Reviewed Preprint
v1 • July 24, 2026
Not revised

For correspondence:
nicholas.tolwinski@duke-nus.edu.sg

Competing interests: No competing interests declared

Funding: See page 17

Reviewing editor: Roel Nusse, Stanford University, United States

Junctional β-Catenin Stabilization Links Wnt Signaling and Force Generation

Agimaa Otgonbaatar[1], Sujithra Shankar[1], Prameet Kaur[2], Prabhat Tawari[2], Nicholas S Tolwinski[2]

[1]Program in Cancer and Stem Cell Biology, Duke-NUS Medical School, Singapore, Singapore • [2]Division of Science, Yale-NUS College, Singapore, Singapore

eLife Assessment

This **important** study by Otgonbaatar and colleagues employs advanced live microscopy, optogenetics, and an endogenous fluorescent timer system to investigate short- and long-term stabilization dynamics of β-catenin/Armadillo (Arm) during Drosophila development. The authors identify an unexpected and functionally relevant enrichment of stabilized junctional Arm in leading-edge cells during dorsal closure, providing evidence for a stabilization mechanism that appears independent of canonical Wingless signaling. These findings are significant because they expand current understanding of β-catenin/Arm beyond its canonical signaling functions and suggest a role in tissue mechanics and force transmission during dorsal closure. The proposed model represents a key advance in the field, but the strength of evidence is currently **incomplete**: the main conclusions regarding Wingless independence, JNK-mediated regulation, and the mechanical role of stabilized Arm are only partially supported by the available data and would benefit from further experimental testing and corroboration.

https://doi.org/10.7554/eLife.111825.1.sa3

Abstract

β-catenin plays two fundamental roles in animal tissues: it acts as a transcriptional effector of canonical Wnt signaling and as a core structural component of adherens junctions that mediate cell–cell adhesion. In canonical Wnt signaling, the post-transcriptional regulation of β-catenin abundance, primarily through regulated phosphorylation, ubiquitination, and proteasomal degradation, determines whether the pathway is “off” or “on.” Despite the central importance of β-catenin stabilization, *in vivo* measurements of β-catenin protein lifetime and stabilization dynamics during development remain limited. Here, we measure the stability of endogenous β-catenin *in vivo* using tandem fluorescent protein timers (tFPs; “Timers”) inserted as minimally disruptive cassettes within the endogenous locus. Timers allow simultaneous visualization of a newly synthesized, rapidly accumulating pool (fast-maturing GFP) and a long-lived, stabilized pool (slow-maturing RFP). Surprisingly, the strongest stabilization does not occur in canonical Wnt patterning stripes; instead, we observe marked stabilization of junctional β-catenin at the leading edge during dorsal closure, a force-generating morphogenetic process. This stabilization is not explained by canonical Wnt ligand input and seems to reflect a stability program linked to β-catenin’s adhesive function in adherens junctions. We suggest that a stable junctional pool of β-catenin is vital for dorsal closure mechanics and provide evidence that this stabilization is regulated by Dishevelled and JNK, thus connecting Wnt pathway components to mechanotransduction.

Introduction

β-catenin at the intersection of adhesion, signaling, and morphogenesis

The molecular basis of multicellularity is inseparable from the evolution of stable cell– cell adhesion. The discovery of cadherins as transmembrane adhesion molecules[1,2] and the demonstration that their intracellular domains are required for stable adhesion[3] established the framework for adherens junctions. Cadherins associate with intracellular catenins—α-catenin, β-catenin, and γ-catenin—forming a multi-protein complex that links cell–cell adhesion to the actin cytoskeleton.[4] In this complex, β– and γ-catenin bind directly to the cadherin cytoplasmic tail,[5–7] whereas α-catenin, which is homologous to actin-binding proteins, supports cytoskeletal coupling.

Historically, β-catenin was first recognized as a structural component in adherens junctions. This perspective changed considerably when β-catenin was identified as the vertebrate equivalent of the *Drosophila* segment polarity gene *armadillo*,[8–11] a gene discovered through classic developmental patterning screens.[12,13] Later, as β-catenin was confirmed as the main cytoplasmic-to-nuclear effector of canonical Wnt signaling, the field's focus shifted to signal transduction: pathway components, post-translational modifications, transcriptional partners, and downstream gene targets. A lingering question remained: is β-catenin primarily a membrane or cytoplasmic protein with occasional nuclear signaling, or does its signaling activity arise from a separate, distinct pool?

Dynamic adherens junctions and the Wnt–polarity–force interface

Adherens junctions are now understood to be dynamic and spatially regulated rather than purely structural. In *Drosophila* embryos, junctions are redistributed asymmetrically during convergent extension[14,15] and are regulated by epithelial polarity[16,17] and influenced by non-canonical Wnt polarity signaling[18,19]. Across systems, junction remodeling is tightly coupled to force-generating machinery and morphogenesis.[20–25] This raises a key question for β-catenin biology: are β-catenin's nuclear and junctional roles separate outputs, or do they converge through shared regulatory mechanisms during mechanically active tissue remodeling?

Measuring β-catenin dynamics in vivo with engineered endogenous alleles

Several technical advances now enable direct in vivo analysis of protein dynamics at endogenous levels. Genome engineering allows modification of endogenous genes to insert intragenic cassettes encoding fluorescent proteins or functional modules.[26–30] Optogenetics enables perturbation of protein behavior with light, enabling temporally controlled functional assays. In our earlier work, we used overexpression in normal and mutant backgrounds to probe signaling dynamics and timing.[31–33] By combining genome engineering and optogenetic tools, we can now both observe and perturb pathway components in living embryos.[34,35]

A persistent challenge is that endogenously tagged β-catenin reporters often primarily reveal the abundant membrane-associated pool and are less effective at reporting the canonical signaling pool *in vitro*.[36,37] To overcome this limitation, we inserted a tandem fluorescent protein timer into the endogenous *armadillo* gene. Timers combine a fast-maturing fluorescent protein (here, superfolder GFP) with a slow-maturing RFP, enabling inference of protein lifetime and stability from the GFP/RFP ratio[38–42].

Canonical Wnt regulation of β-catenin

In canonical Wnt signaling, β-catenin is the central relay from the membrane to the nucleus.[43] In the absence of Wnt ligand, cytoplasmic β-catenin is phosphorylated by CK1 and GSK3 and targeted for ubiquitination by the destruction complex (Axin, APC, CK1, GSK3), keeping cytoplasmic β-catenin levels low.[44,45] When Wnt binds Frizzled (Fz) and LRP5/6 (Arrow in *Drosophila*),

Dishevelled (Dsh) is recruited to the membrane and nucleates assembly of the Wnt signalosome, recruiting Axin and kinases. This disables the destruction complex, allowing β-catenin to accumulate, enter the nucleus, and activate transcription through TCF.[46,47]

Importantly, β-catenin's junctional and signaling roles are both regulated by phosphorylation at distinct sites. Destruction-complex phosphorylation targets β-catenin for degradation. Other kinases (e.g., JNK, Src) have been proposed to regulate junctional function by phosphorylating β-catenin and thereby modulating cadherin association.[48,49]

Dorsal closure: a force-driven morphogenetic context to study junctional β-catenin stabilization

We focus on dorsal closure, a classic morphogenetic process in embryogenesis. After germ-band retraction, an epidermal gap covered by the amnioserosa occupies the dorsal side (Stage 13). During dorsal closure, lateral epidermal sheets converge and the amnioserosa contracts, closing the dorsal opening and restoring a continuous epidermis (Stage 17).[50–52] The leading edge of the epidermis forms an actin cable coupled through adherens junctions. Signaling and force-generating processes must be coordinated, and Armadillo is required. Mutations in *arm* and *wg* show dorsal closure defects[53,54], but the mechanistic basis remains unclear. Here, we ask why β-catenin/Arm is strongly stabilized at the leading edge during dorsal closure and what molecular mechanisms govern this stabilization.

Results

Arm localizes to the leading edge and becomes highly stabilized during dorsal closure

Attempts to visualize endogenously tagged β-catenin primarily detect membrane– and cytoplasmic pools and do not robustly report the nuclear/signaling pool *in vitro*, including during embryogenesis, where expected Wnt stripes are subtle or absent with conventional exon traps.[36,37] We therefore enhanced endogenous visualization by inserting a tandem fluorescent protein timer (tFP) into *arm*. The timer uses fast-folding superfolder GFP and slow-maturing RFP, so that GFP highlights newly synthesized or recently accumulated protein, while RFP indicates protein that has persisted long enough to mature—an operational readout of increased stability/longer lifetime.[38–42]

In toto imaging revealed broadly distributed Arm-GFP. However, the earliest clear appearance of a stabilized (RFP-positive) pool occurred specifically in the first row of epidermal cells bordering the dorsal opening—the leading edge—during dorsal closure. The combined GFP and RFP signals at the leading edge produced a bright yellow overlap, indicating that Arm is not only abundant but also strongly stabilized in these cells during dorsal closure (Figure 1).

Arm activity is necessary for dorsal closure

Previous genetic studies reported dorsal closure defects in arm mutants.[54,55] However, strong arm loss often disrupts epithelial cohesion earlier in development, complicating interpretation because the adhesive and signaling functions cannot be separated when the epithelium fails prematurely.[56,57]

To test Arm function with temporal control, we inserted an optogenetic cassette (Cryptochrome 2 and mCherry) into *arm* using MiMIC/RMCE. Blue-light exposure (488 nm) induces CRY2 oligomerization, which functionally inactivates Arm fused to CRY2.[31] Arm-CRY2-mCherry localized normally to the leading edge in the absence of activation (Figure 2A), indicating that the insertion did not disrupt localization or baseline function. In contrast, blue-light activation caused a severe dorsal closure defect (Figure 2A'), demonstrating that Arm activity is required during dorsal closure. While this result is consistent with an adhesive role, it does not, on its own, distinguish adhesion from signaling.

Figure 1. **The arm stabilizes at the leading edge.**

(A) Green fluorescence indicates newly accumulating Arm, as only the GFP has had time to mature. Higher levels of Arm localize at the leading edge (the first row of epithelial cells of the dorsal gap). (B) Red fluorescence shows stable Arm protein with time for RFP to mature. (C) The leading edge exhibits bright yellow fluorescence, showing colocalization of mainly within dorsal closure.

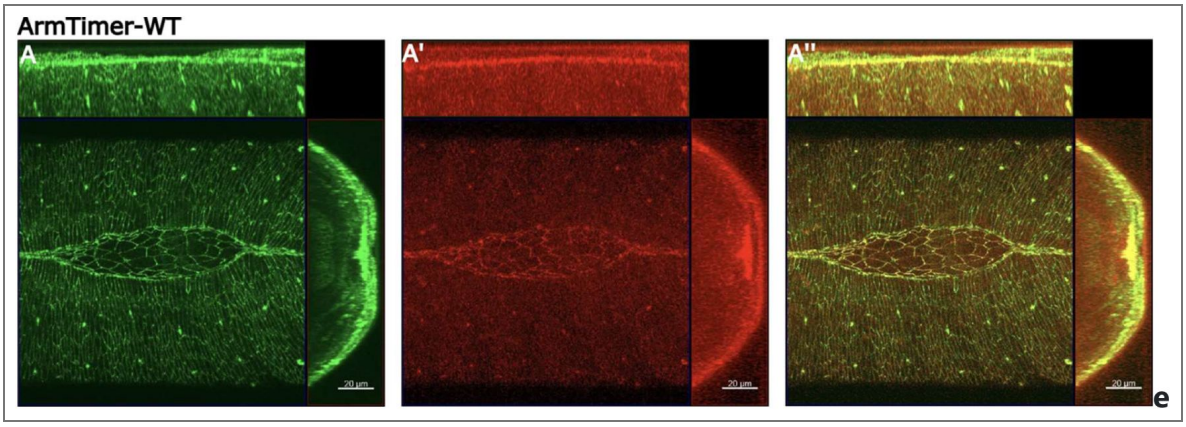
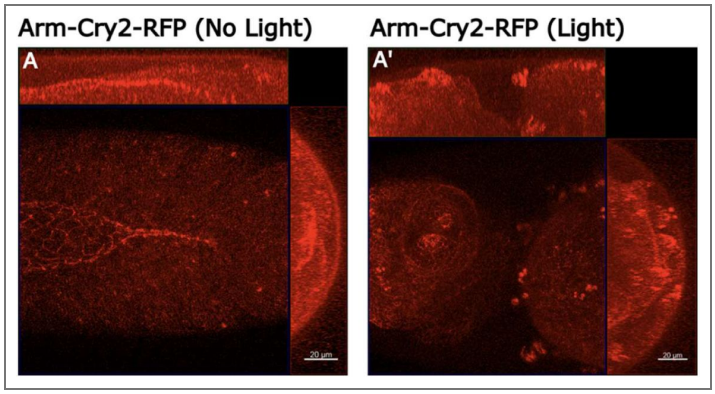


Figure 2. **Arm is required for dorsal closure.**

(A) Arm-CRY2-mCh without blue-light activation localized at the leading edge of dorsal closure. The insertion of CRY2 and RFP did not perturb its localization or function. (A') Light-induced oligomerization of Arm-CRY2-mCh through the application of blue light led to a disruption in dorsal closure.



