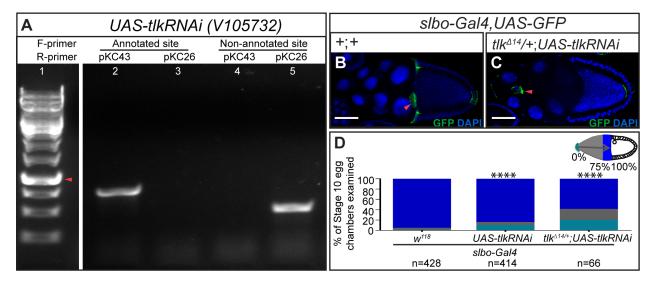
Supplemental Materials Molecular Biology of the Cell

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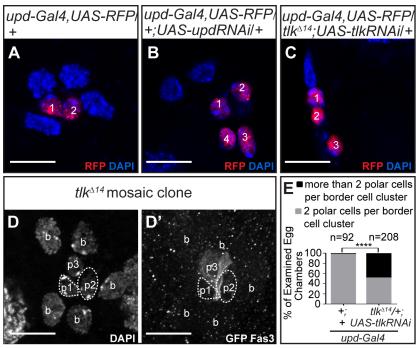
SUPPLEMENTAL MATERIAL



Supplemental Figure 1. PCR-based diagnosis of *UAS-tlkRNAi* (*V105732*) and phenotype test in border cells. (A) Lane 1 shows the 1kb ladder. Red arrowhead points to 1kb. The forward primer for lanes 2 and 3 is from the so-called annotated site. Reverse primers are for the pKC43 vector (lane 2) and the pKC26 vector (lane 3). In lanes 4 and 5, the forward primer is from the so-called non-annotated site and reverse primers are from pKC43 vector (lane 4) and pKC26 vector (lane 5). The white line in between lanes 1 and 2 indicates the deletion of some irrelevant lanes. (B, C) Confocal micrographs of Stage 10 egg chambers of the indicated genotypes. Red arrowheads indicate border cell clusters. Scale bars, 50 μ m. (D) Quantification of migration defects in the indicated genotypes. Flies were fattened at 29°C. We adopted Mann-Whitney U test to analyze the statistics significance of border cell migration defect, and **** indicates p<0.0001.

Cell type	Genotype	% of cCasp-3 ⁺ egg chambers	Ν
Polar cells	upd-Gal4,UAS-RFP	0	101
	tĺk ^{∆14} /upd-Gal4,UAS- RFP,UAS-tlkRNAi	0	71
Border cells	tlk ^{∆14} /upd-Gal4,UAS- RFP,UAS-tlkRNAi	0	71
Other follicle cells	FRT101 clone	2.44	41
	<i>tlk^{∆14}</i> clone	42.86	84

Supplemental Table 1. Tlk is not required for polar cell or border cell viability.



Supplemental Figure 2. *tlk* mutation in polar cells causes extra polar cells. (A-D') Confocal micrographs of border cell clusters in indicated genotypes. (A-C) RFP and numerals label polar cells. (D and D') A border cell cluster containing two *tlk* mutant polar cells (p1 and p2, GFP-negative and Fas3-positive, circled by white dotted lines) and one wild type polar cell (p3, GFP-positive and Fas3-positive). Polar cells and border cells are labeled with "p" and "b", respectively. Scale bars, 10 µm. (E) Quantification of egg chambers containing more than two polar cells per cluster. 52% of *upd-Gal4>UAS-tlkRNAi* egg chambers contained more than two anterior polar cells. We used t-test for statistical significance, and **** indicates p<0.0001.

Gene	RNAi lines	Border cell	Additional	Border cell	Mutant alleles	Border cell
	tested in 1°	defect	RNAi lines	defect	tested	defect
	screen		tested			
tlk	V105732	+	B33983	+	tlk ^{∆14}	+
			B36102	+	N.D.	
			B35298	+	N.D.	
dom	V7787	+	N.D.		dom ¹	+
	V7789	+	N.D.		dom³	+
asf1	V23737	+	N.D.		N.D.	
	V23738	-	N.D.		N.D.	
	V107711	+	N.D.		N.D.	
HDAC4	V20522	+	N.D.		N.D.	
not	V45775	+	N.D.		N.D.	
	V45776	+	N.D.		N.D.	
Usp7	V18231	+	N.D.		N.D.	
Mt2	V37815	+	N.D.		N.D.	
phol	V26196	+	N.D.		N.D.	
piwi	V22235	+	N.D.		N.D.	
N.D. not	determined					

Supplemental Table 2. Candidate border cell migration genes identified in the RNAi screen.