

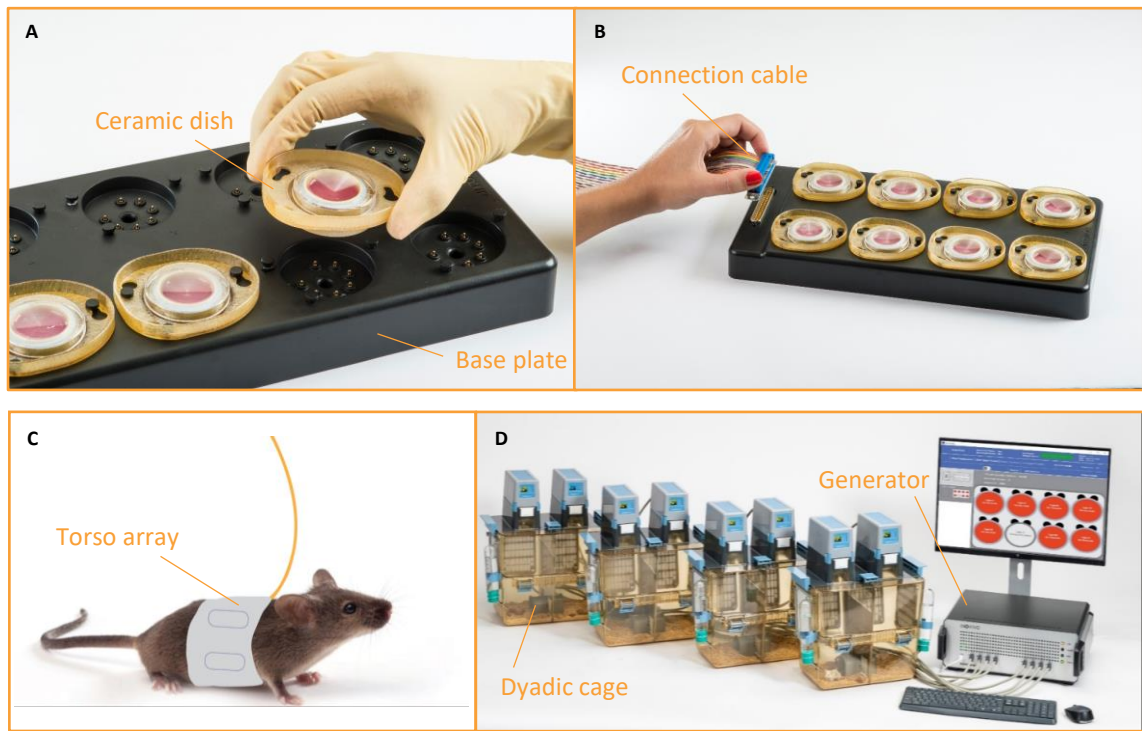


Figure S1. Lab tools for applying TFields to cells and mice.

(A-B) The *in vitro* system for applying TFields to cells. Cells are seeded in individual ceramic dishes that are connected to the base plate, which is connected via a cable to the electric field generator (not shown). **(C-D)** The *in vivo* system for applying TFields to mouse torso. Mice are wrapped around their torsos with electrodes and placed within dedicated cages allowing individual housing while maintaining dyadic interaction. The electrodes are connected to the field generator, allowing connection of a total of 8 animals (in 4 dyadic cages).

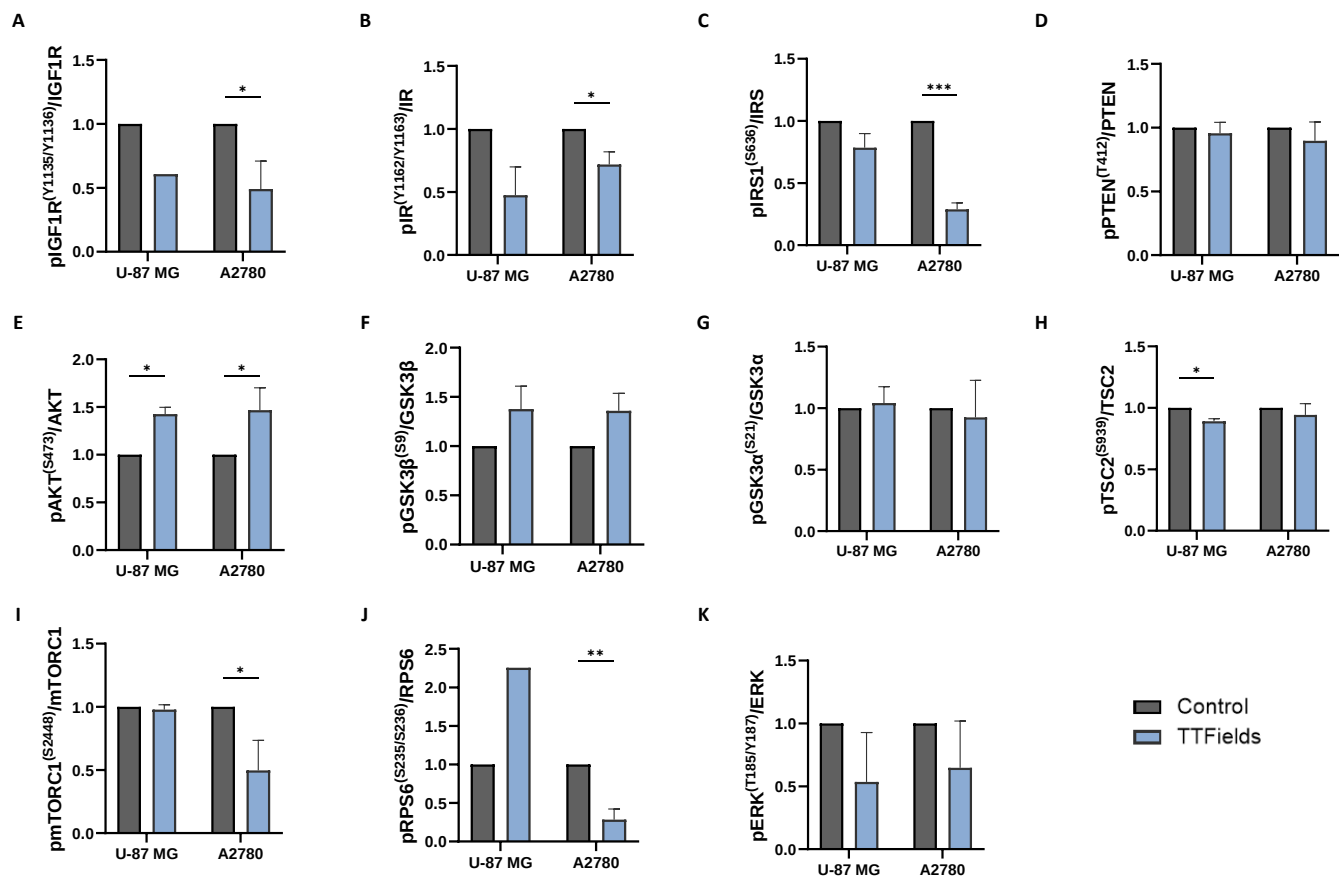


Figure S2. Supporting data for the Luminex assay.

Luminex assay of lysates from control and TTFields-treated (72h) U-87 MG and A2780 cells. Fold change of phosphorylated to total protein ratio relative to the control is presented for: **(A)** IGF1R (Tyr1135/Tyr1136); **(B)** IR (Tyr1162/Tyr1163); **(C)** IRS1 (Ser636); **(D)** PTEN (Thr412); **(E)** AKT (Ser473); **(F)** GSK3β (Ser9); **(G)** GSK3α (Ser21); **(H)** TSC2 (Ser939); **(I)** mTORC1 (Ser2448); **(J)** RPS6 (Ser235/Ser236); and **(K)** ERK (Thr185/Tyr187). Data are presented as mean ± SEM; *p < 0.05; **p < 0.01; and ***p < 0.001; multiple unpaired t-test N ≥ 2.

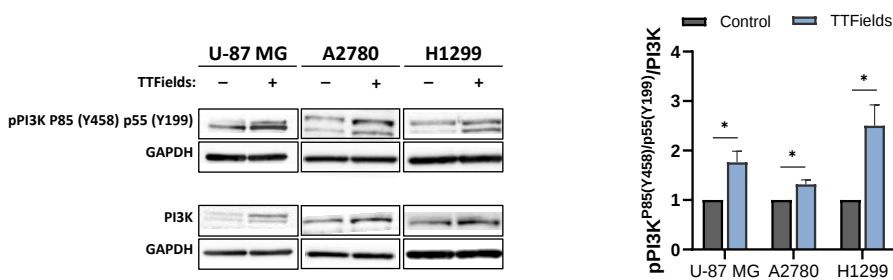


Figure S3. PI3K activation following TTFields application.

