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Supplemental Information

Dynamic Imaging of LDH Inhibition in Tumors Reveals Rapid *In Vivo* Metabolic Rewiring and Vulnerability to Combination Therapy

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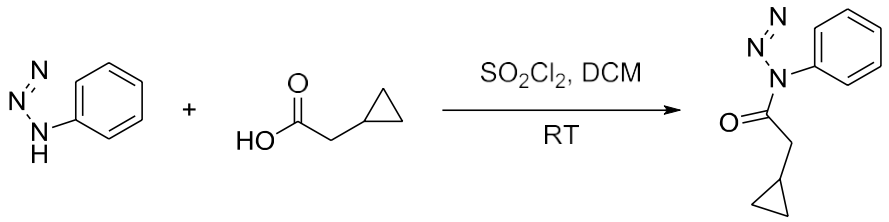
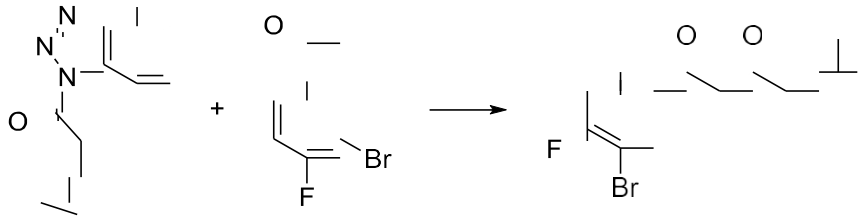
Method S1. Synthesis and purity of NCI-006. Related to STAR Methods.

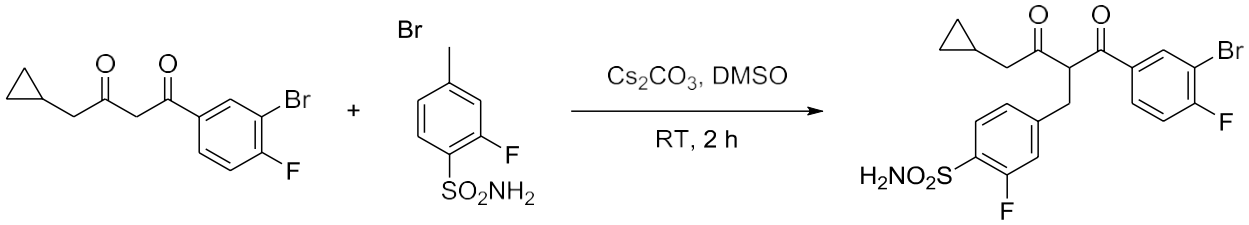


Synthesis of 1-(1H-benzo[d][1,2,3]triazol-1-yl)-2-cyclopropylethan-1-one: This compound was prepared as described in our previous paper[1]. To the solution of 1H-benzo[d][1,2,3]triazole (476 g, 3995 mmol, 4 eq) in DCM (600 mL) was added thionyl chloride (72.9 ml, 999 mmol, 1eq) and stirred at room temperature for 0.5 h then carefully added 2-cyclopropylacetic acid (93 ml, 999 mmol, 1 eq) upon cooling in an ice water bath (for larger scale cooling necessary due to exothermic reaction, if the reaction mixture forms thick precipitate and difficult to stir then add more DCM) and stirred for 6 h. The reaction was filtered, and the filter cake was washed with DCM. The filtrate was added bicarbonate solution slowly and stirred for 30 minutes then transferred to a separatory funnel. The organic layer was subsequently washed with bicarbonate solution and brine solution. The organic layer was dried under sodium sulfate and concentrated to get a thick oil. The crude product was purified on a CombiFlash system using a 340 g silica column eluting with 0-20 % EA in hexanes over 10 column volumes (divided into six batches due to large quantity). The first peak was collected, concentrated and dried to get oil which eventually solidifies into white solid (Yield 92 %). LC-MS Retention Time: = 3.51 min. (Standard Gradient 4% to 100% Acetonitrile 0.05% TFA over 3 minutes; Luna C18 3 micron 3 x 75mm).

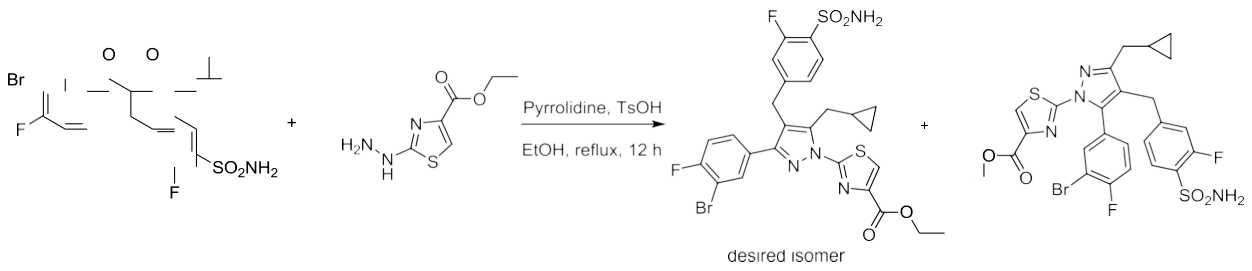


Synthesis of 1-(3-bromo-4-fluorophenyl)-4-cyclopropylbutane-1,3-dione: This compound was prepared as described in our previous paper[1]. 3'-Bromo-4-fluoroacetophenone (754 mmol, 1eq) and 1-(1H-benzo[d][1,2,3]triazol-1-yl)-2-cyclopropylethan-1-one (167 g, 829 mmol, 1.2 or 1.5 eq) was charged 1000 mL DCM then added magnesium bromide diethyl etherate (487 g, 1884 mmol, 2.5 eq) in one portion in a 4 necked flask set up with overhead stirrer. The reaction

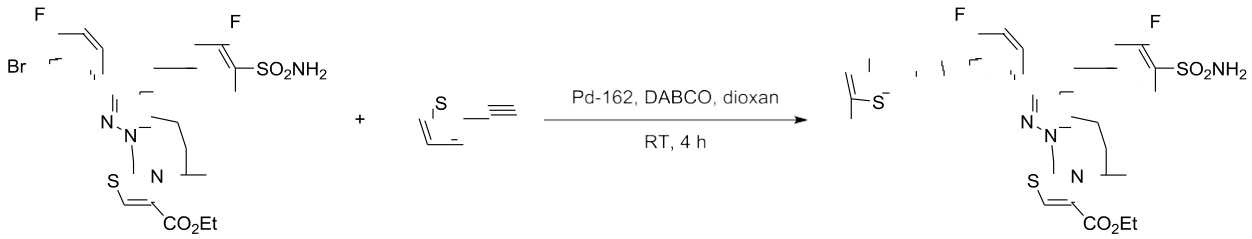
was cooled in an ice bath then added Hunig's base (395 ml, 2261 mmol, and 3 eq) dropwise over 15 minutes through a dropping funnel. The reaction was stirred overnight. The reaction was placed in an ice bath then added ice cubes slowly while stirring during which heat generated (caution for exothermic reaction and slow addition is essential). The addition of ice continued until no more exothermic reaction then added 1 molar HCl dropwise under ice cooling added few ml of 6 molar HCl to acidify then extracted with DCM, the organic layer was washed with brine. The organic layer was dried with magnesium sulfate and concentrated. The crude product was purified on a flash system using 340 g Biotage columns eluting with gradient elution 0-30 % ethyl acetate in hexanes over 20 column volumes (divided into 8 columns due to large quantity) to get yellow liquid as a first peak. LC-MS Retention Time = 3.9 min; $(M+H)^+ = 300$ (usually as in keto enol form another peak around 3.5 min); (Standard Gradient 4% to 100% Acetonitrile 0.05% TFA over 3 minutes; Luna C18 3 micron 3 x 75mm).



