Supplemental methods

Viruses, cells, and semen. Recombinant VSV expressing the EBOV glycoprotein and mCherry (rVSV-EboGP-mCherry) has been previously described[43,44]. To generate rVSV-EboGP-mCherry stocks, Vero CCL81 cells (gift from Susan Weiss, U. Pennsylvania) were infected at an MOI of 0.001 for 3 days; clarified supernatant was buffered with 25 mM HEPES, aliquoted, frozen at -80°C, and titered by $TCID_{50}$ on Vero CCL81 cells. EBOV/"Zaire 1995" (EBOV/H.sap-tc/COD/95/Kik-9510621) was used in authentic virus studies (1). HeLa, A549, and THP1 cell lines and macrophages differentiated from purified human blood monocytes (UPenn Human Immunology Core) were used as target cells for infections. 293T cells were used for transfection. Seminal plasma was obtained from the Penn Medicine Division of Reproductive Endocrinology and Infertility. Semen was centrifuged at 4000g for 10 min to remove cells, then frozen. To remove amyloid fibrils, seminal plasma was spun through a Amicon Ultra (100 kDa cutoff (2)) for 45 min at 4°C and the filtrate was used; the retentate was discarded. Primary cell and semen samples are considered to be a secondary use of deidentified human specimens and are exempt via Title 55 Part 46, Subpart A of 46.101 (b) of the Code of Federal Regulations. 293T, HeLa, and A549 cells (ATCC) were maintained in DMEM with 4.5 g/L glucose and no sodium pyruvate supplemented with 10% FBS (Sigma). THP1 cells were maintained in RPMI-1640 supplemented with 10% FBS and 50 mM β-mercaptoethanol. Human monocyte-derived macrophages were maintained in RPMI-1640 with glutamine, 10% FBS, and penicillin/streptomycin, and were differentiated from peripheral blood monocytes by incubation in 20 μg/mL MCSF (Gemini) for 7 days.

Peptides and Fibrils. SEVI, SEVI-Ala, PAP85-120, SEM1, and SEM2 fibrils were generated by dissolving peptides (Keck Biotechnology Resource Laboratory, Yale University) in PBS, filtering through a 0.2 μm filter, seeding with 1% preformed amyloid, and incubating at 37°C with shaking

at 1400 rpm. Amyloid formation was confirmed by assessing thioflavin T fluorescence. Aliquots were stored at -80°C and working stocks kept at 4°C. Peptide sequences are available in Supplementary Table 3.1.

Infection assays. rVSV-EboGP-mCherry was diluted into DMEM-10 alone or supplemented with amyloid fibrils or soluble peptides and incubated at 37°C for 15 minutes. 20 μL of the infection mixture was added to each well of target cells in a 96-well plate (plated at 1.5e4 cells/well the previous day in 100 μL DMEM-10) and incubated at 37°C for 1 h, then the media was replaced with fresh DMEM-10 and incubated at 37°C for a total of 12 h. Cells infected in the presence of SEVI were harvested by trypsinization, fixed in 2% paraformaldehyde (PFA), and analyzed by flow cytometry for mCherry expression. For experiments containing amyloids other than SEVI, the cells were fixed, permeablized with 0.1% saponin in FACS buffer (1% BSA, 0.01% sodium azide in PBS), and stained for 1 h with a combination of 1:1000 mouse monoclonal anti-VSV(M) primary antibody (gift from Robert Doms, U. Pennsylvania) and 1:5000 goat anti-mouse secondary antibody labeled with AF488 (Life Technologies) before analysis by flow cytometry. For each experiment, the average of triplicate technical replicates was log-transformed, and transformed percents infection of biological replicates were compared by repeated measures ANOVA with *post hoc* analysis using false discovery rate analysis to correct for multiple comparisons (GraphPad Prism). Monocyte-derived macrophages were treated with B18R (Abcam) for 24 h prior to as well as during infection to inhibit the interferon response.

For authentic virus infections, peptides were diluted to 5-50 μg/ml and pre-incubated with EBOV for 15 minutes. HeLa cells were exposed to peptide/virus inoculum at an MOI of 2.0 or 0.2

PFU/cell for 1 h, after which peptide/virus inoculum was removed and fresh culture media added. At 24-48 h post-infection, cells were formalin-fixed, removed from containment, and immunostained using the 13F6 antibody (3) at 2 μg/ml. Infection was quantified using automated fluorescence microscopy as described (4).