Upstream activation of canonical Wnt does not affect dorsal closure or the stabilized Arm pool

Embryos lacking Wnt signaling (e.g., wingless mutants) exhibit dorsal closure defects with an anterior break in zippering.[54] We therefore tested whether increasing Wg ligand levels could enhance Arm stabilization at the leading edge and/or perturb dorsal closure.

In wild-type embryos, Wg is expressed in anterior–posterior stripes[58] oriented perpendicular to the dorsal closure leading edge, where stable Arm accumulates (Figure 3A). Overexpression of Wg specifically in the amnioserosa (C381-Gal4) did not disrupt dorsal closure (Figure 3B). Uniform overexpression of Wg likewise did not alter Arm-RFP levels at the leading edge or cause a dorsal closure phenotype (Figure 3B'). These results indicate that the stabilized leading-edge Arm pool is not simply increased by ectopic upstream canonical Wnt ligand input.

Downregulating canonical Wnt downstream, or forcing Arm stabilization, causes dorsal closure defects

Because Wg overexpression did not affect Arm stabilization or dorsal closure, we tested inhibition downstream at the level of Arm stability control. Axin is a rate-limiting scaffold of the β-catenin destruction complex.[44,59] Overexpression of Axin prevents Arm stabilization and inhibits Wnt signaling. Axin overexpression produced a strong dorsal closure defect (Figure 3C), indicating that perturbing the stability machinery can disrupt dorsal closure.

We then tested stabilized versions of Arm, or those resistant to phosphorylation-dependent degradation. ΔArm lacks the N-terminus required for destruction-complex phosphorylation and degradation.[60] ArmS56A is a gain-of-function allele that blocks phosphorylation by substituting the first serine with alanine in the degradation motif, thereby preventing normal degradation. Expression of either ΔArm or ArmS56A caused severe dorsal closure defects (Figure 3D,E). Because Wg overexpression did not cause a phenotype, these results are consistent with the possibility that dorsal closure is sensitive to direct perturbations of Arm stability/structure (bypassing normal regulation) rather than to ligand-mediated canonical activation. Additionally, ΔArm lacks the α-catenin-binding site, suggesting that impaired junctional coupling contributes to the phenotype.

Adherens junction components co-localize at the leading edge, and α-catenin is required for dorsal closure

To assess whether leading-edge Arm stabilization reflects adherens junction reinforcement, we imaged E-cadherin during dorsal closure and observed strong colocalization with Arm at junctions (Figure 4A). We also visualized the actin cytoskeleton with LifeActin-mKO and observed the characteristic actin cable and distribution aligned with the leading edge, consistent with force generation and junctional coupling during zippering (Figure 4B).

Because genetic disruption of adherens junction components often causes early defects that complicate interpretation during dorsal closure, we used an optogenetic approach targeting α-catenin. We inserted CRY2 and mCherry into α-catenin via MiMIC-RMCE, generating α-cat-CRY2. Without activation, α-catenin localized normally to the leading edge, and dorsal closure proceeded (Figure 4C). Blue-light activation reduced α-catenin activity and caused dorsal closure failure (Figure 4C'). Notably, the α-cat-CRY2 phenotype closely resembled Arm-CRY2, ΔArm, and ArmS56A phenotypes, particularly in the failure of zippering forces at the two closure edges. Together, these results support the conclusion that adherens junction function—specifically involving Arm and α-catenin—is essential for dorsal closure.

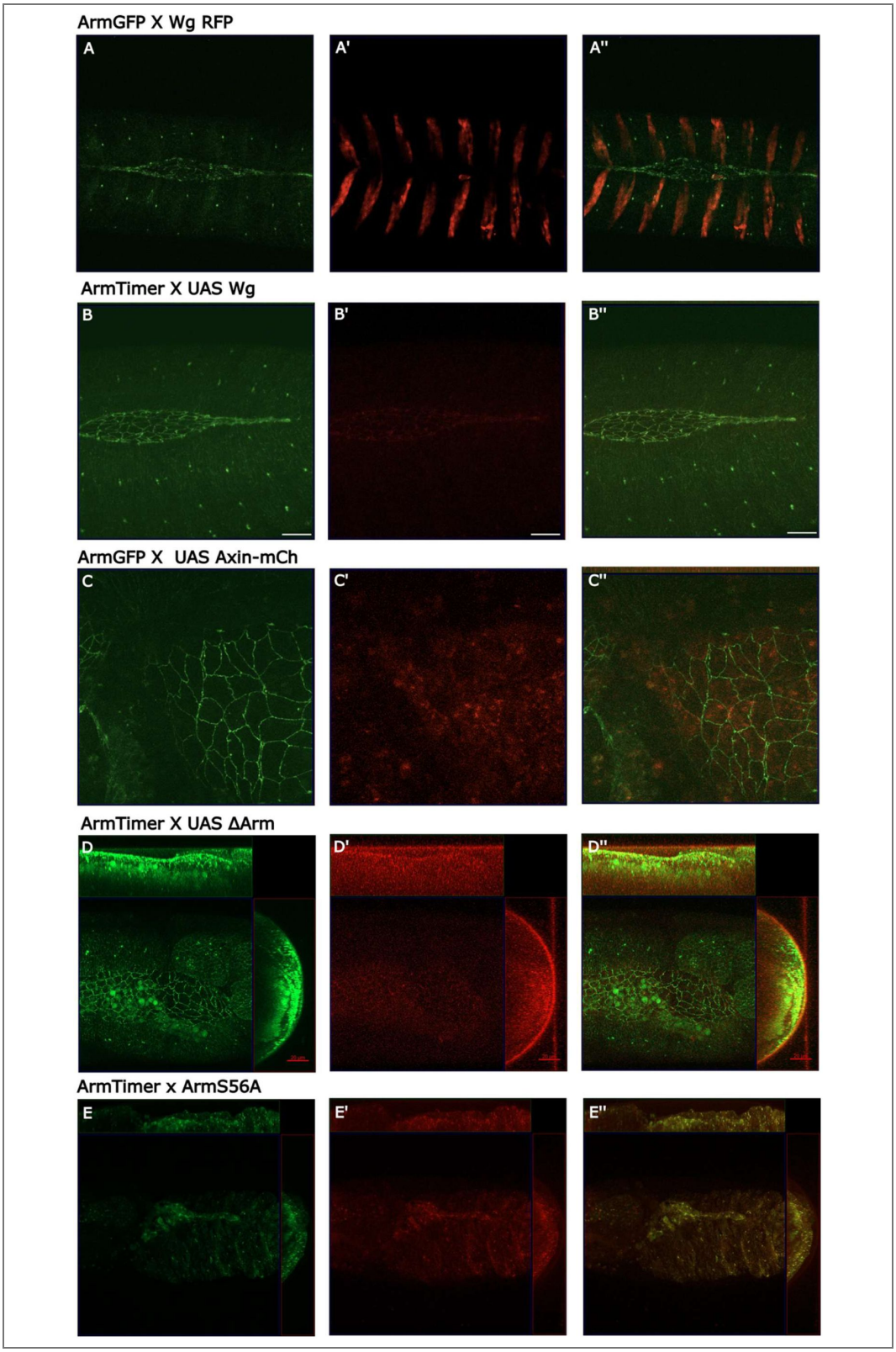


Figure 3. Canonical Wnt activation does not affect dorsal closure, while inactivation of Wnt results in defective dorsal closure.

(A–A'') Wingless (C381-Gal4 X UAS-Wg, UAS-RFP), the primary Wnt pathway ligand, is expressed perpendicular to the leading edge cells (Arm-GFP), and does not affect dorsal closure. (B–B'') Wnt activation through uniform Wg overexpression (UAS Wg) does not affect dorsal closure. (C–C'') Wnt signaling inactivation through Axin overexpression results in dorsal closure defect. (D–D'') Expression of the gain-of-function allele of Arm, ΔArm resulted in a dorsal closure defect. (E–E'') Gain of function allele ArmS56A also showed a dorsal closure defect.

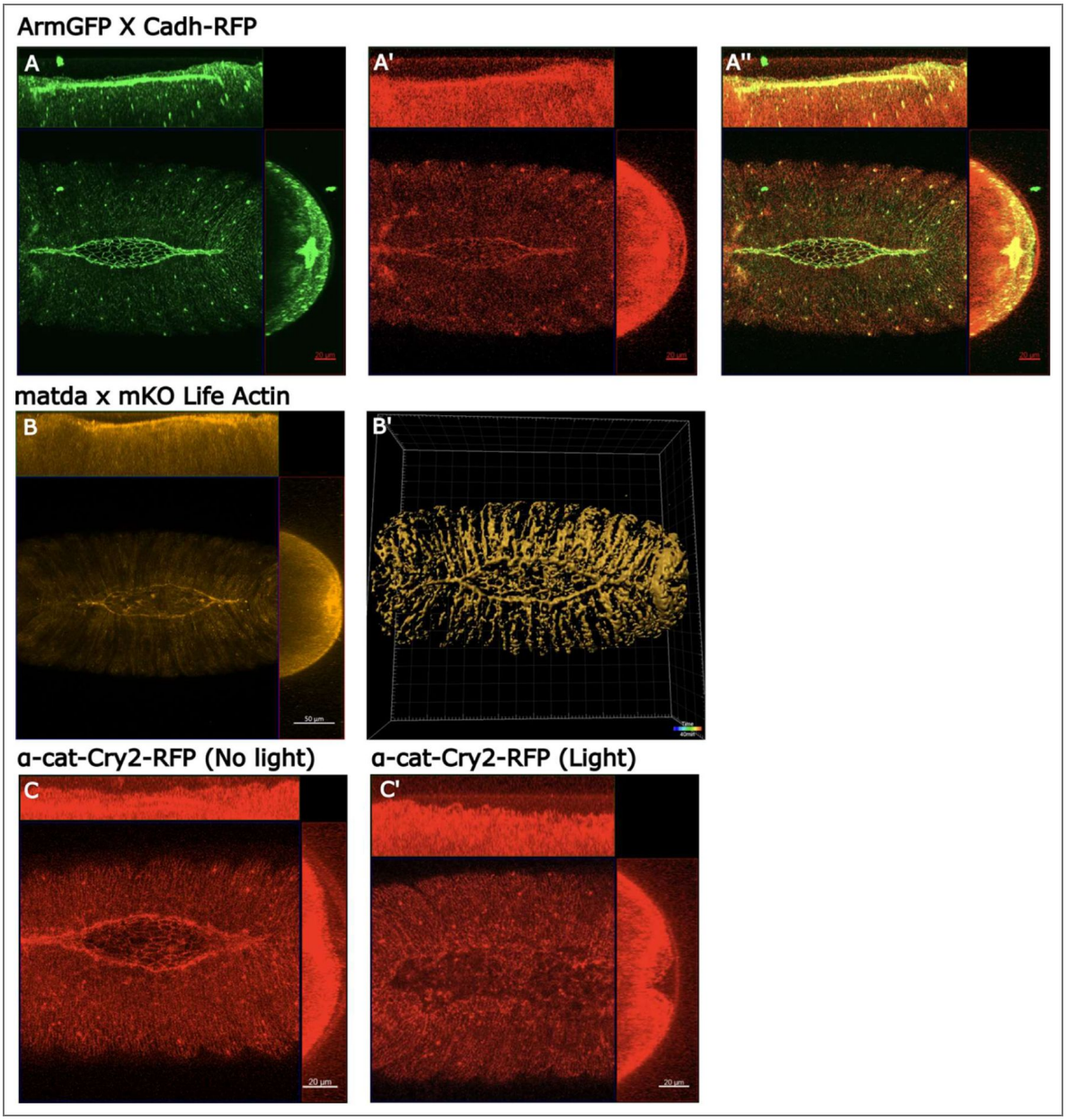


Figure 4. E-cadherin, actin and α-catenin co-localize with Arm at the leading edge, and α-catenin is necessary for dorsal closure.

(A–A'') E-cadherin (red) colocalizes with Arm (green) at junctions. (B, B') Filamentous actin follows a similar pattern to adhesive junctions (B mKO tagged LifeActin, B' projection using IMARIS software). (C) In the absence of blue light, α-catenin (red) localizes at the leading edge. (C, C') Activation of CRY2 with blue light led to a loss of localization of α-catenin and a severe dorsal closure defect.

