Supplementary Material: Movie S1 and supplementary figures S1-S4

Movie S1. Neural tube closure defect in an embryo unilaterally depleted of GEF-H1. Time-lapse images were taken every 10 min for an embryo unilaterally injected with 25 ng of GEF-H1 MO during early neurula stages (st. 13 to st. 16).

Fig. S1. Neural tube defects in GEF-H1-depleted embryos.

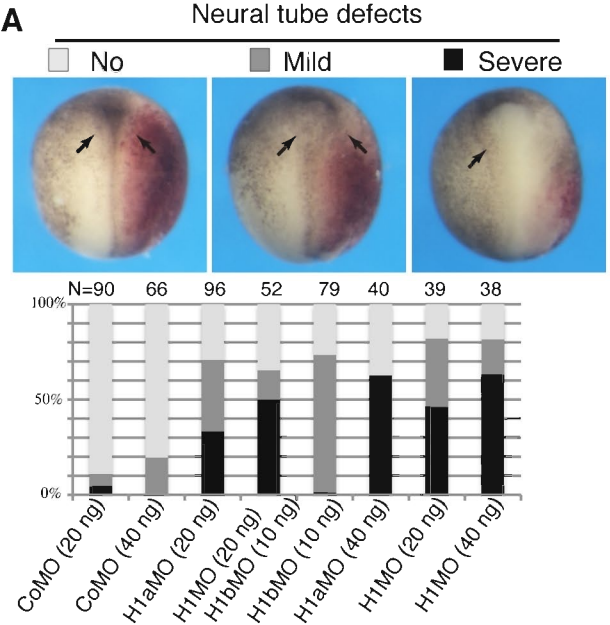
(**A**) Neural fold defects in GEF-H1- and CoMO-injected embryos. Four-cell embryos were injected twice unilaterally with control MO (20 or 40 ng), GEF-H1a (20 or 40 ng), GEF-H1b (10 ng), or GEF-H1 (20 or 40 ng) MO as indicated. Neural fold defects were assigned at stage 16-18 to the following categories: no defects (no difference from uninjected control embryo); mild defects (one neural fold is further away from the midline than the other); or severe defects (one neural fold does not form or severely defective). Representative embryos are shown. (**B**) GEF-H1a and GEF-H1 MOs efficiently block GEF-H1 mRNA translation in vivo. All blastomeres of four-cell embryos were injected with *Xenopus* GEF-H1-HA RNA (30 pg) with 10 ng of GEF-H1 or control MOs as indicated. Embryo lysates prepared at stage 10 were probed with anti-HA antibody. α-Tubulin is a loading control. (**C, C'**) Cross-section of a representative embryo unilaterally injected with GEF-H1a MO (40 ng) and membrane-bound RFP (mRFP) RNA (150 pg) as a lineage tracer, as described in Fig. 1 legend. Anti-β-catenin antibody reveals cell boundaries (C, merged). C', red channel. Arrow points to the defective neural fold at the site of injection, marked by mRFP. N, notochord; S, somite. (**D-F**) Embryos were injected with control MO (40 ng) and GFP RNA (200 pg) as described in Fig. 3. Immunostained frozen embryo sections (stage 16) are shown. The uninjected side serves as a control. Midline (M) is indicated by broken line. (**D**) phosphorylated myosin II light chain (pMLC), (**E**) F-actin, (**F**) Rab11. Bar, 20 μm.

