Supplemental Figures

Supplemental Figure 1. Mcm2-7 chromatin association is abolished in Dup/Cdt1 RNAi and G2 arrested cells.

A. FACs profiles of DNA content for each cell population used to determine Mcm2-7 chromatin loading. **B.** Cytoplasmic and nuclear fractions were assayed for Orc2 and Mcm2-7 by western blot. A non-specific band is indicated by an asterisk (*). **C.** The Mcm2-7/ORC ratios from each condition were normalized to the HU ratio for each independent blot and plotted on a $\log_{10}$ scale. **D.** Representative cell cycle profile from an asynchronous *Drosophila* cell population treated with nonspecific control RNAi.

Supplemental Figure 2. Immunofluorescence of nuclear Mcm2-7 in an asynchronous cell population.

A. H4K20me1 is specifically enriched in late S-phase and G2/M. FACS analysis of H4K20me1 (red) as a function of DNA content. **B.** Mcm2-7 and H4K20me1 are mutually exclusive. H4K20me1 signal as a function of Mcm2-7 signal. Cells enriched for H4K20me1 (late S-phase, G2/M) have low levels of Mcm2-7**. C.** Representative panel of cells stained with DAPI (blue), Mcm2-7 (green) and H4K20me1 (red). G1 and early S-phase staining cells (low H4K20me1) exhibit a range of nuclear Mcm2-7. The small-localized region of Mcm2-7 in H4K20me1 positive cells represents cells that are in late S-phase as the Mcm2-7 is confined to the heterochromatic and late replicating DAPI bright chromocenter.

Supplemental Figure 3. ORC and Mcm2-7 ChIP enrichment mark early activating origins of replication.

The log2 enrichment of ORC (red) and Mcm2-7 (green) are plotted relative to the peaks of BrdU incorporation from cells arrested with HU. BrdU incorporation is limited to the sequences immediately adjacent to early origins. 93% of the early origin peaks contain significant ORC and Mcm2-7 peaks.

Supplemental Figure 4. Genome-wide location analysis of ORC and Mcm2-7 for multiple cell cycle points.

A. Genome-wide analysis of ORC localization by ChIP-chip from an asynchronous cell population. ORC enrichment from asynchronous cells is depicted for a 5 Mb section of

chromosome 2L. **B.** Genome-wide analysis of ORC localization by ChIP-chip from cells arrested in HU. ORC enrichment from HU arrested cells is depicted for a 5 Mb section of chromosome 2L. **C.** Genome-wide analysis of Mcm2-7 localization in early G1 by ChIP-chip. Mcm2-7 enrichment from cyclin E RNAi depleted cells is depicted for a 5 Mb section of chromosome 2L. **D.** Genome-wide analysis of Mcm2-7 localization in HU arrested cells by ChIP-chip. Mcm2-7 enrichment from HU arrested cells is depicted for a 5 Mb section of chromosome 2L.

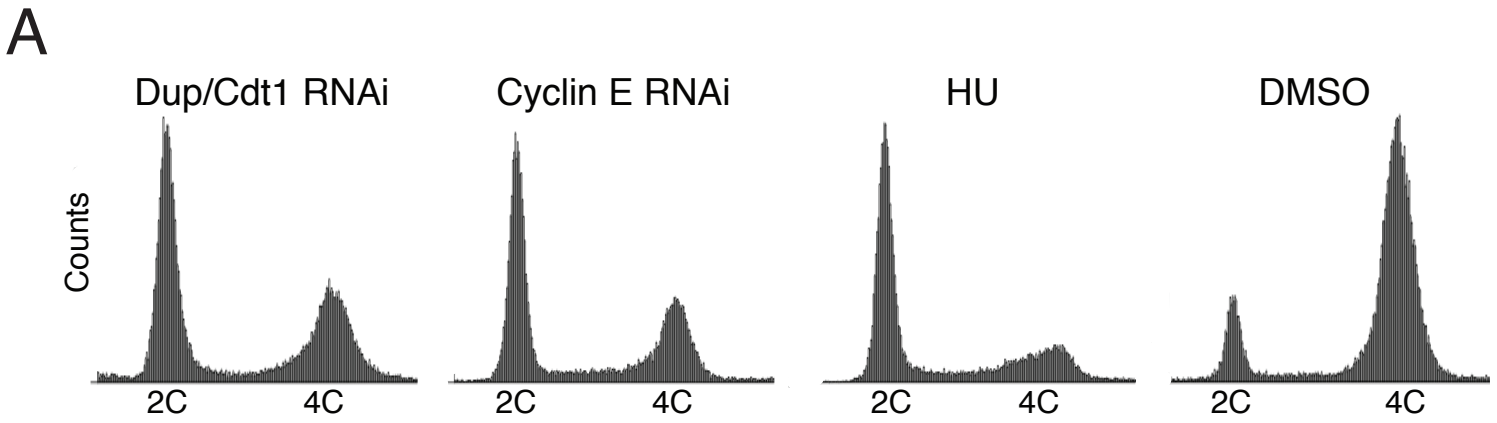
Supplemental Figure 5. Bimodal enrichment of Mcm2-7 at active and inactive transcripts is not dependent on early origin activation.
Box-plots of Mcm2-7 enrichment for actively transcribed and non-transcribed genes grouped by their overlap with early origins of DNA replication.

