Supplementary Materials: New Insights into the Type II Toxins from the Sea Anemone *Heteractis crispa*

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Table S1. Hot spots of δ-SHTX-Hcr1f interchain non-covalent interactions with rNav1.2.

| Type  | δ-SHTX-Hcr1f | rNav1.2 | Energy contribution, kcal/mol | rNav1.2 region              |
|-------|--------------|---------|-------------------------------|-----------------------------|
| DIH   | Lys46        | Asp317  | -28.909                       | Ex1a-Ex1b linker            |
| DIH   | Lys48        | Lys355  | -27.764                       | Eβ1b                        |
| DIH   | Glu31        | Lys355  | -26.446                       | Eβ1b                        |
| DIH   | Asp7         | Lys355  | -23.855                       | Eβ1b                        |
| DIH   | Lys48        | Asp317  | -23.828                       | Ex1a-Ex1b linker            |
| DIH   | Lys48        | Glu318  | -19.798                       | Ex1b                        |
| DIH   | Lys46        | Glu318  | -13.38                        | Ex1b                        |
| DH    | Lys32        | Glu1616 | -8.574                        | S3 VSD-IV                   |
| DH    | Lys47        | Cys353  | -8.414                        | Eβ1b                        |
| DH    | Asp7         | Asn340  | -6.453                        | Ex1b-Eβ1b linker            |
| DH    | Lys32        | Ser1621 | -5.907                        | S4 VSD-IV                   |
| DH    | Arg45        | Asn285  | -5.338                        | Ex1a-Ex1b linker            |
| DH    | Asn25        | Glu1616 | -4.618                        | S3 VSD-IV                   |
| DH    | Val21        | Asn303  | -4.282                        | Ex1a-Ex1b linker            |
| DH    | Asn25        | Glu1613 | -3.713                        | S3 VSD-IV                   |
| DH    | Lys46        | Trp316  | -2.845                        | Ex1a-Ex1b linker            |
| D     | Asp8         | Ile352  | -2.838                        | Eβ1b                        |
| D     | Arg45        | Trp316  | -2.784                        | Ex1a-Ex1b linker            |
| DH    | Asp11        | Asn340  | -2.194                        | Ex1b-Eβ1b linker            |
| DH    | Glu28        | Val311  | -2.139                        | Ex1a-Ex1b linker            |
| DH    | Arg13        | Val1620 | -2.127                        | S3-S4 linker, VSD-IV         |
| DH    | Ser2         | Leu300  | -1.981                        | Ex1a                        |
| D     | Trp30        | Trp302  | -1.515                        | Ex1a                        |
| D     | Val36        | Lys1617 | -1.492                        | S3 VSD-IV                   |
| D     | Trp24        | Trp1565 | -1.47                         | S2 VSD-IV                   |
| D     | Asp6         | Val1620 | -1.238                        | S3-S4 linker, VSD-IV         |
| DH    | Asp8         | Cys353  | -1.199                        | Eβ1b                        |

1H - hydrogen bonds, I - ionic, D - distance (hydrophobic, van der Waals).

Table S2. δ-SHTX-Hcr1f residue Arg13 non-covalent interchain with phospholipid, adjacent to VSD-IV helix S4.

| Non-covalent interaction type | δ-SHTX-Hcr1f Arg13 atoms | DPPC atoms | Energy contribution, kcal/mol |
|-------------------------------|--------------------------|------------|------------------------------|
| Hbond                         | NH1                      | O13        | -8.1                         |
| Hbond                         | NH2                      | O13        | -7.3                         |
| Ionic                         | NH2                      | O13        | -6.707                       |
| Ionic                         | NH1                      | O13        | -6.152                       |
| Ionic                         | NH1                      | O14        | -2.119                       |
| Distance                      | NH2                      | P          | -0.361                       |
| Distance                      | CG                       | C13        | -0.316                       |
| Distance                      | CG                       | C15        | -0.272                       |
| Distance                      | CZ                       | P          | -0.171                       |
| Distance                      | CZ                       | C15        | -0.16                        |
| Distance                      | NH2                      | NC3        | -0.151                       |
| Distance | OD2     | C15     | -0.112 |
|---------|---------|---------|--------|
| Distance | CZ      | O14     | -0.07  |
| Distance | OD2     | C13     | -0.053 |
| Distance | OD2     | NC3     | -0.017 |
| Distance | OD1     | C13     | -0.016 |

1 HBond - hydrogen bonds, Distance (hydrophobic, van der Waals).