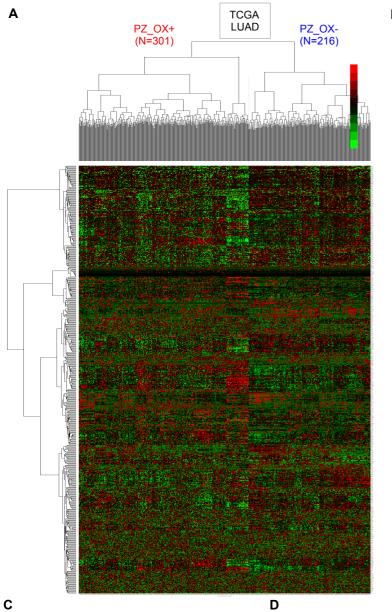


Figure S1: Proteomic comparison of PZ_{OX+} and PZ_{OX-} LUAD tumors. A) Comparative proteomics between PZ_{OX+} and PZ_{OX-} tumors using label free proteomics. The proteins that differed between the two groups are shown in the Volcano plot (Anova; p<0.05). B) The cellular functions associated with the proteins differentially expressed in PZ_{OX+} versus PZ_{OX^-} were analyzed using IPA (Qiagen) and ranked by significance (Fisher's Exact Test with multiple corrections). C) Glucose metabolizing proteins downregulated in OX+_LUADs. The reduction is shown as percentage of inhibition (PZ _{OX-} as % of PZ _{OX+}).



В

PZ_OX+

Ribosome						
Oxidative phosphorylation						
Parkinson's disease			•			
Alzheimer's disease		••••••				
Huntington's disease		••••••				
Metabolic pathways		•••••				
Metabolism of xenobiotics by cytochrome P450		•				
Systemic lupus erythematosus)				
Non-alcoholic fatty liver disease (NAFLD)						
Cell cycle	• • • • • •					
Alcoholism	• • • • • • • • • • • • • • • • • • • •					
Cardiac muscle contraction	•••••					
Maturity onset diabetes of the young	• • • • • • • • • • • • • • • • • • • •					
Chemical carcinogenesis	•••••					
Pyrimidine metabolism	••••					
Biosynthesis of amino acids	••••					
Drug metabolism – cytochrome P450	• • • • • • • • • • • • • • • • • • • •					
Phenylalanine metabolism	• • • •					
Steroid hormone biosynthesis	• • • • • • • • • • • • • • • • • • • •					
Glycine, serine and threonine metabolism	• • • • • • • • • • • •					
Ascorbate and aldarate metabolism						
Arginine and proline metabolism						
Pentose and glucuronate interconversions	• •					
DNA replication	•••					
Glutathione metabolism	•••					
Drug metabolism – other enzymes						
Oocyte meiosis	• •					
Alanine, aspartate and glutamate metabolism	•					
Tyrosine metabolism	• • • • • • • • • • • • • • •					
Purine metabolism	•					
	0	5	10	15	20	25

Ε

PZ-OX-

-log10 FDR

.

•

•

•

.

