

Supplemental information

**Loop stacking organizes genome folding
from TADs to chromosomes**

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Figure S1 related to Figure 1

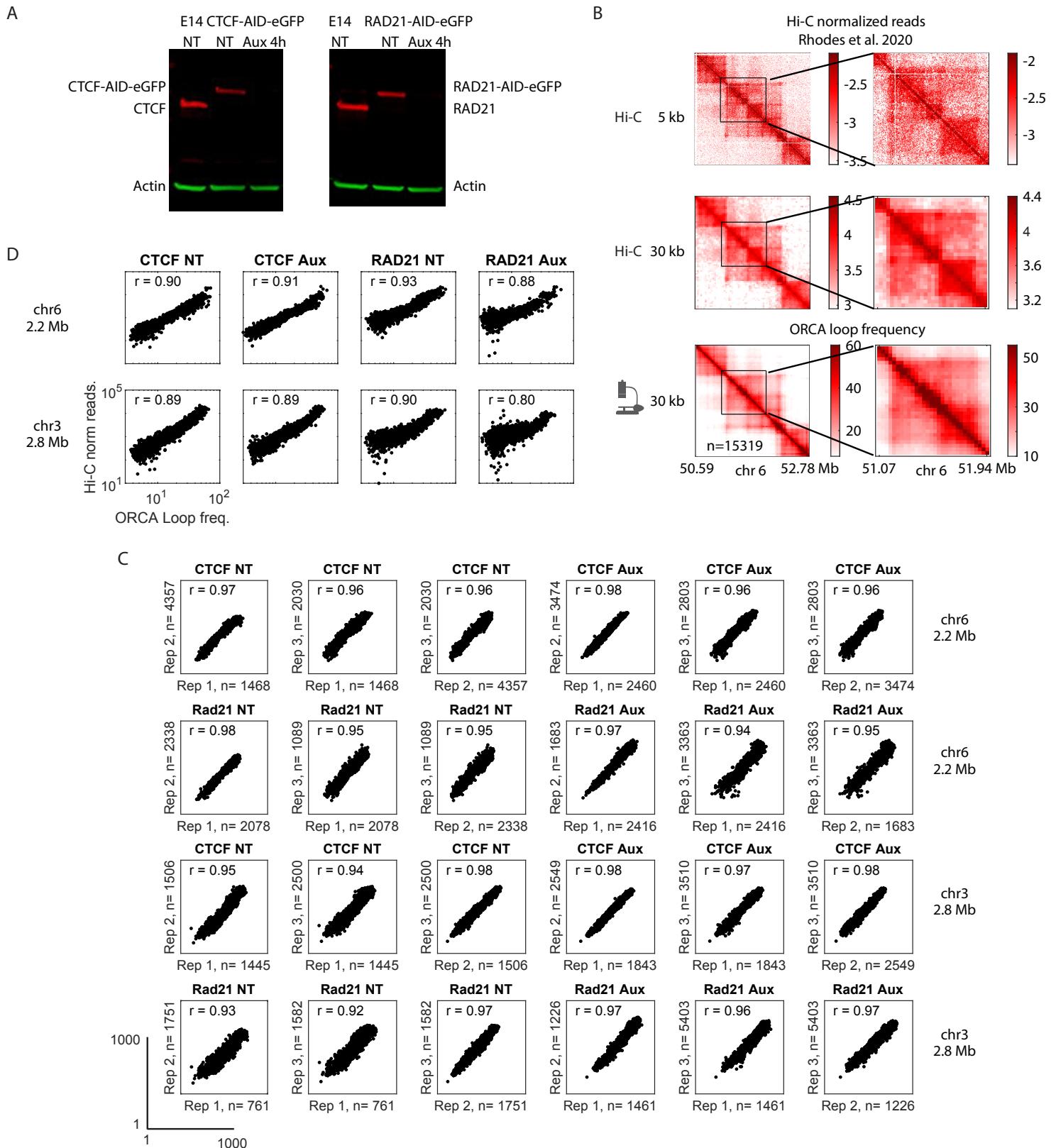


Figure S2 related to Figure 2

