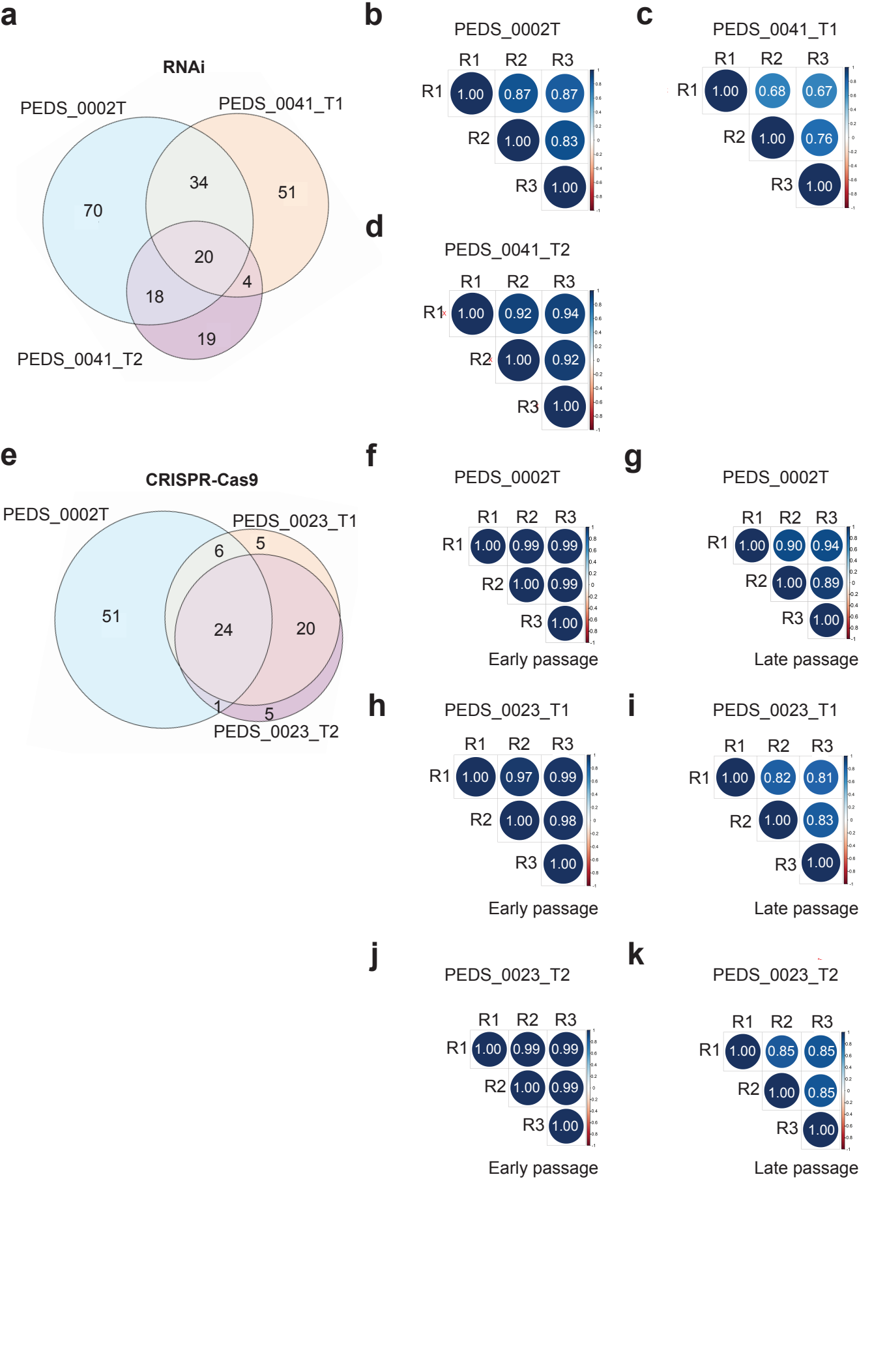
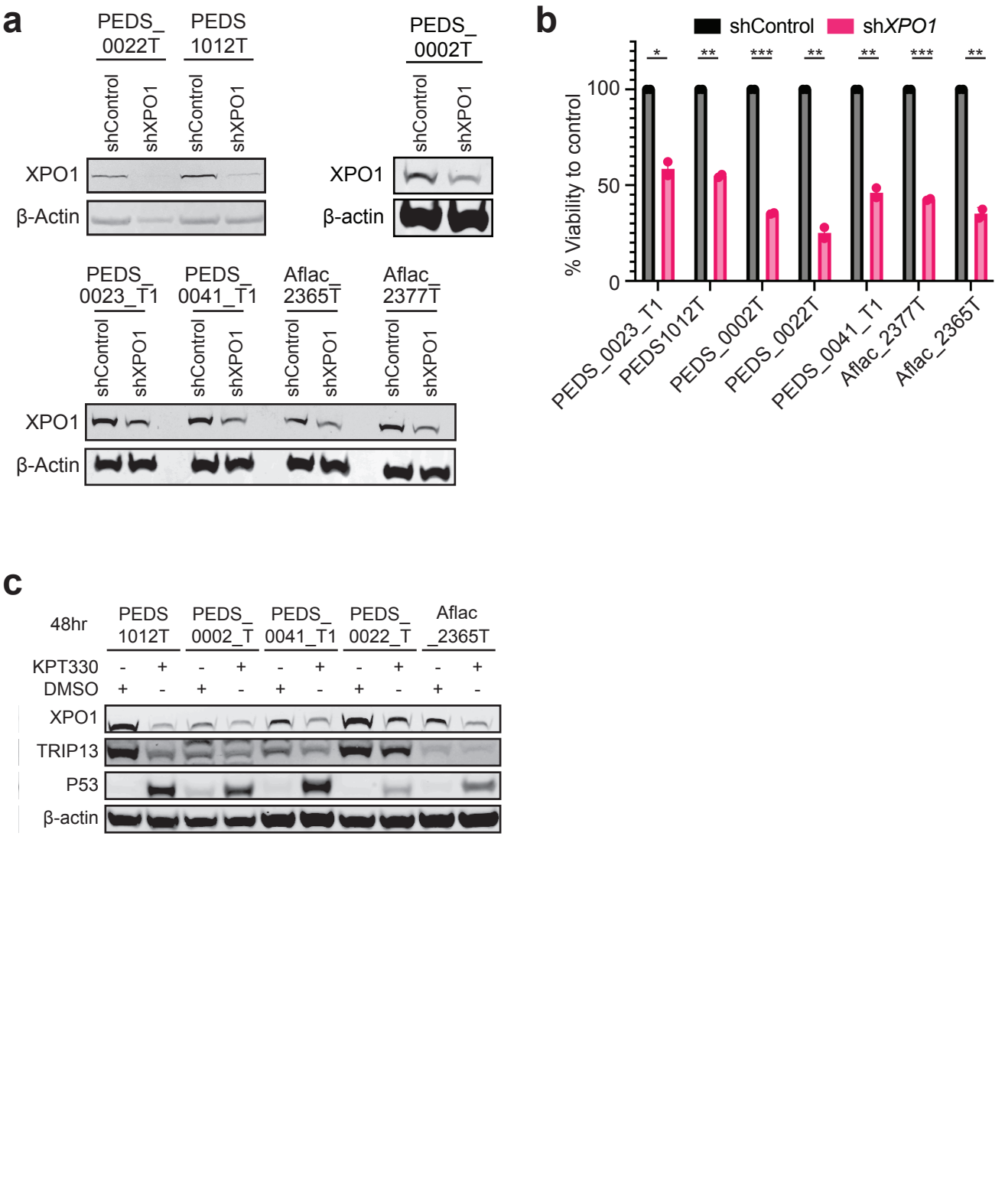
Supplementary Figure 1: Correlation between shRNA and sgRNA loss of function screens among biological replicates.

RNAi screens by cell lines represented in (a). Correlations of RNAi screens in (b) PEDS_0002T, (c) PEDS_0041_T1, (d) PEDS_0041_T2. CRISPR-Cas9 screens used early and late passages to determine change in abundance of sgRNAs summarized by cell lines in (e). as shown in f-k. (f) PEDS_0002T early passage, (g) PEDS_0002T late passage, (h) PEDS_0023_T1 early passage, (i) PEDS_0023_T1 late passage, (j) PEDS_0023_T2 early passage, (k) PEDS_0023_T2 late passage. Right color gradient delineates the Pearson's correlation with anticorrelation as red and correlation as blue.


a
RNAi
PEDS_0002T
PEDS_0041_T1
PEDS_0041_T2
70
34
51
20
4
18
19
b
PEDS_0002T
R1 R2 R3
R1 1.00 0.87 0.87
R2 1.00 0.83
R3 1.00
c
PEDS_0041_T1
R1 R2 R3
R1 1.00 0.68 0.67
R2 1.00 0.76
R3 1.00
d
PEDS_0041_T2
R1 R2 R3
R1 1.00 0.92 0.94
R2 1.00 0.92
R3 1.00
e
CRISPR-Cas9
PEDS_0002T
PEDS_0023_T1
PEDS_0023_T2
51
6
5
24
20
1
5
f
PEDS_0002T
R1 R2 R3
R1 1.00 0.99 0.99
R2 1.00 0.99
R3 1.00
Early passage
g
PEDS_0002T
R1 R2 R3
R1 1.00 0.90 0.94
R2 1.00 0.89
R3 1.00
Late passage
h
PEDS_0023_T1
R1 R2 R3
R1 1.00 0.97 0.99
R2 1.00 0.98
R3 1.00
Early passage
i
PEDS_0023_T1
R1 R2 R3
R1 1.00 0.82 0.81
R2 1.00 0.83
R3 1.00
Late passage
j
PEDS_0023_T2
R1 R2 R3
R1 1.00 0.99 0.99
R2 1.00 0.99
R3 1.00
Early passage
k
PEDS_0023_T2
R1 R2 R3
R1 1.00 0.85 0.85
R2 1.00 0.85
R3 1.00
Late passage

Supplementary Figure 2: Inhibition of nuclear export in FHWT by KPT-330 or sh*XPO1*.

