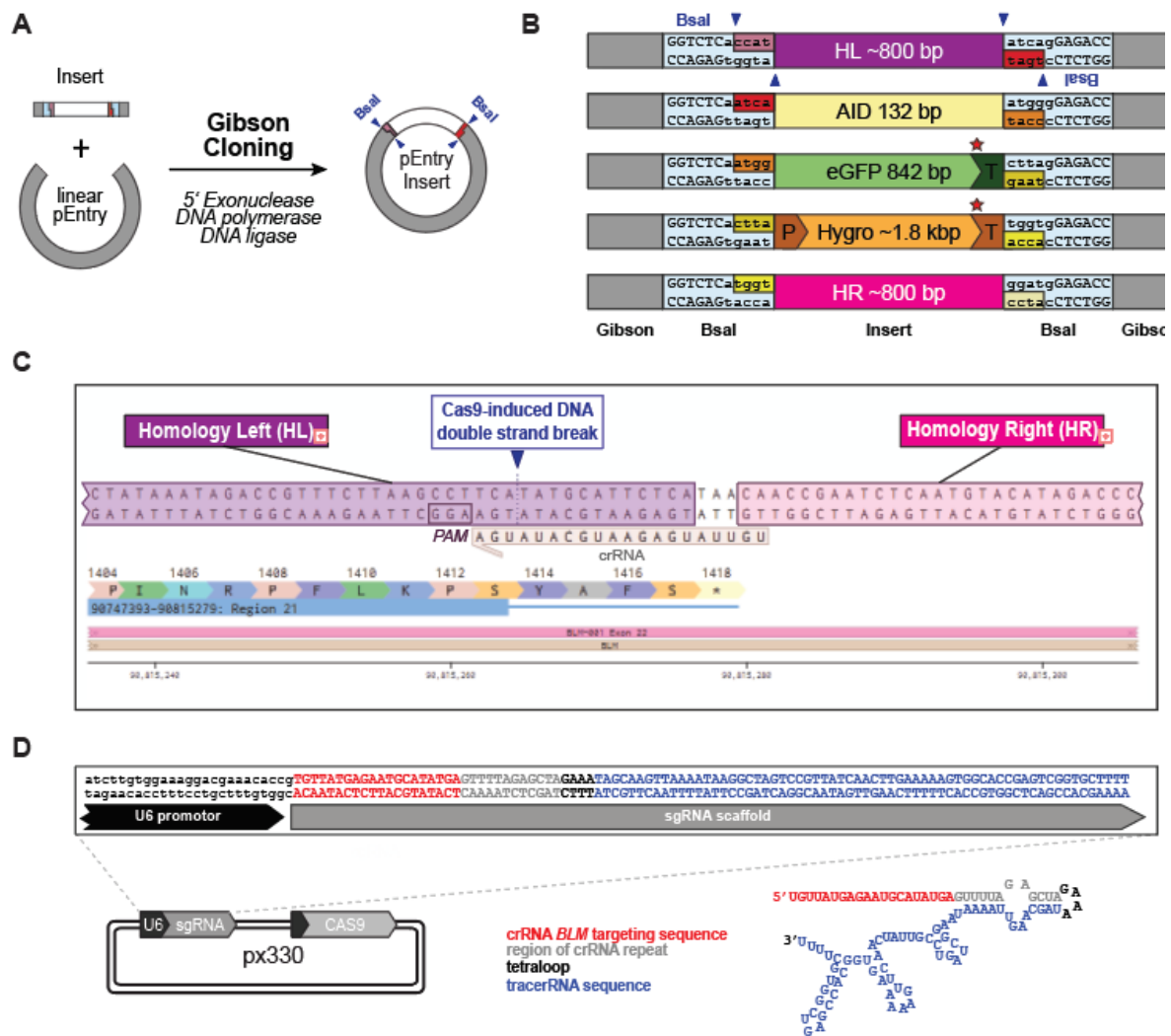
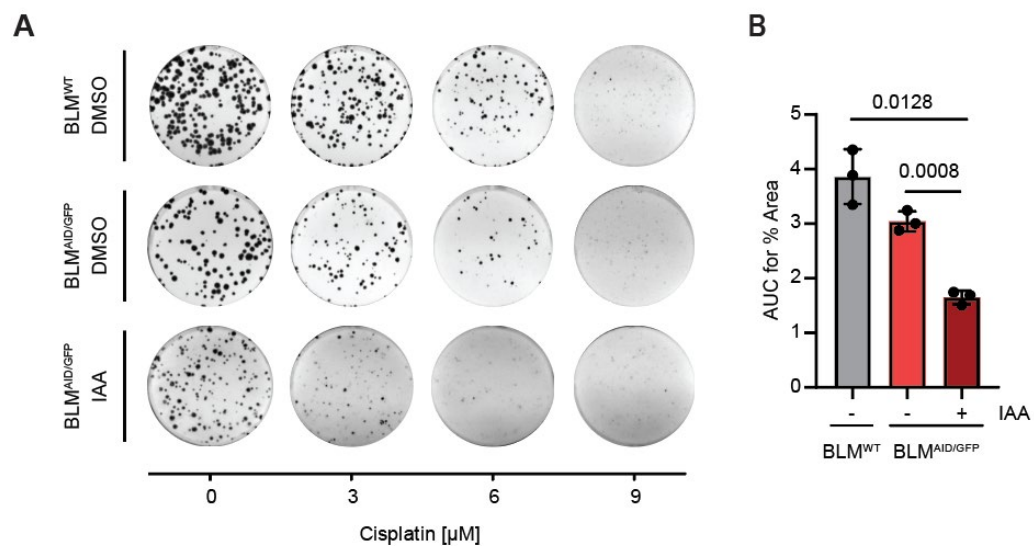


