

Supplementary Table S1. Primers for RT-qPCR and bisulfite sequencing.

| Primer | Sequence | Purpose |
|----------------|-------------------------------------|-------------------------------|
| GAPDH_Fw | GGTGAAGGTCGGAGTCAACGGA | RT-qPCR |
| GAPDH_Rv | GAGGGATCTCGTCCTGGAAGA | RT-qPCR |
| PRKCZ_Fw | ACATGTGTCGTCTGCACCAG | RT-qPCR |
| PRKCZ_Rv | GTGCTCGGAAAAACATGAAT | RT-qPCR |
| ATM_Fw | CCGAGTGCAGTGACAGTG | RT-qPCR |
| ATM_Rv | TTGACGGCAGCAGATAAGC | RT-qPCR |
| COL2A1_Fw | CAAACTGCCAACGTCCAGAT | RT-qPCR |
| COL2A1_Rv | CTGCTTCGTCCAGATAGGCAAT | RT-qPCR |
| ACAN_Fw | AGGCAGCGTGATCCTTACC | RT-qPCR |
| ACAN_Rv | GGCCTCTCCAGTCTCATTCTC | RT-qPCR |
| PRKCZ_Fw | AATTGGATCCAGGATTGGTTTAGGTG | Targeted bisulfite sequencing |
| PRKCZ_Rv | GGGGGAATTCAAAACTCCAAATAAAACC | Targeted bisulfite sequencing |
| PRKCZ_cDNA1_Fw | TAGCTCTAGATGCCCAGCAGGACC | Vector Construction |
| PRKCZ_cDNA1_Rv | GTACGAATTCACACCGACTCCTCGGT | Vector Construction |
| PRKCZ_cDNA2_Fw | GCTTCGAATTCTGCAGATGCCCAGCAGGACCGGC | Vector Construction |
| PRKCZ_cDNA2_Rv | CGGGCCCGCGGTACCGCACACCGACTCCTCGGTGG | Vector Construction |

Fw, Forward. Rv, Reverse. PRKCZ_cDNA1 primers were inserted to pCDH-EF1-MCS-IRES-Puro. PRKCZ_cDNA2 primers were inserted to pEGFP-N1-FLAG.

Supplementary Table S2. Antibodies for western blotting.

| Antibody | Dilution rate | Manufacturer | Catalog number |
|---------------------|----------------------|---------------------------|-----------------------|
| Actin | 1:5000 | Millipore | MAB1501 |
| PRKCZ | 1:500 | Sigma Aldrich | HPA021851 |
| ATM | 1:1000 | Cell Signaling Technology | 2873 |
| phospho-ATM (S1981) | 1:1000 | Cell Signaling Technology | 5883 |
| CHK2 | 1:1000 | Cell Signaling Technology | 6334 |
| phospho-CHK2(T68) | 1:1000 | Cell Signaling Technology | 2197 |
| MRE11 | 1:1000 | Cell Signaling Technology | 4874 |
| RAD50 | 1:1000 | Cell Signaling Technology | 3427 |
| NBS1 | 1:1000 | Cell Signaling Technology | 14956 |
| cleaved caspase-3 | 1:500 | Cell Signaling Technology | 9661 |
| cleaved PARP | 1:500 | Promega | G7341 |
| GFP | 1:1000 | Cell Signaling Technology | 2956 |
| mouse IgG | 1:5000 | Santa Cruz Biotechnology | 2005 |
| rabbit IgG | 1:5000 | Santa Cruz Biotechnology | 2004 |

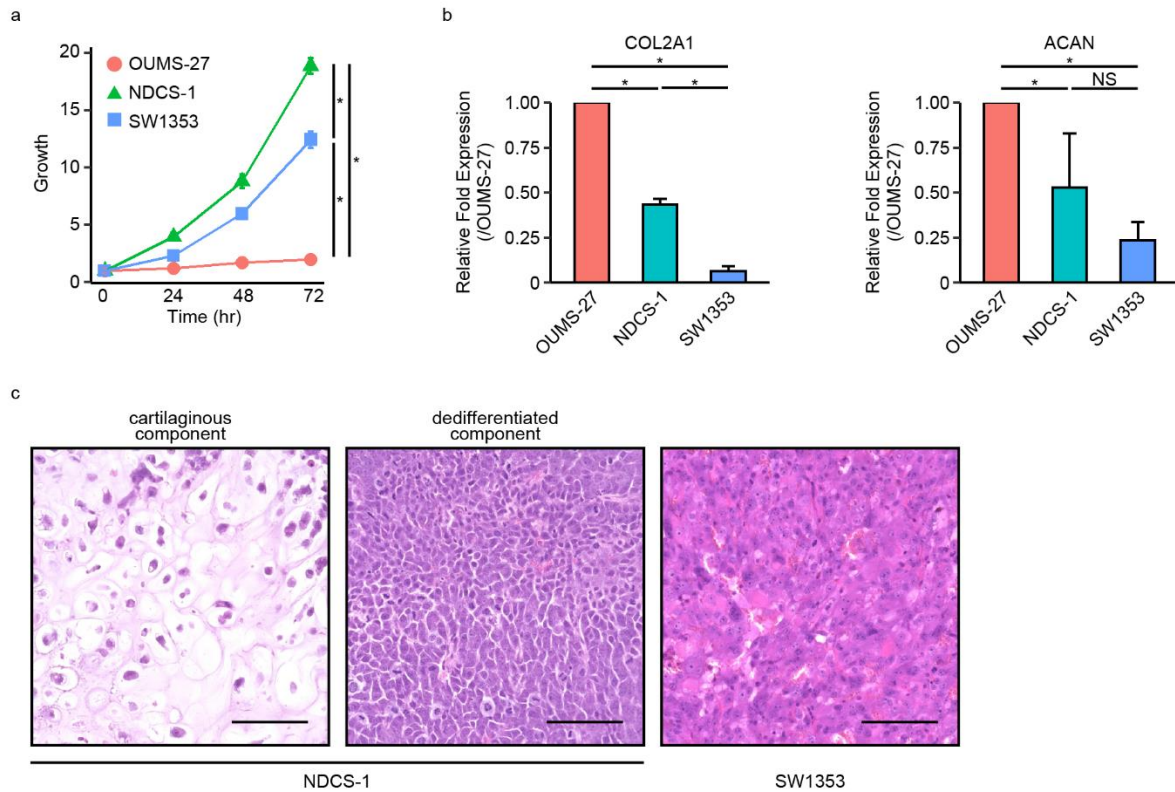
Supplementary Table S3. Clinical demographics of chondrosarcoma samples.

