

1 **Functional classification of noncoding RNAs associated with distinct**
2 **histone modifications by PIRCh-seq**

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32 **ABSTRACT**

33

34 Many long noncoding RNAs (lncRNAs) regulate gene transcription through
35 binding to histone modification complexes. Therefore, a comprehensive study of nuclear
36 RNAs in a histone modification-specific manner is critical to understand their regulatory
37 mechanisms. Here we develop a method named Profiling Interacting RNAs on Chromatin
38 by deep sequencing (PIRCh-seq), in which we profile chromatin-associated transcriptome
39 in 5 different cell types using antibodies recognizing histone H3 and 6 distinct histone
40 modifications associated with active or repressive chromatin states. PIRCh-seq identified
41 chromatin-associated RNAs with substantially less contamination by nascent transcripts,
42 as compared to existing methods. We classified chromatin-enriched lncRNAs into 6
43 functional groups based on the patterns of their association with specific histone
44 modifications. LncRNAs were enriched with different chromatin modifications in
45 different cell types, suggesting lncRNAs' regulation may also be cell type-specific. By
46 integrating profiles of RNA secondary structure and RNA m⁶A modification, we found
47 that RNA bases which bind to chromatin tend to be more single stranded. We discovered
48 hundreds of allele-specific RNA-chromatin interactions, nominating specific single
49 nucleotide variants that alter RNA association with chromatin. These results provide a
50 unique resource to globally study the functions of chromatin-associated lncRNAs and
51 elucidate the basic mechanisms of chromatin-RNA interaction.

52

53 **INTRODUCTION**

54

55 RNAs are both the product of transcription and major regulators of the
56 transcriptional process. In particular, long noncoding RNAs (lncRNAs) are numerous in
57 eukaryotes and function in many cases as transcription regulators^{1,2,3}. With the
58 development of next-generation sequencing (NGS), tens of thousands of lncRNAs have
59 been revealed in both murine and human genomes, and have emerged as important
60 regulators for different biological processes^{4,5}. However, among all expressed lncRNAs,
61 only a small subset are shown to be cell essential⁶ or important for development⁷ or
62 immune responses⁸. Strategies to annotate biochemical properties of lncRNAs will be
63 helpful to prioritize lncRNA candidates for functional analyses. Some well-studied cases
64 have indicated that one major mechanism of lncRNAs is their ability to function through
65 binding to histone-modifying complexes^{9,10}. LncRNAs can either recruit chromatin
66 modifiers to regulate the chromatin states or directly regulate the process of transcription
67 through chromosome looping to bridge distal enhancer elements to promoters^{11,12}.
68 Thereby, a genome-wide identification of chromatin-associated lncRNAs may reveal
69 functions and mechanisms of lncRNAs in mediating chromatin modification and
70 regulating gene transcription.

71

72 A considerable amount of literature has been published concerning protein-RNA
73 interactions. The advent of technologies such as RIP¹³, CLIP¹⁴ and fRIP¹⁵ have led to the
74 discovery of multiple protein-associated RNAs, including many chromatin regulators.
75 Conversely, nuclear extraction methods followed by RNA-seq have enabled the detection
76 of lncRNAs which are physically associated with chromatin¹⁶⁻¹⁸. In addition, more
77 recently reported methods like GRID-seq¹⁹, MARGI²⁰, and SPRITE²¹ can be used to
78 capture pair-wise RNA interactions with DNA. However, these approaches are not
79 capable of revealing which chromatin modifications are associated with specific
80 lncRNAs, and are thus limited in the ability to elucidate their potential regulatory
81 functions. For instance, a large number of lncRNAs are associated with Polycomb
82 Repressive Complex 2 (PRC2), a key mammalian epigenetic regulator, to silence gene
83 transcription by targeting its genomic loci and trimethylating histone H3 lysine 27
84 (H3K27me3)²². Therefore, lncRNAs associated with PRC2 complex may be enriched on
85 heterochromatin regions with H3K27me3 modification. On the other hand, a new class of
86 lncRNAs called super-lncRNAs were recently characterized. These lncRNAs target
87 super-enhancers which have potential to regulate enhancer activities and transcription²³.
88 These super-lncRNAs may be enriched on euchromatin and active DNA regulatory
89 elements with histone H3 lysine 27 acetylation (H3K27ac), H3 lysine 4 monomethylation
90 (H3K4me1) and trimethylation (H3K4me3). Therefore, we believe it will be helpful to
91 develop an experimental technology to distinguish different histone modification-
92 associated lncRNAs, as well as analytical approaches to classify them and predict
93 lncRNA functions based on their chromatin association patterns. Another technical
94 challenge in studying chromatin associated lncRNAs is avoiding interference from
95 abundant nascent transcripts on chromatin. For example, results from GRID-seq¹⁹ or
96 MARGI²⁰, approaches recently developed to identify in situ global RNA interactions with
97 DNA, contain significant amounts of nascent transcripts, making it difficult to distinguish
98 whether the detected RNA is truly chromatin-associated or merely captured during the
99 process of transcription.

100
101 To address these questions, we developed a new method named Profiling
102 Interacting RNAs on Chromatin followed by deep sequencing (PIRCh-seq), which
103 enriches chromatin associated RNAs in a histone modification-specific manner and
104 classifies functional lncRNAs based on the patterns of their attachment to nucleosomes
105 with specific chemical modifications. Compared to current techniques for detecting
106 chromatin-RNA association, PIRCh-seq efficiently reduces the influence of nascent
107 transcripts with a significantly lower number of intronic reads. Through performing
108 PIRCh-seq with histone H3 and a number of different histone modification antibodies on
109 different cell types, we identified cell type-specific relationships between lncRNAs and
110 epigenetics. We found that chromatin-associated lncRNAs can be classified into 6
111 functional groups based on their association with chromatin modifications, which

112 undergo dynamic changes with cell differentiation. In addition, we found that bases on
113 lncRNAs attached to chromatin tend to be more single stranded in an allele-specific
114 manner. Overall, our PIRCh-seq data provides novel insights into global functional and
115 mechanistic studies of chromatin-associated lncRNAs.

116

117 **RESULTS**

118

119 **PIRCh-seq identifies RNA association with specific histone modifications in living** 120 **cells**

121

122 We conceived of PIRCh-seq as the inverse of ChIRP, a previously developed and
123 robust method to crosslink endogenous RNA-chromatin interactions in living cells²⁴. In
124 the PIRCh-seq work flow, living cells are chemically crosslinked by glutaraldehyde and
125 quenched with glycine, which prevents chromatin-associated RNA from further
126 degradation. Chromatin is extracted and sonicated to 300-2000 basepair (bp) size, and
127 then immunoprecipitated (IP) by histone modification-specific antibodies. Residual DNA
128 and proteins are removed, and retrieved RNAs are then subjected to deep sequencing
129 (**Figure 1A**). We tested the possibility that glutaraldehyde crosslinking may alter the
130 pull-down specificity of antibodies targeting histone modification. Using SNAP-ChIP²⁵, a
131 pool of modified mono-nucleosomes with known histone tail modifications individually
132 tagged with DNA barcodes, we found that glutaraldehyde crosslinking did not affect
133 antibody specificity (**Figure S1A-C**). The input control for PIRCh is the lysate obtained
134 after crosslinking and sonication but not subject to IP, which we also analyzed deep
135 sequencing. RNAs that are retrieved by a histone modification over input beyond that
136 expected by chance are considered PIRCh-seq hits. In this study, we generated and
137 analyzed 26 high-resolution PIRCh-seq datasets from 2 different species: human and
138 mouse; 5 cell types: human H9 embryonic stem cells (H9), human female fibroblasts
139 (HFF), mouse V6.5 embryonic stem cells (mESC), mouse embryonic fibroblasts (MEF),
140 and mouse neuronal precursor cells (NPC), targeting histone H3 and 6 histone
141 modifications (namely H3K4me1, H3K4me3, H3K27ac, H3K27me3, H3K9me3 and
142 H4K16ac) and input as control with 2 replicates for each experiment (**Figure S1D**). The
143 expression distributions of the input RNAs extracted from our study were similar to that
144 of the RNAs from nuclear extraction, but differed from cytoplasmic and total RNA (data
145 obtained from GSE57231 & GSE32916 in the same cell line) (**Figure S1E**), suggesting
146 that our chosen input could serve as a reasonable baseline for chromatin-associated RNA
147 identification. Correlation analysis of these samples indicates the high reproducibility of
148 PIRCh-seq experiments ($R=0.900-0.988$, **Figure S1F-M**).

149

150 As a proof of principle, we first examined PIRCh-seq signal of the well-
151 characterized lncRNA *XIST*, which coats the inactive X chromosome in female cells, and

152 is known to be associated with heterochromatin with repressive histone modifications²⁶.
153 Indeed, we observed that the PIRCh-seq signal of *XIST* is highly enriched on histone H3
154 over input in human female fibroblast cells (**Figure 1B**), as was histone H3 PIRCh
155 followed by qRT-PCR for *Xist* in female murine neural stem cells (NSCs) and intact adult
156 brain (**Figure 1C**). These results suggest that PIRCh-seq is not only capable of enriching
157 chromatin-associated lncRNAs, but may be applied to study brain tissue *in vivo*.
158 Similarly, the lncRNA *KCNQ1OT1*, which is involved in imprinting in Beckwith-
159 Wiedemann syndrome by silencing lineage-specific transcription through chromatin
160 regulation²⁷, is also enriched on histone H3 over input, as expected (**Figure S2A**).
161 Additionally, the imprinted oncofetal lncRNA *H19*²⁸ was also enriched by histone H3
162 PIRCh-seq (**Figure S2B**). On the other hand, abundant protein-coding and house-keeping
163 mRNAs, such as *ACTB* or *EEF2*, did not show PIRCh-seq enrichment as expected for
164 cytoplasmic mRNAs (**Figure S2C-D**).
165

166 Next, we checked whether PIRCh-seq could enrich for RNAs associated with
167 specific histone modifications. We performed PIRCh-seq on female NPCs with 3
168 ENCODE consortium validated antibodies targeting H3K4me3, H4K27ac and
169 H3K27me3. PIRCh-seq in female NPCs demonstrated that *Xist* RNA was enriched by
170 H3K27me3, a repressive mark enriched on the inactive X-chromosome, but not by active
171 histone marks H3K4me3 nor H3K27ac that are depleted on the inactive X (**Figure 1D**).
172 Interestingly, from *Xist*'s PIRCh-seq signal it is possible to infer which domain of this
173 lncRNA is associated with chromatin. Within the *Xist* locus, the 5' domain of *Xist*
174 displays significantly more substantial enrichment in H3K27me3 PIRCh-seq as compared
175 to other regions along the RNA (highlighted by the gray box, **Figure 1D**), consistent with
176 previous findings that this is the domain potentially associated with chromatin (repC
177 domain)^{29,30,31}. Conversely, coding genes such as *Actb* and *Eef2* were not enriched on
178 chromatin with the same set of modifications (**Figure S2E-F**). These results were
179 obtained from 3 different cell lines in 2 species and indicate that PIRCh-seq is able to
180 identify histone modification specific chromatin-associated lncRNAs transcriptome-wide.
181

182 PIRCh-seq can also be utilized to identify novel histone modification-specific
183 chromatin-enriched lncRNAs. In our NPC PIRCh-seq, a lncRNA upstream of the *Nr2f1*
184 gene, *lnc-Nr2f1*, was retrieved by the promoter marks histone H3K4me3 ($P < 0.05$), but
185 not enhancer-associated nor repressive modifications (H3K27ac and H3K27me3),
186 indicating that this lncRNA may preferentially associate with H3K4me3 regions (**Figure**
187 **1E**). Recently, *lnc-Nr2f1* was reported to play a critical role in regulating
188 neurodevelopmental disorders³². In order to further validate the chromatin-RNA
189 association of this lncRNA, we retrieved *lnc-Nr2f1* RNA and mapped its associated DNA
190 elements in NPCs (ChIRP-seq experiment). Overlaying *lnc-Nr2f1* ChIRP-seq with ChIP-
191 seq data of the histone modifications confirmed that *lnc-Nr2f1* does bind to genomic

192 locations with H3K4me3 (**Figure 1F-G**), further confirms that the PIRCh approach can
193 retrieve lncRNAs specifically associated with certain modifications. In addition, gene
194 ontology analysis of *lnc-Nr2f1* ChIRP-seq peaks using GREAT³³ suggests that *lnc-Nr2f1*
195 regulates cerebellar cortex development (**Figure S2G**, $P < 10^{-5}$), consistent with previous
196 findings regarding the function of this lncRNA. These results not only demonstrate the
197 reliability of PIRCh-seq in identifying chromatin-associated ncRNAs, but also suggest
198 potential application of the histone modification-specific PIRCh-seq approach in
199 predicting their functions.

