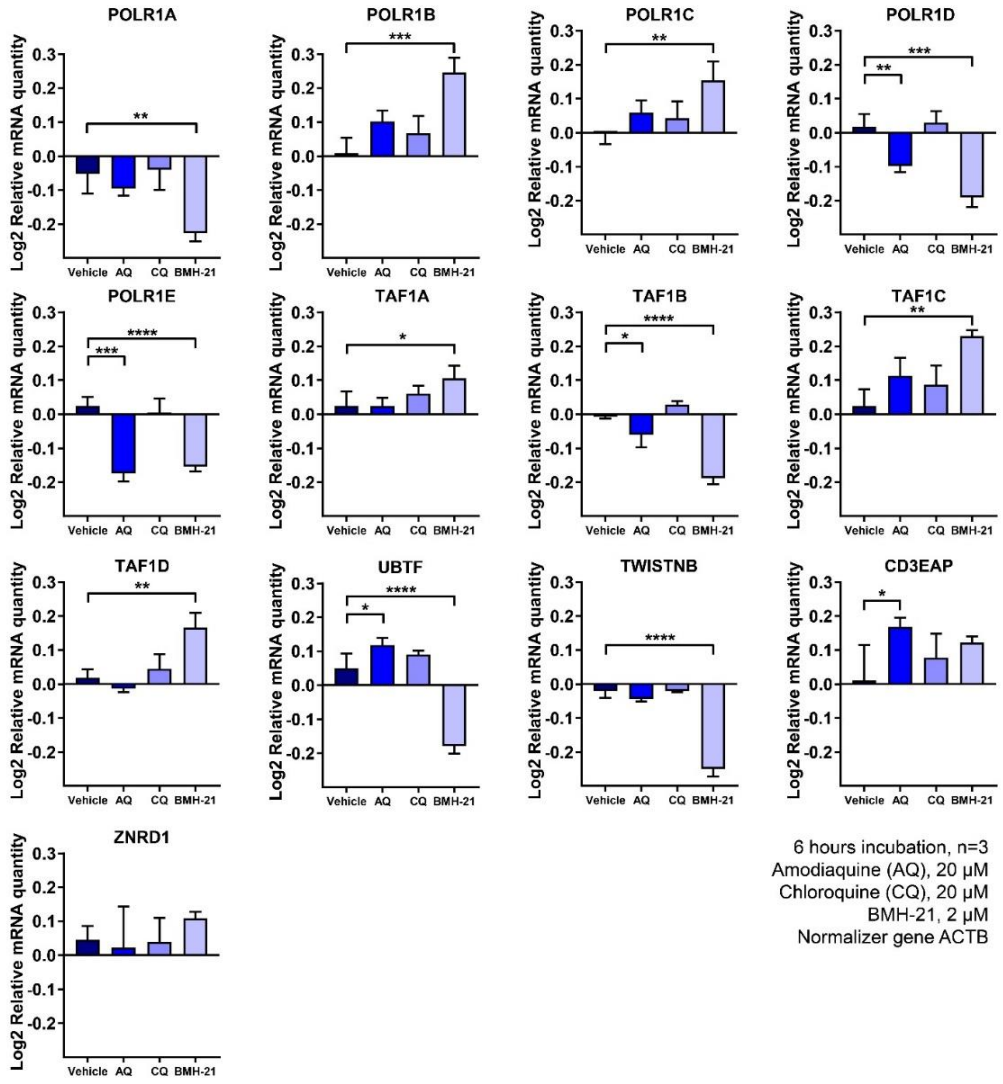
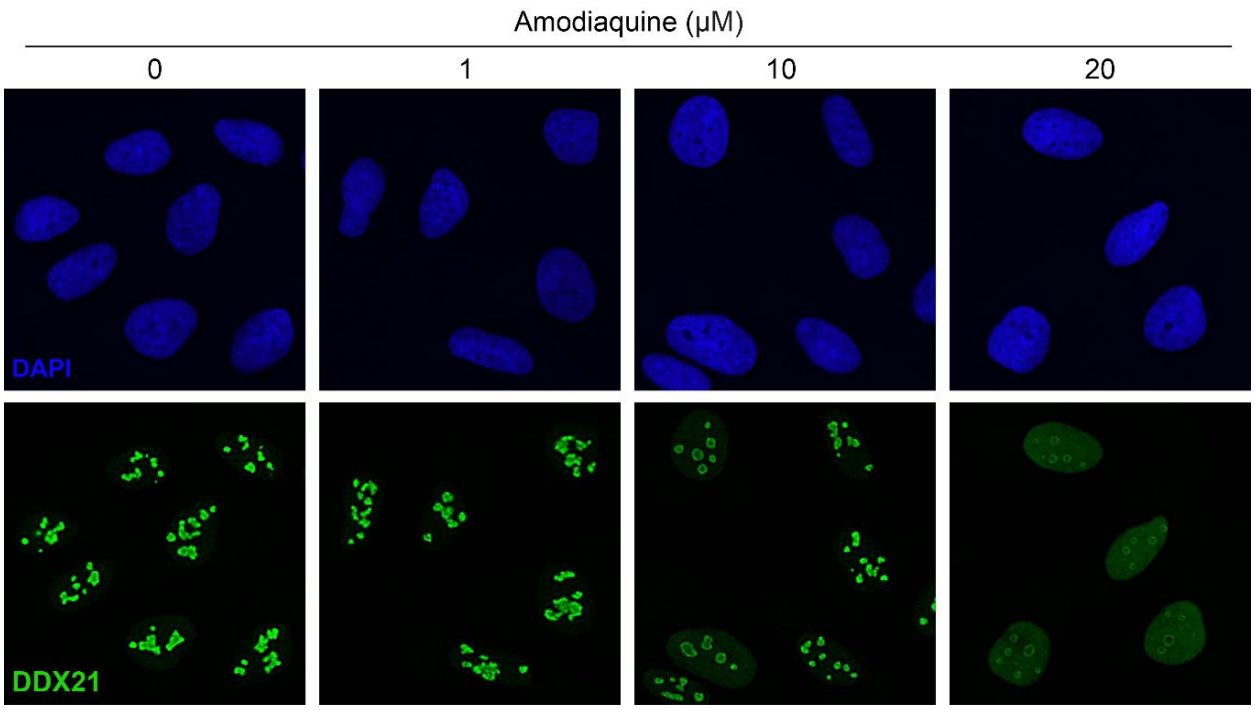
**Supplementary information**

