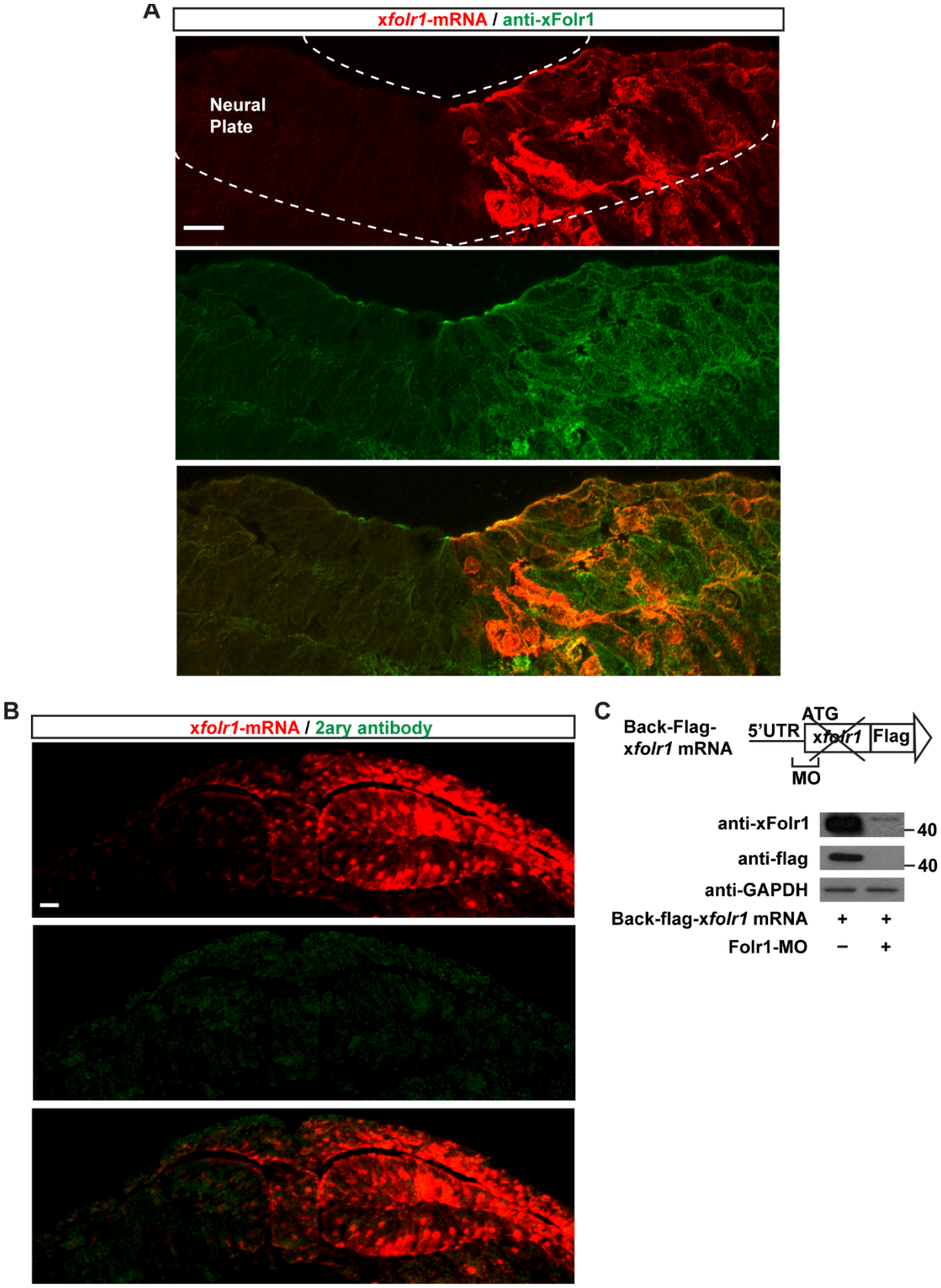


**Fig. S1. Specificity of *Xenopus* folate receptor 1 antibody.** (A,B) Two-cell-stage embryos were unilaterally injected with 700 pg x*folr1* mRNA+GFP mRNA (A) or Alexa Fluor 594-dextran conjugate (B), paraffin-sectioned and processed for xFolr1 immunostaining with (A) or without (B) Folr1 primary antibody added. Scale bar: 20 μm. (C) Schematic of construct to demonstrate specificity of xFolr1 antibody and representative Western blot from whole cell homogenates from embryos injected at the 2-cell stage with the indicated constructs. xFolr1: *Xenopus laevis* folate receptor 1, Folr1-MO: xFolr1-targeted translation blocking-morpholino.

