Supplementary Materials.

Recombinant murine protein production.

Plasmid pDR2ΔEF1α was obtained from Dr I. Anegon (University of Nantes, Nantes, France) (65) and a new multiple-cloning site was engineered through restriction enzyme digestion of the plasmid with Xba I-HF and EcoR V (NEB, UK) and insertion of DNA oligonucleotides introducing Nhe I and Not I sites (see supplementary table 2). Oligonucleotides were heated to 95 °C in PCR buffer and allowed to cool to room temperature before being ligated into a pre-digested pDR2 plasmid, using T4 DNA ligase according to manufacturer's instructions (NEB, UK), and thus creating pDR2-nMCS-3. For recombinant mouse C3 (rmC3) production, a synthetic DNA construct was ordered (Eurofin MWG Operon, Germany). It contained a Nhe I site, bp -14 to bp 72 of mC3 followed by the FLAG Tag sequence (see supplemental table 3), pp 104 to bp 390 of murine C3 (mC3) (ending with the internal Kpn I site), a short six-bp linker (GGAGGA) then (beginning with the internal Sal I site) bp 4625 to 4988 mC3 followed by a PME I site, a thrombin cleavage site, a 7x histidine tag (see supplementary table 3) and finally, the mC3 stop codon, bp 4989 to 5032, plus a Not I restriction site. This synthetic construct was released from the supplied vector using the flanking *Nhe* I-HF and *Not* I-HF restriction sites and cloned into pDR2-nMCS-3. A Kpn I to Sal I fragment of mouse C3 cDNA was then isolated from a commercially available Image clone (5134713), supplied by Source Bioscience (UK; IRAV p968G0388D; pCMV-Sport6) that contained the full mouse cDNA coding for C3 (-42 to 5133 NCBI sequence reference BC043338.1 v NM_009778.3). Site-directed mutagenesis was carried out using the primers in supplementary table 1 and according to manufacturer's instructions (Quikchange XL, Stratagene, UK). Recombinant mouse FH SCRs 1-5 and minimal mouse Factor H (mini-mFH), SCR1-5 fused to SCR18-20, were PCR amplified from a pBluescript plasmid containing the DNA coding for full-length

murine

CFH (a gift from Dr Elena Goicoechea de Jorge, Madrid, Spain) with flanking restriction sites, i.e. Xba I to Bam HI (plus 6x Histidine tag and stop, for mFH1-5) and Bam HI to 6x Histidine tag, stop, Nhe I (for minimal mouse FH), respectively. Bam HI-digested PCR products were ligated and used as template for the 5' Xba I and 3' Nhe I-containing primers. The resulting PCR product was double digested and ligated into cut and de-phosphorylated pDR2-nMCS-3. All plasmids were Sanger sequenced (using overlapping primers as illustrated for rmC3 in supplemental figure 2) before being transfected into Chinese hamster ovary (CHO) cells using the JetPEI reagent according to the manufacturer's (Polyplus transfection) instructions. Inclusion of hygromycin B (600 µg/ml) and limiting dilution allowed the selection of clonal transfectants. The various recombinant proteins were purified from tissue-culture supernatant using immobilized metal-ion or FLAG-tag affinity chromatography, as required, using a standard imidazole gradient (up to 500 mM) or 0.1 M glycine, pH 2.5, elution. The resultant recombinant mature mouse C3 containing the Flag/His-tags (rmC3) had a predicted molecular weight of 187247 Daltons, a predicted pI of 6.31, and a calculated extinction coefficient of 184525 as determined from the amino acid sequence using ExPASy ProtParam tool (https://web.expasy.org/protparam/). Purity was confirmed using 10% non-reducing and reducing SDS-PAGE (Supplementary data figure 3). The human FH19-20 fragment was produced in *Pichia Pastoris* as previously described (34). Recombinant soluble mouse CRRY (mCR11[NM 013499.2]/Thrombin/10xHis) was designed in house and a plasmid generated by VectorBuilder (Cyagen Biosciences, CA, USA) before being transfected into CHO cells and then purified from tissue culture supernatant using HIS-tag affinity chromatography, using a standard imidazole gradient (up to 500 mM).

Expression of recombinant human C3 (rhC3,WT) or rhC3 Asn1115.

WT and p.D1115N human C3 plasmids were prepared previously (29). Plasmids were transformed and amplified into E. Coli DH5 α , purified using a Maxiprep kit (QIAGEN). The mutant and WT C3 DNAs were transiently transfected into HEK 293F cells (Invitrogen) using polyethylenimine transfection reagent based on an established protocol (66), using FreeStyle 293 expression medium. 3 days after transfection, the supernatants were collected and adjusted to pH 7.5 using Trizma base solution (pH 9.0). The purification processes were based on (67), the pH adjusted supernatants were loaded on a 5 ml Hitrap Q column, the C3 containing fractions were identified and purified further via a mono-S cation exchange column. Concentrated C3 fractions were hydrolysed by incubating with 50 mM methylamine for 2 hours, at 37 degree, in PBS pH 8.2. The resulted C3(H₂O) was dialysed against HBST buffer prior the Biacore analysis. Human FH was purified from plasma via affinity chromatography using a method previously described. Purified MCP domain 1-4 was a prepared previously (29).

Each version of human hydrolysed C3 was immobilised (1000 ±50 RU) on separate flow cells of a CM5 chip using standard amine coupling. A two-fold dilution series of purified human FH (10 to 0 μ M) or recombinant human MCP SCR 1-4 (2.984 to 0 μ M) were made in HBST buffer (10 mM HEPES, 150 mM NaCl, and 0.005% Tween 20, pH 7.4) and flowed across either chip surface in the same buffer. All analytes were injected in duplicate (30 μ l/min for 190 *s*), followed by running buffer for 300 *s* and a regeneration phase involving injection of regeneration buffer (10 mM sodium acetate, 1 M NaCl, pH 4.5) for 60 *s*. The equilibrium dissociation constant K_D and standard error were calculated using steady-state model in BIAcore S200 Evaluation Software.

