# Supplementary Information

# *In vitro* modulation of complement activation by therapeutically prospective analogues of the marine polychaeta arenicin peptides

**Ilia A. Krenev [1], Pavel V. Panteleev [2], Ekaterina S. Umnyakova [1,3], Nikolay P. Gorbunov [4], Valeria A. Kostevich [4], Sergey V. Balandin [2], Tatiana V. Ovchinnikova [2,5], Galina M. Aleshina [1] and Mikhail N. Berlov [1,*]**

[1] Department of General Pathology and Pathological Physiology, Institute of Experimental Medicine, Acad. Pavlov Str. 12, 197376 Saint Petersburg, Russia

[2] M.M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya Str., 16/10, 117997 Moscow, Russia

[3] Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland

[4] Department of Molecular Genetics, Institute of Experimental Medicine, Acad. Pavlov Str. 12, 197376 Saint Petersburg, Russia

[5] Department of Biotechnology, I.M. Sechenov First Moscow State Medical University, Trubetskaya Str., 8-2, 119991 Moscow, Russia

* Correspondence: berlov.mn@iemspb.ru; Tel.: +7-911-936-9839

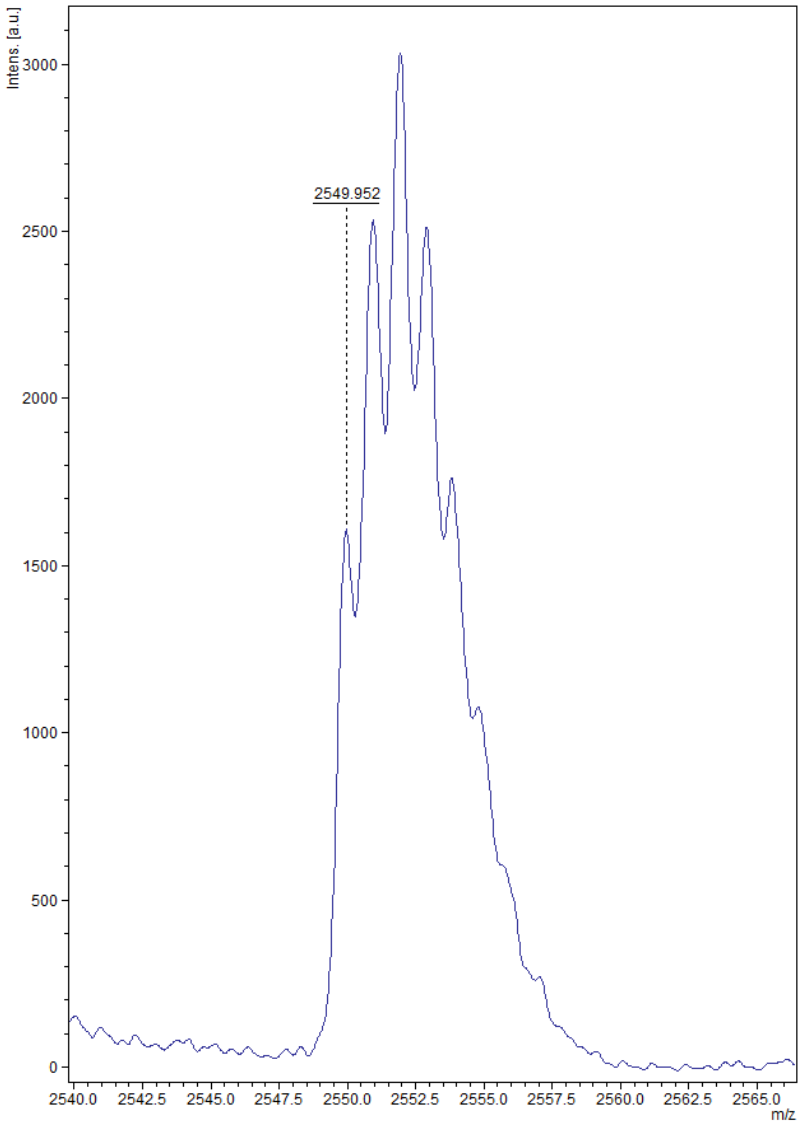


**Figure S1.** MALDI-MS analysis of the recombinant AA139. Calculated [M+H]+ monoisotopic molecular mass of AA139 is of 2549.17.

