

Abstract

Upon injury, cardiac fibroblasts respond to stress-induced signals by undergoing a cell state transition to activated fibroblasts. This process underlies the maladaptive remodeling and poor outcomes seen in heart failure. However, the mechanisms regulating pathologic activation of cardiac fibroblasts remain poorly understood. Previous studies have demonstrated that small molecule inhibitors of bromodomain and extraterminal (BET) proteins can ameliorate cardiac fibrosis. Our prior study revealed that the BET family plays a role in activating gene programs required for fibroblast activation, with the transcription factor MEOX1 as an upstream regulator. Bromodomain-containing protein 4 (BRD4) is a BET family member with diverse functions as a transcriptional coactivator, and our lab recently discovered a novel role of BRD4 in maintaining genome folding by stabilizing the cohesin complex, which is necessary for forming DNA loops that allow for enhancer-promoter interactions.

We hypothesize that BRD4-mediated genome folding establishes the enhancer-promoter interaction and regulates subsequent expression of *Meox1* upon fibroblast activation.

Our data provide novel mechanistic understanding of the establishment of genome organization during the cell state transition of fibroblast activation. These studies will expand our knowledge on how genome folding regulates fibroblast plasticity, and inform on new therapeutic approaches for reversing and/or preventing pathologic remodeling.

