Title:

Genome-wide screen for new components of the *Drosophila melanogaster* Torso receptor tyrosine kinase pathway

Authors:

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Abstract

Patterning of the *Drosophila* embryonic termini by the Torso (Tor) receptor pathway has long served as a valuable paradigm for understanding how Receptor Tyrosine Kinase (RTK) signalling is controlled. However, the mechanisms that underpin the control of Tor signalling remain to be fully understood. In particular, it is unclear how the Perforin-like protein Torso-like (Tsl) localises Tor activity to the embryonic termini. To shed light on this, together with other aspects of Tor pathway function, we conducted a genome-wide screen to identify new pathway components that operate downstream of Tsl. Using a set of molecularly-defined chromosomal deficiencies, we screened for suppressors of ligand-dependent Tor signalling induced by unrestricted Tsl expression. This approach yielded 59 genomic suppressor regions, 11 of which we mapped to the causative gene, and a further 29 that were mapped to less than 15 genes. Of the identified genes, six represent previously unknown regulators of embryonic Tor signalling. These include *twins*, which encodes an integral subunit of the protein phosphatase 2A complex, and *α-tubulin at 84B*, a major constituent of the microtubule network, suggesting that these may play an important role in terminal patterning. Together these data comprise a valuable resource for the discovery of new Tor pathway components. Many of these may also be required for other roles of Tor in development, such as in the larval prothoracic gland where Tor signalling controls the initiation of metamorphosis.
Introduction

The first events that define the major axes and termini of the early *Drosophila* embryo are governed by genes expressed in the mother (St Johnston & Nüsslein-Volhard, 1992). Patterning of the termini is achieved by spatially localised activation of the receptor tyrosine kinase (RTK) Torso (Tor) and signal transduction via the highly conserved Ras/mitogen-activated protein kinase (MAPK) cascade. This pathway leads to the transcriptional derepression of key zygotic target genes including *tailless* (*tll*) and *huckebein* (*hkb*) to drive terminal cell fate specification (Brönner & Jäckle, 1991). Since its discovery, the Tor pathway/terminal patterning system has been a valuable paradigm for understanding fundamental aspects of how RTK signalling is controlled in space and time (for review see Li, 2005).

Despite much interest, however, several key features of the terminal patterning system remain unclear. This includes the mechanism by which Tor is activated by its ligand Trunk (Trk) only at the ends of the embryo. A significant body of work suggests that Tor activation is controlled by Torso-like (Tsl), a protein localised to the early embryo termini (Savant-Bhonsale & Montell, 1993; Martin *et al.*, 1994). For example, Tor signalling is not activated in the absence of Tsl (Sprenger *et al.*, 1993), and unrestricted expression of Tsl induces ubiquitous Tor signalling (Savant-Bhonsale & Montell, 1993; Martin *et al.*, 1994).

Tsl is a member of the perforin-like superfamily of proteins best characterised in terms of their membrane pore-forming roles in vertebrate immune defence (Rosado *et al.*, 2008; Law *et al.*, 2010; Dudkina *et al.*, 2016). Current hypotheses for Tsl function include: the control of Trk proteolysis and activation (Casanova *et al.*, 1995; Casali & Casanova, 2001), Trk secretion (Johnson *et al.*, 2015), and facilitating Tor dimerisation or the binding of Trk to Tor (Amarnath *et al.*, 2017). For each of these possibilities it is highly likely that
additional pathway components remain undiscovered, and their identification could provide valuable insights into the mechanism controlling localised Tor activation.