200

201 **PIRCh-seq enriches lncRNAs on chromatin with low nascent transcription**

202

203 Various techniques have been developed to study ncRNA functions on chromatin.
204 For instance, ChIRP²⁴, CHART³⁴, and RAP³⁵ are RNA-centric methods that profile DNA
205 binding sites genome-wide of one target RNA at a time. Many investigators have isolated
206 chromatin-associated RNAs from stringent nuclear or chromatin fractionation^{16,18}. In
207 addition, recent methods such as GRID-seq and MARGI can be applied in mapping the
208 global RNA-chromatin interactome^{19,20}. Comparatively, chromatin fractionation and
209 sequencing detects chromatin-associated RNA without delineating the specific chromatin
210 states that specific RNAs prefer. Furthermore, proximity ligation methods predominantly
211 detect nascent RNAs co-transcriptionally tethered to chromatin by RNA polymerase,
212 confounding signals from the functional chromatin-associated ncRNAs and background
213 signal from all RNAs in the process of transcription. Thus, to evaluate the level of
214 nascent transcription from PIRCh, we compared our PIRCh-seq results in H9 and HFF
215 with that from GRID-seq¹⁹, che-RNA isolation (named CPE “chromatin pellet extract”
216 for experiment and SNE “soluble-nuclear extract” for background control)¹⁸, and
217 chromatin-associated RNAs (CAR)¹⁶. These experiments were all performed in human
218 cell lines. We found that the ratios of intronic reads in PIRCh-seq profiles were
219 significantly lower than those from previously reported methods ($P < 0.01$, T-test), and
220 were almost comparable with input RNAseq from bulk cultured cells (**Figure 2A**).
221 Moreover, by averaging signals over the entire transcriptome centered by introns from all
222 the existing methods, we found PIRCh was more effective in obtaining mature RNAs
223 than extant chromatin-RNA enrichment methods, based on the higher signal over exons
224 than introns (**Figure 2B**). We obtained similar findings in other cell types and with every
225 tested histone modifications (**Figure 2C**, PIRCh-seq of V6.5 mouse ES cells with histone
226 H3 and 6 histone modifications). These results demonstrate that PIRCh-seq consistently
227 generates a significantly lower level of intronic reads with multiple histone modifications
228 than existing methods, and therefore is able to preserve regulatory interactions in trans
229 between lncRNAs and chromatin.

230

231 To further estimate the level of nascent transcription, we then integrated each
232 histone modification-specific PIRCh-seq profile with its corresponding ChIP-seq dataset
233 in the same cell line (V6.5 mESCs), and asked whether the PIRCh-seq signal of each
234 RNA correlated with the nearby ChIP-seq signal carrying the corresponding modification
235 (see **Methods**). The ChIP-seq profiles of each histone modification in mESC were
236 obtained from ENCODE. Our results suggest that there was no significant correlation
237 with these two sets of signals (**Figure S3A-E**), confirming that the nascent transcription
238 from PIRCh-seq is negligible. These results suggest that the majority of PIRCh-seq
239 enriched chromatin-associated RNAs are mature RNAs with introns spliced out, which
240 allows PIRCh-seq to identify more chromatin-associated RNAs with low abundance,
241 such as many ncRNAs.

242

243 **PIRCh-seq identifies ncRNAs associated with specific histone modifications**

244

245 Because PIRCh-seq enables transcriptome-wide annotation of chromatin-RNA
246 association, we next determined whether various types of RNA (especially coding RNAs
247 versus ncRNAs) are differentially affiliated with chromatin. We first applied the limma
248 package³⁶ in R to normalize the RNA read counts in PIRCh-seq and input samples
249 (**Figure S4A-B**). We then defined a PIRCh enrichment score by dividing the normalized
250 read counts in PIRCh over input, and ranked all the transcripts by their enrichment scores
251 in H3 PIRCh-seq. To test whether ncRNAs were enriched on chromatin, we performed a
252 gene set enrichment analysis (GSEA)³⁷ of the annotated coding and noncoding RNAs.
253 We found that ncRNAs, but not coding RNAs, were indeed highly enriched on chromatin
254 and many known ncRNAs were top ranked in terms of chromatin enrichment scores
255 (**Figure 2D**). Next, we performed PIRCh-seq with antibodies specific to distinct histone
256 modifications in mESCs. Similar to the enrichment on histone H3, we expect that
257 ncRNAs should be highly ranked by the average fold enrichment of the histone
258 modification-specific PIRCh-seq signal versus the corresponding input, among all the
259 expressed genes. Indeed, compared with mRNAs, we found that in most cases (22 out of
260 28) the average enrichment scores of the annotated lncRNAs, pre-miRNAs, snoRNAs, as
261 well as other ncRNAs were significantly higher on H3 and multiple histone modified
262 chromatin than coding genes (**Figure 2E**, $P < 0.05$, T-test). We then checked the
263 distributions of the expressed and chromatin associated RNAs on histone H3 and
264 chromatin with other modifications in mESCs, and found that ncRNAs were significantly
265 more frequent on chromatin compared with mRNAs (**Figure S4C**), serving as additional
266 evidence that ncRNAs are more enriched on chromatin in general. Furthermore, when we
267 defined a variation score which measured the standard deviation of the chromatin
268 association enrichment scores across each histone modification for every expressed RNA,
269 we concluded that ncRNAs are significantly more variable than mRNAs (**Figure 2F**,
270 $P < 0.001$, T-test). This suggests that non-coding transcripts are more differentially

271 enriched at distinct chromatin states, consistent with the potential regulatory function
272 divergence of lncRNAs, and naturally prioritizes downstream studies of lncRNAs by
273 activity.

274

275 We then sought to characterize the ncRNAs significantly on chromatin in mESC
276 from their PIRCh-seq profiles. We considered PIRCh-seq biological replicates versus the
277 inputs in limma³⁶ and defined an RNA with chromatin association by P -value <0.05
278 (**Methods**). Using this cutoff, we identified 258 chromatin associated ncRNAs in mESC
279 which were enriched in at least one of the 6 histone modification-specific PIRCh-seq
280 profiles (**Table S1**). To further evaluate the performance of the PIRCh approach, we
281 compared our PIRCh-seq enriched lncRNA results with 96 published RNA-chromatin
282 association profiles from ChIRP/CHART/RAP/GRID-seq datasets, collected by
283 LnChrom³⁸. We found a total of 23 lncRNA, including *Xist*, *Firre*, *Rmrp*, *Tug1* and etc.
284 were also expressed in our mESCs. All 23 lncRNAs were positively enriched in PIRCh,
285 and 14 were significant with $P<0.05$, reaffirming the sensitivity of the PIRCh approach in
286 identifying chromatin associated lncRNAs. Furthermore, we wanted to validate whether
287 the PIRCh lncRNA enrichment patterns were consistent with results obtained from
288 published orthogonal methods. We hypothesized that if a lncRNA is able to associate
289 with DNA elements marked by a specific histone modification, its genomic binding sites
290 from ChIRP/CHART/RAP/GRID-seq experiments should greatly overlap with
291 corresponding ChIP-seq peaks associated with the same modification. We then obtained
292 the genomic binding sites (peaks) of the 23 lncRNAs from the aforementioned
293 experiments, and found the ratio of this overlap from published data (**Figure 2G**) is
294 highly correlated with the corresponding PIRCh-seq signal among most of the lncRNAs
295 (**Figure 2H**). The Spearman correlation coefficients of the ratio of the overlap ChIP-seq
296 peaks³⁹ with the lncRNA's PIRCh-seq enrichment scores in the same cell line were
297 significantly higher than random permutations (**Figure 2I**, $P<0.0001$). These results
298 further confirm that PIRCh-seq reliably identifies chromatin associated lncRNAs.

299

300 Conversely, we hypothesized that certain ncRNAs are enriched at chromatin with
301 distinct types of DNA regulatory elements, and asked whether gene regulatory elements
302 could be naturally differentiated via chromatin-ncRNA association. We then calculated
303 the pairwise Pearson correlation of all chromatin states based on the PIRCh-seq
304 enrichment scores of 258 chromatin associated ncRNAs. It is clear that the enhancer-like
305 states (H3K27ac, H4K16ac, H3K4me1) clustered together, then the promoters
306 (H3K4me3), while the repressive histone modifications (H3K27me3, H3K9me3) were
307 grouped in a distinct cluster (**Figure 2J**). Interestingly, the PIRCh-seq signal of histone
308 H3 clustered closest with H3K4me1 (Pearson correlation $r=0.89$). We observed that
309 H3K4me1 ChIP-seq signal from the same cells as above covers three to four times the

310 genomic regions than other chromatin modifications, which may reflect the differential
311 sensitivities of the different antibodies for ChIP (**Figure S4D**).

312

313 **PIRCh-seq classifies functional ncRNAs via chromatin association**

314

315 Different gene regulatory elements--such as enhancers, promoters, insulators, and
316 silenced elements carry distinctive and characteristic histone and DNA modifications
317 (**Figure 3A**)⁴⁰. We noticed that 14-25 ncRNAs in HFF and H9 respectively were also
318 reported as “essential” ncRNAs with functions through CRISPRi screening⁶. We then
319 hypothesized that specific modification enriched ncRNAs regulate each of these elements,
320 and thereby the functions of ncRNAs can be classified by their divergent chromatin
321 modification enrichment. Hence, PIRCh-seq is anticipated to classify and associate
322 ncRNAs with functions such as promoter, enhancer, silencer, or insulator etc. To test this
323 hypothesis, we analyzed *7sk*, a well-known regulator of RNA polymerase II elongation
324 that resides at enhancers, promoters, and super enhancers⁴¹, consistent with its role in
325 enhancer-promoter interactions. From *7sk* ChIRP-seq data in mESC, we noticed that its
326 chromatin occupancy sites greatly overlapped with ChIP-seq peaks of H3K4me1,
327 H3K4me3, and H3K27ac in the same cell type (**Figure 3B**), confirming an active
328 function of *7sk*. Consistently, PIRCh-seq signal of *7sk* in mESC was also enriched at
329 chromatin carrying these three histone modifications, but depleted of repressive
330 modifications such as H3K27me3 and H3K9me3 (**Figure 3C**), suggesting the possibility
331 to extrapolate lncRNA function using PIRCh-seq.

332

333 We then analyzed all 258 PIRCh enriched ncRNAs and sought to categorize their
334 functions based on their PIRCh-seq signals. We found that these ncRNAs associate with
335 chromatin in a combinatorial pattern, similar to those observed in ChIP-seq performed on
336 histone modifications (**Figure S5A**). H3K27ac, H3K4me3 and H3K4me1 were the top 3
337 most favored chromatin states that interacted with ncRNAs, consisting of 88% of the
338 enriched ncRNAs in mESC. As we know from histone ChIP-seq, instead of each
339 individual modification, a combinatorial pattern of multiple modifications better
340 classifies the functions of DNA elements⁴². A machine learning strategy employing
341 hidden Markov model, named chromHMM, which automatically learns the major
342 combinatorial patterns, was applied successfully to classify DNA elements based on
343 histone modifications^{43,44}. We then inquired if a similar strategy could be used to classify
344 chromatin-associated ncRNAs and examine if the functions of these ncRNAs could be
345 distinguished based on their association with histone modifications. To investigate this
346 relationship transcriptome-wide, we started from a 258 by 6 matrix of enrichment scores
347 in mESC, where each row was an enriched ncRNA as defined above, each column was a
348 histone modification, and each element of the matrix represented the enrichment score of
349 the corresponding ncRNA on the specific modified chromatin (**Methods**). We then

350 applied K-means clustering on the matrix, where the number of Ks was determined by
351 the Silhouette method⁴⁵. This analysis yielded 6 distinct groups of chromatin-associated
352 ncRNAs, which were visualized in a 2-dimensional projection of t-distributed stochastic
353 neighbor embedding (tSNE) (**Figure 3D**). Within these 258 chromatin associated
354 ncRNAs, 247 are lncRNAs, and many well-studied ncRNAs, such as *7sk*⁴¹, *Neat1*¹⁰ and
355 *Malat1*⁴⁶, and *Dancr*⁴⁷ naturally clustered into groups with distinct function. Interestingly,
356 14 lncRNAs were also reported to have a biological function based on LncRNADB⁴⁸,
357 and 8 out of 56 were predicted bivalent in mESCs (**Figure S5B**, odds-ratio=4.4, $P < 0.01$,
358 Chi-square test). In addition, for each cluster we evaluated the relative contributions of
359 each histone modification based on the enrichment pattern of the chromatin associated
360 RNAs, and defined the clustered states by active promoters, heterochromatin, weak
361 promoter, strong enhancer, bivalent, and weak enhancer (**Figure 3E**). Overall, we
362 partially recapitulated the chromatin classifications based on chromHMM algorithm to
363 ChIP-seq profiles⁴³. These results suggest that the chromatin association of ncRNAs can
364 be used to classify ncRNAs that might have functional implications.

