Supplementary Material

to

A Novel C5a-neutralizing Mirror-image (L-)Aptamer Prevents Organ Failure and Improves Survival in Experimental Sepsis

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Short title: C5a-neutralizing L-aptamer improves sepsis

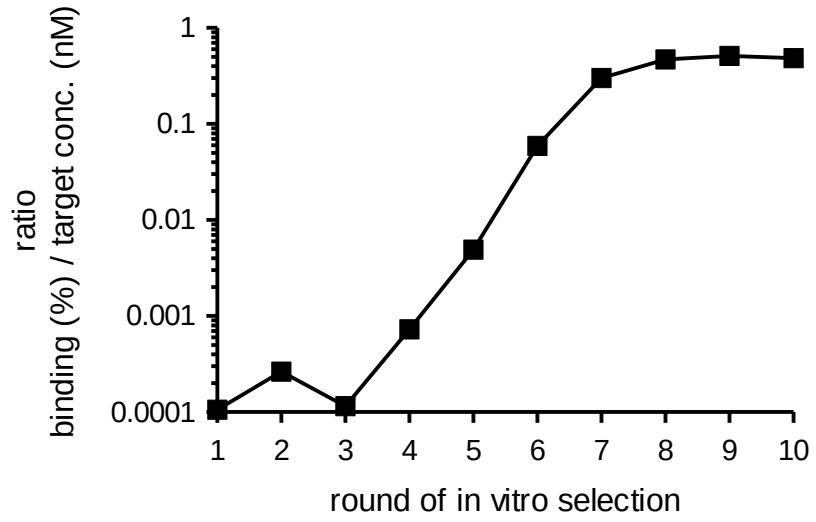


Figure S1. Enrichment of bio-D-mC5a binding aptamers from a random RNA library. The ratio of the percentage of target-bound aptamers and the concentration of target applied to the respective selection round was used as a measure for the frequency and affinity of target-binding aptamers in the library.

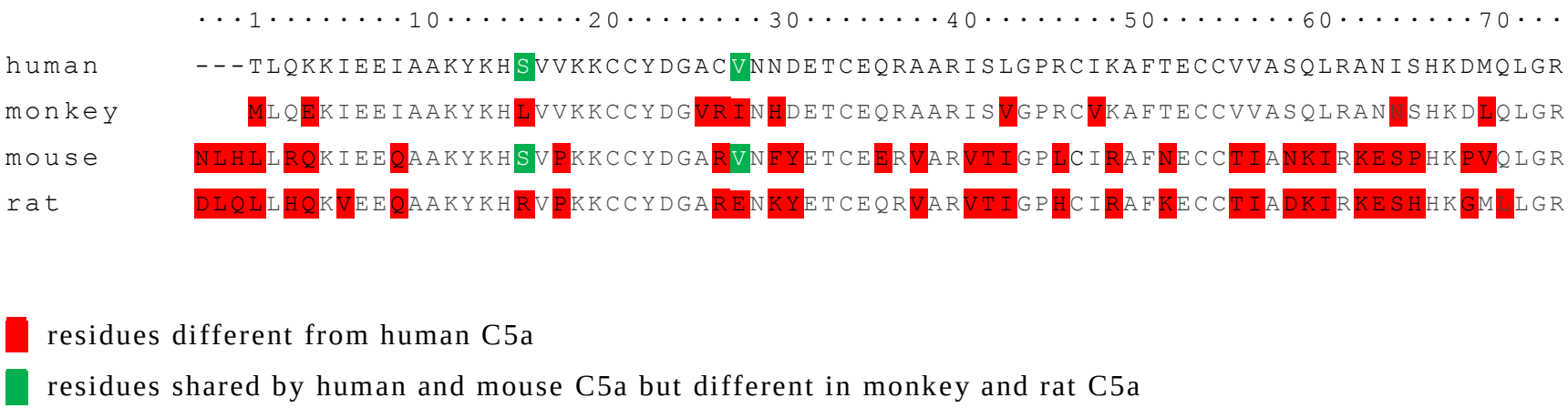


Figure S2. C5a sequence alignment. Alignment of C5a protein sequences from human (UniProt ID: P01031), rhesus/cynomolgus monkey, mouse (UniProt ID: P06684), and rat (UniProt ID: P08650). Protein sequence of C5a from rhesus monkey was predicted from the genomic sequences (NCBI reference sequence: XP_001095750.2). Identity of cynomolgus monkey C5a was confirmed by RT-PCR and sequencing.

