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Genome Res. published online December 12, 2025
Access the most recent version at doi:[10.1101/gr.280832.125](https://doi.org/10.1101/gr.280832.125)

P<P	Published online December 12, 2025 in advance of the print journal.
Accepted Manuscript	Peer-reviewed and accepted for publication but not copyedited or typeset; accepted manuscript is likely to differ from the final, published version.
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1 Transcription and potential functions of a novel *XIST* isoform in male peripheral glia

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38 Abstract:

39

40 The *XIST* RNA is known for its critical roles in X Chromosome inactivation (XCI). It is thought

41 to be expressed exclusively from one copy of the X Chromosome and silence it by recruiting

42 various chromatin factors in female cells. In this study, we find *XIST* expression in male

43 peripheral glia after integrated analyses of single cell RNA-seq data from multiple human tissues

44 and organs. Single cell epigenomic data further indicate that the expression is likely driven by an

45 alternative promoter at the end of the first exon, resulting in at least one shorter transcript

46 (referred to as *sXIST*) that is active in Schwann cells and, moreover, at a higher level in non-

47 myelinating Schwann cells. This promoter exhibits similar activity in female glia. Multiple lines

48 of evidence from bulk transcriptomic and epigenomic data from peripheral nerve tissues further

49 support these findings. Genes co-expressed positively and strongly with *sXIST* in male glia show

50 functional enrichment in axon assembly and cilia signaling, with many of them sharing putative

51 miRNA binding sites with *sXIST*, while the negatively correlated genes are enriched for52 processes important for neuromuscular junctions. This suggests possible functions of *sXIST* in

53 modulating glia-neuron interactions, perhaps via competitive miRNA binding. This idea is also

54 supported by overexpression analysis of a partial *sXIST* sequence and the finding of significant55 *XIST* expression changes in human cardiomyopathy and polyneuropathy. In summary, the current56 study suggests a novel, non-XCI role of *XIST* in peripheral Schwann cells that is mediated by a

57 newly recognized transcript.

58

59

60 Introduction:

61
62 X-inactive specific transcript (*XIST*) is a long non-coding RNA that serves as an important
63 regulator of X Chromosome-inactivation (XCI) in mammalian XX females (Loda and Heard
64 2019). Through interactions between its functional domains (repeat regions A-E) and various
65 proteins, such as Polycomb Repressive Complexes (PRC1/2), CIZ1, and YY1, the *XIST*
66 ribonucleoprotein complex silences one copy of the X Chromosome in female somatic tissues
67 (Loda and Heard 2019; Jacobson et al. 2022). Since the 1990's, *XIST* expression has been
68 continuously shown to be female-specific and exclusively expressed from the inactive X
69 Chromosome in mammals (Borsani et al. 1991; Brockdorff et al. 1991; Brown et al. 1991). It has
70 also been described to unambiguously target the X Chromosome to silence gene expression
71 (Panning 2008; Cerase et al. 2015). However, recent work indicated that *XIST* may also have
72 roles in regulating autosomal genes in female cells (Dror et al. 2024; Yao et al. 2024). Moreover,
73 some studies in cancers further reported that *XIST* could function as a “miRNA sponge” by
74 sequestering multiple miRNAs and thus affecting the expression of their mRNA targets
75 (Marshall et al. 2019; Jiang et al. 2021; Liu et al. 2021; Yan et al. 2022).

76
77 Along with these findings of new *XIST* functions beyond XCI, the canonical view of its
78 exclusive expression in female cells has also been challenged. A few studies have reported rare
79 instances of *XIST* expression in male cells, mostly in cancers. For example, two groups
80 independently found *XIST* expression in male samples from multiple types of cancers, such as
81 testicular cancer (Looijenga et al. 1997) and hepatocellular carcinoma (Sadagopan et al. 2022).
82 Similarly, among the 54 human tissues analyzed by bulk RNA sequencing (RNA-seq) in the
83 GTEx consortium from hundreds of healthy individuals (Lonsdale et al. 2013), robust *XIST*
84 expression was observed in the testes and male tibial nerves (Lonsdale et al. 2013; Sadagopan et
85 al. 2022). While *XIST* expression in the testes during spermatogenesis is well-documented
86 (Salido et al. 1992; McCarrey et al. 2002), its regulation and potential function in male tibial
87 nerve remains unexplored. In male cancers, however, Sadagopan *et al.* suggested that *XIST*
88 expression could lead to repression of genes on the X Chromosome (Sadagopan et al. 2022).
89 Thus, additional studies are needed to address whether this occurs in normal human tissues, if
90 repression also occurs for autosomal genes, and if so, in what cell types and to what extent. *XIST*

91 RNA may have entirely new tissue or cell type-specific roles in males, which further highlights
92 the need for investigation into its non-canonical functions.

93

94 Towards this, we performed a systematic analysis of single cell RNA-seq (scRNA-seq) and
95 chromatin accessibility (scATAC-seq) data to characterize *XIST* expression in male and female
96 tissues from healthy human individuals, including data from child and adult heart (Sim et al.
97 2021; Mehdiabadi et al. 2022; Kanemaru et al. 2023), skeletal muscle (Kedlian et al. 2024), and
98 many other tissues (Zhang et al. 2021). We also analyzed transcriptomic data from multiple
99 studies of mouse peripheral nerves to determine if *XIST/Xist* expression in male nerves is
100 conserved. We further performed transcriptomic profiling to characterize genes and pathways
101 that may be affected by the expression of a new *XIST* isoform (termed “*sXIST*”) that we
102 discovered in male peripheral glia. Using these systematic approaches, we aim to investigate: a)
103 the cell/tissue type and disease relevance of *sXIST* expression in male cells, b) distinct
104 transcriptional regulation of the *XIST* expression in male and female cells, and c) the potential
105 molecular mechanisms of *sXIST* functions in peripheral glia. The overarching goal is to
106 understand the potential roles of *XIST* in peripheral nerves and its relevance to the functions and
107 diseases of peripheral nervous and cardiovascular systems.

108

109 **Results:**

110

111 *XIST* expression in human male and female Schwann cells

112

113 To study the effects of sex chromosomes (XX vs XY) on gene programs in heart tissues at the
114 single cell level, we integrated existing scRNA-seq (or single nucleus RNA-seq, snRNA-seq)
115 data from 20 healthy child and adult human hearts, which were collected from a variety of
116 sources (Sim et al. 2021; Mehdiabadi et al. 2022; Kanemaru et al. 2023) (**Supplemental Table**
117 **S1**). We unexpectedly observed *XIST* expression in male hearts. To study this in more detail, we
118 clustered the integrated data to define major cell types in human hearts, from which glial
119 (referred to as “neural” in some previous reports) cells were extracted and further subclustered to
120 identify non-myelinating and myelinating Schwann cells (**Fig. 1**) using conventional markers
121 from the literature, such as *SCN7A* and *SLC35F1* for Schwann cells (Karlsson et al. 2021),
122 *NRXN1/2* as pan-glial markers (Diehl et al. 1986), *TUBB3* for neurons (Diehl et al. 1986), and
123 *PLP1* as a myelination marker (Diehl et al. 1986) (**Fig. 1; Supplemental Fig. S1**). Based on the

124 lack of neuronal marker expression, we determined that the previously annotated “neural” cells
125 were glia, in agreement with the authors of the Heart Cell Atlas (Kanemaru et al. 2023). Our
126 analysis showed that male glia expressed *XIST* robustly (**Fig. 1A,B,C**). To study if this is specific
127 to the heart, we then obtained human skeletal muscle scRNA-seq data for fibroblasts and neural
128 cells (Kedlian et al. 2024), with non-myelinating and myelinating Schwann cells annotated by
129 the original authors (**Fig. 1D,E,F**). In both tissues, male glia showed strong *XIST* expression
130 (**Fig. 1C,F**). More specifically, a greater percentage of male non-myelinating Schwann cells
131 (33.63% in heart, 31.13% in skeletal muscle) expressed *XIST* compared to their myelinating
132 counterparts (20.29% in heart, 21.71% in skeletal muscle) (heart: p -value = 1.7×10^{-10} ; skeletal
133 muscle: $p < 2.2 \times 10^{-16}$; Fisher’s exact test). The average expression of *XIST* in male non-
134 myelinating Schwann cells was also higher than in myelinating cells, determined at either the
135 single cell level (heart: adjusted p -value = 2.4×10^{-7} , AvgLog₂FC (fold-change) = 1.2; skeletal
136 muscle: adjusted p -value = 3.3×10^{-33} , AvgLog₂FC = 8.9, Wilcoxon rank-sum test) (**Fig. 1C,F**) or
137 pseudobulk level with cells from the same samples pooled (heart: adjusted p -value = 2.1×10^{-6} ,
138 log₂FC = 2.8; skeletal muscle: adjusted p -value = 2.8×10^{-8} , log₂FC = 5.6; negative binomial test).
139 In general, a greater percentage of male glia expressed *XIST* compared to other cell types (<
140 0.55%) in both datasets (**Supplemental Table S2, Supplemental Fig. S2**). Additionally, even
141 among cell types with *XIST* RNA detected, *XIST* abundance remained significantly lower in the
142 male compared to female glia (**Fig. 1C,F**).

143
144 To expand our finding, we analyzed *XIST* expression in the Human Cell Atlas using the CZ
145 CELLxGENE Discover Gene Expression tool (CZI Cell Science Program et al. 2024), which
146 enabled us to quantify the percentages of cells expressing *XIST* across a large number of cell
147 types in aggregated datasets from multiple tissues. Among all the cell types in the atlas, we found
148 significant *XIST* expression in male Schwann cells (58% of 2955 cells) and non-pigmented
149 ciliary epithelial cells (54% of 3011 cells) of the eye, pancreatic stem cells (59% of 492 cells),
150 and fibro/adipogenic progenitor cells of adipose tissue (51% of 1154 cells) (**Supplemental Table**
151 **S3**). Although *XIST* expression was sporadically detected in other male cell types, these were the
152 only cell types with expression in > 50% of cells, which accounted for just 0.15% (5/3151) of all
153 cell type-tissue combinations that we extracted from the database up to January 2025. There were
154 still only 0.98% (31/3151) of all cell type-tissue combinations if we reduced the detection

155 percentage to >30%, which was approximately the percentage of male cells expressing *XIST* in
156 the heart and skeletal muscle data (**Fig. 1C,F**). In the central nervous system, 6% of male
157 differentiation-committed oligodendrocyte precursors in both the spinal cord and brain expressed
158 *XIST*, but only 0.4% of mature oligodendrocytes in the spinal cord and 0.3% in the brain
159 expressed *XIST*. Male *XIST* expression was barely detectable in two large cohorts of human
160 brains (Velmeshev et al. 2019; Wamsley et al. 2024).

161
162 Taken together, our analysis found that *XIST* is consistently expressed in male Schwann cells,
163 while its male expression in other cell types is much sparser and probably needs stronger
164 evidence from more studies (**Supplemental Fig. S3; Supplemental Table S3**). Note that our
165 finding of *XIST* expression in male cardiac glia was independently described by a recent report
166 from Gorin and Goodman (Gorin and Goodman 2025).

167
168 *Characterization of XIST transcript sequence and regulation in peripheral nerves*

169
170 Next, we sought to investigate how *XIST* achieves such specific expression by comparing
171 chromatin accessibility data from Assay for Transposase-Accessible Chromatin using sequencing
172 (ATAC-seq) across cell types. We first turned to scATAC-seq data from the Human Heart Cell
173 Atlas, which were generated using the 10X Multiome platform (Kanemaru et al. 2023). We re-
174 called ATAC-seq peaks using MACS2 (Zhang et al. 2008) for each of the broad cell types
175 defined by the authors, except we renamed their “neural” cells as “glia” because they lacked
176 expression of the neuronal markers in **Figure 1**. This identified two ATAC peaks at the *XIST*
177 locus, with a peak called only for the cardiac glia ~10kb downstream from the main *XIST*
178 promoter. The main *XIST* promoter is constitutively active in female cells but not in male cells
179 (**Fig. 2A**). The glia-specific peak (ChrX:73,841,364-73,841,611; hg38) is located in a region
180 suggested to be an active promoter in several cell lines by the FANTOM5 project. Their data is
181 from a combination of cell lines and tissues (including Schwann cells, optic nerve, and skeletal
182 muscle), with the sample metadata indicating 32% males, 32% females, and 36% as other/NA
183 (Noguchi et al. 2017) (**Supplemental Fig. S4A**). Additionally, the binding sites for a variety of
184 transcription factors (TFs), such as *FOXD3* and *REST*, are found in this region according to
185 motifs in the JASPAR database (Rauluseviciute et al. 2024) (**Supplemental Fig. S4B,**
186 **Supplemental Table S4**). Among the predicted TFs, one (*FOXO1*) was expressed higher in both

187 skeletal muscle and heart non-myelinating Schwann cells compared to myelinating, while 11
188 (*NFATC3*, *SOX13*, *PBX1*, *RREB1*, *SOX10*, *TCF4*, *ETS1*, *SREBF1*, *CEBPB*, *KLF2*, and *ETV5*)
189 were higher in either skeletal muscle or heart non-myelinating Schwann cells. Analysis of the
190 transcription regulatory programs by the SCENIC software predicted that *FOXO1* (and *YY1*)
191 could regulate *XIST* in heart and skeletal muscle Schwann cells, with greater activity in non-
192 myelinating cells (**Supplemental Fig. S5**). This suggests that *FOXO1*, among other TFs, may
193 regulate a previously uncharacterized promoter (or enhancer) of *XIST*.

194
195 After separating male from female cells, we observed that all female cell types show high
196 chromatin accessibility at the main *XIST* promoter, consistent with constitutive *XIST* expression
197 in females; however, it became clear that the alternative promoter is likely active in both males
198 and females. Silencing of the main *XIST* promoter in males allowed us to correlate the increased
199 accessibility of the alternative promoter with increased *XIST* expression (**Fig. 2A-B**). There were
200 significantly more scATAC fragment counts detected over the alternate promoter region
201 (ChrX:73,841,364-73,841,611 +/- 100 bases) in glia compared to all other cell types for both
202 males ($p\text{-value} = 2.83 \times 10^{-14}$, $\text{AvgLog}_2\text{FC} = 3.98$, Wilcoxon rank-sum) and females ($p\text{-value} =$
203 1.92×10^{-11} , $\text{AvgLog}_2\text{FC} = 2.84$, Wilcoxon rank-sum). This was associated with significantly
204 increased *XIST* expression in male glia ($p\text{-value} < 2.23 \times 10^{-308}$, $\text{AvgLog}_2\text{FC} = 10.51$, Wilcoxon
205 rank-sum test) and to a lesser degree in female glia ($p\text{-value} = 1.24 \times 10^{-4}$, $\text{AvgLog}_2\text{FC} = 0.37$)
206 compared to all other cell types, likely masked by the higher expression of the full-length *XIST* in
207 female cells.

