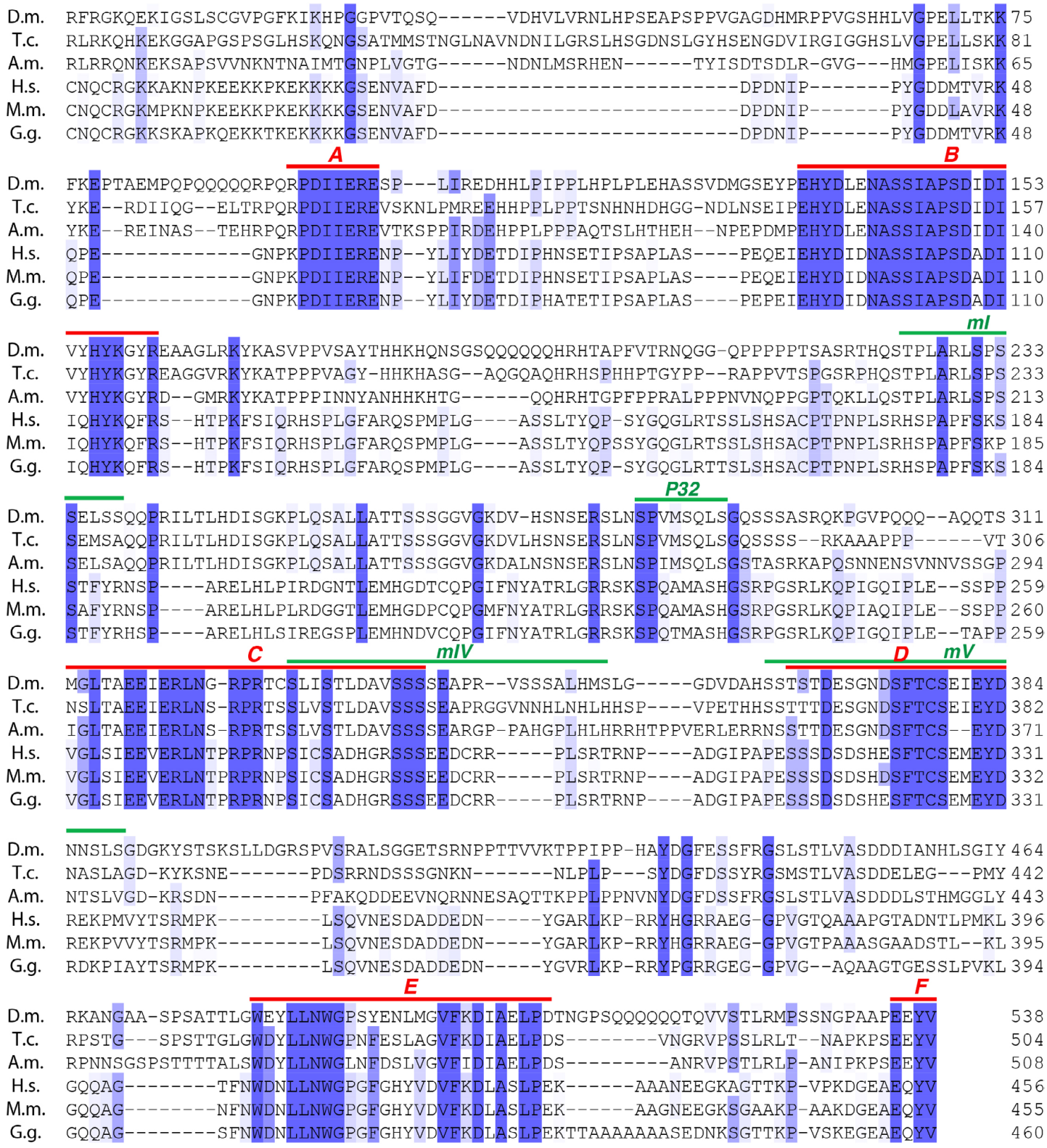

Fig. S1. Alignment of Fat ICD sequences. ClustalW alignment of intracellular domains (generated at the NCBI web site) of three insect Fat sequences (D.m., *Drosophila melanogaster*; T.c., *Tribolium castaneum*; A.m., *Apis mellifera*) and three vertebrate Fat4 sequences (H.s., *Homo sapiens*; M.m., *Mus musculus*; G.g., *Gallus gallus*). Based on transmembrane domain predictions, the ICD in *D. melanogaster* Fat is 538 amino acids, and begins at amino acid 4610. Sequence identity is indicated by relative shading. Conserved motifs deleted by mutations are indicated by red lines and regions affected by point mutations are indicated by green lines; the specific Ser and Thr residues changed in these point mutations are indicated in Fig. S5.