Western blot analysis of PI3K, pPI3K (Tyr458 of the PI3K p85 regulatory subunit), and GAPDH in lysates from control and TTFields-treated (72h) U-87 MG, A2780, and H1299 cells. Densitometric analysis of phosphorylation fold change is shown as mean ± SEM. * p < 0.05; multiple unpaired t-tests; N = 2.

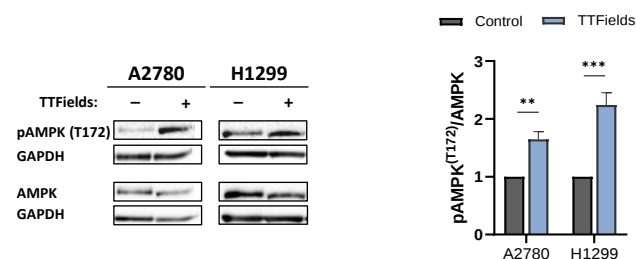


Figure S4. AMPK activation following TTFields application.

Western blot analysis of AMPK, pAMPK (Thr172), and GAPDH in lysates from control and TTFields-treated (72h) A2780 and H1299 cells. Densitometric analysis is shown as mean $\pm$ SEM. ** $p < 0.01$; and *** $p < 0.001$; multiple unpaired t-tests; $N = 2$.

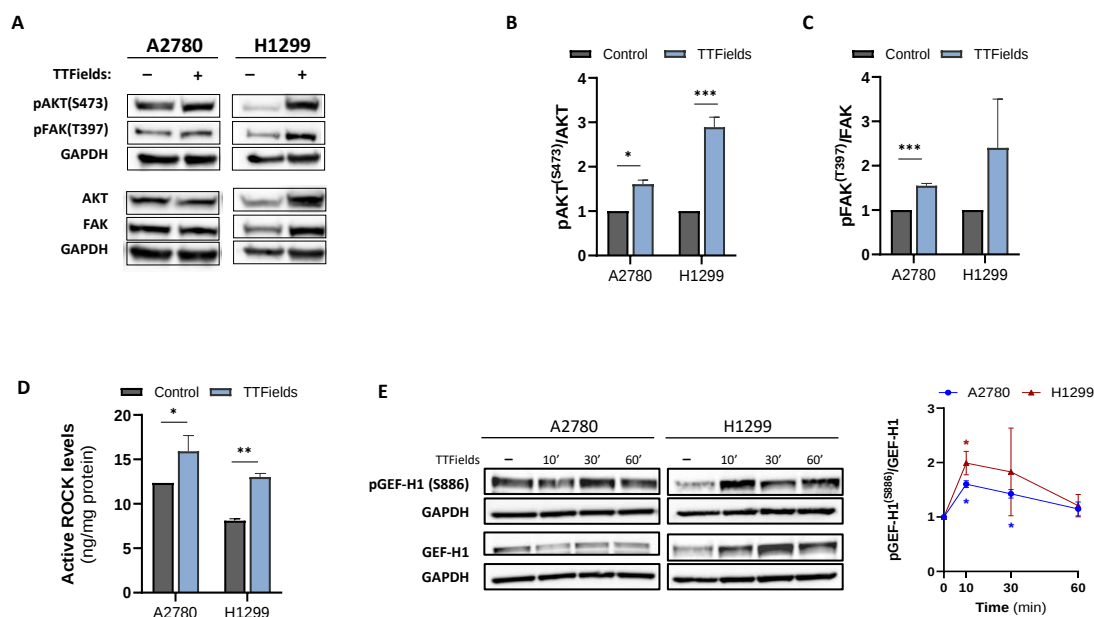


Figure S5. AKT, FAK, ROCK, and GEF-H1 activation following short-term TTFields application at low cell confluency.

(A-C) Western blot analysis of AKT, pAKT (Ser473), FAK, pFAK (Tyr397), and GAPDH in lysates from control and TTFields-treated (10min) A2780 and H1299 cells. Densitometric analysis of phosphorylation fold change is shown as mean $\pm$ SEM; $N = 2$. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; multiple unpaired t-tests. **(D)** ROCK activity from control and TTFields-treated (10 min) A2780 and H1299 cells, shown as mean $\pm$ SEM. * $p < 0.05$, and ** $p < 0.01$; multiple unpaired t-tests, $N = 2$. **(E)** Western blot analysis of for GEF-H1, pGEF-H1 (Ser886), and GAPDH in lysates from control and TTFields-treated (10, 30 or 60 min) A2780 and H1299 cells. Densitometric analysis is shown as mean $\pm$ SEM. * $p < 0.05$; multiple unpaired t-tests; $N = 2$.

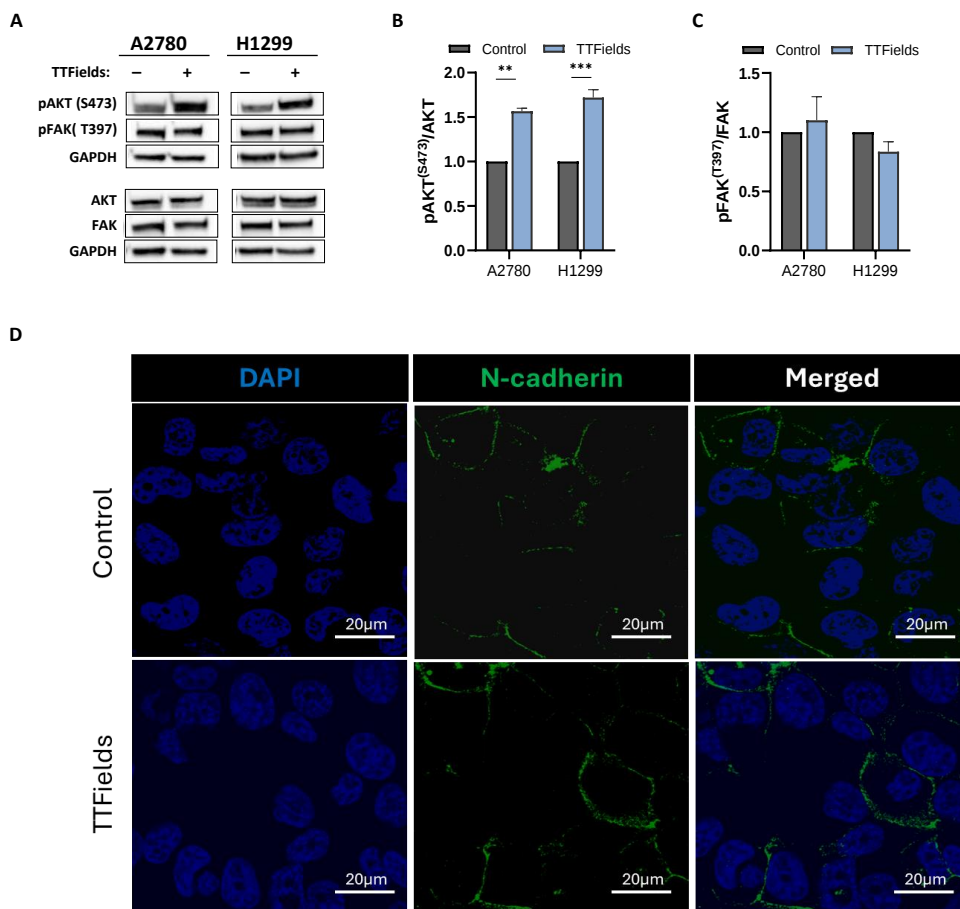


Figure S6. N-cadherin activation and FAK lack of activation following short-term TTFields application at high cell confluency.

(A-C) Western blot analysis of AKT, pAKT (Ser473), FAK, pFAK (Tyr397), and GAPDH in lysates from control and TTFields-treated A2780 and H1299 cells, performed following 2h treatment of 20×10^4 cells/coverslip. Densitometric analysis of phosphorylation fold change is shown as mean $\pm$ SEM; $N = 2$. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; multiple unpaired t-tests. **(D)** Confocal fluorescence microscopy images (x63 magnification) of N-cadherin in the control and TTFields-treated H1299 cells, performed following 2h treatment of 20×10^4 cells/coverslip.

