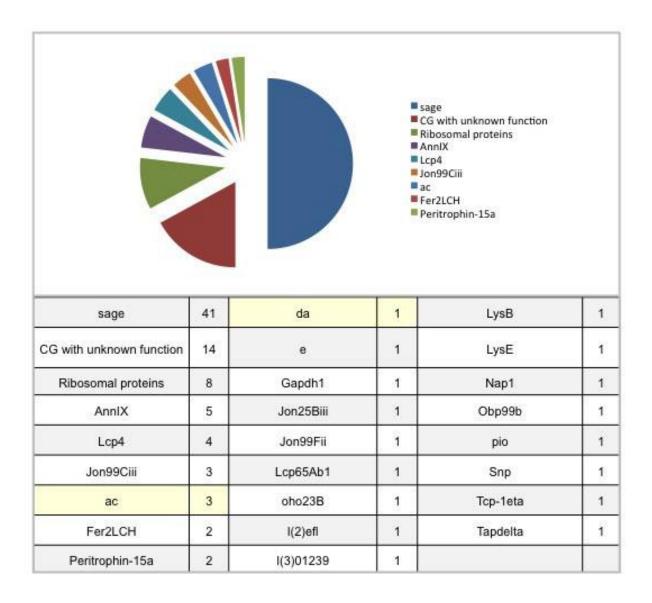
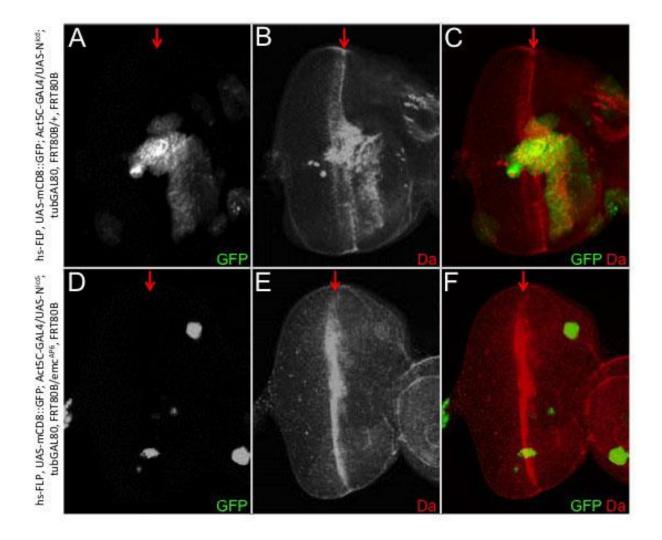


Supplemental Figure 1: Analysis of the genomic region surrounding the *emc* transcriptional start site. (A) Depiction of the genomic region upstream the *emc* transcriptional start site located on 3L and the subgenomic regions that were tested for the ability to drive expression at the midline. The genomic regions shown in purple were isolated and fused to GAL4 by Gerald Rubin's laboratory (Jory et al., 2012; Manning et al., 2012). We cloned the regions in orange and fused them directly to a *lacZ* reporter. (B-I) Light microscope images of third instar eye discs. Dorsal side is up and anterior is to the right. The red arrow in panels B-I indicates the position of the morphogenetic furrow. All discs were photographed at 10X magnification. Expression patterns driven by the eight genomic fragments in late third instar eye discs. (B,F) Putative enhancers E1 and GMR10H11 both show anterior compartment expression.



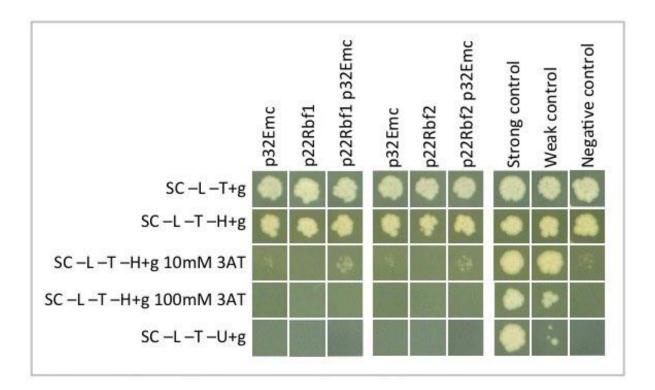
## Supplemental Figure 2: Results from an unbiased Y2H screen for binding partners of Emc.

An unbiased Y2H screen for protein interactions with Emc was conducted. Plasmids from 100 colonies were isolated and sequenced. Of the 100 colonies, sequence data was recovered for 99 putative interacting genes. In this figure a list of putative interacting proteins is presented along with the number of times we recovered each factor. From our screen, we identified Da and Ac, two bHLH proteins that are known to interact with Emc (coded in yellow). We also identified Sage (41 times), a bHLH protein that is expressed exclusively in the salivary glands. The pie chart is a graphical representation of the chart - note that only candidates that were recovered more than once are represented within the pie chart.

