Supplementary Figure 1: ADAMs of the Meltrin family can shed Cadherin-11.
(A) Transfected Cos-7 cells over-expressing Cad-11 with different ADAMs were extracted and Cad-11 processing was visualized by western blot analysis using the 1B4 monoclonal antibody. In addition to the 120 kDa full length protein, an 80 kDa Cad-11 cleavage fragment is present in cells co-transfected with ADAM9 and ADAM13, but not with their proteolytic-inactive E/A mutants. Co-transfection of Cad-11 with ADAM10 (A) or ADAM19 (B) does not stimulate Cad-11 cleavage. (A lane was removed between 10E/A and 13 corresponding to ADAM11 a non-proteolytic ADAM that did not cleave cadherin-11 and had no relation to this study).

Supplementary Figure 2: ADAM13 binds to Cadherin-11 in cell culture and in embryos.
(A) Cos-7 overexpressing Cad-11 alone (lane 1), with ADAM13 (lane 2), with ADAM13 E/A (lane 3), or ADAM13 alone (lane 4) were extracted and immunoprecipitated for Cad-11. Western blot analysis for ADAM13 shows that both pro (120 kDa) and mature (100 kDa) forms of ADAM13 coimmunoprecipitate with Cad-11 (lanes 2 and 3). ADAM13 is not detected in Cos-7 that were not co-transfected with Cad-11 (lane 4). (B) A similar experiment was performed on Stage 19 embryos overexpressing Cad-11 alone (lane 1), Cad-11 and ADAM13 (lane 2), Cad-11 and ADAM13 E/A (lane 3), ADAM13 alone (lane 4), or nothing (lane 5) (10 embryos/lane). As was shown in Cos-7, both pro (120 kDa) and mature (100 kDa) forms of ADAM13 co-immunoprecipitate with overexpressed Cad-11 (top panel, lanes 2 and 3). Only the mature form of overexpressed ADAM13 immunoprecipitates with endogenous Cad-11 (lane 4). This membrane was stripped and re-probed for Cad-11 (lower panel). As expected the amount of full-length (120 kDa), and cleavage fragment (80 kDa), increase when Cad-11 is overexpressed in embryos (lanes 1-3 vs. lanes 4-5).

Supplementary Figure 3: Cadherin-11 protein is mostly restricted to the CNC. Ten embryos at stages 17, 21, 23 and 26, were dissected into three domains: The head, the dorsal, and the ventral part of the trunk. Cadherin-11 was immunoprecipitated with mAb

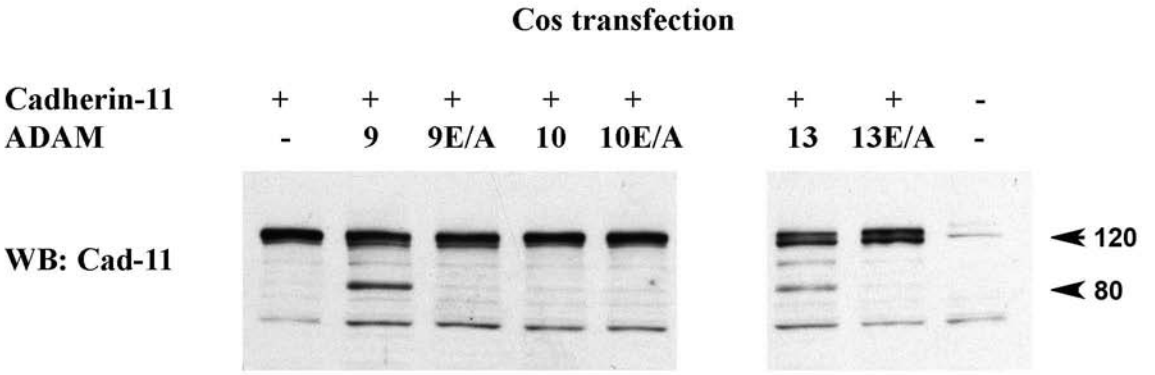
1B4 and detected with the rabbit polyclonal antibody to Cadherin-11. The result shows that the vast majority of Cadherin-11 is present in the head but not the trunk of embryos during CNC migration. β-catenin was used as a loading control and is present in every part of the embryos. As a comparison, ten whole embryos were also extracted and immunoprecipitated for Cadherin-11. Together with in situ hybridization data published previously these results suggest that most of the Cadherin-11 is expressed in CNC cells during their migration.

