Supplementary Materials and Methods

Flies and genetics

To isolate *Tnks* mutants, the transposase encoded by *P{Δ2-3}* (Robertson et al., 1988) (Bloomington Drosophila Stock Center (BDSC)) was used to mobilize the *P* element *G9172*, an EP insertion (Rorth, 1996) provided by KAIST, South Korea. We screened for deletions within the *Tnks* locus by PCR, which were confirmed by sequencing. $Tnks^{503}$ contains a deletion of 1859 nucleotides (-20 to +1839 with reference to the *Tnks* transcriptional start site). Approximately 3.3K nucleotides from *G9172* remain at this site. A deletion of the entire *Tnks* gene, $Tnks^{19}$, was isolated by hybrid element insertion (Pare et al., 2009; Parks et al., 2004), using *P{Δ2-3}HoP* (BDSC) to simultaneously mobilize the *P* elements *G9172* and *KG00687* (Bellen et al., 2004), which flank the *Tnks* gene. Prior to performing this screen, the recessive marker *scarlet (st)* was combined in *cis* with *KG00687*, and *claret (ca)* was combined in *cis* with *G9172* using meiotic recombination. To isolate potential deletions, we screened for *st ca* recombinants. $Tnks^{19}$ contains a deletion of 9089 nucleotides (-20 to +9069 with reference to the *Tnks* transcriptional start site).

Antibodies

To generate Axin polyclonal antibodies, a PCR fragment encoding amino acids 43 to 358 of the Axin protein was amplified by PCR from the *Axin* cDNA in *pAc5.1-Daxin-3xHA* (Huang et al., 2009), and ligated at the EcoRI site of *pPROEX HTb* vector (Invitrogen). To generate Tnks polyclonal antibody, a fragment encoding amino acids 423 to 885 (in the Ankyrin repeat region) of the Tnks protein was amplified by PCR from the *Tnks cDNA LD22548* (Drosophila Genomics Research Center), and inserted at the NcoI and NotI sites of the *pPROEX HTa* vector (Invitrogen). His-tag fusion proteins were purified

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with TALON metal affinity resin (Clontech) and used as an immunogen in guinea pigs (Cocalico Biologicals). The Axin GP90 and Tnks GP96 antisera were used at a 1:1000 dilution for immunoblots.

RT-qPCR

The following primers were used to examine relative gene expression by RT-qPCR:

upd2: Forward primer: 5'-TGGTATTCGCTCATCGTGA-3'

Reverse primer: 5'-GGCAAATCA GAGATCCCG-3'

upd3: Forward primer: 5'-AGGCCATCAACCTGACCAAC-3'

Reverse primer: 5'-ACGCTTCTCCATCAGCTTGC-3'

krn: Forward primer: 5'-GTTGCTCCGCTAACAATGCT-3'

Reverse primer: 5'-GAACGATGGCACCTGCT-3'

dpp: Forward primer: 5'-TCTGCTGACCAAGTCGG-3'

Reverse primer: 5'-GCGGGAATGCTCTTCAC-3'

rpl32: Forward primer: 5'-AGGCCCAAGATCGTGAAGAA-3'

Reverse primer: 5'-TGTTGCACCAGGAACTTCTTGAA-3'

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Supplementary References

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Huang, S.M., Mishina, Y.M., Liu, S., Cheung, A., Stegmeier, F., Michaud, G.A., Charlat, O., Wiellette, E., Zhang, Y., Wiessner, S., *et al*. (2009). Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. Nature *461*, 614-620.

Pare, A.C., Dean, D.M., and Ewer, J. (2009). Construction and characterization of deletions with defined end points in Drosophila using P elements in trans. Genetics *181*, 53-63.

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Robertson, H.M., Preston, C.R., Phillis, R.W., Johnson-Schlitz, D.M., Benz, W.K., and Engels, W.R. (1988). A stable genomic source of P element transposase in Drosophila melanogaster. Genetics *118*, 461-470.