Binding/Internalization assays. rVSV-EboGP-mCherry was pretreated with or without SEVI then bound to HeLa cells on ice. Cells were either lysed in 1% Triton for 10 minutes on ice after 1 h (binding) or warmed to 37°C for 1 h to allow viral internalization, washed 3x in PBS with Ca^{2+} and Mg^{2+}, trypsinized for 10 minutes at 37°C, and lysed with 1% Triton for 10 minutes on ice (internalization). Lysates were separated on a 12% Criterion TGX gel, transferred to nitrocellulose for 1 h, and blocked with TBS Odyssey Blocking Buffer (Li-Cor). Membranes were probed for VSV M (1:1000, Ab as above) and GAPDH (1:2000, rabbit polyclonal, Santa Cruz Biotechnology) in TBS Blocking Buffer/0.2% Tween simultaneously for 1 h, then with IRDye 800CW goat anti-mouse and IRDye 680RD goat anti-rabbit (1:15,000, Li-Cor) in TBS blocking buffer/0.2% Tween. Membranes were then analyzed by quantitative Western blotting by comparing VSV M signal to GAPDH signal for each sample.

Virus-like particle generation. 293T cells were plated in 15 cm plates the day before transfection. Cells were transfected with 7.5 μg each of pCAGGS-EboGP, pCAGGS-VP40, and either pCAGGS-VP40(luc) or pCAGGS-VP40(GFP) with polyethylenimine[48]. Supernatants were collected at 24 and 48 h after transfection, concentrated through a 20% sucrose cushion by ultracentrifugation, resuspended in 1% BSA, 50 mM HEPES-buffered PBS, and frozen at -80°C until use.

VLP binding assay. HeLa cells were plated in a 96-well plate at 1.5e4 cells/well the day before the assay and incubated on ice for 30 min prior to the experiment. SEVI fibrils were diluted to 35 μg/mL in DMEM-10 and mixed with 3 μL concentrated EBOV VLP (VP40-luc) and incubated at 37°C for 10 min. 20 μL of the mixture was added to triplicate wells and spun at 1200g for 30 min at 4°C. After spinning, the cells were washed 5X with cold DMEM-10 and lysed with Bright-Glo luciferase assay buffer (Promega). Luciferase activity was read on a Luminoskan Ascent (Thermo) 10 minutes after addition of assay buffer, and after background subtraction, readings were normalized to 0 μg/mL SEVI condition. Statistical significance was determined by paired t-test of log-transformed data (StataIC 14).

VLP internalization assay. The VLP internalization assay was done similarly to what has been previously described (5). HeLa cells were plated in a 96-well plate at 1.5e4 cells/well the day before the assay. Cells treated with EIPA were pretreated with 100 μM EIPA for 1 h prior to the beginning of the experiment and incubated on ice for 30 minutes prior to the beginning of the experiment. SEVI fibrils were diluted to 35 μg/mL in DMEM-10 and mixed with 2 μL EBOV VLP (VP40-GFP) and incubated at 37°C for 10 min. After incubation, 20 μL of this mixture was added to triplicate wells and spun at 1200g for 30 min at 4°C. The plate was then shifted to 37°C for 1 h. The cells were then trypsinized, fixed in 2% PFA, and analyzed by flow cytometry for geometric mean fluorescence intensity in the GFP channel. Statistical significance was determined by repeated measured ANOVA with false discovery rate correction (GraphPad Prism).

Dextran uptake assay. HeLa cells were plated in a 96-well plate at 1.5e4 cells/well the day before the assay. Cells treated with EIPA were pretreated with 100 μM EIPA for 1 h. Culture medium was removed from each well and replaced with indicated concentrations of SEVI diluted in DMEM-10 with or without 100 μM EIPA in triplicate. The cells were incubated at 37°C for 20

minutes, then 2.5 μL of 20 mg/mL FITC-dextran (70 kDa in DMSO, Invitrogen) was added to each well for 10 minutes at 37°C. Afterward, the medium was removed and replaced with 100 μL of PBS pH 4.9 to bleach any uninternalized FITC. Cells were trypsinized and fixed in 2% PFA, washed 3X with FACS buffer (1% BSA in PBS, 0.1% sodium azide) and analyzed by flow cytometry. Data were analyzed with FlowJo to determine geometric mean fluorescence intensity in the FITC channel. Statistical significance was assessed by linear regression analysis or repeated measures ANOVA with false discovery rate correction (GraphPad Prism).

