Materials/Methods

Preparation of STX-ge: To a solution of STX–hexylamine tris-heptafluorobutyrate (1.5 mg, 1.4 µmol) in 250 µL of a 1:3 mixture of pH 9.5 buffer (0.1 M NaHCO₃/Na₂CO₃) and MeCN was added the glycerol ether N-hydroxysuccinimide ester (1.9 mg, 4.6 µmol, 3.3 equiv). The solution was stirred for 2.25 h at room temperature, then acidified with 1 mL of 0.1% aqueous trifluoroacetic acid (TFA). The mixture was filtered through a Fisher 0.2 µm PTFE syringe filter and the filter washed with an additional 0.5 mL of 0.1% TFA. The filtrate was purified by reverse phase HPLC (Altima C18, 10 µm, 22 x 250 mm column, eluting with a gradient flow over 40 min of 25:75 MeCN/0.1% aqueous CF₃CO₂H → 80:20 MeCN/0.1% aqueous CF₃CO₂H, 214 nm UV detection). At a flow rate of 12 mL/min, STX-ge had a retention time of 21.4–22.8 min and was isolated following lyophilization as a white powder (0.7 mg, 0.75 µmol, 54%).

¹H NMR (D₂O, 500 MHz) δ 4.67 (s, 1H), 4.22 (dd, 1H, J = 10.0, 10.0 Hz), 4.03-3.98 (m, 1H), 3.80-3.74 (m, 2H), 3.68-3.64 (m, 1H), 3.62-3.46 (m, 11H), 3.14 (dd, 2H, J = 6.5, 6.5 Hz), 3.12-3.06 (m, 2H), 2.43-2.26 (m, 2H), 2.21 (dd, 2H, J = 7.0, 7.0 Hz), 1.62-1.43 (m, 12H), 1.36-1.28 (m, 10H), 0.89 (t, 6H, J = 7.0, 7.0 Hz) ppm; HRMS (ES⁺) calculated for C₃₃H₆₂N₈O₈ 698.4691 found at m/z 699.4760 (MH⁺).

Human tissue: Human skin samples were obtained from four patients undergoing plastic surgery at Stanford Medical School. All patients gave written informed consent following approved IRB protocols. Within minutes of surgical resection, the excess fat was carefully removed from the inner layers of the
skin specimen, which was then placed in a shallow dish partially filled with synthetic interstitial fluid. The surface of the skin was carefully handled and dried prior to compound application. Confined circular treatment areas (d = 1 cm) were created by delineating the application area with ink and surrounding the site with Vaseline. For each application site, 25 nmol of compound was applied in 25 µL of vehicle. After 1, 4 or 10 hours of application, the skin was frozen in a -80° C freezer. Five different compounds were tested on human skin: lidocaine (Sigma Aldrich), aconitine, saxitoxin, STX-ge, and BTX-A derivative. The two vehicles tested were ethanol (Fisher) and DMSO (Fisher).

**UV burn injury:** Confined circular treatment areas of the human skin samples were exposure to UV (300 to 450 nm, 3100mJ/cm²) irradiation before application of the compound. UV light was directly applied to skin over 25s using the BlueWave® 200 Version 3.0 UV Curing Spot Lamp with Intensity Adjustment (Dymax Inc., Torrington, CT). After UV exposure, 25 nmol of compound was applied in 25 µL of vehicle to each application site. After 4 hours of application, the skin was frozen in a -80° C freezer.

**Animals:** Animal experiments were conducted using ~300 g male Sprague Dawley rats. The hair on the back of the rats was removed 24 h before drug application. Under urethane anesthesia, confined circular treatment areas (d = 1 cm) were created by marking the application area with ink and surrounding the application area with Vaseline. For each application site, 25 nmol of the compound was applied in 25 µL of vehicle. After 4 hours of application the animal was sacrificed and its skin removed and frozen in -80° C freezer. The following compounds were tested in-vivo rat skin: 1) Saxitoxin, 2) Saxitoxinol, 3) STX-ge and 4) Aconitine in the following vehicles: water, ethanol, DMSO and a cream formulation.