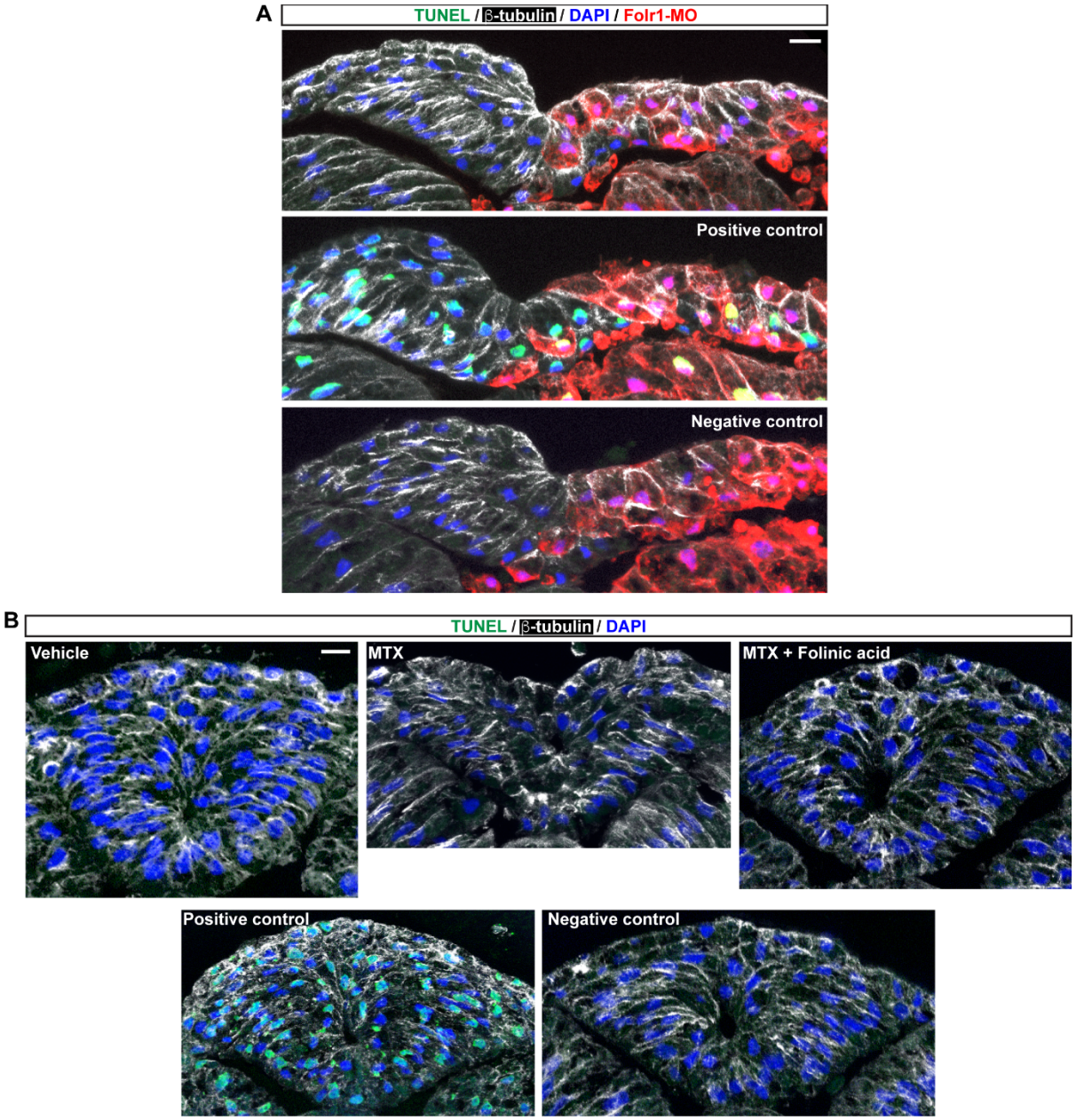



**Fig. S2. Folr1-MO or methotrexate does not induce apoptosis of neural plate cells during neurulation.** (A) Two-cell-stage embryos were unilaterally injected with 10 pmol folate receptor 1 morpholino (Folr1-MO) and Alexa 594-conjugated dextran and grown until neural plate stage 17 (18.75 hpf). (B) Early neural plate stage embryos (stage 13, 14.75 hpf) were incubated in the absence (Control) or presence of 1-1.5 mM methotrexate (MTX) until closure of the neural tube in controls (stage 20, 21.75 hpf). (A,B) Embryos were then processed for TUNEL assay, immunostaining for β-tubulin and nuclear labeling with DAPI. Shown are representative transverse sections of neural tissue from aforementioned