

A

fragment 001 (≈ 130 kDa)	Position (aa)		25-666 75 kDa	667-1663 120 kDa	667-1663 120 kDa		1304-1320 2 kDa	1321-1663 42 kDa
			β (C3, C3b, iC3b, C3c)	α (C3)	α1-dg (iC3b)	α1 (C3c)		
LREQHRR	734	740						
NRVYFQTIK	880	887						
LLWENGNLLR	1311	1320						X
SGSDEVQAGQQR	1571	1582						X
VMQDFFIDLR	825	834						
GICVADPYEIR	814	824						
AFIDCCNHITK	723	733						
GRG TLSVVAVYHAK	1338	1351						X
AAVFNHFISDGVKK	914	927						
FYHPEKDDGMLSK	1492	1504						X
AVLFNYREQEELK	849	861						
KCCEDGMRDIPMR	692	704						
VHQYFNVGLIQPGSVK	1463	1478						X
SELEEDIPEEDIISR	749	764						
LEEPYLGKFLNTAKDR	1202	1217						
SHFPQSWLWTIEELK	765	779						
VDVPAADLSDQVPD T DSETR	960	979						
IILQGSPPVQMAEDAVDGER	980	999						
TDPGHEAKIRYYTYLVMNK	479	497	X					
QKPDGVFQEDGPVIHQEMIGGFR	1112	1134						
IRAFYEHAPK	207	216	X					
VYSYNNLEESCTR	1479	1491						X
TLKVVPPEGMR	928	937						
IRYYTYLVMNK	487	497	X					
MELKPGDNLNPNFHLRTDPGHEAK	463	486	X					

B

fragment 002 (≈ 130 kDa)	Position (aa)		25-666 75 kDa	667-1663 120 kDa	667-1663 120 kDa		1304-1320 2 kDa	1321-1663 42 kDa
			β (C3, C3b, iC3b, C3c)	α (C3)	α1-dg (iC3b)	α1 (C3c)		
LLWENGNLLR	1311	1320	X					
IRAFYEHAPK	207	216	X					
GICVADPYEIR	814	824						
VMQDFFIDLR	825	834						
IRYYTYLVMNK	487	497	X					
AAVFNHFISDGVKK	914	927						
AVLFNYREQEELK	849	861						
AFSNKNTLIYLEK								
VYSYNNLEESCTR	1479	1491						
VHQYFNVGLIQPGSVK	1463	1478						X
SELEEDIPEEDIISR	749	764						X
LEEPYLGKFLNTAKDR	1202	1217						
VELLHNPFCSMATAKNR								
IILQGSPPVQMAEDAVDGER	980	999						
TDPGHEAKIRYYTYLVMNK	479	497	X					
GRG TLSVVAVYHAK	1338	1351						X
IRYYTYLVMNK	487	497	X					
FYHPEKDDGMLSK	1492	1504						X
KCCEDGMRDIPMR	692	704						
VDVPAADLSDQVPD T DSETR	960	979						
QKPDGVFQEDGPVIHQEMIGGFR	1112	1134						
AGEYIEASYMNLQRPYTVAIAGYALALMKNK	1172	1201						

