#### Supplemental data

#### Methods.

### C3bB and CVFB assembly in the presence and absence of lufaxin, analysis by SPR

Surface plasmon resonance measurements were performed using a Biacore T200 instrument. C3b and CVF were immobilized in pH 5.0 sodium acetate on CM5 chip surfaces at levels of 300-3000 RU using amine coupling methodology. FB and lufaxin, either individually or together, were injected over the surfaces in 10 mM HEPES, pH 7.4, 150 mM NaCl (HBS) buffer containing either 5 mM MgCl<sub>2</sub> or no divalent cation. For gel filtration and SDS-PAGE the C3bB-lufaxin-fXa complex was formed by mixing 0.56 nmol human C3b, 0.6 nmol FB, 1 nmol lufaxin and 1 nmol fXa in HBS containing 5 mM MgCl<sub>2</sub>.

#### Assays of complement activation and thrombin generation

The mHam assay, conventional Ham assay, thrombinoscopy and thromboelastography were conducted as previously described<sup>1-4</sup>. All patients gave written informed consent. This study was approved by the institutional review board and conducted in accordance with the Declaration of Helsinki.

#### mHAM assay

TF1*PIGA*null cells were maintained at a density of 500,000 cells per milliliter daily. For the assay, the cells were seeded in a round-bottom 96-well plate at a density of 6700 cells per well in gelatin veronal buffer with Ca and Mg (GVB<sup>++</sup>, Complement Technology, Inc). Serum either with or without inhibitors was diluted in gelatin veronal buffer and added to the wells. Cells were washed as described previously<sup>5</sup> and incubated with the cell proliferation reagent 4-[3-(4-lodophenyl)-2-(4-nitrophenyl)-2H-5-

tetrazolio]-1.3-benzene disulfonate/WST-1 as previously described<sup>5</sup>. Absorbance was measured at 490 nm with a reference wavelength of 595 nm.

### Ham assay

Acidified NHS was added to erythrocytes (5 x 10<sup>7</sup> cells/well) from a healthy or PNH individual as described previously<sup>6</sup> and 0.5mM EDTA was added to acidified NHS as a negative control. Samples were treated as described previously and the absorbance at 405 nm was measured in supernatants in a 96-well flat bottom plate using a plate reader<sup>6</sup>. The sample absorbance value was normalized by subtracting the absorbance of a blank sample containing erythrocytes. Hemolysis in each sample was compared to erythrocyte lysis in water (100 % value). Percent hemolysis was calculated as previously described.<sup>6</sup>

### Crystallization and structure solution of lufaxin

Lufaxin was crystallized using the hanging drop vapor diffusion method from 100 mM HEPES, pH 7.0, 10 % PEG 6000. Crystals were soaked in the crystallization solution containing 15 % glycerol and flash cooled in liquid N<sub>2</sub> for X-ray diffraction. Crystals were prepared with 0.5 M potassium bromide added to the cryoprotectant solution of 100 mM HEPES, pH 7.0, 20 % PEG 6000. Diffraction data were collected at beamline 22-ID of the Southeast Regional Collaborative Access Team, APS, Argonne National Laboratory. Diffraction data were processed using XDS<sup>7</sup> and the structure of lufaxin was determined using single anomalous diffraction methods with the bromide-soaked crystals. Phasing using Phenix Autosol<sup>8</sup> initially produced a map that was not easily interpretable. Phase extension in Phenix Autobuild<sup>8</sup> using a native data set and incorporating non-crystallographic symmetry for two molecules in the asymmetric unit greatly improved the map and allowed for model building using Phenix Autobuild. The initial structure was then refined against the native data set and completed using iterative cycles of manual rebuilding using Coot <sup>9</sup> and refinement using Phenix Refine <sup>8</sup>.

### CryoEM sample preparation and data collection

The C3bB-lufaxin and C3bB-lufaxin-fXA complexes were prepared by mixing 0.57 nmol C3b, 0.57 nmol FB and 1.1 nmol of lufaxin in 1.0 mL HBS buffer containing 5 mM MgCl<sub>2</sub>. For the C3bB-lufaxin-fXa complex 1.1 nmol of fXa was added to the above mixture. The sample was then concentrated to give a total protein concentration of 1.2-1.5 mg/mL using Amicon-Ultra centrifuge concentrators.

To prepare grids of the C3bB-lufaxin and C3bB-lufaxin-fXa complexes, 3 μL of protein sample were applied to freshly glow-discharged (easiGLow) C-flat grids (Protochips, CF1.2/1.3-3Au). Blotting was done at 6 °C and 100 % humidity using a Vitrobot Mark IV (Thermo-Fisher), with a 2.5 s blotting time and 4 pN blotting force for C3bB-lufaxin and a 4 s blotting time and 5 pN blotting force for C3bB-lufaxin-FXa. Grids were vitrified by plunging into liquid ethane and stored in liquid nitrogen before examination by cryo-EM. Images were recorded on a Glacios TEM (Thermo Fisher) at 200 kV and recorded at 36,000X magnification for C3bB-lufaxin and 45,000X magnification for C3bB-lufaxin and 45,000X magnification for C3bB-lufaxin and ethace and store for C3bB-lufaxin-fXa, with a defocus range of - 0.3 to -2.2 μm on K3 direct electron detector (Gatan) in super-resolution mode.

## **Cryo-EM Image processing**

Movies of the C3bB-lufaxin and C3bB-lufaxin-fXa complexes were processed with MotionCor2<sup>10</sup>, during which dose weighting was applied and the pixel size was binned to 1.12 Å/pixel for C3bB-lufaxin and0.92 Å/pixel for C3bB-lufaxin-FXa. The CTF (contrast transfer function) was estimated in Ctffind4<sup>11</sup>. Particle picking was conducted in Gautomatch (<u>http://www.mrc-lmb.cam.ac.uk/kzhang/Gautomatch/</u>) using references generated with EMAN2<sup>12</sup> resulting in 1,547,893 particles for C3bB-lufaxin and 713,201 for C3bB-lufaxin-fXa. These were extracted in RELION-3.0.8 or 3.2<sup>13</sup> with a box size of 220 x 220 pixels for C3bB-lufaxin and 300 x 300 pixels for C3bB-lufaxin-fXa. The picked-particles were subjected to 2D classification in RELION-3.0.8 to remove bad particles (Fig. S1). A previously calculated map was used as an initial reference for 3D classification. The best class was selected for subsequent gold-standard refinement (FSC=0.143) in both RELION-3.0.8 and cisTEM <sup>14</sup> for C3bB-lufaxin and RELION 3.2 for C3bB-lufaxin-fXa (Fig. S1).

### Model building and refinement of C3bB and C3bB-lufaxin complexes

Models of C3bB in the closed and open conformations were built from components of the C3bB complex crystallized in the presence of nickel ion (PDB accession code 2XWJ, <sup>15</sup>), the C3bB-FD complex crystallized in the presence of magnesium ion (PDB accession code 2XWB, <sup>15</sup>), the crystal structure of free, wild-type FB (PDB accession code 2OK5, <sup>16</sup>) and the structure of lufaxin described above. Positioning was assisted by the Phenix dock-in-map application <sup>8</sup>. For the C3bB-lufaxin-fXa complex, the structure of fXa was added to the model using coordinates from PDB depositions 1HCG<sup>17</sup> and 1FAX<sup>18</sup>. Models were refined using real-space methods in Phenix combined with manual rebuilding using Coot <sup>9</sup>.

# Alphafold2 structure predictions.

For the prediction of C3bB-lufaxin and lufaxin-fXa complex structures, Alphafold 2.3.1 was run in multimer mode on the NIH Biowulf HPC cluster using sequences for the mature peptides of each component.

# Analysis of CVFB and CVFB-lufaxin complexes.

CVFB complexes were formed by mixing 0.6  $\mu$ M CVF and 1.1  $\mu$ M FB in 10 mM Hepes pH 7.4, 0.15 M NaCl (HBS) containing 5 mM MgCl<sub>2</sub>, NiCl<sub>2</sub> or no divalent cation. In experiments testing the role of lufaxin binding, the inhibitor was also added to a concentration of 1.2  $\mu$ M. After 20 min incubation the samples were injected onto a Superdex 200 increase (10/30) column and eluted with HBS containing the appropriate divalent cation. Fractions (0.5 mL) were collected, separated by SDS-PAGE gels (NuPage) in MES running buffer and stained using silver (Thermo Scientific).

### Lufaxin cleavage by FXa

Recombinant lufaxin containing a 6-His tag (1.9  $\mu$ M) was incubated with fXa (3.8  $\mu$ M) in the presence or absence of 5mM CaCl<sub>2</sub>, in Tris-buffered saline pH 7.4, at 30°C for 3 hours. Immediately after incubation sample was mixed with NuPage LDS sample buffer and NuPAGE sample reducing agent, heated for 10 minutes at 94°C, and loaded in 4-12% BisTris gels. Two identical gels were run in parallel at 160 V using MES Buffer. After running, one of the gels was stained with Coomassie R-250, while the proteins from the other were transferred to a nitrocellulose membrane using an iBlot device.