embryos. Positive control is a sample treated with DNase; negative control is a sample in which no labeling enzyme (Terminal Deoxynuclotidyl Transferase) was added. Scale bars: 20 μm.

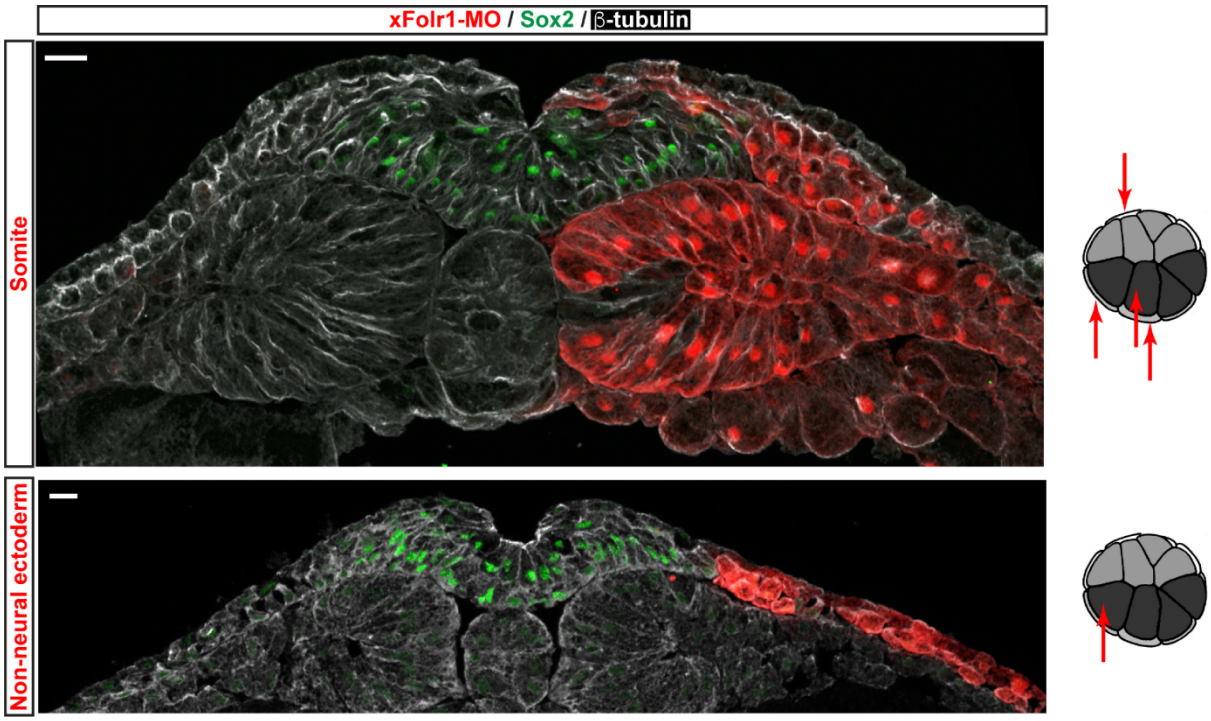



**Fig. S3. Knockdown of folate receptor 1 in mesoderm or non-neural ectoderm does not induce neural tube defects.** Targeted blastomeres of 16-cell-stage embryos were unilaterally microinjected with 3 pmol/cell morpholino against the *Xenopus laevis* Folr1 (Folr1-MO) along with Alexa 594-dextran conjugate in indicated blastomeres (left). Neural plate stage embryos were then sectioned and processed for immunostaining with anti-β-tubulin (grayscale) and Sox2 (green). Red indicates MO-containing cells. Scale bars: 20 μm.