| Case | Diagnosis | Age | Gender | Location | Experiment |
|------|-----------|-----|--------|-------------------------------|--------------|
| 1 | DDCS | 59 | Male | Pelvis | IHC |
| 2 | DDCS | 38 | Male | Pelvis | IHC |
| 3 | DDCS | 53 | Female | Femur | IHC |
| 4 | DDCS | 71 | Male | Pelvis | IHC |
| 5 | DDCS | 71 | Female | Mesentery of descending colon | IHC |
| 6 | DDCS | 59 | Male | Humerus | IHC |
| 7 | DDCS | 54 | Female | Pelvis | IHC |
| 8 | DDCS | 71 | Male | Femur | IHC |
| 9 | DDCS | 44 | Male | Femur | IHC, RT-qPCR |
| 10 | DDCS | 73 | Male | Femur | IHC |
| 11 | DDCS | 38 | Female | Humerus | IHC |
| 12 | DDCS | 79 | Male | Rib | IHC |
| 13 | DDCS | 56 | Male | Femur | IHC |
| 14 | DDCS | 62 | Female | Femur | IHC |
| 15 | DDCS | 67 | Male | Femur | IHC, RT-qPCR |
| 16 | DDCS | 68 | Female | Femur | IHC |
| 17 | DDCS | 81 | Male | Spine | IHC, RT-qPCR |
| 18 | CCS | 42 | Female | Pelvis | RT-qPCR |
| 19 | CCS | 27 | Female | Scapula | RT-qPCR |
| 20 | CCS | 49 | Female | Pelvis | RT-qPCR |
| 21 | CCS | 22 | Male | Scapula | RT-qPCR |
| 22 | CCS | 66 | Female | Femur | RT-qPCR |
| 23 | CCS | 89 | Female | Rib | RT-qPCR |
| 24 | CCS | 88 | Male | Femur | RT-qPCR |
| 25 | CCS | 57 | Female | Spine | RT-qPCR |
| 26 | CCS | 33 | Male | Pelvis | RT-qPCR |

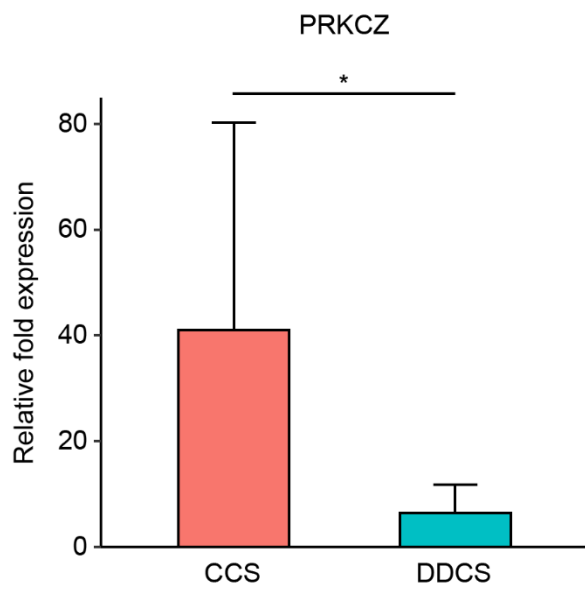
DDCS, dedifferentiated chondrosarcoma. CCS, conventional chondrosarcoma. IHC, immunohistochemistry. RT-qPCR, quantitative reverse transcription polymerase chain reaction.

Supplementary Table S4. Detail data of whole-genome bisulfite sequence.

