Cheng et al., http://www.jcb.org/cgi/content/full/jcb.201101042/DC1

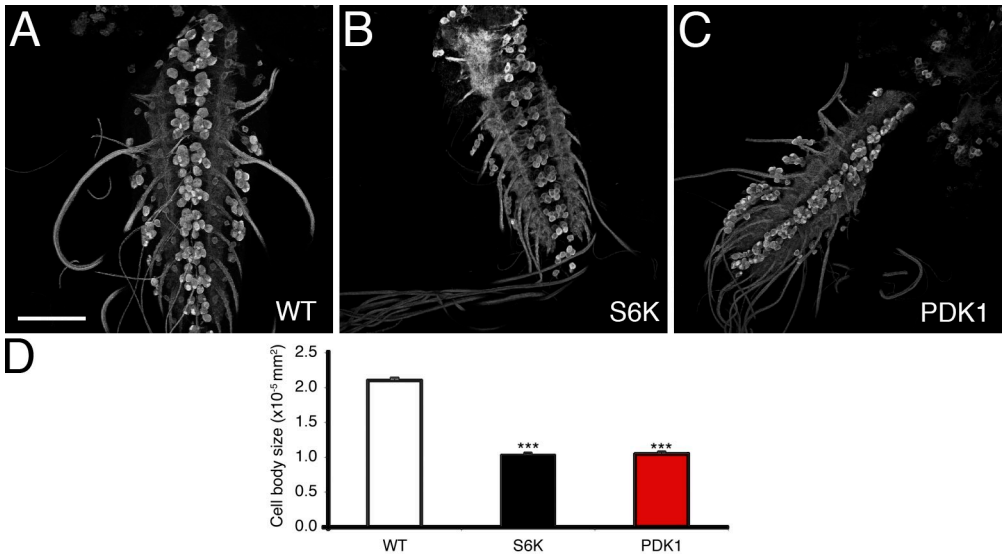

Figure S1. **Loss of S6K and PDK1 results in smaller cell size of motoneurons.** (A–C) Confocal images of third instar ventral nerve cords. Motoneurons in the ventral nerve cord are labeled with membrane-targeted *UAS-CD8::GFP* driven by motoneuron-specific *OK371-Gal4*. *OK371-Gal4,UAS-CD8::GFP/+* (A), *OK371-Gal4,UAS-CD8::GFP/+;S6K^{l-1}/ Df(3L)CH18* (B), and *OK371-Gal4,UAS-CD8::GFP/+;PDK1^{33}/PDK1^{33}* (C) are shown. (D) Quantifications of cell body size of motoneurons are shown. Cell body size of 30–50 motoneurons per larva was quantified, and 8–10 larvae were analyzed for each genotype. WT, wild type. ***, P < 0.0001. Error bars show SEM. Bar, 75 µm.

