

Supplementary Results

Standalone FUS Fails to Downregulate NF- κ B and Akt in CRPC Cells

According to western blot analysis, the C4-2B cells constitutively expressed NF- κ B but not Akt (untreated control group, Fig. S1AC), while the more aggressive DU-145 cells expressed both of these transcription factors (Fig. S1BC). Furthermore, the DU-145 cells had significantly higher ($p < 0.05$) expression of NF- κ B (0.21 ± 0.06 per GAPDH) than the C4-2B cells (0.05 ± 0.01 ; Fig. S1C, left). Standalone FUS did not decrease the expression of NF- κ B and Akt in the surviving C4-2B cells (0.96 ± 0.30 and 1.1 ± 0.93 of the untreated control level; Fig. S1D, left) and had no effect on NF κ B expression in the surviving DU-145 cells (1.1 ± 0.28). A significant ($p < 0.001$) increase in Akt expression was observed in the DU-145 cells (3.4 ± 0.52).

The expression of phosphorylated (active) NF- κ B was slightly stronger in the DU-145 cells than that in the C4-2B cells (0.17 ± 0.03 vs. 0.14 ± 0.05 ; Fig. S1C, right). Standalone FUS at level H4 did not diminish NF- κ B signaling in the DU-145 cells (1.0 ± 0.31) and, in fact, it increased NF- κ B activity in the C4-2B cells (2.5 ± 1.3 ; Fig. S1D, right) and enhanced the activity of Akt in the DU-145 cells (1.8 ± 0.65). These data indicate that FUS enables survival and rapid recurrence of PCa cells due to the increased expression and activity of NF κ B and Akt pro-survival signaling pathways.

Supplementary Figures

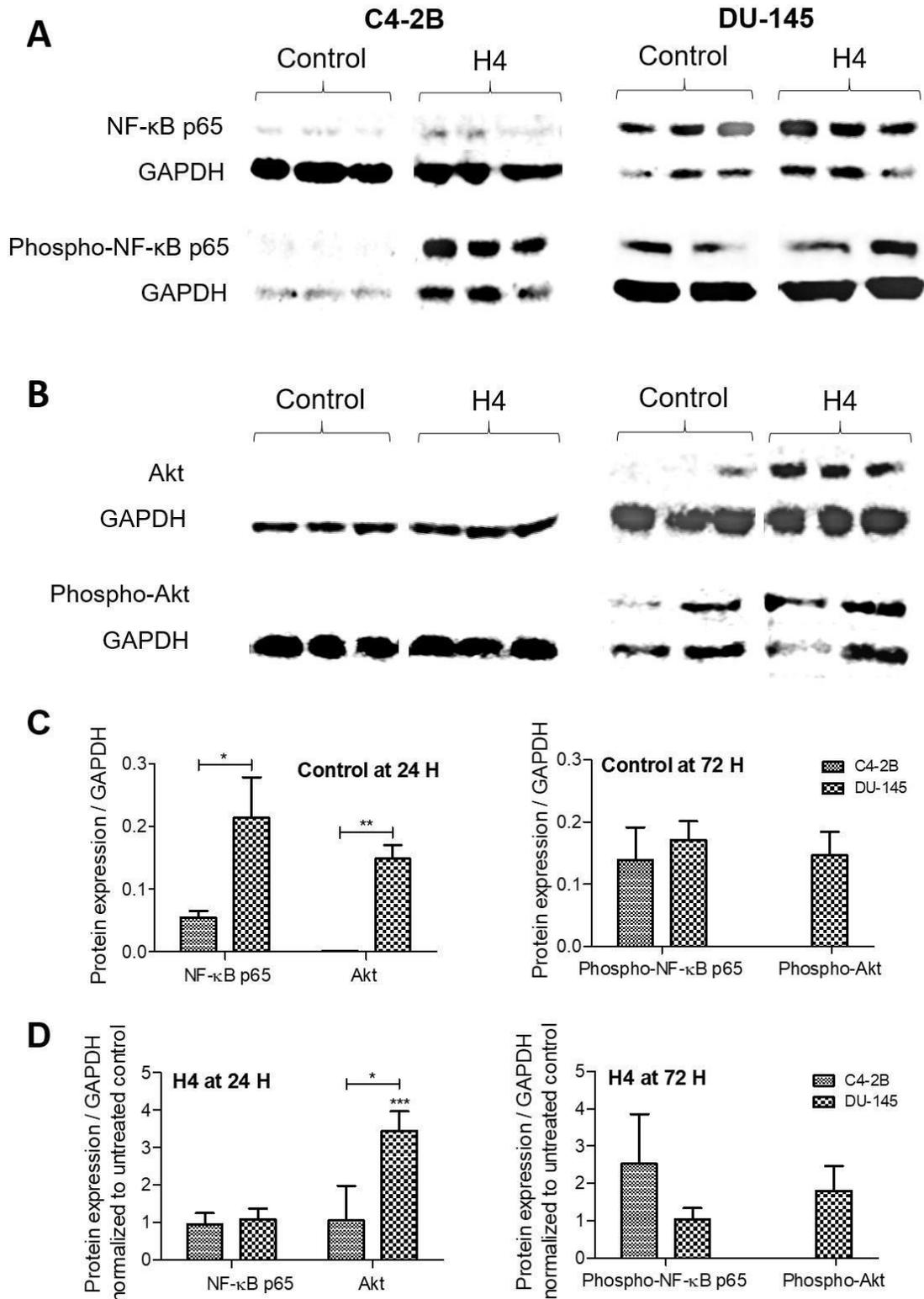


Figure S1. Standalone FUS at safe physiologic doses fails to downregulate the expression and activity of pro-survival markers NF-κB and Akt in CRPC cells. **A** and **B**, Shown are representative western blot images of the expression of total and phosphorylated NF-κB p65 (**A**) and Akt (**B**) in C4-2B and DU-145 cells, exposed or not to FUS at level H4. **C**, Expression of NF-κB p65 and Akt (left) and their phosphorylated forms (right) per GAPDH level for untreated C4-2B and DU-145 cells. **D**, Expression of NF-κB p65 and Akt (left) and their phosphorylated forms (right) per GAPDH and untreated control group levels for C4-2B and DU-145 cells treated by FUS at level H4. Total protein

expression data were collected at 24 h post-treatment, while phosphorylated protein expression was measured at 72 h. Values are mean \pm SEM of 3-6 independent tests. * $p < 0.05$. ** $p < 0.01$, *** $p < 0.001$.