Supplementary Data

Dual specificity phosphatase 1 expression inversely correlates with NF-κB activity and expression in prostate cancer and promotes apoptosis through a p38 MAPK dependent mechanism

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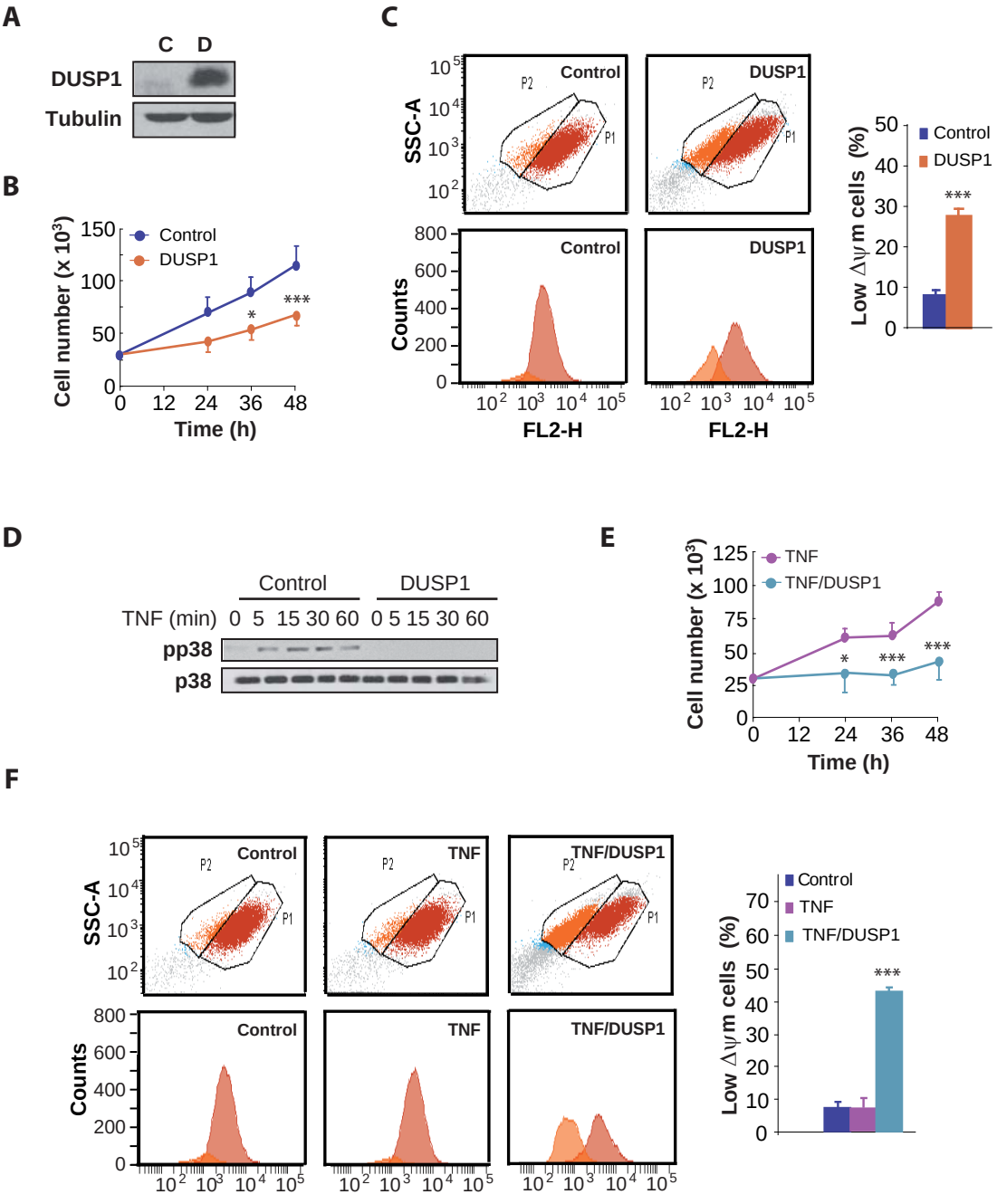
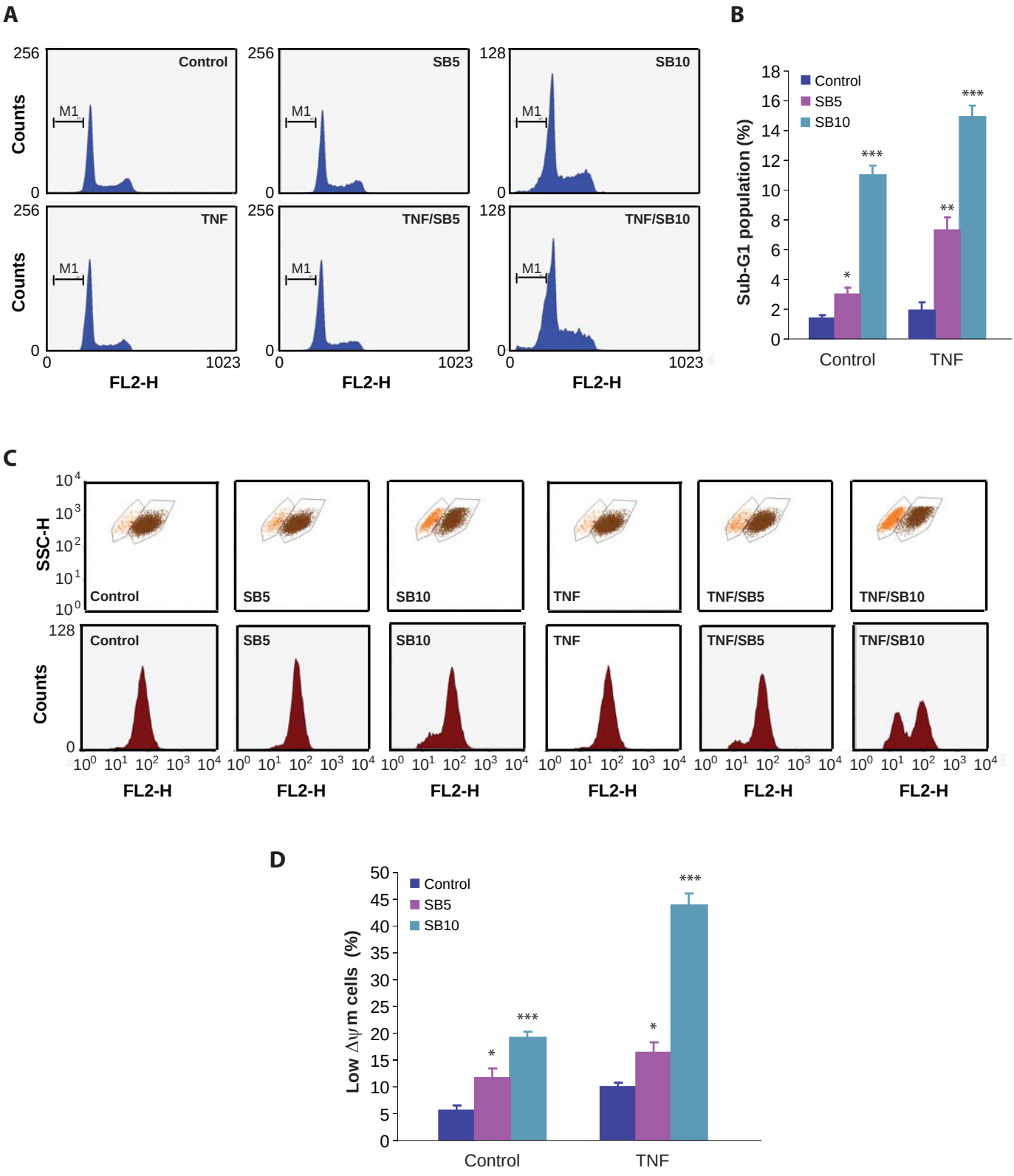
Supplementary data includes 4 Supplementary Figures