365

366 Although tens of thousands noncoding transcripts were discovered in the past few
367 years, only a small portion that function through chromatin organization were
368 consolidated. More recently, evidence has accumulated that indicates many lncRNAs
369 may regulate gene expression in cis^{49,50}. Since the PIRCh approach cannot pinpoint the
370 exact binding sites of chromatin-associated lncRNAs, it does not directly predict whether
371 each lncRNA is functioning in cis or trans. Instead, PIRCh provides more information
372 about the epigenetic function of the lncRNA, in context of the histone modifications it
373 associates with. Our analysis suggests that chromatin-associated lncRNAs function both
374 in trans and cis. For example, when we calculated the nearby (+/-100Kb) coding gene
375 expression of the PIRCh clustered ncRNAs in **Figure 3D**, we observed that lncRNAs
376 were monotonically decreasing from the more active to more repressive groups;
377 additionally, the nearby coding gene expression of the “Active Promoter” and “Strong
378 Enhancer” lncRNA groups were significantly higher than that of the group “Repressed”
379 ncRNAs (**Figure 3F**, $P < 0.05$, T-test). However, when the chromatin associated ncRNAs
380 were grouped based on their enrichment with each histone modification, no significant
381 expressional differences were observed from nearby coding genes. (**Figure S5C**), e.g.
382 compared H3K27me3 vs H3K27ac. No similar trends were observed in the expression
383 patterns of the ncRNAs themselves (**Figure S5D**). These results not only indicate that the
384 chromatin-associated ncRNAs may function through a combinational pattern of the
385 histone modifications instead of an individual modification, but also favors the argument
386 that the chromatin associated ncRNAs may function in cis in general. Nevertheless, not
387 all the lncRNAs enriched in our PIRCh experiment function in cis. When we integrated
388 each histone modification specific PIRCh-seq profile with its corresponding ChIP-seq

389 signal at the genomic loci of the chromatin-enriched ncRNAs, no statistical correlation
390 was observed (**Figure S3**), suggest that some lncRNAs can function in trans.

391

392 **Cell type-specific chromatin association of ncRNAs**

393

394 It is known that ncRNAs are differentially expressed in distinct cell types and
395 perform specific cellular functions. Therefore, we sought to check whether the patterns of
396 ncRNA-chromatin association diverge in distinct mouse cell types, and how these
397 patterns contribute to their cell type-specific functions. We then performed PIRCh-seq on
398 MEF cells and analyzed the profiles in an identical fashion to the mESC data. Similar to
399 the mESC results, we observed that PIRCh-seq identified lncRNAs enriched on
400 chromatin with low nascent transcription (**Figure S6A**), and non-coding transcripts were
401 consistently more enriched on chromatin compared with protein coding gene in MEF
402 cells (**Figure S6B-C**), validating these conclusions in distinct cell types. We then
403 performed a similar enrichment analysis on MEF and NPC PIRCh-seq profiles and
404 obtained 200 and 110 chromatin associated ncRNAs respectively ($P<0.05$). The
405 combinatorial patterns of the MEF enriched ncRNAs are predominantly similar to those
406 from mESC (**Figure S6D-E**). As a negative control, the IgG PIRCh was tested in tandem
407 with the other chromatin modification PIRCh experiments performed in MEF.
408 Differential analysis of PIRCh-groups over IgG control revealed that only 1 out of 200
409 PIRCh-enriched ncRNA over input was also enriched in IgG, evincing the high
410 specificity of our method in identifying the chromatin associated ncRNAs (**Figure S6F**).
411 In our analysis, a total of 458 chromatin enriched ncRNAs were identified in three cell
412 types, 20 of which were enriched in all three cell types (**Figure 4A**). We then calculated
413 the Pearson correlation coefficient matrix based on the enrichment scores of these 458
414 ncRNAs. Unsupervised clustering of this correlation matrix suggested that the cell type
415 specificity was the dominant factor which determines ncRNA chromatin association
416 (**Figure 4B**).

417

418 Embryonic stem cells are characterized by their pluripotency - the ability to give
419 rise to multiple cell types. The chromatin state in ES cells is reported to be more flexible
420 than those of differentiated cells⁵¹. Interestingly, compared with those of the more
421 differentiated cells (MEF and NPC), the ncRNA chromatin association in mESCs showed
422 a higher correlation coefficient among distinct histone modifications, suggesting the
423 specificity of chromatin-associated ncRNAs in mESCs is more plastic than those in
424 differentiated cells (**Figure 4B**). In addition, we analyzed the percentage of enriched
425 ncRNA versus total expressed ncRNA in each cell type for every tested chromatin
426 modification, and found significantly more ncRNAs enriched on chromatin with
427 H3K9me3 in ES cells when compared with MEF (**Figure 4C**, $P<0.05$, Chi-square test),
428 but fewer on chromatin with H4K16ac. This result may reflect the joint presence of

429 activating and repressive histone marks on genome regions, termed bivalent⁵² and
430 trivalent chromatin domains⁵³ in ES cells. We identified ncRNAs which were associated
431 with both active and repressive histone marks consistent with bivalency, while others
432 associated with strictly active or repressive marks (**Figure 4D**). Since PIRCh-seq enabled
433 us to identify cell-type and histone modification specific ncRNA-chromatin associations,
434 we first screened for ncRNAs which were enriched at both active and repressive
435 chromatin in ES cells but only enriched in either active or repressive markers in
436 differentiated cells. We found several ncRNAs of this description. For example, ncRNA
437 *uc008bcq.1* is broadly enriched in ES cells with high PIRCh-seq signals associated
438 H3K4me1, H3K4me3, H3K27me3 and H3K9me3 modifications, but enriched only on
439 active chromatin of H3K4me1 in MEF and repressive chromatin of H3K27me3 in NPC,
440 implying lineage-specific resolution of chromatin associations (**Figure 4E**). Interestingly,
441 there were dozens of such ncRNAs that are distinctly enriched in certain cell types. Since
442 ES cells possess a higher potential to differentiate into multiple lineages, and hence more
443 poised chromatin states, we expected more bivalent-enriched (“bi-enriched” for short)
444 and fewer mono-enriched ncRNAs in mESC compared with more differentiated cells
445 such as MEF and NPC. In mESC, we found 30 bi-enriched and 33 mono-enriched
446 ncRNAs; while in MEF, we found only 8 bi-enriched but 32 mono-enriched ncRNAs;
447 lastly, in NPC, we found 2 bi-enriched and 11 mono-enriched ncRNAs (**Figure 4F**,
448 $P < 0.01$ for MEF and $P < 0.05$ for NPC, Chi-square test). These results indicate that
449 ncRNAs may play distinct functional roles by either enhancing or repressing gene
450 expression or both in certain cell types, conducted by affixing to either active or
451 repressive chromatin or both.

452

453 **Single-stranded RNA regions as candidate mediators of chromatin association**

454

455 As a key player in the central dogma of biological regulation, RNA and its ability
456 to adopt specific structures is intimately involved in every step of gene expression.
457 Previously, multiple approaches have been described in order to probe RNA secondary
458 structure transcriptome-wide *in vitro*⁵⁴ and *in vivo*^{31,55} in mammalian cells, revealing
459 structural principles of RNA-protein interactions. Correspondingly, we noted that RNA
460 enrichment on chromatin occurs in a domain-specific manner based on our PIRCh-seq
461 data. For instance, the repC domain of *Xist* is dramatically more enriched on chromatin
462 carrying H3K27me3 modifications (highlighted by the gray box, **Figure 1D**). *Malat1* is
463 another well-studied chromatin-associated lncRNA which binds to active chromatin¹⁰.
464 Instead of attaching to histone proteins across the entire transcript, we noticed from the
465 H3K4me3 PIRCh-seq signal that there were certain regions on *Malat1* which were more
466 closely associated with chromatin than the rest bases on the transcript (**Figure 5A**).
467 Interestingly, these regions tend to be single-stranded according to both 2' hydroxyl
468 acylation profiling experiments (icSHAPE data) and RNA secondary structure

469 predictions from RNAfold⁵⁶ (**Figure 5B**). This led us to investigate whether there are
470 structural preferences involved in RNA-chromatin association (**Figure 5C**). We first
471 obtained a transcriptome-wide and per-base RNA secondary structure profile from
472 icSHAPE data measured in mESCs⁵⁵. A high icSHAPE score suggests a greater
473 probability that a base is single stranded. We then applied a 5-base sliding window
474 method to identify the enriched sites (peaks) on each RNA which interacted with
475 chromatin from PIRCh-seq, compared with our input control (see **Methods**). We then
476 overlaid the structural profiles from icSHAPE on top of all the histone modification
477 specific PIRCh-seq peaks centered by the peak summits and generated an average
478 structural profile for each modification. Our results show that bases ~5-10 nt upstream of
479 the chromatin associated peaks are more likely to be single stranded (**Figure 5D**). To test
480 the significance of this single-strand preference, we performed 2-tailed Welch's T-tests
481 by comparing all the icSHAPE scores of the bases from PIRCh-seq peaks with those
482 from a randomly selected background, and found this phenomenon was significant with
483 $P < 10^{-5}$. We then asked whether RNAs containing a greater number of single-stranded
484 bases are more likely to be associated with chromatin. We separated expressed RNAs
485 into two groups based on chromatin enrichment or depletion, and calculated the average
486 icSHAPE scores for every RNA in each group. We noticed that, on average, RNAs
487 enriched on chromatin tended to be more single-stranded with higher icSHAPE scores
488 (**Figure 5E**, $P < 0.001$, T-test). Similarly, we took the top 100 most single-stranded RNAs
489 and top 100 most double-stranded RNAs based on their average icSHAPE scores and
490 confirmed that the average chromatin enrichment scores of the most single-stranded
491 RNAs were significantly higher than those of the double-stranded RNAs (**Figure 5F**,
492 $P < 0.01$, T-test). These results suggest that RNAs containing more single stranded regions
493 are more likely to associate with chromatin.

