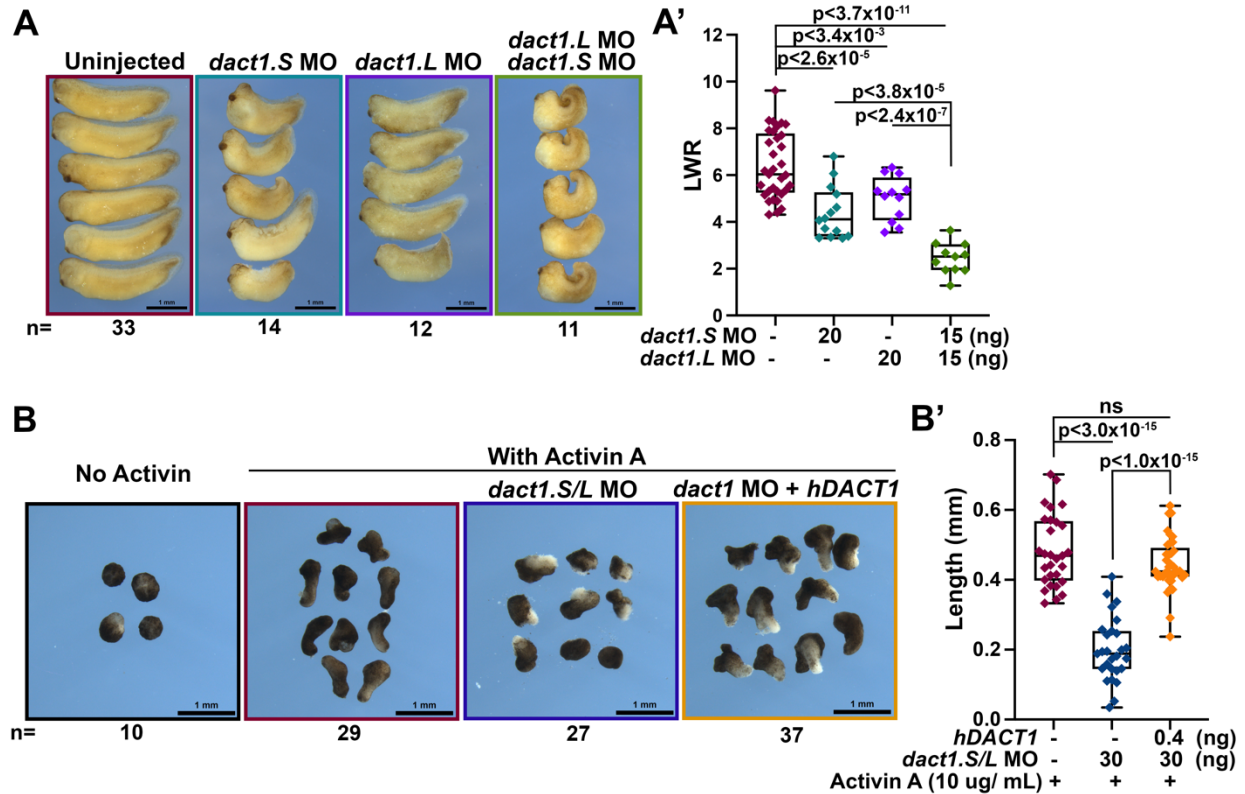
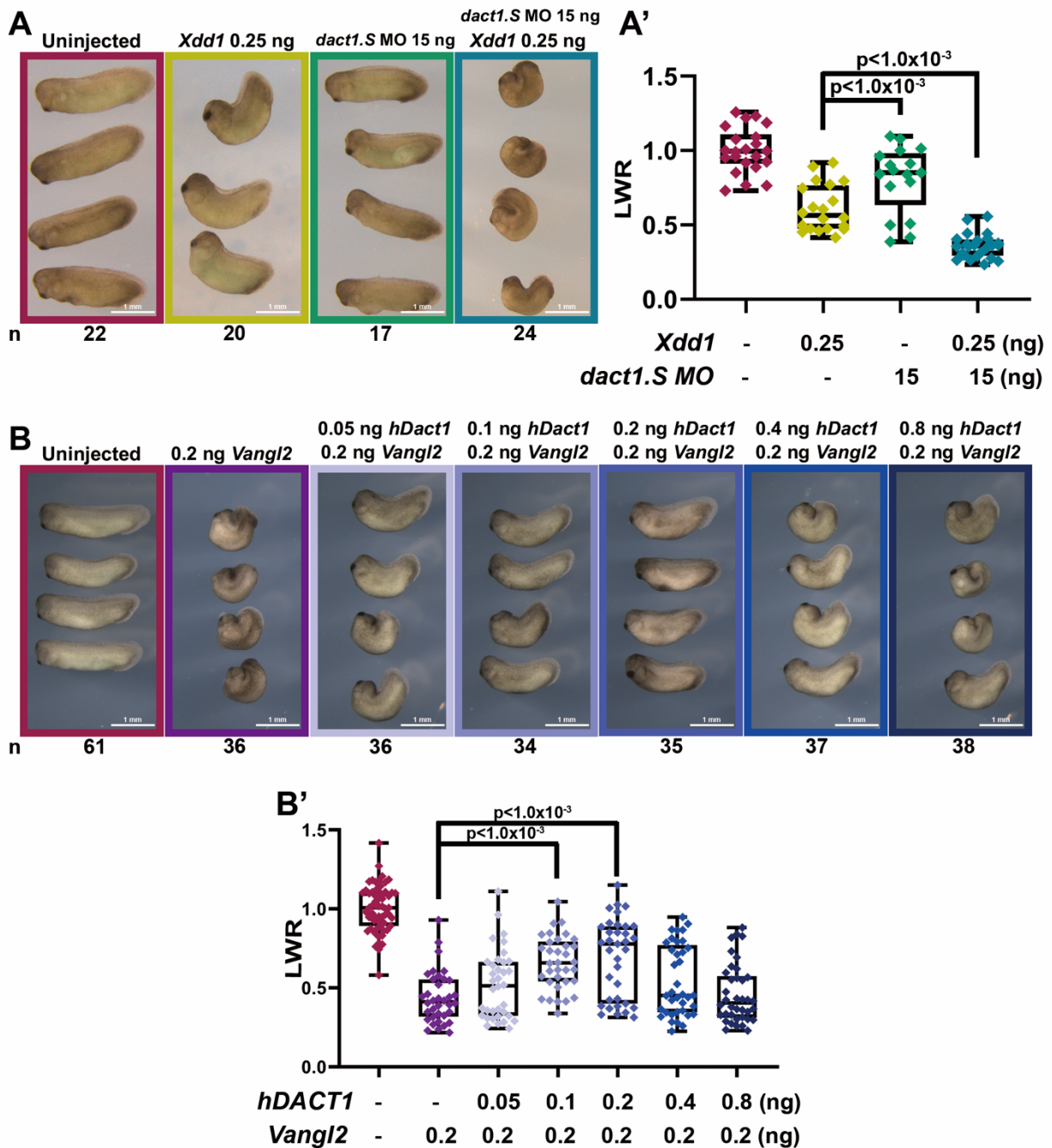




Supplementary Figure 1. Loss of *Xenopus dact1* induced CE defects can be rescued by human *DACT1*.

