Science Translational Medicine
AAAS

Supplementary Materials for

Exportin 1 inhibition prevents neuroendocrine transformation through SOX2 down-regulation in lung and prostate cancers

Alvaro Quintanal-Villalonga *et al.*

Corresponding author: Email: Alvaro Quintanal-Villalonga, quintaa1@mskcc.org; Charles M. Rudin, rudinc@mkcc.org

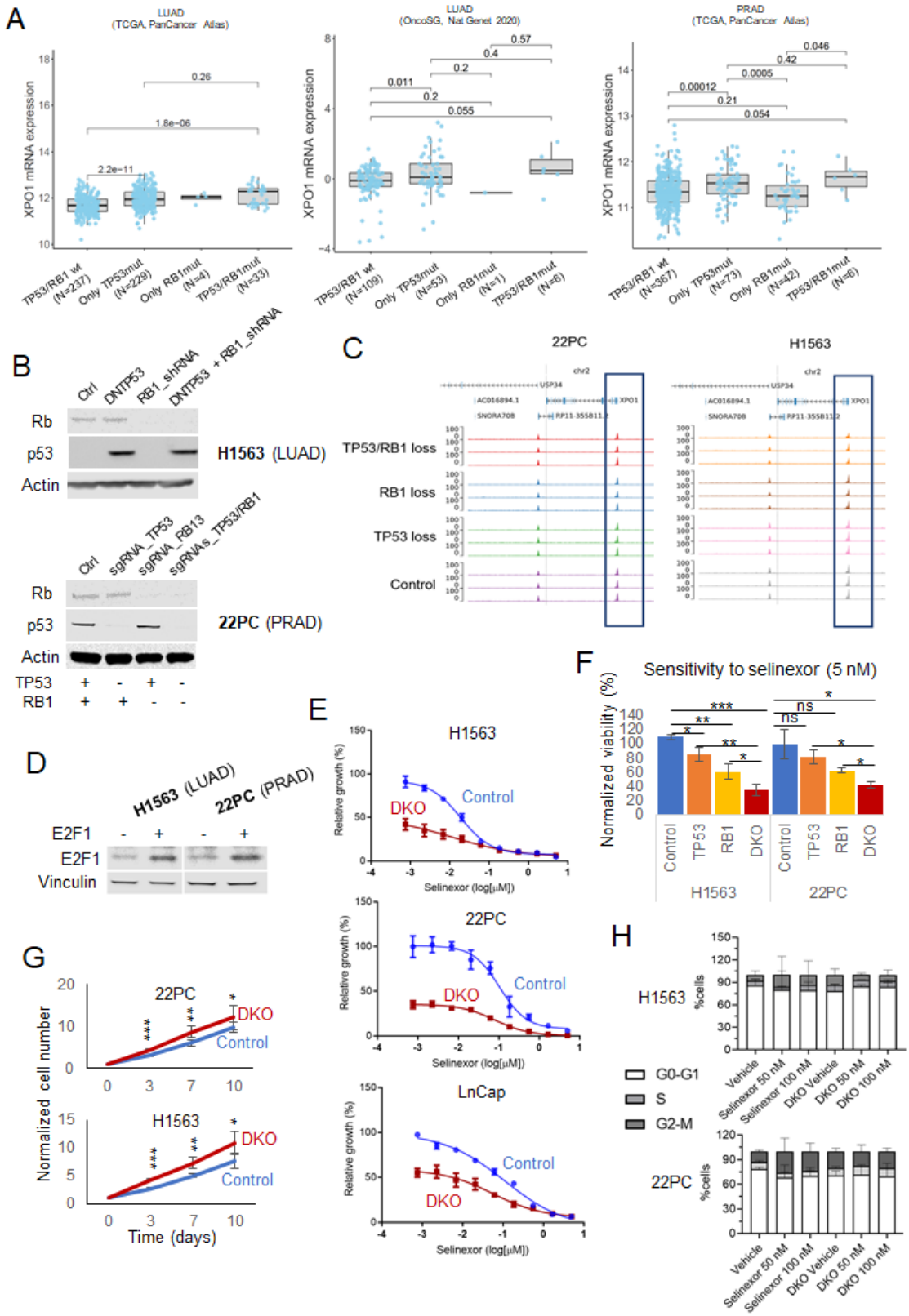
Sci. Transl. Med. **15**, eadf7006 (2023)
DOI: 10.1126/scitranslmed.adf7006