Supplementary data



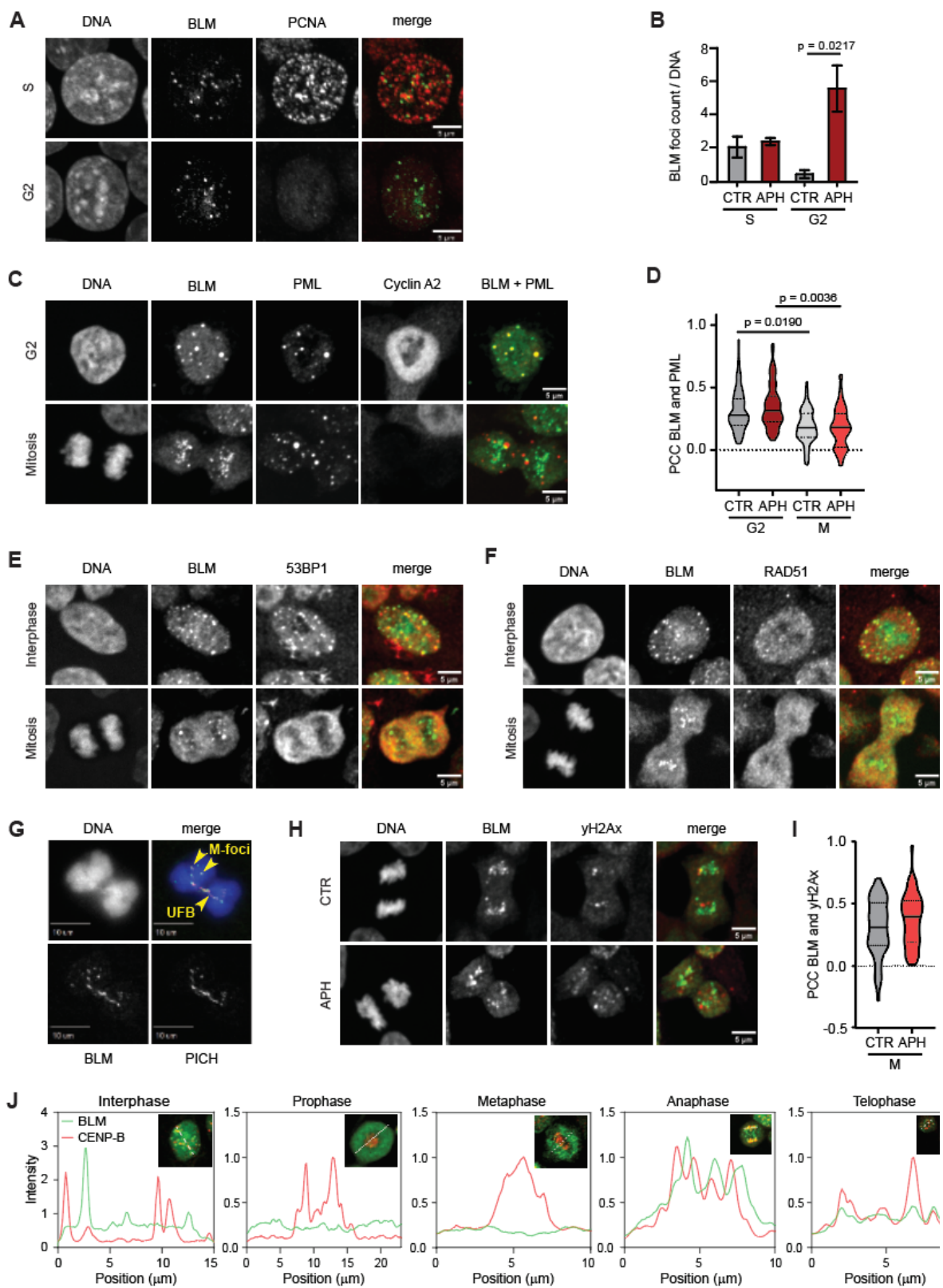
Supplementary Figure 1. CRISPR/Cas9 gene editing of BLM for endogenous AID-eGFP tagging.

(A) Schematic illustration of the entry plasmid cloning. (B) Individual inserts of the pEntry vectors. HL – homology left, HR – homology right, P – promoter, T – terminator, Gibson overhangs used for the Gibson cloning are shown in gray. Red stars indicate stop codons, blue arrows indicate cuttings sites of the Bsal restriction endonuclease. See Material and Methods for details. (C) Benchling view of the genomic *BLM* locus around the stop codon depicting the design of the sgRNA. (D) Schematic of the px330 plasmid with the cloned sgRNA targeting *BLM* and providing Cas9.



