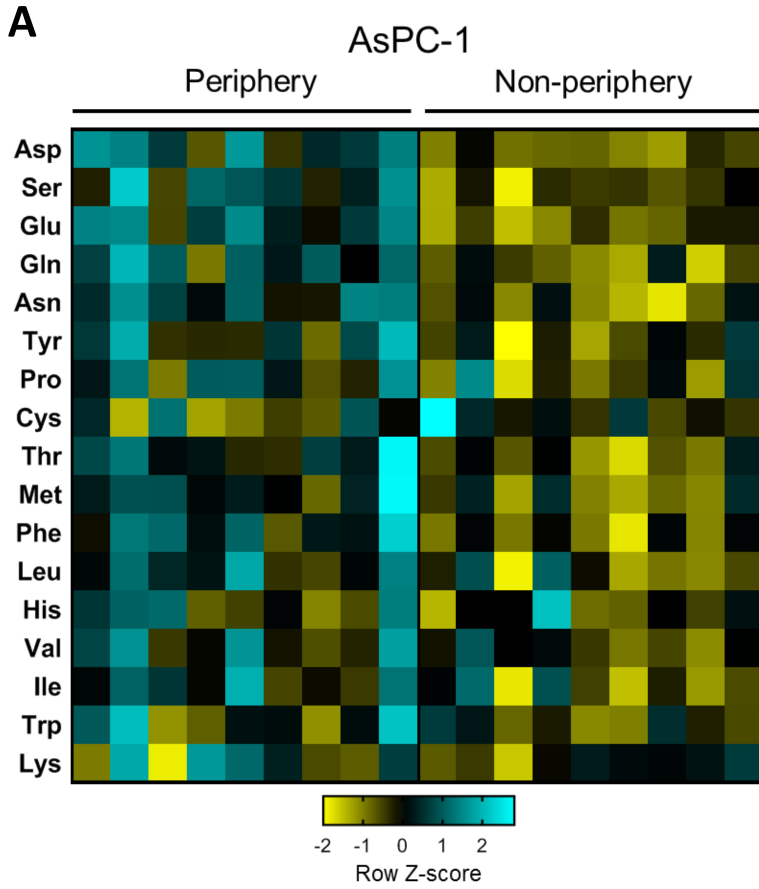
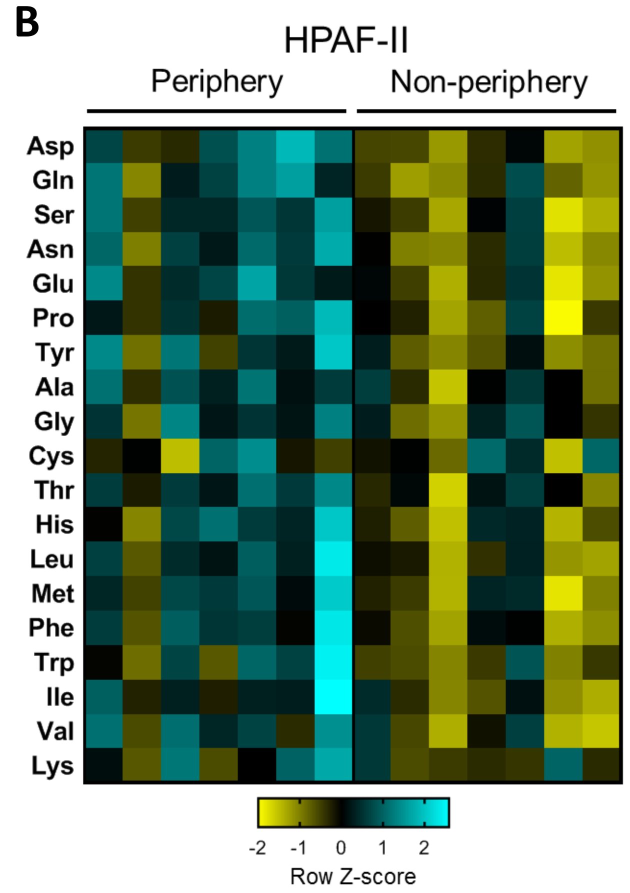

A
AsPC-1
Periphery
Non-periphery
Asp
Ser
Glu
Gln
Asn
Tyr
Pro
Cys
Thr
Met
Phe
Leu
His
Val
Ile
Trp
Lys
-2 -1 0 1 2
Row Z-score


B
HPAF-II
Periphery
Non-periphery
Asp
Gln
Ser
Asn
Glu
Pro
Tyr
Ala
Gly
Cys
Thr
His
Leu
Met
Phe
Trp
Ile
Val
Lys
-2 -1 0 1 2
Row Z-score

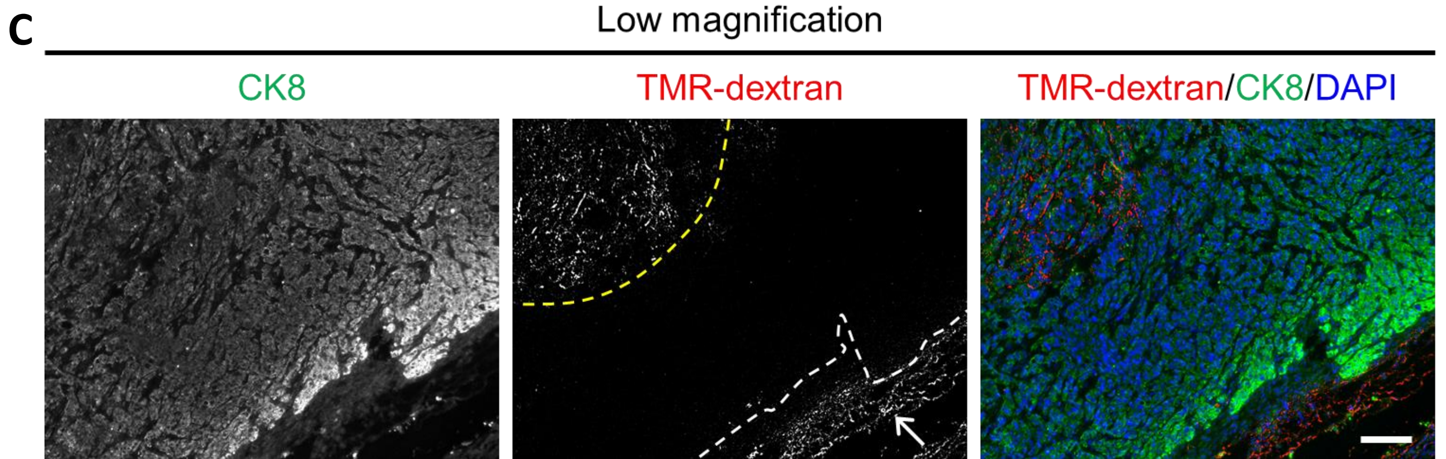

C
Low magnification
CK8
TMR-dextran
TMR-dextran/CK8/DAPI

Figure S1. Regional amino acid deficiencies within PDAC tumors coincide with enhanced levels of macropinocytosis, Related to Figure 1.

