Cell Reports, Volume *42*

Supplemental information

Diverse logics and grammar encode notochord enhancers

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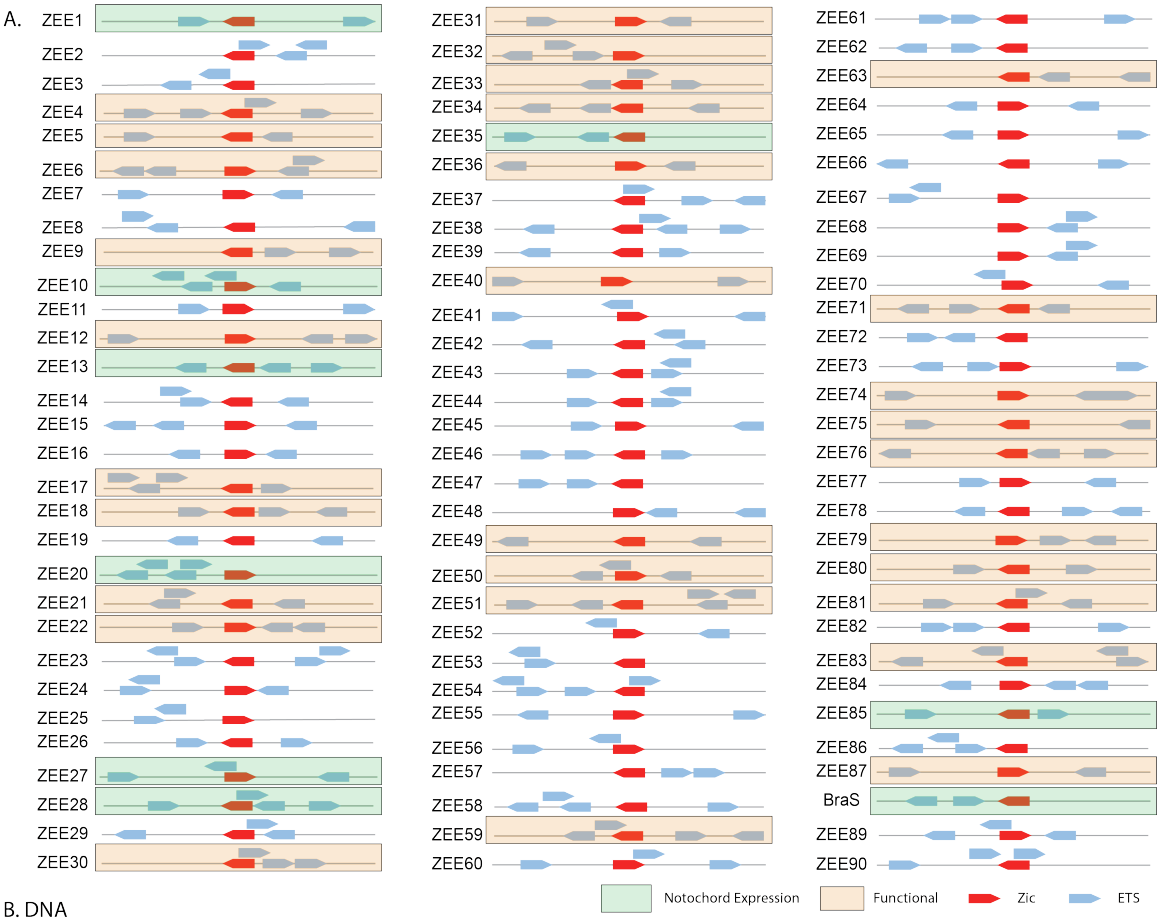
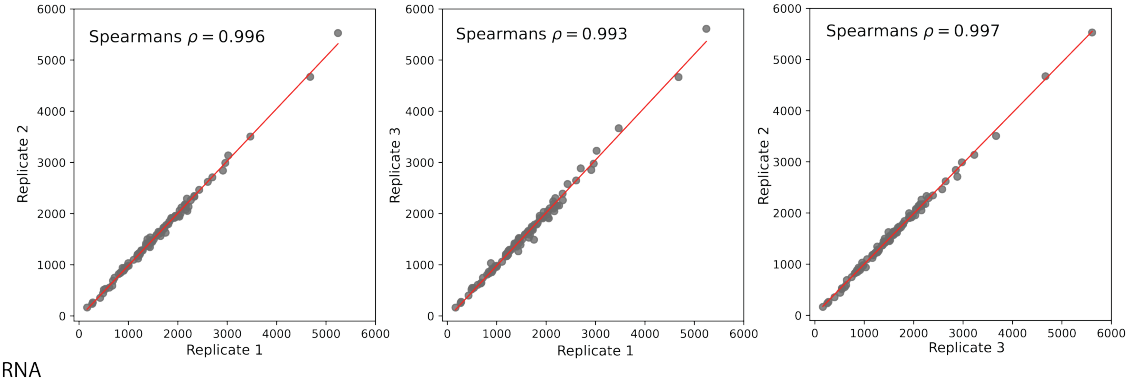
Supplementary figures:



B. DNA



C. RNA

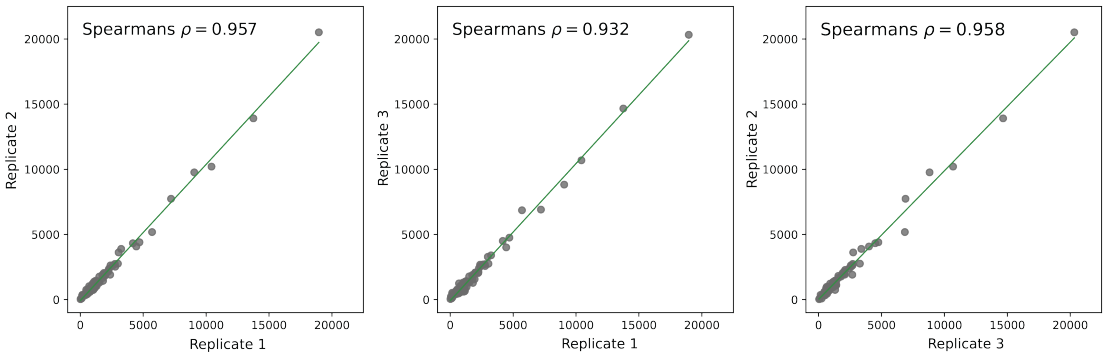


Figure S1. ZEE elements screened and the experimental reproducibility of the library electroporated into embryos, related to Figure 2. **A**. Schematic of each ZEE element tested within our MPRA assay. Zic sites are colored red and ETS sites are colored blue. ZEE elements that were functional are boxed in orange. ZEE elements that drove notochord expression are boxed in green. **B.** Correlation of DNA plasmids detected between replicates was plotted. All Spearman correlations between replicates were >0.99. **C.** Correlation of mRNA barcodes detected between replicates was plotted. All Spearman correlations between replicates were >0.9. Three biological replicates were performed of the library screen.