208
209 To study if the newly discovered alternative promoter leads to alternative *XIST* transcripts, we
210 analyzed bulk RNA-seq data because most of the scRNA-seq technologies sequence only the 3'
211 end of RNAs. Unfortunately, we were unable to find bulk RNA-seq data for purified male
212 Schwann cells. Thus, we turned to publicly available bulk RNA-seq data from peripheral nerve
213 samples because this tissue type contains a relatively high proportion of Schwann cells, which
214 are functionally required for supporting/myelinating peripheral neurons. In a previous study,
215 Sandy-Hindmarch *et al.* performed strand-specific bulk RNA-seq on Morton's neuroma and
216 control samples (Sandy-Hindmarch *et al.* 2025) from males and females independently. These
217 samples were collected from a variety of digital, upper limb, and lower limb nerves, allowing us
218 to examine the distribution of RNA-seq reads along the *XIST* locus in both strands. The data

219 showed that reads from male samples were significantly enriched at the last exon (i.e., exon 6 at
220 the 3' of *XIST*) (**Fig. S6**). While RNA-seq reads were also mapped to other exons (exons 2-5),
221 only a few reads were aligned to the end of the first exon in males. A comparison of the ratios of
222 RNA-seq reads mapped to the 1st and 6th exons in male and female samples support a significant
223 bias of reads to the latter in males (p -value = 0.044, t -test) (**Supplemental Fig. S6A**).
224 Importantly, the region at the end of the first exon with only a few RNA-seq reads in male
225 samples is near the cardiac glia-specific accessible region discussed above (**Fig. 2**).

226

227 To seek additional, independent support for the activation of the alternative promoter, we next
228 utilized the Cis-element atlas database (CATlas) (Zhang et al. 2021) to study chromatin
229 accessibility for other cell types at the *XIST* locus because it curates comprehensive reference
230 maps of *cis*-regulatory elements from thousands of cell types. Moreover, it has a heart dataset
231 with both snRNA-seq data and scATAC-seq peaks from males and females. We found that cells
232 annotated as “nerve” showed increased chromatin accessibility at the alternative *XIST* promoter,
233 with concordant snRNA-seq reads in the same region, but other cell types did not (**Fig. 3A**).

234

235 We also analyzed tibial nerve bulk RNA-seq and ChIP-seq data (two male samples) and sciatic
236 nerve ATAC-seq data (one male sample) available from the ENCODE Project (The ENCODE
237 Project Consortium 2012; Luo et al. 2020). The results support open chromatin accessibility at
238 the alternative promoter, with its activity further supported by active histone modifications
239 H3K4me3 (marks promoters) and H3K27ac (marks both promoters and enhancers). Moreover,
240 the data strongly support that *XIST* expression in males occurred after (3' to) the alternative
241 promoter as seen from enriched signals of RNA-seq and H3K36me3, a histone mark for
242 transcription elongation (**Fig. 3B**). Like in the above analysis of Morton's neuroma data, we
243 again found that RNA-seq reads exhibited a significantly greater bias to exon 6 (vs exon 1) in the
244 male nerve samples when compared to the male brain samples (p -value = 0.0011, t -test), which
245 do not have Schwann cells (**Supplemental Fig. S7**). This biased trend was also in female data,
246 but the difference was not statistically significant (p -value = 0.22).

247

248 To address the concern of inferring RNA transcript sequences from short read technology, we
249 searched the ENCODE database for *XIST* transcripts from long-read sequencing technology.

250 There was no such data from nerve samples, but we found RNA transcripts that aligned with the
251 presence of *sXIST* transcripts in human hearts (**Supplemental Fig. S8**, red arrows; a male and a
252 female sample).

253
254 Lastly, for additional regulation evidence, we examined another CATlas dataset derived from
255 integration of multiple male and female tissues and found that adult Schwann cells contained an
256 ATAC-seq peak (called by the authors) at ChrX:73,841,318-73,841,717, which was essentially
257 the exact same region where we called an ATAC-seq peak for cardiac glia (**Fig. 2**). The same
258 peak was called for cardiac fibroblasts, peripheral nerve stromal cells, vascular smooth muscle
259 cells, and type II skeletal myocytes in CATlas (**Fig. 3C**), potentially related to sparse *XIST*
260 expression in non-glial heart cells (**Fig. 1**). Nevertheless, the peaks were the highest in Schwann
261 and peripheral nerve stromal cells. Note that no peak at this region was called for the remaining
262 106 adult cell types or any of the 111 fetal cell types in CATlas.

263
264 Taken together, our systematic analysis uncovers multiple lines of evidence that an alternative
265 *XIST* promoter is active in peripheral glia, most likely Schwann cells, resulting in a shorter *XIST*
266 transcript (or transcripts) in both males and females, which we referred to as *sXIST*.

267

268 *Functional implications of XIST activation in male Schwann cells/nerves*

269

270 To study possible roles of *sXIST* expression, we first addressed whether it is involved in
271 repressing X-Chromosome genes in males. We divided the genes on the X Chromosome into
272 groups: genes undergoing X inactivation (“X non-escapees”), genes escaping inactivation (“X
273 escapees”) (Wainer Katsir and Linial 2019), and genes in the pseudoautosomal region (PAR)
274 (Weng et al. 2016). As controls, we also included Y Chromosome genes that are not in the
275 pseudoautosomal region (Y non-PAR) and all autosomal genes. We compared their expression
276 between myelinating and non-myelinating Schwann cells using DESeq2 (Love et al. 2014) and
277 the pseudobulking method, dividing samples by sex (**Fig. 4A,B**).

278

279 If *sXIST* participates in X Chromosome silencing in males as it does in females, we would expect
280 a general decrease in the expression of X non-escapee genes in male non-myelinating (higher
281 *sXIST*) compared to myelinating Schwann cells, manifesting as a leftward shift in the
282 distributions of their log₂FC values in **Figure 4A,B**. No global shift was observed in the

283 comparisons of skeletal muscle cells (**Fig. 4A**). Although the mean \log_2FC value of this
284 comparison in the heart data was -0.81 with a slight leftward shift (i.e. most non-escapee genes
285 reduced expression in nmSchwann cells), this trend was also seen in our control groups
286 (autosomal genes and X-escapee genes) and similarly in the comparisons of female samples.
287 Thus, it is unlikely that *sXIST* leads to selective repression of X-Chromosome genes. Because
288 male cells have only one X Chromosome, we also expected a decrease in X non-escapee
289 expression in males (leftward shift) when comparing male to female nmSchwann cells. Instead,
290 the distribution of \log_2FC values were centered around 0 without apparent skewness for both
291 human and skeletal muscle datasets. The same trends were observed when male and female
292 mSchwann cells were compared. The expected and observed trends are summarized at the
293 bottom of **Figure 4A/B** and the mean \log_2FC values for each comparison can be found in
294 **Supplemental Figure S9**. Furthermore, no evidence for a global reduction of non-escapee genes
295 was observed in male or female Schwann cells when compared to all the other cell types
296 (**Supplemental Fig. S10**).

297
298 Only PAR genes showed a consistent expression reduction in nmSchwann relative to mSchwann
299 cells in males for both heart and skeletal muscle datasets (shown as left-shift in **Fig. 4A,B** top),
300 indicating a global decrease in PAR gene expression in non-myelinating Schwann cells (heart:
301 mean $\log_2FC = -0.87$; skeletal muscle: mean $\log_2FC = -0.27$). PAR genes are thought to escape
302 XCI (Helena Mangs and Morris 2007), but we found that they exhibited higher expression in
303 males compared to females irrespective of Schwann cell type (**Fig. 4A,B** bottom). This trend
304 appears the same in other tissues from our analysis of bulk GTEx data (Lonsdale et al. 2013)
305 (**Supplemental Fig. S11**). The increased PAR expression in males is consistent with previous
306 reports (Tukiainen et al. 2017). Thus, the increased PAR expression in male compared to female
307 Schwann cells can be attributed to sex biases while decreased PAR gene expression in non-
308 myelinating vs myelinating Schwann cells (in males) may be cell type dependent and related to
309 *sXIST* expression.

310
311 In short, we did not find convincing evidence for the involvement of *sXIST* in the general
312 suppression of X Chromosome genes in the male non-myelinating Schwann cells. This contrasts
313 with previous reports in male cancer cells (Looijenga et al. 1997; Sadagopan et al. 2022).

314 Nevertheless, our results are consistent with the consensus view in the literature that XCI needs
315 the functional domains in *XIST* exon 1 (Brockdorff 2002), which are absent in *sXIST*.

316
317 Next, we reasoned that genes potentially affected or regulated by *sXIST* would exhibit a stronger
318 correlation with *XIST* expression in male cells than in female cells because the correlation in
319 female cells captures the combined expression of *sXIST* and the full *XIST* transcript, with the
320 latter at a much higher level, thus masking *sXIST* effects in this analysis. To investigate this, we
321 used the Morton's neuroma bulk RNA-seq dataset from peripheral nerves (Sandy-Hindmarch et
322 al. 2025) discussed above (**Supplemental Fig. S6**) because scRNA-seq data is too sparse for this
323 purpose. We first randomly selected 100 genes and calculated the Pearson correlation
324 coefficients between each of them and all the other human genes across all male ($n = 11$) and
325 female ($n = 22$) samples separately to obtain a null distribution defined by fitting an ellipse
326 around 99% of the data points (**Supplemental Fig. S12A-B**). Adding *XIST*, we found that 2.93%
327 of all genes correlated with *XIST* were outside the ellipse (*i.e.*, outliers), compared to just 0.81%
328 in null background (p -value $< 2.2 \times 10^{-16}$, odds ratio (OR) = 3.68, Fisher's Exact Test), thus
329 representing genes with significantly different correlations with *XIST* between male and female
330 samples. In the end, we determined that 195 genes (0.76% of all genes) had a stronger and
331 positive correlation ("SPC") with *XIST* in males than in females, compared to 0.17% of control
332 gene pairs (p -value $< 2.2 \times 10^{-16}$, odds ratio = 4.61, Fisher's Exact Test), and 206 genes (0.80% of
333 all genes) had a stronger and negative correlation ("SNC") with *XIST* in males, compared to
334 0.16% of control comparisons (p -value $< 2.2 \times 10^{-16}$, odds ratio = 4.92, Fisher's Exact Test) (**Fig.**
335 **4C**). Two examples, *DNAAF1* (SPC) and *KCNAB1* (SNC), are shown in **Figure 4D**.

336
337 Among the 206 SNC genes, many have roles in neuromuscular system development and
338 function, including *CYFIP2* (involved in synapse formation and plasticity), *FLYWCH1* (linked to
339 neuronal migration and axon guidance), ion channel genes (e.g., *SLC2A4*, *KCNQ4*, *KCNAB1* and
340 *KCNMB1*), calcium signaling genes (*CACNA1S*, *CACNA1C* and *CACNB2*), extracellular matrix
341 genes (*COL4A2* and *ITGA8*) and genes related to muscle structure and function (*ACTA1/2* and
342 *ACTG2*). Overrepresentation analysis (using ToppFun (Chen et al. 2009)) of the SNC genes also
343 revealed a significant enrichment (adjusted p -value < 0.05) for functions related to muscle
344 system process, calcium ion homeostasis, skeletal muscle development, actin filament-based
345 movement, and action potential (**Fig. 4E**). Among the 195 SPC genes, many have functions in

346 neurodevelopment and synaptic function (e.g., *FOXP2*, *NPASI*, and *CNTNAP4*), signaling
347 transduction (e.g., *NFATC1*, *WNT9A*, and *RASGRF2*), ciliary function (e.g., *DNAH9* and
348 *DNAAF1*), and immune response (e.g., *CXCR4* and *IL18*). Unbiased enrichment analysis of the
349 SPC genes showed a significant enrichment of functions related to cilium, extracellular transport,
350 and axoneme assembly (**Fig. 4F**). Although research in this area is relatively sparse, primary cilia
351 are signaling hubs, exist in glia (and neurons), exhibit impairment in some neurodegenerative
352 diseases, and may be involved in a variety of functions related to nerve regeneration signaling in
353 Schwann cells (Ki et al. 2021). Note that neither SPC nor SNC genes were enriched on the X
354 Chromosome and very few exhibited a significant expression difference between male and
355 female samples. Interestingly, both SPC and SNC genes were enriched for putative targets of
356 multiple miRNAs (**Fig. 4E,F**). Previous studies have reported that *XIST* can act as a molecular
357 sponge to inhibit miRNA function (Marshall et al. 2019; Jiang et al. 2021; Liu et al. 2021; Yan et
358 al. 2022), which will lead to a positive correlation among genes sharing miRNA targets because
359 of fewer miRNAs binding to individual targets (Hausser and Zavolan 2014). Among the top 15
360 miRNAs predicted to target SPC genes, 5 of them (miR-361-3p, miR-32, miR-149-3p, miR-122,
361 and miR-367) have reported evidence for interactions with *XIST* (Li et al. 2018; Cheng et al.
362 2020; Yang et al. 2020; Jiang et al. 2021; Wang et al. 2023).

363
364 To relate this result back to Schwann cells, we wondered how the SPC and SNC genes were
365 differentially expressed between non-myelinating and myelinating Schwann cells. First, we
366 found that two SPC genes (*RNF144A* and *PCBP3*) were significantly upregulated (adjusted *p*-
367 value < 0.05 from DESeq2) when comparing all heart non-myelinating to myelinating Schwann
368 cells. For skeletal muscle, three SPC genes (*CCND3*, *ANO4*, and *PYGL*) were upregulated. For
369 SNC genes, heart non-myelinating Schwann cells showed significant downregulation of 31 (e.g.,
370 *ILK*, *FLNA*, and *CYFIP2*) while no SNC genes were downregulated in skeletal muscle. We next
371 used biomaRt to extract all genes in the “epithelial cilium movement involved in extracellular
372 fluid movement” (GO:0003351, 43 genes) and “actin filament-based movement” (GO:0030048,
373 133 genes) categories because SPC and SNC genes were enriched for these terms, respectively.
374 We used these genes to compute module scores for non-myelinating, myelinating, and “other”
375 glia in the integrated human heart scRNA-seq data discussed above (but not skeletal muscle due
376 to the relatively low number of total Schwann cells). The module scores using the cilia-related

377 gene set were significantly higher in non-myelinating Schwann cells compared to myelinating
378 and “other” for both males and females (p -value < 0.05 , Wilcoxon rank-sum) (**Supplemental**
379 **Fig. S12C**). However, the module scores using the “actin filament-based movement” gene set
380 were not consistently lower in non-myelinating Schwann cells compared to myelinating
381 (**Supplemental Fig. S12D**).

382
383 Overall, these results indicate that *XIST*-expressing male non-myelinating Schwann cells do not
384 exhibit evidence of XCI; instead, they suggest *sXIST* may have other functions potentially
385 involving miRNAs, including some targeting cilia-related genes.

386
387 *Assessing the effects of sXIST overexpression in a human cell line*

388
389 To further assess if *sXIST* has non-XCI functions, we over-expressed a subsequence of *sXIST* in a
390 human cell line (HEK-293T), more specifically the second half of *XIST* exon 6 (**Supplemental**
391 **Methods; Supplemental Fig. S13**). The construct lacks domain E, which is required for
392 anchoring *XIST* RNA to the inactive X Chromosome through CIZ1 interactions (Sunwoo et al.
393 2017), and other domains needed for XCI. We carried out strand-specific RNA-seq analysis
394 comparing *sXIST* overexpression (“*XIST* OE”) to GFP controls ($n=3$; **Fig. 5**), and identified a few
395 hundred differentially expressed genes (DEGs), including *XIST* itself (**Fig. 5A,F; Supplemental**
396 **Table S5**). Among the various gene groups described in **Figure 4**, the SPC genes and especially
397 the 10 genes in cilia-related pathways exhibited strong, increased expression in the *XIST* OE
398 samples (**Fig. 5B**). However, the other groups of genes did not exhibit a group-level trend, except
399 for a reduction of PAR genes in males (**Fig. 5B, Supplemental Fig. S13C**). Thus, our over-
400 expression data are consistent with our bioinformatic results in **Figure 4**.

