Data S1. Additional materials and methods

Immunostainings and antibodies

Mouse monoclonal anti-GFP antibody was used at 1:1000 (Clontech), mouse monoclonal anti-PY100 antibodies were used at 1:1000 (Cell Signalling), rabbit anti-β-gal antibodies were used at 1:150 (ICN Biomedicals), rabbit anti-Vasa antibodies (Lasko and Ashburner, 1990) were used at 1:500 (a kind gift from P. Lasko). Mouse monoclonal 2A12 antibody, which recognises an unknown tracheal luminal component was used at 1:5 (Developmental Hybridoma Bank). Anti-CREB antibodies (Andrew et al., 1997) were used at 1:15,000 (kind gift from D. Andrew), guinea pig anti-ALK antibody was used at 1:1000, mouse monoclonal anti-Fas3 antibodies were used at 1:50, mouse monoclonal anti-Elav (958A9) was used at 1:1000 and mouse monoclonal 22C10 was used at 1:50 (Developmental Hybridoma Bank). DAPI was used at a final concentration of 0.5   g/ml.

Generation of Fak56 transgenic flies

The pBluescript:Fak56 (Palmer et al., 1999) was used as a template to create the Fak56-GFP fusion protein. A C-terminal segment of Fak56, corresponding to amino acids 1121-1204, was amplified by standard PCR using forward (5′-TTCTGGAGATTTGCTTCA-3′) and reverse (5′-TGCTCGAGCACTGTGCGGTAACTGTG-3′) primers. The resulting PCR fragment was digested with *Bsa*BI/*Bam*HI, and subcloned in frame with GFP in the pEGFP vector (Clonetech) (digested with *Bsa*BI/*Bam*HI), to create pEGFP:Fak56 aa1135-1204-GFP. Subsequently, pEGFP:Fak56 aa1135-1204-GFP was digested with *Mun*I/*Xba* I and the 964 bp fragment, encoding Fak56 aa1135-1204-GFP, subcloned back into the vector encoding full-length Fak56 (pBluescript:Fak56) (digested with *Mun*I/*Xba*I). This construct, named pBluescript:Fak56-GFP was confirmed by sequence analysis. To create pUAST:Fak56-GFP, pBluescript:Fak56-GFP was digested with *Xho*I/*Xba* I and the resulting 4434 bp fragment subcloned into pUAST vector (digested with *Xho*I/*Xba*I). The *Fak56* genomic rescue transgene was generated by subcloning a 10,189bp *Apa*I-*Alw*NI genomic DNA fragment containing 1,883 bp upstream of the nucleotide corresponding to the initiation AUG codon in the *Fak56* gene, and 2795 bp downstream of the polyA site, into the P-element transformation vector pCasper4. P element transformation was performed by microinjection together with a delta 2.3 transposase source containing plasmid, into *white*^{1118} embryos.

Immunostainings of ovarioles

Ovaries from Fak56 mutant and wild-type fattened flies were dissected in PBS, fixed in PBS containing 4% formaldehyde for 20 minutes, rinsed, and blocked in NP40 block buffer (50 mM Tris pH 7.4, 150 mM NaCl, 0.5% NP40, 5 mg/ml BSA) overnight at 4°C (Bai et al., 2000). Egg chambers were incubated with rhodamine-phalloidin (Molecular Probes) and DAPI at a final concentration of 0.5   g/ml for 1 hour at room temperature. After three washes, egg chambers were mounted in PVA/DABCO mounting solution and analysed by confocal microscopy.