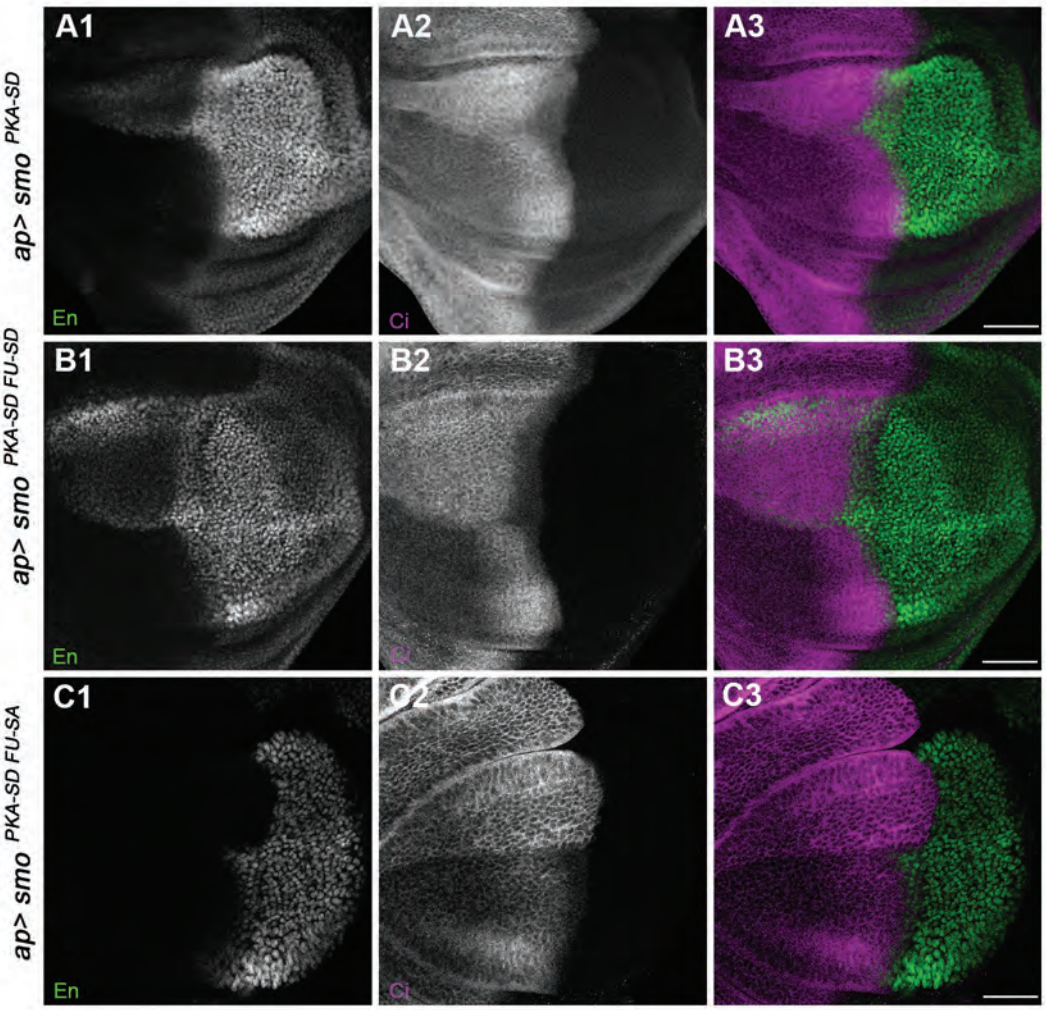

Fig. S1. Shifting the domain of anterior *en* expression causes a corresponding shift in the domain of low level Ci protein. Wing discs expressing activated Smo (*PKA-SD* and *PKA-SD FU-SD*) or attenuated Smo (*PKA-SD FU-SA*)(Sanial et al., 2017) in the dorsal compartment using *ap-GAL4*(ap^{md544}) and stained with antibodies against En (A1,B1,C1, green) and Ci (A2,B2,C2, magenta). Merge A3,B3,C3. (A) $UAS\text{-}smo^{PKA\text{-}SD}$, (B) $UAS\text{-}smo^{PKA\text{-}SD\ FU\text{-}SD}$ and (C) $UAS\text{-}smo^{PKA\text{-}SD\ FU\text{-}SA}$. The scale bars represent 50 um

