Supplementary Materials and Methods

PCR Conditions

1.0µl w^{1118} genomic DNA
31.5 µl dH_2O
10.0 µl Phusion Buffer (5X)
3.0 µl Forward primer (0.2 µM final concentration)
3.0 µl Reverse primer (0.2 µM final concentration)
1.0 µl dNTPs (25 mM each)
0.5 µl Phusion DNA Polymerase

Step 1: 98°C 30 seconds
Step 2: 98°C 10 seconds
Step 3: 68°C 30 seconds
Step 4: 72°C 30 seconds/kilobase
Step 5: 72°C 10 minutes
Steps 2 through 4 cycled 35 times

Cloning Strategy

Fragment E1 was amplified from w^{1118} genomic DNA using primers (Listed in Table S1) that added a StuI and XbaI restriction enzyme site to the 5' and 3' end of the fragment sequence, respectively. The amplified PCR product was digested with StuI and XbaI restriction enzymes (NEB Catalog R0187S and R0145S), as was the expression vector placZ-attB. Both the digested fragment region PCR product and the digested placZ-attB vector were purified using the Thermo Scientific GeneJET PCR Purification Kit (Product K0701). The purified, digest placZ-attB vector was phosphatase-treated prior to ligation (NEB Catalog M0289S). The phosphatase enzyme was inactivated by high-temperature incubation but the reaction was not further purified. The digested Fragment E1 PCR product was ligated into the phosphatase-treated placZ-attB plasmid using T4 DNA Ligase (NEB Catalog M0202S). The ligation reaction was used to transform DH5α Subcloning Efficiency Competent Cells (Life Technologies Catalog 18265-017). Cultures were started from the transformation colonies, mini-prepped, and the purified plasmids were sequenced to ensure proper ligation.

Fragments E2 and E3 were both amplified from w^{1118} genomic DNA using primers (Listed in Table S1) that added the Gateway recombination sequences to the 5' and 3' end of the fragment region. The amplified fragment PCR products were cloned into the pDONR201 vector using the Life Technologies Gateway BP Clonase Kit (Product 11789013). The recombined plasmids were used to transform DH5α Subcloning Efficiency Competent Cells (Life Technologies Catalog 18265-017). Cultures were started from the transformation colonies, mini-prepped, and the purified plasmids were sequenced to ensure proper integration of the fragment. The two pDONR201-fragment plasmids were then used to move the enhancer region into the final destination vector pglacZ-attB using the Life Technologies Gateway LR Clonase Kit (Product 11791019). The recombined plasmids were used to transform DH5α Subcloning Efficiency Competent Cells (Life Technologies Catalog 18265-017). Cultures were started from the transformation colonies, mini-prepped, and the purified plasmids were digested to ensure proper integration of the fragment.

Fragment E1 Sequence

CCCATCATGTGTGTGAAATACAATCGTGGATTTCTTGCCCAGAAACCCTGCAACTTCTTCGGTGCAATG
GAGTGGGAGAGAAGTCGAGGCGCCTCGAACTCTTATGTGTGTTCAAGATGTCGTGGACTCAAATTAAG
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CCACTGATAACGACTAGTTTTCCATTGAACGGAGGCGCATAACGCTTATCTTTGTTAGGAATAAATATG
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ATCATTTTGCATCTCGTGGAGAGTATTCAGATTTGTTTGGAGAGTTTCGGAGAGGAACTCGTTAAGCCG
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TCTCGGGTAGAAGTCAATGAGGTGGATGTTCGGGCACACAGGCTGTAGAACTTGTTGGATATTTTGTC
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CCACATTCTTGTCCACCAAAGCAGACCTCTCCGCAACTCACCTCTTAGGAACTCTCCTAAATTCCGGTT
TCAAGTTGTGGTCCAAGTTCCACATGGACACCAATGAGACACATTCCCCAGTCTCCACTCTCCAACCA
AGTACATATCTCTCTCCAGCCGAACGAACCCGAGAGCGAAGACTCCGCCTATCGGATTCGGAAATGCC
CGTTCGGGGGGGTATAAAAGCGGGCGCTCTGAGCGAGCACCACTC