A number of screening approaches have previously been used to identify genes involved in terminal patterning. The terminal class genes were first discovered in early mutagenesis screens that identified many components of the major developmental pathways required for embryonic patterning (Schupbach & Wieschaus, 1986; Nusslein-Volhard et al., 1987; Schupbach & Wieschaus, 1989). However, a major limitation of this approach is that the maternal effect of a given allele could only be examined if homozygous mothers were viable. Zygotic lethal maternal effect screens and dominant modifier screens have been used to overcome this problem (Strecker et al., 1991; Perrimon et al., 1996; Bellotto et al., 2002; Li & Li, 2003; Luschnig et al., 2004). Unlike the saturating whole-genome mutant screens, however, zygotic lethal screens are low-throughput and hence were performed on a smaller scale. Furthermore, this approach precludes the detection of genes that perform critical roles during oogenesis and prior to pattern formation. In contrast, dominant modifier screens are not limited in this regard and have been successfully performed using the phenotype produced by tor gain-of-function alleles to find new genes that act downstream of Tor (Strecker et al., 1991; Li & Li, 2003). However, use of these alleles does not permit the discovery of potential interactors involved in generating the active Tor ligand (ie. acting upstream of tor).

Since we were interested in the events upstream of Tor, we conducted a genome-wide screen to identify suppressors of the phenotype produced upon ectopic tsl expression. This screen has the ability to identify new pathway components that function either upstream or downstream of tor and led to the identification of six genes that have not previously been associated with embryonic terminal patterning. In addition, we performed detailed mapping
for a further 48 genomic suppressor regions that may contain novel regulators of ligand-dependent Tor signalling.

Materials and Methods

Drosophila stocks

The following stocks were used: \( w^{1118}\) (BL5905), \( P\{GawB\}c355 \) (called \( c355\)-Gal4, from BL3750), \( P\{tubP-GAL80^{ts}\} \) (from BL7018), \( UAS-tsl \) (Johnson et al., 2013), \( nos-Cas9 \) (CAS-0001) and \( P\{nos-\Phi C31\} \) lines (TBX-0002 and TBX-0003; Kondo & Ueda, 2013), and the BDSC deficiency kit (Cook et al., 2012). All non-kit deficiency lines used for mapping are in Table 2 and alleles tested in Table S2. All flies were maintained on standard media at 25°C unless otherwise stated.

Screening crosses

Autosomal deficiencies or mutant alleles were tested by crossing virgin females from the screen line (\( c355\)-Gal4; Gal80\(^{ts}\); UAS-tsl) to deficiency males. X chromosome deficiencies or mutant alleles were tested by crossing deficiency females to males from the screen line. In all cases crosses were performed at 22°C and cultures shifted to 29°C approximately 92h post egg lay. From each cross, at least 10 F1 females carrying the deficiency chromosome and screening transgenes were placed in a vial containing apple juice agar supplemented with yeast paste and allowed to mate with \( w^{1118}\) males.

Cuticle preparations and scoring
Adults were allowed to lay for 24 h and embryos were left to develop for a further 24 h. Embryos were then dechorionated in 50% (vol/vol) bleach and mounted on slides in a mixture of 1:1 (vol/vol) Hoyer’s solution: Lactic acid. Slides were incubated overnight at 65°C and imaged using dark field optics (Leica). Three consecutive cuticle preps were performed for each deficiency and if suppression was observed the deficiency was retested at least twice. Suppression strength was qualitatively assessed based on the number of embryos showing central segment gain and the number of segments gained compared to controls (screen line crossed to w^{1118}).

**Immunostaining and imaging**

Ovaries were dissected in phosphate buffered saline (PBS) while on ice and fixed in 4% paraformaldehyde in PBS for 1 hour. Ovaries were then washed 5 times in PBS with 0.1% Triton-X (PTx) followed by blocking in 5% goat serum in PTx and incubation with anti-Tsl (1:500) overnight period at 4°C. Anti-Tsl was raised in a rabbit against a peptide consisting of the C-terminal 18 residues of Tsl and affinity purified (Genscript). We note that this antibody does not detect endogenous Tsl and only recognises overexpressed Tsl in fixed tissue. Ovaries were then washed 5 times with PTx and incubated with anti-rabbit Alexa568 (Thermofisher Scientific, 1:500) secondary antibody in PTx. Following washing in PTx, ovarioles were further dissected and mounted on slides in VectaShield (Vector Laboratories). Single confocal sections of stage 9 egg chambers were captured on a Nikon C1 confocal microscope at 20X magnification.