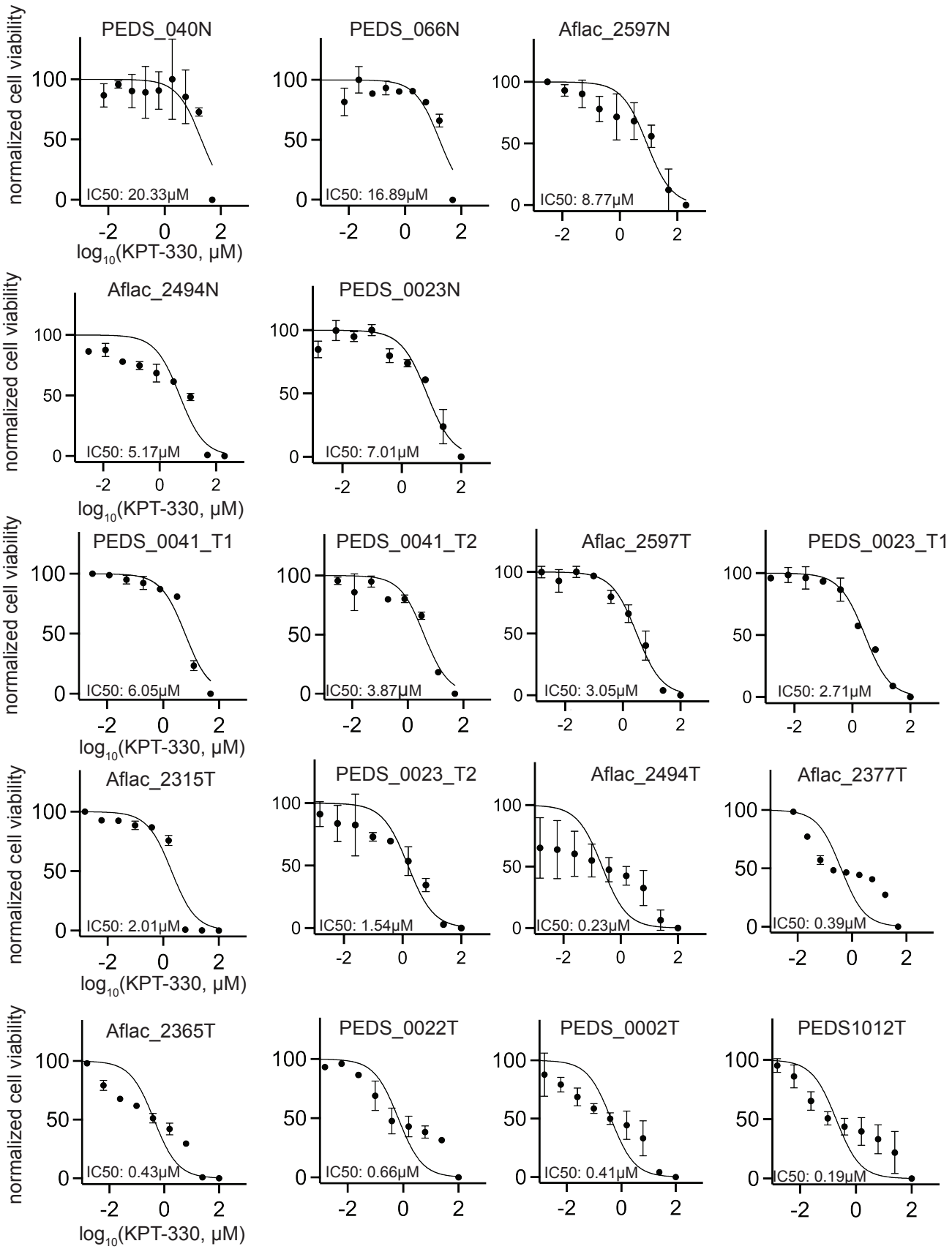
(a) Suppression of XPO1 by immunoblot (b) Change in viability using sh*XPO1* across FHWT cell lines as compared to shControl. * p-value <0.05, ** p-value <0.005, *** p-value <0.0005. Error bars represent mean ± SD. (c) Additional replicate of the KPT-330 treated (5μM treat for 48 hours) Wilms tumor cells across 5 different cell lines.


a
PEDS_ 0022T
PEDS 1012T
PEDS_ 0002T
shControl
shXPO1
XPO1
β-Actin
β-actin
PEDS_ 0023_T1
PEDS_ 0041_T1
Aflac_ 2365T
Aflac_ 2377T
b
shControl
shXPO1
% Viability to control
100
50
0
*
**
***
PEDS_0023_T1
PEDS1012T
PEDS_0002T
PEDS_0022T
PEDS_0041_T1
Aflac_2377T
Aflac_2365T
c
48hr
PEDS 1012T
PEDS_ 0002_T
PEDS_ 0041_T1
PEDS_ 0022_T
Aflac _2365T
KPT330
DMSO
XPO1
TRIP13
P53
β-actin

Supplementary Figure 3: Dose response curves to KPT-330 across cells lines

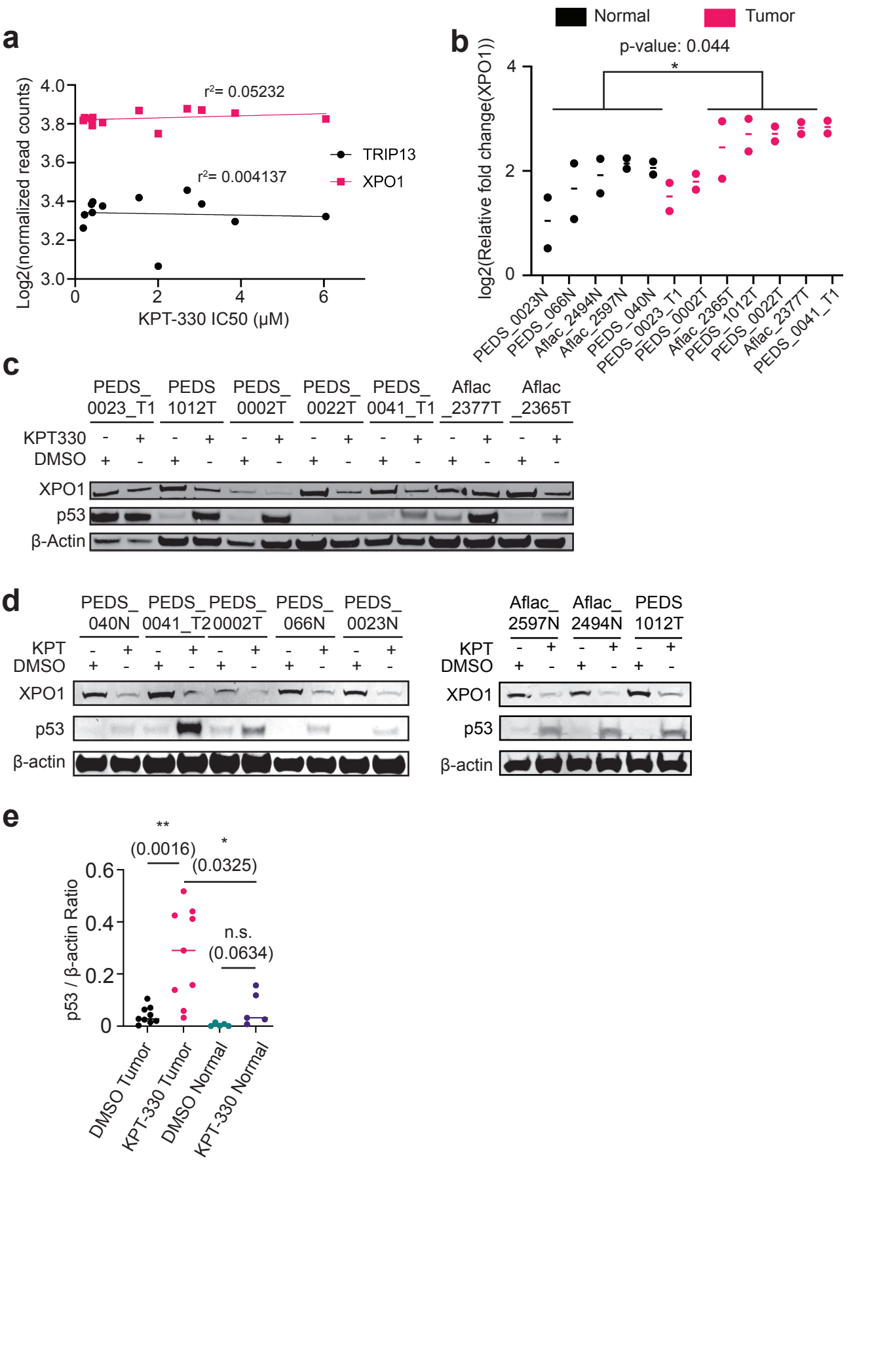
KPT-330 IC50 data from all cell lines depicted in Fig 3d. Matched tumor normal pairs are plotted together for three of the cell lines. Error bars represent mean ± SD from two biological replicates.