Rorth, P. (1996). A modular misexpression screen in Drosophila detecting tissue-specific phenotypes. Proceedings of the National Academy of Sciences of the United States of America *93*, 12418-12422.

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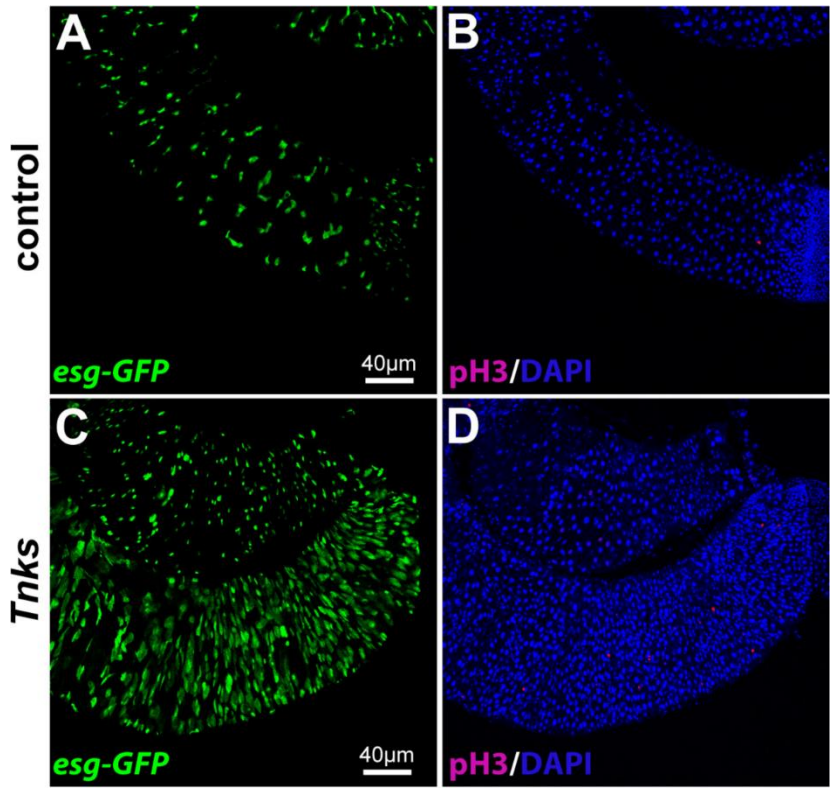

Fig S1: ISC overproliferation resulting from loss of Tnks increases in severity with age

Midguts of 14-day-old female flies expressing *esg>GFP* were stained with anti-phospho-histone H3 (magenta) and anti-GFP (GFP) antibodies. $Tnks^{19/503}$ mutants (C and D) have increased numbers of *esg-GFP*$^{+}$ and pH3^{+} cells compared with control flies ($Tnks^{503}$/+) (A and B).