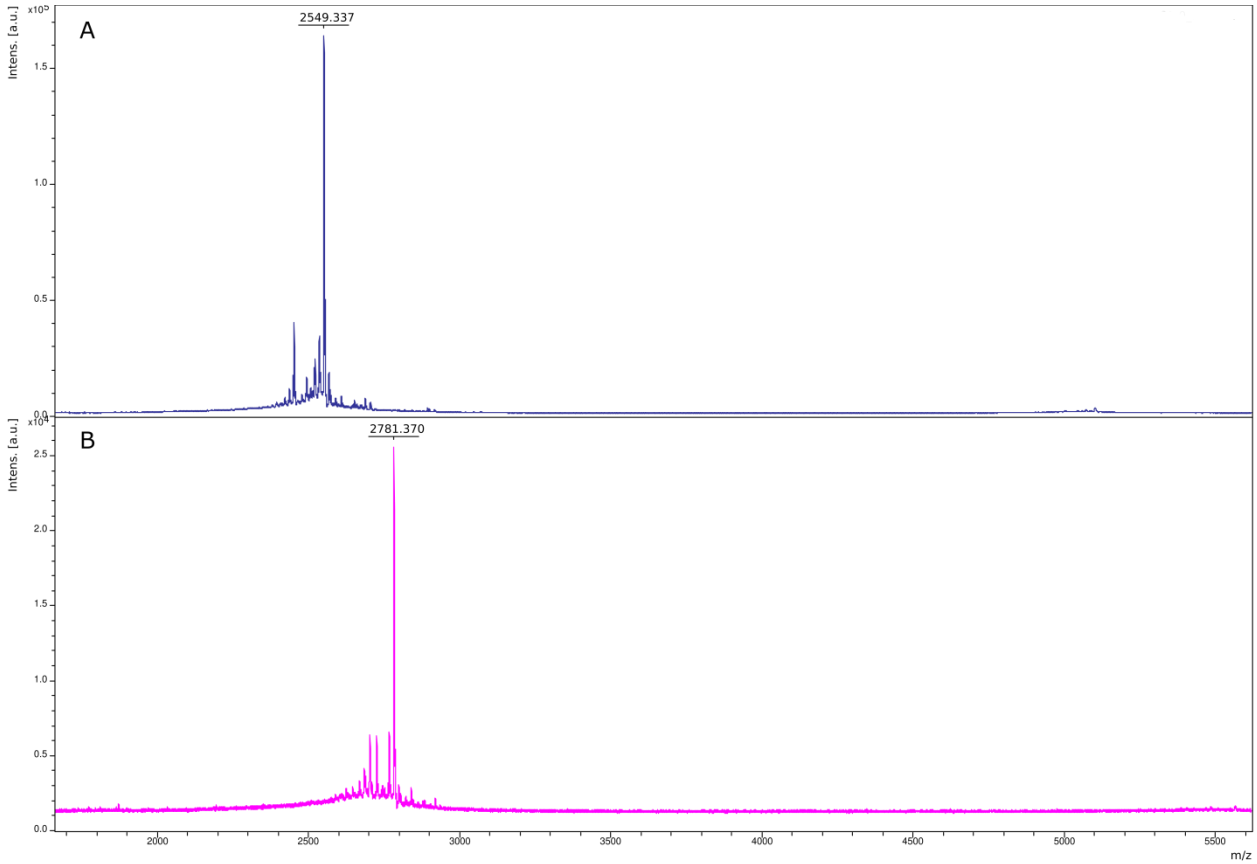


**Figure S2.** MALDI-MS analysis of the recombinant AA139 after incubation with iodoacetamide (IAA) w/o (A) or after (B) the addition of DTT. The difference between the two m/z values (232 Da) corresponds to 4 acetamide residues which indicates the absence of free thiol groups in the recombinant peptide.

