Supplementary Information

Chromatin condensation and recruitment of PHD finger proteins to histone H3K4me3 are mutually exclusive

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Keywords: PHD finger, phospho/methyl switch, epigenetics, mitosis

Supplementary Figures

Figure S1. The electrostatic surface potentials of the PHD fingers bound to H3K4me3 peptide (yellow sticks) are shown. PDB codes 2F6J, 4L7X, 2QIC, 2G6Q, 2PNX, 3C6W, 4L58, 3LQJ, 3KQI, 3KV4, 3OT4, 3O7A, 2V83 and 2K17. (b) Ribbon diagrams of the indicated PHD fingers in complex with H3K4me3 peptides.

Figure S2. Superimposed $^{1}H,^{15}N$ HSQC spectra of the DIDO PHD finger (left) and the PHF8 PHD finger (right) collected upon titration with indicated peptides.

Figure S3. DIDO (a) and PHF8 (b) are excluded from mitotic chromatin. Random cycling RPE-1 cells were labeled with antibodies against DIDO or PHF8 (red), and H3T3ph (upper panels, green), H3T6ph (middle panels, green) or H3K4me3 (lower panels, green), respectively. DNA was counterstained with DAPI (blue). Single confocal layers corresponding to interphase or mitotic phases are shown, and mitotic phases are indicated. The less visually obvious exclusion of DIDO from mitotic chromatin is due to its association with the mitotic spindle. Scale bars, 5 μm.

Figure S4. (a) Superimposed $^{1}H,^{15}N$ HSQC spectra of the ING1 PHD finger collected upon titration of the triple PTM-containing H3T3phK4me3T6ph peptide (left). Spectra are color coded according to the protein:peptide molar ratio up to 1:4, for comparison with the titrations of H3T3phK4me3 and H3K4me3T6ph peptides (right, also depicted in Fig. 1d). (b) Full titration experiment as in (a), with the protein:peptide ratio up to 1:10 is shown. (c) Representative binding curves used to determine the K_d values by NMR.

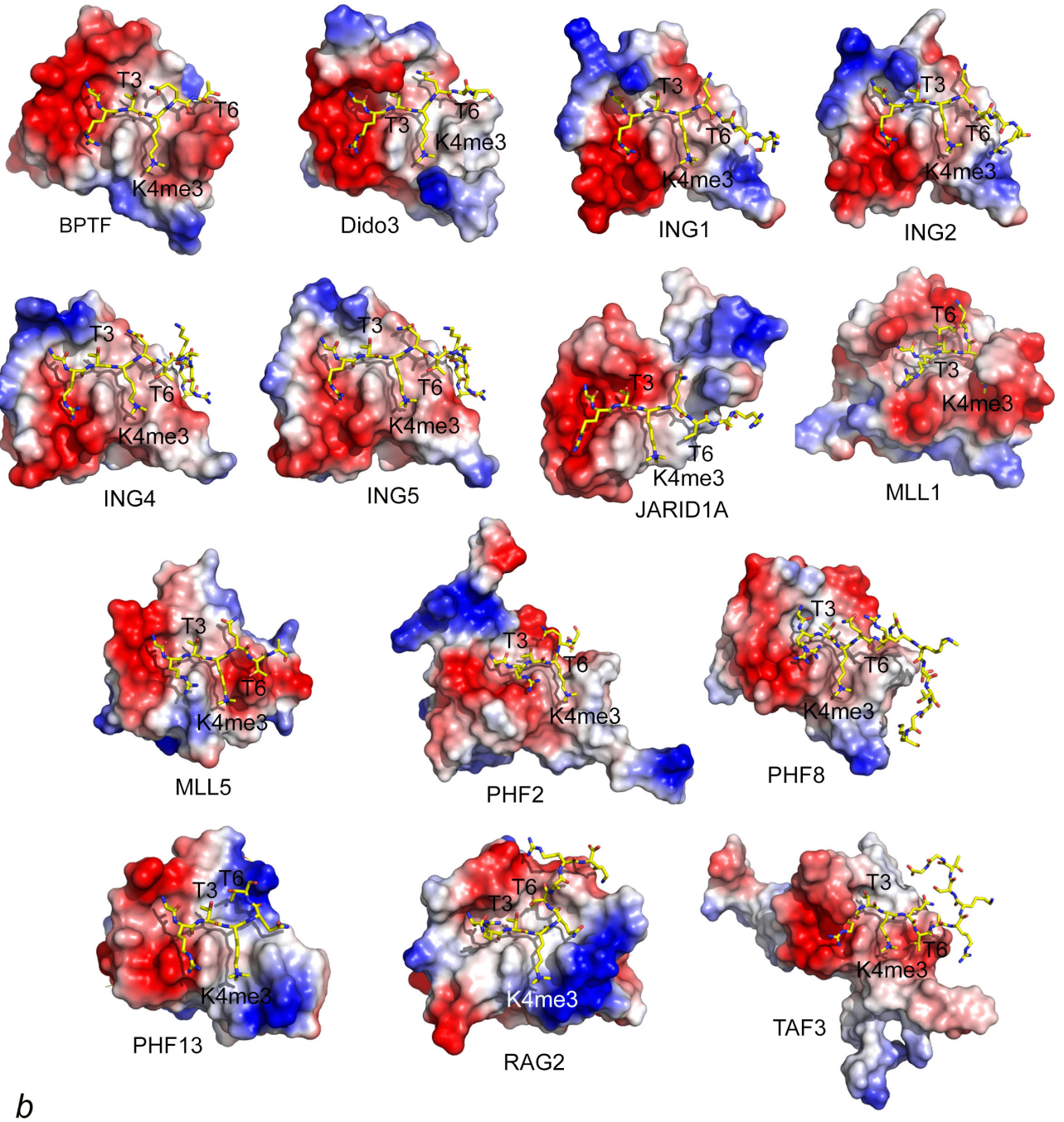
Figure S5. Haspin inhibition results in DIDO (a) and ING1 (b) re-association with telophase chromatin. RPE-1 cells were treated with 1 μM CHR6494 for 24 hours, and labeled as in Figure S3. Control cells were treated with DMSO only. Scale bars, 5 μm.

