Expanded View Figures

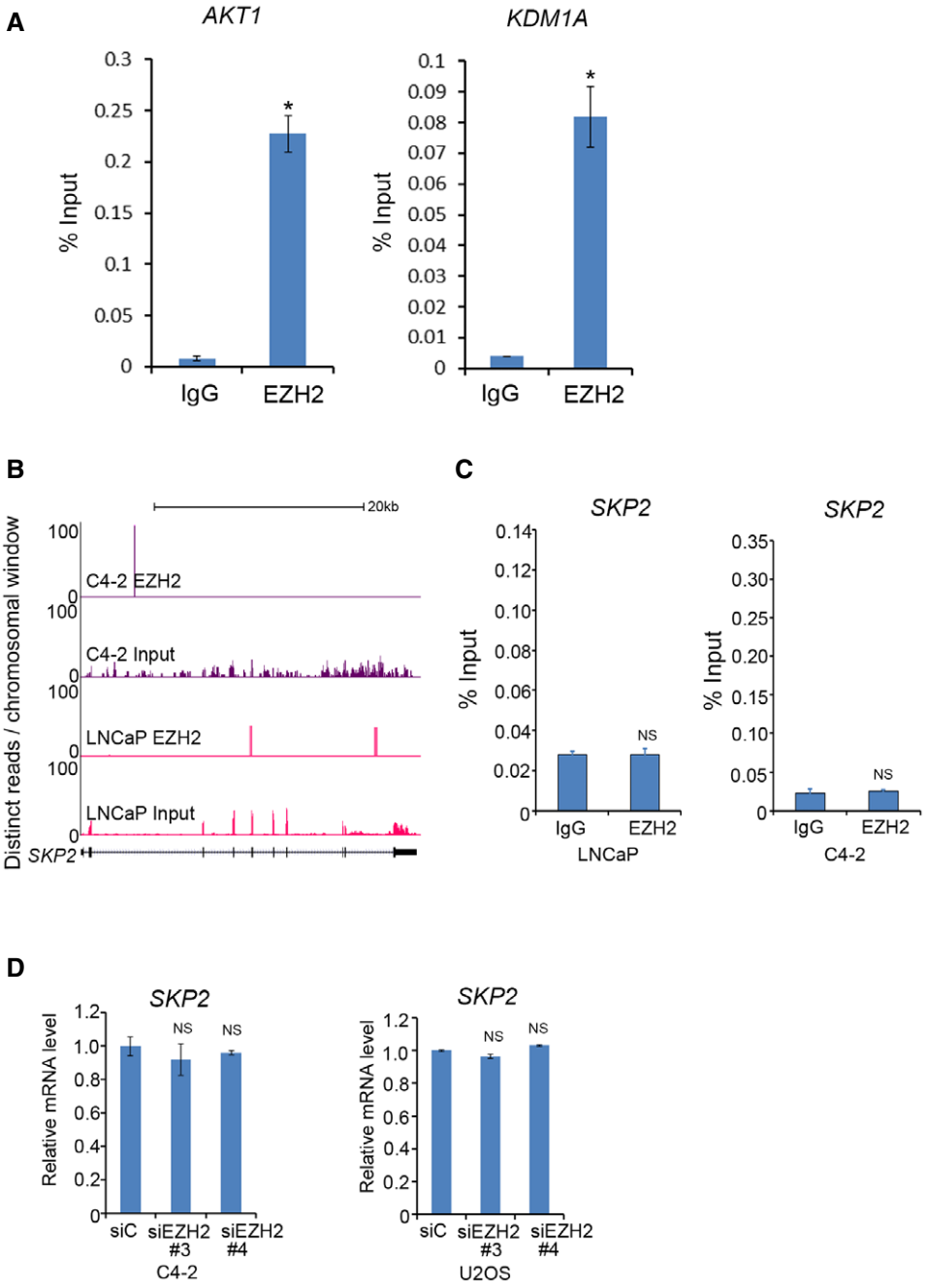


Figure EV1. Assessment of binding and regulation of mRNAs by EZH2. Related to Fig 1.