Fragment E2 Sequence

GACTTCTATGTGGCTTCCATCGCAACTATTACTTAGCGCTCCTTTGATGAATATTTTTATGTATATTTTTC
TGCTTCACTAGAATGCTTGACTTATAGTTATTGGTCCCATGATCTGCATACCCCTAACGCAAATCAATC
GGCCCATTGCGAGTCTGAAATCCAGCTGAGCTCGGCAGTTATACCCATGCGTAAACAAAAAAAAAGGC
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AAAGAAGTGGATGTTTACCCAAGTTTCTGCTCAAGATTTAGTTTTAACCGAAATGGTGAAGTGGAGAGC
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CGTGTCGTTTTTCACAAACAACGACGCGCCCGCTCATTTTCAATGAAAATTTCCACAATTTCCTCAATG
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TTATGAATATTTACTCTTCTTATTTTCAAATGATTTAAAATTTTCACCATTCCACAGCGATTAAAATCTAAT
TTACCCATGCATATATTTAATATTCATTCTGTAATTAATCAAGCCCTTGTTGAACCACTCGCATTGTC

Fragment E3 Sequence

CATTGTTAATCGTATCCTGCGTAATAGCATAATAATCCCTAATCGCATCTGATCAGTTCACCTGGTTGAA
ACACTATCCCTTTCTCTCTCTCGCTCAATTCCCCGTCCCTGTCCCGCACACTATCGAAACGCCGGCTG
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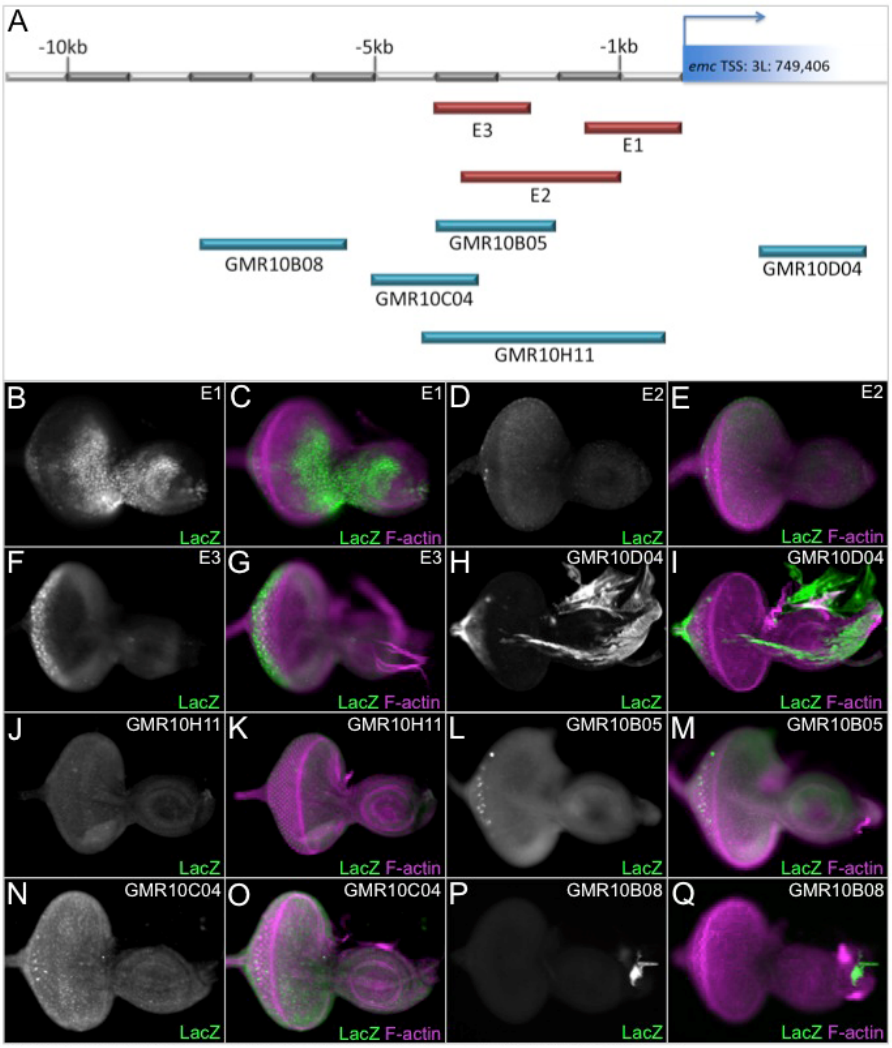
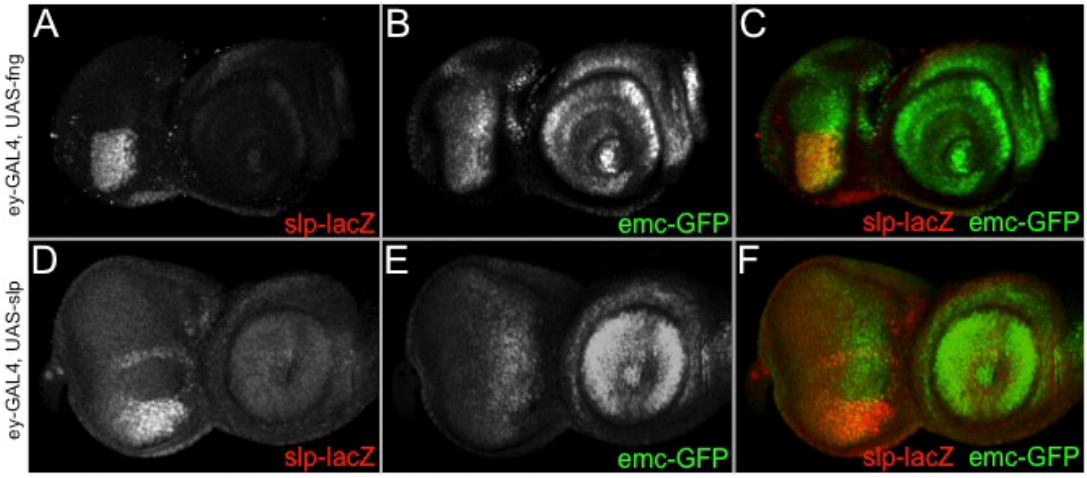
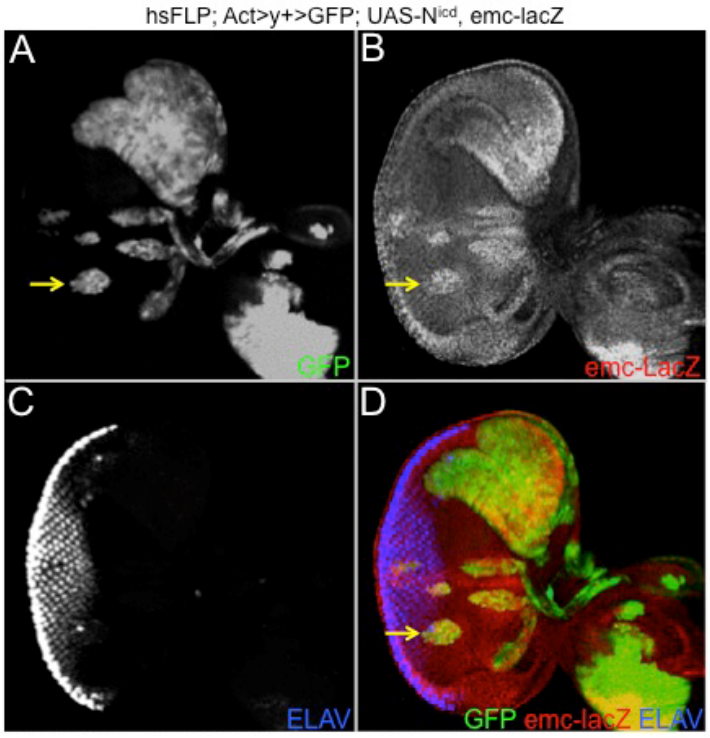


