Supplementary Figure Legends

Supplementary Fig S1 Kinase assay of the Fj^{ANE} mutant
Histograms show the results of kinase assays, in fmol ATP transferred in a 15 min reaction, using secreted wild-type Fj or secreted Fj with the DNE site mutated to ANE. Assays were performed as described previously [1], using Ds2-3 as a substrate. As a negative control, the assay was performed in the absence of substrate. The DNE to GGG mutation used has been characterized previously [1].

Supplementary Fig S2 Conservation of Fj phosphorylation sites
Amino acid sequence alignments of 20 aa portions of the cadherin domains (Cad#) of Fat from *Drosophila melanogaster* (Dm, P33450), Fat from *Tribolium castaneum* (Tc, XP_971084), Fat4 form *Gallus gallus* (Gg, XP_420617), Fat4 *Mus musculus* (Mm, Q2PZL6), and Fat4 *Homo sapiens* (Hs, Q6V0I7). The Gg sequence lacks the first cadherin domain. The complete sequences were aligned by clustalW, and then the best matches to the calcium-binding linker motif (DXND(N/H)) were identified by manual scanning. Biochemical characterization of cadherin domain phosphorylation identified a loose consensus sequence that is necessary, but not sufficient, for phosphorylation by Fj [1]. This consensus sequence requires a Ser or Thr at the seventh amino acid of a cadherin domain, counting from the first amino acid after the DXND(N/H) motif. We note that not all cadherin domains in Fat and Ds contain good matches to this calcium binding consensus sequence, which might be expected to affect the rigidity of the extracellular domain (e.g., as observed for EGF domains [2]). For ease of comparison, the seventh amino acid is underlined in the Dm sequence, and amino acids that conform to the Fj consensus sequence are highlighted in blue. Biochemical characterization confirmed Fj-mediated phosphorylation (highlighted in bold) of Dm Fat Cad3, Cad5, Cad11, and Cad13, whereas phosphorylation of Cad10 was not detected, and other sites have not been evaluated. The alignments identify Cad3 as the only conserved site within Fat1-10, although the sites at Cad13 and Cad22 are also conserved.

Supplementary Fig S3 Effect of S273A mutation on full-length Fat binding to Ds
The S273A mutation significantly reduces the Ds binding activity of Fat. S2 cells were transiently transfected with either wild-type or mutant pMT-Fat (8μg) and either pMT-Fj:V5 (2μg) or the control plasmid pMT/V5-His/*lac*Z (2μg) and assayed for their ability to bind Ds:AP. The histograms show the average of two replicate binding assays with each sample. Bound AP activity is expressed as milli-OD/min. Bars indicate the deviation between the replicates. Similar results were obtained in three independent transfection experiments. Transfections were done using Cellfectin (Invitrogen).

References

1. Ishikawa, H.O., Takeuchi, H., Haltiwanger, R.S., and Irvine, K.D. (2008). Four-jointed is a Golgi kinase that phosphorylates a subset of cadherin domains. Science 321, 401-404.

2. Hambleton, S., Valeyev, N.V., Muranyi, A., Knott, V., Werner, J.M., McMichael, A.J., Handford, P.A., and Downing, A.K. (2004). Structural and functional properties of the human notch-1 ligand binding region. Structure 12, 2173-2183.

Supplemental Figure 1

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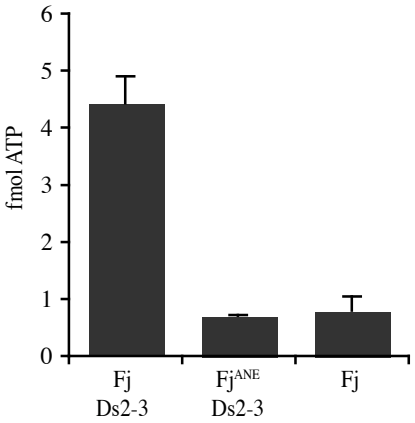

Supplemental Figure 2

Supplemental Figure 3

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