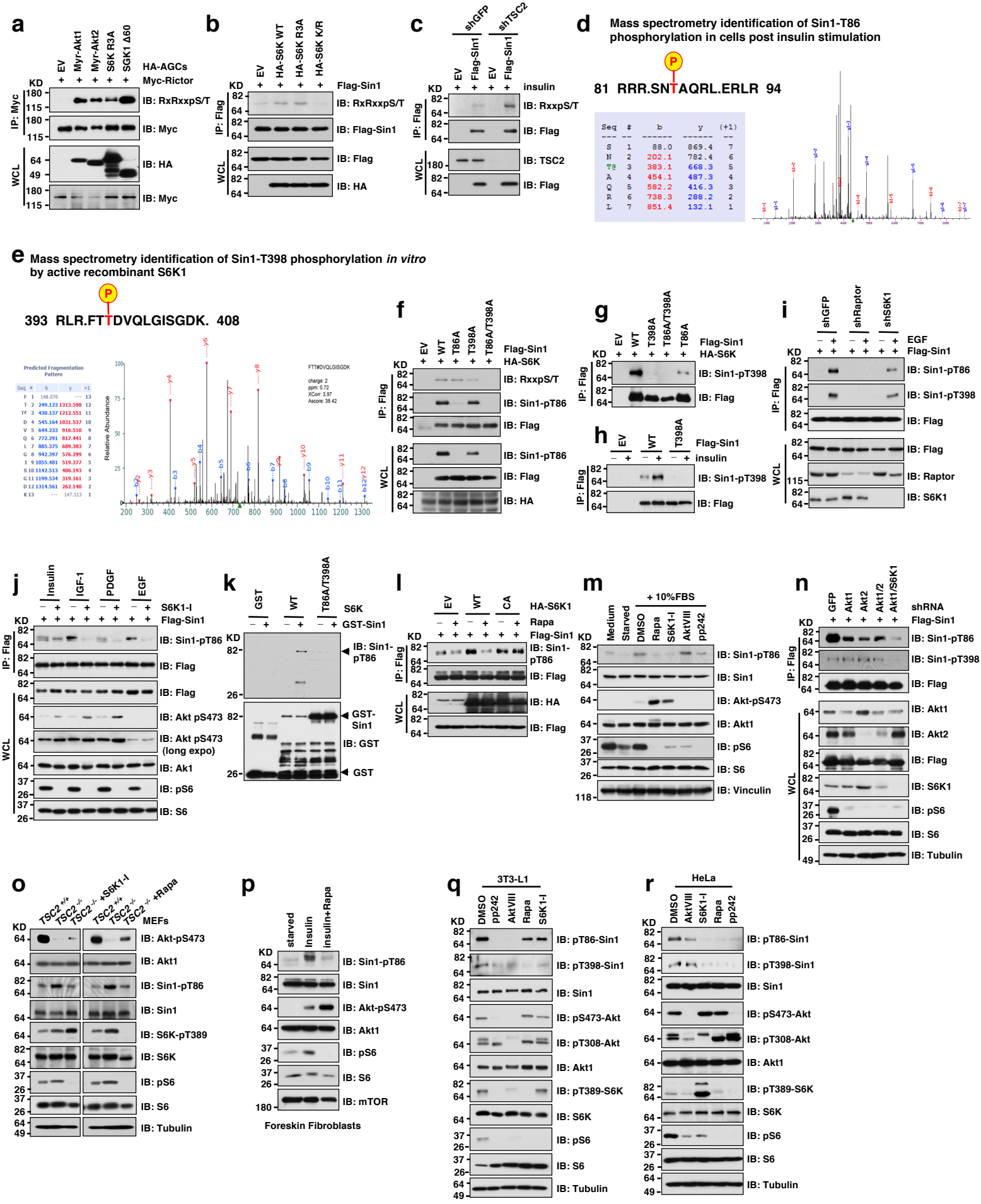
Figure S2


a
EV
Myr-Akt1
Myr-Akt2
S6K R3A
SGK1 Δ60
HA-AGCs
Myc-Rictor
KD
IP: Myc
IB: RxRxxpS/T
IB: Myc
WCL
IB: HA
IB: Myc
b
EV
HA-S6K WT
HA-S6K R3A
HA-S6K K/R
Flag-Sin1
IP: Flag
IB: RxRxxpS/T
IB: Flag-Sin1
WCL
IB: Flag
IB: HA
c
shGFP
shTSC2
EV
Flag-Sin1
insulin
IP: Flag
IB: RxxpS/T
IB: Flag
IB: TSC2
WCL
IB: Flag
d
Mass spectrometry identification of Sin1-T86 phosphorylation in cells post insulin stimulation
81 RRR.SNTAQRL.ERLR 94
Seq # b y (+1)
S 1 88.0 869.4 7
N 2 202.1 782.4 6
T@ 3 383.1 668.3 5
A 4 454.1 487.3 4
Q 5 582.2 416.3 3
R 6 738.3 288.2 2
L 7 851.4 132.1 1
e
Mass spectrometry identification of Sin1-T398 phosphorylation in vitro by active recombinant S6K1
393 RLR.FTTDVQLGISGDK. 408
Predicted Fragmentation Pattern
FTT#DVQLGISGDK
charge: 2
ppm: 0.72
XCorr: 3.97
Ascore: 38.42
Relative Abundance
f
EV
WT
T86A
T398A
T86A/T398A
Flag-Sin1
HA-S6K
IP: Flag
IB: RxxpS/T
IB: Sin1-pT86
IB: Flag
WCL
IB: Sin1-pT86
IB: Flag
IB: HA
g
EV
WT
T398A
T86A/T398A
T86A
Flag-Sin1
HA-S6K
IP: Flag
IB: Sin1-pT398
IB: Flag
h
EV
WT
T398A
Flag-Sin1
insulin
IP: Flag
IB: Sin1-pT398
IB: Flag
i
shGFP
shRaptor
shS6K1
EGF
Flag-Sin1
IP: Flag
IB: Sin1-pT86
IB: Sin1-pT398
IB: Flag
WCL
IB: Flag
IB: Raptor