**Generation of Rab3-GEF**

Transgenic flies expressing two guide-RNAs targeting Rab3-GEF (535: 5’-ATC GGT TCG GGA TAG TCT TC-3’; 5244: 5’-AGT CAG GAG CGT GAT ATG AT-3’) were made by cloning annealed guide sequence oligos into the pBFv-U6.2 vector (Kondo & Ueda, 2013) followed by genomic integration via ΦC31-mediated transgenesis (attP40 and attP2 landing sites). These flies were crossed to the Cas9 source and single lines carrying deletions between the two guide-RNA target sites were established. The genomic deletion in line 5 (coordinates: X:15,097,551-15,102,259 inclusive, D. melanogaster release 6.18) removes 4708bp and is predicted to truncate the Rab3-GEF protein (PC isoform) at residue L546 (of 2084 amino acids) and add a short out-of-frame carboxy-terminal extension of 12 residues. We note that these flies are homozygous viable and fertile.

**Data and reagent availability statement**

Data and reagents are available upon request. Table S1 contains a list of the Bloomington Drosophila stock centre deficiency kit deficiencies that do not suppress the ectopic Tsl phenotype and Table S2 lists the alleles of genes within suppressor regions that were screened.
Results

Normally tsl expression is restricted to subpopulations of follicle cells located at the anterior and posterior ends of the developing oocyte (Savant-Bhonsale & Montell, 1993; Martin et al., 1994). Expressing tsl ectopically in all follicle cells (c355-Gal4; UAS-tsl, Figure 1) causes a highly consistent maternal embryonic lethal cuticle phenotype whereby the terminal regions are expanded and all central segments are lost (known as spliced, Savant-Bhonsale & Montell, 1993; Martin et al., 1994). Importantly, Tor activation in this scenario is ligand-dependent and thus may also be sensitive to loss of genes that function upstream of tor, such as those involved in ligand generation and trafficking, in addition to those that act downstream.

In order to conduct an F1 suppressor screen using this background we first needed to overcome the maternal sterility associated with ectopic tsl expression. This was necessary to enable the screening line to be crossed directly to chromosomal deficiencies and mutant alleles and examine embryos produced by the offspring. We achieved this using the temperature-sensitive (ts) repressor of Gal4, Gal80ts (McGuire et al., 2004). When females (c355-Gal4; Gal80ts; UAS-tsl) were raised at 22°C, Gal80ts efficiently prevented ectopic maternal tsl expression (Figure 1B) and females produced embryos with wildtype cuticles (Figure 1C). Raising females at 29°C, either from the third instar larval stage or shifting adults raised at 22°C to this temperature, fully restored ectopic tsl expression (Figure 1D) and induced the spliced phenotype (Figure 1E).

To test whether this line was suitable for a suppressor screen we introduced one copy of a strong hypomorphic allele of trk (trk3), a terminal-class gene known to be limiting with respect to Tor signalling (Sprenger & Nusslein-Volhard, 1992). This strongly suppressed the ectopic tsl phenotype as evidenced by restoration of multiple central segments in almost all
embryos (Figure 1F). A similar degree of suppression was observed using one copy of a chromosomal deficiency that deletes a genomic region including \( trk \) (Df[2L]BSC143; Figure 1G). Taken together these data suggest that the ectopic \( tsl \) phenotype is sensitive to \( tor \) pathway gene dosage and may serve as a discovery tool for new pathway components including genes that act upstream of \( tor \).