The nitrocellulose membranes were blocked with 1% blotting grade milk in Tris Buffer Saline pH 8.0 with 0.05% Tween 20 (TBS-T) overnight at 4°C. Membrane was then incubated with 6x-His Tag monoclonal antibody (Invitrogen # MA-1-21315) diluted 1:3,000 in blocking buffer, for 1 hour at room temperature. After three washes with TBS-T, membrane was incubated with secondary antibody conjugated to alkaline phosphatase (anti-mouse IgG AP conjugated, SIGMA #A3562) diluted 1:10,000 in blocking buffer. Finally, after three washes with TBS-T membrane was incubated with Western Blue® stabilized substrate for alkaline phosphatase (Promega #S3841).

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**Supplemental Figures** 



**Figure S1.** Cryo-EM of C3bB-lufaxin and C3bB-lufaxin-fXa in the presence of  $Mg^{2+}$  ion. **A** - 2D class averages from processing of C3bB-lufaxin images. **B** – FSC plot for C3bB-lufaxin after masking. **C** – Orientation plot for C3bB-lufaxin particle distribution. **D** – Workflow for processing of the C3bB-lufaxin data. **E** - 2D class averages from processing of C3bB-lufaxin-fXa images. **F** - FSC plot for C3bB-lufaxin-fXa after masking. **G** – Orientation plot for C3bB-lufaxin-fXa particle distribution. **H** – Workflow for processing of C3bB-lufaxin-fXa data.



**Figure S2.** Assembly of C3bB complex components and lufaxin on a C3bB SPR surface (3000 RU). SPR analysis of alternative C3 convertase assembly in the presence and absence of lufaxin on an immobilized C3b surface. Black- 57 nM FB, 500 nM lufaxin injected in the absence of divalent cation, purple- FB-lufaxin mixture injected in the presence of 5 mM Mg<sup>2+</sup>, blue-FB with 5 mM Mg<sup>2+</sup>, green- lufaxin with Mg<sup>2+</sup>, red – FB without divalent cation, brown – lufaxin without divalent cation. The green, red and brown lines run near zero on the Y-axis making them difficult to distinguish from one another. Apparent first order rate constants ( $k_{app}$ ) for the association phase are shown on the left side of the corresponding curve, and first order rate constants (k) for the dissociation phase are shown on the right side of the corresponding curve.



**Figure S3.** Structure of  $\alpha L/\alpha 7$  and the scissile bond region in the C3bB-lufaxin structure. **A** - Stereoview of map density covering helices  $\alpha L$  and  $\alpha 7$  of the vWA domain of FB contoured at 4.0 r.m.s.d. **B** - Stereoview of map density covering the scissile bond loop region of FB contoured at 4.0 r.m.s.d. Arg 234, the residue at the "P1" cleavage position of the SP domain is colored in red.



**Figure S4.** Stereoview of map density covering the interaction interface between lufaxin and the CCP3 domain of FB contoured at 2.5 r.m.s.d. Lufaxin residues are colored light blue and labeled in black, while FB residues are colored in cyan and labeled in cyan. Hydrogen bonds are shown as red dashed lines.

Majority	VXDDGDEYXLGKX-DNSDEELLYXTFDFKKDPCQKV-KXKCXNNXTHFILNYVXXKKXCISSIKVTSYPDIXQX							
	10	20	30	40	50	60	70	80
L_longipalpus L_ayacuchen L_olmeca P_ariasi P_perniciosus P_duboscqi P_papatasi	DGD NGD NGD 	EYFIGKYKEKD- EHFLGKYSQND- EHYLGKHVEPGS EYLLGKP-ANSI EYLLGKP-DNTI EYQIGKF-DNSI EYQIGKF-DNSI	ETLFFASYG EKLFFGTYG SERLFYAMYG DELLYSTFD DELLYSTFD DEQFLDTDFD DEFLDTDFD	LKRDPCQVQIVLGY UKKDSCQQVQHY VKKDSCQQVLNH FQRDPCSKS-YV FIKNTCANP-KM FIEKPY-KP-KL FIEKPY-KS-KM	KCSNNOTHFV RCFNNKTHFI RCFNNKTHFI KCTNNNTHFI KCTNNATHFV DSIYNGTHFI NSIYTGTHFI	'LNFKTNK <mark>K</mark> SC 'VNYHANKKFC 'LDFYDPKKRC 'LDFSDPKKRC 'LNYVGTPKTC 'LNYVGRP <mark>K</mark> TC	ISAIKLTSY ISTIKLTSY ISAIKLTTY ISSIHVFSY ISSIHVFST ISSITVQSY ISSITVLSY	PKINQN 70 PTINRD 70 PVISEH 72 PDRPPS 76 PDGPVN 76 EDDTQK 76 EDDTQK 77
Majority	XXXXXXPSX	IYCQKGGIGXNN	ICLLVFRKRX	RREXAXVEIFGI	PAXK-CSFKE	RYTGXDPKHX	DAYGLXYQF	DKE-DG
	+ 90	100	+ 110	120	130	140	+ 150	+ 160
L_longipalpus L_ayacuchen L_olmeca P_ariasi P_perniciosus P_duboscqi P_papatasi	SD-LTRN WS-SRLR STTLIRR FEEKRIP-SKSA LEEENKPRSKSS -TPTQNPKKKSK -KSTQNPKK-SK	LYCQTGGIGTDN FYCQKGGIGTNN FYCQKGGIGTNN IYCQKGGIGKSI IYCQVGGIGQS IWCQKGGIGKKI IWCQKGGIGKN	ICKLVFKKRK ICVLVFEKSK ICVLVVRKDS ICLLVFRKKE ICLLVFKKKE ICLLVFRMRT	RQ <mark>IA</mark> ANIEIYGI EQIAVGIEIYGI RHIAVNVEIFGI PREDALVDIRGI RREDALVDIRGL KREDARVKIFGV KRENARVKIFGV	PAKK-CSFKE PTKQ-CSFKE RSKK-CSLKQ PADQTCSLKE KTCSLKE RGPCSFKE KEPCSFKE	RYIGADPLHV RYIGNDPQHI GLIGSDPLHT RYTSGDPKKT RYTSGDPKKT RYLNKNPRQI RYLNKIPKQI	DSYGLSYQF DAYGLPYIF DIYGLPYEF DAYGMAYQF DAYGMAYKF DAYGQHYQY DAYGQRYQF	DQE-HG 142 DKE-DG 142 DKD-DG 145 DRK-DD 154 DKN-DN 152 SEDYPY 153 SKEYPN 153
Majority	WNXERTXIKXYK	RXXNEIFYXKNO	GLFNTQIXYL	AXXDKFTEAREL	VVKDNKKK	FXMDFSNXGQ	XRISFLDIY	WFQESM
Majority	WNXERTXIKXYK + 170	RXXNEIFYXKN0 180	LENTQIXYL	AXXDKFTEAREL  200	VVKDNKKK + 210	FXMDFSNXGQ 220	XRISFLDIY + 230	WFQESM + 240
Majority L_longipalpus L_ayacuchen L_olmeca P_ariasi P_perniciosus P_duboscqi P_papatasi	WNXERTXIKXYK 170 170 WNLERNNIFKDT WNSERIGVHKIK WSIERTKIPKYA WHIQRTGIKTWK WSIKREGVKQWK WNLPRTNLKQYN WNLPRANMKQYQ	RXXNE IFYXKN( 	SLFNTQIXYL 	AXXDKFTEAREL 200 AEEDSFSEARE I AEDDSFTEAREV AQDDTFVEATEL SKFDKYTVTREL SKFDKYTVTREM DESDKLTAVREL DKSDKFTMAREK	VVKDNKKK + TAKDIKKK SFKKINKKKK VGKAVQNKMK VVKNNAKK VVKHRAKK FVPDNTLK FVPDNTLK	FXMDFSNXGQ 220 FSILPNEEY FSLSISNEGK FSLKIPNKGK FTLEFSNFRQ FTMDFSNYGQ FVMDFKNSGQ	XRISFLDIY 230 + KRISFLDVY KRISFLDIY YRISFLDIY YRISFLDIY YRISFLDIY YRISFLDIY YRISFLDIY	WFQESM + WFQETM 220 WFQESM 222 WFQESM 225 WFQESV 230 WFQESV 230 WYQQSK 230 WYQQSE 230
Majority L_longipalpus L_ayacuchen L_olmeca P_ariasi P_perniciosus P_duboscqi P_papatasi Majority	WNXERTXIKXYK 170 170 WNLERNNIFKDT WNSERIGVHKIK WSIERTKIPKYA WHIQRTGIKTWK WSIKREGVKQWK WNLPRTNLKQYN WNLPRANMKQYQ KSKPKLPYIYYN	RXXNE IFYXKN( 	SLFNTQIXYL  SLFNTQ <sup>I</sup> TYL SLFNTQ <sup>V</sup> TYL SIFNTQMSYL SLMNHQIRYL SLFNIQKNYL SLFNVQRNYL SLFNVQENYL SLFNTDEPI	AXXDKFTEAREL 200 AEEDSFSEARE I AEDDSFSEARE I AQDDTFVEAIEL SKFDKYTVTREL SKFDKYTVTREL DKSDKFTVAREL DKSDKFTMAREK TYAFVKVFSNPD	VVKDNKKK 210 	FXMDFSNXGQ 220 FSIILPNEEY FSLSISNEGK FSVKIPNKGK FTLEFSNFRQ FTMDFSNYGQ FVMDFKNSGP FTMDFTNSGQ LGRG	XRISFLDIY 230 XRISFLDVY NRISFLDVY KRISFLDIY YRISFLDIY YRISFLDIY YRISFLDIY YRISFLDIY	WFQESM + WFQETM 220 WFQETM 225 WFQESQ 232 WFQESV 230 WYQQSK 230 WYQQSE 230
Majority L_longipalpus L_ayacuchen L_olmeca P_ariasi P_perniciosus P_duboscqi P_papatasi Majority	WNXERTXIKXYK 	RXXNE IFYXKN( 	SLFNTQIXYL 190 SLFNTQITYL SLFNTQVTYL SLFNTQMSYL SLMNHQIRYL SLFNVQRNYL SLFNVQRNYL QLVFDTDEPI 270	AXXDKFTEAREL 200 AEEDSFSEAREI AEDDSFTEAREV AQDDTFVEAIEL SKFDKYTVTREL SKFDKYTVTREL DKSDKFTMAREK TYAFVKVFSNPD 	VVKDNKKK 210 	FXMDFSNXGQ 220 FSILPNEEY FSLSISNEGK FSVKIPNKGK FTLEFSNFRQ FTMDFSNYGQ FTMDFKNSGP FTMDFTNSGQ LGRG- 300	XRISFLDIY 230 	WFQESM + WFQETM 220 WFQESM 222 WFQESQ 232 WFQESQ 232 WFQESV 230 WYQQSE 230 WYQQSE 230
Majority L_longipalpus L_ayacuchen L_olmeca P_ariasi P_berniciosus P_duboscqi P_papatasi Majority L_longipalpus L_ayacuchen L_olmeca P_ariasi P_perniciosus P_duboscqi	WNXERTXIKXYK 170 170 WNLERNNIFKDT WNSERIGVHKIK WSIERTKIPKYA WHIQRTGIKTWK WSIERTKIPKYA WHIQRTGIKTWK WNLPRINKQYQ KSKPKLPYIYYN KSKPKLPYIYYN KEKPKYPFIYFN KKKPKYPFIYFN KHKPKLPYIYYN KHKPKLPYIYYN	RXXNE I FYXKN( 	SLFNTQIXYL 190 SLFNTQUTYL SLFNTQVTYL SIFNTQMSYL SLFNTQRSYL SLFNTQRNYL SLFNTQRNYL SLFNTQRNYL 2LVFDTDEPI 270 CLVFDTDEIM SLIFDTDEQI ELIFDTDEQI ELIFDTDEQI ELIFDTDEPI QUFDDEPI QVFDDEPI QVFDDEPI	AXXDKFTEAREL 200 AEEDSFSEARE I AEDDSFTEAREV AQDDTFVEAIEL SKFDKYTVTREM DESDKLTAVREL DKSDKFTMAREK TYAFVKVFSNPD 280 TYALVKVFSNTG TYALVKVFSNTG TYALVKVFSNPD TYAFVKVFSNPD TYAFVKVFSNPD SYVFVKVFRNED	VVKDNKKK  210  TAKDIKKK SFKKINKKK VGKAVQNKMK VVKNNAKK VVKNNAKK VVKNNAKK VVKNNAKK VVKNNAKK VVKDNTLK PVPDNTLK PVPDNTLK 290  SDGSRLREKI VDGSRLREKI HNEPRLRHEI HNEPRLRHEI HNEPRLRHEI	FXMDFSNXGQ 220 FSILPNEEY FSILSNEGK FSVKIPNKGK FTMDFSNYGQ FTMDFSNYGQ FTMDFKNSGP FTMDFTNSGQ LGRG	XRISFLDIY 230 	WFQESM + WFQETM 220 WFQESM 222 WFQESQ 232 WFQESV 230 WFQQSK 230 WYQQSK 230 WYQQSE 230 284 284 280 284 280 284 290 288 290

