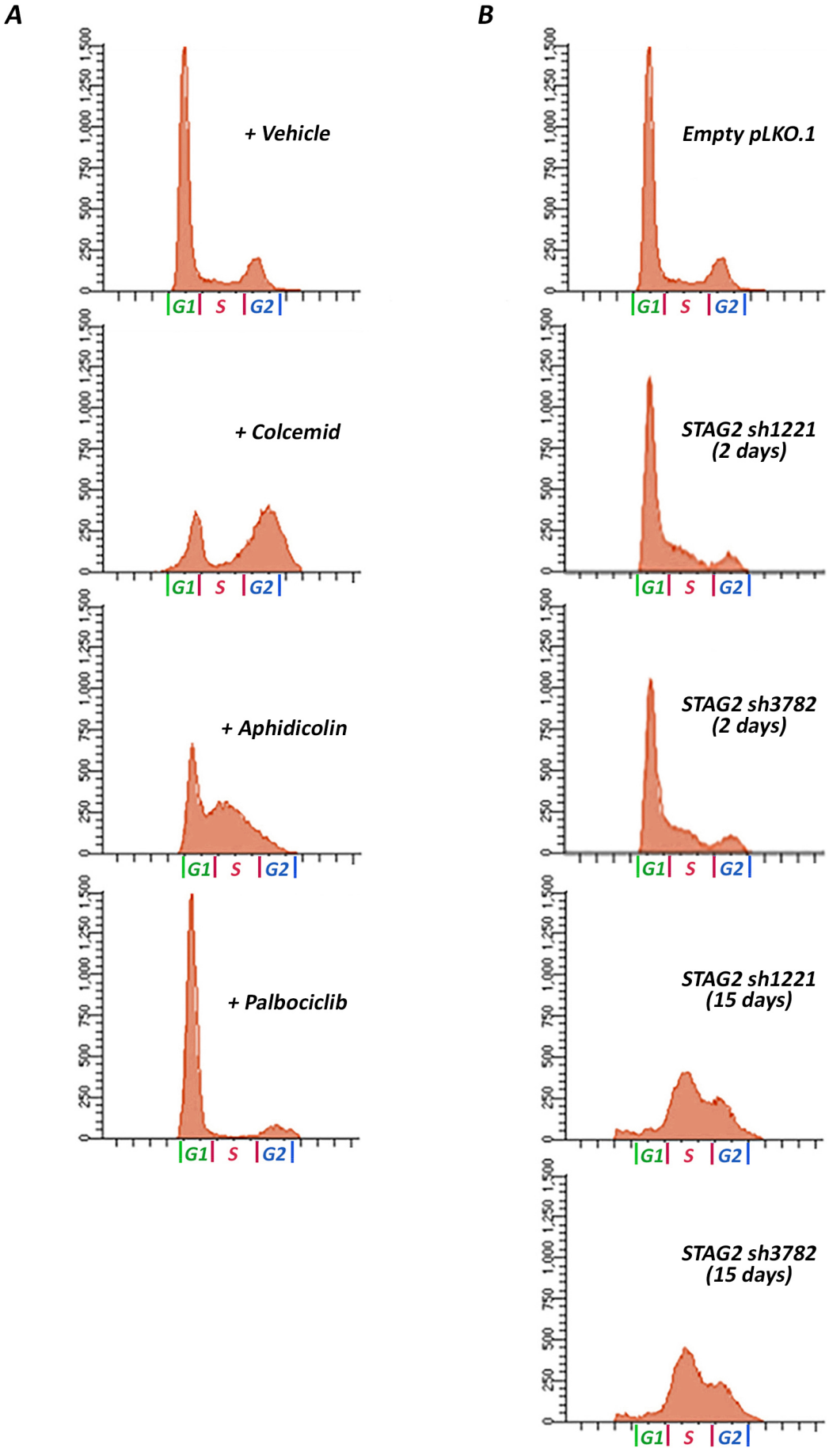
Supplementary Information for:

Mondal et al.

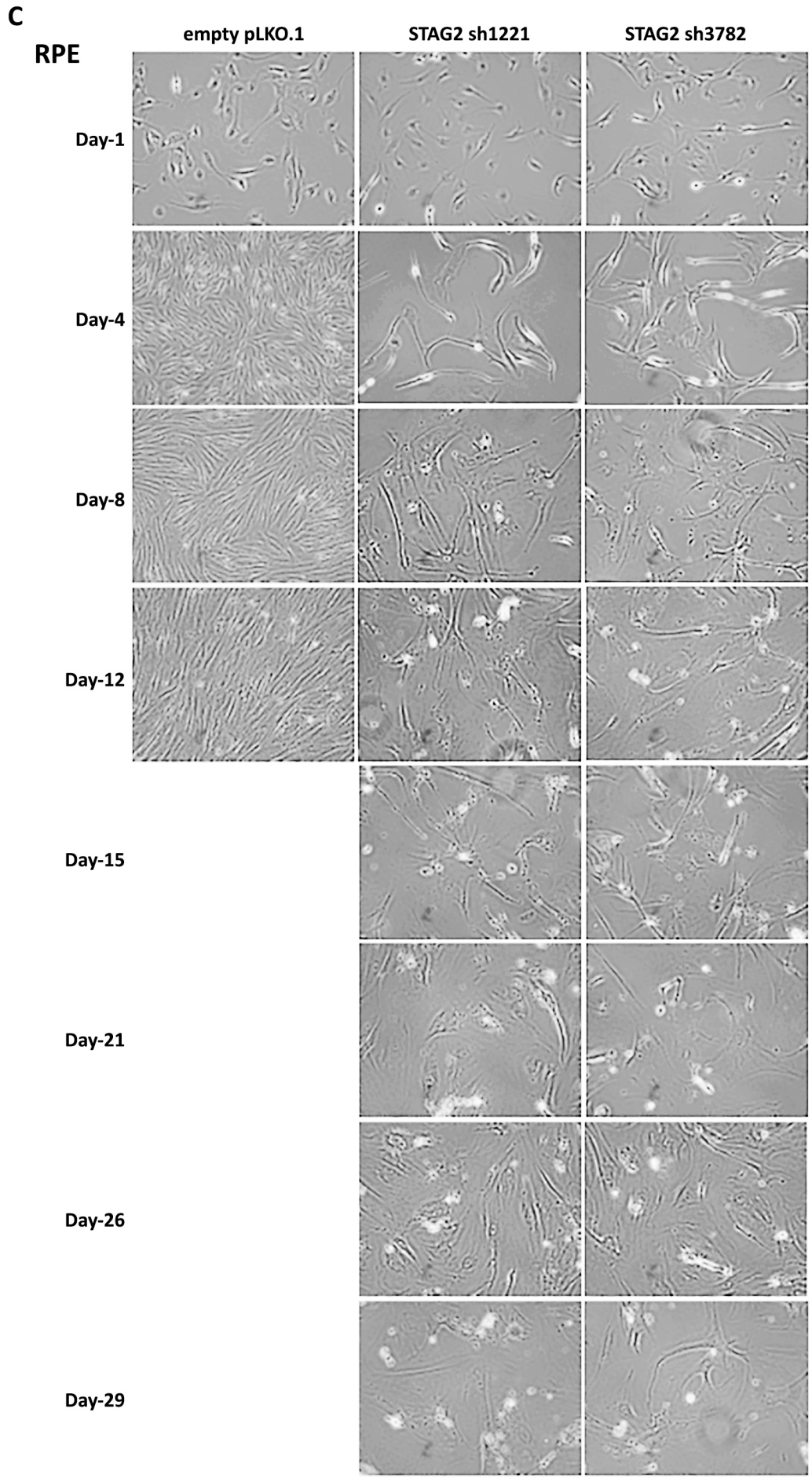
A requirement for STAG2 in replication fork progression creates a targetable synthetic lethality with DNA repair factors in cohesin-mutant cancers

*Nature Communications*

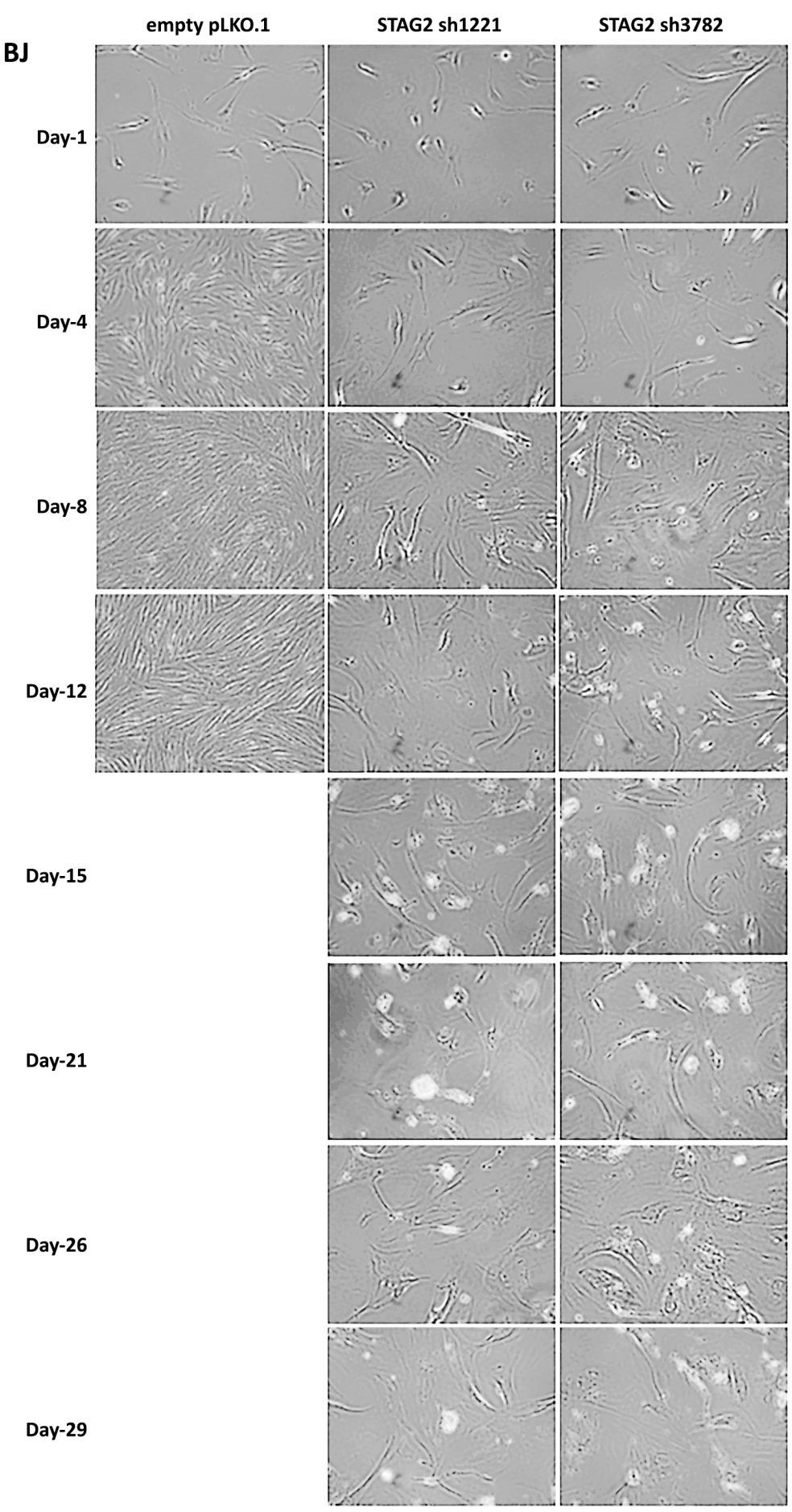
Contents:



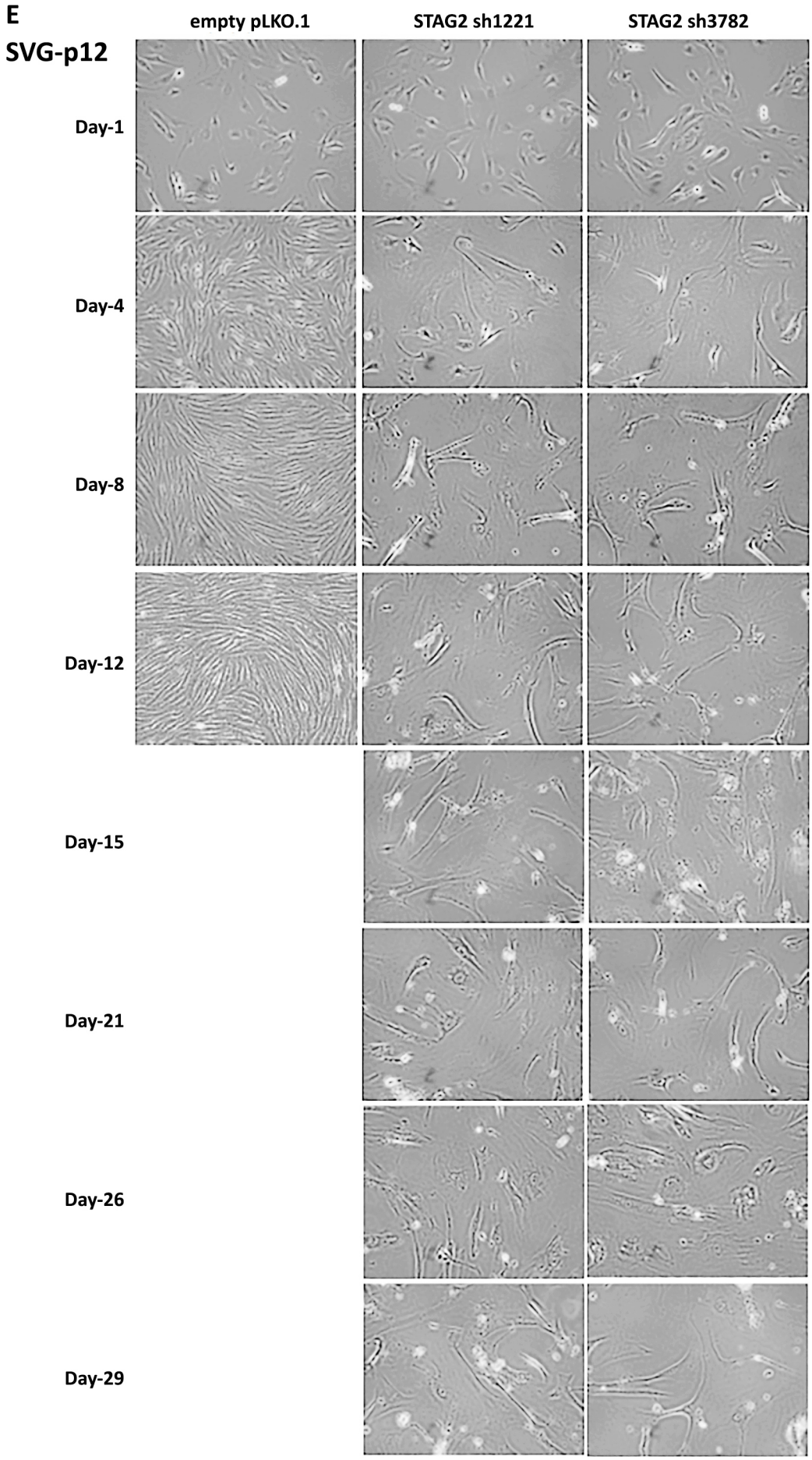

Supplementary Figure 1



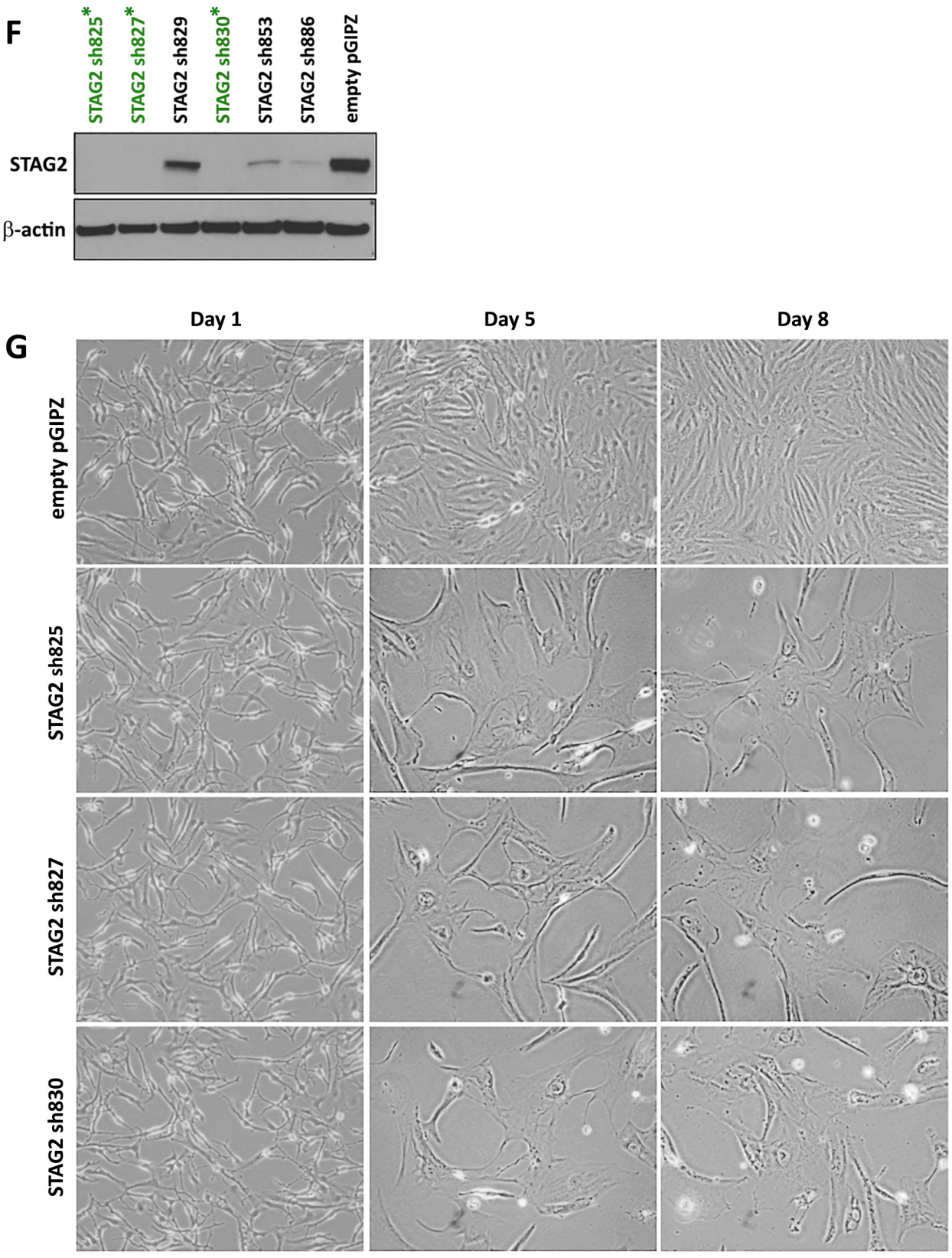

Supplementary Figure 1




Supplementary Figure 1

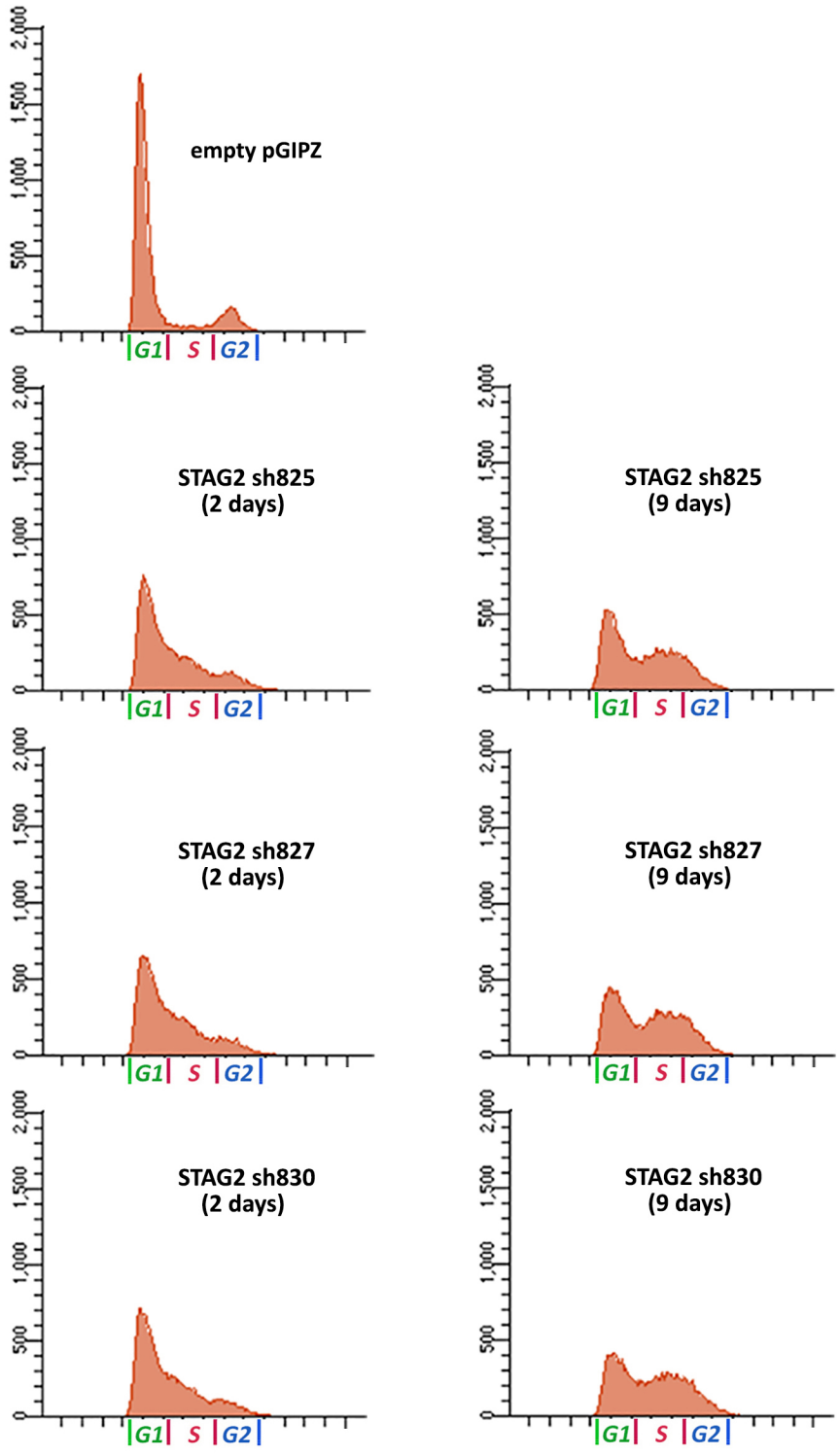



Supplementary Figure 1




Supplementary Figure 1

**H**

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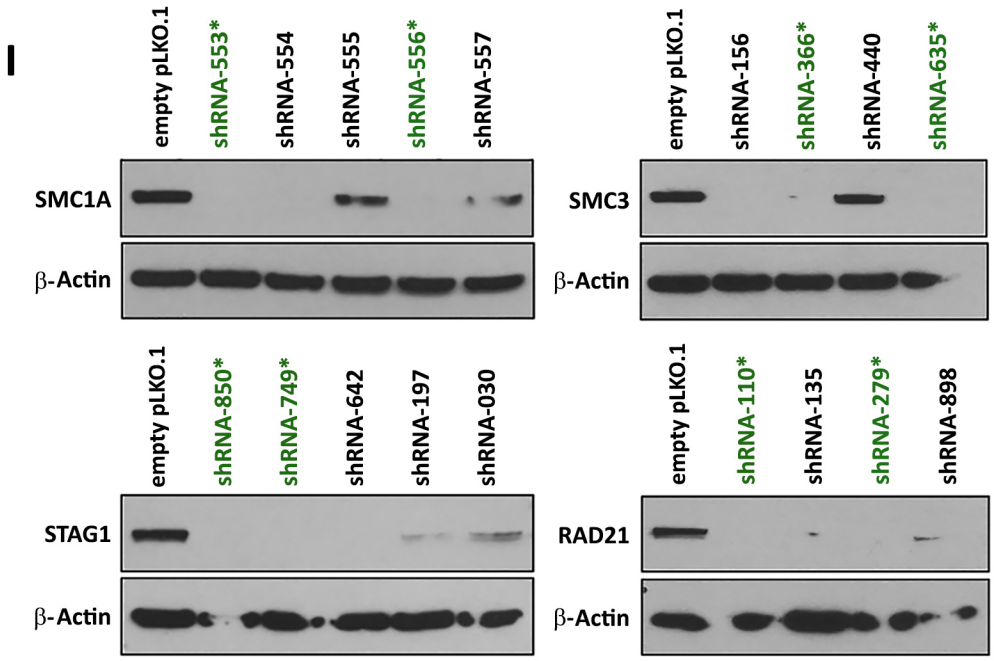
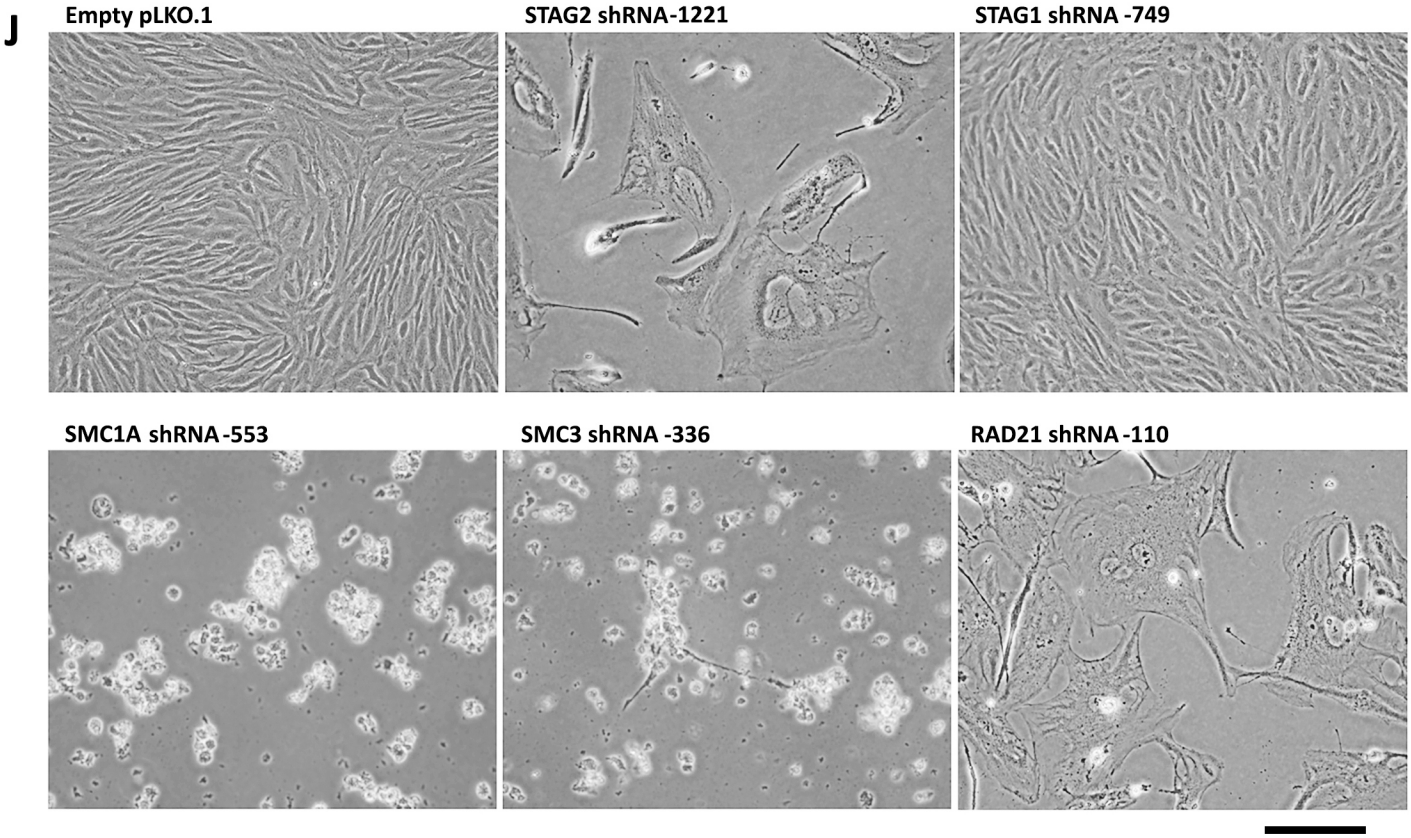
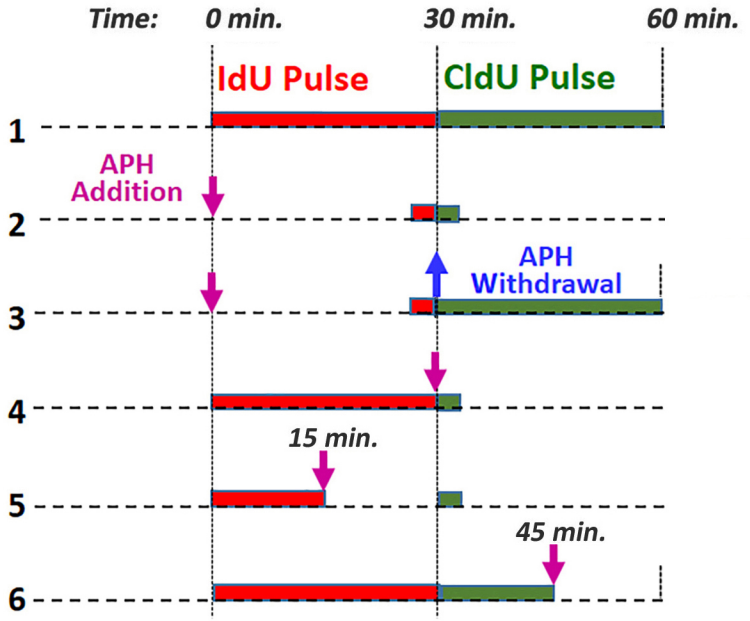



Supplementary Figure 1

**Supplementary Figure 1.** *STAG2* depletion in primary human cells results in intra-S-phase cell cycle arrest, whereas depletion of the core cohesin subunits *SMC1A*, *SMC3*, and *RAD21* results in cell death. **A,** Flow cytometry plots of RPE cells after treatment with DMSO vehicle, a microtubule polymerization inhibitor (colcemid), a DNA polymerase inhibitor (aphidicolin), and a cyclin-dependent kinase 4/6 inhibitor (palbociclib). Y-axis, cell count. X-axis, propidium iodide intensity. **B,** Flow cytometry plots of RPE cells after lentiviral transduction with empty pLKO.1 and two independent *STAG2* shRNAs, which demonstrate an abnormal accumulation of cells within S-phase after STAG2 depletion. **C-E,** Phase contrast image of RPE cells (**C**), BJ cells (**D**), and SVG p12 cells (**E**) following lentiviral transduction with empty pLKO.1 or *STAG2* shRNA demonstrating failure to proliferate and morphologic features of senescence. **F,** Validation of lentiviral shRNA mediated depletion of STAG2 using the pGIPZ vector system. Shown are immunoblots of total lysate from U87MG cells following lentiviral transduction with empty pGIPZ or multiple independent shRNA sequences against *STAG2*. The three shRNAs that produced the most effective protein depletion (highlighted in green) were used to assess cellular phenotypes in primary human cells. **G,** Phase contrast image of RPE cells following lentiviral transduction with empty pGIPZ or *STAG2* shRNA demonstrating failure to proliferate and morphologic features of senescence. **H,** Flow cytometry plots of RPE cells after lentiviral transduction with empty pGIPZ and three independent *STAG2* shRNAs, which demonstrate an abnormal accumulation of cells within S-phase after STAG2 depletion. **I,** Validation of lentiviral shRNA mediated depletion of cohesin ring components. Shown are immunoblots of total lysate from U87MG cells following lentiviral transduction with empty pLKO.1 or multiple independent shRNA sequences against the cohesin subunits *SMC1A*, *SMC3*, *STAG1*, and *RAD21*. The two shRNAs that produced the most effective protein depletion (highlighted in green) were used to assess cellular phenotypes in primary human cells. **J,** Phase contrast image of RPE cells at seven days following lentiviral transduction with empty pLKO.1 or shRNAs against cohesin components.