494

495 **Single nucleotide variants and RNA modifications that alter chromatin association**

496

497 Genetic variation can alter RNA structure and function *in vivo*. Single nucleotide
498 polymorphisms (SNPs) comprise the most prevalent source of variation, and SNPs that
499 alter RNA secondary structures, termed “riboSnitches” (a fusion of SNP and riboswitch),
500 are a recently appreciated source of noncoding variants associated with human diseases⁵⁷.
501 We therefore asked whether different alleles of the same RNA may differentially
502 associate with chromatin; and if so, how is it related to the RNA structure? In order to
503 answer those questions, we performed PIRCh-seq in the NPC line that is derived from the
504 F1 hybrid offspring of two mouse parental lines (129S1 and CAST) with a high density
505 of SNPs across the genome (~1 SNP per 100 nucleotides). We first built the reference
506 genomes for each mouse line and aligned the raw reads to 129S1 and CAST separately
507 with 0 mismatches to reduce false positive hits. Reads mapped to either 129S1 or CAST
508 were counted to construct the allele-specific RNA expressions and chromatin

509 enrichments profiles (see **Methods**). First, we looked at whether allelic RNA chromatin
510 association is related to allelic expression. From allele-specific PIRCh-seq analysis, we
511 found that for most RNAs, allelic or unbiased expression from the two alleles determines
512 allelic or unbiased chromatin association pattern. For example, it is known that only the
513 129S1 version of the lncRNA *Xist* is expressed in this cell line. Consistent with allelic
514 expression, we found that enrichment of *Xist* in H3K27me3 modification is much higher
515 in 129S1 versus the CAST version of the lncRNA (**Figure 5G**). An additional example is
516 the lncRNA *Malat1* in which both the 129S1 and CAST alleles are almost equally
517 expressed. As predicted, we observed unbiased enrichment on chromatin for both alleles.
518 Moreover, we discovered several lncRNAs that are enriched on chromatin in an allele-
519 specific manner independent of the expression levels from the two alleles (**Table S2**). For
520 example, *Gas5* is a lncRNA that binds to PRC2 complex and mediate transcriptional
521 repression⁵⁸. We found that *Gas5* is enriched in H3K27me3 modification, consistent with
522 its understood repressive function. Notably, even though the CAST version of *Gas5* was
523 3-fold more expressed in the input sample, the 129S1 allele was 4-fold more enriched on
524 chromatin carrying H3K27me3 modification (**Figure 5G**, $P < 0.05$, T-test), suggesting that
525 129S1 allele of *Gas5* preferentially associates with chromatin. To further investigate the
526 mechanism under *Gas5* allele-specific enrichments, we predicted the secondary structure
527 of the 129S1 and CAST version of *Gas5* using RNAfold (**Figure S7A, B**), and found that
528 several riboSnitches (1774 T/C, 1804 C/T, 1810 T/A, 1812 T/G, 1887 T/C, CAST(mm9)
529 /129S1) converted one of the chromatin binding sites of *Gas5* from single-stranded in
530 129S1 to double-stranded in CAST and thus depleted its association with repressive
531 chromatin in the latter (**Figure 5H**). Consistent with this prediction, when we calculated
532 the icSHAPE score obtained from mESC containing 129S1 allele⁵⁹ for the *Gas5* region
533 allelic enriched in H3K27me3, we concluded that the region is more likely to be single
534 stranded (**Figure S7C**).

535

536 Another major factor that can influence RNA structure is RNA modification, such
537 as the N6-methyladenosine (m⁶A) modification. Previous studies have shown that m⁶A
538 can alter base-pairing thermodynamics and destabilize RNA duplexes^{55,60,61}. We also
539 evaluated whether RNA modifications affect RNA-chromatin association. We integrated
540 PIRCh-seq data with the transcriptome-wide profiles of RNA m⁶A modifications in
541 mESCs from our previous study⁶², and found the distribution of PIRCh-seq peaks along
542 the transcripts is similar to that of m⁶A modified regions (**Figure S8A**). When we
543 overlaid m⁶A signals on top of PIRCh-seq peaks, we found that RNA bases associated
544 with chromatin are generally more m⁶A modified ($P < 10^{-5}$ in H3, **Figure S8B-C**). These
545 results may reflect that the tendency of m⁶A to induce RNA single-stranded regions that
546 coincide with elements for chromatin association, or due to additional mechanisms that
547 jointly impact chromatin association and RNA modification.

548

549 **DISCUSSION**

550

551 **PIRCh-seq identifies chromatin associated RNAs genome-wide**

552

553 A large and growing body of literature has investigated protein-RNA interactions.
554 The development of approaches such as RIP¹³, CLIP¹⁴ and fRIP¹⁵ have enabled the
555 successful elucidation of many RNAs associated with proteins, including multiple
556 chromatin regulators. Studies have also shown that many lncRNAs function through
557 DNA/chromatin interaction. Previously described techniques such as ChIRP-seq and
558 CHART-seq have been used to identify genome-wide binding sites of specific lncRNA to
559 chromatin. However, these methods require prior knowledge of which particular
560 lncRNAs are capable of binding to chromatin before ChIRP-seq or CHART-seq can be
561 applied. Furthermore, ChIRP or CHART are limited to examining one chromatin
562 associated RNA at a time. In this study, we describe a new technology, PIRCh-seq, which
563 enables a global profiling of chromatin-associated RNAs through a robust method to
564 crosslink endogenous RNA-chromatin interactions in living cells. Compared with current
565 methods which predominantly detect nascent RNAs co-transcriptionally tethered to
566 chromatin by RNA polymerase, PIRCh-seq significantly reduces the influence of nascent
567 transcripts, and more clearly reveals relationships between chromatin-associated ncRNAs.
568 Although the PIRCh approach cannot pinpoint the exact binding sites of the chromatin
569 associated lncRNAs, and therefore does not inform whether each lncRNA is functioning
570 in cis or trans, PIRCh is able to provide a significantly higher ratio of mature RNAs and
571 thereby preserve the regulatory interactions in trans between lncRNAs and chromatin.
572 Examples of some well-studied cases, such as *Xist*, *7sk*, *H19* and *KCNQ1OT1* etc.
573 demonstrate that PIRCh-seq is likely generalizable to the majority of ncRNA.
574 Additionally, PIRCh-seq identifies novel chromatin-associated lncRNAs and not only
575 provides potential targets for mechanistic studies using ChIRP and CHART, but could
576 also be extended to reveal the function and mechanisms of lncRNAs which are disease-
577 relevant. However, the PIRCh-seq approach, like RIP/CLiP-seq like methods, may also
578 be heavily contaminated with co-purified mRNA species that often compose more than 50%
579 of RNA material. Therefore, further experimental and analytical improvements are
580 required to truly capture chromatin-associated ncRNAs.

581

582 **PIRCh-seq classifies ncRNA putative function via histone modification and cell**
583 **type-specific chromatin-RNA association**

584

585 Another major advantage of the PIRCh method is that it utilizes antibodies to pull
586 down chromatin with specific chemical modifications and thereby enables the
587 classification of chromatin-associated ncRNAs with putative functions such as promoter,
588 enhancer, silencer or bivalent. Since we performed PIRCh-seq with various histone

589 modification antibodies and in different human and murine cell types, the dataset
590 provides rich resources to study chromatin-associated ncRNAs in mammalian cells. In
591 addition, different cell types and histone modifications did not show much technical
592 variation, confirming that PIRCh-seq may be a useful technology to perform profiling of
593 epigenetic-associated ncRNAs. Analogous to the types of gene regulatory elements
594 bearing distinctive histone and DNA modifications, we developed a bioinformatics
595 method to classify the putative biological functions of ncRNAs based on their enrichment
596 patterns on chromatin with different histone modifications. Our method successfully
597 arranged several well studied lncRNAs in the correct functional category, and predicted
598 functions for hundreds of other ncRNAs from their chromatin association patterns. More
599 importantly, when a similar analysis was performed on multiple cell types, chromatin
600 state-specific ncRNA enrichment patterns were generally conserved, suggesting this is a
601 reliable method for functional classification. Since ncRNA-chromatin interaction is likely
602 a widespread epigenetic regulation mechanism in many cell types, our integrative
603 approach in identifying and classifying chromatin-associated ncRNAs can be broadly
604 applicable to many other cell types to deeper investigate ncRNA functions. However,
605 chromatin association does not guarantee that a ncRNA will have a biological function;
606 furthermore, the histone modification specific PIRCh-seq approach can only predict
607 putative functions. As such, the true function of each ncRNA still requires further
608 investigation beyond PIRCh-seq.

609

610 **RNA secondary structure affects RNA-chromatin interaction**

611

612 We observed that RNAs attach to chromatin in a domain-specific manner.
613 However, when we surveyed the enriched sites of chromatin-associated RNAs linked to
614 various histone modifications in different cell types, we did not find significant sequence
615 motifs, suggesting the existence of a complex mechanism responsible for the RNA-
616 chromatin interaction. On the other hand, when we integrated PIRCh-seq signals with
617 RNA structural information from previous icSHAPE and RNA modification m⁶A profiles
618 and further evaluated structural information regarding the enriched domains, we found
619 that ncRNAs were likely to bind to chromatin through single-stranded region or bases
620 with m⁶A methylation. This may possibly be explained by the supposition that RNA-
621 dependent recruitment of transcriptional activators and repressors may occur within a
622 double-stranded structural region, and therefore, single-stranded regions are made more
623 accessible to chromatin. In addition, chromatin interactions may also be allele-specific,
624 especially when certain alleles result in distinct RNA secondary structures. In conclusion,
625 when taken as a whole, these results open new avenues of inquiry and require further
626 investigation to fully elucidate the molecular mechanisms of ncRNA-chromatin
627 interaction.

628

629 **Author Contributions**

630

631 KQ, QM, CC, HYC conceived the project. QM, CC, LL, PJB, KEMT, RL performed
632 PIRCh-seq library generation and qPCR experiments. JF performed all data analysis with
633 assistance from BH, PC, QM, JX and PD. KQ, HYC, JF, and QM wrote the manuscript
634 with inputs from all authors.

635

636 **Data and code availability**

637

638 The PIRCh-seq and ChIRP-seq data generated in this study can be obtained from NIH
639 GEO with the accession number GSE119006 and is available by go to the following
640 website <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE119006>, and entering
641 token “ulgmesubpmdbkx” in the box. Other published data sets used in this study are
642 available and described in the Reporting Summary file. All in house developed
643 codes/scripts were uploaded to Github website (<https://github.com/QuKunLab/PIRCh>).

644

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646

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656

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659

660 **REFERENCES**

661

- 662 1. Rinn, J. L. & Chang, H. Y. Genome Regulation by Long Noncoding RNAs.
663 *Annual Review of Biochemistry* **81**, 145–166 (2012).
- 664 2. Fu, X.-D. Non-coding RNA: a new frontier in regulatory biology. *National*
665 *Science Review* **1**, 190–204 (2014).
- 666 3. Khalil, A. M. *et al.* Many human large intergenic noncoding RNAs associate with
667 chromatin-modifying complexes and affect gene expression. *Proceedings of the*
668 *National Academy of Sciences* **106**, 11667–11672 (2009).

- 669 4. Flynn, R. A. & Chang, H. Y. Long Noncoding RNAs in Cell-Fate Programming
670 and Reprogramming. *Cell Stem Cell* **14**, 752–761 (2014).
- 671 5. Djebali, S. *et al.* Landscape of transcription in human cells. *Nature* **489**, 101–108
672 (2012).
- 673 6. Liu, S. J. *et al.* CRISPRi-based genome-scale identification of functional long
674 noncoding RNA loci in human cells. *Science* **355**, aah7111 (2017).
- 675 7. Sauvageau, M. *et al.* Multiple knockout mouse models reveal lincRNAs are
676 required for life and brain development. *eLife* **2**, e01749 (2013).
- 677 8. Xu, J. & Cao, X. Long noncoding RNAs in the metabolic control of inflammation
678 and immune disorders. *Cellular and Molecular Immunology* **16**, 1–5 (2019).
- 679 9. Tsai, M.-C. *et al.* Long noncoding RNA as modular scaffold of histone
680 modification complexes. *Science* **329**, 689–693 (2010).
- 681 10. West, J. A. *et al.* The Long Noncoding RNAs NEAT1 and MALAT1 Bind Active
682 Chromatin Sites. *Molecular Cell* **55**, 791–802 (2014).
- 683 11. Lai, F. *et al.* Activating RNAs associate with Mediator to enhance chromatin
684 architecture and transcription. *Nature* **494**, 497–501 (2013).
- 685 12. Wang, K. C. *et al.* A long noncoding RNA maintains active chromatin to
686 coordinate homeotic gene expression. *Nature* **472**, 120–U158 (2011).
- 687 13. Zhao, J. *et al.* Genome-wide identification of polycomb-associated RNAs by RIP-
688 seq. *Molecular Cell* **40**, 939–953 (2010).
- 689 14. Darnell, R. B. HITS-CLIP: panoramic views of protein-RNA regulation in living
690 cells. *Wiley Interdiscip Rev RNA* **1**, 266–286 (2010).
- 691 15. G Hendrickson, D., Kelley, D. R., Tenen, D., Bernstein, B. & Rinn, J. L.
692 Widespread RNA binding by chromatin-associated proteins. *Genome Biology* **17**,
693 674 (2016).
- 694 16. Mondal, T., Rasmussen, M., Pandey, G. K., Isaksson, A. & Kanduri, C.
695 Characterization of the RNA content of chromatin. *Genome Research* **20**, 899–907
696 (2010).
- 697 17. Bhatt, D. M. *et al.* Transcript Dynamics of Proinflammatory Genes Revealed by
698 Sequence Analysis of Subcellular RNA Fractions. *Cell* **150**, 279–290 (2012).
- 699 18. Werner, M. S. & Ruthenburg, A. J. Nuclear Fractionation Reveals Thousands of
700 Chromatin-Tethered Noncoding RNAs Adjacent to Active Genes. *Cell Report* **12**,
701 1089–1098 (2015).
- 702 19. Li, X. *et al.* GRID-seq reveals the global RNA-chromatin interactome. *Nature*
703 *Biotechnology* **35**, 940–950 (2017).
- 704 20. Sridhar, B. *et al.* Systematic Mapping of RNA-Chromatin Interactions In Vivo.
705 *Current Biology* **27**, 602–609 (2017).
- 706 21. Quinodoz, S. A. *et al.* Higher-Order Inter-chromosomal Hubs Shape 3D Genome
707 Organization in the Nucleus. *Cell* **174**, 744–757.e24 (2018).
- 708 22. Margueron, R. & Reinberg, D. The Polycomb complex PRC2 and its mark in life.
709 *Nature* **469**, 343–349 (2011).
- 710 23. Soibam, B. Super-lncRNAs: identification of lncRNAs that target super-enhancers
711 via RNA:DNA:DNA triplex formation. *RNA* **23**, 1729–1742 (2017).
- 712 24. Chu, C., Qu, K., Zhong, F. L., Artandi, S. E. & Chang, H. Y. Genomic maps of
713 long noncoding RNA occupancy reveal principles of RNA-chromatin interactions.
714 *Molecular Cell* **44**, 667–678 (2011).

