

Supplemental Figures

Figure S1 Misexpression of CtBP^{Mono} leads to a significant enhancement in the expression of *Dll-lacZ* reporter levels. (A-C) Scheme of quantification of the *Dll-lacZ* reporter expression in the anterior and posterior regions at the D/V boundary. A region of interest was selected (ovals) close to A/P boundary (arrow) in wing discs with (A) no ectopic CtBP (+) or (B) CtBP^{WT} and (C) CtBP^{Mono} ectopically expressed in the posterior region of the disc using EnGal4. (D) Levels of *Dll-lacZ* were significantly enhanced upon expression of CtBP^{WT} and CtBP^{Mono} in the posterior region of the discs. Each bar represents a mean pixel intensity from the region of interest in the wing imaginal discs (n = 5) (\pm S.E.) (* P<0.0005, Student's t-test).

Figure S2 *nkd* WREs are repressed by *CtBP* in the absence of signaling. (A) Reporter assay showing derepression of WREs *nkd-UpE1* and *nkd-UpE2*, derived from the region upstream of the *nkd* transcription start site. In the absence of signaling knockdown of *CtBP* leads to a much higher derepression of *UpE1* compared to *UpE2* and knockdown of *TCF* leads to derepression of *UpE1* but not *UpE2*. (B) Reporter assay showing that a WRE from the first intron of the *nkd* gene (*nkd-IntE*) is derepressed to a much smaller degree upon knockdown of *CtBP* or *TCF* when compared to *nkd-UpE1*. Each bar represents a mean of luciferase values from cultures transfected in duplicate (\pm S.E.) with the result representative of at least three independent experiments.

Figure S3 CtBP is recruited to the *nkd-UpE1 WRE* in the absence of signaling. CtBP binding to chromatin was assayed by ChIP with an antibody against endogenous CtBP. CtBP is enriched at *UpE1* compared to the coding region (ORF) of the *nkd* gene. Each bar represents a mean of quantitative PCR values in duplicate, from cultures transfected in duplicate (\pm S.E.). The result shown here is representative of two independent experiments.

Figure S4 CtBP^{Acidic} and CtBP^{Basic} efficiently form heterooligomers. (Top panel) When coexpressed, Flagged tagged CtBP^{Basic} can immunoprecipitate HA-tagged CtBP^{Acidic} at comparable levels (lane 4) as similarly tagged versions of CtBP^{WT} (lane 2). (Bottom panel) Flag-tagged CtBP^{WT} and CtBP^{Basic} were pulled down at similar levels (compare lanes 2 and 4). Inputs (10% of total) for each co-IP are shown in lanes 1 and 3 of each panel.

Figure S5 Misexpression of CtBP transgenes does not affect Wg expression in the wing primordium. (A-R) Confocal images of third instar larval wing imaginal discs showing Wg expression (red) at the D/V boundary of the presumptive wing blade (A, D, G, J, M and P). *Dpp-Gal4* driven expression of CtBP^{WT} (n=21), CtBP^{Acidic1/Basic1} (n=7), CtBP^{Acidic2/Basic2} (n=12), CtBP^{Acidic1/Acidic2} (n=12), CtBP^{Basic1/Basic2} (n=14) and CtBP^{Mono} (n=11) transgenes (green) at the A/P boundary (B, E, H, K, N and Q). Note that CtBP^{Acidic2/Basic2} and CtBP^{Basic1/Basic2} were expressed at lower levels compared to other transgenic combinations (compare H and N to B, E, K and Q) but no combinations affect Wg expression.

Figure S6 Wg signaling does not detectably influence the oligomerization of CtBP. (A) The top panel shows an immunoblot showing co-IP of CtBP^{WT}-HA with CtBP^{WT}-Flag without (lane 2) or with (lane 4) expression of Arm*. Arm* had no detectable change in the degree of co-IP observed. The bottom panel displays the degree of IP of the CtBP^{WT}-Flag protein. Inputs are in lanes 1 and 3. (B) Immunoblots showing the co-IP of CtBP^{WT}-V5 (middle Panel) with CtBP^{WT}-Flag (bottom panel). No signal was observed if CtBP^{WT}-V5 was left out of the transfection (middle panel, lane 1). There is no change detected in the amount of CtBP^{WT}-V5 co-IPed in the absence (middle panel, lane 2) or presence (middle panel, lane 3) of Arm*. CtBP^{WT}-V5 is expressed at similar levels in the absence (top panel, lane 2) or presence (top panel, lane 3) of Arm*.

