Supplementary File 1

High glucose induced c-Met activation promotes aggressive phenotype and regulates expression of glucose metabolism genes in HCC cells

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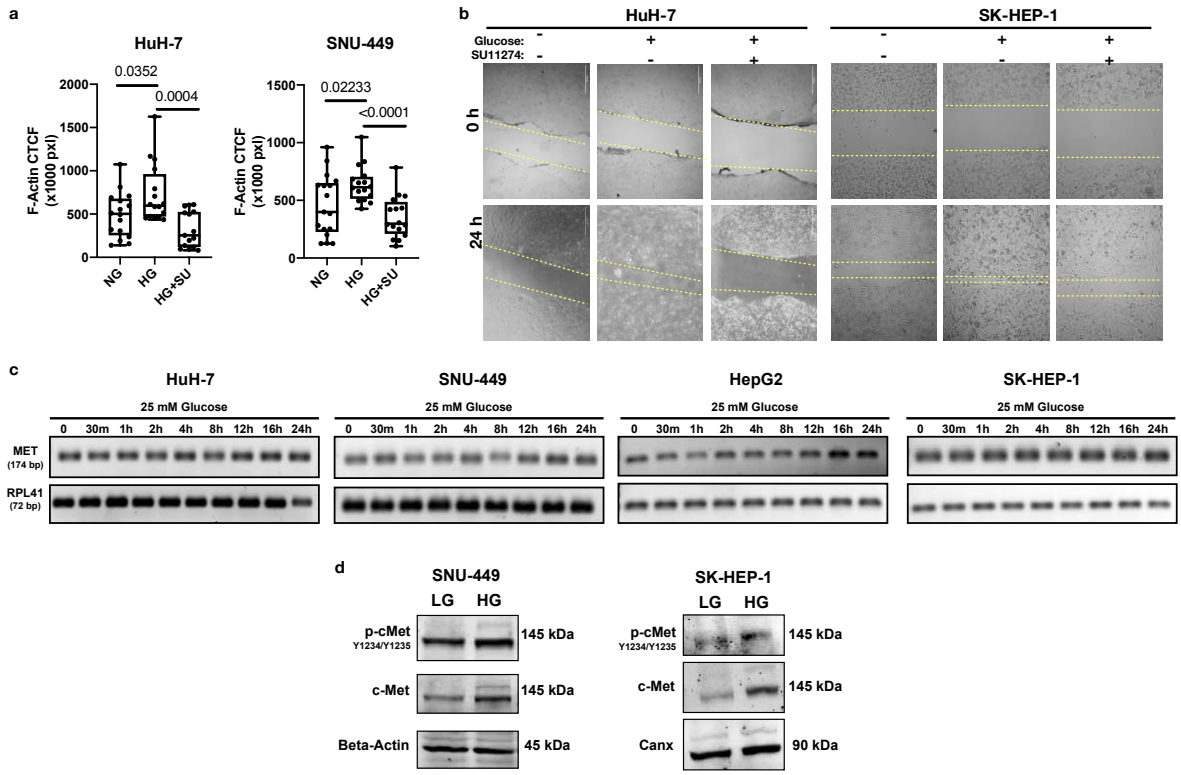
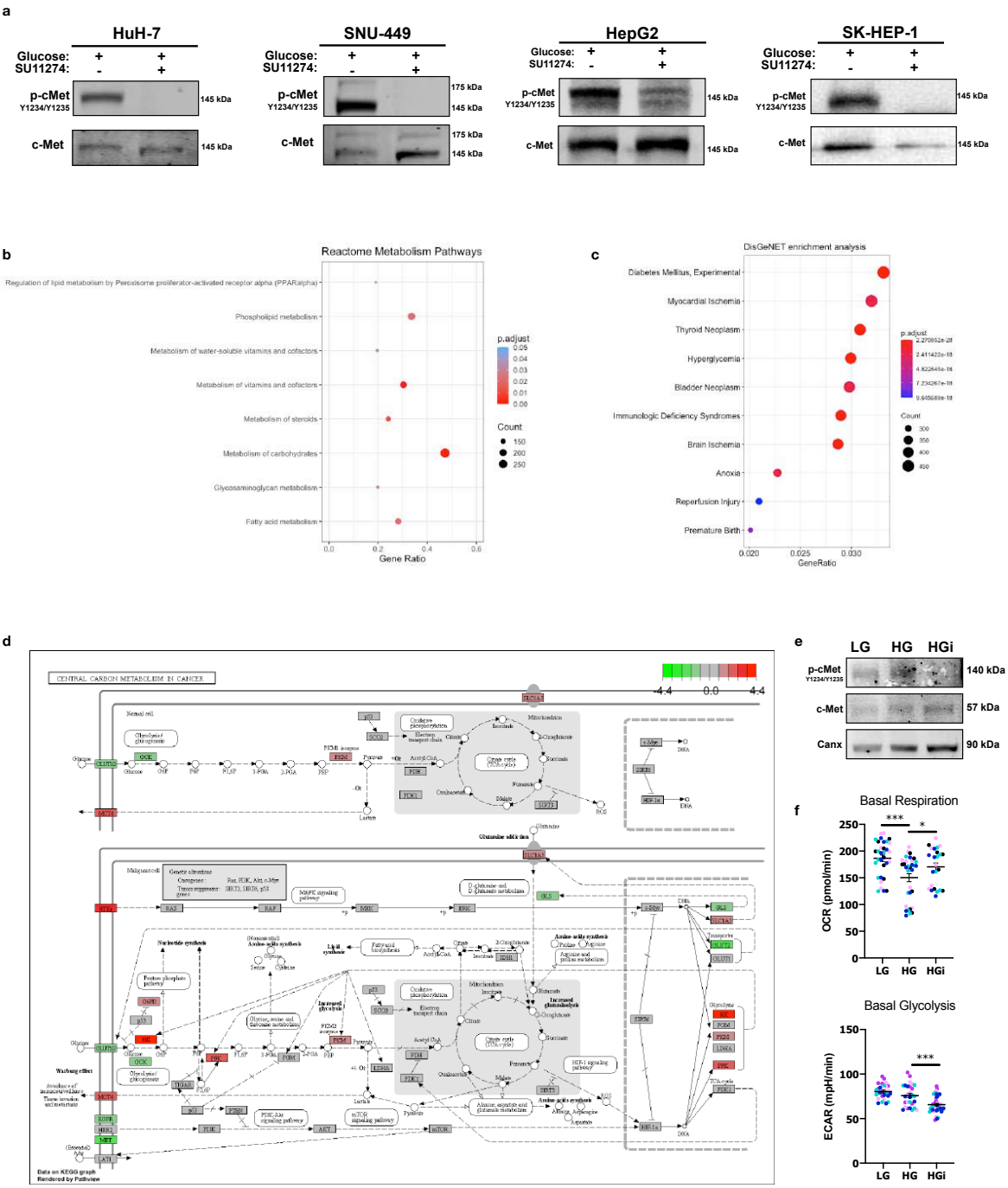


