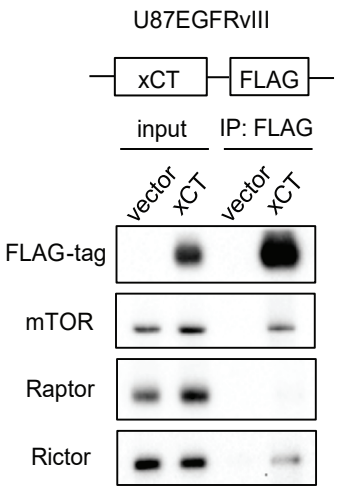
Figure S1.

A


U87EGFRvIII
xCT
FLAG
input
IP: FLAG
vector
xCT
vector
xCT
FLAG-tag
mTOR
Raptor
Rictor

B

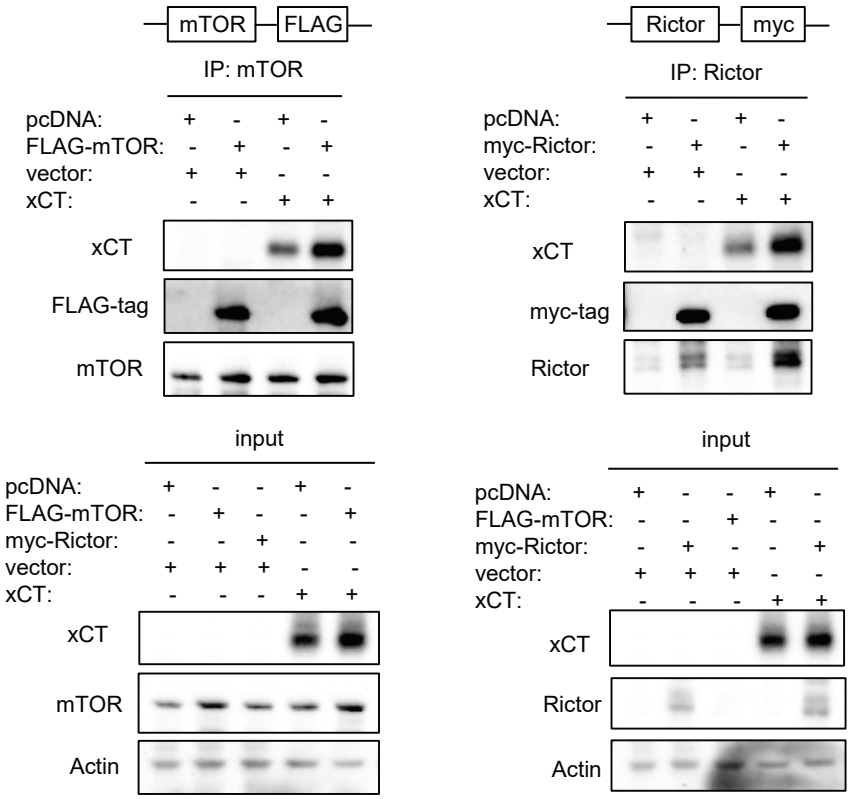

mTOR
FLAG
IP: mTOR
pcDNA: + - + -
FLAG-mTOR: - + - +
vector: + + - -
xCT: - - + +
xCT
FLAG-tag
mTOR
input
pcDNA: + - - + -
FLAG-mTOR: - + - - +
myc-Rictor: - - + - -
vector: + + + - -
xCT: - - - + +
xCT
mTOR
Actin
Rictor
myc
IP: Rictor
pcDNA: + - + -
myc-Rictor: - + - +
vector: + + - -
xCT: - - + +
xCT
myc-tag
Rictor
input
pcDNA: + - - + -
FLAG-mTOR: - - + - -
myc-Rictor: - + - - +
vector: + + + - -
xCT: - - - + +
xCT
Rictor
Actin

Figure S2.