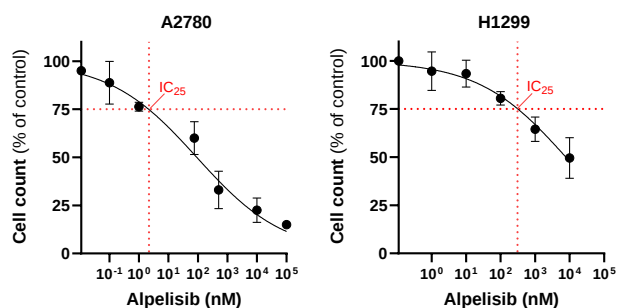


Figure S7. Alpelisib dose-response curves.

Cell count of A2780 and H1299 cells treated (72h) with escalating alpelisib doses. The intersection of the dashed lines indicates the IC_{25} . Values are mean $\pm$ SEM. $N = 2$.

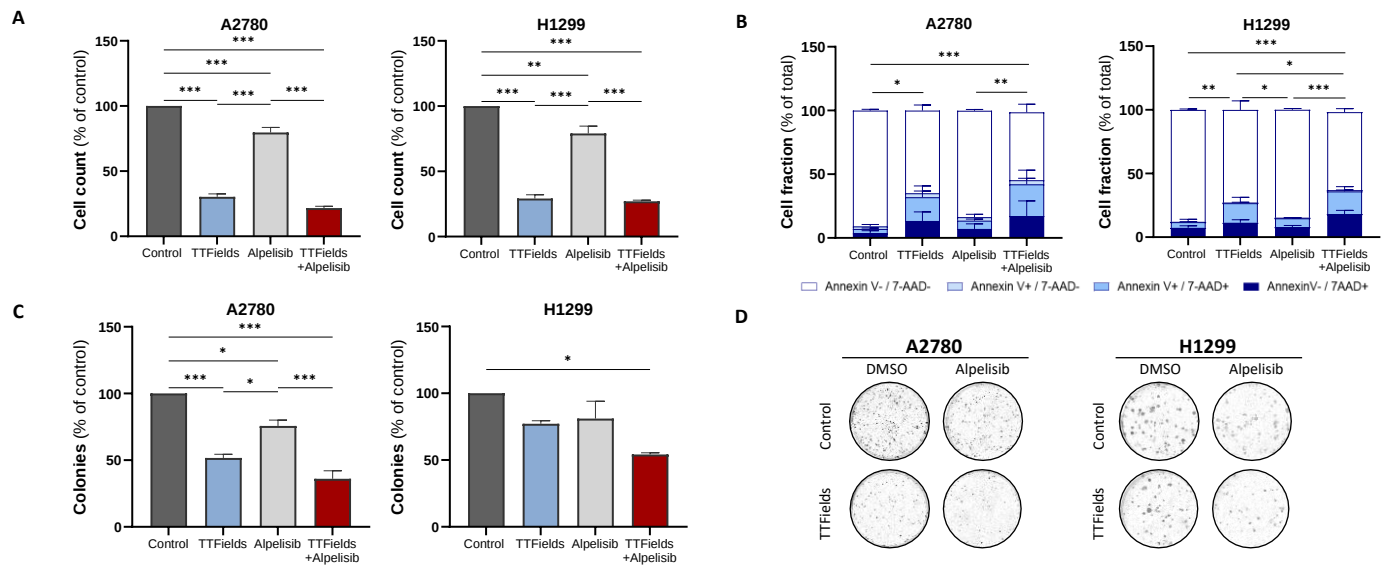


Figure S8. Concomitant treatment with TTFields and alpelisib *in vitro*.

Cell counts (A), apoptosis [Live: Annexin V-/7AAD-; early apoptosis: Annexin V+/7AAD-; late apoptosis: Annexin V+/7AAD+] (B), and clonogenic survival (C-D) of control and TTFields-treated (72h) A2780 and H1299 cells, with or without concomitant alpelisib throughout. Results are shown as mean $\pm$ SEM; $N = 2$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$; one-way ANOVA followed by Tukey's post hoc test for cell count and clonogenic survival; two-way ANOVA followed by Tukey's post hoc test for apoptosis, with significance within shown for the live cell fraction.

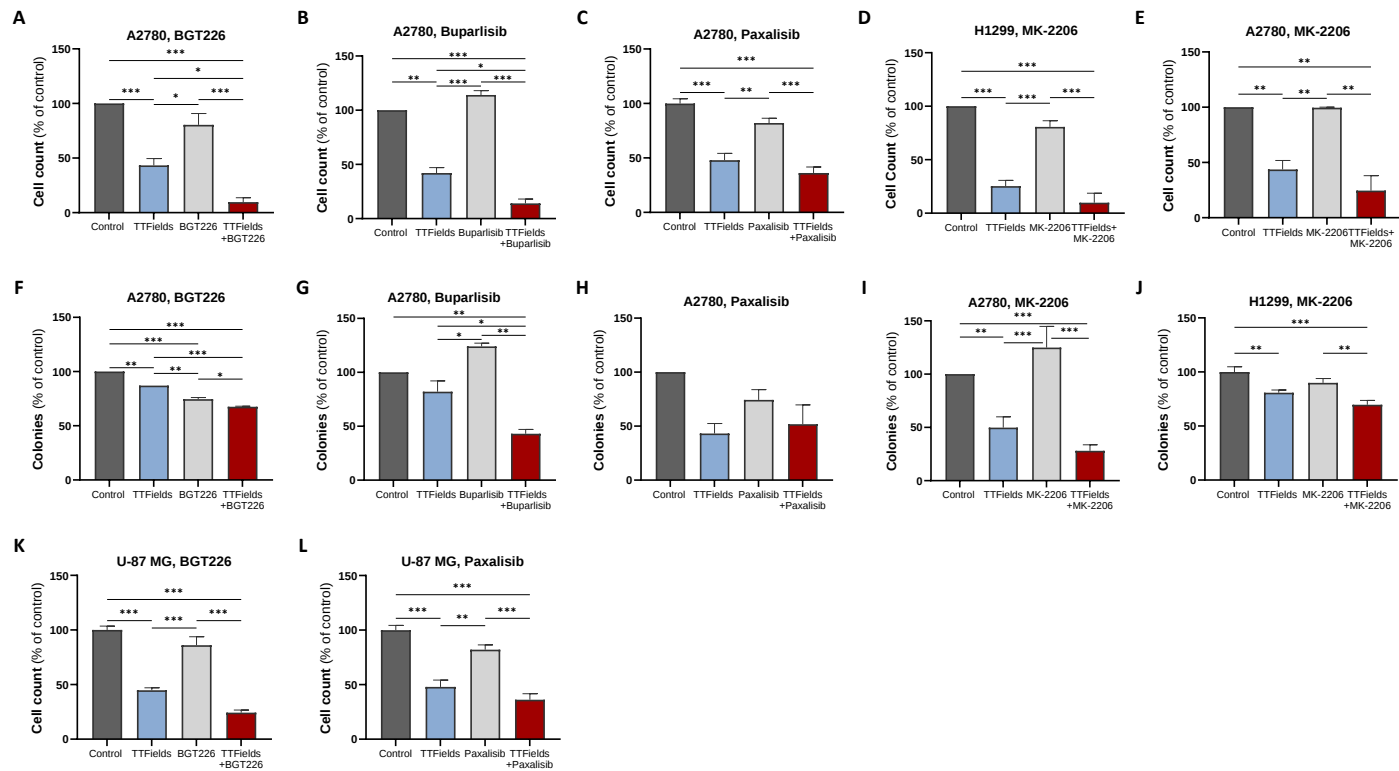
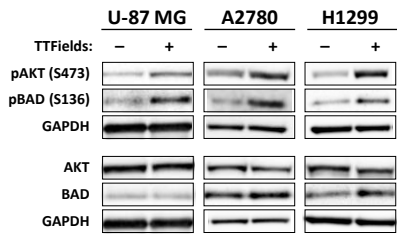


Figure S9. Concomitant treatment with TTFields and PI3K/AKT inhibitors *in vitro*.

Cell counts (A-E) and clonogenic survival (F-J) of control and TTFields-treated (144h) A2780 (A-D and F-I) or H1299 cells (E and J), with or without concomitant BGT226 (A and F), buparlisib (B and G), paxalisib (C and H), or MK-2206 (D, E and I, J) (during the first 72h). Cell counts (K-L) of control and TTFields-treated (240h) U-87 MG cells, with or without concomitant BGT226 (G), or paxalisib (L) (during the first 72h). Results are shown as mean $\pm$ SEM; $N = 2$. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; one-way ANOVA followed by Tukey's post hoc test for cell count and clonogenic survival.

