Zhang et al., http://www.jcb.org/cgi/content/full/jcb.201306012/DC1

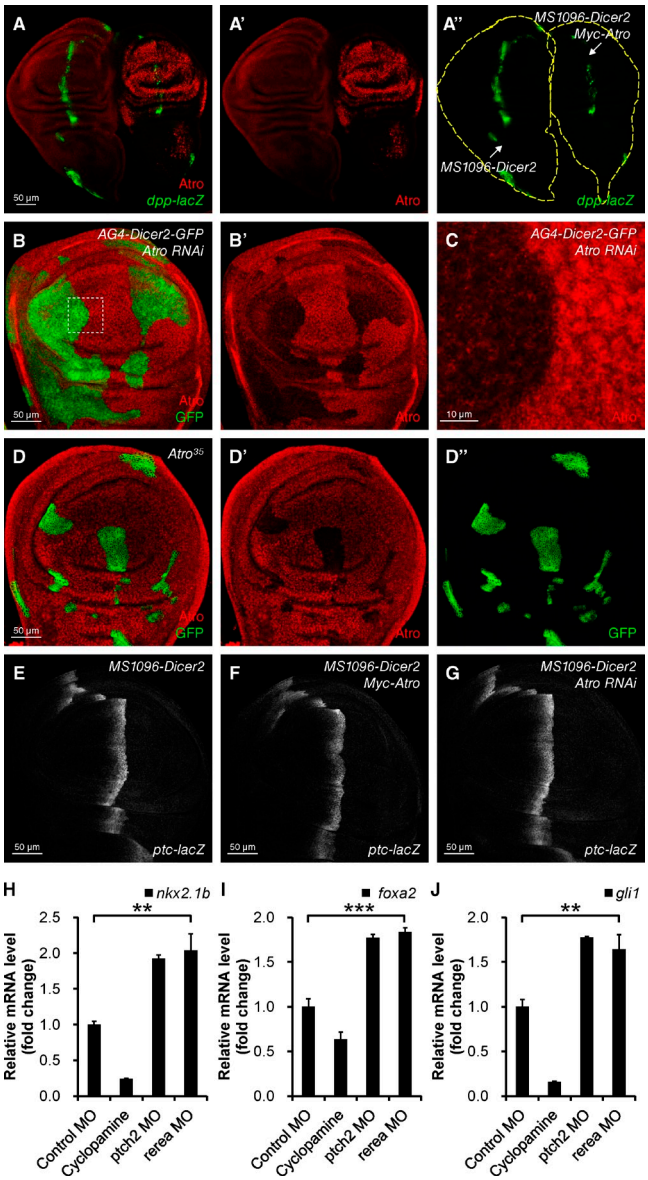

Figure S1. **An Hh signaling repressor function of Atro in *D. melanogaster* and Rerea in zebrafish.** (A–A″) To make a reliable comparison between different staining, fly imaginal discs from MS1096-Dicer2 × Myc-Atro/TM6B were dissected to make both the wild-type control and Atro overexpression discs existing in a same tube. Atro antibody (red) was used to identify wild-type (left) and Atro overexpression (right) discs. Atro overexpression reduced *dpp-lacZ* staining (green). (B and C) Low (B and B′) and high (C) magnifications of a wing disc expressing *Atro RNAi* with *AG4-Dicer2-GFP* immunostained with Atro (red) and GFP (green) antibodies. *Atro* knockdown (marked by GFP-positive cells) resulted in a reduction of endogenous Atro levels. (D–D″) A wing disc carrying $Atro^{35}$ mutant clones was immunostained to show the expression of Atro (red) and GFP (green). $Atro^{35}$ mutant clones (marked by GFP-positive cells) exhibited diminished Atro levels. (E–G) A wild-type wing disc (E) or wing discs expressing *Myc-Atro* (F) or *Atro RNAi* (G) with *MS1096-Dicer2* Gal4 were immunostained to show the expression of *ptc-lacZ*. No obvious changes of *ptc-lacZ* levels were observed. (H–J) Relative mRNA levels of *nkx2.1b*, *foxa2*, and *gli1* from 24-hpf zebrafish embryos that were injected with indicated MOs or treated with cyclopamine (10 μM) at 24 hpf (mean ± SD; $n \geq 3$). P-values were obtained by student's *t* test between two groups (**, $P < 0.01$; ***, $P < 0.001$).

