

Article

Impact of Wnt/ β -Catenin Inhibition on Cell Proliferation through *CDC25A* Downregulation in Soft Tissue Sarcomas

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Supplementary Figures

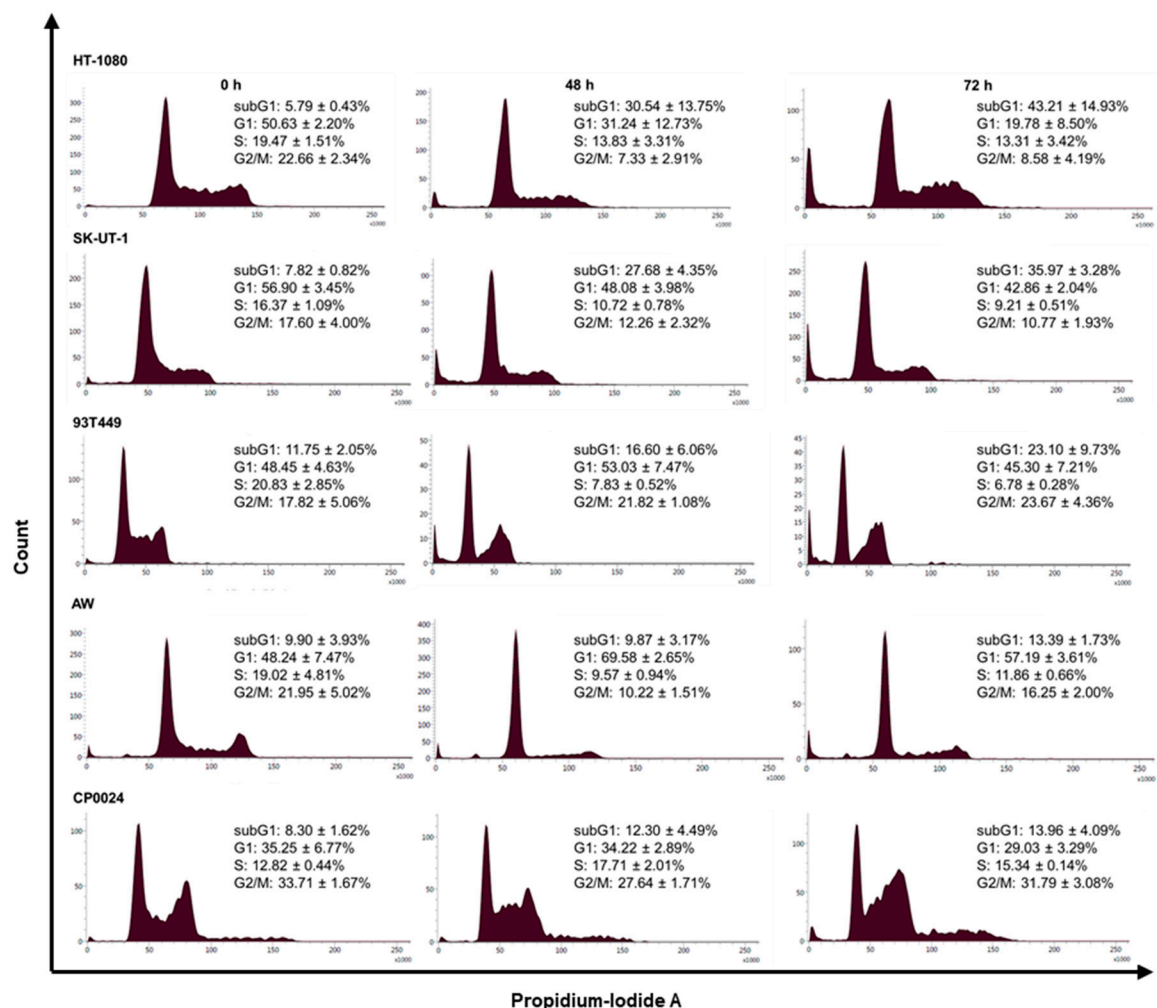


Figure S1. Inhibition of CBP/ β -catenin interaction promotes cell death in STS cells. Cells were treated with PRI-724 (10 μ M), fixed with ethanol, stained with propidium iodide, and DNA content was determined by flow cytometry. Histograms of PRI-724-treated cells for 0, 48 and 72 h are shown. The fluorescence values (count) used to calculate the peaks corresponding to the sub-G1, G1 and the G2/S/M phase are indicated on each histogram.