The PDF file includes:

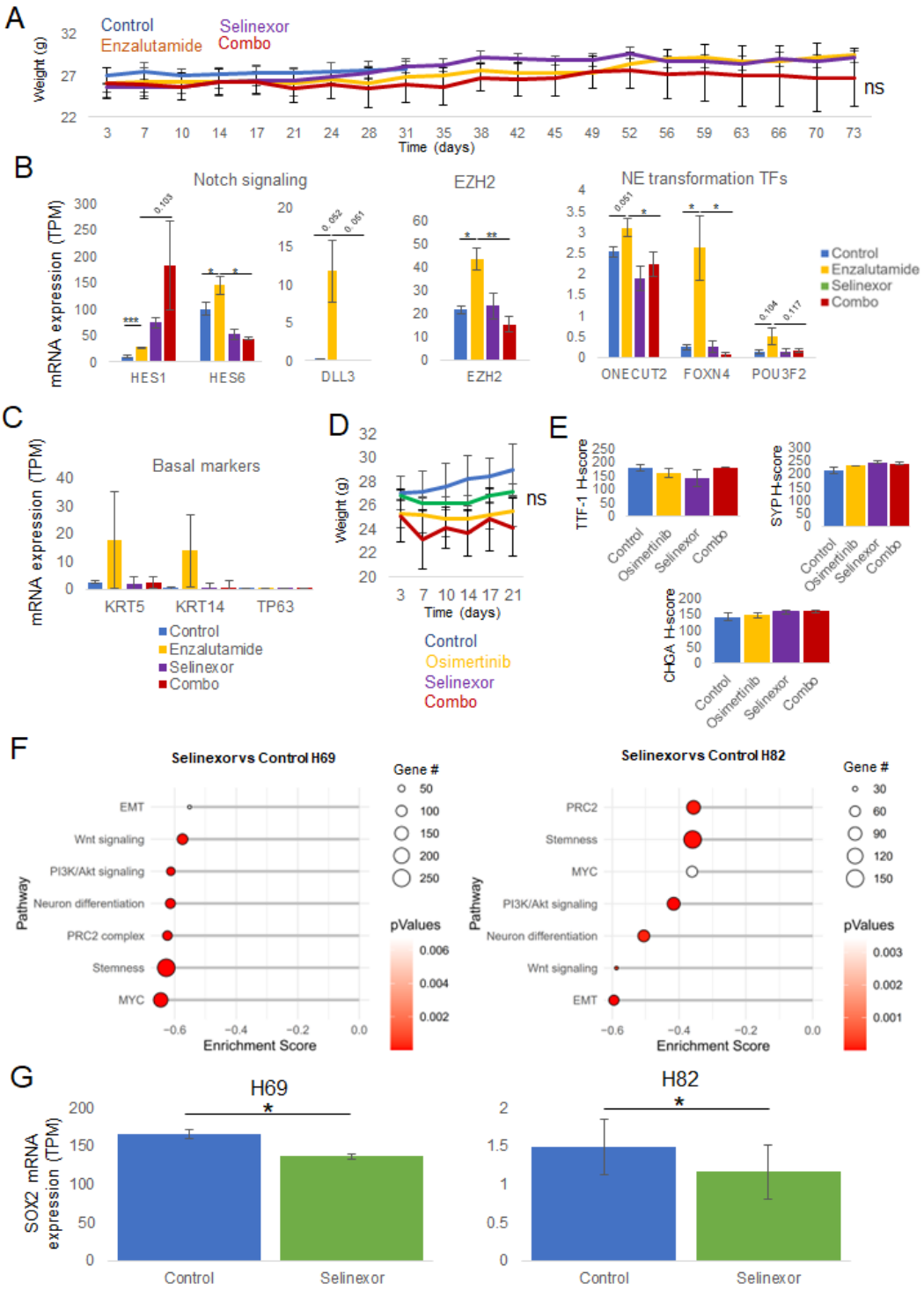
Figs. S1 to S3

Other Supplementary Material for this manuscript includes the following:

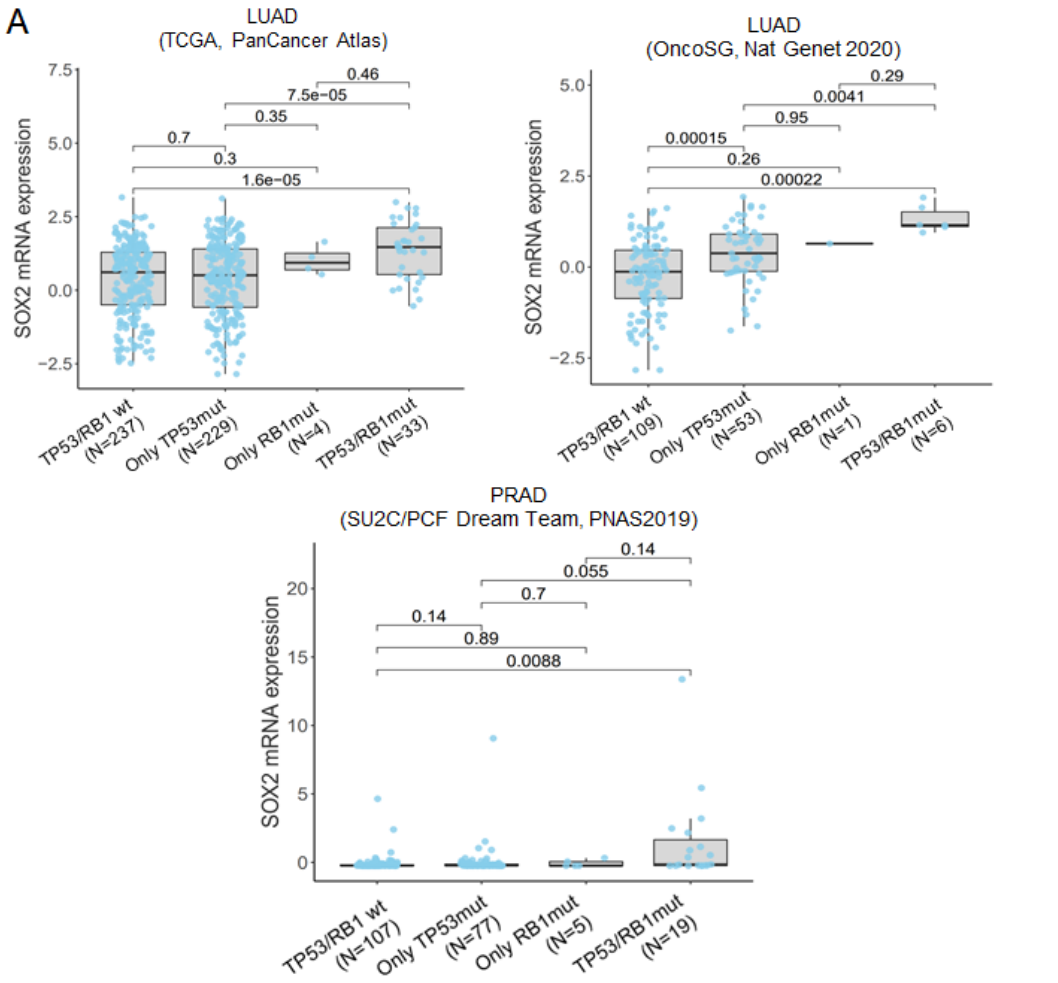
Data file S1
MDAR Reproducibility checklist

