Supplementary Information

Spatiotemporal expression of regulatory kinases directs the transition from mitosis to cellular morphogenesis in *Drosophila*

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Running Title: Bsd activates Polo during myogenesis
Supplementary Figures

Supplementary Figure 1, related to Figure 1. (A) *in silico* expression analysis of *Drosophila* and zebrafish protein kinases. Embryonic expression patterns for all kinases were queried in Flybase and Zfin, and those with reported expression patterns were graphed. Expression patterns were subdivided by embryonic stage (Stages 0-5 in *Drosophila* occur prior to gastrulation), and classified as Tissue/Group Enriched, (meaning the kinase was expressed in a single tissue or a group of tissues but not all tissues), Ubiquitous, or Low Expression (in which the expression pattern was too faint to categorize). No Data indicates the expression pattern is known for only a subset of embryonic stages. See Table S1 for individual expression patterns. (B) *bsd* deficiency mapping. Live Stage 16 embryos that expressed Myosin Heavy Chain (MHC)-GFP. *bsd*/*Df(2R)BSC199 and *bsd*/*Df(2R)BSC699 embryos phenocopied *bsd* embryos. A control (*w*1118) embryo is shown for comparison. (C) Phylogenetic analysis of human, mouse, and fly Vrk proteins. The homology tree was generated using observed divergency as the distance method. The percent homology among species is shown. (D) Protein alignment of the Vrk proteins from (C); black shading shows identical residues in all species, pink shading shows residues that are the same in most species, blue shading shows residues that are the same in at least 50% of species. The position of the *bsd* Q545*"* mutation is given (red box). Residues in the previously defined Kinase Domain (orange line) and ATP binding pocket (violet) for human Vrk1 are shown. Note that the Bsd kinase domain is split by a unique linker region (green line), and the C-termini of the Vrk proteins show low sequence homology. (E) Two-sided Fisher’s exact test of the myogenic phenotypes shown in Fig. 1B. Significance indicates pairwise comparison between two genotypes. (*wt*, *n*=60; *bsd*+, *n*=54; *bsd*+,61F090, *n*=60; *bsd*+,61F090 out cross, *n*=60). (F) Stage 12 5053>GFP and slou>GFP embryos labeled for GFP. Control (5053>GFP, *n*=7; slou>GFP, *n*=53) and *bsd*+ (5053>GFP, *n*=6; slou>GFP, *n*=35) embryos showed an equivalent number of GFP+ founder cells. Significance was determined by two-sided unpaired student’s t test. Error bars represent SEM.
Supplementary Figure 2, related to Figure 2. (A-C) Validation of a Bsd antibody. (A) Western blots of control (w^{1118}), Df(2R)BSC199, and rp298>Bsd embryo lysates using the Bsd antibody. UAS/Gal4 expressed Bsd was detectable, but we could not detect endogenous Bsd by Western blot. (B) Western blot of S2 cell lysates transfected with control or Bsd.Myc expressing plasmids. The Bsd and Myc antibodies recognized proteins of the same molecular weight. (C) Stage 12 embryos immunolabeled with the Bsd antibody (green) and Mef2 (violet). Signal from the Bsd antibody was not observed in Df(2R)BSC199 body wall muscle precursors. The embryos shown were prepared in parallel. (D) Complete embryonic expression of Bsd. Embryos were immunolabeled as in (C). Multiple confocal projections are shown for each embryo to compare expression in the ectoderm and the mesoderm. Bsd was ubiquitously expressed prior to gastrulation (Stage 5), and Bsd expression remained high in the ectoderm after gastrulation. Bsd expression in the mesoderm increased from Stage 11 to Stage 12, and then decreased. (E) Relative Bsd expression in the mesoderm, normalized to Stage 11 (St11, n=215; St12, n=421; St14, n=286; St16, n=292). (F) Embryos immunolabeled with the Bsd antibody (green) and membrane localized Mef2>CD8.RFP (violet). Bsd localization was enriched in the nucleus of Mef2+ cells through Stage 14. Significance was determined by one-way ANOVA. Error bars represent SEM.
Figure a shows an immunoblot (IB) for Bsd protein in control and Df(2R)BSC199 mutant embryos. The Western blot analysis demonstrates a decrease in Bsd expression in the mutant strain compared to the control.

Figure b displays the results of an immunoprecipitation (IP) experiment. Two blots are shown, one for Bsd and Myc and the other for Bsd and Bsd. The IP with Myc and Bsd antibodies shows a difference in band intensities between the control and mutant samples.

Figure c presents confocal images of wild-type and Df(2R)BSC199 embryos at stages 12 and 12. Dotted white boxes highlight regions of interest. For both genotypes, the images are labeled w1118 and Df(2R)BSC199. The Bsd expression appears to be more intense in the wild-type embryos.

Figure d contains images of stage 5 and 9 embryos, with lateral and ventral views. Ectoderm and mesoderm regions are indicated by green and purple colors, respectively. The images show the expression patterns of Bsd and Bsd/Mef2/DAPI in these stages.

