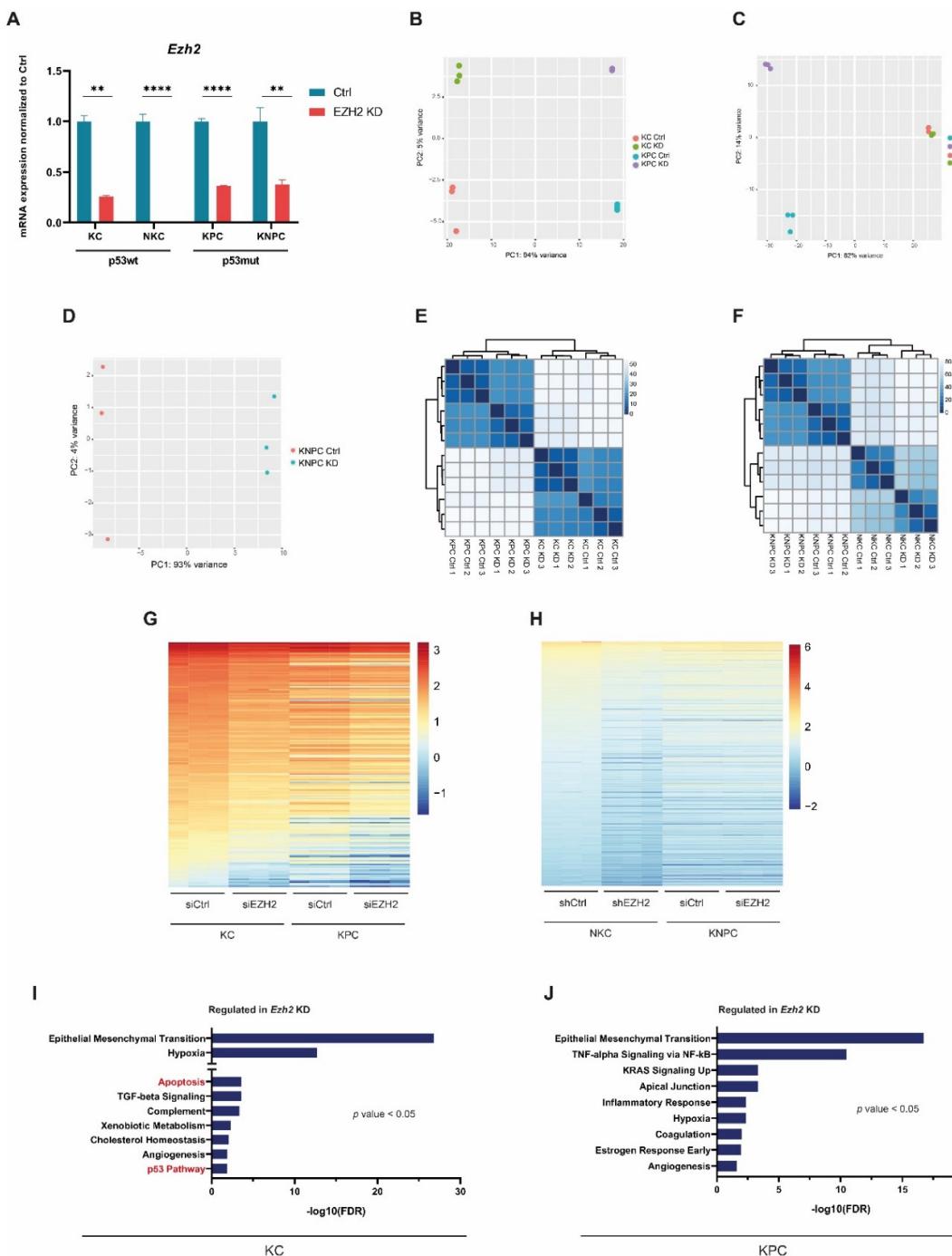
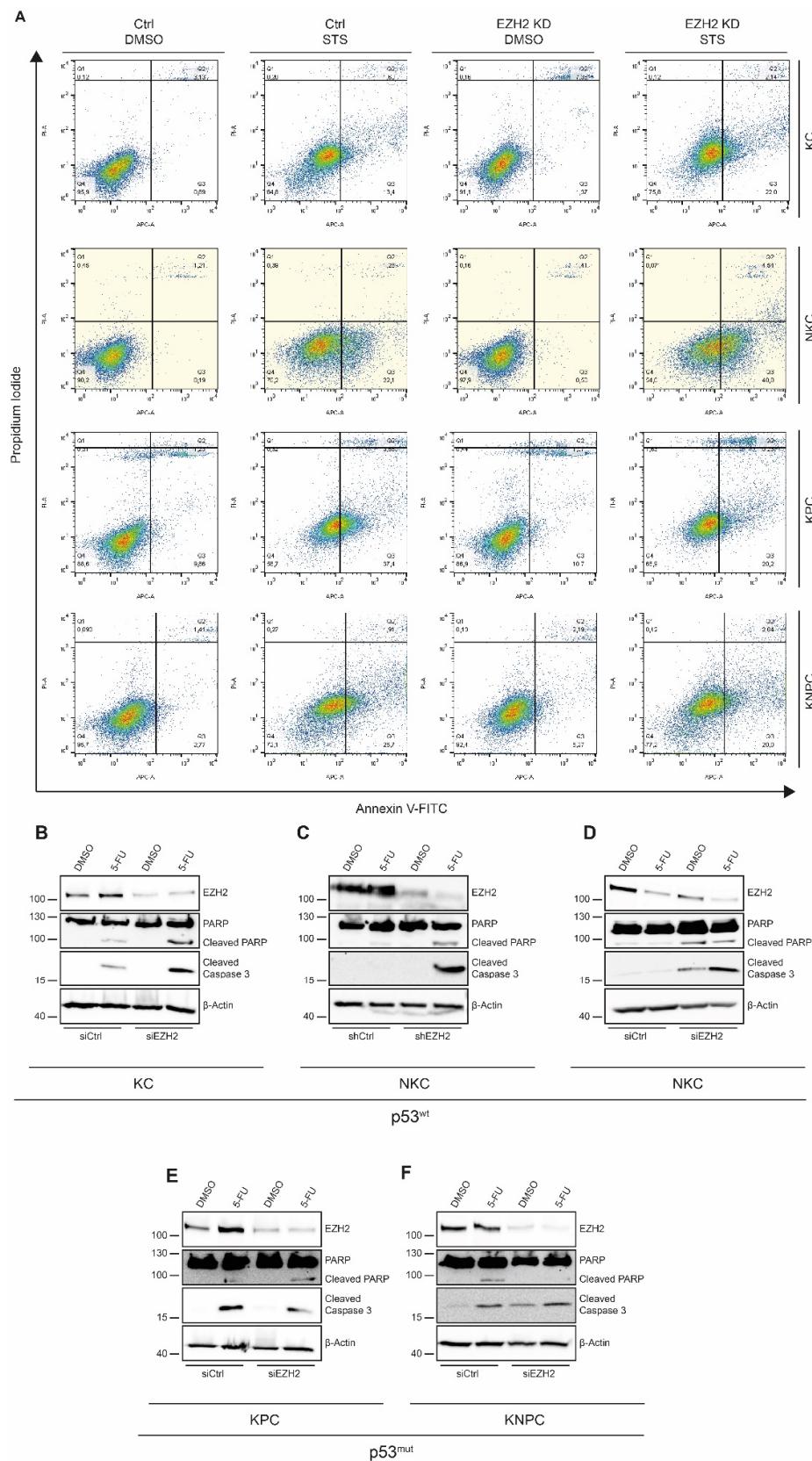


