

## Supporting Information

# Chromatin Hierarchical Branching at Nanoscale by Electron Microscopy

Zhongwu Zhou<sup>a,b</sup>, Rui Yan<sup>c,d</sup>, Wen Jiang<sup>c,\*</sup>, Joseph M.K. Irudayaraj<sup>a,e,\*</sup>

a. Bindley Bioscience Center, Department of Agricultural and Biological Engineering, Purdue University, West Lafayette, IN 47907, USA

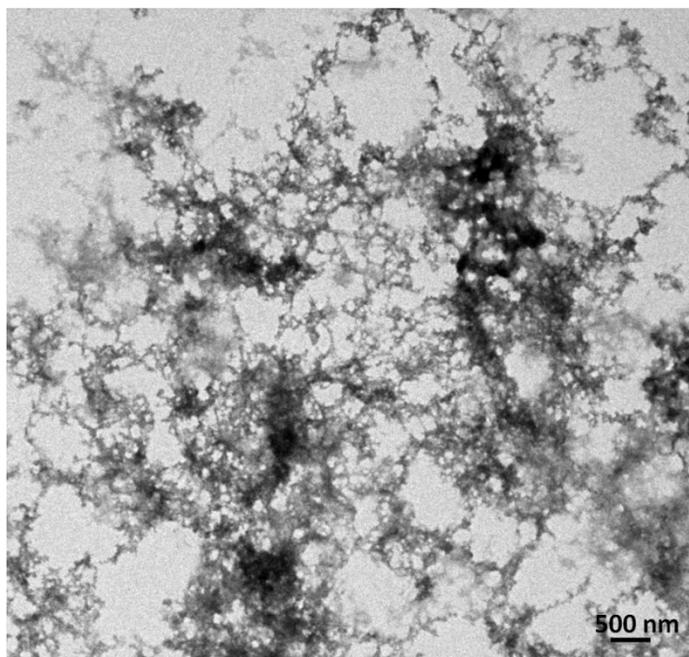
b. The University of Texas at Austin, NHB 4.120, 100 E. 24th St. Austin, TX 78712, USA

c. Markey Center for Structural Biology, Department of Biological Science, Purdue University, West Lafayette, IN 47907, USA

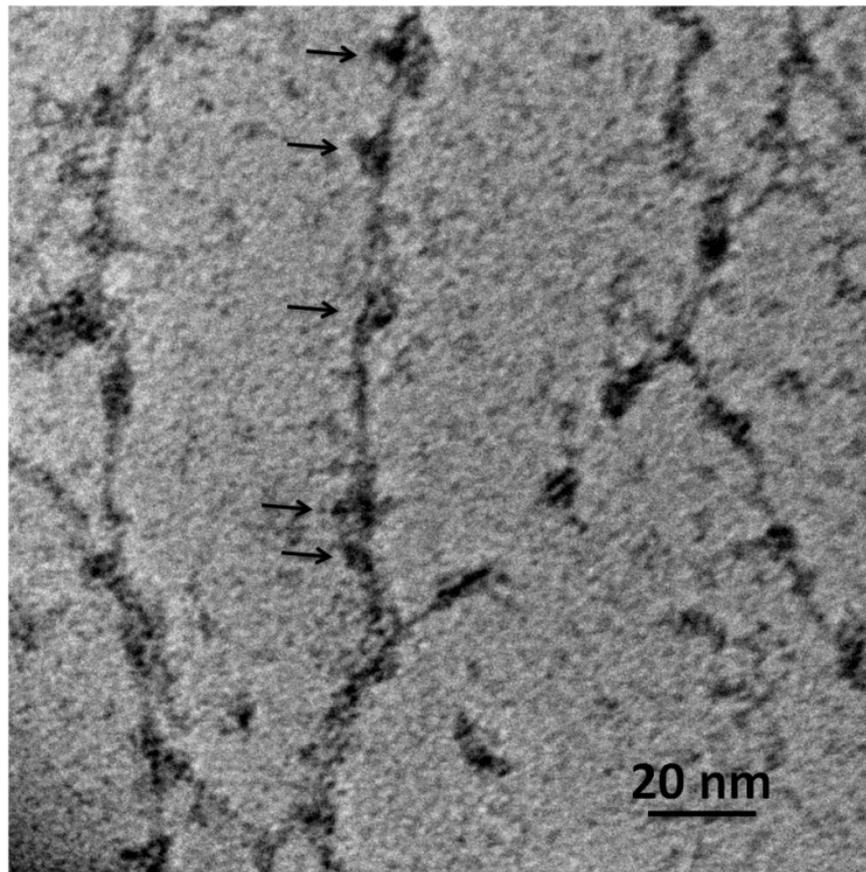
d. Howard Hughes Medical Institute, Janelia Research Campus, 19700 Helix Drive, Asburn, 20147, Virginia, USA

