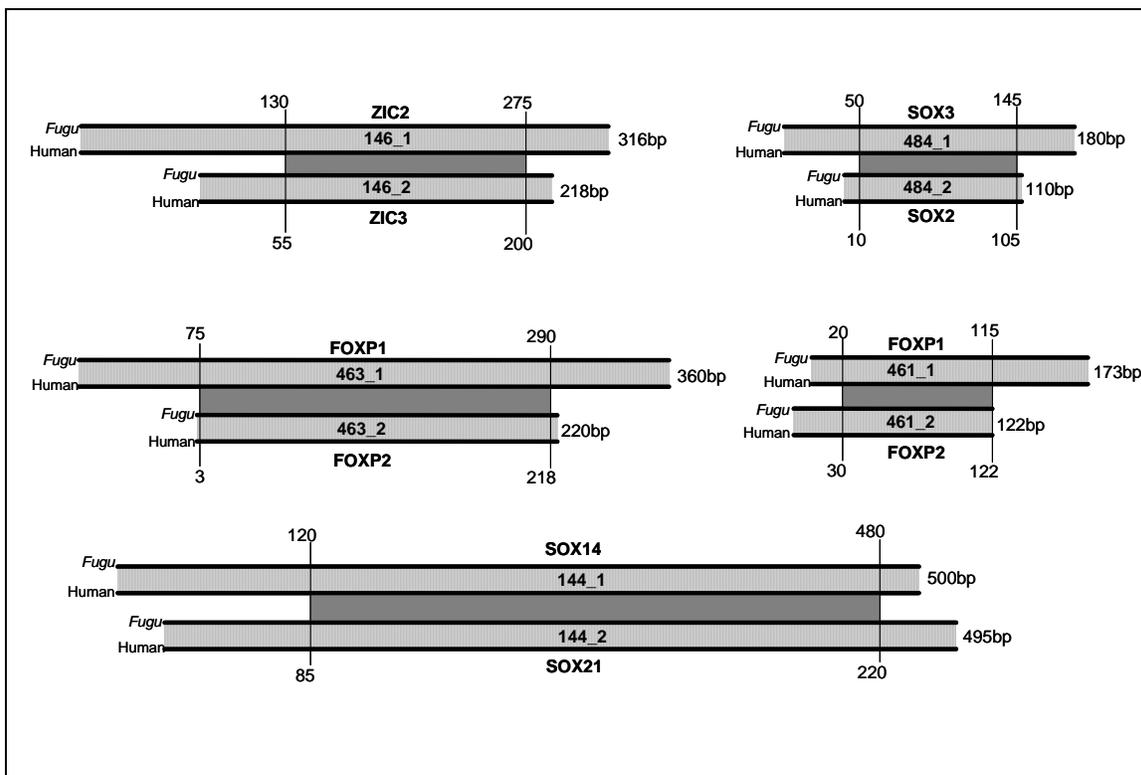


### Supplemental Figure S3.

(A). **Length comparison of dCNE families used in the functional analysis.** Regions of sequence conservation between orthologous *Fugu* and human dCNEs are indicated by light grey shading, with lengths of the elements indicated on the right hand side. Regions of sequence similarity between paralogous dCNEs are denoted by dark grey shading and start and finish of this overlap are indicated. The dCNE family IDs (which can be related back to data on Supplemental table S1) and their associated paralog are given in each case.



**(B).** Alignments of dCNE sequences used in functional analysis. Alignments show the conservation between human and *Fugu* of each dCNE family member followed by the alignment between both family members in both species.

