Differential mechanical coupling between cardioblasts guides heart morphogenesis

Timothy Saunders

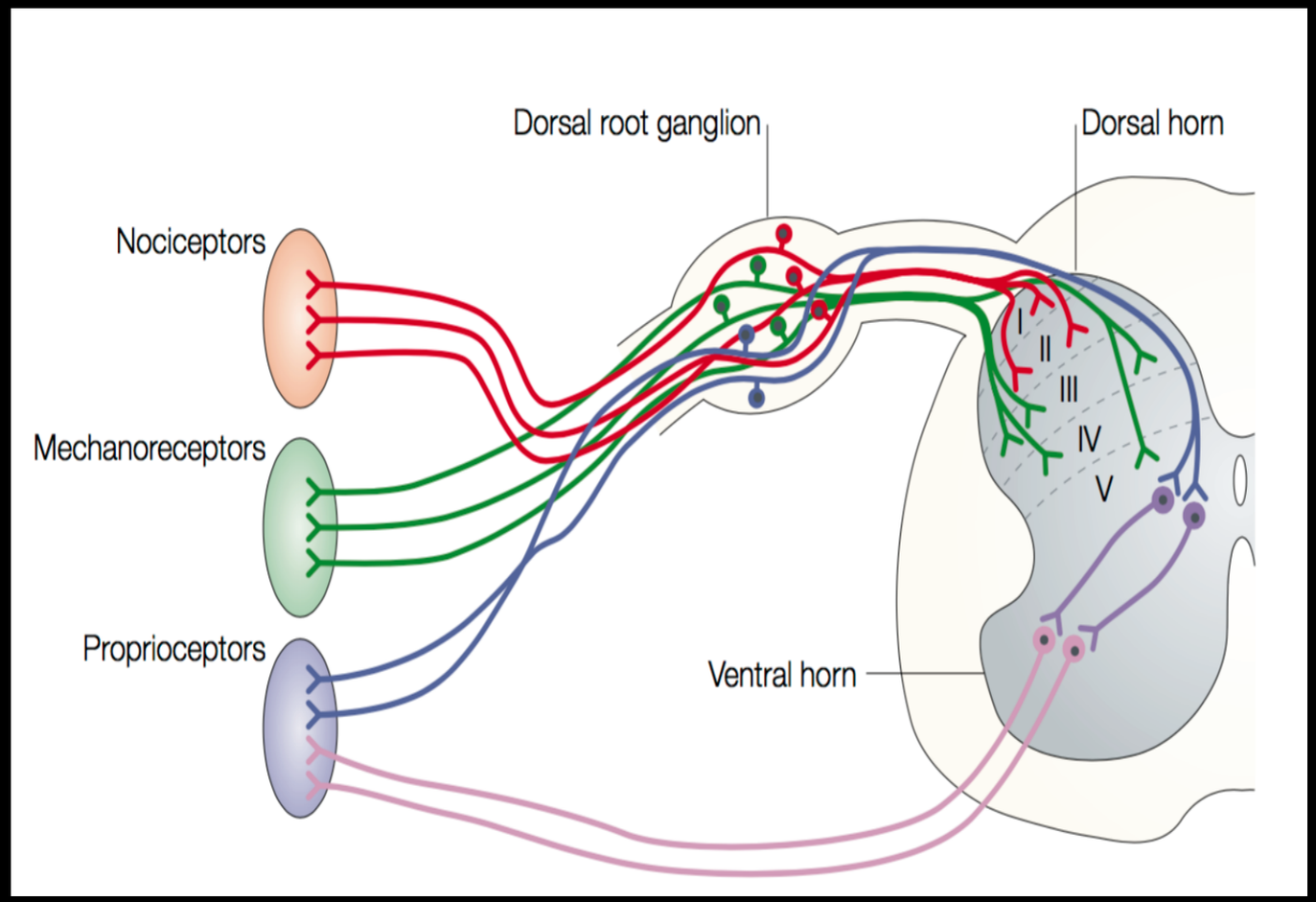
MBI
MECHANOBIOLOGY INSTITUTE
National University of Singapore

NUS National University of Singapore
Department of Biological Sciences
Faculty of Science

A*STAR
Institute of Molecular and Cell Biology

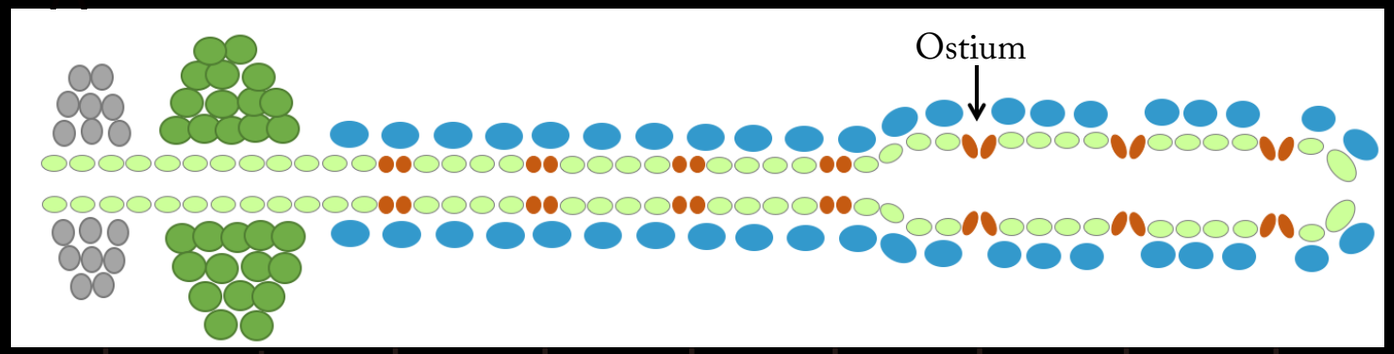
Cell matching is essential in development

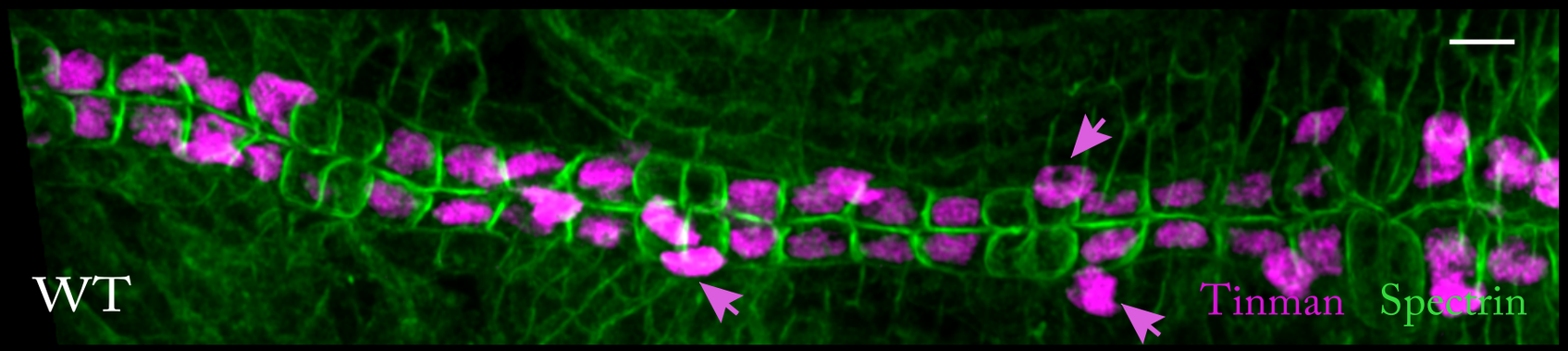
Caspary & Anderson, Nature Reviews Neuroscience 2003

