SoxB1-Mediated Chromatin Remodeling Promotes Sensory Neuron Differentiation in Planarians

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Abstract

Understanding how adult stem cells generate neurons is critical for advancing regenerative medicine. However, few in vivo models enable studying how stem cell fates are specified as neurons in an adult body. The planarian *Schmidtea mediterranea* provides a powerful system for investigating these mechanisms, owing to its abundant adult pluripotent stem cells, termed neoblasts, and its capacity to regenerate a molecularly complex nervous system. The SoxB1 family of transcription factors is broadly implicated in ectodermal lineage commitment. In planarians, the SoxB1 homolog *soxB1-2* has been shown to promote neural and epidermal differentiation. However, the mechanisms by which *soxB1-2* influences chromatin dynamics and transcriptional programs during adult neurogenesis remain unknown. To address this, we performed ATAC-seq and RNA-seq on neural-rich head tissues to assess how *soxB1-2* RNAi knockdown alters chromatin accessibility and gene expression. Disrupting *soxB1-2* resulted in reduced chromatin accessibility and transcriptional downregulation at neural and epidermal loci, consistent with a pioneer-like role in chromatin priming. We identified 31 candidate downstream targets with concordant accessibility and expression changes, including the transcription factors *castor* and *mecom*, which regulate mechanosensory and ion transport genes. Head tissue sampling enabled the detection of *soxB1-2*-responsive genes within rare neural subtypes that were missed in our previous whole-body RNA-seq experiments. These findings offer mechanistic insight into adult ectodermal lineage specification and establish a framework for understanding chromatin-mediated neurogenesis in regenerative systems.

Introduction

The nervous system is necessary for most of the physiological and behavioral activities of metazoans. However, many adult organisms lack the ability to regenerate lost neurons *de novo*. Understanding the molecular programming of stem cells during adult neurogenesis may inform strategies to induce neural fates and restore damaged or lost neurons. One highly conserved class of transcription factors that has roles in neural programming during development includes the SoxB1 group proteins. Diversification of the SoxB family into SoxB1 and SoxB2 subfamilies occurred before diversification of vertebrates, and the ancestral bilaterian had both a SoxB1 and SoxB2 gene (Zhong et al., 2011). Thus, members of the SoxB1 family are conserved across bilaterians, including SoxB1 family members Sox1, 2, and 3 in mammals, of which Sox2 is critical for maintaining stem cell potency and directing the specification of multiple neural progenitor lineages (Zhang and Cui, 2014). Beyond development, SoxB1 genes are expressed in adult tissues and contribute to regenerative processes across diverse organisms (Kamachi and Kondoh, 2013; Reiprich and Wegner, 2015; She and Yang, 2015).

SoxB1 homologs regulate neurogenesis across various neural lineages by modulating transcription factor networks that drive terminal fate decisions (Miyagi et al., 2009). They are thought to function as pioneer transcription factors, capable of binding to closed chromatin and altering accessibility, thus facilitating the binding of downstream factors and initiating broad regulatory cascades (Stevanovic et al., 2021). Despite their well-characterized roles in development, the genomic activity and function of SoxB1 genes influencing cell fate specification and differentiation during adult neural regeneration remain poorly understood.

Integrating epigenomic and transcriptomic approaches offers a path to dissect how SoxB1 genes orchestrate adult stem cell-driven neurogenesis. This approach enables identification of

SoxB1 regions targeted for chromatin alteration and links the changes in chromatin dynamics to gene regulation, offering mechanistic insight into SoxB1-driven cell fate specification. One such strategy involves comparing chromatin accessibility and gene expression profiles in systems where SoxB1 activity is perturbed to controls. Characterization of these target loci can illuminate how chromatin-level regulation underlies SoxB1-mediated neurogenesis.

The planarian *Schmidtea mediterranea* presents an ideal model for investigating neural regeneration. *S. mediterranea* harbors a large population of pluripotent stem cells, enabling complete tissue regeneration, including a molecularly complex nervous system (Cebrià, 2007; Rink, 2013, 2018; Ross et al., 2017). Previous work has shown that inhibition of the planarian SoxB1 gene, *soxB1-2*, results in severe phenotypes, including seizure-like behaviors and loss of mechanosensation (Ross et al., 2018). Co-expression analyses, conducted via in situ hybridization with cell-type-specific markers, along with pseudotime analysis of single-cell RNA sequencing data, suggested that *soxB1-2*$^{+}$ stem cells constitute ectodermal progenitors destined for epidermal or neuronal fates (Ross et al., 2018).

Functional screens from our lab have identified *soxB1-2* downstream targets, including the BRN3/POU4 homolog *pou4-2*, which plays a role in specifying a subset of mechanosensory neurons (McCubbin et al., 2025). Knockdown of *soxB1-2* leads to depletion of the *pou4-2*$^{+}$ mechanosensory population, suggesting a positive regulatory relationship (McCubbin et al., 2025). Additionally, RNAi of *pou4-2* results in loss of the sensory neuron terminal gene *pkd1L-2*, a phenotype also observed following *soxB1-2* disruption (McCubbin et al., 2025; Ross et al., 2018). However, the mechanisms by which *soxB1-2* regulates sensory neural fate trajectories remain unclear. One hypothesis is that *soxB1-2* acts as a pioneer transcription factor in planarians, binding to silent chromatin and modifying the chromatin landscape at target loci to

permit binding of additional transcription factors that direct the differentiation of terminal sensory and epidermal lineages.

In this study, we tested the hypothesis that *soxB1-2* primes chromatin for the acquisition of sensory and epidermal progenitor fates through the integration of epigenetic and transcriptomic approaches focused on isolated planarian head tissues to enrich for neuronal cell populations. We assessed changes in chromatin accessibility (via ATAC-seq) and gene expression (via RNA-seq) following *soxB1-2* RNAi knockdown to identify direct and functional targets. Our findings revealed that loss of *soxB1-2* leads to reduced chromatin accessibility at genomic regions associated with sensory neuronal and epidermal genes, some of which encode transcription factors. Among these, we identified *mecom* and *castor* transcription factor genes, which we found are involved in the regulation of mechanosensory signaling genes. Together, these results provide mechanistic evidence that *soxB1-2* functions as a pioneer factor in priming chromatin for the emergence of sensory neuronal and epidermal progenitor fates.

Results

Neural genes exhibit reduced chromatin accessibility following *soxB1-2* RNAi

To investigate how *soxB1-2* activity influences chromatin accessibility, we performed ATAC-seq (Buenrostro et al., 2015) on head fragments (amputated in the "neck" region anterior to the pharynx and posterior to the auricles; see Supplemental Figure 1A) from *soxB1-2*(*RNAi*) and *gfp*(*RNAi*) (control) animals. These fragments were selected to enrich for neural cell populations (Supplemental Figure 1A). The optimal RNAi feeding regimen and timepoint for obtaining *soxB1-2* knockdown samples were determined based on prior studies conducted in our laboratory (Ross et al., 2018). Sequencing data were aligned to the *S. mediterranea* genome assembly

(S3h1) (Ivankovic et al., 2024), and peaks were mapped to the nearest annotated gene model (see Methods).

Peak calling identified thousands of accessible chromatin sites in controls and *soxB1-2(RNAi)* samples. Subsequent differential accessibility analysis using Csaw (see Methods) identified 264 significantly differentially accessible (DA) peaks. The majority of these (257 peaks) were present in controls but significantly reduced in *soxB1-2(RNAi)* animals (down). In comparison, only seven peaks were significantly increased in *soxB1-2(RNAi)* animals relative to controls (up) (Supplemental Table 1). This overall reduction in accessible peaks in the *soxB1-2(RNAi)* animals was also observed as a cumulative decrease in signal across all gene bodies and promoter regions in the *soxB1-2* RNAi groups (Figure 1A-D). Thirteen genes associated with transcription factor binding were identified within DA chromatin regions, such as *castor* (Figure 1D-E), which has been linked to neurodevelopmental processes in both invertebrate and vertebrate models (Isshiki et al., 2001). Most of the peaks that were shared between the *gfp* and *soxB1-2* RNAi groups were located in distal intergenic regions (54.7% and 57.54%, respectively). Promoter regions accounted for 22% of peaks in controls and 21.25% in *soxB1-2* RNAi samples, while internal intronic regions contained 17.53% and 16.23%, respectively. Only a small fraction mapped to internal exonic regions, 3% in controls and 2.56% in *soxB1-2* RNAi. In contrast, DA peaks showed a markedly different distribution: only 25.52% were in distal intergenic regions, while 36.39% mapped to internal intronic regions and 9.96% to internal exonic regions (Supplemental Figure 1C).

To validate ATAC-seq results and determine whether genes that lose chromatin accessibility are downstream targets of *soxB1-2*, we selected two genes, *dd_26266* and *castor,* for i*n situ* hybridization following *soxB1-2* RNAi. These genes are expressed in the same neural

or ciliated neural clusters as *soxB1-2* (Fincher et al., 2018). *In situ* analysis revealed reduced expression of *dd_26266* and *castor* in the head margin (Figure 1E), a region enriched in sensory neurons (Okamoto et al., 2005; Reho et al., 2022; Wang et al., 2016). Additionally, *castor* exhibited further expression loss in the dorsal epidermis and dorsal ciliated stripe (an organ associated with water flow sensing in planarians) (Farnesi and Tei, 1980; Hyman, 1951; Ross et al., 2018) (Figure 1E). The loss of chromatin accessibility and gene expression in sensory neuron-rich regions supports a key role of *soxB1-2* in regulating downstream genes with potential roles in neuronal specification or terminal differentiation, including other transcription factors.

soxB1-2 **RNAi results in loss of sensory and neural gene expression**

To examine the relationship between gene regulation and chromatin accessibility in the *soxB1-2* RNAi amputation scheme, we repeated *soxB1-2* RNAi experiments and performed RNA-seq on RNA extracted from animal heads (Supplemental Figure 1A). RNA-seq analysis identified 533 downregulated genes meeting our significance cut-offs (fold change ≥ 1.4, $p\text{-}adj \leq 0.1$) (Figure 2A, Supplemental Table 2). Among these, we observed a significant reduction in genes previously shown to be downstream targets of *soxB1-2* that are involved in neural and mechanosensory function. These included *pou4-2, pkd1L-1, pkd1L-2, pkd1L-3, pkd2L-1, pkd2L-2, pkd2-1,* and *pkd2-4* (Figure 2A) (McCubbin et al., 2025; Ross et al., 2024).

To validate the differential expression results, we isolated all genes that were significantly differentially expressed between *gfp* controls and *soxB1-2* RNAi-treated animals and generated a heatmap (Supplemental Figure 2D). This visualization confirmed consistent expression patterns across replicates, with upregulated genes in the control group showing

corresponding downregulation in the RNAi group, and vice versa, indicating robust sample clustering for this gene set.To further investigate these expression changes and validate patterns observed in our dataset, we selected *no mechnoreceptor protein channel c* (*nompc*)*, dd_17839, formimidoyltransferase cyclodeaminase* (*ftcd*)*, ms310138,* and *receptor-type tyrosine-protein phosphatase S* (*ptprs*) as representative genes downregulated in the *soxB1-2*(*RNAi*) group. These genes were co-expressed with *soxB1-2* specifically within neural and ciliated neural populations in published single-cell RNA-seq datasets (Wurtzel et al., 2015). To assess whether they were potential downstream targets of *soxB1-2*, we performed *in situ* hybridization on *gfp* and *soxB1-2* RNAi animals. Consistent with the RNA-seq results, these genes exhibited reduced expression following *soxB1-2* RNAi, particularly in sensory neuron-enriched regions concentrated in the head rim and dorsal ciliated stripe (Figure 2B).

The observed transcriptional changes following *soxB1-2* RNAi prompted us to examine how these patterns compared to those in previous RNA-seq datasets. Given that our prior studies were conducted on RNA extracted from whole worms (Ross et al., 2018), we sought to determine whether broader tissue sampling may have masked gene expression changes in rare neural populations in the whole-worm datasets. Comparing this head fragment dataset to previous whole-worm *soxB1-2* RNAi RNA-seq results identified 134 shared downregulated genes (Supplemental Table 2). Gene Ontology (GO) enrichment analysis indicated that shared genes are primarily associated with 'ion homeostasis', 'transmembrane transport regulation', and 'cell communication', with key processes including 'negative regulation of ion and cation transport', 'sodium ion transmembrane transport', and 'response to metal ions' (Supplemental Figure 2B). Additionally, the presence of electrical coupling-related terms suggests a role in direct cell-to-cell communication, particularly in excitable tissues such as neurons and muscle.