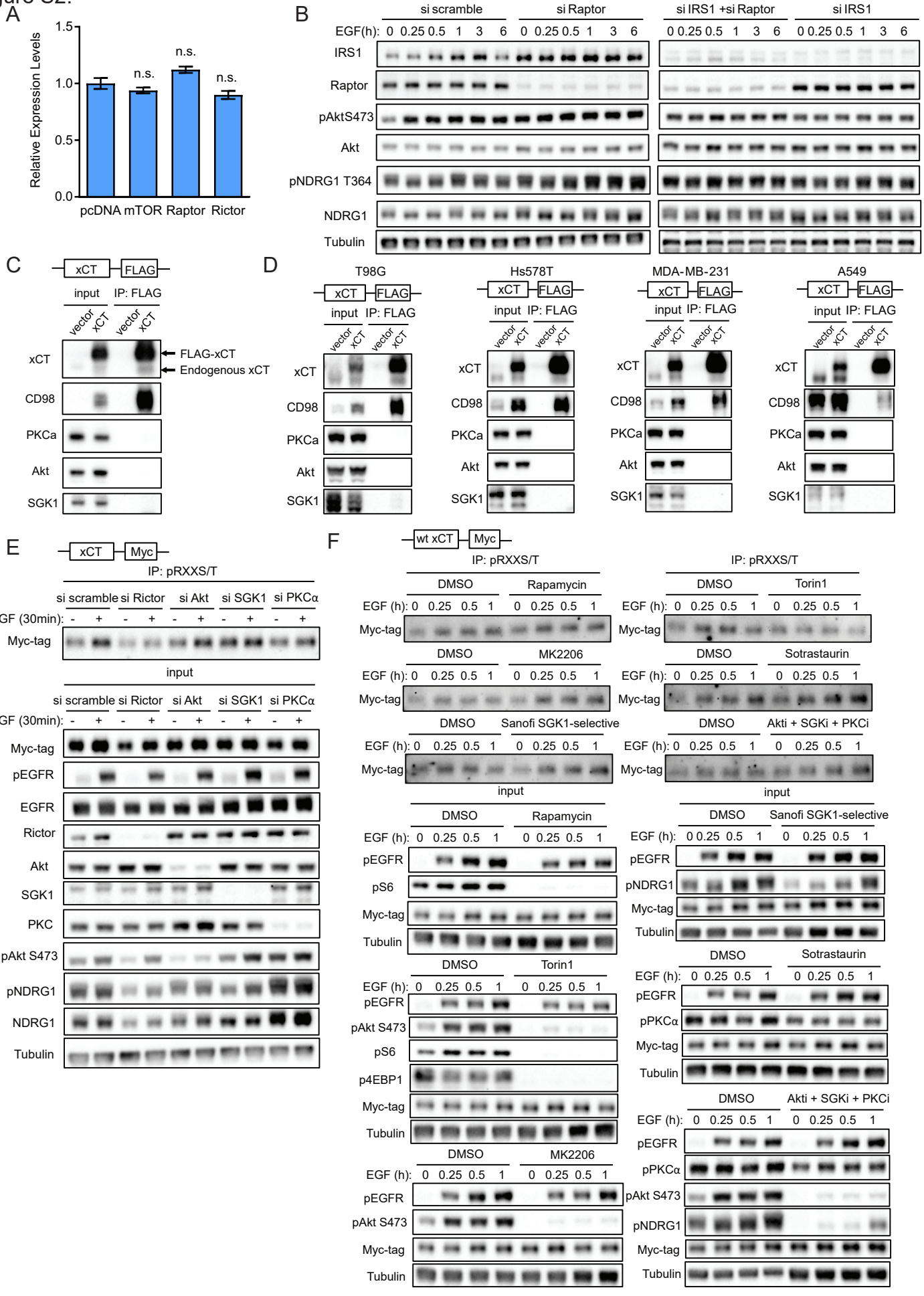
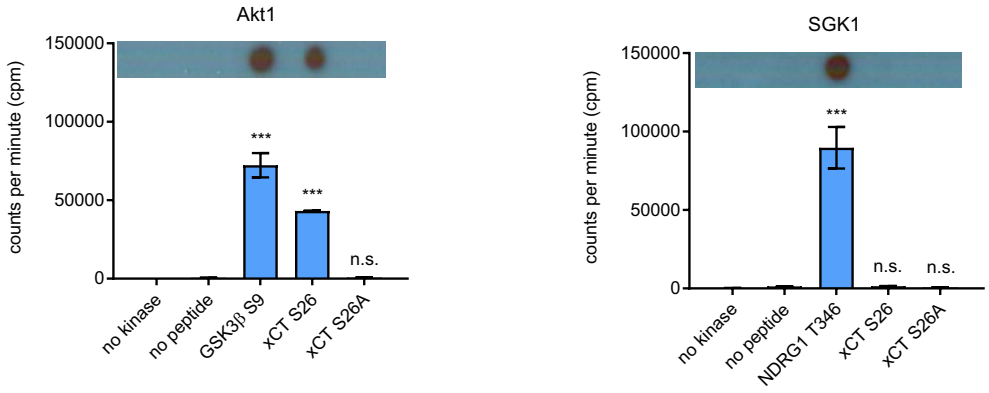