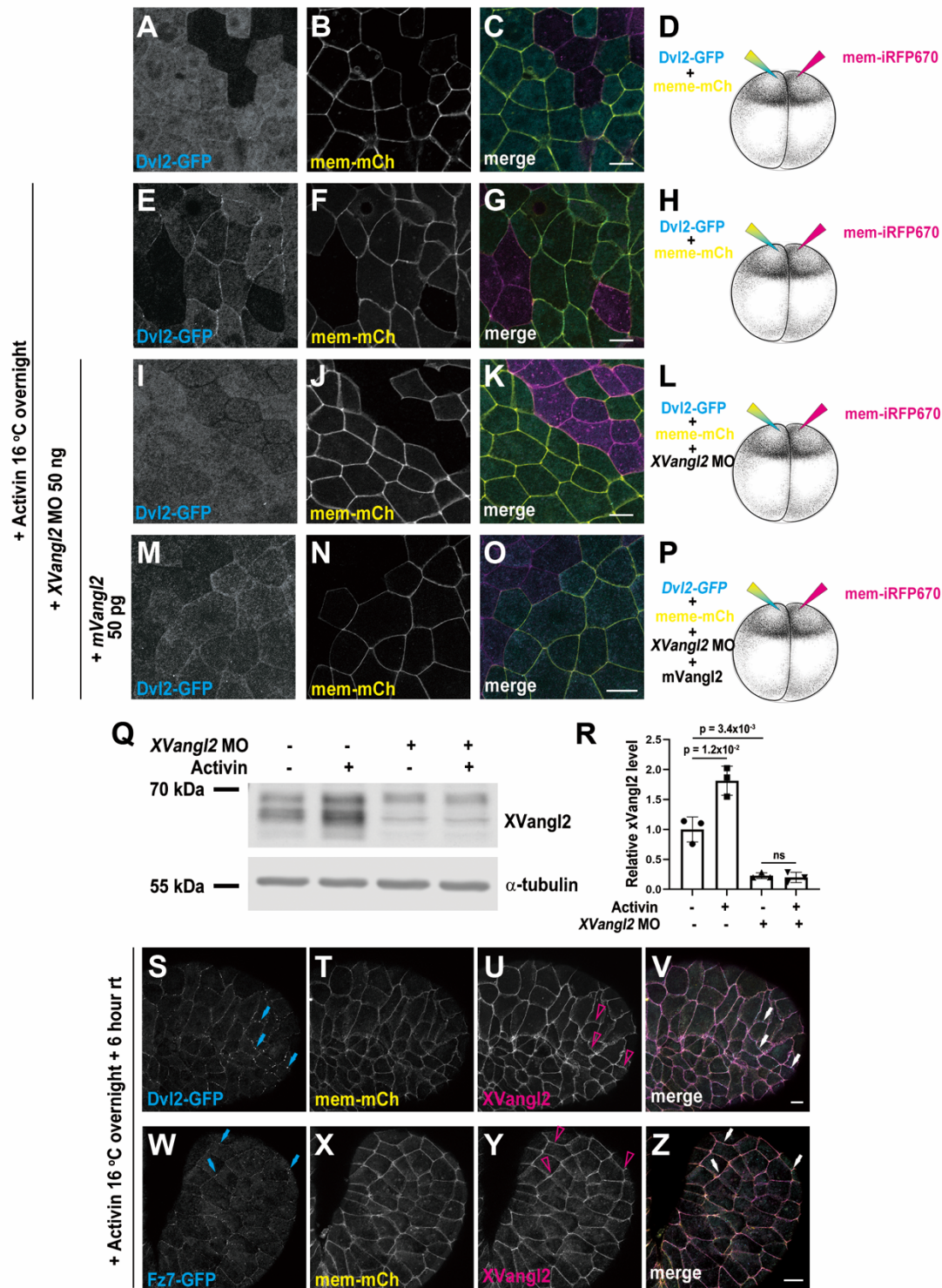
(A) DMZ injection of 20 ng morpholino (MO) against either *Xenopus dact1.S* or *dact1.L* induces moderate CE defects, and co-injection of 15 ng of each MO causes a significantly more severe CE defect. (A') Quantification of the severity of CE defects by measuring the length-to-width ratio (LWR) of the injected embryos. (B) Animal cap explants can be induced to elongate by treating with 10 ug/ml of Activin A, but the elongation is blocked in explants injected with *dact1.S* and *dact1.L* morpholinos (15 ng each; 30 ng total). Co-injection of 0.4 ng *mSc-hDACT1* mRNA can rescue the elongation defect in *dact1.S/L* morphant explants. (B') Quantification of the length of the animal cap explants in each group. Parametric student's t-test was used to compare injected groups with controls. ns indicates no statistical significance between compared groups. Source data are provided as "Supplementary Figure 1 source data" file.



Supplementary Figure 2. *Dact1* synergizes with *Dvl* but antagonizes *Vangl*.

(A) DMZ injection of either 0.25 ng *Xdd1* mRNA or 15 ng *dact1.S* MO induces only moderate CE defects, whereas their co-injection induces severe CE defect. (A') Quantification of the length-to-width ratio (LWR) of the injected embryos. (B) Severe CE defect induced by *Vangl2* overexpression in the DMZ (0.2 ng mRNA injection) can be rescued by co-overexpression of