In contrast, 396 genes were uniquely identified in the RNA-seq dataset from head tissue compared to our previously published day-14 whole worm dataset (Supplemental Table 2). GO analysis of these genes revealed enrichment in 'ciliary and flagellar dependent cell motility', 'movement', 'axoneme assembly', and 'microtubule bundle formation' (Supplemental Figure 2C). These features are fundamental not only for locomotion and fluid movement in the ventral epidermis but also for sensory-neural functions given the presence of cilia in sensory neurons (Azimzadeh and Basquin, 2016; Rompolas et al., 2013).

These findings support the role of *soxB1-2* in sensory neural gene expression and indicate that its loss leads to widespread transcriptional changes, particularly in mechanosensory genes. RNA-seq analysis of head tissue fragments revealed additional downregulated genes that were previously undetected in whole-worm RNA-seq, many of which are likely involved in ciliated sensory neuron function, suggesting a more specialized role for *soxB1-2* in these populations. Therefore, we further investigated the genome-wide overlap between the RNA-seq and ATAC-seq datasets.

Integrating ATAC-seq and RNA-seq identifies candidate downstream targets of *soxB1-2* in neural and ciliated neural cells

To uncover potential downstream targets of *soxB1-2*, we integrated our head region ATAC-seq and RNA-seq datasets. This combined approach allowed us to identify genes that not only exhibit changes in chromatin accessibility but also corresponding changes in transcriptional output. While we found consistent signals across both assays in some genes, others were detected only by one method, underscoring the complementary nature of chromatin and transcriptomic profiling.

While differences between ATAC-seq and RNA-seq datasets are expected due to the distinct regulatory layers they capture, their integration revealed a set of 31 genes exhibiting both decreased chromatin accessibility and transcriptional downregulation (Figure 3A), highlighting them as strong candidates for direct downstream targets of *soxB1-2*. We assessed enrichment of the 31 candidate genes in neural and ciliated neural cell clusters and examined their co-expression with *soxB1-2* using single-cell RNA-seq datasets. Single-cell databases indicated most of the 31 genes were either enriched in the same neural and ciliated neural clusters where *soxB1-2* is also enriched or co-expressed with *soxB1-2* within these populations (Fincher et al., 2018; Wurtzel et al., 2015). Exceptions included *dd_380,* enriched in epidermal clusters, and *dd_8471* and *dd_8567*, which lacked specific cluster enrichment and exhibited broad co-expression across all cell types (Supplemental Table 3).

Among the genes identified, we were particularly interested in transcription factors, as these are likely to act as intermediate regulators within the broader *soxB1-2* gene regulatory network. By integrating ATAC-seq and RNA-seq, we could prioritize transcription factors that were either losing accessibility and downregulated at the chromatin and transcriptional levels or uniquely detected through chromatin accessibility changes, suggesting potential regulatory priming. This allowed us to expand the architecture of the *soxB1-2* pathway by highlighting candidate nodes acting downstream of *soxB1-2*.

Notably, transcription factors, including *castor*, were exclusively detected through ATAC-seq assays (Figure 1D); they were not identified as differentially expressed in RNA-seq following *soxB1-2* RNAi treatment (Table 1), potentially due to low transcript abundance or subtle expression changes. However, some transcription factors, such as *mecom* and *foxj1-5,* exhibited significant decreases in both chromatin accessibility and expression in *soxB1-2*(*RNAi*)

animals (Figure 3B). The *mecom* gene is a neural transcription factor that is expressed in the same neural and ciliated neural clusters as *soxB1-2* (Fincher et al., 2018; Wurtzel et al., 2015). ATAC-seq and RNA-seq peaks at the *mecom* locus in both *soxB1-2*(*RNAi*) and control animals are shown in Figure 3B, demonstrating a significant reduction in chromatin accessibility following RNAi of *soxB1-2*.

GO analysis revealed distinct enrichment patterns between the ATAC-seq and the RNA-seq datasets (Figure 3C-D). ATAC-seq enrichment was associated with direct neural functions, with 'forebrain development' as the top term, alongside pathways such as 'potassium ion transport', which is critical for neural processes like action potential generation and neurotransmitter release (Meir et al., 1999; Wang et al., 2023). Additionally, ATAC-seq revealed enrichment for 'synaptic development and assembly' (Figure 3C). In contrast, RNA-seq data GO enrichment predominantly involved cilium-related processes, including movement, assembly, and development (Figure 3D), consistent with our previous findings on gene expression associated with ciliated neurons and the epidermis (Ross et al., 2018). The enrichment of cilium-related terms aligns with the observed loss of chromatin accessibility and gene downregulation following *soxB1-2* RNAi in genes that have sensory or mechanosensory-related functions. Additionally, this gene enrichment was reflected in spatial expression data, as in situ hybridization of these potential *soxB1-2* target genes showed a decrease in expression in body regions strongly associated with ciliated sensory neurons (Figures 1E, 2B, 4, and Supplemental Table 3).

In addition to neural gene regulation, we observed overlapping chromatin accessibility loss and downregulation of multiple genes enriched in epidermal progenitor and late epidermal lineages, as well as genes shared by both neural and epidermal populations. These include

candidates potentially involved in epidermal fate determination, such as *dd_12765*, *foxj1-5, dd_13134*, and *dd_380* (Supplemental Table 3) (Fincher et al., 2018; Pascual-Carreras et al., 2021; Vij et al., 2012). The presence of both neural and epidermal signatures suggests that *soxB1-2* plays a broader role in ectodermal lineage identity. However, because this study primarily focuses on its function in neuronal lineages, our downstream analyses were concentrated on genes enriched in neural populations.

To validate that genes showing overlap in both datasets are regulated by *soxB1-2*, we cloned 14 of the 31 candidate targets and assessed their expression using in situ hybridization in both control and *soxB1-2* RNAi animals. All tested genes were expressed in the head margin and other regions enriched for sensory neurons, and all showed reduced or absent expression following *soxB1-2* knockdown (Figure 4, Supplemental Figure 3A). These results support the model that *soxB1-2* activity regulates these genes and provide strong functional validation of our integrated RNA-seq and ATAC-seq approach for identifying *soxB1-2* targets in neural and ciliated neural populations. Furthermore, the validated genes include several with known or predicted roles in neural development or function (Figure 3C). For example, *kcnka*, a potassium channel homolog, was previously shown to be downregulated following *coe* RNAi, a neural transcription factor involved in nervous system development in *S. mediterranea* (Cowles et al., 2014). *mecom* serves as a marker for medial motor column neurons in mammals and is expressed in the embryonic spinal column of various species, including mice, chickens, and humans (Liau et al., 2023). In the context of sensory neural development, *mecom* overexpression has been shown to promote supporting cell proliferation and hair cell differentiation by modulating Tgf-β, Notch, and Wnt signaling pathways (Chen et al., 2022). The identification of *soxB1-2* downstream targets exhibiting both chromatin accessibility loss and transcriptional

downregulation highlights its regulatory role in neural and ciliated neural populations, further supporting the involvement of genes like *mecom* in these developmental processes

Distinct and overlapping roles of *mecom* and *castor* in mechanosensory signaling, ion transport, and ciliary regulation

Based on the significant loss of chromatin accessibility at the *mecom* and *castor* loci and their downregulation following *soxB1-2* RNAi, we investigated these genes as candidate downstream transcription factor targets of *soxB1-2* activity. While only *mecom* was significantly reduced in the *soxB1-2*(RNAi) RNA-seq dataset, we observed a reduction in expression for both genes in sensory regions by in situ hybridization (Figures 1E and 4). Combined with their known roles in neural and sensory development in other model systems, these observations prompted us to define the regulatory targets of *mecom* and *castor* to better understand their contribution to the *soxB1-2*-dependent transcriptional network. To determine the optimal RNAi timepoints for transcriptomic analysis, we performed a time-course experiment across ten RNAi feedings and monitored *mecom* and *castor* expression levels. *mecom* transcript depletion was consistently observed by RNAi day 24 (Supplemental Figure 4A). *castor* transcript loss occurred by day 37 (Supplemental Figure 5A). RNA-seq analysis revealed that knockdown of *mecom* and *castor* resulted in the significant downregulation of 25 and 391 genes, respectively (Supplemental Tables 4 and 5). The relatively small number of *mecom*-dependent genes detected is consistent with its low baseline expression in a small number of cells (Figure 4). Among the downregulated genes, *nompc* was the only mechanosensory receptor reduced in both *mecom* and *castor* RNAi samples. *pkd2-1*, another known *soxB1-2* target involved in mechanosensation, was downregulated exclusively in the *mecom* dataset (Figure 5A and Figure 6A). In contrast, *castor*

knockdown led to downregulation of additional mechanosensory and ion channel-related genes, including *transient receptor potential ankyrin 1* (*trpa1*) and *pkd* family members *pkd1L-1, pkd1L-3, and pkd2L-1* (Figure 6A), which were all previously implicated in *soxB1-2*-dependent mechanosensory regulation (Ross et al., 2024). In addition to mechanosensory and ion channel-related genes, *castor* RNAi resulted in the downregulation of numerous genes associated with ciliary structure and function, including *dystonin* (*dst*) and multiple dynein component homologs. These findings suggest that while *mecom* and *castor* regulate distinct gene sets, both contribute to a mechanosensory and ion channel gene expression network downstream of *soxB1-2*.

GO enrichment analysis of downregulated genes in *mecom* RNAi revealed significant enrichment for 'ion transporter activity,' consistent with the functional roles of several affected genes. Some of these genes encode TRP channels, which detect mechanical stimuli and mediate ion flux across membranes, including *pkd2-1* and *nompc*. Additionally, genes involved in ion channel regulation, such as *cav3*, which encodes a homolog to human *caveolin 3*, were also downregulated (Supplemental Table 4), establishing a potential role for *mecom* in planarians in mechanosensory signaling and ion transport regulation. In contrast, although multiple mechanosensory and ion channel-related genes were downregulated in the *castor* RNA-seq dataset following RNAi, enriched GO terms were primarily associated with ciliary functions, including 'cilium movement', 'microtubule bundle formation', 'axoneme assembly', and 'microtubule-based movement' (Figure 6B). Given the role of cilia in planarian sensory neurons, this enrichment is consistent with *castor's* potential role in sensory regulation. GO terms related to the detection of mechanical stimuli were also enriched, supporting the observed downregulation of mechanosensory genes, such as *nompc* and members of the PKD gene family, in planarians. Additionally, GO terms enriched among castor-regulated genes overlapped with

those identified in the *soxB1-2* RNAi dataset (Figures 3D and 6B). Together, these findings suggest that *castor* plays a key role in regulating genes involved in mechanosensation, ion transport, and ciliary function, positioning it as a critical component of the *soxB1-2* downstream regulatory network in planarian mechanosensory biology.

Discussion

Evidence for a pioneer-like role of *soxB1-2* in chromatin remodeling during neural and epidermal specification

Planarians provide a powerful *in vivo* model for investigating adult neurogenesis and lineage specification. Leveraging this system, we have begun to dissect the role of *soxB1-2* in directing adult stem cell fate toward ectodermal lineages, with a particular focus on neural differentiation. Although prior studies established that *soxB1-2* promotes ectodermal identity in planarians (Ross et al., 2018), the molecular mechanisms underlying this process had not been explored. In both vertebrate and invertebrate systems, SoxB1 family members are known to play critical roles in maintaining pluripotency, sustaining progenitor identity, and promoting ectodermal development (Buescher M., 2002; Kurtova et al., 2024; Meulemans and Bronner-Fraser, 2007). However, except for mammalian SOX2 homologs, few studies have addressed the mechanisms by which SoxB1 factors regulate these processes (Iwafuchi-Doi and Zaret, 2014; Soufi et al., 2015).

Here, we show that loss of *soxB1-2* leads to a marked reduction in chromatin accessibility at numerous neuronal and epidermal loci, some of which encode transcription factors. These findings suggest that *soxB1-2* may function as a pioneer transcription factor, initiating chromatin remodeling to prime progenitor cells for neural and epidermal differentiation. While this study provides evidence that *soxB1-2* influences chromatin accessibility and regulates downstream

transcription factors such as *mecom*, *foxJ1-5*, and *castor*, we do not present direct evidence of *soxB1-2* binding to closed chromatin. To address this, it will be necessary to develop a SoxB1-2-specific antibody for potential use in future chromatin profiling assays such as CUT&RUN and ChIP-seq. Integration of these assays with ATAC-seq will enable us to determine whether *soxB1-2* directly binds inaccessible chromatin regions, further elucidating its potential pioneer role.

Mechanistic insights into *soxB1-2* function via ATAC-seq and RNA-seq integration post RNAi

ATAC-seq has enhanced our understanding of the chromatin landscape in planarian neoblasts and throughout regeneration (Ivankovic et al., 2024; Pascual-Carreras et al., 2023; Poulet et al., 2023). When combined with RNAi-mediated perturbations, this approach has enabled detailed investigation of the epigenomic effects resulting from disruption of key regulators, including the stem cell marker *smedwi-2*, the chromatin remodeler BPTF (a core subunit of the Nucleosome Remodeling Factor, NuRF), and BAF complex components such as *brg1* and *smarcc2* (Li et al., 2021; Verma et al., 2025; Wiggans et al., 2023).