Figure S1.
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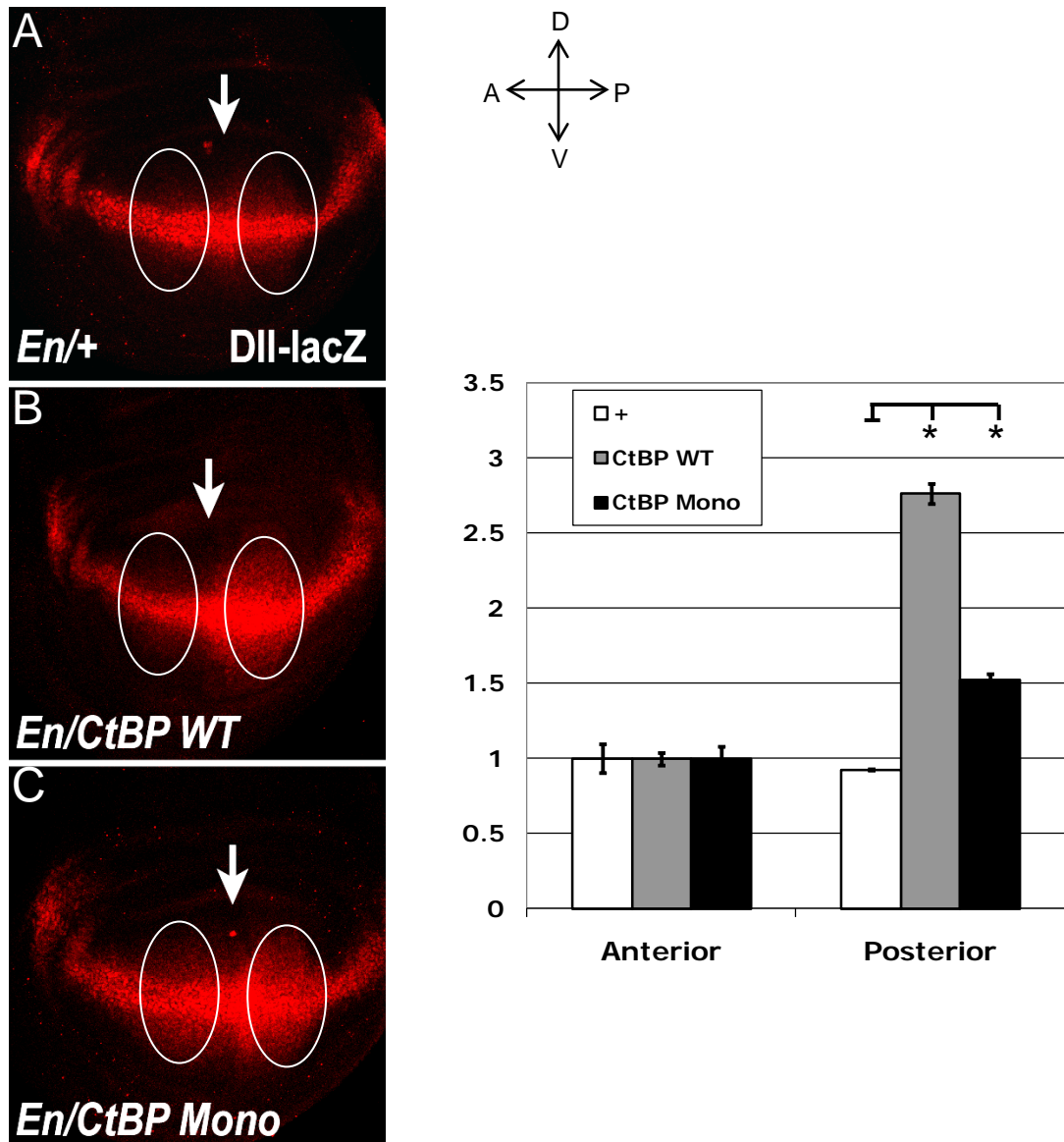