A RT–qPCR analysis of mRNAs of AKT1 and KDM1A immunoprecipitated by IgG or EZH2 antibody in C4-2 cells. These genes are on the top of the list of mRNAs highly enriched by anti-EZH2 antibody. Data shown are mean values ± SD from three replicates. *$P < 0.01$.

B Screen shots from UCSC genome browser showing signal profiles of SKP2 mRNA immunoprecipitated by anti-EZH2 antibody in LNCaP and C4-2 cells. Total RNA was used as the input control of RIP-seq.

C RIP-qPCR analysis of SKP2 mRNA immunoprecipitated by IgG and anti-EZH2 antibody in LNCaP and C4-2 cells. Data shown are mean values ± SD from three replicates.

D Effect of EZH2 on SKP2 mRNA expression. C4-2 prostate cancer and U2OS osteosarcoma cell lines were transfected with non-specific control (siC) or two independent EZH2-specific siRNAs. 48-h post-transfection expression of SKP2 mRNA was measured by RT–qPCR. Error bar showing SD from three replicates. NS, no significance.

Figure EV2. EZH2 regulation of expression of p53 downstream target genes. Related to Fig 1.

A EZH2 fragment binding to p53 5′UTR determined by RNA EMSA. Different doses of GST-EZH2 recombinant proteins (GST-EZ3) were incubated with 1 μg of biotin-labeled p53 mRNA 5′UTR probe for 1 h on ice. The RNA–protein complex (RPC) was detected by PAGE followed by immune blotting with HRP-conjugated streptavidin.

B pcDNA3.1-based expression vectors for Flag-p53 FL and/or Flag-p53/47 in combination with empty vector or Myc-EZH2 were transfected into PC3 cells. Forty-eight hours after transfection cells was lysed in RIPA buffer for Western blots with indicated antibodies. ERK2, a loading control.

C PC3 cells were transfected with indicated plasmids. Forty-eight hours after transfection cells was lysed for Western blot.

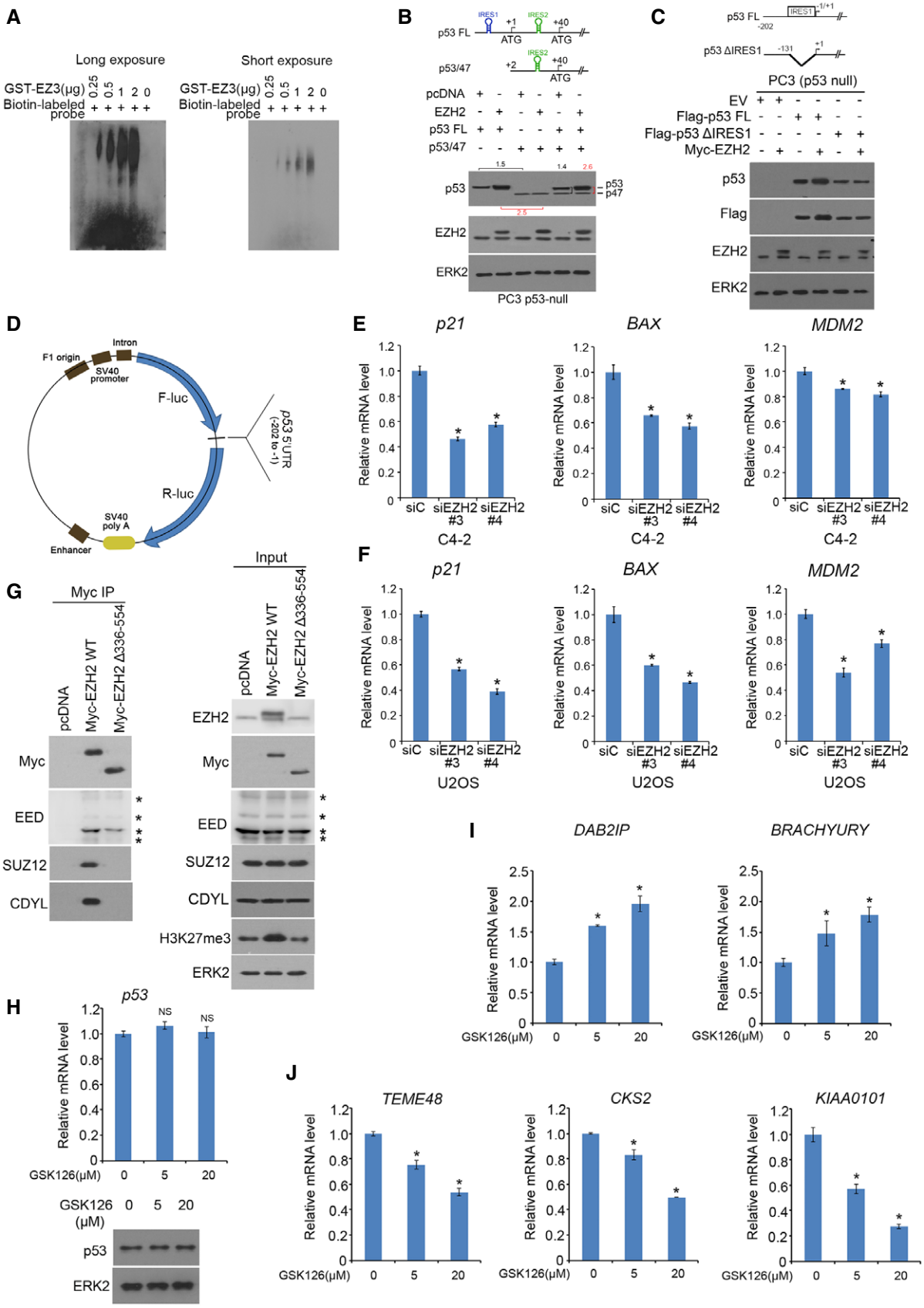
D Diagram of the map for *pp53*-5′UTR-F/Rluc vector. F means firefly luciferase gene. R means Renilla luciferase gene.

