Supporting Information

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SI Experimental Procedures

***Drosophila* Genetics.** All crosses were performed according to standard procedures at 25 °C. Fly strains used in this study were *en-Gal4 UAS-GFP*, *ap-Gal4*, *omb-Gal4*, *UAS-flp*, *ey-FLP*, *dpp-lacZ*, w^{1118} (as wild type) (Bloomington *Drosophila* Stock Center), *UAS-hipk* (II), *UAS-hipk* (III) (1), *UAS-hipk* ^{RNAi} (II), *UAS-hipk*^{RNAi} (III) (Vienna *Drosophila* RNAi Center), *slimb*^{P1493} *FRT82* (2), *UAS-Daxin*$^{A2\text{-}4}$ (3), *UAS-FLAG-Dcu1l* (4), *UAS-slimb* (5), *Tub > Myc-Slimb* (6), *hipk*^{4} *FRT79* (7), *Daxin*^{S044230} *FRT82/TM6B* (8), *Dcul1*^{EX} *FRT40* (9). In assays examining the interaction between two *UAS* transgenes, control crosses were performed with *UAS-lacZ* to rule out suppressive effects caused by titration of the Gal4 protein. MARCM clones were generated by crossing *y w hsflp UAS-GFP tubGal4; FRT42D tubGal80;* + or *y w hsflpUAS-GFP tubGal4; +; FRT82B tubGal80* (gift from Bruce Edgar) females to males of the particular genotype for 24 h and the progeny were then heat-shocked at 38 °C for 90 min at 48 h AEL. Loss-of-function clones for *hipk* in the wing disk were generated by crossing *omb-Gal4/FM7; hipk*^{4} *FRT79/TM6B* females to *UAS-flp; GFP,FRT79/TM6B* males.

DNA Constructs. The following plasmids were used in this study: pUAST-Hipk (1), pGEX-GST-Hipk (10), pMK33-HA-Sgg (11), pMK33-HA-CK1 (12), β-TrCP-Myc-pCS2+ (13), pCDNA3-Hipk2^{WT}-FLAG and pCDNA3-Hipk2^{K221R}-FLAG (14), pCDNA3-Myc-Ubiquitin (15), pDA-FLAG-Ci, pDA-FLAG-Hh-N, pDA-RL (16), ptcΔ136-Luc and ptcΔ136-mut (17).

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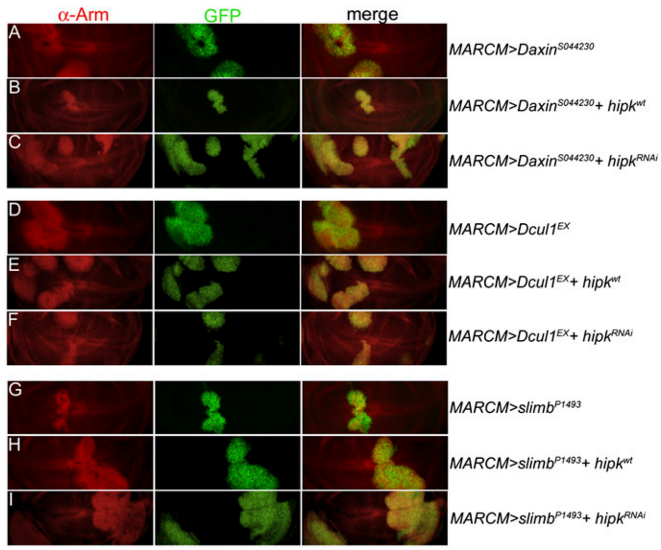

Fig. S1. Modulation of *hipk* has no effect on completely stabilized Arm. (*A*) The generation of positively GFP-marked loss-of-function MARCM clones for *Daxin* prevents the degradation of Arm and stabilizes the entire cytosolic pool. Once all cytosolic Arm is completely stabilized, the effects of overexpression of *hipk* (*B*) or $hipk^{RNAi}$ (*C*) in this background are negligible. (*D*) Loss-of-function clones for *Dcul* also stabilizes all cytosolic Arm anywhere in the wing disk. Overexpression of *hipk* (*E*) or $hipk^{RNAi}$ (*F*) in this context does not enhance or decrease the levels of stabilized Arm. (*G*) Similar to *Daxin* and *Dcul1*, loss-of-function clones for *slimb* result in the complete stabilization of Arm anywhere in the wing disk, which is affected by neither an increase (*H*) nor a decrease (*I*) in Hipk levels.

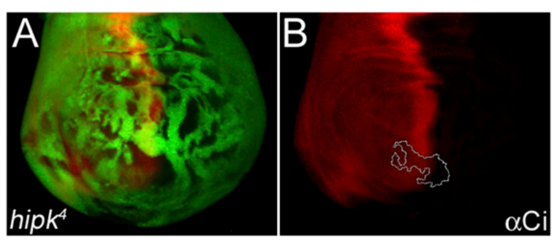

Fig. S2. Full-length Ci is reduced in loss-of-function clones for *hipk* in the wing disk. (*A* and *B*) Clonal analysis of *hipk* (indicated by a loss of GFP) shows a reduction in full-length Ci in certain parts of the wing disk.