Title: p27/Kip1 functions as a tumor suppressor and oncoprotein in osteosarcoma.

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Supplementary dataset File

Supplementary Table S1

AZD1775 (nM)	Gemcitabine (nM)	Fa	CI
100	1.0	0.80	1.49
100	3.2	0.35	0.25
100	6	0.24	0.16
100	30	0.07	0.05
100	100	0.14	0.22
30	6	0.45	0.22
60	6	0.35	0.19
250	6	0.17	0.24
500	6	0.13	0.34

Table S1: Concentrations of AZD1775 and gemcitabine that were used for titrations to determine synergy of agents.

Fa – Fraction affected
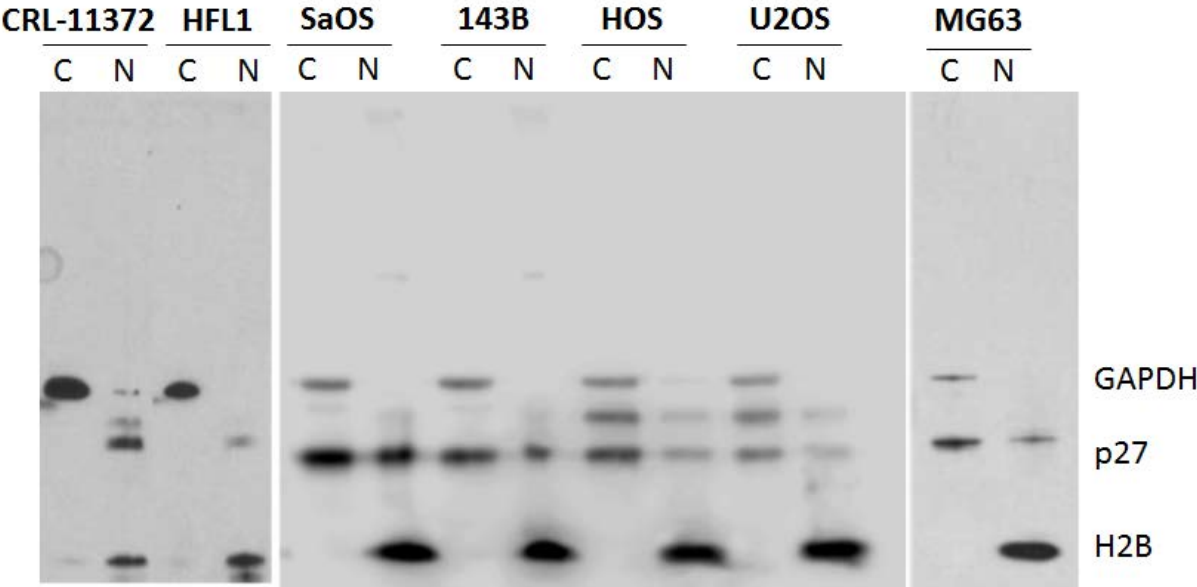
CI – Combination Index