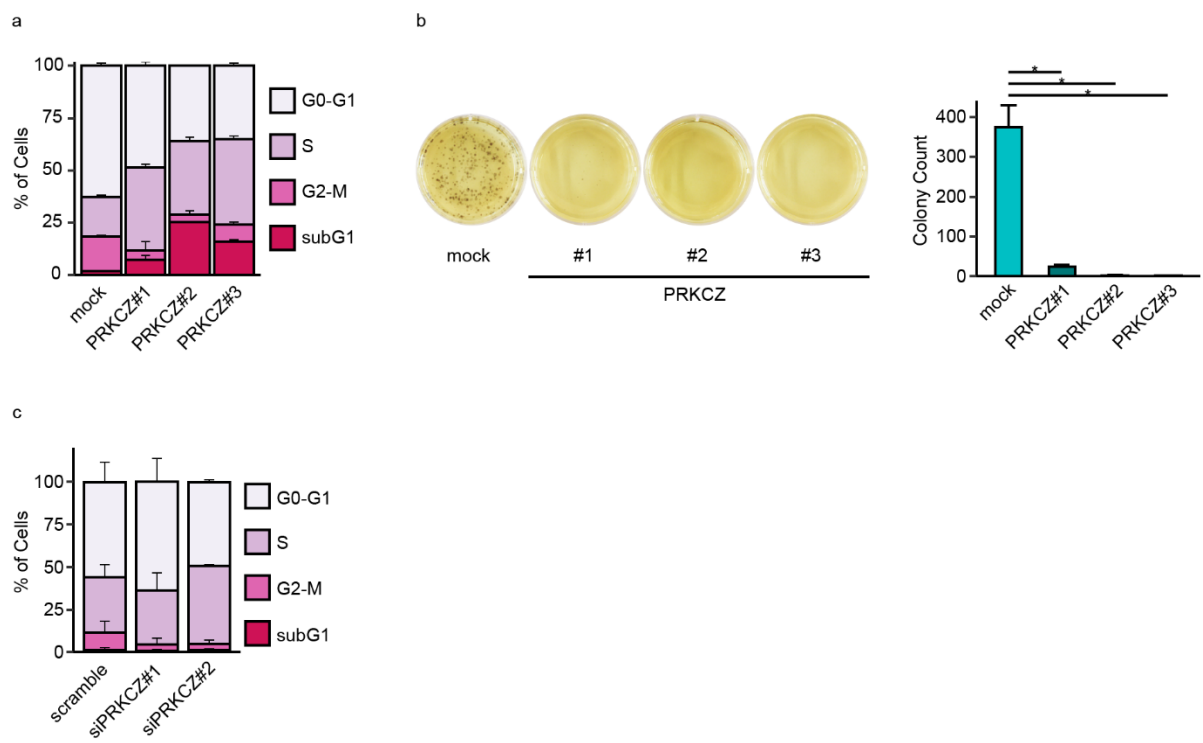
| Cell line | Total sequence pairs | Mapping efficiency | Total number of C's analysed | C methylated in CpG context |
|------------------|-----------------------------|---------------------------|-------------------------------------|------------------------------------|
| NDCS-1 | 590,950,972 | 47.5 % | 15,249,768,806 | 57.2 % |
| OUMS-27 | 581,096,690 | 49.9 % | 15,674,058,312 | 63.6 % |



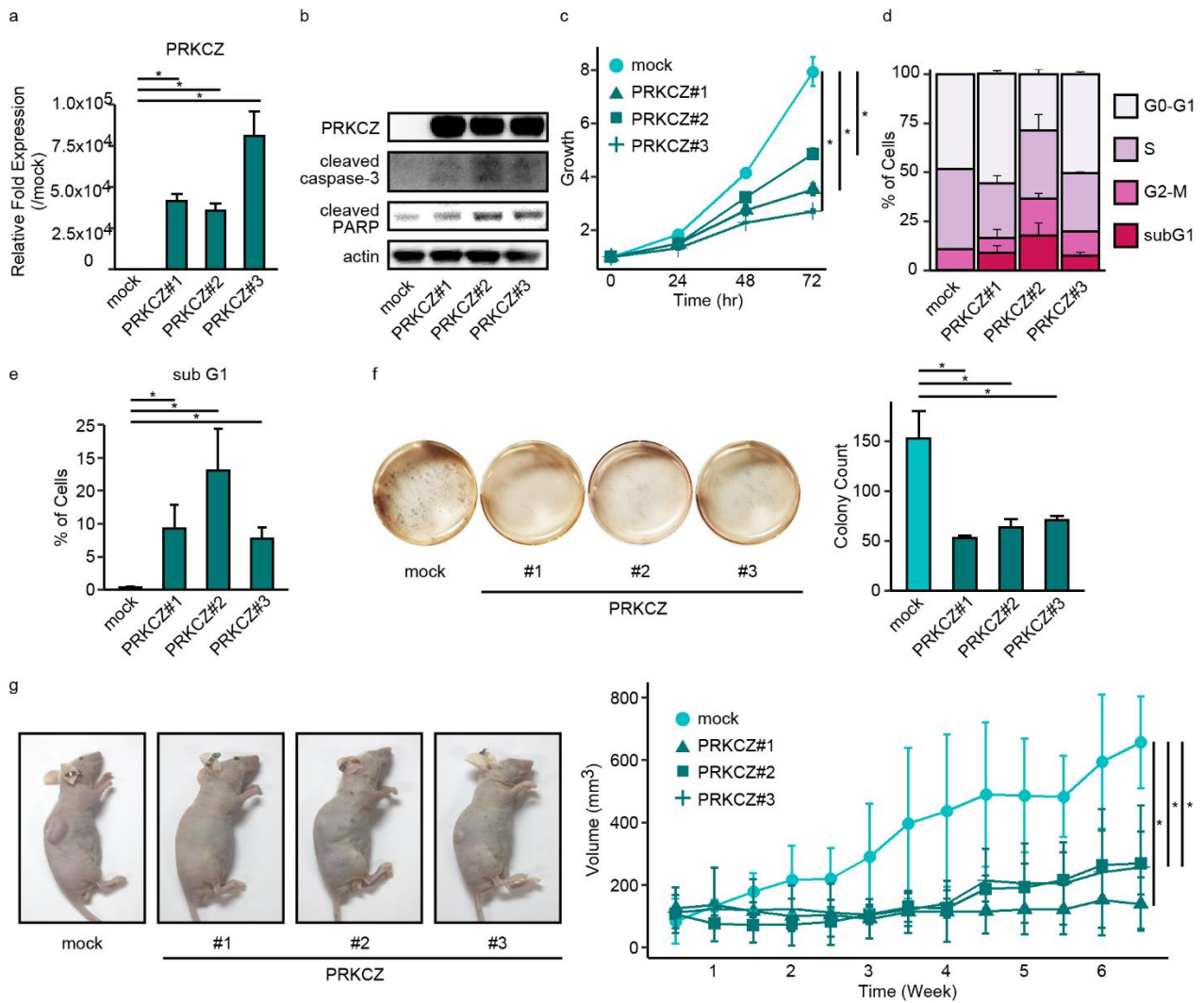
Supplementary Figure S2. Phenotype of each cell line; OUMS-27, NDCS-1, and SW1353. **a.** Proliferation assay. NDCS-1 and SW1353 showed relatively rapid growth compared to OUMS-27. **b.** Expression of chondrogenic differentiation-related genes determined by RT-qPCR. OUMS-27 expressed significantly higher level of chondrogenic differentiation-related genes than the others. **c.** Representative images of HE stains in xenografts of NDCS-1 and SW1353. The xenograft of SW1353 was close to dedifferentiated component rather than cartilaginous component of NDCS-1. Scale bars, 100 μ m. In **a** and **b**, the data are means \pm SD; n = 3. Two-tailed t tests. *, p < 0.05. NS, not significant.