**A**




*1) No block*

*2) APH Block throughout both (IdU and CldU) pulses*

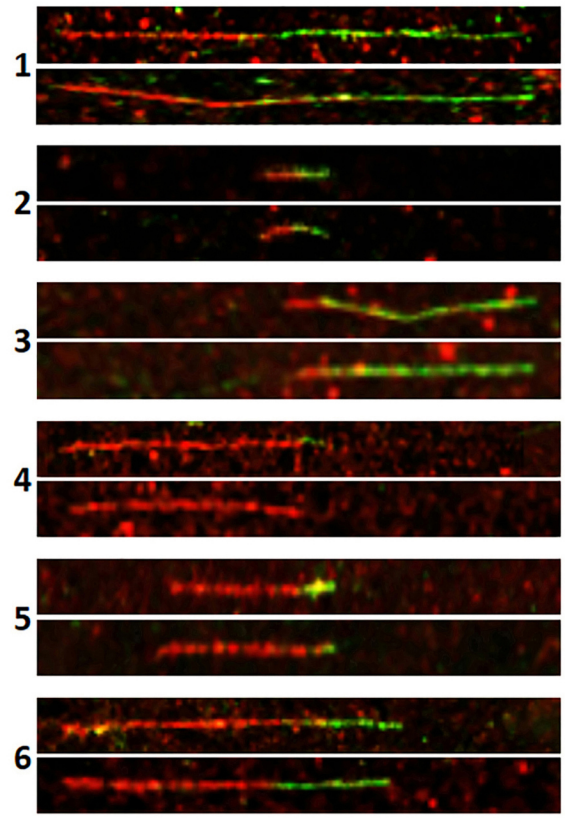
*3) APH Block during first (Idu) pulse only*

*4) APH Block during second (CldU) pulse only*

*5) APH Block after first 15 minutes of (IdU) pulse*

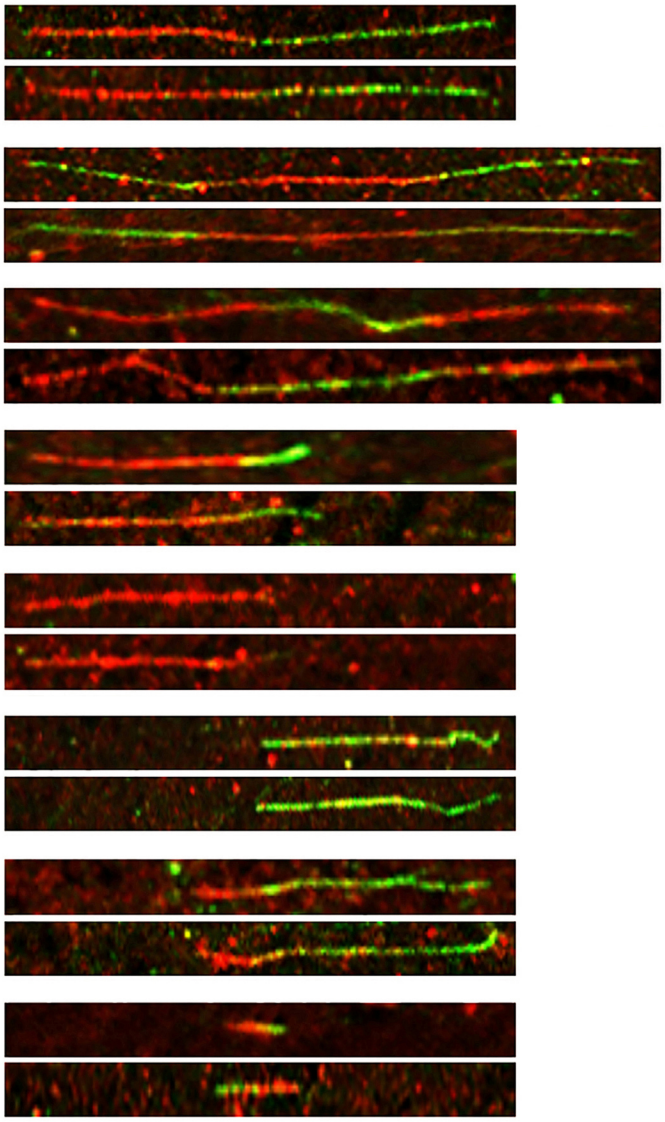
*6) APH Block after first 15 minutes of (CldU) pulse*

**B**




Supplementary Figure 2

C



Unidirectional elongating fork (normal)

Bi-directional Replication (normal)

Completion/ Termination (normal)

Stalling (or Termination) during second pulse (abnormal)

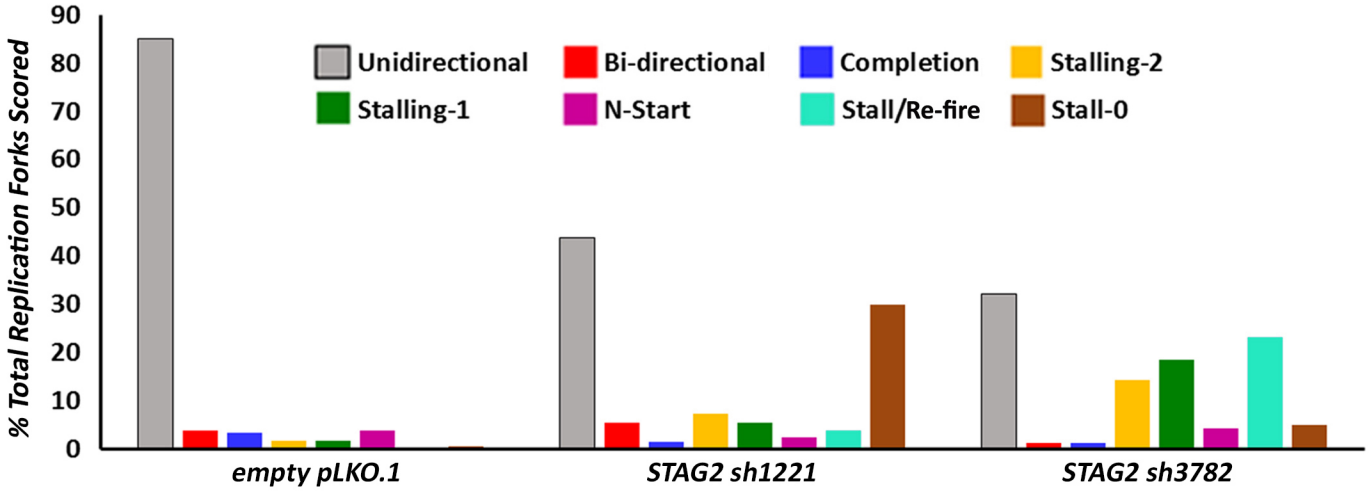
Stalled fork (or Termination) during first pulse (abnormal)

New origin of replication during second pulse (indeterminate)

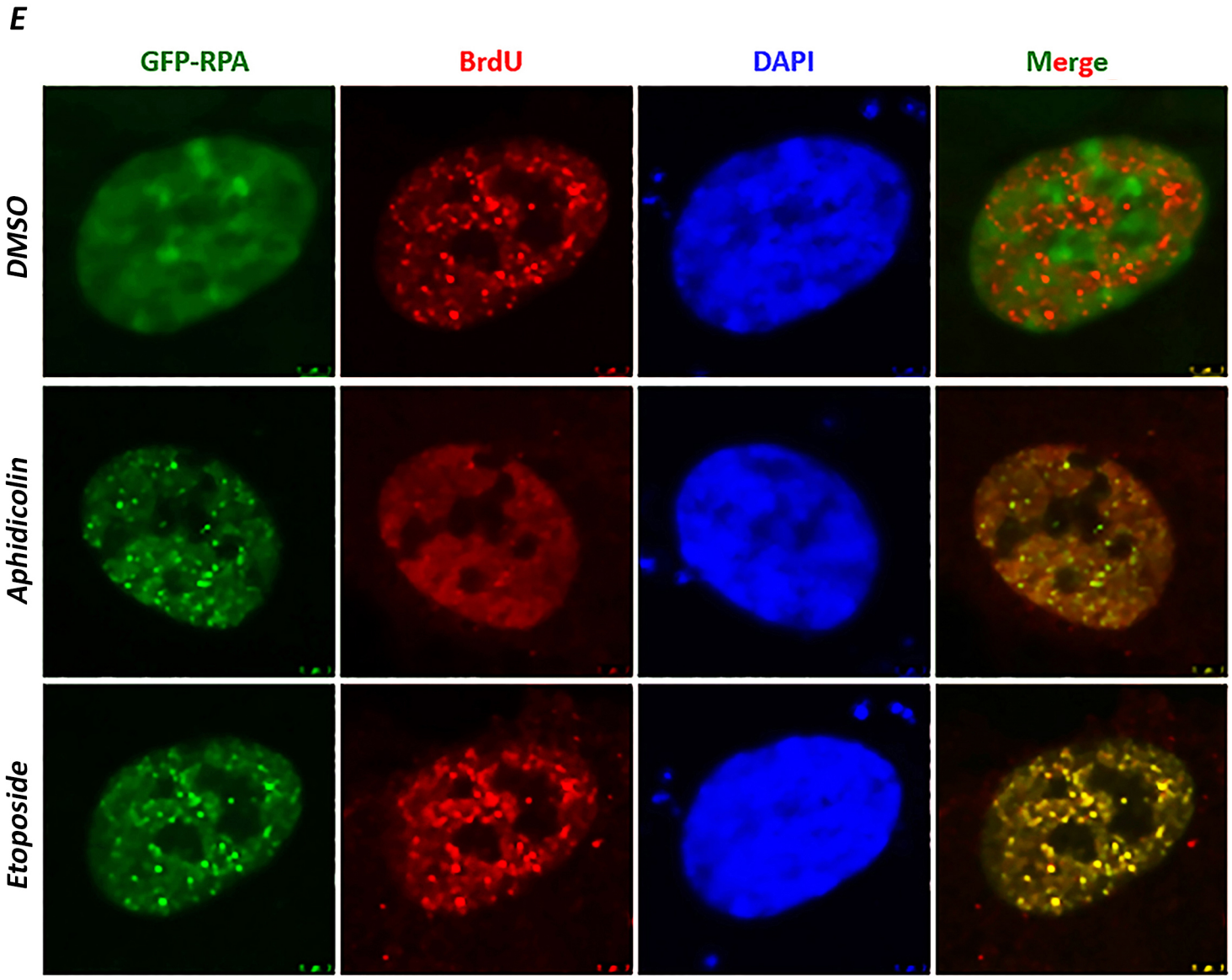
Stalling / Re-fire (or new Initiation) during 1st pulse (abnormal)

Stalling fork throughout both pulse (abnormal)

D




Supplementary Figure 2

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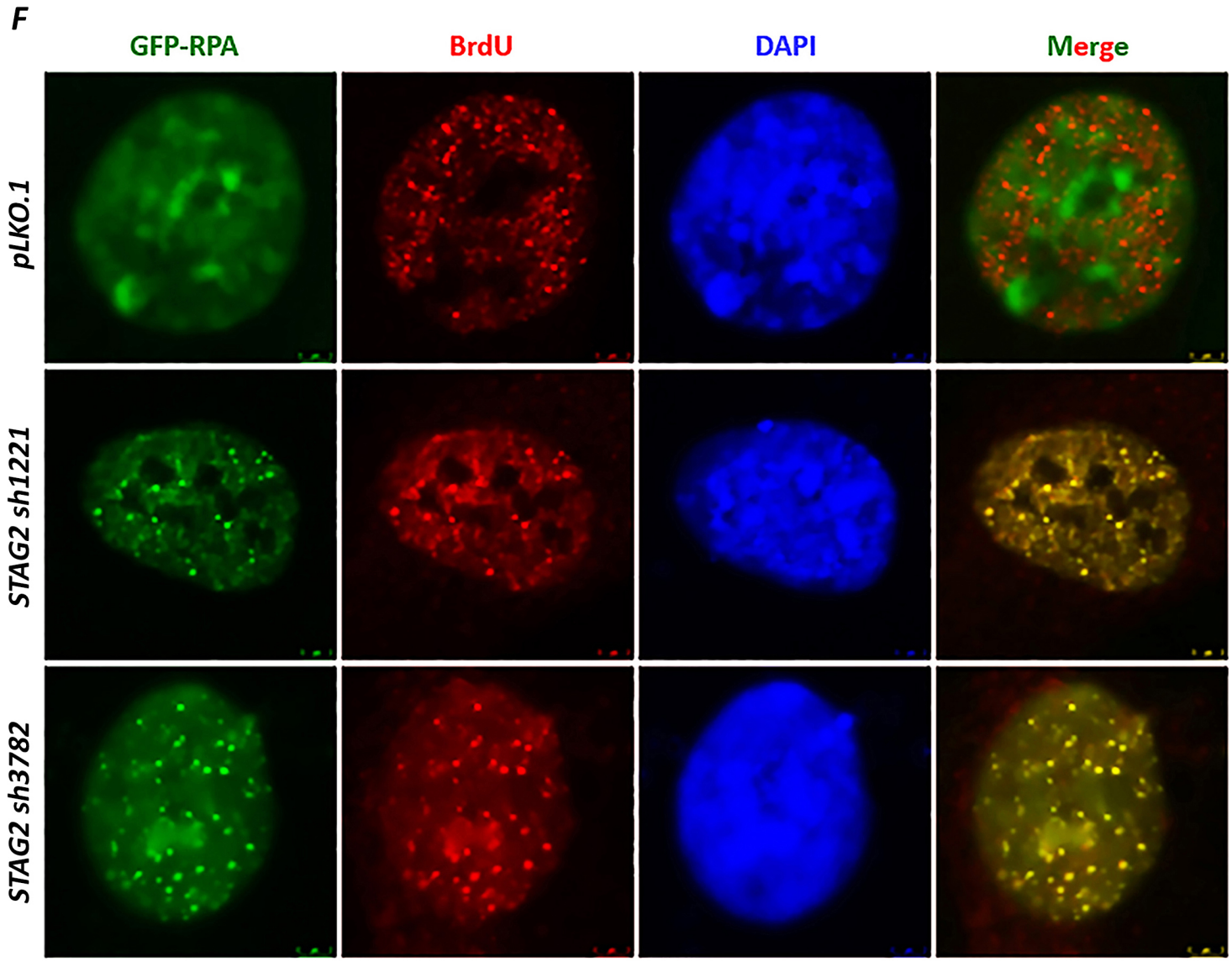
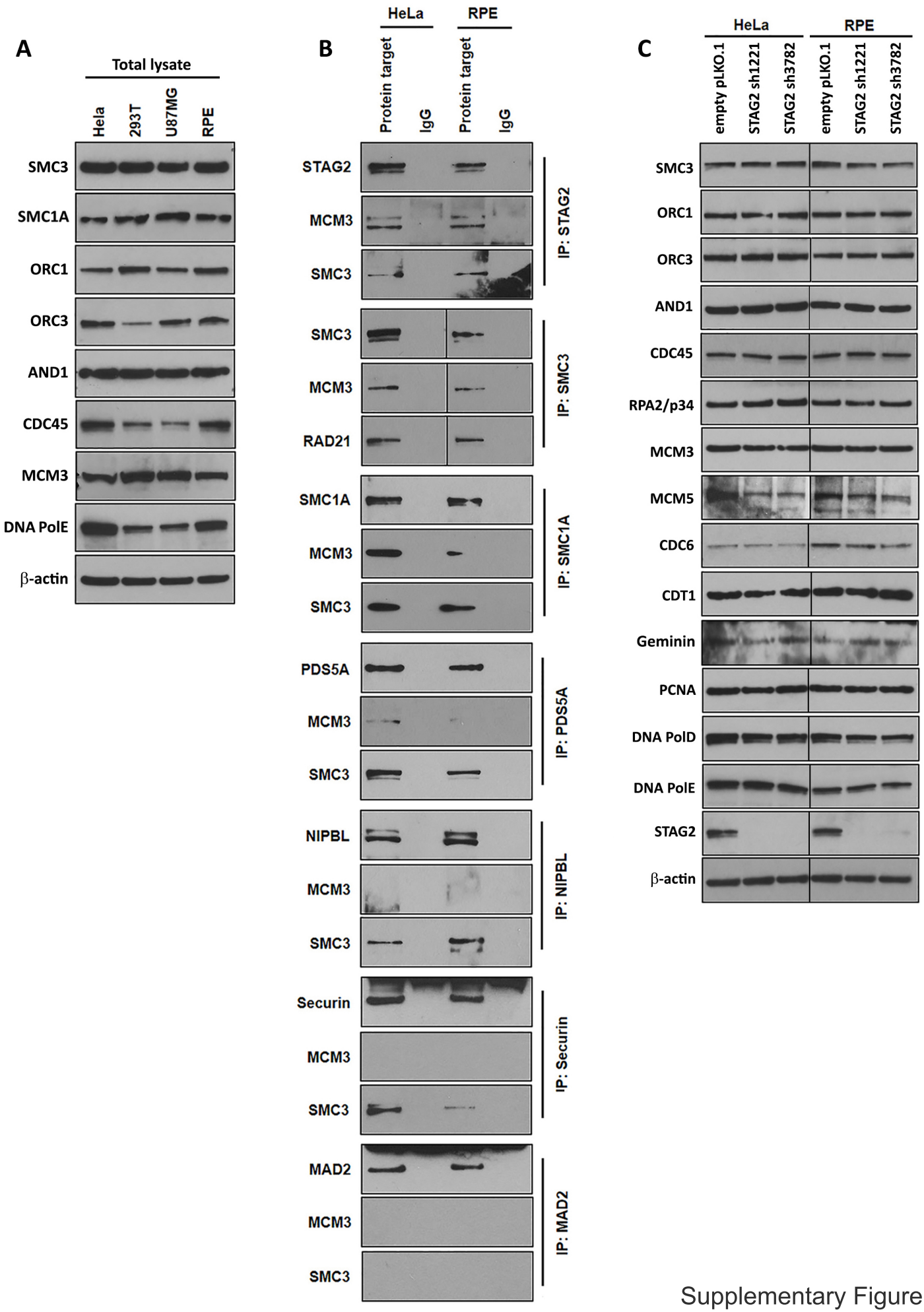