Synthesis of 4-(2-(3-bromobenzoyl)-4-cyclopropyl-3-oxobutyl)-2-fluorobenzenesulfonamide: A mixture of 1-(3-bromo-4-fluorophenyl)-4-cyclopropylbutane-1,3-dione (23.68 g, 79 mmol) in DMSO (100 mL) was added Cs_2CO_3 (35.2 g, 108 mmol) and stirred for 5 minutes. Added 4-(bromomethyl)-2-fluorobenzenesulfonamide (19.29 g, 72.0 mmol) portion wise upon cooling in ice water and stirred at RT for 1 h. The reaction was diluted with ethyl acetate and filtered through celite. The filtrate was washed with 1 molar HCl, and saturated ammonium chloride 2 times. The organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified on isco flash system using a 220 g silica normal column eluting with 20-50 % EA in hexanes over 20 column volumes. The last peak was pooled to get a white solid. Yield 26.1 g (74.6%). LC-MS Retention Time: = 3.36 min $(M+H)^+ = 488$. (Standard Gradient 4% to 100% Acetonitrile (0.05% TFA) over 3 minutes; Luna C18 3 micron 3 x 75mm).

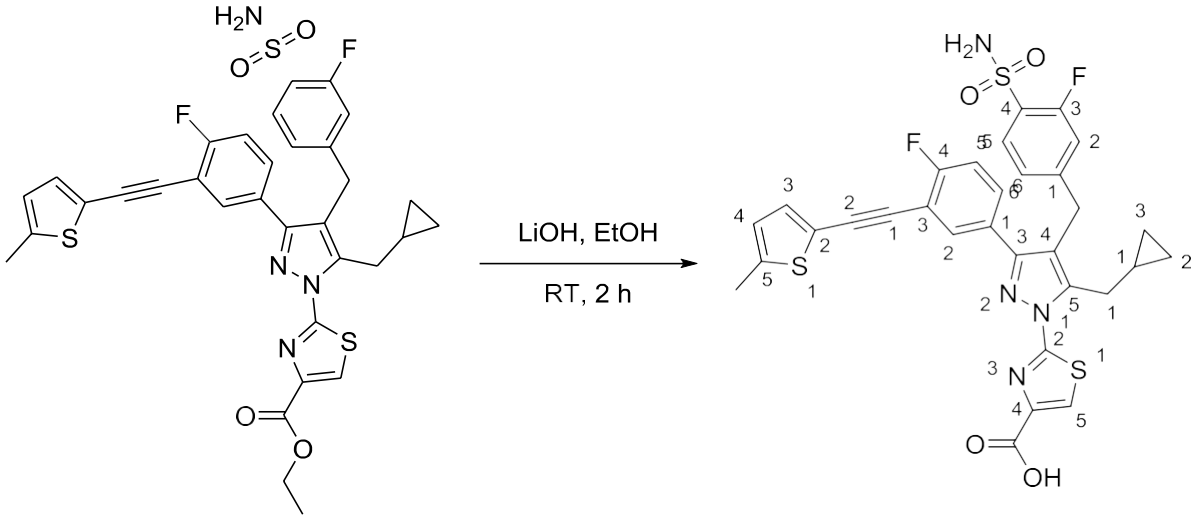


Synthesis of ethyl 2-(3-(3-bromo-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate: A mixture of 4-(2-(3-bromo-4-fluorobenzoyl)-4-cyclopropyl-3-oxobutyl)-2-fluorobenzenesulfonamide (25 g, 51.4 mmol, 1 eq) and tosic acid (4.89 g, 25.7 mmol, 0.5 eq) in ethanol (200 mL) was added pyrrolidine (2.126 ml, 25.7 mmol, 0.5 eq) and refluxed for 1 h. Cooled and then added ethyl 2-hydrazinylthiazole-4-carboxylate (12.51 g, 66.8 mmol) and refluxed overnight. The reaction was concentrated and the residue was taken in DCM and immediately loaded to a silica loading cartridge. The compound was purified on an isco flash system using 330 g gold column eluting with 20-40 % ethyl acetate in hexanes over 20 column volumes. The pure product containing a mixture of 2 regioisomers was further separated on a reverse phase isco using a 415 g gold column eluting with 60-100 % ACN (0.1 TFA) in water over column volumes. The 2nd peak was pooled and concentrated the solid was stirred with a clear solution of bicarbonate. The precipitate formed was collected by filtration. The filter cake was thoroughly washed with water and air dried and finally in a vacuum desiccator under P_2O_5 to get pure white solid. Yield 11.2 g (34.2 %). LC-MS Retention Time = 6.924 min $(M+H)^+$ = 639. (Long Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes, Agilent Eclipse XDB-C18 3 micron 3 x 75mm).



Synthesis of Ethyl 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-((5-methylthiophen-2-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate: A mixture of ethyl 2-(3-(3-bromo-4-fluorophenyl)-5-(cyclopropylmethyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate (9 g, 14.12 mmol), 2-ethynyl-5-

methylthiophene (2.242 g, 18.35 mmol), [P(tBu)3] Pd(crotyl)Cl (http://jmcct.com/products-services/product_p429.html) (cat # Pd-162) (0.141 g, 0.353 mmol) and DABCO (3.17 g, 28.2 mmol) in dioxan (30 mL) was stirred at RT for 4 h. After completion of the reaction, Pd scavenger silia DMT was added and stirred for 2 h at RT. The reaction mixture was diluted with ethyl acetate and filtered through a plug of silica. The filtrate was concentrated and purified on an isco flash system using a 330 g gold column eluting with 15-40 % ethyl acetate in hexanes over 20 column volumes. The product had some yellow color (pure by LC) which is further purified in isco flash reverse phase using a 415 g gold column eluting with 60-100 % ACN (0.1 % TFA) in water (0.1 % TFA) over 25 column volumes (elutes with around 80 % ACN). The fractions pooled and concentrated then neutralized with bicarbonate solution. The precipitate was collected by filtration and washed with water then air dried followed by vacuum drying under P_2O_5 to get white solid. Yield 8.65 g (90 %). LC-MS Retention Time = 7.111 min $(M+H)^+$ = 679. (Long Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes, Agilent Eclipse XDB-C18 3 micron 3 x 75mm).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (s, 1H), 7.74 (dd, J = 6.9, 2.3 Hz, 1H), 7.70 – 7.58 (m, 2H), 7.57 (s, 2H), 7.37 (t, J = 9.0 Hz, 1H), 7.29 (dd, J = 3.6, 0.5 Hz, 1H), 7.16 (dd, J = 11.3, 1.5 Hz, 1H), 7.06 (dd, J = 8.1, 1.6 Hz, 1H), 6.85 (dq, J = 3.4, 0.9 Hz, 1H), 4.32 (q, J = 7.1 Hz, 2H), 4.18 (s, 2H), 3.18 (d, J = 6.9 Hz, 2H), 2.49 (t, J = 1.3 Hz, 3H), 1.33 (t, J = 7.1 Hz, 3H), 1.22 – 1.09 (m, 1H), 0.44 – 0.32 (m, 2H), 0.29 – 0.21 (m, 2H).



