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## Mitotic gene bookmarking by RUNX1 contributes to stabilization of the normal mammary epithelial phenotype — [Source link](#)

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1 **RUNX1 mitotically bookmarks target genes that are important for the mammary**  
2 **epithelial-to-mesenchymal transition**

3

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23 **Key Words:** Mammary phenotype, epithelial phenotype, RUNX1, mitotic gene  
24 bookmarking.

25

26

27 **Significance:**

28           This study elucidates mitotic gene bookmarking as a potential epigenetic  
29 mechanism that impacts breast epithelial cell growth and phenotype and has potential  
30 implications in breast cancer onset.

31 **ABSTRACT**

32           RUNX1 has recently been shown to play an important role in determination of  
33 mammary epithelial cell identity. However, mechanisms by which loss of the RUNX1  
34 transcription factor in mammary epithelial cells leads to epithelial-to-mesenchymal  
35 transition (EMT) are not known. Here, we report mitotic bookmarking of genes by RUNX1  
36 as a potential mechanism to convey regulatory information through successive cell  
37 divisions for coordinate control of mammary cell proliferation, growth, and identity.  
38 Genome-wide RUNX1 occupancy profiles for asynchronous, mitotically enriched, and  
39 early G1 breast epithelial cells reveal RUNX1 is retained during the mitosis to G1  
40 transition on protein coding and long non-coding RNA genes critical for mammary  
41 epithelial proliferation, growth, and phenotype maintenance. Disruption of RUNX1 DNA  
42 binding and association with mitotic chromosomes alters cell morphology, global protein  
43 synthesis, and phenotype-related gene expression. Together, these findings show for the  
44 first time that RUNX1 bookmarks a subset of epithelial-related genes during mitosis that  
45 remain occupied as cells enter the next cell cycle. Compromising RUNX1 DNA binding  
46 initiates EMT, an essential first step in the onset of breast cancer.

47

## 48 INTRODUCTION

49 Breast cancer arises from a series of acquired mutations and epigenetic changes  
50 that disrupt normal mammary epithelial homeostasis and create multi-potent cells that  
51 can differentiate into biologically unique and clinically distinct subtypes (1-6). Epithelial-  
52 to-mesenchymal transition (EMT)—a trans-differentiation process through which  
53 mammary epithelial cells acquire the aggressive mesenchymal phenotype—is a key  
54 driver of breast cancer progression, invasion and metastasis (7-12). Transcription factors  
55 Snail, Slug, Twist, and Zeb1/2 contribute to EMT during early, normal development and  
56 have also been implicated in invasion (13-16). Despite accumulating evidence that  
57 defines a broad understanding of EMT regulation and maintenance of the epithelial  
58 phenotype (7-12), the mechanism(s) by which mammary cells maintain their epithelial  
59 phenotype is unknown.

60 Runt-Related Transcription Factor 1 (RUNX1/AML1) is required for hematopoietic  
61 lineage specification during development and hematopoiesis throughout life (17-30). In  
62 addition to the recognized role in hematological malignancies, RUNX1 has been recently  
63 identified as a key player in breast cancer development and tumor progression (31-38).  
64 Findings from our group (39), reinforced by studies from others (40, 41), have shown that  
65 RUNX1 plays a critical role in maintaining breast epithelial phenotype and prevents EMT  
66 through transcriptional regulation of genes (e.g., the EMT marker and a key cell adhesion  
67 protein E-cadherin) involved in fundamental cellular pathways. However, mechanisms by  
68 which RUNX1 prevents EMT have not been identified.

69 Mitotic gene bookmarking, i.e. transcription factor binding to target genes during  
70 mitosis for transcriptional regulation following cell division, is a key epigenetic mechanism

71 to convey and sustain regulatory information for cell proliferation, growth, and cell identity  
72 from parent to progeny cells (42-49). We have established that RUNX proteins, as well  
73 as other phenotypic transcription factors that include MYOD and CEBP $\alpha$ , mitotically  
74 bookmark RNA Pol I- and II-transcribed genes in osteoblasts and leukemia cells for  
75 coordinate control of cell growth, proliferation and phenotype (50-57). It is increasingly  
76 evident that mitotic gene bookmarking by transcription factors is a key mechanism to  
77 determine and maintain cell fate across successive cell divisions (58-70).

78 We addressed the hypothesis that RUNX1 maintains the breast epithelial  
79 phenotype by mitotic bookmarking of genes that support mammary epithelial proliferation,  
80 growth, and phenotype for expression in the next cell cycle. Fluorescence confocal  
81 microscopy of fixed and live mammary epithelial cells revealed that RUNX1 is present on  
82 chromosomes throughout mitosis and colocalizes with upstream binding transcription  
83 factor (UBF), a subunit of RNA Pol I transcriptional machinery (71). To identify genes  
84 occupied by RUNX1, we performed chromatin immunoprecipitation coupled with high  
85 throughput sequencing (ChIP-Seq) using a RUNX1-specific antibody on mitotic, G1, and  
86 asynchronous normal mammary epithelial MCF10A cells. As expected, ribosomal RNA  
87 genes, regulated by the RNA Pol I transcriptional machinery, were occupied by RUNX1.  
88 A fluorescence-based, global protein synthesis assay showed reduced protein synthesis  
89 when RUNX1 DNA binding was perturbed using a small molecule inhibitor. Importantly,  
90 ChIP-Seq revealed that, in mitosis, RUNX1 associates with RNA Pol II regulated genes  
91 specifically involved in maintenance of the epithelial phenotype and EMT progression.  
92 Strikingly, disruption of RUNX1 DNA binding, which is required for association with mitotic  
93 chromosomes (56), results in loss of the epithelial phenotype and acquisition of

94 mesenchymal properties that are accompanied by changes in expression of associated  
95 genes and pathways and represent early events in the onset of breast cancer. These  
96 findings implicate RUNX1 occupancy of target genes at the mitosis into G1 transition in  
97 regulating the normal breast epithelial phenotype.

98

## 99 **RESULTS**

### 100 **RUNX1 associates with mitotic chromatin and occupies target genes**

101 To investigate subcellular localization of RUNX1 in normal mammary epithelial  
102 cells, we performed immunofluorescence (IF) microscopy in actively proliferating  
103 MCF10A cells and imaged cells undergoing spontaneous mitoses. We observed that  
104 RUNX1 is localized on mitotic chromatin at all topologically identified substages of mitosis  
105 (Fig. 1; top panels). Two distinct types of foci are detectable on mitotic chromosomes: 2-  
106 8 large punctate foci that appear to be allelic as well as numerous smaller foci that are  
107 distributed across the chromosomes (Fig. 1; bottom panels, white arrowheads). In all  
108 replicates, important secondary-antibody-only controls were included to confirm  
109 specificity of RUNX1 signal on mitotic chromosomes (Suppl. Fig. 1). In the interphase  
110 nuclei, RUNX1 exhibited the characteristic punctate distribution (Fig. 1; interphase panel).  
111 To ensure reproducibility of our findings, the IF experiments were repeated at least 3  
112 times and, at the minimum, 20 interphase and mitotic cells were imaged.

113 Multiple reports have indicated that formaldehyde fixation can prevent regulatory  
114 protein detection on mitotic chromosomes (58, 69). To further confirm that association of  
115 RUNX1 with mitotic chromosomes is not under-represented because of formaldehyde  
116 fixation, we examined the localization of RUNX1-EGFP in actively proliferating, unfixed  
117 MCF10A cells. Consistent with our findings in fixed and synchronized cells, RUNX1-  
118 EGFP was associated with chromosomes in live MCF10A cells undergoing mitosis  
119 (Suppl. Fig. 2.). Together, these findings establish that RUNX1 foci are present on  
120 chromosomes at all stages of mitosis under physiological conditions in actively dividing,



121 unfixed breast epithelial cells and, in agreement with our previous findings, are equally  
122 distributed into resulting progeny cells (57).

123 To experimentally address whether RUNX1 presence on mitotic chromosomes  
124 reflects occupancy of target genes, MCF10A cells were synchronized in mitosis using  
125 nocodazole (50ng/mL; Supp. Fig. 3A). Nocodazole dose and treatment were empirically  
126 determined to minimize toxic effects of the drug, while maximizing mitotic enrichment.  
127 Mitotic cells were collected by mitotic shake-off and purity of harvested cells was  
128 confirmed by the presence of H3pS28 in >70% of cells. We chose the H3pS28 mark to  
129 identify mitotic cells because this histone mark is highly specific to condensed  
130 chromosomes during mitosis; more commonly used H3pS10 mark is additionally  
131 observed in early G1 cells and has also been associated with replicating centers in S-  
132 phase (72, 73). A parallel, nocodazole-treated cell population was released into early G1  
133 by replacing nocodazole-containing growth medium with fresh, nocodazole-free, growth  
134 medium and was harvested 3 hours post-release (Suppl. Fig. 3B). Western blot analysis  
135 of whole cell lysates from the three cell populations showed expected levels of expression  
136 for cell cycle-specific proteins Cyclin B and CDT1 (Suppl. Fig. 3C). FACS profiles of the  
137 cell populations confirmed the characteristic enrichment of blocked cells in mitosis (Supp.  
138 Fig. 3D; Mitotic) and release into G1 upon media replacement (Supp. Fig. 3D; G1) when  
139 compared to asynchronous cells (Supp. Fig. 3D; Asynch). To determine whether RUNX1  
140 remains bound to target genes during mitosis, ChIP-Seq was performed on Asynch,  
141 Mitotic, and G1 MCF10A cells using a RUNX1 specific antibody (Fig. 2A). Enriched  
142 regions (peaks called by MACS2) were compared using k-means clustering (k=4) of  
143 normalized enrichment profiles of the three cell populations. This analysis revealed

144 subsets of genes that were either shared across the three groups or were specific for  
145 each, indicating dynamic binding of RUNX1 during and immediately after mitosis (Fig.  
146 2A). Peak calling identified RUNX1 occupancy of both protein coding and long non-coding  
147 RNA (lncRNA) genes. Specifically, RUNX1 occupied 1070 genes in cell population not in  
148 G1 or M phase (Fig. 2A; green bar) and 1095 genes in G1-enriched cells (Fig. 2A; light  
149 red bar). Importantly, RUNX1 occupied 551 genes (413 protein coding and 138 lncRNAs)  
150 in mitotically enriched MCF10A cells (Fig. 2A; blue bar).

151 Functional relevance of RUNX1 occupancy in the three cell populations was  
152 determined by comparing RUNX1-occupied genes with those that are differentially  
153 regulated upon shRNA-mediated RUNX1 knockdown (39). Of the 1070 genes occupied  
154 by RUNX1 in cell populations not in G1 or M phases, 353 genes were deregulated upon  
155 RUNX1 depletion (Fig. 2A). Importantly, RUNX1 depletion deregulated 399 of 1268  
156 RUNX1-bound genes in the M and G1 populations. These findings reveal that several  
157 hundred target genes are bookmarked by RUNX1 during mitosis and transcriptionally  
158 regulated in normal mammary epithelial cells.

159 To identify cellular processes and pathways that are comprised of RUNX1-  
160 bookmarked genes, we performed gene set enrichment analysis (GSEA) on genes bound  
161 by RUNX1 during mitosis or G1, or not bound in either cell cycle stage (Fig. 2B).  
162 Interestingly, most genes bookmarked by RUNX1 during mitosis were associated with  
163 negative regulation of gene expression and metabolic process (Fig. 2B; blue box).  
164 Consistent with a cellular requirement to reattach and enter the next cell cycle and fully  
165 resume transcription, genes bound during G1 were primarily enriched in biological  
166 processes involving cell anchorage, protein localization and positive regulation of gene

167 expression (Fig. 2B; red box). CHIP-seq results were further validated by motif analysis  
168 of RUNX1-bound peaks, which showed that the RUNX motif was the most enriched motif  
169 in all three cell populations (Fig. 2C). Importantly, RUNX1-bound genomic regions were  
170 also enriched in motifs for transcription factors (e.g., Fra1, JunB, ETS) known to  
171 cooperate with RUNX1 for gene regulation (74)(Fig. 2C). Together, these findings indicate  
172 that RUNX1 occupies genes involved in cell proliferation, growth, and phenotype during  
173 mitosis in normal mammary epithelial cells.

174

### 175 **RUNX1 mitotically bookmarks RNA Pol I-transcribed genes that control cell growth**

176 Our CHIP-Seq results revealed that RUNX1 occupies rDNA repeats in MCF10A  
177 mammary epithelial cells; all three MCF10A cell populations (Asynch, Mitotic and G1)  
178 exhibited significant fold enrichment within the promoter region of hrDNA (Fig. 3A),  
179 suggesting a potential role for RUNX1 in regulating rRNA genes in MCF10A cells. We  
180 confirmed this finding in actively proliferating MCF10A cells by immunofluorescence  
181 microscopy for antibodies specific against RUNX1 and upstream binding factor (UBF), a  
182 transcriptional activator that remains bound to rRNA genes during mitosis (75). We  
183 observed large RUNX1 foci colocalizing with UBF throughout each stage of mitosis (Fig  
184 3B and Suppl. Fig. 4). Colocalization between RUNX1 and UBF was validated by confocal  
185 microscopy. Line scans of MCF10A cells show that although RUNX1 and UBF occupy  
186 distinct nuclear microenvironments in interphase (n=15), both proteins substantially  
187 colocalize in metaphase (n=15)(Suppl. Fig. 4). Taken together, these findings establish  
188 RUNX1 binding to ribosomal DNA repeat regions by CHIP-Seq (Fig. 3A) and confirmed  
189 at the cellular level by confocal microscopy (Fig 3B & Suppl. Fig. 4).

190 We experimentally addressed the hypothesis that RUNX1 regulates ribosomal  
191 RNA gene expression by using a pharmacological inhibitor of RUNX1. The small  
192 molecule inhibitor AI-14-91, which has been extensively characterized by several groups,  
193 interferes with RUNX1-CBF $\beta$  interaction and disrupts RUNX1 DNA binding (76, 77). We  
194 examined the effect of RUNX1 inhibitor on pre-rRNA expression and found that pre-rRNA  
195 expression was significantly increased at 12hr and 48hr time points after treatment of  
196 asynchronous cells with the specific RUNX1 inhibitor but not the control inactive  
197 compound AI-4-88, indicating that RUNX1 suppresses rRNA gene expression in normal  
198 mammary epithelial cells (Fig. 3C). Because levels of rRNA directly correlate with global  
199 protein synthesis, a fluorescent-based detection method was used to measure newly  
200 synthesized proteins. Cells treated with AI-14-91 for 24hr or 48hr showed a moderate  
201 change in levels of global protein synthesis in comparison to AI-4-88 control-treated cells  
202 under identical conditions (n=3; Fig. 3D). Together, our results demonstrate that RUNX1  
203 bookmarks RNA Pol I regulated rRNA genes during mitosis and transcriptionally  
204 represses them during interphase with moderate impact on global protein synthesis in  
205 normal mammary epithelial cells.

206

### 207 **RUNX1 occupies RNA Pol II-transcribed genes involved in hormone-** 208 **responsiveness and cell phenotype during mitosis**

209 Using RUNX1-bookmarked genes, gene set enrichment analysis (GSEA) was  
210 performed to identify regulatory pathways (Fig. 4A). In agreement with known roles of  
211 RUNX1 (78-82), the top 10 pathways identified were those involved in regulation of G2M  
212 Checkpoint, E2F targets, p53, and DNA repair (Fig. 4A). Consistent with our finding that

213 RUNX1 bookmarks and regulates rRNA genes, one of the pathways identified is mTOR  
214 signaling, a pathway that is required for cell growth and is a therapeutic target in breast  
215 cancers (83, 84). Relevant to the normal mammary epithelial phenotype, both early and  
216 late estrogen responsive gene sets significantly overlap with RUNX1 mitotically  
217 bookmarked genes (Fig. 4A). Because estrogen plays vital roles in promoting proliferative  
218 phenotypes of mammary epithelial cells (85-87), we interrogated RUNX1 bookmarked  
219 genes to identify those bound by RUNX1 and ER $\alpha$  in MCF7 cells, where RUNX1  
220 contributes to higher order genome organization (Fig. 4B) (88, 89). Using publicly  
221 available datasets of ER $\alpha$  genome-wide occupancy and estradiol-regulated gene  
222 expression (GSE40129)(90), we find that a subset of genes mitotically bookmarked by  
223 RUNX1 is also bound by ER $\alpha$ , and either up or down regulated in response to estradiol.  
224 These findings indicate that RUNX1-bookmarked genes are involved in pathways that  
225 control hormone-responsiveness, proliferation and growth of normal mammary epithelial  
226 cells (Fig. 4B).

227 We identified a novel subset of genes that are bookmarked by RUNX1 and relate  
228 to regulatory pathways involved in cellular phenotype including TNF $\alpha$ , Apical Junction  
229 and Notch signaling (Fig. 4A). Furthermore, NEAT1 and MALAT1, lncRNAs often  
230 deregulated in breast cancer (91, 92), were also mitotically bookmarked by RUNX1. Of  
231 the 413 RUNX1 bookmarked protein coding genes, *TOP2A*, *MYC*, *HES1*, *RRAS*, *H2AFX*,  
232 and *CCND3* are representative of RNA Pol II-transcribed genes involved in phenotype  
233 maintenance and cell fate decisions (See Suppl. Table 1 for complete list). Recently,  
234 *HES1* and *H2AFX* have been identified as regulators of breast epithelial phenotype (93-  
235 95). In our ChIP-seq dataset, *HES1* and *H2AFX* show significant fold enrichment of

236 RUNX1 occupancy between the three populations of MCF10A cells (Fig. 4C; top panels).  
237 Expression of *HES1* increased upon inhibition of RUNX1 DNA binding (Fig. 4C; left  
238 panel—bar graph), indicating that RUNX1 represses *HES1*. In contrast, *H2AFX*  
239 expression at 24hr and 48hr of inhibitor treatment was decreased, suggesting RUNX1  
240 activates H2AFX expression (Fig. 4C; right panel—bar graph). These results suggest that  
241 RUNX1 bookmarks both protein coding and non-coding genes that are critical  
242 determinants of epithelial lineage identity as a potential mechanism to stabilize the  
243 mammary epithelial phenotype.