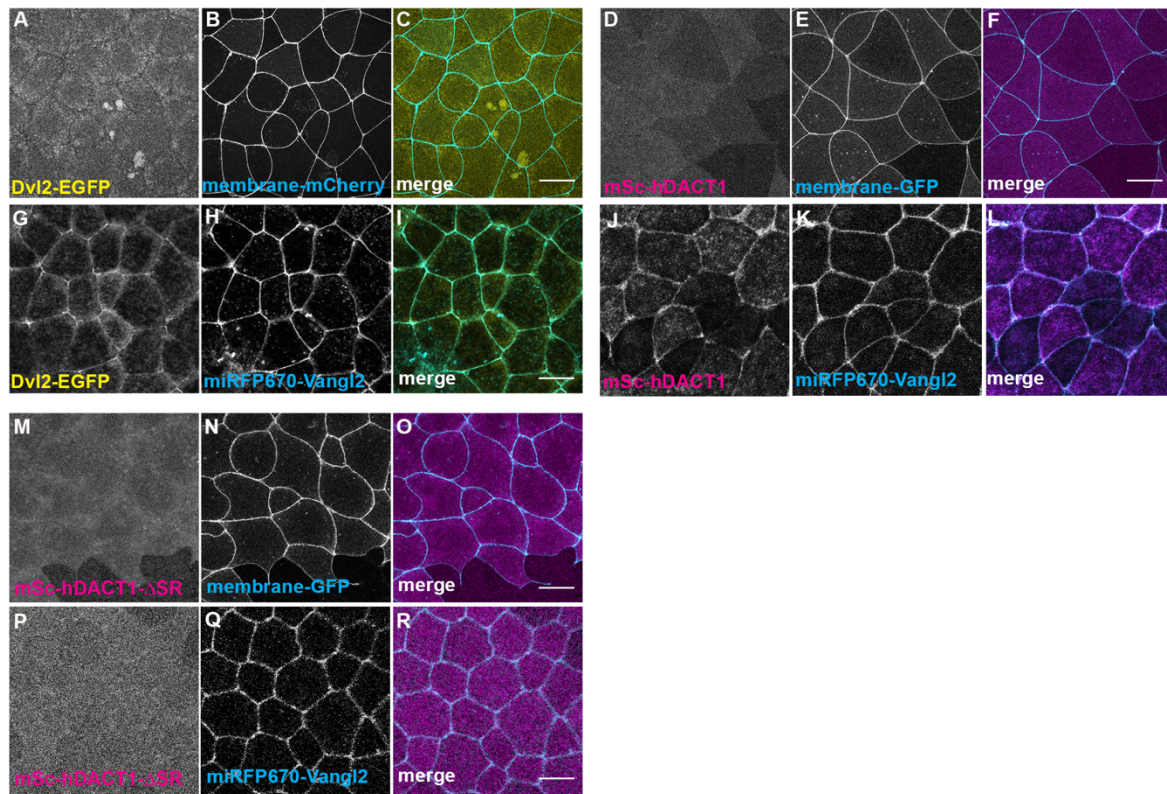
hDACT1 in a dose-dependent manner: 0.1 or 0.2 ng of *hDACT1* mRNA co-injection provides the best rescue, but the rescue efficiency is decreased when either less (0.05 ng) or more (0.4, or 0.8 ng) mRNA is used. **(B')** Quantification of the CE defects of the injected embryos by the length-to-width ratio (LWR) measurement. Experiments were repeated at least three times and the total number of embryos analyzed is indicated below each panel. Parametric student's t-test was used to compare injected groups with controls. Source data are provided as "Supplementary Figure 2 source data" file.



Supplementary Figure 3. Analyses of endogenous *Xenopus* Vangl2.

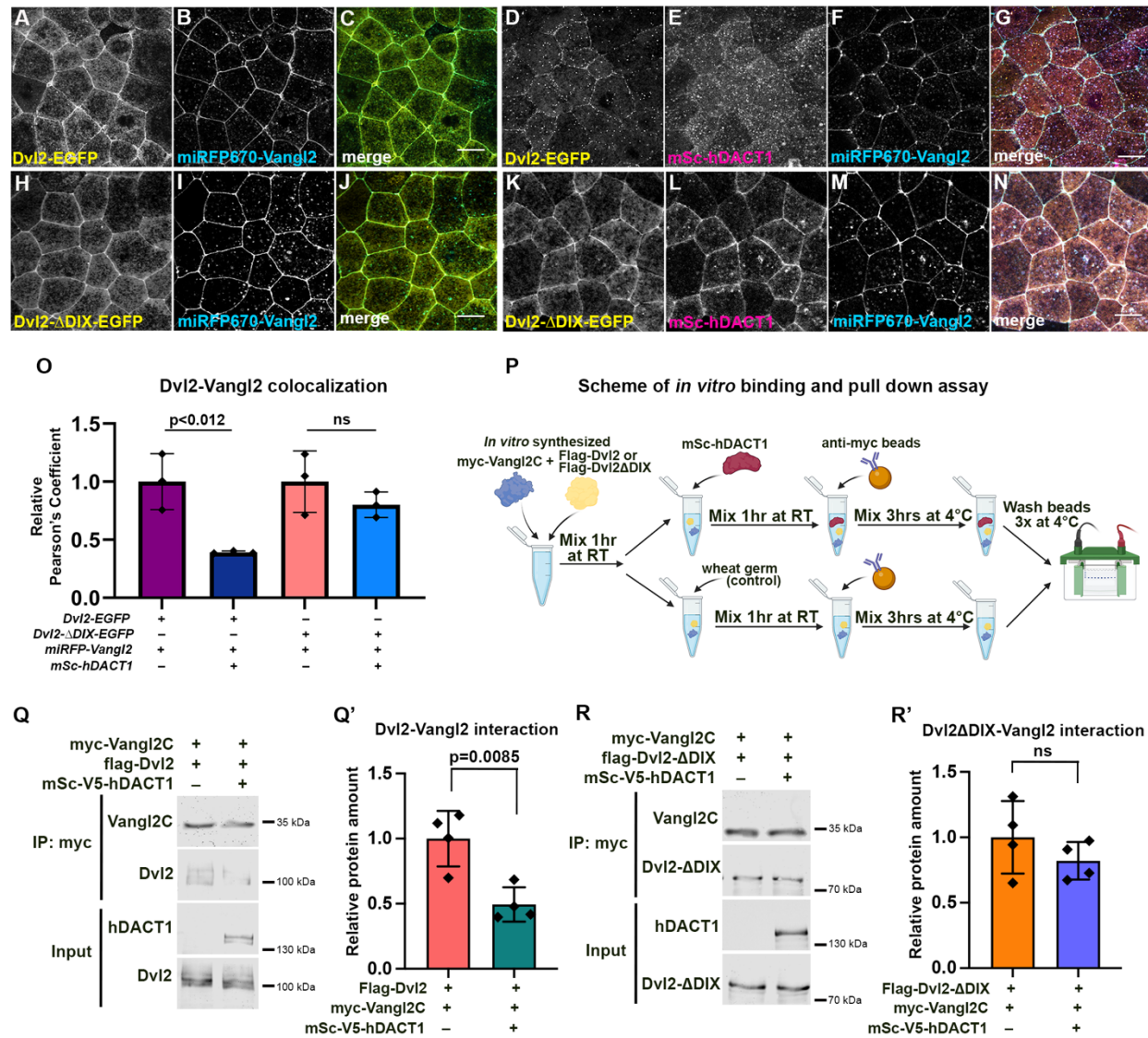
One blastomere of two-cell stage *Xenopus* embryos was injected with 30 ng *Dvl2-GFP* mRNA, a dose that produces no morphogenetic defect and is over 15 folds less than that needed to induce

