Blastomere reaggregation assay
Morpholino oligos and/or mRNAs were injected into both blastomeres at the 2-cell stage near the animal pole region. Animal caps were dissected from injected embryos at stage 8 and dissociated in 1× calcium/magnesium-free modified Barth’s solution (CMF-MBS: 88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO	extsubscript{3}, and 10 mM Hepes, pH 7.5) and incubated for 1 h in 35-mm Petri dishes coated with 1% agarose in the aforementioned buffer. Cells were re-aggregated at RT on an orbital bench-top shaker at 60 rpm for 1 h in 4 mM Ca	extsuperscript{2+} in the CMF-MBS buffer. Cell reaggregation was observed by digital capture of random cell populations from each sample using a dissection microscope (model SMZ-U; Nikon).

Confocal immunofluorescence microscopy of animal caps
Embryos were injected with standard control morpholino or depleted of xARVCF and/or Xp120 (40 ng respective morpholino injected into animal hemisphere of each cell of 2-cell embryos). Animal caps were subsequently isolated from stage 9 (late blastula) embryos and fixed in MEMFA (3.7% formaldehyde, 4 mM MgO, 2 mM EGTA, and 1 mM MgSO	extsubscript{4}, pH 7.4) for 1 h at RT. Fixed embryos underwent serial methanol dehydration and were blocked with 20% donkey serum (Jackson ImmunoResearch Laboratories) for 1 h, followed by overnight incubation with primary anti–C-Cadherin rabbit polyclonal sera at 1:500 dilution with rotation at 4°C overnight. The resulting immunocomplexes were sedimented at 14,000 x g for 10 s. The precipitates were washed in prechilled 1% TX buffer followed by 4 mM Mops, 2 mM EGTA, and 1 mM MgSO	extsubscript{4}, pH 7.4 for 1 h at RT. Fixed embryos underwent serial methanol dehydration and were blocked with 20% donkey serum (Jackson ImmunoResearch Laboratories) for 1 h, followed by overnight incubation with primary anti–C-Cadherin rabbit polyclonal sera at 1:500 dilution, and donkey anti–rabbit FITC Fab′2 secondary antibody at 1:500 dilution (Jackson ImmunoResearch Laboratories). Coverslips were mounted on glass slides using VectaShield mounting medium with DAPI (Vector Laboratories). Images were obtained from outer ectoderm cells of animal caps using an inverted microscope (model IX-70, Olympus) fitted with an Olympus FV5-PSU multiple-laser confocal imaging system. Mounted animal caps were visualized with an UplanFL 40× oil immersion objective, and the resulting FITC channel images were digitally captured using Olympus Fluoview FV500 software (version 4.3).

Immunoprecipitation and Western blotting of xARVCF–RhoA and Xp120–RhoA complexes
Embryos were injected at the 2-cell stage with 6× Myc and 3× HA epitope–tagged constructs. The injected embryos were harvested at stage 11, and whole embryos lysated prepared by pipetting with prechilled 1% TX buffer (10 mM Hepes, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, and 1% Triton X-100, pH 7.4) supplemented with a protease inhibitor cocktail consisting of 1 mM PMSF, 4 μg/ml aprotinin, 1 μg/ml pepstatin A, 2 μg/ml leupeptin, 10 μg/ml antipain, 50 μg/ml benzamide, 10 μg/ml soybean trypsin inhibitor, 100 μg/ml soybean serine protease inhibitor, and 10 μg/ml TLCK. The extract was cleared by centrifugation at 14,000 g, 4°C for 30 min. Lysates were incubated with anti-xARVCF or Xp120 polyclonal antibodies, or with anti-Myc or HA monoclonal antibodies at 1:500 dilution with rotation at 4°C for 1–3 h. Protein A– and G–Sepharose 4B beads (Sigma-Aldrich) were added and incubated with rotation at 4°C overnight. The resulting immunocomplexes were sedimented at 14,000 g, 4°C for 10 s. The precipitates were washed in prechilled 1% TX buffer followed by 10 mM Hepes buffer, pH 7.4, and resuspended in SDS sample buffer (125 mM Tris, 4% SDS, 20% glycerol, 2% β-mercaptoethanol, and 1% Bromphenol blue, pH 6.8). The samples were electrophoresed on 8% polyacrylamide gels and transferred to nitrocellulose membranes. Blots were probed with anti-Myc or anti-HA polyclonal antibodies at 1:5,000 dilution, followed by a second incubation with goat anti–rabbit or –mouse antibodies (Bio-Rad Laboratories) at 1:3,000 dilution. The signal was detected using ECL Western blotting detection reagents (Amersham Biosciences).

Healing/exogastrulation assay
Embryos were injected with morpholinos (40 ng) directed against xARVCF and/or Xp120 into both cells at the 2-cell stage. Injected embryos were partially dissected at stage 9, turning the animal caps outwards while remaining attached (Heasman et al., 1994). Initial healing (blastula stages) and subsequent exogastrulation processes were monitored as the embryos were incubated in 1× Marc’s Modified Ringers solution (100 mM NaCl, 2 mM KCl, 2 mM CaCl	extsubscript{2}, 1 mM MgCl	extsubscript{2}, and 5 mM Hepes, pH 7.4) over a time course of 3 and 6 h at RT. Images were obtained using a standard binocular dissecting microscope.

Low-resolution visualization of blastomere association/tissue integrity in dissected embryos
Morpholinos directed against xARVCF and/or Xp120 were injected into each cell of 2-cell stage embryos. Injected embryos were dissected at stage 9 to remove the animal caps. Images were collected from both unfixed (monolayer imaging) and fixed embryo samples (multilayer imaging). Blastomere association and tissue integrity were visualized in unfixed samples using a standard binocular dissecting microscope. Fixed samples were examined using a stereomicroscope (model MZ FLIII; Leica; Leica PLAN APO 1.0× objective) with an associated Motor-Focus System (Leica). Image processing was conducted using Image-Pro Plus (Media Cybernetics, Inc.) and Optronics Magnafire (Image Content Technology LLC) software. The cell–cell interactions of xARVCF- and/or Xp120-depleted embryos appear unperturbed relative to standard control–injected embryos.

C-Cadherin constructs used to rescue Xp120-depleted embryos
Native (full-length) Xenopus laevis C-cadherin (spanning nucleotides 1–2643) was a gift of B. Gumbiner (University of Virginia, Charlottesville, VA). The C-cadherin juxtamembrane (JMR) domain construct (nucleotides 2185–2334) was generated by fusing the NH2-terminus of the JMR domain to the c-Src myristylation motif MGSSKSKPKD, to direct the construct to the inner plasma membrane (Paulson et al., 2000). The C-cadherin ectodomain construct (includes ecto and transmembrane domains spanning nucleotides 1–2190), the C-cadherin catenin-binding domain deletion (ΔCJb) construct (spanning nucleotides 1–2470), and the C-cadherin JMR domain deletion (ΔJM) construct (nucleotides 1–2200 + 2470–2643) were gifts of C. Nielsen (University of Cologne, Cologne, Germany). To generate C-cadherin triple-point-mutant constructs that abrogate xARVCF/Xp120 association (Thoreson et al., 2000), PCR-mediated mutagenesis was conducted using the QuikChange site-directed mutagenesis kit (Stratagene; PCR primer pair, 5′-ATGGGAGGAAAAGCTGAGCAGCAGAGGAGATCAGG-3′ and 5′-ATCATGATCCTCTCTGCTGACGTTTCTCCTCC-3′), resulting in the substitution of three alanines for three glycines (amino acid positions 771–773).
References