**The antimalarial drug amodiaquine stabilizes p53 through ribosome biogenesis stress, independently of its autophagy-inhibitory activity**

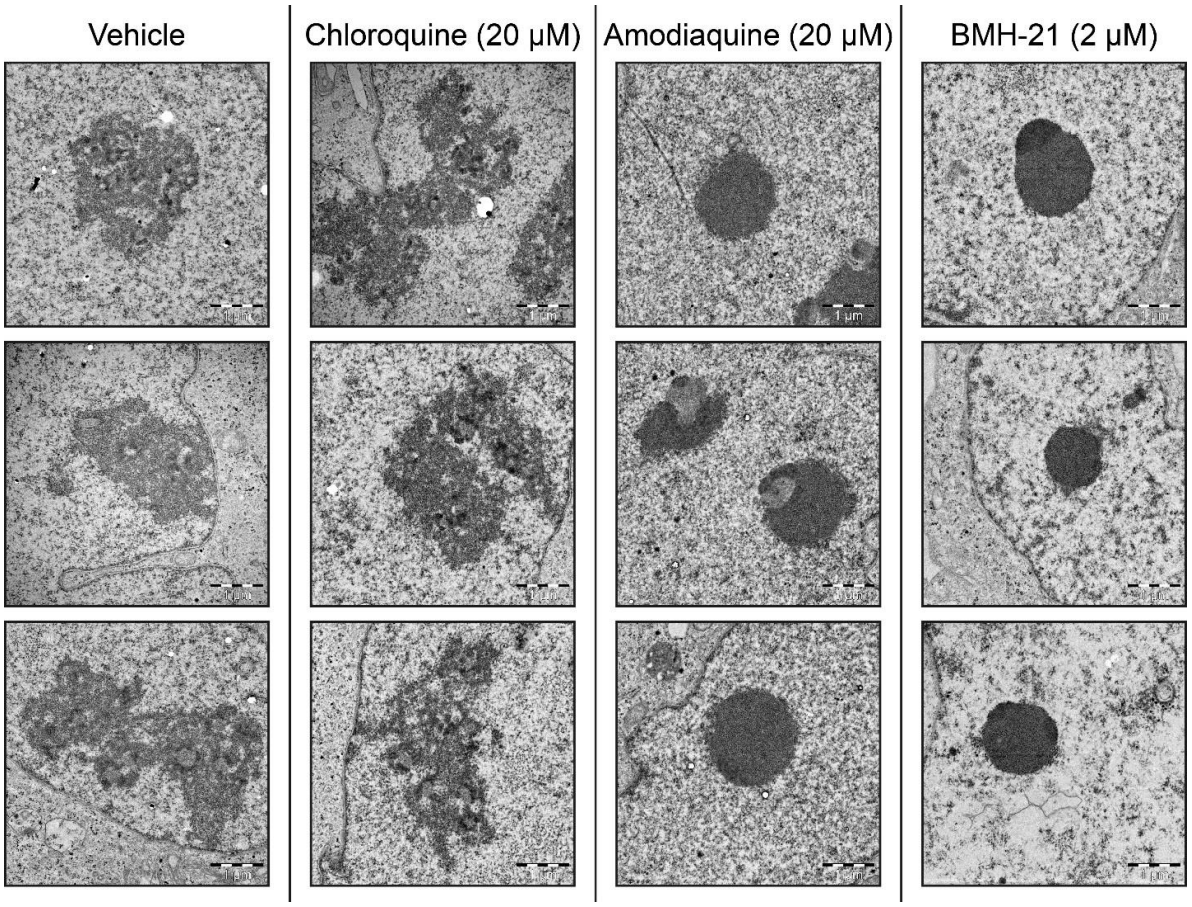
Espinoza *et al,* Cell Death & Differentiation, 2019