Figure S6. RPE-1 cells were incubated with 2 μM CHR6494 for 24 hours, and labeled as in Fig. 4c. H3K4me3, proteins (DIDO and ING1), and DNA are green, red and blue, respectively. Scale bars, 5 μm.

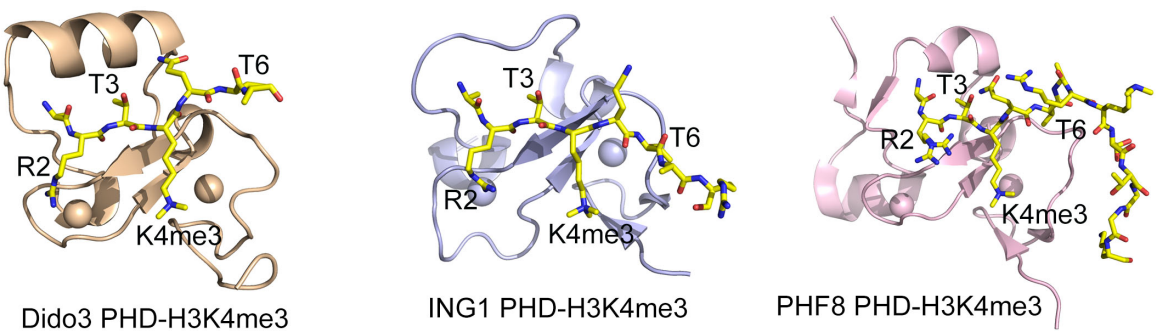
Figure S7. Images of the same section from peptide microarrays probed with the indicated proteins. Red spots indicate protein binding to peptides containing H3K4me3. The spots containing H3T3pK4me3 and H3K4me3T6p are boxed in white and blue, respectively.

