# Developmentally Regulated GTP binding protein 1 modulates ciliogenesis through an interaction with *Dishevelled.*

**Moonsup Lee**, Yoo-seok Hwang, Jaeho Yoon, Jian Sun, Adam Harned, Kunio Nagashima and Ira O. Daar

Cancer & Developmental Biology Laboratory (CDBL), CCR, NCI, NIH MD

## Abstract

Cilia are critical for proper embryonic development and maintaining homeostasis. Although extensively studied, there are still significant gaps regarding the proteins involved in regulating ciliogenesis. Using the Xenopus laevis embryo, we show that Dishevelled (Dvl), a key Wnt signaling scaffold that is critical to proper ciliogenesis, interacts with Drg1 (developmentally regulated GTP-binding protein 1). The loss of Drg1 or disruption of the interaction with Dvl reduces the length and number of cilia and displays defects in basal body migration and docking to the apical surface of multiciliated cells (MCCs). Moreover, Drg1 morphants display abnormal rotational polarity of basal bodies and a decrease in apical actin and RhoA activity that can be attributed to disruption of the protein complex between Dvl and Daam1. These results support the concept that the Drg1-Dvl interaction regulates apical actin polymerization and stability in MCCs. Thus, Drg1 is a newly identified partner of Dvl in regulating ciliogenesis.

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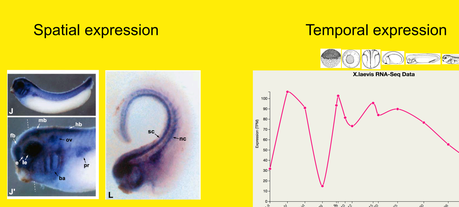
## Introduction




Reported roles of Drg1
1. control cell growth
2. promote tumor progression
3. spindle formation

Unknown role in embryonic development

## Summary

1. Drg1 associates with Dvl2 in Xenopus embryos and human cell lines.
2. The DEP domain and the contiguous C-terminal region (DEP+C) domain of Dvl and the TGS domain of Drg1 are required for Dvl2–Drg1 interactions.
3. Drg1 localizes to the basal body area in MCCs.
4. Drg1 is required for ciliogenesis in MCCs.
5. Drg1 is required for planar polarization and apical docking of basal bodies in MCCs.
6. An interaction between Drg1 and Dvl is required for apical actin meshwork formation.
7. Drg1 is required for proper basal body and Dvl2 and Daam1 localization in MCCs.
8. Active Daam1 expression is sufficient to suppress Drg1knockdown phenotypes in MCCs.

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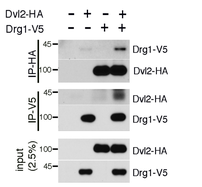
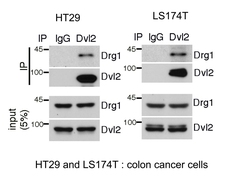
## Result

### 1. Mass-spec validation

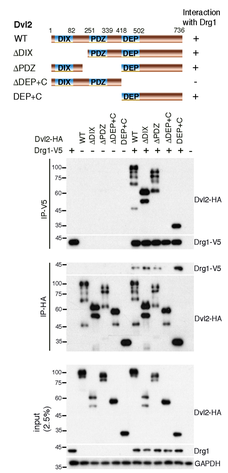
| MASS-SPEC | Dvl2 (bait) |
|---|---|
| Drg1 | 10 (6 kinds) |



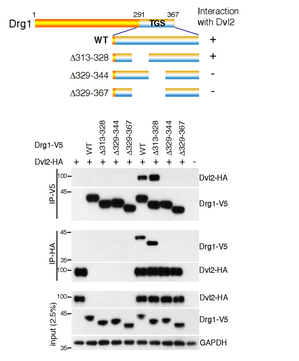




HT29 and LS174T : colon cancer cells

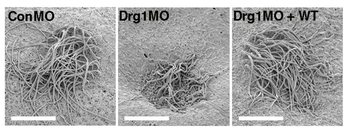
### 2. Drg1 interaction domain mapping of Dvl2.