Fluid phase co-factor activity assays

For studies on recombinant mouse C3 (rmC3), 5 µg of affinity-purified rmC3b and mutants Val1072, Asn1115 were incubated with mouse mini-FH and serum-derived mouse complement factor I for increasing time at 37 °C. Reduced samples (1/10 diluted) were applied to 10% PAGE gels, Western blotted and exposed to an anti-His tag antibody (Penta-His 1/1000, cat. 34660; Qiagen) followed by 1/500 SA-HRPO. For experiments where C3 was isolated from mice, a 30-µl reaction volume was used. Either purified WT or murine C3 Asn1115 was converted to C3b as follows. In brief, 3 µg of either recombinant or plasma-derived murine C3 was incubated with 1 µg of human complement factor B, and 0.01 µg of human complement factor D in the presence of 5 mM MgCl₂ at 37 °C for one hour (human complement factor B and D purchased from Comptech, Texas, USA). After the reaction was stopped by the addition of 10 mM EDTA, plasma purified murine FH and human FI were added to final concentrations of 200 nM and 20 nM respectively. The reaction was incubated at 37 °C, and 4-µl sample was taken at 0, 5, 10, 20, 30, 40 and 60 min and analyzed by anti-murine C3 Western blotting.

References for supplementary methods (ordered per main article).

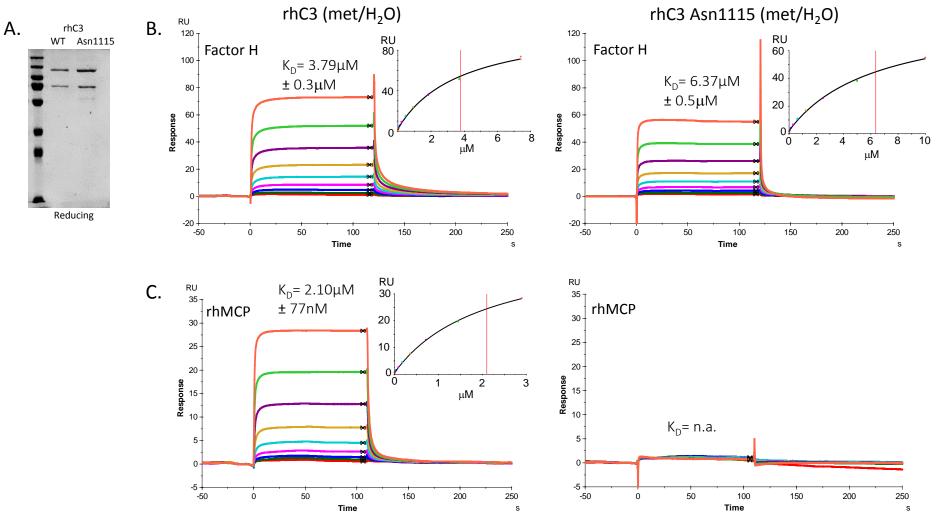
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Supplementary Figure 1.



(A) A 10% reducing SDS-PAGE gel of purified recombinant human C3 (rhC3,WT) or rhC3Asn1115 produced via transient expression in HEK293 (Invitrogen, UK) is shown. SPR analysis was performed after methylamine treatment of the rhC3 variants and coupling to a CM5 biosensor chip (1000 \pm 10 RU). Doubly diluted concentration series of (B) purified human FH (0 to 10 μ M) or (C) rhMCP (0 to 3 μ M), were flowed across either chip surface. The equilibrium dissociation constant K_D was calculated using steady state model in BiacoreTM evaluation package, indicated as the black vertical line.

Supplementary Figure 2A.

•	·	24-aa signal sequence	
Mouse	1	MGPASGSQLLVLLLLASSPLALGIPMYSIITPNVLRLESEETIVLEAHDAQGDIPVTVT	60
Human	1	TPSTHLSIMV 22-aa signal sequence	58
Mouse	61	VQDFL-KRQVLTSEKTVLTGASGHLRSVSIKIPASKEFNSDKE <mark>G</mark> HKYVTVVANFGETVVE	119 G79
Human	59	.HPG.KLSP.TN.MGN.TFTNRK.E.G <mark>R</mark> N.FQ.TTQ	118 R80
Mouse	120	KAVMVSFQSGYLFIQTDKTIYTPGSTVLYRIFTVDNNLLPVGK <mark>T</mark> VVILIETPDGIPVKRD	179 T139
Human	119	.V.LL	178 T140
 Mouse	360	KTPKFFKPAMPFDLMVFVTNPDGSPASKVLVVTOGSNA-KALTODDGVAKLSINTPNSRO	418 P346
Human	359	YG.	418 P347
		·····	
Mouse	539	GASGQREVVADSVWVDVKDSCIGTLVVK-GDPRDNHLAPGQQTTLRIEGNQGA <mark>R</mark> VGLVAV	597 R567
Human	539		598 R570
 Na	CE0	<mark>cleavage</mark> DLECTKPAA <mark>RRRR</mark> SVQLMERRMDKAGQYTDKGLRKCCEDGMRDIPMRYSCQRRARLITQG	715
Mouse Human	658 659	$\mathbf{E} \cdot \mathbf{Q} \cdot \mathbf{P} \mathbf{Q} \cdot \mathbf{Q} \cdot \mathbf{M} \mathbf{E} \cdot \mathbf{K} \mathbf{M} \mathbf{D} \mathbf{K} \mathbf{A} \mathbf{G} \mathbf{Q} \mathbf{H} \mathbf{D} \mathbf{K} \mathbf{G} \mathbf{G} \mathbf{K} \mathbf{K} \mathbf{C} \mathbf{C} \mathbf{E} \mathbf{D} \mathbf{G} \mathbf{M} \mathbf{K} \mathbf{D} \mathbf{I} \mathbf{D} \mathbf{K} \mathbf{G} \mathbf{G} \mathbf{K} \mathbf{K} \mathbf{D} \mathbf{I} \mathbf{D} \mathbf{K} \mathbf{G} \mathbf{G} \mathbf{K} \mathbf{D} \mathbf{I} \mathbf{D} \mathbf{U} \mathbf{U} \mathbf{U} \mathbf{U} \mathbf{U} \mathbf{U} \mathbf{U} U$	715
IICINEIII	000	cleavage ANA domain (C3a)	111
Mouse	718	ENCIKAFIDCCNHITKLREQHRRDHVLGLARSELEEDIIPEEDIISRSHFPQSWLWTIEE	777 R711
Human	718	.A.K.V.LYER.A.ASHN.DAN.VEENV.D	777 R713
	0.20		0.07
Mouse Human	838 838	SVVRNEQVEIRAVLFNYREQEELKVRVELLHNPAFCSMATAKNRYFQTIKIPPKSSVAVP	897 Y830 897 Y832
numan			
Mouse	898	YVIVPLKIGQQEVEVKAAVFNHFIS <mark>DG</mark> VKKTLKVVPEGMRINKTVAIHTLDPEKLGQGGV	957 ADG
Human	898		957 ADG
 Mouse	1078	LTAYVVKVFSLAANLI <mark>A</mark> IDSHVLCGAVKWLILEKQKP <mark>D</mark> GVFQEDGPVIHQEMIGGFRNAK	1137 A1070, D1091
Human	1078		1137 A1072, D1093
Mouse	1138	EADVSLTAFVLIALQEARDICEGQVNSLPGSINKAGEYIEASYMNLQRPYTVAIAGYALA	1197 Q1137
Human	$1138 \\ 1138$	K.MA SKE.	1197 Q1137 1197 Q1139
	1100		
Mouse	1438	FSNKNTLIIYLEKISHTEEDCLTFKV <mark>H</mark> QYFNVGLIQPGSVKVYSYYNLEESCTRFYHPEK	1497 H1440
Human	1438	DRD.VS.DA <mark>.</mark> EAAA	1497 H1442
 Mouse	1618	TSYIIGKDTWVEHWPEAEECODOKYOKOCEELGAFTESMVVYGCPN 1663	
Mouse Human	1618	L	
	1010		