Figure e illustrates the relative Bsd expression (% of stage 11) across different stages (St 1, St 2, St 4, St 16). The y-axis represents the relative expression level, while the x-axis represents the stage. The plot includes a line graph and a bar chart with statistical significance indicated by p-values.

Figure f shows immunofluorescence images of stage 11, 12, 14, and 16 embryos. The images are labeled CD8:RFP and Bsd, with scale bars 10 µm. The images demonstrate the spatial distribution of Bsd protein expression across these stages.
Supplementary Figure 3, related to Figure 3. (A) Coomassie stained control gel for the GST-based precipitation experiment described in (Fig. 3A). Bsd-bound GST beads (lane 5; red shading) recovered a large number of unique bands compared to control GST beads (lane 3; blue shading). (B) Immunoprecipitation of S2 cell lysates transfected with Bsd and candidate proteins identified by MS. Bsd interacted with all five proteins, but Polo showed the strongest interaction. (C) Myogenic phenotypes in polo mutants. Stage 16 embryos labeled with Tropomyosin. DT1, LO1, and VL1 muscles are pseudocolored orange, green, and violet. The polo alleles showed stronger phenotypes in trans to Df(3L)BSC447, as expected for hypomorphic alleles. Heat maps and Fisher's exact test of the myogenic phenotypes are as described in Figs. 1B and S1E. (D) Histogram of polo' VL1 phenotypes (n=54). Significance was determined by Fisher's exact test. (E) Stage 10 control embryos and polo' embryos labeled for Mef2. (F) Statistical analysis of embryos shown in (E) (For each group, n=24). Each data point represents the number of Mef2+ cells in a single hemisegment; polo' embryos had 14.2% fewer Mef2+ cells than controls. The vertical lines in the boxplots indicate the median, the boxes indicate the first and third quartiles, and the whiskers indicate minima and maxima. (G) Stage 12 5053>GFP embryos and slou>GFP embryos labeled for GFP. Control (5053>GFP, n=7; slou>GFP, n=50) and polo' (5053>GFP, n=6; slou>GFP, n=39) embryos showed an equivalent number of GFP+ cells. (H) Statistical analysis of embryos shown in (G). Significance was determined by two-sided unpaired student’s t test. (I) Two-sided Fisher's exact test of the myogenic phenotypes shown in Fig. 3F (bsd', n=54; polo', n=96; bsd';polo', n=60). Error bars represent SEM.
Supplementary Figure 4, related to Figure 4. (A) Lysates from S2 cells transfected with a control plasmid or a plasmid expressing Bsd.Myc were immunoblotted with anti-phosphothreonine (pThr). Lysates showed equivalent levels of pThr. (B) Lysates from S2 cells transfected with Polo and a control plasmid or Bsd.I129A were immunoblotted with pThr. The amount of phosphorylated Polo was not significantly different between cells transfected with Bsd.I129A and controls. (C) Lysates from S2 cells transfected with Polo and a control plasmid or wild-type Bsd were immunoblotted with anti-PLK1-phospho-T210 (Polo<sup>pT182</sup>). There was a significant increase in the amount of phosphorylated Polo in cells transfected with Bsd compared to controls. (D) S2 cells transfected with Bsd.Myc and Polo, immunolabeled for pThr210-PLK1 (Polo<sup>pT182</sup> green) and Myc (red). Bsd transfected cells (arrows, n=45) showed significantly more Polo<sup>pT182</sup> than controls (n=76). (E) Purified GST-Bsd and GST-Polo were used for cell free in vitro kinase assays. Polo<sup>pT182</sup> was detected by immunoblotting with anti-pThr210-PLK1 antibody. Bsd directly phosphorylated Polo. (F) Early and late Stage 12 embryos immunolabeled for activated Polo<sup>pT182</sup> (green) and Mef2 (violet). Polo<sup>pT182</sup> increased during Stage 12 in control (bsd<sup>1</sup> heterozygous) but not bsd<sup>1</sup> embryos. Control and bsd<sup>1</sup> embryos were labeled in the same preparation. (G) Stage 12 embryos with endogenous GFP-tagged Polo (Polo<sup>GFP</sup>) that expressed rP298.nLacZ were immunolabeled for GFP (green) and lacZ (red). Fluorescent intensity and cellular localization of total Polo protein was comparable between control and bsd<sup>1</sup> embryos. Graph plots Polo and nlacZ fluorescent intensity across myoblasts (n≥32 cells from 6 or more embryos per genotype). The median and interquartile ranges are shown (dotted horizontal lines). Significance was determined by two-sided unpaired student’s t test (B-E). Error bars represent SEM.
