

576 **Figure 1-figure supplement 1. Transcriptional inhibition after irradiation and**
577 **transcriptional restart after DNA repair in U2OS cells. A.** U2OS cells were either incubated
578 with EU or not, as indicated, and were irradiated (10 Gy) or not as indicated, followed by
579 detection of the EU by click chemistry of a fluorophore, and immunofluorescence staining of
580 gamma H2AX in the same cells and the DNA was detected by Hoechst staining.

581

582 **Figure 1-figure supplement 2. Confirmation of knockdown of HIRA (A) and H3.3 (B).** The
583 samples were from the same experiments shown in Figure 1.

584

585 **Figure 2-figure supplement 1. The mean intensities of the EU peaks shown in Figure 2D are**
586 **indicated.** The mean value of each sample was calculated by Flowjo software.

587

588 **Figure 3-figure supplement 1. Nascent transcript levels before and after 30 minutes of IR.**

589 The samples from two independent experiments were used for the EU-seq and analyses in Fig. 3
590 and 4.

591

592 **Figure 3-figure supplement 2. Screen shot from the UCSC browser of nascent transcripts.**

593 **A.** Nascent transcripts before and after IR over ribosomal DNA (rDNA). **B.** Nascent histone
594 transcripts before and after IR over histone cluster 1.

595

596 **Figure 3-figure supplement 3. Validation of nascent transcript levels of DEGs from EU-**

597 **RNA seq by real-time quantitative RT-PCR.** Samples from three independent experiments

598 were used for the analyses. Ct value is presented to show the absolute amounts of EU labeled

599 RNA transcripts from the same number of cells before and after 30 minutes of IR (10 gray).

600 Significant difference after IR compared to before IR are indicated by asterisks, where **

601 indicates $p < 0.01$, * indicates $p < 0.05$ and ns indicates non-significant by students T-test.

602

603 **Figure 5-figure supplement 1. CRISPR-Cas9 screen identifies genes promoting**

604 **transcriptional inhibition after IR.** A volcano plot of guide RNA changes between Eu high

605 cells and unsorted cells. Labeled genes are some of those that have $P_{\text{adjust}} \leq 10^{-6}$.

606

607 **Figure 5-figure supplement 2. Confirmation of knockdown of Nae1.** gEV is an empty vector.

608 The samples were from the same experiments shown in Figure 5D.

609

610 **Figure 6-figure supplement 1. Analysis of CUL4A and CUL4B depletion. A.** The western
611 blot shows the CUL4A and CUL4B levels from the experiment shown in Fig. 6B. Additionally
612 gRNAs were used to deplete CUL4A in cul4b cells and CUL4B in cul4a cells and their western
613 blot analyses are also shown. **B.** The EU analysis of these double depleted cells is shown. **C.** Cell
614 cycle analysis of the experiment shown in A and B and Fig. 6B.

615

616 **Figure 6-figure supplement 2. Cell cycle analysis of cells treated with MLN4924 and**
617 **RO3306.** MLN4924 or RO3306 treatment leads cell cycle arrest in G₂ phase.

618

619 **Figure 7-figure supplement 1. Quantification of cells with TOPBP1 expression** before and
620 after IR (10 Gy) in cells treated with MLN4924 and RO-3306. The samples were from the same
621 experiments shown in Fig. 7F, 7G.

622

623 **Supplementary File 1.** Nascent RNA profiles of each gene using EU RNA-seq.

624

625 **Supplementary File 2.** Significantly enriched GO terms for up-regulated gene after irradiation.

626

627 **Supplementary File 3.** Significantly enriched GO terms for down-regulated genes after
628 irradiation.

629

630 **Supplementary File 4.** Whole genome CRISPR-Cas9 screen detects the abundance of all
631 gRNAs and target genes for EU high cells and unsorted cells.

632

633 **Figure 1-figure supplement 2 Source Data 1.** Original file for the Western blot analysis in
634 Figure 1-Figure Supplement 1A (anti-HIRA and anti-GAPDH).

635

636 **Figure 1-figure supplement 2 Source Data 2.** PDF containing Figure 1-Figure Supplement 1A
637 and original scans of the relevant Western blot analysis (anti-CUL4A, anti-CUL4B and anti-
638 GAPDH) with highlighted bands and sample labels.

639

640 **Figure 1-figure supplement 2 Source Data 3.** Original file for the Western blot analysis in
641 Figure 1-Figure Supplement 1B (anti-H3.3 and anti-GAPDH).