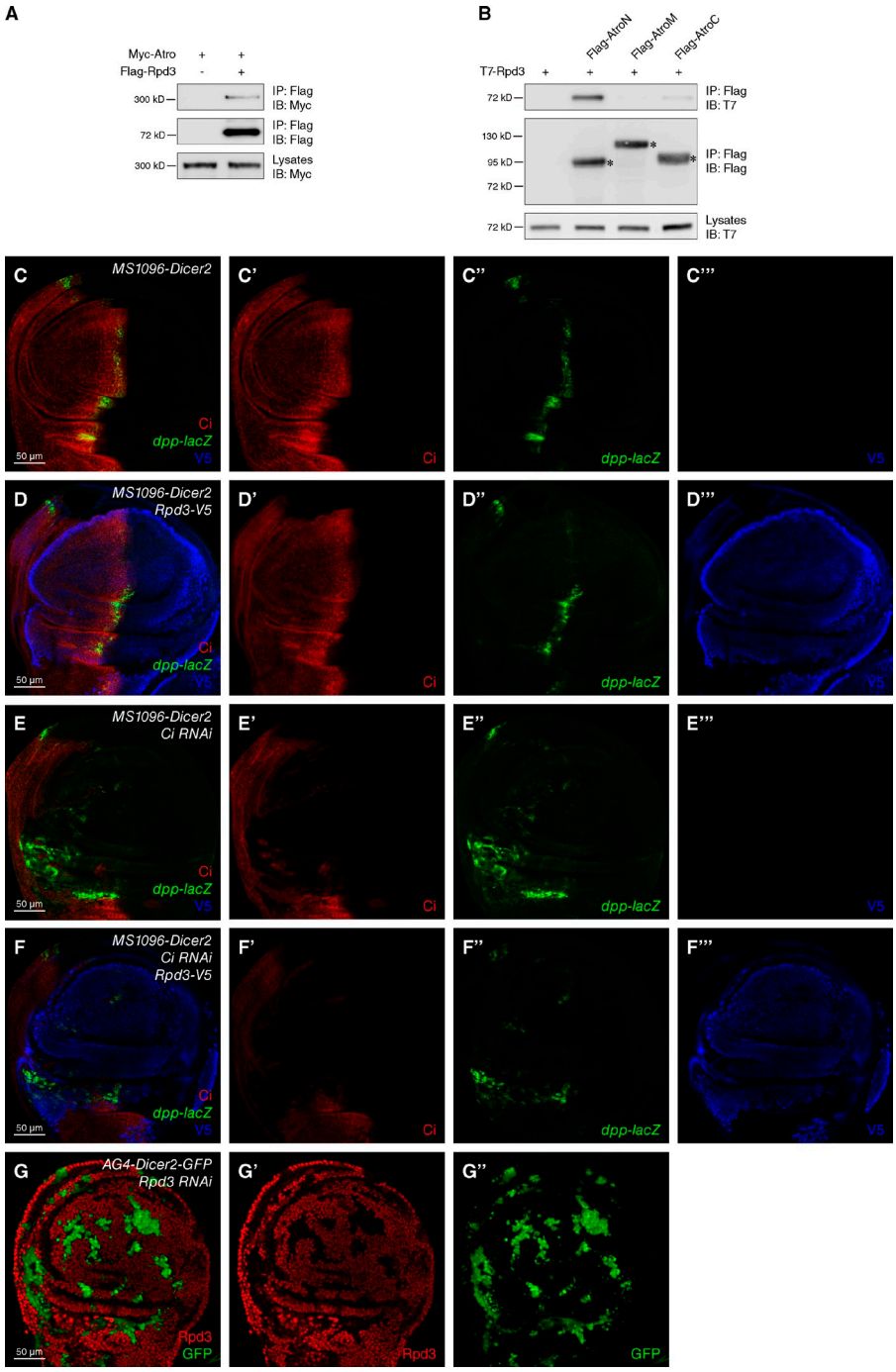

Figure S2. **The repressor function of Rpd3 in Hh signaling depends on Ci.** (A and B) Western blots of immunoprecipitates (top two panels) or lysates (bottom) from S2 cells expressing the indicated proteins. Rpd3 interacted with the N-terminal region of Atro. Asterisks indicate the target proteins. (C–F‴) Wing discs expressing the indicated transgenes were immunostained to show the expression of *dpp-lacZ* (red), Ci (green), and V5 (blue). Rpd3 overexpression failed to inhibit the expression of *dpp-lacZ* in the absence of Ci. (G–G″) A wing disc expressing *Rpd3 RNAi* with *AG4-Dicer2-GFP* was immunostained with Rpd3 (red) and GFP (green) antibodies. *Rpd3* knockdown (marked by GFP-positive cells) resulted in a reduction of endogenous Rpd3 levels.

