Supplemental material

Rosen et al., https://doi.org/10.1083/jcb.201808176

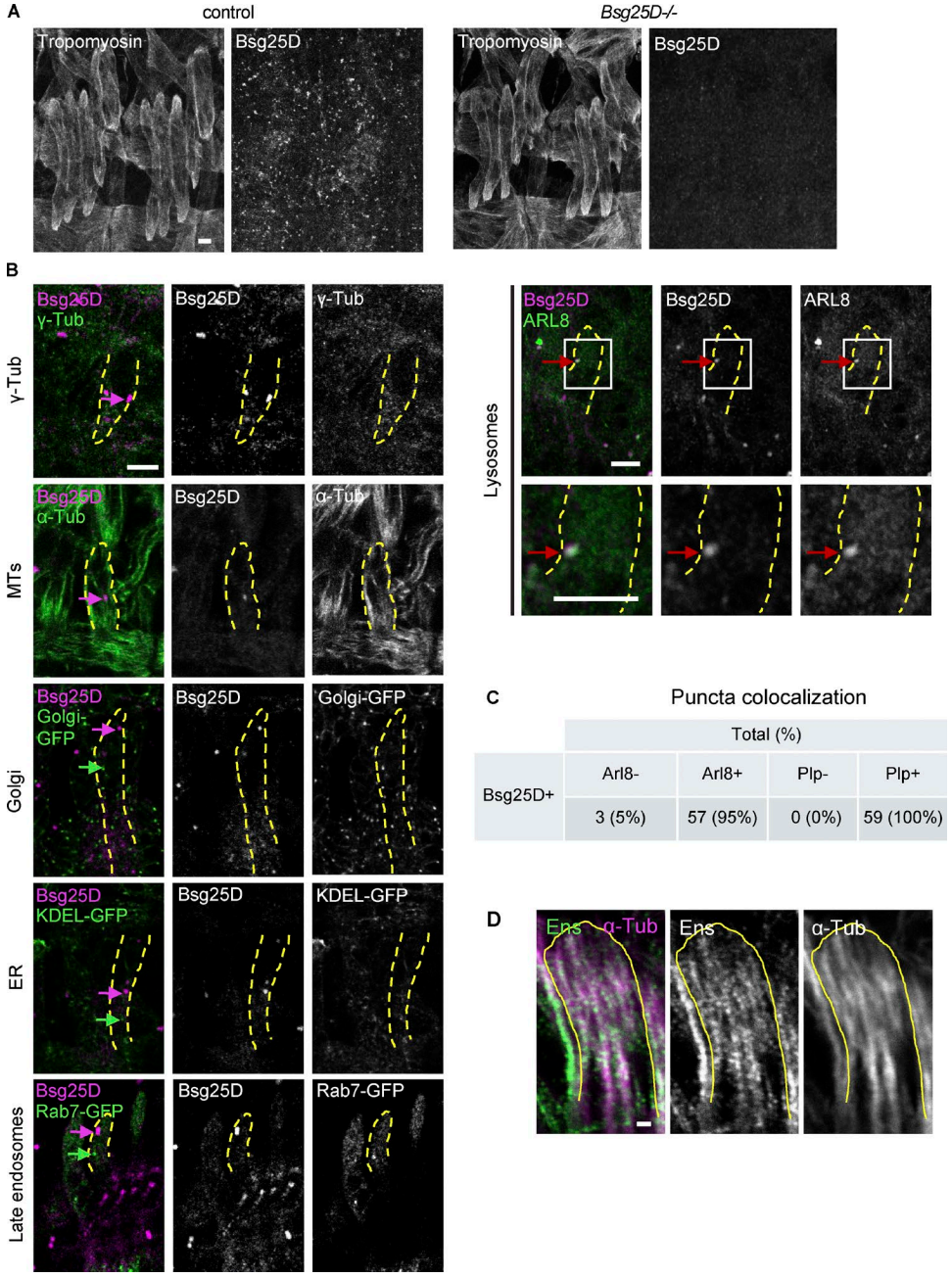


Figure S1. **Bsg25D puncta in developing myotubes localizes with Ens and the lysosome marker Arl8, but not with markers of other subcellular compartments. (A)** Bsg25D antibody labels puncta in control, but not *Bsg25D*$^{-/-}$, embryos. Focused projections of stage 16 embryos stained for Tropomyosin and Bsg25D. Scale bar = 5 µm. **(B)** Bsg25D does not colocalize with γ-Tubulin (γ-Tub), MT, Golgi apparatus, ER, or endosome markers in myotubes, but does colocalize with a lysosome marker. Images of single slices from confocal stacks of stage 16 embryos stained for Bsg25D and various markers or organelle-GFP reporters. Yellow dashed lines indicate individual lateral transverse muscles, as determined by costaining for Tropomyosin (not depicted). Bsg25D, magenta; marker proteins, green. Magenta and green arrows point to puncta positive for Bsg25D or marker proteins, respectively. Scale bars = 5 µm. **(C)** Table of colocalization data of Bsg25D puncta with listed proteins. For each condition, *n* = 3 embryos. **(D)** Ens localization in control myotubes. Single slice from confocal stack of a stage 16 embryo stained for Ens and α-Tub. Ens, green; α-Tub, magenta; colocalization, white. Yellow line indicates a lateral transverse muscle, as determined by costaining with phalloidin (not depicted). Scale bar = 1 µm.

