Supplementary Fig.S1

Fig. 1A WB quantification

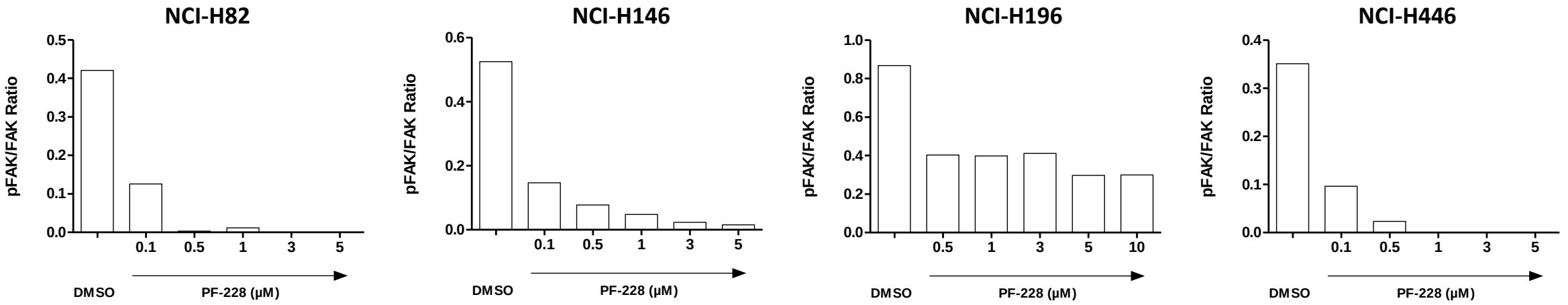
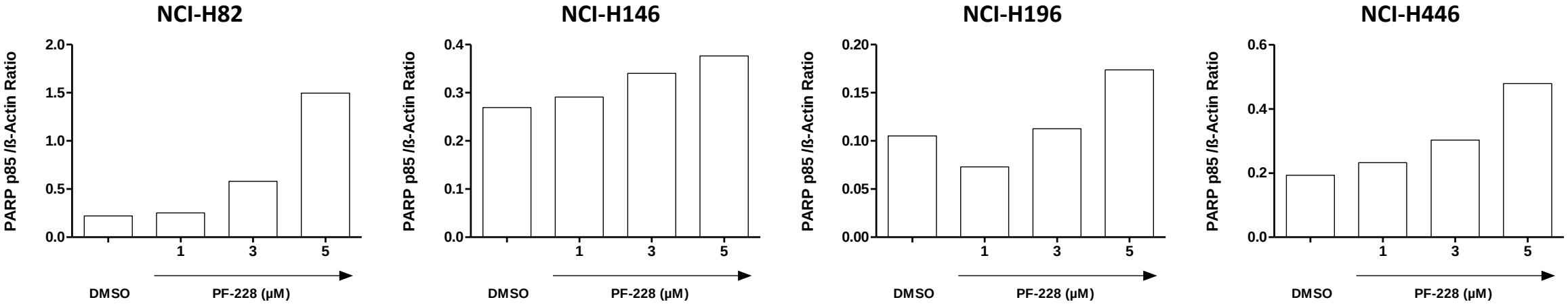


