

Fig. S1. Denticle placement defects in *fat* and *dachshous* mutants. (A-D) Cuticle preparations of wild-type (WT) (A), *fat*^{NY1} (B), *ds*^{UAO71} (C) and *fat*^{Grv} *ds*^{UAO71} (D) embryos. A majority of denticles point posteriorly in WT, with the exception of columns 1 and 4 (arrows), which point anteriorly. Misoriented denticles are highlighted in red. (E-G) Stage 15 denticle belts stained for F-actin (phalloidin, green) and E-cadherin (red) in WT (E), *fat*^{Grv m/z} mutants that lack maternal and zygotic *fat* activity (F) and *fat*^{NY1} zygotic mutants (G). Ventral views, anterior left. (H) Percentage of misplaced denticle precursors by column in WT, *fat*^{NY1} zygotic mutants, *fat*^{Grv m/z} mutants, *fat*^{Grv} zygotic mutants, *ds*^{UAO71} zygotic mutants and *fat*^{Grv} *ds*^{UAO71} double zygotic mutants. All mutants were significantly different from WT in columns 4-6 ($P < 0.01$). All mutant genotypes except *fat*^{Grv} *ds*^{UAO71} were significantly different from WT in column 3 ($P < 0.05$). Denticle placement defects in embryos lacking maternal and zygotic *fat*^{Grv} activity were not significantly different from *fat*^{Grv} zygotic mutants except in