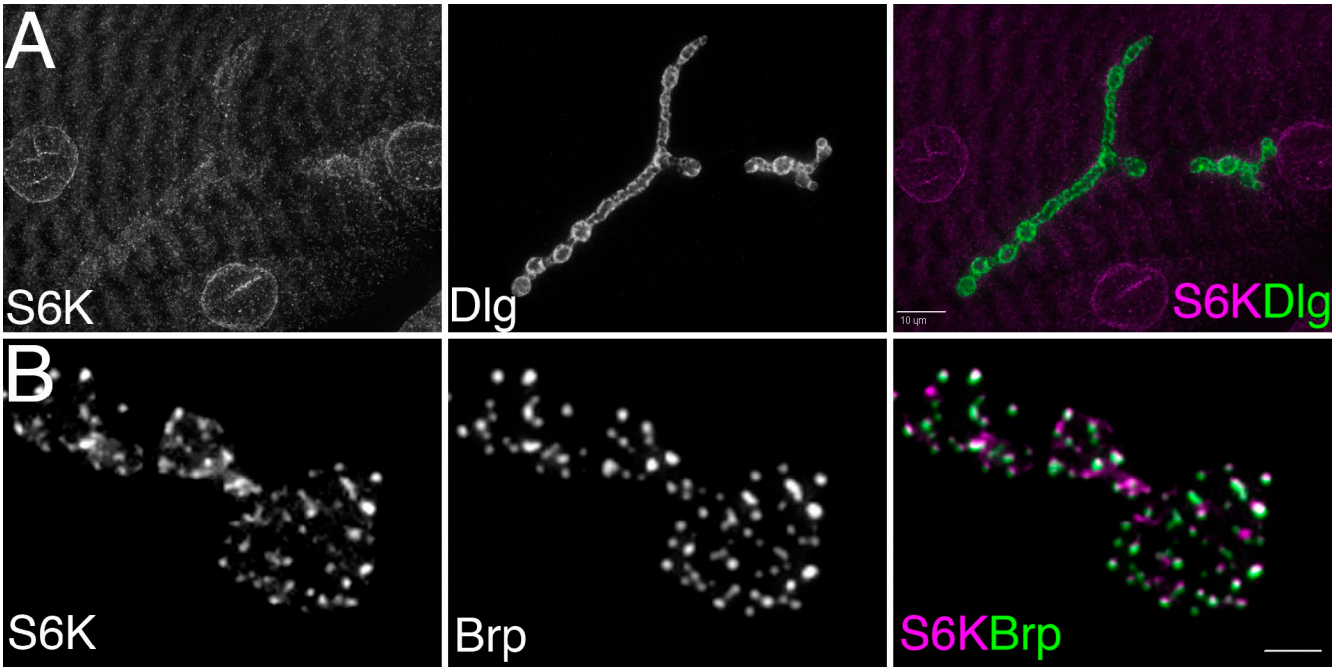

Figure S2. **Localization of postsynaptically expressed S6K and presynaptically expressed Venus-tagged S6K.** (A) Z-series projection of *BG57-Gal4/UAS-3xFLAG-S6K* NMJs on muscle 4. S6K localization in the muscle was labeled with anti-FLAG and colabeled with subsynaptic reticulum marker anti-Dlg. Bar, 10 µm. (B) Z-series projection of *OK371-Gal4/+;UAS-Venus-S6K/+* synapses. This is the same construct used in Fig. 2 (E and F). Venus-S6K expressed at the synapses was colabeled with anti-Brp. Bar, 2 µm.

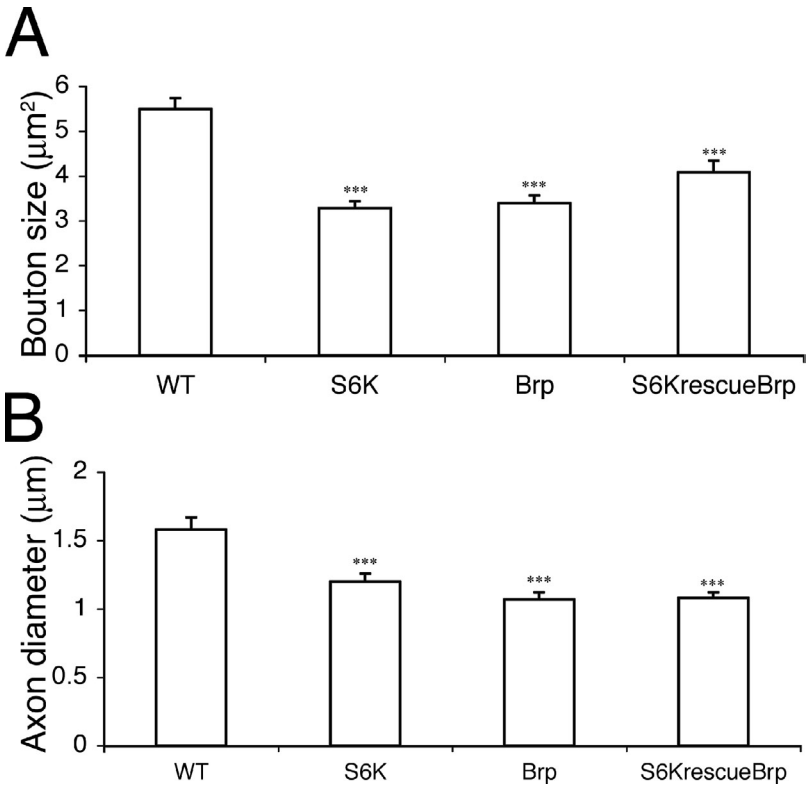

Figure S3. **Loss of Brp results in smaller boutons and axons.** (A and B) Quantification of bouton size (A) and axon diameter (B) for wild type (WT; *w1118*), S6K (*S6K$^{l\text{-}1}$/ Df(3L)CH18*), Brp (*Brp^{69}/Brp^{69}*), and S6K overexpression in the *brp* mutant background (*OK371-Gal4,Brp^{69}/Brp^{69};UAS-Venus-S6K/+*). ***, P < 0.0001. Error bars show SEM.

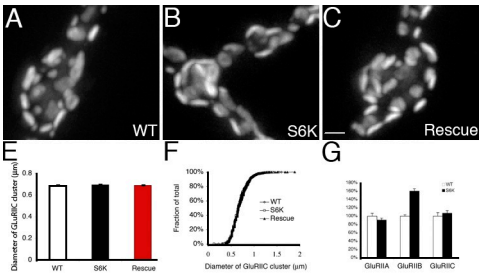

Figure S4. **Analysis of postsynaptic glutamate receptor clusters.** (A–C) Z-series projections of glutamate receptor clusters at the third instar NMJs labeled with anti-GluRIIC. Wild type (WT; A), *S6K$^{l\text{-}1}$/Df(3L)CH18* (B), and *C155/+;UAS-S6K/+;S6K$^{l\text{-}1}$/ Df(3L)CH18* (C) are shown. Bar, 1 µm. (D and E) Quantifications of the diameter of GluRIIC clusters are shown as the mean (D) and as a histogram (E). (F) Quantification of GluRIIC, GluRIIB, and GluRIIA synaptic immunofluorescence shows only a change in the levels of GluRIIB. Error bars show SEM.

