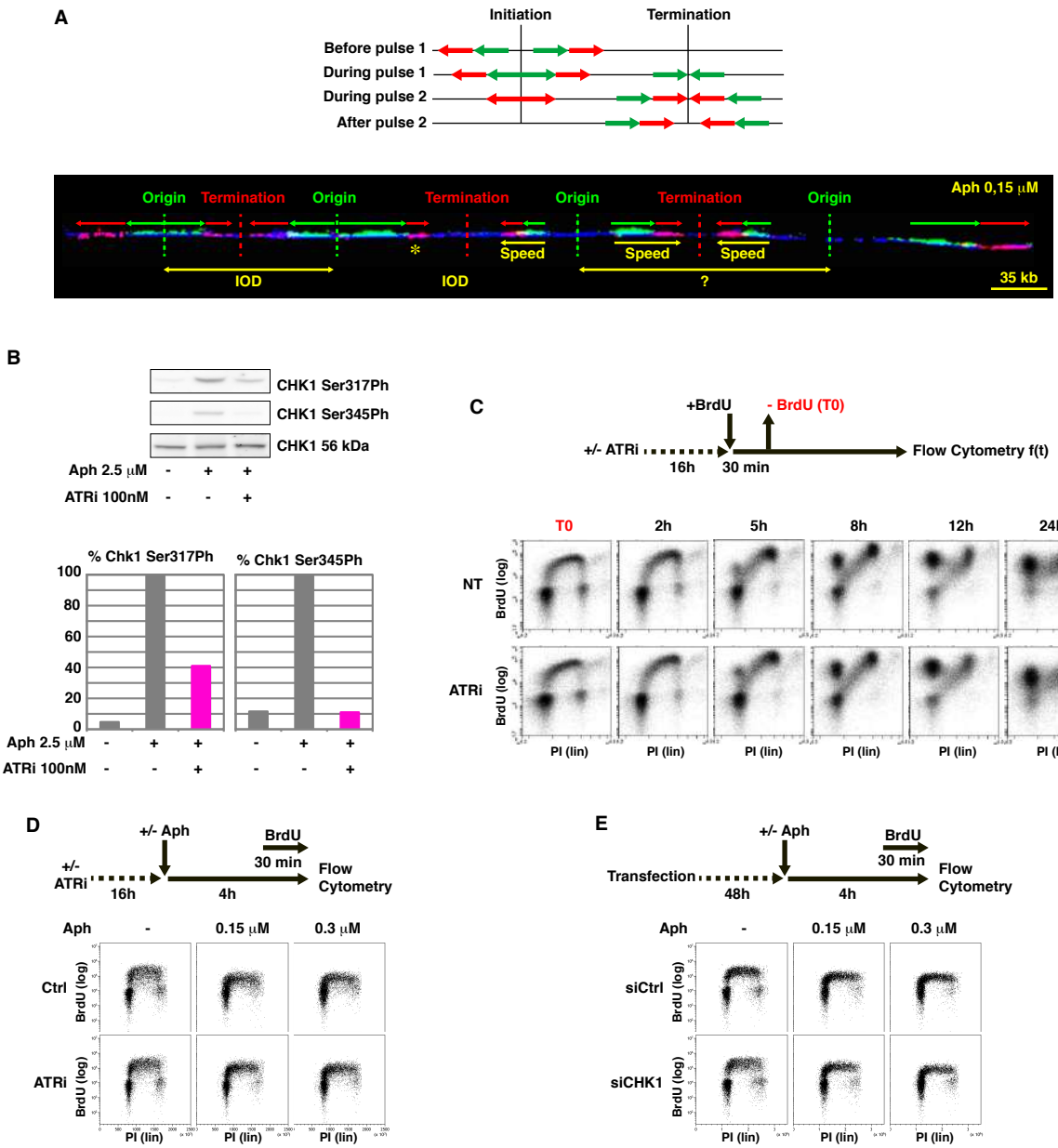

Figure S1: Compensation efficiency relies on ATR- but not CHK1-activation.
