

Fig. S1. JNK is required for the localization of adherens junction components during *Xenopus* gut morphogenesis. (A-L) Embryos were exposed to DMSO or SP600125 from stages 35 to 46, fixed, sectioned and immunohistochemically processed to reveal E-cadherin (Ecad, green; A-D), the adherens junction components α -catenin (α cat, red; A-F) and β -catenin (β cat, red; G-L), the gut mesodermal marker, smooth-muscle actin (green, as a counterstain; G-J) and DAPI-stained nuclei (blue) in the developing gut tube, as indicated. Asterisks indicate the inner population of endoderm cells. Scale bars: 50 μ m.

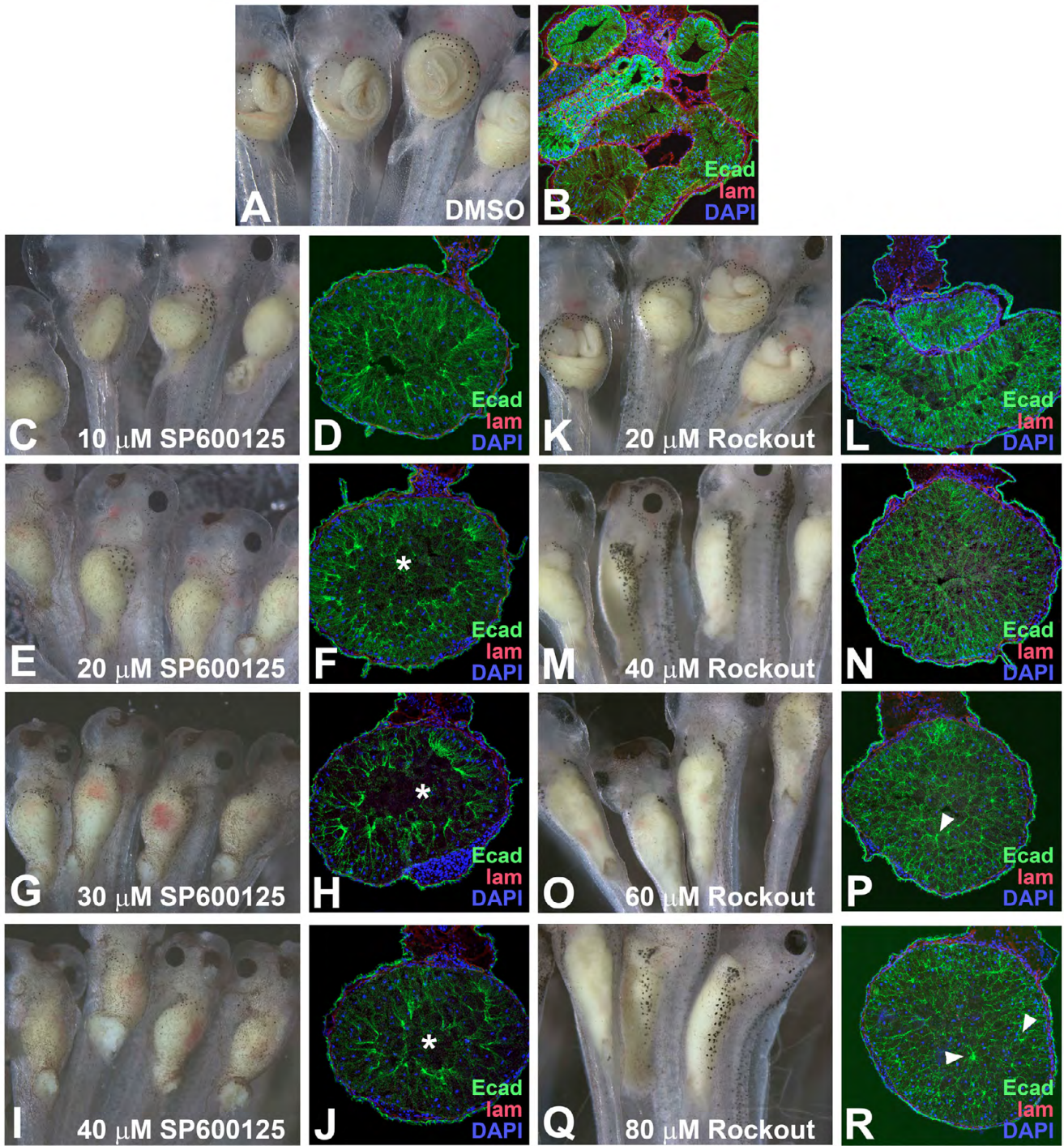


Fig. S2. Increasing concentrations of SP600125 and Rockout elicit increasingly short guts, but with distinct changes in tissue architecture. (A-R) Embryos were exposed to DMSO (A), SP600125 (C,E,G,I) or Rockout (K,M,O,Q) from stage 35 through 46, at the concentrations indicated. Transverse sections of a representative embryo exposed to DMSO (B) and each concentration of SP600125 (D,F,H,I) or Rockout (L,N,P,R) were stained to reveal the presence of E-cadherin (green; cell-cell adhesion) and laminin (red; basement membrane). Increasing levels of SP600125 elicit more severe gut elongation defects accompanied by decreasing levels of E-cadherin and a bilaminar endoderm architecture. Increasing levels of Rockout also elicit progressive defects in gut elongation, but E-cadherin levels remain unaffected, except for the appearance of foci of upregulated adhesive contacts (arrowheads).