column 1 ($P = 0.008$). A single value was obtained for each column in each embryo (400-640 denticles in four to six embryos/genotype). The mean \pm s.e.m. of these values is shown. Scale bars: 5 μ m.

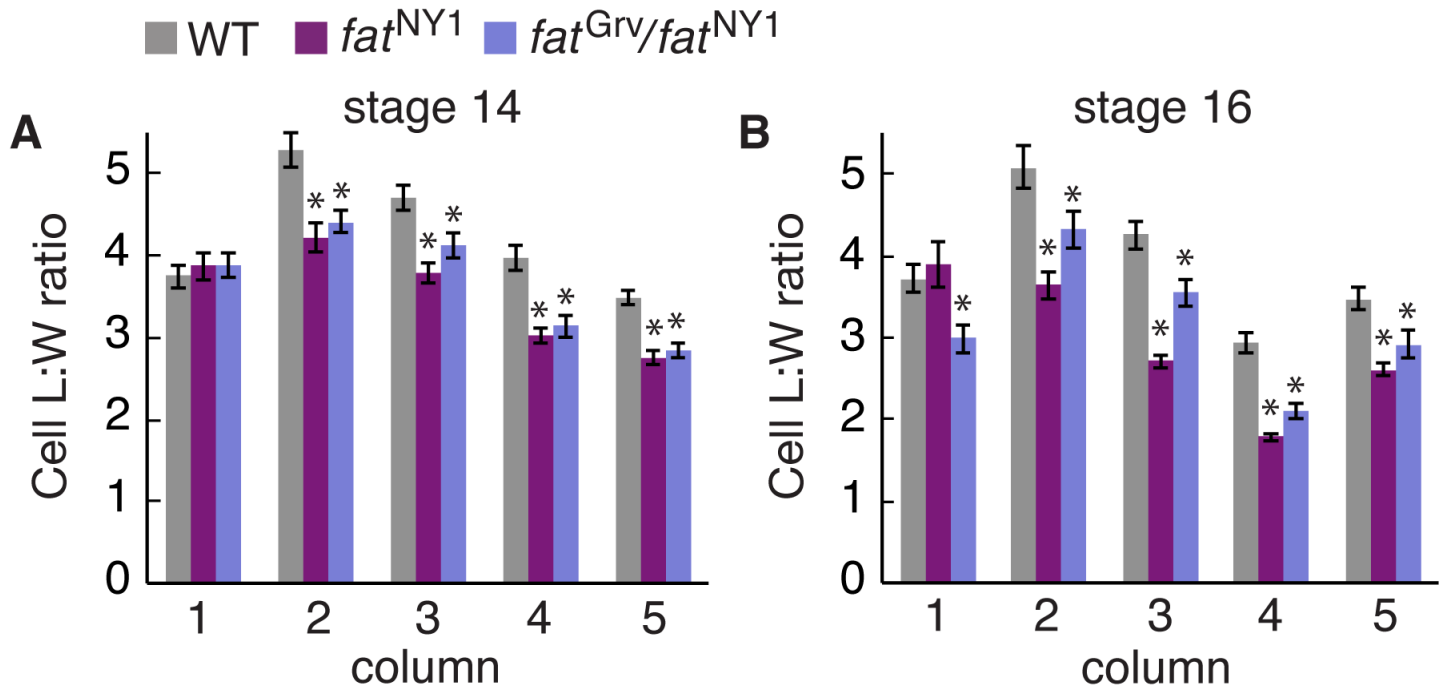


Fig. S2. Cell shape defects in *fat* mutants. Cell length:width (L:W) ratios (the ratio of cell length along the DV axis to cell width along the AP axis) plotted by column in stage 14 wild type (WT), *fat*^{NY1} zygotic mutant and *fat*^{Grv}/*fat*^{NY1} transheterozygous embryos. **(A)** Stage 14 cell L:W ratios were decreased in *fat*^{NY1} in columns 2-5 ($P < 0.001$), and in *fat*^{Grv}/*fat*^{NY1} in columns 2 ($P < 0.001$), 3 ($P = 0.01$), 4 ($P < 0.001$) and 5 ($P < 0.001$). **(B)** Stage 16 cell L:W ratios were decreased in *fat*^{NY1} in columns 2-5 ($P < 0.001$) and in *fat*^{Grv}/*fat*^{NY1} in columns 1 ($P < 0.01$), 2 ($P < 0.05$), 3 ($P < 0.01$), 4 ($P < 0.001$) and 5 ($P = 0.012$). A ratio was measured for each cell (40-75 cells in four embryos/column). The mean \pm s.e.m. of these values is shown. Asterisks indicate statistical significance.

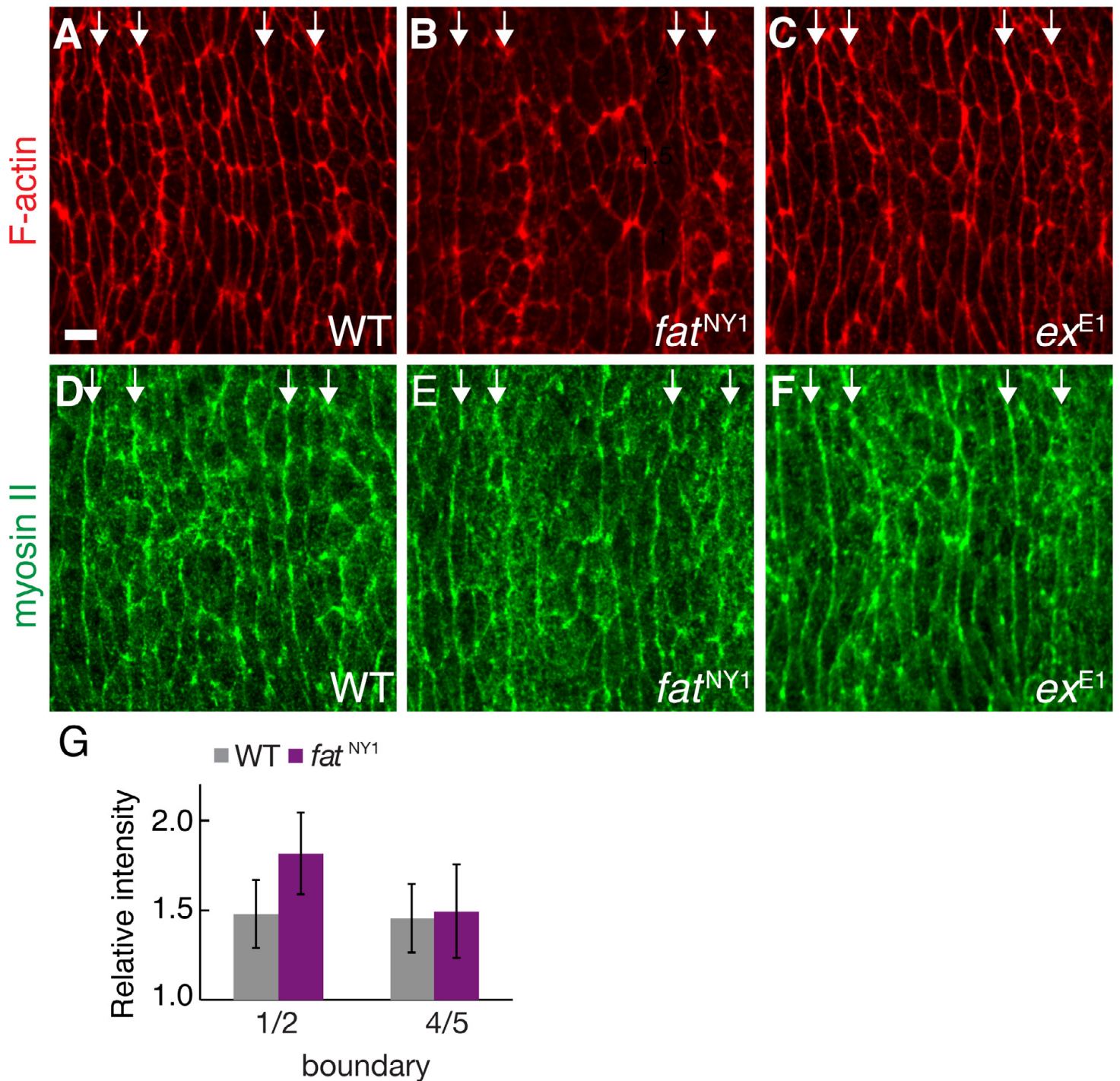