(A, B) Heatmap profiles for all detected intratumoral amino acids.

(C) Low-magnification images of xenograft tumor tissue with macropinosomes labeled with TMR-dextran (red in overlay) and tumor cells immunostained with anti-CK8 (green in overlay). Nuclei are labeled with DAPI (blue in overlay). Non-tumor sheath cells (CK8-negative) at the periphery of the tumor show strong macropinocytic capacity and mark the tumor edges (white arrow). The CK8 staining that abuts the sheath cells was used to demarcate the tumor periphery for microscopic analysis (white dashed line). The yellow dashed line indicates the outer boundary of the tumor non-peripheral region. Scale bar, 200 μm.

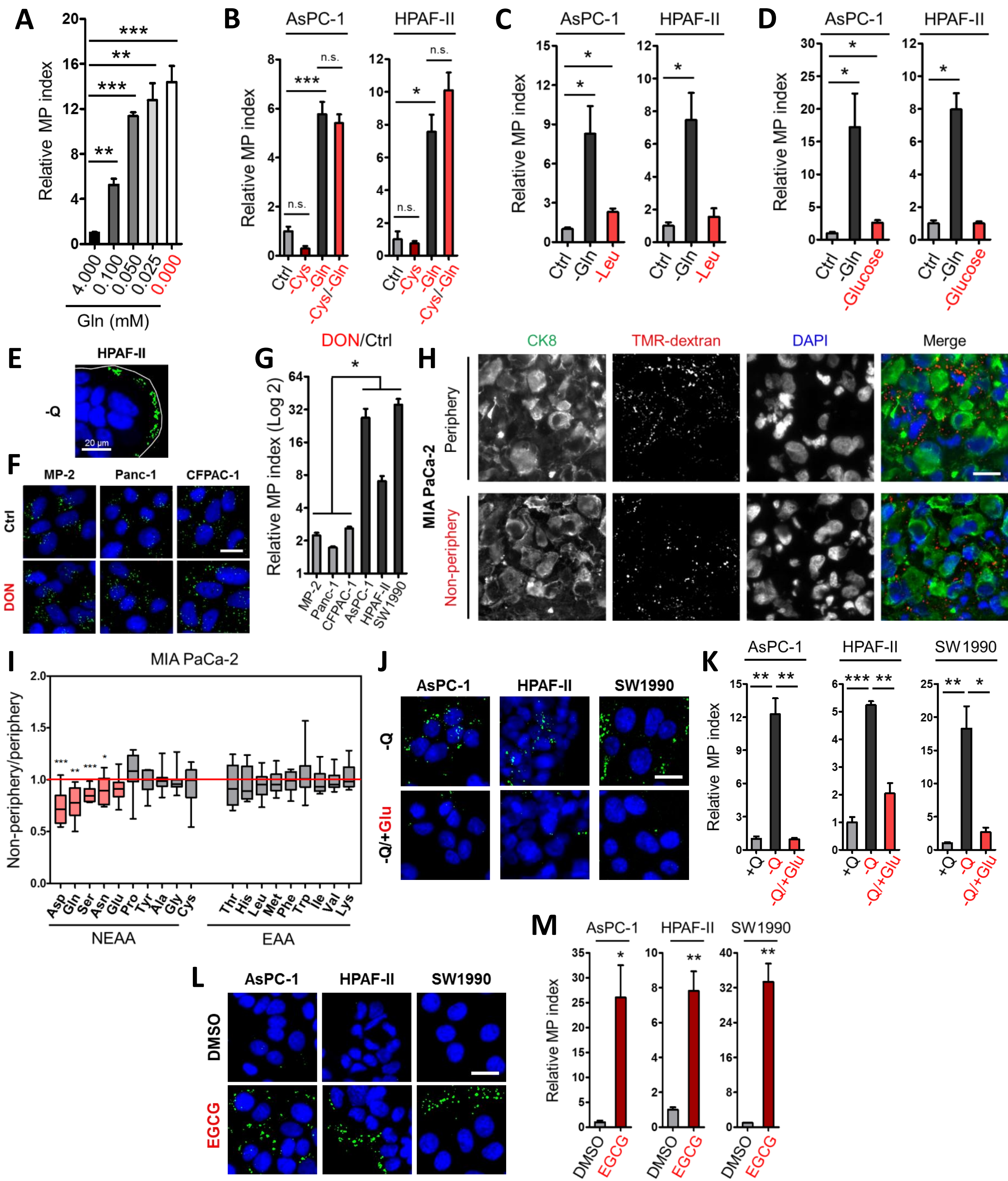

A
Relative MP index
Gln (mM)
4.000
0.100
0.050
0.025
0.000
B
AsPC-1
HPAF-II
Relative MP index
n.s.
Ctrl
-Cys
-Gln
-Cys/-Gln
C
Ctrl
-Gln
-Leu
D
-Glucose
E
HPAF-II
-Q
20 μm
F
MP-2
Panc-1
CFPAC-1
Ctrl
DON
G
DON/Ctrl
Relative MP index (Log 2)
MP-2
Panc-1
CFPAC-1
AsPC-1
HPAF-II
SW1990
H
CK8
TMR-dextran
DAPI
Merge
MIA PaCa-2
Periphery
Non-periphery
I
MIA PaCa-2
Non-periphery/periphery
Asp
Gln
Ser
Asn
Glu
Pro
Tyr
Ala
Gly
Cys
Thr
His
Leu
Met
Phe
Trp
Ile
Val
Lys
NEAA
EAA
J
AsPC-1
HPAF-II
SW1990
-Q
-Q/+Glu
K
SW1990
+Q
-Q
-Q/+Glu
L
DMSO
EGCG
M
DMSO
EGCG

Figure S2. Glutamine metabolism regulates macropinocytic induction in a subset of PDAC cells, Related to Figure 2.

(A) Quantification of macropinocytosis in AsPC-1 cells in media containing the indicated concentrations of glutamine (Gln). Data are presented relative to the values obtained for the full glutamine (4 mM) condition.

(B–D) Quantification of macropinocytosis in the indicated cells deprived of the indicated nutrients. Data are presented relative to the values obtained for the nutrient-replete control (Ctrl) condition.

(E) The white line marks the cell boundary of an island of HPAF-II cells from Figure 2B.