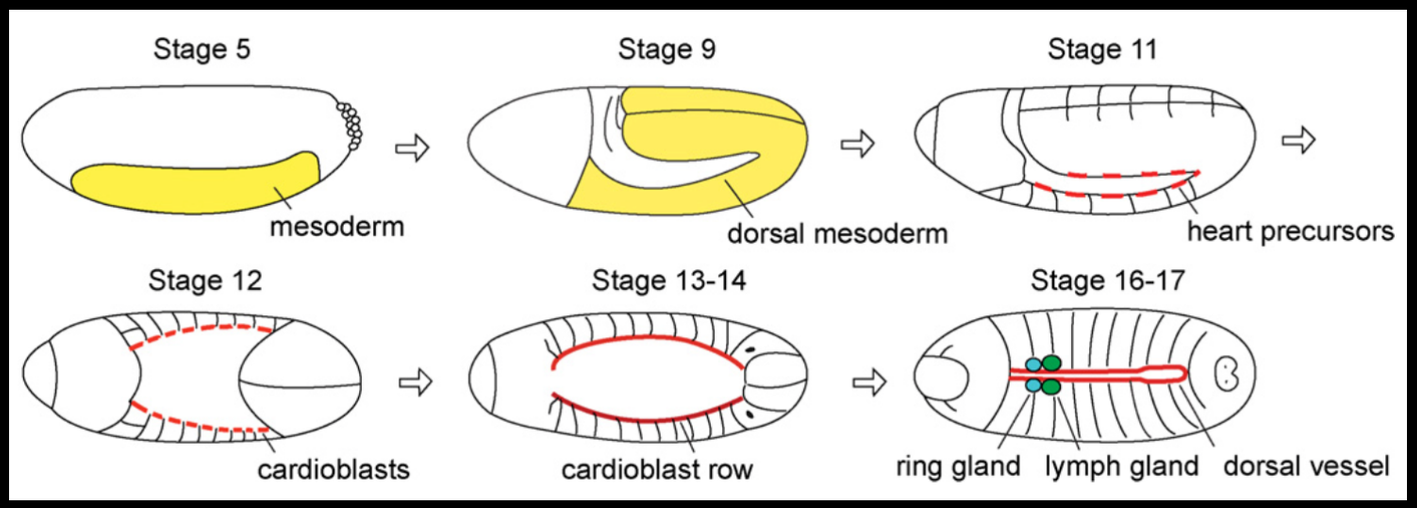


https://www.youtube.com/watch?v=A9zLKmt2nHo

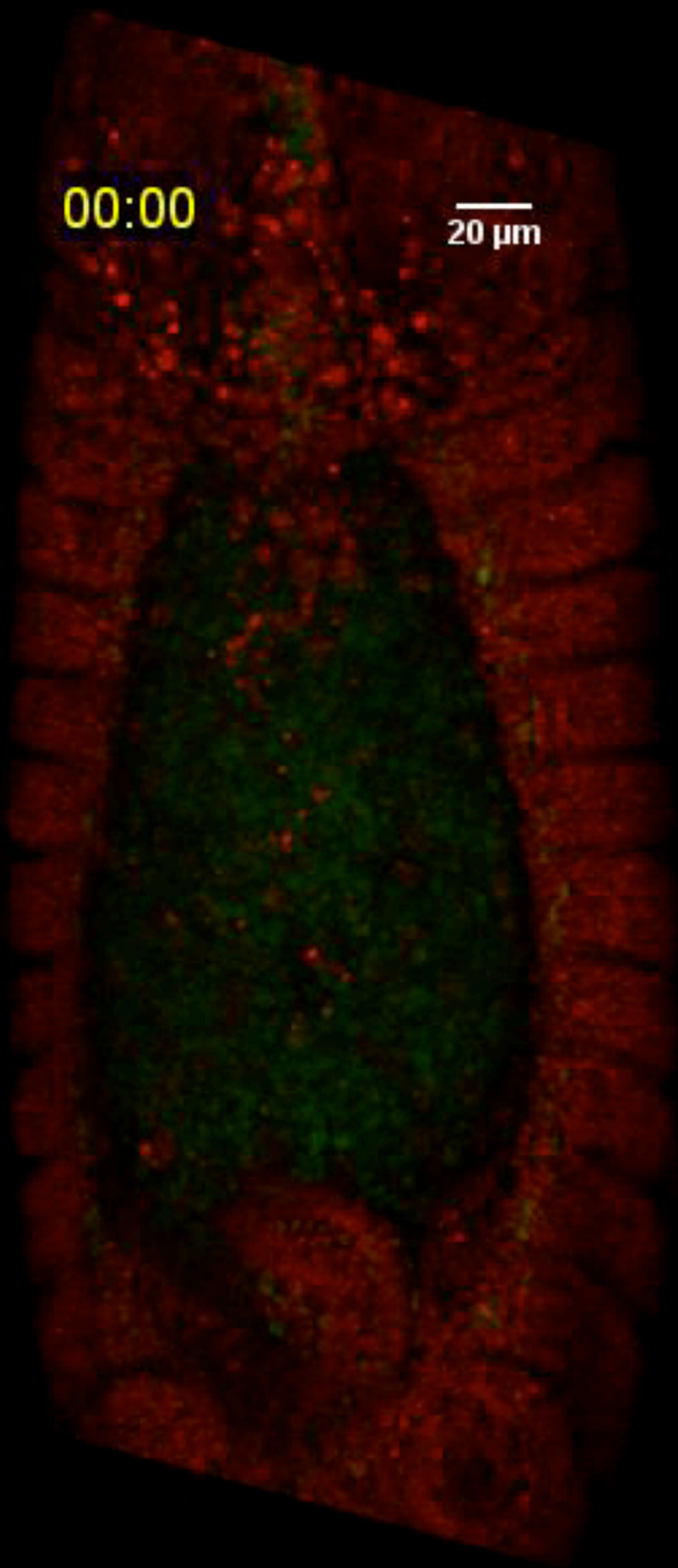
Drosophila cardiogenesis as simple matching system