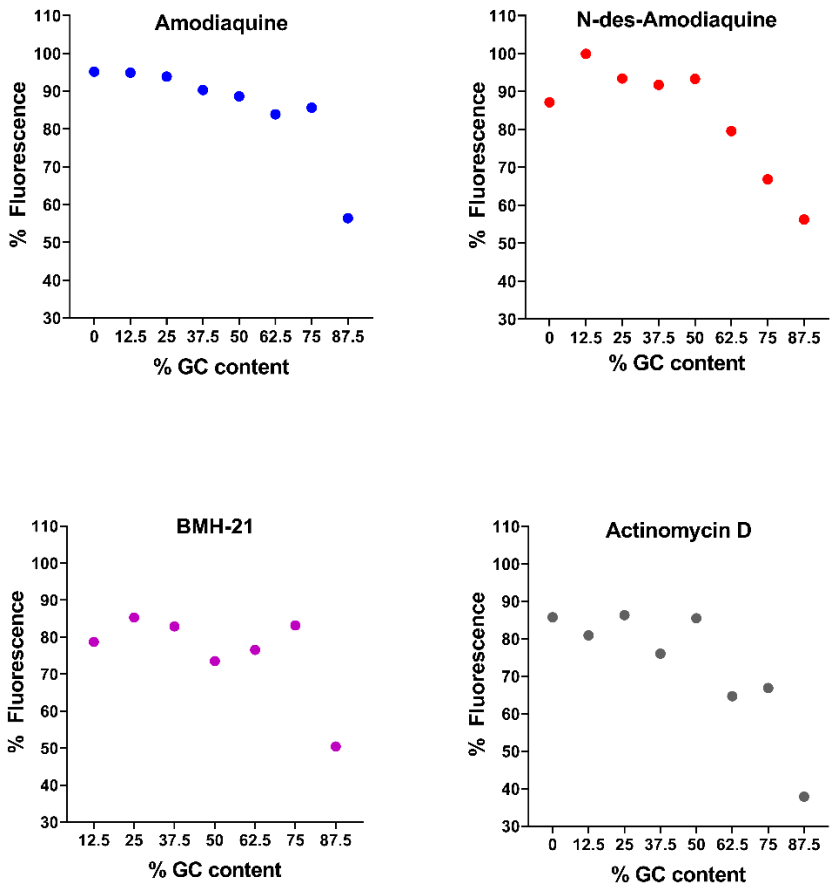

**Supplementary Figure 1. Transcriptional changes of genes that structure the preinitiation complex and RNA Pol I complex**. mRNA levels were assessed in U2OS cells after exposure to CQ, AQ and BMH-21 during 6 hours. Data shown as mean ± SD of triplicate wells and are representative of triplicate treatments; Statistical significance was calculated by one-way ANOVA using log- transformed data and Dunnett's multiple test comparison (* p<0.05, ** p<0.01, *** p<0.001, **** p<0.001)



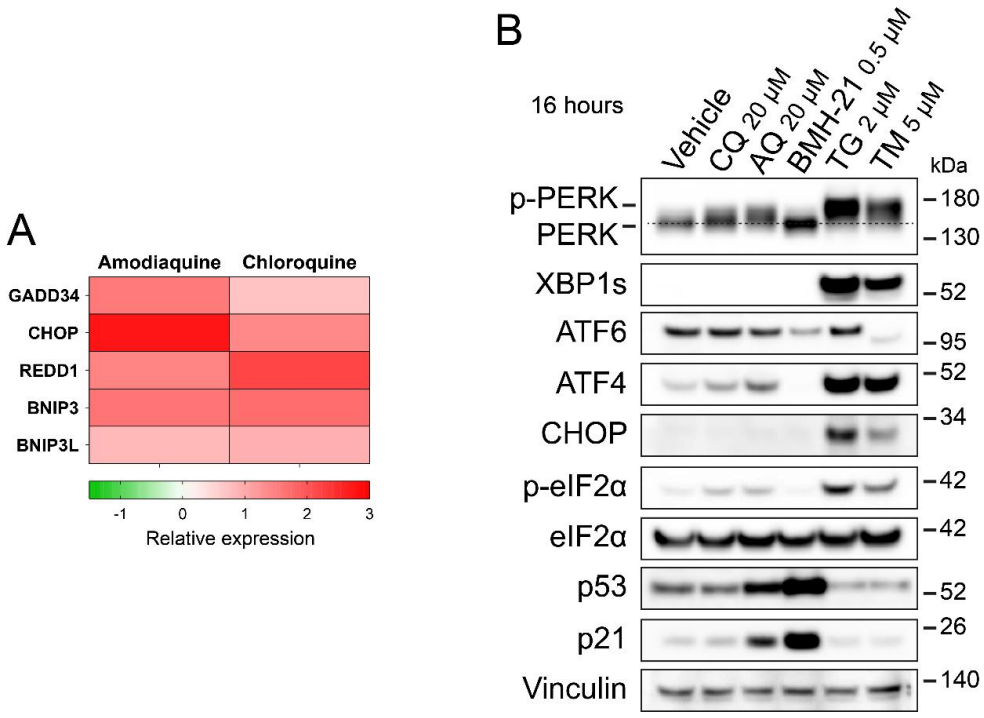

**Supplementary Figure 2**. **Amodiaquine treatment causes RNA helicase DDX21 translocation into the nucleoplasm**. Immunofluorescence analysis of U2OS cells treated with increasing doses of AQ (6 hours) and immunostained for DDX21.