Pairwise alignment (BLAST) of selected sequences from mouse and human C3. Residue numbering includes signal sequences (labeled and shaded) but mutations (highlighted in magenta) are numbered (on the right), based on the mature mouse and human protein sequences as used in Figure 1B. The cleavage site (generating chains B and A of mature C3) and the ANA domain (excised during production from C3 of C3b) are labeled and shaded in red and green, respectively.

Supplementary Figure 2B. Alignment of C3 proteins and disease associated mutants

Sequence Name	< Pos = 1										
+											
Consensus	MGPTSGPSLLL	LLLASLPLAL	GSPMYSI	TVTI KI	PASKEFKSDK-	GHKFVTVVATF	GNTVVEKVV	RIFTVI	DHKLLPVGQT	VVVTI ETPDGI	PVKRDS
6 Sequences	10	20	30	90	100	110	120	150	160	170	180
Bovine C3	MKPTSGPSLLL	LLLASLPMAL	GNP MY S M	TVTI KI	PASKELKSDK-	GHKFVTVVATF	GNVQVEKVV	RVFTV	DHKLLPVGQT	VFI TI ETPDGI	PVKRDS
Pig C3	MGSTSGP RLLL	LLLTSLPLAL	GDPI YTI I	TVNI KI	PASKEFKSEK-	GHKFVTVQALF	GNVQVEKVV	RIFTVI	DHKLLPVGQTI	VVTI ETPEGI	DI KRDS
GP C3	MGPAAGP SLLL	LLLASVSLAL	GDP MY SI	TVTI KI	PASKEFKSDK-	GRKLVVVQAAF	GGTQLEKVV	RIFTVI	DSDLLPVGRTI	I VTI ETPDGI	PI KRDT
Rat c3	MGPTSGSQLLVLL	LLLASSLLAL	GSPMYSI	IRVELKI	PASKEFNADK-	GHKYVTVVANF	GATVVEKAV	RIFTVI	DNNLLPVGKT	VVI VI ETPDGV	PI KRDI
human C3	MGPTSGPSLLL	LLLTHLPLAL	GSPMYSII	SNVTFTI	PANREFKSEK	RNKFVTVQATF	GTQVVEKVV	IRI FTVI	NHKLLPVGR	VMVNI ENPEGI	PVKQDS
mouse C3	MGPASGSQLLVLL	LLLASSPLAL	GI PMYSI	SVSI KI	PASKEFNSDKE	GHKYVTVVANF	GETVVEKAV	RIFTVI	DNNLLPVGKT	VVI LI ETPDGI	PVKRDI
I						.02/G103			T162	2/3	

R102/G103

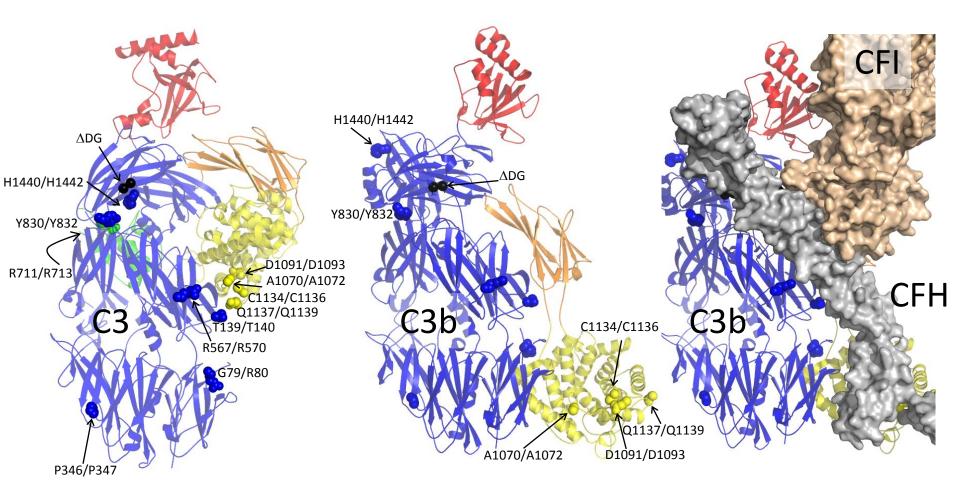
		··	
Sequence Name			
+			
Consensus	HFTKTPKFFKPAMPFDLMVFVTNPDC	GGKDDR-QHAPGQQMTLRIEGNQGARVGLVAVDKGVFVLN	ARFI SQGEACVKAFLDCCNYI TKLRQQHRRDHVL
6 Sequences	360 370 380	580 590 600 610	20 730 740 750
Bovine C3	HFTKTPKFFKPAMPFDLMVYVTNPDC	GGKEEK- HHRPGQQI TLKI EADQGARVGLVAVDKGVFVLN	AQFI LQGDACVKAFLDCCEYI TQLRQQHSRDGAL
Pig C3	HFTKTPKFFKPAMPFDLMVYVTNPDC	GGKQDK-QHRPGQQMTLEIQGERGARVGLVAVDKGVFVLN	AQFI QHGDACVKAFLDCCEYI AKLRQQHSRNKPL
GP C3	HFTKTPKYFKPAMPFEI MVLVTNPDC	DGQDKRQQHLPRQQMTLRIEGNQGARVGLVAVDKGVFVLN	ARYVSLGEACVKAFLDCCTYMAQLRQQHRREQNL
Rat c3	HFTKTPKFFKPAMPFDL MVFVTNPD0	DPRDNR- QPAPGHQTTLRI EGNQGARVGLVAVDKGVFVLN	ARLI TQGESCLKAFMDCCNYI TKLREQHRRDHVL
human C3	HFTKTPKYFKPGMPFDLMVFVTNPD(GQSEDR-QPVPGQQMTLKIEGDHGARVVLVAVDKGVFVLN	TRFI SLGEACKKVFLDCCNYI TEL RQHARASHL
mouse C3	HFTKTPKFFKPAMPFDLMVFVTNPDC	DPRDNHLAPGQQTTLRIEGNQGARVGLVAVDKGVFVLN	ARLI TQGENCI KAFI DCCNHI TKLREQHRRDHVL
1	P369/70	R592/1	R735