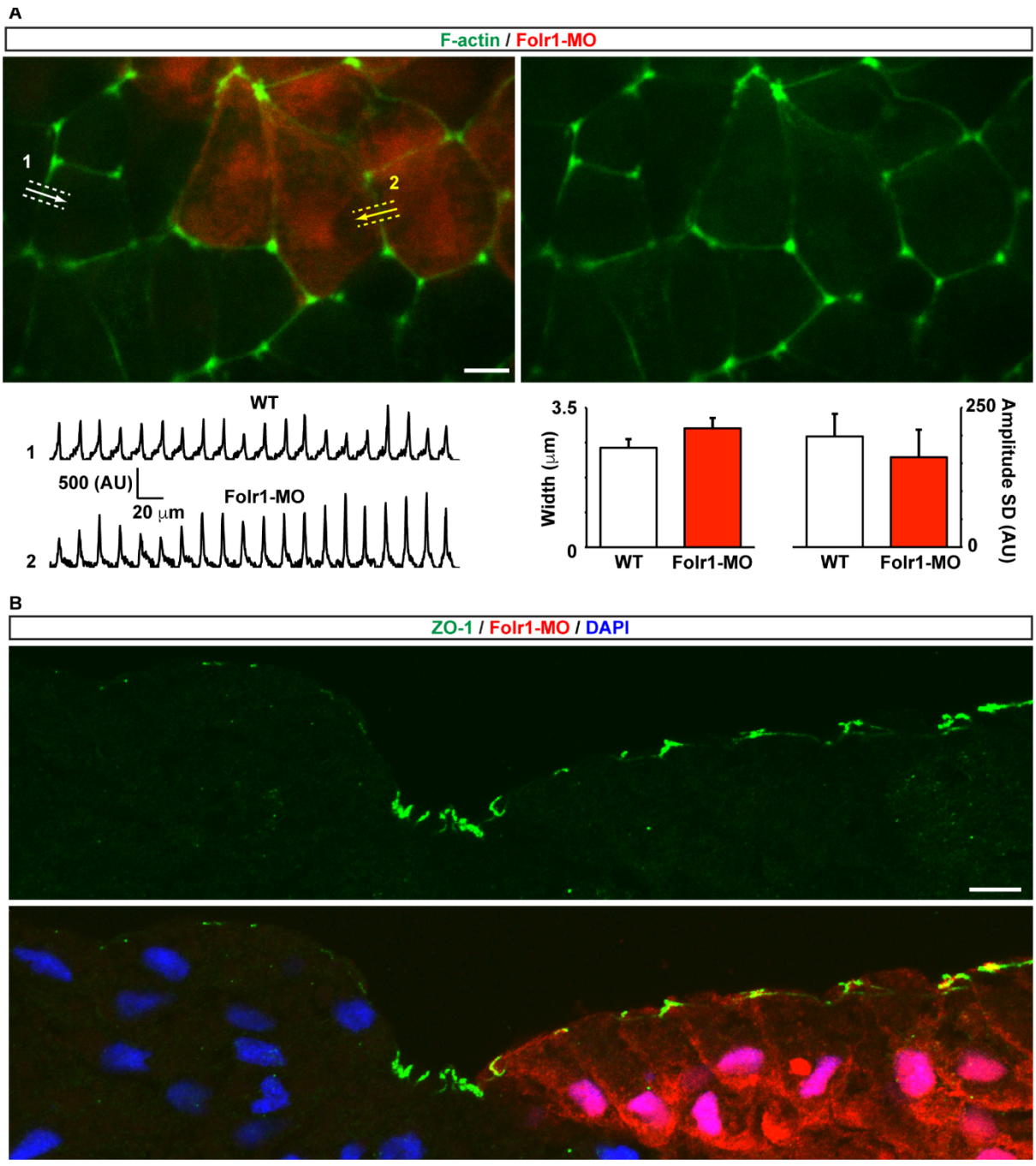



**Fig. S4. Folr1 knockdown does not induce changes in actin dynamics or in overall apicobasal polarity in the folding neural plate.** (A) Two-cell-stage embryos were unilaterally microinjected with Folr1-MO along with Alexa Fluor 594-dextran conjugate (red) and bilaterally injected with mCherry-UtropinCH (F-actin reporter, green). Superficial medial neural plate was time-lapse imaged from whole embryos at a rate of 1-2 $min^{-1}$. Fluorescence intensity profiles (arrows) across cell-cell borders were measured among wild-type (WT, 1) and Folr1-MO (2) containing cells during 15 min imaging. Scale bar, 10 μm. Intensity profiles were fitted with Gauss function using R software after background substraction. Bar graph shows mean±SEM peak width at 50% maximum intensity (left) and standard deviation (SD) of normalized maximum intensity during 15 min recording (right), n: 28 cell-cell borders per group, t-test. (B) Unilateral Folr1-MO-containing neural plate stage embryos were sectioned and processed for immunostaining with anti-ZO-1 (green). Scale bar, 20 μm.