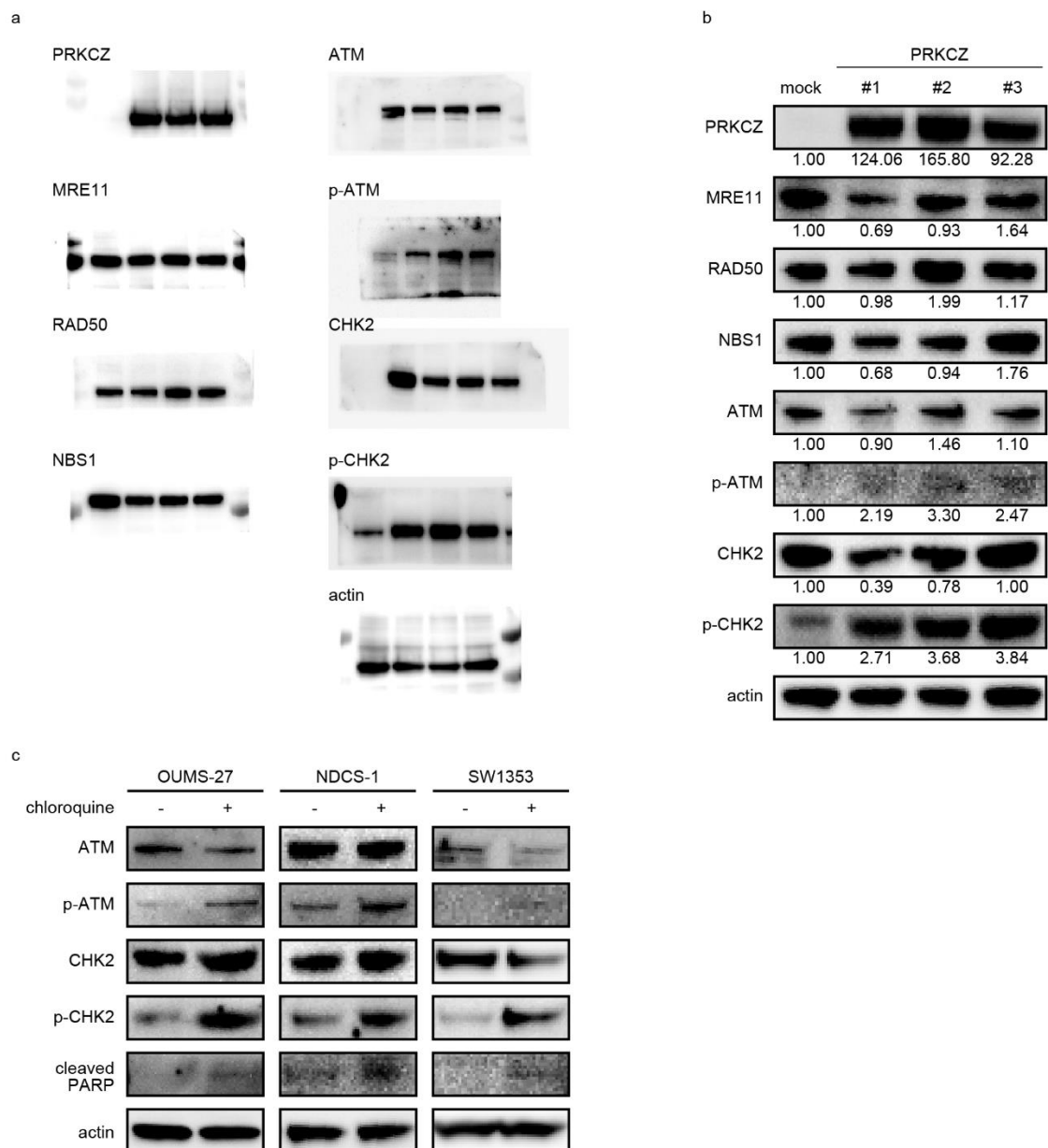
Supplementary Figure S3. Comparison of PRKCZ expression levels in clinical samples. Nine samples of CCS and three samples of DDCS were analyzed by RT-qPCR. The relative fold expression was calculated based on the lowest expression level among all samples. Two-tailed t tests. *, $p < 0.05$.