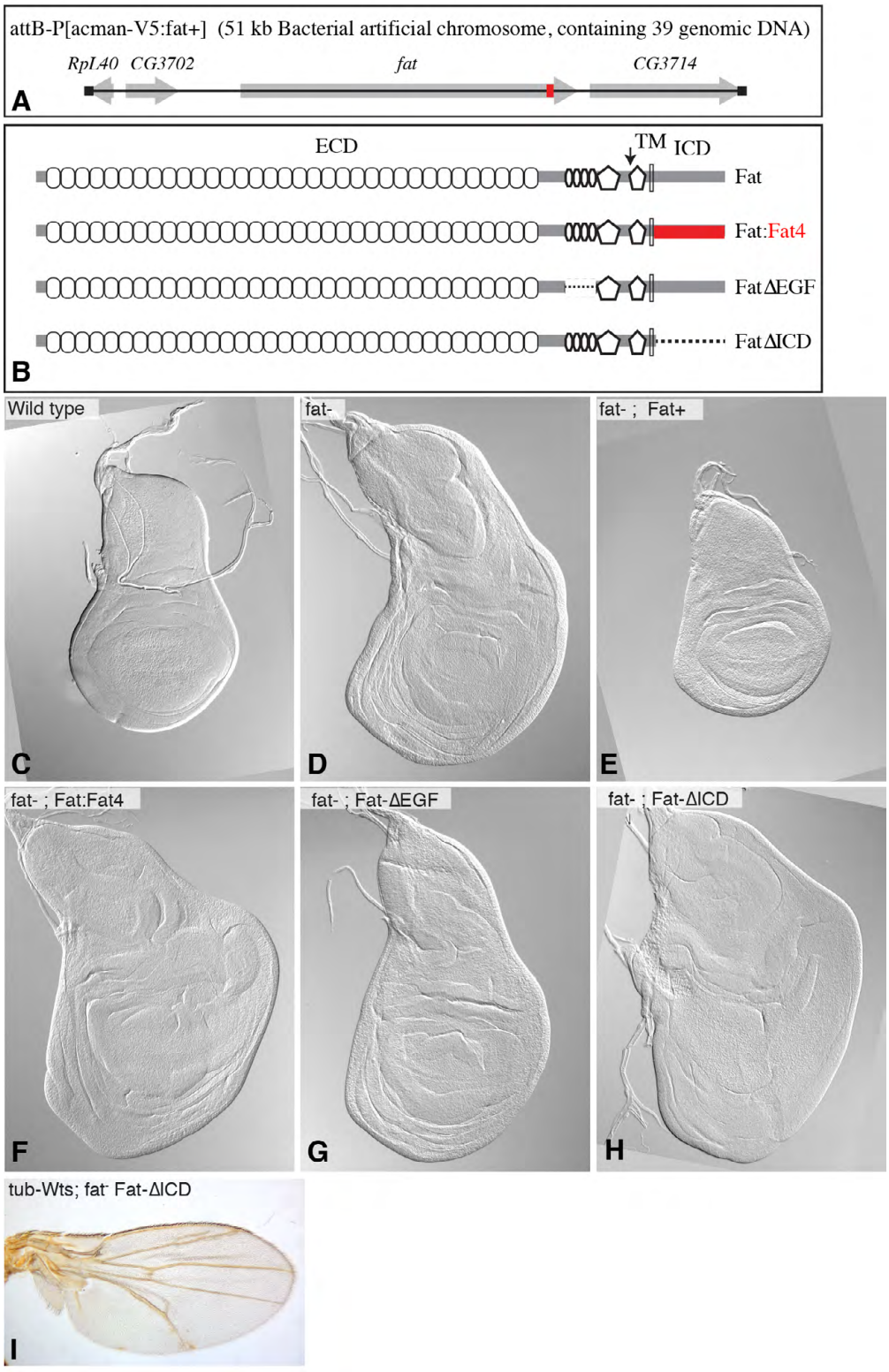

Fig. S2. Additional analysis of Fat constructs that fail to rescue *fat* mutant lethality. (**A**) The *fat* locus genomic rescue construct, with the region encoding the ICD highlighted in red. (**B**) Wild-type (top) and three mutant forms of Fat examined; red indicates replacement of the *Drosophila* ICD by the human FAT4 ICD. (**C-H**) Examples of wing discs from representative late third instar larvae of the indicated genotypes. Lack of Fat activity results in overgrown wing discs and increased folding in the proximal wing. (**I**) Adult wing from $fat^{8}/fat^{G\text{-}rv}$ expressing *tub-Gal4 UAS-wts* and *P[acman] V5:fatΔICD[68A4]*.

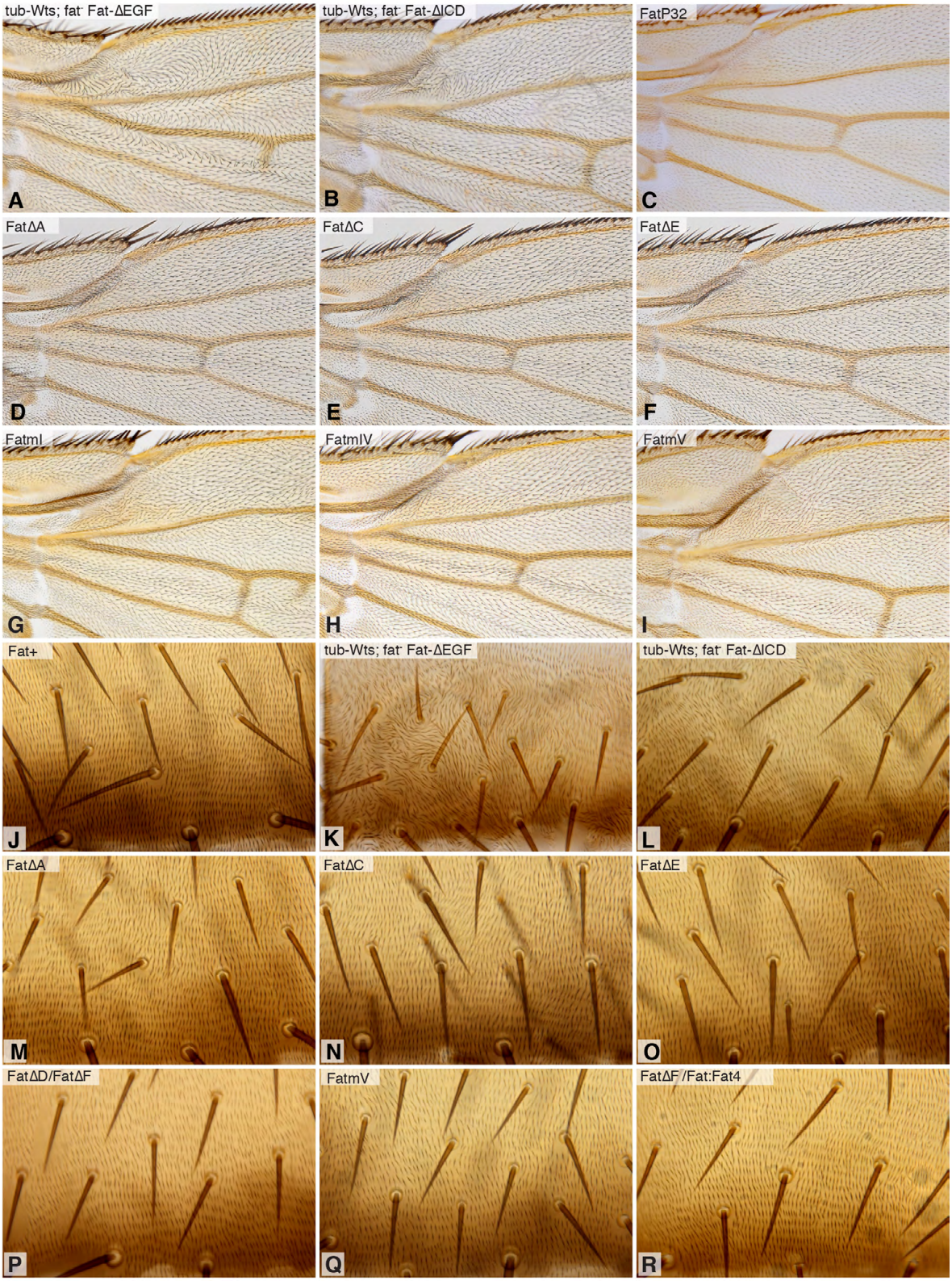

Fig. S3. Additional analysis of hair polarity phenotypes associated with Fat ICD motif mutations. (**A-I**) Proximal anterior wings from $fat^{8}/fat^{G\text{-}rv}$ expressing (A) *tub-Gal4 UAS-wts P[acman]V5:fatΔEGF[68A4],* (B) *tub-Gal4 UAS-wts P[acman]V5:fatΔICD[68A4],* (C) *P[acman]V5:fatP32[68A4]*, (D) *P[acman]V5:fatΔA[68A4],* (E) *P[acman]V5:fatΔC[68A4],* (F) *P[acman]V5:fatΔE[68A4],* (G) *P[acman]V5:fat-mI[68A4],* (H) *P[acman]V5:fat-mIV[68A4],* (I) *P[acman]V5:fat-mV[68A4]*. (**J-R**) Abdomens from $fat^{8}/fat^{G\text{-}rv}$ expressing (J) *P[acman]V5:fat[68A4],* (K) *tub-Gal4 UAS-wts P[acman]V5:fatΔEGF[68A4],* (L) *tub-Gal4 UAS-wts P[acman]V5:fatΔICD[68A4,* (M) *P[acman]V5:fatΔA[68A4],* (N) *P[acman]V5:fatΔC[68A4],* (O) *P[acman]V5:fatΔE[68A4],* (P) *P[acman]V5:fatΔD[68A4]/ P[acman]V5:fatΔF[68A4],* (Q) *P[acman]V5:fat-mV[68A4],* (R) *P[acman]V5:fatΔF[68A4]/P[acman]V5:fat:Fat4[68A4].*