Supplemental Figure 1

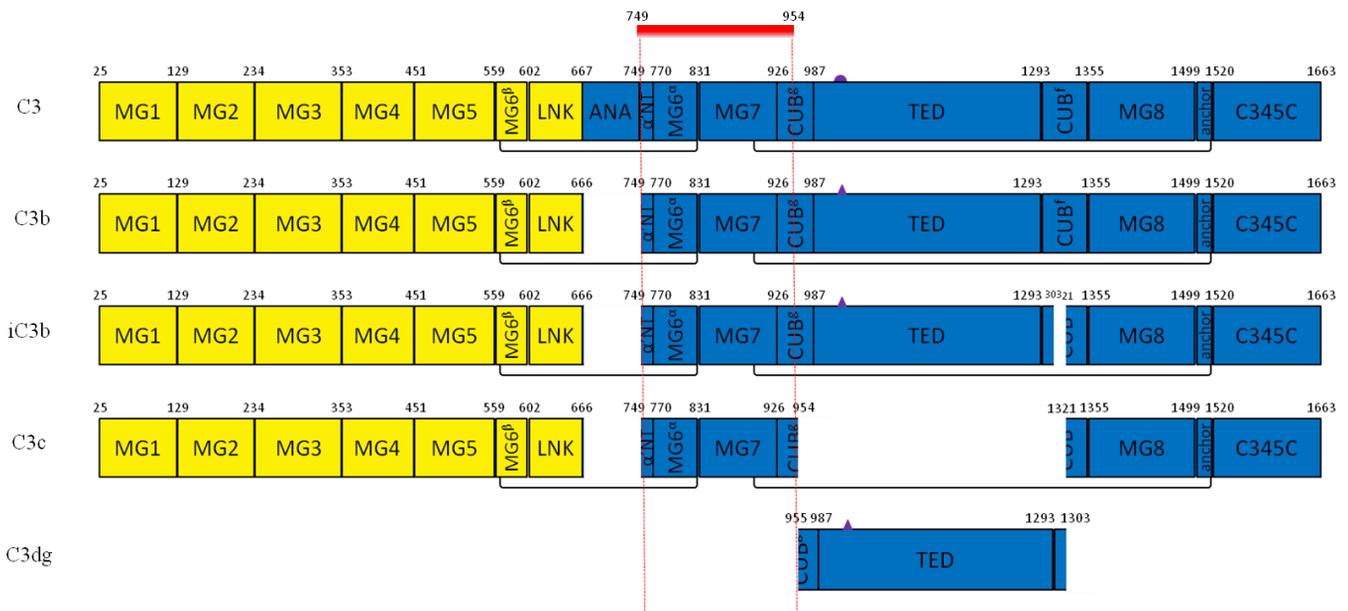
C

fragment 007 (≈ 75 kDa)	Position (aa)		β (C3, C3b, iC3b, C3c)	667-748 10 kDa C3a	749-954 26 kDa α1 (C3c)	1002-1303 35 kDa C3d	667-1663 120 kDa α (C3)	749-1663 110 kDa α' (C3b)	1304-1320 2 kDa C3f	1321-1663 42 kDa α2 (iC3b, C3c)
	Start	End								
NRYFQTIK	880	887			X					
DFDSVPPVVR	1245	1254				X				
VMQDFIDLR	825	834			X					
GICVADPYEIR	814	824			X					
IRYYTYLVMNK	487	497	X							
DSCIGTLVVKGDPR	557	570	X							
NYAGVFMDAGLAFK	633	646	X							
AAVFNHFISDGVKK	914	927			X					
AFIDCCNHITKLR	723	735		X						
SELEEDIPEEDIISR	749	764			X					
AVLFNYREQEELKVR	849	863			X					
SHFPQSWLWTEELK	765	779			X					
TVAIHTLDPEKLGQGGVQK	941	959			X			X		
IILQGSPPVQMAEDAVDGER	980	999						X		
TDPGHEAKIRYYTYLVMNK	479	497	X							
QKPDGVFQEDGPMHQEMIGGFR	1112	1134				X				
YLGVDVATMSLIDISMMTGFAPDTK	1392	1416								X
VMNIFLKD SITTWEI LAVSLSDKK	790	813			X					
DICEGQVNSLPGSINK	1156	1171				X				