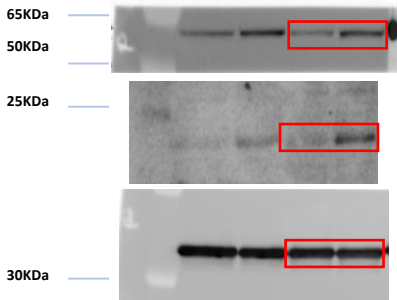
Figure 1

B



U-87 MG

TTFields: - +



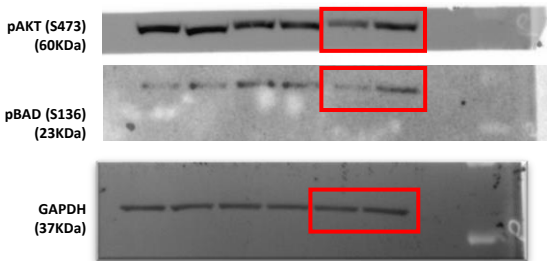
pAKT (S473)
(60KDa)

pBAD (Ser136)
(23KDa)

GAPDH
(37KDa)

A2780

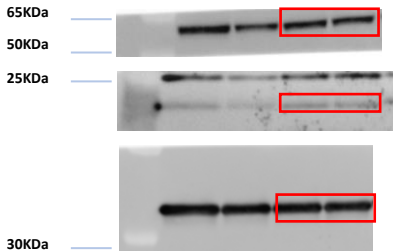
TTFields: - +



pAKT (S473)
(60KDa)

pBAD (S136)
(23KDa)

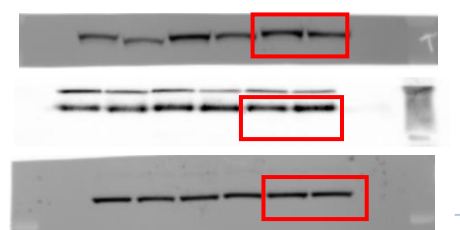
GAPDH
(37KDa)



AKT
(60KDa)

BAD
(23KDa)

GAPDH
(37KDa)



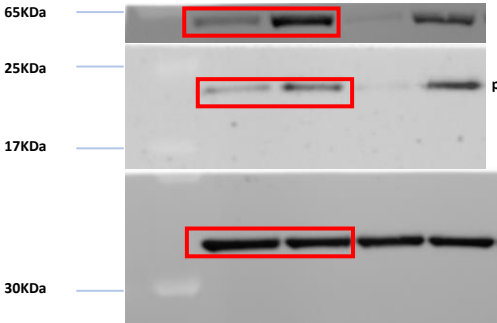
AKT
(60KDa)

BAD
(23KDa)

GAPDH
(37KDa)

H1299

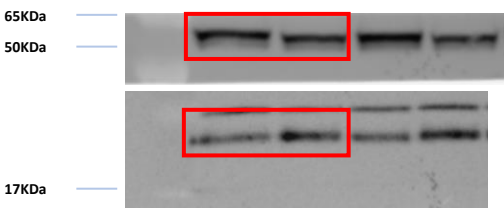
TTFields: - +



pAKT (S473)
(60KDa)

pBAD (Ser136)
(23KDa)

GAPDH
(37KDa)



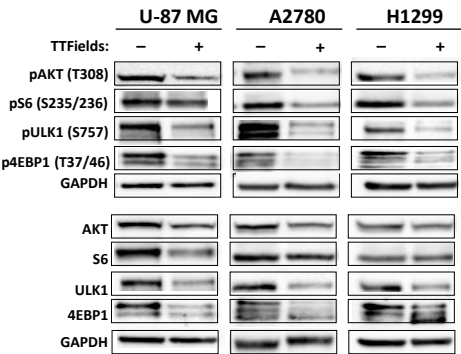
AKT
(60KDa)

BAD
(23KDa)

GAPDH
(37KDa)

Figure 1

E



U-87 MG

A2780

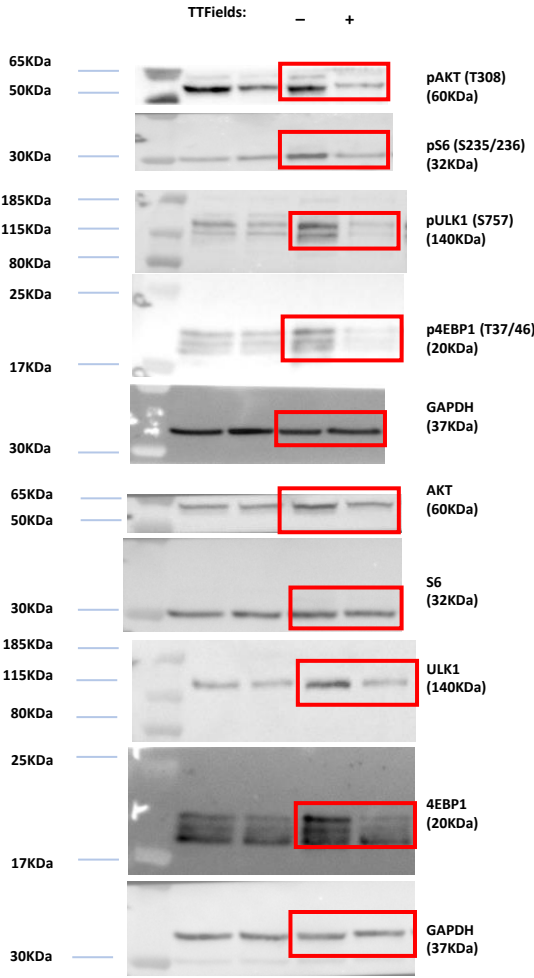
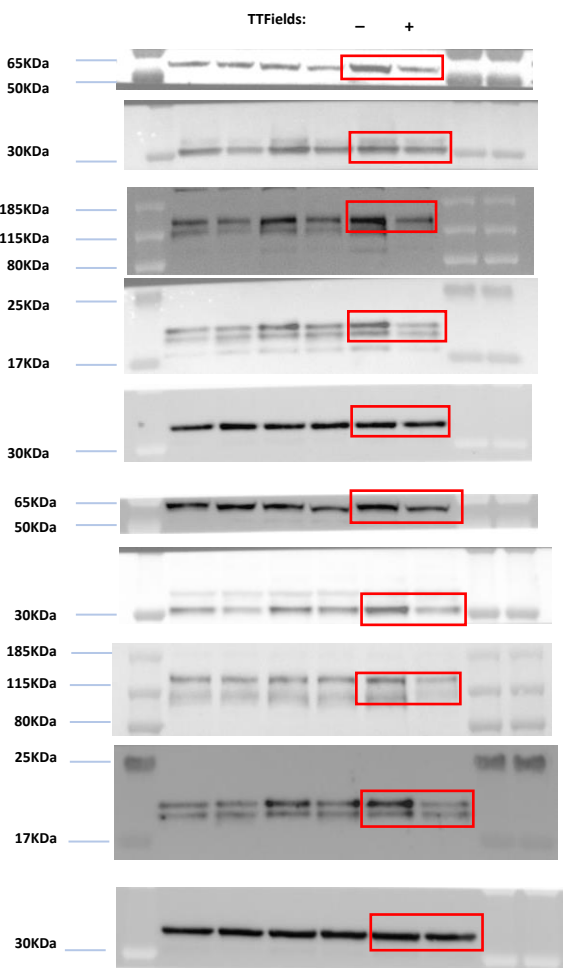


