SUPPLEMENTARY CONTENT

Synthesis of glycerolipids containing simple linear acyl chains or aromatic rings and evaluation of their Mincle signaling activity

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GENERAL METHODS

$^1$H NMR spectra were recorded at 400 MHz or 500 MHz and $^{13}$C NMR spectra were recorded at 100 MHz or 125 MHz on JEOL ECS400, ECX400, and ECA500 instruments. The chemical shifts are expressed in ppm downfield from the internal solvent peaks CDCl$_3$ (7.26 ppm, $^1$H NMR), CD$_3$OD (3.31 ppm, $^1$H NMR), CDCl$_3$ (77.0 ppm $^{13}$C NMR), and CD$_3$OD (49.0 ppm $^{13}$C NMR), and $J$ values are expressed in hertz. The coupling patterns are expressed as s (singlet), d (doublet), t (triplet), m (multiplet), br (broad) or brs (broad singlet). ESI mass spectra were recorded on a Waters ACQUITY UPLC/Xevo G2-S QTof or electron spray ionization quadrupole time of flight (ESI-QTOF) mass spectrometer (micrOTOF-QII-HC; BRUKER). Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60F254 plates. Flash column chromatography was performed on Fji Sylysia silica gel PSQ 60B.

2,3-Dihydroxypropyl behenate (2a)

![Chemical structure of 2,3-Dihydroxypropyl behenate (2a)](image)

To a stirred solution of behenic acid (500 mg, 1.47 mmol) in toluene (1.5 mL) was added SOCl$_2$ (19.9 µL, 2.20 µmol) and 1 drop of DMF. After stirring for 2 h at 40 °C, the mixture was evaporated, and toluene was added and evaporated again three times. The residue was concentrated under reduced pressure to give the desired acid chloride, which was used for the next reaction without further purification.

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (182 µL, 1.47 mmol) in DCM (5 mL) was added pyridine (237 µL, 2.94 mmol) and the afforded acid chloride at 0 °C. After stirring overnight at the same temperature, the mixture was supplemented with 1 M HCl and extracted with CHCl$_3$. The organic layer was dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (n-hexane/EtOAc = 8:1) to yield an isopropylidene glycerolipid.

To a stirred solution of isopropylidene glycerolipid in DCM (1.0 mL) and MeOH (1.0 mL) was added amberlyst (100 mg). After stirring for 12 h at room temperature, the mixture was filtered and washed with MeOH. The organic layer was dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure to give a crude product, which was purified...
by silica gel column chromatography (n-hexane/EtOAc = 6:1) to yield a mixture of mono acyl glycerolipids (35.6 mg, 29% over 2 steps). The major compound is a title compound: 1H NMR (400 MHz, CDCl3) δ (ppm): 4.23-4.13 (ddd, J = 3.6, 9.2, 15.2 Hz, 2H), 3.96-3.91 (m, 1H), 3.72-3.68 (m, 1H), 3.62-3.57 (m, 1H), 2.35 (t, J = 7.3 Hz, 2H), 1.63 (m, 2H), 1.30-1.25 (complex m, 36 H), 0.88 (t, J = 6.8 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ (ppm): 174.4, 70.2, 65.2, 63.3, 34.1, 31.9, 29.7-29.1 (many unresolved C’s), 24.9, 22.7, 14.1; HRMS-ESI (m/z): [M+Na]+ calc’d C31H62O4Na, 437.3601 found, 437.3609.

2,3-Dihydroxypropyl tetracosanoate (2b)

To a stirred solution of tetracosanoic acid (100 mg, 271 µmol) in toluene (271 µL) was added SOCl2 (43.0 µL, 596.7 µmol) and 1 drop of DMF. After stirring for 2.5 h at 40 °C, the mixture was evaporated, and toluene was added and evaporated again. The residue was concentrated under reduced pressure to give the desired acid chloride, which was used for the next reaction without further purification.

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (30.5 µL, 247 µmol) in DCM (4.9 mL) was added pyridine (21.9 µL, 271 µmol) and the afforded acid chloride at 0 °C. After stirring for 14.5 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with CHCl3. The organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (n-hexane/EtOAc = 6:1) to yield an isopropylidene glycerolipid.