Sequence Name											
+											
Consensus	PYSVVRNEQVEL	RAVLYNYREAE	ELKVRVELLH	INPAF	VKVF	SLAANLI	AI DSQVLC	GAVKWLI	LEKQKPDG	FQEDGPVI	HQEMI GGF
6 Sequences	850	860	870	880		1100	1110	6	1120	1130	1140
Bovine C3	PYSVVRNEQVEI	RAI LYNYREAE	NLKVRVELL	NPAF	VKVF	ALAANLI	AI DSKDLC	ETVKWLI	LEKQKPDG	FQEDGPVI	HQEMI GGF
Pig C3	PYSVVRNEQVEI	RAI LYNYREAE	DLKVRVELL	NPAF	VKVF	AMAANLI	AI DSQVLC	GAVKWLI	LEKQKPDG	FEENGPVI	HQEMI GGF
GP C3	PYSVVRNEQVEI	RAVLYNYREAG	SLKVRVELLH	INPAF	VKVF	SLAANLI	GI DSEVLC	GAVKWLI	LEKQKPDG	FQEDGPVI	HQEMI GGV
Rat c3	PYSVVRNEQVEI	RAVLFNYREQE	KLKVRVELLH	INPAF	NSRSF	SLAANLI	AI DSQVLC	GAVKWLI	LEKQKPDG	FQEDGPVI	HQEMI GGF
human C3	PYSVVRNEQVEI	RAVLYNYRQNG	ELKVRVELLH	INPAF	VKVF	SLAVNLI	AI DSQVLC	GAVKWLI	LEKQKPDG	FQEDAPVI	HQEMI GGL
mouse C3	PYSVVRNEQVEI	RAVLFNYREQE	ELKVRVELLH	INPAF I	VKVF	SLAANLI	AI DSHVLC	GAVKWLI	LEKQKPDG	FQEDGPVI	HQEMI GGF
I	II	Y854				A10	94		D1115		

Sequence Name				_						
+										
	VLI ALQEAKDI	CEGQVNSL	PGSI NKAGDF	LDKVSHSEE	DCLSFKVHQ	YENVGLI Q	PGSVKVYSYY	LECQDEENQKQC	EDLGAFTEN	AVVF GCPN-
6 Sequences	1160	1170	1180	1460	1470	1480	1490	1650	1660	1670
Bovine C3	VLI ALHEAKDI	CEAQVNSL	GRSI AKAGDF	LDKVSHTVE	DCLSFKVHQ	YFNVGLIQ	PGAVKVYSYY	LEECODEENOKO	EDLANFTEN	AVVFGCPN
Pig C3	VLI ALQEAKDI	CEPQVNSL	LRSI NKARDF	LDKI SHTLE	DCI SFKVHQ	YENVGLIQ	PGSVKVYSYY	DVCQDEENQKQC	ODLANFSENM	AVVFGCPN
GP C3	VLI ALQEAKDI	CRAQVNNL	EANI NKAGDY	LDKVSHSEE	ECLSFKIHQ	FFNVGLIQ	PGSVKVYSYY	AEECQDEENQQQQ	ODLGTFTENM	AVVFGCPN
Rat c3	VLI ALQEARDI	CEGQVNSL	PGSI NKAGEY	LEKI SHSEE	DCLSFKVHQ	FFNVGLIQ	PGSVKVYSYY	AEERQDQKNQKQC	CEDLGAFTETM	AVVFGCPN
human C3	VLI SLQEAKDI	GEEQVNSL	PGSI TKAGDE	LDKVSHSED	DCLAFKV	YENVELIQ	PGAVKVYAYY	DECODEENQKQC	CODLGAFTESM	NV VF GCPN.
mouse C3	VLI ALQEARDI	CEGQVNSL	PGSI NKAGEY	LEKI SHTEE	DCLTFKVHQ	FNVGLIQ	PGSVKVYSYY	AEECODOKYOKO	CEELGAFTESM	IVVYGCPN.
	C115	58 Q1161			H146	4			V	1654

Pairwise alignment (BLAST) of selected sequences from various species. Bovine (Q2UVX4), Pig (P01025), Guinea Pig (GP;P12387), Rat (P01026), Human (P01024) and Mouse C3 (P01027) protein sequences taken from Uniprot (<u>http://www.uniprot.org/uniprot/</u>) were aligned using ClustralW in DNAstar. Met +1. Human position/mouse position of selected mutants are noted under aligned sequence.

Supplementary Figure 2C.



Locations of mutations in C3 and C3b. Representations of human C3 (pdb = 2a73), and of human C3b taken from the crystal structure of C3b in complex with "mini"-FH (FH SCR domains 1-4 and 19-20 joined by a flexible linker) and FI (pdb = 5o32). C3 and C3b are drawn as cartoons and mini-FH and FI are represented by their surfaces. FI and mini-FH are omitted from the central panel, for clarity. The side-chains of mutated residues are shown as spheres and numbered as in the mature sequences of mouse/human C3; except for the Asp-Gly deletion identified by black spheres representing Ca atoms. The C345C domains are red, the CUB domains are orange, the ANA domain (in C3 only) is green and the thioester domains are yellow; other C3 and C3b domains are blue.

Supplementary Figure 3.

