

# 1       **Single Cell Analysis Reveals Partial Reactivation of X-chromosome Instead of** 2       **Chromosome-wide Dampening in Naïve Human Pluripotent Stem Cells**

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## 10       **Abstract**

11       Recently, a unique form of X-chromosome dosage compensation has been demonstrated in  
12       human preimplantation embryos, which happens through the dampening of X-linked gene  
13       expression from both X-chromosomes. Subsequently, X-chromosome dampening has also  
14       been demonstrated in female human pluripotent stem cells (hPSCs) during the transition from  
15       primed to naïve state. However, the existence of dampened X-chromosomes remains  
16       controversial in both embryos and hPSCs. Specifically, in preimplantation embryos it has  
17       been shown that there is inactivation of X-chromosome instead of dampening. Here, we have  
18       performed allelic analysis of X-linked genes at the single cell level in hPSCs and found that  
19       there is partial reactivation of the inactive X-chromosome instead of chromosome-wide  
20       dampening upon conversion from primed to naïve state. In addition, our analysis suggests  
21       that the reduced X-linked gene expression in naïve hPSCs might be the consequence of  
22       erasure of active X-chromosome upregulation.

23  
24       **Keywords:** X-chromosome dampening; X-chromosome inactivation; X-chromosome  
25       upregulation, Human pluripotent stem cells; Naïve & primed pluripotency; Human embryos;  
26       *XIST*

## 32 **Introduction**

33 In therian mammals, to balance the X-chromosome dosage between males and females, one  
34 X-chromosome becomes inactivated in female cells (Lyon, 1961). The dosage imbalance  
35 between a single active X-chromosome and two copies of autosomes (AA) is compensated  
36 through upregulation of the active-X chromosome in both males and females (Deng et al.,  
37 2011; Larsson et al., 2019; Ohno S, 1967). Recently, another form of X-chromosome dosage  
38 compensation has been demonstrated in human preimplantation embryos, termed as X-  
39 chromosome dampening. Based on single cell transcriptome analysis of human  
40 preimplantation embryos, Petropoulos *et al.* (2016) found that X-linked gene expression  
41 gradually decreased from morula to blastocyst stage, while both X-chromosomes were  
42 maintaining active state (Petropoulos et al., 2016). Based on these, they proposed that  
43 dampening of X-linked gene expression from both X-chromosome as a likely dosage  
44 compensation mechanism during human pre-implantation development. However,  
45 dampening phenomenon in human embryos remains controversial (De Mello et al., 2017;  
46 Saiba et al., 2018). De Mello *et al.* (2017) found evidence of inactivation of the X-  
47 chromosome instead of dampening in preimplantation embryos when they reanalyzed the  
48 same transcriptome dataset of Petropoulos *et al.* (2016) with more stringency. In addition,  
49 Sahakyan *et al.* (2017) showed that naïve human pluripotent stem cells (hPSCs) also exhibit  
50 the X-chromosome dampening found in embryos (Sahakyan et al., 2017a). However, similar  
51 to the human embryos, X-chromosome states in hPSCs remains unclear (Kaur et al., 2019;  
52 De Mello et al., 2017). Conventional hPSCs derived from blastocysts represent a primed state  
53 instead of naïve state and are therefore unable to recapitulate the preimplantation X-  
54 chromosome states (Nichols and Smith, 2009; Sahakyan et al., 2017b). To model the  
55 preimplantation X-chromosome state, Sahakyan *et al.* (2017) converted primed hPSCs to the  
56 naïve state using 5iLAF culture condition (Fig. 1A) (Theunissen et al., 2014). The primed cell  
57 line used for their study, UCLA1, harbored one active-X chromosome and one inactive-X  
58 chromosome. The transition of primed to naïve state happened through an intermediate early  
59 naïve state (Fig. 1A). Primarily based on RNA-sequencing analysis using bulk cell  
60 population, they suggested that the inactive X-chromosome was reactivated upon transition  
61 from primed to the early naïve state and this was followed by X-chromosome dampening in  
62 late naïve cells (Fig. 1A). However, considering the heterogeneity of cell states during the  
63 conversion process, in this study, we have analyzed available single cell RNA-Seq (scRNA-

64 Seq) dataset of early and late naïve cells from Sahakyan *et al.* (Sahakyan et al., 2017a), to  
65 gain better insight into X-chromosomal states (Fig. 1A).

## 66 **Results**

### 67 **Increased *XIST* expression and reduction in X-linked gene expression upon conversion** 68 **of early to late naïve state**

69 First, in early and late naïve cells, we quantified the expression of *XIST*, a master regulator of  
70 X-inactivation. We found that a majority of the early naïve cells had very low level of *XIST*  
71 expression, whereas late naïve cells mostly showed higher level of *XIST* expression (Fig.  
72 S1A). Overall our analysis revealed that transition of early to late naïve state was associated  
73 with significant increase of *XIST* expression (Fig. S1B). Next, based on quality (RPKM sum  
74 and mean) and *XIST* expression level, we selected the top 28 early cells having low level of  
75 *XIST* and the top 30 late cells having higher level of *XIST* expression for further analysis (Fig.  
76 1B). Again, comparison of *XIST* expression in these cells (28 early vs 30 late) showed a  
77 significant increase in late naïve cells compared to early naïve cells (Fig. 1B). In addition, we  
78 found that there was a significant reduction in X-linked gene expression in late naïve cells  
79 compared to early naïve cells (Fig. 1C; Supplementary file1). Altogether, our analysis of  
80 scRNA-Seq data showed increased *XIST* expression and reduction in X-linked gene  
81 expression upon transition from the early to late naïve state.

82

### 83 **Reduction in X-linked gene expression in late naïve cells is independent of *XIST***

84 It has been shown that X-chromosome dampening in preimplantation embryos is associated  
85 with the expression of *XIST* from both X-chromosomes (Petropoulos et al., 2016). In fact, X-  
86 dampening initiates concomitantly with the initiation of *XIST* expression and therefore it is  
87 thought that *XIST* might have an important role in the dampening process. To test this, we  
88 examined what fraction of cells show *XIST* expression from both X-chromosomes in late  
89 naïve cells and if the reduction in X-linked gene expression is restricted to the biallelically  
90 *XIST* expressed cells. We found about 26% cells (9 of 35) expressed *XIST* from both X-  
91 chromosomes (Fig. 2A; Supplementary file 2). Interestingly, comparison of the global X-  
92 linked gene expression level of *XIST* biallelic vs monoallelic cells did not show any  
93 significant difference (Fig. 2B; Supplementary file 2). Moreover, we observed that there was  
94 no significant difference in X-linked gene expression when we compared against the *XIST*

95 negative cells (Fig. 2B; Supplementary file 2). Based on these data, we concluded that  
96 reduction in X-linked gene expression in late naïve cells was independent of *XIST*.