To a stirred solution of isopropylidene glycerolipid in DCM (440 µL) and MeOH (440 µL) was added amberlyst (86 mg). After stirring for 1 h at 60 °C, the reaction was cooled to room temperature. The mixture was filtered and washed with MeOH. The organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (n-hexane/EtOAc = 6:1) to yield a mixture of mono acyl glycerolipids (41.5 mg, 38% over 2 steps). Major compound is a title compound: 1H NMR (500 MHz, CDCl3) δ (ppm): 4.24-4.10 (m, 2H), 3.93 (m, 1H), 3.69 (m, 1H), 3.61 (m, 1H), 2.35 (t, J = 7.5 Hz, 2H), 1.63 (m, 2H), 1.29-1.22 (complex m, 40 H), 0.88 (t, J = 7.5 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ (ppm): 174.4, 70.2, 65.2, 63.3, 34.1, 31.9, 29.7-29.1 (many unresolved C’s), 24.9, 22.7, 14.1; HRMS-ESI (m/z): [M+Na]+ calc’d C27H54O4Na, 465.3914 found, 465.3927.

2,3-Dihydroxypropyl hexacosanoate (2c)

To a stirred solution of tetracosanoic acid (100 mg, 252 µmol) in toluene (252 µL) was added SOCl2 (40.0 µL, 555 µmol) and 1 drop of DMF. After stirring for 1.5 h at 40 °C, the mixture was evaporated, and toluene was added and evaporated again. The residue was concentrated under reduced pressure to give the desired acid chloride, which was used for the next reaction without further purification.
To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (28.3 µL, 229 µmol) in DCM (4.6 mL) was added pyridine (18.5 µL, 229 µmol) and the afforded acid chloride at 0 °C. After stirring for 15.5 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with CHCl₃. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to yield a crude product, which was purified by short silica gel column chromatography (n-hexane/EtOAc = 6:1) to give an isopropylidene glycerolipid.

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (24.5 µL, 198 µmol) in DCM (4 mL) was added pyridine (16.0 µL, 198 µmol) and the afforded acid chloride at 0 °C. After stirring for 15.5 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with CHCl₃. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to yield a mixture of mono acyl glycerolipids (22.4 mg, 21% over 2 steps). The major compound is a title compound: ¹H NMR (400 MHz, CDCl₃) δ (ppm); 4.23-4.13 (m, 2H), 3.93 (m, 1H), 3.70 (m, 1H), 3.60 (m, 1H), 2.35 (t, J = 7.3 Hz, 2H), 1.63 (m, 2H), 1.30-1.25 (complex m, 44 H), 0.88 (t, J = 7.3 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ (ppm); 174.5, 70.4, 65.3, 63.5, 34.3, 32.1, 29.9-29.3 (many unresolved C’s), 25.1, 22.9, 14.3; HRMS-ESI (m/z): [M+Na]⁺ calcd C₂₀H₃₈O₃Na, 493.4227 found, 493.4240.

2,3-Dihydroxypropyl octacosanoate (2d)

To a stirred solution of octacosanoic acid (92.5 mg, 218 µmol) in toluene (500 µL) was added SOCl₂ (34.6 µL, 518 µmol) and 1drop of DMF. After stirring for 1.5 h at 40 °C, the mixture was evaporated, and toluene was added and evaporated again. The residue was concentrated under reduced pressure to yield the desired acid chloride, which was used for the next reaction without further purification.

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (24.5 µL, 198 µmol) in DCM (4 mL) was added pyridine (16.0 µL, 198 µmol) and the afforded acid chloride at 0 °C. After stirring for 15.5 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with CHCl₃. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (n-hexane/EtOAc = 8:1) to yield a mixture of isopropylidene glycerolipid.

To a stirred solution of isopropylidene glycerolipid in DCM (300 µL) and MeOH (300µL) was added amberlyst (37 mg). After stirring for 3 h at 60 °C, the reaction was cooled to room temperature. The mixture was filtered and washed with MeOH. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (n-hexane/EtOAc = 4:1) to yield a mixture of mono acyl glycerolipids (9.8 mg, 10% over 2 steps). The major compound is a title compound: ¹H NMR (400 MHz, CDCl₃) δ (ppm); 4.23-4.13 (m, 2H), 3.93 (m, 1H), 3.68 (m, 1H), 3.61 (m, 1H), 2.35 (t, J = 7.3 Hz, 2H), 1.63 (m, 2H), 1.30-1.25 (complex m, 48 H), 0.88 (t, J = 6.8 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ (ppm); 174.4, 70.2, 65.2, 63.3, 34.1, 31.9, 29.7-29.1 (many unresolved C’s), 24.9, 22.7, 14.1; HRMS-ESI (m/z): [M+Na]⁺ calcd C₃₁H₆₂O₃Na, 521.4540 found, 521.4545.