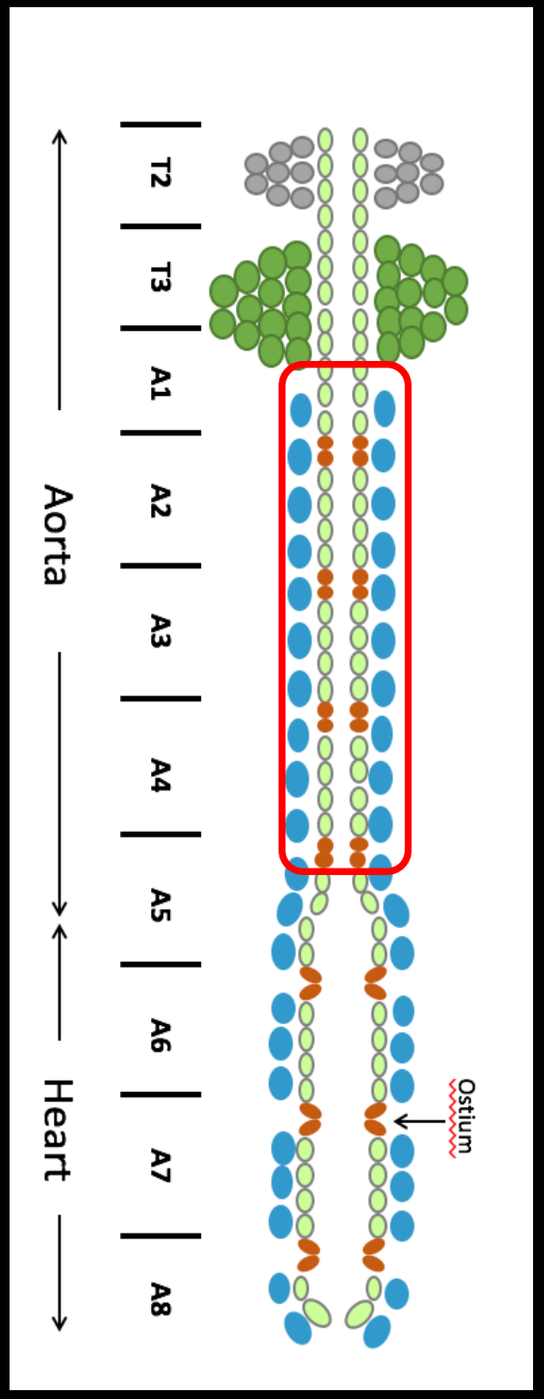


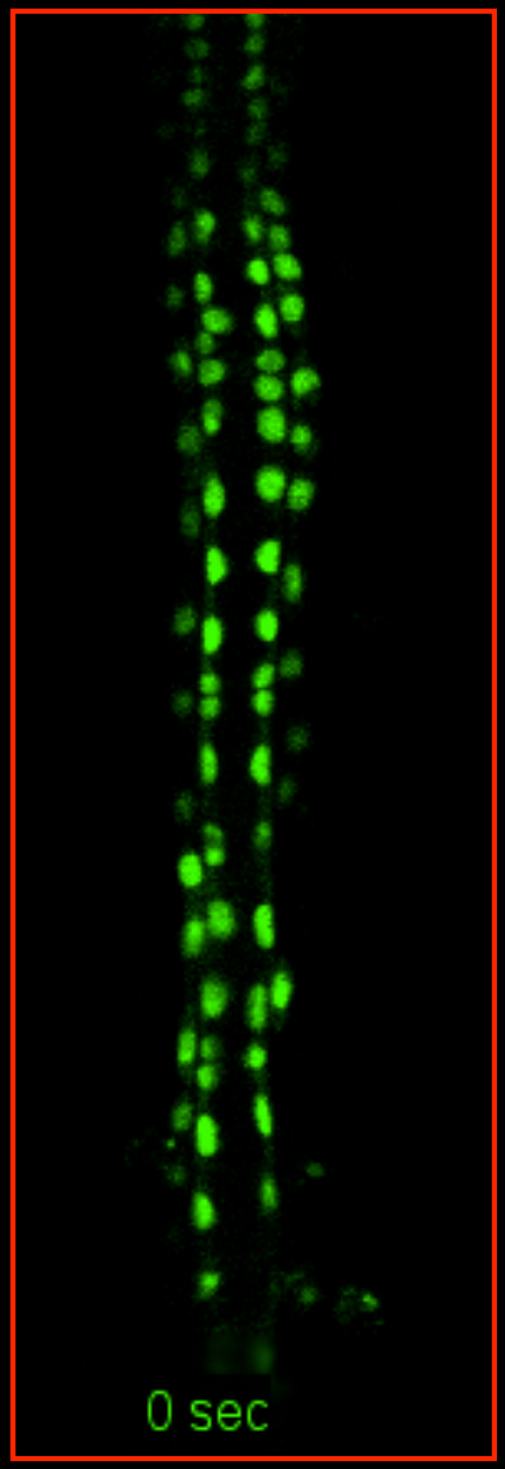
Tao & Schulz, Sem. Cell Dev. Biol. 2007

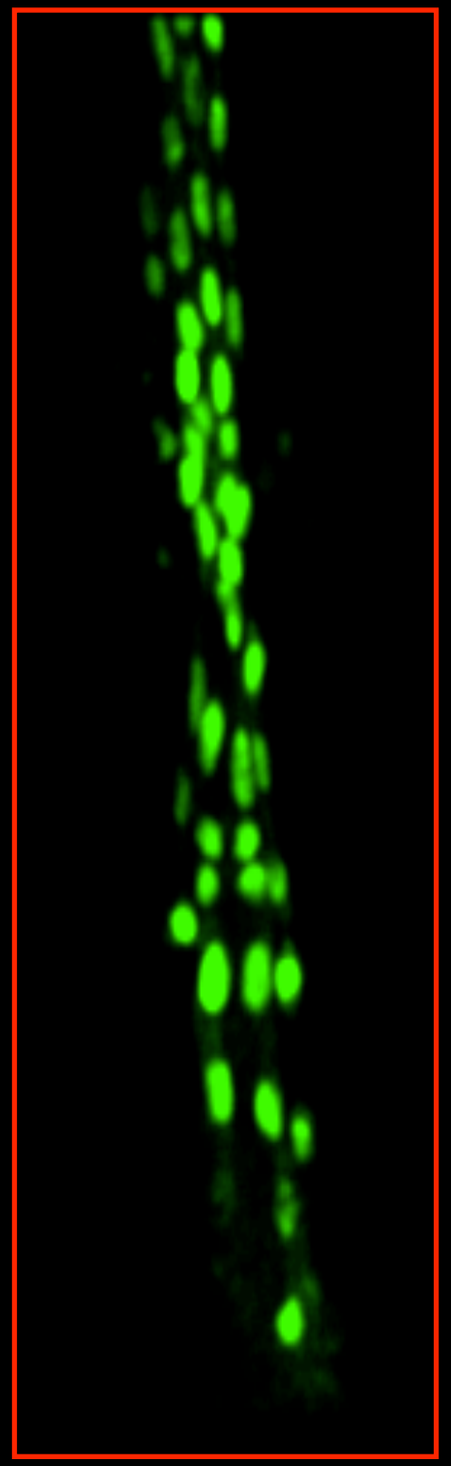


Hand>GFP, H2b-mCherry

Mismatched hearts have beating defects



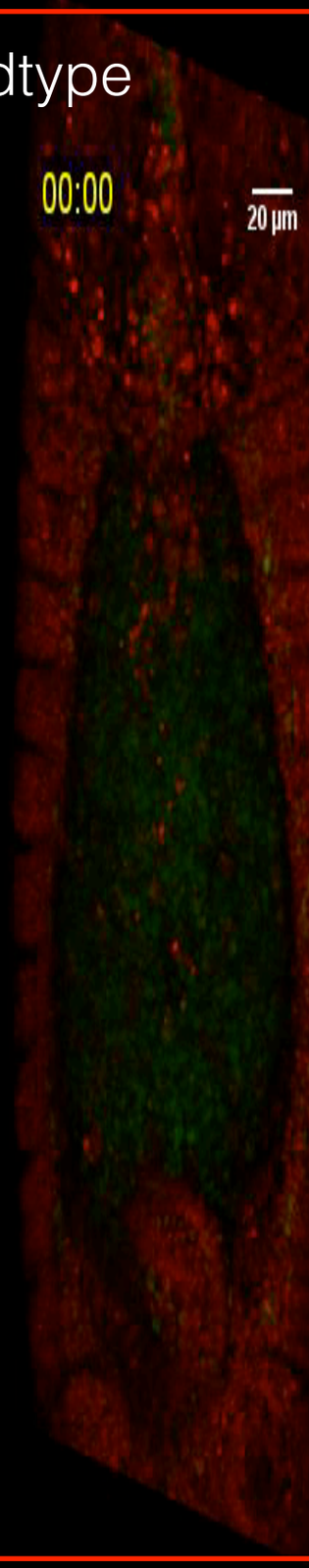




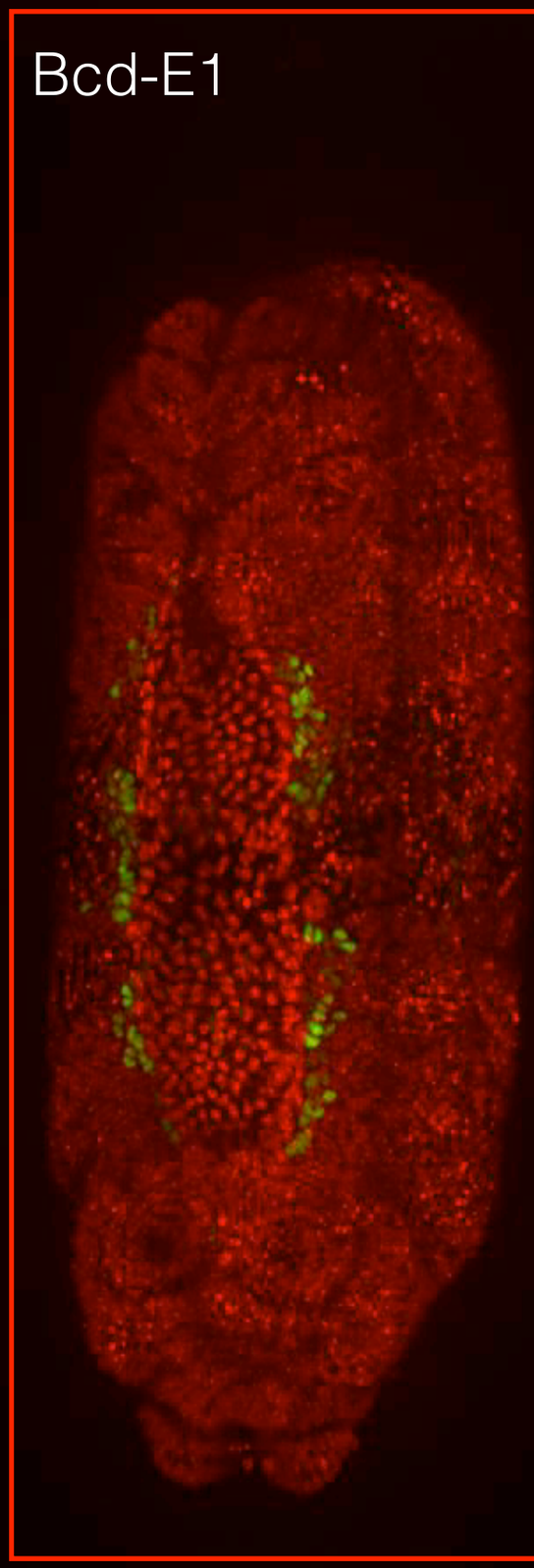
Hand-GFP

Cell matching is an active process