Fig. S3. Myosin and F-actin are correctly enriched at specific column boundaries in *fat* mutants. (A-C) Mid-stage 13 denticle belts in wild type (WT) (A), *fat*^{NY1} (B) and *ex*^{E1} (C) embryos stained with phalloidin to visualize F-actin. (D-F) Mid-stage 13 denticle belts in WT (D), *fat*^{NY1} (E) and *ex*^{E1} (F) embryos expressing a myosin II regulatory light chain GFP fusion (*sqh*:GFP) visualized with antibodies to GFP. Myosin II and F-actin accumulate at the column 1/2 and 4/5 boundaries (arrows) to a similar extent in WT, *fat* and *ex*. Ventral views, anterior left. (G) Enrichment of myosin II at AP edges compared with DV edges at the column 1/2 and 4/5 boundaries in WT and *fat*^{NY1}. In WT, myosin was enriched 1.5 \pm 0.2-fold at the column 1/2 boundary and 1.5 \pm 0.2-fold at the column 4/5 boundary. In *fat*^{NY1}, myosin was enriched 1.8 \pm 0.2-fold at the column 1/2 boundary and 1.5 \pm 0.3-fold at the column 4/5 boundary. A single value was obtained for each embryo by averaging the ratio of the average AP intensity to the average DV intensity for six to seven cells in two denticle belts/embryo (three to four embryos/genotype). The mean \pm s.e.m. of these values is shown. Scale bar: 5 μ m.

