Supplementary Information

Supplemental Materials and Methods

Fly Stocks

$ebd1^{240}$ and $Df(3L)5$ were isolated by imprecise excision of the P element $EY01876$ (Bellen et al, 2004). $ebd1^{240}$ has a 2925 base pair (bp) deletion (+308 to +3232 with reference to the $ebd1$ transcriptional start site) and retains approximately 3 kb of $EY01876$. $Df(3L)5$ has a 2821 bp deletion (-1922 to +899 with reference to the $ebd1$ transcriptional start site). $CG13895^{190}$, isolated by imprecise excision of the P element $KG05495$ (Bellen et al, 2004), has an 8187 bp deletion (+228 to +8414 with reference to the $CG13895$ transcriptional start site) and retains 5 kb of the P element. $ebd2^{136}$ was isolated by excising the P element $EY11662$ (Bellen et al, 2004) and has a 1077 bp deletion in $CG12972$ (+560 to +1636 with reference to the start of translation). Four hundred and seventy seven base pairs of the P element remain in $ebd2^{136}$. $CG12346^{14A}$ was isolated by imprecise excision of the P element $KG06212$ and has a 1070 bp deletion (+217 to 1286 with reference to the $CG12346$ transcriptional start site), which deletes the entire $CG12346$ coding region. 30 bp of the P element remain in $CG12346^{14A}$.

$Df(3L)4125$, $Df(3L)4157$, $Df(3L)4136$ and $Df(3L)9698$ were isolated by flipase-mediated recombination of the FRT-containing P elements 5-SZ-3592 and $CB-5432-3$, 5-HA-1830 and $CB-5430-3$, 5-HA-1830 and $CB-0798-3$, and 5-HA-1830 and $CB-6672-3$, respectively.
(all P elements and nomenclature for deficiencies from DrosDel; Ryder et al, 2004). 
Df(3L)4238 and Df(3L)4196 were obtained from DrosDel (Ryder et al, 2007).


To analyze the phenotype of flies in which all five CENPB domain proteins are eliminated simultaneously, we generated a quintuple mutant of this genotype: CG1234614A /CG1234614A; Df(3L)4136 ebd2136 / Df(3L)9698 ebd2136. The Df(3L)4136 and Df(3L)9698 deletions eliminate three clustered genes encoding CENPB proteins: ebd1, CG13895, and CG13894.

**Plasmids**

ebd1 cDNA (GH13810) was obtained from the Drosophila Genomics Resource Center (DGRC, Indiana University). Ninety two bp of coding sequence are missing from the 3′
end of the DGRC plasmid. To generate a full-length *ebd1* cDNA, we isolated an XhoI-XbaI fragment that includes this missing sequence from genomic DNA and inserted this fragment into *GH13810*, at the XhoI site. The resulting DNA was inserted into the EcoRI and XbaI sites of *pUAST* (Brand & Perrimon, 1993). PCR amplification was used to add epitope tags to Ebd1, creating *pUAST-ebd1-HA* and *pUAST-FLAG-ebd1*. The *QF1* mutation was introduced into *pUAST-ebd1-HA* by PCR-based mutagenesis, to generate *pUAST-ebd1^{QF1}-HA*. For mammalian expression, *FLAG-ebd1* cDNA was cloned into the EcoRI and EcoRV sites of *pFLAG-CMV-5a* (Sigma). To make the *ebd1-Gal4* driver, genomic DNA (2161 bp upstream to 2200 bp downstream of the *ebd1* coding sequence (CDS)) was amplified by PCR and cloned into the NotI and XbaI sites of *pW8* (Klemenz et al, 1987). The *ebd1* CDS was then replaced by the *Gal4* CDS.

cDNAs for human *jerky* (IMAGE clone 6156875), *TIGD1* (IMAGE clone 5248661), *TIGD2* (IMAGE clone 4006771), *TIGD4* (IMAGE clone 5273281) and *CENP-B* (IMAGE clone 6470289) were obtained from Open Biosystems. *TIGD4* cDNA was amino-terminally FLAG-tagged and cloned into the EcoRI and PstI sites of *pFLAG-CMV-5a*. *jerky* cDNA was amino-terminally FLAG-tagged or V5-tagged, and cloned into the EcoRI and BglII sites of *pFLAG-CMV-5a* and *pUAST*, and into the NheI and XbaI sites of *pcDNA3.1(+)*, respectively. Silent mutations to generate a cDNA refractory to siRNA knockdown were introduced into *pFLAG-CMV-5a-FLAG-Jerky* by PCR-based mutagenesis. The following mutations (in bold) were introduced: GAG GTT GTG TGG AGT TCA GAA changed to GAA GTA GTT TGG TCT AGC GAG.
The first 33 amino acids of mouse Lef1 were removed from \textit{pCMV5c-Flag-Lef1} (Miller et al, 2009) by PCR-based mutagenesis, to generate \textit{pCMV5c-Flag-Lef1-ΔN}. \textit{pCMV5c-Flag-Lef1-ΔN/ΔC341} and \textit{pCMV5c-Flag-Lef1-ΔN/ΔC303} were generated using the Smal and XmnI-Smal sites in \textit{pCMV5c-Flag-Lef1-ΔN}, respectively. \textit{pCMV5c-Flag-Lef1-Δ35-304} was generated using the XmnI sites in \textit{pCMV5c-Flag-Lef1}. Lef1 was amino-terminally V5-tagged and cloned into NheI and XbaI sites of \textit{pcDNA3.1(+)}.