Synthesis of 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-((5-methylthiophen-2-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylic acid: Ethyl 2-(5-(cyclopropylmethyl)-3-(4-fluoro-3-((5-methylthiophen-2-yl)ethynyl)phenyl)-4-(3-fluoro-4-sulfamoylbenzyl)-1H-pyrazol-1-yl)thiazole-4-carboxylate (8.65 g, 12.74 mmol) in EtOH (150 mL) was added LiOH (42.5 ml, 63.7 mmol, 5 eq) and stirred at RT for 2 h. After completion of the reaction, most of the solvent was removed and the residue was diluted with water (50 mL). The reaction mixture was acidified with 1 molar HCl. The precipitate formed was stirred at RT for 1 h then collected by filtration. The filter cake was thoroughly washed with cold water. The milky precipitate was further suspended in hot water and stirred for 30 minutes and again filtered to collect the precipitate. The precipitate was washed with hot water and with cold ethanol to get the white solid which was further dried under air overnight and finally in a vacuum oven overnight at 80 ºC. Yield 7.3 g pure white solid (88 %). LC-MS Retention Time = 6.133 min $(M+H)^+$ = 651. (Long Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes, Agilent Eclipse XDB-C18 3 micron 3 x 75mm). 1H NMR (400 MHz, DMSO-d6) δ 13.14 (s, 1H), 8.29 (s, 1H), 7.71 (dd, J = 6.9, 2.3 Hz, 1H), 7.66 – 7.54 (m, 4H), 7.35 (dd, J = 9.4, 8.7 Hz, 1H), 7.26 (dd, J = 3.6, 0.5 Hz, 1H), 7.14 (dd, J = 11.3, 1.6 Hz, 1H), 7.03 (dd, J = 8.1, 1.6 Hz, 1H), 6.83 (dq, J = 3.6, 1.0 Hz, 1H), 4.15 (s, 2H), 3.15 (d, J = 6.9 Hz, 2H), 2.47 – 2.45 (m, 3H), 1.20 – 1.06 (m, 1H), 0.38 – 0.29 (m, 2H), 0.24 – 0.15 (m, 2H); 13C NMR (101 MHz, DMSO-d6) δ 161.58, 160.94, 159.18, 150.73, 147.20, 147.12, 144.47, 144.44, 143.30, 133.59, 131.80, 129.81, 129.73, 129.50, 129.35, 128.51, 128.48, 128.39, 126.25, 125.90, 123.59, 123.56, 118.47, 116.75, 116.28, 116.23, 116.07, 116.02, 110.99, 84.86, 27.99, 14.90, 10.15, 4.34; HRMS (ESI) m/z (M+H)+ calcd. for $C_{31}H_{25}F_2N_4O_4S$; 651.1001 found 651.1003.

Instrument: AG09 Datafile: Z:\PurGroup\Instr\AG09Raw\bantukallug\02-18\200218-GRB061-0151-13477.D

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Sample Name       : GRB061-015                     Location  : P1-D-03
Acq. Operator     : Ganesha Rai                        Inj : 1
Spec. Reported    : UV Integration                 Inj Volume : 3 ul
Acq. Method       : C:\Chem32\1\METHODS\FINAL_GRAD.M
Analysis Method   : C:\Chem32\1\METHODS\FINAL_GRAD.M
Sample Info : Easy-Access Method: 'FINAL_GRAD'  650.00
Method Info : Long Gradient 4% to 100% Acetonitrile (0.05% TFA) over 7 minutes
              Agilent Eclipse XDB-C18 3 micron 3 x 75mm
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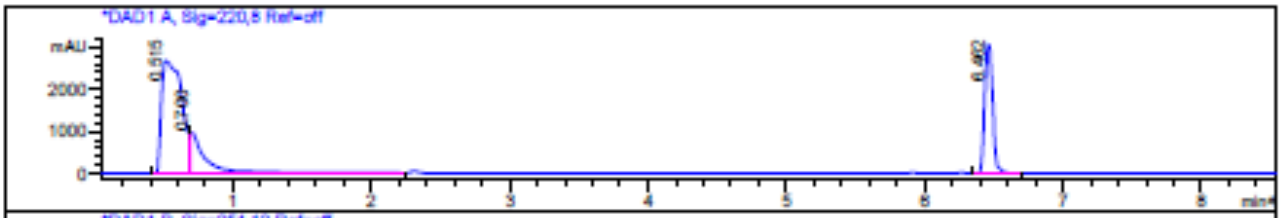
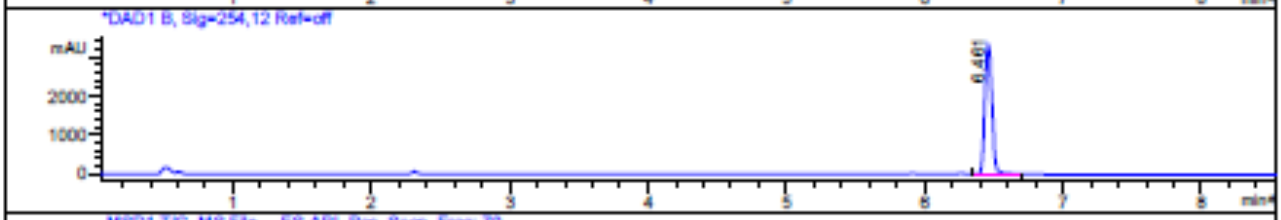
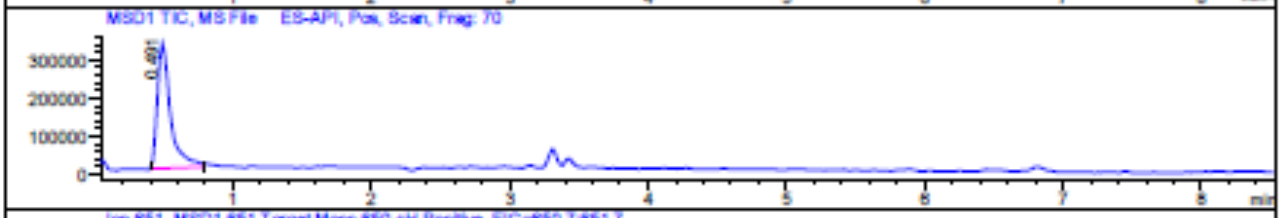
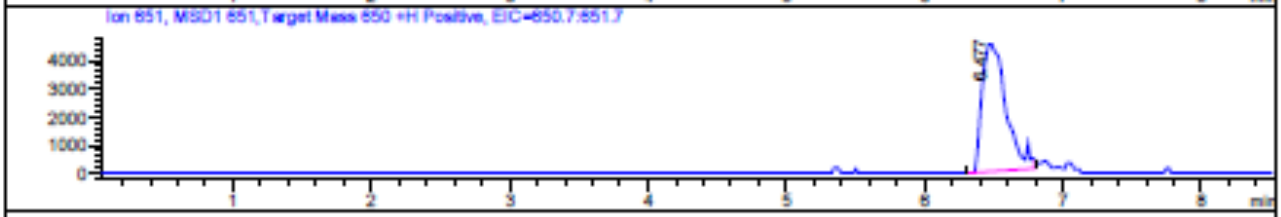








Integration Results for DAD1 A, Sig=220,8 Ref=off

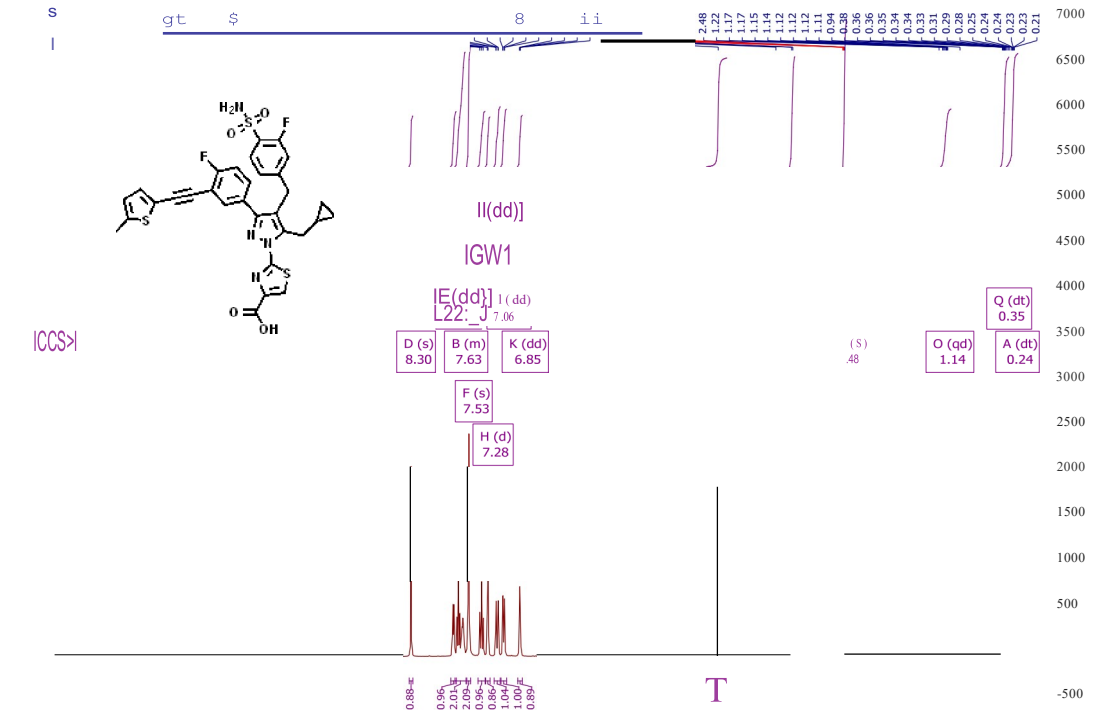
RetTim	Width	Area	Height	Area%	MS(+)
0.52	0.14	27394.09	2676.13	56.91	179
0.70	0.14	8205.07	967.65	17.05	179
6.46	0.07	12533.18	3103.14	26.04	651