A
Relative Expression Levels
1.5
1.0
0.5
0.0
n.s.
n.s.
n.s.
pcDNA mTOR Raptor Rictor
B
si scramble
si Raptor
si IRS1 +si Raptor
si IRS1
EGF(h): 0 0.25 0.5 1 3 6 0 0.25 0.5 1 3 6 0 0.25 0.5 1 3 6 0 0.25 0.5 1 3 6
IRS1
Raptor
pAktS473
Akt
pNDRG1 T364
NDRG1
Tubulin
C
xCT
FLAG
input
IP: FLAG
vector
xCT
vector
xCT
xCT
CD98
PKCa
Akt
SGK1
FLAG-xCT
Endogenous xCT
D
T98G
Hs578T
MDA-MB-231
A549
xCT
FLAG
input
IP: FLAG
vector
xCT
vector
xCT
xCT
CD98
PKCa
Akt
SGK1
E
xCT
Myc
IP: pRXXS/T
si scramble
si Rictor
si Akt
si SGK1
si PKCα
EGF (30min): - + - + - + - + - +
Myc-tag
input
si scramble
si Rictor
si Akt
si SGK1
si PKCα
EGF (30min): - + - + - + - + - +
Myc-tag
pEGFR
EGFR
Rictor
Akt
SGK1
PKC
pAkt S473
pNDRG1
NDRG1
Tubulin
F
wt xCT
Myc
IP: pRXXS/T
DMSO
Rapamycin
EGF (h): 0 0.25 0.5 1 0 0.25 0.5 1
Myc-tag
DMSO
MK2206
EGF (h): 0 0.25 0.5 1 0 0.25 0.5 1
Myc-tag
DMSO
Sanofi SGK1-selective
EGF (h): 0 0.25 0.5 1 0 0.25 0.5 1
Myc-tag
input
DMSO
Rapamycin
EGF (h): 0 0.25 0.5 1 0 0.25 0.5 1
pEGFR
pS6
Myc-tag
Tubulin
DMSO
Torin1
EGF (h): 0 0.25 0.5 1 0 0.25 0.5 1
pEGFR
pAkt S473
pS6
p4EBP1
Myc-tag
Tubulin
DMSO
MK2206
EGF (h): 0 0.25 0.5 1 0 0.25 0.5 1
pEGFR
pAkt S473
Myc-tag
Tubulin
IP: pRXXS/T
DMSO
Torin1
EGF (h): 0 0.25 0.5 1 0 0.25 0.5 1
Myc-tag
DMSO
Sotrastaurin
EGF (h): 0 0.25 0.5 1 0 0.25 0.5 1
Myc-tag
DMSO
Akti + SGKi + PKCi
EGF (h): 0 0.25 0.5 1 0 0.25 0.5 1
Myc-tag
input
DMSO
Sanofi SGK1-selective
EGF (h): 0 0.25 0.5 1 0 0.25 0.5 1
pEGFR
pNDRG1
Myc-tag
Tubulin
DMSO
Sotrastaurin
EGF (h): 0 0.25 0.5 1 0 0.25 0.5 1
pEGFR
pPKCα
Myc-tag
Tubulin
DMSO
Akti + SGKi + PKCi
EGF (h): 0 0.25 0.5 1 0 0.25 0.5 1
pEGFR
pPKCα
pAkt S473
pNDRG1
Myc-tag
Tubulin

Figure S3.

A

Akt S473: GYFPQFSYSASGRRRRR
GSK3β S9: GYGRPRTTSFAGRRRRR
NDRG1 T346: GYTRSRSHTSEGRRRRR
xCT S26: GYGRLPSLGNKGRRRRR
xCT S26A: GYGRLPALGNKGRRRRR

