# SUPPORTING INFORMATION

# Novel Scorpion Toxin ω-Buthitoxin-Hf1a Selectively Inhibits Calcium Influx via Cav3.3 and Cav3.2 and Alleviates Allodynia in a Mouse Model of Acute Postsurgical Pain

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## 1. Supplementary Results

### *1.1. High-throughput FLIPR venom screening at Cav3.2*

55 crude venoms from 52 scorpion species of 20 genera across 6 families of scorpions were selected from the collected venom library, and screened at Cav3.2 FLIPR window current assays with 10 μg/well (Figure S1). Inhibitory crude venoms with >50% inhibition are presented in Table S1.

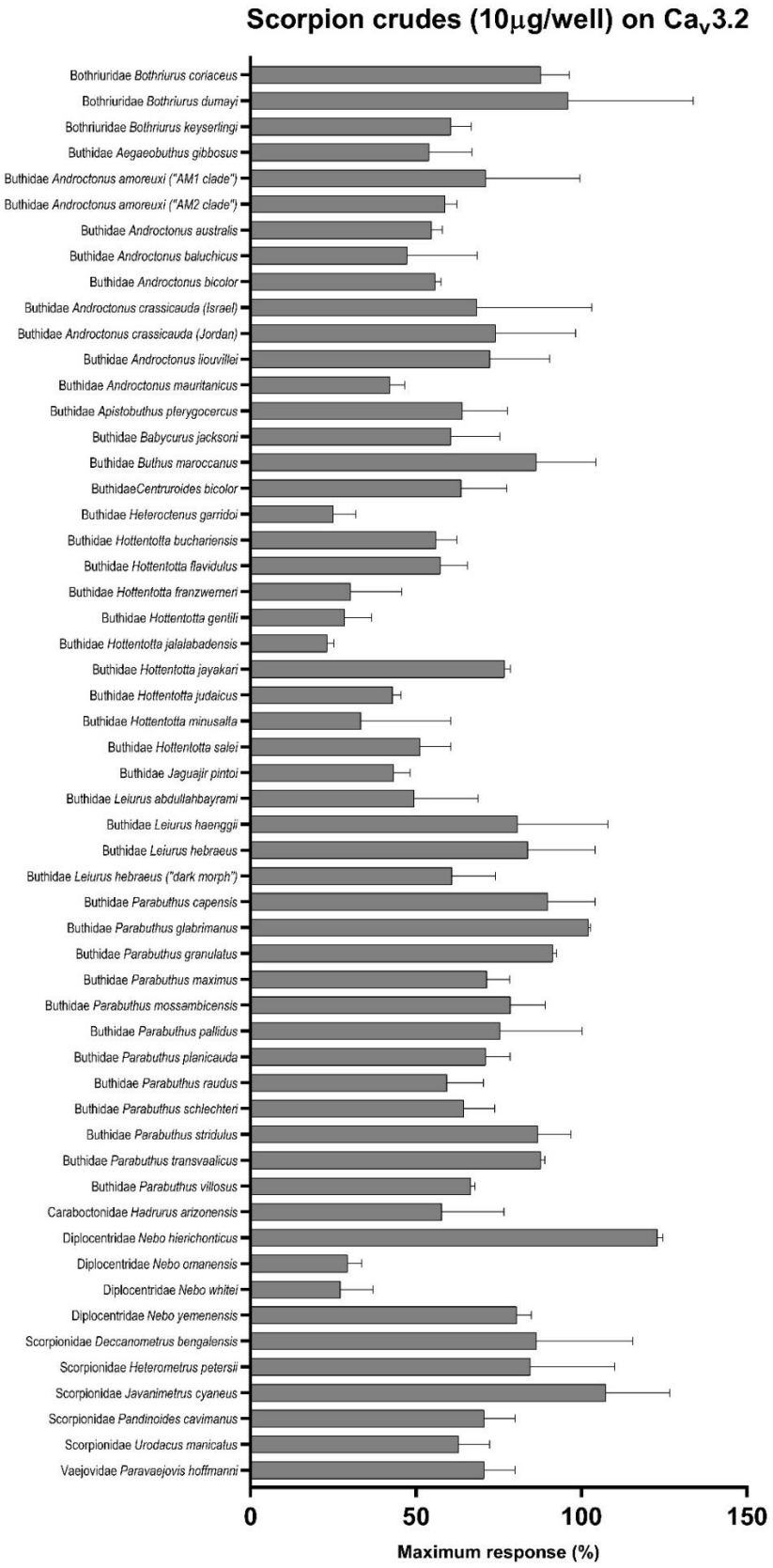


**Figure S1.** Activity of 55 scorpion crude venoms at Cav3.2 expressed in HEK293 cells. Data are means ± SEM of single experiments per venom performed in duplicate.