Figure 1

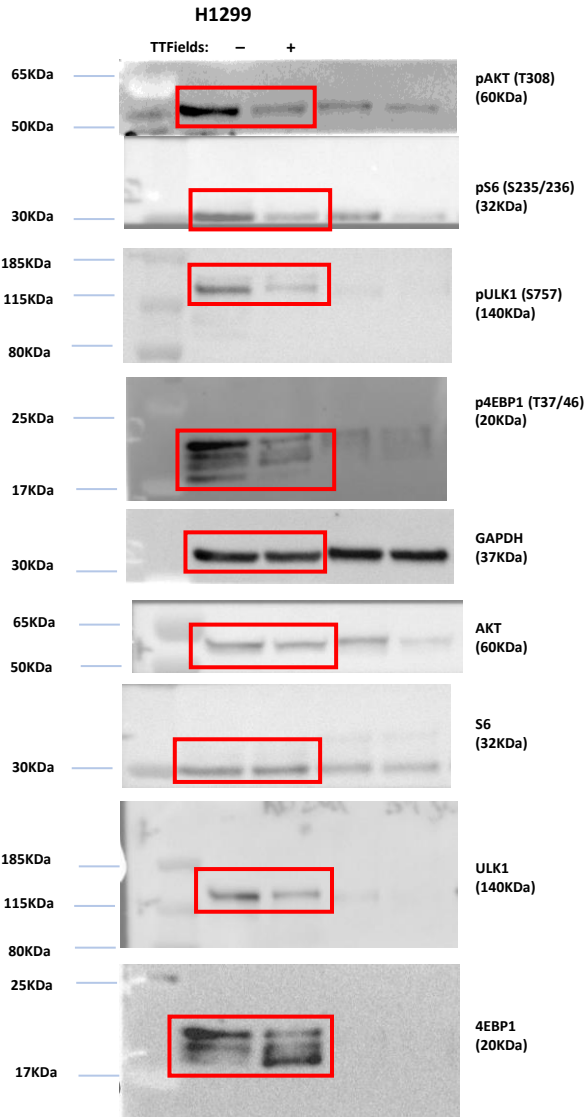
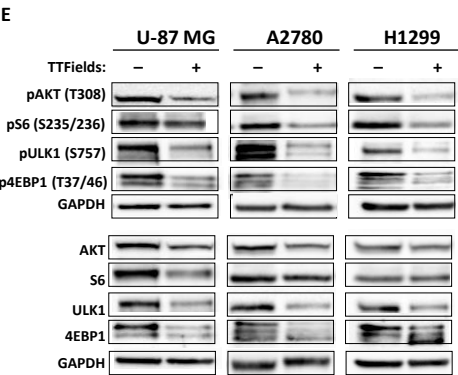
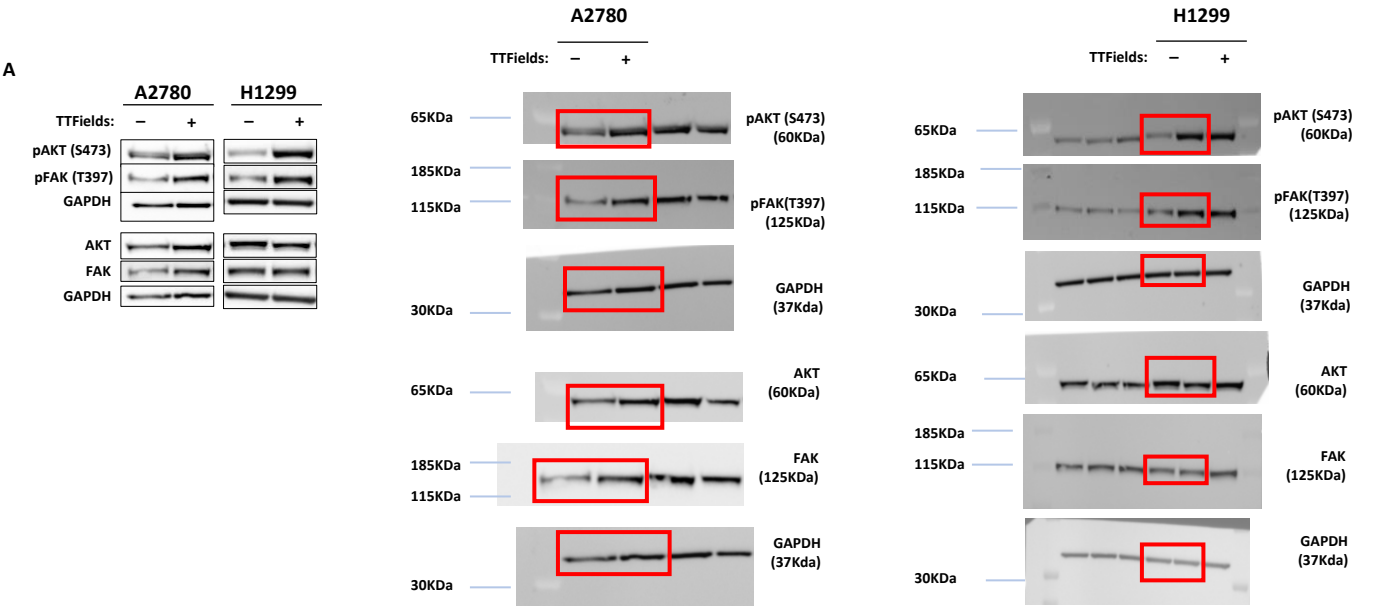
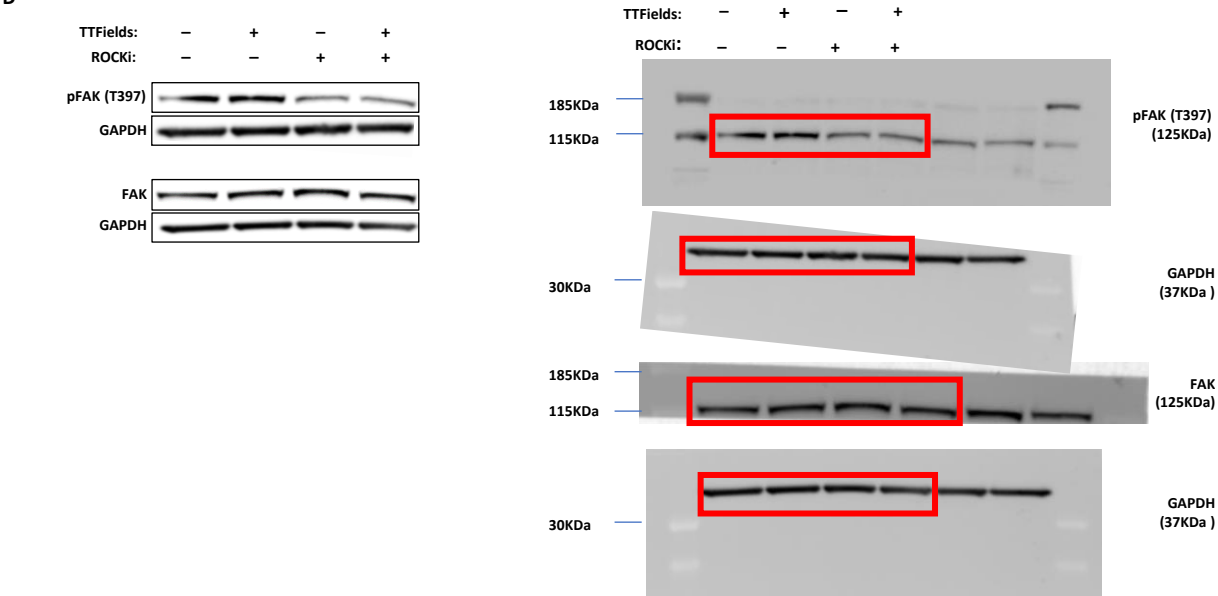


