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Supporting Information

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The Binding of Fluorophores to Proteins Depends on the Cellular Environment**

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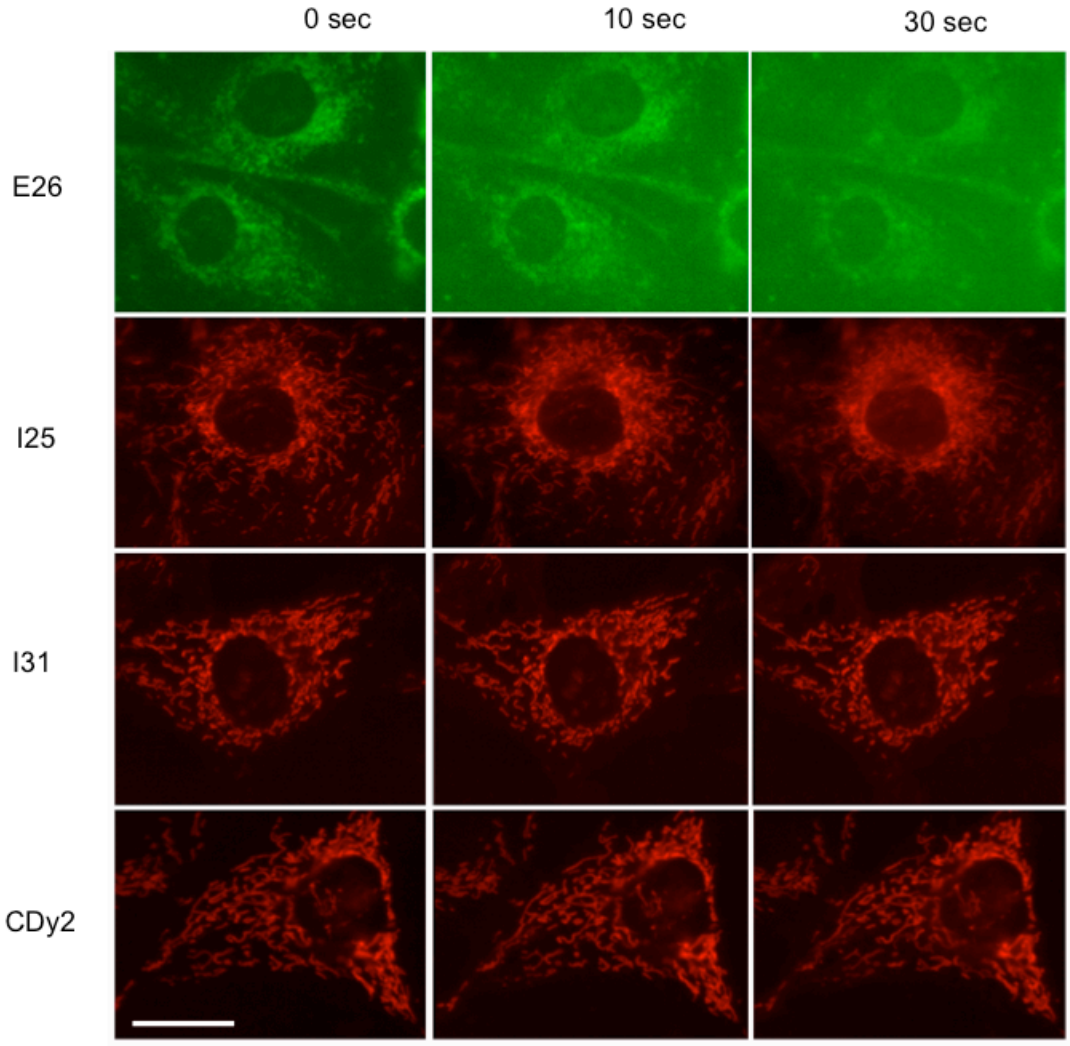
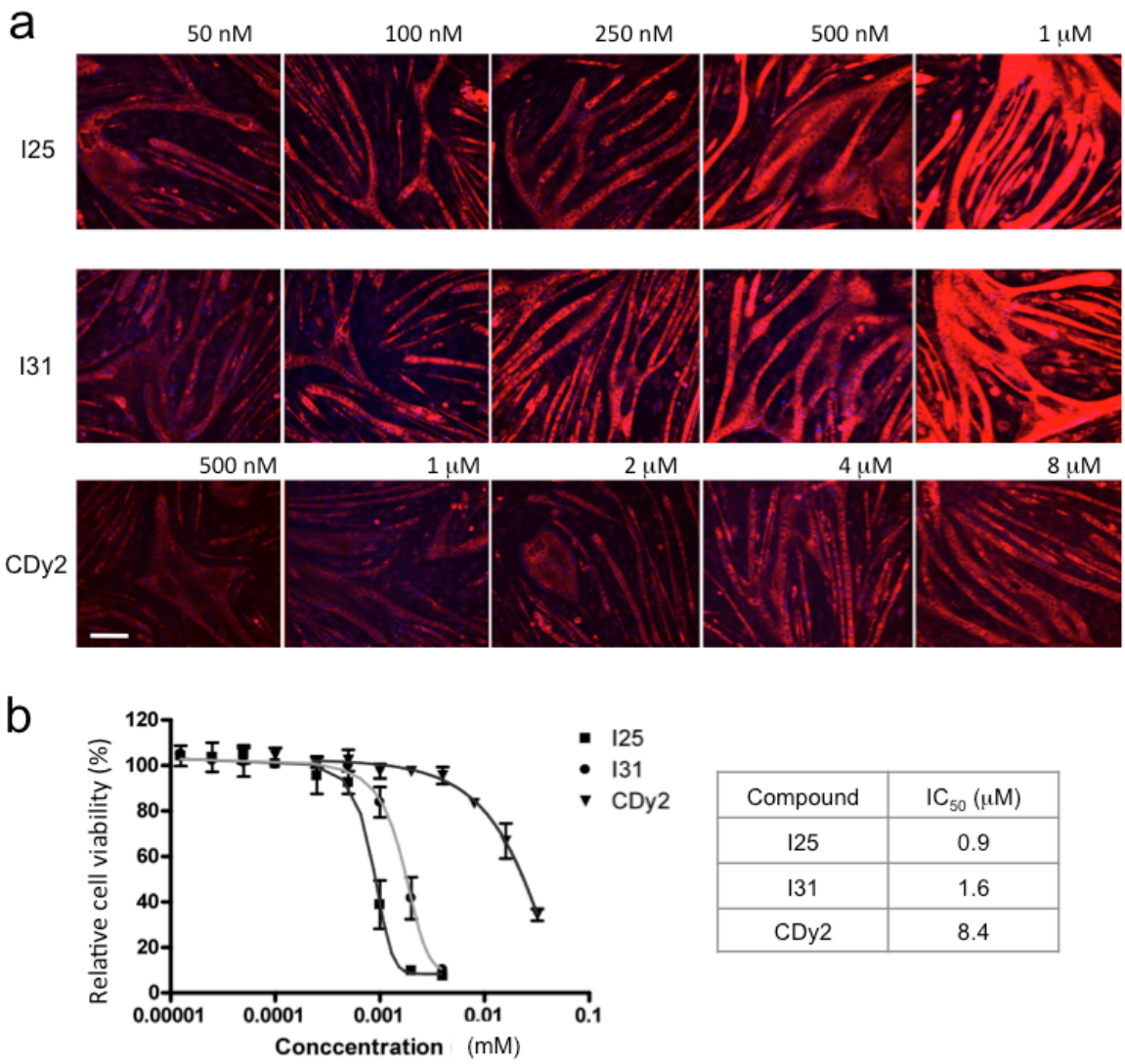
Supplementary Information