**Table S1.** Crude venoms with >50% inhibition of Cav3.2 FLIPR responses

| Inhibition | Genus | Species | Sex | Family | Organism |
|---|---|---|---|---|---|
| >70% | *Hottentotta* | *franzwerneri* | m | Buthidae | scorpion |
| >70% | *Hottentotta* | *gentili* | m | Buthidae | scorpion |
| >70% | *Hottentotta* | *jalalabadensis* | f | Buthidae | scorpion |
| >70% | *Heteroctenus* | *garridoi* | m | Buthidae | scorpion |
| >70% | *Nebo* | *omanensis* | f | Diplocentridae | scorpion |
| >70% | *Nebo* | *whitei* | f | Diplocentridae | scorpion |
| >60% | *Hottentotta* | *minusalta* | f | Buthidae | scorpion |
| >50% | *Androctonus* | *baluchicus* | m | Buthidae | scorpion |
| >50% | *Androctonus* | *mauritanicus* | n/a | Buthidae | scorpion |
| >50% | *Jaguajir* | *pintoi* | n/a | Buthidae | scorpion |

*1.2. Determination of peptide mass and NMR structure*

Figure S2 shows the main results in MALDI TOF MS of scorpion *Hottentotta franzwerneri* fractions I (1195.4 Da) and II (1196.4 Da) in positive ion mode. After the purity (>98%) of synthetic Hf1a-$NH_2$ being confirmed by HPLC and correct mass confirmed by electrospray ionization mass spectrometry (ESI MS) (Figure S3), its NMR statistics data was acquired and summarized in Table S2.

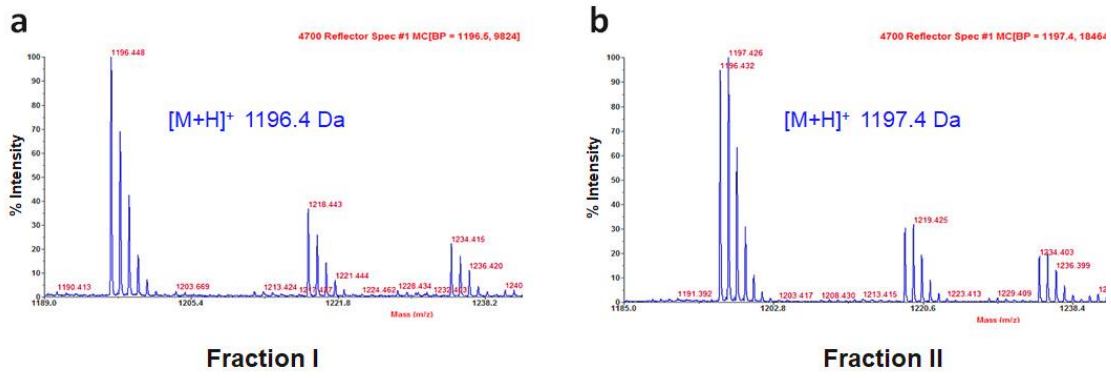


**Figure S2.** (**a**) MALDI TOF MS result of scorpion *H. franzwerneri* fraction I with a main average mass of 1195.4 Da. (**b**) MALDI TOF MS result of scorpion *H. franzwerneri* fraction II with a main average mass of 1196.4 Da.