Supplementary Figure S4. Additional data for Figure 4. a. Cell cycle profiles in PRKCZ-overexpressing NDCS-1 vs. mock-transfected NDCS-1. **b.** Colony counts in PRKCZ-overexpressing NDCS-1 vs. mock-transfected NDCS-1, as determined by colony formation assay. Representative images are shown. Two-tailed t tests. *, $p < 0.05$. **c.** Cell cycle profiles in siPRKCZ-transfected OUMS-27 vs. scrambled siRNA-transfected OUMS-27.

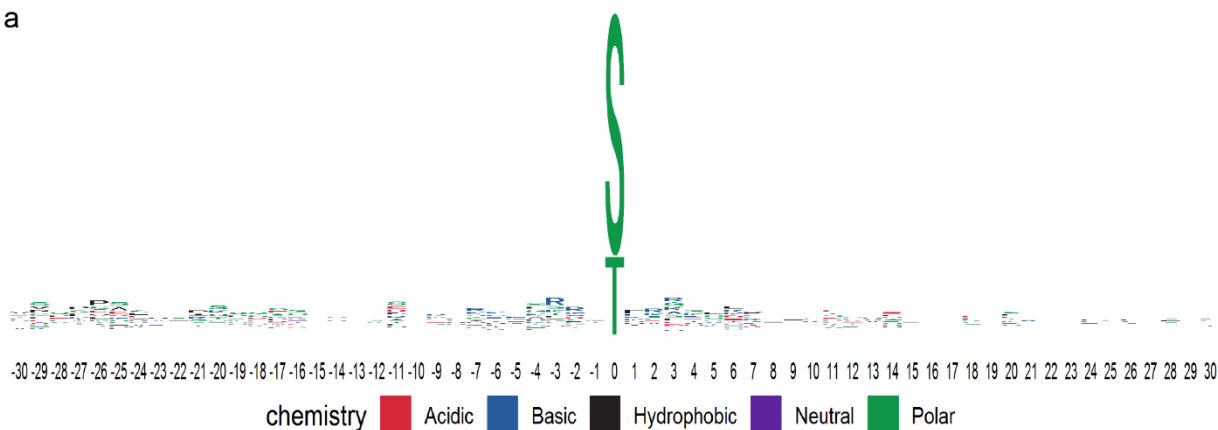


Supplementary Figure S5. PRKCZ overexpression decreases proliferation and induces apoptosis in SW1353. In **a–g**, PRKCZ–overexpressing SW1353 vs. mock–transfected SW1353. **a**. PRKCZ expression determined by RT–qPCR. **b**. PRKCZ and apoptosis–related proteins expression determined by western blotting. **c**. Proliferation assay. **d** and **e**. Cell cycle profiles, and sub G1 fraction rate determined by cell cycle assay. **f**. Colony counts determined by colony formation assay. Representative images are shown. **g**. Time course of tumor volume of xenograft transplantation. Images were obtained at the time of sacrifice; representative images are shown. In **a**, **c**, **e**, **f**, and **g**, data are means \pm SD; $n = 3$. Two–tailed t tests. *, $p < 0.05$.



Supplementary Figure S6. Validation for relationship between PRKCZ expression and ATM/CHK2 activation. a. The original images of western blotting shown in Figure 5b. **b.** Expression of ATM/CHK2-related proteins in PRKCZ-overexpressing SW1353 vs. mock-transfected SW1353. Band intensities were quantified and normalized using actin levels. The relative intensities compared to mock are shown under each band. **c.** Expression of ATM/CHK2-related proteins and apoptosis-related protein in chloroquine-treated each cell line.

a



b

ATM

| Position | Code | Kinase | Peptide | Score | Cutoff |
|----------|------|--------------------|------------------|--------|--------|
| 21 | T | AGC/PKC/PKCi/PRKCZ | QLEHDRATERKKEVE | 51.196 | 41.793 |
| 47 | S | AGC/PKC/PKCi/PRKCZ | IKHLDRHSDSKQGKY | 45.588 | 41.793 |
| 474 | S | AGC/PKC/PKCi/PRKCZ | DKRSNLESSQKSDLL | 43.131 | 41.793 |
| 571 | S | AGC/PKC/PKCi/PRKCZ | CEVNRSFSLKESIMK | 44.304 | 41.793 |
| 1306 | S | AGC/PKC/PKCi/PRKCZ | AYEGTRDSGMAQQRE | 50.485 | 41.793 |
| 1635 | S | AGC/PKC/PKCi/PRKCZ | MVDIMRASQDNPQDG | 47.287 | 41.793 |
| 1695 | S | AGC/PKC/PKCi/PRKCZ | IQHSDKASYTEKALKL | 45.944 | 41.793 |
| 2114 | S | AGC/PKC/PKCi/PRKCZ | MQWDHCTSVSKEVEG | 44.723 | 41.793 |
| 2549 | S | AGC/PKC/PKCi/PRKCZ | NNLISRISMDHPHHT | 47.727 | 41.793 |

CHK2

| Position | Code | Kinase | Peptide | Score | Cutoff |
|----------|------|--------|---------|-------|--------|
|----------|------|--------|---------|-------|--------|