**Figure S5**. Sequence alignment of lufaxin and orthologs from new- and old-world sand fly species. L\_longipalp: *Lutzomyia longipalpis* (lufaxin); L\_ayacuchen: *Lutzomyia ayacuchensis*; L\_olmeca: *Lutzomyia olmeca*; P\_ariasi: *Phlebotomus ariasi*; P\_pernicios: *Phlebotomus perniciosus*; P\_duboscqi: *Phlebotomus duboscqi*; P\_papatasi: *Phlebotomus papatasi*. N-terminal domain residues interacting (as indicated by PISA) with the Ba fragment are highlighted in cyan, those interacting with the vWA domain are highlighted in black and those interacting with the CUB domain are highlighted in green. In the C-terminal domain residues interacting with the SP domain of fXa are highlighted in yellow.

Majority	TPWSLARPQXSCSLEG	VEIKGGSFR	LLQXGQALI	EYVCPSGFYI	PYPVQTRTCRS	STGSWSXLKTX	DQKXVRKAEC	CRAIR
		+	+ 30	+	+	+	+	+
	++	+	+	+	+	+	+	+
human	TPWSLARPQGSCSLEG	VEIKGGSFR	LLQEGQALI	EYVCPSGFY	YPVQTRTCRS	STGSWSTLKT	DQKTVRKAE	CRAIH 77
mus	TPVLEARPQVSCSLEG	VEIKGGSFQ	LLQGGQALI	EYLCPSGFYI	PYPVQTRTCRS	STGSWSDLQTF	DQKIVQKAEC	CRAIR 77
elephas	TPLPAAQPRSLCSLEG	JIEITGGSFR	LLQKGQALI	EYVCPSGFYI	PYPVQTRVCRS	SWGSWSALQAF	RGEKIVKKAEC	CRAIR 77
notechis	TPWSLARPQGSCSLEG	VEIKGGSFR	LLQEGQALI	EYVCPSGFYI	PYPVQTRTCRS	STGSWSTLKTQ	DOKTVRKAEC	CRAIH 77
xenopus	VQCDLTF	VAIIGGSYT	VSDGGNVGSKVI	EYQCPKGKYI	PYPKYTRECQY	INGFWTDQKA-	KTIC	CKDVR 64
Majority	CPRPXDFENGEYWPRS	PYYNVSDXI	SFXCYDGYTLR	SANRTCOV	IGRWSGOTAIC	CONGAGYCPNE	GIPIGTRKVG	SOYR
	+	+	+	+	+	+	+	+
	90	100	110	120	130	140	150	160
h		+		+			+	+
numan	CPRPHDFENGEIWPRS	PIINVSDEL	SFHCIDGITLR	SANKTCOVI	ICRWSGQTAI		GIPIGTRKVG	SQIR 157
elephas	CPRPODEENGEEWPRA	AYYNLSDOT	SFOCYDGYTTR	SANRTCOV	GRWDGQIAIC	DNGAGYCPNE	CTPICTRKV	SOYB 157
notechis	CPRPHDFENGEYWPRS	SPYYNVSDEI	SFHCYDGYTLR	SANRTCOVI	IGRWSGOTAI	DNGAGYCSNE	GIPIGTRKVO	SSOYR 157
xenopus	CPRPVTFEDGDYEPRO	PFYKVGDTL	YFECYSGFTM <mark>K</mark> (	GPQNRTCQEN	VAKWTGETTIC	D <mark>NNGYCP</mark> NE	GIPIGASKS	SSSYK 144
			_					
Majority	LEDSVTYHCSRGLTLF	GSQRRTCQE	GGSWSGTEPSC	DSFMYDTPG	2EVAEAFLSSI	TETIEGXDAE	DGHXPGEQQI	KRKIV
	170	180	190	200	210	220	230	240
	+	+	+	+	+	+	+	+
human	LEDSVTYHCSRGLTLF	GSQRRTCQ <mark>E</mark>	GGSWSGTEPSC(	DSFMYDTPG	2EVAEAFLSSI	TETIEGVDAE	DGHGPGEQQI	RKIV 237
mus	LEDIVTYHCSRGLVLF	RGSQKRKCQ <mark>E</mark>	<mark>GGS</mark> WSGTEPSC(	DSFMYDSPG	2EVAEAFLSSI	LT <mark>BT</mark> IEGADAE	DGHSPGEQQH	RKIV 237
elephas	LEDRVTYYCNRGLTLF	RGSQQRTCL <mark>E</mark>	GGS WSGTEPSC(	QDSFMYDTPG	2EVAEAFLSSI	LT DT IEGADAE	DGHSPGEQQH	RKIV 237
notechis	LEDSVTYHCSRGLTLF	RGSQRRTCQE	GGSWSGTEPSC	2DSFMYDTPG	2EVAEAFLSSI	TEGVDAE	EDGHGPGEQQH	KRKIV 237
xenopus	MENKVSYNCQQGLVME	GSKERECL <mark>E</mark>	DKSWSGTEPSCI	KQWYTYDTPI	LEVAKTE'SSSN	ILISN VDTTNLE	DRSI	DRSVR 218
Majority	LDPSGSMNIYLVLDGS	DSIGASNET	GAKXCLXNLIE	<b>KVASYGVKP</b> I	RYGLVTYATYI	KIXVXVSDXI	SSNADWVTEG	<b>ZINEI</b>
Majority	LDPSGSMNIYLVLDGS	DSIGASNFT	GAKXCLXNLIE	KVASYGVKPI	NYGLVTYATYI	KIXVXVSDXI	SSNADWVTEG	OTNEI
Majority	LDPSGSMNIYLVLDGS  250	DSIGASNFT( + 260	GAKXCLXNLIER	KVASYGVKP + 280	YGLVTYATY + 290	RIXVXVSDXI + 300	SSNADWVTEG + 310	QLNEI + 320
Majority	LDPSGSMNIYLVLDGS 250	260	GAKXCLXNLIEI 270 +	XVASYGVKP +	<b>RYGLVTYATY</b> 290	PKIXVXVSDXI 300	SSNADWVTEG + 310 +	2LNEI + 320 +
Majority human	LDPSGSMNIYLVLDGS 250 	DSIGASNFT 260 DSIGASNFT SDSIGASNFT	GAKXCLXNLIEN 270 GAKKCLVNLIEN CAKBCLVNLIEN	XVASYGVKP 280 + XVASYGVKP	RYGLVTYATYI 290 RYGLVTYATYI RYGLUTYATYI	PKIXVXVSDXI 300 PKIWVKVSEAL	DSSNADWVTEG + 310 + DSSNADWVTKG RSSDADWVTKG	QLNEI + 320 + QLNEI 317 KINOI 317
Majority human mus elephas	LDPSGSMNIYLVLDGS 250 	SDSIGASNFT 260 SDSIGASNFT SDSIGSSNFT SDSIGSSNFT	GAKXCLXNLIE 270 GAKKCLVNLIE GAKKCLVNLIE GAKRCLTNLIE GAKRCLTSLIE	KVASYGVKPF + 280 + KVASYGVKPF KVASYGVRPF KVASYGVKPF	YGLVTYATYI 290 XYGLVTYATYI YGLLTYATVI YALVTYATDI	PKIXVXVSDXI 300 PKIWVKVSEAL PKIWVKVSEAL PKVLVRVSDEF PNILVRVSDEF	DSSNADWVTEC 310 DSSNADWVTKC RSSDADWVTER	2LNEI 320 + 2LNEI 317 KLNQI 317 DLNKI 317
Majority human mus elephas notechis	LDPSGSMNIYLVLDGS 250 	DSIGASNFT 260 DSIGASNFT DSIGSSNFT DSIGSSNFT DSIGASNFT	GAKXCLXNLIEJ 270 GAKKCLVNLIEJ GAKKCLVNLIEJ GAKRCLTNLIEJ GAKKCLVNLIEJ	KVASYGVKP 280 280 KVASYGVKP KVASYGVRP KVASYGVRP KVASYGVKP	RYGLVTYATYI 290 RYGLVTYATYI RYGLLTYATVI RYGLLTYATVI RYGLVTYATJI RYGLVTYATYI	PKIXVXVSDXI 300 PKIWVKVSEAI PKULVRVSDEF PNILVRVSDEF PKIWVKVSEAI	DSSNADWVTEG 310 DSSNADWVTKG RSSDADWVTEG RSSNADWVTEG DSSNADWVTKG	2LNEI + 320 + 2LNEI 317 KLNQI 317 2LNKI 317 2LNEI 317
Majority human mus elephas notechis xenopus	LDPSGSMNIYLVLDGS 250 LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS	SDSIGASNFT 260 SDSIGASNFT SDSIGSSNFT SDSIGASNFT SDSIGASNFT SDSIGASNFT	GAKXCLXNLIEI 270 GAKKCLVNLIEI GAKKCLTNLIEI GAKRCLTSLIEI GAKKCLVNLIEI EAKSASILFIEI	KVASYGVKP 280 + KVASYGVKP KVASYGVRP KVASYGVRP KVASYGVKP KMSNYDIKP	AYGLVTYATYI 290 AYGLVTYATYI AYGLLTYATYI AYGLUTYATYI AYGLVTYATYI AYCIISYASKA	PKIXVXVSDXI 300 PKIWVKVSEAI PKULVRVSDEF PKULVRVSDEF PKILVRVSDEF PKIWVKVSEAI AISVVSLRDPI	DSSNADWVTEG 310 	2LNEI + 2LNEI 317 KLNQI 317 2LNKI 317 2LNKI 317 2LNEI 317 HLEEF 298
