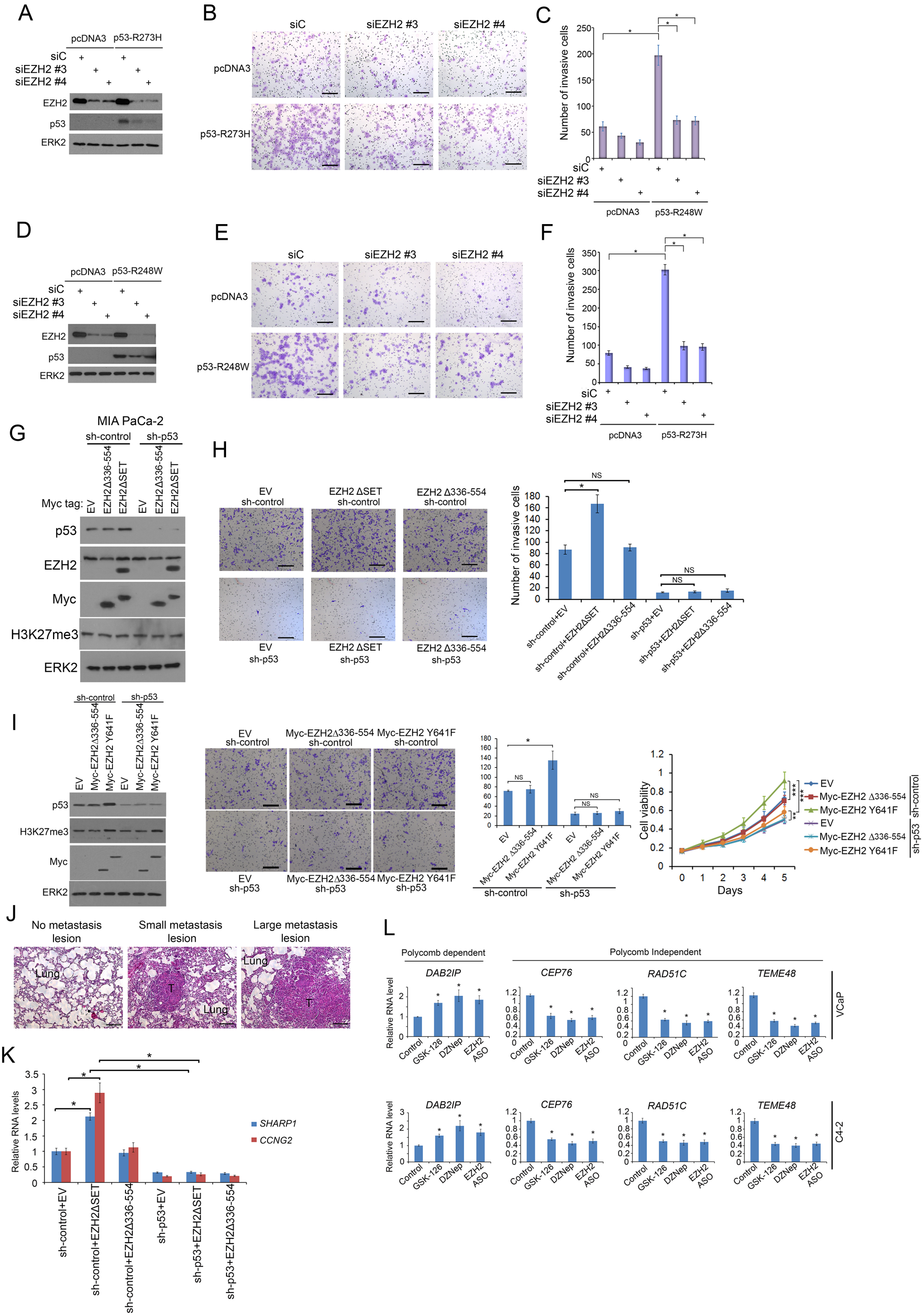
Appendix

Appendix Fig S1
Appendix Fig S2

Appendix Figure S1



Appendix Figure S1. EZH2 enhances p53 mutant-mediated cancer cell growth and invasion independently of its methyltransferase activity. Related to Figs 6 and 7.

(A-F) p53-null PC-3 cells stably expressing empty vector pcDNA3 or R248W (or R273H) mutant-p53 full-length mRNA were transfected with non-specific control (siC) or EZH2-specific siRNAs. 48 h after transfection cells were used for western blot analysis (A and D) and matrigel invasion assay (B, C, E and F). Representative microscopic (100× magnification) pictures of invaded cells are shown in (B and E) and quantification data are shown in (C and F). Scale bars, 50 μm. Data are means ± S.D. from experiments with three replicates. Statistical significance was determined by two-tailed Student's *t*-test. * $P <$ 0.01. ERK2 was used as a loading control.