### 97 **No evidence of X-chromosome inactivation or dampening**

98 We then looked into possible mechanisms behind the reduction in X-linked gene expression  
99 in late naïve hPSCs. First, we postulated that it could be due to the either X-chromosome  
100 inactivation or dampening. To test for X-inactivation, we profiled allelic expression of  
101 multiple X-linked genes distributed across the X-chromosome based on single nucleotide  
102 polymorphisms (SNPs) in early and late cells (Fig. 3; Supplementary file 3). We found that  
103 the majority of early cells showed monoallelic expression of many genes along with  
104 biallelically expressed X-linked genes, which indicated incomplete reactivation of X-linked  
105 genes (Fig.3A & 3B). Moreover, we found significant variation in the proportion of biallelic  
106 vs monoallelic genes between these cells (Fig. 3B). Interestingly, late naïve cells showed a  
107 similar pattern of allelic expression as early cells. However, there was a significant increase  
108 in the fraction of SNPs showing biallelic expression in late naïve cells compared to early  
109 naïve cells. (Fig. 3B). Altogether, these data indicated that late naïve cells do not harbor  
110 inactive X-chromosome but rather they have partially reactivated X-chromosome. If the late  
111 naïve cells underwent X-inactivation, then monoallelic expression of most of the X-linked  
112 genes would be expected. In contrast, we observed increased biallelic expression upon  
113 transition from early to late stage. Next, we examined whether X-dampening was causing  
114 reduction of X-linked gene expression in late naïve cells. From the allelic analysis of X-  
115 linked gene expression it was clear that late naïve cells harbor partially reactivated X-  
116 chromosome. If these cells harbored dampened X-chromosomes, we would have expected  
117 biallelic expression of most of the genes chromosome -wide, which was not observed. In  
118 addition, we compared median expression of biallelically expressed genes of early cells to  
119 that of the late naïve cells. If X-dampening was occurring then we would have expected a  
120 significant decrease in median expression of biallelically expressed genes in late cells.  
121 However, significant differences were not observed (Fig. 3C). Taken together, we concluded  
122 that there was neither X-inactivation nor dampening upon conversion of early to late naïve  
123 cells. Second, to determine whether loss of an X-chromosome in late cells is causing the  
124 reduction in X-linked gene expression in late naïve cells, we explored X-chromosome ploidy  
125 of these cells. It was clear that cells harbored two X-chromosomes as evident by the biallelic  
126 expression of some X-linked genes (Fig. 3A). However, the possibility existed that these cells  
127 may lose part (s) of the X-chromosome. To test for this, the gene expression ratio of X-linked

128 genes across the X-chromosome was analyzed, but significant differences between early vs  
129 late naïve cells were not identified (Fig. 3D; Supplementary file 4). Therefore, we confirmed  
130 that loss of a portion of the X-chromosome is not the cause of reduction in X-linked gene  
131 expression in late naïve cells.

132

### 133 **Erasure of active-X upregulation might be the cause of reduced X-linked gene** 134 **expression in late naïve cells**

135 Next, we investigated if the erasure of active X-chromosome upregulation might be causing  
136 the reduction in X-linked gene expression in late naïve cells as proposed by De Mello *et*  
137 *al.* (De Mello et al., 2017). In recent years, the existence of upregulated active X-chromosome  
138 has been extensively demonstrated in mammals as hypothesized by Ohno (Deng et al., 2011,  
139 2013; Li et al., 2017; Lin et al., 2011; De Mello et al., 2017; Sangrithi et al., 2017). Although  
140 some studies have found lack of active-X upregulation (Chen and Zhang, 2016; Xiong et al.,  
141 2010). To probe this further, we analyzed X to autosomal (X:A) gene expression ratio of 7  
142 different male primed hPSC lines. If a diploid male cell has upregulated active-X and then the  
143 X:A ratio should be more than 0.5 and closer to 1. Indeed, we found the X:A ratio of all male  
144 primed cells was greater than 1, indicating that primed hPSCs harbor an upregulated active  
145 X-chromosome (Fig. 4A; Supplementary file 5). We then asked whether the active-X  
146 upregulation becomes erased in naïve hPSCs. To test this, we compared the X:A ratio of male  
147 primed cells against different male naïve cell lines. Interestingly, a significant reduction of  
148 X:A ratio in naïve cells compared to the primed cells was observed, suggesting erasure of  
149 active X-chromosome upregulation in naïve cells (Fig. 4A). We made sure that the X:A ratio  
150 was not impacted by the difference between X-linked and autosomal gene expression  
151 distribution for each dataset (Fig. 4B). We focused on male cells for analysis of active-X  
152 upregulation as in female cells X-linked gene expression is often confounded with X-  
153 chromosome inactivation / reactivation / erosion. However, we profiled the X:A ratio in 3  
154 different primed female cells (including UCLA1), which are known to harbor one inactive-X  
155 chromosome (Fig. 4C). We found the X:A ratio of female primed cells was above 1, which  
156 indicated that these cells harbor an upregulated active-X chromosome (Fig. 4C; Fig. S2B).  
157 Next, we examined the X:A ratio dynamics during the primed to naïve conversion of UCLA1  
158 female cells. An significant increase in the X:A ratio upon transition from primed to early  
159 naïve state was observed (Fig. 4D; Fig. S2B). Considering the partial X-reactivation upon

160 transition of primed to early naïve cells, it is obvious that the X:A ratio should increase  
161 provided that the active-X upregulation is not completely erased. If there was complete  
162 erasure of active-X upregulation, the X:A ratio should not have an observable increase.  
163 Therefore, this data indicated that erasure of active-X upregulation was incomplete in the  
164 early naïve state. Conversely, a decrease in the X:A ratio was observed in the late naïve state  
165 compared to the early naïve cells, which suggested that erasure of active-X upregulation was  
166 occurring (Fig. 4D; Fig. S2B). In this scenario, it is possible that the erasure of active-X  
167 upregulation led to the overall reduction in X-linked gene expression in late naïve cells.  
168 However, the decrease in X:A ratio from early to late naïve state was not significant as  
169 expected. We think that a significant increase in biallelic X-linked gene expression from early  
170 to late naïve state was masking this to some extent. In summary, our data suggests that X-  
171 dampening is not the determining factor for the reduction in X-linked gene expression in late  
172 naïve cells, and erasure of active-X upregulation might be leading to the decrease of X-linked  
173 gene expression (Fig. 4E).

