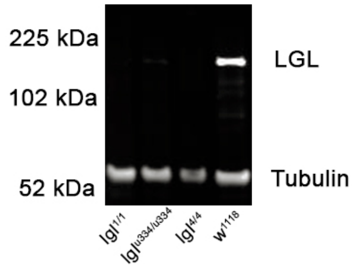

Fig. S1. Anti-LGL western blot analysis of *lgl* mutant alleles. Representative anti-LGL western blot comparing the w^{1118} genetic background control with a *lgl* mutant allelic series. The single ~110kDa band corresponding to the predicted molecular weight of LGL in the w^{1118} control is undetectable in both $lgl^{1/1}$ and $lgl^{4/4}$ mutants, but a very faint band is present in the lgl^{u334} homozygous mutant. These data are consistent with the published characterizations of the lgl^{1} and lgl^{4} alleles as protein nulls and lgl^{u334} as a hypomorphic allele (Mechler et al., 1985).

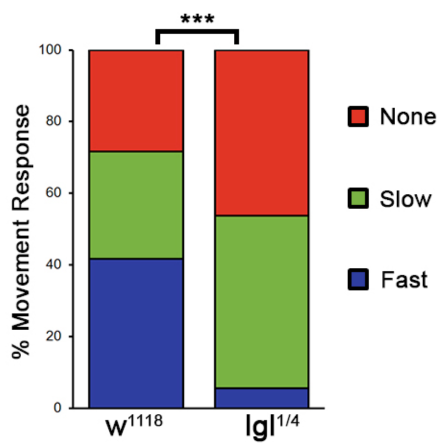

Fig. S2. Null *lgl* mutant larvae exhibit impaired behavioral movement activity. Loss of LGL reduces the coordinated motor response in a well-characterized movement escape behavior. Wandering third instars were stimulated with a 45°C probe, and movement response time assayed (see Materials and Methods). In the w^{1118} genetic background control, 42% of larvae exhibited the stereotyped escape response in ≤1 second (fast; Blue), 30% responded between 2–10 seconds (slow; Green) and 28% failed to respond (none; Red). In *lgl* nulls ($lgl^{1/4}$), only 6% initiated a fast response, 48% exhibited a delayed response, and 46% failed to respond. Statistical comparison Chi^2 test of independence ($\chi^2 = 20.004$, P<0.0001 (***); N=114, DF=2).

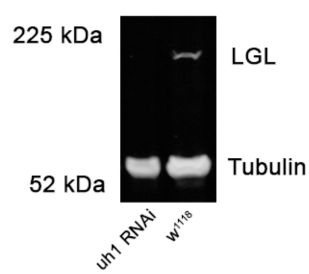

Fig. S3. Anti-LGL western blot analysis of *lgl* mutant alleles. Representative anti-LGL western blot comparing the uh1-Gal4/+ genetic background control with uh1-Gal4 × lgl RNAi. The ~110kDa band in the uh1-Gal4/+ control corresponds to the predicted molecular weight of LGL. This band is undetectable following RNAi knockdown.

