Supplemental Figure 1 Chromatin immunoprecipitation of TAP-tagged ORC2 with IgG coupled sepharose beads confirms dORC2 polyclonal antibody ChIP results. (A) Comparison of ChIP signal from biological replicates for the dORC2 antibody, ORC2-TAP, and an IgG control. Chromatin extracts from ORC2-TAP expressing or control cells were immunoprecipitated with IgG sepharose beads. Log2 enrichment of MA2C normalized signal is depicted for a 2 Mb region of chromosome X. Although the pattern of enrichment is similar between ORC2-TAP and the dORC2 ChIP, the signal and sensitivity is decreased in the ORC2-TAP experiment which is likely due to ORC2-TAP being assembled into a limited subset of ORC complexes. (B) Comparison of individual replicates for dORC2 ChIP. (C) Comparison of individual replicates for ORC2-TAP ChIP. (D) Comparison of individual replicates for IgG control ChIP.

Supplemental Figure 2 Distribution of Affymetrix expression scores from *Drosophila* Kc167 cells. Density plot of the distribution of scores. Transcripts were grouped into 5 equal bins based on their expression level. Each bin is marked by a colored rectangle at the bottom of the graph.

Supplemental Figure 3 Chromatin profiling of ORC associated sequences. (A) H2Av is enriched at ORC sequences that overlap with promoter elements. The average enrichment of H2Av is plotted relative to the position of ORC peaks which were proximal (overlapping) or distal to a promoter. The overall decrease in H2Av signal is likely due to the decrease in gene density. (B) Chromatin profiling with salt at ORC binding sites. Average enrichment of salt extracted chromatin fractions relative to ORC binding sites.

Supplemental Figure 4 Cell-cycle arrest of *Drosophila* Kc167 cells. (A) Asynchronous cells. (B) Cells arrested at G1/S with 1 mM HU. (C) Cells arrested at G2 with 3% DMSO. (D) Cells arrested at G1 by RNAi depletion of DUP for 24 hours. (E) Western blot analysis of DUP depletion. Cells were treated with RNAi directed against *dup* or a non-specific control (pUC) for the indicated time. ORC2 is shown as a loading control. B4 equals before treatment. (F) Relative abundance of pre-RC components and cohesin complex members from whole cell extract prior to the chromatin spin down assay.

Supplemental Figure 5 Predicted nucleosome occupancy and ORC binding for the *Drosophila* genome. The background shows the average ORC ChIP enrichment (blue illustrates low enrichment, pink high enrichment). The 1-probability of predicted nucleosome occupancy is plotted as a black curve. Peaks in the curve represent regions of predicted nucleosome depletion. The nucleosome predictions were computationally derived for the *Drosophila* genome by Kaplan and colleagues (Kaplan et al., 2009). ORC only localizes to a small subset of potential open chromatin in the *Drosophila* genome.