To identify regions of the genome that contain novel Tor pathway genes, we performed a screen using the Bloomington Drosophila Stock Centre (BDSC) deficiency kit. This is a collection of 467 lines each containing a genomic deletion with molecularly defined breakpoints, collectively covering approximately 98% of euchromatic genes in \( Drosophila melanogaster \) (Cook et al., 2012). Of the 467 lines, data was obtained for 429 lines, equating to approximately 90% of the genome (Table 1). The remaining lines were mostly deficiencies on the X chromosome that were problematic due to poor culture health. The screen identified 65 deficiencies that consistently suppressed the ectopic \( tsl \) phenotype to varying degrees (Table 2), and 363 deficiencies that showed no suppression (Table S1). Most of the observed suppressor deficiencies were moderate in strength (gain of at least one segment, 44 deficiencies in the primary screen), while 17 were scored as strong suppressors (gain of multiple segments), and one was a weak suppressor. Very strong suppression (gain of multiple segments and partially restored viability) was observed for three deficiencies: \( Df(1)ED7005 \) and \( Df(1)ED7289 \), neither of which span previously known \( tor \) pathway genes, and \( Df(2L)BSC143 \), which spans \( trk \). We note that 7 suppressor deficiencies overlap in the regions they delete and hence these may represent a single suppressor region. After such cases were accounted for, we estimated there to be at least 59 individual suppressors (hereon referred to as suppressor regions) across the three major chromosomes (Table 1). To reduce the sizes of the regions and narrow the list of candidate suppressor genes further, mapping was conducted using available additional deficiency lines (Table 2). This markedly reduced
the number of candidate genes, leaving 15 genes or less in 29 of the regions and five genes or less in 9 of the regions.

We next wanted to identify the genes responsible for the observed suppression in each of these regions. To do this we used available known loss-of-function and potential loss-of-function mutant alleles, as well as mutant alleles that we generated (Rab3-GEF$^5$, this study and trk$^4$, Henstridge et al., 2014), and tested whether they too could suppress the ectopic tsl phenotype. In total we tested mutant alleles of 88 genes (Table S2) and successfully identified the causative suppressor gene in 10 of the regions (in addition to trk, Table 3). Four of these genes; Ras oncogene at 85D (Ras85D), Son of sevenless (Sos), tailless (tll) and C-terminal Binding Protein (CtBP) have known roles in terminal patterning (Pignoni et al., 1990; Simon et al., 1991; Lu et al., 1993; Cinnamon et al., 2004) and thus further validate the screening approach. In each of these cases, the strength of suppression caused by the original deficiency was moderate to strong and this matched well with the alleles we tested. Notably, many deficiencies that include other previously identified terminal class genes were not identified as suppressors. This suggests that these genes may not be dosage limiting for the ectopic tsl phenotype (Table 4). The remaining six genes that we identified have no previously known involvement in terminal patterning and thus represent potentially novel regulators of the Tor pathway. These genes are: Tetraspanin 3A (Tsp3A), Ribosomal protein S21 (Rps21), Ribosomal protein S26 (Rps26), Protein disulphide isomerase (Pdi), α-Tubulin at 84B (αTub84B) and twins (tws). As suppressors of the ectopic tsl phenotype, our findings suggest that these genes are haploinsufficient with respect to unrestricted Tor signalling.

Discussion
We were initially surprised by the large number of suppressor regions detected in our screen. A previous screen performed using the tor\(^{Y9}\) gain-of-function allele found only 26 suppressor regions (Li & Li, 2003). While this study used a different deficiency kit with cytologically defined breakpoints and larger deleted regions, the difference between these figures could also reflect differences in our approaches. Our approach is both ligand-dependent and also sensitive to dosage limited genes that act either upstream or downstream of tor. It is therefore possible that the additional suppressor regions we detected contain genes required for generation of the active Tor ligand. In addition, because our screening strategy relied upon Gal4/UAS driven expression of a tsl transgene, genes required for production of ectopic Tsl (i.e. involved in protein translation, etc) could act as suppressors. Indeed, the suppressor genes RpS21 and RpS26 that we identified may be haploinsufficient in this regard. Both genes encode crucial structural constituents of the ribosome and are required for protein translation (Marygold et al., 2007). Despite this, five of the 11 genes that we identified in our screen are known terminal patterning genes. Thus, many of the suppressor regions remaining are likely to house genes relevant to Tor signalling rather than suppressors of Gal4 driven tsl expression. A brief discussion of each identified suppressor gene in the context of this screen is given below.