Supplementary Figure 1. Graphical presentation of (**a**) fluorescent intensity measurements for Phalloidin staining. Microscopic image of (**b**) wound closure of HuH-7 and SK-HEP-1 cells after 24-hours incubation in no-, high-glucose and c-Met inhibitor supplemented culture conditions. (**c**) RT-PCR agarose gel electrophoresis image of time-dependent MET and RPL41 expressions in response to high glucose (25 mM) treatment of HuH-7, SNU-449, HepG2 and SK-HEP-1 cells. (**d**) Immunoblotting of c-Met activity phosphorylations (Y1234/Y1235) and c-Met protein in low-glucose (5.5 mM) and high-glucose (25 mM) supplemented conditions of SNU-449 and SK-HEP-1 cells. Boxplot graphs were generated and statistical analyses were performed with the GraphPad Prism version 8.2.1 for MacOS, GraphPad Software, San Diego, California USA, www.graphpad.com. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$



Supplementary Figure 2. (**a**) Immunoblot images of phospho-Met and total c-Met proteins of HuH-7, SNU-449, HepG2 and SK-HEP-1 cells. (**b**) Dot-plot graph of metabolism pathways that were over-represented in pathway enrichment analysis of low-*MET* expressing quartile (Q1) and high-*MET* expression quartile (Q4) of TCGA HCC tissue samples. (**c**) Dot-plot graph of gene-disease association enrichment analysis of low-*MET* expressing quartile (Q1) and high-*MET* expression quartile (Q4) of TCGA HCC tissue samples. (**d**) Visualization of fold

changes of differentially expressed genes in KEGG pathway "central carbon metabolism in cancer". (**e**) Immunoblot images of phospho-Met, total c-Met and Calnexin proteins of Hepa1-6 cells cultured in low-glucose (LG, 5.5 mM), high-glucose (HG, 25 mM) and high glucose together with c-Met kinase activity inhibitor SU11274 (HGi). (f) Graphs of basal OCR and ECAR levels of Hepa1-6 cells in LG, HG and HGi conditions. Graphs were generated and statistical analyses were performed with the GraphPad Prism version 8.2.1 for MacOS, GraphPad Software, San Diego, California USA, www.graphpad.com. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

Supplementary Table 1. Table represents differential expression tendency of gene of interests in RT-qPCR array, RT-qPCR experiments and bioinformatic analyses with TCGA-LIHC dataset. The effect of gene expressions on overall patient survival in TCGA-LIHC dataset is represented in the last (right) column. Glucose metabolism genes that are downregulated in both HCC cell line and upregulated in High-*MET* expressing group of TCGA data are marked with red fonts. Genes that are not investigated in indicated condition/dataset is presented with "hyphen (-)". "N.S.": not significant.

GENE	c-Met Activity Inhibition			TCGA-LIHC		
	RT-qPCR Array	SNU-449	SK-HEP-1	Normal vs. HCC Tissues	Low-MET vs. High MET	SURVIVAL
GYS1	Downregulated	Downregulated	N.S.	Upregulated	N.S.	Significantly Lower
H6PD	Downregulated	Downregulated	Upregulated	Upregulated	Higher in High-MET group	-
HK1	-	Downregulated	-	-	Lower in High-MET group	-
HK2	Downregulated	Downregulated	Downregulated	Upregulated	Lower in High-MET group	Significantly Lower
MDH1B	Downregulated	Downregulated	Downregulated	Upregulated	N.S.	-
PDK2	Downregulated	Downregulated	N.S.	N.S.	Higher in High-MET group	-
PDK4	Downregulated	Downregulated	N.S.	Upregulated	Higher in High-MET group	-

PDPR	Downregulated	Downregulated	N.S.	Upregulated	Higher in High-MET group	-
PFKL	Downregulated	Downregulated	Downregulated	Upregulated	N.S.	-
PGAM2	Downregulated	Downregulated	-.	Upregulated	N.S.	-
PHKG1	Downregulated	Downregulated	N.S.	Upregulated	N.S.	-
PKLR	Downregulated	Downregulated	N.S.	Upregulated	Higher in High-MET group	-
PYGM	Upregulated	Downregulated	-	N.A.	N.S.	-
SLC2A1	Downregulated	Downregulated	Downregulated	Upregulated	Lower in High-MET group	Significantly Lower
SLC2A2	-	-	-	-	Higher in High-MET group	-
SLC2A4	-	-	-	-	N.S.	-

Cell Culture Media and Supplements:

RPMI 1640 Medium, no glucose, no glutamine (Biological Industries, #01-101-1A)

RPMI 1640 Medium, no glutamine (Biological Industries, #01-104-1A)

DMEM, no glucose, no glutamine (Biological Industries, #01-057-1A)

DMEM, High Glucose (Biological Industries, #01-055-1A)

DMEM, low glucose, no glutamine (Biological Industries, # 01-050-1A)

Glucose Solution (Gibco, #A2494001)

Trypsin EDTA Solution B (0.25%), EDTA (0.05%) (Biological industries, #03-052-1B)

L-Glutamine Solution (200 mM) (Biological industries, #03-020-1B)

MEM Non-Essential Amino Acids Solution (100X) (Biological industries, #01-340-1B)

Sodium Pyruvate (Biological industries, #02-042-1B)

Penicillin-Streptomycin Solution (Biological industries, #01-031-1B)

Fetal Bovine Serum, qualified, heat inactivated (Gibco, #10500064)

Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-free, LDEV-free, 10 mL (Corning, #356231)

Supplementary Table 2. Primer sequences used in RT-qPCR analyses.