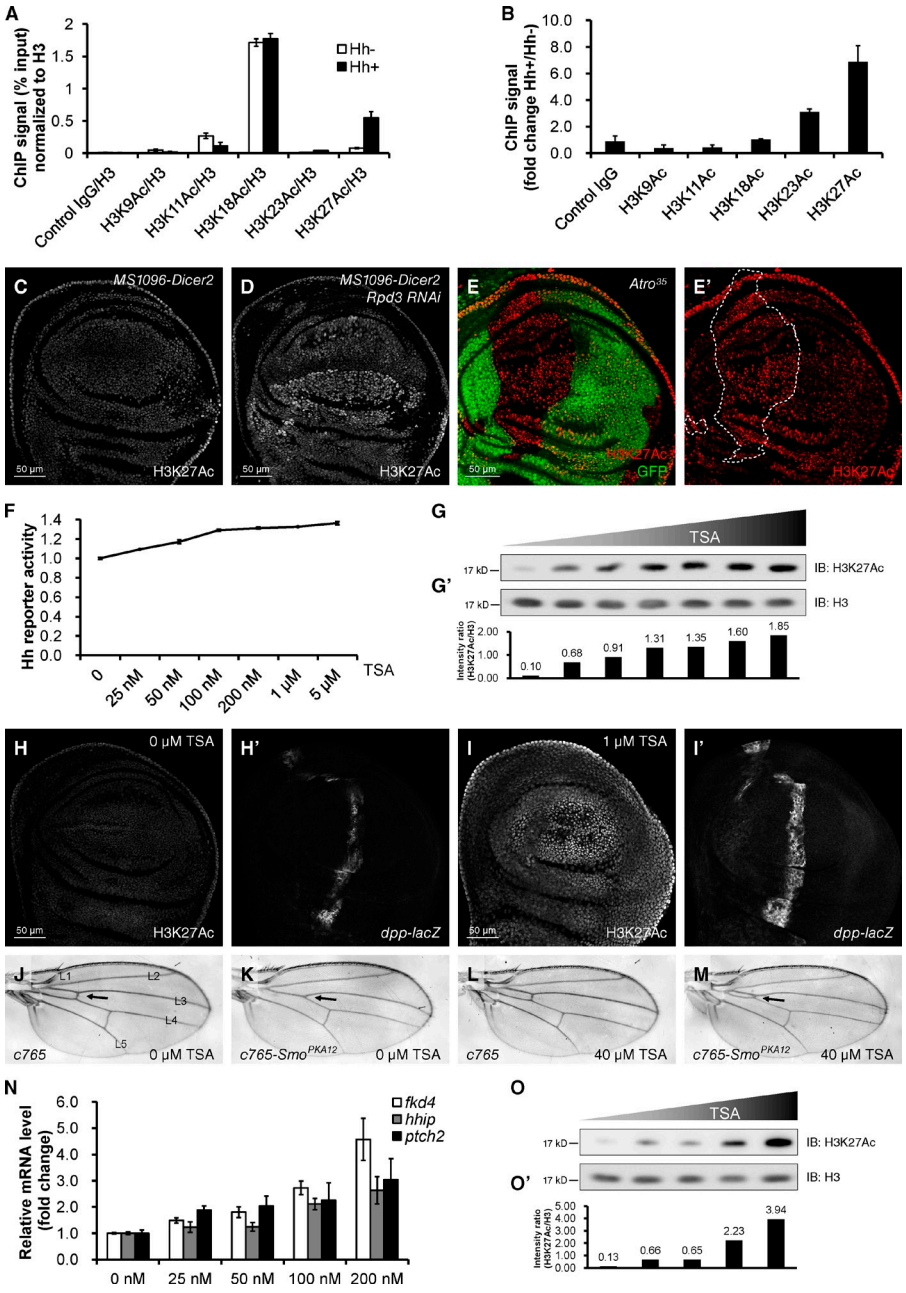

Figure S3. **The correlation between Hh activation and increased histone H3 acetylation.** (A and B) ChIP for H3K9Ac, H3K11Ac, H3K18Ac, H3K23Ac, and H3K27Ac around the transcriptional start region of *dpp* gene in CI.8 cells with/without Hh treatment. Data of ChIP signals were normalized to 1/10 of input and then to histone H3 in A, and were further shown as the fold change of Hh+ to Hh− groups in B (mean ± SD; $n = 3$). (C and D) A wild-type wing disc (C) or wing disc expressing *Rpd3 RNAi* (D) with *MS1096-Dicer2* Gal4 were immunostained by H3K27Ac antibody. (E and E′) A wing disc carrying $Atro^{35}$ mutant clones (marked by GFP-negative cells in dashed lines) was immunostained to show the expression H3K27Ac (red) and GFP (green). (F–G′) S2 cells treated with gradient concentrations of TSA showed increased Hh reporter activity (F) and H3K27Ac level (G and G′). Data are represented as mean ± SD; $n = 3$. (H–I′) Wing discs treated with TSA in vitro were immunostained to show the expression of *dpp-lacZ* and H3K27Ac. (J–M) Adult wings of indicated flies were fed with foods supplemented with TSA. TSA feeding partially rescued "fused" phenotype (arrows) of $c765\text{-}Smo^{PKA12}$. (N–O′) 24-hpf zebrafish embryos treated with gradient concentrations of TSA exhibited the up-regulated mRNA level of Hh-responsive genes (N) and H3K27Ac level (O and O′). Data are represented as mean ± SD; $n \geq 3$.