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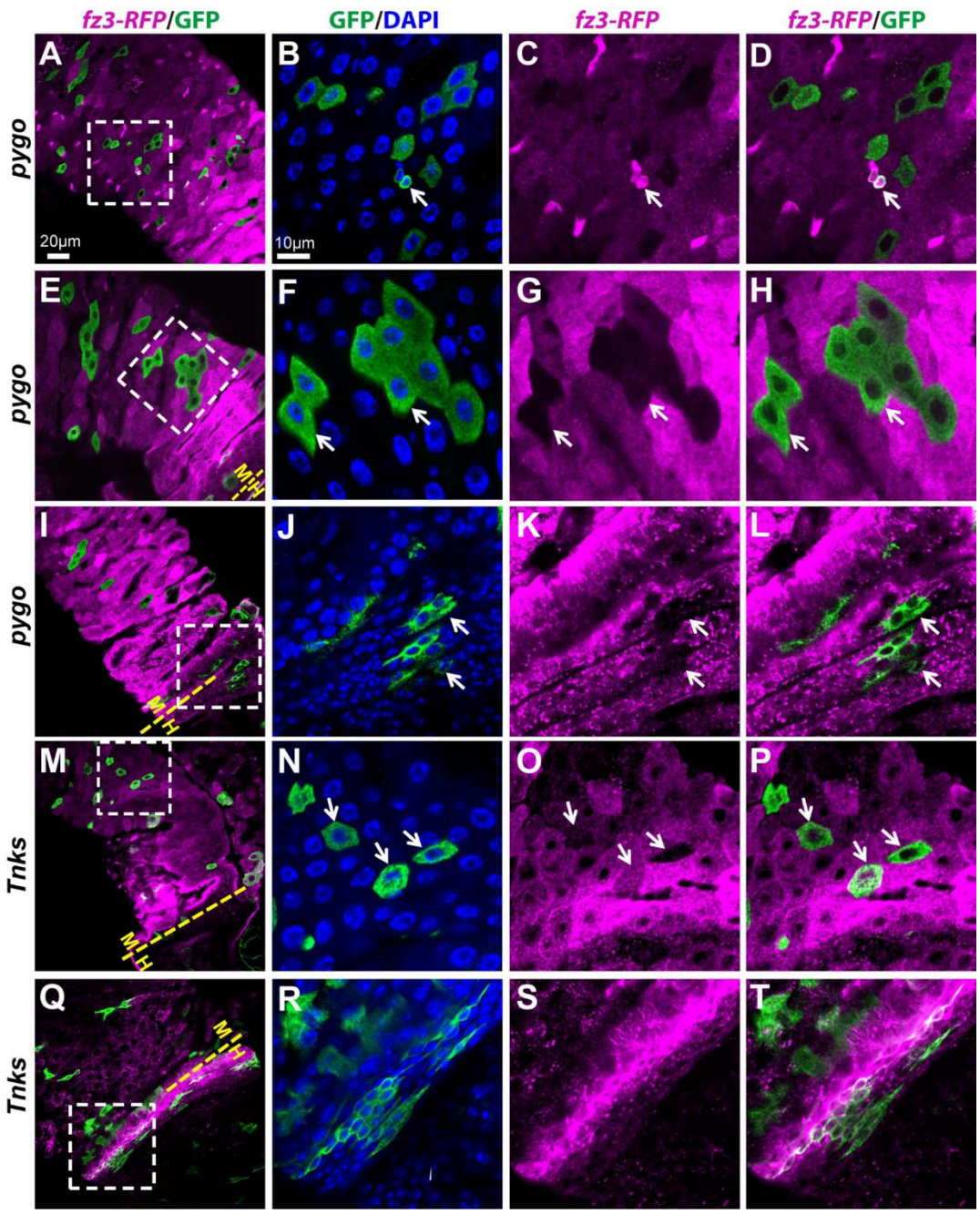

Fig S2: Tnks promotes Wingless target gene activation in regions where the pathway activity is relatively low

(A-D) *fz3-RFP* expression in ISCs is not dependent on Wingless signaling activation. $pygo^{S123}$ clones (marked by GFP) were induced in flies expressing *fz3-RFP*. *fz3-RFP* (magenta) remains the same in $pygo^{S123}$ mutant ISCs (arrow). (E-L) *fz3-RFp* expression in

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ECs is dependent on Wingless signaling activation. *fz3-RFP* (magenta) is absent in *pygo*^{S123} mutant clones (arrows) both away from the compartment boundary (E-H) and near the boundary (I-L). (M-T) Tnks is required for *fz3-RFP* expression in regions away from the compartment boundary (M-P), but is dispensable near the boundary where Wingless pathway activity is high (Q-T). Mutant clones (marked by GFP) were induced in 3rd instar larvae and examined 1-2 days after eclosion. Low magnification images are shown in A, E, I, M and Q with yellow dashed line indicating the midgut/hindgut boundary. Higher magnification views of the boxed areas are shown on the right.