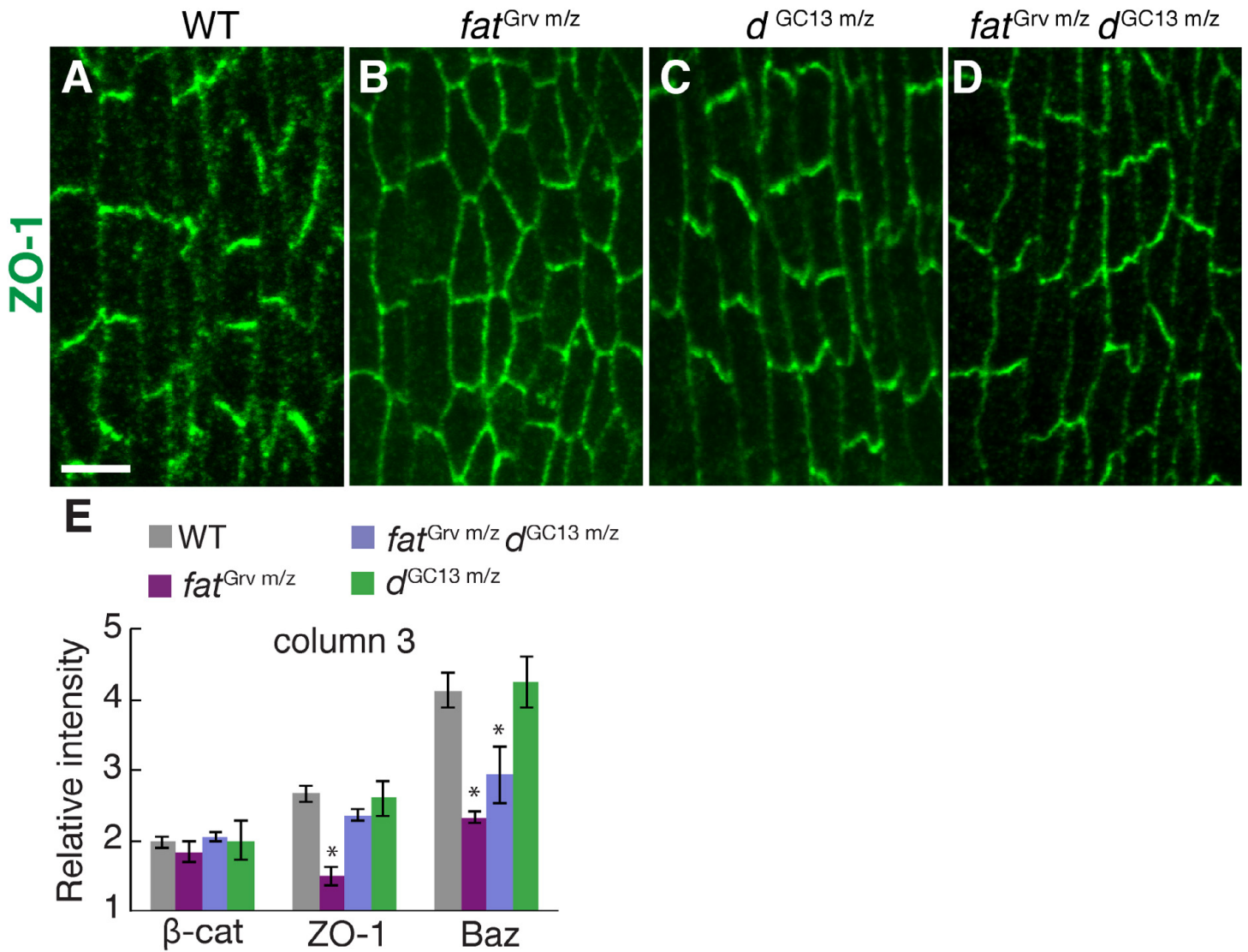


Fig. S4. Loss of *dachs* suppresses a subset of the junctional defects in *fat* mutants. (A-D) Stage 14 denticle belts stained for ZO-1 (green) in wild type (WT) (A) and embryos maternally and zygotically mutant for *fat*^{Grv m/z} (B), *d*^{GC13 m/z} (C) and both *fat*^{Grv m/z} and *d*^{GC13 m/z} (D). Scale bar: 5 μ m. (E) Enrichment of junctional proteins at DV edges in column 3 of stage 14 embryos of the indicated genotypes. The enrichment of ZO-1 and Baz was significantly reduced in *fat*^{Grv m/z} ($P < 0.001$ for ZO-1 and $P < 0.02$ for Baz). *d*^{GC13 m/z} was not significantly different from WT. Embryos doubly mutant for *fat* and *dachs* had WT ZO-1 localization ($P = 0.2$ compared with WT, $P = 0.04$ compared with *fat*^{Grv m/z}). However, Baz enrichment was significantly reduced in *fat dachs* double mutants ($P = 0.02$ compared with WT), indicating that *dachs* does not suppress all aspects of the *fat* mutant phenotype. A single value was calculated for each embryo by measuring the ratio of average DV intensity to average AP intensity for five to seven cells in two denticle belts/embryo; $n = 4$ embryos for each genotype. The mean \pm s.e.m. of these values is shown.

