
Molecular machines stimulate intercellular calcium waves and cause muscle contraction

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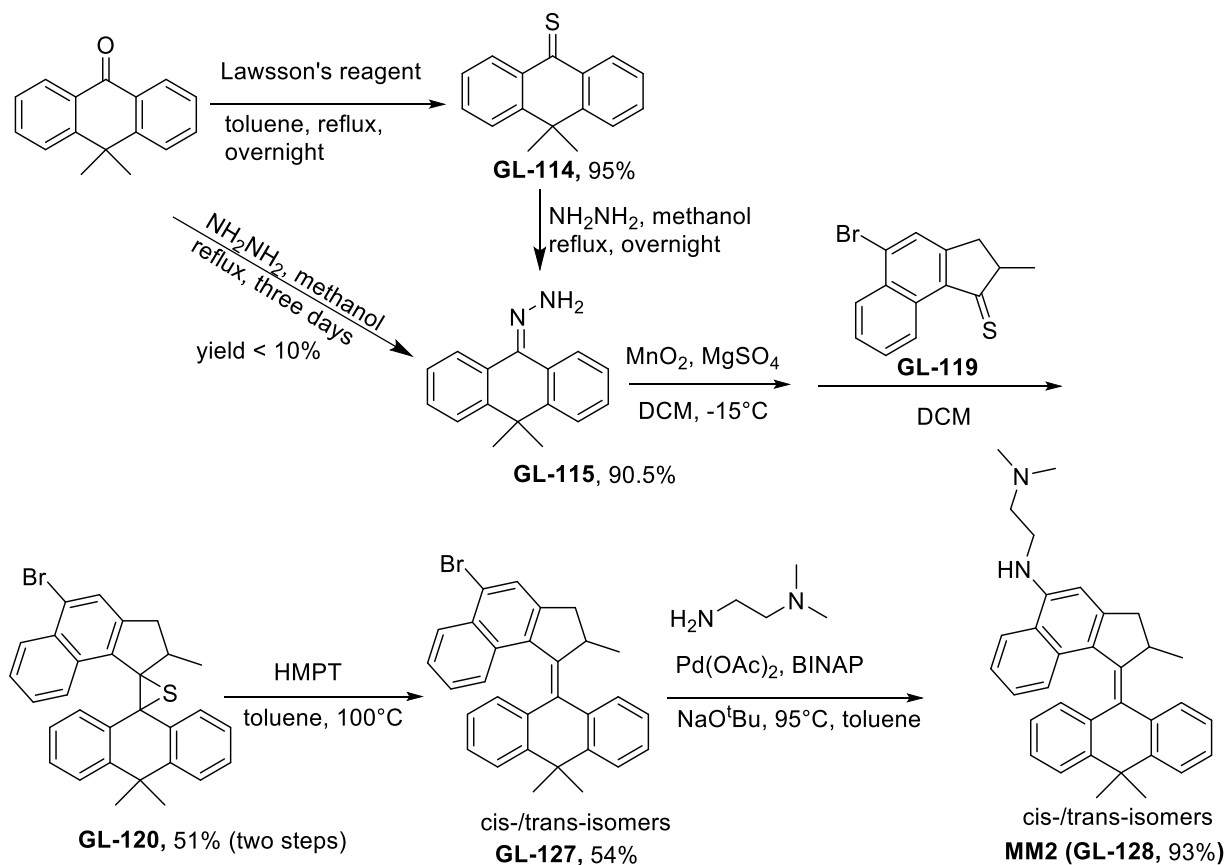
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Materials and Methods

Synthesis of MM 2

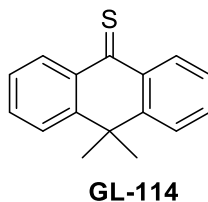
Final product characterization of MM 2 is reported in Supplementary Figs. 34-36.



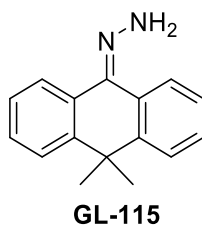
Supplementary Scheme 1. Synthesis of MM 2 (herein denoted GL-128).

General Methods. All glassware was oven-dried prior to use. Reagent grade dichloromethane (DCM, CH_2Cl_2) was distilled from calcium hydride (CaH_2) under a nitrogen atmosphere. All reactions were carried out under a nitrogen atmosphere unless otherwise noted. All other chemicals were purchased from commercial suppliers and used without further purification. Flash column

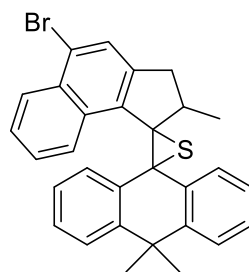
chromatography was performed using 230-400 mesh silica gel from EM Science. ^1H NMR and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively. Chemical shifts (δ) are reported in ppm from tetramethylsilane (TMS). Compound GL-119 was synthesized according to the previous literature²⁵.



10,10-Dimethyl-9(10H)-anthracenone (1.67 g, 7.5 mmol) and Lawesson's reagent (6.08 g, 15 mmol) were mixed in a solution of toluene (30 mL). The solution was heated to reflux for 14 h. The solution was permitted to cool to room temperature. The toluene was removed by rotary evaporation. The residue was purified by silica gel chromatography using ether/hexanes (1/4), affording the title compound GL-114 as a blue solid in 95% yield (1.58 g). FTIR (KBr): 3060, 2974, 2928, 2862, 1723, 1594, 1578, 1558, 1478, 1443, 1310, 1301, 1277, 1226, 1204, 1165, 1023, 903, 785, 759, 637 cm^{-1} . ^1H NMR (600 MHz, CDCl_3) δ 8.61 (m, 2H, Ar-*H*), 7.64 (m, 4H, Ar-*H*), 7.35 (m, 2H, Ar-*H*), 1.72 (s, 6H, CH_3) ppm. ^{13}C NMR (150.8 MHz, CDCl_3) δ 144.4, 138.0, 132.9, 130.3, 126.7, 126.6, 126.1, 125.6, 38.9, 32.9, 32.1 ppm. HRMS (ESI) calcd for $\text{C}_{16}\text{H}_{15}\text{S}$ $[\text{M}+\text{H}]^+$: 239.0894. Found: 239.0901.



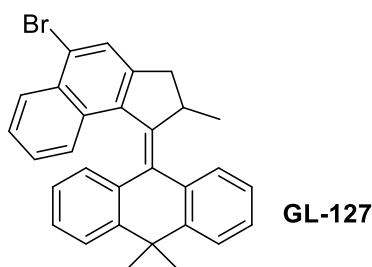
Hydrazine (20 mL) was added into a solution of **GL-114** (1.36 g, 5.71 mmol) in methanol (30 mL) in a round-bottom flask. The solution was heated to reflux for 16 h. After permitting to cool to room temperature, the solvents were removed by rotary evaporation. The residue was purified by silica gel chromatography using ether/hexanes (1/4) as eluent, affording the title compound **GL-115** in 90.5% yield (1.28 g). FTIR (KBr): 3389, 3206, 3064, 3029, 2971, 2922, 1658, 1622, 1597, 1570, 1468, 1447, 1385, 1360, 1343, 1291, 1264, 1185, 1094, 1040, 944, 765, 734, 654 cm^{-1} . ^1H NMR (600 MHz, CDCl_3) δ 8.18 (m, 1H, Ar-*H*), 8.02 (m, 1H, Ar-*H*), 7.35 (m, 1H, Ar-*H*), 7.27 (m, 1H, Ar-*H*), 7.14-7.09 (m, 2H, Ar-*H*), 7.06-7.03 (m, 2H, Ar-*H*), 5.35 (s, 2H, NH_2), 1.40 (s, 6H, CH_3) ppm. ^{13}C NMR (150.8 MHz, CDCl_3) δ 148.2, 143.9, 141.1, 136.6, 128.6, 128.4, 127.9, 127.7, 127.5, 127.3, 126.5, 126.3, 125.3, 124.9, 124.4, 122.7, 39.1, 29.7 ppm. HR-MS: (ESI) calcd for $\text{C}_{16}\text{H}_{17}\text{N}_2$ $[\text{M}+\text{H}]^+$: 237.1392. Found: 237.1389.



120

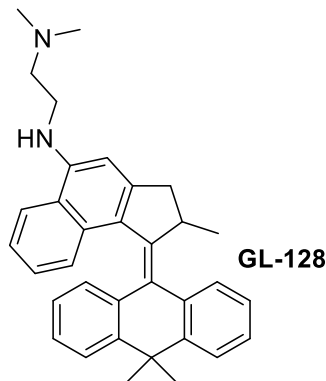
To an oven dried Schlenk flask charged with **GL-115** (0.708 g, 3.0 mmol) and MgSO_4 (200 mg), dichloromethane (20 mL) was added. The mixture was cooled down to $-15\text{ }^\circ\text{C}$ and MnO_2 (1.3 g, 15 mmol, Millipore-Sigma >90%) was added. The resulting mixture was stirred in a cold bath at $\sim -20\text{ }^\circ\text{C}$ for 2 h. The solid was filtered. Thione **GL-119** (0.24 g, 1 mmol) was subsequently added portion wise into the filtrate. The mixture was warmed to room temperature and stirred overnight. The solvents were removed by rotary evaporation. The crude product was purified by silica gel chromatography using hexane as the eluent, affording the title compound **GL-120** in 51% yield (0.76 g). FTIR (KBr): 3062, 3027, 2972, 2922, 2864, 1602, 1580, 1559,

1508, 1463, 1445, 1385, 1360, 1323, 1281, 1256, 1213, 1156, 1046, 952, 919, 894, 793, 760, 712, 680 cm^{-1} . ^1H NMR (600 MHz, CD_2Cl_2) δ 9.47 (d, J = 8.38 Hz, 1H), 8.09-8.08 (m, 1H), 7.94-7.93 (m, 1H), 7.74-7.73 (m, 1H), 7.51-7.49 (m, 1H), 7.40 (s, 1H), 7.38-7.35 (m, 1H), 7.33-7.30 (m, 1H), 7.26-7.23 (m, 1H), 7.21-7.18 (m, 1H), 7.15-7.13 (m, 1H), 6.89-6.87 (m, 1H), 6.81-6.78 (m, 1H), 2.90 (m, 1H), 2.23 (d, J = 16.86 Hz, 1H), 1.97 (m, 1H), 1.66 (s, 3H), 1.10 (d, J = 7.15 Hz, 3H), 1.02 (s, 3H). ^{13}C NMR (150.8 MHz, CD_2Cl_2) δ 147.76, 147.55, 143.27, 137.81, 133.79, 132.77, 132.76, 131.88, 131.01, 127.96, 127.59, 127.39, 127.28, 126.72, 126.15, 125.97, 125.49, 125.02, 124.77, 124.21, 124.19, 123.89, 123.07, 73.74, 62.41, 39.36, 39.06, 37.21, 33.23, 26.51, 22.49.



To an oven dried Schlenk flask charged with **GL-120** (0.200 g, 0.40 mmol) and toluene (20 mL), 2 mL of hexamethylphosphoramide (HMPT) was added. The yellow solution was stirred and heated at 100 °C for overnight. The solution was permitted to cool to room temperature and the solvents were removed by rotary evaporation. The residues were purified by column chromatography on silica gel using hexanes/DCM (10/1) as the eluent, affording compound **GL-127** as a yellow solid (100 mg, 54%). FTIR (KBr): 3065, 3021, 2955, 2923, 2862, 1599, 1571, 1557, 1506, 1449, 1461, 1383, 1358, 1347, 1285, 1214, 1156, 1083, 952, 904, 890, 870, 759, 713, 579 cm^{-1} . ^1H NMR (600 MHz, CDCl_3) δ 8.07 (d, J = 8.37 Hz, 1H), 7.76-7.74 (m, 1H), 7.72 (s, 1H), 7.50-7.44 (m, 2H), 7.22-7.15 (m, 3H), 7.00-6.96 (m, 2H), 6.75-6.73 (m, 1H), 6.63-6.60 (m, 1H), 6.51-6.49 (m, 1H), 4.45 (m, 1H), 3.58 (dd, J_1 = 15.24, J_2 = 5.93 Hz, 1H), 2.53 (d, J = 15.24

Hz, 1H), 1.89 (s, 3H), 1.72 (s, 3H), 0.76 (d, $J = 6.81$ Hz, 3H). ^{13}C NMR (150.8 MHz, CDCl_3) δ 147.21, 146.16, 145.56, 142.48, 140.09, 139.51, 136.35, 131.10, 130.21, 130.02, 128.28, 127.43, 127.37, 127.08, 126.98, 126.57, 126.27, 125.52, 125.36, 125.34, 124.89, 123.85, 123.38, 123.24, 40.54, 39.39, 37.73, 29.53, 24.68, 18.68. HRMS (ESI) calcd for $\text{C}_{30}\text{H}_{25}\text{Br}$ [M]: 464.1140. Found: 464.1133.

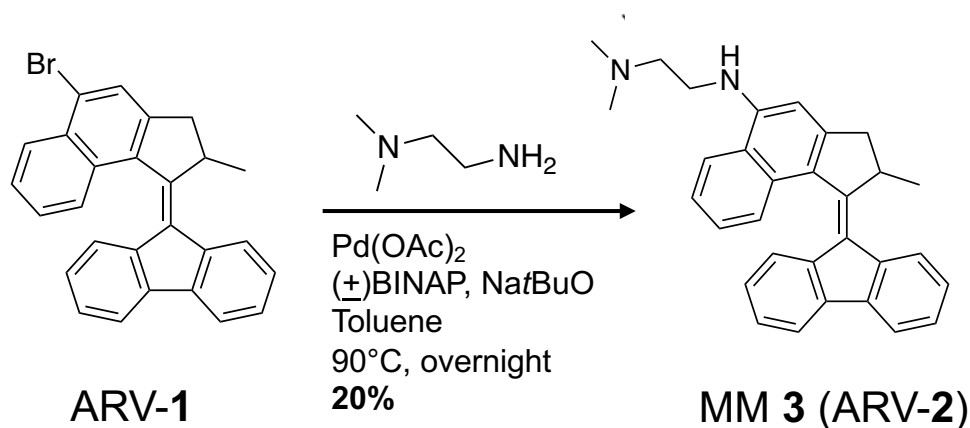


($\pm$)-2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl (BINAP) (15 mg, 0.024 mmol) and palladium(II) acetate (1.8 mg, 0.008 mmol) were mixed in toluene (5 mL). This solution was stirred for 20 min at room temperature. After that, NaO^tBu (82 mg, 0.90 mmol), bromo-substituted motor GL-127 (76 mg, 0.164 mmol) and *N*1,*N*1-dimethylethane-1,2-diamine (89 mg, 0.714 mmol) were added. The mixture was stirred and heated at 90°C for overnight. The reaction mixture was permitted to cool to room temperature, and H_2O (10 mL) and DCM (10 mL) were added. The organic phase was separated with a separation funnel. The aqueous phase was extracted with DCM (3×10 mL), and the organic phases were combined and washed with H_2O (2×10 mL). After the solvents were removed by rotary evaporation, the residue was purified by column chromatography on silica gel using MeOH/DCM (1/9), obtaining **GL-128** as a yellow-green solid in 93% yield. FTIR (KBr): 3062, 3022, 2951, 2859, 2820, 2771, 1583, 1563, 1526, 1471, 1413, 1357, 1347, 1265, 1258, 1160, 1041, 761, 738, 711 cm^{-1} . ^1H NMR (500 MHz, CD_2Cl_2) δ 7.89 (dd, $J_1 = 7.63$, $J_2 = 1.25$ Hz, 1H), 7.76 (d, $J = 8.61$ Hz, 1H), 7.58-7.54 (m, 2H), 7.29-7.23 (m, 2H), 7.18 (t, 1H),

7.08-7.05 (m, 1H), 7.03 (d, J = 8.28 Hz, 1H), 6.79-6.77 (m, 2H), 6.63-6.61 (m, 2H), 4.48 (m, 1H), 3.63 (m, 1H), 3.36 (m, 2H), 2.76 (t, 2H), 2.35 (s, 6H), 0.85 (d, J = 6.77 Hz, 3H) ppm. ^{13}C NMR (125 MHz, CD_2Cl_2) δ 148.37, 147.21, 145.67, 145.49, 144.16, 141.27, 140.32, 129.77, 127.66, 127.30, 127.11, 126.92, 126.11, 125.73, 125.53, 125.48, 125.10, 124.95, 124.23, 124.01, 123.10, 122.99, 122.92, 122.78, 120.03, 101.71, 57.76, 57.54, 44.82, 40.86, 40.37, 39.97, 39.27, 37.84, 37.38, 29.69, 28.76, 24.47, 18.73, 18.34. HRMS (ESI) calcd for $\text{C}_{32}\text{H}_{37}\text{N}_2$ $[\text{M}+\text{H}]^+$: 473.2957. Found: 473.2952.

Synthesis of MM 3

Final product characterization of MM 3 is reported in Supplementary Figs. 37-39.



Supplementary Scheme 2. Synthesis of MM 3 (herein denoted ARV-2).

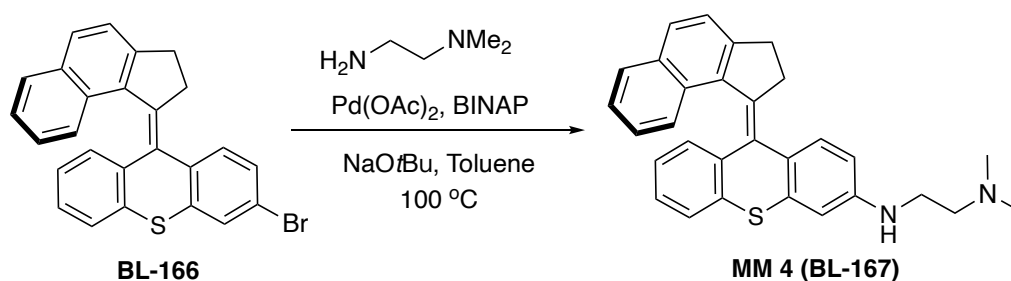
ARV-1 was synthesized according to the previous literature⁵⁰. ARV-1 (247 mg, 0.58 mmol) and NaOtBu (288 mg, 2.99 mmol) were added to an oven-dried screw-cap tube. The tube was evacuated and refilled with argon three times. To an oven-dried round bottom flask, (+) BINAP (50 mg, 15 mol %) and Pd(OAc)₂ (13 mg, 5 mol %) were added and the flask was evacuated and refilled with argon three times. Anhydrous toluene, degassed by bubbling with argon for 30 min, was added and the catalyst solution was stirred at room temperature for 30 min. At this time, the catalyst solution was transferred into the screwcap tube containing **13** and the NaOtBu. *N,N*-dimethylethylenediamine (375 μ L, 3.41 mmol) was added, the tube was capped, and the reaction was stirred at 90 °C overnight. Upon completion, the reaction was quenched with the addition aqueous ammonium chloride, extracted with DCM, and dried over sodium sulfate. The crude

product was purified with column chromatography (2% MeOH in DCM) followed by preparatory TLC (2% MeOH in DCM) to afford **7** as a dark orange solid (50 mg, 20% yield). ^1H NMR (500 MHz, DCM-d_2) 8.00 (d, $J=7.7$ Hz, 2H), 7.87 (dd, $J_1=7.4$ Hz, $J_2=0.8$ Hz, 1H), 7.81-7.79 (m, 1H), 7.66 (d, $J=8.3$ Hz, 1H), 7.44-7.34 (m, 3H), 7.31-7.28 (m, 1H), 7.18 (td, $J_1=7.3$ Hz, $J_2=0.9$ Hz), 6.71 (d, $J=7.9$ Hz, overlaps with signal at 6.69 ppm, 2H total), 6.69 (s, overlaps with signal at 6.71 ppm, 2H total), 6.00 (br s, 1H) 4.30-4.25 (m, 1H), 3.50 (dd, $J_1=15.2$ Hz, $J_2=5.7$ Hz, 1H), 3.44-3.42 (m, 2H), 2.88-2.75 (m, 2H), 2.71 (d, $J=15.13$ Hz, 1H), 2.39 (s, 6H), 1.40 (d, $J=6.9$ Hz, 3H). ^{13}C NMR (126 MHz, CD_2Cl_2) δ 153.39, 151.00, 147.01, 140.00, 139.01, 138.59, 137.40, 130.69, 127.93, 126.57, 126.51, 125.80, 125.58, 125.49, 125.47, 125.31, 124.43, 124.00, 123.48, 122.23, 121.04, 119.40, 118.71, 101.52, 57.28, 44.73, 44.69, 42.20, 40.53, 19.77. HRMS (ESI) m/z calculated for $[\text{M}+\text{H}^+]$ $\text{C}_{31}\text{H}_{30}\text{N}_2$ 431.2409; found, 431.2472.

FTIR (cm^{-1}): 3347, 2932, 2853, 2823, 2786, 1617, 1551, 1522, 1475, 1445, 1427, 1336, 1314, 1279, 1261, 1222, 1178, 1145, 1024, 956, 932, 831, 846, 773, 762, 730, 705, 673, 648.

Synthesis of MM 4

Final product characterization of MM 4 is reported in Supplementary Figs. 40-42.



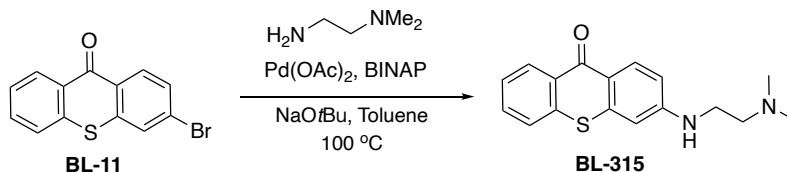
Supplementary Scheme 3. Synthesis of MM 4 (herein denoted **BL-167**).

BL-166 was synthesized according to the previous literature⁵¹. BINAP (87 mg, 0.14 mmol) and palladium(II) acetate (15 mg, 0.07 mmol) were dissolved in dry toluene (15 mL). This solution was stirred for 30 min at room temperature, where upon it turned from dark red to dark orange. After this period NaOtBu (456 mg, 4.75 mmol, 5 equiv) was added, followed by **BL-166** (420 mg, 0.95 mmol, 1 equiv) and *N*^l, *N*^l-dimethylethane-1,2-diamine (419 mg, 4.75 mmol, 5 equiv). The mixture was stirred at 100 °C for 2 d. Subsequently, the reaction mixture was poured into DCM (50 mL). After filtration, the solvents were evaporated. The crude product was dissolved in a small amount of DCM and purified using column chromatography (10% MeOH in DCM) to yield MM 4 as a yellow solid product (319 mg, 75% yield): ¹H-NMR (600 MHz, CD₂Cl₂) δ 7.78 (d, *J* = 7.8 Hz, 0.5H), 7.75–7.62 (m, 2H), 7.61–7.52 (m, 1.5H), 7.47 (dd, *J* = 8.2, 2.9 Hz, 1H), 7.35 (t, *J* = 7.5 Hz, 0.5H), 7.24–7.09 (m, 2H), 7.01– 6.96 (m, 0.5H), 6.95–6.97 (m, 1H), 6.86–6.76 (m, 1.5H), 6.68–6.63 (m, 1H), 6.57 (t, *J* = 7.5 Hz, 0.5H), 6.46 (d, *J* = 8.4 Hz, 0.5H), 5.89 (dd, *J* = 8.4, 2.4 Hz, 0.5H), 4.80–4.00 (m, 2H), 3.25–3.17 (m, 4H), 3.05 (t, *J* = 5.9 Hz, 1H), 2.60 (t, *J* = 5.8 Hz, 1H),

2.50 (t, $J = 5.8$ Hz, 1H), 2.27 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (151 MHz, CD_2Cl_2) δ 147.92, 147.84, 147.81, 147.38, 141.35, 139.99, 139.66, 139.63, 138.10, 137.82, 136.90, 136.69, 135.86, 135.67, 133.31, 133.26, 129.65, 129.58, 129.28, 129.18, 129.08, 128.75, 128.56, 128.48, 128.21, 128.11, 128.08, 128.06, 127.84, 127.51, 127.09, 126.64, 126.57, 126.38, 126.21, 126.12, 125.04, 124.99, 124.42, 124.39, 123.59, 123.57, 112.14, 112.07, 110.93, 110.90, 58.08, 57.96, 45.18, 45.13, 41.44, 41.31, 34.33, 34.30, 32.63, 32.59. ESI-MS: m/z 449.2 $[\text{M}+\text{H}]^+$; HRMS (ESI) calculated for $[\text{M}+\text{H}, \text{C}_{30}\text{H}_{29}\text{N}_2\text{S}]^+$: 449.1973, found: 449.2042. FTIR (cm^{-1}): 3052, 2938, 2852, 2819, 2762, 1596, 1581, 1560, 1494, 1455, 1437, 1374, 1309, 1249, 1054, 1039, 956, 938, 812, 755, 743, 707.

Synthesis of **BL-315**

Final product characterization of MM **4** is reported in Supplementary Figs. 43-45.



Supplementary Scheme 4. Synthesis of **BL-315**.

3-((2-(dimethylamino)ethyl)amino)-9H-thioxanthen-9-one (BL-315). BINAP (150 mg, 0.24 mmol, 14 mol%) and palladium (II) acetate (27 mg, 0.12 mmol, 7 mol%) were dissolved in dry toluene (30 mL). This solution was stirred for 30 min at room temperature, where upon it turned from dark red to dark orange. After this period NaOtBu (826 mg, 8.6 mmol, 5 equiv) was added, followed by **BL-11** (500 mg, 1.72 mmol, 1 equiv) and *N*¹, *N*¹-dimethylethane-1,2-diamine (774 mg, 8.6 mmol, 5 equiv). The mixture was stirred at $100\text{ }^\circ\text{C}$ for overnight. Subsequently, the reaction mixture was poured into DCM (50 mL). After filtration, the solvents were evaporated. The crude product was dissolved in a small amount of DCM and purified using column chromatography (5% MeOH in DCM) to afford **BL-315** (421 mg, 82% yield).