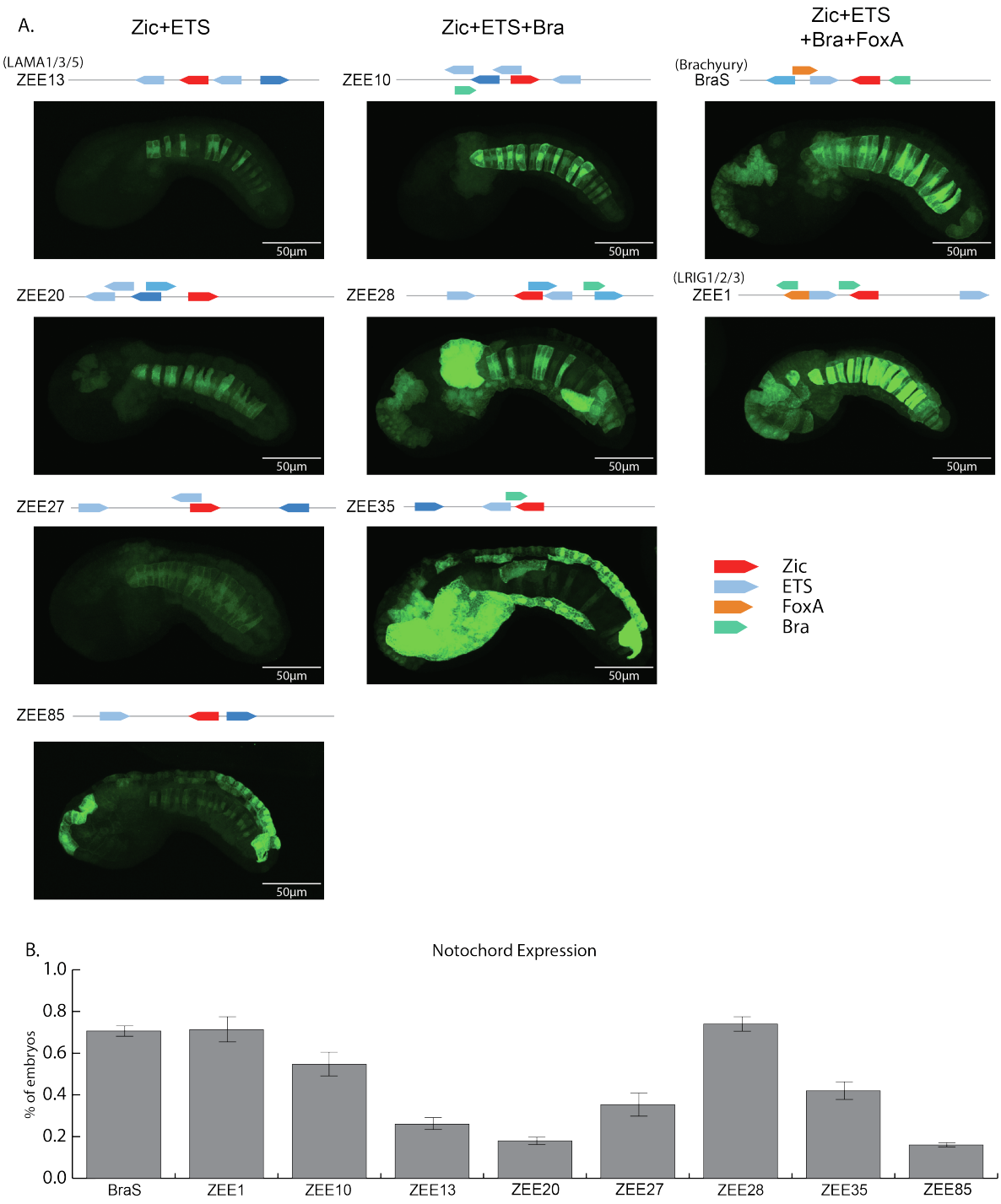


Figure S2, related to Figure 3. Nine ZEE elements drive notochord expression. A. Images and schematics of the nine notochord enhancers in the ZEE library. Zic (red), ETS (blue), FoxA (orange), and Bra sites (green) are annotated. Dark blue ETS sites have an affinity of greater than 0.5, light blue sites have an affinity of less than 0.5. **B.** Counting data for nine ZEE elements showing the percentage of embryos with notochord expression. Three biological replicates were performed with 50 embryos per replicate analyzed.