More broadly, the integration of ATAC-seq with RNA-seq has become a widely adopted strategy for linking chromatin accessibility to gene expression, with applications spanning neural development, cancer biology, and chromatin remodeling dynamics (Bai et al., 2024; Xu et al., 2023). Building on this, recent studies have begun applying this integrative strategy in planarians following RNAi (Bai et al., 2024). In our work, combining ATAC-seq and RNA-seq enabled a mechanistic analysis of *soxB1-2*–driven lineage specification and the regulation of its direct downstream targets. We identified 31 genes that exhibited both differential chromatin

accessibility and altered transcript levels following *soxB1-2* knockdown. These genes were either enriched in or co-expressed with *soxB1-2* across multiple cell types, including ciliated neural cells, general neural populations, early epidermal progenitors, and neoblasts. Among these, ciliated neural cells showed the highest levels of enrichment and co-expression (Supplemental Table 3). These patterns support a model in which *soxB1-2* activity regulates ectodermal progenitor states through coordinated control of chromatin accessibility and gene expression (Figure 7).

Furthermore, in situ hybridization of select candidate targets (Figure 4, Supplemental Figure 3A, Supplemental Figure 3B) confirmed reduced or ablated expression of the putative target genes following *soxB1-2* RNAi, supporting their status as downstream, transcriptionally regulated genes. Interestingly, changes in chromatin accessibility following *soxB1-2* RNAi did not always correspond to changes in gene expression; some loci showed decreased accessibility without changes in transcript levels, while others exhibited altered expression despite stable accessibility. Independent validation of several of these loci (Figure 1E and Figure 2B) supports a model in which *soxB1-2* initiates chromatin remodeling that may precede or occur independently of transcriptional activation. This model aligns with findings from *Xenopus* spinal cord regeneration, where chromatin changes have been shown to prime cells for future gene expression (Kakebeen et al., 2020). These findings highlight the power of combining ATAC-seq and RNA-seq to reveal regulatory mechanisms that underlie lineage specification and transcriptional control during planarian tissue homeostasis or regeneration.

Enhanced resolution of neural regulation via targeted analysis of head tissue

Similar to previous studies (Nakazawa et al., 2003; Roberts-Galbraith et al., 2016; Wang et al., 2016), using of head tissue enriched for neural and ciliated neural cell populations improved our ability to detect expression changes in neurons. This spatially targeted sampling revealed gene expression changes in sensory neurons undetectable in previous whole-worm RNA-seq datasets (Ross et al., 2018), particularly within rare or spatially restricted cell types. For example, *mecom* exhibited significantly reduced chromatin accessibility and transcript levels following *soxB1-2* RNAi in the head tissue dataset. These changes were not observed in the whole-body RNA-seq dataset. In both our whole-body and head tissue RNA-seq datasets and head tissue ATAC-seq datasets the samples represent heterogeneous cell populations, not isolated neural or *soxB1-2*$^{+}$ cells. This heterogeneity inherently introduces noise and likely obscures additional SoxB1-2 targets or changes in rare cell populations, even in our neural-enriched head tissue. Notably, we did not detect changes in chromatin accessibility or transcript levels in sensory populations marked by *pou4-2*, despite research in our lab showing strong downregulation following *soxB1-2* RNAi (McCubbin et al., 2025). This may reflect indirect regulation or a dilution effect due to the limited expression of *pou4-2* and the reduced sensitivity of bulk assays to capture changes in rare cell types. Future studies employing single-cell RNA-seq or FACS-based enrichment of *soxB1-2*$^{+}$ populations, pending the development of a validated antibody, will be critical for resolving these questions.

mecom and _castor_ are downstream of _soxB1-2_ and are putative mechanosensory signaling, synapse, and ion transport regulators

Chromatin accessibility at the regulatory regions of *mecom* and *castor* was markedly reduced following *soxB1-2* RNAi, in situ hybridization revealed a loss of *mecom*-expressing cells and a

marked reduction in *castor*-expressing cells after *soxB1-2* RNAi, providing evidence that both factors act downstream of *soxB1-2*. GO analysis of *mecom*-downregulated genes showed enrichment for ion transport processes. Among downregulated genes were *pkd2-1* and *nompc*, both encoding TRP channels involved in sensory function. Genes downregulated after *castor* RNAi were enriched for GO terms related to ciliary function, synapse transport, and mechanosensation. These included TRP channel genes *pkd1L-2, pkd1L-3, pkd2L-1, nompc*, and *trpa1*.

As cilia mediate both motility and mechanical sensing in planarians, these findings suggest that *mecom* and *castor* regulate mechanosensory populations downstream of *soxB1-2*, which is consistent with known roles of this gene in other organisms, For example, Mecom promotes supporting cell proliferation and sensory hair cell differentiation in neonatal mouse cochlear cultures, while Castor is required for specification of early-born E-PG and P-EN neurons in the *Drosophila* central complex, a region essential for sensory integration and motor control (Chen et al., 2022; Dillon and Doe, 2024; Plath and Barron, 2015). Elucidating conserved *mecom*- and *castor*-mediated pathways could inform future therapeutic strategies for sensory disorders such as hearing loss caused by hair cell damage (Chen et al., 2022). In planarians, *mecom* and *castor* likely mark subsets of ciliated neural cells, as evidenced by co-expression with *soxB1-2* and their in situ hybridization patterns (Figures 1E and 4).

Although transcript knockdown was confirmed by in situ hybridization prior to RNA-seq (Supplemental Figures 3C and D), RNAi targeting *mecom* did not result in significant downregulation of *mecom* transcript levels in the RNA-seq data. This may be due to its low baseline expression or its localized enrichment at the head tip in both control and experimental samples. Because *mecom* is an uncharacterized gene in planarians, we performed RNA-seq on

whole worms, which may have diluted expression signals from this restricted domain. Future studies should employ spatially enriched sampling, such as head dissections, to improve detection sensitivity. Alternatively, the lack of statistical significance may reflect the use of stringent analytical thresholds. Similarly, *castor* did not meet RNA-seq significance cutoffs following *soxB1-2* RNAi, despite clear changes in chromatin accessibility. Such discrepancies between ATAC-seq and RNA-seq are not uncommon and may reflect regulatory complexity—chromatin accessibility may change prior to detectable transcript-level effects, or *castor* mRNA may be unusually stable and persist despite transcriptional repression. Despite these caveats, the data highlight *mecom* and *castor* as likely components of a conserved regulatory program linking *soxB1-2* to sensory specialization (summarized in Figure 7). Future work will be critical for understanding the specific functions and cell-type contexts of *mecom* and *castor* in this regulatory axis.

Conclusion

Our study demonstrates that *soxB1-2* plays a critical role in lineage specification in *S. mediterranea* by modulating chromatin accessibility and gene expression. Integration of ATAC-seq and RNA-seq revealed that *soxB1-2* knockdown reduces chromatin accessibility at neural and epidermal loci, supporting the idea that *soxB1-2* functions as a pioneer-like transcription factor that primes chromatin for differentiation. We identified *mecom* and *castor* as downstream targets implicated in mechanosensory signaling, ion transport, and synapse formation, thereby expanding the regulatory network governed by *soxB1-2*. Beyond planarian biology, these findings contribute to a broader understanding of adult neurogenesis and chromatin remodeling in regeneration-competent systems. The regulatory principles uncovered here provide a

conceptual framework that could inform stem cell reprogramming strategies in less regenerative organisms, including humans, where restoring neurogenic capacity remains a critical therapeutic challenge.

Methods

Animal husbandry

Asexual *S. mediterranea* (clonal strain CIW4) planarians were kept in 9-cup Ziplock reusable containers in 1x Montjuïc salts (1.6 mM NaCl, 1.0 mM $CaCl_2$, 1.0 mM $MgSO_4$, 0.1 mM $MgCl_2$, 0.1 mM KCl, 1.2 mM $NaHCO_3$) dissolved in ultrapure filtered water at 20°C. Animals were fed beef calf liver weekly, as previously described (Merryman et al., 2018). Animals were starved for 1 week prior to all experiments.

RNA interference

Double-stranded RNA (dsRNA) was expressed in HT115 *E. coli* containing cDNA inserts in either pJC.53.2 (Collins et al., 2010) or pPR-T4P (Liu et al., 2013) as described previously (Ross et al., 2018). Worms measuring 3-4 mm (for in situ hybridization) or 5-7 mm (for ATAC-seq and RNA-seq) were fed 0.5 µg dsRNA per worm, mixed 1:3 with a paste composed of 200 µL ultrapure water, 500 µL pureed calf liver, and 40 µL food dye. Animals were cleaned with 1X Montjuïc salt exchanges after each feeding and again the following day. Feedings were performed twice per week, for a total of five to eight feedings, using *green fluorescent protein* (*gfp*) dsRNA as a negative control. For *soxB1-2* RNAi experiments, animals were amputated anterior to the pharynx three days after the fifth feeding for RNA-seq and ATAC-seq or fixed for in situ hybridization seven days after the fifth feeding. For *mecom* and *castor* RNAi experiments,

whole worms (3-4 mm) were fixed for in situ hybridization or processed for RNA-seq seven days after the sixth feeding or tenth feeding, respectively.

ATAC-seq assay and analysis

Two independent replicates of both *soxB1-2* RNAi and *gfp* control groups (30 heads per replicate) were placed directly into ATAC-seq lysis buffer (10 mM Tris pH 7.4, 10 mM NaCl, 3 mM MgCl, 0.1% Digitonin, 0.1% Tween-20, 0.1% NP-40, in water) and pipetted up and down until homogeneous. The slurry was run through a 50-micron filter, and the ATAC-seq assay was performed as previously described (Corces et al., 2017) with the Illumina tagment DNA enzyme and buffer kit (cat# 20034197). Samples were purified using Zymo DNA Clean and Concentrator-5 Kit (cat# D4014) and stored at -20°C until sequencing. Samples were quality-checked on an Agilent 2100 Bioanalyzer and sequenced to a read depth of 38 million paired-end reads on the Illumina NovaSeq 6000 at the University of California, San Diego IGM Genomics Center, Inc. (San Diego, CA). The ATAC-seq sequencing data have been deposited in NCBI under BioProject accession PRJNA1292954.

ATAC-seq libraries were assessed for quality using FastQC (v0.11.9) (Andrews, 2010), and fragment size distributions were evaluated with ATACseqQC (v1.26.0) (Ou et al., 2018). Adapter trimming and merging of forward and reverse reads were performed using NGmerge (v0.3) (Gaspar, 2018). Trimmed reads were aligned to both the S3h1 and S2F2 *Schmidtea mediterranea* genome assemblies using Bowtie2 (v2.4.4) (Langdon, 2015). Samtools (v1.2.0) software was used to remove mitochondrial and improperly paired reads and to generate BAM files (Li et al., 2009). Replicates were subsampled based on library complexity, as described previously (Reske et al., 2020). Picard (v2.26.4) was used to remove PCR duplicates.

Deduplicated BAM files were used for peak calling with Genrich (BioGrids) using parameters -j -v -p 0.01 -a 150. Peaks were called for individual and pooled replicates. Genrich-generated BedGraph files were converted to BroadPeak format and processed as previously described (Reske et al., 2020). Consensus peaks were mapped to gene annotation models for both genomes using BEDTools (Quinlan and Hall, 2010). RPKM-normalized coverage tracks (bigWig) and profile plots were generated using deepTools (v3.5.1) (Ramirez et al., 2014). Differential accessibility analysis between *gfp* controls and *soxB1-2* RNAi samples was performed using csaw (v1.36.0) (Lun and Smyth, 2016), as described previously (Reske et al., 2020). Gene Ontology enrichment was performed using enrichGO from clusterProfiler (v4.12.6) (Yu et al., 2012). The resulting datasets were compared to the human proteome using BLASTX (cut-off e-value < $1e^{-3}$). Human UniProt IDs (UniProt, 2015) were used as input for annotation and overrepresentation analysis. Statistical analyses were carried out in R (v4.4.0) (Team, 2024), and figures were generated using ggplot2 (Wickham, 2009).

Gene identification and cloning

The *soxB1-2* cDNA used in all experiments was previously cloned into pJC.53.2 (Collins et al., 2010; Ross et al., 2018) in our lab. All other gene sequences were identified through PlanMine (Rozanski et al., 2019) using the dd_Smed_v6 transcriptome and cloned from *S. mediterranea* cDNA into pPR-T4P (Liu et al., 2013) using ligation-independent cloning and gene-specific primers as described in Adler and Alvarado (2018) (all primer sequences and gene identifiers listed in Supplemental Table 6). In the text, dd_Smed_v6 transcripts are referred to as *dd_transcript no.* for brevity.