Wildtype

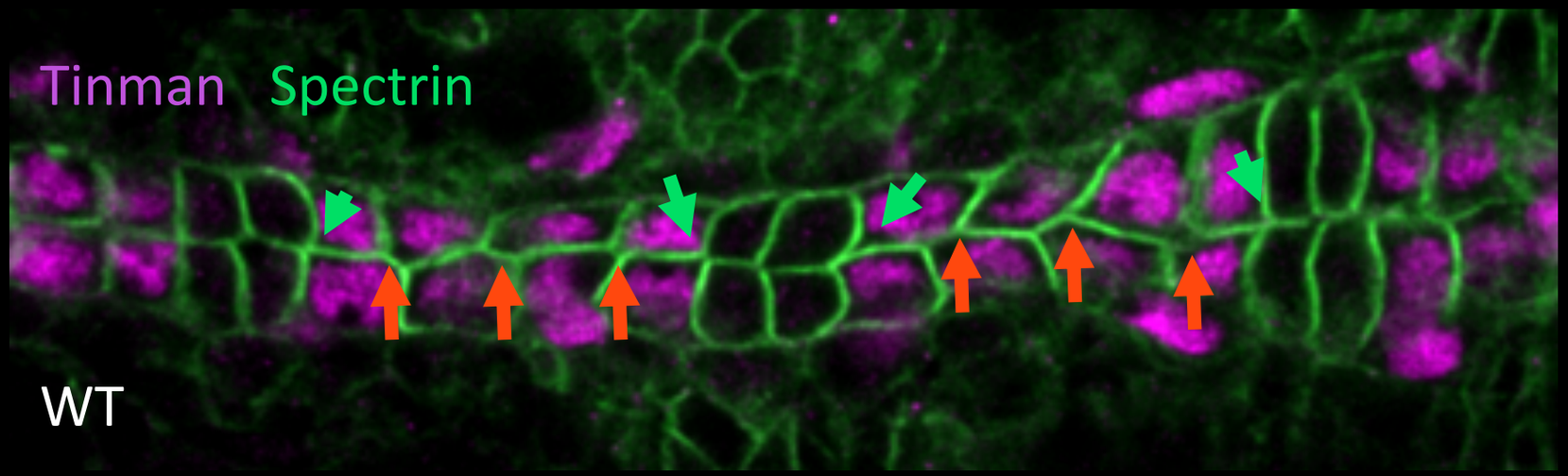


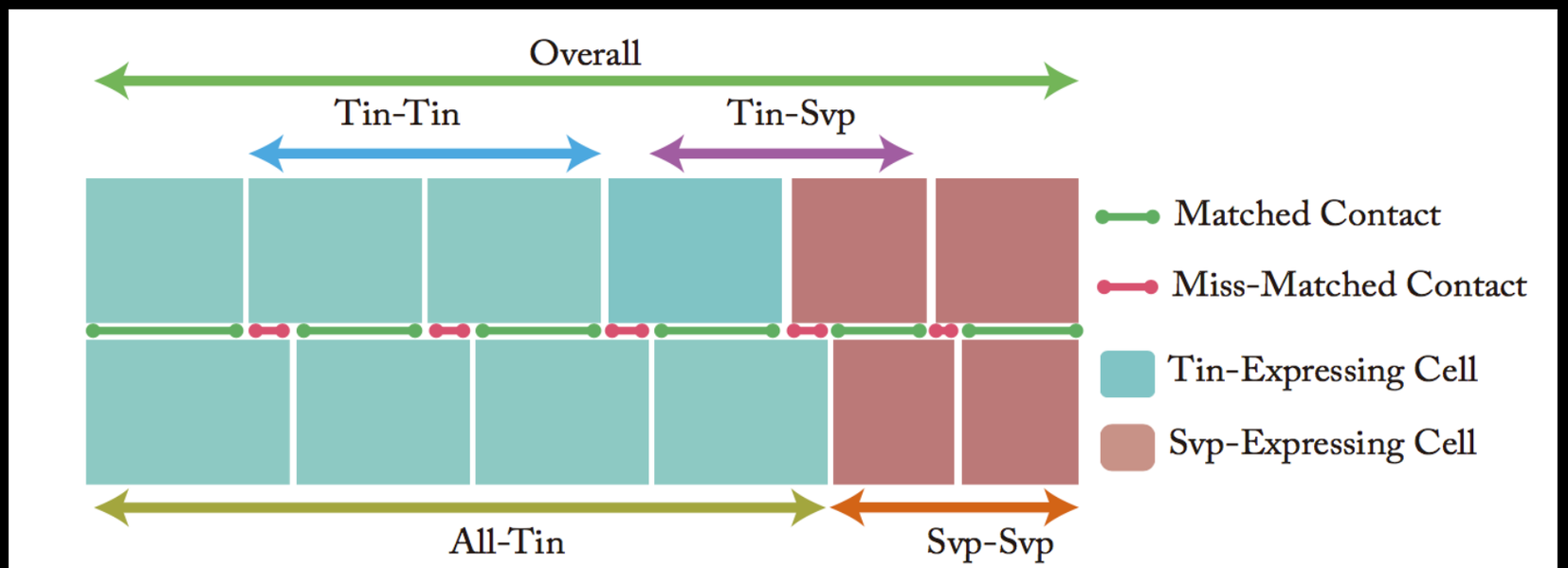
Bcd-E1



Hand-GFP
Histone-mCherry

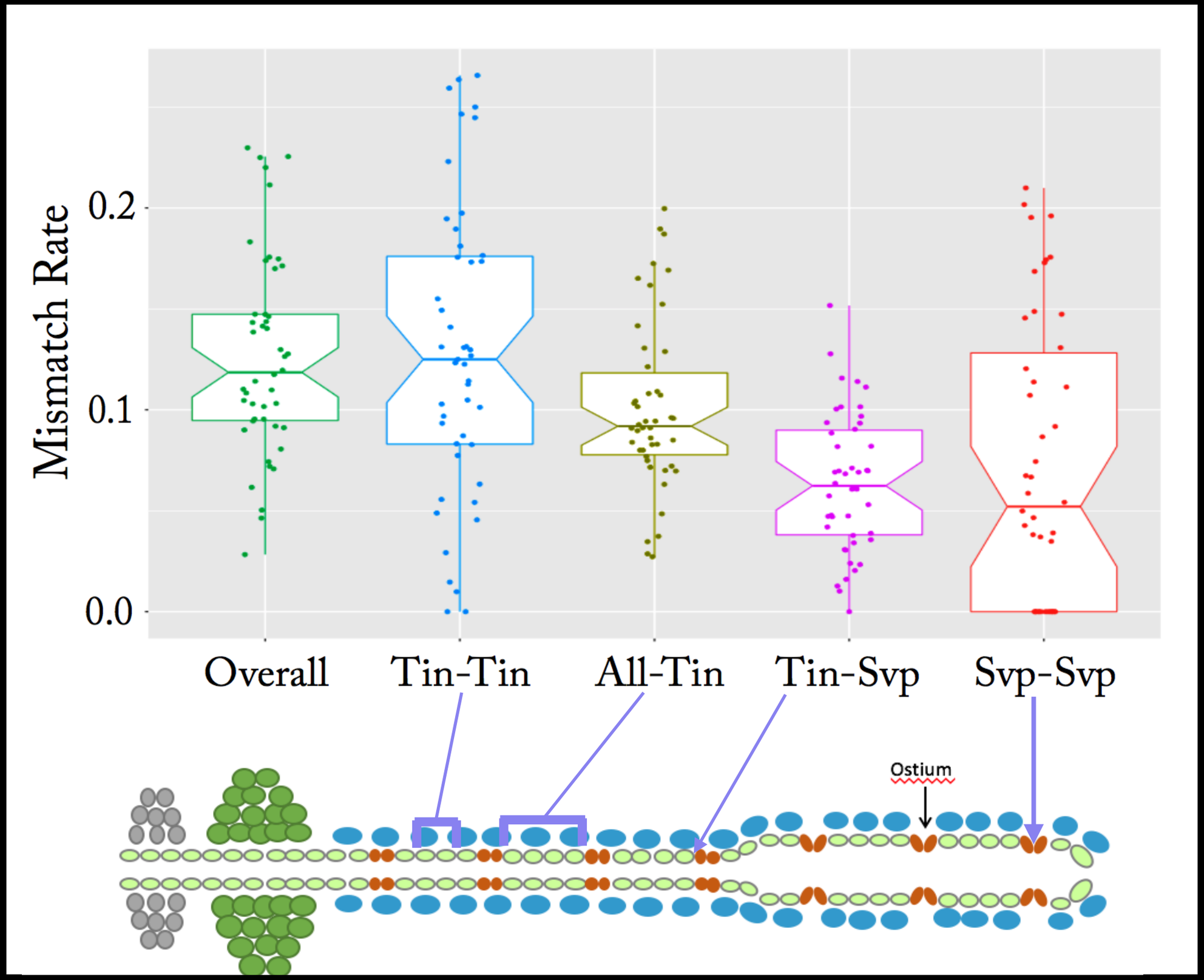
Quantifying cell matching



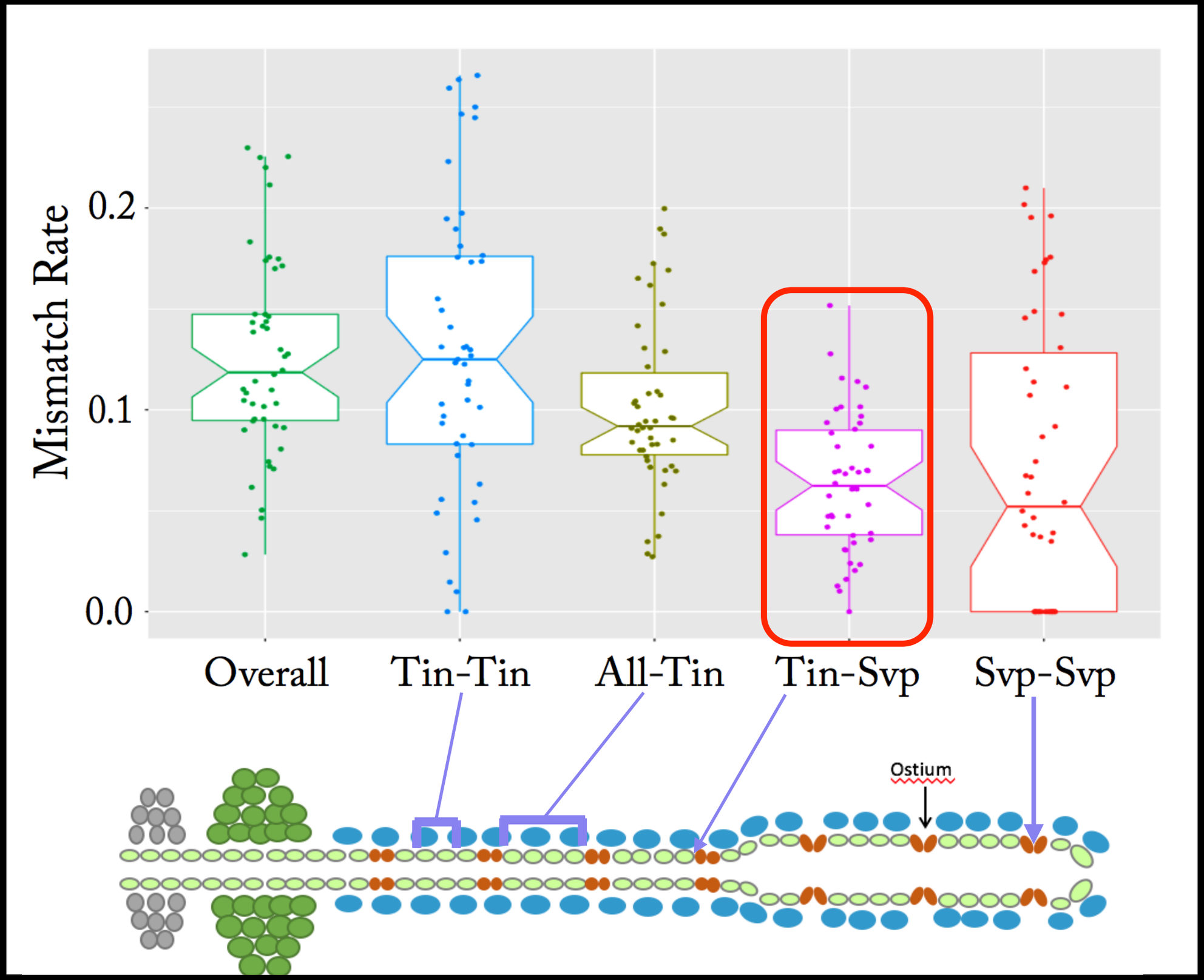


$$\frac{\text{Mis-Matched Contact Length}}{\text{Total Contact Length}} = \text{Mismatch}$$