Contents



Supplementary Fig. 1. Photo-stability and mitochondria localization.

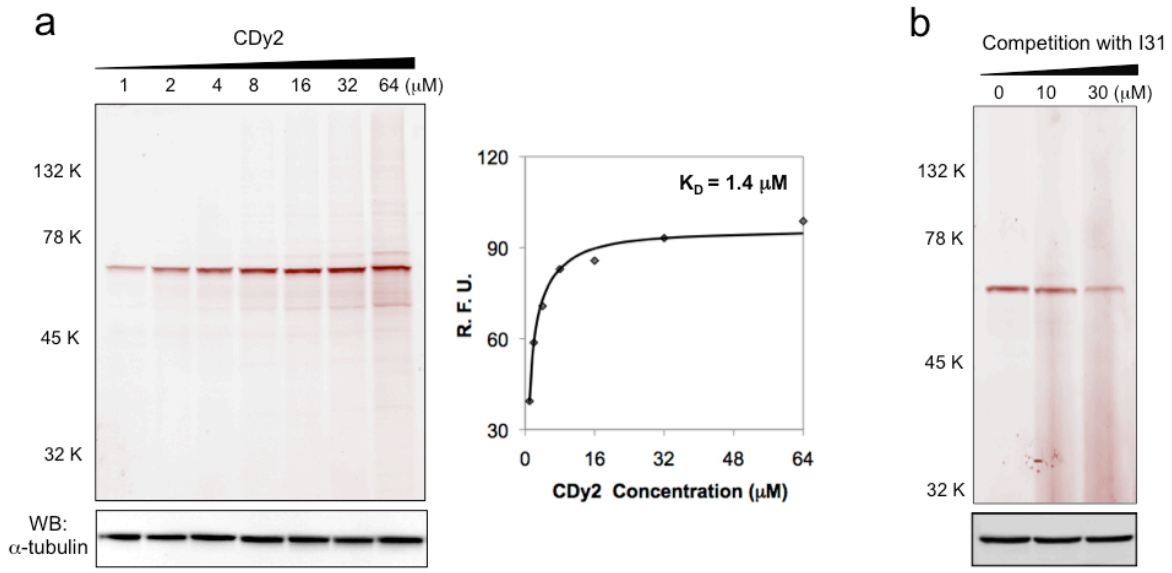
Myoblasts were incubated with the active compounds (500 nM) for 30 min. Then, cells were continuously exposed to light sources for 30 seconds to investigate photo-stability of the compounds (E26; λ_{ex} = 480/40 nm and I25, I30, and CDy2; λ_{ex} = 545/30 nm). Images were taken every 5 seconds. While E26 showed significant photo-bleaching at 10 seconds, I31 and its derivative, CDy2 (compound of designation yellow 2), showed strong photo-stability even after 30 sec. Scale bar = 20 μm.



