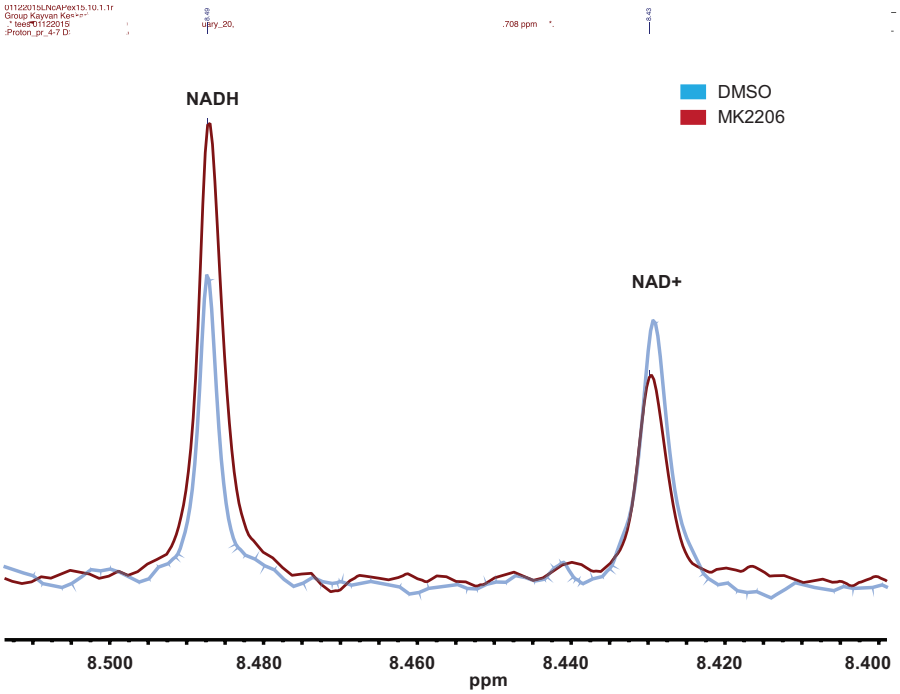
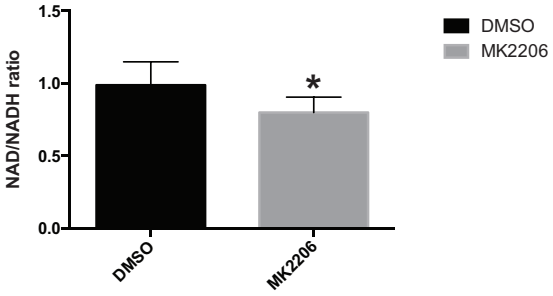
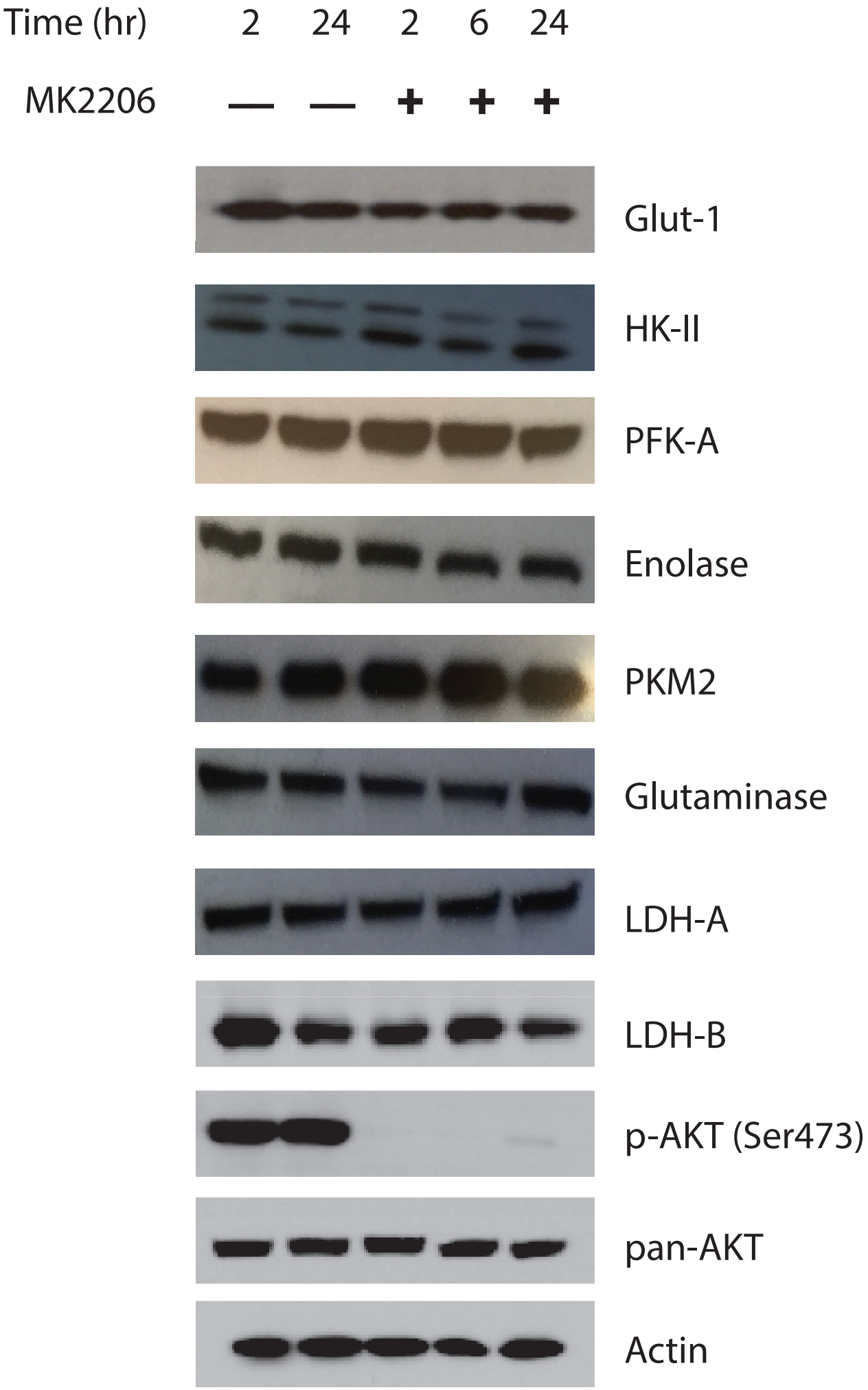