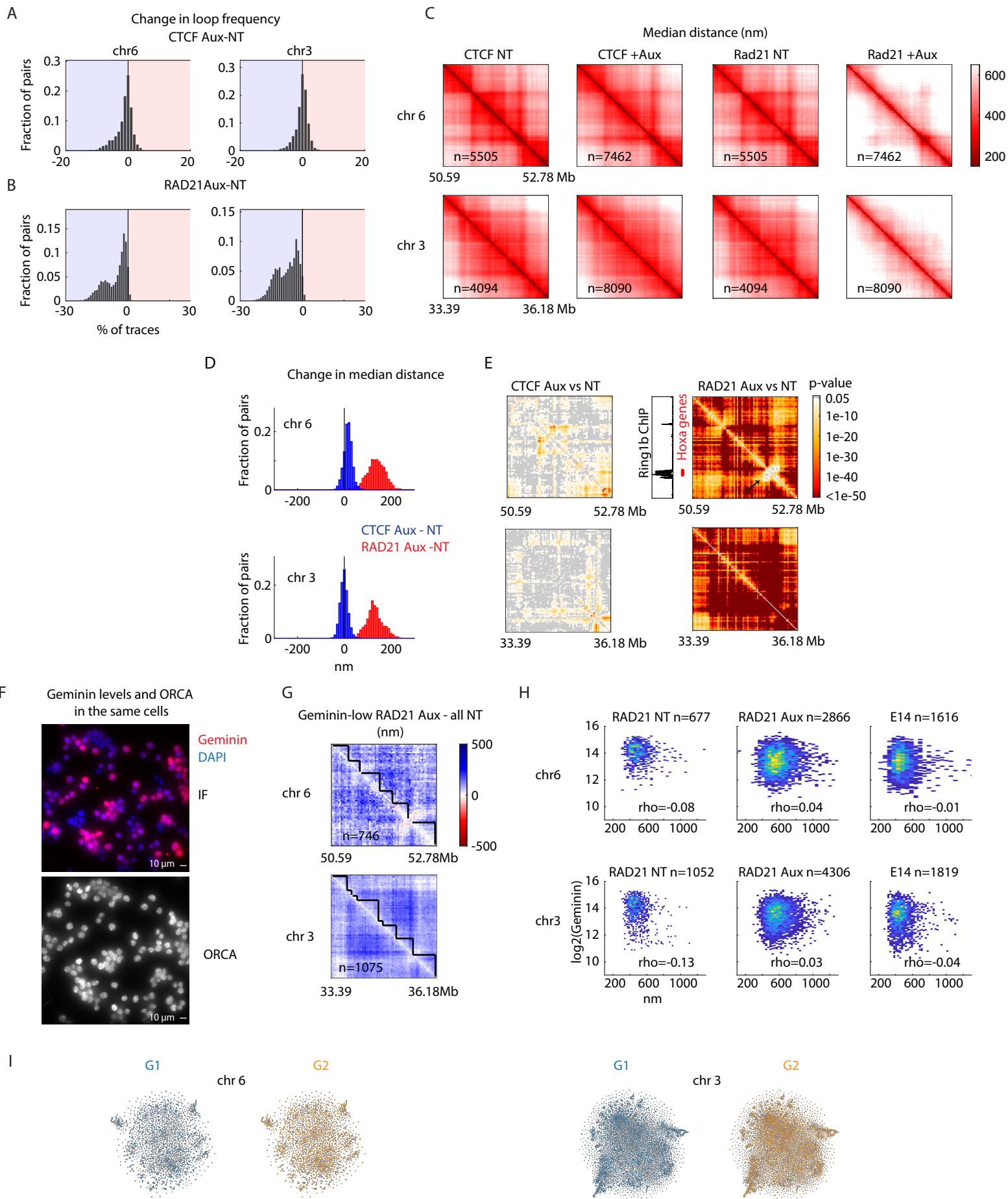


Figure S3 related to Figure 3

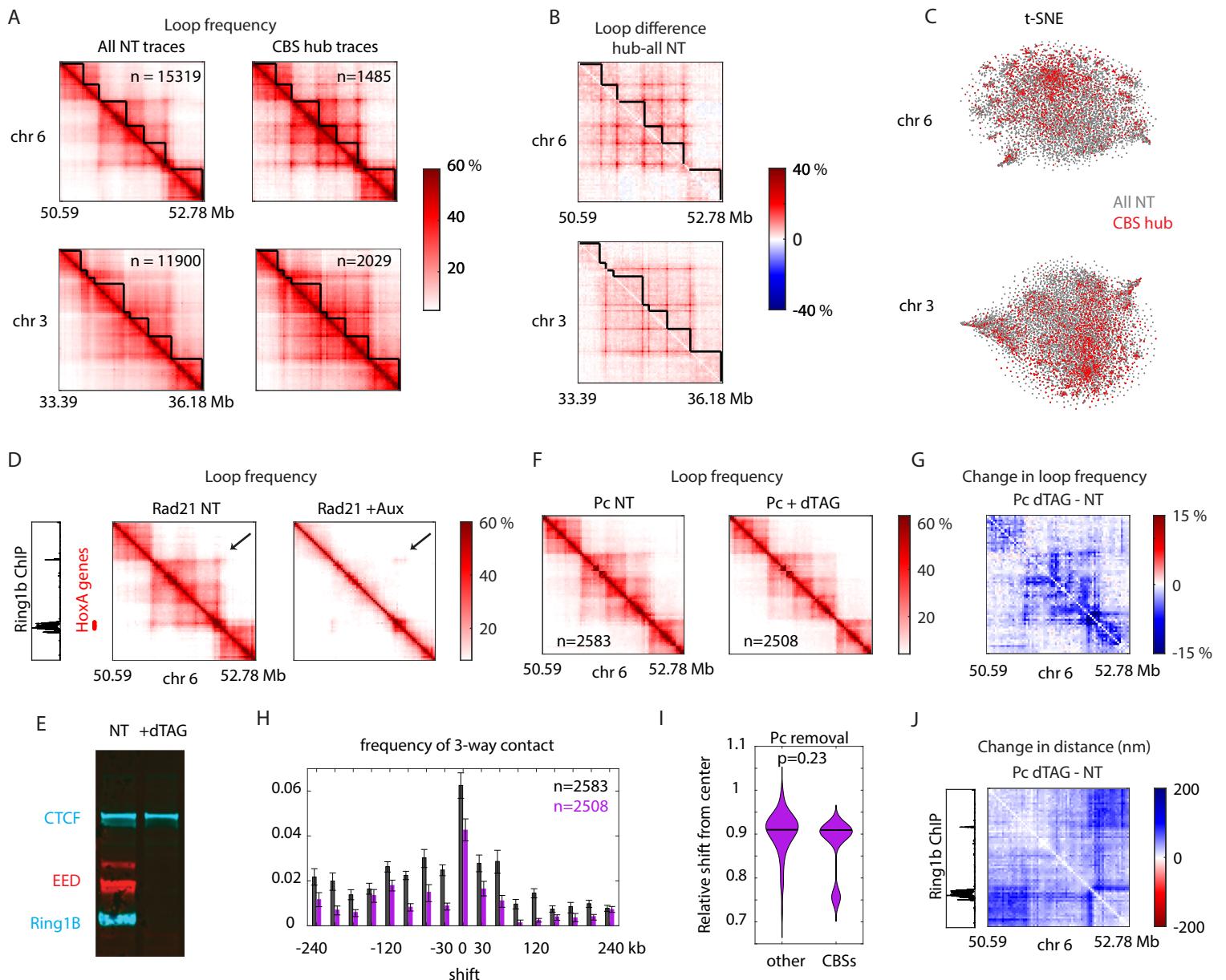


Figure S4 related to Figure 4

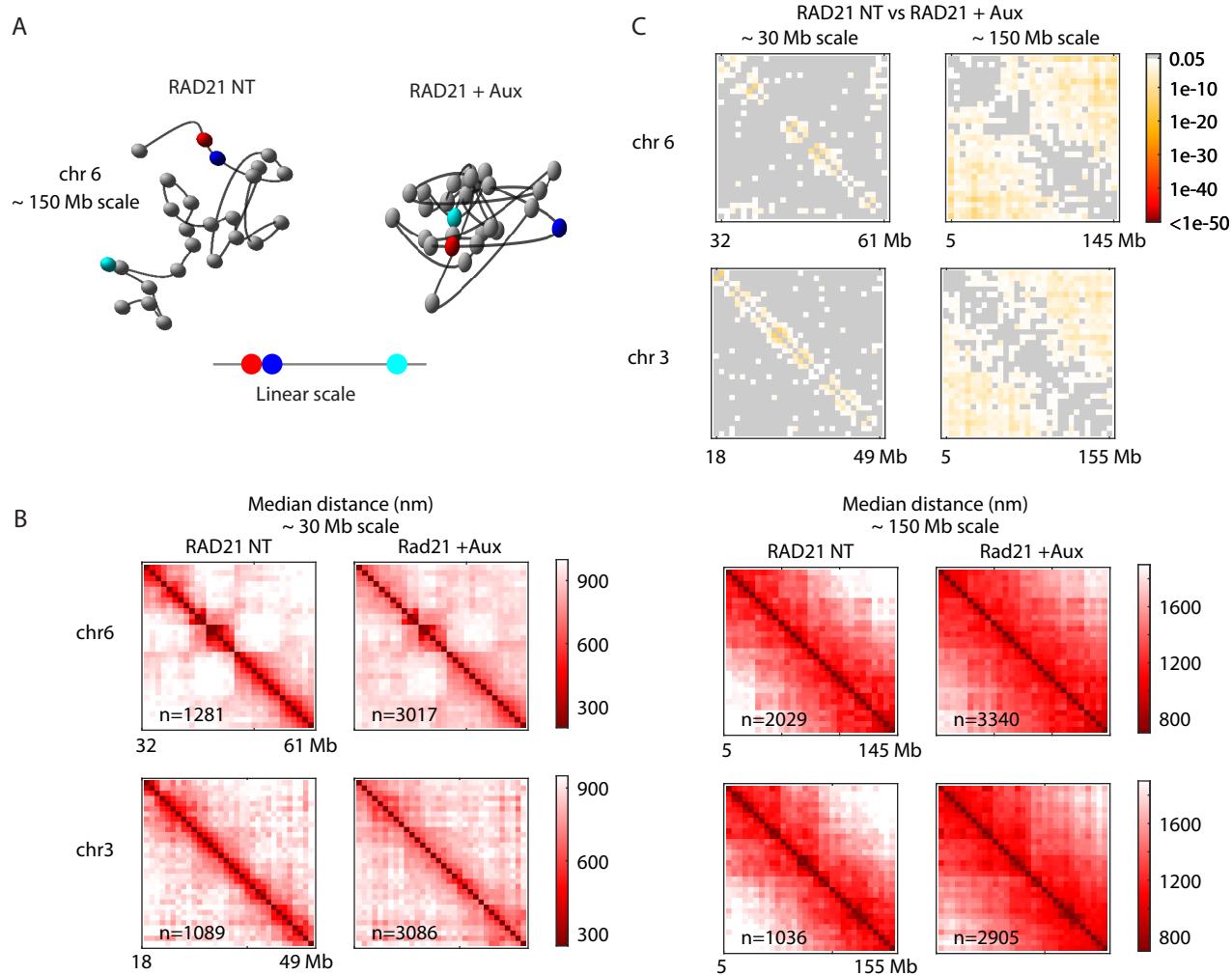


Figure S5 related to Figure 5

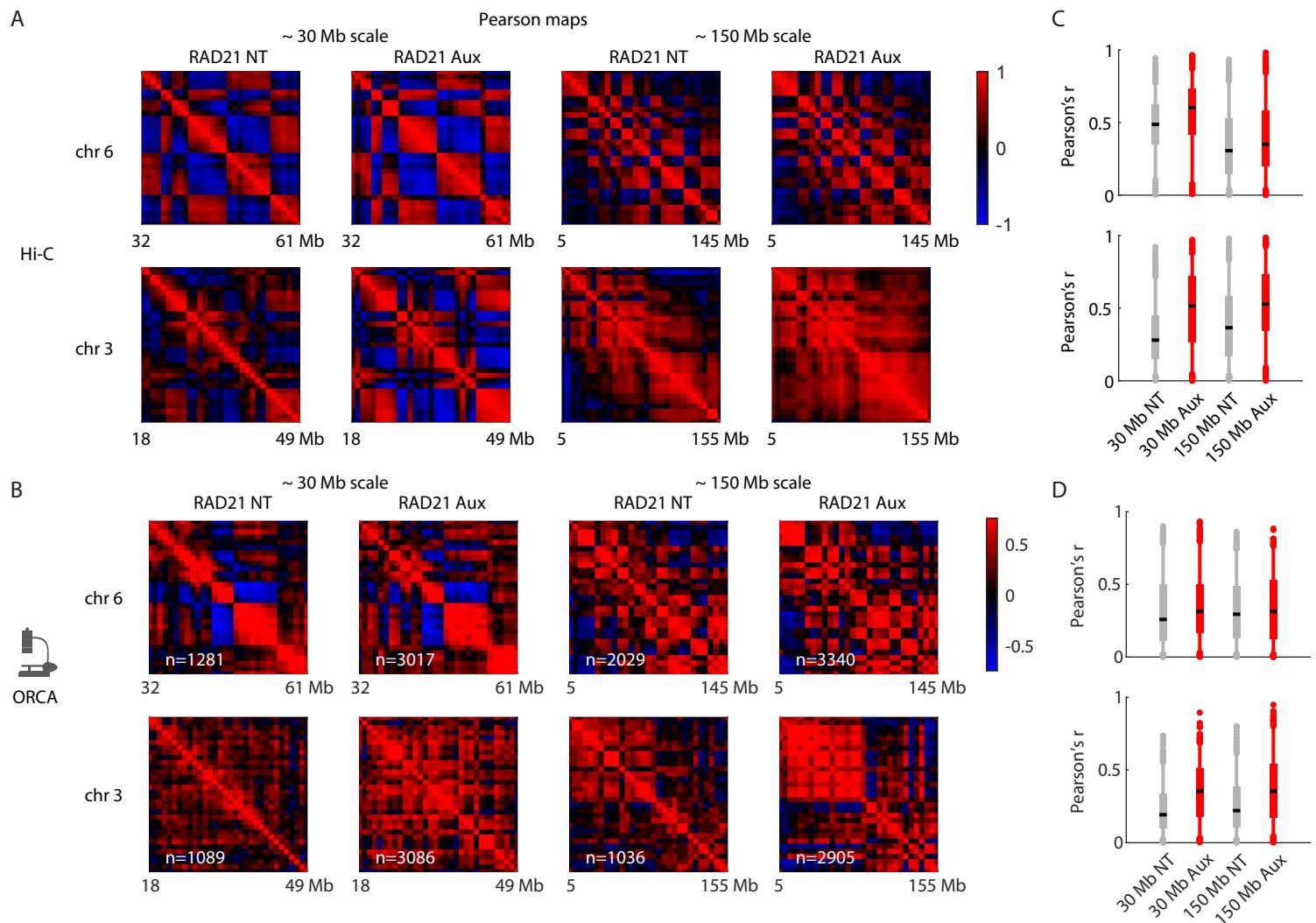