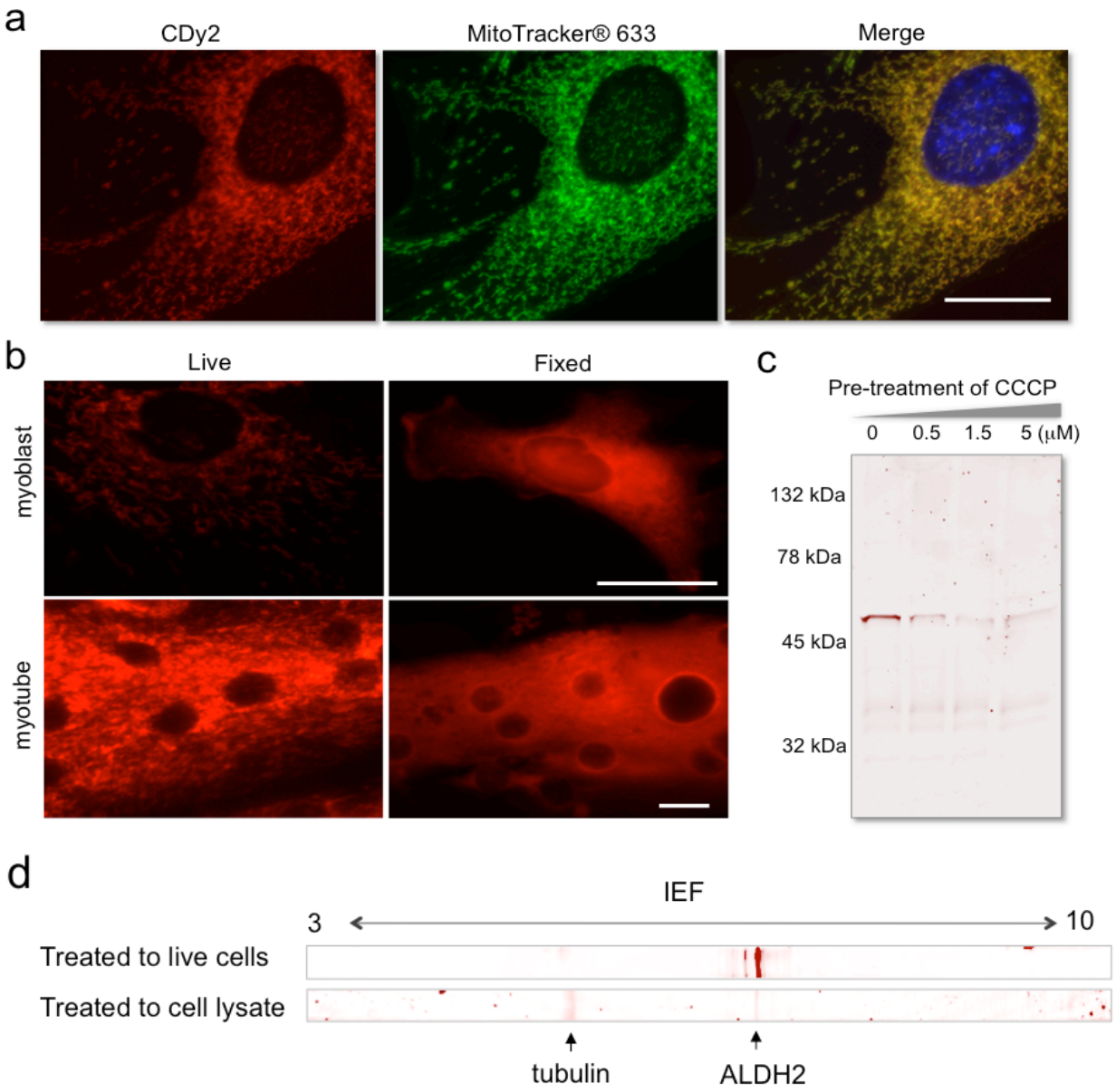
Compound	IC_{50} (μM)
I25	0.9
I31	1.6
CDy2	8.4

Supplementary Fig. 2. Dose-dependent myotube staining and MTS assay.

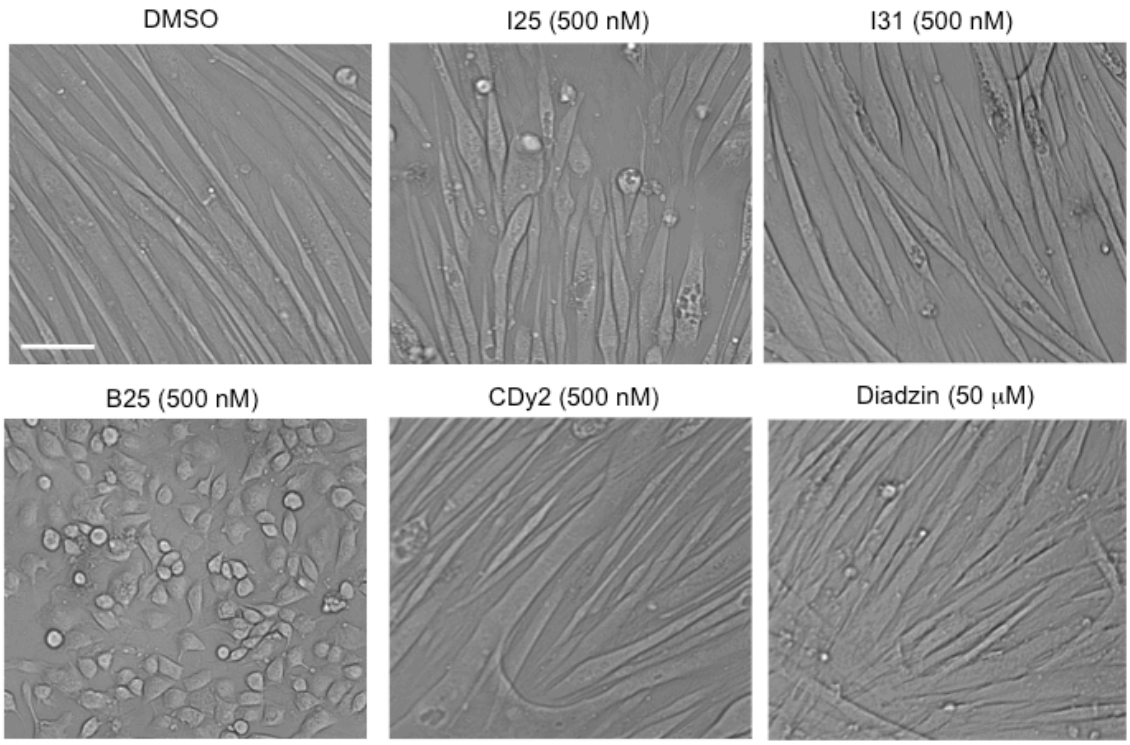
(a) Myotubes were incubated with each compound for 30 min and imaged. Scale bar = 100 μm. **(b)** To measure cytotoxicity, myoblasts grown in 96-well plates were incubated with various concentrations of each compound in growth medium. After 24 hours, an MTS assay was performed and analyzed according to the manufacturer's instructions (Promega, CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay). DMSO was used as negative control and taxol (10 μM) was used as a positive control for MTS assay. Error bars represent standard deviation of duplicated experiments. Growth inhibitory concentration (IC_{50}) was calculated by using Prism software (nonlinear regression curve fitting).



Supplementary Fig 3. Concentration-dependent labeling of CDy2 and competition assay. (**a**) Concentration-dependent labeling of CDy2. Myotubes were incubated with various concentration of CDy2 for 1 hour. The intensities of CDy2-labeled ALDH2 bands were quantified with ImageJ software (Wayne Rasband, NIH) and the dissociation constant (K_d) was determined with Prism software (one-site competition equation). (**b**) For competition assay, myotubes were pre-incubated with I31 for 30 min, then labeled with CDy2 (500 nM) for 20 min. The strongest band at 54 kDa is efficiently disappeared upon competition with unmodified I31. Tubulin immuno-blotting indicates equal loading.