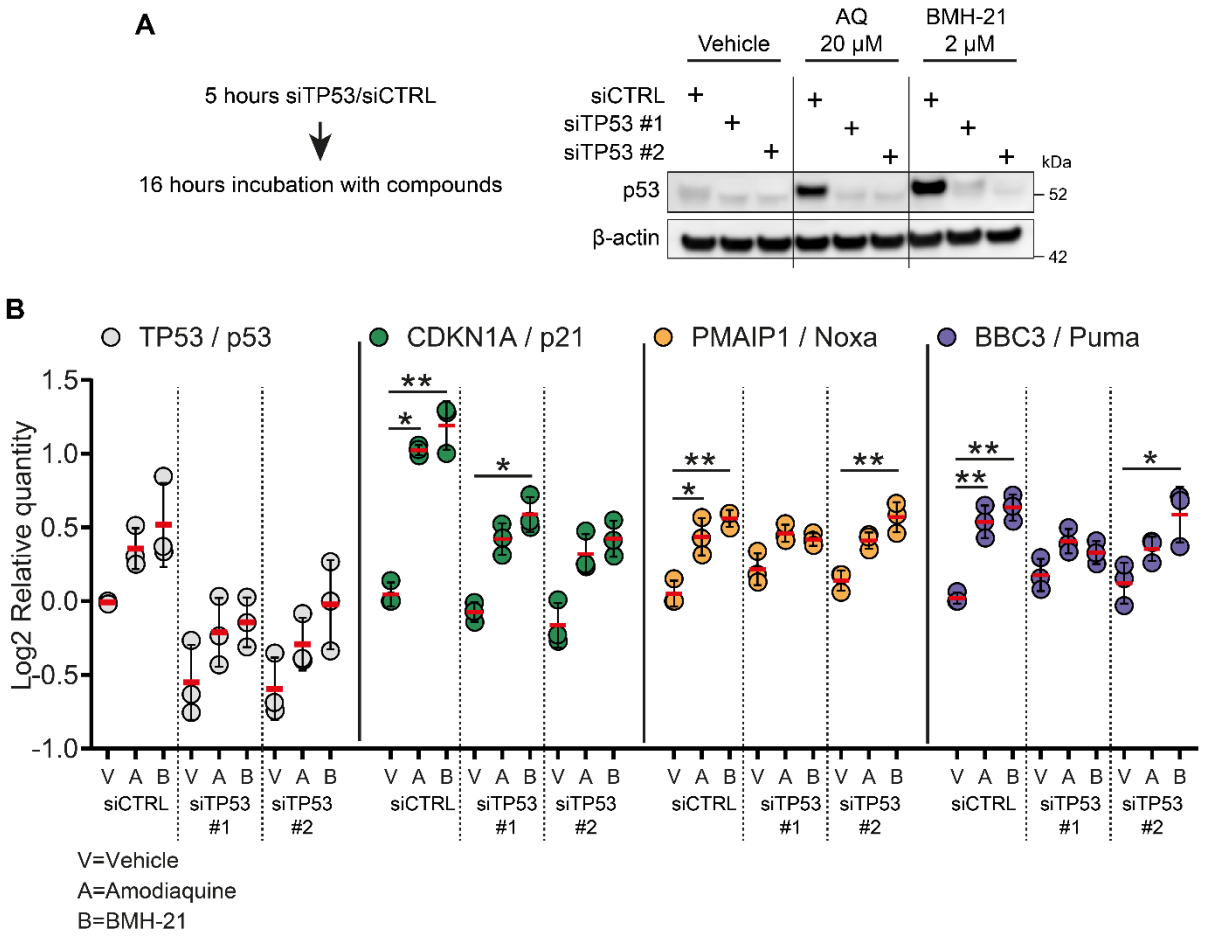

**Supplementary Figure 3**. Additional images showing the ultrastructural changes in the nucleolus after exposure to CQ, AQ and BHM-21 during 6 hours (Bar= 1 µM).



