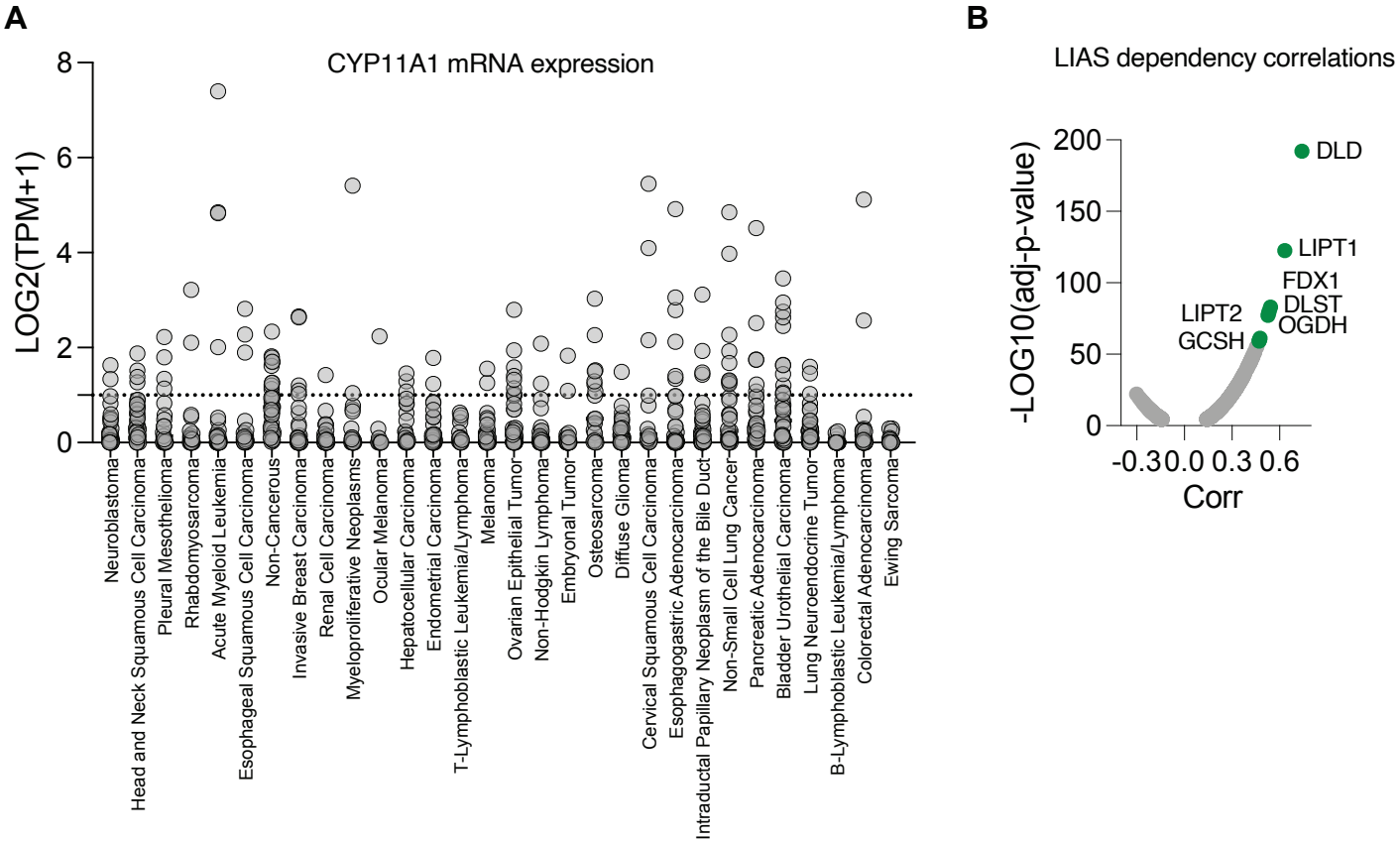
A
CYP11A1 mRNA expression
LOG2(TPM+1)
8
6
4
2
0
Neuroblastoma
Head and Neck Squamous Cell Carcinoma
Pleural Mesothelioma
Rhabdomyosarcoma
Acute Myeloid Leukemia
Esophageal Squamous Cell Carcinoma
Non-Cancerous
Invasive Breast Carcinoma
Renal Cell Carcinoma
Myeloproliferative Neoplasms
Ocular Melanoma
Hepatocellular Carcinoma
Endometrial Carcinoma
T-Lymphoblastic Leukemia/Lymphoma
Melanoma
Ovarian Epithelial Tumor
Non-Hodgkin Lymphoma
Embryonal Tumor
Osteosarcoma
Diffuse Glioma
Cervical Squamous Cell Carcinoma
Esophagogastric Adenocarcinoma
Intraductal Papillary Neoplasm of the Bile Duct
Non-Small Cell Lung Cancer
Pancreatic Adenocarcinoma
Bladder Urothelial Carcinoma
