Supplementary materials

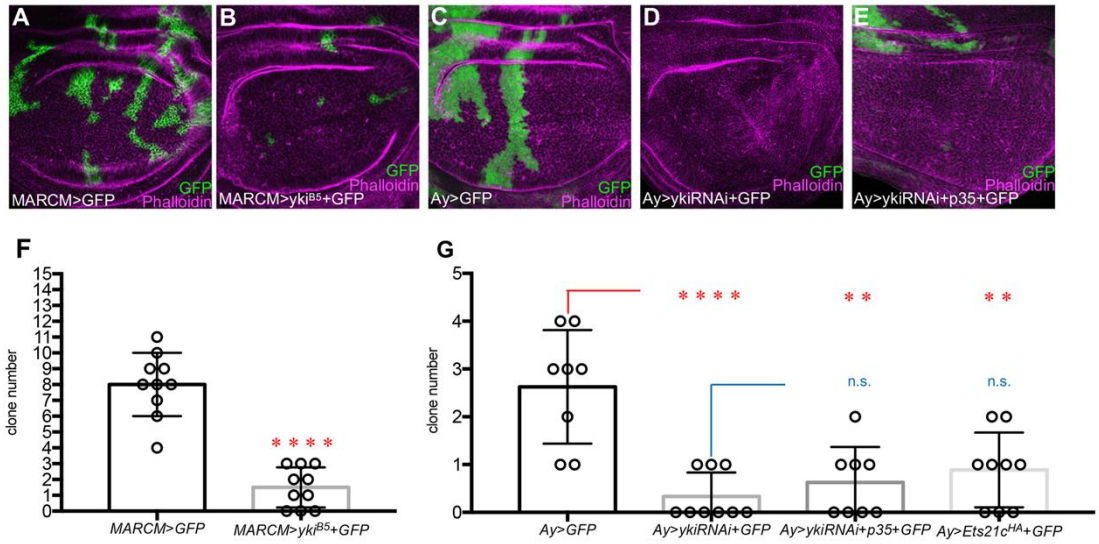

Figure S1. Lack of *yki* induced small and rare clones in the wing discs

(A) *GFP*-marked MARCM control clones in the *Drosophila* wing disc (n=). (B) *yki*^{B5} mutant clones were small and rare (n=). (C) *GFP* expressing clones (n=). (D) *ykiRNAi* clones were very rarely observed in the wing disc pouch (n=). (E) *ykiRNAi* and *p35* co-expressing clones were still rare and small (n=). (F and G) Quantification of clone numbers in A–E and Fig 5A. Data are presented as mean + SD. P values were calculated using two-tailed Student's t tests. **p < 0.01;; ****p < 0.0001; n.s., no significant difference.

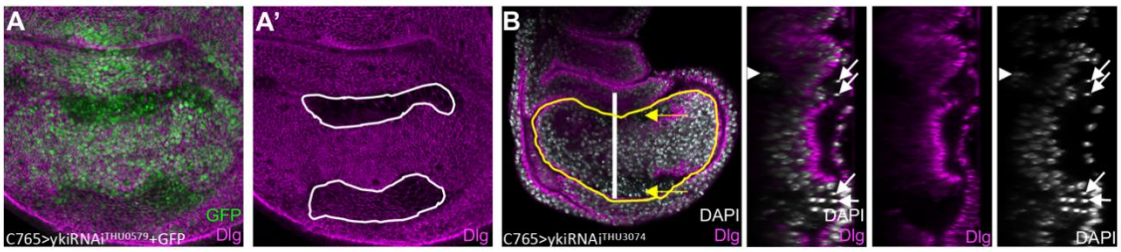

Figure S2. Apical extrusion of cells occurred intensively in two stripe regions close to the wing hinge

(A and A') x-y views close to the apical section of the wing disc showed two stripe regions with reduced Dlg staining. (B) Knocking-down *yki* in another independent *ykiRNAi* line (*UAS-ykiRNAi*^{THU3074}) induced the same apical cell extrusion (arrows) and basal cell extrusion (arrowhead) phenotypes in the wing epithelia. ACE occurred (arrows) in the pouch of the wing disc (yellow circle). *UAS-ykiRNAi*^{THU0579} and *UAS-ykiRNAi*^{THU3074} were constructed using two different vectors: VALIUM20 and VALIUM10. Furthermore, the RNAi target sequences in these two lines differ.