Supplementary Figure 4: ADAM MO do not increase Cadherin-11 transcription. Embryos injected with a control Morpholino or antisense to ADAM9, ADAM13, ADAM19 and a combination of ADAM13 and 19 were grown until stage 22. Following mRNA purification and reverse transcription the cDNA were analyzed using primer specific to alpha tubulin (control) and Cadherin-11. The relative expression calculated using the 2ΔCT method and represented as a Log2 (fold change) is presented. The relative expression of Cadherin-11 mRNA appear to decrease following each MO injection when compared to the control MO. Results are from 3 independent injection experiments. This shows that the increase of uncleaved Cadherin-11 Protein level is not due to increased transcription.

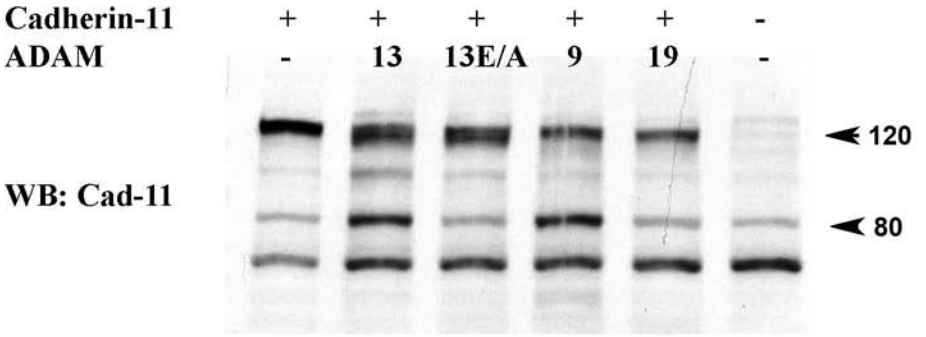
Supplementary Figure 5: ADAM13 does not disrupt the interaction between Cadherin-11 and β-catenin. (A) Co-Immunoprecipitation experiments of endogenous β-catenin with Cad-11. Embryos were injected with synthetic mRNA (1 ng each) for Cad-11 (lane 1), Cad-11 and ADAM13 (lane 2), Cad-11 and ADAM13-E/A (lane 3), no mRNA (lane 4), ADAM13 (lane 5), or ADAM13-E/A (lane 6). Extracted embryos (10 embryos/lane) were immunoprecipitated for Cad-11 and bound β-catenin was detected by western blot analysis. (B) Co-Immunoprecipitation of endogenous β-catenin with overexpressed full-length Cadherin-11 or an extracellular truncated form (ΔEC1-3) Cadherin-11 designed to mimic the C-terminal portion of cleaved Cadherin-11. The truncated form of Cadherin-11 is still capable of binding to β-catenin. (C) Cleavage of Cadherin-11 does not activate transcriptional markers downstream of β-catenin signaling. Q-PCR analysis on cDNA made from embryos injected with mRNA encoding Cad-11,

Cad-11+ADAM13 (A13), C11+A13E/A, A13, GFP, or had been UV irradiated (Ventralized), or treated with 0.1M LiCl. (Dorsalized) xTwist, Cyclin-D1, c-myc, and Sox8 expression levels were normalized to Actin. Transcript quantities for each injection set are presented relative to GFP injected embryos (considered 100% expression). ADAM13 does not rescue expression of Twist, Cyclin-D1, c-myc and Sox8 that are down regulated by Cadherin-11 overexpression (Compare Cad-11 to Cad-11+A13). In fact by itself, ADAM13 appears to reduce Twist expression and Cad-11+A13 have lower Twist expression level than Cad-11 alone. The rescue of Twist expression would be expected if ADAM13 induced the cleavage of Cadherin-11 and its release from β-catenin. The released β-catenin could translocate to the nucleus and activate gene transcription.