Arm point mutants indicate that Arm–α-catenin interaction is required for dorsal closure

To dissect the mechanism underlying Arm function during dorsal closure, we engineered point-mutant *arm* alleles. We used MiMIC/RMCE to knock in mutated Arm alleles fused to the Timer construct into the first coding intron, followed by a stop codon to prevent translation of the endogenous gene. We validated this method with a hypomorphic Arm allele (ArmF1a), which has reduced activity but normal stability.[59] Imaging ArmF1a-Timer revealed a mild dorsal closure defect in which the dorsal gap fails to fully close, supporting the effectiveness of this allele-replacement strategy (Figure 5B).

We next examined four phosphorylation sites: two tyrosines (Y150, Y667) implicated in regulating E-cadherin binding and junctional dynamics[49,61–65], and two threonines (T111, T121) within the α-catenin-binding region that influence Arm–α-catenin binding.[16,66] Each engineered allele includes the Timer tag to enable simultaneous visualization of "new" (GFP) and "stabilized" (RFP) pools.

For the tyrosine sites, we generated two variants:

- ArmTimer-FF, where Y150 and Y667 are replaced with phenylalanine, preventing phosphorylation at these sites (phospho-resistant).
- ArmTimer-EE, where Y150 and Y667 are replaced with glutamic acid, intended to mimic phosphorylation by introducing a negative charge (phosphomimetic).

Both ArmTimer-FF (Figure 5C) and ArmTimer-EE (Figure 5D) completed dorsal closure without gross morphological defects, yet both showed markedly reduced fluorescence intensity compared to wild-type ArmTimer. Quantification confirmed that both mutants exhibited significantly reduced GFP and RFP signals (Figure 5F). Notably, the pattern of reduction differed between the two mutants: ArmTimer-EE showed lower GFP intensity but comparable RFP intensity relative to wild type, whereas ArmTimer-FF exhibited reduced RFP intensity but similar GFP intensity compared to wild type. Interpreting the timer signals, this suggests that ArmTimer-EE may reduce overall production/accumulation (lower GFP) while preserving a stabilized pool (RFP), whereas ArmTimer-FF more strongly affects stabilization (reduced RFP) without strongly affecting the rapidly accumulating pool (GFP).

Although these tyrosine mutants did not cause overt closure failure, they did influence the duration of dorsal closure (Figure 5G). Specifically, ArmTimer-EE showed a significantly longer closure duration compared to wild type, while ArmTimer-FF did not significantly alter closure timing.

In contrast to the tyrosine mutants, altering the threonine sites within the α-catenin binding region produced a clear functional phenotype. ArmTimer-AA (T111A, T121A), which prevents phosphorylation at these threonines and reduces affinity for α-catenin, disrupted dorsal closure dynamics (Figure 6). The phenotype was characterized by defective and asymmetric zippering, resulting in an uneven dorsal gap and a substantially prolonged closure process compared to wild-type ArmTimer. Both ArmTimer-AA and ΔArm disrupt Arm–α-catenin interaction, but to different extents: ΔArm removes the binding region entirely, while ArmTimer-AA reduces binding affinity by modifying two regulatory sites. The graded phenotypes match this expectation: ΔArm causes failure of closure, while ArmTimer-AA produces slower and asymmetric zippering rather than complete failure. Together, these results map dorsal closure robustness onto the integrity of Arm–α-catenin coupling.

Dishevelled regulates Arm during dorsal closure

Dishevelled (Dsh) is a key upstream component of both canonical and non-canonical Wnt signaling pathways that regulate polarity, adhesion, and migration. Null dsh mutants exhibit dorsal closure defects, including dorsal holes.[54] Canonically, Dsh is recruited to the membrane downstream of Frizzled activation, where it helps recruit Axin and associated kinases to the

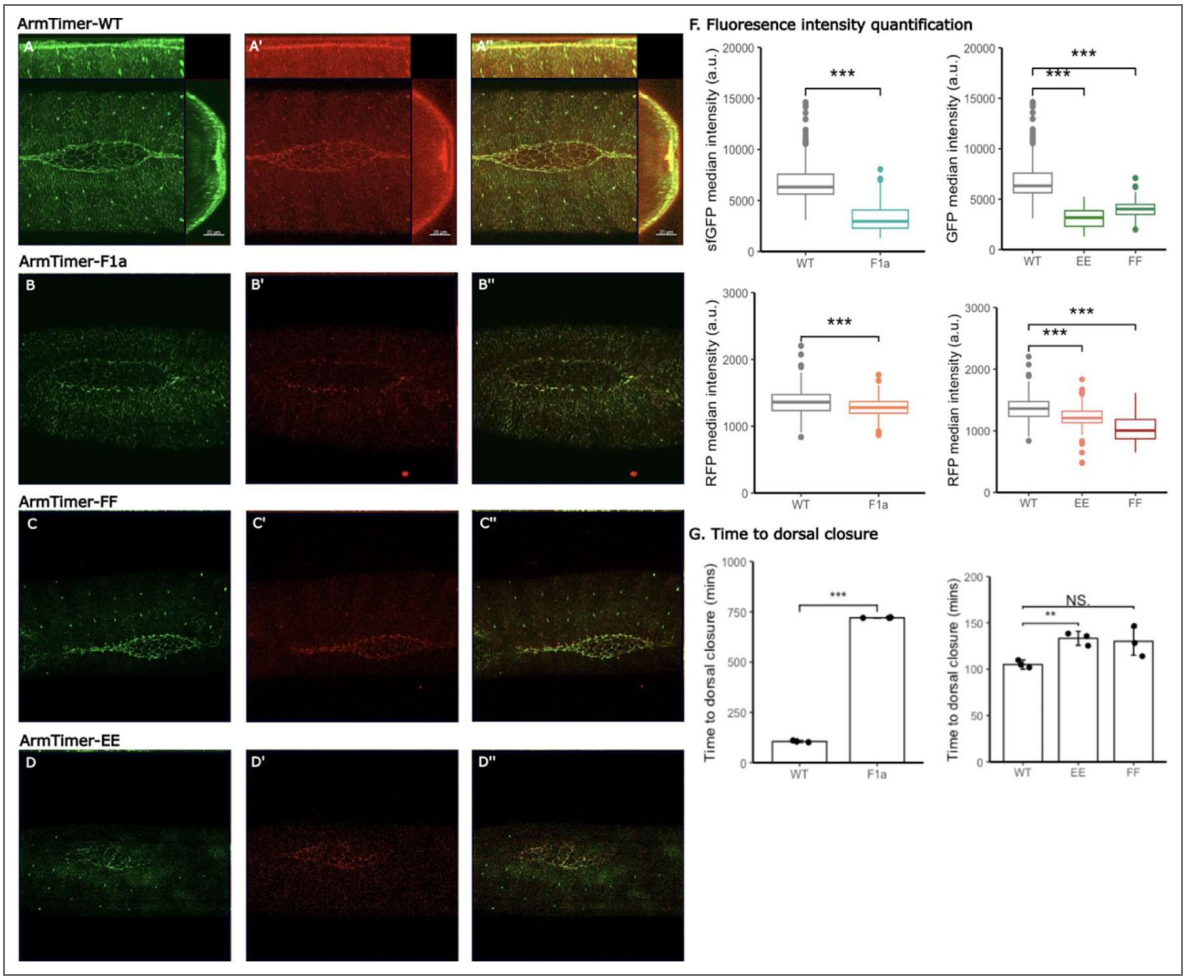


Figure 5. Functional dissection of point mutations in ArmTimer during dorsal closure.

(A–A'') Wild-type ArmTimer control shows high production and stabilization at the leading edge, as indicated by green (GFP) and red (RFP) fluorescence. (B–B'') ArmTimer-F1a shows a mild dorsal closure defect and reduced fluorescence, validating the allele-replacement strategy. (C–C'') ArmTimer-FF (Y150F, Y667F) shows no overt dorsal closure defect but reduced GFP and RFP intensities. (D–D'') ArmTimer-EE (Y150E, Y667E) shows no overt defect but further reduced GFP and RFP intensities. (F) Quantification of GFP/RFP intensities comparing wild type, –EE, and –FF. (G) Dorsal closure duration is increased in ArmTimer-EE.

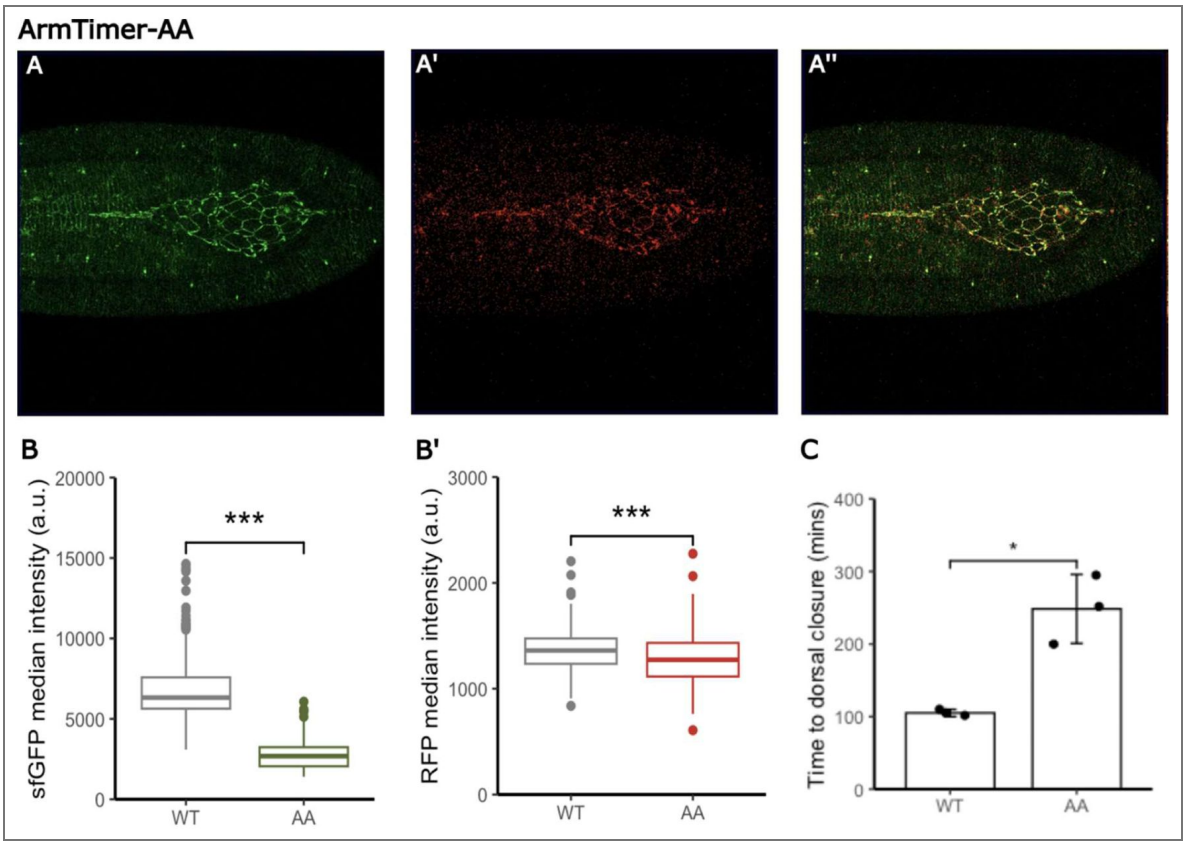


Figure 6. ArmTimer-AA disrupts zippering dynamics, supporting the requirement for Arm–α-catenin interaction.

(A–A'') ArmTimer-AA (T111A, T121A) shows asymmetric zippering and an uneven dorsal gap. (B) GFP comparison indicates lower Arm production/accumulation in ArmTimer-AA relative to wild type at the same time point. (B') RFP comparison indicates similar stabilization signal to wild type. (C) Dorsal closure duration is significantly increased for ArmTimer-AA.

activating "signalosome," thereby antagonizing the destruction complex. Non-canonically, Dsh can activate Rho/Rac pathways and downstream JNK signaling, depending on interacting partners and domain usage.[18]

Because we did not have a fluorescently tagged Dsh line suitable for live imaging, we used an indirect "frankenbody" strategy: HA-tagged Dsh is co-expressed with a fluorescent anti-HA nanobody, enabling live visualization of the HA-tagged protein.[67] Using this approach, we observed that Dsh accumulates in the leading-edge cells where Arm is stabilized (Figure 7A–A"), placing Dsh in the correct spatial domain to influence Arm dynamics during dorsal closure.