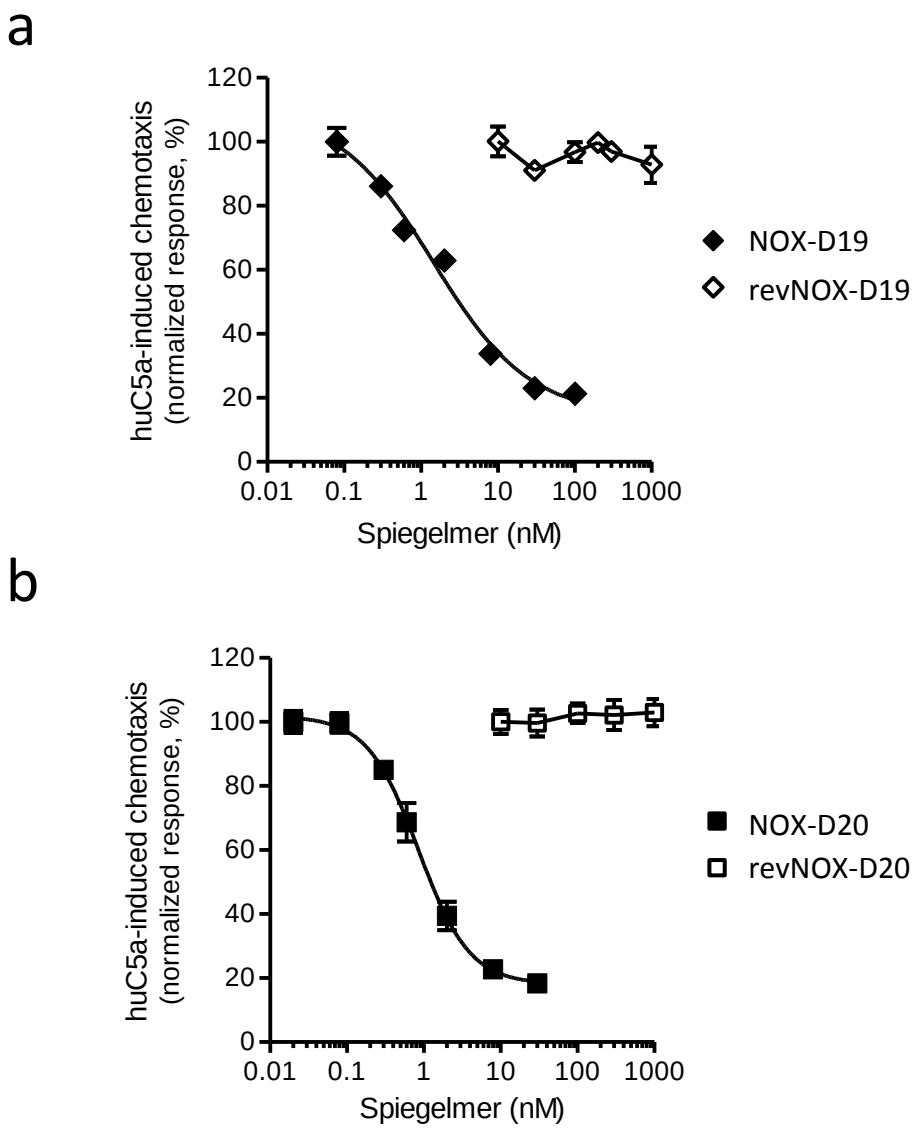
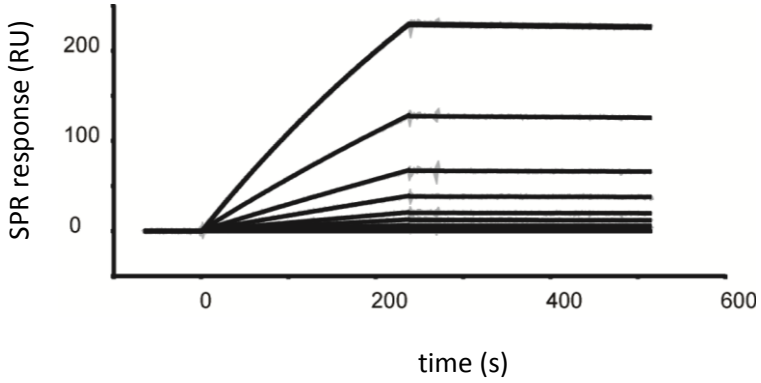