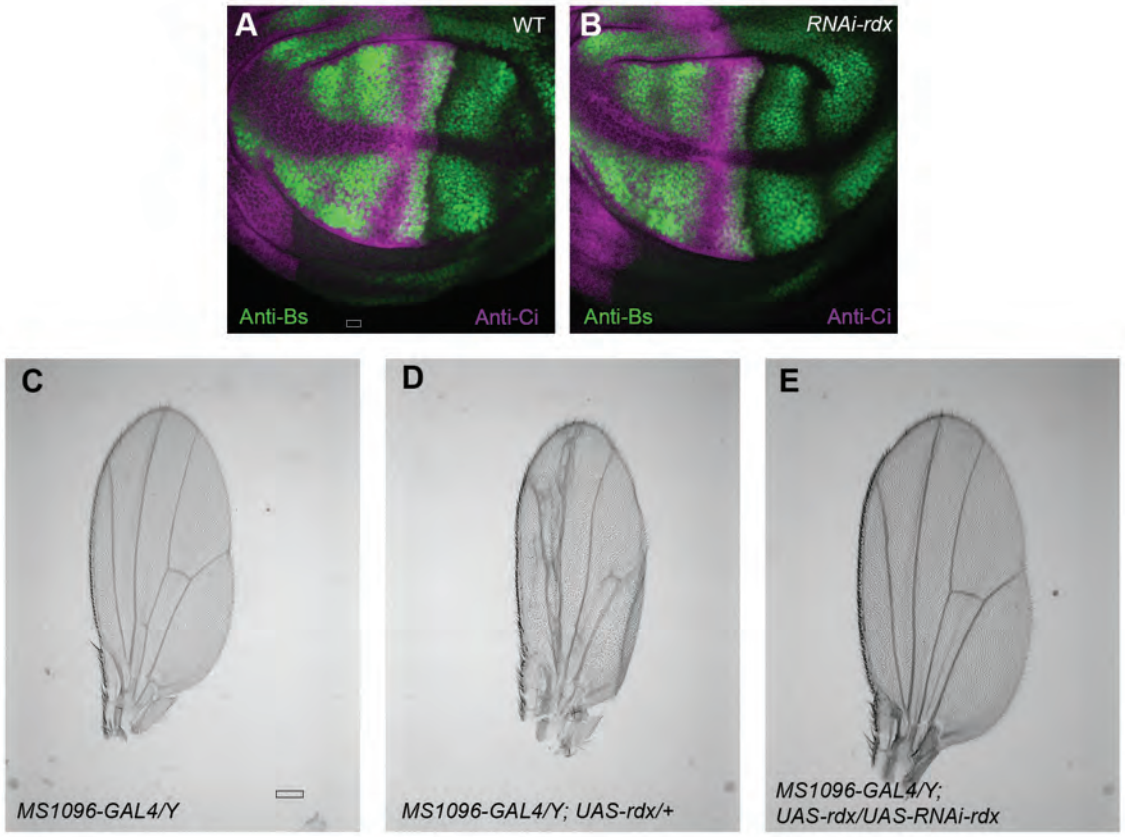

Fig. S2. RNAi targeting *rdx* does not significantly alter the position of the third wing vein primordium. (A and B) Wing discs were double labeled with anti-Bs (green) to visualize cells destined to make wing intervein and anti-Ci (magenta) to define the anterior compartment (anterior to the left). LV4 at the right edge of Ci expression marks the boundary between the anterior and posterior compartment. (A) Wild type wing disc n=6. (B) *yw*; *ptc-GAL4* (*ptc*$^{559.1}$)/*UAS-RNAi-rdx*(v28798)/+ n=4. Scale bar for panels A and B is in panel A and is 10 um. (C) Male fly wing hemizygous for *MS1096-GAL4*/Y. (D) *w MS1096-GAL4*/Y; *UAS-rdx-myc*/+. (E) *w MS1096-GAL4*/Y; *UAS-RNAi-rdx*(v28798), *UAS-rdx-myc*/+. The wing phenotypes were very consistent for each genotype and at least 10 wings were mounted for each genotype. Scale bar for panels C,D,E is in panel C and is 100 um.