Figure S6 related to Figure 6

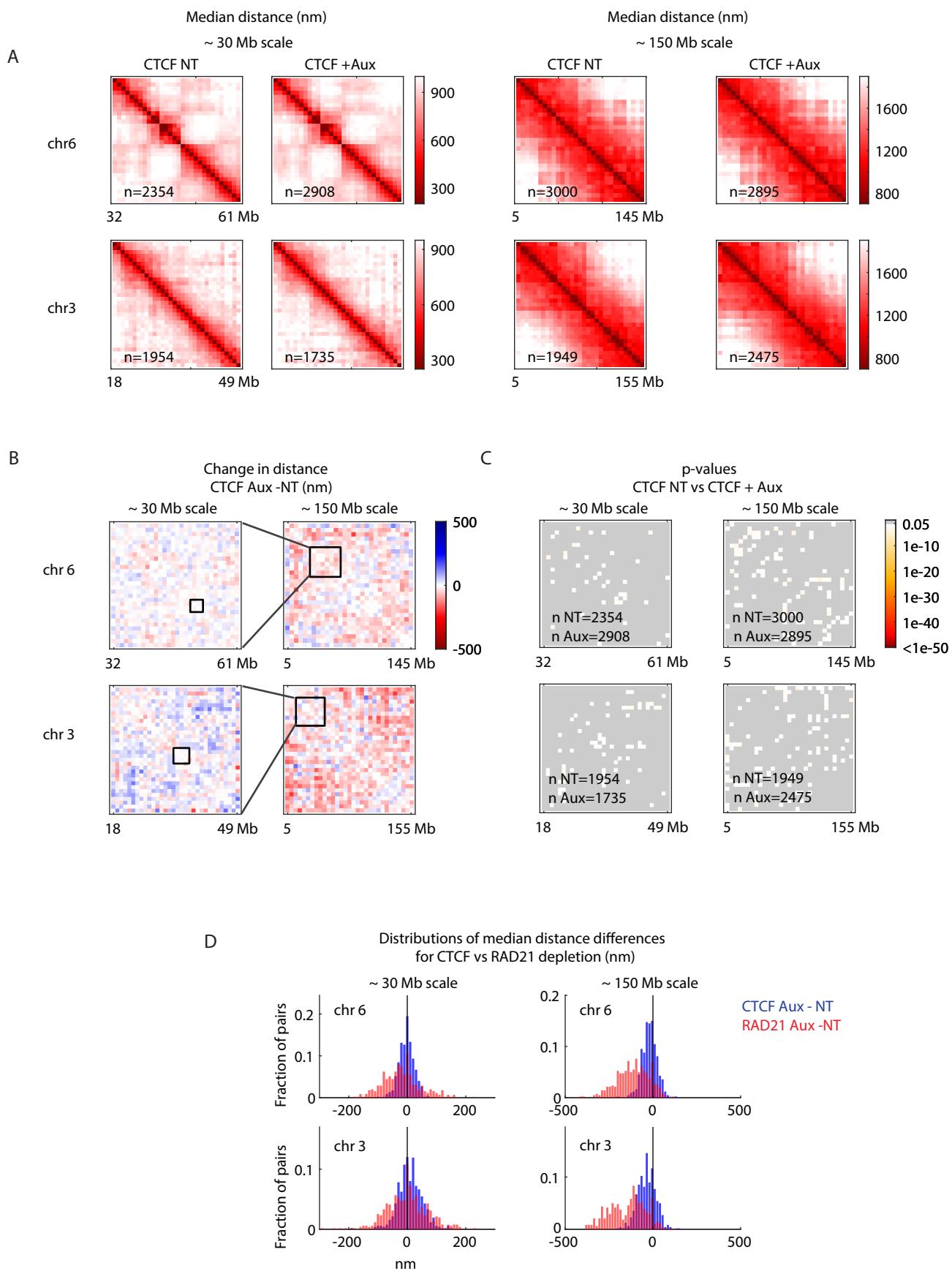


Figure S1 | Comparison across ORCA replicates and with Hi-C, Related to Figure 1. **A.** Western blot labeling Actin (loading control) and either CTCF or Cohesin in the parental E14 cell line and in the CTCF-AID-eGFP or RAD21-AID-eGFP cells, with or without auxin treatment. **B.** Hi-C data for chr 6, 2.2 Mb region and a ~900 kb region from 4 at either 5 kb or 30 kb resolution. The same regions are shown for the ORCA data. **C.** Scatter plots of inter-barcode distances, in nanometers, comparing reproducibility among three replicates labeling the chr6 or chr3 domains. Median distance is plotted for each barcode pair against the pairwise median distance in a different replicate. Pearson's correlation coefficient, r , is shown on the plot. The lower left plot shows the axis scale and the same scale was used for all plots. **D.** Scatter plots for ORCA loop frequencies vs Hi-C contact with Pearson's r is indicated on the plots.