Majority human mus elephas notechis xenopus	LDPSGSMNIYLVLDGS 250 	DSIGASNFT 260 DSIGASNFT DSIGSSNFT DSIGASNFT SDSIGASNFT SDSIGASNFT	GAKXCLXNLIEI 270 GAKKCLVNLIEI GAKKCLTNLIEI GAKRCLTSLIEI GAKKCLVNLIEI EAKSASILFIEI	XVASYGVKP 280 XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP	YGLVTYATYI 290 YGLVTYATYI YGLLTYATVI XYGLUTYATDB XYGLVTYATYI RYCIISYASKA	PKIXVXVSDXI 300 PKIVVKVSEAI PKILVRVSDEF PKILVRVSDEF PKILVRVSDEF PKILVRVSDEF PKILVRVSLRDPI	DSSNADWVTEG 310 	2LNEI + 2LNEI 317 (LNQI 317 2LNKI 317 2LNKI 317 4LEEF 298
Majority human mus elephas notechis xenopus Majority	LDPSGSMNIYLVLDGS 250 LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS XYEDHKLKSGTNTKKZ	DSIGASNFT 260 250SIGASNFT DSIGASNFT DSIGASNFT SDSIGASNFT SSIGASNFT SKSVGQNRFD	3AKXCLXNLIEI 270 3AKKCLVNLIEI 3AKRCLTNLIEI GAKKCLVNLIEI GAKKCLVNLIEI EAKSASILFIEI MMSWPXDVP1	XVASYGVKP 280 XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XMSNYDIKP PEGWNRTRHY	XYGLVTYATYI 290 XYGLVTYATYI XYGLLTYATVI XYGLLTYATVI XYGLVTYATYI XYCIISYASKA VIIIMTDGLHY	PKIXVXVSDXI 300 PKIWVKVSEAI PKIWVKVSEAI PKILVRVSDPF PKIWVKVSEAI AISVVSLRDPI MGGDPXTVII	DSSNADWVTEG 310 +	2LNEI + 320 + 2LNEI 317 XLNQI 317 2LNKI 317 2LNEI 317 1LEEF 298 XDRKN
Majority human mus elephas notechis xenopus Majority	LDPSGSMNIYLVLDGS 250 LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS XYEDHKLKSGTNTKKZ 330	DSIGASNFT 260 DSIGASNFT DSIGSSNFT DSIGASNFT SDSIGASNFT	GAKXCLXNLIEI 270 GAKKCLVNLIEI GAKKCLTNLIEI GAKKCLTNLIEI GAKKCLVNLIEI EAKSASILFIEI MMSWPXDVP1 	XVASYGVKP 280 XVASYGVKP XVASYGV XVASY	XYGLVTYATYI 290 XYGLVTYATYI XYGLLTYATVI XYGLLTYATVI XYGLVTYATJI XYGLVTYATYI XYCIISYASKA /IILMTDGLHN 	PKIXVXVSDXI 300 KIWVKVSEAI PKIWVKVSEAI PKIWVKVSEAI AISVVSLRDPI MGGDPXTVII 380	DSSNADWVTEG 310 DSSNADWVTKG RSDADWVTEG SSNADWVTEG DSSNADWVTEG DSSNADWVTKG DSNNADAVMEH DEIRDLLDIG3 	2LNEI + 320 + 2LNEI 317 XLNQI 317 2LNEI 317 2LNEI 317 HLEEF 298 XDRKN + 400
Majority human mus elephas notechis xenopus Majority	LDPSGSMNIYLVLDGS 250 LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS LDPSGSMNIYLVLDGS XYEDHKLKSGTNTKKZ 330	DSIGASNFT 260 DSIGASNFT DSIGSSNFT DSIGASNFT SDSIGASNFT SSUGASNFT SSUGASNFT SSUGASNFT SAUGANYS	GAKXCLXNLIEI 270 GAKKCLVNLIEI GAKKCLVNLIEI GAKKCLVNLIEI GAKKCLVNLIEI EAKSASILFIEI MMSWPXDVPI 	XVASYGVKP 280 XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XMSNYDIKP PEGWNRTRH 2EGWNRTRH 360	XYGLVTYATYI 290 XYGLVTYATYI XYGLLTYATVI XYGLUTYATVI XYGLUTYATYI XYCIISYASKA VIILMTDGLHN 370	PKIXVXVSDXI 300 PKIWVKVSEAL PKIWVKVSEAL PKIWVKVSEAL AISVVSLRDPI MGGDPXTVII 380	DSSNADWVTEG 310 DSSNADWVTKG RSSDADWVTEG DSSNADWVTEG DSSNADWVTEG DSSNADWVTKG DSNNADAVMEH DEIRDLLDIG3 	2LNEI + 2LNEI 317 CLNQI 317 2LNKI 317 2LNEI 317 HLEEF 298 KDRKN + 400 +
Majority human mus elephas notechis xenopus Majority human	LDPSGSMNTYLVLDGS 250 LDPSGSMNTYLVLDGS LDPSGSMNTYLVLDGS LDPSGSMNTYLVLDGS LDPSGSMNTYLVLDGS LDPSGSMNTYLVLDGS LDPSGSMNTYLVLDGS XYEDHKLKSGTNTKKF 330	SDSIGASNFT 260 SDSIGASNFT SDSIGAS	GAKXCLXNLIEI 270 GAKKCLVNLIEI GAKKCLVNLIEI GAKKCLVNLIEI GAKKCLVNLIEI EAKSASILFIEI MMSWPXDVPI 350 +	XVASYGVKP 280 XVASYGVKP XVASYGV XVASYGV XVASYGV XVASYGV XVASYGV XVASYGV XVASYGV XVASYGV XVASYGV XVASYG XVASY	XYGLVTYATYI 290 XYGLVTYATYI XYGLLTYATVI XYGLUTYATVI XYGLUTYATYI XYCIISYASKZ //IILMTDGLHN 370 +	PKIXVXVSDXI 300 PKIWVKVSEAL PKIWVKVSEAL PKIWVKVSEAL AISVVSLRDPI MGGDPXTVII 380 +	DSSNADWVTEG 310 	2LNEI 320 + 2LNEI 317 XLNQI 317 2LNEI 317 2LNEI 317 HLEEF 298 XDRKN + 400 + XDRKN 392
Majority human mus elephas notechis xenopus Majority human mus	LDPSGSMNTYLVLDGS 250 	260 260 250SIGASNFT 3D	3AKXCLXNLIEI 270 3AKKCLVNLIEI 3AKKCLTNLIEI GAKKCLVNLIEI EAKSASILFIEI MMSWPXDVPI 	XVASYGVKPI 280 XVASYGVKPI XVASYGVKPI XVASYGVKPI XVASYGVKPI XVASYGVKPI XMSNYDIKPI PEGWNRTRHY PEGWNRTRHY PEGWNRTRHY	XYGLVTYATYI 290 XYGLVTYATYI XYGLUTYATVI XYGLUTYATVI XYGLUTYATYI XYCIISYASKA //IILMTDGLHN //IILMTDGLHN //IILMTDGLHN	PKIXVXVSDXI 300 PKIVVKVSEAI PKILVRVSDEF PKILVRVSDEF PKILVRVSDEF PKIVVKVSEAI AISVVSLRDPI MGGDPXTVII 380 MGGDPITVII MGGDPITVII	DSSNADWVTEG 310 	2LNEI 320 + 2LNEI 317 XLNQI 317 2LNEI 317 2LNEI 317 ALEEF 298 CORKN + 400 + CORKN 392 200 200 200 200 200 200 200 2
Majority human mus elephas notechis xenopus Majority human mus elephas	LDPSGSMNTYLVLDGS 250 	DSIGASNFT 260 DSIGASNFT DSIGASNFT DSIGASNFT SDSIGASNFT SDSIGASNFT SDSIGASNFT 305IGASNFT	3AKXCLXNLIEI 270 3AKKCLVNLIEI 3AKRCLTNLIEI GAKKCLVNLIEI EAKSASILFIEI MMSWPXDVPI 	XVASYGVKP 280 XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XXXASYGVKP XXXASYGVKP XXXASYGVKP XXXXXX XXXX XXXXX XXXXXXXXXXXXX XXXXXX	XYGLVTYATYI 290 XYGLVTYATYI XYGLVTYATYI XYGLVTYATYI XYGLVTYATYI XYCIISYASK //IILMTDGLHN //IILMTDGLHN //IILMTDGLHN //IILMTDGLHN	PKIXVXVSDXI 300 PKIVVKVSEAI PKIVVKVSEAI PKILVRVSDEF PKILVRVSDEF PKIVVKVSEAI AISVVSLRDPI RMGGDPXTVII 380 MGGDPITVII MGGDPVTVIQ MGGDPVTVIQ	DSSNADWVTEG 310 	2LNEI 320 + 2LNEI 317 (LNQI 317 2LNEI 317 2LNEI 317 2LNEI 317 HLEEF 298 CORKN + 400 + 400 + CORKN 392 CORKN CORKN CORKN CORKN CORKN CORKN CORKN CORKN CORKN CORKN CORKN CORKN CORKN CORKN C