2,3-Dihydroxypropyl triacontanoate (2e)
To a stirred solution of mellisic acid (21.3 mg, 47.0 µmol) in DCM (500 µL) was added (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (5.8 µL, 47.0 µmol), DMAP (0.3 mg, 0.24 µmol) and WSCI (10.0 mg, 52.3 µmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was purified by short column chromatography (CHCl₃) to afford an acylated compound (9.4 mg, 35%). To a stirred solution of acylated compound (7.9 mg, 13.9 µmol) in a mixture of AcOH (0.75 mL) and H₂O (0.25 mL) was heated to 65 °C and stirred at overnight. After cooling to room temperature, the solvent was removed in vacuo. The residue was purified by flash column chromatography (Hex/EtOAc 1/1) to yield a mixture of mono acyl glycerolipid (4.0 mg, 55%). The major compound is a title compound: ¹H NMR (400 MHz, CDCl₃) δ (ppm); 4.24-4.13 (m, 2H), 3.96-3.91 (m, 1H), 3.70 (m, 1H), 3.72-3.68 (dd, J = 4.0, 11.6 Hz, 1H), 3.62-3.58 (dd, J = 6.0, 11.6 Hz, 1H), 2.35 (t, J = 7.6 Hz, 2H), 1.63 (m, 2H), 1.25-1.30 (complex m, 4H), 0.88 (t, J = 6.8 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ (ppm); 174.9, 70.4, 65.7, 63.4, 34.1, 31.9, 29.9-29.3 (many unresolved C’s), 24.9, 22.7, 14.1; HRMS-ESI (m/z): [M+Na]^+ calcd C₃₃H₆₆O₄Na, 549.4853 found, 549.4860.

(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl benzoate (4a)

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (500 mg, 3.78 mmol) in DCM (75.6 mL) was added pyridine (335 µL, 4.16 mmol) and benzoyl chloride (479 µL, 4.16 mmol) at 0 °C. After stirring for 26 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with CHCl₃, and the obtained organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (n-hexane/EtOAc = 2:1) to yield a title compound (206.8 mg, 23% yield). ¹H NMR (400 MHz, CDCl₃) δ (ppm); 8.05 (m, 2H), 7.57 (m, 1H), 7.45 (t, 2H), 4.40 (complex m, 3H), 4.16 (m, 1H), 4.16 (m, 1H), 3.89 (m, 1H), 1.46 (s, 3H), 1.40 (s, 3H), HRMS-ESI (m/z): [M+Na]^+ calcd C₁₀H₁₂O₄Na, 219.0628 found, 219.0832.

2,3-Dihydroxypropyl benzoate (5a)

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl)methyl benzoate in DCM (936 µL) and MeOH (936 µL) was added amberlyst (185 mg). After stirring for 1 h at 60°C, the reaction was cooled to room temperature. The mixture was filtered and washed with MeOH. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (n-hexane/EtOAc = 1:1) to yield a mixture of mono acyl glycerolipids (80.4 mg, 85% yield). The major compound is a title compound: ¹H NMR(400
To a stirred solution of 4-(octyloxy) benzoic acid (214.0 mg, 809.5 µmol) in Toluene (809 µL) was added SOCl₂ (128.4 µL, 1.78 mmol) and 1 drop of DMF. After stirring for 2.5 h at 40 °C, the mixture was evaporated, and added Toluene and evaporated again. Then the residue was concentrated under reduced pressure to give a desired acid chloride, which was used to the next reaction without further purification.

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl)methanol (90.9 µL, 735.9 µmol) in DCM (7.4 mL) was added pyridine (89.5 µmol) and 4-(octyloxy) benzoyl chloride at 0 °C. After stirring for 19 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with CHCl₃, and the obtained organic layer was washed by H₂O. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (n-hexane/EtOAc = 2:1) to yield a title compound (73.4 mg, 63%)

1H NMR (500 MHz, CDCl₃) δ (ppm): 7.99 (d, J = 9.2 Hz, 2H), 6.91 (d, J = 8.6 Hz, 2H), 4.38 (complex m, 3H), 3.75 (m, 1H), 3.68 (m, 1H), 1.80 (m, 2H), 1.46 (m, 2H), 1.4-1.23 (complex m, 10H), 0.89 (t, J = 6.9 Hz, 3H) HRMS-ESI (m/z): [M+Na]⁺ calcd C₁₂H₂₃O₅Na, 387.2142 found, 387.2147.