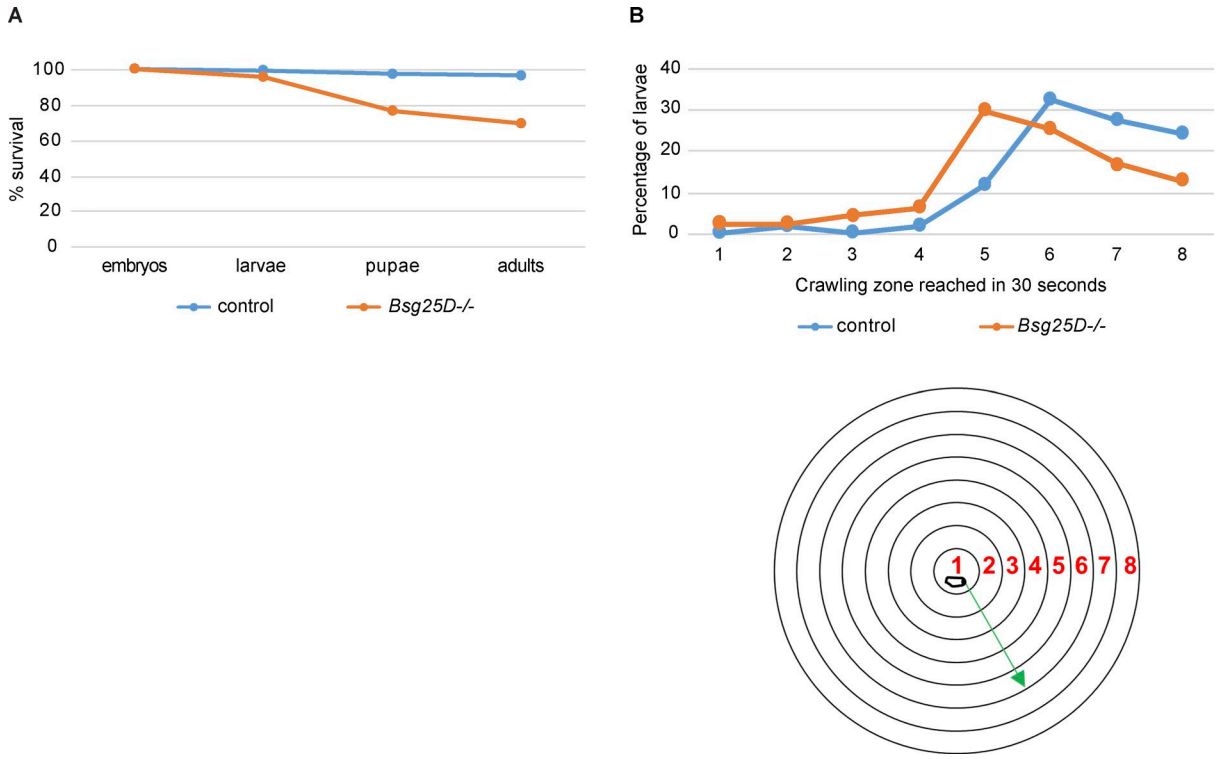


Figure S2. ***Bsg25D*$^{-/-}$ individuals have mild decreases in viability and motility. (A)** Viability of control and *Bsg25D*$^{-/-}$ individuals during development. 96% of control embryos (n = 107) and 70% of *Bsg25D*$^{-/-}$ embryos (n = 119) survived to adulthood. **(B)** Motility of control *Bsg25D*$^{-/-}$ third-instar larvae. Top: Frequency histogram showing how far larvae of indicated genotypes could crawl in 30 s. Bottom: Diagram of experimental setup. The experimenter placed individual larvae in the center of an apple juice plate (crawling zone 1) and recorded what zone they reached in 30 s. For control and *Bsg25D*$^{-/-}$ larvae, the modes were zone 6 and zone 5, respectively. Control, n = 61 larvae; *Bsg25D*$^{-/-}$, n = 47 larvae.