^1H -NMR (500 MHz, CDCl_3) δ 8.56 (d, $J = 7.9$ Hz, 1H), 8.39 (d, $J = 8.9$ Hz, 1H), 7.52–7.48 (m, 1H), 7.47–7.44 (m, 1H), 7.42–7.37 (m, 1H), 6.68 (dd, $J = 8.9, 2.3$ Hz, 1H), 6.51 (d, $J = 2.3$ Hz, 1H), 5.11 (t, $J = 4.7$ Hz, 1H), 3.22–3.15 (m, 2H), 2.59–2.53 (m, 2H), 2.24 (s, 6H);

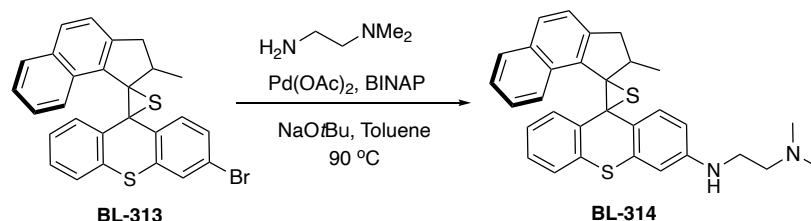
^{13}C NMR (126 MHz, CDCl_3) δ 178.65, 151.31, 140.08, 136.91, 131.52, 131.44, 129.74, 129.57, 125.91, 125.65, 119.65, 113.78, 104.75, 57.34, 45.10, 40.21.

HRMS (ESI) calculated for $[\text{M}+\text{H}, \text{C}_{17}\text{H}_{19}\text{N}_2\text{OS}]^+$: 299.1140, found: 299.1212.

FTIR (cm^{-1}): 3325, 3056, 2943, 2859, 2821, 2771, 1664, 1620, 1582, 1553, 1519, 1459, 1436, 1320, 1252, 1176, 1138, 1115, 1058, 1034, 928, 830, 788, 742, 670.

Synthesis of **BL-314**

Final product characterization of **BL-314** is reported in Supplementary Figs. 46-48.



Supplementary Scheme 5. Synthesis of **BL-314**.

*N*¹, *N*¹-dimethyl-*N*²-(2-methyl-2,3-dihydrodispiro[cyclopenta[a]naphthalene-1,2'-thiirane-3',9''-thioxanthen]-3''-yl)ethane-1,2-diamine (**BL-314**). BINAP (3.3 mg, 0.0053 mmol, 2.5 mol%) and palladium (II) acetate (1.2 mg, 0.0053 mmol, 2.5 mol%) were dissolved in dry toluene (15 mL). This solution was stirred for 30 min at room temperature, where upon it turned from dark red to dark orange. After this period NaOtBu (101 mg, 1.05 mmol, 5 equiv) was added, followed by **BL-313** (100 mg, 0.21 mmol, 1 equiv) and *N*¹, *N*¹-dimethylethane-1,2-diamine (92.4 mg, 1.05 mmol, 5 equiv). The mixture was stirred at $90\text{ }^\circ\text{C}$ for overnight. Subsequently, the reaction mixture was poured into DCM (50 mL). After filtration, the solvents were evaporated. The crude product was dissolved in a small amount of DCM and purified using column chromatography (5% MeOH in DCM) to yield **BL-314** as a yellow solid product (69 mg, 68% yield, **MM 1 = 9.2:1 analyzed by ¹H-NMR**):

¹H-NMR (600 MHz, CD_2Cl_2) δ 8.97–8.91 (m, 1H), 7.81 (dd, $J = 7.7, 1.4\text{ Hz}$, 0.5H), 7.59 (dd, $J = 7.7, 1.5\text{ Hz}$, 0.5H), 7.55 (d, $J = 8.4\text{ Hz}$, 0.5H), 7.51–7.48 (m, 1H), 7.46–7.42 (m, 1H), 7.36–7.32 (m, 1H), 7.20 (td, $J = 7.5, 1.3\text{ Hz}$, 0.5H), 7.17–7.06 (m, 3.5H), 6.88 (dd, $J = 7.6, 1.2\text{ Hz}$, 0.5H), 6.81 (td, $J = 7.4, 1.0\text{ Hz}$, 0.5H), 6.67 (td, $J = 7.5, 1.4\text{ Hz}$, 0.5H), 6.61 (d, $J = 2.4\text{ Hz}$, 0.5H), 6.46 (dd, $J = 8.4, 2.4\text{ Hz}$, 0.5H), 6.18 (d, $J = 2.3\text{ Hz}$, 0.5H), 6.13 (dd, $J = 8.4, 2.4\text{ Hz}$, 0.5H), 4.47 (brs,

0.5H), 4.07 (brs, 0.5H), 3.43 (dd, $J = 15.1, 6.4$ Hz, 0.5H), 3.36 (dd, $J = 15.1, 6.4$ Hz, 0.5H), 3.04 (t, $J = 5.9$ Hz, 1H), 2.80–2.74 (m, 1H), 2.49–2.45 (m, 1H), 2.34–2.25 (m, 2H), 2.16 (s, 3H), 2.04 (s, 3H), 1.62–1.57 (m, 0.5H), 1.45–1.40 (m, 0.5H), 0.99–0.93 (m, 3H);

^{13}C NMR (151 MHz, CD_2Cl_2) δ 147.57, 143.21, 143.17, 140.73, 137.55, 137.06, 136.89, 136.47, 136.24, 133.17, 133.11, 132.54, 132.36, 131.89, 131.74, 131.55, 130.92, 129.82, 129.78, 129.43, 129.34, 128.78, 128.03, 127.94, 127.88, 127.29, 127.00, 126.77, 126.66, 126.52, 125.98, 125.51, 125.31, 124.58, 124.55, 124.46, 124.14, 124.06, 124.04, 111.44, 111.20, 110.56, 110.49, 72.85, 72.82, 62.45, 62.39, 58.07, 57.85, 45.22, 45.12, 41.45, 41.31, 41.27, 41.19, 38.40, 38.38, 21.81, 21.73.

HRMS (ESI) calculated for $[\text{M}+\text{H}, \text{C}_{31}\text{H}_{31}\text{N}_2\text{S}_2]^+$: 495.1850, found: 495.1956.

FTIR (cm^{-1}): 3383, 3050, 2926, 2818, 2769, 1734, 1600, 1494, 1455, 1436, 1316, 1253, 1158, 1111, 1036, 941, 810, 784, 768, 742, 690.

Supplementary Text

Density Functional Theory Calculation of Motor Energies

The geometries of the motor cores were optimized at the TPSS/TPSS(GD3BJ)/def2-SVP level in Gaussian 16 with the ultrafine integration grid and using density fitting to accelerate computations⁵²⁻⁵⁹. Previously, it was found that this level of theory was sufficient for qualitative accuracy²⁸. Frequency calculations were performed to ensure that the minima found had no imaginary frequencies, and that all transition states had a single negative eigenvalue. Then, intrinsic reaction coordinate calculations were performed to ensure that the correct minima connected to the transition states were found. The coordinates of the geometries are listed in this document. Metastable, stable, and transition conformations are labeled as A, B, and TS, correspondingly. The energies calculated are listed in Supplemental Table 1. The appended aniline groups were not included in the energy calculations.

Although the results are sufficient for us to obtain a qualitative understanding of the motors, quantitative accuracy was not achieved. The difference between the thermal helix inversion energy barriers of MM **1** and MM **2** is sufficiently small that it is beyond the accuracy of most practical DFT methods. Although in theory double hybrids can achieve sufficient quantitative accuracy, the calculations will be computationally expensive due to the number of atoms in the molecules. However, the results do support that the difference in barriers to thermal helix inversion is sufficiently small for MM **1** and **2** that other factors, such as solvent effects and temperature, are likely to be significant.

The thermal helix inversion energy barrier for MM **3** was found to be larger than that of MM **1** and **2**. The calculated energy barrier is approximately twice as large. By examining the driving energy difference between the metastable and stable state, we see that the increase in

thermal helix inversion energy barrier for MM **3** is due to the relatively more stabilized metastable state in MM **3**, which is primarily attributable to the differences in the stator.

For MM **4**, we find that, in addition to the usual thermal helix inversion transition state (analogous to the transition states found for MM **1**, **2**, and **3**), there is an additional transition state. This additional pathway for the metastable state to relax is available because of the lack of the methyl group in the rotor (as compared to MM **1**, **2**, and **3**). Additionally, given that we are comparing different conformations of the same molecule, this alternative relaxation pathway was calculated to be likely slightly more favorable than the thermal helix inversion pathway, although the energy difference is small. Therefore, from the metastable state, MM **4** can relax through either transition state to any of the stable states with the same change in energy since the stable states are all energetically the same for MM **4** due to symmetry. In this case, as usual, the driving energy is denoted as the energy difference between the metastable state and the stable state, but there is no directional bias because each metastable state can lead to two energetically equivalent stable states related by symmetry.

Excitation in a Non-Scanning Laser Regime

In typical experiments, the exciting laser for stimulation was rastered across a defined region-of-interest approximated as a circle of 5 μm diameter. We employed a second stimulation set-up on an epifluorescence microscope to verify that laser rastering is not necessary for excitation. Experiments with a non-rastering laser beam required similar stimulation powers to elicit responses from MM-treated cells ($\sim 10^2 \text{ W cm}^{-2}$).

We adapted the optical setup from a single cell fluorescence sensing optical system⁶⁰ to deliver a non-scanning laser beam (Coherent Chameleon Discovery) to the specimen from the top, at the excitation wavelength of the MM. The fluorescence signal is collected by an inverted fluorescence microscope from the bottom of the specimen, while an electronic shutter is used to release optical pulses for MM triggering at the desired frequency during fluorescence recording.

Assessment of Stimulation Toxicity

The purpose of this present work was to use MM to interact with cell signaling. However, several previous studies by our group have explored the same or similar molecules for inducing cell permeabilization and death^{21,25,27-28,61}. These studies used minutes-long light doses rather than the millisecond-scale doses employed in this work. Nevertheless, assessing the effects of MM stimulation on cell viability remains imperative.

Potentially toxic effects of MM stimulation on cells were assessed in several ways. The results of these experiments are reported in Supplementary Figs. 8-10. First, qualitative analysis of stimulated cells was used to assess toxicity. For these experiments, HEK293 cells were treated with MM 1 and prepared as was typical for stimulation experiments (see *Methods*). At very high stimulation powers, cells exhibited membrane blebbing and runaway calcium accumulation (Supplementary Fig. 10a). By reducing the light power, we were able to find a more suitable light dose that elicited strong calcium responses from cells without observable toxicity (Supplementary Fig. 10b).

Second, once a suitable window of stimulation power had been identified, the effects of stimulation on cells were compared to healthy cells and several positive controls representing mechanisms of cell death. For these experiments, cells were loaded with Fluo-4, CellMask cell membrane labeling dye, and MM 1 and were imaged in the presence of cell-impermeant viability dye propidium iodide (PI; 1 $\mu\text{g mL}^{-1}$). The results of these experiments are shown in Supplementary Fig. 9. Apoptosis was initiated by prolonged exposure of cells to blue light, and the onset of apoptotic cell death in exposed colonies was confirmed by the observation of pyknosis (cell shrinkage)⁶², the uptake of small amounts of PI, and the enhanced fluorescence of Fluo-4 in the colony (Supplementary Fig. 8a). Necrotic cell death was observed by activating MM 1 *in situ*

using a SOLA LED fed through a DAPI excitation filter (395/25 nm, 166 mW cm⁻²) for 5 min (Supplementary Fig. 8b). Similar conditions have been shown to induce necrotic cell death in our previous work²⁵. Using this protocol, cells do not shrink in the 5 min of stimulation, but they uptake large amounts of PI. Such changes were not observed in healthy, unperturbed cells (Supplementary Fig. 8c) or cells exhibiting MM-driven calcium responses driven by typical protocols (250 ms pulse to a 5 µm diameter at 3.2×10² W cm⁻² stimulation power; Supplementary Fig. 8d).

Finally, to account for potential aftereffects of stimulation, we grew isolated HEK293 colonies in a grid pattern by photolithographically patterning polydimethylsiloxane onto Ibidi imaging dishes. Colonies were seeded with 25,000 cells cm⁻². Two days after seeding, cells were treated with MM 1 and Fluo-4 using typical protocols (see *Methods*), and 10 selected cells were stimulated in selected colonies. Results from these experiments are shown in Supplementary Fig. 9. After 24 h, these colonies were imaged again in the presence of 1 µg mL⁻¹ PI to observe any effects of MM stimulation on growth, cell stress, or viability. The PI uptake of stimulated colonies was indistinguishable from untreated colonies. As a positive control, several colonies were irradiated with UV light (SOLA LED fed through a DAPI excitation filter (395/25 nm, 166 mW cm⁻²) for 10 min on Day 3 of growth (MM 1 was not present). UV irradiation caused PI uptake into cells immediately and distinguished the UV-treated cells from cells that had been stimulated with MM 1 and light the day before.

In summary, qualitative assessment of the effects of MM stimulation on cells reveal that stimulation does not incur toxicity either by apoptosis or necrosis so long as the light dose administered is appropriately controlled.

Effects of Rotation Speed and Directionality *In Vitro* and *In Vivo*

The propensity for unidirectional MM to rotate and exert mechanical force on their environment depend principally on two factors: the rotation rate and the photoconversion efficiency. Both of these depend on MM structure. The rotation rate is determined by the half-life of the thermal helix inversion, which is driven by the steric interactions governing the passage of the rotor over the stator²⁴. The photoconversion efficiency, also known as the quantum yield of photoisomerization, represents the proportion of photons absorbed by the molecule that drive molecular rotation (as opposed to fluorescence or non-radiative decay processes). The quantum yield of photoisomerization is primarily influenced by the electronic structure. MM directionality can also be influenced by structure, as the driving force for unidirectional rotation depends upon the presence of a stereogenic center.

In Vitro

The selection of different MM of similar physicochemical character allowed us to probe the effects of altered MM speed or directionality on the signaling behavior elicited. MM **1**, which rotates at ~3 MHz, elicited the strongest signaling responses *in vitro*. ICW elicited by MM **1** peaked ~10-20 s after stimulation and decayed over the next minute. Slow-rotating MM **3** did not elicit any noticeable responses *in vitro*, whereas bidirectional MM **4** elicited inconsistent and weak responses. These results mirror those previously observed when tracking MM diffusion in solution²⁰ and when tracking their ability to permeabilize phospholipid bilayers²¹. In these experiments, slow-rotating MM were indistinguishable from solvent-only controls, whereas bidirectional MM were distinguishable from controls but did not exert as pronounced an effect as fast, unidirectional MM.

MM 2, which has a reported rotation rate of ~43 MHz, also elicited strong responses *in vitro*, but did not elicit the same release kinetics, instead causing release of calcium to a stable level without decay until several minutes after stimulation. Given that ICW elicited by MM 1 reached a higher amplitude than those elicited by MM 2, alongside the aforementioned difference in release and recovery kinetics, we conclude that MM 1 imparts a stronger stimulatory effect than MM 2 despite its slower rotation rate. The observed recovery by cells treated with MM 1 but not with MM 2 can be attributed to various mechanisms of cellular recovery, including SERCA activity and RyR closing probability, being enhanced by a larger cytosolic concentration of calcium¹. If MM 2 does not elicit sufficient calcium release to cause accelerated cellular recovery, ICW elicited by MM 2 would persist at stable levels for a longer timeframe than those elicited by MM 1.

Since both MM 1 and MM 2 operate in the MHz regime, several factors may contribute to the observed difference in the signaling they elicit:

- (1) MM 1 possesses a higher photoisomerization quantum yield than MM 2.
- (2) The intracellular environment changes the rotary properties of MM 1 and MM 2 so as to affect their relative speeds.
- (3) Increasing speed beyond a certain threshold value does not result in a higher potency for eliciting signaling cascades.

Previous studies of overcrowded alkene motor dynamics have shown that motors bearing similar core stator structures to MM 2 suffer from lower quantum yields compared to sulfur-bearing core stators⁶³. We preliminarily investigated the relative order of the photoisomerization efficiencies of the molecules employed in this study using fluorescence spectroscopy. Fluorescence competes with photoisomerization; therefore, a higher quantum yield of fluorescence

would imply (but not necessarily prove) a lower quantum yield of photoisomerization. The quantum yield of fluorescence is proportional to the integrated intensity of fluorescence emission divided by the absorbance of the test solution at the employed wavelength⁶⁴ by the following equation: $\phi = \frac{\phi_s F_u (OD_s) n_u^2}{F_s (OD_u) n_s^2}$, where “s” denotes a reference with known quantum yield at the tested wavelength, “u” represents the sample with unknown quantum yield, n represents the refractive index of the solvent, OD represents the optical density of the solution, and F represents the integrated intensity of fluorescently emitted photons. Supplementary Fig. 15 shows the fluorescence of MM **1-4** normalized to their molar absorptivity, revealing that MM **2** likely possesses the highest fluorescence quantum yield. This supports the hypothesis that the differences in the elicited signaling between these two fast motors is correlated to their photoisomerization efficiency. Future studies will expand on the relative potencies of different classes of fast motors and explore the other possible explanations for this observation.

In Vivo

In *Hydra*, several different trends are observed when compared to *in vitro* studies. Most notably, MM **4** is able to drive enhanced signaling behavior *in vivo*. MM **2** was also highly effective at driving *Hydra* contraction, and MM **1** and MM **2** were similar in ability to drive body column ICW. Small responses were observed even to stimulation with MM **3**, and the *Hydra* were a much more sensitive model system than HEK293 cells or cardiac myocytes.

We considered several possible explanations for the differences between our *in vitro* and *in vivo* systems, and specifically why slow-rotating molecules like MM **3** elicited small responses in *Hydra*:

- (1) A change in the environment of the slow motors causes a shift in the rotation speed, resulting in sufficient motion to generate small responses in *Hydra*.
- (2) The protein machinery expressed in *Hydra* lends itself to be much more sensitive to mechanical stimulation, either due to a higher expression of IP₃ pathway components, a higher expression of voltage-gated channels or gap junctions which amplify and propagate signals, or a difference in the mechanical properties of *Hydra* tissue.
- (3) Other factors, such as the propensity for MM to bind to individual receptors, generation of photodecomposition products, or photothermal effects, contribute to observed responses in *Hydra*.

As indicated in the main text, we hypothesize that factors besides molecular rotation contribute to the responses we see *in vivo*, and that these factors are either not present or have a negligible effect *in vitro*. To investigate this hypothesis, we conducted experiments with several other slow-rotating or non-rotating molecules.

First, we used a slow MM, analogous to MM **3**, but with a higher molar absorptivity than MM **1** and without the aniline group present on the molecule (**DL-4-762**, Supplementary Fig. 25a). The synthesis of this molecule was previously reported²⁵. Recent findings from our group show that the presence of an aniline can cause shifts in the rotation speed of a motor that depend on the solvation environment of the molecule⁴⁶. Hence, **DL-4-762** allowed us to exclude solvent effects when considering the speed of MM **3**. **DL-4-762** absorbed more light than MM **1** (Supplementary Fig. 25b) and was inactive *in vitro* (Supplementary Fig. 25c), but elicited small calcium transients *in vivo* (Supplementary Fig. 25d), similar to MM **3**.

Second, we attempted a non-rotating molecule. We first explored the rotor of MM **3** without the stator (**ARV-3-056**, Supplementary Fig. 26a). Although this molecule was rotationally inactive, it also did not absorb light at the stimulation wavelength (Supplementary Fig. 26b). This molecule did not elicit any appreciable responses *in vitro* (Supplementary Fig. 26c). Some responses were seen in *in vivo* experiments, but the molecule's average response profile was indistinguishable from solvent-only controls (Supplementary Fig. 26d), indicating that this is likely attributable to spontaneous calcium activity in *Hydra*. Therefore, it is likely that any mechanism contributing to the quantifiable *in vivo* responses we observe depends on the absorption of light at the stimulation wavelength.

We then synthesized a visible light-absorbing motor portion by appending an aniline group to the stator of MM **1** (**BL-315**, Supplementary Fig. 27a). This molecule absorbed visible light (Supplementary Fig. 27b) and was completely inactive *in vitro* (Supplementary Fig. 27c) but was too toxic *in vivo* after a 24 h incubation period to reliably test (Supplementary Fig. 27d).

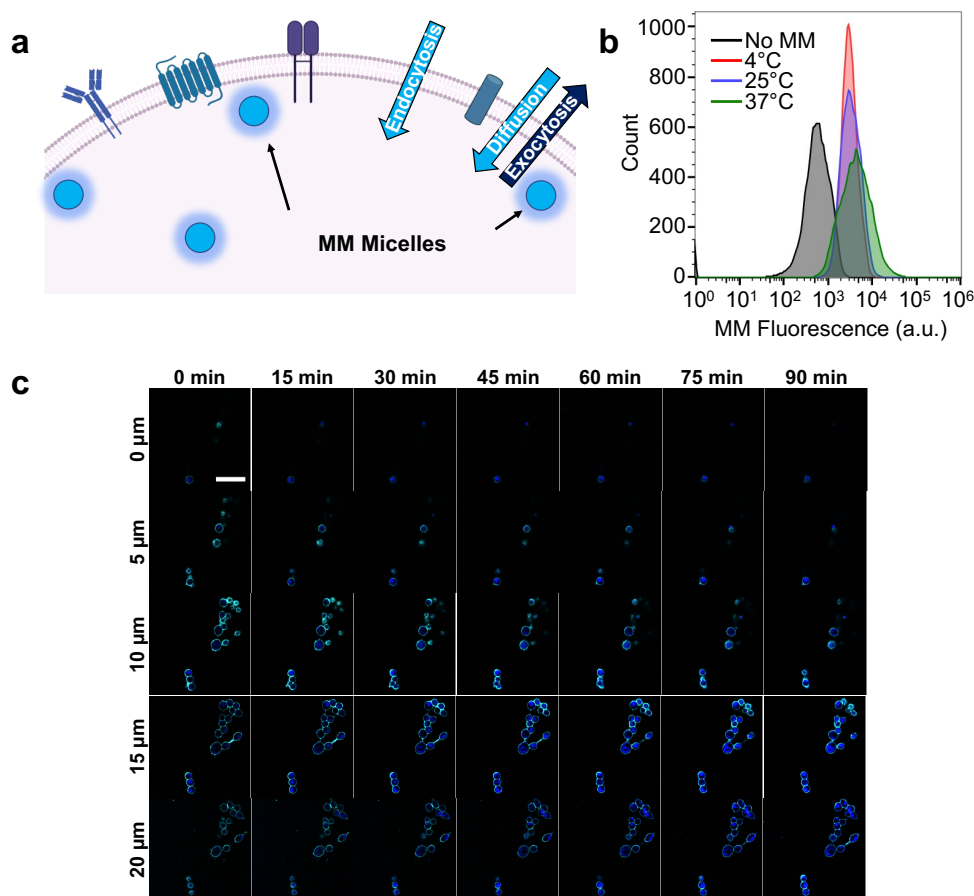
Finally, we synthesized an episulfide-locked analog of MM **1** (**BL-314**, Supplementary Fig. 28a). This molecule, in principle, should be completely rotationally inert. However, the episulfide moiety is unstable and prone to elimination reactions, which form MM **1**. The pure form of this compound was present in a 9.2:1 mixture with MM **1** by ¹H NMR. The molecule absorbed visible light (Supplementary Fig. 28b) and exhibited a decrease in activity *in vitro* relative to MM **1** (Supplementary Fig. 28c) that was consistent with a reduced concentration of the active molecule. However, **BL-314** stimulated more potent calcium transients *in vivo* than analogous concentrations of MM **1** (Supplementary Fig. 28d-e). Hence, it is unlikely that all the activity of this molecule could be attributed to MM **1**, and it is likely that the episulfide-locked moiety contributes to the

responses observed *in vivo*. It is also possible that the episulfide eliminates *in situ* and generates MM 1 *in vivo*.

In addition to these experiments with slow and non-rotating controls, we attempted several experiments to block cell-to-cell transmission and amplification of observed responses either by voltage-gated channels or gap junctions (Supplementary Fig. 24). These treatments had no observable effect on the amplitude of *Hydra* responses.

In summary, our experiments indicate (i) that other mechanisms besides molecular rotation contribute to *in vivo* calcium responses, (ii) that these mechanisms are driven by the absorption of visible light, and (iii) that molecular rotation likely serves to contribute or amplify existing responses to a regime sufficient for behavioral modulation. Non-molecular rotation driven responses could easily arise *via* the activation of, for example, a thermally sensitive calcium channel such as those in the TRPA and TRPM family⁶⁵. Alternatively, if MM possessed a high binding affinity for proteins present only in *Hydra*, such as the HyNaC family of neuropeptide-gated sodium channels present in the *Hydra* nerve net⁶⁶, then their activation by visible light might serve to modulate the activity of these channels.

However, the responses observed from *Hydra* treated with fast, unidirectional MM are markedly faster than those observed from *Hydra* treated with non-unidirectional MM, and noticeably faster and more intense than those observed from *Hydra* treated with slow, unidirectional MM. Furthermore, only the *Hydra* treated with fast-rotating MM experience responses sufficient to drive whole-body contraction in behavioral experiments. The contribution of rapid molecular rotation could arise by either a direct stimulatory effect, such as the mechanotransduction processes we characterize *in vitro* or by other mechanisms, such as enhancing the diffusion of a secondary messenger.



Supplementary Fig. 1. Monitoring the uptake process of MM.

(a) A scheme depicting processes that contribute to the flux of MM across the cell membrane.