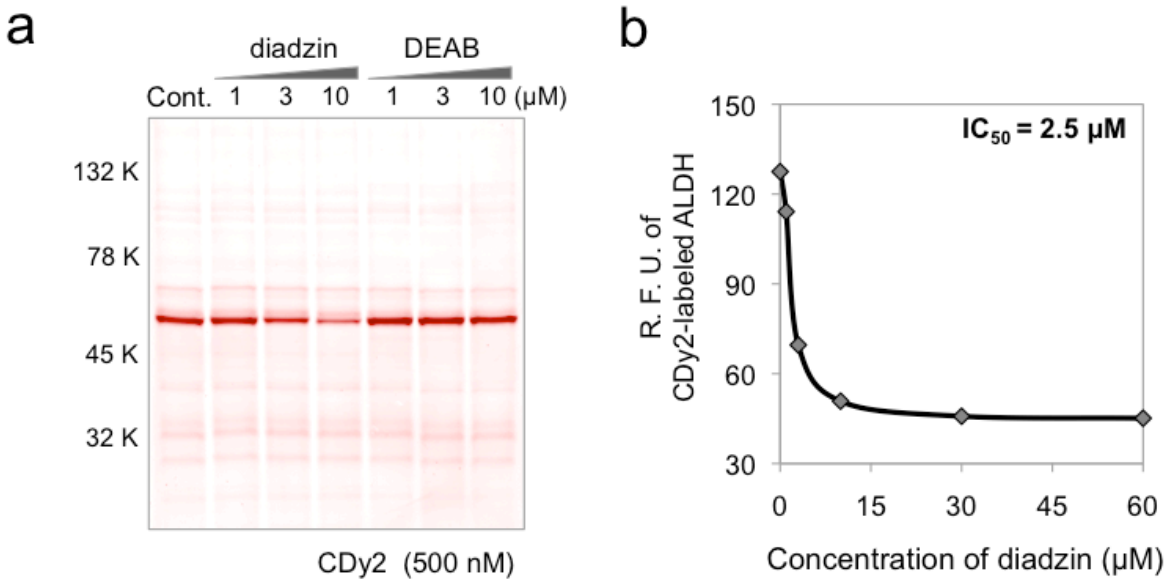


Supplementary Fig. 4. CDy2 localizes to the mitochondria based on the mitochondrial membrane potential. (**a**) A C2C12 myoblast labeled with CDy2 and co-stained with MitoTracker® Deep Red 633 (false-colored green here). (**b**) Live-cell versus fixed-cell images of CDy2. For live-cell images, myoblasts and myotubes were incubated with CDyellow2CA (500 nM) for 30 min. For fixed-cell images, cells were fixed with formaldehyde before labeled with CDy2. Scale bar = 20 μm. (**c**) To disrupt mitochondrial membrane potential, myotubes were pre-incubated with CCCP for 30min before CDy2 labeling. Cell lysates were subjected to in-gel fluorescence analysis. CDy2 labeling was effectively decreased with the pretreatment of CCCP. (**d**) Due to the difficulty of distinguishing ALDH2 and tubulin by size, isoelectric focusing (IEF) was conducted. When treated to living cells CDy2 strongly labeled ALDH2 (pI 7), but the band was almost disappeared when treated to cell lysate. Instead, a new band was appeared around pI 5, which was identified to be tubulin.



Supplementary Fig. 5. Inhibiting ALDH2 does not affect myotube formation.

Myoblasts were differentiated in the presence of each compound for 5 days. The treatment of I31, CDy2 or diadzin, a well-known inhibitor of ALDH2, did not affect myoblast differentiation, though I25-treated myoblasts formed shortened myotubes. This result suggests that the enzymatic activity of ALDH2 is not directly related to myogenesis. As a positive control we used B25, a rosamine library compound that blocks muscle differentiation.[15] Scale bar = 100 μm.



Supplementary Fig. 6. Competition assay with ALDH inhibitors.

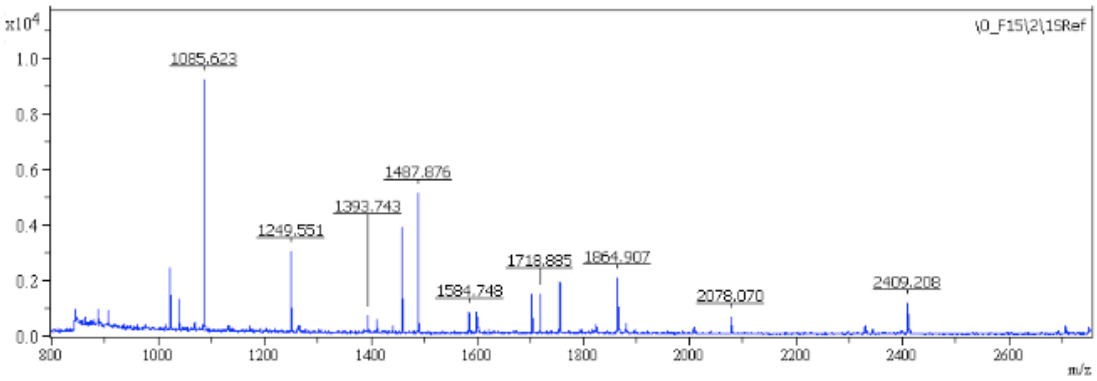
(**a**) DEAB is an inhibitor of ALDH1 and diadzin is an inhibitor of ALDH2. For competition assay, C2C12 myotubes were pre-incubated with various concentrations of daidzin or DEAB for 2 hours. Then cells were labeled with CDy2 for 20 min and lysed for in-gel fluorescence visualization. The pretreatment of daidzin, but not DEAB, selectively reduced the amount of CDy2-labeled ALDH2. (**b**) To determine the half inhibitory concentration (IC_{50}) of diadzin in CDy2-labeling, myotubes were incubated with various concentration of diadzin (1, 3, 10, 30, and 60 uM), then labeled with CDy2 (500 nM). After in-gel fluorescence visualization, the intensities of CDy2-labeled ALDH2 bands were quantified with ImageJ software (Wayne Rasband, NIH) and IC_{50} was determined with Prism software (one-site competition equation).