KPT-330 IC50 Curves

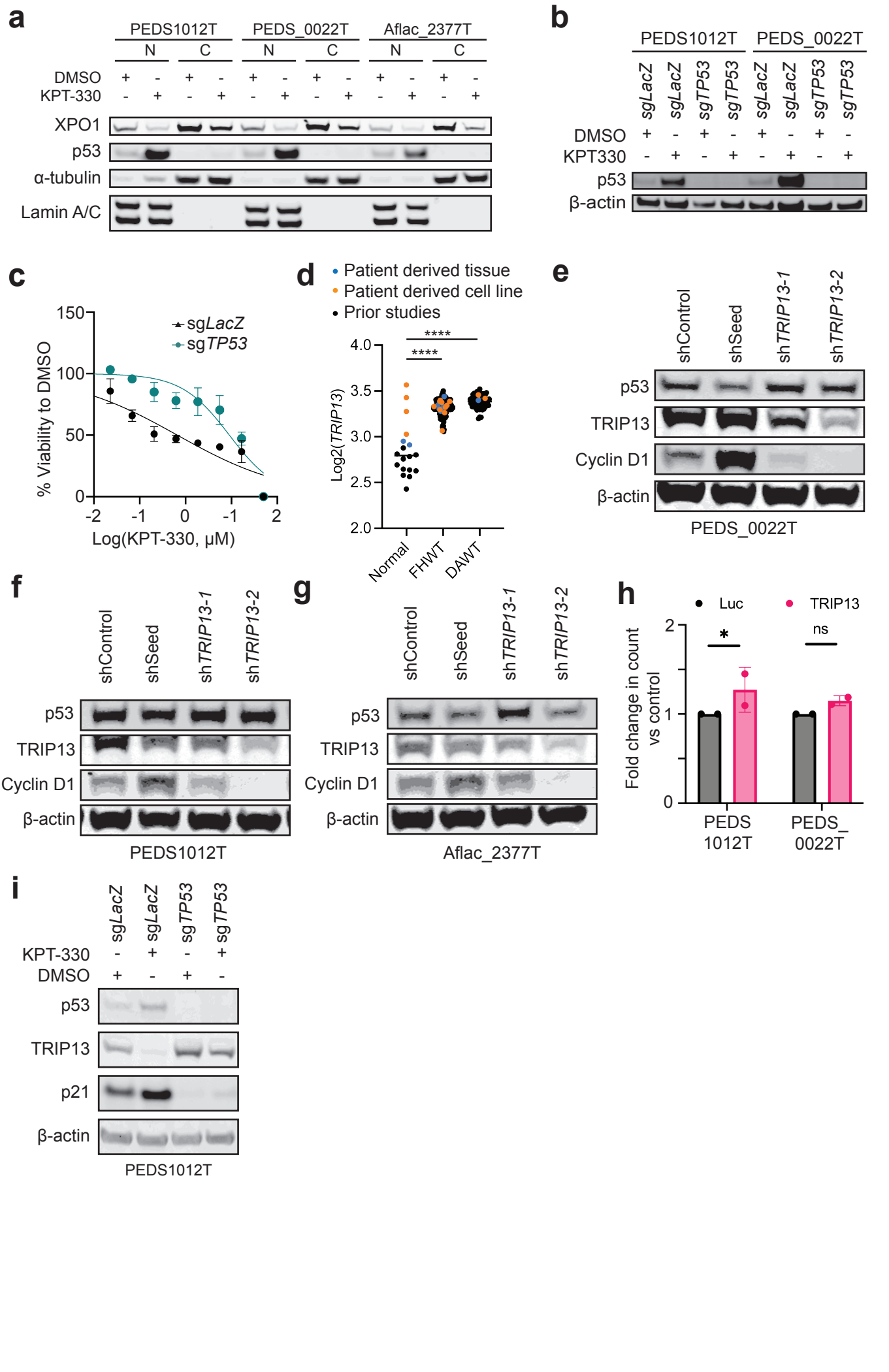


Supplementary Figure 4: Response to KPT-330 Treatment.

(a) Plot measuring correlation between XPO1 expression levels and corresponding KPT-330 IC50. (b) qRT-PCR analysis of *XPO1* in KPT-330 and DMSO treated cells. Data was normalized by the amount of TBP expressed relative to the corresponding value for all the cells and are means ± SD from at least two biological replicates. * p-value<0.05, all comparisons represent a Student's unpaired two-sided t-test. (c) Cell lines were treated with 5μM of KPT-330 for 24 hours, XPO1/CRM levels are suppressed with accumulation of p53 across a majority of cell lines with exception to PEDS_0023_T1 which harbors a TP53 mutation. (d) Normal cell lines were treated with 5μM of KPT-330 for 24 hours and accumulation of p53 was assessed. (e) Immunoblots from Supp Fig 4c and 4d were quantified to determine the accumulation of p53 relative to β-actin, excluding PEDS_0023_T1. ** p-value<0.005, all comparisons represent a Student's t-test.


a
Log2(normalized read counts)
4.0
3.8
3.6
3.4
3.2
3.0
0
2
4
6
KPT-330 IC50 (μM)
r²= 0.05232
r²= 0.004137
TRIP13
XPO1
b
Normal
Tumor
p-value: 0.044
*
log2(Relative fold change(XPO1))
4
2
0
PEDS_0023N
PEDS_066N
Aflac_2494N
Aflac_2597N
PEDS_040N
PEDS_0023_T1
PEDS_0002T
Aflac_2365T
PEDS_1012T
PEDS_0022T
Aflac_2377T
PEDS_0041_T1
c
PEDS_ 0023_T1
PEDS 1012T
PEDS_ 0002T
PEDS_ 0022T
PEDS_ 0041_T1
Aflac _2377T
Aflac _2365T
KPT330 - + - + - + - + - + - + - +
DMSO + - + - + - + - + - + - + -
XPO1
p53
β-Actin
d
PEDS_ 040N
PEDS_ 0041_T2
PEDS_ 0002T
PEDS_ 066N
PEDS_ 0023N
KPT - + - + - + - + - +
DMSO + - + - + - + - + -
XPO1
p53
β-actin
Aflac_ 2597N
Aflac_ 2494N
PEDS 1012T
KPT - + - + - +
DMSO + - + - + -
XPO1
p53
β-actin
e
**
(0.0016)
*
(0.0325)
n.s.
(0.0634)
p53 / β-actin Ratio
0.6
0.4
0.2
0
DMSO Tumor
KPT-330 Tumor
DMSO Normal
KPT-330 Normal

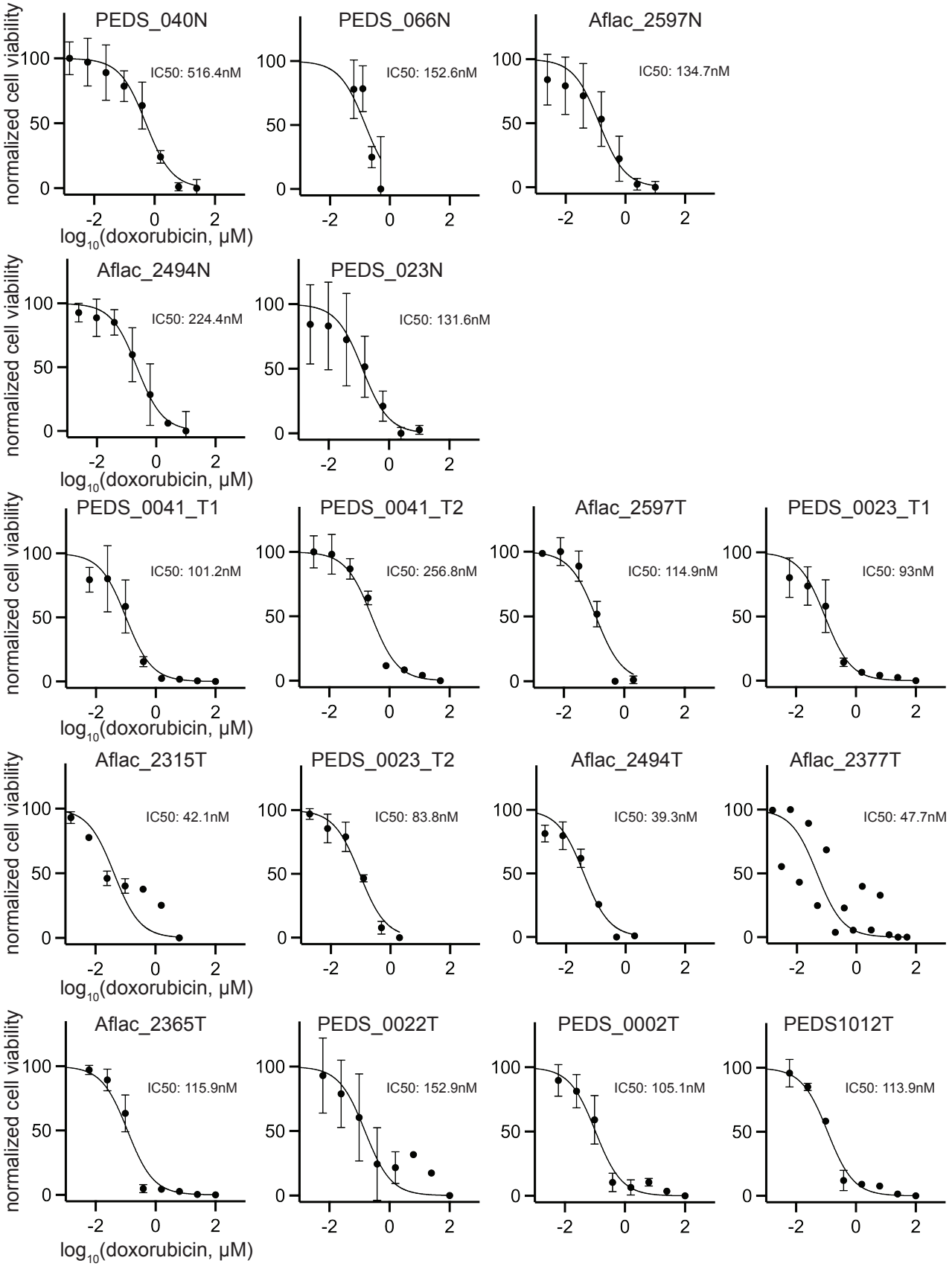
Supplementary Figure 5: Inhibition of nuclear export and TRIP13 in FHWT cell lines. (a) Immunoblots depicting the decrease in protein levels of XPO1 in the cytoplasmic lysates and nuclear accumulation of p53 in the nuclear lysates upon treatment with KPT-330 at 24 hr. (b) We introduced sgRNAs targeting either LacZ or TP53 and confirmed increase in p53 following treatment with KPT-330 in the LacZ controls. (c) Dose-response curves for the sgTP53 and sgLacZ cells for KPT-330 in PEDS_0022T. Error bars represent mean ± SD and represent biological replicates. (d) Dot plots representing the expression levels of TRIP13 in Wilms tumor when compared with the normal matched kidney tissue. Immunoblot depicting suppression of TRIP13 in (e) CCLF_PEDS1012T (f) CCLF_PEDS_0022T, and (g) Aflac_2377T. (h) Overexpression of TRIP13 leads to modest increase in proliferation as compared to luciferase. (i) p53 and p21 protein levels increase while TRIP13 levels decrease upon KPT-330 treatment in TP53 wildtype cells.