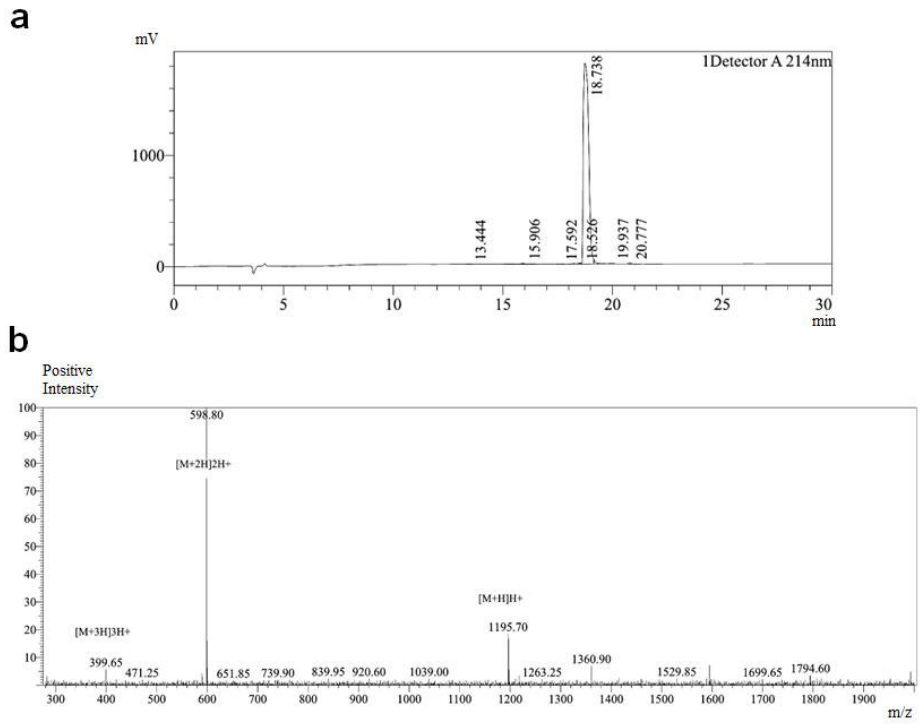


**Figure S3.** (**a**) RP-HPLC trace of synthetic Hf1a-$NH_2$ using the gradient of 5–35% solvent B over 30 min, where Solvent A consists of 0.1% trifluoroacetic acid (TFA) in milli-Q water, while solvent B consists of 0.1% TFA in acetonitrile. (**b**) ESI MS result of synthetic Hf1a-$NH_2$ with observed mass of 1195.6 Da.

**Table S2.** NMR statistics data for Hf1a-$NH_2$

| **Energies (kcal/mol)** | **Hf1a-$NH_2$** |
|---|---|
| Overall | -275.67 ± 12.79 |
| Bonds | 3.84 ± 0.45 |
| Angles | 9.31 ± 1.42 |
| Improper | 3.15 ± 1.18 |
| Dihedral | 43.31 ± 1.01 |
| Van der Waals | -27.21 ± 4.17 |
| Electrostatic | -308.23 ± 11.80 |
| NOE | 0.033 ± 0.010 |
| Cdih | 0.118 ± 0.134 |
| **MolProbity Statistics** | |
| Clashes (>0.4 Å / 1000 atoms) | 2.13 ± 3.13 |
| Poor rotamers | 0.05 ± 0.22 |
| Ramachandran Outliers (%) | 0 |
| Ramachandran Favoured (%) | 100 |

| | |
|---|---|
| MolProbity score | 0.87 ± 0.42 |
| Molprobity score percentile | 99.3 ± 0.92 |
| Residues with bad bonds | 0.15 ± 0.37 |
| Residues with bad angles | 0 |
| **Atomic RMSD (Å)** | |
| Mean global backbone (residues 2-9) | 0.43 ± 0.13 |
| Mean global heavy (residues 2-9) | 1.06 ± 0.21 |
| **Experimental Restraints** | |
| **Distance restraints** | |
| Short range (i-j < 2) | 55 |
| Medium range (/i-j/ < 5) | 10 |
| Long range (/i-j/ > 5) | 3 |
| Hydrogen bonds | 2 |
| Total | 70 |
| **Dihedral angle restraints** | |
| ϕ | 7 |
| ψ | 4 |
| Total | 11 |
| **Violations from experimental restraints** | |
| Total NOE violations exceeding 0.2 Å | 0 |
| Total Dihedral violations exceeding 2.0° | 0 |

*1.3. Evaluation of the effects of ω-Buthitoxin-Hf1a on I–V curves of Cav3.2 and Cav3.3*

Current-voltage (*I–V*) relationships of Cav3.2 and Cav3.3 in the presence of 1 μM Hf1a-$NH_2$ revealed that block was not accompanied by shifts in the *I–V* relationship (Figure S4).