Supplementary Figure S1 Gil-Araujo *et al.*



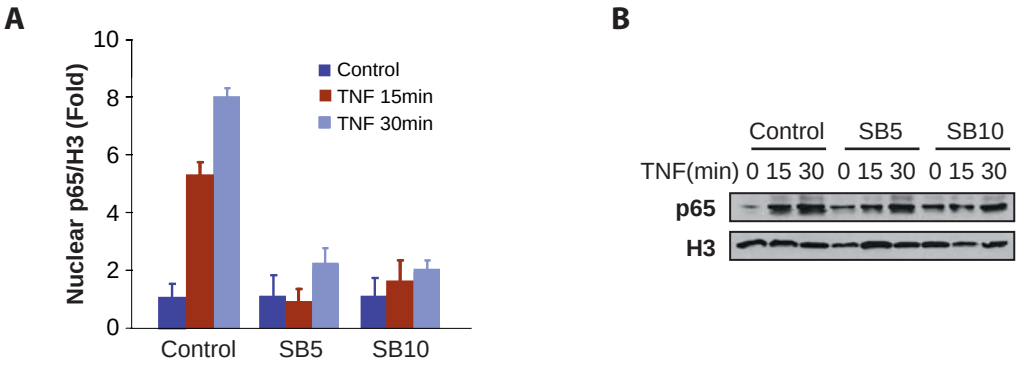
Supplementary Figure S1 – DUSP1 induces apoptosis in PC3 cells. (A) Cells were transiently transfected for 48 h with a control vector (C) or a vector encoding DUSP1 (D), and total cellular protein lysates were analysed by western blotting with antibodies against DUSP1 and tubulin, to assess equal loading protein. **(B)** Cells were transfected for the indicated times as in *(A)*, and counted. The asterisks show the statistical significance of differences between the groups: DUSP1 *vs.* control. **(C)** Cells were transfected for 48 h as in *(A)*, and apoptosis was determined by the TMRM assay. **(D)** Cells were transfected for 48 h as in *(A)*, incubated with TNF-α (10 ng/ml) for the indicated times and total cell lysates were analysed by western blotting with antibodies against both phosphorylated and total p38 MAPK. **(E)** Cells were transfected for the indicated times as in *(A)*, incubated in the absence or presence of TNF-α, and counted for the indicated times. The asterisks show the statistical significance of differences between the groups: DUSP1 plus TNF-α *vs.* TNF-α. **(F)** Cells were transfected as in *(A)*, incubated with TNF-α for 48 h, and apoptosis was determined by the TMRM assay.

Supplementary Figure S2 Gil-Araujo *et al.*



Supplementary Figure S2 - Effect of SB202190 inhibitor on cellular apoptosis. DU145 cells were incubated for 48 h with SB202190 (5 or 10 μM) in the absence or presence of TNF-α (10 ng/ml). **(A-B)** Measurement of sub-G1 population by PI staining and flow cytometry. The graph shows the quantification of the results from two independent experiments performed in duplicate. **(C-D)** Measurement of population with low ΔΨm by the TMRM assay. The graph shows the quantification of the results from two independent experiments performed in duplicate.

Supplementary Figure S3 Gil-Araujo *et al.*



Supplementary Figure S3 - Effect of SB202190 inhibitor on p65/NF-κB nuclear translocation. DU145 cells were incubated for 48 h with SB202190 (5 or 10 μM), and treated for the indicated times in the absence or presence of TNF-α (10 ng/ml). Nuclear extracts were analysed by western blotting using antibodies against p65. The levels of histone H3 were determined as control to validate the integrity of the nuclear fractions. **(A)** The graph shows the quantification of the ratio of nuclear p65/histone H3 levels from two independent experiments with similar results. **(B)** Representative immunoblot showing nuclear p65 and H3 levels.

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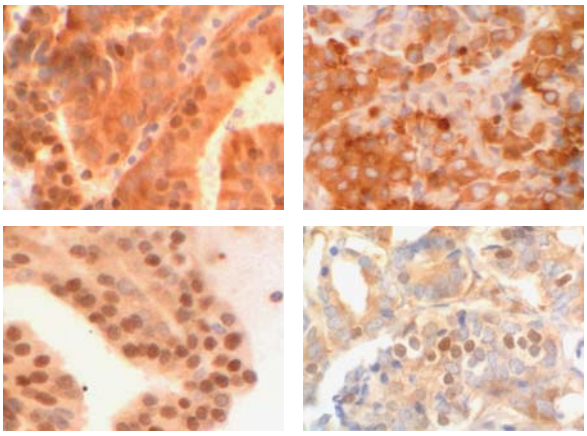

Supplementary Figure S4 – Inmunohistochemical analysis of p65 expression levels from human prostate cancer specimens. Details of p65 nuclear localization in samples from advanced disease.