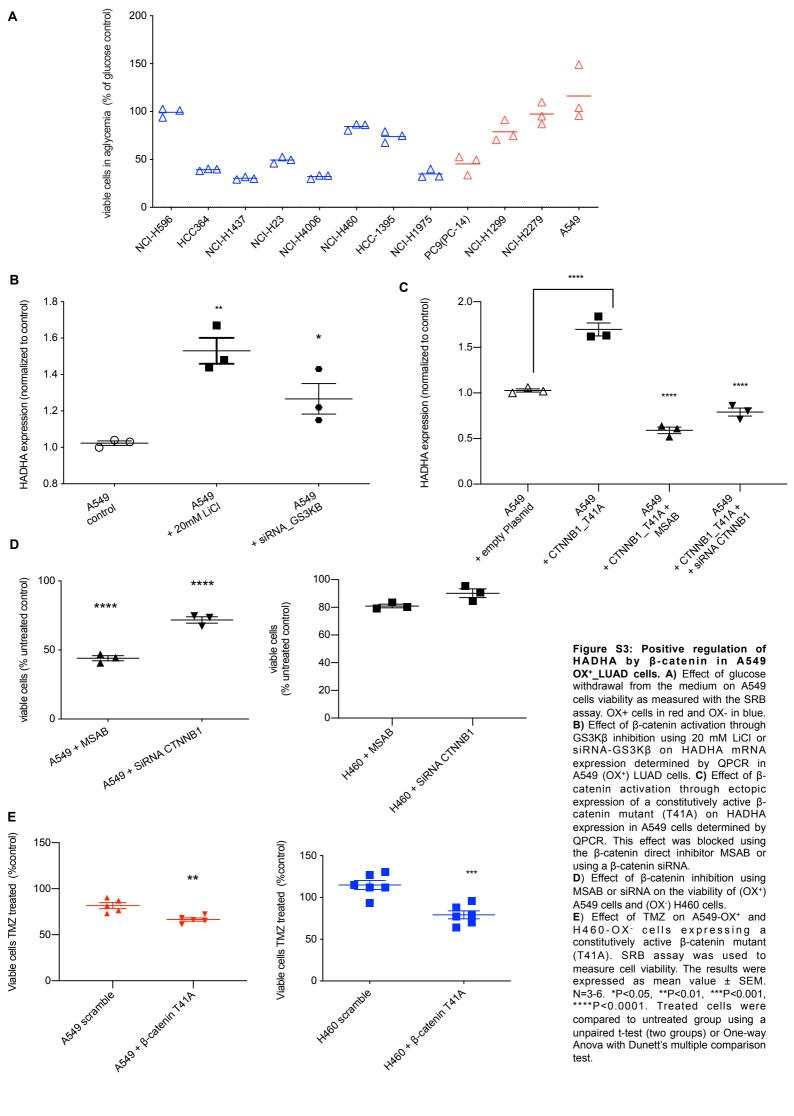
0 2 4 6 8 10

-log10 FDR

		PZ	<u>z_0</u>	X+
SRP-dependent cotranslational protein targeting to membrane translation viral transcription				•
nuclear-transcribed mRNA catabolic process, nonsense-mediated decay translational initiation			•	•
rRNA processing mitochondrial translational elongation		•	•	
mitochondrial translational termination mitochondrial ATP synthesis coupled proton transport cell division		, • ,		
mitochondrial electron transport, NADH to ubiquinone mitotic nuclear division))		
protein heterotetramerization DNA replication-dependent nucleosome assembly telomere organization	· · · •			
regulation of gene silencing ATP biosynthetic process	· · · •			
neuropeptide signaling pathway anterior/posterior pattern specification mitotic sister chromatid segregation				
insulin secretion proximal/distal pattern formation				
cytoplasmic translation mitochondrial electron transport, cytochrome c to oxygen				
mitochondrial respiratory chain complex I assembly chromatin silencing at rDNA acute-phase response	••••			
embryonic skeletal system morphogenesis ATP synthesis coupled proton transport spliceosomal snRNP assembly	• •			
	ι 0	5	10	15
		-lo	g10 FD	R

	PZ_OX-	
cell adhesion	•	ECM-receptor interaction
extracellular matrix organization	• • • • • • • • • • • • • • • • • • • •	Protein digestion and absorption
inflammatory response	•	Neuroactive ligand-receptor interaction
leukocyte migration	•	Focal adhesion
immune response	•	Hematopoietic cell lineage
integrin-mediated signaling pathway	•	Cell adhesion molecules (CAMs)
cilium movement	• • • • • • • • • • • • • • • • • • • •	Platelet activation
positive regulation of cytosolic calcium ion concentration	• • • • • • • • • • • • • • • • • • • •	PI3K–Akt signaling pathway
cell chemotaxis	• • • • • • • • • • • • • • • • • • • •	Hypertrophic cardiomyopathy (HCM)
collagen catabolic process	• • •	Cytokine-cytokine receptor interaction
calcium ion transport into cytosol	••••	Osteoclast differentiation
chemokine-mediated signaling pathway	•	Dilated cardiomyopathy
transmembrane receptor protein tyrosine kinase signaling pathway	•	Arrhythmogenic right ventricular cardiomyopathy (ARVC)
homophilic cell adhesion via plasma membrane adhesion molecules	•	Amoebiasis
regulation of immune response	•	Calcium signaling pathway
chemotaxis	•	Primary immunodeficiency
cell surface receptor signaling pathway	•	Malaria
calcium ion transmembrane transport	•	Ras signaling pathway
adaptive immune response	•	Complement and coagulation cascades
activation of phospholipase C activity	•	Chemokine signaling pathway
positive regulation of ERK1 and ERK2 cascade	•	cAMP signaling pathway
muscle contraction	•	Leukocyte transendothelial migration
cell-matrix adhesion	•	Staphylococcus aureus infection
positive regulation of phosphatidylinositol 3-kinase signaling	•	Rap1 signaling pathway
regulation of postsynaptic membrane potential	•	Regulation of actin cytoskeleton
positive regulation of GTPase activity	•	cGMP-PKG signaling pathway
positive regulation of T cell proliferation	•	ABC transporters
cellular defense response	•	Adrenergic signaling in cardiomyocytes
signal transduction	•	Oxytocin signaling pathway
leukocyte cell-cell adhesion	•	Phagosome
	5 10 15 20	
	-log10 FDR	