Fig. 2A WB quantification



Supplementary Fig.S1

Fig. 4A WB quantification

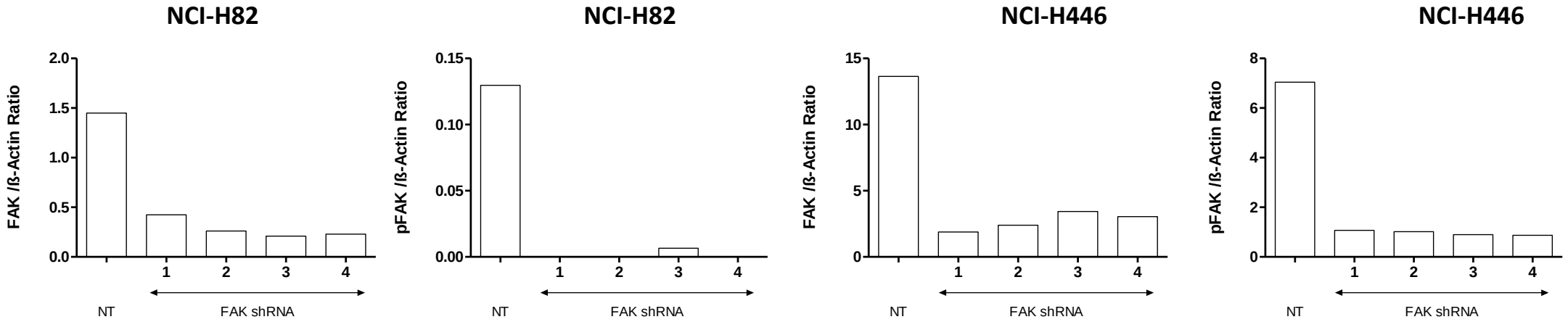
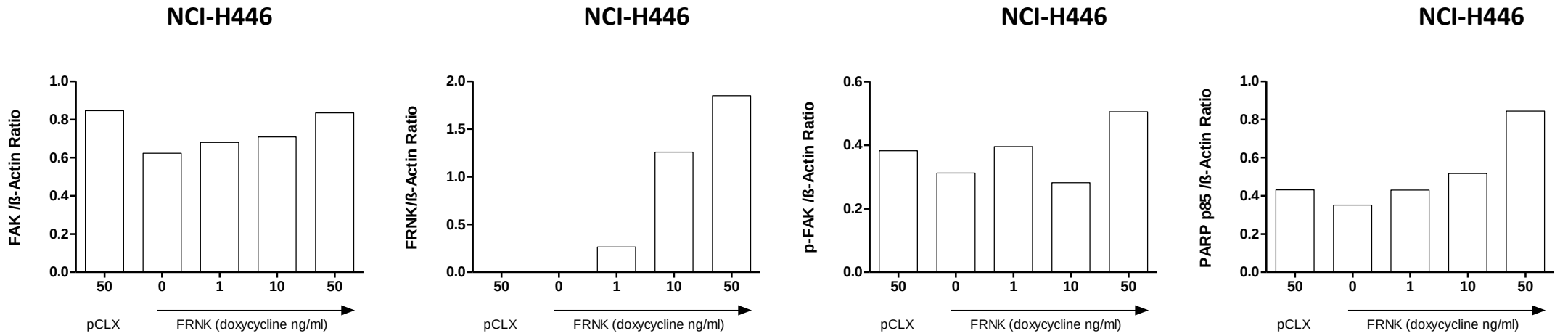
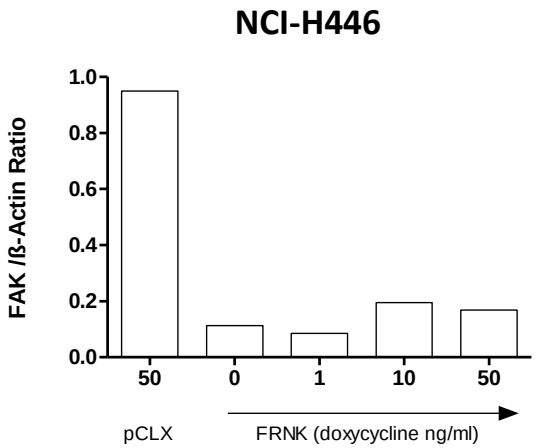


