Supplementary Data

Title:

Selinexor (KPT-330) has antitumor activity against anaplastic thyroid carcinoma *in vitro* and *in vivo* and enhances sensitivity to doxorubicin

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Supplementary Table 1

Selected gene primers for real-time quantitative PCR

Gene name	Forward primer	Reverse primer
XPO1	5'-CTCGTCAGCTGCTTGATTTC-3'	5'-CTCTTGTCCAAGCATCAGGA-3'
CCNB1	5'- CATGGTGCACTTTCCTCCTT-3'	5'- CAGGTGCTGCATAACTGGAA-3'
CCNB1	5'-CCTCCCTTTTCAGTCCGC-3'	5'-CTCCTGTGTCAATATTCTCCAAATC-3'
CDC25C	5'-CTTCCTTTACCGTCTGTCCAG-3'	5'-CCAAGTTTCCATTGTCATCCC-3'
AURKA	5'-ATCTGTGGTGCATTGGAGTG-3'	5'-CATACAAACACACGCACCCG-3'
AURKB	5'-CAGAGAGATCGAAATCCAGGC-3'	5'-CCTTGAGCCCTAAGAGCAGAT-3'
AXL	5'-AACCTTCAACTCCTGCCTTCTCG-3'	5'-CAGCTTCTCCTTCAGCTCTTCAC-3'
GAS6	5' -CATCAACAAGTATGGGTCTCCGT3'	5'- GTTCTCCTGGCTGCATTCGTTGA-3'
MET	5'-TGCAGCGCGTTGACTTATTCATGG-3'	5'-GAAACCACAACCTGCATGAAGCGA-3'
TNF	5'-GGAGAAGGGTGACCGACTCA-3'	5'-CTGCCCAGACTCGGCAA-3'
KLF6	5'-CTGCCGTCTCTGGAGGAGT-3'	5'-TCCACAGATCTTCCTGGCTGTC-3'
IL-6	5′-GGTACATCCTCGACGGCATCT-3′	5′-GT GCCTCTTTGCTGCTTTCAC-3′
SMAD7	5'-GAATCTTACGGGAAGATCAACCC-3'	5'-CGCAGAGTCGGCTAAGGTG-3'
GAPDH	5'-GGTCGGAGTCAACGGAT-3'	5'-GTCATGAGTCCTTCCACGATA-3'

Supplementary Table 2

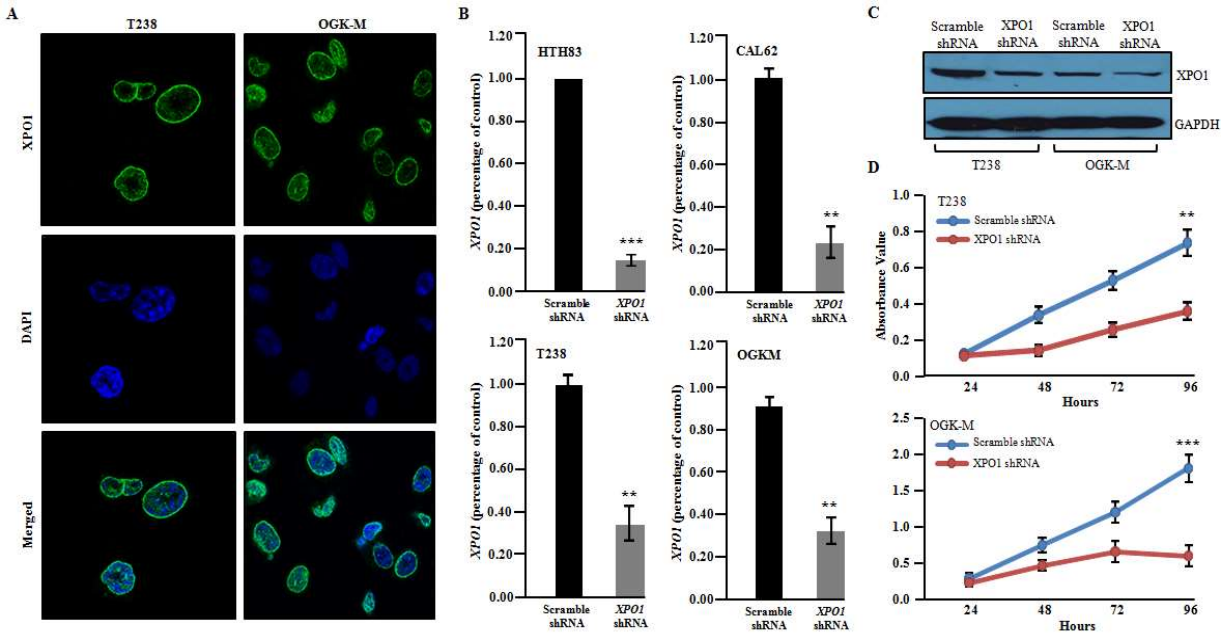
Analysis of murine blood samples after treatment with vehicle and selinexor