Figure S3. Reverse control Spiegelmers do not inhibit huC5a-induced chemotaxis. The oligonucleotide part of the PEGylated control Spiegelmers revNOX-D19 and revNOX-D20 is of the reverse sequence of NOX-D19 and NOX-D20, respectively. Chemotaxis of $CD88^+$ BA/F3 cell was stimulated with 0.1 nM human C5a pre-incubated with (a) NOX-D19 (closed diamonds), revNOX-D19 (open diamonds) and (b) NOX-D20 (closed squares), revNOX-D20 (open squares) at indicated concentrations. Mean ± SD of triplicate measurement are shown. Data is representative for three independent experiments.



k_a (10^6 M^{-1} s^{-1})	k_d (10^{-5} s^{-1})	K_d (= k_d/k_a)
2.44±0.001	4.65±0.09	19 pM

Figure S4. NOX-D20 binds to mouse C5a. SPR measurement of NOX-D20 binding to mouse C5a. Kinetic rate constants are shown as mean ± SEM. Data is representative for at least 3 individual measurements.

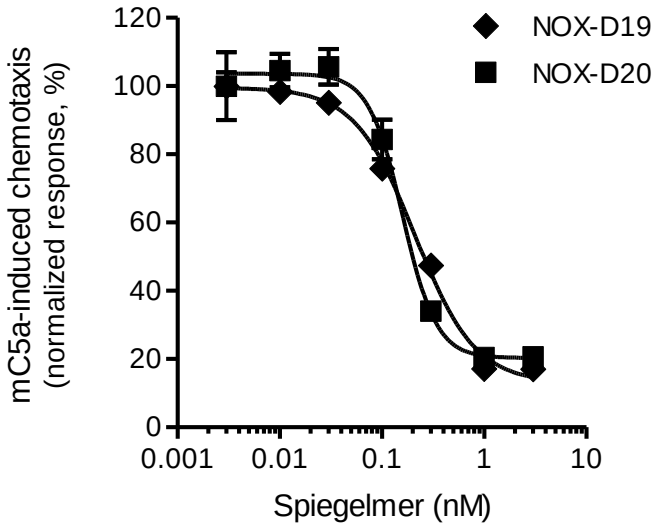


Figure S5. NOX-D19 and NOX-D20 inhibit mouse C5a-induced chemotaxis. Chemotaxis of $CD88^+$ BA/F3 cell was stimulated with 0.3 nM mouse C5a pre-incubated with NOX-D19 (closed diamonds) and NOX-D20 (black squares) at indicated concentrations. Stimulation with 0.3 nM mouse C5a stoichiometrically limits the sensitivity of the assay to IC_{50} = 0.15 nM. Mean ± SD of triplicate measurement are shown. Data is representative for five independent experiments.