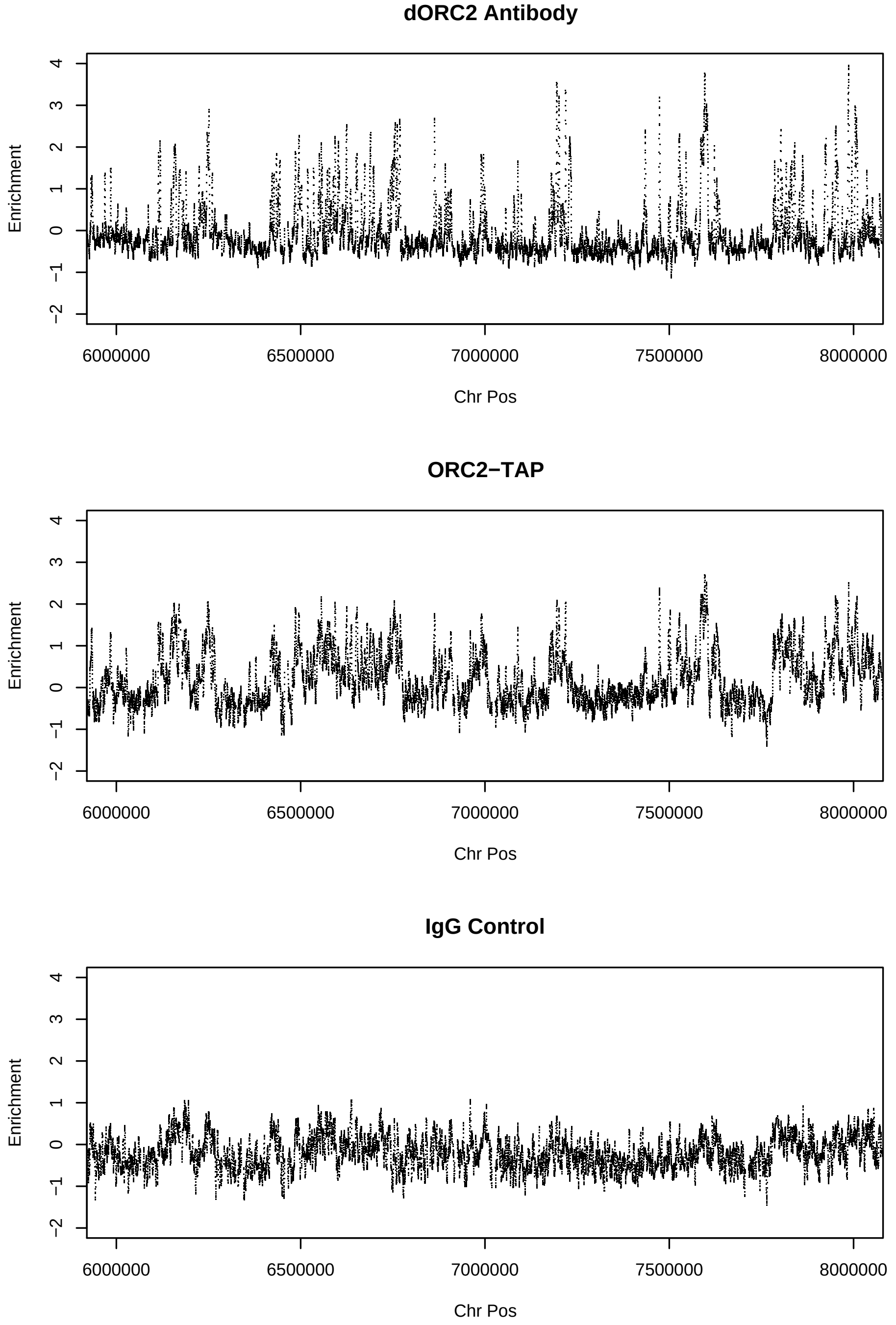
Exp	**Peaks** $p<1\times10^{-3}$	**Peaks** $p<1\times10^{-5}$	**FDR**	**Overlap with dORC2** $p<1\times10^{-3}$	**Overlap with dORC2** $p<1\times10^{-5}$	**Pearson correlation**
dORC2	NA	924	0.24	NA	NA	1
ORC2-TAP	733	183	1.99	557(76%)	174(95%)	0.434
IgG	50	8	36.82	8(16%)	0(0%)	0.042

Table 1: Summary of ChIP statistics for dORC2 antibody, ORC2-TAP and IgG. The number of coincident peaks with the dORC2 antibody dataset ($p<1\times10^{-5}$) is shown at different peak calling thresholds. For example, in the ORC2-TAP experiment we identified 733 peaks at $p<1\times10^{-3}$, 557 of these peaks were also found in the dORC2 antibody ChIP. We also calculated a Pearson correlation between the ChIP signal found at peaks in the dORC2 antibody ChIP versus the same regions in either the ORC2-TAP or IgG control experiments.