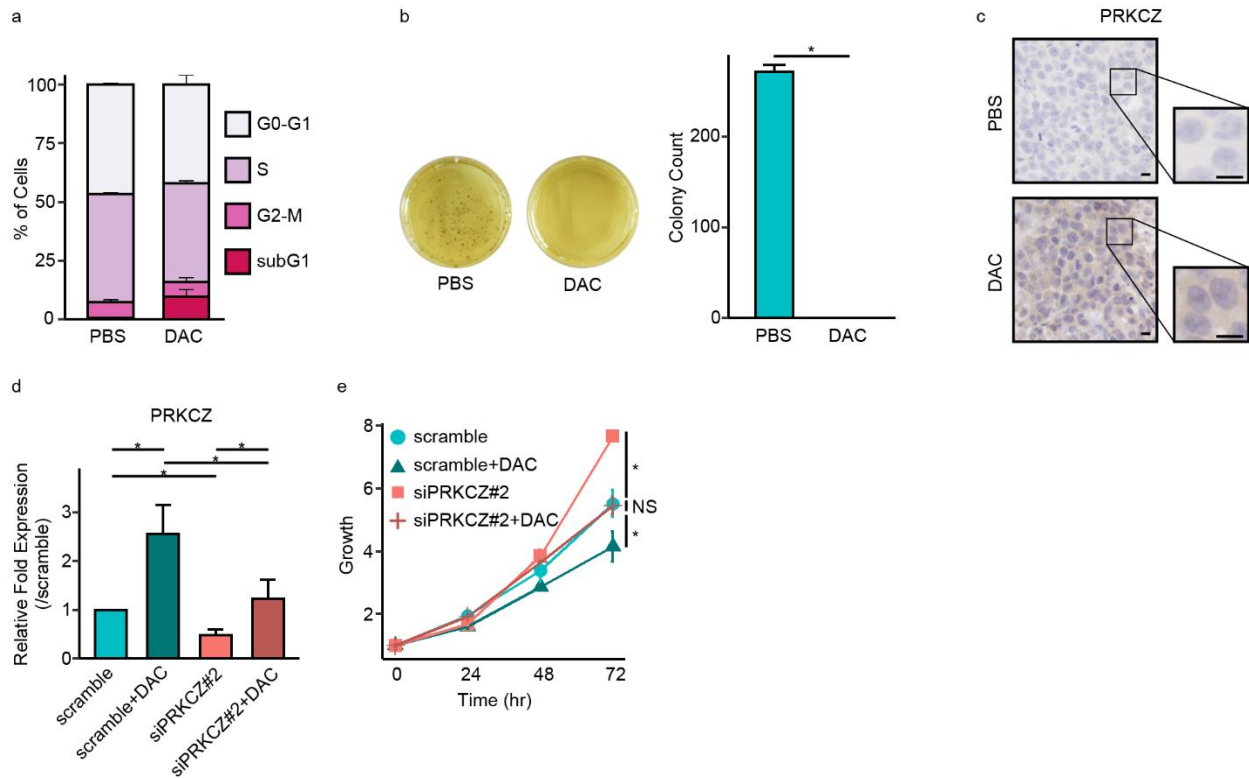
No site was predicted

RAD50

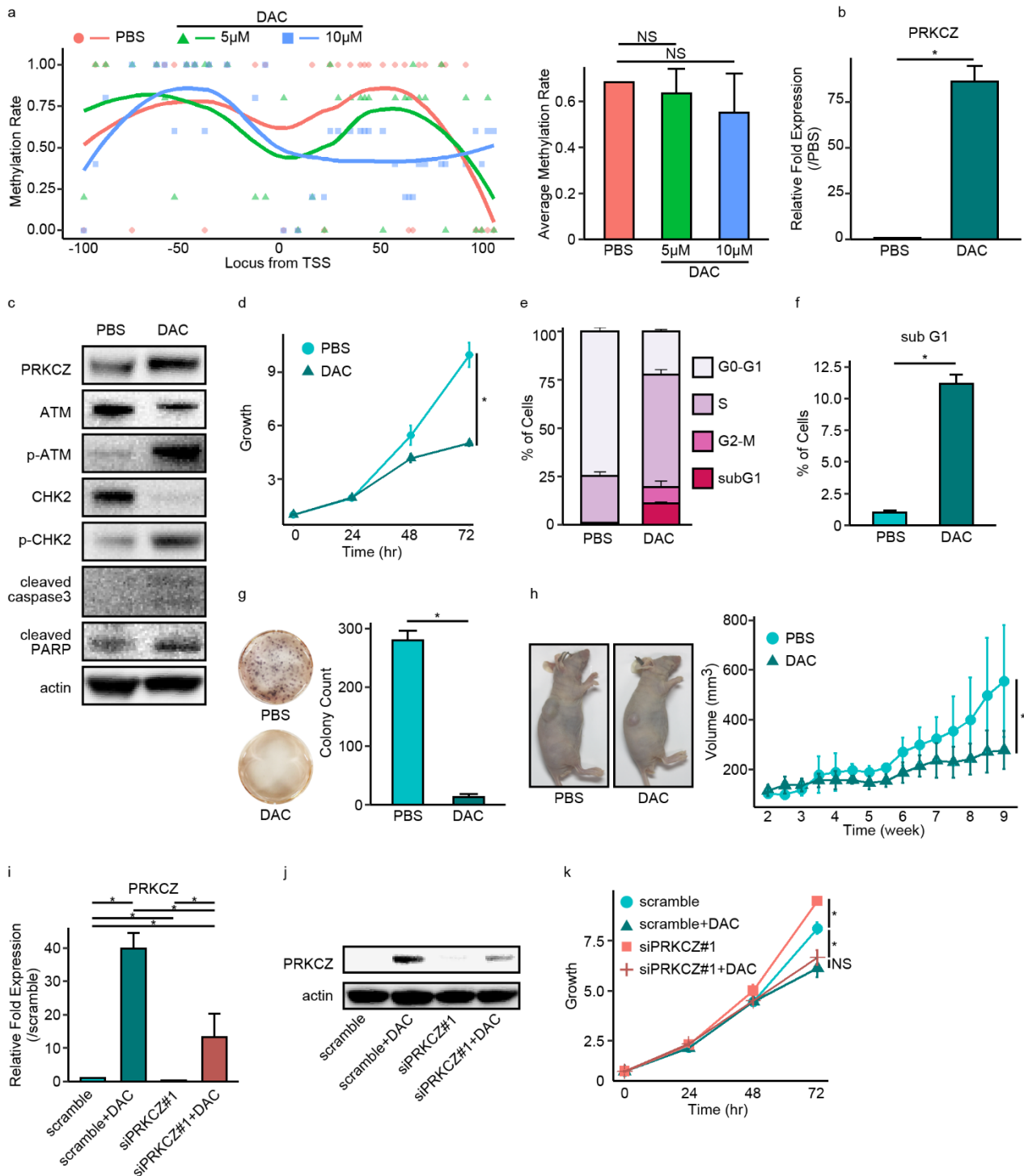
| Position | Code | Kinase | Peptide | Score | Cutoff |
|----------|------|--------|---------|-------|--------|
|----------|------|--------|---------|-------|--------|