Supplementary Figure S1

Figure S1: Full length immunoblot for Figure 1B.
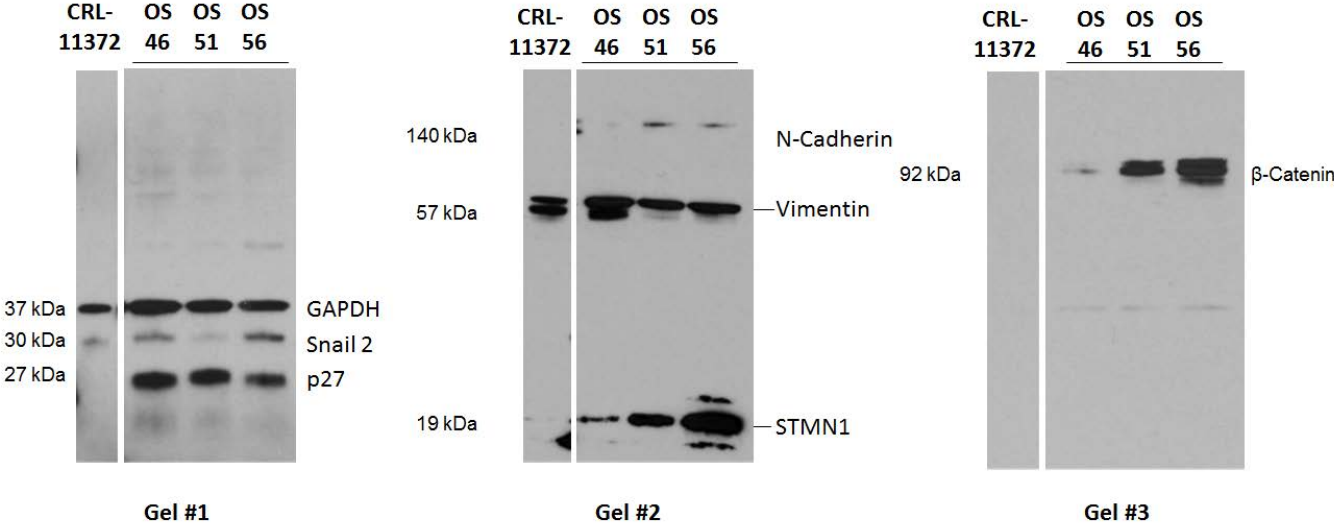
Supplementary Figure S2

Figure S2: Full length immunoblot for Figure 1E.
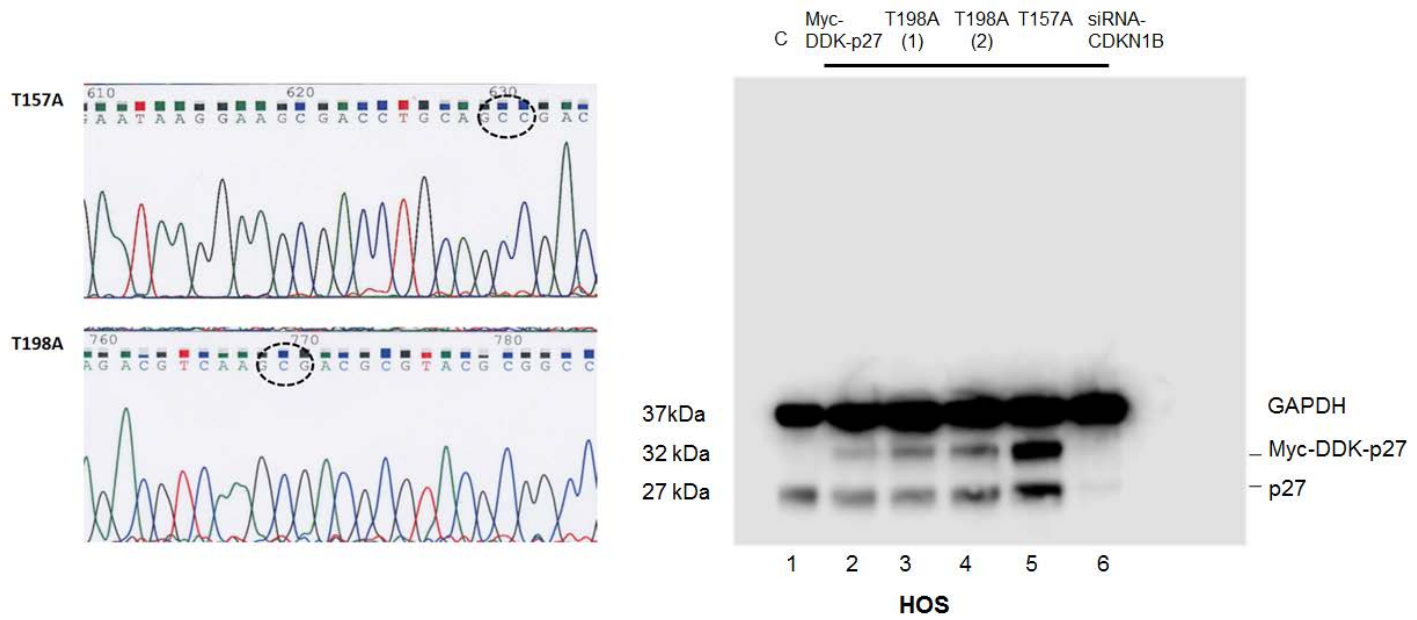
Supplementary Figure S3