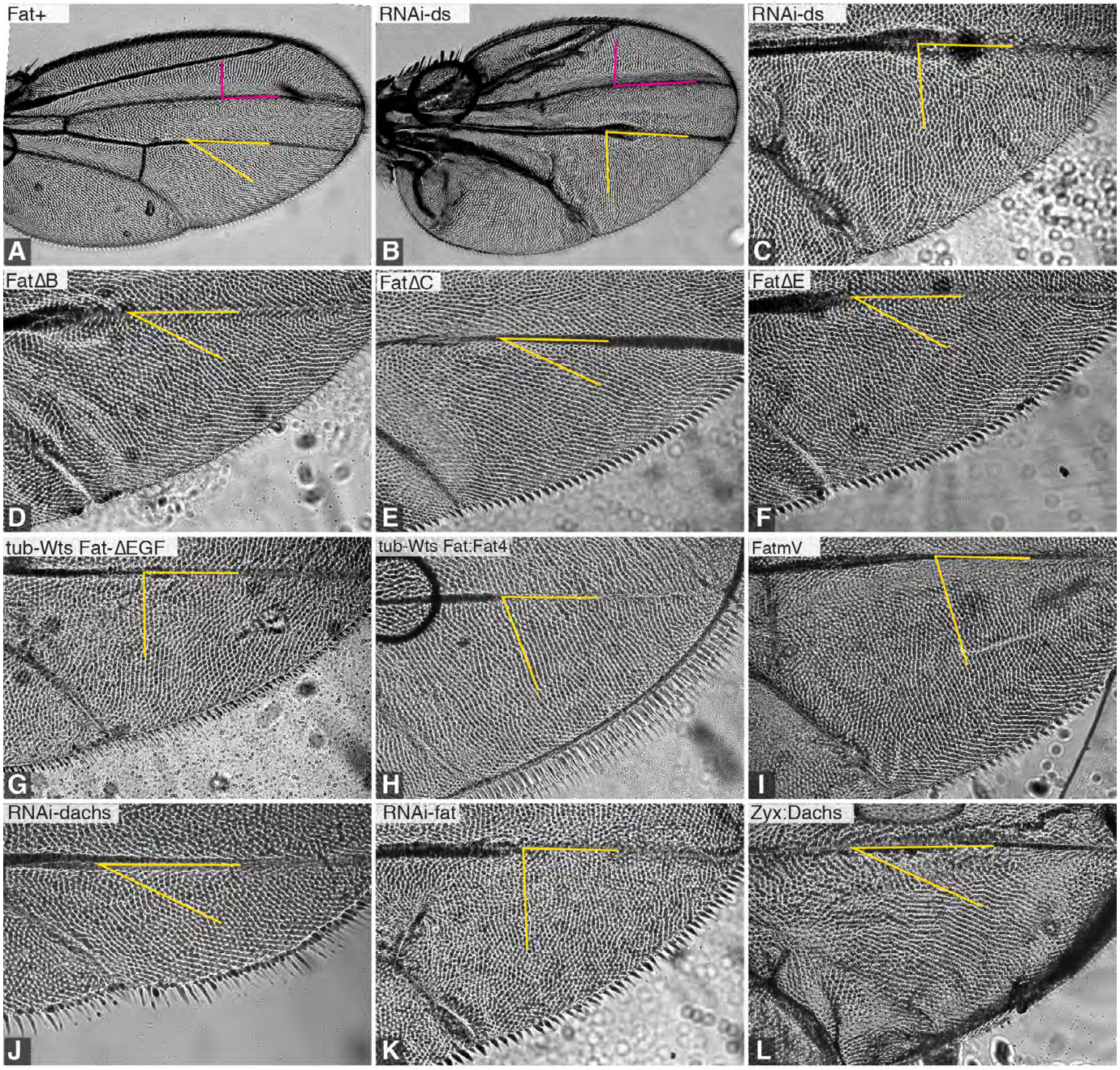

Fig. S4. Additional analysis of ridge polarity. Proximal anterior wings, visualized by cuticle refraction microscopy (Doyle et al., 2008), from $fat^{8}/fat^{G\text{-}rv}$ expressing (**A**) *P[acman]V5:fat[68A4],* (**D**) *P[acman]V5:fatΔB[68A4],* (**E**) *P[acman]V5:fatΔC[68A4],* (**F**) *P[acman]V5:fatΔE[68A4],* (**G**) *tub-Gal4 UAS-wts P[acman]V5:fatΔEGF[68A4],* (**H**) *tub-Gal4 UAS-wts P[acman]V5:fat:Fat4[68A4],* (**I**) *P[acman]V5:fat-mV[68A4],* or from fat^{+} flies expressing *nub-Gal4* and (**B**,**C**) *UAS-RNAi-ds*, (**J**) *UAS-RNAi-dachs*, (**K**) *UAS-RNAi-fat*, (**L**) *UAS-Zyx:dachs*.