Figure S2: A) Hierarchical clustering of the TCGA-LUAD RNAseq data available from the Genomic Data Commons Portal (<u>https://portal.gdc.cancer.gov/</u>) using the full proteomic signature of oxidative tumors (OX⁺PZ/OX⁻PZ) determined from Figure S1A). B) Top 30 KEGG pathways and C) GO terms enriched in PZ_OX⁺_LUAD tumors. D) Top 30 KEGG pathways and E) GO terms enriched in PZ_OX_LUAD tumors. For B-E, differential expression analysis was performed on TCGA RNA-Seq data using DESseq2 between OX+ and OX- LUAD tumors and genes were selected based on adjusted p-value (<0.05) and fold-change (>1.5). DEseq2 compares groups using a Wald test followed by a Bonferroni multiple test correction.



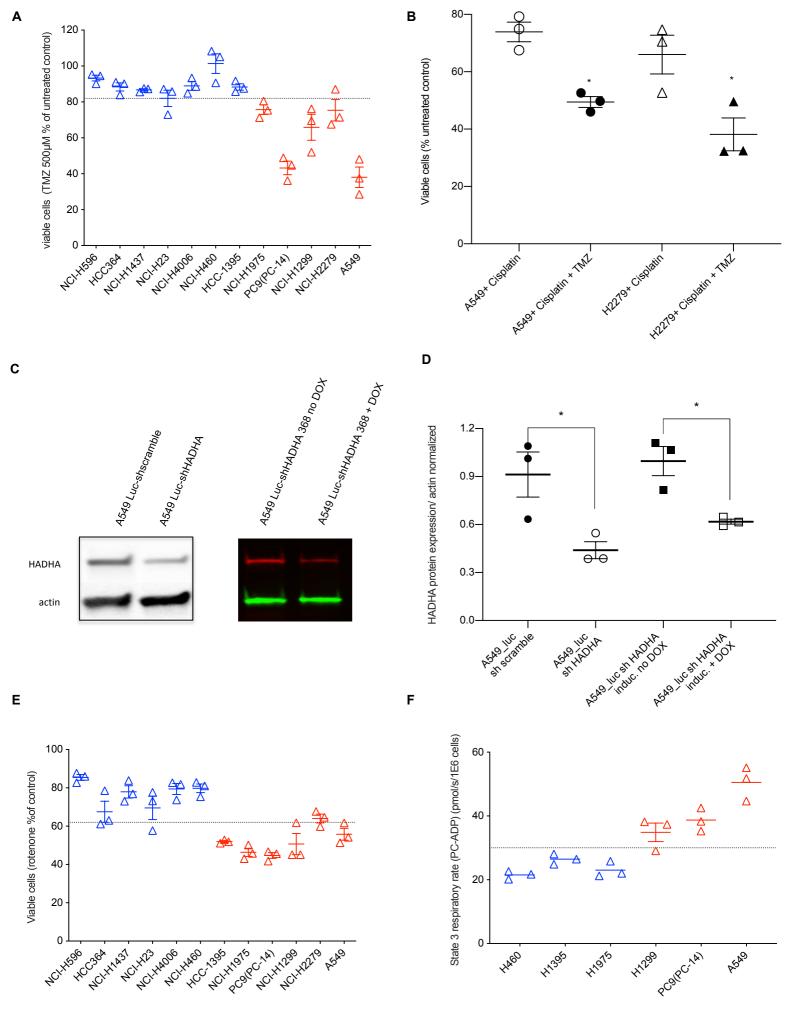


Figure S4: Pharmacological inhibition of HADHA in twelve NSCLC cancer cell lines. (A) The effect of HADHA inhibition by 500µM TMZ (24H) in OX+/HADHA+ (red) and OX-/HADHA- (blue) lung cancer cell lines was determined by measuring cell viability using the SRB assay. **(B)** Effect of TMZ 500µM in co-treatment with Cisplatin 30µM for 48H on cell viability. **C)** HADHA knock-down by shHADHA or by doxycyclin (DOX) inducible shHADHA verified by westernblot. **D)** Westernblot quantification of HADHA levels as normalized to actin level. **E)** Effect of rotenone treatment on OX+ (red) and OX- (blue) NSCLC cell lines. **F)** Rate of fatty-acid oxidation coupled to OXPHOS measured by high-resolution respirometry on permeabilized human lung cancer cells fueled with palmitoyl-carnitine (PC) and ADP. Cells with low capacity of palmitoyl-carnitine oxidation are shown in blue (OX-) and cells with high capacity (OX+) are shown in red. The dashed line shows the median value of palmitate fueled coupled respiration. The data are expressed as the mean value ± SEM . N=3. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. The groups were comparing using a unpaired t-test (two groups) or One-way Anova with Dunett's multiple comparison test.