A



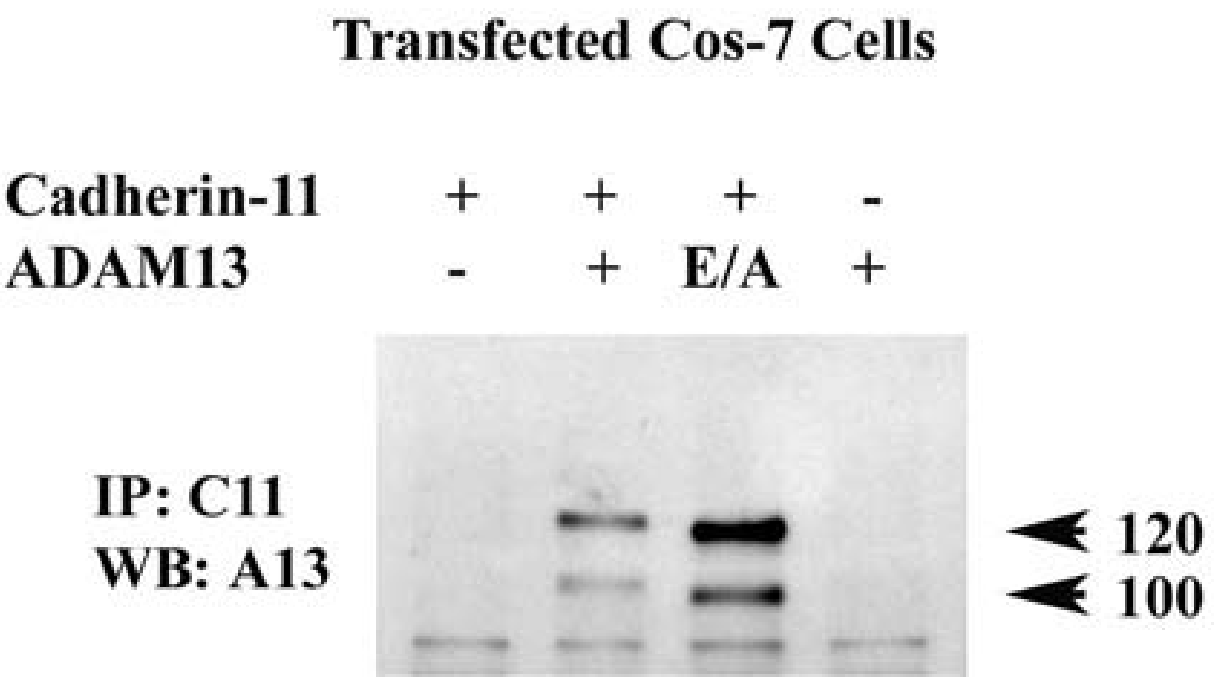
B



SUPPLEMENTARY FIGURE 1

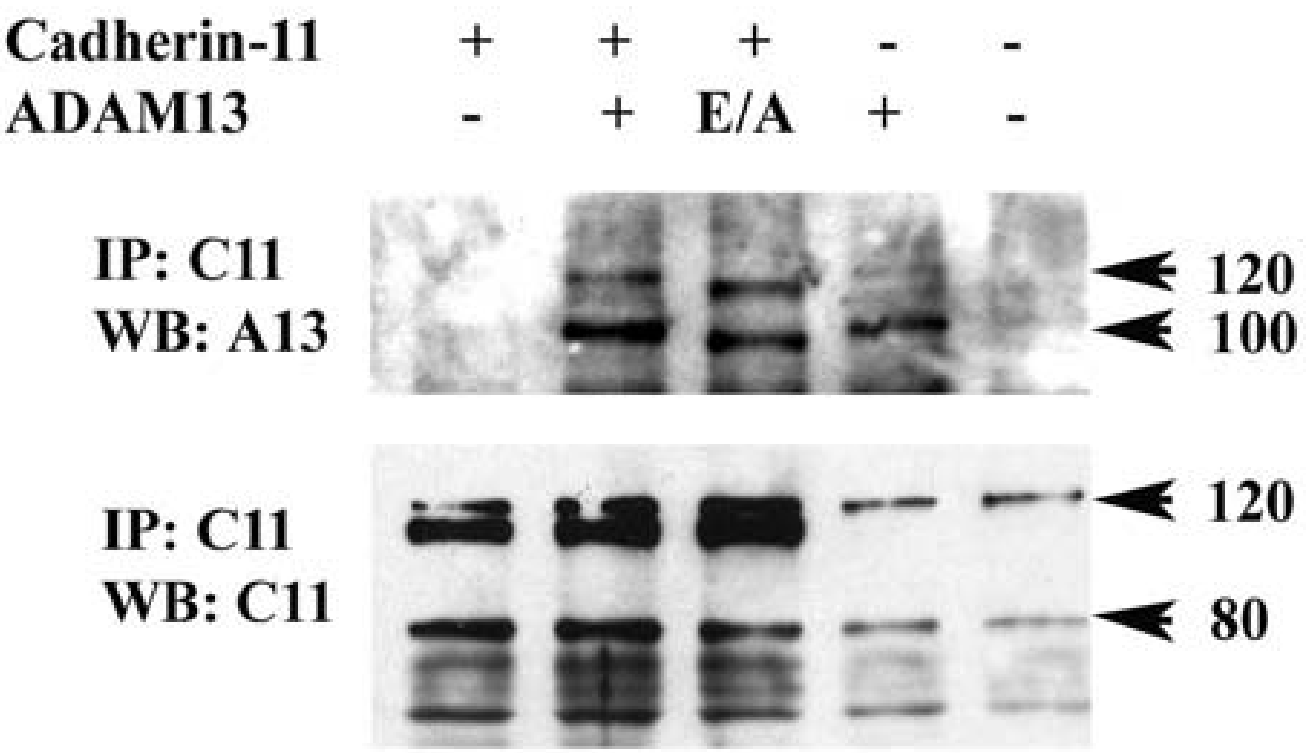
A