\textit{arm} cDNA (Zecca et al, 1996), with an HA tag inserted at the NheI site (amino acid 784), was cloned into the NotI and EcoRV sites of \textit{pFLAG-CMV-5a}. Human \textit{pygo2} cDNA (Kramps et al, 2002) was amino-terminally HA tagged and was cloned into the HindIII and XbaI sites of \textit{pCMV5c}. Human Bcl9 obtained from Open Biosystems (IMAGE clone 7961941), was cloned into the NotI and HindIII sites of \textit{pcDNA3.1(-)} (Invitrogen). \textit{pCMV5b-myc-β-catenin} (Labbe et al, 2000), \textit{pcDNA3.1-HA-Pygo} (Townsley et al, 2004), \textit{pcDNA3.1-V5-Legless} (Townsley et al, 2004), \textit{pcDNA3-Wnt3A-V5} (Banziger et al, 2006), \textit{pCMV-Myc-dTCF} (Zeng et al, 2008), \textit{pMBP-Bem1-His} (Xu & Wickner, 2006), \textit{pGST-HMG-C-clamp} (Chang et al, 2008), \textit{pSuperFOPFlash} (Veeman et al, 2003), \textit{pTOPFLASH}, \textit{pFOPFLASH} (Korinek et al, 1997), \textit{pGST-Jerky} (Liu et al, 2002), \textit{pGST-Jerky 1-168, 1-118, 119-168, 49-557 and 119-557} (Liu et al, 2003), \textit{pCMV5c-FLAG-Lef1} and \textit{pCMV5c-β-catenin-FFLuc} (Miller et al, 2009) have been described. \textit{pSuperTOPFlash} containing 14 TCF binding sites was provided by R. Moon.

For bacterial expression, \textit{ebd1} cDNA was cloned into the EcoRI and XbaI sites of \textit{pGST-Parallel1} (Sheffield et al, 1999), or into the EcoRI and XbaI sites of \textit{pProEx-Hta}.
(Invitrogen), with or without an amino-terminal V5 tag, to produce His-Ebd1 and His-V5-Ebd1. Untagged *arm* and HA-tagged *Pygo* cDNAs were cloned into the BamHI and XhoI sites of a modified *pGEX-4T-1* vector.

**Immunohistochemistry and histology**

Primary antibodies used for immunostaining were rabbit anti-β-Gal 1:5000 (Cappel) and mouse anti-β-Gal (40-1a) 1:10 (Developmental Studies Hybridoma Bank, DSHB), guinea pig anti-Hth 1:500 (Abu-Shaar et al, 1999), mouse (9F8A9) or rat (7E8A10) anti-Elav 1:20 (DSHB), rat anti-HA (3F10) 1:500 (Roche), rabbit anti-Lamin 1:5000 (Smith & Fisher, 1984), rabbit anti-Mef2 1:800 (Bour et al, 1995), anti-Repo 1:20 (Alfonso & Jones, 2002), guinea pig anti-Ebd1 1:4000, and mouse anti-Eve 1:20 (DSHB).

Secondary antibodies used for immunostaining were goat or donkey Alexa Fluor 488 or 568 conjugates 1:200 to 1:400 (Molecular Probes), and goat or donkey Cy3 or Cy5 conjugates 1:200 (Jackson Immunochemicals). Fluorescent images were obtained on a Leica TCS SP UV confocal microscope, except images of polytene chromosomes obtained on a Zeiss Axioskop 2.

Pupal retinas, pupal flight muscles, and embryos were fixed and stained as described previously (Fernandes et al, 2005; Rothwell & Sullivan, 2000; Takacs et al, 2008). Adult eyes and thoraces were fixed as described previously (Cagan & Ready, 1989; Fernandes et al, 2005; Wolff, 2000), embedded in plastic resin (Durcupan, Fluka), sectioned to 1 μm and 5 μm, respectively, and stained with toluidine blue. For flight muscle studies, males
and females were analyzed separately because the severity of the phenotypes varied between the two sexes.