Inhibitor treatments. HeLa cells were plated in a 96-well plate at 1.5e4 cells/well the day before the assay. Cells treated with inhibitors [100 μM EIPA (Toronto Research Chemicals), 1 μM cytochalasin D (Cayman Chemical Company), 0.5 μM 17-hydroxywortmannin (Cayman Chemical Company), 50 μM LY294002 (Cayman Chemical Company), 50 mM NH_4Cl (Fisher), 10 μM Z-FF-FMK (EMD Biosciences), 10 μM leupeptin (Sigma), 10 μM E64 (EMD Biosciences), or 10 μM MDL28170 (Calbiochem)] were pretreated for 1 h before infection. Cells were infected as described earlier. After 1 h of infection, the virus- and inhibitor-containing medium was removed and replaced with medium without inhibitor for the remainder of the incubation. Infections were harvested and analyzed for percent infection as described above. To test for cytotoxic effects, HeLa cells were treated with inhibitors for 2 h before assessment for viability by the CellTiter 96 AQueous One Solution Cell Proliferation assay kit (Promega) according to manufacturer instructions.

Virus stability analysis. rVSV-EboGP-mCherry was diluted from stock concentrations to concentrations of 1e7 $TCID_{50}$/mL in artificial semen simulant, with or without SEVI fibrils or α-synuclein fibrils (35 μg/mL) or 10% seminal plasma/seminal plasma filtrate. For thermal stability experiments, samples were taken immediately (0 h timepoint) or after indicated times of incubation

at 37°C in a thermocycler with heated lid to minimize evaporation. Samples were frozen at -80°C until titration by $TCID_{50}$. For desiccation tolerance experiments, 10 µL of diluted virus in artificial semen simulant was spotted in the bottom of non-tissue-culture-treated 96-well plates and allowed to dry under laminar flow in a biosafety cabinet at room temperature for indicated lengths of time. For comparison, samples from the bulk liquid (maintained at room temperature in sealed tube) were taken at the initial timepoint and the last timepoint. Samples were immediately titered by $TCID_{50}$ after addition of 200 µL of DMEM to recover virus from the dried samples.

$TCID_{50}$. Vero cells were plated the previous day at 1.5e4 cells/well in 96-well plates. Viral samples were serially diluted in serum-free DMEM then added in 8-fold replicate to 96-well plates. After 48-72 h, wells were scored by presence/absence of viral replication as marked by fluorescent protein expression and cytopathic effects. $TCID_{50}$/mL was calculated by the Spearman & Kärber algorithm (6). Data were log-transformed and analyzed for statistical significance by nonlinear regression (GraphPad Prism).

Supplemental Results

To further confirm these finding, EBOV VLP experiments were performed. Binding was further quantified with a binding assay using EBOV virus-like particles (VLPs). These particles show filamentous morphology and replicate steps of the EBOV entry process (7–10). VLPs with a luciferase reporter were preincubated with SEVI and bound to HeLa cells on ice for 1 h, then the cells were washed, lysed in luciferase assay buffer, and luminescence recorded. In agreement with previous results, an approximately 2-fold increase in binding was observed in the presence of SEVI (Fig S4). VLPs labeled with GFP were preincubated with SEVI and bound to HeLa

cells on ice then shifted to 37°C. After trypsinization, cells were analyzed by flow cytometry for GFP signal. An increase in geometric mean fluorescence intensity of approximately 25% was observed in the presence of SEVI relative to its absence, suggesting that cells internalized a significantly higher quantity of VLPs pretreated with SEVI than not (Fig S5). This effect was ablated by the macropinocytosis inhibitor N-(ethyl-N-isopropyl)-amiloride (EIPA), suggesting efficient removal of bound VLPs from the cell surface.

Supplemental Figure Legends

Figure S1. HeLa cells were treated with seminal amyloids and autofluorescence was measured by flow cytometry. The percentage of cells in the *mCherry* gate indicates background autofluorescence*; shifts in the PAP85-120 and SEM1 populations are readily appreciated*.

Figure S2. HeLa cells were infected with rVSV-EboGP-mCherry with each seminal amyloid alone or together (5 μg/mL each amyloid)

Figure S3. HeLa cells were chilled on ice and rVSV-EboGP-mCherry was bound on ice for 1 h. The media was then removed and replaced with DMEM-10 containing SEVI. The cells were warmed for 1 h at 37°C before washing, trypsinizing, centrifuging, lysing, and finally analyzing the amount of internalized virus by Western blotting for VSV M.