CE phenotype¹. The injected blastomere is marked by co-injecting membrane-mCherry (mem-mCh) whereas the control blastomere is marked with membrane iRFP670 (mem-iRFP670) (schematic diagrams in D,H,L,P). In the resulting animal cap explants, Dvl2-EGFP remains even distributed in the cytoplasm when there is no Activin treatment (A-C). By contrast, in explants that have been treated with Activin overnight at 16 °C (to induce the onset of CE), Dvl2-EGFP becomes enriched at the plasma membrane (E-G). Activin-induced Dvl2-EGFP localization at the plasma membrane is abolished by knocking down endogenous XVangl2 with 50 ng MO (I-K), but restored by co-injecting 50 pg mouse *Vangl2* (*mVangl2*) mRNA into *XVangl2* morphant (M-O). Western blot with an anti-Vangl2 antibody (36E3; ref²) shows that the endogenous XVangl2 protein level is significantly increased by Activin treatment but diminished by MO (Q, R). Note that endogenous Vangl2 was reported to have two isoforms, a major isoform translated from the canonical AUG and a less abundant Vangl2-long isoform translated from an alternative translation start codon AUA in the 5' UTR region². The 36E3 anti-Vangl2 detects both isoforms with approximate size of 70 Kd (for Vangl2-Long isoform) and 62 Kd (for the major Vangl2 isoform). Since the *XVangl2* morpholino was designed to target the canonical start codon³, it was efficient at blocking translation of the major Vangl2 isoform but less so of Vangl2-long. The total amount of endogenous Vangl2 under each condition was assessed in [®] by measuring the intensity of both isoforms. (S-Z) After an additional six-hour incubation at room temperature, CE has led to active elongation of Activin treated animal cap explants, in which small amount of injected Dvl2-GFP (0.03 ng, S-V) or Fz7-GFP (0.02 ng, W-Z) becomes punctate on the plasma membrane (blue arrows). Vangl2 immunostaining shows that endogenous XVangl2 remains evenly distributed on the plasma membrane and does not cluster with either Dvl2 or Fz7 into puncta (hollow arrowhead in U and Y). Source data are provided as "Supplementary Figure 3 source data" file. *Xenopus* illustrations in D, H, L, P were adapted from drawings on Xenbase (www.xenbase.org RRID:SCR_003280), which was created by Zahn (© Natalya Zahn (2022) and published in ref.⁴. *Xenopus* illustrations © Natalya Zahn (2022).



Supplementary Figure 4. Vangl2 recruits wild-type Dvl2 and hDACT1, but not hDACT1- Δ SR mutant, to the plasma membrane.

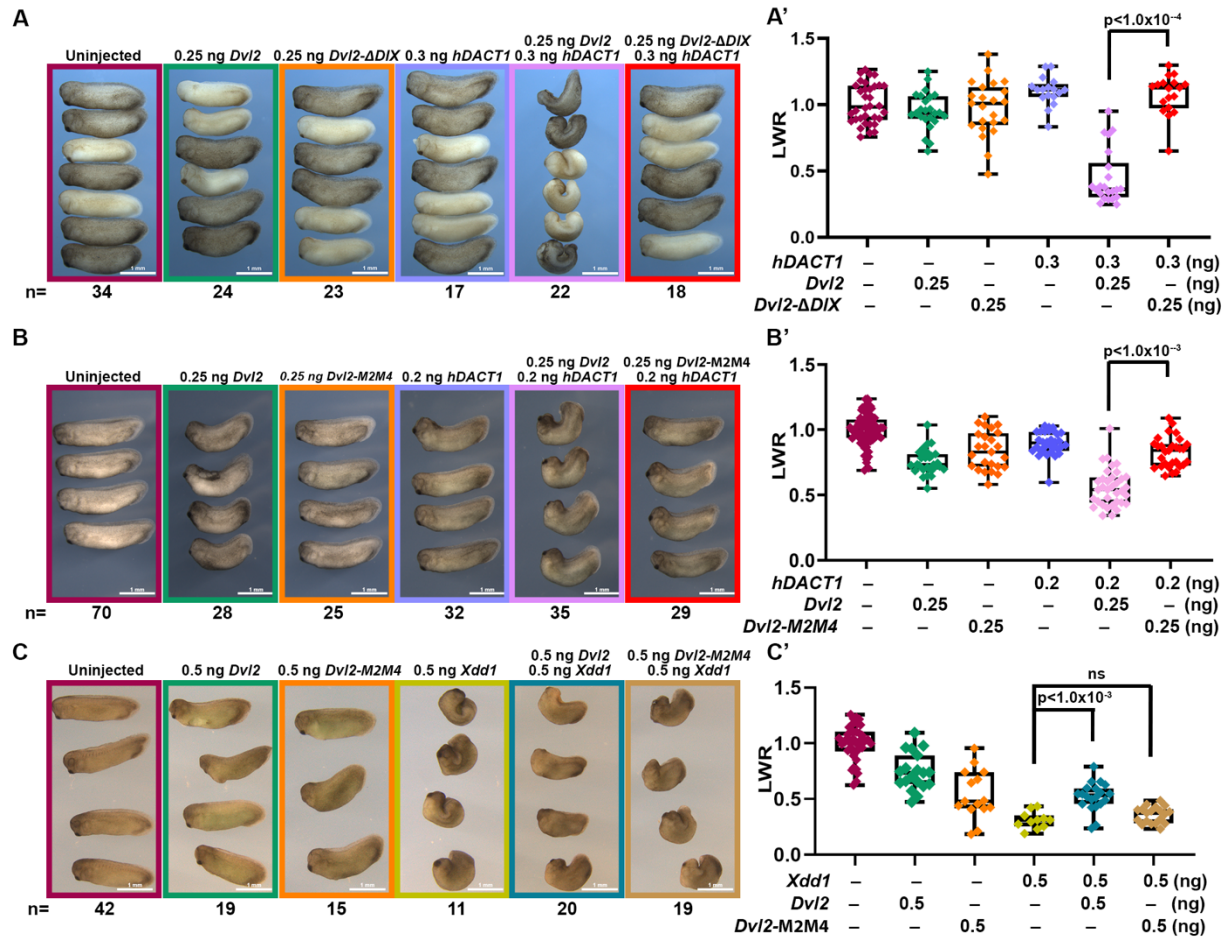
In animal cap explant Dvl2-EGFP displays homogeneous cytoplasmic distribution when co-expressed with membrane-mCherry (A-C), but is recruited to the plasma membrane when co-expressed with miRFP670-Vangl2 (G-I). Similarly, mSc-hDACT1 displays homogenous cytoplasmic distribution when co-expressed with membrane-GFP (D-F), but becomes enriched at the plasma membrane when co-expressed with miRFP670-Vangl2 (J-L). In contrast, mSc-hDACT1- Δ SR mutant displays homogeneous cytoplasmic distribution irrespective of co-expression with membrane-GFP or miRFP670-Vangl2 (M-R). Scale bars indicate 30 μ m.



Supplementary Figure 5. Dact1 removes Dvl2 from Vangl2 by promoting DIX-mediated oligomerization.