2,3-Dihydroxypropyl 4-(octyloxy)benzoate (5b)

To a stirred solution of isopropylidene glycerolipid (31.2 mg, 85.6 µmol) in DCM (251 µL) and MeOH (251 µL) was added amberlyst (50 mg). After stirring for 2 h at 70°C, the reaction was cooled to room temperature. The mixture was filtered and washed with MeOH. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (n-hexane/EtOAc = 1:1) to yield a mixture of mono acyl glycerolipids (13.1 mg, 47% yield). The major compound is a title compound; 1H NMR (500 MHz, CDCl₃) δ (ppm); 7.99 (d, J = 8.6 Hz, 2H), 6.91 (d, J = 8.6 Hz, 2H), 4.41 (m, 2H), 4.02 (complex m, 3H), 3.75 (m, 1H), 3.68 (m, 1H), 1.80 (m, 2H), 1.46 (m, 2H), 1.4-1.23 (complex m, 10H), 0.89 (t, J = 6.9 Hz, 3H) HRMS-ESI (m/z): [M+Na]⁺ calcd C₁₈H₃₈O₇Na, 347.1829 found, 347.1838.

(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 2-phenylacetate (7a)
To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (500 mg, 3.78 mmol) in DCM (75.6 mL) was added pyridine (335 µL, 4.16 mmol) and phenylacetyl chloride (549 µL, 4.16 mmol) at 0 °C. After stirring for 16 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with CHCl₃, and the obtained organic layer was washed by H₂O. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (n-hexane/EtOAc = 3:1) to yield a title compound (768.0 mg, 81% yield).

1H NMR (400 MHz, CDCl₃) δ (ppm): 7.23 (complex m, 5H), 4.31 (m, 1H), 4.16 (m, 2H), 4.03 (m, 1H), 3.69 (complex m, 3H), 1.41 (s, 3H), 1.36 (s, 3H).


2,3-Dihydroxypropyl 2-phenylacetate (8a)

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl)methyl 2-phenylacetate (328 mg, 1.31 mmol) in DCM (3.5 mL) and MeOH (3.5 mL) was added amberlyst (689 mg). After stirring for 1 h at 60°C, the reaction was cooled to room temperature. The mixture was filtered and washed by MeOH. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (n-hexane/EtOAc = 1:1) to yield a mixture of mono acyl glycerolipids (145.1 mg, 53% yield). The major compound is a title compound: 1H NMR (500 MHz, CD₂OD) δ (ppm): 7.26 (complex m, 5H), 4.17 (m, 1H), 4.09 (m, 1H), 3.82 (m, 2H), 3.52 (m, 2H); 13C NMR (100 MHz, CDCl₃) δ (ppm): 172.1, 133.6, 129.3 (2C), 128.6 (2C), 127.2, 69.9, 65.4, 63.2, 41.0.

(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 2-(4-(octyloxy)phenyl)acetate (7b)

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl)methyl 2-phenylacetate (328 mg, 1.31 mmol) in DCM (3.5 mL) and MeOH (3.5 mL) was added pyridine (335 µL, 4.16 mmol) and obtained phenylacetyl chloride (549 µL, 4.16 mmol) at 0 °C. After stirring for 16 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with CHCl₃, and the obtained organic layer was washed by H₂O. The organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (n-hexane/EtOAc = 3:1) to yield a title compound (768.0 mg, 81% yield). 1H NMR (400 MHz, CDCl₃) δ (ppm): 7.23 (complex m, 5H), 4.31 (m, 1H), 4.16 (m, 2H), 4.03 (m, 1H), 3.69 (complex m, 3H), 1.41 (s, 3H), 1.36 (s, 3H).

a crude product, which was purified by short silica gel column chromatography (n-hexane/EtOAc = 2:1) to yield a title compound (165.1 mg, 59% yield). 1H NMR (400 MHz, CDCl3) δ (ppm) 7.18 (d, 2H, J = 8.6 Hz), 6.84 (d, J = 8.6 Hz, 2H), 4.30 (m, 1H), 4.14 (m, 2H), 4.04 (dd, J = 6.9, 1.7 Hz, 1H), 3.93 (dd, J = 6.3 Hz, 2H), 3.70 (dd, J = 6.3, 2.3 Hz, 1H), 3.59 (s, 2H), 1.76 (m, 2H), 1.46 - 1.22 (complex m, 16H), 0.88 (t, J = 7.2 Hz, 3H). HRMS-ESI (m/z): [M+Na]+ calcd C22H34O5Na, 401.2298 found, 401.2304.