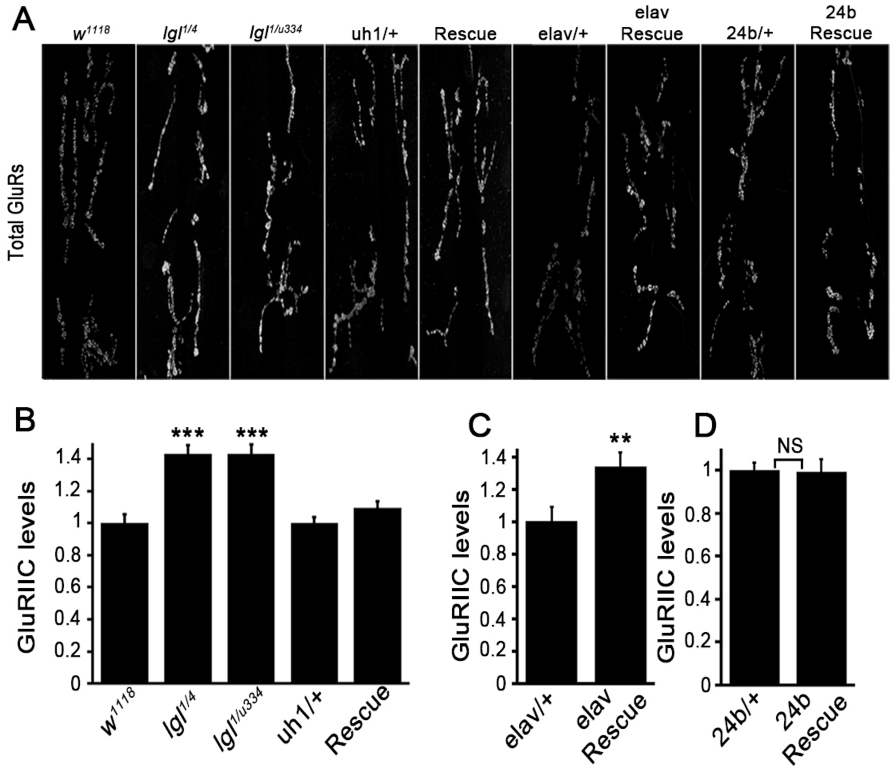

Fig. S4. High magnification image of panels A–D of Fig. 8. Loss of LGL induces a net increase in total glutamate receptors (GluRs) caused by the selective elevation of GluRIIB-containing receptors at wandering third instar NMJ synapses. (**A**) Representative muscle 6/7 NMJ images of anti-GluRIIC labeling in control (w^{1118}) and *lgl* mutant backgrounds, with ubiquitous, neuronal and muscle driven UAS-*lgl* expression. (**B**) Quantification of GluRIIC labeling intensity in w^{1118} and UAS-*lgl*/+; $lgl^{1/u334}$; uh1-Gal4/+ backgrounds compared to *lgl* mutants (N = 12, 12, 12, 13 and 13 NMJs, respectively; Kruskal–Wallis P<0.0001). (**C**) Quantification of GluRIIC intensity in the *elav*-Gal4/+ control versus UAS-*lgl* expression condition (N = 12 NMJs each; P=0.03, Mann–Whitney Test). (**D**) Quantification of GluRIIC intensity in 24b-Gal4/+ control versus UAS-*lgl*/+; $lgl^{1/u33}$; 24b-Gal4/+ condition (N=14 NMJs each; P=0.88).