Blood and serum analysis	Reference range	Vehicle	Selinexor (10mg/kg)
WBC (x 1000 per µl)	1 - 12.2	5.1 ± 0.38	8.0 ± 0.45
Neut (x 1000 per µl)	0 - 2.5	1.9 ± 0.31	2.5 ± 0.3
HCT (%)	34 - 53	39 ± 2.0	46.1 ± 3.7
PLT(x 1000 per µl)	625-2241	2010 ± 202	1891 ± 395
ALB (g/L)	25-30	25.3 ± 2.3	28.4 ± 2.5
AST (U/L	54-298	126.1 ± 30	242.7 ± 45
ALT (U/L	17-77	32 ± 10	60.4 ± 13
CREAT (mg/dl)	0.2-0.9	0.18 ± 0.02	0.35 ± 0.07

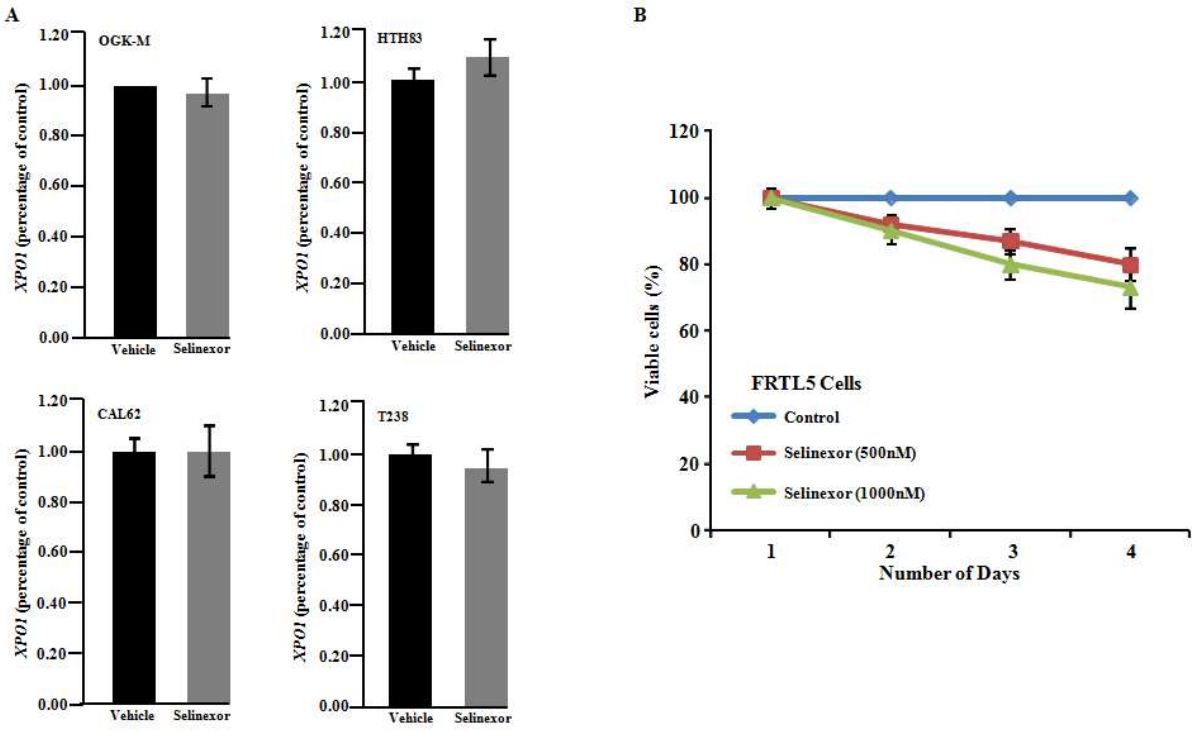
Mice were randomly divided into two groups (six mice per group) and orally treated either with vehicle (0.6% w/v aqueous Pluronic F-68) or Selinexor (KPT-330; 10mg/kg, thrice weekly for 4 weeks). Mice were given Nutri-Cal (tomlyn) during therapy to allow robust nutrition. Data represent mean ± SD of NOD/SCID mice.

Abbreviations: WBC = white blood cell; Neut = neutrophil; HCT = haematocrit; PLT = platelet; ALB = albumin; AST = aspartate aminotransferase; ALT = alanine aminotransferase; CREAT = creatinine.