642

643 **Figure 1-figure supplement 2 Source Data 4.** PDF containing Figure 1-Figure Supplement 1B
644 and original scans of the relevant Western blot analysis (anti-H3.3 and anti-GAPDH) with
645 highlighted bands and sample labels.

646

647

648 **Figure 5-figure supplement 2 Source Data 1.** Original file for the Western blot analysis in
649 Figure 5D (anti-Nae1 and anti-GAPDH).

650

651 **Figure 5-figure supplement 2 Source Data 2.** PDF of Western blot analysis in Figure 5D and
652 original scans of the relevant Western blot analysis (anti-Nae1 and anti-GAPDH) with
653 highlighted bands and sample labels.

654

655 **Figure 6 Source Data 1.** Original file for the Western blot analysis in Figure 6A (anti-CUL4A,
656 anti-CUL4B and anti-GAPDH).

657

658 **Figure 6 Source Data 2.** PDF containing Figure 6A and original scans of the relevant Western
659 blot analysis (anti-CUL4A, anti-CUL4B and anti-GAPDH) with highlighted bands and sample
660 labels.

661

662 **Figure 6 Source Data 3.** Original file for the Western blot analysis in Figure 6C (anti-CUL4B,
663 and anti-GAPDH).

664

665 **Figure 6 Source Data 4.** PDF containing Figure 6C and original scans of the relevant Western
666 blot analysis (anti-CUL4B and anti-GAPDH) with highlighted bands and sample labels.

667

668 **Figure 6-figure supplement 1 Source Data 1.** Original file for the Western blot analysis in
669 Figure 6-figure supplement 1A (anti-CUL4A, anti-CUL4B and anti-GAPDH).

670

671 **Figure 6-figure supplement 1 Source Data 2.** PDF containing Figure 6-figure supplement 1A
672 and original scans of the relevant Western blot analysis (anti-CUL4A, anti-CUL4B and anti-
673 GAPDH) with highlighted bands and sample labels.

Figure 1-figure supplement 1

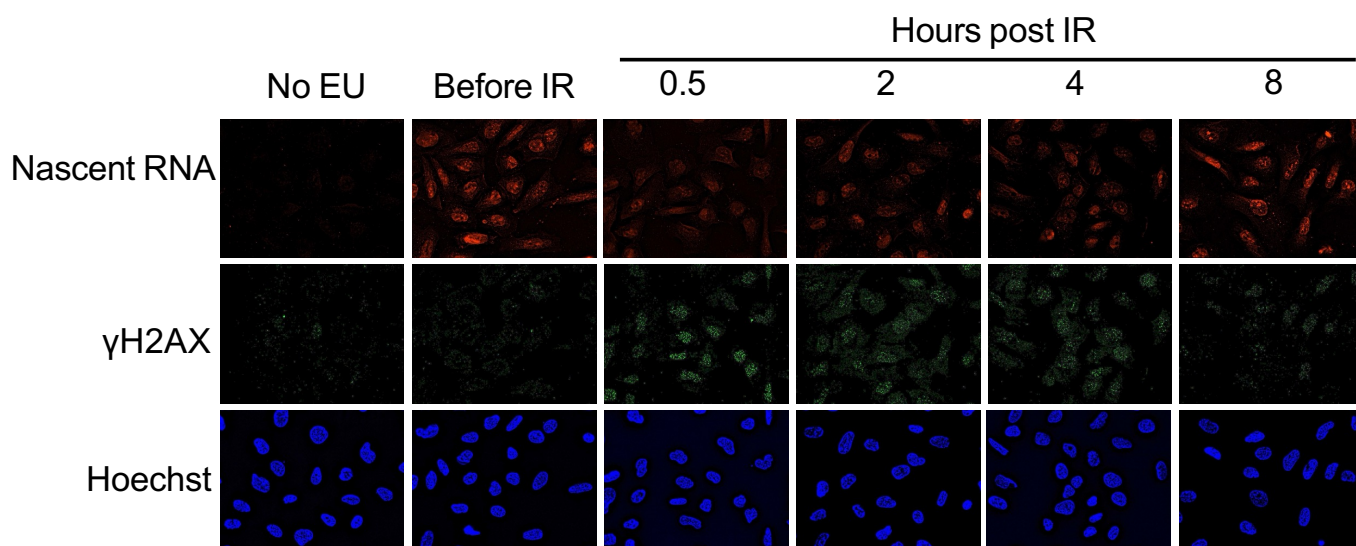
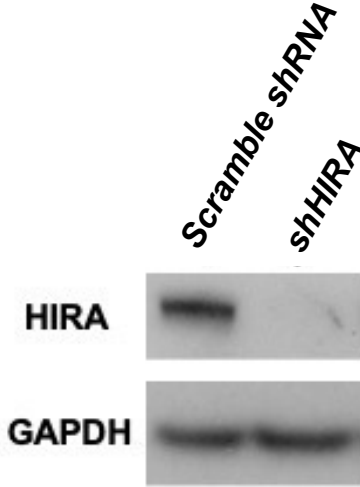