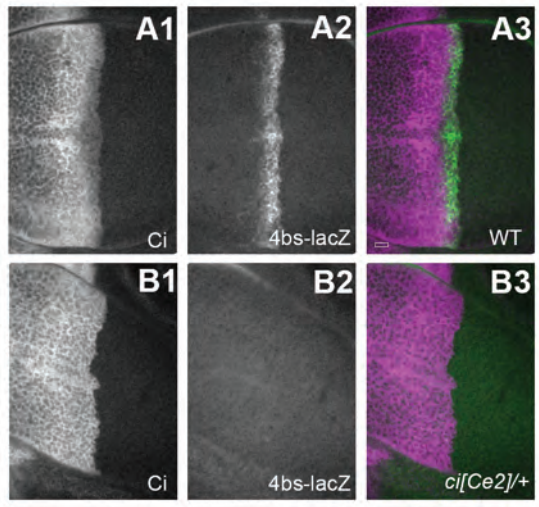

Fig. S3. Presence of a Ci repressor like protein along the compartment boundary prevents the expression of a reporter with perfect Ci binding sites. (A) Wild type animals show expression of *4bs-lacZ* (Hepker et al., 1999), a reporter construct with four perfect Ci binding sites (*4bs-lacZ*/+; ey^{D}/+), along the compartment boundary of the wing pouch (Anterior to the left). (A1) Antibody staining to Ci. (A2) *4bs-lacZ* expression along the compartment boundary. A3 is the merge of A1,2 (Anti-Ci magenta and anti--Gal green) n=4. (B) Animals heterozygous for the ci^{Ce2} mutation (*4bs-lacZ*/+; ci^{Ce2}/+) lose the expression of *4bs-lacZ* along the compartment boundary. (B1) Anti-Ci. (B2) Anti--Gal B3 is the merge of B1,2 (Anti-Ci magenta and anti--Gal green) n=6. In B1 note that there is no attenuation of Ci along the compartment boundary as there is in A1 due to the failure to express *en*. Scale bar for panels A and B is in panel A3 and is 10 um.

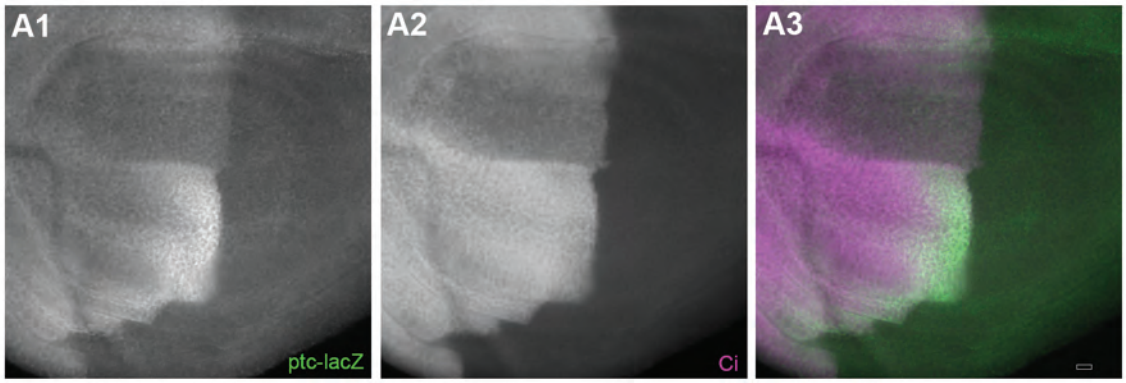

Fig. S4. Overexpression of *rdx* reduces Ci protein levels and the expression of *ptc-lacZ* in a *ci^{Ce2}*/+ background. Wing imaginal disc of the genotype *yw*; *ap-GAL4*(*ap^{md544}*)/*UAS-rdx-myc*; *ptc-lacZ*/+; *ci^{Ce2}*/+ in which *ap-GAL4* drives overexpression of *rdx* in the dorsal compartment (n=5). (anterior to the left, dorsal up) (A1) Anti--Gal visualization of *ptc-lacZ* shows dramatic attenuation of expression in the dorsal compartment. (A2) Anti-Ci. Ci levels are dramatically reduced in the dorsal compartment. (A3) Merge of A1 (anti--Gal green) and A2 (anti-Ci magenta). Scale bar in panel A3 and is 10 um.