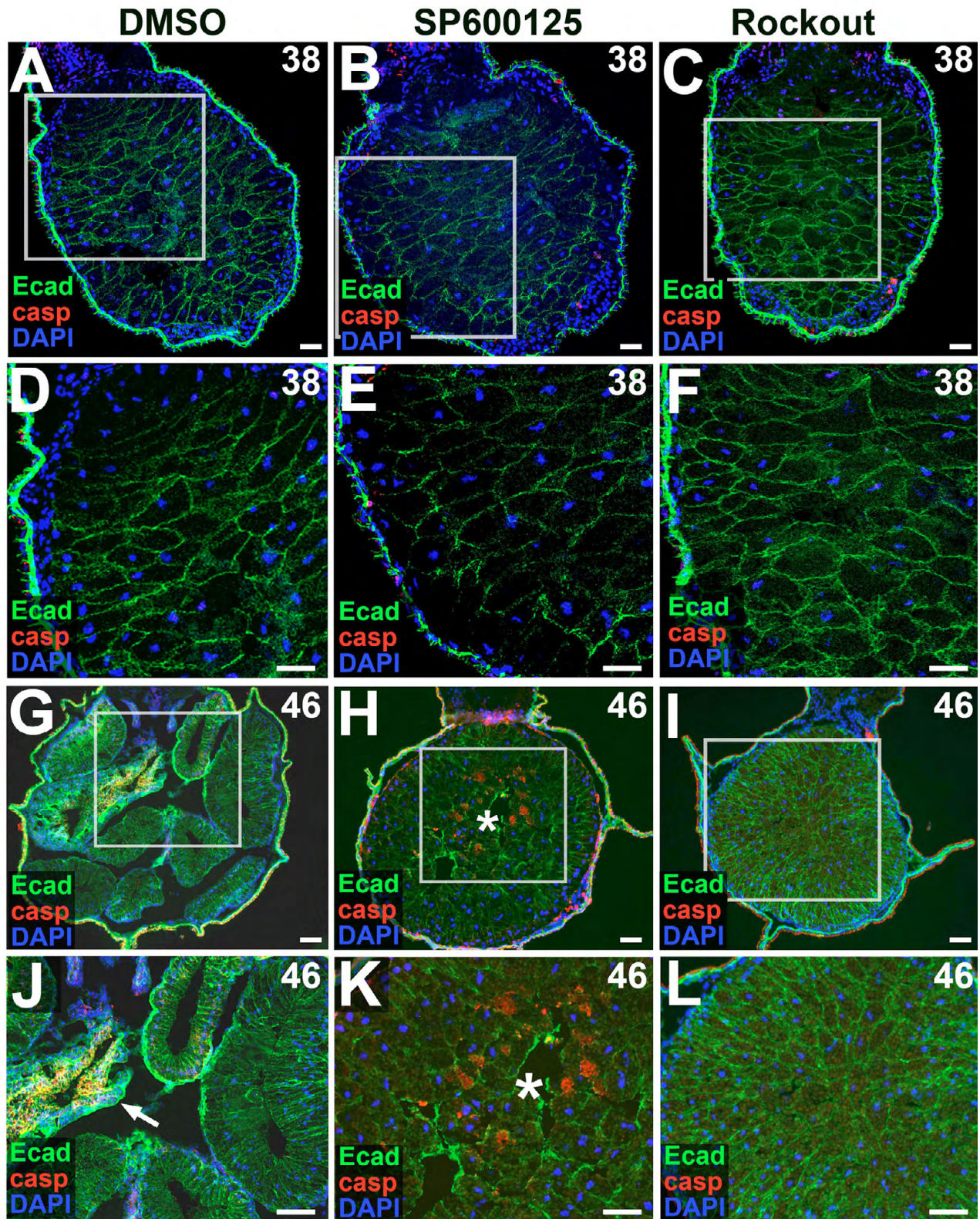


Fig. S3. SP600125-induced defects in adhesion precede apoptosis. (A-L) Embryos were exposed to DMSO, SP600125 or Rockout from stage 35-38 (12 hours; A-F) or 35-46 (48 hours; G-L), fixed, sectioned and immunohistochemically processed to reveal E-cadherin (Ecad, green), activated caspase 3 (casp, red) and DAPI-stained nuclei (blue) in the developing gut tube. Compared with DMSO and RO, SP600125 guts exhibit reduced and irregular E-cadherin as early as 12 hours after exposure to the inhibitor (B,E). Caspase-positive cells are normally found only in the stomach (arrow, J), but are evident in the core of SP600125 guts after 48 hours exposure (H,K). Caspase-positive cells are undetectable in the RO gut endoderm (I,L). Asterisks indicate the inner cells in the core of the gut. D-F and J-L are higher magnification images of the boxed regions in A-C and G-I, respectively. Scale bars: 50 μ m.