Meox1 as a model locus to investigate chromatin looping

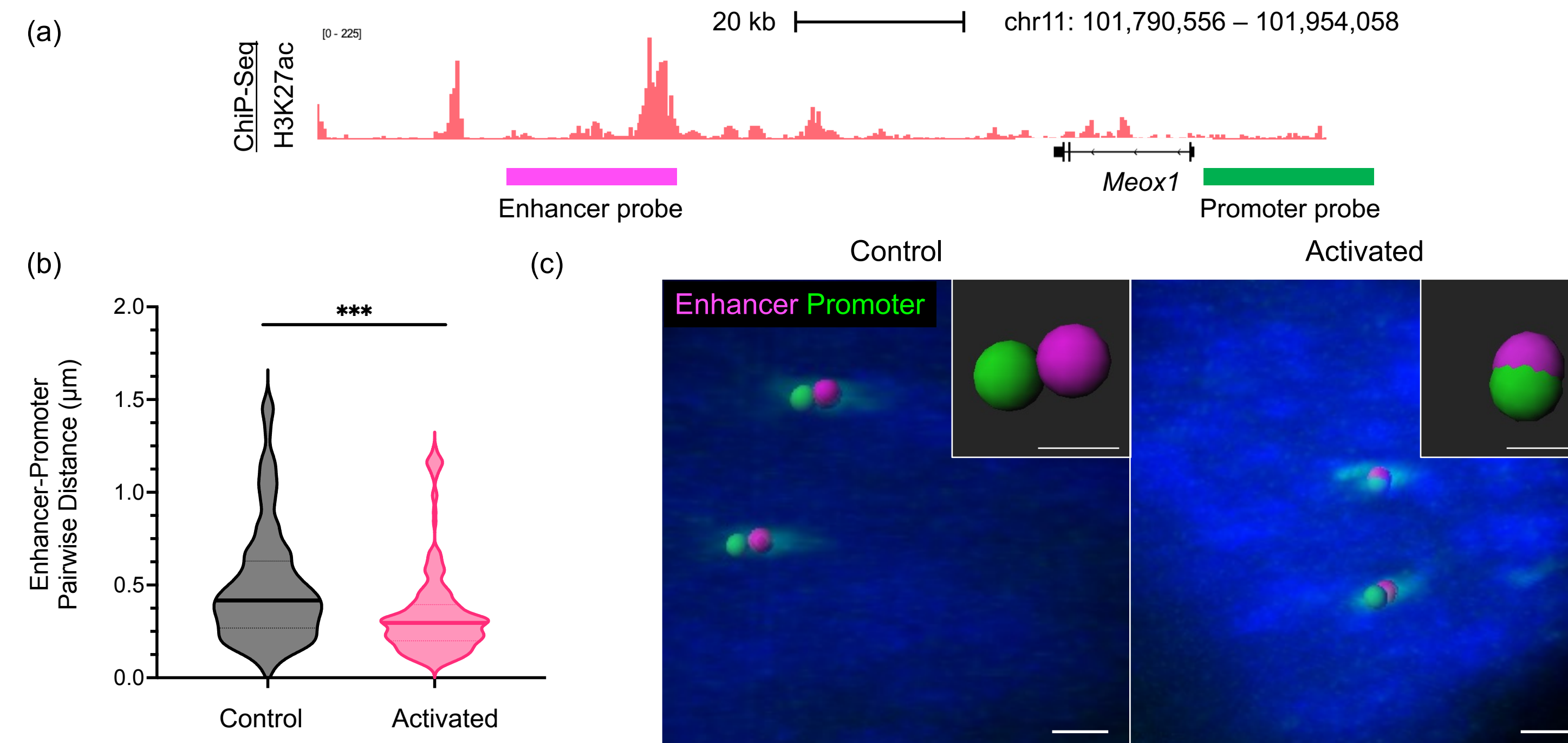


Figure 3. *Meox1* as a model locus for understanding enhancer-promoter looping in fibroblast activation. (a) Design of Oligo-FISH probes for the enhancer, as marked by H3K27ac, and promoter of *Meox1*. (b,c) The pairwise distance between the enhancer and promoter of *Meox1* decreases upon fibroblast activation. Scale bars 1 μ m.