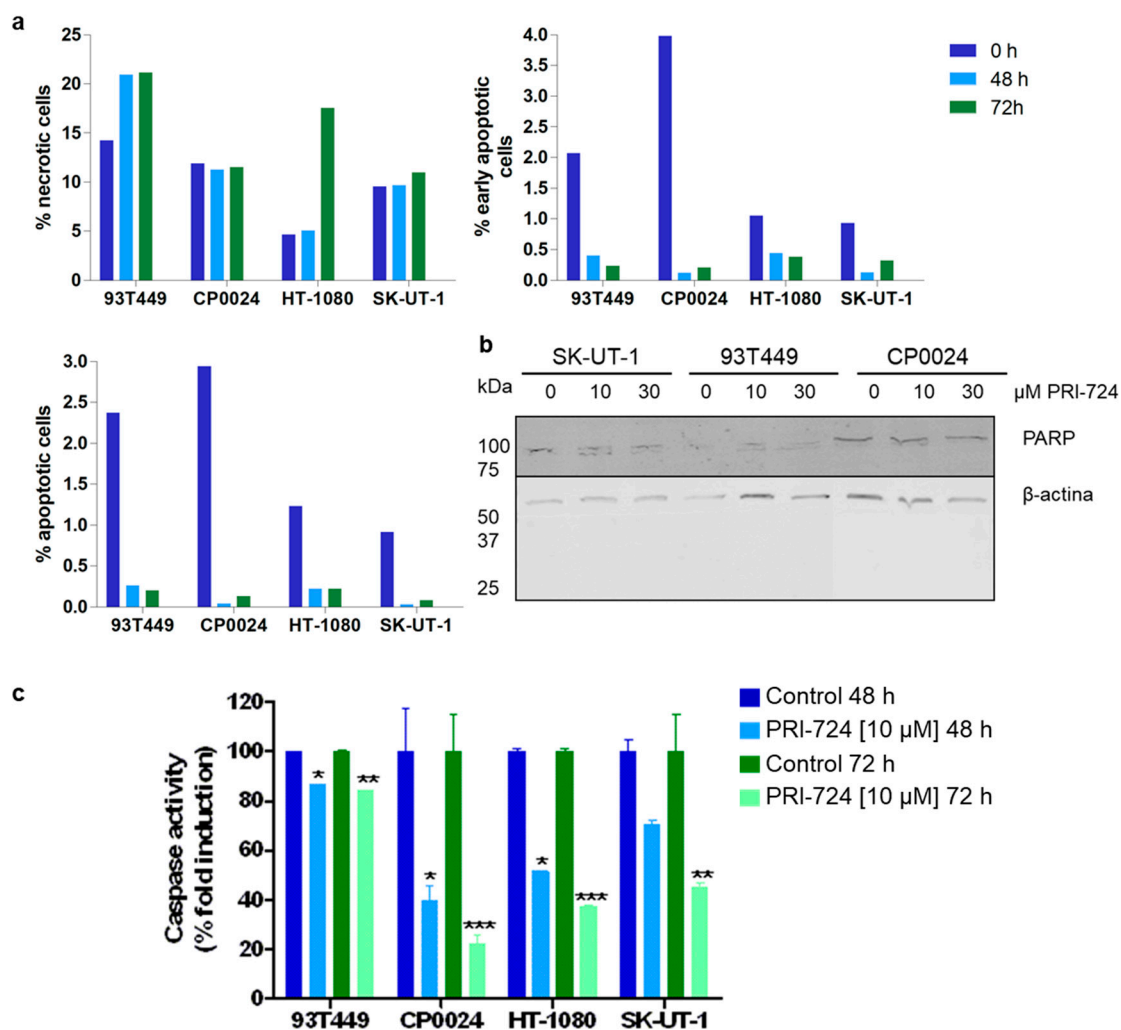


Figure S2. Determination of cellular apoptosis upon treatment with PRI-724 a) Detection of apoptosis by concurrent staining with Annexin V and PI. STS cells were treated with PRI-724 (10 μM) for 48 and 72 h. Cells were subsequently stained with Annexin V and PI and their fluorescence was measured by flow cytometry. Columns show the percentage of necrotic cells, and cells at early or late stage of apoptosis. b) STS cells were treated with PRI-724 (10 μM) for 48 and 72 h. Panel show the immunoreactive bands of PARP and β-actin, used as loading control. c) Caspase 3/7 activation upon treatment with PRI-724. STS cells were treated with PRI-724 (at 10 μM) and incubated for 48 and 72 h. Caspase activity was determined using the Caspase-Glo 3/7 Assay Kit. Caspase activity is represented as fold induction relative to vehicle-treated cells, and data represents mean ± S.E.M. from two independent determinations performed in triplicate. *, $p < 0.05$; **, $p < 0.01$ and ***, $p < 0.001$ compared with vehicle-treated cells.

