iScience, Volume *25*

Supplemental information

IL11 stimulates ERK/P90RSK to inhibit LKB1/AMPK and activate mTOR initiating a mesenchymal program in stromal, epithelial, and cancer cells

Anissa A. Widjaja, Sivakumar Viswanathan, Joyce Goh Wei Ting, Jessie Tan, Shamini G. Shekeran, David Carling, Wei-Wen Lim, and Stuart A. Cook

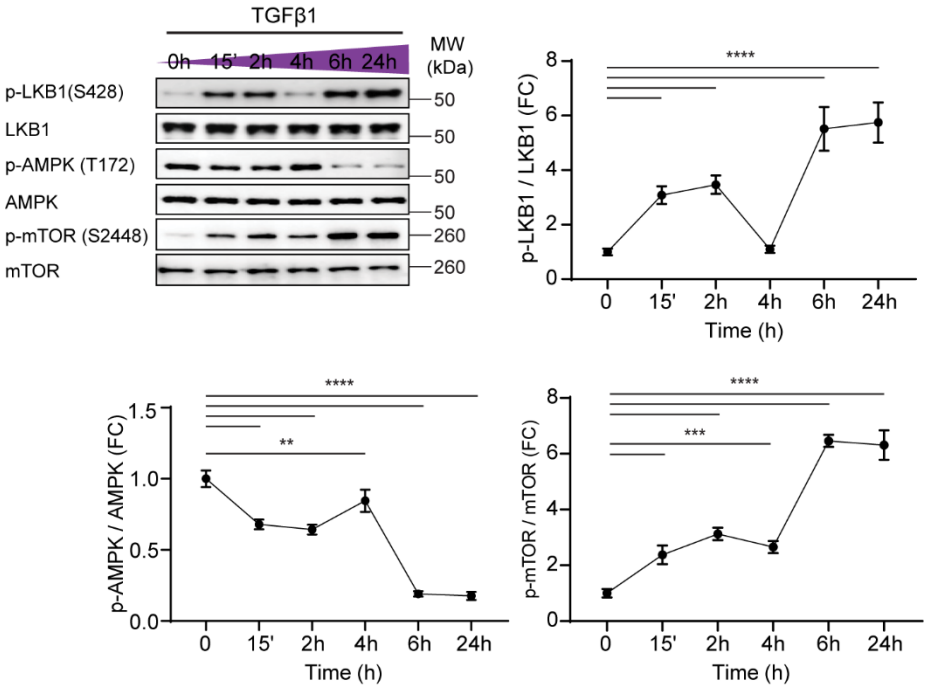


Figure S1. TGFβ stimulation inactivates LKB1 in cardiac fibroblasts, Related to Figure 1.
Western blot and densitometry analyses of phosphorylated (p-) and total LKB1, AMPK and mTOR in human cardiac fibroblasts stimulated with TGFβ1 (10 ng/ml) over a time course. Data are shown as mean±SD (n=3 biological replicates); **p<0.01, ***p<0.001, ****p<0.0001. FC: Fold change.

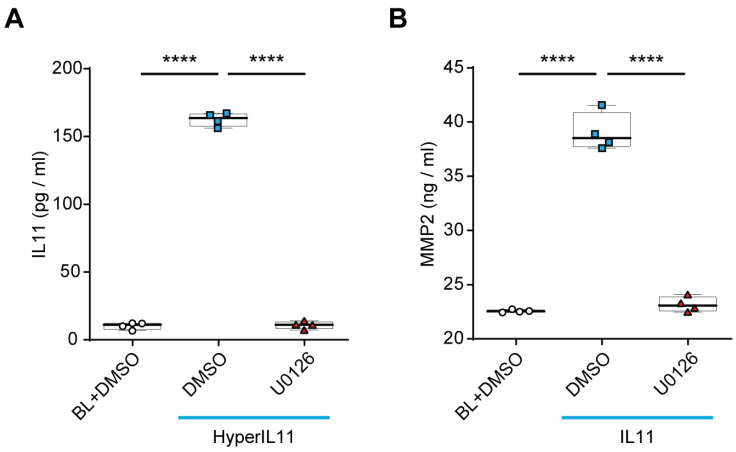


Figure S2. Secretion of IL11 and MMP2 from human cardiac fibroblasts is dependent on ERK activity, Related to Figure 1. (**A-B**) Secreted (A) IL11 or (B) MMP2 from human cardiac fibroblasts stimulated with (A) HyperIL11 or (B) IL11 in the absence or presence of U0126. All conditions were carried out in the presence of 0.1% DMSO. Data are shown as box-and-whisker with median (middle line), 25th–75th percentiles (box) and min-max percentiles (whiskers); one-way ANOVA with Tukey's correction (n=4 biological replicates); ****p<0.0001. BL: Baseline

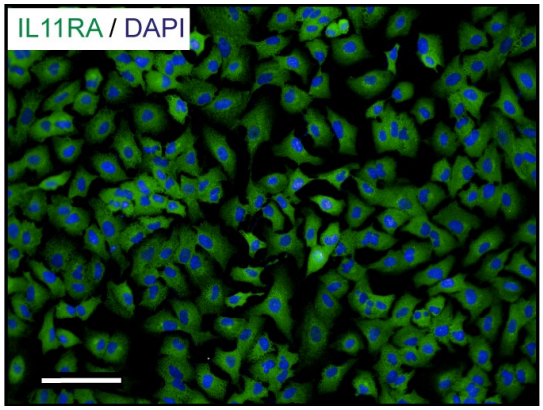


Figure S3. A549 cells express IL11RA, Related to Figure 1. Immunofluorescence image of IL11RA expression in A549 cells (representative dataset from n=3 biological replicates, scale bar, 100 μm).

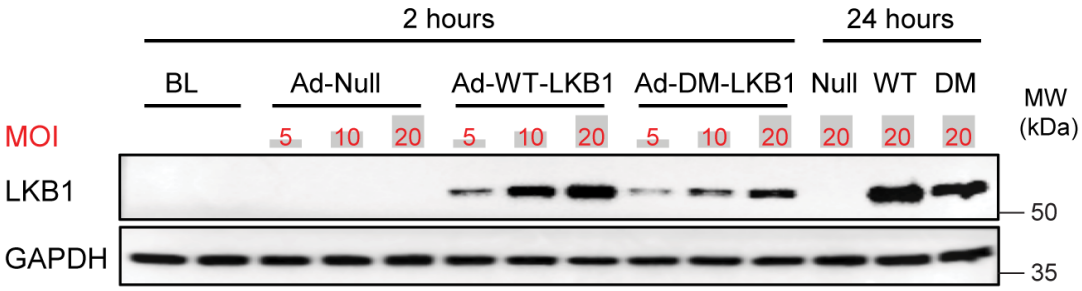


Figure S4. Optimal MOI determination for Ad-WT-LKB1 and Ad-DM-LKB1(S325A/S428A) in A549 cells, Related to Figure 1. Western blots of LKB1 and GAPDH following transduction with either adenovirus encoding empty vector (Null), wild-type (WT)-LKB1, or double mutant (DM)-LKB1 at an MOI of 5, 10 or 20 with incubation period of either 2 or 24 hours in A549 cells (n=1 biological replicate).

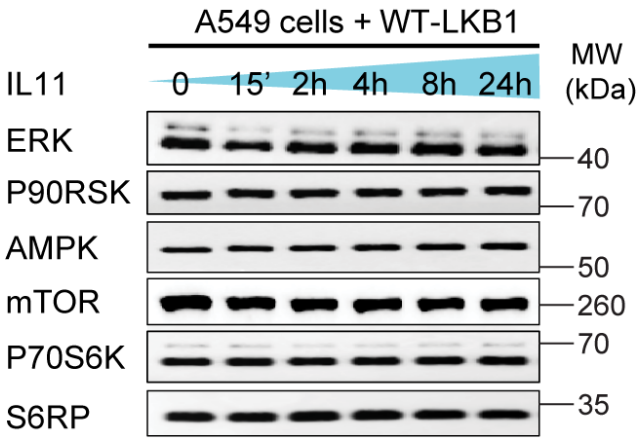


Figure S5. Sequential phosphorylation of LKB1 by ERK and P90RSK, Related to Figure 1. WB of total ERK, P90RSK, AMPK, mTOR, P70S6K and S6RP in IL11(10ng/ml)-stimulated A549 cells infected with adenovirus encoding WT-LKB1 across time. Experiments were conducted concurrently with **Figure 1F** (n=2 biological replicates).

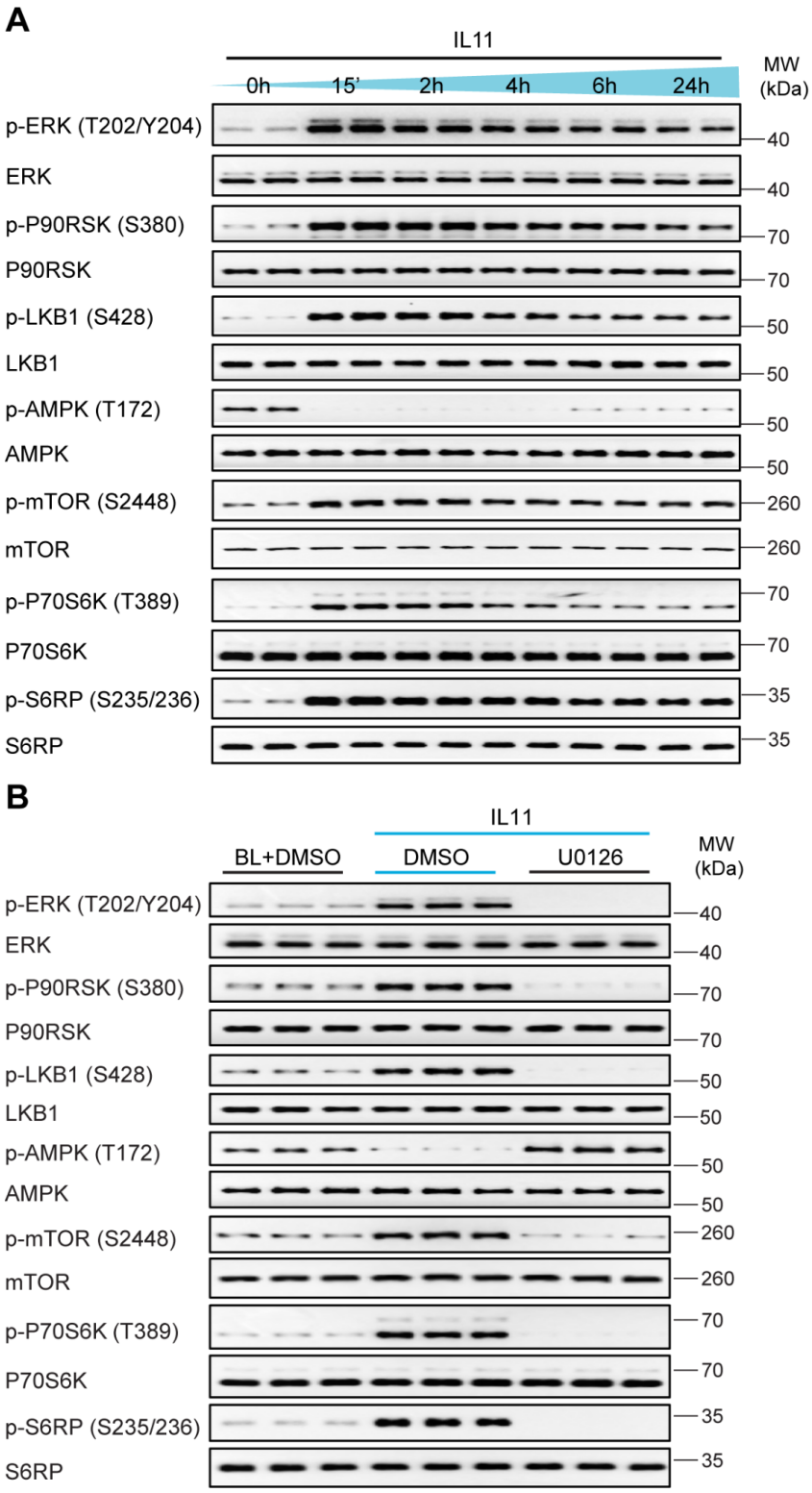


Figure S6. IL11 inhibits LKB1/AMPK signalling in ERK-dependent manner in LKB-expressing lung epithelial cancer cell line (H1792), Related to Figure 1. (A-B) WB of phospho and total ERK, P90RSK, LKB1, AMPK, mTOR, P70S6K and S6RP in IL11(10ng/ml)-stimulated H1792 cells (A) over a time course (n=2 biological replicates) and (B) in the presence of U0126 (10 μM), a MEK inhibitor at a 2-hour time point (n=3 biological replicates).

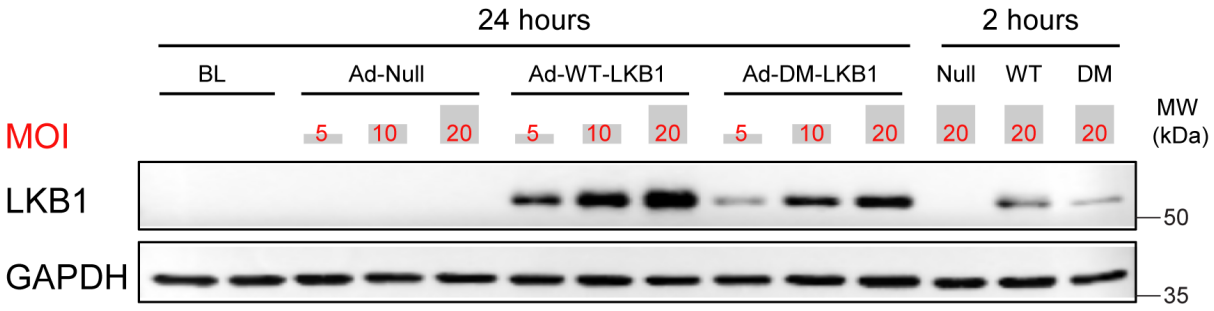


Figure S7. Optimal MOI determination for Ad-WT-LKB1 and Ad-DM-LKB1(S325A/S428A) in HSCs, Related to Figure 2. Western blots of LKB1 and GAPDH following transduction with either adenovirus encoding empty vector (Null), WT-LKB1, or DM-LKB1 at an MOI of 5, 10 or 20 with incubation period of either 2 or 24 hours in HSCs (n=1 biological replicate).

.

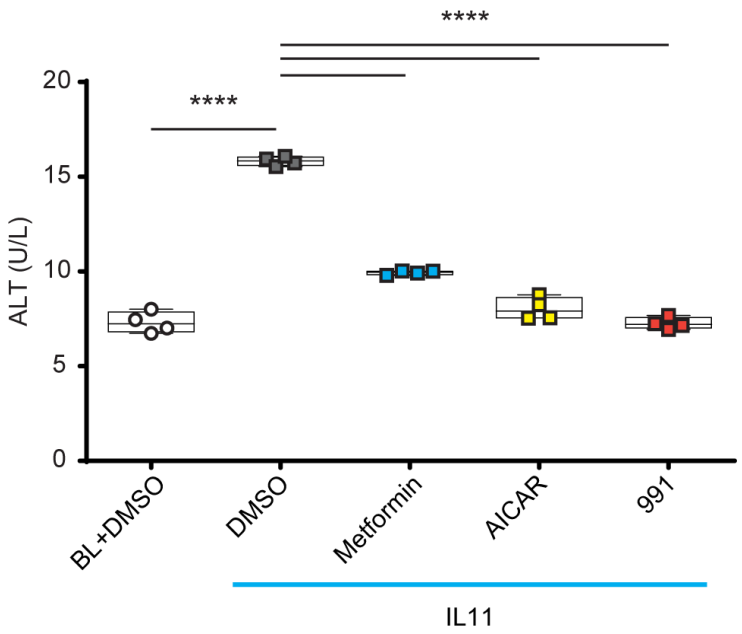


Figure S8. AMPK activation inhibits IL11-driven hepatotoxicity, Related to Figure 3. ALT concentrations in the supernatant of hepatocytes stimulated with IL11 (10 ng/ml, 24 hours) in the presence of DMSO, metformin (1 mM), AICAR (1 mM) or 991 (1 μM); one-way ANOVA with Dunnett's correction. Data are shown as box-and-whisker with median (middle line), 25th–75th percentiles (box) and min-max percentiles (whiskers) (n=4 biological replicates); ****p<0.0001.