Integration Results for DAD1 B, Sig=254,12 Ref=off

RetTim	Width	Area	Height	Area%	MS(+)
6.46	0.06	11868.17	3325.29	100.00	651

Proton NMR and Fluorine NMR

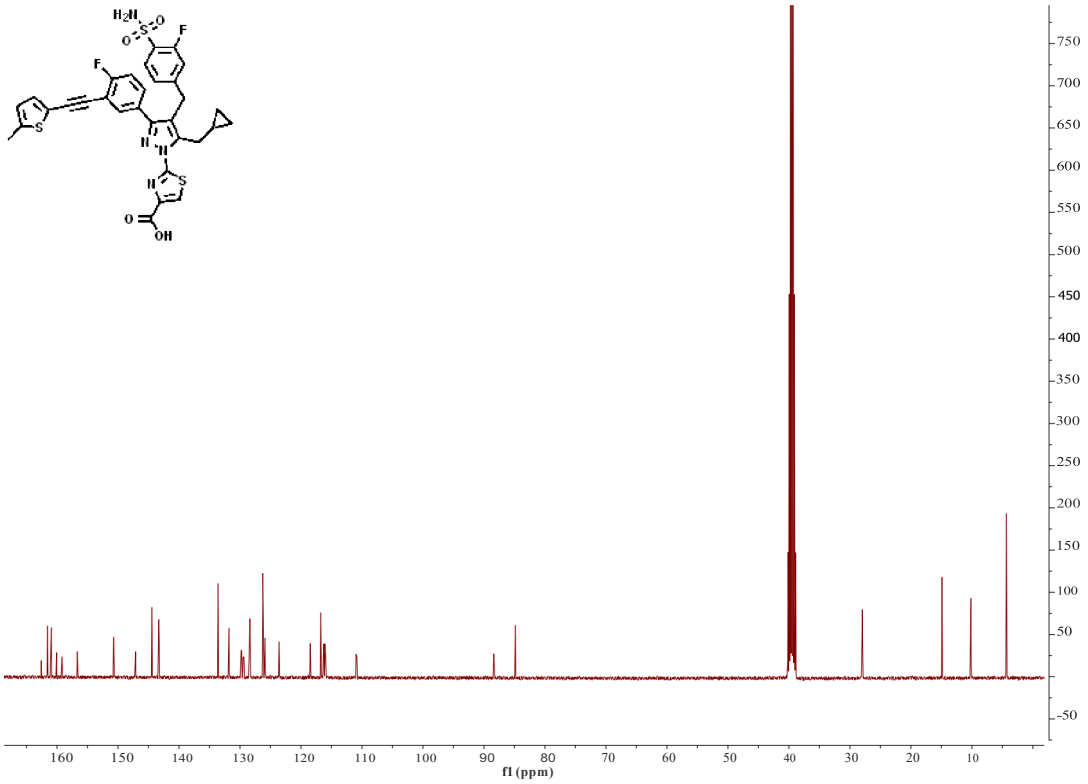
grb061-015 _proton_01



13.5 13.0 12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

grb061-015_fluor i ne_01

55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110 -115 -120 -125 -130 -135 -140 -145 -150 -155 -160 -165 -170 -175
f1 (ppm)