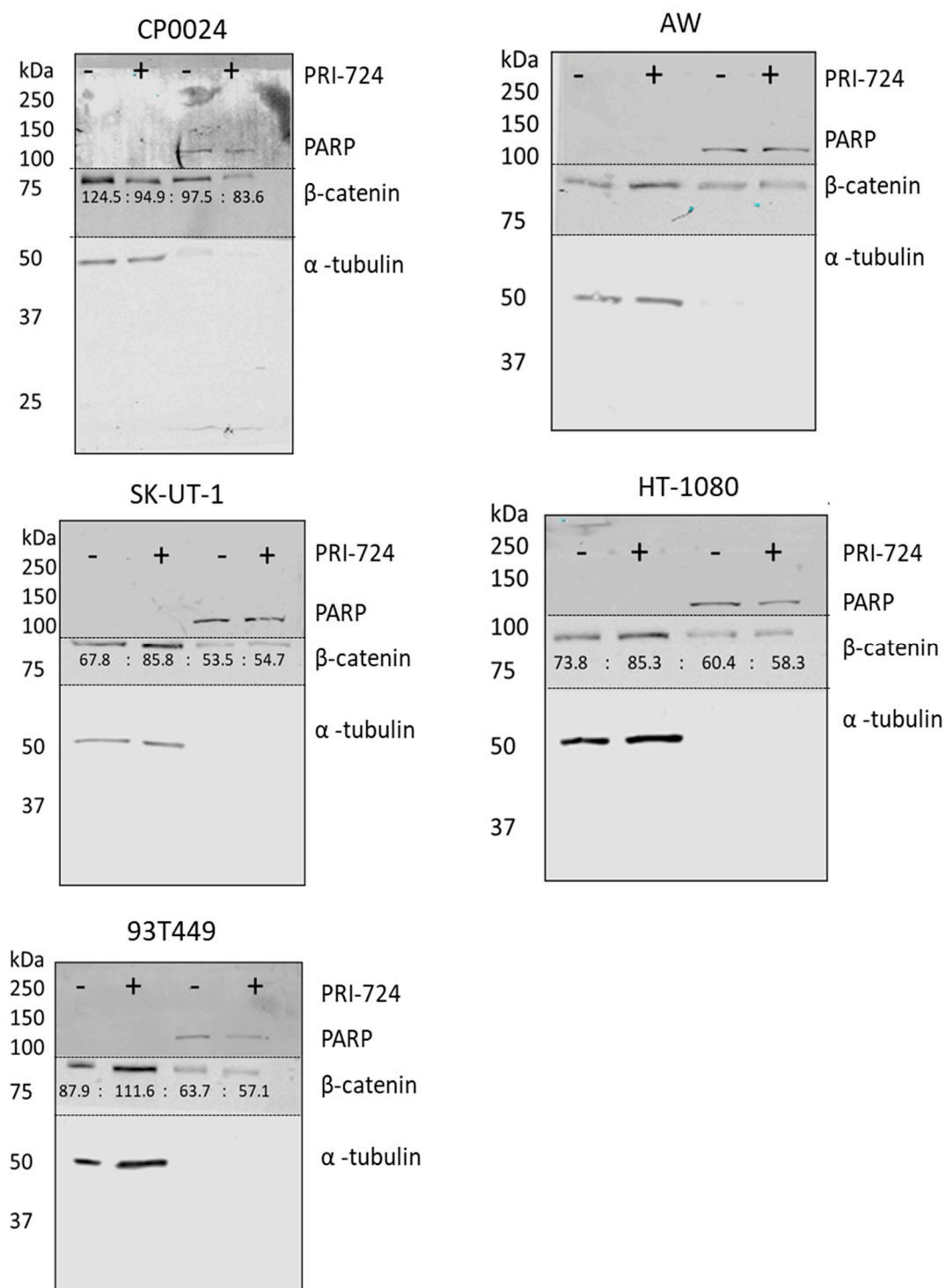


Figure S3. Inhibition of CBP/β-catenin interaction does not alter β-catenin subcellular localization. STS cells treated with PRI-724 (10 μM) for 48 h were subjected to subcellular fractionation. Upper panel show the immunoreactive bands of β-catenin (#8480, Cell Signaling, 92 kDa) in cytoplasmic and nuclear fractions in a representative immunoblot of at least three independent experiments. α-Tubulin (#T9026, Sigma-Aldrich, 50 kDa) and PARP (#9542S, Cell Signaling, 116 kDa) were used as a cytoplasmic and nuclear marker, respectively.