Majority human mus elephas notechis xenopus Majority human mus elephas notechis xenopus	LDPSGSMNTYLVLDGS 250 	DSIGASNFT 260 DSIGASNFT DSIGSSNFT DSIGASNFT SDSIGASNFT	GAKXCLXNLIEI 270 GAKKCLVNLIEI GAKKCLTNLIEI GAKKCLTSLIEI GAKKCLVNLIEI EAKSASILFIEI MMSWPXDVPI MSWPDDVPI MSWPDDVPI MMSWPQDTPI MMSWPDDVPI MMSWPDDVPI MMSWPDDVPI	XVASYGVKP 280 XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XXASYGVK XXXASYGVK XXXX XXXX XXXXX XXXX XXXXXX XXXXXXXXX	YGLVTYATYI 290 YGLVTYATYI YGLLTYATVI YGLUTYATVI XYGLUTYATYI XYCIISYASK 7111LMTDGLHN 7111LMTDGLHN 7111LMTDGLHN 7111LMTDGLHN 7111LMTDGLHN	PKIXVXVSDXI 300 PKIVVKVSEAI PKIVVKVSEAI PKILVRVSDEF PKILVRVSDEF PKIVVKVSEAI AISVVSLRDPI PKIWVKVSEAI AISVVSLRDPI PKIGOPPTVII MGGDPITVII MGGDPITVII MGGDPVTVIQ MGGDPFEFM	DSSNADWVTEG 310 	2LNEI + 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 4LEEF 298 4DRKN + 400  400 + 400 + 400 + 400  400  400  400  400     
Majority human mus elephas notechis xenopus Majority human mus elephas notechis xenopus	LDPSGSMNTYLVLDGS 250 	DSIGASNFT 260 DSIGASNFT DSIGASNFT DSIGASNFT SDSIGASNFT	3AKXCLXNLIEI 270 3AKKCLVNLIEI 3AKRCLTNLIEI GAKKCLVNLIEI GAKKCLVNLIEI EAKSASILFIEI MMSWPXDVPI 	XVASYGVKP 280 XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVR XXX XXX XXX XXXX XXX XXXX XXX	XYGLVTYATYI 290 XYGLVTYATYI XYGLVTYATYI XYGLVTYATYI XYGLVTYATYI XYGLVTYATYI XYGLUTYATYI XY	PKIXVXVSDXI 300 PKIWVKVSEAI PKIWVKVSEAI PKILVRVSDEF PKILVRVSDEF PKILVRVSDEF PKIWVKVSEAI AISVVSLRDPI PKIWVKVSEAI AISVVSLRDPI PKIGDPXTVII MGGDPXTVII MGGDPITVII MGGDPVFVIC MGGDPITVII MGGDPTVII	DSSNADWVTEG 310 	2LNEI 320 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 4LEEF 298 4DRKN 400 + + + + + + + 
Majority human mus elephas notechis xenopus Majority human mus elephas notechis xenopus Majority	LDPSGSMNIYLVLDGS 250 PSGSMNIYLVLDGS PSGSMNIYLVLDGS PSGSMNIYLVLDGS PSGSMNIYLVLDGS PSGSMNIYLVLDGS PSGSMNIYLVLDGS VEDHKLKSGTNTKKA SYEDHKLKSGTNTKKA SYEDHKLKSGTNTKKA QYDRHEDKQGTNTRAA PREDYLDVYVFGVGPI	DSIGASNFT 260 DSIGASNFT DSIGASNFT SDSIGASNFT SDSIGASNFT SKSVGQNRFD SKSVGQNRFD SLQAVYS	GAKXCLXNLIEI 270 GAKKCLVNLIEI GAKRCLTNLIEI GAKRCLTNLIEI GAKKCLVNLIEI EAKSASILFIEI MMSWPXDVPI 	XVASYGVKP 280 XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVKP XVASYGVRTR XVASYGVRTR XEGFMKIHN XVKDMENLEI	XYGLVTYATYI 290 XYGLVTYATYI XYGLUTYATYI XYGLVTYATYI XYGLVTYATYI XYGLVTYATYI XYGLUTYATYI XY	PKIXVXVSDXI 300 PKIWVKVSEAI PKIVVRVSDEI PKIWVKVSEAI AISVVSLRDPI MGGDPXTVII MGGDPXTVII MGGDPITVII MGGDPVTVIQ MGGDPVTVIQ MGGDPITVII MGGDPITVII MGGDPIEEMF 2SLSLCGMVWB	DSSNADWVTEQ 310 DSSNADWVTEK SSDADWVTEK SSDADWVTEK SSNADWVTEK DSSNA	2LNEI 320 + 2LNEI 317 (LNQI 317 2LNEI 317 2LNEI 317 2LNEI 317 1LEEF 298 CORKN + 400 + 400 + 400 CORKN 392 CORKN 392 CORKN 392 CORKN 392 CORKN 378 
Majority human mus elephas notechis xenopus Majority human mus elephas notechis xenopus Majority	LDPSGSMNIYLVLDGS 250 PSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS XYEDHKLKSGTNTKKA 330 NYEDHKLKSGTNTKKA SYEDHKLKSGTNTKKA QYDRHEDKQGTNTRAA PREDYLDVYVFGVGPI	DSIGASNFT 260 DSIGASNFT DSIGASNFT SDSIGASNFT SKSVGQNRFD LQAVYS	GAKXCLXNLIEI 270 GAKKCLVNLIEI GAKRCLTNLIEI GAKRCLTSLIEI GAKRCLVNLIEI EAKSASILFIEI MMSWPXDVPI 	XVASYGVKPI 280 XVASYGVKPI XVASYGVKPI XVASYGVKPI XVASYGVKPI XVASYGVKPI XVASYGVKPI XVASYGVKPI XVASYGVRPI XVASYGVRTRI PEGWNRTRHY PEGWNRTRHY PEGWNRTRHY YCGWNRTRHY XXKDMENLEI	XYGLVTYATYI 290 XYGLUTYATYI XYGLUTYATYI XYGLUTYATYI XYGLVTYATDI XYGLVTYATDI XYGLVTYATDI XYGLUTYATYI XYGUTYATYI XYGU	PKIXVXVSDXI 300 PKIWVKVSEAI AISVVSLRDPI PKIWVKVSEAI AISVVSLRDPI MGGDPXTVII MGGDPYTVIQ MGGDPYTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ	DSSNADWVTEG 310 DSSNADWVTEG SSDADWVTEG SSDADWVTEG DSSN	2LNEI 320 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 1LEEF 298 CORKN + 400 CORKN 392 CORKN 378 CORKN 378 CO
Majority human mus elephas notechis xenopus Majority human mus elephas notechis xenopus Majority	LDPSGSMNIYLVLDGS 250 PSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS XYEDHKLKSGTNTKKZ SYEDHKLKSGTNTKKZ SYEDHKLKSGTNTKKZ QYDRHEDKQGTNTRAZ PREDYLDVYVFGVGPI 410	DSIGASNFT 260 DSIGASNFT DSIGASNFT SDSIGASNFT SUSIGASNFT	GAKXCLXNLIEI 270 GAKKCLVNLIEI GAKRCLTNLIEI GAKRCLTSLIEI GAKKCLVNLIEI EAKSASILFIEI MMSWPXDVPI 	XVASYGVKP 280 XVASYGVKP XVASYG	AYGLVTYATYI 290 AYGLUTYATYI AYGLUTYATYI AYGLUTYATYI AYGLVTYATYI AYGLVTYATYI AYGLUTYATYI AYGLUTYATYI AYGLISYASKA AYGLISYASKA ATILMTDGLHN ATILMTDGLN ATILMTDGLN ATILMTDGLN ATILMTDGLN ATILMTDGLN ATILMTDGLN ATILMTDGLN ATILMT ATILMTDGLN ATILMT	PKIXVXVSDXI 300 PKIWVKVSEAI PKIVVRVSDEF PKIWVKVSEAI AISVVSLRDPI MGGDPXTVII MGGDPXTVII MGGDPITVII MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPITVII MGGDPREEMF QSLSLCGMVWE 460	DSSNADWVTEG 310 DSSNADWVTKG RSSDADWVTEH RSSNADWVTEG DSSNADWVTEG	2LNEI 320 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 317 2LNEI 317 317 2LNEI 317 317 2LNEI 317 317 2LNEI 317 317 2LNEI 317 317 2LNEI 317 317 400 317 317 317 400 317 317 400 317 317 400 317 317 400 317 317 400 317 400 317 400 317 400 317 400 317 400 317 400 317 400 317 400 317 400 317 400 317 400 317 400 317 317 400 400 400 400 400 400 400 40