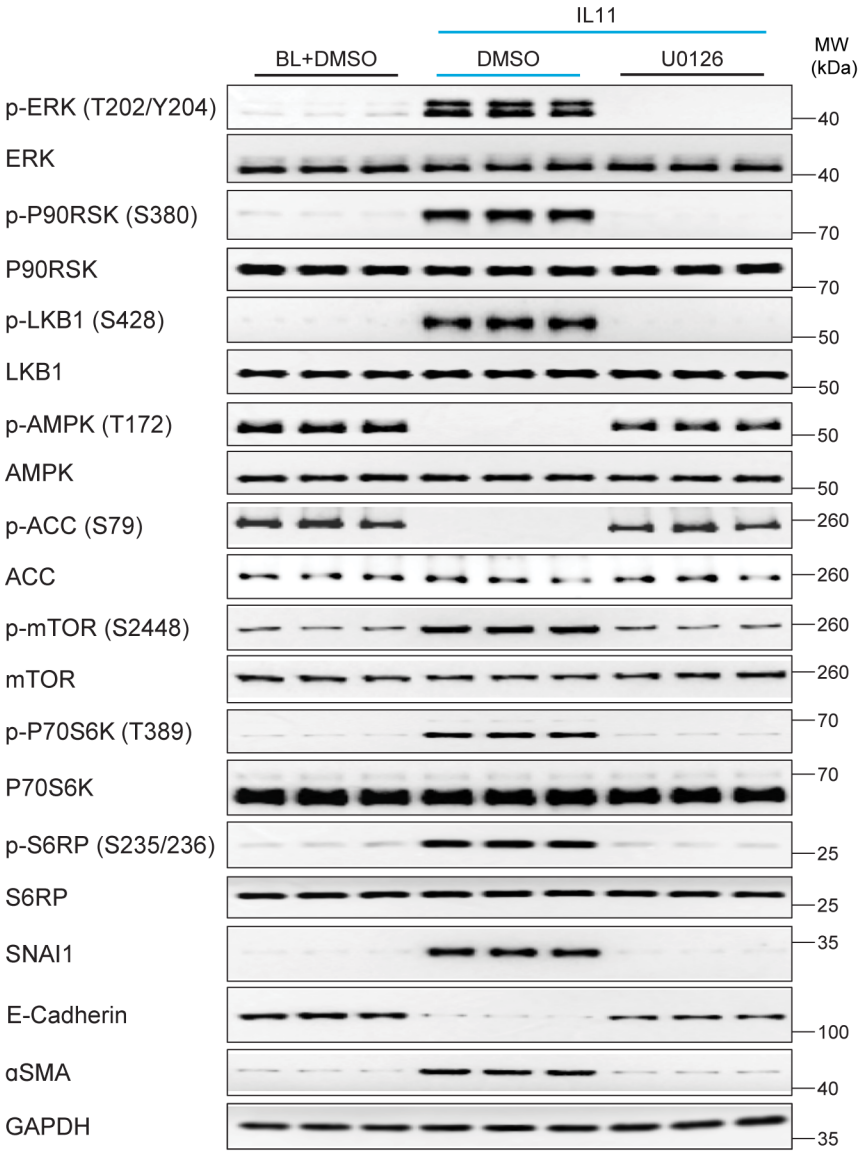


Figure S9. IL11/ERK activity inhibits LKB1/AMPK to cause renal tubular epithelial cell dedifferentiation, Related to Figure 3. WB of p- and total ERK, P90RSK, LKB1, AMPK, ACC, mTOR, P70S6K, S6RP, SNAI1, E-Cadherin, αSMA and GAPDH in TECs stimulated with IL11 (10 ng/ml) in the presence of U0126 (10 μM) or DMSO (n=3 biological replicates).

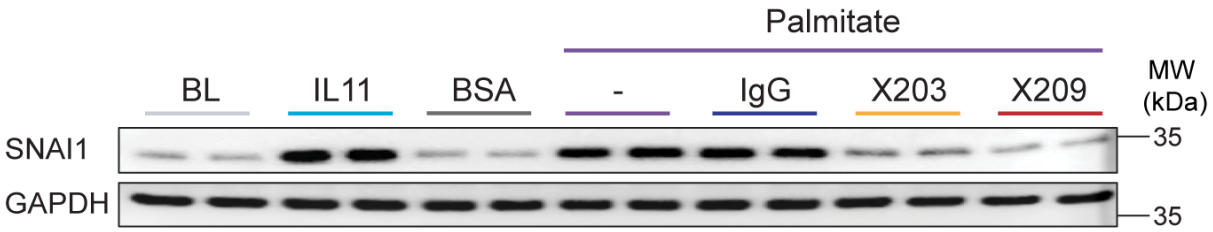
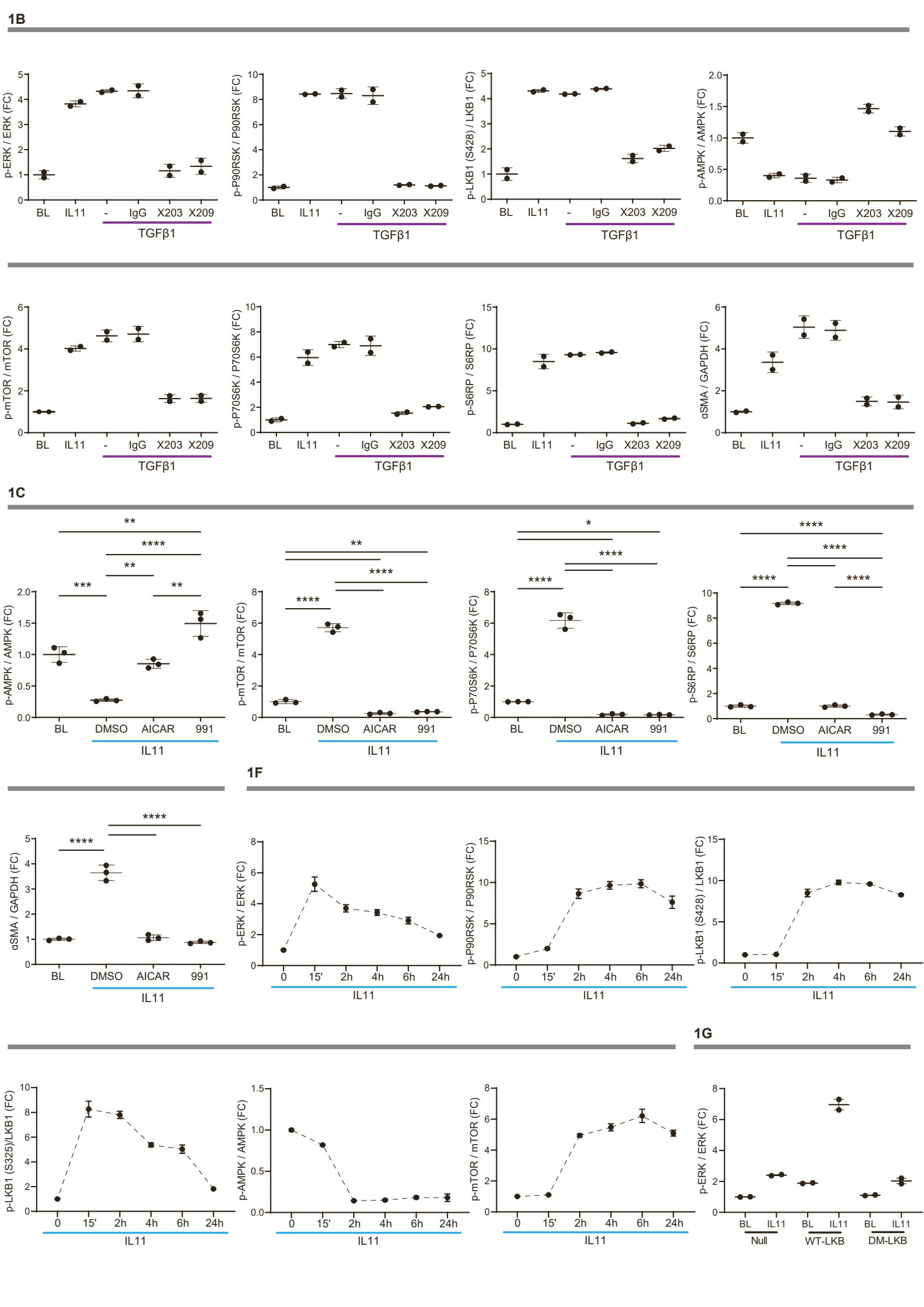
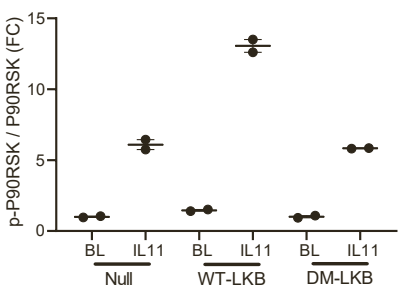


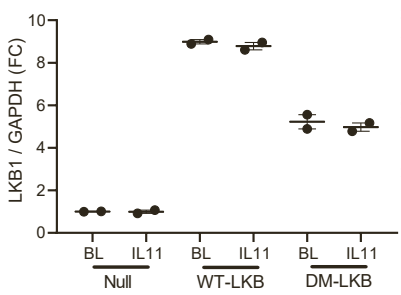
Figure S10. IL11 stimulates hepatocyte SNAI1 expression, Related to Figure 3. WB of SNAI1 and GAPDH in hepatocytes stimulated with IL11 (10 ng/ml) or palmitate in the presence of IgG, X203, or X209 (2 µg/ml) (n=2 biological replicates)

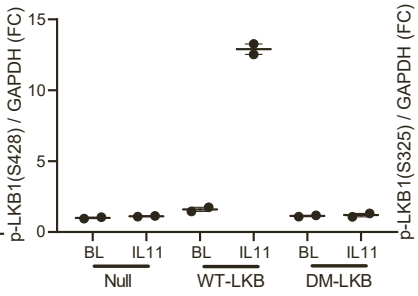
Data S2. Densitometry analyses, Related to STAR Methods.

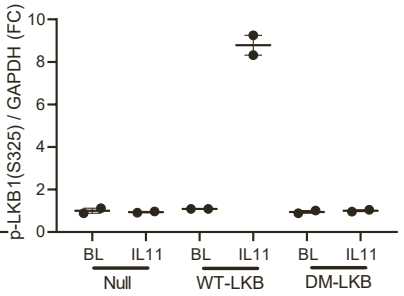

1B
p-ERK / ERK (FC)
p-P90RSK / P90RSK (FC)
p-LKB1 (S428) / LKB1 (FC)
p-AMPK / AMPK (FC)
p-mTOR / mTOR (FC)
p-P70S6K / P70S6K (FC)
p-S6RP / S6RP (FC)
αSMA / GAPDH (FC)
BL
IL11
-
IgG
X203
X209
TGFβ1
1C
p-AMPK / AMPK (FC)
p-mTOR / mTOR (FC)
p-P70S6K / P70S6K (FC)
p-S6RP / S6RP (FC)
DMSO
AICAR
991
IL11
1F
αSMA / GAPDH (FC)
p-ERK / ERK (FC)
p-P90RSK / P90RSK (FC)
p-LKB1 (S428) / LKB1 (FC)
0
15'
2h
4h
6h
24h
p-LKB1 (S325)/LKB1 (FC)
p-AMPK / AMPK (FC)
p-mTOR / mTOR (FC)
1G
p-ERK / ERK (FC)
Null
WT-LKB
DM-LKB