Gatchalian et al., Suppl. Fig. S1

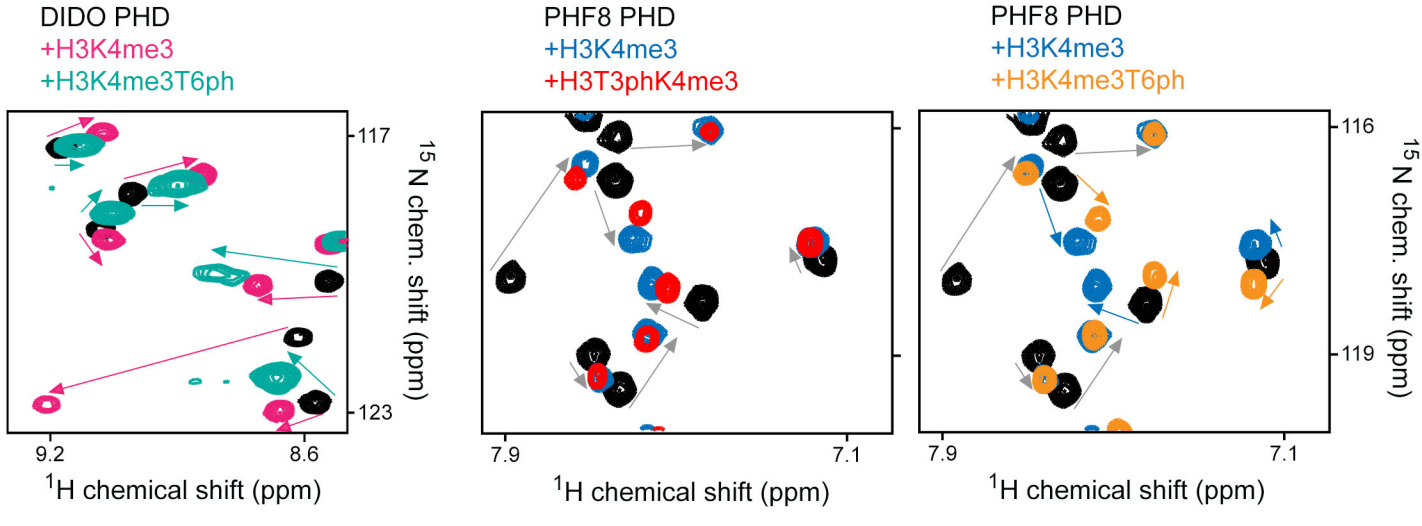
a PHD-H3K4me3 complexes