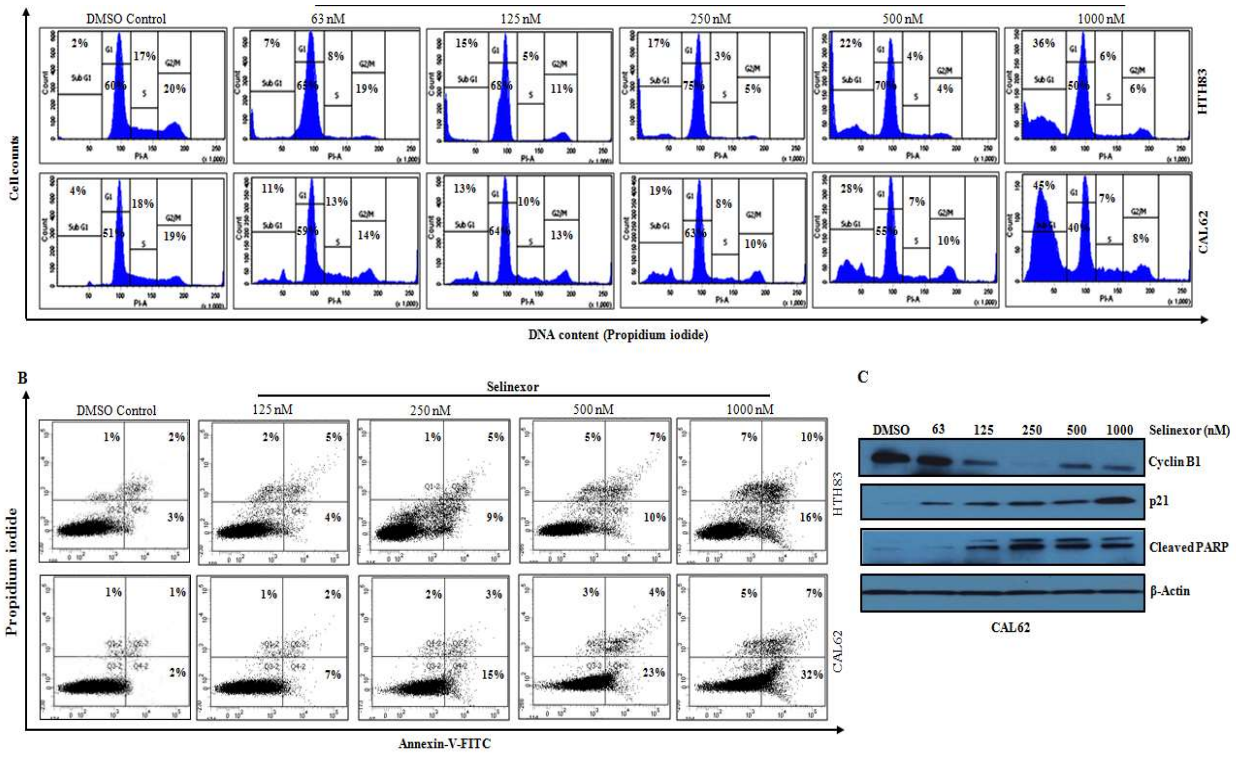
Supplementary Figures



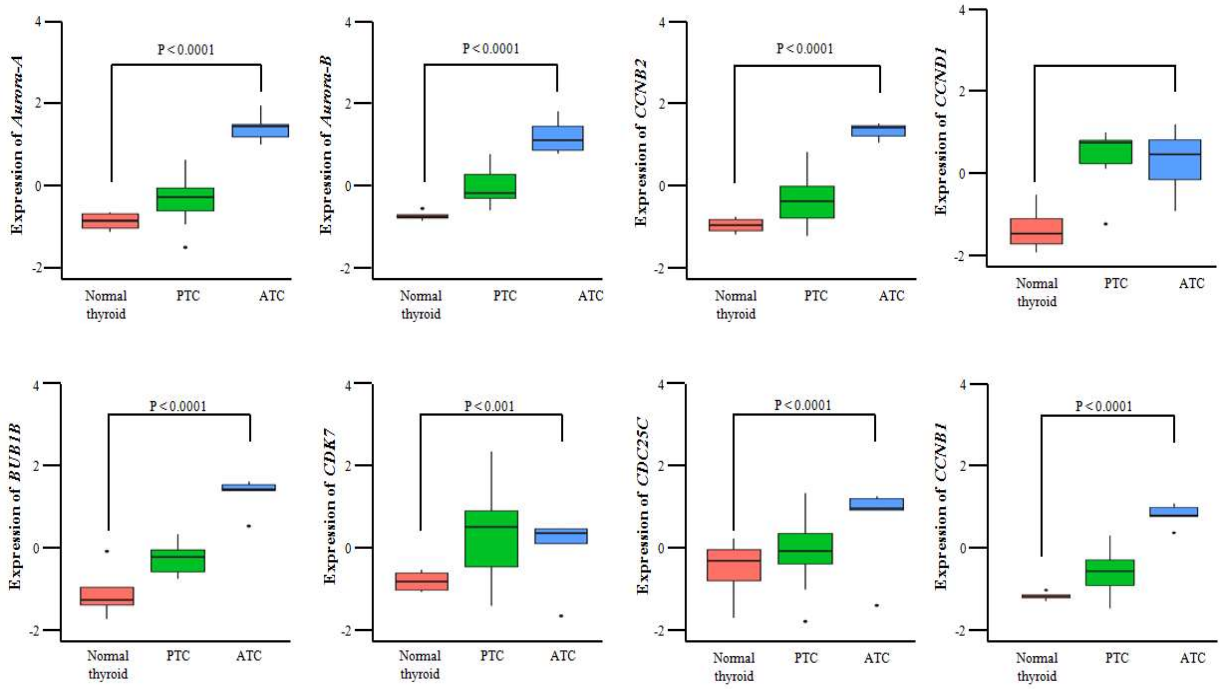
Supplementary Figure 1. Endogenous expression of XPO1 in human ATC tissues and cell lines, and silencing of *XPO1* in ATC cells. (A) Immunofluorescence analysis displayed nuclear localization of XPO1 protein in fixed/permeabilized T238 and OGK-M cell. DAPI was used to stain nuclei. **(B and C)** ATC cells were infected with either *XPO1* shRNA or scrambled shRNA lentiviral particles. Quantitative RT-PCR and western blotting confirmed *XPO1* knockdown in ATC cell lines. (**D)** *XPO1* knockdown reduced cellular proliferation of T238 and OGK-M as measured using MTT assay. Results represent mean ± SD; n=4. ** $P \leq 0.001$; *** $P \leq 0.0001$(Student's t-test).