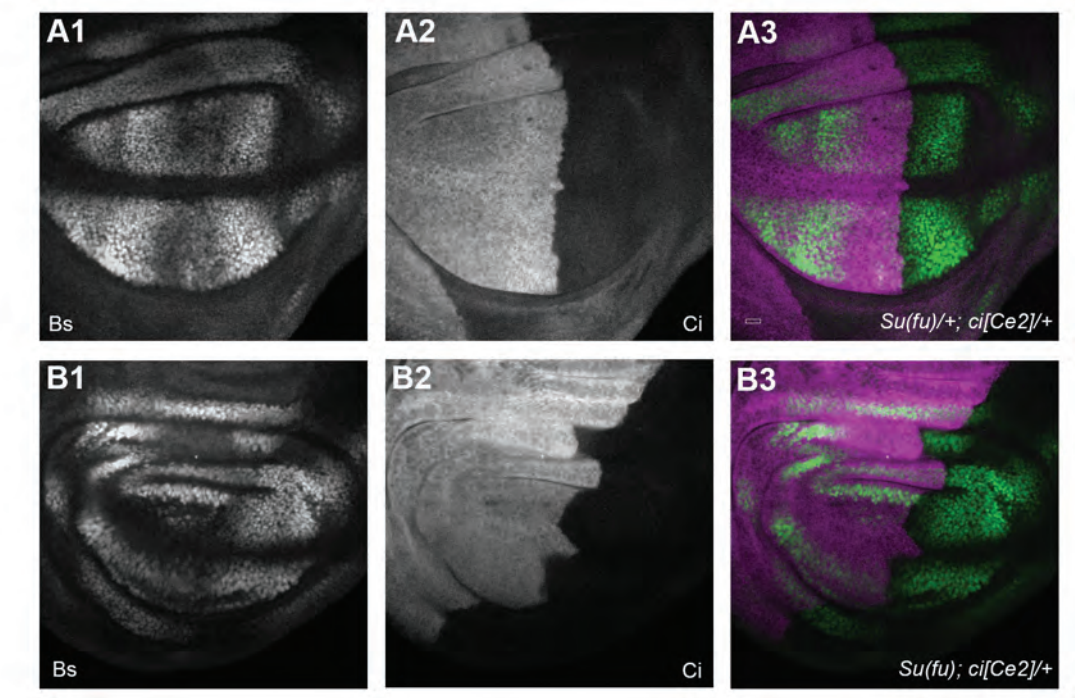

Fig. S5. ***Su(fu)*** **and** ***ci*^{Ce2}** **genetically interact and disrupt the patterning of the wing vein primordia.** (A) In *Su(fu)*^{LP}/+; *ci*^{Ce2}/+ wing discs, the 3/4 intervein region is not well defined as visualized using anti-Bs Antibody (A1). (A2) Anti Ci staining. (A3) merge Bs (green) Ci (magenta) n=4. (B) In *Su(fu)*^{LP}; *ci*^{Ce2}/+ wing discs, a single poorly defined wing vein primordium forms along the compartment boundary as visualized using anti-Bs Antibody (B1). (B2) Anti-Ci. (B3) merge Bs (green) Ci (magenta) n=5. Scale bar for panels A and B is in panel A3 and is 10 um.