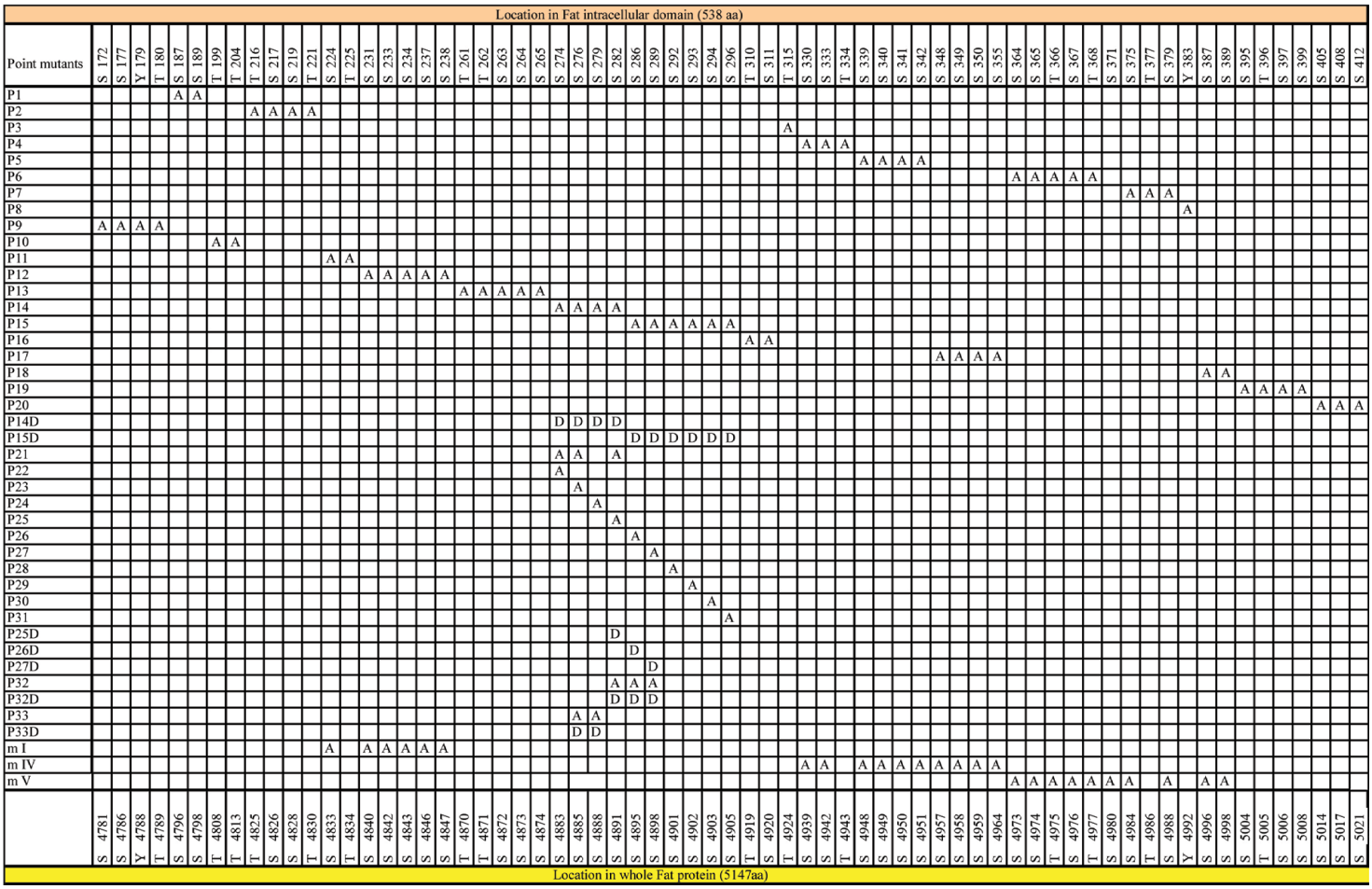

Fig. S5. Potential phosphorylation sites altered in point mutant constructs. Potential phosphorylation sites (Ser, Thr or Tyr) within the Fat intracellular domain were mutated to Ala or Asp, as indicated, within a series of constructs, which were then analyzed for effects on Fat mobility or activity. The first column indicates the name of the construct, top row shows the location of the site within the intracellular domain, bottom row shows location within the entire Fat coding sequence. Middle rows show amino acid mutations within each construct.