Figure S3: Sequencing data for T157 and T198 amino acid mutations (Alanine sequences are shown in dotted black circles) and full length immunoblot for Figure 2C
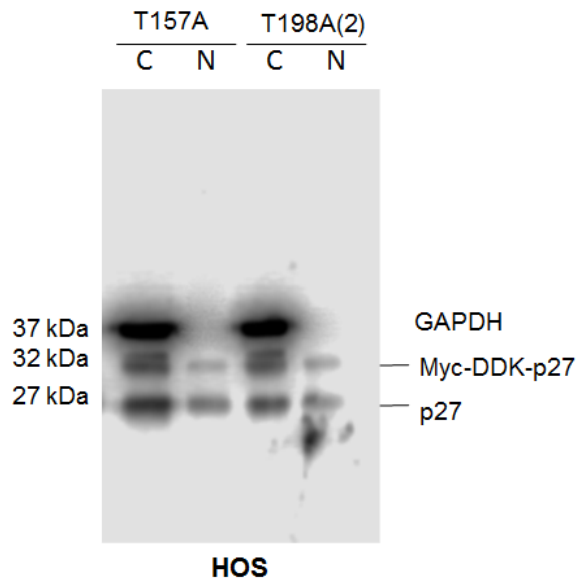
Supplementary Figure S4

Figure S4: Full length immunoblot for Figure 2D.
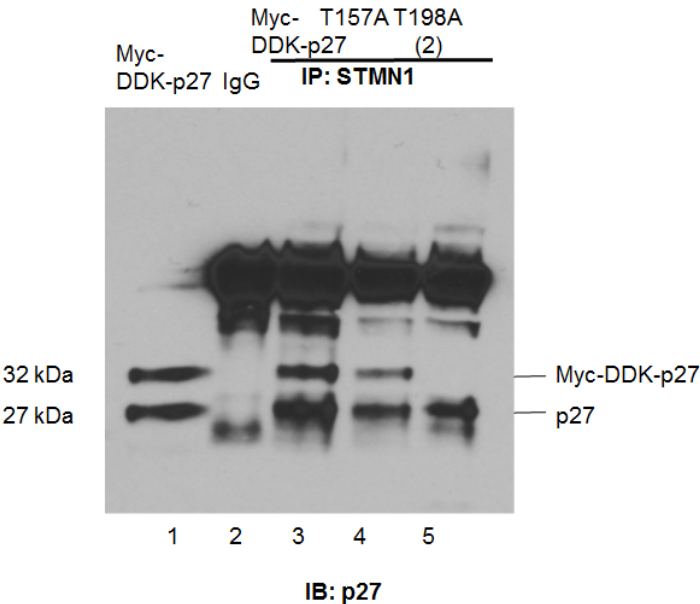
Supplementary Figure S5

