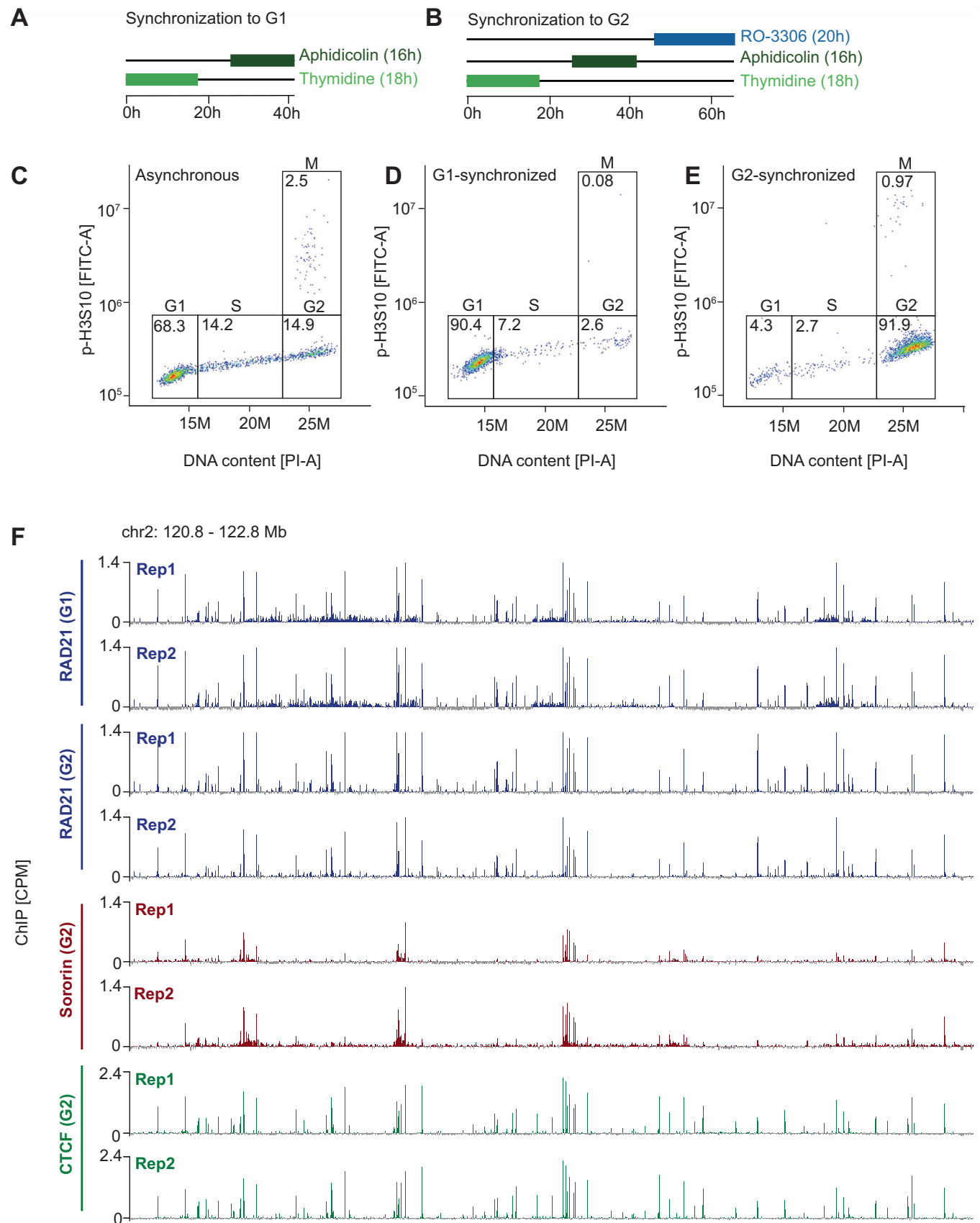


Expanded View Figures

Figure EV1. Characterization of cohesin distribution in G1 and G2 cell cycle phases.