Overexpression of full-length Dsh localized to the leading edge but did not disrupt dorsal closure, consistent with the earlier observation that Wg overexpression does not disrupt closure mechanics. To dissect which Dsh domains are required, we tested membrane-tethered (myristoylated) Dsh constructs with deletions of the conserved DIX, PDZ, and DEP domains.[68] These domains partition Dsh function across pathway branches: PDZ and DEP support membrane recruitment and Frizzled interaction, whereas DIX supports signalosome assembly and interaction with Axin in canonical signaling. In polarity signaling, DEP is critical for engaging small GTPases and JNK pathway outputs.[69]

- Myr-Dsh localized to the leading edge but disrupted amnioserosa integrity, resembling previously described phenotypes[54] (Figure 7B).
- Myr-DshΔDIX, which cannot efficiently engage Axin/canonical signalosome formation, localized to membranes and produced amnioserosa integrity defects but did not block dorsal closure (Figure 7C).
- Myr-DshΔPDZ localized more diffusely across the epithelium but dorsal closure proceeded (Figure 7D).
- Myr-DshΔDEP produced severe early defects including defective germ-band retraction and dorsal closure failure (Figure 7E).

Together, these results indicate that the DEP domain is essential for the morphogenetic program culminating in dorsal closure, consistent with a strong role for the Dsh DEP-dependent non-canonical/polarity branch in this process.

JNK signaling regulates ArmTimer dynamics and is essential for dorsal closure

The requirement for the Dsh DEP domain naturally implicates downstream pathways engaged by DEP, including the Rac/JNK pathway.[69] The Jun N-terminal kinase (JNK) pathway is a mitogen-activated protein kinase (MAPK) cascade required for dorsal closure, regulating leading-edge behaviors, expression of target genes such as dpp and puc, and actin-based protrusions important for zippering.[70–72] Genetic studies have shown that JNK pathway mutants fail dorsal closure.[73,74] However, how JNK activity might translate into Arm stabilization or junctional dynamics remains incompletely defined.

To test whether JNK influences ArmTimer behavior, we modulated JNK levels. Uniform JNK-GFP overexpression did not visibly disrupt dorsal closure, but ArmTimer imaging revealed reduced Arm-RFP intensity, suggesting decreased Arm stabilization or increased turnover at junctions when JNK is elevated (Figure 8A, C). Conversely, JNK knockdown (JNK RNAi) caused a strong early disruption of dorsal closure and altered Arm localization at the leading edge (Figure 8B), consistent with the essential requirement of JNK signaling for this morphogenetic process.

Discussion

This study uses endogenous protein lifetime reporting and optogenetic perturbation to reveal a striking and unexpected stabilization of β-catenin/Armadillo at adherens junctions during a force-generating morphogenetic process *in vivo*. Given the canonical view that β-catenin stabilization is a hallmark of canonical Wnt pathway activation, we expected Arm stabilization to be most

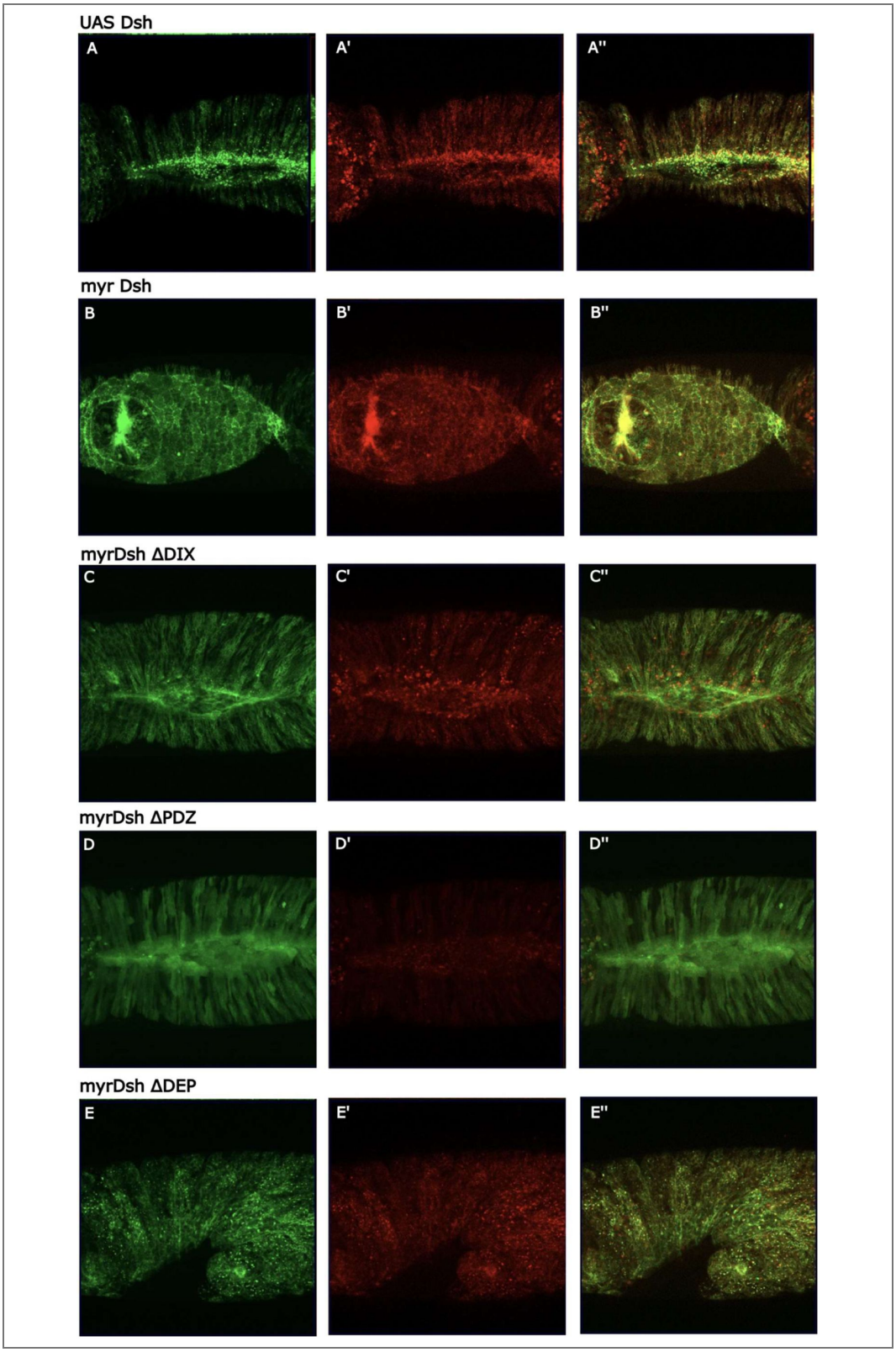


Figure 7. Dishevelled accumulates at the leading edge and the DEP domain is required for dorsal closure.

(A–A'') Dsh visualized with frankenbody reporters accumulates at the leading edge (GFP, RFP, and overlap). (B–B'') Membrane-tethered Dsh localizes to leading edge and amnioserosa and disrupts amnioserosa junction integrity. (C–C'') DshΔDIX localizes to membrane and causes amnioserosa integrity defects without blocking closure. (D–D'') DshΔPDZ is diffuse but dorsal closure proceeds. (E–E'') DshΔDEP results in failure of germ-band retraction and dorsal closure.

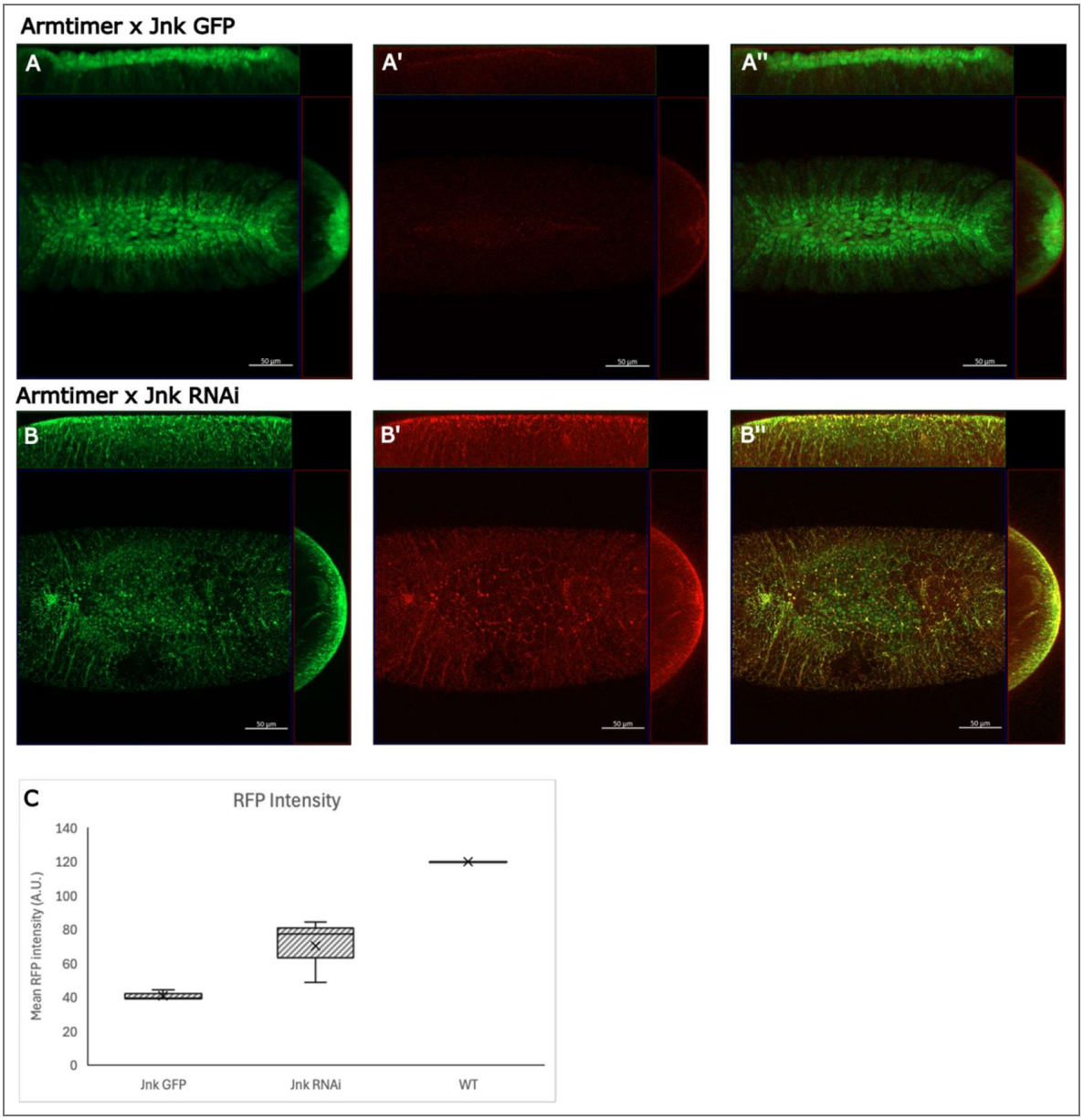


Figure 8. JNK signaling regulates ArmTimer dynamics and is essential for proper dorsal closure.

(A–A'') ArmTimer embryos with uniform JNK-GFP expression show normal closure but reduced Arm-RFP intensity. (B–B'') ArmTimer × JNK RNAi shows disrupted dorsal closure and altered Arm leading-edge localization. (C) Quantification of Arm-RFP intensity across JNK-GFP, JNK RNAi, and wild-type ArmTimer.

prominent in Wingless-expressing stripes during embryonic patterning. Instead, Arm stabilization is highly enriched at the leading edge during dorsal closure, a mechanically active zone that coordinates epithelial migration, actin cable tension, and zippering.

Junctional Arm stabilization is essential for dorsal closure mechanics

Our optogenetic inactivation of Arm shows that Arm activity is required for dorsal closure. Importantly, optogenetic inactivation of α-catenin produces a comparable dorsal closure failure phenotype, indicating that adherens junction function is essential for closure. These observations support a model in which dorsal closure requires robust adherens junction coupling to transmit and coordinate forces across cells at the leading edge.

A central mechanistic inference from our mutant analyses is that Arm–α-catenin interaction is critical. Multiple perturbations that compromise this interaction produce dorsal closure defects: α-catenin inactivation (α-cat-CRY2), Arm inactivation (Arm-CRY2), and ΔArm, which lacks the α-catenin binding region, all display failure of zippering forces. ArmTimer-AA, which reduces α-catenin binding through altered threonine residues in the binding region, produces a milder but clear defect—slower and asymmetric zippering. This graded relationship between the extent of interaction disruption and the severity of closure defects strongly supports the conclusion that Arm–α-catenin coupling is required to stabilize junctions and coordinate force transmission during closure.

Threonine phosphorylation of β-catenin/Arm has previously been linked to polarity by stabilizing Arm–α-catenin binding,[66] and planar polarity in the Drosophila epidermis can be established through junctional Arm dynamics.[16] Our data extend this concept to the dorsal closure context, showing that compromising α-catenin binding specifically disrupts zippering dynamics, consistent with a requirement for stable, polarized junctions at the leading edge to enable mechanical closure.