Majority human mus elephas notechis xenopus Majority Majority Majority	LDPSGSMNIYLVLDGS 250 PSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS XYEDHKLKSGTNTKKZ SYEDHKLKSGTNTKKZ SYEDHKLKSGTNTKKZ QYDRHEDKQGTNTRAZ PREDYLDVYVFGVGPI 410 PREDYLDVYVFGVGPI	DSIGASNFT 260 DSIGASNFT DSIGASNFT SDSIGASNFT SUSIGASNFT	GAKXCLXNLIEI 270 GAKKCLVNLIEI GAKRCLTNLIEI GAKRCLTSLIEI GAKRCLTSLIEI EAKSASILFIEI MMSWPXDVPI 	XVASYGVKP 280 XVASYGVKP XVASYGVK XVASYGV	AYGLVTYATYI 290 AYGLUTYATYI AYGLUTYATYI AYGLUTYATYI AYGLVTYATYI AYGLVTYATYI AYGLUTYATYI AYGLUTYATYI AYGLISYASKA AY	PKIXVXVSDXI 300 PKIWVKVSEAI NILVRVSDEF PKIWVKVSEAI NISVVSLRDPI MGGDPXTVII MGGDPXTVII MGGDPITVII MGGDPVTVIQ MGGDPITVII MGGDPVTVIQ MGGDPITVII MGGDPTVIQ MGGDPITVII MGGDPTVIQ MGGDPITVII MGGDPTVIQ 25LSLCGMVWE 460 	DS SNADWVTEG 310 SSNADWVTKG SSDADWVTEH SSSNADWVTEG DS SNADWVTEG DS SNADWVTEG SSNADWVTEG DS SNADWVTEG SSNADWVTEG DS SNADWVTEG SSNADWTEG SS	2LNEI 320 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 317 2LNEI 317 317 2LNEI 317 317 2LNEI 317 317 2LNEI 317 317 400 392 200 200 200 200 200 200 200 2
Majority human mus elephas notechis xenopus Majority Majority Majority human mus	LDPSGSMNIYLVLDGS 250 PSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS SYEDHKLKSGTNTKKZ SYEDHKLKSGTNTKKZ SYEDHKLKSGTNTKKZ SYEDHKLKSGTNTKKZ QYDRHEDKQGTNTRAZ PREDYLDVYVFGVGPI 	DSIGASNFT 260 DSIGASNFT DSIGASNFT SDSIGASNFT SUSIGASNFT	GAKXCLXNLIEI 270 GAKKCLVNLIEI GAKRCLTNLIEI GAKRCLTSLIEI GAKRCLTSLIEI GAKKCLVNLIEI MMSWPXDVPI 	XVASYGVKP 280 XVASYGVKP XVASYG	AYGLVTYATYI 290 AYGLUTYATYI AYGLUTYATYI AYGLUTYATYI AYGLUTYATYI AYGLUTYATYI AYGLUTYATYI AYGLISYASKA AY	PKIXVXVSDXI 300 PKIWVKVSEAI PKIWVKVSEAI NISVVSLRDPI MGGDPXTVII 380 MGGDPITVII MGGDPITVII MGGDPITVII MGGDPITVII MGGDPITVII MGGDPITVII 2SLSLCGMVWE 2SLSLCGMVWE CSLSLCGMVWE	DS SNADWVTEG 310 SSNADWVTKG SSDADWVTEH RSSNADWVTEG DS SNADWVTEG DS SNADWVTEG SSNADWVTEG DS SNADWVTEG SSNADWVTEG DS SNADWVTEG SSNADWVTEG	2LNEI 320 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2DRKN 392 2DRKN 30 2DRKN 30 2DRK
Majority human mus elephas notechis xenopus Majority Majority Majority human mus elephas	LDPSGSMNIYLVLDGS 250 PSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS XYEDHKLKSGTNTKKA SYEDHKLKSGTNTKKA SYEDHKLKSGTNTKKA QYDRHEDKQGTNTRAA PREDYLDVYVFGVGPI PREDYLDVYVFGVGPI PREDYLDVYVFGVGPI PREDYLDVYVFGVGPI PREDYLDVYVFGVGPI PREDYLDVYVFGVGPI PREDYLDVYVFGVGPI PREDYLDVYVFGVGPI PREDYLDVYVFGVGPI	DSIGASNFT 260 DSIGASNFT DSIGASNFT SDSIGASNFT SDSIGASNFT SKSVGQNRFD SLQAVYS	GAKXCLXNLIEI 270 GAKKCLVNLIEI GAKRCLTNLIEI GAKRCLTSLIEI GAKRCLTSLIEI EAKSASILFIEI MMSWPXDVPI 	XVASYGVKP 280 XVASYGVKP XVASYG	XYGLVTYATYI 290 XYGLUTYATYI XYGLUTYATYI XYGLUTYATYI XYGLVTYATYI XYGLUTYATYI XYGLUTYATYI XYGLISYASKA /IIIMTDGLHN /IIIMTDGLHN /IIIMTDGLHN /IIIMTDGLHN /IIIMTDGLHN /IIIMTDGLHN /IIIMTDGLHN /IIIMTDGLHN /IIIMTDGLHN /IIIMTDGLHN /IIIMTDGLHN /IILMTDGLHN	PKIXVXVSDXI 300 PKIWVKVSEAI ALSVVSLRDPI PKIWVKVSEAI ALSVVSLRDPI MGGDPXTVII MGGDPXTVII MGGDPITVII MGGDPVTVIQ MGGDPITVII MGGDPVTVIQ MGGDPITVII MGGDPTVIQ SLSLCGMVWE SLSLCGMVWE SLSLCGMVWE SLSLCGMVWE SLSLCGMVWE	DS SNADWVTEG 310 SSNADWVTEG SSNADWVTEG SSNADWVTEG SSNADWVTEG DS SNADWVTEG DS SNADWVTEG SSNADWVTEG DS SNADWVTEG SSNADWVTEG DS SNADWVTEG S	2LNEI 320 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 317 2LNEI 317 317 317 400 392 200 200 200 200 200 200 200 2
Majority human mus elephas notechis xenopus Majority Majority Majority human mus elephas notechis	LDPSGSMNIYLVLDGS 250 PSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS DPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS IDPSGSMNIYLVLDGS XYEDHKLKSGTNTKKA 330 	DSIGASNFT 260 DSIGASNFT DSIGASNFT SDSIGASNFT SDSIGASNFT SKSVGQNRFD LQAVYS JLQAVYS LQAVYS LLQAVYS LLQAVYS LLQAVYS LLQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS LQAVYS	GAKXCLXNLIEI 270 GAKKCLVNLIEI GAKRCLTSLIEI GAKRCLTSLIEI GAKRCLTSLIEI EAKSASILFIEI MMSWPXDVPI 	XVASYGVKP 280 280 XVASYGVKP X	XYGLVTYATYI 290 XYGLUTYATYI XYGLUTYATYI XYGLUTYATYI XYGLVTYATYI XYGLVTYATYI XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XYGLYTY XY	PKIXVXVSDXI 300 PKIWVKVSEAI AISVVSLRDPI PKIWVKVSEAI AISVVSLRDPI MGGDPXTVII MGGDPXTVII MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ MGGDPVTVIQ SLSLCGMVWE SLSLCGMVWE SLSLCGMVWE SLSLCGMVWE SLSLCGMVWE	DS SNADWVTEG 310 SSNADWVTEG SSDADWVTEH RSSDADWVTEG DS SNADWVTEG DS SNADWVTEG SSNADWVTEG DS SNADWVTEG SSNADWVTEG DS SNADWVTEG SSNADWVTEG	2LNEI 320 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 2LNEI 317 1LEEF 298 2DRKN 392 2DRKN 30 2DRKN 30

