

## Supplementary Figure Legends

**Supplementary Fig S1 Kinase assay of the Fj<sup>ANE</sup> 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 from *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.

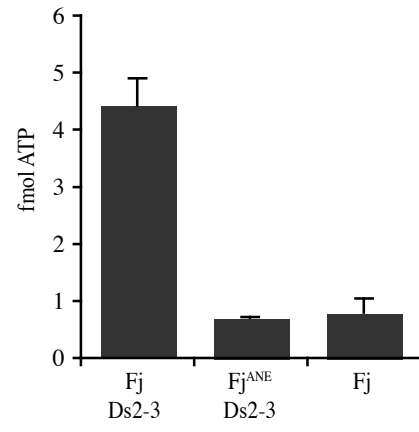
**Supplementary Fig S3 Effect of S273A mutation on full-length Fat binding to Ds**

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/*lacZ* (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.



Cad1		Cad10		Cad19		Cad28	
Dm	QSRVDTSDADFESSSPSGEM	Dm	DVNDHTPVFDH <u>T</u> SYETSLEPE	Dm	DLNDNAPIFDPMYSYSSEVFEE	Dm	DQNDNAPEFEH <u>S</u> FYSFSFPE
Tc	PGDYSTQMQRADVTRVTFEE	Tc	DVNDHTPVFDH <u>T</u> SYETSLEPE	Tc	DLNDNTPLFDPMYSYTNEIFE	Tc	DQNDNAPEFEH <u>S</u> FYSFNFPE
Gg	-	Gg	DVNDNPPVFDQLSYEITILE	Gg	DINDNPPVFSM <u>T</u> SYSTSLME	Gg	DVNDNAPRFTKPSYYLECPE
Mm	LSLLPGSARVQAAEQRVFQ	Mm	DVNDNPPVFDQISYEVTLSE	Mm	DVNDNPPVFSM <u>S</u> SYSTSLME	Mm	DINDNTPRFSRPSYYLDCPE
Hs	LSLLPGQAWVHGAEPRQVFQ	Hs	DVNDNSPVFDQLSYEVTLSE	Hs	DVNDNPPIFSLNSYSTSLME	Hs	DINDNAPRFSR <u>T</u> SYYYLDCPE
Cad2		Cad11		Cad20		Cad29	
Dm	DVNDNSPEFPEPSIAISFSE	Dm	DENDNAPQFTN <u>S</u> TFTFSIPE	Dm	DVNDVPPVVISANETAIMEN	Dm	DAHNNPPKFEQ <u>A</u> EYLAPLPQ
Tc	DINDNPEFPEPSISVSFSE	Tc	DENDNSPEFTNASFSFNIRE	Tc	DVNDMSPEFVTPNETSVAEN	Tc	DANNNAPKFDKPEYLSVVPD
Gg	DLNDNAPVFPDPSIVVTFKE	Gg	DVNDNRPLFNS <u>T</u> NYVFYFEE	Gg	DVNDSPPSFISPKLTYIPEN	Gg	DSNDNAPLFLAPKYFTPVTK
Mm	DLNDNAPVFPDPSIVVTFKE	Mm	DVNDNRPLFNS <u>T</u> NYTFYFEE	Mm	DVNDNPPMFLSPKLTYPEN	Mm	DSNDNPPQFLQNKYFTPVTK
Hs	DLNDNAPVFPDPSIVVTFKE	Hs	DVNDNRPLFNS <u>T</u> NYTFYFEE	Hs	DVNDNPPTFLLSPKLTYPEN	Hs	DSNDNAPQFLK <u>S</u> KYFTPVTK
Cad3		Cad12		Cad21		Cad30	
Dm	DVNDNPPIFDH <u>S</u> DYINVSLNE	Dm	DVNDNAPEFLRAPYHVTISE	Dm	DENDNSPVFDPKQYSASVAE	Dm	GENMDTPRFSVNSYQVIVPE