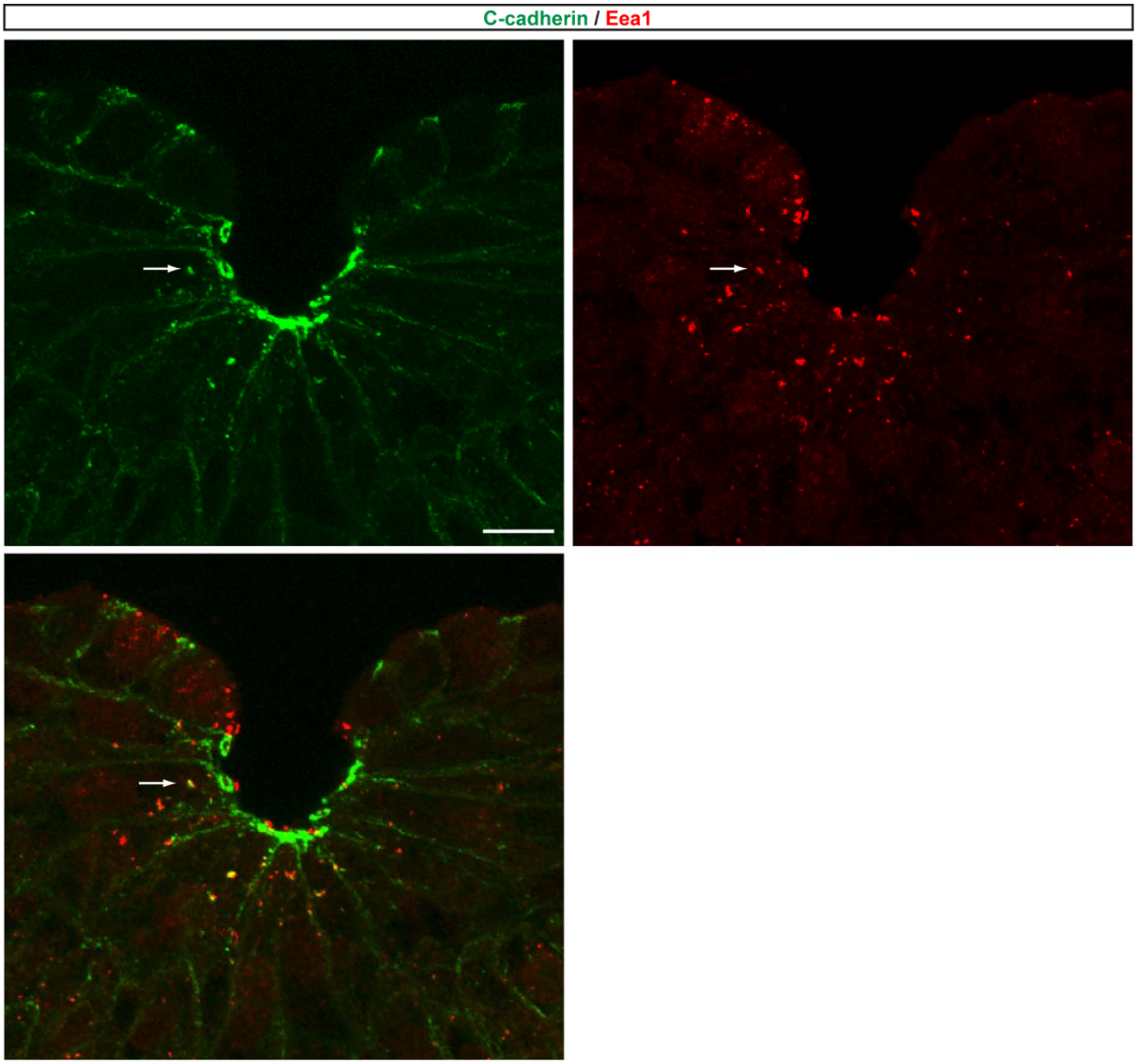
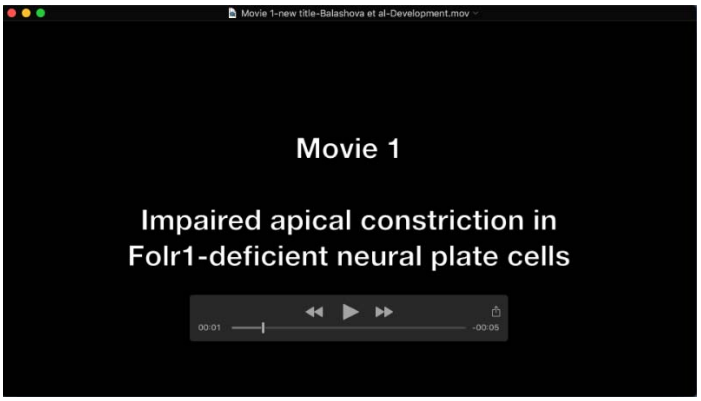
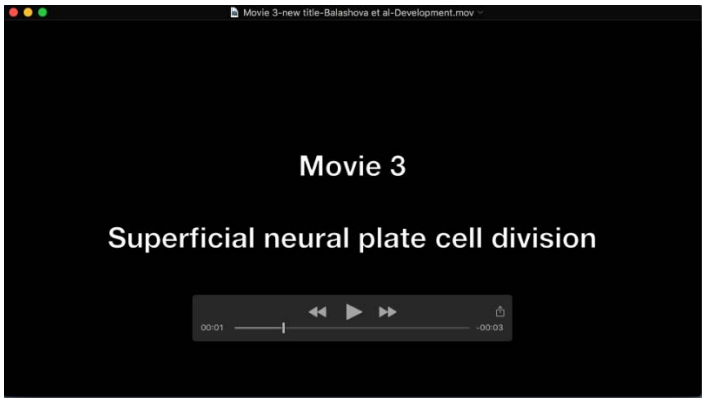