ZEB1	Forward:	GCACCTGAAGAGGACCAGAG
	Reverse:	TGCATCTGGTGTTCCATTTT
ZEB2	Forward:	CGGTGCAAGAGGCGCAAACA
	Reverse:	GGAGGACTCATGGTTGGGCA
HK2	Forward:	GCTGTGGTGGACAGGATACG
	Reverse:	ATTTCGGAGCCAGGTCCTTC
VIM	Forward:	CCTTGACATTGAGATTGCCACCTA
	Reverse:	TCATCGTGATGCTGAGAAGTTTCG

PFKL	Forward:	TCCTCATCTACGAGGGCTATG
	Reverse:	CGAGCGCTGCCAATGATAGT
PDPR	Forward:	ATCTCCCCCAAGAAAGTGGC
	Reverse:	CATGAAGAACAGATGTCCGGT
MDH1B	Forward:	CGGGTAGAGCAGATTGTCCATA
	Reverse:	CGATCCAACAGCTCTCTCCAG
RPL41	Forward:	GAAACCTCTGCGCCATGA
	Reverse:	TCTTTCTTCTTTTGCGCTTCA
GAPDH	Forward:	GAAGGTGAAGGCGGAGTC
	Reverse:	GAAGATGGTGATGGGATTTC
PKLR	Forward:	CAACTGACCCAGGAGCTG
	Reverse:	CTCCACGGAGCGAGATGC
GYS1	Forward:	ATACTGCATGGAAAGGGCGG
	Reverse:	ACTCCTGGATTCGAGCCTTG
PHKG1	Forward:	ACTGAAGATCTGAGCATGACCC
	Reverse:	ATCGCCTGACCACACTGCTAA
PDK2	Forward:	GACATGGCTAAGCTCCTGTGT
	Reverse:	CGCCCTCATGGCATTCTTGA
PDK4	Forward:	CCAAGCCACATTGGAAGCAT
	Reverse:	CGATGTGAATTGGTTGGTCTGG
H6PD	Forward:	TGCCTTTCCGAGACCAGAAC
	Reverse:	GCGAATGACACCGTACTCCT
SLC2A1 (GLUT1)	Forward:	GCGGGTTGTGCCATACTCAT
	Reverse:	ACCCACTTCAAAGAAGGCCA
CDH-2 (N-Cadherin)	Forward:	ATTGGACCATCACTGGGCTTA
	Reverse:	CACACTGGCAAACCTTCACG
MET	Forward:	GTCAGCCTTGTCCCTCCTTC
	Reverse:	GTCCTGCAGTCAATGCCTCT

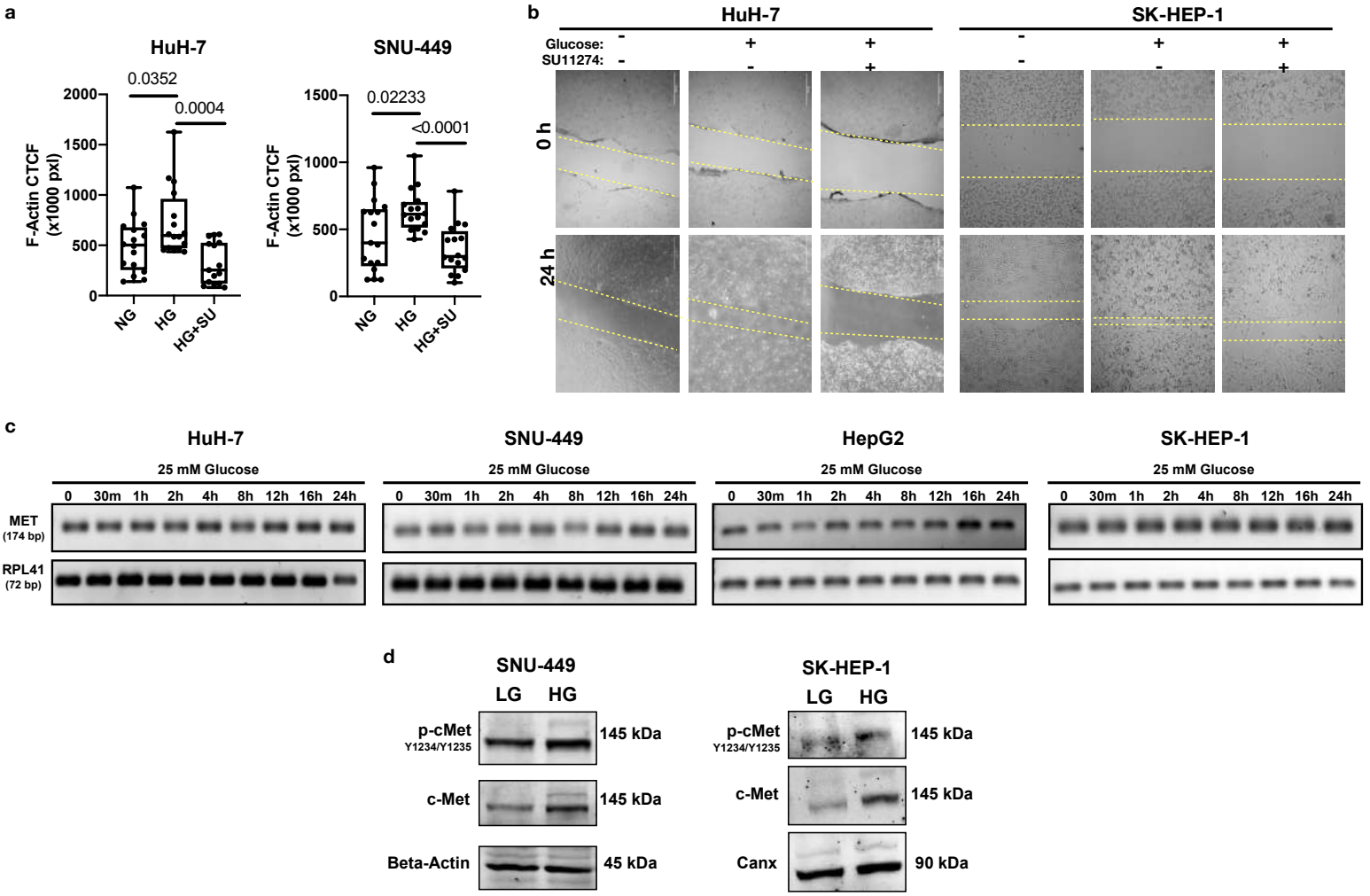
Supplementary Table 3. Antibodies used in IF and WB experiments