Figure 2

A



D



E

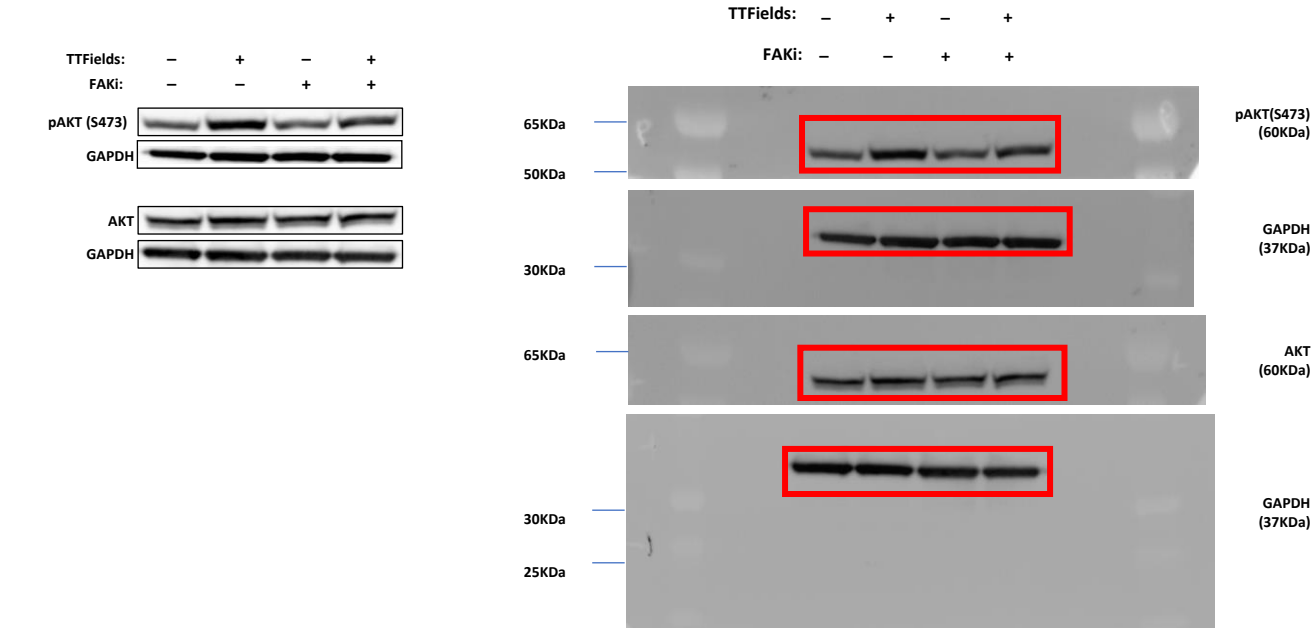


Figure 2

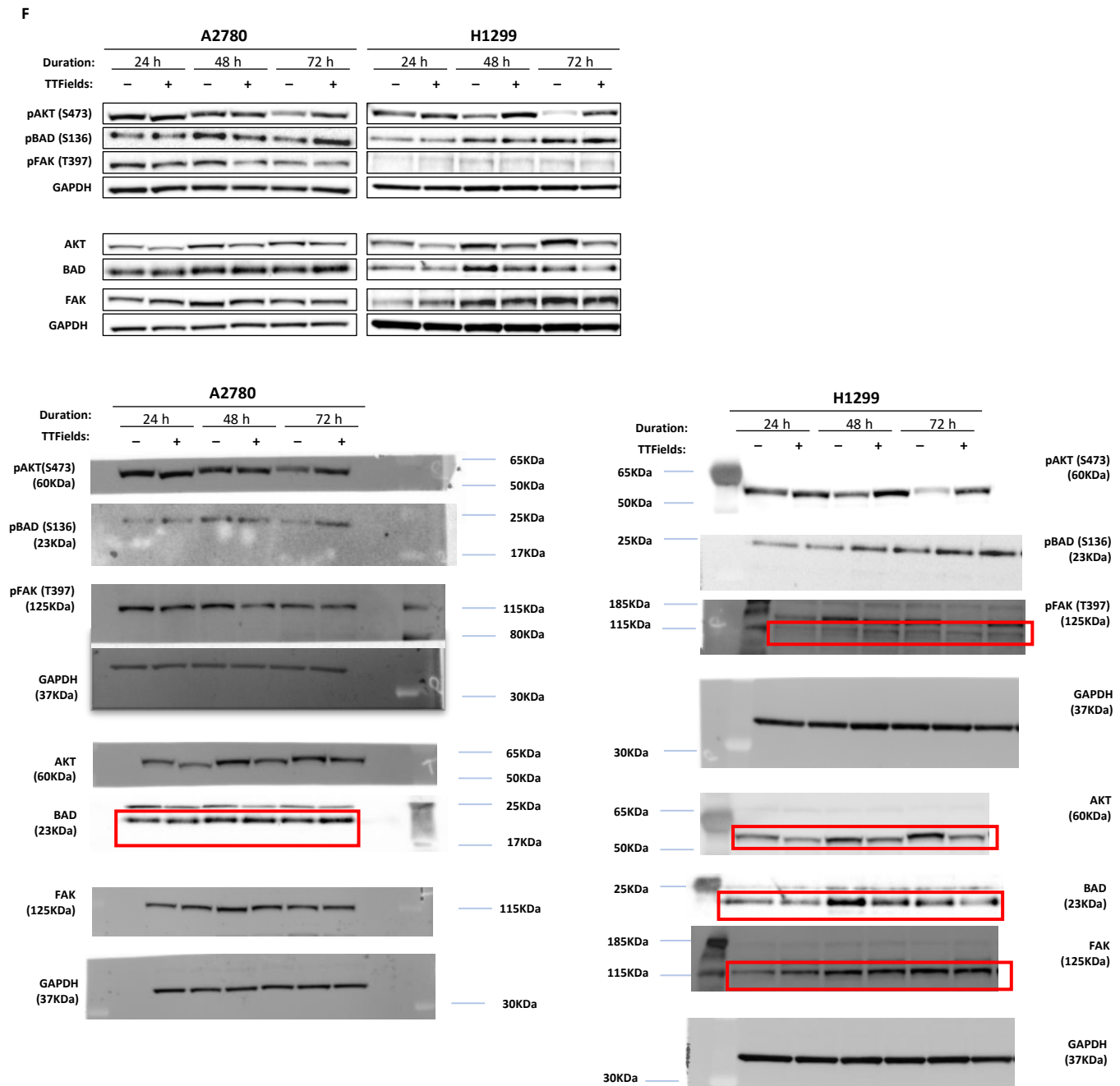


Figure 3

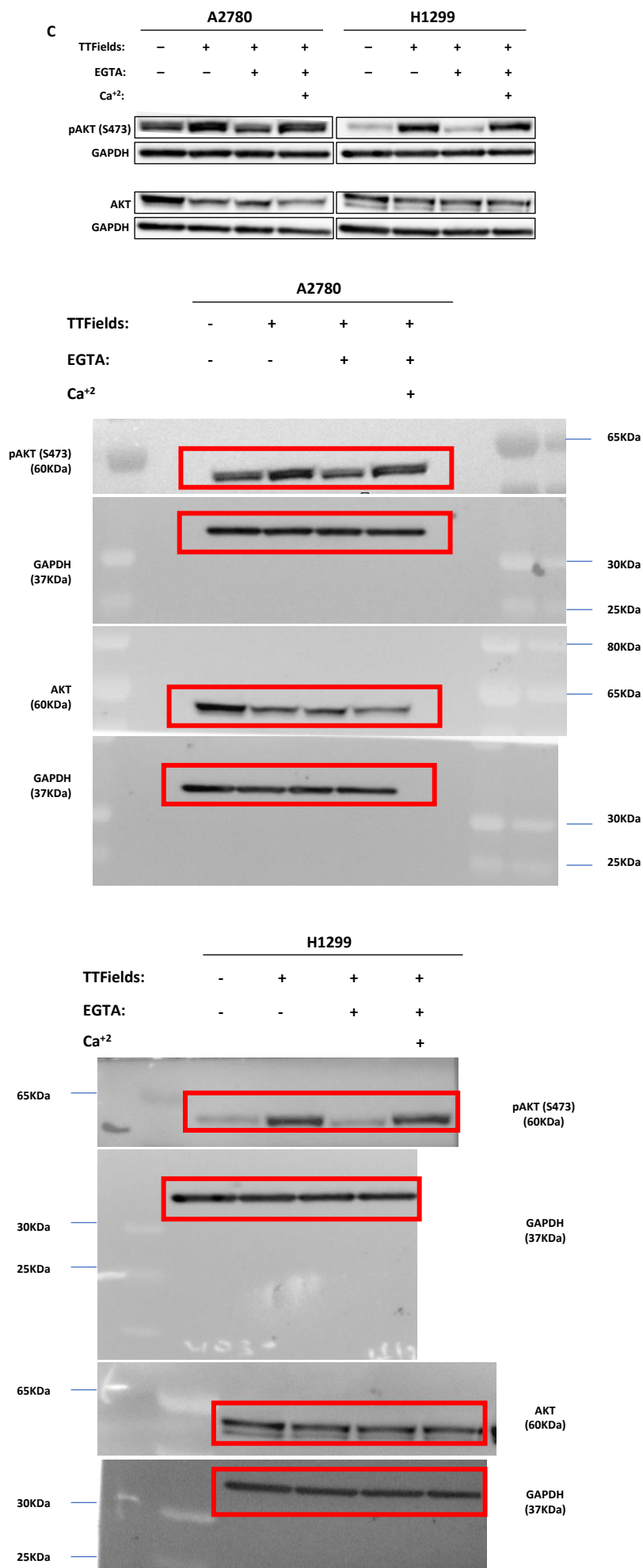


Figure 3