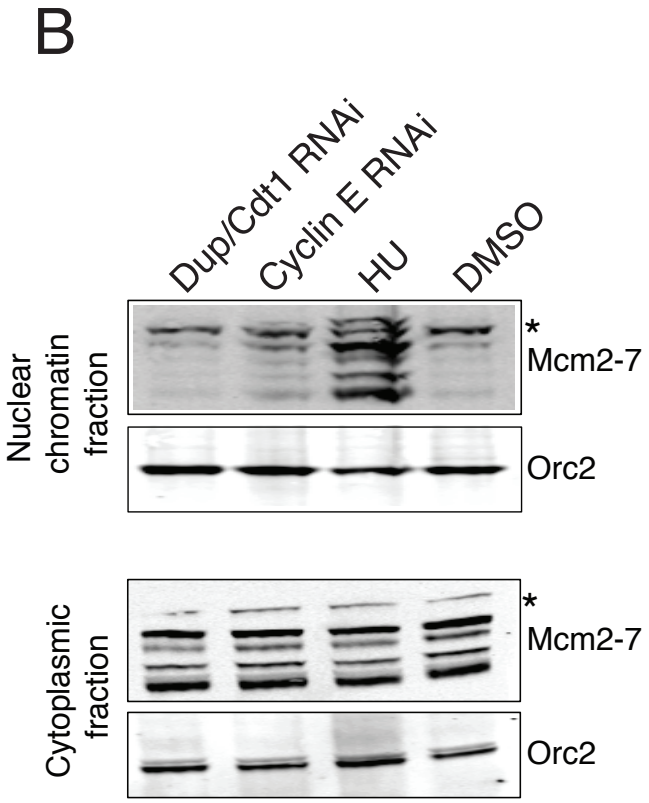
Supplemental Figure 6. Mcm2-7 enrichment in non-transcribed genes is indistinguishable from intergenic levels.
Box-plots of Mcm2-7 enrichment over genic and intergenic regions. Non-transcribed genic regions have a similar level of Mcm2-7 enrichment signal as intergenic regions.

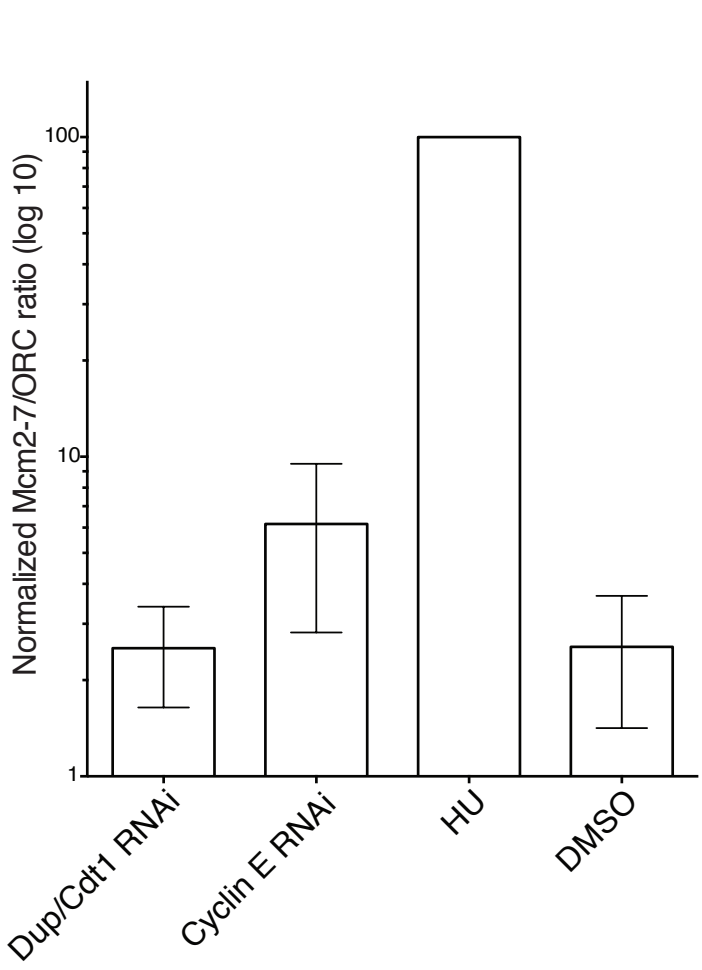
Supplemental Figure 7. Active transcription is necessary for Mcm2-7 depletion from gene bodies.
Box-plots of Mcm2-7 enrichment for actively transcribed and non-transcribed genes in two different cell lines (Kc167 (green) and S2 (red)). Mcm2-7 is displaced from differentially expressed genes in the two different cell lines. Relative Mcm2-7 levels are shown for genes that are transcribed in one cell line and off in the other. Upregulated in S2 are those genes that are on in S2 and off in Kc. Upregulated in Kc are those genes that are on in Kc and off in S2. (Mann-Whitney-Wilcoxon Test; $p<7x10^{-5}$).