a
PEDS1012T
PEDS_0022T
Aflac_2377T
N
C
DMSO
KPT-330
XPO1
p53
α-tubulin
Lamin A/C
b
PEDS1012T
PEDS_0022T
sgLacZ
sgTP53
DMSO
KPT330
p53
β-actin
c
% Viability to DMSO
sgLacZ
sgTP53
Log(KPT-330, μM)
d
Patient derived tissue
Patient derived cell line
Prior studies
Log2(TRIP13)
****
Normal
FHWT
DAWT
e
shControl
shSeed
shTRIP13-1
shTRIP13-2
p53
TRIP13
Cyclin D1
β-actin
PEDS_0022T
f
PEDS1012T
g
Aflac_2377T
h
Luc
TRIP13
Fold change in count vs control
*
ns
PEDS 1012T
PEDS_ 0022T
i
KPT-330
DMSO
p21
PEDS1012T

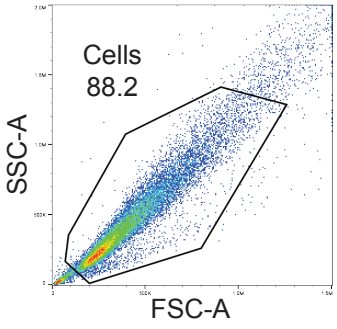
Supplementary Figure 6: Dose response curves to doxorubicin across cells lines

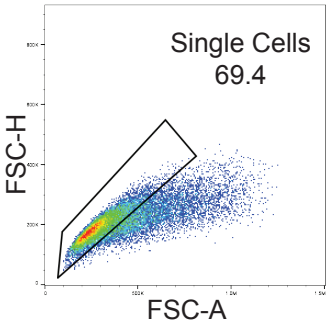
Wilms tumor response to doxorubicin treatment. Doxorubicin IC50 data from all cell lines depicted in Fig 5b. Matched tumor normal pairs are plotted together for three of the cell lines. Error bars represent mean ± SD from two biological replicates.

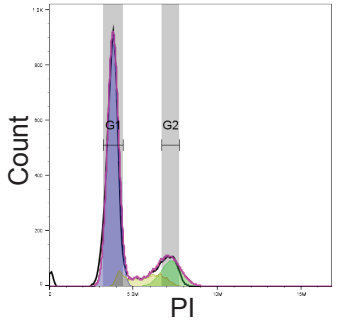
Doxorubicin IC50 Curves



Supplementary Figure 7: Gating Strategy for Flow Cytometry







RMSD: 4.89
%G1: 73.4
%S: 12.3
%G2: 14.3
G1 Mean: 3.77E6
G2 Mean: 7.26E6
G1 CV: 11.9
G2 CV: 11.9
% less G1: 4.10
% greater G2: -0.93

Supplementary Figure 8: The full, unedited blots in Figures.

Fig 3e

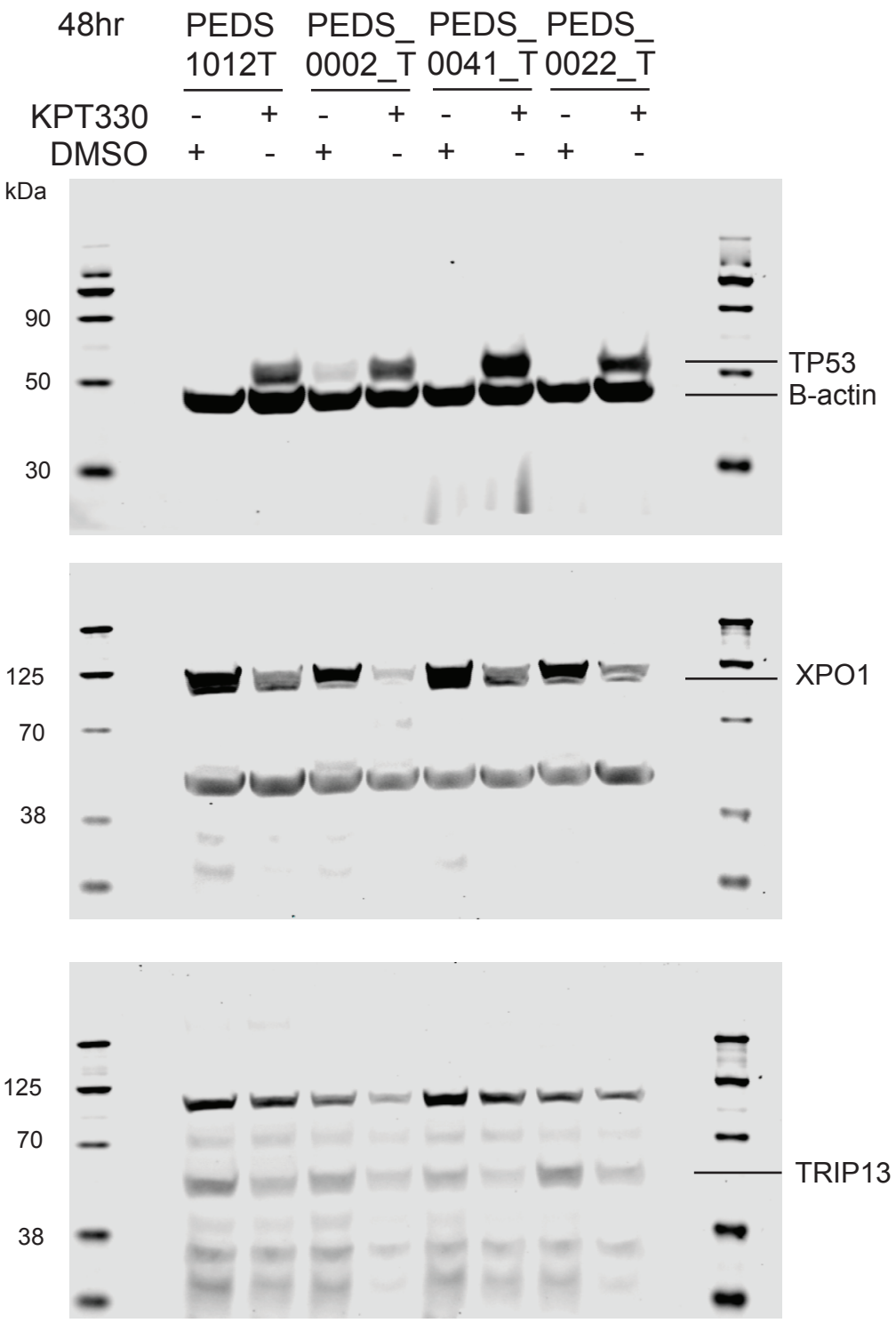
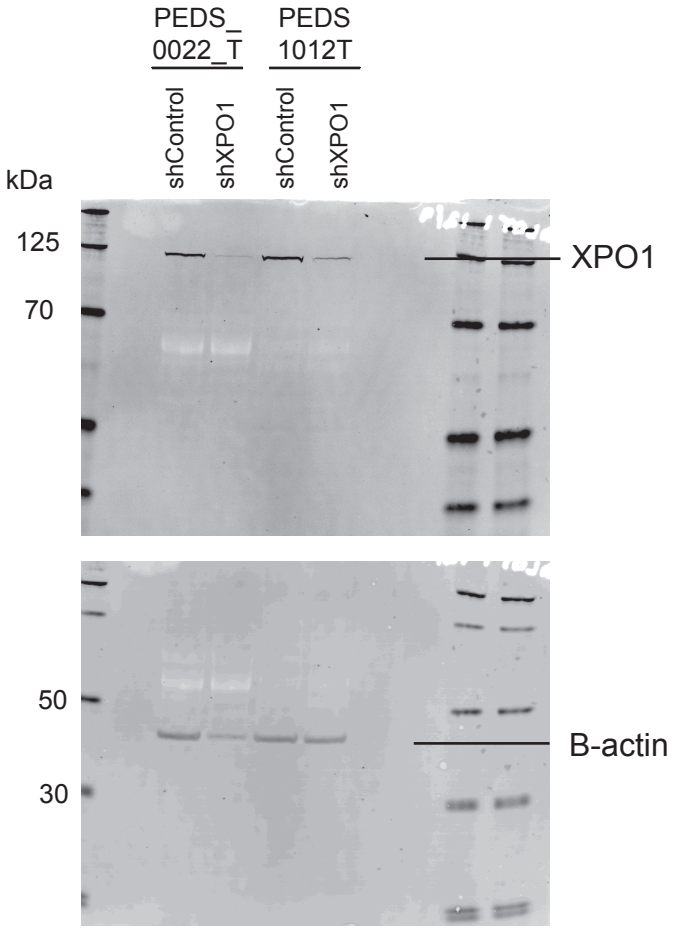
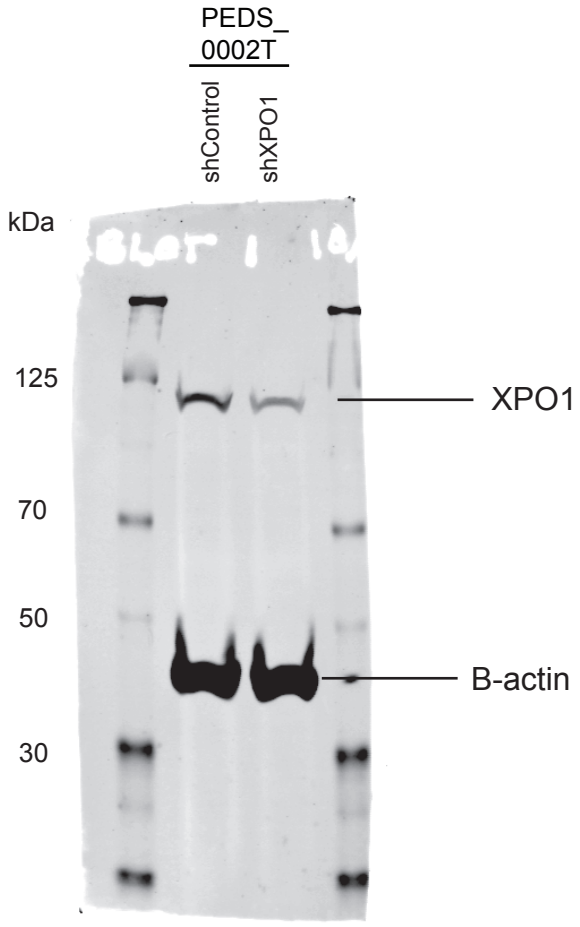