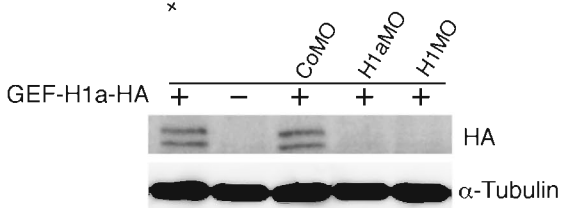
Fig. S2. GEF-H1-depleted embryos reveal cell intercalation defects, but no cell cycle changes. (**A, B**) Cross-sections of representative embryos unilaterally injected with GEF-H1 MO (40 ng) and membrane-bound RFP (mRFP, A) or GFP (mGFP, B) RNA (100-150 pg) as described in Fig. 1 legend. (A) Stage 17 embryo. Note multiple cell layers in lateral ectoderm (E, white bars) and mesoderm (M) at the injected side. White arrow marks defective neural fold. S, somites, N, notochord. Bar, 100 μm. (A'-A"') Magnified sections of the embryo in A. Note the different thickness (4-6 cell layers) of non-neural ectoderm and lateral mesoderm on the injected side (A', merged, A'', red channel only, Bar, 50 μm), as compared to the two-layered ectoderm on the uninjected side (A"'). Tissue boundaries are marked by dashed lines. (B) Stage 12.5 embryo. GEF-H1 MO-injected side contains more ectoderm cell layers (arrow) as compared to the uninjected side. Dorsal is at the top. Bar, 100 μm. (**C, D**). Mitotic cell number is not affected by GEF-H1 depletion. (C, C'). Cross-sections of early neurula embryos (stage 14) injected into two lateral sites at the four-cell stage with mRFP mRNA (not shown) and GEF-H1a or GEF-H1 MO (40 ng each) were immunostained with anti-phospho-Histone-H3 (pH3) antibody to marks cells undergoing mitosis. DAPI labels all nuclei. Representative embryo with GEF-H1a MO-injected side (C) and the uninjected side (C') is shown. Dorsal is to the top. Scale bar, 100 μm. (D) Quantitation of data shown in C and C'. Numbers of pH3-positive cells and total nuclei in dorsal ectoderm were counted on the MO-injected side or uninjected side from 6-10 sections per embryo. Results are presented as mean numbers of pH3-positive cells per 100 nuclei from three embryos +/- s. d. Numbers of nuclei analyzed are on the top of each bar.

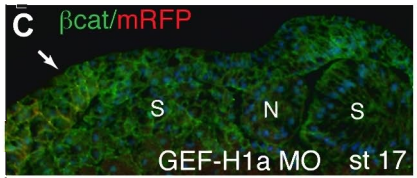
Fig. S3. Rho-GEF activity is necessary for GEF-H1-dependent apical constriction. Four-cell embryos were injected with *Xenopus* wild-type or mutant GEF-H1a RNAs (60 pg per each injection) into two animal blastomeres. Animal views of representative stage 8 embryos are shown. (**A**) Uninjected control embryos. (**B**) Wild-type GEF-H1 triggers the onset of apical constriction; (**C**) Inactive GEF-H1 mutant (Y444A) does not alter normal ectoderm morphology. More than 90 % of injected embryos (n>30) reveled the shown phenotypes in several independent experiments. (**D, E**) The localization of wild-type and inactive GEF-H1 in stage 10 ectoderm. GEF-H1 is distributed throughout the cell and is clearly visible at the cell cortex (D, arrows). GEF-H1-Y444A is more cytoplasmic and less associated with the cortex (E, asterisks). Embryos were injected with Myc-GEF-H1 RNAs as described in Figure 1. Cell boundaries are visualized with anti-β-catenin antibodies. Bar, 20 μm.

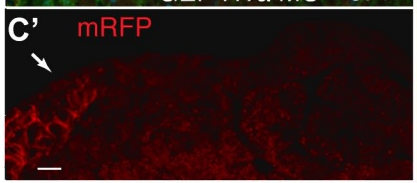
Fig. S4. GEF-H1 is apically polarized in deep ectoderm cells.
Immunostaining for endogenous pMLC (green) in cross-sections of stage 10 control embryos (**A**) or embryos injected with HA-GEF-H1 RNA (5-10 pg, red, **B**). pMLC is distributed at the cell boundary facing to the apical surface and lateral surfaces in control deep ectoderm (A). GEF-H1-expressing cells display elongated morphology and apical protrusions, positive for HA-GEF-H1 (arrow in B, B', B"). Bar, 20 μm. RNA injections were carried out as described in Figure 1.

