectively), 20 µm and 100 µm (**C**, mouse and human, respectively), 100 µm (**D**), and 50 µm (**E**-**H**). CV: central vein; TI: tubulointerstitium.

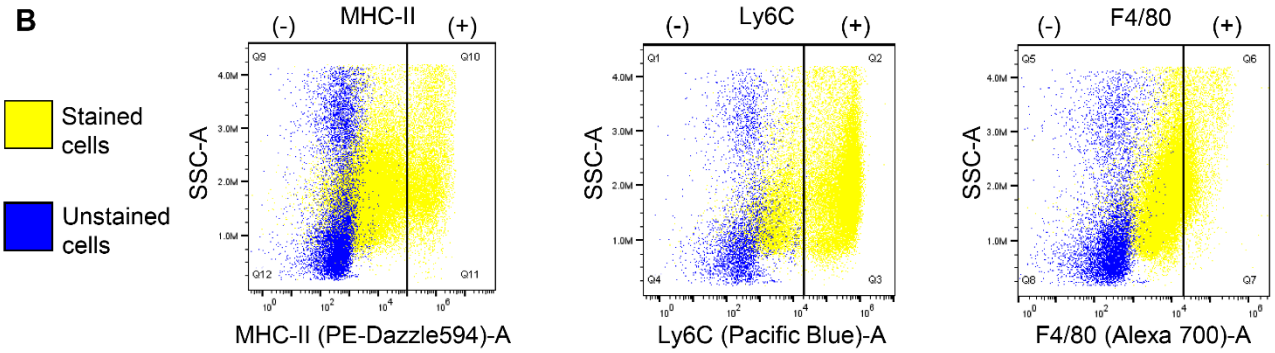
Supplementary Figure S2



Supplementary Figure S2. (**A-B**) C3b deposition is frequently observed colocalizing with COL4 (**A**, orange) and adjacent to MECA-32 (**B**, magenta) in *fH*$^{-/-}$ sinusoids, but not on the large vasculature (vascular lumen denoted by asterisks). Nuclei stained with DAPI (blue). Scale=50 μm (**A** and **B**, left-most images) or 10 μm (all other images); n=5 males (7 months). Representative images shown from four independent experiments.