401
402 Functional enrichment analysis of the 318 significantly upregulated (adjusted p -value < 0.05 and
403 fold change > 1.5) and 119 downregulated (adjusted p -value < 0.05 and fold change < -1.5) (**Fig.**
404 **5C**) genes showed that the up-regulated set was significantly enriched for GO terms related to
405 cilia and axoneme assembly (**Fig. 5E**). Moreover, “Cilium movement” and “axoneme assembly”
406 were in the top five most enriched GO terms for both male SPC and *XIST* OE upregulated genes.
407 Four upregulated genes (*BDKRB2*, *DNAAF1*, *DNAI3*, and *LRRC46*) and two downregulated
408 genes (*COL4A6*, and *P2RX1*) in our over-expression analysis overlapped with SPC and SNC

409 genes, respectively. The small overlap potentially reflects distinct biological contexts of HEK-
410 293T cells and peripheral nerves.

411
412 Targets of multiple miRNAs were enriched in the upregulated genes. Importantly, among the top
413 20 miRNAs, miR-9, miR-651, miR-520a-5p, and miR-218-2 (also called miR-218-3p) were
414 predicted by miRDB (Chen and Wang 2020) to bind to the overexpressed *sXIST* sequence, as
415 was miR-520a-3p but not ranked in the top 20. MiR-9-5p is especially interesting because it is
416 expressed in both HEK-293T (Kavakiotis et al. 2022) and nerve tissue (Rishik et al. 2025),
417 previously implicated in *XIST* sponging (Zheng et al. 2020), and inhibits Schwann cell migration
418 (Zhou et al. 2014). One of its predicted targets is *HMMR* (hyaluronan-mediated motility receptor;
419 **Fig. 5F,G**), which is highly expressed in Schwann cells (Ouasti et al. 2020), important for their
420 motility (Ouasti et al. 2020), plays a role in mitotic spindle positioning (Connell et al. 2017), and
421 supports neural development (Connell et al. 2017). While not predicted to bind to its 3' UTR, a
422 potential hsa-miR-9-5p binding site is found in the final exon of *DNAAF1* (**Fig. 5G**), a cilia-
423 related gene strongly correlated with *XIST* expression in males (**Fig. 4E**). It has been implicated
424 in neural tube defects (Miao et al. 2016) and ciliary dyskinesia due to its important role in
425 dynein-arm complex assembly (Loges et al. 2009).

426
427 As for the other miRNAs, cilia-related genes are predicted targets of miR-520a-5p (*DNAH6*,
428 *DNAI4*), miR-651 (*DNAAF1*), miR-218-2 (*DNAAF1*), and miR-520a-3p (*DNAAF1*). Mir-218-2
429 and miR-34a-5p were also predicted to target *LRRC46*. Additionally, miR-520a-5p, miR-9, and
430 miR-34a have been previously implicated in *XIST* sponging (Liu et al. 2018; Zheng et al. 2020;
431 Liu et al. 2021). To check if these miRNAs are expressed in HEK-293T cells and nerve tissue,
432 we searched the microRNA Tissue Expression Database (Kavakiotis et al. 2022) and confirmed
433 that miR-218-2-3p, miR-34a-3p/5p, miR-651-3p/5p, and miR-9-3p/5p were expressed, with
434 miR-9 showing the strongest expression (top 3% of 1773 expressed miRNAs in 293T cells and
435 top 1% of 1089 expressed miRNAs in peripheral nerves). These miRNAs were also detected in
436 nerve tissue according to the miRNA Tissue Atlas (Rishik et al. 2025).

437
438 Taken together, many genes, including those related to cilia, were upregulated upon
439 overexpression of an *sXIST* fragment and there is no evidence that the DEGs are enriched on the

440 X Chromosome. The overexpression data, however, supports the potential function of *sXIST* as a
441 miRNA sponge.

442
443 *Evaluating XIST expression in glial cells from human cardiomyopathy and polyneuropathy tissue*
444

445 Since *sXIST* exhibited high expression in male Schwann cells, and because Schwann cells are
446 part of the intracardiac nervous system and important for cardiac sympathetic nerve fasciculation
447 (i.e., axon bundling to form nerve tracts) (Hortells et al. 2021), we wondered whether there might
448 be changes in *XIST* expression in the glia of diseased hearts, more specifically from patients with
449 arrhythmogenic cardiomyopathy and dilated cardiomyopathy (up to 38% of hearts with dilated
450 cardiomyopathy exhibit non-sustained ventricular arrhythmias (Spezzacatene et al. 2015)). We
451 thus analyzed a snRNA-seq dataset with 881,081 nuclei from 18 healthy and 61 non-ischemic,
452 failing hearts (Reichart et al. 2022) (**Fig. 6A**). We again found that author-annotated male
453 “neural” cells, which we reclassified to glia based on marker gene expression (**Supplemental**
454 **Fig. S14**), expressed *XIST* at much higher levels compared to other male cell types (adjusted *p*-
455 value $< 2.23 \times 10^{-308}$, $\log_2\text{FC} = 6.44$, Wilcoxon rank-sum) (**Fig. 6B**). We also found that the
456 percentage of cells expressing *XIST* in glia was significantly higher in both cardiomyopathy
457 groups for males and females (*p*-value = 2.2×10^{-16} , OR = 1.14, Fisher’s exact test) (**Fig. 6D**).
458 After pseudobulking by samples, the expression difference of *XIST* between disease groups and
459 controls was only significant for males (**Fig. 6C**). High expression of the full-length *XIST*
460 transcript in female samples could explain the inconsistency in the results from pseudobulk level
461 and single cell level, since *sXIST* would contribute to a fraction of the total pseudobulked
462 expression values.

463
464 Because of *XIST* upregulation in cardiomyopathy, we expected that SPC genes would be
465 upregulated and SNC genes downregulated (**Fig. 4**) in both types of cardiomyopathies. We found
466 this to be true for five genes, which were *RYR2*, *TPM2*, *CACNB2*, *AKAP6*, and *MYOCD*. A full
467 list of the overlap between disease DEGs by SPC/SNC genes, sex, and expression direction
468 relative to normal hearts is provided in **Supplemental Table S6**. We also observed significant
469 differences in *FOXO1* expression, mentioned above as a potential TF regulating *sXIST*
470 expression (*FOXO1* expression was significantly higher in male and female disease group glia,
471 except female arrhythmogenic RV cardiomyopathy) (**Fig. 6E**). *COL4A6*, which is an SNC gene

472 (Fig. 4) and downregulated in the *XIST* OE group (Fig. 5), showed decreased expression in male
473 and female dilated cardiomyopathy glia compared to healthy hearts (Fig. 6F). *RYS2*, another
474 SNC gene that encodes a receptor crucial for cardiac muscle function and involved in cardiac
475 and neuronal disorders (ryanopathies) (Sleiman et al. 2021), was significantly downregulated in
476 both types of cardiomyopathies for male and female glia (Fig. 6G).

477
478 For males and females, we also used SPC and SNC genes to compute module scores for glia
479 from cardiomyopathy and healthy groups. For males, the SPC module scores, as expected, were
480 significantly higher (Wilcoxon rank-sum test p -value < 0.05) in glial cells from both types of
481 cardiomyopathies compared to controls (Fig. 6H). For females, module scores were higher for
482 glial cells from arrhythmogenic RV cardiomyopathy but not dilated cardiomyopathy (Fig. 6H).
483 For both males and females, SNC gene module scores were significantly lower in glial cells from
484 both cardiomyopathies compared to healthy controls (Fig. 6I).

485
486 Likewise, we studied *XIST* and its putatively affected pathways using scRNA-seq data
487 comparing polyneuropathy (PNP) sural nerve samples to controls, which included non-
488 myelinating, myelinating, and repair Schwann cells (Heming et al. 2025). We found that *XIST*
489 was significantly downregulated in non-myelinating Schwann cells from PNP samples (adjusted
490 p -value = 2.98×10^{-2} , AvgLogFC = -1.91, two-sided likelihood ratio test) but not in myelinating
491 or repair Schwann cells (Supplemental Fig. S15, Supplemental Table S7). Heming *et al.* noted
492 that genes upregulated in non-myelinating Schwann cells from PNP samples were associated
493 with cell migration (Heming et al. 2025). We further found that the average log fold change value
494 for genes in the actin filament-based movement (GO:0030048) gene set was 0.56 (high in PNP)
495 comparing PNP non-myelinating Schwann cells vs controls, while the average log fold change
496 values for cilium-related genes (GO:0003351) was -0.19 (lower in PNP).

497
498 Overall, our results indicate that peripheral glia from diseased tissue exhibit significant changes
499 in *XIST* expression, but the direction of change depends on the disease (lower in PNP and higher
500 in cardiomyopathy), suggesting that *XIST* (specifically *sXIST* since it is the only apparent
501 transcript in male hearts) could potentially affect the expression of some genes implicated in
502 heart disease and neuropathy.

503
504 *Xist* expression in mouse male glial cells

505
506 To determine if *Xist* expression is conserved in male mouse Schwann cells, we focused on mouse
507 scRNA-seq data from peripheral nerves (Gerber et al. 2021; Yim et al. 2022). The data were
508 collected after male (n=2) and female (n=2) samples were pooled; thus, sex information was not
509 available for individual cells. We therefore used Y Chromosome gene expression to predict sex,
510 similar to the method described by Twa et al. (Twa et al. 2024). We found that labeling cells with
511 any non-pseudoautosomal Y Chromosome gene expression as from males was 91.18% accurate
512 using human skeletal muscle cells and 75.95% accurate using human heart cells (**Supplemental**
513 **Fig. S16**), but our method is expected to put many true male cells into the female group. We
514 applied this strategy to separate male cells in the scRNA-seq data collected by Gerber *et al.* for
515 peripheral nerves at postnatal day 1 (P1) and 60 (P60). The authors had identified Schwann cells,
516 so we retrieved these cells from their full data and then subclustered. A cell type in P1 called
517 fibroblast-related (FbRel) also expressed Schwann cell-related genes and were therefore
518 included. For both P1 and P60 data, we used the same myelin-related genes in **Figure 1** (*PLP1*,
519 *MPZ*, *MBP*, and *PRX*) to separate cells into myelinating vs non-myelinating cells. It is important
520 to note that all clusters expressed myelinating genes to some degree, but those with lower
521 expression were called non-myelinating Schwann cells (**Supplemental Fig. S17A-B**). The
522 second dataset from Yim *et al.* already had myelinating and non-myelinating Schwann cell
523 subtypes separated, so we directly compared cells in these two subtypes after predicting male
524 and female cells (**Supplemental Fig. S17C**). We should also note that the P60 sciatic nerve
525 sample had only 414 Schwann cells compared to 3,443 at P1 and 9,676 for other peripheral
526 nerves.

527
528 The predicted male Schwann cells from both studies expressed *Xist*, indicating co-expression of
529 *Xist* and Y Chromosome gene(s) in the same cells. Non-myelinating Schwann cells had the
530 greatest percentage of male cells expressing *Xist* in all three groups of samples (**Supplemental**
531 **Fig. S18, S19**). Yim *et al.* data exhibited greater between-sample variation in the percentage of
532 cells expressing *Xist* by cell type for both males and females (**Supplemental Table S8**,
533 **Supplemental Fig. S19**). A summary of these comparisons for each sample is in **Supplemental**
534 **Table S9**, which supports higher *Xist* expression in the non-myelinating compared to myelinating
535 Schwann cells (**Supplemental Fig. S18A-C**), consistent with the human data (**Fig. 1**). Gerber *et*
536 *al.* also provided a tool for their scRNA-seq sciatic nerve atlas (SNAT) data for visualizing gene

537 expression through mouse age and pseudotime by cell type in their Smart-seq2 data. Although
538 *Xist* was not detected in the Smart-seq2 samples (perhaps a technical effect), *Foxo1* (predicted by
539 SCENIC to regulate *XIST* in humans) exhibited higher expression in non-myelinating glia,
540 showed greater expression with increasing mouse age, and demonstrated increasing expression
541 through pseudotime for non-myelinating glia but not myelinating glia (**Supplemental Fig. S20**).
542 *Foxo1* expression was also higher in predicted male and female non-myelinating Schwann cells
543 in the mouse data we analyzed (**Supplemental Fig. S18**).

544
545 To expand our analysis to other mouse tissues, we again utilized the CZ CELLxGENE Discover
546 Gene Expression tool (CZI Cell Science Program et al. 2024) to determine the percentage of
547 male and female cells expressing *Xist* in mice by cell type across tissues (**Supplemental Fig.**
548 **S3B**). Across all tissues, cells in the eye had a particularly high percentage of male cells
549 expressing *Xist*, especially for amacrine cells (45%) and retinal ganglion cells (34%). Several
550 other amacrine cell subtypes also had a high percentage of male cells expressing *Xist* as well as
551 “neural” cells of the eye (7%), which may be glia. A summary of CELLxGene percentages by
552 cell type for mice can be found in **Supplemental Table S10**.

553
554 Taken together, our analysis of mouse RNA-seq data indicate that, like humans, male Schwann
555 cells express *Xist* with a bias toward the non-myelinating lineage. The *Xist* expression in mouse
556 male peripheral glial cells, however, seems less specific than in human data. At this point, it is
557 unclear if this represents a true species difference or a bias in sampling.

558 559 **Discussion**

560
561 *XIST* is the most studied long non-coding RNA (lncRNA) whose main and canonical functions,
562 interacting proteins, and individual functional domains have been dissected by many previously
563 published works. In this study, we found that a shorter *XIST* isoform is expressed in male
564 Schwann cells in both healthy and disease conditions, with its level higher in non-myelinating
565 Schwann cells. Our findings challenge the idea that *XIST* is exclusively female-specific in non-
566 cancerous cells, while our results support potential non-XCI roles of *XIST* in both male and
567 female peripheral glia cells.

568

569 Our data indicate that *XIST* expression in male Schwann cells is driven by a previously
570 uncharacterized promoter at the end of the first exon, leading to a shorter *XIST* RNA. This is
571 based on multiple lines of evidence, including RNA-seq read coverage, H3K36me3
572 modification, and long-read full-length transcripts. We believe this short *XIST* isoform is also
573 expressed in females based on shared scATAC-seq peaks across datasets and long-read RNA-seq
574 data from the heart; however, high expression of the full-length isoform makes it impossible to
575 conclude unambiguously if the same short transcript is present in both male and female glia.
576 Furthermore, although our presentation considered one short isoform, *sXIST*, our data do not
577 exclude the possibility that multiple short, spliced isoforms exist in the Schwann cells since our
578 current data did not have the resolution to resolve alternative splicing. Distinct *XIST* isoforms
579 have been reported (Johnston et al. 1998; Memili et al. 2001; Yue and Ogawa 2018), but the
580 documented isoforms are not related to *sXIST* reported here. We should also mention there is
581 some degree of *sXIST* expression variations across human individuals from whom all the bulk or
582 single cell data were derived. In addition to technical reasons, one possibility is that the tissue
583 samples could have different composition of non-myelinating Schwann cells or moreover *sXIST*
584 expression is affected by the activation state of Schwann cells.