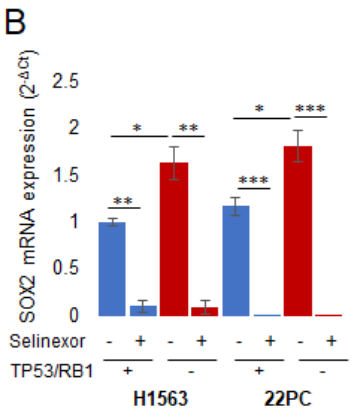

A
LUAD
(TCGA, PanCancer Atlas)
XPO1 mRNA expression
0.26
1.8e−06
2.2e−11
TP53/RB1 wt (N=237)
Only TP53mut (N=229)
Only RB1mut (N=4)
TP53/RB1mut (N=33)
LUAD
(OncoSG, Nat Genet 2020)
0.57
0.4
0.2
0.011
0.2
0.055
TP53/RB1 wt (N=109)
Only TP53mut (N=53)
Only RB1mut (N=1)
TP53/RB1mut (N=8)
PRAD
(TCGA, PanCancer Atlas)
0.046
0.42
0.0005
0.00012
0.21
0.054
TP53/RB1 wt (N=367)
Only TP53mut (N=73)
Only RB1mut (N=42)
TP53/RB1mut (N=6)
B
Ctrl
DNTP53
RB1_shRNA
DNTP53 + RB1_shRNA
Rb
p53
Actin
H1563 (LUAD)
Ctrl
sgRNA_TP53
sgRNA_RB13
sgRNAs_TP53/RB1
22PC (PRAD)
TP53 + - + -
RB1 + + - -
C
22PC
H1563
TP53/RB1 loss
RB1 loss
TP53 loss
Control
D
H1563 (LUAD)
22PC (PRAD)
E2F1 - + - +
E2F1
Vinculin
E
H1563
Control
DKO
Relative growth (%)
Selinexor (log[μM])
22PC
LnCap
F
Sensitivity to selinexor (5 nM)
Normalized viability (%)
Control
TP53
RB1
DKO
H1563
22PC
G
22PC
H1563
DKO
Control
Normalized cell number
Time (days)
H
H1563
22PC
%cells
G0-G1
S
G2-M
Vehicle
Selinexor 50 nM
Selinexor 100 nM
DKO Vehicle
DKO 50 nM
DKO 100 nM

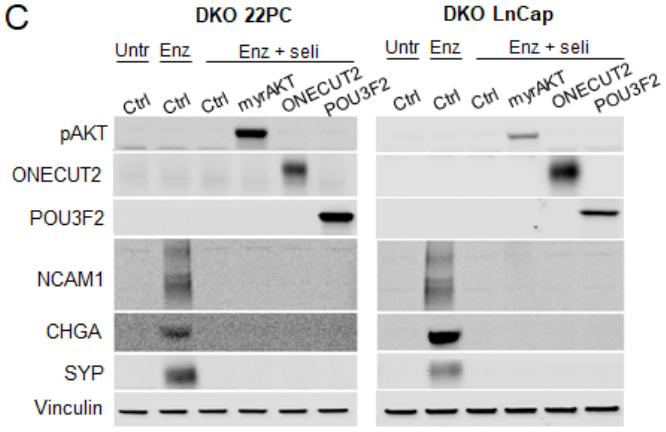
Supplementary Figure S1. (A) *XPO1* mRNA expression in adenocarcinoma clinical specimens, categorized by *TP53/RB1* status. Data obtained from LUAD TCGA (PanCancer, n=237 wild type (wt), 33 mutated), LUAD OncoSG (OncoSG, Nat Genetics 2020, n=109 wt, 6 mutated) and PRAD TCGA (PanCancer, n=367 wt, 6 mutated)*(1, 2)*. (B) Western blots showing TP53 and RB1 expression in our isogenic cell line models with or without induced loss of function of *TP53* and/or *RB1* by shRNA against RB1 and dominant negative TP53 gene overexpression (H1563) or CRISPR/Cas9 knock out (22PC). (C) DNA accessibility ATACseq data from isogenic control and *TP53/RB1*-inactivated H1563 and 22PC isogenic cell lines. The transcription start site for the XPO1 gene is highlighted. (D) Western blot showing E2F1 protein expression in isogenic adenocarcinoma cell lines with ectopic E2F1 overexpression. (E) Dose-response curves for cytotoxic assays of control and *TP53/RB1*-inactivated ("DKO") cell lines with selinexor. (F) Plot showing a representative biological replicate of an experiment assessing viability of control and *TP53*- and/or *RB1-inactivated* H1563 and 22PC cells treated with 5 nM selinexor. (G) Plots showing proliferation of control and *TP53/RB1*-inactivated H1563 and 22PC isogenic cell lines (H) Barplots showing cell cycle analyses on control and *TP53/RB1*-inactivated ("DKO") cell lines, with or without selinexor 4-day treatment at the concentrations stated. p-values were calculated using the Student's t-test (unpaired, heterogeneous variances, two-tailed). p-value legend: *<0.05, **<0.01, ***<0.001.