Tc	DVNDNPPIFDH <u>S</u> DIIVSLNE	Tc	DVNDNPPKFLR <u>T</u> PYRVQVSE	Tc	DENDNSPVFDPKQYSASIAE	Tc	GENKMPVFTALSYQVIVPE
Gg	DINDNPPVFSQ <u>T</u> LYQARVPE	Gg	DINDNAPKFLKDLQATISE	Gg	DINDNPLFAQKLYRVELEE	Gg	EENYHTPEFSQ <u>S</u> HMSVTIPE
Mm	DINDNPPVFGS <u>S</u> HYQAGVPE	Mm	DINDNAPKFLKDFYQATVSE	Mm	DINDNPPVFAQAMYRVQIKE	Mm	EENYHTPEFSQNHISATIPE
Hs	DINDNPPVFGS <u>S</u> HYQAGVPE	Hs	DINDNAPKFLKDFYQATISE	Hs	DINDNPIFAQALYKVEINE	Hs	EENYHTPEFSQ <u>S</u> HMSATIPE
Cad4		Cad13		Cad22		Cad31	
Dm	DTNDHDPFIISFRFFPDGGKV	Dm	DENDNAPEFTQ <u>S</u> SSEVSVLE	Dm	DINDNRPTFLD <u>S</u> PYLARVME	Dm	DVNDNPPVFNHKEYHCYIPE
Tc	DANDHDPVIKFRYFPFNAMF	Tc	DENDNSPEFTQ <u>T</u> NSKISVIE	Tc	DINDNPTFLD <u>S</u> PYLAVVME	Tc	DINDNSPTFNQ <u>S</u> IYEAYIPE
Gg	DVNDNEPRVKFRYFPATSRF	Gg	DENDNSPSFPK <u>S</u> TLSVDVLE	Gg	DVNDYVPTFELS <u>P</u> YVNVVPE	Gg	DVNDNSPTFSPEYFPNVLE
Mm	DVNDNDPVVKFRYFPATSRF	Mm	DENDNTPSFPK <u>S</u> TLFDVDLE	Mm	DMNDFVPVFEL <u>S</u> PYSVNVPE	Mm	DVNDNSPVFVPEFFPTVME
Hs	DVNDNDPVVKFRYFPATSRF	Hs	DENDNTPSFPK <u>S</u> TLFDVDLE	Hs	DINDFVPVFEL <u>S</u> PYSVNVPE	Hs	DVNDNSPVFLSDDYFPTVLE
Cad5		Cad14		Cad23		Cad32	
Dm	DVNDHEPVFEK <u>S</u> EYSAVLSE	Dm	DDNDNPPIFPS <u>T</u> AIVRQIKE	Dm	DINDNDPVFELQSYHATVRE	Dm	GVNEFYPPQLQPVFHFVDFSE
Tc	DVNDHEPVFEK <u>S</u> EYSAILSE	Tc	DANDNPPSFP <u>S</u> TAIVRQIRE	Tc	DVNDHTPEFKRQSYHATINE	Tc	GVNEYYPRIQPVFHFVDFSE
Gg	DINDHPPVFEQ <u>S</u> VYRVNIIIE	Gg	DFNDNPPNFAGDIFKSIIE	Gg	DVNDNVPTFAFNMYSATVPE	Gg	GTNEYVPRFVSKLYFEVSE
Mm	DINDHPPVFEQQVYRVNLSE	Mm	DFNDNPPSFP <u>P</u> GDIFKSIIVE	Mm	DINDNVPTFANNMYLTSIAE	Mm	GTNEYVPRFVSKLYFEVSE
Hs	DINDHPPVFSQQVYRVNLSE	Hs	DFNDNPPSFP <u>P</u> GDIFKSIIVE	Hs	DVNDNVPTFASKAYFTIPE	Hs	GTNEYVPRFVSKLYFEIPE
Cad6		Cad15		Cad24		Cad33	
Dm	DENDEAPQFSQREQNVTLGE	Dm	DINDNAPVFSMNAAILPPK	Dm	DVNDNIPKFD <u>S</u> TYNVAVPE	Dm	DGNDPPEFIKHYYTSTISEA
Tc	DENDEAPRFSQ <u>S</u> KFNVLSE	Tc	DVNDNAPVFSMNSAILPQN	Tc	DENDNSPSFS <u>S</u> TKYEVNISD	Tc	DGNDPPEFLQTLYEVEISEG