**Tissue Sample Preparation:** Frozen skin samples were sectioned at 25 µm thickness using a Leica CM1950 cryostat (Leica Mycrosystems Inc., Buffalo Grove, IL). The tissue sections were thaw mounted onto positively charged glass slides. The cryostat section holder and blade were cleaned with 70% ethanol solution in between the preparation of each tissue section to avoid tissue contamination. The slides were stored at -80° C and prior to analysis dried in a dessicator for approximately 15 minutes.

**DESI Imaging:** A lab-built DESI-MS imaging ion source coupled to a LTQ-Orbitrap mass spectrometer was used for tissue imaging. Mass spectra were acquired in the positive ion mode using Orbitrap as the mass analyzer at 60,000 resolving power in the range m/z 100 - 1100. Pure acetonitrile (Fisher) was used as the histologically compatible solvent system for DESI-MSI at a flow rate of 2 µL/min, and
nitrogen gas pressure of 175 psi. The tissue sections were scanned using a 2D moving stage with spatial resolution of 100 µm or 150 µm. The software ImgGenerator (freeware, http://www.msimaging.net/) was used for converting raw files into 2D images. Spatially accurate ion images were assembled using BioMap software (freeware, http://www.maldi-msi.org/).

**Histology:** After DESI-MS imaging, the same tissue sections were stained using standard hematoxylin and eosin (H&E). H&E stained tissue sections were evaluated by veterinary pathologist Dr. Richard Luong. Optical images of the H&E stained tissue sections were obtained using a microscope Axioskop 2 Plus (Carl Zeiss International, Thornwood, NY) and digitally overlaid with DESI-MS ion images for compounds depth penetration calculation.

**Supporting Tables**

**Table S1.** Tentative identification of ions detected from human skin using high mass resolution/high mass accuracy and tandem mass spectrometry analyses.

