Supplemental Methods

Western blotting

Adult fly heads were homogenized in extraction buffer (10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 5 mM EDTA (pH 8.0), 5 mM EGTA (pH 8.0), 10% glycerol, 0.8 M Urea, cocktail of protease inhibitors (Roche), 50 mM NaF, 1 mM Na3VO4, 5 mM NaPPi, 60 mM β-glycerophosphate, and 5 mM DTT) on ice. The head extracts were centrifuged at 16,000 rpm for 5 minutes and subjected to 8% SDS-PAGE. After SDS-PAGE, proteins were transferred to nitrocellulose membrane (Bio-Rad) and detected with ECL detection reagent (Yeasen). To examine whether 3xFLAG-tagged UAS-cDNA plasmids were expressed in the presence of GAL4 in HEK293T cells, a GAL4-expressing plasmid, pCMV-GAL4 (Addgene plasmid # 24345, a gift from Liqun Luo) (Potter et al. 2010), was used in transfection of HEK293T cells. Transfected HEK293T cells were lysed in lysis buffer (250 mM NaCl, 50 mM Tris-HCl (pH 7.5), 0.5% Triton X-100, 5% glycerol, and cocktail of protease inhibitors) on ice for 30 minutes and sonicated on ice with ultrasound. Cell lysates were centrifuged at 16,000 rpm for 5 minutes and subjected to 8% or 12% SDS-PAGE. 8% SDS-PAGE was used for separating proteins with an expected molecular weight over 49 kDa, whereas 12% SDS-PAGE was used for separating proteins with an expected molecular weight below 49 kDa. Specifically for proteins with an expected molecular weight below 20 kDa, the stacking gel height is about 3 cm from the well bottom to the top of the separating gel. After SDS-PAGE, proteins were transferred to PVDF membrane (Millipore) and detected with ECL detection reagent (Yeasen). The primary antibodies used were: rabbit anti-MYC tag (1:1,000, Cell Signaling Technology, Cat # 2272), mouse anti-V5 (1:500, Thermo Fisher Scientific, Cat # R960-25), mouse anti-FLAG (M2, 1:1000, Sigma, Cat # F1804), mouse anti-β-Tubulin (E7, 1:200, DSHB). Goat anti-mouse IgG conjugated to HRP (1:4,000, Jackson ImmunoResearch, Cat # 115-035-003) and goat anti-rabbit IgG conjugated to HRP (1:4,000, Jackson ImmunoResearch, Cat # 111-035-003) were used as secondary antibodies.

Immunohistochemistry

Immunohistochemistry was performed as previously described (Wang et al. 2011). The antibodies used were: mouse anti-bruchpilot (BRP) (nc82, 1:50, DSHB), rabbit anti-MYC tag (1:200, Cell Signaling Technology, Cat # 2272), rabbit anti-V5 tag (1:200, Novus, Cat # NB600-381), goat anti-mouse IgG conjugated to Alexa 488 (1:2,000, Jackson ImmunoResearch, Cat # 115-545-003), goat anti-rabbit IgG conjugated to Alexa 594 (1:2,000, Jackson ImmunoResearch, Cat # 111-585-003). L3 larval muscles were stained with phalloidin-TRITC (Sigma).