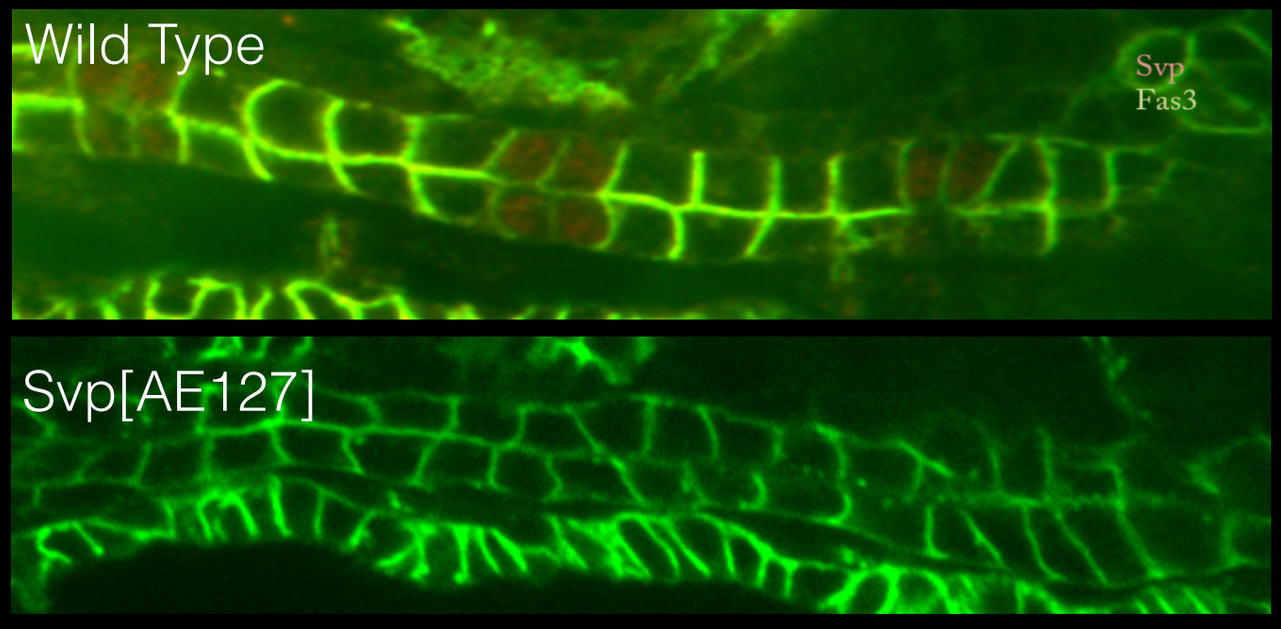
Quantifying cell matching

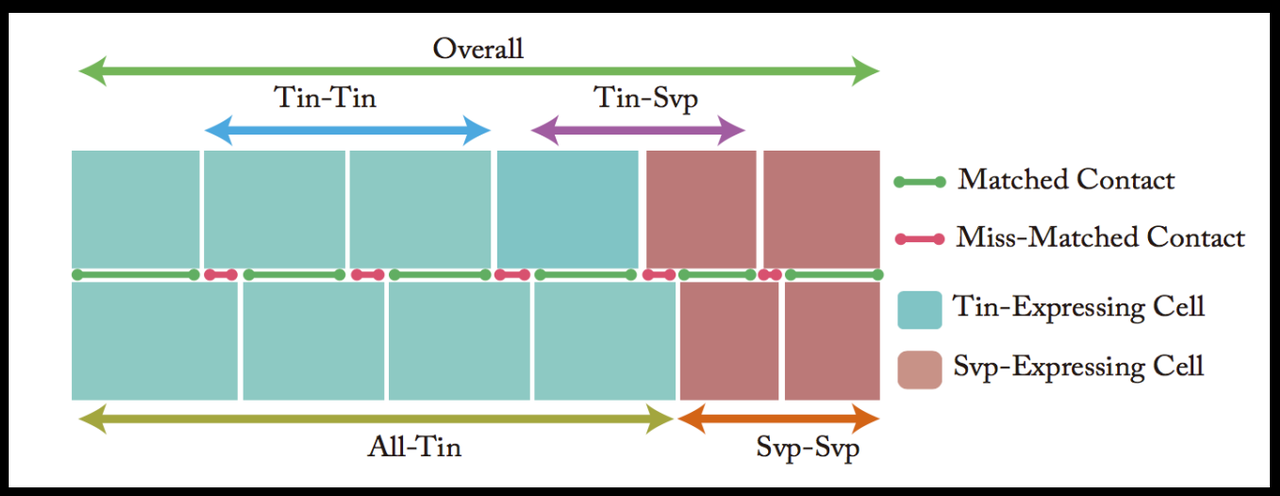


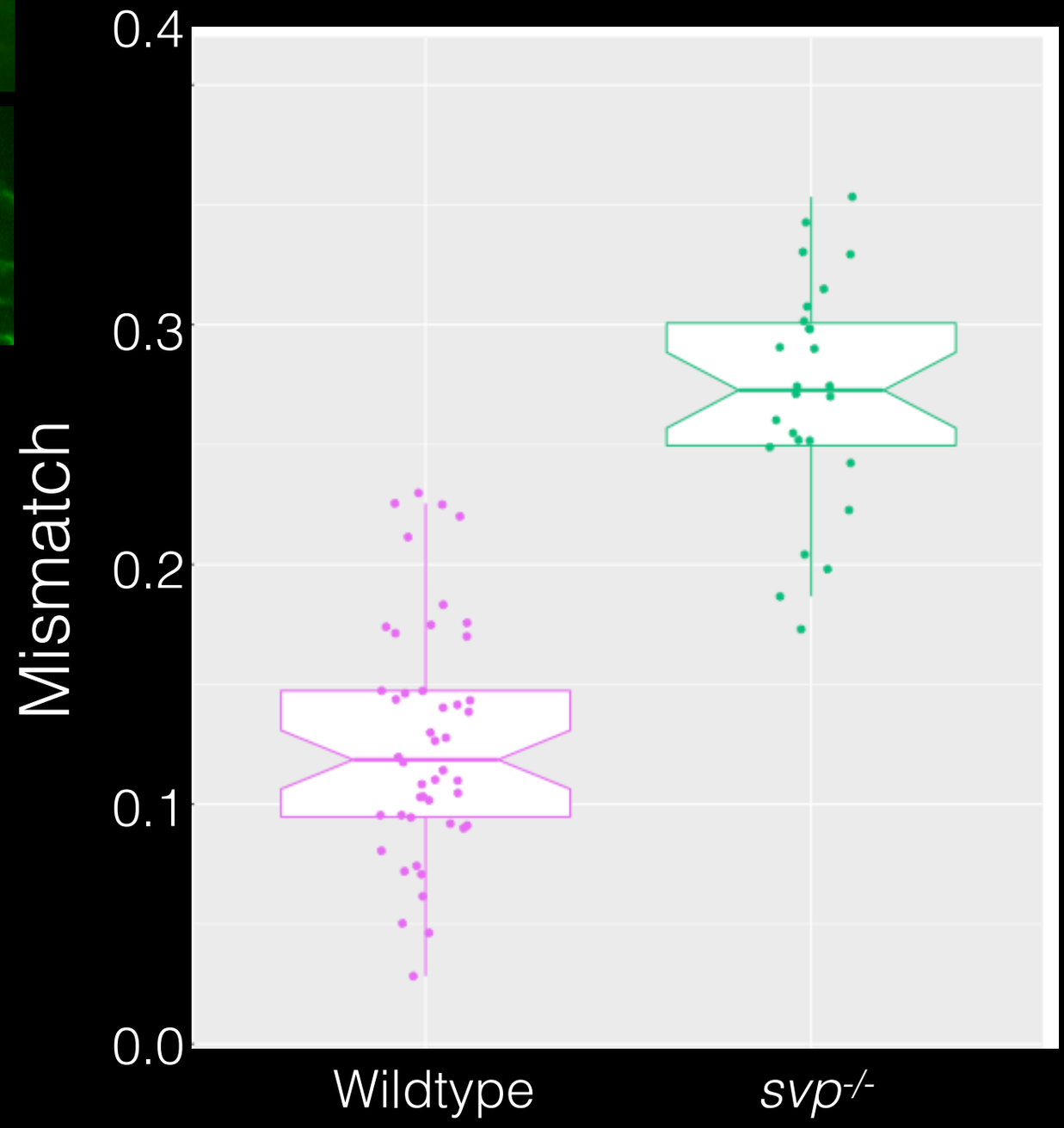
Cell matching robust at boundary of different cell types