Figure S4. HeLa cells were prechilled and treated with EBOV VLPs (VP40-luc) pretreated with or without 35 μg/mL SEVI fibrils. After spinfection, cell-associated luciferase signal was determined and normalized; n=3, mean ± SEM. **$p<0.01$ by paired t test.

Figure S5. HeLa cells were prechilled and treated with EBOV VLPs (VP40-GFP) pretreated with or without 35 μg/mL SEVI fibrils. After allowing internalization, cells were analyzed for

geometric mean fluorescence intensity of GFP. *p<0.05, ***p<0.001 by repeated measures ANOVA.

Figure S6. HeLa cells were treated for 2 h with inhibitors in concentrations as described in Materials and Methods then cell viability was assessed by the CellTiter 96 AQueous One Solution Cell Proliferation assay. n=2, mean±SD.

Figure S7. rVSV-EboGP-mCherry was diluted in artificial semen simulant alone or supplemented with 35 μg/mL SEVI fibrils and titered either immediately or after six hours of incubation in a sealed tube at room temperature by TCID50 on Vero cells. p=0.10 by paired t test.

Figure S8. rVSV-EboGP-mCherry was incubated in artificial semen simulant under desiccating conditions with or without 35 µg/mL SEVI fibrils and titered by TCID50 on Vero cells. n=1.

Figure S9. rVSV-EboGP-mCherry was diluted in DMEM-10 alone or supplemented with 35 µg/mL SEVI fibrils and treated with PBS or chlorine bleach diluted in PBS for a final concentration of 0.005% hypochlorite for 1 minute. The samples were then neutralized with sodium thiosulfate (10-fold excess of 0.01% solution) and titered by TCID50 on Vero cells. n=1, mean±SD.

Supplemental References

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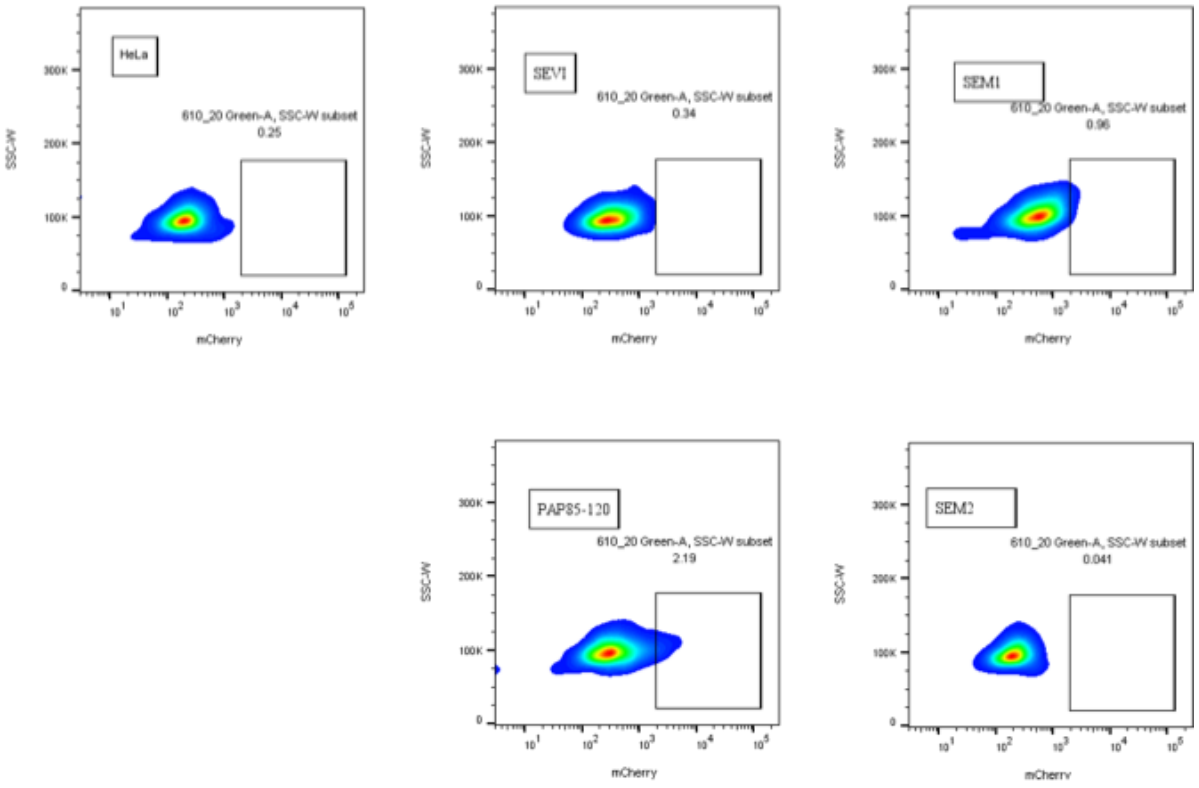
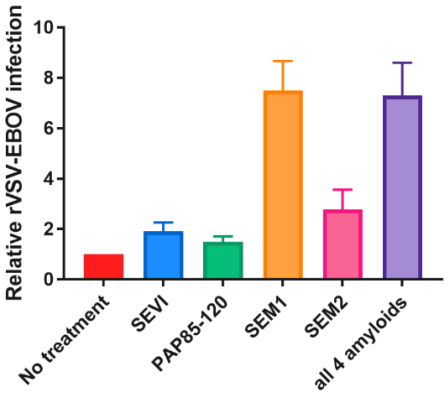
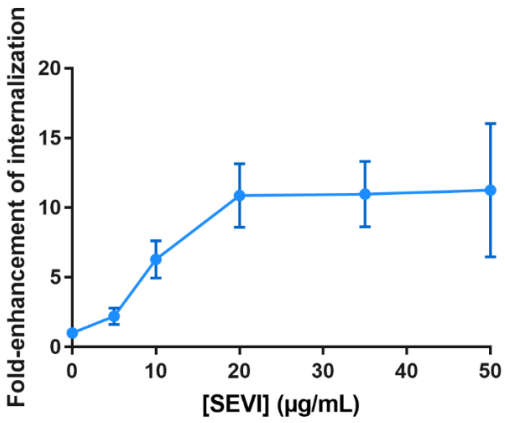
S1