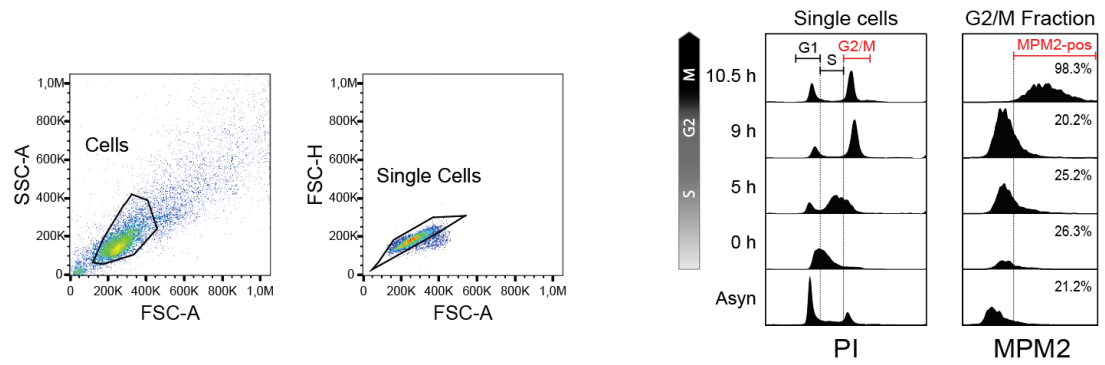
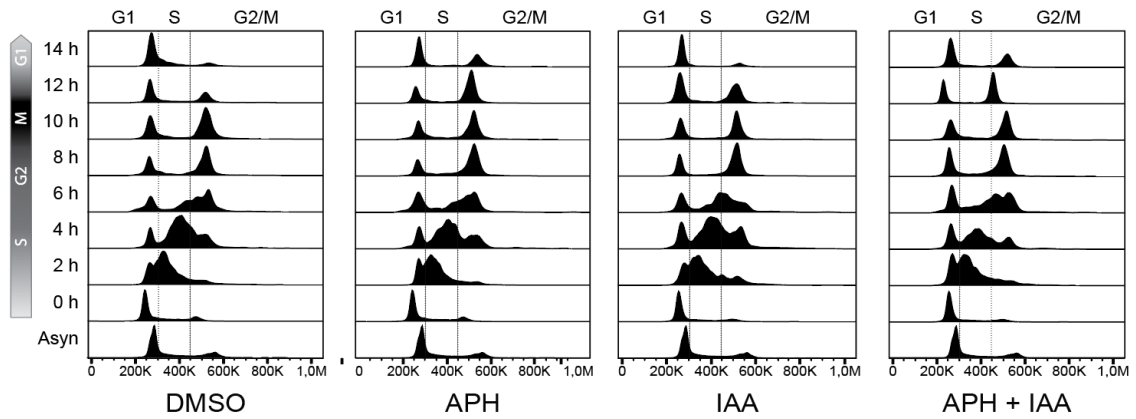
Supplementary Figure 2. Cisplatin sensitivity of parental HCT116 and BLM AID/GFP cells

(A) Clonogenic assay (B) Quantification of the area under the curve (AUC) for Fig 1G. AUC was calculated for all three biological replicates individually. Student's t-test with Welch's correction, mean \pm SD is shown, n = 3



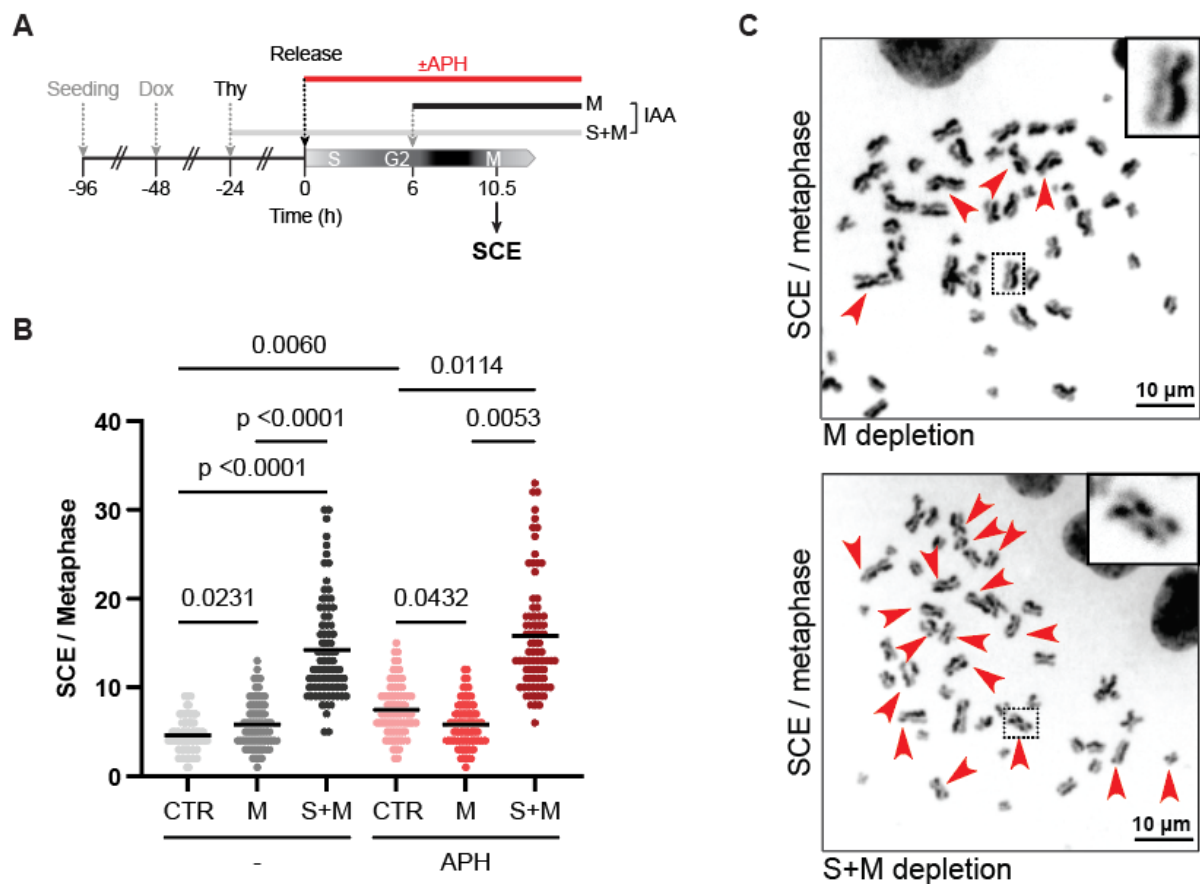
Supplementary Figure 3. Characterization of BLM foci in individual cell cycle phases.