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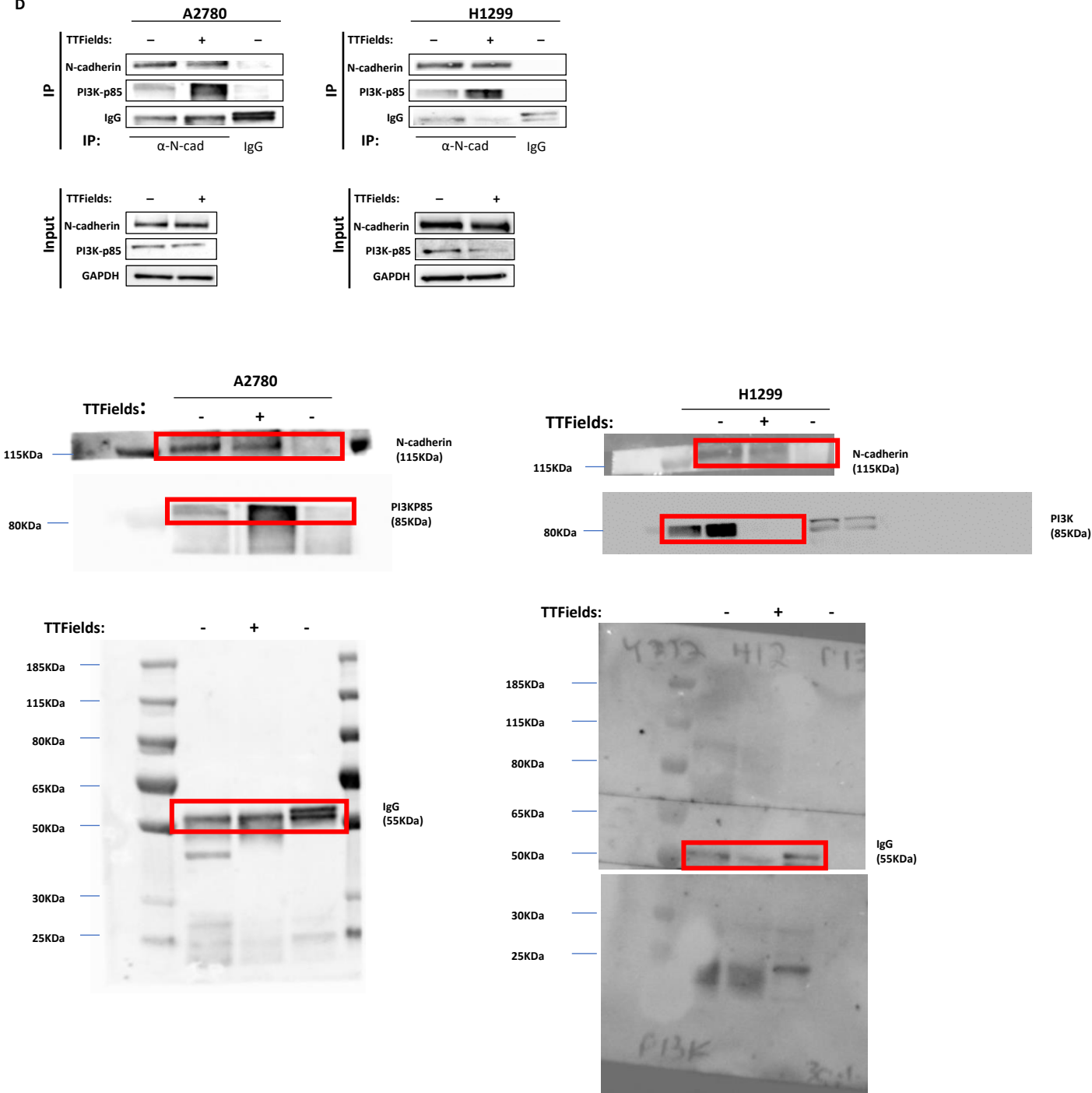


Figure 3

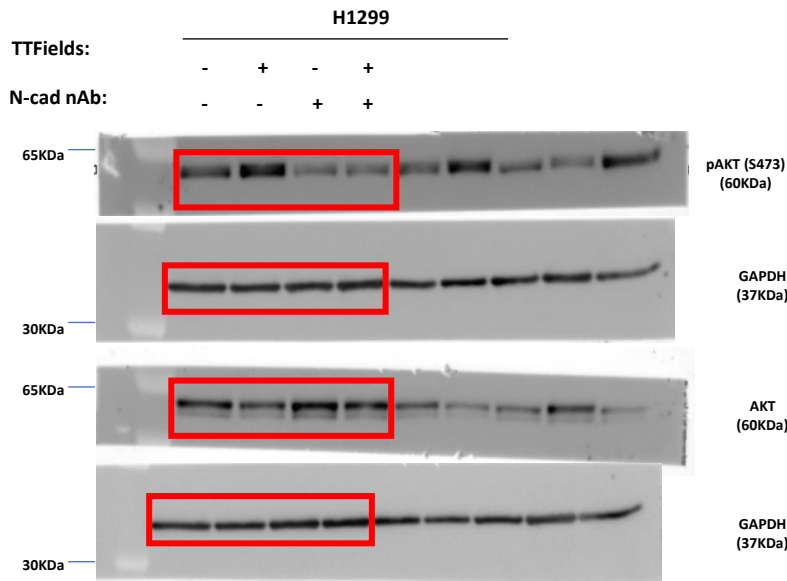
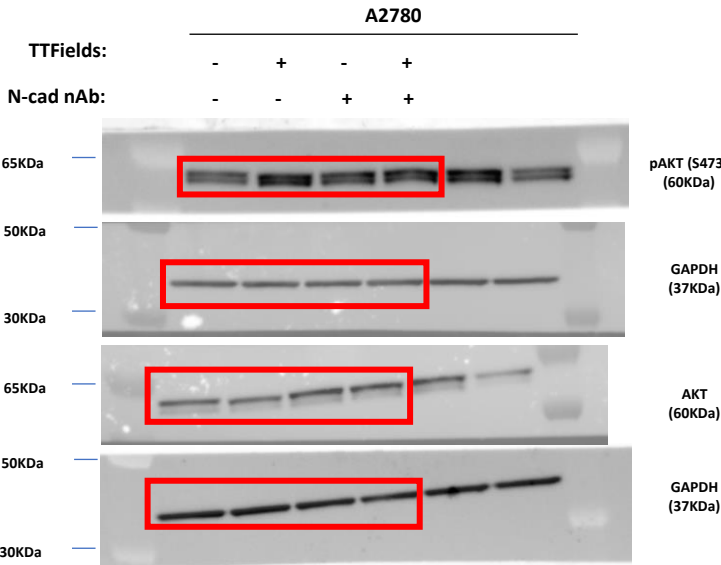
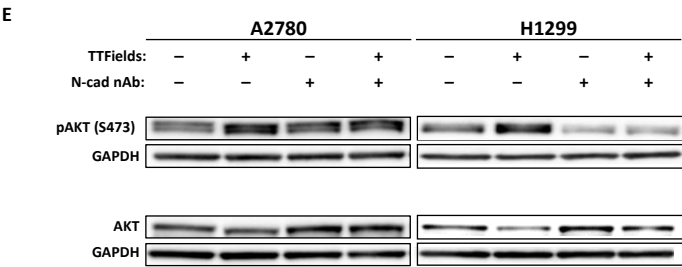