**Fig. S5. Neural plate cell apical constriction is concurrent with endocytosis of C-cadherin.** Neural plate stage embryos were sectioned and processed for immunostaining with anti-C-cadherin (green) and anti-Eea1 (red). Scale bar, 20 μm. Arrow points to a C-cadherin-containing endosome.




**Movie 1. Impaired apical constriction in Folr1-deficient neural plate cells.** Two-cell-stage embryos were bilaterally injected with membrane-GFP (in green) and unilaterally with 10 pmol Folr1-MO + AlexaFluor 594-dextran conjugate (in red). Confocal images of the apical neural plate in live embryos at stage 15-15.5 were taken every 5 min. Shown are z-projections of the sequence of time frames during 1 h recording. Note decrease in cell surface, folding of the neural plate and appearance of non-neural ectoderm (relatively big hexagonal cells) towards the end of the movie in the wild-type side (top, green), while neural plate cells containing Folr1-MO fail to constrict and the neural plate does not fold (bottom, yellow).


**Movie 2. Apical constriction is not affected in CMO-containing neural plate cells.** Two-cell-stage embryos were bilaterally injected with membrane-GFP (in green) and unilaterally with 10 pmol CMO + AlexaFluor 594-dextran conjugate (in red). Confocal images of the apical neural plate in live embryos at stage 15-15.5 were taken every 5 min. Shown are z-projections of the sequence of time frames during 1 h recording. Sample realignment was done after 30-min recording to recenter the field of view on the neural plate midline. Note decrease in cell surface and folding of the neural plate in the wild-type (top, green) and CMO-containing neural plate (bottom, yellow).




**Movie 3. Apical neural plate cell division.** Two-cell-stage embryos were bilaterally injected with membrane-GFP (in green) and unilaterally with 10 pmol Folr1-MO + AlexaFluor 594-dextran conjugate (in red). Confocal images of the apical neural plate in live embryos at stage 15-15.5 were taken every 5 min. Shown are z-projections of the sequence of time frames during 25 min recording. Asterisks indicate dividing and daughter cells. Dividing cells were excluded from the measurements of changes in apical cell surface during neural plate folding (Fig. 6). The proportion of dividing apical cells is small during neural plate folding and is similar in wild type and Folr1-deficient cells (see Results for details).