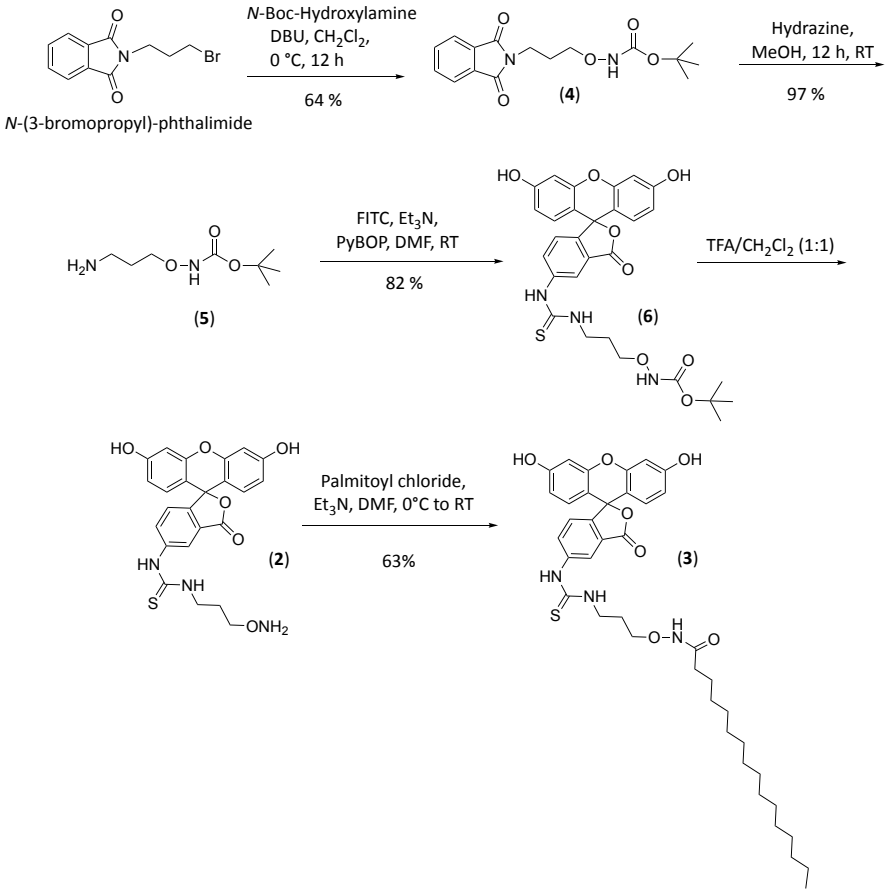
General Information

All commercially available compounds were used as provided without further purifications. Chemicals and solvents were purchased from Sigma Aldrich, Alfa Aesar, Acros Organics. Merck Chemicals, Life Technologies, Santa Cruz Biotechnology and Fisher Scientific. The phospholipids 1, 2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1, 2-di-palmitoyl-snglycero-3-phosphocholine (DPPC) were purchased from Avanti Polar Lipids (Alabaster, AL). Cholesterol (Chol) was from Sigma-Aldrich and the fluorescent lipids N-(lissamine rhodamine B sulfonyl)-1, 2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethyl ammonium salt (N-Rh-DHPE) from Molecular Probes (Invitrogen). Analytical thin-layer chromatography (TLC) was performed on Merck silica gel aluminum plates with F-254 indicator. Compounds were visualized by UV light, potassium permanganate staining or cerium ammonium molybdate (CAM) staining. Column chromatography was performed using silica gel Acros 60 Å (particle size 0.035-0.070 mm). NMR were recorded on a Bruker Avance DRX-400 or 500 (400 or 500 MHz) or on an Agilent Technologies Mercury Inova-600 (600MHz). Chemical shifts (δ) are reported in part per million (ppm) referenced with respect to residual solvent. Signal characterization is described using the next abbreviations: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Coupling constant values are presented in Hertz (Hz). High resolution mass spectra were recorded on an LTQ Orbitrap mass spectrometer coupled to an Accela HPLC-System (flow injection: 50% (containing 0.1% formic acid) water and 50% (containing 0.1% formic acid) acetonitrile, flow rate: 250 µL/ min). Preparative HPLC-MS was performed on an Agilent Series 1100/LC/MSD VL system, using a Macherey & Nagel Nucleodur VP C4 or C18 column (Solution A: 0.1% TFA in water and Solution B 0.1% TFA in acetonitrile). Analytical HPLC-MS was recorded on an Ultimate 3000 (Thermo Fisher Scientific) using a Nucleodur C4 (EC 125/3) or C18 (EC 50/3) column. Mass detection was performed through an LCQ Fleet (Thermo Fisher Scientific) using HESI as ionization technique.

Synthesis of the hydroxylamine 2 and the FAH 3.



Scheme S1. Synthesis of **2** and the corresponding reference compound **3**.

***N*-(3-*tert*-butyl-*N*-propoxycarbamate)phthalimide (4)**

10 g of *N*-(3-bromopropyl)-phthalimide (37.3 mmol, 1 eq) and 9.93 g of *tert*-butyl-*N*-hydroxycarbamate (74.6 mmol, 2 eq) were dissolved in CH_2Cl_2. The mixture was cooled to 0 ° C and 22.26 mL of diazabicycloundecene (149.19 mmol, 4 eq) were added dropwise. The reaction was stirred at room temperature for 12 h, diluted with CH_2Cl_2 and extracted with 1 M HCl (2 times). The organic layer was dried over $MgSO_4$ and concentrated under

low pressure. The product was purified by column chromatography (EtOAc/CH_2Cl_2 (1:9)). Yield: 7.56 g (23.60 mmol, 64 %); R_f: 0.63 MeOH/EtOAc (9:1); HPLC-MS: m/z calculated for $C_{16}H_{20}N_2O_5$: 321.13 $[M+H]^+$, found 321.14 $[M+H]^+$; HR-MS: m/z calculated for $C_{16}H_{20}N_2O_5$: 321.14450 $[M+H]^+$, found 321.14465 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.91 - 7.70 (m, 4H), 7.27 (s, 1H), 3.73 (t, J = 5.8 Hz, 2H), 3.65 (t, J = 7.0 Hz, 2H), 1.85 (q, J = 6.3 Hz, 2H), 1.38 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 167.8, 156.1, 134.2, 131.3, 122.9, 79.5, 72.9, 34.8, 27.9, 26.8.

***tert*-butyl (3-aminopropoxy) carbamate (5)**

7.56 g of *N*-(3-*tert*-butyl-*N*-propoxycarbamate)phthalimide (0.023 mol, 1 eq) were dissolved in methanol and 2.99 mL of hydrazine (0.09 mol, 4 eq) were added dropwise and the reaction was stirred for 12 h at room temperature. The solvent was removed under reduced pressure and the residue was taken up in chloroform. The white precipitate was filtered off and the filtrate was concentrated. The product was purified by column chromatography (MeOH/CH_2Cl_2 (1: 9)). Yield: 4.39 g (0.02 mol, 97 %); HPLC-MS: m/z calculated for $C_8H_{18}N_2O_3$: 191.13 $[M+H]^+$, found 190.87 $[M+H]^+$; HR-MS: m/z calculated for $C_8H_{18}N_2O_3$: 191.13902 $[M+H]^+$, found 191.13903 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.31 (s, 1H), 3.75 (t, J = 6.3 Hz, 2H), 2.64 (t, J = 6.7 Hz, 2H), 1.61 (q, J = 6.5 Hz, 2H), 1.41 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 156.1, 79.3, 73.4, 30.9, 28.0, 27.9.

Fluorescein-ONHBoc (6)

0.87 mL of DIPEA (5.14 mmol, 4 eq) were added to a solution containing 0.61 g *tert*-butyl (3-aminopropoxy) carbamate (**8**) (3.21 mmol, 2.5 eq) and 0.5 g fluorescein isothiocyanate (1.28 mmol, 1 eq) in dry DMF. The mixture was stirred at room temperature for 18 h. The product was purified by column chromatography (MeOH/CH_2Cl_2 (1: 9)). Yield: 0.73 g (1.26 mmol, 98 %); R_f : 0.79 MeOH/CH_2Cl_2 (1:9); HPLC-MS: m/z calculated for $C_{29}H_{29}N_3O_8S$: 580.16 $[M+H]^+$, found 580.02 $[M+H]^+$; HR-MS: m/z calculated for $C_{29}H_{29}N_3O_8S$: 580.17481 $[M+H]^+$, found 580.17654 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.51 (s, 1H), 10.10 (s, 2H), 8.22 (d, J = 6.3 Hz, 1H), 7.90 (dd, J = 9.1, 2.1 Hz, 1H), 7.18 (d, J = 9.2 Hz, 2H), 6.75 (m, 3H), 6.62 (dd, J = 9.1, 2.1 Hz, 2H), 4.03 (t, J = 7.7 Hz, 2H), 2.29 (m, 2H), 1.95 - 1.89 (m, 2H), 1.39 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 179.4, 168.2, 158.3, 154.5, 154.3, 152.6, 152.4, 140.6, 136.2, 134.7, 129.3, 128.7, 128.1, 126.4, 124.8, 109.2, 109.1, 108.1, 107.9, 103.2, 102.7, 85.1, 83.2, 47.1, 41.9, 29.4, 25.3.

Fluorescein-ONH$_2$ (2)

0.73 g of Compound (**12**) (1.26 mmol, 1 eq) were stirred at room temperature for 2 h in a mixture of TFA/CH_2Cl_2 (1:1). Toluene was then added and the solvent was eliminated at reduced pressure, the product was purified by preparative HPLC. Yield: 0.59 g (1.24 mmol, 98 %); R_f : 0.22 MeOH/CH_2Cl_2 (1:9); HPLC-MS: m/z calculated for $C_{24}H_{21}N_3O_6S$: 480.12 $[M+H]^+$, found 480.00 $[M+H]^+$; HR-MS: m/z calculated for $C_{24}H_{21}N_3O_6S$: 480.12238 $[M+H]^+$, found 480.12276 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.51 (s, 1H), 10.10 (s, 2H), 8.22 (d, J = 6.3 Hz, 1H), 7.90 (dd, J = 9.1, 2.1 Hz, 1H), 7.18 (d, J = 9.2 Hz, 2H), 6.75 (m, 3H), 6.62 (dd, J = 9.1, 2.1 Hz, 2H), 4.03 (t, J = 7.7 Hz, 2H), 2.29 (m, 2H), 1.95 - 1.89 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 179.4, 168.2, 154.5, 154.3,152.6, 152.4, 140.6, 136.2, 134.7, 129.3, 128.7, 128.1, 126.4, 124.8, 109.2, 109.1, 108.1, 107.9, 103.2, 102.7, 83.2, 47.1, 41.9, 25.3.

Fluorescein palmitohydroxamic acid (3)

0.13 mL of palmitoyl chloride (0.42 mmol, 2 eq) and 0.16 mL of DIPEA (0.94 mmol, 4.5 eq) were added to a solution of 100 mg of Fluorescein-ONH$_2$ **3** (0.21 mmol, 1 eq) in dry DMF. The reaction mixture was stirred for 18 h at room temperature. The solvent was eliminated at reduced pressure and the product was purified by preparative HPLC. Yield: 94 mg (0.13 mmol, 63 %); R_f: 0.75 MeOH/CH_2Cl_2 (1:9); HPLC-MS: m/z calculated for $C_{40}H_{51}N_3O_7S$: 718.34 $[M+H]^+$, found 718.22 $[M+H]^+$; HR-MS: m/z calculated for $C_{40}H_{51}N_3O_7S$: 718.35205 $[M+H]^+$, found 718.35316 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.51 (s, 1H), 10.10 (s, 2H), 8.22 (d, J = 6.3 Hz, 1H), 7.90 (dd, J = 9.1, 2.1 Hz, 1H), 7.18 (d, J = 9.2 Hz, 2H), 6.75 (m, 3H), 6.62 (dd, J = 9.1, 2.1 Hz, 2H), 4.03 (t, J = 7.7 Hz, 2H), 2.51 (t, J = 7.5 Hz, 2H), 2.29 (m, 2H), 1.95 - 1.89 (m, 2H), 1.42 (m, 2H), 1.22 (m, 24H), 0.86 (t, J = 6.0 Hz 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 179.4, 168.2, 168.1, 154.5, 154.3, 140.6, 136.2, 134.7, 129.3, 128.7, 128.1, 126.4, 124.8, 109.2, 109.1, 108.1, 107.9, 103.2, 102.7, 83.2, 47.1, 43.3, 41.9, 29.9, 29.08, 29.07, 29.06, 29.05, 29.04, 29.03, 29.00, 28.8, 28.73, 28.69, 28.20, 25.3, 22.01, 13.89.