(A) Representative microscopy images of fixed HCT116^{BLM-AID/GFP} cells stained with anti-PCNA antibodies. (B) Quantification of BLM foci in S (positive for PCNA staining) and G2 cells as indicated. (C) Representative microscopy images of BLM and PML co-localization in G2 and M cells. (D) Pearson correlation coefficient of BLM and PML signals in cells shown in (C). Means and quantiles are shown as a black dotted line. 25 cell per biological replicate were evaluated, n = 3. (E) Representative microscopy images of BLM and 53BP1 co-localization in interphase and M cells. (F) Representative microscopy images of BLM and RAD51 co-localization in interphase and M cells. (G) Immunostaining of fixed parental HCT116 cells with antibodies raised against BLM and PICH (H) Representative microscopy images of BLM and γ H2AX co-localization in anaphase cells. (I) Pearson correlation coefficient of BLM and γ H2AX signals in anaphase cells. Means and quantiles are shown as a black dotted line. 25 cells per biological replicate were evaluated, n = 3. (J) Co-localization of BLM and CENPB in different cell-cycle phases. The fluorescence intensity is plotted along an arbitrary line randomly placed across the cell nucleus (see white dotted line in insets). In except (G) cells via the fluorescence of GFP.

A**B**

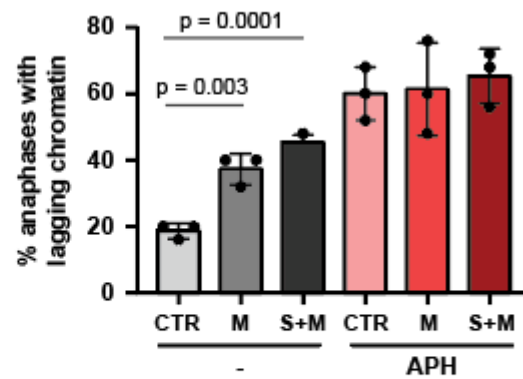
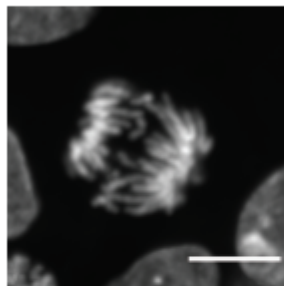
Supplementary Figure 4. Cell cycle phase specific depletion of BLM helicase.

(A) Flow cytometry analysis for determining cell cycle profiles of the BLM^{AID/GFP} cells released from single thymidine block (see Fig. 3A). G2/M cells were gated as shown and analyzed for binding the MPM2 antibody, which specifically recognizes mitotically phosphorylated proteins. (B) Cell cycle analysis of BLM^{AID/GFP} cells released from a single thymidine block into media containing DMSO, APH, IAA or a combination of APH and IAA. Note that IAA was added together with thymidine at the start of the synchronization ("S+M" depletion). IAA – auxin.

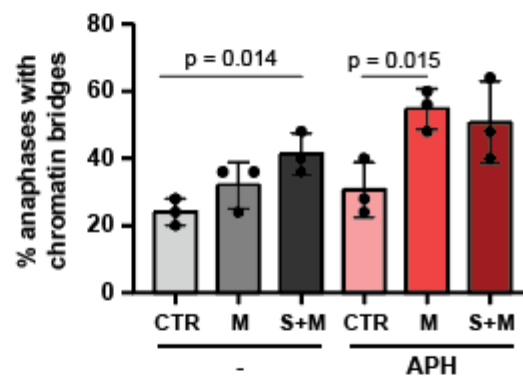
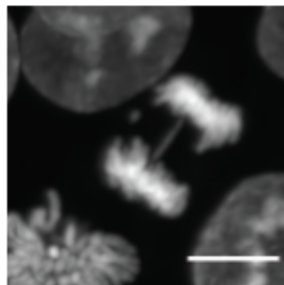


Supplementary Figure 5. “S+M” Depletion of BLM increases the rate of sister chromatid exchanges. **(A)** Synchronization scheme. **(B)** Quantification of sister chromatid exchange events observed on chromosome spreads of metaphase arrested cells (see Methods for details). Statistical significance was assessed using an unpaired Student’s t-test on three replicates, each containing measurements from 25 individual cells. Black bars represent the mean of three replicates (N=3). **(C)** Representative pictures of chromosome spreads from cells with continuous (“S+M”) or mitotic (“M”) depletion of the BLM helicase.

