

Supporting Information

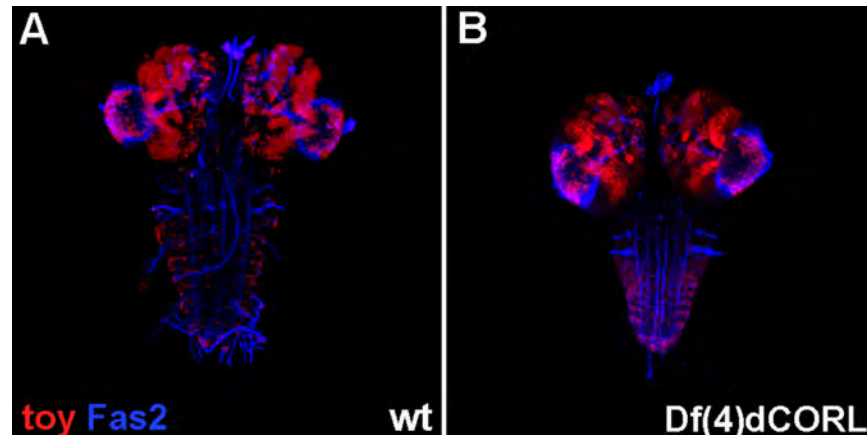


Fig. S1. Toy larval brain expression is unaffected in the dCORL deletion mutant. Dorsal view of larval brains at scale with anterior up displaying Toy (red) and Fas2 (blue). A) Wild type and B) *Df(4)dCORL*. Both show Toy expression in the brain that appears identical while there may be a slight increase in ventral cord expression in the mutant.

Table S1. Eleven markers that do not coexpress with *dCORL* reporters in the third instar CNS.

Antibody	Source (concentration & animal)	LacZ reporters co-stained	Co-stained antibodies (concentration & animal)	Representative citation - antibody expression
Engrailed (En)	4D9 DSHB (1:250 M)	AH, BA (see Figure 2)	lacZ (1:1000 Rab)	Siegler & Jia (1999)
Even-skipped (Eve)	John Reinitz (1:200 GP)	full set (11)	FasII (1:75 M) lacZ (1:1000 Rab)	Frasch et al. (1987)
Twin of eyeless (Toy)	Uwe Walldorf (1:500 GP)	full set	FasII (1:75 M) lacZ (1:1000 Rab)	Furukubo-Tokunaga et al. (2009)
Bursicon (Burs)	Ben White (1:2000 Rab)	full set	FasII (1:75 M) lacZ (1:1500 Rat)	Peabody et al. (2008)
Crustacean cardioactive peptide (Ccap)	Abnova; Taiwan (1:1000 Rab)	intergenic set (7)	FasII (1:75 M) lacZ (1:1500 Rat)	Dulcis et al. (2005)
Dimmed (Dimm)	Paul Taghert (1:1000 GP)	intergenic set	FasII (1:75 M) lacZ (1:1000 Rab)	Park et al. (2008)
Trio	9.4A DSHB (1:300 M)	intergenic set	lacZ (1:1000 Rab)	Awasaki et al. (2000)
Dachshund (Dac)	1-1 DSHB (1:750 M)	intergenic set	lacZ (1:1000 Rab)	Hasegawa et al. (2011)
Tailless (Tll)	John Reinitz (1:750 Rab)	KH, AH	EcR-B1 (1:50 M) lacZ (1:1500 Rat)	Kurusu et al. (2009)
Reversed polarity (Repo)	8D12 DSHB (1:50 M)	AH	Tll (1:250 GP) lacZ (1:1000 Rab)	Campbell et al. (1994)
Fasciclin II (FasII)	1D4 DSHB (1:75 M)	full set	See above	Landgraf et al. (2003)

Table S2. Conservation of intergenic Hox, bHLH, EcR and Octamer binding sites across 12 *Drosophila* species predicts five *dCORL* or *toy* cis-regulatory modules (CRM).

CRM#1

235bp = 3 conserved **Hox** in 12 species + 1 Hox missing only in *mojavensis* chromosome 4 981675bp (Ref Seq NC_004353.4)
CACGCGTtttCa**TATTA**Ttcc**TGCCGGAT**a**CGGAAATATTA**TTTTAAATAACT**GCGCAC**ATCACgaa**AG**gaaatattttcccgtgg
 gttcgtcaagggactgagagtgtgtatatatatatttctcggacctaactgcgcttgcccttataaaagcggtaataagaaagga
 a**GAGTG****TGCGTGTGCACGGGAAATTA**Aa**AG****AGCGTGC**a**GAATCTGATTC**g**TAATAT****TGCTTA**ttctcgc**TTATTAATT**
ATT 981899bp