Fluorescein laurohydroxamic acid (7)

0.042 mL of dodecanoyl chloride (0.17 mmol, 2 eq) and 0.064 mL of DIPEA (0.38 mmol, 4.5 eq) were added to a solution of 40 mg Fluorescein-ONH$_2$ (0.08 mmol, 1 eq) in dry DMF. The reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated and the product was purified by preparative HPLC. Yield: 8.55 mg (0.01 mmol, 16 %); HPLC-MS: m/z calculated for $C_{36}H_{43}N_3O_7S$: 662.28 $[M+H]^+$, found 662.32 $[M+H]^+$; HR-MS: m/z calculated for $C_{36}H_{43}N_3O_7S$: 662.2894, $[M+H]^+$ found 662.2759 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.52 (s, 1H), 10.11 (s, 2H), 8.24 (d, J = 6.3 Hz, 1H), 7.91 (dd, J = 9.1, 2.1 Hz, 1H), 7.19 (d, J = 9.2 Hz,

2H), 6.76 (m, 3H), 6.63 (dd, *J* = 9.1, 2.1 Hz, 2H), 4.05 (t, *J* = 7.7 Hz, 2H), 2.53 (t, *J* = 7.5 Hz, 2H), 2.28 (m, 2H), 1.96 - 1.87 (m, 2H), 1.43 (m, 2H), 1.25 (m, 16H), 0.87 (t, *J* = 6.0 Hz 3H); ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ 179.4, 168.2, 168.1, 154.5, 154.3, 140.6, 136.2, 134.7, 129.3, 128.7, 128.1, 126.4, 124.8, 109.2, 109.1, 108.1, 107.9, 103.2, 102.7, 83.2, 47.1, 43.3, 41.9, 29.9, 29.07, 29.06, 29.03, 28.87, 28.73, 28.69, 28.20, 25.3, 22.01, 13.89.

Fluorescein myristohydroxamic acid (8)

0.045 mL of myristoyl chloride (0.17 mmol, 2 eq) and 0.064 mL of DIPEA (0.38 mmol, 4.5 eq) were added to a solution of 40 mg Fluorescein-ONH$_2$ in dry DMF. The reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated and the product was purified by preparative HPLC. Yield: 12.175 mg (0.02 mmol, 21 %); HPLC-MS: m/ calculated for $C_{38}H_{47}N_3O_7S$: 690.32 $[M+H]^+$, found 690.28 $[M+H]^+$; HR-MS: m/z calculated for $C_{38}H_{47}N_3O_7S$: 690.3163, $[M+H]^+$ found 690.3168 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 10.52 (s, 1H), 10.11 (s, 2H), 8.24 (d, *J* = 6.4 Hz 1H), 7.91 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.19 (d, *J* = 9.2 Hz, 2H), 6.76 (m, 3H), 6.63 (dd, *J* = 9.1, 2.1 Hz, 2H), 4.05 (t, *J* = 7.7 Hz, 2H), 2.53 (t, *J* = 7.5 Hz, 2H), 2.28 (m, 2H), 1.96 - 1.87 (m, 2H), 1.43 (m, 2H), 1.25 (m, 20H), 0.87 (t, *J* = 6.0 Hz 3H); ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ 179.4, 168.2, 168.1, 154.5, 154.3, 140.6, 136.2, 134.7, 129.3, 128.7, 128.1, 126.4, 124.8, 109.2, 109.1, 108.1, 107.9, 103.2, 102.7, 83.2, 47.1, 43.3, 41.9, 29.9, 29.07, 29.06, 29.05, 29.03, 28.8, 28.87, 28.73, 28.69, 28.20, 25.3, 22.01, 13.89.

Fluorescein palmitoleohydroxamic acid (9)

0.059 mL of palmitoleic acid (0.21 mmol, 2 eq), 0.054 g of PyBOP (0.10 mmol, 1eq) and 0.08 mL of DIPEA (0.47 mmol, 4.5 eq) were added to a solution of 50 mg Fluorescein-ONH$_2$ (0.10 mmol, 1 eq) in dry DMF. The reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated and the product was purified by preparative HPLC. Yield: 26.32 mg (0.04 mmol, 36 %); HPLC-MS: m/z calculated for $C_{40}H_{49}N_3O_7S$: 716.32 $[M+H]^+$, found 716.34 $[M+H]^+$; HR-MS: m/z calculated for $C_{40}H_{49}N_3O_7S$: 716.33640, $[M+H]^+$found 716.33380 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 10.51 (s, 1H), 10.10 (s, 2H), 8.22 (d, *J* = 6.3 Hz, 1H), 7.90 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.18 (d, *J* = 9.2 Hz, 2H), 6.75 (m, 3H), 6.62 (dd, *J* = 9.1, 2.1 Hz, 2H), 5.43 (m, 2H), 4.03 (t, *J* = 7.7 Hz, 2H), 2.51 (t, *J* = 7.5 Hz, 2H), 2.29 (m, 4H), 1.95 - 1.89 (m, 2H), 1.42 (m, 2H), 1.22 (m, 20H), 0.86 (t, *J* = 6.0 Hz 3H);^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ 179.4, 168.2, 168.1, 154.5, 154.3, 152.6, 140.6, 136.2, 134.7, 130.06, 130.3, 128.7, 128.1, 126.4, 124.8, 109.2, 109.1, 108.1, 107.9, 103.2, 102.7, 83.2, 69.3, 47.1, 41.9, 32.3, 31.3, 29.4, 28.3, 27.1, 25.3, 22.5, 21.9, 14.7.

Fluorescein stearohydroxamic acid (10)

0.075 mL of stearoyl chloride (0.21 mmol, 2 eq) and 0.08 mL of DIPEA (0.47 mmol, 4.5 eq) were added to a solution of 50 mg of Fluorescein-ONH$_2$ (0.10 mmol, 1 eq) in dry DMF. The reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated and the product was purified by preparative HPLC. Yield: 16.77 mg (0.02 mmol, 23 %); HPLC-MS: m/z calculated for $C_{42}H_{55}N_3O_7S$: 746.37 $[M+H]^+$, found 746.19 $[M+H]^+$; HR-MS: m/z calculated for $C_{42}H_{55}N_3O_7S$: 746.38335, $[M+H]^+$ found 746.38116 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 10.51 (s, 1H), 10.10 (s, 2H), 8.22 (d, *J* = 6.3 Hz, 1H), 7.90 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.18 (d, *J* = 9.2 Hz 2H), 6.75 (m, 3H), 6.62 (dd, *J* = 9.1, 2.1 Hz, 2H), 4.03 (t, *J* = 7.7 Hz, 2H), 2.51 (t, *J* = 7.5 Hz, 2H), 2.29 (m, 2H), 1.95 - 1.89 (m, 2H), 1.42 (m, 2H), 1.22 (s, 28H), 0.86 (t, *J* = 6.0 Hz 3H); ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ 179.4, 168.2, 168.1, 154.5, 154.3, 140.6, 136.2, 134.7, 129.3, 128.7, 128.1, 126.4, 124.8, 109.2, 109.1, 108.1, 107.9, 103.2, 102.7, 83.2, 47.1, 43.3, 41.9, 29.9, 29.08, 29.07, 29.06, 29.05, 29.04, 29.03, 29.01, 29.00, 28.87, 28.82, 28.73, 28.69, 28.20, 25.3, 22.0, 13.9.

Fluorescein oleohydroxamic acid (11)

0.070 mL of oleoyl chloride (0.21 mmol, 2 eq) and 0.08 mL DIPEA (0.47 mmol, 4.5 eq) were added to a solution of 50 mg Fluorescein-ONH$_2$ in dry DMF. The reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated and the product was purified by preparative HPLC. Yield: 31.76 mg (0.04 mmol, 41 %); HPLC-MS: m/z calculated for $C_{42}H_{53}N_3O_7S$: 744.36 $[M+H]^+$, found 744.18 $[M+H]^+$; HR-MS: m/z calculated for $C_{42}H_{53}N_3O_7S$: 744.36770,$[M+H]^+$ found 744.36816 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ 10.51 (s, 1H), 10.10 (s, 2H), 8.22 (d, *J* = 6.3 Hz, 1H), 7.90 (dd, *J* = 9.1, 2.1 Hz, 1H), 7.19 (d, *J* = 9.2 Hz, 2H), 6.75 (m, 3H), 6.62 (dd, *J* = 9.1, 2.1 Hz, 2H), 5.36 - 5.27 (m, 2H), 4.03 (t, *J* = 7.7 Hz, 2H), 3.82 (t, *J* = 7.5 Hz, 2H), 2.51 (t, *J* = 7.5 Hz, 2H), 2.29 (m, 4H), 1.95 - 1.89 (m, 2H), 1.42 (m, 2H), 1.22 (m, 20H), 0.86 (t, *J* = 6.0 Hz 3H); ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ 179.4, 168.2, 168.1, 154.5, 154.3, 152.6, 140.6, 136.2, 134.7, 130.71, 130.06, 130.3, 128.7, 128.1, 126.4, 124.8, 109.2, 109.1, 108.1, 107.9, 103.2, 102.7, 83.2, 69.3, 47.1, 41.9, 32.3, 31.3, 29.42, 28.31, 27.1, 25.3, 22.5, 21.9, 14.7.

Fluorescein arachidohydroxamic acid (12)

52.14 mg of arachidic acid (0.17 mmol, 2 eq) 0.054 g of PyBOP (0.10 mmol, 1eq) and 0.08 mL of DIPEA (0.47 mmol, 4.5 eq) were added to a solution of 50 mg Fluorescein-ONH$_2$ (0.10 mmol, 1 eq) in dry DMF. The reaction

mixture was stirred for 18 h at room temperature. The solvent was evaporated and the product was purified by preparative HPLC. Yield: 11.24 mg (0.014 mmol, 15 %); HPLC-MS: m/z calculated for $C_{44}H_{59}N_3O_7S$: 774.41 $[M+H]^+$, found774.53 $[M+H]^+$; HR-MS: m/z calculated for $C_{44}H_{59}N_3O_7S$: 774.4146, $[M+H]^+$ found 774.3981 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.51 (s, 1H), 10.10 (s, 2H), 8.22 (d, J = 6.3 Hz 1H), 7.90 (dd, J = 9.1, 2.1 Hz, 1H), 7.18 (d, J = 9.2 Hz, 2H), 6.75 (m, 3H), 6.62 (dd, J = 9.1, 2.1 Hz, 2H), 4.03 (t, J = 7.7 Hz, 2H), 2.51 (t, J = 7.5 Hz, 2H), 2.29 (m, 2H), 1.95 - 1.89 (m, 2H), 1.42 (m, 2H), 1.22 (m, 34H), 0.86 (t, J = 6.0 Hz 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 179.4, 168.2, 168.1, 154.5, 154.3, 140.6, 136.2, 134.7, 129.3, 128.7, 128.1, 126.4, 124.8, 109.2, 109.1, 108.1, 107.9, 103.2, 102.7, 83.2, 47.1, 43.3, 41.9, 29.9, 29.09, 29.08, 29.07, 29.06, 29.05, 29.04, 29.03, 29.00, 28.9, 28.8, 28.87, 28.73, 28.69, 28.20, 25.3, 22.01, 13.89.

Fluorescein arachidonohydroxamic acid (13)
0.055 mL of arachidonic acid (0.17 mmol), 0.054 g of PyBOP (0.10 mmol) and 0.08 mL of DIPEA (0.47 mmol, 4.5 eq) were added to a solution of 50 mg Fluorescein-ONH_2 (0.10 mmol) in dry DMF. The reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated and the product was purified by preparative HPLC. Yield: 8.14 mg (0.01 mmol, 10 %); HPLC-MS: m/z calculated for $C_{44}H_{51}N_3O_7S$: 766.35 $[M+H]^+$, found 766.58 $[M+H]^+$; HR-MS m/z calculated for $C_{44}H_{51}N_3O_7S$: 766.3476,$[M+H]^+$: found 766.3462 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.51 (s, 1H), 10.10 (s, 2H), 8.22 (d, J = 6.3 Hz, 1H), 7.90 (dd, J = 9.1, 2.1 Hz, 1H), 7.18 (d, J = 9.2 Hz, 2H), 6.75 (m, 3H), 6.62 (dd, J = 9.1, 2.1 Hz, 2H), 5.49 – 5.31 (m, 8H), 4.03 (t, J = 7.7 Hz, 2H), 2.51 (t, J = 7.5 Hz, 2H), 2.29 (m, 2H), 1.95 - 1.89 (m, 2H), 1.42 (m, 2H), 1.22 (m, 16H), 0.87 (t, J = 6.0 Hz 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 179.4, 168.2, 168.1, 154.5, 154.3, 140.6, 136.2, 134.7, 129.3, 128.7, 128.1, 127.8, 127.7, 127.5, 127.4, 126.9, 126.7, 126.4, 124.8, 109.2, 109.1, 108.1, 107.9, 103.2, 83.2, 47.1, 43.3, 37.9, 37.8, 37.7, 29.9, 29.00, 28.73, 28.69, 28.20, 25.3, 22.01, 13.89.

Fluorescein heptadecanohydroxamic acid (14)
0.071 ml of heptadecanoic acid chloride (0.21 mmol, 2 eq) and 0.08 mL DIPEA (0.47 mmol, 4.5 eq) were added to a solution of 50 mg Fluorescein-ONH2 (0.10 mmol, 1 eq) in dry DMF. The reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated and the product was purified by preparative HPLC. Yield: 31.75 mg (0.04 mmol, 42 %); HPLC-MS: m/z calculated for $C_{41}H_{53}N_3O_7S$: 732.36 $[M+H]^+$, found 732.11 $[M+H]^+$; HR-MS: m/z calculated for $C_{41}H_{53}N_3O_7S$: 732.36770,$[M+H]^+$ found 732.36928 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.51 (s, 1H), 10.10 (s, 2H), 8.2 (dd, J = 6.3 Hz, 1H), 7.90 (dd, J = 9.1, 2.1 Hz, 1H), 7.18 (d, J = 9.2 Hz, 2H), 6.75 (m, 3H), 6.62 (dd, J = 9.1, 2.1 Hz, 2H), 4.03 (t, J = 7.7 Hz, 2H), 2.51 (t, J = 7.5 Hz, 2H), 2.29 (m, 2H), 1.95 - 1.89 (m, 2H), 1.42 (m, 2H), 1.22 (m, 28H), 0.86 (t, J = 6.0 Hz 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 179.4, 168.2, 168.1, 154.5, 154.3, 140.6, 136.2, 134.7, 129.3, 128.7, 128.1, 126.4, 124.8, 109.2, 109.1, 108.1, 107.9, 103.2, 102.7, 83.2, 47.1, 43.3, 41.9, 29.9, 29.08, 29.07, 29.06, 29.05, 29.04, 29.03, 29.00, 28.8, 28.87, 28.73, 28.69, 28.20, 25.3, 22.01, 13.89.

