Sfyroera, Ricklin et al., M373 mutation in C3 affects complement activation

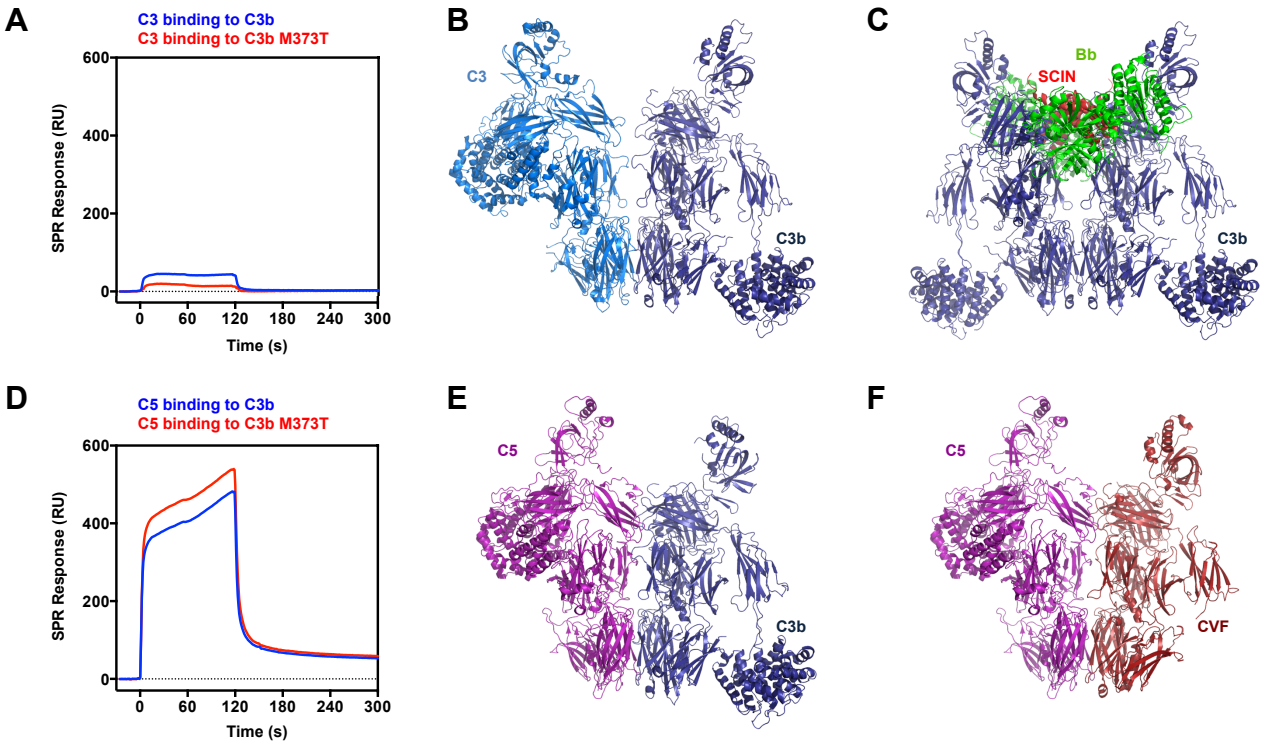
Supplemental Material

Supplemental Table 1

SUPPLEMENTAL TABLE 1. Primers used for DNA sequencing studies.