A: Schematic representation of IdU/CldU patterns observed in cells labeled as in Fig. 1A. Upper panel; schematic representation of signals identifying origins and terminations according to when the considered event had occurred relative to the pulses. Lower panel; typical example of molecules (DNA counterstaining in blue) observed in lymphoblasts treated with Aph 0.15 µM. The question-mark illustrates a situation in which the localization of the rightmost origin is too uncertain to permit reliable IOD measurement. ⁎ Example of an asymmetric fork. **B**: Western blot analysis and corresponding quantification showing how ATRi affects CHK1 phosphorylation on the indicated serines in response to treatment with a high Aph concentration (experiment done once). **C**: Upper panel; experimental procedure for time-course analysis of the impact of ATRi on cell cycle progression. Lower panels; flow-cytometry analysis of cells recovered at the indicated times after BrdU chase, then grown in fresh medium supplemented with ATRi when present at the beginning of the experiment. **D-E**: Cells treated as indicated (upper panels) were analyzed by flow-cytometry (lower panels), each experiment was done once.

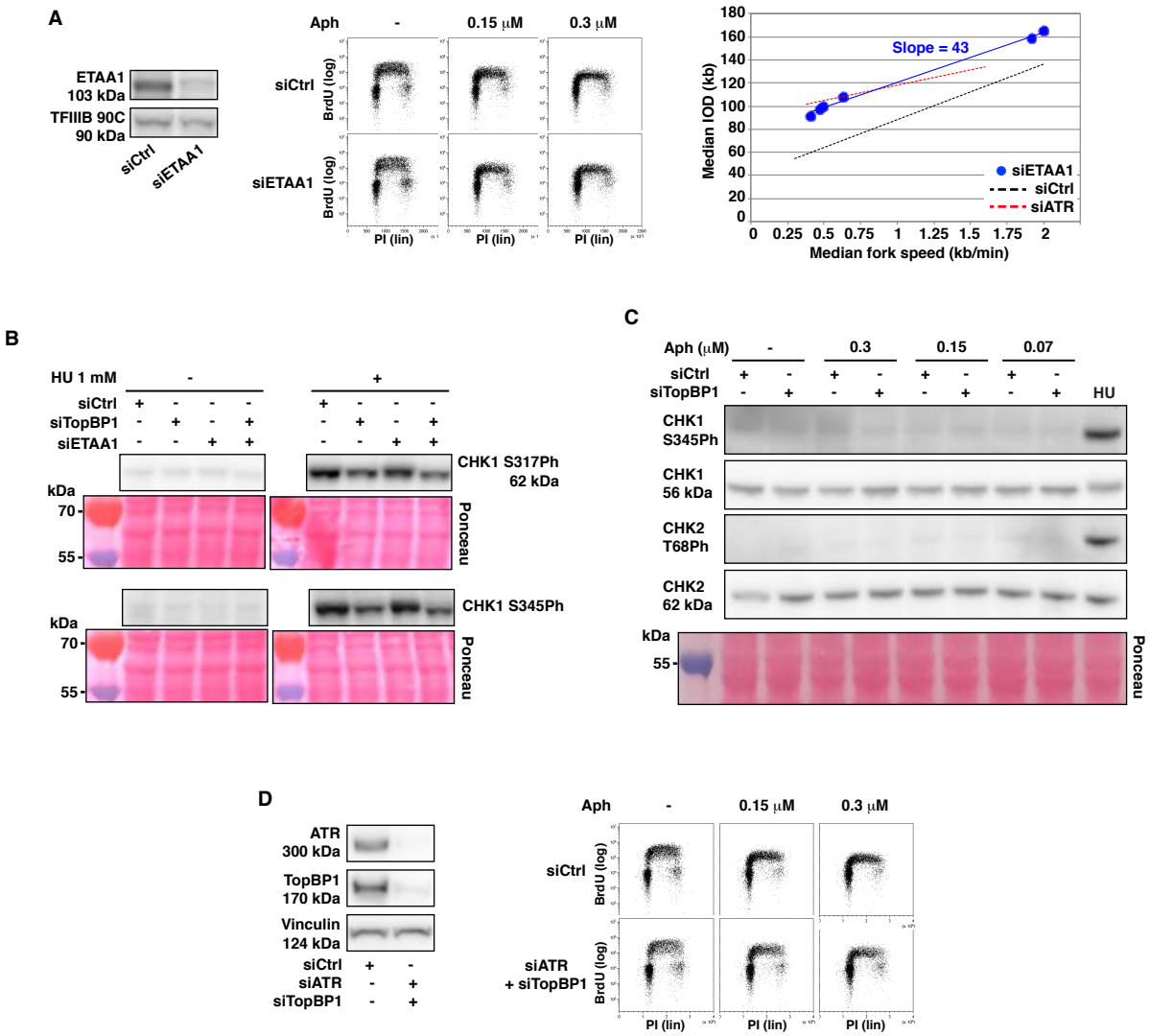

Figure S2: Impact of ETAA1 or TopBP1 depletion on compensation and DRC.