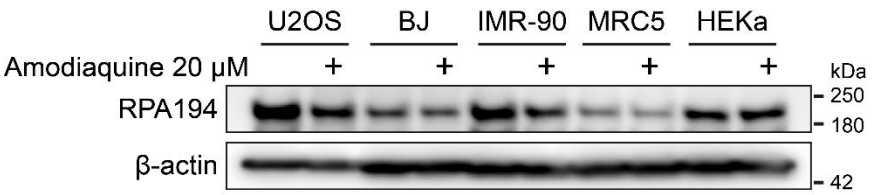

**Supplementary Figure 4.** Fluorescent intercalator displacement (FID) assay of amodiaquine, N-desethyl-amodiaquine, BMH-21 and actinomycin D. Plots show percentage of fluorescence according to the GC content.



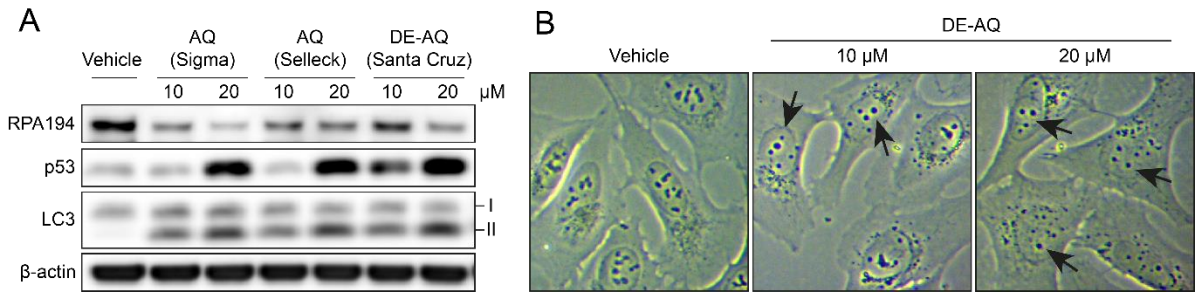

**Supplementary Figure 5. Amodiaquine and chloroquine induce a mild ER-stress.** A) Amodiaquine and chloroquine induce expression of ER-stress related genes; RNAseq data, relative expression compared to untreated control. B) Immunoblot analysis of ER stress markers. U2OS cells were treated during 16 hours with the compounds using the indicated concentrations. PERK hyperphosphorylation is observed as a shift in molecular size. Thapsigargin (TG) and Tunicamycin (TM) were used as positive controls of ER stress. CQ=chloroquine; AQ=Amodiaquine.



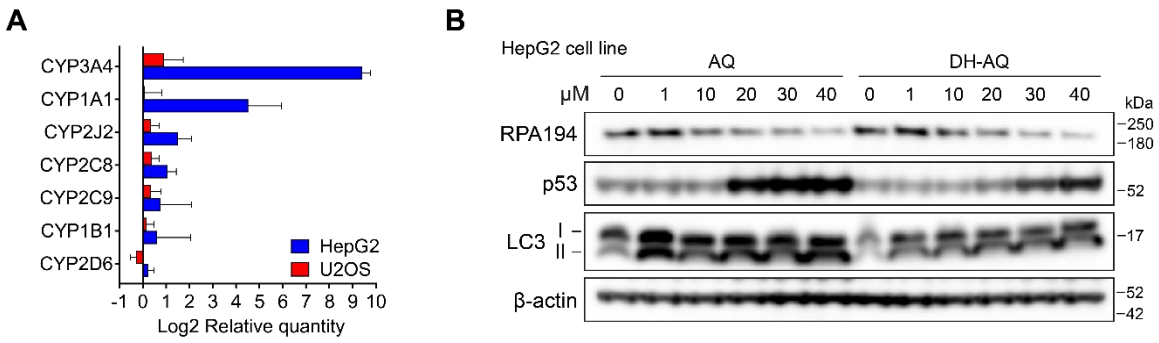

**Supplementary Figure 6**. **Expression of p53-regulated genes induced by Amodiaquine and BMH-21**. A) U2OS cells were treated with 20 nM siRNA during 5 hours prior to treatment with Amodiaquine and BMH-21. Immunoblot analysis showed no p53 activation after 16 hours of incubation with compounds in siTP53 treated cells. B) RT-qPCR analysis of TP53, CDKN1A, PMAIP1 and BBC3 mRNAs in U2OS cells treated as in A.




**Supplementary Figure 7. RPA194 degradation in non-transformed cells treated with amodiaquine**. Immunoblot analysis of RPA194 in BJ fibroblasts, IMR-90 fibroblasts, MRC5 fibroblasts and HEKa Primary Epidermal Keratinocytes treated with amodiaquine for 6 hours. U2OS cancer cell line is included as control.




**Supplementary Figure 8**. **The DE-AQ metabolite has similar nucleolar disruptive activity as AQ.** A) DE-AQ induces RPA194 degradation, activation of p53 and accumulation of LC3-II. U2OS cells were treated during 6 hours with the compounds. B) Brightfield images of U2OS cells treated with DE-AQ. Black arrows show nucleoli with altered morphology.




**Supplementary Figure 9.** A) HepG2 expresses higher levels of CYP genes than U2OS assessed by RT-qPCR analysis, (n=3). B) Immunoblot analysis of RPA194, p53 and LC3 in HepG2 cells treated with increasing doses of AQ and its non-reactive analog DH-AQ during 6 hours.

