# Identification and characterization of potential enhancers of Robo2 in the *Drosophila* embryonic CNS

Muna Abdal-Rhida[1,2,4] Gina Hauptman[1,3], Stephanie Greene[1,2,] and Tim Evans[1]
1Department of Biological Sciences, University of Arkansas, Fayetteville AR
2Cell and Molecular Biology graduate program
3Fulbright College Undergraduate Honors program
4Wasit University/ Iraq

## *Axon guidance roles of Drosophila Robo2*

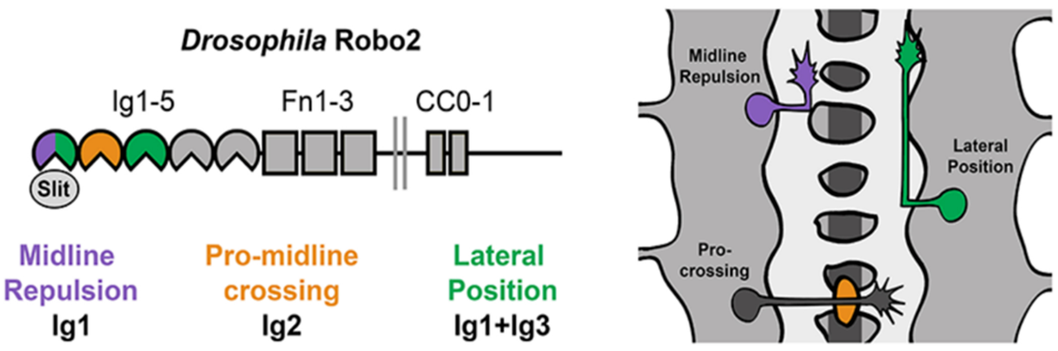



**Figure 2.1**: Structure and function of Robo2 receptor. Left panel, Robo2 schematic structure, Robo2 is a transmembrane protein consists of extracellular portion contains five Ig like domains and three fibronectin-like domain, and cytoplasmic portion consists of two short conserved motifs (CC0, and CC1). Right panel, schematic of the nerve cord of *drosophila*. It shows the three distinct axon guidance decisions controlled by Robo2 in preventing midline crossing by binding to slit (purple), mediating longitudinal pathway formation in the lateral neuropile (green), and antagonizing slit-robo1 repulsion and promoting midline crossing(orange).

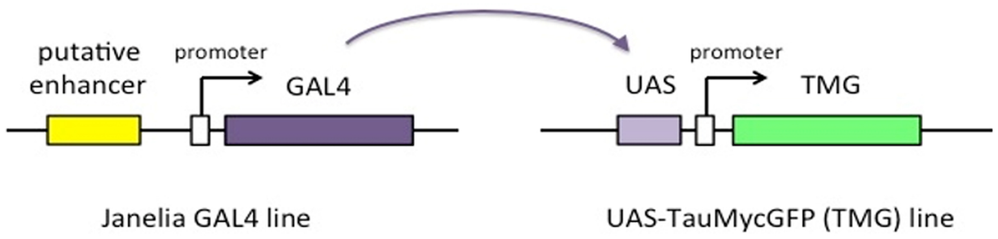
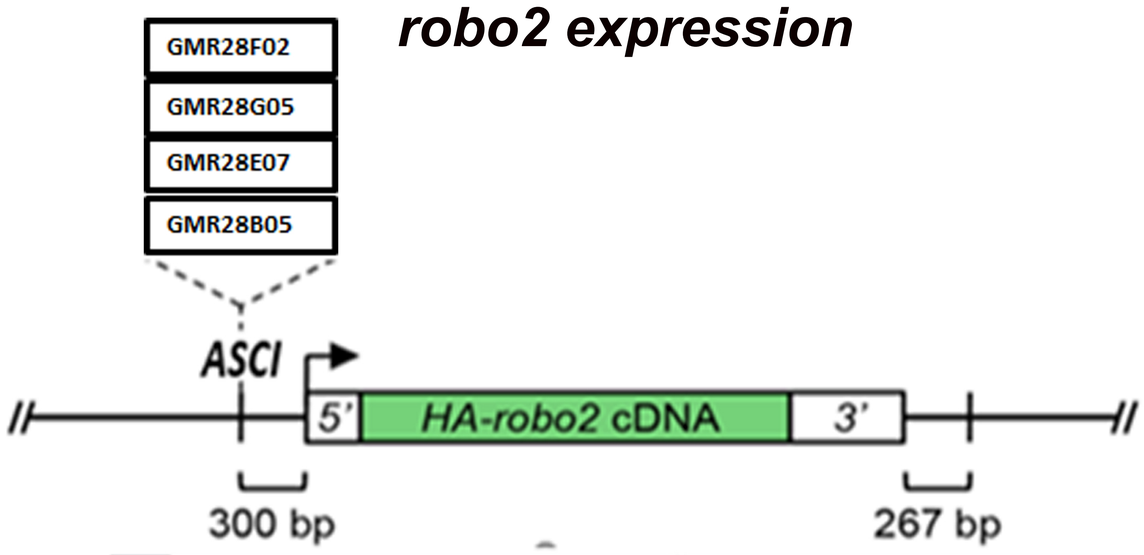
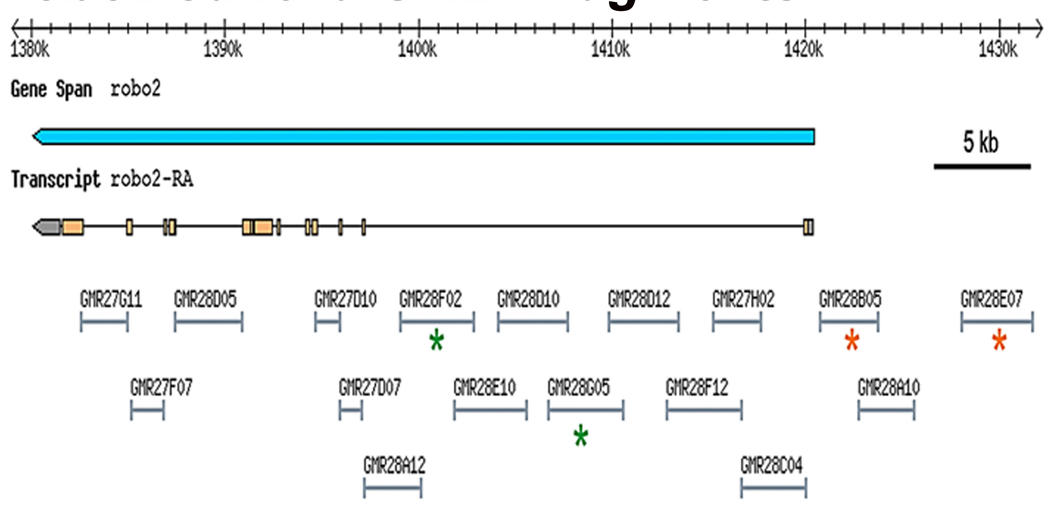



