Motor neuron apoptosis and neuromuscular junction perturbation are prominent features in a *Drosophila* model of Fus-mediated ALS

Ruohan Xia[1], Yajuan Liu[1], Liuqing Yang[2], Jozsef Gal[2], Haining Zhu[2*], and Jianhang Jia[1,2*]

[1]Markey Cancer Center, [2]Department of Molecular and Cellular Biochemistry, College of Medicine, University of Kentucky, Lexington, KY 40536, USA.

[*]Correspondence to J. Jia: Jianhang.jia@uky.edu, Phone: (859) 323-6236, Fax: (859) 257-6030; and H. Zhu: haining@uky.edu, Phone: (859) 323-3643, Fax: (859) 323-5505.

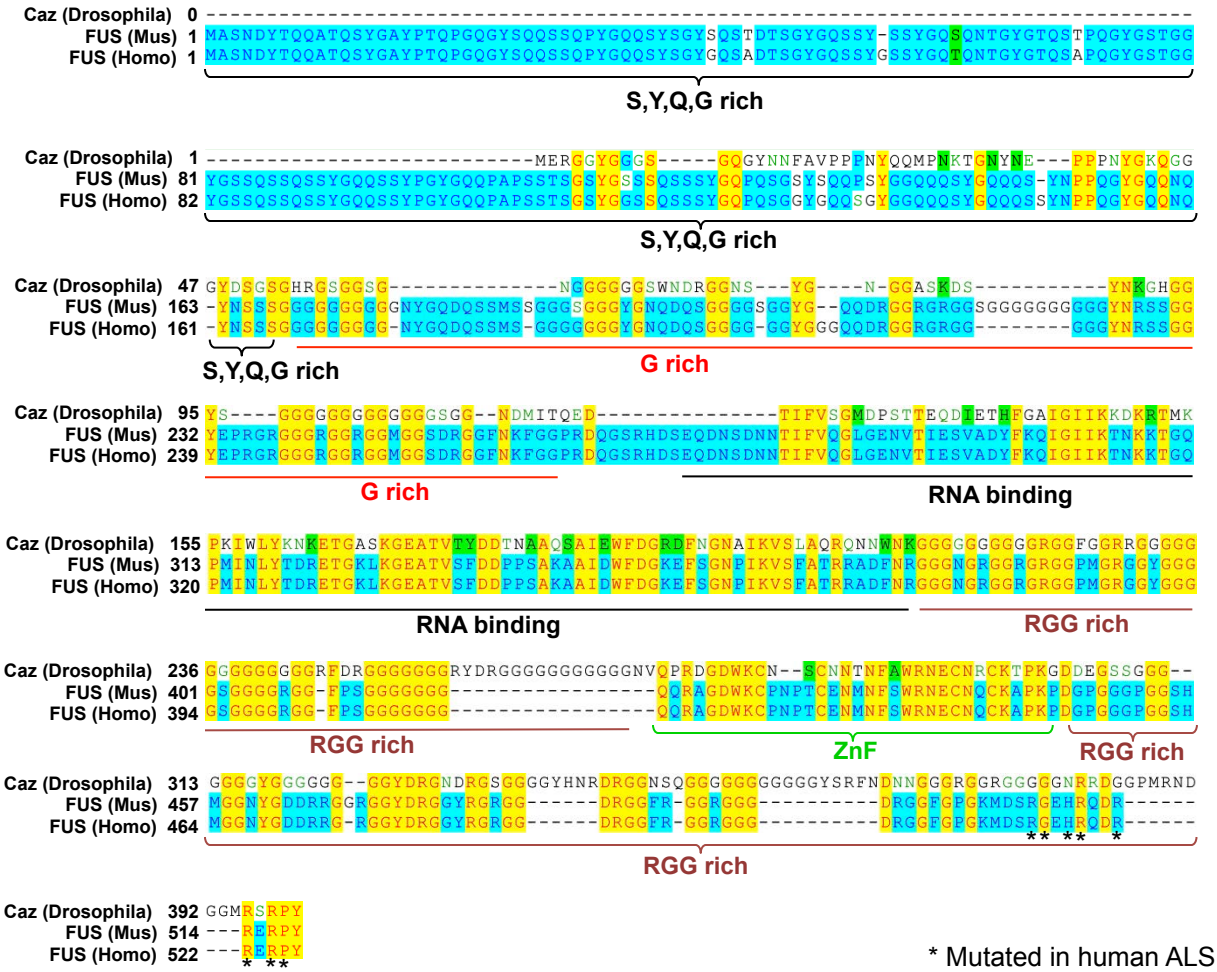
Supplemental Figure S1. The *caz* gene encodes the *Drosophila* homolog of Fus. A sequence alignment between Caz, mouse Fus, and human Fus is shown here. The SYQG domain, G-rich domain, RNA binding domain, Zn-finger domain, and RGG-rich domains are underlined. The asterisks indicate the ALS-related mutations that occur in patients.

Supplemental Figure S2. Large magnification of the motor neuronal cells indicating the localization of the proteins expressed in VNCs from Fig. 5. Fus, Caz, and FusΔ32NLS are exclusively localized in the nucleus. FusR521G shows slightly increased staining in the cytosol, but FusΔ32 and FusNES are mainly localized in the cytoplasm.

Supplemental Figure S3. Mutating *caz* in flies causes severe defects in the eye. (A) A female *yw* fly showing adult wild-type morphology. (B) A *caz* heterozygous female fly shows the balancer marker and intact eye structure. (C) A *caz* homozygous female fly shows growth defects in the eye and exhibits a normal body. (D) A male *yw* fly showing adult wild-type morphology. (E) A *caz* mutant male fly shows growth defects in the eye and exhibits a normal body. Mutating *caz* causes severely disrupted locomotive ability (Fig. 8) without obvious changes in body size, suggesting that Caz is likely involved in neuron development.