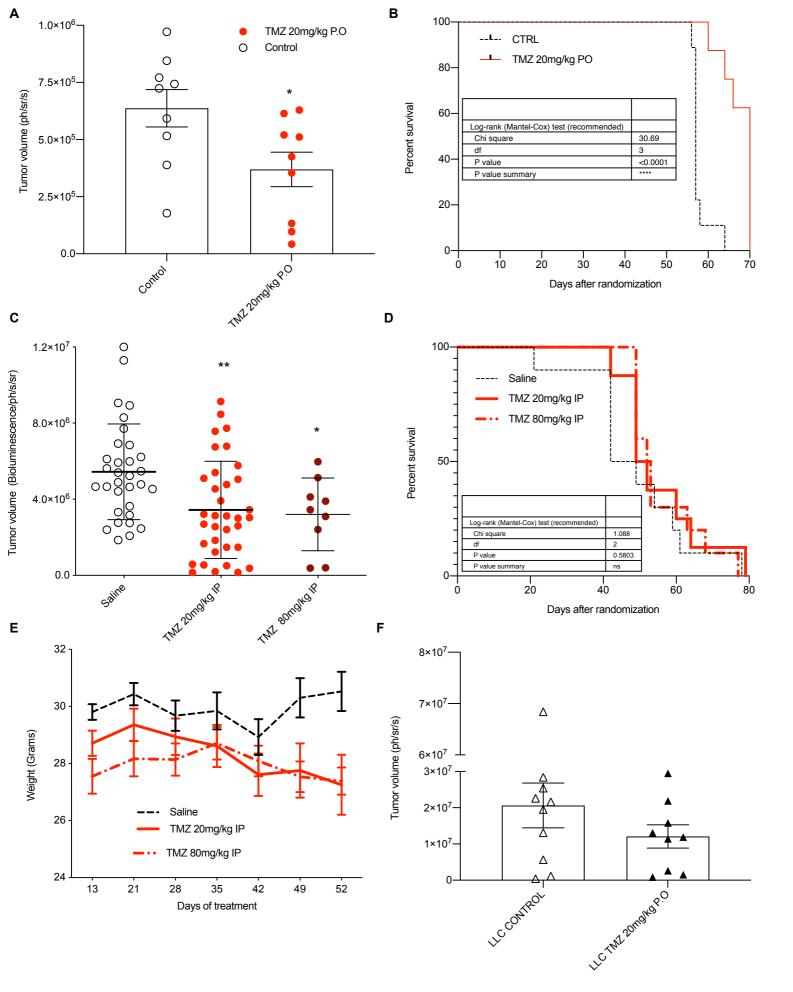


Figure S5. Anticancer effect of HADHA pharmacological inhibition using Trimetazidine administered by IP or PO on OX⁺_LUAD . A) Mice bearing orthotopic human lung adenocarcinomas obtained with OX⁺-LUAD_A549 cells were treated with TMZ 20mg/kg per oral (P.O). The volume of orthotopic A549luc-OX⁺_LUAD tumors was determined *in vivo* non-invasively using the luminescence signal. **B**) Survival curve obtained with TMZ 20mg/kg P.O treatment on A549 OX+_LUADs. **C**) Effect of intraperitoneal injection (IP) of TMZ on OX⁺_A549luc_LUAD volume. TMZ 20mg/kg or TMZ 80mg/kg was injected daily by IP. **D**) Survival curve obtained