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Supporting Information

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Control of Germline *torso* Expression by the BTB/POZ Domain Protein Pipsqueak Is Required for Embryonic Terminal Patterning in *Drosophila*

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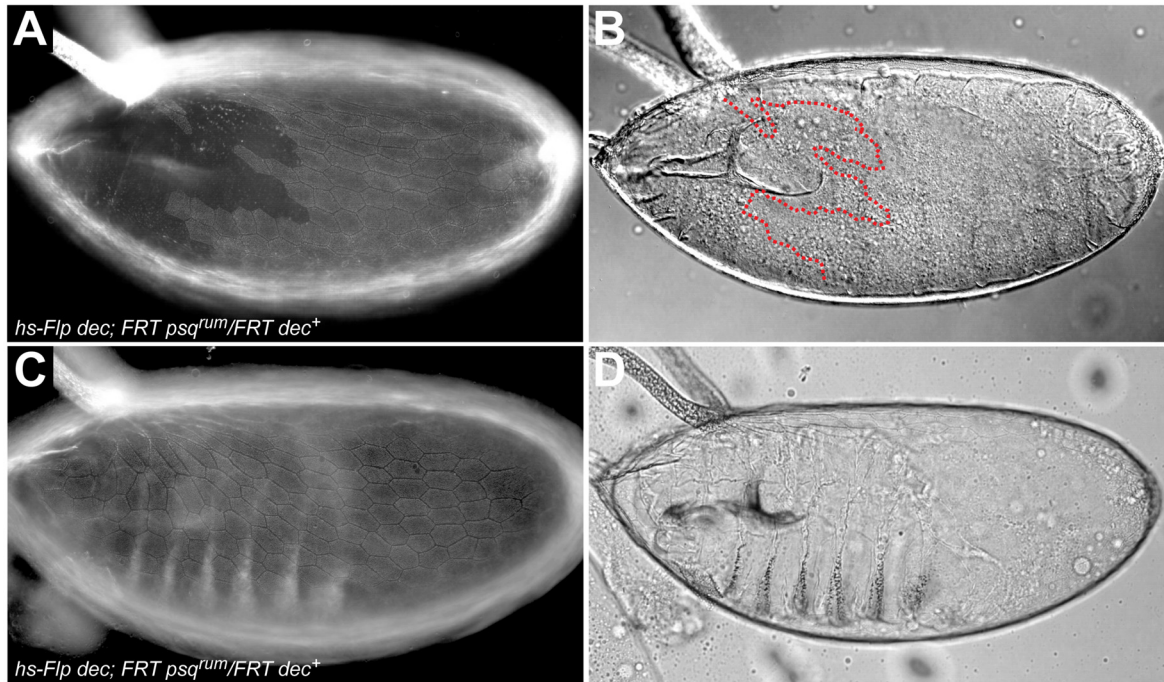


FIGURE S1.—*psq^{rum}* mutant follicle cell clones do not affect terminal patterning. (A,B) *psq^{rum}* somatic clones in the follicle epithelium do not affect terminal regions in the embryo. *psq^{rum}* mutant follicle cells are marked by the loss of the *dec⁺* marker, resulting in a transparent chorion. A large *dec⁻; psq^{rum}* clone covers the anterior part of the egg shown in (A). Note the presence of a normal head skeleton in the phase contrast view shown in (B). The border of the clone is indicated by a hatched red line in (B). (C,D) Conversely, *psq^{rum}* germline clones produce terminal phenotypes even when somatic follicle cells are wild-type as detected by the absence of *dec⁻* clones. (A) and (C) are dark-field images. (B) and (D) are phase-contrast images of the embryos shown in (A) and (C), respectively.