| Ion detected (m/z) | Tentative Attribution[a] | Molecular Formula[b] | Mass error (ppm)[c] |
|-------------------|--------------------------|----------------------|---------------------|
| 140.0679          | 4 aminopentanoic acid    | C₅H₁₁NO₂Na           | 2.40                |
| 156.0417          | 4 aminopentanoic acid    | C₅H₁₁NO₃K            | 2.60                |
| 184.0729          | Phosphocholine            | C₅H₁₅NO₄P            | 2.18                |
| 518.3205          | Lyso-PC(16:0)             | C₂₄H₅₀NO₇PNa          | 1.99                |
| 534.2945          | Lyso-PC(16:0)             | C₂₄H₅₀NO₇PK           | 1.36                |
| 615.4940          | DG(34:2)                  | C₃₇H₆₈O₅             | 2.90                |
| 639.4947          | DG(36:4)                  | C₃₉H₆₈O₅Na           | 1.88                |
| 641.5092          | DG(36:3)                  | C₃₉H₇₀O₅Na           | 3.60                |
| 672.4198          | PC(25:0)                  | C₃₃H₆₄NO₉PNa          | 1.80                |
| 688.3937          | PC(25:0)                  | C₃₃H₆₄NO₉PK           | 1.80                |
| 715.5622          | DG(40:2)                  | C₄₃H₈₂O₅K            | 2.10                |
| 717.5781          | DG(40:1)                  | C₄₃H₈₂O₅K            | 1.70                |
| 719.5941          | DG(40:0)                  | C₄₃H₈₄O₅K            | 1.30                |
| 725.5557          | SM(34:1)                  | C₃₉H₇₉N₂O₆PNa         | 0.88                |
| 741.5302          | SM(34:1)                  | C₃₉H₇₉N₂O₆PK          | 0.70                |
| 756.5502          | PC(32:0)                  | C₄₀H₈₀NO₅PNa          | 0.98                |
772.5240  PE(38:5)  C_{43}\text{H}_{76}\text{NO}_7\text{PNa}  1.10
772.5241  PC(32:0)  C_{40}\text{H}_{80}\text{NO}_8\text{PK}  0.91
780.5505  PC(34:2)  C_{42}\text{H}_{80}\text{NO}_8\text{PNa}  0.90
782.5681  PC(34:1)  C_{42}\text{H}_{82}\text{NO}_8\text{PNa}  0.02
785.5681  PG(36:0)  C_{42}\text{H}_{83}\text{O}_9\text{PNa}  1.80
787.5839  PG(36:0)  C_{42}\text{H}_{85}\text{O}_9\text{NaP}  2.10
796.5240  PC(34:2)  C_{42}\text{H}_{80}\text{NO}_8\text{PK}  1.22
798.5406  PC(34:1)  C_{42}\text{H}_{82}\text{NO}_8\text{PK}  0.11
804.5502  PC(36:4)  C_{44}\text{H}_{80}\text{NO}_8\text{PK}  1.10
806.5673  PC(36:3)  C_{44}\text{H}_{82}\text{NO}_8\text{PNa}  0.79
808.5819  PC(36:2)  C_{44}\text{H}_{84}\text{NO}_8\text{PNa}  0.72
811.5840  PG(38:2)  C_{44}\text{H}_{85}\text{O}_9\text{NaP}  2.20
813.5999  PG(38:2)  C_{44}\text{H}_{87}\text{O}_9\text{NaP}  2.60
824.5556  PC(36:2)  C_{44}\text{H}_{84}\text{NO}_8\text{PK}  0.72
825.6926  TG(48:2)  C_{51}\text{H}_{94}\text{O}_6\text{Na}  2.00
828.5358  PC(38:6)  C_{46}\text{H}_{80}\text{NO}_7\text{PK}  7.50
832.5816  PC(38:4)  C_{46}\text{H}_{84}\text{NO}_8\text{PNa}  1.18
848.5555  PC(38:4)  C_{46}\text{H}_{84}\text{NO}_8\text{PK}  1.37
849.6932  TG(50:4)  C_{53}\text{H}_{94}\text{O}_6\text{Na}  1.27
851.7086  TG(50:3)  C_{53}\text{H}_{96}\text{O}_6\text{Na}  2.02
851.7091  TG(50:3)  C_{53}\text{H}_{96}\text{O}_6\text{Na}  0.94
853.7252  TG(50:2)  C_{53}\text{H}_{98}\text{O}_6\text{Na}  0.50
855.7417  TG(50:1)  C_{53}\text{H}_{100}\text{O}_6\text{Na}  0.57
867.6822  TG(50:3)  C_{53}\text{H}_{98}\text{O}_6\text{K}  1.90
869.6984  TG(50:2)  C_{53}\text{H}_{98}\text{O}_6\text{K}  1.30
875.7086  TG(50:5)  C_{55}\text{H}_{96}\text{O}_6\text{Na}  1.54
877.7244  TG(50:4)  C_{55}\text{H}_{98}\text{O}_6\text{Na}  1.36
879.7406  TG(54:6)  C_{55}\text{H}_{100}\text{O}_6\text{Na}  0.57
881.7582  TG(54:6)  C_{55}\text{H}_{102}\text{O}_6\text{Na}  2.25
891.6826  TG(52:5)  C_{55}\text{H}_{98}\text{O}_6\text{K}  1.20
893.6984  TG(52:4)  C_{55}\text{H}_{98}\text{O}_6\text{K}  1.20
895.7150  TG(52:3)  C_{55}\text{H}_{100}\text{O}_6\text{K}  0.09
897.7321  TG(54:5)  C_{55}\text{H}_{102}\text{O}_6\text{K}  1.70
| Mass (m/z) | Formula          | Tentative Assignments |
|-----------|------------------|-----------------------|
| 899.7084  | TG(54:5)         | C₅₅H₁₀₂O₆K           | 2.00 |
| 901.7244  | TG(54:6)         | C₅₇H₉₈O₆Na           | 1.38 |
| 903.7415  | TG(54:5)         | C₅₇H₁₀₀O₆Na           | 0.98 |
| 905.7570  | TG(56:7)         | C₉₉H₁₀₀O₆H            | 2.50 |
| 907.7733  | TG(54:3)         | C₅₇H₁₀₄O₆Na           | 1.56 |
| 907.7735  | TG(56:8)         | C₉₉H₁₀₃O₆             | 1.70 |
| 917.6985  | TG(56:7)         | C₅₇H₉₈O₆K            | 1.10 |
| 919.7145  | TG(54:5)         | C₅₇H₁₀₀O₆K            | 0.76 |
| 921.7309  | TG(54:4)         | C₅₇H₁₀₂O₆K            | 0.11 |
| 923.7472  | TG(54:3)         | C₅₇H₁₀₄O₆K            | 0.99 |
| 929.7557  | TG(56:6)         | C₉₉H₁₀₂O₆Na           | 0.40 |
| 943.7137  | TG(56:5)         | C₉₉H₁₀₀O₆K            | 0.70 |
| 945.7298  | TG(56:6)         | C₉₉H₁₀₂O₆K            | 0.22 |
| 947.7459  | TG(56:7)         | C₉₉H₁₀₄O₆K            | 0.22 |

