

Supplementary information, Figure S5 Hh affects Su(fu) level in S2 cells. (A) The expression of *hh*, *ci*, *hib*, *crn* and *su(fu)* in S2 cells were shown by RT-PCR assay. Obviously, ci expression is relatively limited in S2 cells. The RT-PCR product from ci mRNA corresponds to the region from 1500 to 3513bp of its cDNA, RT-PCR products from other genes' mRNAs correspond to their full-length cDNAs. (B-C) hib expression was measured in S2 cells through Q-PCR analysis. Stimulation with Hh conditioned medium and simultaneous overexpression of UAS-Ci or overexpression of UAS-Ci alone increased hib expression (B) whereas knockdown of *hh* reduced *hib* expression (C). (D) *ci* expression was compared between S2 cells and wing discs through Q-PCR analysis. The mRNA level of ci in wing discs is much higher than that of S2 cells. (E-F"") HA-Crn was localized in the nucleus when co-expressed with UAS-Hh in wing discs (E-E""); however, treatment with MG132, HA-Crn was mainly localized in cytoplasm (F-F""). The nuclei were stained with DAPI (blue) and the membrane was stained with TRITC-labeled phalloidin (red). (G-H"") HA-Crn localization with Hh treatment wasn't dramatically changed with or without MG132 treatment in S2 cells. HA-Crn was mainly located in the cytoplasm and barely in the nucleus. (I) Treatment with Hh conditioned medium doesn't dramatically affect endogenous Su(fu) level in S2 cells. Actin serves as a loading control. (J) Knockdown of hh by its dsRNA upregulated Su(fu) protein level. The effect of hh RANi was assessed by RT-PCR. (K) Quantification of the western blot analysis of endogenous Su(fu) protein level (J) as determined by ImageJ. (L) hh knockdown didn't change *su(fu)* mRNA level in S2 cells by Q-PCR.