Figure S5: Full length immunoblot for Figure 2E.
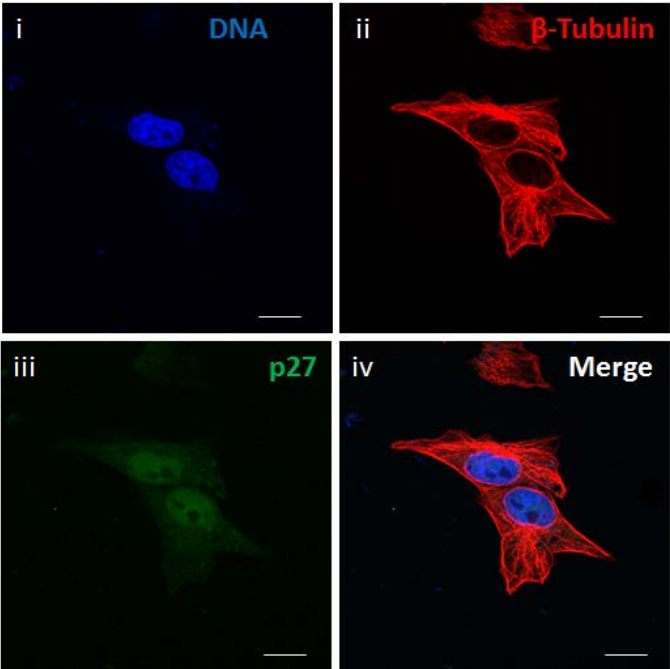
Supplementary Figure S6

Figure S6: Immunofluorescence staining of 143B cells transfected with pCMV- Myc-DDK empty vector control
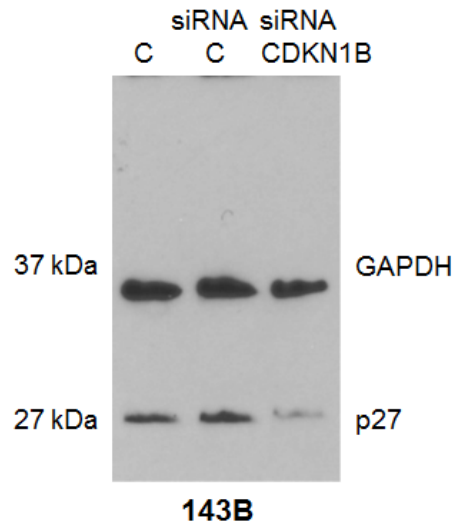
Supplementary Figure S7

Figure S7: Immunoblot analysis for lysates of 143B cells treated with siRNA non-targeting (siRNA C) and siRNA*CDKN1B* oligonucleotides using p27 antibody. GAPDH was loading control.
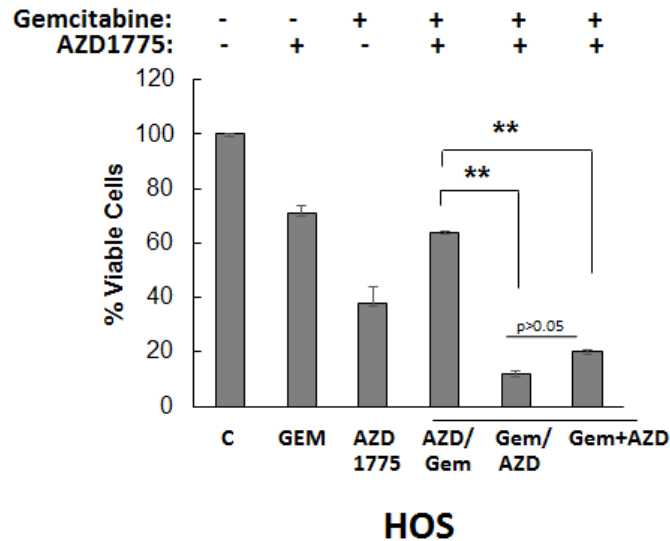
Supplementary Figure S8