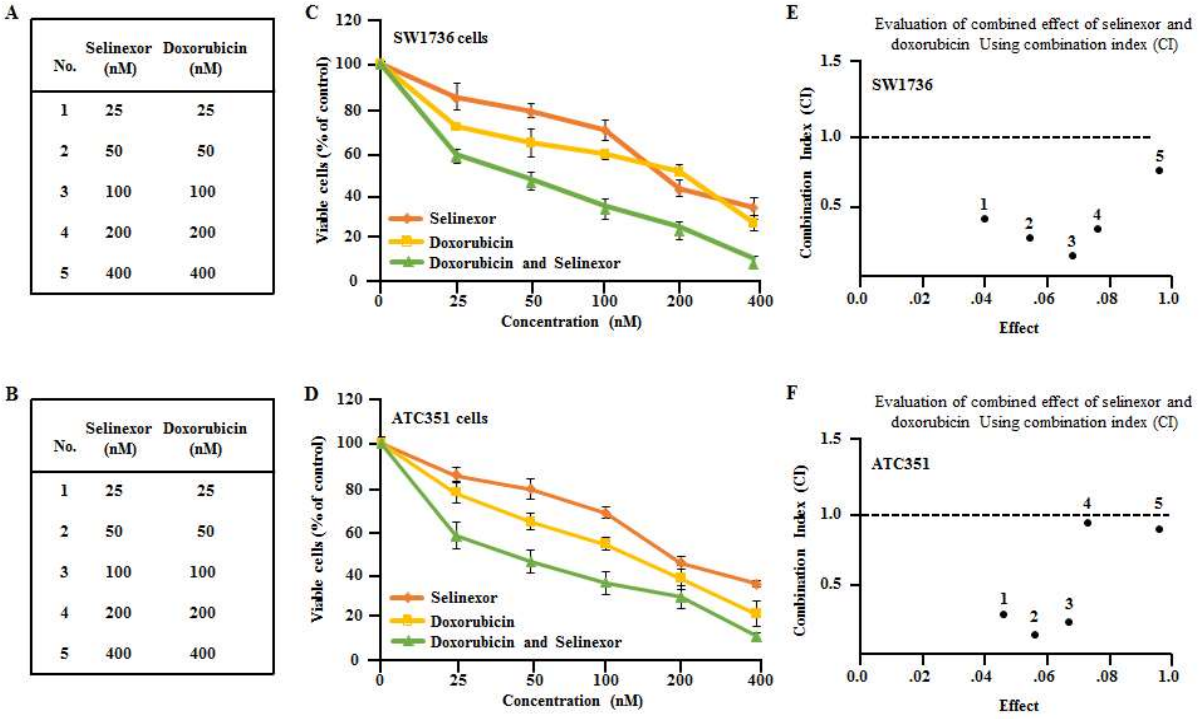
Supplementary Figure 2. Effect of selinexor (KPT-330; *XPO1* inhibitor) on the expression of *XPO1* mRNA. (A) OGK-M, HTH83, CAL62 and T238 ATC cells were exposed to either vehicle or selinexor (1000 nM) for 48 h for quantitative RT-PCR analysis. Results represent mean ± SD of three experiments done in triplicates. *XPO1* expression was normalized to *GAPDH* as a reference. **(B)** Anti-proliferative effect of selinexor (500, 1000 nM) against FRTL5 cells in liquid culture. Proliferation assay measured cell viability of FRTL5 cells. Results represent the mean ± SD of three independent experiments with quadruplicate wells per experiment point.



Supplementary Figure 3. Inhibition of XPO1 by selinexor induced cell cycle arrest and apoptosis of ATC cells. Selinexor treatment (0-1000 nM, 48 h) leads to cell cycle arrest in ATC cells. (**A)** Fluorescent activated cell sorter evaluated the percentage of ATC cells in each phase of the cell cycle. The arrest of the cell cycle at G1 phase along with accumulation sub-G1 phase was observed in selinexor treatment ATC cells in a dose-dependent fashion. The figures are representative of three independent experiments. (**B)** Flow cytometry profile upon selinexor treatment (Annexin V-FITC staining on the X-axis and PI staining on the Y-axis). Upper left quadrants display the necrotic cells, upper right quadrants show late apoptotic cells, lower right quadrants show early apoptotic cells and lower left quadrants display the live cells. Selinexor induces apoptosis of ATC cells in a dose-dependent manner. (**C)** Western blot analysis shows decreased expression of cyclin B1 and increased expression of p21 and cleaved PARP protein in selinexor (63-1,000 nM) treated ATC cells (CAL62).