585
586 Our finding brings up more questions than we could address here. The central question is
587 whether *sXIST* has a non-XCI functional role in Schwann cells, and more generally in peripheral
588 nerves, or if its expression is merely transcriptional noise. Since the *sXIST* transcript(s) does not
589 contain the tandem repeat domains required for XCI, we think its expression does not lead to
590 XCI, supported by our analysis and data. Instead, we suggest that *sXIST* RNA may regulate the
591 expression of autosomal genes involved in cilia-related processes and others that are important
592 for neuromuscular development and in male and female peripheral nerves. LncRNA functions,
593 however, are diverse and challenging to define (Statello et al. 2021; Mattick et al. 2023). Among
594 many other potential mechanisms, we suggest that *sXIST* could act as a miRNA sponge. This
595 hypothesis also considers the possibility of *sXIST* RNA existing as circular RNAs (circRNAs)
596 because it contains short repeat sequences. In fact, many circRNAs mapped to the locus are
597 recorded in the circAtlas (Wu et al. 2020), e.g., *hsa-XIST_0011*. Therefore, it is possible that
598 *sXIST* RNAs can sequester miRNAs that target genes in processes important for non-myelinating
599 Schwann cell functions, such as cell-cell signaling through primary cilia, which exist in glia and

600 may be involved in a variety of functions related to nerve regeneration signaling (Ki et al. 2021).
601 This is in line with the main functions of Schwann cells in supporting nerve development and
602 regeneration after injury. We also noticed that many zinc-finger transcription factors were up-
603 regulated in our *sXIST* overexpressing cells (**Supplemental Fig. S13E**), but the functional
604 implication is unclear.

605

606 Among the top 15 miRNAs predicted to target SPC genes, 5 of them (miR-361-3p, miR-32,
607 miR-149-3p, miR-122, and miR-367) have been reported to interact with *XIST*, although in those
608 studies the specific *XIST* isoforms were not addressed and were all in cancer contexts (Li et al.
609 2018; Cheng et al. 2020; Yang et al. 2020; Jiang et al. 2021; Wang et al. 2023).

610

611 From our *sXIST* overexpression data, we similarly found that three miRNAs (miR-520a-5p, miR-
612 9, and miR-34a) predicted to target upregulated genes were previously reported to interact with
613 *XIST*, with miR-520a-5p described in non-small cell lung cancer resistance (Liu et al. 2021) and
614 miR-34a in thyroid cancer (Liu et al. 2018). As for miR-9, it is a known regulator of
615 neurogenesis (Coolen et al. 2013), was shown to inhibit Schwann cell migration (Zhou et al.
616 2014), and is targeted by *XIST* to regulate mesenchymal stem cell differentiation in the bone
617 marrow (Zheng et al. 2020). If *XIST* acts as a sponge for miR-9, its downregulation in
618 neuropathy (**Figure S15**) may exacerbate symptoms because the excessive miR-9 could lead to
619 reduced Schwann cell migration to damaged nerves. It is therefore compelling that *HMMR* (also
620 known as *RHAMM* or *CDI68*) shares a binding site with miR-9-5p at its 3' UTR (**Fig. 5G**).
621 *HMMR* is important for the motility of Schwann/Schwann-like cells (Ouasti et al. 2020) and is
622 involved in the PLK1-dependent pathway that stabilizes astral microtubules coming from the
623 centrosome (Connell et al. 2017). Centrosomes and the microtubules they produce are important
624 for primary cilia development (Dantas 2020), so we wonder if *HMMR* and other cilia-related
625 genes affected by *XIST* expression could affect primary cilia production/maintenance.

626

627 Future studies are needed to address this and, more importantly, whether these miRNAs and
628 others predicted from our analysis bind to *sXIST* RNA in Schwann cells and lead to altered
629 expressions of their other targets, either at the transcript or protein level. We should note that
630 miRNAs can regulate their targets both negatively and positively (Hausser and Zavolan 2014).

631
632 It is important to point out that the model of *XIST* functioning as a miRNA sponge will depend
633 on its location in the cytoplasm, but some miRNAs are located in nuclei (Marshall et al. 2019;
634 Morey et al. 2025). The full length *XIST* RNA contains sequences that retain it to nuclei, but
635 these sequences are mostly absent in the *sXIST* transcript. Thus, although we did not have data to
636 resolve the sub-cellular location of *sXIST* RNA, conceivably it can be in the cytoplasm, nucleus,
637 or both (Politz et al. 2006; Leung 2015; Turunen et al. 2019). Future studies will be needed to
638 pinpoint *sXIST*'s cytoplasmic and/or nuclear distribution and determine its direct interactions
639 with key miRNA targets.

640
641 Our finding of *XIST* expression changes in cardiomyopathy and polyneuropathy is also
642 interesting. *XIST* and its miRNA sponging capacity are suggested in a variety of
643 cardiovascular/circulatory system-related diseases, such as myocardial ischemia, cardiac
644 hypertrophy, myocardial infarction, atherosclerosis, deep vein thrombosis, pulmonary arterial
645 hypertension, and more (Almalki 2024). Gorin and Goodman similarly showed that *XIST*
646 expression was higher in cardiomyopathy (Gorin and Goodman 2025), but we are unaware of
647 other literature directly linking *XIST* to this disease. Our results revealed that *RYR2*, which
648 exhibited greater negative correlation with *XIST* in male peripheral nerves and therefore may be
649 (either directly or indirectly) downregulated by the *sXIST* transcript, was significantly
650 downregulated in glial cells of cardiomyopathy hearts. Because *RYR2* is an important cardiac
651 muscle-related gene involved in multiple diseases (Sleiman et al. 2021), we wonder whether
652 ryanopathies are partly influenced by aberrant *sXIST* expression. If so, specifically targeting
653 *sXIST* could upregulate *RYR2* and modulate disease progression. However, more work is needed
654 to prove that *sXIST* directly influences *RYR2* expression.

655
656 Another critical question is whether the expression, regulation and function of *XIST* is conserved
657 across mammalian species. We tried to address this, but mining sex-specific gene expression
658 from published mouse RNA-seq data is extremely difficult because almost all studies pool
659 tissues from both sexes. We attempted to overcome this limitation by predicting the sex of
660 individual cells, but the approach will not work for bulk RNA-seq data. Based on our analysis
661 and some limited data in the mouse ENCODE Project, we are confident that *Xist* is expressed in

662 peripheral nerves in male mice, but we cannot determine if the alternative promoter and resulting
663 transcript(s) are conserved between humans and mice.

664

665 Finally, our analysis of the CELLxGENE data indicated that several other cell types, such as
666 pancreatic stem cells, ciliary epithelial cells, and fibro/adipogenic progenitor cells also exhibit
667 active *XIST* expression in male cells. In future studies, we need to address if *sXIST* is activated
668 and functional in these cell types like in Schwann cells.

669

670 In conclusion, our study suggests that *sXIST* is highly specific to peripheral glia and potentially
671 acts as a molecular sponge to sequester miRNAs that are involved in modulating various
672 Schwann cell functions. Further work is needed to confirm the exact region of *sXIST* that
673 regulates the expression of nervous system-related processes and whether this indeed occurs
674 through miRNA binding. While we focus our discussion on miRNA sponge function in this
675 study, the *XIST* functions in peripheral glia are very likely more complex. For example, we do
676 not know if the short and full *XIST* transcripts interact in female cells.

677

678 **Methods**

679

680 *Overexpression of sXIST in the HEK-293T cell line and RNA sequencing*

681

682 The last 3,323 base pair (bp) sequence of *XIST* exon 6 (ChrX:73,820,656-73,823,979(-)) was
683 cloned into pLV-eGFP vector and transfected into *HEK-293T* cells for overexpression. Total
684 RNA was isolated from the *sXIST* overexpression cells and controls for strand-specific RNA
685 sequencing (see **Supplemental Methods**). RNA-seq reads were mapped to the human genome
686 (GRCh38) with the GENCODE annotation (v46) (Mudge et al. 2025) by the STAR aligner
687 (v2.7.9a) (Dobin et al. 2013). Gene counts were determined using RSEM (v1.2.28) (Li and
688 Dewey 2011). Differential expression analysis was performed using DESeq2 (Love et al. 2014).

689

690 *Public bulk and single cell data*

691

692 *Human peripheral nerve bulk RNA-seq data:* The data were collected by Sandy-Hindmarch *et*
693 *al.* from 11 controls (4 females, 7 males) and 21 Morton's neuroma samples (18 females, 4
694 males) to understand the molecular signatures of nerve injury (Sandy-Hindmarch et al. 2025). All
695 Morton's neuroma samples were from the plantar digital nerves whereas control samples were

696 collected from a variety of upper and lower limb nerves (3 plantar digital, 3 gracilis motor, 4
697 posterior interosseous sensory, and 1 intercostal sensory). We downloaded the raw count
698 expression data and FASTQ files (generated from strand-specific RNA sequencing) from the
699 Gene Expression Omnibus (GEO): accession number GSE250152. We mapped the RNA-seq
700 reads with STAR (Dobin et al. 2013) to the GRCh38/hg38 genome build, as the authors did. The
701 alignment BAM files for individual samples were then merged by sex and neuroma status and
702 subsequently indexed using SAMtools v1.14 (Danecek et al. 2021). Then reads mapped to the
703 *XIST* locus (Chr X:73,820,656-73,852,714) were extracted and visualized in IGV (Robinson et
704 al. 2011). For expression difference between the 1st and 6th exon, the number of reads were
705 obtained using SAMtools for exon 6 (ChrX:73,820,548-73,828,166) and exon 1
706 (ChrX:73,841,317-73,852,768) for each sample. The ratios of the exon 6 reads to exon 1 reads
707 for male and female samples were compared by *t*-tests.

708
709 *Human tibial and sciatic nerve bulk RNA-seq, ATAC-seq and ChIP-seq data:* The data were
710 downloaded from the ENCODE Project (The ENCODE Project Consortium 2012) data portal,
711 including RNA-seq and ChIP-seq (H3K4me3, H3K4me1, H3K27ac, H3K36me3, H3K27me3,
712 and H3K9me3) data from the tibial nerve of 37 and 54-year-old males, and ATAC-seq data from
713 the sciatic nerve of a 26 year-old male and 16 and 47-year-old females. For positive (+) and
714 negative (-) strand RNA-seq bigWig files, we used bigWigSummary from the UCSC Genome
715 Browser (Perez et al. 2025) to get the average signal for exon 6 (ChrX:73,820,548-73,828,166)
716 and exon 1 (ChrX:73,841,317-73,852,768), which we then multiplied by the exon length to get
717 the total signal. We used these signal values to calculate the ratio of exon 6 to exon 1 signal for
718 each strand and sample. The accession numbers for the data used for results presented in **Figure**
719 **3** and **Supplemental Figure S7** are listed in **Supplemental Table S1**.

720
721 *Human heart long-read RNA-seq data:* The data were downloaded from the ENCODE Project
722 (The ENCODE Project Consortium 2012) data portal. BAM files for accession numbers
723 ENCFF330VRX and ENCFF327SFR were downloaded and filtered for reads on the X
724 Chromosome.

725
726 *Human heart sc/snRNA-seq data:* The data were from three different sources (Sim et al. 2021;
727 Mehdiabadi et al. 2022; Kanemaru et al. 2023) (**Supplemental Table S1**; **Supplemental**

728 **Methods**). These three datasets were re-analyzed using Seurat (Hao et al. 2024) (version 5) to
729 manage sample-level metadata (age/age range, sex, and dataset) and filter for cells/nuclei with >
730 200 detected genes and percent mitochondrial gene expression < 10% for scRNA-seq data and
731 5% for snRNA-seq data. Next, we normalized raw counts for all sc/snRNA-seq data with
732 Seurat's NormalizeData function, followed by FindVariableFeatures, ScaleData, and RunPCA
733 functions. We also used Scrublet (Wolock et al. 2019) to predict and remove doublets from these
734 data. Next, we used reciprocal principal component analysis (RPCA) (Hao et al. 2024) to
735 integrate data from all samples, followed by clustering for consistent cell type identification
736 using the original samples' metadata and cell type markers in the three original studies.

737
738 *Human Skeletal Muscle Atlas data*: The data were obtained from
739 <https://www.muscleageingcellatlas.org/> and they were collected by Kedlian *et al.* (Kedlian et al.
740 2024). We used only the “Fibroblasts and Schwann cells” dataset, which was converted from an
741 h5ad object to a Seurat object in R using the anndata (github.com/dynverse/anndata) and sceasy
742 (github.com/cellgeni/sceasy) R packages. This data was derived from the intercostal muscles of
743 12 (four females, eight males) deceased donors between the ages of 15 and 75 years-old. The
744 data was already preprocessed using SCANPY (Wolf et al. 2018), where the authors first used
745 CellBender (Fleming et al. 2023), which removes potential technical artifacts from sc/snRNA-
746 seq data. They then filtered for cells/nuclei with mitochondrial gene expression < 10% for
747 scRNA-seq data and <5% for snRNA-seq data, along with other gene detection and counts
748 filters, such as a count range between 400 and 400,00. The authors also shared their cell type
749 annotations, including non-myelinating and myelinating glia and the corresponding markers
750 (Kedlian et al. 2024).

751
752 *Cardiomyopathy heart snRNA-seq data*: These data were obtained from a previous study by
753 Reichart *et al.* The samples were collected from donor ventricles to characterize the cell type and
754 gene program changes that occur in hearts with arrhythmogenic right ventricular and dilated
755 cardiomyopathy (Reichart et al. 2022). Data for 18 controls and 61 cardiomyopathy hearts were
756 downloaded from [https://cellxgene.cziscience.com/collections/e75342a8-0f3b-4ec5-8ee1-
757 245a23e0f7cb/private](https://cellxgene.cziscience.com/collections/e75342a8-0f3b-4ec5-8ee1-245a23e0f7cb/private). They were uploaded by the authors and processed after filtering for
758 counts between 300 and 15,000, 300 – 5,000 detected genes for gene detection, and < 1%
759 mitochondrial expression. Cell type annotation was also provided. Because there were no male

760 cells in the non-compact cardiomyopathy samples, we eliminated this group from our
761 analysis, leaving us with normal, arrhythmogenic right ventricular cardiomyopathy, and dilated
762 cardiomyopathy groups.

763
764 *Mouse snRNA-seq data:* These data were from multiple peripheral nerves (GSE182098; snRNA-
765 seq) (Yim et al. 2022) and sciatic nerves (GSE137870; scRNA-seq) (Gerber et al. 2021) and
766 available as processed Seurat objects. The data by Yim *et al.* came from a combination of sciatic,
767 sural, vagus, and peroneal nerve collections (Yim et al. 2022). For both datasets, multiple mice,
768 regardless of sex, were pooled. Both provided detailed assessments of glial cell types/subtypes.
769 For Yim et al. data, we used their processed data and cell type annotations without further
770 filtering. For Gerber et al., we subclustered their labeled Schwann cells to help us differentiate
771 myelinating from non-myelinating cells. For the data by Gerber *et al.*, they were preprocessed by
772 filtering for cells with 500 - 5000 genes, while Yim *et al.* removed nuclei with < 500 genes and
773 with > 5% mitochondrial gene expression.

774
775 *Mouse and Human Cell Atlas:* The tissue and cell-type level expression data for *XIST/Xist* were
776 obtained using the CELLxGENE Discover Gene Expression tool (CZI Cell Science Program et
777 al. 2024). We summarize all cell-type-tissue combinations with *XIST/Xist* detection in > 30% of
778 male cells in **Supplemental Figure S3** and provide the percentages for all other cell types by sex
779 and tissues in **Supplemental Tables S3** and **S10**. These data came from many sources and
780 studies, but were re-processed consistently by the CELLxGENE team, including removal of
781 duplicate cells, cells with < 500 genes, and keeping only data derived from sequencing assays
782 that do not require gene-length normalization, such as 10x 3' and 5' kits, Drop-Seq, Seq-Well,
783 and CEL-Seq. The data can be obtained from <https://cellxgene.cziscience.com/gene-expression>.