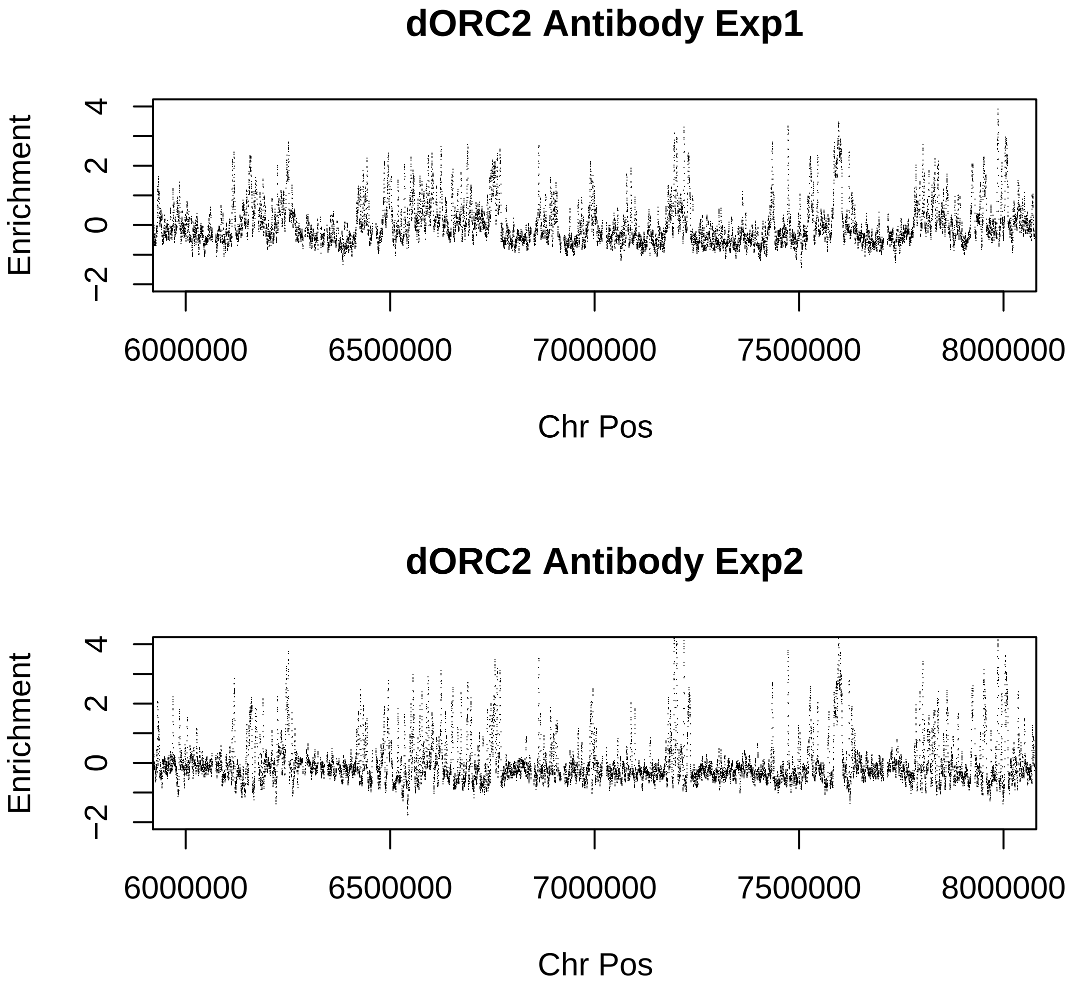
Category	**Number**	**Fold-enrichment**	**P-value**
Nucleus	196	2.48	8.98e-33
Nucleotide-binding	186	2.49	2.59e-31
Alternative splicing	133	2.76	6.45e-26
Hydrolase	238	2.00	1.56e-25
Cytoplasm	103	3.23	2.25e-25
Transferase	169	2.27	4.13e-23
ATP-binding	139	2.35	5.28e-20
Developmental protein	117	2.46	4.86e-18
Ribonucleoprotein	55	3.80	5.26e-16
Transcription	90	2.67	8.37e-16
Transcription regulation	89	2.65	2.32e-15
Ribosomal protein	49	4.00	4.32e-15
Metal-binding	152	1.95	1.26e-13
Zinc	122	2.09	9.49e-13
Zinc-finger	94	2.34	1.96e-12
Phosphoprotein	72	2.63	1.20e-11
Mitochondrion	48	3.01	2.44e-09
Kinase	68	2.35	1.76e-08
Membrane	156	1.67	2.66e-08
GTP-binding	44	2.79	2.96e-07

Table 2: ORC-associated promoters map to broad functional gene ontology categories.