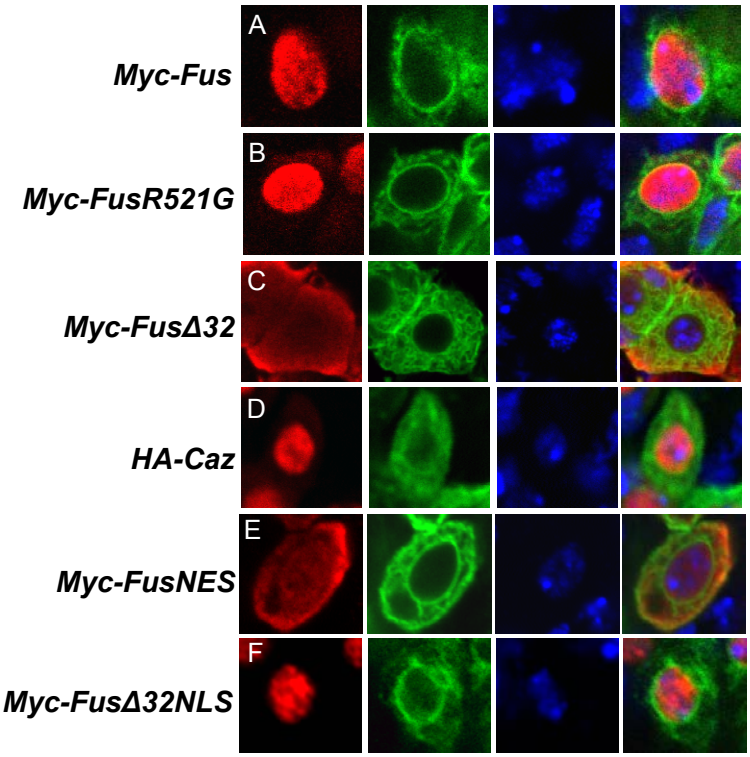
Supplemental Table S1. Characterization of Fus toxicity in *Drosophila*. Shown here is a diagram illustrating the domain structure of Fus along with truncation and point mutation constructs that are expressed in flies by the indicated Gal4 lines. The levels of toxicity are determined in the wing (by *MS1096*-Gal4), the eye (by *GMR*-Gal4), the entire body (by the *act5C*-Gal4), and the neuron (by *Elav*-Gal4 and *D42*-Gal4). More "+" symbols indicates more severe phenotypes.

Xia_Fig. S1

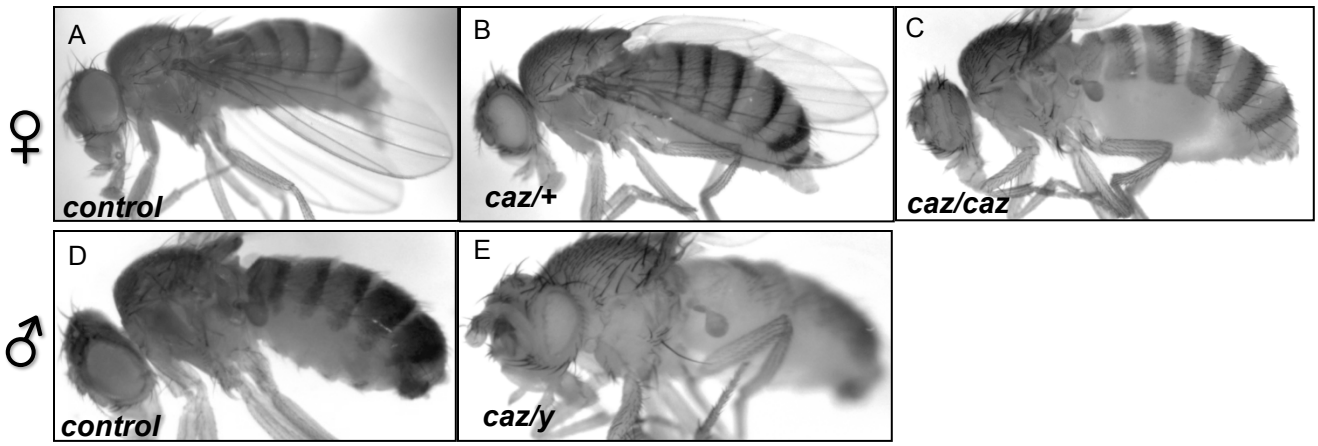


* Mutated in human ALS

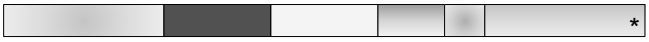
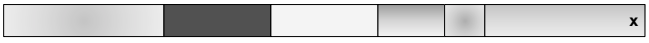
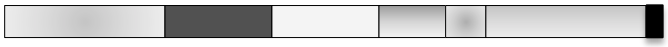
Xia_Fig. S2


A
Myc-Fus
B
Myc-FusR521G
C
Myc-FusΔ32
D
HA-Caz
E
Myc-FusNES
F
Myc-FusΔ32NLS

Xia_Fig. S3



Xia_Table S1

Construct name	Diagram of the construct	Wing phenotype by *MS1096*-Gal4	eye phenotype by *GMR*-Gal4	Adult viability by *act5C*-Gal4 *Elav*-Gal4 *D42*-Gal4
Fus		**+++++**	**+++++**	**+**
FusR521G		**++++**	**++++**	**++**
FusR521C		**++++**	**++++**	**++**
FusΔ32		**+**	**+**	**+++++**
Fus1-453		**–**	**–**	**+++++**
FusY526F		**++**	**++**	**+++**
FusNES		**+**	**+**	**+++++**
FusΔ32NLS		**++++**	**++++**	**++**