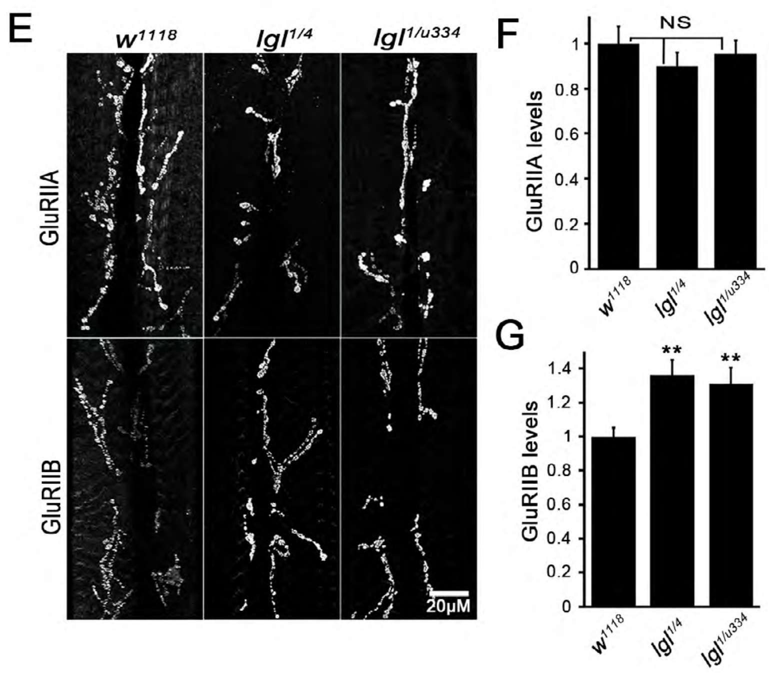

Fig. S5. High magnification image of panels E–G of Fig. 8. (**E**) Representative NMJ 6/7 images of anti-GluRIIA/B labeling in control and *lgl* mutants. (**F**) Quantification of GluRIIA intensity in control and *lgl* mutant backgrounds (N=12 NMJs each; ANOVA P=0.62, not significant (NS)). (**G**) Quantification of GluRIIB intensity in control and *lgl* mutants (N=12 NMJs each; ANOVA P=0.005). Significance shown as not significant (NS), P<0.01 (**) and P<0.001 (***).

Table S1. Post-hoc pairwise comparisons of all experiments analyzed by One-Way ANOVA. Table showing all pairwise comparisons of individual genotypes and their significance values. (**A**) Fig. 2: All results of Tukey–Kramer post-hoc tests for branch number (left) and bouton number (right). (**B**) Fig. 4: All results for Tukey–Kramer post-hoc tests for evoked junctional current responses for *lgl* mutants, rescue, and controls (left), and Student–Newman–Keuls multiple comparisons for pre- and postsynaptic LGL rescue and RNAi (right). (**C**) Fig. 5: All pairwise comparisons of *lgl* mutants, rescue, and control mEJC data. Dunn's non-parametric pairwise test for mEJC amplitude (left) and Tukey–Kramer pairwise test for mEJC frequency (right). (**D**) Fig. 6: Results of pairwise comparisons for active zone size (left) and active zone number (right) by Tukey–Kramer. (**E**) Fig. 7: Results of Student–Newman–Keuls multiple comparisons test for FM1-43 unloaded intensity (left) and unloaded/loaded ratio (right). (**F**) Fig. 8: Dunn's Multiple comparisons test of *lgl* mutants, rescue, and controls for GluRIIC intensity (left) and Student–Newman–Keuls multiple comparisons test for *lgl* mutants and control for GluRIIB intensity.

(A) Fig. 2. Structure

Tukey–Kramer — Branch number

w^{1118}	vs	$lgl^{1/4}$	***	P<0.001
w^{1118}	vs	lgl1/u334	**	P<0.01
w^{1118}	vs	uh1-Gal4/+	ns	P>0.05
w^{1118}	vs	uh1-Gal4 Rescue	ns	P>0.05
$lgl^{1/4}$	vs	lgl1/u334	ns	P>0.05
$lgl^{1/4}$	vs	uh1xw	***	P<0.001
$lgl^{1/4}$	vs	uh1-Gal4 Rescue	***	P<0.001
$lgl^{1/u334}$	vs	uh1-Gal4/+	**	P<0.01
$lgl^{1/u334}$	vs	uh1-Gal4 Rescue	**	P<0.01
uh1-Gal4/+	vs	uh1-Gal4 Rescue	ns	P>0.05

Tukey–Kramer — Bouton number

w^{1118}	vs	$lgl^{1/4}$	*	P<0.05
w^{1118}	vs	$lgl^{1/u334}$	*	P<0.05
w^{1118}	vs	uh1xw	ns	P>0.05
w^{1118}	vs	uh1-Gal4 Rescue	ns	P>0.05
$lgl^{1/4}$	vs	$lgl^{1/u334}$	ns	P>0.05
$lgl^{1/4}$	vs	uh1-Gal4/+	*	P<0.05
$lgl^{1/4}$	vs	uh1-Gal4 Rescue	**	P<0.01
$lgl^{1/u334}$	vs	uh1-Gal4/+	*	P<0.05
$lgl^{1/u334}$	vs	uh1-Gal4 Rescue	**	P<0.01
uh1-Gal4/+	vs	uh1-Gal4 Rescue	ns	P>0.05