Cell Signaling	9272	Akt
Cell Signaling	4060	Phospho-Akt (Ser-473)
Santa Cruz Biotechnologies	Sc-11397	Calnexin
Cell Signaling	9101	Phospho-Erk 1/2 p44/42 (MAPK) (Thr202/Tyr204)
Cell Signaling	4377	Phospho-Erk 1/2 p44/42 (MAPK) (Thr202/Tyr204)
Cell Signaling	4696	Erk 1/2 p44/42
Cell Signaling	3127	Met
Santa Cruz Biotechnologies	Sc-161	Met
Cell Signaling	3129	Phospho-Met (Y-1234/1235)
Santa Cruz Biotechnology	Sc-101033	Egr-1
Invitrogen	A-11005	Alexa594-conjugated goat anti-mouse secondary antibody
Invitrogen	A-11037	Alexa594-conjugated goat anti-rabbit secondary antibody
LI-COR Biosciences	926-68071	IRDye 680RD Goat anti-Rabbit IgG Secondary Antibody

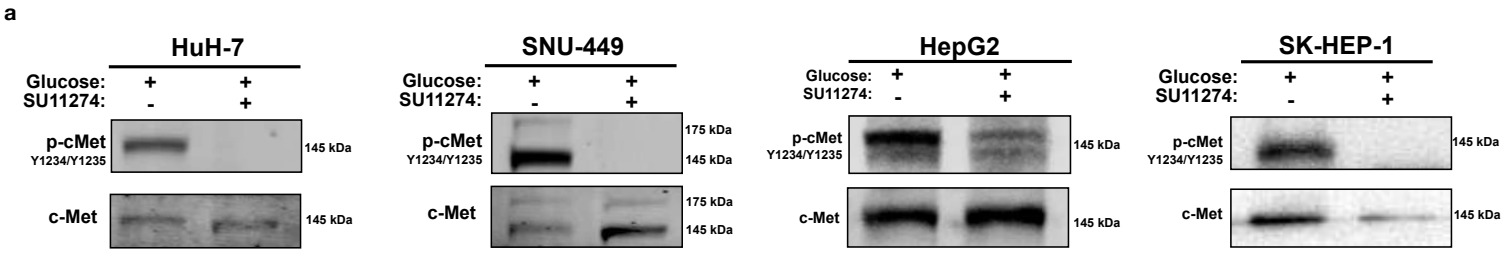
LI-COR Biosciences	926-32210	IRDye® 800CW Goat anti-Mouse IgG Secondary Antibody

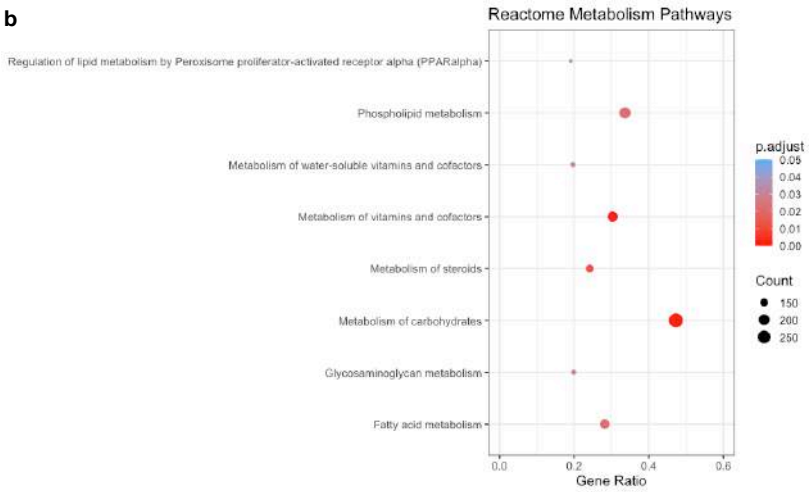
PDF files of supplementary figures and raw membrane images of immunoblots are presented in the following pages of this document.