No site was predicted

Supplementary Figure S7. Group-based phosphorylation site prediction system (<http://gps.biocuckoo.cn>) identified predicted phosphorylation sites of PRKCZ. a. Sequence logo of PRKCZ. b. Predicted phosphorylation sites of ATM, CHK2, and RAD50. CHK2 and RAD50 have no predicted phosphorylation sites.

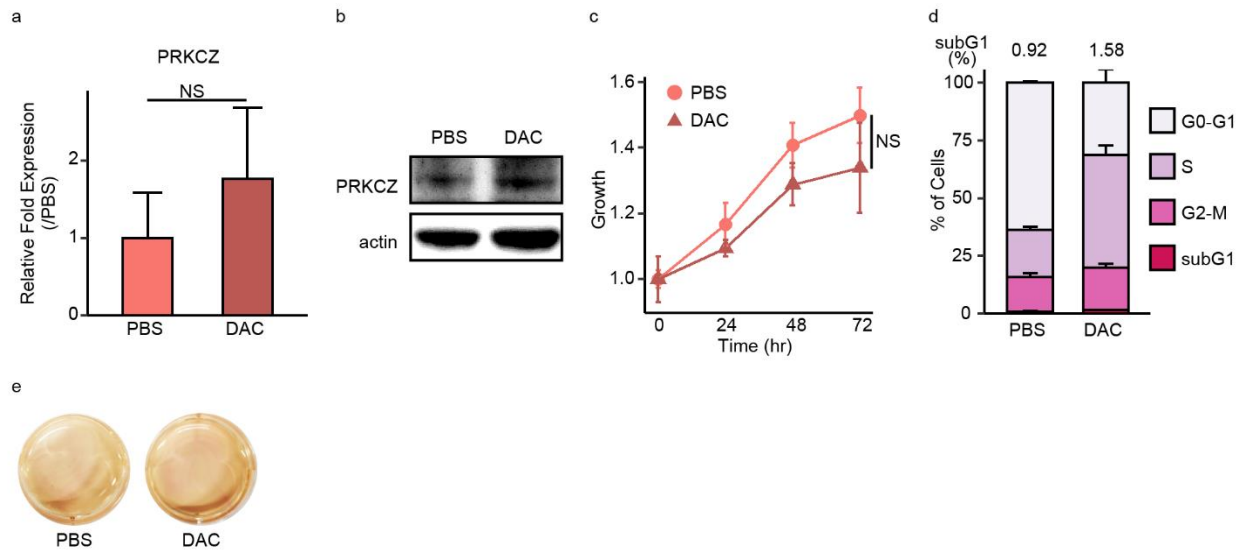


Supplementary Figure S8. Additional data for Figure 6. **a.** Cell cycle profiles in 10 μ M of decitabine–treated NDCS–1 vs. PBS–treated NDCS–1. **b.** Colony counts in 10 μ M of decitabine–treated NDCS–1 vs. PBS–treated NDCS–1, as determined by colony formation assay. Representative images are shown. **c.** Representative images of PRKCZ IHC staining in xenograft. Scale bars, 10 μ m. **d.** PRKCZ expression determined by RT–qPCR. **e.** Proliferation assay. In **b**, **d**, and **e**, data are means \pm SD; n = 3. Two–tailed t tests. *, p < 0.05. NS, not significant. DAC, decitabine.