**Supplementary table S1**. Cell lines

| Cell line | Tissue of origin | Species | Medium |
|---|---|---|---|
| U2OS | Sarcoma | Human | DMEM 10% FBS |
| MEF | Fibroblast | Mouse | DMEM 10% FBS |
| BJ | Fibroblast | Human | DMEM 10% FBS |
| MRC5 | Fibroblast | Human | DMEM 10% FBS |
| IMR-90 | Fibroblast | Human | DMEM 10% FBS |
| HepG2 | Hepatic cancer | Human | DMEM 10% FBS |
| A375 | Melanoma | Human | DMEM 10% FBS |
| A549 | Lung carcinoma | Human | DMEM 10% FBS |
| MCF7 | Breast adenocarcinoma | Human | DMEM 10% FBS |
| HT-29 | Colorectal adenocarcinoma | Human | DMEM 10% FBS |
| RKO | Colorectal adenocarcinoma | Human | DMEM 10% FBS |
| RKO ATM -/- 2.1 | Colorectal adenocarcinoma | Human | DMEM 10% FBS |
| RKO ATM -/- 6.3 | Colorectal adenocarcinoma | Human | DMEM 10% FBS |
| SK-CO-1 | Colorectal adenocarcinoma | Human | DMEM 10% FBS |
| SW948 | Colorectal adenocarcinoma | Human | DMEM 10% FBS |
| SW837 | Colorectal adenocarcinoma | Human | DMEM 10% FBS |
| SW1116 | Colorectal adenocarcinoma | Human | DMEM 10% FBS |
| T84 | Colorectal adenocarcinoma | Human | DMEM 10% FBS |
| LoVo | Colorectal adenocarcinoma | Human | DMEM 10% FBS |
| HCT116 | Colorectal adenocarcinoma | Human | DMEM 10% FBS |
| LS123 | Colorectal adenocarcinoma | Human | DMEM 10% FBS |
| HEKa | Primary Epidermal Keratinocytes | Human | Dermal Cell Basal Medium + Keratinocyte Growth Kit (ATCC, PCS-200-040 |

153 **Supplementary table S2**. Antibodies

| Antibodies | Company | Clone | Catalogue No | Application (dilution) |
|---|---|---|---|---|
| RPA194 (POLR1A) C-1 | Santa Cruz | C-1 | sc-48385 | WB (1:2,000); IF (1:500); Wes (1:50) |
| p53, | Abcam | DO-1 | ab1101 | WB (1:2,000): Wes (1:50) |
| p21 | Cell Signaling | DCS60 | 2946 | WB (1:1,000) |
| Beclin-1 | Cell Signaling | D4OC5 | 3495 | WB (1:2,000) |
| p62 (SQSTM1) | Cell Signaling | D5L7G | 88588 | WB (1:2,000) |
| p62 (SQSTM1) | Santa Cruz | D-3 | sc-28359 | WB (1:2,000) |
| LC3B (D11) XP® | Cell Signaling | D11 | 3868 | IF (1:500) |
| LC3B | Cell Signaling | Polyclonal | 2775S | WB (1:2,000) |
| β-actin | Abcam | AC-15 | ab6276 | WB (1:20,000); Wes (1:150) |
| phospho-H2AX (ser139) | Millipore | JBW301 | 05-636 | WB (1:1,000) / IF (1:500) |
| ATM | Abcam | 2C1 | ab78 | WB (1:1,000) |
| phospho-ATM (Ser1981) | Abcam | D6H9 | 5883S | WB (1:1,000) |
| Nucleolin | Abcam | Polyclonal | ab22758 | WB (1:2,000) / IF (1:500) |
| RPL5 | Bethyl Laboratories | Polyclonal | A303-933A | WB (1:2,000) |
| RPL11 | Thermo | 3A4A7 | 37-3000 | WB (1:500) |
| LAMP1 | Cell Signaling | D4O1S | 15665 | IF (1:500) |
| LAMP1 | Cell Signaling | C54H11 | 3243S | WB (1:1,000) |
| GAPDH | Millipore | Polyclonal | AB2302 | WB (1:5,000) |
| DDX21 | Novus Biologicals | Polyclonal | NBP1-83310 | IF (1:500) |
| KAP1 | Abcam | 20C1 | ab22553 | WB (1:1,000) |
| phospho-KAP1 (Ser824) | Abcam | EPR5248 | ab133440 | WB (1:1,000) |
| UBF | Santa Cruz | F-9 | sc-13125 | IF (1:500) |
| Fibrillarin | Abcam | Polyclonal | ab5821 | IF (1:500) |
| Anti-amodiaquine | Thermo | 6D10 | HYB 320-04-02 | Wes (1:25) |
| PERK | Cell Signaling | D11A8 | 5683 | WB (1:1,000) |
| XBP-1s | Cell Signaling | D2C1F | 12782 | WB (1:1,000) |
| ATF6 | Cell Signaling | D4Z8V | 65880 | WB (1:1,000) |
| ATF4 | Cell Signaling | D4B8 | 11815 | WB (1:1,000) |
| CHOP | Cell Signaling | D46F1 | 5554 | WB (1:1,000) |
| Phosphor-eIF2α (Ser51) | Cell Signaling | Polyclonal | 9721 | WB (1:1,000) |
| eIF2α | Cell Signaling | Polyclonal | 9722 | WB (1:1,000) |
| Vinculin | Abcam | EPR8185 | ab129002 | WB (1:1,000) |
| Anti-chicken HRP | Thermo | Polyclonal | A16054 | WB / 1:5000 |
| Anti-mouse Alexa-647 | Thermo | Polyclonal | A-21235 | IF / 1:1000 |
| Anti-mouse Alexa-488 | Thermo | Polyclonal | A-11029 | IF / 1:1000 |
| Anti-rabbit Alexa-647 | Thermo | Polyclonal | A-21244 | IF / 1:1000 |
| Anti-rabbit Alexa-488 | Thermo | Polyclonal | A-11008 | IF / 1:1000 |
| Goat Anti-Mouse IgG (Cy3) | Abcam | Polyclonal | ab97035 | WB / 1:1000 |
| Goat Anti-Rabbit IgG (Cy5) | Abcam | Polyclonal | ab97077 | WB / 1:1000 |