E, F Expression of mRNAs for $p21^{CIP1}$, *BAX*, and *MDM2* genes was measured by RT–qPCR in C4-2 (E) and U2OS (F) cells 48 h after transfection with non-specific control (siC) or two independent EZH2-specific siRNAs. *GAPDH* was used as internal control. Data shown are mean values ± SD (error bar) from three replicates. $*P < 0.01$ comparing EZH2 siRNA-transfected with siC-transfected cells.

G C4-2 cells were transfected with indicated plasmids for 24 h, and cells were harvested for co-IP and Western blot analysis. Asterisks indicate different EED isoforms.

H–J C4-2 cells treated with vehicle (DMSO) or different concentrations (5 and 20 μM) of GSK126 for 24 h were harvested for analysis of expression of p53 mRNA and protein using RT–qPCR and Western blot, respectively (H), mRNA expression of EZH2 repressed genes *DAB2IP* and *BRACHYURY* (I), and EZH2-activated genes *TEME48*, *CKS2*, and *KIAA0101* (J). The *GAPDH* was used as internal control. Data shown are mean values ± SD (error bar) from three replicates. $*P < 0.01$ comparing GSK126-treated with mock-treated cells.

Source data are available online for this figure.


A
Long exposure
Short exposure
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Biotin-labeled probe + + + + +
B
p53 FL
p53/47
IRES1
IRES2
ATG
pcDNA
EZH2
p53 FL
p53/47
p53
p47
EZH2
ERK2
PC3 p53-null
C
p53 FL
p53 ΔIRES1
PC3 (p53 null)
EV
Flag-p53 FL
Flag-p53 ΔIRES1
Myc-EZH2
p53
Flag
EZH2
ERK2
D
Intron
F1 origin
SV40 promoter
F-luc
R-luc
SV40 poly A
Enhancer
p53 5'UTR (-202 to -1)
E
p21
BAX
MDM2
Relative mRNA level
siC
siEZH2 #3
siEZH2 #4
C4-2
F
U2OS
G
Myc IP
Input
pcDNA
Myc-EZH2 WT
Myc-EZH2 Δ336-554
Myc
EED
SUZ12
CDYL
EZH2
H3K27me3
ERK2
H
p53
NS
GSK126(μM) 0 5 20
I
DAB2IP
BRACHYURY
J
TEME48
CKS2
KIAA0101

Figure EV2.