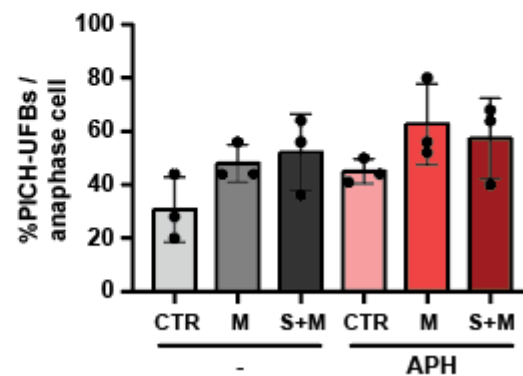
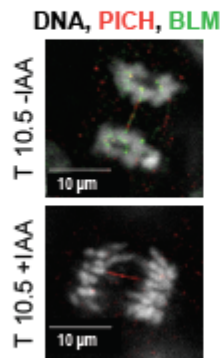
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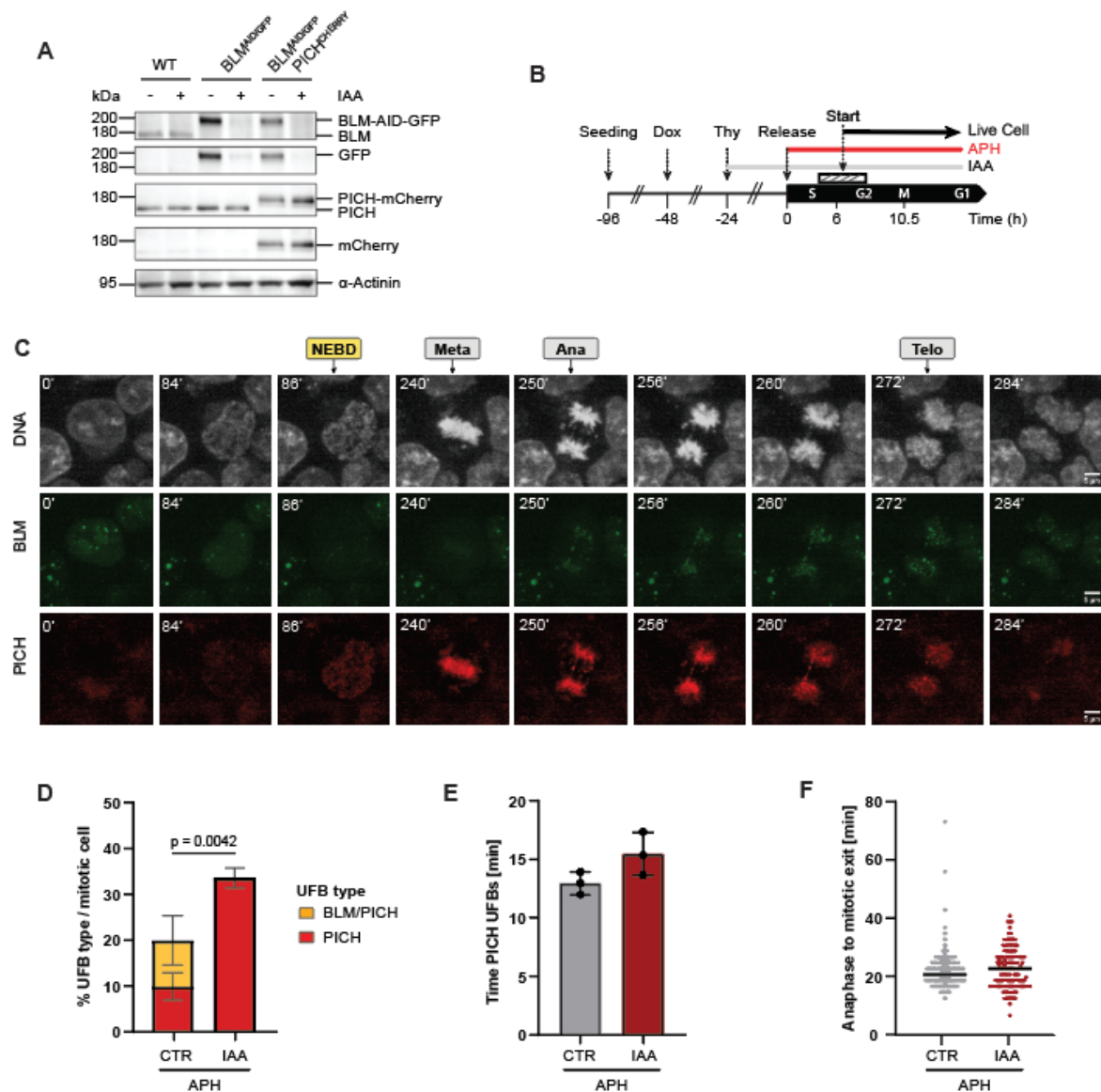