Wholemount in situ hybridization

Antisense probes were generated from DNA templates amplified from pJC.53.2 (Collins et al., 2010; Ross et al., 2018) or pPR-T4P (Liu et al., 2013), incorporating digoxigenin-labeled UTPs, as previously described (Pearson B. J., 2009). Samples were incubated in 5% N-acetyl cysteine (NAC) in 1× PBS for five minutes to euthanize the animals, then fixed in 4% formaldehyde in 1× PBS with 0.1% Triton X-100 for 25 minutes. Animals were processed for in situ hybridization as described previously (King R. S., 2013), except for *mecom*, a low-abundance transcript, for which hybridization and heated washes were performed at 58°C.

RNA-seq assay and analysis

For *soxB1-2* RNAi experiments, head fragments from four independent *gfp* control and six independent *soxB1-2* RNAi replicates were homogenized in TRIzol (Invitrogen), and RNA was extracted and purified according to the manufacturer's protocol. For *mecom* and *castor* RNAi experiments, the same procedure was applied to whole worms, using five independent *gfp* controls and five *mecom* or castor RNAi replicates. RNA was treated with the TURBO DNA-free Kit and column-purified using the Zymoclean Miniprep Kit (cat. no. 11-330). RNA libraries were sequenced on an Illumina NovaSeq 6000 platform to a depth of 24 million reads per sample (12 million paired-end reads) at MedGenome, Inc. (Foster City, CA). Reads were pseudo-aligned to the S2F2 transcriptome using kallisto (Bray N., 2016), and differential gene expression analysis was performed using Bioconductor (Huber W., 2015) and DESeq2 (Love M., 2014) with an FDR cut-off value ≤ 0.05 and fold-change threshold of 1.4. The RNA sequencing data have been deposited in NCBI under BioProject accession numbers PRJNA1292856 and PRJNA1292967. Gene Ontology enrichment was conducted using enrichGO from clusterProfiler

(v4.12.6) (Yu et al., 2012). Gene sets were compared to the human proteome using BLASTX (e-value cut-off < $1e^{-3}$). Resulting UniProt human gene IDs were used for annotation and GO overrepresentation analysis. Statistical analyses were carried out in R (v4.4.0) (Team, 2024), and figures were generated using ggplot2 (Wickham, 2009).

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Tables

Table 1. Transcription factors with differential ATAC-seq accessibility but not differentially expressed in RNA-seq following *soxB1-2* RNAi.

Gene	Transcription factor domain classification
Pknox2 (dd_Smed_v6_8606)	Homeobox
Msh1 (dd_Smed_v6_18505)	Homeobox
Meox1 (dd_Smed_v6_12317)	Homeobox
Phtf2 (dd_Smed_v6_8465)	Homeobox
Castor (dd_Smed_v6_6778)	Zinc finger
Tcf-3 (dd_Smed_v6_8152)	T-cell specific & High mobility group
dd_Smed_v6_4717	Ets-1
Tcf-2 (dd_Smed_v6_9140)	T-cell specific & High mobility group
Smad9 (dd_Smed_v6_6050)	SMAD
Islet1 (dd_Smed_v6_8820)	LIM & Homeobox

Figure Legends

Figure 1. *soxB1-2* RNAi reduces chromatin accessibility in *S. mediterranea*. (A) Histogram of $\log_2$ fold changes ($\log_2$FC) in differentially accessible ATAC-seq peaks between *gfp* and *soxB1-2* RNAi groups, with 269 peaks exhibiting decreased accessibility (blue) and eight peaks showing increased accessibility (orange) (FDR < 0.05). (B) Profile plot of global ATAC-seq signal across gene bodies in *gfp* control (blue) and *soxB1-2* RNAi (orange) groups. TSS, transcription start site; TES, transcription end site. (C) Profile plot of global ATAC-seq signal across promoter regions in *gfp* control (blue) and *soxB1-2* RNAi (orange) groups. TSS, transcription start site. (D) Genome tracks showing reduced chromatin accessibility at the *mecom* and dd_*26266* locus. (E) In situ hybridization of genes enriched in neural and ciliated neural cell populations that display reduced chromatin accessibility post-*soxB1-2* RNAi. Blue arrows indicate regions of in situ signal loss following *soxB1-2* RNAi, with specific anatomical areas marked using red arrows. Sample size: For controls, N ≥ 3 worms were analyzed, and all samples showed consistent expression patterns. For RNAi-treated animals, the number of biological replicates (N) is indicated at the top left of each treatment group.

Figure 2. Genes identified by ATAC-seq are regulated by *soxB1-2*. (A) Volcano plot of differentially expressed genes in *gfp* control vs. *soxB1-2* RNAi groups, with downregulated (blue) and upregulated (red) genes (FC ≥ 1.4, p < 0.1). (B) In situ hybridization of putative sensory neural genes identified by RNA-seq following *soxB1-2* RNAi. Blue arrows indicate regions of in situ signal loss following *soxB1-2* RNAi. Red arrows mark anatomical regions with undetectable signal; red asterisk denotes loss of expression in the tip of the head. Sample size: For controls, N ≥ 3 worms were analyzed, and all samples showed consistent expression

patterns. For RNAi-treated animals, the number of biological replicates (N) is indicated at the top left of each treatment group.

Figure 3. ATAC-seq and RNA-seq reveal coordinated loss of chromatin accessibility and downregulation of neural genes. (A) Heatmap of differentially expressed genes identified through RNA-seq (FC ≥ 1.4, $p < 0.1$) in *gfp* control vs. *soxB1-2* RNAi groups. Z-scores indicate upregulation (2), no change (0), and downregulation (-2). Labeled genes are putative neural genes identified from both ATAC-seq and RNA-seq experiments. (B) Genome tracks showing reduced chromatin accessibility and gene downregulation following *soxB1-2* RNAi at putative neural gene loci (*foxn(p)*, *foxj1-5*, and *stumble*) in *S. mediterranea*. (C) Gene Ontology (GO) term enrichment analysis of genes associated with reduced chromatin accessibility following *soxB1-2* knockdown. (D) Gene Ontology (GO) term enrichment analysis of downregulated genes in RNA-seq experiments following *soxB1-2* knockdown.

Figure 4. In situ hybridization validation of neural genes identified in ATAC-seq and RNA-seq. (A) In situ hybridization of neural genes expressed in the head rim, dorsal ciliated stripe, and periphery in *gfp* control (top) and *soxB1-2* RNAi (bottom) animals. Sample size: For controls, N ≥ 3 worms were analyzed, and all samples showed consistent expression patterns. For RNAi-treated animals, the number of biological replicates (N) is indicated at the top left of each treatment group.

Figure 5. *mecom* RNAi disrupts mechanosensory and ion channel-like gene expression. (A) (A) Volcano plot of differentially expressed genes in *gfp* control vs. *mecom* RNAi groups, with downregulated (blue) and upregulated (red) genes (FC ≥ 1.4, $p < 0.1$). (B) Gene Ontology (GO) term enrichment analysis of downregulated genes following *mecom* knockdown.

Figure 6: *castor* RNAi disrupts genes involved in synapse and ciliary function. (A) Volcano plot of differentially expressed genes in *gfp* control vs. *castor* RNAi groups, with downregulated (blue) and upregulated (red) genes (FC ≥ 1.4, $p < 0.1$). (B) Gene Ontology (GO) term enrichment analysis of downregulated genes following *castor* knockdown.

Figure 7: Hypothetical model of SoxB1-2-regulated neuronal cell fates uncovered by integrating epigenomic and transcriptomic approaches. Previous work showed that *soxB1-2* is first expressed in a *piwi-1*$^{+}$ progenitor population that acquires epidermal or neuronal cell fates that terminally differentiate into ciliated sensory neurons or epidermis (Ross et al., 2018). Subsequent work has focused on understanding the mechanisms downstream of SoxB1-2 involved in specifying sensory neuron subclasses. McCubbin et al. (2025) found that the Pou4 homolog, *pou4-2,* regulates the differentiation of ciliated sensory neurons in the ciliated stripes. In this study, we show that *mecom* and *castor* also function in the *soxB-2* regulatory network by regulating neuronal gene expression. Created in https://BioRender.com.

Supplemental Figure Legends

Supplemental Figure S1. ATAC-seq analysis reveals differences in chromatin accessibility following *soxB1-2* knockdown. (A) Schematic of the assay used to assess chromatin accessibility in *gfp* control and *soxB1-2* RNAi conditions. (B) Distribution of ATAC-seq peaks across annotated genomic regions of the *S. mediterranea* genome, comparing all *gfp* control and *soxB1-2* RNAi replicates. "DA peaks" indicate regions with detectable accessibility in *gfp* controls that are absent following *soxB1-2* knockdown. (C) Fragment size distributions from each ATAC-seq replicate. Forward and reverse reads are shown in red and blue, respectively.

Supplemental Figure S2. Co-expression of *soxB1-2* and select genes identified by RNA-seq. (A) Single-cell expression profiles of cells co-expressing *soxB1-2* and representative RNA-seq–identified genes that are significantly downregulated following *soxB1-2* RNAi. Cell type abbreviations: sNB, sigma neoblast; zNB, zeta neoblast; gNB, gamma neoblast; EEP, early epidermal progenitor; LEP, late epidermal progenitor; E1, epidermis 1; E2, epidermis 2; Gut, intestine; PN, protonephridia; PP, parapharyngeal; Mu, muscle; N, neural; N-C, neural-ciliated. (B) Gene Ontology (GO) term enrichment of 134 genes shared between RNA-seq datasets from whole-worm and head-only samples. (C) Gene Ontology (GO) term enrichment of 148 downregulated genes uniquely identified in whole-worm RNA-seq datasets. (D) Heatmap of isolated differentially expressed genes (FC $\geq$ 1.4, $p < 0.05$) in *gfp* control vs. *soxB1-2* RNAi groups. Z-scores indicate upregulation (2), no change (0), and downregulation (-2). Labeled genes represent putative sensory neural genes selected for validation via in situ hybridization.

Supplemental Figure S3. Expression patterns of genes with reduced chromatin accessibility or transcription following *soxB1-2* RNAi, and confirmation of *mecom* transcript loss after RNAi. (A) Decreased or absent expression of genes identified through ATAC-seq or RNA-seq as downregulated following *soxB1-2* RNAi. *dd_11972* shows reduced expression in the head tip and across the epidermis, while *dd_7770*, *dd_45275*, *dnai1*, and s*lc25a-21* exhibit complete loss of expression. *pkd1L-2* serves as a positive control for confirming the knockdown of *soxB1-2* downstream targets via in situ hybridization. (B) Expression of genes enriched in the central nervous system that appeared in ATAC-seq or RNA-seq datasets but did not meet statistical cut-offs, yet exhibited reduced brain size phenotypes. *dd_2708* additionally showed loss of expression in sensory cells. Black arrows indicate regions with reduced or absent expression. (C) Validation of *mecom* transcript loss at the time point used for RNA-seq sample collection. (D) Validation of *castor* transcript loss at the time point used for RNA-seq sample collection. Sample size: For controls, $N \geq 3$ worms were analyzed, and all samples showed consistent expression patterns. For RNAi-treated animals, the number of biological replicates (N) is indicated at the top left of each treatment group.

Supplemental Figure S4. Experimental design for *mecom* RNA-seq and heatmap of candidate targets meeting differential expression cut-offs. (A) Schematic of the assay used to assess differential gene expression in *gfp* control and *mecom* RNAi conditions. (B) Heatmap of differentially expressed genes ($FC \geq 1.4$, $p < 0.1$) in *gfp* control vs. *mecom* RNAi groups. Z-scores indicate upregulation (2), no change (0), and downregulation (-2). Labeled genes are

putative mechanosensory genes identified in RNA-seq experiments performed on *mecom* RNAi samples.

Supplemental Figure S5. Experimental design for *castor* RNA-seq and heatmap of candidate targets meeting differential expression cut-offs. (A) Schematic of the assay used to assess differential gene expression in *gfp* control and *castor* RNAi conditions. (B) Heatmap of differentially expressed genes (FC ≥ 1.4, $p < 0.1$) in *gfp* control vs. *castor* RNAi groups. Z-scores indicate upregulation (2), no change (0), and downregulation (-2). Labeled genes are putative mechanosensory genes identified in RNA-seq experiments performed on *castor* RNAi samples.