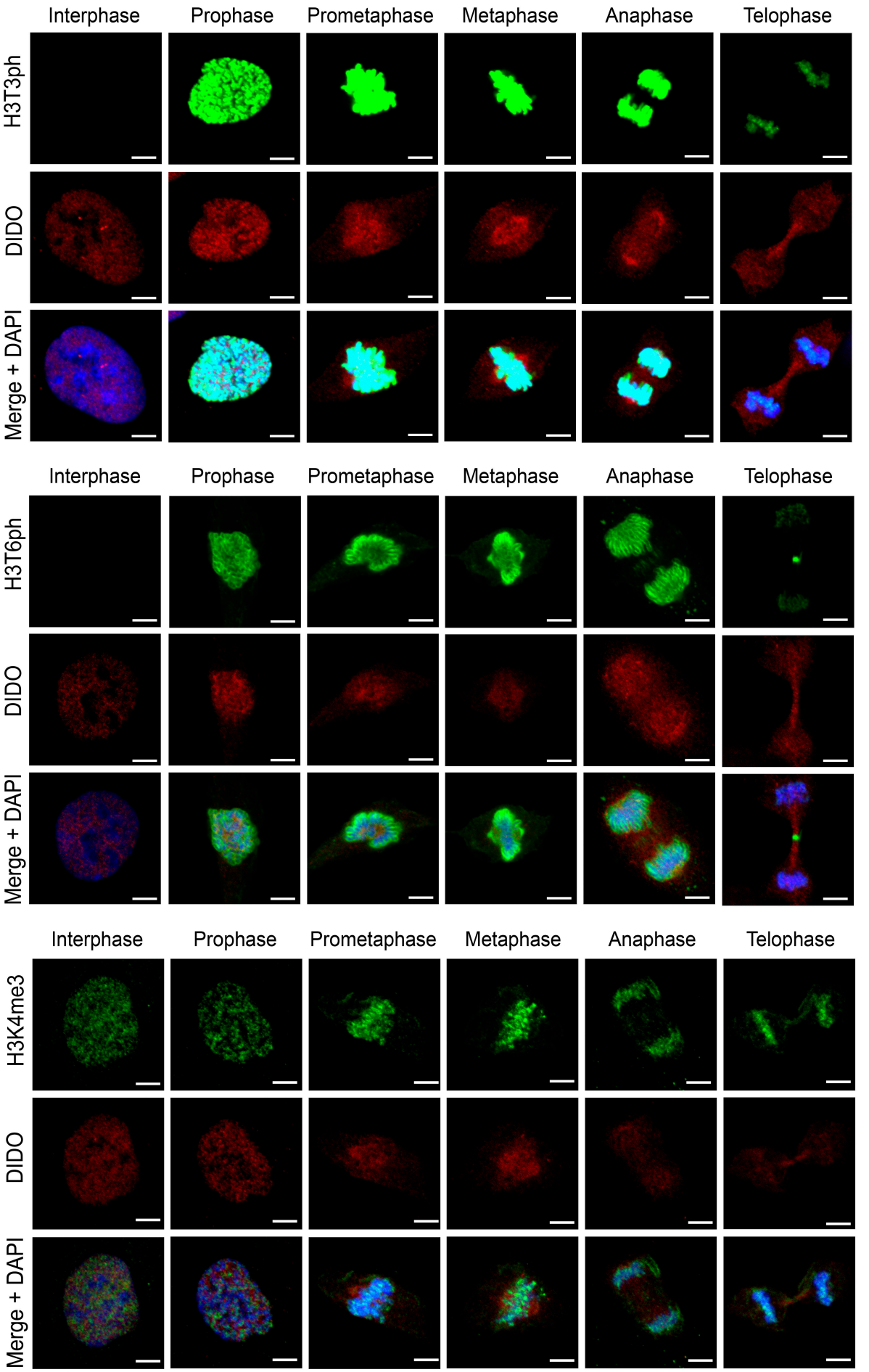
b



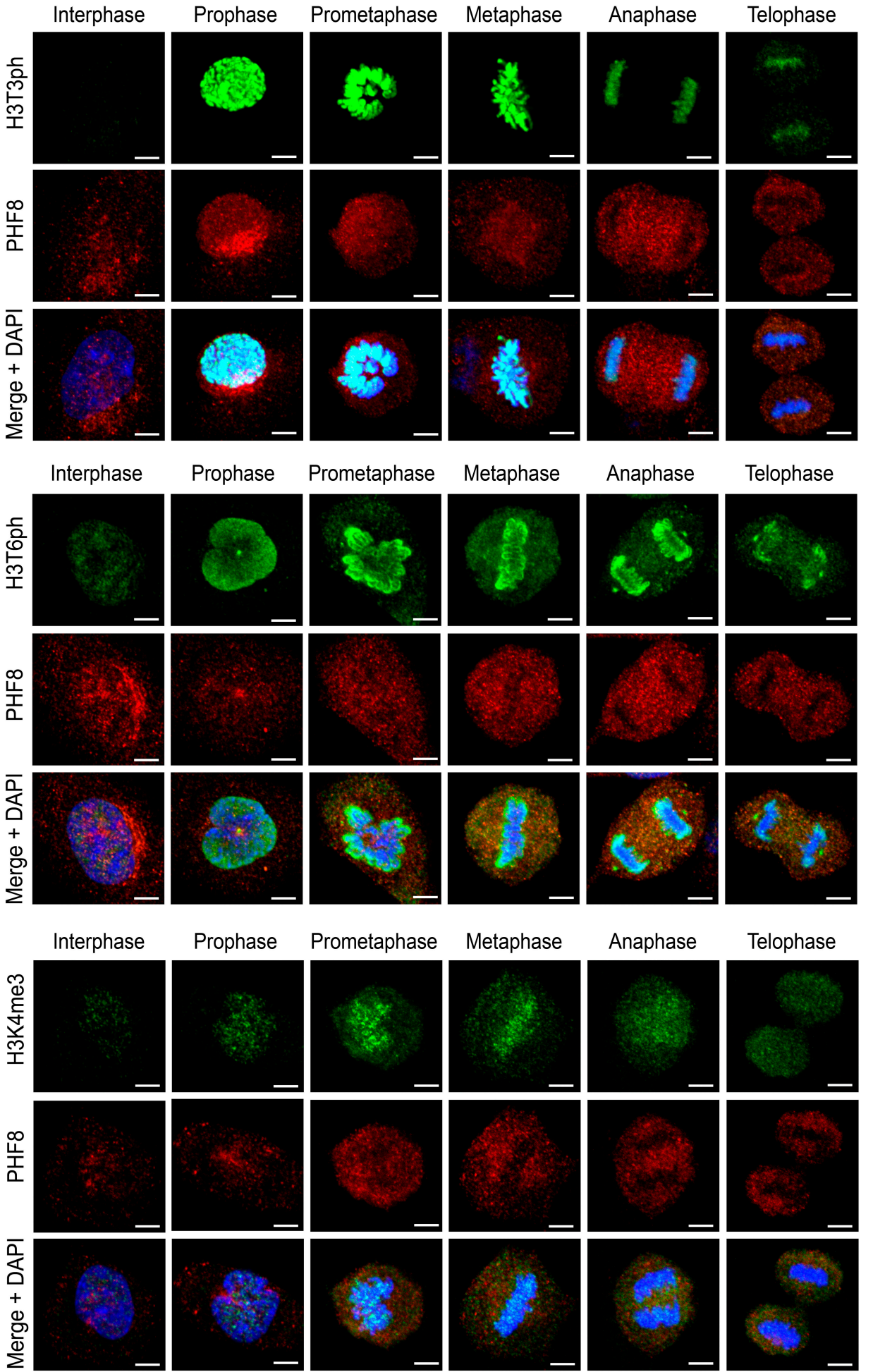
Gatchalian et al., Suppl. Fig. S2