(A, B) Schematic of cell synchronization to G1 (A) and G2 (B) cell cycle phases. (C-E) Representative cell cycle profiles (phospho-H3S10 vs. DNA content) of asynchronous (C), G1-synchronized (D), and G2-synchronized (E) wild-type HeLa cells. The fraction of cells in the different cell cycle stages is shown. (F) RAD21 ChIP-Seq signal tracks (1 kb resolution) from G1-synchronized wild-type HeLa cells and RAD21, Sororin and CTCF ChIP-Seq tracks from G2-synchronized wild-type HeLa cells at the locus shown in Fig. 1A. Individual biological replicates are shown. RAD21 G1- and G2-synchronized replicates were downsampled to a matching sequencing depth (60 mln). ChIP-Seq data are CPM-normalized and centered by subtracting the genome-wide mean.



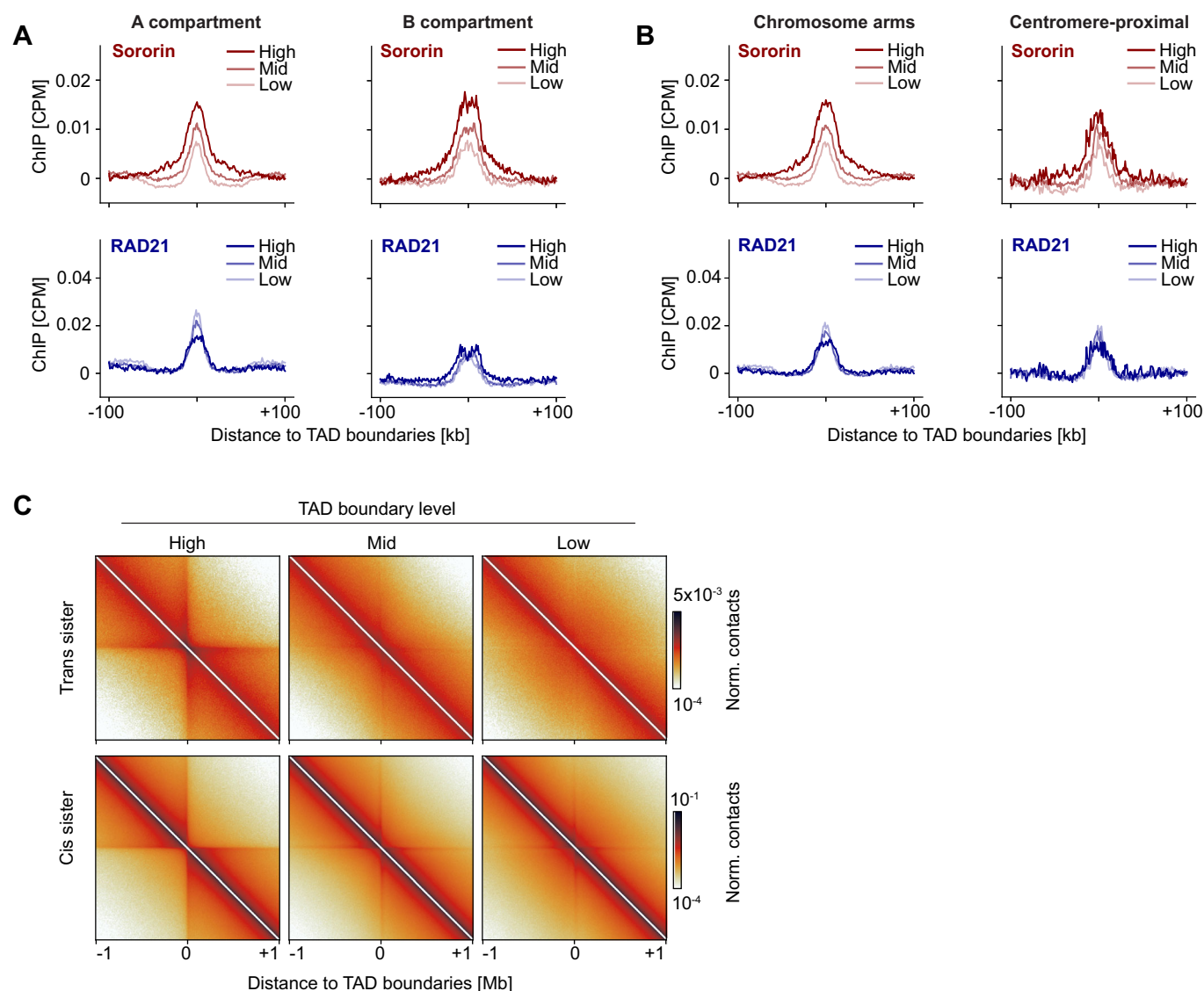
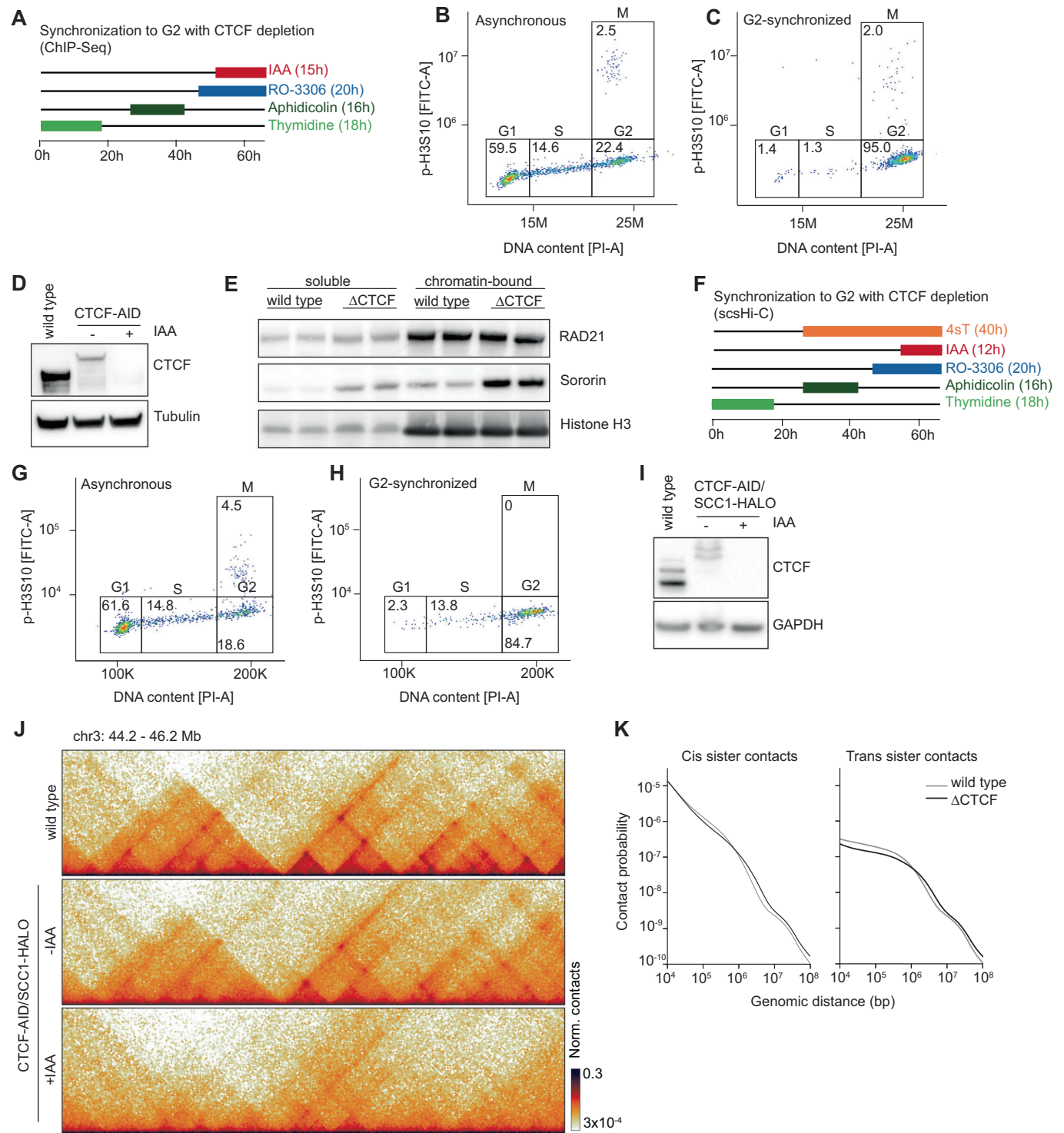


