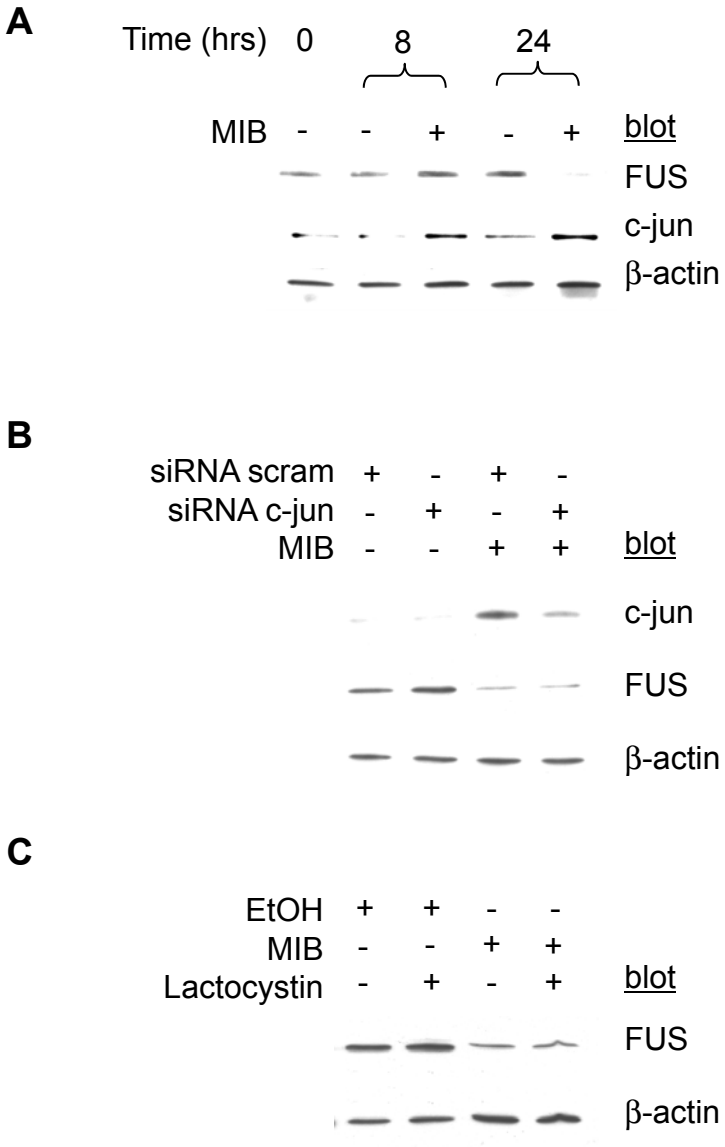
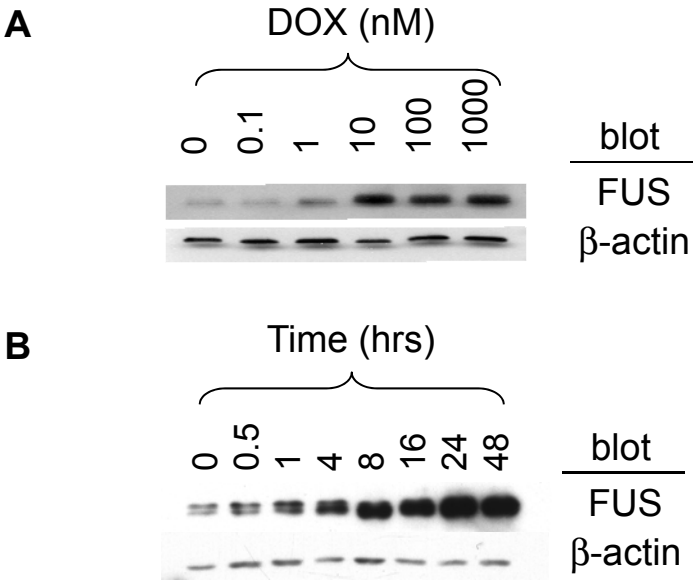
Brooke et al. Supplemental Figure 1



Supplemental Figure 1. The down-regulation of FUS in response to androgen is independent of c-jun and proteasomal degradation. (A) LNCaP cells were treated with MIB (mibolerone) for 8 and 24 hours. Cells were harvested and c-jun and FUS levels investigated using immunoblotting. **(B)** LNCaP cells were transfected with an siRNA pool to target c-jun. Cells were incubated for 72hours before ligand was added and the cells left for an additional 48 hours. **(C)** LNCaP cells were treated with mibolerone for 32 hours, 10μM Lactocystin added and cells incubated for a further 16 hours.