Tyrosine-site mutants suggest altered Arm dynamics without overt failure

Our results with the tyrosine mutants (ArmTimer-EE and ArmTimer-FF) are more ambiguous with respect to a simple, direct model of E-cadherin binding regulation. Both mutants show reduced fluorescence intensity and altered Timer signatures but do not lead to obvious dorsal closure failure. Both tyrosine mutants show reduced stabilization and/or abundance, suggesting these residues influence Arm dynamics. However, the directionality does not map cleanly onto a simple phosphomimetic (less stable) versus phospho-resistant (more stable) expectation.

Prior literature has reported complex and sometimes conflicting roles for tyrosine phosphorylation in regulating β-catenin/E-cadherin affinity and junction stability. Phosphorylation of Y150 reduces affinity for E-cadherin, whereas Y667 has been reported to either increase or decrease affinity depending on context.[49,64,75,76] In our study, ArmTimer-EE's preserved RFP but reduced GFP suggests altered production/turnover with a stable fraction maintained, whereas ArmTimer-FF's reduced RFP suggests reduced stabilization despite normal GFP. These results imply that the tyrosine sites likely influence more than one aspect of Arm behavior— potentially the balance among membrane retention, junction remodeling, and degradation—rather than acting as simple binary switches.

ArmTimer-EE also increases the duration of dorsal closure, suggesting that phosphomimetic Arm sufficiently alters junction dynamics to slow closure without preventing it. Thus, while tyrosine phosphorylation does not appear essential for completion of dorsal closure under our conditions, it likely modulates the kinetics and stability of junctional Arm.

Dishevelled and JNK provide a link between Wnt components and mechanical morphogenesis

Canonical Wnt activation by Wg overexpression does not perturb dorsal closure or increase leading-edge Arm stability. However, inhibiting Arm stabilization by Axin overexpression causes closure defects, suggesting that the stability machinery regulating Arm abundance remains relevant. At the same time, Dsh accumulates at the leading edge, and perturbing the Dsh DEP domain causes severe morphogenetic failure. Because the DEP domain is central to non-canonical Wnt signaling outputs that engage Rac/JNK pathways, these observations support the notion that Wnt pathway components contribute to dorsal closure primarily through polarity/force-regulatory modules rather than through canonical ligand-driven transcriptional activation.

Consistent with this, JNK knockdown causes severe dorsal closure defects, and JNK overexpression alters ArmTimer RFP levels, suggesting that JNK activity modulates Arm stabilization and/or junction turnover. One interpretation is that increased JNK accelerates junction remodeling and turnover (reducing the stable Arm pool), whereas reduced JNK disrupts cytoskeletal tension, adhesion coordination, and leading-edge dynamics required for closure. Together, our results support a model in which Dsh (via DEP) and JNK signaling regulate junctional Arm stability and/or junction remodeling at the leading edge, thereby providing a functional link between Wnt components and mechanotransduction during dorsal closure.

Future directions: mechanotransduction and Hippo pathway integration

Since dorsal closure is inherently mechanical, these findings suggest broader links between mechanosensation and mechanotransduction links involving junctions. We observed stable Arm expression in other tissues undergoing mechanotransduction later in embryogenesis, including the gut and trachea (Supplementary Figure S1 ↗).[77–79] This suggests that junctional β-catenin stabilization may be a recurring feature of force-driven morphogenesis. Given that α-catenin has been implicated as a mechanosensor and that the Hippo pathway is force-regulated and central to controlling epithelial growth, a compelling future direction is to test whether Hippo signaling interacts with adherens junction stabilization during dorsal closure. Such research could clarify whether force generation increases Arm stabilization as part of a mechanical reinforcement process or if Arm stabilization itself facilitates effective force transmission and successful closure.

Materials and methods

Fly lines, MiMIC/RMCE, and expression of UAS constructs

All crosses for transgene expression were performed as previously described.[31] Expression of transgenes was driven by the daughterless, C381, and armadillo-GAL4 drivers (Kaur et al., 2017b). Additional stocks were obtained from the Bloomington Drosophila Stock Center (NIH P40OD018537). The tandem fluorescent protein timer recombination insertion construct was synthesized by SynbioTech (Monmouth Junction, NJ, USA) and injected by Bestgene (Chino Hills, CA, USA) following MiMIC-RMCE protocols.[27–29,80] and timer protocols.[40,81]

Fly Line	Reference
ArmTimer: Mi[MIC]armMI08675 - RMCE sfGFP-RFP Phase 1	This study
OptoArm: armMI08675 MiMIC RMCE Cry2-mCherry Phase 1	This study
Mi(PT-GFSTF.1)armMI08675-GFSTF	BDSC 60561
ArmTimerWT: Mi[MIC]armMI08675 - RMCE ArmWT - sfGFP-RFP	This study
ArmTimerFF: Mi[MIC]armMI08675 - RMCE ArmY150F, Y667F - sfGFP-RFP	This study
ArmTimerEE: Mi[MIC]armMI08675 - RMCE ArmY150E, Y667E - sfGFP-RFP	This study
ArmTimerAA: Mi[MIC]armMI08675 - RMCE ArmT111A, T121A - sfGFP-RFP	This study
ArmTimerF1a: Mi[MIC]armMI08675 - RMCE ArmR394H - sfGFP-RFP	This study
OptoαCat: Mi[MIC]α-CatMI02577 - RMCE Cry2-mCherry Phase 0	This study
OptoDlg: Mi[MIC]dlg1MI06353 - RMCE Cry2-mCherry Phase 0	This study
UAS- LifeActin-mKO	This study
Arm RMCE CRY2-mCh, UbiGFP, FRT19A (for germline clones)	This study
UAS Franken (anti-HA epitope nanobody)-GFP-mCherry	Murakawa et al, 2022[82]
UAS myr-Dsh-HA	Kaur et al, 2017[68]
UAS myr-Dsh ΔDIX-HA	Kaur et al, 2017
UAS myr-Dsh ΔPDZ-HA	Kaur et al, 2017
UAS myr-Dsh ΔDEP-HA	Kaur et al, 2017
UAS-Dsh-HA	Kaur et al, 2017
UAS-Wingless	BDSC 2075
UAS-RFP	BDSC 30555
UAS-Axin-Cry2-mCherry	Bunnag et al. 2020[35]
UAS-ΔArm	Zecca et al. 1996[60]
UAS-ArmS56A-sfGFP-RFP	This study
UAS-E-cadherin RFP	Gift Timothy Saunders

The frankenbody approach was used to visualize overexpression and mutants of Dishevelled.[67] This system uses an α-HA nanobody fused to GFP, mCherry, or both, co-expressed with any HA-tagged transgene, enabling live imaging of HA-tagged proteins.[82] Transgene expression was driven using the daughterless (da), C381, and armadillo (arm)-GAL4 drivers. Unless otherwise indicated, all additional fly stocks were obtained from the Bloomington Drosophila Stock Center (NIH P40OD018537).

The tandem fluorescent protein timer recombination insertion construct was synthesized by SynbioTech (Monmouth Junction, NJ, USA) and injected by Bestgene (Chino Hills, CA, USA).

Imaging

Arm-Timer embryos were imaged using a Zeiss Lightsheet Z.1 microscope (Carl Zeiss, Germany). Embryos were dechorionated with bleach, rinsed twice in water, dried, and loaded into a capillary containing 1% low-melting agarose (Type VII-A) in water (Sigma-Aldrich, St. Louis, MO, USA).[31,83] For imaging, the agarose plug was extruded from the capillary, and the sample was suspended in the imaging chamber filled with water. Images were acquired using a water-immersion W Plan-Apochromat 40×/1.0 UV-VIS detection objective (Carl Zeiss, Jena, Germany). Samples were imaged every 5 minutes for 200 cycles, using 5% laser power at 488 nm and 15% laser power at 561 nm. A late developmental time point was selected for analysis. Background fluorescence was automatically calculated in ZEN 2014 using the background correction function (Carl Zeiss). Deep, out-of-focus z-stacks were excluded, and maximum intensity projections were generated from in-focus stacks for each embryo. Embryos were dechorionated with bleach, rinsed twice with water, and mounted in water in an imaging dish.

Dorsal closure imaging was performed on a Zeiss LSM 800 (Carl Zeiss, Germany) with acquisition every 5 min for 60 cycles, using 3% laser power at 488 nm and 5% laser power at 561 nm. Dishevelled imaging was performed on the same system using 5% laser power at 488 nm and 10% laser power at 561 nm, also every 5 min for 60 cycles. Images were processed using the maximum intensity projection function in ZEN 2014 SP (Carl Zeiss). RFP and GFP cluster intensities were quantified using IMARIS 9.0 (Bitplane AG, UK). Z-stacks from the LSM800 were rendered using the Surface module, and fluorescence intensities were extracted for plotting. All time points were analyzed, and all channels were rendered using identical brightness and sensitivity settings. Statistical analyses were performed in R (v4.2.2), and plots were generated using ggpubr (v0.6.0).

Acknowledgements

Stocks obtained from the Bloomington Drosophila Stock Center (NIH P40OD018537) were used in this study. We used *Flybase.org* release FB2026_01.[84]

Additional information

Author Contributions

Conceptualization, N.S.T.; methodology, A.O., S.S., P.K., P.T., N.S.T; investigation, A.O., S.S., P.K., P.T.; writing—original draft preparation, A.O., and N.S.T.; writing—review and editing A.O., S.S., P.K., P.T., N.S.T,; supervision and funding acquisition, N.S.T. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the Singapore Ministry of Education, AcRF grants IG21-SG103 and FY2022-MOET1-0003 to N.S.T.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used OpenAI GPT-5.2 to improve the manuscript's readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Funding

Funder	Grant reference number	Author
Ministry of Education - Singapore (MOE)	FY2022-MOET1-0003	Nicholas S Tolwinski

Author ORCID iDs

Nicholas S Tolwinski: https://orcid.org/0000-0002-8507-2737

Additional files

Supplementary file 1.

Supplementary file 2.

Video 1–37.

References

1. **Kemler R,** Babinet C, Eisen H, Jacob F (1977) Surface antigen in early differentiation. *Proceedings of the National Academy of Sciences* **74**:4449-4452 https://doi.org/10.1073/pnas.74.10.4449 | PubMed
2. **Takeichi M** (1977) Functional correlation between cell adhesive properties and some cell surface proteins. *The Journal of cell biology* **75**:464-474 https://doi.org/10.1083/jcb.75.2.464 | PubMed

3. **Nagafuchi A,** Takeichi M (1988) Cell binding function of E-cadherin is regulated by the cytoplasmic domain. *The EMBO journal* **7**:3679-3684 https://doi.org/10.1002/j.1460-2075.1988.tb03249.x | PubMed
4. **Ozawa M,** Baribault H, Kemler R (1989) The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. *The EMBO journal* **8**:1711-1717 https://doi.org/10.1002/j.1460-2075.1989.tb03563.x | PubMed
5. **Herrenknecht K,** Ozawa M, Eckerskorn C, Lottspeich F, Lenter M, Kemler R (1991) The uvomorulin-anchorage protein alpha catenin is a vinculin homologue. *Proceedings of the National Academy of Sciences* **88**:9156-9160 https://doi.org/10.1073/pnas.88.20.9156 | PubMed
6. **Nagafuchi A,** Takeichi M, Tsukita S (1991) The 102 kd cadherin-associated protein: similarity to vinculin and posttranscriptional regulation of expression. *Cell* **65**:849-857 https://doi.org/10.1016/0092-8674(91)90392-c | PubMed
7. **Nagafuchi A,** Ishihara S, Tsukita S (1994) The roles of catenins in the cadherin-mediated cell adhesion: functional analysis of E-cadherin-alpha catenin fusion molecules. *J Cell Biol* **127**:235-245 https://doi.org/10.1083/jcb.127.1.235 | PubMed
8. **Peifer M,** Pai LM, Casey M (1994) Phosphorylation of the Drosophila adherens junction protein Armadillo: roles for wingless signal and zeste-white 3 kinase. *Dev Biol* **166**:543-556 https://doi.org/10.1006/dbio.1994.1336 | PubMed
9. **Wieschaus E,** Nüsslein-Volhard C, Jürgens G (1984) Mutations affecting the pattern of the larval cuticle inDrosophila melanogaster. *Wilhelm Roux's archives of developmental biology* **193**:296-307 https://doi.org/10.1007/BF00848158 | PubMed
10. **Riggleman B,** Schedl P, Wieschaus E (1990) Spatial expression of the Drosophila segment polarity gene armadillo is posttranscriptionally regulated by wingless. *Cell* **63**:549-560 https://doi.org/10.1016/0092-8674(90)90451-j | PubMed
11. **Wieschaus E,** Riggleman R (1987) Autonomous requirements for the segment polarity gene armadillo during Drosophila embryogenesis. *Cell* **49**:177-184 https://doi.org/10.1016/0092-8674(87)90558-7 | PubMed
12. **Nüsslein-Volhard C,** Wieschaus E (1980) Mutations affecting segment number and polarity in Drosophila. *Nature* **287**:795-801 https://doi.org/10.1038/287795a0 | PubMed
13. **Wieschaus E,** Nüsslein-Volhard C (2016) The Heidelberg screen for pattern mutants of Drosophila: a personal account. *Annual review of cell and developmental biology* **32**:1-46 https://doi.org/10.1146/annurev-cellbio-113015-023138 | PubMed
14. **Zallen JA,** Wieschaus E (2004) Patterned gene expression directs bipolar planar polarity in Drosophila. *Developmental cell* **6**:343-355 https://doi.org/10.1016/s1534-5807(04)00060-7 | PubMed
15. **Blankenship JT,** Backovic ST, Sanny JS, Weitz O, Zallen JA (2006) Multicellular rosette formation links planar cell polarity to tissue morphogenesis. *Developmental cell* **11**:459-470 https://doi.org/10.1016/j.devcel.2006.09.007 | PubMed
16. **Colosimo PF,** Tolwinski NS (2006) Wnt, Hedgehog and junctional Armadillo/beta-catenin establish planar polarity in the Drosophila embryo. *PLoS One* **1**:e9 https://doi.org/10.1371/journal.pone.0000009 | PubMed
17. **Kaplan NA,** Tolwinski NS (2010) Spatially defined Dsh-Lgl interaction contributes to directional tissue morphogenesis. *J Cell Sci* **123**:3157-3165 https://doi.org/10.1242/jcs.069898 | PubMed
18. **Schlessinger K,** Hall A, Tolwinski N (2009) Wnt signaling pathways meet Rho GTPases. *Genes Dev* **23**:265-277 https://doi.org/10.1101/gad.1760809 | PubMed
19. **Liu S,** Haghani S, Petretto E, Madan B, Harmston N, Virshup DM (2026) Identification of Wnt-regulated genes that are repressed by, or independent of, β-catenin. *The FEBS Journal* https://doi.org/10.1111/febs.70417 | PubMed