Transfected Cos-7 Cells

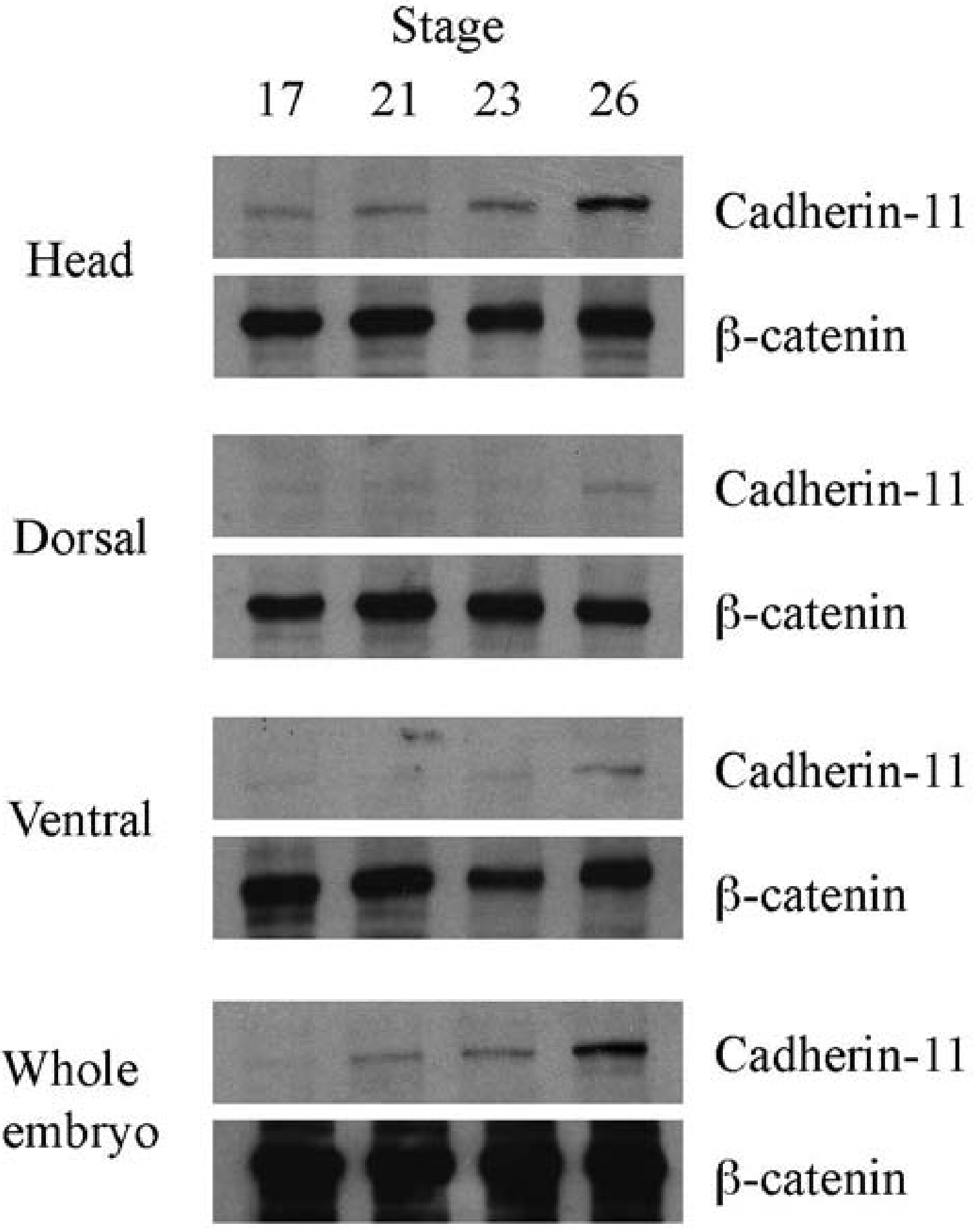


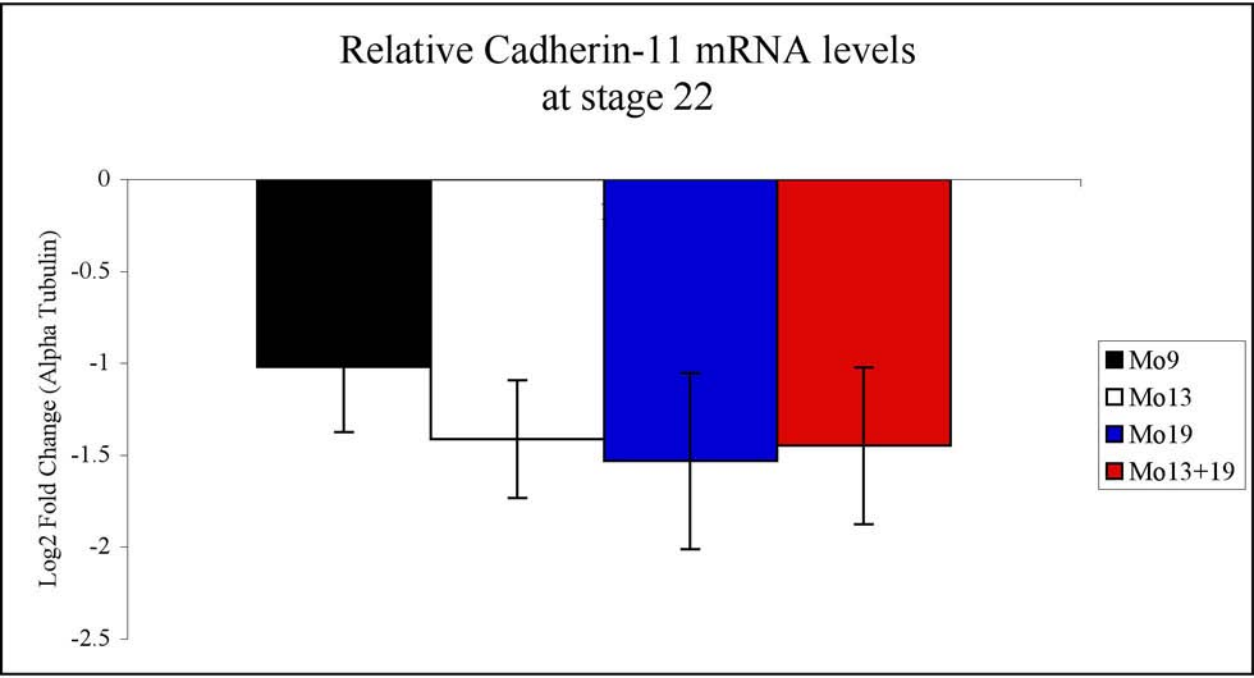
B

Injected Embryos