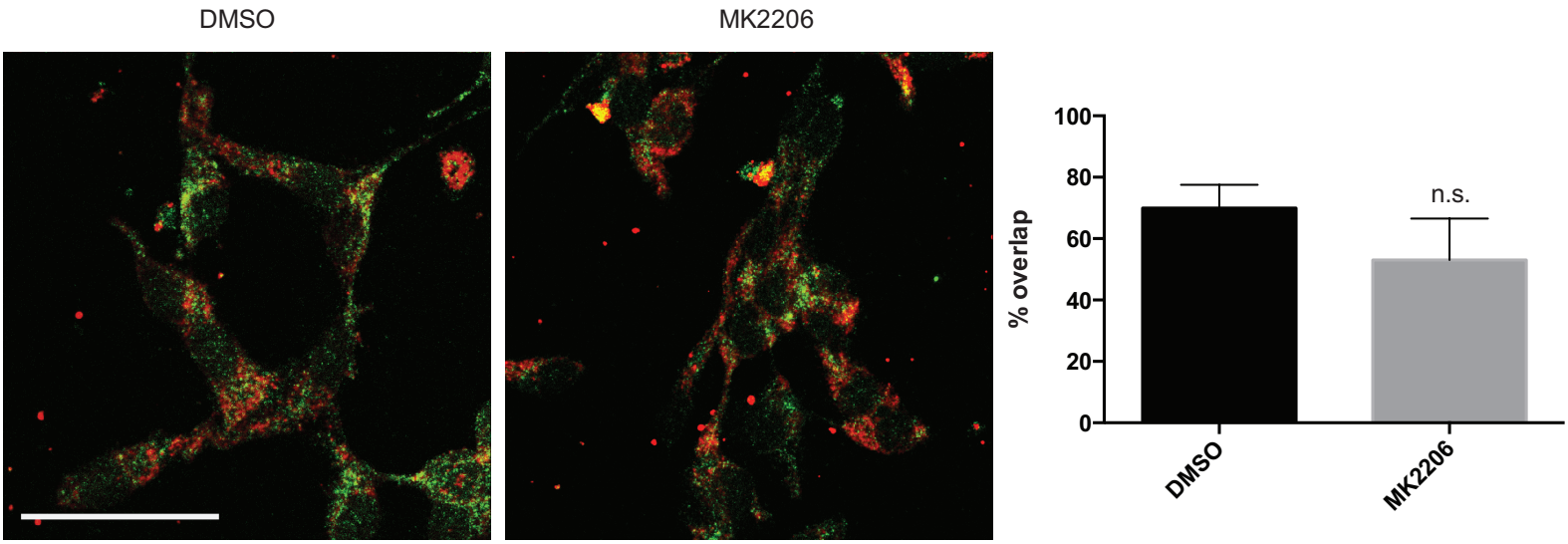
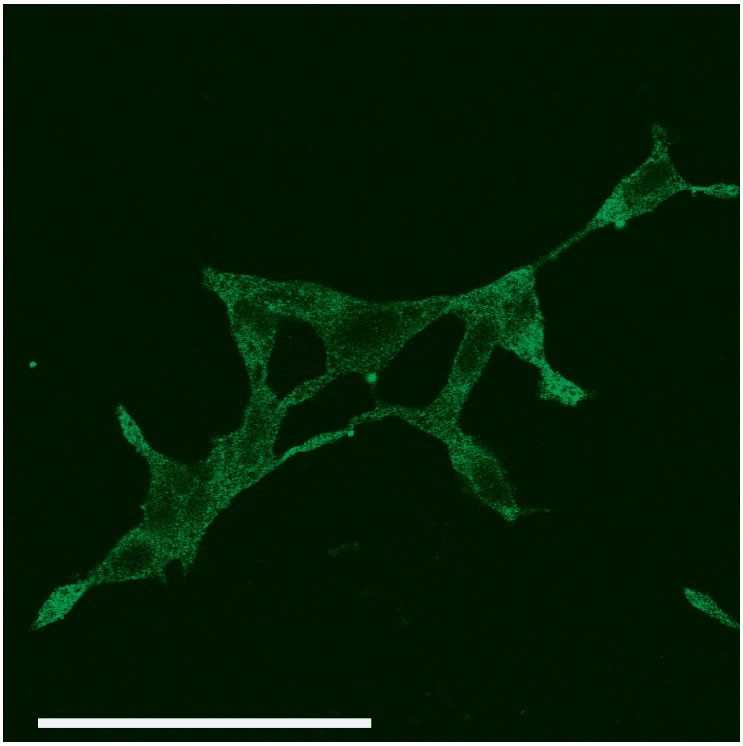