- 715 25. Shah, R. N. *et al.* Examining the Roles of H3K4 Methylation States with
716 Systematically Characterized Antibodies. *Molecular Cell* **72**, 162–177.e7 (2018).
- 717 26. Simon, M. D. *et al.* High-resolution Xist binding maps reveal two-step spreading
718 during X-chromosome inactivation. *Nature* **504**, 465–469 (2013).
- 719 27. Pandey, R. R. *et al.* Kcnq1ot1 Antisense Noncoding RNA Mediates Lineage-
720 Specific Transcriptional Silencing through Chromatin-Level Regulation.
721 *Molecular Cell* **32**, 232–246 (2008).
- 722 28. Monnier, P. *et al.* H19 lncRNA controls gene expression of the Imprinted Gene
723 Network by recruiting MBD1. *Proceedings of the National Academy of Sciences*
724 **110**, 20693–20698 (2013).
- 725 29. Royce-Tolland, M. E. *et al.* The A-repeat links ASF/SF2-dependent Xist RNA
726 processing with random choice during X inactivation. *Nature Structural &*
727 *Molecular Biology* **17**, 948–U55 (2010).
- 728 30. Chu, C. *et al.* Systematic Discovery of Xist RNA Binding Proteins. *Cell* **161**, 404–
729 416 (2015).
- 730 31. Lu, Z. *et al.* RNA Duplex Map in Living Cells Reveals Higher-Order
731 Transcriptome Structure. *Cell* **165**, 1267–1279 (2016).
- 732 32. Ang, C. E. *et al.* The novel lncRNA lnc-NR2F1 is pro-neurogenic and mutated in
733 human neurodevelopmental disorders. *eLife* **8**, 377 (2019).
- 734 33. McLean, C. Y. *et al.* GREAT improves functional interpretation of cis-regulatory
735 regions. *Nature Biotechnology* **28**, 495–501 (2010).
- 736 34. Simon, M. D. *et al.* The genomic binding sites of a noncoding RNA. *Proceedings*
737 *of the National Academy of Sciences* **108**, 20497–20502 (2011).
- 738 35. Engreitz, J. M. *et al.* The Xist lncRNA Exploits Three-Dimensional Genome
739 Architecture to Spread Across the X Chromosome. *Science* **341**, 767–+ (2013).
- 740 36. Ritchie, M. E. *et al.* limma powers differential expression analyses for RNA-
741 sequencing and microarray studies. *Nucleic Acids Research* **43**, e47–e47 (2015).
- 742 37. Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach
743 for interpreting genome-wide expression profiles. *Proceedings of the National*
744 *Academy of Sciences* **102**, 15545–15550 (2005).
- 745 38. Yu, F. *et al.* LnChrom: a resource of experimentally validated lncRNA-chromatin
746 interactions in human and mouse. *Database* **2018**, 354 (2018).
- 747 39. Zviran, A. *et al.* Deterministic Somatic Cell Reprogramming Involves Continuous
748 Transcriptional Changes Governed by Myc and Epigenetic-Driven Modules. *Cell*
749 *Stem Cell* **24**, 328–341.e9 (2019).
- 750 40. Rando, O. J. & Chang, H. Y. Genome-Wide Views of Chromatin Structure.
751 *Annual Review of Biochemistry* **78**, 245–271 (2009).
- 752 41. Flynn, R. A. *et al.* 7SK-BAF axis controls pervasive transcription at enhancers.
753 *Nature Structural & Molecular Biology* **23**, 231–238 (2016).
- 754 42. Wang, Z. *et al.* Combinatorial patterns of histone acetylations and methylations in
755 the human genome. *Nature Genetics* **40**, 897–903 (2008).
- 756 43. Ernst, J. *et al.* Mapping and analysis of chromatin state dynamics in nine human
757 cell types. *Nature* **473**, 43–49 (2011).
- 758 44. Ernst, J. & Kellis, M. ChromHMM: automating chromatin-state discovery and
759 characterization. *Nature Methods* **9**, 215–216 (2012).

- 760 45. Rousseeuw, P. J. Silhouettes: A graphical aid to the interpretation and validation of
761 cluster analysis. *Journal of Computational and Applied Mathematics* **20**, 53–65
762 (1987).
- 763 46. Hirata, H. *et al.* Long Noncoding RNA MALAT1 Promotes Aggressive Renal Cell
764 Carcinoma through Ezh2 and Interacts with miR-205. *Cancer Research* **75**, 1322–
765 1331 (2015).
- 766 47. Jia, J. *et al.* Long noncoding RNA DANCR promotes invasion of prostate cancer
767 through epigenetically silencing expression of TIMP2/3. *Oncotarget* **7**, 37868–
768 37881 (2016).
- 769 48. Quek, X. C. *et al.* lncRNADB v2.0: expanding the reference database for functional
770 long noncoding RNAs. *Nucleic Acids Research* **43**, D168–73 (2015).
- 771 49. Wang, X. *et al.* Molecular analysis of PRC2 recruitment to DNA in chromatin and
772 its inhibition by RNA. *Nature Structural & Molecular Biology* **24**, 1028–1038
773 (2017).
- 774 50. Beltran, M. *et al.* The interaction of PRC2 with RNA or chromatin is mutually
775 antagonistic. *Genome Research* **26**, 896–907 (2016).
- 776 51. Meshorer, E. *et al.* Hyperdynamic plasticity of chromatin proteins in pluripotent
777 embryonic stem cells. *Developmental Cell* **10**, 105–116 (2006).
- 778 52. Bernstein, B. E. *et al.* A Bivalent Chromatin Structure Marks Key Developmental
779 Genes in Embryonic Stem Cells. *Cell* **125**, 315–326 (2006).
- 780 53. Wapinski, O. L. *et al.* Hierarchical Mechanisms for Direct Reprogramming of
781 Fibroblasts to Neurons. *Cell* **155**, 621–635 (2013).
- 782 54. Kertesz, M. *et al.* Genome-wide measurement of RNA secondary structure in
783 yeast. *Nature* **467**, 103–107 (2010).
- 784 55. Spitale, R. C. *et al.* Structural imprints in vivo decode RNA regulatory
785 mechanisms. *Nature* **519**, 486–490 (2015).
- 786 56. Hofacker, I. L. Vienna RNA secondary structure server. *Nucleic Acids Research*
787 **31**, 3429–3431 (2003).
- 788 57. Wan, Y. *et al.* Landscape and variation of RNA secondary structure across the
789 human transcriptome. *Nature* **505**, 706–709 (2014).
- 790 58. Sun, D. *et al.* LncRNA GAS5 inhibits microglial M2 polarization and exacerbates
791 demyelination. *EMBO reports* **18**, 1801–1816 (2017).
- 792 59. Sun, L. *et al.* RNA structure maps across mammalian cellular compartments.
793 *Nature Structural & Molecular Biology* **26**, 322–330 (2019).
- 794 60. Roost, C. *et al.* Structure and Thermodynamics of N⁶-Methyladenosine in RNA:
795 A Spring-Loaded Base Modification. *Journal of the American Chemical Society*
796 **137**, 2107–2115 (2015).
- 797 61. Liu, N. & Pan, T. N⁶-methyladenosine–encoded epitranscriptomics. *Nature*
798 *Structural & Molecular Biology* **23**, 98–102 (2016).
- 799 62. Batista, P. J. *et al.* m⁶A RNA Modification Controls Cell Fate Transition in
800 Mammalian Embryonic Stem Cells. *Cell Stem Cell* **15**, 707–719 (2014).
- 801
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805

806 **FIGURE LEGENDS**

807

808 **Figure 1. PIRCh-seq enables effective chromatin-RNA association *in vivo***

809 **A.** Schematic representation of PIRCh approach followed by high-throughput sequencing.

810 **B.** Normalized input and histone H3 PIRCh-seq profiles of lncRNA *XIST* in human
811 female fibroblasts.

812 **C.** PIRCh-qPCR analysis in mouse neuronal stem cells (NSCs, orange) and adult brain
813 (purple) shows that *Xist* is attached to chromatin H3 compared with Actin control.

814 **D-E.** Normalized input and PIRCh-seq profiles with histone modifications of H3K4me3,
815 H3K27ac, and H3K27me3 at the lncRNA *Xist* (**D**) and *lnc-Nr2f1* (**E**) locus in mouse
816 neuronal precursor cells (NPC).

817 **F.** Normalized input and ChIRP-seq profiles of lncRNA *lnc-Nr2f1* in NPC, and
818 H3K4me3, H3K27ac, H3K4me1 and H3K27me3 ChIP-seq profiles in NPC. Showing
819 *Cln4* gene locus as an example.

820 **G.** Average coverage of ChIP-seq signal (Reads per million over Input) around (+/-10kb)
821 *lnc-Nr2f1* ChIRP-seq peaks in NPC.

822

823 **Figure 2. ncRNAs are enriched on chromatin compare with protein coding**
824 **transcripts**

825 **A.** Ratio of intronic over exonic reads obtained from different chromatin-RNA
826 association sequencing technologies (GRID, CPE, CAR, and PIRCh) versus input
827 controls in multiple cell lines.

828 **B.** Normalized average read coverage around introns from different chromatin-RNA
829 association sequencing technologies (GRID, CPE, CAR, and PIRCh) versus input
830 controls in multiple cell lines.

831 **C.** Normalized average read coverage around introns from histone modification specific
832 PIRCh-seq profiles (colored) and inputs (black) in mouse embryonic stem cells (mESCs).

833 **D.** Gene set enrichment analysis (GSEA) shows highly statistical enriched (FDR=0,
834 P<0.0001) of non-coding genes (Green) and depleted of coding genes (Blue) on histone
835 H3 in mESCs. Genes were ranked by their histone H3 PIRCh enrichment scores.

836 **E.** Average fold enrichment (calculated by limma in R) of the coding gene, lncRNA, pre-
837 miRNA, snoRNA and other ncRNA from histone modification specific PIRCh-seq
838 profiles (namely H3, H3K4me1, H3K4me3, H3K27ac, H3K27me3, H3K9me3, and
839 H4K16ac) in mESC. Error bar shows the standard deviation from the mean.

840 **F.** Average variation score of the PIRCh-seq signals for the coding versus non-coding
841 genes (****P<0.0001, two-tailed Welch's T-test). Error bar shows the standard deviation
842 from the mean.

843 **G.** Heatmap displaying the ranking of the ChIP-seq enrichment of the chromatin binding
844 sites of 23 lncRNA. The 23 lncRNAs are chromatin enriched from PIRCh-seq and the
845 chromatin binding sites are obtained from ChIRP/CHART/RAP/GRID-seq profiles from
846 the LnChrom database. Colors represent ranking from 1-5.