**Supplementary Figure S1.** Quantification of (immuno-) histological stainings depicted in Figure 1G. A-C Quantification of Ki-67 positive staining (A), Masson's Trichrome staining (B), and  $\alpha$ SMA expression (C) of PDAC from orthotopically transplanted Panc1 cells (upper panel) and KrasG12D;Trp53R172H/+ (KPC) cells (lower panel). D Calculation of the activated stroma index by dividing the  $\alpha$ SMA positive area by Masson's Trichrome blue area (collagen). For Masson's Trichrome and  $\alpha$ SMA staining percentage of positively stained area of ten representative images of each tumor and for Ki-67 staining percentage of Ki-67 positive cells of ten representative images were measured using ImageJ Fiji. Each dot represents one mouse. Values represent mean  $\pm$  SD. Significance was determined by two-tailed unpaired Student's t test; \*\*,  $p \leq 0.01$ ; ns, non-significant.

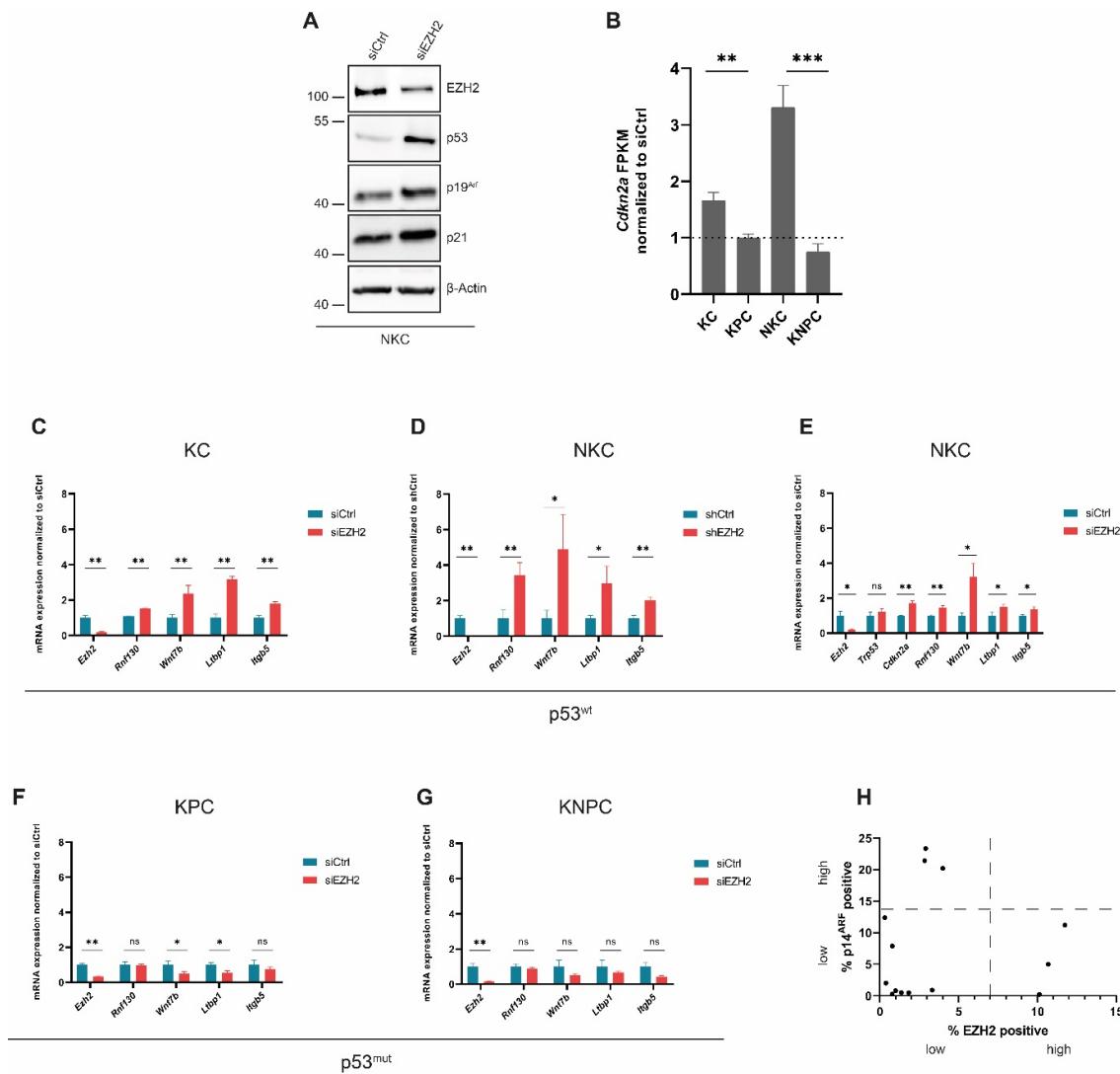


**Supplementary Figure S2.** TP53-status dependent EZH2 target gene regulation. **A** qRT-PCR analysis in the indicated p53wt and p53mut PDAC cells confirming knockdown of EZH2 ( $n = 3$ ). Values represent mean  $\pm$  SD. Significance was determined by Student's *t* test; \*\*,  $p \leq 0.01$ ; \*\*\*\*,  $p \leq 0.0001$ . **B-D** Principal component analysis (PCA) upon RNA-sequencing of KC and KPC cells (**B**) and NKC and KNPC cells (**C, D**) after EZH2 knockdown displaying distinct clusters of triplicates. **E, F** Sample-to-sample distances upon RNA-seq analysis of KC and KPC cells (**E**) and NKC and KNPC cells (**F**) upon EZH2 knockdown. **G, H** Heatmap illustrating genes being significantly downregulated ( $FPKM > 0.01$ ,  $\log_{2}FC < -0.5$ ,  $q < 0.05$ ) upon knockdown of EZH2 in the indicated p53wt cells (KC: 188 genes, NKC: 964 genes) and its consequences on the expression of these genes in the respective p53mut cells. **I, J** Gene ontology (GO) analysis to reveal significantly upregulated pathways upon EZH2 depletion in the indicated PDAC cells ( $p < 0.05$ ).