Figure S8: HOS cells were incubated with gemcitabine (GEM) and AZD1775 as indicated and cell viability was measured by the cell titer blue assay. Data is shown as mean + SE. * represents $p < 0.05$; n=6.
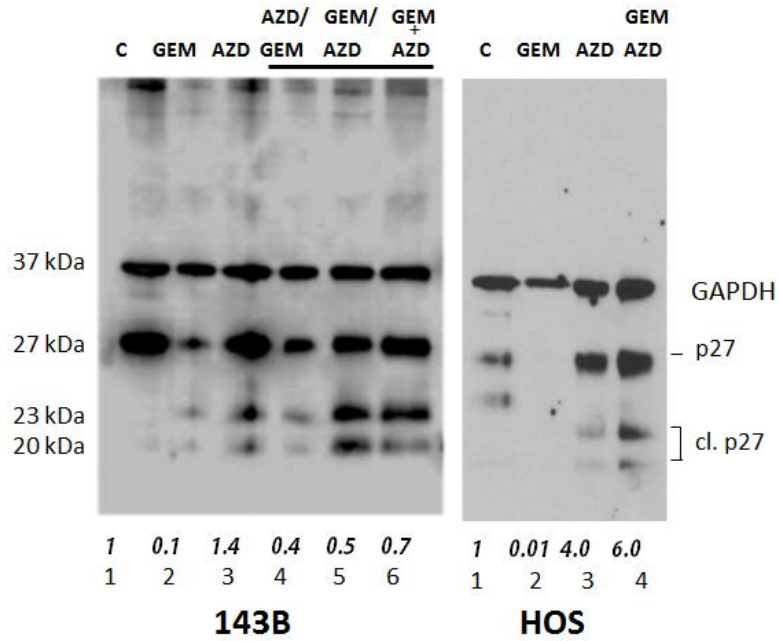
Supplementary Figure S9

Figure S9: Full length immunoblot for Figure 4C.
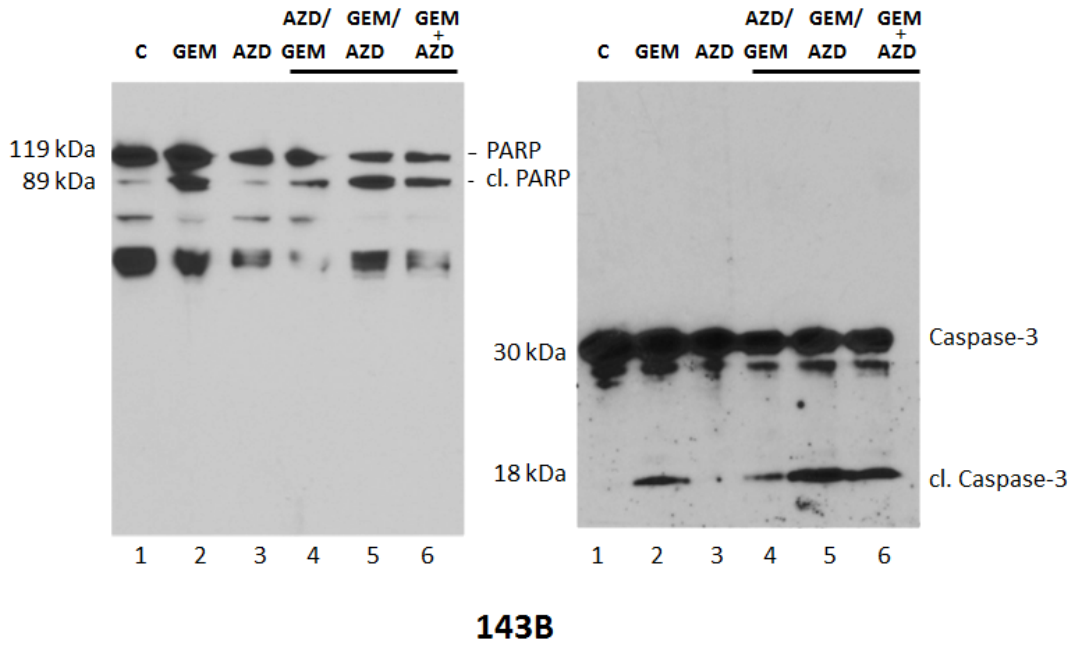

Supplementary Figure S10

Figure S10: Full length immunoblot for Figure 4D.