Figure 4

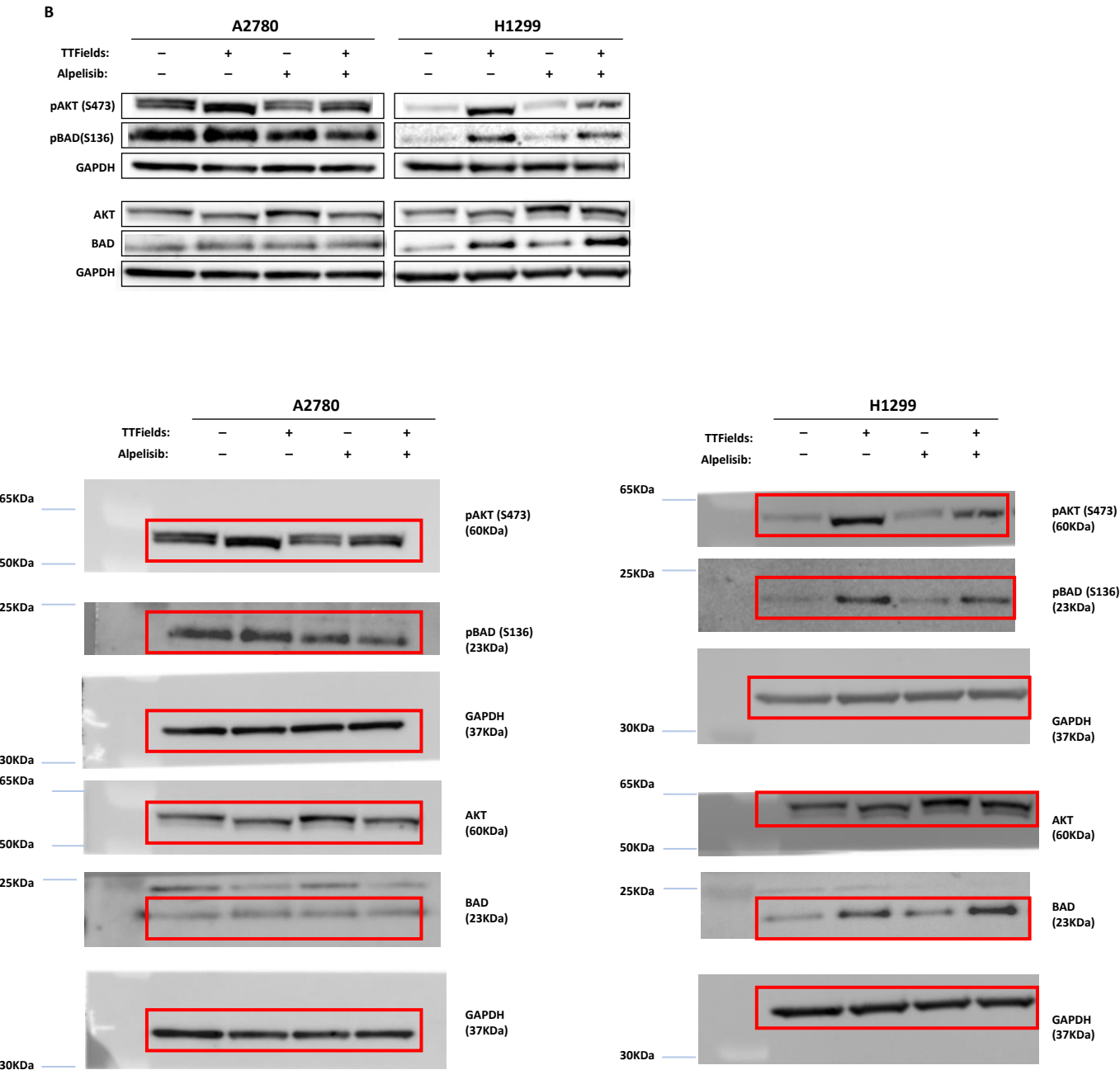


Figure S3

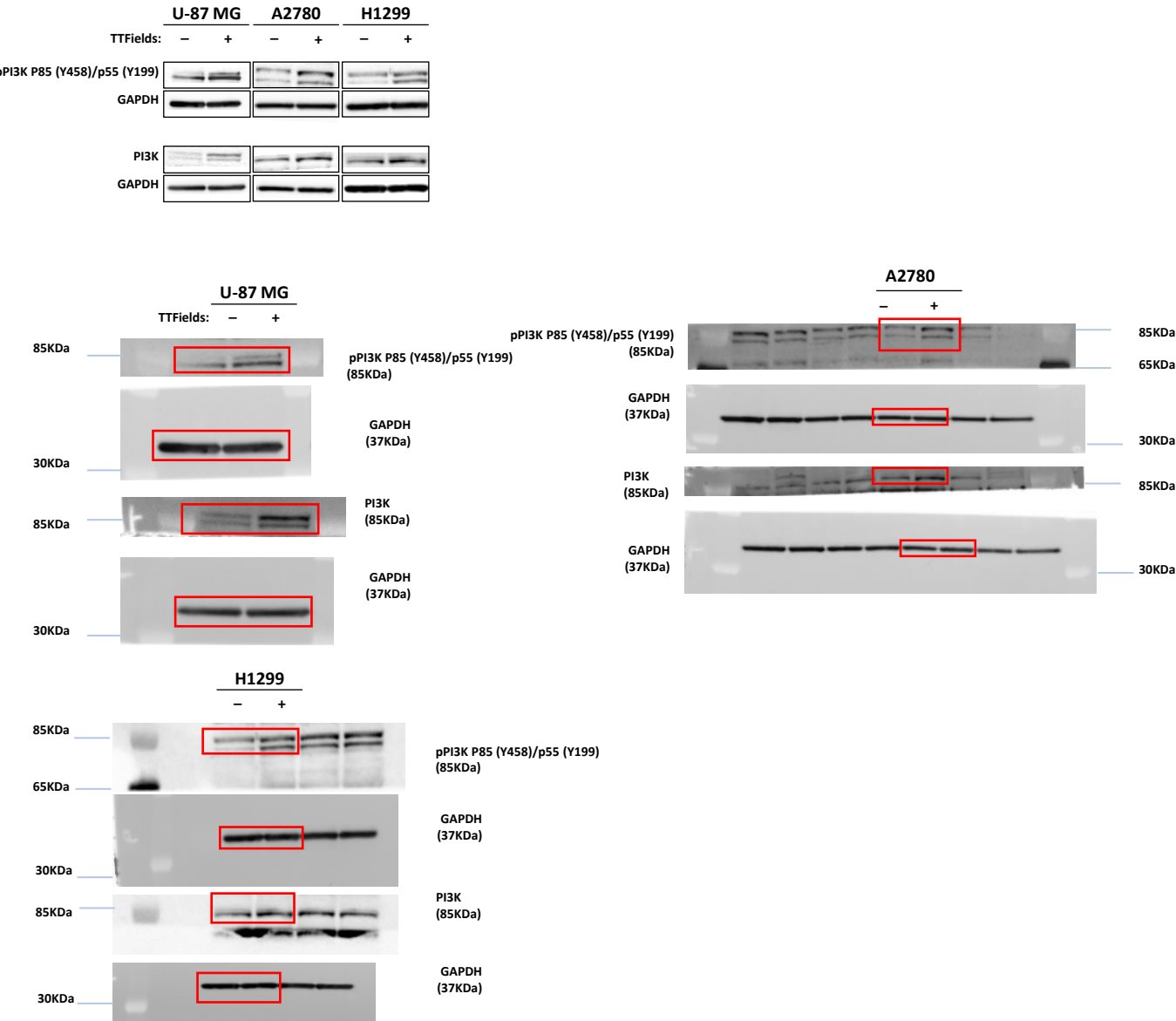


Figure S4

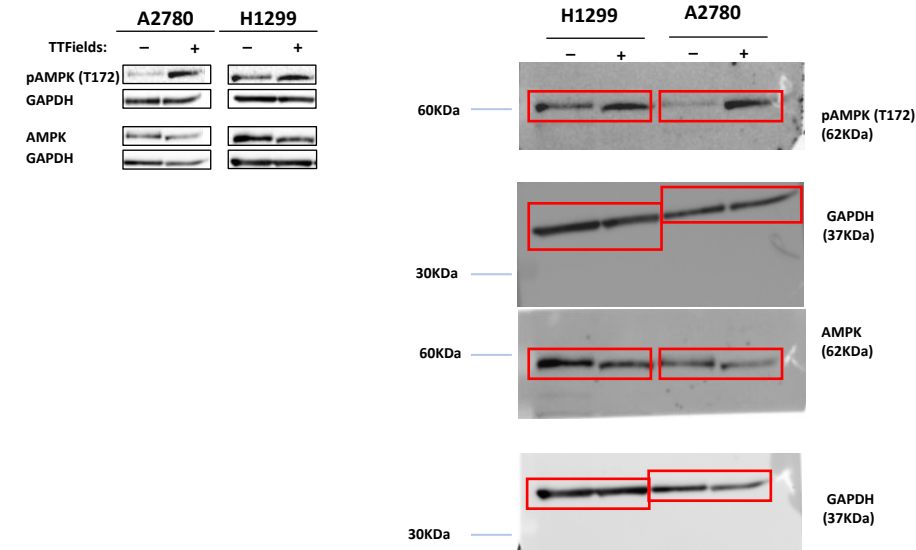
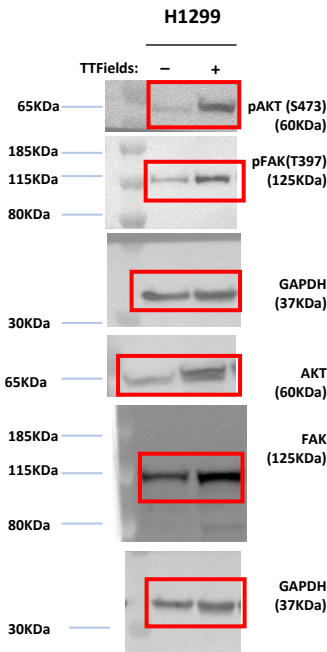
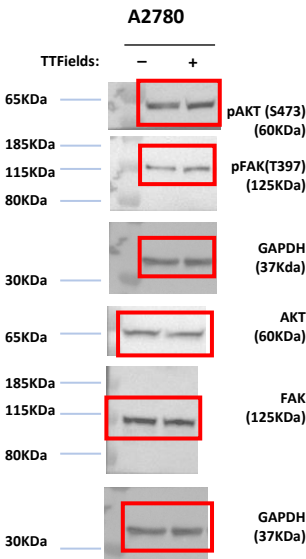
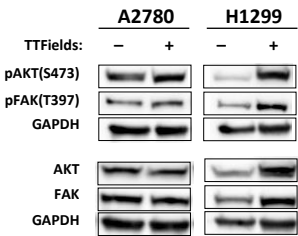


Figure S5

A



D