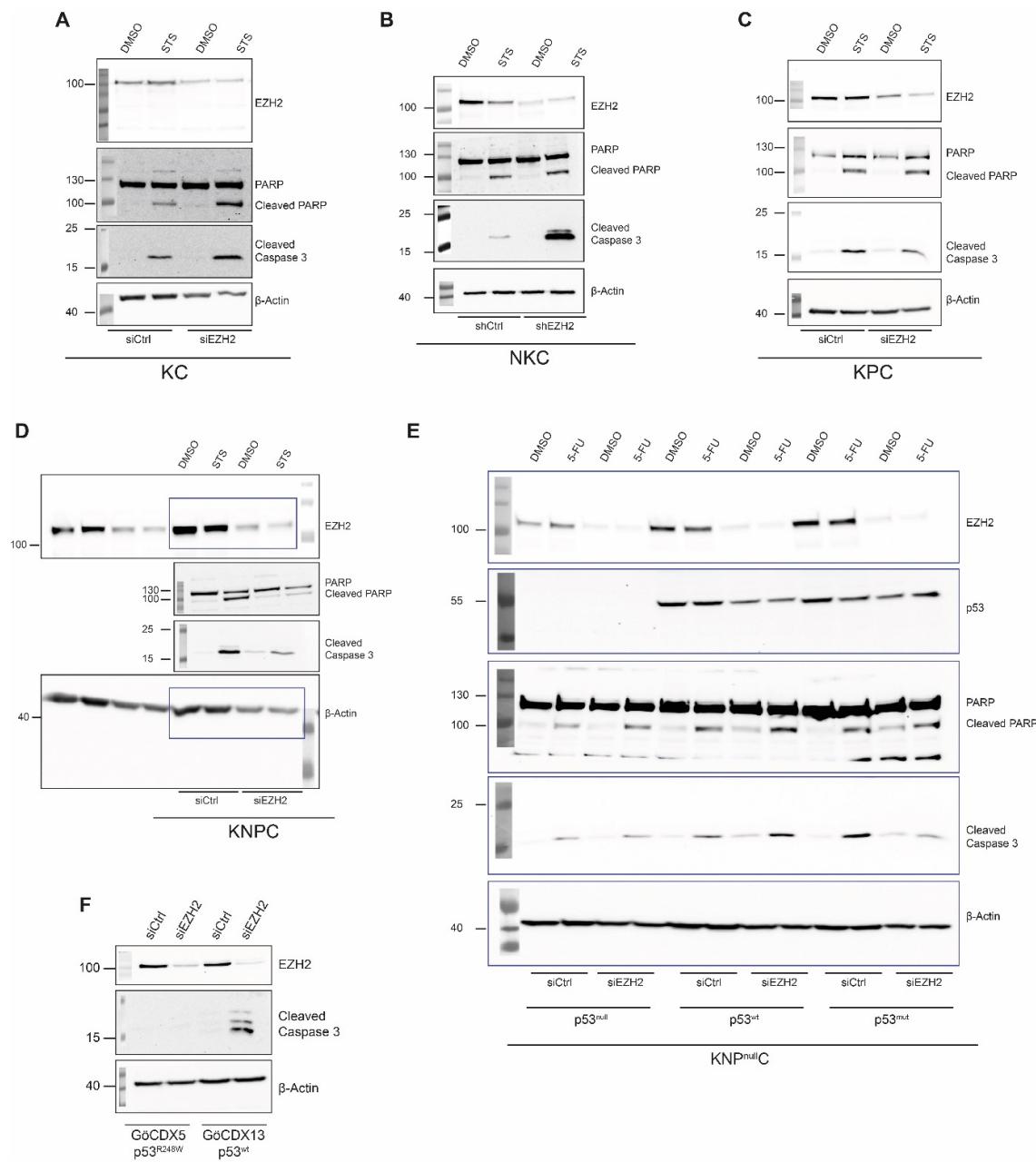


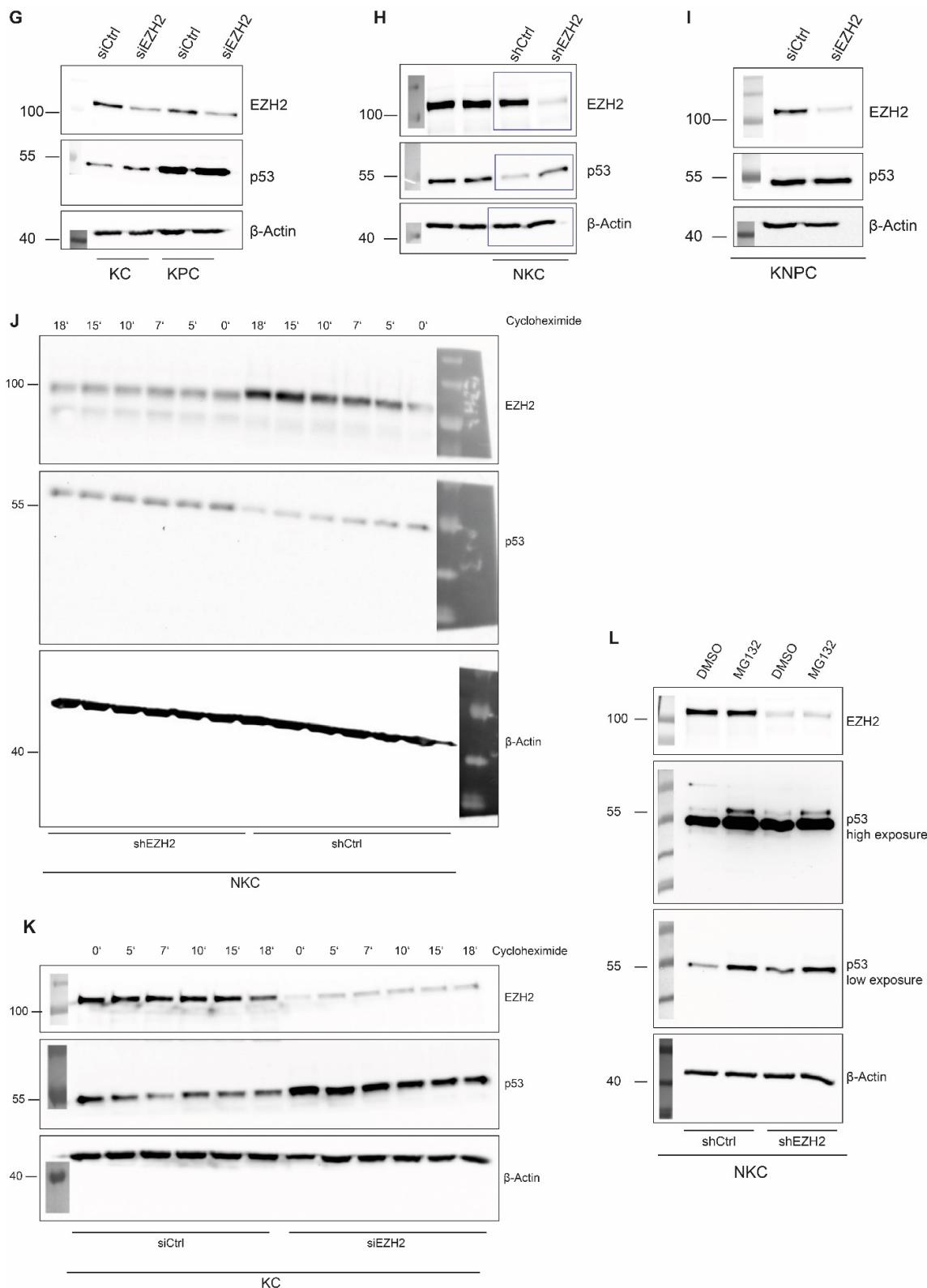
**Supplementary Figure S3.** depletion induces apoptosis-related protein expression in p53wt PDAC, but not in cells bearing mutations in the *Trp53* gene. A Knockdown of EZH2 in PDAC cells with