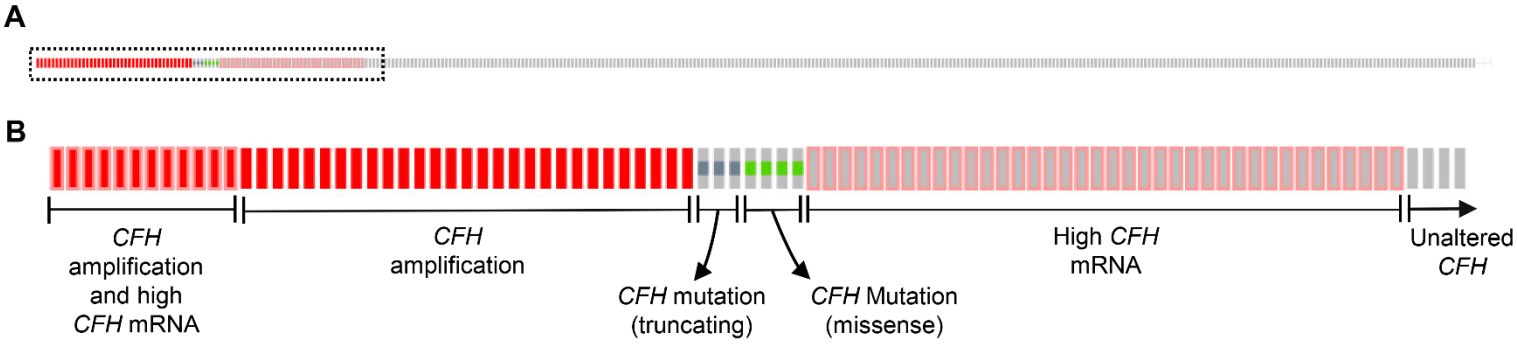
Supplementary Figure S3

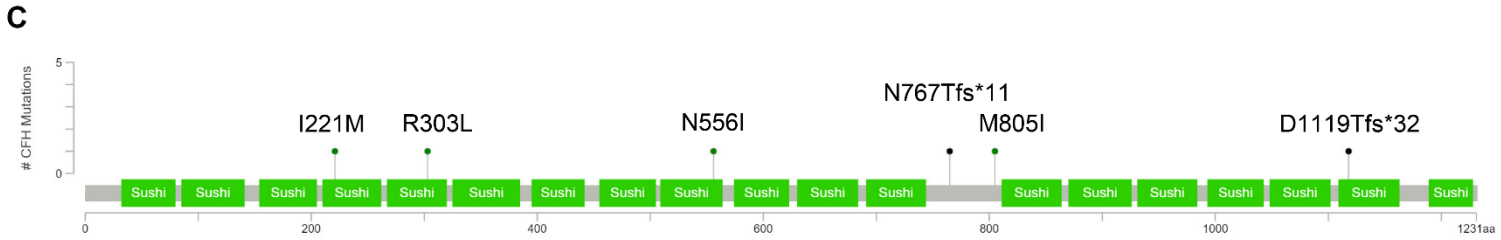




Supplementary Figure S3. Liver myeloid cell multispectral flow cytometry gating strategy. (**A**) Spectrally unmixed hepatic single cell suspensions were first gated on CD45 positivity, followed by cell viability and selection of single cells. Next, polymorphonuclear cells were excluded based on $CD11b^+$ and $Ly6G^+$ expression, leaving the $CD45^+CD11b^+Ly6G^-$ myeloid cell population. Myeloid cells were further characterized based on Ly6C and MHC-II expression followed by back gating on F4/80. (**B**) Pseudo color dot plot illustrating the respective positive/negative gates for MHC-II (PE-Dazzle594), Ly6C (Pacific Blue), and F4/80 (Alexa fluor 700). Negative, unstained cells (blue) and positive, stained cells (yellow).

Supplementary Figure S4





D

Protein Change	Mutation Type	Copy #	Exon
I221M	Missense Mutation	Gain	6
R303L	Missense Mutation	Gain	7
N556I	Missense Mutation	Gain	11
D1119Tfs*32	Frame Shift Deletion	Shallow Deletion	15
M805I	Missense Mutation	Gain	16
N767Tfs*11	Frame Shift Deletion	Gain	21

Supplementary Figure S4. CFH alterations in human HCC data. (**A**) Cartoon depicting human HCC TCGA data accessed at cbioportal.org. A total of 442 tumors from 440 patients in the Liver Hepatocellular Carcinoma (TCGA Firehose Legacy) study is represented, each by a hash mark. (**B**) Magnified view of the 86 tumors (23% of the group) with a *CFH* genetic alteration. Amplifications (derived from copy-number analysis) are shown in red, followed by truncating mutations (blue stripe), missense mutations (green stripe), and high CFH mRNA expression (pink outline). (**C**) Cartoon depicting the human CFH protein with "lollipop" markers identifying each mutation and its location. (**D**) Six identified *CFH* mutations observed in HCC tumors from this study, detailing mutation type and location, protein change, and the exon within which each mutation occurs.