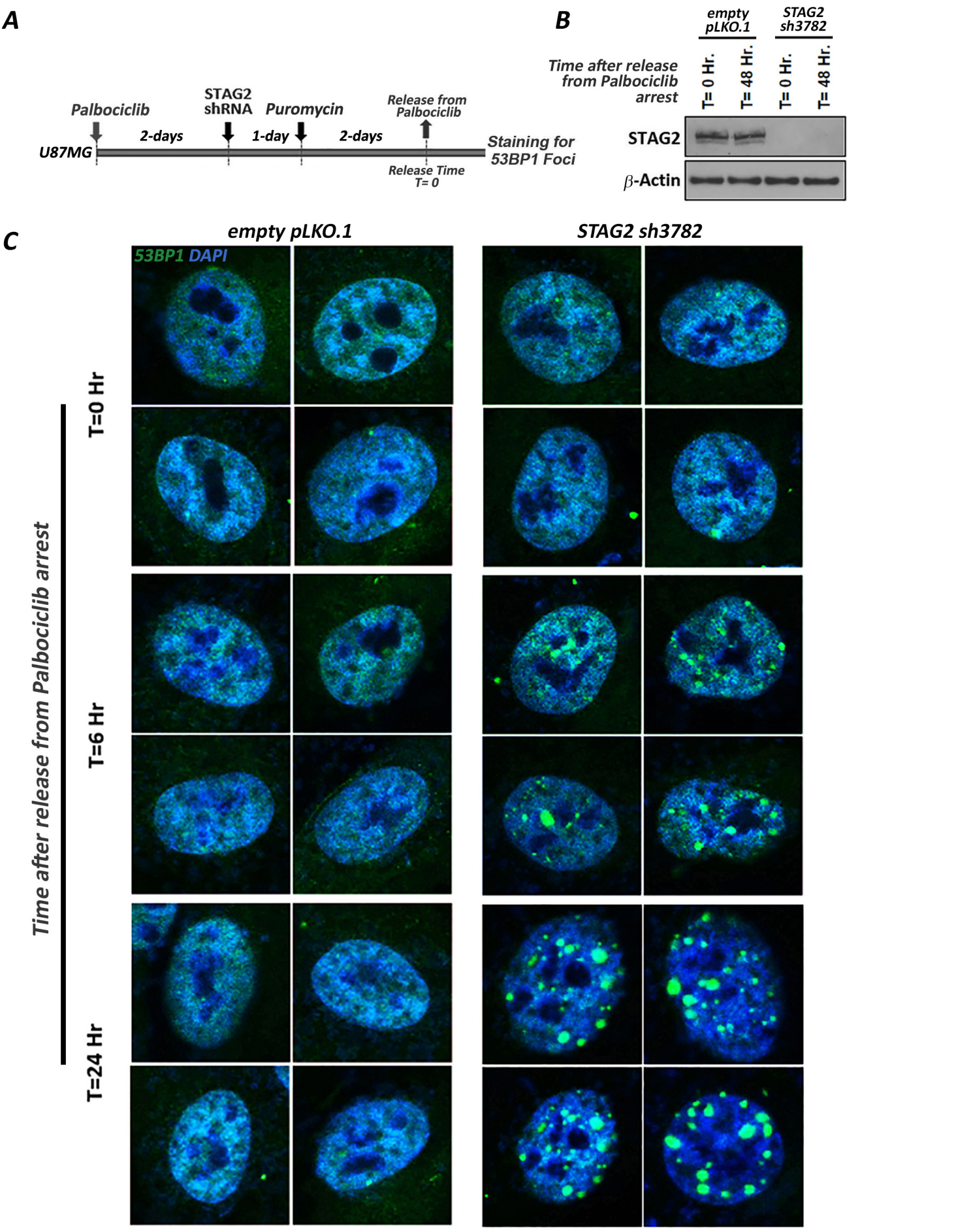

Supplementary Figure 2

**Supplementary Figure 2.** Inactivation of *STAG2* in primary human cells causes replication fork stalling. **A,** Schematic diagram of DNA combing assay utilizing the DNA polymerase inhibitor aphidicolin (10 μg/mL) at various time points, which was performed to validate this methodology for assessment of replication fork progression. **B,** Microscopic images of representative single DNA fibers showing normal replication fork progression (1) and replication fork stalling at various timepoints induced by aphidicolin block (2-6). **C,** Microscopic images of representative single DNA fibers showing normal and various abnormal replication fork progression in RPE cells following lentiviral transduction with empty pLKO.1 or two independent *STAG2* shRNAs. **D,** Quantitation of normal and abnormal replication forks scored from DNA combing assay of RPE cells following lentiviral transduction with empty pLKO.1 or two independent *STAG2* shRNAs. More than 200 individual replication forks were evaluated from two independent experiments for each condition. **E,** Representative images of parental RPE cells showing localization of GFP-tagged RPAp34 during unchallenged DNA replication (DMSO vehicle), replication block (aphidicolin), and topoisomerase inhibition (etoposide). RPAp34 foci accumulation is only present upon DNA replication fork stalling. **F,** Representative images of RPE cells showing accumulation of GFP-tagged RPAp34 at stalled replication forks following lentiviral transduction with *STAG2* shRNA but not empty pLKO.1.




Supplementary Figure 3

**Supplementary Figure 3.** Inactivation of *STAG2* in primary human cells causes disruption of cohesin interaction with the replication machinery. **A,** Western blots of total cell lysate from HeLa, 293T, U87MG, and RPE cells for cohesin complex, origin recognition complex, pre-replication complex, and replication machinery components. **B,** Immunoprecipitation using antibodies against cohesin and cohesion regulatory factors in HeLa and RPE cells demonstrating a specific interaction of the MCM helicase subunit MCM3 with STAG2, SMC3, SMC1A, and PDS5A, but not the cohesin regulatory factors NIPBL and Securin. **C,** Western blots of total cell lysate from HeLa and RPE cells for origin recognition complex, pre-replication complex, and replication machinery components following depletion of STAG2 with two independent shRNAs.

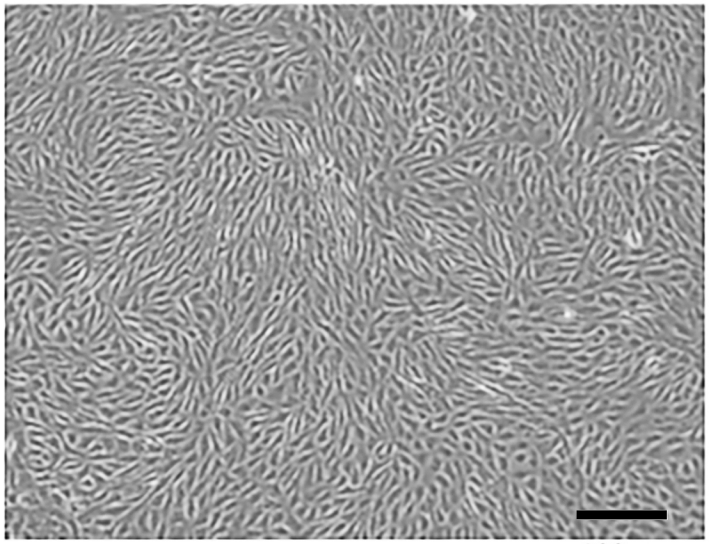



Supplementary Figure 4

**Supplementary Figure 4.** The replication fork stalling caused by *STAG2* depletion leads to fork collapse and DNA double-strand breaks. **A,** Schematic diagram of palbociclib arrest experiment to determine the requirement of cell cycle progression on the formation of 53BP1 foci following STAG2 depletion. **B,** Immunoblot demonstrating STAG2 depletion in RPE cells upon lentiviral shRNA transduction after palbociclib arrest. **C,** Representative immunofluorescence images of cells following sequential arrest in G1 using palbociclib, lentiviral transduction with empty pLKO.1 or *STAG2* shRNA, and then release from palbociclib arrest.