Created with BioRender. **(b)** Average MM fluorescence of individual cells treated with MM 1

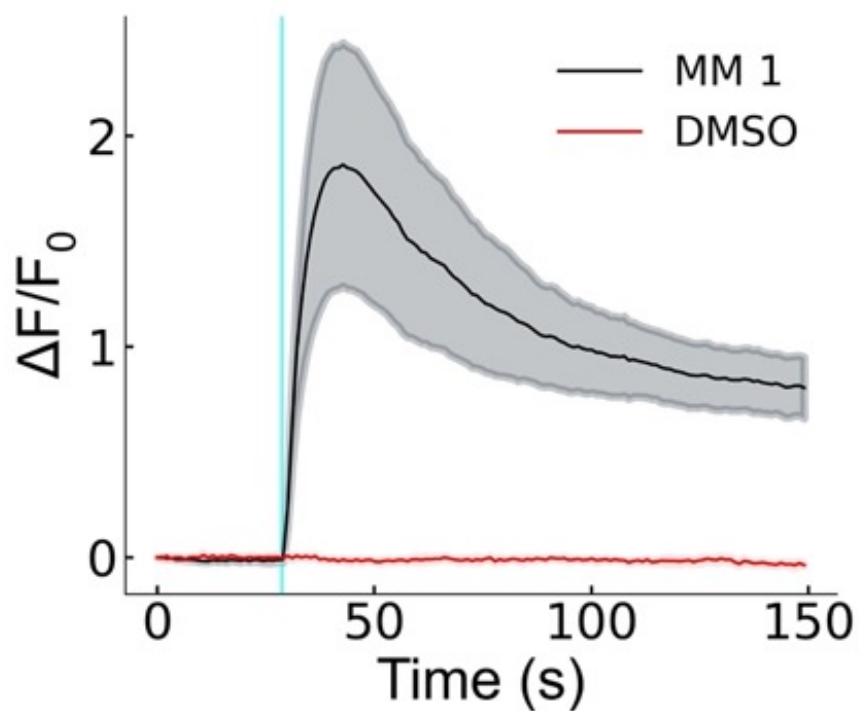
for 45 min at varying temperature. **(c)** Confocal microscope z-stack images of HEK293 cells

treated with MM 1 (8 μ M, blue) and cellMask (400 nM, cyan). The timestamps indicate the time

from which imaging began. MM were added to cells and immediately taken to the microscope

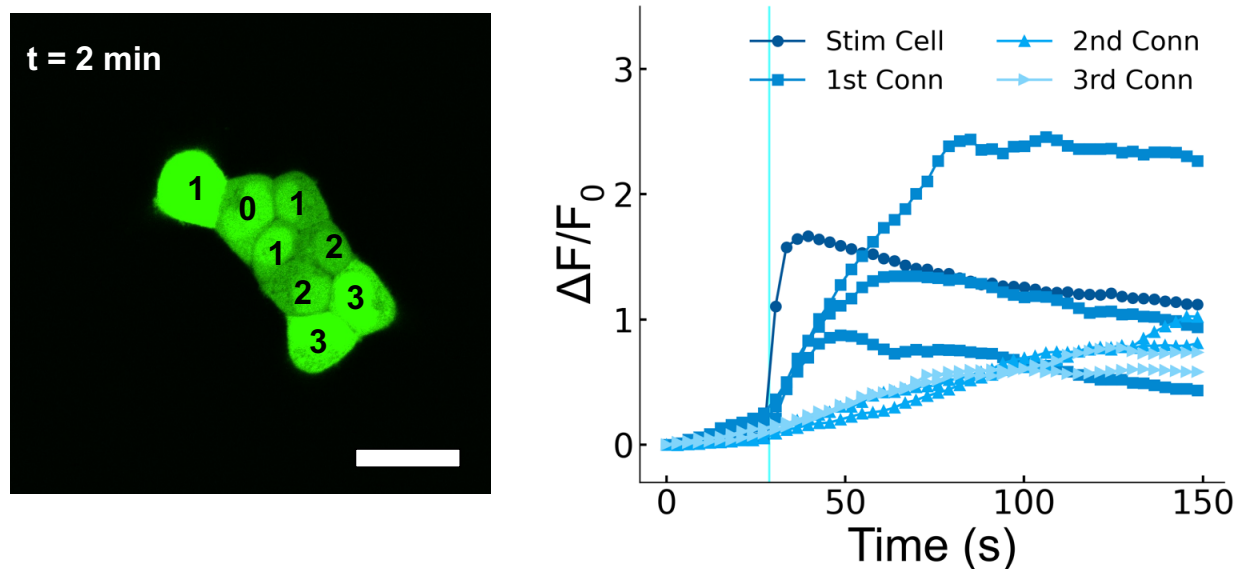
(delay time of $\sim$ 2 min). Experiments were performed in imaging medium as described in

Methods in a controlled environment chamber at 37 C and 5% CO₂.



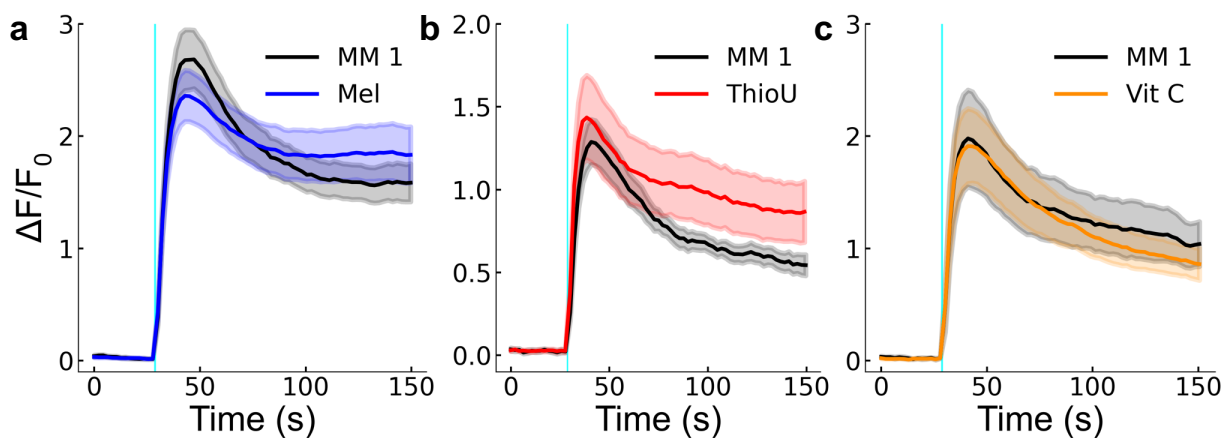
Supplementary Fig. 2. Comparison to solvent control.

Normalized fluorescence intensity traces of Fluo-4 in HEK293 cells treated with either MM **1** (8 μ M) or DMSO solvent (0.1% v/v). The solid lines represent the average of $n=6$ cells. The shaded area represents the standard error of the mean.



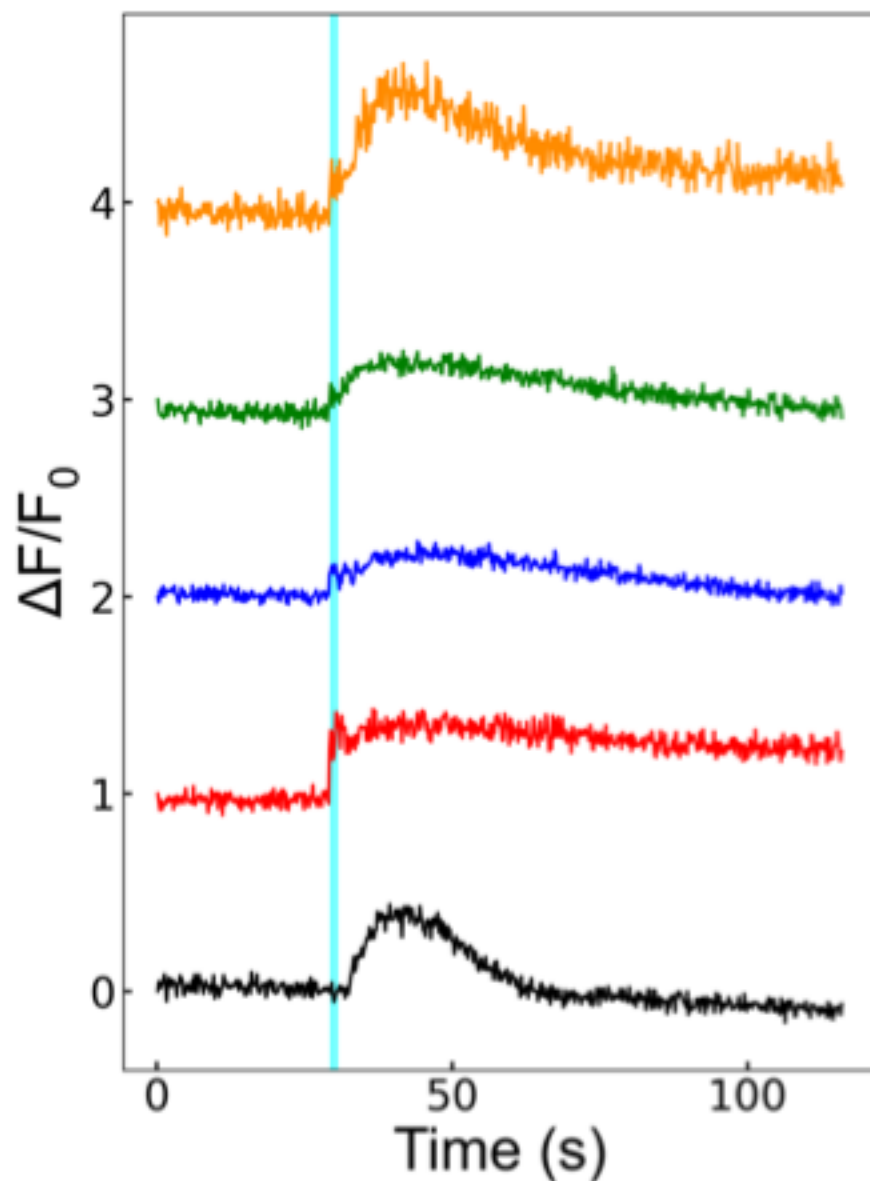
Supplementary Fig. 3. MM-induced ICW propagate according to the degree of electrical connection in stimulated tissue.

Fluo-4 fluorescence traces of HEK293 cells treated with MM **1** from a single colony (corresponding to Figure 1c), showing propagation of signal to surrounding cells over the timespan of several minutes. The cell marked “0” was stimulated with a 250 ms pulse of 400 nm light delivered to a 5 μm diameter circular region of interest at $3.2 \times 10^2 \text{ W cm}^{-2}$. The image on the left shows the cells from which the traces are derived. The numbers on each individual cell denote the separation, in number of cells, from the stimulated cell. The plot on the right shows normalized intensity traces of both the stimulated cell and the other cells in the colony. The cells marked “1st Conn” were directly connected to the stimulated cell and correspond to the cells marked “1” in the left image. The cells marked “2nd Conn” were connected to a cell designated “1st Conn” and correspond to cells marked “2” in the left image. The cell marked “3rd Conn” were connected to cells designated “2nd Conn” and correspond to the cells marked “3” in the left image. Scale bar is 20 μm .



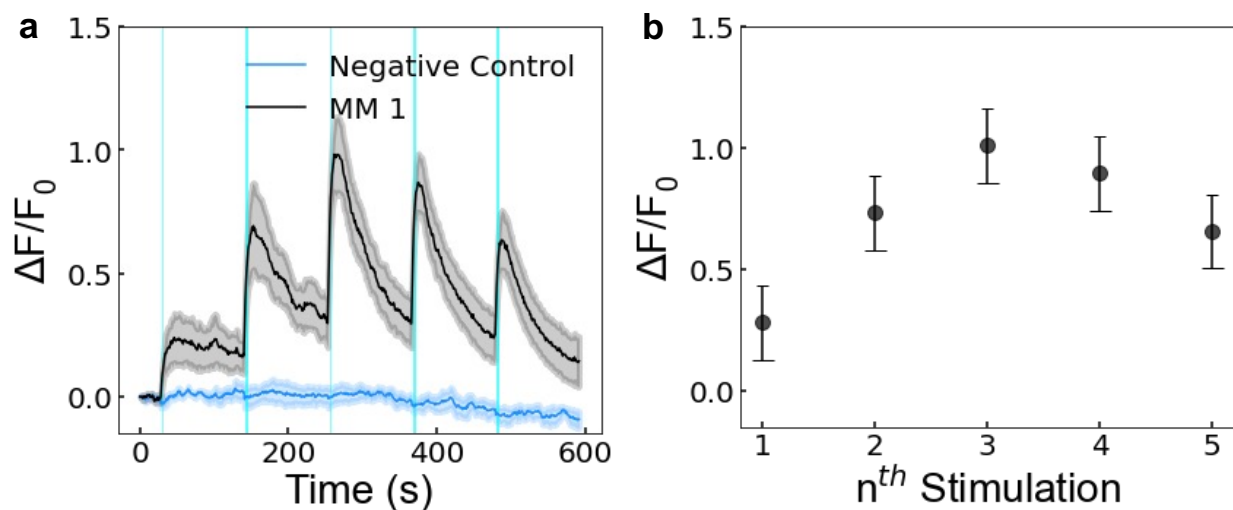
Supplementary Fig. 4. Administration of ROS scavengers does not affect MM induced ICW.

Normalized fluorescence intensity traces of HEK293 cells treated with MM **1** and Fluo-4 and administered **(a)** melatonin (Mel; 100 μ M), **(b)** thiourea (ThioU; 50 mM), and **(c)** L-ascorbic acid (Vit C; 2 mM). The solid line represents the average of $n = 6$ stimulation attempts. The shaded region indicates the standard error. Dark traces indicate positive controls collected prior to treatment. Stimulation was administered at $4.5 \times 10^2 \text{ W cm}^{-2}$. Cells were incubated with chosen ROS scavengers for 1 h prior to stimulation.



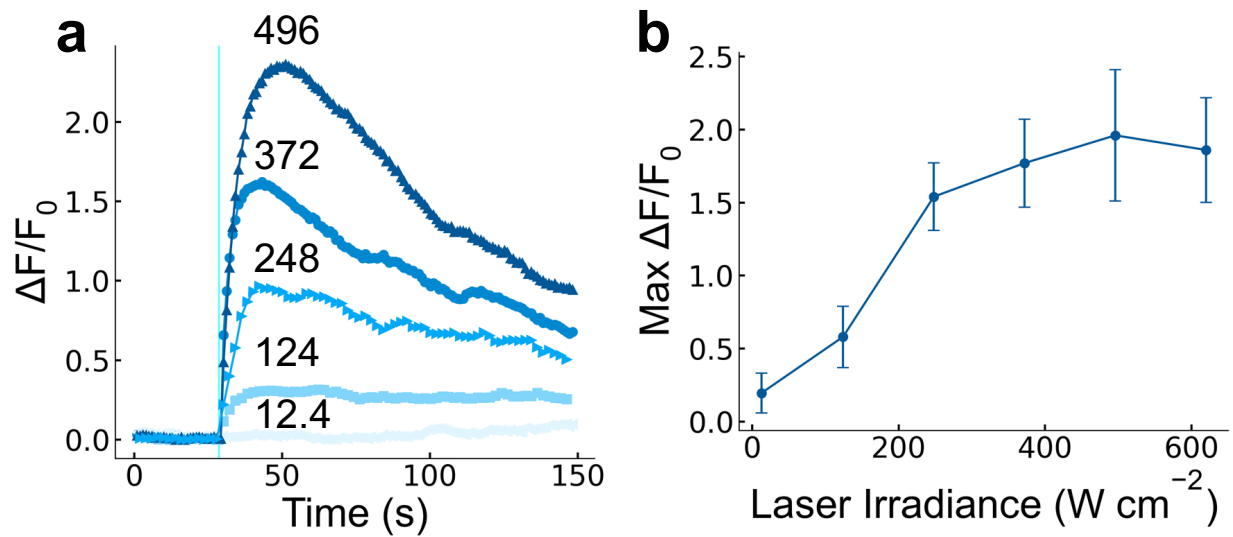
Supplementary Fig. 5. Using an alternative calcium tracking dye.

Normalized fluorescence intensity traces of X-Rhod-1 in HEK293 cells treated with MM 1 and stimulated with a 250 ms pulse of 400 nm light delivered to a 5 μm diameter circular region of interest at $3.2 \times 10^2 \text{ W cm}^{-2}$. Different colors represent distinct cells. X-Rhod-1 was loaded into cells at 2 μM over a period of 45 min and excited using 561 nm laser light in a Nikon A1 Rsi fluorescence microscope.



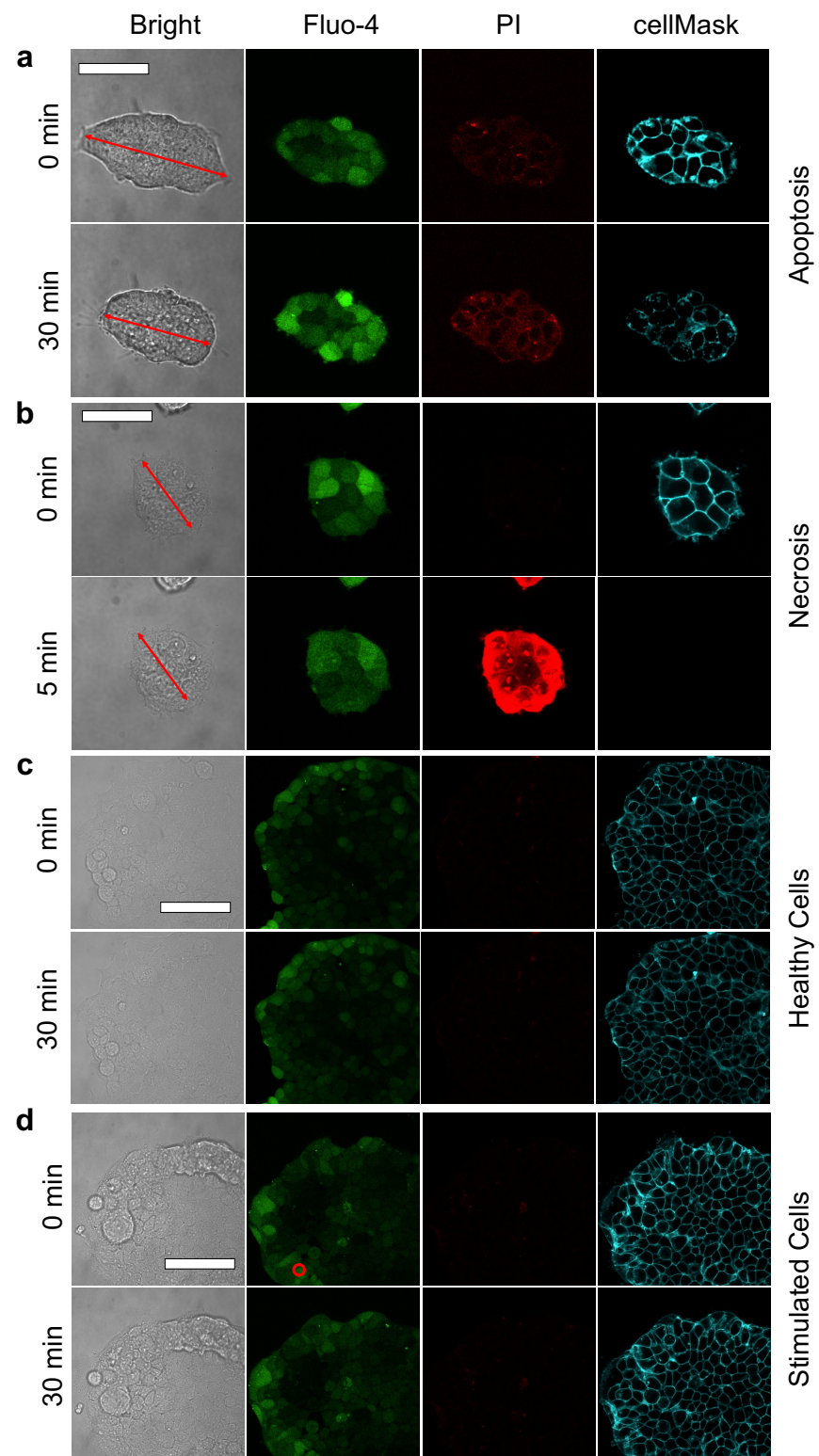
Supplementary Fig. 6. Calcium responses to MM are repeatable.

(a) Fluorescence traces of Fluo-4 in HEK293 cells treated with MM **1** as described in the Methods section and stimulated multiple times ($3.2 \times 10^2 \text{ W cm}^{-2}$, $5 \text{ }\mu\text{m}$ diameter, 250 ms pulse time). The “negative control” represents solvent-treated (0.1% DMSO) cells that were subjected to the same irradiation regime as MM-treated cells. The shaded region represents the standard error of the mean across $n=6$ cells. **(b)** Magnitude of changes in intracellular calcium levels in MM **1**-treated cells exposed to multiple light stimulations. Data points represent the average and error bars represent the standard error of $n=6$ cells. The cyan line indicates the time of stimulus presentation.



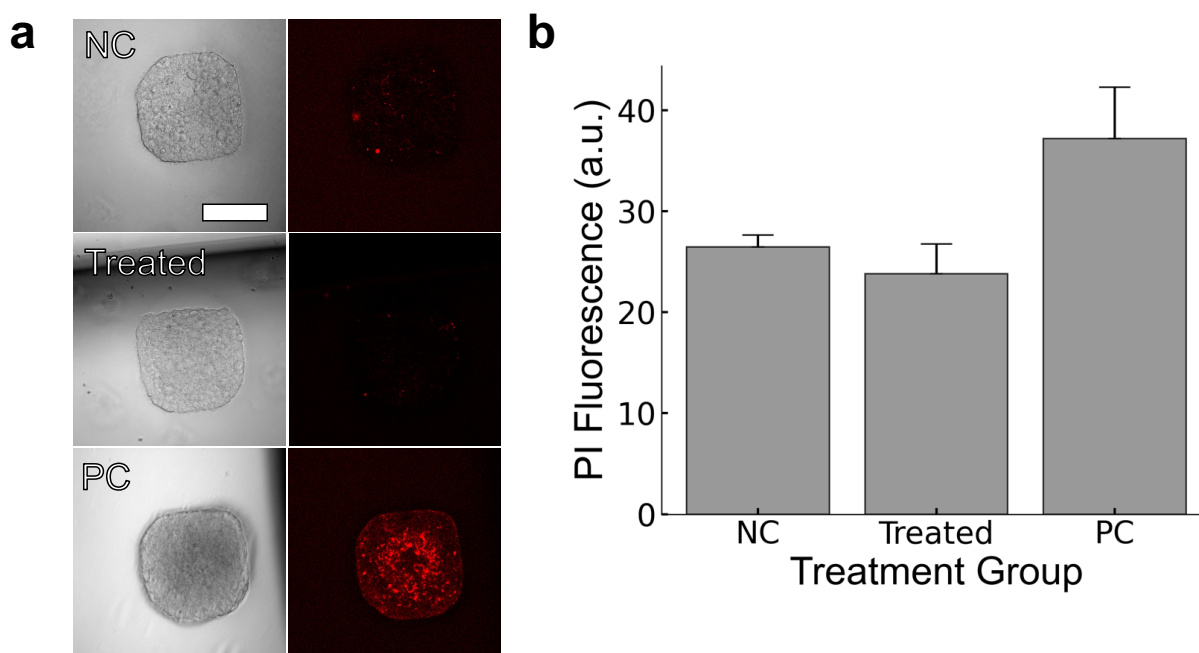
Supplementary Fig. 7. Light power dependence of the responses of HEK293 cells to MM 1.

(a) Representative fluorescent intensity traces of Fluo-4 over time at varying laser duty cycles, showing higher response amplitude at higher irradiance. Numbers in (a) depict the irradiance of each experiment in W cm^{-2} . Stimuli were delivered as a 250 ms pulse to a $5 \mu\text{m}$ diameter circular area. **(b)** Dose-response curve showing higher calcium response amplitudes at higher light intensities. Values shown represent the peak response amplitude. Error bars represent the standard error of the mean of at least six individual cells.



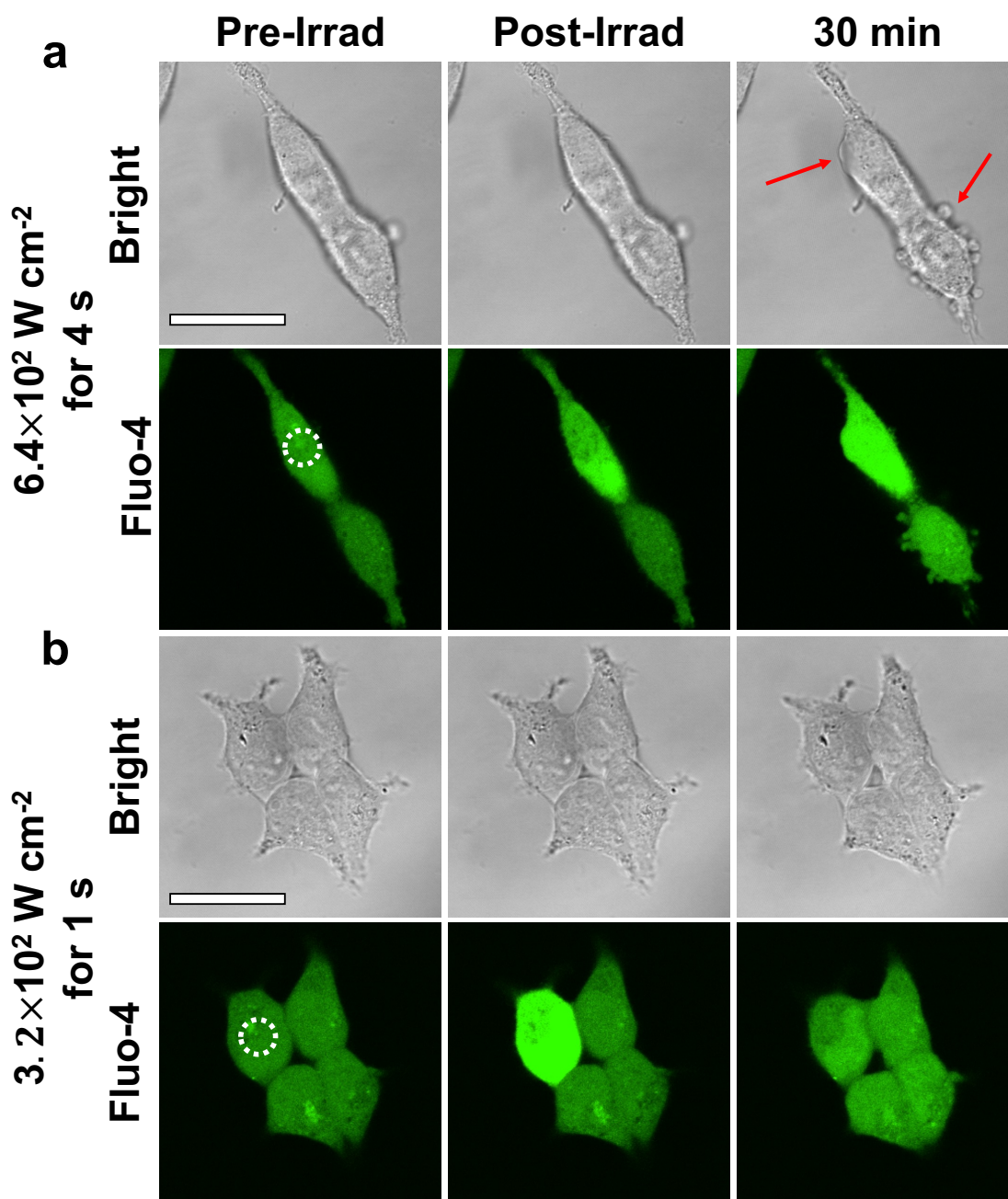
Supplementary Fig. 8. MM-induced ICW do not cause apoptosis or necrosis.