154

**Supplementary table S3**. Chemicals

| Chemical | Company | Catalogue code | Solvent |
|---|---|---|---|
| Amodiaquin dihydrochloride dihydrate | Sigma-Aldrich | A2799 | Water |
| Amodiaquin dihydrochloride dihydrate | Selleck Chemicals | S4589 | Water |
| N-Desethyl Amodiaquine Hydrochloride | Santa Cruz Biotechnology | sc-212178 | Water |
| Chloroquine diphosphate salt | Sigma-Aldrich | C6628 | Water |
| Hydroxychloroquine sulfate | Sigma-Aldrich | H0915 | Water |
| Doxorubicin | Sigma-Aldrich | D1515 | DMSO |
| KU-60019 | Selleck Chemicals | S1570 | DMSO |
| Aloxistatin | Selleck Chemicals | S7393 | DMSO |
| Pepstatin A | Selleck Chemicals | S7381 | DMSO |
| Bafilomycin A1 | Selleck Chemicals | S1413 | DMSO |
| Lys05 trihydrochloride | MedKoo Biosciences | 406969 | DMSO |
| BMH-21 | Sigma-Aldrich | SML1183 | DMSO |
| MG-132 | Selleck Chemicals | S2619 | DMSO |
| CX-5461 | Cellagen Technologies | C2954 | DMSO |
| Cycloheximide | Sigma-Aldrich | C7698 | DMSO |
| Actinomycin D | Sigma-Aldrich | A1410 | DMSO |
| Thapsigargin | Abcam | ab120286 | DMSO |
| Tunicamycin | Cayman chemicals | 11445 | DMSO |
| Clotrimazole | Sigma-Aldrich | C6019 | DMSO |