Figure EV3. The effect of the EZH2-specific enzymatic inhibitor GSK126 on p53 mRNA expression and stability. Related to Fig 3.

A C4-2 cells transfected with Myc-EZH2 WT for 24 h were lysed for co-IP and Western blot analysis.

B C4-2 cells were lysed in IP buffer and treated with DNaseI in 37°C for 15 min followed by co-IP and Western blot analysis. Genomic DNA in input samples was assessed by PCR using primers for *TP53* promoter.

C GST, GST-eIF4G2, and GST-PABP1 proteins were purified from BL21 bacteria. Myc-EZH2 was produced using Quick coupled transcription/translation kit through T7 promoter *in vitro*. p53 full-length (FL) mRNA was transcribed by T7 enzyme *in vitro*. These purified proteins were used for GST pull-down assay in the presence or absence of p53 FL mRNA, followed by Western blot analysis.

D UV-RIP-qPCR analysis of p53 mRNA immunoprecipitated by IgG, anti-EED, anti-SUZ12, or anti-EZH2 antibody from lysate of C4-2 cells treated with 4SU for 8 h. Data shown are mean values ± SD from three replicates. $^{**}P < 0.01$ comparing EZH2 IP with IgG IP. NS, no significance.

E C4-2 (Left) and VCaP (Right) cells were infected with lentivirus for non-specific control (sh-Control) or two independent EED (or SUZ12)-specific shRNAs for 48 h followed by Western blot analysis. ERK2, a loading control.

F RNA was extracted from sucrose gradient fractions of C4-2 cell lysate. 40S, 60S, 80S, and polyribosomes were indicated above fraction numbers. A254, absorbance at 254 nm.

G C4-2 cells were infected with lentivirus for si-control or siEZH2#3 or siEZH2#4 for 48 h followed by RT–PCR analysis of p53 pre-mRNA expression (Left) and ChIP-PCR analysis of Pol II (N20) occupancy (Right).

H Control or EZH2 knockdown C4-2 cells were treated with cycloheximide (CHX, 50 mg/ml) for different periods of time and harvested for Western blot analysis. β-TUBULIN, a loading control.

I Control or EZH2 knockdown C4-2 cells were treated with MG132 (20 mg/ml) or chloroquine (CQ 25 mg/ml) for 8 h followed by Western blot analysis. β-TUBULIN, a loading control.

J Expression of p53 mRNA was measured by RT–qPCR in C4-2 cells 48 h after transfection with non-specific control (siC) or two independent EZH2-specific siRNAs. GAPDH was used as internal control. Data shown are mean values ± SD from three replicates. $^{*}P < 0.01$.

K Expression of p53 mRNA was measured by RT–qPCR in C4-2 cells 48 h after transfection with non-specific control (siC) or EZH2-specific siRNAs in combination with or without empty vector (EV) or siRNA-resistant Myc-EZH2 wild-type (WT) or mutants. GAPDH was used as an internal control. Data shown are mean values ± SD from three replicates. $^{*}P < 0.01$.

L, M p53 (L) and SKP2 (M) mRNA stability was assessed in C4-2 and U2OS cells transfected with non-specific siRNA (siC) or EZH2-specific siRNA followed by actinomycin D treatment. GAPDH was used as internal control. Mean values ± SD from three replicates. $^{*}P < 0.01$.

Source data are available online for this figure.