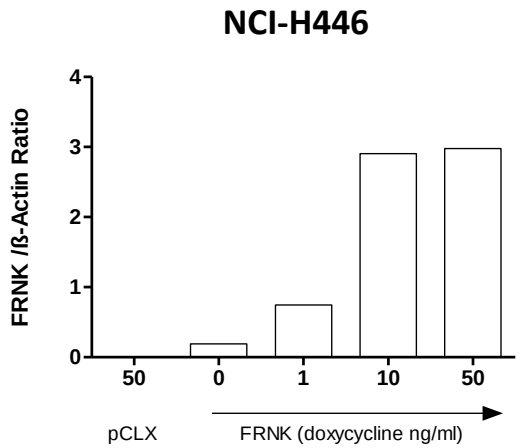
Fig. 4B WB quantification

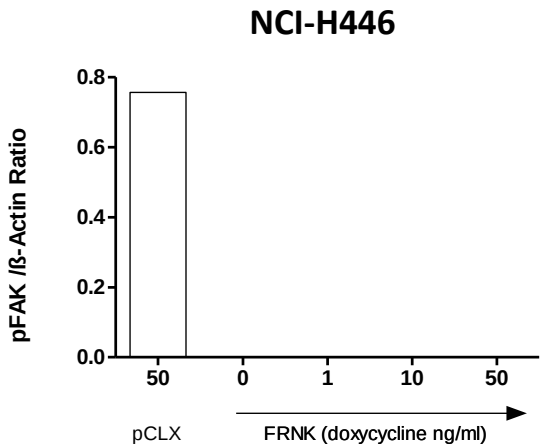


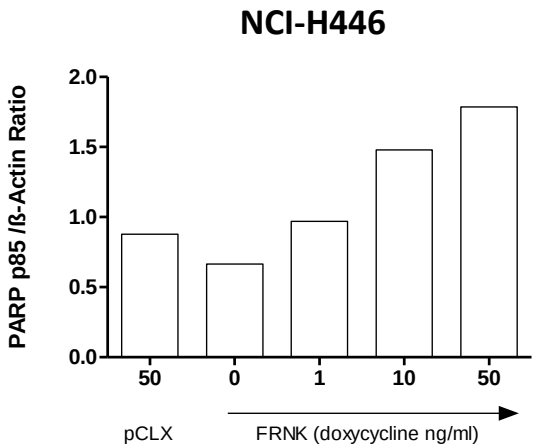
Supplementary Fig.S1

Fig. 5A WB quantification







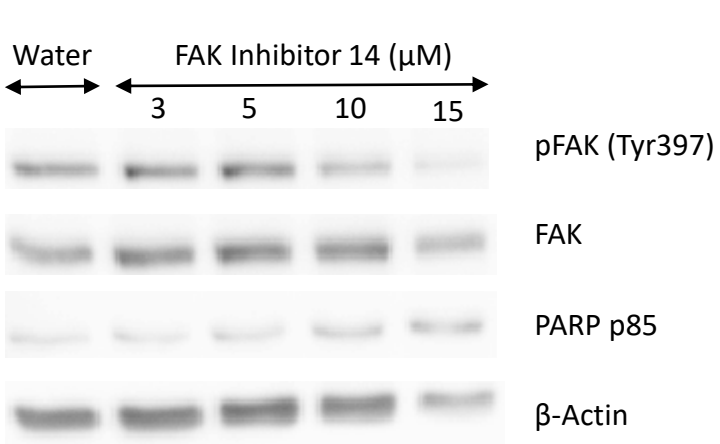


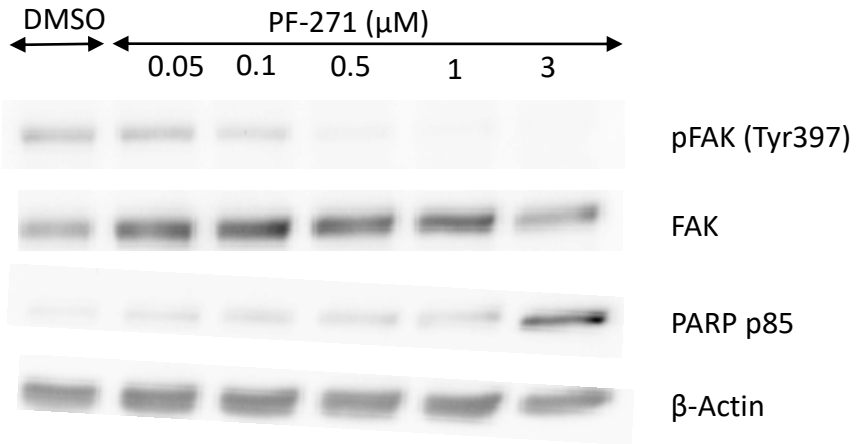
Supplementary Figure S1: Western blot (WB) densitometric quantifications: Densitometric quantification of the WB displayed in Figures 1A, 2A, 4A, 4B, and 5A was performed using the Quantity One analysis software (Bio-Rad). The Y-axis represents the level of protein of interest normalized to β-Actin. The X-axis represents experimental conditions. These graphs confirm the conclusions drawn in the manuscript based on the WB pictures.