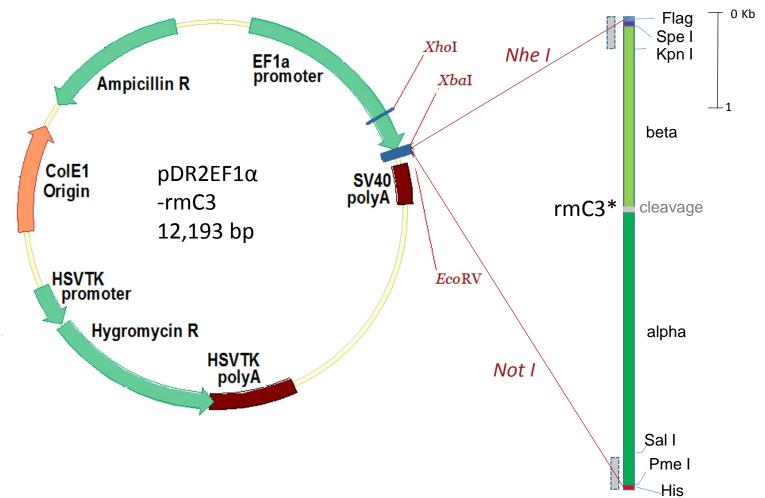
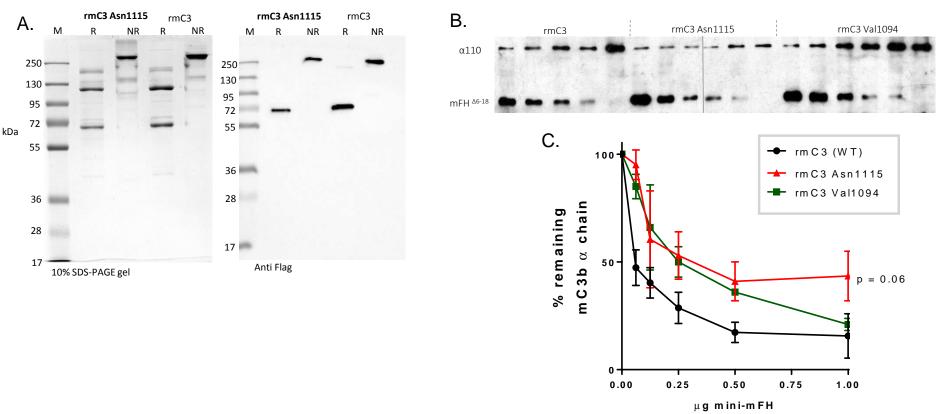


Diagram of the genetic organisation of the recombinant mouse (rm)C3 cDNA in the eukaryotic expression vector pDR2\DeltaEF1a. Restriction digest sites and purification tags are indicated. Mouse C3 was restriction digest cloned from a pCMV-Sport6 vector containing image clone 5124713* (with NCBI accession number = BC043338.1; Source Bioscience) using Kpn I and Sal I restriction sites. His = refers to a 7x histidine repeat and Flag to the DYKDDDDK amino acid sequence engineered into sequence as outlined in the supplementary methods (a guide to the synthetic DNA generated to allow construction of the tagged rmC3 is illustrated by grey dashed boxes). Primers used to confirm the integrity of the plasmid are fully outlined in supplemental Table 3.

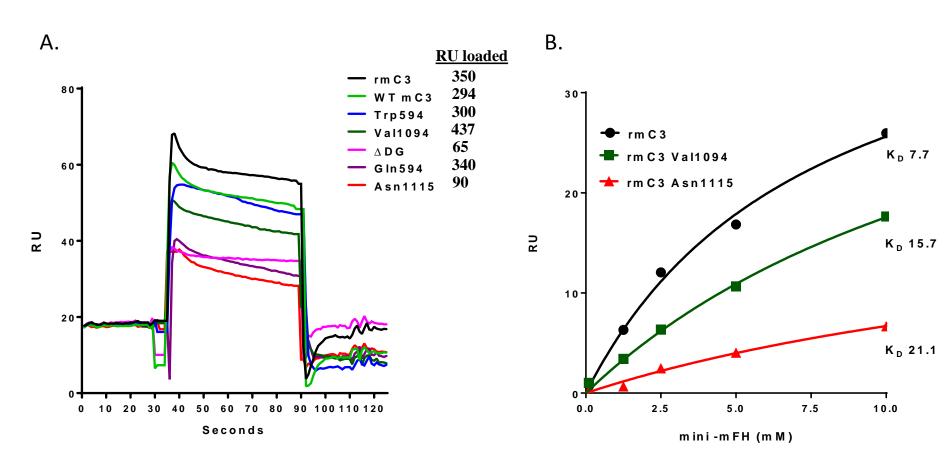
Supplementary Figure 4.



Analysis of recombinant mouse C3. (**A**) Recombinant wild type mouse C3 (rmC3) and recombinant mutant protein (rmC3^{D1115N}) were purified from CHO cell supernatant using FLAGTm tag affinity chromatography and elution using 0.1M glycine (immediately neutralised with 1M Tris pH 8). A 10% SDS-PAGE with loaded with $2\mu g$ of rmC3^{D1115N} or rmC3 non-reduced (NR) or reduced (R) to confirm purity. Western blot analysis ($0.2\mu g/ml$ loaded) using monoclonal anti-FLAG antibody M2 (Sigma, UK) confirms the presence of intact C3 and C3 β chain under NR and R conditions, respectively. Pre-stained markers were included (M; Frementas, UK) and sizes are indicated on the left of the SDS-PAGE gel. (**B**) 5 μg of affinity purified recombinant mouse c3 (rmC3) and mutants Val1094, Asn1115 (Met +1) were incubated with decreasing amounts of mini-mFH (minimal mouse FH, SCR1-5 linked to 18-20) and serum derived mouse FI for 30 minutes at 37°C. Reduced samples (1/10 diluted) were applied to 10% page, western blotted and exposed to an anti-his tag antibody (Penta-His 1/1000, Qiagen) followed by 1/500 SA-HRPO (representative of 2 experiments). Dashed line indicates the division between two gels/westerns that were run back to back.

(C) Densitometry analysis of intact alpha chain, standardised to Beta chain load, as determined by Image Studio v5.2. N = 2 independent experiments, mean +/- SEM. Wilcoxon's rank sum was used to establish significance of difference between rmC3 and rmC3 Asn1115.

Supplementary Figure 5.