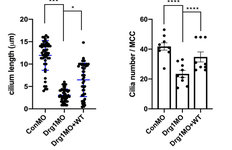


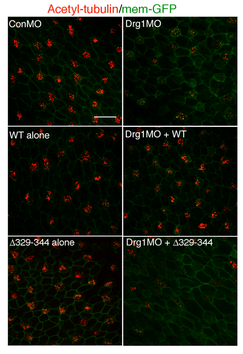

### 3. Dvl2 interaction domain mapping of Drg1

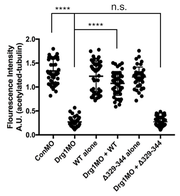



### 4. Drg1 knockdown disrupts ciliogenesis in MCCs.

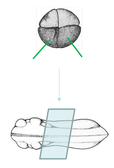


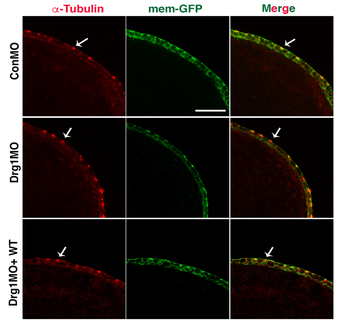



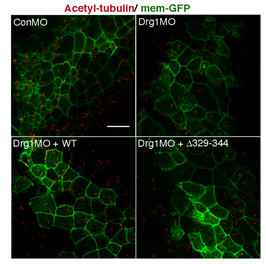



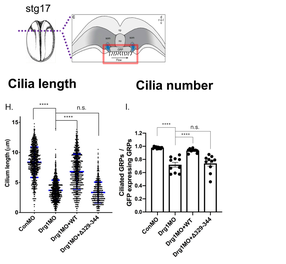

### 5. Drg1 knockdown does not affect migration of MCC progenitors



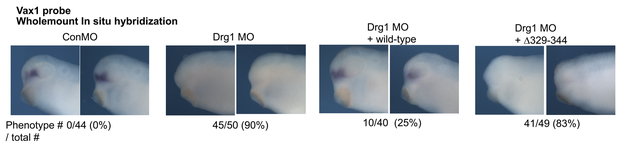



### 6. Drg1 knockdown causes ciliogenesis defect in gastrocoel roof plate.

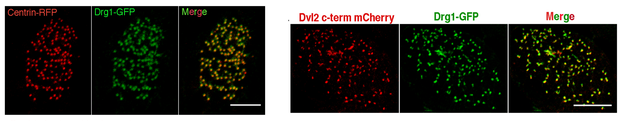


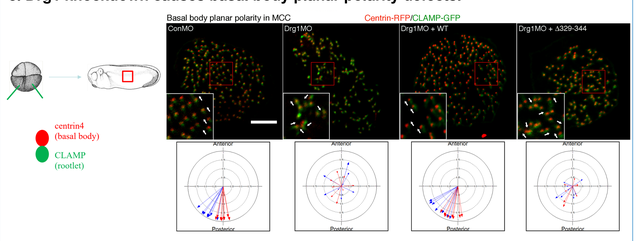

### Drg1 knockdown decreases Vax1 (*shh* downstream target) transcripts.



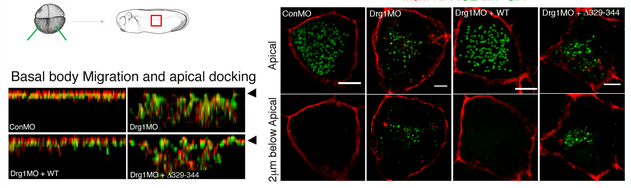

### 7. Drg1 localizes to basal body area in MCCs, and colocalized with Dvl2.



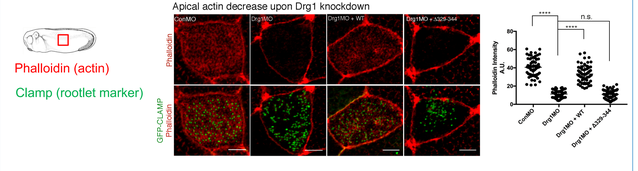

### 8. Drg1 knockdown causes basal body planar polarity defects.



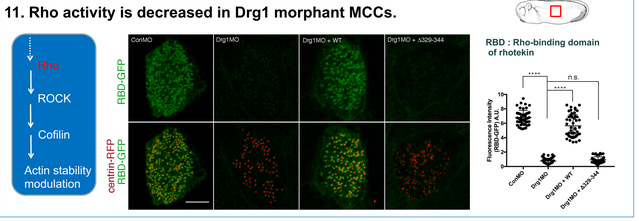

### 9. Drg1 knockdown causes basal body migration and docking defects in MCCs.



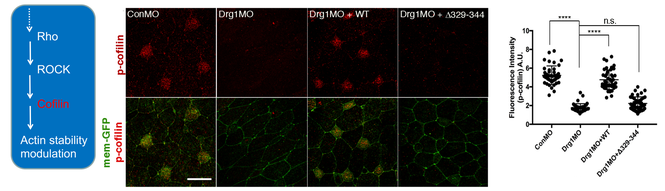