(B) Fig. 4. EJC

Tukey–Kramer

w^{1118}	vs	$lgl^{1/u334}$	**	P<0.01
w^{1118}	vs	$lg^{lu334/u334}$	*	P<0.05
w^{1118}	vs	$lgl^{1/1}$	*	P<0.05
w^{1118}	vs	uh1-Gal4/+	ns	P>0.05
w^{1118}	vs	uh1-Gal4 Rescue	ns	P>0.05
$lgl^{1/u334}$	vs	$lg^{lu334/u334}$	ns	P>0.05
$lgl^{1/u334}$	vs	$lgl^{1/1}$	ns	P>0.05
$lgl^{1/u334}$	vs	uh1-Gal4/+	**	P<0.01
$lgl^{1/u334}$	vs	uh1-Gal4 Rescue	*	P<0.05
$lg^{lu334/u334}$	vs	$lgl^{1/1}$	ns	P>0.05
$lg^{lu334/u334}$	vs	uh1-Gal4/+	*	P<0.05
$lg^{lu334/u334}$	vs	uh1-Gal4 Rescue	*	P<0.05
$lgl^{1/1}$	vs	uh1-Gal4/+	*	P<0.05
$lgl^{1/1}$	vs	uh1-Gal4 Rescue	*	P<0.05
uh1-Gal4/+	vs	uh1-Gal4 Rescue	ns	P>0.05

Student–Newman–Kuels multiple comparisons test

24b Rescue	vs	24b-Gal4/+	*	P<0.05
24b Rescue	vs	24b RNAi	ns	P>0.05
24b RNAi	vs	24b-Gal4/+	*	P<0.05
ELAV Rescue	vs	ELAV-Gal4/+	**	P<0.01
ELAV Rescue	vs	ELAV RNAi	ns	P>0.05
ELAV RNAi	vs	ELAV-Gal4/+	*	P<0.05

(C) Fig. 5. mEJC

Dunn's Multiple comparisons test (non-parametric) — Amplitude

w^{1118}	vs	$lgl^{1/u334}$	***	P<0.001
w^{1118}	vs	$lg^{lu334/u334}$	**	P<0.01

Tukey–Kramer — Frequency

w^{1118}	vs	$lgl^{1/u334}$	***	P<0.001
w^{1118}	vs	$lg^{lu334/u334}$	***	P<0.001

w^{1118}	vs	lgl1/1	***	P<0.001	w^{1118}	vs	$lgl^{1/1}$	**	P<0.01
w^{1118}	vs	uh1-Gal4/+	ns	P>0.05	w^{1118}	vs	uh1-Gal4/+	ns	P>0.05
w^{1118}	vs	uh1-Gal4 Rescue	ns	P>0.05	w^{1118}	vs	uh1-Gal4 Rescue	ns	P>0.05
$lgl^{1/u334}$	vs	$lg^{lu334/u334}$	ns	P>0.05	$lgl^{1/u334}$	vs	$lg^{lu334/u334}$	ns	P>0.05
$lgl^{1/u334}$	vs	lgl1/1	ns	P>0.05	$lgl^{1/u334}$	vs	$lgl^{1/1}$	ns	P>0.05
$lgl^{1/u334}$	vs	uh1-Gal4/+	***	P<0.001	$lgl^{1/u334}$	vs	uh1-Gal4/+	***	P<0.001
$lgl^{1/u334}$	vs	uh1-Gal4 Rescue	**	P<0.01	$lgl^{1/u334}$	vs	uh1-Gal4 Rescue	**	P<0.01
$lg^{lu334/u334}$	vs	$lgl^{1/1}$	ns	P>0.05	$lg^{lu334/u334}$	vs	$lgl^{1/1}$	ns	P>0.05
$lg^{lu334/u334}$	vs	uh1-Gal4/+	**	P<0.01	$lg^{lu334/u334}$	vs	uh1-Gal4/+	***	P<0.001
$lg^{lu334/u334}$	vs	uh1-Gal4 Rescue	*	P<0.05	$lg^{lu334/u334}$	vs	uh1-Gal4 Rescue	**	P<0.01
$lgl^{1/1}$	vs	uh1-Gal4/+	***	P<0.001	$lgl^{1/1}$	vs	uh1-Gal4/+	**	P<0.01
$lgl^{1/1}$	vs	uh1-Gal4 Rescue	**	P<0.01	$lgl^{1/1}$	vs	uh1-Gal4 Rescue	*	P<0.05
uh1-Gal4/+	vs	uh1-Gal4 Rescue	ns	P>0.05	uh1-Gal4/+	vs	uh1-Gal4 Rescue	ns	P>0.05