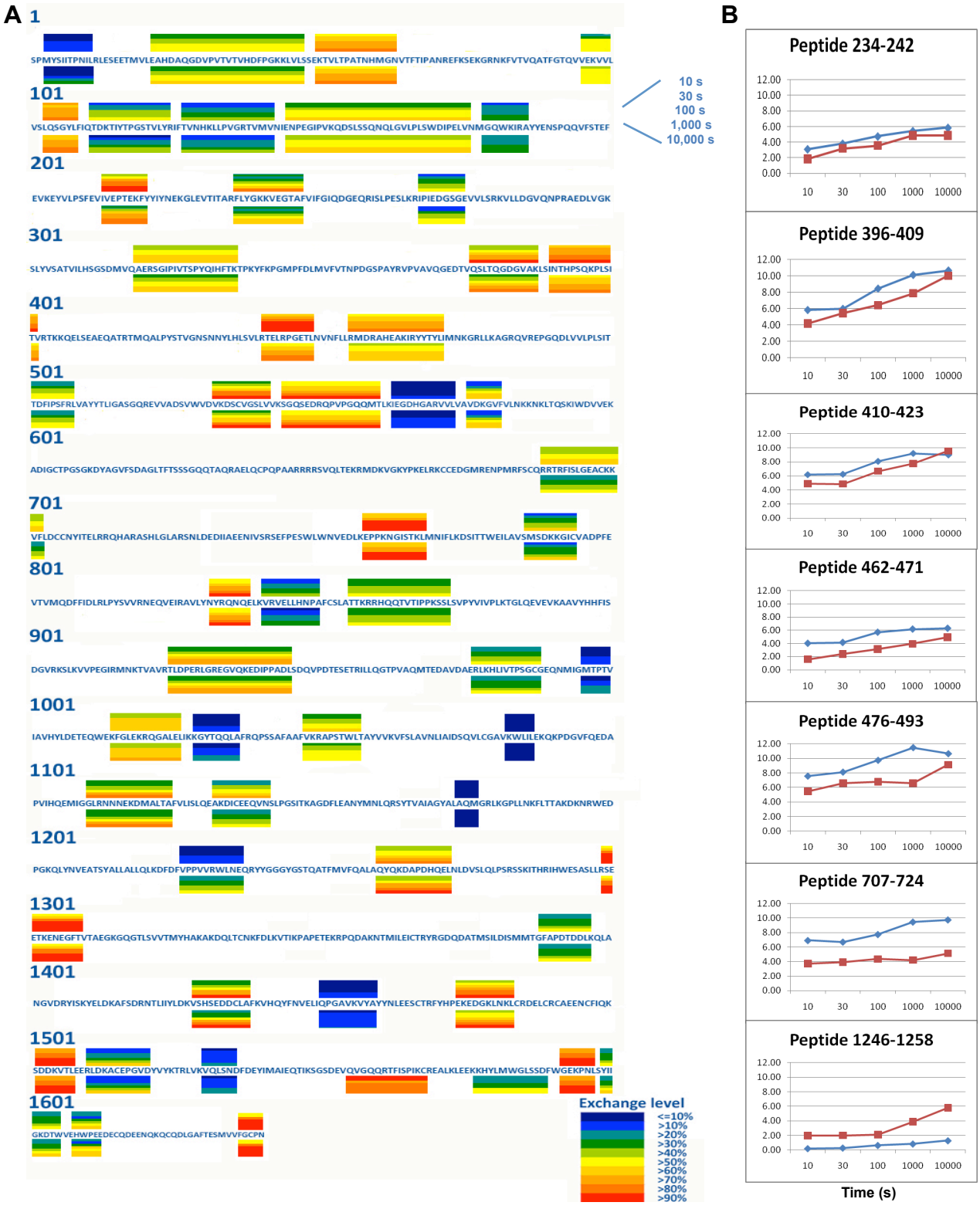
Exon	Forward (5' – 3')	Reverse (5' – 3')
1	ATCCTCTCCCTCTGTCCCTCTGT	CACCCCAGAACCAGCCCTGTTTGT
2	ACTGGCGTCTCACATCCGTGGAATGA	AAAGGGAGAAGACAGAAGGGGAG
3	AAAACGGCCACCTCGGAAGACCAAGAA	AAAGAGGCCTCGTGAGACCCTA
4	ATCTACACCCCTGGCTCCACA	TCTTTGCCTCTCCTAAGCCTGTGC
5	AGCCTCCGAAGAGCCACTTTATCCT	GATCTTCCACTGGCCCATGCTGTGA
6	AGGCCTGAGAGACCACTCATC	GGCCCTTCTCGTTATAGATGTAGTAG
7	AGGCCTGAGAGACCACTCATC	TGGTCCCTCACCTGGCTCTTACCT
8-9	TCCAGTCTTCAGCACAGGCAGGAGAA	TTTCTCTTCTGACCTGGTCTCCCC
10-11	CCAGAAGCCTACACTCACAGCTGGT	CTGTACCGTCTTCCCAGTGATGG
12	TGTTAAGAAAGGCCACCAATTCCCA	TGCGGAAGAAACAAGGAGGAGGCG
13	GCCTCCTCCTTGTTTCTTCCGCA	GAGAGAGAGAGAGGAGTAGGGAGA
14	CTCTCTCCCAGGGCTGACTTCTTT	TTGGGTCACTGGCCCTTACCTTACT
15	TCATTTGGGAAGAGATACACAGGT	TTTCCAGAGCTCTGCTCCCAGCATCT
16	AACCGAGGACACCTAGTAGGTGCCT	AGTGGGTAGGAAAGGCTCCCACCTTT
17	TCACGGAGAAGCGAATGGACAAAG	CGCAGCTCTGTGATGTAGTTGCA
18	TGCAGGTAACCTGGATGAGGACAT	ATGCTCACAGCCAGAATCTCCCA
19	AGGAATTGGGTGACCTACATGGAGGCAC	TGTGGTAACCAGGGCAGAGAATA
20	TTGACAGCGTTTAGTTCACAGGC	CAGGCTGCAGAAGGCTGGATTGT