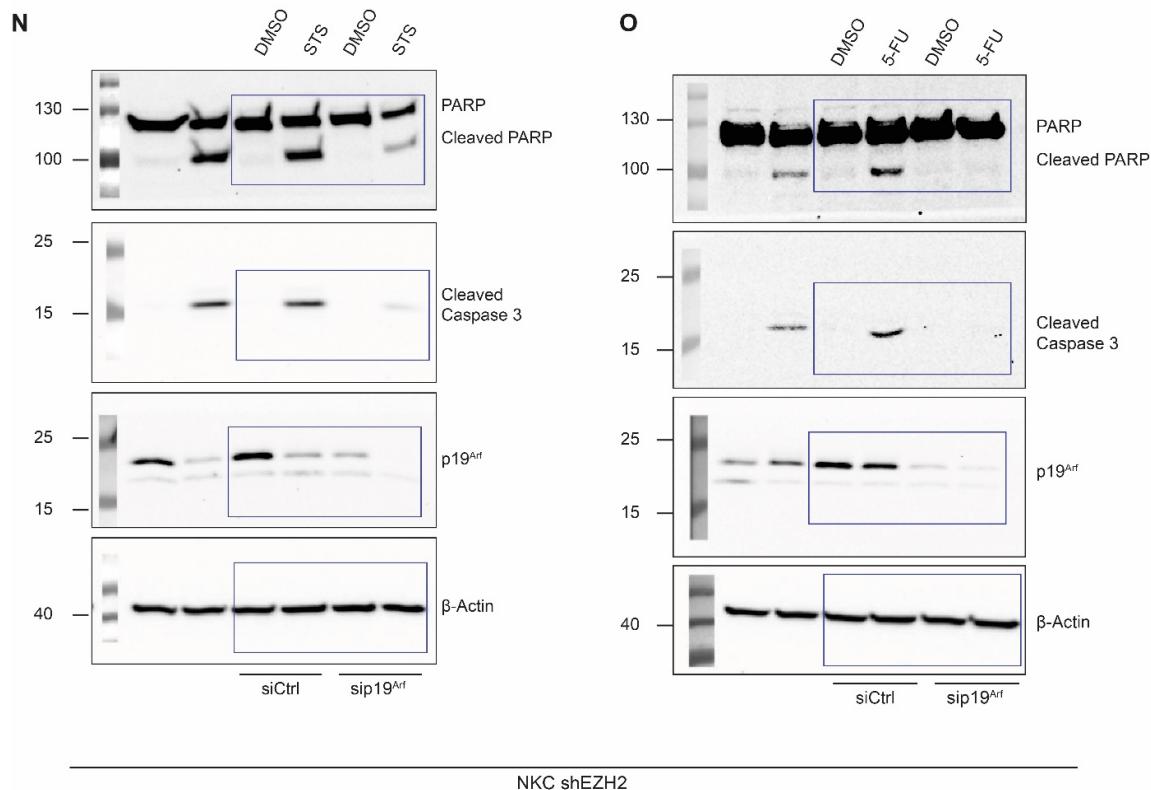
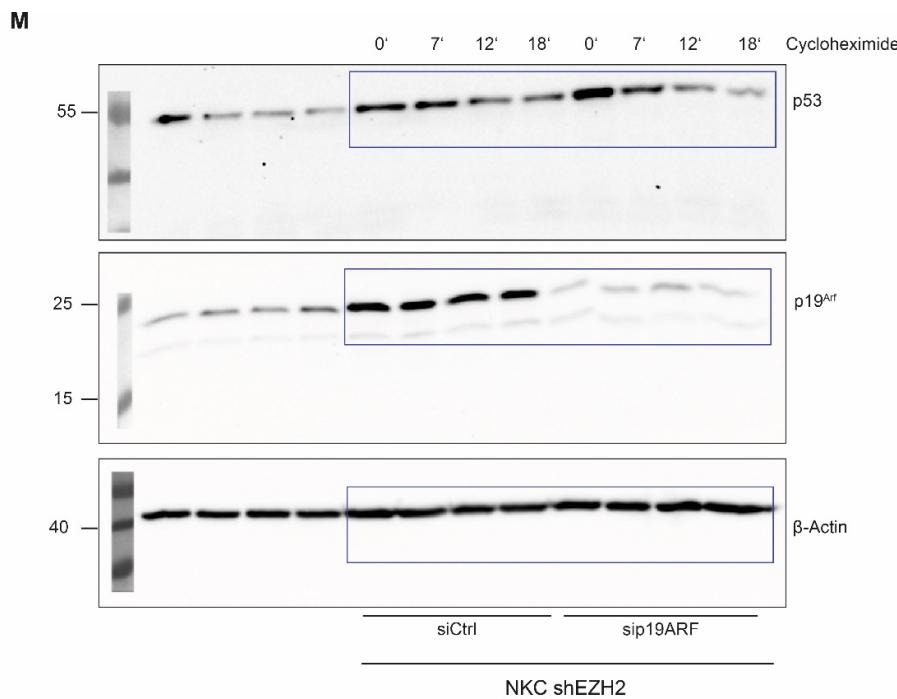
p53wt (KC, NKC) and p53mut (KPC, KNPC) expression and simultaneous treatment with staurosporine (STS). Representative flow cytometry results with the respective gating strategy upon Annexin-V/propidium iodide staining for each cell line referring to Figure 3. **B–F** Western blot analysis of apoptosis-related proteins upon knockdown of EZH2 and simultaneous treatment with 5-FU in the indicated p53wt and p53mut PDAC cells.