(F) Representative images of macropinocytotic uptake (green) in the indicated cells treated without or with 6-diazo-5-oxo-L-norleucine (DON) in glutamine-containing media.

(G) The macropinocytic index in response to DON treatment relative to non-treated control (Ctrl) for the indicated cell lines. Data are presented on a Log2 scale.

(H) Representative images from sections of MIA PaCa-2 xenograft tumor tissue with macropinosomes labeled with TMR-dextran (red) and tumor cells immunostained with anti-CK8 (green). Nuclei are labeled with DAPI (blue).

(I) Intratumoral amino acid levels in the non-peripheral regions relative to the periphery of xenograft tumors derived from MIA PaCa-2 cells (n=8 tumors). Data are expressed as box-and-whiskers plots. Horizontal lines represent median; boxes range from the 25th to 75th percentiles; vertical lines extend down to the 10th percentile and up to the 90th percentiles. NEAA, non-essential amino acids. EAA, essential amino acids.

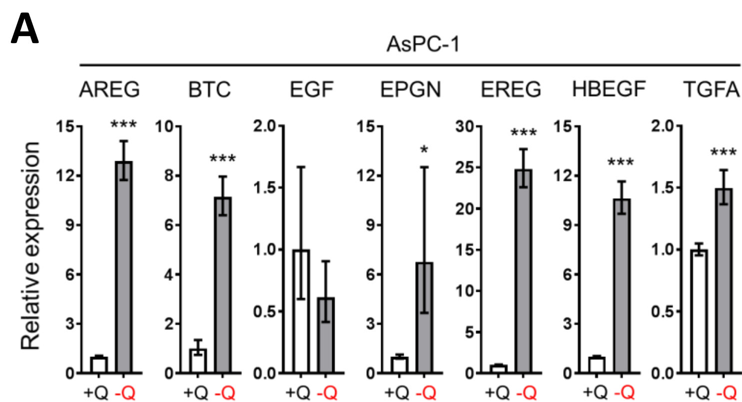
(J) Representative images of macropinocytosis (green) in the indicated cells treated without or with a cell-permeable form of glutamate (Glu) in glutamine-free media.

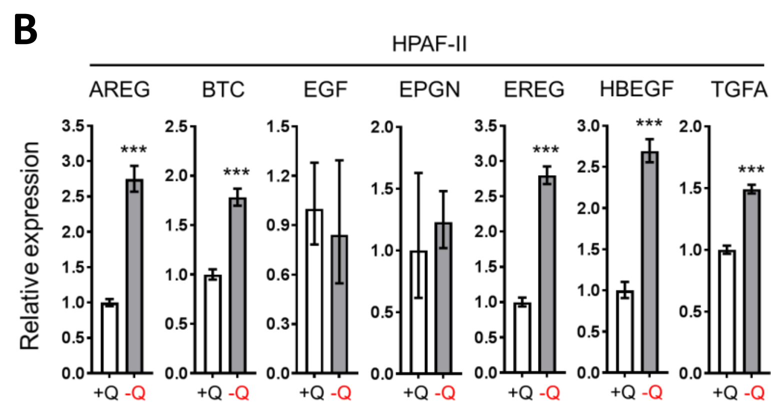
(K) Quantification of macropinocytosis in the indicated cells in glutamine-containing media and the conditions described in (J). Data are presented relative to the values obtained for the +Q condition.

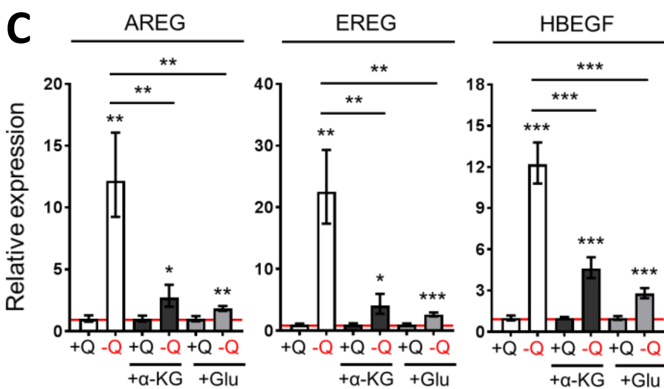
(L) Representative images of macropinocytosis (green) in the indicated cells treated with vehicle (DMSO) or epigallocatechin gallate (EGCG) in glutamine-containing media.

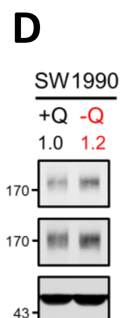
(M) Quantification of macropinocytosis under the conditions described in (L). Graphs are presented relative to the values obtained for the DMSO condition.

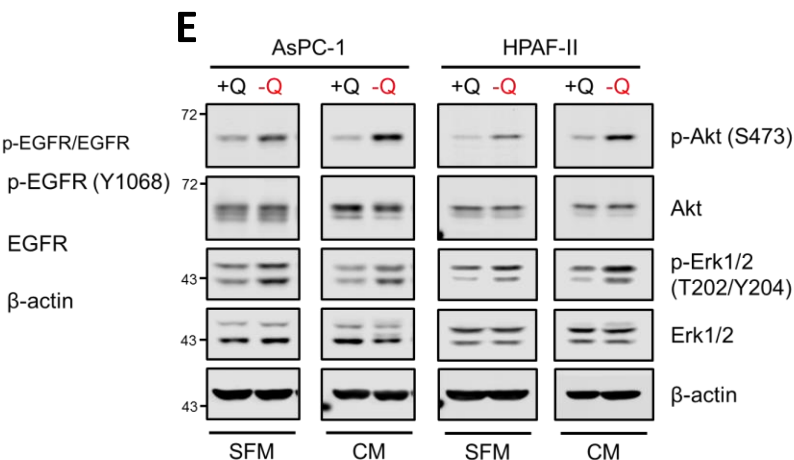
All data are representative of at least 3 independent experiments. All error bars represent s.e.m. of n=3 replicates with at least 300 cells scored per condition. *p<0.05, **p<0.01, ***p<0.001 by unpaired two-tailed Student’s t-test. Gln, Q, glutamine; Cys, cystine; Leu, leucine; n.s., not significant. Scale bar, 20 μm.