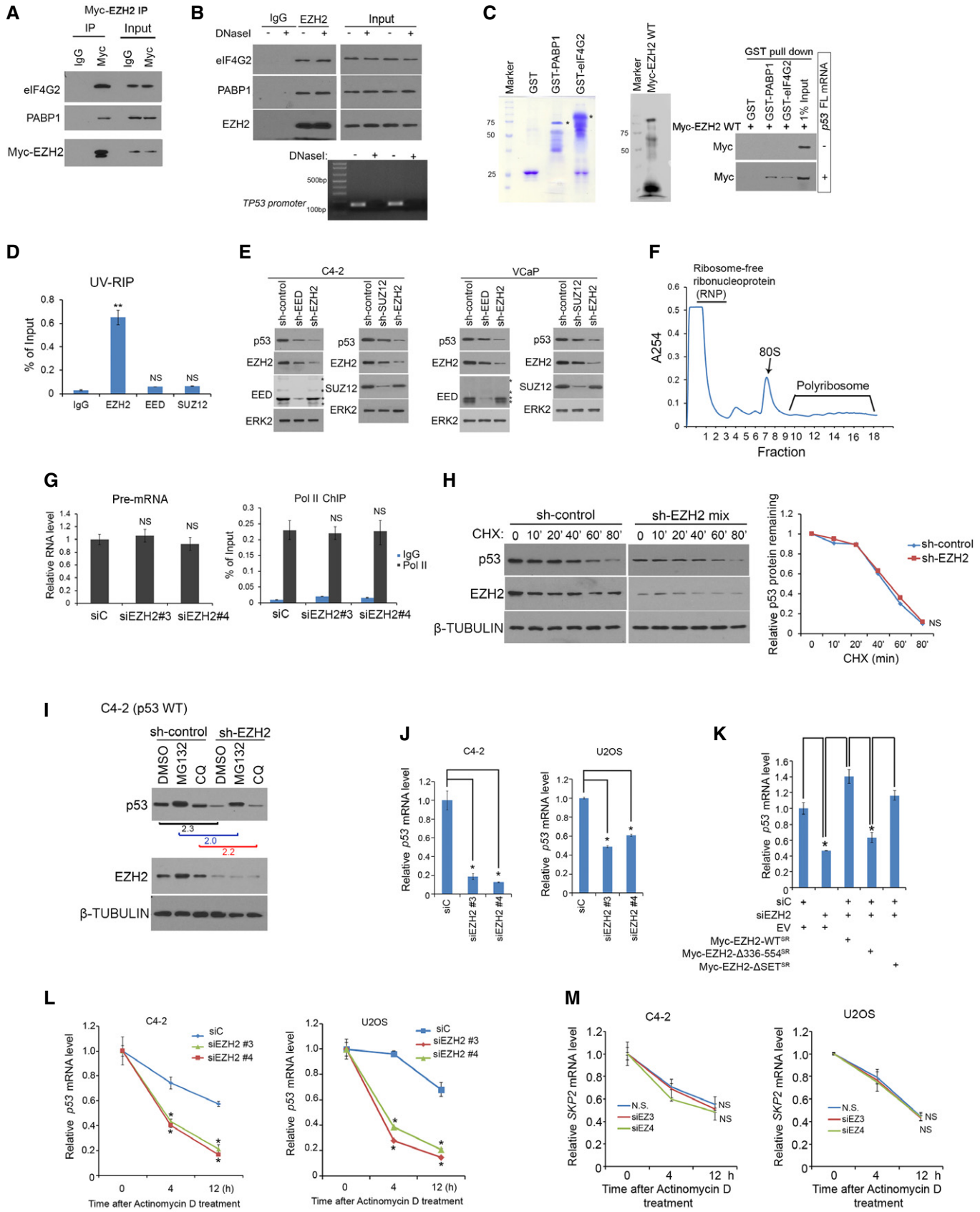
A
Myc-EZH2 IP
IP
Input
IgG
Myc
eIF4G2
PABP1
Myc-EZH2
B
IgG
EZH2
Input
DNaseI
eIF4G2
PABP1
EZH2
DNaseI:
500bp
100bp
TP53 promoter
C
Marker
GST
GST-PABP1
GST-eIF4G2
Marker
Myc-EZH2 WT
GST pull down
GST
+ GST-PABP1
+ GST-eIF4G2
+ 1% Input
p53 FL mRNA
Myc-EZH2 WT
Myc
D
UV-RIP
% of Input
IgG
EZH2
EED
SUZ12
NS
E
C4-2
VCaP
sh-control
sh-EED
sh-EZH2
sh-SUZ12
p53
EZH2
EED
SUZ12
ERK2
F
Ribosome-free ribonucleoprotein (RNP)
80S
Polyribosome
A254
Fraction
G
Pre-mRNA
Relative RNA level
Pol II ChIP
% of Input
IgG
Pol II
siC
siEZH2#3
siEZH2#4
H
sh-control
sh-EZH2 mix
CHX: 0 10' 20' 40' 60' 80'
p53
EZH2
β-TUBULIN
Relative p53 protein remaining
sh-control
sh-EZH2
CHX (min)
I
C4-2 (p53 WT)
sh-control
sh-EZH2
DMSO
MG132
CQ
p53
EZH2
β-TUBULIN
J
C4-2
U2OS
Relative p53 mRNA level
siC
siEZH2 #3
siEZH2 #4
K
Relative p53 mRNA level
siC
siEZH2
EV
Myc-EZH2-WTSR
Myc-EZH2-Δ336-554SR
Myc-EZH2-ΔSETSR
L
C4-2
U2OS
Relative p53 mRNA level
siC
siEZH2 #3
siEZH2 #4
Time after Actinomycin D treatment
M
C4-2
U2OS
Relative SKP2 mRNA level
N.S.
siEZ3
siEZ4
NS
Time after Actinomycin D treatment