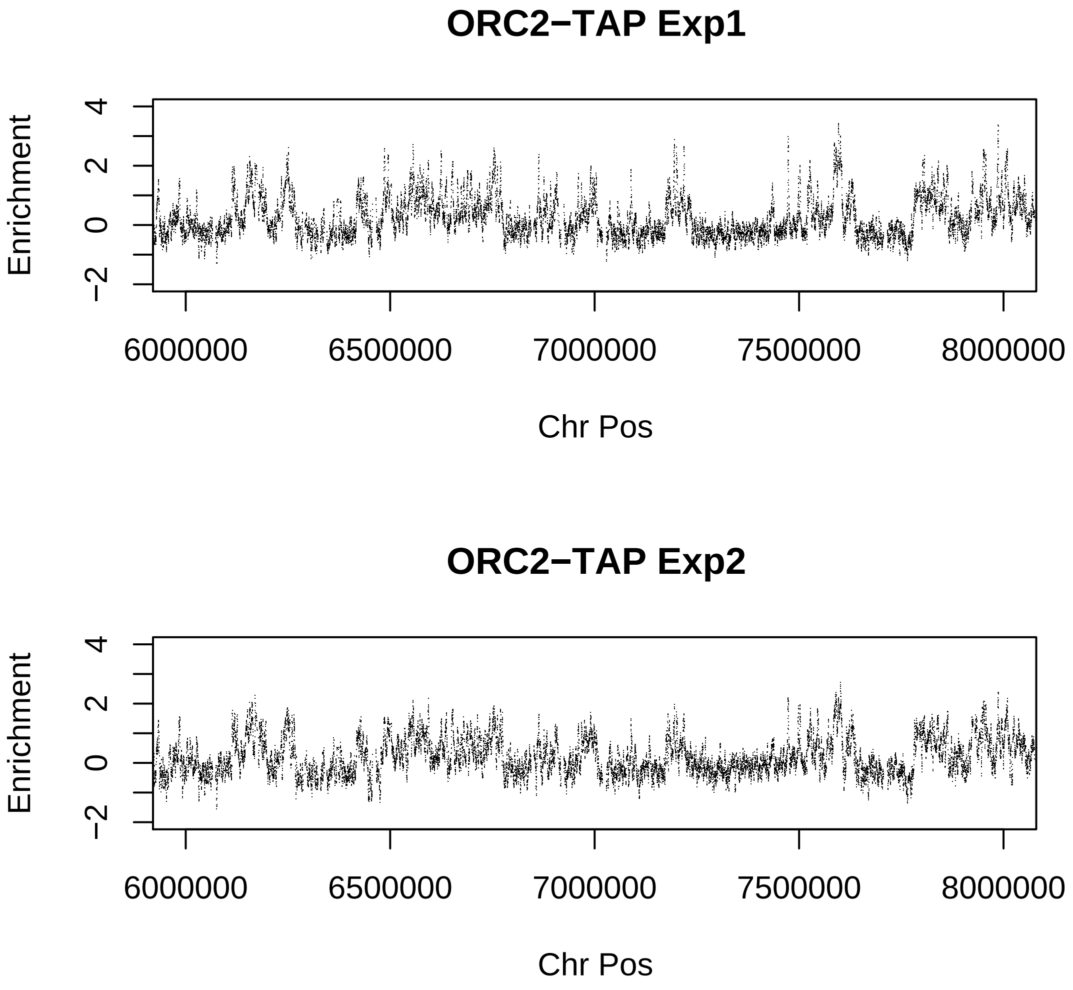
Supplemental Figure 1A



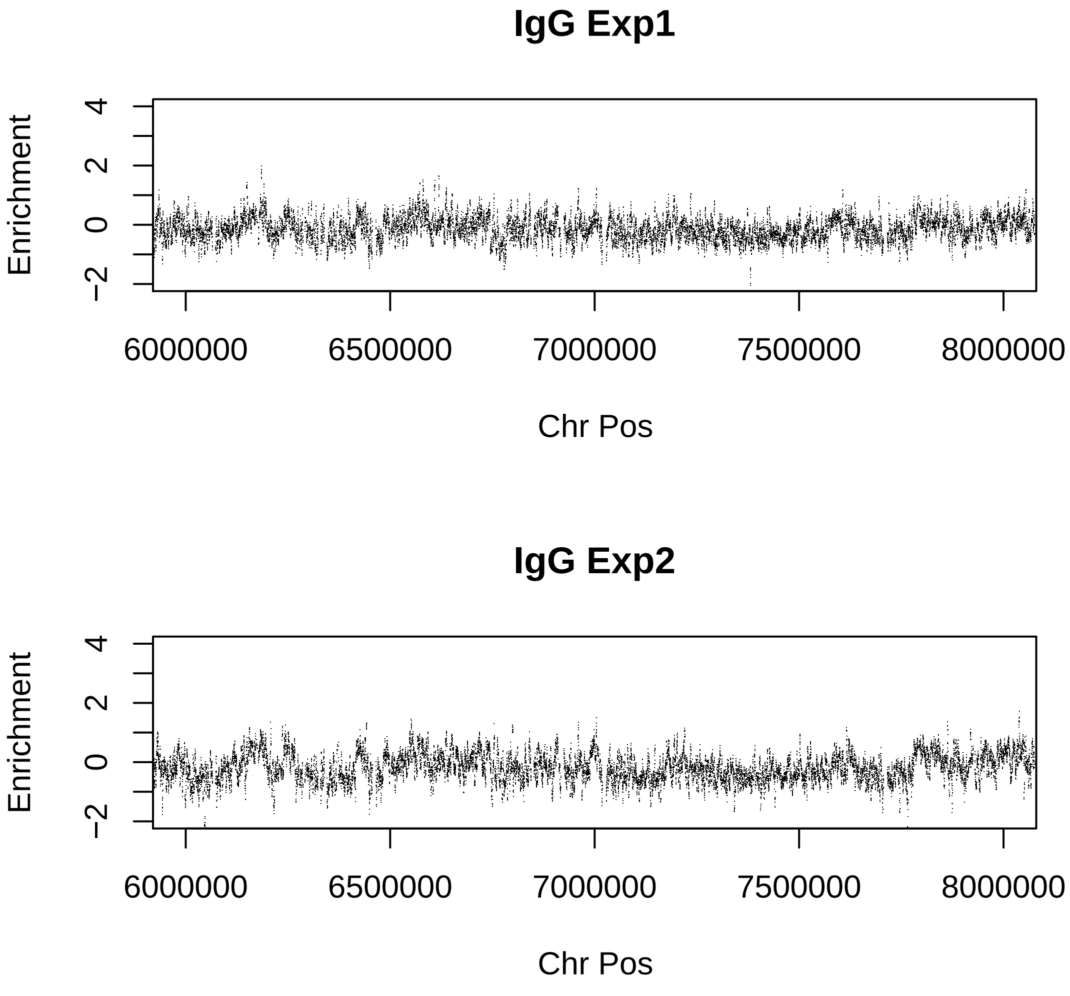