Figure S2 | Quantification of effects of CTCF or cohesin depletion on genome folding, Related to Figure 2. **A-B.** Distribution of difference in loop frequency upon CTCF depletion (A) or cohesin depletion (B). **C.** Median distance maps for CTCF NT, CTCF +Aux, RAD21 NT and RAD21 +Aux conditions. **D.** Comparison of median distance differences between NT or +Aux conditions for either CTCF (blue) or RAD21 (red). **E.** P-values computed using Wilcoxon rank sum for each pairwise distance distribution between auxin treated and untreated cells. Arrows point to the Ring1B coated region on the heatmap that did not significantly change upon cohesin depletion and the corresponding Ring1B (member of the Polycomb complex) ChIP signal from 21 as well as the position of Hoxa genes. **F.** Example field of view of DAPI and immunofluorescent staining of Geminin and ORCA fiducial staining for the same cells. **G.** Difference in median distance between the Geminin-low population for the RAD21 auxin treated cells (number of cells on the plot) and all RAD21 untreated cells. **H.** Scatter plot of median distance (over all pairs) in each chromosome relative to Geminin levels in the same cell. Spearman's correlation coefficient is indicated for each condition. **I.** t-SNE plots (as in Figure 1) with colors corresponding to either Geminin-low (G1) cells or Geminin high (G2) cells.

Figure S3 | Quantification of effects of Polycomb depletion on looping, 3D distance and multi-way interactions, Related to Figure 3. **A.** Loop frequency for all NT traces vs Loop frequency of only hub-chromosomes. **B.** Loop difference between hub-chromosomes and All NT traces. **C.** t-SNE analysis of all NT cells with CBS-hub traces in red. **D.** Loop frequency for RAD21 NT and RAD21 +Aux conditions and the corresponding Ring1B (member of the polycomb complex), ChIP signal from 21 as well as the position of Hoxa genes. Arrow points to the loop-dot between two Pc domains that remains after cohesin depletion. **E.** Western blot showing depletion of Ring1B and EED following dTAG treatment (8h, 500nM) reproduced from ²⁵. **F.** Loop frequency from Pc NT or Pc depleted cells. **G.** Difference in loop frequency between Pc depleted and NT cells. **H.** Quantification of 3-way contacts in Pc NT (black) and in Pc +dTAG

cells (purple), as in Figure 3B. **I.** Fold change in central positioning upon Pc depletion for CBSs relative to other readouts. **J.** Difference in median distance between Pc dTAG and Pc NT cells.

Figure S4 | Cohesin depletion affects 3D structure at ~30 Mb and ~150 Mb scale, Related to Figure 4. **A.** Example of a trace at the 150 Mb scale from NT or RAD21 +Aux cells. Red and blue spheres mark positions of adjacent readouts (5 Mb apart), while the cyan sphere is 100 Mb away. Tube radius is 15 nm, sphere radius is 100 nm. **B.** Median distance maps for RAD21 NT and RAD21+Aux cells at ~30 Mb and ~150 Mb scales. **C.** P-values computed using Wilcoxon rank sum for each pair of barcodes between auxin-treated and untreated cells.

Figure S5 | ORCA, like Hi-C, shows compartment strengthening after depletion of cohesin, Related to Figure 5. **A.** Hi-C and **B.** ORCA Pearson maps for the ~30 Mb and ~150 Mb scales in RAD21 untreated or auxin treated cells. **C-D.** Distribution of Pearson correlation coefficients for Hi-C⁴ (C) and ORCA (D).

Figure S6 | CTCF depletion leads to less change in physical distance than cohesin depletion, Related to Figure 6. **A.** Median distance maps for ~30 Mb and ~150 Mb scales for CTCF NT or CTCF +Aux conditions. **B.** Median distance difference maps for CTCF Aux - CTCF NT cells at ~30 Mb and ~150 Mb scales. **C.** P-values computed using Wilcoxon rank sum for each pair between CTCF auxin-treated and untreated cells at different genomic scales. **D.** Distribution of median distance differences between auxin-treated and untreated cells for either CTCF (blue) or RAD21 (red).