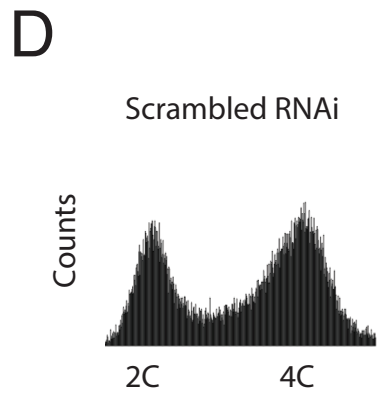
Supplemental Figure 8. Chromatin fractionation optimization.
A. 1 mM HU and 3% DMSO treated cells were subjected to chromatin fractionation. For each cell cycle condition, the following fractions are shown. Lane 1, cytoplasmic fraction; lane 2, soluble fraction in high salt buffer (480 mM KCl); lane 3, pellet fraction from high salt buffer (480 mM KCl); lane 4, pellet fraction following a wash in low salt buffer (20 mM KCl). **B.** Chromatin fractionation and solubilization with MNase. Lane 1, whole cell extract; lane 2, cytoplasmic fraction; lane 3, supernatant from wash of nuclear

pellet in low salt buffer; lane 4, nuclear pellet fraction; lane 5, soluble fraction following treatment of nuclear pellet with MNase, lane 6, soluble fraction following treatment of nuclear pellet without MNase. Orc2, Mcm2-7 and Histone H3 are shown for the fractions.

