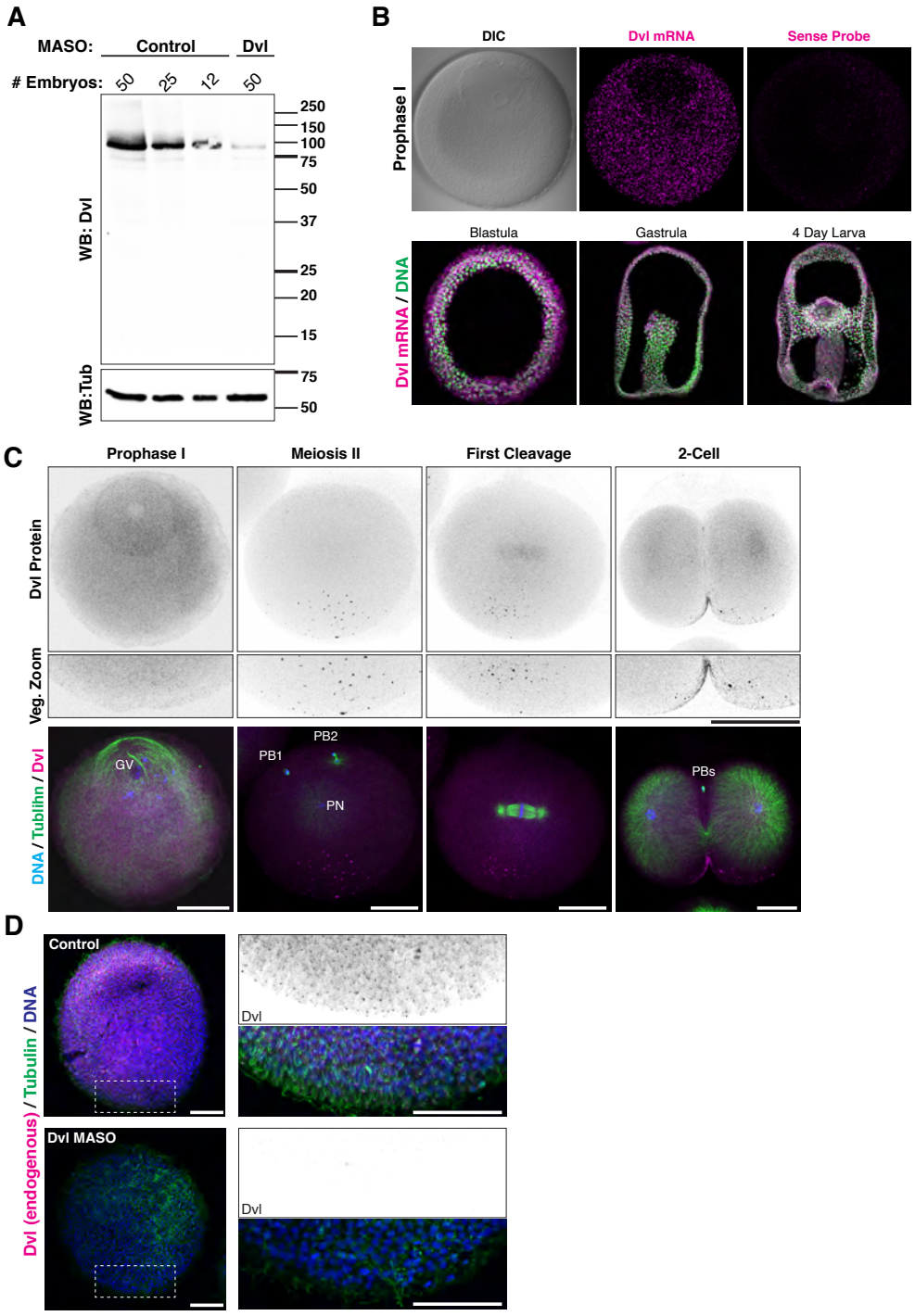

Figure S1. Localization of Dishevelled in meiosis and development, related to Figure 1. (A) Western blot for Dvl on 24-hour embryos following control or Dvl morpholino injection. (B) Fluorescent in situ hybridization for Dvl mRNA in Prophase I arrest of meiosis, blastula, gastrula, and 4-day larvae. Dvl transcripts are detected throughout the oocyte cytoplasm and embryo. Sense control probe signal is provided to the top right. (C) Immunofluorescence for endogenous Dvl protein and microtubules in meiosis and the first embryonic cleavage. Images are scaled individually to better visualize Dvl localization. Upper images are of Dvl channel alone by inverted grayscale. (D) Immunofluorescence for endogenous Dvl and microtubules in 24 hour control or Dvl-knockdown embryos. Dvl channel is scaled equivalently between images. Scale bars = 50 μm.