Lung Neuroendocrine Tumor
B-Lymphoblastic Leukemia/Lymphoma
Colorectal Adenocarcinoma
Ewing Sarcoma
B
LIAS dependency correlations
-LOG10(adj-p-value)
200
150
100
50
0
DLD
LIPT1
FDX1
DLST
OGDH
LIPT2
GCSH
-0.3
0.0
0.3
0.6
Corr

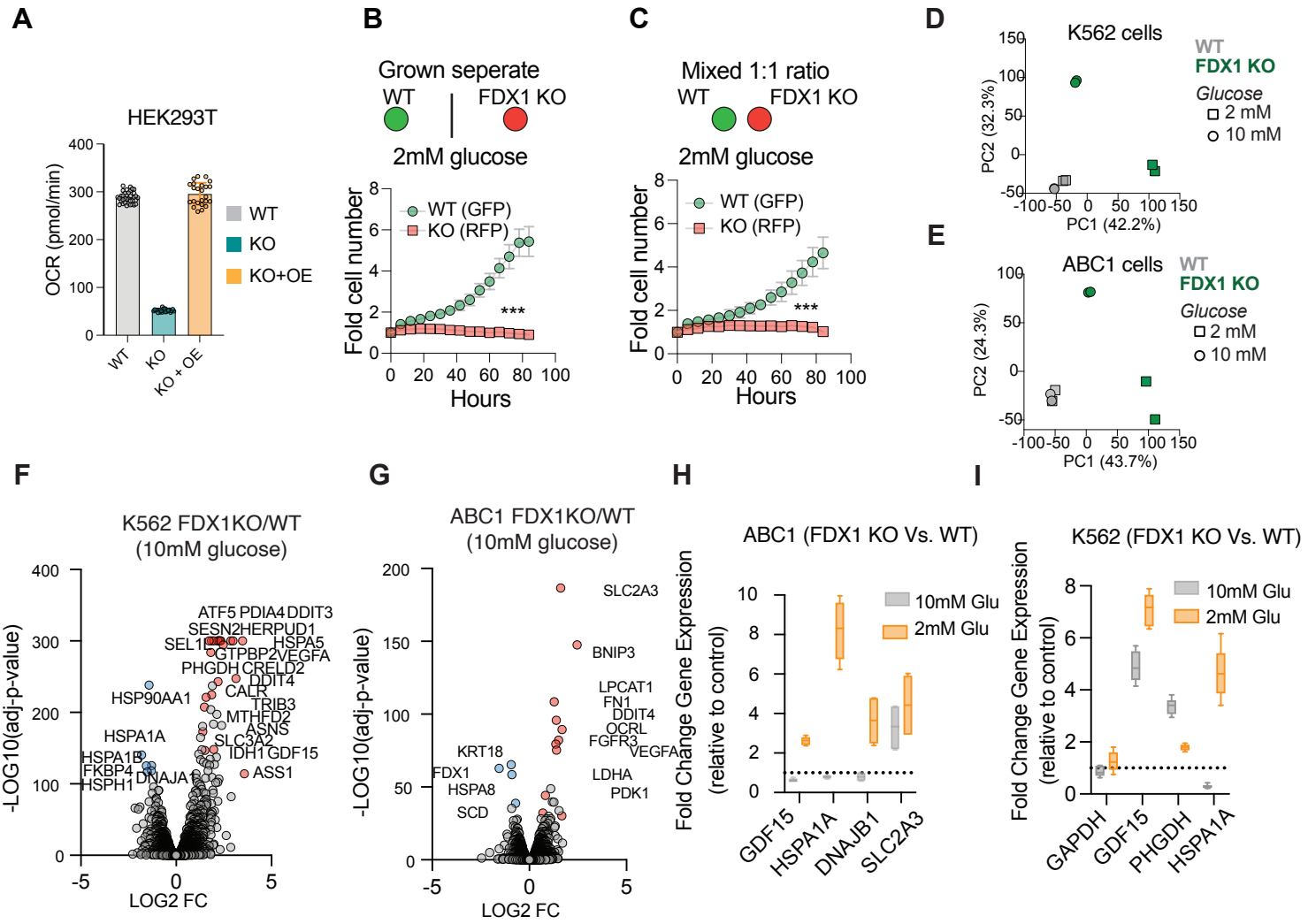
A
HEK293T
OCR (pmol/min)
WT
KO
KO+OE
B
Grown seperate
WT
FDX1 KO
2mM glucose
Fold cell number
WT (GFP)
KO (RFP)
***
Hours
C
Mixed 1:1 ratio
WT
FDX1 KO
2mM glucose
Fold cell number
WT (GFP)
KO (RFP)
***
Hours
D
K562 cells
WT
FDX1 KO
Glucose
2 mM
10 mM
PC2 (32.3%)
PC1 (42.2%)
E
ABC1 cells
WT
FDX1 KO
Glucose
2 mM
10 mM
PC2 (24.3%)
PC1 (43.7%)
F
K562 FDX1KO/WT
(10mM glucose)
-LOG10(adj-p-value)
LOG2 FC
ATF5 PDIA4 DDIT3
SESN2 HERPUD1