CRM#2

189bp = 2 conserved Hox + 2 conserved **bHLH** in 12 species chromosome 4 983052bp
CACACC**GCCTGCTG**T**TGCCA**C**AACT**c**GAA**g**G**gccgcccga**AACAGC**ggtt**gtGCCACGTG**g**CGCTGACAACTGCTTAAAC**
ACGCCa**TTGATGATTCAAATA**tagtctggttgggtctctagcgcgctgctcagacggttctcccttttcgggctcaacg**AATT**
ACACACTTTGCGGCATTTTGCCTTTTAAACATTTTATTGTTATCGATCGgc**GGTCGCC**a**GGCTGCCAATTA**c**CAAGTG**Ct**AG**
 Ag**CAGA** 983241bp

CRM#1 and CRM#2 are nearby. Located 3186bp distal to *dCORL* exon1, CRM#1 is 235bp and contains 4 Hox sites. Located 4563bp distal to *dCORL* exon1, CRM#2 is 189bp and contains 2 Hox and 2 bHLH sites. Both are near the center of the intergenic region where BB.lacZ, BA.lacZ and BpK.lacZ overlap (Fig. 6A). CRM#1,2 could activate or repress *dCORL* embryonic ventral cord expression.

CRM#3

345bp = 4 conserved Hox in 12 species + 1 bHLH missing only in *mojavensis* + inverted repeat of **Octamer** binding sites with the forward site overlapping the third **Hox** site + **ECR** site overlapping both the third Hox and forward Octamer sites chromosome 4 984737bp
GCAgc**AAATTAATTATTGTA**ca**ACAAATGT**T**CG**g**CAATTTTCATGAATTCAAAA**c**CATTGA**g**ATGAAA****TAATGAAAATC**
CCAAAAACAATCAAAAGGATACCCA**tacc**cagttctgaaccctgctgcattcattcccattgctcatgcttctttgcttctacc
 agaaacacatccctagctctacaaatatatttcggtctgctcctggacctgaccctttttgtatggttgacttggct**TTGGGG**
TGTc**GT**c**Tat**G**ACGCGTGC**GT**G**a**TTGTTGGTGAGCCACACA**a**ATT**Tgcgtcacttcagc**AT**ca**ATATGAAA**gt**AGGCGTAT**
CTg**Tac****TGAT**g**AAGTTAAATTAGC** 985052bp

Located 6246bp distal to *dCORL* exon1, CRM#3 is 345bp and contains 4 Hox, 1 bHLH, 1 EcR and an inverted repeat of Octamer sites. It is distal to CRM#1,2 where BA.lacZ, BgK.lacZ and BpK.lacZ overlap (Fig. 6A). CRM#3 could repress *dCORL* embryonic ventral cord or activate *dCORL* larval brain or repress *dCORL* adult brain expression.

CRM#4

108bp = 1 conserved Hox in 12 species + 1 Hox missing only in *mojavensis* chromosome 4 985342bp
TGAAATGAACAAacgca**TT**gtttaa**TAT**tttaacaacgaatgcaataataaacaacacaaaataatttaggcaacaatgtga
 tgcgcgccctaggaa**GAC**gat**GACAATTACAATGAG**a**ATTT**a**GC**C**G**CT**GCGGTGGATGG**ttgt**C****TCATCA**cggacacag
 actgaagcccgttggctgt**AGC**cg**G**gt**CTGCCA**TT**ACTGGGCAATTA**gg**CA**T**G**act**GGAAAAG**a**AAAAGGGGAGCT**c**A**
 gcagcagcagcatccagcaaaaatgatcaactaaa**TTTGTGA**gc**TCT**ga**A****CCTAGC** 985450bp

CRM#5

121bp = 1 conserved Hox + 3 conserved bHLH in 12 species + 1 EcR site overlapping the third bHLH site chromosome 4 986318bp
CAACAAGTGTT**GGTTGACAGCATCATTTCC**g**CATTTTCA**TTT**TGTGTC****ATTAATTTT**g**GAGT**C**G**g**A**a**AACCGCA**tt**GTGAAAA**
TAACAGCTGATCCcccc**TT**tccc**TTT**tat**TTTGTGCTCG**g**C**atggttggttcgaatcgccg**ACTGGA**ACT**AAAT****GTGACATG**
TGATAAGAATTTT**c****AGATAAATCTC** 986439bp

CRM#4 and CRM#5 are nearby. Located 6835bp distal to *dCORL* exon1, CRM#4 is 108bp and contains 2 Hox sites. Located 7829bp distal to *dCORL* exon1, CRM#5 is 121bp and contains 1 Hox, 3 bHLH and 1 EcR site overlapping the third bHLH site. Both are distal to CRM#3 where AH.lacZ, BgK.lacZ and BpK.lacZ overlap but are not in KH.lacZ (Fig. 6A). CRM#4,5 could activate or repress *dCORL* embryonic or larval or adult brain expression or activate *toy* embryonic or larval ventral cord expression.

Supplemental References

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