**Figure 2.3:** Janelia GAL4 lines. A schematic shows crosses between UAS-GFP and Janelia GAL4 lines containing Robo2 fragments. The expression patterns of the GFP is directed by presence or absence of enhancers in each Robo2 fragment. The Robo2 transcription factors in the CNS bind to the Robo2 putative enhancers present in each fragment upstream of the GAL4. As GAL4 protein produces, it binds to UAS enhancer and promotes GFP expression. The GFP expression reflects the expression governed by Robo2 transcriptional elements.

## *Enhancers showing specific patterns of robo2 expression*

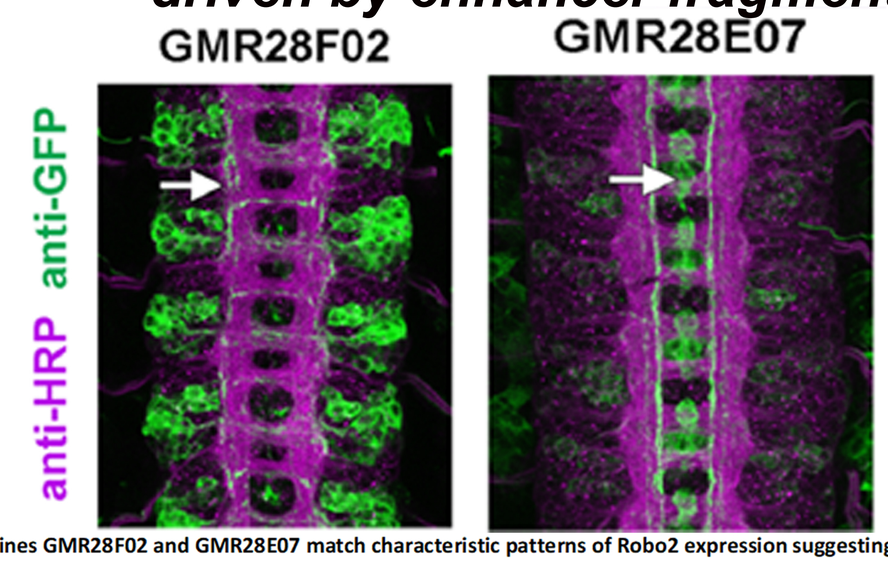



## *robo2 Janelia GAL4 fragments*

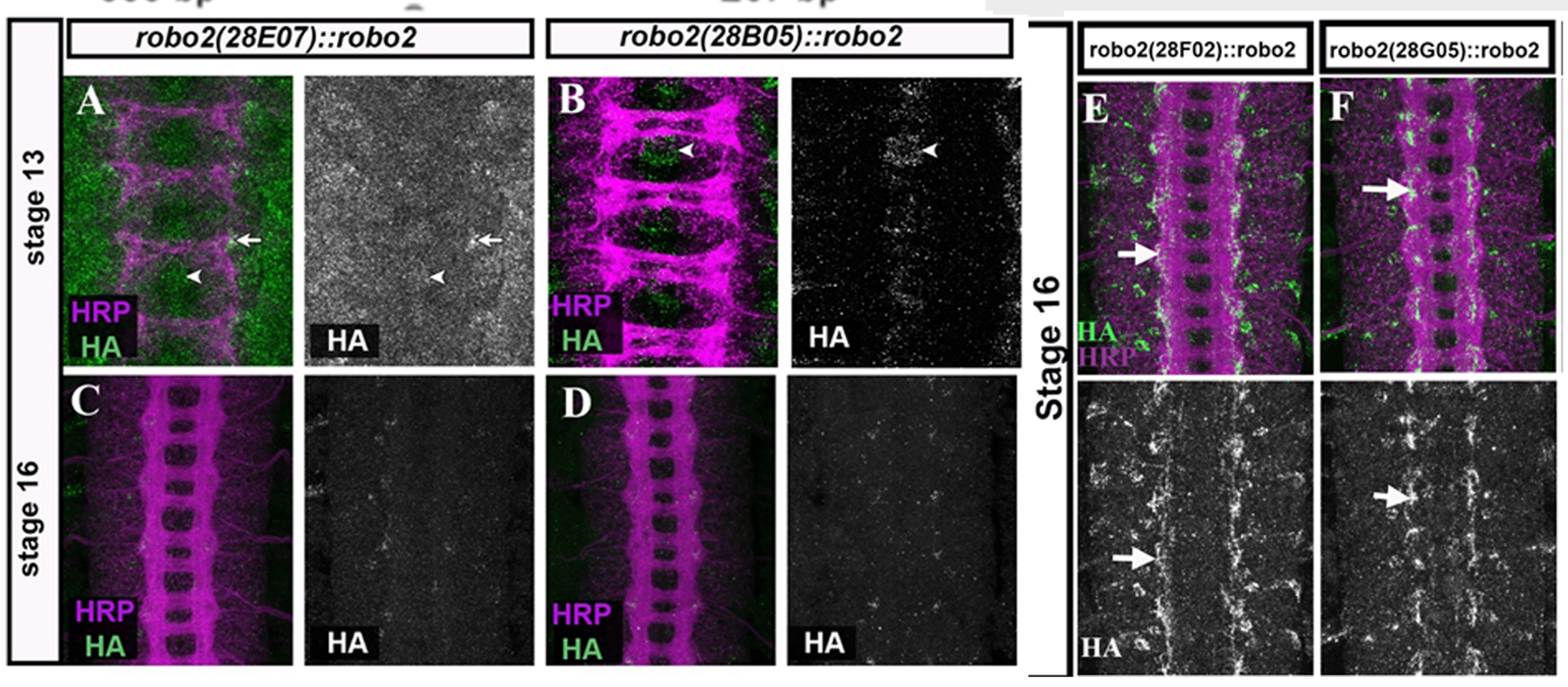



The structure of the *robo2* gene and mRNA transcript (orange and grey boxes indicate exons). Bracketed regions below the gene indicate putative enhancer regions cloned by Pfeiffer et al. Each region represents a ~ 3 kb fragment of non-coding DNA found either upstream of the *robo2* promoter or within a *robo2* intron. Each fragment was individually fused to the coding sequence of the yeast GAL4 gene and used to create a Janelia GAL4 transgenic line.

## *Expression patterns of rescue constructs driven by enhancer fragments*




**Lines GMR28F02 and GMR28E07 match characteristic patterns of Robo2 expression suggesting the presence of enhancers**. We identified lines GMR28F02 and GMR28E07 as showing expression patterns that reproduce specific aspects of Robo2 expression. We stained the lines with fluorescent antibodies to further clarify the GFP expression patterns in the CNS. GMR28F02 promotes expression in longitudinal axons, which may indicate the 28F02 robo2 fragment contains an enhancer regulating longitundinal axon expression. GMR28E07 promotes expression in midline cells, including midline glia and neurons with axons in medial and intermediate axon pathways.




**Enhancer fragments can drive robo2 expression in a rescue construct**. We built a robo2 rescue construct in which expression of an HA-tagged robo2 cDNA is under the control of cloned enhancer fragments. Fragment 28F02 and 28G05 promote robo2 expression in lateral neurons in stage 16 embryos (E and F arrows). Fragments 28E07 and 28B05 promote transient expression in midline cells (B, arrowhead) and some neurons (A and B, arrows) in stage 13 embryos. but staining is undetectable in the ventral nerve cord of older embryos (C).

www.evansflylab.com

presenting author's email