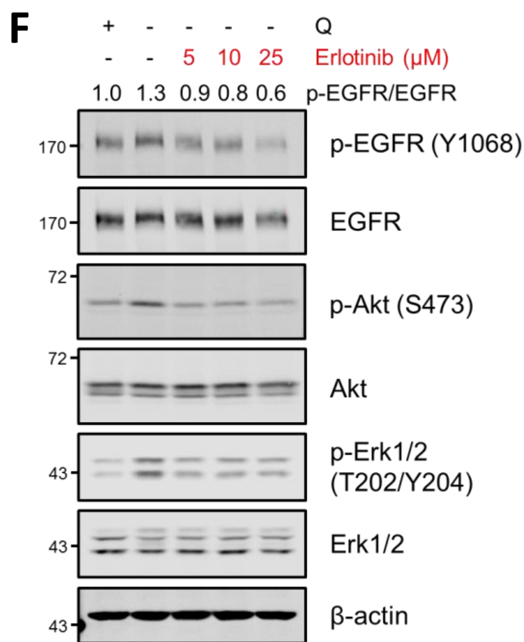

A
AsPC-1
AREG
BTC
EGF
EPGN
EREG
HBEGF
TGFA
Relative expression
+Q -Q

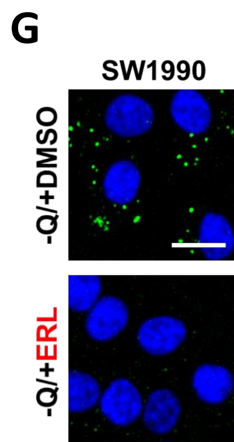

B
HPAF-II
AREG
BTC
EGF
EPGN
EREG
HBEGF
TGFA
Relative expression
+Q -Q

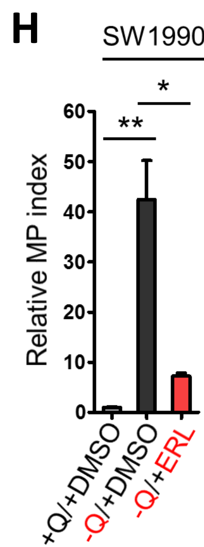

C
AREG
EREG
HBEGF
Relative expression
+α-KG
+Glu

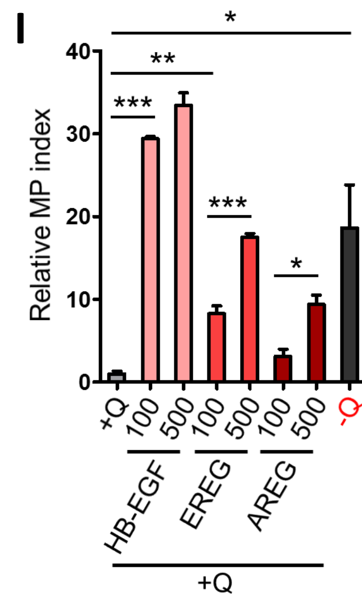

D
SW1990
+Q -Q
1.0 1.2 p-EGFR/EGFR
p-EGFR (Y1068)
EGFR
β-actin

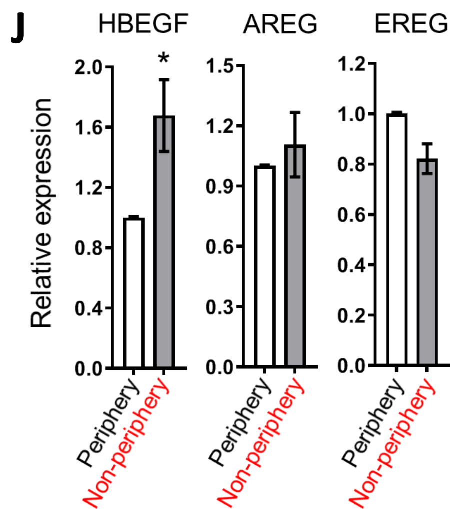

E
AsPC-1
HPAF-II
p-Akt (S473)
Akt
p-Erk1/2 (T202/Y204)
Erk1/2
β-actin
SFM
CM


F
Q
Erlotinib (μM)
1.0 1.3 0.9 0.8 0.6 p-EGFR/EGFR
p-EGFR (Y1068)
EGFR
p-Akt (S473)
Akt
p-Erk1/2 (T202/Y204)
Erk1/2
β-actin


G
SW1990
-Q/+DMSO
-Q/+ERL


H
SW1990
Relative MP index
+Q/+DMSO
-Q/+DMSO
-Q/+ERL


I
Relative MP index
HB-EGF
EREG
AREG
+Q
-Q


J
HBEGF
AREG
EREG
Relative expression
Periphery
Non-periphery

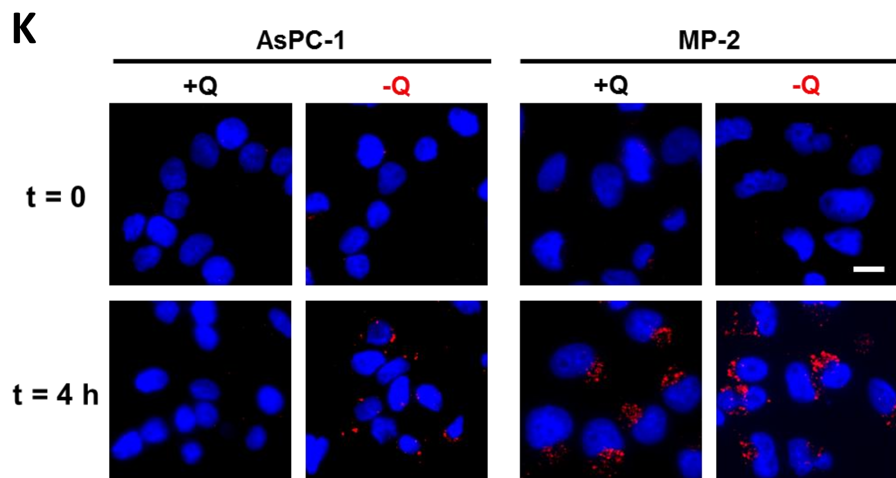

K
AsPC-1
MP-2
+Q
-Q
t = 0
t = 4 h

Figure S3. EGFR signaling is upregulated in response to glutamine deprivation, Related to Figure 3.

(A, B) Relative expression of the indicated genes determined by qRT–PCR in AsPC-1 (A) or HPAF-II (B) cells under glutamine-containing or glutamine-free conditions. Data are presented relative to the values obtained for the +Q condition. Data are representative of at least 3 independent experiments. Error bars represent s.d. of n=3 replicates.

(C) Relative expression of the indicated genes determined by qRT–PCR in AsPC-1 cells cultured in glutamine-containing or glutamine-free media without or with supplementation with a cell-permeable form of glutamate (Glu) or α-ketoglutarate (α-KG). Data are presented relative to the values obtained for each +Q condition (indicated by the red lines). Error bars represent s.d. of n=3 independent experiments.