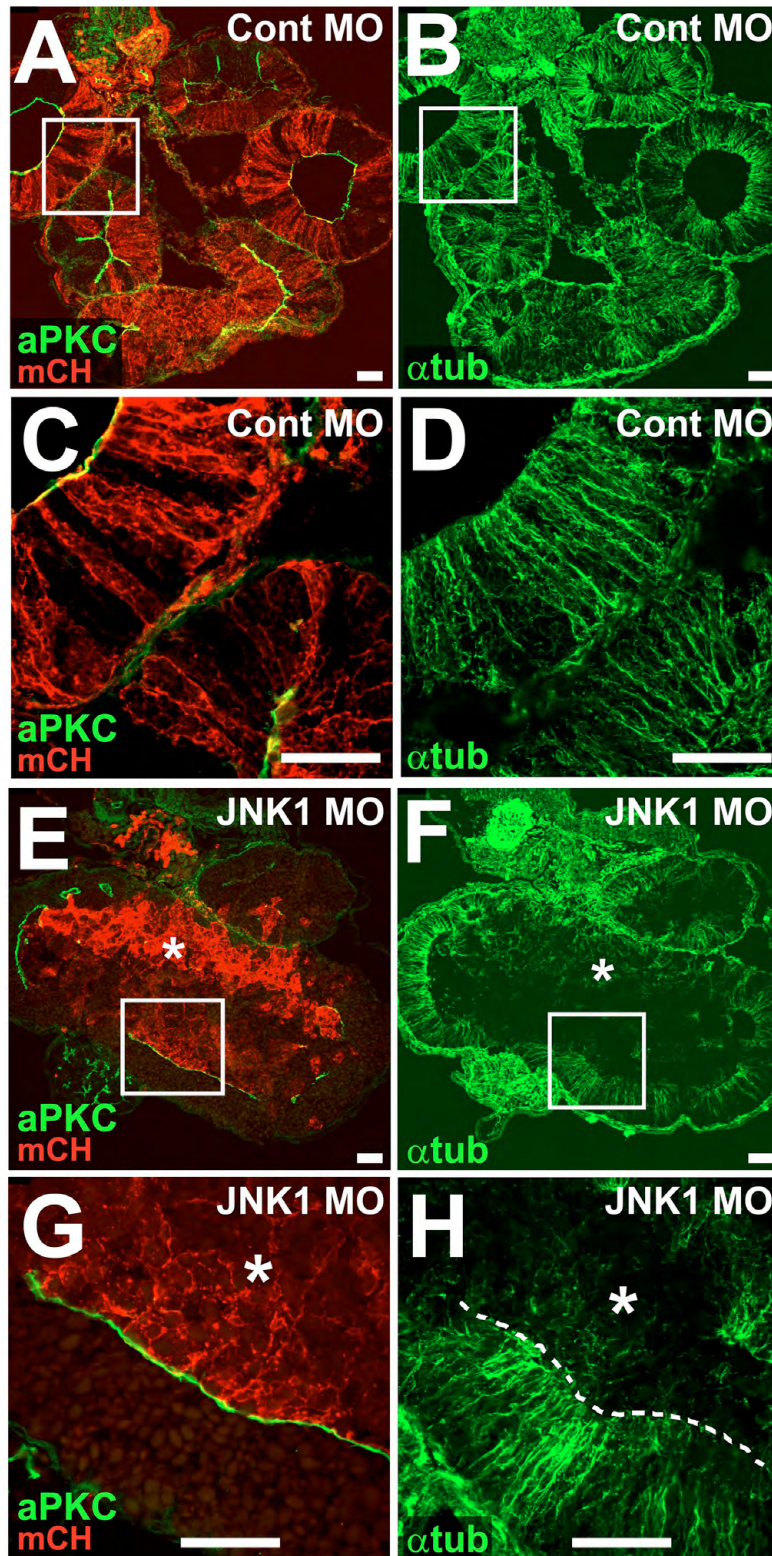


Fig. S4. Morpholino knockdown of JNK1 disrupts endoderm microtubule (MT) architecture. (A-H) Embryos were co-injected with control morpholino (Cont MO; A-D) or JNK1 morpholino (JNK1 MO; E-H) and mCherry mRNA (as a lineage tracer). Sections of injected embryos (stage 46) reveal the localization of atypical protein kinase C and mCherry (aPKC, green; mCh, red; A,C,E,G) or α -tubulin (green; B,D,F,H) in the gut. Cont MO-injected epithelium (red cells in A,C) exhibits normal MT architecture (B,D). By contrast, in the JNK1 MO-injected population (red cells in E,G), MTs are sparse and disorganized (F,H). Asterisks in E-H indicate an inner population of MO-injected cells. (C,D,G,H) Higher magnification images of boxed regions in A,B,E,F, respectively. Images in A,C,E,G have been reproduced from Fig. 5. Scale bars: 50 μ m.