IB: S6K1
j
Insulin
IGF-1
PDGF
EGF
S6K1-I
Flag-Sin1
IP: Flag
IB: Sin1-pT86
IB: Flag
IB: Flag
WCL
IB: Akt pS473
IB: Akt pS473 (long expo)
IB: Ak1
IB: pS6
IB: S6
k
GST
WT
T86A/T398A
S6K
GST-Sin1
IB: Sin1-pT86
IB: GST-Sin1
IB: GST
GST
l
EV
WT
CA
HA-S6K1
Rapa
Flag-Sin1
IP: Flag
IB: Sin1-pT86
IB: Flag
WCL
IB: HA
IB: Flag
m
Medium
Starved
+ 10%FBS
DMSO
Rapa
S6K1-I
AktVIII
pp242
IB: Sin1-pT86
IB: Sin1
IB: Akt-pS473
IB: Akt1
IB: pS6
IB: S6
IB: Vinculin
n
GFP
Akt1
Akt2
Akt1/2
Akt1/S6K1
shRNA
Flag-Sin1
IP: Flag
IB: Sin1-pT86
IB: Sin1-pT398
IB: Flag
WCL
IB: Akt1
IB: Akt2
IB: Flag
IB: S6K1
IB: pS6
IB: S6
IB: Tubulin
o
TSC2 +/+
TSC2 -/-
TSC2 -/- +S6K1-I
TSC2 +/+
TSC2 -/-
TSC2 -/- +Rapa
MEFs
IB: Akt-pS473
IB: Akt1
IB: Sin1-pT86
IB: Sin1
IB: S6K-pT389
IB: S6K
IB: pS6
IB: S6
IB: Tubulin
p
starved
Insulin
insulin+Rapa
IB: Sin1-pT86
IB: Sin1
IB: Akt-pS473
IB: Akt1
IB: pS6
IB: S6
IB: mTOR
Foreskin Fibroblasts
q
3T3-L1
DMSO
pp242
AktVIII
Rapa
S6K1-I
IB: pT86-Sin1
IB: pT398-Sin1
IB: Sin1
IB: pS473-Akt
IB: pT308-Akt
IB: Akt1
IB: pT389-S6K
IB: S6K
IB: pS6
IB: S6
IB: Tubulin
r
HeLa
DMSO
AktVIII
S6K1-I
Rapa
pp242
IB: pT86-Sin1
IB: pT398-Sin1
IB: Sin1
IB: pS473-Akt
IB: pT308-Akt
IB: Akt1
IB: pT389-S6K
IB: S6K
IB: pS6
IB: S6
IB: Tubulin