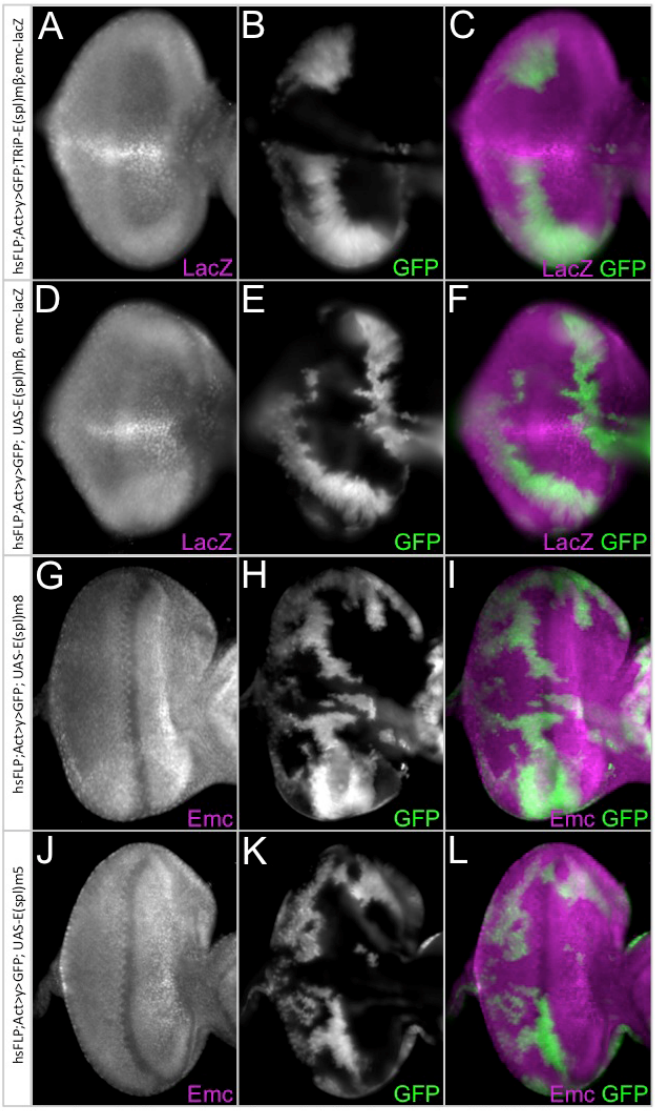
Supplemental Figure 1. Preliminary search for enhancers driving midline expression. (A) Depiction of the genomic region upstream the *emc* transcriptional start site located on 3L and the sub-genomic regions that were tested for the ability to drive expression at the midline. The genomic regions shown in aqua were isolated and fused to GAL4 by Gerald Rubin's laboratory at Janelia Farm. We cloned the regions in red and fused them directly to a lacZ reporter. (B-Q) Expression patterns driven by the eight genomic fragments in early third instar eye discs. None appear to direct reporter expression to the D/V midline. Dorsal side is up and anterior is to the right.