Supplementary Note 1. Target identification by affinity pull-down assay

(a) Affinity pull-down assay. For affinity matrix experiments, 100 µL of packed beads were washed three times with 1 mL of PBS containing protease inhibitor cocktail. The beads were resuspended in 100 µL of the buffer and then 400 µL of 1 mg/mL myotube extract was added to the bead suspension; the tubes were incubated for 1 hr at 4°C with gentle shaking. After removing the supernatant, beads were washed 4 times with PBS and resuspended in 100 µL of Laemmli sample buffer (Bio-Rad). After SDS-PAGE separation, a protein band around 54 Kda were excised from the gel for mass analysis.

(b) Trypsin digestion and MALDI-TOF/TOF MS and MS/MS analyses. The excised band was washed with Mili-Q water, and then washed with 200 µl of 50% acetonitrile/25mM ammonium bicarbonate buffer (pH7.8) three times. After dehydrated with 100% acetonitrile, the gel pieces were rehydrated with trypsin solution in 25 mM ammonium bicarbonate buffer (pH8.0) and digested for 16 hr at 37 ^{o}C. The peptides were extracted sequentially with 20 mM ammonium bicarbonate buffer and with 50% acetonitrile in 0.1% TFA, the polled peptides were dried and dissolved in 4 µl of 0.1% TFA. Tryptic digested peptides were spotted onto Prespotted AnchorChip target plate (Bruker Daltonics) according to manufacturer's instruction. The peptide mass fingerprint was acquired in UltraFlex III TOF-TOF (Bruker Daltonics) with Compass 1.2 software package.

(c) MASCOT search result of affinity pull down assay. Peptide mass fingerprint (PMF) was obtained using MALDI-TOF/TOF mass spectrometry (Daltonics Ultraflex III, Bruker Inc.). The assigned peaks were used for MASCOT search, and it was identified to be tubulin.



Match to: Tubulin, alpha-1_MOUSE Score: 134 Expect: 2.3e-09

Matched peptides shown in **Bold Red**

1 MRECISIHVG QAGVQIGNAC WELYCLEHGI QPDGQMPSDK TIGGGDDSFN

51 TFFSETGAGK HVPRAVFVDL EPTVIDEVRT GTYRQLFHPE QLITGKEDAA

101 NNYARGHYTI GKEIIDLVLD RIRKLADQCT GLQGFLVFHS FGGGTGSGFT

151 SLLMERLSVD YGKKSKLEFS IYPAPQVSTA VVEPYNSILT THTTLEHSDC

201 AFMVDNEAIY DICRRNLDIE RPTYTNLNRL IGQIVSSITA SLRFDGALNV

251 DLTEFQTNLV PYPRIHFPLA TYAPVISAEK AYHEQLSVAE ITNACFEPAN

301 QMVKCDPRHG KYMACCLLYR GDVVPKDVNA AIATIKTKRT IQFVDWCPTG

351 FKVGINYQPP TVVPGGDLAK VQRAVCMLSN TTAIAEAWAR LDHKFDLMYA

401 KRAFVHWYVG EGMEEGEFSE AREDMAALEK DYEEVGVDSV EGEGEEEGEE

451 Y

(**d**) None of the peptide-peaks from the affinity pull-down assay (List I) matches to calculated masses of tryptic fragments of ALDH2 (List II).

List I. Peptide peaks from the affinity matrix (m/z>800)							
m/z	Intensity	Signal/Noise	Area	m/z	Intensity	Signal/Noise	Area
844.993	799	6.6	87	**1756.963**	1849.13	28.1	640
887.444	844	7	110	**1864.907**	1861.14	29.6	733
906.434	797	6.7	98	**2008.903**	278	4.5	41
1023.454	2329	20.7	264	**2078.07**	478.18	8.9	218
1039.617	1192.24	10.6	201	**2330.011**	277	5	45
1085.623	8809.13	83	1733	**2331.011**	322	5.8	66
1249.551	2983	31.2	630	**2343.976**	188	3.4	24
1393.743	644.37	7.5	186	**2409.202**	732	13.5	140
1410.771	541.37	6.4	176	**2705.167**	318.5	6.4	66
1457.865	3845.92	47.5	1023	**2750.288**	251.5	5.1	55
1487.876	5135.17	64	1438	**2766.298**	170.5	3.5	32
1584.748	816.61	11.2	253	**2799.302**	178.5	3.6	34
1598.764	801.94	11.1	244	**2833.214**	212.5	4.4	55
1701.906	1330.57	19.7	471	**3338.71**	258	5.7	63
1718.885	1384.23	19.8	526				

List II. Calculated masses of ALDH2 fragments (m/z > 800)					
804.3846	1040.5775	1470.7627	2191.0699	3157.674	3862.6539
816.4825	1060.5204	1506.707	2248.0914	3173.669	3894.6437
829.4778	1132.5898	1531.7427	2286.2088	3368.6044	4033.7182
902.4941	1369.7871	1548.7176	2663.3625	3425.6259	
973.4414	1385.782	1599.79	2679.3574	3720.7611	
990.5003	1403.7603	1774.8282	2720.3839	3762.7717	
1003.4989	1419.7552	1844.0527	2961.5104	3777.7826	

Supplementary Note 2. Target identification by 2DE-MALDI-TOF-TOF

(a) Protein Extraction C2C12 myotubes were treated with CDy2 (1 μM) for one day, and then washed three times with cold PBS and harvested. The cell pellet was resuspended in protein lysis buffer (40mM Trizma, 7M Urea, 2M thiourea and 4% CHAPS) containing Protease Inhibitor Cocktail and 50 μg/ml DNase I and 50 μg/ml RNase A (Roche). After sonication (10 seconds), the supernatant was collected by centrifugation (20,000 g) for 45 min at 10 ^{o}C.

(b) Two dimensional gel electrophoresis (2-DE). Isoelectric focusing (IEF) was performed by using PROTEAN IEF Cell (Bio-Rad) with 18 cm ReadyStrip pH 3-10NL(Bio-Rad) followed by manufacturer's instruction. Then, the IEF strips were seperated on 12 % SDS-PAGE gel for 2D analysis. 2D fluorescence image was acquired using the Typhoon 9400 scanner (GE healthcares) at excitation/emission wavelengths of 532nm/580nm. The fluorescence-labeled spots were excised from the gel for mass analysis.