48hr
PEDS 1012T
PEDS_ 0002_T
PEDS_ 0041_T
PEDS_ 0022_T
KPT330 - + - + - + - +
DMSO + - + - + - + -
kDa
90
50
30
TP53
B-actin
125
70
38
XPO1
125
70
38
TRIP13

Fig S2A


PEDS_
0022_T
PEDS
1012T
shControl
shXPO1
shControl
shXPO1
kDa
125
70
XPO1
50
30
B-actin


PEDS_
0002T
shControl
shXPO1
kDa
125
XPO1
70
50
B-actin
30

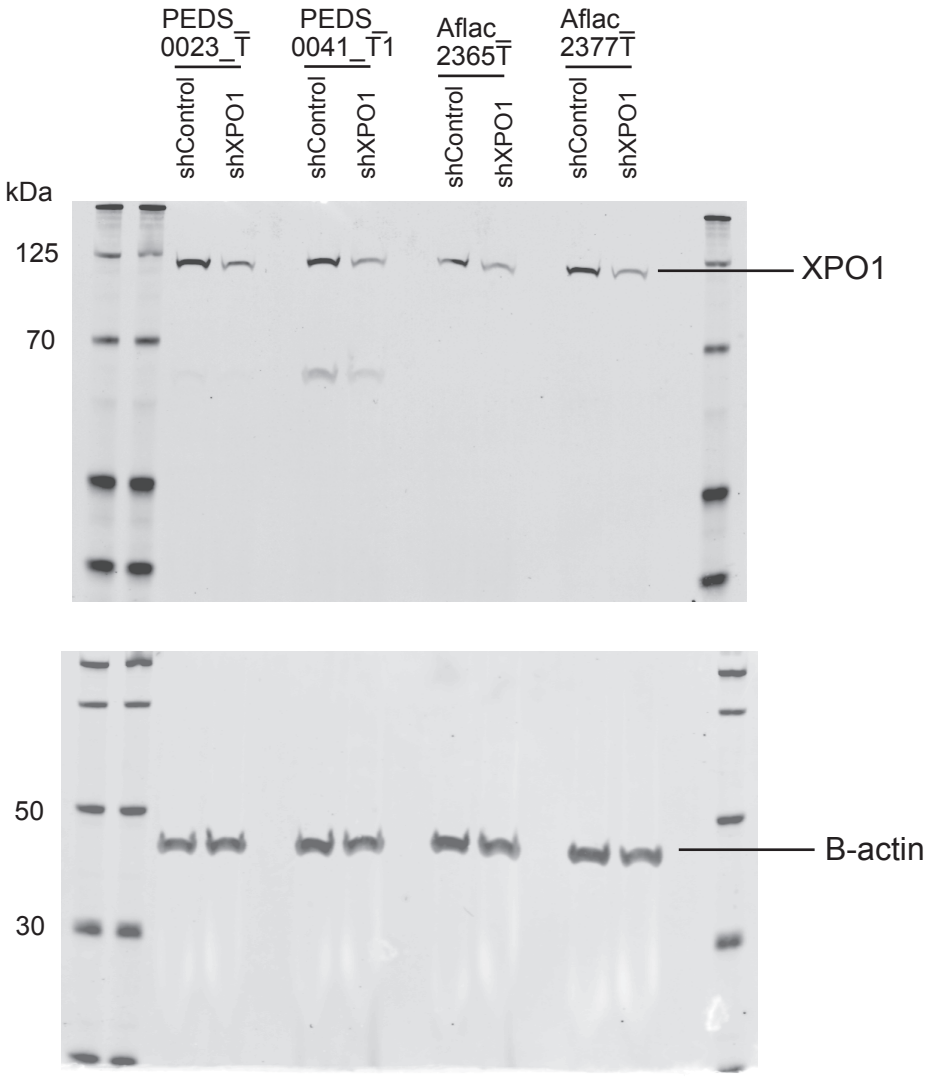
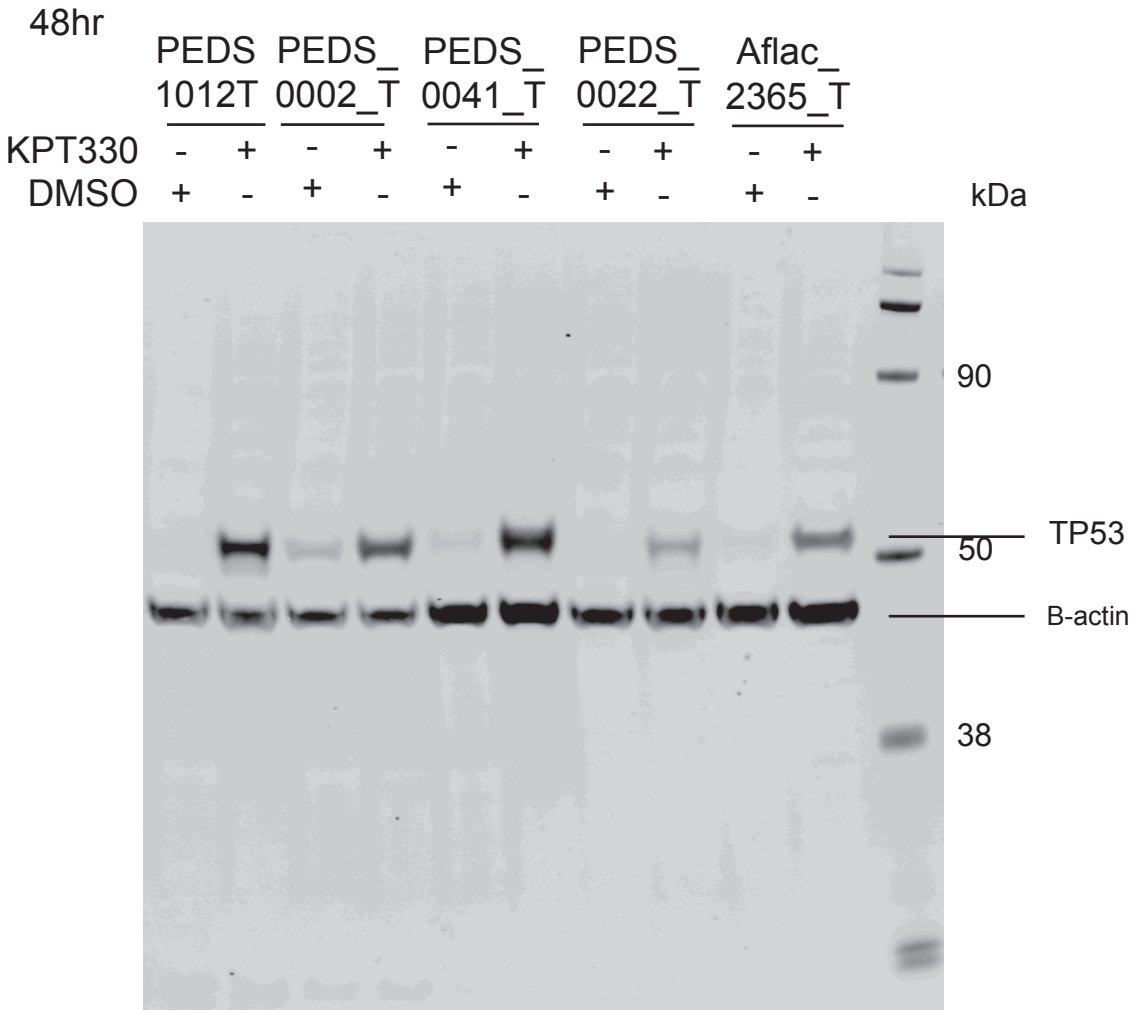

PEDS_
0023_T
PEDS_
0041_T1
Aflac_
2365T
Aflac_
2377T
shControl
shXPO1
shControl
shXPO1
shControl
shXPO1
shControl
shXPO1
kDa
125
XPO1
70
50
B-actin
30

Fig S2C


48hr
PEDS 1012T
PEDS_ 0002_T
PEDS_ 0041_T
PEDS_ 0022_T
Aflac_ 2365_T
KPT330 - + - + - + - + - +
DMSO + - + - + - + - + -
kDa
90
50
38
TP53
B-actin