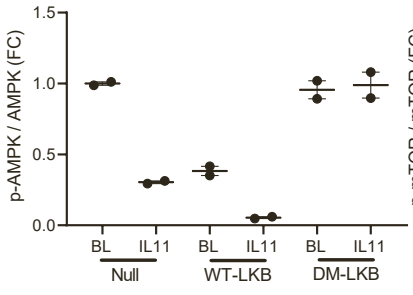
1G

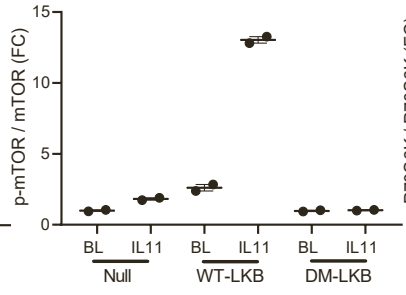

p-P90RSK / P90RSK (FC)
BL
IL11
Null
WT-LKB
DM-LKB

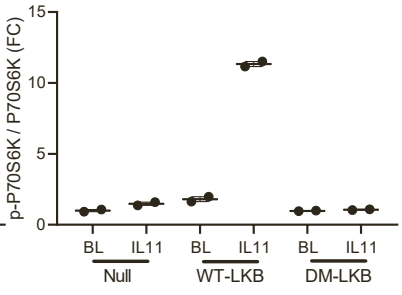

LKB1 / GAPDH (FC)
BL
IL11
Null
WT-LKB
DM-LKB

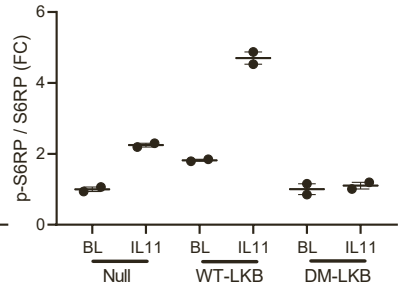

p-LKB1(S428) / GAPDH (FC)
BL
IL11
Null
WT-LKB
DM-LKB


p-LKB1(S325) / GAPDH (FC)
BL
IL11
Null
WT-LKB
DM-LKB

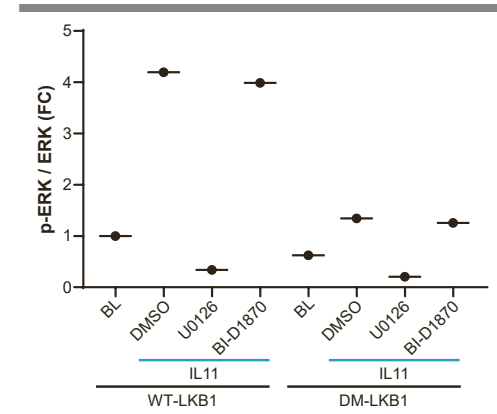

p-AMPK / AMPK (FC)
BL
IL11
Null
WT-LKB
DM-LKB

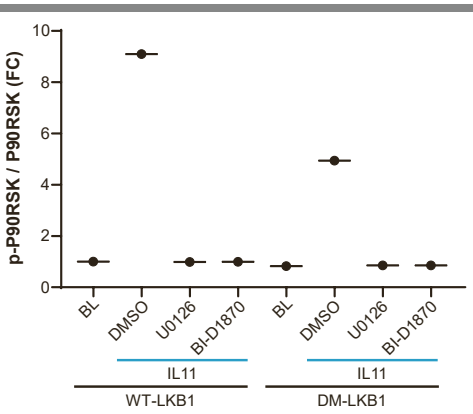

p-mTOR / mTOR (FC)
BL
IL11
Null
WT-LKB
DM-LKB

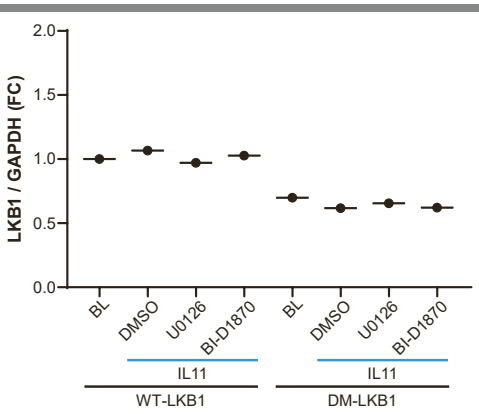

p-P70S6K / P70S6K (FC)
BL
IL11
Null
WT-LKB
DM-LKB

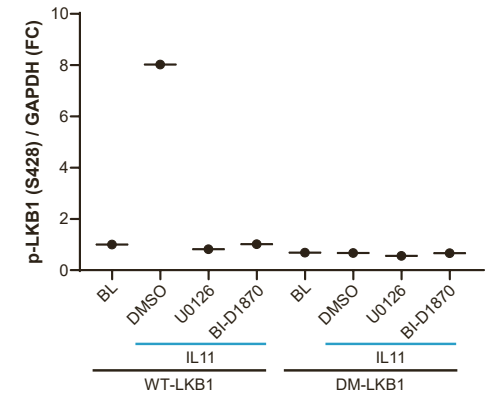

p-S6RP / S6RP (FC)
BL
IL11
Null
WT-LKB
DM-LKB

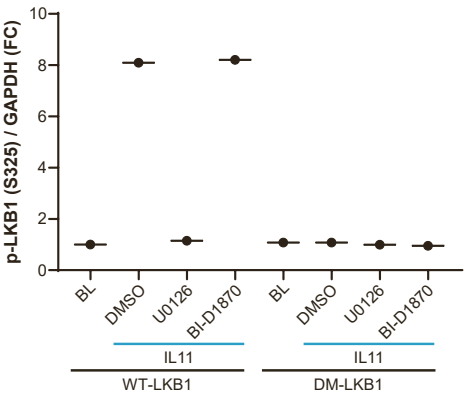
1H

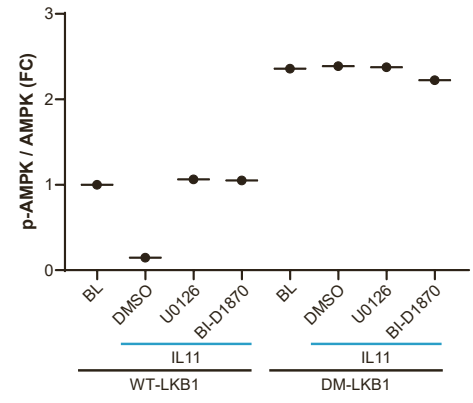

p-ERK / ERK (FC)
BL
DMSO
U0126
BI-D1870
IL11
WT-LKB1
DM-LKB1

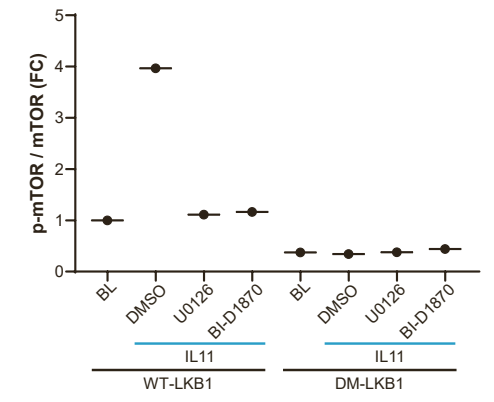

p-P90RSK / P90RSK (FC)
BL
DMSO
U0126
BI-D1870
IL11
WT-LKB1
DM-LKB1

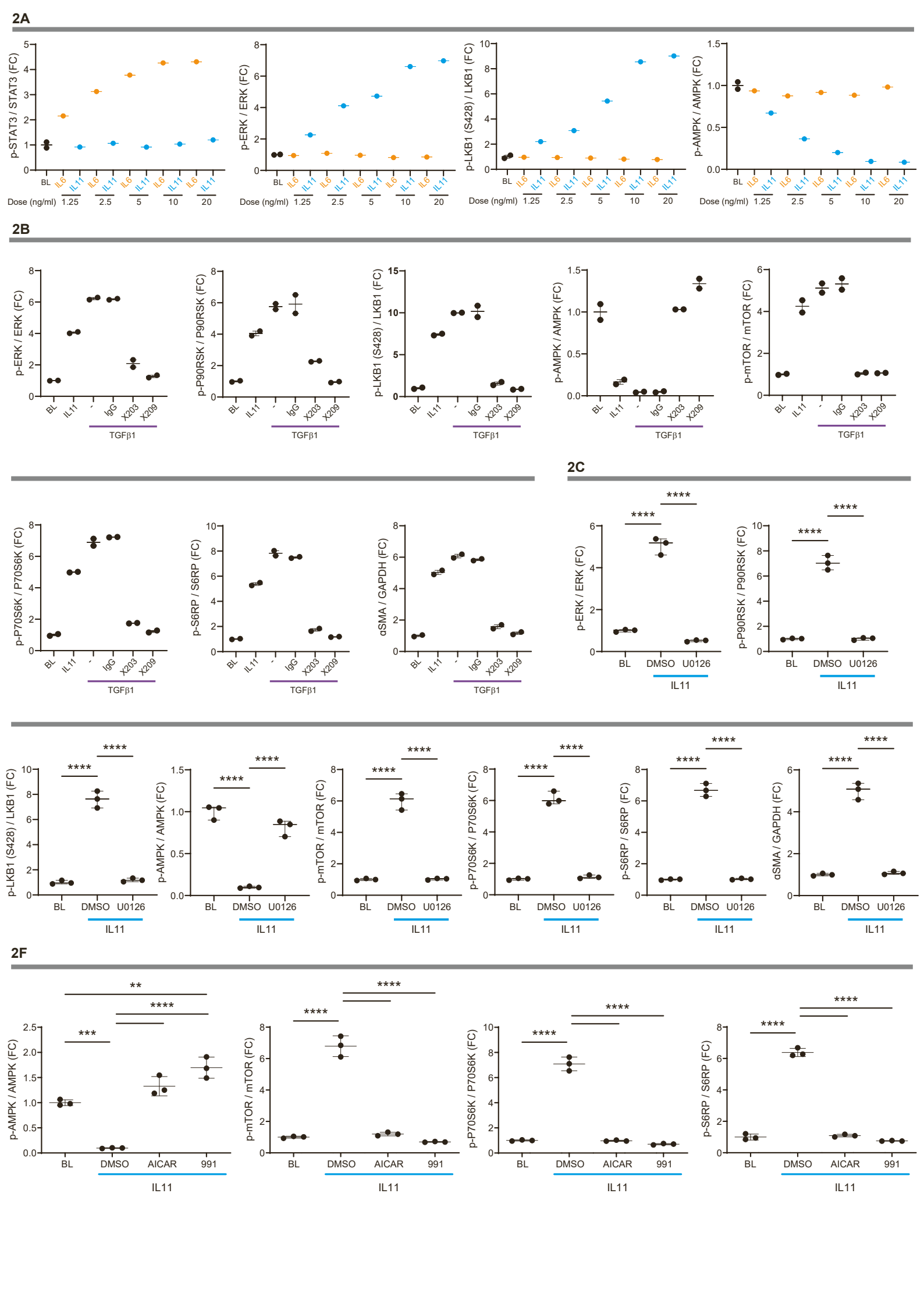

LKB1 / GAPDH (FC)
BL
DMSO
U0126
BI-D1870
IL11
WT-LKB1
DM-LKB1


p-LKB1 (S428) / GAPDH (FC)
BL
DMSO
U0126
BI-D1870
IL11
WT-LKB1
DM-LKB1

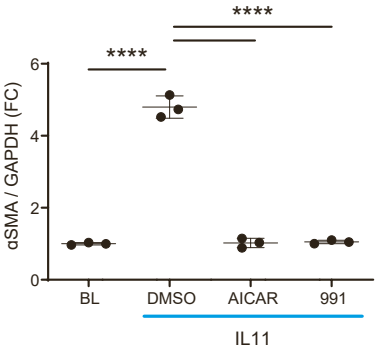

p-LKB1 (S325) / GAPDH (FC)
BL
DMSO
U0126
BI-D1870
IL11
WT-LKB1
DM-LKB1


p-AMPK / AMPK (FC)
BL
DMSO
U0126
BI-D1870
IL11
WT-LKB1
DM-LKB1

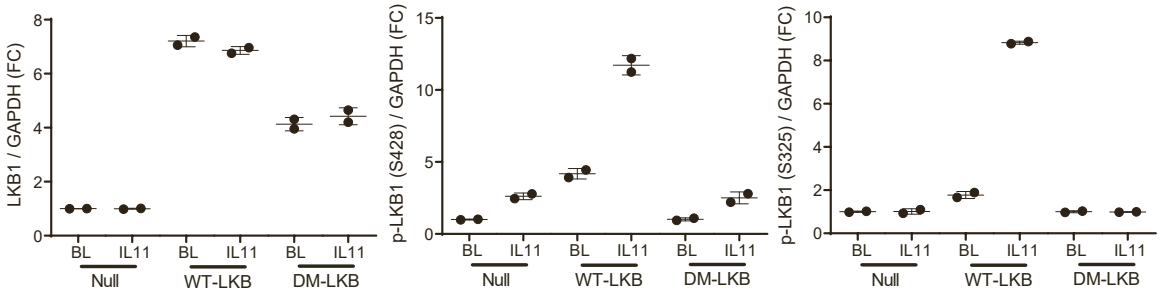

p-mTOR / mTOR (FC)
BL
DMSO
U0126
BI-D1870
IL11
WT-LKB1
DM-LKB1


2A
p-STAT3 / STAT3 (FC)
p-ERK / ERK (FC)
p-LKB1 (S428) / LKB1 (FC)
p-AMPK / AMPK (FC)
BL IL6 IL11 IL6 IL11 IL6 IL11 IL6 IL11 IL6 IL11
Dose (ng/ml) 1.25 2.5 5 10 20
2B
p-ERK / ERK (FC)
p-P90RSK / P90RSK (FC)
p-LKB1 (S428) / LKB1 (FC)
p-AMPK / AMPK (FC)
p-mTOR / mTOR (FC)
BL IL11 - IgG X203 X209
TGFβ1
p-P70S6K / P70S6K (FC)
p-S6RP / S6RP (FC)
αSMA / GAPDH (FC)
2C
p-ERK / ERK (FC)
p-P90RSK / P90RSK (FC)
BL DMSO U0126
IL11
p-LKB1 (S428) / LKB1 (FC)
p-AMPK / AMPK (FC)
p-mTOR / mTOR (FC)
p-P70S6K / P70S6K (FC)
p-S6RP / S6RP (FC)
αSMA / GAPDH (FC)
****
2F
p-AMPK / AMPK (FC)
p-mTOR / mTOR (FC)
p-P70S6K / P70S6K (FC)
p-S6RP / S6RP (FC)
BL DMSO AICAR 991
IL11
**
***
****

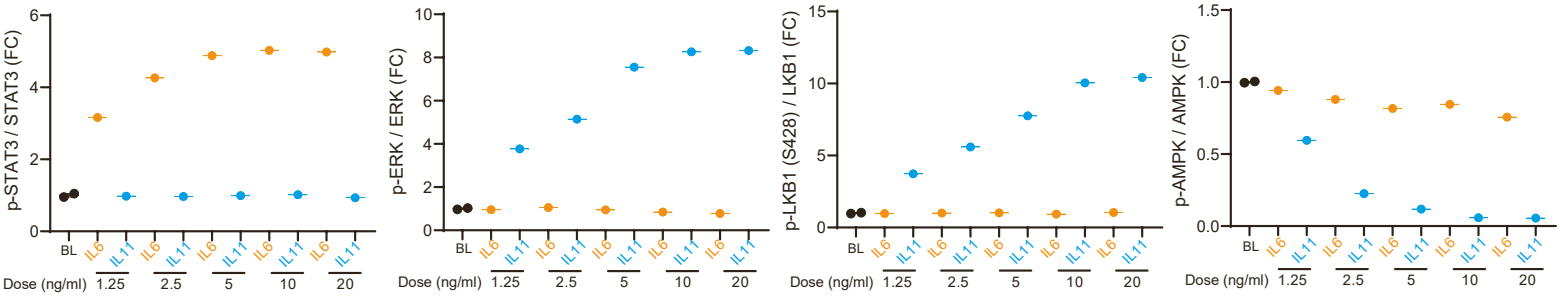
2F


****
****
αSMA / GAPDH (FC)
0
2
4
6
BL
DMSO
AICAR
991
IL11

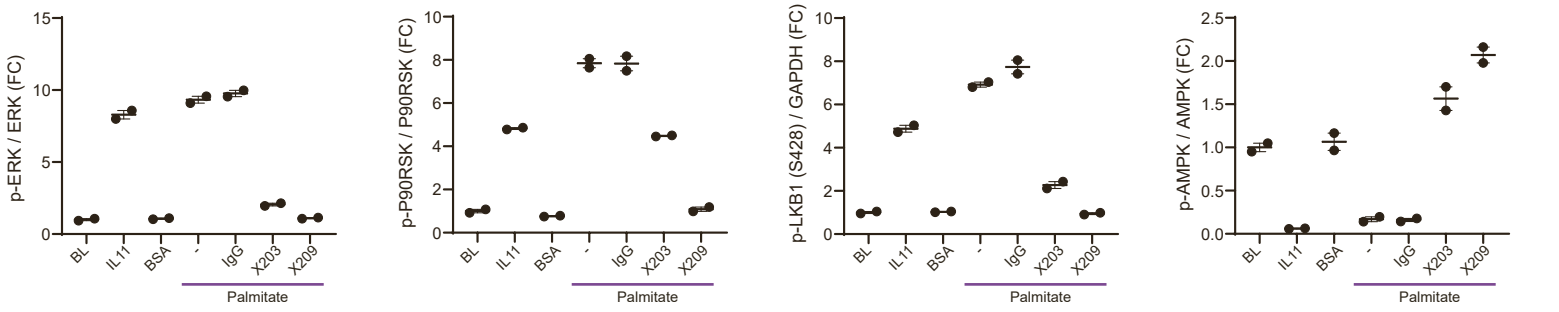
2I

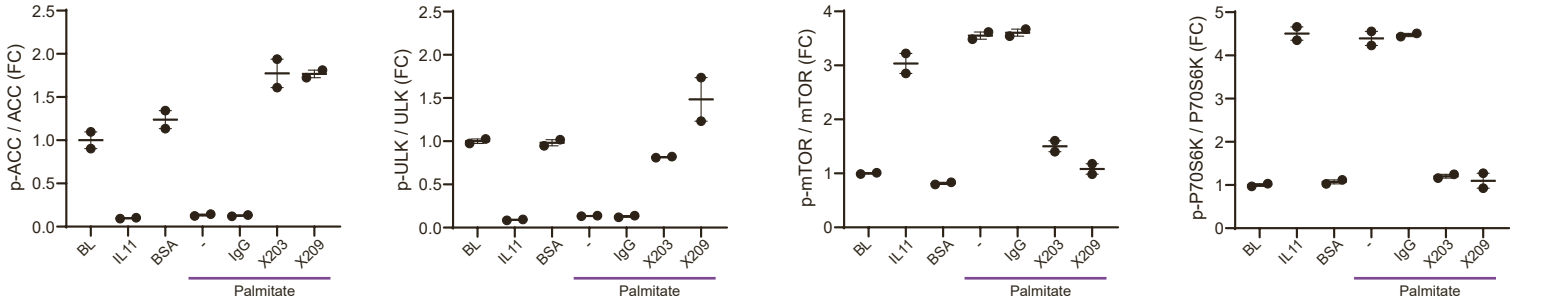

LKB1 / GAPDH (FC)
0
2
4
6
8
BL
IL11
Null
WT-LKB
DM-LKB
p-LKB1 (S428) / GAPDH (FC)
0
5
10
15
p-LKB1 (S325) / GAPDH (FC)
0
2
4
6
8
10

3A

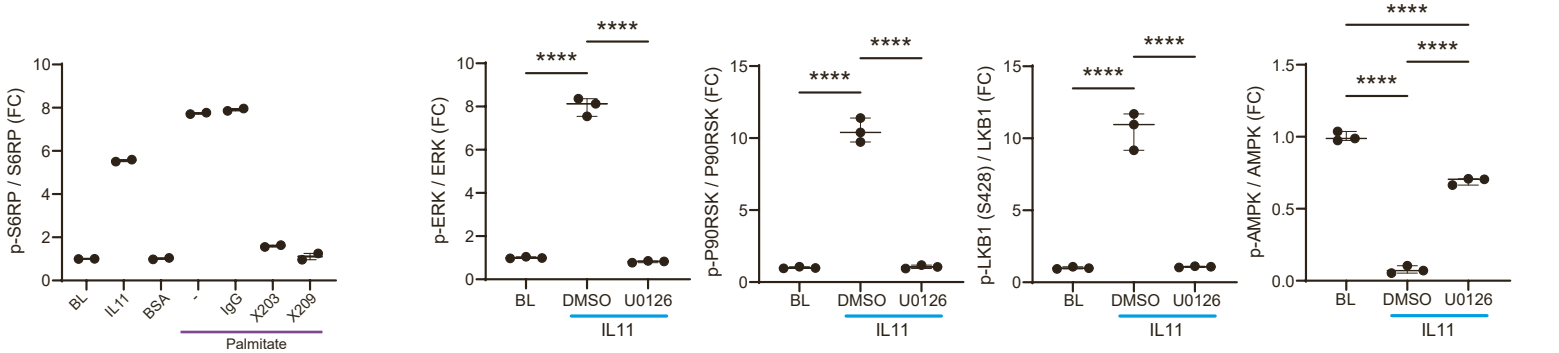

p-STAT3 / STAT3 (FC)
p-ERK / ERK (FC)
p-LKB1 (S428) / LKB1 (FC)
p-AMPK / AMPK (FC)
BL
IL6
IL11
Dose (ng/ml) 1.25 2.5 5 10 20

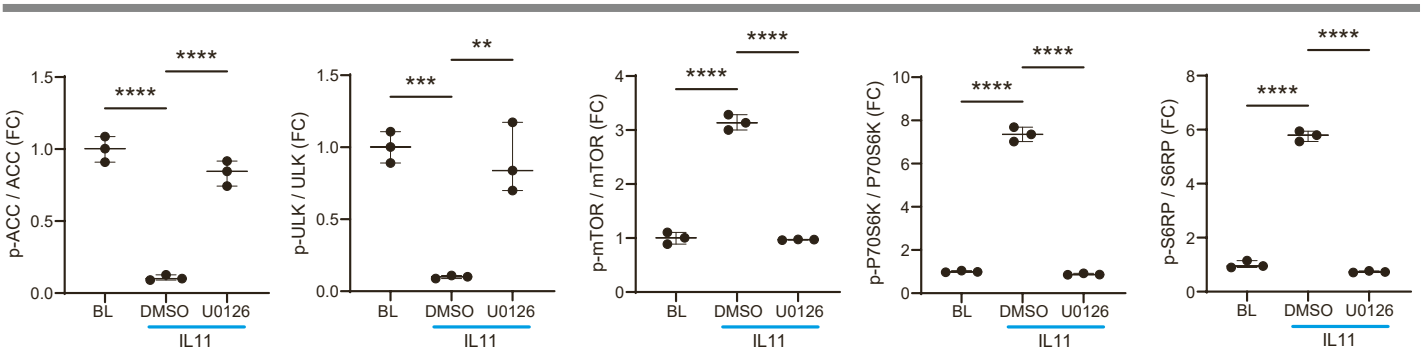
3B


p-ERK / ERK (FC)
p-P90RSK / P90RSK (FC)
p-LKB1 (S428) / GAPDH (FC)
p-AMPK / AMPK (FC)
BL
IL11
BSA
-
IgG
X203
X209
Palmitate

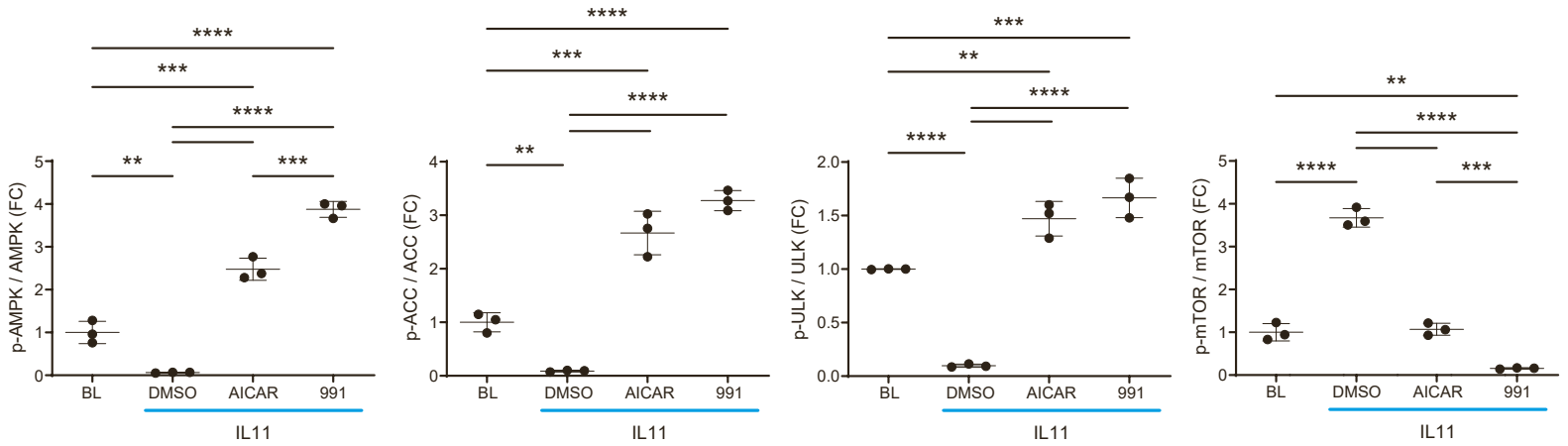

p-ACC / ACC (FC)
p-ULK / ULK (FC)
p-mTOR / mTOR (FC)
p-P70S6K / P70S6K (FC)
BL
IL11
BSA
-
IgG
X203
X209
Palmitate