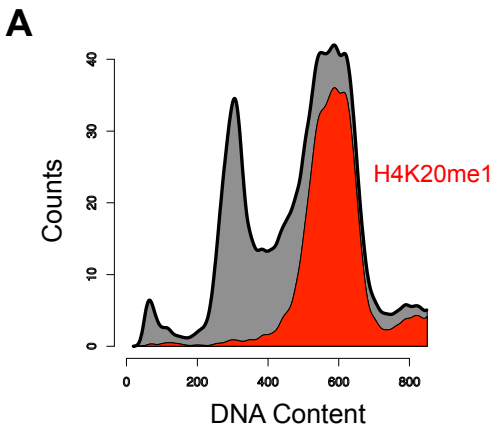


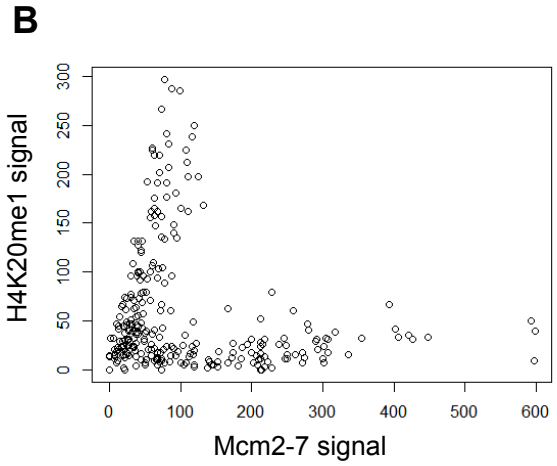


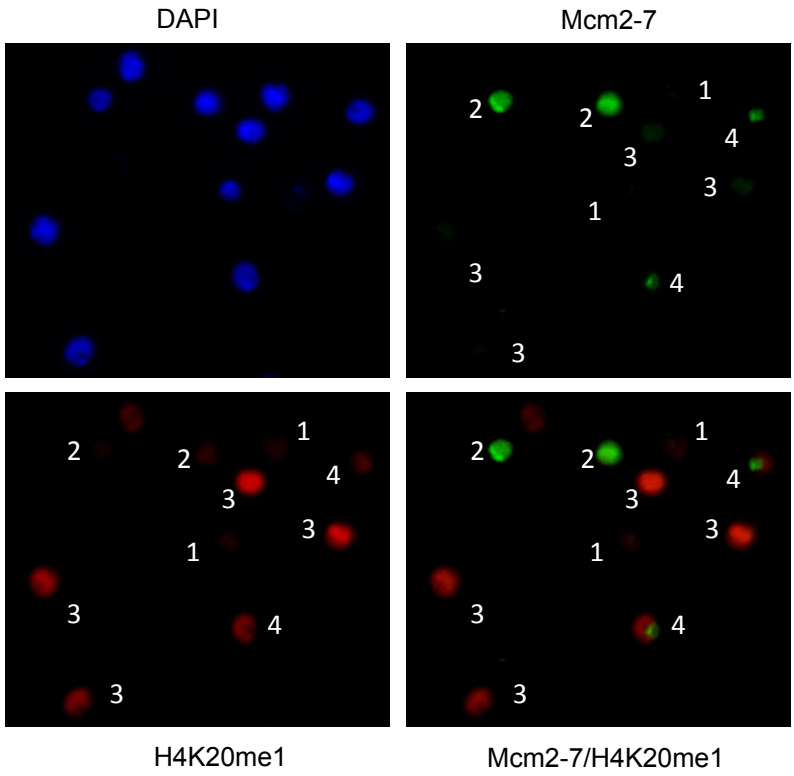