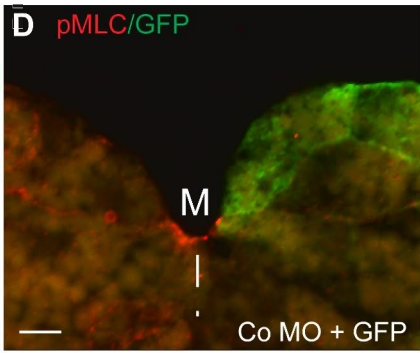
Fig. S1

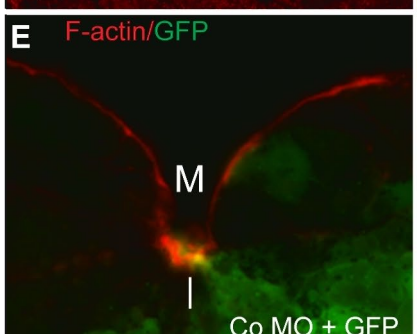

A
Neural tube defects
No
Mild
Severe
N=90
66
96
52
79
40
39
38
100%
50%
0%
CoMO (20 ng)
CoMO (40 ng)
H1aMO (20 ng)
H1MO (20 ng) + H1bMO (10 ng)
H1bMO (10 ng)
H1aMO (40 ng)
H1MO (20 ng)
H1MO (40 ng)