Supplemental Figure 1B



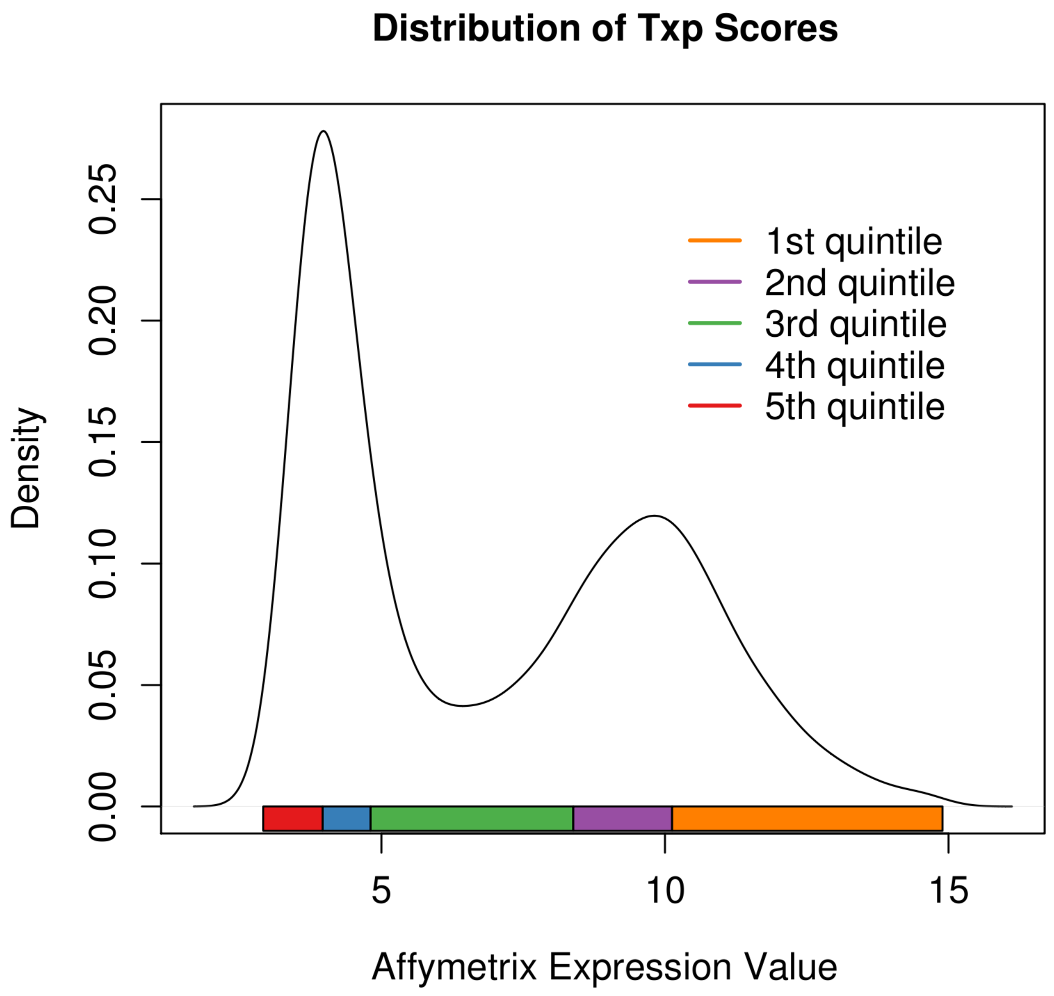