**Luciferase assay**

HEK293T cells were seeded at 20% confluence in 24-well plates and each well was transfected with 0.1 µg and 0.001 µg of the luciferase and *Renilla* reporters, respectively, 0.01 µg of the Wnt3A expression plasmid, and 0.05 µg of the other cDNA-encoding plasmids.

DLD-1 cells were seeded at 20% confluence in 24-well plates and each well was transfected with 0.1 µg and 0.01 µg of the luciferase and *Renilla* reporters, respectively, and 0.05 to 0.2 µg of the *jerky* cDNA-encoding plasmids. 1 nM of Jerky siRNA (GGUUGUGUGAGUUCAGAdTdT) or control siRNA (Control Non-Targeting siRNA#1, Dhharmacon) was used.

**Immunoprecipitation and immunoblotting,**

Extracts were immunoprecipitated with anti-FLAG M2 antibody (Sigma), anti-HA 3F10 antibody (Roche), anti-V5 antibody (Invitrogen), anti-mouse Jerky antiserum (Liu et al, 2002), normal mouse IgG (Santa Cruz Biotechnology) and anti CENP-B H-65 (Santa Cruz Biotechnology).

Immunoblotting was performed with anti-FLAG, anti-HA, anti-V5, anti-β-catenin (BD transduction laboratories), anti-c-Myc 9E10 (Santa Cruz Biotechnology), anti-TCF4 6H5-
3 (Millipore), anti-histone H3 (Millipore), anti-Ets-1 C-20 (Santa Cruz Biotechnology), anti-α-tubulin (Sigma), anti-BCL9 (Novus Biologicals), anti-His (Qiagen), anti-GST Z5 (Santa Cruz Biotechnology), anti-mouse Jerky and anti-CENP-B antibodies.

**GST interaction assays**

For GST interaction assays, GST-Ebd1, GST-HMG-C-clamp fusion, and GST-Jerky proteins were purified using Glutathione Sepharose 4 Fast Flow beads (GE Healthcare) and incubated with cell lysates from transfected HEK293T cells. Associated proteins were detected by immunoblotting with anti-HA or anti-FLAG antibody.

**Reverse transcription**

Total RNA from flies or fly heads was extracted using Trizol reagent (Invitrogen), DNAse treated and purified using RNeasy columns (Qiagen). The mRNA was reverse transcribed with M-MLV reverse transcriptase (Invitrogen), and cDNA levels were analyzed by QPCR. The following primers were used to detect cDNA levels: GAAGCTGACGGAGATTATCAAC and TCTTGCCCAGTAGCTGTTTGC for *GPDH*, ATGAAGCTACTGTCTTCTATCGA and CAAAATCATGTCAAGGTCTTCTC for *Gal4*, AAAATATCATCTGAGAUCTTATTAGTT and CTTGGAGAACCATTGCCATACG for *dTCF*, TTACATGCCAGCCCAGAATCG and GAGGGTCTGCTCCTTGGCG for *Arm*, CGGACCGGCCGCGCATGAAC and ACACTTGAACTTGACCATGGGAA for *Pygo*.
Supplemental References


**Supplemental Figures**

**Supplemental Figure 1** Identification of ebd1 as a suppressor of apoptosis induced by Apc1 loss.

(A) Schematic representation of deficiencies at cytological position 61C used to map ebd1. Deficiencies that suppress apoptosis (+) or do not suppresses apoptosis (-) in the Apc1Q8 mutant are indicated on right.

(B) The location of ebd1 was narrowed down to a 57 kb region deleted in the Df(3L)4136 deficiency. Weak suppression of apoptosis in the Apc1Q8 mutant is seen in flies heterozygous for Df(3L)4136.

(C) To identify which gene deleted in Df(3L)4136 is responsible for the suppression, P elements within the deleted region were examined for their ability to suppress the apoptosis phenotype. One P element, EY01876, inserted within CG3371, dominantly suppressed apoptosis, suggesting that the QF1 mutation may be in the CG3371 gene.

(D) A more complete suppression of apoptosis is observed in Apc1 flies transheterozygous for Df(3L)4136 and ebd1EY01876.

(E) Genomic organization of CG3371/ebd1 and surrounding genes (adapted from Flybase). The P element EY01876 (insertion site indicated by arrowhead) was
imprecisely excised to generate the deletions \(Df(3L)5\) and \(Df(3L)240\). \(Df(3L)240\) retains a fragment of \(EY01876\).