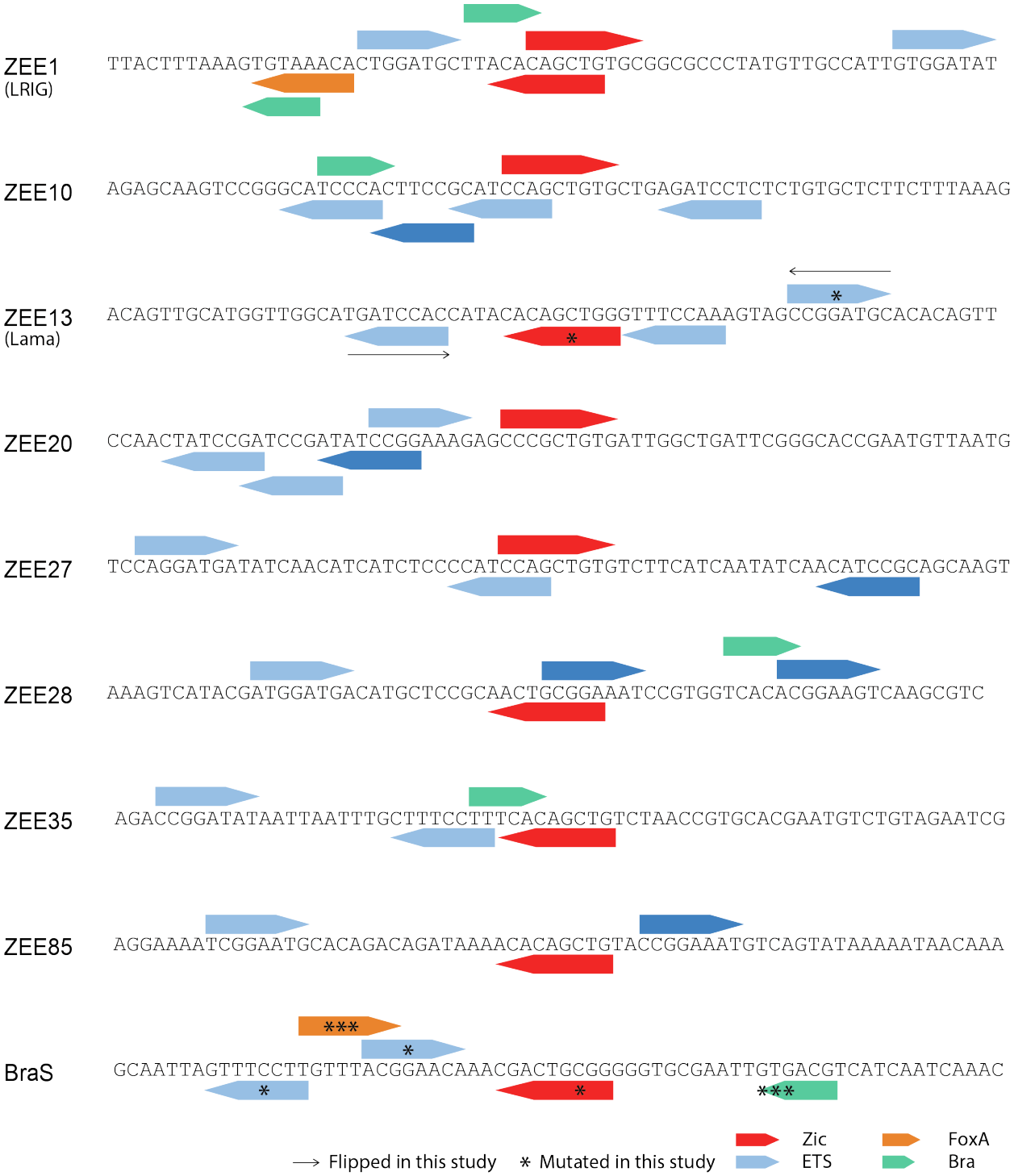
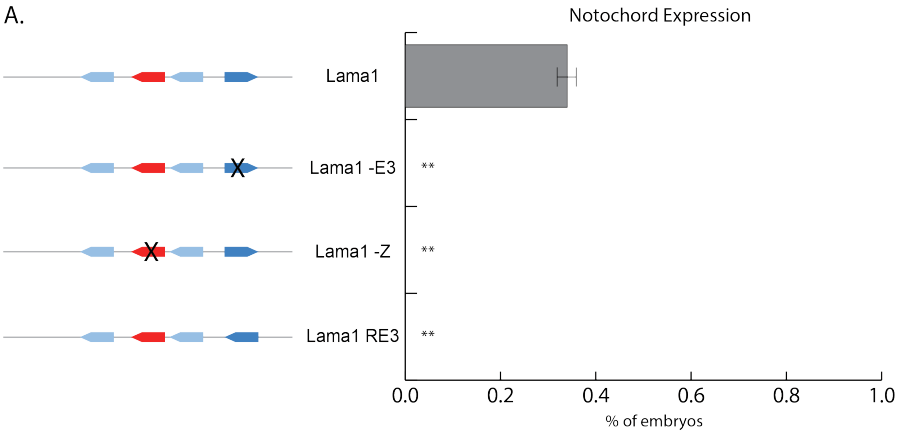