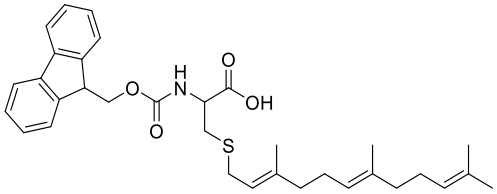
Fluorescein nonadecanohydroxamic acid (15)
0.070 mL of nonadecanoic acid (0.21 mmol, 2 eq), 0.054 g of PyBOP (0.10 mmol, 1eq) and 0.08 mL of DIPEA (0.47 mmol, 4.5 eq) were added to a solution of 50 mg Fluorescein-ONH_2 (0.10 mmol, 1 eq) in dry DMF. The reaction mixture was stirred for 18 h at room temperature. The solvent was evaporated and the product was purified by preparative HPLC. Yield: 12.68 mg (0.02 mmol, 16 %); HPLC-MS: m/z calculated for $C_{43}H_{57}N_3O_7S$: 760.39 $[M+H]^+$, found 760.38 $[M+H]^+$; HR-MS: m/z calculated for $C_{43}H_{57}N_3O_7S$: 760.3945,$[M+H]^+$ found 760.3948 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.51 (s, 1H), 10.10 (s, 2H), 8.22 (d, J = 6.3 Hz 1H), 7.90 (dd, J = 9.1, 2.1 Hz, 1H), 7.18 (d, J = 9.2 Hz, 2H), 6.75 (m, 3H), 6.62 (dd, J = 9.1, 2.1 Hz, 2H), 4.03 (t, J = 7.7 Hz, 2H), 2.51 (t, J = 7.5 Hz, 2H), 2.29 (m, 2H), 1.95 - 1.89 (m, 2H), 1.42 (m, 2H), 1.22 (m, 32H), 0.86 (t, J = 6.0 Hz 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 179.4, 168.2, 168.1, 154.5, 154.3, 140.6, 136.2, 134.7, 129.3, 128.7, 128.1, 126.4, 124.8, 109.2, 109.1, 108.1, 107.9, 103.2, 102.7, 83.2, 47.1, 43.3, 41.9, 29.9, 29.09, 29.08, 29.07, 29.06, 29.05, 29.04, 29.03, 29.00, 28.8, 28.87, 28.73, 28.69, 28.20, 25.3, 22.01, 13.89.

Fmoc-Cys(Pal)-OH (16)
3 g of Fmoc Cys-(Trt)-OH (0.01 mol, 1 eq) were dissolved in 65 mL of CH_2Cl_2. 5% trifluoroacetic acid and 2.23 mL triethylsilane (0.01 mol, 1.6 eq) were added and the reaction solution was stirred at room temperature for 2 h. The residue was co-evaporated with toluene and the recovered residue was washed with pentane to remove the resulting triphenylmethane. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 and 5.63 mL of chlorotrimethylsilane (0.03 mol, 3.65 eq) were added. The mixture was refluxed for 3 h and 4.51 mL of palmitoyl chloride were then added to the cooled solution. 20 mL of a mixture of 1.12 mL of triethylamine in 19.88 mL of CH_2Cl_2 were then added dropwise to the reaction mixture under inert gas for 3 h. After an additional hour, the solvent was removed under reduced pressure. The crude product was purified by column chromatography (CH_2Cl_2/MeOH/HCO_2H (98:2:1)). Yield: 3.46 g (0.01 mol, 68 %); HPLC-MS: m/z calculated for $C_{34}H_{47}NO_5S$: 582.31 $[M+H]^+$, found 581.90 $[M+H]^+$; HR-MS: m/z calculated for $C_{34}H_{47}NO_5S$: 582.32477, $[M+H]^+$ found

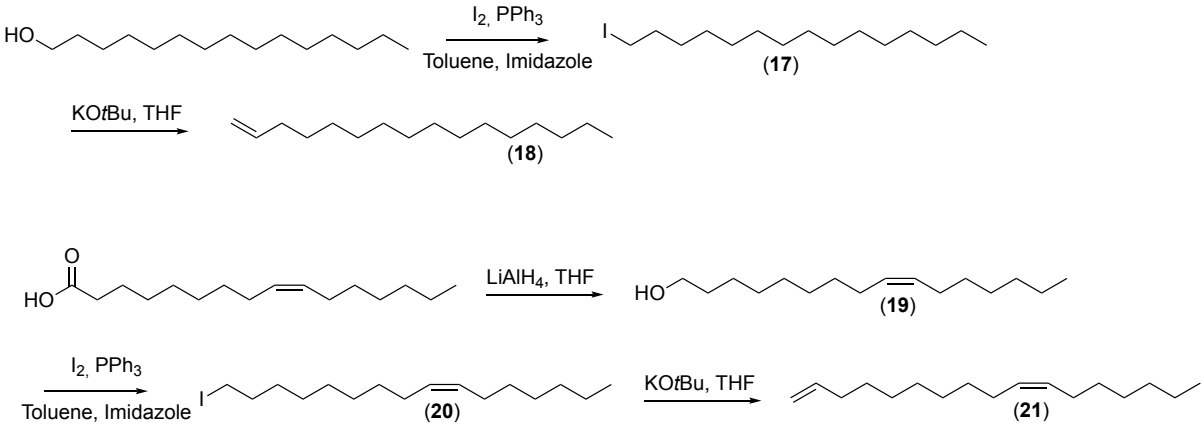
582.32546 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 7.88 (d, *J* = 7.5 Hz, 2H), 7.70 (d, *J* = 7.4 Hz, 2H), 7.44 (m, 3H), 7.32 (dd, *J* = 7.4 Hz, 2H), 4.18 (m, 3H), 4.04 (m, 1H), 3.44 (dd, *J* = 12.9, 4.1 Hz, 1H), 3.03 (dd, *J* = 13.4, 9.1 Hz, 1H), 2.51 (t, *J* = 8.0 Hz, 2H), 1.47 (m, 2H,), 1.20 (m, 24H,), 0.84 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 198.1, 171.8, 155.9, 143.7, 140.7, 127.5, 127.0, 125.2, 120.0, 65.8, 53.4, 46.6, 43.3, 31.3, 29.9, 29.08, 29.07, 29.06, 29.05, 29.04, 29.03, 29.00, 28.8, 28.73, 28.69, 28.2, 25.0, 22.1, 13.9;
$[\alpha]^{20}$D = -2.2 ($CHCl_3$, c = 1.16).

Synthesis of the lipidated Cysteines

N-(9-Fluorenylmethoxycarbonyl)-*S*-farnesyl-L-cysteine (Fmoc-Cys(Far)-OH)

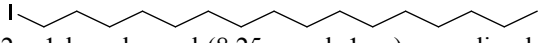


5 g of L-cysteine hydrochloride monohydrate (31.72 mmol, 1 eq) were dissolved in 50 mL of methanol and cooled to 0 °C. 68 mL of a 7 N ammonia-methanol solution were then added and the mixture was stirred for 5 minutes. 8.3 mL of trans, trans-farnesyl chloride (31.72 mmol, 1eq) were then added, and the mixture was stirred at 0 °C for 3 hours and at room temperature for a further hour. The solvent was eliminated at reduced pressure and the crude product was washed several times with n-pentane. The washed solid was taken up in CH_2Cl_2 and cooled again to 0 °C. After addition of 10.36 g of Fmoc-OSu (30.72 mmol, 1 eq) and 4.26 mL of triethylamine (30.72 mmol, 1 eq), the mixture was stirred overnight at room temperature. The solvent was removed and purified by column chromatography (CH_2Cl_2/methanol, 98: 2). Yield: 14.73 g (0.03 mol, 88 %); HPLC-MS: m/z calculated for $C_{33}H_{47}NO_4S$: 548.75 $[M+H]^+$, found 548.11 $[M+H]^+$; HR-MS: m/z calculated for $C_{33}H_{47}NO_4S$: 548.2835 $[M+H]^+$, found 548.04 $[M+H]^+$; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 7.89 (d, *J* = 7.5 Hz, 2H), 7.72 (t, *J* = 6.8 Hz, 3H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 5.17 (t, *J* = 7.7 Hz, 1H), 5.05 (m, 2H), 4.28 (m, 3H), 4.15 (m, 1H), 3.17 (m, 2H), 2.86 (dd, *J* = 13.9, 4.5 Hz, 1H), 2.67 (dd, *J* = 13.6, 9.6 Hz, 1), 1.93 (m, 8H), 1.62 (s, 3H), 1.61 (s, 3H), 1.54 (s, 6H); ^{13}C NMR (101 MHz, DMSO- *d*$_6$) δ 172.4, 156.0, 143.8, 140.7, 138.4, 134.5, 130.6, 127.6, 127.0, 125.2, 124.1, 123.6, 120.2, 120.1, 65.7, 53.9, 46.6, 39.7, 39.1, 32.0, 28.8, 26.2, 25.9, 25.4, 17.5, 15.76, 15.73; $[\alpha]^{20}$D = -9.29 ($CHCl_3$, c = 1.19).



Scheme S2. Synthesis of the required alkenes **18** and **21**

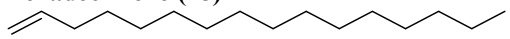
1-Iodohexadecane (17)



2 g 1-hexadecanol (8.25 mmol, 1 eq) were dissolved in 40 mL of dry toluene and 0.91 mL of imidazole (16.50 mmol, 2 eq), 3.77 g iodine (0.01 mol, 1.8 eq) and 4.32 g PPh_3 (0.02 mol, 2 eq) were slowly added to the solution. The reaction mixture was stirred for 3 hours at room temperature. After addition of saturated $Na_2S_2O_3$ solution and ethyl acetate, the organic phases were extracted and washed with brine. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The product was purified by column chromatography. Yield: quant.; GC-MS: m/z calculated for $C_{16}H_{33}I$: 353.33 $[M+H]^+$, found 353.25 $[M+H]^+$; HR-MS: m/z calculated for $C_{16}H_{33}I$: 353.163 $[M+H]^+$, found 353.166 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.56 (t, *J* = 7.2, 3H), 1.51-1.63

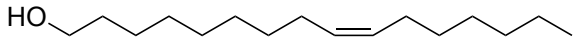
(m, 2H), 1.24-1.39 (m, 26H), 0.89 (t, J = 6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 34.5, 33.3, 32.2, 30.0, 29.9, 29.8, 29.7, 29.5, 29.1, 28.2, 27.2, 23.1, 14.5.

Hexadec-1-ene (18)



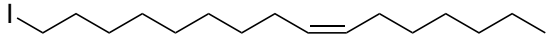
3 g 1-iodohexadecane (8.51 mmol, 1 eq) were dissolved in THF and 5.25 g KOtBu (0.05 mol, 5.5 eq) were added to the solution and stirred at room temperature. After addition of water and ethyl acetate, the organic phases were extracted and washed again with water and brine. The organic phase was dried over $MgSO_4$ and concentrated in vacuum. The product was purified by column chromatography. Yield: 1.84 g (0.01 mol, 96 %); GC-MS: m/z calculated for $C_{16}H_{32}$: 225.42 $[M+H]^+$, found 225.35 $[M+H]^+$; HR-MS: m/z calculated for $C_{16}H_{32}$: 225.2504 $[M+H]^+$, found 225.2510 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.93 (m, 1H), 5.14 (m, 1H), 4.89 (m, 1H), 2.56 (t, J = 7.2, 3H), 1.51-1.63 (m, 2H), 1.24-1.39 (m, 26H), 0.89 (t, J = 6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.2, 116.5, 34.5, 33.3, 32.2, 30.0, 29.9, 29.8, 29.7, 29.5, 29.1, 28.2, 27.2, 23.1, 14.5.

(*Z*)-Hexadec-9-en-1-ol (19)



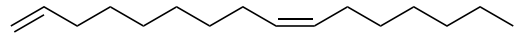
2 g palmitoleic acid (7.86 mmol, 1 eq) were dissolved in THF and added dropwise to a cooled suspension (0 °C) of 0.59 g $LiAlH_4$ (15.72 mmol, 2 eq) in THF. The mixture was refluxed for 1 h and then stirred overnight at room temperature. Subsequently, 2 M NaOH solution was added to unreacted $LiAlH_4$. The organic phase was washed three times with Brine and dried over $MgSO_4$. The solvent was removed under reduced to yield compound. Yield: 1.86 g (7.74 mmol, 98 %); GC-MS: m/z calculated for $C_{16}H_{32}O$: 241.42 $[M+H]^+$, found 241.05 $[M+H]^+$; HR-MS: m/z calculated for $C_{16}H_{32}O$: 241.2525 $[M+H]^+$, found 241.2526 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43 - 5.31 (m, 2H), 3.56 (t, J = 7.2, 2H), 2.21-2.08 (m, 4H), 1.24-1.39 (m, 18H), 0.89 (t, J = 6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 130.6, 130.5, 62.9, 34.5, 33.3, 32.2, 30.0, 29.9, 29.8, 29.7, 29.5, 29.1, 28.2, 27.2, 23.1, 14.5.