**Supplementary table S4**. RT-qPCR primer sequences

| Target | Forward | Reverse |
|---|---|---|
| 47S_5´ETS* | GAACGGTGGTGTGTCGTT | GCGTCTCGTCTCGTCTCACT |
| 18S_5'-junction* | GCCGCGCTCTACCTTACCTACCT | CAGACATGCATGGCTTAATCTTTG |
| 18S_3'-junction* | AGTCGTAACAAGGTTTCCGTAGGT | CCTCCGGGCTCCGTTAAT |
| 5.8S_5'-junction* | TACGACTCTTAGCGGTGGATCA | TCACATTAATTCTCGCAGCTAGCT |
| 5.8S_3'-junction* | GAATTGCAGGACACATTGATCATC | GGCAAGCGACGCTCAGA |
| 28S_5'-junction* | CCGAGACGCGACCTCAGAT | TCCGCTGACTAATATGCTTAAATTCA |
| 18S* | GATGGTAGTCGCCGTGCC | GCCTGCTGCCTTCCTTGG |
| 5.8S* | ACTCGGCTCGTGCGTC | GCGACGCTCAGACAGG |
| 28S* | GTGACGCGCATGAATGGA | TGTGGTTTCGCTGGATAGTAGGT |
| GAPDH | ACCTGACCTGCCGTCTAGAAAA | CAAAGTGGTCGTTGAGGGCAAT |
| ACTB | TCACAATGTGGCCGAGGACTTT | AGAAGTGGGGTGGCTTTTAGGA |
| POLR1A | TTTGCCGTGTATGGCATCGC | TGTCATCTGCTGTAGCGGGG |
| POLR1B | TGAGTGGGGCGGAATATGGG | CTGCGGTTTCGGTTTCTCGG |
| POLR1C | TTGCCCGGGTTCGAGATCATT | TGATGGCTTCACTCACCAGCA |
| POLR1D | TGCACGAGGAAGACCATACCC | ACAGCTGGAAGGGTACCTCG |
| POLR1E | GTGACTGCTCTGGTCAGCGA | ACACGTCTTCAGGCTTGGCT |
| TAF1A | GCAGGAGGAGTTACGAGCCG | TCCGGCCCACGTGAAGAAAT |
| TAF1B | CCCCGTCAGCAAAGCATCAC | GGCAAGTGTCTGTGGCATGG |
| TAF1C | CTCCCTCACTGGCTGACCTG | CTGCATTGCCTGCAGAAGGG |
| TAF1D | TGGGCCACGGAAATAGCCAA | CTGGCCTGGTGTCCTAGAGC |
| UBTF | TCATAAGGCCCTGGAGCAGC | ACTTGTCCTTGAGCTGGCGT |
| TWISTNB | GAGTTGCCGACTTATGCCGC | CTGTTCTCGAATGCCGGTGC |
| CD3EAP | GCTCCCGGATGATGCCCTAC | AACCGAGCAGCATCCTCACC |
| ZNRD1 | AACTCCCTCCTCAGACCCGA | GTGACCGTATCCTGAGCCCC |
| CYP3A4 | AGCACCGAGTGGATTTCCTTCAG | GCTCCAGATCGGACAGAGCTTT |
| CYP1A1 | TACCTACCCAACCCTTCCCTGAAT | TCAATCAGGCTGTCTGTGATGTCC |
| CYP2J2 | CATCACCTTCGGAGAACGCT | GGGTCCAGGCAGGAATTTCA |
| CYP2C8 | CTTGGTTGGCACTGTAGCTGATCT | TCCTGGACTTTAGCTGTGACCTCT |
| CYP2C9 | TCTATGGCCCTGTGTTCACTCTG | CAGGGCTTCCTTCACTGCTTCATA |
| CYP1B1 | ACCAGGTATCCTGATGTGCAGACT | GCTTCATAAAGGAAGGCCAGGACA |
| CYP2D6 | ATTCATGAGGTGCAGCGCTTTG | CTCATCCTTCAGCACCGATGACA |
| TP53 (p53) | CAGTTGGGCAGCTGGTTAGG | ATCCTCCAGGGTGTGGGATG |
| CDKN2A (p21) | AATCGTCCAGCGACCTTCCT | CTGACTCCTTGTTCCGCTGC |
| PMAIP1 (Noxa) | TACCGCTGGCCTACTGTGAA | ATGTGCTGAGTTGGCACTGA |
| BBC3 (Puma) | GCGATTGCGATTGGGTGAGA | TACTTCCTGCCCTGCTCTGG |

*Sequences obtained from Kwon *et al*, 2014.

**Supplementary table S5**. ChIP-qPCR primer sequences

| rDNA region | Position | Forward | Position | Reverse |
|---|---|---|---|---|
| upstream | -988 | GCTTCTCGACTCACGGTTTC | -798 | GGAGCTCTGCCTAGCTCACA |
| upstream | -410 | GATCCTTTCTGGCGAGTCC | -272 | GGAGCCGGAAGCATTTTC |
| promoter | -48 | GAGGTATATCTTTCGCTCCGAGTC | -14 | CAGCAATAACCCGGCGG |
| promoter | -46 | GGTATATCTTTCGCTCCGAG | 13 | AGCGACAGGTCGCCAGAGGA |
| 5'ETS | 851 | GAACGGTGGTGTGTCGTT | 961 | GCGTCTCGTCTCGTCTCACT |
| 5'ETS | 1297 | CAGGTGTTTCCTCGTACCG | 1483 | GCTACCATAACGGAGGCAGA |
| 18S | 4013 | AAACGGCTACCACATCCAAG | 4148 | CCTCCAATGGATCCTCGTTA |
| 18S | 4446 | CCCGAAGCGTTTACTTTGAA | 4612 | CGGTCCAAGAATTTCACCTA |
| 28S | 10319 | GAACTTTGAAGGCCGAAGTG | 10450 | ATCTGAACCCGACTCCCTTT |
| 28S | 12293 | TGGGTTTTAAGCAGGAGGTG | 12472 | AACCTGTCTCACGACGGTCT |
| IGS | 18449 | TGGTGGGATTGGTCTCTCTC | 18572 | CAGCCTGCGTACTGTGAAAA |
| IGS | 30541 | ACTGGCGAGTTGATTTCTGG | 30621 | CGAGACAGTCGAGGGAGAAG |

*Sequences obtained from Peltonen *et al*, 2014.

**Supplementary table S6. FID oligo hairpins**

| Sequence (5'-3') | GC content (%) |
|---|---|
| TTTATATAAAAAATATATAAA | 0 |
| CAATAAATAAAAAATTTATTG | 12.5 |
| CATAATTCAAAAAGAATTATG | 25 |
| CGAATAACAAAAAGTTATTCG | 37.5 |
| CGAGAATCAAAAACATTCTCG | 50 |
| CGATGCACAAAAAGTGCATCG | 62.5 |
| CGCGAACCAAAAAGGTTCGCG | 75 |
| CGGCCTGCAAAAAGCAGGCCG | 87.5 |