(c) MASCOT search result of live-cell target identification by CDy2
Match to: ALDH2_MOUSE Score: 185 Expect: 5.1e-15
Sequence Coverage: 34%
Matched peptides shown in Bold Red

1 MLRAALTTVR RGPRLSRLLS AAATSAVPAP NHQPEVFCNQ IFINNEWHDA
51 VSRKTFPTVN PSTGEVICQV AEGNKEDVDK AVKAARAAFQ LGSPWRRMDA
101 SDRGRLLYRL ADLIERDRTY LAALETLDNG KPYVISYLVD LDMVLKCLRY
151 YAGWADKYHG KTIPIDGDFF SYTRHEPVGV CGQIIPWNFP LLMQAWKLGP
201 ALATGNVVVM KVAEQTPLTA LYVANLIKEA GFPPGVVNIV PGFGPTAGAA
251 IASHEGVDKV AFTGSTEVGH LIQVAAGSSN LKRVTLELGG KSPNIIMSDA
301 DMDWAVEQAH FALFFNQGQC CCAGSRTFVQ ENVYDEFVER SVARAKSRVV
351 GNPFDSRTEQ GPQVDETQFK KILGYIKSGQ QEGAKLLCGG GAAADRGYFI
401 QPTVFGDVKD GMTIAKEEIF GPVMQILKFK TIEEVVGRAN DSKYGLAAAV
451 FTKDLDKANY LSQALQAGTV

Supplementary Methods

Myogenic cell culture and differentiation. The C2C12 myoblast cell line was obtained from American Type Culture Collection (Rockville, MD). Undifferentiated myoblasts were grown in DMEM supplemented with 10% fetal bovine serum at 37°C in the presence of 5% CO_2. After 2 days, myoblasts were stimulated to differentiate by replacing the medium with DMEM supplemented with 2% heat-inactivated horse serum. Differentiation was allowed to continue for 8 days to prepare differentiated myotubes, with media replacement every two days.

Screening. Myoblasts and myotubes grown in 96-well plates were incubated with library compounds at 500 nM for 1 hr. Fluorescence images were taken in three optical channels using a Leica 2000 fluorescence microscope (λ_{ex} = 545/30 nm and λ_{em} = 610/70nm; λ_{ex} = 480/40 nm and λ_{em} = 527/30nm; λ_{ex} = 420-490 nm, $\lambda_{em} \geq$ 515 nm).

Compound treatment and fluorescence microscopy. For live-cell imaging, myoblasts and myotubes grown in a black optical 96-well plate were incubated with 300 nM of free compound, or 500 nM of CDy2, for 30 min. Fluorescence images of active compounds were taken by using a Leica 2000 fluorescence microscope (λ_{ex} = 545/30 nm and λ_{em} = 610/70nm).

CDy2 labeling for SDS-PAGE analysis. For CDy2 labeling, 500 nM of CDy2 was added to the culture medium of live myoblasts or myotubes, incubated for 20 min, and washed three times with phosphate-buffered saline (DPBS, Sigma). Total cell lysates were prepared in mammalian cell lysis buffer (Sigma, C2978) containing protease inhibitor cocktail (Sigma, P8340), and protein concentrations were measured by Bradford assay (Bio-Rad). 10 μg aliquots of total protein were run on a 10-14% SDS-PAGE gel to visualize CDy2-labelled proteins. To visualize concentration-dependent staining patterns of CDy2, live myotubes were incubated with different concentrations of CDy2 for 30 min, and 10 μg of the lysates were evenly loaded on an SDS-PAGE gel. Fluorescence of SDS-PAGE gels was visualized by Typhoon 9400 scanner (GE Healthcare).

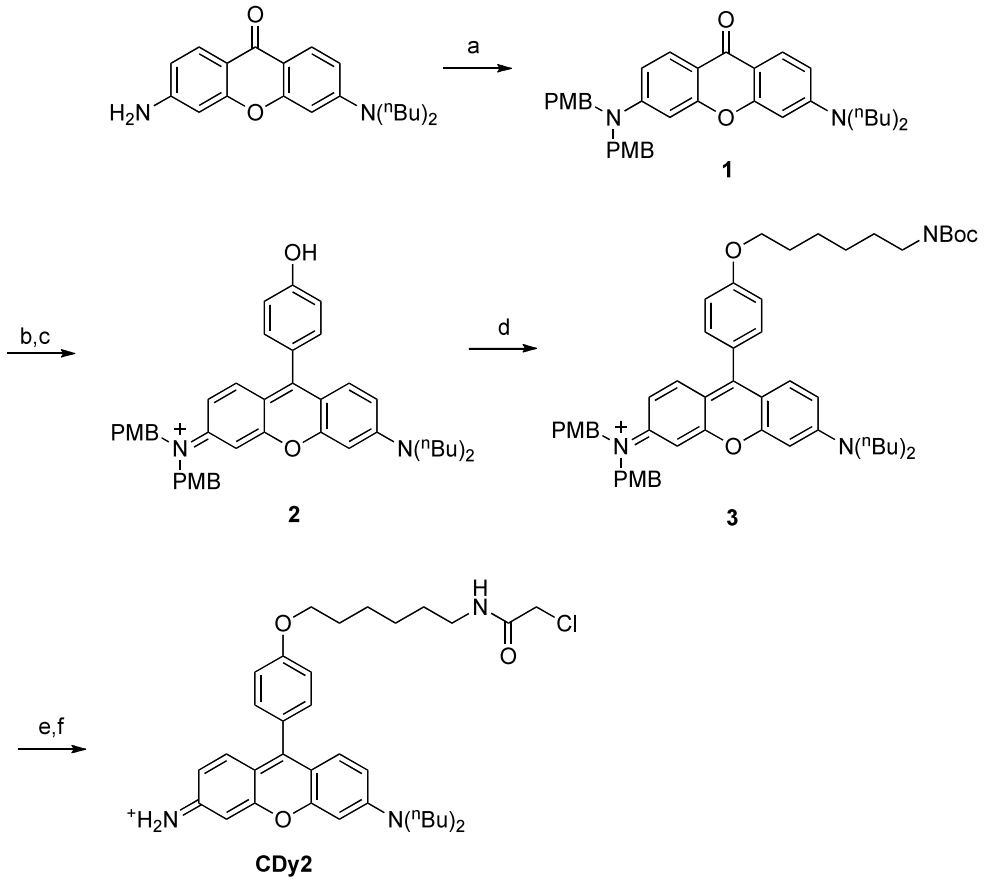
Immunoblotting. Protein samples were separated by SDS-PAGE and transferred to a PVDF membrane. For tubulin immunohistochemistry, a mouse anti-α-tubulin antibody (GeneTex., Inc.) was used; for ALDH2 immunohistochemistry, goat anti-ALDH2-K15 (Santa Cruz Biotechnology, Inc.) was used.