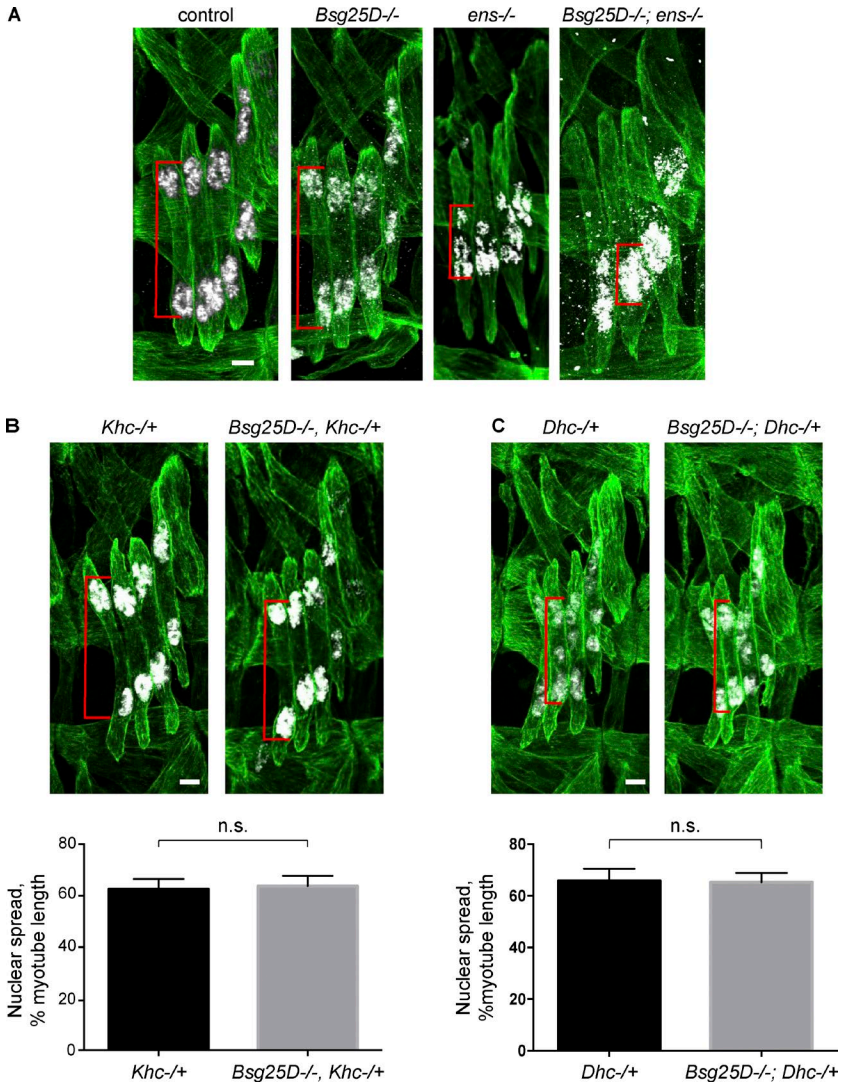


Figure S3. ***Bsg25D*$^{-/-}$ does not enhance the *ens*$^{-/-}$ phenotype and does not genetically interact with *Khc* or *Dhc* to regulate myonuclear positioning.** **(A)** *Bsg25D*$^{-/-}$;*ens*$^{-/-}$ double mutants have myotubes with a single cluster of myonuclei, like *ens*$^{-/-}$ single mutants. Control and *Bsg25D*$^{-/-}$ images are reproduced from Fig. 2. The *ens*$^{-/-}$ image is reproduced from Fig. 3. Scale bar = 5 µm. **(B and C)** *Bsg25D* does not genetically interact with *Khc* or *Dhc* in myonuclear positioning. Top: extended focus projections of representative stage 16 hemisegments of indicated genotypes. Tropomyosin, green; nuclei, white. Scale bars = 5 µm. Note that myotubes with reduced *Dhc* are shorter, as has previously been shown (Folker et al., 2012). Bottom: Bar graphs showing mean nuclear spread and SD. For each genotype, the number of hemisegments is *Khc*$^{-/+}$, *n* = 31 and *Bsg25D*$^{-/-}$;*Khc*$^{-/+}$, *n* = 30 (A); *Dhc*$^{-/+}$, *n* = 45 and *Bsg25D*$^{-/-}$;*Dhc*$^{-/+}$, *n* = 43 (B). P values were calculated by Student's *t* test.

