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Supplemental Material to:

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Cell cycle-dependent chromatin shuttling of HBO1–JADE1 histone acetyl transferase (HAT) complex

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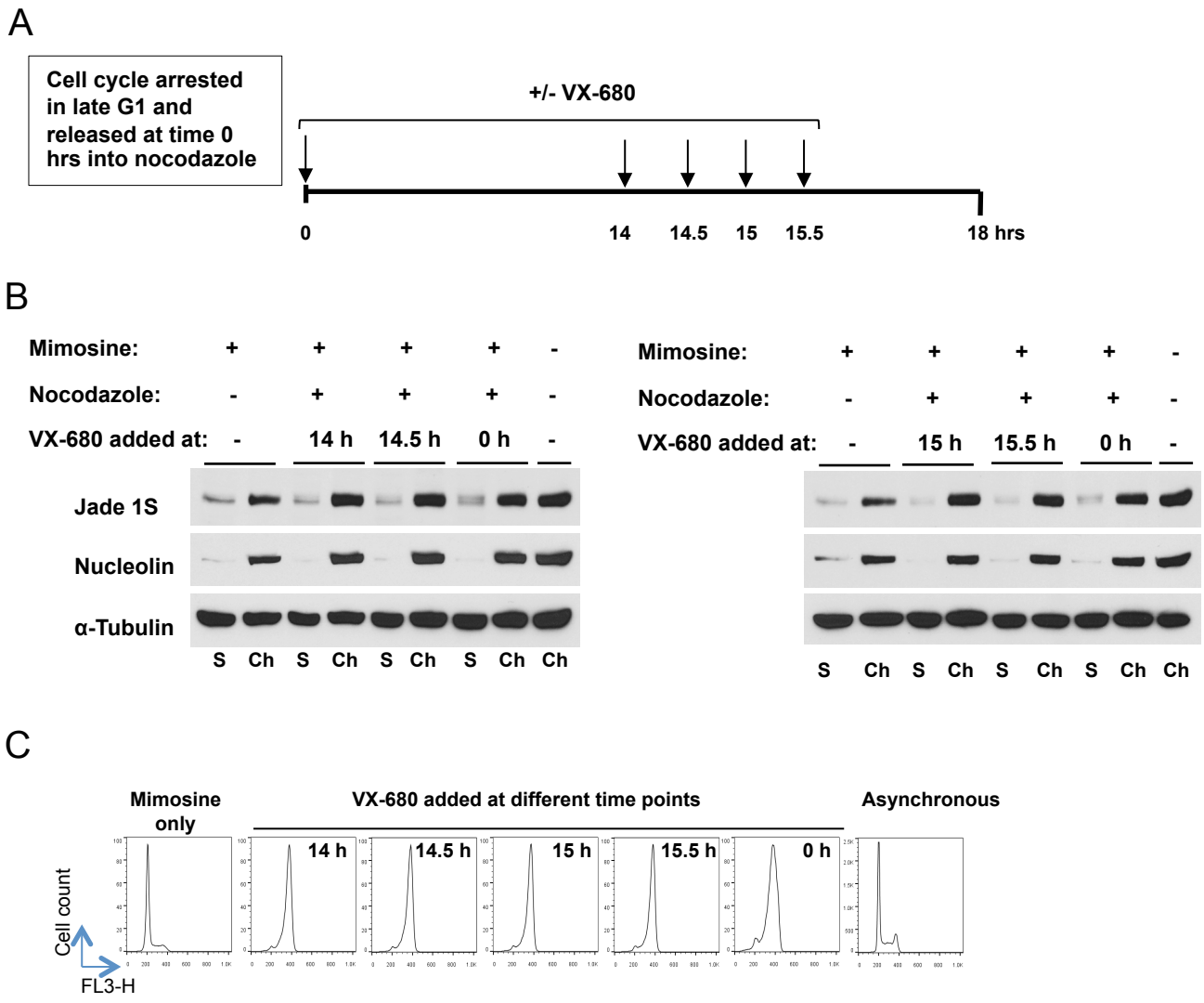
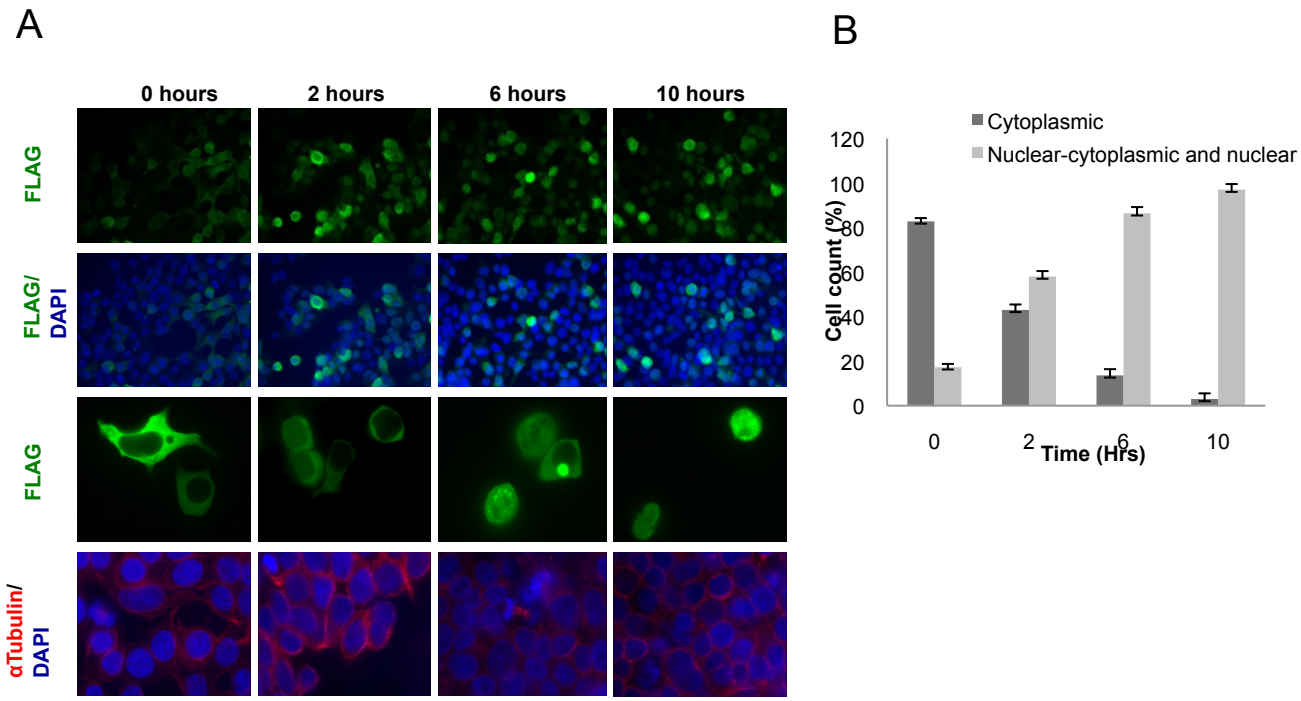