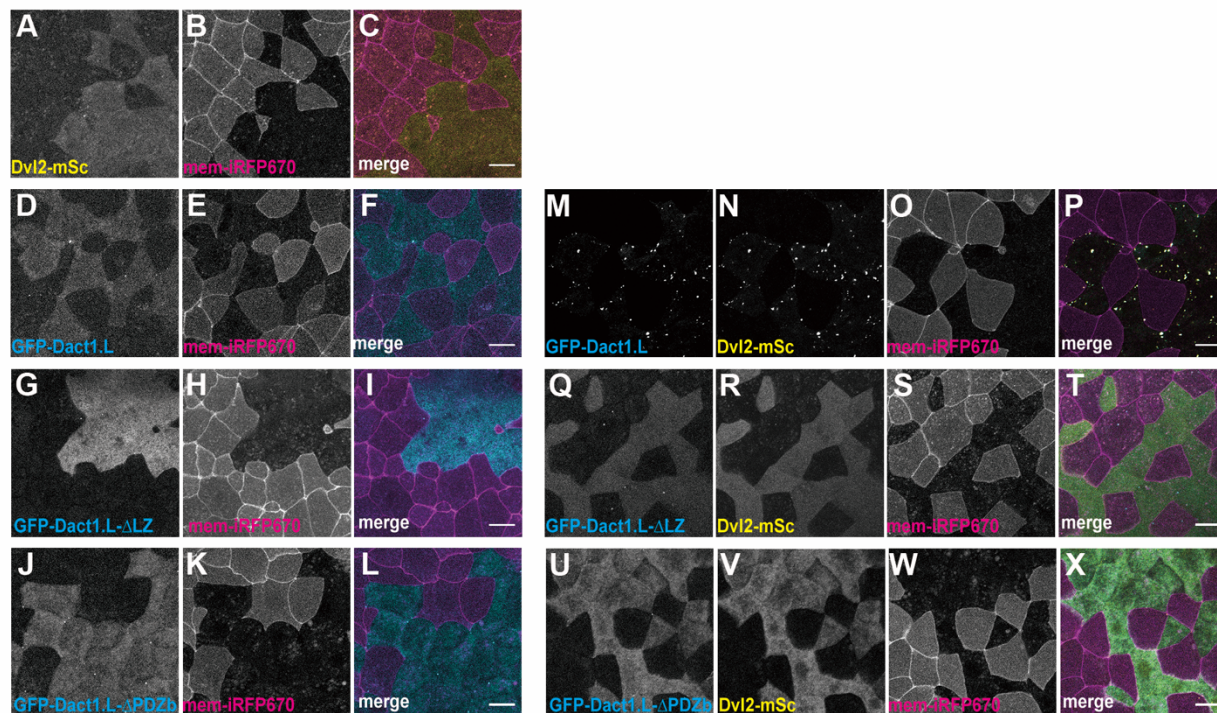
In animal cap cells, EGFP tagged wild-type Dvl2 (A-C) and Dvl2-ΔDIX mutant (H-J) can both be recruited to the plasma membrane by Vangl2 (tagged with miRFP670) and display co-localization with Vangl2. Co-overexpression of mSc-hDACT1 decreases Dvl2-Vangl2 colocalization on the plasma membrane with simultaneous formation of puncta (D-G). But hDACT1 did not induce Dvl2-ΔDIX to form puncta when co-expressed, and both Dvl2-ΔDIX and hDACT1 are co-localized with Vangl2 on the plasma membrane (K-N). (O) Quantification of Pearson's coefficient shows that

that hDACT1 decreased Vangl2 co-localization with Dvl2 but not with Dvl2- Δ DIX. (P) *In vitro* binding and co-immunoprecipitation (co-IP) assay (illustration created in BioRender. Angermeier, A. (2025) <https://BioRender.com/o68y049>). *In vitro* synthesized Myc-Vangl2C is first incubated with Flag-Dvl2 or Flag- Δ DIX for an hour, followed by addition of hDACT1 or wheat-germ extract (as control) for another hour before anti-Myc co-IP and western blot analysis. Western blots and quantification show that Flag-Dvl2 pulled down by Myc-Vangl2 is significantly reduced by the second incubation with hDACT1 (Q, Q'), whereas Flag- Δ DIX is not (R, R'). Scale bars indicate 30 μ m in panels A-N. Source data are provided as "Supplementary Figure 5 source data" file.



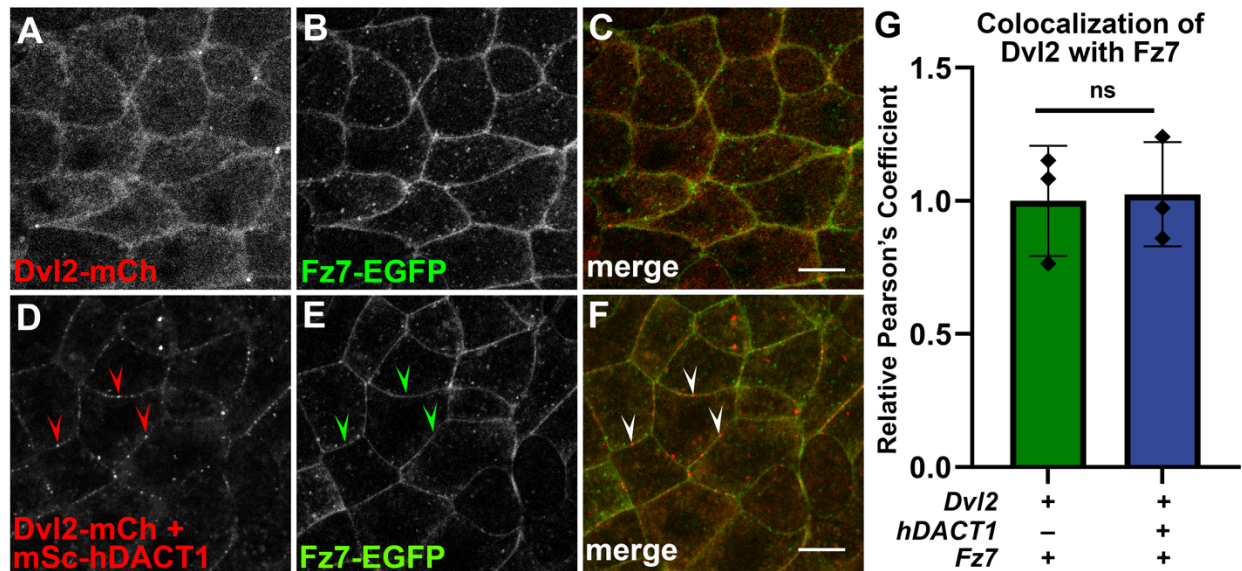
Supplementary Figure 6. DIX-mediated oligomerization is required for Dvl to synergize with hDACT1 and to rescue *Xdd1* induced defects during CE.

DMZ injection of four-cell stage embryos shows that hDACT1 can synergize with wild-type Dvl2 to disrupt CE, but the synergy is not observed with Dvl2-ΔDIX or Dvl2-M2M4 mutant (A-B). DMZ injection of 0.5 ng *Xdd1* induces severe CE defects, which can be rescued by co-injection with 0.5 ng wild-type *Dvl2* but not *DVL2-M2M4* (C). (A', B' and C') CE defects are quantified by measuring the length-to-width ratio (LWR) of the injected embryos, and parametric student's t-test was used to compare different groups. Scale bars indicate 1 mm in panel A, B and C. Source data are provided as "Supplementary Figure 6 source data" file.