244

#### 245 **Inhibition of RUNX1 DNA binding causes epithelial to mesenchymal transition and** 246 **alters the associated transcriptome**

247 To experimentally address whether disruption of RUNX1 bookmarking leads to a  
248 change in epithelial phenotype, we treated cells with RUNX1 DNA binding inhibitor and  
249 the inactive control compound and monitored changes in cell morphology (Fig. 5A).  
250 Consistent with RUNX1 bookmarking and regulation of genes critical for epithelial  
251 phenotype (Figs. 2 & 4), disruption of RUNX1 DNA binding for 48 hours resulted in  
252 mesenchymal morphology. We next examined whether long-term inhibition of RUNX1  
253 caused a permanent change in cell phenotype. Longer term treatment (5 days) of actively  
254 proliferating MCF10A cells showed significant apoptosis, although a small sub-population  
255 of cells survived and exhibited an altered phenotype (Fig. 5B). The surviving sub-  
256 population at day 5 was recovered by culturing cells in media without the inhibitor. By day  
257 3-4 following media replacement, cells clearly showed a mesenchymal morphology (Fig.  
258 5B), indicating that interfering with RUNX1 DNA binding causes loss of the normal

259 mammary epithelial phenotype. Consistent with changes in cell morphology, we find  
260 alterations in expression and localization of the cytoskeletal F-actin protein (Fig. 5C).  
261 These observations were confirmed by examining the expression of epithelial markers  
262 (e.g., E-cadherin (Fig. 5D)), as well as mesenchymal markers (e.g., Vimentin (Fig. 5D)).  
263 E-cadherin was largely unchanged; however, Vimentin expression was significantly  
264 increased, confirming an epithelial-to-mesenchymal transition upon inhibition of the  
265 RUNX1-CBF $\beta$  interaction.

266 To identify transcriptome-wide changes associated with EMT upon inhibition of  
267 RUNX1 DNA binding activity that is required for retention of the protein with mitotic  
268 chromosomes (56), we performed RNA sequencing (in triplicates) at each of the indicated  
269 time points (Day 1 and 2 in Crisis Phase and Day 4 and 7 in Recovery Phase). Heatmap  
270 analysis of all time points identified substantial changes in gene expression as cells  
271 transition from epithelial to mesenchymal phenotype (Fig. 6A). A differential gene  
272 expression analysis between the crisis and recovery phases uncovered significant  
273 changes in expression of genes associated with EMT (e.g., IL32, SERPINB2 etc.; Fig.  
274 6B). Importantly, a subset of differentially expressed genes was occupied by RUNX1  
275 during mitosis. We performed pathway analysis on differentially expressed genes (Fig.  
276 6C and Suppl. Table 2). Consistent with phenotypic changes, we found that multiple  
277 signaling pathways that include TNF alpha, Interferon Gamma and estrogen  
278 responsiveness were altered during EMT caused by RUNX1 inhibition.

279 We next determined the effect of the inhibition of RUNX1-CBF $\beta$  interaction on  
280 mitotic retention of the protein. Actively proliferating MCF10A cells were treated with the  
281 inhibitor (AI-14-91) for 6hr, 12hr, 24hr, and 48hr at 20 $\mu$ M; a structurally similar but inactive

282 compound (AI-4-88) was used as a control under identical conditions. Cells were  
283 subjected to immunofluorescence microscopy followed by detection of RUNX1 and UBF  
284 as described above. Although RUNX1 signal was detected in all mitotic sub-stages  
285 (Suppl. Fig. 5), we observed a substantial decrease in RUNX1 signal intensity on  
286 metaphase chromosomes (white arrows; Fig. 6D), indicating that RUNX1-CBF $\beta$   
287 interaction and RUNX1 DNA binding activity play a key role in mitotic gene bookmarking.  
288 These changes were more pronounced for smaller RUNX1 foci and were not observed in  
289 control-treated cells; appreciable signal for large RUNX1 foci that colocalize with UBF  
290 (Fig. 3B & Suppl. Fig. 4) remained detectable in all sub-stages of mitosis (Fig. 6D and  
291 Suppl. Fig. 5). Together, these findings indicate that RUNX1 mitotic bookmarking of  
292 genes related to epithelial cell growth, proliferation, and lineage is associated with  
293 epithelial cell identity, and disruption of RUNX1 DNA binding leads to mesenchymal  
294 transition.



## 295 **DISCUSSION**

296           This study identifies retention of RUNX1 on mitotic chromosomes and occupancy  
297 of target genes as a potential epigenetic mechanism for coordinate regulation of RNA Pol  
298 I- and II-transcribed genes that are critical for mammary epithelial proliferation, growth,  
299 and phenotype maintenance. Pharmacological inhibition of RUNX1 DNA binding causes  
300 transition of mammary epithelial cells to a mesenchymal phenotype, indicating that  
301 RUNX1 bookmarking of target genes contributes to stabilizing the normal breast epithelial  
302 phenotype.

303           Our findings are the first to examine RUNX1 bookmarking of target genes during  
304 mitosis in mammary epithelial cells and to report that RUNX1 coordinately controls cell  
305 growth-related ribosomal RNA (rRNA) genes and a large subset of cell  
306 proliferation/phenotype-related genes in these cells. One target gene of interest is hairy  
307 and enhancer of split-1 (*HES1*). *Hes1* is a transcription factor that represses genes  
308 involved in cellular development and is regulated primarily by NOTCH signaling, one of  
309 our top ten overlapping hallmark gene sets bookmarked by RUNX1 (Fig. 4)(96, 97). *HES1*  
310 was recently shown to have a prominent role in proliferation and invasion of breast cancer  
311 cells, and its silencing led to downregulation of p-Akt signaling and ultimately prevented  
312 EMT (93). Our findings indicate that RUNX1 stabilizes the normal mammary epithelial  
313 phenotype, in part, by bookmarking *HES1* and suppressing its expression.

314           Another important RNA Pol II-transcribed gene mitotically bookmarked by RUNX1  
315 and critical for maintaining cellular phenotype is histone variant H2AFX (*H2AFX*).  
316 Silencing *H2AFX* in breast epithelial cells leads to induction of EMT through activation of  
317 *SNAIL2/SLUG* and *TWIST1* (95). Upon inhibition of the RUNX1-CBF $\beta$  interaction, we find

318 a decrease in *H2AFX* expression and a concomitant, significant increase in  
319 *SNAIL2/SLUG* expression. These data identify RUNX1 as a novel upstream regulator of  
320 *H2AFX* expression; RUNX1 bookmarking and activation of *H2AFX* and subsequent  
321 suppression of *SNAIL2/SLUG* prevents EMT in breast epithelial cells.

322 Several groups have shown that RUNX1 interacts with ER $\alpha$  at both enhancer  
323 regions and transcriptional start sites (TSSs) for regulation of specific genes (34, 88). Our  
324 ChIP-Seq results, coupled with publicly available data sets, reveal a novel finding: RUNX1  
325 bookmarking of a subset of ER $\alpha$ -occupied, hormone-responsive genes during mitosis  
326 may be critical for maintenance of the breast epithelial phenotype. Future studies will be  
327 required to investigate mechanistic significance of this observation in estrogen receptor  
328 positive mammary epithelial and breast cancer cells.

329 Our findings suggest that mitotic gene bookmarking by RUNX1 contributes to  
330 regulation of the mammary epithelial phenotype. Equally important, our study shows that  
331 inhibition of RUNX1 DNA binding specifically elicits an epithelial-to-mesenchymal  
332 transition that accompanies changes in critical genes and pathways involved in EMT.  
333 These findings are further supported by RUNX1 mitotic occupancy of cell growth-related  
334 rRNA genes, and together highlight a key role of RUNX1 in coordinating cell proliferation,  
335 growth and phenotype. Because RUNX1 interacts with multiple co-activators and co-  
336 repressors, additional in-depth studies are required to determine contributions of RUNX1  
337 co-regulatory proteins to mitotic gene bookmarking.

338 Another novel contribution of the current study is mitotic bookmarking of lncRNAs  
339 by a transcription factor. RUNX1 was recently shown to regulate lncRNAs NEAT1 and  
340 MALAT1 (89, 91), which have critical roles in the onset and progression of breast cancer

341 (92). Our findings show that, in addition to occupying protein coding genes, RUNX1  
342 bookmarks several lncRNAs for post-mitotic regulation. It will be important to determine  
343 if RUNX1-bookmarked lncRNAs have G1-specific roles in maintaining the normal  
344 mammary epithelial phenotype and/or in the onset and progression of breast cancer.

345         In summary, this study shows that RUNX1 occupies RNA Pol I- and II-transcribed  
346 genes during mitosis for coordinate regulation of normal mammary epithelial proliferation,  
347 growth, and phenotype. Disruption of RUNX1 DNA binding leads to the epithelial-to-  
348 mesenchymal transition, a key event in breast cancer onset, and implicates RUNX1  
349 mitotic gene bookmarking as an epigenetic mechanism to physiologically sustain the  
350 mammary epithelial phenotype.

## 351 **MATERIALS AND METHODS**

### 352 **Cell Culture Techniques**

353 Breast epithelial (MCF10A) cells were cultured in DMEM/F-12 50/50 mixture  
354 (Corning™, Corning, NY). Culturing media was also supplemented with horse serum to  
355 5% (GIBCO, Grand Island, NY), human insulin to 10µg/mL (Sigma Aldrich, St. Louis, MO),  
356 human epidermal growth factor to 20ng/mL (PeproTech, Rocky Hill, NJ), cholera toxin to  
357 100ng/mL (Thomas Scientific, Swedesboro, NJ), hydrocortisone to 500ng/mL (Sigma  
358 Aldrich, St. Louis, MO), Penicillin-Streptomycin to 100U/mL (Thermo Fisher Scientific,  
359 Ashville, NC), and L-Glutamine to 2mM (Thermo Fisher Scientific, Ashville, NC).

360 For mitotic arrest of parental MCF10A cells, culturing media was supplemented  
361 with 50ng/mL of Nocodazole (Sigma Aldrich, St. Louis, MO) and incubated with cells for  
362 16hrs. Supplementing culturing media with equivalent volumes of DMSO (Sigma Aldrich,  
363 St. Louis, MO) served as a control. DMSO-treated and mitotically arrested populations of  
364 MCF10A cells were harvested following the 16hr incubation. For G1 (released from  
365 mitotic arrest) populations of MCF10A cells, the nocodazole-supplemented media was  
366 replaced with normal media and cells were incubated for 3hrs, after which the released  
367 cell population was harvested for subsequent analyses that include western blotting,  
368 qPCR, FACS and ChIP-seq.

369

### 370 **Western Blot Analyses**

371 Protein lysates were prepared by incubating cells in RIPA buffer on ice for 30min,  
372 followed by sonication using Q700 Sonicator (QSonica, Newtown, CT). Proteins were  
373 resolved by SDS-PAGE and transferred to PVDF membrane using standard protocols.

374 Following primary antibodies were used at a 1:1000 dilution (except Lamin B, which was  
375 used at 1:2000 dilution) in this study: UBF (sc-13125, Santa Cruz Biotechnology, Dallas,  
376 TX); RUNX1 (4334S, Cell Signaling Technologies, Danvers, MA); Cyclin B (4138S, Cell  
377 Signaling Technologies, Danvers, MA); Beta-Actin (3700S, Cell Signaling Technologies,  
378 Danvers, MA), and CDT1 (ab70829, AbCam, Cambridge, UK); Lamin B1 (ab16048,  
379 AbCam, Cambridge, UK). Horseradish peroxidase conjugated secondary antibodies used  
380 in this studies were: goat anti-mouse IgG at 1:5000 dilution (31460, Invitrogen, Carlsbad,  
381 CA), goat anti-rabbit IgG HRP conjugated (31430, Thermo Fisher Scientific, Ashville, NC)  
382 at 1:1000, 1:2000, or 1:5000 dilutions. Blots were developed using Clarity Western ECL  
383 Substrate (Bio-Rad, Hercules, CA) and imaged using Molecular Imager<sup>®</sup> Chemi doc<sup>™</sup>  
384 XRS+ Imaging System (Bio-Rad, Hercules, CA) aided by Image Lab Software Version  
385 5.1 (Bio-Rad, Hercules, CA).

386

### 387 **Confocal Microscopy, Image Acquisition, Processing and Analyses**

388 MCF10A cells were plated on gelatin-coated coverslips in 6-well plates at 175,000  
389 cells/mL and processed for immunofluorescence 24 hrs after plating using standard  
390 protocol. Briefly, cells were washed twice with sterile-filtered PBS on ice and cell were  
391 fixed in 1% MeOH-free Formaldehyde in PBS for 10min. After permeabilization in 0.25%  
392 Triton X-100-PBS solution, cells were sequentially incubated with primary and Alexa  
393 fluorophore conjugated secondary antibodies for 1hr each at 37°C in a humidified  
394 chamber with extensive washes after each incubation. Primary antibodies used were:  
395 RUNX1 at 1:10 dilution (4334S, Cell Signaling Technologies, Danvers, MA), and  
396 Upstream Binding Transcription Factor (UBF) at 1:200 dilution (F-9 sc-13125, Santa Cruz

397 Biotechnology, Dallas, TX). Secondary antibodies used were goat anti-rabbit IgG  
398 conjugated with Alexa Fluor 488 (A-11070, Life Technologies, Carlsbad, CA) and goat  
399 anti-mouse IgG conjugated with Alexa Fluor 594 (A-11005, Life Technologies, Carlsbad,  
400 CA) diluted 1:800. Cells were counterstained with DAPI to visualize DNA and coverslips  
401 were mounted onto slides in ProLong Gold Antifade Mountant (Thermo Fisher Scientific,  
402 Ashville, NC). Images were captured using a Zeiss Axio Imager.Z2 fluorescent  
403 microscope and Hamamatsu ORCA-R2 C10600 digital camera. Images were processed  
404 using ZEN 2012 software.

405 To examine mitotic localization of RUNX1 in unfixed cells, an expression plasmid  
406 containing RUNX1-EGFP was introduced using either nucleofection or Lipofectamine  
407 2000 transfection reagent in actively proliferating MCF10A cells grown on gelatin-coated  
408 coverslips. After 16 hours of nucleofection, cells were washed once with 1X PBS and  
409 stained with Hoechst dye to visualize DNA. Coverslips were mounted using the ProLong  
410 Gold Antifade Mountant and subjected to confocal microscopy.

411 Cells were initially imaged with a Zeiss LSM 510 META confocal laser scanning  
412 microscope (Carl Zeiss Microscopy, LLC., Thornwood, NY, USA). The DAPI signal was  
413 excited with a 405 nm laser and collected with a 425-475 nm band pass filter, Alexa 488  
414 was excited with a 488 nm laser and collected with a 500-550 nm band pass filter, and  
415 Alexa 568 with a 561 nm laser and collected with a 570-620 nm band pass filter. Images  
416 were captured with a Plan-Fluor 40X (1.3 NA) objective lens. The confocal pinhole was  
417 initially set to 1.2 Airy Unit diameter for the 561 nm excitation giving an optical section  
418 thickness of 0.41  $\mu\text{m}$ . Images were acquired at 12-bit data depth, and all settings,  
419 including laser power, amplifier gain, and amplifier offset were established using a look

420 up table to provide an optimal gray-scale intensities. All images were acquired using  
421 matching imaging parameters. The acquired images were subjected to colocalization  
422 analysis via Volocity version 6.3.0 (Perkin Elmer, Waltham, MA, USA). At least 15  
423 interphase and 15 metaphase cells were identified in captured images and appropriate  
424 thresholds were manually determined to eliminate background fluorescence for  
425 calculating Pearsons and Manders correlation coefficients between RUNX1 and UBF.

426 To ensure the specificity of our observations, additional samples were imaged with  
427 a Nikon A1R-ER laser scanning confocal microscope (Nikon, Melville, NY, USA). Images  
428 were acquired with the galvano scanner at a frame size of 1024 X 1024 pixels with an  
429 Apo TIRF 60X objective lens (N.A. 1.49) zoom of 2 and 1.2 Airy Unit pinhole setting.  
430 Images were also viewed in NIS Elements version 5.02.01 and analyzed using the line  
431 profiling tool. Overlaying DAPI, RUNX1, and UBF fluorescent intensities from individual  
432 channels along the line profile revealed overlapping peak intensities between the RUNX1  
433 and UBF channels, thus indicating colocalization.

434

### 435 **RNA Isolation, cDNA Synthesis and Quantitative PCR**

436 Total RNA was isolated from MCF10A cells using TRIzol™ Reagent (Invitrogen,  
437 Carlsbad, CA) and Direct-Zol™ RNA MiniPrep isolation kit (Zymo Research, Irvine, CA)  
438 following manufacturer instructions. cDNA was created using SuperScript IV® First-  
439 Strand Synthesis System for RT-PCR (ThermoFisher, Asheville, NC). Resulting samples  
440 were quantified on a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA) and diluted to  
441 500pg/μL. Equal amounts of DNA template were loaded for samples analyzed by qPCR.  
442 At least three independent biological replicates were analyzed for expression of RUNX1

443 bookmarked genes by qPCR. Student's t-test was used to determine the significance of  
444 changes in transcript levels under different biological conditions.

445

446 **Chromatin Immunoprecipitation, Library Preparation, Sequencing and**  
447 **Bioinformatics Analyses**

448 Asynchronous (Asynch), mitotically arrested (M), and released from mitosis (G1)  
449 MCF10A breast epithelial cells were subjected to chromatin immunoprecipitation using a  
450 modified Farnahm' protocol {O'Geen, 2010 #2713}. Sonication parameters for each  
451 population of cells was as follows: Peak Watt 140W, Duty Factor 10, Cycle/Burst 200  
452 using a S220 focused ultra-sonicator (Covaris, Matthews, NC). M and G1 populations of  
453 cells were sonicated for 28min total, whereas asynchronous populations of cells were  
454 sonicated for 36min. An aliquot of sonicated lysates was boiled in 100°C for 15min with  
455 NaCl and elution buffer and DNA was purified using PureLink™ PCR Purification Kit  
456 (K310001, ThermoFisher, Ashville, NC). Purified DNA was resolved on a 1.5% agarose  
457 gel to confirm optimal sonication (bulk of fragments between 200-400bp) prior to  
458 performing CHIP. In parallel, an aliquot was also quantified via Qubit 2.0 Fluorometer  
459 (Invitrogen, Carlsbad, CA) and analyzed by using a High Sensitivity DNA Kit on a  
460 Bioanalyzer 2100 (Agilent, Santa Clara, CA).