Table S1. Sequences and frequencies of RNA aptamers identified after 10 rounds of in vitro selection.

name	sequence[a]	frequency
	1 10 20 30 40 50 60 70 80	
274-D5	ggagcucagaccguacaccUGUGCCUGAUGUGGUGUUGAGGGGUUGUGGGGUGUCGACGCACAggcugcagugucgguuccag	16.7 %
274-H6	ggagcucagaccguacaccUGUGCCUGAUGUGGUGUUGAGGGGCUGUGGGGUGUCGACGCACAggcugcagugucgguuccag	16.7 %
274-C8	ggagcucagaccguacaccUAUGCCUGAUGUGGUGUUGAAGGGUUGUUGGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %
274-C5	ggagcucagaccguacaccUGUGCCUGAUGUGGUGGUGAGGGGUUGUGGGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %
274-B5	ggagcucagaccguacaccUAUGCCUGAUGUGGUGUUGAAGGGUUGUGGGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %
274-F5	ggagcucagaccguacaccUGUGCCUGAUGUGAUGUUGAGGGGUUGUGGGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %
274-A5	ggagcucagaccguacaccUGUGCCUGAUGUGGUGUUGAGGGGCUGUGGGGUGUCGACGCAUAggcugcagugucgguuccag	4.2 %
274-B6	ggagcucagaccguacaccUGUGCCUGAUGUGAUGUUGAGGGGUUGUUGGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %
274-G8	ggagcucagaccguacaccUGUGCCUGAUGUGGUGGUGAGGGGCUGUGGGGUGUCGACGCAUAggcugcagugucgguuccag	4.2 %
274-G7	ggagcucagaccguacaccUGUGCCUGUUGUGGUGGUGAGGGGCUGUGGGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %
274-G6	ggagcucagaccguacaccUGUGCCUGAUGUGGUGGUGAGGGGAUGUGGGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %
274-H7	ggagcucagaccguacaccUGUGCCUGAUAUGGUGCUGAGGGGCUGUGGGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %
274-F6	ggagcucagaccguacaccUGUGCCUGAUAUGGUGUUGAGGGGCUGUGGGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %
274-H5	ggagcucagaccguacaccUGUGCCUGAUGUGGUGUUGAGGGGCUGUGCGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %
274-F8	ggagcucagaccguacacccGUGCCUGAUGUGGUGGUAAGGGACUGUGGGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %
274-A6	ggagcucagaccguacaccUAUGCCUGAUGUGGUGGUGAGGGGCUGUGGGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %
274-C6	ggagcucagaccguacaccUUUGCCUGAUGUGGUGUUGAGGGGUUGUGGGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %
274-D7	ggagcucagaccguacaccUAUGCCUGAUAUGGUGUUGAGGGGCUGUGGGGUGUCGACGCACAggcugcagugucgguuccag	4.2 %

[a] Fixed primer-binding sites are in lower case. Point mutations are highlighted.

Table S2. Truncation of NOX-D19001-6xDNA.

name	sequence	length	affinity (K_d)[a]
NOX-D19001-6xDNA	**GCCUG** AUG(dU)GGUGGU(dG)(dA)AGGGUUGUUGGG(dU)G(dU)CGACGCA **(dC)AGGC**	44 nt	0.4 nM
NOX-D19001-6xDNA-011	**GC-UG** AUG(dU)GGUGGU(dG)(dA)AGGGUUGUUGGG(dU)G(dU)CGACGCA **(dC)A-GC**	42 nt	0.3 nM
NOX-D19001-6xDNA-018	**GCC-G** AUG(dU)GGUGGU(dG)(dA)AGGGUUGUUGGG(dU)G(dU)CGACGCA **(dC)-GGC**	42 nt	0.3 nM
NOX-D19001-6xDNA-012	**G--UG** AUG(dU)GGUGGU(dG)(dA)AGGGUUGUUGGG(dU)G(dU)CGACGCA **(dC)A--C**	40 nt	0.8 nM
NOX-D19001-6xDNA-020 **= NOX-D20001**	**G-C-G** AUG(dU)GGUGGU(dG)(dA)AGGGUUGUUGGG(dU)G(dU)CGACGCA **(dC)-G-C**	40 nt	0.4 nM
NOX-D19001-6xDNA-033	**G---G** AUG(dU)GGUGGU(dG)(dA)AGGGUUGUUGGG(dU)G(dU)CGACGCA **(dC)---C**	38 nt	5.5 nM

[a] Equilibrium dissociation constants (K_d) for Spiegelmers binding to huC5a measured by SPR.