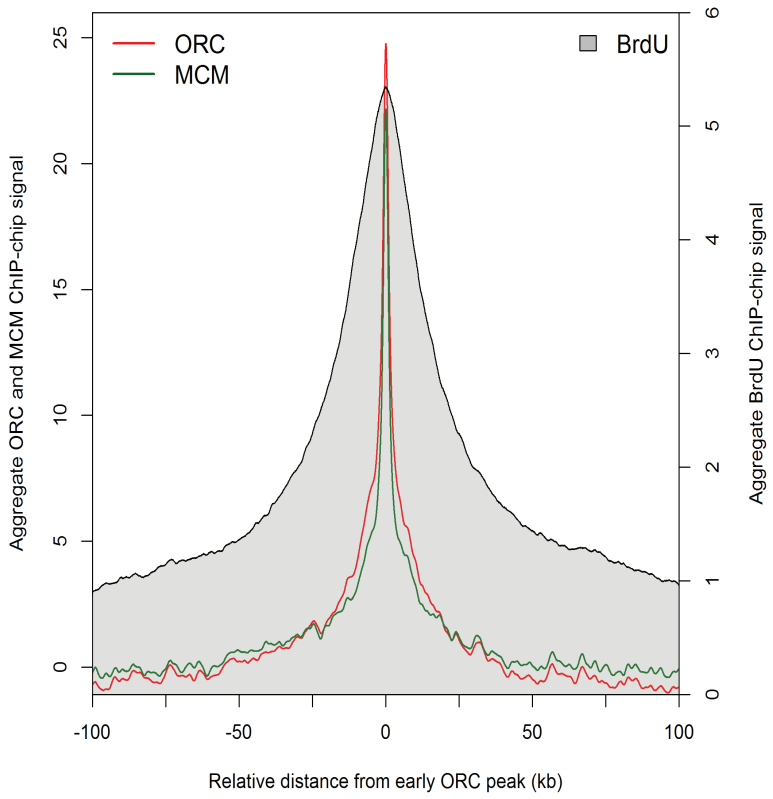
Supplemental Figure 1



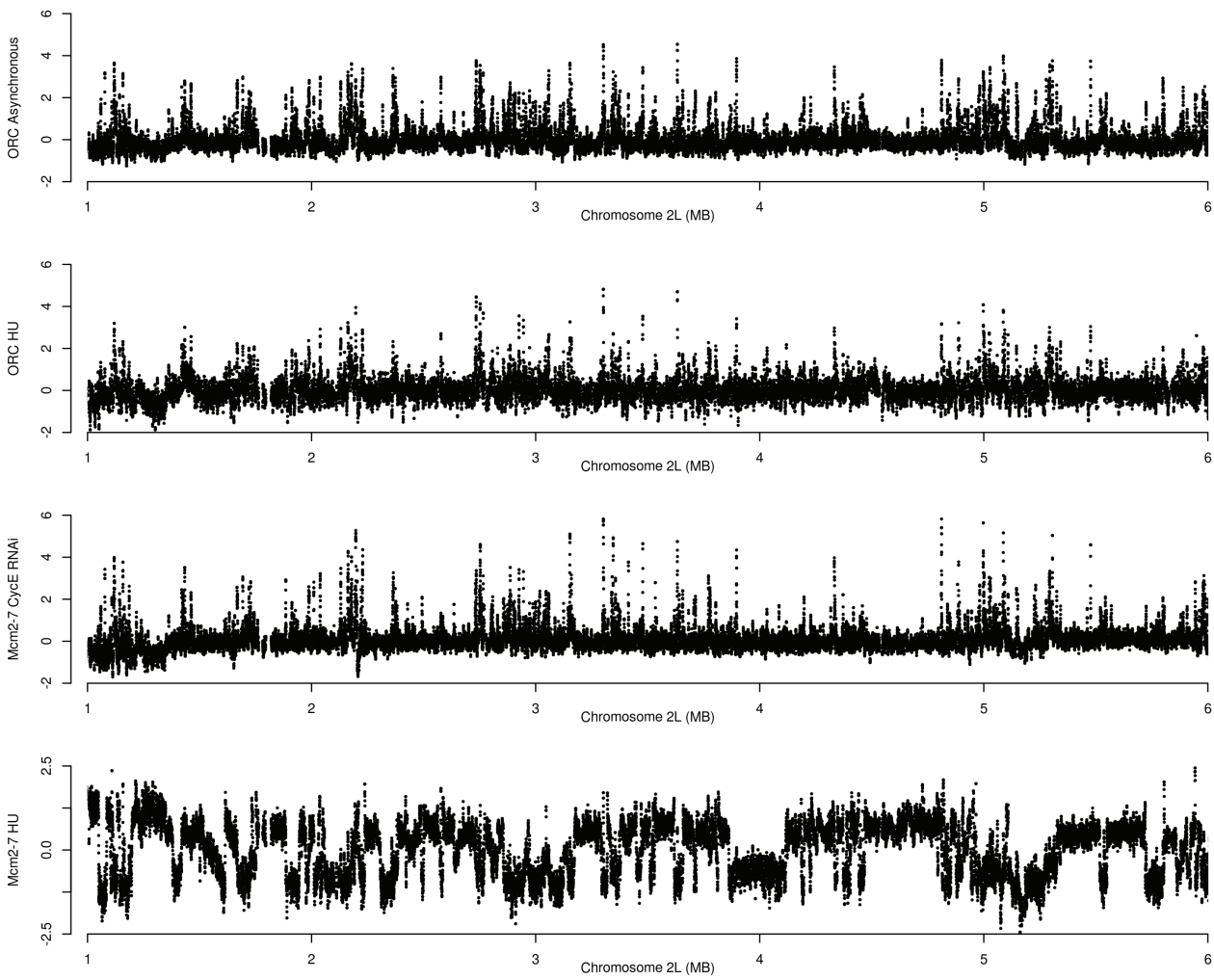




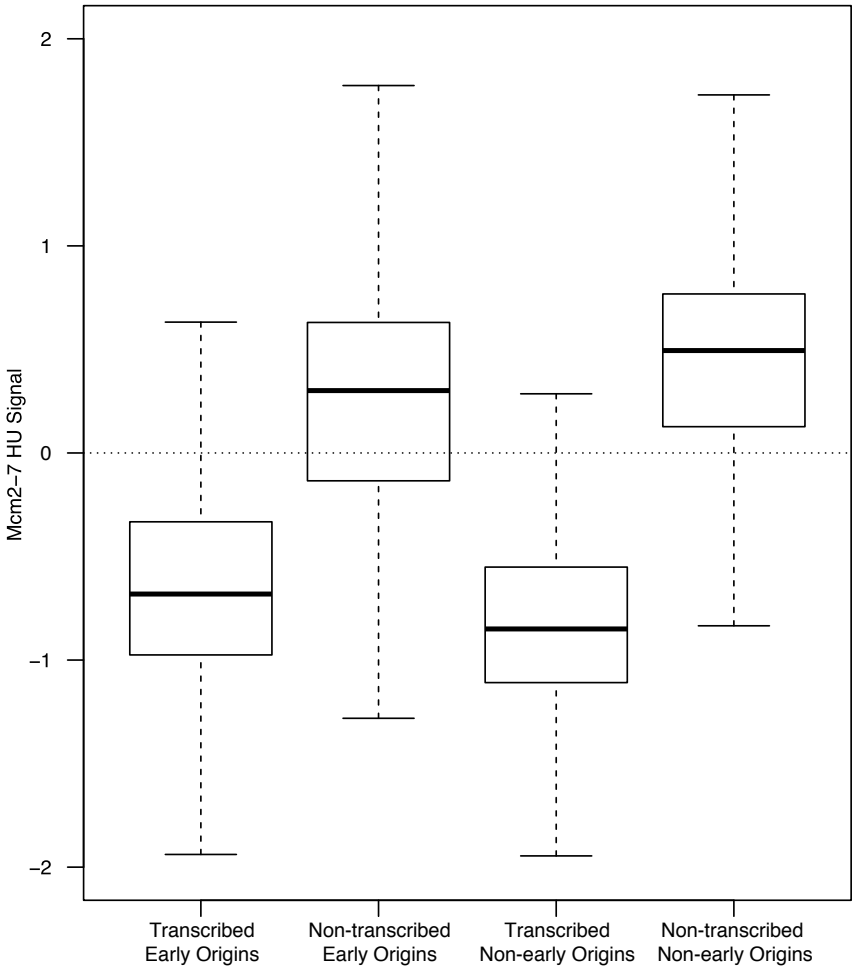
1. Low Mcm2-7/Low H4K20me1 = Early G1
2. High Mcm2-7/Low H4K20me1 = Late G1/Early S
3. Low Mcm2-7/High H4K20me1 = G2/M
4. Punctate Mcm2-7/High H4K20 me1 = Late S