^{13}C NMR spectra

Fig. S1

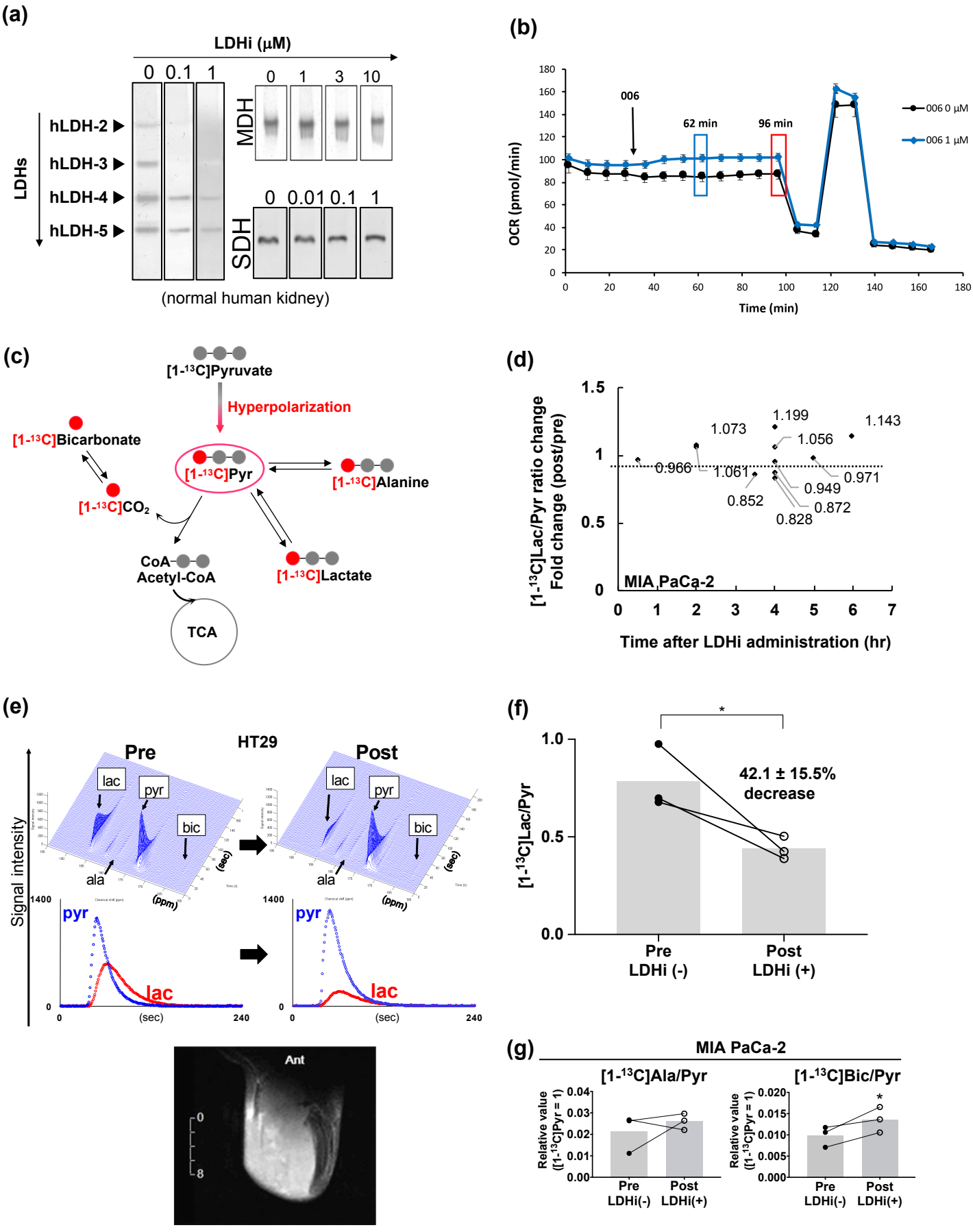

(a)
LDHi (μM)
0 0.1 1
0 1 3 10
MDH
0 0.01 0.1 1
SDH
LDHs
hLDH-2
hLDH-3
hLDH-4
hLDH-5
(normal human kidney)
(b)
006
62 min
96 min
006 0 μM
006 1 μM
OCR (pmol/min)
Time (min)
(c)
[1-13C]Pyruvate
Hyperpolarization
[1-13C]Bicarbonate
[1-13C]Pyr
[1-13C]Alanine
[1-13C]CO2
CoA
Acetyl-CoA
[1-13C]Lactate
TCA
(d)
[1-13C]Lac/Pyr ratio change
Fold change (post/pre)
1.073
1.199
1.056
1.143
0.966
1.061
0.971
0.949
0.852
0.872
0.828
MIA PaCa-2
Time after LDHi administration (hr)
(e)
HT29
Pre
Post
lac
pyr
bic
ala
(ppm)
(sec)
Signal intensity
1400
0
240
(f)
*
42.1 ± 15.5%
decrease
[1-13C]Lac/Pyr
Pre
LDHi (-)
Post
LDHi (+)
(g)
MIA PaCa-2
[1-13C]Ala/Pyr
[1-13C]Bic/Pyr
Relative value
([1-13C]Pyr = 1)
Pre
LDHi(-)
Post
LDHi(+)
Ant
0
8

Figure S1 (related to Figures 1, 3) : **(a)** Native gel dehydrogenase assays demonstrate LDHi specificity. The bands indicate redox reactions by human (h) LDH isoforms in the gels. NCI-006 suppresses redox reactions by all hLDH isoforms in a dose dependent manner but does not affect either malate dehydrogenase (MDH) or succinate dehydrogenase (SDH). The proteins were obtained from human kidney tissue. Ten µg protein was applied in each lane. **(b)** Control and 1 µM LDHi tracings from Fig. 1g are replotted to demonstrate the small but significant increase in basal OCR caused by 1 µM LDHi. Data from the boxed time points were reanalyzed and are displayed in Fig. 1h. **(c)** Schematic representation of possible metabolic fluxes of HP [1-^{13}C]pyruvate. **(d)** [1-^{13}C]Lac/Pyr ratio change in MIA PaCa-2 xenografts after orally administered LDHi (50 mg/kg). The study was performed as in Fig.3a. Mice bearing MIA PaCa-2 tumors were orally administered 50 mg/kg NCI-006. At 0.5, 2, 3.5, 4, 5, 6 h after LDHi administration, HP ^{13}C-MR spectroscopy was performed. The "pre-treatment" [1-^{13}C]Lac/Pyr ratio was set to 1, and the relative values of [1-^{13}C]Lac/Pyr ratio at each time point are shown. Significantly decreased [1-^{13}C]Lac/Pyr ratio in tumor xenografts was not observed with orally administered LDHi at any of the time points examined. **(e)** Representative ^{13}C-MR spectra and signal intensity curves of [1-^{13}C]Pyr, [1-^{13}C]Lactate (Lac), [1-^{13}C]Alanine (Ala) and H^{13}CO3- (Bic) detected in a HT29 xenograft after HP [1-^{13}C]Pyr injection. Lactate production is suppressed following intravenous LDHi administration. The scale bar of the MR image is 8 mm. This scale is the same for all MR images shown. **(f)** [1-^{13}C]Lac/Pyr ratio change calculated by ^{13}C MR spectra in non-tumor bearing leg. [1-^{13}C]Lac/Pyr ratio significantly decreased after LDHi administration (50 mg/kg, IV), but the change in ratio was less than that seen for MIA-PaCa-2 and HT29 tumor xenografts (Fig. 3g). **(g)** [1-^{13}C]Ala/Pyr and [1-^{13}C]Bic/Pyr ratios in MIA PaCa-2 were calculated using ^{13}C MR spectra. [1-^{13}C]Ala/Pyr ratio in MIA PaCa-2 tumors was not changed significantly by LDHi administration, while [1-^{13}C]Bic/Pyr ratio in MIA PaCa-2 was significantly increased (* $p < 0.05$, t-test). Data are presented as mean value with individual values overlaid.

Fig. S2

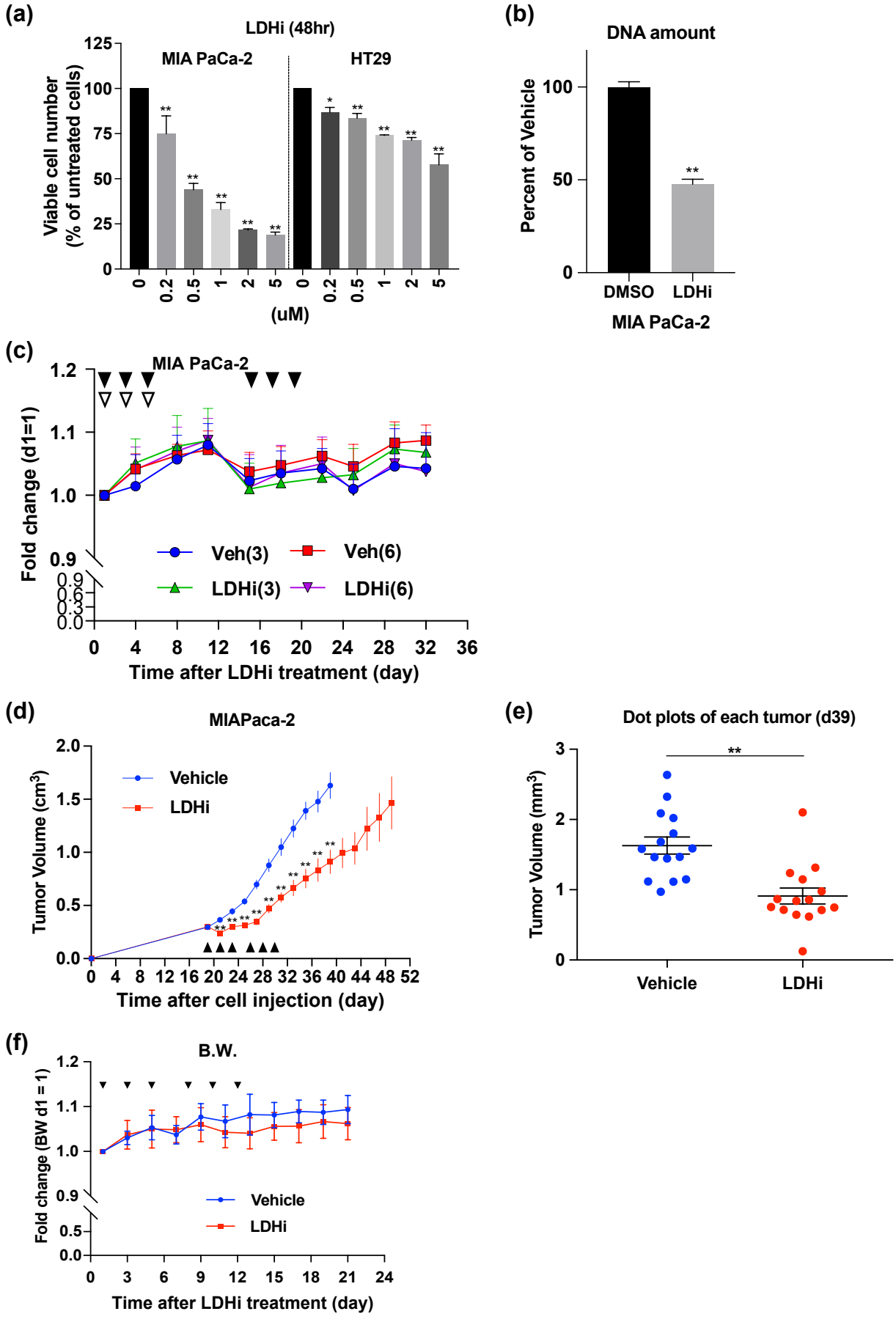

(a)
LDHi (48hr)
MIA PaCa-2
HT29
Viable cell number (% of untreated cells)
125
100
75
50
25
0
0 0.2 0.5 1 2 5 0 0.2 0.5 1 2 5
(uM)
(b)
DNA amount
Percent of Vehicle
100
50
0
DMSO LDHi
MIA PaCa-2
(c)
MIA PaCa-2
Fold change (d1=1)
1.2
1.1
1.0
0.9
0.9
0.6
0.3
0.0
Veh(3)
Veh(6)
LDHi(3)
LDHi(6)
0 4 8 12 16 20 24 28 32 36
Time after LDHi treatment (day)
(d)
MIAPaca-2
Tumor Volume (cm3)
2.0
1.5
1.0
0.5
0.0
Vehicle
LDHi
0 4 8 12 16 20 24 28 32 36 40 44 48 52
Time after cell injection (day)
(e)
Dot plots of each tumor (d39)
Tumor Volume (mm3)
3
2
1
0
Vehicle
LDHi
(f)
B.W.
Fold change (BW d1 = 1)
1.2
1.1
1.0
0.9
0.5
0.0
Vehicle
LDHi
0 3 6 9 12 15 18 21 24
Time after LDHi treatment (day)