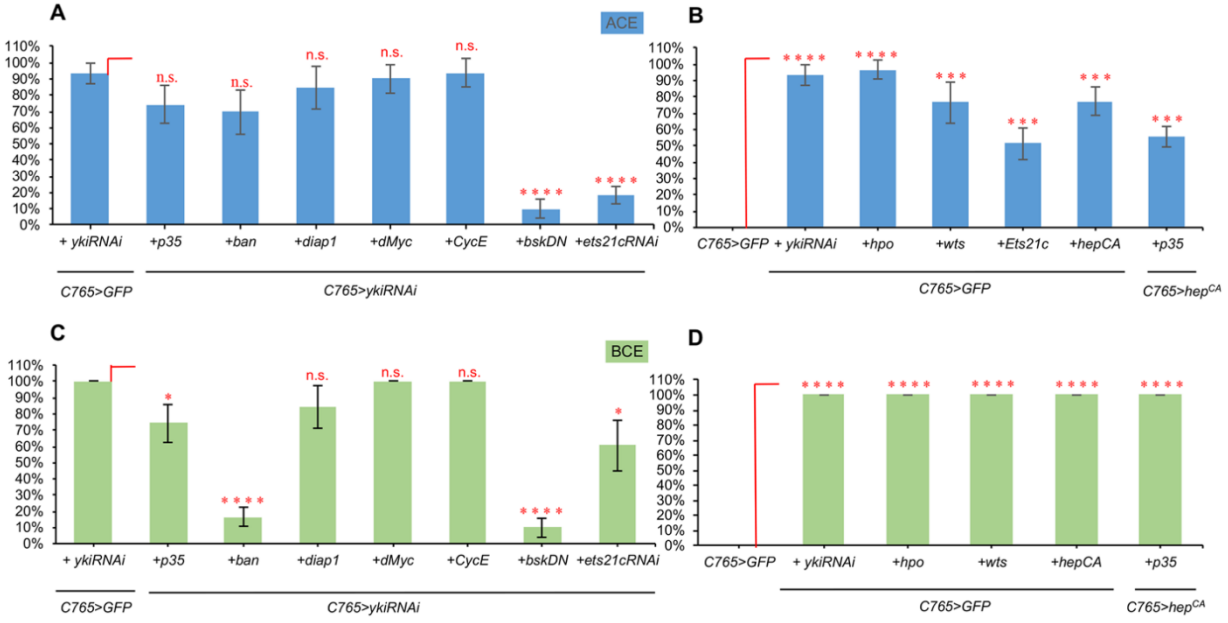

Figure S3. The percentage of wing discs with ACE and BCE

We used C765-Gal4 to drive gene expression. (A) Percentage of wing discs with ACE: *ykiRNAi+GFP* (93.3%, n=30) (n refer to average wing disc number of three replications), *ykiRNAi+p35* (74.2%, n=31), *ykiRNAi+ban* (70%, n=30), *ykiRNAi+diap1* (84.4%, n=32), *ykiRNAi+dMyc* (90%, n=30), *ykiRNAi+CycE* (93.8%, n=32), *ykiRNAi+bsk*^{DN} (10%, n=30), and *ykiRNAi+Ets21cRNAi* (18.2%, n=33). (B) Percentage of wing discs with ACE: *GFP* (0%, n=28), *hpo+GFP* (96.8%, n=31), *wts+GFP* (76.5%, n=34), *Ets21c+GFP* (51.5%, n=33), *hep*^{CA}*+GFP* (77.4%, n=31), and *hep*^{CA}*+p35* (55.6%, n=36). (C) Percentage of wing discs with BCE (the wing discs used for this analysis are the same as those used in Fig. s3. A): *ykiRNAi+GFP* (100%, n=30), *ykiRNAi+p35* (74.2%, n=31), *ykiRNAi+ban* (16.7%, n=30), *ykiRNAi+diap1* (84.4%, n=32), *ykiRNAi+dMyc* (100%, n=30), *ykiRNAi+CycE* (100%, n=32), *ykiRNAi+bsk*^{DN} (10%, n=30) and *ykiRNAi+Ets21cRNAi* (60.6%, n=33). (D) Percentage of wing discs with BCE (the wing discs used for this analysis are the same as those used in Fig. s3. B): *GFP* (0%, n=28), *hpo+GFP* (100%, n=31), *wts+GFP* (100%, n=34), *hep*^{CA}*+GFP* (100%, n=31), and *hep*^{CA}*+p35* (100%, n=36). Results are shown as mean + SD. P values were calculated using two-tailed Student's t-test. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001; n.s., no significant difference.