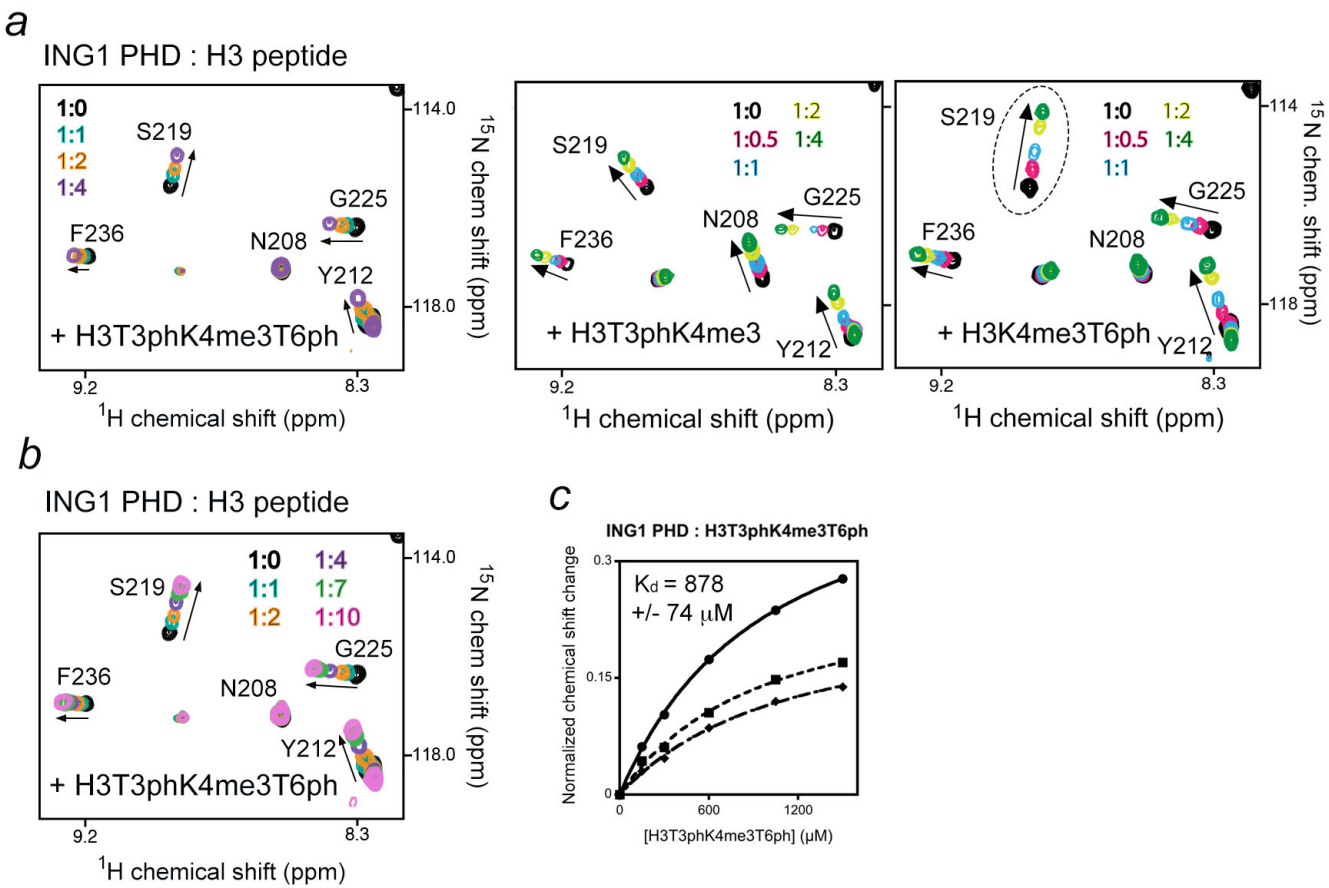
Gatchalian et al., Suppl. Fig. S3a