HeLa
610_20 Green-A, SSC-W subset
0.25
SEVI
610_20 Green-A, SSC-W subset
0.34
SEM1
610_20 Green-A, SSC-W subset
0.96
PAP85-120
610_20 Green-A, SSC-W subset
2.19
SEM2
610_20 Green-A, SSC-W subset
0.041
SSC-W
mCherry

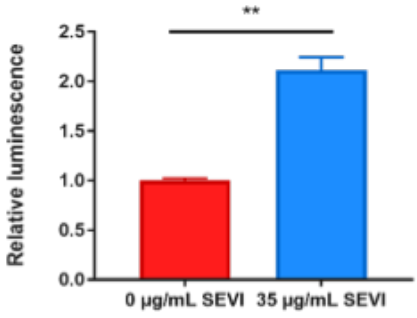
S2


Relative rVSV-EBOV infection
No treatment
SEVI
PAP85-120
SEM1
SEM2
all 4 amyloids

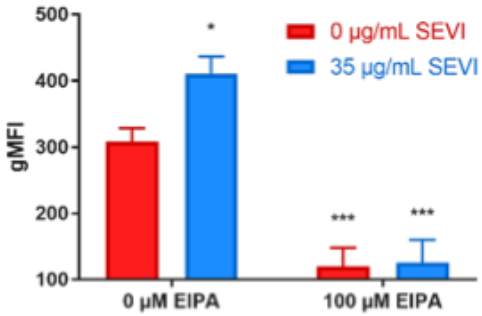
S3


Fold-enhancement of internalization
[SEVI] (µg/mL)