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FIGURE 1

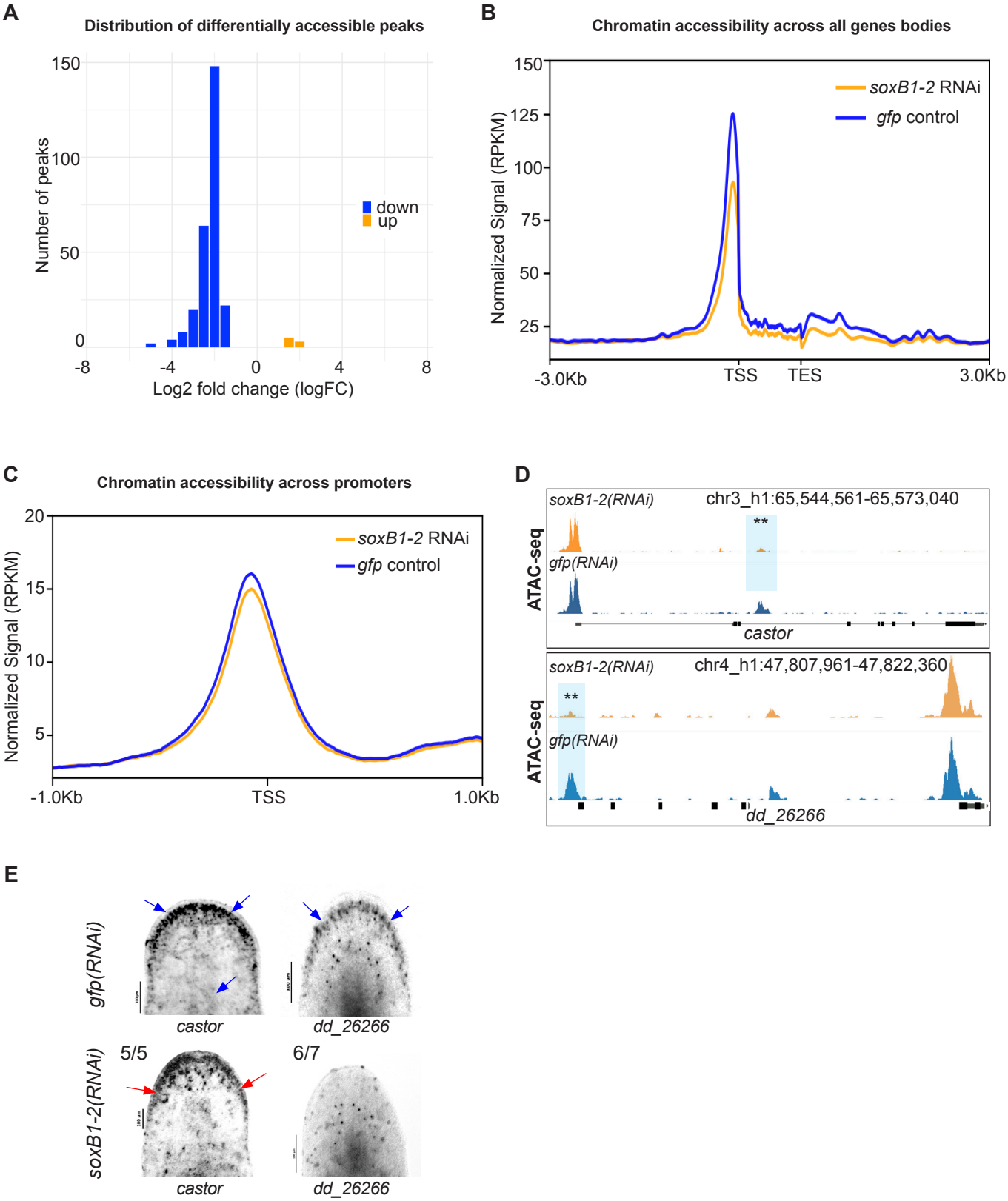

A
Distribution of differentially accessible peaks
Number of peaks
150
100
50
0
-8
-4
0
4
8
Log2 fold change (logFC)
down
up
B
Chromatin accessibility across all genes bodies
Normalized Signal (RPKM)
150
125
100
75
50
25
-3.0Kb
TSS
TES
3.0Kb
soxB1-2 RNAi
gfp control
C
Chromatin accessibility across promoters
Normalized Signal (RPKM)
20
15
10
5
-1.0Kb
TSS
1.0Kb
soxB1-2 RNAi
gfp control
D
soxB1-2(RNAi)
chr3_h1:65,544,561-65,573,040
**
gfp(RNAi)
ATAC-seq
castor
soxB1-2(RNAi)
chr4_h1:47,807,961-47,822,360
**
gfp(RNAi)
ATAC-seq
dd_26266
E
gfp(RNAi)
castor
dd_26266
5/5
6/7
soxB1-2(RNAi)
castor
dd_26266

FIGURE 2

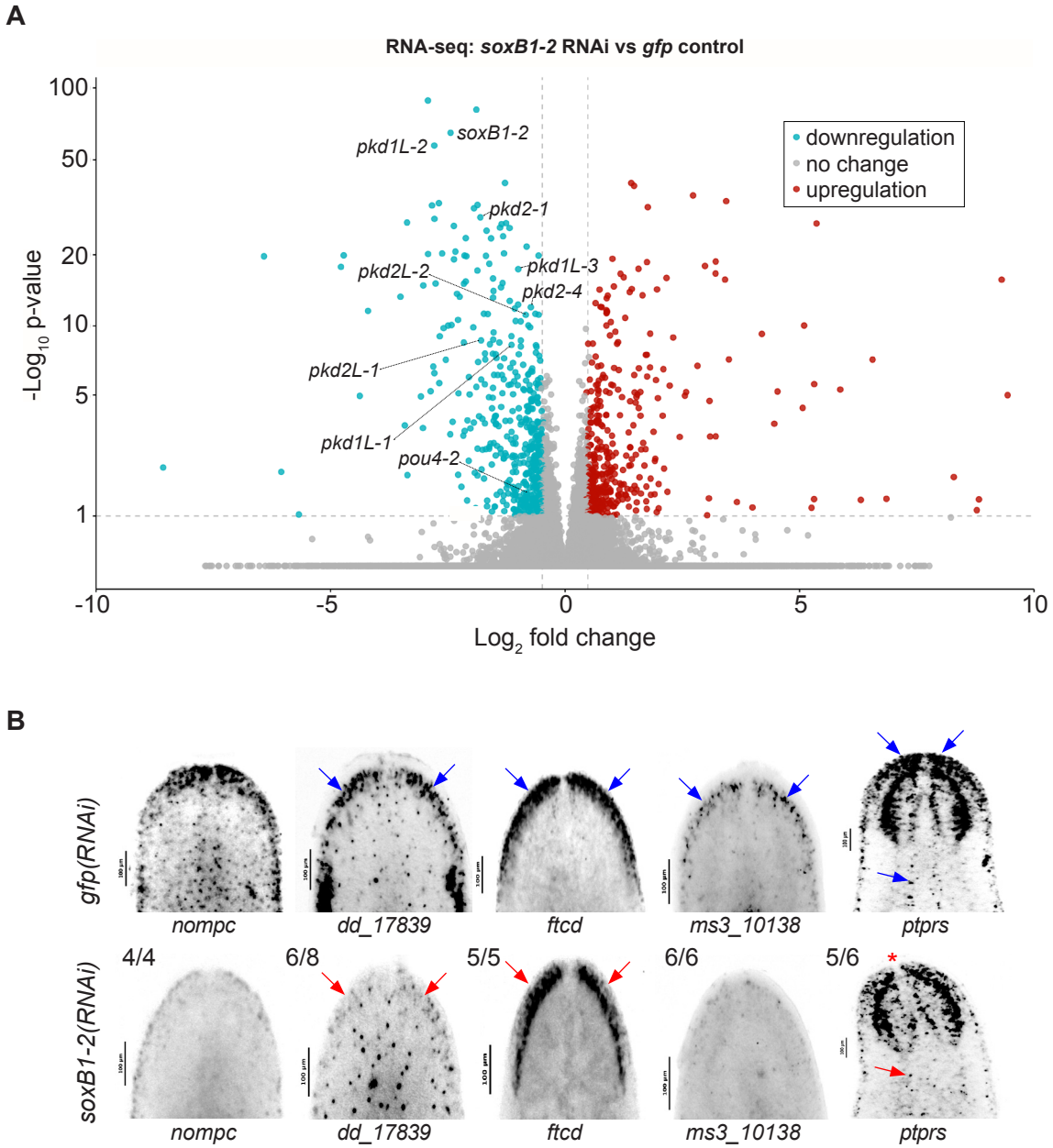

A
RNA-seq: soxB1-2 RNAi vs gfp control
downregulation
no change
upregulation
soxB1-2
pkd1L-2
pkd2-1
pkd1L-3
pkd2-4
pkd2L-2
pkd2L-1
pkd1L-1
pou4-2
-Log10 p-value
Log2 fold change
B
gfp(RNAi)
soxB1-2(RNAi)
nompc
dd_17839
ftcd
ms3_10138
ptprs
4/4
6/8
5/5
6/6
5/6

FIGURE 3

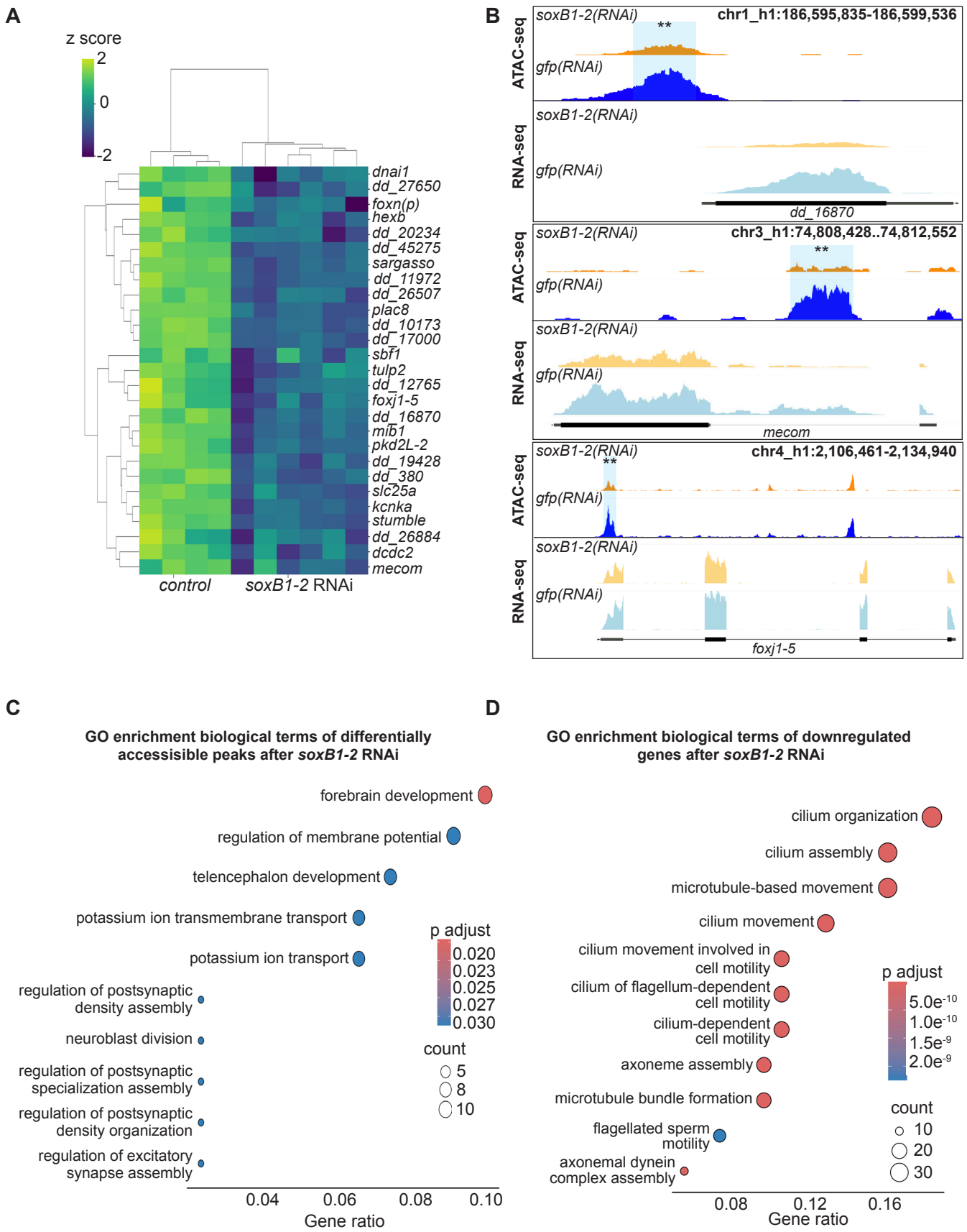

A
z score
2
0
-2
dnai1
dd_27650
foxn(p)
hexb
dd_20234
dd_45275
sargasso
dd_11972
dd_26507
plac8
dd_10173
dd_17000
sbf1
tulp2
dd_12765
foxj1-5
dd_16870
mib1
pkd2L-2
dd_19428
dd_380
slc25a
kcnka
stumble
dd_26884
dcdc2
mecom
control
soxB1-2 RNAi
B
ATAC-seq
RNA-seq
soxB1-2(RNAi)
gfp(RNAi)
chr1_h1:186,595,835-186,599,536
dd_16870
chr3_h1:74,808,428..74,812,552
mecom
chr4_h1:2,106,461-2,134,940
foxj1-5
C
GO enrichment biological terms of differentially accessible peaks after soxB1-2 RNAi
forebrain development
regulation of membrane potential
telencephalon development
potassium ion transmembrane transport
potassium ion transport
regulation of postsynaptic density assembly
neuroblast division
regulation of postsynaptic specialization assembly
regulation of postsynaptic density organization
regulation of excitatory synapse assembly
p adjust
0.020
0.023
0.025
0.027
0.030
count
5
8
10
0.04
0.06
0.08
0.10
Gene ratio
D
GO enrichment biological terms of downregulated genes after soxB1-2 RNAi
cilium organization
cilium assembly
microtubule-based movement
cilium movement
cilium movement involved in cell motility
cilium of flagellum-dependent cell motility
cilium-dependent cell motility
axoneme assembly
microtubule bundle formation
flagellated sperm motility
axonemal dynein complex assembly
p adjust
5.0e-10
1.0e-10
1.5e-9
2.0e-9
count
10
20
30
0.08
0.12
0.16
Gene ratio