Figure 1-figure supplement 2

A



B

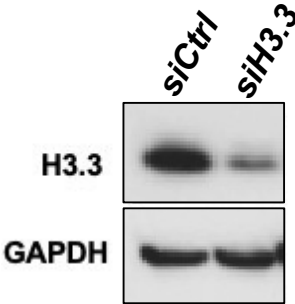


Figure 2-figure supplement 1

Dose	No EU	No IR	15 m	30 m	1 h	2 h	4 h
2 Gy	314	2690	1440	1261	1282	1640	2349
5 Gy	314	2690	1379	1169	1101	1345	2004
10 Gy	314	2690	1224	1094	1053	1347	1803

Figure 3-figure supplement 1

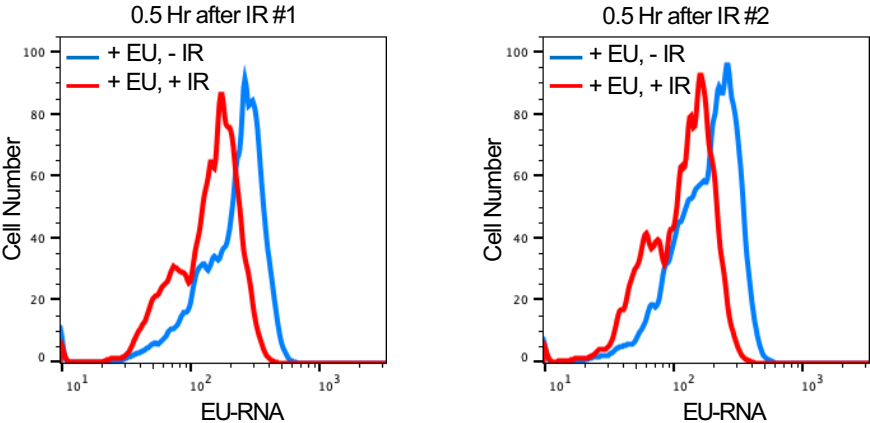


Figure 3-figure supplement 2

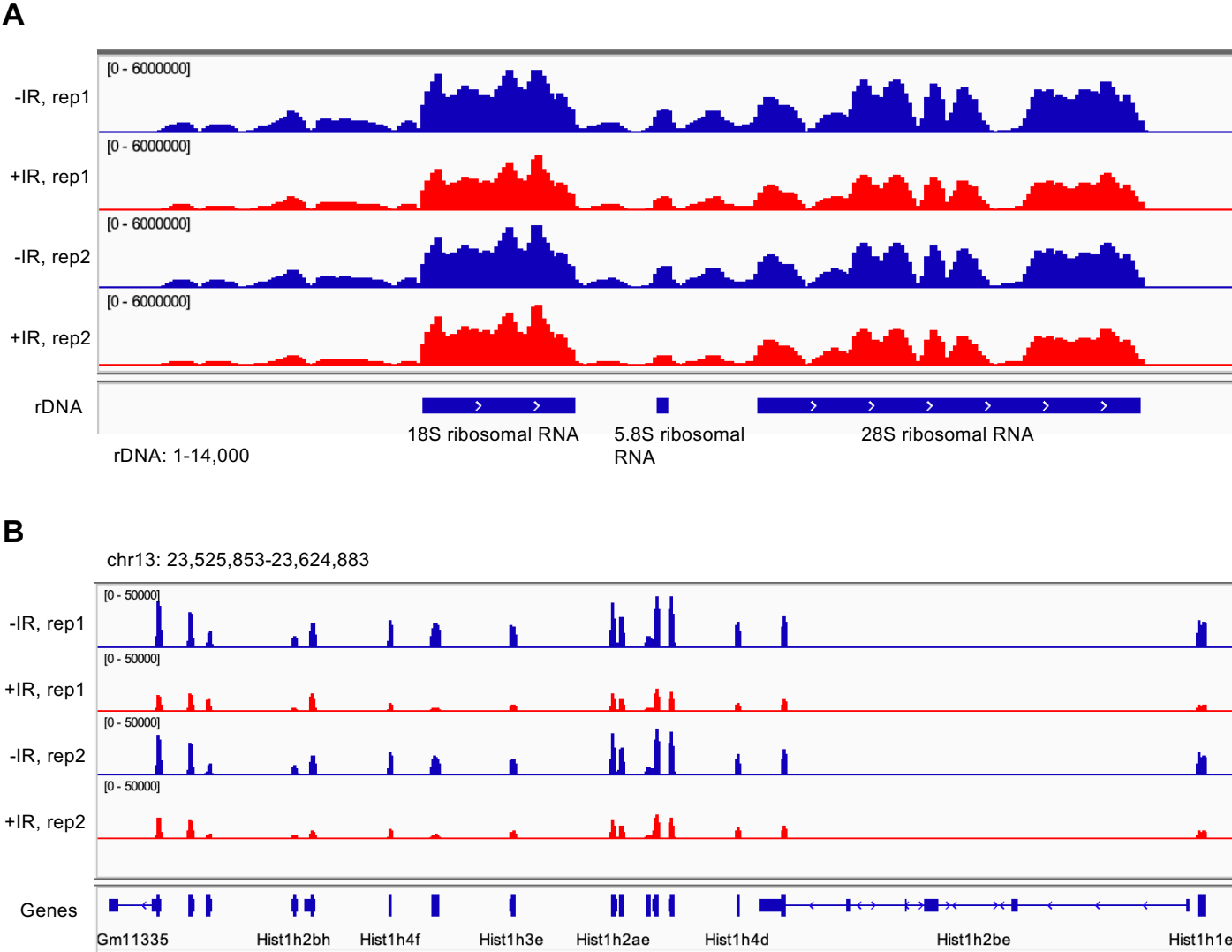


Figure 3-figure supplement 3

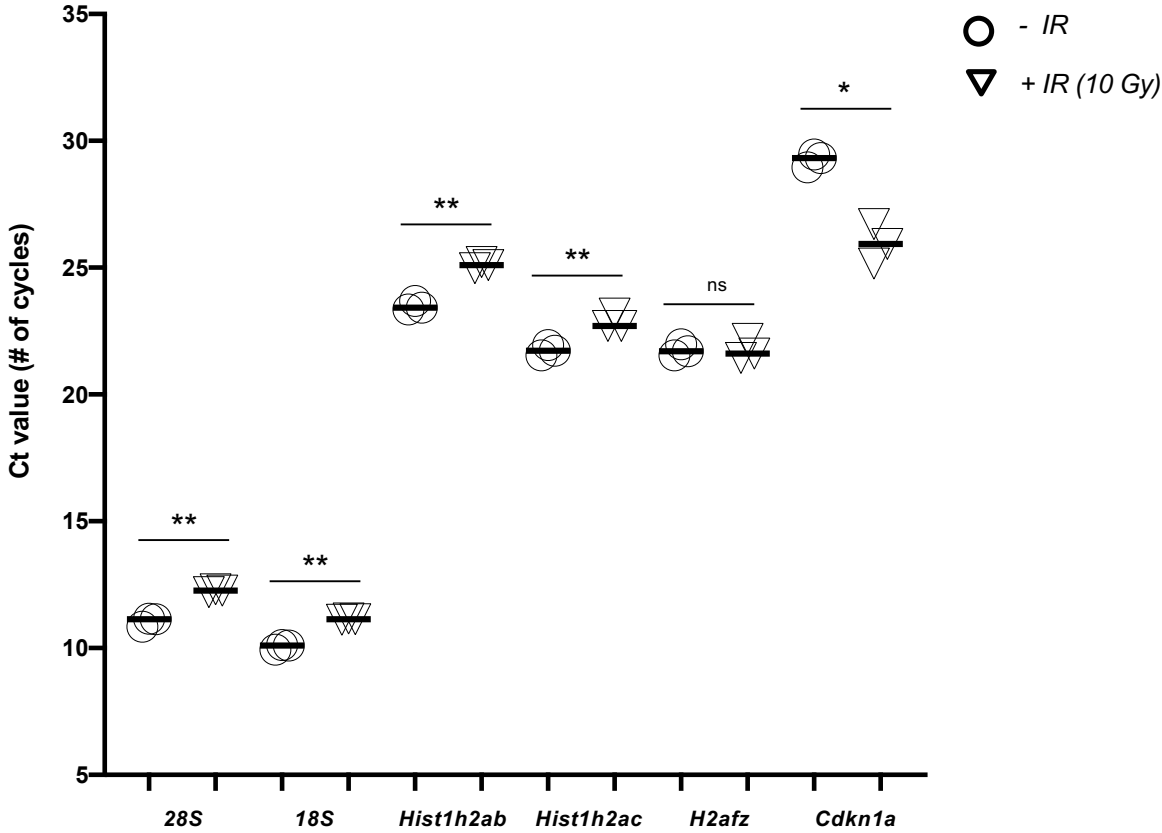