(G and H) MIA PaCa-2 cells, infected with lentivirus for sh-control or p53-specific shRNAs in combination with or without empty vector (EV) or Myc-tagged EZH2 mutants, were harvested for assessment of expressions of p53, Myc-tagged EZH2, H3K27me3 and total EZH2 protein by western blot (G) or plated into 6-well plate for invasion assay (H). ERK2 was used as the loading control. Invaded cells were photographed (H, Left) and quantified (H, Right). Scale bars, 50 μm. Data are means ± S.D. from experiments with three replicates. Statistical significance was determined by two-tailed Student's *t*-test. * P<0.01; NS, no significance.

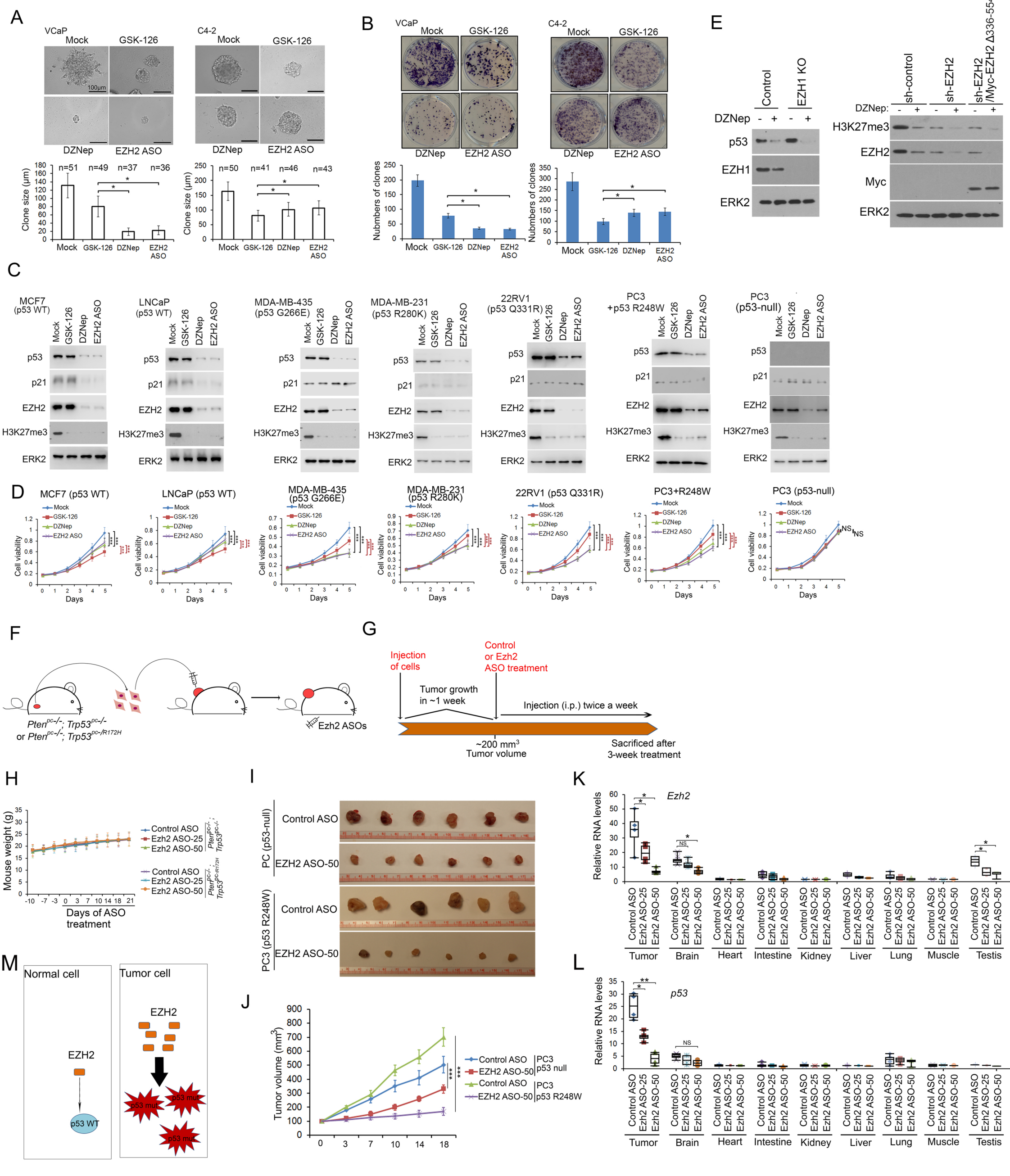
(I) Mia-PaCa2 cells were infected with lentivirus for indicated shRNAs and/or mutated EZH2 for 48 h followed by western blot analysis, invasion chamber assay and MTS assay. Data are means ± S.D. from experiments with three replicates. * $P < 0.05$, NS, no significance. ERK2 was used as a loading control.