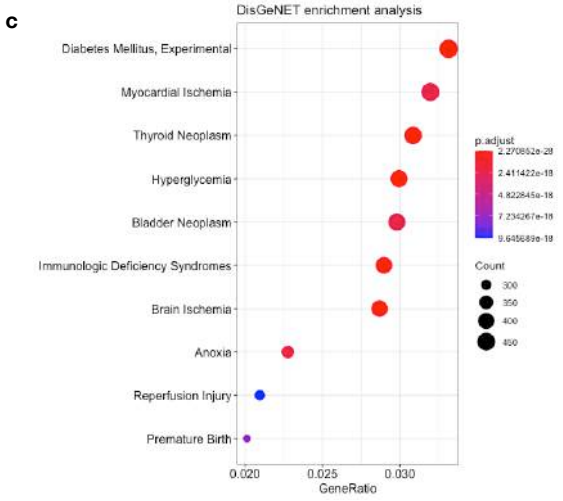
Supplementary Figure 1

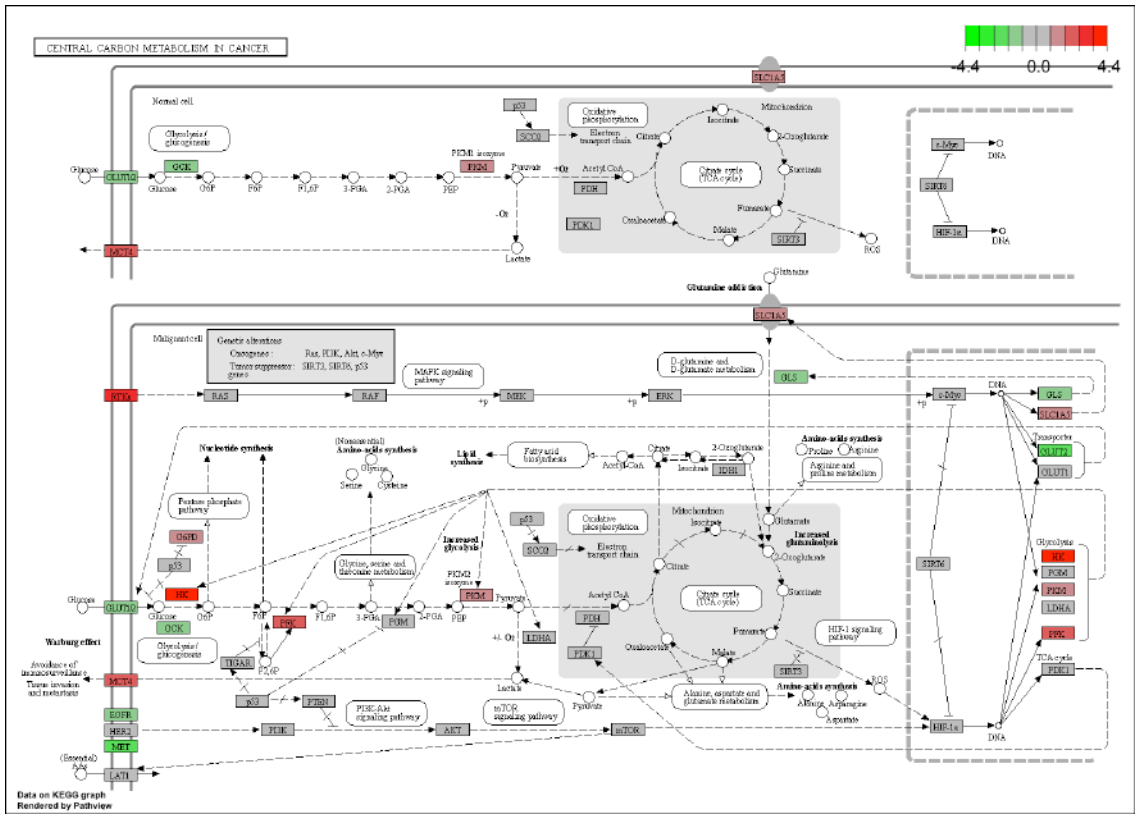


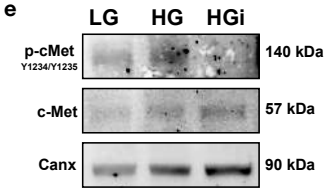
Supplementary Figure 2











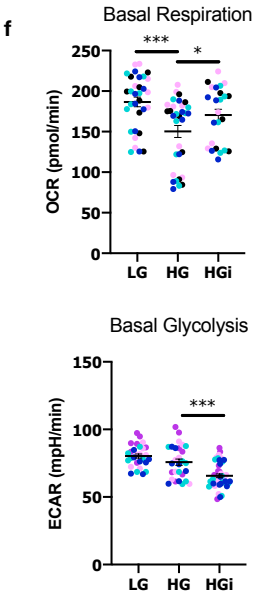
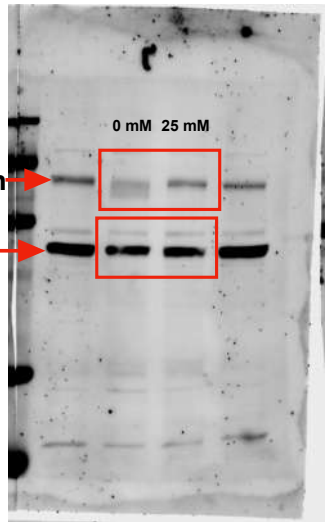
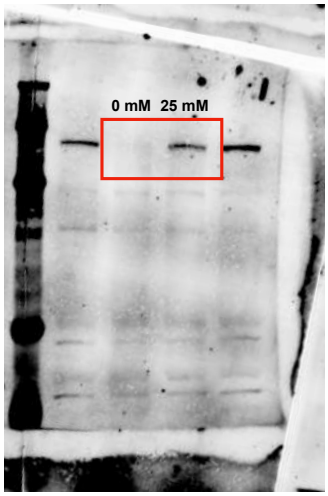