847 **H.** Heatmap shows the ranking of PIRCh-seq enrichment of the same lncRNAs in **G**.
848 Colors represent ranking from 1-5.

849 **I.** Bar plot of the Spearman correlation coefficients between the ranking in **G** and **H** for
850 each lncRNA versus random permutation (****P<0.0001, two-tailed Welch's T-test).

851 **J.** Unsupervised clustering of the Pearson correlation coefficients matrix of the histone
852 modification specific PIRCh-seq profiles based on the enrichment scores from the 258
853 chromatin associated ncRNAs in mESC.

854

855 **Figure 3. PIRCh-seq classifies functional ncRNAs via chromatin state association** □

856 **A.** Summary of histone modifications representing distinct regulatory patterns.

857 **B.** The enrichment of the *7sk* ChIRP-seq peaks overlap with different histone
858 modification ChIP-seq peaks in the same cell line (mESC). A positive value indicates the
859 ChIRP-seq peaks are highly enriched with ChIP-seq peaks compare to random, and a
860 negative value indicates depletion.

861 **C.** The PIRCh enrichment score of the lncRNA *7sk* in mESC from distinct histone
862 modification specific PIRCh-seq experiments. A positive value means enriched, and a
863 negative value means depleted.

864 **D.** Classification of the PIRCh-seq identified chromatin associated ncRNAs (n=258) in
865 mESC. Scatter plot shows the t-SNE result on PIRCh-seq enrichment score matrix and
866 annotated by K-means clustering.

867 **E.** Functional classification of histone specific chromatin-RNA association patterns
868 defined by chromHMM algorithm.

869 **F.** Box plot of the expression of the coding genes nearby (+/-100Kb) each group of
870 PIRCh clustered ncRNAs defined in **D**. Center lines represent mean values; box limits
871 represent the interquartile range; whiskers each extend 1.5 times the interquartile range;
872 dots represent outliers. The expression of the coding genes that close to the ncRNAs in
873 the “repressed” group is significantly lower than those in the “active promoter/enhancer”
874 group (P<0.05, two-tailed Welch's T-test). Genes close to un-enriched ncRNAs are
875 shown as control.

876

877 **Figure 4. Cell type specific chromatin association of ncRNAs**

878 **A.** Number of chromatin enriched RNAs in mESC, MEF and NPC.

879 **B.** Unsupervised clustering of the Pearson correlation coefficients matrix of the histone
880 modification specific PIRCh-seq profiles in mESC, MEF and NPC, based on the
881 enrichment scores from the 458 chromatin associated ncRNAs in each cell type.

882 **C.** Ratio of the chromatin enriched ncRNA under each chemical modification over the
883 total number of enriched ncRNAs in mESC, MEF, and NPC.

884 **D.** Schematic illustration of how RNAs enriched on both the repressive and active
885 chromatin (bi-chromatin enriched) and either the repressive or active chromatin (mono-
886 chromatin enriched).

887 **E.** UCSC tracks of the normalized PIRCh-seq signal at the lncRNA *uc008bcq.1* locus in
888 mESC, MEF and NPC. *uc008bcq.1* is bi-chromatin enriched in mESC, but mono-
889 chromatin enriched in MEF and NPC.

890 **F.** Number of ncRNAs that are bi-chromatin enriched or mono-chromatin enriched in
891 mESC, MEF and NPC (***P<0.001, *P<0.05, Chi-square test).

892

893 **Figure 5. RNA with single strand are more likely to associate with chromatin.** □

894 **A.** UCSC track of the normalized input (black) and H3 (red) and H3K4me3 (green)
895 PIRCh-seq signals of lncRNA *Malat1* in mESC. Bottom peaks are chromatin enriched
896 sites on *Malat1*.

897 **B.** Structure profile from icSHAPE and structural prediction from RNAfold around a
898 zoom in chromatin associated peak on lncRNA *Malat1*.

899 **C.** Computational workflow to integrate RNA secondary structure information from
900 icSHAPE and chromatin enrichment information from PIRCh-seq to study the structural
901 preference of chromatin-RNA association.

902 **D.** Average diagram of icSHAPE scores around all PIRCh-seq peaks under different
903 histone modifications (colored solid line) versus a randomly selected background (grey
904 solid line). P-values (colored dash line) were estimated by using two-tailed Welch's T-
905 test on every position between PIRCh-seq profiles over background.

906 **E.** Box-plot of the icSHAPE score of PIRCh-seq enriched vs depleted RNAs
907 (**P<0.001, two-tailed Welch's T-test). Center lines represent mean values; box limits
908 represent the interquartile range; whiskers each extend 1.5 times the interquartile range;
909 dots represent outliers.

910 **F.** Box-plot of the PIRCh-seq enrichment scores of the top 100 most single stranded
911 RNAs versus the top 100 most double stranded RNAs based on icSHAPE scores
912 (**P<0.01 two-tailed Welch's T-test). Center lines represent mean values; box limits
913 represent the interquartile range; whiskers each extend 1.5 times the interquartile range;
914 dots represent outliers.

915 **G.** Relative allele specific RNA expression and chromatin enrichment of lncRNAs *Xist*,
916 *Gas5* and *Malat1* in the 129S1 allele versus the CAST allele of NPC. The 129S1 version
917 of lncRNA *Xist* is highly expressed and also enriched at chromatin with H3K27me3
918 modification. Both alleles of lncRNA *Malat1* were almost equally expressed and
919 enriched. The 129S1 version of *Gas5* was lowly expressed but highly enriched on
920 chromatin compared to the CAST version of the same gene.

921 **H.** Normalized allele specific input and histone H3K27me3 PIRCh-seq signals in the
922 129S1 and CAST alleles. Top shows single nucleotide polymorphisms (SNP) positions
923 that distinguish the alleles.

924

925 **Supplementary Figure 1. Quality control of histone modification specific PIRCh-seq**
926 **experiments on distinct cell types.**

927 **A-C.** The specificity of IP of different antibodies H3K4me1 (A), H3K4me3 (B), and
928 H3K27me3 (C) after glutaraldehyde crosslinking using modified mononucleosomes with
929 barcodes. 7 different mononucleosomes with barcodes were tested.

930 **D.** Table summarizing PIRCh-seq experiments performed in this paper.

931 **E.** Kernel density estimation (KDE) plot of the gene expression from different subcellular
932 RNA sequencing data and PIRCh-seq data.

933 **F-M.** Scatter plots of expressed transcripts (log2) in two PIRCh-seq replicates with
934 correlation score R on different histone modification, H3, H3K4me1, H3K4me3,
935 H3K27ac, H3K27me3, H3K9me3, and H4K16ac respectively.

936

937 **Supplementary Figure 2. PIRCh-seq effectively and finely enriches RNA associated**
938 **with chromatin**

939 **A-B.** Normalized UCSC tracks of input and histone H3 PIRCh-seq signals on lncRNA
940 *KCNQ1OT1*(**A**) in human female fibroblast cells and *H19* (**B**) in human H9 embryonic
941 stem cells.

942 **C-D.** Normalized UCSC tracks of input and histone H3 PIRCh-seq signals on protein
943 coding genes *ACTB* (**C**), and *EEF2* (**D**) in human female fibroblast cells.

944 **E-F.** Normalized UCSC tracks of input and histone modification specific PIRCh-seq
945 signals on protein coding gene *Actb* (**E**) and *Eef2* (**F**) in mouse neuronal precursor cells.

946 **G.** Top 5 enriched gene ontology of the *lnc-Nr2f1* ChIRP-seq peaks using GREAT.

947

948 **Supplementary Figure 3. PIRCh-seq captures low nascent transcription**

949 **A-E.** Scatter plot of the PIRCh-seq (y-axis) signal over input vs the corresponding ChIP-
950 seq (x-axis) signal over input for all the expressed genes in mESC, with linear regression
951 (red dotted line). Colors represent the density of point.

952

953 **Supplementary Figure 4. ncRNAs are more enriched on chromatin than protein**
954 **coding genes.**

955 **A-B.** Box-plots of the PIRCh-seq signal before (**A**) and after (**B**) normalization using the
956 limma algorithm in R. cpm represents count per million, and log scale is shown. Center
957 lines represent mean values; box limits represent the interquartile range; whiskers each
958 extend 1.5 times the interquartile range; dots represent outliers.

959 **C.** Circle plots showing the distribution of the expressed (inner circle) and PIRCh
960 enriched (outer circle) RNA types associated with different histone modifications.
961 ncRNAs are highly enriched in PIRCh compared with coding genes.

962 **D.** Total length of the genomic regions (in bp) covered by each histone modification
963 ChIP-seq peak in mESC.

964

965 **Supplementary Figure 5. The chromatin-RNA association of ncRNAs give a hint of**
966 **cis regulation.**

967 **A.** Bar chart showing the number of ncRNAs enriched at chromatin with specific histone
968 modifications in mESC.

969 **B.** The odds ratio of the PIRCh enriched ncRNAs overlap with the chromatin enriched
970 ncRNAs defined in lncRNADB. “Yes” means the ncRNA is both PIRCh enriched and
971 found in lncRNADB, and “No” means PIRCh enriched but was not identified in
972 lncRNADB.

973 **C.** Box-plot of the expression of the coding genes near (+/-100Kb) each group of histone
974 modification specific PIRCh-seq enriched ncRNAs. Center lines represent mean values;
975 box limits represent the interquartile range; whiskers each extend 1.5 times the

976 interquartile range; dots represent outliers. The expression of the coding genes that close
977 to the ncRNAs enriched on active chromatin shows no significant difference between that
978 with repressed chromatin.

979 **D.** Box-plot of the expression of each groups of PIRCh cluttered ncRNAs defined in
980 **Figure 3D**. Center lines represent mean values; box limits represent the interquartile
981 range; whiskers each extend 1.5 times the interquartile range; dots represent outliers.

982

983 **Supplementary Figure 6. Pattern of ncRNA chromatin association is generally**
984 **conserved in distinct cell types.**

985 **A.** Normalized average read coverage around introns from histone modification specific
986 PIRCh-seq profiles (colored) and inputs (black) in MEF.

987 **B.** Gene set enrichment analysis (GSEA) shows highly statistical enriched (FDR=0,
988 $P < 0.0001$) of non-coding genes (Green) and depleted of coding genes (Blue) on histone
989 H3 in MEF. Genes were ranked by their histone H3 PIRCh enrichment scores.

990 **C.** Average fold enrichment (calculated by limma in R) of the coding gene, lncRNA, pre-
991 miRNA, snoRNA and other ncRNA from histone modification specific PIRCh-seq
992 profiles (namely H3, H3K4me1, H3K4me3, H3K27ac, H3K27me3, H3K9me3, and
993 H4K16ac) in MEF. Error bar shows the standard deviation from the mean.

994 **D.** Functional classification of histone specific chromatin-RNA association patterns
995 defined by chromHMM algorithm.

996 **E.** Classification of the PIRCh-seq identified chromatin associated ncRNAs (n=200) in
997 MEF. Scatter plot shows the t-SNE result on PIRCh-seq enrichment score matrix and
998 annotated by K-means clustering.

999 **F.** Normalized input, Igg and H3 PIRCh-seq profiles of lncRNA *Pvt1* in MEF.

1000

1001 **Supplementary Figure 7. Allele specific RNA secondary structure and chromatin**
1002 **enrichment of lncRNA *Gas5*.**

1003 **A-B.** RNAfold predicted the secondary structure of the 129S1 (**A**) and CAST (**B**) allele
1004 of *Gas5*. RiboSNithes are noted in blue and bases attached to chromatin (peaks) are
1005 shown in red.

1006 **C.** Structural information of 129S1 allele around the PIRCh enriched peak region. Data
1007 obtained from icSHAPE experiments on mESC.

1008

1009 **Supplementary Figure 8. RNA m⁶A methylation affects chromatin-RNA association.**

1010 **A.** Distribution of histone H3 and other chemical modification PIRCh-seq peaks along
1011 scaled transcripts.

1012 **B-C.** Average diagrams of m⁶A modification scores around bases attached to chromatin
1013 (peaks) from histone H3 (**B**) and all chemical modification specific (**C**) PIRCh-seq
1014 profiles, versus that from randomly selected background.