Figure EV3.

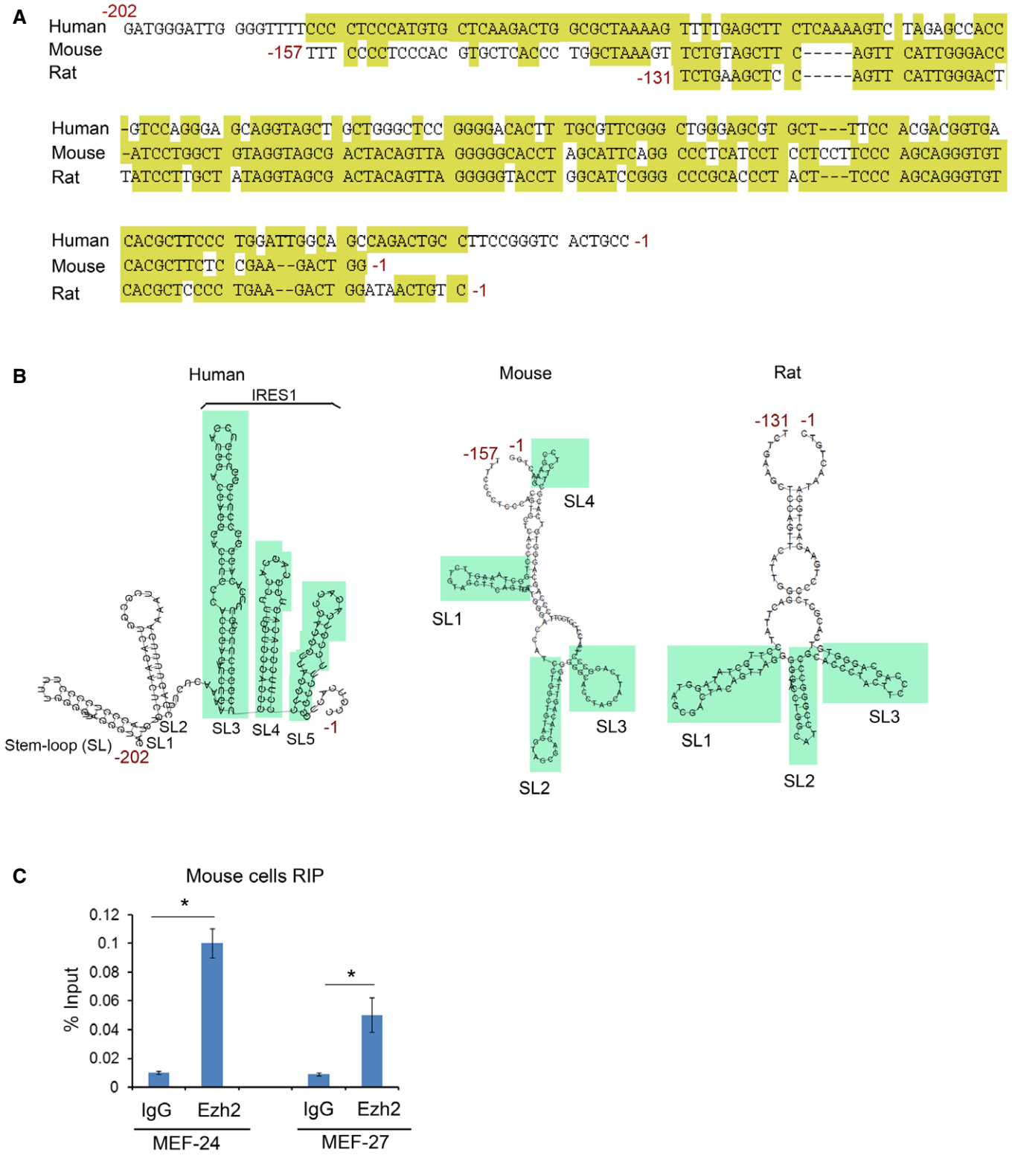

Figure EV4. Ezh2 binds to p53 mRNA in murine cells. Related to Fig 4.

A Alignment of nucleotide sequence of the 5′UTR of p53 mRNA of human, mouse, and rat. The conserved nucleotides are highlighted in yellow.

B The predicted secondary structure of 5′UTR of p53 mRNA of human, mouse, and rat. SL, stem loop.

C RIP assay showing p53 mRNA immunoprecipitated by anti-EZH2 antibody in MEF-27 and MEF-24 cells as measured by RT–qPCR. Data shown as means ± SD (n = 3). *P < 0.01.

Figure EV5. Positive correlation between EZH2 and p53 mRNA expression in different types of human cancer. Related to Figs 5 and 6.

A–H Analysis of correlation between EZH2 and p53 mRNA expression in published datasets obtained from different types of human cancer. The correlation coefficient (Pearson's *r*) and *P* value were shown in each figure.

I Correlation analysis of EZH2 and p53 (WT or mutant) mRNA level in primary prostate cancer specimens of the TCGA cohort.

J–M Western blot and RT–qPCR analysis of p53 protein and mRNA expression in VCaP (J), DU145 (K), glioblastoma U251 (L), and T98 (M) cells at 48 h after transfected with non-specific control (siC) or EZH2-specific siRNAs. GAPDH was used as internal control. Data shown are mean values ± SD from three replicates. $*P < 0.01$ comparing EZH2 siRNA-transfected with siC siRNA-transfected cells.

N PC3 cells were transfected with plasmids for R273H (Left)- or R248W (Right)-mutated Flag-tagged p53 full-length or ΔIRES1 mutant for 48 h followed by Western blots.