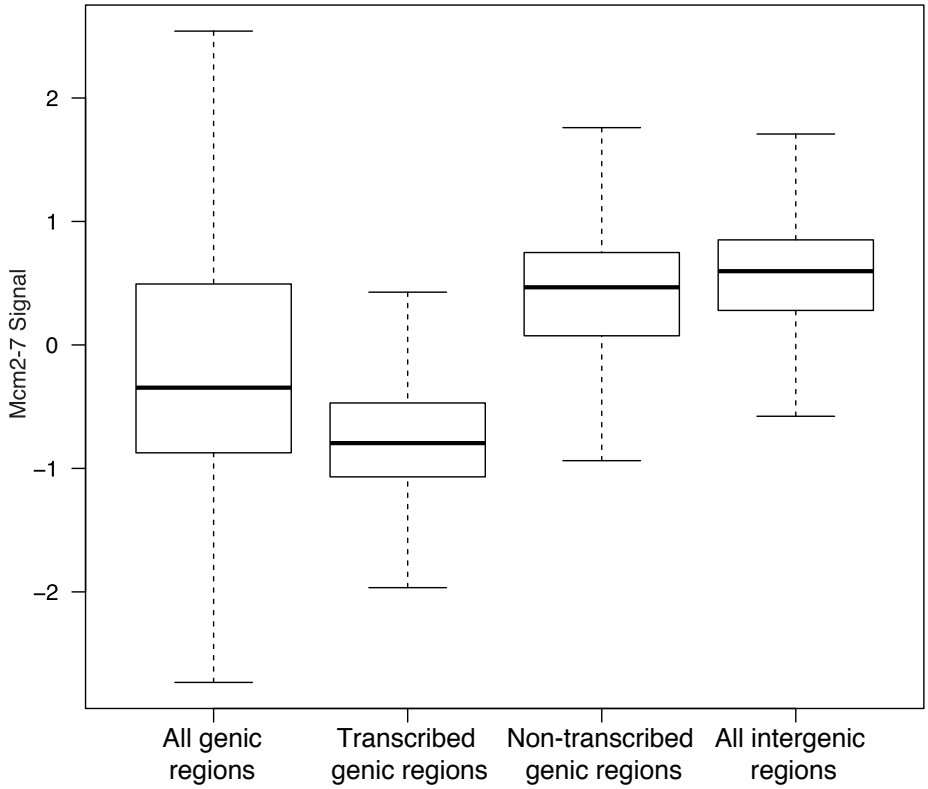
Supplemental Figure 3



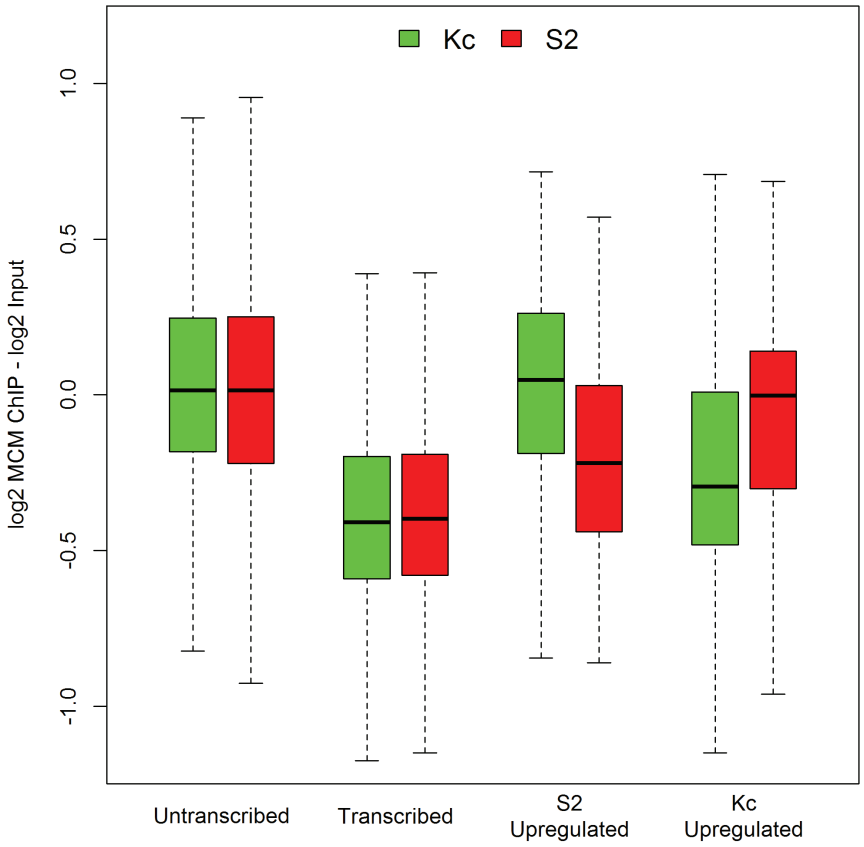
Supplemental Figure 4



Supplemental Figure 5

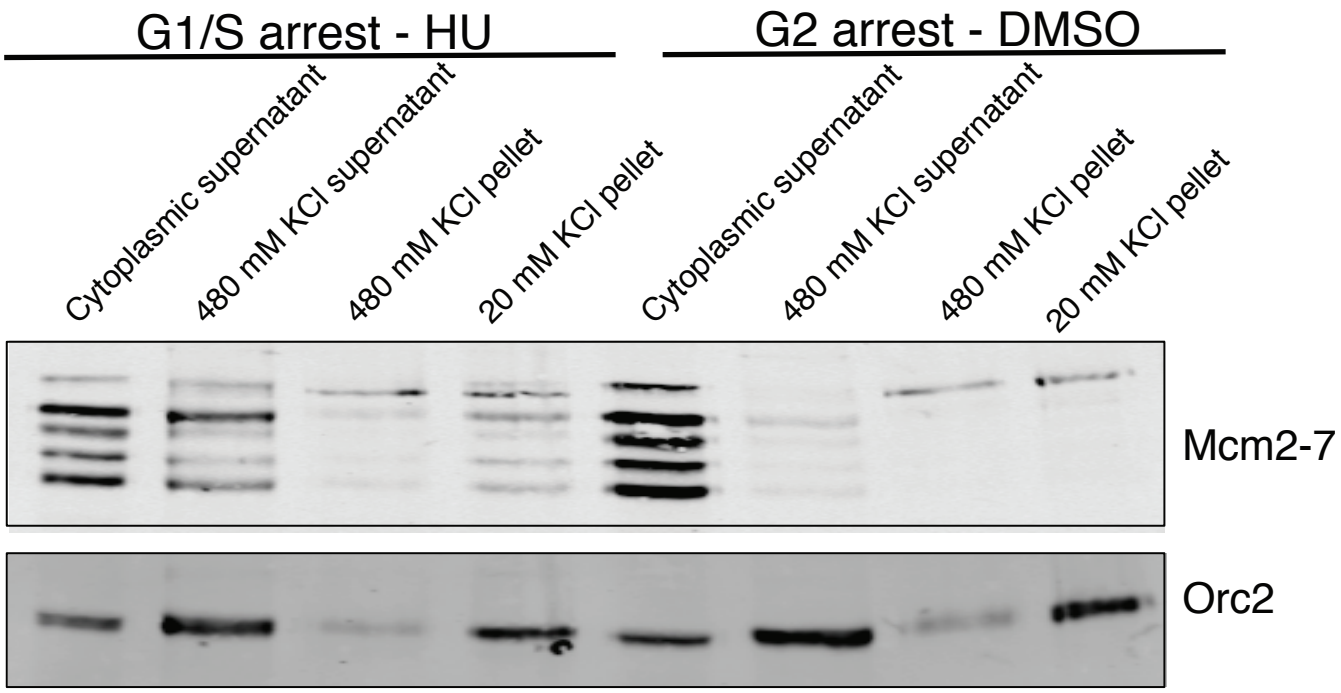