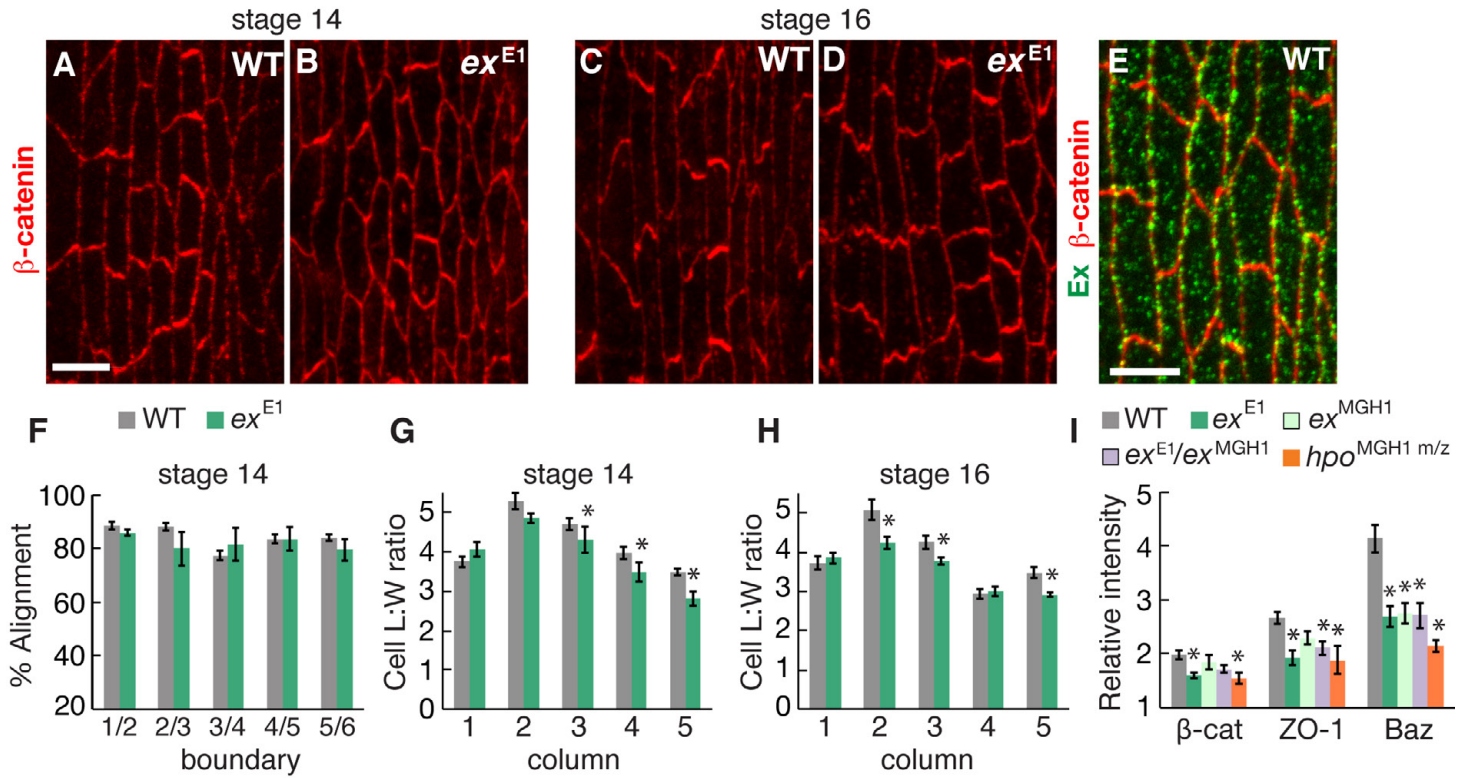


Fig. S5. Cell-shape defects in *expanded* mutants. (A-D) Denticle belts stained for β -catenin in wild type (WT) (A,C) and ex^{E1} (B,D) embryos at stage 14 (A,B) or stage 16 (C,D). Ventral views, anterior left. Scale bar: 5 μ m. (E) WT denticle belt stained for Ex (green) and β -catenin (red) at stage 14. (F) Edge alignment in WT (gray) and ex^{E1} (green) at stage 14. Alignment in ex^{E1} was not significantly different from WT. A single value was obtained for each column boundary in each embryo by averaging all angles measured for that boundary (40 angles in four embryos/boundary). The mean \pm s.e.m. of these values is shown. (G,H) Cell length:width (L:W) ratios (the ratio of cell length along the DV axis to cell width along the AP axis) plotted by column in WT and ex^{E1} at stage 14 (G) and stage 16 (H). Cell L:W ratios were decreased in stage 14 ex^{E1} embryos compared with WT in columns 3 ($P=0.02$), 4 ($P=0.04$) and 5 ($P<0.001$), and in stage 16 ex^{E1} embryos in columns 2 ($P=0.006$), 3 ($P=0.01$) and 5 ($P<0.001$). A ratio was