e. Cancer Center at Illinois, Department of Bioengineering, College of Engineering, University of Illinois at Urbana-Champaign. Urbana, IL 61801, USA

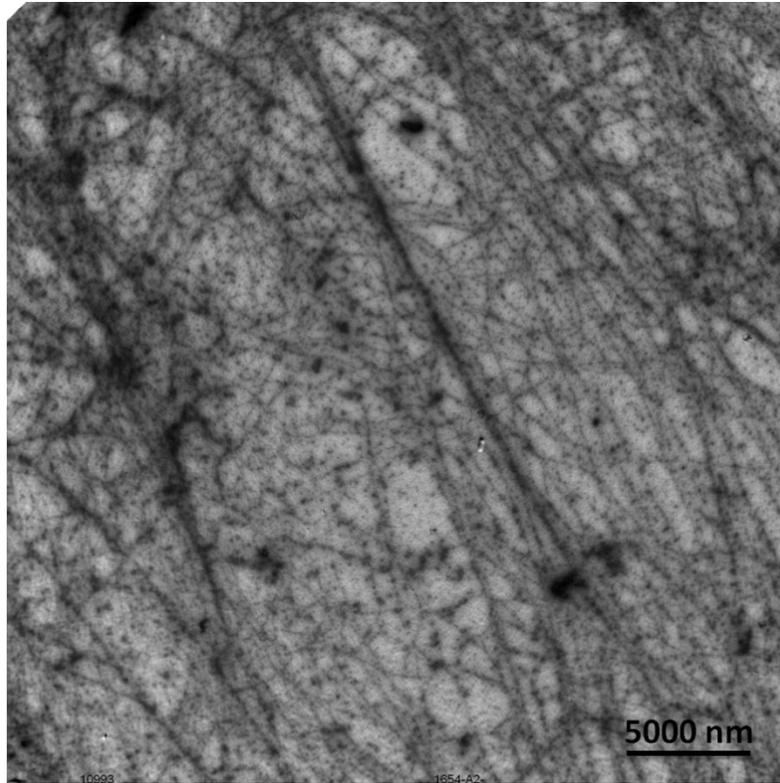
\* To whom correspondence may be addressed: [jiang12@purdue.edu](mailto:jiang12@purdue.edu) or [jirudaya@illinois.edu](mailto:jirudaya@illinois.edu)