B



C

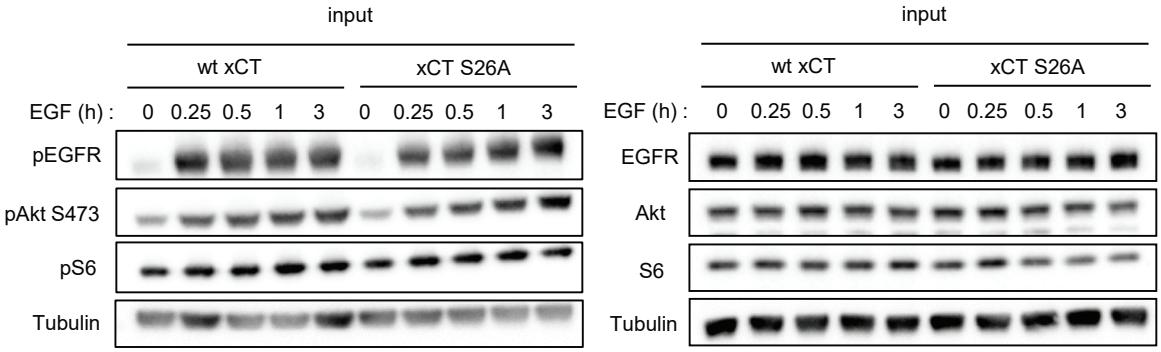


Figure S4.