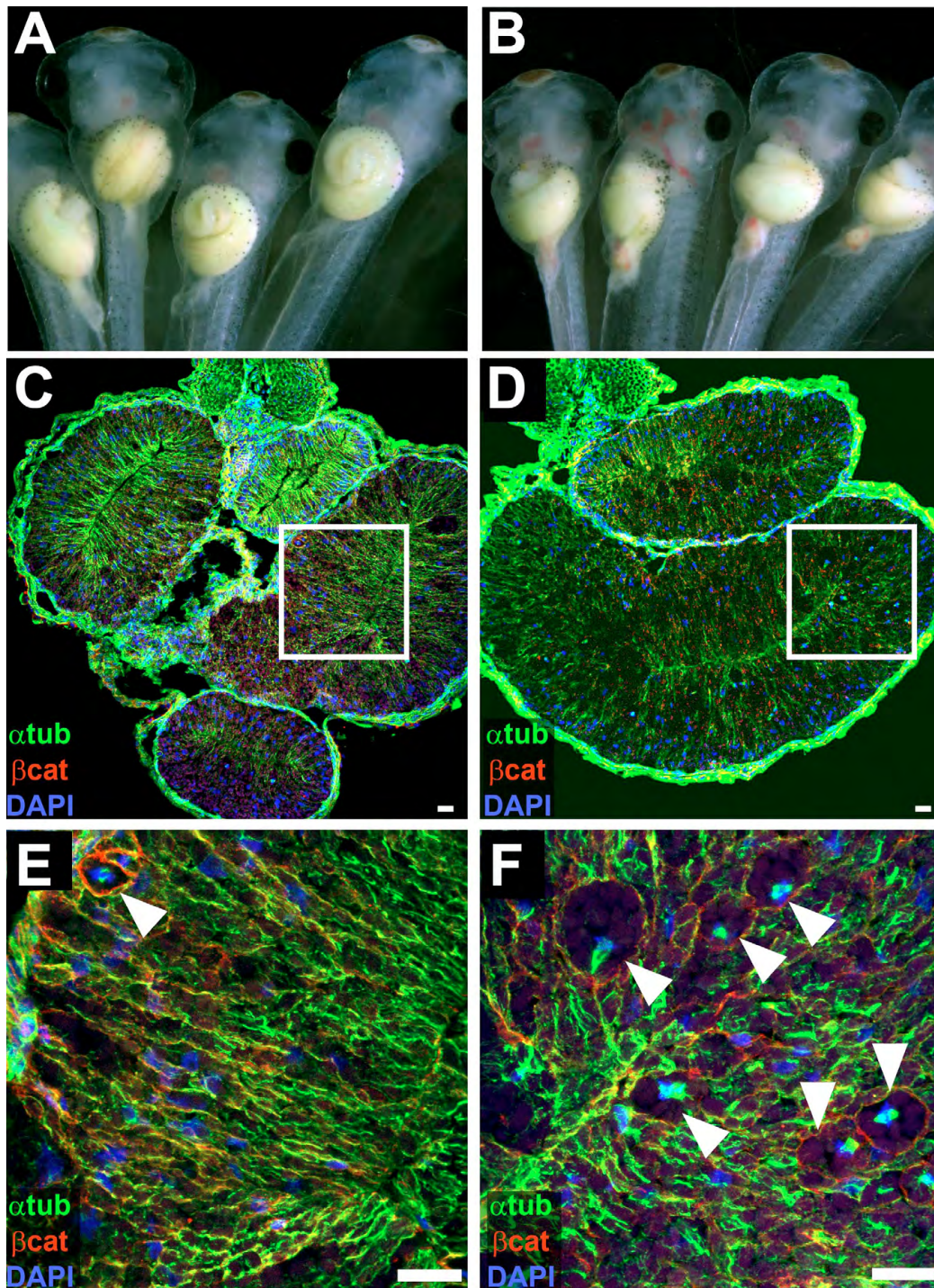


Fig. S5. Microtubule (MT) stabilization does not phenocopy JNK inhibition. (A-F) Embryos were exposed to DMSO (A) or etoposilone B (B) from stage 35 through to stage 46. Transverse sections of a representative embryo exposed to DMSO (C,E) and Etoposilone B (D,F) were stained to reveal the presence of α -tubulin (green; MTs), β -catenin (red; membrane) and nuclei (blue). An increased number of M-phase stalled mitotic figures (arrowheads) can be observed in the etoposilone-treated gut, but gut elongation is only mildly affected; MT arrays and cell-cell adhesion are normal.

