## Supplementary Information

**RAD51 inhibition induces R-loop formation in early G1 phase of the cell cycle**

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**Table S1. The sequences of primers used for qPCR**

| Amplicon | Forward | Reverse |
|---|---|---|
| H42 | AGAGGGGCTGCGTTTTCGGCC | CGAGACAGATCCGGCTGGCAG |
| H42.9 | CCCGGGGGAGGTATATCTTT | CCAACCTCTCCGACGACA |
| H0.1 | TCTGGCGACCTGTCGTCGGA | CTCGGACGCGCGAGAGAACAG |
| H0.4 | CAGGCGTTCTCGTCTCCG | CACCACATCGATCGAAGAGC |
| H4 | CGACGACCCATTCGAACGTCT | CTCTCCGGAATCGAACCCTGA |
| H6 | CAGCTAGCTGCGAGAATTAATG | CGATTGATCGGCAAGCGAC |
| H8 | AGTCGGGTTGCTTGGGAATGC | CCCTTACGGTACTTGTTGACT |
| H11 | GGACCAGGGGAATCCGAC | CGCTTCATTGAATTTCTTCAC |
| H13 | ACCTGGCGCTAAACCATTCGT | GGACAAACCCTTGTGTCGAGG |
| H18 | GTTGACGTACAGGGTGGACTG | GGAAGTTGTCTTCACGCCTGA |
| H27 | CCTTCCACGAGAGTGAGAAGCG | CTCGACCTCCCGAAATCGTACA |

| | |
|---|---|
| MCS-BamH1_forward | TC**GGATCC**ACTTCAAGAACCTGATCTGGC |
| MCS-BamH1_reverse | AGC**GGATCC**CTATGCGTAATCCGGTACATC |

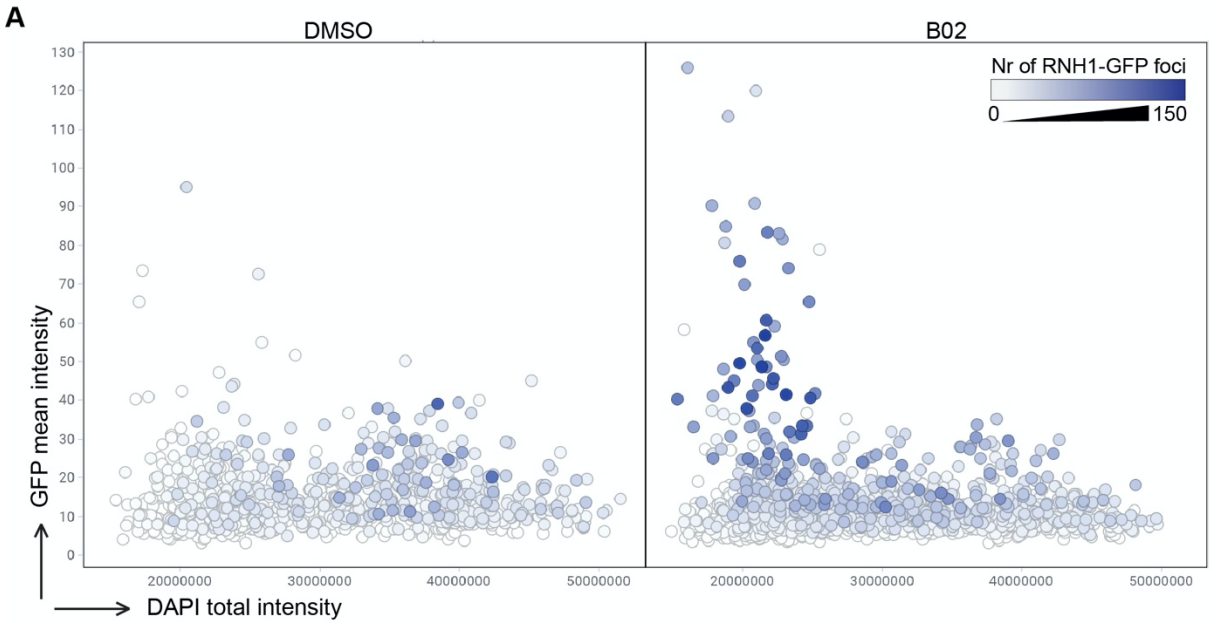


**Figure S1. B02-induced R-loop formation in asynchronous cell population**

(**A**) U-2-OS T-Rex [RNH1(D210N)-GFP] cells were treated with doxycycline (1 ng/ml) for 24 h. B02 (20 μM) was added for the last 6 h of doxycycline treatment. Cell were pre-extracted before fixation, counterstained with DAPI and subjected to image-based quantification of GFP intensity and number of GFP foci per cell. The cell cycle distribution was evaluated based on the DAPI intensity and is shown on x axis and GFP mean intensity is shown on y axis. The number of RNH1(D210N)-GFP foci per cell is increasing with an increasing intensity of blue color.

