

Supplementary Materials

Quantitative analysis of complement membrane attack complex proteins associated with extracellular vesicles

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Figure S1. The amino acid sequences of COM1 and COM2 QconCATs. Peptides (marked red) with 6 amino acid residues flanking sequences on both sides (marked black) from proteins listed below were randomly concatenated in COM1 and COM2 sequences, respectively. 6 x His tag and linker sequence (marked orange) were from expression vector

COM1

Number of amino acids: 552

Molecular weight for ¹⁵N-labeled: 64532

Theoretical pI: 9.23

MLGGEYRVLFFYVDSEKLKQNDNFNSVEEKKCKSSGWHFVVKFSSHGCDSTCERLYYG
DDEKYFRKPYNFLKYHFEALQRVKRLPLEYSYGEYRDLFRDFVIRNRDVVLTTFVDDI
KALPTTYEKGEYFAFSFGFKIPGIFELGISSQSDRGKHYIRFTTKAKDLHLSDFLKA
HLPLEYNSALYSRIFDDFGKDFYRLSGNVLSYTFQVKINNDFNKLSEKHEGSFIQGA
SISLIRGGRSEYGAALAWEEKGSSGLEEKTFSWLESVKENPAVIEHLRHTSLGPLEAK
RQNLRRIRSVRGGSSGWSGGLAQNRSTITYRKQNLERAIEDYINEFSVRKCHTCQIFR
SRSIEVFGQFNGKRCTDAVFLHARSDLEVAHYKLKPRSLNTRFRKPYNVESYTPQTQG
KYEFILKRGEGRAVNITSENLDDVSLIRGGTRKYAGNKRRYSAWAESVTNLPQVIKQ
KLTPLYELVKEVPCASGEKNFRTEHYEEQIEAFKSIIQEKAKNDFKIGGAIEEVYVSLGV
SVGKCRGILNKLAAALEHHHHHH

Complement component C6 (P13671, 22-934 aa)

1-2. FTTKAKDLHLSVDVFLKALNHLPLEYNSALYSRIFDDFG (1185.6, 1860.0)

3-6.

NKLSEKHEGSFQGAEKSSISLIRGGRSEYGAALAWKEGSSGLEEKTFSEWLESVKENP
AVI (1201.6, 1223.6, 805.4, 1224.6)

Complement component C7 (P10643, 23-843 aa)

1. GKDFYRLSGNVLSYTFQVKINNDNFN (1454.8)

2-4. LGGEYRVLFYVDSEKQKQNDNFNSVEEKCKSSGWVHFVVKFSSHGC (1098.6,
1208.5, 1045.5)

5-6. AGNKRRYSAWAESVTNLPQVIKQKLTPLYELVKEVPCAS (1805.0, 1074.6)

Complement component C8 alpha chain (P07357, 31-584 aa)

1-2. DSTCERLYYGDDKEYFRKPYNFLKYHFEAL (1001.4, 908.5)

3. IISVRVGGSSGWSGGLAQNRSTITYR (1332.6)

4. IHEVLRHTSLGPLEAKRQNLRR (1051.6)

Complement components C8 beta chain (P07358, 55-591 aa)

1. LNTRFRKPYNVESYTPQTQGGKYEFILK (1738.9)

2. FSFGFKIPGIFELGISSQSDRGKHYIR (1617.8)

3. VFLHARSDLEVAHYKLKPRSL (1060.5)

4. LQRVKRLPLEYSYGEYRDLFRDF (1388.7)

5. AKNDFKIGGAIEEVYVSLGVSVGKCRGILN (1776.0)

Complement component C9 (P02748, 22-559 aa)

1. QMFRSRSEVFGQFNGKRCTDAV (1224.6)

2. GEKNFRTEHYEEQIEAFKSIIQEK (1522.7)

3-4. FVMRNRDVVLTFTTTFVDDIKALPTTYEKGEYFAF (1464.8, 921.5)

