Supplementary Fig. S1. VaaSPH-1 almost completely abolishes formation of thrombin in blood. Thrombin generation assay was used to assess the capacity of VaaSPH-1 to suppress the generation of thrombin. Human plasma was incubated with 0.77 µM VaaSPH-1 or Milli-Q water (control) for 2 minutes at 37°C. By addition of less than 5 pM TF, we activated both the intrinsic and the extrinsic pathway of blood coagulation. Thrombin activity was measured fluorimetrically using Technothrombin TGA substrate and Victor X2 multilabel plate reader (Perkin Elmer, Finland). Fluorescence was induced at 355 nm and recorded at 460 nm. From the intensity of the emitted fluorescence, concentration of thrombin was calculated using Technoclone software (www.technoclone.com). The area under the concentration curve corresponds to the total thrombin potential. Each experiment was performed twice (blue and violet trace).

### Binding of VaaSPH-1 to lipids

**Binding of VaaSPH-1 to Lipids by Sedimentation Assay**

Phospholipid multilamellar vesicles (MLVs) were prepared in a 10-mL vacuum flask. Phospholipids (PLs) in the final concentration of 10 mg/mL were dissolved in chloroform. Solutions of PLs were pipetted in the vacuum flask in a way that the final mass of MLVs in 1 mL was 5 mg. MLVs consisted of sphingomyelin (SM) and phosphatidylcholine (PC); SM and cholesterol (CH); phosphatidylserine (PS); PC and CH; and PS and CH in all cases in the molar ratio 1:1. Applying vacuum, chloroform was evaporated from the flask and a lipid film was formed on the bottom of the flask. This was dried for 2 additional hours. MLVs were formed from lipid films by addition of 1 mL of buffer C (20 mM Tris, 5 mM EDTA, 140 mM NaCl, pH 7) and intensive vortexing in the presence of acid-washed glass beads. Note that 20 µL of MLV suspension was added to
Supplementary Fig. S2 VaaSPH-1 binds to negatively charged PLs. The binding of VaaSPH-1 to PLs and its charge selectivity was explored using three different experimental approaches, PL vesicle sedimentation assay (A), thin-layer chromatography (TLC) – blotting (B) and surface plasmon resonance (SPR) (C). Sedimentation assay showed that VaaSPH-1 binds to multilamellar vesicles (MLV) or not. MLV varied in their PL composition, consisting of sphingomyelin (SM) + phosphatidylcholine (PC); SM + cholesterol (CH); phosphatidylinositol (PI) + CH; PC + phosphatidylserine (PS); PC + CH and PS + CH in the molar ratio 1:1. VaaSPH-1 was present in sedimented MLVs of PS + PC, PS + CH and PI + CH composition. If mixed with the MLV of other composition, VaaSPH-1 was found in the supernatant after the centrifugation (A). Individual PLs were chromatographed on a thin layer of silica gel. From the developed TLC plate, PLs were transferred/blotted onto the PVDF membrane, and analysed for binding of VaaSPH-1 using detection with anti-Vaa serum. By overlaying immunostained PVDF membrane and the silica gel plate, we concluded that VaaSPH-1 bound to negatively charged PS and PI, but not with SM, PC or CH (B). The selective binding of VaaSPH-1 to membranes containing the negatively charged PS or PI was confirmed using SPR (C). VaaSPH-1 interacted only with SUV of PS + CH and PI + CH, while no binding was observed with SUV of other compositions (PC + PS, SM + CH, SM + PC, PC + CH). Using this technology, we also determined the dissociation constant (Kd) for the interaction of VaaSPH-1 with SUV consisting of PS + CH and PI + CH in the molar ratio 1:1. In the first instance, Kd was 189 nM, while in the second 481.3 nM (C).

20 µL of 0.5 mg/mL VaaSPH-1 in buffer C and incubated at the RT with shaking for 30 minutes. In negative controls, the same amount of VaaSPH-1 was added in three-fold Milli-Q water and buffer C. Following incubation, the samples were centrifuged at 60,000 g for 60 minutes at 4°C. Supernatants were transferred to fresh test tubes, supplemented with 12.5 µL of 100% trichloroacetic acid and incubated for 10 min on ice. Then the test tubes were centrifuged at 16,100 g for 12 minutes at 4°C. In each test tube, supernatant was removed and 300 µL of ice-cold acetone was added to the sediment. Samples were centrifuged at 16,100 g for 5 minutes at 4°C, followed by removal of supernatant and addition of ice-cold acetone. After the centrifugation at 16,100 g for 5 minutes at 4°C, supernatants were removed and sediments air-dried. Sediment and supernatant were analysed with 12.5% SDS-PAGE, under reducing conditions. Proteins were visualized by silver staining, as described in the ‘Material and Methods section’.