Figure S2 (related **to Figure 5): (a**) Cell proliferation assay *in vitro* with LDHi treatment. A total of $3x10^5$ cells were plated in six-well plates on day 1 and NCI-006 was added on day 2. After 48h, viable (trypan blue excluding) cells were counted. 0.02% (V/V) DMSO was used as vehicle control. **(b)** Cell proliferation assay *in vitro* assessed by total DNA content. A total of $3x10^5$ cells were plated in six-well plates on day 1, and medium was changed to a medium containing 200 nM NCI-006 (or 0.02% DMSO) on day 2. After 48 h, DNA content was determined. LDH inhibitor significantly reduced DNA content compared to vehicle controls (n=8/group, **$p < 0.01$). **(c)** Mouse body weights during treatment with NCI-006. The day mice received the first IV administration of NCI-006 is set to day 1. Arrowhead (white) denotes each intravenous administration in Veh-3 and LDHi-3 groups, and arrowhead (black) denotes each intravenous administration in Veh-6 and LDHi-6 groups (see Fig. 5). Body weight of each mouse at day 1 is set to 1. The data are presented as the mean ± SEM. (**d**) Tumor growth with or without LDHi treatment. A total of 3 x 10^6 MIA PaCa-2 cells was subcutaneously injected into the right leg of athymic mice. LDHi treatment started 18 days after cell inoculation. 50 mg/kg NCI-006 was intravenously injected three times (every other day) per week (one cycle), and the mice were treated for two cycles (6 total IV injections). MIA PaCa-2 tumor growth in LDHi-treated mice is significantly lower than that in control. Data are presented as the mean ± SEM (** $p < 0.01$, t-test at each day). (**e**) Dot plot of individual tumor volumes on day 39 after cell inoculation. The mean tumor volume of the LDHi-treated mice is significantly lower than that of the vehicle treated mice. Data are presented as mean ± SEM **(**** $p < 0.01$, t-test). (**f**) Comparison of body weight in LDHi treated mice compared to vehicle treated mice.

Fig. S3

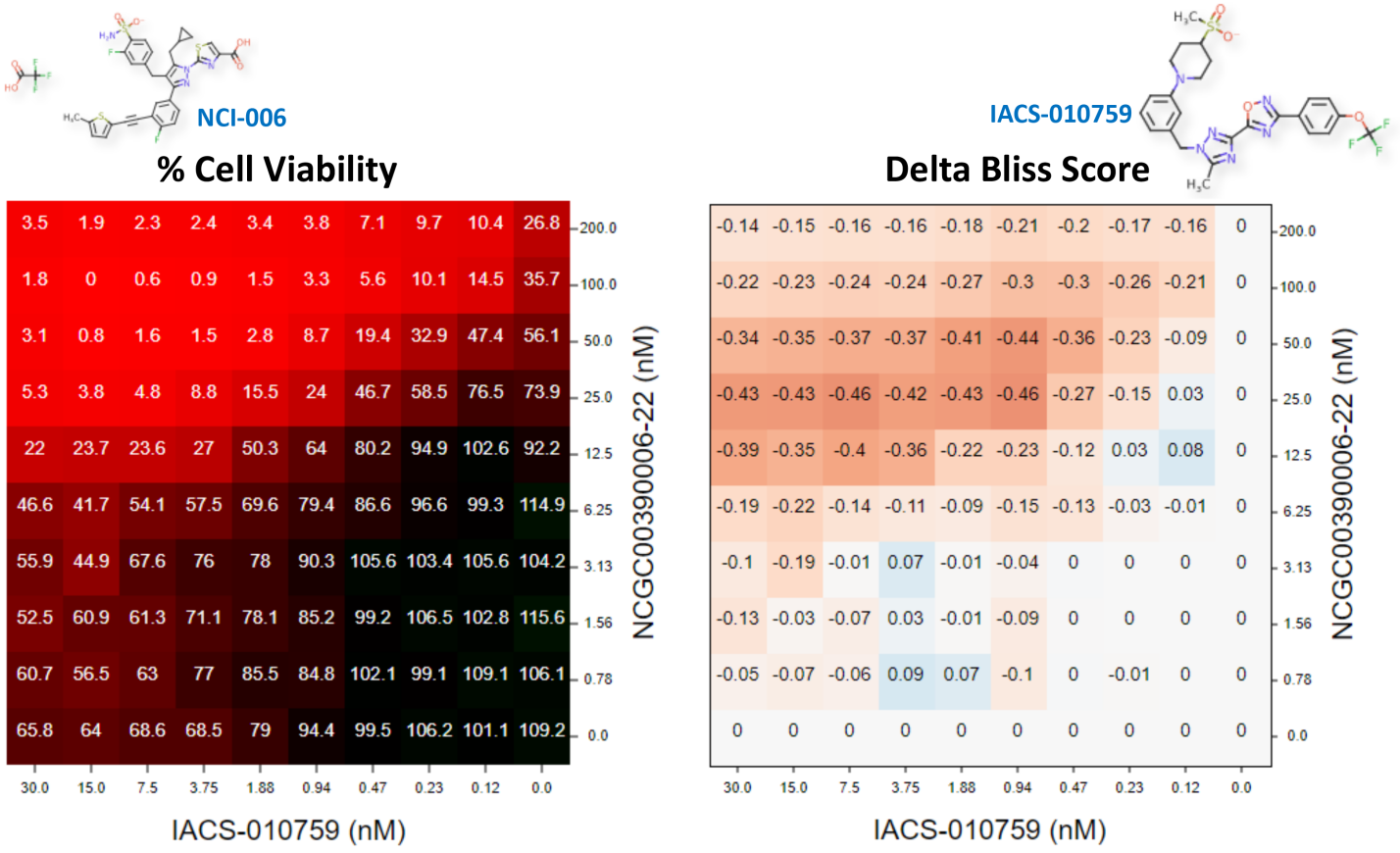


Figure S3 (related to Figure 6): An *in vitro* synergy screen demonstrating synergy between the mitochondrial complex 1 inhibitor IACS-010759 and the LDH inhibitor NCI-006 in causing MIA PaCa-2 cell death. Synergistic drug combinations affecting cell viability are shown in the left panel. The bright red squares reflect synergistic drug concentrations. In the right panel, the darker the salmon color of the squares (depicting various combinatorial drug concentrations) the greater the significance of the synergy. Please see STAR Methods for more detail.