using TMZ 20mg/kg or 80mg/kg IP showing no significant effect. **E**) Body weight alteration in the animals treated with TMZ 20mg/kg or 80mg/kg by IP injection. **F**) Effect of TMZ 20mg/kg per oral (PO) administration in a immunocompetent mouse LUAD model obtained with murine LLC lung cancer cells in syngenic animals. Values represent mean ± SEM; N=10-30 for each group. *P<0.05, **P<0.01, ***P<0.001. Treated animals were compared to untreated group using One-way Anova with Dunett's multiple comparison test. Survival curves were compared using Log-rank (Mantel-Cox) test.

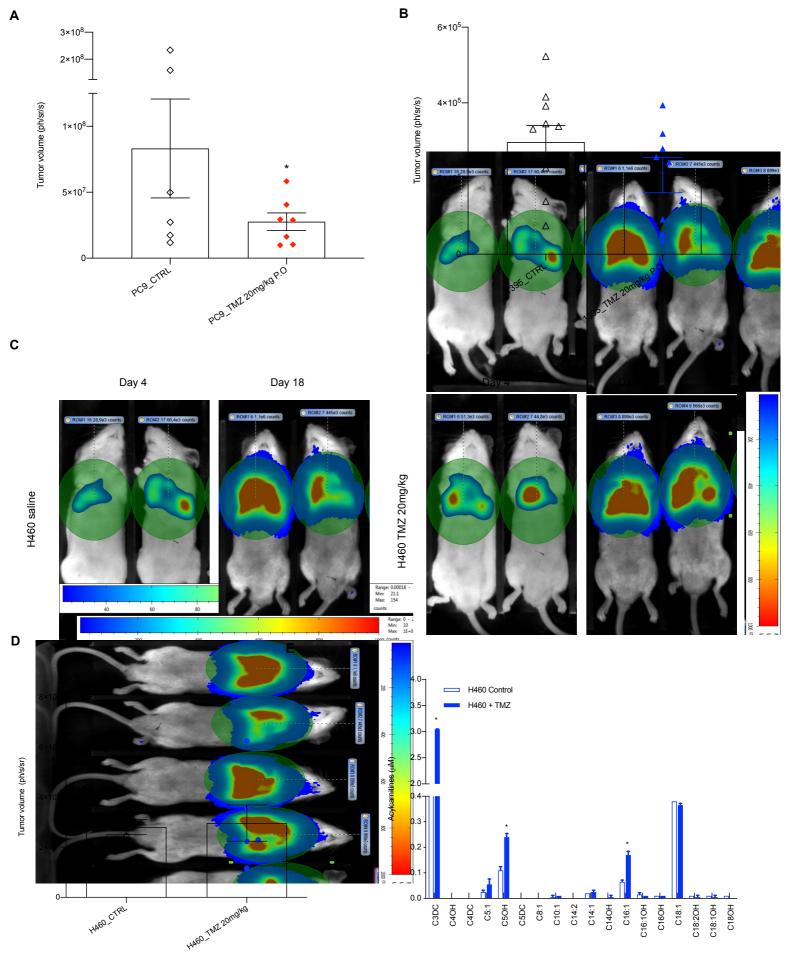
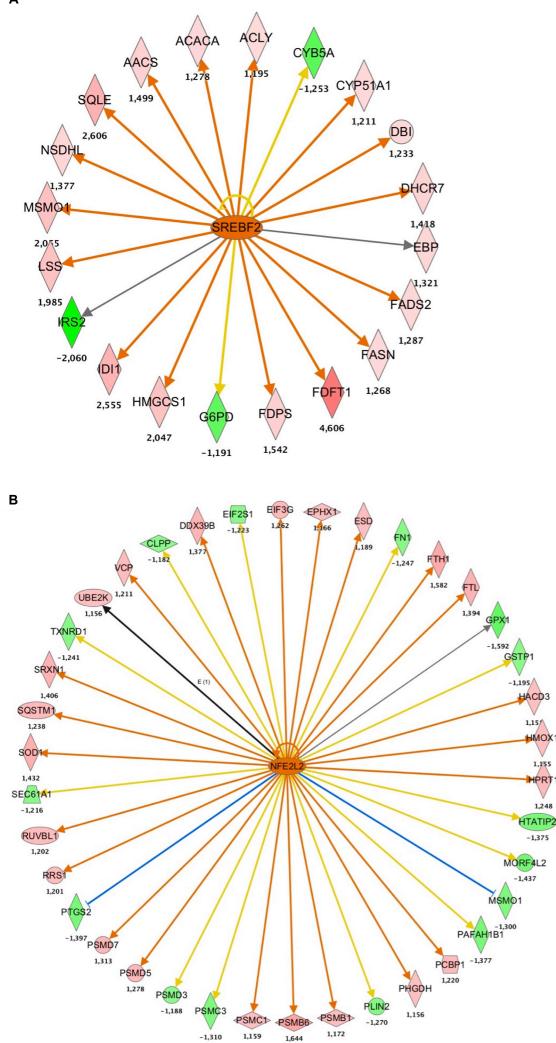