Supplementary Fig.S2

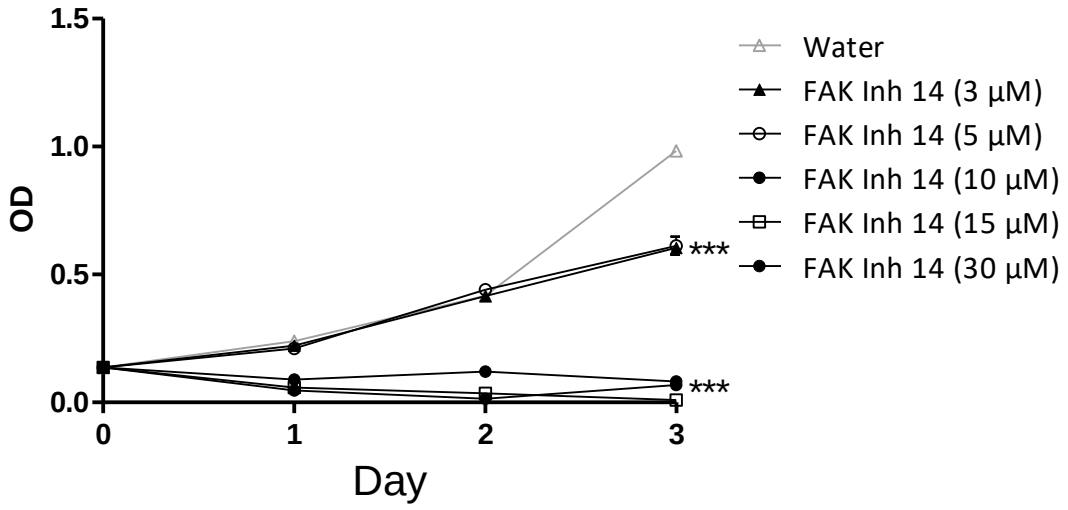
NCI-H446

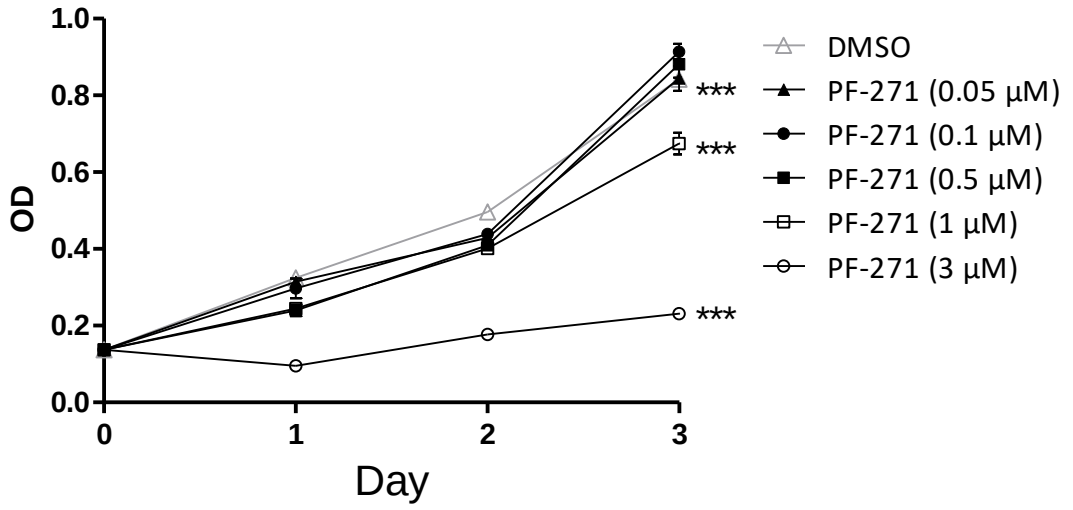
A





B





Supplementary Figure S2: FAK Inhibitor 14 (Inh14) and PF-562,271 (PF-271)'s effects on FAK expression/activity, cell proliferation, and apoptosis in NCI-H446 SCLC cell lines:

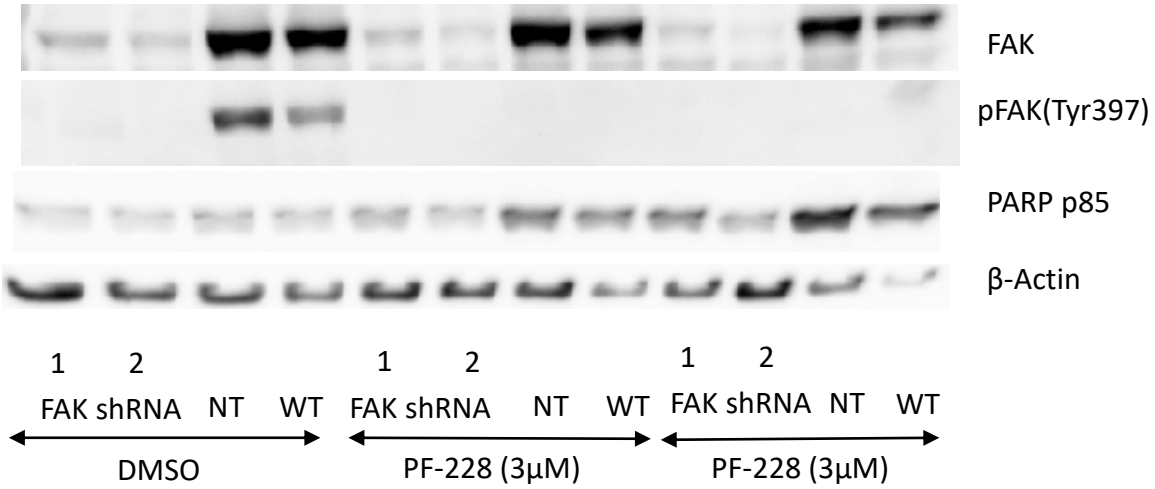
A. FAK expression and phosphorylation and PARP p85 expression evaluation by Western blot (WB). Whole cell lysates from NCI-H446 SCLC cell lines treated with Inh14, PF-271, or control for 60 min. (except 48h for evaluation of apoptosis via PARP p85 expression) were resolved by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blots were incubated with antibodies against total FAK (125 kD), phospho-FAK (Tyr397) (125 kD), PARP p85 (85 kD), and β-Actin (45 kD) for normalization. Dose-dependent inhibition of FAK phosphorylation (Tyr397) and dose-dependent increase of PARP p85 expression, marker of apoptosis, was observed by WB in cell lines treated with Inh 14 or PF-271 as compared to those treated with control, while total FAK expression was not modified.

B. Cell proliferation evaluation by WST-1 assay. NCI-H446 SCLC cell lines were cultured for three days in presence of Inh14, PF-271, or control. Dose-dependent inhibition of proliferation was observed by WST-1 assay in cells treated with Inh14 or PF-271 as compared to those treated with control. Optical density (OD) in Y-axis reflects the proportion of metabolically active cells. Error bars represent mean +/- standard deviation (SD) (n=3). All the graphs represent one of three independent experiments with similar results. *** $P \leq 0.001$.