Figure EV2. Trans sister contacts accumulate at high-level boundaries.

(A) Average profiles of Sororin (upper row) and RAD21 (lower row) ChIP-Seq signal (1 kb resolution) at ± 100 kb regions centered at high-, mid- and low-level boundaries within A (left column) and B compartments (right column). (B) Average profiles of Sororin (upper row) and RAD21 (lower row) ChIP-Seq signal (1 kb resolution) at ± 100 kb regions centered at high-, mid- and low-level boundaries on chromosome arms (left column) and near centromeres (right column). (C) Average maps of trans sister (upper row) and cis sister (lower row) scsHi-C contacts (10 kb resolution) at ± 1 Mb regions centered at high-, mid-, and low-level boundaries. Wild-type G2-synchronized HeLa scsHi-C data were reanalyzed from (Mitter et al, 2020; Data ref: Mitter et al, 2020a).



◀ **Figure EV3. Characterization of CTCF depletion for scsHi-C and ChIP-Seq experiments.**

(A) Schematic of G2 cell synchronization and CTCF depletion for ChIP-Seq. (B, C) Representative cell cycle profiles (phospho-H3S10 vs. DNA content) of asynchronous (B) and G2-synchronized (C) HeLa CTCF-AID cells corresponding to (A). The fraction of cells in the different cell cycle stages is shown. (D) Western blot confirming CTCF depletion upon indole-3-acetic acid (IAA) treatment in G2-synchronized CTCF-AID cells corresponding to (A–C). Tubulin serves as a loading control. (E) Western blot confirming the presence of RAD21 and Sororin on chromatin in G2-synchronized CTCF-depleted (Δ CTCF, CTCF-AID) cells, as shown in (A, C). Histone H3 serves as a loading control. Data were representative of $n = 2$ independent experiments. (F) Schematic of G2 cell synchronization and CTCF depletion for scsHi-C. (G, H) Representative cell cycle profiles (phospho-H3S10 vs. DNA content) of asynchronous (G) and G2-synchronized (H) CTCF-AID/SCC1-HALO cells corresponding to (F). The fraction of cells in the different cell cycle stages is shown. (I) Western blot confirming CTCF depletion upon indole-3-acetic acid (IAA) treatment in G2-synchronized CTCF-AID/SCC1-HALO cells corresponding to (F–H). GAPDH serves as a loading control. (J) Representative Hi-C contact maps (10 kb resolution, 1 Mb separation) from wild-type cells, and CTCF-AID/SCC1-HALO cells in the presence and absence of indole-3-acetic acid (IAA). (K) Distance-dependent contact probability ($P(s)$) curves for cis and trans sister scsHi-C-contacts from G2-synchronized wildtype and CTCF-depleted (Δ CTCF, CTCF-AID/SCC1-HALO) cells. scsHi-C data from G2-synchronized CTCF-AID/SCC1-HALO cells without target depletion (–IAA) and upon CTCF depletion (Δ CTCF, +IAA) are merged from $n = 3$ technical replicates and $n = 5$ independent experiments, respectively. Wild-type G2-synchronized scsHi-C data were reanalyzed from (Mitter et al, 2020; Data ref: Mitter et al, 2020a).