21	CTCTGTTGTTCGAAACGAGCAGGTG	CTCTGCTTCTGAATTCGTGGGATTCC
22-23	AATGCTAGGGTGATCCTAAGGACA	TGCGGGTTAAACACCTCCAGAATGA
24	AGTCACATCCTCCCCAGTCCT	TAGGAGAGAAGGTGGAGCCTCAG
25	CTTTTTAAGGTCCCCTCCTCCCAGAA	TGCTTAAGGATGCTTAATGACCGCC
26	CATACAACAGCTGGGTCCTGG	AGTGACGCCTCTGGCTCATATATCT
27	CCTTCCTAGGGTAGCGGGGTAACA	CAGTGATGTCTGTTATTGCACTGGG
28	CTAAGACAGGTGTGTCCCAAGGAC	CAGCTCAGGGTTATGCATCCCAGCTT
29	AATTGAGAGCCTCGAATGTCAGCC	GGCTCAACACACAGCTTGGAGTAC
30	AACCTCTTTATCCCCCACTGGATTC	TTGGGAATTAAGCCTTCTGCTGCCT
31	CAGCCTCCTGCGATCAGAAGA	GTGAGTTGATCTTTGGCCTTAGCATGG
32	TCACAGCTGAAGGAAAAGGCCAAGG	TAGTGTTCTTGGCATCCTGAGGCCTC
33	AGGTCACCATAAAACCAGCACCGGA	AGGACTGTCTGTGTTGTCTACCTCATG
34	TAACGCCCCTCTGTGCTGCTATGT	TATCGGAGAAGGCTTTGTCCAGCTCAT
35	TGCTCCAGACACAGATGACCTGAAGCA	GGAGGCAAATGGTCAGAAGATGAGATG
36	GGTAGTCTCACTGTGAGTCTAGG	CAACTCATAGATCCTTGGGACCCTC
37	GATCAGAAAGTAGAGGGGGTCTTG	TGGGGTGGAGACAGGGTCTAAGT
38	TACCCAGGTCATCTATCCCATGGTCA	TTCCCCACACAATCATGGCCCCTCACAA
39	ACTGTGTGTGGATGGTGACATGGGAC	AAATCGGAGGAGAGACCCCACATG
40	ACTGTGTGTGGATGGTGACATGGGAC	TCCTTCCCGATGATGTAGCTGAG
41	CATGTGGGGTCTCTCCTCCGATTT	GGAATGGGGTGTGGTCAGTTG