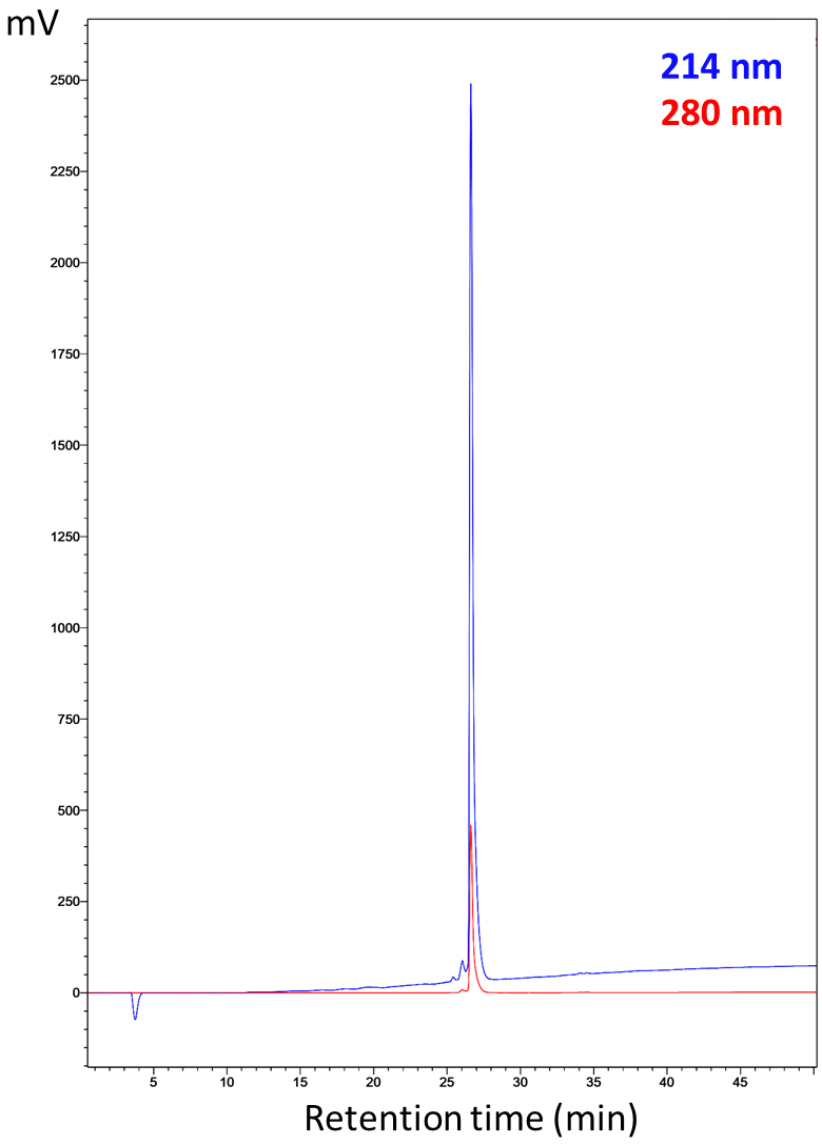


**Figure S3.** Repurification of the recombinant AA139. RP-HPLC was performed using the analytical column (Symmetry 300 C18) at a flow rate of 1 mL/min in a linear gradient of solution B (80% acetonitrile, 0.1% TFA) in solution A (5% acetonitrile, 0.1% TFA): 0-100% for 50 min.

**Table S1.** Evaluation of the hemolytic activity of arenicin analogues in heat-inactivated serum. Peptides at 160 μg/mL were incubated with $Er^{sh}$ or $Er^{rab}$ in presence of heat-inactivated serum at +37°C for 30 minutes, then the samples were diluted with PBS as described in section 4.3. For 100% lysis, distilled $H_2O$ was added to the samples instead of PBS. The average values from the two experiments are shown.

| | $OD_{414}$ | |
|---|---|---|
| | $Er^{sh}$ | $Er^{rab}$ |
| 100% lysis | 0.995 | 0.878 |
| Without peptide | 0.006 | 0.002 |
| Ar-1[V8R] | 0.003 | 0.002 |
| ALP1 | 0.005 | 0.004 |
| AA139 | 0.003 | 0.003 |