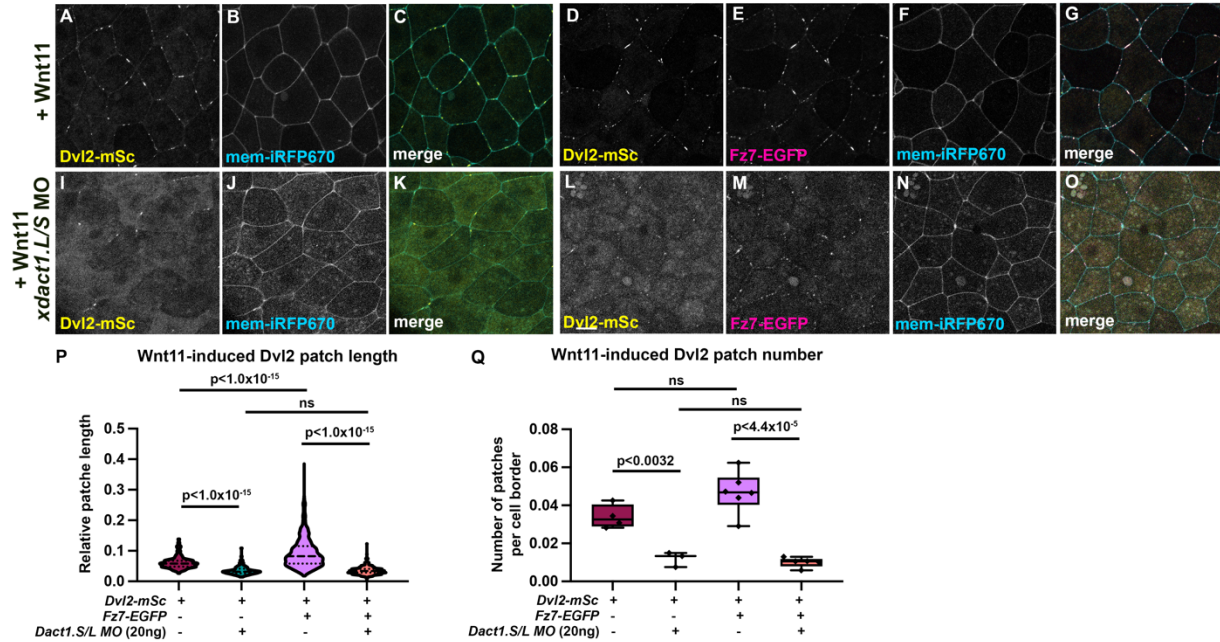
Supplementary Figure 7. Both the PDZb and LZ domains of Dact1 are required for it to induce Dvl2 puncta formation.

One blastomere of two-cell stage *Xenopus* embryos was injected with membrane-iRFP670 (mem-iRFP670), whereas the adjacent blastomere was injected with mScarlet-I tagged mouse Dvl2 (A-C), GFP tagged wild-type *Xenopus* Dact1.L (D-F), or Dact1.L mutants lacking either the leucine zipper domain (Dact1.L-ΔLZ, G-I) or the PDZ-binding domain (GFP-Dact1.L-ΔPDZb, J-L). When co-injected, wild-type Dact1.L induces Dvl2-mSc to form puncta in which Dact1.L and Dvl2 co-localize (**M-P**). In contrast, Dact1.L-ΔLZ (**Q-T**) and Dact1.L-ΔPDZb (U-X) mutants do not induce co-injected Dvl2 to form puncta. Scale bars indicate 30 μm.



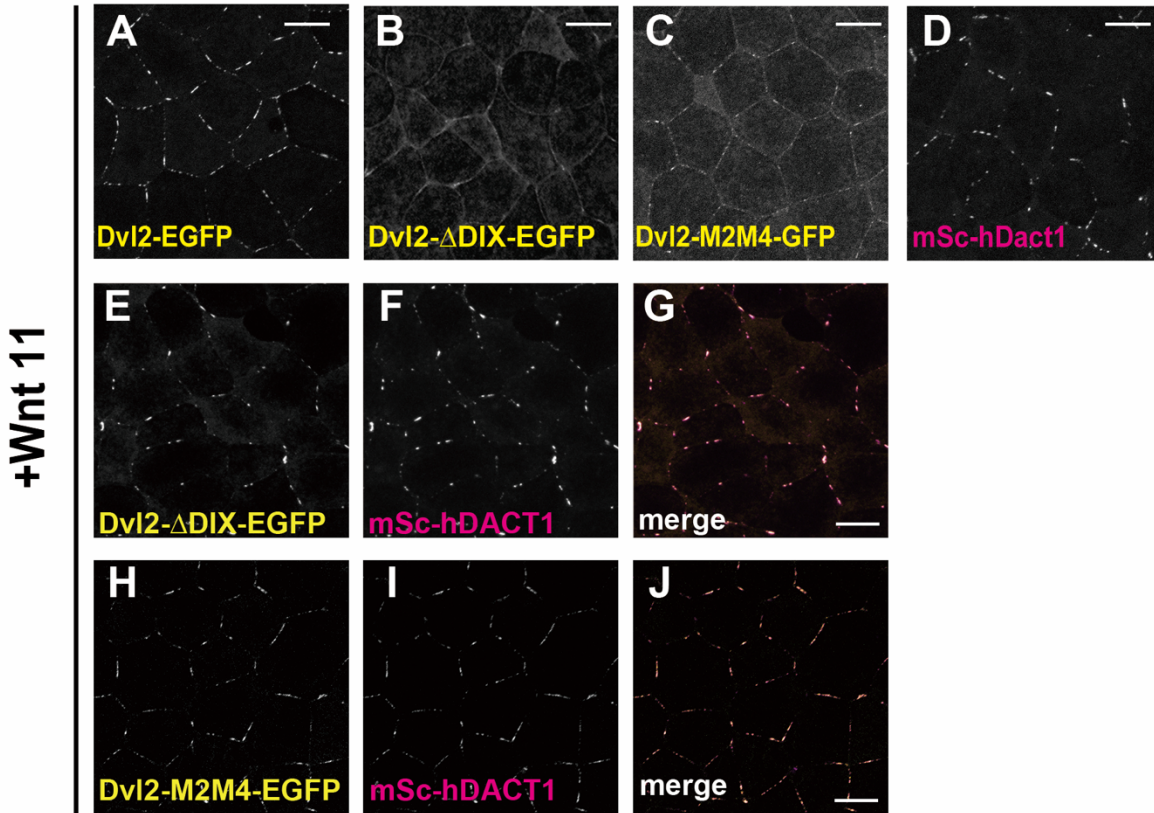
Supplementary Figure 8. Dact1-induced Dvl2 puncta are retained on the plasma membrane with Fz.