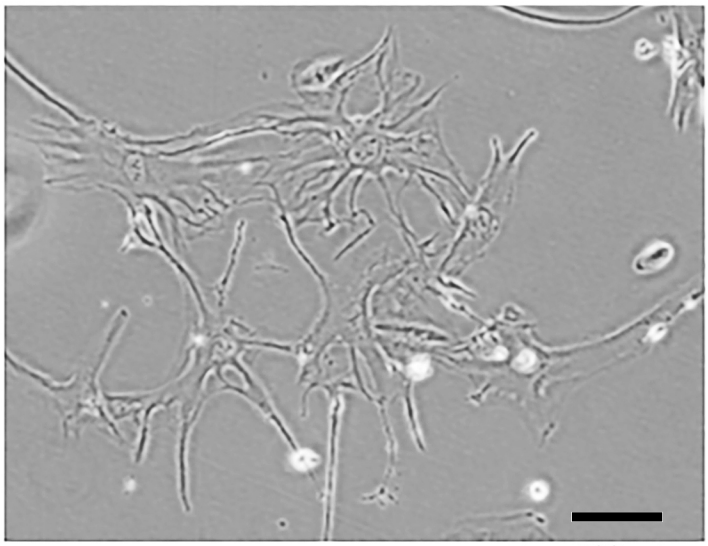
***A***

***Empty pLKO.1***



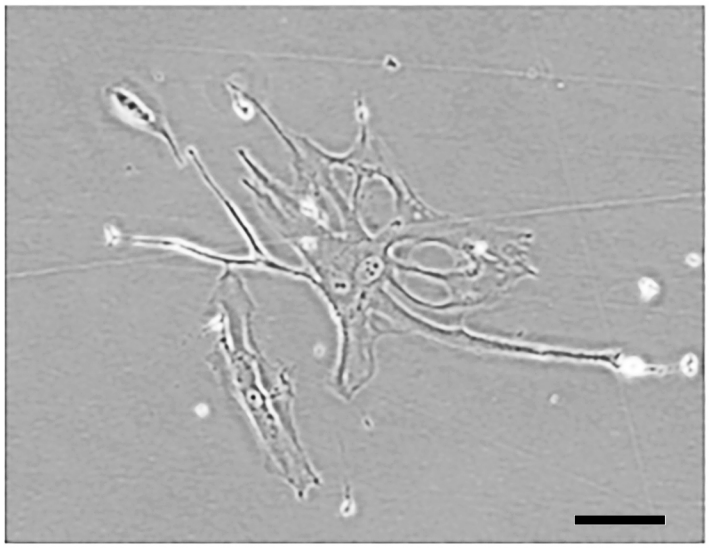
***100 μm***

***STAG2 sh1221 alone***



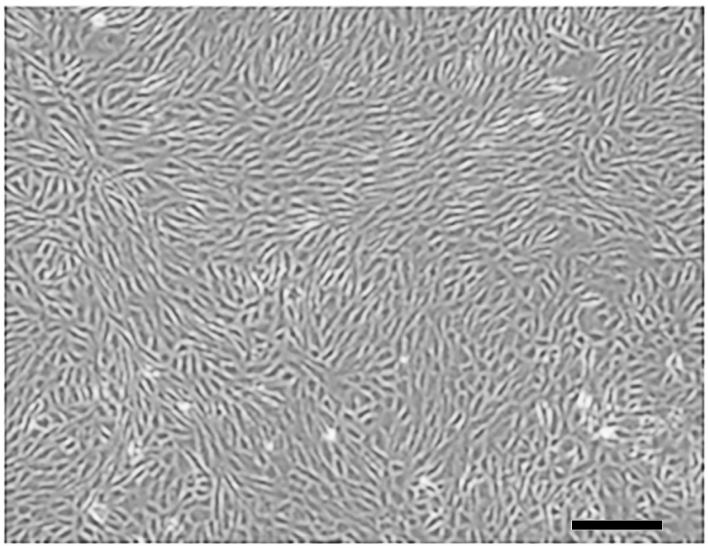
***100 μm***

***STAG2 sh3782 alone***



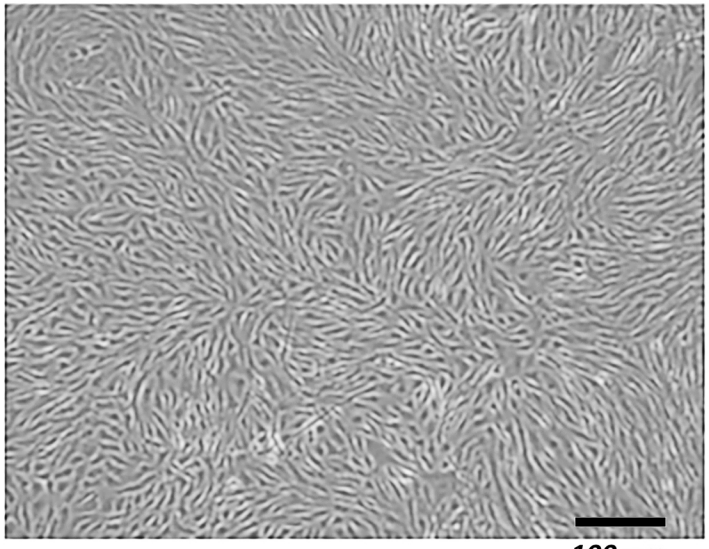
***100 μm***

***STAG2 sh1221 + shTP53***



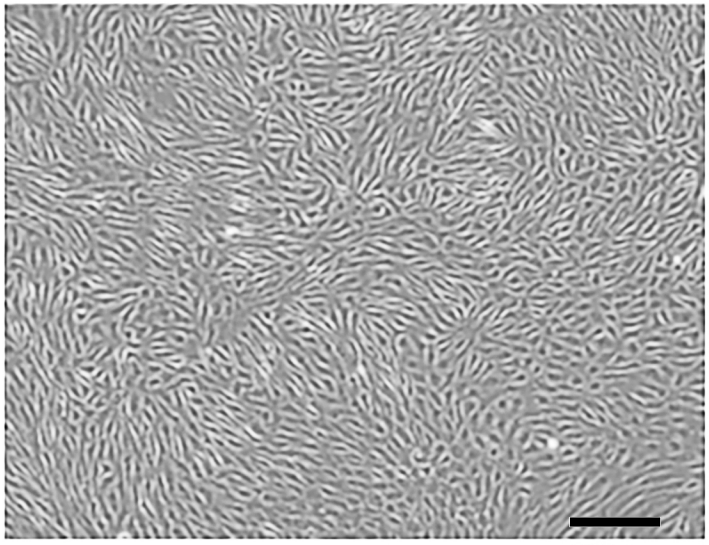
***100 μm***

***STAG2 sh3782 + shTP53***



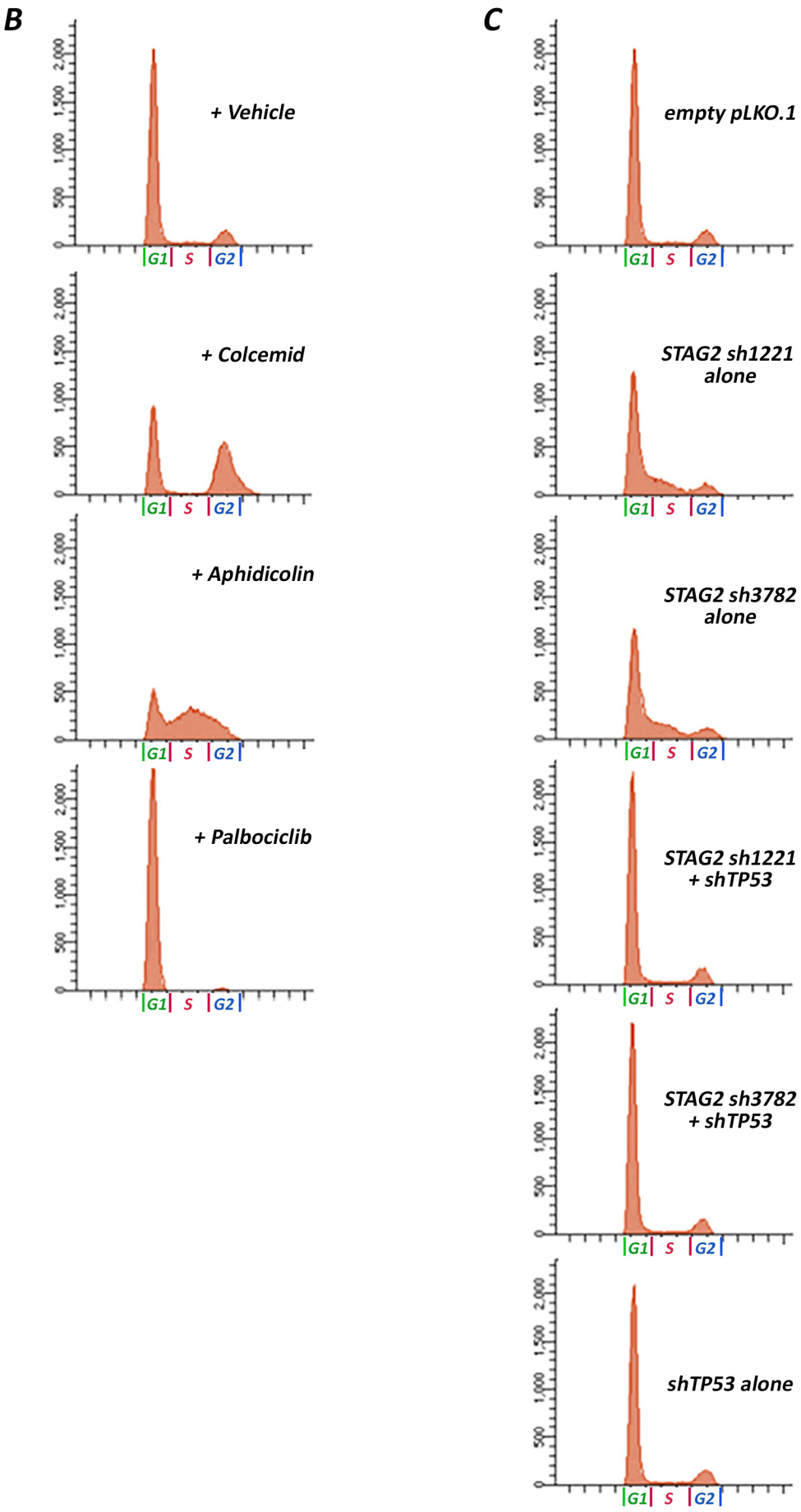
***100 μm***

***shTP53 alone***



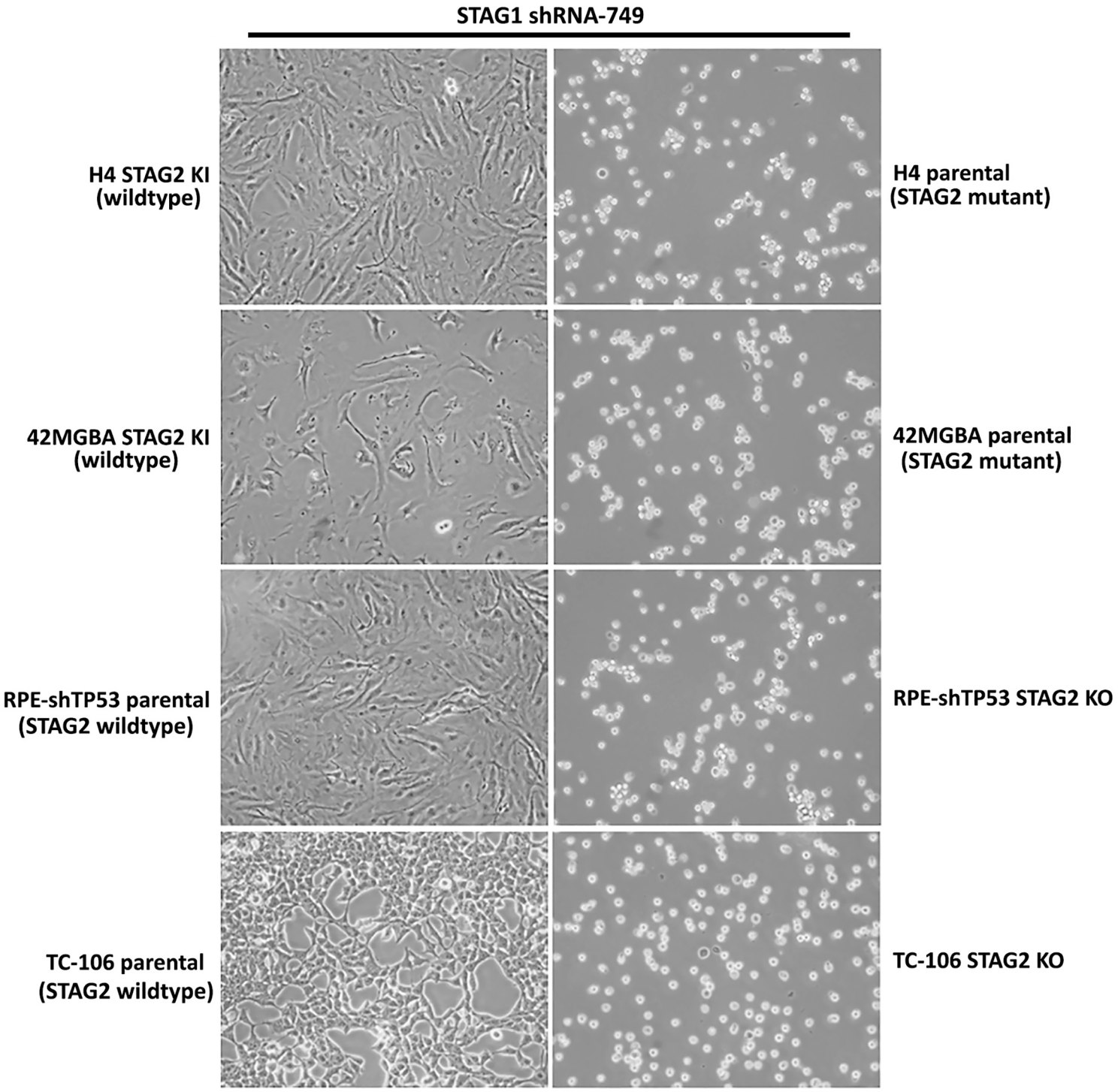
***100 μm***

Supplementary Figure 5



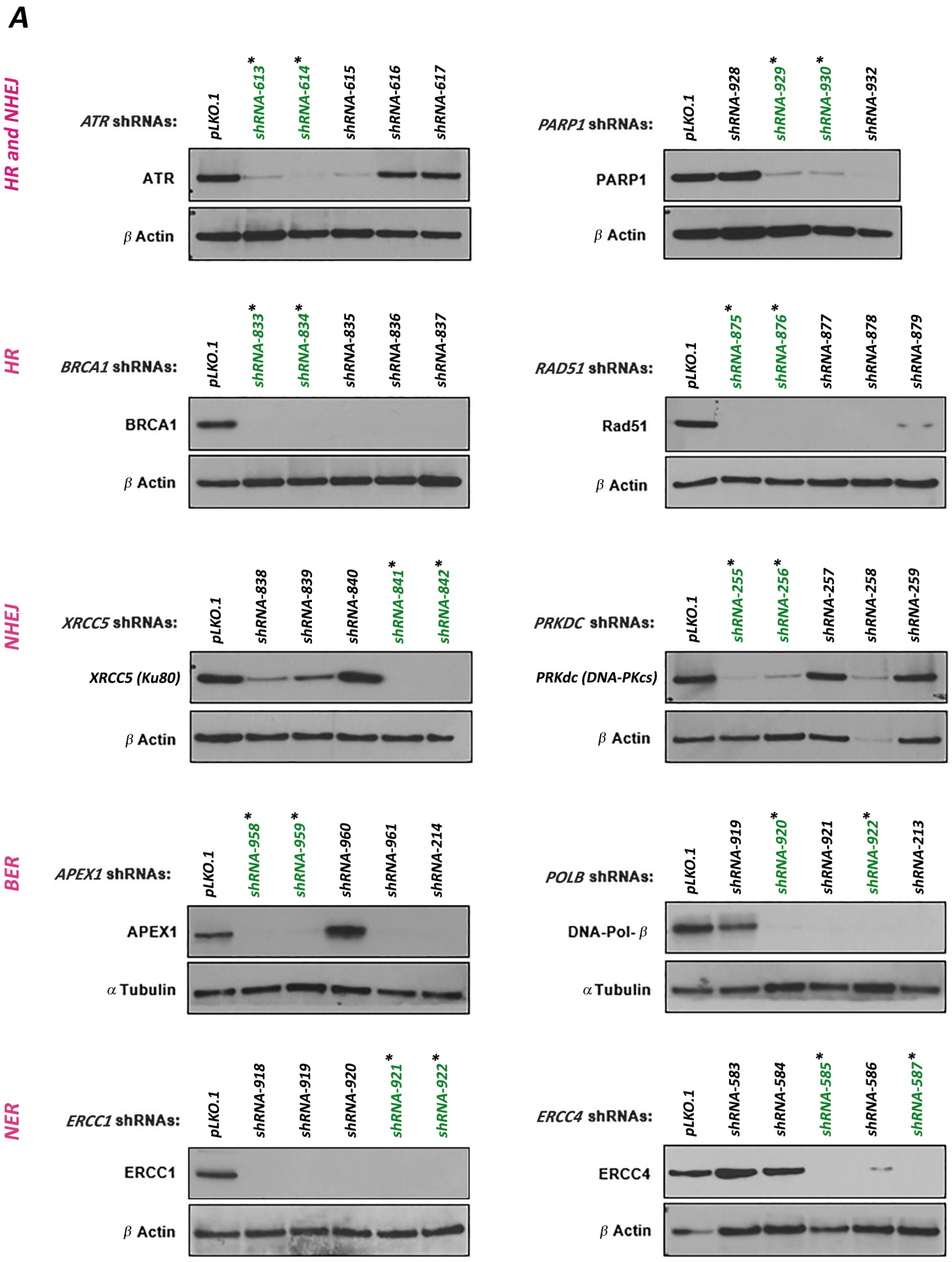

Supplementary Figure 5

**Supplementary Figure 5.** The intra-S-phase cell cycle arrest induced by *STAG2* inactivation is p53 dependent. **A,** Phase contrast image of RPE cells following lentiviral transduction with *STAG2* shRNA in combination with *TP53* shRNA demonstrating cellular proliferation and abrogation of the senescence induced by STAG2 depletion alone. **B,** Flow cytometry plot of parental RPE cells after treatment with DMSO vehicle, a microtubule polymerization inhibitor (colcemid), a DNA polymerase inhibitor (aphidicolin), and a cyclin-dependent kinase 4/6 inhibitor (palbociclib). Y-axis, cell count. X-axis, propidium iodide intensity. **C,** Flow cytometry plots of parental RPE cells following lentiviral transduction with *STAG2* shRNA in combination with *TP53* shRNA demonstrating bypass of the S-phase arrest induced by STAG2 depletion alone.

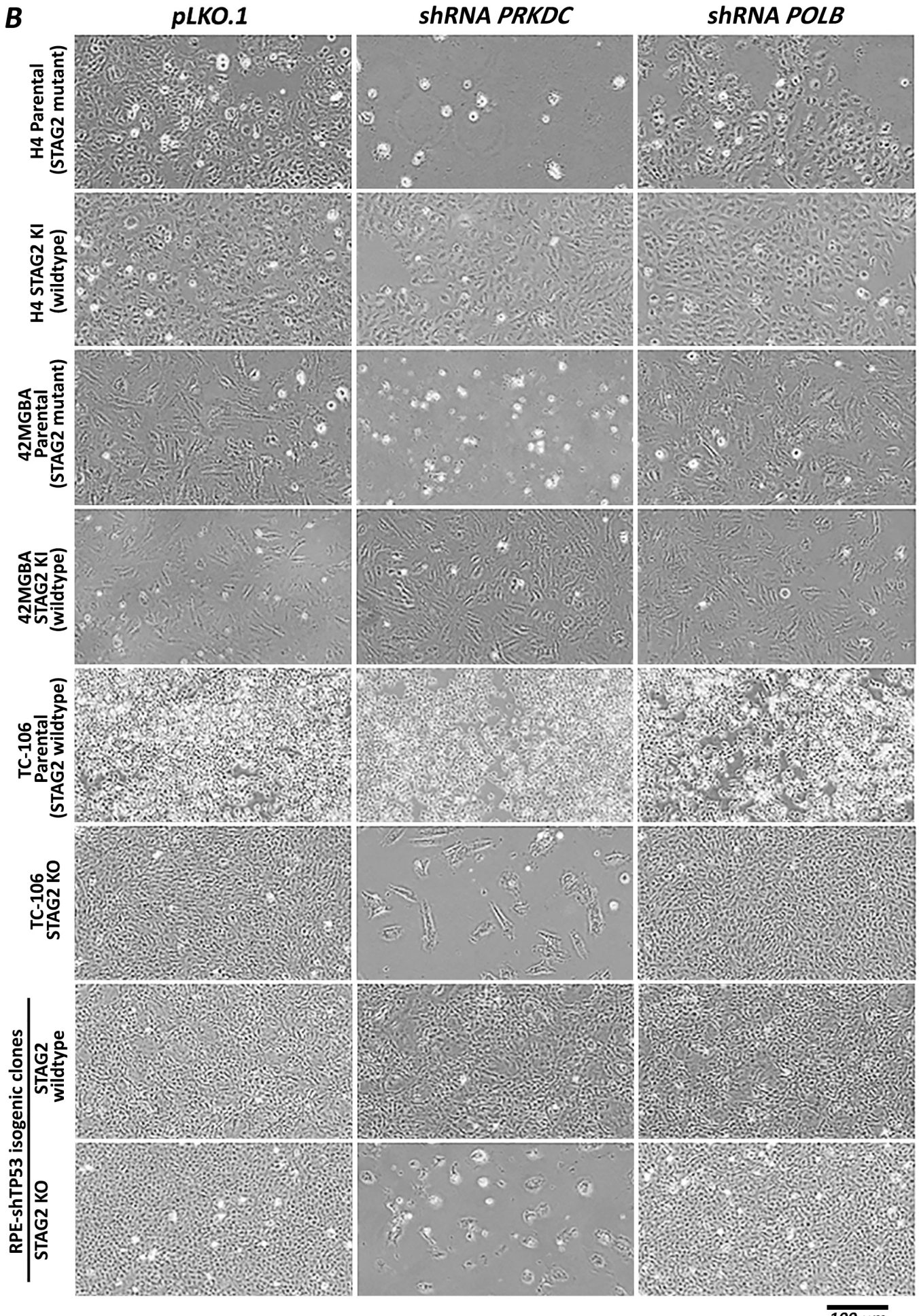



Supplementary Figure 6

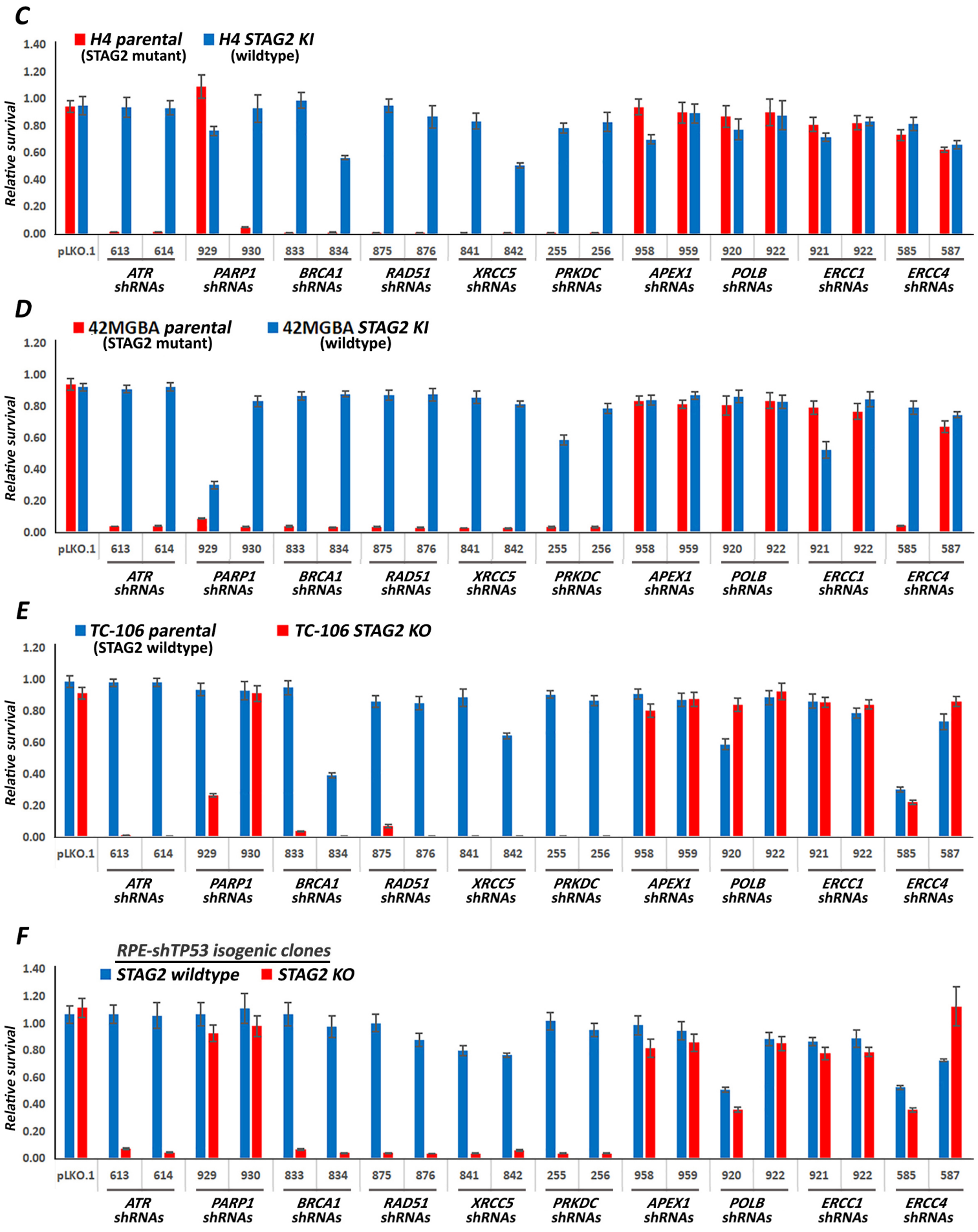
**Supplementary Figure 6.** *STAG2* deficiency in human cancer cells creates a synthetic lethality with the cohesin subunit *STAG1*. Representative phase contrast images of *STAG2* isogenic H4, 42MGBA, TC-106, and RPE-sh*TP53* cells showing synthetic lethality of *STAG2* inactivation with shRNA depletion of *STAG1*.