Supplementary Table S1

P value legend

Adverse outcome	
P ≤ 0.05	NS
Favorable outcome	
P ≤ 0.05	NS

	CFH Mutation			High *CFH* mRNA		
	Frequency / cases profiled	Median Months Survival * ^	Median Months Disease-Free Progression * ^	Frequency / cases profiled	Median Months Survival * ^	Median Months Disease-Free Progression * ^
	P value	*P* value	*P* value	*P* value	*P* value	*P* value
CFH mutation associated with adverse outcome; high _CFH_ mRNA associated with favorable outcome						
Hepatocellular Carcinoma	2% / 373	-35.05 0.0332	-11.27 0.0824	13% / 373	NA, 51.25 0.0219	36.65 0.002254
Lymphoid Neoplasm Diffuse Large B-Cell Lymphoma	4% / 48	-169.94 0.089	-109.43 0.062	4% / 48	NA, 211.07 0.634	NA, 120.53 0.579
Lung Adenocarcinoma	7% / 230	-2.33 0.947	-23.33 0.0112	4% / 517	NA, 49.24 0.274	11 0.107
Esophageal Carcinoma	5% / 185	-4.96 0.886	-4.99 0.669	7% / 184	5.78 0.75	9.52 0.765
CFH mutation and/or high _CFH_ mRNA associated with adverse outcome						
Brain - Lower Grade Glioma	1% / 286	-38.07 0.0177	-38.5 0.0822	1% / 286	-83.68 0	NA, 47.7 NaN
Bladder Urothelial Carcinoma	3% / 238	-14.52 0.0682	-7.49 0.854	6% / 408	-15.47 0.588	-9.19 0.401
Head and Neck Squamous Cell Carcinoma	3% / 510	-36.37 0.0975	-15.14 0.463	5% / 520	-19.22 0.931	-32.98 0.943
Sarcoma	2% / 252	-42.05 0.835	-29.76 0.546	2.7% / 252	-51.38 0.471	-27.69 0.309
Kidney Renal Cell Carcinoma	1% / 451	-18.86 0.763	NA, 123.72 0.343	4% / 534	-37.42 0.398	NA, 123.72 0.88
Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma	3% / 194	-113.27 0.0164	NA, NA NaN	9% / 304	-39.35 0.64	NA, NA 0.527
Adrenocortical Carcinoma	2.2% / 90	39.55, NA 0.687	11.53, 37.75 0.243	6% / 79	-59.99 0.216	-20.34 0.474
Uveal Melanoma	0% / 80	--	--	1.3% / 80	31.04, NA 0.0205	-34.96 0.0452
Acute Myeloid Leukemia	0% / 197	--	--	2.3% / 173	-16.06 0.000008084	NA, NA --
Colorectal Adenocarcinoma	4% / 223	NA, NA 0.492	NA, NA 0.315	5% / 379	-45.63 0.0427	NA, 74.57 0.756
Stomach Adenocarcinoma	7% / 395	NA, 30.88 0.57	NA, 55.06 0.134	2% / 415	-21.91 0.0355	-33.94 0.756
Cholangiocarcinoma	0%	--	--	11% / 36	-24.77 0.522	-7.62 0.785
Breast Invasive Carcinoma	1% / 982	NA, 129.6 0.314	NA, 214.72 0.258	1% / 1093	-15.87 0.238	NA, 214.72 0.722
Testicular Germ Cell Cancer	0.7% / 155	NA, NA 0.869	NA, 142 0.604	5% / 156	NA, NA 0.723	-176.61 0.108
CFH mutation and/or high _CFH_ mRNA associated with favorable outcome						
Uterine Corpus Endometrial Carcinoma	6% / 248	NA, NA 0.0711	NA, NA 0.0335	0% / 248	--	--
Cutaneous Melanoma	11% / 368	13.1 0.397	5.32 0.453	2% / 368	56.74 0.495	71.06 0.27
Pancreatic Adenocarcinoma	1% / 150	0.16 0.686	2.14 0.787	3% / 179	23.82 0.697	-3.59 0.785
Glioblastoma Multiforme	0.7% / 290	3.32 0.829	4.5 0.599	4% / 528	0.49 0.272	-0.23 0.37
Prostate Adenocarcinoma	2% / 498	NA, NA 0.77	48.39, NA 0.0339	4% / 498	NA, NA 0.544	69.12, NA 0.903
Kidney Chromophobe	0% / 66	--	--	8% / 66	NA, NA 0.31	NA, 151.84 0.317
Undetermined or no apparent affect on outcome with either _CFH_ mutation and/or increased _CFH_ mRNA						
Lung Squamous Cell Carcinoma	4% / 179	18.33 0.834	-30.94 0.157	2.4% / 501	-16.1 0.883	NA, 58.9 0.513
Ovarian Serous Cystadenocarcinoma	0% / 316	-- --	-- --	4% / 307	-3.25 0.406	0.3 0.3
Mesothelioma	0% / 87	Not available	Not available	13% / 87	Not available	Not available
Thymoma	0% / 123	--	--	3% / 120	NA, NA 0.644	NA, NA 0.408
Thyroid Carcinoma	0% / 405	--	--	4% / 509	NA, NA 0.139	NA, NA 0.875
Uterine Carcinosarcoma	0% / 57	Not available	Not available	7% / 57	Not available	Not available
Pheochromocytoma and Paraganglioma	0.6% / 184	Not available		4% / 184	Not Available	