784
785 *Multomic heart data:* 10X multiomic scRNA/ATAC-seq data was also obtained from the Heart
786 Cell Atlas (Kanemaru et al. 2023). ATAC fragments and peaks from ten (four female, six male)
787 adult hearts (between 20 and 75 years-old) were obtained from <https://www.heartcellatlas.org/>.
788 Author-provided metadata was used for labeling major cell types and sex. We used the Signac R
789 package (Stuart et al. 2021) and followed the standard analysis procedures to merge data for each
790 of the samples. Peaks were originally called based on high resolution cell types that the authors
791 annotated. We thus utilized MACS2 (Zhang et al. 2008) to re-call peaks after merging data to the

792 level of broad cell types. We also analyzed the expression data after retaining only cells present
793 in the ATAC dataset based on barcode. We then used Signac’s built-in plotting functions to create
794 ATAC coverage plots over the *XIST* locus.

795

796 *CATlas data*: The Cis-element Atlas (CATlas) (Zhang et al. 2021) is a crowdsourcing resource
797 that curates human and mouse *cis*-regulatory element reference maps. We downloaded and used
798 bigWig files without further processing. We obtained these cell-type level bigWig files for
799 snRNA and ATAC-seq data from their curated heart and multi-tissue datasets.

800

801 *sc/snRNA-seq data cell type identification*

802

803 For sc/snRNA-seq data without cell type annotations for direct usage, we first computed cell
804 type markers using Seurat’s FindAllMarkers function, which performs a Wilcoxon Rank-Sum
805 Test between cells in each cluster and all other cells. We then compared the top markers with
806 known glia, myelination, and neuronal markers, adapted from Kanemaru et al. (Kanemaru et al.
807 2023), to further identify glial subtypes (myelinating, non-myelinating, or other) and to ensure
808 that they were negative for neuronal markers. *SCN7A* was identified as a Schwann cell marker in
809 the Human Protein Atlas (Karlsson et al.) (Karlsson et al. 2021) and is more highly expressed in
810 non-myelinating Schwann cells by Yim et al. (Yim et al. 2022), along with *SLC35F1*. Thus, these
811 genes were included as Schwann cell markers. *NRXN1*, *NRXN2*, *NCAM2*, *ALDH1A1*, and *S100B*,
812 like by Kanemaru et al., were used as glial cell markers. Kanemaru et al. considered *MBP*, *MPZ*,
813 and *PRX* as general Schwann cell markers; however, we used these myelinating genes in addition
814 to *PLP1*, which encodes a major component of myelin (Diehl et al. 1986), to specifically identify
815 myelinating Schwann cells. We also added *XIST*, *CADM2*, and *SMYD3*, which were markers for
816 cardiac non-myelinating Schwann cells from our integrated data. *NGF* was used to identify
817 “other” glia since *NGF*+ heart glia clustered separately. Because *NRXN1* and *NRXN2* are also
818 expressed in neurons, we needed to include genes to ensure that glia were not neurons. For this,
819 we used the same neuronal markers as Kanemaru *et al.*, which included *PRPH*, *STMN2*,
820 *CACNA1B*, *NEFL*, *NEFM*, *NEFH*, *DLG4*, and *TUBB3*. The markers listed here were also used to
821 identify Schwann cells in non-heart datasets when such cell types were not defined or shared by
822 the original authors.

823

824 *Differential expression analysis*

825

826 For sc/snRNA-seq datasets, pseudobulking by cell type or subcluster was performed prior to
827 differential expression analysis with DESeq2 (Love et al. 2014) in order to use the sample
828 replicates appropriately. For the integrated human heart data, a group variable was introduced to
829 account for batch effects (e.g., labs) observed across datasets. All other parameters were set to
830 default. For more information on density plots, gene set enrichment analysis, and
831 overrepresentation analysis, please refer to **Supplemental Methods**.

832

833 *Defining genes with strong expression correlation with sXIST*

834

835 To predict genes that may be regulated by the *sXIST* transcript, we applied co-expression analysis
836 to the human peripheral nerve bulk RNA-seq data from Sandy-Hindmarch *et al.* To control for
837 the effect of expression level, we first needed to obtain a list of genes with similar levels of
838 expression as *XIST*. Based on the DESeq2-normalized count data, we filtered for genes with
839 mean expression values across samples within ± 3 of *XIST*'s mean. After, we randomly selected
840 100 of such genes and calculated the Pearson's correlation between them (and *XIST*) and all
841 other genes across male and female samples, separately. Thus, for each of these 101 genes, we
842 obtained male and female correlation coefficient values with the rest of the (expressing) human
843 genes. The values were used to prepare a female vs male correlation coefficient 2D plot. We next
844 fit an ellipse around 99% of the data points to obtain a background null distribution. Applying
845 this ellipse to the correlation coefficients for *XIST*, we obtained genes whose expression
846 correlation with *XIST* were higher than the null background, i.e., outliers located outside of the
847 ellipse. We further filtered for genes with an absolute difference > 0.3 between their correlation
848 coefficients in male and female samples and grouped them into two groups: Stronger Negative
849 Correlation (SNC) genes (male correlation coefficient (r) < 0 and p -value < 0.05), and Stronger
850 Positive Correlation (SPC) genes (male correlation coefficient (r) > 0 and p -value < 0.05).

851

852 *Pooled mouse scRNA-seq sex prediction*

853

854 To predict each cell's sex (XX/XY) for pooled mouse sc/snRNA-seq samples, we first obtained a
855 list of all Y Chromosome genes using the biomaRt (Durinck et al. 2009) R package. We then
856 annotated a cell as male if it showed expression of any of the Y Chromosome genes. Changing
857 this to stricter criterion, e.g., increasing the expression counts to 3, did not affect our results. For

858 the mixed peripheral nerve data by Yim *et al.*, we eliminated Y Chromosome genes starting with
859 “Gm” from this filtering step, which were abundant in the dataset (perhaps due to their
860 abundance in snRNA-seq). To assess the positive rate of this cell-level sex prediction, we used
861 integrated heart and the Skeletal Muscle Atlas single cell/nucleus data, described above.

862 863 **Data Access**

864
865 RNA-seq data from HEK-293T cells were deposited to the GEO database (accession number
866 GSE309419). All analysis codes in R (R Core Team 2023) are publicly available at
867 <https://github.com/bioinfoDZ/sXIST> and included in **Supplemental Codes**.

868
869 **Conflict of Interest:** none declared.

870 871 **Acknowledgements**

872
873 The authors would like to thank the Einstein High Performance Computing and members in the
874 Zheng Lab for providing valuable suggestions. This study was supported partially by the
875 National Heart, Lung, and Blood Institute (NHLBI) grants R01HL153920 and R01HL163667
876 (B.M., D.Z) and by the National Institute of Arthritis and Musculoskeletal and Skin Diseases
877 (NIAMS) grants R01AR069078 and P30AR079200 (E.E).

878 879 **Author contributions**

880
881 K.O. and D.Z. conceived and designed the study. K.O. performed all the bioinformatics data
882 analysis, with assistance from K.J. M.L. and E.E. performed the *sXIST* overexpression assay.
883 L.S. and B.M. contributed to the mouse data analysis. K.O. and D.Z. wrote the manuscript. All
884 authors reviewed and edited the manuscript.

885 886 887 **References**

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1139 967-976.
- 1140 **Figure Legends:**
1141
1142 **Figure 1: High XIST expression in non-myelinating Schwann cells from male human heart**
1143 **and skeletal muscle tissue. A)** Clusters of 510,171 human heart cells from 11 male and 9 female
1144 individuals. Cells within the dashed box are glia, which are colored by subtypes. EC, endothelial
1145 cells; FB, fibroblasts; aCM, atrial cardiomyocytes; vCM, ventricular cardiomyocytes;

1146 mSchwann, myelinating Schwann; nmSchwann, non-myelinating Schwann. **B/E)** A variety of
 1147 positive and negative markers used to identify male and female myelinating and non-myelinating
 1148 Schwann cells. **C/F)** Seurat-normalized *XIST* expression across all cells, grouped by cell types
 1149 and split by sexes. The asterisk represents a significant difference in expression between male
 1150 non-myelinating (nm) and myelinating (m) Schwann cells. **D)** Clusters of 20,611 skeletal muscle
 1151 fibroblast and neural cells from 8 male and 4 female individuals, colored by cell annotations
 1152 provided by the original authors.

1153

1154 **Figure 2: Chromatin accessibility at the *XIST* locus across human heart cell types. A)**
 1155 Multiomic scATAC/RNA-seq signal and expression (split by sex) across cell types. MACS2-
 1156 called peaks are represented as “cell-type peaks.” The normalized signal across all cells and
 1157 fragments for 100 random cells are presented above the *XIST* gene locus, with introns and exons
 1158 as thin and thick green lines, respectively. **B)** A zoomed-in view of scATAC-seq signal for male
 1159 (blue) and female (brown) glia. **A-B)** The red highlighted region represents the glia-specific peak
 1160 (ChrX:73,841,364-73,841,611) called by MACS2.

1161

1162 **Figure 3: Assessment of ATAC, RNA, and ChIP-seq read coverages over the *XIST* locus. A)**
 1163 snRNA/ATAC-seq tracks from CATlas heart dataset. **B)** RNA-seq (+/- strands), H3K4me3,
 1164 H3K4me1, H3K27ac, and H3K36me3 ChIP-seq from two human male tibial nerve samples and
 1165 ATAC-seq tracks from one human male and two female sciatic nerve samples in the ENCODE
 1166 database. **C)** snATAC-seq tracks from CATlas multi-tissue dataset. **A-C)** All plots show the
 1167 region at ChrX:73,790,905-73,854,216 from the hg38 reference genome. The 3' region of *XIST*
 1168 exon 1 is enclosed by two red dashed lines. *TSIX* gene location spanning ChrX:73,792,205-
 1169 73,829,231 on the + strand is shown as a blue bar, indicating the RNA-seq reads were from *XIST*
 1170 but not *TSIX*. For each track, cell types and/or sequencing modality are shown on the left while
 1171 the data range is shown in the right. Additional data for the tibial and sciatic nerve samples are in
 1172 **Supplemental Figure S7.**

1173

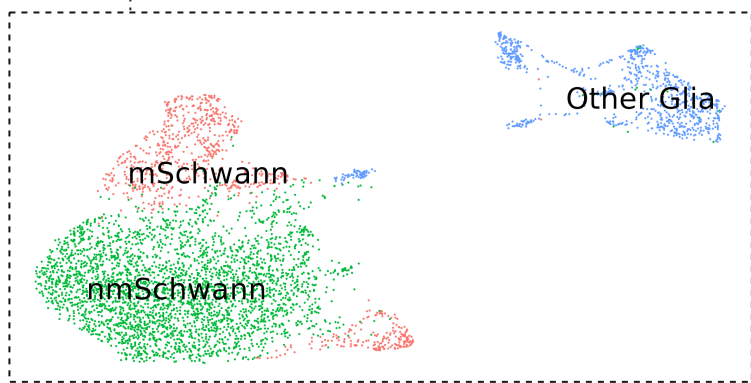
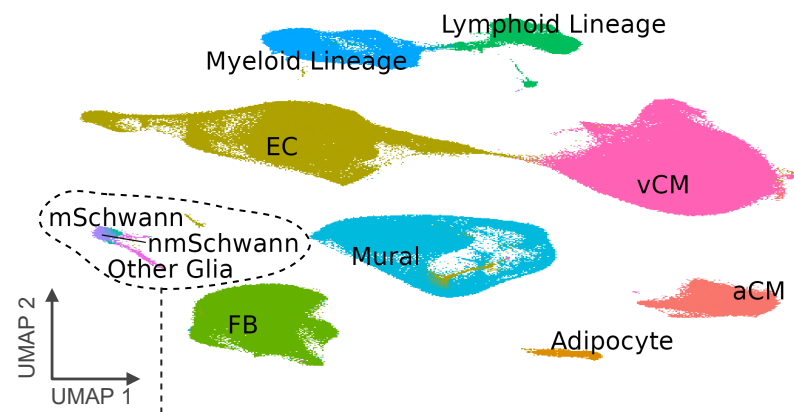
1174 **Figure 4: Potential roles of *XIST* in peripheral nerve. A-B)** Density plots of \log_2FC from a
 1175 variety of gene group comparisons using human skeletal muscle data (**A)** and human heart data
 1176 (**B)** along with their expected and observed trends. For X Chromosome non-escapee genes, the
 1177 expected trend is based on the presumption that *sXIST* participates in XCI. In all comparisons,
 1178 the \log_2FC values were computed from $\log_2(\text{sample-on-right}/\text{sample-on-left})$. Gene groups with
 1179 too few genes for robust density analysis in a comparison were not included in these plots.
 1180 Expected and observed trends for **A** and **B** are summarized at the bottom. **C)** Pearson correlation
 1181 coefficients (r) between *XIST* and all other genes computed separately for males (x-axis) and
 1182 females (y-axis) using peripheral nerve bulk RNA-seq data. In this plot, each dot is a gene, and
 1183 the predicted 99% confidence ellipse was overlaid (refer to **Supplemental Figure S12**). Outlier
 1184 genes with a male Pearson's correlation p -value < 0.05 and absolute difference > 0.3 between
 1185 male and female correlation coefficients were colored red if male $r > 0$ and blue if male $r < 0$. **D)**

1186 Two gene examples (*DNAAF1* and *KCNAB1*) exhibiting stronger positive (red) and negative
 1187 (blue) expression correlation with *XIST* in males. Expression values are provided as
 1188 $\log_2(\text{DESeq2-normalized counts} + 1)$. **E-F** Overrepresentation analysis for genes with stronger
 1189 negative male correlations (**E**) or stronger positive male correlations (**F**). Dot size represents the
 1190 number of query genes in each term and color is $-\log(\text{FDR adjusted } p\text{-value})$.