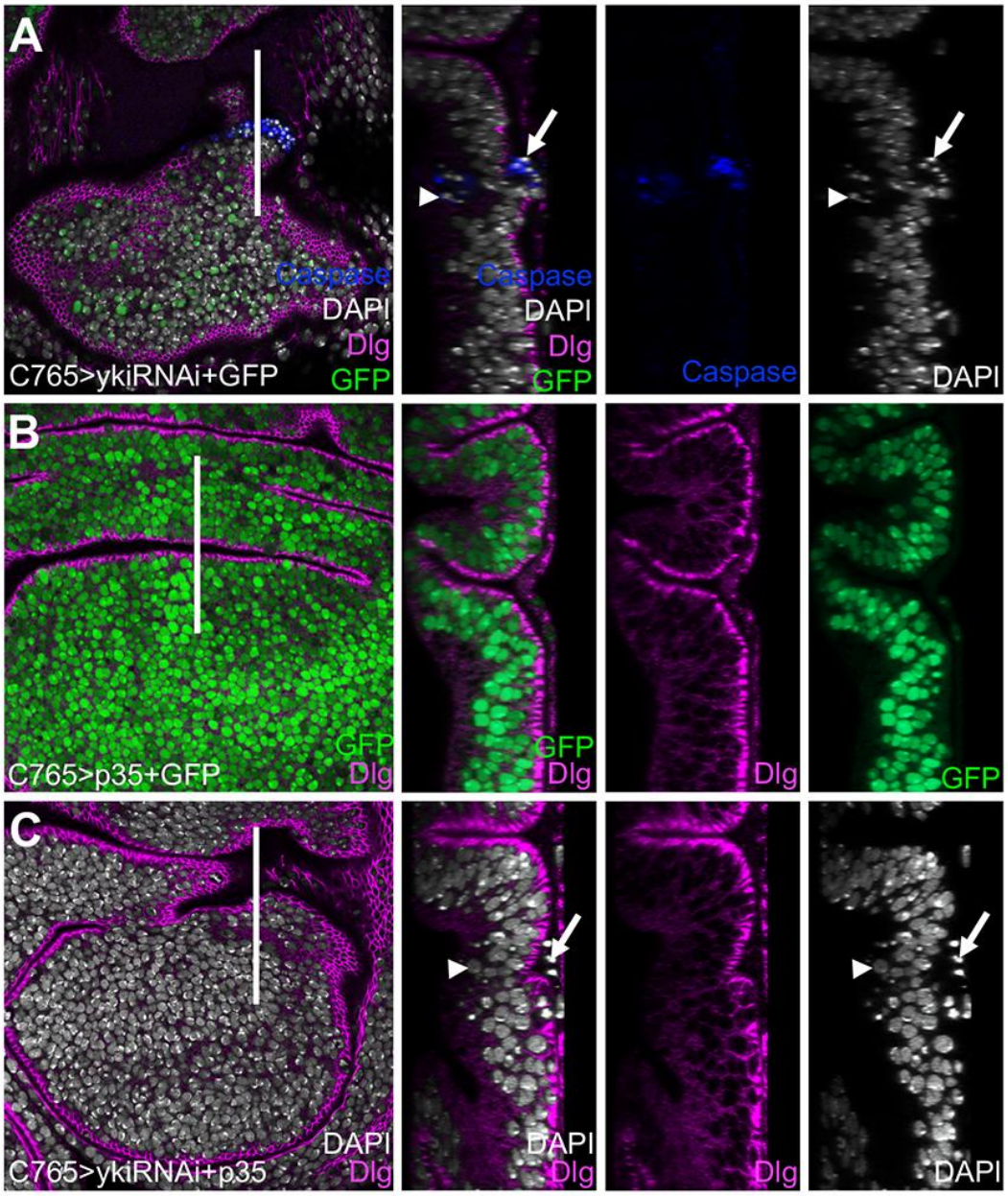

Figure S4. Suppressing apoptosis did not block *ykiRNAi* induced cell extrusion

(A) *ykiRNAi* induced cell extrusion and cleaved caspase-3 staining. (B) Control experiment, *p35* expression. (C) Co-expressing *p35* and *ykiRNAi* did not repress *ykiRNAi*-induced cell extrusion.