P values presented as logrank

* Median months survival and disease-free progression of patients with *CFH* alteration compared to those without

^ When two values are given: the first corresponds to patients with *CFH* alteration, the second to those without.

NS: not significant; NA: not applicable

Supplementary Table S2

Name	Supplier or Citation	Catalog Number	Clone	Source and isotype (if applicable)	Experimental Concentration (µg/ml) or Dilution
Human/Mouse Glypican 3	Abcam	ab66596	pAb	Rabbit	1
Mouse Collagen IV	EMD Millipore	AB756P	pAb	Rabbit	4
MECA-32 Panendothelial cell antigen (Alexa Fluor 647)	Novus Biologicals	NB100-77668AF647	--	Rat $IgG_{2a}κ$	7.5
Mouse C3b (FITC)	MP Biomedicals	55500	pAb	Goat	5
Mouse CD46	Hycult Biotech	HM1118	MM10	Rat IgG_{2a}	2
Mouse/Human iC3b/C3d (Alexa Fluor 647)	*(25)*	--	C3d29	Mouse IgG_{2a}	1
Mouse/Human iC3b/C3d (DyLight 555)	*(25)*	--	C3d29	Mouse IgG_{2a}	1
Rat $IgG_{2a}κ$ Isotype control (FITC)	Southern Biotechnology	0117-02	KLH/G2a-1-1	Goat	5
ChromePure Rabbit IgG	Jackson ImmunoResearch	011-000-003	pAb	Rabbit	1
ChromePure Goat IgG (FITC)	Jackson ImmunoResearch	005-090003	pAb	Goat	5
Rat $IgG_{2a}κ$ Isotype control (Alexa fluor 647)	Southern Biotechnology	0117-31	KLH/G2a-1-1	Goat	7.5
Rat IgG_{2a} Isotype control	BD Bioscience	553928	R35-95	Rat	2
Mouse IgG_2 Isotype (Alexa fluor 647)	Produced and validated in house	--	10-5C6	Mouse IgG_2	1
CD3ε	Cell Signaling Technology	99940S	D4V8L	Rabbit	0.86
FoxP3	ebioscience	14-5773	FJK-16s	Rat $IgG_{2a}κ$	2.5
CD4	ebioscience	14-9766-82	4SM95	Rat $IgG_1κ$	2.5
CD8a	ebioscience	14-0808-80	4SM15	Rat $IgG_{2a}λ$	5
F4/80	Cell Signaling Technology	70076S	D2S9R	Rabbit	4.35
B220 (CD45R)	BD Biosciences	557390	RA3-6B2	Rat $IgG_{2a}κ$	0.5
Mouse CD45 (APC/Cy7)	Biolegend	103115	30-F11	Rat $IgG_{2b}κ$	0.2
Mouse Ly-6G (FITC)	BD Bioscience	551460	1A8	Rat $IgG_{2a}κ$	0.2 (FC) / 5 (IF)
Mouse CD11b (BV510)	BD Bioscience	562950	M1/70	Rat DA/HA $IgG_{2b}κ$	0.2
Mouse F4/80 (Alexa fluor 700)	BioRad	MCA497A700T	A3-1	Rat IgG_{2b}	0.2
Mouse Ly-6C (Pacific Blue)	BioRad	MCA2389PB	ER-MP20	Rat IgG_{2a}	0.2
Mouse I-A/I-E [MHC class II] (PE/Dazzle 594)	BioLegend	107647	M5/114.15.2	Rat $IgG_{2b}κ$	0.2
Alexa Fluor 647 F(ab')2 fragment donkey anti-rabbit IgG (H+L)	Jackson ImmunoResearch	711-606-152	pAb	Donkey	3.75
Cy5 AffiniPure goat anti-rat IgG (H+L)	Jackson ImmunoResearch	112-175-143	pAb	Goat	3.75
Rat-on-Mouse HRP Polymer	Biocare	RT517L	pAb	Rat	Per supplier instructions
Anti-rabbit HRP	Akoya Biosciences	ARR1001KT	pAb	Rabbit and mouse cocktail	1:4
Mouse CD16/CD32 Fc Block	BD Biosciences	553142	2.4G2	Rat $IgG_{2b}κ$	1
AffiniPure donkey anti-mouse IgG (H+L)	Jackson ImmunoResearch	715-005-151	pAb	Donkey	10
Opal 520 reactive fluorophore	Akoya Biosciences	FP1487001KT	N/A	N/A	1:150
Opal 540 reactive fluorophore	Akoya Biosciences	FP1494001KT	N/A	N/A	1:100
Opal 570 reactive fluorophore	Akoya Biosciences	FP1488001KT	N/A	N/A	1:300
Opal 620 reactive fluorophore	Akoya Biosciences	FP1495001KT	N/A	N/A	1:150
Opal 650 reactive fluorophore	Akoya Biosciences	FP1496001KT	N/A	N/A	1:150
Opal 690 reactive fluorophore	Akoya Biosciences	FP1497001KT	N/A	N/A	1:50

