

Supporting Information

Immunofluorescence Imaging Strategy for Evaluation of the Accessibility of DNA 5-Hydroxymethylcytosine in Chromatins

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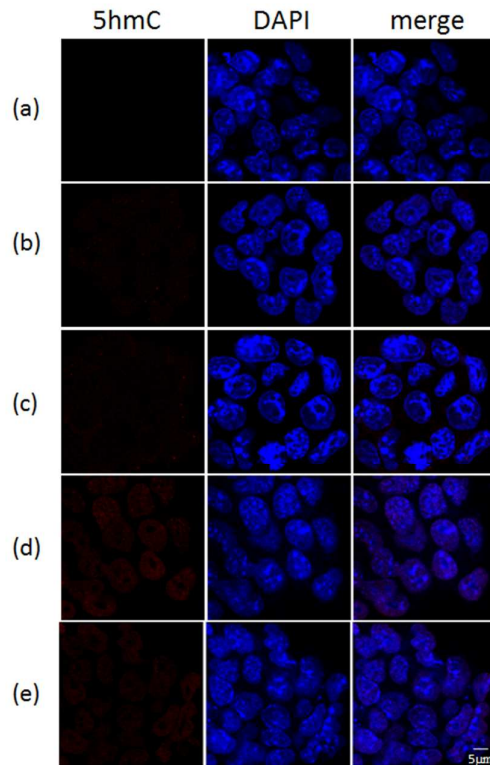


Figure S1. Absence of DNA 5hmC in *Tet1/Tet2* double knockout (DKO) mouse ES cells. (a) negative control, the cells were incubated with no 5hmC primary antibody but with only second antibody; (b) the cells were not pretreated with HCl acidification; (c) the cells were pretreated without HCl acidification but with RNase; (d) the cells were pretreated with HCl acidification; (e) the cells were pretreated both with HCl acidification and RNase.

Note, we analyzed the DNA 5hmC in *Tet1/Tet2* double knockout mouse ES cells, there was no 5hmC foci being observed in the case without or with HCl treatment (Figure S1), since Tet1/Tet2 enzymes mainly contribute to oxidation of 5mC to form 5hmC.

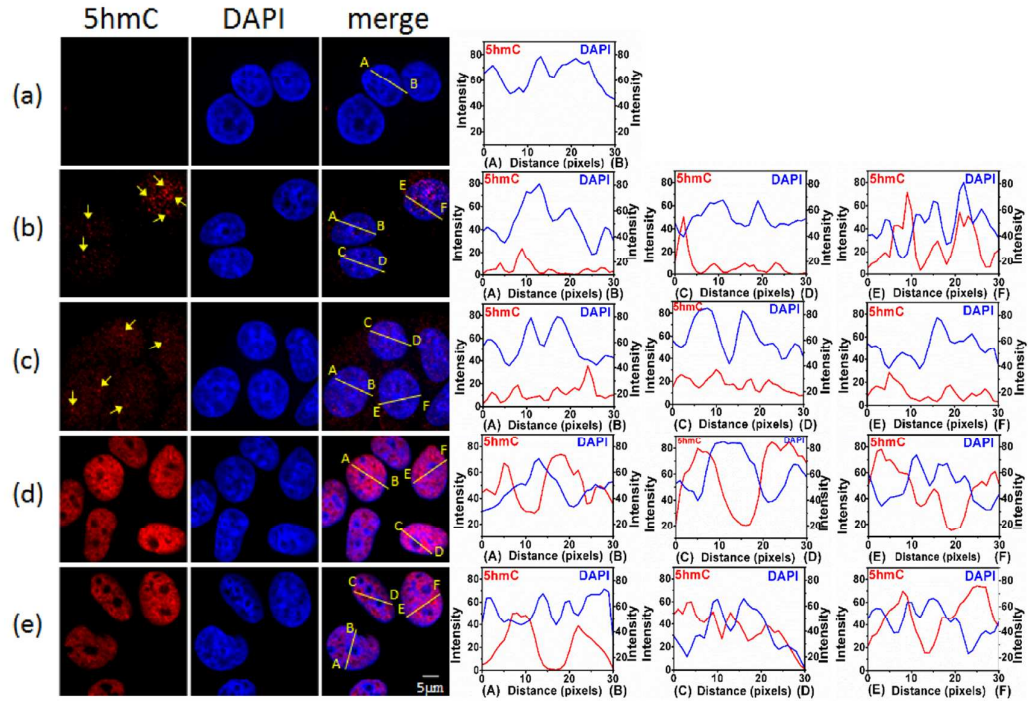


Figure S2. Accessibility of DNA 5hmC in MCF-7 cells. (a) negative control, the cells were incubated with no anti-5hmC primary antibody but with only second antibody; (b), (c), (d), (e): the cells were pretreated without HCl acidification, no HCl acidification but with RNase, HCl acidification, both HCl acidification and RNase, respectively. Arrows indicate fluorescent highlights (yellow arrow). Line scan graphs of 5hmC (red) and DAPI (blue) fluorescence intensities in cells are shown (right).

Left Y axis is shown for 5hmC, and right Y axis is shown for DAPI.

Note, we also evaluated the accessibility of DNA 5hmC in MCF-7 cells. Without HCl acidification treatment, we observed a few of accessible DNA 5hmC foci (Figure S2b), which did not disappear after RNase treatment (Figure S2c). Consistent with the

distribution of accessible DNA 5hmC in WT mES cells, the accessible DNA 5hmC also mainly distributed in the weak DAPI staining regions in MCF-7 cells. With HCl treatment, we observed increased immunofluorescent signals of DNA 5hmC (Figure S2d and S2e).