B
CoMO
H1aMO
H1MO
GEF-H1a-HA
+ − + + +
HA
α-Tubulin


C
βcat/mRFP
S
N
S
GEF-H1a MO st 17


C'
mRFP


D
pMLC/GFP
M
Co MO + GFP


E
F-actin/GFP
M
Co MO + GFP

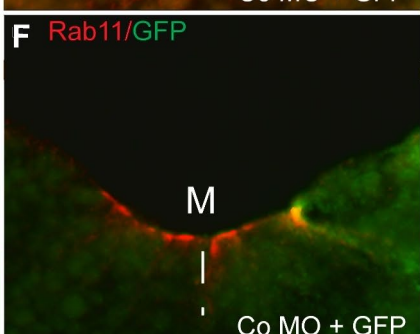

F
Rab11/GFP
M
Co MO + GFP

Fig. S2

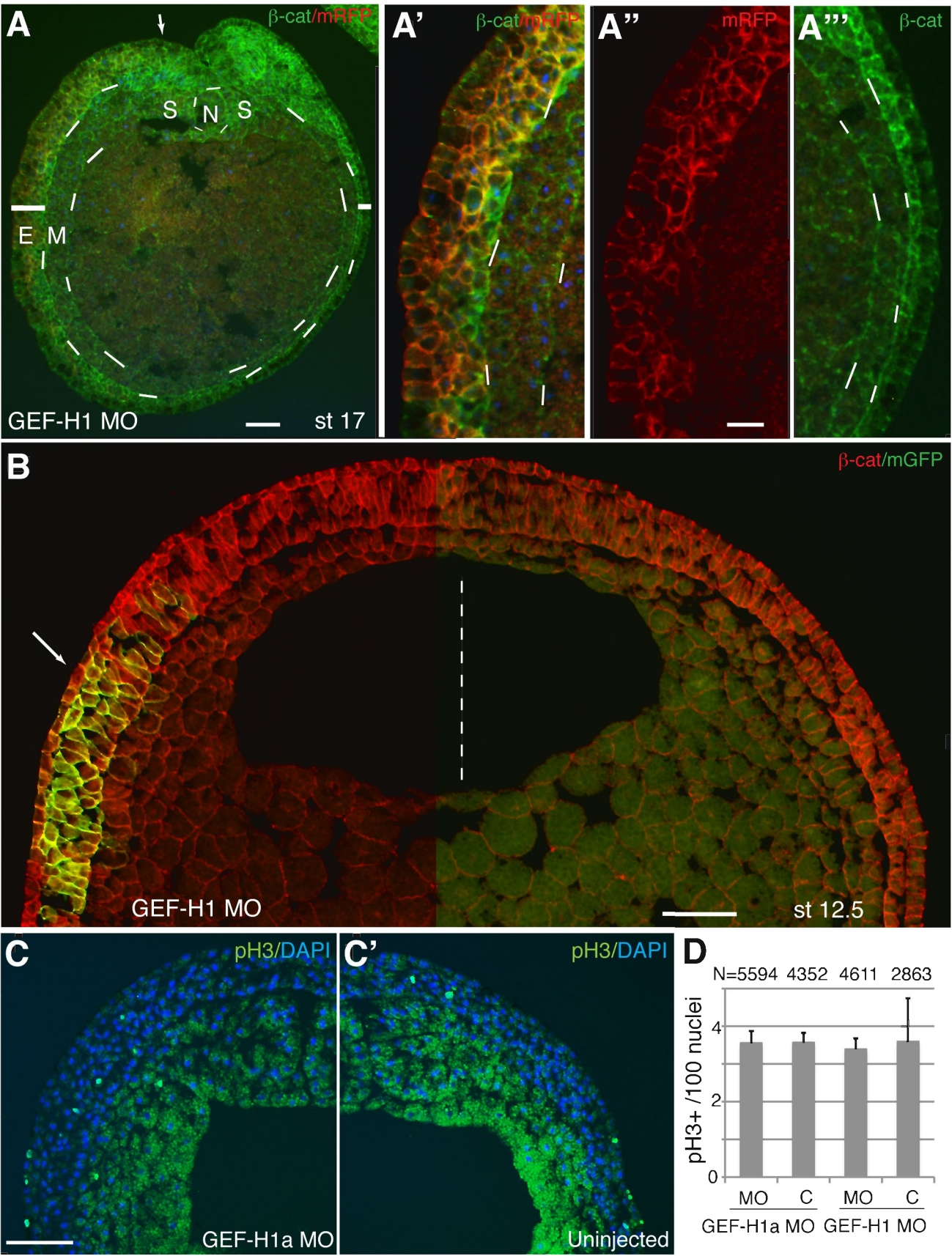

A
β-cat/mRFP
S N S
E M
GEF-H1 MO
st 17
A'
β-cat/mRFP
A''
mRFP
A'''
β-cat
B
β-cat/mGFP
GEF-H1 MO
st 12.5
C
pH3/DAPI
GEF-H1a MO
C'
pH3/DAPI
Uninjected
D
N=5594 4352 4611 2863
pH3+ /100 nuclei
0
2
4
MO
C
MO
C
GEF-H1a MO
GEF-H1 MO