(J) Representative images of H&E staining of lung tissues from mice (shown in Figure 6G) tail-vein injected with tumor cells.

(K) Expression of p53 GOF mutant target genes as measured by RT-qPCR in MIA PaCa-2 cells infected with lentivirus as in (G). GAPDH was used as internal control. Data shown are mean values ± SD from three replicates. Statistical significance was determined by two-tailed Student's *t*-test. * $P < 0.01$.

(L) VCaP and C4-2 cells were treated with three different EZH2 inhibitory agents including 10μM GSK-126, DZNep, and EZH2 ASO. 48 h after treatment, cells were harvested for analysis of expression of EZH2 repressed and activated genes by RT-qPCR. Statistical significance was determined by two-tailed Student's *t*-test. * $P < 0.01$ comparing gene expression in cells treated with EZH2 inhibitory agents with vehicle-treated (control) cells.

Appendix Figure S2



Appendix Figure S2. EZH2 enhances p53 GOF mutant-mediated tumor growth. Related to Fig 7.

(A and B) VCaP and C4-2 cells were treated with GSK-126, DZNep, and EZH2 ASO, followed by 3D culture (A) and colony formation assay (B). Statistical significance was determined by two-tailed Student's *t*-test. * $P < 0.05$.

(C and D) MCF7, LNCaP, MDA-MB-435, MDA-MB-231, 22RV1, PC3 transfected with p53 R248W and parental PC3 cells were treated with GSK-126, DZNep, and EZH2 ASO, followed by western blot analysis with indicated antibodies (C) and MTT assay (D). Statistical significance was determined by two-tailed Student's *t*-test. *** $P < 0.001$, NS, no significance.

(E) Control and EZH1 knockout (KO) VCaP cells (Left) or parental VCaP cells were infected with indicated lentivirus expressing shRNAs and/or genes for 48 h (Right) were treated with or without DZNep (5 μM) for 12 h followed by western blot. ERK2 was used as a loading control.

(F and G) Schematic diagram illustrating the process of generation of mouse allografts (F) and scheme for mouse treatment with Ezh2 ASO (G). i.p., intraperitoneal.

(H) Control and Ezh2 ASOs had no significant effect on animal weight. Body weight of mice bearing murine prostate cancer allografts was measured twice a week following treatment of mice with control ASOs at 50 mg kg^{-1} (n=10) or Ezh2 at ASOs 25 mg kg^{-1} (n=10) or 50 mg kg^{-1} (n=10). Data are means ± S.D. from 10 mice.

(I and J) PC3 cells (5x106) infected with lentivirus as indicated were injected subcutaneously into NSG mice (n = 6/group). Mice were treated with control ASO (50 mg kg^{-1}) or EZH2 ASO (50 mg kg^{-1}). Tumors were measured by caliper twice a week. Data are shown as means ± SD (I), and tumors at the end point of measurement were isolated and photographed (J). Statistical significance was determined by two-tailed Student's *t*-test for tumors at day 18 of treatment of ASO. *** $P < 0.001$.

(K and L) RT-qPCR analysis of mRNA expression of Ezh2 and p53 genes in 9 different tissues analyzed of $Pten^{pc-/-}$;$Trp53^{pc-/R172H}$ mice. Gapdh was used as internal control. The lowest expression level of Ezh2 or p53 gene in all tissues examined was arbitrarily set to 1 and expression levels in other tissues were

normalized by the lowest expression value. Data shown are mean values ± SD from tissues of four different mice. Statistical significance was determined by two-tailed Student's *t*-test. * $P< 0.05$, ** $P< 0.01$, NS, no significance.

(M) Schematic diagram illustrating the drastic effect of high level expression of EZH2 on the level and function of mutated p53 in tumor cells, implying that such tumor-specific effect could be harnessed for EZH2-targeted therapy of p53-mutated tumors.