Fig. S4

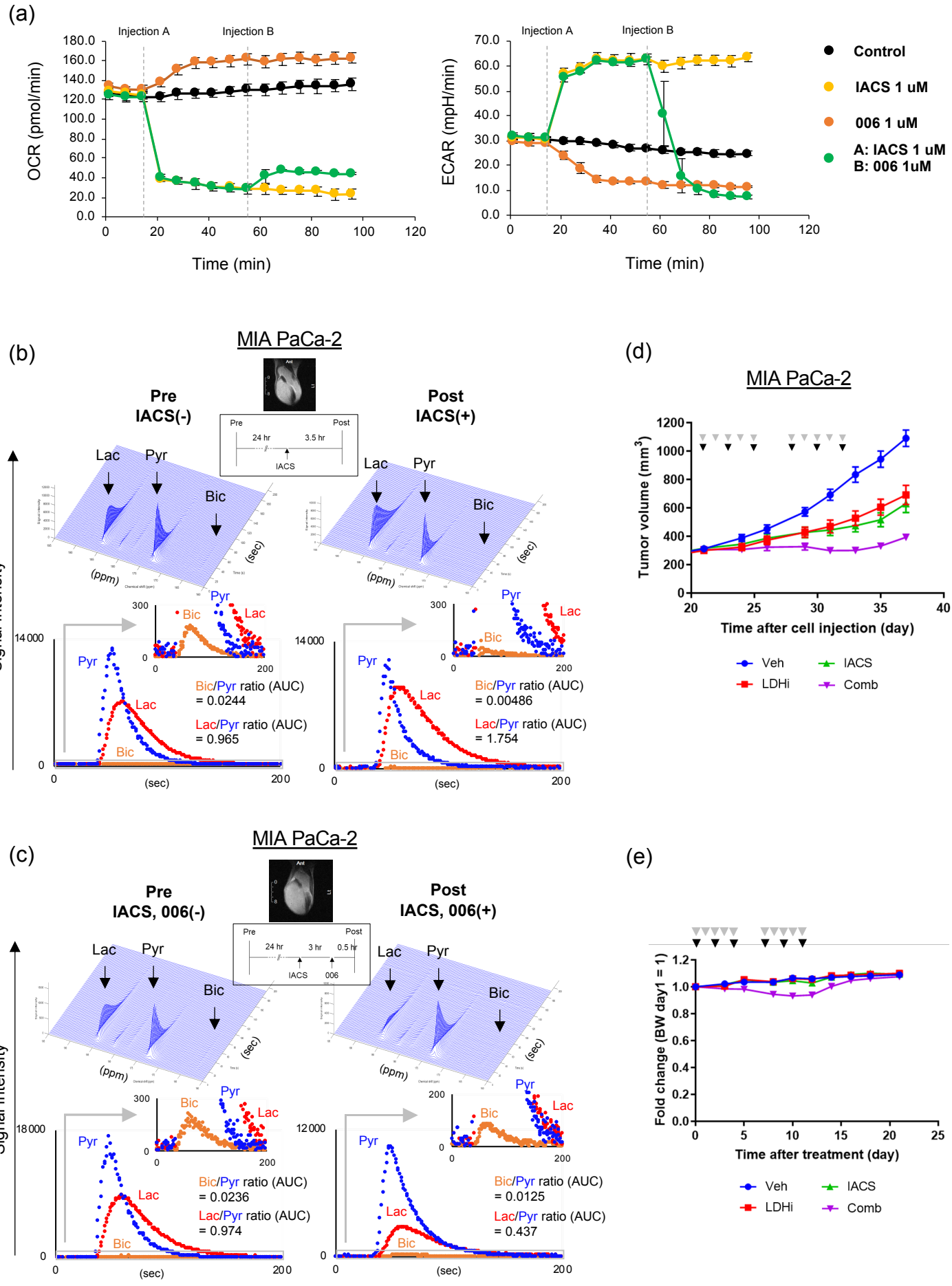

(a)
Injection A
Injection B
OCR (pmol/min)
Time (min)
ECAR (mpH/min)
Control
IACS 1 uM
006 1 uM
A: IACS 1 uM
B: 006 1uM
(b)
MIA PaCa-2
Pre
IACS(-)
Post
IACS(+)
Pre
Post
24 hr
3.5 hr
IACS
Lac
Pyr
Bic
(ppm)
(sec)
Signal intensity
Bic/Pyr ratio (AUC)
= 0.0244
Lac/Pyr ratio (AUC)
= 0.965
Bic/Pyr ratio (AUC)
= 0.00486
Lac/Pyr ratio (AUC)
= 1.754
(d)
MIA PaCa-2
Tumor volume (mm3)
Time after cell injection (day)
Veh
IACS
LDHi
Comb
(c)
MIA PaCa-2
Pre
IACS, 006(-)
Post
IACS, 006(+)
Pre
Post
24 hr
3 hr
0.5 hr
IACS
006
Bic/Pyr ratio (AUC)
= 0.0236
Lac/Pyr ratio (AUC)
= 0.974
Bic/Pyr ratio (AUC)
= 0.0125
Lac/Pyr ratio (AUC)
= 0.437
(e)
Fold change (BW day1 = 1)
Time after treatment (day)
Veh
IACS
LDHi
Comb

Figure S4 (related to Figure 6): (a) The levels of OCR and ECAR were evaluated with or without the drug treatments in MIA PaCa-2 cells. IACS-010759 (1 uM) decreased the OCR and increased the ECAR, whereas NCI-006 (1 uM) increased the OCR and decreased the ECAR. The levels of OCR and ECAR were both decreased with the combination of IACS-010759 (1 uM; injection A) and NCI-006 (1uM; injection B). (**b**) Representative ^{13}C MR spectra (upper) and signal intensity curves (lower) in a MIA Paca-2 xenograft before and after IACS-010759 treatment. IACS-010759 (2.5 mg/kg) was orally administered 3.5 h before HP ^{13}C MR spectroscopy. [^{13}C] Lac/Pyr ratio was increased and [^{13}C] Bic/Pyr ratio was decreased after IACS-010759 administration. The scale bar of the MR image is 8 mm. **(c)** Representative ^{13}C MR spectra (upper) and signal intensity curves (lower) in a MIA PaCa-2 xenograft before and after the combination treatment of IACS-010759 and NCI-006. The scale bar of the MR image is 8 mm. [^{13}C] Lac/Pyr ratio and [^{13}C] Bic/Pyr ratio were both decreased after combination treatment. IACS-010759 (2.5 mg/kg) was orally administered 3 h before NCI-006 administration, and NCI-006 (50 mg/kg) was intravenously administered 30 min before HP ^{13}C MR spectroscopy. **(d)** Combination treatment with NCI-006 and IACS-010759 produced the most profound reduction in tumor growth when compared with vehicle and either monotherapy (same experiment as Fig. 6e and 6f). Drug treatments are delineated by black arrowhead (NCI-006, 50 mg/kg, IV) and gray arrowhead (IACS-010759, 20 mg/kg, oral). The data are presented as mean ± SEM. **(e)** Mouse body weights during the treatments. Mice treated with combination therapy lost weight (maximum weight loss < 10%) but recovered immediately upon treatment cessation (same experiment as Fig. 6e, 6f, S4d). Drug treatments are delineated by black arrowhead (NCI-006, 50 mg/kg, IV) and gray arrowhead (IACS-010759, 20 mg/kg, oral). The data are presented as mean ± SEM.