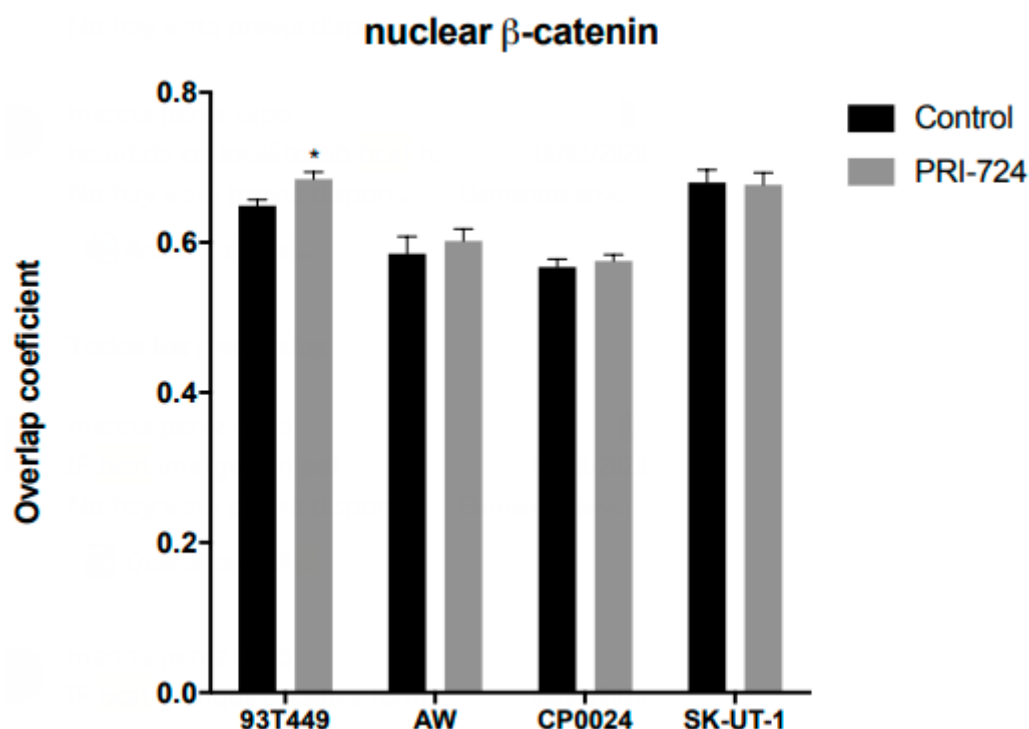


Figure S4. Inhibition of CBP/β-catenin interaction does not alter β-catenin nuclear levels in STS cells. 48h after treatment with PRI-724 (10 μM), cells were fixed with methanol: acetone (1:1) and incubated with the primary antibody β-catenin (D10A8) [(XP Rabbit mAb #8480, Cell Signaling Technology)] followed by the addition of the secondary antibody Alexa Fluor 488 (Goat a-R A11008, Invitrogen). DAPI was added to visualize the nuclei. Images were taken with confocal microscope (ZEISS LSM 710, with a 40X objective and power of 512 × 512 pixels) and colocalization analysis was made using ZEN 2011 software to detect the presence of β-catenin in the nucleus. PRI-724 treated cells presented similar protein levels of nuclear β-catenin to vehicle-treated cells. Each column represents means ± SEM of at least two independent experiments performed by duplicate. *, $p < 0.05$ —compared with vehicle-treated cells.

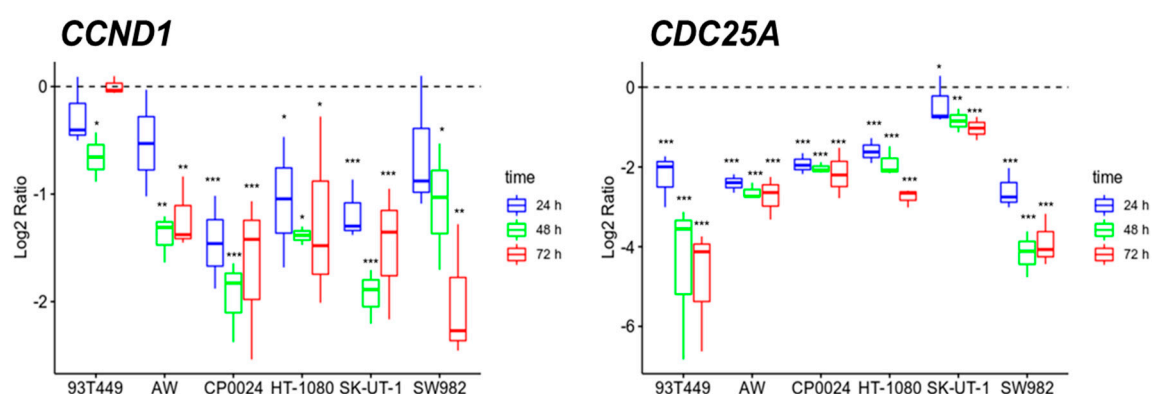


Figure S5. Inhibition of CBP/β-catenin interaction decreases the expression of TCF/β-catenin dependent target genes. *CCND1* and *CDC25A* mRNA expression levels in STS cells treated with PRI-724 (10 μM) for 24, 48 and 72 h. Each column represents means ± SEM of three independent determinations performed by duplicate. *, $p < 0.05$, **, $p < 0.01$ and ***, $p < 0.001$ compared with vehicle-treated cells.