461 For chromatin immunoprecipitation (ChIP) reactions, 150ug of sonicated  
462 chromatin was incubated with 10ug of RUNX1 antibody (4336BF, Cell Signaling  
463 Technologies, Danvers, MA), diluted 1:10 in IP dilution buffer, and incubated overnight  
464 (16-18hrs) at 4°C with mild agitation. Following incubation, 150uL of Protein A/G magnetic  
465 beads (Thermo Scientific – Pierce, Waltham, MA) per ug of antibody used were added to



466 each IP reaction and incubated for 2-4hrs at 4°C with mild agitation. Beads were  
467 extensively washed with IP wash buffers and resuspended in Elution Buffer to extract  
468 immunoprecipitated chromatin, which was subsequently purified using PureLink™ PCR  
469 Purification Kit. At least 3 biological replicates were performed for each cell population  
470 and each antibody.

471 ChIP libraries were generated using Accel-NGS® 2S Plus DNA Library kit (Swift  
472 Biosciences, Ann Arbor, MI) following manufacturers protocol. Input and RUNX1 ChIP  
473 samples were normalized to 1ng prior to library generation. Libraries were amplified in an  
474 optional PCR step for 12 total cycles. Finalized libraries were double size selected using  
475 AMPure XP beads (0.8X and 0.2X volume ratios to sample), resulting in the majority  
476 fragments sized between 250-400bp. Next generation sequencing of pooled ChIP  
477 libraries was performed by the University of Vermont Cancer Center - Vermont Integrated  
478 Genomics Resource (VIGR).

479 Single end, 50bp reads (SE50) were processed pre-alignment by removing  
480 adapter reads (Cutadapt v1.6) and trimming low quality base calls from both ends  
481 (FASTQ Quality Trimmer 1.0.0; min score  $\geq$  20, window of 10, and step size of 1).  
482 Because we were specifically investigating rDNA, a customized build of hg38 was  
483 constructed that included normally masked regions of rDNA (Gencode U13369). Since  
484 some (although not complete) rDNA sequence is present in the hg38 assembly, we  
485 masked all parts of hg38 that would normally be attributed to rDNA sequences (bedtools  
486 v2.25.0 maskfasta) based on alignment positions of 50 bp *in silico* reads generated  
487 across U13369. Finally, we appended the complete rDNA sequence as a distinct

488 sequence (chrU13369.1) to the masked hg38 FASTA resulting in the hg38\_rDNA  
489 assembly used for analysis.

490 Resulting reads were aligned to hg38\_rDNA (STAR v2.4; splicing disabled with '--  
491 alignIntronMax 1'). Next, we called peaks and generated fold-enrichment (FE) bedGraph  
492 files (MACS2 v2.1.0.20140616; callpeak at p-value e-5; and bdgcmp with FE  
493 method)(98). Irreproducible Discovery Rate (IDR) was conducted using unpooled  
494 replicates with all peaks in pooled samples passing an IDR cutoff of 0.5 (99). To reduce  
495 artificial peaks, we calculated strand cross-correlation for all peaks at a shift of 95 bp (the  
496 mean observed fragment size of 180 bp minus the read size of 85bp) and unshifted (100).  
497 We eliminated peaks that exhibited low shifted correlation (shifted correlation <0.7) and  
498 those that exhibited high unshifted correlation relative to shifted (shifted – unshifted  
499 correlation < 0.1). This increased retrieval of the RUNX1 motif and improved agreement  
500 with other RUNX1 datasets. Passing peaks were annotated separately to mRNA and  
501 lncRNA transcript start sites (TSSs) using Gencode v27 with a distance cutoff of 5000 bp.  
502 Regional distribution of peaks was determined using the same annotation reference  
503 limited to the “basic” tag for exons and promoters.

504

## 505 **Inhibitor Treatment and Assessment of Global Protein Synthesis**

506 Core binding factor – Beta (CBF $\beta$ ) inhibitors AI-4-88 and AI-14-91 were kindly  
507 provided by John H. Bushweller (University of Virginia) and used to evaluate RUNX1  
508 DNA-binding inhibition in MCF10A cells. Protein synthesis evaluation by  
509 immunofluorescence was conducted following manufacturer protocol (K715-100,  
510 BioVision, San Francisco, CA). To examine effects of inhibiting the RUNX1-CBF $\beta$

511 interaction, MCF10A cells were treated with active or inactive compound for 48 hours.  
512 Culture medium containing the active or inactive compounds was replaced with fresh  
513 medium without the compounds and cells were harvested 4- and 7-days post medium  
514 change.

515

### 516 **RNA-Sequencing, Differential Expression Analysis, and Pathway Analysis:**

517 RNA was isolated using Direct-zol RNA MiniPrep (Zymo Research, Irvine, CA,  
518 USA) and was quantified and assayed for RNA integrity by Bioanalyzer (Agilent  
519 Technologies, Inc., Santa Clara, CA, USA). Following the removal of ribosomal RNA, the  
520 RNA pool was reverse transcribed, amplified, purified, and bound to strand-specific  
521 adaptors following the manufacturer's protocol (SMARTer Stranded Total RNA Sample  
522 Prep Kit, Takara Bio, Mountain View, CA, USA). cDNA libraries were assayed for quality  
523 control by Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). After cDNA  
524 quality validation, generated libraries were sequenced. 24 hour and 48-hour counts were  
525 grouped together into one "crisis" category and the day 4 recovery and day 7 recovery  
526 counts were grouped together into one "recovery" category. Treatment groups were  
527 compared with untreated MCF10A cells. After demultiplexing and quality filtering, reads  
528 were aligned to hg38 using Gencode (GRCh38.p13). As a reference, annotation with  
529 STAR (v2.5.2a)(101) aligned reads were then counted using HT-Seq (102). Differential  
530 gene expression was analyzed using DESeq2 in R v.3.5.1 (103). Parameters for  
531 significant differential expression were base mean expression greater than five, absolute  
532 log<sub>2</sub> fold change greater than one, and a p-value less than 0.05. Pathway analysis was

533 performed using Gene Set Enrichment Analysis v6.3 (Broad Institute, Inc., MIT, UC San  
534 Diego).

535

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544

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546 No competing interests declared.

547

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559

560 **DATA AVAILABILITY**

561 GEO accession number for the sequencing data generated in this study is  
562 GSE121370.

563 **FIGURE LEGENDS**

564 **Figure 1. RUNX1 associates with DNA during interphase and remains bound**  
565 **throughout mitosis in the form of major and minor foci.** Representative  
566 immunofluorescent images of interphase and mitotic MCF10A breast epithelial cells  
567 showing subcellular localization of RUNX1, identified using a specific antibody,  
568 throughout mitosis. Mitotic cells were further classified into substages of mitosis based  
569 on DAPI topology. RUNX1 – Green (top row), DAPI – Blue (second row from top).  
570 Merged channel images (third row from top) contain an outlined region magnified in the  
571 bottom row labeled “inset”. White arrows highlight major Runx1 foci on mitotic  
572 chromatin. Three independent biological replicates were performed, and at least 20  
573 cells for each mitotic substage were analyzed.

574

575 **Figure 2. RUNX1 occupies protein coding genes and long non-coding RNAs**  
576 **across asynchronous, mitotic, and G1 populations of MCF10A breast epithelial**  
577 **cells.** A) Heatmaps showing peaks called between A, M, and G1 MCF10A cells (left,  
578 middle, and right respectively). Cumulative occupancy of RUNX1 is shown as line  
579 graphs and genes that occupied by RUNX1 in each of the three cell populations are  
580 shown. Shown also are genes that are deregulated upon RUNX1 downregulation. For  
581 example, 1070 genes are bound by RUNX1 in cells that are neither in M nor G1 cells  
582 (green bar graph) and of those, 353 genes are deregulation upon RUNX1 deregulation.  
583 B) Gene ontology analysis of RUNX1-bound genes identifies key regulatory pathways  
584 that are similar or unique in the three cell populations. C) Motif analysis of A, M, and G1  
585 MCF10A cells reveals RUNX motif as one of the top motifs in all cell populations.

586 Binding sites for key transcription factors that are known to cooperate with RUNX1 are  
587 also identified.

588 **Figure 3. RUNX1 bookmarks rDNA promoter repeat regions and affects both pre-**  
589 **rRNA and global protein expression.** A) ChIP-Seq tracks of A, M, and G1 (top,  
590 middle, bottom respectively) MCF10A cells mapped against rDNA repeat regions. B) A  
591 representative metaphase MCF10A cell, stained for RUNX1 (green) and UBF1 (red)  
592 localization, is shown demonstrating that the two proteins colocalize during mitosis  
593 (merged). Cells are also counter stained with DAPI to visualize DNA (blue) and identify  
594 mitosis substages. C) qRT-PCR data of pre-rRNA in actively proliferating MCF10A cells  
595 treated with either active (AI-14-91) or inactive (AI-4-88) compounds for 6, 12, 24, or  
596 48hrs. Expression of pre-rRNA was normalized relative to Beta Actin expression. Graph  
597 represents three independent biological replicates. Asterisks represents a  $p$  value of  
598  $<0.05$ . D) Representative fluorescence microscopy images of global protein synthesis  
599 from MCF10A cells treated with either AI-4-88 (left) or AI-14-91 (right) for 24hr at  $20\mu\text{M}$   
600 ( $n=2$ ). Intensity of red fluorescence at 580nm emission indicates nascent protein  
601 synthesis. All images were taken with 1000ms exposures.

602 **Figure 4. RUNX1 bookmarks RNA Pol II-transcribed genes involved in**  
603 **maintenance of breast epithelial phenotype.** A) Gene Set Enrichment (GSE) analysis  
604 from interrogating mitotically bookmarked genes (i.e. RUNX1 mitotically occupied)  
605 against Hallmark Gene sets from Molecular Signatures Database (MSigDB). The top 10  
606 most significantly overlapping gene sets are shown from top to bottom. B) Scatter plot of  
607 genes identified to be up or down regulated in response to estradiol treatment, that are  
608 also bound by estrogen receptor  $\alpha$  ( $\text{ER}\alpha$ ) and RUNX1 (empty circles, blue for

609 downregulated and red for upregulated). Scatter plot also illustrates up or down  
610 regulated genes in response to estradiol treatment that are bound by ER $\alpha$  and  
611 mitotically bookmarked by RUNX1 (filled in circles, blue for downregulated and red for  
612 upregulated). C) Top panel: ChIP-Seq tracks of *HES1* (left) and *H2AFX* (right) from  
613 asynchronous (top-red), mitotic (middle-green), and G1 (bottom-blue). Bottom panel:  
614 qRT-PCR data of *HES1* (left) and *H2AFX* (right) in asynchronous MCF10A cells treated  
615 with either active (AI-14-91) or inactive (AI-4-88) inhibitors for 6hr, 12hr, 24hr and 48hr  
616 at 20 $\mu$ M. Expression of target genes were normalized relative to beta actin.

617 **Figure 5. Disrupting RUNX1 mitotic gene bookmarking in MCF10A cells leads to a**  
618 **transformed cellular phenotype and EMT.**

619 A) Phase contrast microscopy images of MCF10A cells treated with AI-4-88 or A-14-91  
620 for 48 hours at 20 $\mu$ M. Left panel – 20X magnification, right panel – 40X magnification.  
621 The outlines rectangle in the left panel is the resulting 40X magnification in the right  
622 panel. B) Top panel: Experimental schematic depicting treatment schedule for the  
623 “crisis” and “recovery” stages. Bottom panel: Phase contrast microscopy images from  
624 Day 0, 1, and 2 or crisis where MCF10A cells were treated with AI-14-91 at 20 $\mu$ M (top –  
625 left, middle, right respectively). Phase contrast images from Day 0, 4, and 7 or recovery  
626 following a media replacement. C) Morphological changes upon inhibition of the  
627 cytoskeletal protein F-actin. When compared to inactive compound (left panel), cells  
628 treated with the active compound show substantial alteration in cytoarchitecture (right  
629 panel). D) Western blot for RUNX1, RUNX2, epithelial marker E-cadherin,  
630 mesenchymal marker Vimentin, and loading control Beta-actin (top panel to bottom



631 panel respectively) in MCF10A whole cell lysate harvested from the crisis 24 hour and  
632 48-hour timepoints and recovery day 4 and day 7 timepoints.

633 **Figure 6. Differential expression and pathway analysis of RNA-Seq shows**  
634 **changes in key regulatory pathways involved in cell proliferation, metabolism,**  
635 **cell cycle control, estrogen response, and EMT.** A) Expression heatmap of three  
636 biological replicates of 24-hour and 48-hour crisis timepoints and Day 4 and Day 7  
637 recovery timepoints. B) Scatterplot of log<sub>2</sub> fold change between crisis and recovery  
638 phases. Most changed genes in each stage are indicated. C) Table of overlapping  
639 pathways specific to crisis and recovery stages. D) Representative immunofluorescence  
640 images of the active compound (AI-14-91)-treated MCF10A cells in prophase and  
641 metaphase are shown. A substantial decrease in smaller RUNX1 foci (green) during  
642 mitosis is observed when compared to the inactive (AI-4-88) compound. Large RUNX1  
643 foci that colocalize with UBF (red) are detectable at all substages of mitosis (white  
644 arrows) in the presence of either active or inactive compounds.

645 **Supplementary Figure 1. Secondary antibody controls for immunofluorescence**  
646 **microscopy.** To ensure the specificity of RUNX1 signal on mitotic chromosomes,  
647 actively proliferating mammary epithelial MCF10A cells, grown on gelatin-coated  
648 coverslips, were subjected to immunofluorescence microscopy procedure as described  
649 in Materials and Methods section with one modification: no primary antibody was added,  
650 but secondary antibodies were used at the same dilution as in all IF experiments. Nuclei  
651 were counterstained with DAPI. Two different fields are shown, confirming that the  
652 RUNX1 signal we observe on mitotic chromosomes is specific.

653 **Supplementary Figure 2. RUNX1 associates with chromosomes during all stages**  
654 **of mitosis in unfixed live MCF10A cells.** Mammary epithelial MCF10A cells were  
655 transiently transfected with EGFP-RUNX1 and imaged by confocal microscopy without  
656 fixation (see Materials and Methods section for details). Top panels show RUNX1  
657 (green) association with mitotic chromosomes in unfixed, live MCF10A cells. Cells were  
658 counterstained with Hoechst (middle panels; blue) to visualize DNA in live cells and to  
659 identify mitotic cells. Merged images (bottom panels) were generated to confirm  
660 localization of RUNX1 signal with DNA. Arrow heads indicate punctate RUNX1 foci  
661 retained on mitotic chromosomes.

662 **Supplementary Figure 3. Determination of optimal nocodazole dosage for**  
663 **maximum mitotic block.** A) Micrographs of MCF10A cells, treated with various doses  
664 of nocodazole for 16 hours, are shown (bottom panels). DMSO control treatment is also  
665 included (top panels). B) Experimental schematic depicting mitotic arrest and harvest of  
666 each treated MCF10A cell population: Asynchronous – A, Mitotic – M, and Released –  
667 G1. C) Western blot of each harvested MCF10A population for cell cycle specific  
668 markers to evaluate mitotic arrest and synchronization procedure. D) Fluorescence-  
669 activated cell sorting (FACS) analysis of harvested A, M, and G1 MCF10A populations  
670 to determine mitotic purity and DNA content (n=2 biological replicates per group).

671 **Supplementary Figure 4. RUNX1 colocalizes with RNA Pol I subunit, upstream**  
672 **binding factor (UBF) on mitotic chromatin.** A) Immunofluorescence microscopy  
673 images of RUNX1 (green – top row), UBF (red – 2<sup>nd</sup> row from top), DAPI (blue – 2<sup>nd</sup> row  
674 from bottom), and the three channels merged (bottom row) in MCF10A cells. Images  
675 were captured of spontaneously dividing MCF10A cells in different substages of mitosis.

676 B) Representative images of line profiles taken on interphase vs metaphase cells (n=15  
677 each).

678 **Supplementary Figure 5. The RUNX1-CBF $\beta$  inhibitor reduces RUNX1 association**  
679 **with mitotic chromosomes.** MCF10A cells, treated with 20 $\mu$ M inactive control  
680 compound or active inhibitor for 12 hours, were stained for localization of endogenous  
681 RUNX1 (green) to mitotic chromosomes. RUNX1 retention on mitotic chromosomes,  
682 particularly in smaller foci, was substantially reduced in cells treated with active inhibitor  
683 of RUNX1-CBF $\beta$  interaction, which disrupts RUNX1 DNA binding activity.

684 **Dataset S1 (separate file).** List of genes occupied by RUNX1 in asynchronous, mitotic  
685 and G1 cell populations in mammary epithelial cells.

686 **Dataset S2 (separate file).** Gene enrichment analysis of genes sensitive to RUNX1-  
687 CBF $\beta$  inhibition during crisis and recovery phases of epithelial to mesenchymal  
688 transition in MCF10A cells.