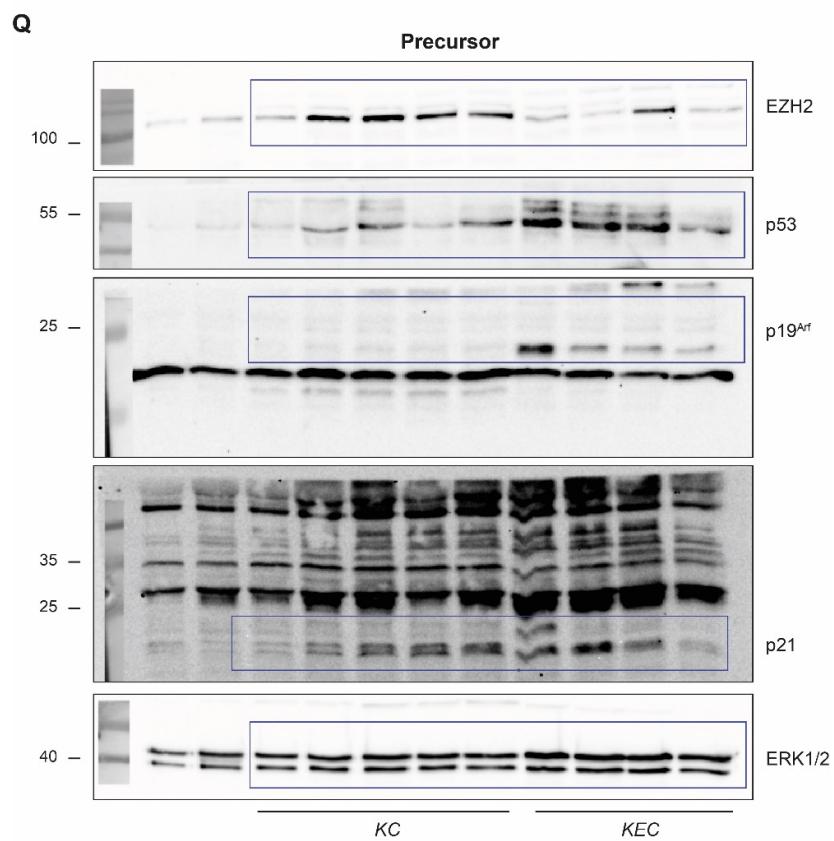
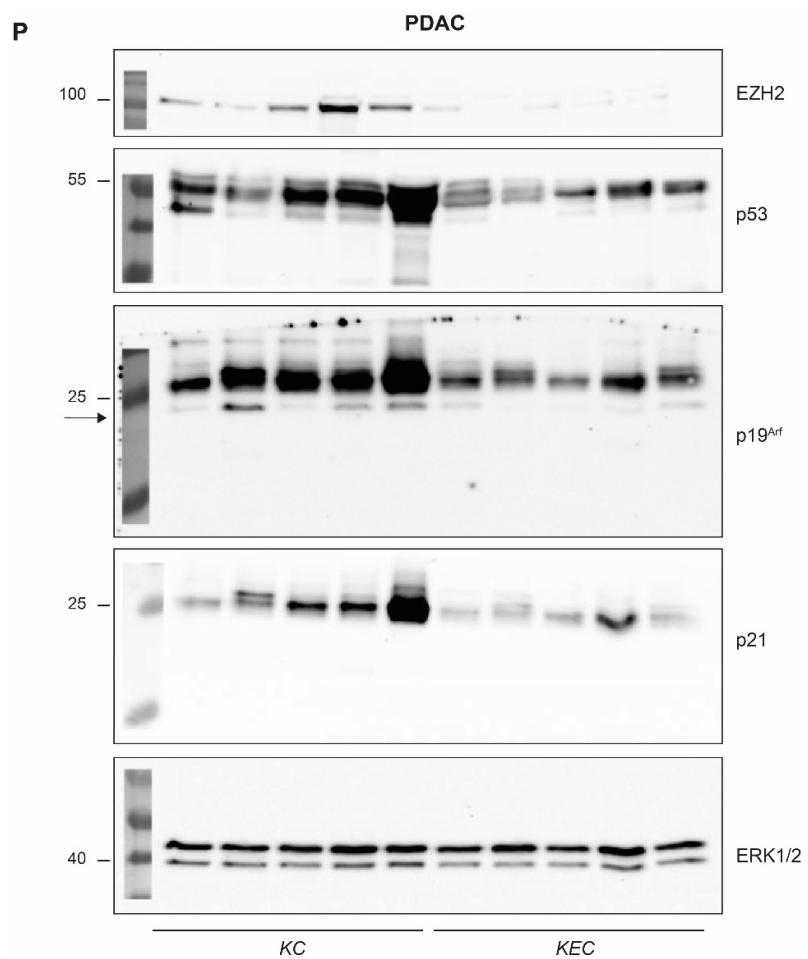


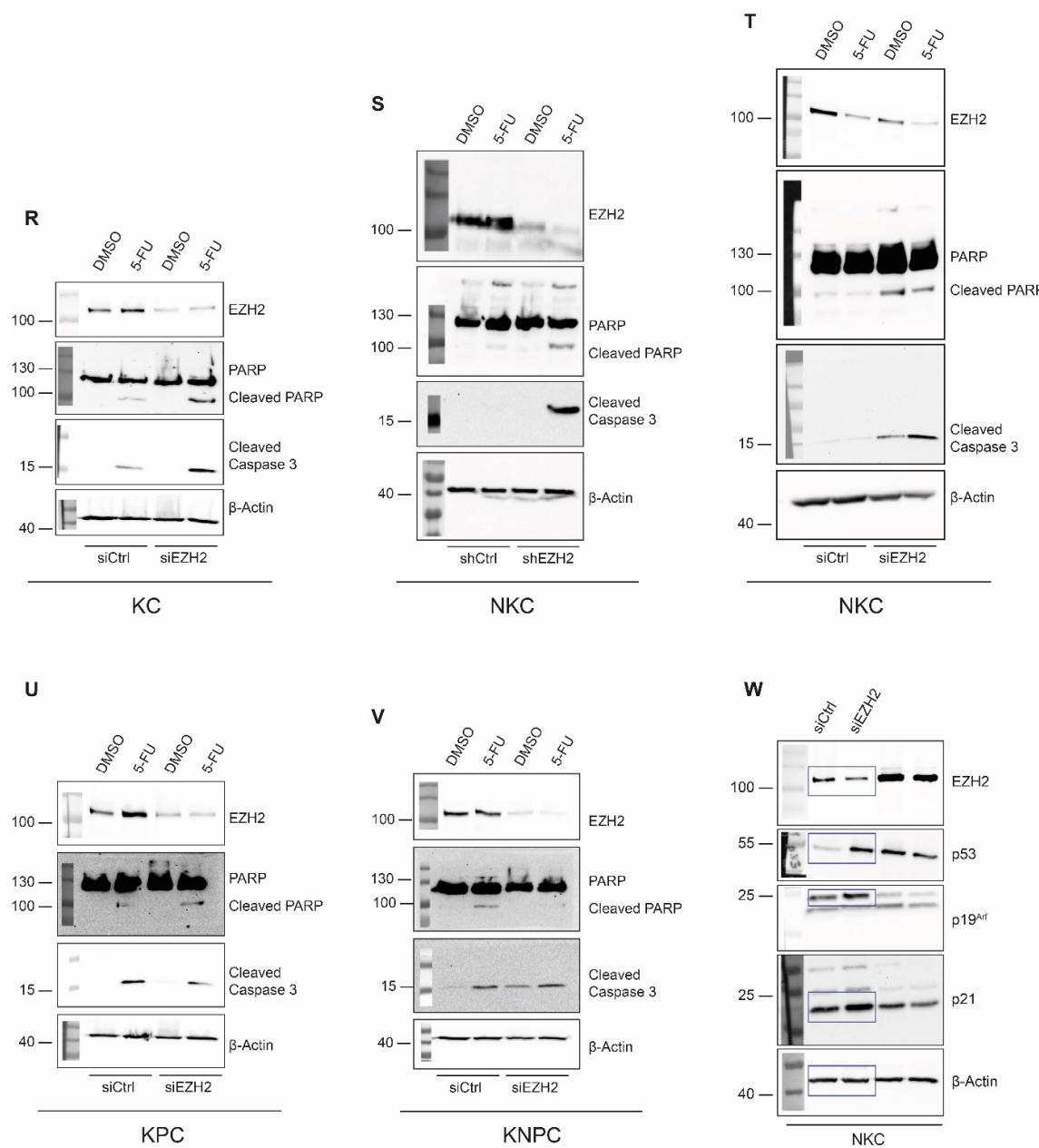
**Supplementary Figure S4.** EZH2-dependent target gene regulation differs in p53wt and p53mut PDAC. **A** Knockdown of EZH2 in p53wt NKC cells using siRNA following western blot analysis to investigate the consequences of EZH2 KD on the indicated proteins. **B** Normalized FPKM values of Cdkn2a expression after knockdown of EZH2 as revealed by RNA-seq in the indicated cell lines. Values represent mean  $\pm$  SD. Significance was determined by Student's t test; \*\*,  $p \leq 0.01$ ; \*\*\*,  $p \leq 0.001$ . **C–G** QRT-PCR analysis in the indicated PDAC cells upon knockdown of EZH2 validating the upregulation of these genes in p53wt cells ( $n = 3$ ). Values represent mean  $\pm$  SD. Significance was determined by Student's t test; \*,  $p \leq 0.05$ ; \*\*,  $p \leq 0.01$ ; ns, non-significant. **H** Table displaying number of patients in the indicated EZH2low/high and p14ARF low/high groups based on IHC staining.