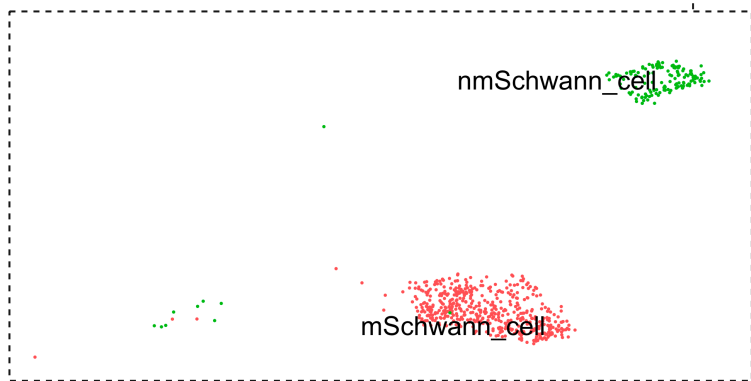
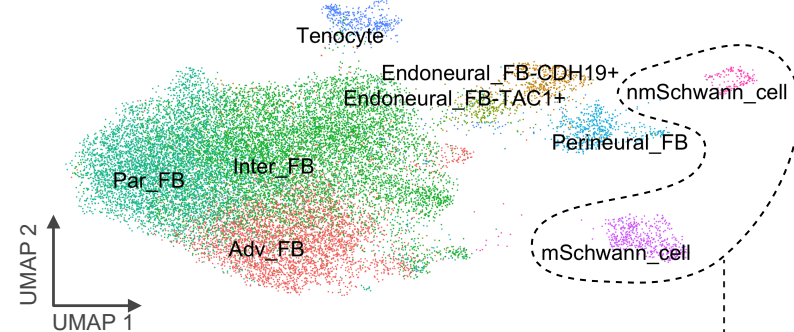
1191
 1192 **Figure 5: RNA-seq analysis of *sXIST* overexpression.** **A)** IGV tracks showing RNA-seq reads
 1193 over ChrX:73,790,905-73,854,216, with *TSIX* and *XIST* indicated. Samples overexpressing the
 1194 second half of exon 6 (*XIST* OE) are shown in red while GFP controls (Ctrl) are in blue. **B)**
 1195 Density and boxplots showing $\log_2\text{FC}$ values across gene groups. Values above boxplots are the
 1196 mean $\log_2\text{FC}$ for that group. **C)** Heatmap of the DEGs (adjusted p -values < 0.05 and $|\text{fold}$
 1197 $\text{change}| > 1.5$). **D-E)** ToppFun results from downregulated (**D**) and upregulated (**E**) genes in **C**.
 1198 **F)** Boxplots showing the expression of *XIST*, *DNAAF1*, and *HMMR*. **G)** Cloned *XIST*, *DNAAF1*,
 1199 and *HMMR* transcript sequence alignments with hsa-miR-9-5p.

1200
 1201 **Figure 6: *XIST* expression in arrhythmogenic and dilated cardiomyopathy.** **A)** UMAP of
 1202 881,081 heart nuclei from snRNA-seq data performed on 61 heart failure patients and 18 healthy
 1203 controls (Reichart et al. 2022). Cells originally labeled “neural” were renamed to “glia” based on
 1204 expression of glial markers (**Supplemental Figure S14**). **B)** Normalized *XIST* expression across
 1205 all nuclei, grouped by cell type and split by sex. For males, the percentages of cells expressing
 1206 *XIST* are shown at the top. **C-I)** Cells were subset by glia cell type annotation and grouped based
 1207 on arrhythmogenic (arrhyth) RV cardiomyopathy, dilated cardiomyopathy, and normal (healthy)
 1208 annotations and sex. Normalized *XIST* (**C**), *FOXO1* (**E**), *COL4A6* (**F**), and *RYR2* (**G**) expression
 1209 after pseudobulking glial cells by donor. **D)** The percentage of glial cells that express *XIST* for
 1210 each sample by disease group and sex. **H-I)** Module scoring was performed on SPC (**E**) and
 1211 SNC (**I**) genes from **Figure 4**. Asterisks indicate a significant difference using Fisher’s exact test
 1212 (**D**) or DESeq2 negative binomial test (**C,E-I**).

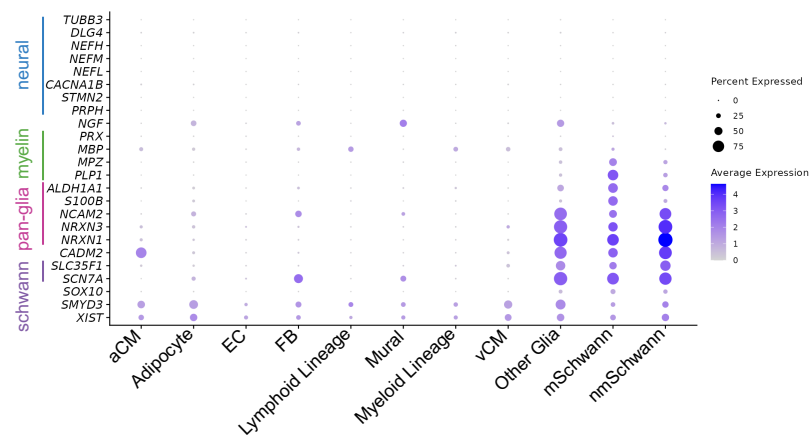
A Integrated Human Heart



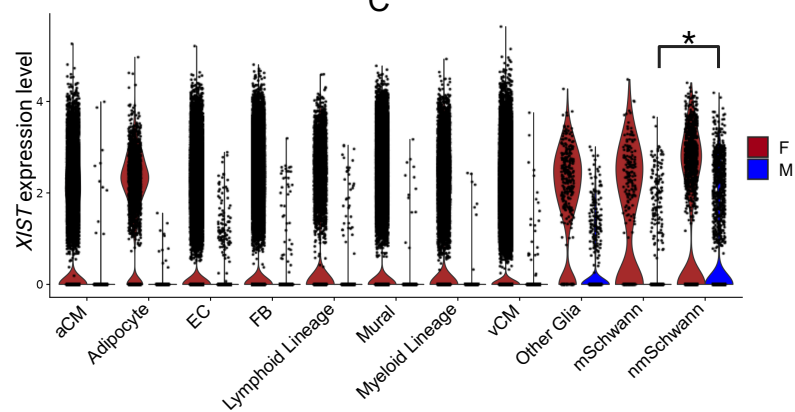
D Human Skeletal Muscle Atlas



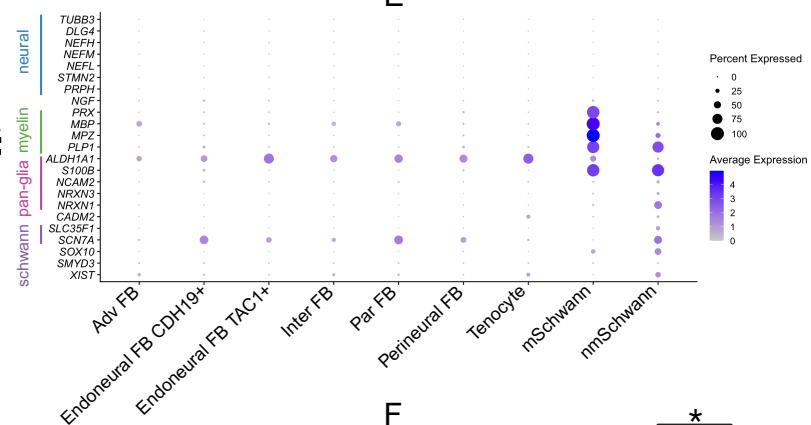
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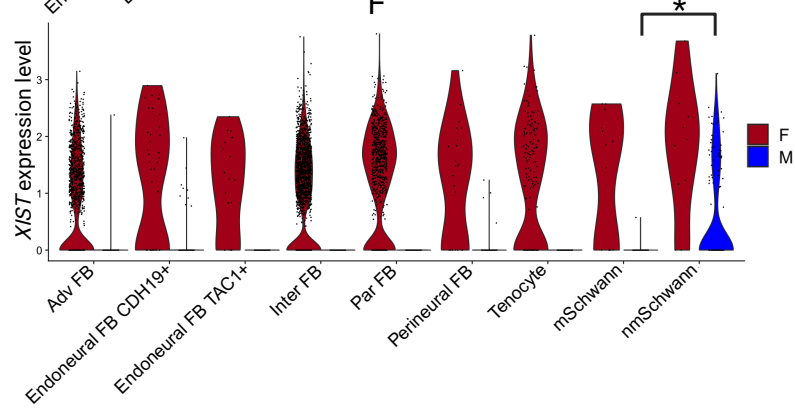
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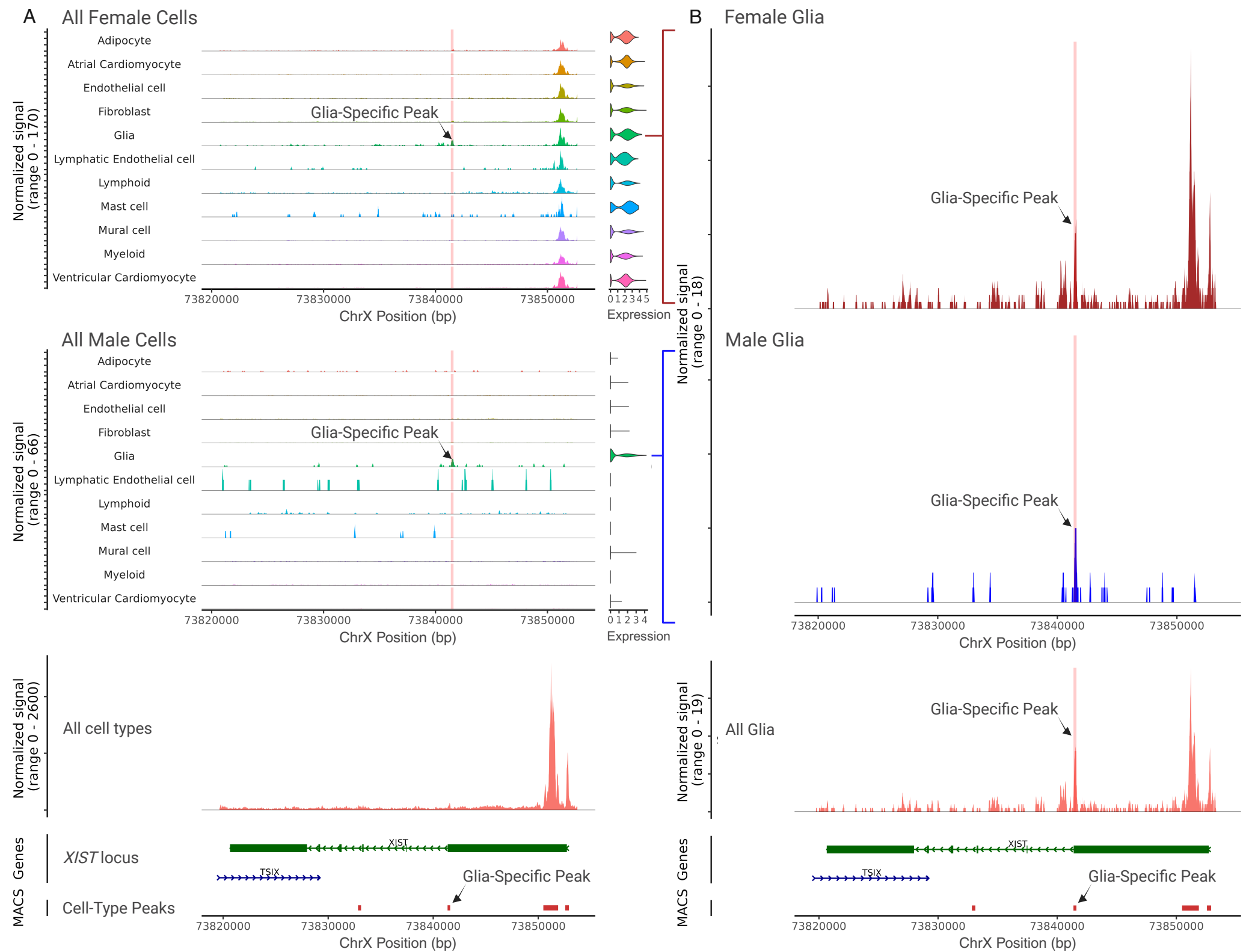