A: ETAA1 depletion does not impact compensation. Left panel; quality-control of ETAA1 depletion 48 hours after transfection. Middle panel; lymphoblasts transfected with the indicated siRNAs were treated with Aph as described in Figure 1D (upper panel) and analysed for cell cycle progression by flow-cytometry (experiment done once). Right panel; stressline of cells transfected with ETAA1 siRNAs (n: as in Fig. 1B, dotted stresslines: as in Fig. 1G). **B**: Impact of TopBP1 or/and ETAA1 depletion on full DRC activation. Western blots show the phosphorylation status of CHK1 at S317 and S345 in cells treated as in Fig. 2C. Ponceau staining is shown as loading control. A second biologically independent experiment gave consistent results (quantifications in Fig. 2C). **C**: Impact of TopBP1 depletion on loose DRC activation. Western blot showing the phosphorylation status of CHK1 and CHK2 in cells treated as described in Fig. 2D. Growth conditions, transfected siRNAs and phospho-sites are indicated. Normalization was done relative to the total amount of CHK1 or CHK2, respectively. HU is shown as positive control. A second biologically independent experiment gave consistent results (quantifications in Fig. 2D). **D**: Co-depletion of TopBP1 and ATR. Left panel; quality-control of the depletions, vinculin is shown as loading control. Right panel; flow-cytometry analysis of the impact of dual depletion on cell cycle progression of cells treated with Aph as in Fig. 2D (experiment done once).

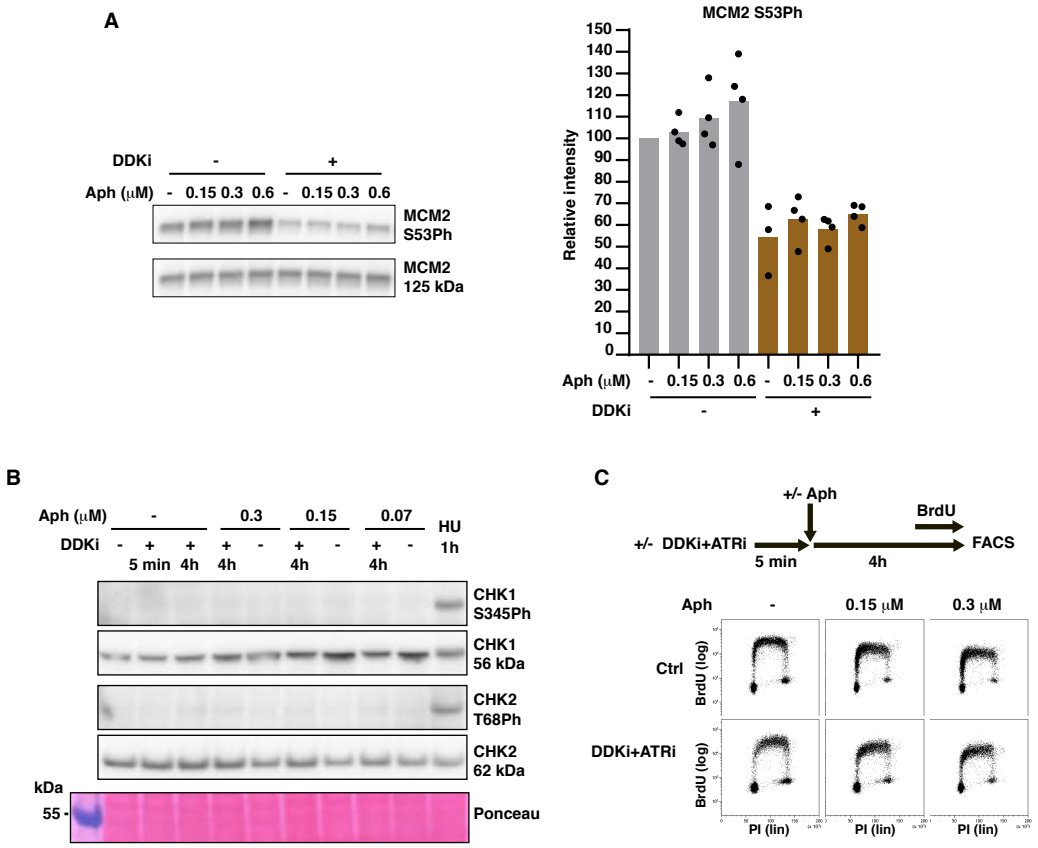

Figure S3: Impact of DDKi on cell fitness.