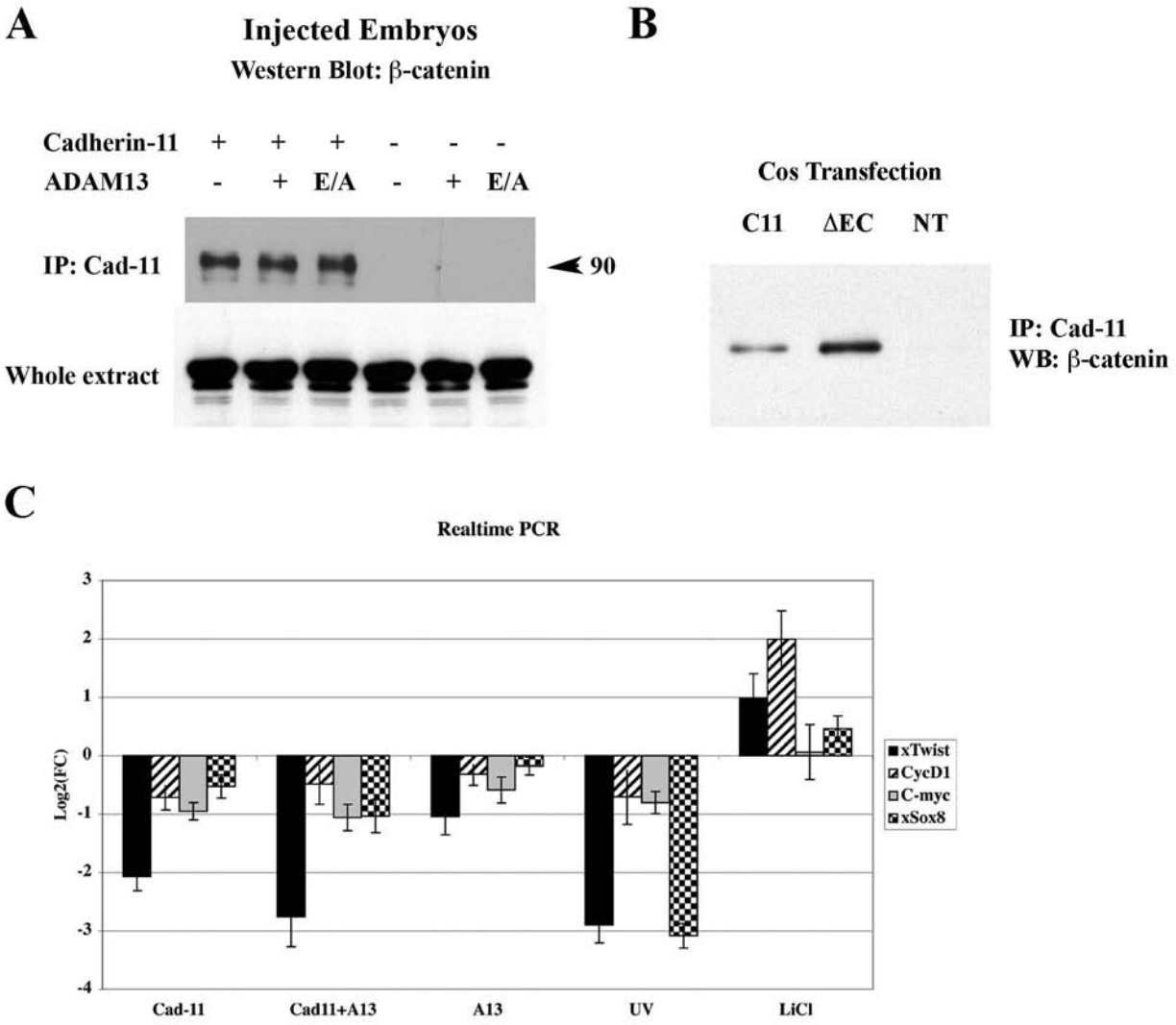


SUPPLEMENTARY FIGURE 2


Stage
17
21
23
26
Head
Cadherin-11
β-catenin
Dorsal
Cadherin-11
β-catenin
Ventral
Cadherin-11
β-catenin
Whole embryo
Cadherin-11
β-catenin



SUPPLEMENTARY FIGURE 4



SUPPLEMENTARY FIGURE 5