SEL1L HSPA5
GTPBP2 VEGFA
PHGDH CRELD2
DDIT4
HSP90AA1 CALR
TRIB3
MTHFD2
HSPA1A ASNS
SLC3A2
HSPA1B IDH1 GDF15
FKBP4 DNAJA1 ASS1
HSPH1
G
ABC1 FDX1KO/WT
(10mM glucose)
-LOG10(adj-p-value)
LOG2 FC
SLC2A3
BNIP3
LPCAT1
FN1
DDIT4
OCRL
FGFR3
VEGFA
KRT18
FDX1
HSPA8
SCD
LDHA
PDK1
H
ABC1 (FDX1 KO Vs. WT)
Fold Change Gene Expression
(relative to control)
10mM Glu
2mM Glu
GDF15
HSPA1A
DNAJB1
SLC2A3
I
K562 (FDX1 KO Vs. WT)
Fold Change Gene Expression
(relative to control)
10mM Glu
2mM Glu
GAPDH
GDF15
PHGDH
HSPA1A

Figure S2

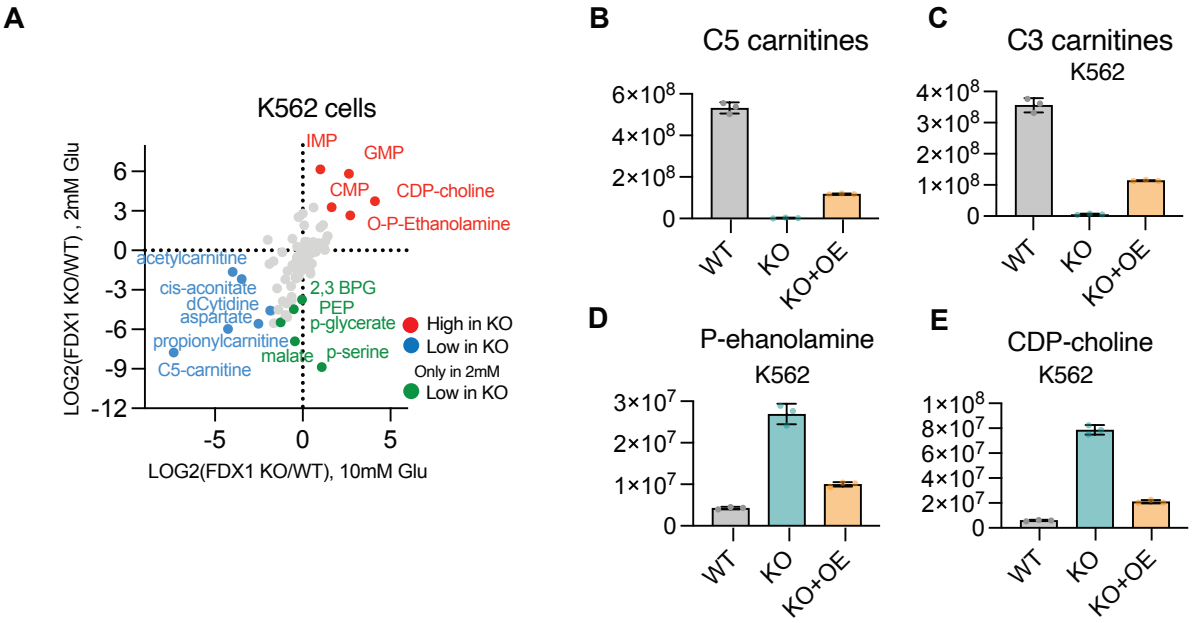
A
K562 cells
IMP
GMP
CMP
CDP-choline
O-P-Ethanolamine
acetylcarnitine
cis-aconitate
dCytidine
aspartate
propionylcarnitine
C5-carnitine
malate
2,3 BPG
PEP