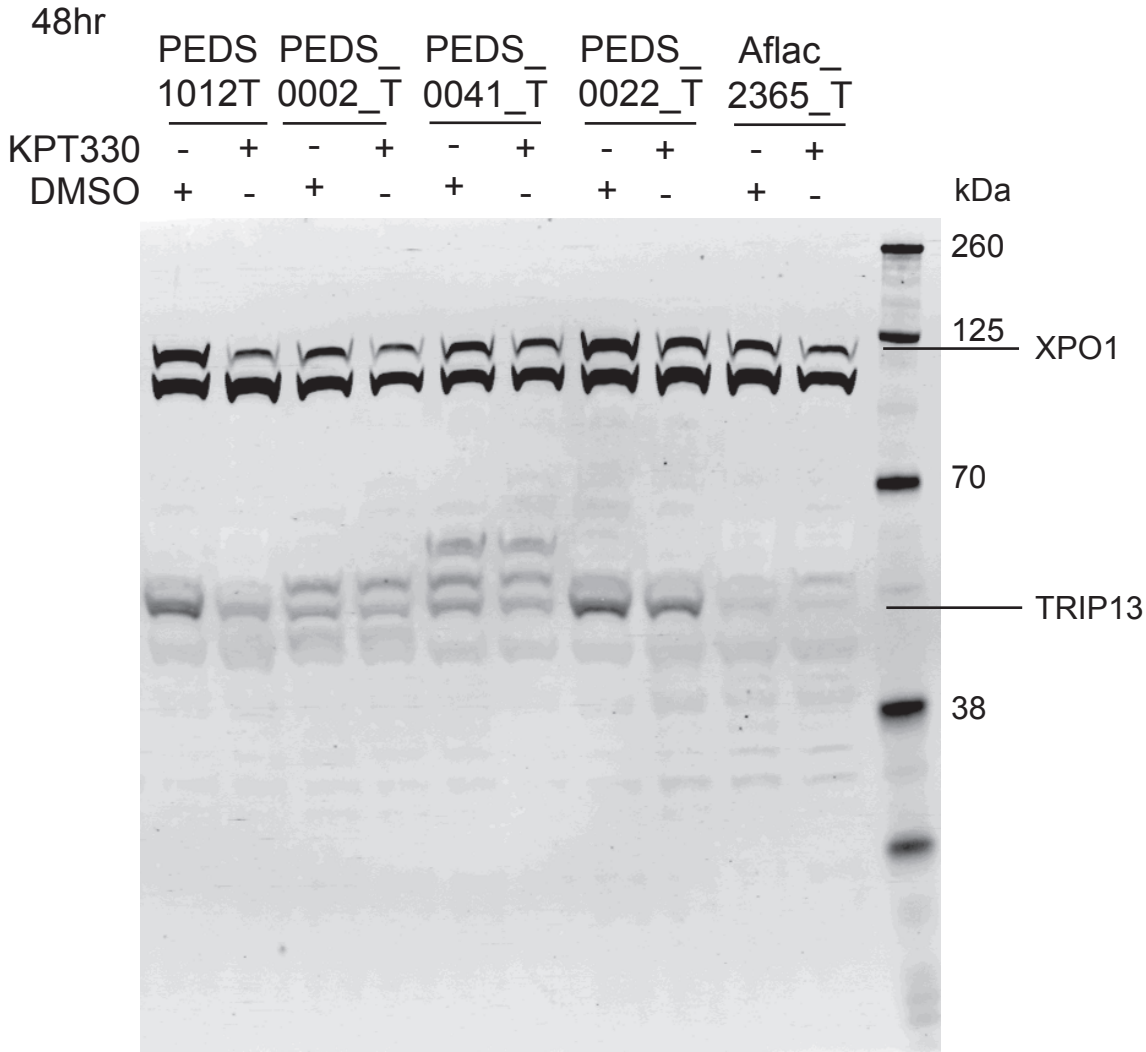

48hr
PEDS 1012T
PEDS_ 0002_T
PEDS_ 0041_T
PEDS_ 0022_T
Aflac_ 2365_T
KPT330 - + - + - + - + - +
DMSO + - + - + - + - + -
kDa
260
125
70
38
XPO1
TRIP13