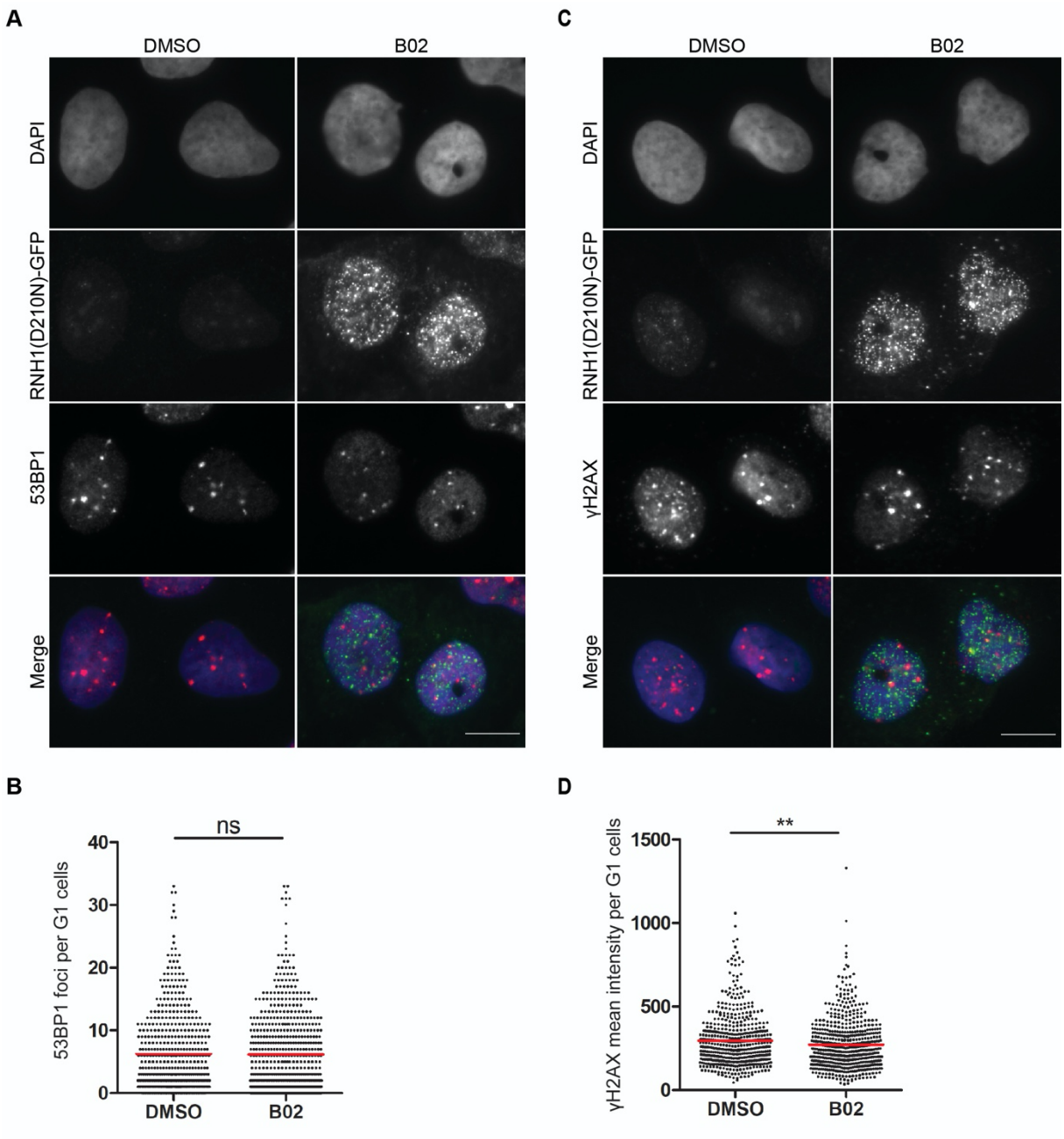


**Figure S2. DNA damage markers upon B02 treatment**

(**A-D**) U-2-OS T-Rex [RNH1(D210N)-GFP] cells were treated with doxycycline (1 ng/ml) for 24 h, and nocodazole (100 ng/ml) for the last 20 h of doxycycline treatment. B02 (20 μM) was added for 3 h post-release. Cells were pre-extracted before fixation and subjected to immunostaining of DNA damage markers including 53BP1 and γH2AX. Representative images are shown in (**A**) and (**C**) alongside with the image-based quantification of number of 53BP1 foci (**B**) and γH2AX intensity (**D**) per G1 cell. Scale bar in (**A**) and (**C**) represents 10 μm. Data in (**B**) and (**D**) are pooled from 3 individual experiments. Statistical significance was determined using Mann-Whitney test (**** $p<0.0001$, *** $p<0.005$, ** $p<0.01$, * $p<0.05$, ns, not significant).

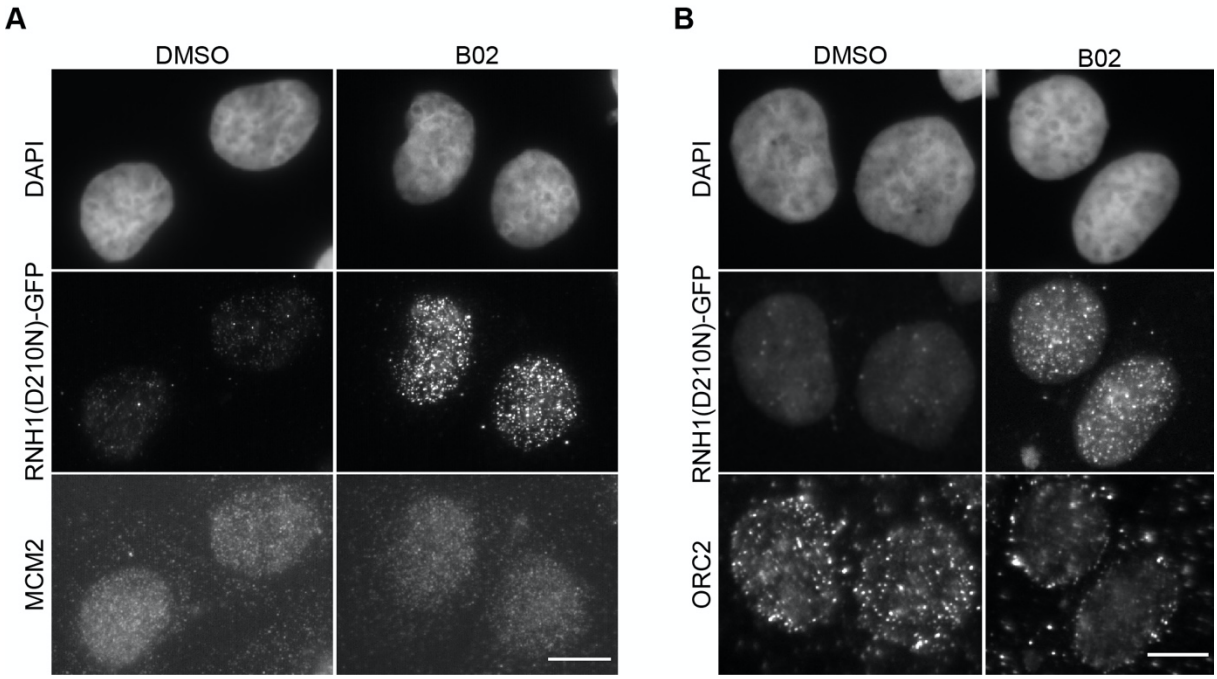


**Figure S3. Staining of replication origin licensing proteins**

(**A-B**) U-2-OS T-Rex [RNH1(D210N)-GFP] cells were treated with doxycycline (1 ng/ml) for 24 h, and nocodazole (100 ng/ml) for the last 20 h of doxycycline treatment. B02 (20 μM) was added for 3 h post-release. Cells were pre-extracted before fixation and subjected to immunostaining of MCM2 in (**A**) or ORC2 in (**B**). Scale bar represents 10 μm.