C



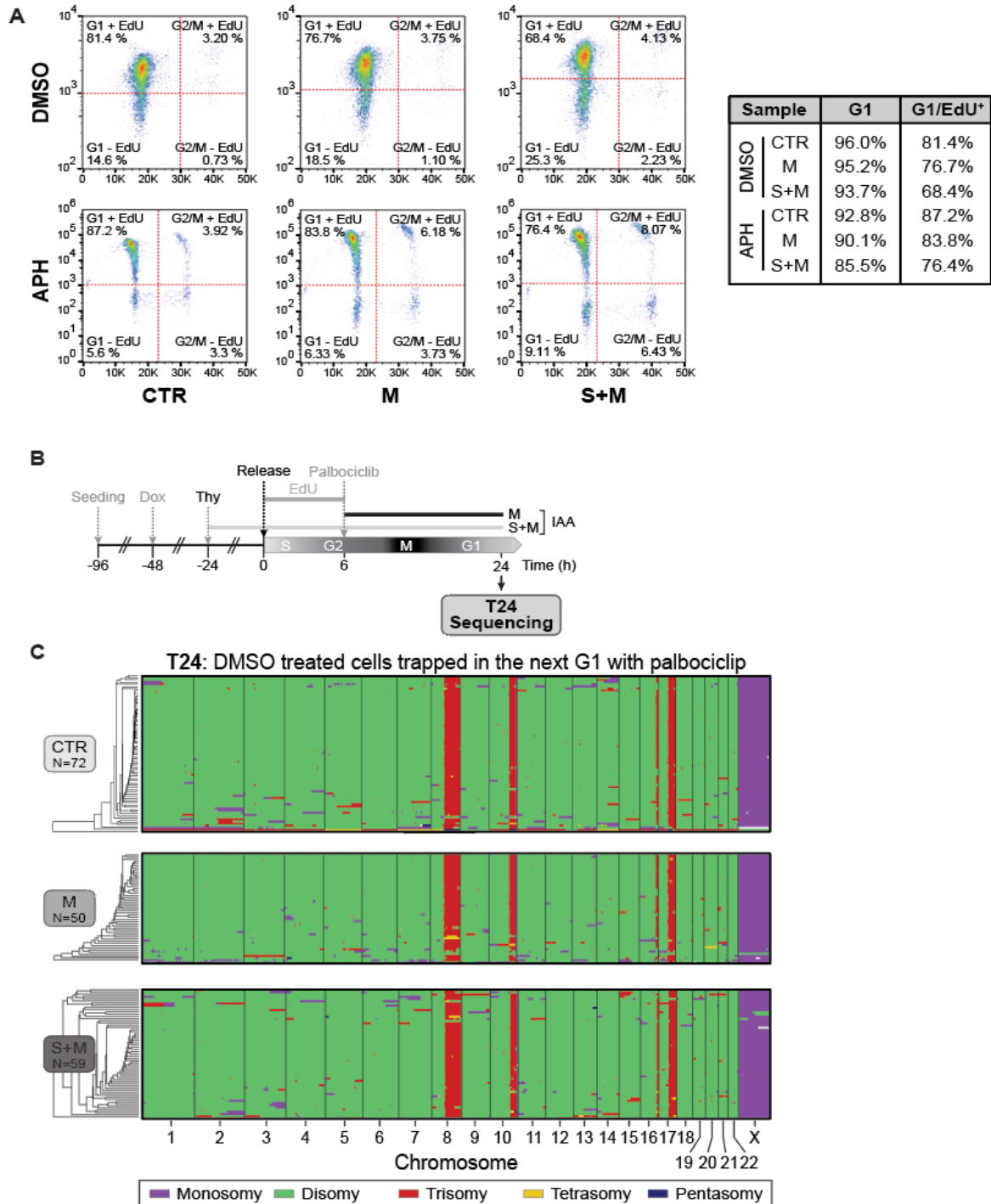
Supplementary Figure 6. Cell cycle-specific depletion of BLM increases the frequency of mitotic aberrations.

(A-C) Quantification of cells with lagging chromatin, chromatin bridges or PICH positive UFBs in control and BLM depleted cells. Cells were synchronized with a single thymidine block and released into media with or without 100 nM aphidicolin, as shown in Fig. 3A except that IAA was added 6 h after the thymidine release. 25 anaphase cells were counted in each of three independent experiments (N=3). Student's t-test, mean \pm SD is shown.