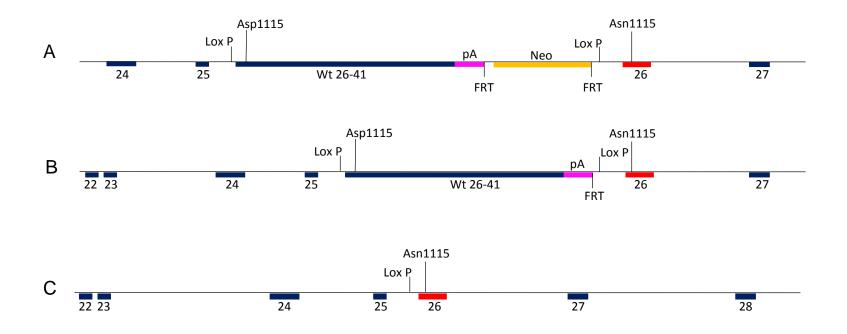


Analysis of recombinant mouse C3 using surface plasmon resonance (BIAcore).

A). Zeroed line graph of response units (RU) interaction of 20μ M mini-mFH (mouse FH SCR1-5-18-20) binding to methylamine treated wild type, recombinant wild type or mutant proteins (Met + 1) C3 pre-captured onto a CM5 chip coated with 1000 RU of monoclonal antibody C3d11 (kind gift Prof M Holers, UCD, Denver, USA). A target of ~300 RU of each mouse protein was achieved (with the exception of Δ DG and Asp1115, as indicated) and given 300 seconds to stabilise. Mouse FH bound rmC3 more readily than WT mC3. Trp594 and Val1094 were comparable to WT C3, while Gln594, Δ DG and Asn1115 demonstrated the lowest binding capacity. Representative of 2 replicates. Data exported and analysed in Graphpad v7.

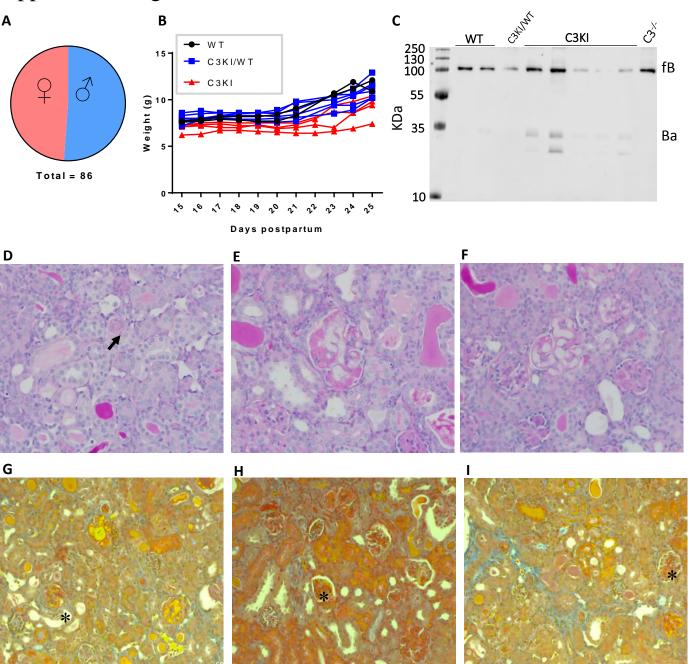
B). Increasing concentrations of mini-mFH were flowed over a CM5 chip pre-coated as above and the response (in RU) determined. K_D value were calculated by non-linear regression curve fit using Graphpad v7.

Supplementary Figure 6.



Making C3^{D1115N} conditional and constitutive KI mice. (A) Map of genetic organisation of the conditional knock-in (ConKI) construct used to transfect ES cells; wt26-41 sequence was obtained from source bioscience, image clone 5134713 (B) map showing genetic organisation after cross with Flpe mice (C3-ConKI-Flp) (C) Map showing genetic organisation of C3 KI mice after cross breed with OzCre mouse. Exons: Blue boxes; Neo: neomycin cassette for selection of ES cells; FRT; recognition sequence for Flp recombinase mediated neo removal; LoxP: recognition sequence for cre recombinase mediated wt mouse C3 cDNA

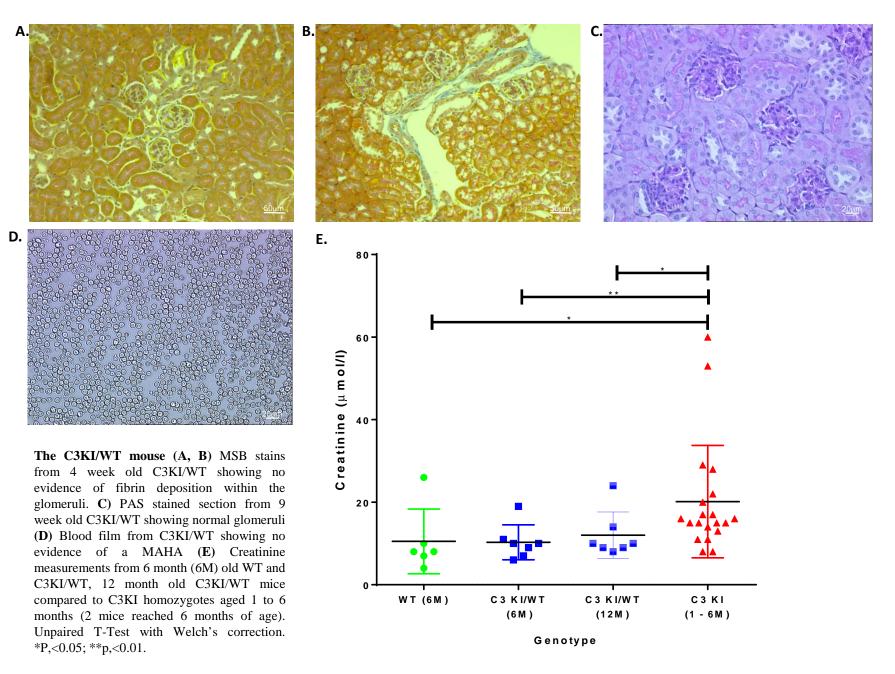
Supplemental Figure 7.



Additional analysis of the C3KI mice.

(A) No sex differences in the number of C3KI bred. (B) C3KI (red triangles) show a failure to thrive in comparison to their litter mate controls. (C) Western blot of murine plasma (freshly collected into EDTA and run under non-reduced conditions). A polyclonal goat anti-human factor B (1/1000, Comptech, Tyler, Tx) and donkey anti-goat HRPO (1/5000, Jackson Labs, Stratech, UK) was used to visualise murine factor B; C3 knockout mouse plasma was used as control for factor B breakdown. (D-F) PAS stain of C3KI mice showing an intravascular thrombus (arrow) (28 days old), representative images of chronic TMA in the C3KI mice. (G-I) MSB stains from C3KI mice showing a TMA with fibrin deposition within the glomeruli (asterix).