BRD4 physically interacts with NIPBL

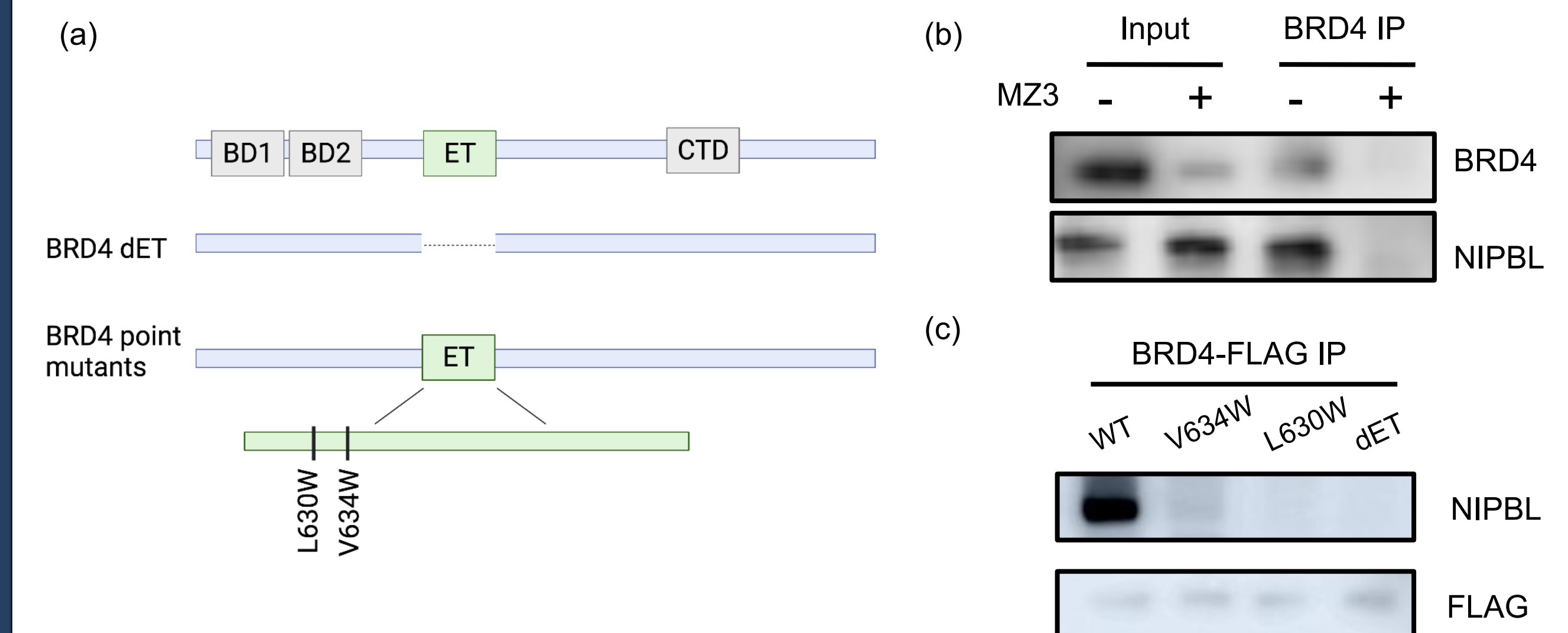


Figure 6. BRD4 physically interacts with NIPBL through the ET domain. (a) Domain structure of BRD4 peptides with mutated ET domain. (b) Co-immunoprecipitation of BRD4 demonstrates a fraction of NIPBL that is bound to BRD4. Treatment with MZ3, a small molecule degrader of BRD4, was used as a control. (c) Western blotting demonstrates that ET-domain mutant BRD4 peptides lose ability to bind NIPBL.