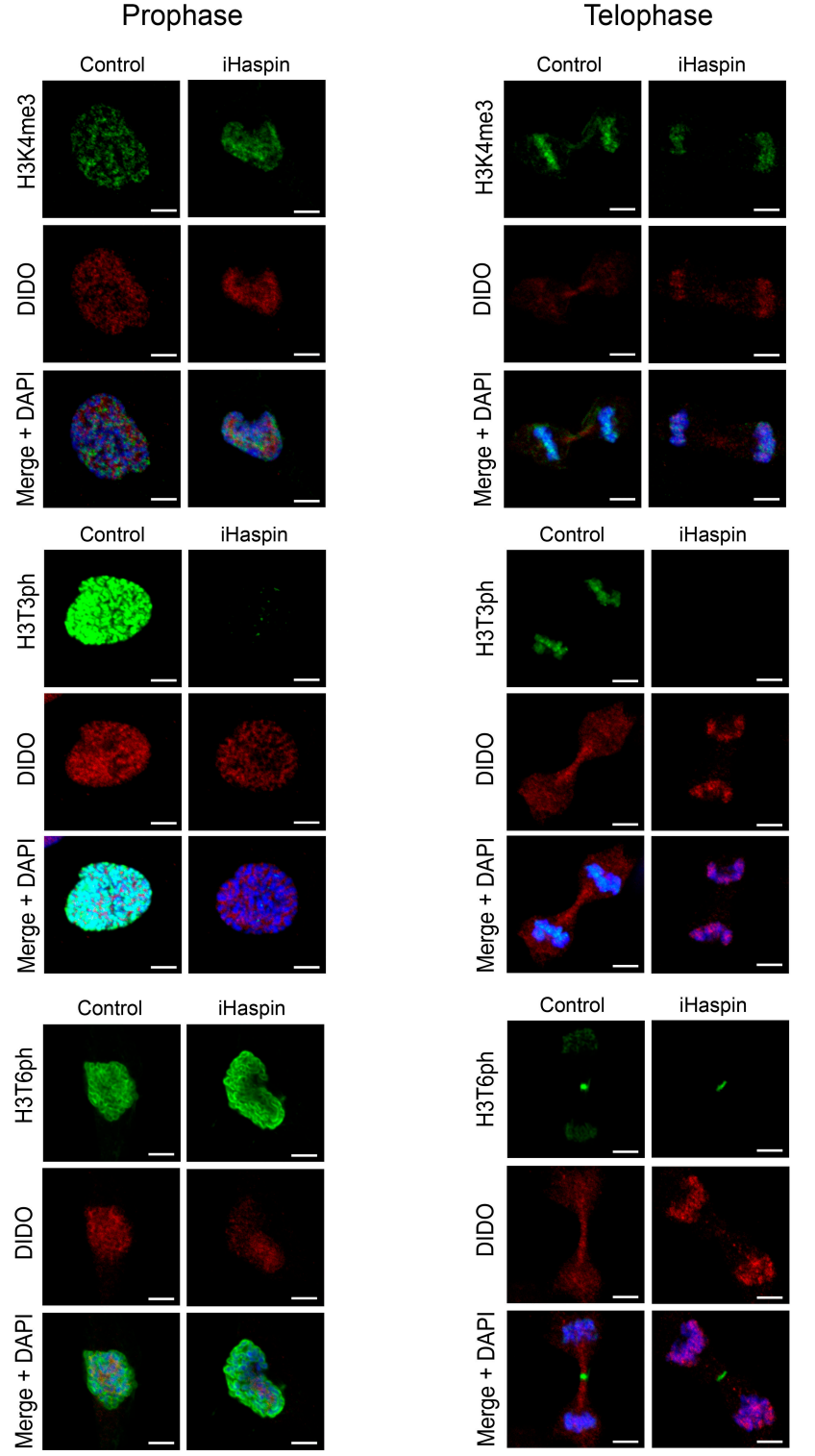
Gatchalian et al., Suppl. Fig. S3b



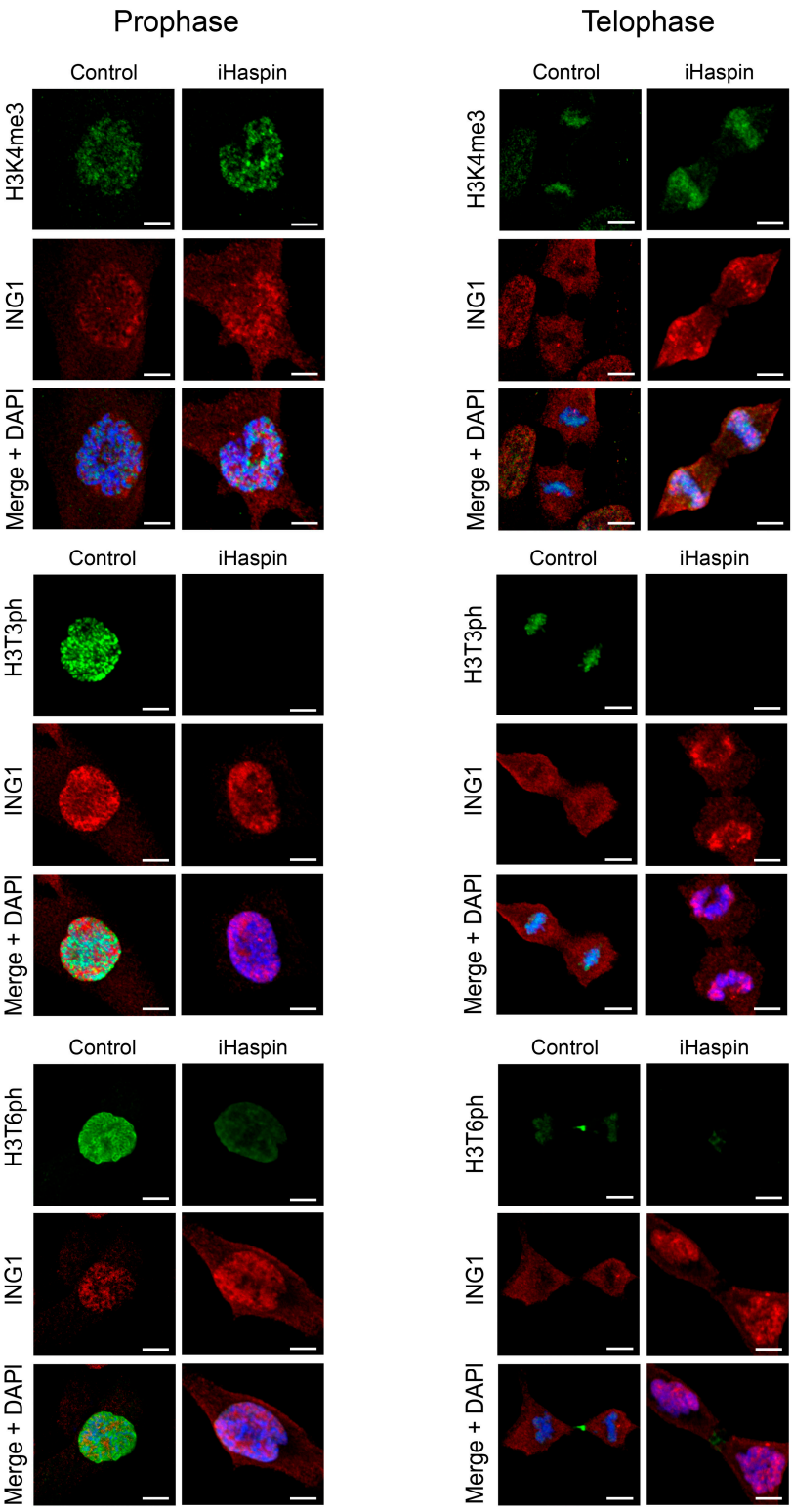