Supplemental Figure 8.



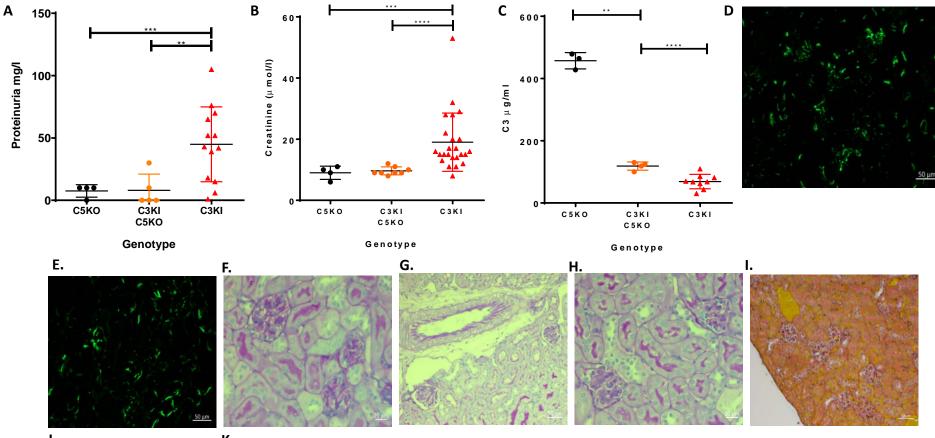
Supplemental Figure 9.

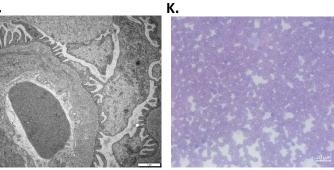
А В Weight (male) Weight (fem ale) W T/W T W T/W T C3KI + BB5.1 16 16 C3KI + Isotype C3KI + BB5.1 Grams Grams C3KI + Isotype 12 12 8 8 Λ 12 14 16 18 20 22 24 26 28 30 12 14 16 18 20 22 24 26 28 30 Days postpartum Days postpartum

Weight Trajectory of C3KI mice.

(A) Weight gains of male C3KI mice in both treatment arms in comparison to wild type. (B) Weight gains of female C3KI mice in both treatment arms in comparison to wild type. Mice were weighted daily from post-partum day 12. N = 6 mice on average for each group.

Supplemental Figure 10.





The C3KI.C5KO 2 month old mouse. (A) No evidence of proteinuria in 8 week old C3 C3KI.C5KO (orange circles), in comparison to aged matched C5KO controls (black circles) (B) No evidence of renal impairment evidenced through normal creatinine values (orange circles). (C) Plasma C3 levels are lower in the C3KI.C5KO in comparison to age matched C5KO but higher than C3KI whom exhibited spontaneous disease (red triangles). (D) C3 immunofluorescence 2 month old C5KO or (E) C3KI.C5KO. (F, G & I) PAS stained sections of C3KI.C5KO showing no glomerular pathology. (H) MSB stain showing no evidence of fibrin deposition. (J) Electron microscopy of a 6 month old FH knockout mice showing thickened glomerular basement membranes and ill-defined electron dense material on the sub-endothelial side, original magnification x80002 (K) Blood film from 8 week old C3KI.C5KO showing no evidence of a MAHA. *p,<0.05, **p, <0.01; ***p<0.0005. Unpaired t-test with Welch's correction (A,B, C).

Supplemental Table 1: Forward SDM oligonucleotide sequences used in the study

Name	Sequence (5' to 3')
G103R	GGAATTCAACTCAGATAAGGAG <u>C</u> GGCACAAGTACGTGACAGTGG
T163K	GCCCGTGGGCAAGA <u>A</u> AGTCGTCATCCTC
T163R	GCCCGTGGGCAAG <u>CG</u> AGTCGTCATCCTC
P370S	TTCAAGCCAGCCATG <u>C</u> CCTTTGACCTCATGGTGTT
R594Q	GGAAACCAGGGGGCCC <u>AG</u> GTGGGGCTAGTGGC
R594W	GGAAACCAGGGGGCC <u>TGG</u> GTGGGGCTAGTGGC
R735W	GCTGCAACCACATCACCAAGCTG <u>T</u> G <u>G</u> GAACAACACAG
(923)∆DG	GTCTTCAATCACTTCATCA^GTGTCAAGAAGACACTGAAG
A1094V	CTCTCTAGCTGCCAACCTCATCG <u>T</u> CATCGACTCTCACGTCC
D1115N	GGAGAAACAGAAGCCG <u>A</u> ATGGTGTCTTTCAGGAGG
Q1161K	CCAGGGACATCTGTGAGGGG <u>A</u> AGGTCAATAGCCTTCCTGGG
H1462D	CCTGACCTTCAAAGTT <u>G</u> ACCAGTACTTTAATGTGGGACTTATCCAGCCC

Notes: Reverse complement sequence used for reverse SDM oligo. Altered bases, compared with consensus sequence are underlined and ^ indicates the deletion of sequence, as appropriate. Numbering from Met+1 according to consensus alignment in supplementary Figure 1A.

Supplemental Table 2: Multiple cloning site 3 oligonucleotides

Name	Sequence (5' to 3')
nMCS-3 F	CTAGAGCTAGCTGATCACTAGTCGACCCGGGATCCGGTACCAAGCTTGC GGCCGCATCGAT
nMCS-3 R	ATCGATGCGGCCGCAAGCTTGGTACCGGATCCCGGGTCGACTAGTGATC AGCTAGCT