Gg	DINDNKPRFSQPEGYQVSLA	Gg	DQNDNVVVFISQNALAADPS	Gg	DVNDNPPKFQHHPPYVTHVPS	Gg	DANDPPVFTLGTYNIQISEG
Mm	DVNDKPVFSQPEGYEVSVV	Mm	DLNDNVPMFISQNALAADPS	Mm	DVNDNPPRFQHHPPYVTHIPS	Mm	DANDPPVFSLSYRVQISEG
Hs	DVNDKPVFSQPEGYDVSVV	Hs	DLNDNVPMFISQNALAADPS	Hs	DVNDNPPRFQHHPPYVTHIPS	Hs	DANDPIFTLNIYSVQISEG
Cad7		Cad16		Cad25		Cad34	
Dm	DVNDNDPQFYPRHYIYSLAD	Dm	SSVPQFEQRKLSGVSVEENE	Dm	SKAELTVILRPP <u>E</u> LFPFTFAY	Dm	DVNDNGPTFT <u>P</u> EGLNGYISE
Tc	DVNDNSPEFYPLNYFVAVPE	Tc	SARGPSFESGLFTGSVFENE	Tc	DSTELRVFLRPDHLFPFSFTS	Tc	DINDNGPTFDPRVVGKVLN
Gg	DVNDNSPVFYPVQYFAHIQE	Gg	GVDGPIFTQPKYITILKEGE	Gg	DSTTVTVRFVNRAEFPQVQA	Gg	DINDNGPTLSTRQGEVMENN
Mm	DINDNSPVFYVQYFAHIQE	Mm	GLDGPVFTQPKYITILKEGE	Mm	DSTTVTVRFANKADFPKQVRA	Mm	DINDNGPVLTV <u>S</u> EGEVLENK
Hs	DINDNSPVFYVQYFAHIKE	Hs	GLDGPVFTQPKYITILKEGE	Hs	DSTTVTVRFVNKADFPKQVRA	Hs	DINDNGPVLTV <u>S</u> EGEVLENK
Cad8		Cad17		Cad26			
Dm	SKLEMLECGQAQAGGYEFQM	Dm	DKNDSPPQFLD <u>T</u> PFVYNVSE	Dm	DANDNAPVMEQ <u>L</u> IYNAEVL		
Tc	RDLEELLFDNYGYEFKIVED	Tc	DKNDSPPSFKD <u>T</u> PLYYSISE	Tc	DANDNSPVFNNSLYNASILE		
Gg	DTQDNPPVFSQMGYGFVVFE	Gg	DINDNPPVFPDMLDLTVEE	Gg	DVNDNAPEFEQDPFIAEIVE		
Mm	DTQDNPPVFSQAAYSFVVFE	Mm	DINDNPPVFPDMLDLTVEE	Mm	DINDNAPTFEEDPFVSEILE		
Hs	DTQDNPPVFSQVAYSFVVFE	Hs	DINDNPPVFPDMLDLTVEE	Hs	DVNDNAPIFKEDPFISEILE		
Cad9		Cad18		Cad27			
Dm	DLNDNAPVFDALDRESEPTIS	Dm	DTNDNPPFLFED <u>T</u> VYSFDIPE	Dm	DKNDNPPKFTRLFSLNVTEN		
Tc	DLNDNKPVFDRDKDEVKLAEE	Tc	DTNDNPPAFLE <u>T</u> AYSFDIPE	Tc	DKNDNPPRFSRLFSVNVNEN		
Gg	DLNDNSPHFIHAVESVNVVVE	Gg	DVNDHIPPFSKPVYSFDIPE	Gg	DENDNAPRFSQIFSAVSVEN		
Mm	DLNDNAPHFLQAVESINAVE	Mm	DVNDHTPRFSRPVYSFDIPE	Mm	DENDNAPRFSQIFSAVSVEN		
Hs	DLNDNSPHFLQAIESVNVVVE	Hs	DVNDHTPKFSRPVYSFDIPE	Hs	DENDNAPRFSQIFSAHVSVEN		

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