(D) Immunoblot assessing phosphorylation of EGFR under glutamine-containing or glutamine-free condition in SW1990 cells. β-actin was used as a loading control. The p-EGFR/EGFR ratios are shown.

(E) Immunoblots assessing phosphorylation of Akt and Erk1/2 in AsPC-1 or HPAF-II cells under glutamine-containing or glutamine-free condition in serum-free media (SFM) or complete media (CM). β-actin was used as a loading control. Data are representative of at least 3 independent experiments.

(F) Immunoblots assessing phosphorylation of EGFR, Akt and Erk1/2 in AsPC-1 cells treated with vehicle (DMSO) or erlotinib at the indicated concentrations for 2 hours in glutamine-containing or glutamine-free media. β-actin was used as a loading control. The p-EGFR/EGFR ratios are shown. Data are representative of at least 3 independent experiments.

(G) Representative images of macropinocytosis (green) in SW1990 cells treated with vehicle (DMSO) or erlotinib (ERL) in glutamine-free media. Scale bar, 20 μm.

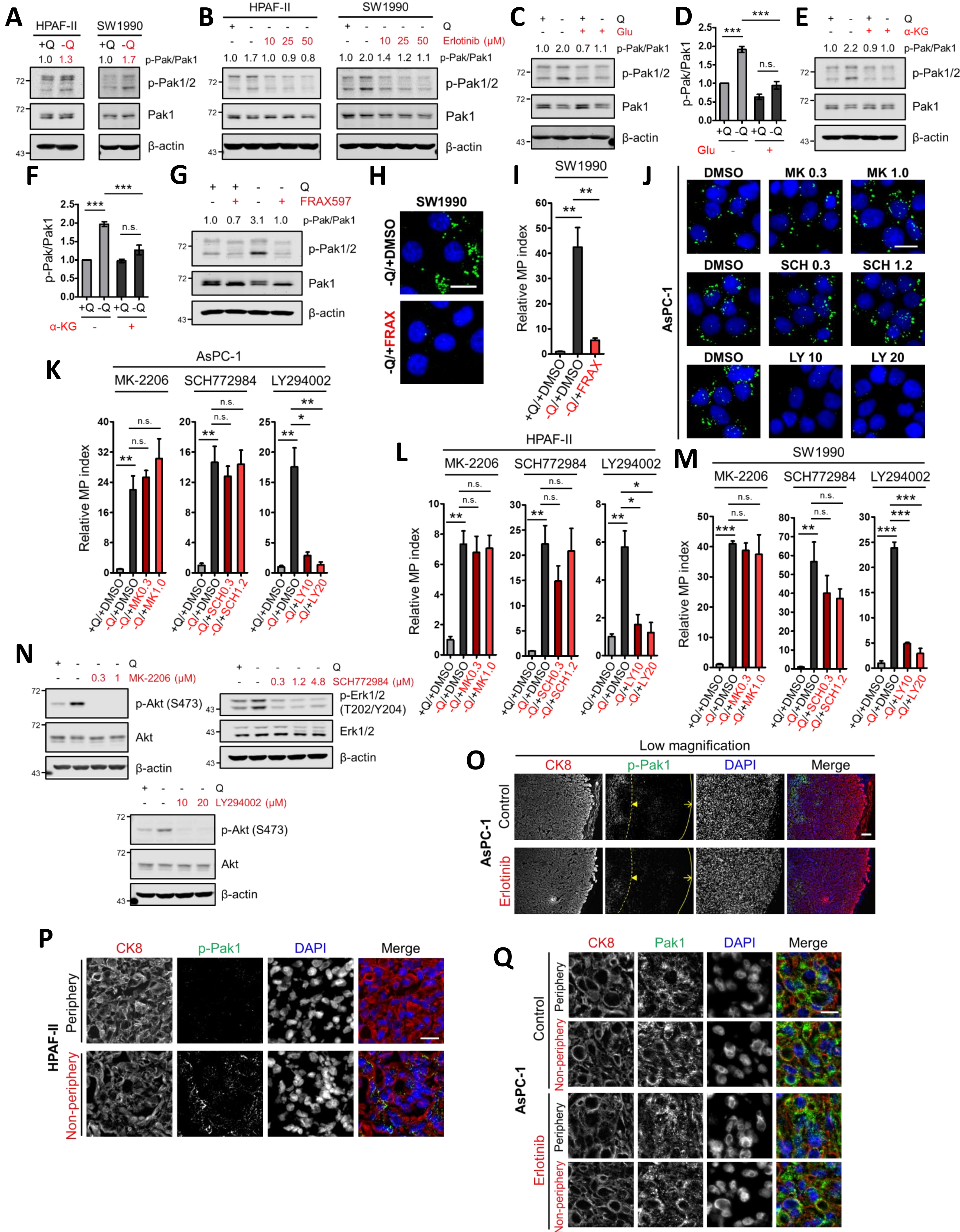
(H) Quantification of macropinocytosis in SW1990 cells treated with DMSO in glutamine-containing media and under the conditions described in (G). Data are presented relative to the values obtained for the +Q/+DMSO condition and representative of at least 3 independent experiments. Error bars represent s.e.m. of n=3 replicates with at least 150 cells scored per condition.

(I) Quantification of macropinocytosis in AsPC-1 cells treated without or with the indicated EGFR ligand at the indicated concentrations (ng/mL) for 5 min in glutamine-containing media. Glutamine-free condition (-Q) is shown as a control. Data are presented relative to the values obtained for the +Q condition. Data are representative of at least 3 independent experiments. Error bars represent s.e.m. for n=3 replicates with at least 900 cells scored per condition.

(J) Relative expression of the indicated genes determined by qRT–PCR in the non-peripheral regions relative to the periphery of AsPC-1 xenograft tumors. Error bars represent s.e.m. of n=6 tumors.

(K) Analysis of DQ-BSA fluorescence in the indicated cells that were incubated with DB red BSA for 1 hour and fixed immediately at the end of the incubation (t = 0) or following a 4-hour chase in media free of DQ-BSA (t = 4 h) under glutamine-containing or glutamine-free conditions. The fluorescent signal emanating from DQ-BSA is an indication of albumin degradation. Images shown are representative of at least 3 independent experiments. Scale bar, 20 μm.

*p<0.05, **p<0.01, ***p<0.001 by unpaired two-tailed Student's t-test. Q, glutamine.