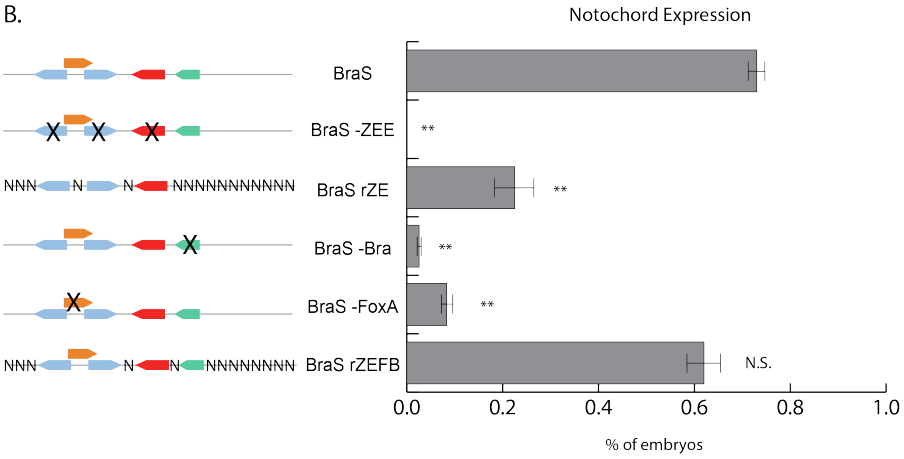
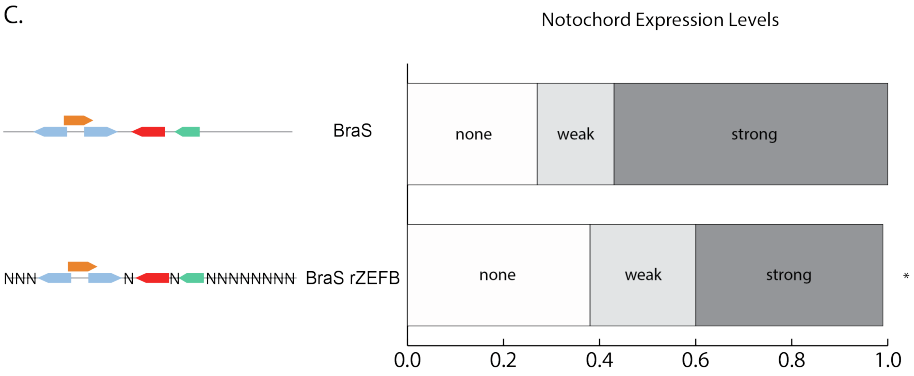