Cells were treated with MM **1** (8 μ M) as described in Methods and stained with Fluo-4 (green) to track calcium flux, propidium iodide (PI; red) to track membrane poration and cell death, and cellMask plasma membrane stain (cyan) to distinguish and track individual cells. **(a)** Positive control for apoptotic cell death with characteristic cell shrinkage (pyknosis) incurred by consecutive cell exposure to blue light (488 nm, irradiance of ~ 60 W cm⁻² rastered across the entire image for a period of 30 min). **(b)** Positive control for necrotic cell death incurred by treatment of cells with MM **1** and irradiation with UV light (365-385 nm, ~ 0.16 W cm⁻² for 5 min). Cell death is indicated by rapid PI uptake. **(c)** Negative control showing morphology of undisturbed, healthy cells. **(d)** A small colony of cells shown just before and 30 min after a typical stimulation experiment with the site of stimulation marked (red circle, second image on top row). All scale bars are 50 μ m and apply to the images in their sub-figure.



Supplementary Fig. 9. MM-induced ICW do not have toxic effects on stimulated cell populations.

Ibidi microscope dishes were photolithographically patterned with polydimethylsiloxane to prevent cell attachment except for in a gridded array pattern, resulting in isolated HEK293-colonies of defined size and geometry⁶⁷. These colonies were imaged 3 days after being seeded following i) no treatment (negative control; NC), ii) stimulation with MM 1 and light on day 2 (10 single-cell stimulations per colony, $3.2 \times 10^2 \text{ W cm}^{-2}$; Treated) , or iii) irradiation with UV light (365-385 nm, $\sim 0.16 \text{ W cm}^{-2}$) for 5 min on day 3 (positive control; PC). **(a)** Bright field (left) and fluorescent (right) images of representative colonies from each treatment showing the onset of PI uptake into colonies in the PC group and no substantial uptake post stimulation in the treated cells. Scale bar is 200 μm and applies to all images. **(b)** Calculated PI fluorescent intensity. The bar height indicates the average of 3 colonies. Error bars indicate standard deviation.

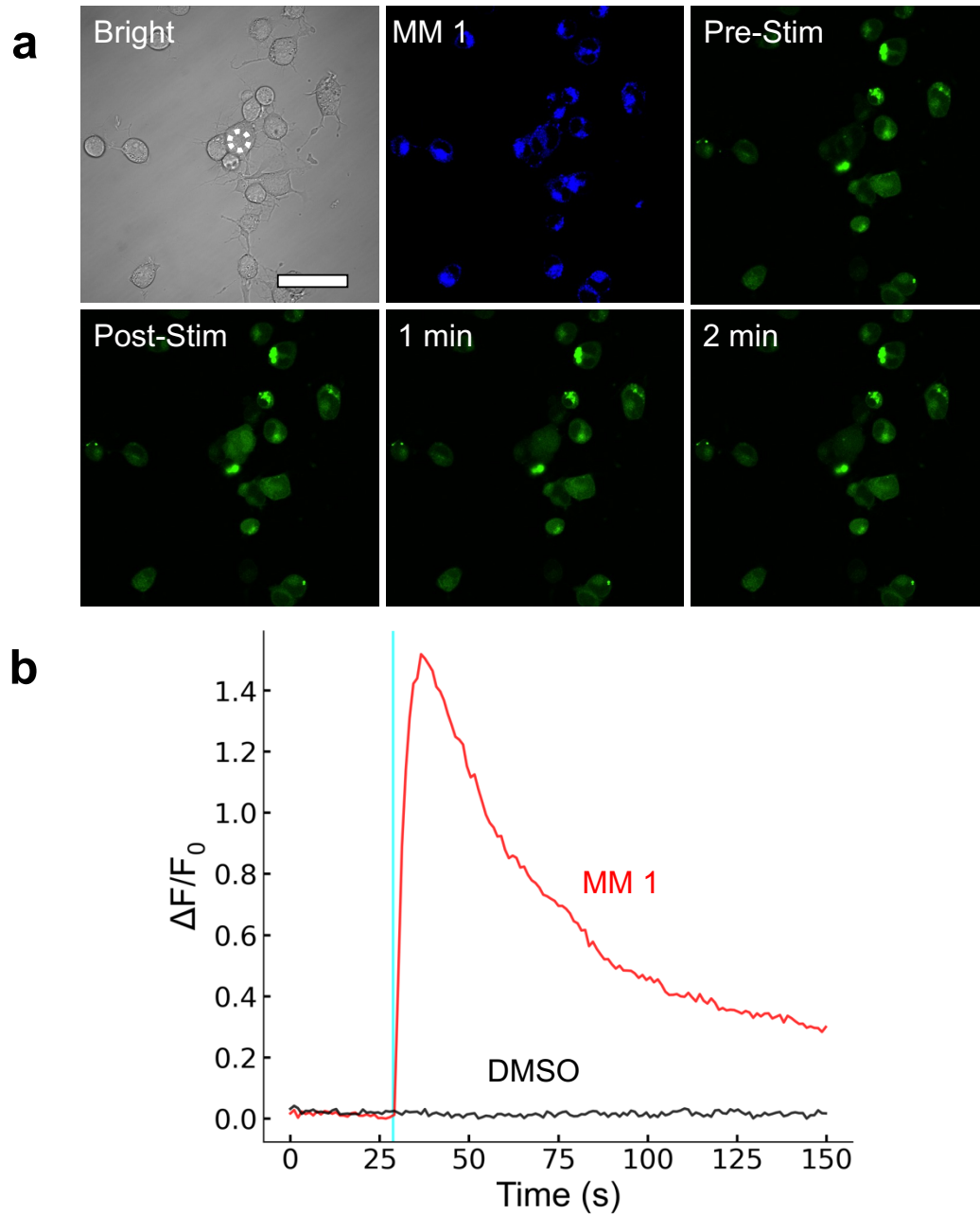


Supplementary Fig. 10. Toxicity at high exposure times and high laser power.

(a) Confocal microscope images of HEK293 cells treated with MM 1 and Fluo-4 calcium tracking dye after a 4 s light pulse of 400 nm light at $6.4 \times 10^2 \text{ W cm}^{-2}$. Thirty minutes after treatment, the calcium signal has not returned to homeostasis, and membrane blebbing is apparent (red arrows).

(b) Confocal images of HEK293 cells treated with MM 1 and Fluo-4 calcium tracking dye after

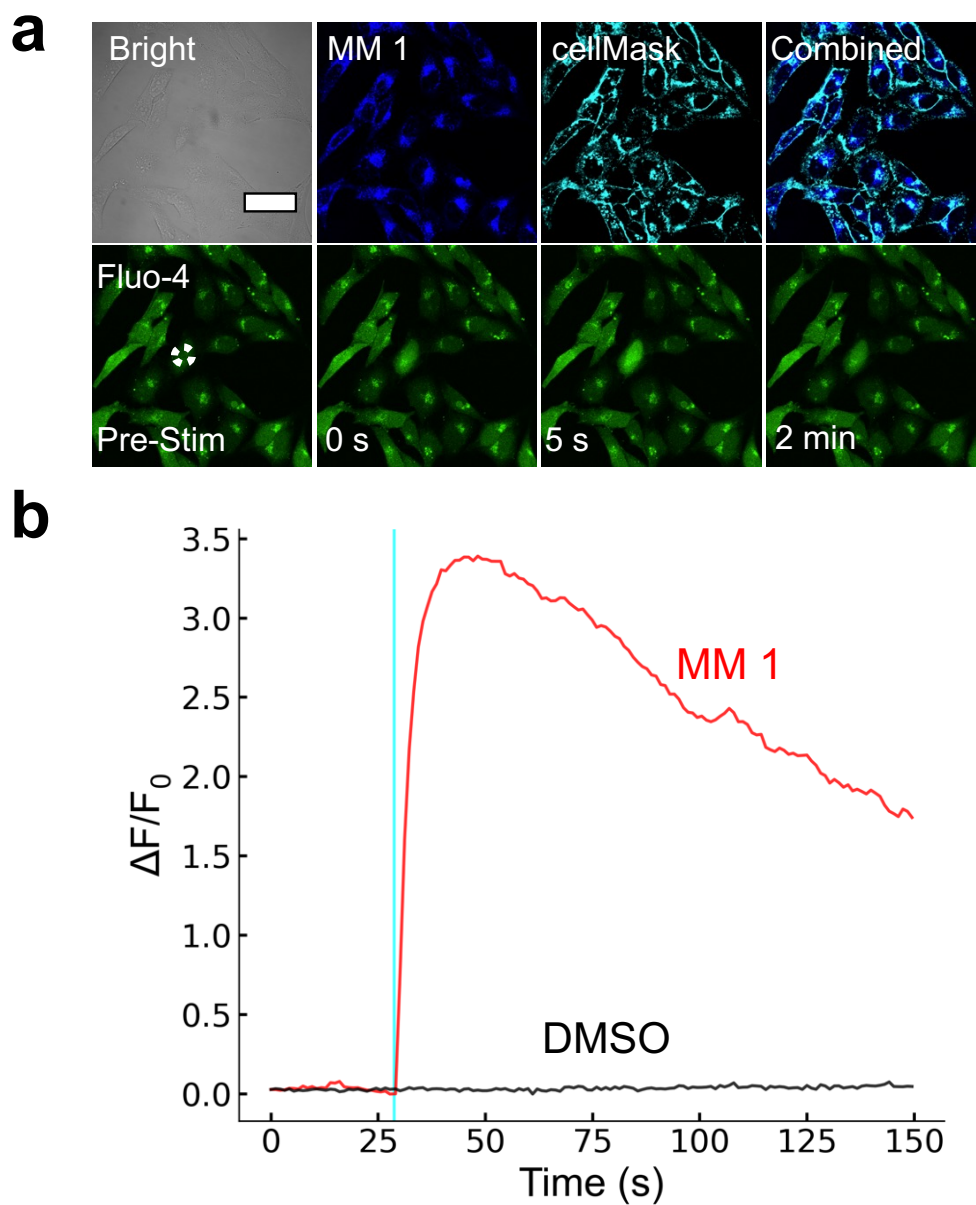
laser pulses of $3.2 \times 10^2 \text{ W cm}^{-2}$ for 1 s. After 30 min, the calcium signal has returned to homeostatic levels, and the stimulated cell and surrounding cells display no apparent damage. Scale bars are 50 μm and apply to all images in their sub-figure. The white dotted circles indicate the area of stimulation.



Supplementary Fig. 11. ICW in N2A cells after stimulation with MM 1 and light.

(a) Confocal images of N2A cells treated with MM 1 (blue) and calcium tracking dye (Fluo-4; green). The white circle in the top-left image depicts the area to which stimulus was delivered. The scale bar is 50 μm and applies to all images. **(b)** Fluo-4 fluorescence traces of N2A cells treated

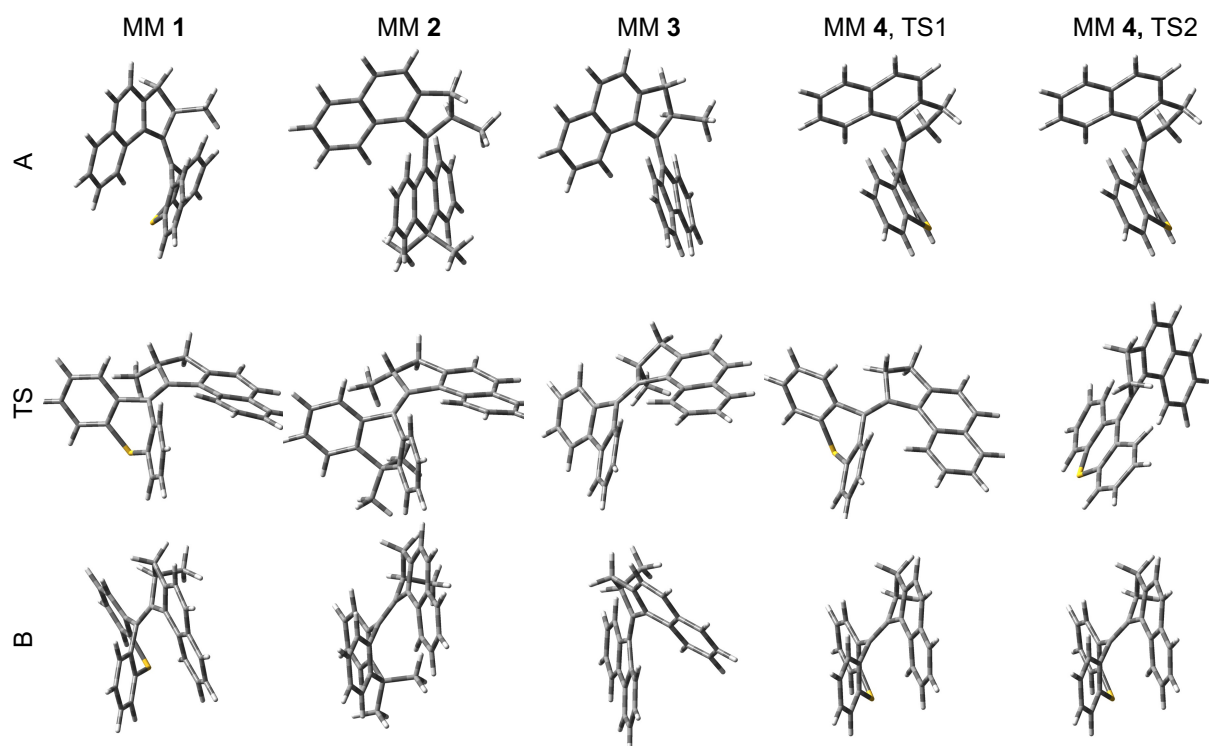
with MM **1** and stimulated with 250 ms pulse of 400 nm light delivered to a 5 μm diameter circular region of interest at $3.2 \times 10^2 \text{ W cm}^{-2}$ stimulation power.



Supplementary Fig. 12. ICW in HeLa cells after stimulation with MM 1 and light.

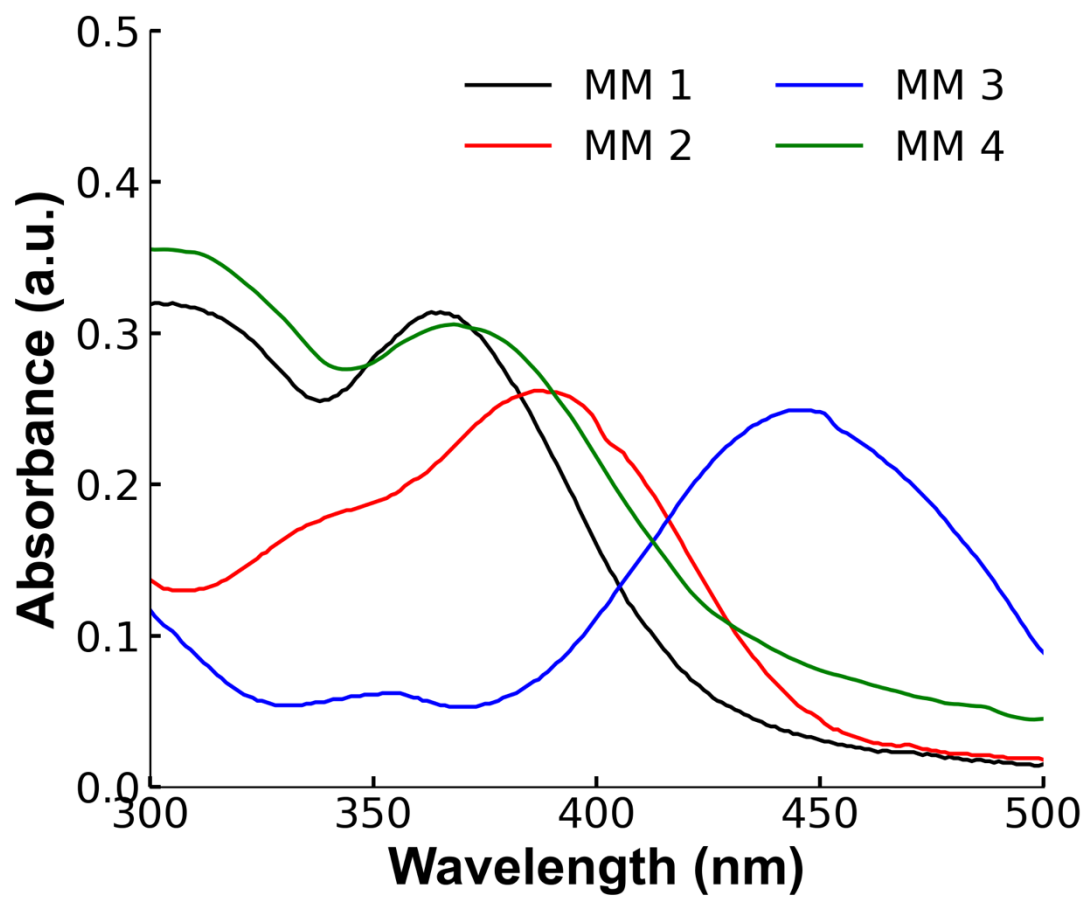
(a) Confocal images of HeLa cells treated with MM 1 (blue) and Fluo-4 calcium tracking dye (green). The white circle in the bottom-left image depicts the area to which light stimulus was delivered. Scale bar is 50 μm and applies to all images. **(b)** Fluo-4 fluorescence traces of HeLa

cells treated with MM **1** and stimulated with 250 ms pulse of 400 nm light delivered to a 5 μm diameter circular region of interest at $3.2 \times 10^2 \text{ W cm}^{-2}$ stimulation power.



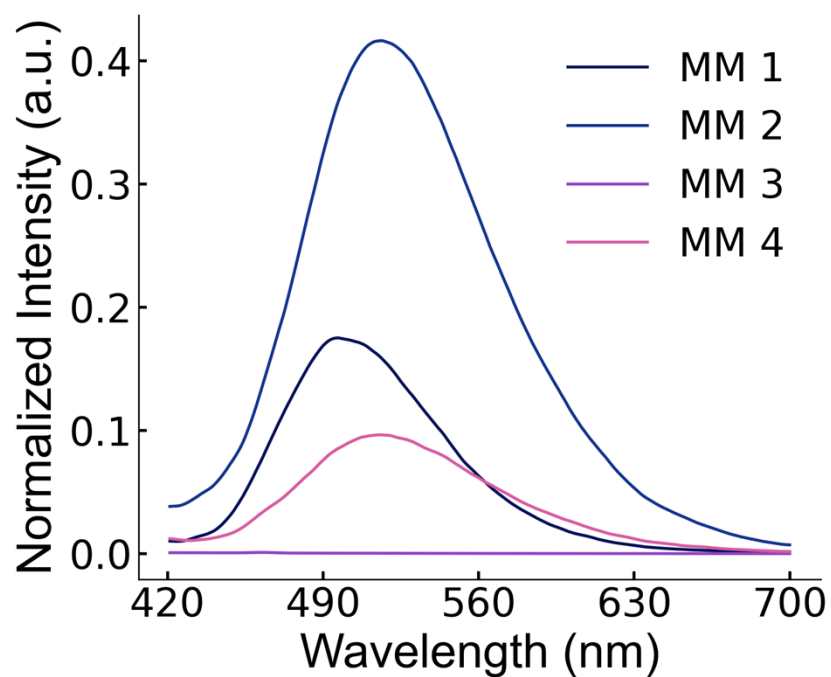
Supplementary Fig. 13. Snapshots of density functional theory simulations.

Thermal helix inversions of MM 1-4 and a second transition state in MM 4 (TS2) that interrupts the thermal helix inversion. Metastable, stable, and transition conformations are labeled as A, B, and TS,



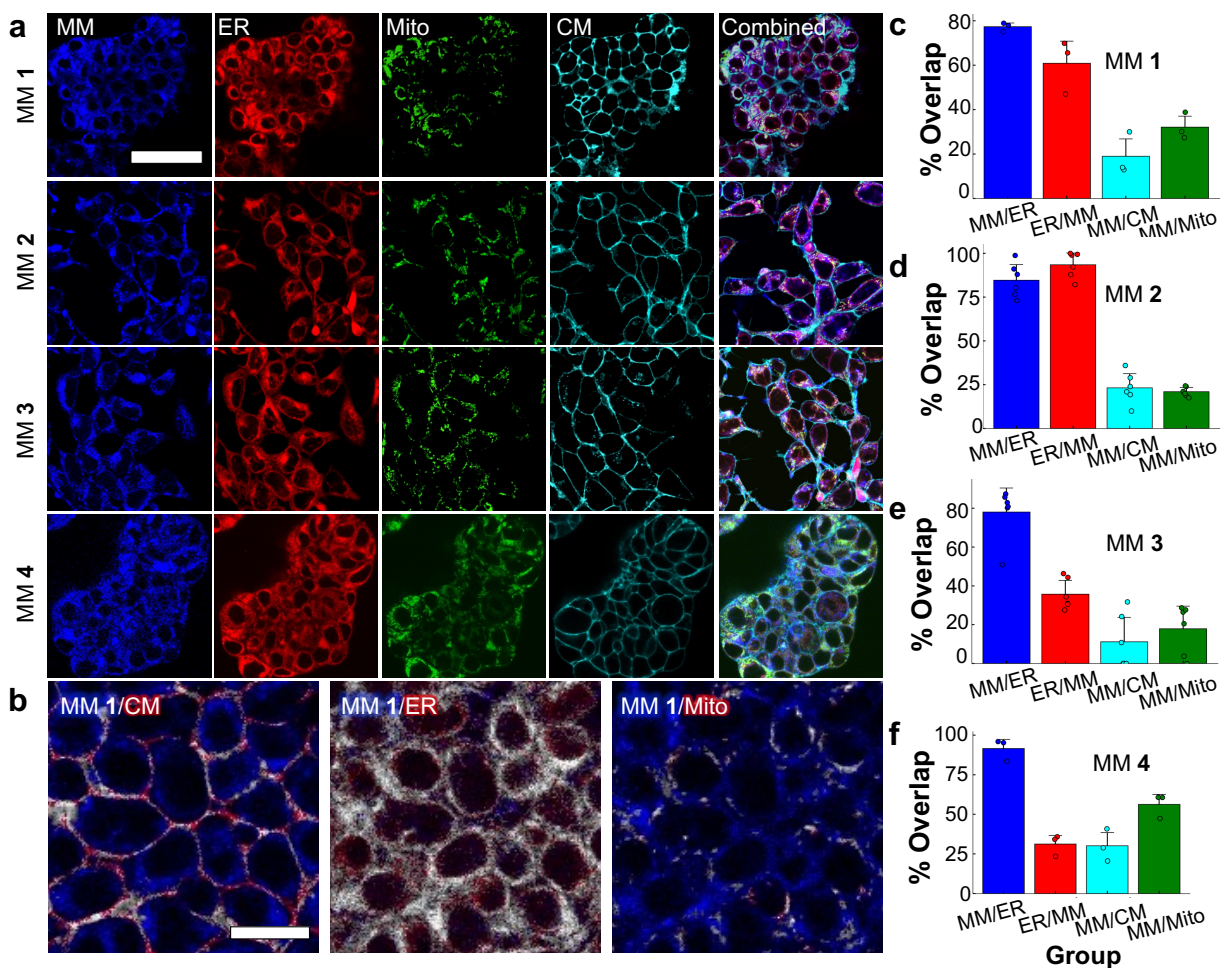
Supplementary Fig. 14. UV-Vis spectra.

Sample UV-Vis spectra of each MM taken at 16 μM in spectral-grade water.



Supplementary Fig. 15. Normalized fluorescence intensity.

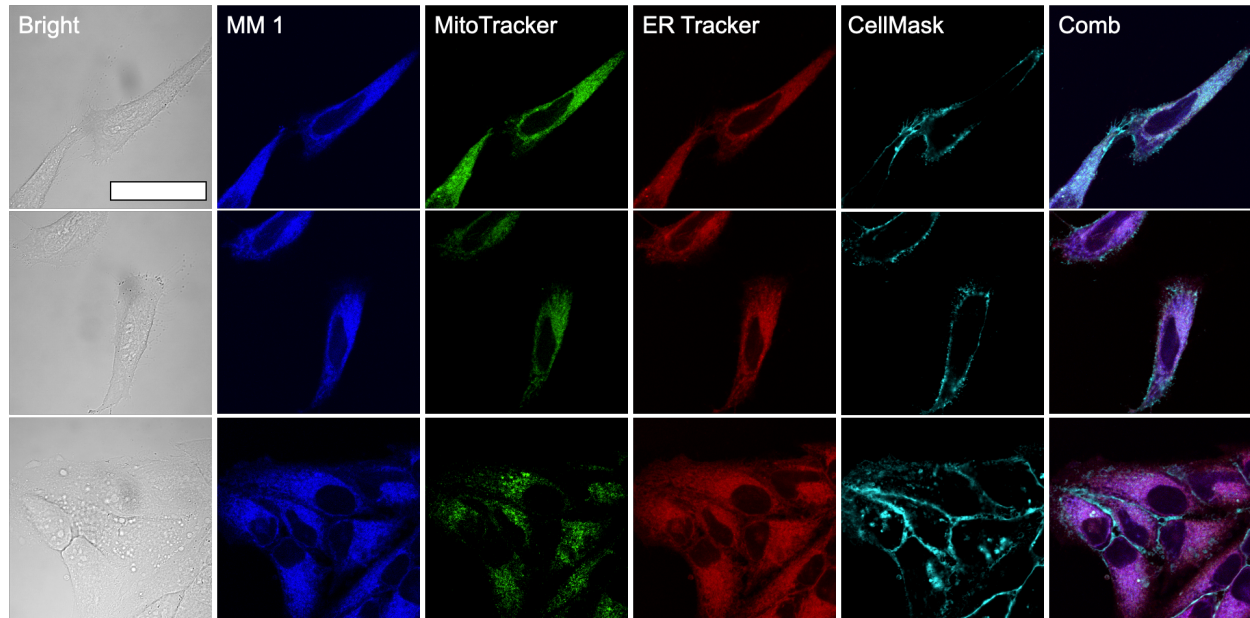
Fluorescence spectra normalized to molar absorptivity of **MM 1-4**. Spectra were taken at 8 μM concentrations in spectral-grade water.



Supplementary Fig. 16. Intracellular location of each MM.