**Figure S6**. Amino alignment of FB (minus the SP domain) from vertebrate species representing mammals (human, *Mus* and *Elephas*), reptiles (*Notechis*) and amphibians (*Xenopus*). Residues in human FB from the Ba fragment (cyan) vWA domain (black) that interact with lufaxin are highlighted along with corresponding residues in other species.

Majority	VAXPYEIX	VMKDFFIDLR	LPYSVVRNEQ	VEIRAVLYNY	R-NEXIKVRV	ELLHNPAFCS	XSTAKQRYRQX	XTIPXLSSRA	V
		810	820 -+	830	840 -+	850 -+	860 -+	870	+ 880 +
human mus thamnophis xenopus cobra_ven_fact	VADPFEVI VADPYEIR VAEPYEII VGQPYEIK VAEPYEIR	VMQDFFIDLR VMQDFFIDLR VMKDFFIDLR VMKDFFIDLK VMKVFFIDLQ	LPYSVVRNEQ LPYSVVRNEQ VPYSVVKNEQ LPYSVVRNEQ MPYSVVKNEQ	VEIRAVLYNY VEIRAVLFNY VEIRAVLYNY VEIRAILYNY VEIRAILHNY	RQNQELKVRV REQEELKVRV A-NNDIYVRV R-NDRIKVRV V-NEDIYVRV	ELLHNPAFCS ELLHNPAFCS ELLYNPAFCS ELTHNPEFCS ELLYNPAFCS	LATTKRRHQQI MATAKNRYFQI ASTETQRYREÇ LSTAKKRYRQI ASTKGQRYRQÇ	VTIPPKSSLS IKIPPKSSVA FTIPALSSRA VWIGGLSSTA FPIKALSSRA	V 874 V 872 V 865 V 872 V 872 V 858
Majority	PXVIVPLK	QGLHDVEVKA	SVXGHFI	SDGVRKKLKV	VPEGMRXNXX	VXIVTLDPEX	KGVGGVQXEDV	PAAXLXDQVP	D +
		890 -+	900 -+	910 -+	920 970-+	930 -+	940 940-+	950 +	960 +
human mus thamnophis xenopus cobra_ven_fact	PYVIVPLK PYVIVPLK PFVIVPLQ PIVIVPLT PFVIVPLE	TGLQEVEVKA LIGQQEVEVKA QGLHDIEVKA LGLHDIEVKA QGLHDVEIKA	AVYHHFI AVFNHFI SVRGVLA SVSAQSGFFG SVQEALW	SDGVRKSLKV SDGVKKTLKV SDGVRKKLKV ADGVRKKLKV SDGVRKKLKV	VPEGIRMNKT VPEGMRINKT VPEGERKE-I VPEGMRIAQD VPEGVQKS-I	VAVRTLDPER VAIHTLDPEK MTIIELDPAT VKTVILEPEV VTIVKLDPRA	LGREGVQKEDI LGQGGVQKVD\ KGVGGTQEQI\ KGKGGVQEEK\ KGVGGTQLEVI	PPADLSDQVP PAADLSDQVP QANKLDDKVP EALNPKNVVP KARKLDDRVP	D 951 D 949 D 941 R 952 D 934
Majority	TEIETXII	LQGXPVAQMI	EDAIDGXXLX	HLIVTPSGCG	EQNMIGMTPX	VIATXYLDAT	EQWEKXGXXRF	XEALXXIKKG	Y +
		970	980	990	-+	-+	1020	1030	1040 +
human mus thamnophis xenopus cobra_ven_fact	TESETRIL TDSETRII SEIETKIS TDIDTTIT TEIETKII	LQGTPVAQMT: LQGSPVVQMA VQGDRVAQMI LQGTPISQMV IQGDPVAQII	EDAVDAERLK EDAVDGERLK EDSIDGSKLR EDAIDGNNMN ENSIDGSKLN	HLIVTPSGCG HLIVTPAGCG HLIITPSGCG HLIVVPAGCG HLIITPSGCG	EQNMIGMTPT EQNMIGMTPT EQNMITMTPS EQNMISTTPS EQNMIRMAAP	VIAVHYLDET VIAVHYLDQT VIATYYLDAT VIATRYLDAS VIATYYLDTT	EQWEKFGLEKF EQWEKFGIEKF GQWESLGVDRF GQWERIGVNRF EQWETLGINRF	QGALELIKKG QEALELIKKG RTEAVNQIMKG REQALKNMRQG RTEAVNQIVTG	Y 1031 Y 1029 Y 1021 Y 1032 Y 1014
Majority	XQQMAFKK	PDXSYAAFTN	RAXSTWLTAY	VVKVFXMAAN	LIA-IXXEVL -+	CGXVKWLILE	KQKPDGVFQEX -+	APVIHQEMIG	G +
		1050	1060 -+	1070 -+	1080 -+	1090 -+	1100 -+	1110	1120 +
human mus thamnophis xenopus cobra_ven_fact Majority	TQQLAFRQ TQQLAFKQ NQQMVYKK AQQMAFRK AQQMVYKK IRX-XAEX	PSSAFAAFVKI PSSAYAAFNNI EDHSYAAFTHI PDNSYAAWKDI ADHSYAAFTNI EDVSLTAFVLI.	RAPSTWLTAY RPPSTWLTAY RASSTWLTAY RPASTWLTGY RASSSWLTAY ALLEXKDICN	VVKVFSLAVN VVKVFSLAAN VVKVFAMATN VAKVFGMAQE VVKVFAMAAK EXVNSLXXSI	LIA-IDSQVL LIA-IDSHVL IAKDISHEII FID-IEANVL MVAGISHEII NKAGDYLXXK	CGAVKWLILE CGAVKWLILE CGGVKWLILN CGSVKWLILE CGGVRWLILN YXNLQRPYTX	KQKPDGVFQEI KQKPDGVFQEI KQQPDGVFKEI KQKPDGLFQEN RQQPDGAFKEN AITXYALALAG	DAPVIHQEMIG DGPVIHQEMIG IAPVIHGEMLG IAPVIHQEMVG IAPVLSGTMQG XLKDXXKL	G 1110 G 1108 G 1101 G 1111 G 1094 L
		1130	-+ 1140	-+ 1150	-+ 1160	-+ 1170	-+ 1180	+ 1190	+ 1200
human mus thamnophis xenopus cobra_ven_fact	LRN-NNEK FRN-AKEA TIGAEF ITTGAAEG IQGAEE	DMALTAFVLI DVSLTAFVLI EVSLTAFVLV DSSLTAFILI EVYLTAFILV	-+ SLQEAKDICE ALQEARDICE SLLESKSICN AMLECQRTCN ALLESKTICN	-+ EQVNSLPGSI GQVNSLPGSI QHINILDSSI EHVNNLQVSI DYVNSLDSSI	-+ TKAGDFLEAN NKAGEYIEAS NKAADYLLKK DKASSYLVGQ KKATNYLLKK	-+ YMNLQRSYTV YMNLQRPYTV YEQLQRPYTT YPGLKKPYSI YEKLQRPYTT	-+AIAGYALAQMG AIAGYALALM ALTAYALATAF AITSYALATAF AITSYALALAG ALTAYALAAAF	+ RLKGPLLNKF IKLEEPYLGKF RLKDDRVL SKLPNTNKL OQLNDDRVL	+ L 1189 L 1187 M 1177 L 1189 M 1170
Majority	AASXGRXR	WEEPGKXXYN	XEXTSYALLA -+	LLKMKXFDXX	XPVVRWLNEQ -+	RYYGGXYGST -+	QATXMVFQALZ	QYQXDXPXHK	D +
		1210	1220 -+	1230	1240 -+	1250 -+	1260 -+	1270	1280 +
human mus thamnophis xenopus cobra_ven_fact	TTAKDKNR NTAKDRNR AASTGGDR SASIGNTH AASTGRDH	WEDPGKQLYN WEEPDQQLYN WEEYGSRTYN WEEPGKRFIS WEEYNAHTHN	VEATSYALLA VEATSYALLA IEGTSYALLA LETTSYALLT IEGTSYALLA	LLQLKDFDFV LLLLKDFDSV LLKMKKFEAA LLKMKEFDLT LLKMKKFDQT	PPVVRWLNEQ PPVVRWLNEQ DRVVRWLTNQ GGIVRWLNEQ GPIVRWLTDQ	RYYGGGYGST RYYGGGYGST NYYGGTYGQT RYYGAVYGST NFYGETYGQT	QATFMVFQAL# QATFMVFQAL# QATVMSFQAL# QATVMSFQAL# QATVMAFQAL#	QYQKDAPDHQ QYQTDVPDHK EYQIQRPTYK QYQTDIPSVN EYEIQMPTHK	E 1269 D 1267 D 1257 E 1269 D 1250
Majority	LNLDVSXH	LPXRXSPXTY	RINWENALLA	RSXETKLNED	FTVTAXGKGX	GTMXVVTVYH	AXLKEKEXXCN	KFXLXVSVXX	x +
		1290	1300	1310	1320	1330	1340	1350	1360 +
human mus thamnophis xenopus cobra_ven_fact	L <mark>N</mark> LDVSLÇ LNMDVSFH LNLDIVIK LNLDVSLH LNLDITIF	2LPSR <mark>SSKITH</mark> ILPSR <mark>SSATTF</mark> ILPER <mark>ELPLNY</mark> ILPER <mark>QQPLTY</mark> LPDR <mark>EVPIRY</mark>	RIHWESASLL RLLWENGNLL RIDGNNAVLA RINLENALLA RIN <mark>YE</mark> NALLA	RSEETKENEG RSEETKQNEA RTAETKLNED RSAETRLNOD RTVETKLNOD	FTVTAEGKGQ FSLTAKGKGR FTVSASGDGK FVVKAKGKGQ ITVTASGDGK	GTLSVVTMYH GTLSVVAVYH AKMTVLTVYN GTMRVVTVYH ATMTILTFYN	AKAKDQLT-CI AKLKSKVT-CI ALLREEENVCI ALVTEKERKCI AQLQEKANVCI	IKFDLKVTIKP KKFDLRVSIRP IKFELDVSVEE INFELSVNVKE IKFHLNVSVEN	A 1348 A 1346 V 1337 V 1349 I 1330