RNA interference. Control and ALDH2 siRNA were purchased from Santa Cruz Biotechnology, Inc. Cells were transfected with siRNA followed by manufacture's instruction. After 3 days of transfection, cells were labeled with CDy2 (500 nM) for 20min.

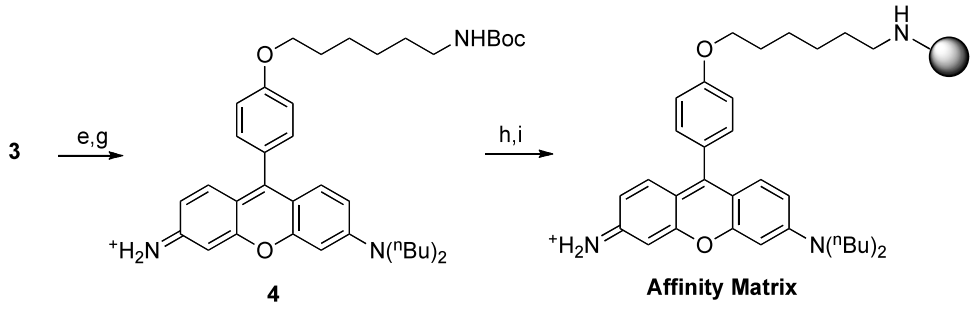
Over-expression of ALDH2-GFP and Tubulin GFP. pAC-GFP-alpha tubulin (human) was purchased from Clontech and pAC-GFP-ALDH2 (mouse) was purchased from OriGene Technologies, Inc. For transfection, HEK293 were plated in 1 mL of medium at 1×10^6 to cells per 35-mm culture plate. The medium was replaced 24 h later with 1 mL of DMEM without serum, and transfected with plasmid DNA mixed with Lipofectamin^{TM}2000 (Invitrogen). After 24 hours of transfection, medium was replaced to normal growth medium containing 10 % FBS and antibiotics. Another 24 hours later, cells were incubated with CDy2 (500 nM) for 20min for lysed for SDS-PAGE analysis.

Affinity matrix and in-vitro competition assay. For affinity matrix experiments, 100 µL of packed beads were washed three times with 1 mL of PBS containing protease inhibitor cocktail. The beads were resuspended in 100 µL of the buffer and then 400 µL of 1 mg/mL myotube extract was added to the bead suspension; the tubes were incubated for 1 hr at 4°C with gentle shaking. After removing the supernatant, beads were washed 4 times with 1 mL of PBS containing protease inhibitor cocktail and resuspended in 100 µL of Laemmli sample buffer (Bio-Rad). For competition assays, cell lysates were pre-incubated with 100 µM of free compound for 30 min before the affinity matrix was added. Protein identification by mass spectrometry was performed.

Synthesis of affinity matrix and CDy2



a. p-methoxybenzyl bromide, NaH, THF/DMF; b. 4-(2-Tetrahydro-2H-pyranoxy)phenylmagnesium bromide, THF; c. 1N HCl / THF; d. 6-(Boc-amino)hexyl bromide, K_2CO_3, DMF; e. TFA; f. Chloroacetic anhydride, pyridine, DCM



e. TFA; g. Boc_2O, pyridine, DCM; h. 5% TFA / DCM; i. Agarose bead, DIEA, DMSO

Compound 1: Sodium hydride (78 mg, 3.25 mmol) was added to an ice cooled solution of Xanthone (110mg, 0.32mmol) in DMF/THF (1:10) (11mL). After incubated for 10 min, p-methoxybenzyl chloride (0.26ml, 1.95mmol) was added and the mixture and stirred overnight. Then, the reaction mixture was diluted with DCM and washed with aq. 1N HCl, aq.$NaHCO_3$, and brine sequentially. The organic phase was dried over anhydrous sodium sulfate, concentrated and purified by silica gel column chromatography to give **1** (72mg, 38%). ^{1}H-NMR ($CDCl_3$) δ 8.05 (dd, J = 4.53,9.06, 2H), 7.15 (d, J = 8.71, 4H), 6.88 (d, J = 8.71, 4H), 6.76 (dd, J = 2.09, 9.06, 1H), 6.59 (dd, J = 2.09, 9.06, 1H), 6.56 (d, J = 2.44, 1H), 6.34 (d, J = 2.09, 1H), 4.66 (s, 4H), 3.80 (s, 6H); 3.33 (t, J=7.32, 4H), 1.65 (m, 2H), 1.31 (m, 6H), 0.97 (t, J=7.32, 6H). ^{13}C-NMR ($CDCl_3$) δ 174.76, 158.85, 158.41, 158.23, 153.67, 152.50, 127.85, 127.68, 114.24, 112.71, 109.25, 108.77, 97.68, 96.30, 55.28, 53.20, 50.90, 29.30, 20.24, 13.91ppm. MS (ESI): m/z 579 (M+1)