A
Control
Enzalutamide
Selinexor
Combo
Weight (g)
ns
Time (days)
B
Notch signaling
EZH2
NE transformation TFs
mRNA expression (TPM)
HES1
HES6
DLL3
EZH2
ONECUT2
FOXN4
POU3F2
Control
Enzalutamide
Selinexor
Combo
C
Basal markers
mRNA expression (TPM)
KRT5
KRT14
TP63
Control
Enzalutamide
Selinexor
Combo
D
Weight (g)
ns
Time (days)
Control
Osimertinib
Selinexor
Combo
E
TTF-1 H-score
SYP H-score
CHGA H-score
Control
Osimertinib
Selinexor
Combo
F
Selinexor vs Control H69
Selinexor vs Control H82
Gene #
pValues
Pathway
EMT
Wnt signaling
PI3K/Akt signaling
Neuron differentiation
PRC2 complex
Stemness
MYC
Enrichment Score
G
H69
H82
SOX2 mRNA expression (TPM)
Control
Selinexor

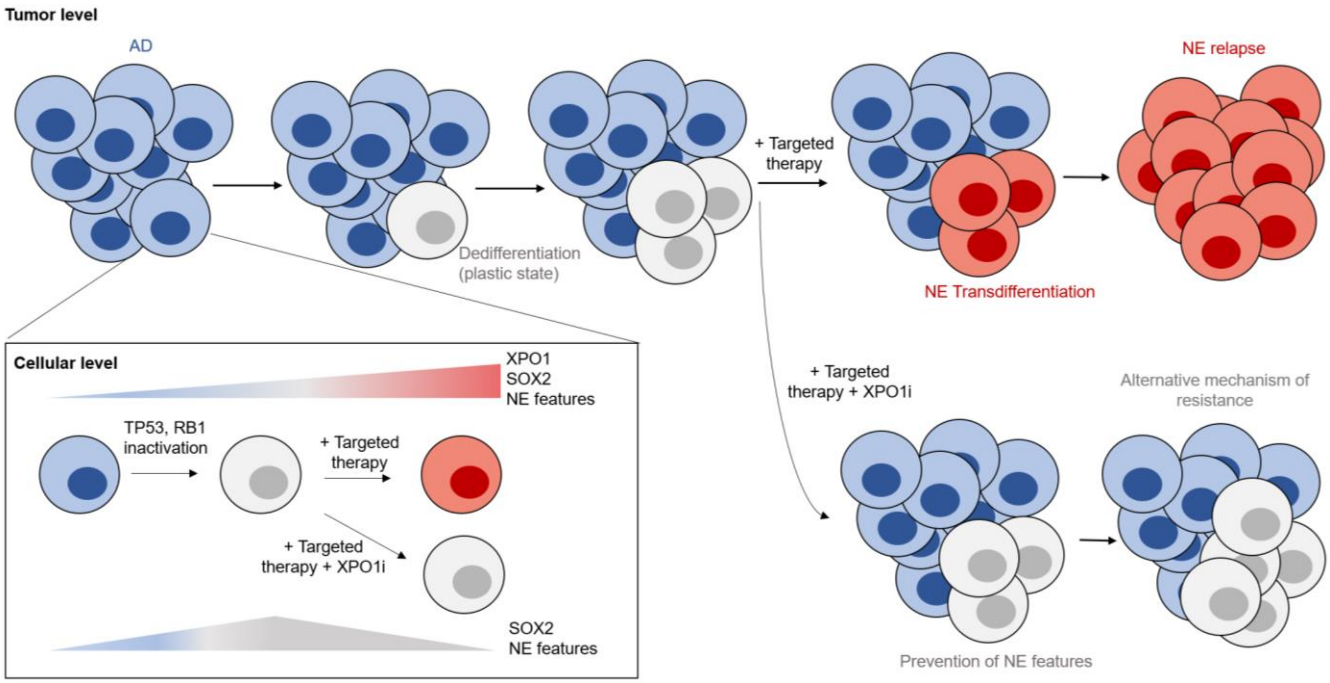
Supplementary Figure S2. (A) Body weight for mice treated with enzalutamide, selinexor or their combination in experiment shown in Figure 4A. RNA sequencing data from tumors from Figure 4A collected at control arm experimental endpoint (day 31), showing mRNA expression levels for genes of interest, involved in NE transformation (B) or basal markers (C), divided by treatment arm (n=4, 3, 3 and 3 tumors for the control, enzalutamide-, selinexor- and combo-treated tumors). In vivo treatment of cell line xenografts for *TP53/RB1* DKO 22PC PRAD cells was performed with enzalutamide, selinexor or their combination. 5-10 female athymic nude mice were subcutaneously engrafted per treatment arm and until tumors reached 100-150 mm^3. At that point, mice were randomized into groups and treated with either vehicle (n=7 for 22PC and n=4 for LnCap/AR), selinexor (10 mg/kg p.o. QDx3, n=7 for 22PC and n=4 for LnCap/AR), enzalutamide (10 mg/kg p.o. QDx5, n=7 for 22PC and n=5 for LnCap/AR), or the combinations of enzalutamide + selinexor at the previously mentioned doses (n=9 for 22PC and n=5 for LnCap/AR). Mice weights and tumor volumes were measured twice a week and mice were sacrificed when tumors reached humane endpoint (volume = 1000 mm^3). (D) Body weight for mice treated with osimertinib, selinexor or their combination in experiment shown in Figure 4I. (E) H-scores for IHC assessment of TTF-1, SYP and CHGA in MSK_Lx_151 tumors from Figure 4I. (F) Pathway enrichment analysis on DEGs from selinexor-treated versus control untreated *de novo* SCLC cell lines H69 and H82. Data was obtained from Quintanal-Villalonga et al., 2022*(3)*. (G) SOX2 mRNA expression on control and selinexor-treated de novo SCLC cell lines H69 and H82. Data for (F) and (G) was obtained from *(3)*. p-values were calculated using the Student's t-test (unpaired, heterogeneous variances, two-tailed). p-value legend: *<0.05, **<0.01, ***<0.001.