5. KRGEGRAVNITSENLIDDVVSIRGGTRKY (1970.1)

6. KQNLERAIEDYINEFSVRKCHTCQ (1454.7)

COM2

Number of amino acids: 675

Molecular weight for ¹⁵N-labeled: 76502

Theoretical pI: 8.30

MSSSENKFQNSAILTIQPKQLPGGQEKGRNKFVTVQATFGTQVVEKVVVLSLEFNVKTD
APDLPEENQAREGYRAIEQTIKSGSDEVQVGQQRFTFISPIAYAYKVSITSITVENVVKY
KATLLRYFYNKVVTEADVYITFGIREDLKDDNGSFKENSQYQPIKLQGTLPVEARENSL
YLTAFTVIGIRKAFDICSFYLKIDTQDIEASHYRGYGNSDCLAFKVHQYFNVELIQPGAVK
VYAYYNEEDLKALVEGVLDQLFTDYQIKDGHVILVQAERSGIPIVTSPIYQIHFTKTPKYFK
AAFVKRAPSTWLTAYVVKVFLAVTIPPKSSLSVPYVIVPLKTGLQEVEVKAAYVHHFIS
DGVVRKSLKVVFIQTDKTIYTPGSTVLYRIFTVNLHLGLARSNLDEDIIEENIVSRSEFPES
YSVWKGGASTWLTAFALRVLGQVNWTDNHLKALLVGEHLNIIVTPKSPYIDKITHYNYLI
LSKGKIIHRSEETKENEGFTVTAEGKGQGTLSFIPSFRLVAYYTLIGASGQREVVDWSV
VDVKDSCVGSFLYGGKVEGTAFVIFGIQDGEQRISLPESLKRIPEDGSGEVVLSRKVLL
DGVQNPRAEDLVGDGVAKLSINTHPSQKPLSITVTRTKKQELGRLHIKTLLPVSKPEIRSY
FPESKLAAALEHHHHHHH

Complement C5 beta chain (P01031, 19-673 aa)

1. LSSSENK**FQNSAILTIQPK**QLPGGQ (1358.8)
2. RYFYNK**VVTEADVYITFGI**REDLKDD (1581.8)
3. LEFNVK**TDAPDLPEENQARE**GYRAI (1454.7)
- 4-5. WTDN**HKALLVGEHLNIIVTPK**SPYIDK**ITHYNYLILSK**GKIIHR (1616.0, 1363.8)

Complement C5 alpha chain (P01031), 678-1676 aa)

1. GRLHMK**TLLPVSKPEIR**SYFPES (1251.8)
2. SYSVWK**GGASATWLTAFALR**VLGQVN (1436.7)
- 3-5. DNGSFK**ENSQYQPIKLQGTLPVEARENSLYLTAFTVIGIRK**AFDIC (1105.5, 1082.6, 1695.9)
6. CSFYLK**IDTQDIEASHYR**GYGNSD (1446.7)
7. NEEDLK**ALVEGVDQLFTDYQIK**DGHVIL (1837.9)
8. IAYAYK**VSITSITVENVFVKYKATLL** (1534.9)

Complement C3 beta-chain (P01024, 23-667 aa)

1. EKGRNK**FVTVQATFGTQVVEK**VVLVSL (1652.9)
2. FIQTDK**TIYTPGSTVLYRIFT**VNH (1369.7)
- 3-5. FLYGKK**VEGTAFVIFGIQDGEQRISLPESLKR**PIEDGS**GEVVL**SRKV**L**LDGVQNPRAEDLVG (1864.9, 1469.8, 1109.6)
6. MVQAER**SGPIV**TSPYQ**IHFTK**TPKYFK (1787.0)
7. GDGVAK**LSINTHPSQKPLSITV**RTKKQEL (1890.1)
- 8-9. FIPSFRLVAYYTLIGAS**GQREV**VADSWVD**VKD**SCVGS (1510.8, 1344.7)

Complement C3 alpha-chain (P01024, 672-1663 aa)

1. HLGLAR**SNLDE**IIAEENIVSRSEFPES (1815.9)
- 2-4. VTIPPK**SSL**SVPYVIVPLKTGLQE**VEVKA**AVYHHFISD**GVRK**SLKV (1400.8, 1001.5, 1470.7)
5. AAFVKR**APSTWLTAYVVK**VFSLAV (1334.7)
6. RSEETK**ENEGFTVTAEGK**GQGTL (1280.6)
7. DCLAFK**VHQYFNVELIQPGAVK**VYAYYN (1841.0)
8. IEQTIK**SGSDEVQVGQQR**TFISPI (1288.6)

Table S1. Transitions used for quantification.