S1. JADE1S phosphorylation and chromatin dissociation is hindered by pharmacological inhibitor of Aurora A kinase. (A) Experimental design: HeLa cells were synchronized by arresting cell cycle at late G1 with mimosine. At 0 hrs cell cycle was released by refreshing media supplemented with nocodazole. At indicated time points VX-680 was added into the media with nocodazole (0, 14, 14.5, 15 and 15.5 hrs) and at 18hrs samples were collected for analysis as described in Fig 4 except here, the total cell population was collected (no mitotic shake off). (B) Soluble (S) and chromatin-enriched (Ch) fractions were analyzed for endogenous JADE1S, β-Actin (loading control) and nucleolin (nuclear marker) by western blots. Soluble fraction after nocodazole and chromatin fraction of asynchronous cells was used as a reference for high and low molecular mass specie of JADE1S, respectively. Note that the band shift and the chromatin dissociation corresponding to JADE1S was inhibited by the addition of VX-680. (C) Cell cycle profiles of the samples by FACS analysis. All samples originated from the same experiment. Due to space limitation samples were run simultaneously on two separate gels using same conditions and transferred onto membranes in the same plane. Each blot contains appropriate controls to enable comparison.



S2. Leptomycin B induces JADE1S accumulation in the cell nucleus. (A) Cells (293T/17) were transfected with FLAG-JADE1S, treated with Leptomycin B for a time course and processed for IF. Different composite images DAPI/JADE1S and DAPI/Tubulin are chosen for better visualization. Magnification: upper two rows, 20x; lower two rows, 60x. (B) Quantitation of (A). The error bars represent standard deviation.