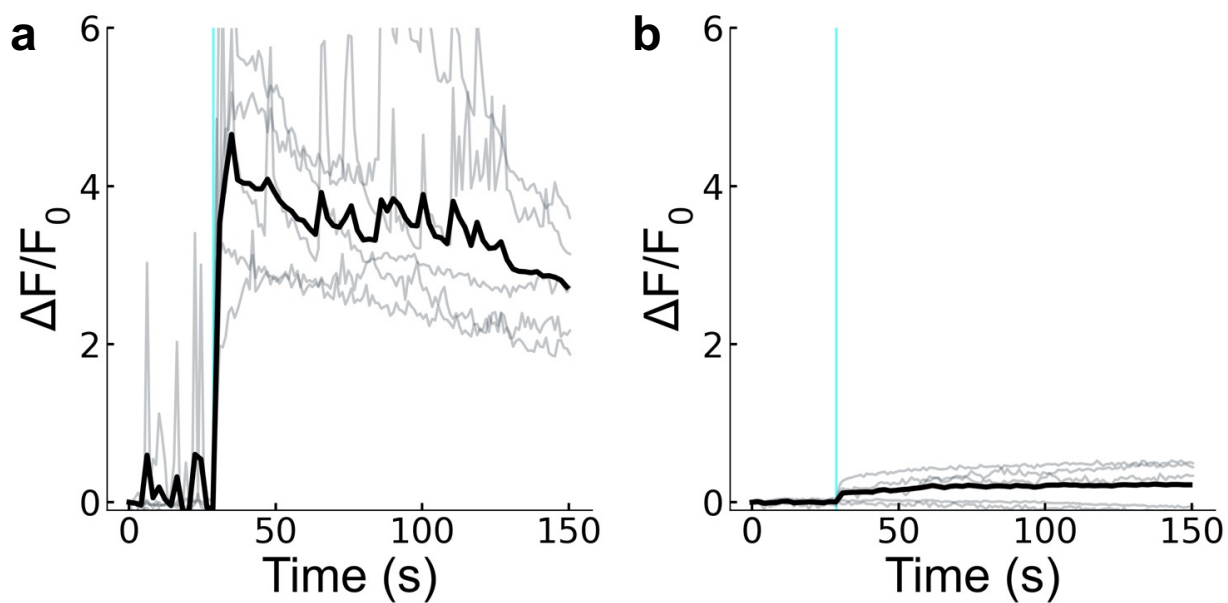
(a) Confocal images of HEK293 cells incubated with each MM, and different organelle-targeting dyes, namely ER-Tracker Red (ER), MitoTracker Green (mitochondria), and cellMask (plasma membrane), showing that MM preferentially distribute to the ER. The 50 μm scale bar applies to all images in the first four rows. **(b)** Pixel overlap maps of MM 1 fluorescence paired with fluorescence from organelle-targeted dyes. Blue color indicates pixels with fluorescence from MM 1 but not dye. Red color indicates pixels with fluorescence from dye but not MM 1. Gray color indicates pixels with overlapping fluorescence from both MM 1 and dye. Left: cell membrane; middle: ER; right: mitochondria. Scale bar is 20 μm and applies to all images. Percent volumetric

coverage of each organelle by **(c) MM 1**, **(d) MM 2**, **(e) MM 3**, and **(f) MM 4**. MM/ER depicts the percentage of MM found in the ER, while ER/MM depicts the percentage of ER covered with MM. MM/CM and MM/Mito depict the proportion of MM found in the cell membrane and mitochondria, respectively. Error bars represent the standard error, $n = 3, 6, 5$, and 3 z-stacks for (c), (d), (e), and (f), respectively.

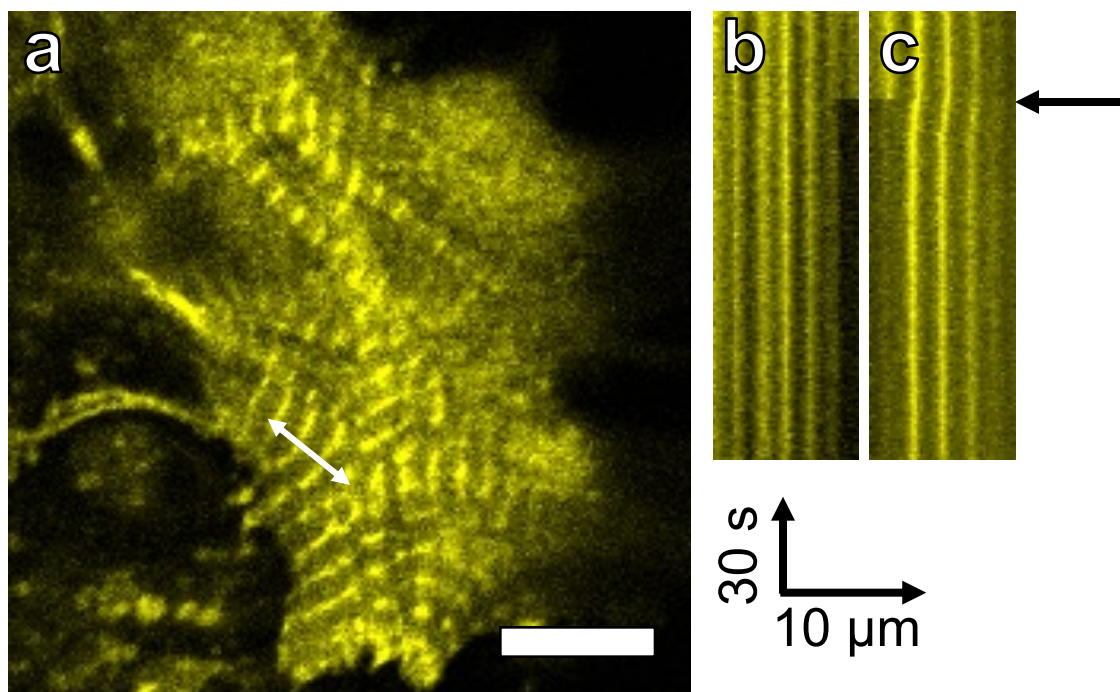


Supplementary Fig. 17. Location of MM in HeLa cells.

Microscopy images revealing the intracellular localization of MM **1** (blue), MitoTracker Green (green), ER-Tracker-Red (red), and cellMask plasma membrane dye (cyan) in HeLa cells. Scale bar is 50 μm and applies to all images.

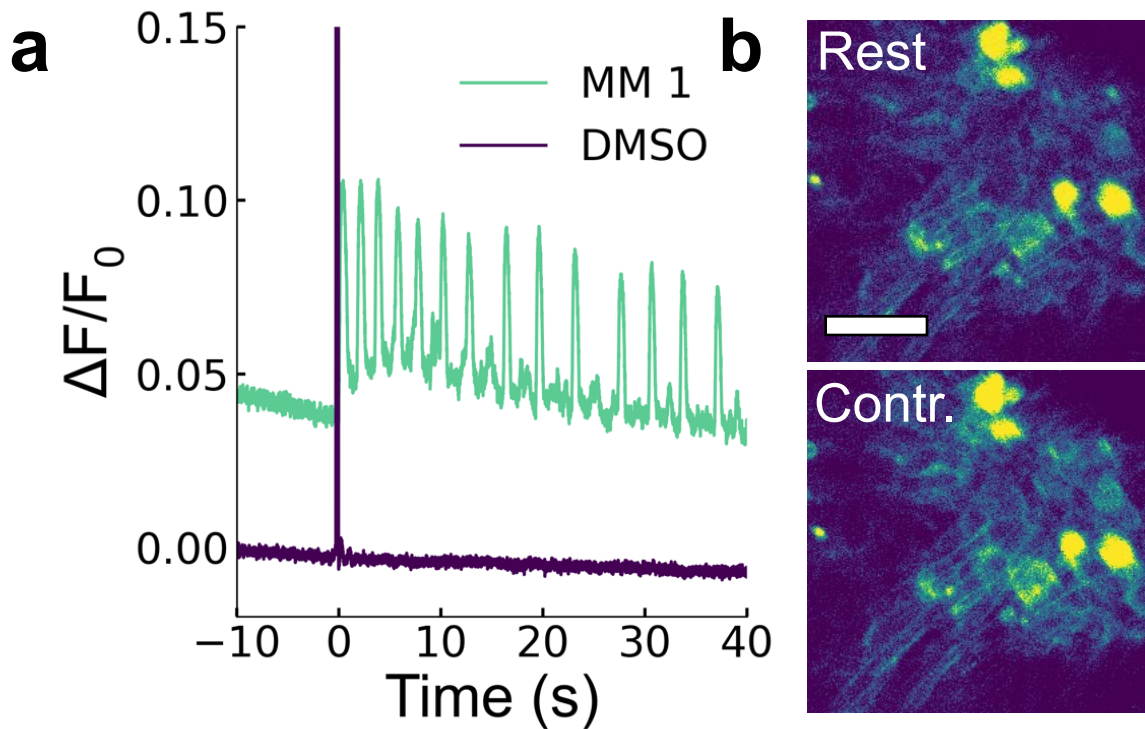


Supplementary Fig. 18. Normalized fluorescence intensity traces of cardiomyocytes treated with MM 1 (8 μM) and Fluo-4 (2 μM) and stimulated with 400 nm light at $5.0 \times 10^2 \text{ W cm}^{-2}$ **(a)** before and **(b)** after treatment with PLC inhibitor U-73 (10 μM).



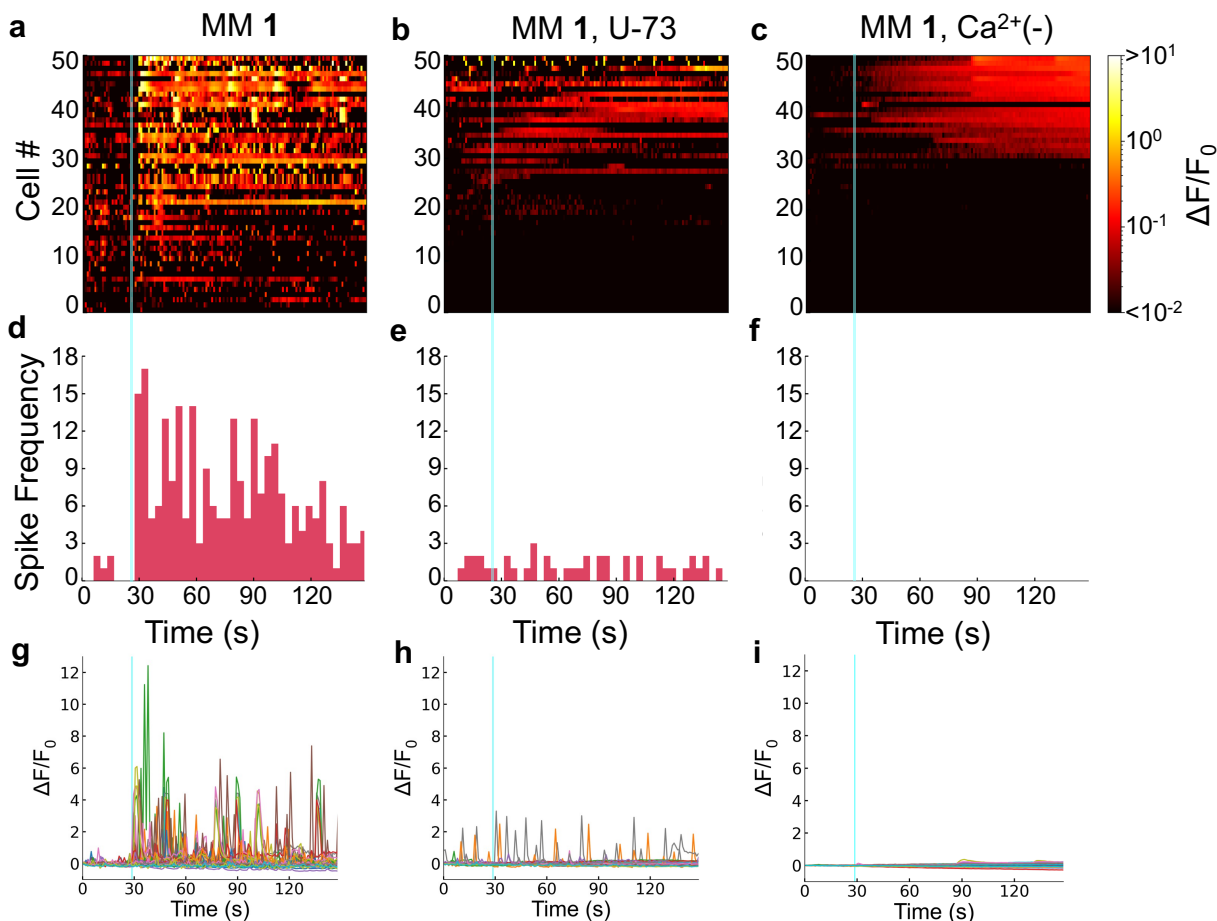
Supplementary Fig. 19. Actin dynamics during cardiomyocyte contraction.

(a) A cardiomyocyte treated with cellLight GFP-Actin BacMam 2.0 and MM **1**. Signal arises from the actin label and has been pseudocolored yellow to avoid confusion with Fluo-4. Scale bar is 10 μm . The arrow represents the area of the cell from which the kymograph is taken. **(b-c)** Kymograph during a typical fluorescence experiment in a cell treated with **(b)** DMSO (0.1% v/v) and **(c)** MM **1** (8 μM). The arrow indicates the time of stimulation. Streaks of light indicate individual z-lines of sarcomeres. The dark region results from laser photobleaching in the stimulated region. The rightward drift of the lines after stimulation indicates z-line migration and contraction. Stimulation was applied after 30 s at $5.0 \times 10^2 \text{ W cm}^{-2}$ to an area of 5 μm diameter for 250 ms at a wavelength of 400 nm.



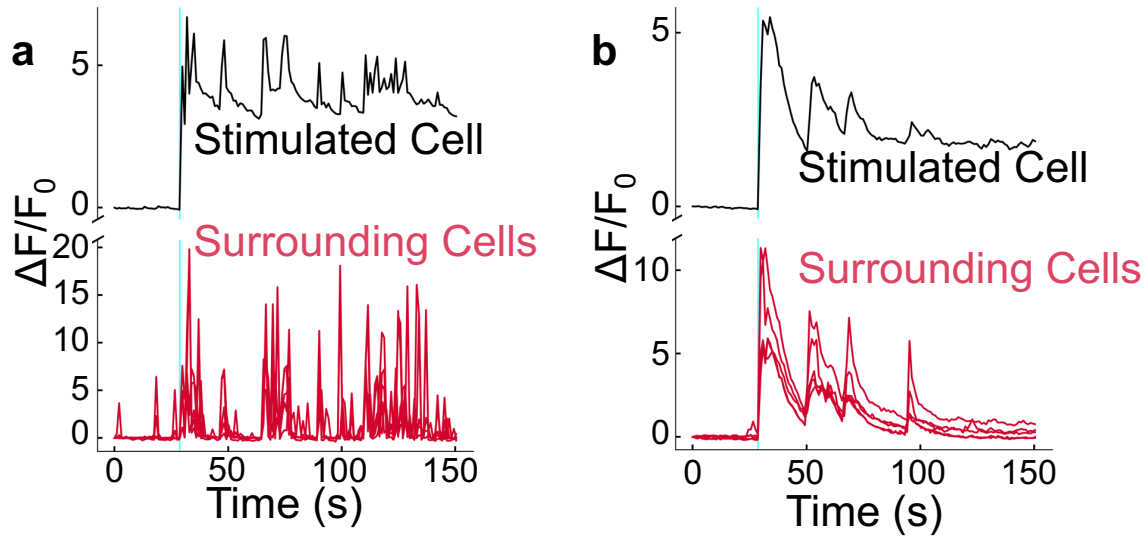
Supplementary Fig. 20. Cardiomyocyte contraction arises from transmembrane currents during AP firing.

(a) Normalized fluorescence intensity traces of cardiomyocytes treated with MM 1 (8 μM) or DMSO (0.1% v/v) and voltage-sensitive, membrane-bound fluorescent dye di-8-Anneps (1 $\mu\text{g mL}^{-1}$). A 405 nm laser stimulus ($3.2 \times 10^2 \text{ W cm}^{-2}$) was applied at $t = 0 \text{ s}$. Traces were taken at 100 fps. The “MM 1” trace was offset vertically for clarity. **(b)** Fluorescent images of the cardiomyocytes at rest and during contraction. Signal in the image comes from di-8-Anneps. Scale bar is 50 μm and applies to all images.



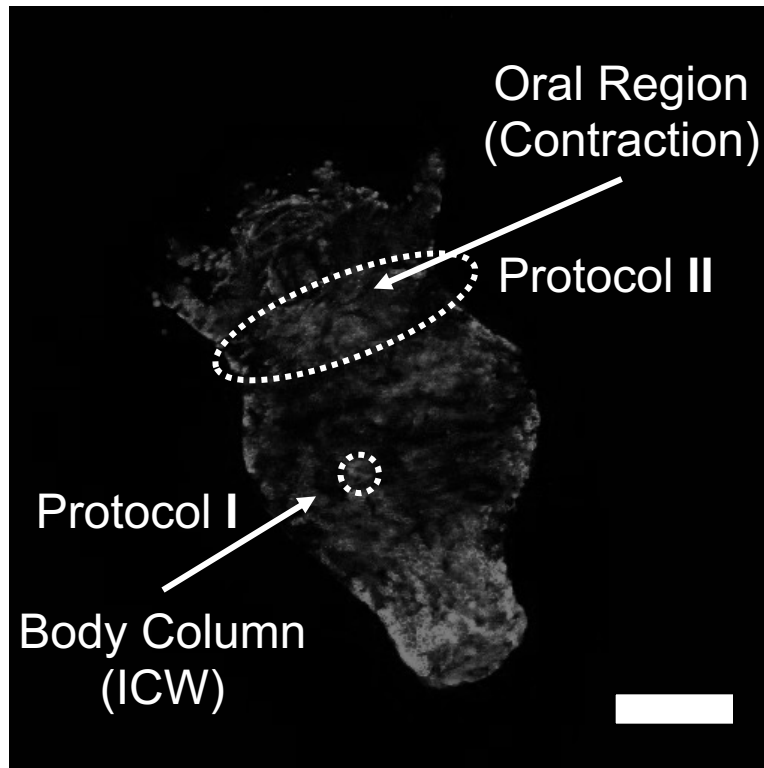
Supplementary Fig. 21. Analysis of signaling activity of cardiomyocytes in colonies connected to cells stimulated by MM 1.

Heatmap plots (**a-c**), temporal spike frequencies (**d-f**), and individually plotted traces (**g-i**) of cardiomyocytes adjacent to a cell stimulated with (**a,d,g**) MM 1 + light, (**b,e,h**) MM 1 + light in 10 μ M U-73, and (**c,f,i**) MM 1 + light in PBS. Light stimulation was performed using a 5.1×10^2 W cm⁻² 250 ms pulse of 400 nm laser light delivered to a 5 μ m diameter area. The histograms in (d-f) indicate the time distributions of calcium spikes in cardiomyocytes identified by a peak detection algorithm (see Methods). Different colors in the individual traces in (g-i) indicate individual cells.



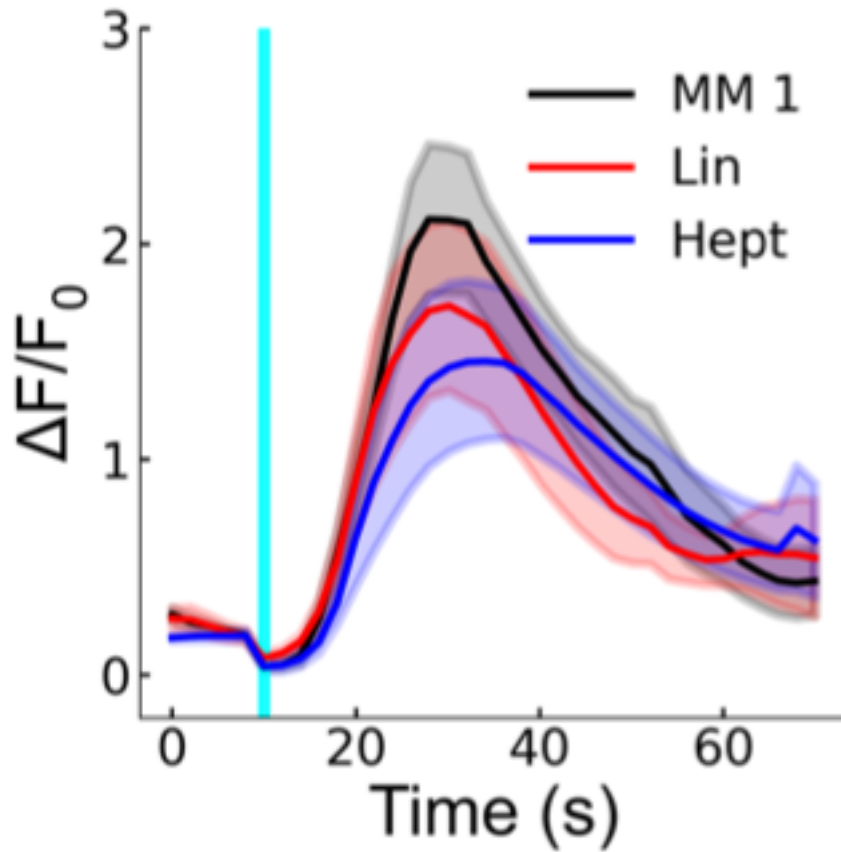
Supplementary Fig. 22. Different phenotypes of cardiac myocyte activation in surrounding cells.

Response phenotypes of individual colonies of cardiac myocytes showing (a) enhanced firing due to membrane depolarization and (b) synchronous calcium increase. Black traces indicate the stimulated cell. Red traces indicate adjacent cells in a colony. Light stimulation was performed using a $5.1 \times 10^2 \text{ W cm}^{-2}$ 250 ms pulse of 400 nm laser light delivered to a 5 μm diameter area.



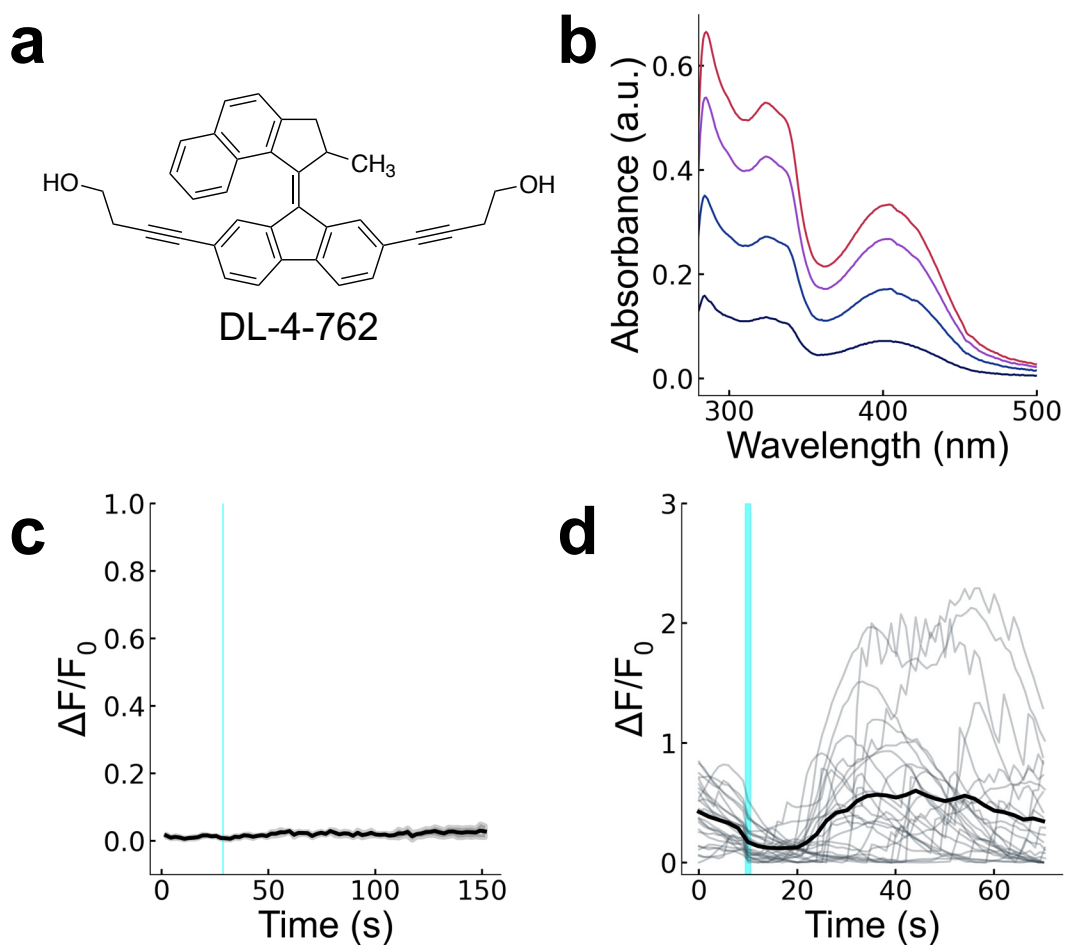
Supplementary Fig. 23. Different stimulation protocols for regional ICW and whole-body contraction.

A *Hydra* expressing GCaMP7b in endodermal epitheliomuscular cells. The stimulation regions used for Protocols I and II are marked. Protocol I was used for body column stimulation (1 s pulse to a 10 μm diameter circular area) to trigger regional ICW. Protocol II was used for oral region stimulation (2 s pulse to the oral region, $\sim 1000\text{-}2000\ \mu\text{m}^2$) to trigger whole-body contraction. The scale bar is 100 μm .