FIGURE 4

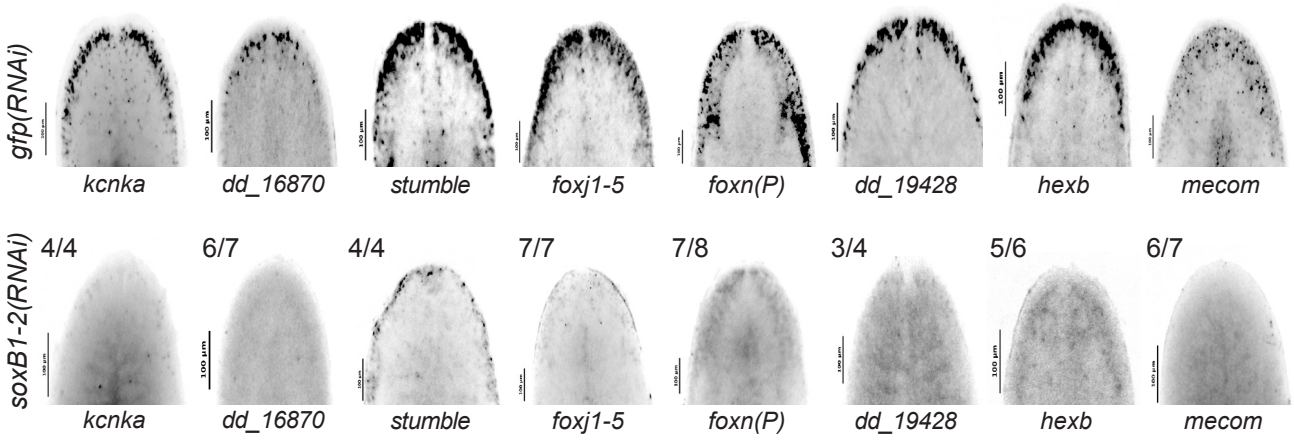
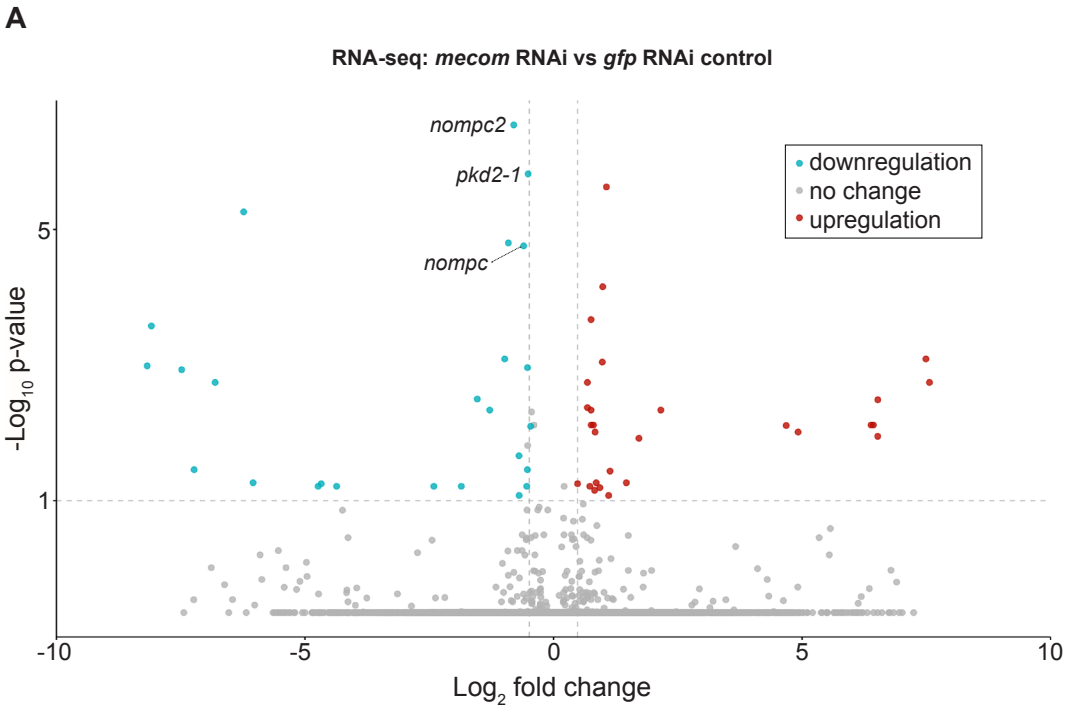

gfp(RNAi)
kcnka
dd_16870
stumble
foxj1-5
foxn(P)
dd_19428
hexb
mecom
soxB1-2(RNAi)
4/4
6/7
4/4
7/7
7/8
3/4
5/6
6/7
kcnka
dd_16870
stumble
foxj1-5
foxn(P)
dd_19428
hexb
mecom

FIGURE 5


A
RNA-seq: mecom RNAi vs gfp RNAi control
nompc2
pkd2-1
nompc
downregulation
no change
upregulation
-Log10 p-value
5
1
-10
-5
0
5
10
Log2 fold change

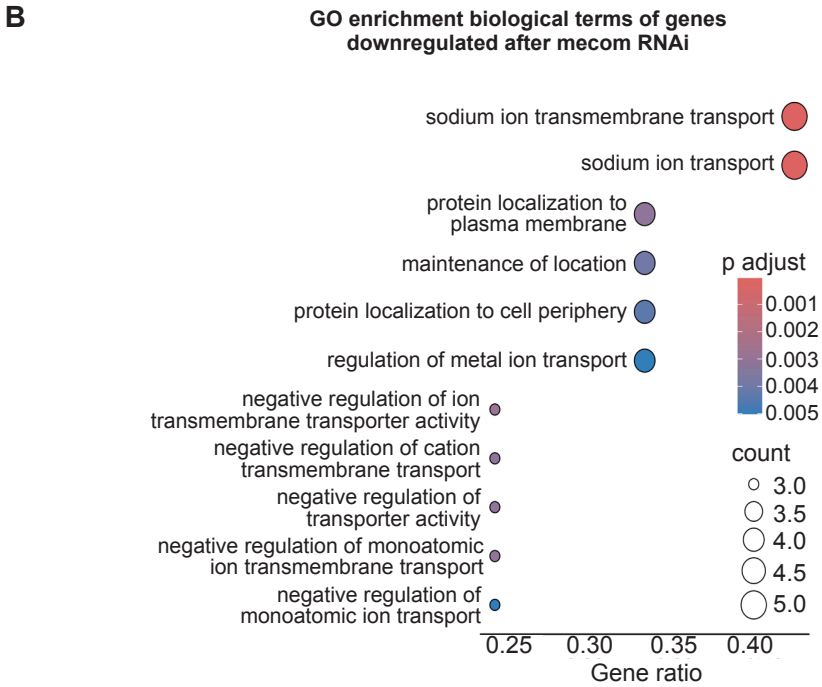
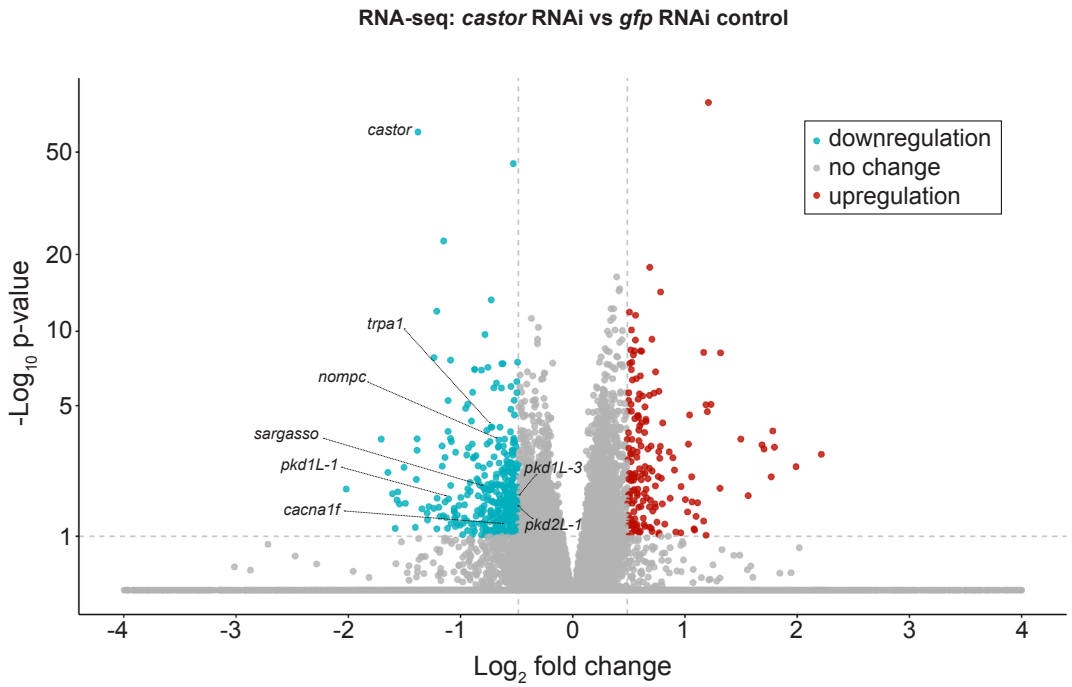

B
GO enrichment biological terms of genes downregulated after mecom RNAi
sodium ion transmembrane transport
sodium ion transport
protein localization to plasma membrane
maintenance of location
protein localization to cell periphery
regulation of metal ion transport
negative regulation of ion transmembrane transporter activity
negative regulation of cation transmembrane transport
negative regulation of transporter activity
negative regulation of monoatomic ion transmembrane transport
negative regulation of monoatomic ion transport
p adjust
0.001
0.002
0.003
0.004
0.005
count
3.0
3.5
4.0
4.5
5.0
0.25
0.30
0.35
0.40
Gene ratio

FIGURE 6

A


RNA-seq: *castor* RNAi vs *gfp* RNAi control
downregulation
no change
upregulation
castor
trpa1
nompc
sargasso
pkd1L-1
cacna1f
pkd1L-3
pkd2L-1
-Log10 p-value
50
20
10
5
1
-4
-3
-2
-1
0
1
2
3
4
Log2 fold change