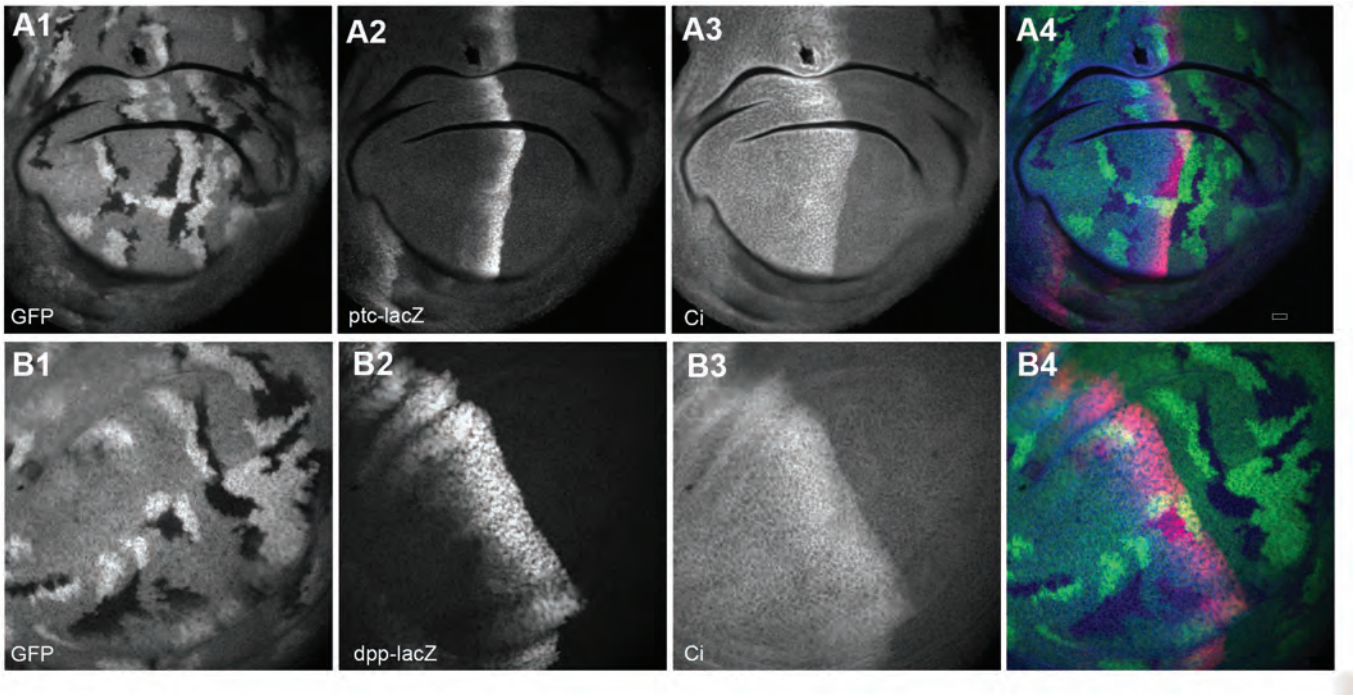

Fig. S6. On its own loss of *Su(fu)* has little effect on Hh target gene expression. (A) Clones mutant for *Su(fu)* do not have a profound effect on the expression of *ptc* in a wild type background (*yw hs-FLP*/+ or Y; *ptc-lacZ*/+; *FRT82B Su(fu)^{LP}/FRT82B ubi-GFP*; *ey^{D}*/+) (Anterior to the left). (A1) *Su(fu)* mutant clones marked by the loss of GFP. (A2) Expression of *ptc-lacZ*. (A3) Antibody staining of Ci. A4 is the merge of A1-3 (GFP in green, β-Gal in red, Ci in Blue) n=6. (B) Clones mutant for *Su(fu)* do not have a profound effect on the expression of *dpp* in a wild type background (*yw hs-FLP*/+ or Y; *dpp^{10638} (lacZ)*/+; *FRT82B Su(fu)^{LP}/FRT82B ubi-GFP*; *ey^{D}*/+) (Anterior to the lower left). (B1) *Su(fu)* mutant clones marked by the loss of GFP. (B2) Expression of *dpp-lacZ*. (B3) Antibody staining of Ci. B4 is the merge of B1-3 (GFP in green, βGal in red, Ci in Blue) n=8. Scale bar for panels A and B is in panel A4 and is 10 um.