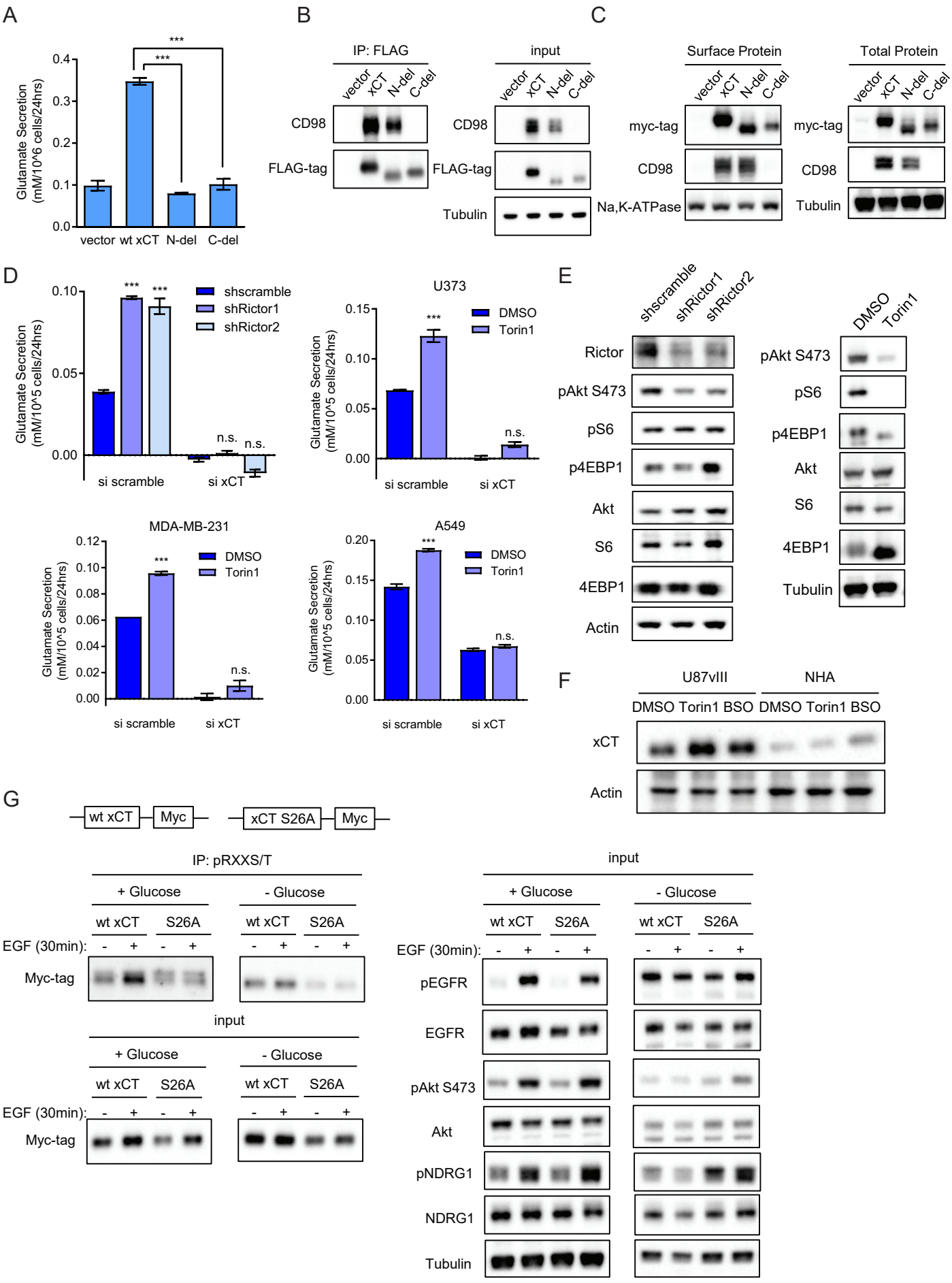

A
B
C
D
E
F
G
Glutamate Secretion (mM/10^6 cells/24hrs)
vector
wt xCT
N-del
C-del
IP: FLAG
input
Surface Protein
Total Protein
CD98
FLAG-tag
Tubulin
myc-tag
Na,K-ATPase
shscramble
shRictor1
shRictor2
Glutamate Secretion (mM/10^5 cells/24hrs)
si scramble
si xCT
n.s.
U373
MDA-MB-231
A549
DMSO
Torin1
Rictor
pAkt S473
pS6
p4EBP1
Akt
S6
4EBP1
Actin
U87vIII
NHA
DMSO Torin1 BSO
xCT
wt xCT
Myc
xCT S26A
IP: pRXXS/T
+ Glucose
- Glucose
S26A
EGF (30min):
Myc-tag
pEGFR
EGFR
pNDRG1
NDRG1

Figure S1. Related to Figure 1
(A) Co-immunoprecipitation (Co-IP) was performed to validate mTOR and Rictor as xCT binding proteins in U87EGFRvIII cells.
(B) Reverse Co-IP was performed to verify mTOR and Rictor as xCT binding proteins. U87EGFRvIII cells with stable xCT overexpression or vector control were transiently transfected with pcDNA vector control, FLAG-mTOR (left) or myc-Rictor (right). After 48 h of transfection, protein lysates were collected and incubated with Dynabeads Protein A pre-incubated with mTOR or Rictor antibodies. Eluates were analyzed by electrophoresis and immunoblotting.

Figure S2. Related to Figure 2
(A) RT-PCR analysis of xCT mRNA levels in U87 cells transiently transfected with vector (pcDNA) control, mTOR, Raptor or Rictor. Cells were collected after 72 h of transfection and mRNAs were extracted. Results were calculated from four independent replicates and data were shown as mean ± SEM. Statistical analysis was performed using one-way ANOVA and compared to the mean of pcDNA as control. n.s. refers to not statistically significant.
(B) Western blot analysis of mTORC2 reactivation (indicated by pAkt S473 and pNDRG1 T346) upon siRNA mediated knockdown of Raptor and IRS1.
(C-D) Co-IP experiment was performed using GBM cell lines U87EGFRvIII (C), T98G triple negative breast cancer cell lines Hs578T and MDA-MB-231, and lung cancer cell line A549 (D) stably expressing FLAG-tagged xCT or vector control to detect xCT binding with PKCα, Akt and SGK1.
(E) U87 cells stably expressing EGFR and myc-tagged xCT were transfected with siRNA targeting Rictor or mTORC2 downstream AGC kinases (si Rictor, 20 nM; si Akt, 10 nM si Akt1 + 10 nM si Akt2 + 10 nM si Akt3; si SGK1, 20 nM; si PKCα, 20 nM). 24 h post-transfection cells were serum starved for an additional 24 h and then stimulated with 25 ng/ml EGF for 30 min before protein lysates were collected and subjected to pRXXS/T IP and western blotting analysis.
(F) U87 cells stably expressing EGFR and myc-tagged xCT were serum starved for 24 h in the presence of DMSO or indicated kinase inhibitors (Rapamycin: 10 nM, Torin1: 250 nM; MK2206: 1 μM; Sanofi-SGK1-selective compound: 2 μM; Sotrastaurin: 5 μM; Akti + SGKi +PKCi refers to combination treatment with MK2206, Sanofi-SGK1-selective compound and Sotrastaurin at the same concentration as individual drug treatment mentioned above) and then stimulated with 25 ng/ml EGF before protein lysates were collected at indicated time points and subjected to pRXXS/T IP and western blotting analysis. Inhibition of kinase activity were analyzed as shown in the input panels.

Figure S3. Related to Figure 3
(A) Sequence of peptides used in the in vitro kinase assays in Fig.3G and Fig.S3B.
(B) Similar in vitro kinase assay as shown in Fig.3G was performed using recombinant Akt1 or SGK1 kinase with known substrates GSK3β S9 or NDRG1 T346 as positive controls. Scintillation counts from three independent replicates were presented as mean counts per minute (cpm) ± SEM. Statistical analysis was performed using one-way ANOVA. *** refers to p value < 0.001. n.s. refers to not statistically significant.
(C) Western blot analysis of EGFR downstream signaling pathway activation through mTORC1 and mTORC2 using input samples from the IP experiment in Fig.3H.