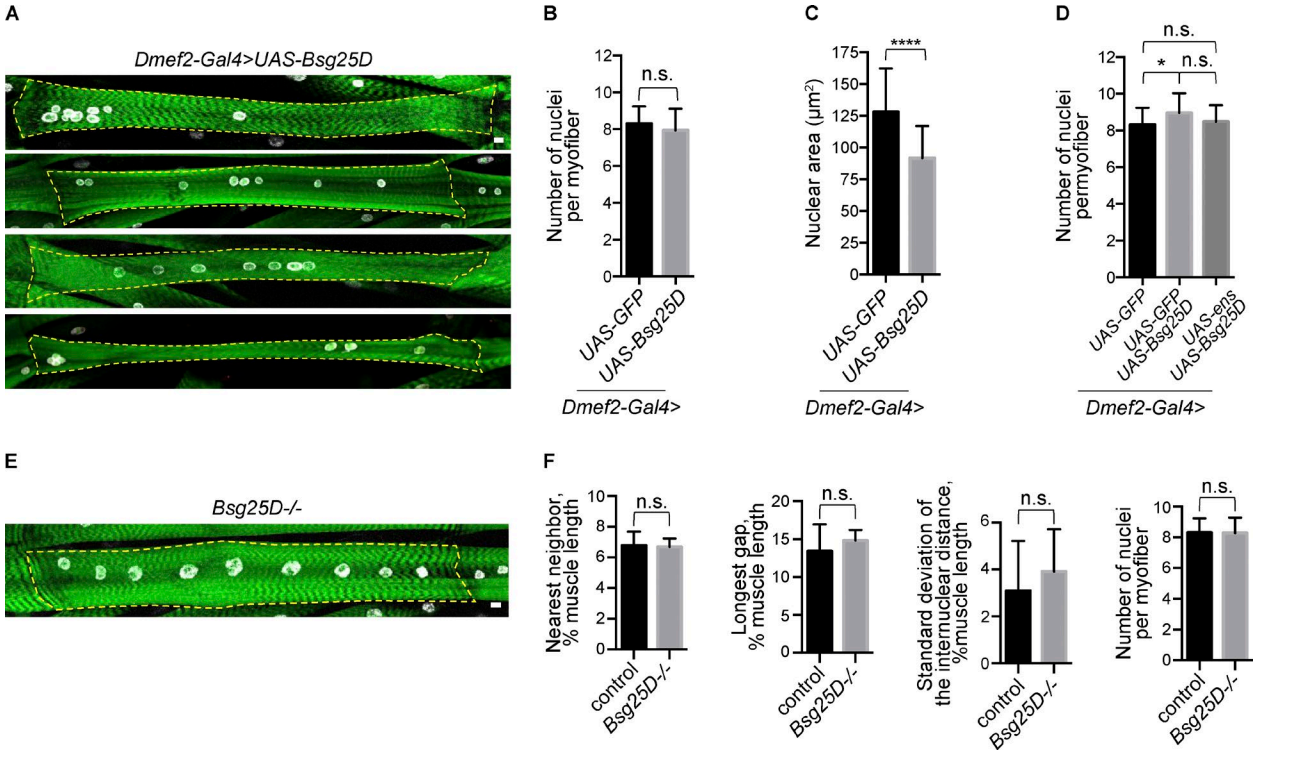


Figure S4. **Bsg25D overexpression induces myonuclear clusters of variable size and position and reduces nuclear size without affecting the number of nuclei per myofiber. (A)** Four examples of Bsg25D-overexpressing myofibers with clustered myonuclei. Note that the cellular position of clusters, the number of myonuclei per cluster, the number of clusters per myofiber, and the location of the longest gap are variable. Phalloidin, green; nuclei, white. Scale bar = 10 µm. Yellow dashed line indicates myofiber edges. **(B)** Bsg25D overexpression does not affect the number of myonuclei per myofiber relative to GFP control. For each genotype, the number of myofibers is as follows: *Dmef2-Gal4>UAS-GFP*, *n* = 40; *Dmef2-Gal4>UAS-Bsg25D*, *n* = 45. **(C)** Mean nuclear area in larval myofibers of larvae expressing either GFP or Bsg25D in muscle. Measurements were done on extended focus projections of Z-stacks. For each genotype, the number of nuclei is as follows: *Dmef2-Gal4>UAS-GFP*, *n* = 82; *Dmef2-Gal4>UAS-Bsg25D*, *n* = 