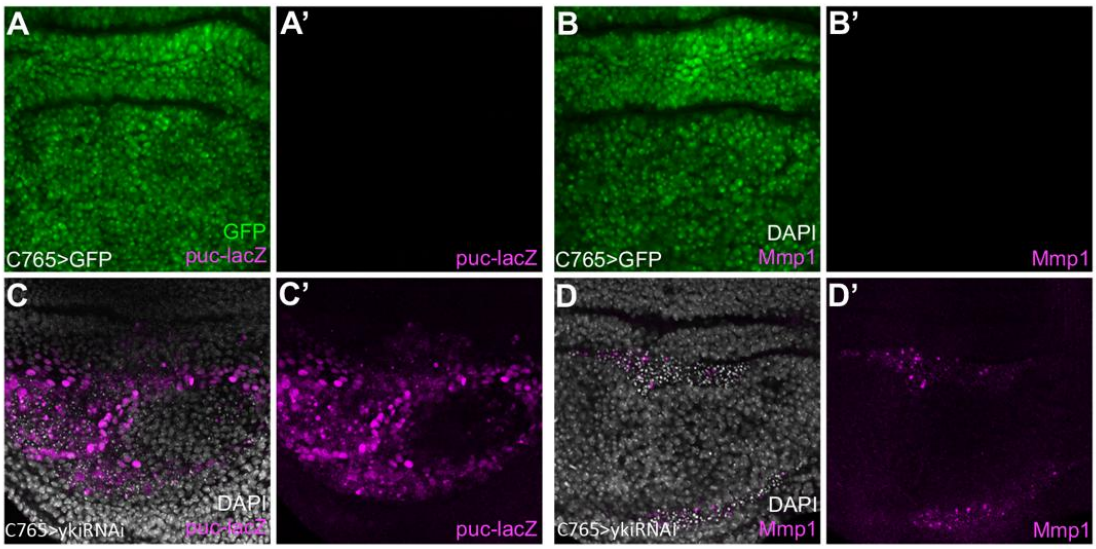

Figure S5. JNK signaling was activated in *ykiRNAi* expressing wing discs

puc (A') and *Mmp1* (B') were not expressed in control wing discs. (C and C') *puc* expression was activated in *ykiRNAi* expressing wing discs. (D, D') *Mmp1* expression was activated in *ykiRNAi* expressing wing discs.

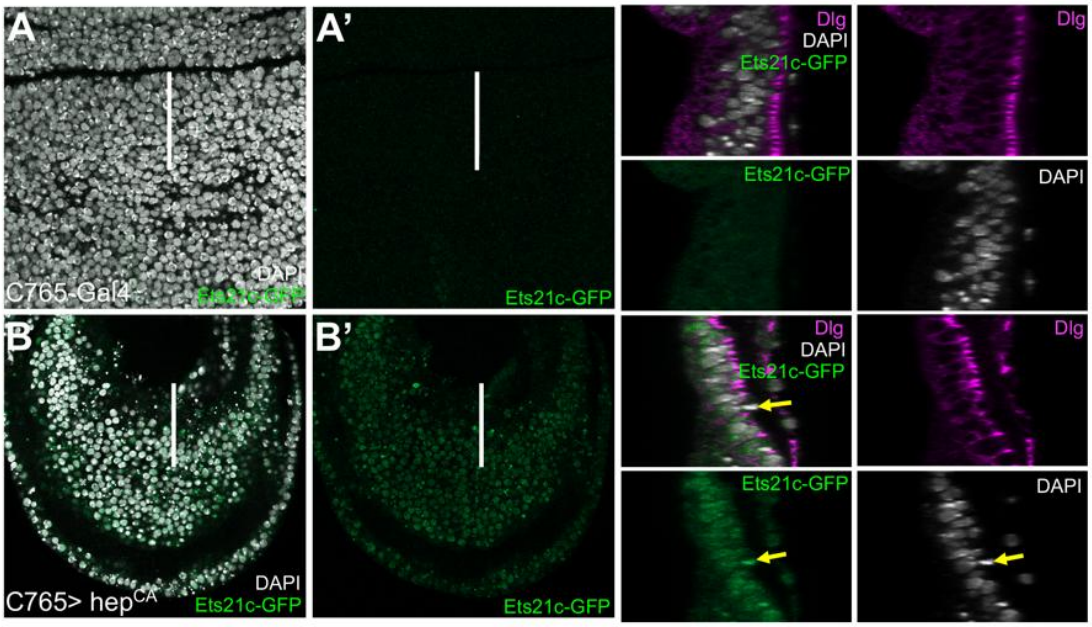

Figure S6. Ets21c-GFP level was increased when JNK signaling was activated

(A, A') *Ets21c-GFP* level is low in control wing discs. (B, B') Expression of hep^{CA} increased wing disc *Ets21c-GFP* levels including in the apically extruded cells (yellow arrow).