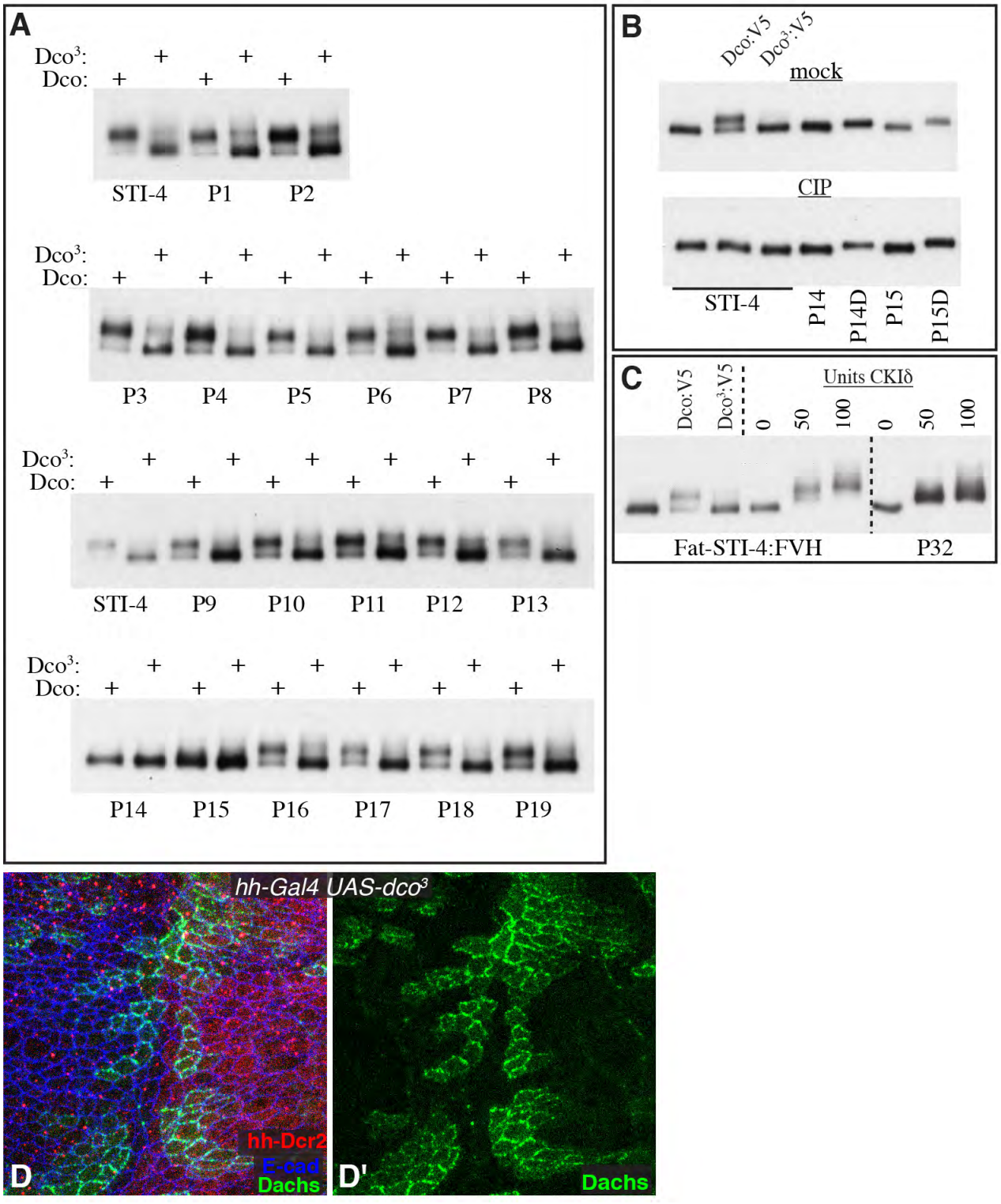

Fig. S6. Additional analysis of Fat ICD phosphorylation. (**A**) Western blots on S2 cell lysates expressing Fat-STI-4:FVH (STI-4) and point mutant derivatives, together with Dco or Dco^3, as indicated. The amino acids mutated in each construct are indicated in Fig. S5. Of the mutants shown here, only P14 and P15 fail to exhibit the Dco-mediated mobility shift. (**B**) The mobility shift induced by Ser to Asp mutations is not reversed by phosphatase treatment (CIP). (**C**) Western blot of products of a kinase assay with vertebrate CKId shows that purified Fat-STI-4:FVH can be directly phosphorylated by CKI *in vitro*, with the extent of phosphorylation proportional to the amount of enzyme; for comparison, protein phosphorylated *in vivo* was run on the same gel. The S-to-A triple mutant Fat-STI-4-P32:FVH is still a substrate for CKId, but the degree of phosphorylation, as assayed by mobility shift, is reduced. (**D**,**D′**) Overexpression of *dco^3* in posterior cells, under *hh-Gal4* control (red), does not affect Dachs:Cit staining (green); Dachs:Cit in anterior cells serves as an internal control. Overexpression of Dco^3 mimics *dco^3* mutation (Feng and Irvine, 2009); this confirms earlier studies of the lack of effect of *dco^3* mutant clones on Dachs localization (Feng and Irvine, 2009).

Table S1. Wing measurement statistics

Rescue constructs	Normalized mean wing area	Standard deviation	Number measured	Standard error of the mean	*P*-value for *t*-test to Fat$^+$
Fat$^+$	1.00	0.030	29	0.006	
FatΔA	0.96	0.034	19	0.008	1.03E–04
FatΔB	1.05	0.043	16	0.011	1.65E–04
FatΔC	0.98	0.044	29	0.008	4.52E–02
FatΔD	1.29	0.051	34	0.009	1.23E–33
FatΔE	1.08	0.043	37	0.007	3.34E–13
FatΔF	1.04	0.057	32	0.010	2.89E–04
FatΔF/FatΔD	1.07	0.037	12	0.011	1.12E–05
FatΔD/FatΔD	1.17	0.042	15	0.011	3.15E–12
FatΔF/FatΔF	0.96	0.068	16	0.017	2.46E–02
Fat$^+$/FatΔD	0.96	0.027	10	0.009	9.53E–04
Fat$^+$/FatΔF	0.94	0.019	10	0.006	1.99E–08
FatΔD/FAT4	1.33	0.029	8	0.010	6.55E–12
FatΔF/FAT4	1.22	0.040	12	0.012	1.02E–11
Fat$^+$/FAT4	0.99	0.028	12	0.008	2.56E–01
FatP32/	1.05	0.029	23	0.006	4.63E–07
FatmI/	1.18	0.036	16	0.009	8.49E–16
FatmIV/	1.08	0.014	12	0.004	5.14E–14
FatmV/	1.35	0.047	28	0.009	1.79E–33
FatmV/FatΔD	1.30	0.034	11	0.010	1.46E–14
FatΔF/FatmV	1.12	0.028	12	0.008	3.73E–11

Rescue constructs	Normalized cross-vein distance	Standard deviation	Number measured	Standard error of the mean	*P*-value for *t*-test to Fat$^+$
Fat$^+$	1.00	0.039	19	0.009	
FatΔA	1.00	0.027	18	0.006	6.53E–01
FatΔB	0.97	0.039	16	0.010	1.27E–02
FatΔC	1.04	0.038	29	0.007	2.07E–04
FatΔD	0.73	0.046	30	0.008	1.41E–31
FatΔE	0.87	0.037	31	0.007	3.17E–20
FatΔF	0.27	0.055	28	0.010	6.78E–45
FatΔF/FatΔD	0.62	0.055	11	0.016	1.28E–11
FatΔD/FatΔD	0.64	0.053	14	0.014	4.21E–15
FatΔF/FatΔF	0.28	0.048	16	0.012	2.92E–26
Fat$^+$/FatΔD	0.94	0.036	10	0.011	4.91E–04
Fat$^+$/FatΔF	0.92	0.037	10	0.012	1.75E–05
FatΔD/FAT4	0.77	0.044	6	0.018	1.26E–05
FatΔF/FAT4	0.66	0.043	7	0.016	5.04E–08

Fat⁺/FAT4	1.03	0.033	12	0.010	2.69E–02
FatP32/	0.97	0.033	23	0.007	1.68E–03
FatmI/	0.77	0.042	14	0.011	1.65E–14
FatmIV/	1.07	0.040	12	0.012	2.61E–05
FatmV/	0.79	0.065	28	0.012	3.20E–18
FatmV/FatΔD	0.70	0.045	7	0.017	2.93E–07
FatΔF/FatmV	0.63	0.053	9	0.018	1.71E–09

Rescue Constructs	Normalized width/length ratio	Standard deviation	Number measured	Standard error of the mean	*P*-value for *t*-test to Fat⁺
Fat⁺	1.00	0.009	29	0.002	
FatΔA	0.99	0.008	18	0.002	1.83E–02
FatΔB	1.01	0.007	16	0.002	2.31E–07
FatΔC	1.00	0.012	26	0.002	9.87E–02
FatΔD	1.16	0.016	30	0.003	2.38E–42
FatΔE	1.05	0.013	31	0.002	1.94E–24
FatΔF	1.12	0.025	31	0.004	5.05E–26
FatΔF/FatΔD	1.12	0.010	12	0.003	6.85E–19
FatΔD/FatΔD	1.12	0.010	12	0.003	3.21E–15
FatΔF/FatΔF	1.18	0.021	14	0.006	1.57E–14
Fat⁺/FatΔD	1.12	0.021	16	0.005	5.05E–05
Fat⁺/FatΔF	1.02	0.010	9	0.003	4.89E–09
FatΔD/FAT4	1.02	0.006	10	0.002	5.12E–10
FatΔF/FAT4	1.18	0.016	8	0.006	1.84E–09
Fat⁺/FAT4	1.19	0.014	7	0.005	1.13E–08
FatP32/	1.01	0.003	12	0.001	1.93E–04
FatmI/	1.15	0.013	16	0.003	4.73E–24
FatmIV/	0.99	0.007	12	0.002	1.85E–03
FatmV/	1.25	0.024	26	0.005	4.78E–32
FatmV/FatΔD	1.19	0.026	10	0.008	6.76E–10

The normalized width/length ratio was calculated by measuring the width and length as indicated in the image below, and then normalizing to the average value in wild-type wings.