(*Z*)-16-Iodohexadec-7-ene (20)

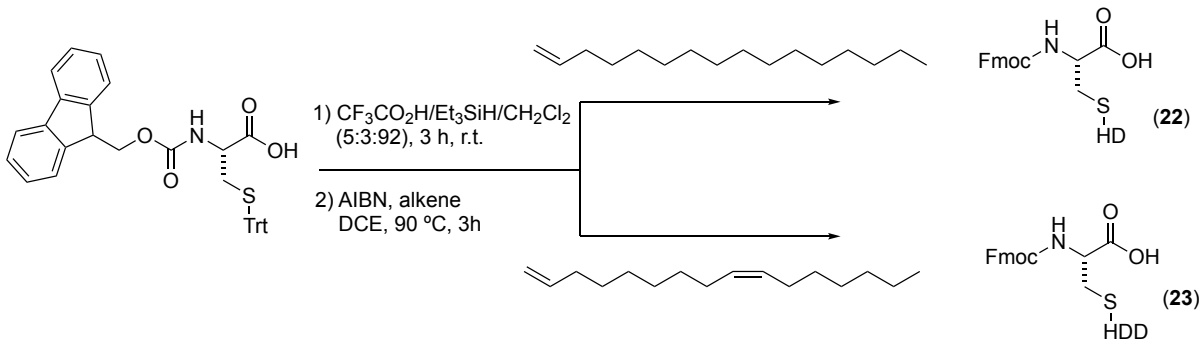


2 g (Z)-hexadec-9-en-1-ol (8.32 mmol, 1eq) were dissolved in 65 mL dry toluene and 1.13 g imidazole (0.02 mol, 2 eq), 3.8 g of iodine (0.01 mol, 1.8 eq) and 4.36 g PPh_3 (0.02 mol, 2 eq) were slowly added to the solution. The reaction mixture was stirred for 3 hours at room temperature. After addition of saturated $Na_2S_2O_3$ solution and ethyl acetate, the organic phases were extracted and washed again with Brine. The organic phase was dried over $MgSO_4$ and concentrated under low pressure. Triphenylphosphine oxide was precipitated in petroleum ether and the product was obtained as a yellowish oil. Yield: 2.61 g (7.44 mmol, 89 %); GC-MS: m/z calculated for $C_{16}H_{31}I$: 351.14 $[M+H]^+$, found 351.21 $[M+H]^+$; HR-MS: m/z calculated for $C_{16}H_{31}I$: 351.1470 $[M+H]^+$, found 351.1775 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43 - 5.31 (m, 2H), 2.91 (m, 2H), 2.21-2.08 (m, 4H), 1.84 (m, 2 H), 1.24-1.39 (m, 18H), 0.89 (t, J = 6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 130.6, 130.5, 34.5, 33.3, 32.2, 30.0, 29.9, 29.8, 29.7, 29.5, 29.1, 28.2, 27.2, 23.1, 14.5.

(*Z*)-Hexadecen-1,9-diene (21)

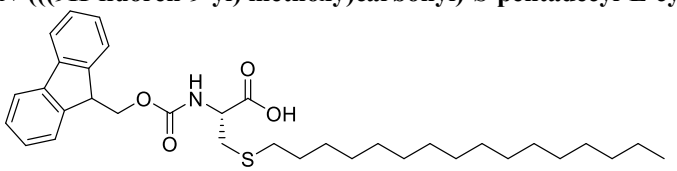


2.0 g iodohexadec-7-ene (5.71 mmol, 1 eq) were dissolved in 40 mL THF and 3.52 g KOtBu (0.03 mol, 5.5 eq) were added in small amounts over a period of 2 h and the solution was stirred at room temperature. 40 mL water were added and the organic phase was extracted with ethyl acetate (3 × 40 mL). The combined organic phases were washed with Brine (2 × 40 mL) and water (2 × 40 mL) and dried over $MgSO_4$. The solvent was removed in vacuo and the product was obtained by column chromatography. Yield: 0.7 g (3.44 mmol, 60 %); GC-MS: m/z calculated for $C_{16}H_{30}$: 223.41 $[M+H]^+$, found 223.25 $[M+H]^+$; HR-MS $[M+H]^+$: m/z calculated for $C_{16}H_{30}$: 223.2348 $[M+H]^+$, found 223.2351 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.93 (m, 1H), 5.43 - 5.31 (m, 2H), 5.14 (m, 1H), 4.89 (m, 1H), 2.56 (t, J = 7.2, 3H), 2.29-2.09 (m, 6H), 1.24-1.39 (m, 16H), 0.89 (t, J = 6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.2, 130.6, 130.5, 116.5, 34.5, 33.3, 32.2, 30.0, 29.9, 29.8, 29.7, 29.5, 29.1, 28.2, 27.2, 23.1, 14.5.



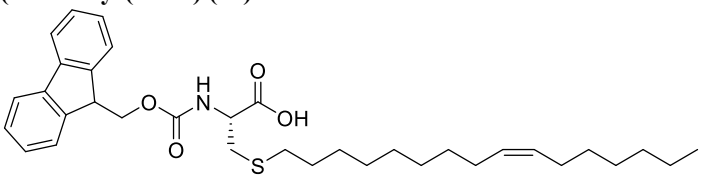
Scheme S3. Synthesis of Fmoc-Cys(HD)-OH (**22**) and Fmoc-Cys(HDD)-OH (**23**).

***N*-(((9H-fluoren-9-yl) methoxy)carbonyl)-S-pentadecyl-L-cysteine (Fmoc-Cys(HD)-OH) (22)**



3 g Fmoc-L-Cys (Trt)-OH were dissolved in 30 mL of a mixture of CH_2Cl_2/TFA/TES (50/45/5) and stirred for 3 hours at room temperature. The solvent was removed, the residue was taken up in CH_2Cl_2, the white solid was removed by filtration and co-evaporated with toluene. For the radical alkylation by means of 2,2'-azobis (2-methylpropionitrile) (AIBN), dichloroethane was degassed for 15 min under an argon stream in an ultrasonic bath. 1.2 g AIBN (1.75 mmol, 0.5 eq) were added with stirring to a solution of 1.2 g unprotected cysteine (3.49 mmol, 1 eq) and 1.65 g hexadec-1-ene (0.01 mol, 2 eq). The mixture was refluxed for 3 hours at 90 ° C and the reaction was then cooled down to room temperature. The solvent was removed under reduced pressure and the resulting residue was purified by column chromatography (CH_2Cl_2/ MeOH (95: 5)). Yield: 1.84 g (3.34 mmol, 96 %); HPLC-MS: m/z calculated for $C_{33}H_{49}NO_4S$: 568.33 $[M+H]^+$, found 567.95 $[M+H]^+$; HR-MS for $[M+H]^+$: m/z calculated for $C_{33}H_{49}NO_4S$: 568.34551 $[M+H]^+$, found 568.34524 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.2 Hz, 2H), 7.61 (d, *J* = 7.2 Hz, 2H), 7.40 (dd, *J* = 7.2 Hz, J = 7.2 Hz, 2H), 7.31 (dd, *J*= 7.2 Hz, *J* = 7.2 Hz, 2H), 5.82 (m, 1H), 4.67 (m, 1H), 4.42 (m, 2H), 4.24 (t, *J* = 7.0 Hz, 1H), 3.05 (m, 2H), 2.56 (t, *J*= 7.2, 3H), 1.51-1.63 (m, 2H), 1.24-1.39 (m, 26H), 0.89 (t, *J* = 6.8, 3H);^{13}C NMR (101 MHz, $CDCl_3$) δ 175.3, 156.2, 143.8, 141.3, 127.6, 127.0, 125.0, 119.8, 67.7, 53.8, 47.4, 34.5, 33.3, 32.2, 30.0, 29.9, 29.8, 29.7, 29.5, 29.1, 28.2, 27.2, 23.1, 14.5; $[\alpha]^{20}D$ = 6.2° ($CHCl_3$, c = 1).

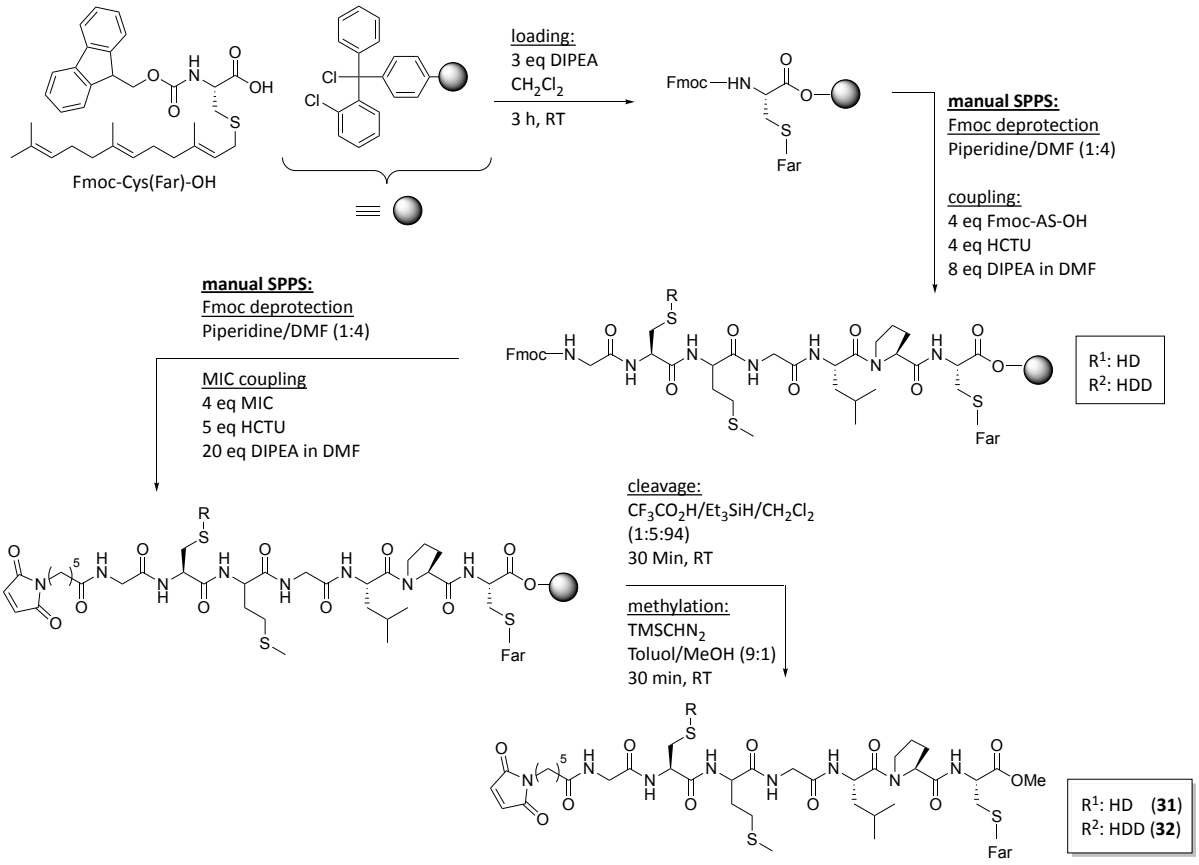
***N*-(((9H-fluoren-9-yl)-methoxy)carbonyl)-*S*-(hexadec-9-en-1-yl)-L-cysteine (Fmoc-Cys(HDD) (23)**



3 g Fmoc-L-Cys (Trt)-OH were dissolved in 30 mL of a mixture of CH_2Cl_2/ TFA/ TES (50/45/5) and stirred for 3 hours at room temperature. The solvent was removed, the residue was taken up in CH_2Cl_2, the white solid was removed by filtration and co-evaporated with toluene. For the radical alkylation by means of 2,2'-azobis (2-methylpropionitrile) (AIBN), dichloroethane was degassed for 15 min under an argon stream in an ultrasonic bath and 1.2 g AIBN (1.75 mmol, 0.5 eq) were added. 1.2 g of the unprotected cysteine (3.49 mmol, 1 eq) and 1.55 g of the octadeca-1,11-diene (0.01 mol, 2 eq) were dissolved in the degassed dichloroethane. The mixture was refluxed for 3 hours at 90 ° C and the reaction was then cooled down to room temperature. The solvent was removed under reduced pressure and the resulting residue was purified by column chromatography (CH_2Cl_2/ MeOH (95: 5)). Yield: 1.68 g (2.98 mmol, 85 %); HPLC-MS: m/z calculated for $C_{33}H_{47}NO_4S$: 566.32 $[M+H]^+$, found 566.12 $[M+H]^+$; HR-MS: m/z calculated for $C_{33}H_{47}NO_4S$: 566.32986 $[M+H]^+$, found 566.32957 $[M+H]^+$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.2 Hz, 2H), 7.61 (d, *J* = 7.2 Hz, 2H), 7.40 (dd, *J* = 7.2 Hz, *J* = 7.2 Hz, 2H), 7.31 (dd, *J* = 7.2 Hz, *J* = 7.2 Hz, 2H), 5.82 (m, 1H), 5.43 - 5.31 (m, 2H), 4.67 (m, 1H), 4.42 (m, 2H), 4.24 (t, *J* = 7.0 Hz, 1H), 3.05 (m, 2H), 2.56 (t, *J* = 7.2, 2H), 1.51-1.63 (m, 2H), 1.24-1.39 (m, 20H), 0.89 (t, *J* = 6.8, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.3, 156.2, 143.8, 141.3, 130.6, 130.5, 127.6, 127.0, 125.0, 119.8, 67.7, 53.8, 47.4, 34.5, 33.3, 32.2, 30.0, 29.9, 29.8, 29.7, 29.5, 29.1, 28.2, 27.2, 23.1, 14.5.