Supplemental Figure 1



SUPPLEMENTAL FIGURE 1. Influence of the M373T mutation on the differential binding of C3b with normal C3 and C5. **(A)** Interaction of normal human C3 (500 nM) with immobilized normal C3b (blue) and the C3b M373T mutant (red) as determined by SPR. **(B-C)** Proposed model of the complex between C3 and C3b (B) based on the crystal structure of the dimeric AP C3 convertase stabilized by the bacterial inhibitor SCIN (C; PDB accession no. 2WIN; Ref. 39). The structure of C3 (PDB 2A73; Ref. 38) was aligned with one of the dimeric C3b molecules of the convertase structure in PyMOL to achieve the model shown in panel B. **(D)** Interaction of human C5 (500 nM) with immobilized normal C3b (blue) and C3b M373T (red). **(E-F)** Hypothetical model of the complex between C5 and C3b (E) based on the generalized convertase binding mode suggested in Ref. 43, which was derived from the crystal structure of the complex between C5 and cobra venom factor (F; PDB 3PVM) and the dimeric AP C3 convertase as mentioned above. The structure of C3b (PDB 2I07) was aligned with the structure of cobra venom factor (CVF) in the C5 complex to achieve the model shown in panel F.

Supplemental Figure 2



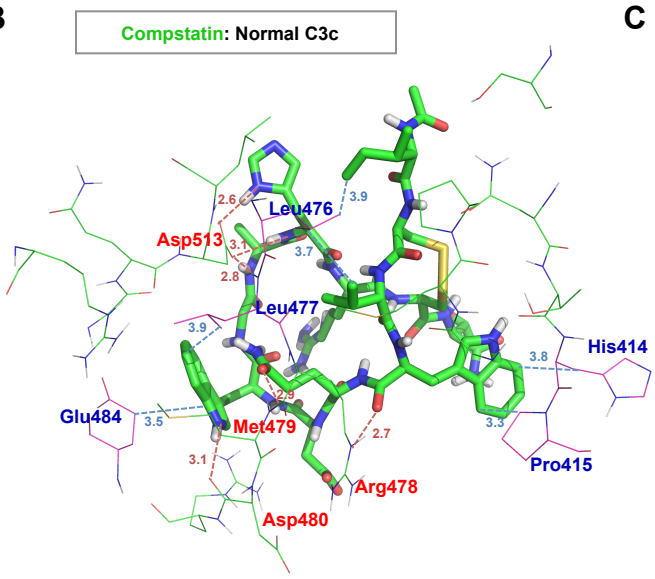
SUPPLEMENTAL FIGURE 2. Comparative hydrogen-deuterium exchange mass spectrometry (HDX-MS) analysis of normal C3 and C3 M373T. (A) Time-resolved exchange levels and sequence coverage plotted above (normal C3) or below (C3 M373T) the sequence of C3. (B) HDX plots of peptides with significantly (≥10%) increased or decreased exchange rates between the normal (blue) and mutated (red) form of C3. The y axis reflects the number of deuterons taken up by each peptide.

Supplemental Figure 3

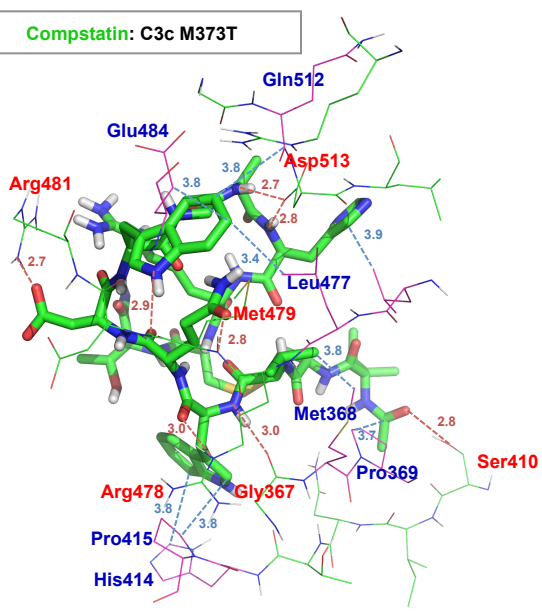
A

Residue	Normal C3c			Mutated C3c (M373T)		
	Structure	Φ (º)	Ψ (º)	Structure	Φ (º)	Ψ (º)
Met369	Turn	-135.96	163.30	β-Strand	-112.87	154.79
Thr413	Coil	-87.87	155.32	β-Strand	-111.99	143.16
Arg481	Turn	-90.96	-63.63	α-Helix	-55.40	-43.51
Ala482	Turn	-66.87	-25.46	α-Helix	-60.72	-51.33
His483	Turn	-69.39	-9.72	α-Helix	-69.79	-43.23
Glu484	Turn	-61.00	-60.40	α-Helix	-42.20	-51.04
Ala485	Turn	-79.22	-7.10	α-Helix	-66.76	-37.39
Lys486	Turn	-67.85	-49.63	α-Helix	-79.61	-21.92

B



C



SUPPLEMENTAL FIGURE 3. Analysis of the compstatin binding site on normal and mutated C3c after molecular dynamics (MD) simulation based on the co-crystal structure of compstatin analog 4W9A with C3c (PDB 2QKI). **(A)** Secondary structure assignment and main-chain dihedral angles (Φ, Ψ) of active site residues for both normal C3c the M351T mutant as determined using STRIDE. **(B-C)** Comparison of the contact network of compstatin 4W9A (stick representation) with the binding site on normal C3c (B) and C3c M373T (C; created by in silico mutagenesis of Met-373, see Methods) determined from the lowest-energy structure extracted from the trajectories of the MD simulations. Hydrogen bonds and hydrophobic interactions between the ligand and protein are represented as red and blue dashed lines, respectively, with distances in Å shown next to each line. Residues of complement C3c, which have hydrophobic interactions with compstatin, are indicated in magenta.