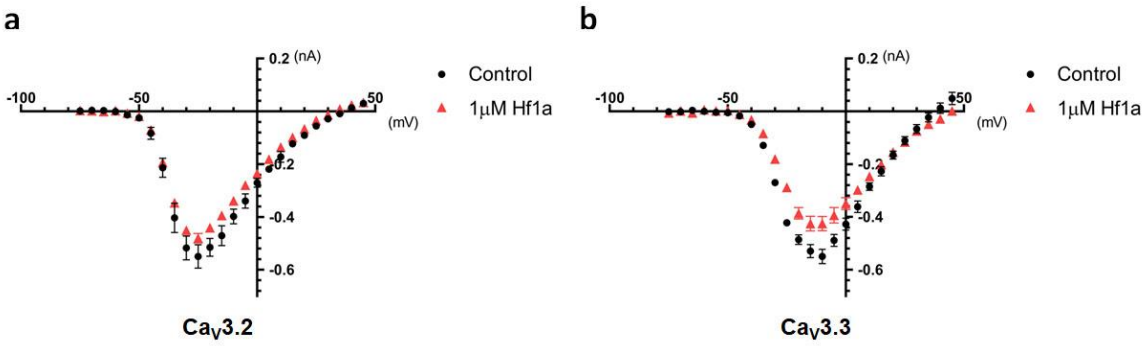


**Figure S4.** Comparison of the *I–V* relationships of Cav3.2 and Cav3.3 before (black) and after (red) the addition of 1 μM Hf1a-$NH_2$. The cells were holding at –100 mV before applying 50 ms pulses to potentials from –75 to +45 mV every 5 s in 5 mV increments. (**a**) *I–V* relationships of Cav3.2 ($n$ = 3) plotted from –75 mV to +45 mV, $V_{max}$ at –25 mV. (**b**) *I–V* relationships of Cav3.3 ($n$ = 3) plotted from –75 mV to +45 mV, $V_{max}$ at –10 mV. Error bars represent the SEM.

*1.4. Locomotor Performance Assessment*

Locomotor effects of 24h post administration of Hf1a-$NH_2$ and MVIIA were measured using a Process Control Treadmill apparatus by assessing distance covered and motor function of the mice. The distance covered for Hf1a-$NH_2$ and MVIIA treated mice showed no difference from vehicle treated mice, and no abnormal motor functions like foot slips were noticed during the run (Figure S5).

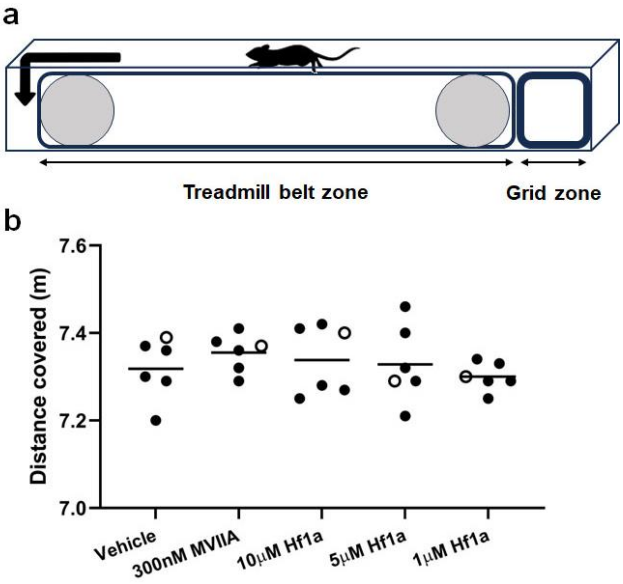


**Figure S5.** Locomotor behavior of mice assessed 48 h post-surgery. (**a**) Side view of the treadmill instrument; (**b**) There are no differences in the mean distance covered among Hf1a (1μM, 5μM and 10 μM Hf1a; 20 μL) injected mice, control peptide MVIIA (300 nM; 20 μL) injected mice and vehicle mice.

*1.5. Evaluation of insecticidal activities of ω-Buthitoxin-Hf1a against sheep blowflies*

Hf1a-NH2 and Hf1a-OH toxicity was tested against sheep blowflies (*Lucilia cuprina*), at doses1, 4 and 10 μg per fly, and no insecticidal activity was observed.

**2. Supplementary Method**

*1.1. Evaluation of insecticidal activities against sheep blowflies*

Peptide insecticidal toxicity was tested using 1, 4 and 10 μg peptide for three groups of Australian sheep blowflies (*Lucilia cuprina*), respectively, each group with 10 flies. Peptide was prepared in milliQ water, and a maximum volume of 2 μL was injected per fly using a 1.0 mL Terumo Insulin syringe (B-D Ultra-Fine, Terumo Medical Corporation, MD, USA) with a fixed 29-gauge needle fitted to an Arnold hand micro-applicator (Burkard Manufacturing Co. Ltd., Rickmansworth, UK). Flies were individually housed in 2 mL Eppendorf tubes and paralytic effects determined 0.5, 1, 2 and 24 h after injection. 20 flies injected with only milliQ water were set as control.