174

## 175 **Discussion**

176 X-chromosome states in female human preimplantation embryos remains elusive till date.  
177 While it has been reported that preimplantation embryos carry dampened X-chromosomes,  
178 other studies have provided evidence of inactivation of one of the X-chromosomes. One of  
179 the major challenges to resolve this issue is the lack of availability of surplus number of  
180 human embryos for experimentation. Therefore, hPSCs derived from human embryos serve  
181 as an alternative system. However, conventional hPSCs represent the primed state instead of  
182 the naïve state of preimplantation embryos (Davidson et al., 2015; Sahakyan et al., 2017b).  
183 Recently, Sahakyan *et al.* (2017) converted primed hPSCs to naïve state to model the X-  
184 chromosome states of preimplantation embryos and suggested that naïve hPSCs also carry  
185 dampened X-chromosomes. However, our analysis indicates that the conversion of primed  
186 hPSCs to naïve state is associated with the partial reactivation of the inactive-X chromosome  
187 instead of the chromosome-wide dampening (Fig. 4E). The main reason behind the dissimilar  
188 outcomes between our study and Sahakyan *et al.* (2017) is likely that their conclusion is  
189 primarily based on analysis of bulk RNA-sequencing of cell population, whereas our  
190 conclusion is based on analysis of single cell RNA-Seq dataset. Since, single cell RNA-Seq  
191 provides better clarity on cellular state and gene expression to distinguish the heterogeneity

192 among cells in a population, we believe that our analysis provides better insight into X-  
193 chromosome states of hPSCs. In addition, our study suggests that erasure of active-X  
194 upregulation might be causing the reduction in X-linked gene expression in late naïve cells  
195 (Fig. 4E).

196 Although two studies led to the dissimilar outcomes, several observations in our analyses  
197 were consistent with findings by Sahakyan *et al.* (2017). For example, we also found that  
198 early naïve cells were mostly *XIST* negative and transitioned to *XIST* positive in the late naïve  
199 state which was accompanied by a reduction in X-linked gene expression (Fig. 1). Moreover,  
200 about 26% of cells in late naïve state showed *XIST* expression from both X-chromosomes,  
201 which was similar to what was reported by Sahakyan *et al.* (2017). We should point out that  
202 in preimplantation embryos majority of the cells (~85%) express *XIST* from both X-  
203 chromosomes (Okamoto *et al.*, 2011) and it is believed that *XIST* might have an important  
204 role in X-dampening. However, we found that reduction in X-linked gene expression was  
205 independent of *XIST* as *XIST*-biallelic, -monoallelic and -negative cells showed almost  
206 similar level of gene expression in late naïve cells (Fig. 2B).

207 On the other hand, allelic analysis of X-linked gene expression at the single cell level  
208 revealed striking differences between the two studies. We found that early and late naïve cells  
209 harbored partially reactivated X-chromosome as indicated by monoallelic expression of many  
210 genes in each cell of both cell states (Fig. 3). This was contrary to Sahakyan *et al.* (2017)  
211 where they found most of the genes had biallelic expression. Importantly, we found  
212 significant variations in allelic patterns of gene expression in different cells within a  
213 population, such as same genes, which were monoallelic in some cells, showed biallelic  
214 expression in other cells. In this scenario, bulk cell population analysis will always show  
215 biallelic gene expression, which was might be the case for Sahakyan's observation.  
216 Interestingly, while Sahakyan *et al.* (2017) interpreted the reduction in X-linked gene  
217 expression in late naïve cells as dampening, we found lack of dampening since there was no  
218 significant difference in the median expression of biallelically expressed genes between early  
219 and late naïve cells (Fig. 3C). In fact, Theunissen *et al.* (2016) found that female naïve cells  
220 had significantly higher X-linked gene expression compared to male naïve cells, which also  
221 suggested that naïve female cells harbored active X-chromosomes instead of dampened X-  
222 chromosomes (Theunissen *et al.*, 2016). In addition, we observed that the proportion of  
223 biallelically expressed genes increased significantly in late naïve cells compared to early  
224 naïve cells, which indicated that the cells were still undergoing the reactivation process.

225 Some observations by Sahakyan *et al.* also indicated that there was an incomplete erasure of  
226 epigenetic memory on the inactive X in the naïve state. They found, upon differentiation,  
227 naïve hPSCs underwent non-random X-inactivation dissimilar to the normal development,  
228 and the same X-chromosome that was inactive in the primed hPSCs was again inactivated.  
229 Even, naïve cells showed an accumulation of H3K27me3 repressive marks on one of the X-  
230 chromosomes, that had not been observed in preimplantation blastocysts (Okamoto *et al.*,  
231 2011). We think the presence of H3k27me3 is the result of incomplete erasure of inactive-X  
232 marks, which is consistent with our observation of a partially reactivated X-chromosome.  
233 Taken together, these findings suggest that naïve cells were still in the process of removing  
234 inactive-X epigenetic marks.

235 Our study suggests that erasure of active-X upregulation might be causing the reduction in  
236 X-linked gene expression upon transition from early to late naïve state. Many recent studies  
237 have demonstrated the existence of upregulated active-X chromosomes in mammals (Deng *et al.*  
238 *et al.*, 2013; Larsson *et al.*, 2019). We also found evidence for upregulated active-X in 7  
239 different male primed hPSC lines (Fig. 4A). Importantly, our analysis of male primed and  
240 naïve cells strongly indicates that there is erasure of active-X upregulation upon conversion  
241 of primed to naïve state (Fig. 4A). Erasure of upregulation has been demonstrated previously  
242 in spermatids, during oogenesis and germ cell reprogramming of both sexes (Di and  
243 Disteche, 2006; De Mello *et al.*, 2017; Sangrithi *et al.*, 2017). Moreover, our observation is  
244 consistent with some other studies in mouse, which showed that naïve ESC has lower X:A  
245 ratio compared to the differentiated cells (Lin *et al.*, 2007; Marks *et al.*, 2015). Specially,  
246 during female ESC differentiation, upregulation increases concomitantly with the initiation of  
247 X-inactivation (Larsson *et al.*, 2019). In fact, Theunissen *et al.* (2016) found a reduction in X-  
248 linked gene expression in male naïve hPSCs compared to that in primed, which may be due  
249 to the erasure of active X-chromosome upregulation (Theunissen *et al.*, 2016). Furthermore, it  
250 has been shown that male blastocyst had significantly lower X:A ratio compared to the  
251 primed hPSCs (De Mello *et al.*, 2017). Taken together, we believe that primed to naïve  
252 conversion of hPSCs is accompanied by erasure of active-X upregulation. In addition, our  
253 data indicate that the erasure of upregulation for female UCLA1 is might still ongoing in the  
254 early naïve state and only reaches near the completion in the late naïve state, thereby leading  
255 to the reduction in X-linked gene expression upon transition from early to late naïve state  
256 (Fig. 4D). In fact, during germ cell reprogramming it has been shown that erasure of



257 upregulation and reactivation of inactive-X does not occur simultaneously rather loss of  
258 upregulation occurs later than loss of X-inactivation (Sangrithi et al., 2017).