Figure S3, related to Figure 3. Annotated sequences of the nine ZEE elements that drive notochord expression. Zic (red), ETS (blue), FoxA (orange), and Bra sites (green) are annotated. Asterisk denotes nucleotide that was mutated in this study, arrow denotes a binding site that was flipped. Dark blue ETS sites have an affinity of greater than 0.5, light blue sites have an affinity of less than 0.5.


A.
Notochord Expression
Lama1
Lama1 -E3
Lama1 -Z
Lama1 RE3
**
**
**
0.0
0.2
0.4
0.6
0.8
1.0
% of embryos


B.
Notochord Expression
BraS
BraS -ZEE
BraS rZE
BraS -Bra
BraS -FoxA
BraS rZEFB
NNN
N
N
NNNNNNNNNN
NNN
N
N
NNNNNNNN
**
**
**
**
N.S.
0.0
0.2
0.4
0.6
0.8
1.0
% of embryos


C.
Notochord Expression Levels
BraS
BraS rZEFB
NNN
N
N
NNNNNNNN
none
weak
strong
none
weak
strong
*
0.0
0.2
0.4
0.6
0.8
1.0

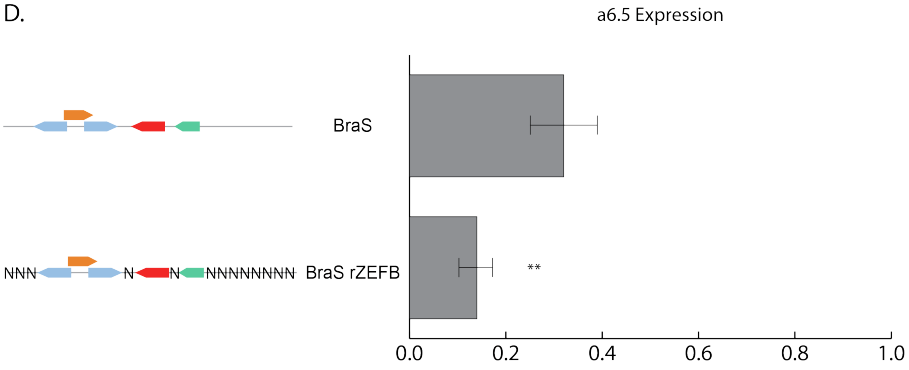
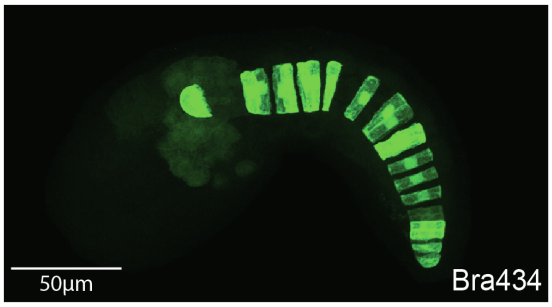

D.
a6.5 Expression
BraS
BraS rZEFB
NNN
N
N
NNNNNNNN
**
0.0
0.2
0.4
0.6
0.8
1.0

Figure S4, related to Figures 4 and 5. Scoring of manipulated notochord enhancers. **A.** Scoring of notochord expression for embryos electroporated with the *laminin alpha* (Lama) enhancer, Lama -E3, Lama -Z, and Lama RE3. Lama -E3, Lama -Z, and Lama RE3 all show no notochord expression. **B.** Scoring of notochord expression for embryos electroporated with Bra Shadow (BraS), BraS -ZEE, BraS rZE, BraS -Bra, BraS – FoxA, and BraS rZEFB. BraS -ZEE, BraS rZE, BraS -Bra, and BraS –FoxA all show statistically significant less notochord expression compared to BraS, while BraS rZEFB is not significantly different. **C.** Scoring of levels of expression in the notochord for embryos electroporated with BraS and BraS rZEFB. BraS rZEFB shows less notochord expression levels compared to BraS **D.** Scoring of a6.5 expression for embryos electroporated with BraS and BraS rZEFB. BraS rZEFB shows statistically significant less a6.5 expression compared to BraS. P values calculated by chi-squared test for expression levels and Fischer's exact test for all other comparisons, *P<0.05, ** P < 0.01. Dark blue ETS sites have an affinity of greater than 0.5, light blue sites have an affinity of less than 0.5. For counting data in A, we conducted three biological repeats analyzing 50 embryos per replicate. For counting data shown in B, C and D, we conducted two biological repeats analyzing 50 embryos per replicate.