**Tetraspanin 3A**

*Tsp3A* is one of 35 known tetraspanin genes in the *Drosophila* genome (Fradkin et al., 2002). Members of the tetraspanin superfamily are characterised by the presence of four transmembrane segments and associate with each other and other transmembrane proteins to carry out cellular functions such as migration, fusion, adhesion, immune response, and intracellular vesicle trafficking (for review see Zhang & Huang, 2012). However, the mechanism of tetraspanin function remains poorly understood (Zhang & Huang, 2012). The
allele of Tsp3A (Tsp3A<sup>e03287</sup>) responsible for suppression of the ectopic tsl phenotype is homozygous viable and fertile, and exhibits no terminal patterning defects (not shown). This could possibly be explained by functional redundancy between Tsp3A and other tetraspanin-encoding genes during normal development, and Tsp3A dosage sensitivity in our screening background. In this regard, Tsp26A and Tsp86D are known to act redundantly with Tsp3A in the promotion of Notch signalling for ovarian border cell migration (Dornier et al., 2012). Whether tetraspanins including Tsp3A play a similar role at the embryo plasma membrane for a Tor signalling factor will be interesting to determine.

**Protein disulphide isomerase**

Pdi encodes one of the two major Protein Disulphide Isomerases found in *Drosophila*. The primary function of these enzymes is to form and break disulfide bonds between cysteine residues as proteins fold in the endoplasmic reticulum (ER, Freedman, 1989; Noiva & Lennarz, 1992). In our screen Pdi was identified as a strong suppressor and thus may play an important role in terminal patterning. How might it achieve this? The Pdi-like protein Windbeutel (Thioredoxin/ERp29 superfamily; Konsolaki & Schupbach, 1998) is required for the trafficking of Pipe through the ovarian follicle cell ER to assist in localising the signal for dorsoventral patterning (Sen et al., 2000). Thus Pdi may be essential for the correct folding and/or transport of one or more key Tor signalling components. This could include genes that function at the level of the ligand, receptor or downstream pathway components.

**α-Tubulin at 84B**

α- and β-Tubulin proteins are the major structural constituents of the eukaryotic microtubule (MT, Nogales, 2001). *Drosophila melanogaster* has three α- and four β-Tubulin
encoding genes, however no other tubulin genes were found to be suppressors of the ectopic tsl phenotype ($\beta$Tub85D and $\beta$Tub97EF were not tested). MTs are cylindrical organelles essential for many cellular processes, including intracellular transport, signal transduction, mitosis, and maintenance of cell shape (Luduena, 1998; Gundersen & Cook, 1999; Nogales, 2001). Interestingly, members of the MAPK signalling cascade, including the extracellular signal regulated kinases (Erks), were first identified as microtubule-associated protein kinases (Reszka et al., 1995; Morishima-Kawashima & Kosik, 1996). These interactions are thought to regulate kinase activity and permit intracellular protein transport for efficient signal transduction (Parker et al., 2014). One possibility therefore is that $a$Tub84B is important for promoting MAPK signalling downstream of Tor.

**twins**

$tw$ encodes one of three different B sub-unit classes of the heterotrimeric serine/threonine phosphatase complex known as Protein Phosphatase 2A (PP2A, Mumby & Walter, 1993; Walter & Mumby, 1993). PP2A is composed of a scaffold A sub-unit, a regulatory B sub-unit, and a catalytic C sub-unit that together form the active holoenzyme (Janssens & Goris, 2001). The B sub-units provide temporal and spatial specificity for PP2A activity (Zolnierowicz et al., 1994; Janssens & Goris, 2001; Seshacharyulu et al., 2013).

$tw$ has been shown to play roles in a wide variety of cellular processes in *Drosophila*, including cell fate determination (Mumby & Walter, 1993), cell cycle regulation (Walter & Mumby, 1993), centriole amplification (Brownlee et al., 2011), and neuroblast proliferation and self-renewal (Chabu & Doe, 2009). Most notably, $tw$ has been identified as a suppressor of a constitutively active form of Sevenless, an RTK with strong homology to Tor (Maixner et al., 1998). It is possible that $Tws$ acts similarly downstream of Tor in terminal patterning.
The maternal role of *tws* has previously been investigated in three different studies, however the results of these are conflicting. Germline clone analyses using the *tws*\(^{j11C8}\) allele by Bajpai *et al.* (2004) observed that embryos arrested prior to segmentation, whereas Perrimon *et al.* (1996) observed patterning defects resulting in the variable deletion of segments using the *tws*\(^{02414}\) allele that we used here. Finally, Bellotto *et al.* (2002) reported terminal defects associated with *l(3)S027313*, a lethal but unmapped P-element insertion. This insertion was later mapped to the *tws* locus (Deak *et al.*, 1997), thus further supporting the idea that *tws* is essential for terminal patterning.