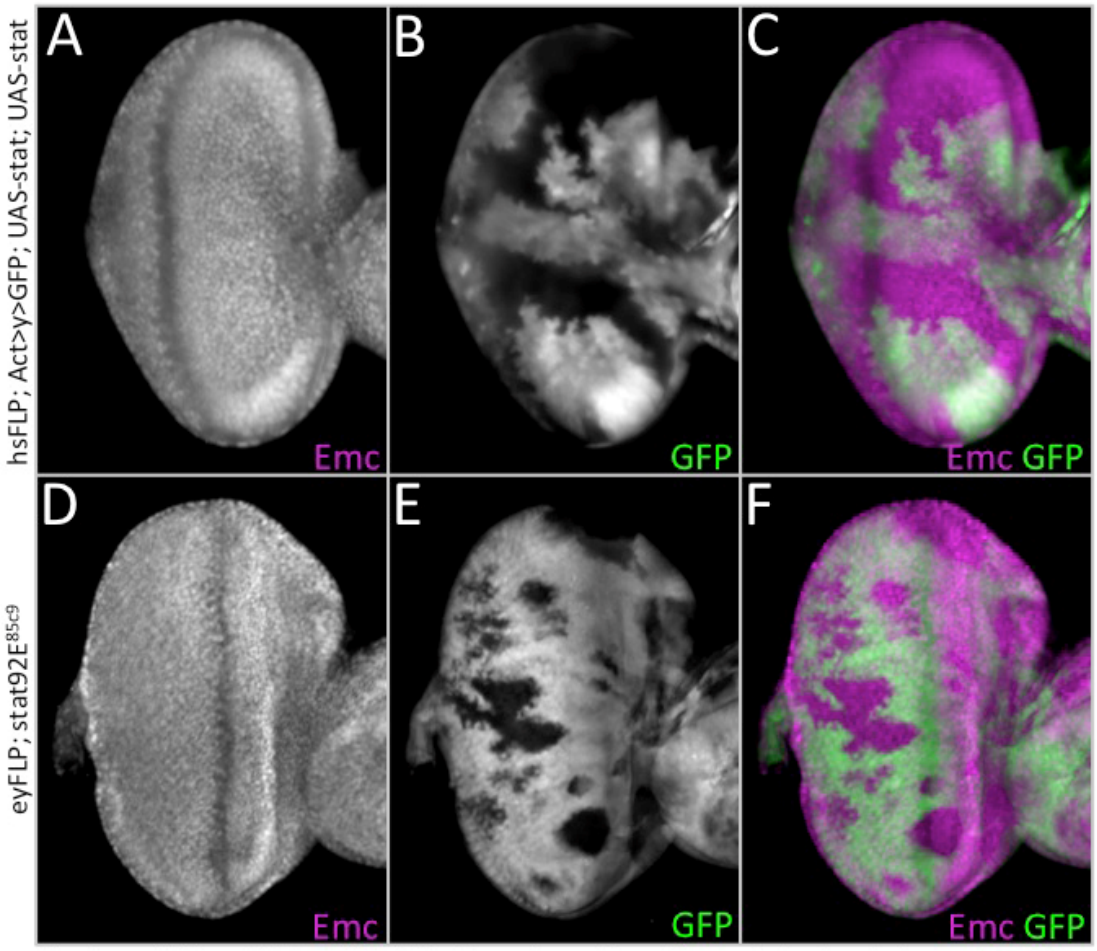
Supplemental Figure 2. Expression of *fng* and *slp* throughout the eye with *ey-GAL4* eliminates enrichment of *emc* expression at the midline. (A-C) Over-expression of *fng* throughout the entire disc using *ey-GAL4*. (D-F) Over-expression of *slp1* throughout the entire eye disc with *ey-GAL4*. In both experiments the enrichment of *emc* expression at the midline is abolished. Dorsal side is up and anterior is to the right.