(F) Suppression of apoptosis is observed in flies transheterozygous for \(Df(3L)5\) and \(Df(3L)240\). \(ebd1\) is the only gene completely disrupted in both \(Df(3L)5\) and \(Df(3L)240\).

Supplemental Figure 2  Inactivation of \(ebd1\) suppresses \(Apc1\) and \(Apc2\) mutant phenotypes.

(A-G, L, M) Confocal images of pupal retinas stained with antibodies against Homothorax (Hth; green) and Elav (blue; marking all photoreceptors). Retinas in B-G contain a \(svp-lacZ\) insertion (\(\beta\)-gal; magenta) allowing demarcation of the dorsal-ventral equator (indicated by a solid white line in C, E, and G). In all panels, dorsal is oriented to the top.

(A) The dorsal margin of the retina, extending up to the dorsal-ventral equator, contains dorsal rim area (DRA) ommatidia, which function as polarized light sensors. DRA ommatidia are normally restricted to one or two rows at the dorsal edge of the retina and are characterized by \(homothorax\) (\(hth\)) expression in R7 and R8 (Tomlinson, 2003; Wernet et al, 2003). Wingless signaling is important for DRA specification (Tomlinson, 2003; Wernet et al, 2003).

(B, C) In \(Apc1\) mutant flies, \(hth\) expression expands throughout the dorsal half of the retina, either up to the equator, or one row of ommatidia above the equator (Benchabane et al, 2008).

(D-G) Inactivation of \(ebd1\) partially prevents this ectopic Hth expression. Hth expression is no longer seen in both R7 and R8 and is not present in ommatidia close to the equator.
in retinas transheterozygous for ebd1QF1 and Df(3L)4136 (D-E) or in retinas transheterozygous for Df(3L)4136 and Df(3L)27-3 (F-G). No suppression of hth expression is seen in flies heterozygous for Df(3L)4136 (data not shown), indicating suppression of the Apc1 phenotype is dependent on homozygous inactivation of ebd1.

(H-K) In wild-type, retinal photoreceptors extend though the apical to basal length of ommatidia. However, in Apc1 mutants, inhibition of apoptosis by expression of the caspase inhibitor p35 (H, I) or by elimination of the cell death effectors reaper, grim and hid, results in the presence of shortened photoreceptors, resulting from ectopic Wingless signaling (Ahmed et al, 1998; Benchabane et al, 2008). The surviving Apc1 mutant photoreceptors are seen at the apical surface of the retina (H) but do not extend to its base (I). In contrast, inactivation of ebd1 results in photoreceptors of wild-type length, extending from the base of the retina to its apical surface (J, K).

(L-N) Apc2 also prevents ectopic DRA specification and hth expression (Benchabane et al, 2008). Inactivation of Apc2 leads to an aberrant increase in the number of second row ommatidia expressing hth and adopting a DRA fate (L, N; arrowheads). Inactivation of both copies of ebd1 in the Apc2 mutant reduces the number of second row ommatidia to less than wild-type numbers (M, N; arrowheads). (N) Bar graph showing the number of second row ommatidia with DRA fate in WT, Apc223 mutant and ebd1240 Apc233 /Df(3L)4136 Apc233 mutant flies.

**Supplemental Figure 3** Ebd1 is expressed in glial cells and nephrocytes.

(A-D) Confocal images of a late embryo showing Ebd1 expression in glial cells. The embryo is stained for Ebd1 (green; B, D). Glial cells are marked with anti-repo (magenta;
A, C, D). Higher magnification of the embryo is shown in B-D. A merged image is shown in D.

(E-H) Confocal images of a late embryo in which β-galactosidase is expressed under control of the duf enhancer (duf-lacZ). Ebd1 expression is shown in green (F, H). duf-lacZ expression marks muscle founder cells and garland cells (nephrocytes) (Ruiz-Gomez et al, 2000) (magenta; E, G, H). Higher magnification of the embryo is shown in F-H. Arrow points to garland cells (G). A merged image is shown in H.

**Supplemental Figure 4** Ebd1 is expressed in wing disc-associated myoblasts, neurons and glial cells.

(A-C) Confocal images of a larval third instar wing imaginal disc stained for Ebd1 (green; A, C). Myoblasts are marked with anti-Mef2 (magenta; B, C). A merged image is shown in C.

(D-F) Confocal images of a larval third instar eye imaginal disc stained for Ebd1 (green; D, F). Photoreceptor neurons are marked with anti-Elav (magenta; E, F). A merged image is shown in F.

(G-J) Confocal images of larval third instar brain stained for Ebd1 (green; G, J). Neurons and glial cells are marked with anti-Elav (magenta; H, J) and anti-Repo (blue; I, J), respectively. A merged image is shown in J.