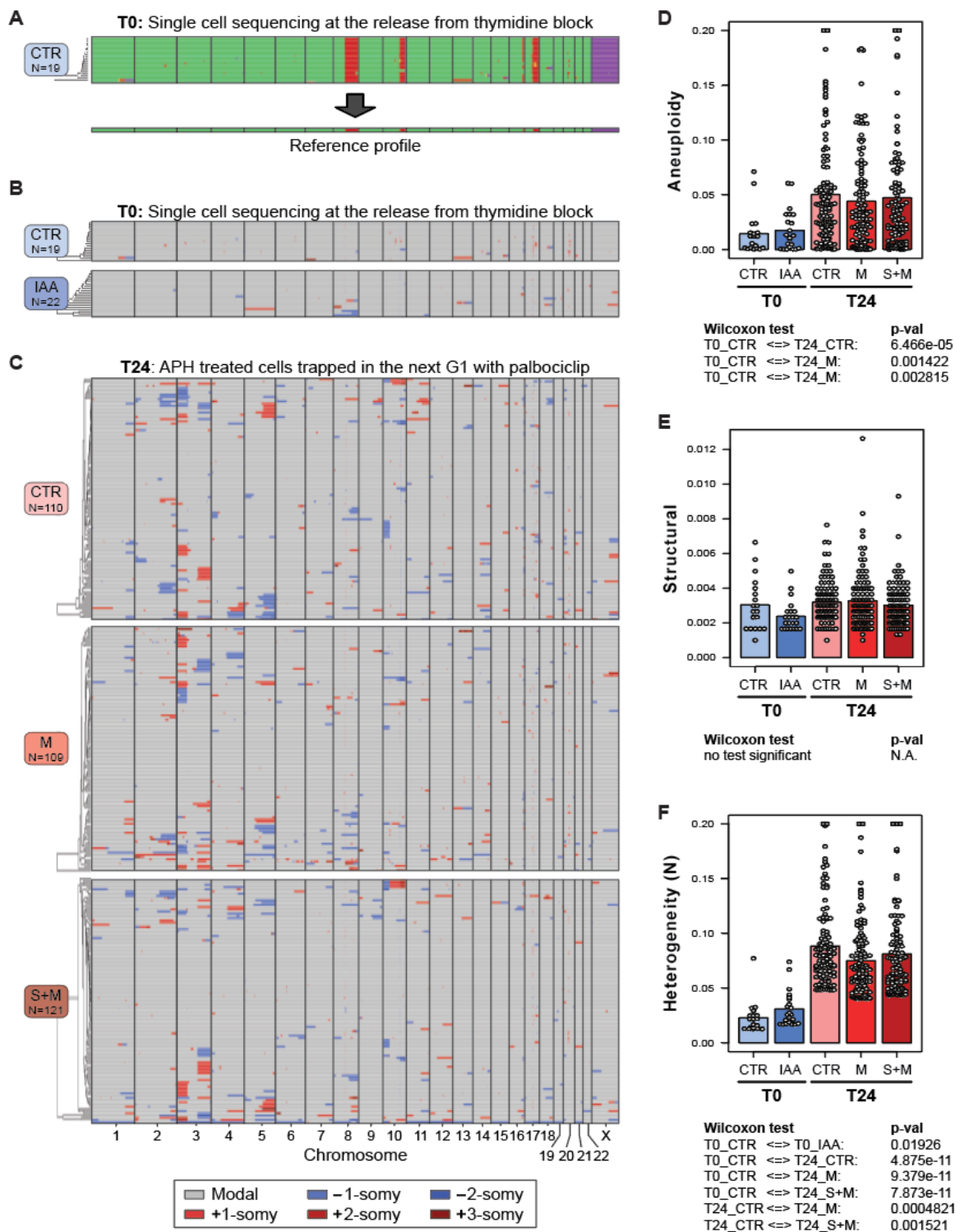


Supplementary Figure 8. Validation of mCherry tagging of the endogenous PICH protein in BLM^{AID/GFP} cells.

(A) Detection of untagged and tagged versions of BLM and PICH in the parental HCT116 cells, BLM^{AID/GFP} cells or BLM^{AID/GFP} with mCherry tagged PICH. Membranes were blotted with antibodies raised against BLM, GFP, PICH, mCherry or α-Actinin, respectively. α-Actinin served as a loading control. (B) Experimental scheme for the live cell microscopy experiment shown in (C). Imaging was started app. 6 h after the released from a single thymidine block (hashed bar). APH – aphidicolin, UFB - ultrafine bridges, MN – micronuclei, IAA – auxin, Thy – Thymidine, Dox – doxycycline. (C) Representative microscopy images from movie M3A-B. Movies were acquired from HCT116 cells expressing AID/GFP tagged BLM and mCherry tagged PICH treated as shown in (B). Labels indicate cells at nuclear envelope breakdown (NEBD), in metaphase (Meta), at the start of anaphase (Ana) or at the end of telophase (Telo). DNA was labeled with SPY 555, BLM and PICH were visualized via the fluorescence of eGFP and mCherry, respectively. (D) Fraction of anaphase cells with UFBs. More than 30 cells per biological replicate were analysed. Student's t-test, mean ± SD is shown, n = 3. IAA – auxin, APH - Aphidicolin. The student's t-test was calculated for the comparison between all PICH positive UFBs in control and IAA treated cells including also PICH/BLM double positive UFBs. (E) Average time for which the PICH UFBs persist. At least 20 anaphases in each biological replicate (n = 3) were analysed. Student's t-test, mean ± SD is shown. (F) Time cells spend from beginning of anaphase until mitotic exit. Mitotic exit was defined here as the time, when DNA appeared to be fully decondensed, as indicated in (A). More than 200 cells per replicate were evaluated. Student's t-test, mean ± SD is shown, n = 2.

