Supplemental Figures

Figure S1  Misexpression of CtBP<sup>Mono</sup> 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<sup>WT</sup> and (C) CtBP<sup>Mono</sup> ectopically expressed in the posterior region of the disc using EnGal4.  (D) Levels of Dll-lacZ were significantly enhanced upon expression of CtBP<sup>WT</sup> and CtBP<sup>Mono</sup> 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) (+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 (+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 (±S.E.). The result shown here is representative of two independent experiments.

**Figure S4** CtBP\(^{\text{Acidic}}\) and CtBP\(^{\text{Basic}}\) efficiently form heterooligomers. (Top panel) When coexpressed, Flagged tagged CtBP\(^{\text{Basic}}\) can immunoprecipitate HA-tagged CtBP\(^{\text{Acidic}}\) at comparable levels (lane 4) as similarly tagged versions of CtBP\(^{\text{WT}}\) (lane 2). (Bottom panel) Flag-tagged CtBP\(^{\text{WT}}\) and CtBP\(^{\text{Basic}}\) 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 tranregenes 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\(^{\text{WT}}\) (n=21), CtBP\(^{\text{Acidic1/Basic1}}\) (n=7), CtBP\(^{\text{Acidic2/Basic2}}\) (n=12), CtBP\(^{\text{Acidic1/Acidic2}}\) (n=12), CtBP\(^{\text{Basic1/Basic2}}\) (n=14) and CtBP\(^{\text{Mono}}\) (n=11) transgenes (green) at the A/P boundary (B, E, H, K, N and Q). Note that CtBP\(^{\text{Acidic2/Basic2}}\) and CtBP\(^{\text{Basic1/Basic2}}\) 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.
Bhambhani et al.

A

En/+ Dll-lacZ

B

En/CtBP WT

C

En/CtBP Mono

Anterior

Posterior

![Graph showing data comparison between different conditions.](image)
Figure S2.
Bhambhani et al.

**A**

![Bar chart](image)

RNAi:
- Ctrl
- CtBP
- TCF

Fold derepression

<table>
<thead>
<tr>
<th>nkd: WRE</th>
<th>UpE1</th>
<th>UpE2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CtBP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**B**

![Bar chart](image)

RNAi:
- Ctrl
- CtBP
- TCF

Fold derepression

<table>
<thead>
<tr>
<th>nkd: WRE</th>
<th>UpE1</th>
<th>IntE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CtBP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure S3.
Bhambhani et al.
Figure S4.
Bhambhani et al.

CtBP HA: WT & Acidic
CtBP Flag: WT & Basic

IP: α Flag
IB: α HA

 IB: α Flag

← CtBP HA
← IgG H
← CtBP Flag
Figure S5.
Bhambhani et al.

A. Dpp>CtBP

B. α-Wg

C. α-CtBP Merge

D. Acidic1/Basic1

E. Acidic2/Basic2

F. Acidic1/Basic2

G. Acidic1/Basic1

H. Acidic2/Basic1

I. Acidic1/Basic2

J. Basic1/Basic2

K. Basic1/Basic2

L. Mono
Figure S6.
Bhambhani et al.

A

<table>
<thead>
<tr>
<th>Arm*</th>
<th>CtBP WT HA</th>
<th>CtBP WT Flag</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Input | IP | Input | IP |

IP: α-Flag
IB: α-HA

IB: α-Flag

B

<table>
<thead>
<tr>
<th>Arm*</th>
<th>CtBP WT V5</th>
<th>CtBP WT Flag</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

WT

WT

Input

IB: α V5

IP: α Flag

IB: α V5

IP: α Flag

IB: α Flag