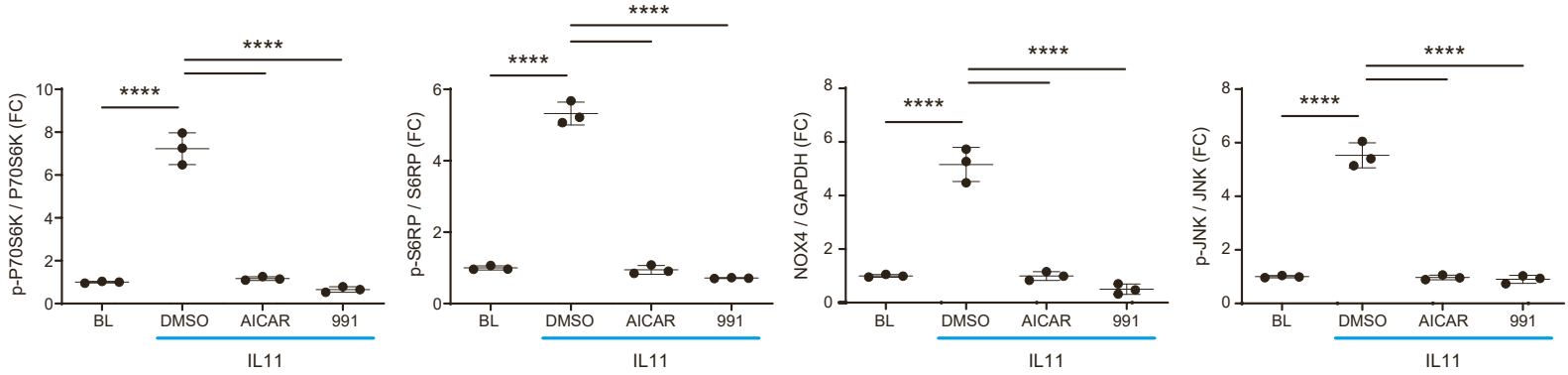
3C


p-S6RP / S6RP (FC)
p-ERK / ERK (FC)
p-P90RSK / P90RSK (FC)
p-LKB1 (S428) / LKB1 (FC)
p-AMPK / AMPK (FC)
BL
IL11
BSA
-
IgG
X203
X209
Palmitate
DMSO
U0126
****

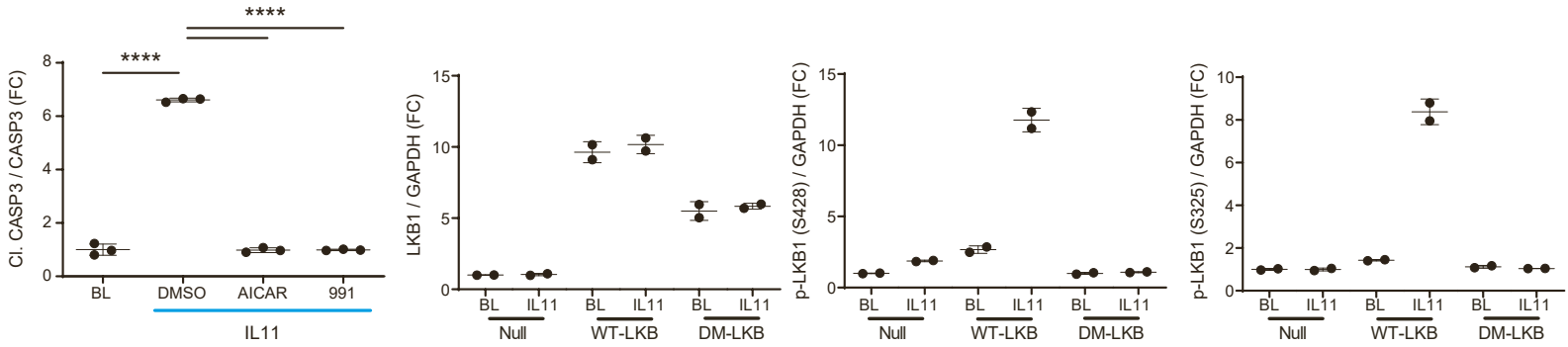

p-ACC / ACC (FC)
p-ULK / ULK (FC)
p-mTOR / mTOR (FC)
p-P70S6K / P70S6K (FC)
p-S6RP / S6RP (FC)
BL
DMSO
U0126
IL11
****
***
**

3F

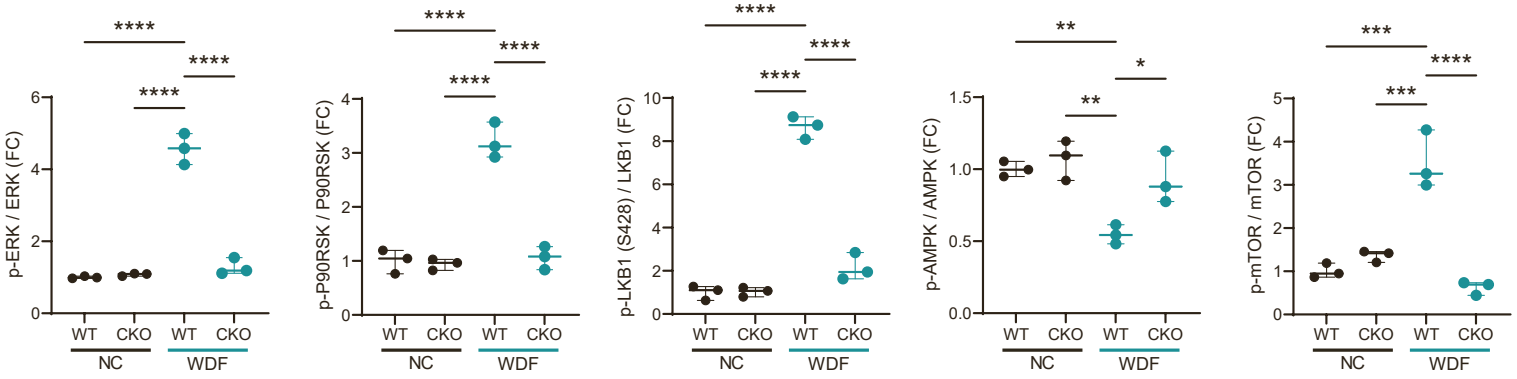

p-AMPK / AMPK (FC)
p-ACC / ACC (FC)
p-ULK / ULK (FC)
p-mTOR / mTOR (FC)
BL
DMSO
AICAR
991
IL11

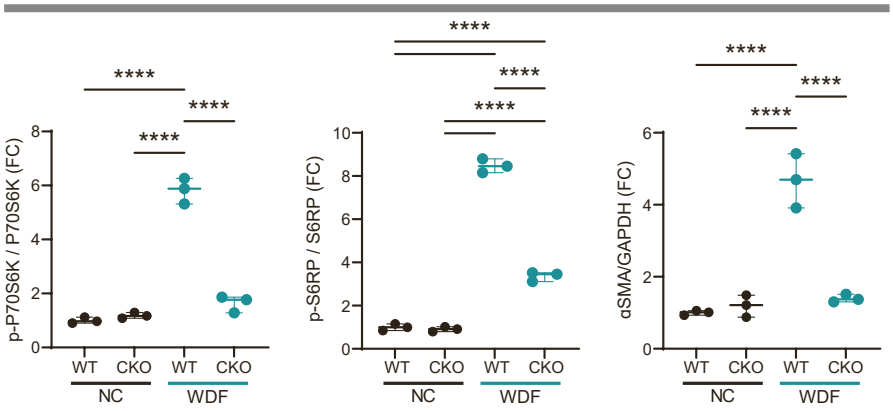

p-P70S6K / P70S6K (FC)
p-S6RP / S6RP (FC)
NOX4 / GAPDH (FC)
p-JNK / JNK (FC)
BL
DMSO
AICAR
991
IL11

3K

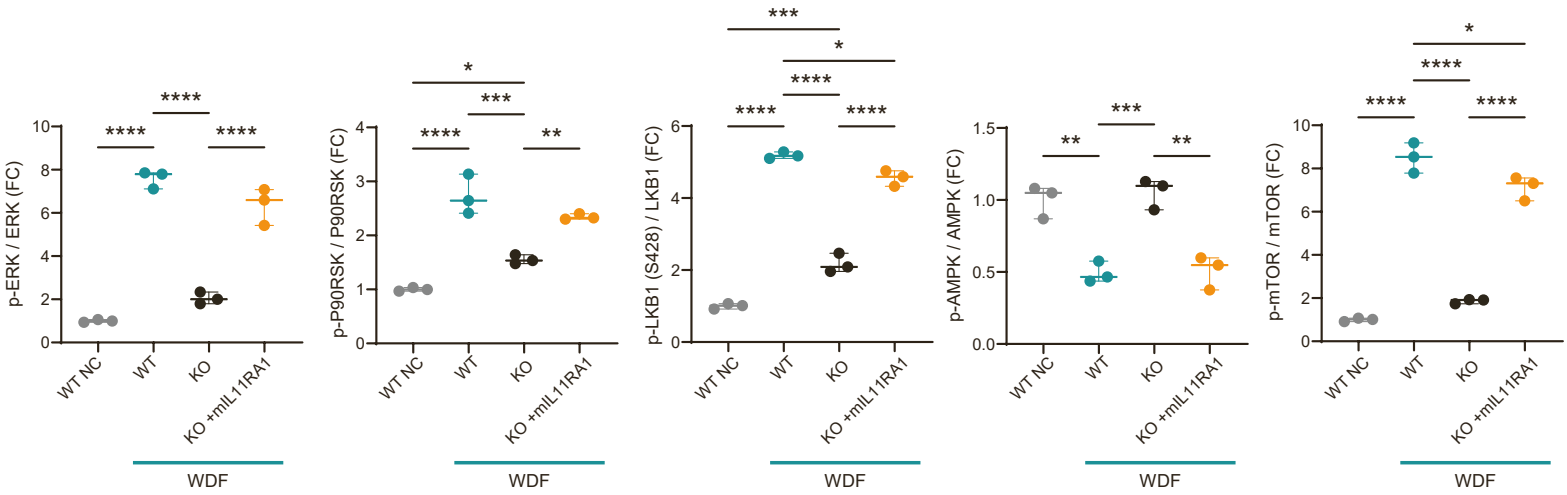

Cl. CASP3 / CASP3 (FC)
LKB1 / GAPDH (FC)
p-LKB1 (S428) / GAPDH (FC)
p-LKB1 (S325) / GAPDH (FC)
BL
IL11
Null
WT-LKB
DM-LKB

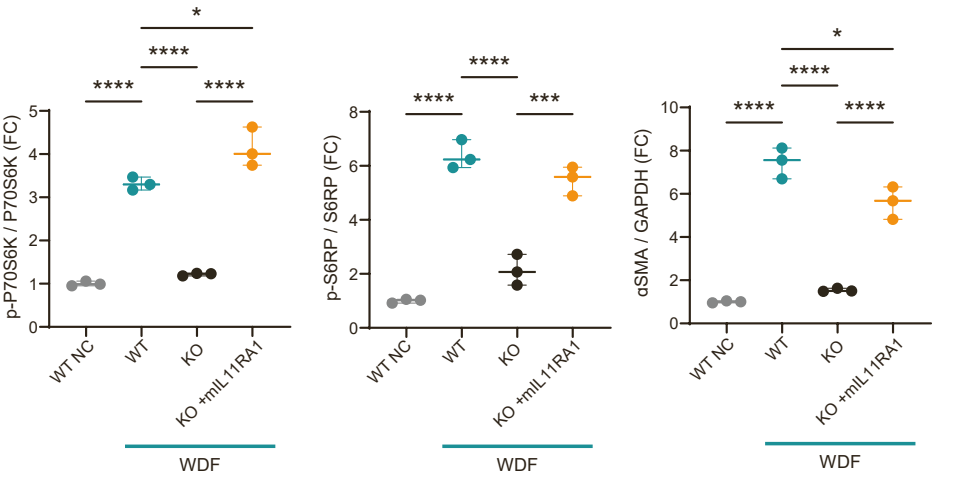
4A


p-ERK / ERK (FC)
p-P90RSK / P90RSK (FC)
p-LKB1 (S428) / LKB1 (FC)
p-AMPK / AMPK (FC)
p-mTOR / mTOR (FC)
WT
CKO
NC
WDF

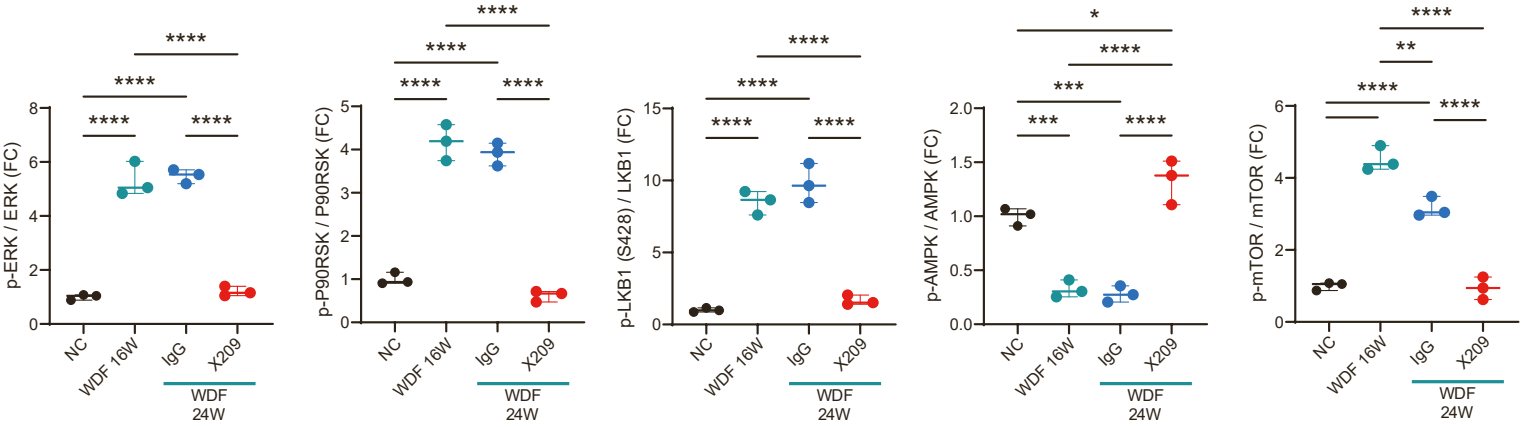

p-P70S6K / P70S6K (FC)
p-S6RP / S6RP (FC)
αSMA/GAPDH (FC)
WT
CKO
NC
WDF

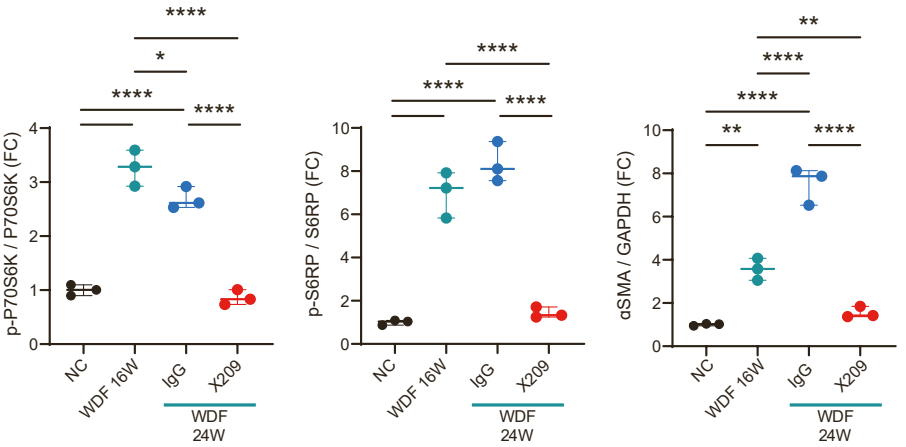
4B


p-ERK / ERK (FC)
p-P90RSK / P90RSK (FC)
p-LKB1 (S428) / LKB1 (FC)
p-AMPK / AMPK (FC)
p-mTOR / mTOR (FC)
WT NC
WT
KO
KO +mIL11RA1
WDF

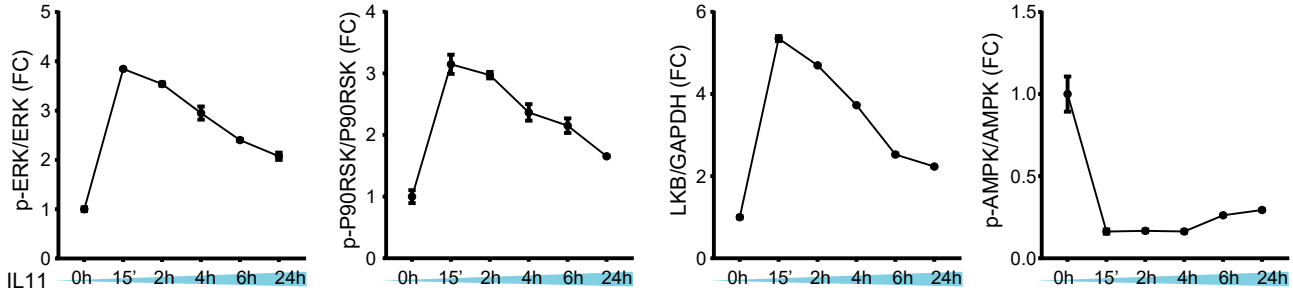

p-P70S6K / P70S6K (FC)
p-S6RP / S6RP (FC)
αSMA / GAPDH (FC)
WT NC
WT
KO
KO +mIL11RA1
WDF

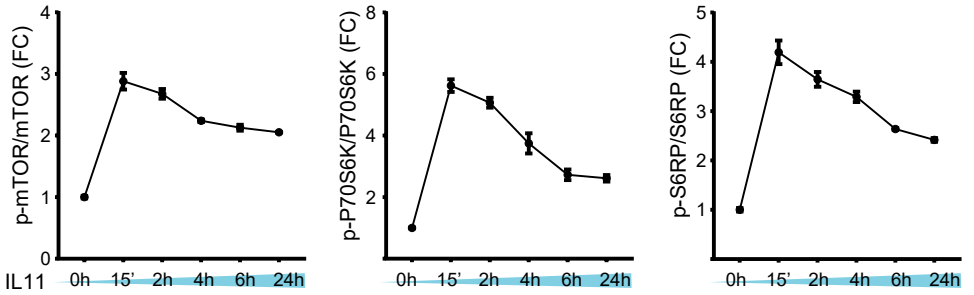
4C


p-ERK / ERK (FC)
p-P90RSK / P90RSK (FC)
p-LKB1 (S428) / LKB1 (FC)
p-AMPK / AMPK (FC)
p-mTOR / mTOR (FC)
NC
WDF 16W
IgG
X209
WDF 24W