BRD4-cohesin-WAPL balance genome folding

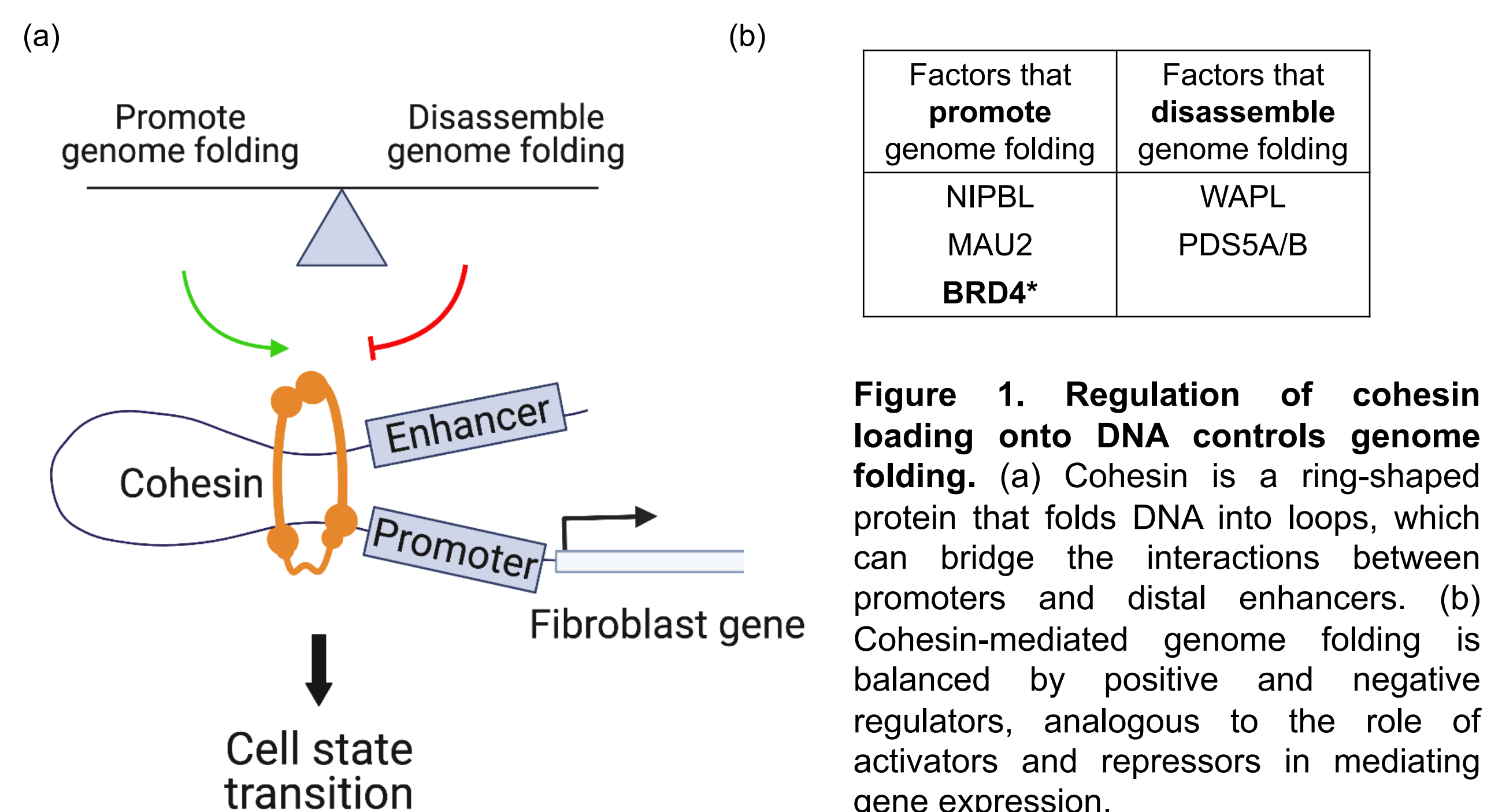


Figure 1. Regulation of cohesin loading onto DNA controls genome folding. (a) Cohesin is a ring-shaped protein that folds DNA into loops, which can bridge the interactions between promoters and distal enhancers. (b) Cohesin-mediated genome folding is balanced by positive and negative regulators, analogous to the role of activators and repressors in mediating gene expression.

BRD4 is efficiently and rapidly degraded in degon system

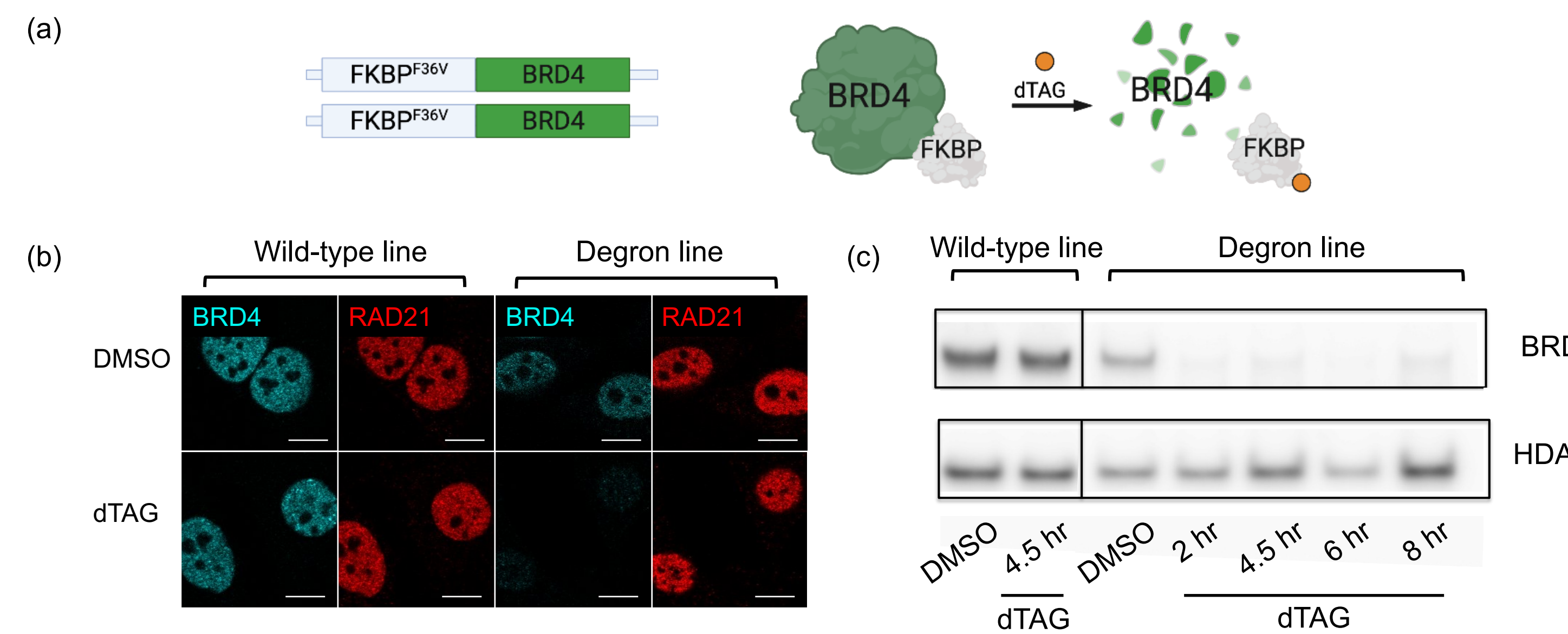


Figure 4. BRD4 is rapidly degraded with CRISPR-mediated insertion of degon epitope at *Brd4* locus. (a) Schematic of degon system in which addition of dTAG molecule targets BRD4 protein for degradation by ubiquitin-proteasome pathway. Immunofluorescence (b) and western blotting (c) of BRD4 in degon conditions demonstrates rapid and efficient degradation. Scale bars 10 μ m.