104. **(D)** Larva overexpressing either GFP or Ens with Bsg25D had the same number of nuclei per myofiber, allowing for straightforward comparisons of myonuclear positioning. For each genotype, the number of myofibers is as follows: *Dmef2-Gal4>UAS-GFP*, *n* = 40; *Dmef2-Gal4>UAS-GFP;UAS-Bsg25D*, *n* = 23; *Dmef2-Gal4>UAS-ens;UAS-Bsg25D*, *n* = 19. **(E and F)** *Bsg25D*$^{-/-}$ larvae have normal myonuclear positioning. **(E)** Extended-focus projection of representative *Bsg25D*$^{-/-}$ myofiber. Phalloidin, green; nuclei, white. Scale bar = 10 µm. Yellow dashed line indicates myofiber edges. **(F)** Quantification of myonuclear positioning and nuclear number in control and *Bsg25D*$^{-/-}$ myofibers. Control data are *Dmef2-Gal4>UAS-GFP* from Fig. 5. For all analyses, *n* = 40 myofibers for control and *n* = 17 myofibers for *Bsg25D*$^{-/-}$. P values were calculated by Student's *t* test. *, P < 0.05; ****, P < 0.0001.

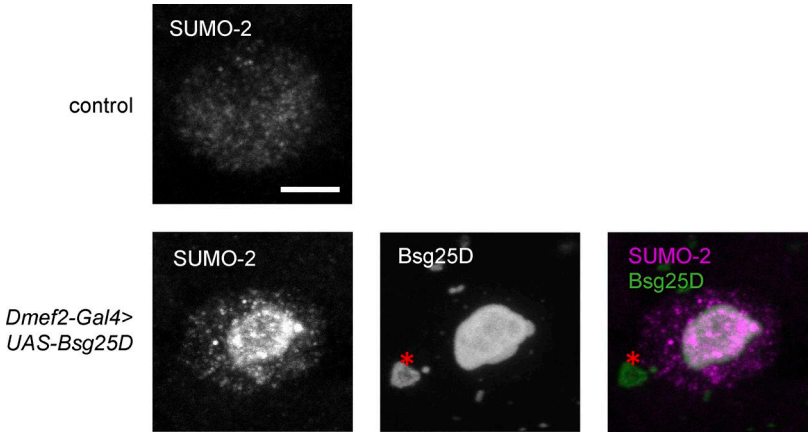
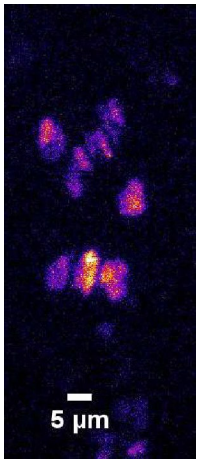
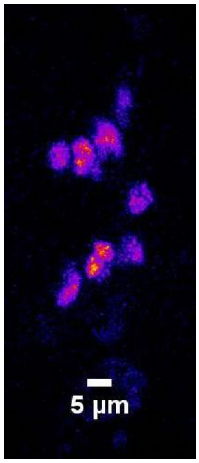