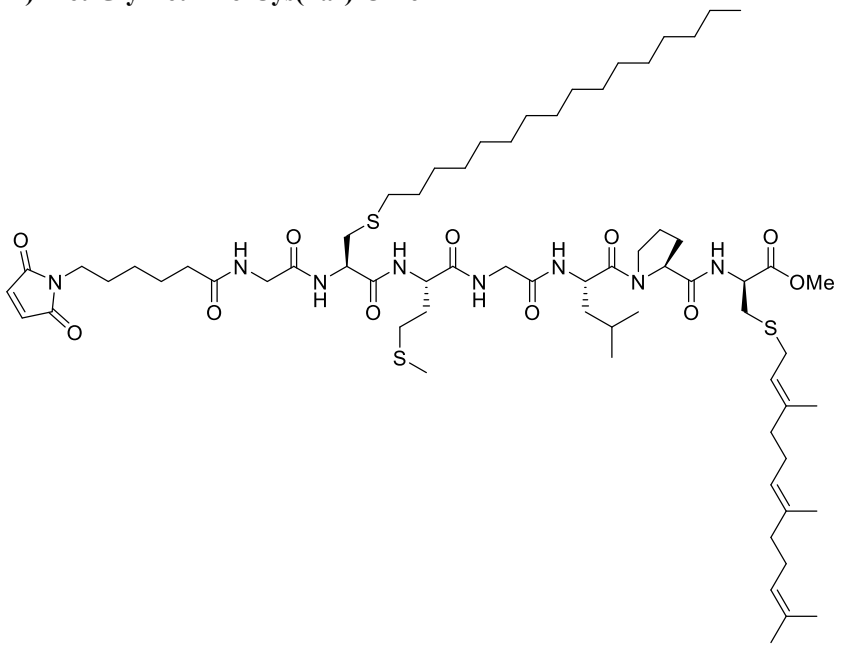
Synthesis of MIC-Peptides for *N*-Ras partitioning studies:

Peptide synthesis was carried out manually. All the solutions used were degassed for 30 min in an ultrasonic bath under inert atmosphere before their usage. The synthesis was carried out by using standard Fmoc chemistry under argon atmosphere. Yields and scales of the solid-phase reactions were calculated with respect to the loading of the first amino acid, here Fmoc-Cys(Far)-OH, coupled to the 2-chlorotrityl resin. The peptide sequence itself, was constructed in iterative cycles of deprotection and coupling of the amino acids. The coupling of the respective Fmoc-protected amino acid was carried out twice and in each case over a period of 1 h. Therefore, a mixture of the Fmoc-AS-OH (4 eq), HCTU (4 eq) and DIPEA (8 eq) dissolved in DMF was added to the preloaded rein. After removal of the coupling solution, the solid phase was washed three times with DMF for 5 min before the next cycle started. After the coupling of the maleimidocaproic acid (5 eq), the peptide was cleaved from the polymeric support by shaking for 30 minutes with a 10 mL of a solution of trifluoroacetic acid, triethylsilane and CH_2Cl_2 (1:4:95). After two additional rounds of cleavage, the resin was washed three times with CH_2Cl_2 and the collected solutions were coevaporated several times with toluene. The residue was dissolved in a small amount of methanol and precipitated in cold Et_2O. The precipitate was collected by centrifugation, dissolved in water and lyophilized. The lyophilized peptide was examined by analytical LC-MS and purified by preparative HPLC-MS. The combined product fractions were subsequently lyophilized. For the final methylation of the free acid, a mixture of 0.9 eq $TMSCHN_2$ (2M in hexane) in toluene /methanol (9:1) was slowly added to the peptide dissolved in toluene /methanol (9:1). The solvent was eliminated under reduced pressure and the desired peptide could be obtained after purification by means of preparative HPLC-MS.



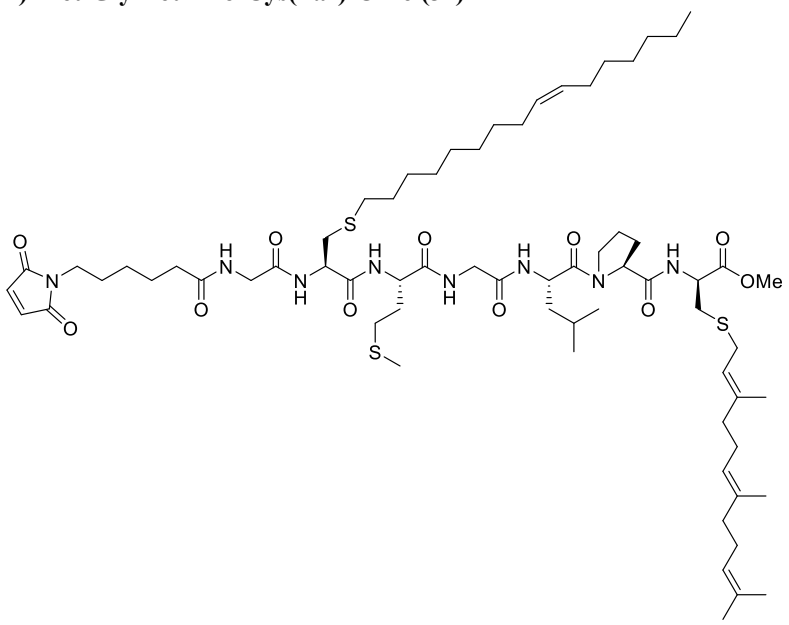
Scheme S4. Synthesis of MIC-tagged lipidated peptides.

MIC-Gly-Cys(HD)-Met-Gly-Leu-Pro-Cys(Far)-OMe



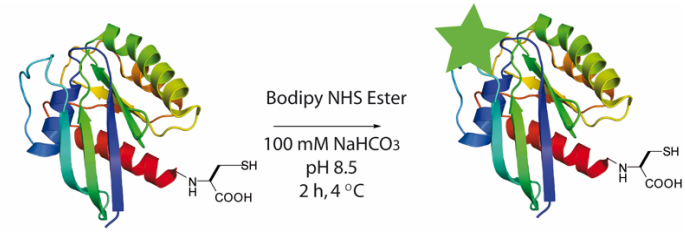
Yield: 25 %; HPLC-MS: m/z calculated for $C_{68}H_{114}N_8O_{11}S_3$: 1315.76 $[M+H]^+$, found 1315.44 $[M+H]^+$; HR-MS for $[M+H]^+$: m/z calculated for $C_{68}H_{114}N_8O_{11}S_3$: 1315.7685 $[M+H]^+$, found 1315.7594 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.22 (m, 2H), 8.10 (d, *J* =7.60 Hz, 1H), 8.01 (m, 1H), 7.92 (m, 2H), 5.13 (t, *J* =8.08 Hz, 1H), 5.03 (d, *J* =6.65 Hz, 2H),4.53 (m, 1H), 4.46-4.29 (m, 2H), 4.23 (m, 1H), 4.15 (q, *J* =5.7 Hz, 1H), 3.68 (m, 2H), 3.60 (t, *J* =9.80 Hz, 1H), 3.45 (s, 3H), 2.63 - 2.83 (m, 6H), 2.53-2.51 (m, 6H), 2.09 (t, *J* =7.41, 2H), 2.09 (s, 3H), 1.98-1.78 (m, 2 H), 1.64 (m, 1H), 1.61 (s, 6H), 1.54 (s, 6H), 1.47 (m, 5H), 1.31-1.14 (m, 30H), 0.84 (m, 9H), 0.74 (t, *J* =6.14 Hz, 6H).

MIC-Gly-Cys(HDD)-Met-Gly-Leu-Pro-Cys(Far)-OMe (32)



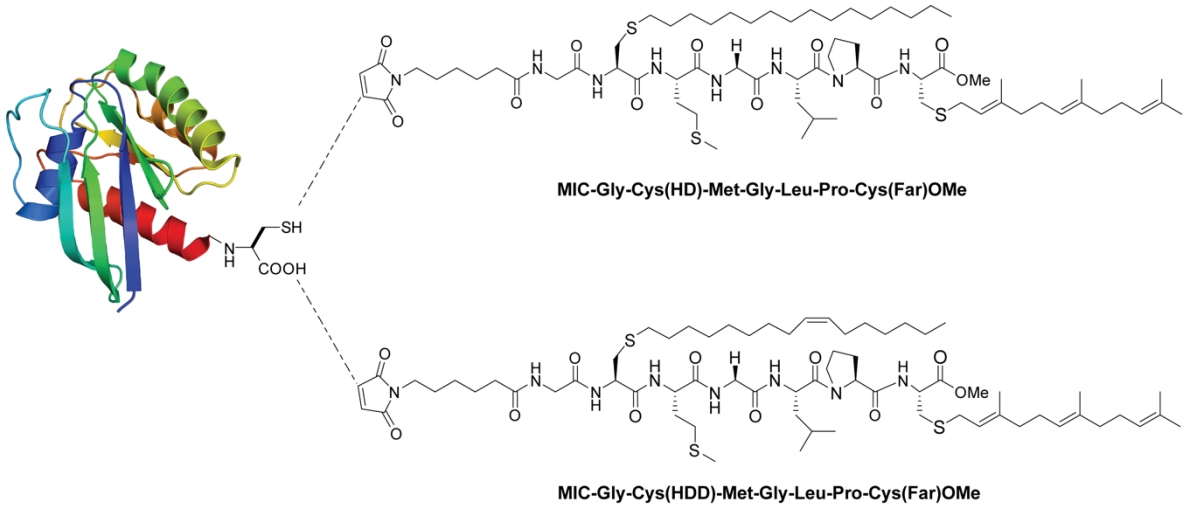
Yield: 21 %; HPLC-MS: m/z $C_{68}H_{112}N_8O_{11}S_3$: 1313.76 $[M+H]^+$, found 1313.58 $[M+H]^+$; HR-MS for $[M+H]^+$: m/z $C_{68}H_{112}N_8O_{11}S_3$: 1313.7641 $[M+H]^+$, found 1313.7654 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.22 (m, 2H), 8.10 (d, *J* =7.60 Hz, 1H), 8.01 (m, 1H), 7.92 (m, 2H), 5.43 - 5.31 (m, 2H), 5.13 (t, *J* =8.08 Hz, 1H), 5.03 (d, *J* =6.65 Hz, 2H),4.53 (m, 1H), 4.46-4.29 (m, 2H), 4.23 (m, 1H), 4.15 (q, *J* =5.7 Hz, 1H), 3.68 (m, 2H), 3.60 (t, *J* =9.80 Hz, 1H), 3.45 (s, 3H), 2.63 - 2.83 (m, 6H), 2.53-2.51 (m, 6H), 2.09 (t, *J* =7.41, 2H), 2.09 (s, 3H), 1.98-1.78 (m, 2 H), 1.64 (m, 1H), 1.61 (s, 6H), 1.54 (s, 6H), 1.47 (m, 5H), 1.31-1.14 (m, 28H), 0.84 (m, 9H), 0.74 (t, *J* =6.14 Hz, 6H).

Labeling of N-Ras (Δ1-181) wt



The shortened N-Ras protein (Δ1-181) expressed in bacteria was converted into 100 mM $NaHCO_3$ buffer (pH 8.5) and purified by size exclusion chromatography. After determination of the protein concentration according to Bradford test, 10 mg of the protein were slowly rotated in 100 mM $NaHCO_3$ buffer (pH 8.5). For the labelling, 1 mg of the Bodipy-NHS ester were added to the protein under argon atmosphere at 4 °C and protected from light. After 2 h, the protein and the free dye were separated by large-scale exclusion chromatography. The labelled protein was transferred to microcons (0.5 mL, MWCO 10 kDa) and the buffer system was changed into the respective ligation buffer (20 mM Tris, 5 mM $MgCl_2$, pH 7.5). Both the protein concentration and the dye concentration were determined by measuring the absorbance at 280 nm (ε = 13660 $M^{-1}cm^{-1}$) and 505 nm (ε = 80000 $M^{-1}cm^{-1}$), respectively.

Ligation of the *N*-terminal lipidated peptides with N-Ras (Δ1-181)



Scheme S5. The shortened N-Ras protein was ligated with the synthesized lipidated peptides to obtain the corresponding lipidated proteins.

2 eq of the double lipidated peptide were dissolved in 300 µL of methanol and mixed with 11% Triton X-114 (1 mL) and 1 eq of the shortened N-Ras protein (Δ1-181, wt). The mixture was rotated overnight at 4 ° C. on a rotary mixer under argon atmosphere and protected from light. Subsequently, 12 ml of buffer were added and the reaction mixture was further incubated at 37 ° C (water bath). To separate the Triton (protein phase) from the buffer phase, the mixture was carefully mixed by inverting the tube 2-3 times and finally centrifuged at 30 °C for 5 minutes at 4000 rpm. This procedure was repeated twice by adding 1 mL of 11% Triton X-114 each time. The collected triton phases were then purified by means of ion exchange chromatography and concentrated in amicons (MWCO 10 kDa). The protein concentration could be further determined by measuring the absorbance at 280 nm (ε = 13660 $M^{-1}cm^{-1}$). Further analysis and characterization of the ligated lipoproteins was carried out by SDS-page (12% gel) and MALDI-TOF. The proteins were further aliquoted and finally frozen and stored at -80 ° C. The same protocol was applied to the BODIPY-labeled N-Ras protein (Δ1-181, wt) to obtain the fluorescently labeled lipidated proteins.