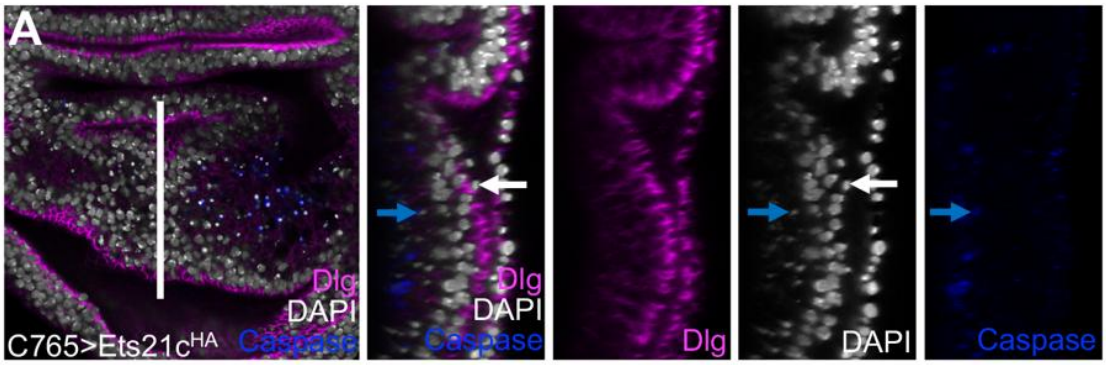

Figure S7. *Ets21c*-induced apical extruded cells were not undergoing apoptosis

(A) An x-y scan close to the basal section of the wing discs. Expression of *Ets21c*^{CA} induced apically extruded cells (arrow) with no cleaved caspase-3 staining. Most cleaved caspase-3 staining (blue arrow) was located on the basal side of the wing discs.

Table S1. Abundance of transcription of third instar larvae

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Table S2. Alterations in mRNA expression of 6 genes involved in Hpo pathway

genes	*Control*	*C765>ykiRNAi*	fold change	regulated
Act57B	17.06863333	30.011	1.591692872	up
Act87E	10.60510521	20.50697756	1.84484084	up
wgn	30.09160511	23.09001711	0.791146608	down
hth	13.89972479	8.764950015	0.710459822	down
ex	68.42301728	34.85187972	0.555330751	down
yki	67.43286075	18.55718096	0.304498467	down

Table S3. Selected genes subjected to quantitative RT-PCR

genes	*Control*	*C765>ykiRNAi*	fold change	regulated
hth	13.89972479	8.764950015	0.710459822	down
kmn1	63.83590667	26.65354333	0.427832717	down
puc	8.850264407	13.10390111	1.405928444	up
rpr	7.154933333	12.48833333	1.551257881	up
yki	67.43286075	18.55718096	0.304498467	down
Ets21c	0.436940337	1.458076081	2.026518117	up

Table S4. Primer sequences used for quantitative RT-PCR

hth-F	5'-CAAACCACCGGAGTTGGGAT-3'
hth-R	5'-AGGGGGTACTCACAACGTCT-3'
kmn1-F	5'-CCGCAGTGACGTACTGGAAT-3'
kmn1-R	5'-GTGCTTGCTTCATAGCGACG-3'
puc-F	5'-TTTCTGCTGACTTGCCACAC-3'
puc-R	5'-ATCCTCTCACACACTCGCTT-3'
rpr-F	5'-CGGGAGTCACAGTGGAGATT-3'
rpr-R	5'-TTTGGGTTTTGGGTTGGCTC-3'
yki-F	5'-GAGCAGGCAGTTACCGAGTC-3'
yki-R	5'-ACGCTAAGCCCAGATTGCAT-3'
Ets21c-F	5'-GTGCCAACAGAGGCCGATTA-3'
Ets21c-R	5'-CTGTTGGTGGGAACTTCCGT-3'
Actin-F	5'-CAGAGCAAGCGTGGTATCCT-3'
Actin-R	5'-CTCATTGTAGAAGGTGTGGTGC-3'

Table S5. Detailed genotypes for each figure

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