<b>FoxP1/FoxP2 dCNE 461 from human and <i>Fugu</i> genomes</b>	
<b>dCNE 461_1 FOXP1</b>	
461_1human	TAGAGGAGCTGCAGTCAAGTGGAAAA--TTTCTGCCTGACCTCTGCCCTT--GCTATTAA
461_1fugu	---GGAGCGGCAGTCAAGTGGAAAAACGTTGTTGCCTGGTCCCCCATCCAGATATTAA *****
461_1human	TTTGCAGGAAAATAATGACACATATACATATTTCCACAAAATTGTTCTAATTGCTAATT
461_1fugu	TTGGCAGGAAAATAATGACACAGATACATATTTCCACCAAATTGTTCTAATTGCTGATT **
461_1human	TGCCAAAGGAGCCAGATTCCAAATTAACATGA--CTAGCCCGTGAGGGAAGGA
461_1fugu	AGCCGAAGGAGGCTTGTTCCAAATTAATATGAGCTCGGACTGTGAGAAAGG-- ***
<b>dCNE 461_2 FOXP2</b>	
461_2human	-AAATCTCAGCTGAGAGGCTCCAGCCAGCTGGAAAATTTTACTGACCTTT-AGTCTCC
461_2fugu	CAAATCTCAAGTGAGAGGCTCTAACCAGCTGGAAAATTCCTCGCTGACCTTTTAGTCCCT *****
461_2human	TTGCTAATTTGG-TGGAATGGTAATGACATTACTACATATTCAAACAAAATTGCTTAAT
461_2fugu	CTGCTAATTTAGCTGGAATGGTAATGACATTGTACATATTCACACCAAATGCTTAAT *****
461_2human	TGC
461_2fugu	TGT **
<b>dCNEs 461_1 FOXP1 and 461_2 FOXP2</b>	
461_2human	-AAATCTCAGCTGAGAGGCTCCAGCCAGCTGGAAAA--TTTACTGACCTTT-AGTC
461_2fugu	CAAATCTCAAGTGAGAGGCTCTAACCAGCTGGAAAA--TTCTCGCTGACCTTTTAGTC
461_1human	-----TAGAGGAGCTGCAGTCAAGTGGAAAA--TTTCTGCCTGACCTT-GCCC
461_1fugu	-----GGAGCGGCAGTCAAGTGGAAAAACGTTGTTGCCTGGTCCCCCATC * * * * *
461_2human	TCCTTGCTAATTTGG-TGGAATGGTAATGACATTACTACATATTCAAACAAAATTGCTTT
461_2fugu	CCTCTGCTAATTTAGCTGGAATGGTAATGACATTGTACATATTCACACCAAATTGCCTT
461_1human	TTGCTATTAATTTGC-AGGAAAATAATGACACATATACATATTTCCACAAAATTGTTCT
461_1fugu	CAGATATTAAT-TGCAGGAAAATAATGACACAGATACATATTTCCACCAAATTGTTCT * * * * *
461_2human	AATTGC-----
461_2fugu	AATTGT-----
461_1human	AATTGCTAATTTGCCAAGGAGCCAGATTCCAAATTAACATGACTAGCCCGTGAGGGA
461_1fugu	AATTGCTGATTAGCCGAAGGAGGCTTGTTCCAAATTAATATGAGCTCGGACTGTGAGA *****
461_2human	----
461_2fugu	----
461_1human	AGGA
461_1fugu	AAGG











**ZIC2/ZIC3 from human and *Fugu* genomes**

**dCNE 146\_1 ZIC2**

```
146_human      GCGGAGAGAAAAGACA-CTTGTGTAGATTTTCAATCTCCTCCGTGTCACCTGGCTGTC
146_lfugu      ----AAGAAGACACTTCTTGTGTGGATTTTCAATCTTCAACATGTCATTTGGCCGGG
                **** * ** ***** ***** * * ***** ***** *
146_human      CTCACCATTGTCTGAGCTCCTTCTCT--TATCCTTTTGTGCTGCAATGAGTTGTGTGTCAT
146_lfugu      AGTGCCATTGTCCCCTTCCCCTTTTCTCCCTGCTCCTTCTGCTGAGACGAGTTGTGTGTCAT
                ***** ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
146_human      TCCCTCATGGTCAGCACTTTTGTGTCTTTGTTTTTGTGCTACCTCAGAAGTCCATCTT
146_lfugu      CCCCTCATGATCAGCACTTTTGTGCTTTGTTTTTGGCCCTACCTCAGAAGTCCATCTT
                ***** ***** * * * * * * * * * * * * * * * * * * * * * * * * *
146_human      TTCTTTCATCTCTTCCCATTCATGCATGAGGGAACAGTTGTGCCCTGTTGTGGGGATGT
146_lfugu      GTCTTTTATGTCTCTGCCTTTCATGCATAAGGGAACAGTTGTGCCCTGTTGTGGGGCTGT
                ***** * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
146_human      TCCGCTGGGTTTCAAACAGTAGGGTTTCTTTTATTCTCTTCTAAGTAGTACAATGGGGC
146_lfugu      TCCAGTGCATTTCCACCAGTGGGGTTTCTTTCATTCTCTTCAAAGTACTACAATGGGGC
                ** * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
146_human      T--GAATGTCTGT-TGCAG
146_lfugu      TCTGAAGGCCTGAATG---
                * * * * * * * * * *
```

**dCNE 146\_2 ZIC3**

```
146_2human     TTTTTCTCCCTCTCTTTTGCCTCAATAGAAAAGA-AAAAAAGTTTGTGTCAGCACTCCTG
146_2fugu      --TTCATTCCATCCTTTTACCACAAAAGGAAAAGAAAAAAGTTTGTGTCAG-ACCTCTG
                ** *** * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
146_2human     TTTCTTTGTTGTTTTCTTTTCTCAGAAGTCTTTTCTGCTTTTATCCTAATCCCAGTC
146_2fugu      TTTCTTTGTTGTTGCTTTCTTCTCTGTG-CTCTTCTACTTTTATCCTAATCCCAGCC
                ***** * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
146_2human     ATGCATGAGAGAACAGTTGTGCTCTGTTGTTTGGCTGTTCTGCTAGTTTGAATCAGTGG
146_2fugu      ATGCATGAGCGATCAGTTGTGCTCTGTTGTTTCACTGTTCTGCTAGTTTTCAGTCAGTAG
                ***** * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
146_2human     GACTCCCTCTTTGTTCTCTTTATTTTCTGAACACAGAA-----
146_2fugu      GGTTCCCTCTTTGTTCTTTTCTTGGCCGAACACAGAAAAGGTAAG
                * *** ***** ** * * * * * * * * * *
```



(C). Sequences used in functional assay. Uppercase letters denote primer sequences.