259 Collectively, our study indicates that the conversion of primed to naïve state is associated  
260 with the incomplete reactivation of X-chromosome rather than X-inactivation or X-  
261 dampening. Importantly, our data also indicates that erasure of active-X upregulation might  
262 be leading the reduction in X-linked gene expression in naïve hPSCs. Although our results  
263 argue against dampening and propose erasure of active-X upregulation is leading to the  
264 reduction in X-linked gene expression in late naïve cells, further work must be done with  
265 better scRNA-Seq data. Finally, better culture conditions are necessary to establish naïve  
266 hPSCs that recapitulate the X-chromosome states of preimplantation embryos.

267

## 268 **Experimental procedures**

269 **Data acquisition:** RNA-Seq datasets were acquired from Gene Expression Omnibus (GEO)  
270 under the accession number GSE87239 (Sahakyan et al., 2017a). For additional datasets see  
271 the supplementary experimental procedures.

272 **Variant calling:** First, reads were mapped to the human genome (hg38) using STAR. To  
273 mark the duplicate reads from the aligned reads of single cells, we used Picard tools  
274 v2.18.11 (<https://broadinstitute.github.io/picard/>). Next, we retrieved the allelic read counts  
275 for SNPs by using GATK (v3.8) “HaplotypeCaller”. We considered those SNPs for our  
276 analysis, which were present in UCLA1 cell line database (GSM2420529). Further we  
277 annotated those SNPs using dbSNP Build 152 (GRCh38.p12).

278 **Allelic expression analyses:** For allelic expression analysis, we considered the SNPs having  
279  $\geq 3$  reads per SNP site in a cell. Further, we proceeded with those SNPs having informative  
280 reads in at least five different cells of each category; early and late naïve.  
281 The allelic expression was calculated by directly counting the allele-specific reads covering a  
282 SNP position mapped to the reference or the alternative allele and then dividing it by the total  
283 number of reads covering that position. A SNP was considered monoallelic if at least 90% of  
284 the allelic reads was coming from only one allele. We only considered allelic ratios of SNPs  
285 for those genes, which had RPKM  $\geq 1$ . Finally, we considered only those SNPs for which the  
286 allelic data was available in at least four cells for each early and late naïve cells. We validated  
287 allele specific expression pipeline through analysis of genes of an autosome (Chr17), which

288 showed mostly biallelic expression of SNPs (Fig. S2A). Moreover, SNPs belongs to the same  
289 gene showed almost similar allelic expression pattern in most of the cells, except for few  
290 cells.

291

292

### 293 **Figure legends**

294 **Figure 1: Transition of early to late naïve state is associated with increased *XIST***  
295 **expression and reduction in X-linked gene expression.** (A) Schematic representation of  
296 different stages and corresponding X-chromosome states of conversion of primed hPSCs to  
297 naïve state as described in Sahakyan *et al.* (2017). In this study, we performed analysis of  
298 scRNA-Seq dataset acquired from the early and late naïve state. (B) Comparison of *XIST*  
299 expression (RPKM) between early naïve (n=28 cells) and late naïve cells (n=30 cells).  
300  $p < 0.00001$  (Mann-Whitney U-test) (C) Comparison of X-linked gene expression (n=57  
301 genes) between early and late naïve cells.  $p < 0.00001$  (Mann-Whitney U-test).

302 **Figure 2: Reduction in X-linked gene expression in late naïve cells is independent of**  
303 ***XIST*** (A) Allelic expression of *XIST* in late naïve cells (n=35 cells). (B) Comparison of X-  
304 linked gene expression among *XIST* -monoallelic, -biallelic and -negative cells. Non-  
305 significant at  $p < 0.05$  (Mann-Whitney U-test).

306 **Figure 3: Partial reactivation of inactive-X chromosome upon conversion of primed to**  
307 **naïve state.** (A) Allelic expression analysis of X-linked genes in early and late naïve cells at  
308 single cell level. Bottom, genomic position on the X-chromosome of the X-linked genes  
309 analyzed (B) Histogram showing the percent of SNPs showing monoallelic and biallelic in  
310 each cell of early and late naïve state.  $p < 0.00001$ ,  $p < 0.01$  (Avg. plot) (Student's t-test) (C)  
311 Comparison of median of expression level of biallelically expressed genes between early and  
312 late naïve cells. Non-significant at  $p < 0.05$  (Mann-Whitney U-test) (D) Ploidy analysis of the  
313 cells of early and late naïve state through the analysis of gene expression ratio across the X-  
314 chromosome.

315 **Figure 4: Reduction in X-linked gene expression in naïve hPSCs might be due to the**  
316 **erasure of active-X upregulation.** (A) Comparison of X:A ratio between male primed and  
317 naïve hPSCs.  $p < 0.001$  (Student's t-test). (B) Histograms representing the distribution of X-  
318 linked and autosomal gene expression for male primed and naïve hPSCs with their different  
10

319 replicates ( $p > 0.05$ , by Kolmogorov-Smirnov test). (C) Analysis of X:A ratio in different  
320 primed female hPSCs. (D) Comparison of X:A ratio in UCLA1 primed, early naïve (cl4) and  
321 late naïve cells (cl9 & cl12).  $p < 0.05$  (E) Proposed model representing the X-chromosome  
322 states during the conversion of primed hPSCs to the naïve state.

323

### 324 **Author's Contribution**

325 SG, SM (Susmita Mandal), DC, and HK conceptualized the study. SG supervised the study.  
326 Bioinformatic analyses was done by SM (Susmita Mandal) and DC. SG, SM (Susmita  
327 Mandal), DC, MA, and SM wrote, edited and proofread the manuscript. Final manuscript  
328 was edited and approved by all the authors.