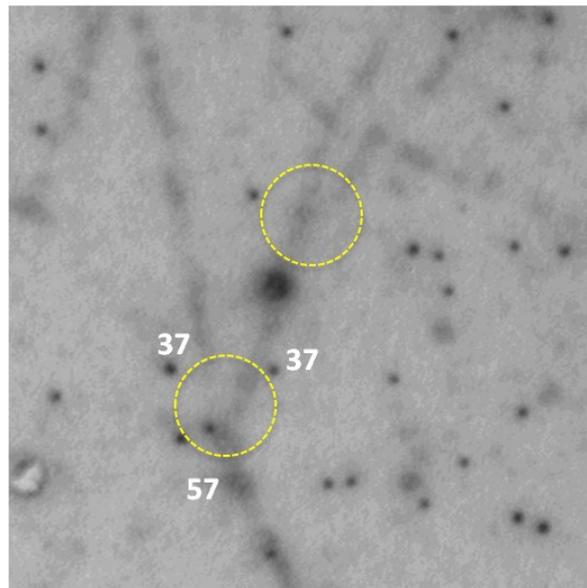
**Figure S1. Chromatin globule-like structure.** Direct mounting of the isolated MCF7 chromatin on continuous carbon coated TEM grids presents the chromatin as a tangled structure with various sized rings stacked on one another.



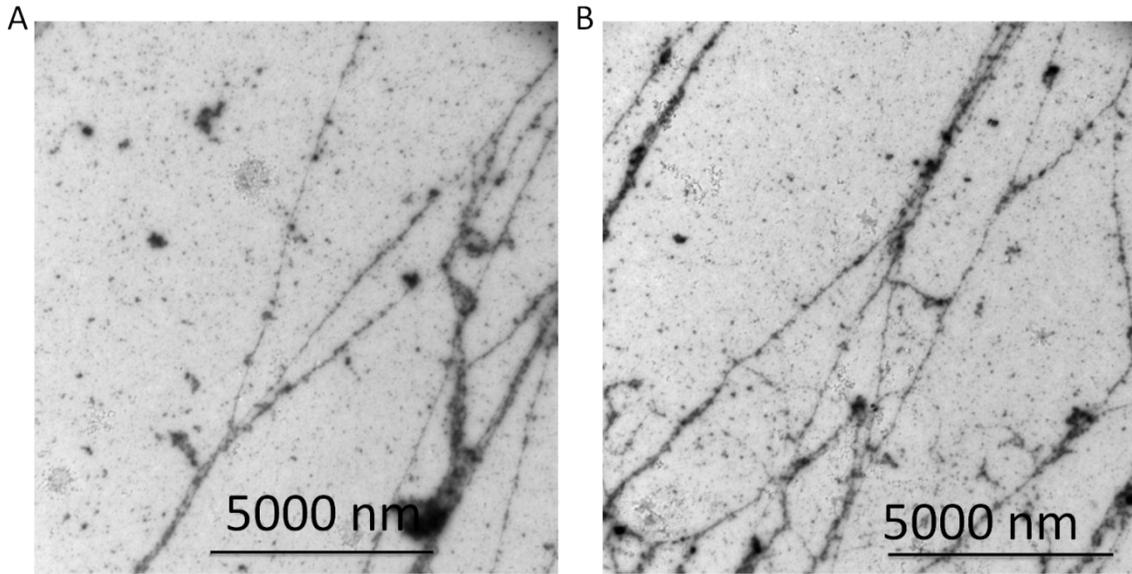
**Figure S2. Chromatin was intact during the buffer alignment process.** After buffer alignment, positively stained MCF7 chromatin was visualized at high magnification by TEM. Nucleosome-like particles were indicated by black arrow. The gentle alignment process well preserved the beads-on-a-string structure indicating the linker DNA was intact.



**Figure S3. Concentrated chromatin sample buffer aligned on continuous carbon coated TEM grids.** Though concentrated chromatin sample could be well aligned by buffer-flow, identification of individual chromatin fiber or the classic bead-on-a-string structure in this crowded environment was infrequent. Thus, in order to observe higher order chromatin organization of individual chromatin by high resolution TEM imaging, it is essential to observe the chromatin *in vitro* in a dispersed state instead of in cell (*in vivo*) concentrated and compacted state.



**Figure S4. The fifth level of MCF7 hierarchical branching structure.** One of the two 37 nm chromatin in Fig. 4B (indicated by yellow circle) further branched into two pieces of chromatin.



**Figure S5. Mouse NSC chromatin has a hierarchical branching structure.**