>dCNE461\_1 FOXP1  
GGAGCGGAGTCAAGTGGaaaaagcttgttgccctgggtccccccatccagatattaattggcaggaaaactaatgacacagatata  
ttccaaccaaattgttctaattgtctgattagccgaaggaggctgtttccaaattaaatATGAGCTCGGACTGTGAGAAAGG

>dCNE461\_2 FOXP2  
CAAATCTCAAGTGAAGGCTCtaaccgactggaaaattcctcctgctgaccttttagtccctctgctaatttagctggaatggtaatga  
cattgtttacataattcaCACCAAATTGCCTTAATTGT

>dCNE4631 FOXP1  
TGTTCCAGCGCTCAGGAGgatgctgctcctcataaagcagcacaagaacagtgtagctgactaatacaaggacatagttcat  
catgacattttctgcaattcccaactgatttagggctcattatggtgggggctgatttatgctgtcatttctctcaccgatcctgca  
tcaatgaaatcctaataatggtaataaagggtgaagattacactgtgtgacactgcaacatttctcaccctcaacagctgagattt  
atgtagccaatttagaggctaacagagaaaattggctggcagaaaaataatattctttatgggtccaaaaacgggggCGTTCTCAGT  
GAAGGGCAAC

>dCNE463\_2 FOXP2  
GAGACATAGTTTCATTGTGACGtttccagttatttctggctgatttagggctcattatgtctccagctctgatttatggtgtcattctcc  
cctcctgctcctgcatcgatagatcctaataatggtaataaagggtgaagattgctggttgacaacaccgcaacatttctcattca  
ttgcagacaagatttatgtaaCCAATTAGGAGCATAACAGGA

>dCNE144\_1 SOX14  
ACCGTTGGCAGTAGCCATCGactggaaggagaagctgccaggagtccttgatggataggctcattttccagccggcagcaagtctc  
ggcgaagcatccgtgtcatttgaatgtcagccaacaaaatgtaatacaaacattccagcgtttaatcacagagggtgctcgagag  
atgattgtagtgcaataataatgctatttcaattatctgtttgtattttatattgacagtgcaagataaaagtggtgaaaataaag  
ttaaaaaataggttaatttcaattcaatcaccocagctacagttgctggccattatgctgattgattacttctgaattaaattgggg  
ctgaatgtaaaagttaattcttcttctctgcaatcagctcccaaggcctcttagtaagtaagtaccatttctatttct  
ttaatgaaaagtaatggcccaaatatgtataaaaTCAGCTGTTGTATGCACCTAGTT

>dCNE144\_2 SOX21  
AGCCGTCAGTAAACACTTAGCTccctttaaaggcagatatttaaccaggccatatttggggcgtgaaactgcaagtgatgctcccttt  
ggaattacattctgaaagaccatcagaccctttaaagctgagtgatttacacaattcattaaagccagtgaaatcctcagccagc  
gtcacattaaatgggtacttactcctcaaatggccccggggagtgatggtggcttgcaaatagataaaagttaacattgaaaag  
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gagtgcttgtttcagtgatataatgctgtgatctgtgatcttctgtgcccgggtgtcaggctggcggccaggggccggggagacatg  
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acagccgggcaagaatggccgcaacaaagtttgcacaataagattattagggocgGTACCTTCCTGCGGCGAAC

>dCNE484\_1 SOX3  
AACGGGAAGCTCCAGGTGAGtttccctcgccaagttttgcagcctgcatgttgtctattctgcacagctatttaaaaaatggtgaa  
tatcaacaatggctttcactggtgcatgtgcaacttttgatgaagcaatcttggtgaaatgagatgggtcCCAGAGCAGGGAGAGC  
TCA

>dCNE484\_2 SOX2  
TGCCACATGTCTATTCTGCTccgctatgtgaaaaatgtgaaatcaacaatggctttcagtggtgattgttgagttttgtatG  
AACTAATCTTGCCCTCAGTGA

>dCNE146\_1 ZIC2  
AAGAAGACTTCTTGTGTGGatttttcaattcttcaacatgtcatttggccgggagtgccattgtcccttcccttttctcctg  
ctccttctgctgagacgagttgtgcatccccatgatcagcacttttggcttctgttttggccctacctcagaagtcacatct  
tgtctttatgtctctgctttcatgcataaggaacagttgtggcctgttggggctgttccagtgcaattccaccagtggggtt  
tcctttcactcttcaaaagtactacaatggGCTCTGAAGGCTGAATG

>dCNE146\_2 ZIC3  
TTCATTCATCCTTTTCACCaacaaaggaaaagaaaaaaagtgtgtcagacctgtttctttgttgtgtgctttctctctgtg  
ctctttctacttttatcctaataccagccatgcatgagcagatcagttgtgctcctgttcttactgttctgtagttttcagtcag  
agggttccctctttgttcttttcttgCCGAACACAGAAAAAGGTAAG