When Dvl2 tagged with mCherry (Dvl2-mCh) is co-expressed with GFP-tagged Fz7 in animal cap cells, they display even co-localization on the plasma membrane (A-C). With additional co-injection of hDACT1, Dvl2-mCh formed puncta that are largely retained on the plasma membrane with Fz (arrowheads, D-F). Pearson's coefficient analyses indicate that Dvl2 co-localization with Fz7 is not reduced by hDACT1 despite the induced puncta formation (G). Source data are provided as "Supplementary Figure 8 source data" file.



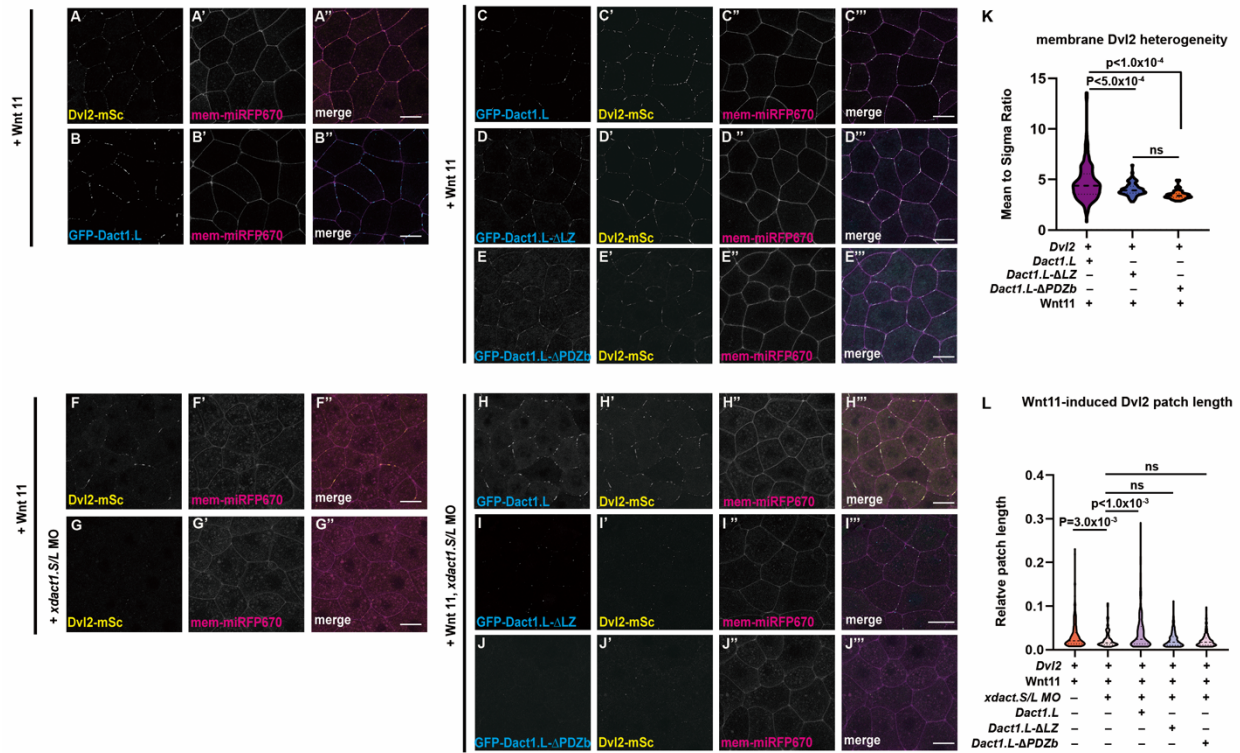
Supplementary Figure 9. Endogenous Dact1 is required for Wnt11 induced formation of Dvl/Fz patches on the plasma membrane.

In animal cap explants, *Xenopus* Wnt11 induces Dvl2-mScarlet-I (Dvl2-mSc) to form distinct patches on the plasma membrane, marked by membrane targeted fluorescent protein mem-miRFP670 (A-C). Co-injection of EGFP-tagged *Xenopus* Fz7 reveals complete overlapping between Wnt11-induced Dvl2 and Fz7 patches (D-G). Partial knock down of *dact1.L/S* with morpholino (20 ng total; 10 ng each for *dact1.L* and *S*) diminishes Wnt11-induced patches of Dvl (I-K) and Dvl/Fz (L-O). Quantification of the Dvl2 patch length (P) and patch number per cell border (Q) show that Fz7 co-injection significantly increases the size but only marginally increases the number of the patches formed. In *dact1* morphants, both the size and number of Dvl2 patches are significantly reduced, either with or without Fz7 co-injection. Scale bars represent 30µm. Source data are provided as “Supplementary Figure 9 source data” file.



Supplementary Figure 10. Overexpression of hDACT1 rescues oligomerization defective Dvl2-ΔDIX and Dvl2-M2M4 mutants to form patches in response to Wnt11.

In animal cap explants, both Dvl2-EGFP and mSc-hDact1 form distinct patches on the plasma membrane in response to Wnt11 (A, D), which is completely blocked by Dvl2-ΔDIX mutation (B) or severely diminished by Dvl2-M2M4 mutation (C). Co-overexpression of mSc-hDACT1 enables Dvl2-ΔDIX and Dvl2-M2M4 mutants to form patches in response to Wnt11 (E, H), and mSc-hDACT1 co-localizes with Dvl2-ΔDIX or Dvl2-M2M4 in these patches (F-G, I-J).