329

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337

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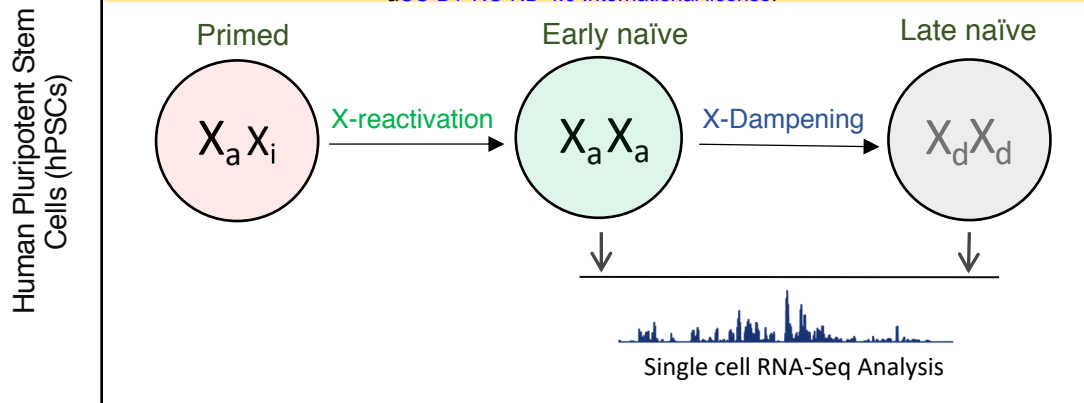
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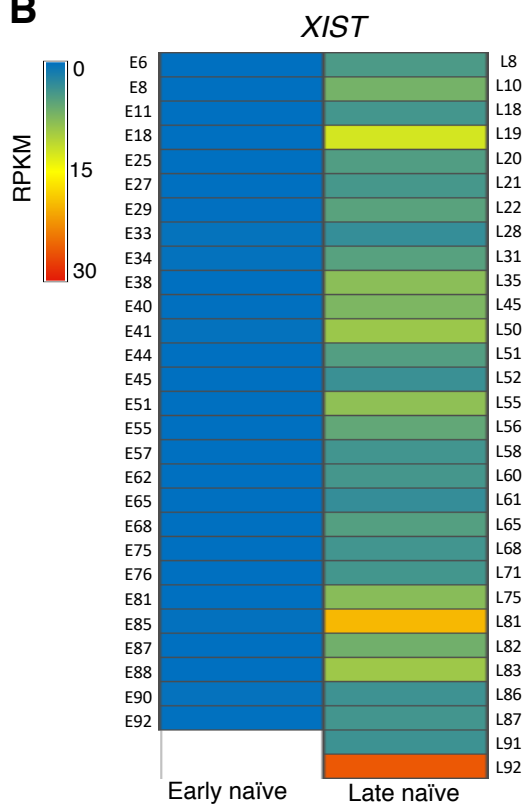
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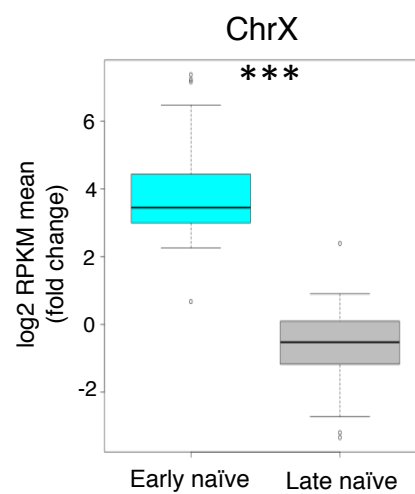
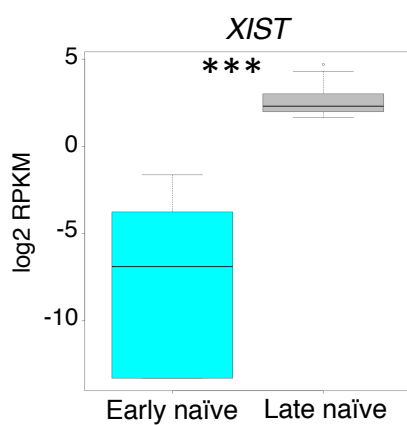
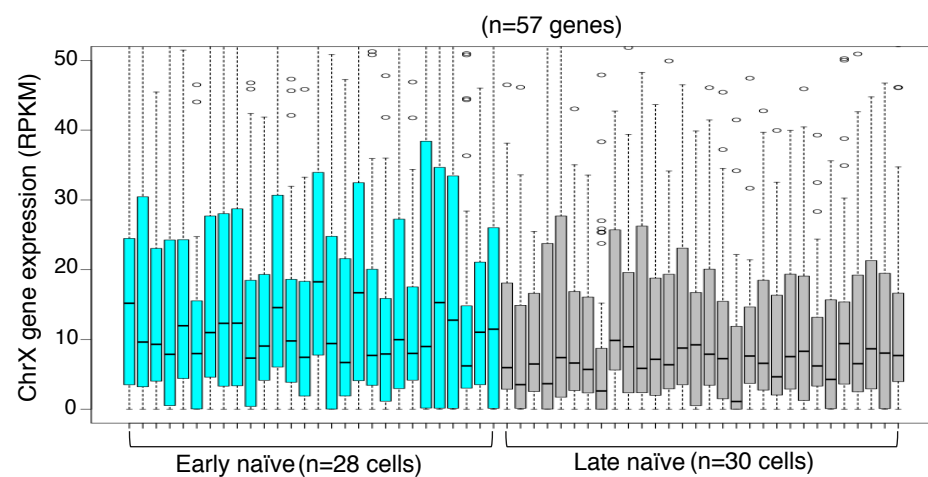
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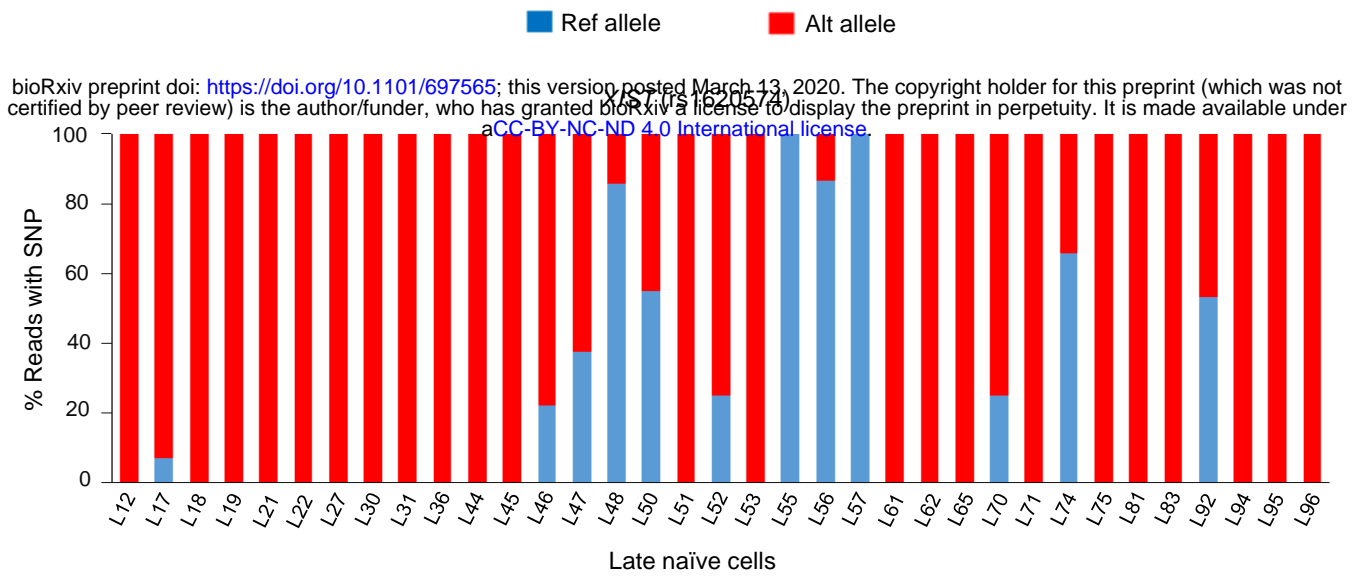