**Supplementary Figure S5.** Un-cut original Western Blot images. Original western blot images from those western blots shown throughout the manuscript. We used the PageRule Prestained Protein Ladder, 10 bis 180 kDa from Thermo Scientific (26616) having bands at 180, 130, 100, 70, 55, 40, 35, 25, 15, and 10 kDa. We did not label all bands of the molecular weight marker but only the most relevant. However, the other molecular weight sizes can be identified accordingly. For protein visualization, we used the Western Lightning Plus (NEL103E001EA) or Ultra (NEL111001EA) chemiluminescent substrate from PerkinElmer. However, the marker cannot be visualized with the chemiluminescent solution and was imaged separately. Subsequently, we added the marker from the raw colorimetric image onto the enhanced chemiluminescent image to compare the molecular weight of the protein of interest with the standard. Please note, that we pre-cut the membranes before antibody incubation around the expected molecular weight of the protein of interest in order to stain different proteins of distinct molecular weight on one membrane. Blue boxes indicate excerpt of protein lanes depicted in the respective Figures. **A** Un-cut western blot images referring to Fig. 3C. **B** Un-cut western blot images referring to Fig. 3E. **C** Un-cut western blot images referring to Fig. 3G. **D** Un-cut western blot images referring to Fig. 3I. **E** Un-cut western blot images referring to Fig. 3K. **F** Un-cut western blot images referring to Fig. 3L. **G** Un-cut western blot images referring to Fig. 4A

and 4C. **H** Un-cut western blot images referring to Fig. 4B. **I** Un-cut western blot images referring to Fig. 4D. **J** Un-cut western blot images referring to Fig. 4F. **K** Un-cut western blot images referring to Fig. 4G. **L** Un-cut western blot images referring to Fig. 4H. **M** Un-cut western blot images referring to Fig. 5E. **N** Un-cut western blot images referring to Fig. 5F. **O** Un-cut western blot images referring to Fig. 5G. **P** Un-cut western blot images referring to Fig. 6B. **Q** Un-cut western blot images referring to Fig. 6H. **R** Un-cut western blot images referring to Supplementary Fig. 3B. **S** Un-cut western blot images referring to Supplementary Fig. 3C. **T** Un-cut western blot images referring to Supplementary Fig. 3D. **U** Un-cut western blot images referring to Supplementary Fig. 3E. **V** Un-cut western blot images referring to Supplementary Fig. 3F. **W** Un-cut western blot images referring to Supplementary Fig. 4A.

### Supplementary tables

**Supplementary Table S1.** sgRNA sequences and validation primers for CRISPR/Cas9-mediated *EZH2* knockout.

	<b>Murine <i>Ezh2</i></b>	<b>Human <i>EZH2</i></b>
<b>sgRNA sequence</b>	GTGGTGGATGCAACCCGAAA	GTGGTGGATGCAACCCGCAA
<b>Forward primers for knockout validation</b>	CCTGTGTAAGTGGGTGTGCT	TGCCTATTCTGTATGTTGGAAG
<b>Reverse primers for knockout validation</b>	GTTTGCTGTCACTGGCTGG	TGTCAACAGCAGGGTGAGAAA

**Supplementary Table S2.** siRNA sequences.

	<b>Sense</b>	<b>Antisense</b>
<b>siEZH2</b>	GGAUACAGCCUGUGCACAU TT	AUGUGCACAGGCUGUAUCCTC
<b>si<math>p19^{Arf}</math></b>	GGCUAGAGAGGAUCUUGAGTT	CUCAAGAUCCUCUCUAGCCTC

**Supplementary Table S3.** Primer for qRT-PCR.

<b>Target</b>	<b>Direction</b>	<b>Sequence</b>
<i>Cdkn2a</i>	forward	CGCAGGTTCTGGTCACTGT
<i>Cdkn2a</i>	reverse	TGTTCACGAAAGGCCAGAGCG
<i>Ezh2</i>	forward	CAACCCGAAAGGGCAACAAA
<i>Ezh2</i>	reverse	ACC AGT CTG GAT AGC CCT CT
<i>Itgb5</i>	forward	GAAGTGGCACCTCGTGTGAA
<i>Itgb5</i>	reverse	GGACCGTGGATTGCCAAAGT
<i>Ltpb1</i>	forward	GGTCGCATCAAGGTGGCTTT
<i>Ltpb1</i>	reverse	GTGGTGGTATTCCCCCTTCTGG
<i>Rnf130</i>	forward	CTGCCCATTCCACGGAGTTG
<i>Rnf130</i>	reverse	CAAGGCATCCACTGTTGA
<i>Rplp0</i>	forward	TGGGCAAGAACACCATGATG
<i>Rplp0</i>	reverse	AGTTTCTCCAGAGCTGGTTGT
<i>Trp53</i>	forward	AGGTGTGCGTAGCACC
<i>Trp53</i>	reverse	CCCCACAACACCAAGTG
<i>Wnt7b</i>	forward	CTTCACCTATGCCATCACGG
<i>Wnt7b</i>	reverse	TGGTTGTAGTAGCCTGCTTCT