689

690 **REFERENCES**

- 691 1. Banerji S, Cibulskis K, Rangel-Escareno C, Brown KK, Carter SL, Frederick AM,  
692 Lawrence MS, Sivachenko AY, Sougnez C, Zou L, Cortes ML, Fernandez-Lopez JC,  
693 Peng S, Ardlie KG, Auclair D, Bautista-Pina V, Duke F, Francis J, Jung J, Maffuz-Aziz  
694 A, Onofrio RC, Parkin M, Pho NH, Quintanar-Jurado V, Ramos AH, Rebollar-Vega R,  
695 Rodriguez-Cuevas S, Romero-Cordoba SL, Schumacher SE, Stransky N, Thompson  
696 KM, Uribe-Figueroa L, Baselga J, Beroukhir R, Polyak K, Sgroi DC, Richardson AL,  
697 Jimenez-Sanchez G, Lander ES, Gabriel SB, Garraway LA, Golub TR, Melendez-  
698 Zajgla J, Toker A, Getz G, Hidalgo-Miranda A, Meyerson M. 2012. Sequence analysis  
699 of mutations and translocations across breast cancer subtypes. *Nature* 486:405-9.
- 700 2. Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhee SK, Pastore A, Zhang H,  
701 McLellan M, Yau C, Kandoth C, Bowlby R, Shen H, Hayat S, Fieldhouse R, Lester SC,  
702 Tse GM, Factor RE, Collins LC, Allison KH, Chen YY, Jensen K, Johnson NB,  
703 Oesterreich S, Mills GB, Cherniack AD, Robertson G, Benz C, Sander C, Laird PW,  
704 Hoadley KA, King TA, Network TR, Perou CM. 2015. Comprehensive Molecular  
705 Portraits of Invasive Lobular Breast Cancer. *Cell* 163:506-19.
- 706 3. Elsheikh SE, Green AR, Rakha EA, Powe DG, Ahmed RA, Collins HM, Soria D,  
707 Garibaldi JM, Paish CE, Ammar AA, Grainge MJ, Ball GR, Abdelghany MK, Martinez-  
708 Pomares L, Heery DM, Ellis IO. 2009. Global histone modifications in breast cancer  
709 correlate with tumor phenotypes, prognostic factors, and patient outcome. *Cancer Res*  
710 69:3802-9.
- 711 4. Li Z, Herold T, He C, Valk PJ, Chen P, Jurinovic V, Mansmann U, Radmacher  
712 MD, Maharry KS, Sun M, Yang X, Huang H, Jiang X, Sauerland MC, Buchner T,

- 713 Hiddemann W, Elkahloun A, Neilly MB, Zhang Y, Larson RA, Le Beau MM, Caligiuri  
714 MA, Dohner K, Bullinger L, Liu PP, Delwel R, Marcucci G, Lowenberg B, Bloomfield  
715 CD, Rowley JD, Bohlander SK, Chen J. 2013. Identification of a 24-gene prognostic  
716 signature that improves the European LeukemiaNet risk classification of acute myeloid  
717 leukemia: an international collaborative study. *J Clin Oncol* 31:1172-81.
- 718 5. Widschwendter M, Jones PA. 2002. DNA methylation and breast carcinogenesis.  
719 *Oncogene* 21:5462-82.
- 720 6. Zhang M, Lee AV, Rosen JM. 2017. The Cellular Origin and Evolution of Breast  
721 Cancer. *Cold Spring Harb Perspect Med* 7.
- 722 7. Chen T, You Y, Jiang H, Wang ZZ. 2017. Epithelial-mesenchymal transition  
723 (EMT): A biological process in the development, stem cell differentiation, and  
724 tumorigenesis. *J Cell Physiol* 232:3261-3272.
- 725 8. Kalluri R, Weinberg RA. 2009. The basics of epithelial-mesenchymal transition. *J*  
726 *Clin Invest* 119:1420-8.
- 727 9. Nieto MA. 2013. Epithelial plasticity: a common theme in embryonic and cancer  
728 cells. *Science* 342:1234850.
- 729 10. Prieto-Garcia E, Diaz-Garcia CV, Garcia-Ruiz I, Agullo-Ortuno MT. 2017.  
730 Epithelial-to-mesenchymal transition in tumor progression. *Med Oncol* 34:122.
- 731 11. Suarez-Carmona M, Lesage J, Cataldo D, Gilles C. 2017. EMT and  
732 inflammation: inseparable actors of cancer progression. *Mol Oncol* 11:805-823.
- 733 12. Thiery JP, Acloque H, Huang RY, Nieto MA. 2009. Epithelial-mesenchymal  
734 transitions in development and disease. *Cell* 139:871-90.

- 735 13. Micalizzi DS, Farabaugh SM, Ford HL. 2010. Epithelial-mesenchymal transition  
736 in cancer: parallels between normal development and tumor progression. *J Mammary*  
737 *Gland Biol Neoplasia* 15:117-34.
- 738 14. Schmalhofer O, Brabletz S, Brabletz T. 2009. E-cadherin, beta-catenin, and  
739 ZEB1 in malignant progression of cancer. *Cancer Metastasis Rev* 28:151-66.
- 740 15. Taube JH, Herschkowitz JI, Komurov K, Zhou AY, Gupta S, Yang J, Hartwell K,  
741 Onder TT, Gupta PB, Evans KW, Hollier BG, Ram PT, Lander ES, Rosen JM,  
742 Weinberg RA, Mani SA. 2010. Core epithelial-to-mesenchymal transition interactome  
743 gene-expression signature is associated with claudin-low and metaplastic breast  
744 cancer subtypes. *Proc Natl Acad Sci U S A* 107:15449-54.
- 745 16. Yang J, Weinberg RA. 2008. Epithelial-mesenchymal transition: at the  
746 crossroads of development and tumor metastasis. *Dev Cell* 14:818-29.
- 747 17. Bidet A, Laharanne E, Achard S, Migeon M, Moreau C, Lippert E. 2016. Analysis  
748 of RUNX1 rearrangements: insights into leukaemogenesis mechanisms. *Br J*  
749 *Haematol* 175:738-740.
- 750 18. Brettingham-Moore KH, Taberlay PC, Holloway AF. 2015. Interplay between  
751 Transcription Factors and the Epigenome: Insight from the Role of RUNX1 in  
752 Leukemia. *Front Immunol* 6:499.
- 753 19. Chin DW, Watanabe-Okochi N, Wang CQ, Tergaonkar V, Osato M. 2015. Mouse  
754 models for core binding factor leukemia. *Leukemia* 29:1970-80.
- 755 20. De Braekeleer E, Douet-Guilbert N, Morel F, Le Bris MJ, Ferec C, De Braekeleer  
756 M. 2011. RUNX1 translocations and fusion genes in malignant hemopathies. *Future*  
757 *Oncol* 7:77-91.

- 758 21. Dowdy CR, Frederick D, Zaidi SK, Colby JL, Lian JB, van Wijnen AJ, Gerstein  
759 RM, Stein JL, Stein GS. 2013. A germline point mutation in Runx1 uncouples its role in  
760 definitive hematopoiesis from differentiation. *Exp Hematol* 41:980-991 e1.
- 761 22. Dowdy CR, Xie R, Frederick D, Hussain S, Zaidi SK, Vradii D, Javed A, Li X,  
762 Jones SN, Lian JB, van Wijnen AJ, Stein JL, Stein GS. 2010. Definitive hematopoiesis  
763 requires Runx1 C-terminal-mediated subnuclear targeting and transactivation. *Hum*  
764 *Mol Genet* 19:1048-57.
- 765 23. Downing JR, Higuchi M, Lenny N, Yeoh AE. 2000. Alterations of the AML1  
766 transcription factor in human leukemia. *Semin Cell Dev Biol* 11:347-60.
- 767 24. Durst KL, Hiebert SW. 2004. Role of RUNX family members in transcriptional  
768 repression and gene silencing. *Oncogene* 23:4220-4.
- 769 25. Friedman AD. 2009. Cell cycle and developmental control of hematopoiesis by  
770 Runx1. *J Cell Physiol* 219:520-4.
- 771 26. Hart SM, Foroni L. 2002. Core binding factor genes and human leukemia.  
772 *Haematologica* 87:1307-23.
- 773 27. Lam K, Zhang DE. 2012. RUNX1 and RUNX1-ETO: roles in hematopoiesis and  
774 leukemogenesis. *Front Biosci (Landmark Ed)* 17:1120-39.
- 775 28. Lichtinger M, Ingram R, Hannah R, Muller D, Clarke D, Assi SA, Lie ALM,  
776 Noailles L, Vijayabaskar MS, Wu M, Tenen DG, Westhead DR, Kouskoff V, Lacaud G,  
777 Gottgens B, Bonifer C. 2012. RUNX1 reshapes the epigenetic landscape at the onset  
778 of haematopoiesis. *EMBO J* 31:4318-33.

- 779 29. Lo Coco F, Pisegna S, Diverio D. 1997. The AML1 gene: a transcription factor  
780 involved in the pathogenesis of myeloid and lymphoid leukemias. *Haematologica*  
781 82:364-70.
- 782 30. Sood R, Kamikubo Y, Liu P. 2017. Role of RUNX1 in hematological  
783 malignancies. *Blood* 129:2070-2082.
- 784 31. Browne G, Dragon JA, Hong D, Messier TL, Gordon JA, Farina NH, Boyd JR,  
785 VanOudenhove JJ, Perez AW, Zaidi SK, Stein JL, Stein GS, Lian JB. 2016.  
786 MicroRNA-378-mediated suppression of Runx1 alleviates the aggressive phenotype of  
787 triple-negative MDA-MB-231 human breast cancer cells. *Tumour Biol* 37:8825-39.
- 788 32. Browne G, Taipaleenmaki H, Bishop NM, Madasu SC, Shaw LM, van Wijnen AJ,  
789 Stein JL, Stein GS, Lian JB. 2015. Runx1 is associated with breast cancer progression  
790 in MMTV-PyMT transgenic mice and its depletion in vitro inhibits migration and  
791 invasion. *J Cell Physiol* 230:2522-32.
- 792 33. Chinge NO, Frenkel B. 2013. The RUNX family in breast cancer: relationships  
793 with estrogen signaling. *Oncogene* 32:2121-30.
- 794 34. Chinge NO, Little GH, Baniwal SK, Adisetiyo H, Xie Y, Zhang T, O'Laughlin A,  
795 Liu ZY, Ulrich P, Martin A, Mhawech-Fauceglia P, Ellis MJ, Tripathy D, Groshen S,  
796 Liang C, Li Z, Schones DE, Frenkel B. 2016. RUNX1 prevents oestrogen-mediated  
797 AXIN1 suppression and beta-catenin activation in ER-positive breast cancer. *Nat*  
798 *Commun* 7:10751.
- 799 35. Ferrari N, Mohammed ZM, Nixon C, Mason SM, Mallon E, McMillan DC, Morris  
800 JS, Cameron ER, Edwards J, Blyth K. 2014. Expression of RUNX1 correlates with  
801 poor patient prognosis in triple negative breast cancer. *PLoS One* 9:e100759.



- 802 36. Janes KA. 2011. RUNX1 and its understudied role in breast cancer. *Cell Cycle*  
803 10:3461-5.
- 804 37. Recouvreux MS, Grasso EN, Echeverria PC, Rocha-Viegas L, Castilla LH,  
805 Schere-Levy C, Tocci JM, Kordon EC, Rubinstein N. 2016. RUNX1 and FOXP3  
806 interplay regulates expression of breast cancer related genes. *Oncotarget* 7:6552-65.
- 807 38. Wang L, Brugge JS, Janes KA. 2011. Intersection of FOXO- and RUNX1-  
808 mediated gene expression programs in single breast epithelial cells during  
809 morphogenesis and tumor progression. *Proc Natl Acad Sci U S A* 108:E803-12.
- 810 39. Hong D, Messier TL, Tye CE, Dobson JR, Fritz AJ, Sikora KR, Browne G, Stein  
811 JL, Lian JB, Stein GS. 2017. Runx1 stabilizes the mammary epithelial cell phenotype  
812 and prevents epithelial to mesenchymal transition. *Oncotarget* 8:17610-17627.
- 813 40. Sokol ES, Sanduja S, Jin DX, Miller DH, Mathis RA, Gupta PB. 2015.  
814 Perturbation-expression analysis identifies RUNX1 as a regulator of human mammary  
815 stem cell differentiation. *PLoS Comput Biol* 11:e1004161.
- 816 41. van Bragt MP, Hu X, Xie Y, Li Z. 2014. RUNX1, a transcription factor mutated in  
817 breast cancer, controls the fate of ER-positive mammary luminal cells. *Elife* 3:e03881.
- 818 42. Festuccia N, Gonzalez I, Owens N, Navarro P. 2017. Mitotic bookmarking in  
819 development and stem cells. *Development* 144:3633-3645.
- 820 43. John S, Workman JL. 1998. Bookmarking genes for activation in condensed  
821 mitotic chromosomes. *Bioessays* 20:275-9.
- 822 44. Kadauke S, Blobel GA. 2013. Mitotic bookmarking by transcription factors.  
823 *Epigenetics Chromatin* 6:6.

- 824 45. Lodhi N, Ji Y, Tulin A. 2016. Mitotic bookmarking: maintaining post-mitotic  
825 reprogramming of transcription reactivation. *Curr Mol Biol Rep* 2:10-16.
- 826 46. Sarge KD, Park-Sarge OK. 2009. Mitotic bookmarking of formerly active genes:  
827 keeping epigenetic memories from fading. *Cell Cycle* 8:818-23.
- 828 47. Zaidi SK, Lian JB, van Wijnen A, Stein JL, Stein GS. 2017. Mitotic Gene  
829 Bookmarking: An Epigenetic Mechanism for Coordination of Lineage Commitment,  
830 Cell Identity and Cell Growth. *Adv Exp Med Biol* 962:95-102.
- 831 48. Zaidi SK, Young DW, Montecino MA, Lian JB, van Wijnen AJ, Stein JL, Stein GS.  
832 2010. Mitotic bookmarking of genes: a novel dimension to epigenetic control. *Nat Rev*  
833 *Genet* 11:583-9.
- 834 49. Zaret KS. 2014. Genome reactivation after the silence in mitosis: recapitulating  
835 mechanisms of development? *Dev Cell* 29:132-4.
- 836 50. Ali SA, Dobson JR, Lian JB, Stein JL, van Wijnen AJ, Zaidi SK, Stein GS. 2012.  
837 A RUNX2-HDAC1 co-repressor complex regulates rRNA gene expression by  
838 modulating UBF acetylation. *J Cell Sci* 125:2732-9.
- 839 51. Ali SA, Zaidi SK, Dacwag CS, Salma N, Young DW, Shakoori AR, Montecino  
840 MA, Lian JB, van Wijnen AJ, Imbalzano AN, Stein GS, Stein JL. 2008. Phenotypic  
841 transcription factors epigenetically mediate cell growth control. *Proc Natl Acad Sci U S*  
842 *A* 105:6632-7.
- 843 52. Ali SA, Zaidi SK, Dobson JR, Shakoori AR, Lian JB, Stein JL, van Wijnen AJ,  
844 Stein GS. 2010. Transcriptional corepressor TLE1 functions with Runx2 in epigenetic  
845 repression of ribosomal RNA genes. *Proc Natl Acad Sci U S A* 107:4165-9.

- 846 53. Bakshi R, Zaidi SK, Pande S, Hassan MQ, Young DW, Montecino M, Lian JB,  
847 van Wijnen AJ, Stein JL, Stein GS. 2008. The leukemogenic t(8;21) fusion protein  
848 AML1-ETO controls rRNA genes and associates with nucleolar-organizing regions at  
849 mitotic chromosomes. *J Cell Sci* 121:3981-90.
- 850 54. Pande S, Ali SA, Dowdy C, Zaidi SK, Ito K, Ito Y, Montecino MA, Lian JB, Stein  
851 JL, van Wijnen AJ, Stein GS. 2009. Subnuclear targeting of the Runx3 tumor  
852 suppressor and its epigenetic association with mitotic chromosomes. *J Cell Physiol*  
853 218:473-9.
- 854 55. Young DW, Hassan MQ, Pratap J, Galindo M, Zaidi SK, Lee SH, Yang X, Xie R,  
855 Javed A, Underwood JM, Furcinitti P, Imbalzano AN, Penman S, Nickerson JA,  
856 Montecino MA, Lian JB, Stein JL, van Wijnen AJ, Stein GS. 2007. Mitotic occupancy  
857 and lineage-specific transcriptional control of rRNA genes by Runx2. *Nature* 445:442-  
858 6.
- 859 56. Young DW, Hassan MQ, Yang XQ, Galindo M, Javed A, Zaidi SK, Furcinitti P,  
860 Lapointe D, Montecino M, Lian JB, Stein JL, van Wijnen AJ, Stein GS. 2007. Mitotic  
861 retention of gene expression patterns by the cell fate-determining transcription factor  
862 Runx2. *Proc Natl Acad Sci U S A* 104:3189-94.
- 863 57. Zaidi SK, Young DW, Pockwinse SM, Javed A, Lian JB, Stein JL, van Wijnen AJ,  
864 Stein GS. 2003. Mitotic partitioning and selective reorganization of tissue-specific  
865 transcription factors in progeny cells. *Proc Natl Acad Sci U S A* 100:14852-7.
- 866 58. Pallier C, Scaffidi P, Chopineau-Proust S, Agresti A, Nordmann P, Bianchi ME,  
867 Marechal V. 2003. Association of chromatin proteins high mobility group box (HMGB)  
868 1 and HMGB2 with mitotic chromosomes. *Mol Biol Cell* 14:3414-26.

- 869 59. Arampatzi P, Gialitakis M, Makatounakis T, Papamatheakis J. 2013. Gene-  
870 specific factors determine mitotic expression and bookmarking via alternate regulatory  
871 elements. *Nucleic Acids Res* 41:2202-15.
- 872 60. Blobel GA, Kadauke S, Wang E, Lau AW, Zuber J, Chou MM, Vakoc CR. 2009.  
873 A reconfigured pattern of MLL occupancy within mitotic chromatin promotes rapid  
874 transcriptional reactivation following mitotic exit. *Mol Cell* 36:970-83.
- 875 61. Caravaca JM, Donahue G, Becker JS, He X, Vinson C, Zaret KS. 2013.  
876 Bookmarking by specific and nonspecific binding of FoxA1 pioneer factor to mitotic  
877 chromosomes. *Genes Dev* 27:251-60.
- 878 62. Deluz C, Friman ET, Strebinger D, Benke A, Raccaud M, Callegari A, Leleu M,  
879 Manley S, Suter DM. 2016. A role for mitotic bookmarking of SOX2 in pluripotency and  
880 differentiation. *Genes Dev* 30:2538-2550.
- 881 63. Festuccia N, Dubois A, Vandormael-Pournin S, Gallego Tejada E, Mouren A,  
882 Bessonard S, Mueller F, Proux C, Cohen-Tannoudji M, Navarro P. 2016. Mitotic  
883 binding of Esrrb marks key regulatory regions of the pluripotency network. *Nat Cell Biol*  
884 18:1139-1148.
- 885 64. Kadauke S, Udugama MI, Pawlicki JM, Achtman JC, Jain DP, Cheng Y,  
886 Hardison RC, Blobel GA. 2012. Tissue-specific mitotic bookmarking by hematopoietic  
887 transcription factor GATA1. *Cell* 150:725-37.
- 888 65. Lake RJ, Tsai PF, Choi I, Won KJ, Fan HY. 2014. RBPJ, the major transcriptional  
889 effector of Notch signaling, remains associated with chromatin throughout mitosis,  
890 suggesting a role in mitotic bookmarking. *PLoS Genet* 10:e1004204.