A
HPAF-II
SW1990
+Q -Q
1.0 1.3
1.0 1.7
p-Pak/Pak1
p-Pak1/2
Pak1
β-actin
B
Erlotinib (μM)
C
Glu
D
p-Pak/Pak1
E
α-KG
F
G
FRAX597
H
-Q/+DMSO
-Q/+FRAX
I
Relative MP index
J
AsPC-1
DMSO
MK 0.3
MK 1.0
SCH 0.3
SCH 1.2
LY 10
LY 20
K
MK-2206
SCH772984
LY294002
L
M
N
p-Akt (S473)
Akt
p-Erk1/2 (T202/Y204)
Erk1/2
MK-2206 (μM)
SCH772984 (μM)
LY294002 (μM)
O
Low magnification
CK8
p-Pak1
DAPI
Merge
Control
Erlotinib
P
Periphery
Non-periphery
Q
Pak1

Figure S4. EGFR signaling modulates macropinocytosis in response to glutamine depletion via Pak1, Related to Figure 4.

(A, B) Immunoblots of Pak1/2 phosphorylation [p-Pak1 (S199/204)/p-Pak2 (S192/197)] in HPAF-II or SW1990 cells under glutamine-containing or glutamine-free conditions. Erlotinib treatment (B) was done at the indicated concentrations for 2 hours. β-actin was used as a loading control. The p-Pak/Pak1 ratios are shown.

(C) Representative immunoblots of Pak1/2 phosphorylation [p-Pak1 (S199/204)/p-Pak2 (S192/197)] in AsPC-1 cells treated without or with a cell-permeable form of glutamate (Glu) in glutamine-containing or glutamine-free media. β-actin was used as a loading control. The p-Pak/Pak1 ratios are shown.

(D) Quantification of p-Pak/Pak1 ratios from immunoblots as (C). Data are presented relative to the values obtained for the +Q condition. Error bars represent s.e.m. of n=4 independent experiments.

(E) Representative immunoblots of Pak1/2 phosphorylation [p-Pak1 (S199/204)/p-Pak2 (S192/197)] in AsPC-1 cells treated without or with a cell-permeable form of α-ketoglutarate (α-KG) in glutamine-containing or glutamine-free media. β-actin was used as a loading control. The p-Pak/Pak1 ratios are shown.

(F) Quantification of p-Pak/Pak1 ratios from immunoblots as (E). Data are presented relative to the values obtained for the +Q condition. Error bars represent s.e.m. of n=6 independent experiments.

(G) Immunoblots of Pak1/2 phosphorylation [p-Pak1 (S199/204)/p-Pak2 (S192/197)] in AsPC-1 cells treated with vehicle (DMSO) or FRAX597 (1 μM, 2 h) under glutamine-containing or glutamine-free condition. β-actin was used as a loading control. The p-Pak/Pak1 ratios are shown.

(H) Representative images of macropinocytosis (green) in SW1990 cells treated with vehicle (DMSO) or FRAX597 (FRAX) in glutamine-free media. Scale bar, 20 μm.

(I) Quantification of macropinocytosis in SW1990 cells treated with DMSO in glutamine-containing media and under the conditions described in (H). Data are presented relative to the values obtained for the +Q/+DMSO condition and representative of at least 3 independent experiments. Error bars represent s.e.m. of n=3 replicates with at least 150 cells scored per condition.

(J) Representative images of macropinocytosis (green) in AsPC-1 cells treated with vehicle (DMSO), MK-2206 (MK), SCH772984 (SCH) or LY294002 (LY) at the indicated concentrations (μM) in glutamine-free media. Treatment with MK-2206 was done for 2 hours, SCH772984 for 4 hours, and LY294002 for 3 hours. Scale bar, 20 μm.

(K–M) Quantification of macropinocytosis in AsPC-1 (K), HPAF-II (L) or SW1990 (M) cells treated with vehicle (DMSO) in glutamine-containing media and under the conditions described in (J). Graphs are presented relative to the values obtained for the +Q/+DMSO condition. Error bars represent s.e.m. for n=3 replicates with at least 850 (K), 400 (L) or 150 (M) cells scored per condition.

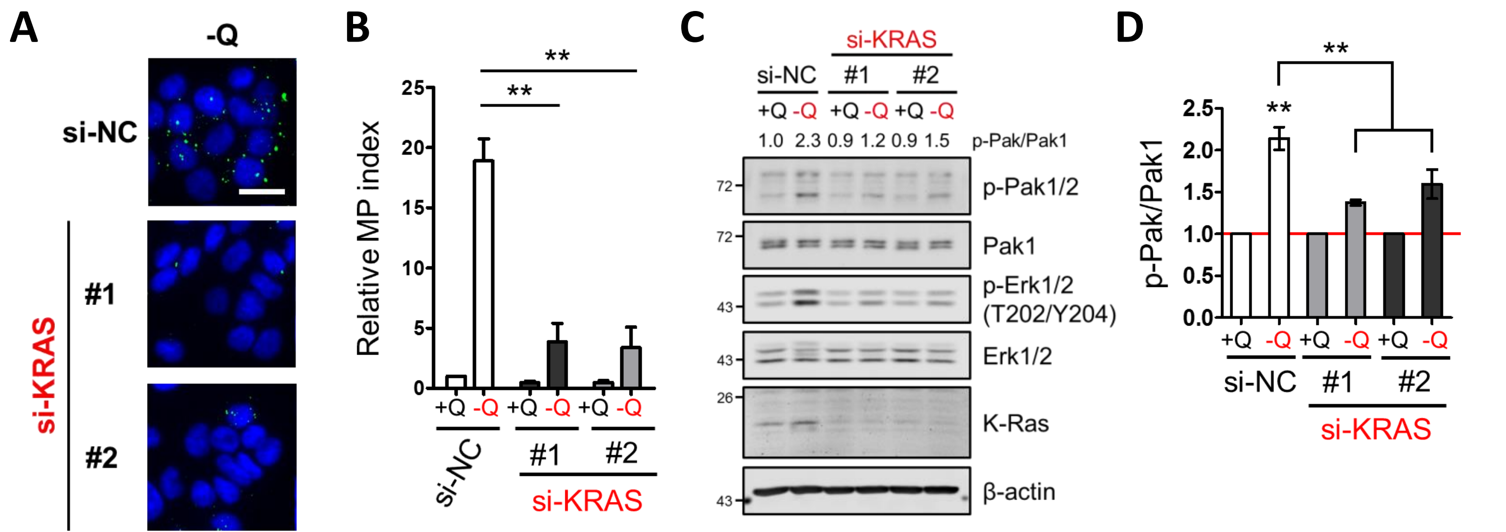
(N) Immunoblots assessing phosphorylation of Akt or Erk1/2 in AsPC-1 cells treated with vehicle (DMSO) or the indicated inhibitor at the indicated concentrations for the time described in (J) in glutamine-containing or glutamine-free media. β-actin or α-tubulin was used as a loading control.