Supplementary Figure 7




Supplementary Figure 7




Supplementary Figure 7

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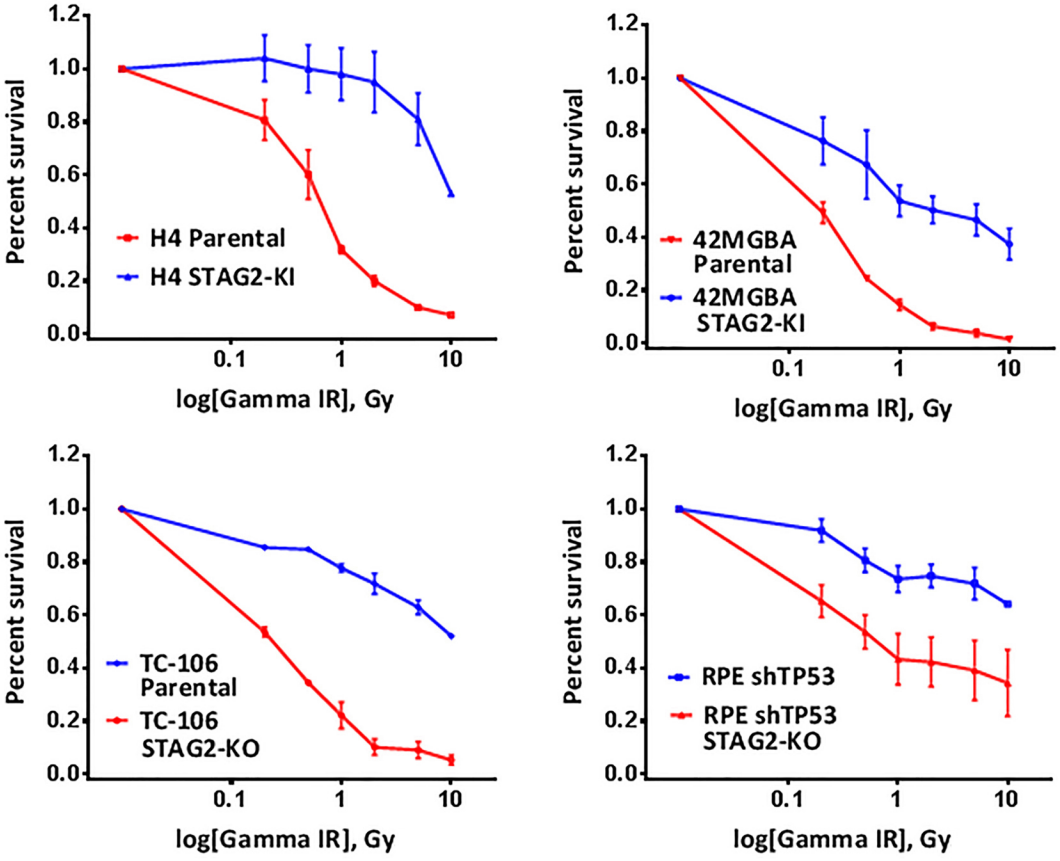


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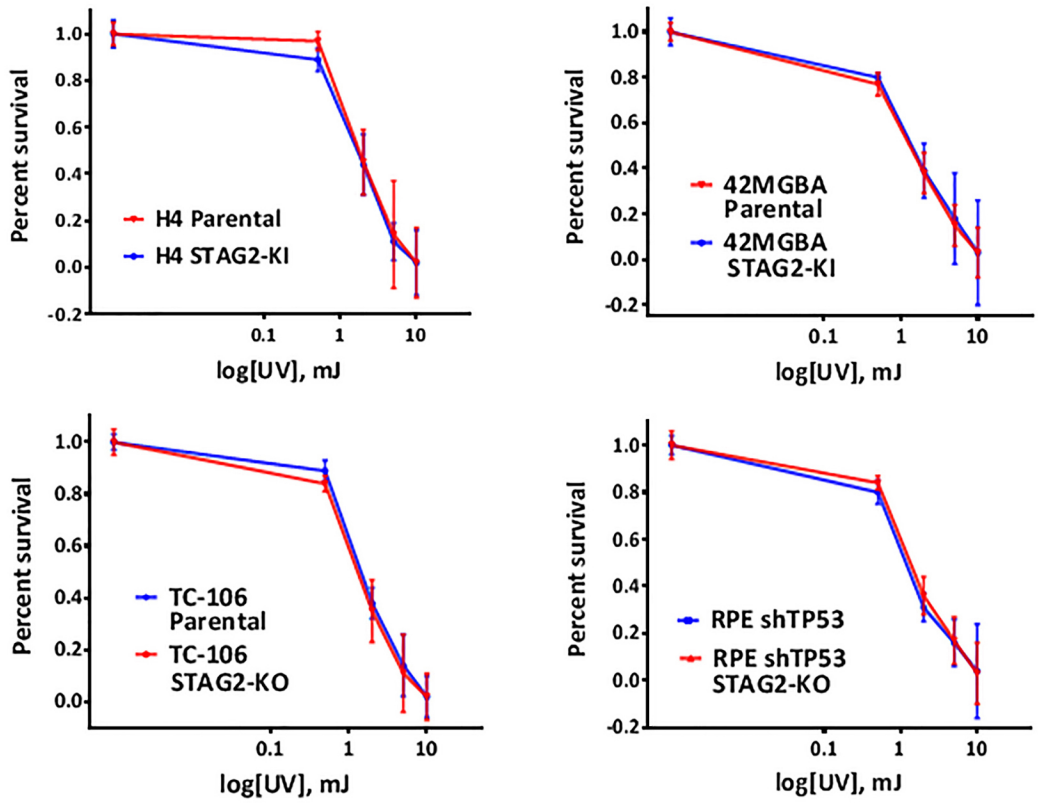
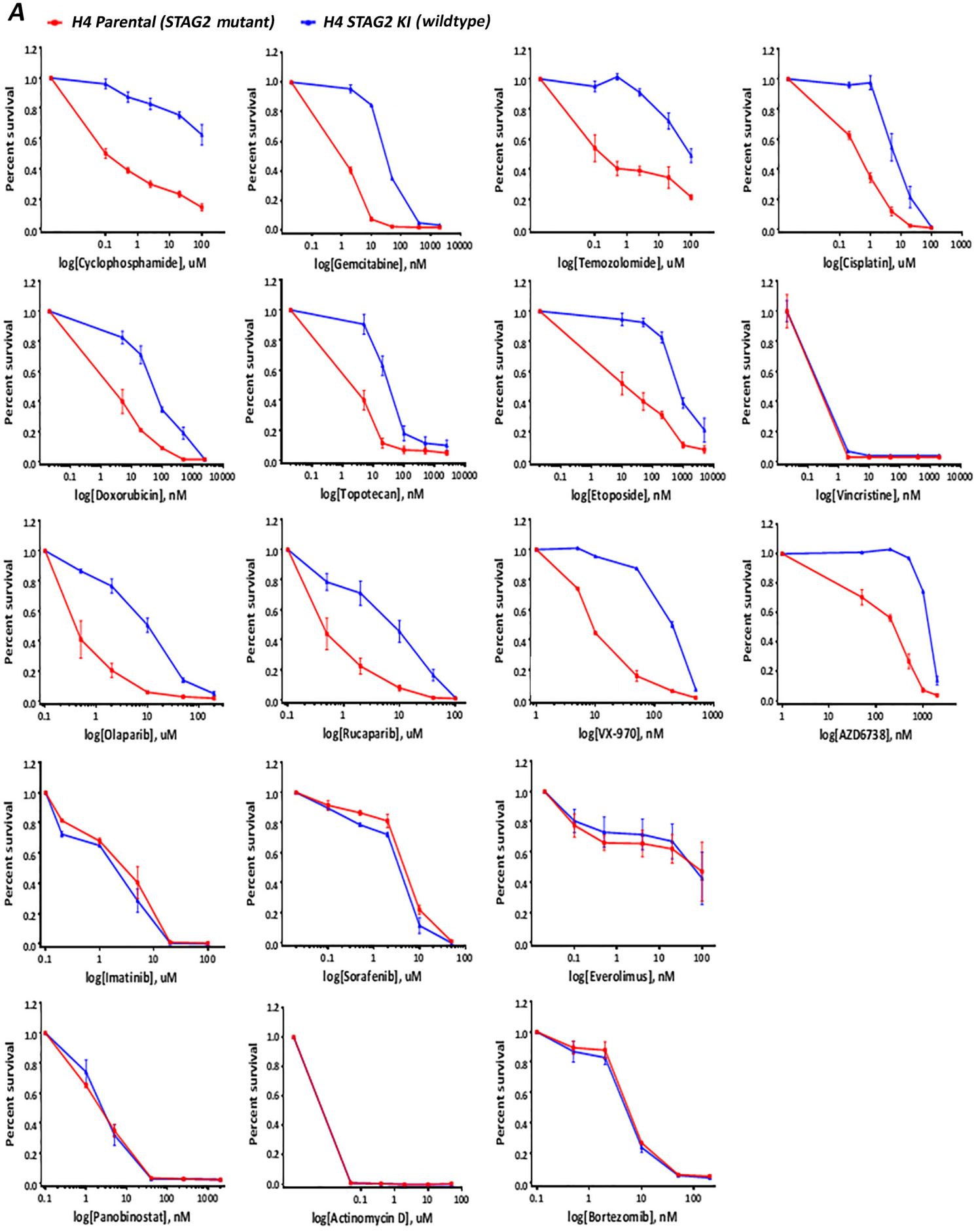
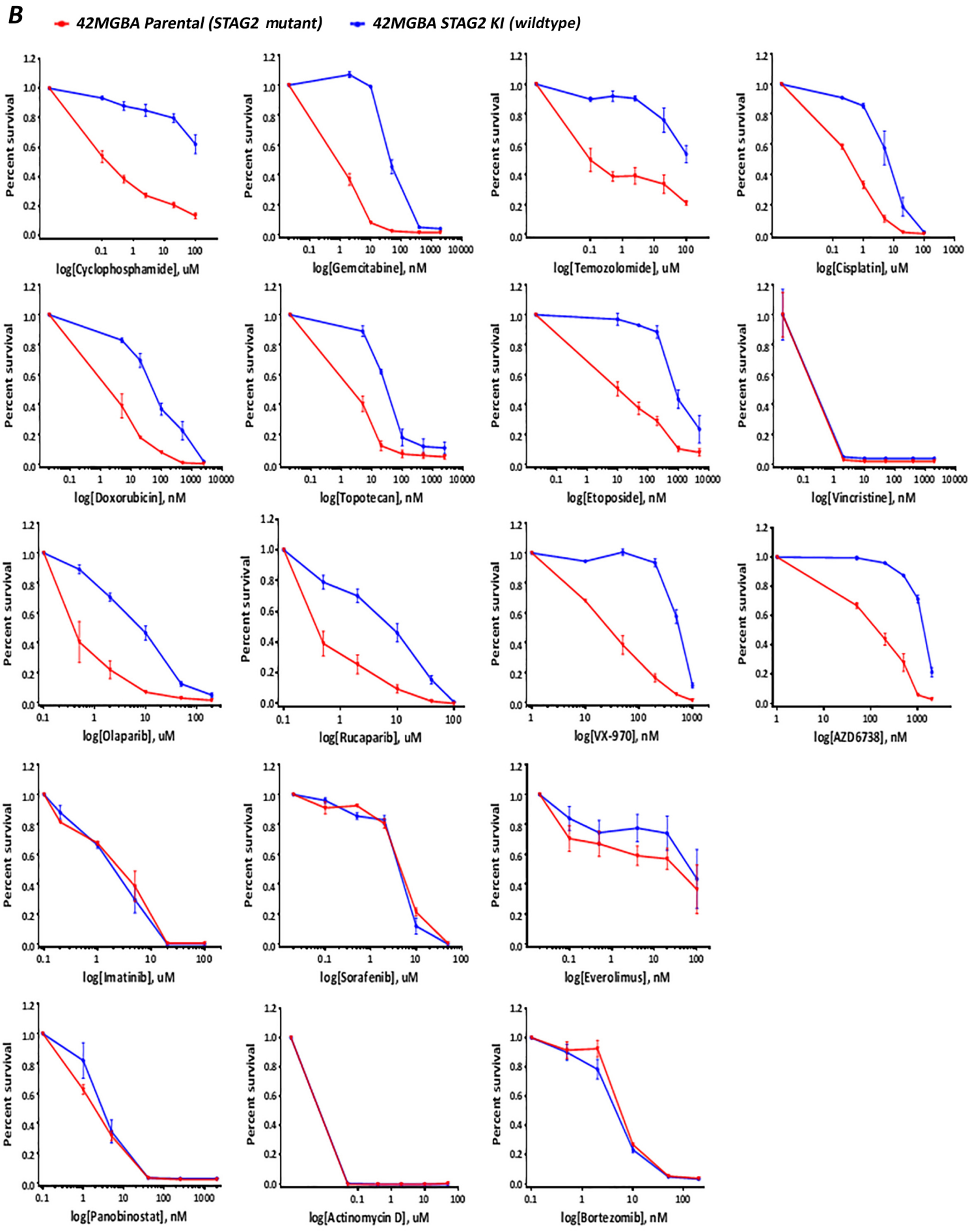



Supplementary Figure 7

**Supplementary Figure 7.** *STAG2* deficiency in human cancer cells creates a synthetic lethality with DNA double-strand break repair factors and increased sensitivity to ionizing radiation. **A,** Validation of lentiviral shRNA mediated depletion of DNA repair factors. Shown are immunoblots of total lysate from U87MG cells following lentiviral transduction with empty pLKO.1 or five independent shRNA sequences against each of 10 DNA repair factors. The two shRNAs that produced the most effective protein depletion (highlighted in green) were used in the shRNA synthetic lethality screen. **B,** Representative phase contrast images of *STAG2* isogenic cell pairs showing synthetic lethality of *STAG2* deficiency with shRNA depletion of the non-homologous end joining factor DNA-PKcs (*PRKDC*), but not the base excision repair factor DNA polymerase β (*POLB*). **C-F,** Quantitation of survival from shRNA synthetic lethality screen in *STAG2* isogenic H4 glioblastoma cells (**C**), 42MGBA glioblastoma cells (**D**), TC-106 Ewing sarcoma cells (**E**), and RPE sh*TP53* cells (**F**). Each data point is the mean of 8 replicates from 2 independent experiments, and error bars show standard error of the mean. **G-H,** Survival plots of four *STAG2* isogenic cell pairs following gamma-irradiation (**G**) or ultraviolet irradiation (**H**). Each data point is the mean of 12 replicates from 3 independent experiments, and error bars show standard error of the mean.