Figure 1 c-d-e

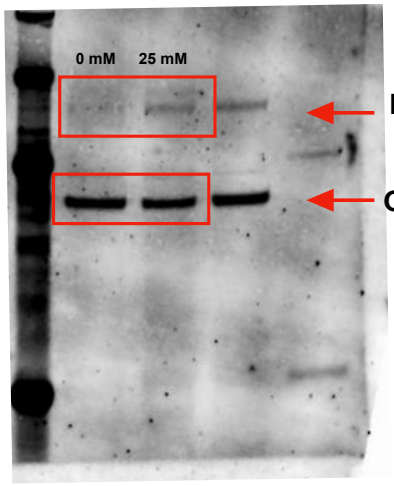
HuH-7-N-Cadherin&Calnexin



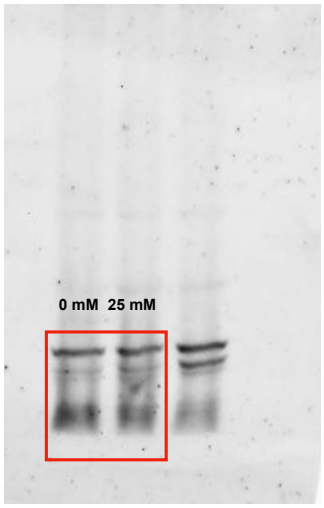
SK-HEP-1— N-Cadherin



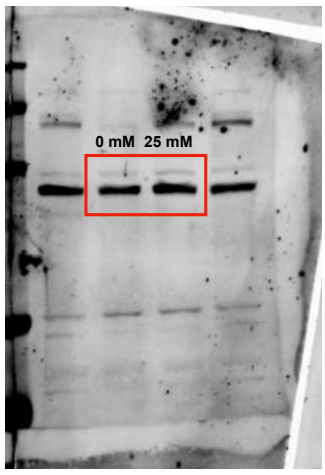
SNU-449— N-Cadherin&Calnexin



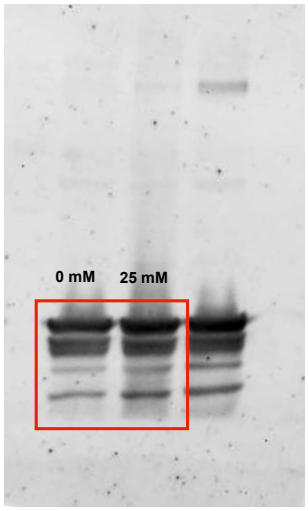
HuH-7 - Vimentin



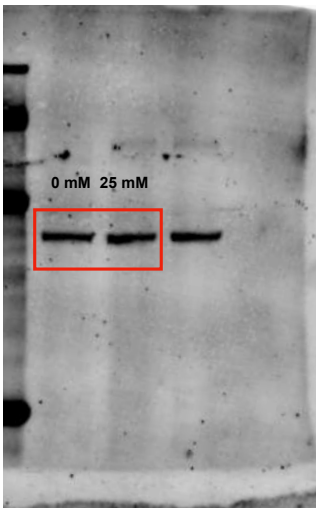
SK-HEP-1— Calnexin



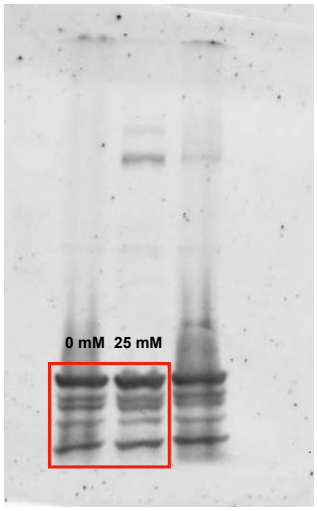
SNU-499—Vimentin



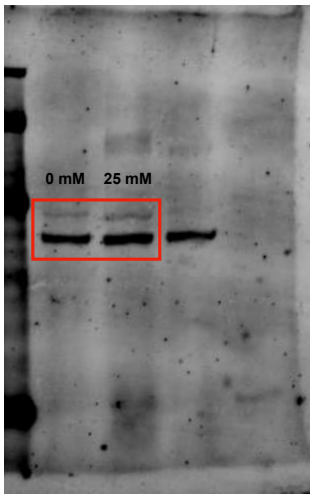