BRD4 is enriched at Meox1 locus upon activation

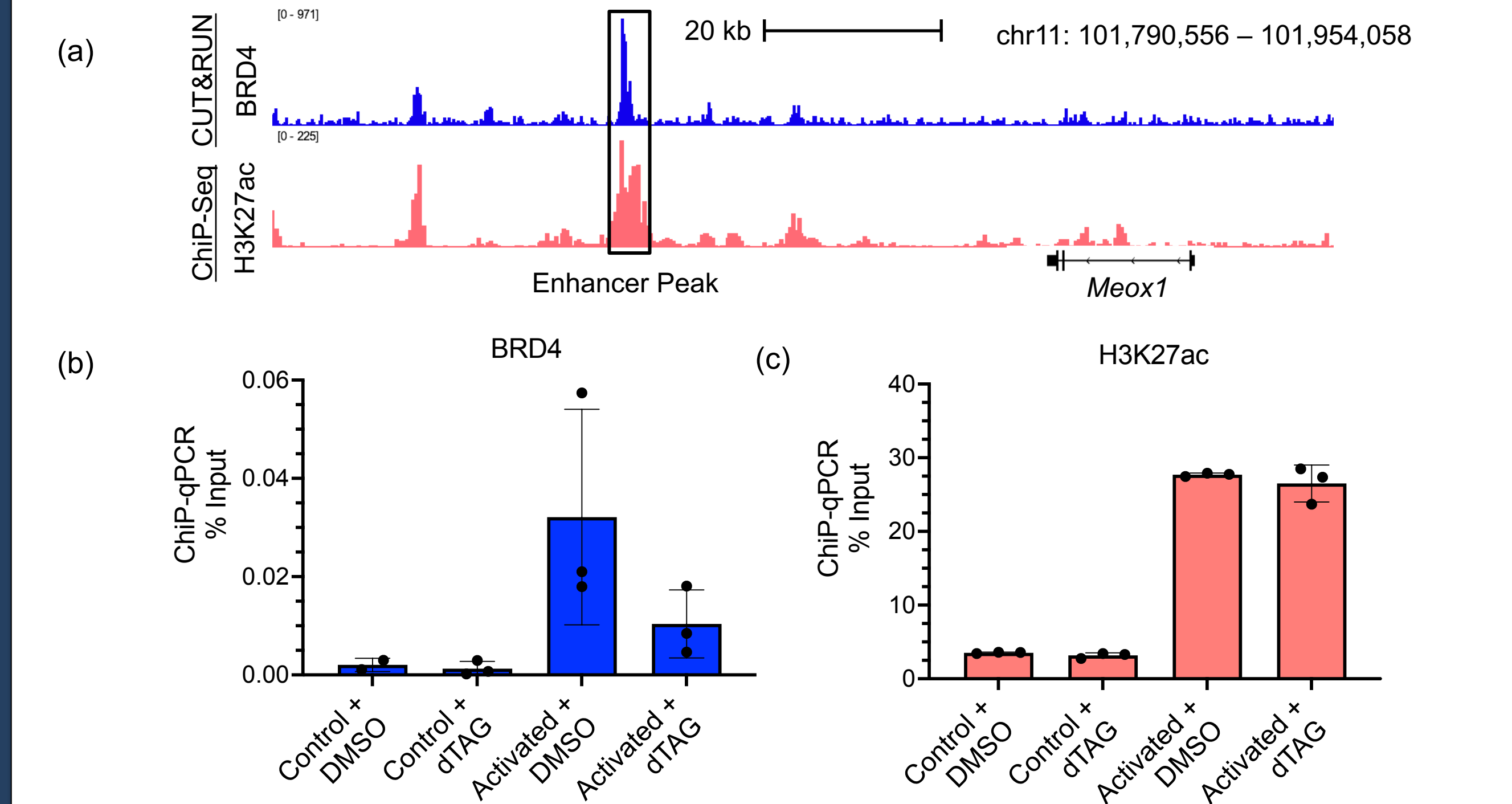


Figure 7. BRD4 occupies the *Meox1* enhancer upon TGF β activation. (a) ChIP-qPCR primer design at enhancer of *Meox1*. BRD4 (b) and acetylated histone (c) occupancy is enriched at *Meox1* enhancer upon TGF β activation.