pAb: polyclonal antibody
FC: flow cytometry
IF: immunofluorescence

Supplementary Table S3

Equipment / Model	Manufacturer	Figure(s)
FV1000 FCS confocal microscope	Olympus Scientific Solutions Americas Corp.	1H-1J; 2A-2C; 5E; 7A-7D, 7F-7G; 8A-8E; 9A; S1; S2
Axio Observer D1 epifluorescent inverted microscope	Carl Zeiss Microscopy, LLC	1E-1G; 2D-2F; 3B;3D, S1A (kidney)
Siemens Inveon microPET/CT scanner	Siemens Medical Solutions	2I
DriChem 7000 Analyzer	Heska	3A
nCounter Max Analysis System	NanoString Technologies	4A-4I
Bond-III Fully Automated IHC and ISH Autostainer	Leica Biosystems	5A-5B
Vectra 3.0 Qualitative Pathology	Perkin Elmer	5A-5B
Aurora multi-spectral flow cytometer	Cytek Biosciences	5D and 6A-6D

Supplementary Table S4

Software	Manufacturer	Version
FV10-ASW	Olympus Scientific Solutions Americas Corp.	04.02.02.09
Zen (Blue edition)	Carl Zeiss Microscopy, LLC	2.6
nSolver	nanoString	4
nCounter Advanced Analysis	nanoString	2.0.115
R	r-project.org	3.3.2
FlowJo	BD (Becton, Dickinson and Company)	10.6.0
Prism	GraphPad	8.4.2
inForm Tissue Finder	Akoya Biosciences	2.4.1
Phenochart Whole Slide Contextual Viewer for Annotation	Akoya Biosciences	1.0.8
SpectroFlo	Cytek Biosciences	2.1.0
Image J	National Institutes of Health http://imagej.nih.gov/ij	1.51w
AsiProVM	Concorde Microsystems	6.7.1.1