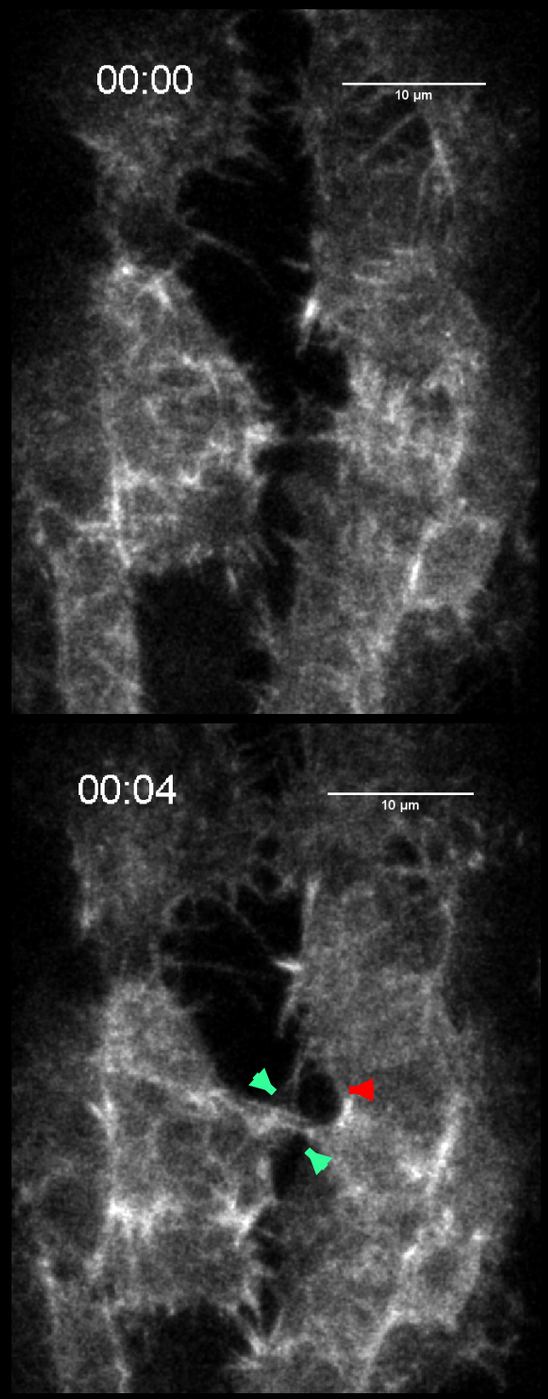
Cell matching imprecise without different cell types