Binding of VaaSPH-1 on the PVDF Membrane-Blotted Lipids
Thin-layer chromatography was performed on the heated aluminium silica gel plate (60 W, F254s: a fluorescent indicator coating silica gel, 20 × 20 cm, Merck, Germany). Note that 60 µg of PLs (SM, PC, PS and CH) were applied. The plate was immersed in a solvent or a mobile phase, consisting of chloroform, methanol and ammonium in the volume ratio 20:20:1, in chromatographic chamber, where the separation was accomplished. After the separation of PLs, the plate was dried to completely remove the solvent. PLs were transferred onto the PVDF membrane (Serva, Germany) by ironing (30 seconds at 180°C). The PVDF membrane was blocked for 2 hours at the RT in 5% (m/v) bovine serum albumin (BSA) (Merck, Germany) in TBS (50 mM Tris/HCl, pH 7.5 and 150 mM NaCl). Then the membrane was incubated for 2 hours with shaking at the RT in the blocking solution supplemented with 2 µg/mL VaaSPH-1. After washing the membrane with TBS, it was incubated with the rabbit anti-Vaa serum, diluted 1:10,000, for 1 hour at the RT in TBS containing 5% (m/v) BSA and then with the horseradish peroxidase (HRP)-conjugated secondary anti-rabbit IgGs (diluted 1:10,000 in TBS with 5% (m/v) BSA) for 1 hour at the RT. PLs remaining on the silica plate were then detected by immersing the plate for 10 minutes in the mixture of 10 mL of methanol containing 30 mg of 4-chloro-1-naphol and 50 mL of 137 mM NaCl, 0.72 mM Na2HPO4, 25 mM Tris and 60 µL of H2O2 in water. Then the plate was sprayed with primulin solution (5 mg primulin in 100 mL 80% (v/v) acetone in water) to visualize PL spots. Processed PVDF membrane and silica plate were captured with G:BOX machine (Syngen, USA) and electronically overlaid to identify the nature of PL to which VaaSPH-1 bound.
Supplementary Fig. S3 Interaction of VaaSPH-1 with different coagulation factors by native PAGE. Each blood coagulation factor was mixed with VaaSPH-1 in the indicated molar ratio, and incubated at 37°C and pH 6.8 for 5 minutes. Samples were analysed by PAGE on continuous 10% (m/v) polyacrylamide gels under non-denaturing conditions to analyse whether VaaSPH-1 and a coagulation factor form the complex or not. Lanes in which only VaaSPH-1 or a coagulation factor was electrophoresed serve to define positions of non-complexed proteins. Appearance of an extra protein band upon analysis of the VaaSPH-1 and a coagulation factor mixture indicated the complexing of the two molecules. The gels were stained with PageBlue. In panel (A), complexing of VaaSPH-1 with FIX, FXa, FVII, FVIIa, FV a and prothrombin are demonstrated. (B) VaaSPH-1 did not associate with FXI, FXIa, kallikrein and, consistently with the negligible influence of VaaSPH-1 on TT, also thrombin.
Small unilamellar vesicles (SUVs) were obtained from MLVs with 30 minutes of sonication (pulse on and off for 15 seconds, amplitude 50%) and 45 minutes of heating at 40°C, followed by centrifugation at 6,000 rpm for 5 minutes. SUVs were prepared in buffer D (20 mM Tris, 140 mM NaCl, 5 mM CaCl₂, pH 7.4). This was also the buffer to perform experiments on the Biacore T-100 apparatus (GE Healthcare BioSciences AB, Sweden). SUVs were attached on the Biacore T-100 L1 chip in a flow cell within 10 minutes at a flow rate 2 µL/min. To prevent the non-specific binding, the chip was subsequently exposed for 2 minutes to 0.1 mg/mL BSA at a flow rate of 10 µL/min. Experiments with VaaSPH-1 in the flow cell 1 have been executed by applying the protein to the chip for 2 minutes at 10 µL/min at different concentrations, from 31.25 nM to 500 nM in case of SUVs containing PS and CH (molar ratio 1:1) and 62.5 nM to 1 µM in case of SUVs containing PI and CH (molar ratio 1:1). Regeneration of the L1 chip was accomplished in the flow cell using 40 mM octyl β-D-glucopyranoside until the baseline was reached. The chip in the reference flow cell 2 was used to record the background signal. It was treated in an identical manner as the chip in the flow cell 1, just without the addition of VaaSPH-1. The dissociation constant Kd was calculated using Biacore T-100 Evaluation Software.