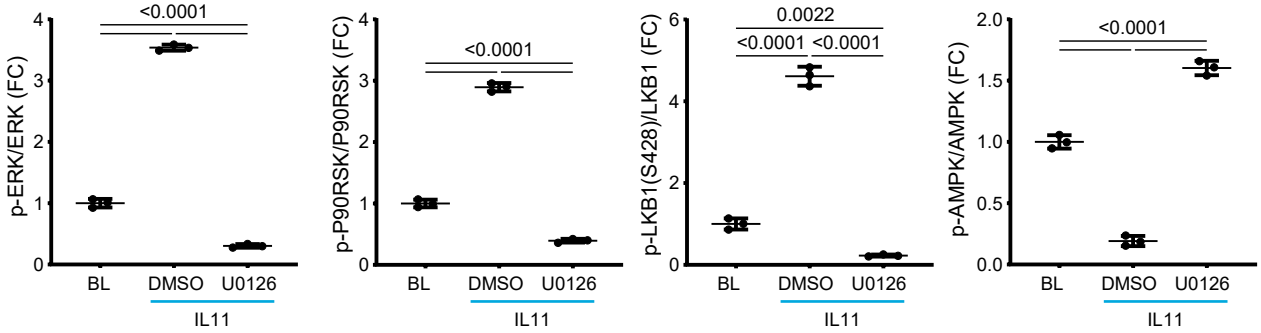

p-P70S6K / P70S6K (FC)
p-S6RP / S6RP (FC)
αSMA / GAPDH (FC)
NC
WDF 16W
IgG
X209
WDF 24W

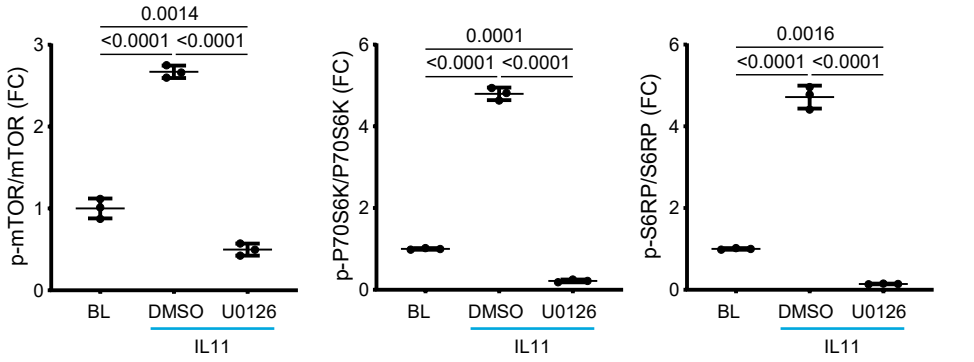
S6A


p-ERK/ERK (FC)
p-P90RSK/P90RSK (FC)
LKB/GAPDH (FC)
p-AMPK/AMPK (FC)
IL11
0h 15' 2h 4h 6h 24h

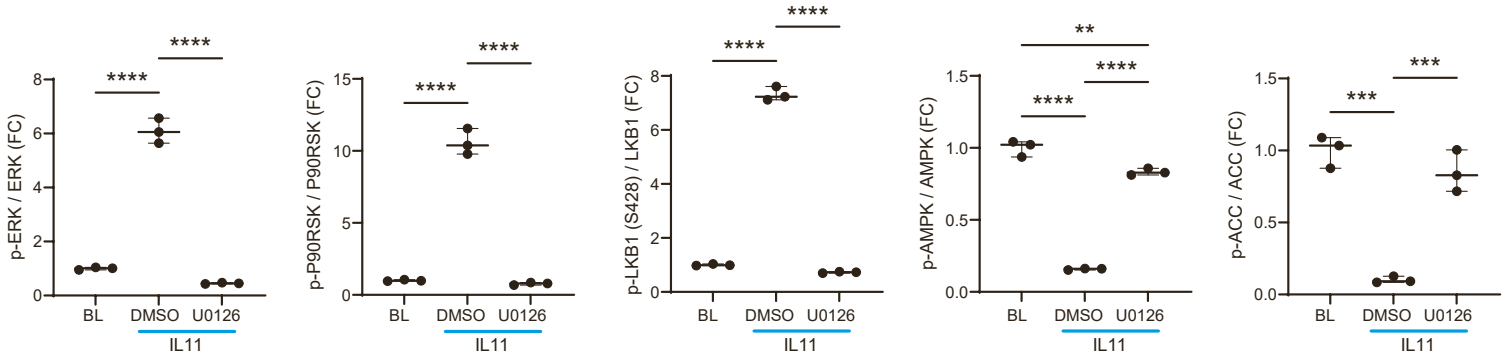

p-mTOR/mTOR (FC)
p-P70S6K/P70S6K (FC)
p-S6RP/S6RP (FC)
IL11
0h 15' 2h 4h 6h 24h

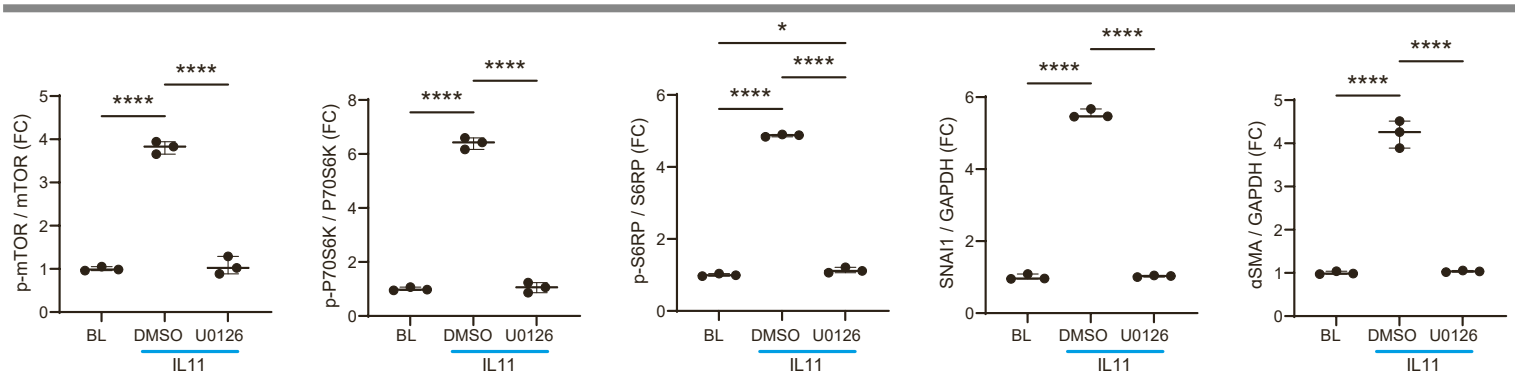
S6B


p-ERK/ERK (FC)
p-P90RSK/P90RSK (FC)
p-LKB1(S428)/LKB1 (FC)
p-AMPK/AMPK (FC)
<0.0001
0.0022
<0.0001 <0.0001
BL DMSO U0126
IL11

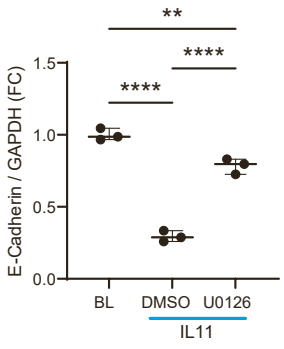

p-mTOR/mTOR (FC)
p-P70S6K/P70S6K (FC)
p-S6RP/S6RP (FC)
0.0014
0.0001
0.0016
<0.0001 <0.0001
BL DMSO U0126
IL11

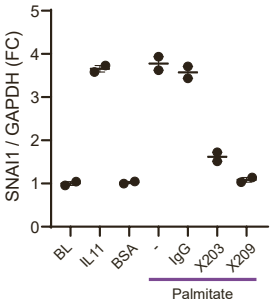
S9


p-ERK / ERK (FC)
p-P90RSK / P90RSK (FC)
p-LKB1 (S428) / LKB1 (FC)
p-AMPK / AMPK (FC)
p-ACC / ACC (FC)
BL
DMSO
U0126
IL11


p-mTOR / mTOR (FC)
p-P70S6K / P70S6K (FC)
p-S6RP / S6RP (FC)
SNAI1 / GAPDH (FC)
αSMA / GAPDH (FC)
BL
DMSO
U0126
IL11

S10


E-Cadherin / GAPDH (FC)
BL
DMSO
U0126
IL11


SNAI1 / GAPDH (FC)
BL
IL11
BSA
-
IgG
X203
X209
Palmitate

Data S3. Uncropped blots, Related to STAR Methods.