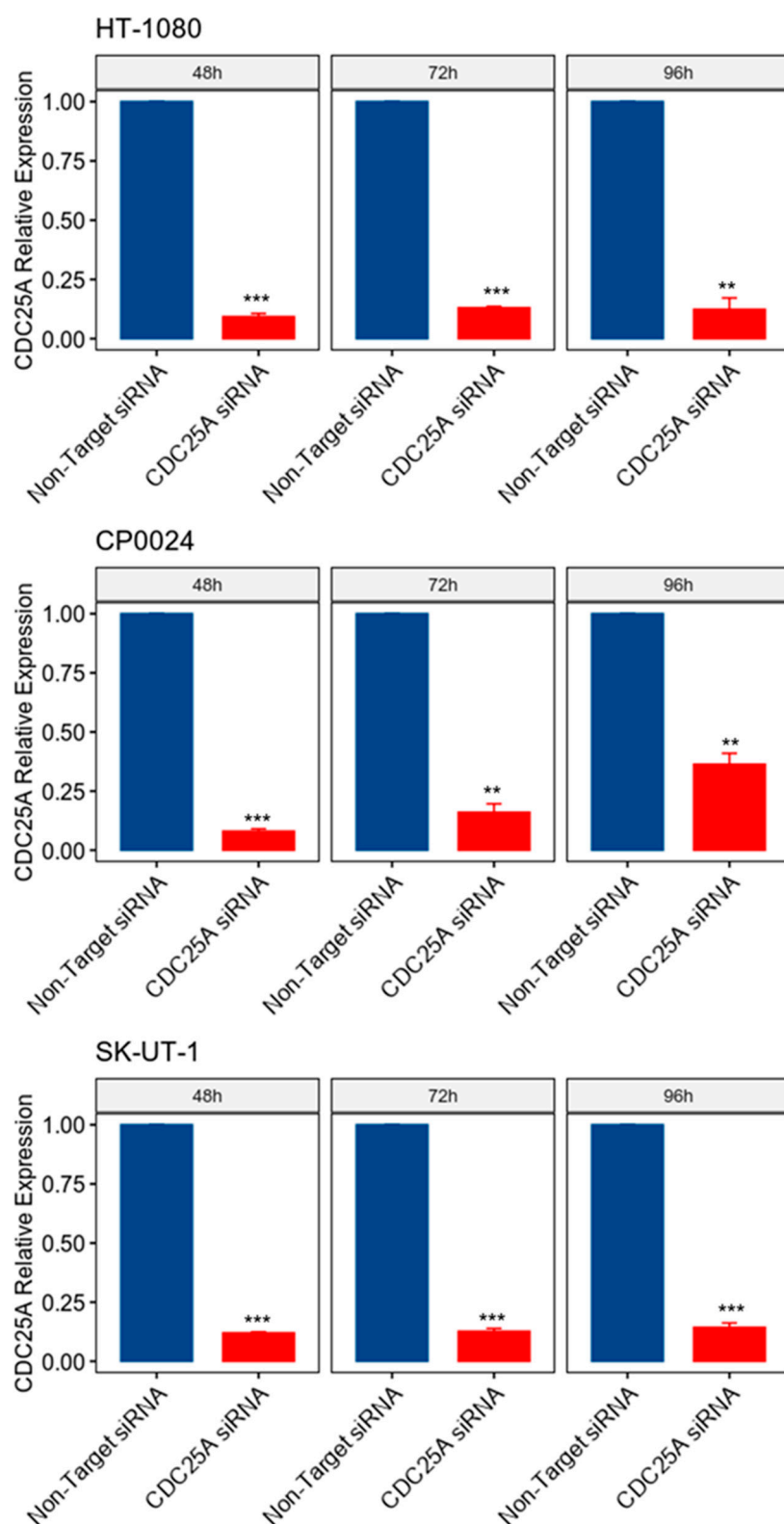


Figure S6. *CDC25A* mRNA expression levels in STS cells after transfection of *CDC25A* siRNA for 48, 72 and 96 h. Each column represents means \pm SEM of three independent determinations performed by duplicate. *, $p < 0.01$ and ***, $p < 0.001$ compared with non-target siRNA transfected cells.