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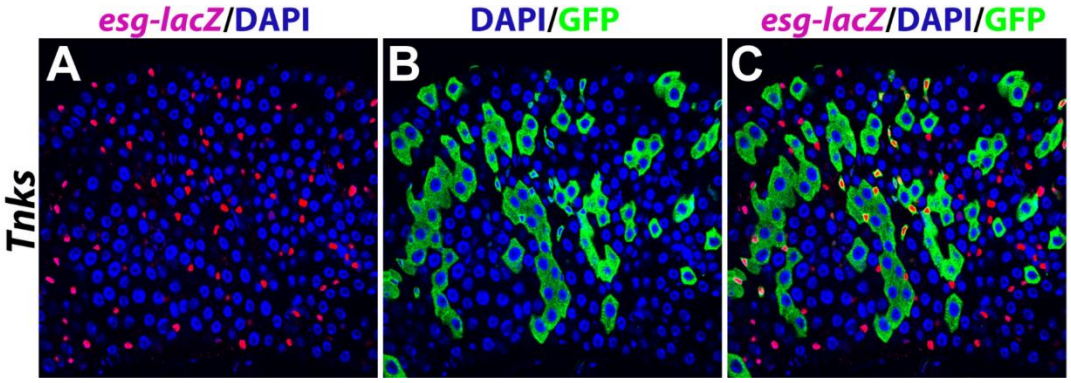

Fig S3: Loss of Tnks during pupal development has no effect on ISC proliferation at eclosion

Tnks clones (marked by GFP) were induced in 3rd instar larvae and analyzed on the day of eclosion. Progenitor cells, indicated by *esg-lacZ*, are evenly scattered along the midgut.

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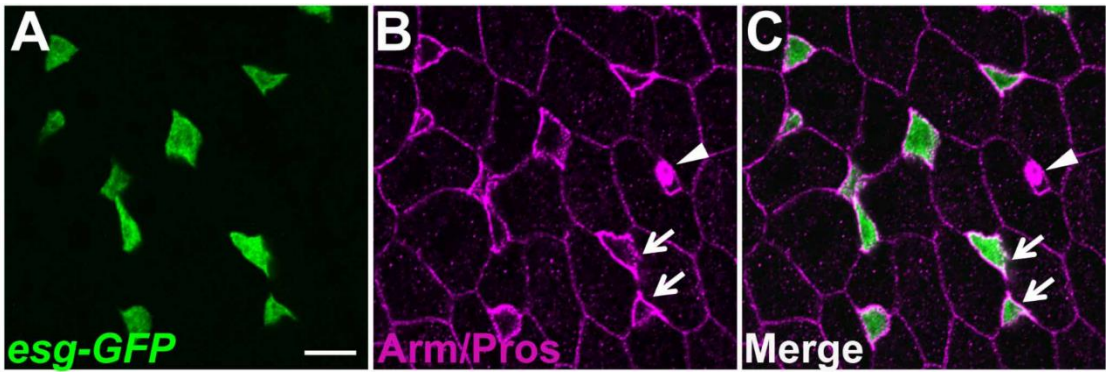

Fig S4: ISCs and EBs can be distinguished by their small size, high levels of membrane-associated Armadillo, and lack of nuclear Prospero

5-day-old female midguts expressing *esg-Gal4, UAS-GFP (esg>GFP)* were stained with anti-GFP (green), anti-Armadillo (Arm) (magenta) and anti-Prospero (magenta) antibodies. Progenitor cells (marked by *esg>GFP*) have smaller cell size and strong membrane-associated Arm staining (arrow). Differentiated EEs can be distinguished by Prospero staining (arrow head). Scale bar: 10μm.