measured for each cell (30-75 cells in four embryos/column). The mean \pm s.e.m. of these values is shown. (I) Enrichment of junctional proteins at DV edges in column 3 of stage 14 WT, ex^{E1} , ex^{MGH1} , ex^{E1}/ex^{MGH1} and $hpo^{MGH1\ m/z}$ embryos. The enrichment of several junctional proteins at DV edges was significantly reduced in ex^{E1} ($P<0.001$ for β -catenin, ZO-1 and Baz), ex^{MGH1} ($P=0.005$ for Baz), ex^{E1}/ex^{MGH1} ($P=0.04$ for ZO-1; $P=0.02$ for Baz) and $hpo^{MGH1\ m/z}$ ($P<0.02$ for β -cat; $P=0.006$ for ZO-1; and $P<0.001$ for Baz). A single value was calculated for each embryo by measuring the ratio of the average DV intensity to the average AP intensity for five to seven cells in two denticle belts/embryo; $n=4$ embryos for each genotype. The mean \pm s.e.m. of these values is shown. Maternal gene products may obscure additional requirements for Ex, as we were unable to obtain embryos that lack maternal *ex* activity.

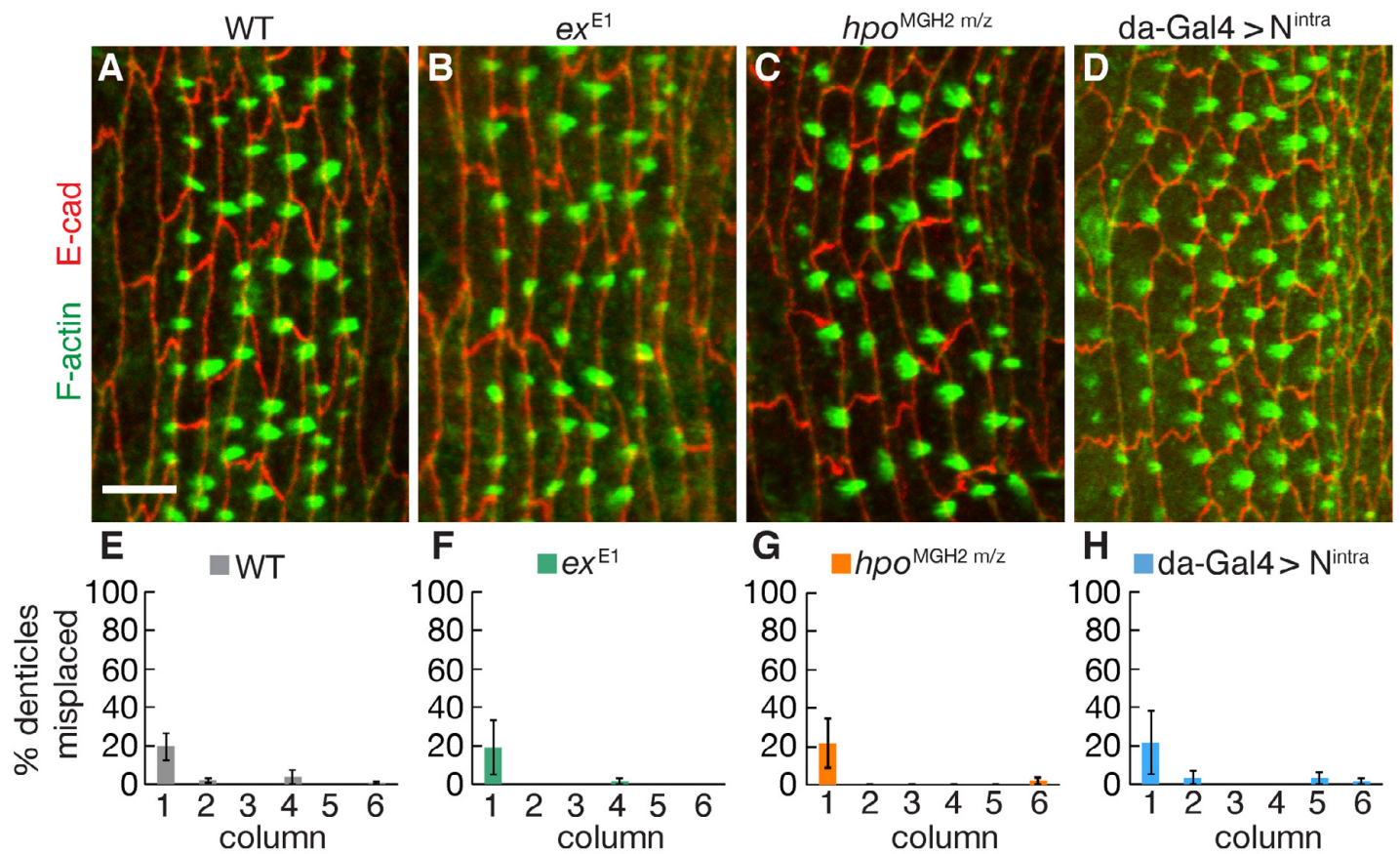