A
LUAD
(TCGA, PanCancer Atlas)
SOX2 mRNA expression
0.46
7.5e−05
0.35
0.7
0.3
1.6e−05
TP53/RB1 wt (N=237)
Only TP53mut (N=229)
Only RB1mut (N=4)
TP53/RB1mut (N=33)
LUAD
(OncoSG, Nat Genet 2020)
SOX2 mRNA expression
0.29
0.0041
0.95
0.00015
0.26
0.00022
TP53/RB1 wt (N=109)
Only TP53mut (N=53)
Only RB1mut (N=1)
TP53/RB1mut (N=6)
PRAD
(SU2C/PCF Dream Team, PNAS2019)
SOX2 mRNA expression
0.14
0.055
0.7
0.14
0.89
0.0088
TP53/RB1 wt (N=107)
Only TP53mut (N=77)
Only RB1mut (N=5)
TP53/RB1mut (N=19)


B
SOX2 mRNA expression (2^-ΔCt)
Selinexor
TP53/RB1
H1563
22PC


C
DKO 22PC
DKO LnCap
Untr
Enz
Enz + seli
Ctrl
myrAKT
ONECUT2
POU3F2
pAKT
ONECUT2
POU3F2
NCAM1
CHGA
SYP
Vinculin

Supplementary Figure S3. (A) *SOX2* mRNA expression in adenocarcinoma clinical specimens, categorized by *TP53/RB1* status. Data obtained from LUAD TCGA (PanCancer, n=237 wt and 33 mutated), LUAD OncoSG (OncoSG, Nat Genetics 2020, n=109 wt and mutated) and PRAD TCGA (PanCancer, n=107 wt and 19 mutated)*(1, 2)*. (B) Barplot showing mRNA expression levels of SOX2 mRNA expression in control and TP53/RB1-inactivated isogenic H1563 (LUAD) and 22PC (PRAD) cell lines treated with selinexor (5 nM, 4 days). (C) Western blot assessment of NE markers in DKO 22PC and LnCap cell lines treated with enzalutamide or enzalutamide + selinexor, including control, myrAKT-, ONECUT2- and POU3F3-overexpressing cell lines. p-values were calculated using the Student's t-test (unpaired, heterogeneous variances, two-tailed). p-value legend: *<0.05, **<0.01, ***<0.001.


Tumor level
AD
Dedifferentiation
(plastic state)
+ Targeted
therapy
NE relapse
NE Transdifferentiation
Cellular level
XPO1
SOX2
NE features
TP53, RB1
inactivation
+ Targeted
therapy
+ Targeted
therapy + XPO1i
SOX2
NE features
+ Targeted
therapy + XPO1i
Alternative mechanism of
resistance
Prevention of NE features

Supplementary Figure S4. Model of XPO1-dependent NE transformation. Loss of *TP53* and *RB1* function may induce a dedifferentiated, plastic state characterized by concomitant *XPO1* and *XPO1*-dependent *SOX2* upregulation, with higher potential for NE transdifferentiation, particularly under the action of targeted therapy. Combination of XPO1 inhibitors with targeted therapy downregulates SOX2 expression, required for NE transdifferentiation, thus interfering with NE relapse and potentially leading to mechanisms of resistances to targeted therapy alternative to NE transformation.