A.



B. Bra434

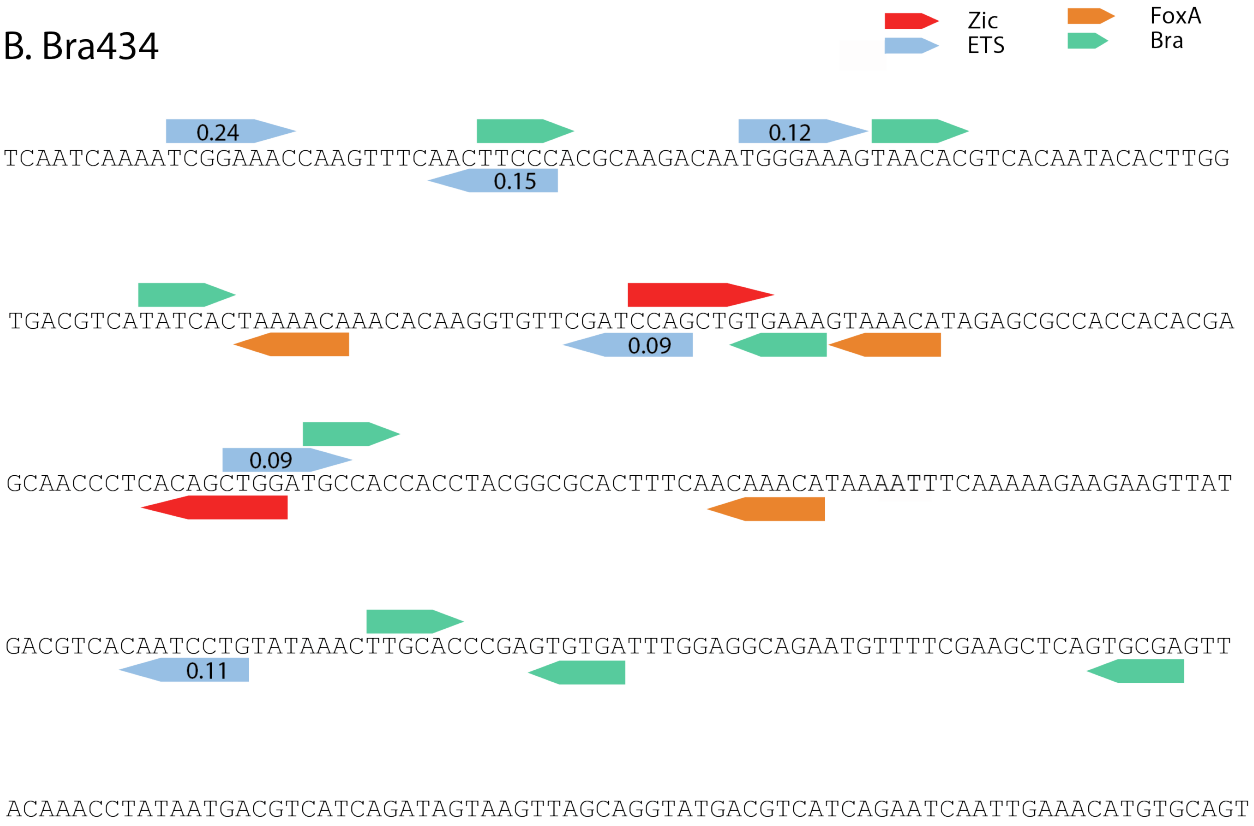


Figure S5, related to Figure 5. Updated annotation of Bra434. **A.** Image of Bra434 electroporated into Ciona embryo. **B.** Annotation of the Bra434 using PBM, EMSA, and crystal structure data[1–13]. Zic sites in red, ETS sites in light blue, FoxA sites in orange, and Bra sites in green. Affinities of ETS calculated from PBM data[7] are labeled.

Supplemental References:

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