Supplementary Table S5

Gene Name	Full Official Name	Gene Accession	Order selected by geNorm	SD after normalization
Ppia - mRNA	peptidylprolyl isomerase A	NM_008907.2	1	0.162
Oaz1 -mRNA	ornithine decarboxylase antizyme 1	NM_008753.4	2	0.167
Rpl19 -mRNA	ribosomal protein L19	NM_009078.2	3	0.160
Hprt -mRNA	hypoxanthine guanine phosphoribosyl transferase	NM_013556.2	4	0.194
Gusb -mRNA	glucuronidase, beta	NM_010368.2	5	0.164
Nubp1 -mRNA	nucleotide binding protein 1	NM_011955.2	6	0.179
Eef1g -mRNA	eukaryotic translation elongation factor 1 gamma	NM_026007.4	7	0.119
Sf3a3 -mRNA	splicing factor 3a, subunit 3	NM_029157.3	8	0.143
Tubb5 -mRNA	tubulin, beta 5 class I	NM_011655.5	9	0.166
Hdac3- mRNA	histone deacetylase 3	NM_010411.2	10	0.151
Tbp -mRNA	TATA box binding protein	NM_013684.3	11	0.178
Abcf1 -mRNA	ATP-binding cassette, sub-family F (GCN20), member 1	NM_013854.1	12	0.177
Polr2a -mRNA	polymerase (RNA) II (DNA directed) polypeptide A	NM_001291068.1	13	0.203
Sdha -mRNA	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	NM_023281.1	14	0.223
Polr1b -mRNA	polymerase (RNA) I polypeptide B	NM_009086.2	15	0.233
Edc3 -mRNA	enhancer of mRNA decapping 3	NM_153799.3	16	0.245
Eif2b4 -mRNA	eukaryotic translation initiation factor 2B, subunit 4 delta	NM_001127355.1	17	0.247
Alas1 -mRNA	aminolevulinic acid synthase 1	NM_020559.2	discarded	0.895
G6pdx -mRNA	glucose-6-phosphate dehydrogenase X-linked	NM_008062.3	discarded	0.340
Sap130 -mRNA	Sin3A associated protein	NM_172965.3	discarded	0.306