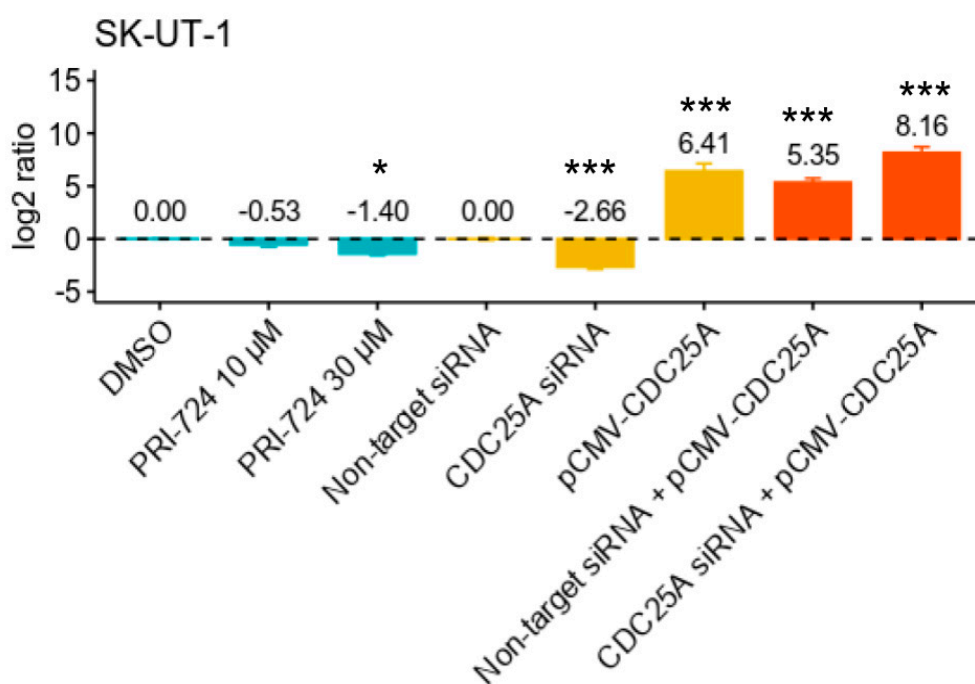


Figure S7. CDC25A mRNA expression levels in STS cells. SK-UT-1 cells were treated with PRI-724 (10 and 30 µM), transfected with CDC25A siRNA or non-target siRNA for 72 h. After 24 h of siRNA transfection, cells were co-transfected with pCMV-CDC25A plasmid or the empty vector pcDNA3.1 for 48 h. Each column represents mean \pm SEM of three independent experiments performed in duplicate. *, $p < 0.05$ and ***, $p < 0.001$ compared with their respective control cells.

Supplementary Tables

Table S1. STS cell lines characteristics and IC₅₀ values for PRI-724 after 48 h.

Cell line	Histology	Mutations		Other mutated genes	PRI-724 IC ₅₀ value (µM)
		APC	β-catenin (CTNNB1)		
93T449	Liposarcoma	NA	NA	MDM2, CDK4, HMGA2	5.18 \pm 0.95
AW	Liposarcoma	NA	NA	NA	8.80 \pm 0.99
HT-1080	Fibrosarcoma	No	No	CDKN2A, IDH1, NRAS	9.35 \pm 1.25
CP0024	Leiomyosarcoma	NA	NA	NA	9.77 \pm 1.01
SW982	Synovial Sarcoma	No	No	BRAF, CDKN2A	20.13 \pm 3.21
SW872	Liposarcoma	No	No	BRAF, CDKN2A, PTEN, TP53	36.03 \pm 5.15
SK-UT-1	Leiomyosarcoma	(c.4661delA) (c.3286C>T)	No	PIK3A, PTEN, RB1, TP53	38.86 \pm 4.31
SW684	Fibrosarcoma	(c.5460C>T)	(c.1213C>T)	TP53	>50
ICP020	Liposarcoma	NA	NA	NA	>50

NA: not available Data actualized from [14].

Table S2. Combination Index (CI) values for combinations of the CBP/β-catenin inhibitor with standard chemotherapeutic agents: trabectedin and doxorubicin in STS cells.

Cell line	PRI-724:Trabectedin			PRI-724:Doxorubicin		
	1:1	1:3	3:1	1:1	1:3	3:1
93T449	0.99	1.44	1.07	0.73	0.96	0.87
AW	0.68	0.77	0.70	1.48	2.44	0.87
HT-1080	0.86	0.90	0.79	0.78	0.85	0.60
CP0024	0.77	1.01	0.75	0.69	0.47	0.69
SK-UT-1	1.23	1.20	1.31	0.75	0.58	0.71

Table S3. Significant top 100 pathways enriched in *CDC25A* high STS samples. Green: DNA repair related pathways, Blue: Cell cycle pathways.