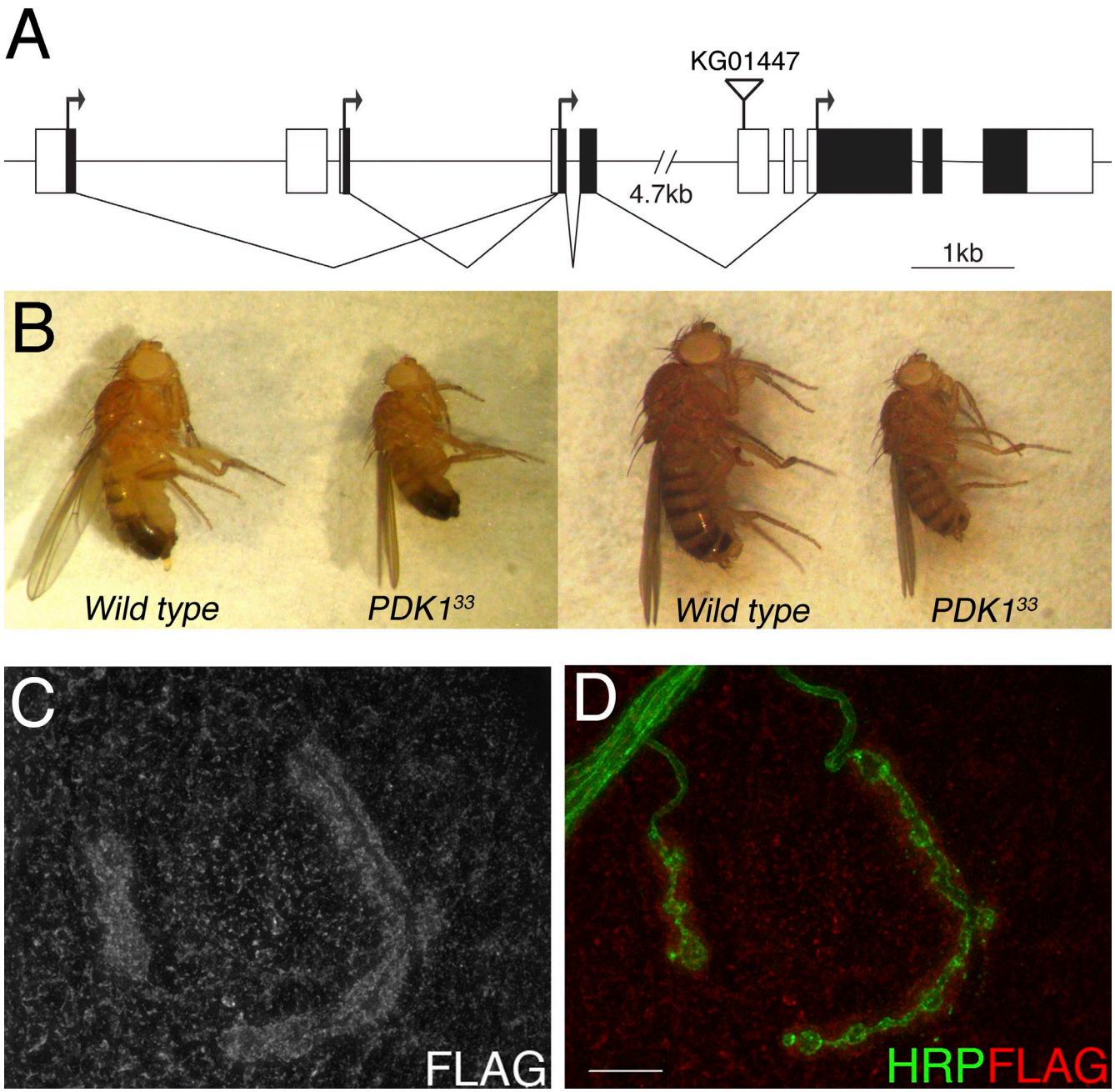

Figure S5. **PDK1 genomic region and its effect on body size.** (A) Genomic region of *dPDK1*. Introns are indicated by horizontal lines, and exons are indicated by boxes. White and black boxes represent untranslated and coding regions, respectively. The translation start sites are indicated by arrows. The insertion site of the *P* element line *P{SUPor-P}KG01447* is indicated as the inverted triangle. Note that the *dPDK1* gene has four protein isoforms with various 5′ sequences and common sequences at the 3′ region. The *P* element was inserted at the 5′ untranslated region of the smallest isoform. PCR mapping revealed that $PDK1^{33}$ had ~600 bp of the original *P* element sequence remaining at the insertion site and is likely to interfere with transcription of some or all of the *dPDK1* isoforms. Based on viability and phenotype analysis, we conclude that $PDK1^{33}$ is a hypomorphic allele of *dPDK1*. (B) Male and female adult flies of wild-type and $PDK1^{33}$ homozygotes. Note that $PDK1^{33}$ homozygous flies, male or female, have smaller body sizes than wild type. (C and D) Z-series projection of *BG57-Gal4/UAS-3×FLAG-PDK1* NMJs on muscle 4. PDK1 localization in the muscle was labeled with anti-FLAG (C) and colabeled with anti-FLAG and subsynaptic reticulum marker anti-Dlg (green; D). Bars, 10 µm.