Gatchalian et al., Suppl. Fig. S4



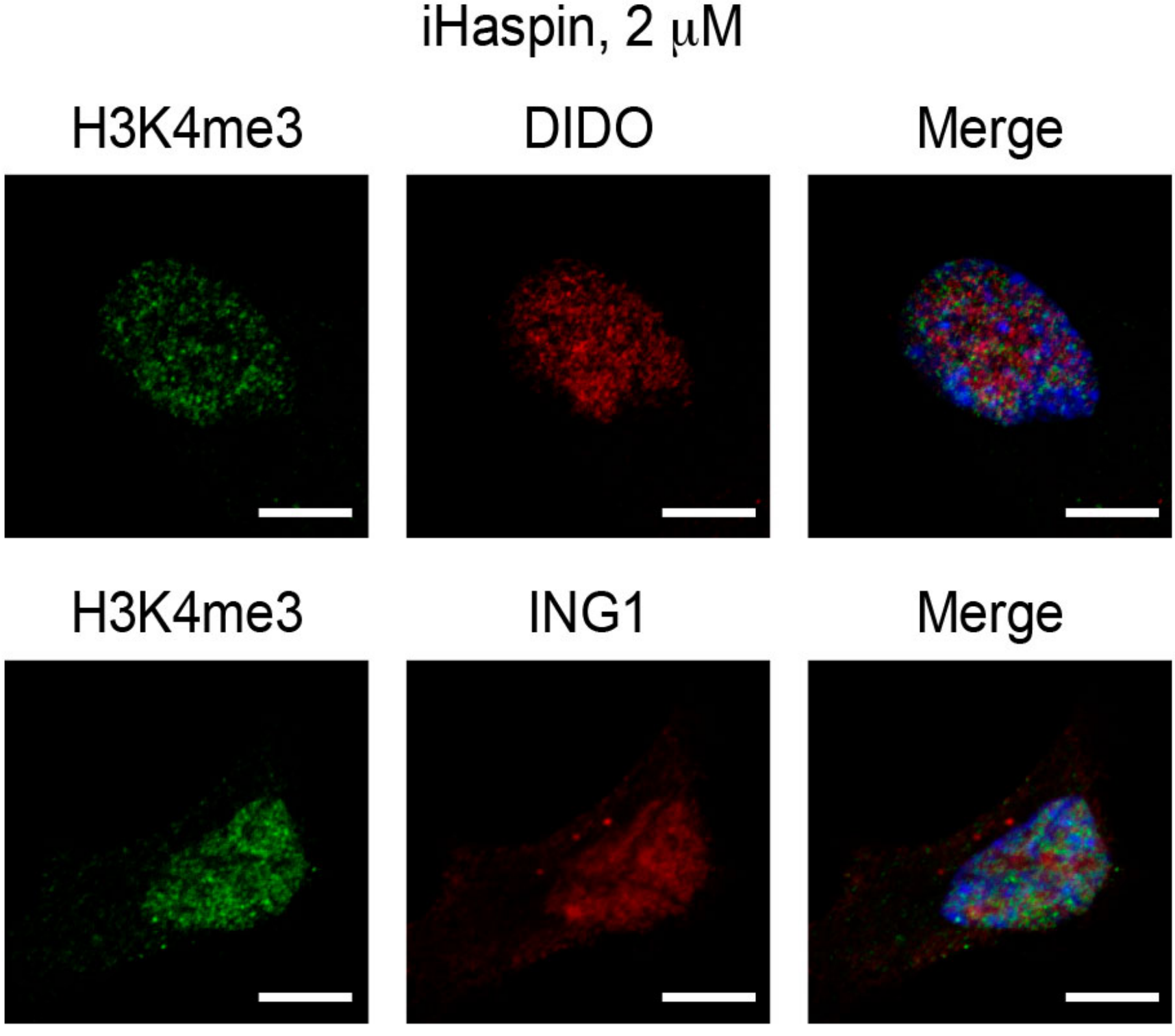
Gatchalian et al., Suppl. Fig. S5a



Gatchalian et al., Suppl. Fig. S5b



Gatchalian et al., Suppl. Fig. S6



Gatchalian et al., Suppl. Fig. S7