**Conclusion**

We have performed a genome-wide suppressor screen for genes that act downstream of *tsl* in embryonic terminal patterning and identified several new Tor pathway regulators. Many of the suppressor regions where the causative gene(s) are yet to be identified have been mapped to a tractable number of candidate genes. Thus, the generation of new loss-of-function alleles for candidate genes in these suppressor regions will permit the rapid discovery of new genes involved in Tor signalling. This will be particularly informative with respect to unanswered questions including how the Tor ligand is generated and, more broadly, how RTK signalling is spatially controlled and transduced.
References


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Acknowledgements

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Author contributions

T.K.J., C.G.W. and J.C.W conceived the experiments, interpreted the data and co-led the work. A.R.J., M.A.H., M.J.S., K.A.M. and T.K.J performed the experiments. A.R.J, M.A.H., J.C.W., C.G.W and T.K.J. wrote the paper.
Figure legends and Tables

Figure 1. Validation of the ectopic Tsl suppressor screen.

(A) Schematics of stage 10 egg chambers showing the endogenous tsl expression pattern (red, left) and ectopic pattern used for screening (UAS-tsl driven by c355-Gal4, right). NCs: nurse cells, BCs: border cells, CCs: centripetal follicle cells, PFCs: posterior follicle cells. (B) A stage 8 egg chamber from a screening line female raised at 22°C and immunostained for Tsl. No ectopic Tsl expression is observed in the perivitelline space (open arrow). oo: oocyte. These flies produce viable offspring with wildtype cuticles containing 8 abdominal segments (numbered) and a filzkorper (closed arrow, C). (D) An egg chamber from a screening line female raised at 29°C showing strong ectopic expression of Tsl. (E) These flies produce embryos with the lethal spliced phenotype (no abdominal segments and expanded termini). Suppression of ectopic tsl phenotype (gain of multiple abdominal segments) is observed upon introduction of either one copy of the amorphic trk allele, trk<sup>3</sup> (F), or Df(2L)BSC143 which deletes one copy of trk (G). Anterior is to the left.
### Table 1. Summary of the ectopic tsl suppressor screen.

The proportion of the Bloomington *Drosophila* Stock Centre (BDSC) deficiency kit screened and number of suppressor deficiencies are shown by chromosome. Minimum number of suppressor regions accounts for deficiencies that overlap and may therefore represent a single suppressor deleted by both deficiencies.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>BDSC kit deficiencies tested / total</th>
<th>Suppressor deficiencies</th>
<th>Minimum number of suppressor regions</th>
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<tr>
<td>1</td>
<td>71/91</td>
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<td>13</td>
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<tr>
<td>2</td>
<td>187/190</td>
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<td>59</td>
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<td>Suppressor region</td>
<td>BDSC kit deficiency</td>
<td>Strength of suppression</td>
<td>Additional deficiencies tested</td>
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<tr>
<td>1</td>
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<td>59</td>
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**Table 2. Genomic regions that contain suppressors of the ectopic maternal Tsl phenotype.** Suppressor strength and the molecular coordinates of the suppressor regions are given when possible. Suppression was scored qualitatively based on segment gain and consistency; weak (+), moderate (++), strong (+++) and very strong (+++++). Mapping was conducted using a combination of the BDSC deficiency kit and additional molecularly defined deficiencies. The number of genes within each candidate region is indicated and in cases where there are five or less, the gene symbols are provided. n.s. no suppression was observed. *denotes that region likely contains multiple suppressor loci.
Table 3. Genes identified as suppressors of the ectopic *tsl* phenotype. Mutant alleles used to identify the gene as a suppressor are indicated. The suppression score is listed with the allele identified as a suppressor for this region. Suppression was scored qualitatively based on