O Control or EZH2 knockdown VCaP cells were treated with 100 μM 4-thiouridine (4SU) for 8 h for Western blot analysis and UV-RIP assay followed by RT–qPCR measurement of p53 mRNA immunoprecipitated by IgG or anti-EZH2 antibody. Data shown as means ± SD ($n = 3$). $**P < 0.01$.

P Expressions of p53, Myc-tagged EZH2, H3K27me3, and total EZH2 protein were measured by Western blot in VCaP cells infected lentivirus for sh-control or p53-specific shRNAs in combination with Myc-tagged empty vector (EV) or EZH2 truncation mutants. ERK2 was used as the loading control. Asterisks indicate that Myc-tagged EZH2Δ336–554 protein cannot be recognized by the anti-EZH2 antibody.

Q VCaP cells were infected lentivirus as in (P) and plated into 6-well plate for colony formation assay. At 14 days after plating, colonies were fixed, stained, photographed (Left), and quantified (Right). $*P < 0.01$; NS, no significance.

R VCaP cells expressing p53 R248W mutant were transfected indicated plasmids followed by Western blot analysis (Left) and MTS assay (Right). Data are means ± SD from experiments with three replicates. $***P < 0.001$. ERK2 was used as a loading control.

S VCaP cells were infected with lentivirus for indicated shRNAs or WT EZH2 or mutants for 48 h followed by colony formation assay (Left and Middle) and Western blot (Right). Data are means ± SD from experiments with three replicates. $**P < 0.01$, NS, no significance. ERK2 was used as a loading control.