Supplemental Figure 1C



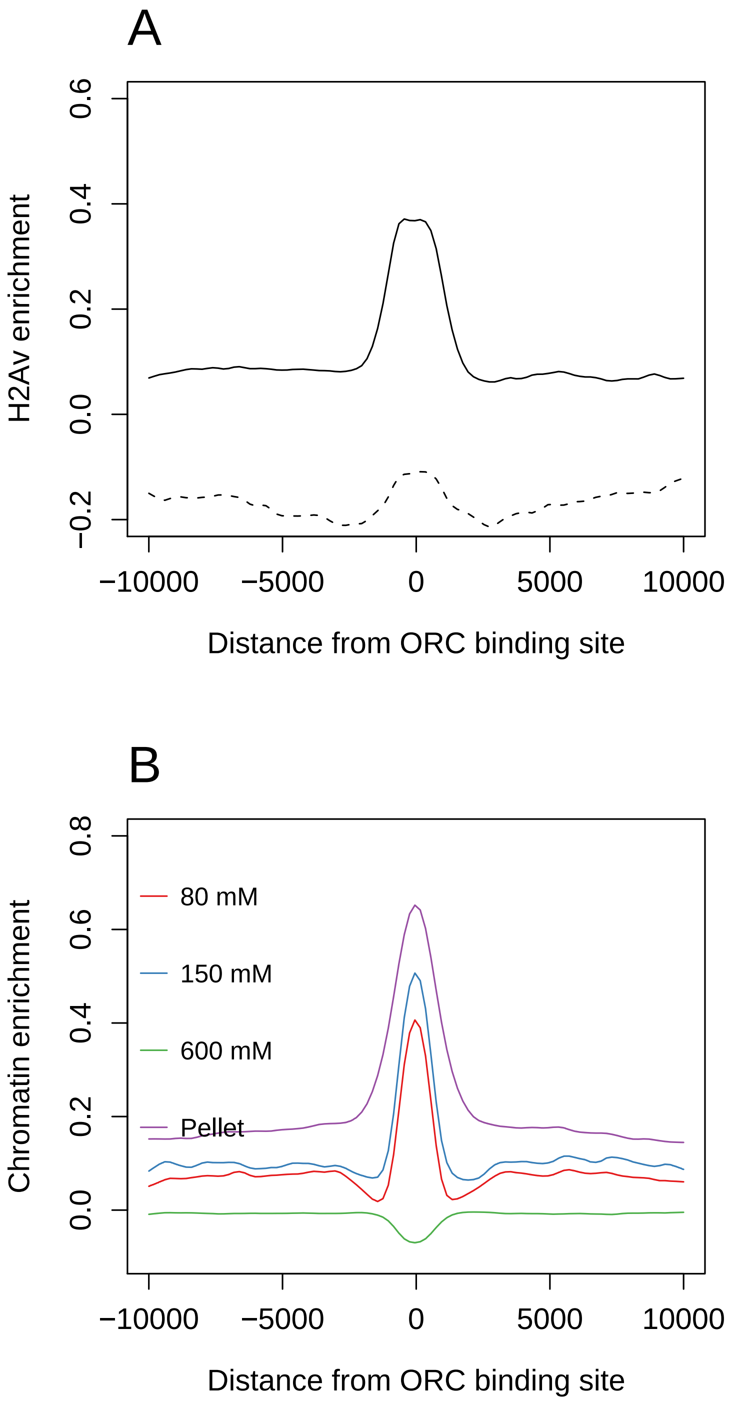
Supplemental Figure 1D