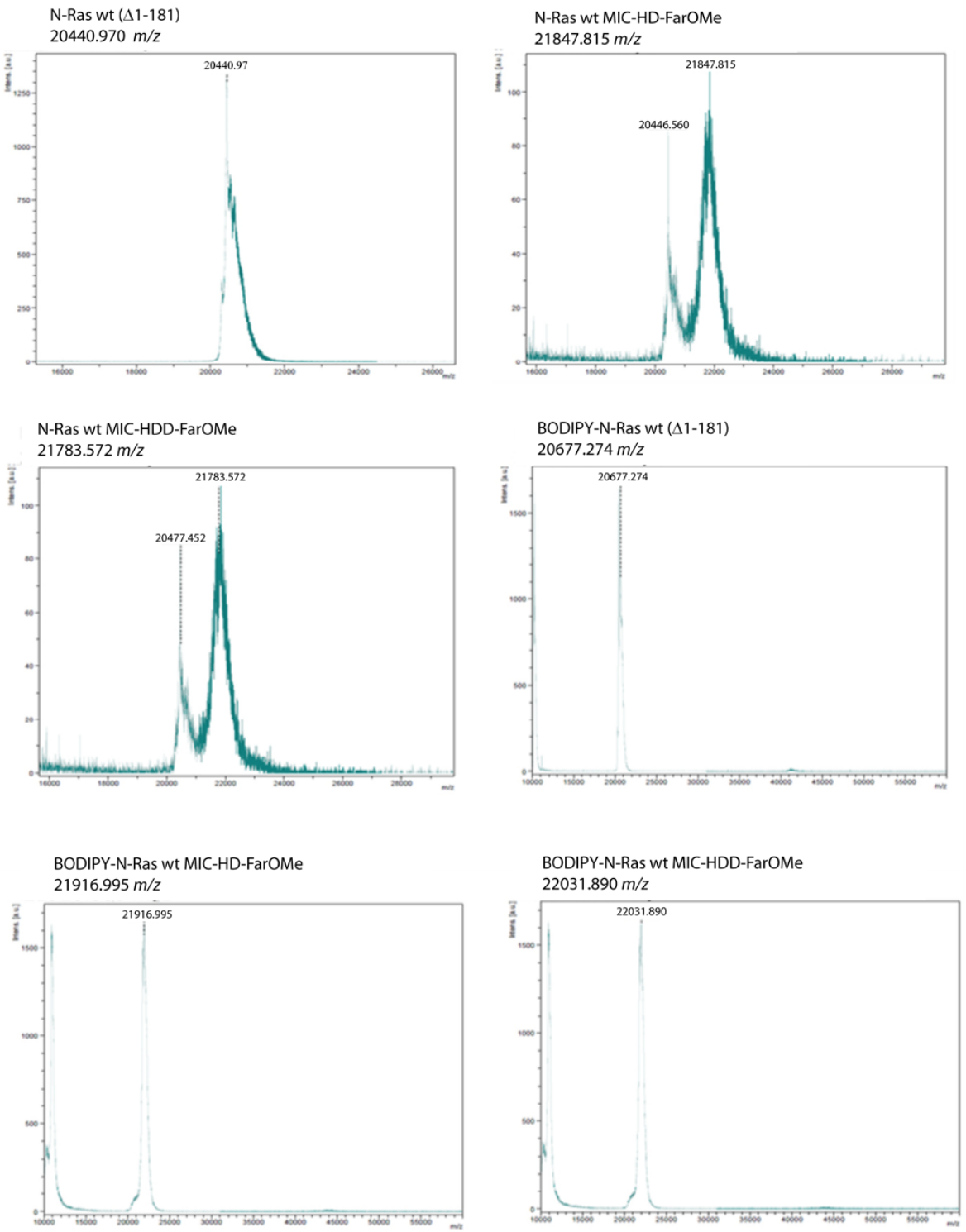


Figure S1. MALDI-TOF mass spectra of the ligated proteins

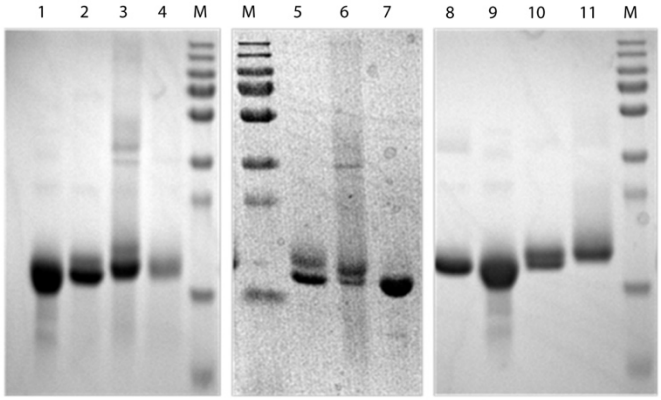


Figure S2. SDS-PAGE analysis of the products from the ligation (Coomassie staining). M: Protein marker, N-Ras wt. (Δ1-181) (lane 1); N-Ras wt. MIC-HDD-FarOMe P1 (lane 2); N-Ras wt. MIC-HDD-FarOMe P2 (lane 3); N-Ras wt. MIC-HDD-FarOMe P3 (lane 4); N-Ras wt. MIC-HD-FarOMe P1 (lane 5), N-Ras wt. MIC-HD-FarOMe P2 (lane 6), Ras wt. (Δ1-181) (lane 7); BODIPY-N-Ras wt. (Δ1-181) (lane 8), Ras wt. (Δ1-181) (lane 9); BODIPY-N-Ras wt. MIC-HDD-FarOMe P2 (lane 10); BODIPY-N-Ras wt. MIC-HDD-FarOMe P3 (lane 11)

Cell culture. Neuro-2a cells (ATCC®CCL-131™, mus musculus, mouse) were cultured in Eagle's Minimum Essential Medium (MEM, PAN BioTech, Aidenbach, GER), supplemented with 10%(v/v) fetal calf serum (FCS, GIBCO-Invitrogen, Darmstadt, GER), sodium pyruvate (PAN BioTech, Aidenbach, GER), *L*-Glutamine and PenStrep-mix (100 U/mL penicillin/100 µg/mL streptomycin (PAN Biotech, Aidenbach GER) and grown at 37°C in a 5% CO_2 incubator. HEK293 cells (ATCC®CRL-1573™, homo sapiens, human) and HeLa cells (ATCC®CCL-2™, homo sapiens, human) were cultured in Dulbecco's Modified Eagle Medium (DMEM 4.5g/L Glucose, PAN BioTech, Aidenbach, GER), supplemented with 10% (v/v) fetal calf serum (FCS, GIBCO-Invitrogen, Darmstadt, GER), sodium pyruvate (PAN BioTech, Aidenbach, GER), MEM-NEAA and PenStrep-mix (100 U/mL penicillin/100 µg/mL streptomycin (PAN Biotech, Aidenbach GER) and grown at 37°C in a 5% CO_2 incubator.

Treatment of cells

Feeding non-natural fatty acids and LE

In order to investigate the uptake and acylation of non-natural fatty acids in cells, the cells were first cultured. From a confluence of 85%, the cells were incubated with a final concentration of 400 µM of nonadecanoic acid (C-19) for at least 24 hours at 37 °C. For this purpose, a fatty acid solution consisting of 78 µL of the fatty acid (12.5 mM in DMSO) and 4 volumes (312 µL) of a solution of 14% (w / v) fatty acid-free BSA in 0.1 M Tris buffer (pH 8.3) was prepared. After an incubation period of one hour, the solution was sterile filtered. This stock solution was diluted 1: 6.25 in the medium. Untreated cells served as a negative control. Subsequently, the cells were harvested and subjected to the lipid extraction protocol and finally analyzed by HRMS.

Table S1 shows the results of the quantification of the released fatty hydroxamic acids in HEK293, HeLa and N2a cells treated with nonadecanoic acid compared to untreated cells. Values are given as pmols of detected fatty hydroxamic acids/millions of cells.

	pmols/10^6 cells					
	HEK293	HEK293+C19:0	HeLa	HeLa+C19:0	N2a	N2a+C19:0
C14:0	6.80	6.18	6.55	5.95	3.94	3.58
C16:0	23.76	21.41	21.24	19.13	8.74	7.87
C16:1	8.76	8.34	8.03	7.65	2.95	2.81
C18:0	-	-	-	-	3.33	2.78
C18:1	6.60	5.50	5.79	4.83	9.68	8.07
C18:2	2.34	1.95	2.81	2.35	1.63	1.36
C20:4	1.74	1.55	1.69	1.50	1.14	1.02
C19:0	-	9.09	-	8.63	-	9.31

Immunoprecipitation

The cells were cultured, harvested and lysed as described before. For the modification of the protein A/ G agarose beads (ThermoFisher Scientific Pierce, 7 mg human IgG/ mL resin), 100 µL were washed three times in 500 µL of PBS buffer and then submerged with 5 µl of the respective primary antibody at 4 °C by head to end rotation overnight. After several washing steps with PBS buffer, 200 µL of the lysate were added to 50 µL of the antibody-modified agarose beads and the mixture was incubated at 4 °C for 2 h. The suspension was then centrifuged (10 sec, 12,000 × g) and the supernatant was carefully removed. After three washing steps with PBS buffer and a final centrifugation step (10 sec, 12,000xg), the supernatant was removed. The resulting pellet was dissolved in 150 µL 4 SB buffer, subjected to the lipid extraction protocol and finally analyzed by HRMS. 10 µL of the mixture were further tested for SDS gel and Western Blot.

Lipid Analysis

Deacylation of Fmoc-Cys(Pal)-OH

5 µL of a solution of Fmoc-Cys(Pal)-OH in DMSO (5 mM, 25 nmol) were treated overnight with 5 µL of a solution of FITC-ONH_2 in 50 mM Tris, 0.2 % Triton X-100, pH 7.4 (50 - 3.75 pmol, 10 - 0.75 µM). 10 µL of the internal standard were then added (0.5 nmol, from a stock solution of 50 µM in Methanol). The samples were dried and solved in 100 µL Methanol (LC-MS *Grade*), 5 µL were taken and analyzed by HR-MS.

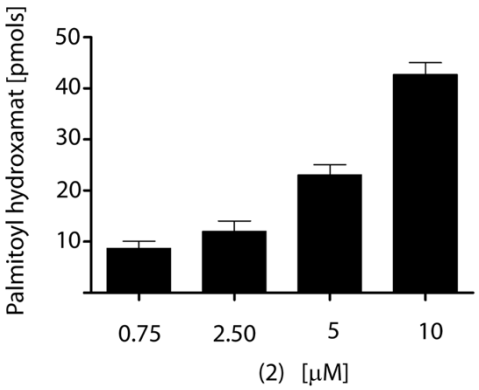


Figure S3: Fmoc-Cys(Pal)-OH was treated with different concentrations of **2** and the released palmitoyl hydroxamic acid was quantified by HRMS. Values shown are mean ± SD (n = 3)

Preparation of cell lysates. Cells were washed with PBS and were incubated with a trypsin/EDTA solution for 3-5 min at 37°C in a 5% CO_2 incubator, washed with PBS and lysed in lysis buffer (50 mM NaCl, 5 mM $MgCl_2$, 5 mM EGTA, 0.1 % NP-40, 0.1 % Tween-20, 0.1 % TritonX-100, Protease-/phosphatase-inhibitors (Complete protease Inhibitor (EDTA free) and PhosSTOP (Phosphatase inhibitor, Roche), pH 7.4) Typically 1–4 × 10^6 cells were employed in each analysis. The cells were unlocked by repeating freeze-thaw cycles and a final ultra-sonication step. The protein concentration of the supernatant was determined by the Bradford method.

All solvents used were LC-MS grade. The cells were cultured (typically 1–4 × 10^6) , harvested and lysed as described above. The final cell pellet was taken up in lysis buffer (500 µL), subjected to five heat/cold shocks, pressed ten times through a 20G cannula and finally cleared by ultrasonication. The proteins were precipitated by adding 500 µL of ice-cold acetone and centrifuged at 13 000 rpm for 10 min at 4 ° C. The precipitated protein pellet was washed three times with cold methanol (500 µL). Non-covalently bound lipids were then removed by a modified Bligh and Dyer lipid extraction protocol. Each sample (200 µL in water) was extracted by sequential addition of methanol (400 µL), chloroform (200 µL) and distilled water (300 µL). Samples were vortexed following each addition.