(O) Low-magnification images of immunofluorescence staining in AsPC-1 xenograft tumors treated with vehicle control or erlotinib. Tumor sections were stained with anti-CK8 (red), anti-p-Pak1 (green) and DAPI (blue). The solid lines (arrows) indicate the tumor boundary. The dashed lines (arrowheads) mark the tumor non-peripheral regions. Scale bar, 200 μm.

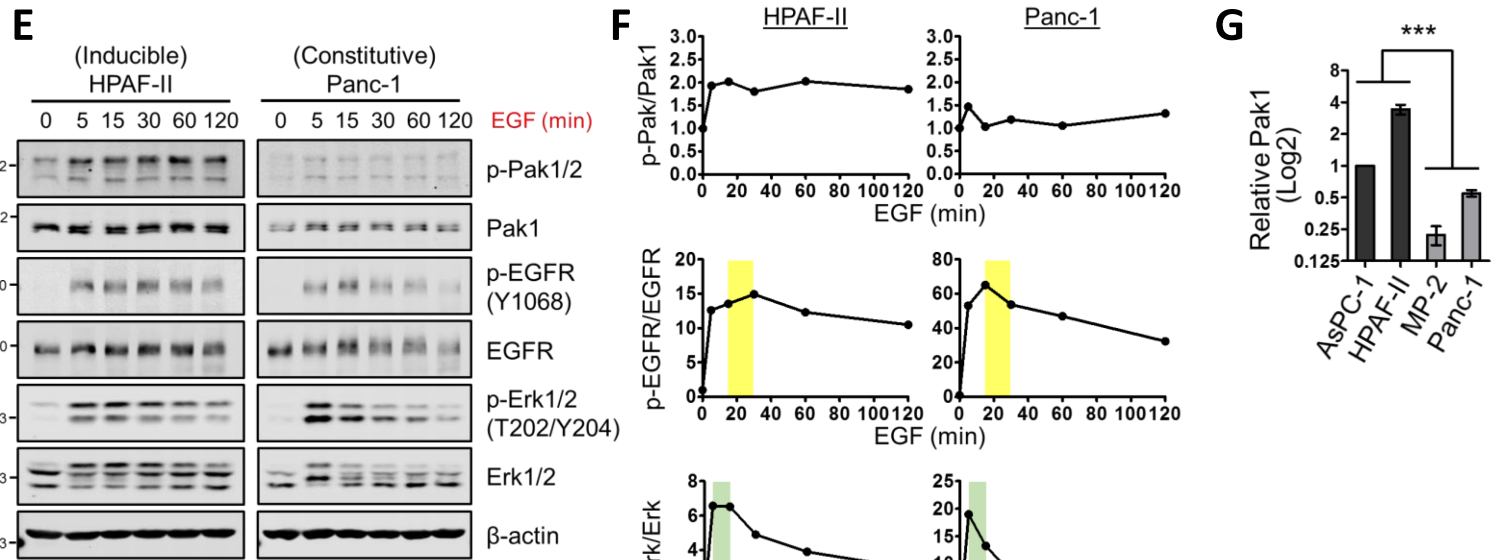
(P) Immunofluorescence staining in HPAF-II xenograft tumors. Tumor sections were stained with anti-CK8 (red), anti-p-Pak1 (green) and DAPI (blue). Scale bar, 20 μm.

(Q) Immunofluorescence staining in AsPC-1 xenograft tumors treated with vehicle control or erlotinib. Tumor sections were stained with anti-CK8 (red), anti-Pak1 (green) and DAPI (blue). Scale bar, 20 μm.

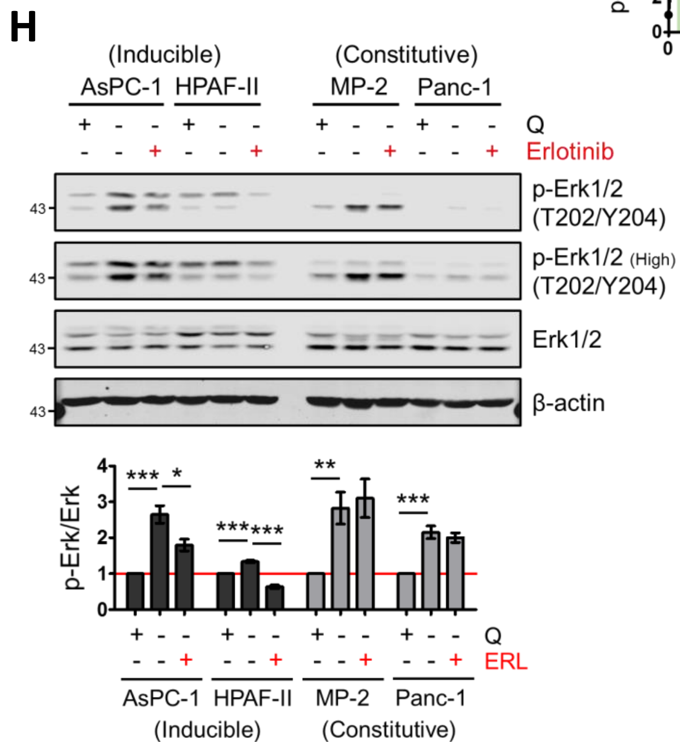
*$p<0.05$, **$p<0.01$, ***$p<0.001$ by unpaired two-tailed Student's t-test. Q, glutamine; n.s., not significant.



A
-Q
si-NC
si-KRAS
#1
#2
B
Relative MP index
**
**
+Q -Q +Q -Q +Q -Q
si-NC
#1
#2
si-KRAS
C
si-KRAS
si-NC
#1
#2
+Q -Q +Q -Q +Q -Q
1.0 2.3 0.9 1.2 0.9 1.5
p-Pak/Pak1
p-Pak1/2
Pak1
p-Erk1/2
(T202/Y204)
Erk1/2
K-Ras
β-actin
D
p-Pak/Pak1
**
**
+Q -Q +Q -Q +Q -Q
si-NC
#1
#2
si-KRAS



E
(Inducible)
HPAF-II
(Constitutive)
Panc-1
0 5 15 30 60 120
EGF (min)
p-Pak1/2
Pak1
p-EGFR
(Y1068)
EGFR
p-Erk1/2
(T202/Y204)
Erk1/2
β-actin
F
HPAF-II
Panc-1
p-Pak/Pak1
p-EGFR/EGFR
p-Erk/Erk
EGF (min)
G
***
Relative Pak1
(Log2)
AsPC-1
HPAF-II
MP-2
Panc-1