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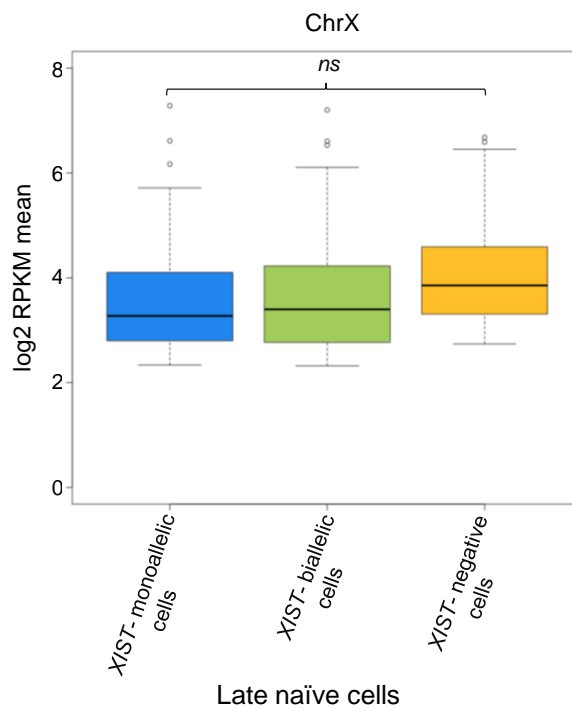
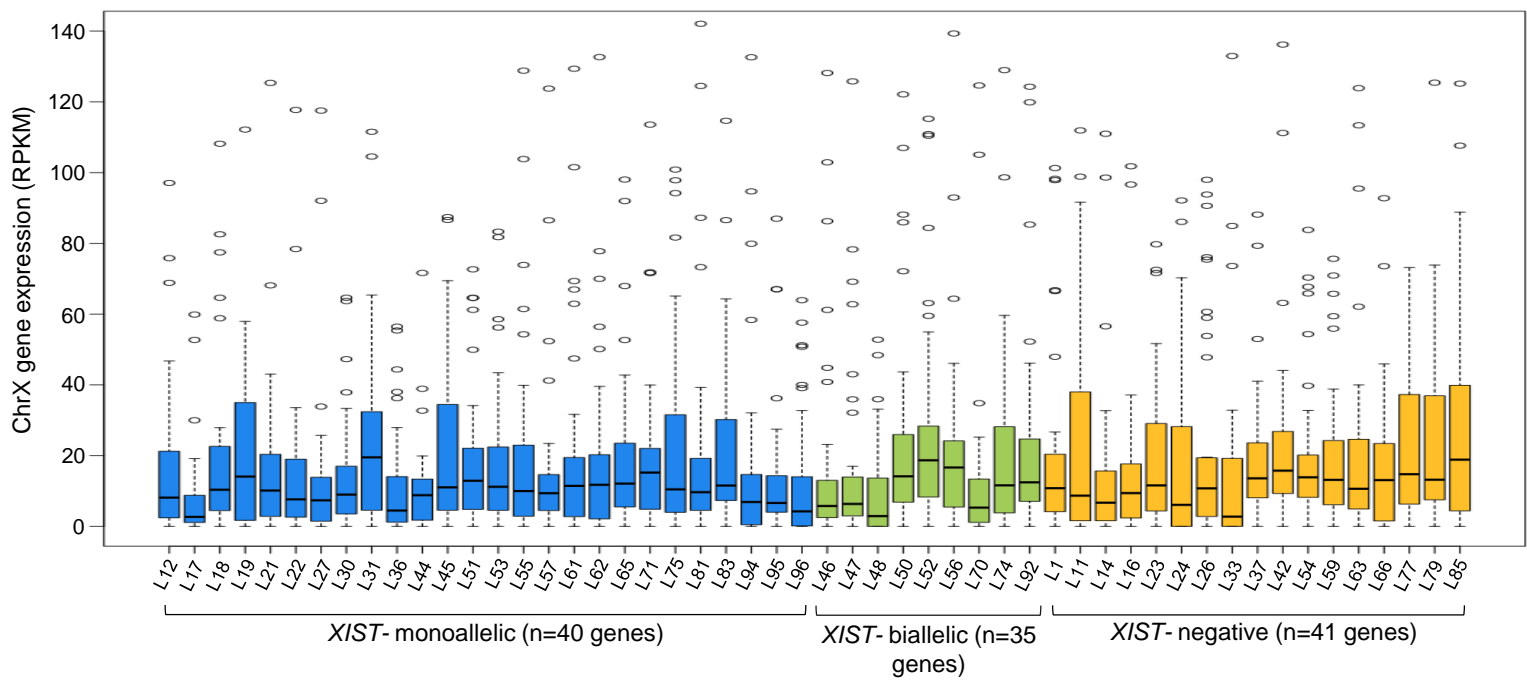
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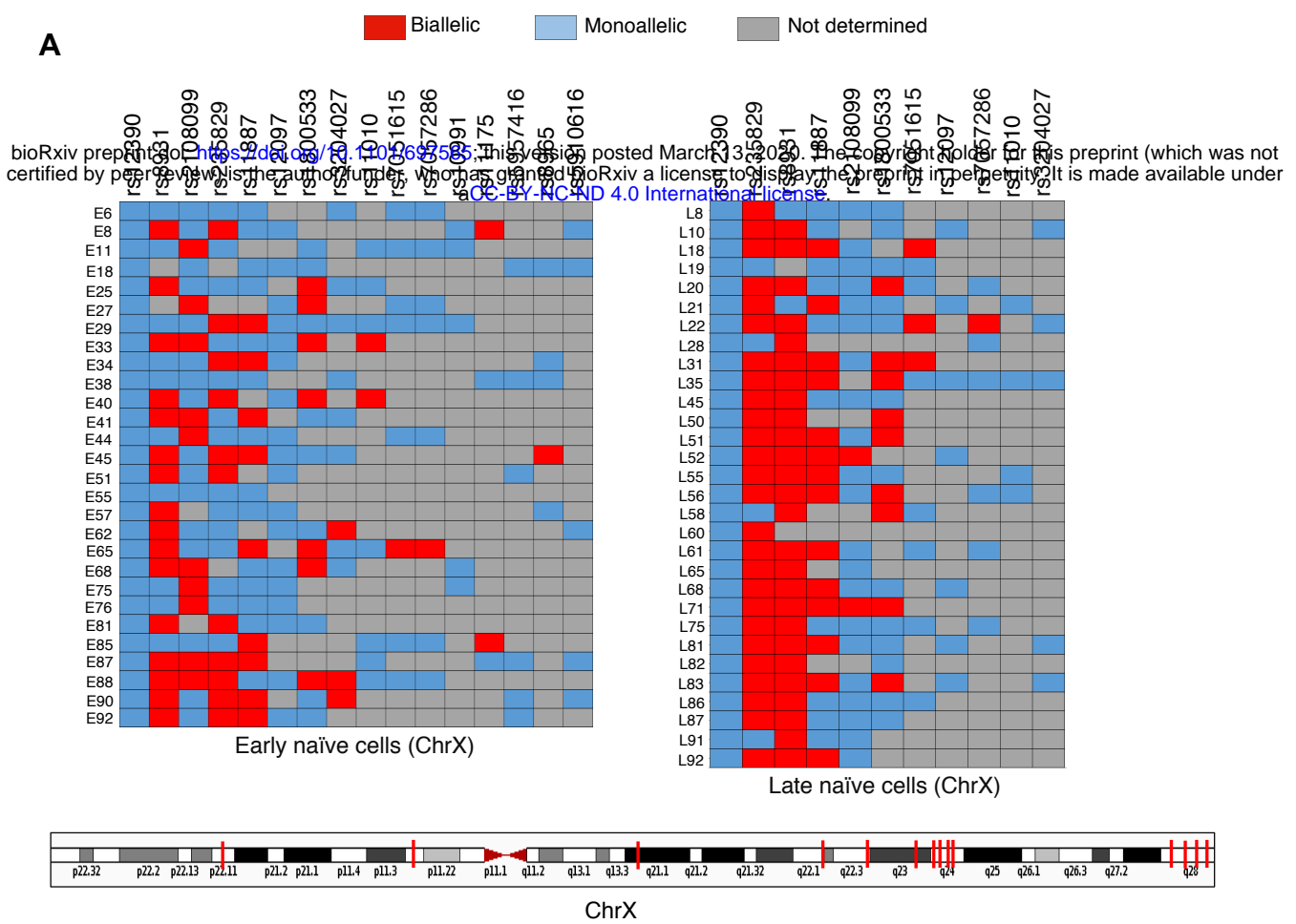
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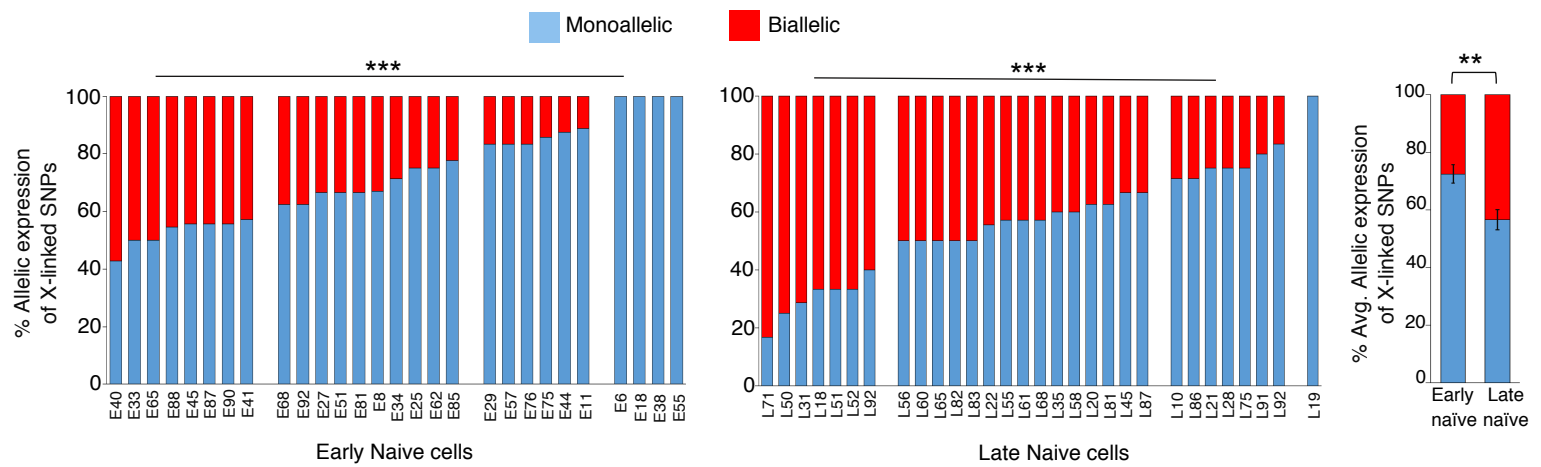
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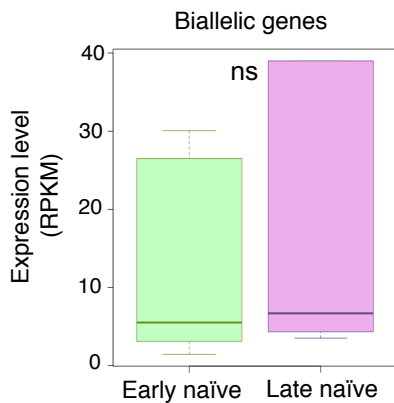
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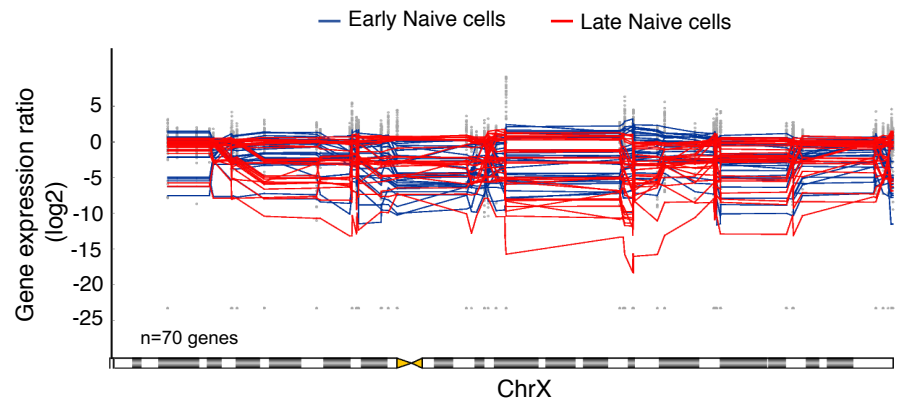
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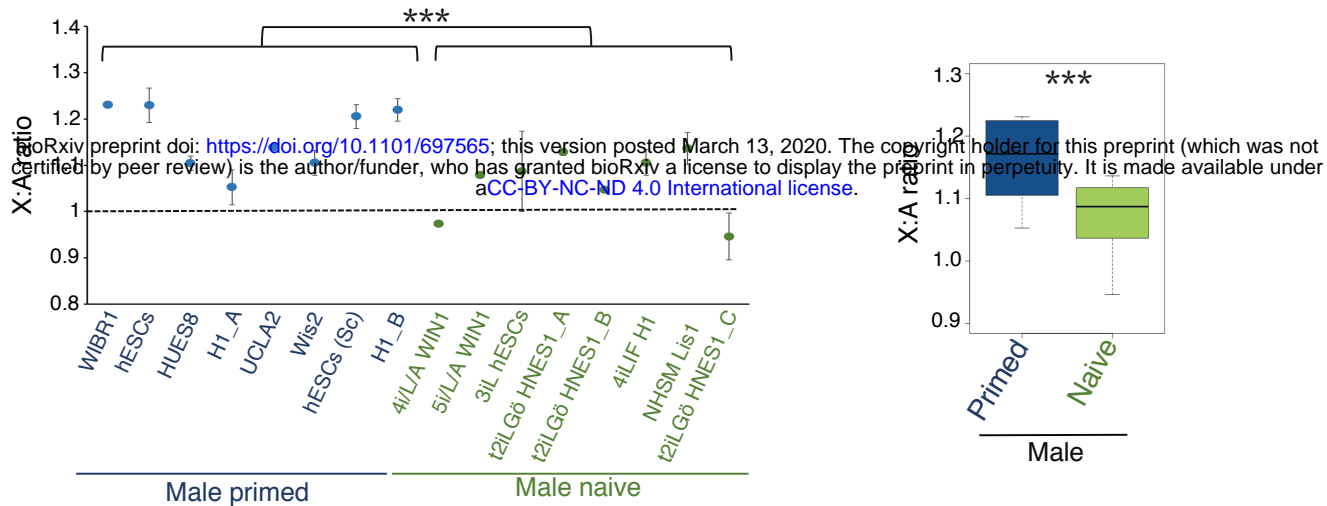