Figure 1A

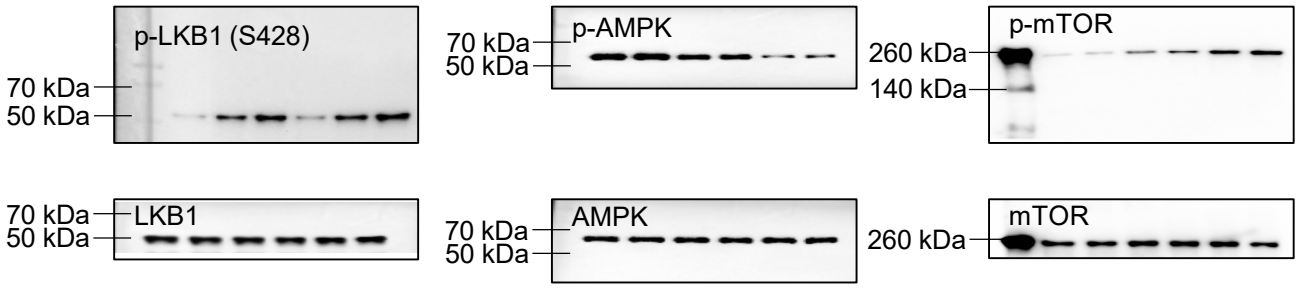


Figure 1B

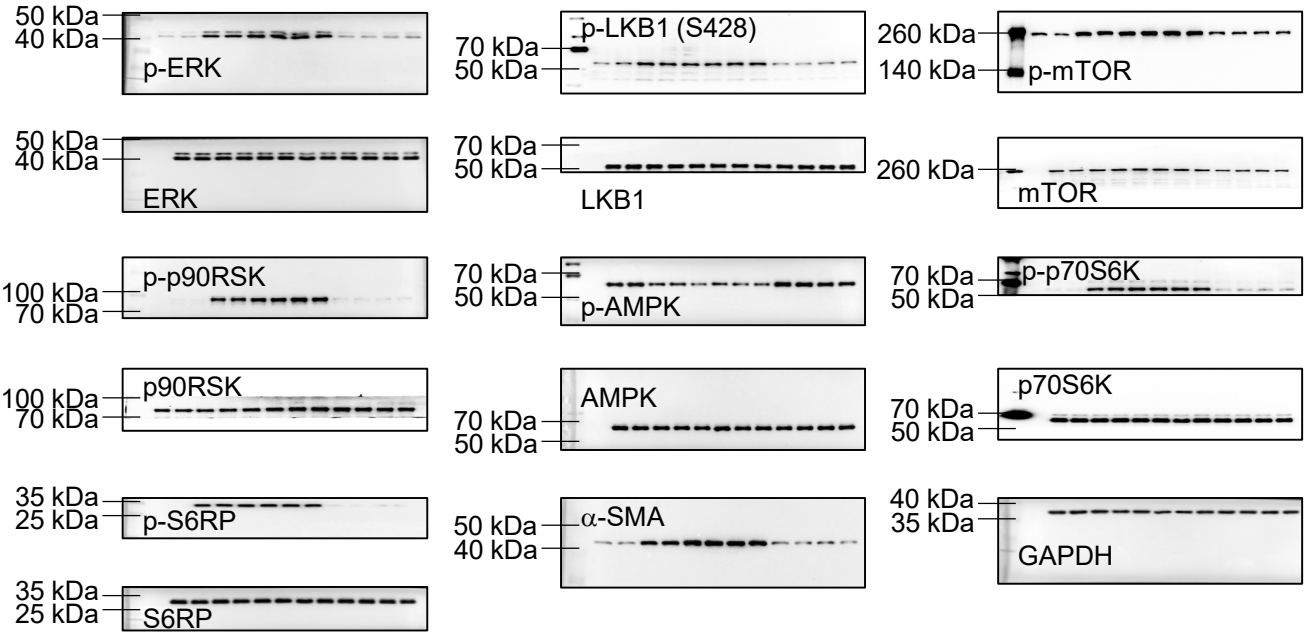


Figure 1C

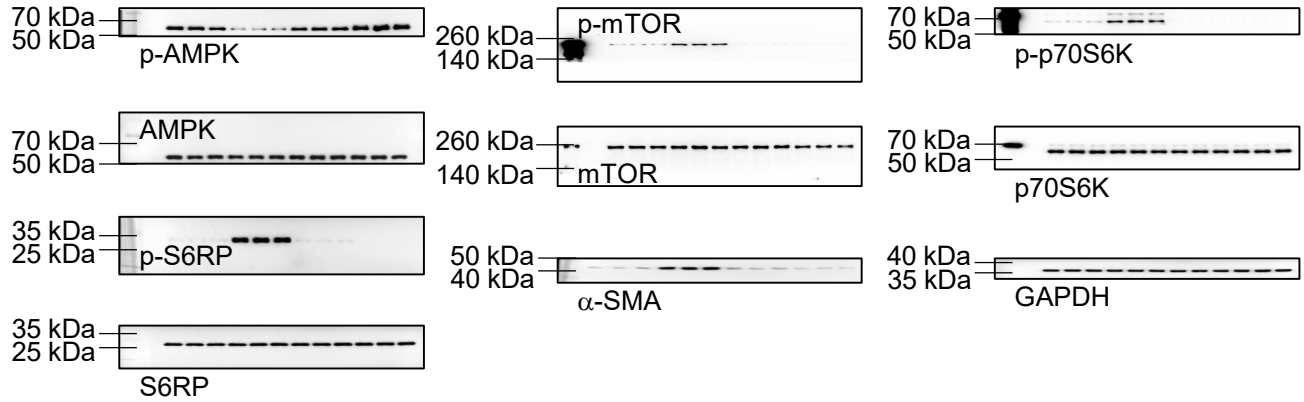


Figure 1F

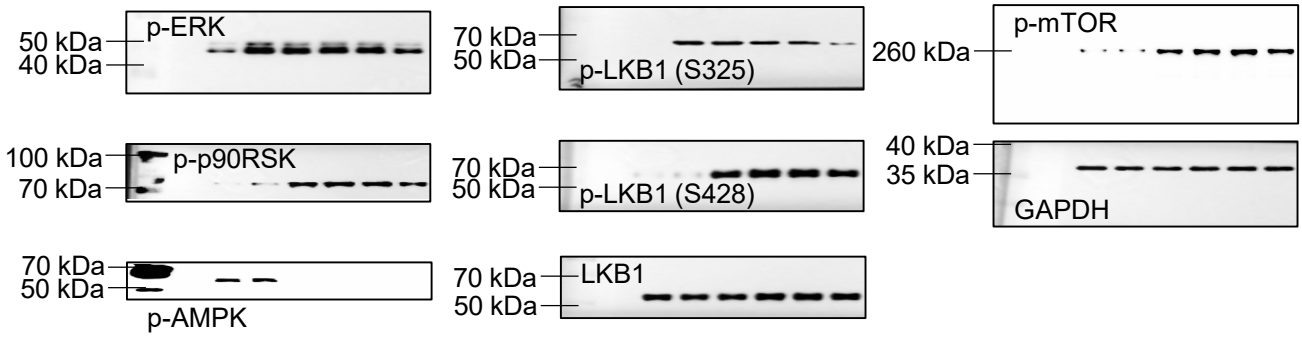


Figure 1G

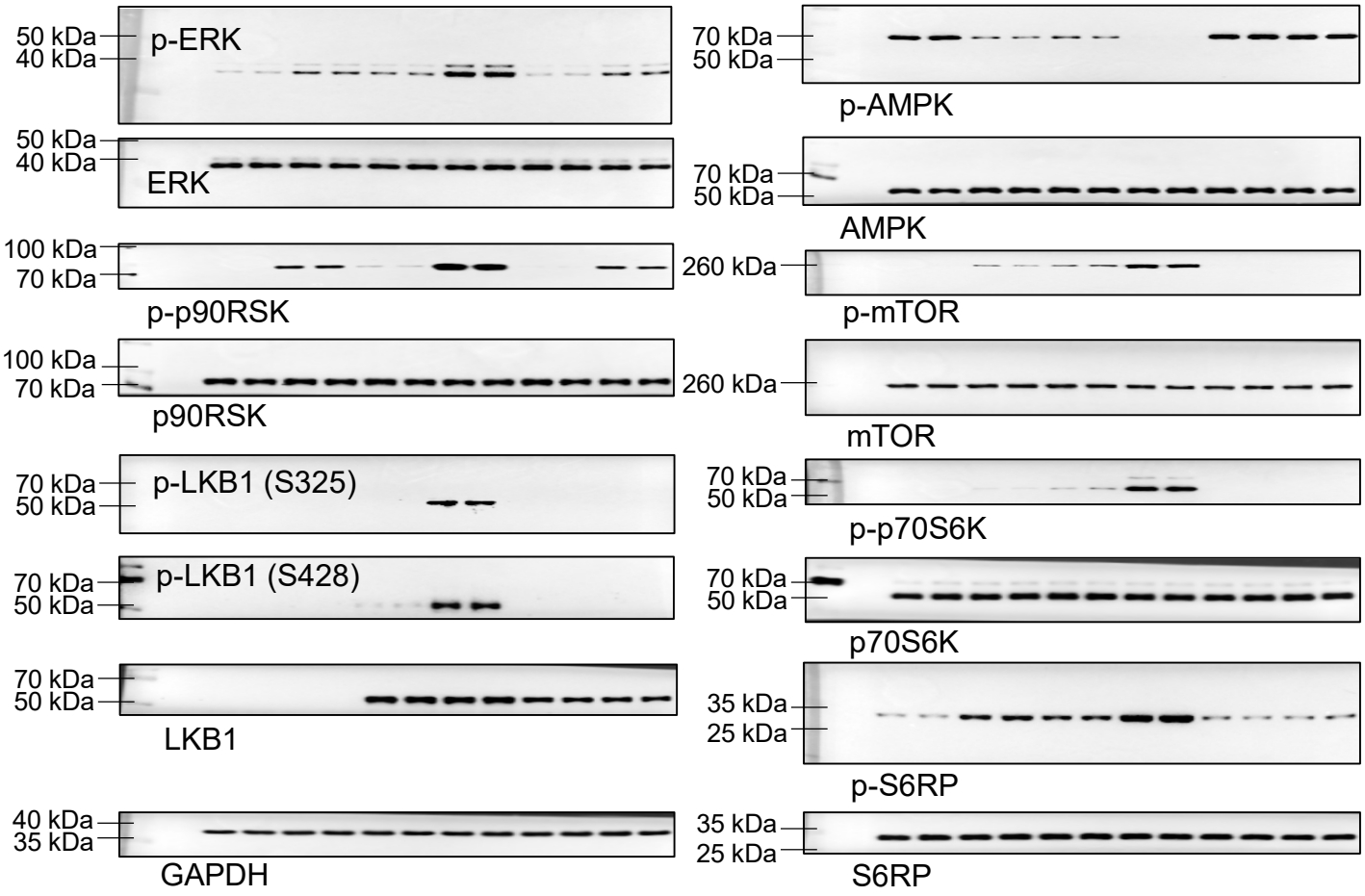


Figure 1H

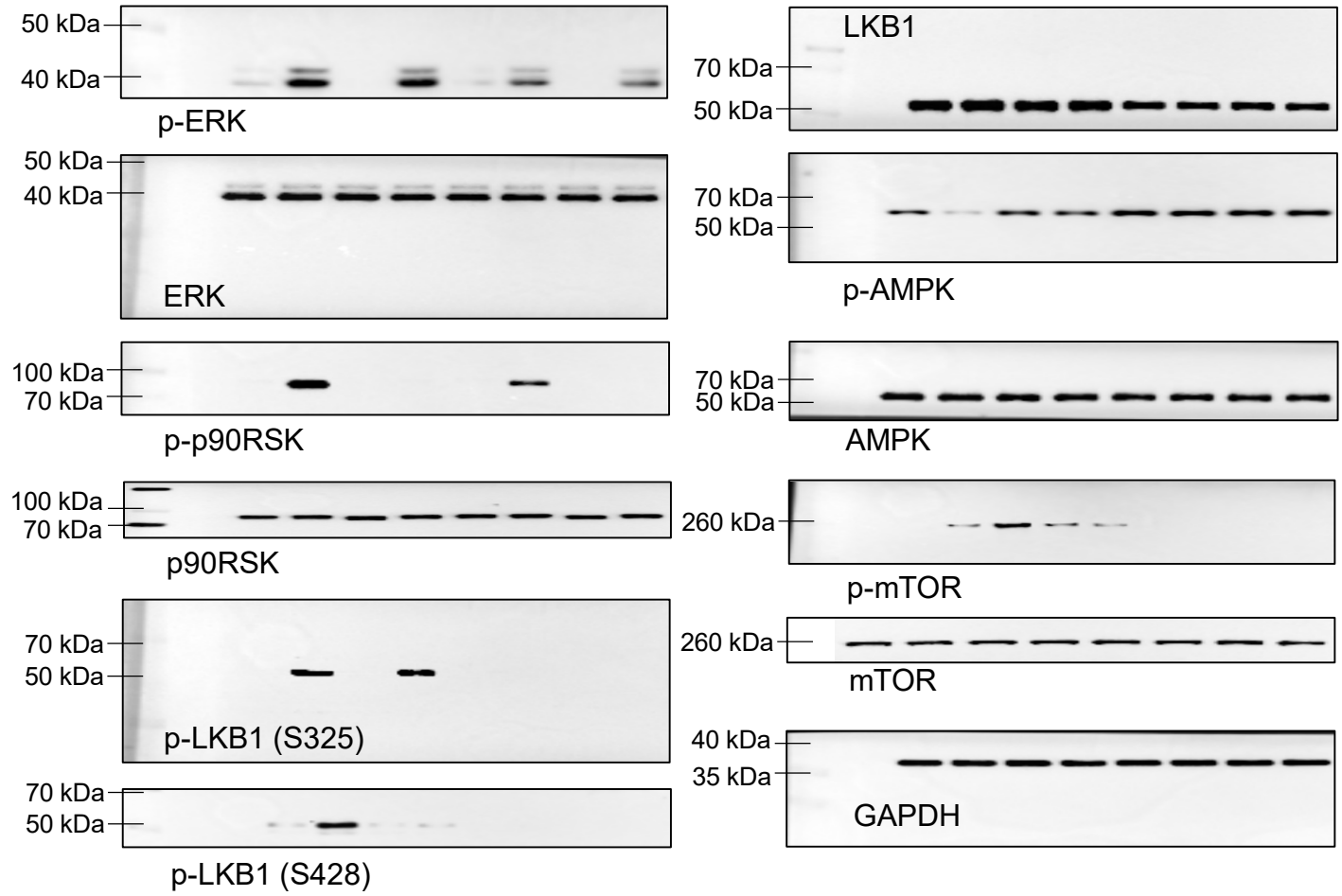


Figure 2A

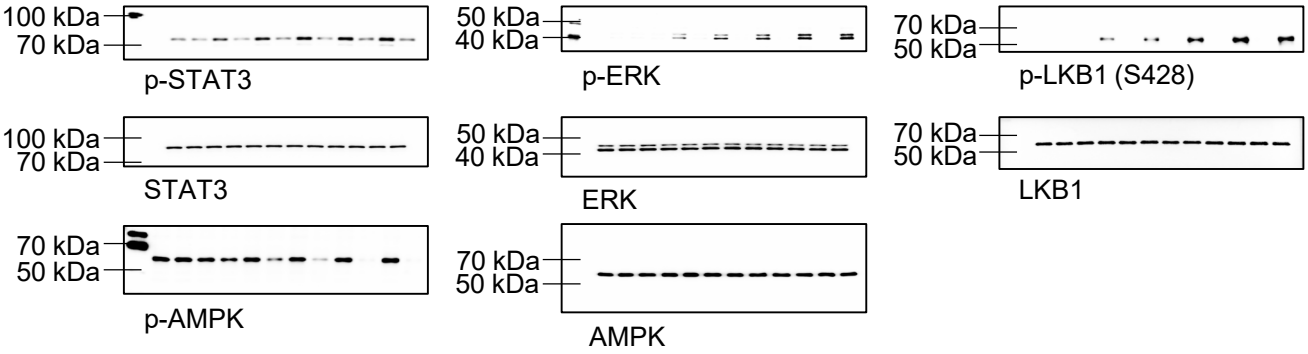


Figure 2B

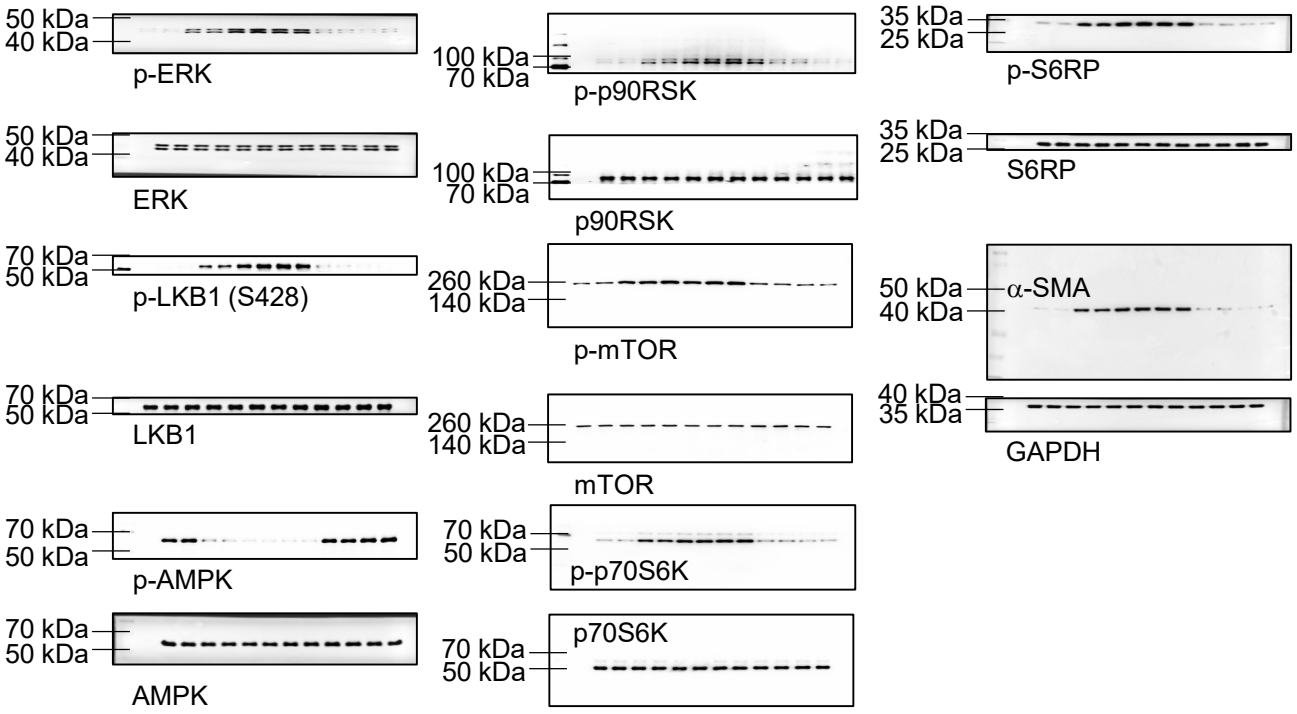


Figure 2C

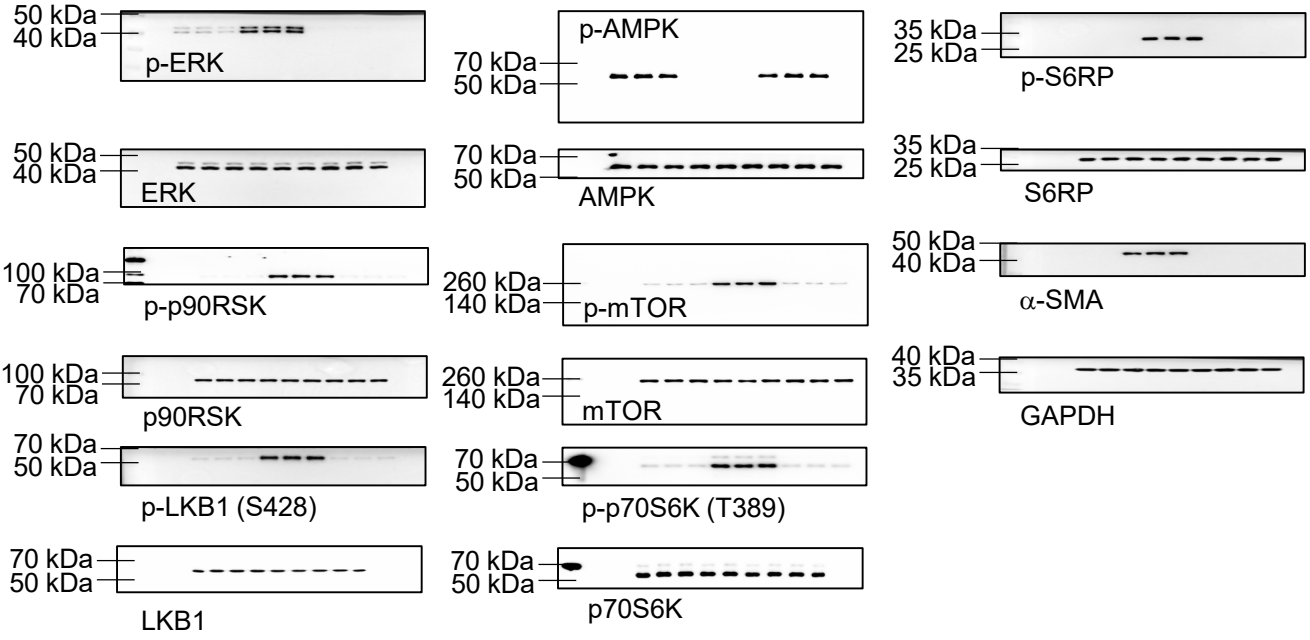


Figure 2F

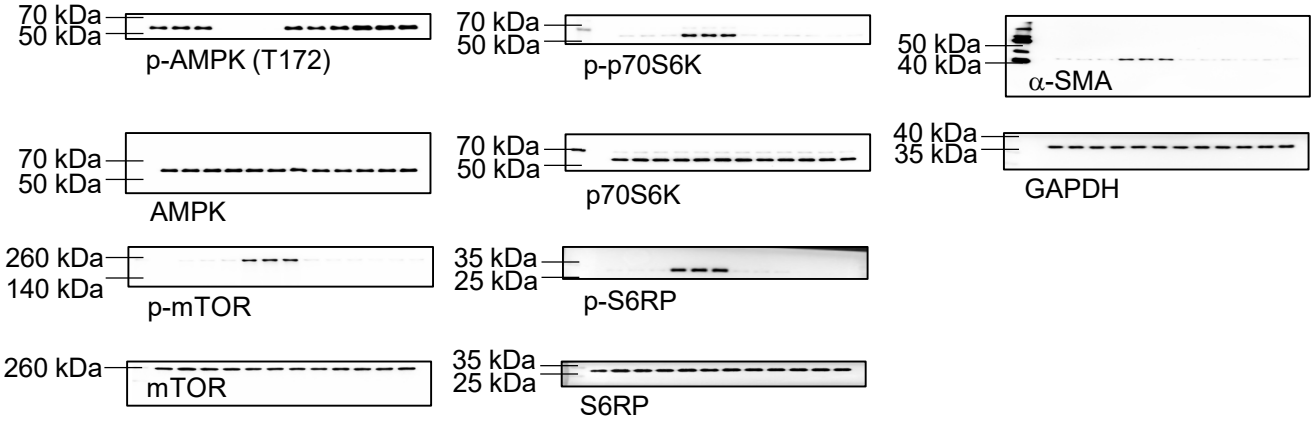


Figure 2I

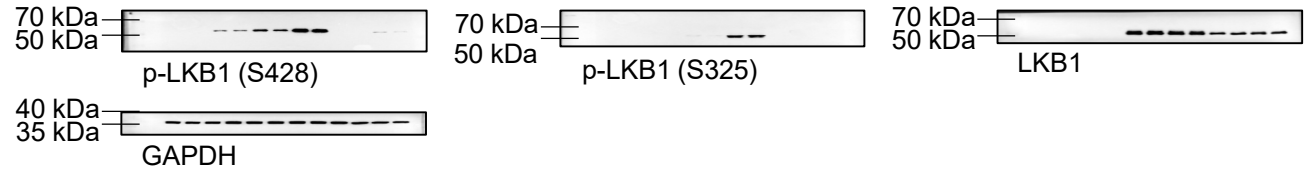


Figure 3A

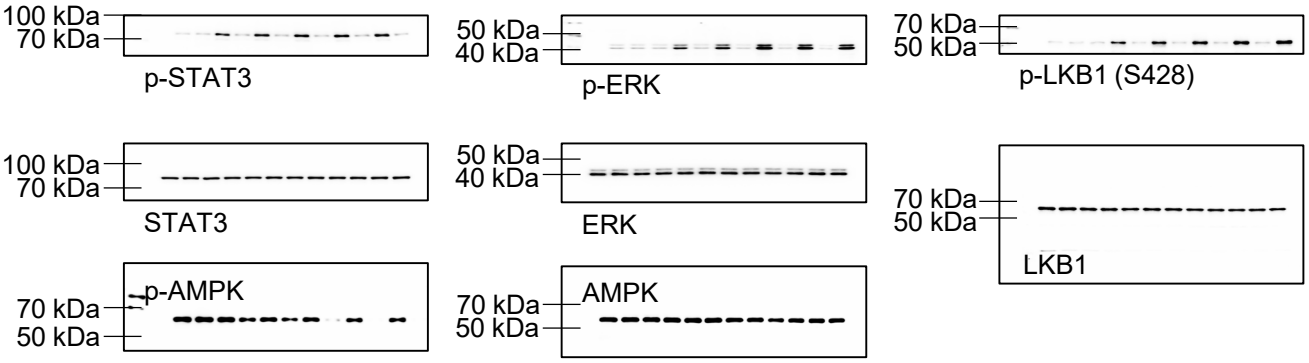


Figure 3B

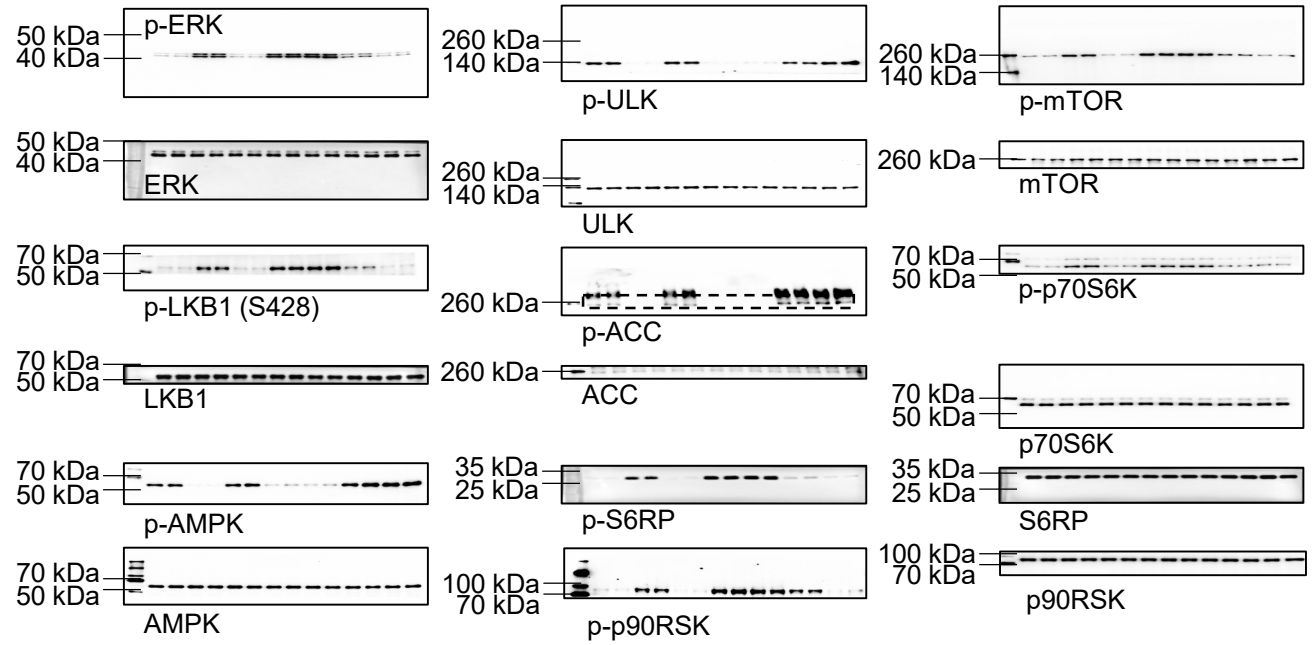


Figure 3C

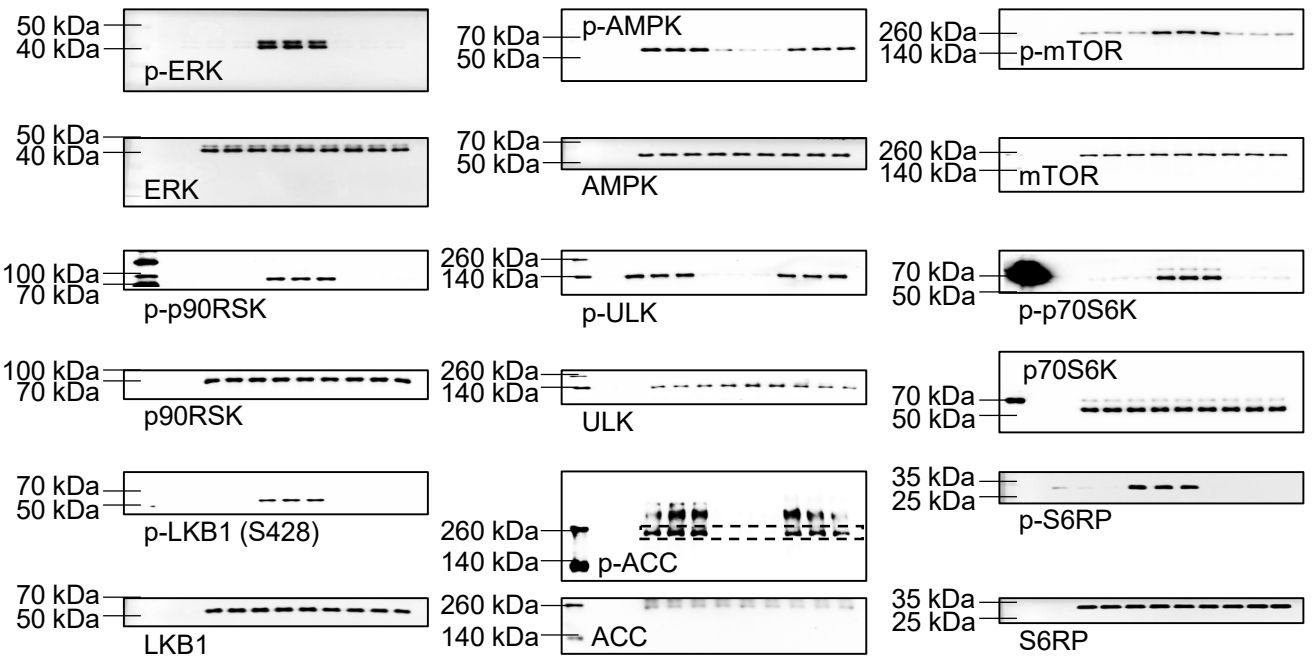


Figure 3F

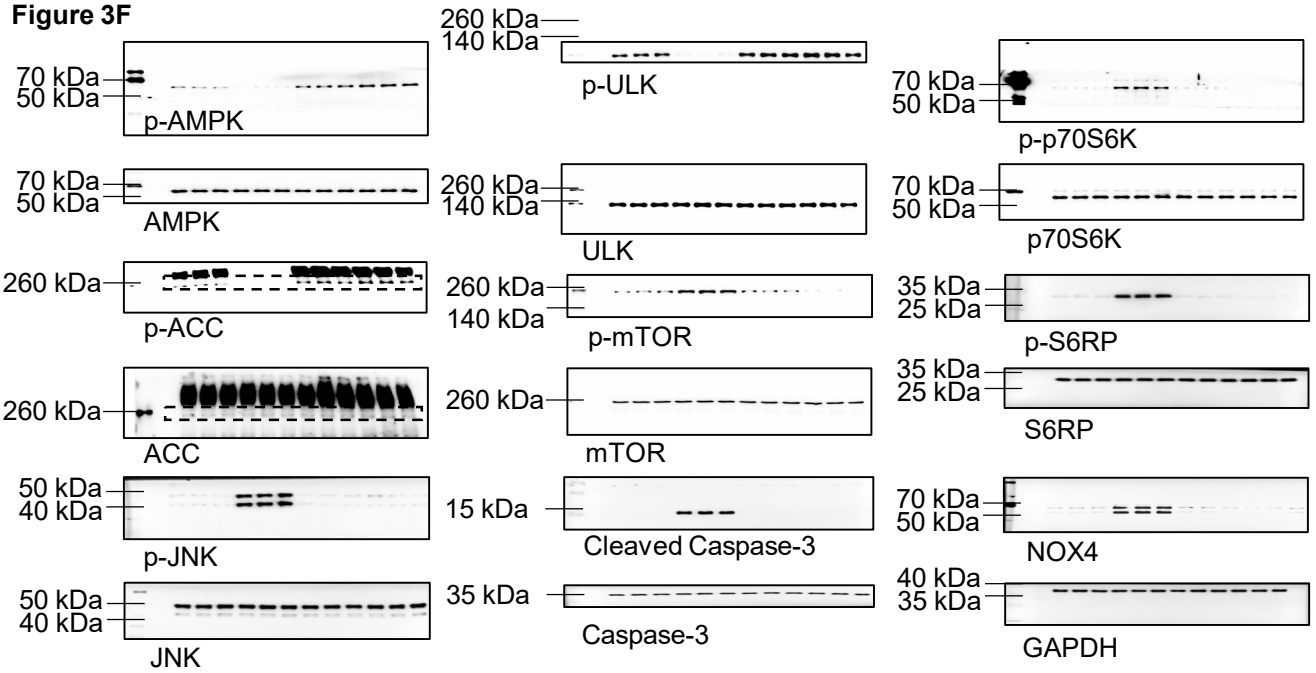


Figure 3K