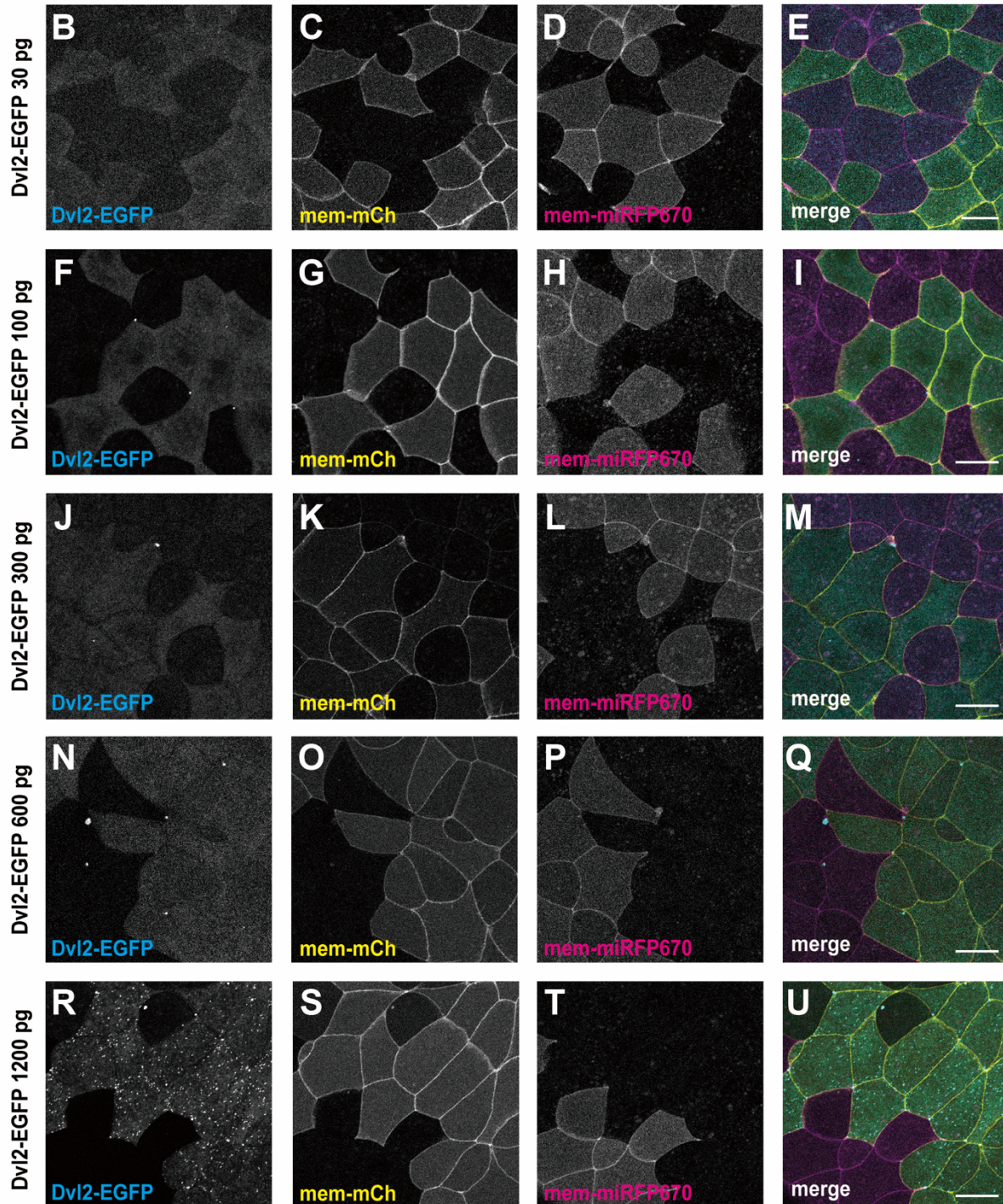
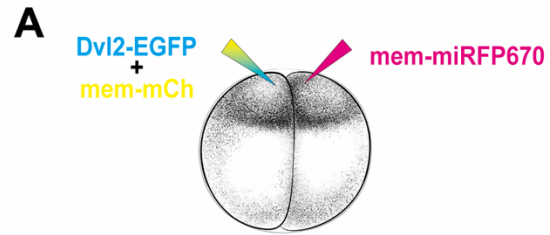


Supplementary Figure 11. Both the PDZb and LZ domains of Dact1 are required for it to mediate Dvl2 patch formation in response to Wnt11.

In animal cap explants, Dvl2-mSc or GFP-Dact1.L can form distinct patches on the plasma membrane in response to co-injected Wnt11 (A-A"; B-B"). When injected together, Dvl2-mSc and GFP-Dact1.L display complete overlap within these Wnt11 induced patches (C-C"). Under this overexpression condition, both Dact1.L-ΔLZ (D-D") and Dact1.L-ΔPDZb (E-E") mutants are still able to form patches that colocalize with Dvl2 on the plasma membrane in response to Wnt11. But the patches formed in the mutants appear less distinct and quantification of signal heterogeneity using the Mean to Sigma Ratio revealed a significant decrease of Dvl2 enrichment in the patches formed with Dact1.L-ΔLZ and Dact1.L-ΔPDZb than with wild-type Dact1.L (K). In *dact1* morphant, Wnt11-induced Dvl2 patch formation (F-F") is disrupted (G-G"); the defect can be significantly rescued by wild-type Dact1.L (H-H") but only marginally rescued by Dact1.L-ΔLZ (I-I") or completely failed to be rescued by Dact1.L-ΔPDZb mutant (J-J"), as quantified by the

average length of Dvl2 patches formed in various explants (L). Scale bars represent 30 μ m.

Source data are provided as "Supplementary Figure 11 source data" file.



Supplementary Figure 12. Over-expressed Dvl2-EGFP can form puncta autonomously in a dose-dependent fashion.

Schematic diagram illustrates the mosaic injection method, where varying dose of *Dvl2-EGFP* is co-injected with membrane-mCherry into one blastomere dorsally at two-cells stage, and membrane-miRFP670 is injected into the adjacent blastomere. Dvl2-EGFP displays diffuse cytoplasmic distribution when injected at 30, 100, 300, or 600 pg (B-Q), and only forms puncta with 1200 pg injection (R-U). *Xenopus* illustration in A was adapted from drawings on Xenbase (www.xenbase.org RRID:SCR_003280), which was created by Zahn (© Natalya Zahn (2022) and published in ref.⁴. *Xenopus* illustrations © Natalya Zahn (2022)

Supplementary Note 1. WL Ratio 4.0(Batch Run Bounding Rectangular)

```
// Ask the user to select a folder
dir = getDirectory("Select a folder containing JPG images");

// Get the list of JPG files in the selected folder
list = getFileList(dir);

// Loop through each file
for (i = 0; i < list.length; i++) {
    if (endsWith(list[i], ".jpg") || endsWith(list[i], ".JPG")) {
// Open the image
        open(dir + list[i]);

// Segmentation the embryo
        run("SIOX: Simple Interactive Object Extraction");
        waitForUser("Generate the Mask");
        waitForUser("select the window to close");
        close();
// Smooth mask
        run("Gaussian Blur...", "sigma=2");
        run("Smooth");
        setOption("BlackBackground", true);
        run("Convert to Mask");
// Obtain the Area and Length
        run("Set Measurements...", "area bounding redirect=None
decimal=2");
        run("Analyze Particles...", "add");
        roiManager("Measure");
        selectWindow("Results");
        Area = Table.getColumn("Area");
        Length = Table.getColumn("Width");

// Show the results
        Array.show("Results", Area, Length);
        String.copyResults();

        waitForUser("copy the results to excel");
        close("Results");

// Close the image and clean up
        selectWindow("Mask");
        close();
        roiManager("Delete");
        run("Close All");
    }
}
```

Supplementary Reference:

- 1 Seo, H. S., Habas, R., Chang, C. & Wang, J. Bimodal regulation of Dishevelled function by Vangl2 during morphogenesis. *Human molecular genetics* **26**, 2053-2061, doi:10.1093/hmg/ddx095 (2017).
- 2 Walton, A. *et al.* A vertebrate Vangl2 translational variant required for planar cell polarity. *J Biol Chem* **300**, 106792, doi:10.1016/j.jbc.2024.106792 (2024).
- 3 Darken, R. S. *et al.* The planar polarity gene strabismus regulates convergent extension movements in *Xenopus*. *EMBO J* **21**, 976-985, doi:10.1093/emboj/21.5.976 (2002).
- 4 Zahn, N. *et al.* Normal Table of *Xenopus* development: a new graphical resource. *Development* **149**, doi:10.1242/dev.200356 (2022).