NADH
NAD+
DMSO
MK2206
8.500
8.480
8.460
8.440
8.420
8.400
ppm


NAD/NADH ratio
1.5
1.0
0.5
0.0
*
DMSO
MK2206
DMSO
MK2206

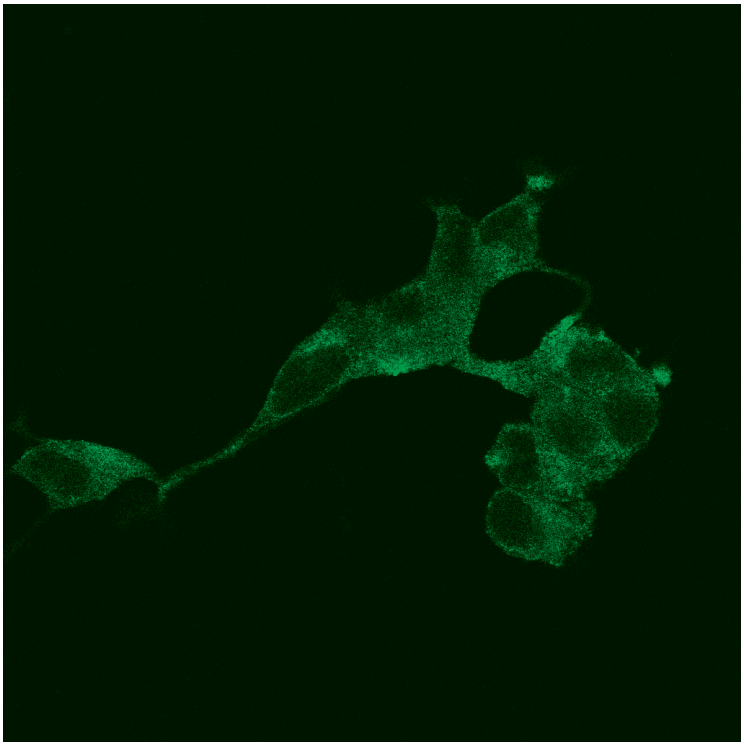

Time (hr)
2
24
2
6
24
MK2206
—
—
+
+
+
Glut-1
HK-II
PFK-A
Enolase
PKM2
Glutaminase
LDH-A
LDH-B
p-AKT (Ser473)
pan-AKT
Actin