- 891 66. Lerner J, Bagattin A, Verdeguer F, Makinistoglu MP, Garbay S, Felix T, Heidet L,  
892 Pontoglio M. 2016. Human mutations affect the epigenetic/bookmarking function of  
893 HNF1B. *Nucleic Acids Res* 44:8097-111.
- 894 67. Liu Y, Pelham-Webb B, Di Giammartino DC, Li J, Kim D, Kita K, Saiz N, Garg V,  
895 Doane A, Giannakakou P, Hadjantonakis AK, Elemento O, Apostolou E. 2017.  
896 Widespread Mitotic Bookmarking by Histone Marks and Transcription Factors in  
897 Pluripotent Stem Cells. *Cell Rep* 19:1283-1293.
- 898 68. Lodhi N, Kossenkov AV, Tulin AV. 2014. Bookmarking promoters in mitotic  
899 chromatin: poly(ADP-ribose)polymerase-1 as an epigenetic mark. *Nucleic Acids Res*  
900 42:7028-38.
- 901 69. Teves SS, An L, Hansen AS, Xie L, Darzacq X, Tjian R. 2016. A dynamic mode  
902 of mitotic bookmarking by transcription factors. *Elife* 5.
- 903 70. Xing H, Wilkerson DC, Mayhew CN, Lubert EJ, Skaggs HS, Goodson ML, Hong  
904 Y, Park-Sarge OK, Sarge KD. 2005. Mechanism of hsp70i gene bookmarking. *Science*  
905 307:421-3.
- 906 71. Jordan P, Mannervik M, Tora L, Carmo-Fonseca M. 1996. In vivo evidence that  
907 TATA-binding protein/SL1 colocalizes with UBF and RNA polymerase I when rRNA  
908 synthesis is either active or inactive. *The Journal of Cell Biology* 133:225-234.
- 909 72. Chen CCL, Goyal P, Karimi MM, Abildgaard MH, Kimura H, Lorincz MC. 2018.  
910 H3S10ph broadly marks early-replicating domains in interphase ESCs and shows  
911 reciprocal antagonism with H3K9me2. *Genome Res* 28:37-51.
- 912 73. Goto H, Tomono Y, Ajiro K, Kosako H, Fujita M, Sakurai M, Okawa K, Iwamatsu  
913 A, Okigaki T, Takahashi T, Inagaki M. 1999. Identification of a novel phosphorylation

- 914 site on histone H3 coupled with mitotic chromosome condensation. *J Biol Chem*  
915 274:25543-9.
- 916 74. Shrivastava T, Mino K, Babayeva ND, Baranovskaya OI, Rizzino A, Tahirov TH.  
917 2014. Structural basis of Ets1 activation by Runx1. *Leukemia* 28:2040-8.
- 918 75. Gébrane-Younès J, Fomproix N, Hernandez-Verdun D. 1997. When rDNA  
919 transcription is arrested during mitosis, UBF is still associated with non-condensed  
920 rDNA. *Journal of Cell Science* 110 ( Pt 19):2429-2440.
- 921 76. Carlton AL, Illendula A, Gao Y, Llana DC, Boulton A, Shah A, Rajewski RA,  
922 Landen CN, Wotton D, Bushweller JH. 2018. Small molecule inhibition of the  
923 CBFbeta/RUNX interaction decreases ovarian cancer growth and migration through  
924 alterations in genes related to epithelial-to-mesenchymal transition. *Gynecol Oncol*  
925 149:350-360.
- 926 77. Illendula A, Gilmour J, Grembecka J, Tirumala VSS, Boulton A, Kuntimaddi A,  
927 Schmidt C, Wang L, Pulikkan JA, Zong H, Parlak M, Kuscu C, Pickin A, Zhou Y, Gao  
928 Y, Mishra L, Adli M, Castilla LH, Rajewski RA, Janes KA, Guzman ML, Bonifer C,  
929 Bushweller JH. 2016. Small Molecule Inhibitor of CBFbeta-RUNX Binding for RUNX  
930 Transcription Factor Driven Cancers. *EBioMedicine* 8:117-131.
- 931 78. Kim W, Barron DA, San Martin R, Chan KS, Tran LL, Yang F, Ressler SJ,  
932 Rowley DR. 2014. RUNX1 is essential for mesenchymal stem cell proliferation and  
933 myofibroblast differentiation. *Proc Natl Acad Sci U S A* 111:16389-94.
- 934 79. Wu D, Ozaki T, Yoshihara Y, Kubo N, Nakagawara A. 2013. Runt-related  
935 transcription factor 1 (RUNX1) stimulates tumor suppressor p53 protein in response to  
936 DNA damage through complex formation and acetylation. *J Biol Chem* 288:1353-64.

- 937 80. Ozaki T, Nakagawara A, Nagase H. 2013. RUNX Family Participates in the  
938 Regulation of p53-Dependent DNA Damage Response. *Int J Genomics* 2013:271347.
- 939 81. Satoh Y, Matsumura I, Tanaka H, Harada H, Harada Y, Matsui K, Shibata M,  
940 Mizuki M, Kanakura Y. 2012. C-terminal mutation of RUNX1 attenuates the DNA-  
941 damage repair response in hematopoietic stem cells. *Leukemia* 26:303-11.
- 942 82. Xu L, Gu ZH, Li Y, Zhang JL, Chang CK, Pan CM, Shi JY, Shen Y, Chen B,  
943 Wang YY, Jiang L, Lu J, Xu X, Tan JL, Chen Y, Wang SY, Li X, Chen Z, Chen SJ.  
944 2014. Genomic landscape of CD34+ hematopoietic cells in myelodysplastic syndrome  
945 and gene mutation profiles as prognostic markers. *Proc Natl Acad Sci U S A*  
946 111:8589-94.
- 947 83. Cargnello M, Tcherkezian J, Roux PP. 2015. The expanding role of mTOR in  
948 cancer cell growth and proliferation. *Mutagenesis* 30:169-76.
- 949 84. Ciruelos Gil EM. 2014. Targeting the PI3K/AKT/mTOR pathway in estrogen  
950 receptor-positive breast cancer. *Cancer Treat Rev* 40:862-71.
- 951 85. Feng Y, Manka D, Wagner KU, Khan SA. 2007. Estrogen receptor-alpha  
952 expression in the mammary epithelium is required for ductal and alveolar  
953 morphogenesis in mice. *Proc Natl Acad Sci U S A* 104:14718-23.
- 954 86. Mallepell S, Krust A, Chambon P, Briskin C. 2006. Paracrine signaling through  
955 the epithelial estrogen receptor alpha is required for proliferation and morphogenesis  
956 in the mammary gland. *Proc Natl Acad Sci U S A* 103:2196-201.
- 957 87. Mueller SO, Clark JA, Myers PH, Korach KS. 2002. Mammary gland  
958 development in adult mice requires epithelial and stromal estrogen receptor alpha.  
959 *Endocrinology* 143:2357-65.

- 960 88. Stender JD, Kim K, Charn TH, Komm B, Chang KC, Kraus WL, Benner C, Glass  
961 CK, Katzenellenbogen BS. 2010. Genome-wide analysis of estrogen receptor alpha  
962 DNA binding and tethering mechanisms identifies Runx1 as a novel tethering factor in  
963 receptor-mediated transcriptional activation. *Mol Cell Biol* 30:3943-55.
- 964 89. Barutcu AR, Hong D, Lajoie BR, McCord RP, van Wijnen AJ, Lian JB, Stein JL,  
965 Dekker J, Imbalzano AN, Stein GS. 2016. RUNX1 contributes to higher-order  
966 chromatin organization and gene regulation in breast cancer cells. *Biochim Biophys*  
967 *Acta* 1859:1389-1397.
- 968 90. Theodorou V, Stark R, Menon S, Carroll JS. 2013. GATA3 acts upstream of  
969 FOXA1 in mediating ESR1 binding by shaping enhancer accessibility. *Genome Res*  
970 23:12-22.
- 971 91. Gutschner T, Hammerle M, Diederichs S. 2013. MALAT1 -- a paradigm for long  
972 noncoding RNA function in cancer. *J Mol Med (Berl)* 91:791-801.
- 973 92. Yu X, Li Z, Zheng H, Chan MT, Wu WK. 2017. NEAT1: A novel cancer-related  
974 long non-coding RNA. *Cell Prolif* 50:e12329.
- 975 93. Li X, Cao Y, Li M, Jin F. 2018. Upregulation of HES1 Promotes Cell Proliferation  
976 and Invasion in Breast Cancer as a Prognosis Marker and Therapy Target via the AKT  
977 Pathway and EMT Process. *J Cancer* 9:757-766.
- 978 94. Strom A, Arai N, Leers J, Gustafsson JA. 2000. The Hairy and Enhancer of Split  
979 homologue-1 (HES-1) mediates the proliferative effect of 17beta-estradiol on breast  
980 cancer cell lines. *Oncogene* 19:5951-3.



- 981 95. Weyemi U, Redon CE, Sethi TK, Burrell AS, Jailwala P, Kasoji M, Abrams N,  
982 Merchant A, Bonner WM. 2016. Twist1 and Slug mediate H2AX-regulated epithelial-  
983 mesenchymal transition in breast cells. *Cell Cycle* 15:2398-404.
- 984 96. Kageyama R, Ohtsuka T, Kobayashi T. 2007. The Hes gene family: repressors  
985 and oscillators that orchestrate embryogenesis. *Development* 134:1243-51.
- 986 97. Rani A, Greenlaw R, Smith RA, Galustian C. 2016. HES1 in immunity and  
987 cancer. *Cytokine Growth Factor Rev* 30:113-7.
- 988 98. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, Nusbaum  
989 C, Myers RM, Brown M, Li W, Liu XS. 2008. Model-based analysis of ChIP-Seq  
990 (MACS). *Genome Biol* 9:R137.
- 991 99. Li QH, Brown JB, Huang HY, Bickel PJ. 2011. Measuring Reproducibility of High-  
992 Throughput Experiments. *Annals of Applied Statistics* 5:1752-1779.
- 993 100. Landt SG, Marinov GK, Kundaje A, Kheradpour P, Pauli F, Batzoglou S,  
994 Bernstein BE, Bickel P, Brown JB, Cayting P, Chen Y, DeSalvo G, Epstein C, Fisher-  
995 Aylor KI, Euskirchen G, Gerstein M, Gertz J, Hartemink AJ, Hoffman MM, Iyer VR,  
996 Jung YL, Karmakar S, Kellis M, Kharchenko PV, Li Q, Liu T, Liu XS, Ma L,  
997 Milosavljevic A, Myers RM, Park PJ, Pazin MJ, Perry MD, Raha D, Reddy TE,  
998 Rozowsky J, Shores N, Sidow A, Slattery M, Stamatoyannopoulos JA, Tolstorukov  
999 MY, White KP, Xi S, Farnham PJ, Lieb JD, Wold BJ, Snyder M. 2012. ChIP-seq  
1000 guidelines and practices of the ENCODE and modENCODE consortia. *Genome Res*  
1001 22:1813-31.

- 1002 101. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P,  
1003 Chaisson M, Gingeras TR. 2013. STAR: ultrafast universal RNA-seq aligner.  
1004 Bioinformatics 29:15-21.
- 1005 102. Anders S, Pyl PT, Huber W. 2015. HTSeq--a Python framework to work with  
1006 high-throughput sequencing data. Bioinformatics 31:166-9.
- 1007 103. Anders S, Huber W. 2010. Differential expression analysis for sequence count  
1008 data. Genome Biol 11:R106.
- 1009