HuH-7 - Calnexin



SK-HEP-1—Vimentin



SK-HEP-1—Calnexin



**Membranes are cut according to the molecular marker after transfer and incubated with antibody of interest.*

Figure 3a

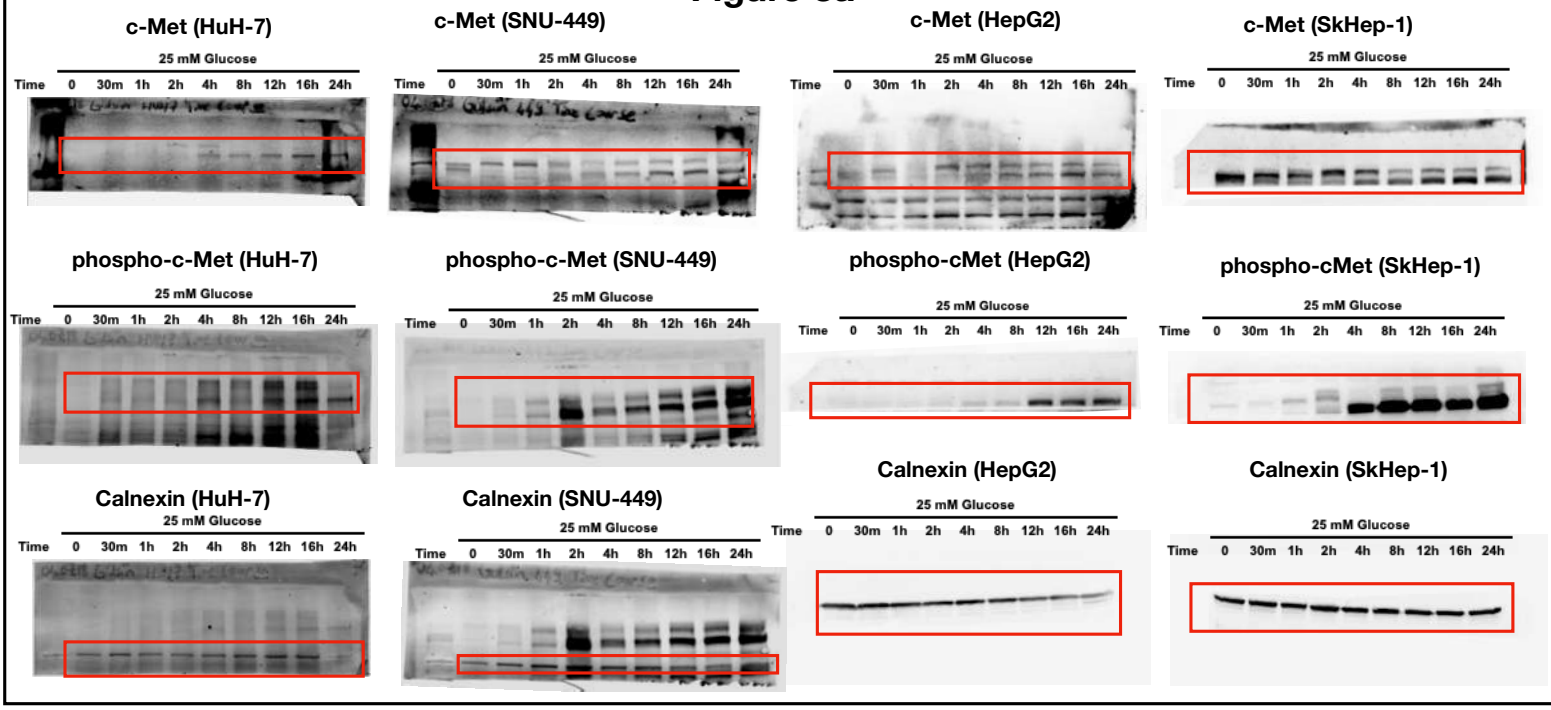


Figure 3d

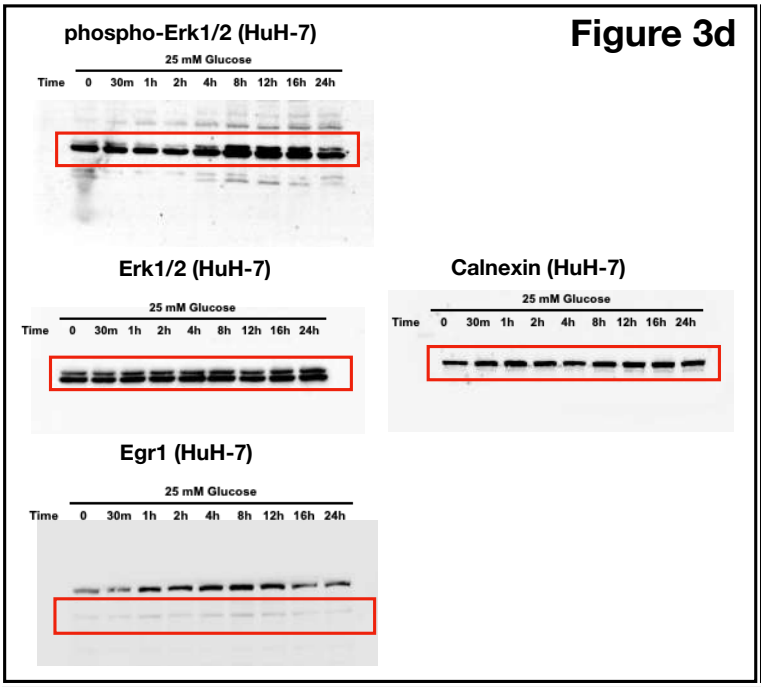


Figure 3e

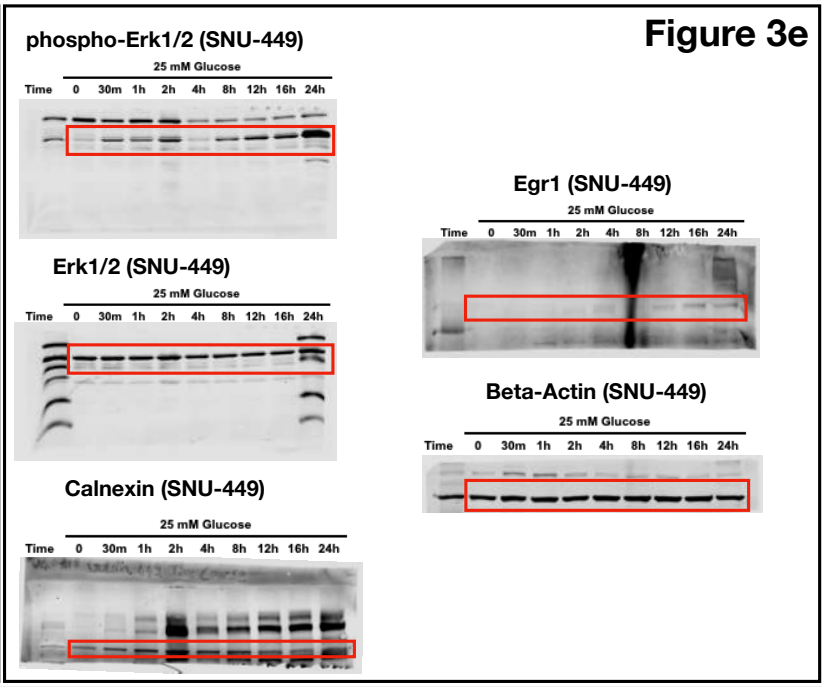
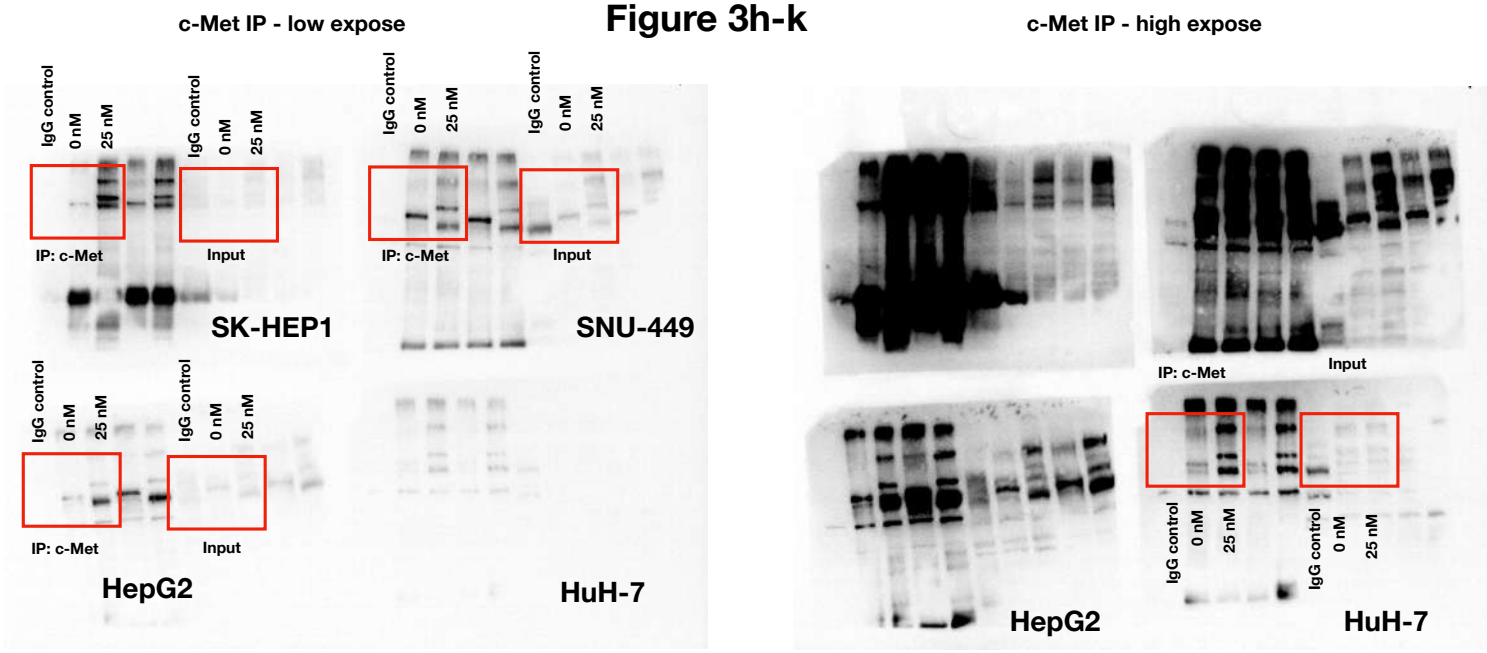
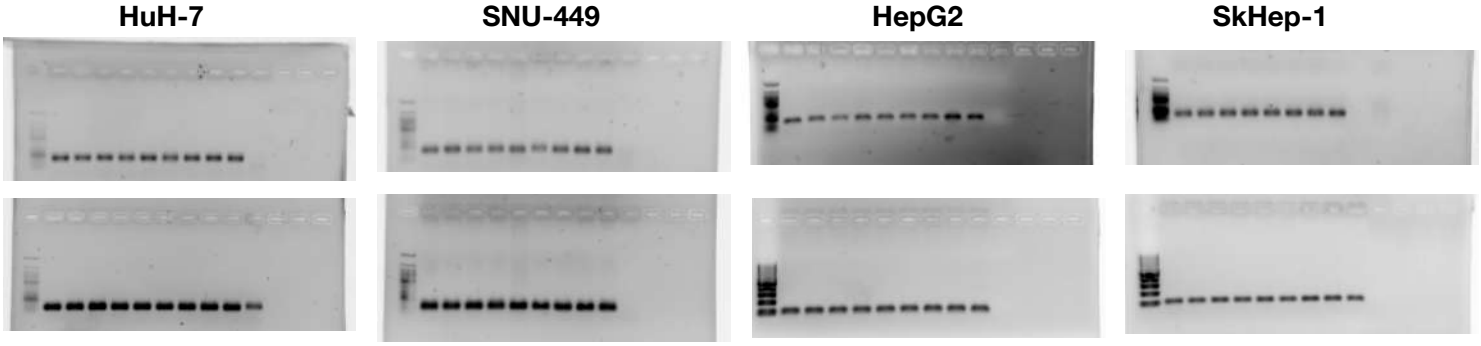