Brooke et al. Supplemental Figure 2



Supplemental Figure 2. Over-expression of FUS in the LNCaP-FUS line. Western blotting of the LNCaP-FUS line to analyze FUS expression in response to **(A)** differing doses of doxycycline (DOX) for 24hrs or **(B)** 10nM DOX for 0-48hrs.

Brooke et al. Supplemental Table 1

A

EtOH

	sub-G1		*G1*		*S*		*G2/M*	
DOX	-	+	-	+	-	+	-	+
4 days	4.5±0.4	4.1±0.2	90.2±0.5	90.5±0.4	1.4±0.2	1.1±0.1	4.0±0.2	4.2±0.1
8 days	2.9±0.3	3.9±0.5	90.2±0.3	89.5±0.9	1.1±0.0	1.0±0.1	5.8±0.2	5.5±0.4

MIB

	sub-G1		*G1*		*S*		*G2/M*	
DOX	-	+	-	+	-	+	-	+
4 days	1.9±0.2	4.3±0.2	88.3±0.4	88.4±0.2	2.9±0.0	1.4±0.1	7.0±0.2	5.8±0.2
8 days	2.7±1.1	31.1±2.5	78.1±0.3	56.3±2.2	4.7±0.0	3.7±0.3	14.4±1.4	8.8±0.4

B

EtOH

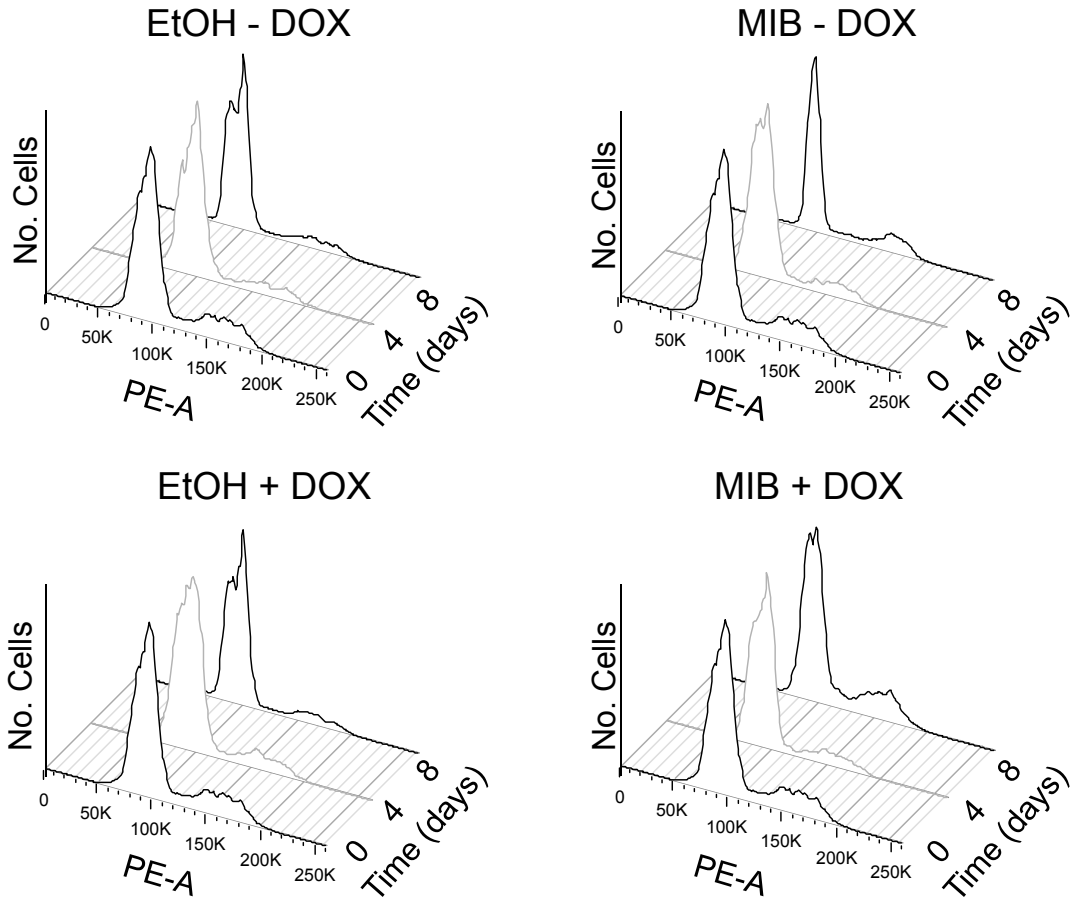
	G1		*S*		*G2/M*	
DOX	-	+	-	+	-	+
4 days	94.4±0.1	94.4±0.2	1.4±0.2	1.2±0.1	4.2±0.3	4.4±0.1
8 days	92.9±0.2	93.2±0.5	1.1±0.0	1.1±0.1	6.0±0.2	5.7±0.4