Figure 5-figure supplement 1

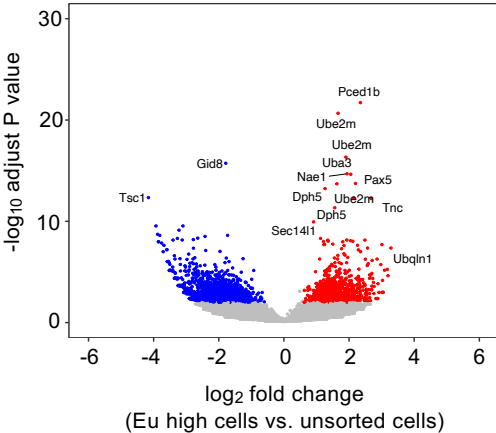


Figure 5-figure supplement 2

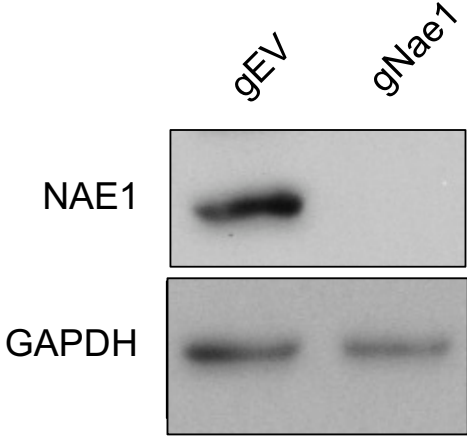
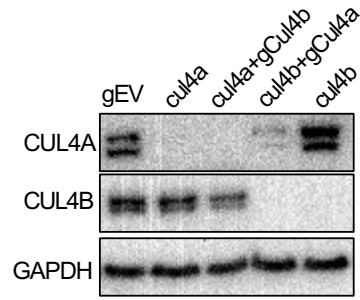
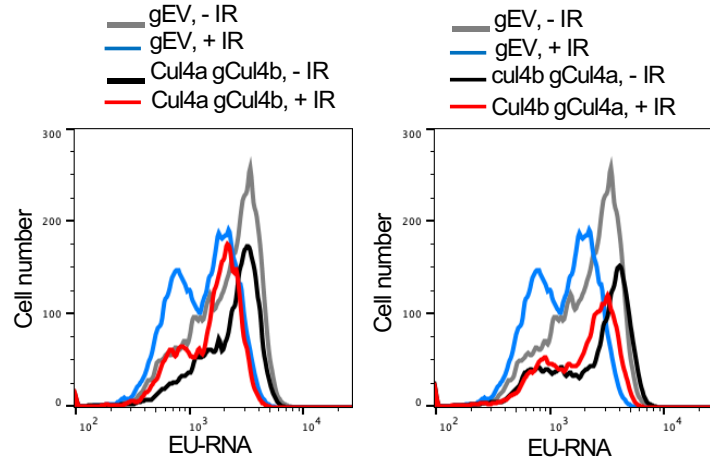