Figure S6: TMZ inhibits specifically OX+ lung tumor growth. Lung tumor growth was determined by the bioluminescence of human cancer cells injected orthotopically in the lung of NSG mice, after 18 days of growth. **A)** Results for the HADHA+/OX+ PC9 NSCLC cells and **B)** for theHADHA-/ OX- 1395 cells. The animals were treated with vehicle or TMZ (20 mg/kg) daily P.O for 18 d (n = 10 mice per group). **C and D**) Relative volume of HADHA-/OX- H460 orthotopic tumors determined in mice treated with vehicle (saline) or TMZ (20 mg/kg) daily for 18 d (n = 10 mice per group). **E)** Lipidomic analysis (mass spectromerty) of fatty-acids accumulating in H460 cells (OX-) cells treated with 500µM TMZ during 48 hours. Values represent mean ± SEM; N=4-6; N=10 *in vivo* experiments for each group. *P<0.05, **P<0.01, ***P<0.01, ***P<0.001. Treated animals were compared to untreated group using a unpaired t-test.





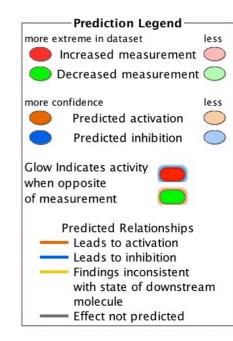


Figure S7: Predicted transcription factors activated following HADHA inhibition using TMZ or shHADHA in human lung adenocarcinoma A549 cells. (A) SREBF2. (B) NRF2. The prediction analysis was performed using IPA (Qiagen). The proteins altered by HADHA inhibition (p<0.05) were analyzed using the 'upstream analysis' module of IPA.

1,15

HMOX1

1,155

HPRT1

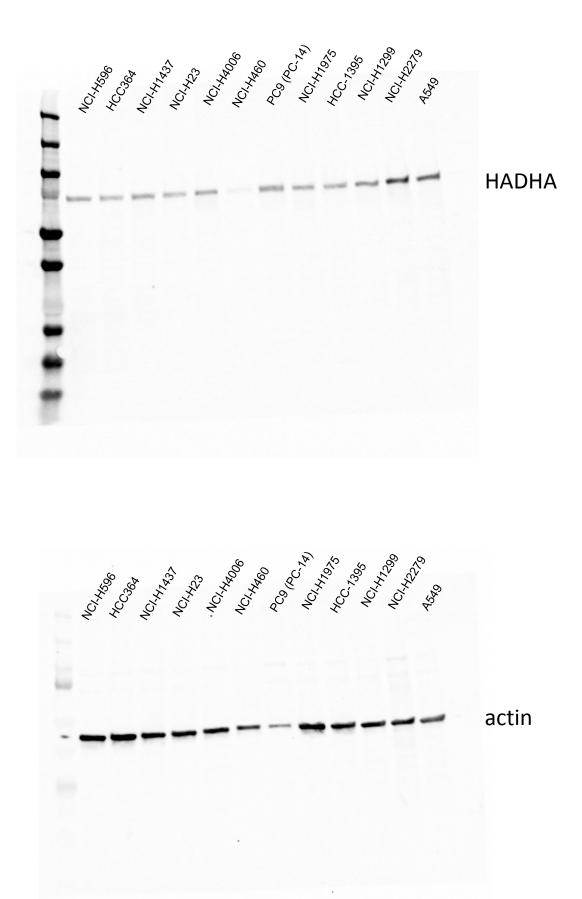
1,248

-1,375

Full unedited gels (Amoedo N.D et al.)