<table>
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<th>Suppressor gene</th>
<th>Allele(s) tested</th>
<th>Strength of suppression</th>
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<td><em>Tetraspanin 3A</em></td>
<td>Tsp3A&lt;sup&gt;e03287&lt;/sup&gt;</td>
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<td>14</td>
<td><em>Ribosomal protein S21</em></td>
<td>RpS2I&lt;sup&gt;03575&lt;/sup&gt;</td>
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<td>RpS2I&lt;sup&gt;k16804a&lt;/sup&gt;</td>
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<td>17</td>
<td>trunk</td>
<td>trk&lt;sup&gt;5&lt;/sup&gt;</td>
<td>++++</td>
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<tr>
<td></td>
<td></td>
<td>trk&lt;sup&gt;D&lt;/sup&gt;</td>
<td>++++</td>
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<tr>
<td>18</td>
<td><em>Son of Sevenless</em></td>
<td>Sos&lt;sup&gt;34Ea-6&lt;/sup&gt;</td>
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<tr>
<td>20</td>
<td><em>Ribosomal protein S26</em></td>
<td>RpS26&lt;sup&gt;KG00230&lt;/sup&gt;</td>
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<td><em>Protein disulfide isomerase</em></td>
<td>P{lacW}I(3)j2A2&lt;sup&gt;jA2A&lt;/sup&gt;</td>
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<td>40</td>
<td><em>α-Tubulin at 84B</em></td>
<td>αTub84B&lt;sup&gt;5&lt;/sup&gt;</td>
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<td>42</td>
<td><em>Ras oncogene at 85D</em></td>
<td>Ras85D&lt;sup&gt;c1B&lt;/sup&gt;</td>
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<td>44</td>
<td>twins</td>
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<td>46</td>
<td><em>C-terminal binding protein</em></td>
<td>CtBP&lt;sup&gt;03463&lt;/sup&gt;</td>
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<td>58</td>
<td>tailless</td>
<td>tll&lt;sup&gt;89&lt;/sup&gt;</td>
<td>+++</td>
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segment gain and consistency; moderate (++), strong (+++) and very strong (++++). Bolded genes have no previously known involvement in terminal patterning.
Table 4

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<td>Jimenez et al., 2002</td>
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<td>fs(1)Nasrat</td>
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<td>Jimenez et al., 2002</td>
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<td>Furin 1 and Furin 2</td>
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<td>Johnson et al., 2015</td>
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<td>Loss of termini</td>
<td>Stevens et al., 1990</td>
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<td>Sprenger et al., 1989</td>
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<tr>
<td>corkscrew</td>
<td>Suppressor of tor GOF</td>
<td>Perkins et al., 1992</td>
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<td>downstream of receptor kinase</td>
<td>Partial loss of termini</td>
<td>Simon et al., 1991</td>
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<td>huckebein</td>
<td>Loss of posterior midgut</td>
<td>Weigel et al., 1990</td>
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<td>sprouty</td>
<td>Suppresses torso GOF</td>
<td>Casci et al., 1999</td>
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<tr>
<td>bicoid</td>
<td>Loss of anterior</td>
<td>Driever &amp; Nusslein-Volhard, 1988</td>
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</table>

Table 4. Terminal class genes not detected as suppressors of the ectopic tsl phenotype.

Terminal class gene names and their previously reported loss-of-function phenotypes are given. Note that only genes known to encode positive regulators of tor signalling are shown (i.e. those expected to be suppressors) and the relevant reference given for each.
A wildtype tsl

BCs

CCs

PFCs

NCs

Oocyte

c355>tsl

B c355-Gal4; Gal80ts; UAS-tsl

22°C

NCs

Tsl

C

22°C

D 29°C

Tsl

E 29°C

F 29°C

trk^3 /+

G Df(2L)BSC143 /+