To separate the phases, the mixture was centrifuged at 4,000 *g* for 20 min in a centrifuge with swinging rotor (room temperature). The proteins precipitated in the interphase of the solvent phases. The upper, aqueous phase (water and methanol) was carefully removed with the pipette and centrifuged again (4,000 × g, 20 min) after the addition of 1.5 volumes of methanol (300 µL). The supernatant (all the remaining liquids) was removed and the protein pellet was subjected to two further CM rounds. After the third and final extraction, the pellet was completely dried in the argon stream and re-suspended in 4 % SDS buffer (4 % SDS, 50 mM Tris, pH 7.4). For this, 200 µL of the buffer were added to the residue and slowly dissolved by shaking (350 rpm, 37 ° C, 10 min). After a final short ultrasonic pulse, the bound fatty acids of the proteins were bound to the hydroxylamine linker by adding the +HA-buffer (200 µL, 0.7 M Hydroxylamin-Linker, 0.2 % Triton X-100, pH 7.4). For this, the

mixture was incubated at room temperature overnight with head to end rotation. The sample for the negative control was incubated with the -HA buffer(200 µL, 50 mM Tris, 0.2 % Triton X-100, pH 7.4) overnight. Subsequently, each sample was provided with an internal standard (C-17; 10 µL, 0.5 nmol, 50 µM stock in methanol) and finally dried at high vacuum overnight. Lipids were resuspended in 100 µL methanol, 5 µL of the samples were directly used for HRMS analysis, without further dilution.

HRMS analysis

High-resolution mass spectrometry was performed on a LTQ Orbitrap Fournier transformation mass spectrometer from Thermo Electron. This spectrometer was coupled to a HPLC device from the same company. The ionization mode was ESI (Electronspray ionization) using a source voltage of 3.8 kV. For the analysis we used an EC125/ 2 Nucledur C4 gravity column and injected 5 µL per sample. The column was maintained at 40 °C using a column oven. All solvents employed were LC-MS grade. Mass range of m/z 50 to 800 was acquired with a resolution of 60.000 for full scan. The two mobile phases were water + 0.1% HCO_2H (A) and acetonitrile + 0.1% HCO_2H (B) at a flow rate of 500 µL/ min. A linear gradient was programmed: 0.0 min: 90% A; 1.00 min 90% A, 6.00 min 25% A, 10.00 min 10% A, 12.00 0% A. Quantification was carried out using the extracted ion chromatogram of each compound. The linear dynamic range was determined by injecting standard mixtures. Positive identification of compounds was based on the accurate mass measurement with an error of 5 ppm and its LC retention time compared to that of a standard.

pmols of Fatty acid hydroxamates were calculated employing the following formula:[2]

pmol (A)= pmol (IS) x (A(A)/A(IS) x (M(IS)/M(A))
(A): Analyte
(IS): Internal standard C17:0
A (A): analyte peak area
A (IS): internal standard peak area
M : slope of the linear regression of the calibration curve

Calibration curves: 5 µL of the reference compounds (50-0.02 pmol; 10 µM-0.002 µM) were spiked with an equal amount of the internal standard (C17:0; 50 pmol). 5 µL of this mixture were injected and analyzed by MS.
Linearity. Stock of the references compounds and internal standard were diluted in MeOH. 5 µL of each serial dilution (25-0.01 pmol; 5 µM-0.001 µM) were analyzed by HRMS and curves were obtained by plotting the peak area versus the amount of pmols present. Linearity was determined from the values of correlation coefficient (*r*). The limit of detection (LOD) and the limit of quantitation (LOQ) were defined as 3 times signal-to-noise and 10 times signal-to-noise, respectively.

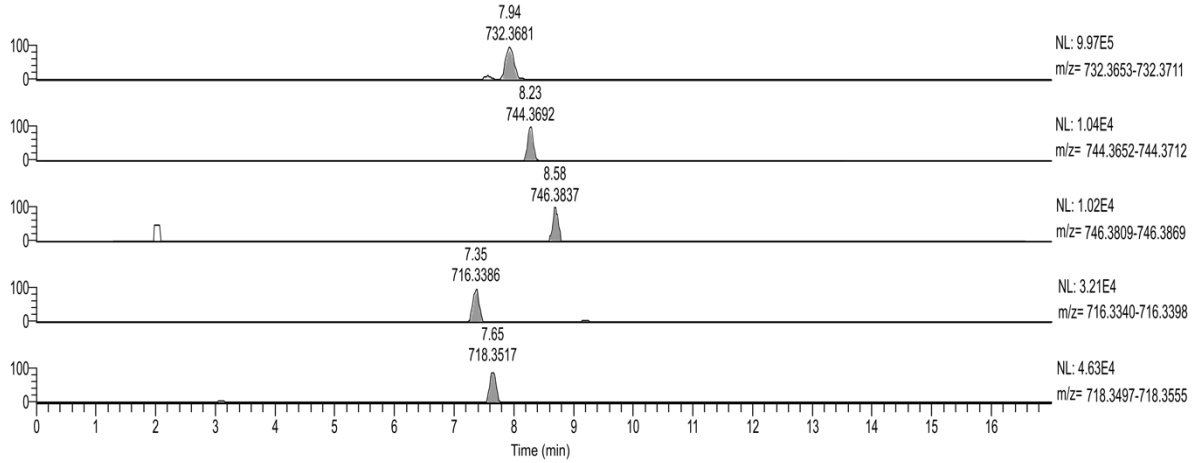
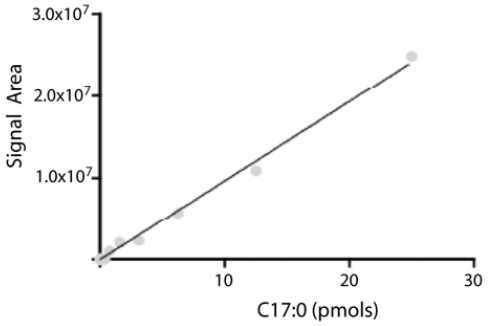
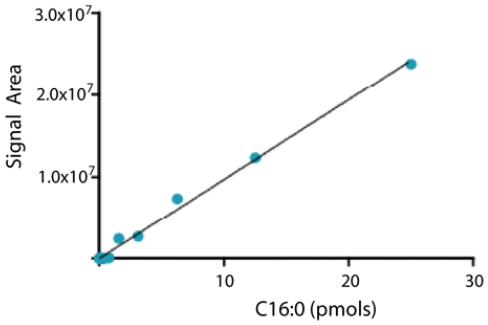
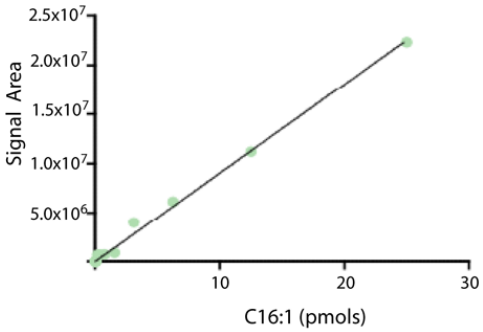
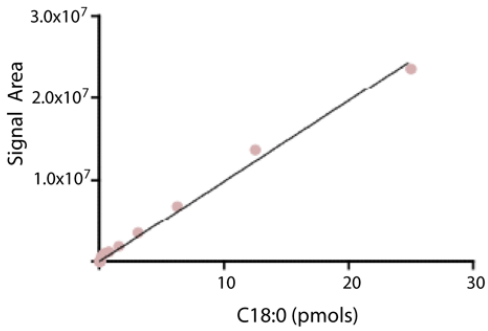


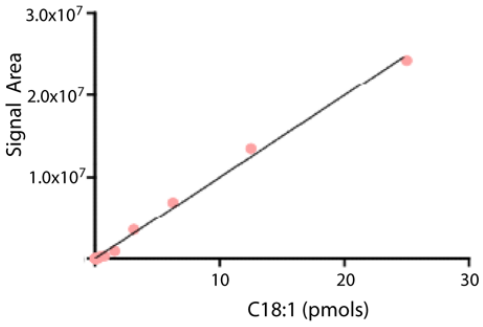
Figure S4. Extracted ion chromatograms (EICs) from positive mode analysis of representative synthesized reference compounds. From top down spiked internal standard C17 (calcd. for $C_{41}H_{53}N_3O_7S$ $[M+H]^+$ = 732.3682), C18:1 (calcd. for $C_{42}H_{53}N_3O_7S$ $[M+H]^+$ = 744.3682), C18:0 (calcd. for $C_{42}H_{55}N_3O_7S$ $[M+H]^+$ = 746.3682), 16:1 (calcd. for $C_{40}H_{49}N_3O_7S$ $[M+H]^+$ = 716.3370), 16:0 (calcd. for $C_{40}H_{51}N_3O_7S$ $[M+H]^+$ = 718.3526).











Reference (FAH)	LOQ [pmol]
Palmitoyl-NH_2OH (**1**)	2.50
Palmitic (C16:0), (**3**)	0.11
Lauric (C12:0), (**7**)	0.11
Myristic (C14:0), (**8**)	0.10
Palmitoleic (C16:1), (**9**)	0.10
Stearic (C18:0), (**10**)	0.13
Oleoic (C18:1), (**11**)	0.17
Arachidic (C20:0), (**12**)	0.17
Arachidonic (C20:4), (**13**)	0.16
Heptadecanoic (C20:4), (**14**)	0.12
Nonadecanoic (C20:4), (**15**)	0.13

Table S2. Limit of Quantification (LOQ) of the fatty acid hydroxamates synthesized as reference compounds determined as 10 times signal-to-noise (S/N) ratio. The attached figure shows the representative curves for the following fatty acid hydroxamates (17:0, 16:0, 16:1, 18:0 and 18:1).

RNA Isolation

The RNA was isolated using the RNeasy mini kit from QIAGEN. To this end, cells were seeded in a 12-well plate in the first step and incubated at 37 °C for 24 h under standard conditions. The cells were then lysed and homogenized (QIA shredder columns). The lysate was mixed 1:1 with 70% ethanol and this mixture was transferred to the RNeasy columns. After several washing steps and a final drying step of the membrane, the RNA was eluted from the column with 30 µL of RNA-free water. All DNA and RNA concentration determination were carried out with the help of the NanoDrop 2000c spectrophotometer at a wavelength of 260 nm in a double-determination. For this purpose, 1 µL of the sample was measured and the baseline (water/ buffer) was subtracted. In addition, the 260/280 nm ratio was employed to determine the purity of the samples.

cDNA (Reverse transcription)

Isolated RNA was transcribed into cDNA using the QuantiTect Reverse Transcription Kit from QIAGEN. First, genomic DNA was eliminated by treating the isolated RNA sample (1 µg) in 12 µLof DNA/ RNA-free water with 2 µL gDNA Wipeout buffer at 42 °C in the PCR thermocycler for 2 min and then placed directly on ice. Next, 6 µL of a mixture containing 1 µL of the Quantiscript reverse transcriptase, 4 µL of the Quantiscript RT buffer and 1 µL of the oligonucleotide mixture was then added to the solution. After incubation at 42 °C for 15 min, the reverse transcription was carried out and terminated by a further incubation of 3 min at 95 °C.

PCR

10 µL of PhusionFlash High-Fidelity PCR master mix (Thermo Scientific), 100 ng of the DNA to be amplified, and 0.5 µM of the respective oligonucleotides were mixed and DNA-free water was added to a total volume of 20 µL. The melting temperatures were adjusted to the length and the GC content of the respective oligonucleotides.
PCR Cycle:
Initial denaturation: 98 ° C for 10 s (1 cycle)
Other denaturations: 98 ° C for 1 s (30 cycles)
Addition of oligonucleotides: individual melting temperature for 5 s
Elongation of oligonucleotides: 72 ° C for 15 s
Final Elongation: 72 ° C for 1 min.

Quantitative PCR

Target	Organism	Orientation	Sequence
NRas	Human	FWD	GTT CCG GGG TCT CCA ACA TT
NRas	Human	REV	CAC CAG CAA GAA CCT CAA GC
NRas	Mouse	FWD	CCA CTT TCA AGC TGC ACT GAC
NRas	Mouse	REV	TTG TGC TGT GGA AGA ACC CA
CLN3	Mouse	FWD	CAA AGG AGA TAT ACC
CLN3	Mouse	REV	CTA GAC CAG TTT AAA

Expression vectors and transfection

The following plasmids were employed: peGFP-C1-N-Ras, pCGN-HA-N-Ras, pOPIN(n)-Cherry-CLN3. pCGN N-Ras wt was a gift from Adrienne Cox (Addgene plasmid #14723).[3] The nucleotide sequence corresponding to the human N-Ras (accession number NM_002524) was amplified using the oligonucleotides: 5'-GTTCCGGGGTCTCCAACATT-3'; 5'-CACCAGCAAGAACCTCAAGC-3' for N-Ras and subsequently subcloned in pEGFP-C1 and pCGN-Hygro, respectively. The human cDNA construct for mCherry-CLN3 was a gift from Stephan Raunser. The nucleotide sequence corresponding to the human mCherry-CLN3 (accession number NM_000086) was amplified using the oligonucleotides: 5'-CAAAGGAGATATACC-3';5'-CTAGACCAGTTTAAA-3' for CLN3 and subsequently subcloned in pOPIN(n).

Transformation into *E. coli*

100 µL of the chemically competent *E. coli* cells (OneShot® OmniMAX ™ 2 T1) were first thawed on ice and then incubated on ice for 30 min with 100 ng of the corresponding DNA. The cells were then incubated at 42 °C for 1 min (heat shock) followed by an incubation for 2 min on ice. 900 µL of LB medium were then added to the mixture. After an incubation at 37 °C for one hour in the shaker, the cells were centrifuged and the supernatant was discarded. The cell pellet was taken up in 100 µL LB medium, plated on two LB agar plates with appropriate selection pressure (antibiotic) and incubated overnight at 37 °C.