Figure S2.
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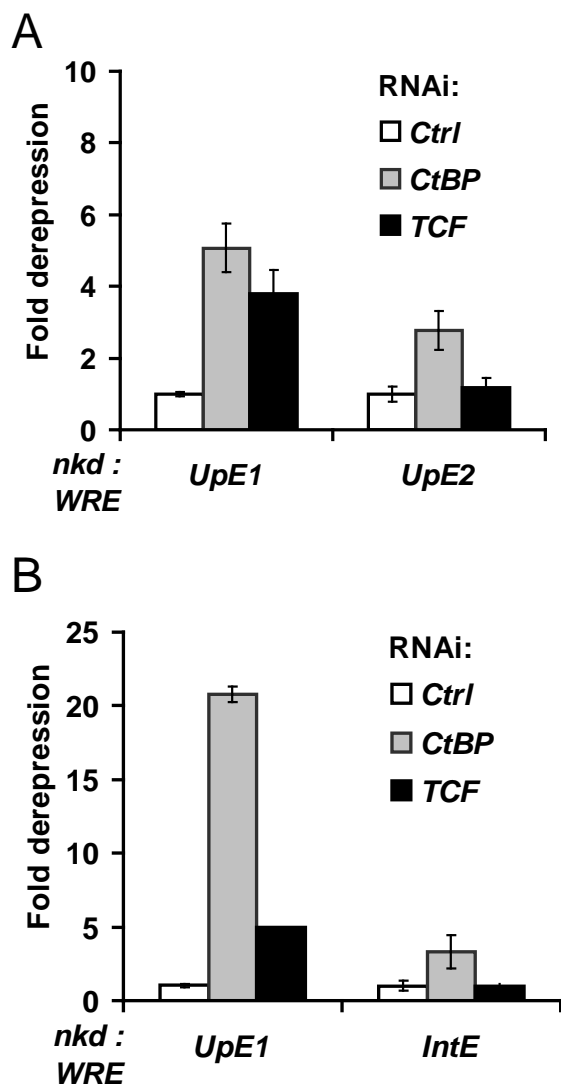


Figure S3.
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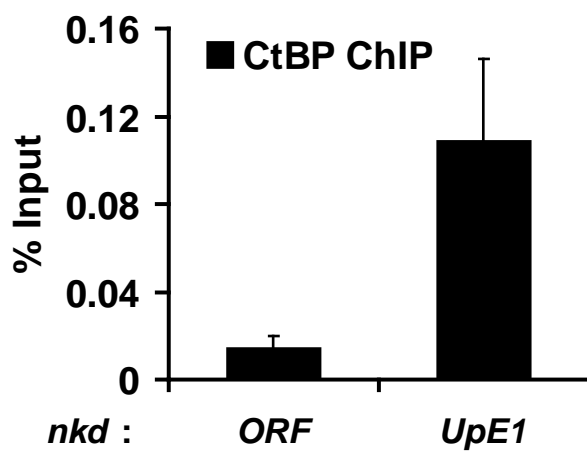


Figure S4.
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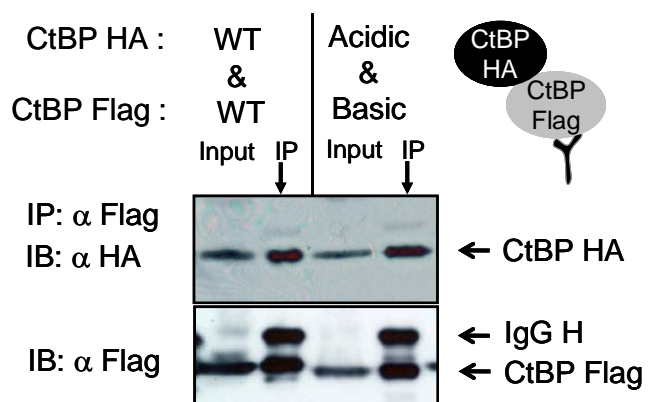