Fig S4C

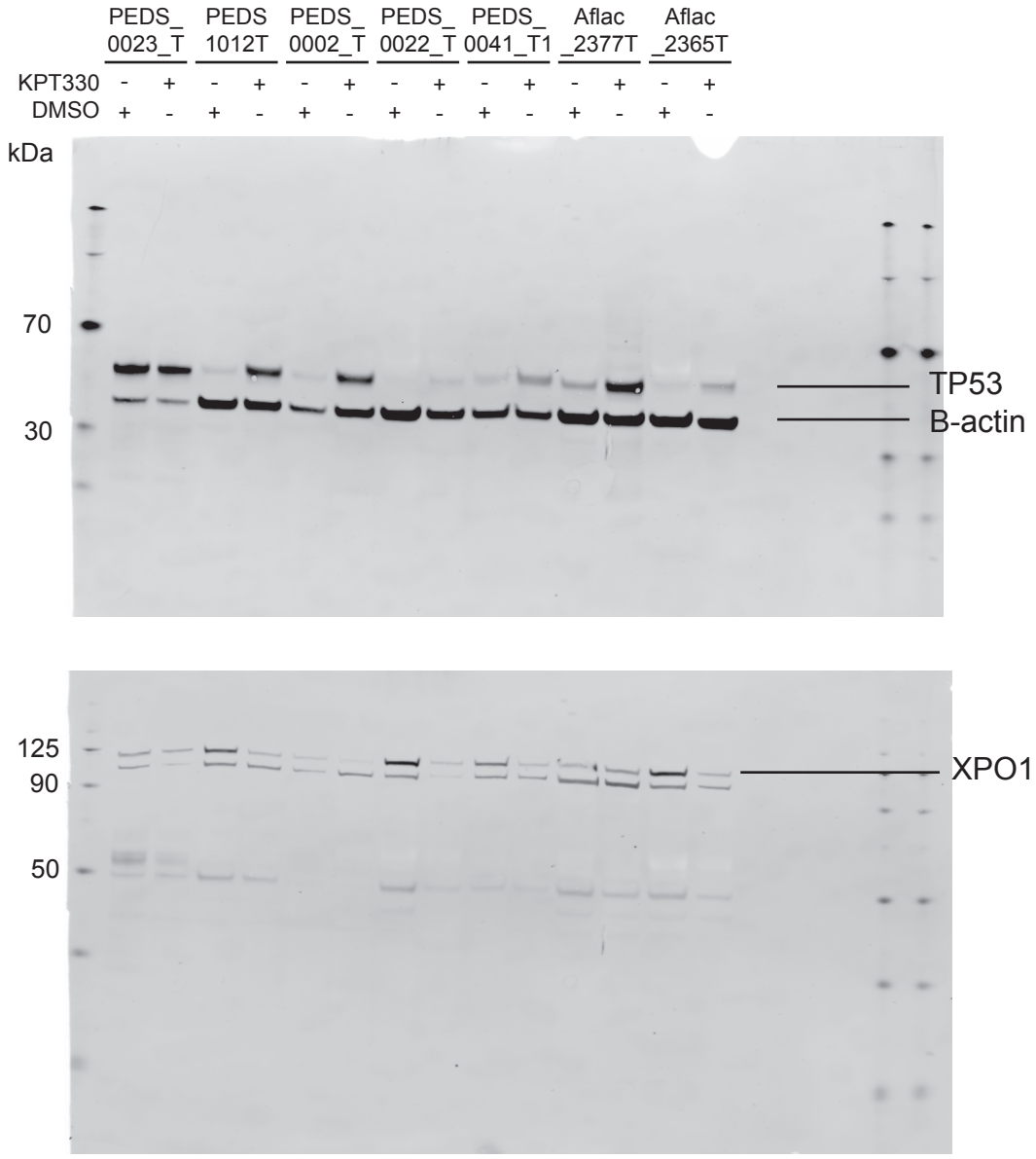


Fig S4D

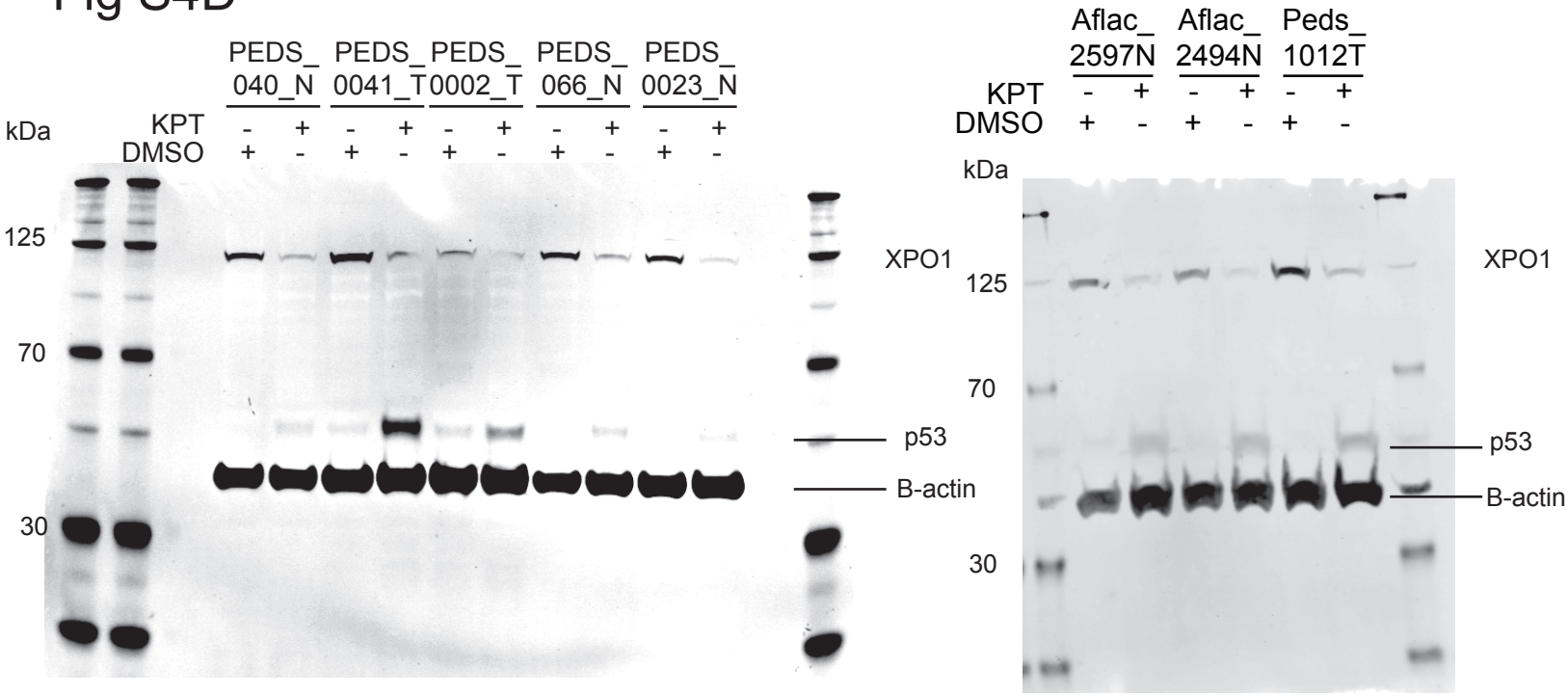


Fig S5A

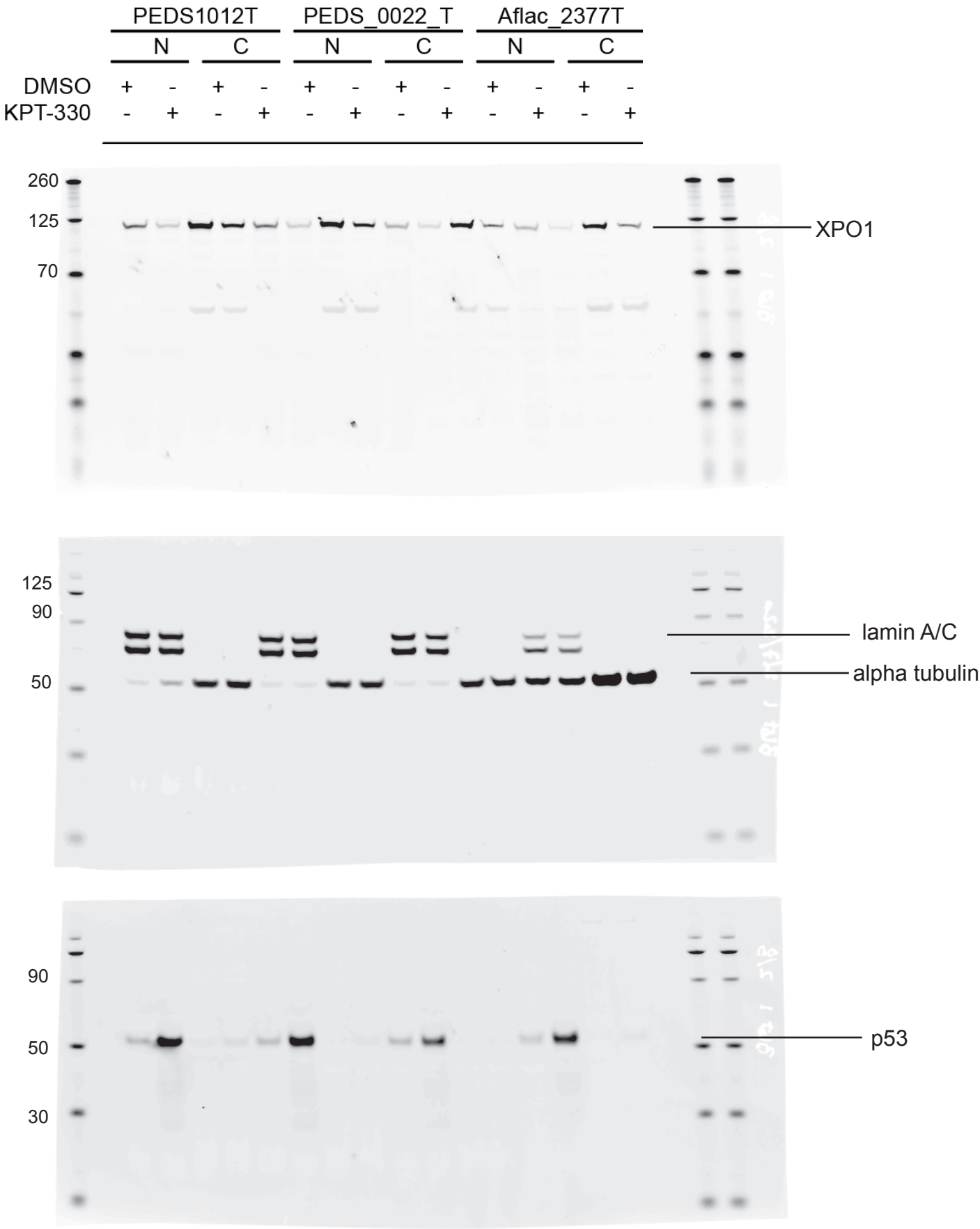

PEDS1012T
PEDS_0022_T
Aflac_2377T
N
C
DMSO
KPT-330
260
125
70
XPO1
125
90
50
lamin A/C
alpha tubulin
90
50
30
p53