Supplementary Fig.S3

NCI-H82

A



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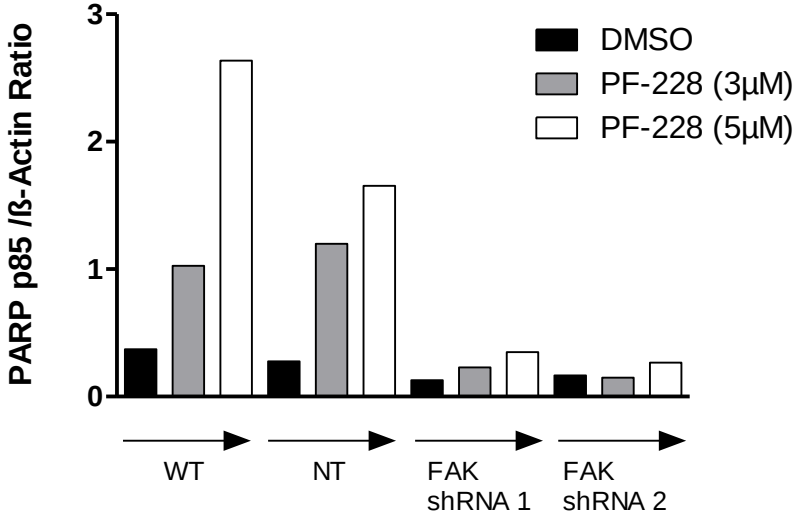
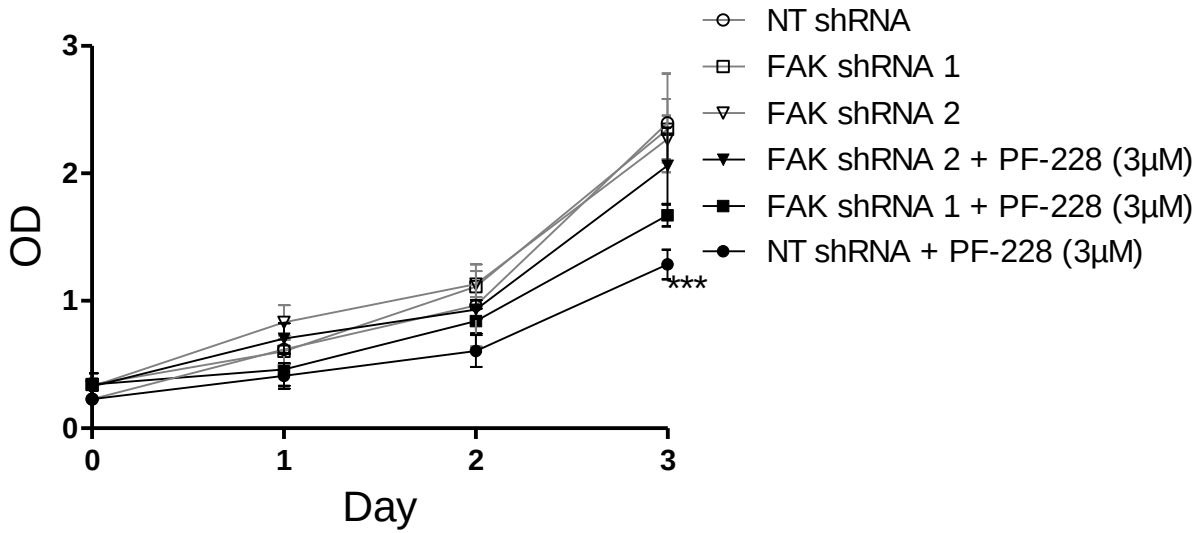


C



Supplementary Figure S3: PF-573,228 (PF-228)'s effects on FAK expression/activity, cell proliferation, and apoptosis in NCI-H82 SCLC cell lines transduced with FAK shRNA:

NCI-H82 SCLC cell lines were stably transduced with FAK shRNA or no-target (NT) shRNA as control, and submitted to puromycin selection for two weeks.

A. FAK expression/activation and PARP p85 expression evaluation by Western blot (WB). Whole cell lysates from NCI-H82 SCLC cell lines transduced with FAK or NT shRNA and treated with PF-228 or DMSO control for 90 min. (except 48h for evaluation of apoptosis via PARP p85 expression) were resolved by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blots were incubated with antibodies against total FAK (125 kD), phospho-FAK (Tyr397) (125 kD), PARP p85 (85 kD), and β-Actin (45 kD) for normalization. Total FAK expression decreased in cell lines transduced with FAK shRNA but not in those transduced with NT shRNA, as compared to wild-type (WT) cell lines, independently of PF-228 treatment. Phospho-FAK expression was absent in cell lines transduced with FAK shRNA and those treated with PF-228, while it was expressed in cell lines transduced with NT shRNA and not treated with PF-228. PARP p85 expression, marker of apoptosis, significantly increased in cell

lines transduced with NT shRNA and treated with PF-228 but not in those transduced with FAK shRNA and treated with PF-228.