Supplemental Table 3: PCR oligonucleotides

Name	Sequence (5' to 3')	Name	Sequence (5' to 3')
mC3 F1	GTGGGTGGATGTGAAGGATTCCTG	mC3 R1	CAACTCTTCTATGGTCCACAACCAGC
mC3 F2a	CCCAGCAGCTGGCCTTCAAACAGCCC	mC3 R2	CATCAGGGCCAGGGCATACCCAGC
mC3 F3	CAAAGCCTTCTCCAACAAGAACACCC	mC3 R3	AGCAGTTCTCTTCAGCACACCGGCAC
mC3 F4	ATGGGACCAGCTTCAGGGTCCCAGCT	mC3 R4	GGATGACGACTGTCTTGCCCACGGGC
mC3 F5	GAAGGAATACGTGCTGCCCAG	mC3 R5	TGTTTCTGGTACTTCTGATCCTGGCATTCT
mC3 F6	CAACCACATCACCAAGCTGCG	mC3 R6	GGGACAACCATAAACCACCATAGATTC
pDEF-NF	CTCAAGCCTCAGACAGTGGTT	mC3 R7	GTTCTTCGCACTGTTTCTGGTACTTCTGAT
FLAG Tag	GATTACAAGGATGACGATGACAAG	mC3 R8	CCACAGCCACTAGCCCCACTC
PME I/ Thrombin	AGTTTAAACCTAGTTCCTCGTGGATCA	mC3 R9	GTGGGTGGATGTGAAGGATTCCTG
cleavage/ HIS Tag	CACCATCACCACCATCACCAC	mC3 R10	CATGTTCTGTTCCCCACAGCC
mFH18 - BF	nFH18 - BF GGGATATGGATCCAAAAACCAAAGTG CCGA		CTGAGGCTTGAGAATGAACC
mFH1- XSF	CACTCTAGAGTCGACCCACGCGTCCG	mFH5 - BR	AGGGATCCAGCCAGTGGGTGTACA
	GAGAGG	mFH20- His-NHR	GCCGCTAGCTTAGTGATGGTGGTGATGGTGGT GTACACAAGTGGGATAATT

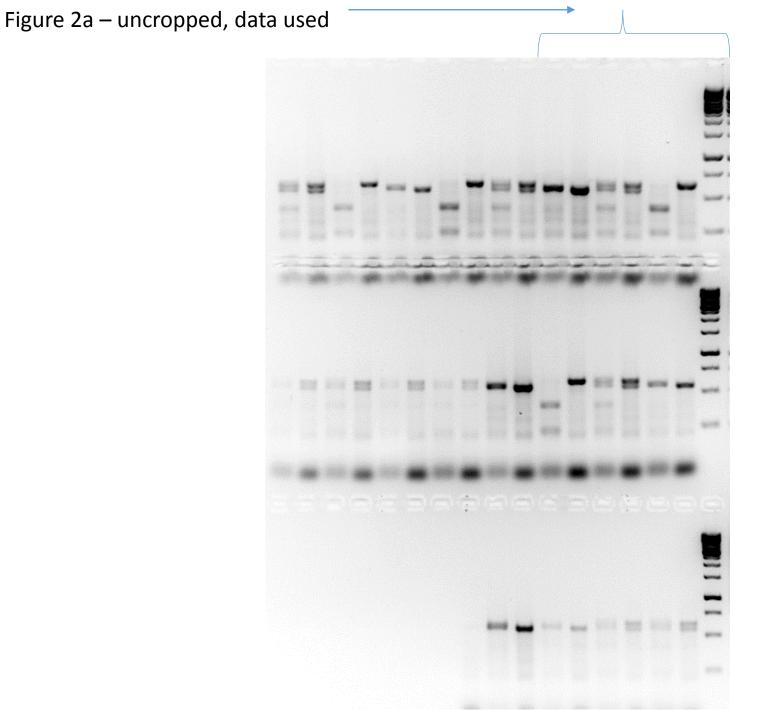
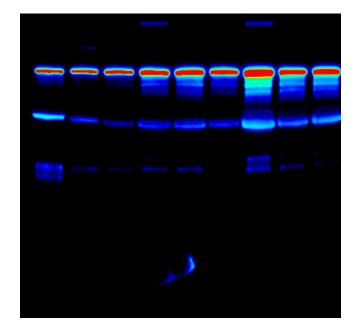


Figure 2C – uncropped, all data used



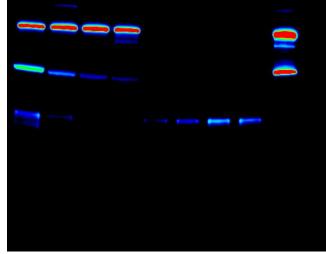
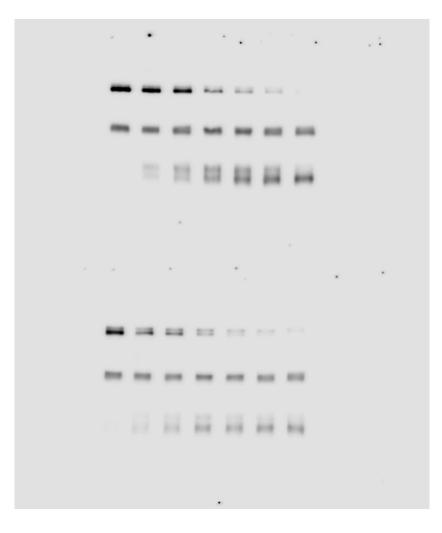
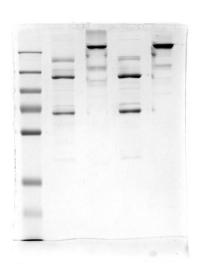


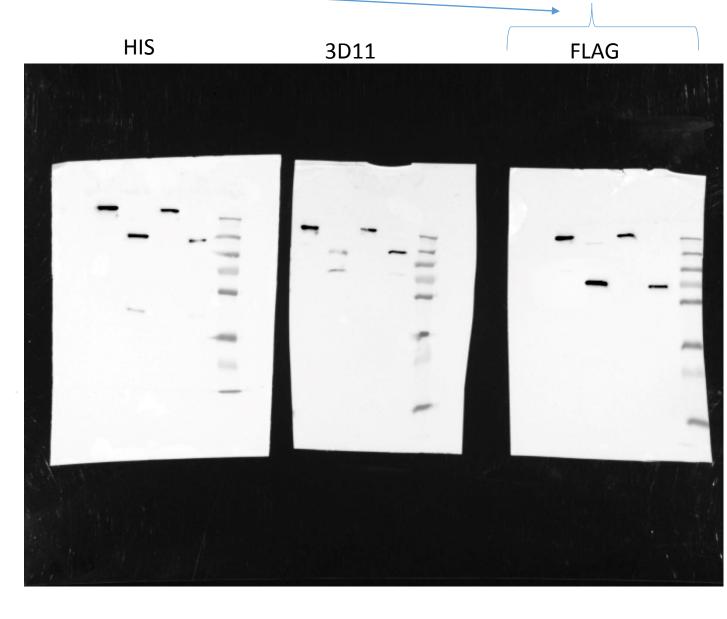
Figure 6E – uncropped, all data used



Supplemental Figure 4a – uncropped,



Bottom of gel cropped in image, all data used



Supplemental Figure 4b – uncropped,