Fibroblasts are activated with TGFβ stimulation

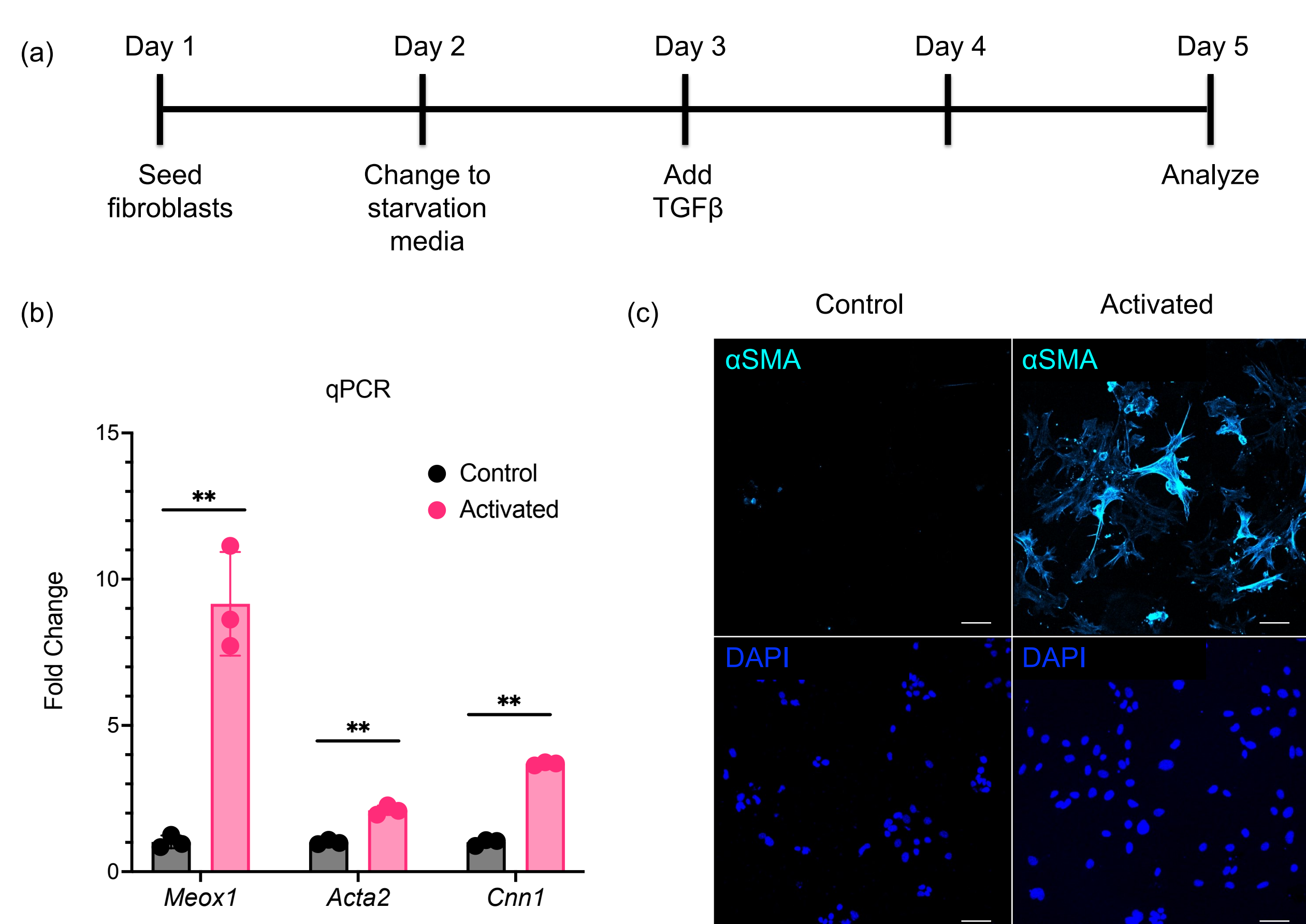


Figure 2. Fibroblasts are activated upon TGF β stimulation. (a) Activation protocol of starving fibroblasts and adding TGF β for 48 hrs. Expression of activated fibroblast markers increases by qPCR (b) and immunofluorescence (c) upon TGF β stimulation. Scale bars 50 μ m.