**Supplementary Table S4.** Antibodies used for western blotting.

<b>Antibody</b>	<b>Company</b>	<b>Number</b>	<b>Dilution</b>
Actin-HRP	Sigma	A3854	1:40000
Cleaved caspase 3	Cell Signaling	9661	1:500
Erk (1/2)	Cell Signaling	9102	1:1000
EZH2	Cell Signaling	5246	1:1000
<i>p19<sup>Arf</sup></i>	Abcam	Ab80	1:1000
<i>p19<sup>Arf</sup></i>	Santa Cruz	32748	1:250
<i>p53</i>	Cell Signaling	2524	1:1000
PARP	Cell Signaling	9542	1:1000
Anti-mouse (IgG) HRP	Cell Signaling	7076	1:6500
Anti-rabbit (IgG) HRP	Cell Signaling	7074	1:6500
Anti-rat (IgG) HRP	Santa Cruz	2006	1:5000

**Supplementary Table S5.** Antibodies used for immunohistochemistry and immunofluorescence.

Antibody	Company	Number	Dilution
EZH2 (mouse)	Cell Signaling	5246	1:100
EZH2 (human)	Leica Biosystems	NCL-L-EZH2	1:50
Ki-67	Thermo Fisher	RM9106	1:600
αSMA	Agilent Dako	M0851	1:100
p14	Cell Signaling	2407	1:400
p19 <sup>arf</sup>	Santa Cruz	32748	1:100
Alexa Fluor 488 donkey anti-rat	Invitrogen	A21208	1:500

**Supplementary Table S6.** Antibodies used for ChIP experiments.

Antibody	Company	Number	Amount
EZH2	Diagenode	C15410039-classic	2 µg
H3K4me3	Cell Signaling	9751	2 µg
Rabbit IgG	Diagenode	C15410206	2 µg

**Supplementary Table S7.** Primer for qRT-PCR following ChIP experiments.

Target	Direction	Sequence
Cdkn2a TSS	forward	GACCGTGAAGTCAGC
Cdkn2a TSS	reverse	GGGGTCGCTTCTCGG

**Supplementary Table S8.** Favorable prognosis genes depicted in Figure 2C/D.

CAMTA2	ABHD8	HCFC1	VPS54	SNAI3	GSE1	TBPL1
TSPYL2	SEMA6C	PPP1R10	MCM3AP	DOHH	LRRK29	ARIH2
ANAPC2	TMEM74B	SAFB	PMM1	RFX6	UAP1L1	RFX2
DEF8	GALT	KLHL36	ATP6V0A1	HERC1	KIF5A	SLC22A5
USP20	COG8	TMEM175	COQ10A	PRPSAP2	SLC27A3	DOPEY1
MICAL1	RAB11FIP3	ABC A5	RAB4B	UNC13B	DYNLL2	BAHCC1
SOCS2	EPOR	SSBP4	NEIL1	GNG7	PNISR	ASPHD1
SLC43A2	DENND4B	BRSK1	RBM4B	SNTA1	NCALD	MPDZ
ARNT2	NECAB3	BBS5	QTRT1	TACO1	INPP5B	MLLT6
WDR37	NAT9	TYK2	SPATC1L	NCDN	RAD51C	SMPD1
INPP5K	NFASC	CBX7	ZBTB46	HNRNPA0	TRMT10B	ULK3
PITPNA	PKD1	RNF166	CXXC4	ALKBH5	TAPT1	XAB2
PPP1R3F	ARMCX2	RBM14	IPO13	PSPH	TSPYL1	PPP6R2
MAMDC4	PNPLA6	ATP1B2	BTBD6	MAVS	POLR3H	MAP2K6
ARMC5	TMEM91	APH1B	WHRN	DVL2	NAP1L2	ANKRD13B
RNF167	B4GAT1	PLD6	PIP5K1C	CD81	INTS3	RBM5
CCDC106	IRGQ	PTPRS	IRF2BP1	GOLGA3	WDR83	RMND5B
MUM1	INCA1	ZFP3	ST3GAL2	FRS3	PFDN5	GAS8
SFXN5	RNASEH2C	GDF11	EFHC1	DIDO1	ANGEL1	PHKA2
TLE2	ACSF3	NCOA5	SDR39U1	IQCD	SYT5	GPX4
CBX6	GADD45G	NICN1	FOXA2	IGP	NELL2	FKBP8
KCND1	CBX8	HDAC4	CCDC130	ADD1	REC8	RNPC3
RBM10	POMT1	KLHDC4	CERK	MAGEE1	HEXDC	ATP6AP1
TBC1D13	ARGLU1	ADGRL1	FN3K	HDAC3	SCG2	MC1R
RAD51D	PELP1	LIPE	SYNGR1	HIRA	STK40	MVB12B
FXR2	OAZ1	CCM2	FAM219A	FASTK	KCNH2	PDE3B
VAMP2	PPP1R12C	MBLAC1	NUDT18	TBC1D17	CALM1	SPECC1L
KLHL22	ZBTB40	DNMT3A	DCLK2	PRKAR2B	NEK9	MZF1
RAB6B	SPATA7	GRK6	RCAN2	SOWAHA	B4GALT6	DCAF15
EVL	MED9	SALL2	NPR1	ENPP5	TEX264	MIS12
TMEM203	KCTD17	RGS11	SLC25A44	NXF1	TMEM150C	TSC1
PRAF2	GPR162	MKS1	ID4	RAB17	CLEC3B	PHKG2
CYB561D1	MCF2L	UNC119B	TRIM39	RANBP3	JMJD6	AKAP8L
ZBTB48	SNAPC4	LUC7L	PIGT	RFXAP	TUBGCP6	PPP1R21
ELMOD3	SNRNP70	RAPGEF4	THEM4	MRPL38	ITGAE	CERS4
PHLDB3	TTC13	TBL1X	CHPF2	DEXI	ATP6VOE2	CUL9
SLC25A11	CCDC92	SPPL2B	TMEM240	ACCS	SF11	FBXO46
VASH1	MTHFR	RGS9	CCND2	HAP1	SMDT1	TECPR1