20. **Bertet C,** Sulak L, Lecuit T (2004) Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature* **429**:667-671 https://doi.org/10.1038/nature02590 | PubMed

21. **Drees F,** Pokutta S, Yamada S, Nelson WJ, Weis WI (2005) α-catenin is a molecular switch that binds E-cadherin-β-catenin and regulates actin-filament assembly. *Cell* **123**:903-915 https://doi.org/10.1016/j.cell.2005.09.021 | PubMed

22. **Etienne-Manneville S,** Hall A (2003) Cdc42 regulates GSK-3β and adenomatous polyposis coli to control cell polarity. *Nature* **421**:753-756 https://doi.org/10.1038/nature01423 | PubMed

23. **Knoblich JA** (2008) Mechanisms of asymmetric stem cell division. *Cell* **132**:583-597 https://doi.org/10.1016/j.cell.2008.02.007 | PubMed

24. **Nelson WJ,** Weis WI (2016) 25 years of tension over actin binding to the cadherin cell adhesion complex: the devil is in the details. *Trends in cell biology* **26**:471-473 https://doi.org/10.1016/j.tcb.2016.04.010 | PubMed

25. **Yamada S,** Pokutta S, Drees F, Weis WI, Nelson WJ (2005) Deconstructing the cadherin-catenin-actin complex. *Cell* **123**:889-901 https://doi.org/10.1016/j.cell.2005.09.020 | PubMed

26. **Kanca O,** Bellen HJ, Schnorrer F (2017) Gene tagging strategies to assess protein expression, localization, and function in Drosophila. *Genetics* **207**:389-412 https://doi.org/10.1534/genetics.117.199968 | PubMed

27. **Li-Kroeger D,** Kanca O, Lee PT, Cowan S, Lee MT, Jaiswal M, Salazar JL, He Y, Zuo Z, Bellen HJ (2018) An expanded toolkit for gene tagging based on MiMIC and scarless CRISPR tagging in Drosophila. *eLife* **7**:e38709 https://doi.org/10.7554/eLife.38709 | PubMed

28. **Nagarkar-Jaiswal S,** DeLuca SZ, Lee P.-T, Lin W.-W, Pan H, Zuo Z, Lv J, Spradling AC, Bellen HJ (2015) A genetic toolkit for tagging intronic MiMIC containing genes. *eLife* **4**:e08469 https://doi.org/10.7554/eLife.08469 | PubMed

29. **Nagarkar-Jaiswal S,** Lee P.-T, Campbell ME, Chen K, Anguiano-Zarate S, Cantu Gutierrez M, Busby T, Lin W.-W, He Y, Schulze KL (2015) A library of MiMICs allows tagging of genes and reversible, spatial and temporal knockdown of proteins in Drosophila. *eLife* **4**:e05338 https://doi.org/10.7554/elife.05338 | PubMed

30. **Venken KJ,** Schulze KL, Haelterman NA, Pan H, He Y, Evans-Holm M, Carlson JW, Levis RW, Spradling AC, Hoskins RA, *et al.* (2011) MiMIC: a highly versatile transposon insertion resource for engineering Drosophila melanogaster genes. *Nat Methods* **8**:737-743 https://doi.org/10.1038/nmeth.1662 | PubMed

31. **Kaur P,** Saunders TE, Tolwinski NS (2017) Coupling optogenetics and light-sheet microscopy, a method to study Wnt signaling during embryogenesis. *Sci Rep* **7**:16636 https://doi.org/10.1038/s41598-017-16879-0 | PubMed

32. **Lim CH,** Kaur P, Teo E, Lam VYM, Zhu F, Kibat C, Gruber J, Mathuru AS, Tolwinski NS (2020) Application of optogenetic Amyloid-beta distinguishes between metabolic and physical damages in neurodegeneration. *eLife* **9** https://doi.org/10.7554/eLife.52589 | PubMed

33. **Yadav V,** Tolwinski N, Saunders TE (2021) Spatiotemporal sensitivity of mesoderm specification to FGFR signalling in the Drosophila embryo. *Sci Rep* **11**:14091 https://doi.org/10.1038/s41598-021-93512-1 | PubMed

34. **Suresh J,** Khor IW, Kaur P, Heng HL, Torta F, Dawe GS, Tai ES, Tolwinski NS (2020) Shared signaling pathways in Alzheimer's and metabolic disease may point to new treatment approaches. *Febs J* https://doi.org/10.1111/febs.15540

35. **Bunnag N,** Tan QH, Kaur P, Ramamoorthy A, Sung ICH, Lusk J, Tolwinski NS (2020) An Optogenetic Method to Study Signal Transduction in Intestinal Stem Cell Homeostasis. *J Mol Biol* **432**:3159-3176 https://doi.org/10.1016/j.jmb.2020.03.019 | PubMed

36. **Ambrosi G,** Voloshanenko O, Eckert AF, Kranz D, Nienhaus GU, Boutros M (2022) Allele-specific endogenous tagging and quantitative analysis of β-catenin in colorectal cancer cells. *eLife* **11** https://doi.org/10.7554/eLife.64498 | PubMed

37. **de Man SM,** Zwanenburg G, van der Wal T, Hink MA, van Amerongen R (2021) Quantitative live-cell imaging and computational modeling shed new light on endogenous WNT/CTNNB1 signaling dynamics. *eLife* **10** https://doi.org/10.7554/eLife.66440 | PubMed

38. **Barry JD,** Dona E, Gilmour D, Huber W (2016) TimerQuant: a modelling approach to tandem fluorescent timer design and data interpretation for measuring protein turnover in embryos. *Development* **143**:174-179 https://doi.org/10.1242/dev.125971 | PubMed

39. **Durrieu L,** Kirrmaier D, Schneidt T, Kats I, Raghavan S, Hufnagel L, Saunders TE, Knop M (2018) Bicoid gradient formation mechanism and dynamics revealed by protein lifetime analysis. *Molecular Systems Biology* **14**:e8355 https://doi.org/10.15252/msb.20188355 | PubMed

40. **Khmelinskii A,** Keller PJ, Bartosik A, Meurer M, Barry JD, Mardin BR, Kaufmann A, Trautmann S, Wachsmuth M, Pereira G, *et al.* (2012) Tandem fluorescent protein timers for in vivo analysis of protein dynamics. *Nat Biotechnol* **30**:708-714 https://doi.org/10.1038/nbt.2281 | PubMed

41. **Revenu C,** Streichan S, Dona E, Lecaudey V, Hufnagel L, Gilmour D (2014) Quantitative cell polarity imaging defines leader-to-follower transitions during collective migration and the key role of microtubule-dependent adherens junction formation. *Development* **141**:1282-1291 https://doi.org/10.1242/dev.101675 | PubMed

42. **Tan QH,** Otgonbaatar A, Kaur P, Ga AF, Harmston NP, Tolwinski NS (2024) The Wnt Co-Receptor PTK7/Otk and Its Homolog Otk-2 in Neurogenesis and Patterning. *Cells* **13** https://doi.org/10.3390/cells13050365 | PubMed

43. **Cadigan KM,** Nusse R (1997) Wnt signaling: a common theme in animal development. *Genes Dev* **11**:3286-3305 https://doi.org/10.1101/gad.11.24.3286 | PubMed

44. **Lee E,** Salic A, Krüger R, Heinrich R, Kirschner MW (2003) The roles of APC and Axin derived from experimental and theoretical analysis of the Wnt pathway. *PLoS Biol* **1**:E10 https://doi.org/10.1371/journal.pbio.0000010 | PubMed

45. **Moldaver S,** Thibeault PE, Robitaille M, Au A, Lin S, MacLeod G, Junge HJ, Gammons MV, Yip CM, Angers S (2026) Wnt-dependent Frizzled clustering is required for Dishevelled phosphorylation but insufficient for β-catenin stabilization. *Science Signaling* **19**:eaec0204 https://doi.org/10.1126/scisignal.aec0204 | PubMed

46. **Gammons M,** Bienz M (2018) Multiprotein complexes governing Wnt signal transduction. *Current Opinion in Cell Biology* **51**:42-49 https://doi.org/10.1016/j.ceb.2017.10.008 | PubMed

47. **Maurice MM,** Angers S (2025) Mechanistic insights into Wnt–β-catenin pathway activation and signal transduction. *Nature Reviews Molecular Cell Biology* **26**:371-388 https://doi.org/10.1038/s41580-024-00823-y | PubMed

48. **Lee MH,** Koria P, Qu J, Andreadis ST (2009) JNK phosphorylates beta-catenin and regulates adherens junctions. *Faseb j* **23**:3874-3883 https://doi.org/10.1096/fj.08-117804 | PubMed

49. **Roura S,** Miravet S, Piedra J, de Herreros AG, Dunach M (1999) Regulation of E-cadherin/Catenin association by tyrosine phosphorylation. *Journal of Biological Chemistry* **274**:36734-36740 https://doi.org/10.1074/jbc.274.51.36734 | PubMed

50. **Hayes P,** Solon J (2017) Drosophila dorsal closure: An orchestra of forces to zip shut the embryo. *Mech Dev* **144**:2-10 https://doi.org/10.1016/j.mod.2016.12.005 | PubMed

51. **Kiehart DP,** Crawford JM, Aristotelous A, Venakides S, Edwards GS (2017) Cell sheet morphogenesis: dorsal closure in Drosophila melanogaster as a model system. *Annual review of cell and developmental biology* **33**:169-202 https://doi.org/10.1146/annurev-cellbio-111315-125357 | PubMed

52. **Kiehart DP,** Galbraith CG, Edwards KA, Rickoll WL, Montague RA (2000) Multiple Forces Contribute to Cell Sheet Morphogenesis for Dorsal Closure in Drosophila. *Journal of Cell Biology* **149**:471-490 https://doi.org/10.1083/jcb.149.2.471 | PubMed

53. **Gorfinkiel N,** Arias AM (2007) Requirements for adherens junction components in the interaction between epithelial tissues during dorsal closure in Drosophila. *Journal of cell science* **120**:3289-3298 https://doi.org/10.1242/jcs.010850 | PubMed

54. **Morel V,** Arias AM (2004) Armadillo/β-catenin-dependent Wnt signalling is required for the polarisation of epidermal cells during dorsal closure in Drosophila. *Development* **131**:3273-3283 https://doi.org/10.1242/dev.01217 | PubMed

55. **McEwen DG,** Cox RT, Peifer M (2000) The canonical Wg and JNK signaling cascades collaborate to promote both dorsal closure and ventral patterning. *Development* **127**:3607-3617 https://doi.org/10.1242/dev.127.16.3607 | PubMed

56. **Tolwinski NS,** Wieschaus E (2004) A nuclear function for armadillo/beta-catenin. *PLoS Biol* **2**:E95 https://doi.org/10.1371/journal.pbio.0020095 | PubMed