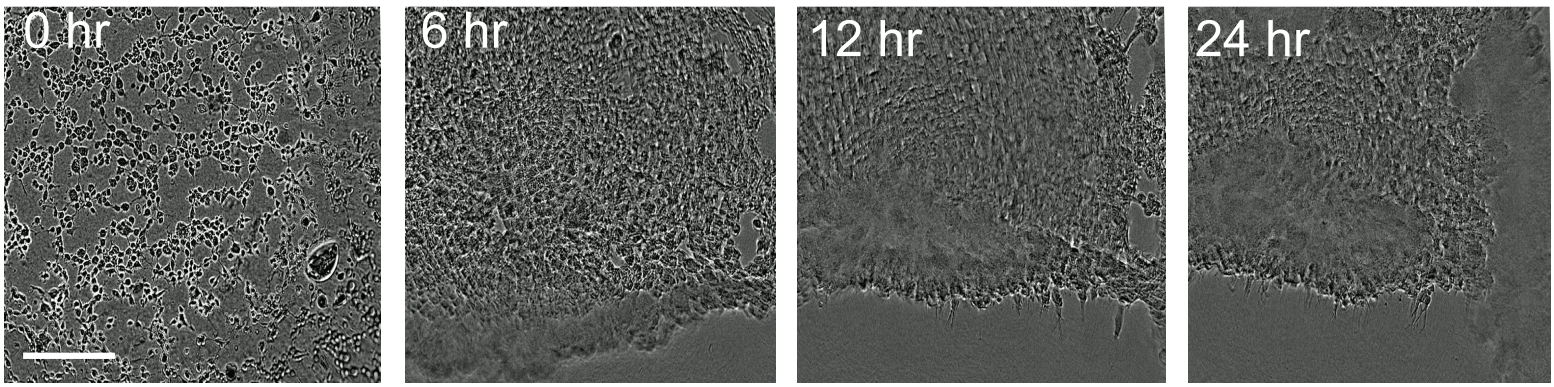

DMSO
MK2206
100
80
60
40
20
0
% overlap
n.s.
DMSO
MK2206

DMSO



MK2206




0 hr
6 hr
12 hr
24 hr

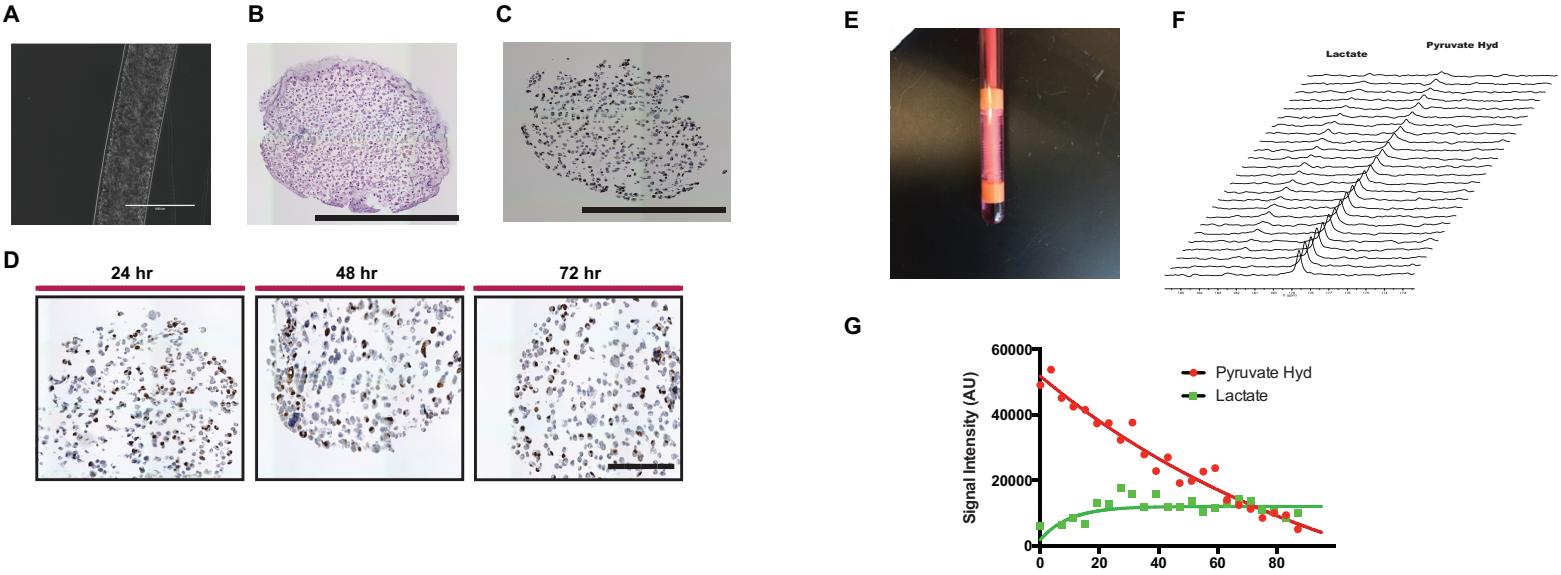


Fig 1: (A) Bright-field image of an alginate thread in culture and (B) H&Estainof the same thread and (C) another cross-section stained with Ki-67.
(D) Representative images of an alginate thread cultured in a rotating plate over a period of 4 days and stained with Ki67 All scale bars represent 500um.

Supplemental Figure Captions

Supp. Figure 1. Proton NMR spectra of LnCAP cell extracts treated either with DMSO or 1uM MK2206 for 24 hr. Co-factor concentrations were quantified as a ratio between the resonance for the lone hydrogen atom on the five membered ring of the adenine moiety in NAD+ that resonates at 8.435 ppm and NADH at 8.486 ppm. Extractions of cells were performed as described in the materials section.

Supp. Figure 2. Western blot of metabolic proteins in LnCAP cells.

Supp. Figure 3. Co-localization analysis of HKII and CoxIV. LnCAP cells were grown on coverslips, fixed and permeablized for detection of HKII and CoxIV, coupled to Alexa488 and Alexa594 secondary antibodies respectively. Co-localization analysis was performed by the MSKCC Molecular Cytology Core using the ImageJ software. Scale bar = 100μm

Supp. Figure 4. Immunofluorescence of Glut-1. LnCAP cells were grown on coverslips, fixed and permeabilized for detection of Glut-1 coupled to Alexa488. Scale bar = 100 μm

Supp. Figure 5. Time-lapse images of spheroid formation. LnCAP cells were grown on a 1:1 mixture of sodium alginate and matrigel in ultra-low adhesion 96-well plates. Time-lapse images were acquired using a 10X objective on the Incucyte cell imager platform located in a humidified cell culture chamber. Scale bar = 1mm

Supp. Figure 6. Hyperpolarized MRS of sodium alginate thread cultures. (A) Bright-field image of an alginate thread in culture and (B) H&E stain of the same thread and (C) another cross-section stained with Ki-67. (D) Representative images of an alginate thread cultured in a rotating plate over a period of 4 days and stained with Ki67 All scale bars represent 500 μm. (E) Thread cultures wound on a custom-printed 3D insert, directly deposited into a 5mm NMR tube metabolizes hyperpolarized [1-^{13}C] pyruvate to lactate after injection of 1mM hyperpolarized pyruvate (F). Quantification of integrals of the lactate and pyruvate hydrate peaks over time (G).