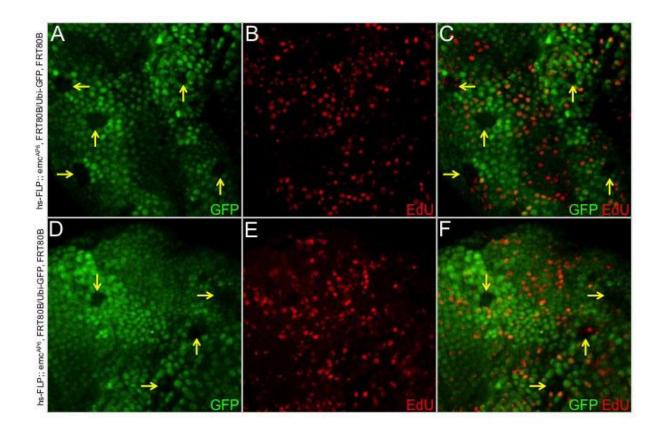


Supplemental Figure 3: Notch activation of *daughterless* is dependent upon extramacrochaetae.

(A-F) Light microscope images of third instar eye-antennal discs containing MARCM clones. Dorsal side is up and anterior is to the right. The red arrow indicates the position of the morphogenetic furrow. All discs were photographed at 10X magnification. (A-C) MARCM clones over-expressing N<sup>ICD</sup> in an otherwise wild type background. Da antibody staining is elevated in response to Notch pathway activation. (D-F) MARCM clones over-expressing N<sup>ICD</sup> in  $emc^{AP6}$  null mutant clones. The reduced size of the clones is consistent with Emc being required for a subset of Notch dependent growth. In addition, da expression is no longer elevated in the  $emc^{AP6}$  mutant clones. This indicates that emc is also required for Notch dependent activation of da expression. Dorsal side is up and anterior is to the right. The red arrow in each panel indicates the position of the morphogenetic furrow.



Supplemental Figure 4: Emc does not bind to *Drosophila* Rbf proteins. The lack of colony growth compared to controls in this directed yeast two-hybrid assay indicates that Emc does not interact with either Rbf1 or Rbf2.



Supplemental Figure 5: EdU incoporation is reduced in *emc*<sup>AP6</sup> null mutant clones. (A-F) Light microscope images of third instar eye discs containing *emc*<sup>AP6</sup> loss-of-function clones. Dorsal side is up and anterior is to the right. All discs were photographed at 40X magnification. Two different (A-C and D-F) high magnification examples of EdU incorporation in *emc*<sup>AP6</sup> null mutant clones. Yellow arrows demarcate null clones. For statistical analysis we examined 48 *emc*<sup>AP6</sup> null clones and observed a reduction in EdU staining within 22.9% of clones. The remaining clones showed EdU incorporation being at comparable levels to surrounding wild type areas.