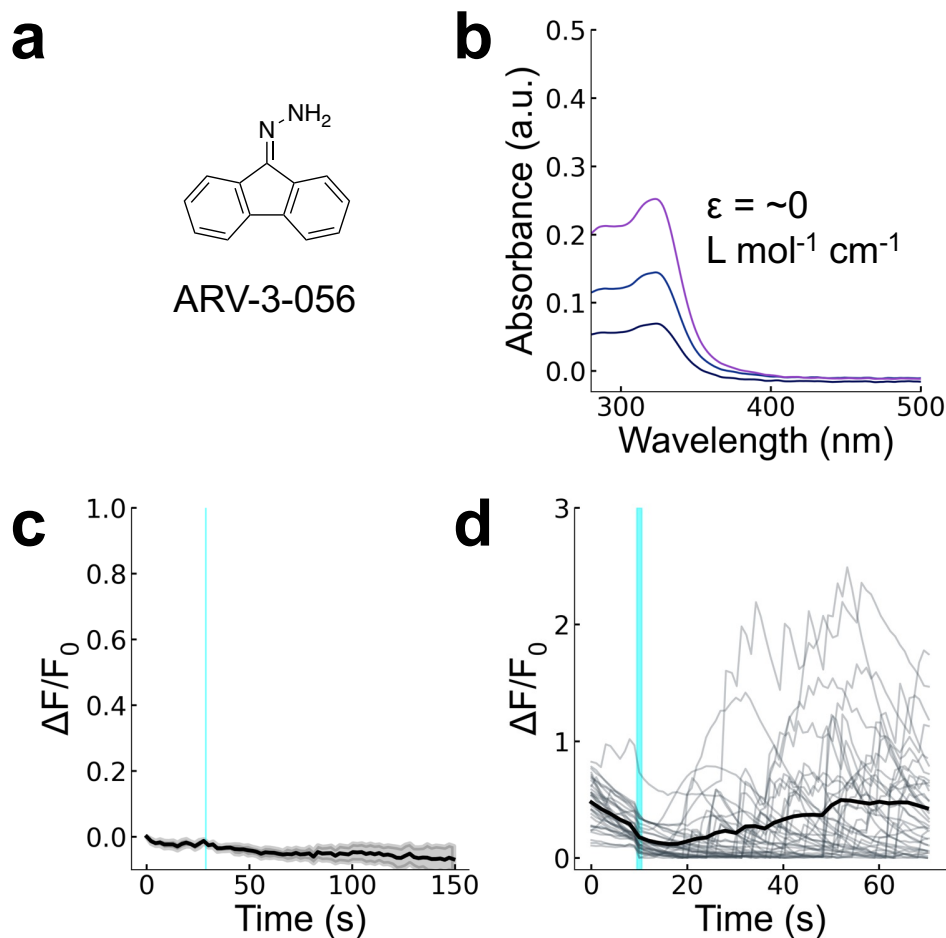
Supplementary Fig. 24. *In vivo* pharmacological investigation.

Normalized fluorescence intensity traces of GCaMP7b showing that ICW elicited from *Hydra vulgaris* in Protocol I experiments were robust to anesthetization with linalool (Lin; conc) and 1-heptanol (Hept; 0.06% v/v). *Hydra* were treated with MM 1 (24 μ M) and stimulated at 9.0×10^2 W cm⁻² power with a 405 nm laser in a 10 μ m diameter circular area for 1 s (Protocol I). The solid line represents the average of all experiments, and the shaded area represents the standard error. The group “MM 1” represents n=13 experiments across 3 *Hydra*. The group “Lin” represents n = 13 experiments across 2 *Hydra*. The group “Hept” represents n = 19 experiments across 3 *Hydra*.



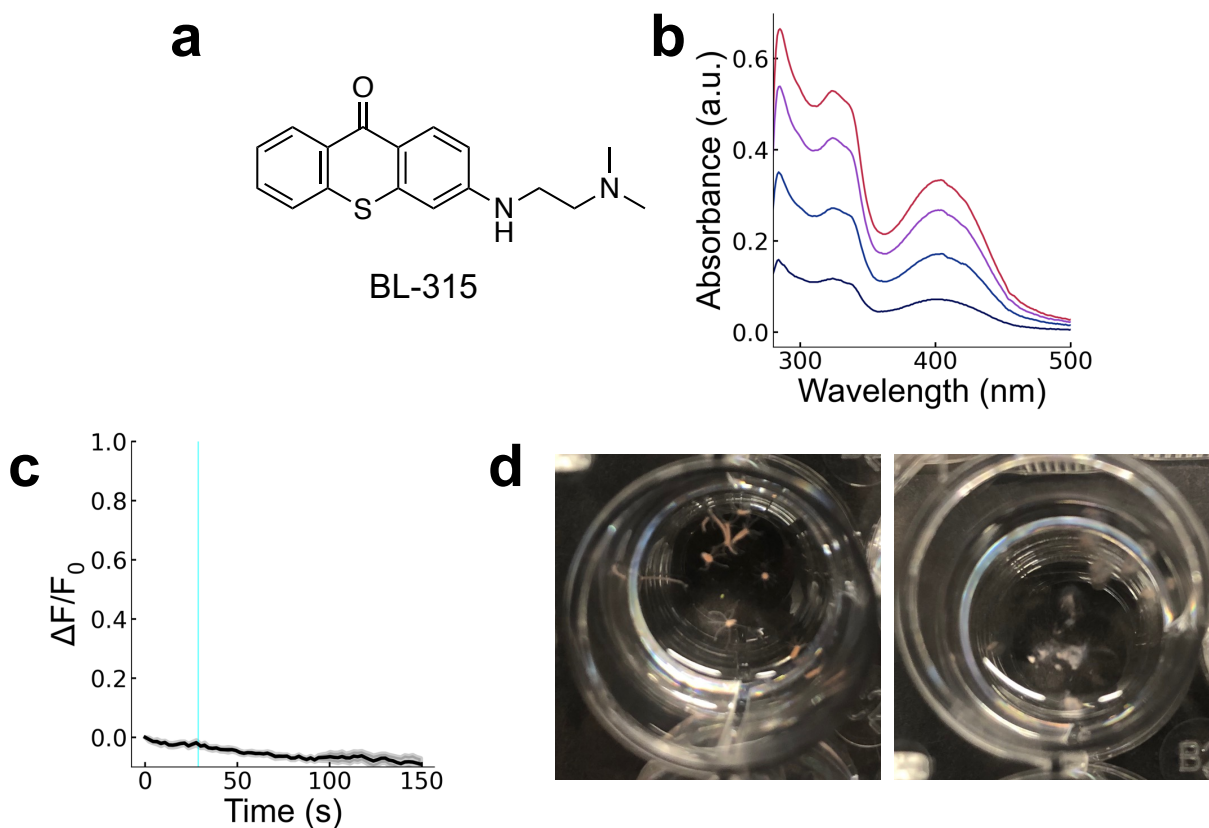
Supplementary Fig. 25. A second slow-rotating control molecule is inactive *in vitro* but elicits small responses *in vivo*.

(a) Structure of **DL-4-762**. **(b)** UV-Vis absorption spectrum at concentrations of 8, 16, 24, and 32 μM . **(c)** Normalized fluorescence intensity traces of HEK293 cells treated with **DL-4-762** (8 μM) and Fluo-4 (2 μM) and stimulated with 400 nm laser light. **(d)** Normalized fluorescence intensity traces of GCaMP7b-expressing *Hydra vulgaris* treated with **DL-4-762** (24 μM) and stimulated according to Protocol I.



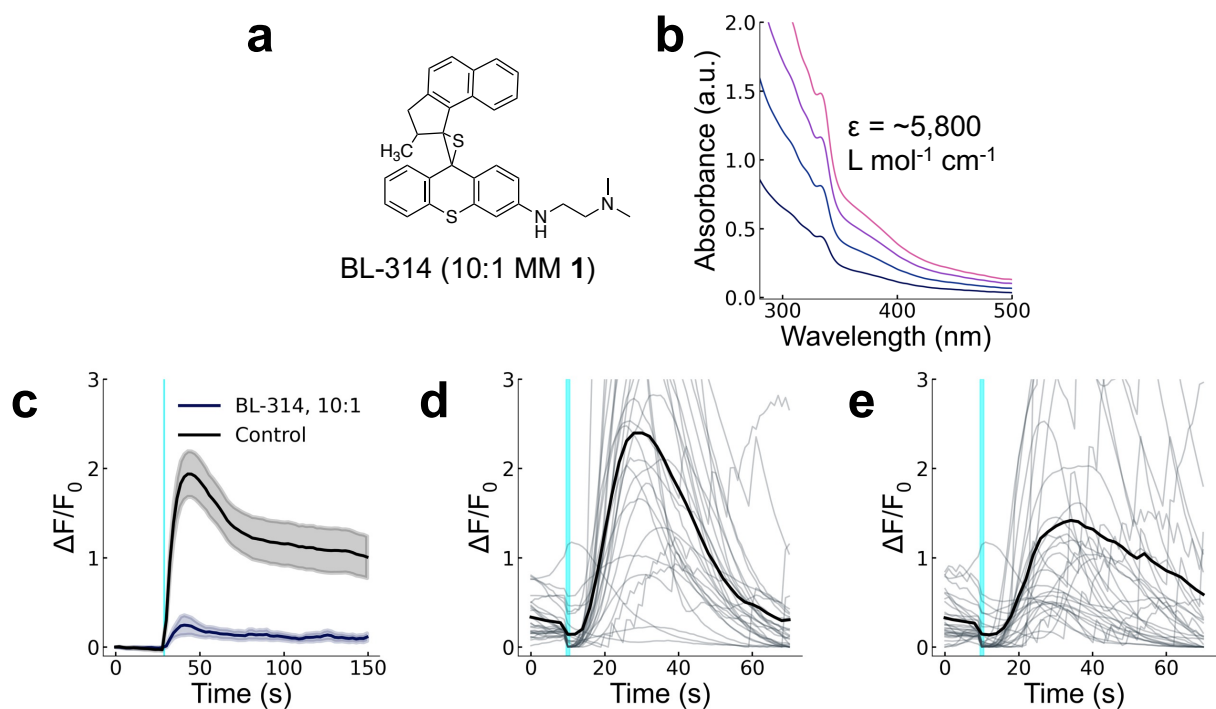
Supplementary Fig. 26. A non-rotating molecule is inactive *in vitro* and mostly inactive *in vivo*, although small responses are occasionally observed.

(a) Structure of **ARV-3-056**. **(b)** UV-Vis of sequential concentrations of the molecule at concentrations of 8, 16, and 24 μM . **(c)** Normalized fluorescence intensity traces of HEK293 cells treated with **ARV-3-056** (24 μM) and Fluo-4 (2 μM) and stimulated with 400 nm light. **(d)** Normalized fluorescence intensity traces of GCaMP7b-expressing *Hydra vulgaris* treated with **ARV-3-056** (24 μM) and stimulated according to Protocol I.



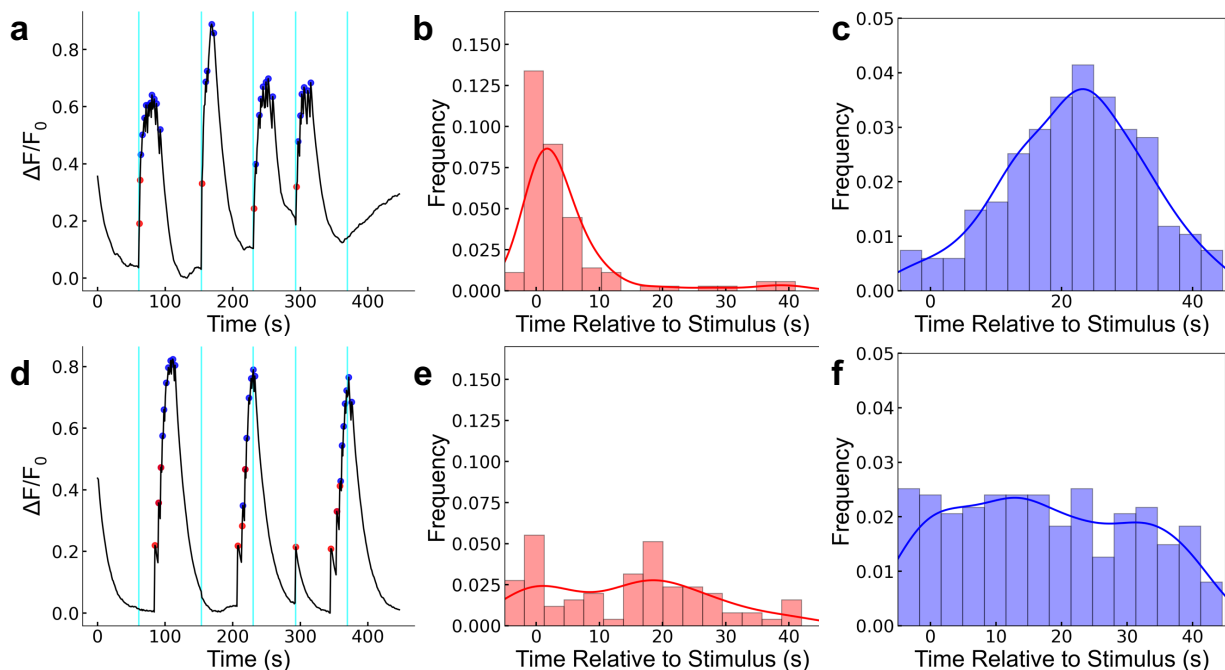
Supplementary Fig. 27. A second non-rotating molecule that absorbs visible light is inactive *in vitro*, but toxic to *Hydra*.

(a) Structure of **BL-315**. **(b)** UV-Vis absorption spectrum at concentrations of 8, 16, 24, and 32 μM . **(c)** Normalized fluorescence intensity traces of HEK293 cells treated with **BL-315** (8 μM) and Fluo-4 (2 μM) and stimulated with 400 nm laser light. **(d)** Images of *Hydra* treated with MM 1 (left) and **BL-315** (right) for 24 h at 24 μM , showing the disintegration of *Hydra* treated with **BL-315**.



Supplementary Fig. 28. A third non-rotating control present as a 10:1 mixture with MM 1 is relatively inactive *in vitro* but more active than a 1:10 dilution of MM 1 *in vivo*.

(a) Structure of **BL-314**. **(b)** UV-Vis absorption spectrum at concentrations of 8, 16, 24, and 32 μM . **(c)** Normalized fluorescence intensity traces of HEK293 cells treated with **BL-314** (8 μM) and Fluo-4 (2 μM) and stimulated with 400 nm laser light compared to MM 1 control (8 μM). **(d)** Normalized fluorescence intensity traces of GCaMP7b-expressing *Hydra vulgaris* treated with **BL-314** (24 μM) and stimulated according to Protocol I. The solid line represents the average, and the shaded region represents the standard error of $n = 6$ experiments. **(e)** Normalized fluorescence intensity traces of GCaMP7b-expressing *Hydra vulgaris* treated with MM 1 (2.4 μM) and stimulated according to Protocol I.



Supplementary Fig. 29. Peak analysis of MM 1-treated *Hydra* (rotation rate of 3 MHz).

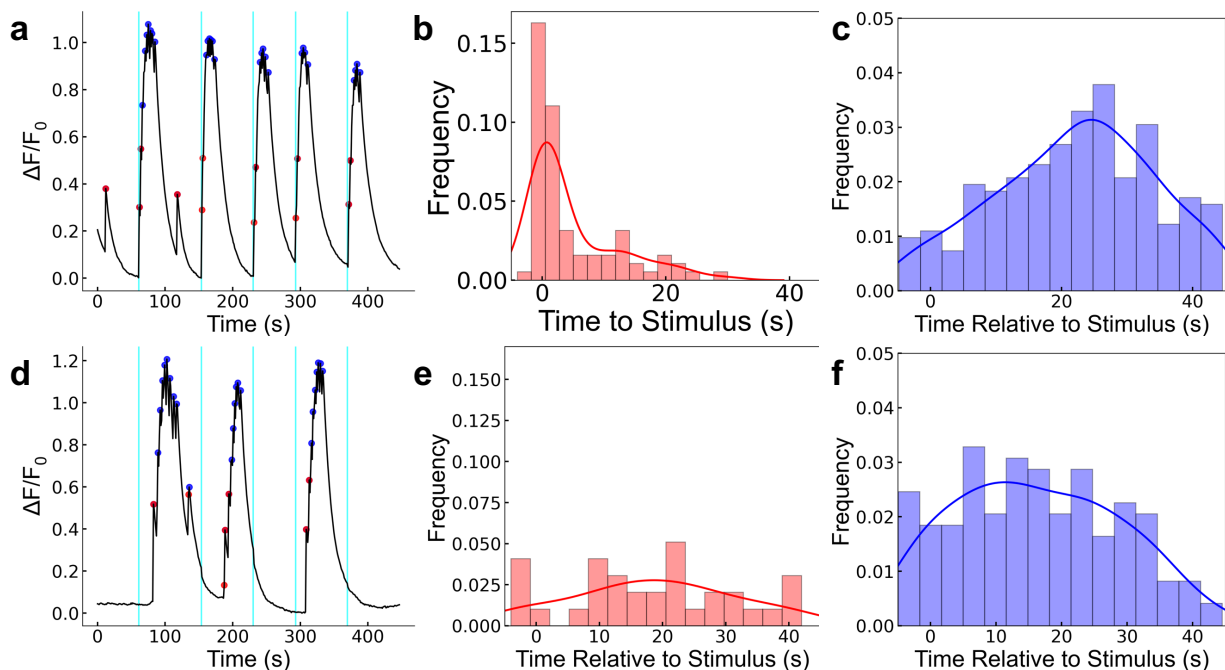
(a) Characteristic GCaMP7b fluorescence trace of *Hydra* treated with MM 1 + light (Protocol II).

The contractions (peaks) most often occur in concert with stimuli (cyan lines). **(b)** Temporal location of contraction onset relative to presentation of light stimulus. **(c)** Temporal location of

contraction burst peaks relative to presentation of light stimulus. **(d)** Characteristic GCaMP7b fluorescence trace of *Hydra* treated with MM 1 and sham light stimulus (same protocol with 0% laser duty cycle). Contractions do not occur in concert with sham stimulus. **(e)** Temporal location

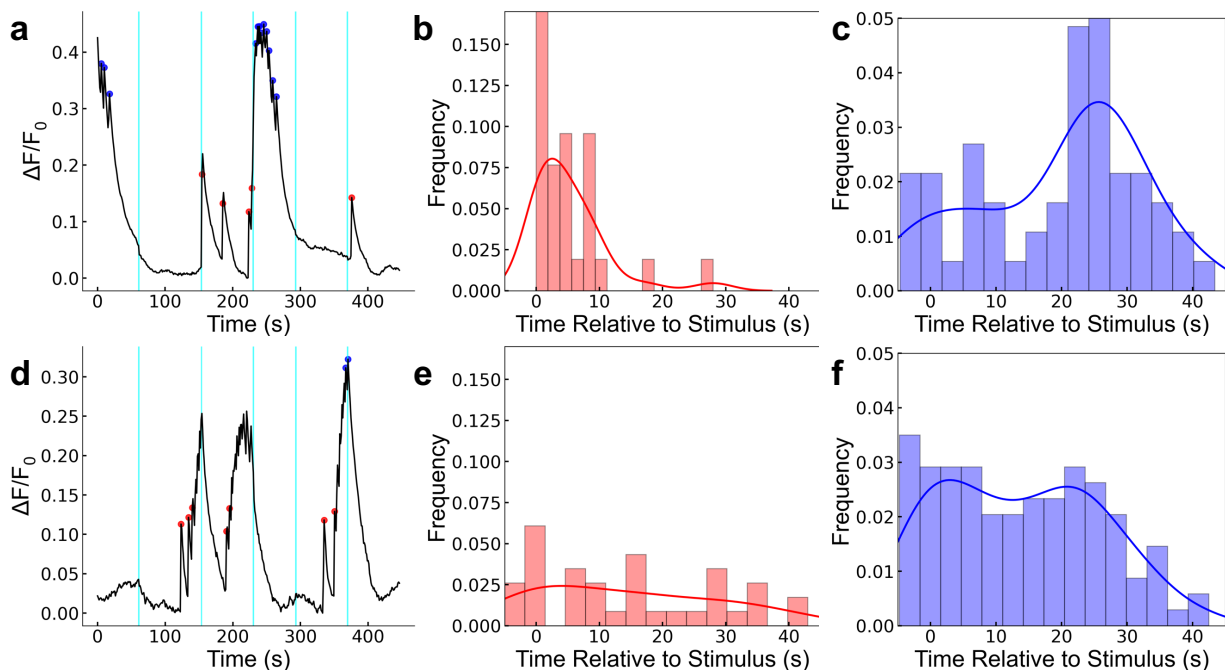
of contraction onset peaks relative to presentation of sham light stimulus. **(f)** Temporal location of contraction burst peaks relative to presentation of sham light stimulus. Histograms were calculated

across all collected data (at least 50 stimulation or sham stimulation attempts in at least 5 *Hydra*).



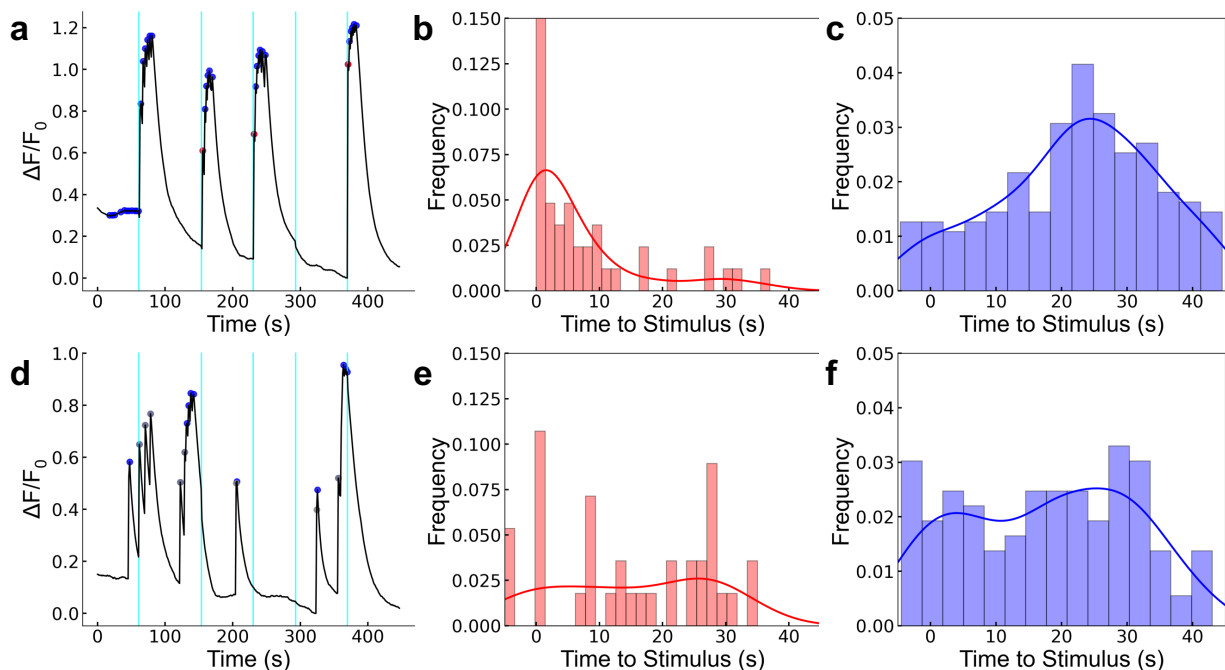
Supplementary Fig. 30. Peak analysis of MM 2-treated *Hydra* (fastest MM; rotation rate of 43 MHz).

(a) Characteristic GCaMP7b fluorescence trace of *Hydra* treated with MM 2 + light (Protocol II). The contractions (peaks) occur in concert with stimuli (cyan lines). **(b)** Temporal location of contraction onset relative to presentation of light stimulus. **(c)** Temporal location of contraction burst peaks relative to presentation of light stimulus. **(d)** Characteristic GCaMP7b fluorescence trace of *Hydra* treated with MM 2 and sham light stimulus (same protocol with 0% laser duty cycle). Contractions do not occur in concert with sham stimulus. **(e)** Temporal location of contraction onset peaks relative to presentation of sham light stimulus. **(f)** Temporal location of contraction burst peaks relative to presentation of sham light stimulus. Histograms were calculated across all collected data (at least 50 stimulation or sham stimulation attempts in at least 5 *Hydra*).



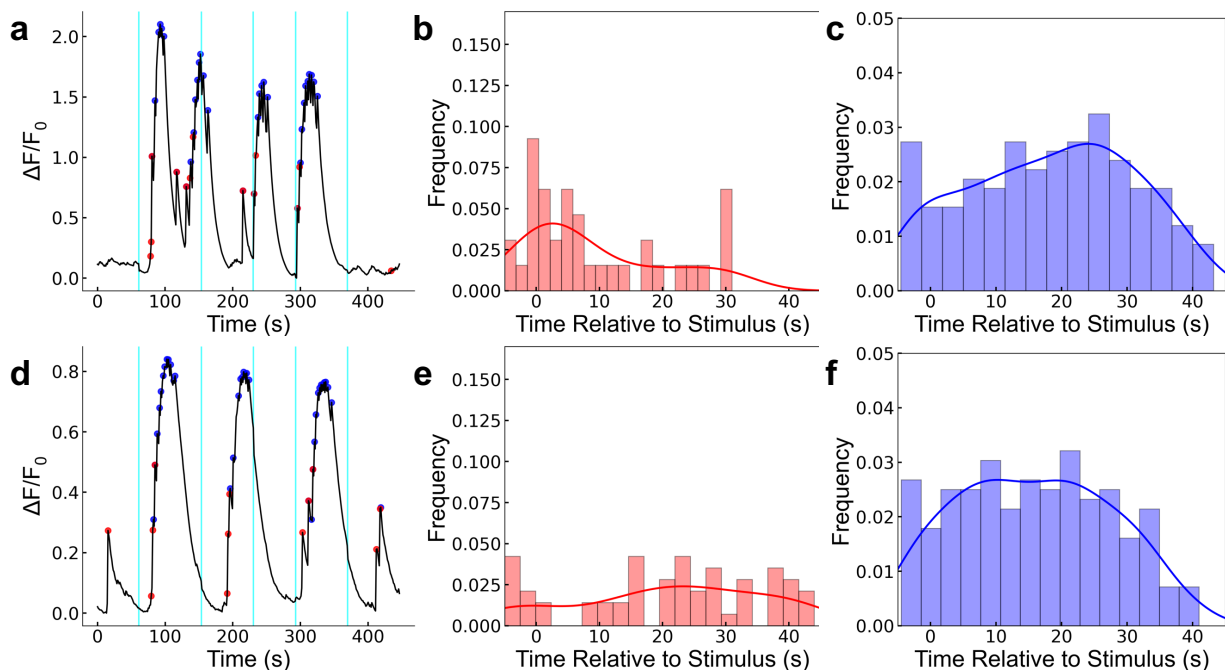
Supplementary Fig. 31. Peak analysis of MM 3-treated *Hydra* (slow MM; rotation rate of 10^{-1} Hz).