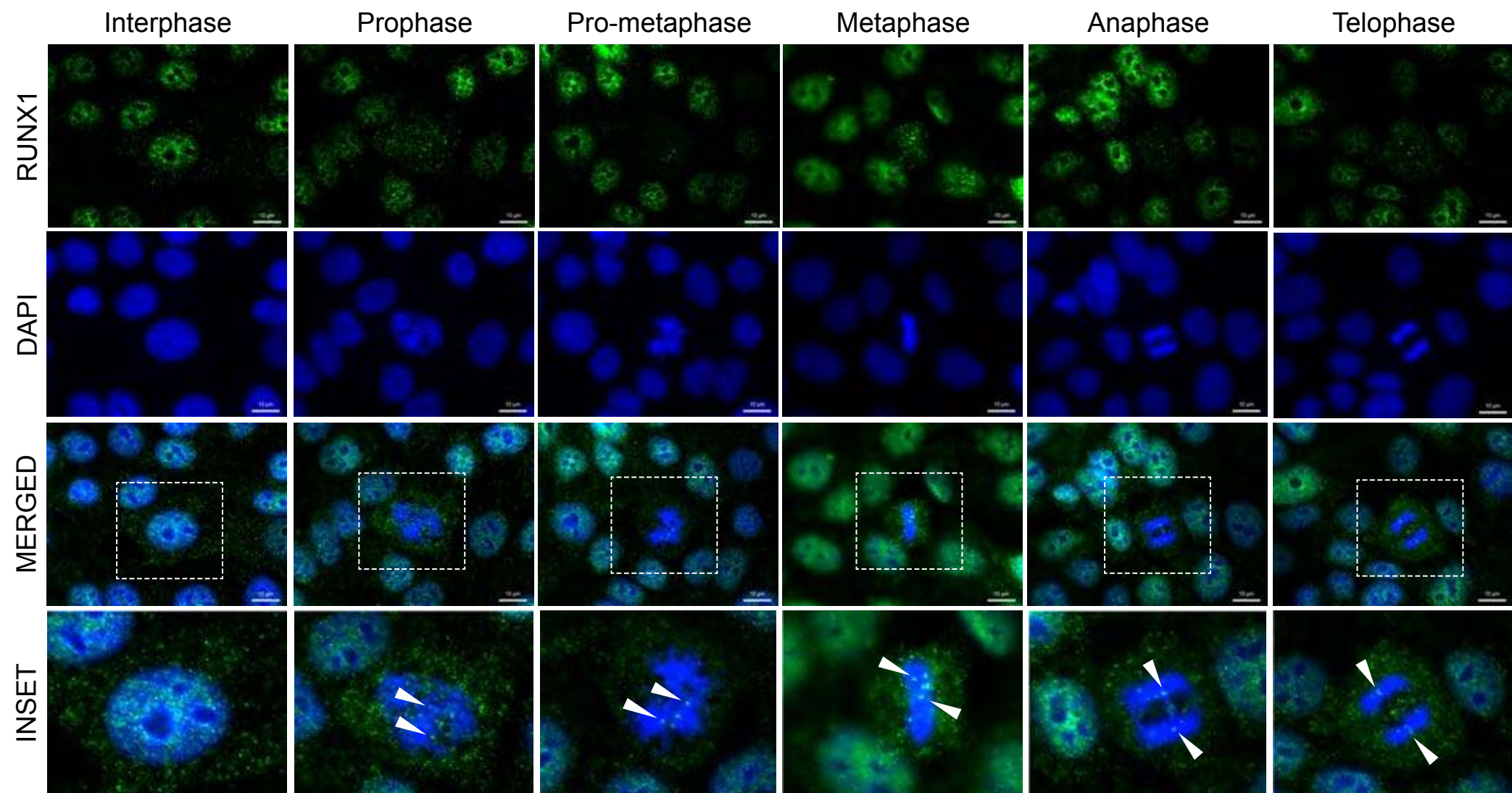


Figure 1

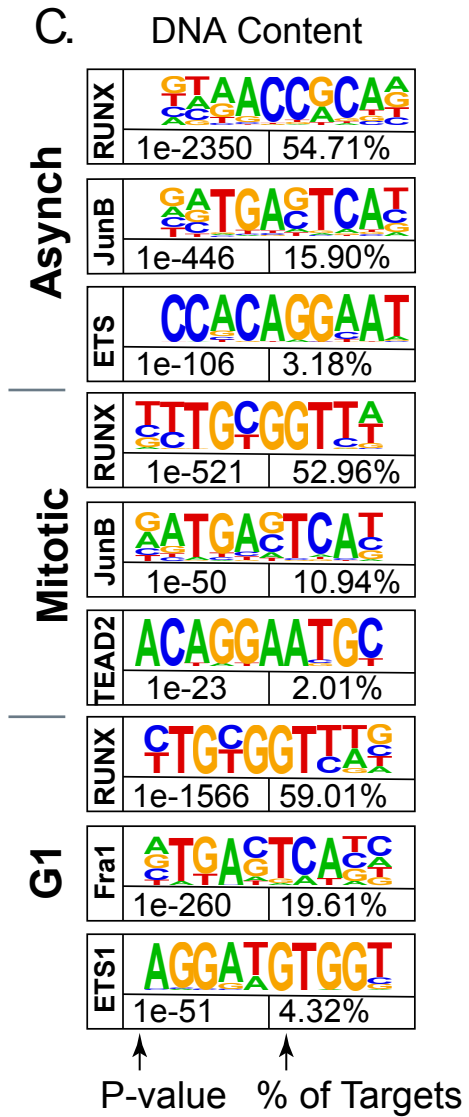
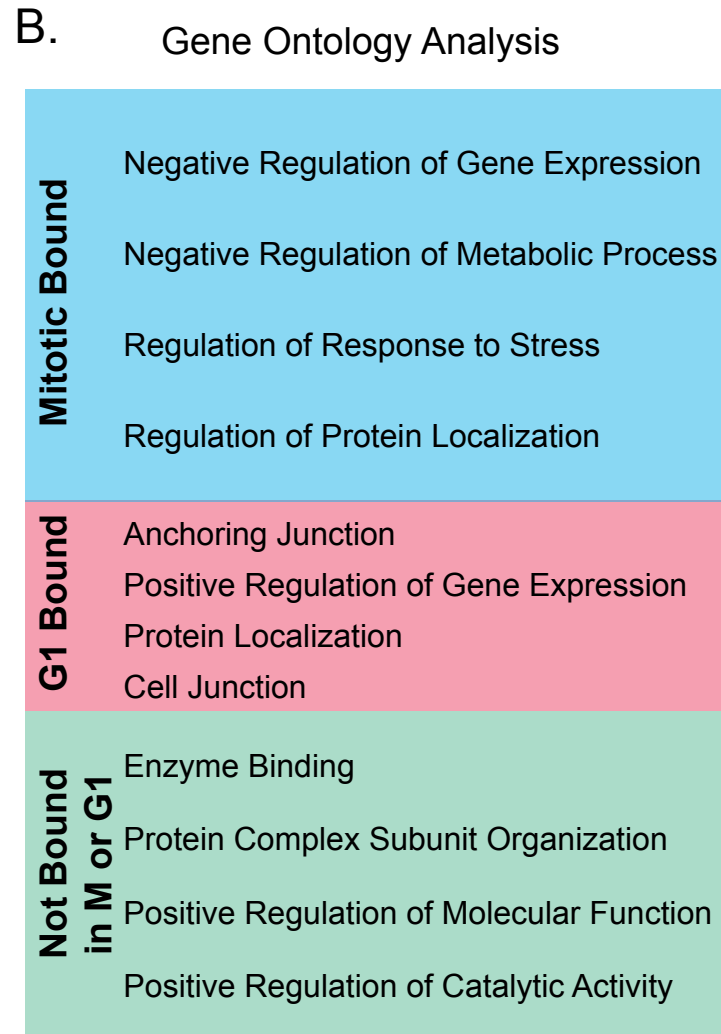
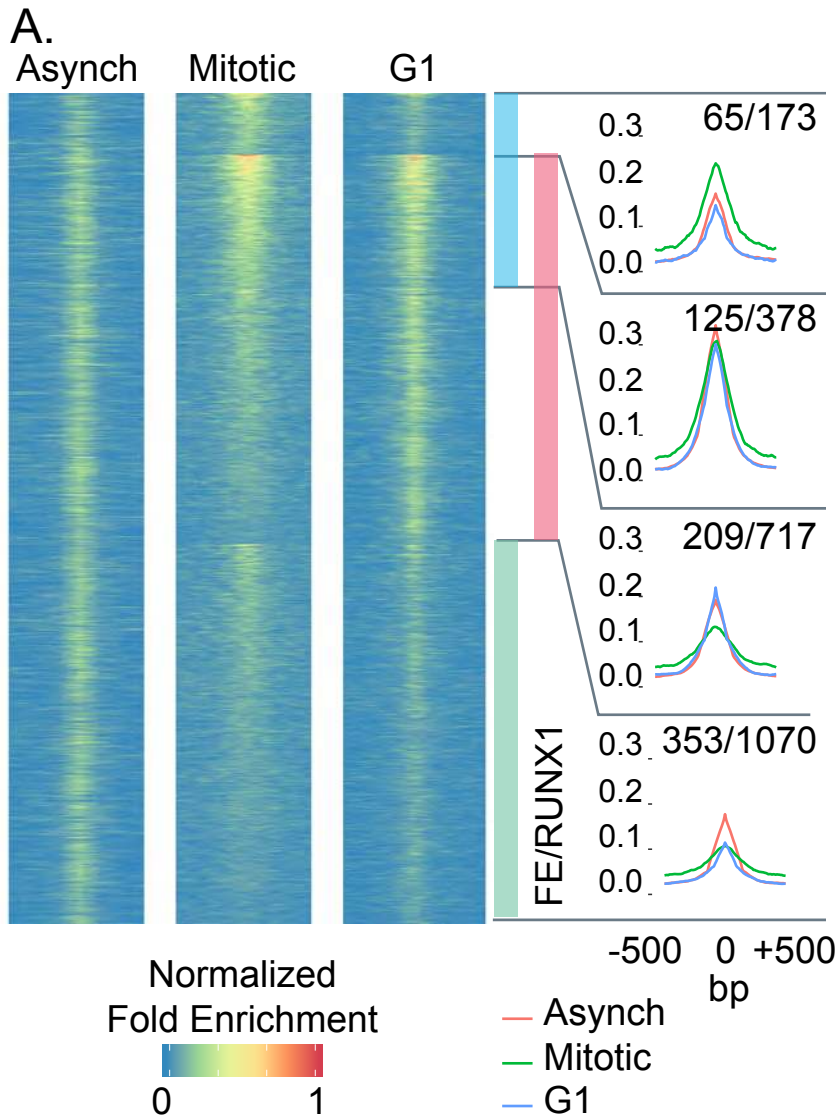
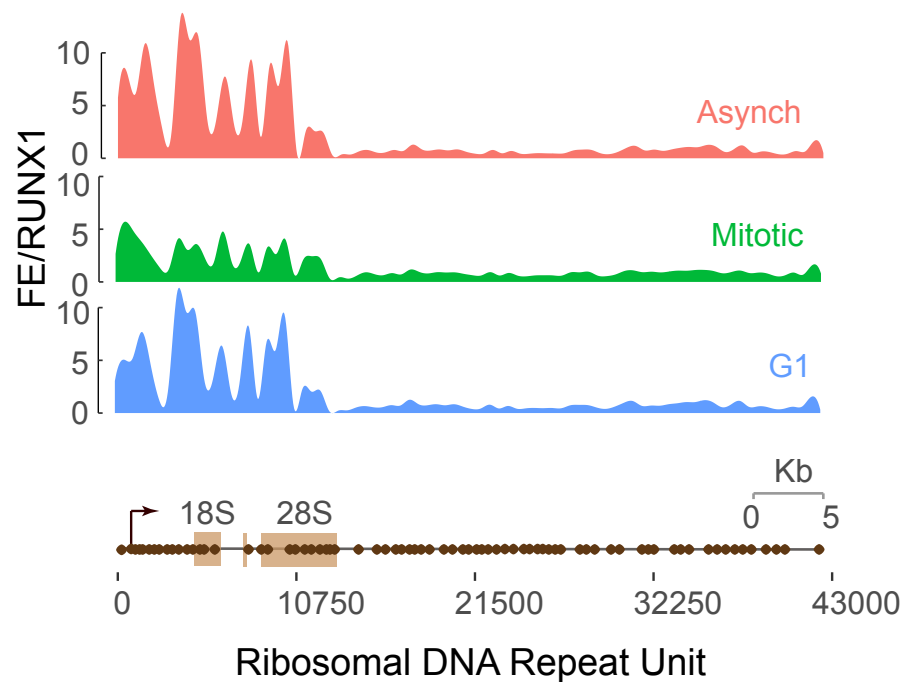
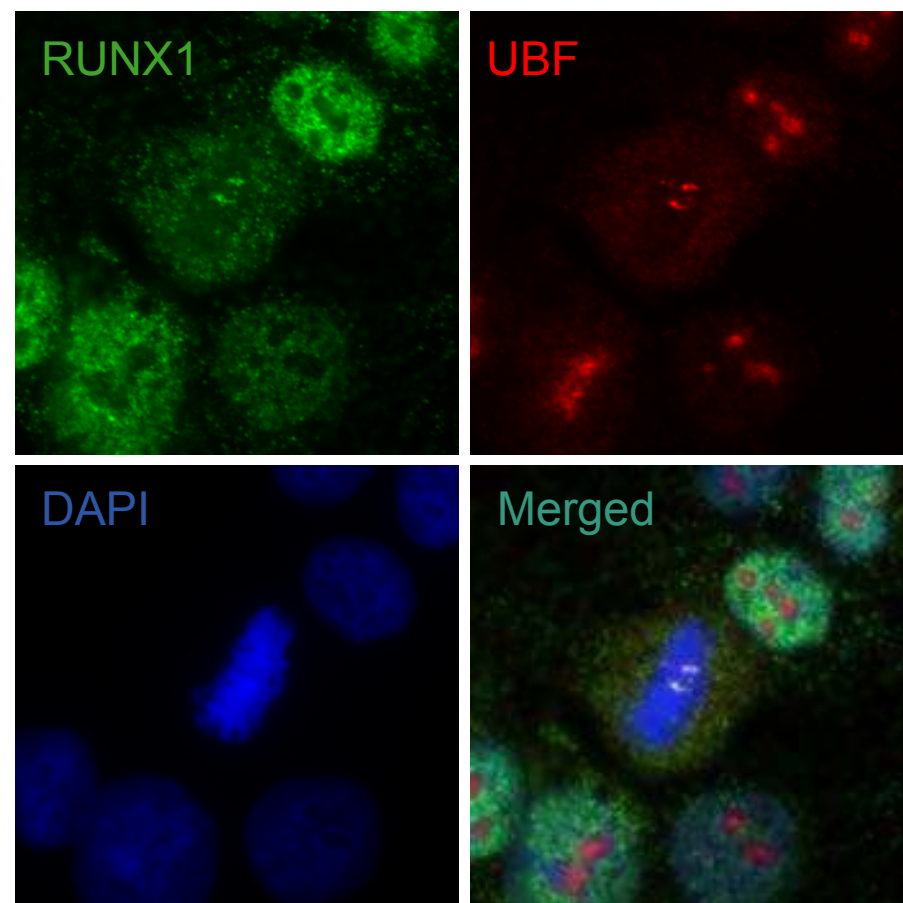


Figure 2

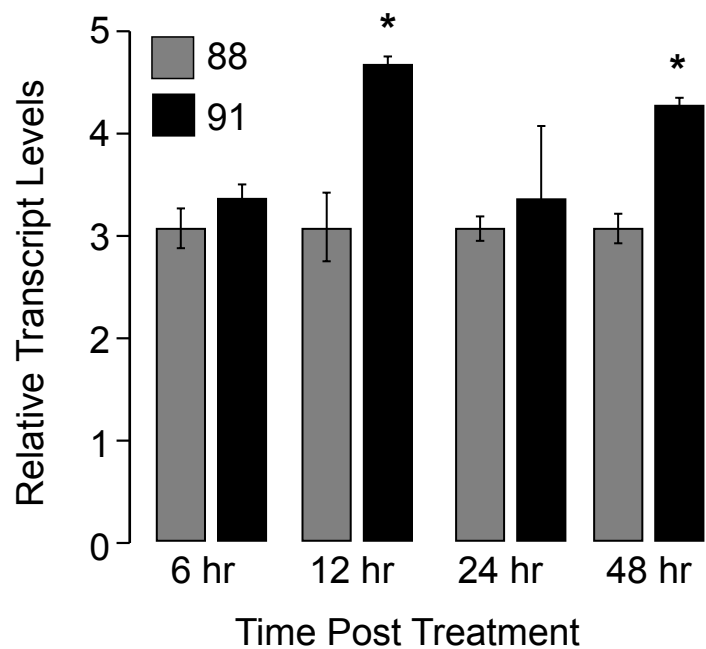
### A Genomic Tracks



### B Immunofluorescence



### C pre-rRNA



### D Protein Label

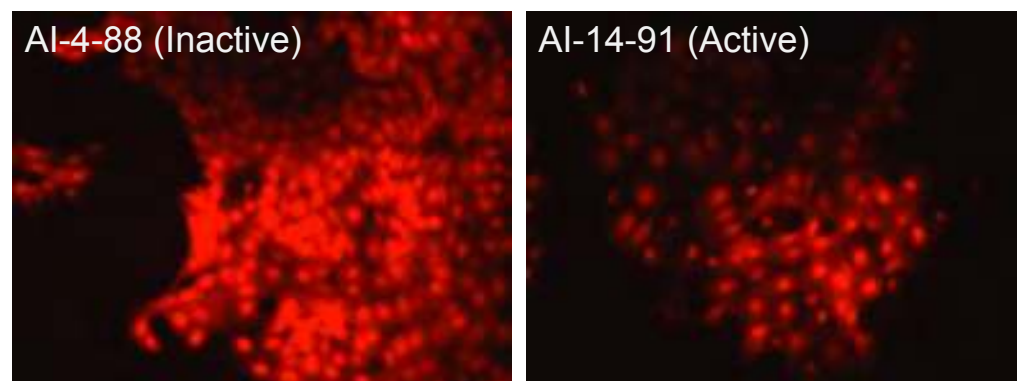


Figure 3

**A**

Hallmark Gene Set	# of Bookmarked Genes
Late Estrogen Response	12
mTOR Signaling	12
TNF $\alpha$ Signaling	12
Apical Junction	11
Early Estrogen Response	10
G2M Checkpoint	10
p53 Pathway	10
DNA Repair	8
E2F targets	9
Notch Signaling	4

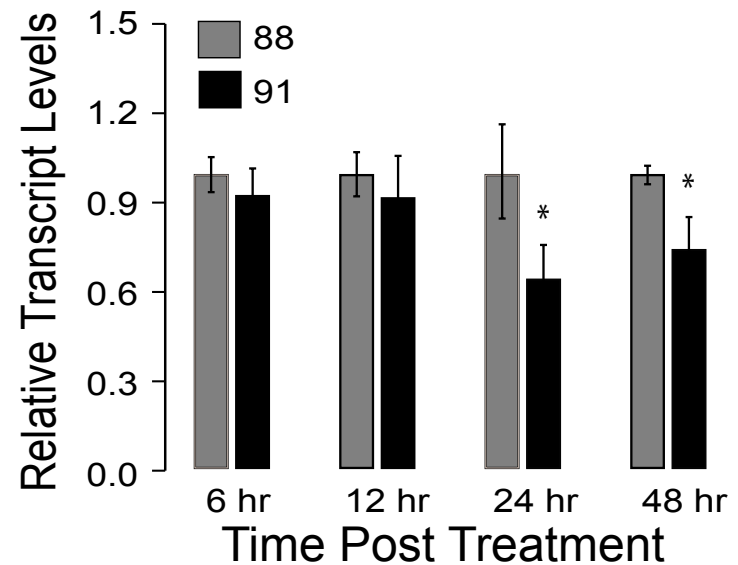
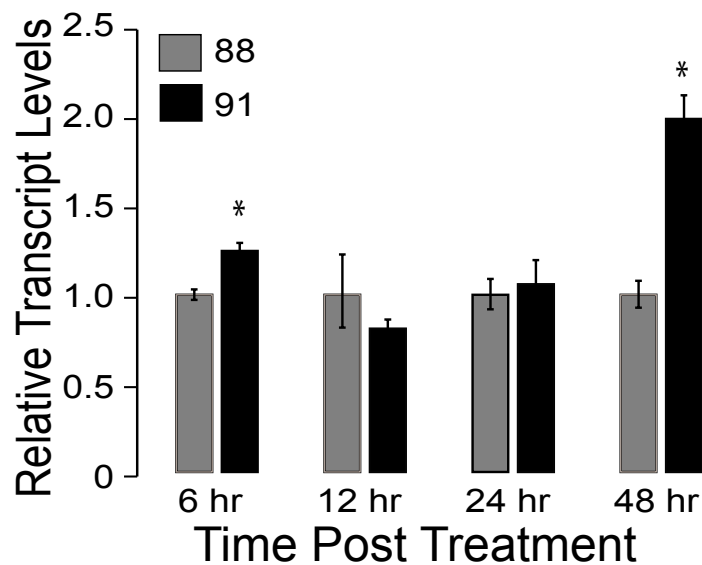
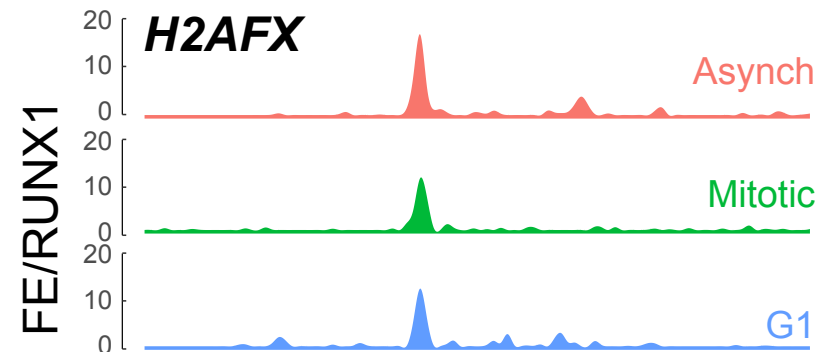
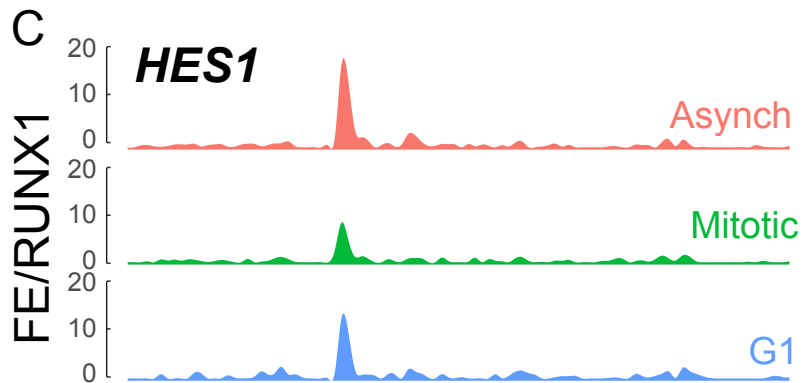
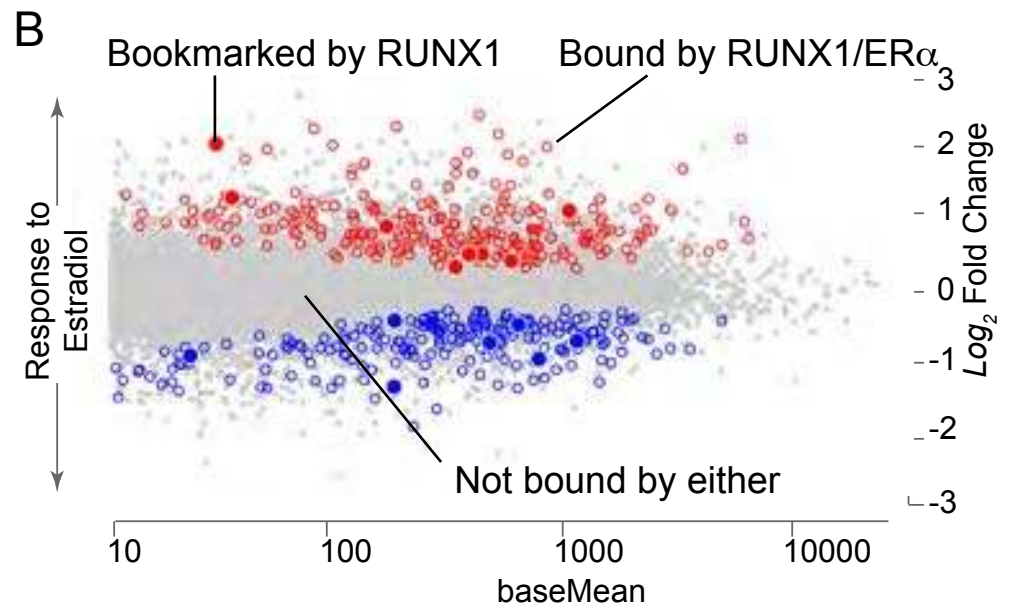