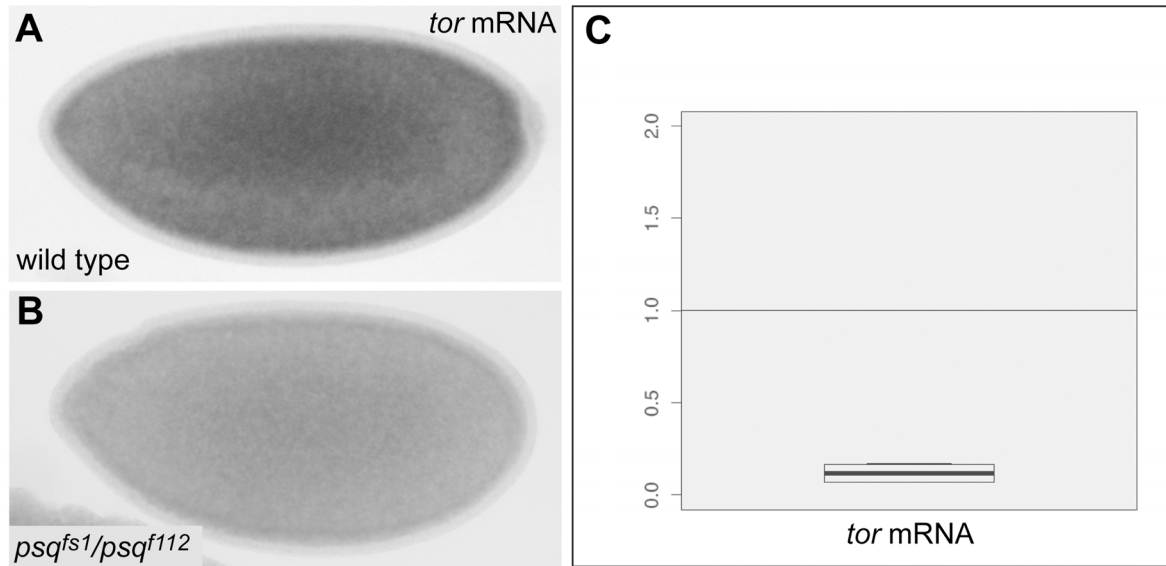


FIGURE S2.—*tor* mRNA levels are reduced in ovaries and embryos from *psq^{fs1}/psq^{f112}* transheterozygous females (A,B) *tor* *in situ* hybridization in wild-type embryo (A) and *psq^{fs1}/psq^{f112}* mutant (B). *tor* mRNA levels are strongly reduced in *psq^{fs1}/psq^{f112}* embryos. (C) *tor* mRNA is eight-fold reduced in *psq^{fs1}/psq^{f112}* ovaries compared to the wild type, as detected by real-time PCR. The Y-axis indicates relative transcript levels as fold-change between wild-type and *psq^{fs1}/psq^{f112}* ovaries.

TABLE S1

Analysis of heteroallelic combinations between *psq^{rum}* and other *psq* alleles or deficiencies uncovering *psq*

| <i>psq</i> alleles or deficiencies | homozygous phenotype | maternal-effect phenotype of heteroallelic combination with <i>psq^{rum}</i> | nature of mutation | reference |
|------------------------------------|---|---|--|---------------------------------|
| <i>psq[rum]</i> | All embryos show terminal defects (lack of structures posterior to A7; n=100). In addition embryos frequently show deletions of one or more abdominal segments. | | EMS-induced; G to A nucleotide substitution affecting the splicing donor site of intron 6, which is common to all known <i>psq</i> transcripts. This leads to partial retention of the intron in the mRNA and is predicted to cause the addition of 16 amino acids followed by a stop codon. | this work |
| <i>psq[8109]</i> | Decreased viability of adults, female sterile, posterior group, dorsalized eggshell and embryo, early oogenesis defects. | 96% of embryos show little cuticle, 4% of embryos develop contiguous cuticle and lack terminal structures (n=100) | P{PZ} insertion into the largest intron of <i>psq-1</i> . Aberrant fusion protein created. | HOROWITZ and BERG 1995 |
| <i>psq[0115]</i> | Decreased viability of adults, female sterile, posterior group, dorsalized eggshell and embryo, early oogenesis defects. | 94% of embryos show little or no cuticle, 6% of embryos develop contiguous cuticle and lack terminal structures (n=100) | P{PZ} insertion into the largest intron of <i>psq-1</i> . Aberrant fusion protein created. | HOROWITZ and BERG 1995 |
| <i>psq[fs1]</i> | Female sterile, posterior group, grandchildless | Embryos do not hatch. 80% of embryos lack segment A8 and/or spiracles (n=50) | P{lacW} insertion into the first intron of <i>psq-1</i> . | SIEGEL <i>et al.</i> 1993 |
| <i>psq[rev2]</i> | n.d. * | Fertile | Isolated as EMS-induced revertant of <i>psq</i> overexpression in the eye. Deletion of 11 bp, resulting in a premature stop codon in the BTB domain. The P{GSV1}lola[GS88A8] insertion is still present on the chromosome. | FERRES-MARCO <i>et al.</i> 2006 |