## Table S1

Figure panel	Abbreviated Genotype in Panel	Full genotype
Figure 1A,B	hs-FLP; emc <sup>AP6</sup> /GFP	hs-FLP <sup>22</sup> w*/+; ; FRT80B emc <sup>AP6</sup> /FRT80B Ubi-GFP
Figure 1C,D	hs-FLP; emc/M <sup>-</sup> , GFP	hs-FLP <sup>22</sup> w*/+; ; FRT80B emc <sup>AP6</sup> / FRT80B Ubi-GFP M(3)i55
Figure 1E,I	emc <sup>AP6</sup> /GFP	hs-FLP <sup>22</sup> w*/+; ; FRT80B emc <sup>AP6</sup> /FRT80B Ubi-GFP
Figure 1F,J	+/GFP	hs-FLP <sup>22</sup> w*/+; ; FRT80B/FRT80B Ubi-GFP
Figure 1G,K	hs-FLP; emc/MT, GFP	hs-FLP <sup>22</sup> w*/+; ; FRT80B emc <sup>AP6</sup> /FRT80B Ubi-GFP M(3)i55
Figure 1H,L	+/M¯, GFP	hs-FLP <sup>22</sup> w*/+; ; FRT80B/FRT80B Ubi-GFP M(3)i55
Figure 1N	WT	hs-FLP <sup>22</sup> y <sup>1</sup> w <sup>*</sup> UAS-mCD8::GFP.L Ptp4E <sup>LL4</sup> /+; Act5C-GAL4/+; FRT80B/FRT80B tub-GAL80
Figure 10	UAS-p35	hs-FLP <sup>22</sup> y <sup>1</sup> w <sup>*</sup> UAS-mCD8::GFP.L Ptp4E <sup>LL4</sup> /+; Act5C-GAL4/UAS-p35; FRT80B/FRT80B tub-GAL80
Figure 1P	emc <sup>AP6</sup>	hs-FLP <sup>22</sup> y <sup>1</sup> w <sup>*</sup> UAS-mCD8::GFP.L Ptp4E <sup>LL4</sup> /+; Act5C-GAL4/+; FRT80B emc <sup>AP6</sup> /FRT80B tub-GAL80
Figure 1Q	UAS-p35, emc <sup>AP6</sup>	hs-FLP <sup>22</sup> y <sup>1</sup> w <sup>*</sup> UAS-mCD8::GFP.L Ptp4E <sup>LL4</sup> /+; Act5C-GAL4/UAS-p35; FRT80B emc <sup>AP6</sup> /FRT80B tub-GAL80
Figure 2A	WT	$P\{PZ\}emc^{04322} ry^{506}/TM3 ry^{RK} Sb^1 Ser^1$
Figure 2B-D	UAS-N <sup>ICD</sup>	hs-FLP <sup>22</sup> w*/+; AyGAL4 UAS-GFP.S65T Myo31DF/UAS-N <sup>CD</sup> ; P{PZ}emc <sup>04322</sup> ry <sup>506</sup> /+
Figure 2E	UAS-N <sup>ICD</sup>	hs-FLP <sup>22</sup> y <sup>1</sup> w <sup>*</sup> UAS-mCD8::GFP.L Ptp4E <sup>LL4</sup> /+; Act5C-GAL4/UAS-N <sup>ICD</sup> ; FRT80B/ FRT80B tub-GAL80
Figure 2F-G	UAS-N <sup>ICD</sup> , emc <sup>AP6</sup>	hs-FLP <sup>22</sup> y <sup>1</sup> w <sup>*</sup> UAS-mCD8::GFP.L Ptp4E <sup>LL4</sup> /+; Act5C-GAL4/UAS-N <sup>ICD</sup> ; FRT80B emc <sup>AP6</sup> /FRT80B tub-GAL80
Figure 4A	UAS-GFP	hs-FLP <sup>22</sup> w*/+; AyGAL4 UAS-GFP.S65T Myo31DF/+
Figure 4B	UAS-da	hs-FLP <sup>22</sup> w*/+; AyGAL4 UAS-GFP.S65T Myo31DF/UAS-da
Figure 4C	UAS-emc	hs-FLP <sup>22</sup> w*/+; AyGAL4 UAS-GFP.S65T Myo31DF/+; UAS-emc⁴ <sup>M</sup> /+
Figure 4D	UAS-da, UAS-emc	hs-FLP <sup>22</sup> w*/+; AyGAL4 UAS-GFP.S65T Myo31DF/UAS-da; UAS-emc <sup>4M</sup> /+
Figure 5A	not labeled	ey-GAL4/UAS-Mnt <sup>T2-33</sup>
Figure 5B	not labeled	ey-GAL4/UAS-Mnt <sup>T2-33</sup>
Figure 5C	not labeled	ey-GAL4/UAS-Mnt <sup>T2-33</sup>
Figure 5F-H	ey-FLP; emcAP6/+	ey-FLP/+; ; FRT80B emc <sup>AP6</sup> /FRT80B Ubi-GFP
Figure 6A,E,I,M	$w^{1118}$	W <sup>1118</sup>
Figure 6 B-D, F-H, J-L, N-P	ey-FLP;; emc <sup>AP6</sup> , FRT80B/Ubi-GFP, FRT80B	ey-FLP/+;; FRT80B emc <sup>AP6</sup> /FRT80B Ubi-GFP
Figure 6 Q-T	hs-FLP; UAS-emc; PCNA::GFP/Act5C>y <sup>+</sup> >RFP	hs-FLP/+; UAS-emc/+; PCNA::GFP/Act5C-GAL4 UAS-RFP.W
Supplemental Figure 1B	E1	emc-E1-lacZ
Supplemental Figure 1C	E2	emc-E2-lacZ
Supplemental Figure 1D	E3	emc-E3-lacZ
Supplemental Figure 1E	GMR10D04	w <sup>1118</sup> /+; UAS-lacZ/+; GMR10D04-GAL4/+
Supplemental Figure 1F	GMR10H11	w <sup>1118</sup> /+; UAS-lacZ/+; GMR10H11-GAL4/+
Supplemental Figure 1G	GMR10B05	w <sup>1118</sup> /+; UAS-lacZ/+; GMR10B05-GAL4/+
Supplemental Figure 1H	GMR10C04	w <sup>1118</sup> /+; UAS-lacZ/+; GMR10C04-GAL4/+
Supplemental Figure 1I	GMR10B08	w <sup>1118</sup> /+; UAS-lacZ/+; GMR10B08-GAL4/+
Supplemental Figure 3 A-C	UAS-N <sup>ICD</sup>	hs-FLP <sup>22</sup> y <sup>1</sup> w <sup>*</sup> UAS-mCD8::GFP.L Ptp4E <sup>LL4</sup> /+; Act5C-GAL4/UAS-N <sup>ICD</sup> ; FRT80B/FRT80B tub-GAL80
Supplemental Figure 3 D-F	emc <sup>AP6</sup> , UAS-N <sup>ICD</sup>	hs-FLP <sup>22</sup> y <sup>1</sup> w <sup>*</sup> UAS-mCD8::GFP.L Ptp4E <sup>LL4</sup> /+; Act5C-GAL4/UAS-N <sup>ICD</sup> ; FRT80B emc <sup>AP6</sup> /FRT80B tub-GAL80
Supplemental Figure 5A-F	ey-FLP; emc/+	ey-FLP/+;; FRT80B emc <sup>AP6</sup> /FRT80B Ubi-GFP