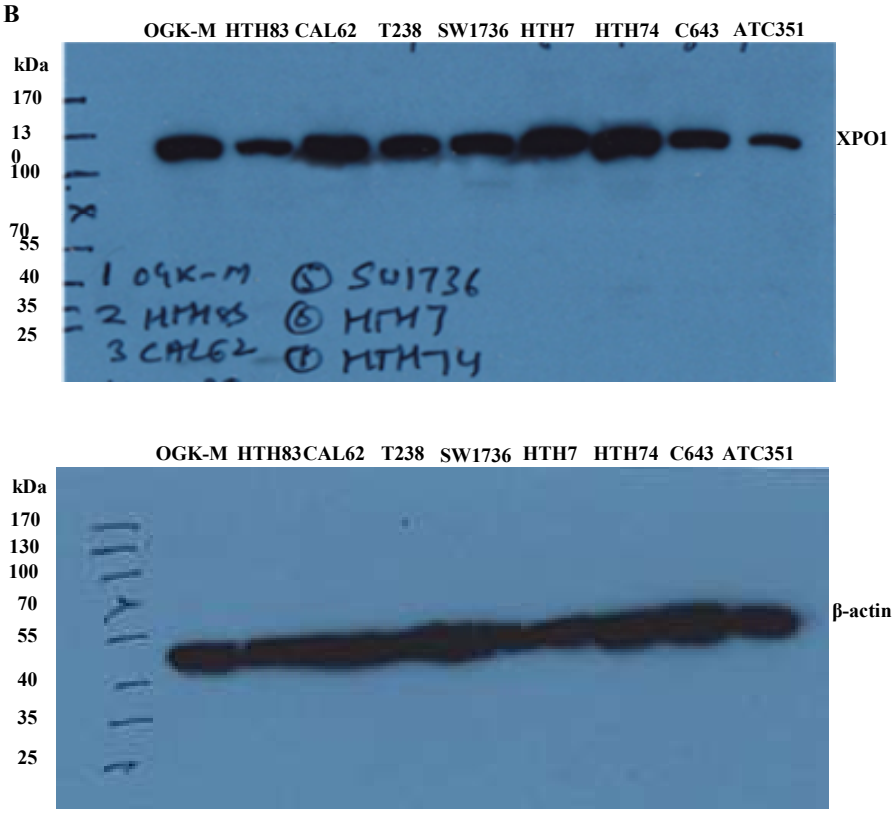
Supplementary Figure 4. Expression of cell cycle genes in human ATC tissue specimens compared to normal thyroid. Microarray data (GSE9115) from GEO database was analyzed and ATC samples showed significantly higher expression of cell cycle related genes compared to normal thyroid.

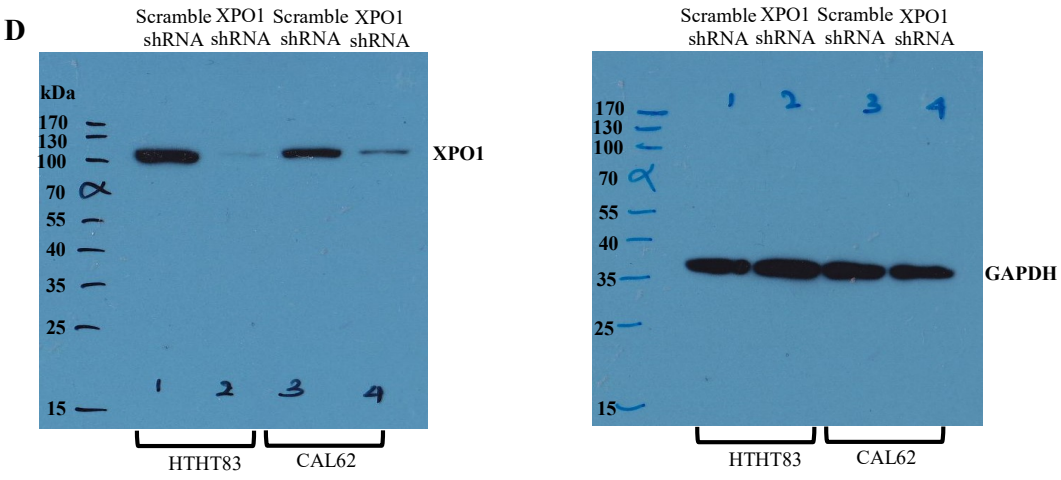


Supplementary Figure 5. Combination of selinexor and doxorubicin synergistically inhibited the growth of ATC cells. (A-D) SW1736 and ATC351 cells were treated with selinexor (0, 25, 50, 100, 200, 400 nM) and/ or doxorubicin (0, 25, 50, 100, 200, 400 nM) for 72 h, and growth inhibition was measured by MTT assay. **(E and F)** Combinational growth inhibition of selinexor and doxorubicin on SW1736 and ATC351 cells displayed as CI. CI defines the interaction between selinexor and doxorubicin as plotted against a fraction of cell viability. CI < 1, CI = 1 and CI > 1 represent synergism, additive, and antagonism of the two compounds, respectively.