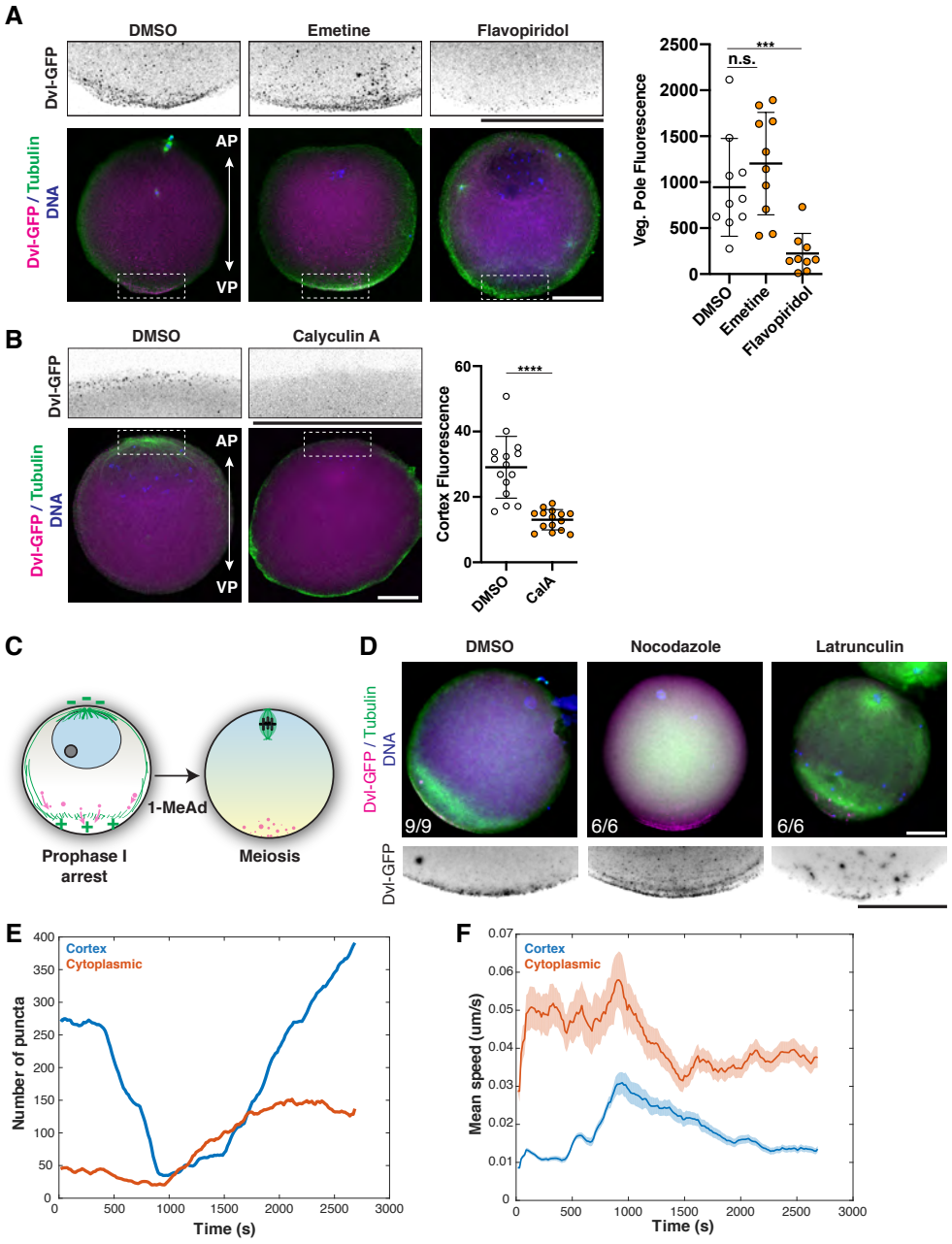

Figure S2. Localization mechanisms of Dishevelled, related to Figure 2. (A) Meiotic oocytes treated with the translational inhibitor emetine, or the CDK inhibitor flavopiridol. Pixel intensity quantification of Dvl-GFP signal within indicated regions of images is provided to the right (DMSO n=10, emetine n=10, flavopiridol n=9 oocytes, ***p=0.0006 Mann-Whitney test). Scale bars = 50 μm. (B) Prophase I-arrested oocytes treated with DMSO or the PP1/PP2A inhibitor Calyculin A. Pixel intensity quantification of indicated regions is provided to the right (DMSO n=15, Calyculin A n=15, ****p<0.0001 Mann-Whitney test). (C) Schematic of a model in which Dvl is actively transported to the vegetal pole along microtubule networks. (D) Fixed