**Supplemental Figure 5** *ebd1 ebd2* double mutant embryos at stage 13-15 display no significant defect in Wingless-dependent specification and migration of RP2 neurons.
Wild type and $ebd1^{240} ebd2^{136}$ mutant embryos stained for Eve. RP2 cells (arrows) differentiated and migrated normally in $ebd1^{240} ebd2^{136}$ double mutants. Anterior is oriented to the top.

**Supplemental Figure 6**  Five CENPB domain-containing proteins are present in Drosophila, some of which function redundantly with Ebd1 in Wingless transduction.

(A) Schematic representation of the Drosophila CENPB domain-containing proteins, indicating the putative CENP-B N and CENPB domains (blue). Three of these CENPB proteins (Ebd1, CG13894 and CG13895) are encoded by genes deleted in $Df(3L)4136$.

(B, C) We tested whether CG13895 and Ebd2 promote Wingless signaling by expressing these genes, along with $lgs^{17E}$, under the control of the salE enhancer. Expression of CG13895 (B) and $ebd2$ (C), like expression of $ebd1$, rescues the attenuation of Wg signaling at the wing margin in $salE>lgs^{17E}$ flies.

(D) Genomic organization of CG13895 (top) and $ebd2/CG12972$ (bottom) (adapted from Flybase). CG13895 was deleted through imprecise excision of the P element KG05495, found within the 5' UTR of the gene (arrowhead). A deficiency, $Df(3L)190$, covering the entire CG13895 gene was obtained. Ebd2 was deleted through imprecise excision of the P element EY11662, located at 3' end of the gene (arrowhead) and a deletion, $Df(3L)136$, covering more than half of $ebd2$ was obtained. Both deficiencies retain a fragment of the P element.

(E) Adult eye section. Partial suppression of apoptosis in the $Apc1$ mutant is observed in flies homozygous for CG13895190. Photoreceptors are seen only in a subset of ommatidia and these ommatidia have less than seven photoreceptors. CG13895 is therefore a weaker
suppressor of the *Apc1* mutant apoptosis than *ebd1*. Correspondingly, we found that expression of *CG13895* under the control of the *GMR* enhancer could rescue the suppression of apoptosis in *Apc1* mutant flies transheterozygous for *Df(3L)ED4136* and *Df(3L)27-3*, but this rescue is weaker than the rescue obtained by expressing *Ebd1* (data not shown).

(F) Apoptosis in *Apc1* mutant flies is weakly suppressed by homozygous inactivation of *ebd2* (arrowheads mark photoreceptor-containing ommatidia).

**Supplemental Figure 7**  Attenuation of Wingless transduction disrupts indirect flight muscle development.

Transverse sections of adult thoraces are shown.

(A) DLMs and DVMs are lost in flies expressing Axin-GFP under the control of the *1151-Gal4* driver (*1151>Axin-GFP*), raised at 25°C (high Axin expression).

(B) This muscle loss phenotype is less severe at 18°C, presumably as a result of decreased Gal4 expression (resulting in lower Axin expression).

(C) Muscles are also lost in flies expressing Axin-GFP under the control of the *ebd1-Gal4* driver, with a concomitant increase in the size of DLMs that remain, similar to that seen upon *ebd1* loss.

(D, E) Anterior (D) and posterior (E) sections from a *wg^{1}/wg^{Il114}* transheterozygous fly displaying indirect flight muscle loss.

(F) Expression of the dominant negative TCF^AB^ protein under control of the muscle specific *1151-Gal4* driver (*1151>dTCF^AB^*) disrupts DLM and DVM development.
**Supplemental Figure 8** Ebd1 and Jerky expression does not affect the activity of the ebd1-GAL4 driver, and disruption of ebd1 and ebd2 does not affect transcription.

(A) Gal4 expression in ebd1-Gal4>GFP-lacZ, ebd1-Gal4>ebd1, or ebd1-Gal4>jerky flies.

(B-E) Glycerol-3-phosphate dehydrogenase (GPDH), dTCF, Arm and Pygo expression in heads of WT or ebd1240 ebd2136 flies. Heads were chosen to enrich for high ebd expression in neurons and to avoid tissue loss due to muscle defects.

(A-E) RNA was reverse transcribed and indicated cDNA was quantified by real-time PCR. Levels of GPDH were used to normalize the results.

**Supplemental Figure 9** Effect of ectopic Jerky expression in DLD1 cells.

Jerky over-expression slightly enhances the SuperTOPFlash, but not the SuperFOPFlash, luciferase reporter in DLD1 cells. DLD1 cells have high levels of endogenous Wnt signaling, which is not increased by ectopic Wnt3a addition.