Full Blots for Figures

Figure-1





Figre-2

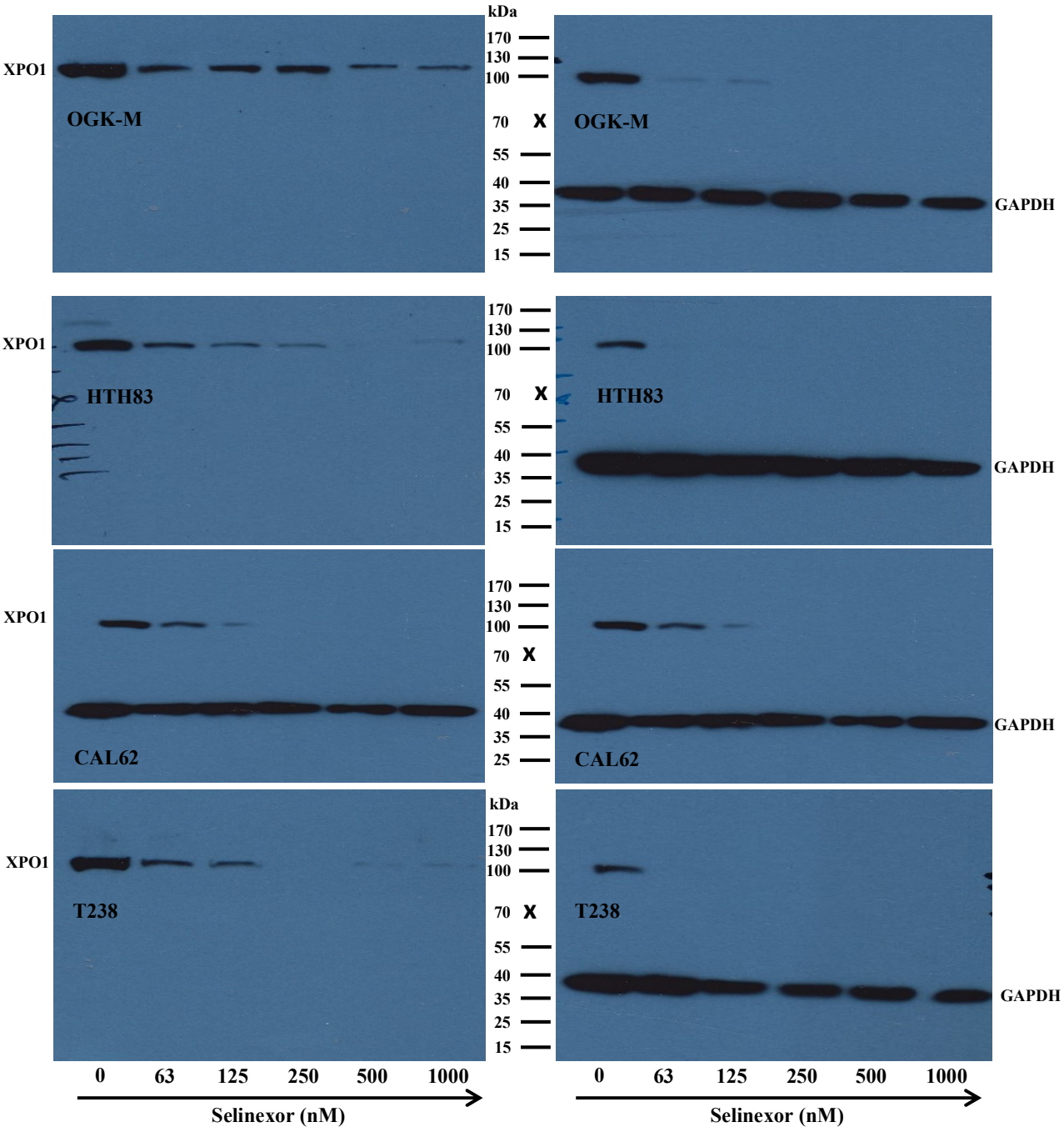
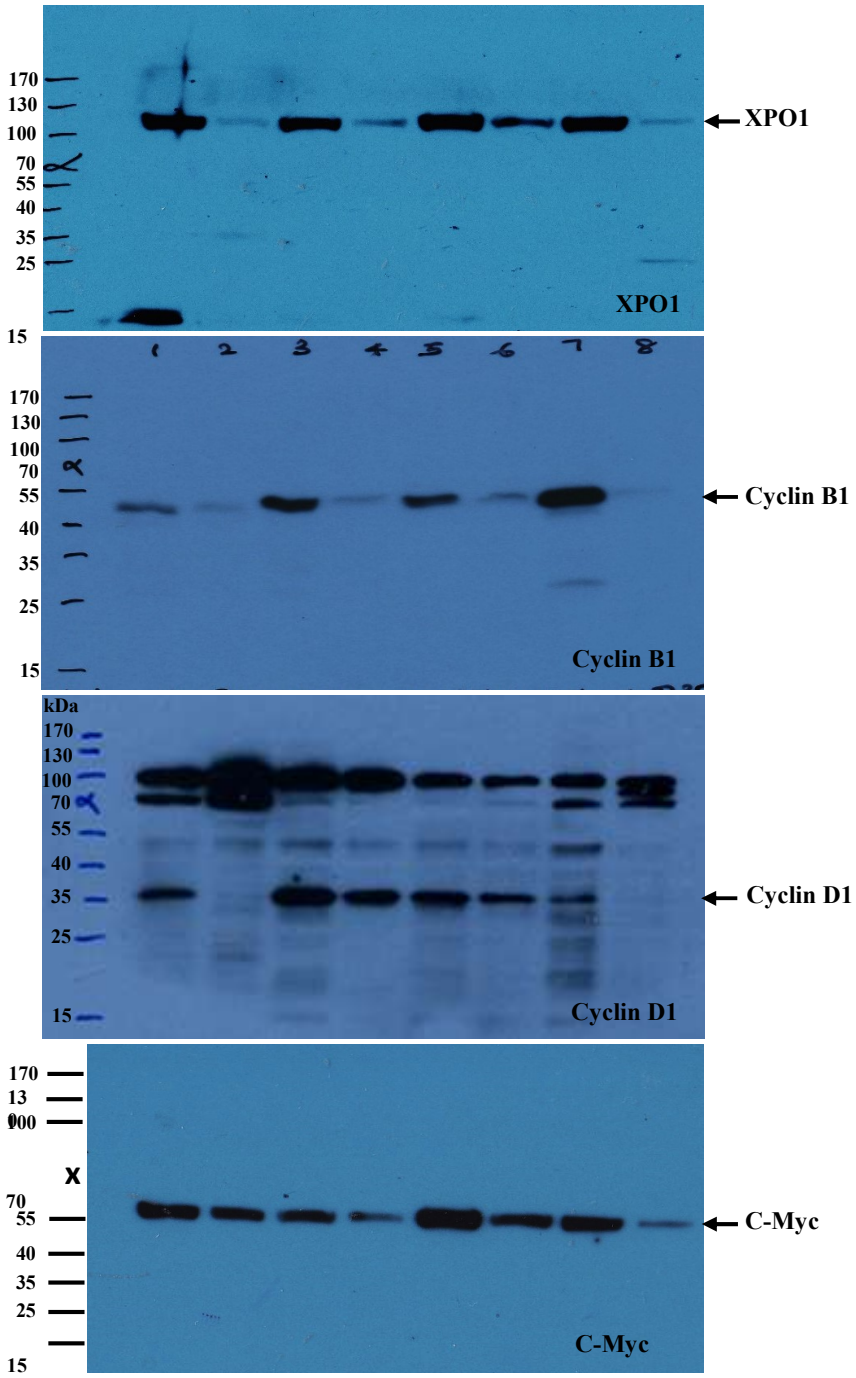