Fig. S5

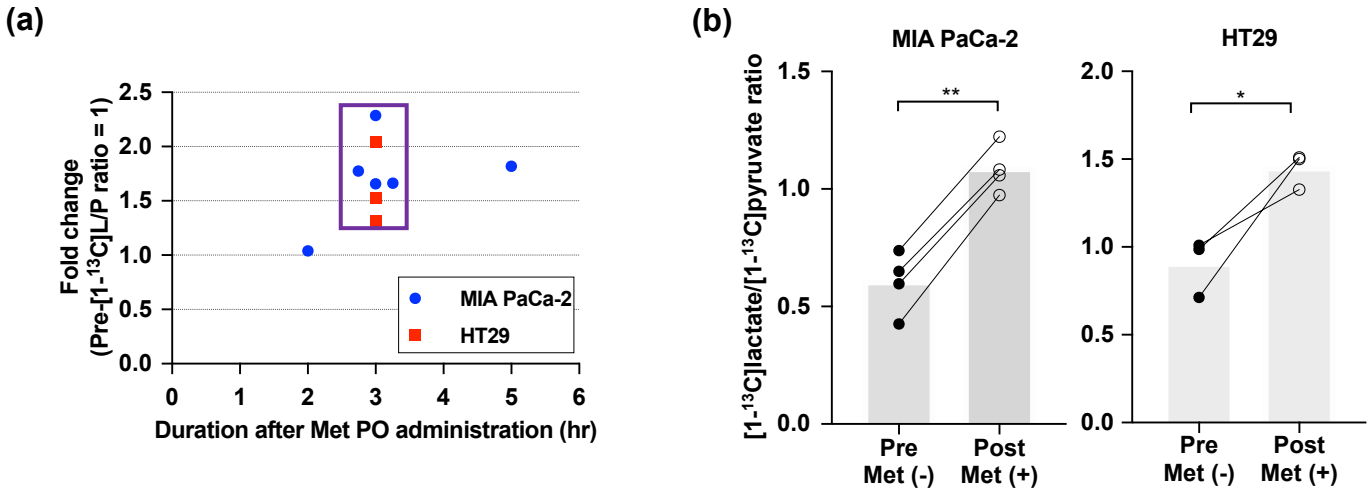


Figure S5 (related to Figure 6): Monitoring the impact of metformin on *in vivo* metabolic flux using HP-MRSI. (a) Change in [13C]Lac/Pyr ratio in MIA PaCa-2 and HT29 xenografts before and after oral administration of 50 mg/kg metformin. [13C]Lac/Pyr ratio in MIA PaCa-2 and HT29 xenografts increases 3 h after administration and remains elevated for up to 5 h. **(b)** [13C]Lac/Pyr ratio is significantly increased 3 h after oral administration of 50 mg/kg metformin in MIA PaCa-2 (n = 4) and HT29 (n = 3) tumors (* $p < 0.05$, ** $p < 0.01$). The data are presented as mean value with individual values overlaid.

Fig. S6

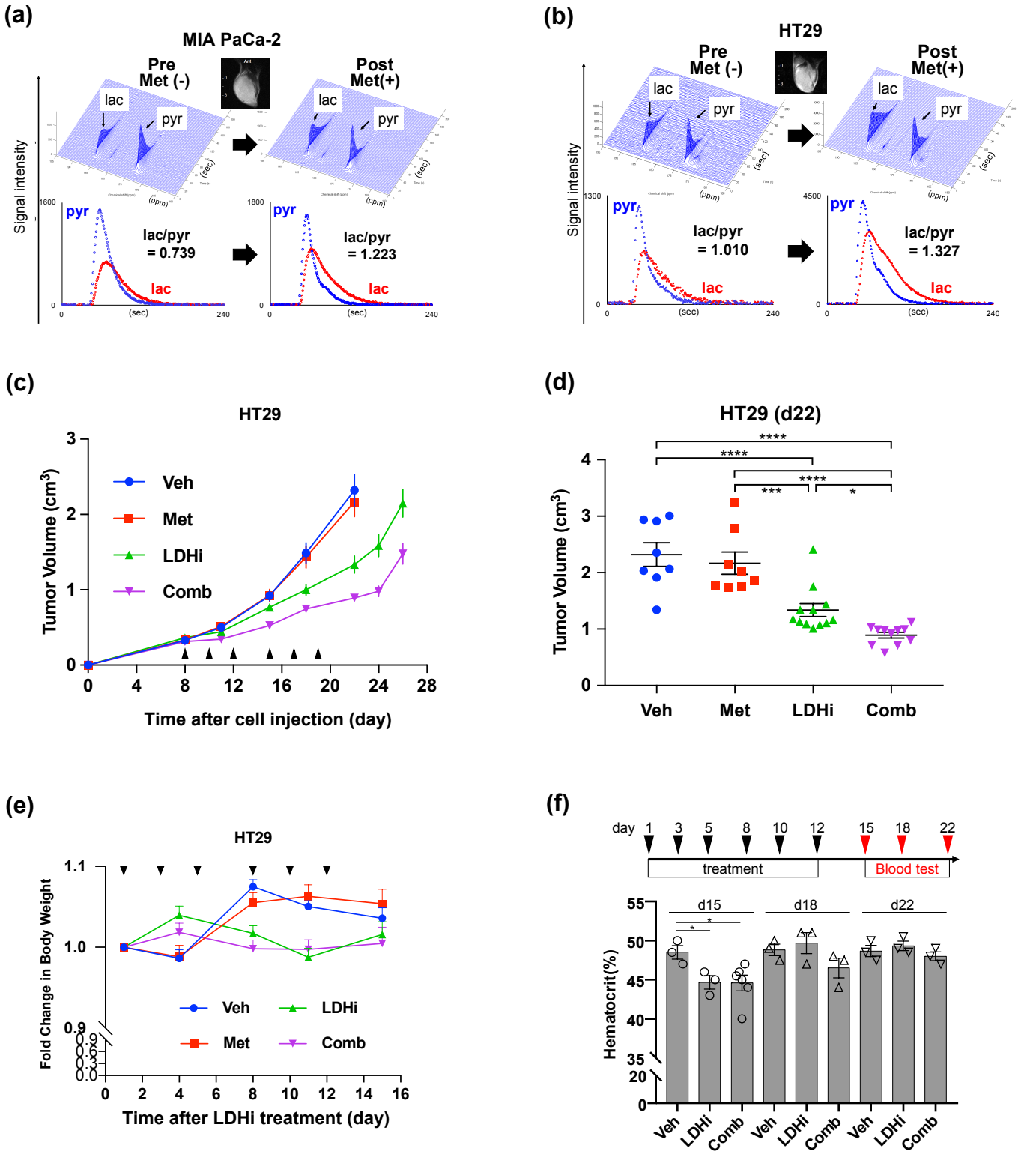

(a)
MIA PaCa-2
Pre Met (-)
Post Met(+)
lac
pyr
Signal intensity
lac/pyr = 0.739
lac/pyr = 1.223
(sec)
(b)
HT29
lac/pyr = 1.010
lac/pyr = 1.327
(c)
HT29
Veh
Met
LDHi
Comb
Tumor Volume (cm3)
Time after cell injection (day)
(d)
HT29 (d22)
(e)
HT29
Fold Change in Body Weight
Time after LDHi treatment (day)
(f)
day 1 3 5 8 10 12 15 18 22
treatment
Blood test
d15
d18
d22
Hematocrit(%)

Figure S6 (Related to Figure 6): (**a**)(**b**) Representative [^{13}C]-MR spectra (upper) and signal intensity curves (lower) in MIA PaCa-2 (**a**) and HT29 (**b**) xenografts obtained from pre-metformin treatment (left) and post-metformin treatment (right). In the "post" evaluations, ^{13}C-MR spectra were collected 3 h after metformin administration. The scale bar of the MR images is 8 mm. Signal level of [^{13}C]Lac is clearly increased by metformin in both MIA PaCa-2 and HT29 tumors. (**c**) 3 x 10^6 HT29 cells were subcutaneously inoculated into the right leg of athymic mice. Drug treatments are delineated by black arrowheads. NCI-006 (30 mg/kg) was administered intravenously and metformin (50 mg/kg) was administered by oral gavage. When given together, metformin was dosed 2 h before NCI-006. Drugs were administered every other day as depicted by the arrowheads, for a total of six treatments. Tumor growth rates are shown. **(d)** Individual tumor volumes measured at day 22 in HT29 are significantly reduced in mice treated with the drug combination compared to the other groups. (* $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; one-way ANOVA with uncorrected Fisher's LSD). Veh: vehicle, Met: metformin, Comb: combination (NCI-006 + metformin). **(e)** Change in body weight during treatment in mice bearing HT29 xenografts. Body weight of each mouse at day 1 is set to 1. Arrowheads indicate each drug administration. The data are presented as mean ± SEM. **(f)** Mouse hematocrit was reduced by < 10 % 3 days after completing 2 cycles (6 IV injections of NCI-006 +/- metformin administered on days 1, 3, 5, 8, 10, 12). When used alone, NCI-006 was administered at 50 mg/kg. When used together with metformin, NCI-006 was administered at 30 mg/kg. All hematocrits returned to normal levels by 6 – 10 days after the last drug dose. * $p < 0.05$, t-test.