Supplementary Table S6

Figure Number	Figure Description	Strain	Sex	Age (months)	Group size	Age (months)	Group size	Age (months)	Group size
1A - 1C and 1D	Macroscopic Evaluation and Tumor Incidence	*WT*	M	**≥ 15**	**29**	**6 - 14**	**21**	**≤ 5**	**13**
				+ hepatic tumor	2	+ hepatic tumor	0	+ hepatic tumor	0
				no tumor	27	no tumor	21	no tumor	13
		fH$^{-/-}$	M	**≥ 15**	**35**	**6 - 14**	**22**	**≤ 5**	**7**
				+ hepatic tumor	19	+ hepatic tumor	2	+ hepatic tumor	0
				no tumor	16	no tumor	20	no tumor	7
		fH$^{-/-}$ *fB*$^{-/-}$	M	**≥ 15**	**10**	**6 - 14**	**0**	**≤ 5**	**0**
				+ hepatic tumor	1	+ hepatic tumor	0	+ hepatic tumor	0
				no tumor	9	no tumor	0	no tumor	0
		WT	F	**≥ 15**	**29**	**6 - 14**	**11**	**≤ 5**	**5**
				+ hepatic tumor	1	+ hepatic tumor	0	+ hepatic tumor	0
				no tumor	28	no tumor	11	no tumor	5
		fH$^{-/-}$	F	**≥ 15**	**26**	**6 - 14**	**17**	**≤ 5**	**5**
				+ hepatic tumor	4	+ hepatic tumor	1	+ hepatic tumor	0
				no tumor	22	no tumor	16	no tumor	5
		fH$^{-/-}$ *fB*$^{-/-}$	F	**≥ 15**	**10**	**6 - 14**	**0**	**≤ 5**	**0**
				+ hepatic tumor	0	+ hepatic tumor	0	+ hepatic tumor	0
				no tumor	10	no tumor	0	no tumor	0
1E-1G	H&E: Injury/HCC	*WT*	M	≥ 15	20				
		fH-/-	M	≥ 15	20				
1H - 1J	GPC3	*WT*	M	≥ 15	20				
		fH-/-	M	≥ 15	20				
2A - 2C	Liver C3b and iC3b/C3d	*WT*	M	3 - 5	10				
		fH$^{-/-}$	M	3 - 5	10				
		fH$^{-/-}$ *fB*$^{-/-}$	M	3 - 5	10				
2D - 2F	Kidney C3b and iC3b/C3d	*WT*	M	3 - 5	5				
		fH$^{-/-}$	M	3 - 5	5				
		fH$^{-/-}$ *fB*$^{-/-}$	M	3 - 5	5				
2G	Quantification: Liver C3b - iC3b/C3d	*WT*	M	3 - 5	10				
		fH$^{-/-}$	M	3 - 5	10				
		fH$^{-/-}$ *fB*$^{-/-}$	M	3 - 5	10				
2H - 2I	PET	*WT*	M	3	4				
		fH$^{-/-}$	M	3	4				
3A	Liver chemistries	*WT*	M	3	5				
		fH$^{-/-}$	M	3	5				
		fH$^{-/-}$ *fB*$^{-/-}$	M	3	5				
3B - 3C	Oil Red O staining / quantification	*WT*	M	3	5 / 5				
		fH$^{-/-}$	M	3	5 / 5				
		WT	M	9	10 / 5				
		fH$^{-/-}$	M	9	10 / 5				
		WT	M	18	10 / 5				
		fH$^{-/-}$	M	18	10 / 5				
3D -3E	COL4 staining / quantification	*WT*	M	14	6				
		fH$^{-/-}$	M	14	6				
4A - 4I	mRNA Analysis	*WT*	M	3	5				
		fH$^{-/-}$	M	3	5				
5A - 5C	Vectra Imaging and Analysis	*WT*	M	3	5				
		fH$^{-/-}$	M	3	5				
5D	Hepatic neutrophils by flow cytometry	*WT*	M	3	3				
		fH$^{-/-}$	M	3	3				
		fH$^{-/-}$	M	4	4				
		fH$^{-/-}$ *fB*$^{-/-}$	M	4	4				
		fH$^{-/-}$ (liver tumors)	M	22 - 24	4				
		fH$^{-/-}$ (no tumors)	M	22 - 24	2				
5E	Neutrophils (IF)	*fH*$^{-/-}$ (liver tumors)	M	≥ 15	10				
6A - 6D	Liver myeloid flow cytometry	*WT*	M	3	3				
		fH$^{-/-}$	M	3	3				
		fH$^{-/-}$	M	4	4				
		fH$^{-/-}$ *fB*$^{-/-}$	M	4	4				
		fH$^{-/-}$ (liver tumors)	M	22 - 24	4				
		fH$^{-/-}$ (no tumors)	M	22 - 24	2				
7A - 7B	rmCFH-Alexa 647 (IF) and quantification	*WT*	M	9	5				
		fH-/-	M	7	5				
7C - 7F	IF and quantification of deposited C3b and iC3b/C3d	*fH*$^{-/-}$ + rmCFH	M	7	5				
		fH-/-	M	7	5				
7G - 7H	rmCFH, COL4, and MECA-32	*fH*$^{-/-}$	M	7	5				
8A	C3b and iC3b/C3d in tumors	*WT*	M	≥ 15	2				
		fH$^{-/-}$	M	≥ 15	10				
8B - 8C	GPC3 and C3b or iC3b/C3d in *fH*$^{-/-}$ tumors	*fH*$^{-/-}$	M	≥ 15	10				
8D - 8E	CD46 and C3b or iC3b/C3d in *fH*$^{-/-}$ tumors	*fH*$^{-/-}$	M	≥ 15	10				
9A	GPC3 and iC3b/C3d in human HCC biopsies	--	unkn	unkn	6				

S1A	C3b and iC3b/C3d in liver and kidney	*fH*$^{+/-}$	M	3 - 5	10 (liver), 5 (kidney)
S1B - S1H	Immunofluorescence Isotypes (murine)	*WT*	M	3, 9, and ≥15	3 each
		fH$^{-/-}$	M	3, 9, and ≥15	3 each
S1B - S1D	Immunofluorescence Isotypes (human)	--	unkn	unkn	3
S2	COL4 + C3b and MECA-32 + C3b	*fH*$^{-/-}$	M	7	5