MIB

	G1		*S*		*G2/M*	
DOX	-	+	-	+	-	+
4 days	89.9±0.3	92.5±0.2	2.9±0.0	1.4±0.1	7.1±0.2	6.1±0.2
8 days	79.2±0.0	81.9±0.6	4.7±0.0	5.3±0.2	16.1±0.1	12.8±0.4

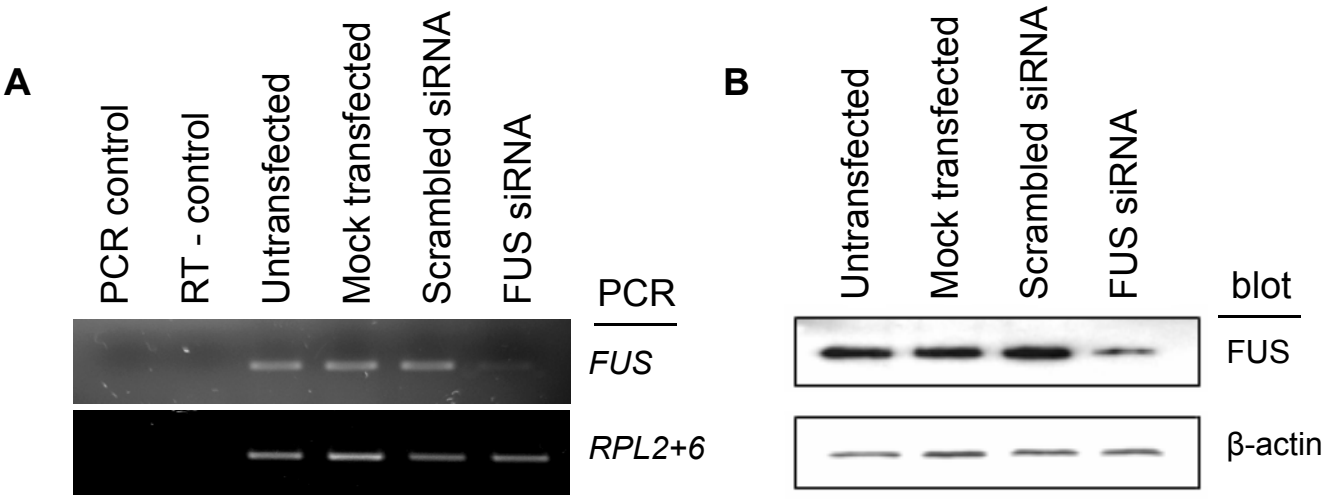
Supplemental Table 1. FUS over-expression results in G1 arrest and an increase in sub-G1. LNCaP-FUS were treated ± doxycycline (DOX) ± mibolerone (MIB) for the indicated times. **(A)** Cells were fixed, propidium iodide stained and cell cycle analyzed using FACs. **(B)** The FACs data was recalculated with the sub-G1 pool removed.