1015

1016 **Supplementary Table 1 Chromatin enriched ncRNAs in all mouse samples.**

1017 **Supplementary Table 2 Allele specific chromatin enrichment in NPC.**

1018

1019 **METHODS**

1020

1021 **Cell culture**

1022 V6.5 mouse ES cells were cultured on 0.2% gelatin-coated plates at 37°C with mES
1023 media: 500ml Knockout DMEM (Gibco), 90ml FBS, 6ml non-essential amino acid
1024 (NEAA, 100x, Gibco), 6ml glutamine or glutamax (200mM stock solution), 6ml
1025 Pen/Strep, 1ml BME and 60µlLIF (Millipore, ESG1106). Mouse embryonic fibroblast
1026 (MEF) cells were cultured at 37 °C and 5% CO₂ in: 450ml DMEM, 50ml FBS, 5ml
1027 Pen/Strep, 5ml NEAA, 5ml pyruvate, 4ul beta-Mercaptoethanol. Mouse Neural Precursor
1028 cells (NPCs) were cultured in N2B27 medium (DMEM/F12 (Invitrogen, 11320-033),
1029 Neurobasal (Gibco, 21103-049), NDiff Neuro-2 Medium Supplement (Millipore,
1030 SCM012), B27 Supplement (Gibco, 17504-044)) supplemented with EGF and FGF (10
1031 ng/ml, each) (315-09 and 100-18B, Peprotech). Cells were passaged using Accutase
1032 (SCR005, Millipore) and cultured on 0.2% gelatin-coated plates. H9 human embryonic
1033 stem cells were seeded in a feeder-free system using Matrigel hESC-Qualified Matrix
1034 (354277, Corning) and were maintained in Essential 8 media (A1517001, Thermo Fisher
1035 Scientific) as described previously¹. Cells were passaged every three days as clumps with
1036 0.5mM EDTA¹. Human Female Fibroblasts (HFF) were cultured at 37 °C and 5% CO₂ in
1037 DMEM supplemented with 1% pen/strep and 10% FBS.

1038 **PIRCh-seq library preparation**

1039 To harvest the cells for PIRCh-seq, approximately 4x10⁷ cells were trypsinized and
1040 pooled into a 50ml falcon tube, after washing with 40ml of cold PBS once. Fresh 1%
1041 glutaraldehyde in room temperature PBS was created from 25% stock and remaining
1042 stock was discarded. The cell pellet was resuspended in 1ml of glutaraldehyde solution
1043 and a p1000 pipette was used to resuspend cells, and to top up to 40ml (1ml 1%
1044 glutaraldehyde / 1 million cells). After inverting several times, the tube was gently
1045 shaken for 10 minutes, and then quenched with 1/10 volume of 1.25M glycine. The tube
1046 was inverted several times, shaken gently for 5 min, and spun down 2000g for 4 min. The
1047 pellet was then washed once with 40ml cold PBS. The pellet was resuspended in
1048 1ml/20million cells of cold PBS. Cells were aliquoted at 1ml each to a fresh eppendorf
1049 tube, and spun down 2000g for 4 min. After, supernatant was carefully aspirated, cell
1050 pellets were flash frozen, and stored at -80°C if necessary.

1051

1052 For sonication, prepared cell pellets were spun down at 2000g for 4 min and any
1053 remaining PBS was removed. Lysis per 20 million cells was performed with 1ml of lysis
1054 buffer (1% SDS, 50mM Tris 7.0, 10mM EDTA, 1mM PMSF, 0.1U/ul Superase-in
1055 (Ambion), 1x Proteinase inhibitor (Roche)). Lysate was then sonicated till the chromatin
1056 size was ~300-2000bp and the lysate was clear. The lysates were spun down at 16000g
1057 for 10 min. Supernatants were flash frozen and stored at -80°C if necessary.

1058

1059 For PIRCh-seq library construction, chromatin was thawed and 10ul was taken as input.
1060 200ul were aliquoted per reaction, and 400ul dilution buffer was added to each reaction.
1061 H3 or a specific histone modification antibody was then added (Dilution buffer: 0.01%
1062 SDS, 1.1% Triton X 100, 1.2 mM EDTA, 16.7 mM Tris 7.0, 167 mM NaCl, 1mM PMSF,
1063 0.1U/ul Superase-in (Ambion), 1x Proteinase inhibitor (Roche)). The reaction was shaken
1064 end-to-end at 4°C overnight. 50ul Protein A dynabeads was used per 5ug antibody IP.
1065 Beads were washed with 5 times the original volume of dilution buffer 4 times. Notice
1066 that it is important not to exceed 200ul original volume of beads per tube. During the last
1067 wash, beads were aliquoted to 1 tube per reaction. The buffer was aspirated and, 200ul of
1068 the IP sample was used to resuspend and transfer beads to the IP sample. The reaction
1069 was shaken end-to-end at room temperature for 2 hours. The beads were then washed
1070 with 1ml wash buffer 4 times, and resuspended in 50ul IP elution buffer (1% SDS, 50mM
1071 NaHCO₃). The reaction was then vortexed at setting 1 for 15 min. The supernatant was
1072 then transferred to a fresh tube and the bead elution was repeated. The supernatant was
1073 combined for a total of 100ul. 5ul 3M NaOAc was immediately added to neutralize pH.
1074 10ul TurboDnase buffer and 1ul TurboDnase (Ambion) were added and the reaction was
1075 incubated 37°C for 30min. 3ul 500mM EDTA was added to eliminate divalent ions. 5ul
1076 Proteinase K (Ambion) was added, and the reaction was incubated at 50°C for 45 min.

1077

1078 To make our sequencing libraries, we extracted RNA using Trizol/chloroform, and
1079 precipitated the RNA with an equal volume of isopropanol. RNA pellet was washed in
1080 1ml 70% EtOH, and pellets were resuspended in 10ul H₂O. 1ul TurboDnase buffer was
1081 added, followed by 1ul TurboDnase, and the reaction at 37°C for 30min. 1.2ul of
1082 TurboDnase inactivating reagents were added. The reaction was vortexed for 3 minutes
1083 and spun down. The 10ul supernatant was heated at 75°C for 10 minutes to kill DNase.
1084 The reaction was purified using a Nugen Ovation v2 kit and eluted in 5uL for library
1085 preparation.

1086

1087 **ChIRP-seq library preparation**

1088 To determine the genome-wide localization of *lnc-Nr2f11* we followed protocols
1089 previously described². ChIRP was performed using biotinylated probes designed against
1090 mouse *lnc-Nr2f1* using the ChIRP probes designer (Biosearch Technologies).
1091 Independent even and odd probe pools were used to ensure lncRNA-specific retrieval as
1092 protocols previously described³. “Even” and “odd” sets of probes shared no overlapping
1093 sequences, as we performed two independent ChIRP-seq experiments with these two sets
1094 of probes separately. Two sets of data were then combined for downstream analysis (see
1095 below). Mouse NPC samples are crosslinked in 3% formaldehyde. RNase pre-treated
1096 samples are served as negative controls for probe-DNA hybridization. ChIRP libraries are
1097 constructed using the NEBNext DNA library preparation kit (New England Biolabs).

1098 Sequencing libraries were barcoded using TruSeq adapters and sequenced on HiSeq or
1099 NextSeq instruments (Illumina).

1100

1101 **Experimental validation of antibody specificity after glutaraldehyde crosslinking**
1102 **using modified mononucleosomes with barcodes**

1103 To ensure that chemical crosslinking with glutaraldehyde did not affect antibody
1104 specificity, we followed previous study to test antibody specificity using SNAP-ChIP⁴.
1105 During IP pulldown, 15 uL of recombinant nucleosomes (SNAP-ChIP, EpiCypher, 19-
1106 1001) were fixed with fresh 1% glutaraldehyde. 1% glutaraldehyde was prepared on the
1107 same day in room temperature PBS from 25% stock. Fixation was performed for 10
1108 minutes at room temperature with gentle shaking. The reaction was then quenched with
1109 1/10 of the original reaction volume of 2.5 M glycine. Tubes were then inverted several
1110 times and incubated for 5 minutes at room temperature with gentle shaking.

1111

1112 500 uL of fixed chromatin were then added to each tube and pipetted up and down
1113 several times to mix well. 10 uL of nucleosomes mixed with chromatin were taken out of
1114 each tube to be used as input during the qPCR. One tablet of Roche cOmplete protease
1115 inhibitor was dissolved (Roche, 11697498001) in 50 mL of DI water to obtain a working
1116 solution of 50x protease inhibitor cocktail. 60 uL of 50x protease inhibitor was added to
1117 3mL of blank dilution buffer (0.01% SDS, 1.1% Triton X100, 1.2 mM EDTA, 16.7 mM
1118 Tris pH 7.0, 167 mM NaCl). 1 mL of dilution buffer with protease inhibitor was then
1119 added to each reaction. 5 ug of appropriate detection antibody for IP pulldown was added
1120 to 300 uL of chromatin mixed with crosslinked nucleosomes for each condition. Samples
1121 were then incubated at 4°C overnight with end-to-end shaking.

1122

1123 IP product was eluted as specified during PIRCH library construction. DNA of
1124 interest was purified using a Zymo DNA Clean and Concentrator-5 kit (Zymo
1125 Research, D4013). The qPCR reaction was performed using Roche's LightCycler and
1126 Brilliant II SYBR® Green QRT-PCR Master Mix (Agilent). We analyzed enrichment for
1127 target histone modifications by amplifying unique DNA barcodes at the 3' end, using
1128 primer sequences provided by EpiCypher.

1129

1130 **RT-qPCR**

1131 For qRT-PCR analysis, we used Roche's LightCycler and Brilliant II SYBR® Green
1132 QRT-PCR Master Mix (Agilent).

1133

1134 **PIRCh-seq data alignment**

1135 Raw reads were uniquely mapped to mm9/hg19 using Tophat with default parameters⁵.
1136 Samtools and BedTools were used to transform the mapped bam file into bedGraph and
1137 bigwig files for visualization on the UCSC genome browser^{6,7}. RPKM and raw reads
1138 count for each gene were calculated by self-designed scripts with ensemble annotation,

1139 Homo_sapiens.GRCh37.75.gtf for human and Mus_musculus.NCBIM37.67.gtf, and a
1140 number of previous publications for mouse samples respectively⁸.

1141

1142 **Calculate exon/intron ratio to estimate nascent transcripts**

1143 To compare the exon/intron ratios between the PIRCh-seq profiles and other chromatin
1144 associated RNA detection technologies, we aligned raw reads to the same hg19 genome
1145 index with Tophat and calculated the reads mapped to intron/exon with ensemble
1146 annotation gtf file as described above⁵. For the average read counts around introns, three
1147 steps were taken: (1) scaled every intron based on its length, and extended 1 intron length
1148 up and down stream of the selected intron; (2) divided the entire region to 300 windows,
1149 and calculate the average number of read counts mapped in each window and then take
1150 log₂ to scale down the values to avoid interferences from the outliers; (3) take average
1151 for all the windows among all introns. To estimate the correlation between the histone
1152 modification-specific PIRCh-seq profile with its corresponding ChIP-seq signals, we
1153 obtained ChIP-seq profiles of each histone modification in mESC from ENCODE. And
1154 then, for each expressed gene in mESC, the histone modification ChIP-seq signal over
1155 input on the gene exon were calculated as the ChIP signal for that gene, and were
1156 compared with the corresponding PRCh-seq enrichment score with the same histone
1157 modification, and our results indicated that there was no significant correlation with these
1158 two sets of signals.

1159

1160 **Gene set enrichment analysis (GSEA)**

1161 GSEA software was downloaded from (<http://software.broadinstitute.org/gsea/index.jsp>)
1162 at the Broad Institute website and was utilized to perform the significant differential
1163 chromatin enrichment from PIRCh-seq against ncRNA versus coding genes⁹. The
1164 ncRNA set was consist of the annotated snoRNA, snRNA, rRNA, lncRNA, miRNA and
1165 miscRNA.