(D) Fig. 6. Active zones

		Tukey–Kramer					**Tukey–Kramer**		
		Active zone size					**Active zone number**		
w^{1118}	vs	$lgl^{1/4}$	***	P<0.001	w^{1118}	vs	$lgl^{1/4}$	**	P<0.01
w^{1118}	vs	$lgl^{1/u334}$	***	P<0.01	w^{1118}	vs	$lgl^{1/u334}$	*	P<0.05
w^{1118}	vs	uh1-Gal4/+	ns	P>0.05	w^{1118}	vs	uh1-Gal4/+	ns	P>0.05
w^{1118}	vs	uh1-Gal4 Rescue	ns	P>0.05	w^{1118}	vs	uh1-Gal4 Rescue	ns	P>0.05
$lgl^{1/4}$	vs	$lgl^{1/u334}$	ns	P>0.05	$lgl^{1/4}$	vs	$lgl^{1/u334}$	ns	P>0.05
$lgl^{1/4}$	vs	uh1-Gal4/+	***	P<0.001	$lgl^{1/4}$	vs	uh1-Gal4/+	**	P<0.01
$lgl^{1/4}$	vs	uh1-Gal4 Rescue	***	P<0.001	$lgl^{1/4}$	vs	uh1-Gal4 Rescue	*	P<0.05
$lgl^{1/u334}$	vs	uh1-Gal4/+	***	P<0.001	$lgl^{1/u334}$	vs	uh1-Gal4/+	*	P<0.05
$lgl^{1/u334}$	vs	uh1-Gal4 Rescue	***	P<0.001	$lgl^{1/u334}$	vs	uh1-Gal4 Rescue	*	P<0.05
uh1-Gal4/+	vs	uh1-Gal4 Rescue	ns	P>0.05	uh1-Gal4/+	vs	uh1-Gal4 Rescue	ns	P>0.05

(E) Fig. 7. FM1-43

Student–Newman–Kuels multiple comparisons test

		Unloaded intensity					**Unloaded/loaded intensity**		
$lgl^{1/4}$	vs	w^{1118}	*	P<0.05	$lgl^{1/4}$	vs	w^{1118}	**	P<0.01
$lgl^{1/4}$	vs	$lgl^{1/u334}$	ns	P>0.05	$lgl^{1/4}$	vs	$lgl^{1/u334}$	ns	P>0.05
$lgl^{1/u334}$	vs	w^{1118}	*	P<0.05	$lgl^{1/u334}$	vs	w^{1118}	*	P<0.05

(F) Fig. 8. GluR expression

		Dunn's multiple comparisons test					**Student–Newman–Kuels multiple comparisons test**		
		GluRIIC intensity					**GluRIIB intensity**		
w^{1118}	vs	$lgl^{1/4}$	***	P<0.001	w^{1118}	vs	$lgl^{1/4}$	**	P<0.01
w^{1118}	vs	$lgl^{1/u334}$	***	P<0.01	w^{1118}	vs	$lgl^{1/u334}$	**	P<0.01
w^{1118}	vs	uh1-Gal4/+	ns	P>0.05	$lgl^{1/u334}$	vs	$lgl^{1/4}$	ns	P>0.05
w^{1118}	vs	uh1-Gal4 Rescue	ns	P>0.05					
$lgl^{1/4}$	vs	$lgl^{1/u334}$	ns	P>0.05					
$lgl^{1/4}$	vs	uh1-Gal4/+	***	P<0.001					
$lgl^{1/4}$	vs	uh1-Gal4 Rescue	*	P<0.05					
$lgl^{1/u334}$	vs	uh1-Gal4/+	***	P<0.001					

lgl$^{1/u334}$	vs	uh1-Gal4 Rescue	*	P<0.05
uh1-Gal4/+	vs	uh1-Gal4 Rescue	ns	P>0.05