B

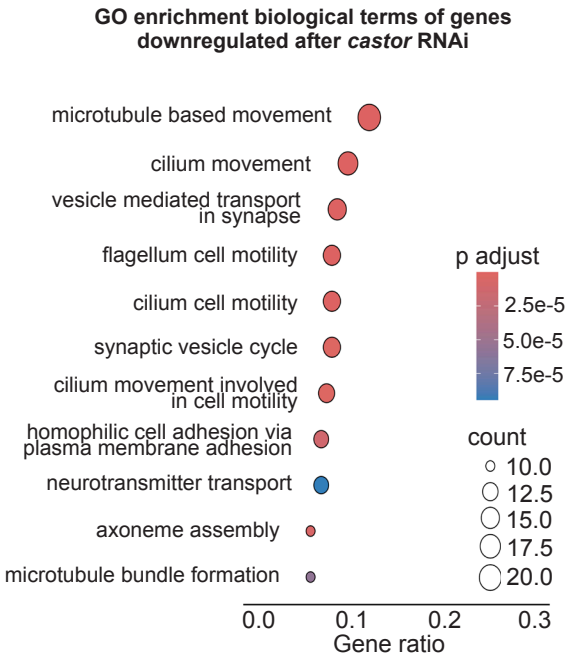
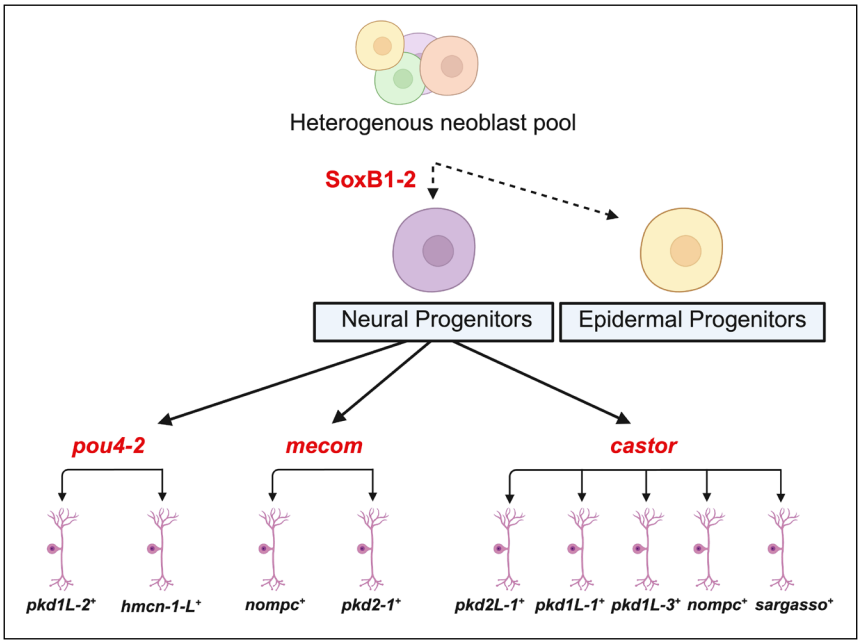
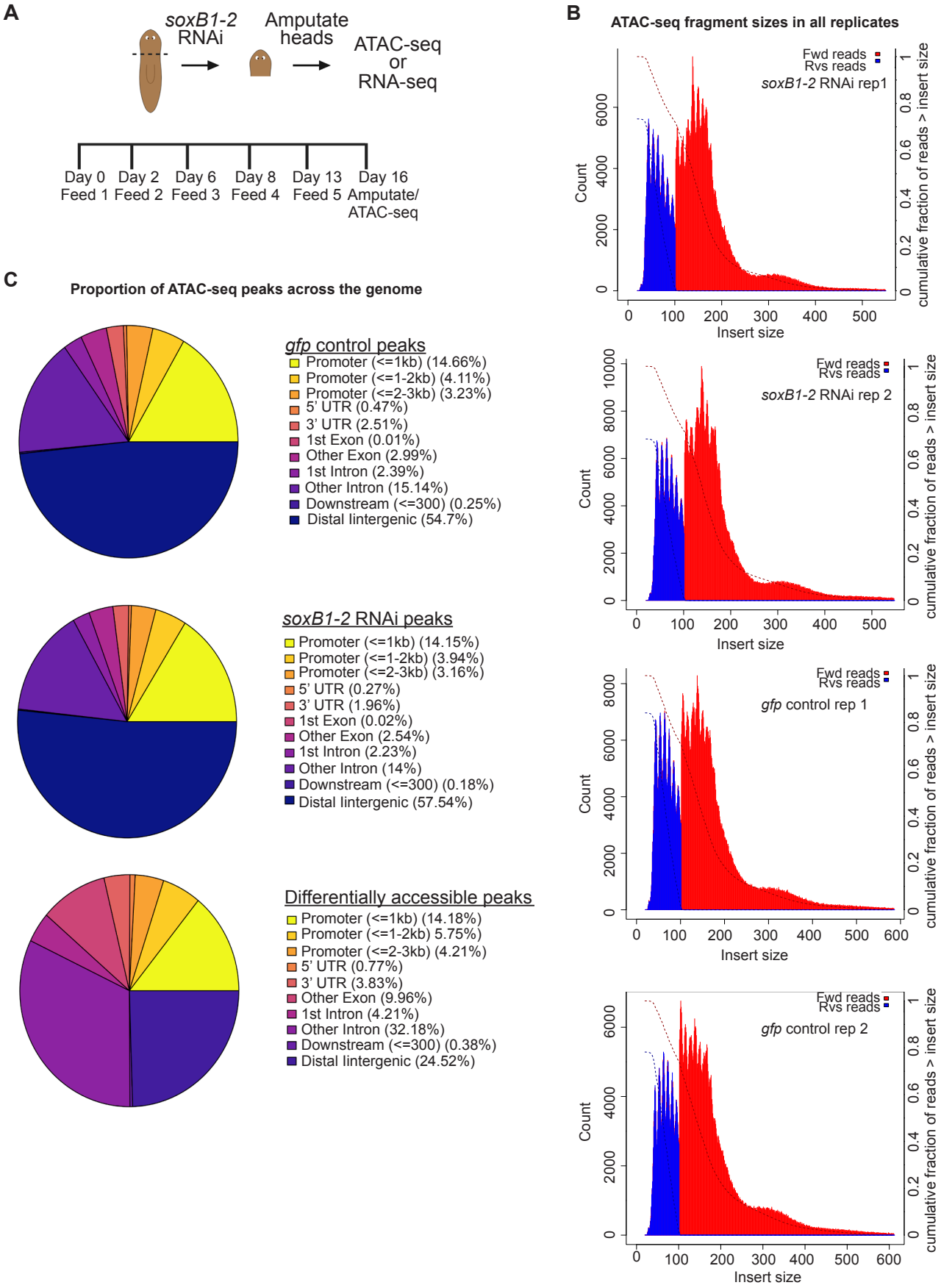

GO enrichment biological terms of genes downregulated after *castor* RNAi
microtubule based movement
cilium movement
vesicle mediated transport in synapse
flagellum cell motility
cilium cell motility
synaptic vesicle cycle
cilium movement involved in cell motility
homophilic cell adhesion via plasma membrane adhesion
neurotransmitter transport
axoneme assembly
microtubule bundle formation
p adjust
2.5e-5
5.0e-5
7.5e-5
count
10.0
12.5
15.0
17.5
20.0
0.0
0.1
0.2
0.3
Gene ratio

FIGURE 7


Heterogenous neoblast pool
SoxB1-2
Neural Progenitors
Epidermal Progenitors
pou4-2
mecom
castor
pkd1L-2+
hmcn-1-L+
nompc+
pkd2-1+
pkd2L-1+
pkd1L-1+
pkd1L-3+
nompc+
sargasso+

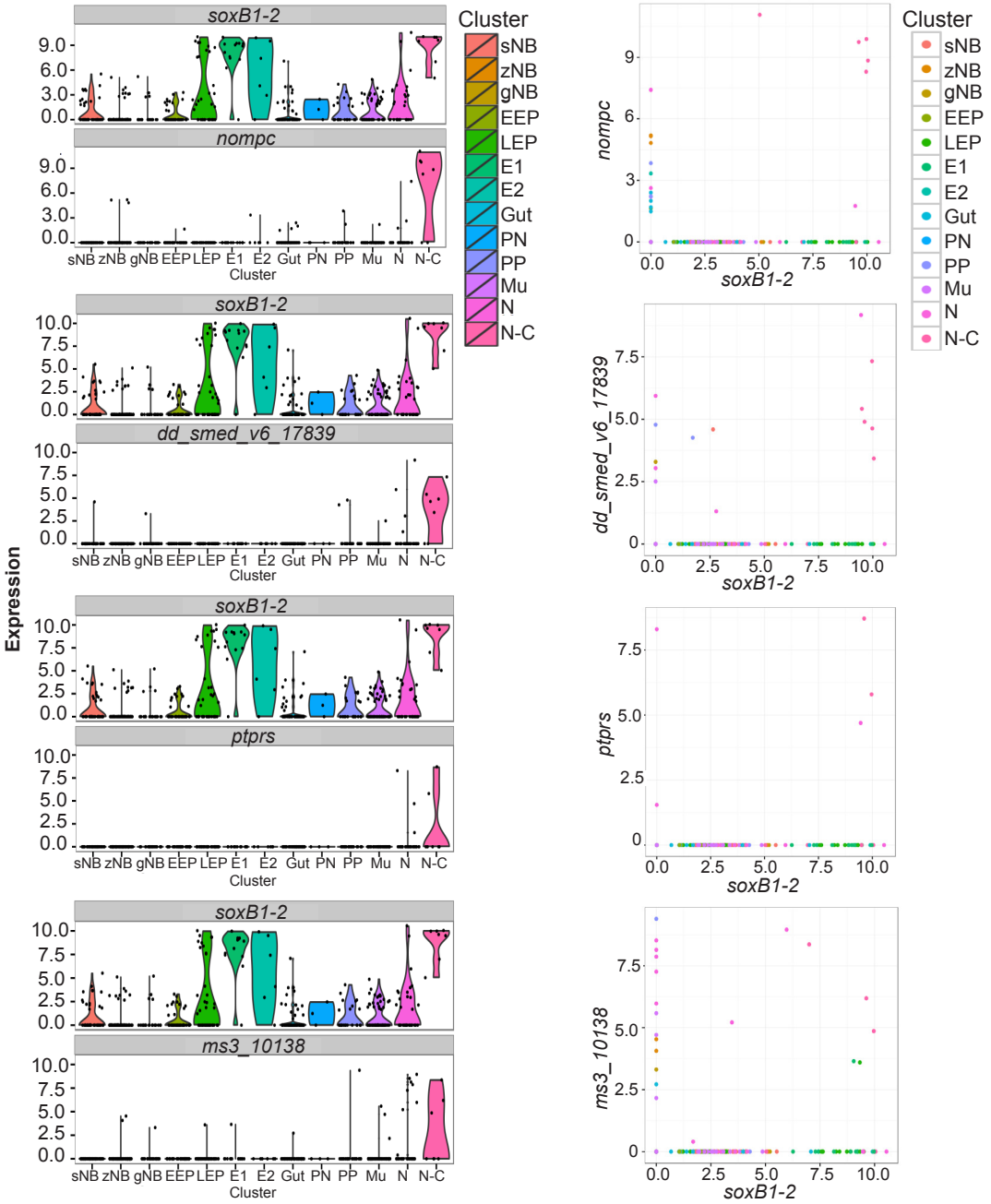
SUPPLEMENTAL FIGURE 1



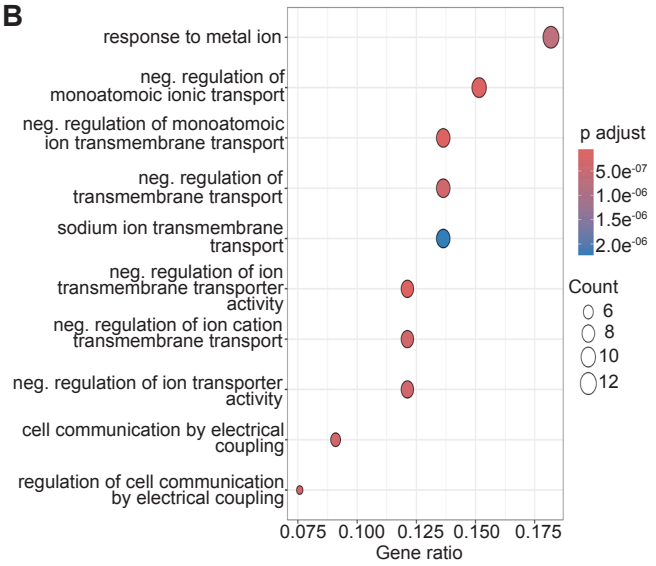
SUPPLEMENTAL FIGURE 2

A

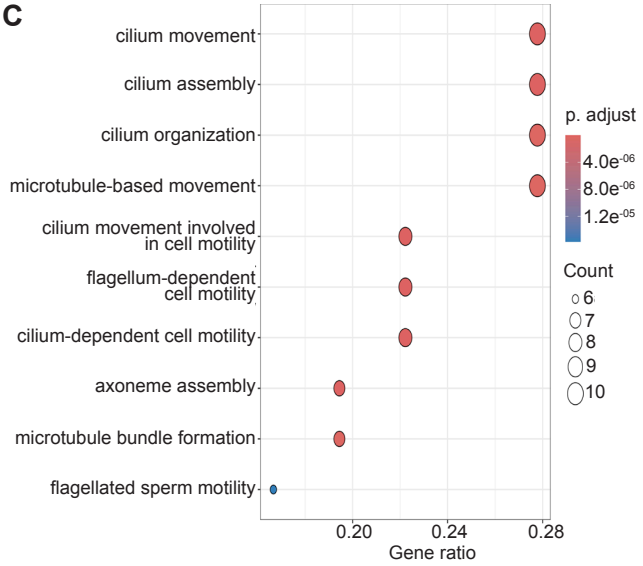
***soxB1-2* coexpression with representative genes downregulated in RNA-seq**