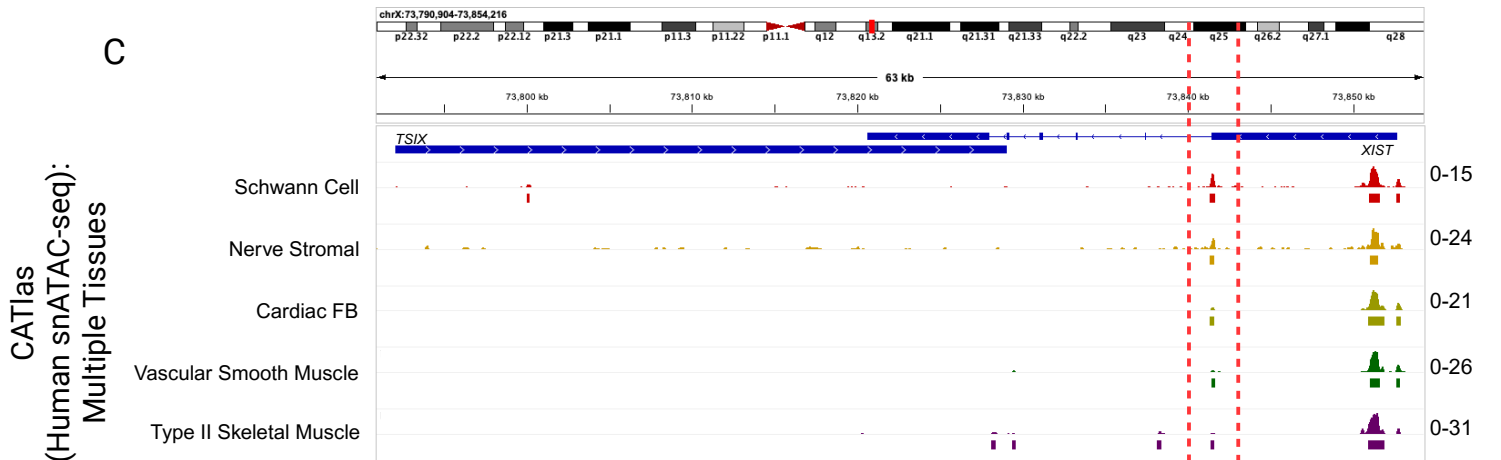
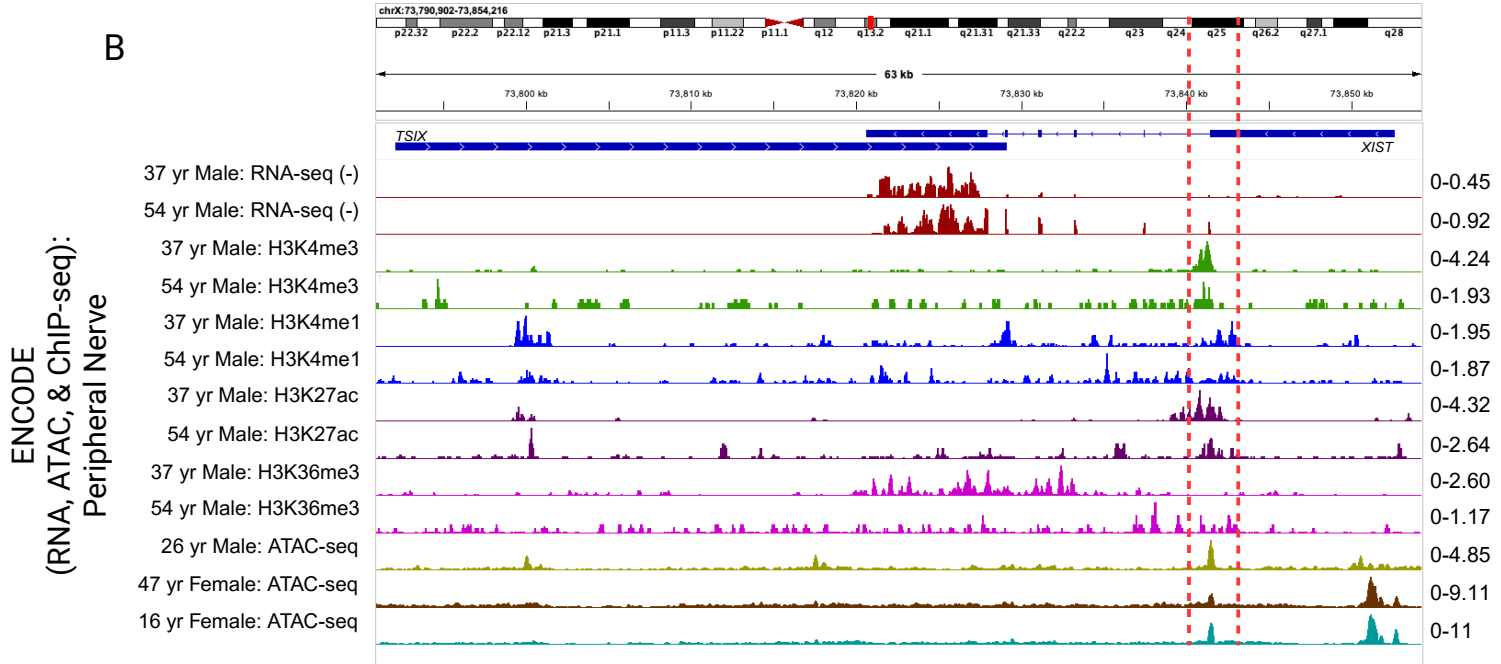
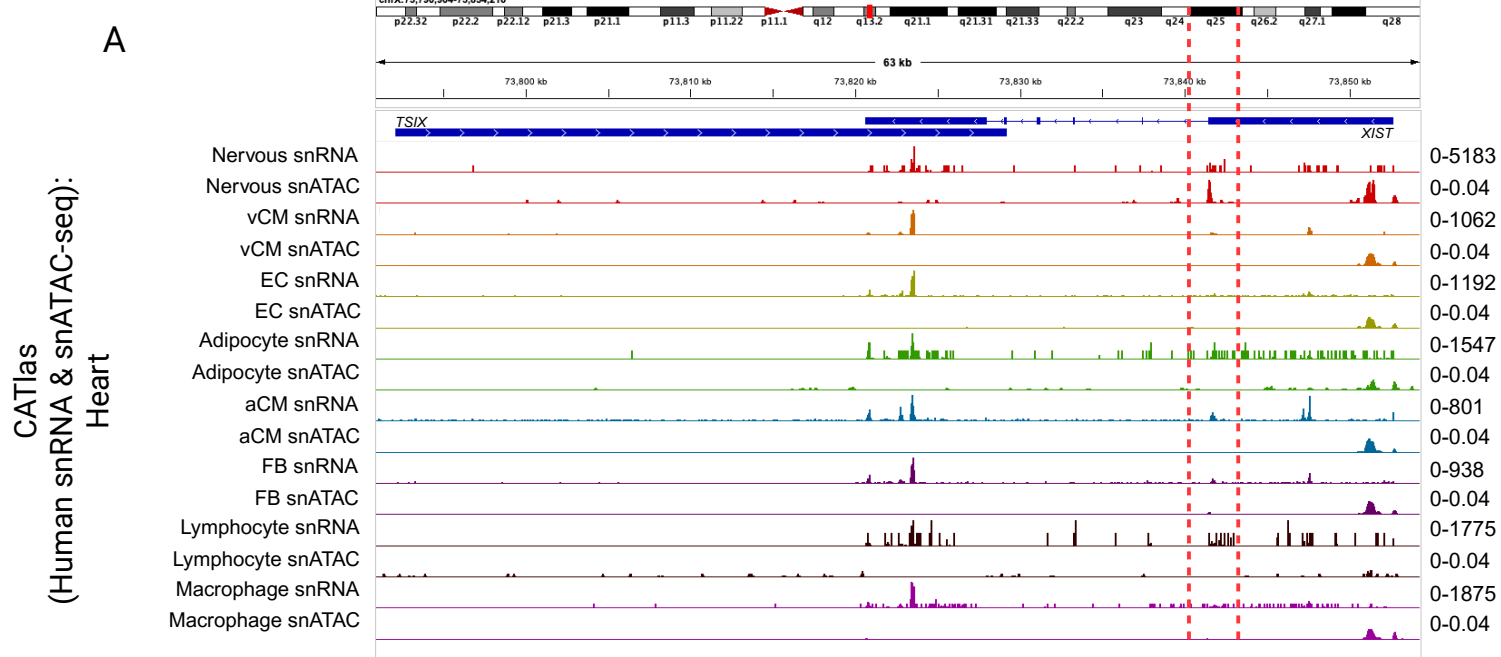
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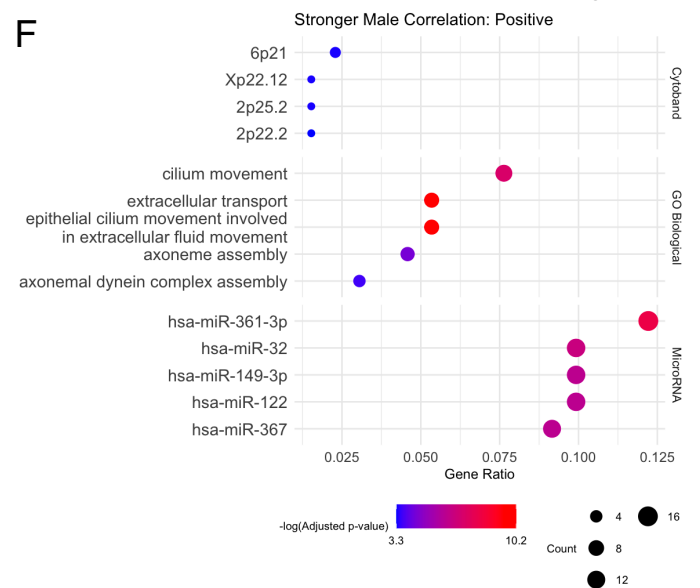
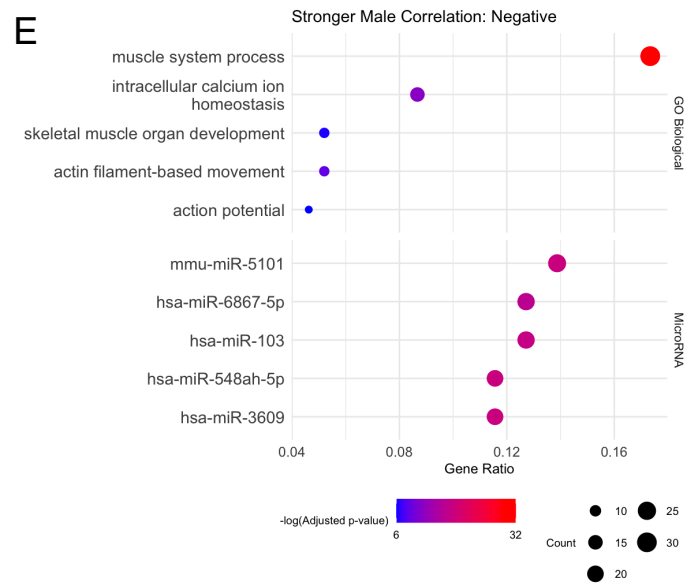
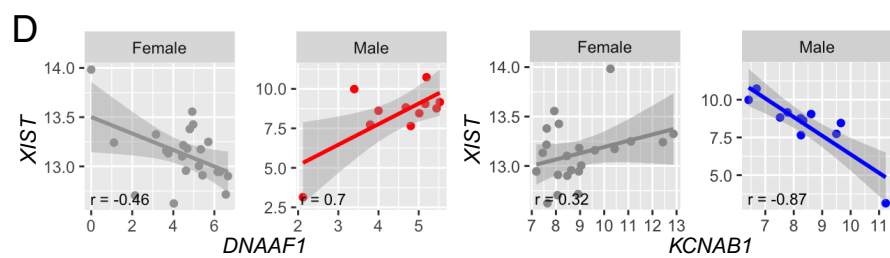
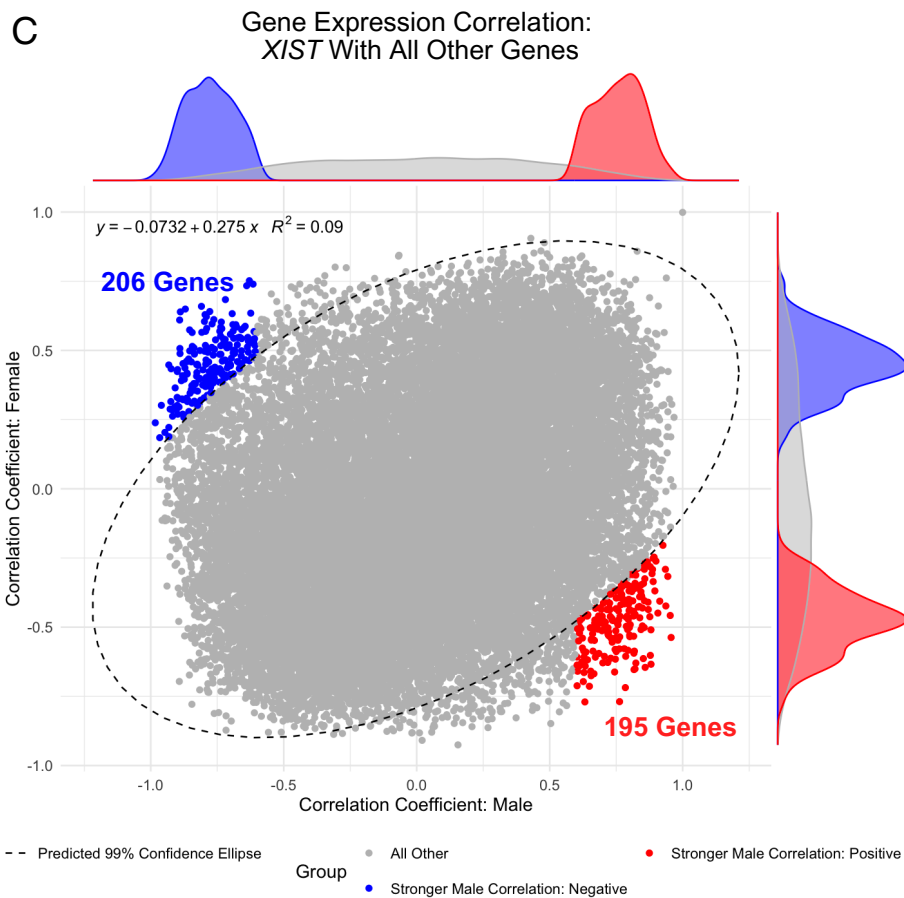
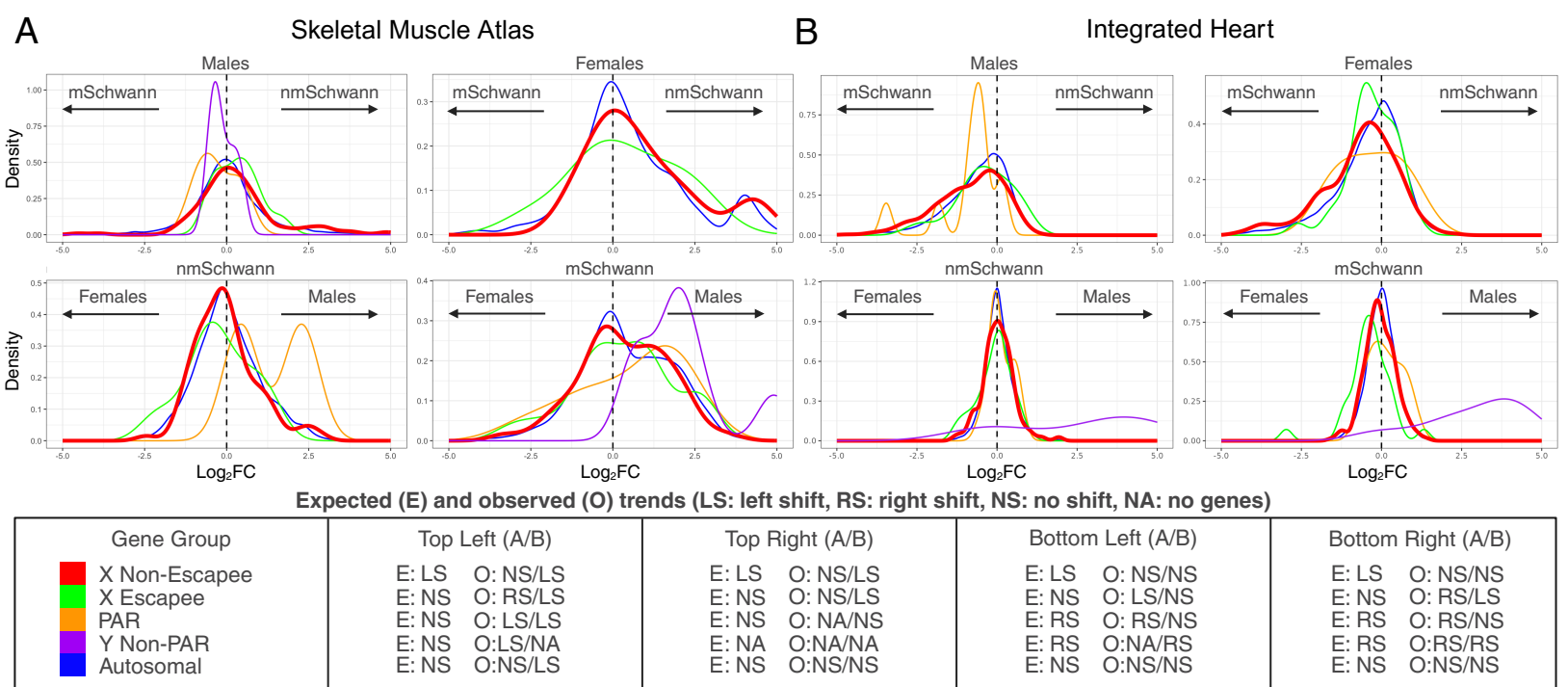