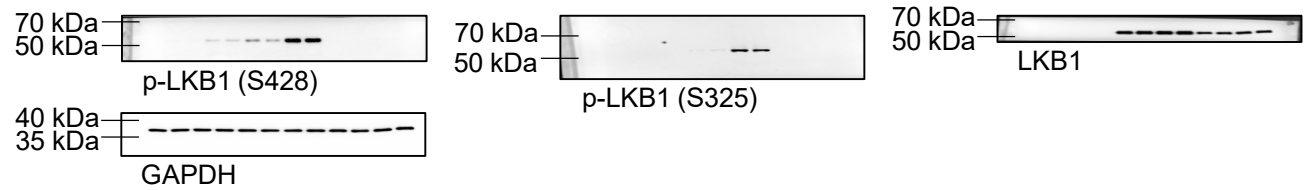


Figure 4A

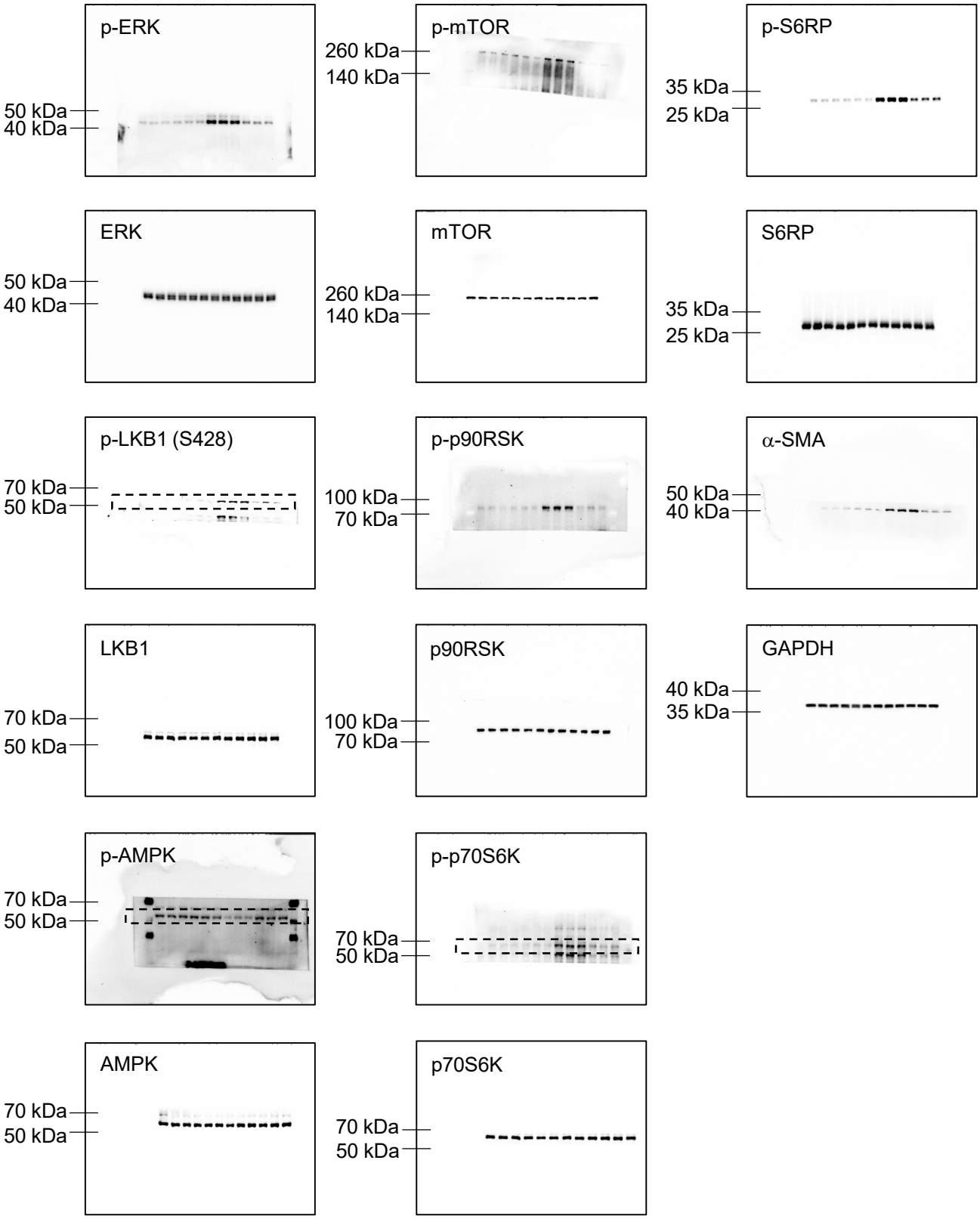

p-ERK
50 kDa
40 kDa
p-mTOR
260 kDa
140 kDa
p-S6RP
35 kDa
25 kDa
ERK
50 kDa
40 kDa
mTOR
260 kDa
140 kDa
S6RP
35 kDa
25 kDa
p-LKB1 (S428)
70 kDa
50 kDa
p-p90RSK
100 kDa
70 kDa
α-SMA
50 kDa
40 kDa
LKB1
70 kDa
50 kDa
p90RSK
100 kDa
70 kDa
GAPDH
40 kDa
35 kDa
p-AMPK
70 kDa
50 kDa
p-p70S6K
70 kDa
50 kDa
AMPK
70 kDa
50 kDa
p70S6K
70 kDa
50 kDa

Figure 4B

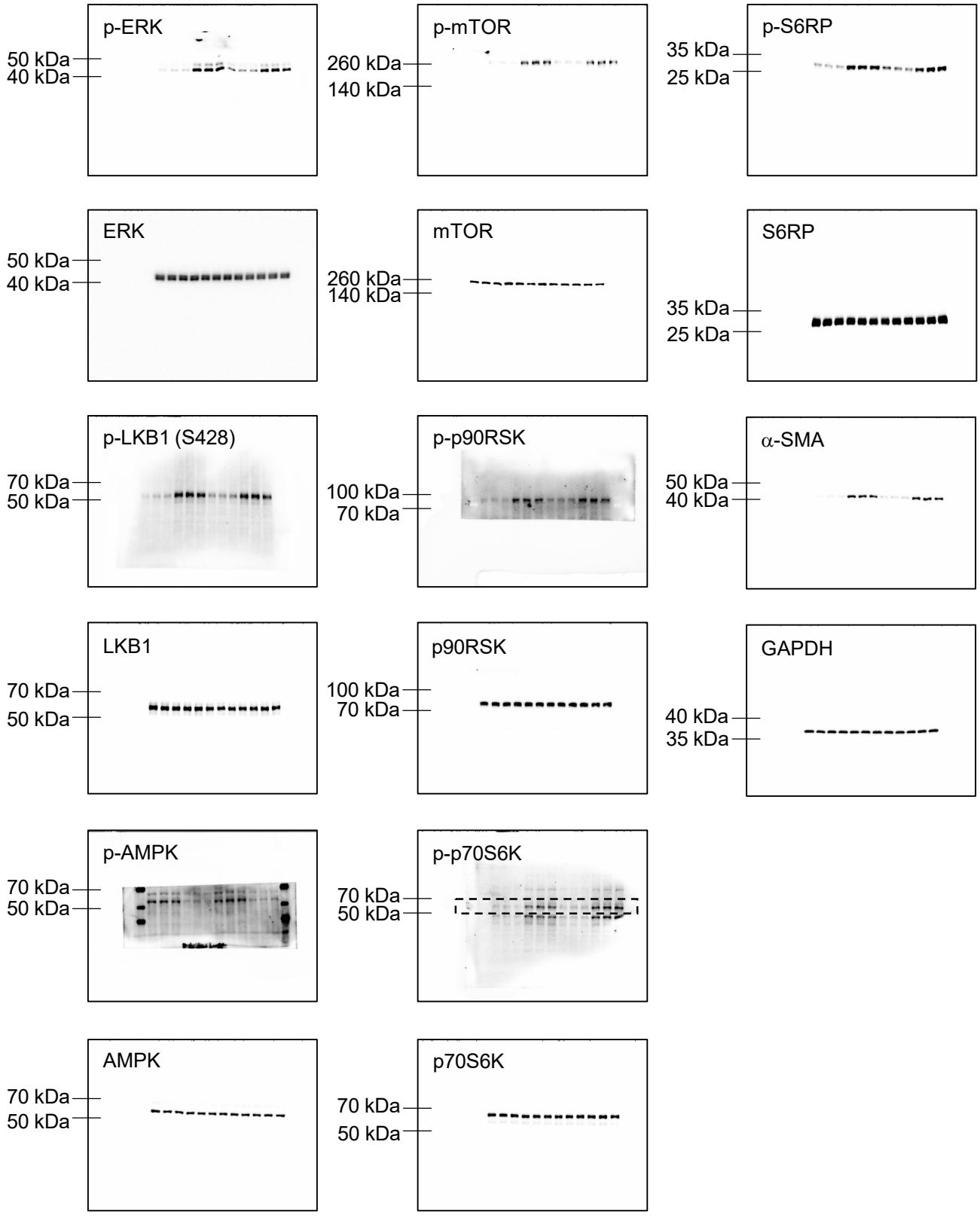

p-ERK
50 kDa
40 kDa
p-mTOR
260 kDa
140 kDa
p-S6RP
35 kDa
25 kDa
ERK
50 kDa
40 kDa
mTOR
260 kDa
140 kDa
S6RP
35 kDa
25 kDa
p-LKB1 (S428)
70 kDa
50 kDa
p-p90RSK
100 kDa
70 kDa
α-SMA
50 kDa
40 kDa
LKB1
70 kDa
50 kDa
p90RSK
100 kDa
70 kDa
GAPDH
40 kDa
35 kDa
p-AMPK
70 kDa
50 kDa
p-p70S6K
70 kDa
50 kDa
AMPK
70 kDa
50 kDa
p70S6K
70 kDa
50 kDa