(a) Characteristic GCaMP7b fluorescence trace of *Hydra* treated with MM 3 + light (Protocol II). The contractions (peaks) occasionally follow stimuli (cyan lines). **(b)** Temporal location of contraction onset relative to presentation of light stimulus. **(c)** Temporal location of contraction burst peaks relative to presentation of light stimulus. **(d)** Characteristic GCaMP7b fluorescence trace of *Hydra* treated with MM 3 and sham light stimulus (same protocol with 0% laser duty cycle). Contractions do not occur in concert with sham stimulus. **(e)** Temporal location of contraction onset peaks relative to presentation of sham light stimulus. **(f)** Temporal location of contraction burst peaks relative to presentation of sham light stimulus. Histograms were calculated across all collected data (at least 50 stimulation or sham stimulation attempts in at least 5 *Hydra*).



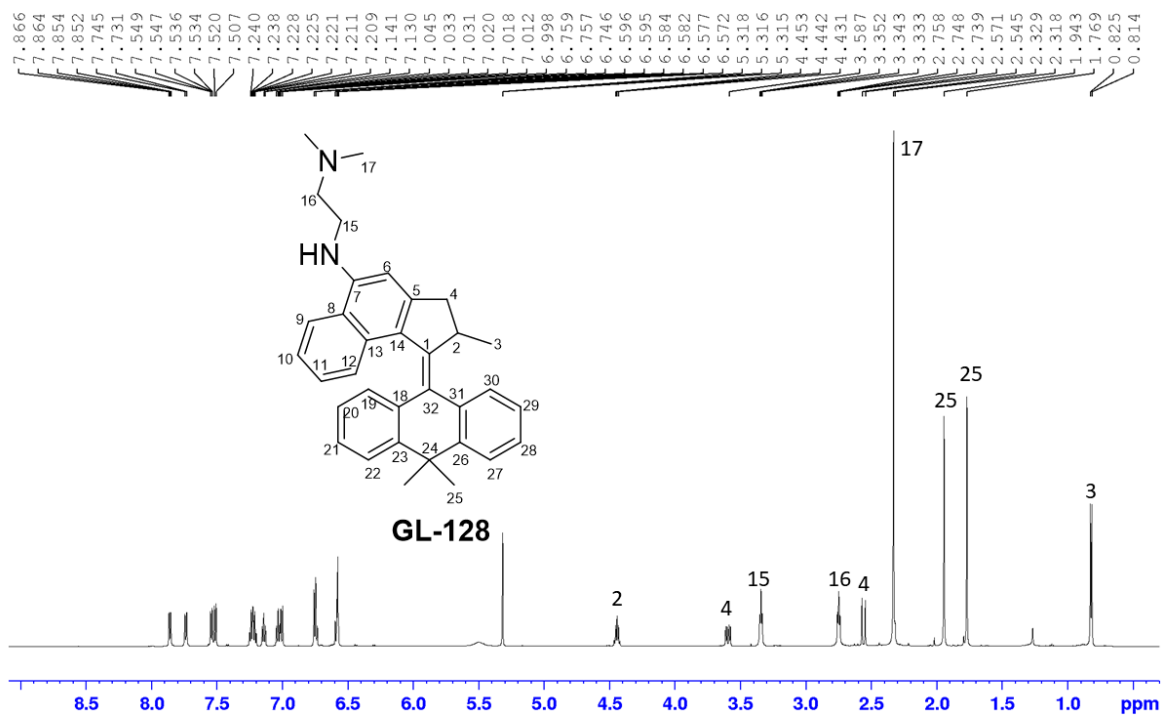
Supplementary Fig. 32. Peak analysis of MM 4-treated *Hydra* (non-unidirectional MM; rotation rate of 3 MHz).

(a) Characteristic GCaMP7b fluorescence trace of *Hydra* treated with MM 4 + light (Protocol II). The contractions (peaks) most often occur in concert with stimuli (cyan lines). **(b)** Temporal location of contraction onset relative to presentation of light stimulus. **(c)** Temporal location of contraction burst peaks relative to presentation of light stimulus. **(d)** Characteristic GCaMP7b fluorescence trace of *Hydra* treated with MM 4 and sham light stimulus (same protocol with 0% laser duty cycle). **(e)** Temporal location of contraction onset peaks relative to presentation of sham light stimulus. Contractions do not occur in concert with sham stimulus. **(f)** Temporal location of contraction burst peaks relative to presentation of sham light stimulus. Histograms were calculated across all collected data (at least 50 stimulation or sham stimulation attempts in at least 5 *Hydra*).

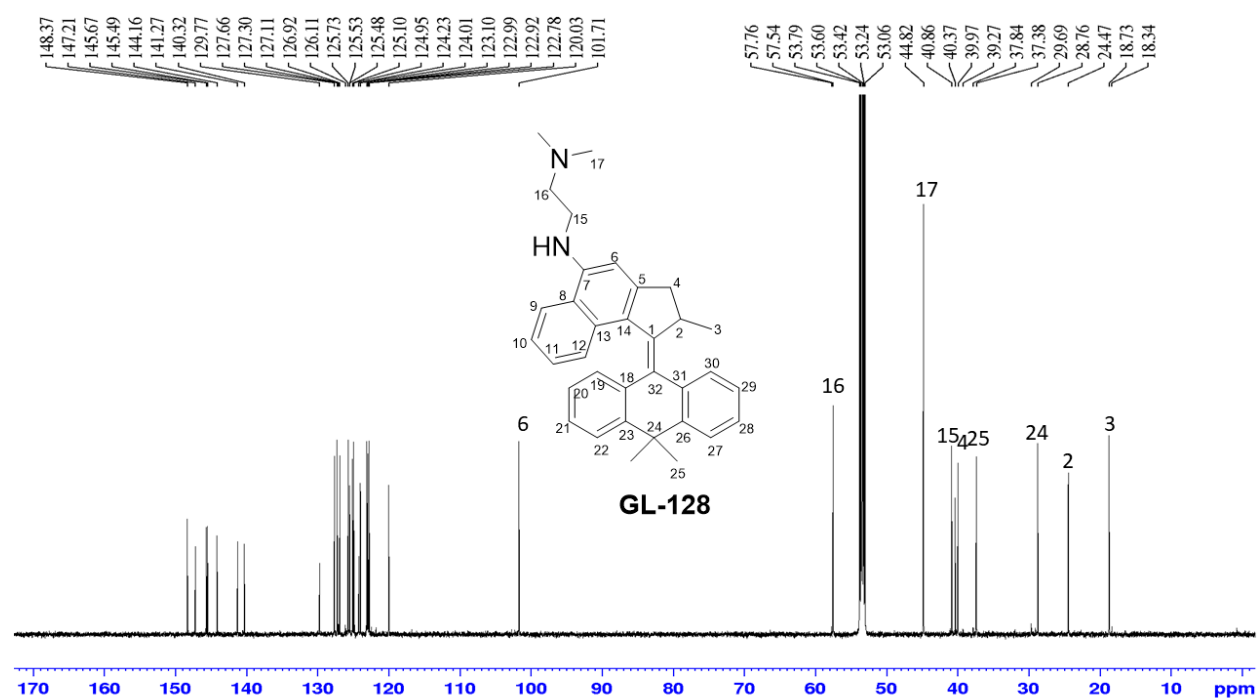


Supplementary Fig. 33. Peak analysis of DMSO-treated control *Hydra*.

(a) Characteristic GCaMP7b fluorescence trace of *Hydra* treated with DMSO (0.3% v/v) + light (Protocol **II**). The contractions (peaks) occasionally follow stimuli (cyan lines) due to the photosensitivity of the oral region. **(b)** Temporal location of contraction onset relative to presentation of light stimulus. **(c)** Temporal location of contraction burst peaks relative to presentation of light stimulus. **(d)** Characteristic GCaMP7b fluorescence trace of *Hydra* treated with DMSO and sham light stimulus (same protocol with 0% laser duty cycle). Contractions do not occur in concert with sham stimulus. **(e)** Temporal location of contraction onset peaks relative to presentation of sham light stimulus. **(f)** Temporal location of contraction burst peaks relative to presentation of sham light stimulus. Histograms were calculated across all collected data (at least 50 stimulation or sham stimulation attempts in at least 5 *Hydra*).



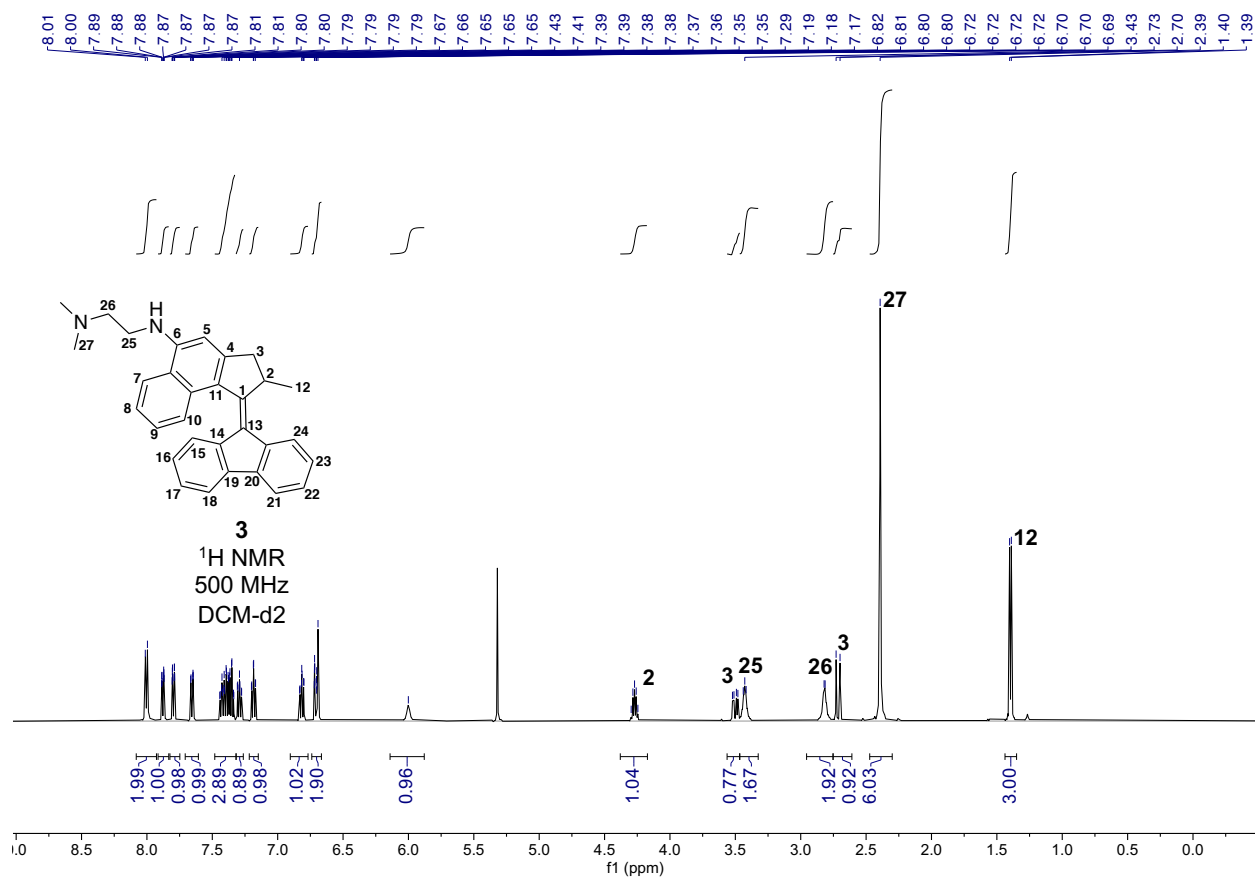
Supplementary Fig. 34. ¹H NMR spectrum of GL-128 (MM 2).



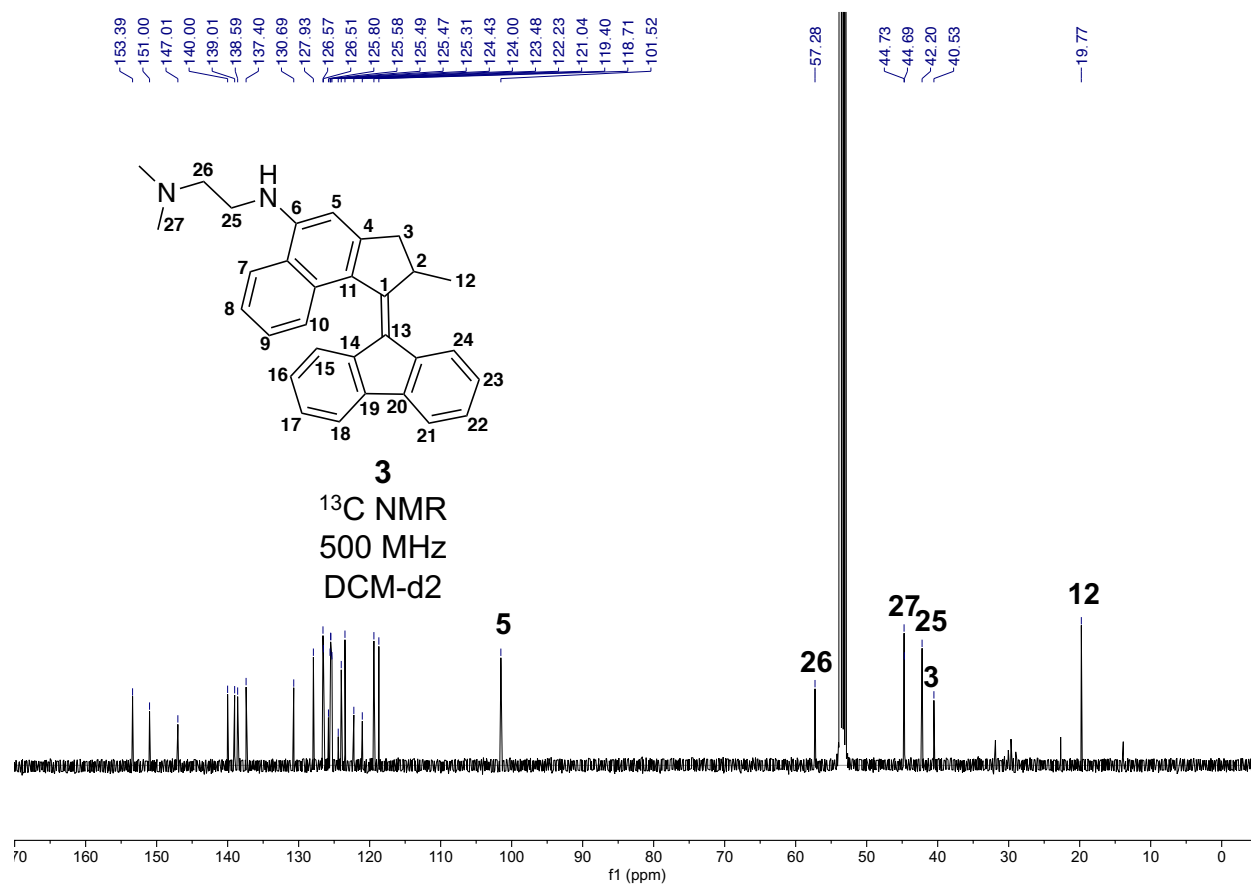
Supplementary Fig. 35. ^{13}C NMR of GL-128 (MM 2).



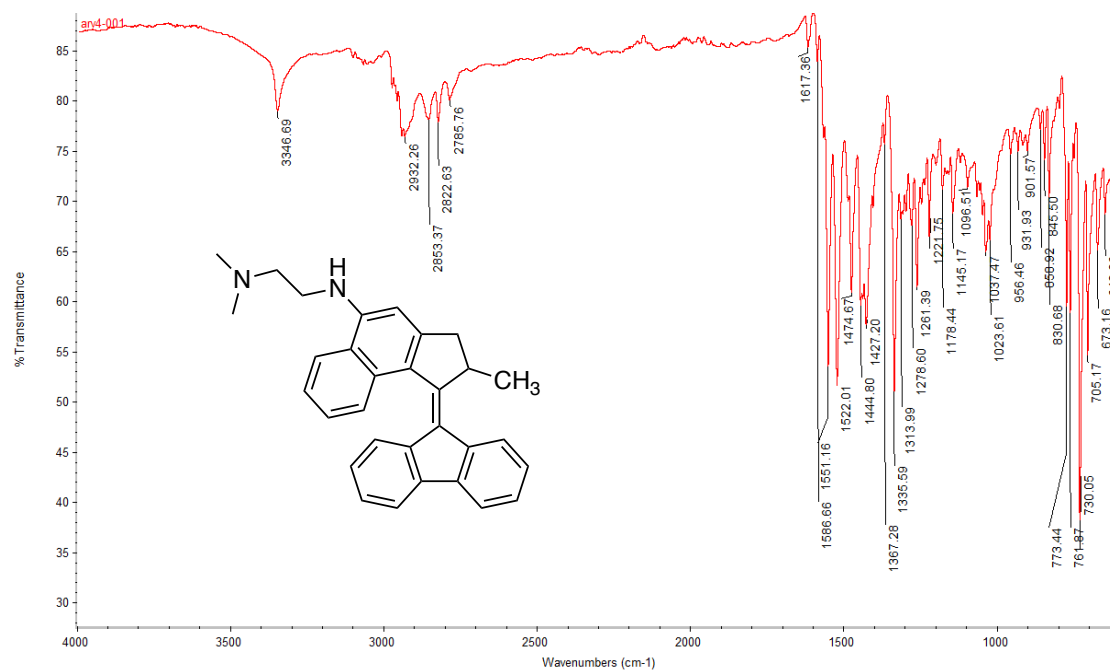
Supplementary Fig. 36. FTIR of GL-128 (MM 2).



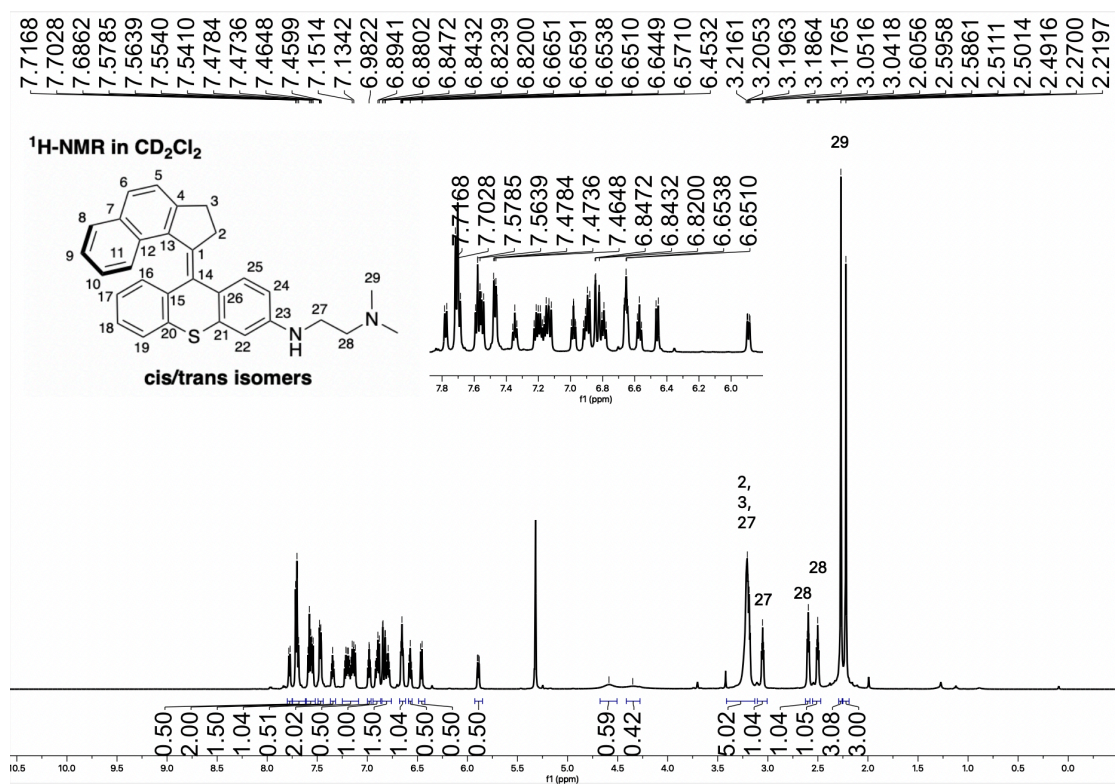
Supplementary Fig. 37. ¹H-NMR spectrum of MM 3 (ARV-1) in CD₂Cl₂



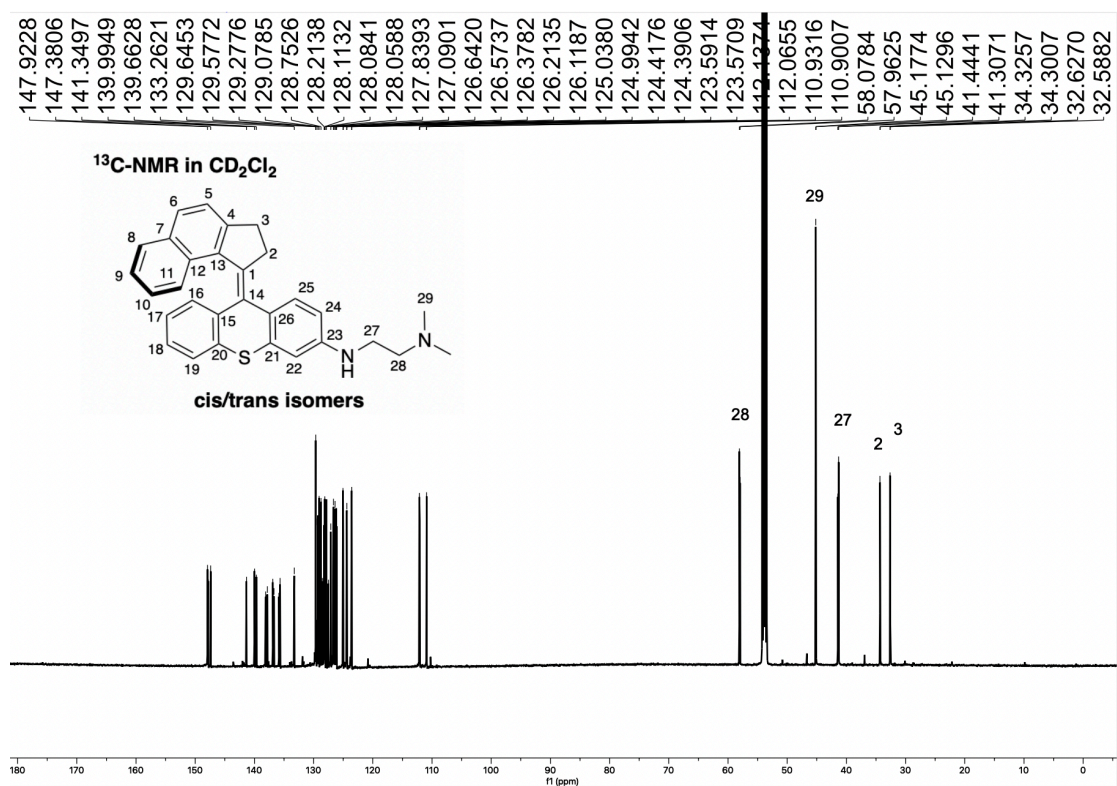
Supplementary Fig. 38. ¹³C-NMR spectrum of MM 3 (ARV-1) in CD₂Cl₂



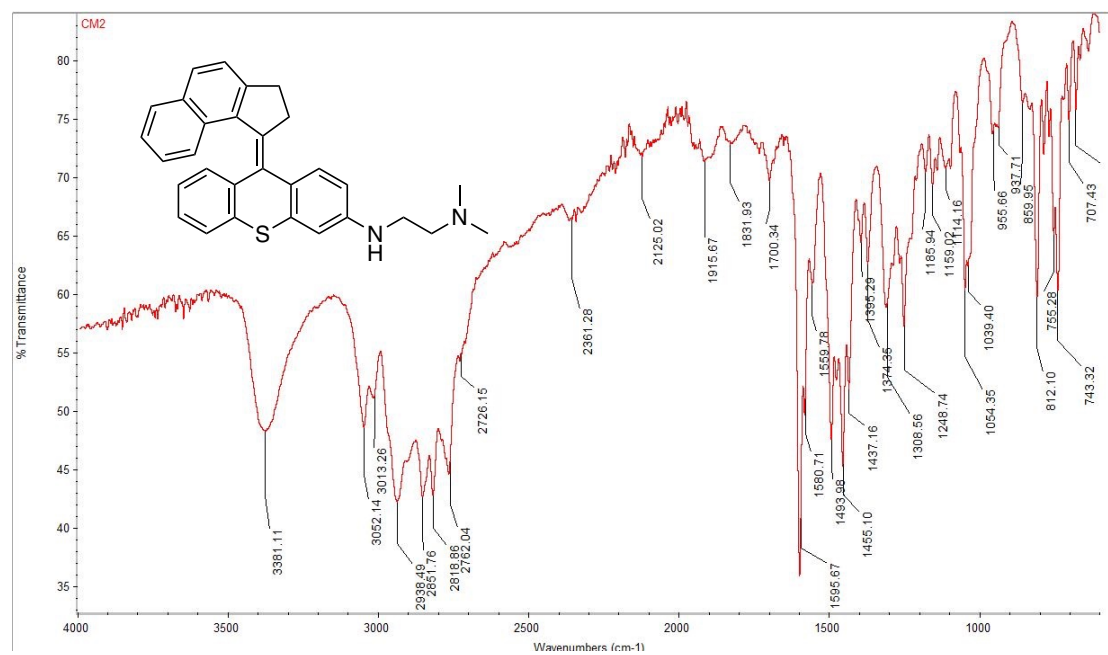
Supplementary Fig. 39. FTIR of ARV4-001 (MM 3).