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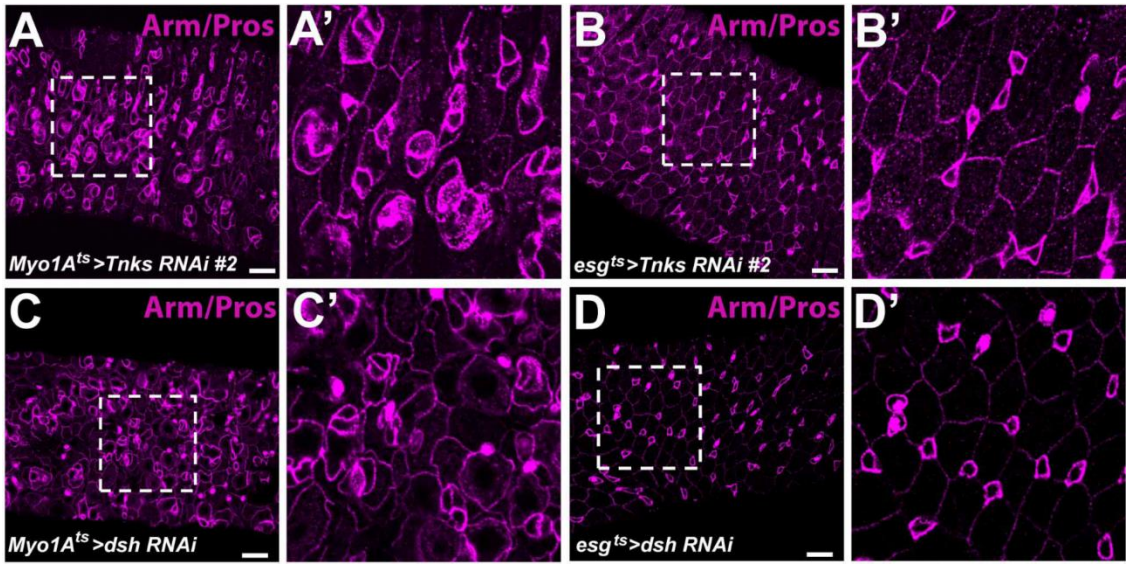

Fig S5: Loss of Tnks or Dishevelled (Dsh) in ECs causes overproliferation of ISCs

Knockdown of Tnks in ECs using the independent *Tnks-RNAi#2* line (A and A') results in overproliferation of ISCs; however, knockdown of Tnks in progenitor cells (B and B') using the same *Tnks-RNAi* line has no effects. Adults expressing *dsh RNAi* (C and C') in ECs display an increased number of progenitor cells, while those expressing the RNAis in progenitor cells (D and D') have no defects. Adult females were shifted to 29°C after eclosion for 7 days and midguts were stained with anti-Arm and anti-Prospero (magenta) antibodies. Scale bar: 20μm.

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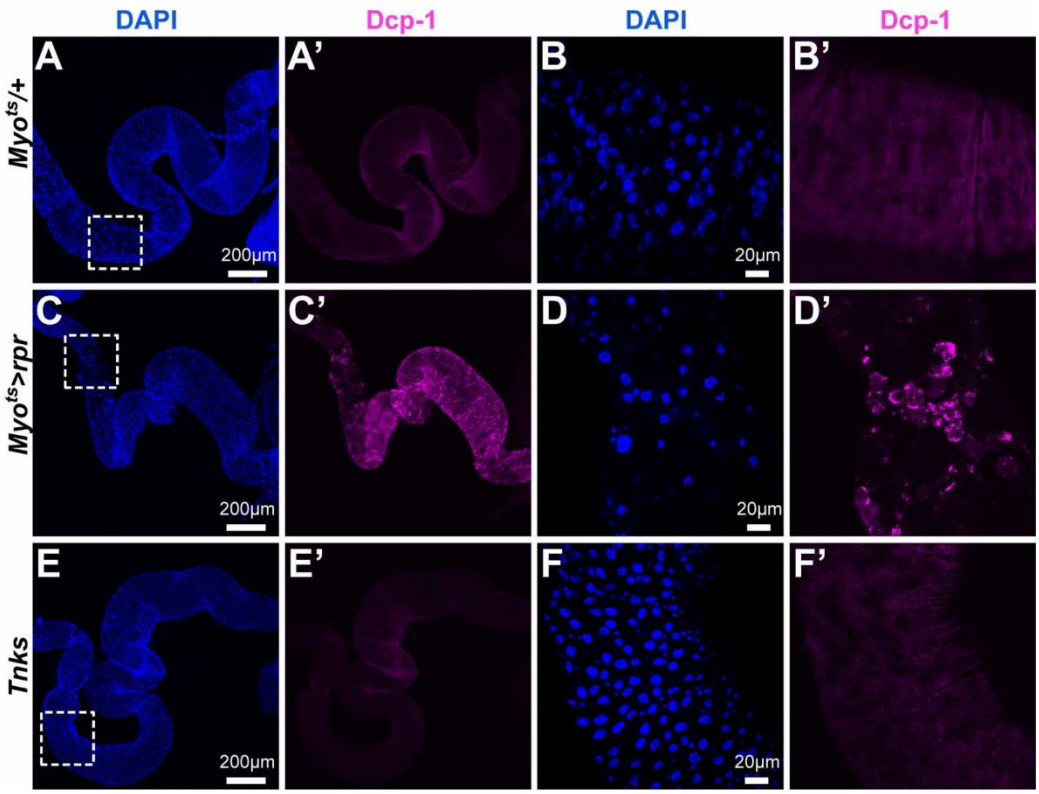