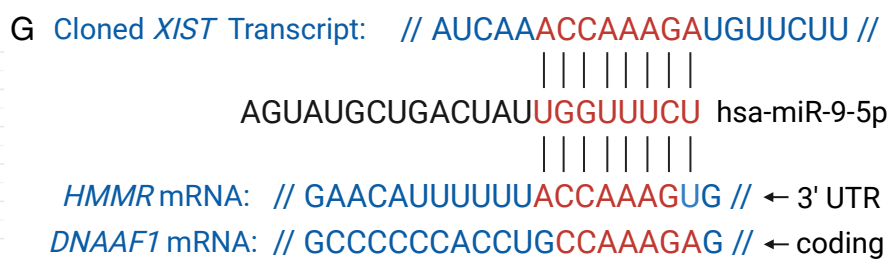
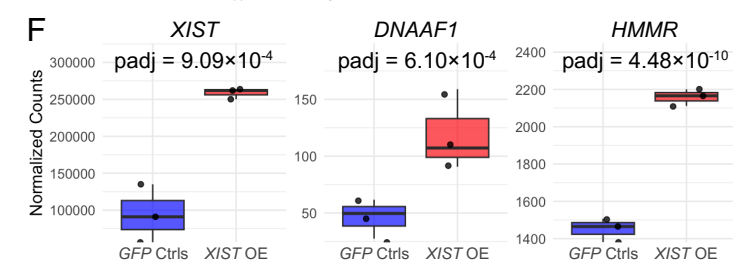
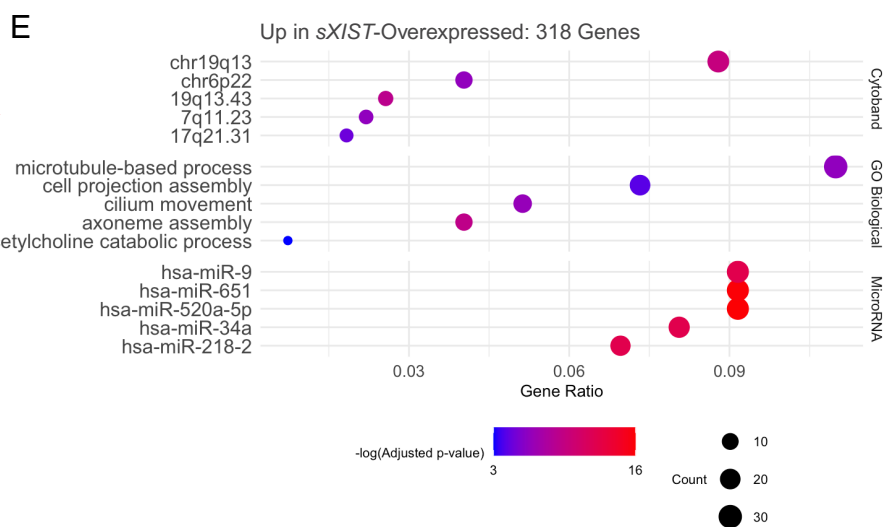
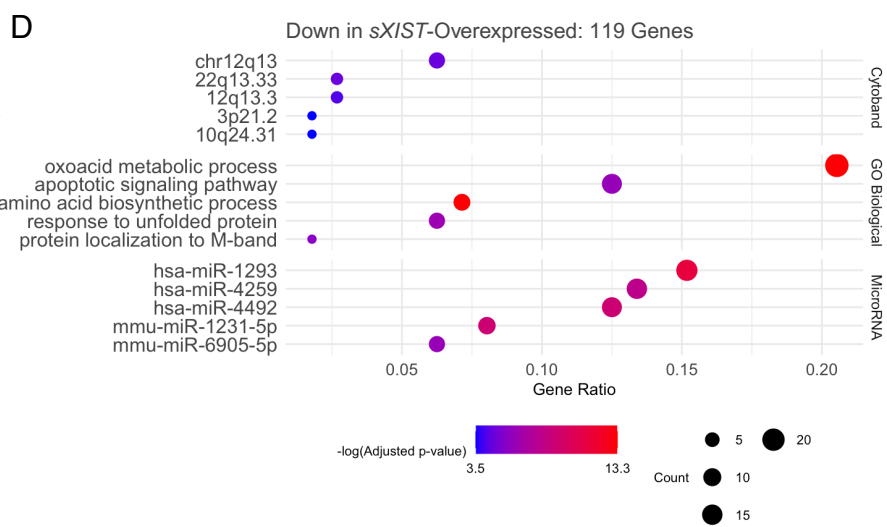
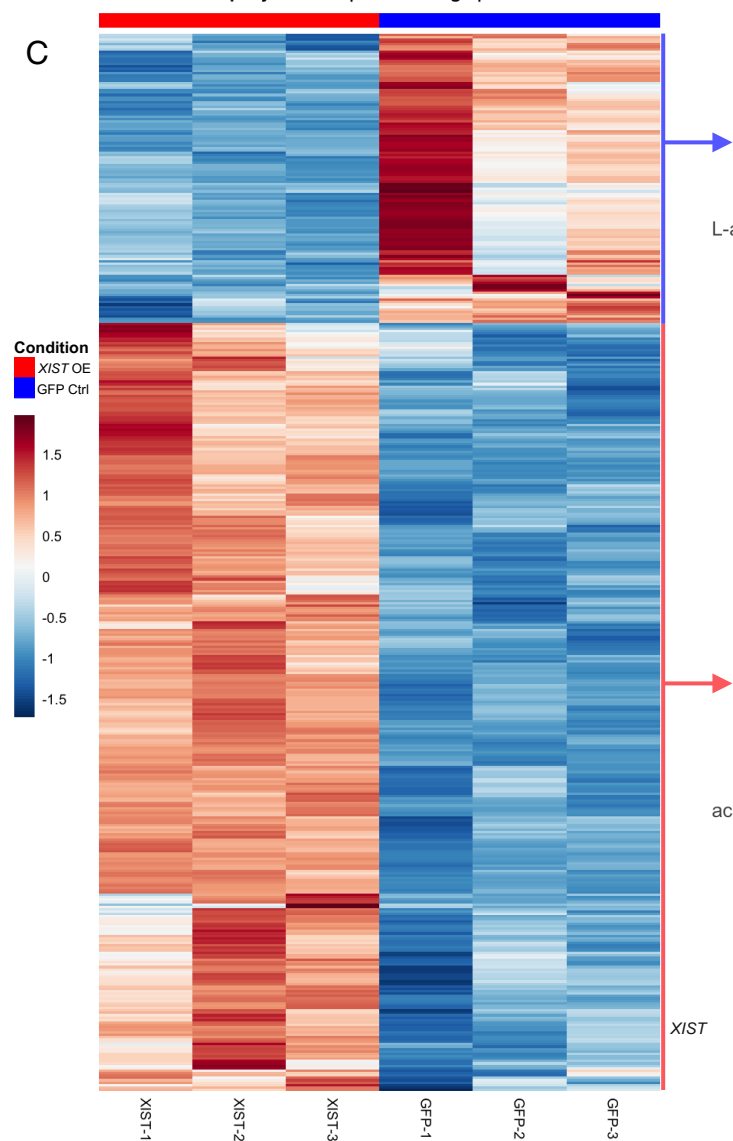
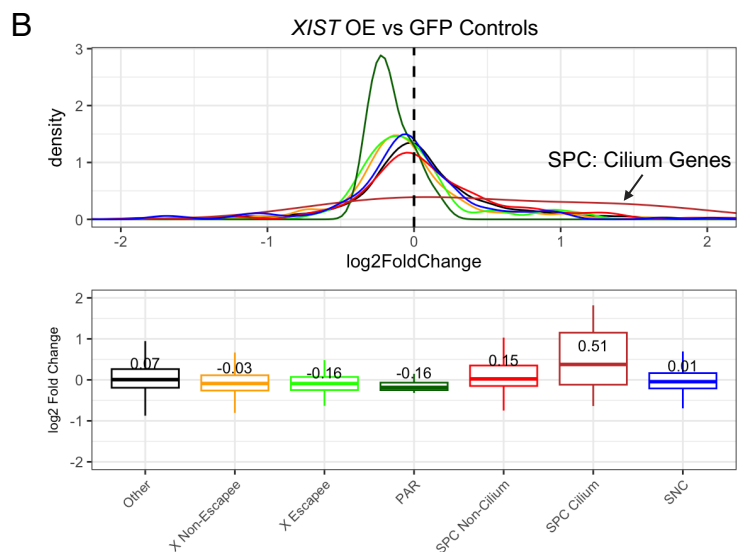
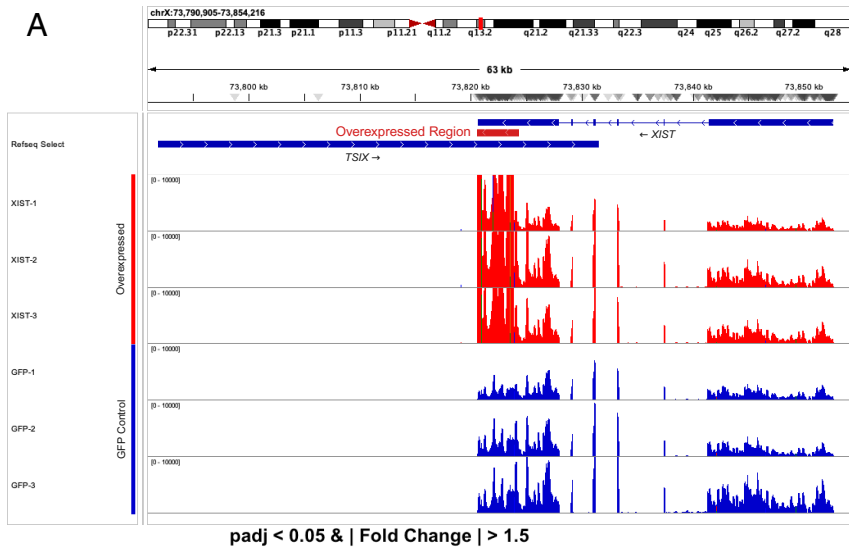
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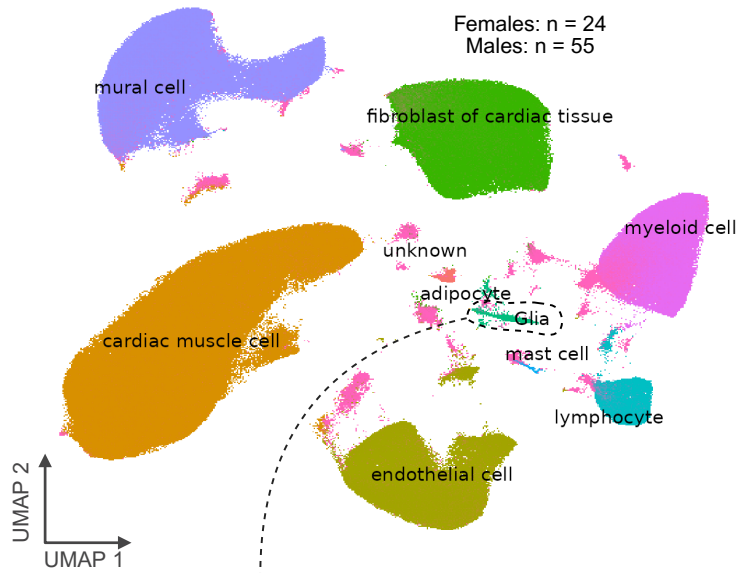




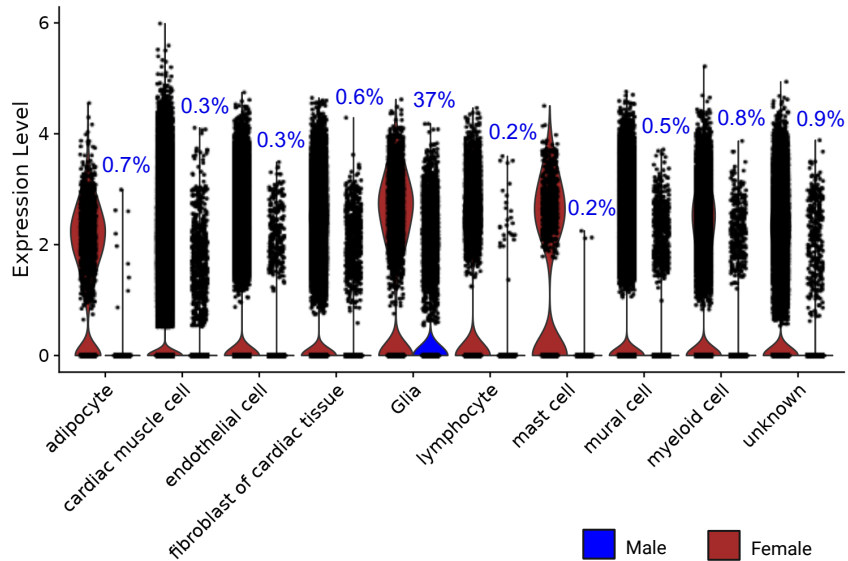




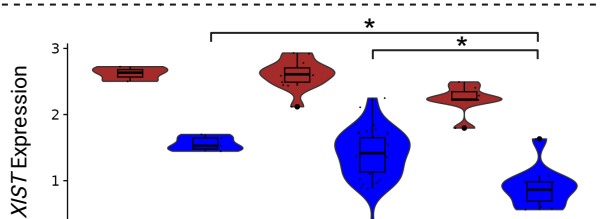
A Healthy and Diseased Human Heart



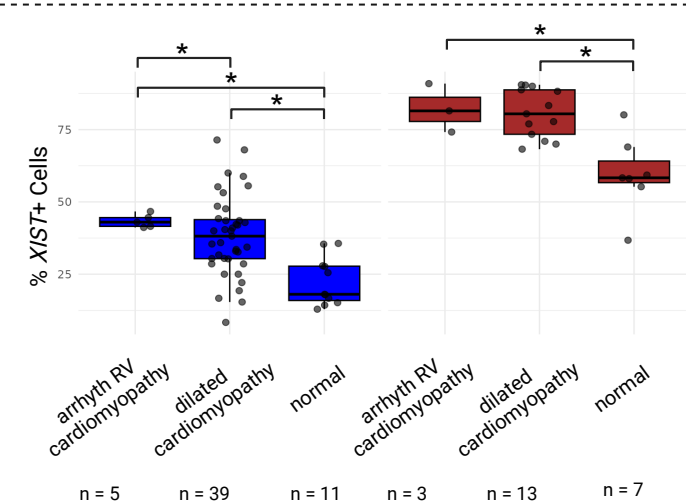
B *XIST*: All Cells



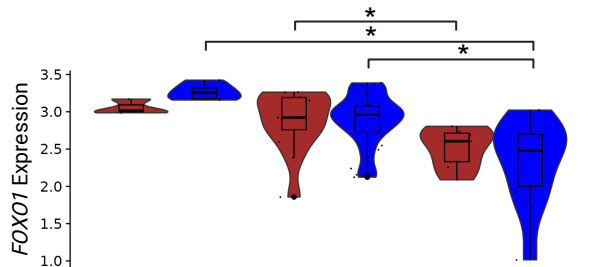
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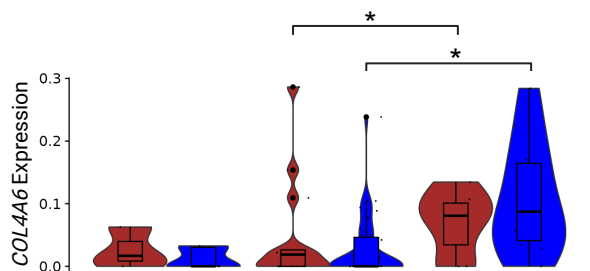
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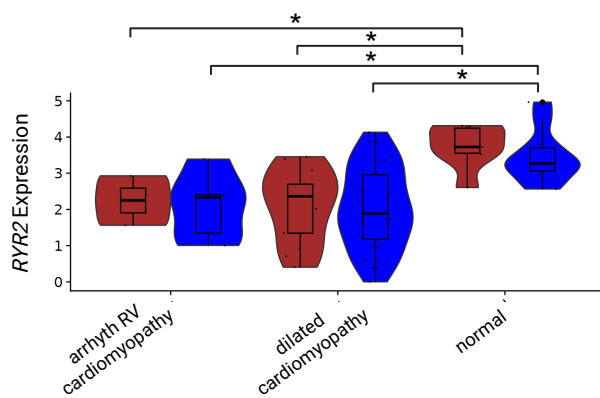
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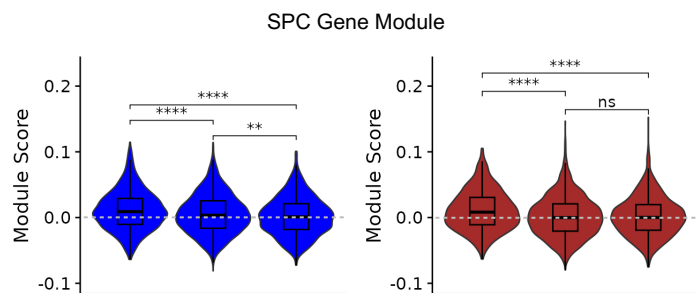


G



Females: n = 3 Males: n = 5 Females: n = 13 Males: n = 39 Females: n = 7 Males: n = 11

H



I