Protein	Peptide		Precursor (m/z)	Product Ions (m/z)		
				1	2	3
C3/C3b	IPIEDGS GEVVL SR	L	735.9	759.4 (y7)	846.5 (y8)	903.5 (y9)
		H	744.4	769.4 (y7)	857.4 (y8)	915.5 (y9)
	VLLDGVQVPR	L	548.3	598.4 (y5)	655.4 (y6)	770.4 (y7)
		H	555.3	607.3 (y5)	665.4 (y6)	781.4 (y7)
C5/C5b	TDAPDLPEENQAR	L	728.3	843.4 (y7)	956.5 (y8)	1168.6 (y9)
		H	737.3	855.4 (y7)	969.4 (y8)	1183.5 (y9)
	FQNSAILTIQPK	L	680.4	699.4 (y6)	812.5 (y7)	883.6 (y8)

		H	688.4	707.4 (y6)	821.5 (y7)	893.5 (y8)
C6	TFSEWLESVK SEYGAALAWEK	L	613.3	761.4 (y6)	890.5 (y7)	977.5 (y8)
		H	619.3	769.4 (y6)	899.4 (y7)	987.5 (y8)
		L	612.8	646.4 (y5)	717.4 (y6)	788.4 (y7)
		H	619.3	653.3 (y5)	725.4 (y6)	797.4 (y7)
C7	VLFYVDSEK QNDFNSVEEK	L	550.3	577.3 (y5)	740.3 (y6)	887.4 (y7)
		H	555.3	583.3 (y5)	747.3 (y6)	895.4 (y7)
		L	605.3	705.3 (y6)	852.4 (y7)	967.4 (y8)
		H	612.3	713.3 (y6)	861.4 (y7)	977.4 (y8)
C8/C8a	HTSLGPLEAK LYYGDDEK	L	526.8	557.3 (y5)	614.4 (y6)	727.4 (y7)
		H	533.3	563.3 (y5)	621.4 (y6)	735.4 (y7)
		L	501.7	506.2 (y4)	563.2 (y5)	726.3 (y6)
		H	506.2	511.2 (y4)	569.2 (y5)	733.3 (y6)
C8/C8b	SDLEVAHYK IPGIFELGISSQSDR	L	531.3	617.3 (y5)	746.4 (y6)	859.5 (y7)
		H	537.2	625.3 (y5)	755.4 (y6)	869.4 (y7)
		L	809.9	849.4 (y8)	962.5 (y9)	1091.5 (y10)
		H	819.4	861.4 (y8)	975.5 (y9)	1105.5 (y10)
C9	ALPTTYEK DVVLTTTFVDDIK	L	461.7	540.3 (y4)	641.3 (y5)	738.4 (y6)
		H	466.2	545.3 (y4)	647.3 (y5)	745.3 (y6)
		L	733.4	736.4 (y6)	837.4 (y7)	938.5 (y8)
		H	740.4	743.4 (y6)	845.4 (y7)	947.5 (y8)
TSG101	GVIDLDVFLK IYLPYLHEWK	L	559.8	621.4 (y5)	734.4 (y6)	849.5 (y7)
		H	565.3	627.3 (y5)	741.4 (y6)	857.4 (y7)
		L	681.4	712.4 (y5)	875.4 (y6)	972.5 (y7)
		H	688.3	721.4 (y5)	885.4 (y6)	983.5 (y7)
flotillin-1	VTGEVLDILTR AQADLAYQLQVAK	L	608.4	617.4 (y5)	730.4 (y6)	829.5 (y7)
		H	615.3	625.3 (y5)	739.4 (y6)	839.5 (y7)
		L	709.9	849.5 (y7)	920.5 (y8)	1033.6 (y9)
		H	718.4	859.5 (y7)	931.5 (y8)	1045.6 (y9)
EHD4	SISVIDSPGILSGEK LDISDEFSEAIK	L	751.4	800.5 (y8)	887.5 (y9)	1002.5 (y10)
		H	759.4	809.4 (y8)	897.5 (y9)	1013.5 (y10)
		L	683.8	547.3 (y5)	694.4 (y6)	823.4 (y7)
		H	690.3	553.3 (y5)	701.4 (y6)	831.4 (y7)
moesin	IGFPWSEIR APDFVFYAPR	L	552.8	690.4 (y5)	787.4 (y6)	991.5 (y8)
		H	559.3	699.3 (y5)	797.4 (y6)	1003.5 (y8)
		L	591.8	653.3 (y5)	752.4 (y6)	899.5 (y7)
		H	598.3	661.3 (y5)	761.4 (y6)	909.4 (y7)
Integrin beta-1	IGGGSFVEK GEVFNELVGK	L	492.3	522.2 (y4)	609.3 (y5)	666.3 (y6)
		H	497.2	527.3 (y4)	615.3 (y5)	673.3 (y6)
		L	546.3	659.4 (y6)	806.4 (y7)	905.5 (y8)
		H	552.3	667.3 (y6)	815.4 (y7)	915.5 (y8)
Integrin alpha-IIb	FGSAIAPLGLDR GEAQVWTQLLR	L	666.4	688.4 (y6)	785.4 (y7)	856.5 (y8)
		H	674.3	697.3 (y6)	795.4 (y7)	867.4 (y8)
		L	650.9	816.5 (y6)	915.5 (y7)	1043.6 (y8)
		H	659.3	827.4 (y6)	927.5 (y7)	1057.6 (y8)
Integrin beta-3	VLEDRPLSDK GSGDSSQVTQVSPQR	L	586.3	559.3 (y5)	715.4 (y6)	830.4 (y7)
		H	593.3	565.3 (y5)	725.4 (y6)	841.4 (y7)
		L	766.9	817.4 (y7)	914.5 (y8)	1042.6 (y9)
		H	777.3	827.4 (y7)	927.5 (y8)	1057.5 (y9)
GP Ib alpha	GLGELQELYLK GVLQGHLESSR	L	631.9	665.4 (y5)	793.4 (y6)	906.5 (y7)
		H	638.3	671.4 (y5)	801.4 (y6)	915.5 (y7)
		L	591.8	728.4 (y6)	785.4 (y7)	913.4 (y8)
		H	600.3	739.3 (y6)	797.4 (y7)	927.4 (y8)
GP Ib beta	LSLTDPLVAER	L	607.3	684.4 (y6)	799.4 (y7)	900.5 (y8)
		H	614.3	693.4 (y6)	809.4 (y7)	911.4 (y8)