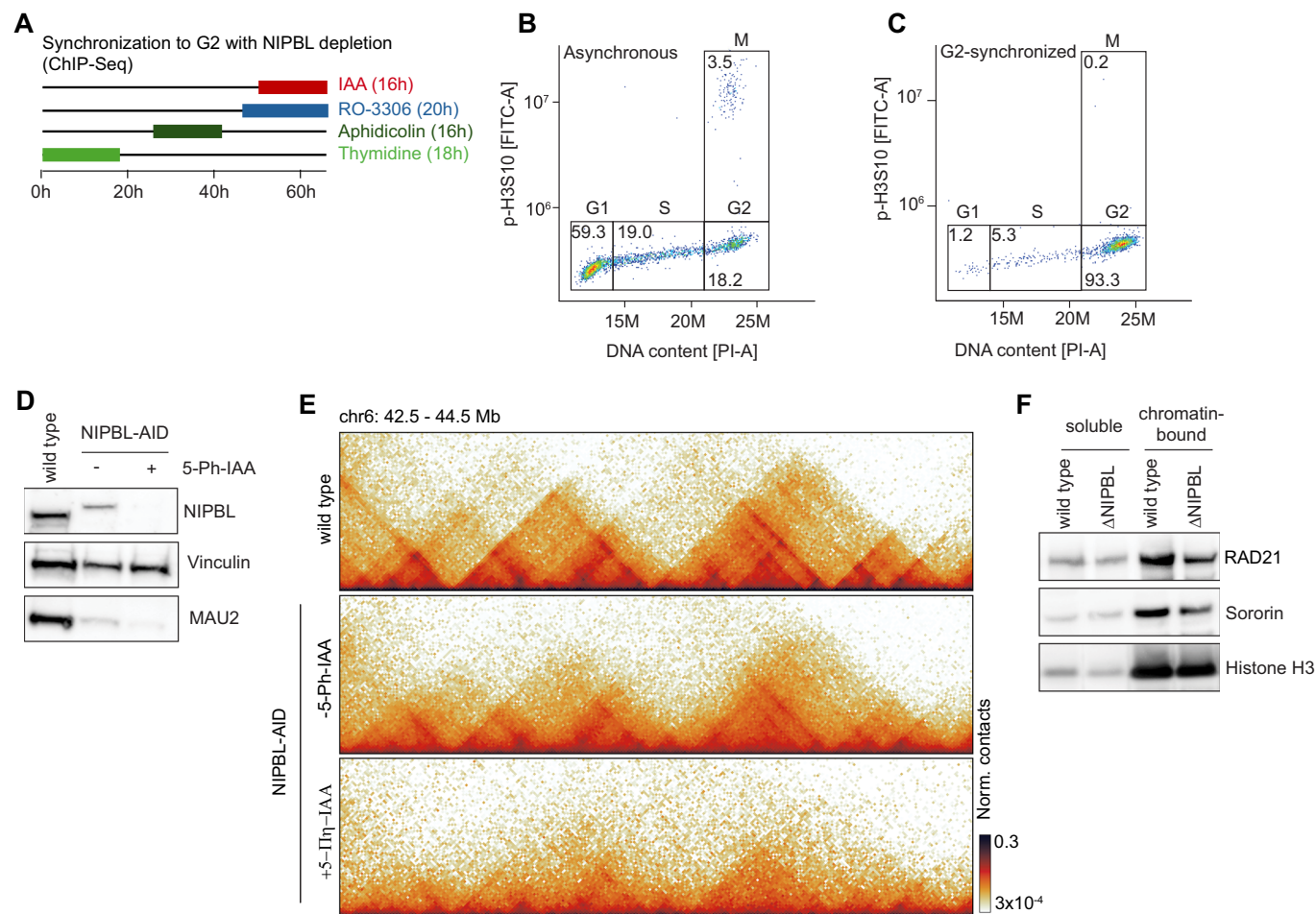


Figure EV4. Characterization of NIPBL depletion for ChIP-Seq experiments.