Fig. S6. Denticle placement in wild-type, *ex* mutant and *N^{intra}*-expressing embryos. (A-D) Stage 15 denticle belts stained for F-actin (phalloidin, green) and E-cadherin (red) in wild type (WT) (A), *ex^{E1}* (B), *hpo^{MGH2 m/z}* (C) and *da-Gal4 > N^{intra}* (D) embryos. Scale bar: 5 μ m. (E-H) Percentage of misplaced denticle precursors plotted by column for WT (E), *ex^{E1}* (F), *hpo^{MGH2 m/z}* (G) and *da-Gal4 > N^{intra}* (H) embryos. Denticle precursors were correctly localized to the posterior cell cortex. Ventral views, anterior left. A single value was obtained for each column in each embryo (354-464 denticles in three to five embryos/genotype). The mean \pm s.e.m. of these values is shown.

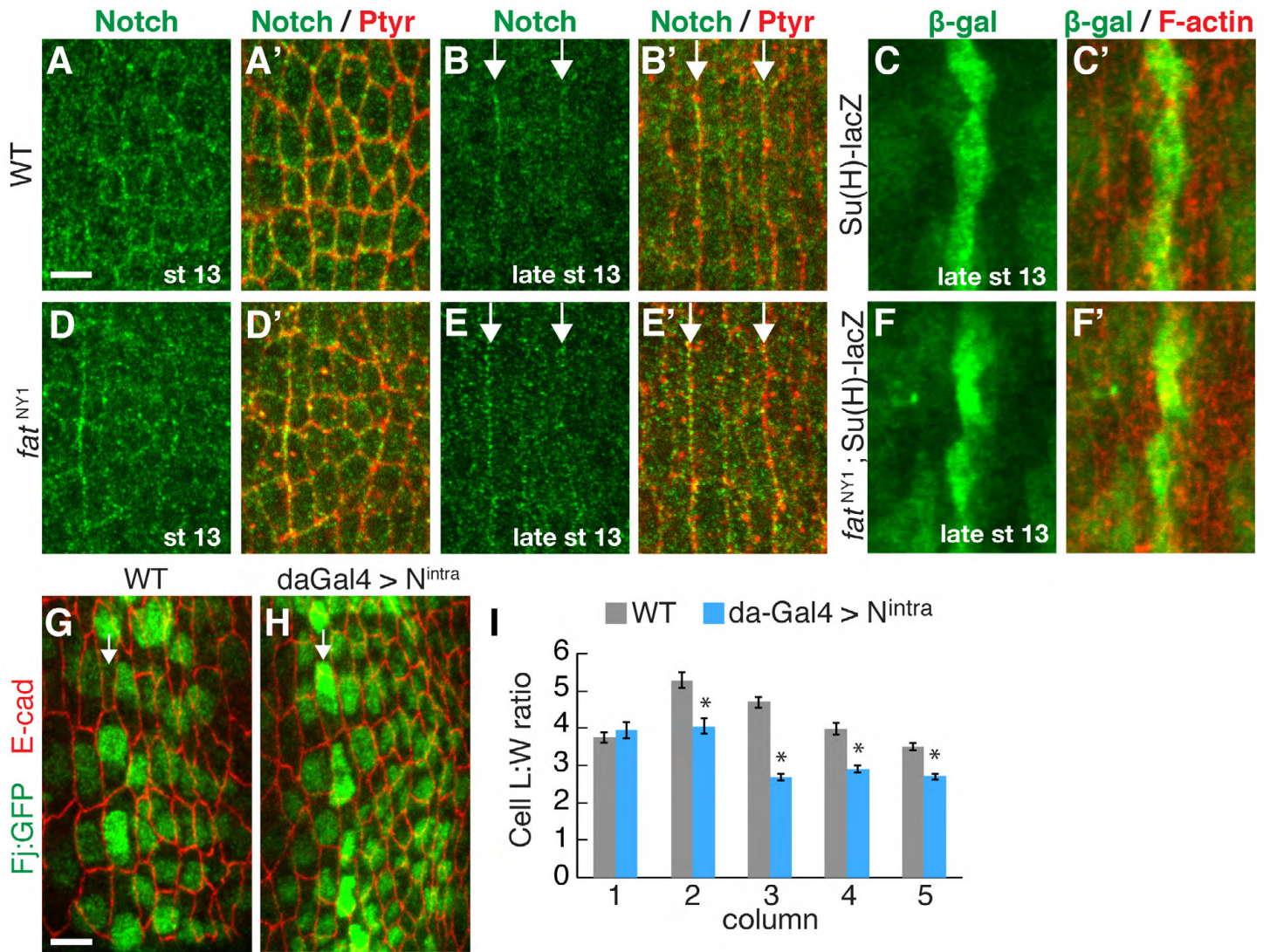


Fig. S7. Notch activation recapitulates the cell-shape defects of *fat* mutants. (A-B', D-E') Denticle belts at the indicated stages stained for Notch (green) and phosphotyrosine (Ptyr, red). (A,D) In early stage 13, Notch localizes to the apical cortex of wild-type (WT) (A) and *fat*^{NY1} (D) embryos. (B) In late stage 13, Notch is enriched at the column 1/2 and 4/5 boundaries (arrows) in WT. (E) This localization occurs normally in *fat*^{NY1}. (C,F) Late stage 13 denticle belts stained for F-actin (phalloidin, red) and β -gal (green) to visualize the expression of the *Su(H)lacZ* Notch activity reporter in WT (C) and *fat*^{NY1} (F). The column 4-specific pattern of *Su(H)lacZ* expression occurs normally in *fat*^{NY1}. Ventral views, anterior left. (G,H) Localization of the endogenous Four-jointed protein using a Four-jointed:GFP (Fj:GFP) trap line visualized with antibodies to GFP. Fj:GFP was expressed throughout the denticle field, with an enrichment in column 2 (arrows). This enrichment of Fj:GFP expression was retained in N^{intra}-expressing embryos, although a broadening of the Fj domain is apparent. (I) Cell length:width (L:W) ratios (the ratio of cell length along the DV axis to cell width along the AP axis) plotted by column in stage 14 WT and da-Gal4>N^{intra} embryos. Cell L:W ratios were decreased in da-Gal4>N^{intra} compared with WT in columns 2-5 ($P < 0.001$). A ratio was measured for each cell (40-100 cells in four embryos/column). The mean \pm s.e.m. of these values is shown. Scale bars: 5 μ m.