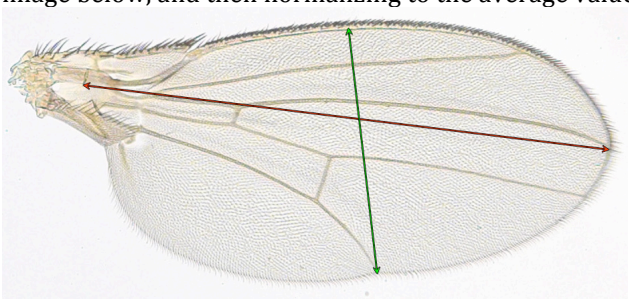

Table S2. Primers

Primers to generate Fat ICD truncates in pUAST-fat plasmid

Construct	PCR template	Primers for 5' fragment		Primers for middle fragment		Primers for 3' fragment	
ft-STI	ft:FVH	ftSPL_NotI5	ftSPL_SOE3			ftL2_SOE5	ftFVH_XbaI3
ft-STI-1	ft-STI	ftSPL_NotI5	STI-1-SOE			FVH_SOE5	ftFVH_XbaI3
ft-STI-2	ft-STI	ftSPL_NotI5	STI-2-SOE			FVH_SOE5	ftFVH_XbaI3
ft-STI-3	ft-STI	ftSPL_NotI5	STI-3-SOE			FVH_SOE5	ftFVH_XbaI3
ft-STI-4	ft:FVH	ftSPL_NotI5	STI-4-SOE			TM-SOE	ftFVH_XbaI3
ft-STI-5	ft-STI	ftSPL_NotI5	ft-TM3	STI5-SOE-A	STI5-SOE-B	FVH_SOE5	ftFVH_XbaI3
ft-STI-6	ft-STI	ftSPL_NotI5	ft-TM3	STI6-SOE-A	STI-2-SOE	FVH_SOE5	ftFVH_XbaI3
ft-STI-7	ft-STI	ftSPL_NotI5	ft-TM3	STI7-SOE-A	STI-3-SOE	FVH_SOE5	ftFVH_XbaI3
ft-STI-8	ft-STI	ftSPL_NotI5	ft-TM3	STI8-SOE-A	STI8-SOE-B	FVH_SOE5	ftFVH_XbaI3
ft-STI-9	ft-STI	ftSPL_NotI5	ft-TM3			STI9-SOE	ftFVH_XbaI3
ft-STI-10	ft-STI	ftSPL_NotI5	ft-TM3	STI5-SOE-A	STI-3-SOE	FVH_SOE5	ftFVH_XbaI3
ft-STI-11	ft-STI	ftSPL_NotI5	ft-TM3	STI6-SOE-A	STI-3-SOE	FVH_SOE5	ftFVH_XbaI3
ft-STI-12	ft-STI	ftSPL_NotI5	ft-TM3			STI5-SOE-A	ftFVH_XbaI3
ft-STI-13	ft-STI	ftSPL_NotI5	ft-TM3			STI6-SOE-A	ftFVH_XbaI3
ft-STI-14	ft-STI	ftSPL_NotI5	ft-TM3			STI7-SOE-A	ftFVH_XbaI3
ft-STI-15	ft-STI	ftSPL_NotI5	ft-TM3			STI8-SOE-A	ftFVH_XbaI3

Primer sequences

ftSPL_NotI5: 5’-TCT GCG GCC GCA TGG AGA GGC TAC TGC TCC-3’

ftSPL_SOE3: 5’-TGA AGT TGA GAG TGC TTC TTC TTC GCG GAA AGG CGG CAT-3’

ftL2_SOE5: 5’-CCG CGA AGA AGA AGC ACT CTC AAC TTC AAC AAA CAG CCC CTG-3’

ftFVH_XbaI3: 5’-GG TCT AGA GAT CA GCG GGT TTA AAC TCA ATG GTG-3’

FVH_SOE5: 5’-ACC GAC TAC AAG GAC GAC GAC GAC AAG-3’

(Overlapping sequence: 5’-CGT CGT CGT CCT TGT AGT CGG TAC C-3’)

STI-1-SOE: 5’- CGT CGT CGT CCT TGT AGT CGG TAC C AAG CGG CGG AAT GGG CAG GTG ATG-3’

STI-2-SOE: 5’- CGT CGT CGT CCT TGT AGT CGG TAC C TTC GCT GTT GCT GTG AAC GTC CTT-3’

STI-3-SOE: 5’- CGT CGT CGT CCT TGT AGT CGG TAC C CTC ACC ACC GCT TAG TGC TCT GGA-3’

STI-4-SOE: 5’-CTT CTT CGC GGA AAG GCG GCA TAT-3’

TM-SOE: 5’-GCC GCC TTT CCG CGA AGA AGA GCC GAT CCT CTC AGC ATT GGC TTC-3’

ft-TM3: 5’-CGA TAG ATA ACA TAG GAG CCC AGT-5’

(Overlapping sequence: 5’-GGG CTC CTA TGT TAT CTA TCG ATT C-3’)

STI5-SOE-A: 5’- GGG CTC CTA TGT TAT CTA TCG ATT C CAG CAA CGT CCC CAG CGA CCC GAT-‘3

STI6-SOE-A: 5’- GGG CTC CTA TGT TAT CTA TCG ATT C AGT GTT CCA CCT GTT TCC GCC

TAC-3’

STI7-SOE-A: 5’- GGG CTC CTA TGT TAT CTA TCG ATT C CAC AGC AAC AGC GAA CGC AGT CTG-3’

STI8-SOE-A: 5’- GGG CTC CTA TGT TAT CTA TCG ATT C AAC AGT CTC AGT GGC GAC GGC AAG-3’

STI9-SOE: 5’- GGG CTC CTA TGT TAT CTA TCG ATT C AAT GGA GCC GCA TCC CCA TCG GCC-3’

(Overlapping sequence: 5’-CGT CGT CGT CCT TGT AGT CGG TAC C-3’)

STI5-SOE-B: 5’- CGT CGT CGT CCT TGT AGT CGG TAC C GGC CTT GTA TTT GCG TAG GCC

AGC-3’

STI8-SOE-B: 5’- CGT CGT CGT CCT TGT AGT CGG TAC C ATT GGC CTT GCG GTA GAT ACC GCT C-3’

Primers to generate human Fat-ICD and *Drosophila* Fat hybrid genomic constructs:

1. Forward: galK_ICD_F: ATT GTC TTC TTC GTC ATT CTG GTG GTG GCT ATA CTG GGC TCC TAT GTT ATC TAT CGA

TTC CCT GTT GAC AAT TAA TCA TCG GCA

1. Reverse: galK_ICD_R:

TGG GGC TCA GAC TTT AGG AAC ACT TTA ACT TTC GTT GAA GAG CAT ACA CAA CAT ATA TTA TCA GCA CTG

TCC TGC TCC TT

2. hsft4_SOE5: GGG CTC CTA TGT TAT CTA TCG ATT C AAC CAG TGC AGG GGG AAG AAG

GCC

2. hsft4_FLAGXhoI3: AAA CTC GAG TCA ACC CTT GTC GTC GTC GTC CTT GTA GTC CAC ATA

3. TM_F: CTCAGCATTGGCTTCACCCTGGTC

3. TM_R: CGATAGATAACATAGGAGCCCAGT

3. FLAGft_dwn_F: GAC TAC AAG GAC GAC GAC GAC AAG GGT TGA TA ATA TAT GTT GTG TAT GCT CTT CAA CG

3. FLAG_R: TCA ACC CTT GTC GTC GTC GTC CTT GTA GTC

Notes: 1. Primers used to insert galK into fly fat ICD region. 2. Primers used to generate fusion protein of human Fat-ICD with *Drosophila* Fat (signal peptide plus transmembrane domain) in pUAST plasmid. 3. Primers used to generate fusion proteins of human Fat-ICD with *Drosophila* Fat (ECD plus transmembrane domain) in genomic rescue construct