(A) Schematic of G2 synchronization and NIPBL depletion for ChIP-Seq. (B, C) Representative cell cycle profiles (phospho-H3S10 vs. DNA content) of asynchronous (B) and G2-synchronized (C) HeLa NIPBL-AID cells corresponding to (A). The fraction of cells in the different cell cycle stages is shown. (D) Western blot confirming NIPBL depletion upon 5-phenyl-indole-3-acetic acid (5-Ph-IAA) treatment in G2-synchronized NIPBL-AID cells corresponding to (A–C). NIPBL depletion was additionally confirmed by immunoblotting for its binding partner MAU2 (Watrin and Peters, 2006; Banigan et al, 2023). Vinculin serves as a loading control. (E) Representative Hi-C contact maps (10 kb resolution, 1 Mb separation) from G2-synchronized wild-type cells, and NIPBL-AID cells in the presence and absence of 5-phenyl-indole-3-acetic acid (5-Ph-IAA). (F) Western blot confirming the presence of RAD21 and Sororin on chromatin in G2-synchronized NIPBL-depleted (Δ NIPBL, NIPBL-AID) cells, as shown in (A, C). Histone H3 serves as a loading control. Data are representative of $n = 2$ independent experiments. scsHi-C data from G2-synchronized NIPBL-AID cells without target depletion (-5-Ph-IAA) are merged from $n = 3$ technical replicates. Wild-type G2-synchronized scsHi-C data were reanalyzed from (Mitter et al, 2020; Data ref: Mitter et al, 2020a). Δ NIPBL G2-synchronized scsHi-C data were reanalyzed from (Batty et al, 2023; Data ref: Batty et al, 2023).