### 10. Drg1 knockdown causes apical actin reduction in MCCs.



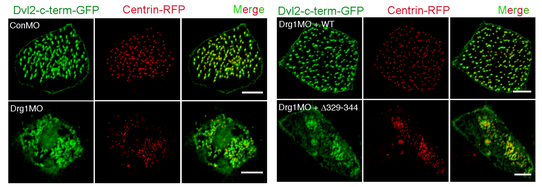

### 11. Rho activity is decreased in Drg1 morphant MCCs.



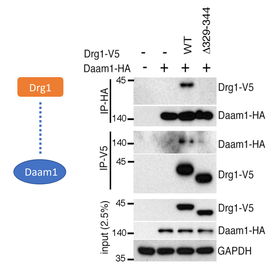

### 12. Drg1 knockdown decreases cofilin phosphorylation in MCCs.



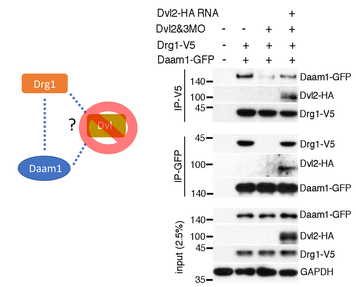

### 13. Drg1 knockdown has a mild effect on Dvl localization to the basal body region in MCCs.



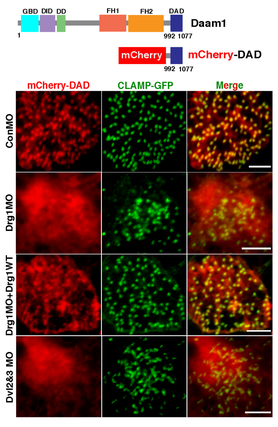

### 14. The amino acids 329-344 of Drg1 is required for Daam1 association.



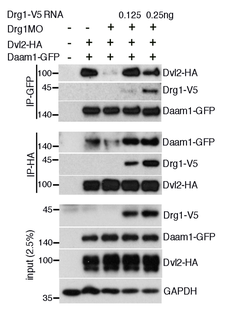

### 15. Dvl knockdown decreases Drg1 interaction with Daam1 in *Xenopus laevis* embryos.



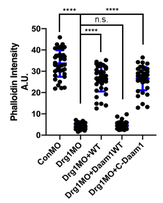

### 16. Knockdown of Drg1 affects localization of Daam1-DAD in MCCs.

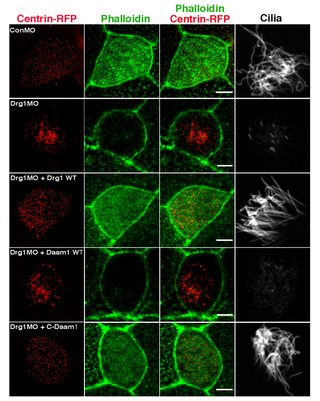



### 17. Drg1 knockdown decreases Dvl2:Daam1 interaction in *Xenopus laevis* embryos.




### 16. Active Daam1 (C-Daam1) expression restores the formation of the apical actin meshwork that was decreased upon Drg1 knockdown.

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