57. **Tolwinski NS,** Wieschaus E (2001) Armadillo nuclear import is regulated by cytoplasmic anchor Axin and nuclear anchor dTCF/Pan. *Development* **128**:2107-2117 https://doi.org/10.1242/dev.128.11.2107 | PubMed

58. **Rijsewijk F,** Schuermann M, Wagenaar E, Parren P, Weigel D, Nusse R (1987) The Drosophila homolog of the mouse mammary oncogene int-1 is identical to the segment polarity gene wingless. *Cell* **50**:649-657 https://doi.org/10.1016/0092-8674(87)90038-9 | PubMed

59. **Tolwinski NS,** Wehrli M, Rives A, Erdeniz N, DiNardo S, Wieschaus E (2003) Wg/Wnt signal can be transmitted through arrow/LRP5,6 and Axin independently of Zw3/Gsk3beta activity. *Dev Cell* **4**:407-418 https://doi.org/10.1016/s1534-5807(03)00063-7

60. **Zecca M,** Basler K, Struhl G (1996) Direct and Long-Range Action of a Wingless Morphogen Gradient. *Cell* **87**:833-844 https://doi.org/10.1016/S0092-8674(00)81991-1 | PubMed

61. **Piedra J,** Martinez D, Castano J, Miravet S, Dunach M, de Herreros AG (2001) Regulation of beta-catenin structure and activity by tyrosine phosphorylation. *J Biol Chem* **276**:20436-20443 https://doi.org/10.1074/jbc.M100194200 | PubMed

62. **Piedra J,** Martínez D, Castaño J, Miravet S, Duñach M, García de Herreros A (2016) Regulation of β-catenin structure and activity by tyrosine phosphorylation. *J Biol Chem* **291**:11463 https://doi.org/10.1074/jbc.A116.100194 | PubMed

63. **Tamada M,** Farrell DL, Zallen JA (2012) Abl regulates planar polarized junctional dynamics through β-catenin tyrosine phosphorylation. *Developmental cell* **22**:309-319 https://doi.org/10.1016/j.devcel.2011.12.025 | PubMed

64. **Tominaga J,** Fukunaga Y, Abelardo E, Nagafuchi A (2008) Defining the function of β-catenin tyrosine phosphorylation in cadherin-mediated cell–cell adhesion. *Genes to Cells* **13**:67-77 https://doi.org/10.1111/j.1365-2443.2007.01149.x | PubMed

65. **van Veelen W,** Le NH, Helvensteijn W, Blonden L, Theeuwes M, Bakker ER, Franken PF, van Gurp L, Meijlink F, van der Valk MA, *et al.* (2011) beta-catenin tyrosine 654 phosphorylation increases Wnt signalling and intestinal tumorigenesis. *Gut* **60**:1204-1212 https://doi.org/10.1136/gut.2010.233460 | PubMed

66. **Bek S,** Kemler R (2002) Protein kinase CKII regulates the interaction of beta-catenin with alpha-catenin and its protein stability. *J Cell Sci* **115**:4743-4753 https://doi.org/10.1242/jcs.00154 | PubMed

67. **Zhao N,** Kamijo K, Fox PD, Oda H, Morisaki T, Sato Y, Kimura H, Stasevich TJ (2019) A genetically encoded probe for imaging nascent and mature HA-tagged proteins in vivo. *Nat Commun* **10**:2947 https://doi.org/10.1038/s41467-019-10846-1 | PubMed

68. **Kaur P,** Lam VYM, Mannava AG, Suresh J, Jenny A, Tolwinski NS (2017) Membrane Targeting of Disheveled Can Bypass the Need for Arrow/LRP5. *Sci Rep* **7**:6934 https://doi.org/10.1038/s41598-017-04414-0 | PubMed

69. **Boutros M,** Paricio N, Strutt DI, Mlodzik M (1998) Dishevelled activates JNK and discriminates between JNK pathways in planar polarity and wingless signaling. *Cell* **94**:109-118 https://doi.org/10.1016/s0092-8674(00)81226-x | PubMed

70. **Harden N** (2002) Signaling pathways directing the movement and fusion of epithelial sheets: lessons from dorsal closure in Drosophila. *Differentiation: REVIEW* **70**:181-203 https://doi.org/10.1046/j.1432-0436.2002.700408.x | PubMed

71. **Noselli S,** Agnès F (1999) Roles of the JNK signaling pathway in Drosophila morphogenesis. *Current opinion in genetics & development* **9**:466-472 https://doi.org/10.1016/s0959-437x(99)80071-9 | PubMed

72. **Jacinto A,** Wood W, Balayo T, Turmaine M, Martinez-Arias A, Martin P (2000) Dynamic actin-based epithelial adhesion and cell matching during Drosophila dorsal closure. *Curr Biol* **10**:1420-1426 https://doi.org/10.1016/s0960-9822(00)00796-x | PubMed

73. **Riesgo-Escovar JR,** Jenni M, Fritz A, Hafen E (1996) The Drosophila Jun-N-terminal kinase is required for cell morphogenesis but not for DJun-dependent cell fate specification in the eye. *Genes Dev* **10**:2759-2768 https://doi.org/10.1101/gad.10.21.2759 | PubMed

74. **Homsy JG,** Jasper H, Peralta XG, Wu H, Kiehart DP, Bohmann D (2006) JNK signaling coordinates integrin and actin functions during Drosophila embryogenesis. *Dev Dyn* **235**:427-434 https://doi.org/10.1002/dvdy.20649 | PubMed

75. **Hamada-Kawaguchi N,** Nishida Y, Yamamoto D (2015) Btk29A-mediated tyrosine phosphorylation of armadillo/β-catenin promotes ring canal growth in Drosophila oogenesis. *PLoS One* **10**:e0121484 https://doi.org/10.1371/journal.pone.0121484 | PubMed

76. **Shomori K,** Ochiai A, Akimoto S, Ino Y, Shudo K, Ito H, Hirohashi S (2009) Tyrosine-phosphorylation of the 12th armadillo-repeat of β-catenin is associated with cadherin dysfunction in human cancer. *International journal of oncology* **35**:517-524 https://doi.org/10.3892/ijo_00000363 | PubMed

77. **Caussinus E,** Colombelli J, Affolter M (2008) Tip-cell migration controls stalk-cell intercalation during Drosophila tracheal tube elongation. *Current Biology* **18**:1727-1734 https://doi.org/10.1016/j.cub.2008.10.062 | PubMed

78. **Farge E** (2003) Mechanical induction of Twist in the Drosophila foregut/stomodeal primordium. *Current biology* **13**:1365-1377 https://doi.org/10.1016/s0960-9822(03)00576-1 | PubMed

79. **Houtekamer RM,** van der Net MC, Maurice MM, Gloerich M (2022) Mechanical forces directing intestinal form and function. *Current Biology* **32**:R791-R805 https://doi.org/10.1016/j.cub.2022.05.041 | PubMed

80. **Lee P.-T,** Zirin J, Kanca O, Lin W.-W, Schulze KL, Li-Kroeger D, Tao R, Devereaux C, Hu Y, Chung V (2018) A gene-specific T2A-GAL4 library for Drosophila. *eLife* **7**:e35574 https://doi.org/10.7554/eLife.35574 | PubMed

81. **Khmelinskii A,** Knop M (2014) Analysis of protein dynamics with tandem fluorescent protein timers. In: Ivanov A (Ed). *Exocytosis and Endocytosis* Springer. pp. 195-210 https://doi.org/10.1007/978-1-4939-0944-5_13 | PubMed

82. **Murakawa T,** Nakamura T, Kawaguchi K, Murayama F, Zhao N, Stasevich TJ, Kimura H, Fujita N (2022) A Drosophila toolkit for HA-tagged proteins unveils a block in autophagy flux in the last instar larval fat body. *Development* **149** https://doi.org/10.1242/dev.200243 | PubMed

83. **Kaur P,** Kibat C, Teo E, Gruber J, Mathuru A, Tolwinski ANS (2020) Use of Optogenetic Amyloid-beta to Monitor Protein Aggregation in Drosophila melanogaster, Danio rerio and Caenorhabditis elegans. *Bio Protoc* **10**:e3856 https://doi.org/10.21769/BioProtoc.3856 | PubMed

84. **Öztürk-Çolak A,** Marygold SJ, Antonazzo G, Attrill H, Goutte-Gattat D, Jenkins VK, Matthews BB, Millburn G, dos Santos G, Tabone CJ, *et al.* (2024) FlyBase: updates to the Drosophila genes and genomes database. *Genetics* **227** https://doi.org/10.1093/genetics/iyad211 | PubMed

Peer reviews

Reviewer #1 (Public review):

In this study, Otgonbaatar and colleagues investigate the stability of Armadillo (Arm) during Drosophila development using a creative tandem fluorescent protein timer approach via endogenous tagging of Arm. The tagging strategy allows for newly synthesised and longer-term stabilised Arm pools to be distinguished from one another. Specifically, the authors address the functional relevance of and mechanism behind the stabilisation of junctional Arm during dorsal closure.

The authors show that Arm is stabilised at the leading edge during dorsal closure. Using a sophisticated optogenetics approach, which allows for acute perturbations, they show that stabilised Arm is functionally required for dorsal closure. Increasing Wg (by overexpression) did not affect dorsal closure or Arm stability, in contrast to Axin overexpression, which reduces Wg/Arm signalling. In line with canonical signalling control of Arm levels being critical, stabilisation of Arm by N-terminal mutations disrupted dorsal closure. However, the same deletion is also expected to affect interaction with alpha-catenin. Co-localisation with E-cadherin and actin suggests a junctional role of leading-edge localised Arm. Optogenetic targeting of alpha-catenin points towards a key role of adherence junctions in dorsal closure. Allele replacement with mutant variants of Arm to affect adherence junction complex assembly further indicates an important contribution of coupling between Arm and alpha-catenin. Using overexpression approaches, the authors suggest that Dsh and Jnk contribute to dorsal closure.

This microscopy- and optogenetics-based study is generally well-conducted and provides strong evidence for stabilised Arm during dorsal closure, as well as its functional importance. This is an important discovery relevant to morphogenesis and potentially mechanotransduction. From a technical perspective, the validated beta-catenin timer provides a valuable tool for the field. The timer has revealed that Arm stabilisation does not coincide with Wg stripes, suggesting a Wg-independent stabilisation mechanism that may instead depend on adherence junction assembly, especially the interaction of Arm with alpha-catenin. However, as N-terminal deletion within Arm and Axin overexpression also disrupted dorsal closure, substantial ambiguity remains. Can suppression of the beta-catenin degradation machinery be ruled out as a regulatory mechanism? An expansion of ArmTimer mutant variants could contribute to testing the authors' conclusion further. Structural insights into junctional interactions involving Arm (e.g., 10.1074/jbc.M114.554709) could, for example, be used for further functional exploration by mutagenesis. The direct mechanistic impact of JNK and its potential link to Dsh in dorsal closure remains less compelling.

In summary, this is a highly relevant and important study, potentially pointing to a novel stabilisation mechanism of beta-catenin in development. Further corroboration of the mechanism, to test whether it is indeed distinct from canonical signalling, would be needed to support the conclusions.

https://doi.org/10.7554/eLife.111825.1.sa2

Reviewer #2 (Public review):

Summary:

Otgonbaatar et al. sought to investigate β-catenin/Arm protein lifetime and stabilization dynamics in vivo during embryonic development. To address this question, the authors developed an endogenous tandem fluorescent protein timer (tFP) system that enables the visualization of newly synthesized versus long-lived Arm protein in vivo. Using this

approach, the authors sought to determine where stabilized Arm accumulates during development and how it contributes to dorsal closure.

Strengths:

A major strength of the study is the development and application of the endogenous Arm timer system, which provides a powerful approach for monitoring protein stabilization dynamics in living tissues. Using this system, the authors unexpectedly found that the strongest Arm stabilization occurs not in Wnt signaling regions, but at the leading edge cells during dorsal closure. The study combines quantitative live imaging, optogenetic perturbation, genetic analysis, and structure-function approaches to demonstrate that stabilized junctional Arm interacts with α-catenin and contributes to tissue mechanics required at the leading edge for dorsal closure. Particularly compelling is the combination of multiple perturbations, including optogenetic disruption of Arm or α-catenin, Axin overexpression, and Arm mutants, which produce consistent dorsal closure defects.

Some conclusions are generally supported by the presented data. The work provides strong evidence that Arm plays an important role in dorsal closure. The identification of a requirement for the Dishevelled DEP domain and JNK signaling supports a non-canonical regulatory mechanism controlling dorsal closure.

Weaknesses:

(1) Conclusions are made regarding force transmission;(however, no experimental evidence is provided to support these conclusions.