1166

1167 **Data normalization and identification of the chromatin enriched RNAs**

1168 The chromatin enriched ncRNAs were identified through the limma algorithm in R¹⁰. First,
1169 a data matrix was obtained, where each raw read was a gene and each column a sample,
1170 and the element of the matrix represented the number of raw reads from PIRCh-seq
1171 experiments and inputs. The values in this matrix were then normalized by the limma-
1172 voom method in R. After that, differential analysis was performed using the limma gene-
1173 wise linear model for each pair of PIRCh replicates over inputs. Non-coding RNAs with
1174 P-value<0.05 and log₂ fold change over inputs>0 were defined as chromatin enriched.
1175 We obtained 258 chromatin enriched ncRNAs in mouse V6.5 cell line, 200 in MEF and
1176 110 in NPC. Variation score of each gene was defined as the standard deviations of the
1177 fold change among all histone modification specific PIRCh-seq profiles. The Pearson

1178 correlation coefficients between each two PIRCh-seq experiments were calculated and
1179 unsupervised clustering of the correlation matrix was performed in Cluster.

1180

1181 **Computational validation of the PIRCh-seq enriched ncRNAs.**

1182 In order to validate the PIRCh-enriched candidates by similar methods, we examined 96
1183 published chromatin-association datasets from ChIRP/CHART/RAP/GRID-seq
1184 experiments collected by the LnChrom database¹¹. We found a total of 23 expressed
1185 lncRNAs in the LnChrom database, including *Xist*, *Firre*, *Rmrp*, *Tug1* and etc., and all of
1186 them were positively enriched in our PIRCh experiment and 14 of which were significant
1187 with P-value<0.05, suggesting the high sensitivity of the PIRCh approach in identifying
1188 chromatin associated lncRNAs. Furthermore, we obtained the genomic binding sites
1189 (peaks) of 23 lncRNAs from the aforementioned experiments, and overlapped them with
1190 the histone ChIP-seq peaks¹² and got a ratio of the overlap for each lncRNA. We then
1191 calculated the Spearman correlation coefficients of these ratios with their corresponding
1192 lncRNA's PIRCh-seq enrichment scores in the same cell line (normalized by the total
1193 number of different ChIP-seq peaks), and found that these correlations were significantly
1194 higher than random permutations. Peak calling was performed by MACS2¹³ with
1195 FDR<0.05.

1196

1197 **The chromatin association states of the enriched ncRNAs**

1198 To cluster chromatin-enriched ncRNAs in distinct groups for functional prediction, we
1199 performed t-SNE and K-mean clustering on the PIRCh enrichment score matrix with the
1200 chromatin associated ncRNAs. The proper K number (K=6) was determined by silhouette
1201 score¹⁴.

1202

1203 **Nearby coding gene expression comparison.**

1204 To further evaluate the functional prediction for chromatin enriched ncRNAs, we first
1205 grouped chromatin enriched ncRNAs by functional classification, and then obtained lists
1206 of the nearby (+/-100Kb) coding genes. We then calculated the gene expressions of these
1207 coding genes and represented them in box-plots. Similarly, we obtained a different list of
1208 nearby coding genes if the chromatin enriched ncRNAs were classified based on their
1209 chromatin enrichment scores on each histone modification. The significance between
1210 each group was estimated by 2-tail Welch's T-test.

1211

1212 **lnc-Nr2f1 ChIP-seq analysis**

1213 To further validate the PIRCh-seq candidates, we performed ChIRP-seq on one of the
1214 H3K4me3 modified PIRCh-seq enriched lncRNAs named *lnc-Nr2f1*. Experimental
1215 methods were mentioned above, where independent "even" and "odd" probe sets were
1216 applied. LncRNA *lnc-Nr2f1* ChIRP-seq data were then analyzed by applying a previously
1217 published pipeline³, where the read alignment was performed in bowtie2 and peak calling

1218 in MACS2. Signals from even and odd ChIRP-seq profiles were then merged to reduce
1219 false positive caused by probes. We confirmed that *Inc-Nr2f1* associated genomic regions
1220 were indeed enriched with H3K4me3 but no other modifications in NPCs, where the
1221 NPC ChIP-seq data was obtained from GSE117289, indicating the high specificity of our
1222 PIRCh-seq approach.

1223

1224 **Allelic specific enrichment analysis in NPC**

1225 We first built the CAST/EiJ and 129S1/SvImJ reference genome. The vcf files containing
1226 the SNPs in the CAST and 129S1 strains were downloaded from the dbSNP database
1227 with the mm9 assembly¹⁵. Their corresponding genome fasta file was made by GATK
1228 toolkit FastaAlternateReferenceMaker and SelectVariants tools¹⁶. After that, the inputs
1229 and PIRCh-seq data in were re-aligned against the CAST and 129S1 indexes by TopHat2
1230 with 0 mismatch (parameter -N 0) to improve the specificity⁵. The allele specific
1231 alignment files were then converted to the bedGraph and bigWigs format using BEDtools.
1232 For each gene, its allele specific expression and enrichment analysis was performed for
1233 every SNP on the list, and estimated the significance between CAST and 129S1 through
1234 the Mann-Whitney-Wilcoxon test, and P-value<0.05 was defined as significant.

1235

1236 **Enriched peak calling from PIRCh-seq profiles**

1237 To further investigate the underlying mechanism of RNA-chromatin association, we
1238 performed peak calling on PIRCh-seq profiles to identify the bases on each enriched
1239 RNA that were mostly affiliated with histone proteins. We first merged data from two
1240 replicates of each gene to minimize the experimental deviation bias, and smoothed the
1241 normalized read counts on each base through a 5bp sliding window, along with a 2bp step
1242 size. Peak calling was performed on the smoothed signal with a home-made script. We
1243 defined a peak in the local maximum that is 5-fold or more amplified relative to the
1244 median read counts of the transcript. Next, we applied a bootstrap method by randomly
1245 sampling 1000 times with reads from the transcripts, and then estimated the P-value of
1246 each peak as the percentage of cases that were more enriched than observed. Finally, we
1247 calculated the relative fold-change of each peak with respect to the input control.
1248 Significant peaks were filtered based on fold change and P-value. Finally, RNA structural
1249 and modification information was integrated with PIRCh-seq peaks for downstream
1250 analysis.

1251

1252 **icSHAPE analysis and structural prediction using RNAfold**

1253 To estimate the structure information around PIRCh peak, we integrate mouse V6.5
1254 icSHAPE data from previous paper^{17,18}. Each transcript's icSHAPE score was calculated
1255 by the original icSHAPE pipeline with default parameter. We used home-made script to
1256 count icSHAPE score around PIRCh peak(+/-200bp) among all transcripts, and the
1257 significance between histone-modification PIRCh peak and random background region

1258 was estimated by by 2-tail Welch's T-test. In terms of *Gas5* in NPC, the structure
1259 information of 129S1 allele was represented by V6.5 icSHAPE data, since they have the
1260 same sequence. Structure prediction of 129S1 allele and CAST allele was performed by
1261 RNAfold web server with default parameter¹⁹. For 129S1 allele, the higher icSHAPE
1262 score at peak region indicate single strand structure, which is similar to the structure
1263 prediction from RNAfold. Furthermore, structure prediction of CAST allele of *Gas5* in
1264 NPC shows that riboSnitches around PIRCh peak might be the cause of the allele specific
1265 enrichment of *Gas5*'s in NPC.

1266

1267 **Statistics**

1268 For data presented in **Figure 1B** (RT-PCR), P-values were calculated via the Mann-
1269 Whitney-Wilcoxon test in Python. For data presented in **Figure 2D & S6B** (GSEA),
1270 enrichment score, P-values and FDR were calculated in GSEA. For data presented in
1271 **Figure S2G**, binomial P-values were calculated by GREAT. For all T-test presented in
1272 this paper, included **Figure 2E, F, I, Figure 3F, Figure S5C, Figure 5D, E, F, G**, P-
1273 values were calculated via two-tailed Welch's T-test in Python. For data presented in
1274 **Figure 4F**, P-values were calculated via the Chi-square Test.

1275

1276 **Data integration**

1277 Mouse v6.5 ChIP-seq results were downloaded from GSE102518¹². Mouse NPC ChIP-
1278 seq data were downloaded from GSE117289. Mouse 7SK ChIRP-seq results were
1279 downloaded from GSE69143²⁰. Murine structural information and RNA modification
1280 information were collected from our previous publications^{18,21}. All RNA binding peaks in
1281 ChIRP/CHART/RAP/GRID-seq experiments were downloaded from LnChrom¹¹. All
1282 mouse data was analyzed using the mm9 assembly and all human data using hg19
1283 assembly.

1284

1285 **Reference:**

1286

- 1287 1. Chen, G. *et al.* Chemically defined conditions for human iPSC derivation and
1288 culture. *Nature Methods* **8**, 424–429 (2011).
- 1289 2. Ang, C. E. *et al.* The novel lncRNA lnc-NR2F1 is pro-neurogenic and mutated in
1290 human neurodevelopmental disorders. *eLife* **8**, 377 (2019).
- 1291 3. Chu, C., Qu, K., Zhong, F. L., Artandi, S. E. & Chang, H. Y. Genomic maps of
1292 long noncoding RNA occupancy reveal principles of RNA-chromatin interactions.
1293 *Molecular Cell* **44**, 667–678 (2011).
- 1294 4. Shah, R. N. *et al.* Examining the Roles of H3K4 Methylation States with
1295 Systematically Characterized Antibodies. *Molecular Cell* **72**, 162–177.e7 (2018).
- 1296 5. Trapnell, C. *et al.* Differential gene and transcript expression analysis of RNA-seq
1297 experiments with TopHat and Cufflinks. *Nature Protocols* **7**, 562–578 (2012).
- 1298 6. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics*
1299 **25**, 2078–2079 (2009).

- 1300 7. Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing
1301 genomic features. *Bioinformatics* **26**, 841–842 (2010).
- 1302 8. Guttman, M. *et al.* Ab initio reconstruction of cell type-specific transcriptomes in
1303 mouse reveals the conserved multi-exonic structure of lincRNAs. *Nature*
1304 *Biotechnology* **28**, 503–510 (2010).
- 1305 9. Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach
1306 for interpreting genome-wide expression profiles. *Proceedings of the National*
1307 *Academy of Sciences* **102**, 15545–15550 (2005).
- 1308 10. Ritchie, M. E. *et al.* limma powers differential expression analyses for RNA-
1309 sequencing and microarray studies. *Nucleic Acids Research* **43**, e47–e47 (2015).
- 1310 11. Yu, F. *et al.* LnChrom: a resource of experimentally validated lincRNA-chromatin
1311 interactions in human and mouse. *Database* **2018**, 354 (2018).
- 1312 12. Zviran, A. *et al.* Deterministic Somatic Cell Reprogramming Involves Continuous
1313 Transcriptional Changes Governed by Myc and Epigenetic-Driven Modules. *Cell*
1314 *Stem Cell* **24**, 328–341.e9 (2019).
- 1315 13. Zhang, Y. *et al.* Model-based analysis of ChIP-Seq (MACS). *Genome Biology* **9**,
1316 R137 (2008).
- 1317 14. Rousseeuw, P. J. Silhouettes: A graphical aid to the interpretation and validation of
1318 cluster analysis. *Journal of Computational and Applied Mathematics* **20**, 53–65
1319 (1987).
- 1320 15. Sherry, S. T., Ward, M. & Sirotkin, K. dbSNP-database for single nucleotide
1321 polymorphisms and other classes of minor genetic variation. *Genome Research* **9**,
1322 677–679 (1999).
- 1323 16. McKenna, A. *et al.* The Genome Analysis Toolkit: a MapReduce framework for
1324 analyzing next-generation DNA sequencing data. *Genome Research* **20**, 1297–
1325 1303 (2010).
- 1326 17. Sun, L. *et al.* RNA structure maps across mammalian cellular compartments.
1327 *Nature Structural & Molecular Biology* **26**, 322–330 (2019).
- 1328 18. Spitale, R. C. *et al.* Structural imprints in vivo decode RNA regulatory
1329 mechanisms. *Nature* **519**, 486–490 (2015).
- 1330 19. Hofacker, I. L. Vienna RNA secondary structure server. *Nucleic Acids Research*
1331 **31**, 3429–3431 (2003).
- 1332 20. Flynn, R. A. *et al.* 7SK-BAF axis controls pervasive transcription at enhancers.
1333 *Nature Structural & Molecular Biology* **23**, 231–238 (2016).
- 1334 21. Batista, P. J. *et al.* m6A RNA Modification Controls Cell Fate Transition in
1335 Mammalian Embryonic Stem Cells. *Cell Stem Cell* **15**, 707–719 (2014).
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