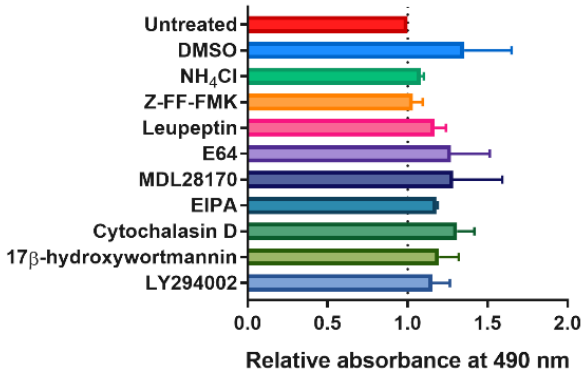
S4


Relative luminescence
0 µg/mL SEVI
35 µg/mL SEVI

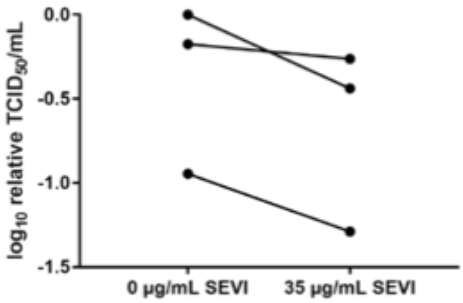
S5


gMFI
0 µg/mL SEVI
35 µg/mL SEVI
0 µM EIPA
100 µM EIPA

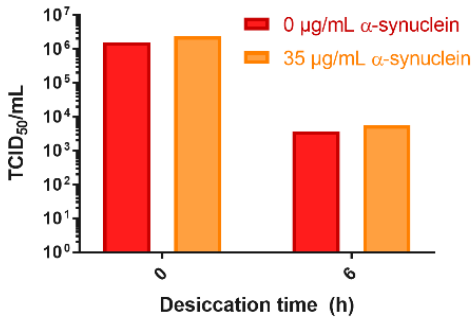
S6


Untreated
DMSO
NH4Cl
Z-FF-FMK
Leupeptin
E64
MDL28170
EIPA
Cytochalasin D
17β-hydroxywortmannin
LY294002
0.0
0.5
1.0
1.5
2.0
Relative absorbance at 490 nm

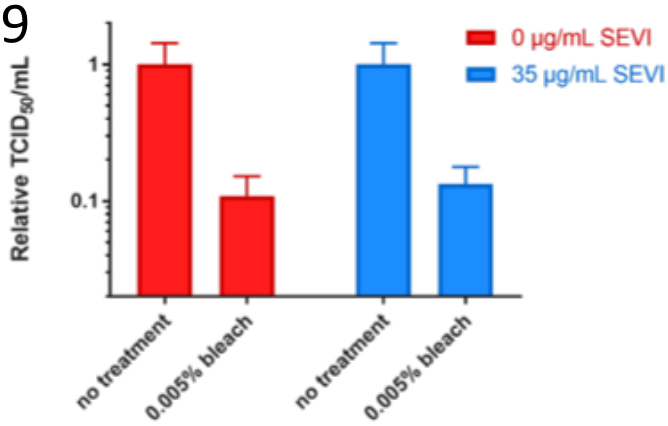
S7


log10 relative TCID50/mL
0.0
-0.5
-1.0
-1.5
0 µg/mL SEVI
35 µg/mL SEVI

S8


TCID50/mL
0 µg/mL α-synuclein
35 µg/mL α-synuclein
Desiccation time (h)

S9


Relative TCID50/mL
0 µg/mL SEVI
35 µg/mL SEVI
1
0.1
no treatment
0.005% bleach
no treatment
0.005% bleach

Supplementary Table 1. Peptide Sequences	
SEVI	GIHKQKEKSRLQGGVLVNEILNHMKRATQIPSYKKLIMY
SEVI-Ala	GIHAQAEASALQGGVLVNEILNHMAAATQIPSYAALIMY
PAP85-120	IRKRYRKFLNESYKHEQVYIRSTDVDRTLMSAMTNL
SEM1 (45–107)	GQHYSGQKGKQQTESKGSFSIQYTYHVDANDHDQSRKSQQYDLNALHKTTKSQRHLGGSQQLL
SEM2 (49-107)	GQKDQQHTKSKGSFSIQHTYHVDINDHDWTRKSQQYDLNALHKATKSKQHLGGSQQLL

Supplementary Table 2. Goodness of fit parameters for Figure 3		
Figure 3A (SEVI-mediated enhancement of stability)		
	R^2	**Overall model p value**
0 µg/mL SEVI	0.9506	0.0007
35 µg/mL SEVI	0.9563	
35 µg/mL SEVI-Ala	0.9665	
35 µg/mL PAP248-286	0.9749	
0 µg/mL SEVI + 35 µg/mL SEVI	0.9689	
Figure 3B (seminal plasma enhancement of stability)		
	R^2	**Overall model p value**
10% seminal plasma	0.8687	0.0003
10% seminal plasma filtrate	0.9533	
Figure 3C (SEVI-mediated enhancement of desiccation tolerance)		
	R^2	**Overall model p value**
0 µg/mL SEVI	0.9821	0.0225
35 µg/mL SEVI	0.9883	
35 µg/mL SEVI-Ala	0.9807	
35 µg/mL PAP248-286	0.9335	
0 µg/mL SEVI + 35 µg/mL SEVI	0.9715	
Figure 3D (seminal plasma enhancement of desiccation tolerance)		
	R^2	**Overall model p value**
10% seminal plasma	0.9884	0.0022
10% seminal plasma filtrate	0.9932	