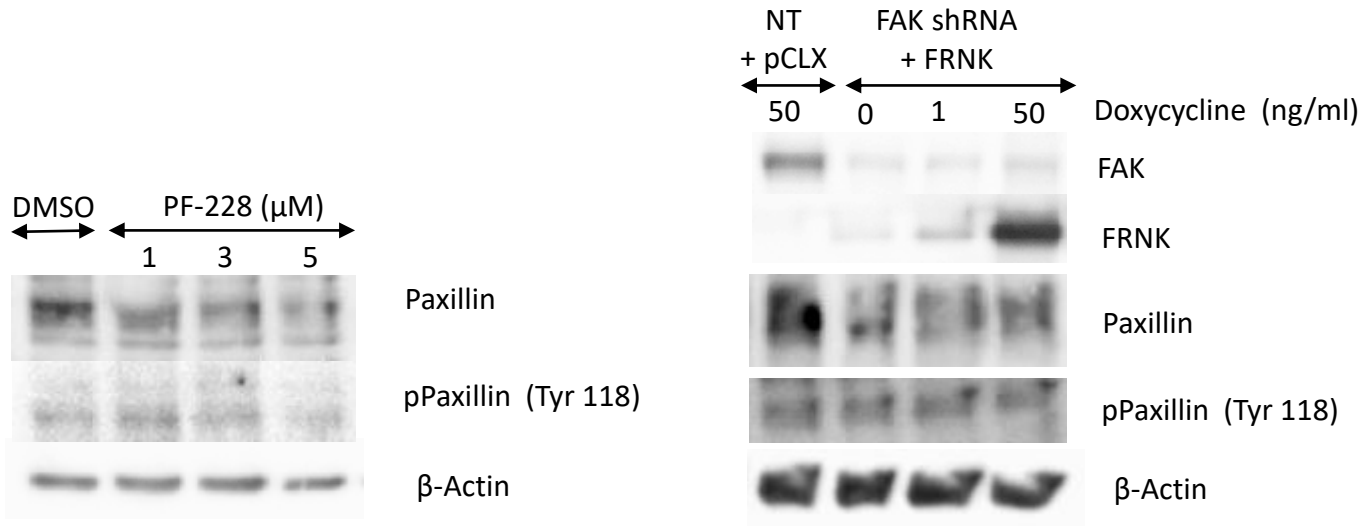
B. WB densitometric quantification of PARP p85 expression. Densitometric quantification of PARP p85 expression on the WB displayed in Supplementary Fig.S3A was performed using the Quantity One analysis software (Bio-Rad). The Y-axis represents the level of each protein of interest normalized to β-Actin. The X-axis represents experimental conditions This graph confirms the observations described in Fig.S3A.

C. Cell proliferation evaluation by WST-1 assay. NCI-H82 SCLC cell lines transduced with FAK or NT shRNA were cultured for three days in presence of PF-228 or DMSO control. A significantly less important inhibition of proliferation was observed after PF-228 treatment in cell lines transduced with FAK shrRNA as compared with those transduced with NT shRNA. Optical density (OD) in Y-axis reflects the proportion of metabolically active cells. Error bars represent mean +/- standard deviation (SD) (n=3). All the graphs represent one of three independent experiments with similar results. *** $P \leq 0.001$.