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|-------------------|--------|--|---|---------------------------------|
| <i>psq[rev4]</i> | n.d. * | Many collapsed eggs. 6.25% of embryos develop contiguous cuticle. These embryos lack abdominal segments (n=80). | Isolated as EMS-induced revertant of <i>psq</i> overexpression in the eye. Nucleotide substitution causing a Q530>stop mutation. The P{GSV1}lola[GS88A8] insertion is still present on the chromosome. | FERRES-MARCO <i>et al.</i> 2006 |
| <i>psq[rev7]</i> | n.d. * | Fertile | Isolated as EMS-induced revertant of <i>psq</i> overexpression in the eye. Amino acid replacement M1I in the start Methionine of Psq1. The P{GSV1}lola[GS88A8] insertion is still present on the chromosome. | FERRES-MARCO <i>et al.</i> 2006 |
| <i>psq[rev9]</i> | n.d. * | Weakly fertile. Adults show narrow blistered wings with extra veins. Embryos do not show terminal or other morphological defects (n=50). | Isolated as EMS-induced revertant of <i>psq</i> overexpression in the eye. G to A nucleotide substitution affecting the splicing donor site of intron 2 (72 bp), leading to retention of the intron in the mRNA. This causes the in-frame addition of 24 amino acids within the BTB/POZ domain, presumably disrupting the conserved BTB pocket domain, which is important for dimerization. The P{GSV1}lola[GS88A8] insertion is still present on the chromosome. | FERRES-MARCO <i>et al.</i> 2006 |
| <i>psq[rev12]</i> | n.d. * | 90% of embryos show little or no cuticle, 10% of embryos develop contiguous cuticle and lack terminal structures (n=80) | Isolated as EMS-induced revertant of <i>psq</i> overexpression in the eye. Amino acid replacement G867D in the third PSQ repeat. The P{GSV1}lola[GS88A8] insertion is still present on the chromosome. | FERRES-MARCO <i>et al.</i> 2006 |

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|---------------------|---|--|--|---------------------------------|
| <i>psq[rev14]</i> | n.d. * | 98% of embryos show little or no cuticle, 2% of embryos develop contiguous cuticle and show posterior and terminal defects (n=100) | Isolated as EMS-induced revertant of <i>psq</i> overexpression in the eye. Nucleotide substitution causing a Q582>stop mutation. The P{GSV1}lola[GS88A8] insertion is still present on the chromosome. | FERRES-MARCO <i>et al.</i> 2006 |
| <i>psq[KG02404]</i> | Viable, female-sterile | Embryos do not hatch, 60% of embryos lack segment A8 and/or spiracles (n=50) | P{SUPor-P} insertion in <i>psq-1</i> 5'-UTR | BELLEN <i>et al.</i> 2004 |
| <i>psq[KG00811]</i> | Lethal | Fertile | P{SUPor-P} insertion in the largest intron of <i>psq-1</i> , 0.7 kb downstream of the transcription start site of <i>psq-3</i> transcripts | BELLEN <i>et al.</i> 2004 |
| <i>psq[EP2011]</i> | Viable, female-sterile. Homozygous females lay no eggs. | Embryos do not develop cuticle (n=100). | P{EP} insertion in the largest intron of <i>psq-1</i> , 71 bp upstream of the transcription start site of <i>psq-3</i> transcripts | BELLEN <i>et al.</i> 2004 |
| <i>Df(2R)47A</i> | | Most embryos undeveloped, few embryos with little cuticle | | FlyBase |
| <i>Df(2R)E3363</i> | | Most embryos undeveloped, few embryos develop and show terminal defects | | FlyBase |

Phenotypes of embryos from females carrying heteroallelic combinations of *psq^{um}* are indicated. *: We were unable to determine homozygous phenotypes of the *psq* revertant alleles, all of which are lethal in homozygous state and in all trans-heterozygous combinations tested. Lethality of these chromosomes is presumably due to the presence of additional mutations or due to the P{GSV1}lola[GS88A8] P-element insertion.

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