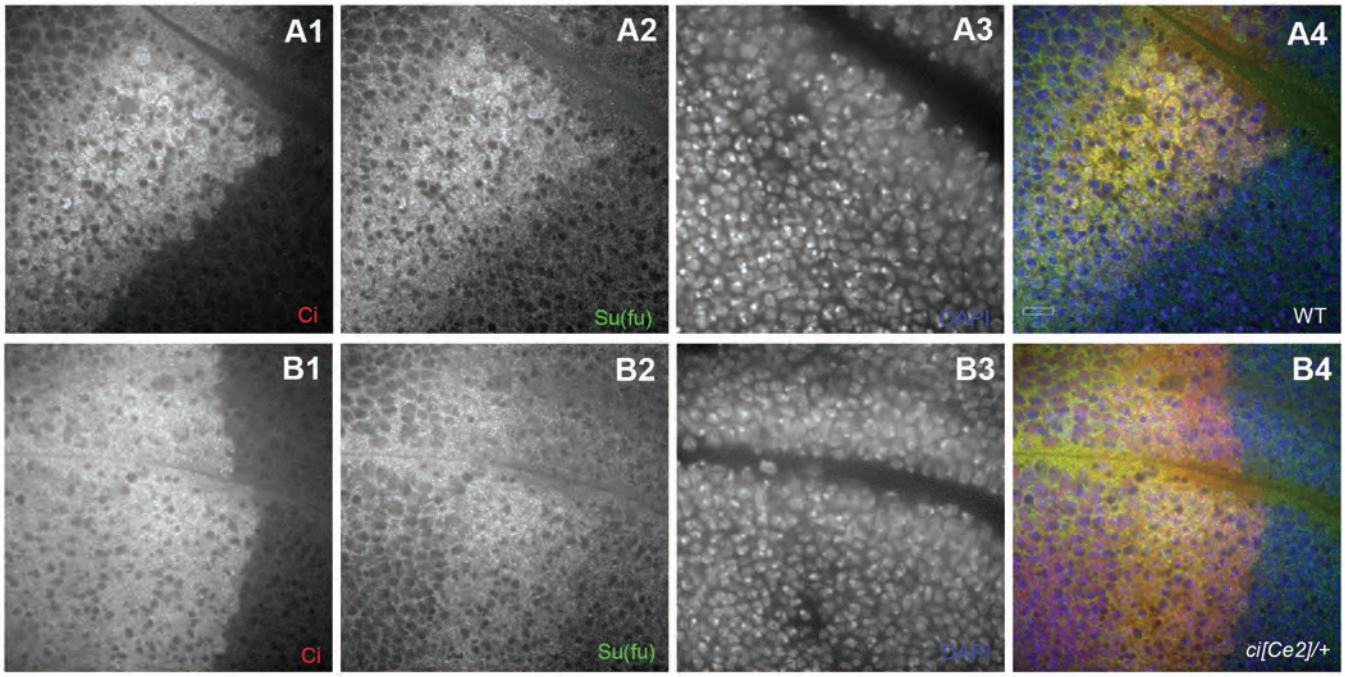

Fig. S7. In response to Hh signaling, full-length Ci accumulates in the nucleus with Su(fu) following treatment with the CRM1 inhibitor leptomycin B whereas Ci^{Ce2} accumulates in the nucleus without Su(fu). (A) *ptc-GAL4* (*ptc$^{559.1}$*)/ *UAS-RNAi-inv*(KK101934); *UAS-RNAi-en*(v35697)/+; *ey^{D}*/+ discs were treated with LMB for 1 hour (Anterior to the upper left). (A1) Antibody staining to Ci. In the domain of Hh signaling, Ci is present throughout the cell including the nucleus. Beyond the domain of Hh signaling Ci is cytoplasmic. (A2) As with Ci, Su(fu) is present throughout the cell in the domain of Hh signaling but is cytoplasmic anterior to the domain of Hh signaling. (A3) Nuclei stained with DAPI. A4 is the merge of A1-3 (Ci red, Su(fu) green, DAPI blue) n=5. (B) *ptc-GAL4* (*ptc$^{559.1}$*)/ *UAS-RNAi-inv*(KK101934); *UAS-RNAi-en*(v35697)/+; *ci^{Ce2}*/+ discs were treated with LMB for 1 hour (Anterior to the left). (B1) Antibody staining to Ci. Full-length Ci is throughout the cell in the domain of Hh signaling but cytoplasmic away from Hh signaling. Ci^{Ce2} is throughout the cell in the entire anterior compartment. (B2) Su(fu) is throughout the cell in the domain of Hh signaling, but away from Hh signaling where only Ci^{Ce2} accumulates in the nucleus, it is cytoplasmic. (B3) Nuclei stained with DAPI. B4 is the merge of B1-3(Ci red, Su(fu) green, DAPI blue) n=3. Scale bar for panels A and B is in panel A4 and is 10 um.