Figure 3 (uncropped)

Gel #1 HADHA NSCLC cell lines



Gel #2 HADHA NSCLC cell lines Figure 3 (uncropped) MCI: HCC: 1540 HADHA 1540 actin

Gel #3 HADHA NSCLC cell lines

Figure 3 (uncropped)

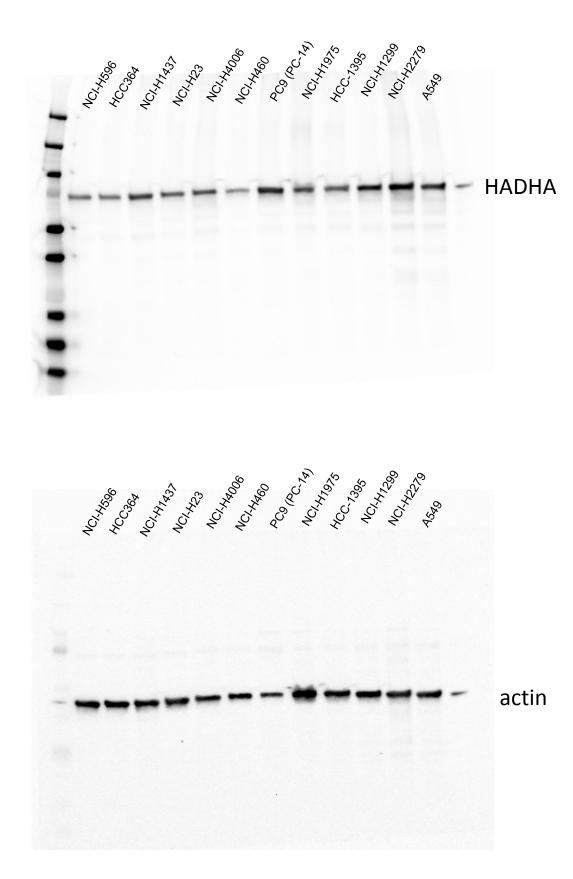


Figure S4 (uncropped)

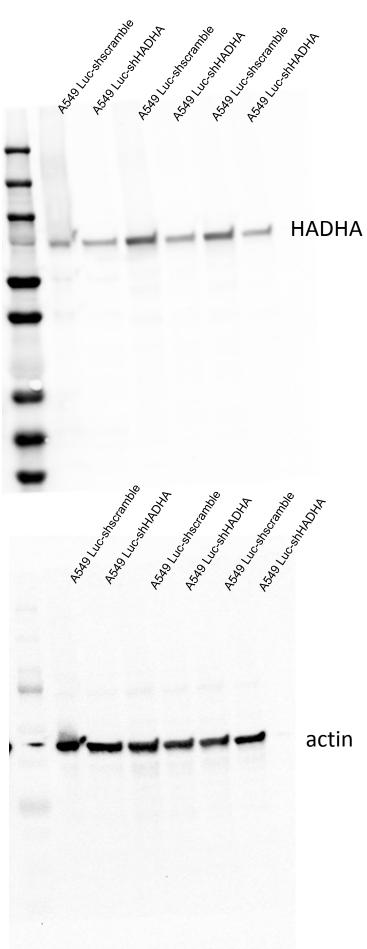
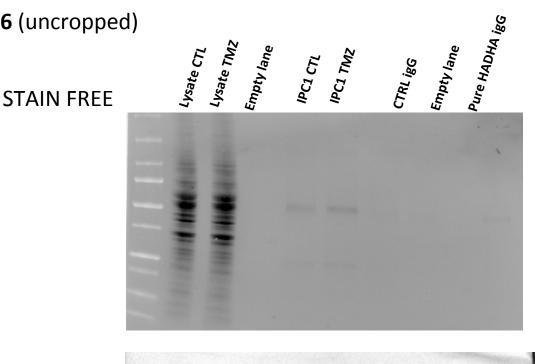


Figure 6 (uncropped)



Western blot

75

Ref. Ac HADHA ab203114 (MW 75kDa)