Figure 6-figure supplement 1

A



B



C

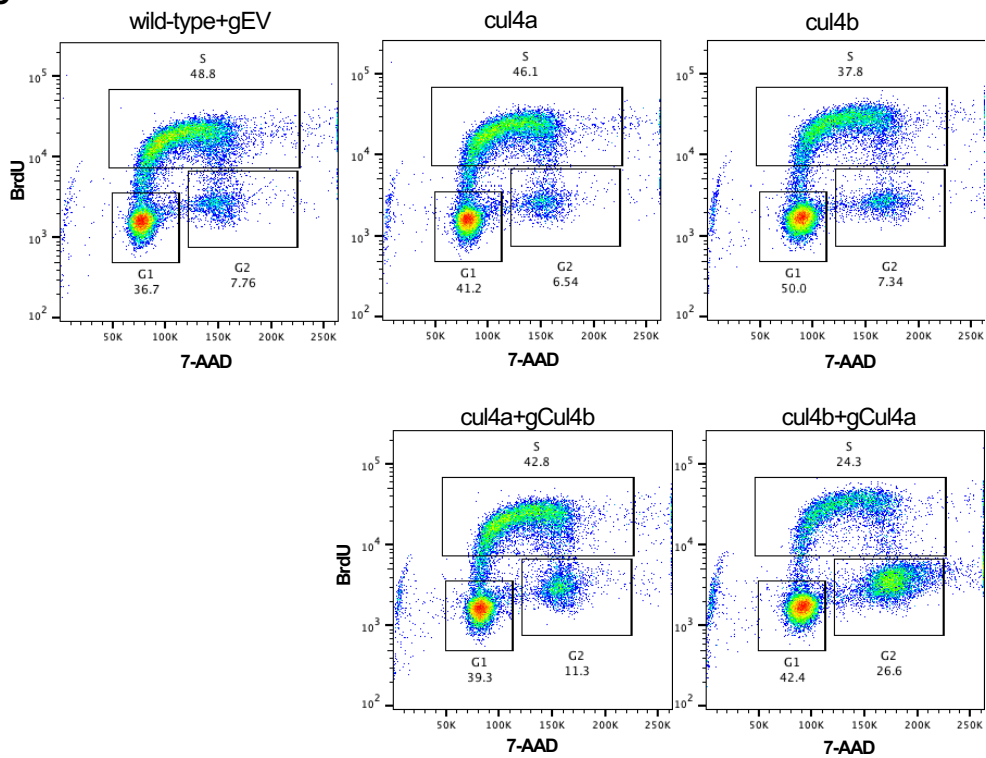


Figure 6-figure supplement 2

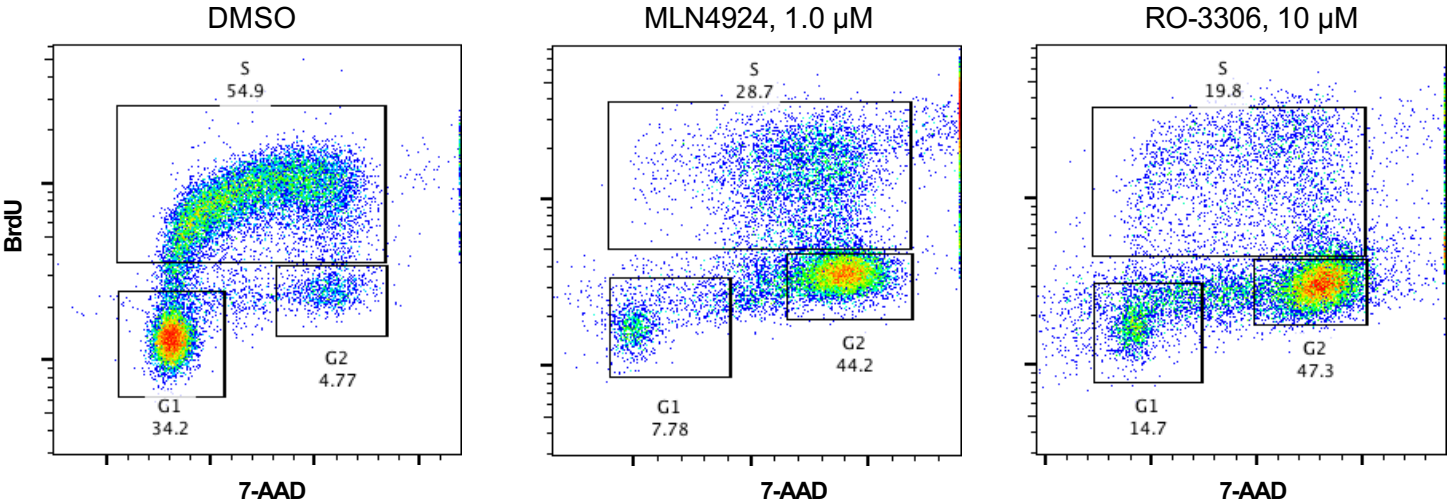


Figure 7-figure supplement 1