[a] Tentative assignments were based on data obtained from tandem mass spectrometry experiments and high mass accuracy measurements. TG = triacylglycerol; PC = glycerophosphocholine; DG = diacylglycerol; SM = sphingomyelins; PG = glycerophosphoglycerols; PE = glycerophosphoethanolamines; (X:Y) denotes the total number of carbons and double bonds in the fatty acid chains.

[b] Molecular formulas of the protonated or sodium/potassium adducts of the assigned molecules.

[c] Mass errors were calculated based on the exact monoisotopic m/z of the protonated or sodium/potassium adducts of the assigned molecules.
Table S2. Average penetration depth in human and rat skins for each compound studied using different vehicles.

### Human Skin

| Vehicle | Aconitine | Lidocaine | STX-ge   | BTX-A   |
|---------|-----------|-----------|----------|---------|
| Ethanol | 3.05 ± 0.91 | 1.24 ± 0.38 | *No penetration* | 2.00 ± 0.61 |
|         | (n = 6)   | (n = 5)   | (n = 7)  | (n = 4) |
| DMSO    | N/A       | N/A       | 0.62 ± 0.03 | N/A     |
|         | (n = 3)   |           |           |         |
| EMLA    | 2.49 ± 0.84 | N/A       | 0.45 ± 0.10 | 1.10 ± 0.20 |
|         | (n = 4)   |           | (n = 4)   | (n = 4) |

### Rat Skin

| Vehicle | Aconitine | STX-ge |
|---------|-----------|--------|
| Ethanol | 1.90 ± 0.40 | 0.90 ± 0.14 |
|         | (n = 7)   | (n = 5) |
| DMSO    | 2.60 ± 0.06 | 1.12 ± 0.42 |
|         | (n = 3)   | (n = 7) |

Supporting Figures

**Figure S1.** Selected positive ion mode DESI-MS ion images of human skin sample and optical image of the same tissue section images by DESI after H&E stain are shown.
Figure S2. Positive ion mode DESI-MS mass spectrum of human skin from $m/z$ 100 - 1000. Inset shows the range $m/z$ 699.30 - 699.60 in which peaks of the compound STX-ge and an endogenous lipid (detected at the same nominal $m/z$) are resolved using high mass resolution DESI-MSI. ($R =$ Orbitrap resolving power at the $m/z$ shown).

Figure S3. DESI-MS ion images showing the penetration of STX-ge using EMLA cream as vehicle in two human skin tissue sections. Ion images showing the penetration of lidocaine and prilocaine, compounds with are both present in the EMLA cream formulation, are also presented for the two sections analyzed.
Figure S4. DESI-MS ion images showing the penetration of BTX-A, aconitine and lidocaine using ethanol as vehicle applied to human skin after UV burn.

Figure S5. DESI-MS ion images showing the penetration of aconitine using ethanol as vehicle after 1, 4 and 10 hours of topical application.

Figure S6. DESI-MS ion images showing the penetration of STX-ge tested in in vivo rat skin using a) ethanol and b) DMSO as vehicles. Optical images of the same tissue sections imaged by DESI after H&E stain are also shown.
References:

[1] F. T. M. C. Vicentini, Y. M. Fonseca, D. L. Pitol, M. M. Iyomasa, M. V. L. B. Bentley, M. J. V. Fonseca, *Journal of Pharmacy and Pharmaceutical Sciences* **2010**, *13*, 274-285.