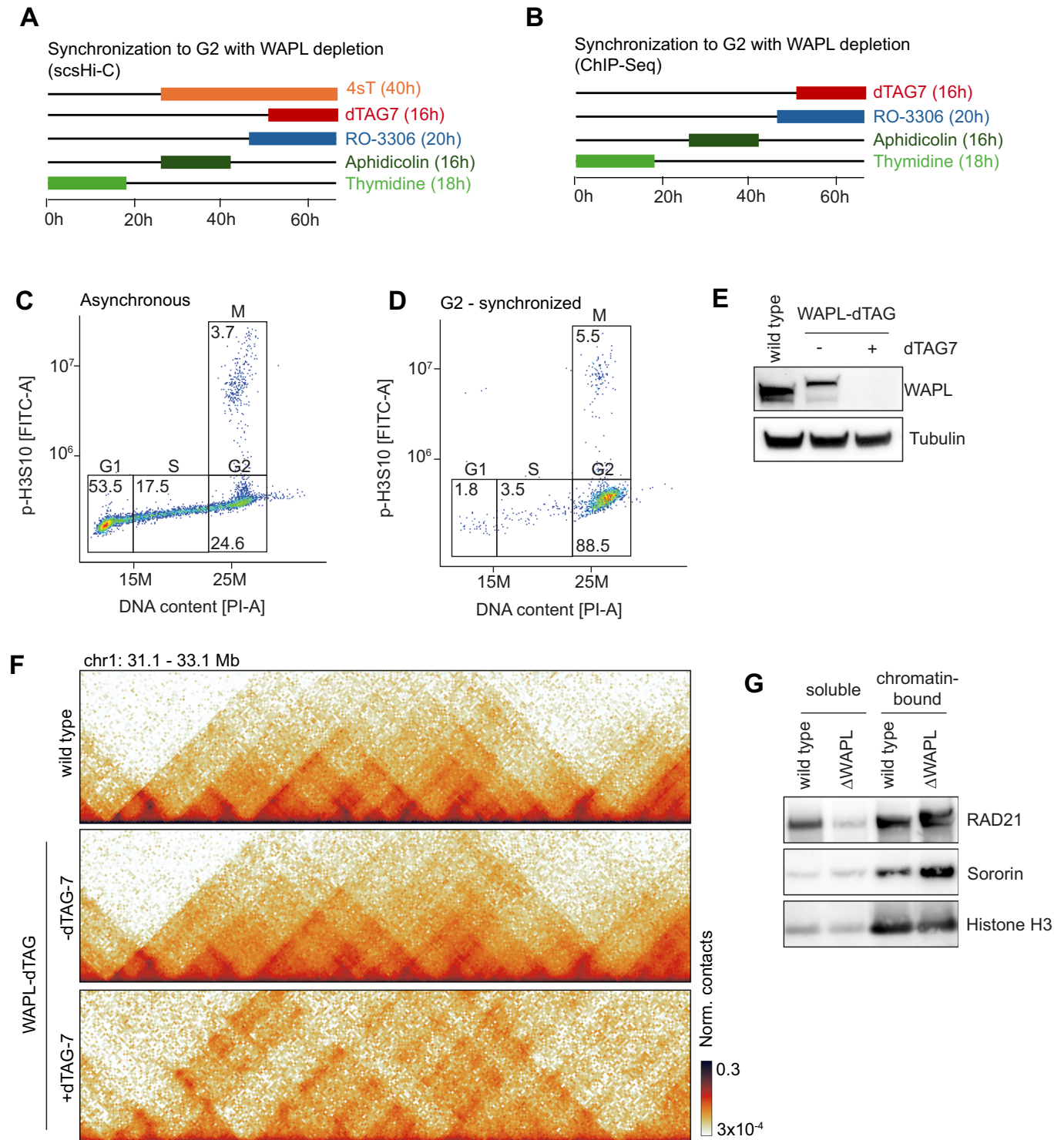


Figure EV5. Characterization of WAPL depletion for scsHi-C and ChIP-Seq experiments.

(A, B) Schematic of G2 synchronization and WAPL depletion for scsHi-C (A) and ChIP-Seq (B). (C, D) Representative cell cycle profiles (phospho-H3S10 vs. DNA content) of asynchronous (C) and G2-synchronized (D) HeLa WAPL-dTAG cells corresponding to (A, B). The fraction of cells in the different cell cycle stages is shown. (E) Western blot confirming WAPL depletion upon dTAG-7 treatment in G2-synchronized WAPL-dTAG cells corresponding to (A, B, D). Tubulin serves as a loading control. (F) Representative Hi-C contact maps (10 kb resolution, 1 Mb separation) from G2-synchronized wild type cells, and WAPL-dTAG cells in the presence and absence of dTAG-7. (G) Western blot confirming the presence of RAD21 and Sororin on chromatin in G2-synchronized WAPL-depleted (Δ WAPL and WAPL-dTAG) cells, as shown in (B, D). Histone H3 serves as a loading control. scsHi-C data from G2-synchronized WAPL-dTAG cells without target depletion (-dTAG-7) and upon WAPL depletion (Δ WAPL, +dTAG-7) are merged from $n=3$ technical replicates and $n=2$ independent experiments, respectively. Wild-type G2-synchronized scsHi-C data were reanalyzed from (Mitter et al, 2020; Data ref: Mitter et al, 2020a).