Compound 2: Compound **1** (40 mg, 0.0692 mmol) in freshly distilled THF (3 mL) was mixed with 4-(2-Tetrahydro-2H-pyranoxy)phenylmagnesium bromide in THF (0.5 M) (3mL). After stirred overnight at room temperature, the reaction mixture was acidified to pH 1-2 by the addition of 1N HCl and stirred for 2 hour. Then, the reaction mixture was diluted with DCM and its organic layer was washed with aq. $NaHCO_3$ and brine, dried over anhydrous sodium sulfate, concentrated, and purified by silica gel column chromatography to give **2** (42 mg, 0.064 mmol). ^{1}H-NMR (MeOD) δ 7.50 (m, 2H) 7.31 (d, J=8.36, 2H), 7.19 (d, J=8.71, 4H), 7.13 (d, J = 8.71, 1H), 7.04 (d, J = 8.36, 2H) 6.97 (d, J = 2.09, 1H), 6.89 (d, J = 8.71, 4H), 6.85 (d, J = 2.09, 1H), 6.70 (d, J = 8.36, 1H), 3.76 (s, 6H), 3.59(t, J = 7.67, 4H), 1.65(m, 4H), 1.44(m, 4H), 0.99(t, J = 7.31, 6H) ^{13}C-NMR (MeOD) δ 160.48, 160.01, 159.35, 158.87, 158.28, 157.33, 156.86, 136.36, 132.71, 132.36, 132.08, 129.43, 129.30, 129.06, 128.29, 128.23, 128.15, 122.97, 115.09, 115.02, 114.85, 114.65, 114.38, 114.26, 114.01, 98.08, 96.73, 54.94, 54.59, 51.80, 29.78, 20.24, 13.37ppm. MS (ESI): m/z 655 (M+1)

Compound 3: Compound **2** (38 mg, 0.0580 mmol) was mixed with potassium carbonate (78 mg, 0.580 mmol), and 6-(Boc-amino)hexyl bromide (162 mg, 0.580 mmol) in DMF (8mL). The mixture was heated at 80^{o}C for 30 min and cooled to room temperature. Then, the reaction mixture was diluted with DCM and washed with 1N HCl, aq. $NaHCO_3$, and brine. The organic layer was dried over anhydrous sodium sulfate, concentrated, and purified by silica gel column chromatography to give **3** (15mg, 0.0175mmol). ^{1}H-NMR (MeOD) δ 7.50 (t, J = 9.86, 2H) 7.38 (d, J=8.55, 2H), 7.18 (m, 7H), 7.06 (d, J = 9.37, 1H), 6.99 (d, J = 2.30, 1H) 6.90 (d, J = 8.71, 4H), 6.88 (m, 1H), 4.89 (s, 4H), 4.57 (brs, 1H), 4.10 (t, J = 6.25, 2H), 3.77 (s, 6H), 3.60 (t, J = 7.57, 4H), 3.05 (t, J = 5.92, 2H), 1.85 (t, J = 6.74, 2H), 1.68 (m, 4H), 1.47 (m, 8H), 1.43 (s, 9H), 1.26 (s, 2H), 0.99 (t, J = 7.23, 6H) ^{13}C-NMR (MeOD) δ 161.06, 159.35, 158.23, 158.19, 157.60, 156.71, 156.26, 136.81, 131.93, 13.58, 131.22, 127.53, 123.48, 114.49, 114.27, 113.62, 113.34, 97.44, 96.10, 78.33, 77.74, 67.84, 67.44, 61.38, 54.29, 53.95, 5115, 39.79, 32.05, 29.44, 29.14, 28.69, 27.30, 26.16, 26.06, 25.33, 25.10, 19.55, 12.68ppm. MS (ESI): m/z 854 (M+1)

CDy2: Compound **3** (12mg, 14.07 μmol) was dissolved in TFA (6 mL) and the solution was stirred for 2 days at room temperature. TFA was removed by evaporation to give a crude oil. Then, the crude oil was dried *in vacuo*, dissolved in DCM (4 mL) and cooled in an ice bath. To the solution, 100 μL of DIEA in

THF/DMF (4mL/2mL) was added and chloroacetic anhydride (21mg) was added squentially. After 1hr of incubation, the reaction mixture was diluted with DCM, washed with 1N HCl, aq. $NaHCO_3$, and brine sequentially, dried over anhydrous sodium sulfate, concentrated, and purified by silica gel column chromatography to give **CDy2** (1.3 mg, 2.5 μmol). ^{1}H-NMR (MeOD) δ 8.25 (s, 1H), 7.70 (m, 2H), 7.51 (m, 3H), 7.34 (d, J = 9.76, 1H), 7.24 (d, J = 8.36, 2H), 7.13 (s, 1H), 4.29 (s, 1H), 4.15 (t, J = 6.27, 2H), 4.00 (s, 2H), 3.75 (t, J = 6.96, 4H), 3.30 (m, 2H), 1.88 (m, 2H), 1.73 (m, 4H), 1.53 (m, 8H), 1.29(s, 2H), 1.04(m,6H) ^{13}C-NMR (MeOD) δ 167.47, 158.72, 156.51, 147.19, 133.87, 132.27, 131.59, 123.52, 118.70, 118.16, 117.81, 115.52, 106.96, 97.24, 68.66, 43.39, 42.53, 40.05, 29.44, 26.86, 26.06, 20.43, 20.29, 13.68ppm. MS (ESI): m/z 591 (M+1)

Compound 4: Compound **4** was prepared in the same procedure as the preparation of CDy2 from compound **3** except using Boc anhydride.

Affinity Matrix: Compound **4** (1.3mg, 2.12 μmol) was dissolved in DCM (1.5mL) and TFA(5%) was added to the solution. After incubated for 1hour, all solvents were removed *in vaccuo*. Then, the boc-deprotected compound **4** was immobilized on affinity matrices; The Affigel (0.5mL, 7.5 μmol) was transferred to a 3 mL cartridge with a 20 um PE frit. The supernatant solvent was drained and the gel was washed with DMSO. A solution of Boc-deprotected 4 (2.12 μmol) in DMSO and DIEA were added to the affigel. After shaking for 3 hr at rt, the solution was drained and the gel was washed with DMSO. Analyzing the eluent mixed with an internal standard by LCMS and comparing the result to the initial reaction mixture determined the loading level. A solution of 50 mM ethanolamine in DMSO (1mL) and DIEA (15uL) were added to the affigel and the mixture was shaken for 3 hr at RT. The solution was drained and the gel was washed with DMSO, water, and 2% aq.