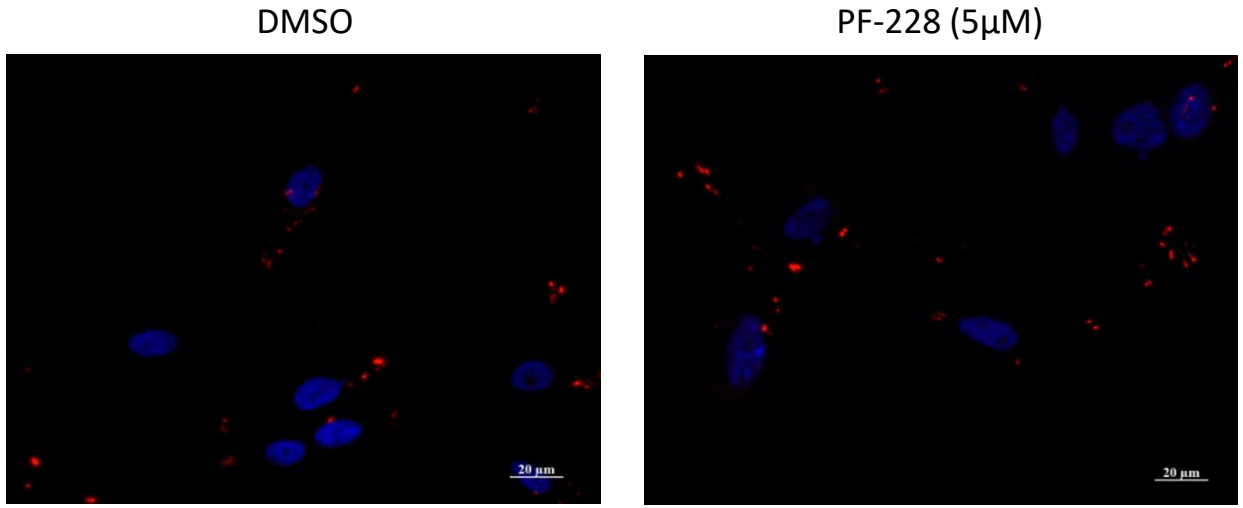
Supplementary Fig.S4

NCI-H446

A



B



- **pPaxillin (Tyr 118) = Alexa555**
- **Nucleus = DAPI**

Supplementary Figure S4: phospho-Paxillin expression in NCI-H446 SCLC cell line where FAK has been inhibited with pharmacological and genetic methods:

A. Phospho-Paxillin expression evaluation by WB. FAK was inhibited in NCI-H446 cell lines with PF-228 for 90 min. or a genetic method. In this last one, cell lines were first stably transduced with FAK shRNA or no-target (NT) shRNA and then stably transduced with doxycycline-inducible FRNK-expression plasmid or pCLX empty vector as control. After transduction, they were submitted to puromycin and blasticidin-selection for two weeks. Finally, they were treated with doxycycline for 48h before the experiments. Whole cell lysates from SCLC cell lines were resolved with SDS-PAGE and blots were incubated with antibodies against total FAK (125 kD), FRNK (41 kD), total Paxillin (68 kD), phospho-Paxillin (68 kD), and β-Actin (45 kD) for normalization. FAK inhibition, either with PF-228 or double-transduction with FAK shRNA and FRNK, did not modify phospho-Paxillin (Tyr118) expression.

B. Phospho-Paxillin expression evaluation by immunofluorescence (IF).

NCI-H446 cell lines treated with PF-228 5µM or DMSO were formol-fixed, stained with an antibody against phospho-Paxillin (Tyr-118) (1/1000, rabbit polyclonal; Cell Signaling Technology), followed by an anti-rabbit polyHRP secondary antibody, and revealed with tyramide Alexa555 (red). Nuclei were stained with DAPI (blue). FAK inhibition with PF-228 did not modify phospho-Paxillin (Tyr118) expression.

Supplementary Video1: PF-228's effect on NCI-446 SCLC cell line migration.

NCI-H446 cell lines were grown to confluence in 12-well plates. Cell monolayers were wounded using a micropipette tip and floating cells were washed off with PBS. After overnight incubation, PF-228 at a concentration of 3µM or DMSO was added to culture medium. Cell movements within wounded area were monitored for 12h starting from the time drug was added using a Zeiss Axiovert 200M microscope (Zeiss, Thornwood, NY) at x200 magnification. Images were captured every 15-minutes from five different fields randomly selected in each well. They show that PF-228 decreased migration velocity in NCI-H446 cell lines.