Supplementary Figure 8

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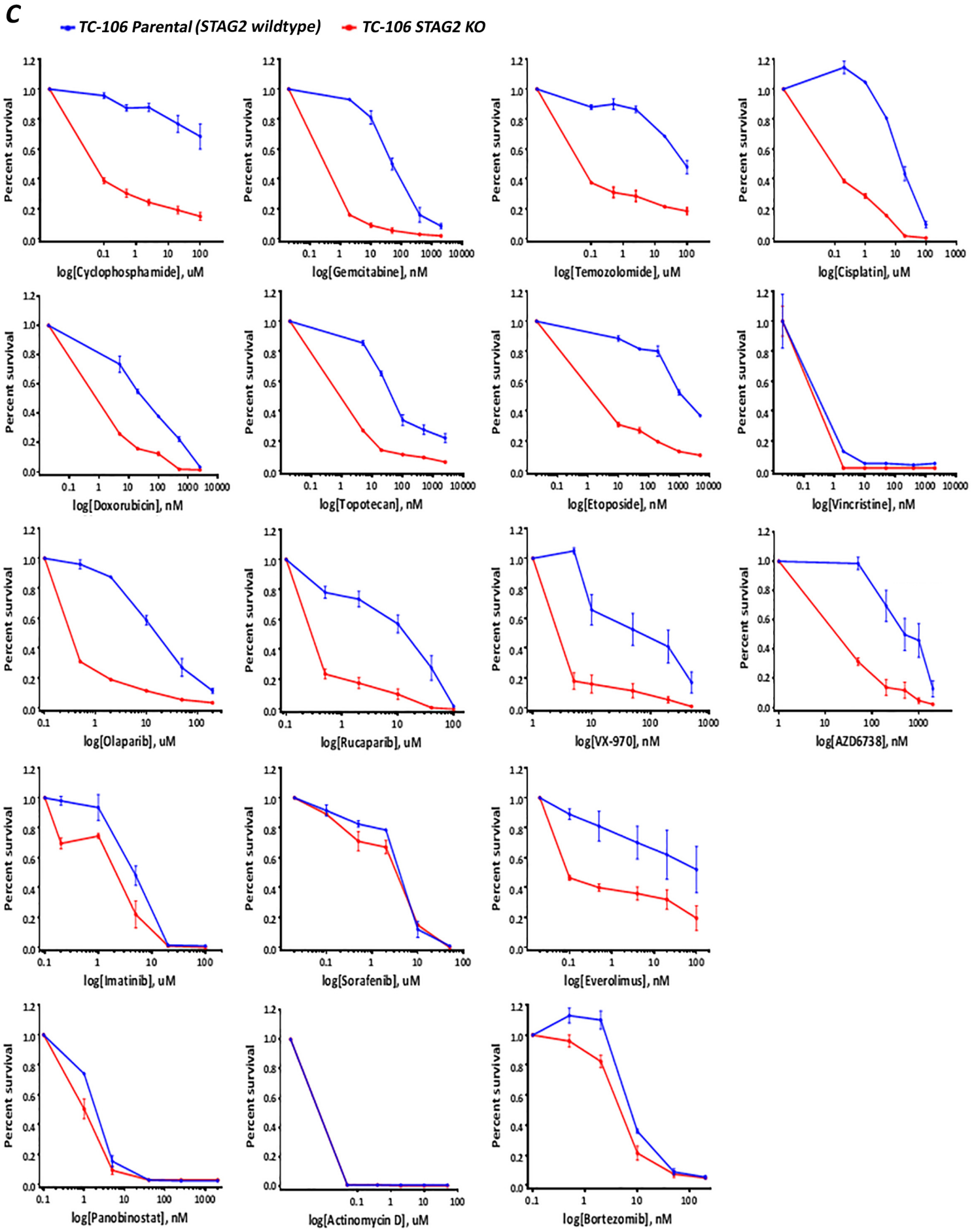


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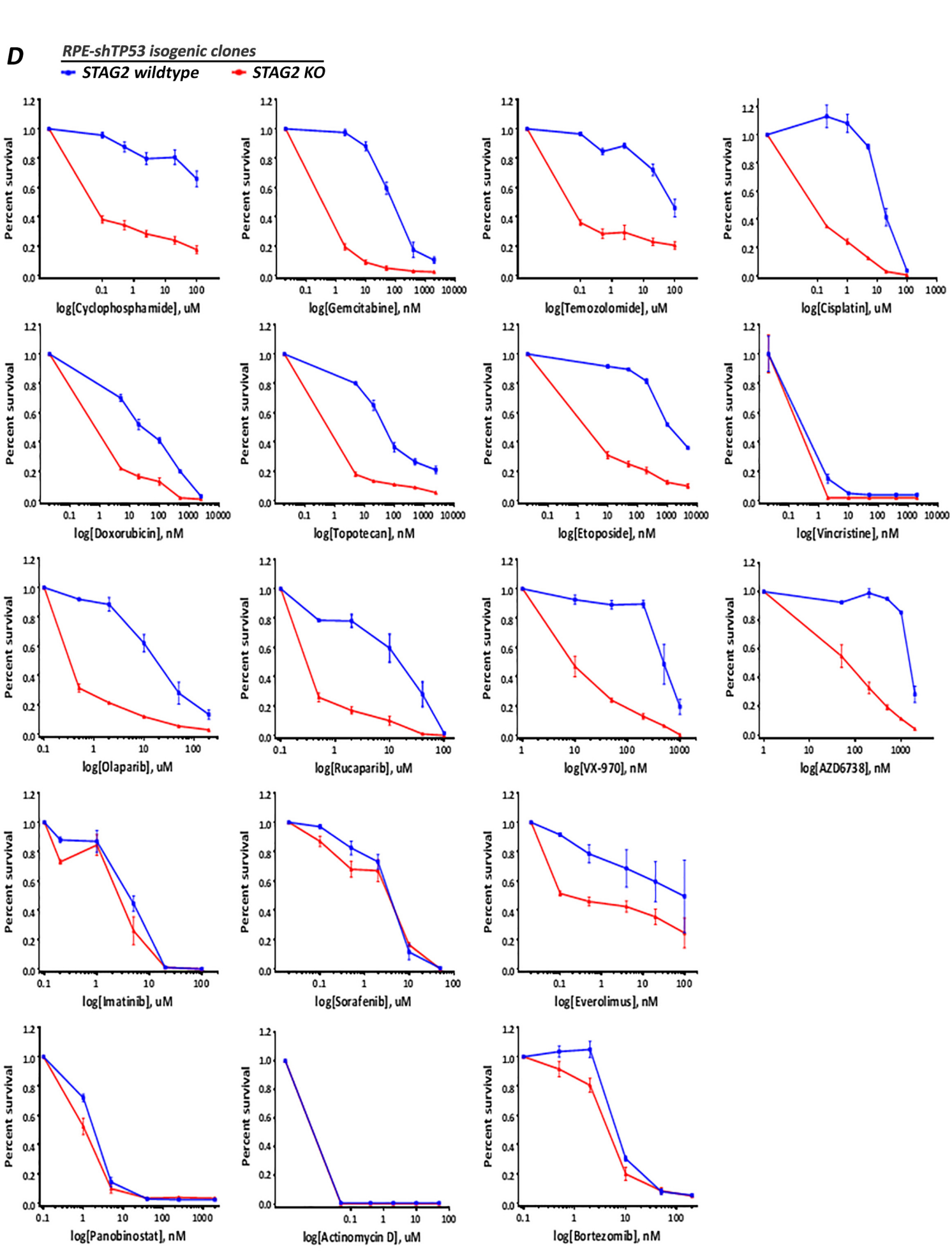
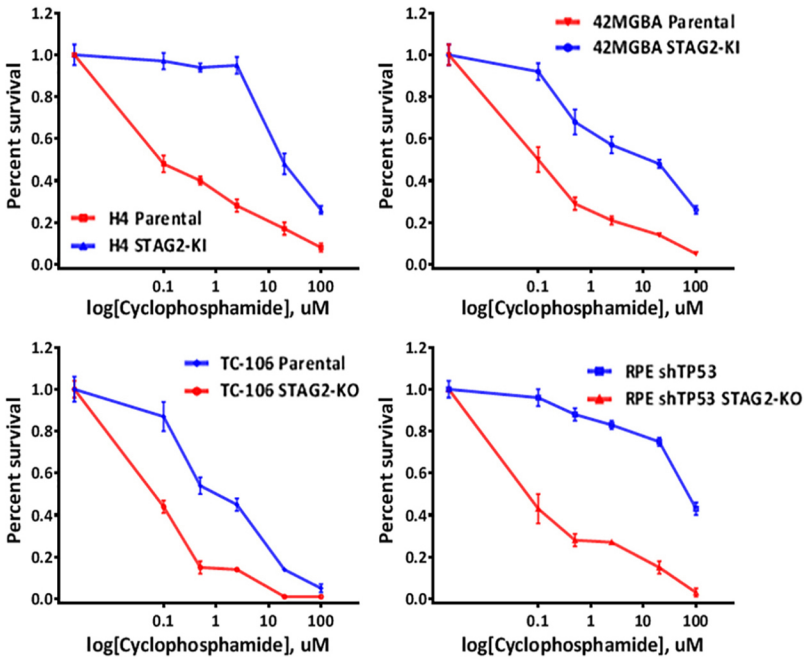



Supplementary Figure 8

**E**

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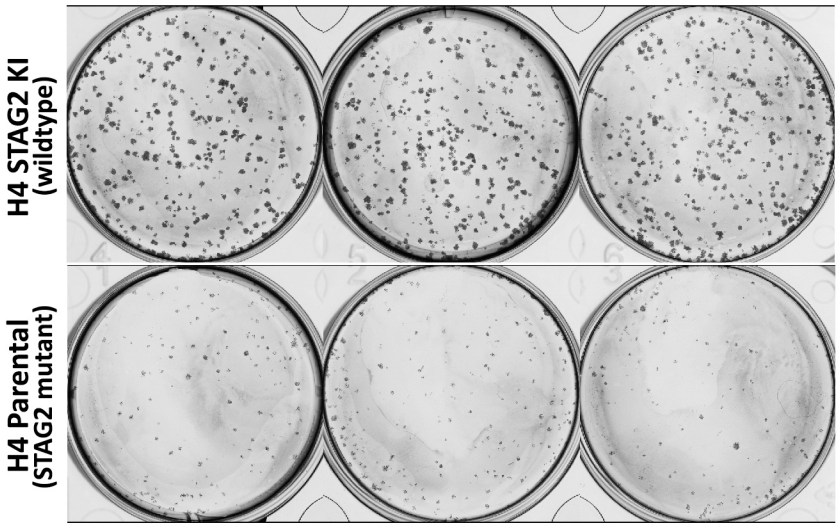


***20 μM Cyclophosphamide***

Supplementary Figure 8

**Supplementary Figure 8.** *STAG2* deficient cancer cells harbor increased sensitivity to cytotoxic chemotherapeutic agents and small molecule inhibitors of DNA double-strand break repair. **A-D,** Survival plots of *STAG2* isogenic H4 glioblastoma cells (**A**), 42MGBA glioblastoma cells (**B**), TC-106 Ewing sarcoma cells (**C**), and RPE sh*TP53* cells (**D**) following treatment with 18 chemotherapeutic agents and small molecule inhibitors. Each data point is the mean of 12 replicates from 3 independent experiments, and error bars show standard error of the mean. **E,** Clonogenic survival assay of four pairs of *STAG2* isogenic human cells upon culture in the presence of the alkylating agent cyclophosphamide. Each data point is the mean of 3 replicates, and error bars show standard error of the mean. **F,** Crystal violet staining of clonogenic survival assay of *STAG2* isogenic H4 glioblastoma cells upon culture in the presence of 20 μM cyclophosphamide.

**Supplementary Table 1.** Cell lines used in this study.

| Cell Line | Description | *STAG2* Status | Source |
|---|---|---|---|
| RPE hTERT | Human hTERT-immortalized retinal pigmented epithelium | Wildtype | ATCC (CRL-4000) |
| RPE hTERT shTP53 | Human hTERT-immortalized retinal pigmented epithelium | Wildtype | This study |
| RPE hTERT shTP53 STAG2 KO | Human hTERT-immortalized retinal pigmented epithelium | CRISPR-induced frameshift mutation | This study |
| BJ-5ta | Human hTERT-immortalized skin fibroblasts | Wildtype | ATCC (CRL-4001) |
| SVG p12 | Human SV40-immortalized fetal astroglial cells | Wildtype | ATCC (CRL-8621) |
| TC-106 | Human Ewing sarcoma | Wildtype | Dr. Timothy Triche (CHLA) |
| TC-106 STAG2 KO | Human Ewing sarcoma | CRISPR-induced frameshift mutation | This study |
| TC-71 | Human Ewing sarcoma | Wildtype | Dr. Jeffrey Toretsky (Georgetown) |
| EW2 | Human Ewing sarcoma | Wildtype | Dr. Suzanne Baker (St. Jude) |
| H4 | Human glioblastoma | Mutant (frameshift) | ATCC (HTB-148) |
| H4 STAG2 KI | Human glioblastoma | Wildtype (corrected by homologous recombination) | Solomon et al. *Science* 2011 |
| 42MGBA | Human glioblastoma | Mutant (nonsense) | DSMZ (ACC 431) |
| 42MGBA STAG2 KI | Human glioblastoma | Wildtype (corrected by homologous recombination) | Solomon et al. *Science* 2011 |
| U87MG | Human glioblastoma | Wildtype | ATCC (HTB-14) |
| 293T | Human embryonic kidney | Wildtype | ATCC (CRL-3216) |

**Supplementary Table 2.** CRISPR guides, primers, and lentiviral shRNAs used in this study.

| Target | Backbone | CRISPR Guide # | Guide Sequence | Source |
|---|---|---|---|---|
| *STAG2* | pCas-Guide-EF1a-GFP | GE200414D | GATGACAGCTTTGGTGAATG | Origene |

| Target | Primer Name | for CRISPR Guide # | Primer Sequence | Source |
|---|---|---|---|---|
| *STAG2* | STAG2 Fwd | GE200414D | TCAATTTTGCCGTGAATGAA | IDT |
| | STAG2 Rev | GE200414D | TCCGCTTTTGTAGCAGGAGT | IDT |

| Target | Backbone | shRNA # | shRNA Sequence | Used in functional studies |
|---|---|---|---|---|
| Empty Vector | pLKO.1 | N/A | N/A | yes |
| Empty Vector | pGIPZ | N/A | N/A | yes |
| *STAG2* | pLKO.1 | TRCN000015**1221** | AAACAAAGCTGTAAGAACTGC | - |
| | pLKO.1 | TRCN000015**3782** | ATTTCGGTAAGACATCAGTGG | - |
| *TP53* | pLKO.1 | TRCN000000**3755** | TTCTGGGAGCTTCATCTGGAC | yes |
| | pLKO.1 | TRCN000000**3756** | ATGTAGTTGTAGTGGATGGTG | yes |
| *ATR* | pLKO.1 | TRCN000003**9613** | ATTGAACAGATACAACCACAG | yes |
| | pLKO.1 | TRCN000003**9614** | TTTACATCTGTAAGTATCCGG | yes |
| | pLKO.1 | TRCN000003**9615** | TAATGTTAGAAGATTAGCGGC | - |
| | pLKO.1 | TRCN000003**9616** | ATAGGCTAGAAATACTTTGGC | - |
| | pLKO.1 | TRCN000003**9617** | TTTGGGTTGTAAATAATCAGC | - |
| *PARP1* | pLKO.1 | TRCN000000**7928** | AAGGCAGACATTCTAACGAAG | - |
| | pLKO.1 | TRCN000000**7929** | AATCTTCGGTTATGAAGCTGC | yes |
| | pLKO.1 | TRCN000000**7930** | TTGAGGTAAGAGATTTCTCGG | yes |
| | pLKO.1 | TRCN000000**7932** | TTGATGTTCCAGATCAGGTCG | - |
| *BRCA1* | pLKO.1 | TRCN000003**9833** | TTTAGAGAAGTAAACTTAGGG | yes |
| | pLKO.1 | TRCN000003**9834** | ATTCAGTACAATTAGGTGGGC | yes |
| | pLKO.1 | TRCN000003**9835** | AATTCAGTACAATTAGGTGGG | - |
| | pLKO.1 | TRCN000003**9836** | AATGATGGGCATTTAGAAGGG | - |
| | pLKO.1 | TRCN000003**9837** | ATCTCGTACTTTCTTGTAGGC | - |
| *RAD51* | pLKO.1 | TRCN000001**8875** | TTATCTTGAGTTAGTCTTAGC | yes |
| | pLKO.1 | TRCN000001**8876** | TAGTCTGTTCTGTAAAGGGCG | yes |
| | pLKO.1 | TRCN000001**8877** | AATGGCGAACATAGCTTCAGC | - |
| | pLKO.1 | TRCN000001**8878** | TAACCGTGAAATGGGTTGTGG | - |
| | pLKO.1 | TRCN000001**8879** | AATCTGTATGATCTCTGACCG | - |
| *XRCC5 (Ku80)* | pLKO.1 | TRCN000003**9838** | TAATCACATCACAAGGGCTGC | - |
| | pLKO.1 | TRCN000003**9839** | TTTCAGGAAGTTGTTAAAGCG | - |
| | pLKO.1 | TRCN000003**9840** | AAAGGCAAGAAGAATTGCAGG | - |
| | pLKO.1 | TRCN000003**9841** | ATAGTTATGCTTGATATGAGG | yes |
| | pLKO.1 | TRCN000003**9842** | TTACTCATGGTAAAGCCCACG | yes |
| *PRKDC (DNA-PKcs)* | pLKO.1 | TRCN000000**6255** | AATGTAATGCTTTCTATCTGC | yes |
| | pLKO.1 | TRCN000000**6256** | TAGAATTAGGATCTTTACCGG | yes |
| | pLKO.1 | TRCN000000**6257** | AATGATTCAGACTTTCACTGG | - |
| | pLKO.1 | TRCN000000**6258** | AATACAAGAGACAAAGGGTGG | - |
| | pLKO.1 | TRCN000000**6259** | ATGTCTTTGTAATAAGGCTGC | - |
| *APEX1* | pLKO.1 | TRCN000000**7958** | AATAGAATGCAGATTTCTCTG | yes |
| | pLKO.1 | TRCN000000**7959** | TATTGATGAGAGAGTCCAGGC | yes |
| | pLKO.1 | TRCN000000**7960** | AATGCAGGTAACAGAGAGTGG | - |
| | pLKO.1 | TRCN000000**7961** | ATCCTTTCTTCTTAATCCAGG | - |
| | pLKO.1 | TRCN000001**1214** | ATTCAGCCACAATCACCCGGC | - |
| *POLB (DNA Polb)* | pLKO.1 | TRCN000000**7919** | TACTCCAGGCAATTTCTTAGC | - |
| | pLKO.1 | TRCN000000**7920** | TTGATTCTGAAGTGAAGCTGG | yes |
| | pLKO.1 | TRCN000000**7921** | TTTGTCTCACCCTTTGACAGG | - |
| | pLKO.1 | TRCN000000**7922** | AAAGTTTGCGAGTTCTGTGAG | yes |
| | pLKO.1 | TRCN000001**1213** | TTCATCTACAAACTTCCTTGC | - |
| | pLKO.1 | TRCN000004**9918** | TAGATCGGAATAAGGGCTTGG | - |

