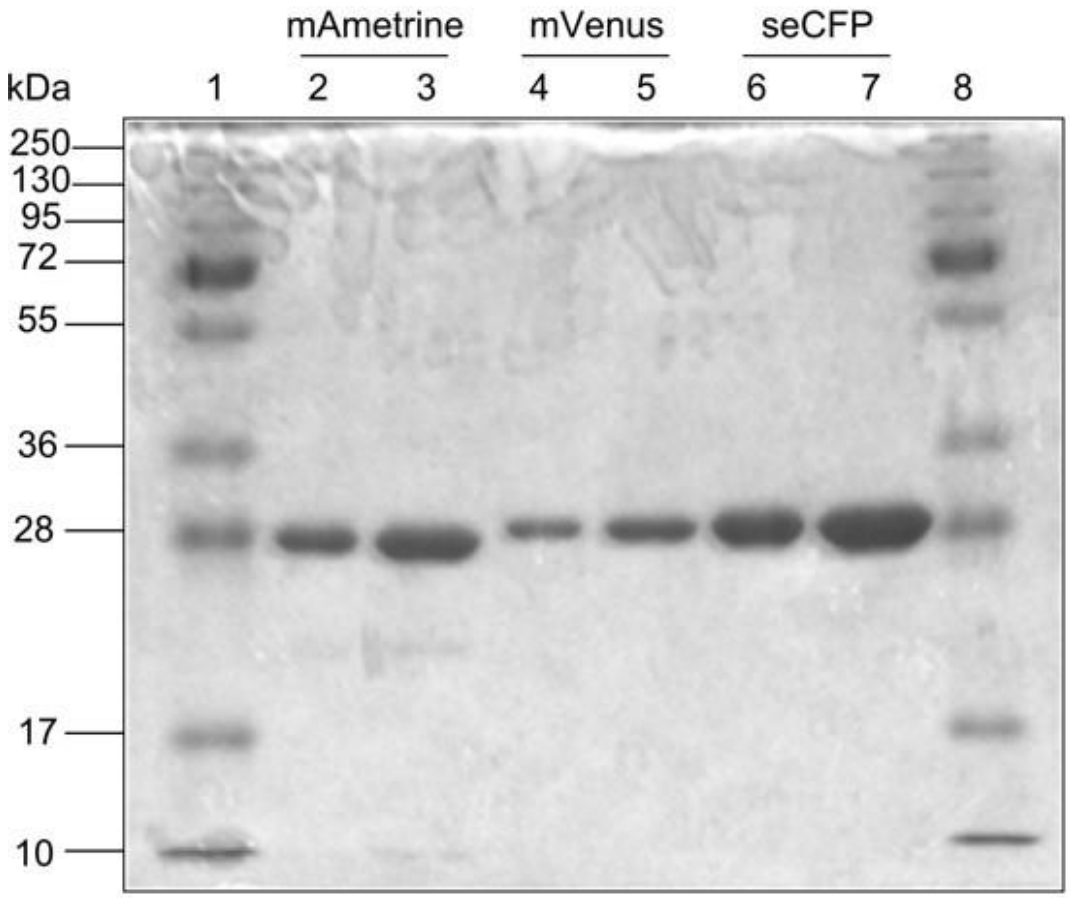


***Supplementary Figure 1***

***SDS-PAGE of the three color variants of recombinant FP-speract.***

Purified FP-speract were analyzed by 15% SDS-PAGE stained with Coomassie blue R-250. Lanes 1 and 8 contain molecular weight markers. Lanes 2 (1.2 µg) and 3 (2.4 µg) were mAmetrine-speract. Lanes 4 (0.75 µg) and 5 (1.5 µg) were mVenus-speract. Lanes 6 (1.9 µg) and 7 (3.7 µg) were eCFP-speract.

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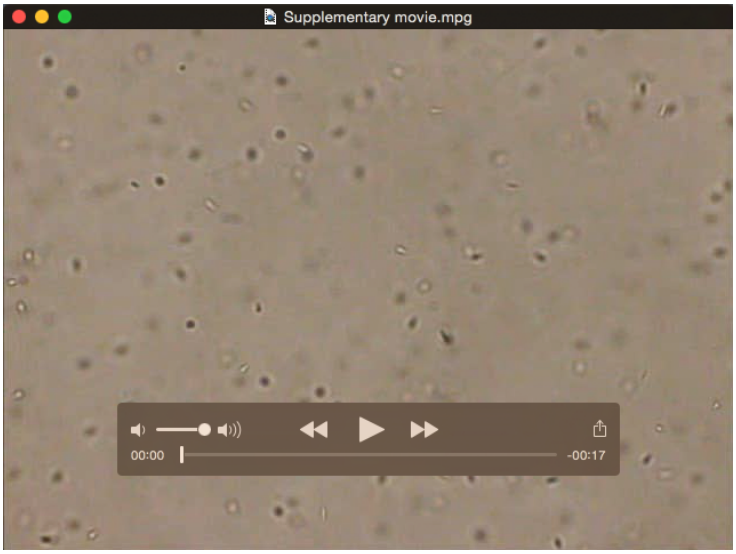


***Supplementary movie***

**Recovery of suppressed sperm motility in acidified ASW by FP-speract**

*L. pictus* spermatozoa suspended in 20 µl of acidified ASW (7.5 mM MES, pH 5.5) were treated with 2 µl of 100 nM of mVenus-speract (10 nM at final) dissolved in the same acidified ASW. Bright field microscopic images were captured using Olympus iX71 (60x objective lens) with Canon Rebel T4i camera using MeCan NY-1S DSLR microscope adapter. It is important to select an appropriate pH of ASW to successfully perform this experiment. The pH value should be acidic enough to suppress the basal sperm motility but should not be too acidic in order to avoid irreversible motility suppression. To easily find the correct pH, it is recommended to prepare several tubes of ASW with different pH (7.5 to 5.5 with 0.2 intervals using HEPES, PIPES and MES depending on the pH range) beforehand. An appropriate pH of ASW will vary depending on individual animal. ASW with $NH_4Cl$ (10 mM at final) should work as positive control of the effect of FP-speract. In high $K^+$ (50 mM) ASW, $NH_4Cl$ can recover the sperm motility, but speract cannot since most of sperm responses will be inhibited in this condition except for an increase in cGMP.

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