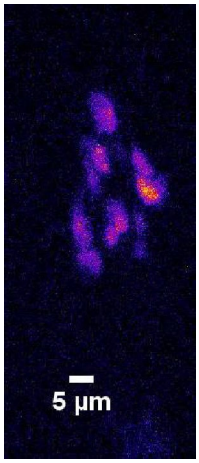
Figure S5. **Overexpressed Bsg25D colocalizes with SUMO-2 in myonuclei.** In control myofibers, an antibody against SUMO-2 labels myonuclei in a punctate pattern. In myofibers overexpressing Bsg25D, SUMO-2 antibody labels the same structures and colocalizes with nuclear Bsg25D. Note that cytoplasmic Bsg25D does not colocalize with SUMO-2 (asterisks). Green, Eos::Bsg25D; magenta, SUMO2; colocalization, white. Scale bar = 5 µm.



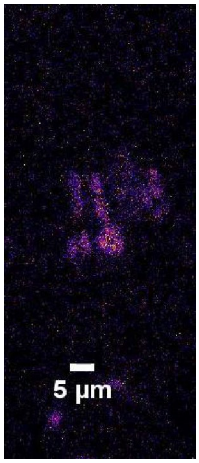
Video 1. **Nuclear movement in control LT myotubes.** Nuclei are labeled and filmed as described in Materials and methods. Representative examples of each listed genotype are included.



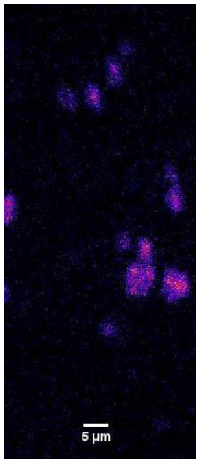
Video 2. **Nuclear movement in *Bsg25D*$^{-/-}$ LT myotubes.** Nuclei are labeled and filmed as described in Materials and methods. Representative examples of each listed genotype are included.



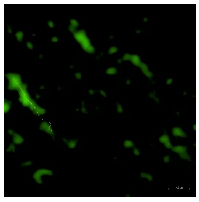
Video 3. **Nuclear movement in*Bsg25D*$^{-/-}$;*ens*$^{-/+}$ LT myotubes.** Nuclei are labeled and filmed as described in Materials and methods. Representative examples of each listed genotype are included.



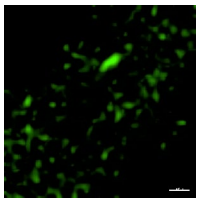
Video 4. **Nuclear movement in *Dmef2-GAL4> UAS-Bsg25D* LT myotubes.** Nuclei are labeled and filmed as described in Materials and methods. Representative examples of each listed genotype are included.



Video 5. **Nuclear movement in *Dmef2-GAL4> UAS-ens* LT myotubes.** Nuclei are labeled and filmed as described in Materials and methods. Representative examples of each listed genotype are included.



Video 6. **EB1-YFP in control myotubes.** Representative EB1-YFP videos for the listed genotypes. See Materials and methods for details.



Video 7. **EB1-YFP in Bsg25D$^{-/-}$ myotubes.** Representative EB1-YFP videos for the listed genotypes. See Materials and methods for details.