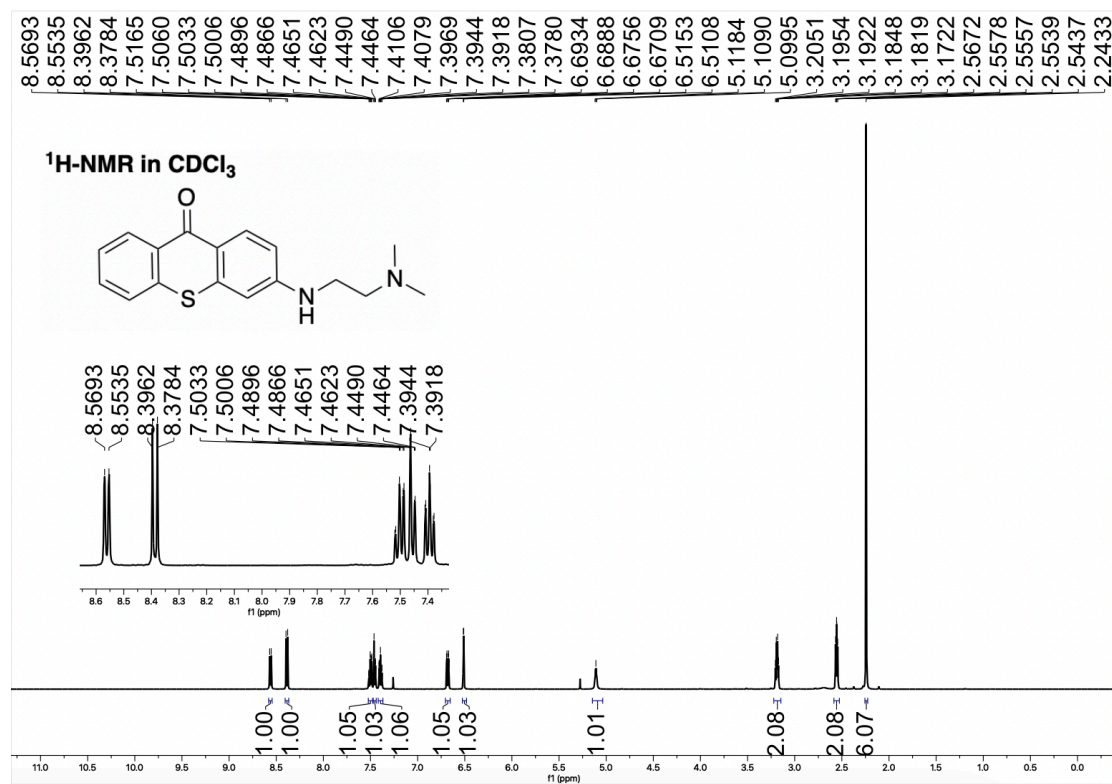
Supplementary Fig. 40. ¹H-NMR spectrum of MM 4 (BL-167) in CD₂Cl₂



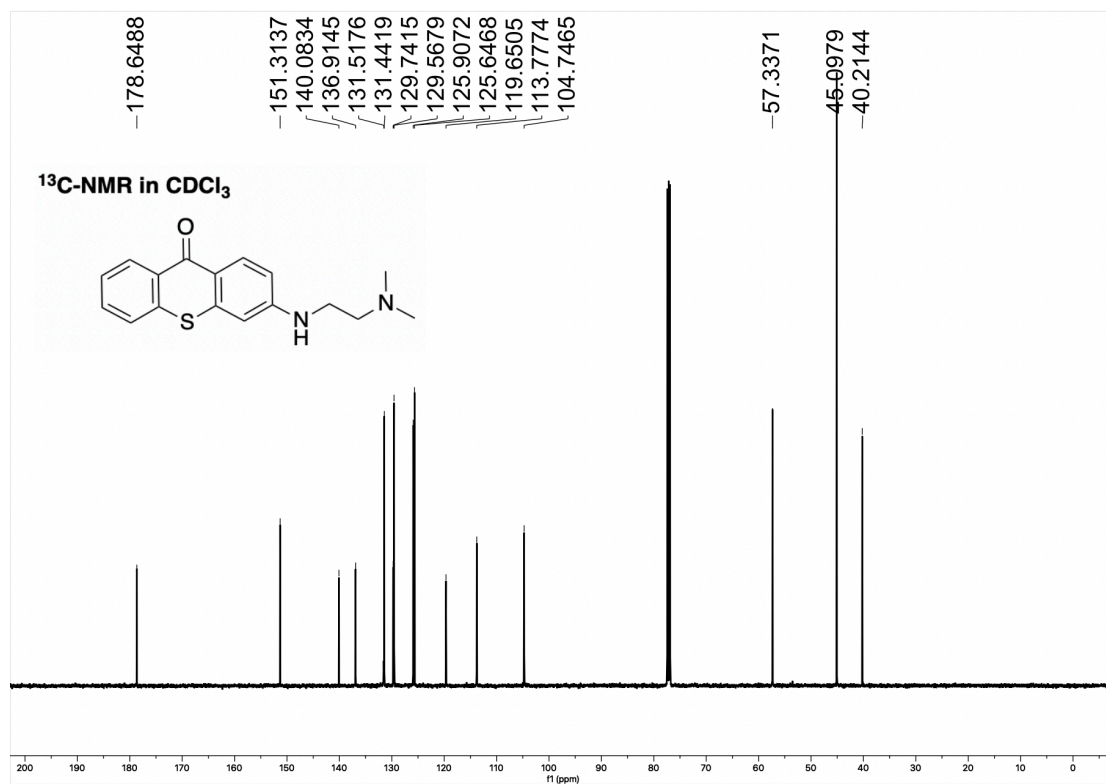
Supplementary Fig. 41. ¹³C-NMR spectrum of MM 4 (BL-167) in CD₂Cl₂



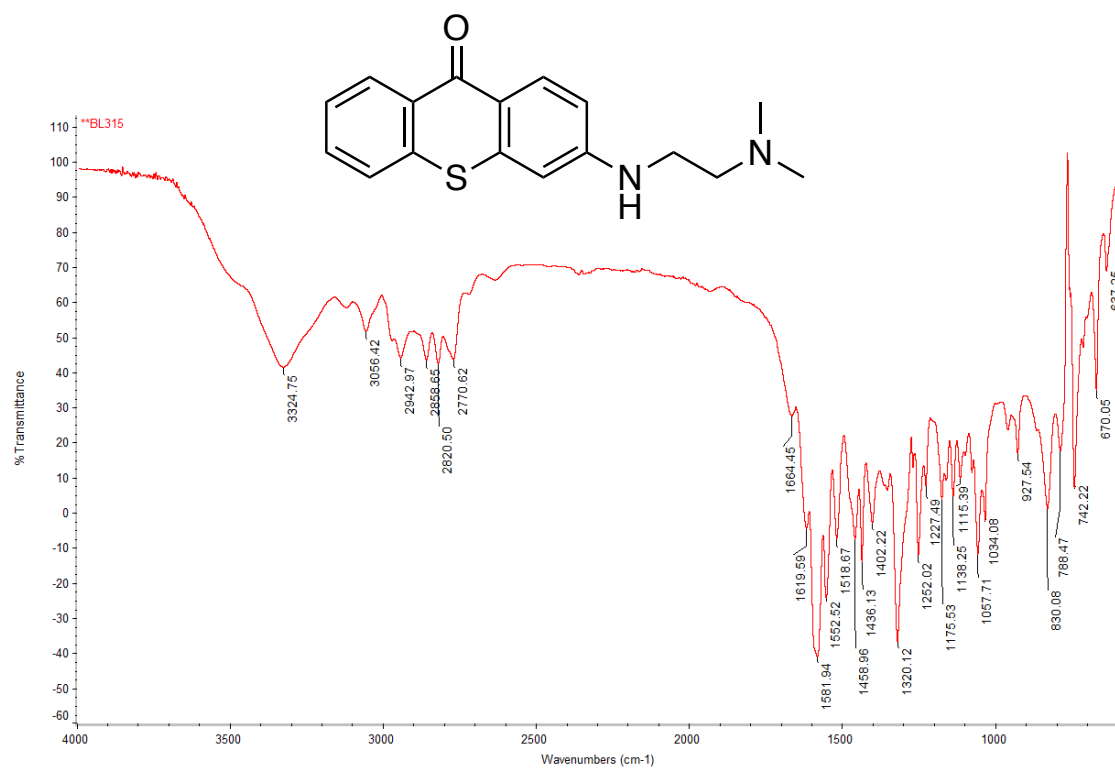
Supplementary Fig. 42. FTIR of MM 4 (BL-167)



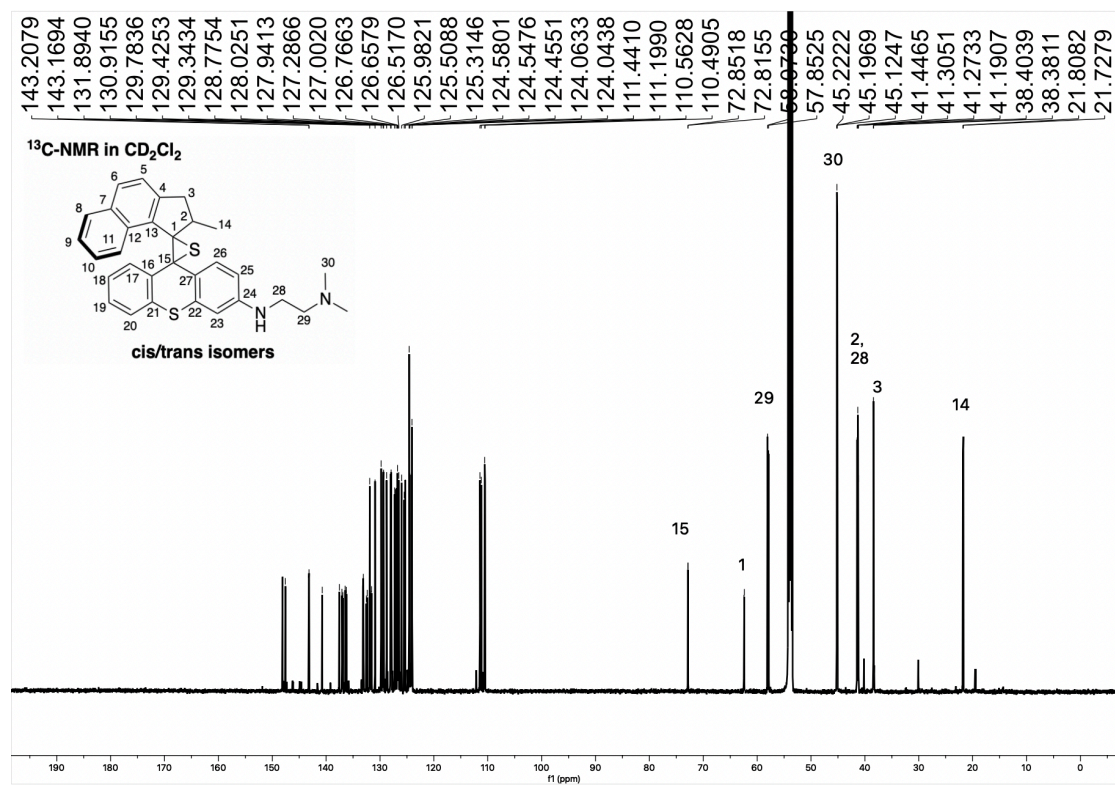
Supplementary Fig. 43. ¹H-NMR spectrum of **BL-315** in CDCl₃



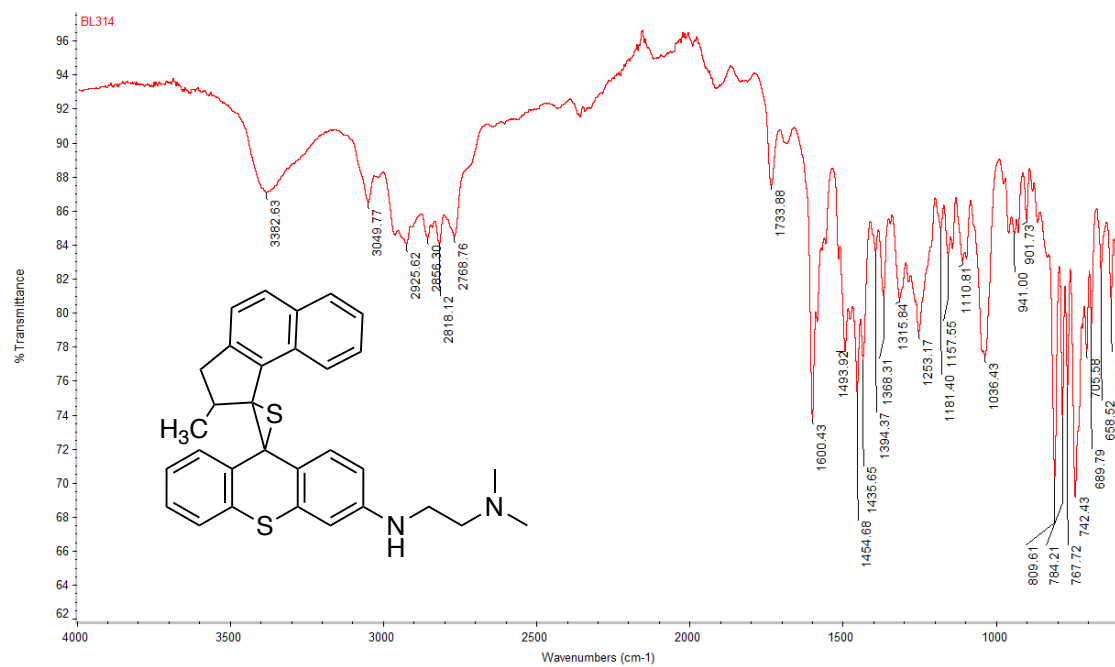
Supplementary Fig. 44. ¹³C-NMR spectrum of BL-315 in CDCl₃



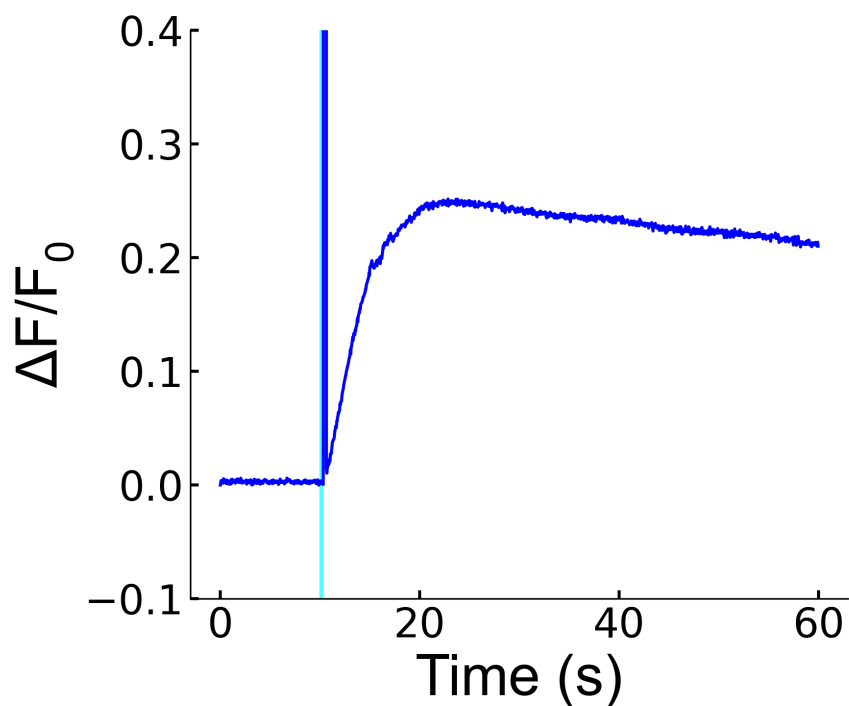
Supplementary Fig. 45. FTIR of BL-315



Supplementary Fig. 47. ¹³C-NMR spectrum of BL-314 in CD₂Cl₂



Supplementary Fig. 48. FTIR of BL-314



Supplementary Fig. 49. ICW from a non-scanning laser.

Normalized fluorescent intensity trace of a HEK293 cell treated with MM **1** as described in the Methods section and excited by a Coherent Chameleon Discovery femtosecond laser operating at $\sim 1.5 \times 10^2 \text{ W cm}^{-2}$ at 405 nm and imaged at 20 fps. The cyan line indicates the time of stimulation. The sharp vertical line likely reflects a fluorescence resonant energy transfer effect between the MM and calcium imaging dye (Fluo-4) during laser excitation, not visible in other experiments because images were not collected during stimulation.

Supplementary Table 1. DFT Simulations.

Energies of the intrinsic reaction coordinate trajectory for each motor calculated using density functional theory. Metastable, stable, and transition conformations are labeled as A, B, and TS,

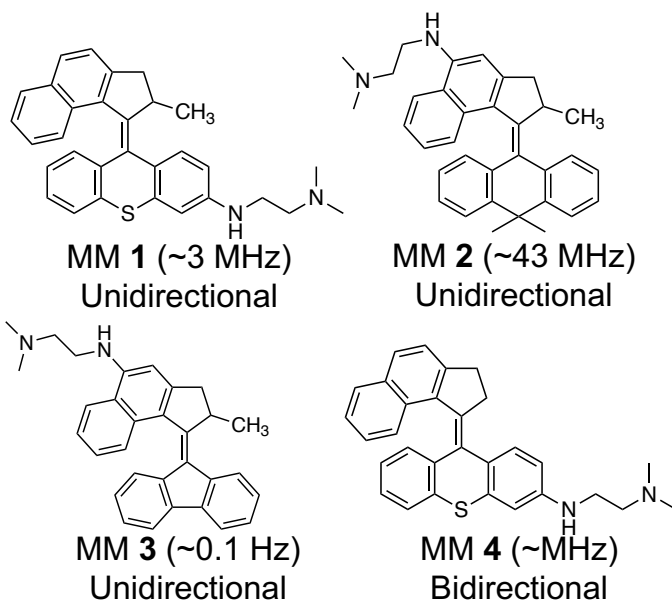
	Hartrees (absolute)	Hartrees (relative to metastable state)	Type of Information
1			
1A	-1438.263904	0	Metastable
1TS	-1438.247104	0.0168	ΔG Activation
1B	-1438.275681	-0.011777	Driving Energy
2			
2A	-1157.977869	0	Metastable
2TS	-1157.95888	0.018989	ΔG Activation
2B	-1157.990884	-0.013015	Driving Energy
3			
3A	-1040.181809	0	Metastable
3TS	-1040.138688	0.043121	ΔG Activation
3B	-1040.185939	-0.00413	Driving Energy

4	THI		
4A	-1398.991162	0	Metastable
4TS1	-1398.984718	0.006444	ΔG Activation
4B	-1399.001979	-0.010817	Driving Energy
	NR		
4A	-1398.991162	0	Metastable
4TS2	-1398.987367	0.003795	ΔG Activation
4B	-1399.001979	-0.010817	Driving Energy

Supplementary Table 2. Properties of MM 1-4.

Partition coefficients and total polar surface area were calculated using the molInspiration online calculator. milogP represents the logarithm of the partition coefficient between octanol and water. Rotation rates were provided using the thermodynamics of the thermal helix inversion step. MM structures provided for reference.

Molecule	Rotation Rate (Hz)	milogP (a.u.)	Total Polar Surface Area (Å ²)	Extinction Coeff @ 400 nm (L mol ⁻¹ cm ⁻¹)
MM 1	3×10^6	7.90	15.27	~11,000
MM 2	43×10^6	7.34	15.27	~17,500
MM 3	0.1	7.31	15.27	~7,500
MM 4	3×10^6	7.90	15.27	~13,000



Supplementary Table 3. Pharmacological blockers and treatments employed.

Treatment	Concentration	Process Affected	Target	Process Notes
Gd³⁺	50 μ M	Extracellular calcium entry	Mechanosensitive Piezo1, Piezo2, TRPC4 ³⁰	Dissolved in iECB
Ruthenium Red (RR)	10 μ M	Extracellular calcium entry	Thermosensitive TRPV1-4 ²⁹	Dissolved in iECB
PBS	N/A	Extracellular calcium entry	Extracellular calcium	Replaced iECB
Thapsigargin (Th)	1 μ M	Intracellular calcium release	Sarco/endoplasmic reticulum pump (SERCA) ³¹	Administered in complete growth medium for 1 h prior to exp.
Ryanodine (Ry)	100 μ M	Intracellular calcium release	Ryanodine receptors (RyR) ³²	Administered in iECB
Xestospongin C (XeC)	20 μ M	Intracellular calcium release	IP3 receptors (IP3R) ³³	Administered in iECB
U-73122	10 μ M	Intracellular calcium release	Phospholipase C (PLC) ⁶⁸	Administered in iECB
Cytochalasin D	2 μ M	F-actin polymerization	Disrupts PLC- >IP ₃ R spacing and communication ³⁴	Administered in complete growth

				medium for 2 h prior to exp.
Gallein	100 μ M	GPCR signaling	GPCR β subunits ⁶⁸	Administered in iECB

Supplementary Table 4. *Hydra* dataset characteristics.

Group	Total Traces	Total <i>Hydra</i> Employed	Total Stim. Attempts	Total Successes*	Total Null**	Success Rate***
MM 1 + light	17	10	85	48	14	68%
MM 1	15	5	75	13	28	29%
MM 2 + light	17	10	85	60	16	87%
MM 2	13	6	65	8	25	20%
MM 3 + light	10	5	50	18	2	38%
MM 3	13	8	65	12	17	12%
MM 4 + light	12	6	60	36	5	65%
MM 4	8	5	45	6	15	20%
DMSO + light	12	7	60	20	15	44%
DMSO	15	6	75	13	23	21%

***Successes** were defined as presentations of stimulus that were paired with a contraction and whole-body calcium spike at most three frames (~3 s) after the stimulus was presented. Contractions were identified using a threshold for the change in body GCaMP7b fluorescence ($\Delta F/F_0 > 1.5\times$ that of the previous data point).

****Null** stimulation attempts were attempts when the stimulus was presented during a period in which the *Hydra* was already contracting ($\Delta F/F_0 > 0.3$).

*****Success rate** was defined as the proportion of stimulation attempts which resulted in a “success”, with null attempts discarded.

For more information see *Methods*.

Supplementary Movie 1.

Animation of the intrinsic reaction coordinate trajectory for the rate-limiting thermal helix inversion step in MM 1.

Supplementary Movie 2.

Animation of the intrinsic reaction coordinate trajectory for the rate-limiting thermal helix inversion step in MM 2.

Supplementary Movie 3.

Animation of the intrinsic reaction coordinate trajectory for the rate-limiting thermal helix inversion step in MM 3.

Supplementary Movie 4.

Animation of the intrinsic reaction coordinate trajectory for the thermal helix inversion step in MM 4.

Supplementary Movie 5.

Animation of the intrinsic reaction coordinate trajectory for an alternative relaxation pathway in MM 4.

Supplementary Movie 6.

Timelapse of Fluo-4 fluorescence in cardiomyocytes treated with MM **1** (8 μM) and Fluo-4 and stimulated with 400 nm laser light for 250 ms at an irradiance of $5.1 \times 10^2 \text{ W cm}^{-2}$. The cell on the left is stimulated at 30 s.

Supplementary Movie 7.

Timelapse of fluorescence images of a cardiomyocyte treated with MM **1** (8 μM) and cellLight Actin-GFP BacMam 2.0 and stimulated with 400 nm laser light for 250 ms at an irradiance of $5.1 \times 10^2 \text{ W cm}^{-2}$. Stimulation was applied at 30 s. The inward movement of the z-lines demonstrates localized contraction at the point of stimulation upon the release of calcium ions from the SR.

Supplementary Movie 8.

Timelapse of bright field images of cardiomyocytes treated with MM **1** (8 μM) and Fluo-4 and stimulated with 400 nm laser light for 250 ms at an irradiance of $5.1 \times 10^2 \text{ W cm}^{-2}$. The cell on the left is stimulated at 30 s.

Supplementary Movie 9.

Timelapse of fluorescence images of a cardiomyocyte treated with MM **1** (8 μM) and di-8-Anneps ($1 \mu\text{g mL}^{-1}$) and stimulated with 400 nm laser light for 250 ms at an irradiance of $5.1 \times 10^2 \text{ W cm}^{-2}$. Stimulation was applied at 30 s. The increase in fluorescence of di-8-Anneps during contraction indicates a change in membrane voltage during an action potential.

Supplementary Movie 10.

A *Hydra* expressing GCaMP7b in epitheliomuscular cells treated with MM 1 (24 μ M) and stimulated with 405 nm laser light for 1 s at an irradiance of 9.0×10^2 W cm⁻² (Protocol I). Stimulation is applied at 10 s. The blue circle shown at 10 s corresponds to the area of stimulation. The trace shown at the bottom of the video corresponds to the intensity of GCaMP7b fluorescence in the area of stimulation across the timescale of the video. The cyan line in the trace represents the time of stimulus presentation, during which no data was collected.

Supplementary Movie 11.

A *Hydra* expressing GCaMP7b in epitheliomuscular cells treated with DMSO solvent solvent (0.3% v/v) and stimulated with 405 nm laser light for 1 s at an irradiance of 9.0×10^2 W cm⁻² (Protocol I). Stimulation is applied at 10 s. The blue circle shown at 10 s corresponds to the area of stimulation. The trace shown at the bottom corresponds to the intensity of GCaMP7b fluorescence in the area of stimulation across the timescale of the video. The cyan line in the trace represents the time of stimulus presentation, during which no data was collected.

Supplementary Movie 12.

A *Hydra* expressing GCaMP7b in epitheliomuscular cells treated with MM 2 (24 μ M) and stimulated with 405 nm laser light for 1 s at an irradiance of 6.8×10^2 W cm⁻² (Protocol I). Stimulation is applied at 10 s at two distinct locations in the *Hydra* indicated by the yellow and blue circles that appear during stimulation. The traces shown at the bottom of the video correspond

to the intensity of GCaMP7b fluorescence in the areas of stimulation across the timescale of the video.

Supplementary Movie 13.

A *Hydra* expressing GCaMP7b in epitheliomuscular cells treated with MM **2** (24 μ M) and stimulated with 405 nm laser light for 2 s at an irradiance of 9.0×10^2 W cm⁻². Light was delivered to the oral region of the *Hydra* (Protocol **II**) at irregular intervals. The yellow circle around the oral region indicates the region to which stimulation was applied. The trace shown at the bottom of the video corresponds to the intensity of GCaMP7b fluorescence in the whole *Hydra* across the timescale of the video. The vertical yellow lines in the trace mark times during which stimulus was presented, during which no data was collected. Contractile and electrophysiological responses were observed upon each presentation of stimulus.

Supplementary Movie 14.

A *Hydra* expressing GCaMP7b in epitheliomuscular cells in the absence of MM or light stimulation. The trace shown at the bottom of the video corresponds to the intensity of GCaMP7b fluorescence in the whole *Hydra* across the timescale of the video.

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