Primers to generate *fat* genomic rescue constructs with deletions:

A. Fat-Delta aa4704-4711-Fgalk: GAACCCACTGCGGAGATGCCACAGCCGCAGCAGCAGCAGCAACGTCCCCAG

CCTGTTGACAATTAATCATCGGCA

A. Fat-Delta aa4704-4711-Rgalk: GGATGAAGCGGCGGAATGGGCAGGTGATGATCCTCCCTTATCAGAGGACT

TCAGCACTGTCCTGCTCCTT

A. Fat ICD F: ATTGTCTTCTTCGTCATTCTGGTGGTGGCTATACTGGGCTCCTATGTTATCTATCGATTC

A. FatDel4704-4711R: GATCCTCCCTTATCAGAGGACTCTGGGGACGTTGCTGCTGCTGC

A. FatDel4704-4711F: GCAGCAACGTCCCCAGAGTCCTCTGATAAGGGAGGATCATC

A. FatDel4921-4959R: GGAAGTTTGCTGCGCCTGCTGCT

B. Fat-Delta aa4745-4770-Fgalk:CTCTCCCGCTGGAGCACGCCAGTTCCGTGGACATGGGTTCCGAGTACCCG

CCTGTTGACAATTAATCATCGGCA

B. Fat-Delta aa4745-4770-Rgalk:

GCGGAAACAGGTGGAACACTGGCCTTGTATTTGCGTAGGCCAGCAGCCTC TCAGCACTGTCCTGCTCCTT

B. Fat ICD F FatDel4745-4770R: TGCGTAGGCCAGCAGCCTCCGGGTACTCGGAACCCATGTCC

B. FatDel4745-4770F: GACATGGGTTCCGAGTACCCGGAGGCTGCTGGCCTACGCAAATA

B. FatDel4921-4959R: GGAAGTTTGCTGCGCCTGCTGCT

C. Fat-Delta aa4921-4959-Fgalk:CCAGCAGGCAAAAGCCCGGAGTGCCACAGCAGCAGGCGCAGCAAACTTCC

CCTGTTGACAATTAATCATCGGCA

C. Fat-Delta aa4921-4959-Rgalk:GAAGTCGAACTATGGGCATCCACATCTCCACCCAGCGACATATGCAGAGC

TCAGCACTGTCCTGCTCCTT

C. ftSTm_checkF:GCCAGTTCCGTGGACATGGGTTCC

C. FatDel4921-4959R: GGAAGTTTGCTGCGCCTGCTGCT

C. FatDel4921-4959F:TGCCACAGCAGCAGGCGCAGCAAACTTCCGCTCTGCATATGTCGCTGGGTGGA

C. ftSTm_checkR:cggaatgggcggtgtcttgaccac

D. Fat-Delta aa4975-4993-Fgalk:GCAGCGCTCTGCATATGTCGCTGGGTGGAGATGTGGATGCCCATAGTTCG CCTGTTGACAATTAATCATCGGCA

D. Fat-Delta aa4975-4993-Rgalk:GAGGAGACTCTTGCTGGTGGAATACTTGCCGTCGCCACTGAGACTGTTATT TCAGCACTGTCCTGCTCCTT

D. ftSTm_checkF: FatDel4975-4993R: GCCGTCGCCACTGAGACTGTTATTCGAACTATGGGCATCCACATCTCCA

D. FatDel4975-4993F: GAGATGTGGATGCCCATAGTTCGAATAACAGTCTCAGTGGCGACGGCA

D. ftSTm_checkR

E. Fat-Delta aa5089-5114-Fgalk:TCTACCGCAAGGCCAATGGAGCCGCATCCCCATCGGCCACCACCCTCGGC CCTGTTGACAATTAATCATCGGCA

E. Fat-Delta aa5089-5114-Rgalk:GTGGACACCACTTGGGTTTGCTGCTGCTGTTGCTGCGACGGTCCATTTGT TCAGCACTGTCCTGCTCCTT

E. Fat-DeltaCDEseq-F: GCAAGGACGTTCACAGCAACAGCG

E. FatDel5089-5114R: GTTGCTGCGACGGTCCATTTGTGCCGAGGGTGGTGGCCGATGGGGA

E. FatDel5089-5114F: CATCGGCCACCACCCTCGGCACAAATGGACCGTCGCAGCAACA

E. FLAG_R:TCAACCCTTGTCGTCGTCGTCCTTGTAGTC

F. Fat_Cterm_galkF: GTG GTG TCC ACG CTA CGA ATG CCA TCA TCG AAT GGA CCG GCG GCT CCA GAG GAG TAC GTG CCT GTT GAC AAT TAA TCA TCG GCA

F. Fat_Cterm_galkR: TGG GGC TCA GAC TTT AGG AAC ACT TTA ACT TTC GTT GAA GAG CAT ACA CAA CAT ATA TTA TCA GCA CTG TCC TGC TCC TT