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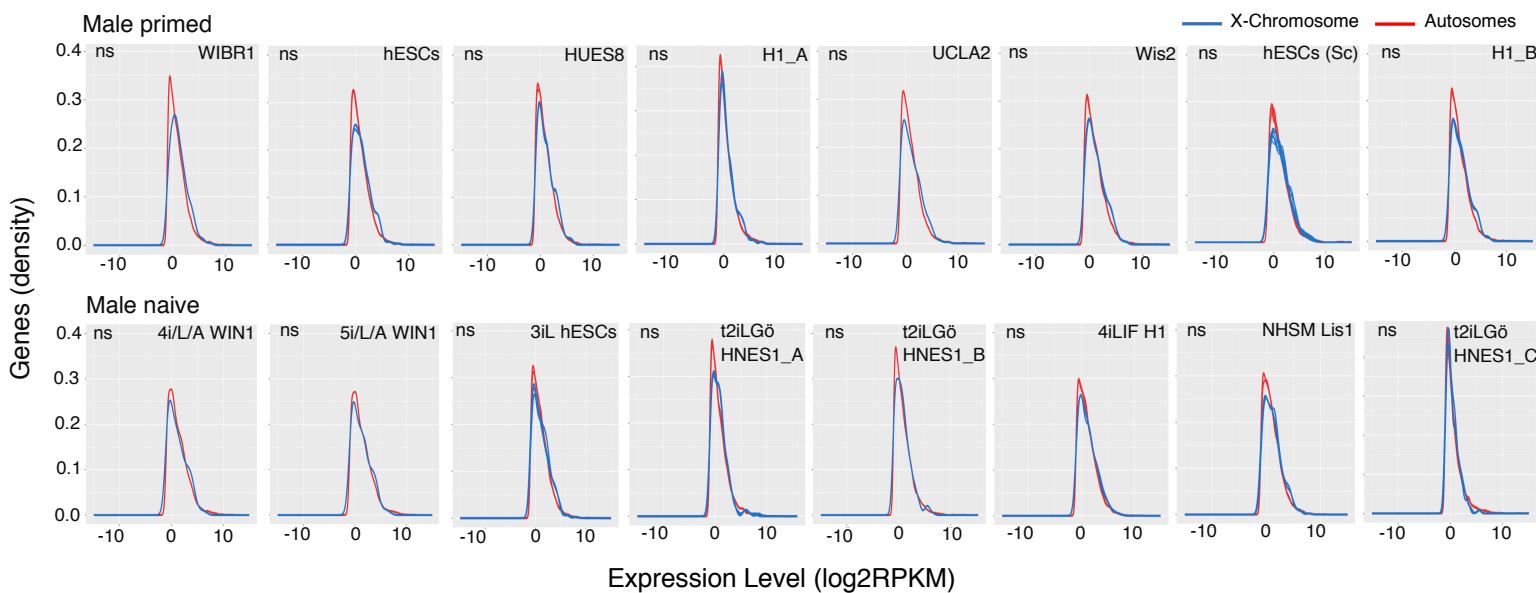