Isolation of Plasmids
Using the EndoFree Plasmid Maxi Kit, the transformed plasmid DNA was isolated using the principle of alkaline lysis. One of the colonies grown under selection pressure was picked and transferred into a liquid culture. For this purpose, the cell colony was placed in 250 mL of an LB medium mixed with the respective selection marker and incubated at 37 ° C in the shaker overnight. After centrifugation of the liquid culture and subsequent re-suspension of the cell pellet, the lysis could be started. In the first step, the cell lysate was neutralized and passed through an ion exchange resin (QIA filter cartridge). Low molecular weight substances (RNA, proteins) can be removed by middle salt concentration washing steps without compromising the binding between plasmid DNA and the column. By increasing the salt concentration (QN buffer), the plasmid DNA can then be eluted. After desalting (isopropanol precipitation), washing steps (endotoxin-free 70% ethanol) and concentration of the product (air drying), the plasmid DNA was re-suspended in 30 µL endotoxin-free water and the concentration could be determined at 260 nm (Nanodrop).

DNA Separation
DNA was separated by gel electrophoresis using agarose gels. For the preparation of the gels, 2% (w /v) agarose was dissolved in a single concentrated TRIS-Acetate-EDTA buffer (TAE buffer) under heating. GelRed ™ (Nucleic Acid Gel Stain) was then added to the cooled mixture. The DNA samples were mixed with 1/6 loading buffer and applied to the gel. Separation in the gel took place at a constant voltage of 130 V. Typically, a DNA standard served as mass control. Subsequently, the DNA and the DNA standard were examined under UV light.

Stable transfections
The Amaxa® CellLine Nucleofector® (Lonza) was used for the stable transfection of cells. Electroporation is a transfection technique based on the instantaneous generation of small pores in the cell membrane by the application of an electrical pulse. The cells were grown to 90 % confluence in the cell culture flask. After removal of the medium and washing steps with sterile PBS buffer, 2 mL of trypsin/ EDTA were added to the cells so that all the cells were briefly covered and incubated at 37 °C until the cells were detached (2-5 min). Subsequently, the cells were taken up in medium and transferred into a 15 mL cultured tube. 3 mL of the cell suspension were centrifuged at 300 rpm for 5 min, the supernatant was removed and the cell pellet was re-suspended in 100 µL of the Amaxa® Cellline Nucleofector® Kit Solvent (82 µL Nucleofector® solution and 18 µL Supplement Solution 1). Immediately thereafter, 2 µg of the plasmid DNA to be transfected was added and the mixture was transferred to the specific Amaxa cuvette. Transfection was carried out after selecting the special transfection program (Hek293: Q-001, N2a: T-024). Subsequently, 500 µL of the respective medium were added to the mixture in the cuvette and pipetted into a tissue culture bottle prepared with medium. The medium was changed after 24 hours and 2-3 days after transfection, selection pressure was initiated.

Immunoblotting
Cells were collected and lysed following the procedure described above. Protein concentration was determined using the Bradford method. IP-beads were modified and treated with cell lysates as described above. To release the proteins from the beads, samples were adjusted to the same protein concentration, mixed with SDS loading buffer (5x, 50% (v/v) glycerol, 250 mM Tris (pH 6.8), 350 mM SDS, 500 mM DTE, 360 µM bromophenol blue) and boiled for 5-10 min at 96°C prior to loading onto the gel. Protein samples were loaded onto a SDS gel. The separation of the proteins was conducted in SDS running buffer (2.5 M glycine, 250 mM Tris, 35 mM SDS) at 40 mA per gel for 1 h at room temperature. Estimation of the protein size was performed in reference to the PageRulerTM Plus Prestained Protein Ladder (Thermo Fisher Scientific). To stain the proteins, the SDS gel was incubated with a fixing Solution (10% (v/v) glacial acetic acid, 40% (v/v) ethanol, in water) for 15 min and immediately transferred into a Coomassie Staining Solution (0.25% (w/v) Coomassie brilliant blue G-250, 45% (v/v) methanol, 10% (v/v) acetic acid) and stained for 1 h. Afterwards the SDS gel was de-stained using a de-staining Solution (30% (v/v) methanol, 10% (v/v) acetic acid) and rinsed with ddH_2O. The gel was imaged using the Gellogic 200 Imaging System (KODAK) and KODAK-Mi software.

The proteins separated by SDS-PAGE were transferred by means of semi-dry electroblotting to a polyvinylidene difluoride (PVDF) membrane (Immobilon® FL- Transfer Membrane, Thermo Scientific, Schwerte, Germany) with a Bio-Rad Trans-Blot semi-dry blotter for 60 min at 25 V prior to the electrotransfer, all the layers were

soaked in freshly prepared transfer buffer (14.41 g/L glycin, 3.03 g/L Tris, 20% (v/v) MeOH, in water). Membranes were blocked for 30 min using 2% (w/v) non-fat dry milk powder in PBS-buffer containing 0.05 % (v/v) Tween-20, pH 7.5 (PBS-T). The appropriate primary antibody was incubated overnight at 4°C in the buffer following vendor's instructions. Anti-α-tubulin was employed to detect α-tubulin as loading control. Unbound antibodies were removed by washing with PBS-T buffer for 10 min (3 times). The corresponding secondary antibody was added according to vendor's instructions and incubated for 1 h at room temperature at constant agitation in the dark. The membrane was again washed twice with PBS-T buffer (10 min) and twice with PBS (10 min) to decrease the detergent concentration. Signals were detected using Super-Signal West Pico Chemiluminescent Substrate (Thermo Scientific) and scanned employing an Odyssey® Fc Imaging System (Li-COR® Bioscience, Bad Homburg, Germany).

Primary Antibody	**Organism**	**Number**	**Brand**
Anti-GFP	Mouse	# G6795	Sigma Aldrich
Anti-Tubulin	Mouse	# F2168	Sigma Aldrich
Anti-CLN3	Mouse	# MABN372	Merck Millipore
Anti-CLN3	Mouse	# WH0001201M3	Sigma Aldrich
Anti-CLN3	Mouse	# sc-398192	Santa Cruz Biotechnology
Anti-panRas	Mouse	# sc-166691	Santa Cruz Biotechnology
Anti-N-Ras	Rabbit	# sc-519	Santa Cruz Biotechnology
Anti-HA	Rat	# 11815016001	ROCHE

Secondary Antibody	**Reactivity**	**Number**	**Brand**
Goat-anti-Mouse HRP	Mouse	# 31430	Thermo Scientific
Ziege-anti-Rabbit HRP	Rabbit	# 31460	Thermo Scientific
Donkey-anti-Mouse IR680	Mouse	# 926-68072	Li-Cor
Donkey-anti-Mouse IR800	Mouse	# 926-32210	Li-Cor
Donkey-anti-Rabbit IR680	Rabbit	# 926-68073	Li-Cor
Donkey-anti-Rabbit IR800	Rabbit	# 926-32213	Li-Cor

Staining and Fixing cells for microscopy

Cells intended for (immuno-) fluorescent stains were plated either in 96 (black wall, clear bottom, Corning), 384 (black wall, clear bottom, Corning) or in standard 24 plates on top of coverslips. The cells were carefully washed with PBS to remove the remaining medium and then bound to the lids by addition of the fixation buffer (3.7% formaldehyde in PBS) for 5-10 min. The fixation buffer was replaced by permeabilization buffer (0.1% (v /v) Triton X-100 in PBS) and the fixed cells were incubated for a further 5 min. Finally, the cells were washed with PBS to remove the remaining buffer. Fluorescent dyes for the direct staining of cellular compartments and structures were used according to the manufacturer's instructions. Washed cells were either dyed directly for the respective application or were first prepared for coloration by fixation and permeabilization, if necessary. After completion of the staining, the cells were washed with PBS for 5 min by gentle stirring to remove the unbound dye residues. The nuclei were stained by the blue DAPI (4′,6-diamidino-2-phenylindole) (Thermo Fisher) in living cells as well as in fixed cells (stock solution 1 mg/ mL in ethanol, 1: 500 to 1: 1000 in PBS, 1-2 h). The dye was removed by washing three times in PBS before the cells were finally fixed, permeabilized and thus used for the staining of further organelles. Fixed cells were blocked for 1 h (2% (v/v) BSA in PBS-T) to minimize nonspecific binding. Rab7 staining: The primary antibody was diluted according to manufacturer instructions (usually PBS-T/ 2% (w/ v) BSA unless otherwise indicated) and added to the cells for 1 hour at room temperature (alternatively at 4 °C overnight). The antibody was removed and stored for reuse at -20 °C. The cells were washed with PBS-T/ 2% (w/v) BSA for 10 min. The secondary antibody (fluorescent conjugate, green) was then added (1: 500 in PBS-T/ 2% BSA) and incubated for 1 h at room temperature. Unbound antibody was removed by washing three times with PBS for 10 min. The cells were fixed and prepared by controlling with nail varnish for light-protected storage. Fluorescent-dyed cells were washed three times for 5 min with PBS under pertinent swirling to remove

the remaining dye. When the cells were grown in plates, a sufficient volume of PBS was allowed per well to keep the cells in a liquid solution. The plates were sealed with aluminum and stored at 4 °C until final microscopy. The cover glasses with the plated cells were carefully removed and then glued onto a slide with a drop of Aqua-Poly / Mount Medium (Polyscience). The coverslips were dried at room temperature with light protection and then stored at 4 °C until microscopy. Fluorescent-dyed cells were washed three times for 5 min with PBS under pertinent swirling to remove the remaining dye. When the cells were grown in plates, a sufficient volume of PBS was allowed per well to keep the cells in a liquid solution. The plates were sealed with aluminum and stored at 4 °C until final microscopy. The cover glasses with the plated cells were carefully removed and then glued onto a slide with a drop of Aqua-Poly/ Mount Medium (Polyscience). The coverslips were dried at room temperature with light protection and then stored at 4 °C until microscopy. Fluorescent tagged proteins, like eGFP-*N*-Ras (green) and mCherry-CLN3 (red) were stained using DAPI, to visualize the nuclei or further stained using WGA-488 (Wheat Germ Agglutinin, Alexa Fluor™ 488 Conjugate, Thermo Fisher) to stain the plasma membrane.

Atomic force microscopy (AFM) studies

The preparation of the supported lipid bilayers and the AFM setup is described in detail in ref[4]. Vesicle fusion on mica was carried out by depositing 70 µL of the large unilamellar vesicles (LUV) solution on freshly cleaved mica and incubation in a wet chamber at 70 °C for 2 h. After vesicle fusion, the samples were rinsed carefully with Tris buffer to remove unspread vesicles. For the lipidated protein-membrane interaction studies, 200 µL of N-Ras protein in 20 mM Tris, 7 mM $MgCl_2$, pH 7.4 (at the desired concentrations) were injected into the AFM fluid cell at room temperature. Measurements were performed on a MultiMode scanning probe microscope with a Nano- Scope IIIa controller (Digital Instruments, Santa Barbara, CA) and use of a J-Scanner (scan size 125 µm). Images were obtained by applying the tapping mode in liquid with oxide-sharpened silicon nitride (DNP-S) or sharp nitride lever (SNL) probes mounted in a fluid cell (MTFML, Veeco (now Bruker), Karlsruhe, Germany). Tips with nominal force constants of 0.24 Nm^{-1} were used at driving frequencies around 9 kHz and drive amplitudes between 200 and 800 mV. Scan frequencies were between 1.0 and 1.94 Hz. Height images of sample regions were acquired with resolutions of 512 × 512 pixels. All measurements were carried out at room temperature and analyzed by using Gwyddion.

Kinetics studies

Preparation of large unilamellar vesicles. The dried lipid mixture was hydrated with 20 mM Tris (Merck, Darmstadt, Germany), 5 mM $MgCl_2$ (Merck, Darmstadt, Germany) buffer at pH 7.4. The hydrated lipid mixture was then vortexed, kept in a water bath at 65 ˚C for 15 min, and sonicated for 10 min. After five freeze–thaw–vortex cycles and brief sonication, large multilamellar vesicles formed and were transformed to unilamellar vesicles of homogeneous size by use of an extruder (Avanti Polar Lipids, Alabaster, USA) with polycarbonate membranes of defined pore size (100 nm) at 65 ˚C.

All the kinetic studies were carried out using fluorescence resonance energy transfer (FRET) based experiments. The FRET-based studies were performed using N-Rh-PE-labeled lipid vesicles as the acceptor and BODIPY-labeled N-Ras was used as donor. Large unilamellar vesicles (LUVs) composed of DOPC/DPPC/Chol (1:2:1) and 0.1 mol% Rh-N-DHPE were prepared in 20 mM Tris, 5 mM $MgCl_2$, pH = 7.4 buffer. Bodipy labelled N-Ras binding to the LUVs caused an increase in the FRET signal which was monitored over time to determine the kinetics of the interaction (protein:lipid = 1:250). Before the measurement, the solution was rigorously mixed in the cuvette which resulted in a dead time of 3 s. The resultant kinetic curve was fitted using a biexponential fitting model.

$$F = F_0 + A_1(1 - \mathrm{e}^{-k_1 t}) + A_2(1 - \mathrm{e}^{-k_2 t})$$

where F is the change in the fluorescence intensity due to the increase in the FRET signal, F_o is the initial fluorescence intensity, t is the time, and k_1 and k_2 are the rate constants which are determined by the fit. It must be noted that similar measurements have been performed before on a stopped flow instrument[5] and the rate constant value that we obtained are in the same order of magnitude.

All fluorescence spectroscopy and anisotropy measurements were performed on a K2 multifrequency phase and modulation fluorometer (ISS, Champaign, IL). Excitation light of 488 nm was provided by a xenon lamp through a monochromator. Single point emission intensity at 591 nm was collected at 90° through a second monochromator.

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