Fig S5b

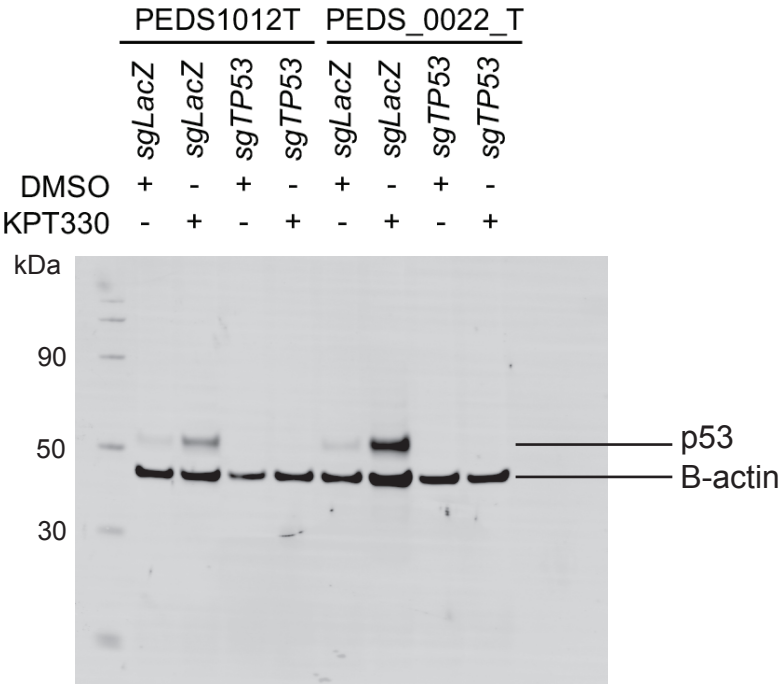


Fig S5e-g

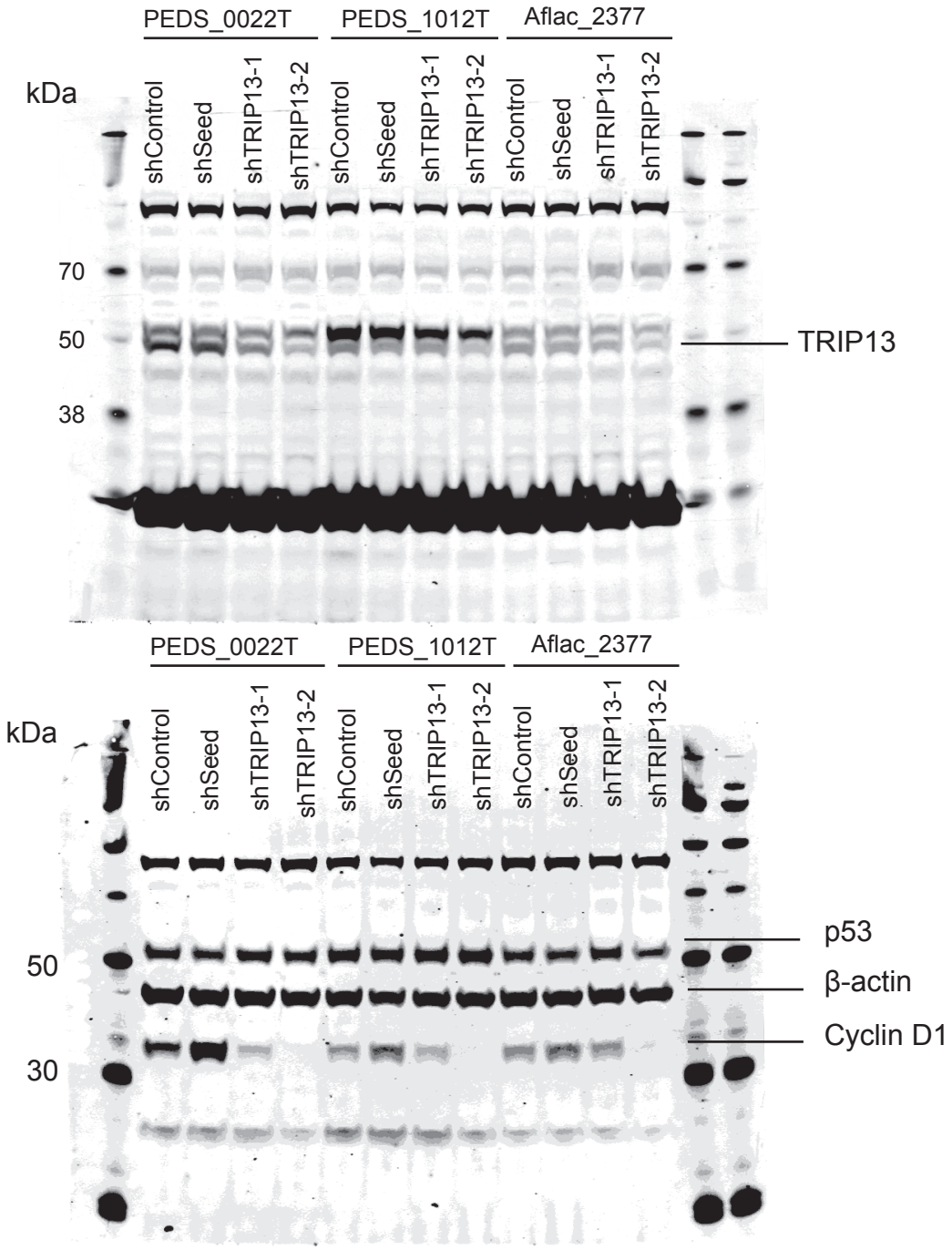
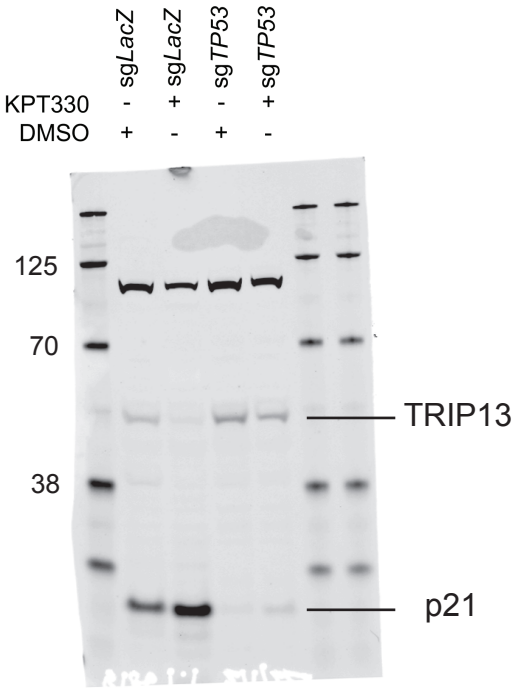
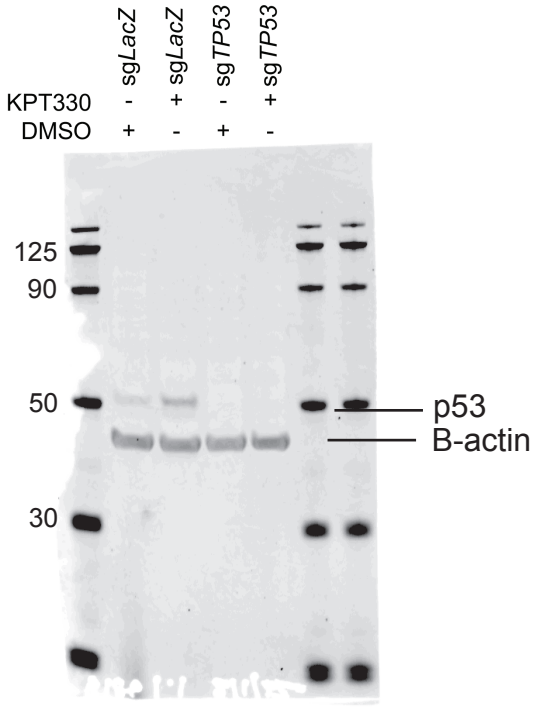


Fig S5I


KPT330
DMSO
- sgLacZ
+ sgLacZ
- sgTP53
+ sgTP53
125
70
38
TRIP13
p21


KPT330
DMSO
- sgLacZ
+ sgLacZ
- sgTP53
+ sgTP53
125
90
50
30
p53
B-actin

Supplementary Table 1:F-Media Components

DMEM	500 mL
DMEM:F12	500 mL
Hydrocortisone/EGF mix	1.0 mL
Insulin (5mg/mL)	1.0 mL
FBS	50 mL
Glutamine	10 mL
Pen/Strep	5 mL
Cholera Toxin (11.7uM)	8.6 uL
Y-27632 (ROCK inhibitor)	0.5 mL

Stock Solutions:

Hydrocortisone/EGF Mix (Sigma # H-0888)	Dissolve hydrocortisone in 100% ethanol to 0.5 ug/mL. Mix 1 mL of this with 19 mL DMEM containing 2.5 ug EGF
Insulin (Sigma # I-550)	Dissolve 100 mg insulin in 20 mL of distilled water contaiing 200 uL glacial acetic acid.
Cholera Toxin (Sigma # C-8052)	Dissolve 1 mg of cholera toxin in 1 mL of distilled water
Y-27632 (Selleck S1049)	Dissolve to stock concentration of 10 mM

Supplementary Table 2: Cell Line Passage Information

Cell Line	Passages before senesc	Media	Detached with	Passage at RNA Sequencing
Aflac_2315_T	14-15	F-media	TrypLE	6
Aflac_2365_T	12-14	F-media	TrypLE	9
Aflac_2377_T	14-15	F-media	TrypLE	4
Aflac_2494_N	10	F-media	TrypLE	3
Aflac_2494_T	38-40	F-media	TrypLE	16
Aflac_2597_N	10	F-media	TrypLE	<5
Aflac_2597_T	9	F-media	TrypLE	<5
PEDS_0002_T	>50	F-media	TrypLE	26
PEDS_0022_N	10	F-media	TrypLE	5
PEDS_0022_T	30	F-media	TrypLE	19
PEDS_0023_T	28-32	F-media	TrypLE	7
PEDS_0023_T2	25-30	F-media	TrypLE	8
PEDS_0041_T1	14-15	F-media	TrypLE	7
PEDS_0041_T2	10	F-media	TrypLE	5
PEDS1012T	40-45	F-media	TrypLE	24

Supplementary Table 3: FACS data

PEDS_1012T	G1			S			G2			< G1			> G2		
DMSO	57.6	60.9	60.8	17.5	17.6	10	17.7	16.5	19.7	7.26	3.52	3.75	0.19	0.049	0.71
KPT330	42.5	47.7	49.4	8.34	7.68	6.84	41.9	35.5	37.7	6.28	5.1	1.73	0.42	0.29	2.25

PEDS_0022_T	G1			S			G2			< G1			> G2		
DMSO	67.3	40.7	64.1	10.9	26.3	13	10.2	13	13.9	3.97	13.4	7.31	0.61	0.85	0.51
KPT330	54.5	70.4	50	3.84	2.17	4.29	35.6	25.2	32.2	1.52	2.2	7.06	0.19	1.62	0.94

Aflac_2377T	G1		S		G2		<G1		>G2	
DMSO	52.5	55.7	18	16.8	13.6	14.5	9.47	8.28	1.48	1.86
KPT330	52.9	61.6	7.83	5.86	24	21.3	10.7	8.86	0.53	0.76

Supp Table 4 - Primer Sequences

Name	Sequence	Type
shTRIP13-1 Fwd	CCGGCGATTATGTGATGACAACTTTCTCGAGAAAGTTGTCATCACATAATCGTTTTTG	shRNA
shTRIP13-1 Rev	AATTCAAAAACGATTATGTGATGACAACTTTCTCGAGAAAGTTGTCATCACATAATCG	shRNA
shTRIP13-2 Fwd	CCGGGCACTGTTGCACTTCACATTTCTCGAGAAATGTGAAGTGCAACAGTGCTTTTTG	shRNA
shTRIP13-2 Rev	AATTCAAAAAGCACTGTTGCACTTCACATTTCTCGAGAAATGTGAAGTGCAACAGTGC	shRNA
shTRIP13-3 Fwd	CCGGCACCTGTAATCCCAGCACTTTCTCGAGAAAGTGCTGGGATTACAGGTGTTTTTG	shRNA - 3'UTR
shTRIP13-3 Rev	AATTCAAAAACACCTGTAATCCCAGCACTTTCTCGAGAAAGTGCTGGGATTACAGGTG	shRNA - 3'UTR
shTRIP13-2 Ctrl Fwd	CCGGGCACTGTTGTGCTTCACATTTCTCGAGAAATGCAAAGTGCAACAGTGCTTTTTG	shRNA Control
shTRIP13-2 Ctrl Rev	AATTCAAAAAGCACTGTTAGTCTTCACATTTCTCGAGAAATGTGAGCAGCAACAGTGC	shRNA Control
TRIP13 Fwd	CGTGCTGATTGATGAGGTGG	qRTPCR
TRIP13 Rev	ACGTCGATCTTCTCGGTGAT	qRTPCR
shXPO1 Ctrl Fwd	CCGGGCTCAAGTTCTACTGACACATCTCGAGGCTCAAGTTCTACTGACACAT TTTTTG	shRNA
shXPO1 Ctrl Rev	AATTCAAAAAGCTCAAGTTCTACTGACACATCTCGAGGCTCAAGTTCTACTGACACAT	shRNA
shXPO1 Fwd	CCGGGCTCAAGAAGTACTGACACATCTCGAGGCTCAAGAAGTACTGACACAT TTTTTG	shRNA
shXPO1 Rev	AATTCAAAAAGCTCAAGAAGTACTGACACATCTCGAGGCTCAAGAAGTACTGACACAT	shRNA
TBP Fwd	TTCGGAGAGTTCTGGGATTGTA	qRTPCR housekeeping
TBP Rev	TGGACTGTTCTTCACTCTTGGC	qRTPCR housekeeping
XPO1 Fwd	GCAGTTGGTTCAATCTCTGGTAAT	qRTPCR
XPO1 Rev	AAATCAAGCAGCTGACGAGC	qRTPCR