**Figure S7**. Amino acid alignment of region of C3 corresponding to the CUB and TED domains from various vertebrate species including mammals (human, *Mus*), reptiles (*Thamnophis*), and amphibians (*Xenopus*) as well as the snake venom component, cobra venom factor. Residues highlighted in green correspond to those of human C3b interacting with lufaxin.



**Figure S8**. Lufaxin binding with CVFB. **A**, **B** – Formation of a CVF complex with FB and lufaxin in the absence and presence of  $Mg^{2+}$ . **A** - Analysis by size exclusion chromatography of Superdex-200 shows formation of a CVFB in the presence of 5 mM  $Mg^{2+}$  (red trace). Addition of lufaxin increases the size of the complex (blue trace). **B** - SDS-PAGE (10% gel) of fractions (with retention volumes on the scale above the gels) shows the formation of CVFB in the absence of lufaxin (upper gel) and the incorporation of lufaxin into the complex when mixed with CVF and FB (lower gel). The gels are stained with silver. **C** - Surface plasmon resonance analysis of FB and lufaxin binding to an immobilized CVF surface in the presence of 5 mM  $Mg^{2+}$ . Blue trace FB (57 nM) + lufaxin (0.5  $\mu$ M), Red trace FB alone, black trace lufaxin alone. **D** – The experiment shown in panel C performed in the absence of  $Mg^{2+}$ . Chromatographic and SPR analyses indicate that lufaxin stabilizes the binding of FB with CVF much like it does with C3b.

Majority	IVGGQECKD	GECPWQALLI	INEENEGFCGG	TILSEFYIL	FAAHCLYQAKF	FKVRVGDRN	TEQEEGGEAVH	EVEVVIKHNR	F
		10	20	30	40	50	60	70 8	+ 80
human bovine porcine	IVGGQECKD IVGGRDCAE IVGGQECKD	GECPWQALLI GECPWQALLI GECPWQALLI	INEEN <mark>EGF</mark> CGG /NEENEGFCGG INEEN <mark>E</mark> GFCGG	TILSEFYIL TILNEFYVL TILSEFYIL	FAA <mark>H</mark> CLY <mark>Q</mark> AKF FAAHCLHQAKF FAA <mark>H</mark> CLY <mark>Q</mark> AKF	RFKVRVGDRN RFTVRVGDRN RFKVRVGDRN	TEQEEGGEAVI TEQEEGNEMAI TAAEEGGEAVI	ievevvikhnr: Ievemtvkhsr: Ievevvikhnr:	F 80 F 80 F 80
Majority	TKETYDFDI	AVLRLKTPI	FRMNVAPACI	PERDWAESTI	MTQKTGIVSG	FGRTHEKGR	QSTRLKMLEVI	YVDRNSCKLS	S +
		90	100	110	120	130	140	150	160
human bovine porcine	T <mark>KE</mark> T <mark>Y</mark> DFDI VKE TYDFDI TKE TYDFDI	AVLRLKTPI AVLRLKTPI AVLRLKTPI	IFRMNVAPACI RFRRNVAPACI IFRMNVAPACI	PERDWAESTI PEKDWAEATI PERDWAESTI	LMTQKTGIVSG LMTQKTGIVSG LMTQKTGIVSG	FG <mark>R THEKGR</mark> FG <mark>R THEKGR</mark> FG <mark>R THEKGR</mark>	QSTRLKMLEVI LSSTLKMLEVI QSTRLKMLEVI	PYVDRNSCKLS PYVDRSTCKLS PYVDRSTCKLS	s 160 s 160 s 160 s 160
Majority	SFIITQNMF	CAGYDTKQEI	DACQGDSGGPE	VTRFKDTYF	TGIVSWGEGC	ARKGKYGIY	TKVTAFLKWII	RSMKTRXXXX	x
		170	180	190	200	210	220	230 :	240
human bovine porcine	S <mark>F</mark> IITQNMF SFTITPNMF S <mark>F</mark> IITQNMF	'CAGYDTKQEI 'CAGYDTQPEI 'CAGYDTKQEI	DACQGDSGGPE DACQGDSGGPE DACQGDSGGPE	VTRFKDTYF VTRFKDTYF VTRFKDTYF	/TGI <mark>VSWGEGC</mark> /TGI <mark>VSWGEGC</mark> /TGI <mark>VSWGEGC</mark>	CARKGKYGIY CARKGKFGVY CARKGKYGIY	TKVTAFLKWII TKVSNFLKWII TKVTAFLKWII	DRSMKTR DKIMKARAGAA DRSMKTRGLPK	235 G 240 A 240
Majority	x	 250							
human bovine porcine	S KSHAPEVIT	SSPLK							235 241 254

**Figure S9**. Amino acid alignment of the heavy chain of FXa from mammal species with regions of the human protein that interact with lufaxin (along with corresponding residues from other species) highlighted in yellow.



**Figure S10**. Factor Xa cleaves lufaxin. Recombinant lufaxin containing a 6-His tag ( $1.9 \mu$ M) was incubated with fXa ( $3.8 \mu$ M) in the presence or absence of 5mM CaCl<sub>2</sub>, in Tris-buffered saline pH 7.4, at 30°C for 3 hours. Same concentrations of lufaxin or FXa alone were used as controls. Immediately after incubation samples (indicated in the figure) were prepared and loaded on 4-12% Bis-Tris gels run in parallel. After running, one of the gels was stained with Coomassie blue (left panel) and the other one was transferred to a nitrocellulose membrane used in western blot assay to detect 6x-his tag (right panel). Lufaxin cleavage by fXa is almost imperceptible if evaluated by its apparent molecular weight in the Coomassie stained gel (left panel), nevertheless it is evident by the loss of the fragment containing the His-tag (right panel), in a reaction that occurs regardless of the presence of CaCl<sub>2</sub>. Pre-stained molecular weight markers (sizes indicated in kDa) were run in the first lane of each gel.



**Figure S11.** Superposition of cryo-EM model (blue) showing lufaxin and fXa (heavy chain) superposed with a prediction produced with alphafold 2.3.1 (green). After its recent release, alphafold 2.3.1 was used to produce a model for the fXa-lufaxin structure that closely resembles the cryo-EM model. Two views of the model are show related by rotation of approximately 180° around the vertical axis. Arg 277 of the C-terminal coil of lufaxin is bound at the S1 subsite of fXa and very similar interactions occur at the autolysis loop and the 220-loop of fXa. The alphafold model is "tighter" than the cryo EM model with the two components lying generally closer to one another at the interface of lufaxin and fXa. Inputs for the prediction were one chain of the lufaxin sequence (mature), one heavy chain of fXa and one light chain of fXa (both mature).