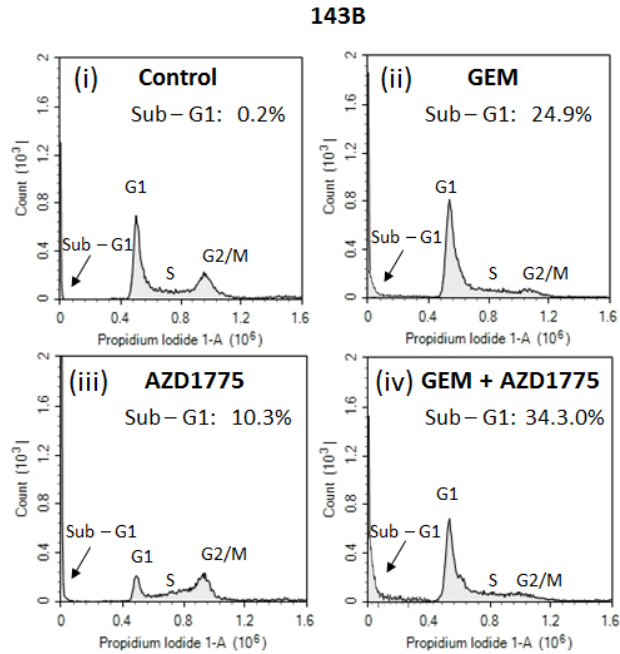**Supplementary Figure S11**

Figure S11: Cell cycle profiles of 143B cells subjected to indicated drug treatments with gemcitabine (GEM) and AZD1775 were measured by flow cytometry. Apoptosis was determined by measuring percent of cells in sub-G1 peak
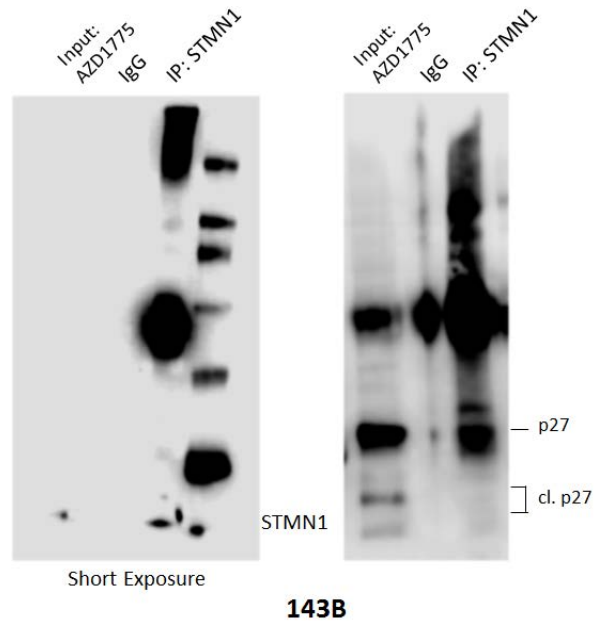
Supplementary Figure S12

Figure S12: Full length immunoblot for Figure 4E.