Figure S4. Related to Figure 4
(A) Glutamate secretion was measured and calculated in U87EGFRvIII cells stably overexpressing vector, wt xCT or N-del and C-del mutant xCT. Results were acquired from three independent repeats and data were presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA. *** refers to p value < 0.001.
(B) Co-IP-western blot to detect binding of wt xCT or N-del and C-del mutant xCT with CD98.
(C) Cell surface proteins were purified and the overexpressed myc-tagged xCT levels were analyzed by western blotting.
(D) The specificity of increased glutamate secretion through xCT upon shRNA mediated Rictor knockdown or Torin1 treatment in different cell lines was confirmed by xCT knockdown. Glutamate secretion was measured after knockdown of xCT with 50 nM siRNA for 48 h followed by 24 h of Torin1 treatment. Glutamate levels in the media was determined by NOVA Bioprofile 400 analyzer and glutamate

secretion was calculated by subtracting blank control and normalized to cell count. Results were obtained from three independent replicates and data are presented as mean ± SEM. *** refers to p value < 0.001. n.s. refers to not statistically significant. Statistical analysis was performed using two-way ANOVA comparing the mean of Torin1 to DMSO control.
(E) Knockdown of Rictor (left) as well as suppression of downstream signaling pathways after 24 h Torin1 treatment in U87EGFRvIII cells (right) was confirmed by western blot.
(F) Changes in xCT protein in response to Torin1 and an glutathione synthesis inhibitor buthionine sulfoximine (BSO) in GBM cells (U87vIII) or normal human astrocytes (NHA) were analyzed by western blot analysis. Cells were treated in corresponding culture media for 24 h before cell lysates were collected using RIPA buffer and proteins were quantified and analyzed by western blot.
(G) xCT phosphorylation on RXXS/T motifs by mTORC2 downstream of growth factor signaling was analyzed by IP-western blot in the presence or absence of glucose. Glucose starvation was performed by incubating cells in glucose/pyruvate free DMEM media for 24 h before cells were stimulated by 25 ng/ml EGF for 30 min and cell lysates were collected and subjected to pRXXS/T IP and western blotting analysis.

Table S3. Additional list of DNA oligos used in this study. Related to STAR Methods

Oligonucleotide Names	Sequence	Reference
Primers used for xCT site-directed mutagenesis and truncation mutants		
pLVX-puro-xCT Forward	AAATGCGGCCATTACGGCCCGAGGAGATCTGCCGCC GCGATCGCCATGG	This paper
pLVX-puro-xCT Reverse	AAATGCGGCCATGGCGGCCTTAAACCTTATCGTCGT CATCCTTGTAATC	
xCT S26A Forward	AACGGGAGGCTGCCTGCCCTGGGC	This paper
xCT S26A Reverse	GCCCAGGGCAGGCAGCCTCCCGTT	
xCT N-del Forward	AAATGCGGCCATTACGGCCCGAGGAGATCTGCCGCC GCGATCGCCATGGTCACTTTACTGAGGGGAGTCTCC	This paper
xCT N-del Reverse	AAATGCGGCCATGGCGGCCTTAAACCTTATCGTCGT CATCCTTGTAATC	
xCT C-del-set1 Forward	AAATGCGGCCATTACGGCCCGAGGAGATCTGCCGCC GCGATCGCCATG	This paper
xCT C-del-set1 Reverse	GAGTTTCTGCTCGAGCGGCCGCGTACGCGTCCATAT AATAAAGAGATAATACGCAGGGAC	
xCT C-del-set2 Forward	ACGCGTACGCGGCCGCTCGAGCAGAAACTC	This paper
xCT C-del-set2 Reverse	AAATGCGGCCATGGCGGCCTTAAACCTTATCGTCGT CATCCTTGTAATC	
RT-PCR primers		
xCT Forward	CAGGAGAAAGTGCAGCTGAA	This paper
xCT Reverse	CTCCAATGATGGTGCCAATG-	This paper
TBP Forward	GAGCTGTGATGTGAAGTTTCC	This paper
TBP Reverse	TCTGGGTTTGATCATTCTGTAG	This paper