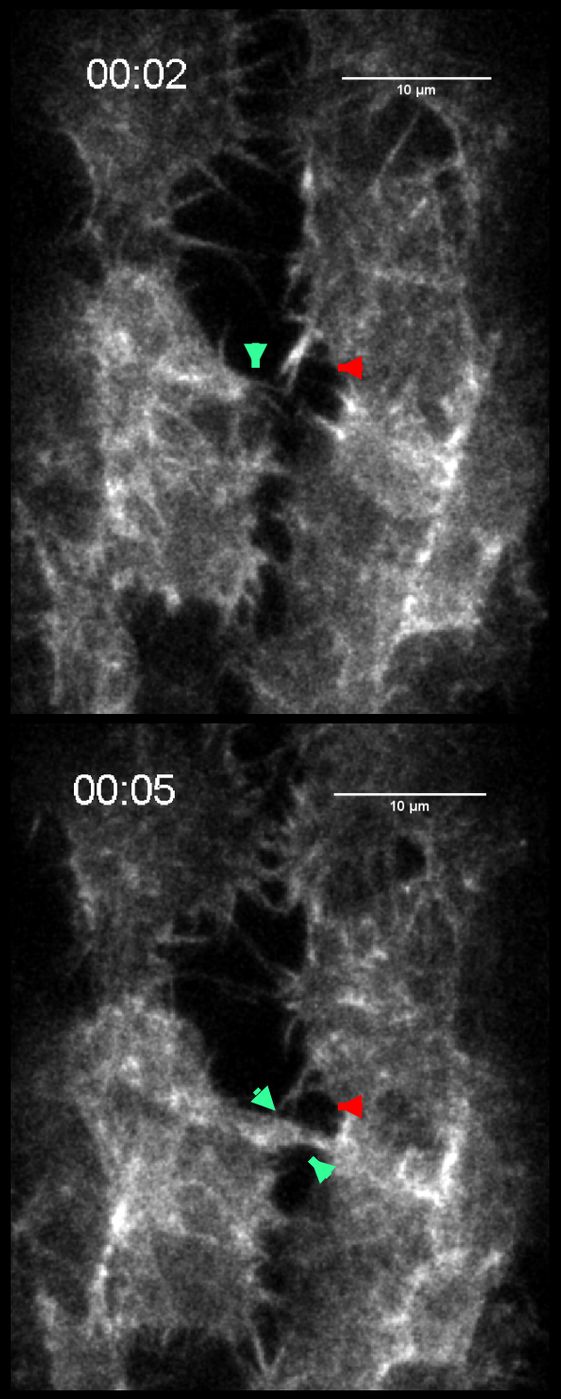


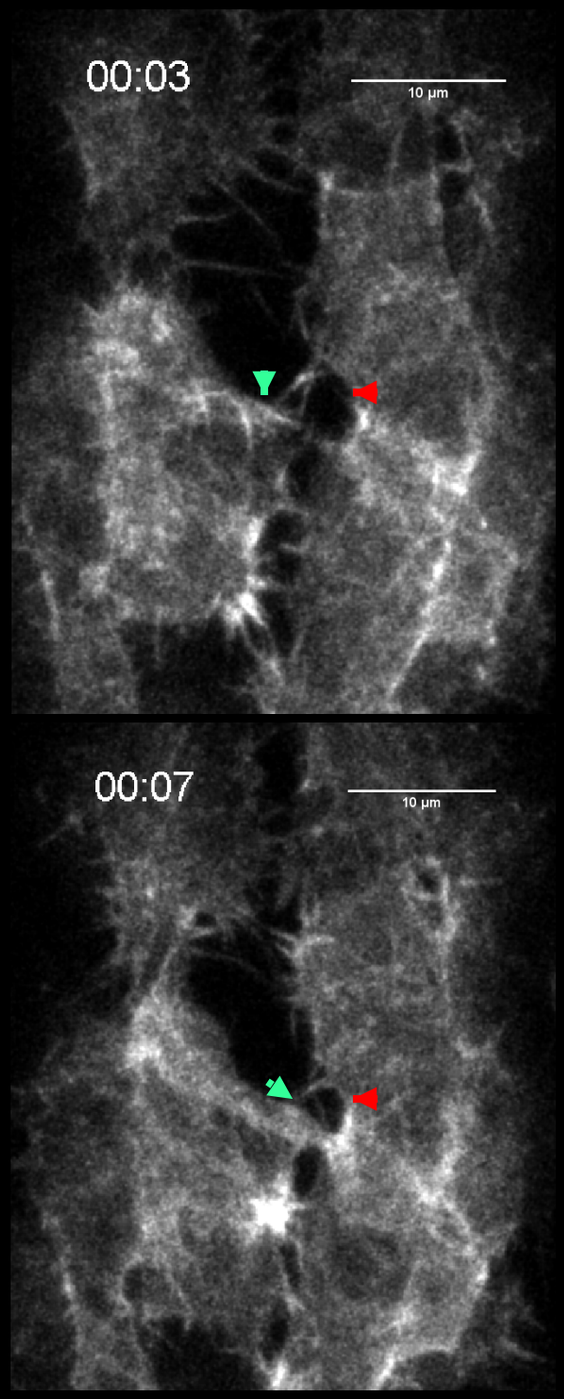




Filopodia are selectively binding to distinct cell types



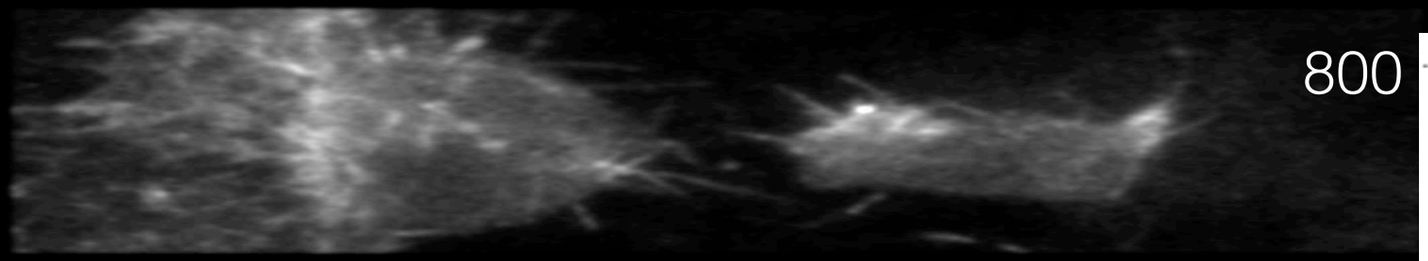


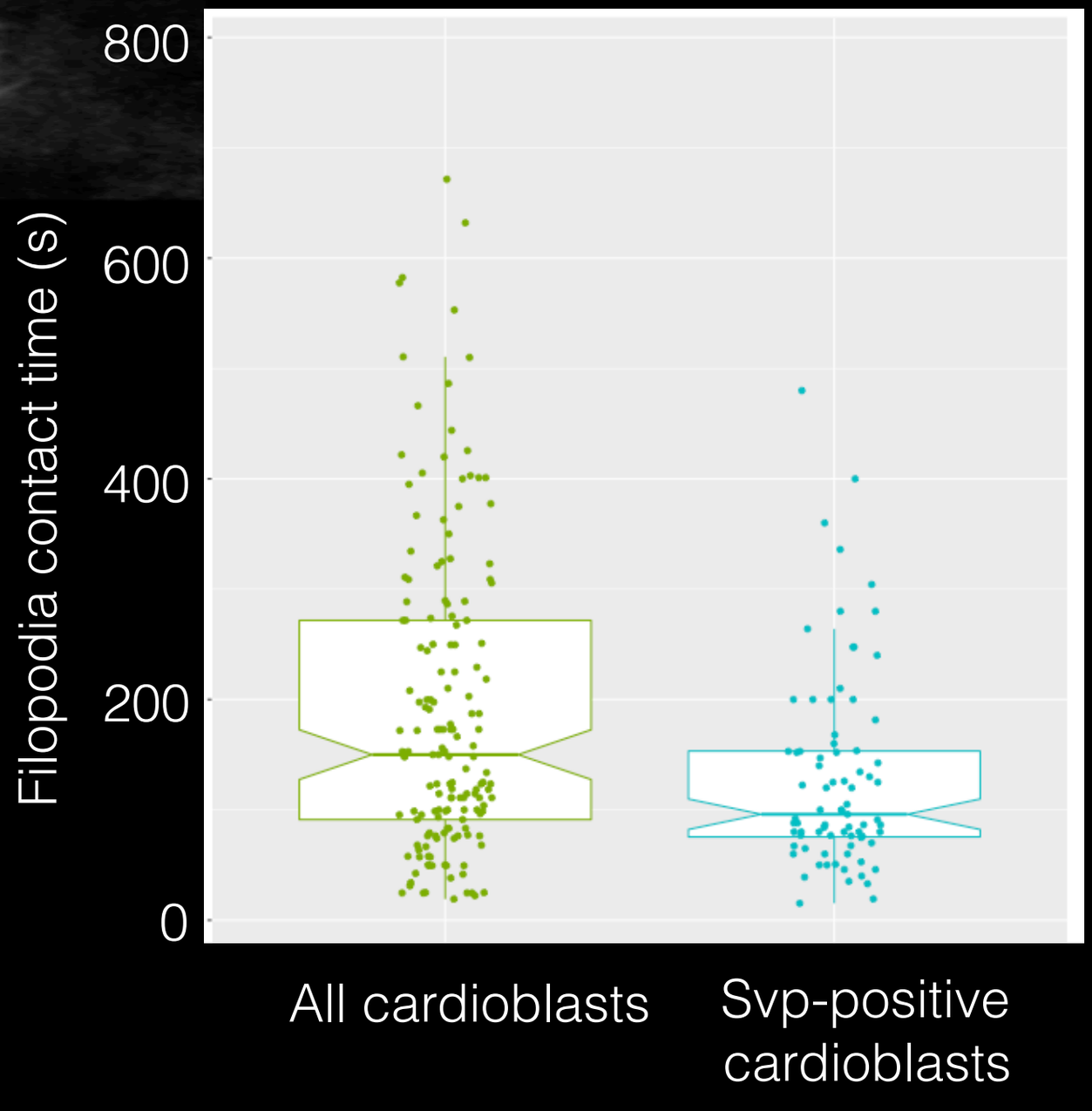


Hand>moesin-GFP

Svp heart cells have weaker filopodia binding

Svp>moesin-GFP

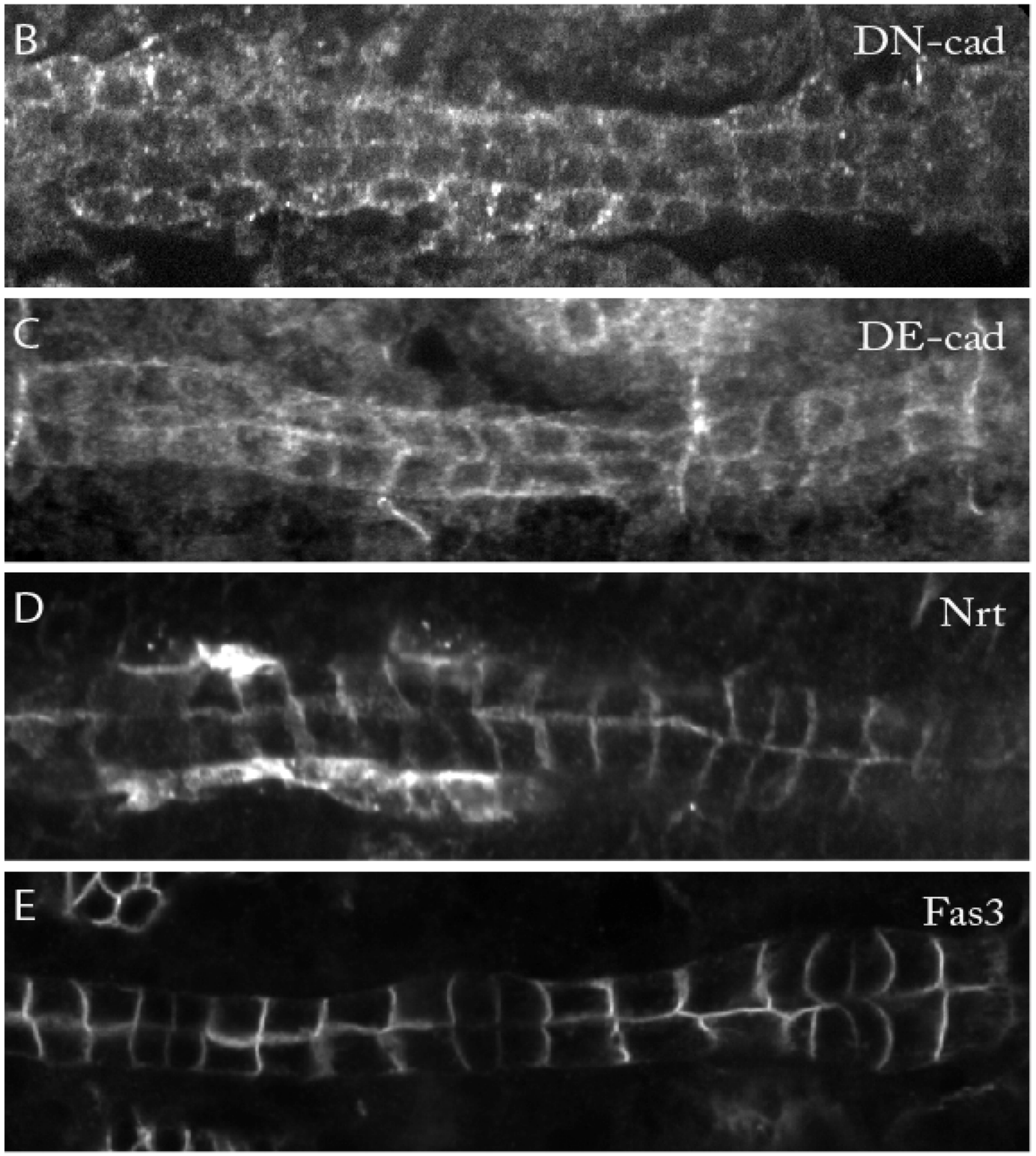




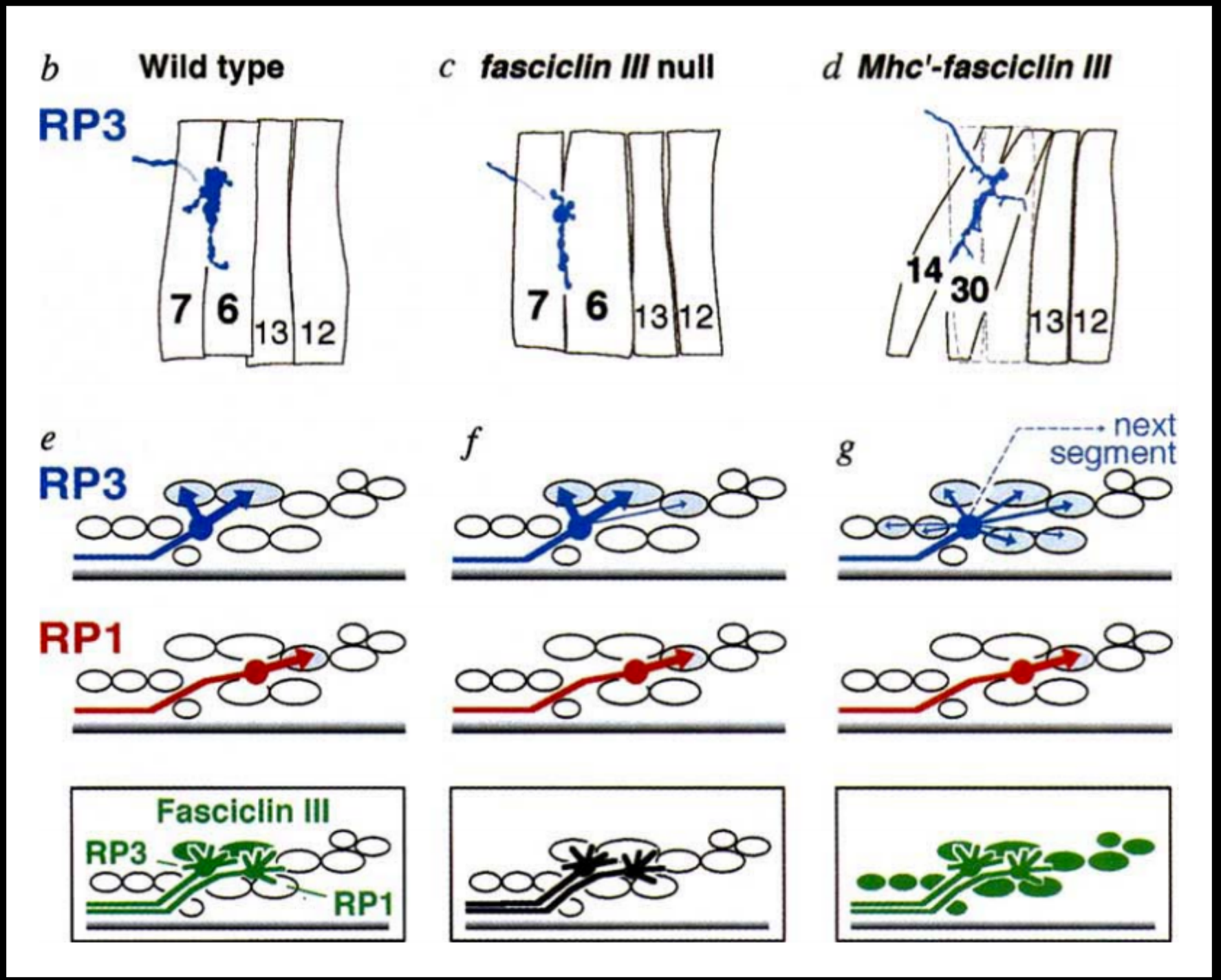
So what is the underlying mechanism?

Screen focusing on known neural cell matching molecules

Gene	Protein