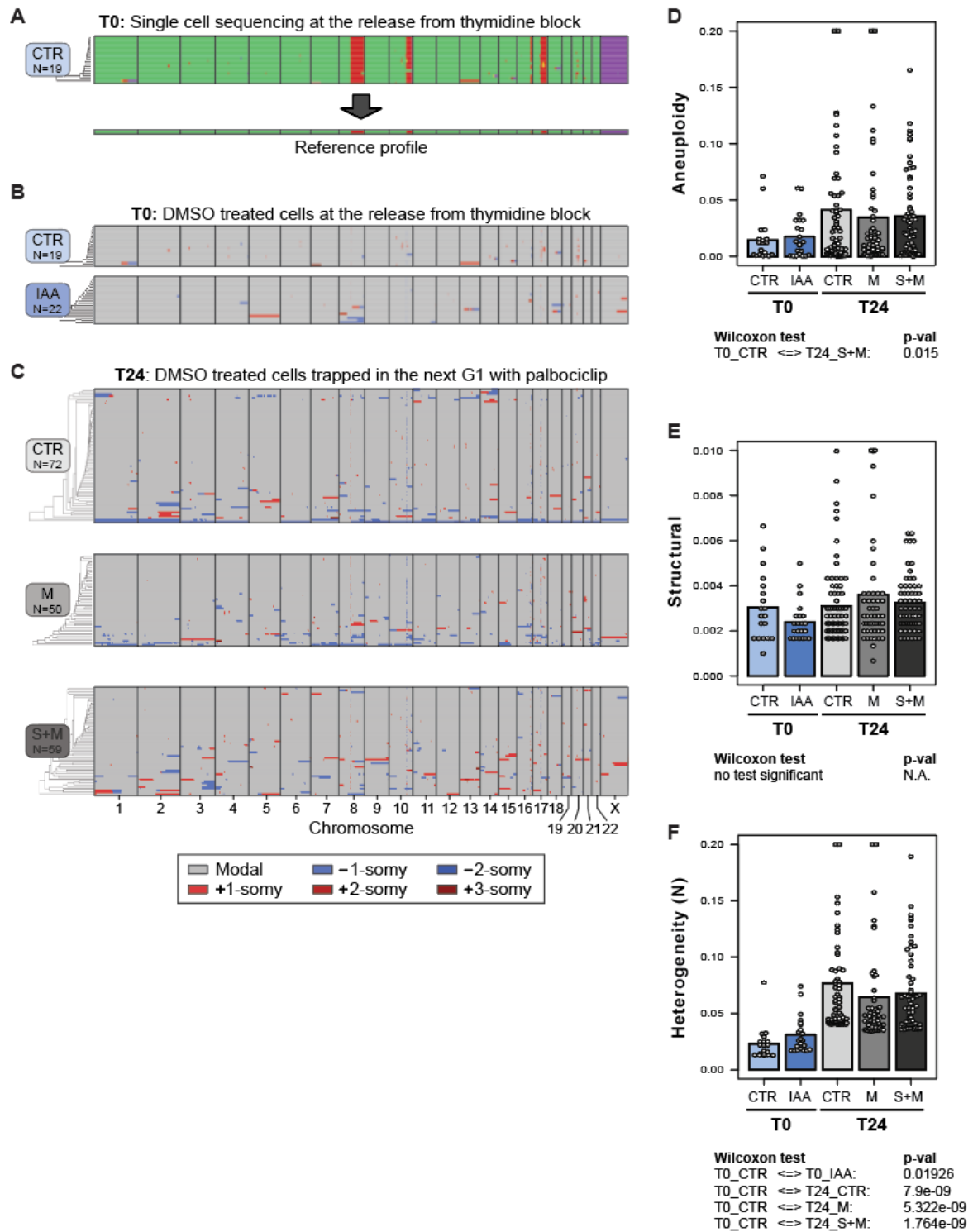


Supplementary Figure 9. EdU incorporation and WGS analysis of BLM depleted cells passing through an unperturbed S-phase. (A) Flow cytometry analysis of EdU incorporation in cells treated as shown in Fig. 6A and Supplementary Fig. 9B. The table on the right specifies the fraction G1 cells scoring positive for EdU. (B) Schematic illustration of the experimental strategy. IAA – auxin, Thy – Thymidine, Dox – doxycycline. (C). Genome-wide copy number plots generated by the Aneupfinder algorithm. Cells treated as shown in (A) were subjected to low coverage scWGS. Sequence reads were aligned to the human reference genome (GRCh38/hg38) and averaged over 1 MB non-overlapping bins. Note that the structural chromosomal changes within t(8p;18q), 10q+, t(9q;16p-) 16q and 17q, as well as chromosome Y loss are characteristic of HCT116.



Supplementary Figure 10. Re-analysis of scWGS data based on the alignment to T0 controls

(A) Schematic illustration of how the T0 controls were median averaged to obtain the reference profiles for DMSO and IAA treated samples, respectively. (B) (C). Genome-wide copy number plots generated by the Aneupfinder algorithm. Data shown in Fig. 6C and D was re-analysed. Sequence reads were aligned to the median average profiles of “T0 – IAA” and “T0 + IAA” and averaged over 1 MB non-overlapping bins. (D-F) Aneuploidy, heterogeneity and structural aberrations scores after re-analysis of the scWGS data (see Methods for details).



Supplementary Figure 11. Re-analysis of scWGS data based on the alignment to T0 controls

(A) Schematic illustration of how the T0 controls were median averaged to obtain the reference profiles for DMSO and IAA treated samples, respectively. (B) (C). Genome-wide copy number plots generated by the Aneupfinder algorithm. Data shown in Fig. 6C and D was re-analysed. Sequence reads were aligned to the median average profiles of “T0 – IAA” and “T0 + IAA” and averaged over 1 MB non-overlapping bins. (D-F) Aneuploidy, heterogeneity and structural aberrations scores after re-analysis of the scWGS data (see Methods for details).