Figure 4

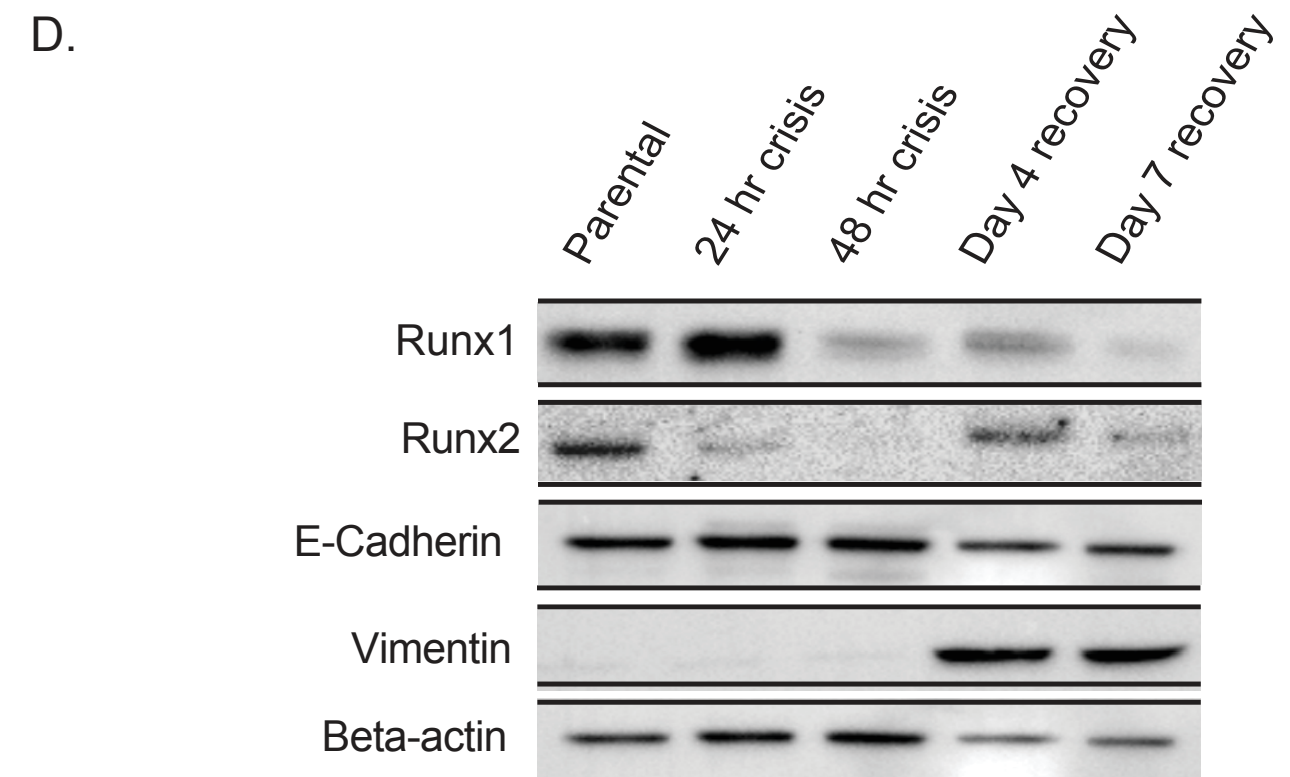
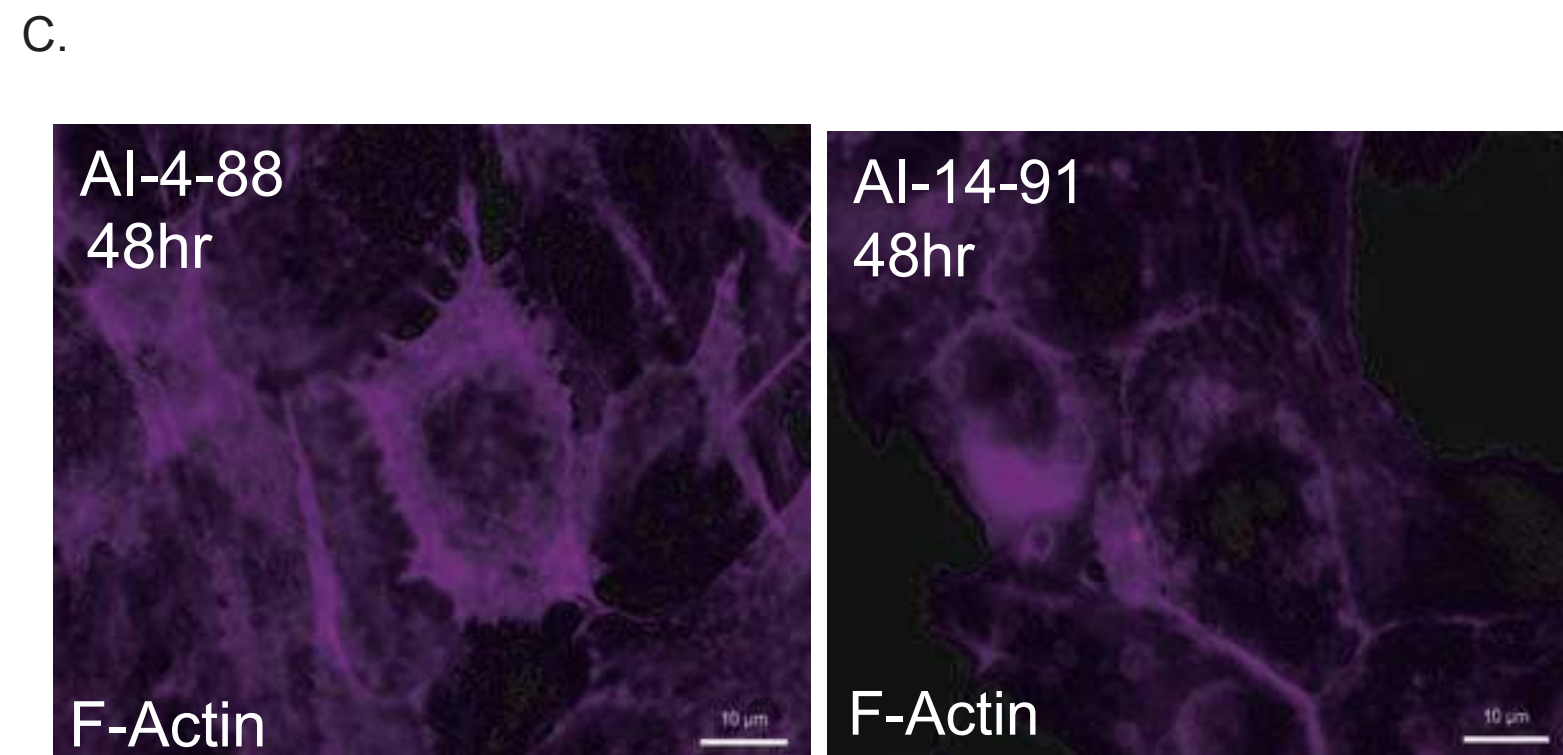
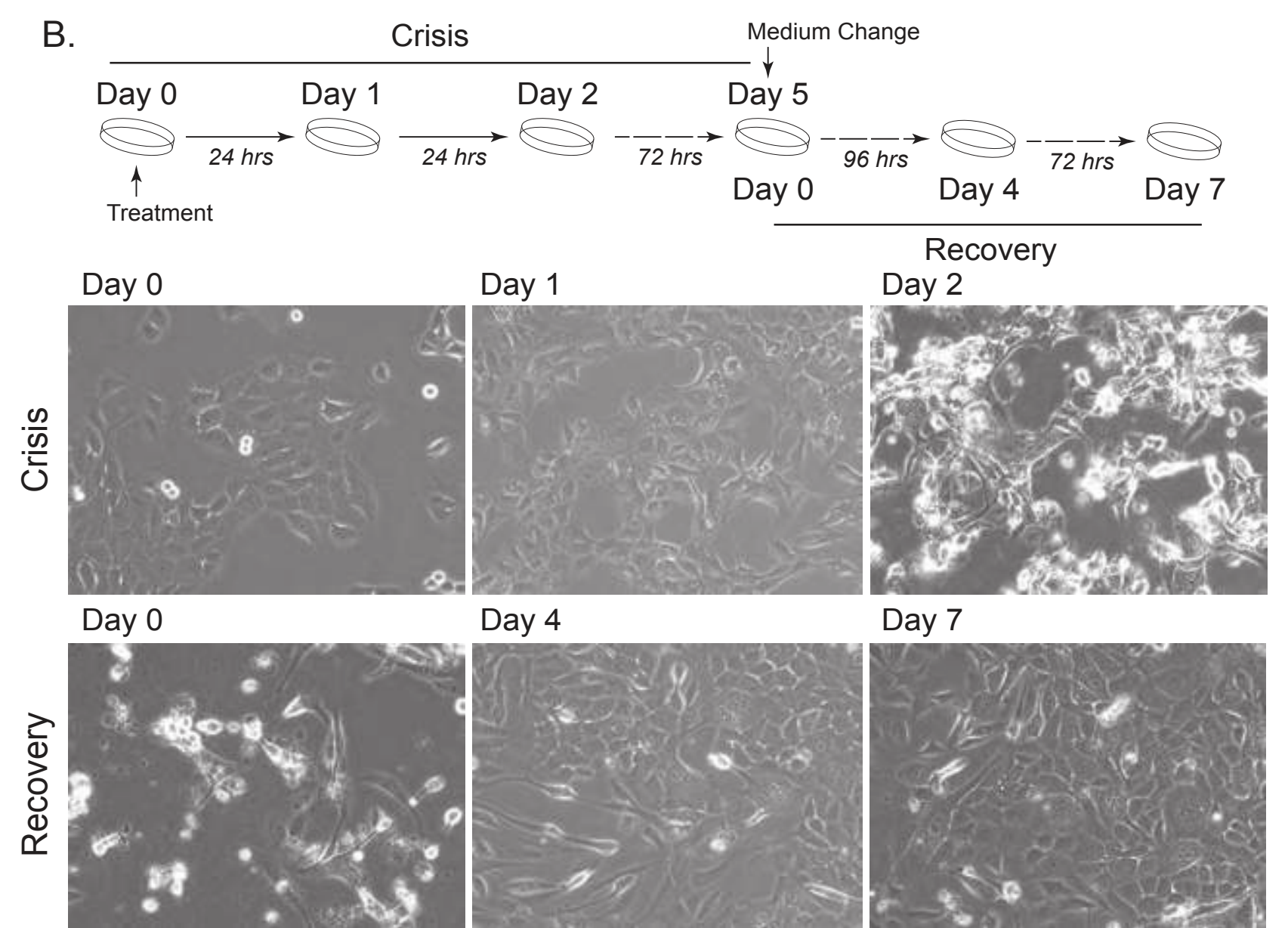
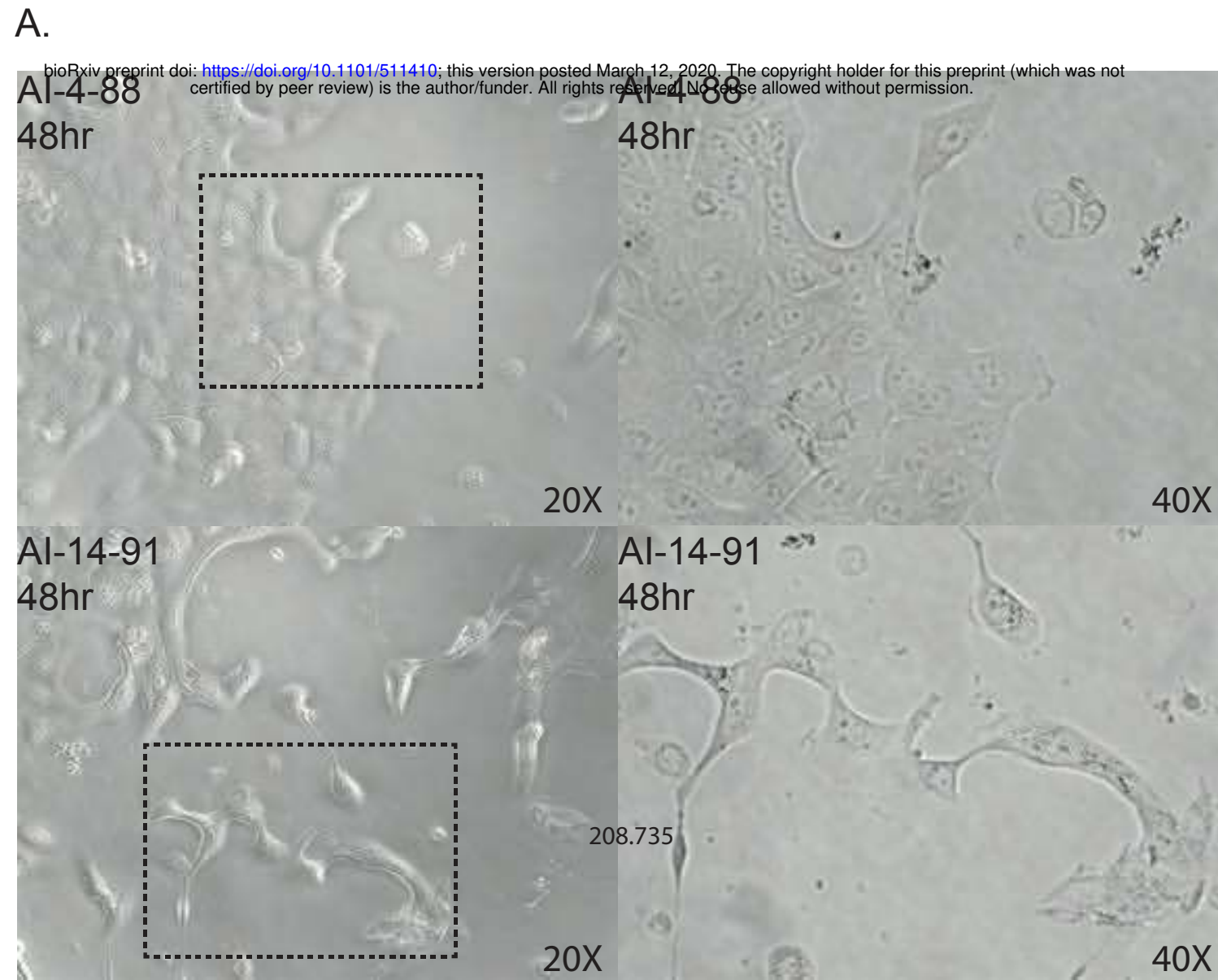


Figure 5

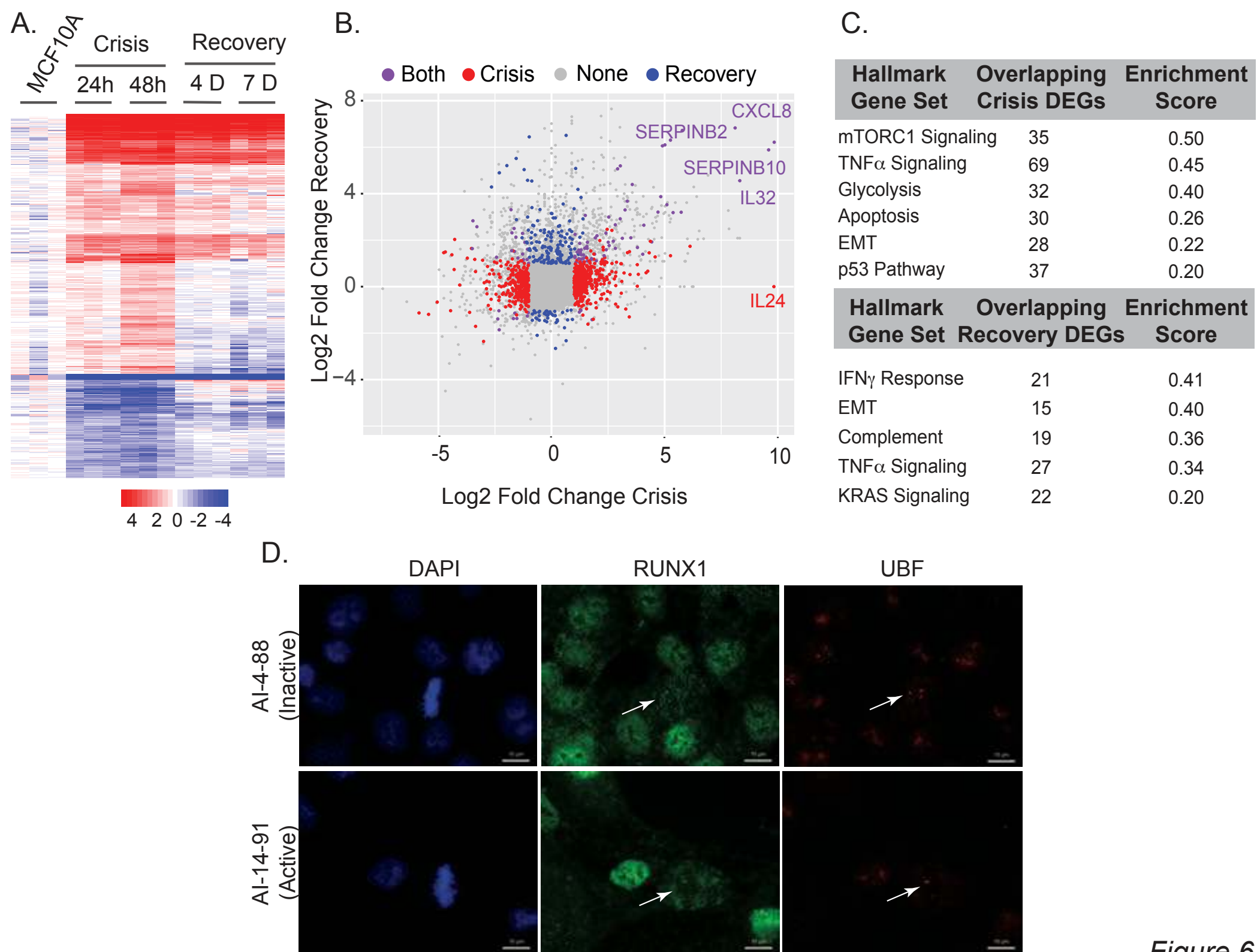


Figure 6



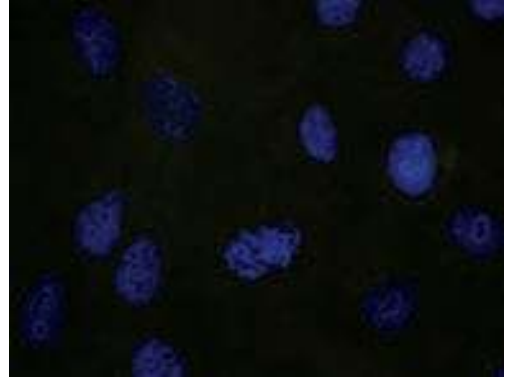
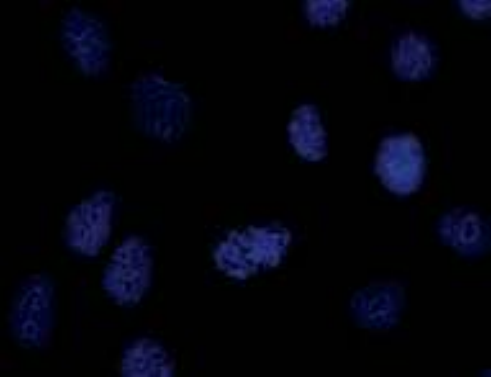
DAPI