Supplemental Figure 6



Supplemental Figure 7

A



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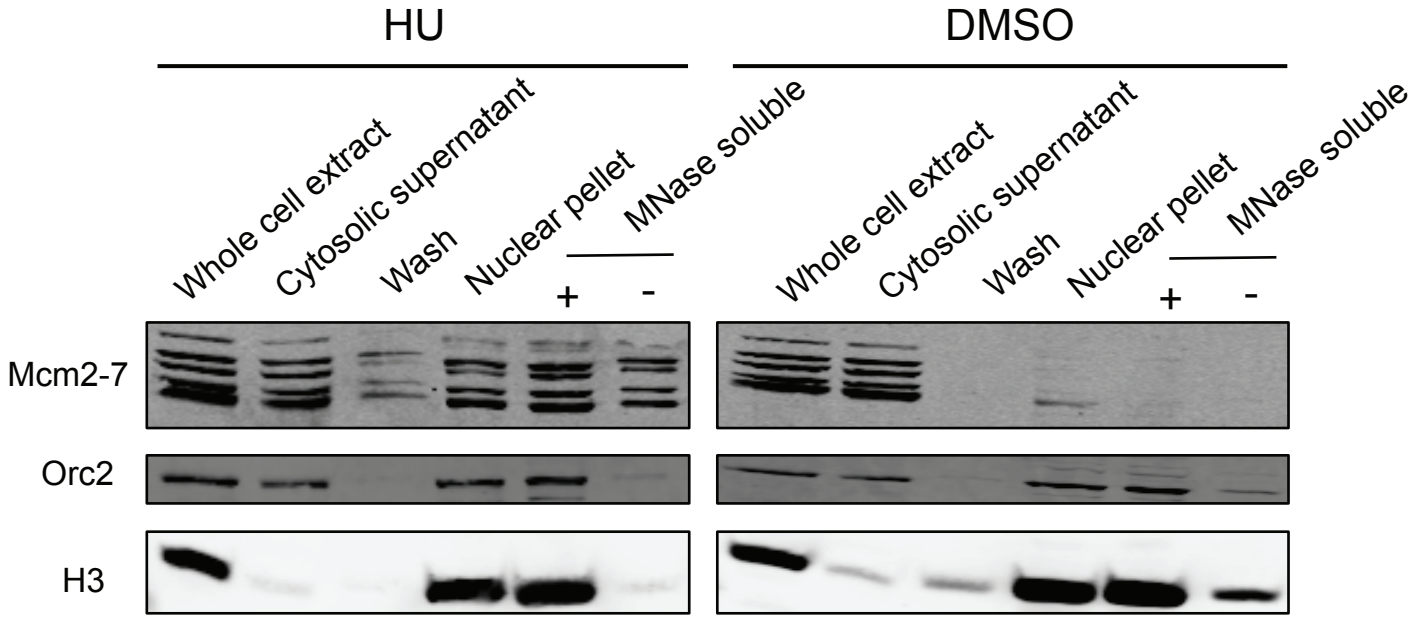

Supplemental Figure 8