Fig S6: Loss of Tnks does not induce ectopic cell death

(A-D') Testing the specificity of Dcp-1 antibody. Three days after eclosion, control flies (*Myo^{ts}-Gal4*/+) or flies expressing *Myo^{ts}-Gal4, UAS-reaper* were shifted to 29°C for 42 hours and stained with Dcp-1 antibody. Ectopic Dcp-1 staining was observed when cell death was induced in ECs (C-D), but not in control flies (A-B). No ectopic expression of Dcp-1 was observed in *Tnks* mutant flies (E-F'). Low magnification images are shown in A, C and E. Higher magnification views of the boxed areas are shown in B, D and F. Images were obtained under the same setting.

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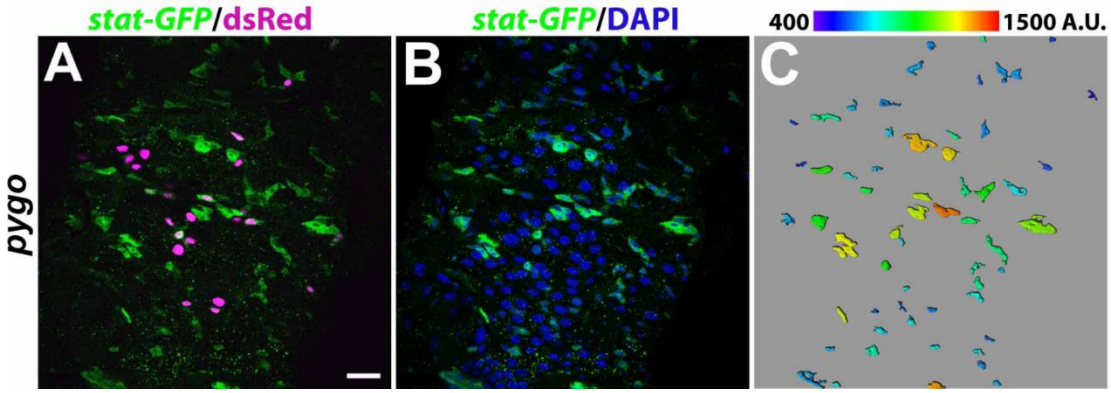

Fig S7: Loss of Pygo non-autonomously activates JAK/STAT signaling

(A-B) Expression of *stat-GFP* in midguts with $pygo^{S123}$ clones (magenta). *stat-GFP* (green) is expressed ectopically in progenitor cells adjacent to $pygo^{S12}$ clones (magenta), as compared to regions farther away from the clones. (C) Color-coded representation of *stat-GFP* intensity. Scale bar: 20µm.

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