A: Left panel; chromatin extracts were prepared from lymphoblasts treated as described in Fig. 3C. Western blot shows DDKI dependent phosphorylation of MCM2 S53 in cells treated as indicated. The experiment has been done three or four times. Right panel; histogram showing signal intensities normalized relative to the total amounts of MCM2. **B**: Example of western blot related to Fig. 3D. Signal intensity of CHK1 phosphorylation on S345 and CHK2 on T68 was normalized relative to the total amount of each protein. Ponceau staining is shown. HU (1mM) is used as positive control. **C**: Upper panel; experimental scheme. Lower panels; flow-cytometry analysis of the impact of dual treatment with ATRi and DDKi on cell cycle progression of lymphoblasts treated with the indicated concentrations of Aph (experiment done once).

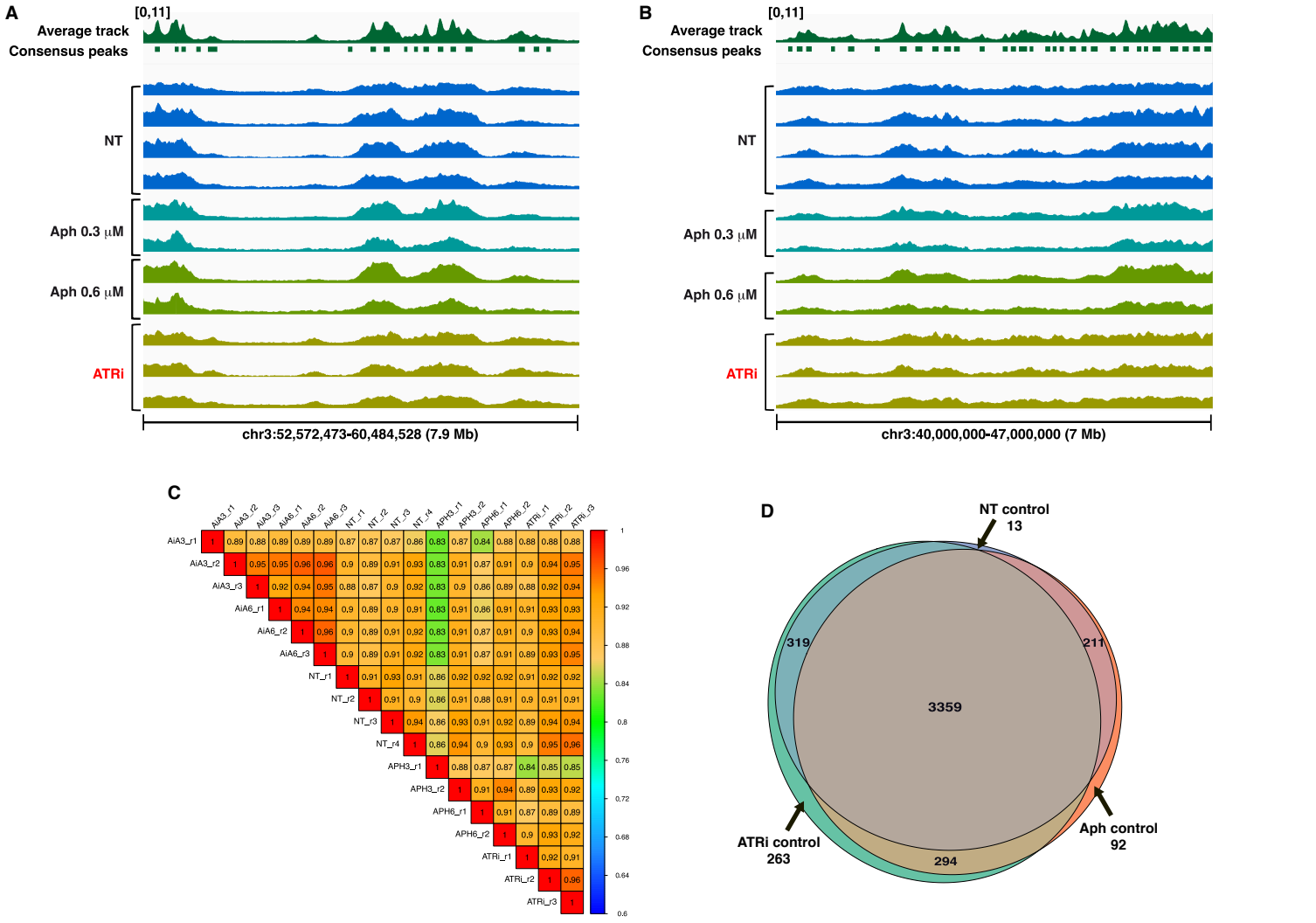

Figure S4 : Fork slowing reveals constitutive origins in ATR deficient cells.
A: Normalized density profiles of Repli-seq experiments (data processed and presented as in Fig 4C). G1/S1 fractions of untreated cells and of cells treated with Aph or ATRi alone along the genomic region shown in Fig. 4B. On top, the corresponding average