**Supplemental Figure 10** Not all proteins with CENPB domains interact with components of the Wnt-dependent transcriptional complex.

(A, B) Jerky does not interact with Bcl9, while Pygo2 does. HEK293T cells were transfected with Bcl9, and with FLAG-tagged Jerky (A) or HA-tagged Pygo2 (B). Cell lysates were immunoprecipitated with anti-FLAG (A) or anti-HA (B) antibody and were analyzed by immunoblotting with anti-BCL9 antibody.

(C, D) Ebd1 does not interact with Legless (Lgs), while Pygo does. HEK293T cells were transfected with V5-tagged Lgs, and with FLAG-tagged Ebd1 (C) or HA-tagged Pygo
(D). Cell lysates were immunoprecipitated with anti-FLAG antibody and analyzed by immunoblotting with anti-V5 (C), or were immunoprecipitated with anti-V5 antibody and analyzed by immunoblotting with anti-HA (D).

(E, F) CENP-B and TIGD4 do not interact with β-catenin. HEK293T cells were transfected with CENP-B (E) or Flag-tagged TIGD4 (F), and with Myc-tagged β-catenin. Cell lysates were immunoprecipitated with an anti-CENP-B (E) or anti-Flag (F) antibody and were analyzed by immunoblotting with anti-β-catenin antibody.

(G-J) CENP-B and TIGD4 do not interact with Lef1 and Pygo. HEK293T cells were transfected with CENP-B (G, I) or Flag-tagged TIGD4 (H, J), and V5-tagged Lef1 (G, H) or HA-tagged Pygo2 (I, J). Cell lysates were immunoprecipitated with anti-V5 (G, H) or anti-HA (I, J) antibody and were analyzed by immunoblotting with anti-CENP-B (G, I) or anti-Flag (H, J) antibody.

Immunoblotting of aliquots of total lysates with anti-FLAG, anti-Bcl9, anti-V5, anti-β-catenin, anti-CENP-B or anti-HA antibodies is shown in lower panels.

**Supplemental Figure 11** Ebd1 interacts with Arm, TCF, and Pygo.

(A-C) Lysates from cells transfected with FLAG-Ebd1 and HA-Arm (A), Myc-dTCF (B) or HA-Pygo (C) were immunoprecipitated with α-FLAG and immunoblotted with α-HA (A, C) or α-Myc (B). Immunoblotting of aliquots of total lysates is shown.

(D, E) Bacterially expressed GST-Ebd1 or the GST control were purified and incubated with lysates from HEK293T cells transfected with HA-tagged Arm (D) or HA-tagged Pygo (E). Proteins bound to GST-Ebd1 or the GST control were detected by immunoblotting with anti-HA antibody.
(F) Bacterially expressed GST-HMG-C-clamp, a fragment of TCF containing the HMG and C-clamp domains fused to GST (Chang et al, 2008), was purified and incubated with lysates from HEK293T cells transfected with FLAG-tagged Ebd1. Ebd1 bound to GST-HMG-C-clamp or the GST control was detected by immunoblotting with anti-FLAG antibody. A schematic representation of TCF and the HMG-C-clamp fragment is shown.

(G) Coomassie staining of GST-fusion proteins used in D-F.

Supplemental Figure 12  Interactions between β-catenin, Lef1, and Jerky are mediated by separable binding sites.

(A, B) Jerky interacts with the amino-terminal half of the Lef1 HMG domain.

(A) Schematic representation of the Lef1 deletion mutants used. The ability of these proteins to interact with Jerky is indicated on the right.

(B) HEK293T cells were transfected with FLAG-tagged Lef1 full length (FL) and mutant proteins and V5-tagged Jerky. Cell lysates were subjected to immunoprecipitation with an anti-FLAG antibody and were analyzed by immunoblotting with anti-V5 antibody. Aliquots of total cell lysates were immunoblotted with anti-V5 and anti-Flag antibodies.

(C, D) The β-catenin and Lef1 binding sites in Jerky are partially separable.

(C) Schematic representation of the Jerky deletion mutants used. The ability of these proteins to interact with β-catenin and Lef1 is indicated on the right.

(D) Bacterially expressed GST-Jerky, full-length or deletion mutants, or the GST control were purified and incubated with lysates from HEK293T cells transfected with Myc-tagged β-catenin or Flag-tagged Lef1. Proteins bound to the GST fusion proteins were detected by immunoblotting with anti-β-catenin or anti-Flag antibodies. β-catenin and
Lef1 interact with full length GST-Jerky, but not Jerky fragments lacking the amino-terminus. Deletions of the Jerky carboxy-terminus do not disrupt Lef1 binding, but markedly attenuate β-catenin binding.