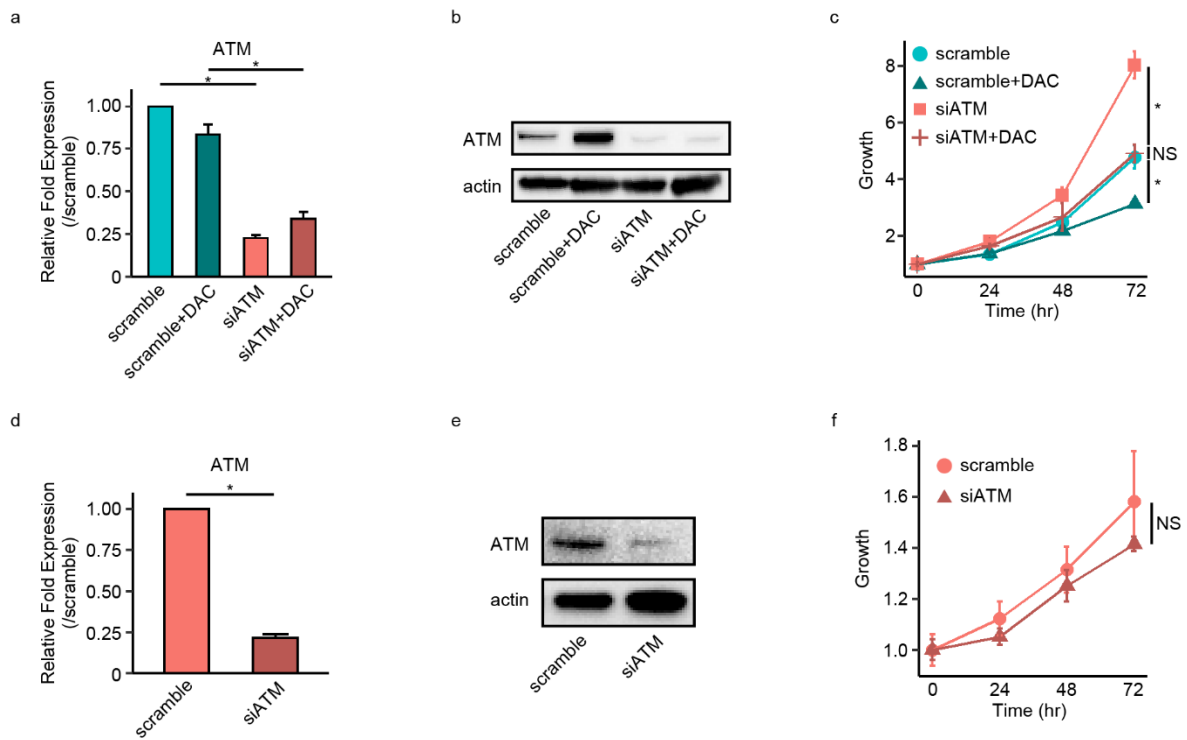


Supplementary Figure S9. Decitabine increases PRKCZ expression and induces apoptosis in SW1353. **a.** DNA methylation levels on the *PRKCZ* promoter in decitabine-treated vs. PBS-treated SW1353, as determined by bisulfite sequencing. Methylation rates of each C in CpG islands are indicated by dots (**left**). Curved lines indicate smooth local regression of methylation rates in each group (**left**). Average methylation rates (**right**). $n = 5$. Decitabine above 10 μ M was not administered because of cytotoxicity. In **b-g**, 10 μ M of decitabine-treated SW1353 vs. PBS-treated SW1353. **b.** *PRKCZ* expression determined by RT-qPCR. **c.** Expression of ATM/CHK2-related proteins and apoptosis-related proteins determined by western blotting. **d.** Proliferation assay. **e** and **f.** Cell cycle profiles, and sub G1 fraction rate

determined by cell cycle assay. **g.** Colony counts determined by colony formation assay. Representative images are shown. **h.** Time course of tumor volume of xenografts in decitabine-treated SW1353 vs. PBS-treated SW1353. Images were obtained at the time of sacrifice; representative images are shown. $n = 5$. In **i–k**, 10 μ M of decitabine-treated SW1353 with or without siPRKCZ. **i.** PRKCZ expression determined by RT-qPCR. **j.** PRKCZ expression determined by western blotting. **j.** Proliferation assay. In **b, d, f, g, i,** and **k**, $n = 3$. In **a, b, d, f–i,** and **k**, data are means \pm SD. Two-tailed t tests. *, $p < 0.05$. NS, not significant. DAC, decitabine.



Supplementary Figure S10. Decitabine does not inhibit proliferation of OUMS-27. In **a–e**, decitabine-treated OUMS-27 vs. PBS-treated OUMS-27. **a**. PRKCZ expression determined by RT–qPCR. **b**. PRKCZ expression determined by western blotting. **c**. Proliferation assay. **d**. Cell cycle profiles. **e**. Colony formation assays showed no colonies in decitabine–treated or PBS–treated OUMS-27. Representative images are shown. In **a** and **c**, the data are means \pm SD; $n = 3$. Two–tailed t tests. NS, not significant. DAC, decitabine.



Supplementary Figure S11. ATM knockdown increases proliferation in NDCS-1 but not in OUMS-27. In **a-c**, 10 μ M of decitabine-treated NDCS-1 with or without siATM. **a**. ATM expression determined by RT-qPCR. **b**. ATM expression determined by western blotting. **c**. Proliferation assay. In **d-f**, siATM-transfected OUMS-27 vs. scrambled siRNA-transfected OUMS-27. **d**. ATM expression determined by RT-qPCR. **e**. ATM expression determined by western blotting. **f**. Proliferation assay. In **a**, **c**, **d**, and **f**, the data are means \pm SD; $n = 3$. Two-tailed t tests. NS, not significant. DAC, decitabine.