B

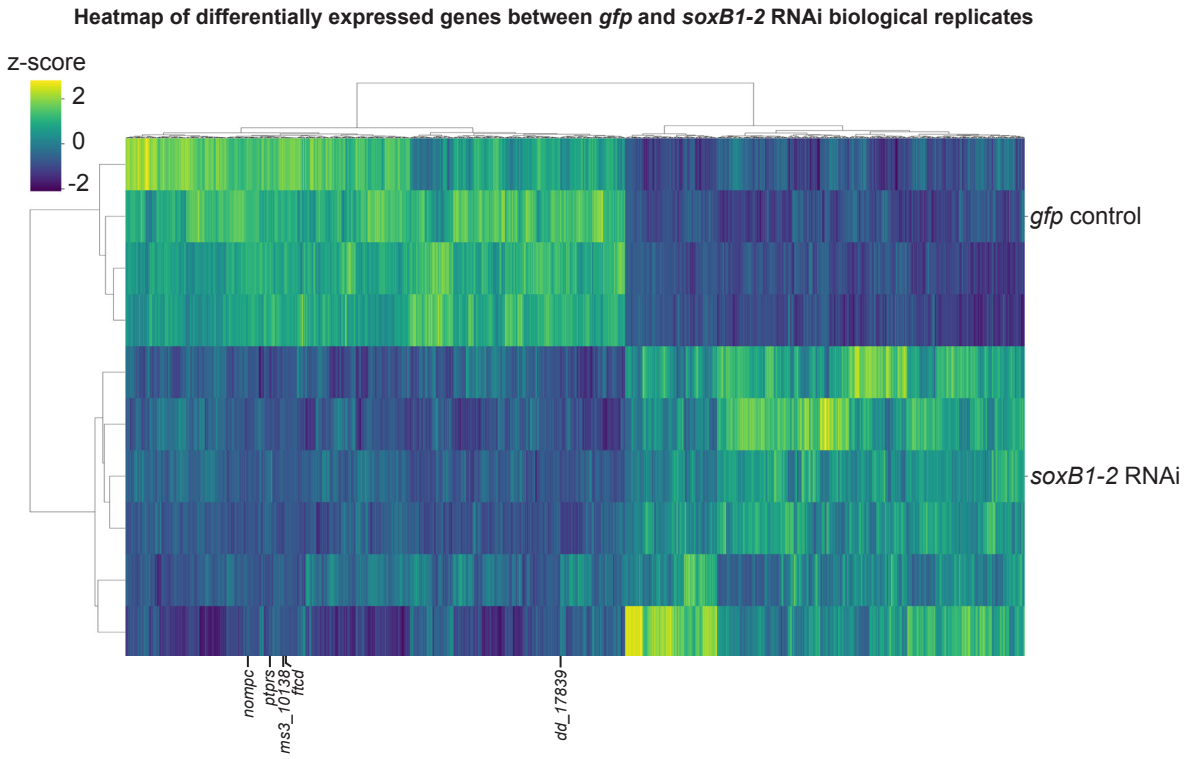


C

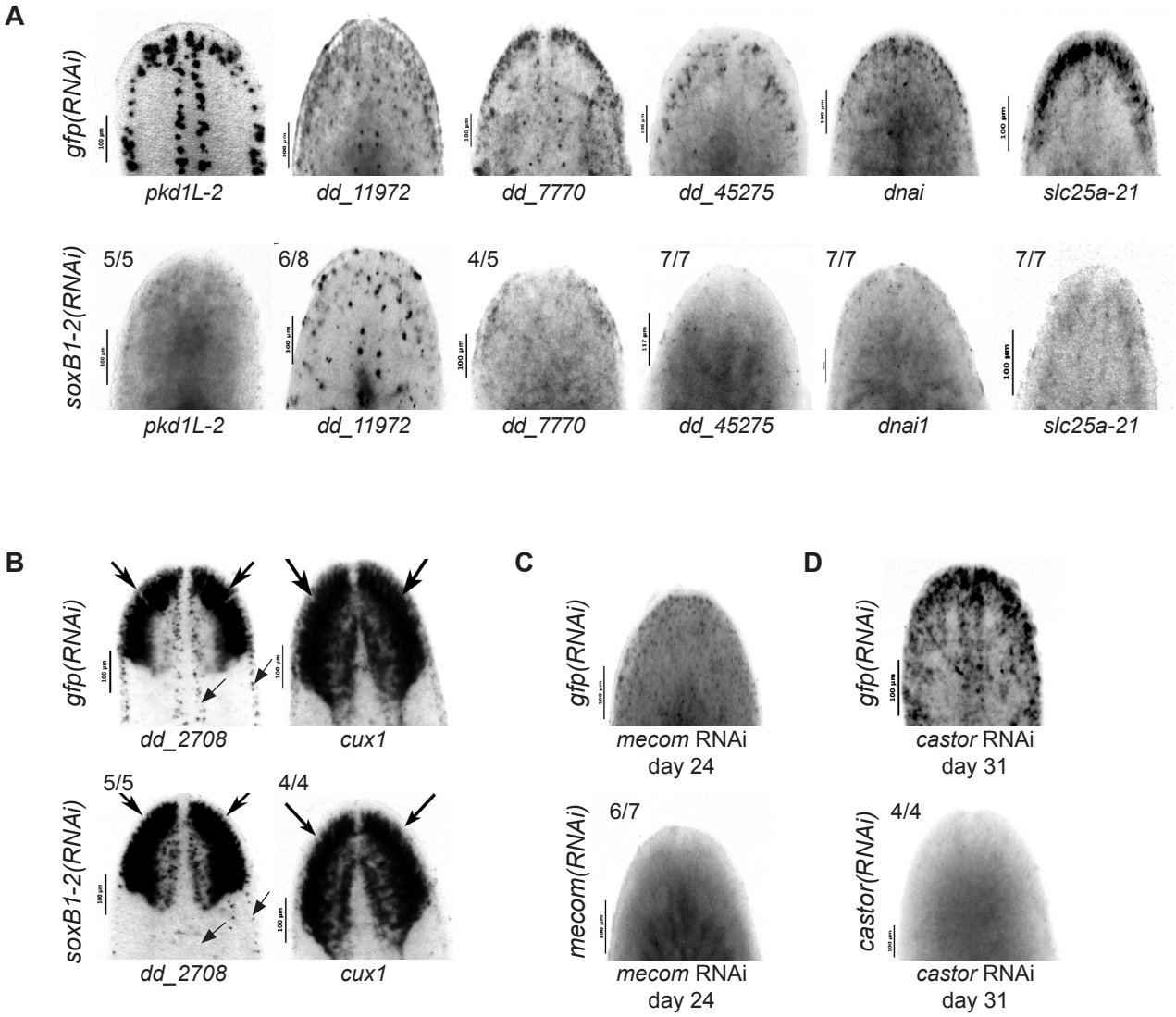


SUPPLEMENTAL FIGURE 2 (continued)

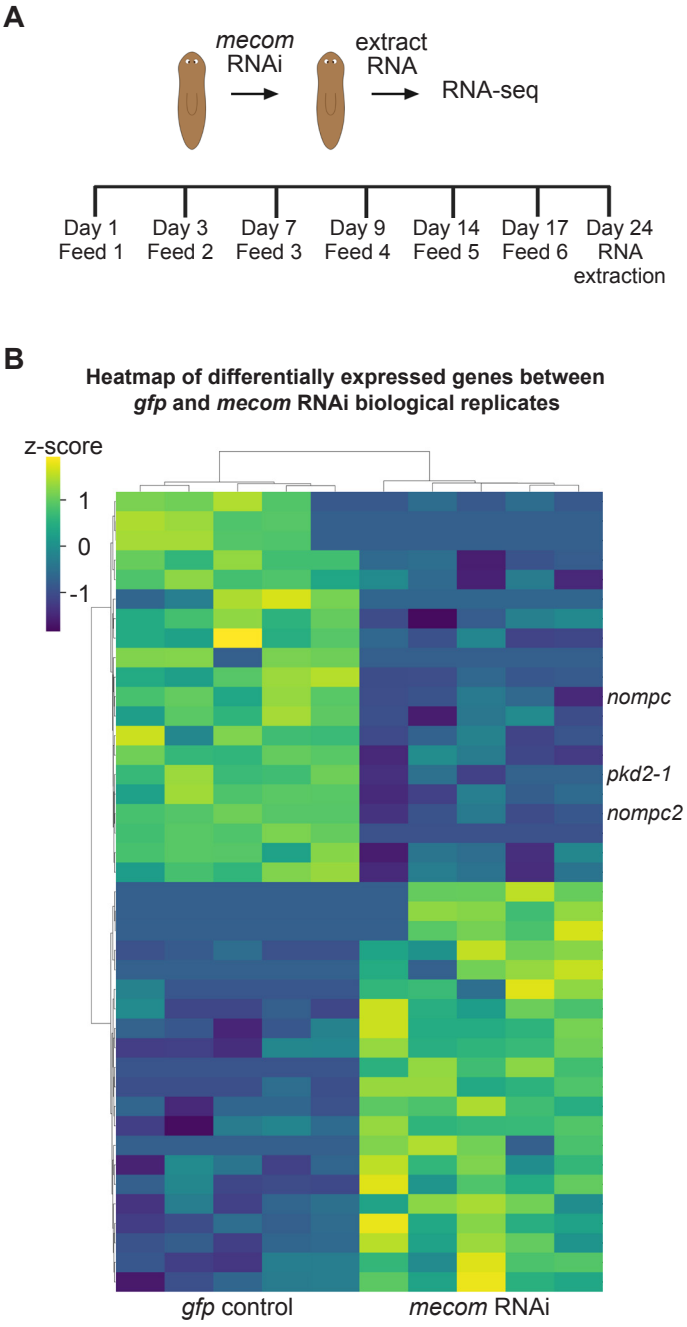
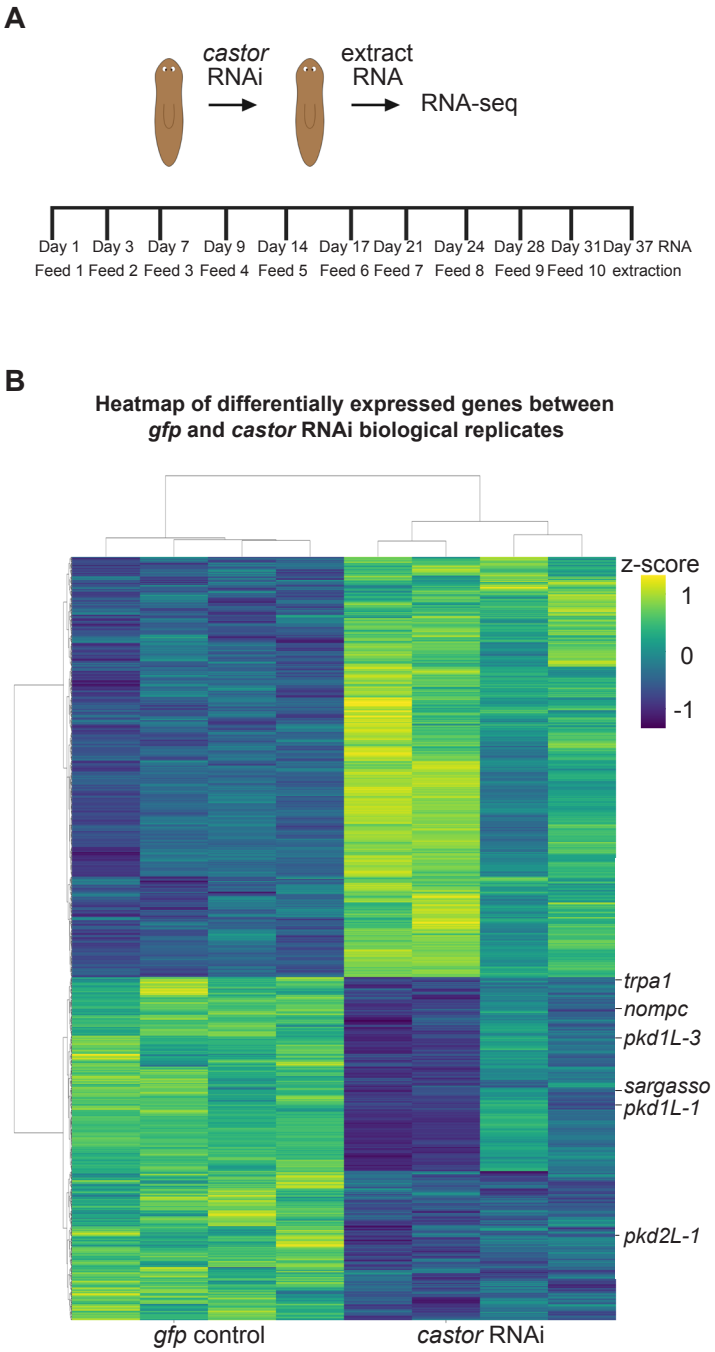
D



SUPPLEMENTAL FIGURE 3



SUPPLEMENTAL FIGURE 4



SUPPLEMENTAL FIGURE 5