Supplementary Figure 5, related to Figure 5. (A) Stage 12 rP298.nLacZ embryo immunolabeled for phosphorylated Histone 3 (pH3, green) and lacZ (violet). lacZ expressing nascent myotubes were not pH3 positive. (B) Stage 12 embryos labeled for aurA (left, green), aurB (right, green) and Mef2 (violet). aurA and aurB expression was excluded from the somatic mesoderm in Stage 12 embryos. (C) Stage 16 embryos labeled with Tropomyosin. DT1, LO1, and VL1 muscles are pseudocolored orange, green, and violet. Muscle patterning was unaffected in aurA\(^{1}\) and aurB\(^{1689/\text{Df}(3L)BSC447}\) embryos. (D) Quantification of muscle phenotypes, as described in Fig. 1B. (E) Stage 11 embryos immunolabeled for pH3 (green) and Mef2 (violet). Mef2>aurB embryos had significantly more pH3/Mef2 double positive cells than control embryos. n\(\geq\)7 embryos per genotype. (F) Quantification of pH3/Mef2 double positive cells in (E). Each data point represents a single hemisegment (wt, n=80; Mef2>aurB, n=56). The median and interquartile ranges are shown (dotted horizontal lines). (G) Quantification of pH3/Mef2 double positive cells in (E), grouped by hemisegment (A2-A9) (n=10 embryos for wild type; n=7 embryos for Mef2>aurB). Error bars represent SEM. (H) Stage 16 embryos immunolabeled for Mef2 (green) and Tropomyosin (violet). (I) Myotubes in Mef2>aurB embryos had significantly more myonuclei than control embryos. n=6 embryos per genotype. The median and interquartile ranges are shown (dotted horizontal lines). Significance was determined by two-sided Fisher’s exact test (D), two-sided unpaired student’s t test (E,H), and two-way ANOVA (F). Error bars represent SEM.
Supplementary Figure 6, related to Figure 6. (A) Stage 16 embryos labeled for slou>GFP (green) and Tropomyosin (violet). DT1, LO1, and VT1 muscle patterning was disrupted in tum^{DH15}, polo^1, and tum^{DH15}polo^1 embryos. (B) Histogram of DT1, LO1, and VT1 phenotypes. The frequency of muscle phenotypes was not significantly different between tum^{DH15} embryos (n=54) and tum^{DH15}polo^1 double mutant (n=42) embryos. For polo^1 embryos, n=60. (C) Stage 16 embryos labeled with Tropomyosin. DT1, LO1, and VL1 muscles are pseudocolored orange, green, and violet. The muscle pattern was disorganized in pav^{B200} embryos, but the frequency of muscle phenotypes was not significantly different between pav^{B200} embryos and tum^{DH15} pav^{B200} embryos. n=36 hemisegments per genotype. Quantification of muscle phenotypes is as described in Fig. 1B. (D) LO1 myotubes from (Fig. 6C) showing F-actin expression by heat map. Scale bar represents the detection range. F-actin levels were equivalent between control and bsd^1 myotubes. (E) Mononucleate LO1 myotubes from Stage 12 slou>GFP embryos labeled for γ-tubulin (green), GFP (violet) and DAPI (blue). Mononucleate, polarized myotubes have just initiated myotube guidance (see Movie 1). Control (bsd^1/Cyo) myotubes showed only cortical γ-tubulin foci. bsd^1 myotubes had an equivalent number of cortical γ-tubulin foci compared to controls. (F) Quantification of γ-tubulin foci in mononucleate Stage 12 myotubes. Embryos used for (E,F) were derived from the same preparation. Significance was determined by two-sided Fisher’s exact test (B,C), and one-way ANOVA (F). Error bars represent SEM.
Supplementary Figure 7, related to Figure 7. (A) C2C12 cells treated with control (scrambled) siRNA or a cocktail of two siRNAs against murine Vrk1, Vrk2, or Vrk3. Cells were fixed after 7 days in differentiation media and labeled for α-actinin (green) to detect differentiated myotubes and DAPI (blue). Vrk3 knockdown myotubes were shorter than controls and often rounded. Vrk1 and Vrk2 knockdown myotubes were indistinguishable from control cells. (B) Quantitative real time PCR of siRNA treated C2C12 cells. Fold change is relative to control treated cells (red line) (VRK1 siRNA, n=2; VRK2 siRNA, n=2; VRK3 siRNA, n=3). (C) Myoblast fusion index shows reduced fusion in Vrk3 siRNA treated C2C12 cells. For each group, n=10. (D) QQ plot of myotube length distribution, related to Figure 7C. Each data point represents the actual length distribution value (x) and the predicted length value for a normal distribution (y). The lengths of the con siRNA (n=13) and DMSO (n=12) treated myotubes fit the normal distribution; the lengths of the Vrk3 siRNA (n=12) and Volasertib (n=10) treated myotubes did not fit the normal distribution. (E) Model of Bsd and the Polo/Tum/Pav cytoskeletal regulatory module. Bsd reorganizes the microtubule cytoskeleton to direct myotube guidance, and likely acts through Tum and Pav. Significance was determined by two-sided unpaired student’s t-test (C) and D’Agostino & Pearson test (D). Error bars represent SEM.