C2: curated gene sets enriched in <i>CDC25A</i> high vs low					
<i>CDC25A</i> high FDR q-val < 0.25 NOMP-val < 0.05	SIZE	ES	NES	NOMP-val	FDR q-val
REACTOME RECRUITMENT OF NUMA TO MITOTIC CENTROSOMES	80	0.88	2.05	0.000	0.014
REACTOME DNA DOUBLE STRAND BREAK REPAIR	116	0.71	2.03	0.000	0.005
KEGG HOMOLOGOUS RECOMBINATION	27	0.77	2.03	0.000	0.005
REACTOME ATRK ACTIVATION BY TPX2	65	0.73	2.03	0.000	0.005
REACTOME HOMOLOGOUS DIRECTED REPAIR	89	0.73	2.01	0.000	0.006
REACTOME REGULATION OF TP53 ACTIVITY	140	0.60	2.00	0.000	0.006
REACTOME DNA REPAIR	242	0.63	1.99	0.000	0.006
KEGG BASE EXCISION REPAIR	30	0.78	1.99	0.000	0.006
REACTOME REGULATION OF TP53 ACTIVITY THROUGH PHOSPHORYLATION	82	0.66	1.98	0.002	0.006
REACTOME ANCHORING OF THE BASAL BODY TO THE PLASMA MEMBRANE	83	0.61	1.97	0.000	0.006
REACTOME RECRUITMENT OF MITOTIC CENTROSOME PROTEINS AND COMPLEXES	69	0.69	1.97	0.000	0.006
REACTOME CELL CYCLE MITOTIC	435	0.70	1.95	0.000	0.007
REACTOME HDR THROUGH HOMOLOGOUS RECOMBINATION HRR	55	0.77	1.94	0.000	0.007
REACTOME REGULATION OF PLK1 ACTIVITY AT G2 M TRANSITION	79	0.67	1.94	0.000	0.007
REACTOME DNA REPLICATION	115	0.78	1.93	0.000	0.007
REACTOME MITOTIC PROMETAPHASE	172	0.72	1.93	0.000	0.007
REACTOME MITOTIC G2 M PHASES	174	0.63	1.93	0.000	0.007
REACTOME PROCESSING OF DNA DOUBLE STRAND BREAK ENDS	57	0.72	1.92	0.002	0.007
REACTOME BASE EXCISION REPAIR	41	0.77	1.92	0.000	0.007
REACTOME TELOMERE MAINTENANCE	39	0.74	1.91	0.000	0.007
REACTOME EXTENSION OF TELOMERES	39	0.74	1.91	0.000	0.007
REACTOME RESOLUTION OF ABASIC SITES AP SITES	34	0.81	1.91	0.000	0.007
REACTOME CELL CYCLE CHECKPOINTS	224	0.71	1.91	0.000	0.007
REACTOME G2 M CHECKPOINTS	122	0.71	1.91	0.002	0.007
REACTOME RESOLUTION OF D LOOP STRUCTURES	27	0.77	1.90	0.000	0.007
REACTOME M PHASE	303	0.64	1.90	0.000	0.007
REACTOME FANCONI ANEMIA PATHWAY	26	0.74	1.90	0.000	0.007
REACTOME CHROMOSOME MAINTENANCE	60	0.77	1.89	0.000	0.008
REACTOME TRANSCRIPTION COUPLED NUCLEOTIDE EXCISION REPAIR TC_NER	72	0.63	1.89	0.000	0.008
REACTOME MITOTIC METAPHASE AND ANAPHASE	198	0.66	1.88	0.002	0.008
REACTOME MITOTIC G1 PHASE AND G1 S TRANSITION	140	0.71	1.88	0.000	0.008
REACTOME HOMOLOGOUS DNA PAIRING AND STRAND EXCHANGE	38	0.79	1.88	0.000	0.008
REACTOME TRNA PROCESSING IN THE NUCLEUS	50	0.62	1.88	0.002	0.008
REACTOME DNA REPLICATION PRE INITIATION	79	0.73	1.88	0.002	0.008
REACTOME S PHASE	142	0.68	1.88	0.002	0.008
REACTOME DUAL INCISION IN TC_NER	59	0.64	1.88	0.000	0.008
REACTOME RHO GTPASES ACTIVATE FORMINS	117	0.67	1.88	0.000	0.008
REACTOME GAP FILLING DNA REPAIR SYNTHESIS AND LIGATION IN GG_NER	23	0.86	1.88	0.000	0.008
REACTOME TRANSCRIPTIONAL REGULATION BY TP53	303	0.53	1.87	0.000	0.007
REACTOME SUMOYLATION OF DNA DAMAGE RESPONSE AND REPAIR PROTEINS	68	0.60	1.87	0.000	0.007
REACTOME RESOLUTION OF D LOOP STRUCTURES THROUGH SYNTHESIS DEPENDENT STRAND ANNEALING SDSA	25	0.78	1.87	0.000	0.007
REACTOME G2 M DNA DAMAGE CHECKPOINT	54	0.74	1.87	0.000	0.008
REACTOME NONHOMOLOGOUS END JOINING NHEJ	32	0.66	1.86	0.002	0.008
REACTOME DUAL INCISION IN GG_NER	38	0.70	1.86	0.002	0.008
KEGG CELL CYCLE	114	0.72	1.85	0.000	0.008
REACTOME RECOGNITION OF DNA DAMAGE BY PCNA CONTAINING REPLICATION COMPLEX	28	0.81	1.85	0.000	0.008
REACTOME MITOTIC SPINDLE CHECKPOINT	91	0.72	1.85	0.000	0.009
REACTOME NUCLEAR ENVELOPE REASSEMBLY	64	0.66	1.84	0.004	0.009
KEGG PROGESTERONE MEDIATED OOCYTE MATURATION	73	0.61	1.84	0.002	0.009
REACTOME SEPARATION OF SISTER CHROMATIDS	160	0.64	1.84	0.004	0.009
REACTOME KINESINS	44	0.79	1.84	0.000	0.009