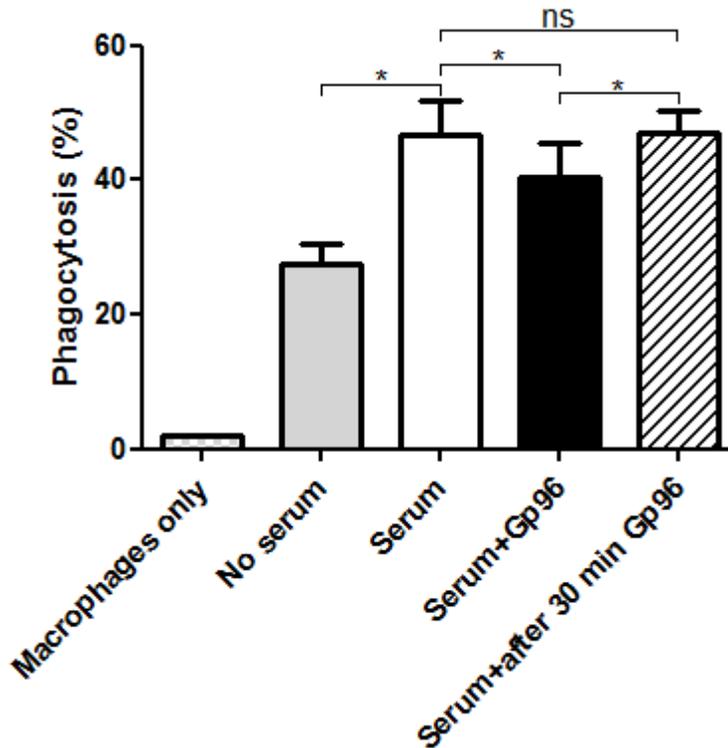
D

fragment 010 (≈ 45 kDa)	Position (aa)		β (C3, C3b, iC3b, C3c)	667-748 10 kDa C3a	749-954 26 kDa α1 (C3c)	1002-1303 35 kDa C3d	667-1663 120 kDa α (C3)	749-1663 110 kDa α' (C3b)	1304-1320 2 kDa C3f	1321-1663 42 kDa α2 (iC3b, C3c)
	Start	End								
SGSDEVGAGQQR	1571	1582								X
GRGTLVAVVYHAK	1338	1351								X
ISHTEEDCLTFK	1451	1462								X
FYHPEKDDGMLSK	1492	1504								X
VYSYNNLEESCTR	1479	1491								X
VHQYFNVGLIQPGSVK	1463	1478								X
TDPGHEAKIRYYTYLVMNK	479	497	X							
LCHSEMCR	1505	1512								X
DLELLASGVDR	1417	1427								X
GTLSVAVVYHAK	1340	1351								X
ACEPGVDYVYK	1536	1546								X
LVAYYTLIGASGQR	531	544	X							
AVLFNYREQEELK	849	861			X					
KCCEDGMRDIPMR	692	704		X						
CAEENCFMQQSQEK	1513	1526								X
VSIRPAPETAKKPEEAK	1365	1381								X
NTLIYILEK	1442	1450								X
TLKVVPEGMR	928	937			X					
NTMFLEICTK	1382	1391								X
KDTLPESRQATK	428	439	X							
DNHLPAPGQQTLLR	571	583	X							
LDKACEPGVDYVYK	1533	1546								X
DTWVEHWPEAECCQDQK	1625	1641								X

Supplemental Figure 1 (continued) C3 associates to Gp96 in mice's sera during GvHD. Mass spectrometry analysis of fragments A 001, B 002, C 007 and D 010 from Figure 3, representing C3. C3 fragments are quoted according to the peptide found.

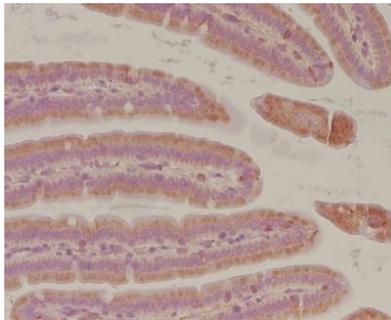


Supplemental Figure 2: Schematic structures of complement C3 and C3 fragments. In yellow the β -chain; in blue the α -chain; in purple the thioester residues; in red the minimal fragment 749-954 interacting with Gp96. α' NT: N-terminus of α' -chain; ANA: anaphylatoxin; CUB: complement C1r/C1s, Uegf, Bmp1; LNK: linker region; MG: macroglobulin; TED: thioester-bond-containing domain

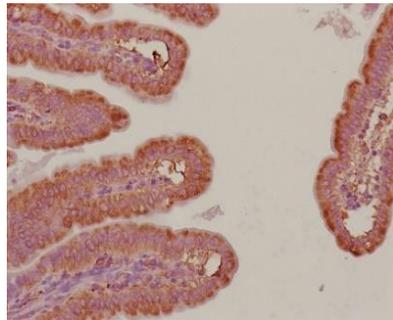


Supplemental Figure 3: Gp96 effect on opsonophagocytosis. Flow cytometry analysis of phagocytosis by human purified macrophages of Alexa Fluor 488-conjugated *Escherichia coli* bioparticles after 1 h opsonization by serum in the absence or presence of Gp96, added either immediately (serum+Gp96) or after 30 min of incubation (serum+after 30min Gp96) of the bioparticles with the serum. Represented is the percentage of living cells showing fluorescence in AF488.

Syn D7



Allo D7



Supplemental Figure 4: BALB/c mice Allo (GvHD) and Syn (no GvHD) were sacrificed at day 7 after hematopoietic stem cell transplantation and small intestine sections were stained for Gp96 expression using a polyclonal anti-Gp96 antibody. A representative image is shown (3 distinct experiments with 5 animals per group). Magnification: x 20.