F. Fat-HomoDomainF-F:GACTACAAGGACGACGACGACAAGGGTTGA

F. Fat_C_chckR:TCCGACATATGCACGATTCTACAC

EGF. Fat-EGF1flank-Fgalk:GAGAGAAGCGTTCAGCGTTTTCGGAACTCCTGCAAAAGGAGGTGATTGTG

CCTGTTGACAATTAATCATCGGCA

EGF. Fat-EGF4flank-Rgalk:GAGCGGGAAAAGTCATGTAGGACAGCGGTTGGAATCCATAGCTAAAACG

TCAGCACTGTCCTGCTCCTT

EGF. Fat33759seq_F: AGAATCCCGCCCAGAGTCAA

EGF. FatDelEGF1-4R: GGAATCCATAGCTAAAACGCACAATCACCTCCTTTTGCA

EGF. FatDelEGF1-4F: GCAAAAGGAGGTGATTGTG CGTTTTAGCTATGGATTCCAA

EGF. Fat34583seq_R: GGTCCCACTTCGTCCGCATA

Notes: Deletions were created by first replacing the relevant region with *galK*. The forward fragment and reverse fragments were then hybridized and extended to generate a DNA fragment without the relevant region but both 5' and 3' homolog arms for recombineering. Primers listed were: (A) for deletion of region A (RPDIIERE, aa4704-4711, cgacccgatatcatagagcgcgag), creating plasmid *attB-P[acman]-V5ftΔA-Flag*; (B) for deletion of region B (EHYDLENASSIAPSDIDIVYHYKGYR, aa4745-4770, gaacactacgacctcgagaacgccagctccattgctccgtccgacattgatatagtctatcattacaagggctatcgt), creating plasmid *attB-P[acman]-V5ftΔB-Flag*; (C) for deletion of region C (MGLTAEEIERLNGRPRTCSLISTLDAVSSSSEAPRVSSS, aa4921-4959, atgggcttgaccgccgaggagattgagagattgaatggcagaccacgaacttgtagcctaatctccaccctggatgccgtctcctccagcagtgaggcgcctcgagtgtcgagcagc), creating plasmid *attB-P[acman]-V5ftΔC-Flag*; (D) for deletion of region D (TSTDESGNDSFTCSEIEYD, aa4975-4993, acttccacggacgaaagcggcaacgacagcttcacgtgctcggagatcgagtacgac), creating plasmid *attB-P[acman]-V5ftΔD-Flag*; (E) for deletion of region E (WEYLLNWGPSYENLMGVFKDIAELPD, aa5089-5114, tgggagtatctgctcaattggggacctagctacgaaaatctgatgggcgtcttcaaggacattgccgagctgccggac), creating plasmid *attB-P[acman]-V5ftΔE-Flag*; (F) For deletion of region F (EEYV, aa5144-5147, gaggagtacgtg), creating plasmid *attB-P[acman]-V5ftΔF-Flag*; (EGF) for deletion of region EGF (GYEPCSEPDVCENGGVCSATMRLLDAHSFVIQDSPALVLSGPRVVHDYSCQCTSGFSGEQCSRRQDPCLPNPCHSQVQCRR LGSDFQCMCPANRDGKHCEKERSDVCYSKPCRNGGSCQRSPDGSSYFCLCRPGFRGNQCESVSDSCRPNPCLHGGLCVSLKP GYKCNCTPGRYGRHCE, aa3950-4128, ggctatgaaccctgcagtgaaccggatgtttgtgaaaatggcggagtctgcagtgccaccatgcgactgctggatgcccatagctttgttatccaagacagtccggccttggtgctgagtggtcctcg ggttgtgcacgactacagctgccagtgcaccagtggattctcgggcgagcagtgcagtcgtcggcaggatccttgcctgcccaatccttgccattcgcaggtccaatgccgtcgcctgggtagcgat ttccagtgcatgtgtcctgccaatcgggatggcaagcactgcgagaaggaacgcagtgacgtgtgctatagcaagccgtgtcgcaatggaggaagttgccaacgcagtccggacggatcctccta

cttttgcctatgtcgtcccggattccgtggcaatcagtgcgagagcgtgtcggactcatgccgacccaatccctgtctgcacggtggcttgtgtgttagtctcaagccaggatacaaatgcaactgcac gccgggacgatatggacgacattgcgag), creating plasmid *attB-P[acman]-V5ftΔEGF-Flag*.

Primers to generate *fat* genomic rescue constructs with deletions:

fatmutrecgalKI-F: AAGGTGGCCAACCGCCACCGCCGCCCACCAGTGCATCCCGCACCCATCAG

CCTGTTGACAATTAATCATCGGCA

fatmutrecgalKVI-R:

CCATTGGCCTTGCGGTAGATACCGCTCAGGTGATTGGCTATATCATCGTC TCAGCACTGTCCTGCTCCTT

I-F: GCA TCC CGC ACC CAT CAG GCC ACT CCA CTG GCC CGA CTC

IV-F: GGC AGA CCA CGA ACT TGT GCC CTA ATC GCC ACC CTG GAT GCC GTC GCC

IV-R: GAG GCG CCT CGA GTG GCG GCC GCC GCT CTG CAT ATG GCG CTG GGT GGA GAT GTG

V1-F: GCT GCC GCG GAC GAA GCC GGC AAC GAC AGC TTC

V1-R: GAG TAC GAC AAT AAC GCT CTC GCT GGC GAC GGC AAG TAT

V-R: GAA GCC GGC AAC GAC GCC TTC ACG TGC GCG GAG ATC GAG TAC GAC

fatmutrecI-F: AAGGTGGCCAACCGCCACCGCCGCCCACCAGTGCATCCCGCACCCATCAG

fatmutrecVI-R: CCATTGGCCTTGCGGTAGATACCGCTCAGGTGATTGGCTATATCATCGTC

Notes: First, the sequence between Fat-mI and Fat-mV was replaced by *galK* using fatmutrecgalKI-F and fatmutrecgalKVI-R to generate plasmid *attB-P[acman]-V5ft-mut-galK-Flag*. Then, nucleotide substitutions were introduced into pUAST-fat-STI plasmid, using I, IV, or V primers to generate pUAST-fat-mutI, pUAST-fat-mutIV and pUAST-fat-mutV. The mutated fragments were PCR amplified from pUAST-fat-mutI, pUAST-fat-mutIV and pUAST-fat-mutV and introduced into *attB-P[acman]-V5ft-mut-galK-Flag* to generate plasmids *attB-P[acman]-V5ft-mutI-Flag*, *attB-P[acman]-V5ft-mutIV-Flag*, *attB-P[acman]-V5ft-mutV-Flag.*

Primers to introduce the ΔD and mV mutations into pUAST_ft_STI4FVH construct:

Fat-STI:4- ΔD:FLAG (pUAS_ft_STI4_ ΔD _FVH)

Fat-STI:4- mutV:FLAG (pUAS_ft_STI4_mutV_FVH)

STI4FVH_ deleteD_F: GATGTGGATGCCCATAGTTCGAATAACAGTCTCAGTGGCGAC

STI4FVH_ deleteD_R: GTCGCCACTGAGACTGTTATTCGAACTATGGGCATCCACATC

Notes: The ΔD or mV mutation was amplified by PCR from corresponding genomic constructs. Then these PCR fragments were introduced into the pUAST_ft_STI4FVH plasmid.

Table S3. Abbreviations for Fat transgenes

Abbreviated name	Full name
Fat⁺	*P[acman]V5:fat[68A4]*
FatΔA	*P[acman]V5:fatΔA[68A4]*
FatΔB	*P[acman]V5:fatΔB[68A4]*
FatΔC	*P[acman]V5:fatΔC[68A4]*
FatΔD	*P[acman]V5:fatΔD[68A4]*
FatΔE	*P[acman]V5:fatΔE[68A4]*
FatΔF	*P[acman]V5:fatΔF[68A4]*
FatΔP32	*P[acman]V5:fat-P32[68A4]*
FatΔmI	*P[acman]V5:fat-mI[68A4]*
FatΔmIV	*P[acman]V5:fat-mIV[68A4]*
FatΔmV	*P[acman]V5:fat-mV[68A4]*
Fat:Fat4	*P[acman]V5:fat:FAT4[68A4]*
FatΔEGF	*P[acman]V5:fatΔEGF[68A4]*