oocytes expressing Dvl-GFP and counterstained for microtubules, treated with DMSO, nocodazole, or latrunculin. Scale bars = 50 μm. (E) The number of Dvl assemblies detected over time at the cortex or within the cytoplasm for the oocyte shown in Figure 2A-D. (F) The mean speed of Dvl assemblies at the cortex or in the cytoplasm over time.

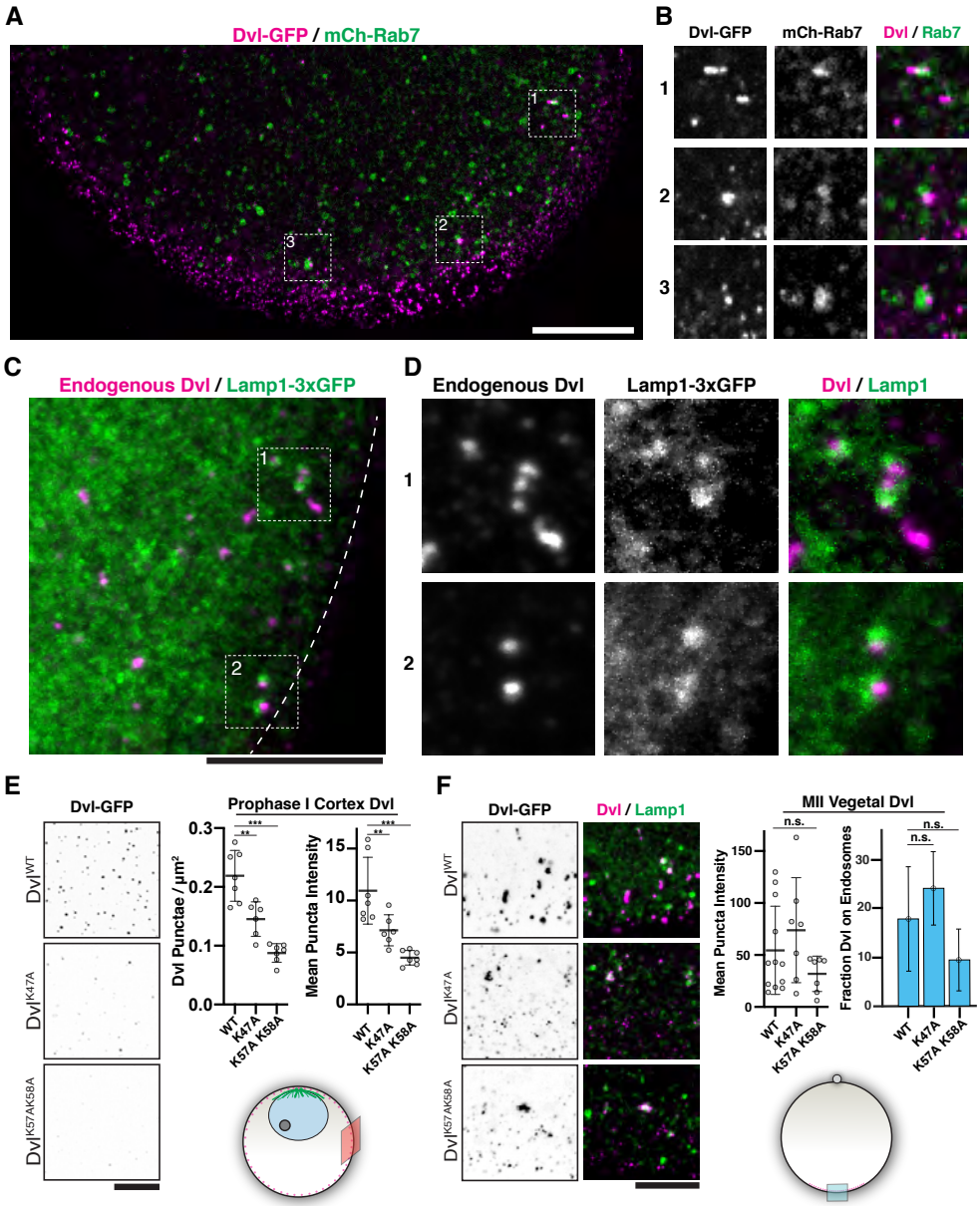

Figure S3. Dishevelled association with Rab7 positive endosomes, related to Figure 3. (A) Vegetal pole view of oocyte expressing Dvl-GFP and mCherry-Rab7. Scale bar = 20 μm. (B) Zoom views of the insets indicated in (A). Each image is 8.67 μm wide. (C) Vegetal pole view of oocyte expressing Lamp1-3xGFP and stained for endogenous Dvl protein. Scale bar = 10 μm. (D) Zoom views of the insets indicated in (B). Each image is 9.23 μm wide. (E) Localization of wild-type Dvl-GFP, Dvl^{K47A}, or Dvl^{K57A,K58A} in Prophase I arrested oocytes. Scale bar = 10 μm. Quantification of puncta density per square micron and mean intensity of Dvl puncta signal provided to right (WT n=7, K47A n=6, K57AK58A n=7 oocytes, **p<0.01, ***p<.001 Mann-Whitney test). (F) Localization of Dvl-GFP, Dvl^{K47A}, or Dvl^{K57A,K58A} and Lamp1-3xGFP in meiosis II oocytes. Scale bar = 10 μm. Quantification of puncta intensity (WT n=12, K47A n=7, K57AK58A n=7 oocytes) and Dvl-GFP fraction co-localized with Lamp1-mCherry (WT n=4, K47A n=3, K57AK58A n=3 oocytes) provided to the right. (n.s. determined by Mann-Whitney test for pixel intensity quantification, and Kolmogorov-Smirnov 2 for colocalization analysis).