(2) The conclusion was made that Wingless does not affect dorsal closure. However, this was based solely on Wingless overexpression in the amnioserosa, and the level of Wingless expression was not quantified. One possibility is that this level was not sufficient to see an effect. Alternatively, Wingless may have a role in migrating epithelium rather than the amnioserosa. Indeed, it is known that wingless mutants display a defect in dorsal closure.

(3) The effect of JNK knockdown on Arm localization maybe is indirect, and due to a secondary consequence on disruption of epithelial morphology rather than a direct effect of JNK on Arm.

(4) Some conclusions rely on overexpression-based perturbations (e.g., Axin or Arm mutants), which may not fully recapitulate endogenous physiological regulation.

(5) The Arm timer was not able to detect Wingless-dependent Arm stabilization in stripes. This finding demonstrates that the timer is not sensitive enough to thoroughly analyze Arm dynamics.

Overall, this work provides important conceptual advances in understanding junctional β-catenin/Arm function during dorsal closure. The endogenous fluorescent timer approach will likely be broadly useful to the community for studying protein stability dynamics in vivo, and the findings expand current views of β-catenin by highlighting its mechanical and junctional functions during tissue morphogenesis.

https://doi.org/10.7554/eLife.111825.1.sa1

Author response:

We are glad the reviewers found the tandem fluorescent timer approach valuable and the leading-edge Arm stabilization finding significant.

We agree with the Assessment that our evidence for three specific claims Wingless-independence, JNK-mediated regulation of Arm stability, and a direct mechanical/force-

transmission role for stabilized Arm is currently incomplete, and we will revise the text throughout to reflect this more precisely rather than overstating the current data. In addition, we commit to two new experiments, both using existing reagents and fly stocks, that speak directly to the two most experimentally tractable points raised by the reviewers:

(1) Re-staining our existing JNK-RNAi and JNK-overexpression embryos for E-cadherin (reagent already validated in Figure 4), to test whether JNK acts directly on junctional architecture or only indirectly, via broader epithelial disruption.

(2) Imaging ArmTimer in a *wingless* loss-of-function background, to directly test Wingless-dependence of leading-edge Arm stabilization as the reciprocal of our existing overexpression data.

We address each public review point below and outline the accompanying text revisions.

On Wingless independence (Reviewer #1; Reviewer #2, Weaknesses #2 and #5; Recommendation #1):

We agree that our current evidence unquantified Wg overexpression restricted to the amnioserosa (C381-Gal4) and uniform overexpression, alongside the absence of detectable Wg-stripe-associated Arm-Timer signal supports a more limited conclusion than "Wingless-independent" as currently stated. We will revise our language throughout the Abstract, Results, and Discussion to state that canonical Wg overexpression does not detectably enhance leading-edge Arm stabilization or perturb dorsal closure under our conditions, rather than asserting pathway independence. As noted above, we commit to imaging ArmTimer in a *wg* mutant background to test this directly, complementing our overexpression data with the reciprocal loss-of-function manipulation.

On the related point that the Timer's failure to detect a Wg-stripe-associated stabilization signal could reflect a sensitivity limitation rather than a true absence of stabilization (Reviewer #2, Weakness #5): we agree and will state this explicitly rather than treating absence of signal as evidence of absence. This does not undermine the positive leading-edge finding, which is not defined relative to the stripe comparison: all embryos, channels, and time points were imaged and rendered using identical laser power and brightness/sensitivity settings, and the leading-edge RFP signal clearly exceeds background under those same acquisition conditions. We also note that detection limits of this kind are a recognized challenge for endogenously tagged reporters of canonical Wnt/β-catenin signaling generally, including in mammalian systems, and cite two studies already in our bibliography that report the same class of limitation: de Man et al. (2021, eLife 10:e66440) and Ambrosi et al. (2022, eLife 11:e64498). We have added this clarification, with these citations, to the Results (paragraph describing Figure 3).

On the mechanistic link between Arm stability and destruction-complex activity (Reviewer #1):

We agree that because both ΔArm and Axin overexpression converge on the destruction complex, our data cannot yet fully separate "escape from degradation" from "impaired α-catenin/junctional coupling" as the operative mechanism. We will revise the Discussion to state this ambiguity explicitly and will treat the ArmTimer-AA result (partial α-catenin-binding disruption via phosphosite mutation, independent of destruction-complex regulation) as the strongest current evidence isolating the junctional-coupling mechanism. We also thank Reviewer 1 for pointing us to Pokutta, Choi, Ahlsen, Hansen & Weis (2014, J Biol Chem 289:13589-13601), which structurally and thermodynamically characterized the mammalian cadherin·β-catenin·α-catenin complex and showed that α-catenin binding to β-catenin is a distinct, allosterically regulated interface cadherin binding increases β-catenin's affinity for α-catenin roughly 10-fold, and α-catenin homodimerization independently

competes with β-catenin binding. We have added this citation to the Discussion as structural support for treating cadherin engagement, α-catenin coupling, and destruction-complex regulation as mechanistically separable interfaces, and note that the crystallized β-catenin·α-catenin interface provides a structural template for future experiments for example, structure-guided point mutations at the homologous interface residues in Arm, or in vitro binding assays comparing wild-type and threonine-mutant (T111A/T121A) Arm affinity for α-catenin.

We do not, however, believe a destruction-complex-independent stabilizing allele of Arm is a tractable experiment to close this gap directly: any allele that stabilizes Arm without engaging the destruction complex is, by definition, a Wnt pathway gain-of-function allele, since destruction-complex-mediated degradation is the very regulatory step that canonical Wnt signaling controls. Nor would restricting the allele to a transcriptionally inactive form of Arm cleanly resolve the confound: Wnt/TCF target loci include dedicated repressive TCF-binding sites (Blauwkamp, Chang & Cadigan, 2008, EMBO J 27:1436-1446), so a transcriptionally "dead" stabilized Arm could still alter transcription by disrupting TCF-mediated repression. We therefore treat this as a genuine, currently unresolvable confound of the overexpression approach, and rely instead on the CRY2 optogenetic and ArmTimer-AA results as the strongest available evidence isolating a junctional-coupling contribution. We have added this reasoning, with both citations, to the Discussion.

On JNK acting on Arm directly vs. indirectly (Reviewer #1; Reviewer #2, Weakness #3 and Recommendation #2):

This is the most actionable point raised by both reviewers. As noted above, we commit to re-imaging and re-staining our existing JNK-RNAi and JNK-overexpression embryos for E-cadherin to determine whether junctional/polarity architecture is broadly disrupted under these conditions (indirect mechanism) or whether E-cadherin localization is comparatively preserved while Arm stabilization is specifically altered (direct mechanism). In the meantime, we note that a direct mechanism is biochemically plausible: in mammalian cells, JNK phosphorylates β-catenin directly and regulates adherens junction integrity, and JNK activity separately controls the binding of α-catenin to the junctional complex (Lee, Koria, Qu & Andreadis, 2009, FASEB J 23:3874-3883; Lee, Padmashali, Koria & Andreadis, 2011, FASEB J 25:613-623). We cite these as precedent that a direct route from JNK to junctional β-catenin/α-catenin regulation exists in another system, while being explicit that this does not establish the same mechanism in *Drosophila* dorsal closure that will be tested directly by the E-cadherin re-staining experiment. We have added these citations and this caveat to the Discussion.

On the Dsh-DEP-to-JNK mechanistic link (Reviewer #1, Public Review #2 and Recommendation #5):

We agree that our data show the Dsh-DEP requirement and the JNK requirement for dorsal closure as parallel, independent findings rather than a demonstrated linear pathway in our system. To provide context for why we consider a DEP-to-JNK connection a reasonable working hypothesis, we searched the literature in both *Drosophila* and vertebrates and will cite six additional studies establishing this link: Axelrod et al. (1998) and Axelrod (2001), establishing that DEP-dependent membrane recruitment and unipolar localization of Dishevelled are specifically required for planar polarity signaling, distinct from Wingless signaling; Paricio et al. (1999) and Fanto et al. (2000), showing Dishevelled acts through Misshapen and Rac1/RhoA to the same JNK module used in dorsal closure; and Moriguchi et al. (1999) and Yamanaka et al. (2002), showing biochemically in vertebrates that the DEP domain of Dvl-1 selectively activates JNK independent of β-catenin/TCF-LEF activity, and that this JNK requirement is conserved in Xenopus convergent extension, the vertebrate process most functionally analogous to dorsal closure. We will state explicitly that this precedent, while now cross-species, comes from planar-cell-polarity and convergent-extension assays

rather than dorsal closure itself, so it supports the plausibility of a Dsh/Dvl-DEP-to-JNK connection without establishing that the identical pathway operates in our system.

On the Dsh DIX/DEP domain-separability argument (Reviewer #1, Recommendation #4):

We thank the reviewer for pointing us to Gammons, Renko, Johnson, Rutherford & Bienz (2016, Mol Cell 64:92-104), which showed that the Wnt signalosome itself is assembled by head-to-tail DEP domain swapping between Dishevelled molecules, and that this DEP-dependent oligomerization is directly required for canonical Wnt pathway activity not restricted to the non-canonical/planar-polarity branch as we had implied. We agree this evidence undercuts our previous interpretation of the DshΔDEP dorsal closure phenotype as evidence for a strong non-canonical/polarity-specific role for the DEP-dependent branch of Dsh. We have revised the Discussion accordingly: we now state that the DEP domain is required for the morphogenetic program culminating in dorsal closure, cite Gammons et al. directly, and note that this requirement does not by itself establish a non-canonical/polarity-specific role, since we cannot rule out a contribution from DEP-dependent canonical Wnt signalosome assembly.

On force transmission (Reviewer #2, Weakness #1):

We agree that we have not directly measured force or tension at the leading edge, and that our current data (colocalization with actin/E-cadherin, and functional requirement shown via CRY2 optogenetics and mutant analysis) are consistent with, but do not directly demonstrate, a role in force transmission. We do not have the in-house expertise to perform direct force/tension measurements (e.g., laser ablation, junctional tension assays), so we will not be adding such an experiment in this revision. Instead, we have revised the language throughout the manuscript including two Discussion section headings that previously stated a mechanical role for stabilized Arm as established fact to consistently present the mechanical/force-transmission role as a hypothesis raised by our data, not a demonstrated conclusion, and we retain a clear statement that direct force measurement (ideally in collaboration with groups with the relevant biophysical expertise) is future work rather than a claim we are making in this manuscript.

On the phosphomimetic threonine mutant (Reviewer #1, Recommendation #2):

ArmTimer-AA (T111A, T121A) was generated with the expectation that the tyrosine phosphosite mutants (ArmTimer-EE, ArmTimer-FF) would be the primary drivers of any dorsal closure phenotype, given their proposed role in E-cadherin binding; the pronounced zippering defect we observed in ArmTimer-AA was therefore an unanticipated finding rather than a predicted result. We have not generated the reciprocal phosphomimetic ArmTimer-EE(Thr) (T111E, T121E) allele. Generating and characterizing this allele is a substantial undertaking we estimate over a year including allele generation, validation, and phenotypic characterization and we will state this explicitly in the Discussion as planned future work rather than part of the current revision.

On confirmation of myristoylated-Dsh membrane targeting (Reviewer #1, Recommendation #3):

We cannot confirm that myristoylation localizes all Dsh protein to the membrane. However, this strategy has extensive prior genetic validation using the identical Src-derived myristoylation sequence: it was originally used to tether Armadillo and shown sufficient for constitutive Wnt pathway activation (Zecca, Basler & Struhl, 1996; Tolwinski & Wieschaus, 2001, 2004), and the same approach was subsequently applied to GSK3 and Dishevelled, in each case producing the expected pathway-activation phenotypes (Mannava & Tolwinski, 2015; Kaur et al., 2017). We have added these citations to the Results where the Myr-Dsh constructs are introduced.

On overexpression-based perturbations versus endogenous regulation (Reviewer #2, Weakness #4):

We would like to clarify that most of the Arm alleles used in this study including all of the point-mutant Timer alleles (ArmF1a, ArmTimer-FF, ArmTimer-EE, ArmTimer-AA) central to our mechanistic conclusions were generated as knock-ins at the endogenous 'arm' locus via MiMIC/RMCE, not overexpressed. The two exceptions are ΔArm and ArmS56A, expressed from UAS constructs because both are gain-of-function alleles anticipated to be lethal if expressed from the endogenous locus, based on prior experience with similarly stabilizing mutations. Axin overexpression was used because no Axin mutant or knock-in allele was generated for this study; we agree an endogenous Axin allele would be the ideal complement and will state this explicitly as a limitation, while noting that our CRY2 optogenetic perturbations of Arm and α-catenin which act acutely on the endogenous proteins provide an orthogonal line of evidence supporting the same conclusions. We have added this clarification to the Discussion.

https://doi.org/10.7554/eLife.111825.1.sa0