	LLPYLADELRL	L	666.4	732.4 (y6)	845.4 (y7)	1008.5 (y8)
		H	673.3	741.3 (y6)	855.4 (y7)	1019.5 (y8)
GP V	SIAPGAFDR	L	467.2	508.3 (y4)	565.3 (y5)	662.3 (y6)
		H	473.2	515.2 (y4)	573.2 (y5)	671.3 (y6)
	LPNLSSLTLR	L	600.9	676.4 (y6)	763.4 (y7)	876.5 (y8)
		H	608.3	685.4 (y6)	773.4 (y7)	887.5 (y8)
GP IX	GHGLTALPALPAR	L	637.4	737.5 (y7)	808.5 (y8)	909.6 (y9)
		H	646.3	747.4 (y7)	819.5 (y8)	921.5 (y9)
	TPEALLQVR	L	513.8	628.4 (y5)	699.5 (y6)	828.5 (y7)
		H	520.3	637.4 (y5)	709.4 (y6)	839.5 (y7)
Integrin alpha-2	FVQGLDIGPTK	L	587.8	630.3 (y6)	743.4 (y7)	700.4 (y8)
		H	594.3	637.3 (y6)	751.4 (y7)	809.4 (y8)
	IGQTSSSVSFK	L	570.8	654.3 (y6)	741.4 (y7)	842.4 (y8)
		H	577.3	661.3 (y6)	749.4 (y7)	851.4 (y8)
CYP 5A1	WYSTSAFSR	L	552.8	567.3 (y5)	668.3 (y6)	755.4 (y7)
		H	559.2	575.3 (y5)	677.3 (y6)	765.3 (y7)
	DELNGFFNK	L	542.3	555.3 (y4)	612.3 (y5)	726.4 (y6)
		H	548.2	561.3 (y4)	619.3 (y5)	735.3 (y6)

Transitions are listed for both unlabeled, light (L) and fully ¹⁵N-labeled, heavy (H) peptides. All precursors were +2 charge and product ions were +1 charge. Additionally, y-ion information is included for reference.