BRD4 mediates Meox1 enhancer and promoter proximity

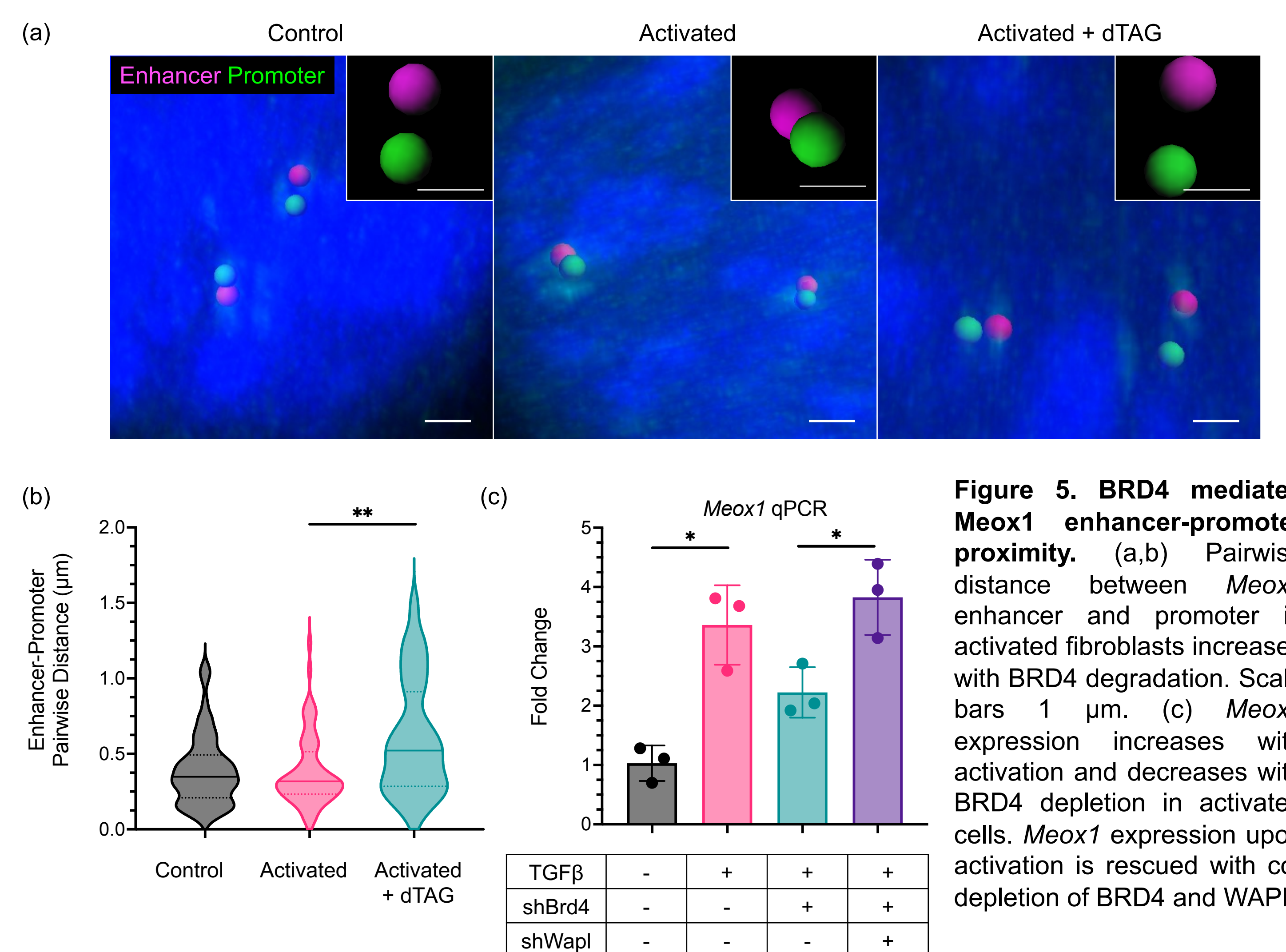
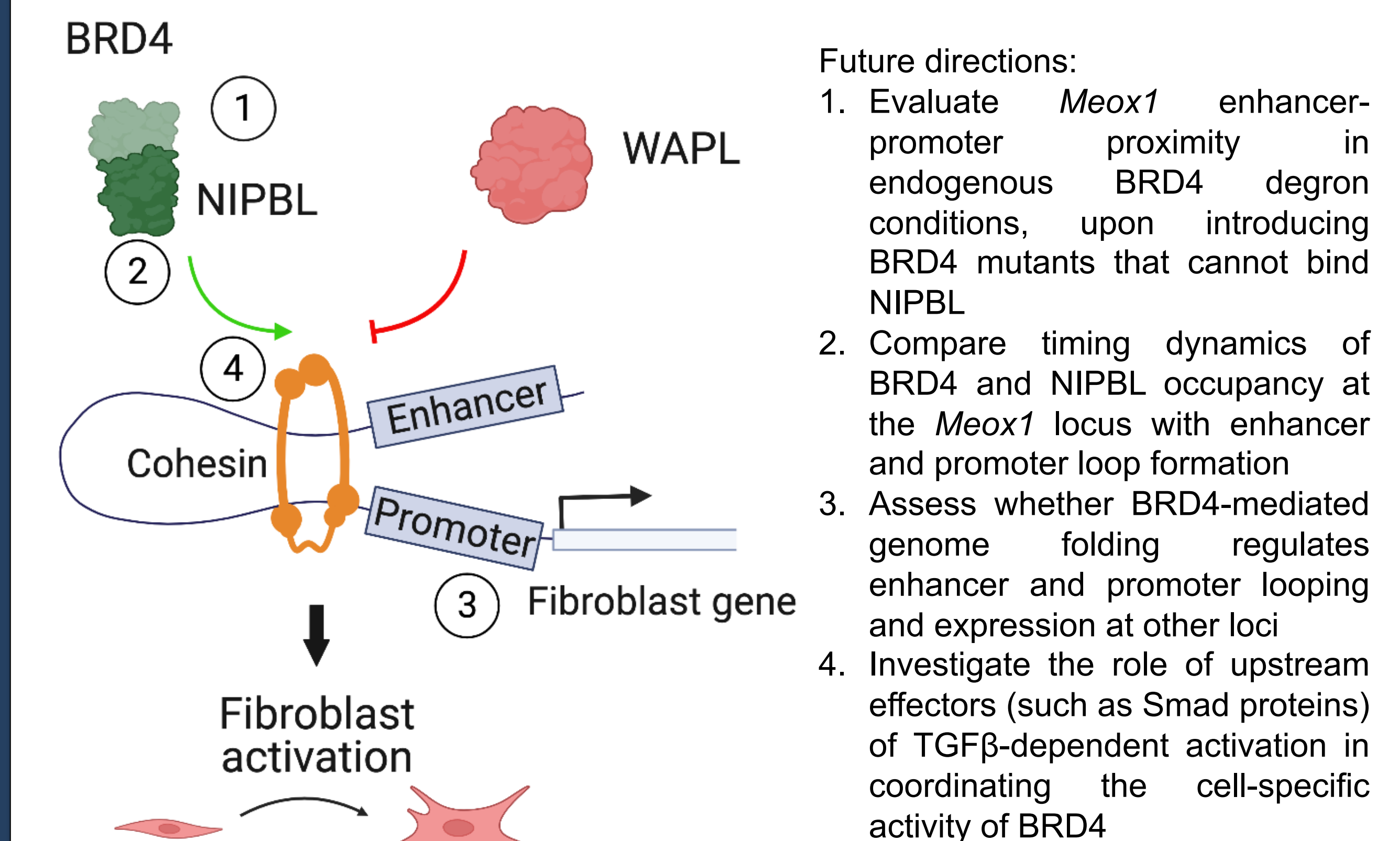


Figure 5. BRD4 mediates *Meox1* enhancer-promoter proximity. (a,b) Pairwise distance between *Meox1* enhancer and promoter in activated fibroblasts increases with BRD4 degradation. Scale bars 1 μ m. (c) *Meox1* expression increases with activation and decreases with BRD4 depletion in activated cells. *Meox1* expression upon activation is rescued with co-depletion of BRD4 and WAPL.

BRD4 mediates chromatin looping at the Meox1 locus



Acknowledgements

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