2,3-Dihydroxypropyl 2-(4-(octyloxy)phenyl)acetate (8b)

HO
O
K
OH

To a stirred solution of isopropylidene glycerolipid (82.5 mg, 218.1 µmol) in DCM (651 µL) and MeOH (651 µL) was added amberlyst (128 mg). After stirring for 2 h at 70˚C, the reaction was cooled to room temperature. The mixture was filtered and washed by MeOH. The organic layer was dried over anhydrous Na2SO4 and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (n-hexane/EtOAc = 1:2) to yield a mixture of mono acyl glycerolipids (61.1 mg, 83% yield). The major compound is a title compound: 1H NMR (400 MHz, CDCl3) δ (ppm) 7.17 (d, J = 9.0 Hz, 2H), 6.85 (d, J = 8.5 Hz, 2H), 4.19 (m, 2H), 3.91 (complex m, 3H), 3.59 (complex m, 4H), 1.77 (m, 2H), 1.44 (m, 2H), 1.22-1.39 (complex m, 8H), 0.88 (t, J = 6.7 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ (ppm) 172.4, 158.3, 130.2 (2C), 125.3, 114.6 (2C), 70.1, 68.0, 65.5, 63.2, 40.2, 31.8, 29.3, 29.2 (2C), 26.0, 22.6, 14.1.; HRMS-ESI (m/z): [M+Na]+ calcd C19H30O5Na, 361.1985 found, 361.1992.

The calculation of the rate of 2-acyl compound in each mono acyl glycerol (2a-2e) described in Fig. 2A

We calculated the rate of the 2-acyl compounds in each inseparable mixture with the integrational ratio of the peak at 3.84 ppm. The peak is ascribed to the methylene (4H) next to the hydroxyl group of 2-acyl glycerol. In the case of the glycerolipid (C22) described in Fig. 2A, the integrational ratio of the peak at 3.84 ppm indicated 0.17 compared to the integrational ratio of hydrogen from the 1-acyl compound in 1H NMR (Fig. S1); the existing rate of the 2-acyl compound was calculated as 4.1% in the mixture. With this method, we calculated the percentage of the 2-acyl compounds in each C22-C30 mono acyl glycerol (2a-2e) shown in Table S1.

Fig. S1 The enlarged view of 1H-NMR spectra of the mixture of dihydroxypropyl behenate (C22).
Table S1

<table>
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<th>The lengths of acyl chain</th>
<th>The integrational ratio of the peak at 3.84 ppm</th>
<th>The percentage of the 2-acyl compounds</th>
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<td>C22</td>
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<tr>
<td>C24</td>
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Complex model
The structural model of Mincle in Fig. 4 was prepared by homology modeling. The high-resolution crystal structures of bovine Mincle in complex with trehalose (PDB code: 4KZV), which coordinates the loop of N170-D177 to open the hydrophobic groove, was used as the starting model. The homology modeling was performed by referring to the amino acid sequence of human Mincle by prime (Schrödinger, LLC). The sequence of C78-E208 in bovine Mincle was used to the homology modeling. The amino acid sequence numbers in the main text and Fig. 4 are based on the corresponding amino acid residues from bovine Mincle. After the ligand was set up on the homology model as mentioned in the text, the ligand performed the minimization by prime (Schrödinger, LLC). The protein surfaces are colored as shown below; dark green: hydrophobic residues (ALA, CYS, ILE, LEU, MET, PHE, TRP, TYR, VAL, PRO), cyan: polar uncharged (SER, THR, HIS, GLN, ASN), blue: positively charged (LYS, ARG), and red: negatively charged (ASP, GLU).

Reporter cell assay
Reporter cell assay was performed as previous reported.1 NFAT-GFP reporter cells expressing human or murine Mincle with FcRγ were prepared. Each glycolipid dissolved in chloroform:methanol (2:1) at 1 mg/ml was diluted in isopropanol, added to 96-well plates at 20 µl per well, followed by the evaporation of the solvent. After drying out, each reporter cell was added to 96-well plates in 200 µl per well (ca. 60000 cells per well). Reporter cells were stimulated for 18 h. Reporter activity was measured by flow cytometry. Error bar indicates ± standard deviation of three independent experiments.

Reference