p-glycerate
p-serine
High in KO
Low in KO
Only in 2mM
Low in KO
LOG2(FDX1 KO/WT) , 2mM Glu
LOG2(FDX1 KO/WT), 10mM Glu
B
C5 carnitines
C
C3 carnitines
K562
D
P-ehanolamine
K562
E
CDP-choline
K562
WT
KO
KO+OE

Figure S3

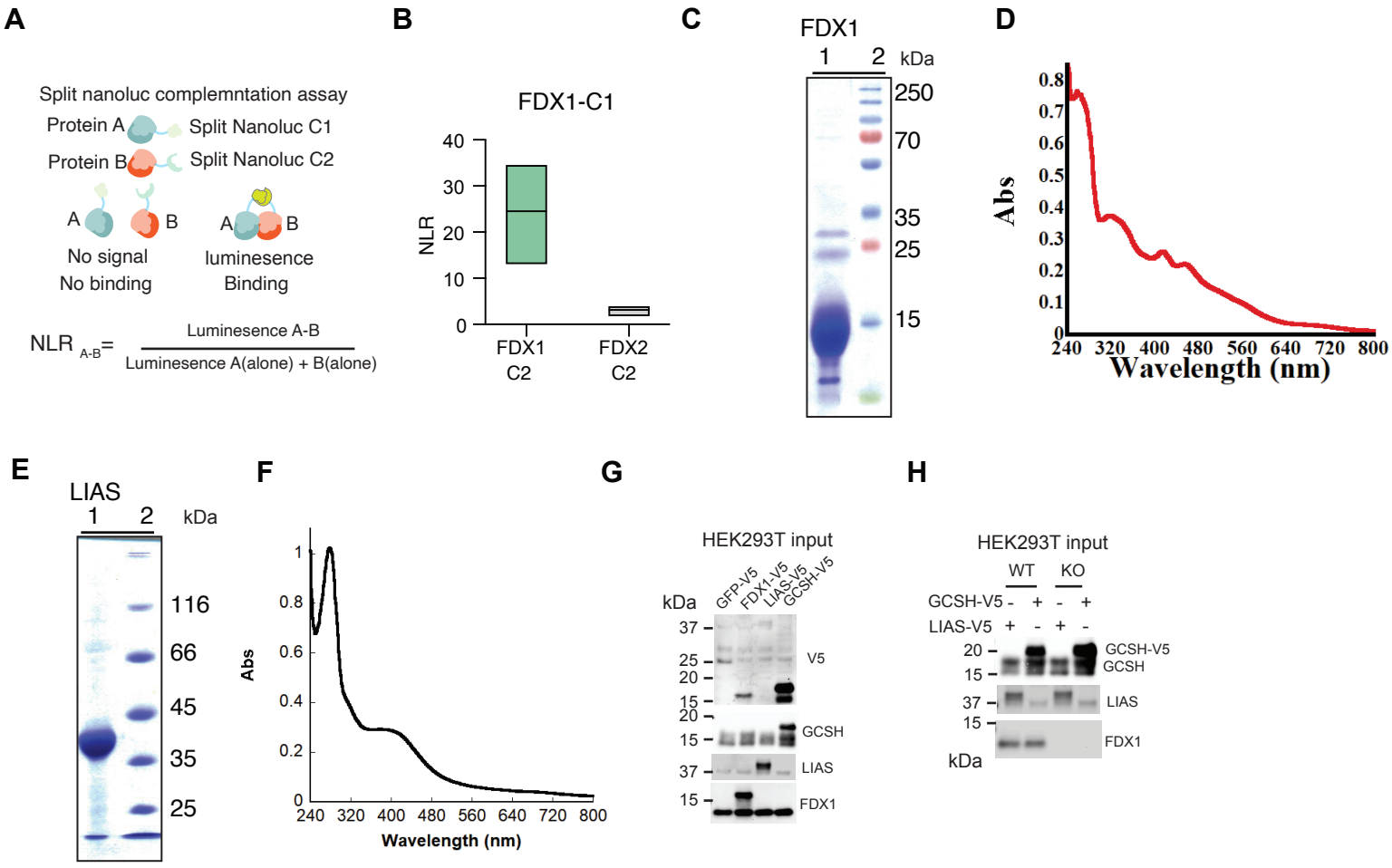
A
Split nanoluc complemntation assay
Protein A
Split Nanoluc C1
Protein B
Split Nanoluc C2
No signal
No binding
luminesence
Binding
NLR A-B= Luminesence A-B / Luminesence A(alone) + B(alone)
B
FDX1-C1
NLR
FDX1 C2
FDX2 C2
C
FDX1
kDa
250
70
35
25
15
D
Abs
Wavelength (nm)
E
LIAS
kDa
116
66
45
35
25
F
Abs
Wavelength (nm)
G
HEK293T input
GFP-V5
FDX1-V5
LIAS-V5
GCSH-V5
V5
GCSH
LIAS
FDX1
H
HEK293T input
WT
KO
GCSH-V5
LIAS-V5
GCSH-V5
GCSH
LIAS
FDX1

Figure S4