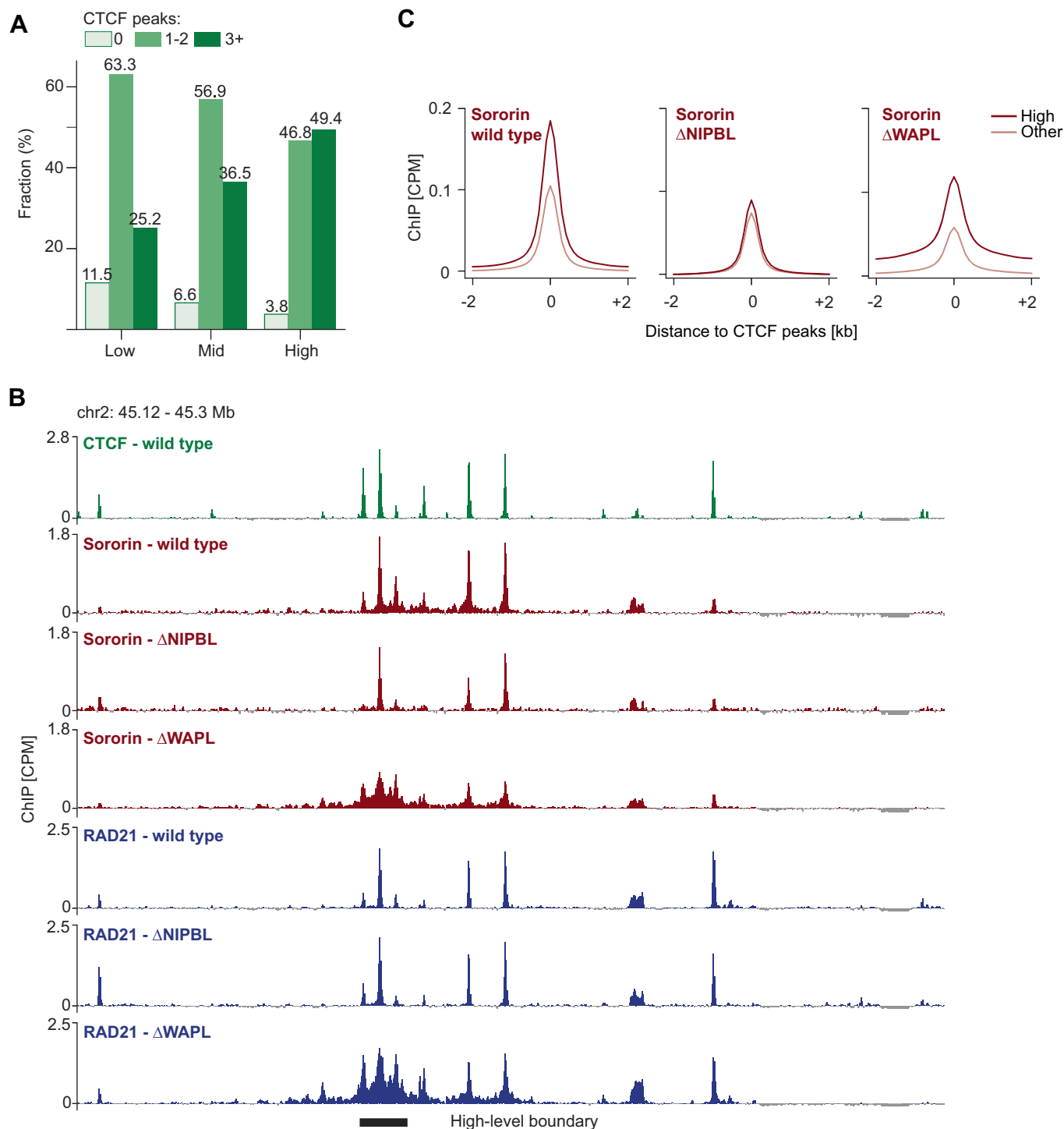


Figure EV6. Characterization of CTCF clusters at high-level boundaries.

(A) Fractions of low-, mid- and high-level boundaries containing 0, 1, 2, or ≥ 3 CTCF peaks. (B) Representative ChIP-Seq tracks (200 bp resolution) of CTCF (green) from G2-synchronized wildtype, and Sororin (red) and RAD21 (blue) from G2-synchronized wild type, NIPBL-depleted (Δ NIPBL, NIPBL-AID) and WAPL-depleted (Δ WAPL, WAPL-dTAG) HeLa cells. Position of a high-level boundary (10 kb) is indicated below. (C) Average profile of Sororin ChIP-Seq signal (100 bp resolution) in G2-synchronized cells under wild type (left panel), NIPBL-depleted (Δ NIPBL, middle panel) and WAPL-depleted (Δ WAPL, right panel) conditions at ± 2 kb regions centered on isolated CTCF peaks (no additional CTCF peak within ± 2.5 kb) and stratified by location: high-level boundaries (High) ($n = 5989$ peaks) or elsewhere in the genome (Other) ($n = 44,600$ peaks). ChIP-seq data were CPM-normalized, centered by subtracting the genome-wide mean and represent merged data from $n = 3$ (CTCF) or $n = 2$ (Sororin, RAD21) independent experiments.