Source data are available online for this figure.

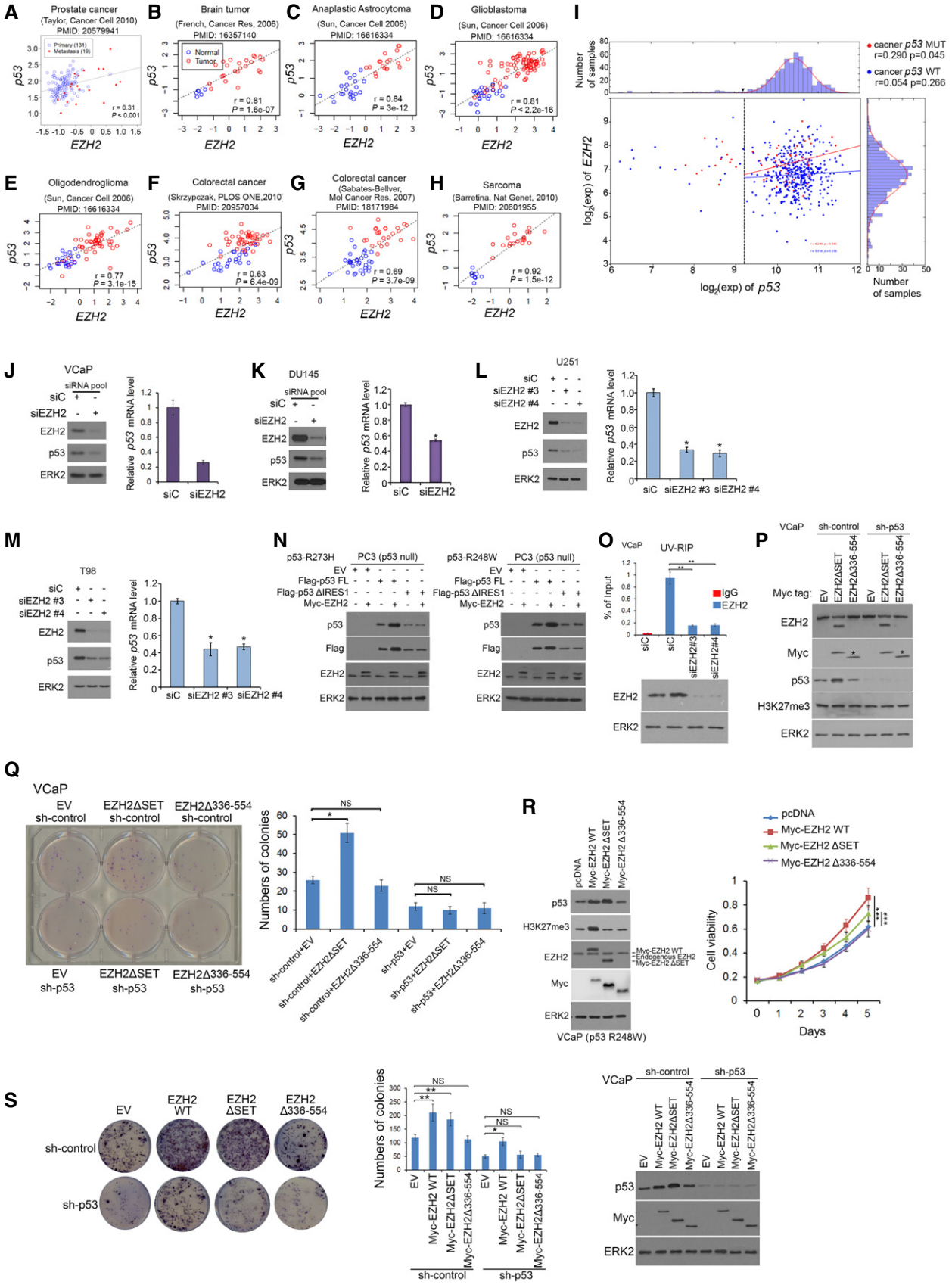

Figure EV5.