Alexa 488

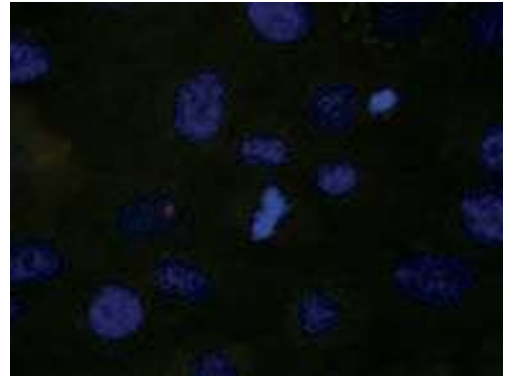
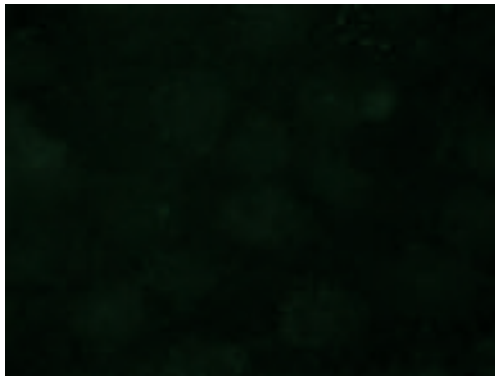
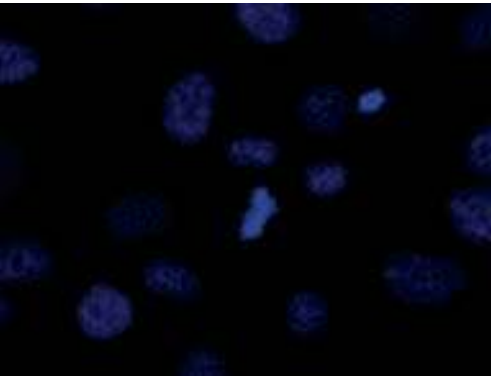
Alexa 568

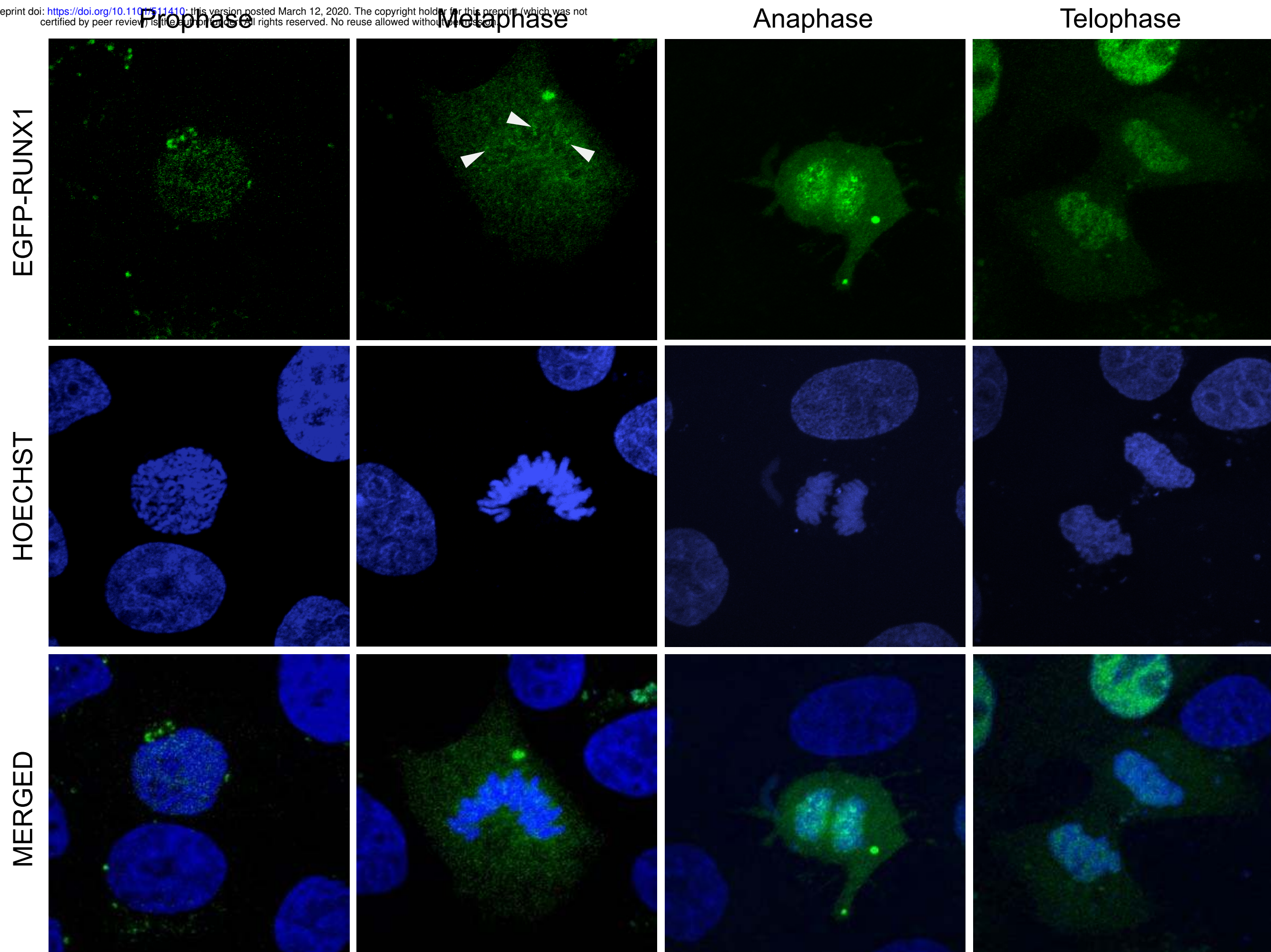
Merged

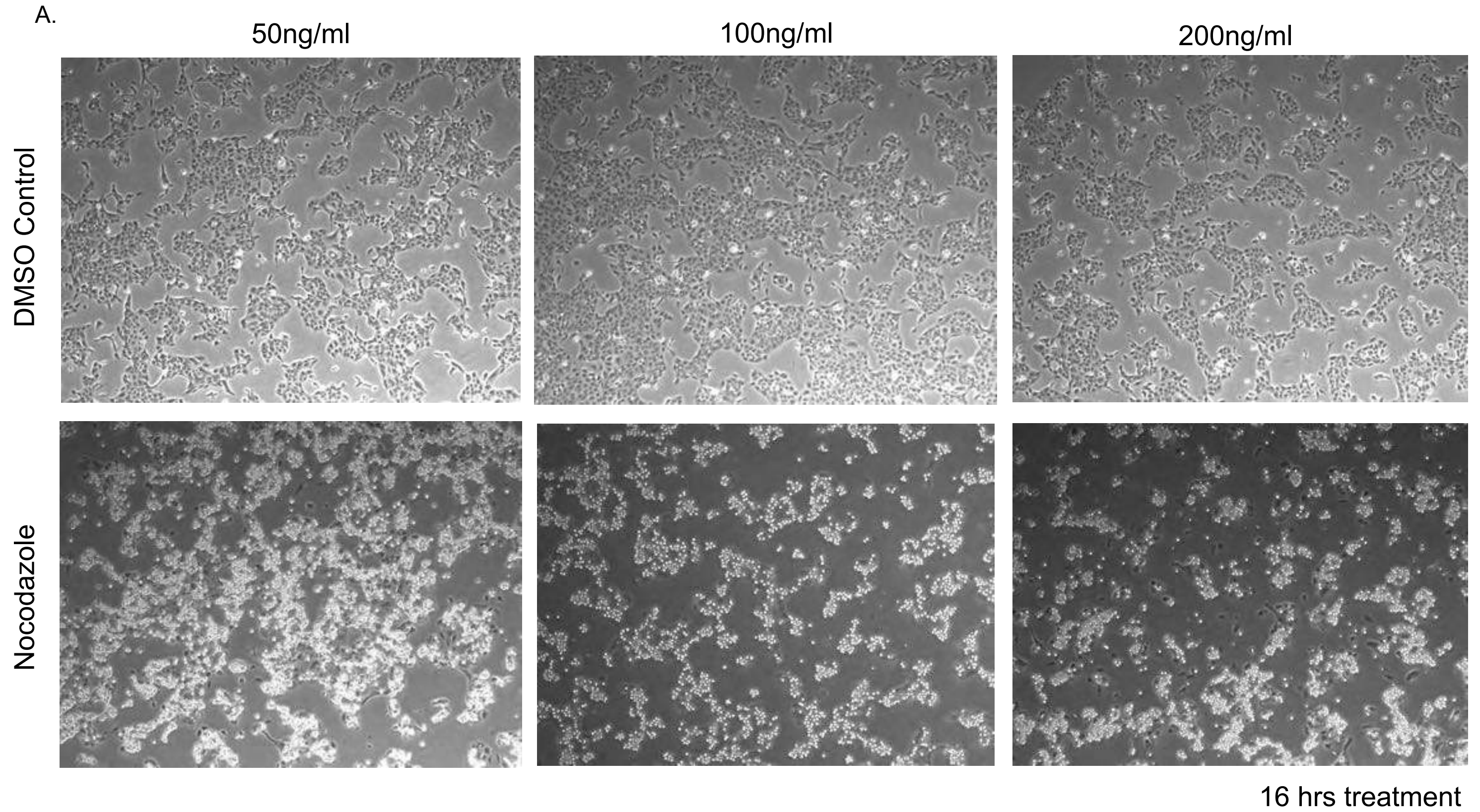
Field 1

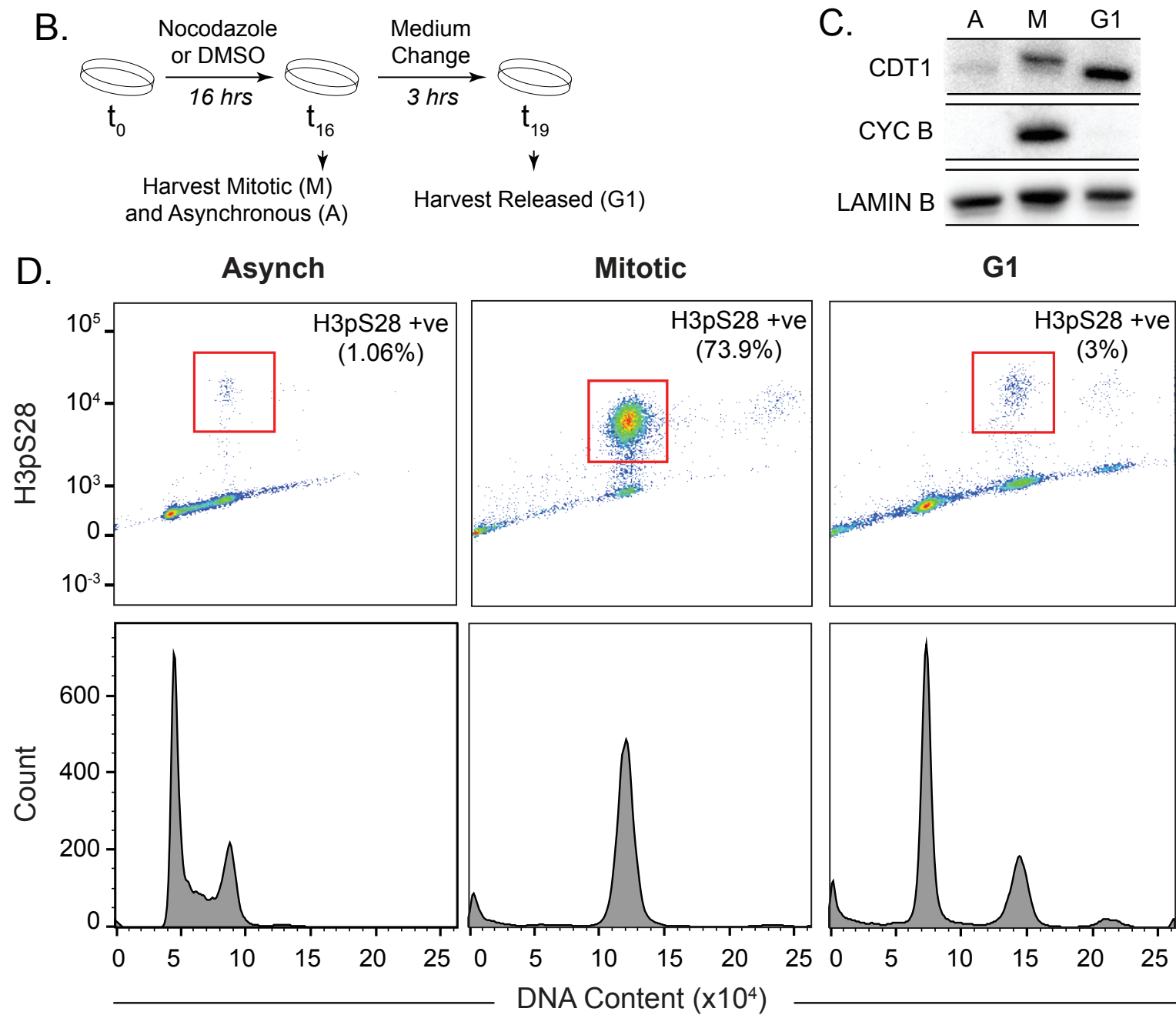


Field 2









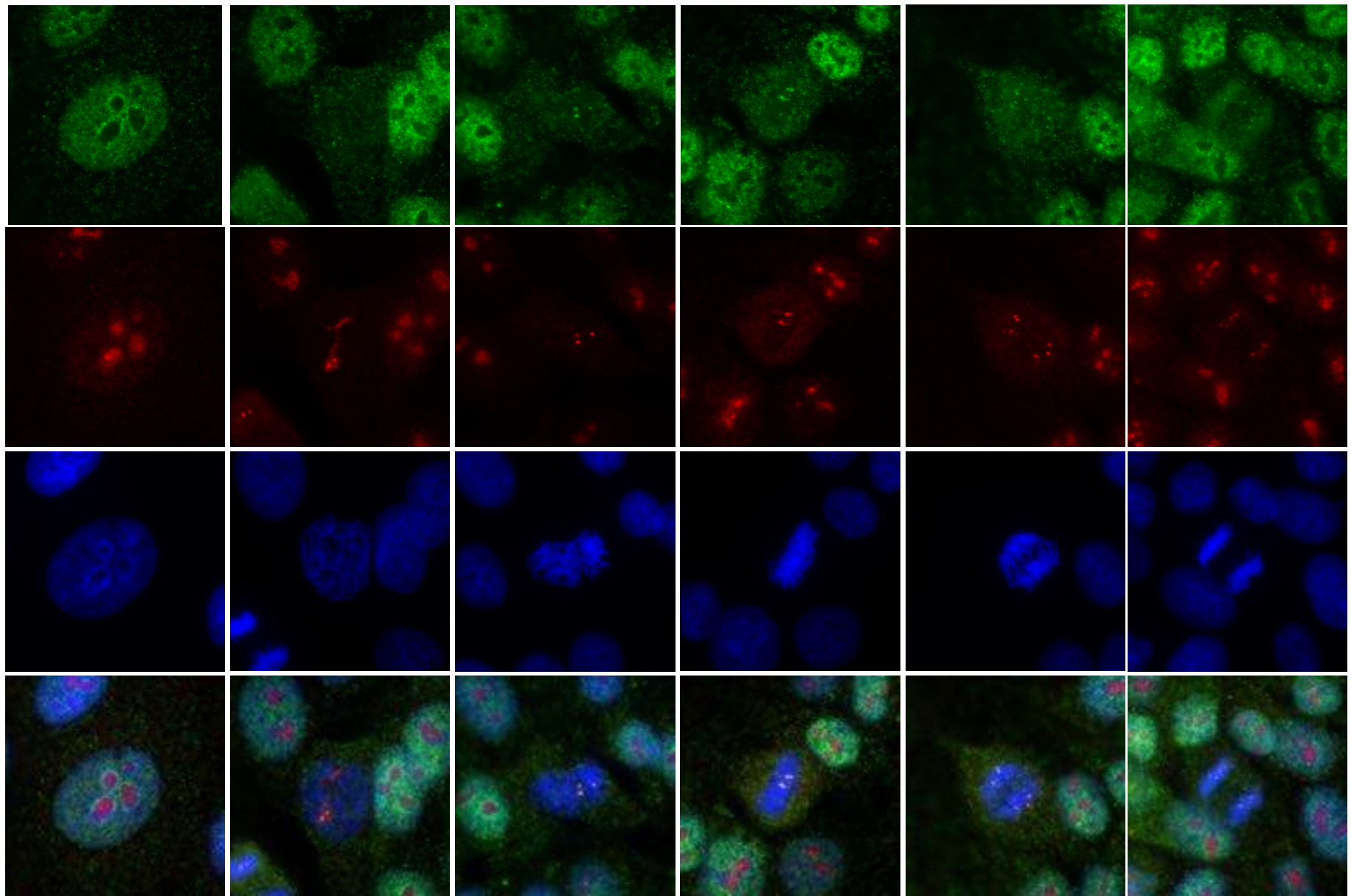
A.

RUNX1

UBF

DAPI

Merged



B.

Interphase

Metaphase