| | | | | |
|---|---|---|---|---|
| *ERCC1* | pLKO.1 | TRCN0000049**919** | TTACGTCGCCAAATTCCCAGG | - |
| | pLKO.1 | TRCN0000049**920** | ATAAGGCCAGATCTTCTCTTG | - |
| | pLKO.1 | TRCN0000049**921** | AGGATACACATCTTAGCCAGC | yes |
| | pLKO.1 | TRCN0000049**922** | AATTTCTTCCTTGCTGGCGGC | yes |
| *ERCC4 (XPF)* | pLKO.1 | TRCN0000078**583** | AAATCACTGATACTCTTGCGC | - |
| | pLKO.1 | TRCN0000078**584** | AAGACTCGATTATTCTGTGGG | - |
| | pLKO.1 | TRCN0000078**585** | ATAGCAGTCTGTATAGCAAGC | yes |
| | pLKO.1 | TRCN0000078**586** | TTGAGTTAAGGTCAACTTCCG | - |
| | pLKO.1 | TRCN0000078**587** | ATAAAGAACCACGTATCTTGG | yes |
| *SMC1A* | pLKO.1 | TRCN0000062**553** | ATTGATTTCATCAATACGCCG | yes |
| | pLKO.1 | TRCN0000062**554** | AAATGGTCCGATAATCTGTCG | - |
| | pLKO.1 | TRCN0000062**555** | TATACTGAATACAGTCCCGGC | - |
| | pLKO.1 | TRCN0000062**556** | AATGGGTCATTTCCTTGTTGG | yes |
| | pLKO.1 | TRCN0000062**557** | TATAGATCTCATCAATGTTGG | - |
| *SMC3* | pLKO.1 | TRCN0000160**156** | ATTGAGATAAGTCTCTACTCG | - |
| | pLKO.1 | TRCN0000160**366** | AACTTCTGGAATACTTCACTG | yes |
| | pLKO.1 | TRCN0000160**440** | TTAAGTTCCTGTTCTACTTGG | - |
| | pLKO.1 | TRCN0000159**635** | TTTAATTCTGTGTTGCACTGC | yes |
| *RAD21* | pLKO.1 | TRCN0000148**110** | TTCTTCAGGTAAAGTAATGGC | yes |
| | pLKO.1 | TRCN0000148**135** | TTGACACTGTCAACAATTAGC | - |
| | pLKO.1 | TRCN0000148**279** | ATGATTTCTGATGCTATCTGG | yes |
| | pLKO.1 | TRCN0000147**898** | AAGCAAACTGATAGATTCAGC | - |
| *STAG1* | pLKO.1 | TRCN0000140**030** | TAATGAGGTTTCCATTCGGGC | - |
| | pLKO.1 | TRCN0000145**197** | TAATTCCAGCTTCAATTCTGC | - |
| | pLKO.1 | TRCN0000142**642** | TAACTCCAACCTTTCATTGGC | - |
| | pLKO.1 | TRCN0000140**749** | TTTGTGAAGGAACTCTCCAGC | yes |
| | pLKO.1 | TRCN0000144**850** | ATCATCTTCTATGATAGCTGC | yes |
| *STAG2* | pGIPZ | V2LHS_198**853** | ATTACAAGCAAATAAATCC | - |
| | pGIPZ | V2LHS_207**886** | TTGCTAATAACTGAGGAAG | - |
| | pGIPZ | V2LHS_391**825** | ACATAGTGAGTACACTGCA | yes |
| | pGIPZ | V2LHS_391**827** | AAATTTCTTCTCTTGTGCA | yes |
| | pGIPZ | V2LHS_391**829** | TACATAGTGAGTACACTGC | - |
| | pGIPZ | V2LHS_391**830** | TCAATGAAGACATGATCCA | yes |

**Supplementary Table 3.** Antibodies used in this study.

| Primary Antibodies | Estimated Size (kDa) | Host | Antibody # | Vendor | Dilution for immunoblot | Dilution for IP | Dilution for IF |
|---|---|---|---|---|---|---|---|
| STAG2 | 141 | Mouse | sc-81852 (J-12) | SCBT | (1:100) | (1:25) | - |
| Beta-Actin | 42 | Mouse | A5441 (AC-15) | Sigma | (1:2000) | - | - |
| Alpha-Tubulin | 50 | Mouse | T5168 (B-5-1-2) | Sigma | (1:2000) | - | - |
| Alpha-Tubulin | 50 | Rabbit | ab24246 | AbCam | (1:1000) | - | - |
| 53BP1 | 220 | Rabbit | ab21083 | AbCam | - | - | (1:1000) |
| ATR | 301 | Rabbit | ab2905 | AbCam | (1:500) | - | - |
| PARP1 | 113 | Mouse | sc-8007 (F-2) | SCBT | (1:200) | - | - |
| BRCA1 | 208 | Mouse | sc-6954 (D-9) | SCBT | (1:200) | - | - |
| RAD51 | 37 | Rabbit | ab63801 | AbCam | (1:500) | - | - |
| Ku80 | 83 | Rabbit | GTX109935 (N3C2) | GeneTex | (1:500) | - | - |
| DNA-PKcs | 469 | Mouse | sc-390698 (E-6) | SCBT | (1:100) | - | - |
| APEX1 | 36 | Rabbit | ab137708 | AbCam | (1:500) | - | - |
| DNA Pol Beta | 38 | Rabbit | ab26343 | AbCam | (1:500) | - | - |
| ERCC1 | 33 | Mouse | sc-17809 (D-10) | SCBT | (1:100) | - | - |
| ERCC4 | 105 | Mouse | sc-136153 (3F2/3) | SCBT | (1:100) | - | - |
| Phospho-ATM (Ser1981) | 350 | Rabbit | 5883 | Cell Signaling | (1:500) | - | - |
| Phospho-ATR (Ser428) | 300 | Rabbit | 2853 | Cell Signaling | (1:500) | - | - |
| Phospho-BRCA1 (Ser1524) | 220 | Rabbit | 9009 | Cell Signaling | (1:500) | - | - |
| Phospho-Chk2 (Thr68) | 62 | Rabbit | 2197 | Cell Signaling | (1:500) | - | - |
| Phospho-p53 (Ser15) | 53 | Mouse | 9286 | Cell Signaling | (1:1000) | - | - |
| SMC3 | 142 | Rabbit | ab9263 | AbCam | (1:500) | (1:50) | - |
| SMC3 | 141 | Mouse | sc-376352 | SCBT | (1:100) | (1:25) | - |
| RAD21 | 72 | Rabbit | ab992 | AbCam | (1:250) | - | - |
| SMC1A | 143 | Rabbit | ab9262 | AbCam | (1:250) | (1:50) | - |
| STAG1 | 144 | Mouse | sc-81851 | SCBT | (1:100) | - | - |
| p21 | 21 | Mouse | 2946 | Cell Signaling | (1:1000) | - | - |
| p53 | 53 | Rabbit | 2527 (7F5) | Cell Signaling | (1:500) | - | - |
| acetyl-Lysine | - | Rabbit | ab21623 | AbCam | (1:1000) | - | - |
| Anti-BrdU | - | Rat | ab6326 [BU1/75 (ICR1)] | AbCam | - | - | (1:400) |
| Anti-BrdU | - | Mouse | 347580 (B44) | BD Biosciences | - | - | (1:500) |
| MCM3 | 91 | Mouse | sc-390480 | SCBT | (1:100) | - | - |
| MCM5 | 82 | Rabbit | ab17967 | AbCam | (1:200) | - | - |
| NIPBL | 316 | Mouse | sc-374625 | SCBT | (1:100) | (1:25) | - |
| PDS5A | 151 | Rabbit | PA5-57756 | Thermo Fisher | (1:200) | (1:50) | - |
| MAD2L1 | 23 | Rabbit | NBP1-31311 | Novus | (1:200) | (1:50) | - |
| Securin | 22 | Rabbit | ab3305 | AbCam | (1:200) | (1:50) | - |
| ORC1 | 97 | Mouse | sc-398734 (F-10) | SCBT | (1:100) | - | - |
| ORC3 | 82 | Mouse | sc-374231 (C-12) | SCBT | (1:100) | - | - |
| RPA2/p34 | 29 | Mouse | R1280 | Sigma | (1:500) | - | - |
| CDC6 | 63 | Rabbit | 3387 | Cell Signaling | (1:500) | - | - |
| CDT1 | 60 | Rabbit | 8064 | Cell Signaling | (1:500) | - | - |
| Geminin | 25 | Rabbit | 5165 | Cell Signaling | (1:500) | - | - |
| PCNA | 29 | Rabbit | 13110 | Cell Signaling | (1:500) | - | - |
| DNA Pol Delta | 124 | Rabbit | ab186407 | AbCam | (1:200) | - | - |
| DNA Pol Epsilon | 220 | Mouse | sc-12728 (3C5.1) | SCBT | (1:100) | - | - |
| Cdc45 | 65 | Rabbit | 11881 | Cell Signaling | (1:500) | - | - |
| AND1 | 126 | Rabbit | NBP1-89091 | Novus | (1:200) | - | - |

| Secondary Antibodies | Estimated Size (kDa) | Host | Antibody # | Vendor | Dilution for immunoblot | Dilution for IP | Dilution for IF |
|---|---|---|---|---|---|---|---|
| anti-mouse HRP | - | horse | 7076 | Cell Signaling | (1:250) | - | - |
| anti-rabbit HRP | - | goat | 7074 | Cell Signaling | (1:250) | - | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| anti-mouse Alexa-488 | - | goat | A11001 | Invitrogen | - | - | (1:500) |
| anti-mouse Alexa-568 | - | goat | A11004 | Invitrogen | - | - | (1:500) |
| anti-rabbit Alexa-488 | - | goat | A11008 | Invitrogen | - | - | (1:500) |
| anti-rabbit Alexa-568 | - | goat | A11011 | Invitrogen | - | - | (1:500) |
| anti-rat Alexa-488 | - | goat | A11006 | Invitrogen | - | - | (1:500) |
| anti-rat Alexa-568 | - | goat | A11077 | Invitrogen | - | - | (1:500) |
| anti-mouse-Cy3 | - | sheep | C2181 | Sigma | - | - | (1:500) |

**Supplementary Table 4.** Drugs used in this study.

| Drug Name | Mol Weight | Target/Mechanism | Cat. No. | Vendor. | Solvent |
|---|---|---|---|---|---|
| Everolimus | 958.22 | mTOR Inh. | S1120 | Selleckchem | DMSO |
| VE-822 (VX-970) | 463.55 | ATR kinase Inh. | S7102 | Selleckchem | DMSO |
| AZD6738 | 412.51 | ATR kinase Inh. | S7693 | Selleckchem | DMSO |
| Cyclophosphamide | 279.1 | DNA Alkylating | S2057 | Selleckchem | DMSO |
| Temozolomide | 194.15 | DNA Alkylating | S1237 | Selleckchem | DMSO |
| Etoposide | 588.56 | Topoisomerase-II Inh. | S1225 | Selleckchem | DMSO |
| Doxorubicin | 579.98 | Topoisomerase-II Inh. | S1208 | Selleckchem | DMSO |
| Topotecan | 457.91 | Topoisomerase-I Inh. | S1231 | Selleckchem | DMSO |
| Gemcitabine | 263.2 | DNA Alkylating | S1714 | Selleckchem | DMSO |
| Cisplatin | 300.05 | DNA-Crosslinking | S1166 | Selleckchem | DMF |
| Vincristine | 923.04 | Microtubule Inh. | S1241 | Selleckchem | DMSO |
| Actinomycin D | 1255.43 | RNA Polymerase Inh. | 1229 | Tocris | DMSO |
| Bortezomib | 384.24 | Proteasome Inh. | S1013 | Selleckchem | DMSO |
| Panobinostat | 349.43 | HDAC Inh. | S1030 | Selleckchem | DMSO |
| Olaparib | 434.46 | PARP Inh. | S1060 | Selleckchem | DMSO |
| Rucaparib | 421.36 | PARP Inh. | S1098 | Selleckchem | DMSO |
| Imatinib | 589.71 | Tyrosine kinase Inh. | S1026 | Selleckchem | DMDO |
| Sorafenib | 637.03 | Tyrosine kinase Inh. | S1040 | Selleckchem | DMDO |
| Palbociclib | 573.66 | CDK4/6 Inh. | S1579 | Selleckchem | Water |
| Aphidicolin | 338.48 | DNA Polymerase Inh. | A0781 | Sigma | Water |
| Colcemid | 371.43 | Microtubule Inh. | 15210-040 | Life Technologies | Water |

**Supplementary Table 5.** Other key reagents used in this study.

| Reagent | Product ID | Vendor |
|---|---|---|
| Beta-galactosidase assay kit | 9860S | Cell Signaling |
| Cell Titer-Glo reagent | G8462 | Promega |
| iBind Flex Solution Kit | SFL2020 | Invitrogen |
| MES SDS Running Buffer | B0002 | Invitrogen |
| Bolt Bis-Tris Plus gels | NW04120/22 | Invitrogen |
| iBlot2 NC Mini Stacks | IB23002 | Invitrogen |
| iBind Flex Card | SFL2010 | Invitrogen |
| NuPAGE LDS Sample Buffer (4X) | NP0007 | Invitrogen |
| Bolt Sample reducing agent (10X) | B0009 | Invitrogen |
| RIPA Buffer | QJ222447 | Pierce |
| Protease and phosphatase Inhibitor | 88668 | Pierce |
| Protease Inhibitor | 88666 | Pierce |
| 5-Iodo-2'-deoxyuridine (IdU) | I7125 | Sigma |
| ImmEdge pen | H-4000 | Vector Labs |
| 5-Chloro-2'-deoxyuridine (CldU) | C6891 | Sigma |
| Propidium Iodide | 81845 | Sigma |
| RNAse A | R6148 | Sigma |
| Polybrene | TR-1003-G | Millipore |
| BCA protein assay kit | 23227 | Pierce |
| Super signal west pico (ECL) | 34078 | Thermo scientific |
| IP Lysis Buffer | 87787 | Pierce |
| Protein A sepharose | ab193256 | Abcam |
| BSA | A4503 | Sigma |
| Puromycin | P8833 | Sigma |
| Ampicilin | A0166 | Sigma |
| Kanamycin | K1377 | Sigma |
| KaryoMAX Colcemid | 15210-040 | Life Technologies |
| DMEM | 10566-016 | Life Technologies |
| Fetal bovine serum | FP-0500-A | Atlas Biologicals |
| Penicillin/streptomycin | 15140163 | Life Technologies |
| FuGENE 6 | E2691 | Promega |
| HiSpeed plasmid midi kit | 12643 | Qiagen |
| Phenol-Chloroform | 15593-049 | Invitrogen |
| Proteinase K | 3115801001 | Roche |
| MyFi DNA polymerase | BIO-21118 | BioLine |
| ExoSAP-IT | AF78201 | Affymetrix |
| Vectashield Mounting Medium with DAPI | H-1200 | Vector Labs |
| Vectashield Mounting Medium | H-1000 | Vector Labs |
| DNase-I | M0303S | NEB |
| Coverslip sealant | 23005 | Biotium |
| Micrococcal Nuclease | 88216 | Thermo scientific |