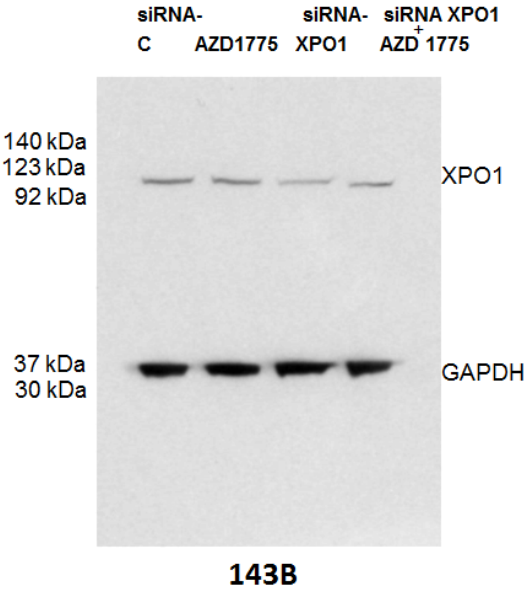## Supplementary Figure S13

Figure S13: Full length immunoblot for Figure 5A

Supplementary Figure S14

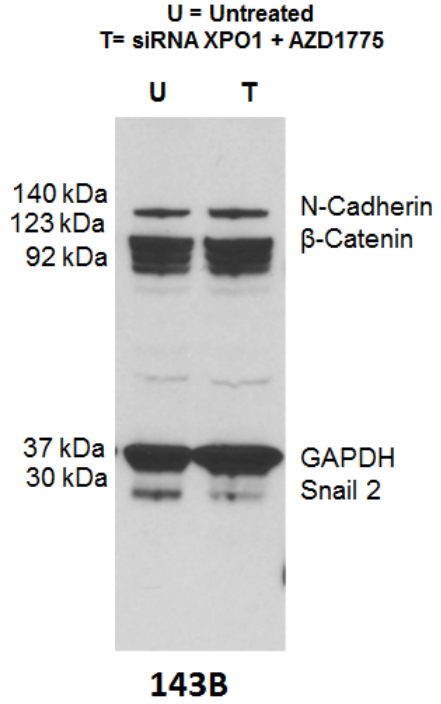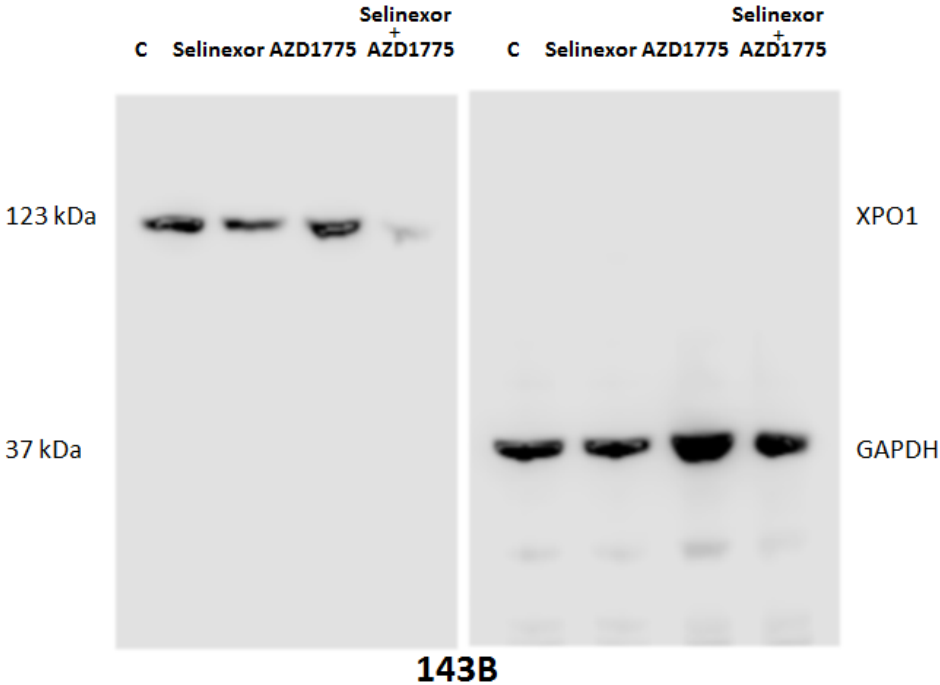**Figure S14:** Full length immunoblot for Figure 5D.

Supplementary Figure S15

143B

Figure S15: Full length immunoblot for Figure 5E.