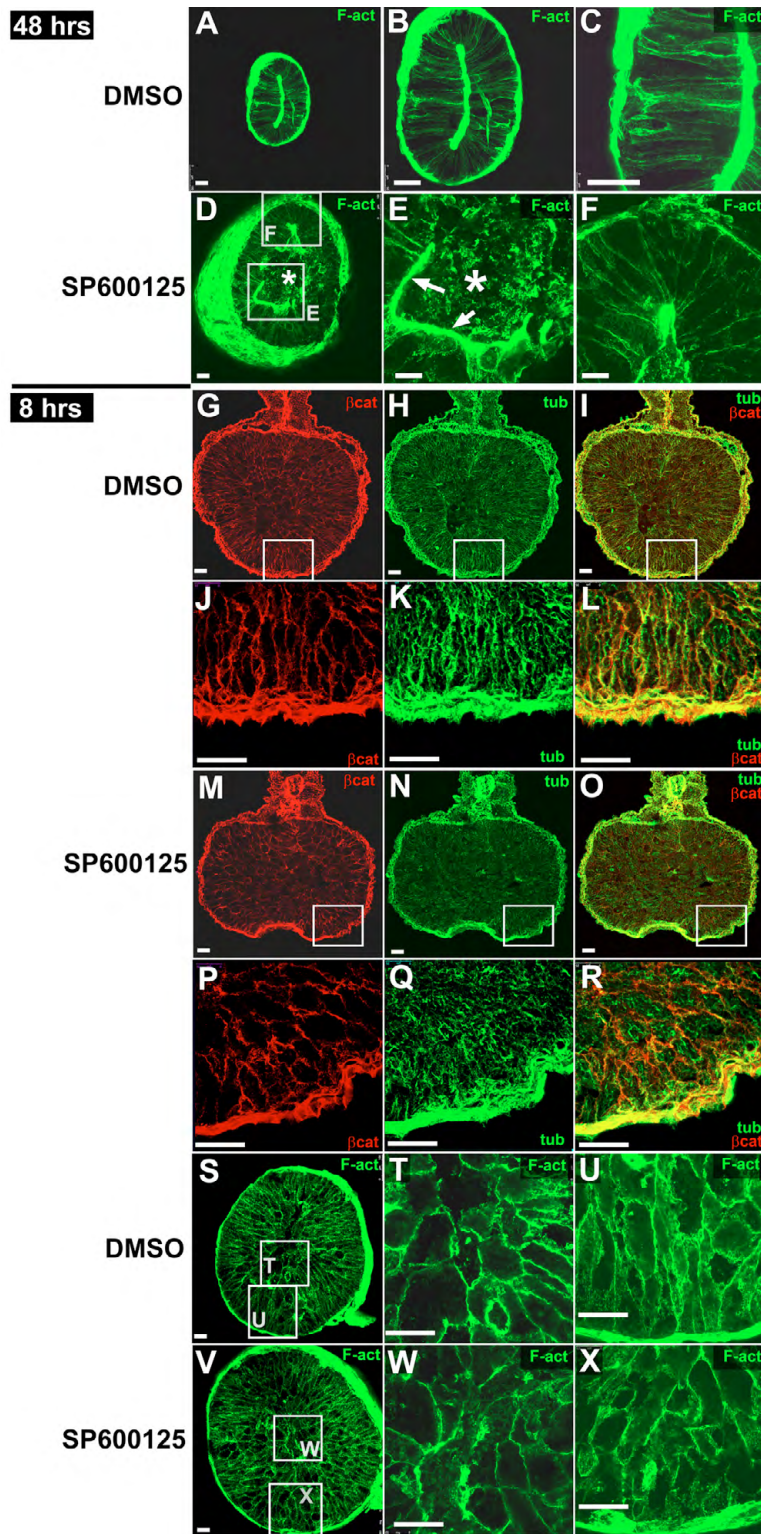


Fig. S6. JNK does not regulate actin polymerization in the gut. (A-F) Embryos were exposed to DMSO (A-C) or SP600125 (D-F) from stage 35 through stage 46 (48 hours). Whole gut tubes were bisected and stained with phalloidin to visualize the distribution of polymerized F-actin. E and F are higher magnifications of the boxed areas in D. Although actin is disrupted in the inner cell population (asterisks in D,E), cortical actin is still evident in the outer cells (F), and a robust actin belt forms at the apical surface of the abnormal epithelium (arrows, E). (G-X) Embryos were exposed to DMSO (G-L,S-U) or SP600125 (M-R,V-X) for 8 hours. Transverse sections of a representative embryo exposed to DMSO (G-L) or SP600125 (M-R) were stained to reveal the presence of α -tubulin (green; MTs) or β -catenin (red; adhesion), as indicated, or whole gut tubes were bisected and stained with phalloidin to visualize F-actin (S-X). Although the 8-hour exposure to SP600125 has disrupted the parallel bundling of MT arrays and the regular distribution of β -catenin at the membrane, there is no observable difference in cortical actin distribution in either the inner (W) or outer (X) endoderm cells. (J-L,P-R) Higher magnification images of the boxed regions in G-I,M-O, respectively. (T-U,W-X) Higher magnifications of the boxed areas in S and V, respectively. Scale bars: 50 μ m.