Figure 4C

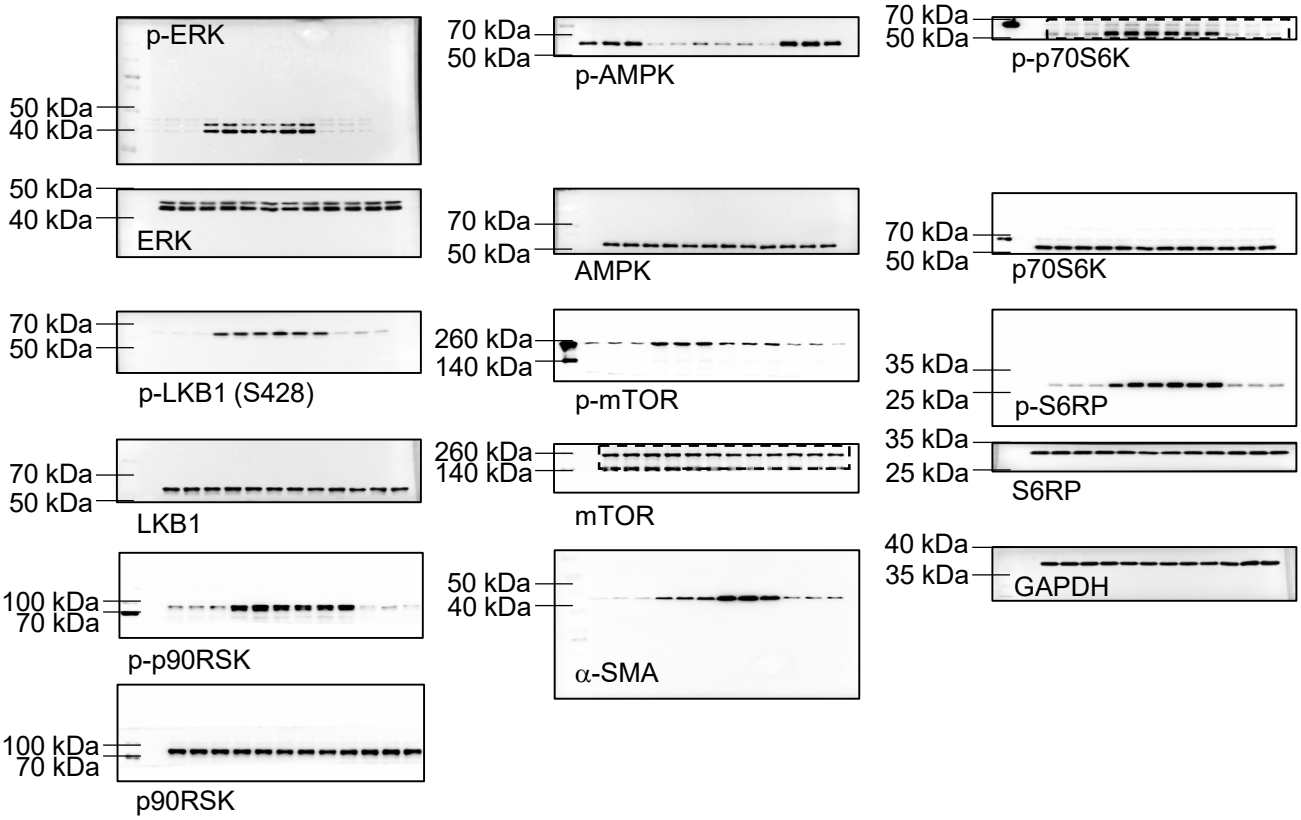

p-ERK
50 kDa
40 kDa
ERK
50 kDa
40 kDa
p-LKB1 (S428)
70 kDa
50 kDa
LKB1
70 kDa
50 kDa
p-p90RSK
100 kDa
70 kDa
p90RSK
100 kDa
70 kDa
p-AMPK
70 kDa
50 kDa
AMPK
70 kDa
50 kDa
p-mTOR
260 kDa
140 kDa
mTOR
260 kDa
140 kDa
α-SMA
50 kDa
40 kDa
p-p70S6K
70 kDa
50 kDa
p70S6K
70 kDa
50 kDa
p-S6RP
35 kDa
25 kDa
S6RP
35 kDa
25 kDa
GAPDH
40 kDa
35 kDa

Figure S1A

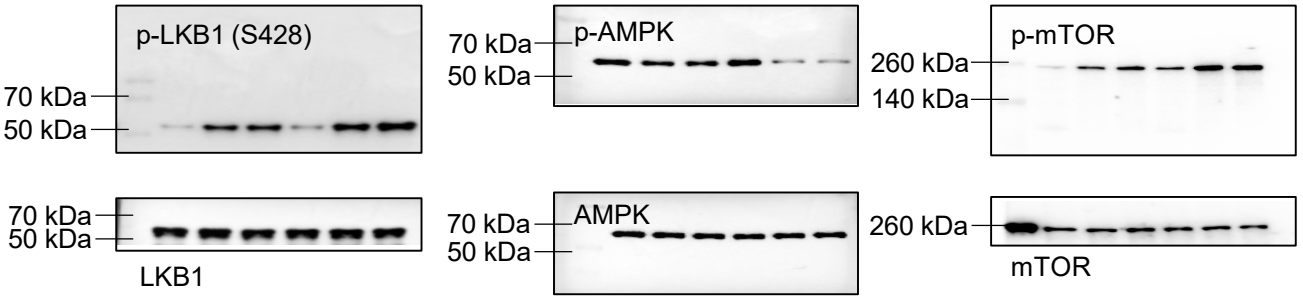


Figure S4

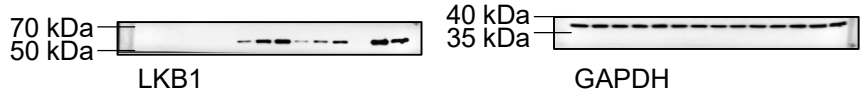


Figure S5

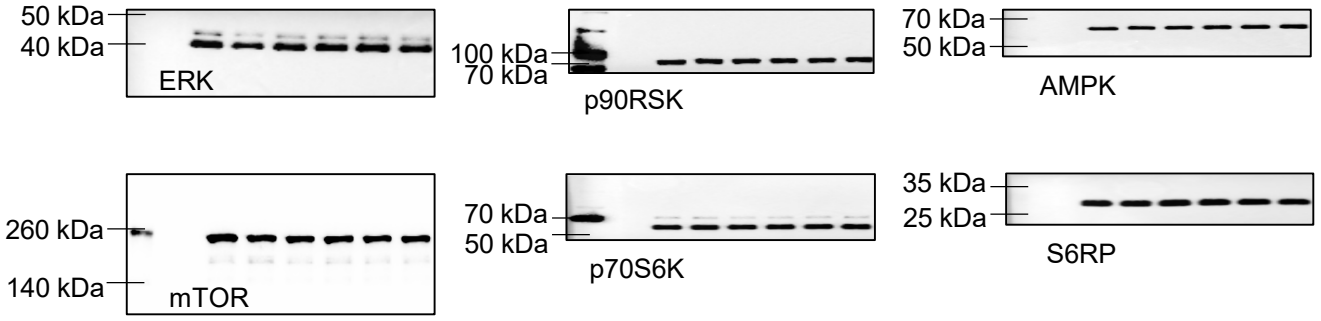


Figure S6A

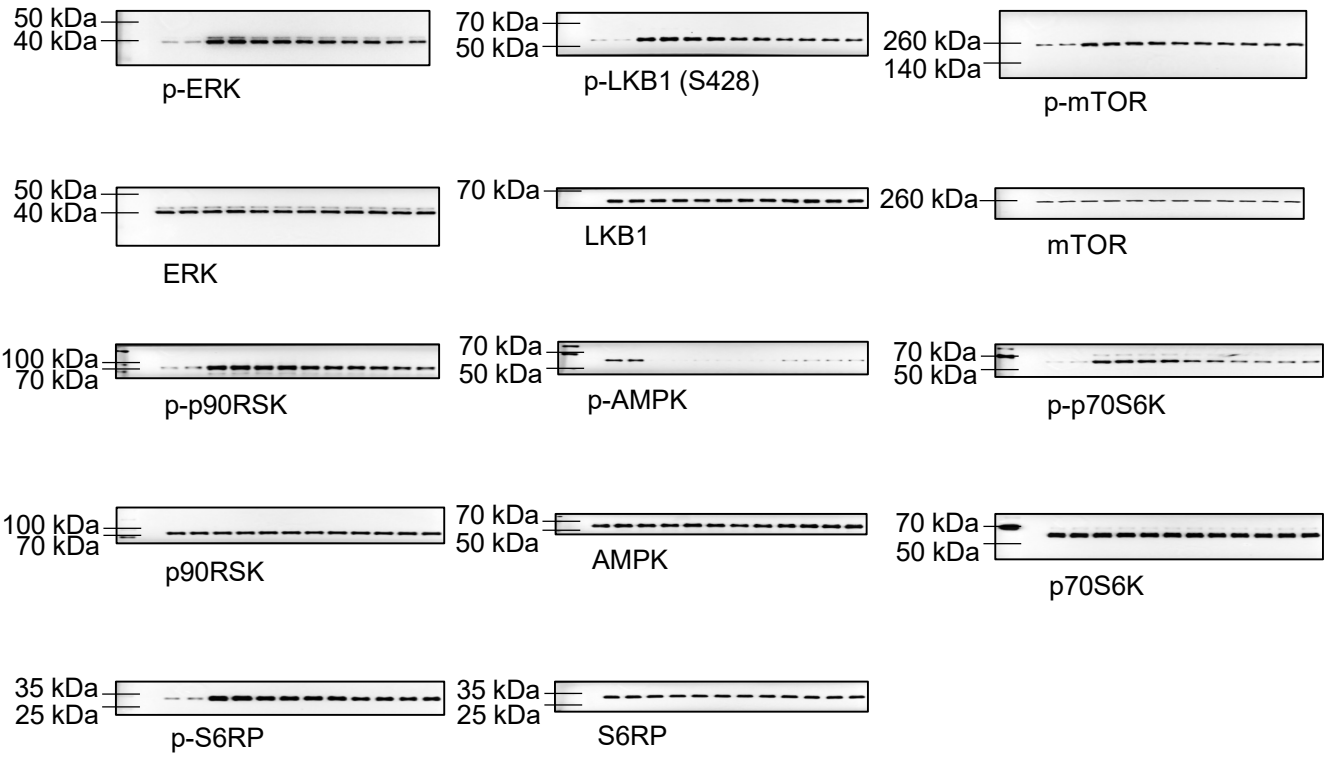


Figure S6B

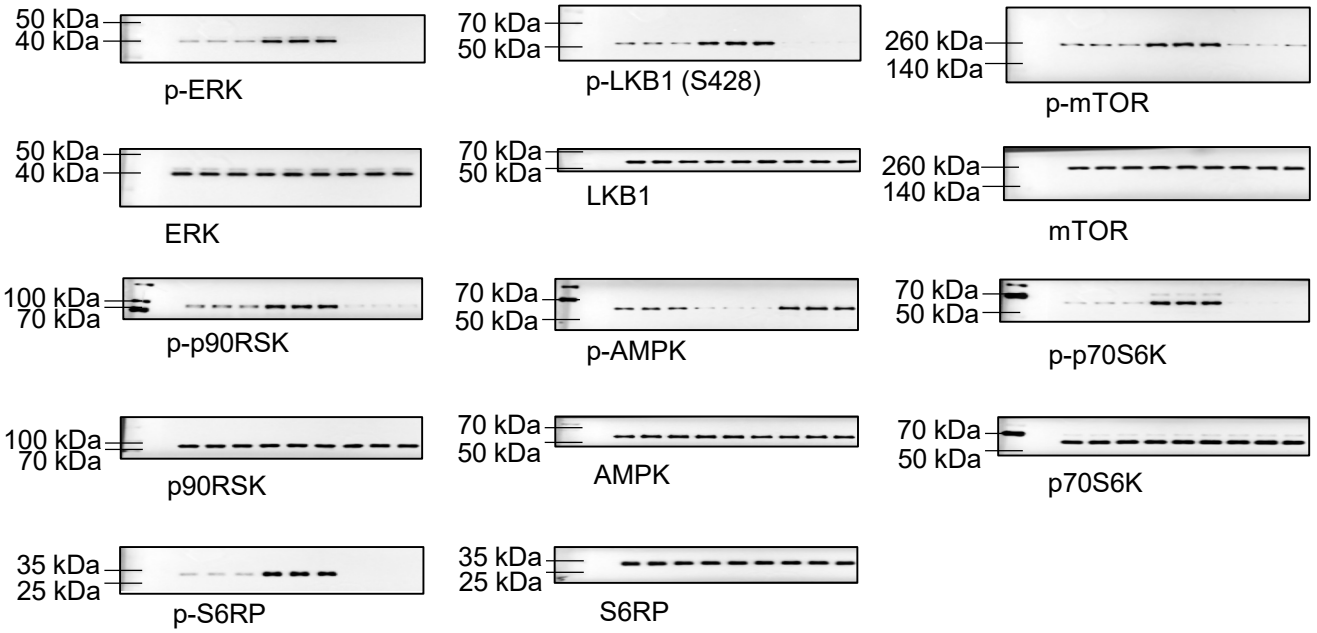


Figure S7

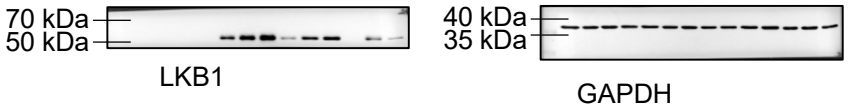


Figure S9

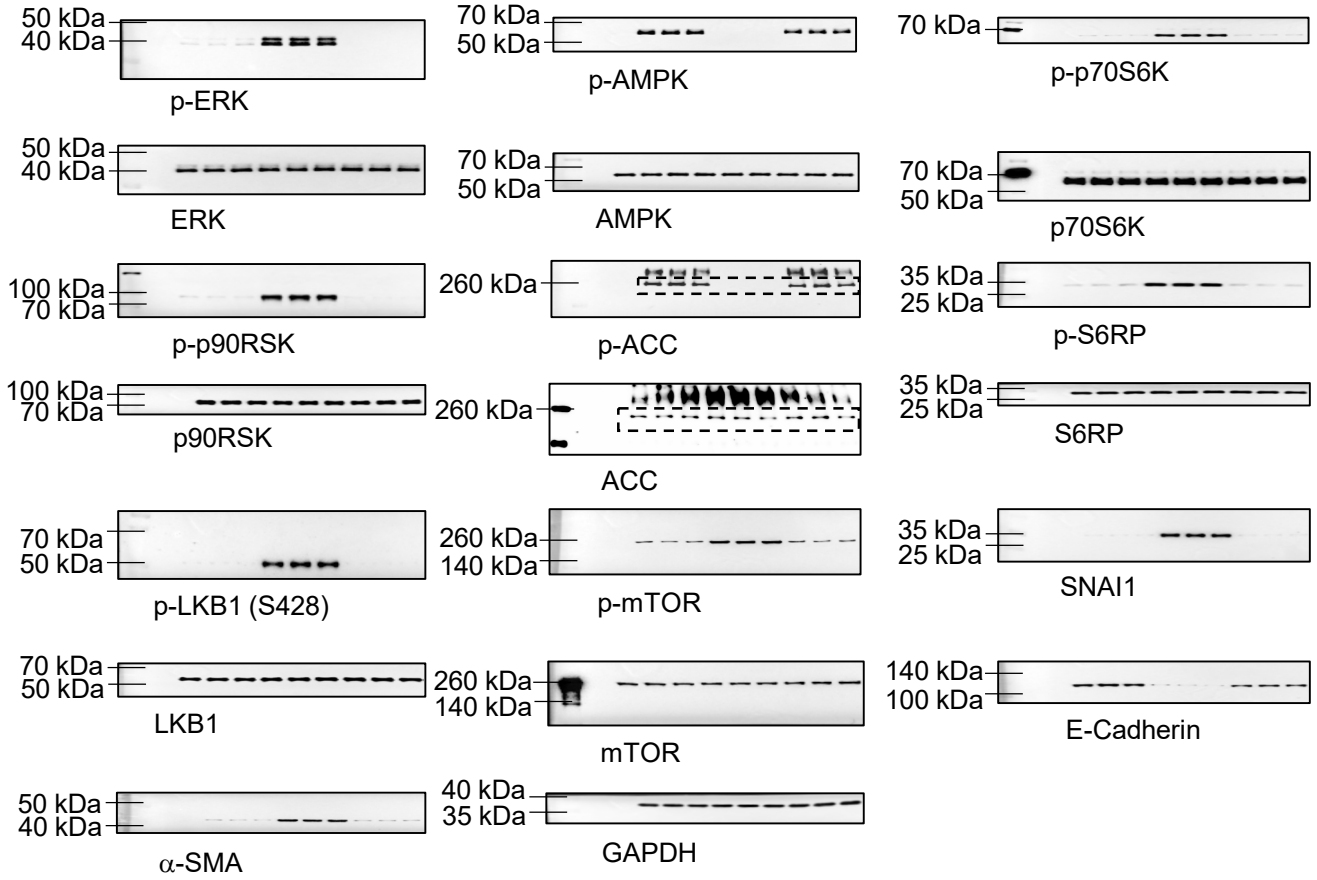


Figure S10