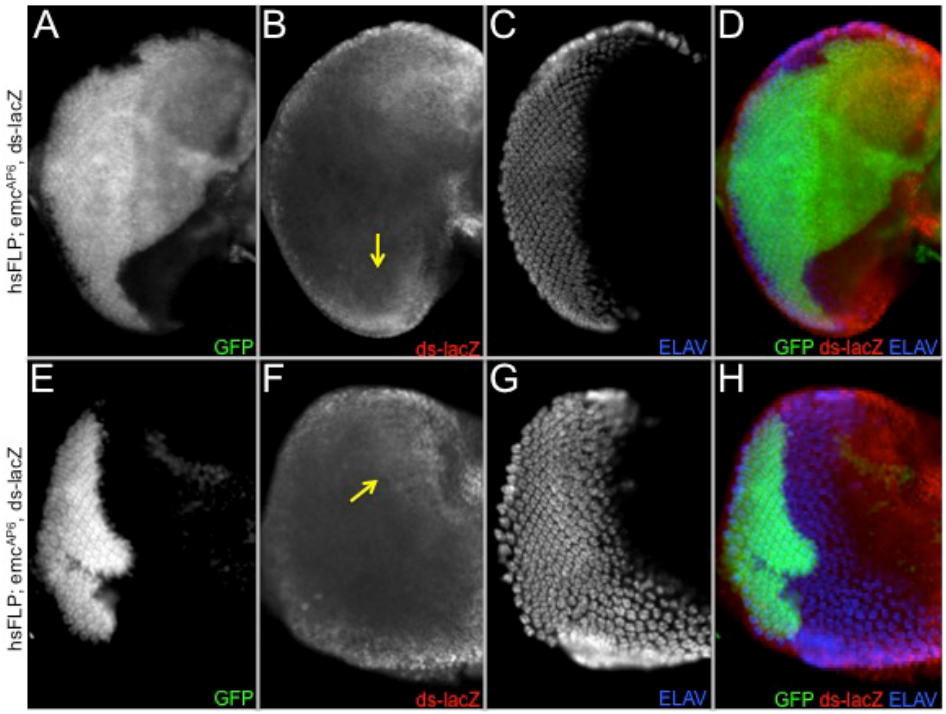
Supplemental Figure 3. Notch signaling can activate *emc* expression. (A-D) Over-expressing the intracellular domain of Notch (*hsFLP/ UAS-N^{icd}; Act>y>GFP/+; emc-lacZ*) in flp-out clones (GFP positive cells in A) show a cell autonomous activation of *emc* expression. Yellow arrow highlights an example of a clone in which *emc-lacZ* is activated in response to higher N signaling. Dorsal side is up and anterior is to the right.



Supplemental Figure 4. *emc* is regulated independently of the E(spl) complex. (A-C) Expression of an RNAi line for *E(spl)mβ* (*hsFLP/+; Act>y>GFP/+; P{TRiP.JF02100}attP2/emc-lacZ*) in flp-out clones (GFP positive cells in B, C) does not alter *emc-lacZ* midline expression. (D-F) Over-expression of *E(spl)mβ* (*hsFLP/+; Act>y>GFP/+; UAS-E(spl)mβ/emc-lacZ*) in flp-out clones (GFP positive cells in E, F) does not alter *emc* levels. (G-I) Flp-out clones of *E(spl)m8* (*hsFLP/+; Act>y>GFP/+; UAS-E(spl)m8/+*: GFP positive cells in H, I) do not affect Emc protein levels in the eye disc. (J-L) Over-expressing *E(spl)m5* (*hsFLP/ UAS-E(spl)m5; Act>y>GFP/+*) in flp-out clones (GFP positive cells in K, L) do not show differences in levels of Emc protein. Dorsal side is up and anterior is to the right.



Supplemental Figure 5. The JAK/STAT pathway does not regulate *emc* in the developing eye. (A-C) Over-expression of *stat92E* in flp-out clones throughout the eye field (GFP positive cells in B, C) has no effect on Emc protein levels. (D-F) Mutant clones of *stat92E* (*eyFLP/+; stat92E^{85c9}, FRT82B/Ubi-GFP, FRT82B*) induced throughout the eye (lack of GFP in E) also do not alter normal Emc protein levels. Dorsal side is up and anterior is to the right.



Supplemental Figure 6. Dachsous expression is not regulated by Emc in the developing eye. Two examples in which *ds-lacZ* expression is examined in *emc*^{AP6} loss-of-function clones. (A-D) Ventral *emc*^{AP6} clone, (E-H) Dorsal *emc*^{AP6} clone. At both margins, ds-lacZ expression appears unaffected. Dorsal side up and anterior is to the right.

SUPPLEMENTARY TABLE

Table S1

Click here to Download Table S1