Fig. S3

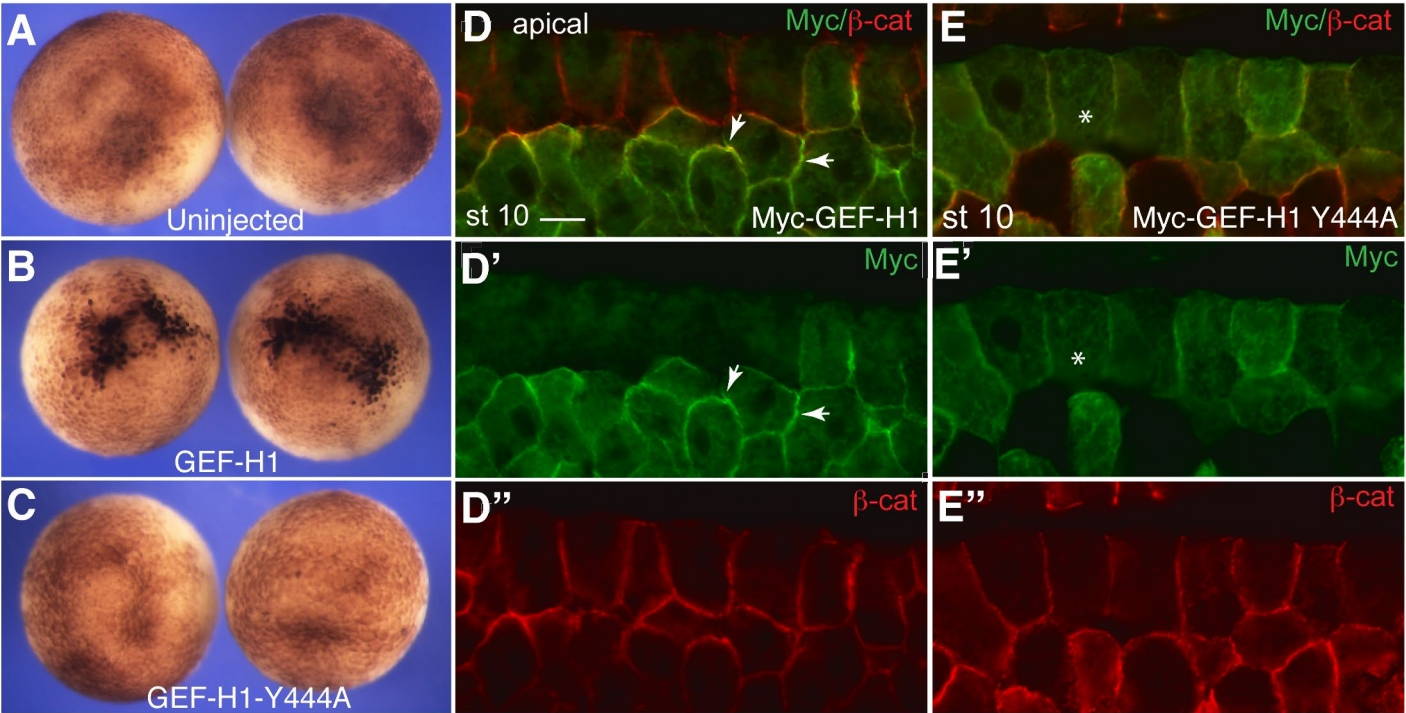
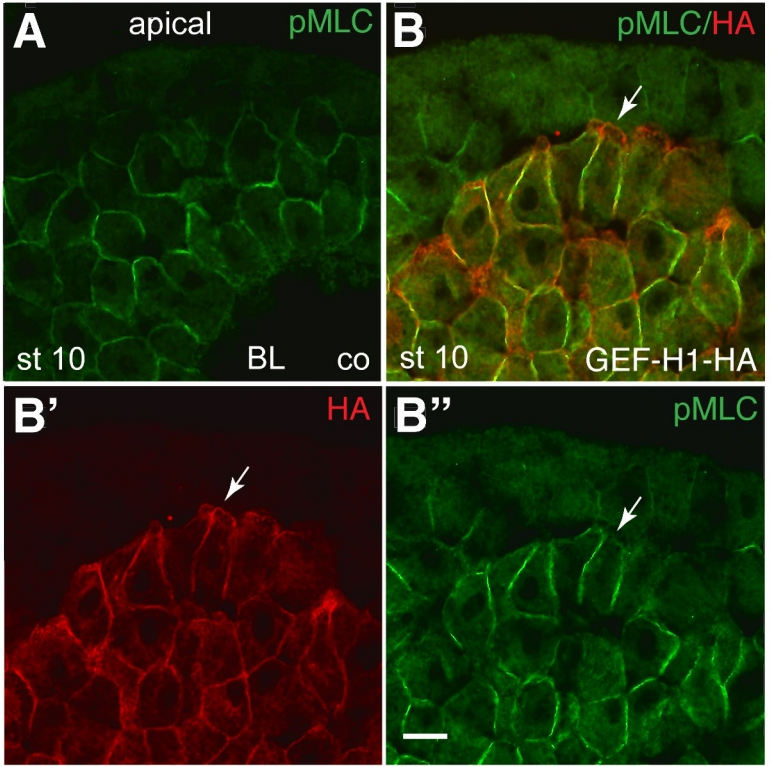

A
Uninjected
B
GEF-H1
C
GEF-H1-Y444A
D apical
Myc/β-cat
st 10
Myc-GEF-H1
D'
Myc
D''
β-cat
E
Myc/β-cat
st 10
Myc-GEF-H1 Y444A
E'
Myc
E''
β-cat

Fig. S4



Movie 1.