H
(Inducible)
(Constitutive)
AsPC-1
HPAF-II
MP-2
Panc-1
Q
Erlotinib
p-Erk1/2
(T202/Y204)
p-Erk1/2 (High)
(T202/Y204)
Erk1/2
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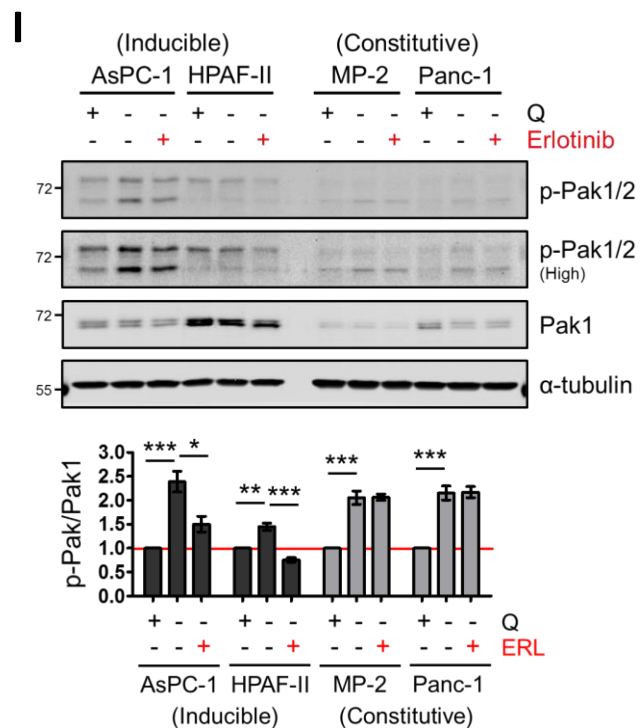
HPAF-II
MP-2
Panc-1
(Inducible)
(Constitutive)



I
(Inducible)
(Constitutive)
AsPC-1
HPAF-II
MP-2
Panc-1
Q
Erlotinib
p-Pak1/2
p-Pak1/2
(High)
Pak1
α-tubulin
p-Pak/Pak1
Q
ERL
AsPC-1
HPAF-II
MP-2
Panc-1
(Inducible)
(Constitutive)

Figure S5. EGFR signaling dynamics in PDAC cells with inducible or constitutive macropinocytosis, Related to Figure 5.

(A) Representative images of macropinocytosis (green) in AsPC-1 cells transfected with a negative control siRNA (si-NC) or two independent siRNAs targeting KRAS (si-KRAS) under glutamine-free conditions. Scale bar, 20 μm.

(B) Quantification of macropinocytosis in the cells described in (A) under glutamine-containing or glutamine-free conditions. Data are presented relative to the values obtained for the +Q condition of si-NC cells. Error bars represent s.e.m. for n=3 independent experiments with at least 1,000 cells scored per condition.

(C) Representative immunoblots assessing phosphorylation of Pak1/2 [p-Pak1 (S199/204)/p-Pak2 (S192/197)] in the cells described in (A) under the conditions described in (B). β-actin was used as a loading control. The p-Pak/Pak1 ratios are shown.

(D) Quantification of p-Pak/Pak1 ratios from immunoblots as (C). Data are presented relative to the values obtained for each +Q condition (indicated by the red line). Error bars represent s.e.m. of n=3 independent experiments.

(E) Immunoblots assessing phosphorylation of Pak1/2 [p-Pak1 (S199/204)/p-Pak2 (S192/197)], EGFR and Erk1/2 in the indicated cells treated with EGF (25 ng/mL) for the indicated time in glutamine-containing media. β-actin was used as a loading control. Results shown are representative of at least 3 independent experiments.

(F) Quantification of phosphoprotein/total protein ratios from the immunoblots in (E). Data are presented relative to the values obtained for each 0 time point (set as 1). The yellow areas highlight the changes of p-EGFR from 15 to 30 min post-EGF treatment. The green areas highlight the changes of p-Erk1/2 from 5 to 15 min post-EGF treatment.

(G) Quantification of Pak1/tubulin ratios in the indicated cells in glutamine-containing media. Data are presented relative to the values obtained for AsPC-1 cells on a Log2 scale. Error bars represent s.e.m. for n=5 independent experiments.

(H, I) Representative immunoblots assessing phosphorylation of Erk1/2 (H) or Pak1/2 (I) in the indicated cells treated with vehicle (DMSO) or erlotinib (25 μM, 2 hours) in glutamine-containing or glutamine-free media. β-actin or α-tubulin was used as a loading control. Graphs are quantification of phosphoprotein/total protein ratios presented relative to the values obtained for each +Q/-ERL condition (indicated by the red line). Error bars represent s.e.m. of at least 3 independent experiments. p-Pak1/2 indicates p-Pak1 (S199/204)/p-Pak2 (S192/197). High, higher intensity.

*p<0.05, **p<0.01, ***p<0.001 by unpaired two-tailed Student's t-test. Q, glutamine.

Table S3. Gene set enrichment analysis, Related to Figure 3.

GENE SET	AsPC-1			HPAF-II		
	NES	p-value	FDR q-value	NES	p-value	FDR q-value
ZWANG_CLASS_3_ TRANSIENTLY_ INDUCED_BY_EGF	2.31856	<0.0001	<0.0001	1.91733	<0.0001	0.00738
NAGASHIMA_EGF_ SIGNALING_UP	2.30442	<0.0001	<0.0001	1.76529	<0.0001	0.03444
ZWANG_CLASS_1_ TRANSIENTLY_ INDUCED_BY_EGF	2.14292	<0.0001	<0.0001	1.71218	<0.0001	0.04638
AMIT_EGF_ RESPONSE_40_ HELA	1.88861	0.00207	0.00507	1.43369	0.04928	0.19052
KOBAYASHI_EGFR_ SIGNALING_ 6HR_DN	1.83111	<0.0001	0.00916	1.39835	0.07605	0.20960
ZWANG_CLASS_2_ TRANSIENTLY_ INDUCED_BY_EGF	1.74537	<0.0001	0.02140	1.58036	0.00594	0.10114
AMIT_EGF_ RESPONSE_60_ MCF10A	1.70951	<0.0001	0.02973	1.35466	0.05848	0.23929
AMIT_EGF_ RESPONSE_60_ HELA	1.68549	0.00409	0.03554	1.43432	0.04762	0.19126
AMIT_EGF_ RESPONSE_40_ MCF10A	1.65841	0.01064	0.04397	1.79912	<0.0001	0.03010

Gene set enrichment analysis (GSEA) was performed using the RNA-Seq data of control and glutamine-starved AsPC-1 and HPAF-II cells. Several gene sets for EGF-induced genes and EGF/EGFR signaling were identified. NES, normalized enrichment score; FDR, false discovery rate.