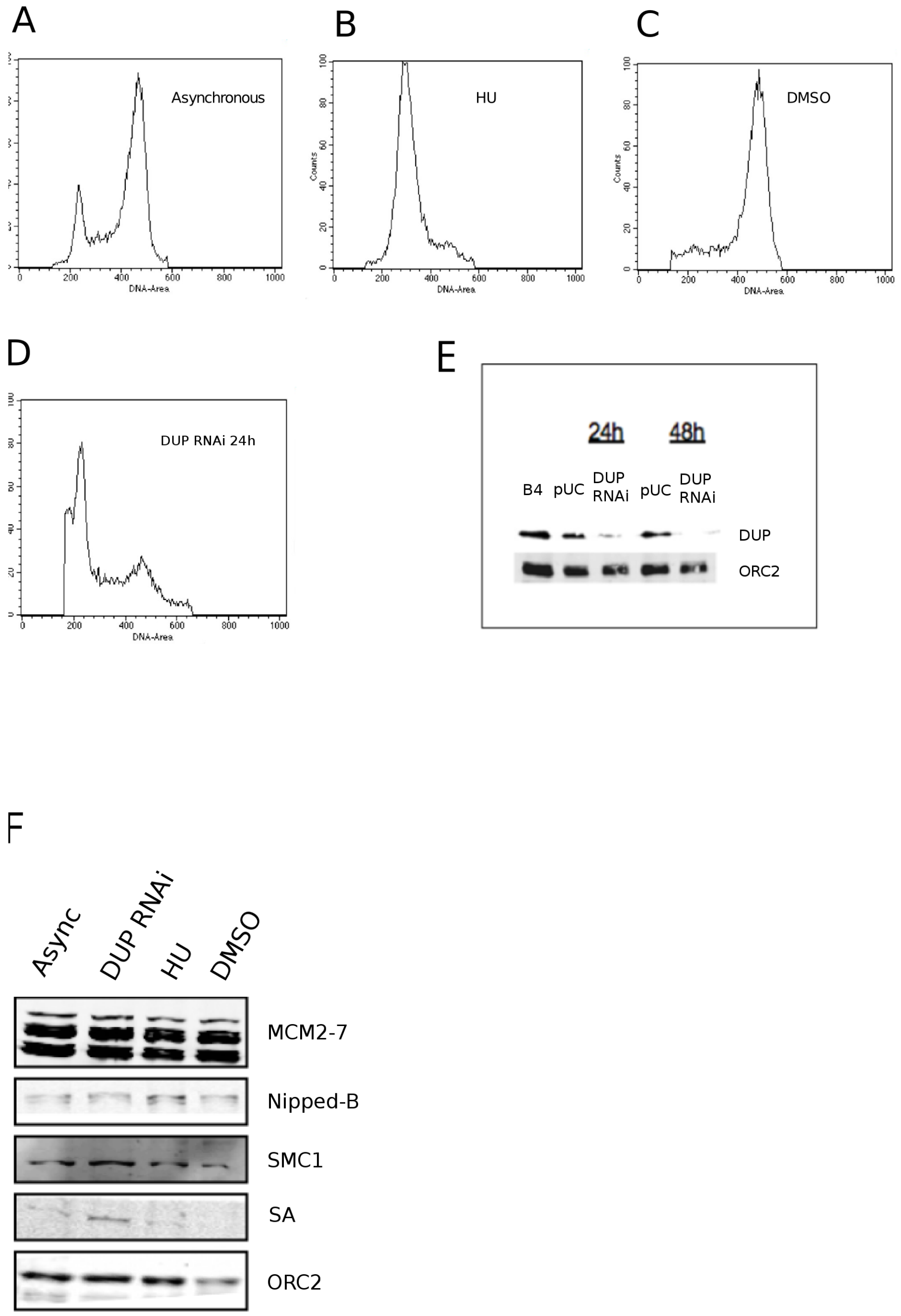
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5