tracks and consensus ATRAP-peaks are shown. **B**: As in A for the region presented in Fig. 4C. **C**: Spearman's correlation of pairwise comparison between G1/S1 Repli-seq fractions of all samples. AiA: ATRi+Aph samples. **D**: Comparison of the differentially enriched peaks resulting from the differential analyses between ATRAP-seq and indicated control samples. Differentially enriched ATRAP-peaks (p-value < 0.05, log2(FC) > 0.45) have been identified for each comparison, i.e. 6*ATRAP-seq vs. 4*NT (purple), 6*ATRAP-seq vs. 4*Aph (orange), 6* ATRAP-seq vs. 3*ATRi (green). Differentially enriched peaks are considered overlapping when at least half of either length is overlapping the other feature.

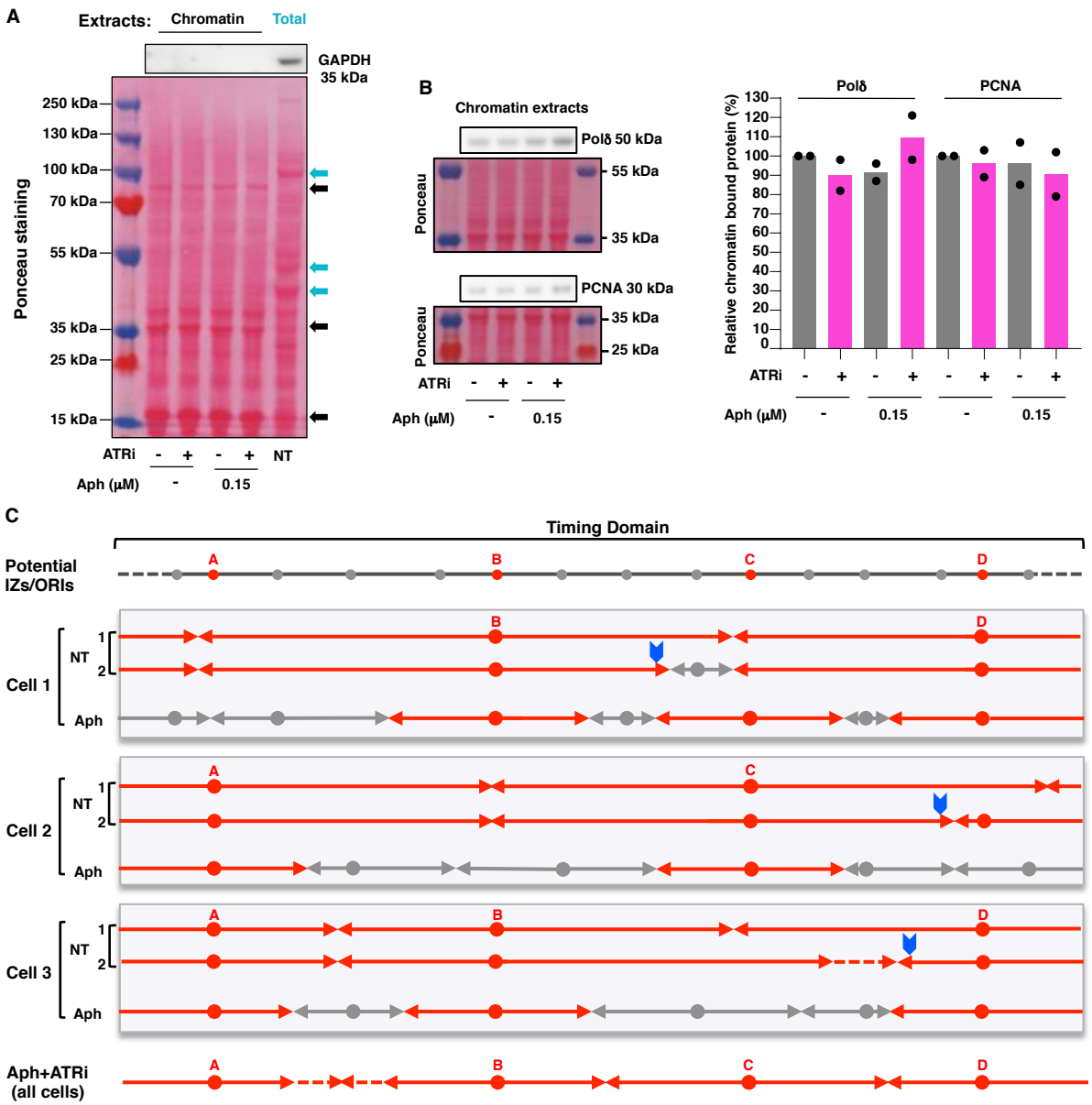

Figure S5: Interplay between passive and active processes controls origin activation.
A: Quality-control of chromatin preparations. GAPDH and Ponceau staining with examples of proteins which amount decreases (blue arrows) or increases (black arrows) in the chromatin fractions. **B:** Biologically independent duplicate of the experiment presented in Fig. 5E and quantification of signals from these replicates (expressed as % of the value found for corresponding protein in untreated cells). **C:** Model for active and passive compensation: A timing domain with four constitutive (red) and eleven dormant (grey) origins is schematized. Cells 1, 2, 3 illustrate cell-to-cell variability of origin usage in untreated cells (NT). NT1; forks progress unimpeded, NT2; one fork occasionally slows/blocks (blue mark). Activation of a dormant (cell 1) or a constitutive (cell 2) origin rescues replication. When no origin is adequately localized (cell 3), increased lifespan of the hosting-focus permits opposite fork to fill the gap (other mechanisms not illustrated here, like re-priming, can also complete replication[1]). Considering the number of domains per cell, the three situations coexist in each of them. Note that cells 2 and 3 solve the problem independently of ATR, *i.e.* passively. In cell grown in Aph, forks slow genome-wide and dormant origin firing becomes the prominent response. The probability of firing of a dormant origin increases with the distance separating it from the nearest constitutive origin. In cells grown in Aph+ATRi, most constitutive origins fire and remaining gaps are filed as in cell 3 (NT2), slowing the pace of later domain activation.