Figure 3h-k

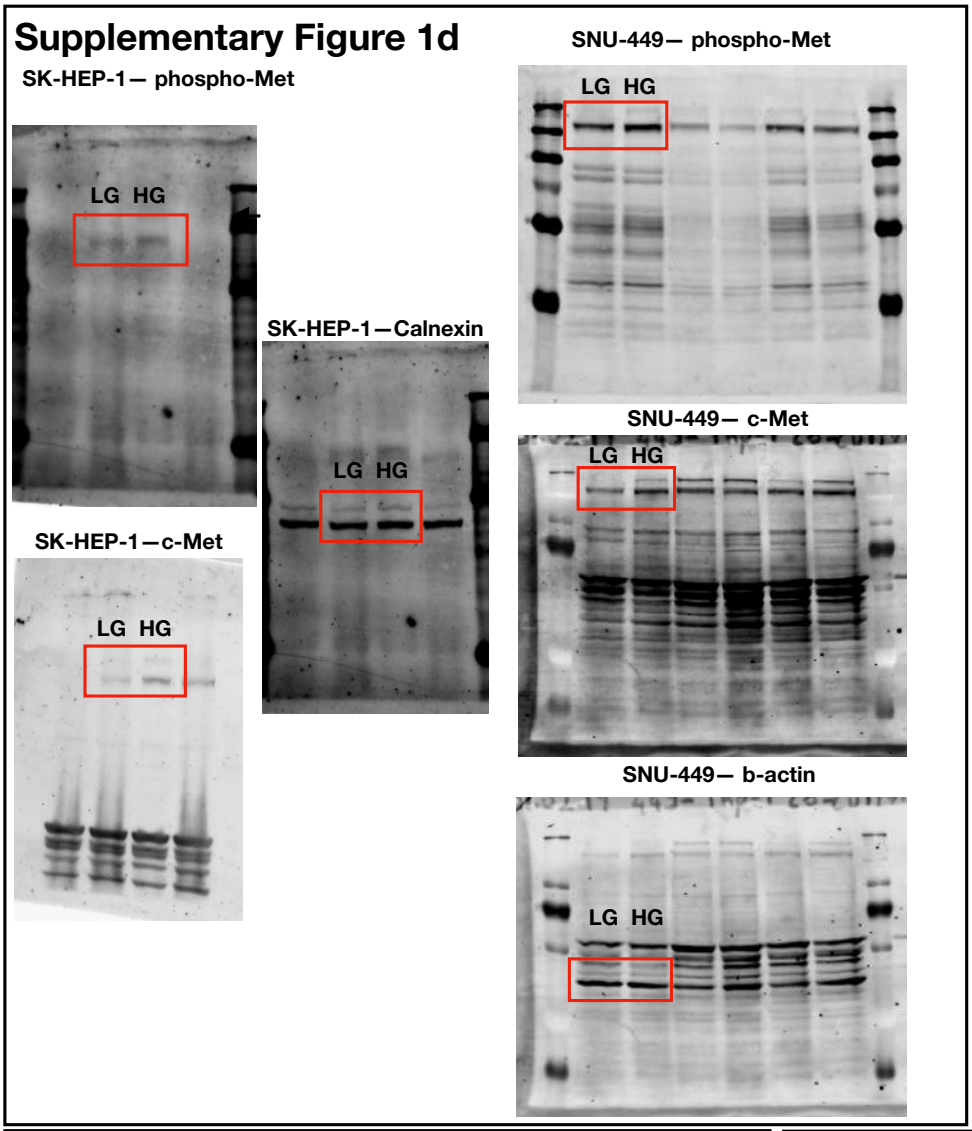


**Membranes are cut according to the molecular marker after transfer and incubated with antibody of interest.*

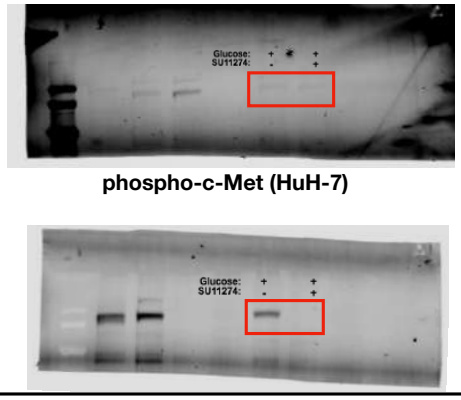
Supplementary Figure 1c (agarose gel images)



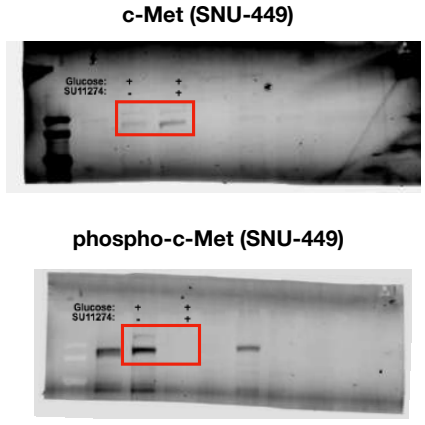
Supplementary Figure 1d



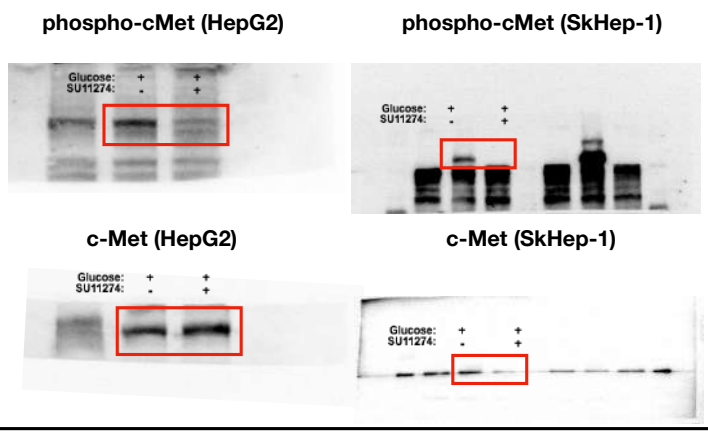
Supplementary Figure 2a



Supplementary Figure 2a



Supplementary Figure 2a



Supplementary Figure 2e