REACTOME TRANSLATION SYNTHESIS BY Y FAMILY DNA POLYMERASES BYPASSES LESIONS ON DNA TEMPLATE	33	0.74	1.84	0.002	0.009
KEGG MISMATCH REPAIR	22	0.85	1.83	0.000	0.009
REACTOME MEIOTIC RECOMBINATION	24	0.66	1.83	0.002	0.009
REACTOME SUMOYLATION OF DNA REPLICATION PROTEINS	43	0.70	1.83	0.000	0.009
REACTOME TRANSCRIPTIONAL REGULATION BY E2F6	30	0.75	1.83	0.000	0.009
REACTOME RESOLUTION OF SISTER CHROMATID COHESION	106	0.73	1.83	0.000	0.009
REACTOME DNA DAMAGE BYPASS	41	0.73	1.82	0.002	0.009
REACTOME HDR THROUGH SINGLE STRAND ANNEALING SSA	33	0.75	1.82	0.000	0.010
REACTOME SUMOYLATION OF RNA BINDING PROTEINS	42	0.60	1.82	0.000	0.010
REACTOME TERMINATION OF TRANSLATION DNA SYNTHESIS	28	0.79	1.82	0.000	0.010
REACTOME NUCLEOTIDE EXCISION REPAIR	99	0.58	1.81	0.002	0.010
REACTOME GENE SILENCING BY RNA	61	0.58	1.81	0.002	0.010
REACTOME INTERACTIONS OF VPR WITH HOST CELLULAR PROTEINS	35	0.68	1.81	0.000	0.010
REACTOME PROCESSING OF CAPPED INTRON CONTAINING PRE mRNA	198	0.65	1.80	0.009	0.010
REACTOME ACTIVATION OF ATR IN RESPONSE TO REPLICATION STRESS	34	0.87	1.80	0.000	0.010
REACTOME POLYMERASE SWITCHING ON THE C STRAND OF THE TELOMERE	15	0.91	1.80	0.000	0.011
KEGG PYRIMIDINE METABOLISM	75	0.59	1.80	0.000	0.011
KEGG DNA REPLICATION	34	0.87	1.79	0.000	0.011
REACTOME TRANSPORT OF MATURE TRANSCRIPT TO CYTOPLASM	65	0.57	1.79	0.002	0.011
REACTOME TRANSCRIPTION OF THE HIV GENOME	64	0.53	1.79	0.008	0.011
REACTOME SNRNP ASSEMBLY	47	0.63	1.79	0.002	0.011
KEGG CYSTEINE AND METHIONINE METABOLISM	32	0.62	1.79	0.000	0.012
KEGG OOCYTE MEIOSIS	92	0.63	1.79	0.002	0.011
REACTOME TRNA PROCESSING	87	0.53	1.78	0.004	0.011
REACTOME HCMV EARLY EVENTS	68	0.60	1.78	0.000	0.012
KEGG NUCLEOTIDE EXCISION REPAIR	42	0.68	1.77	0.002	0.013
REACTOME ACTIVATION OF THE PRE REPLICATIVE COMPLEX	31	0.88	1.77	0.000	0.013
REACTOME TELOMERE C STRAND LAGGING STRAND SYNTHESIS	25	0.83	1.77	0.000	0.013
REACTOME VIRAL MESSENGER RNA SYNTHESIS	42	0.62	1.77	0.002	0.013
REACTOME MRNA SPLICING	154	0.54	1.76	0.022	0.013
REACTOME NUCLEAR ENVELOPE BREAKDOWN	47	0.69	1.76	0.002	0.014
REACTOME TRANSCRIPTIONAL REGULATION BY SMALL RNAs	43	0.61	1.76	0.004	0.014
REACTOME SWITCHING OF ORIGINS TO A POST REPLICATIVE STATE	79	0.66	1.75	0.007	0.014
REACTOME HIV ELONGATION ARREST AND RECOVERY	31	0.59	1.75	0.007	0.015
REACTOME REPRODUCTION	64	0.59	1.75	0.002	0.015
REACTOME RNA POLYMERASE II TRANSCRIPTION TERMINATION	48	0.57	1.75	0.011	0.015
REACTOME RESOLUTION OF AP SITES VIA THE MULTIPLE NUCLEOTIDE PATCH REPLACEMENT PATHWAY	23	0.84	1.75	0.000	0.015
REACTOME ASSEMBLY OF THE PRE REPLICATIVE COMPLEX	63	0.66	1.75	0.009	0.015
REACTOME HIV TRANSCRIPTION INITIATION	43	0.55	1.75	0.008	0.015
REACTOME G0 AND EARLY G1	24	0.83	1.74	0.000	0.015
REACTOME SUMOYLATION OF UBIQUITYLATION PROTEINS	37	0.60	1.74	0.008	0.015
REACTOME GLOBAL GENOME NUCLEOTIDE EXCISION REPAIR GG_NER	76	0.59	1.74	0.008	0.015
REACTOME REGULATION OF GLUCOKINASE BY GLUCOKINASE REGULATORY PROTEIN	30	0.66	1.74	0.002	0.016
REACTOME TRANSLATION SYNTHESIS BY POLH	19	0.74	1.74	0.008	0.015
REACTOME APC C MEDIATED DEGRADATION OF CELL CYCLE PROTEINS	78	0.69	1.74	0.002	0.016
REACTOME COP1 DEPENDENT GOLGI TO ER RETROGRADE TRAFFIC	78	0.65	1.74	0.011	0.016
REACTOME INTERACTIONS OF REV WITH HOST CELLULAR PROTEINS	35	0.65	1.74	0.000	0.016
REACTOME KSRP_KHSRP BINDS AND DESTABILIZES MRNA	15	0.68	1.74	0.008	0.016
REACTOME_PMI1 INTERACTING RNA_PIRNA BIOGENESIS	21	0.66	1.74	0.008	0.016



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