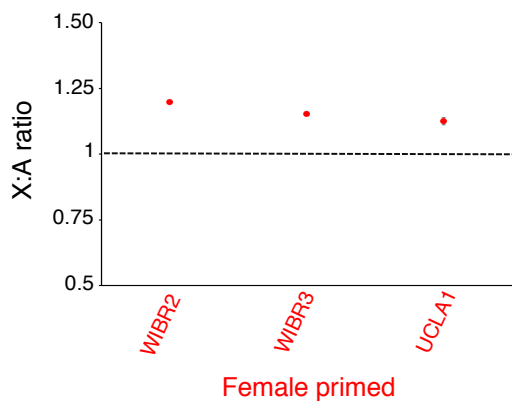
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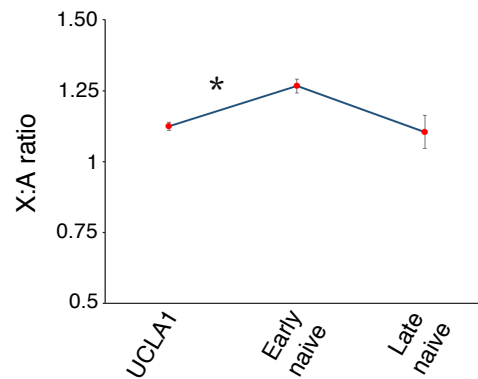
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