kDa
170
130
100
70
55
40
35
25
15
XPO1
OGK-M
GAPDH
HTH83
CAL62
T238
0
63
125
250
500
1000
Selinexor (nM)

Figure-3D


170
130
100
70
55
40
35
25
15
XPO1
XPO1
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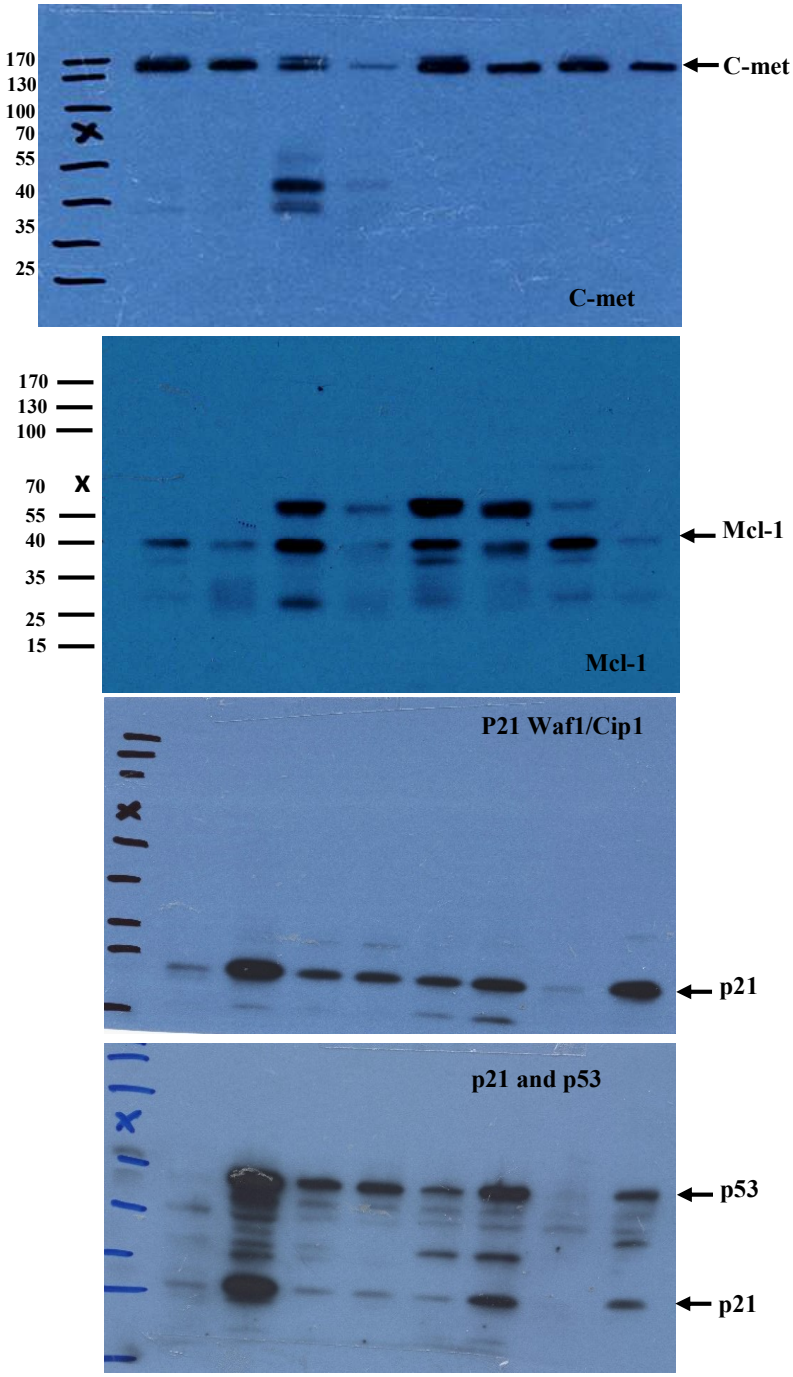
Cyclin B1
Cyclin B1
kDa
Cyclin D1
Cyclin D1
C-Myc
C-Myc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35
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C-met
C-met
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130
100
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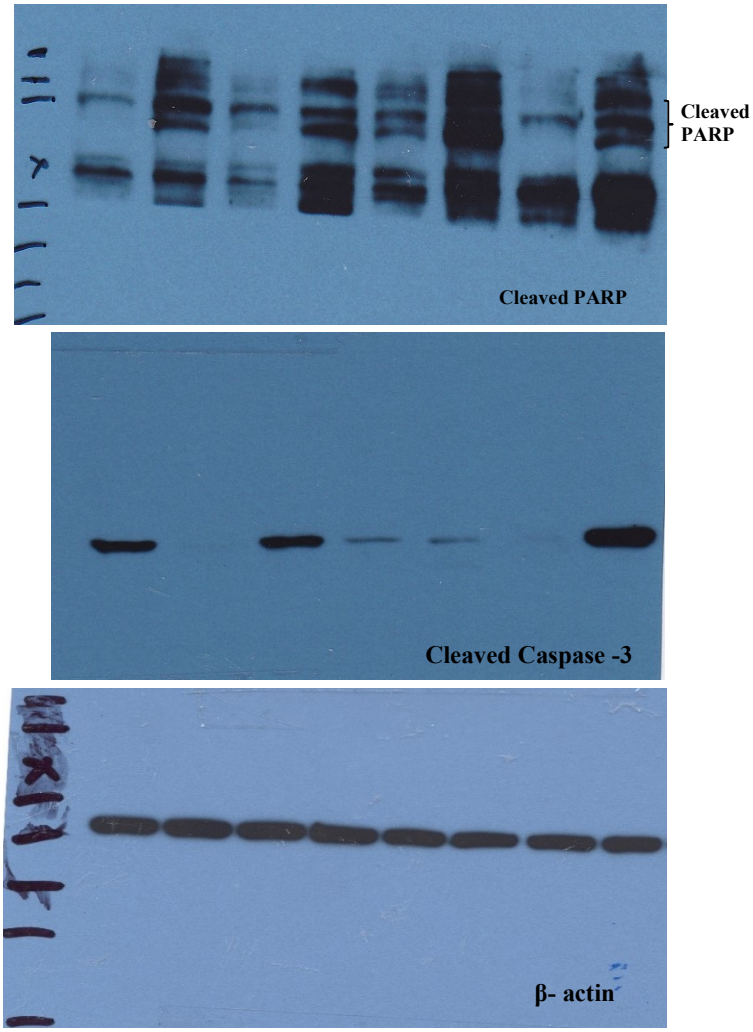
P21 Waf1/Cip1
p21
p21 and p53
p53
p21


Cleaved
PARP
Cleaved PARP
Cleaved Caspase -3
β- actin