Supplemental Figure 13 The QFI mutation in the CENPB domain results in a mis-localization of Ebd1 from nucleus to cytoplasm. (B-I) Confocal images of embryonic ectodermal cells expressing HA-tagged Ebd1 (green; B, D, E) and Ebd1QFI (green; F, H, I) under control of the daughterless (da) enhancer. Nuclear membranes are demarcated by Lamin staining (magenta; C-E, G-I). Merged images (D, E, H, I) and lower magnification (E, I) are shown.
Supplemental Table 1. *ebd1 ebd2* double mutant embryos at stage 13-15 display no significant defect in Wingless-dependent specification and migration of RP2 cells.

<table>
<thead>
<tr>
<th></th>
<th>wild type (n=30)</th>
<th><em>ebd1</em>&lt;sup&gt;240&lt;/sup&gt; <em>ebd2</em>&lt;sup&gt;136&lt;/sup&gt; (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of segments counted</td>
<td>273</td>
<td>135</td>
</tr>
<tr>
<td>Number of segments with RP2 migration defects (%)</td>
<td>17 (6.2)</td>
<td>5 (3.7)</td>
</tr>
<tr>
<td>Number of segments with RP2 differentiation defects (%)</td>
<td>1 (0.4)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Supplemental Table 2. *ebd* mutant flies display DLM defects.

<table>
<thead>
<tr>
<th></th>
<th>CS</th>
<th><em>ebd1</em>&lt;sup&gt;240&lt;/sup&gt;</th>
<th><em>ebd2</em>&lt;sup&gt;136&lt;/sup&gt;</th>
<th><em>ebd1</em>&lt;sup&gt;240&lt;/sup&gt; <em>ebd2</em>&lt;sup&gt;136&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>female (n=12)</td>
<td>male (n=12)</td>
<td>female (n=12)</td>
<td>male (n=12)</td>
</tr>
<tr>
<td>Number of DLM in each semi-thorax</td>
<td>6</td>
<td>6</td>
<td>4.1</td>
<td>5.7</td>
</tr>
<tr>
<td>Percentage of semi-thorax with DLM defects (%)</td>
<td>0</td>
<td>0</td>
<td>92</td>
<td>25</td>
</tr>
</tbody>
</table>
Supplemental Figure 1:

A

61C

klar

εbd1

emc

Df(3L)27-3

Df(3L)4238

Df(3L)4196

Df(3L)4125

Df(3L)4157

Df(3L)4136

Df(3L)9698

B

Df(3L)ED4136 Apc1Q8/Apc1Q8

C

εbd1EY01876 Apc1Q8/Apc1Q8

D

εbd1EY01876 Apc1Q8/Df(3L)ED4136 Apc1Q8

E

CG3386

CG3371(εbd1)

EY01876

CG3344

CG32483

Df(3L)5

Df(3L)240

F

Df(3L)5 Apc1Q8/εbd1240 Apc1Q8
Supplemental Figure 2:

A. Hth 
B. Apc1Q8
C. Hth 
D. ebd1QF1 Apc1Q8 Df(3L)ED4136 Apc1Q8
E. Hth 
F. Apc233 ebd1240 Apc233 Df(3L)ED4136 Apc233

H. Apical
I. Basal
J. GMR-p35 Apc1Q8
K. Df(3L)5 Apc1Q8

L. Hth
M. Apc233 ebd1240 Apc233 Df(3L)ED4136 Apc233

N. Number of second row ommatidia

- 0-8
- 9-12
- 13-16
- 17-20

WT n=37
Apc233 n=51
ebd1240 Apc233 Df(3L)ED4136 Apc233 n=45
Supplemental Figure 4:
Supplemental Figure 5:
Supplemental Figure 6:

A

<table>
<thead>
<tr>
<th>Protein</th>
<th>CENP-B N</th>
<th>CENPB</th>
<th>Length (aa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG3371 (Ebd1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG13895</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG12972 (Ebd2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG12346</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG13894</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


B

salE>lgs^{17E}; UAS-CG13895

C

salE>lgs^{17E}; UAS-ebd2

D

SalE>lgs^{17E}; UAS-CG13895


E

CG13895^{190} Apc^{Q8}

F

ebd^{136} Apc^{Q8}
Supplemental Figure 7:

A. 1151->Axin-GFP, 25°C
B. 1151->Axin-GFP, 18°C
C. ebd1->Axin-GFP

D. wg^I/wg^{L114}
E. wg^I/wg^{L114}
F. 1151->dTFE^{ΔN}
Supplemental Figure 8:

A

![Graph showing relative mRNA levels for GFP-LacZ, Ebd1, and Jerky](image1)

B

![Graph showing relative mRNA levels for GPDH](image2)