LRSAM1	LPCAT1	MRPL53	SAFB2	POMGNT1	DXO	LENG1
CBFA2T2	PKIG	SCN1B	ZBTB4	NAA60	GPANK1	SRR
FAM53B	USF2	RWDD2A	CLDN15	CACNA1A	DNAJC18	OTUD5
BRF1	RNF146	TOP3A	EID2B	RECQL5	IGHMBP2	FZD8
NUAK2	MAP1LC3A	GCH1	MAGEH1	IKZF4	GNAO1	EFNB3
TBKBP1	STRADA	RBM6	CXXC1	SIN3B	CEP164	WRAP53
DLG4	TRIM3	CCDC159	ENGASE	SH2B1	GFOD2	MRPL57
ATOH8	CCDC57	DTX3	ELP5	CRY2	FXYD6	CHD3
SGSM2	MCOLN1	PCGF3	FBXO44	EMC10	NEU3	CNPY2
NCAM1	PRR3	RABL6	PBXIP1	RANGRF	ZDHHC1	FIZ1
CEP250	DCUN1D2	TERF2IP	MAP1S	UCKL1	GBA2	KATNB1
B3GNT8	SCAMP5	NINL	CAMKK1	SIRT3	SLC30A4	STARD3NL
SLC2A8	MADD	SLC16A13	NT5C3B	FLCN	EPC1	SHC2
TMEM86B	PTCH2	APBA3	ASPSCR1	SLC29A4	PLEKHB1	SDSL
NAIF1	VPS16	MAP3K12	CIRBP	TMOD2	MEGF8	KDM2B
SEC61A2	PCED1A	PEBP1	CDK10	ALDH9A1	LUC7L3	PAF1
CCDC28B	LLGL2	EXOC3	FAM120B	AP2A2	ZZEF1	R3HCC1
PHF10	SAT2	ASB1	MIF4GD	ARFGAP1	ELMO2	VPS18
CLSTN3	MOCS1	ATP8B2	WDR19	ATP5D	RGL2	TMEM59L
ATP6V0D1	ANKRD39	ING5	METTL3	ELMO1	SLC25A27	TPGS1
BCAS3	ATP1A3	MYBBP1A	KRBA1	ACBD4	ANKRD54	IRX2
PI4KB	SENP3	STK11IP	RAI2	TATDN2	GPR137	TSPAN33
KCTD2	RNF216	STAT5B	MINK1	FBXW4	SUOX	FKRP
CHST12	MTG2	LIN37	MAGED2	DCXR	LRRC56	NPM2
GIGYF1	CTNS	IZUMO4	SLC25A45	DCAF8	GPATCH3	SREBF1
MXD4	CTC1	TSPAN7	FAAP20	PLCG1	RABEP1	KCTD13
SLC26A11	IFFO1	DPY19L3	SLC25A29	ASCL2	PLD3	IQSEC1
SLC16A11	TNRC6C	PGPEP1	SLC38A10	TFEB	MED22	HELQ
SOBP	UNK	GTPBP6	PACS2	TAOK2	PRKACA	KLHDC3
VPS53	PPP3CB	TNS2	CENPT	FYN	POLR3F	TRAPPC12
ZMAT1	GKAP1	ARHGEF11	TANGO2	DIRAS1	CLPP	PTOV1
ABHD17A	UPF3A	TMEM220	SMARCA1	MAN1C1	DPH7	HDAC10
MBD3	SLC23A2	SLC22A17	METTL16	FAM193A	PAPSS2	SPHK2
GPRASP1	NMNAT3	KDM8	SGSH	EGLN2	AXIN2	USP27X
DPH1	ARMCX1	ITGA7	PPP1R9B	RFNG	AKT1S1	TDRP
CRTC1	PGS1	TAF1C	TBCB	FAM222A	POU6F1	ZFAND2B
UBOX5	FBXL16	ZBTB49	SEPHS1	INTS1	KBTBD7	FAM83F
APBB1	LRRC75A	ZSWIM1	TFPT	GGA1	SSTR2	RASIP1
FLYWCH2	GPS2	ZSWIM7	TMEM8B	NCKAP5L	PKNOX1	LYRM9
EXOG	AHI1	CFD	NPDC1	MRPL34	IMMP1L	GTF3C1
ARID3A	RAB11B	SLC25A14	ATP6V0B	CCDC96	KLC1	ATOX1
TMEM104	CYB5D2	LRRC14	PIN1	SORBS3	PGBD1	AGFG2
ARAP1	CAPN10	CTDNEP1	KCTD7	LSM10	APBB3	SGK3
SAP30L	PMPCA	FAM117A	ERCC2	NAAA	WBP1	CISH
ENPP2	RUFY3	INPP5E	MON1A	WNT4	IP6K1	NDUFA3
VPS37D	UNC119	FBXO9	WDR81	P3H3	TRMU	PABPN1
CDIP1	IQCC	CPT1C	ABCB8	SPG7	ACADVL	EFCC1
TRAF1	DPP7	DUSP28	TRIM25	RFX1	TMEM198	ROGDI
MARK1	TSPYL4	UQCR11	CSAD	PDZRN3	NAGLU	TIMM13
PNPLA7	ORA12	CYB561D2	ACADS	DISP1	PPP1R26	WIP12
GPR173	U2AF1L4	YIF1B	MAP6	SMIM4	ZDHHC14	STXBP1
NEURL4	TXLNA	NISCH	RGAG4	STIM2	UBXN6	PALM
TRAF3	NRIP2	RNASEK	AGER	RSAD1	MAST3	ATG4D
EBF4	LRWD1	TOM1L2	SLC25A4	CPLX1	RXRA	CHGB
CASKIN2	FBXO31	TRO	TIMM22	TSC2	PIAS4	CHMP6
GABBR1	DMPK	ZCWPW1	MTERF4	MTG1	ATG16L2	SMPD2
TSEN54	FAM69B	ACACB	LMBR1L	MTMR4	SCAP	
CD99L2	MTA1	KLHDC1	ZSCAN2	IFT88	MST1	
HSD17B14	GABARAP	ZFP57	CALY	CLCN5	TBXA2R	
PDZD4	CSNK1D	ANKRD16	KAT2A	RPAIN	GRIK5	

**Supplementary Table S9.** Genes being significantly upregulated in p53wt but not in p53mut cells upon EZH2 knockdown.

<i>Anxa6</i>	<i>Dtna</i>	<i>Igf2bp3</i>	<i>Maged2</i>	<i>Ppbp</i>	<i>Slfn2</i>	<i>Tpm2</i>
<i>Camk2b</i>	<i>Efemp2</i>	<i>Igf2r</i>	<i>Map1lc3a</i>	<i>Rdh10</i>	<i>Spon2</i>	<i>Trp53inp2</i>
<i>Cdkn2a</i>	<i>Fhdc1</i>	<i>Itgb5</i>	<i>Masp1</i>	<i>Rnf130</i>	<i>Sprr1a</i>	<i>Tuft1</i>
<i>Ddah2</i>	<i>Foxg1</i>	<i>Ltbp1</i>	<i>Neat1</i>	<i>Serpinb6b</i>	<i>Tbc1d16</i>	<i>Wnt7b</i>
<i>Dnaaf9</i>	<i>Fzd6</i>	<i>Ltbp3</i>	<i>Palld</i>	<i>Slc44a2</i>	<i>Tcf24</i>	<i>Zfpm2</i>