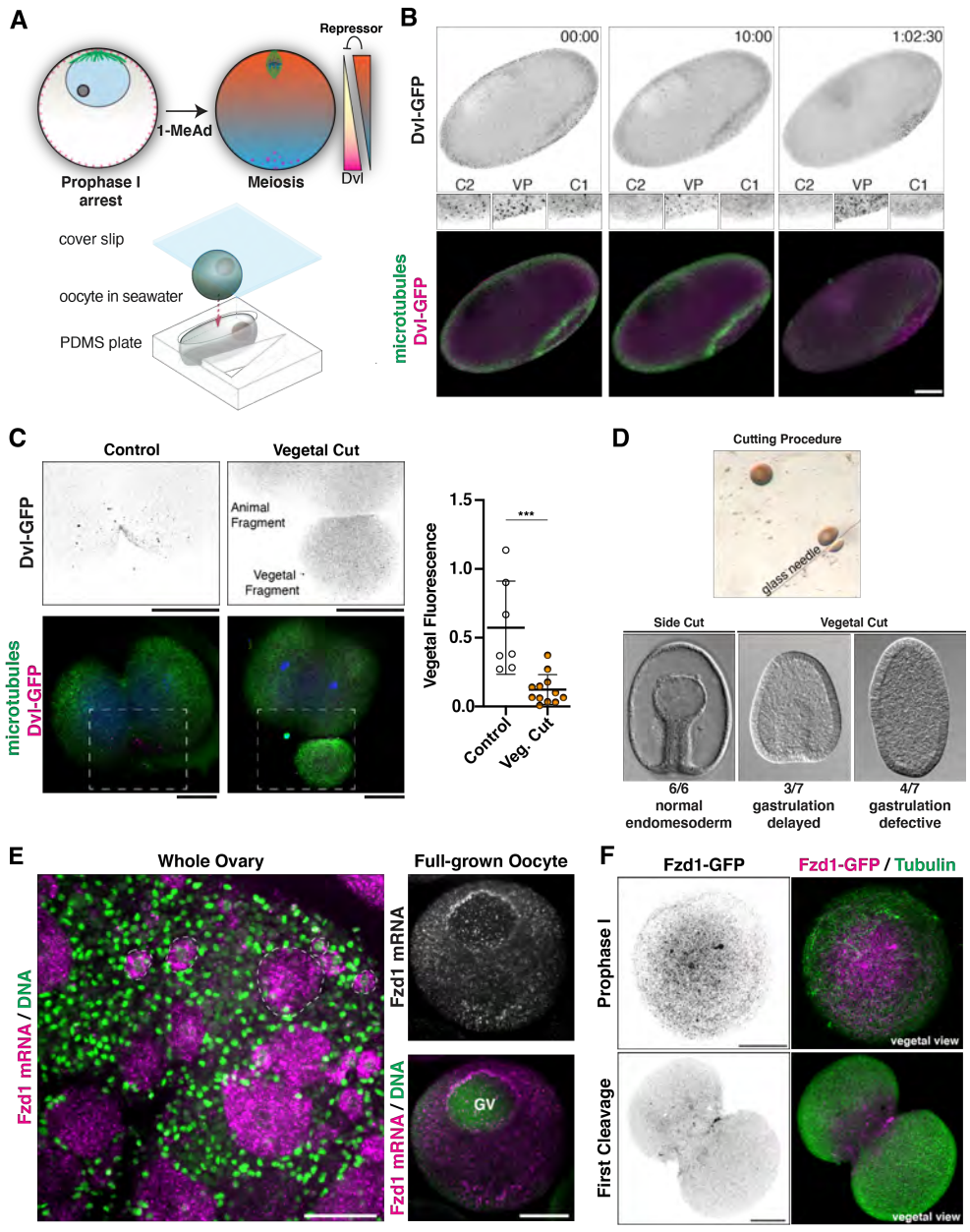

Figure S4. Determinants of Dvl localization, related to Figure 4. (A) Schematic of a regulatory gradient model of Dvl localization and PDMS shape mold for modifying the geometry of the oocyte. (B) Time lapse stills of an oocyte in an oval shape mold expressing Dvl-GFP and EMTB-mCherry, representative of 4 individual oocytes. Upper images are of Dvl-GFP alone in inverted grayscale. (C) Localization of endogenous Dvl in control or vegetal pole-removed oocytes. Scale bar = 50 μm. Pixel intensity quantification of Dvl signal is provided to the right (control n=7, veg. cut n=12, ***p=0.0003 Mann-Whitney test). (D) Development of oocytes with either vegetal poles or sides cut as a control at 48 hours post fertilization (control n=6, vegetal pole cut n=7). The cutting procedure by glass needle is shown above. (E) Distribution of Fzd1 mRNA in intact ovaries (left) or isolated full-grown oocytes (right). Several example immature oocytes are indicated with white dashed lines. Scale bars = 50μ. (F) Views from the vegetal pole of Prophase I arrested oocyte and 2-cell embryos expressing Fzd1-GFP and stained for tubulin. Scale bars = 50 μm.