Figure S5.
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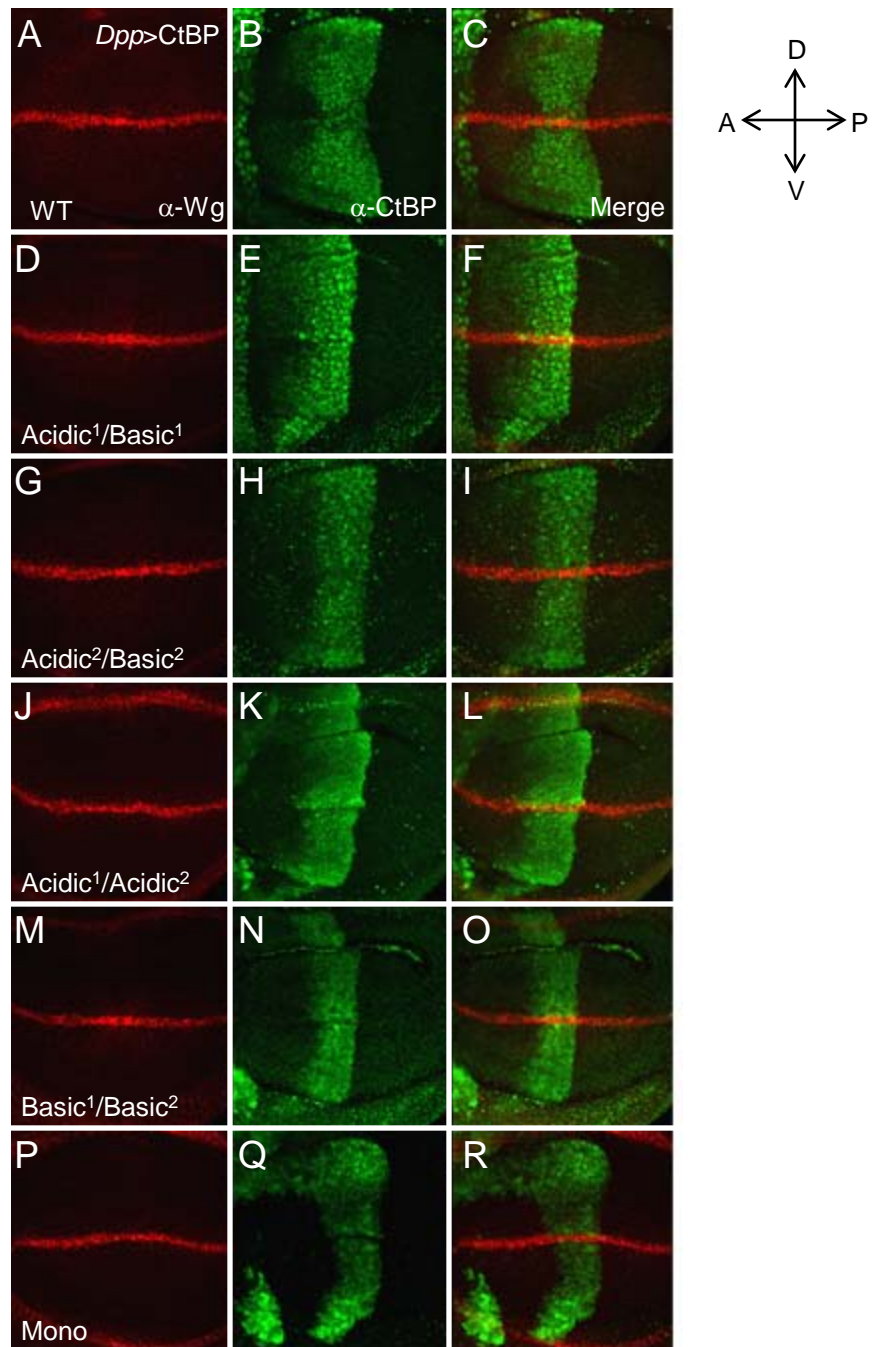
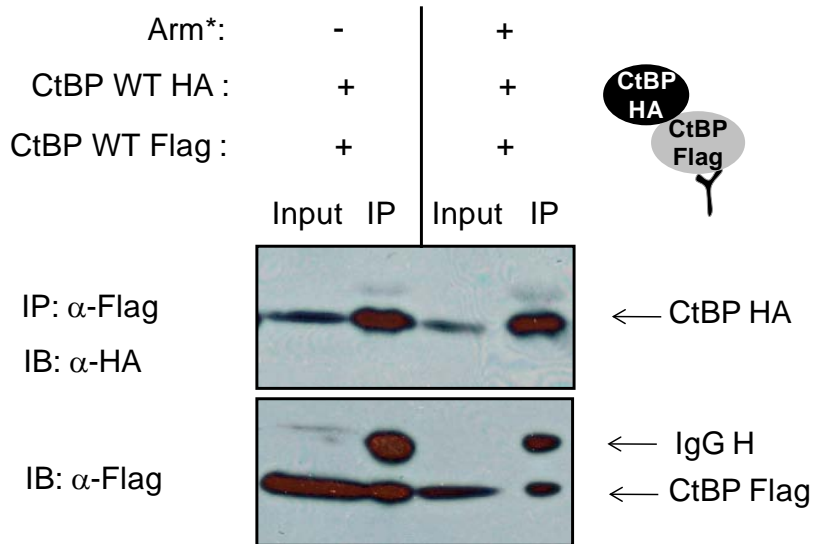


Figure S6.
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A



B