C

![Graph showing relative mRNA levels for dTCF](image3)

D

![Graph showing relative mRNA levels for Arm](image4)

E

![Graph showing relative mRNA levels for Pygo](image5)
Supplemental Figure 9:

A) SuperTOP

B) SuperFOP

Relative luciferase units vs. Jerky treatment.
Supplemental Figure 10:

A

Bcl9 + +
Flag-Jerky - +

α-Bcl9
α-Flag IP

Flag-Jerky
Bcl9 -

Total

B

HA-Pygo2 - +
Bcl9 + +

α-Bcl9
α-Flag

Flag-Ebd1
V5-Lgs

α-Flag IP

C

Flag-Ebd1 - +
V5-Lgs - +

α-Flag IP

α-V5

D

V5-Lgs - +
HA-Pygo - +

α-V5 IP

α-Flag
α-V5

E

Myc-β-cat - + +
CENP-B + - -

α-β-cat
α-CENP-B IP

Flag-Jerky
Myc-β-cat -

α-Flag IP

α-V5 IP

Myc-β-cat
CENP-B -

Total

F

Flag-Jerky - + - - +
Flag-TIGD4 + - - - +
Myc-β-cat - - + + +

α-β-cat
α-Flag IP

α-V5 IP

Flag-TIGD4
Flag-Jerky
Myc-β-cat -

Total

G

CENP-B - + +
V5-Lef1 + - +

α-CENP-B
α-V5 IP

α-V5

H

Flag-Jerky - + - - +
Flag-TIGD4 + - - - -
V5-Lef1 - - + + +

α-Flag
α-V5 IP

α-Flag
α-V5

I

CENP-B - + +
HA-Pygo2 + - +

α-CENP-B
α-HA IP

α-V5

Flag-Jerky
Flag-TIGD4

α-Flag IP

α-Flag
α-HA

J

Flag-Jerky - + - - +
Flag-TIGD4 - - + - -
HA-Pygo2 - - + + +

α-Flag
α-HA IP

α-V5 IP

α-Flag
α-HA

Total
Supplemental Figure 11:

A

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>+</th>
<th>α-HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm-HA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flag-Ebd1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>α-Flag IP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arm-HA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flag-Ebd1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>+</th>
<th>α-Myc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myc-dTCF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flag-Ebd1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>α-Flag IP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myc-dTCF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flag-Ebd1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>+</th>
<th>α-HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA-Pygo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flag-Ebd1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>α-Flag IP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA-Pygo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flag-Ebd1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST-Ebd1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arm-HA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>α-HA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pull down</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Totals</td>
<td></td>
</tr>
</tbody>
</table>

E

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST-Ebd1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA-Pygo</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>α-HA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pull down</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Totals</td>
<td></td>
</tr>
</tbody>
</table>

F

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST-HMG-C-clamp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flag-Ebd1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST-HMG-C-clamp</td>
<td>α-Flag</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pull down</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Totals</td>
<td></td>
</tr>
</tbody>
</table>

G

<table>
<thead>
<tr>
<th></th>
<th>+</th>
<th>-</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST-Ebd1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST-HMG c-Clamp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST-Ebd1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST-HMG c-Clamp</td>
<td>GST</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GA 752 aa

HMG-C-clamp

aa 271-408
Supplemental Figure 12:

A

<table>
<thead>
<tr>
<th>Protein</th>
<th>Δ35-304</th>
<th>ΔN</th>
<th>ΔN/ΔC341</th>
<th>ΔN/ΔC303</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flag-Lef1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ35-304</td>
<td>34</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔN</td>
<td>34</td>
<td>34</td>
<td>340</td>
<td></td>
</tr>
<tr>
<td>ΔN/ΔC341</td>
<td>34</td>
<td>34</td>
<td>340</td>
<td>340</td>
</tr>
<tr>
<td>ΔN/ΔC303</td>
<td>34</td>
<td>34</td>
<td>340</td>
<td>340</td>
</tr>
</tbody>
</table>

Interaction:
- +
- +
- +
- +
- -

B

Flag-Lef1: FL - FL Δ35-304 ΔN ΔN ΔN/ΔC341 ΔN/ΔC303

V5-Jerky

Interaction with: β-catenin

<table>
<thead>
<tr>
<th>Protein</th>
<th>β-catenin</th>
<th>Lef1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jerky 1-557</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>1-168</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>1-118</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>119-168</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>49-557</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>119-557</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

D

GST-Jerky

Pull down

α-GST

α-β-catenin

α-Flag

α-Flag IP
Supplemental Figure 13:

**da>ebd1-HA**

**da>ebd1\textsuperscript{QFT}-HA**