Brooke et al. Supplemental Figure 3



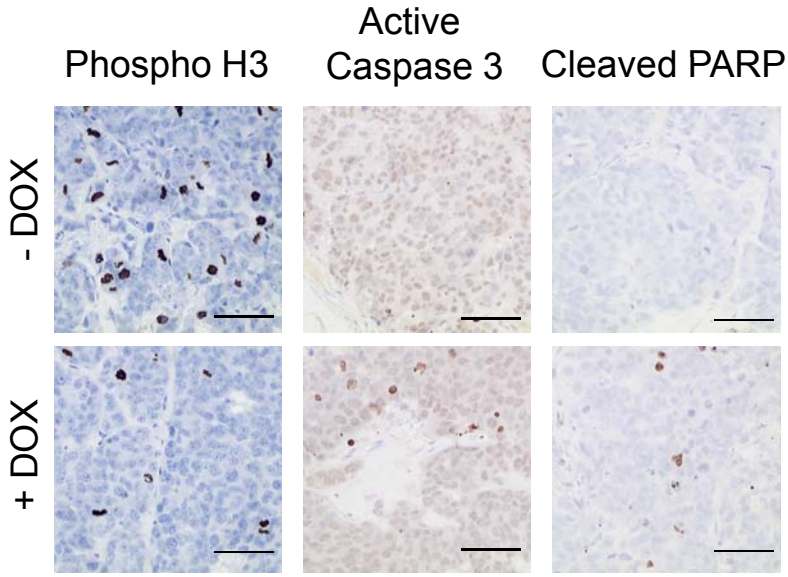
Supplemental Figure 3. Doxycycline has little effect upon the parental LNCaP-TR2 line. LNCaP-TR2 were treated ± doxycycline (DOX) ± mibolerone (MIB) for the indicated times. Cells were fixed, propidium iodide stained and cell cycle analyzed using FACs.

Brooke et al. Supplemental Figure 4



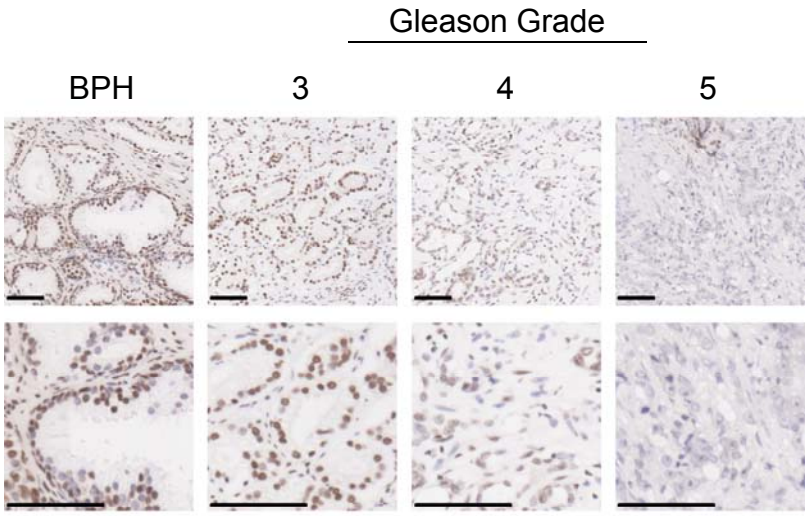
Supplemental Figure 4. FUS levels can be successfully reduced using siRNA. LNCaP cells were either mock, siRNA scrambled or siRNA FUS transfected and left for 3 days. **(A)** RNA was harvested, reverse transcription performed and *FUS* levels analyzed using PCR. **(B)** FUS expression at the protein level was analyzed by immunoblotting.

Brooke et al. Supplemental Figure 5



Supplemental Figure 5. Over-expression of FUS results in a reduction in a marker of proliferation and an increase in apoptotic markers. Xenograft tumours were harvested after 4 days of doxycycline treatment and immunohistochemistry performed to investigate the number of cells expressing phospho histone H3, active Caspase 3 and cleaved PARP. Bars = 100μm.

Brooke et al. Supplemental Figure 6



Supplemental Figure 6. FUS expression is inversely correlated with Gleason grade. Immunohistochemistry was performed upon a prostate cancer tissue microarray to investigate FUS expression in relation to Gleason grade. Bars = 100μm.