shg	DE-cadherin
CadN	DN-cadherin
Nrt	Neurotactin
Nrg	Neuroglian
Con	Connectin
Fas1	Fascilin I
Fas2	Fascilin II
Fas3	Fascilin III
Ten-m	Tenascin major
Ten-a	Tenascin accessory
Dscam 1	Down syndrome cell adhesion molecule 1
Pvf3	PDGF- and VEGF-related factor 3



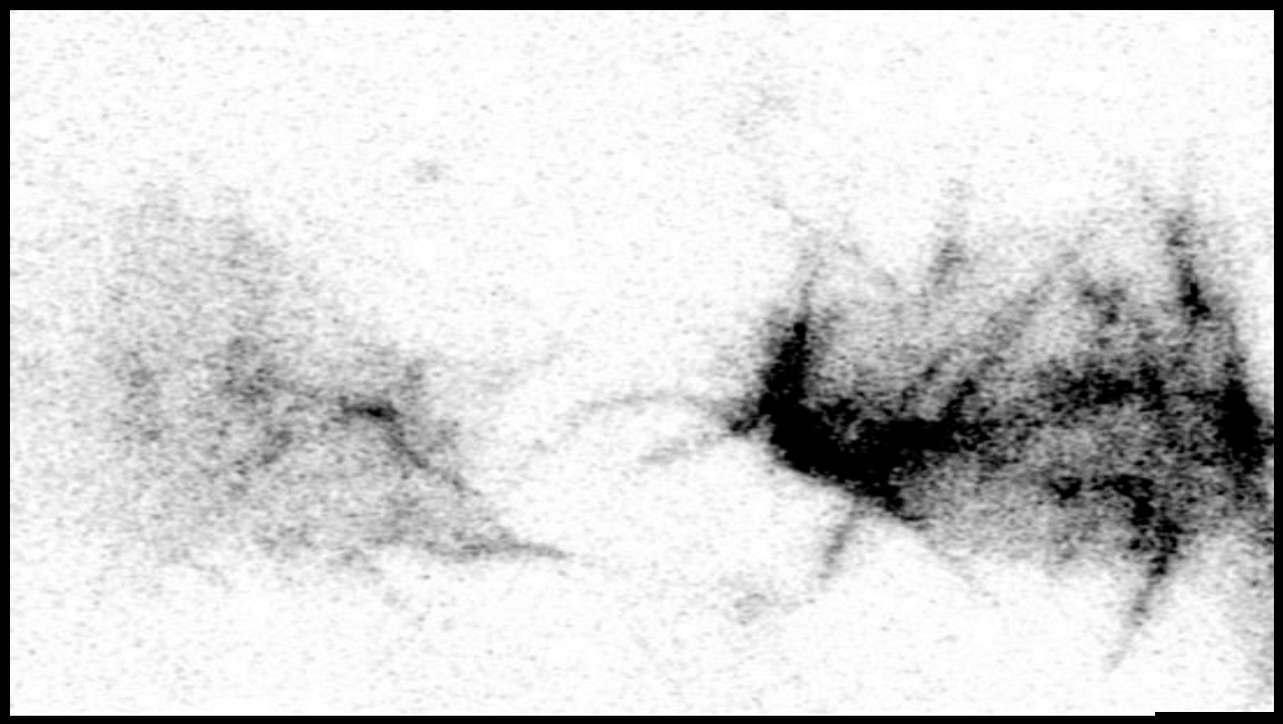
Fas3 is involved in neuronal cell matching



No observed phenotype in null mutant

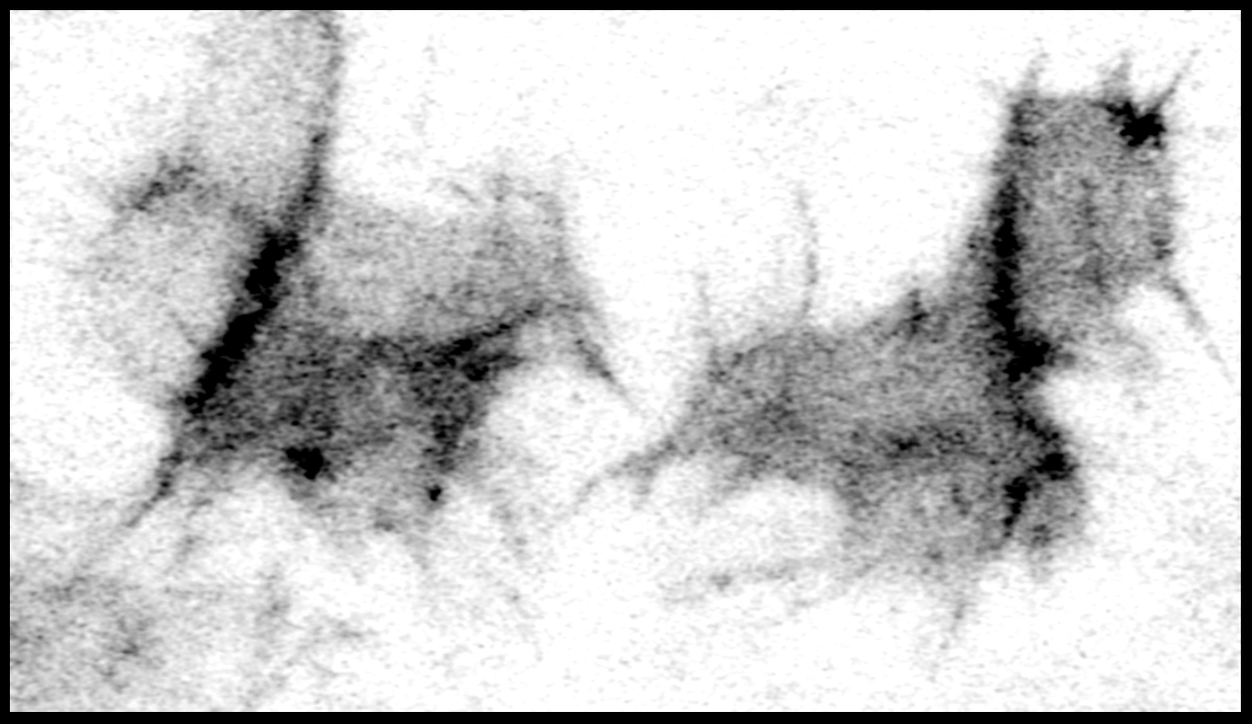
Chiba *et al.* Nature 1995

Altering Fas3 expression effects filopodia contact time



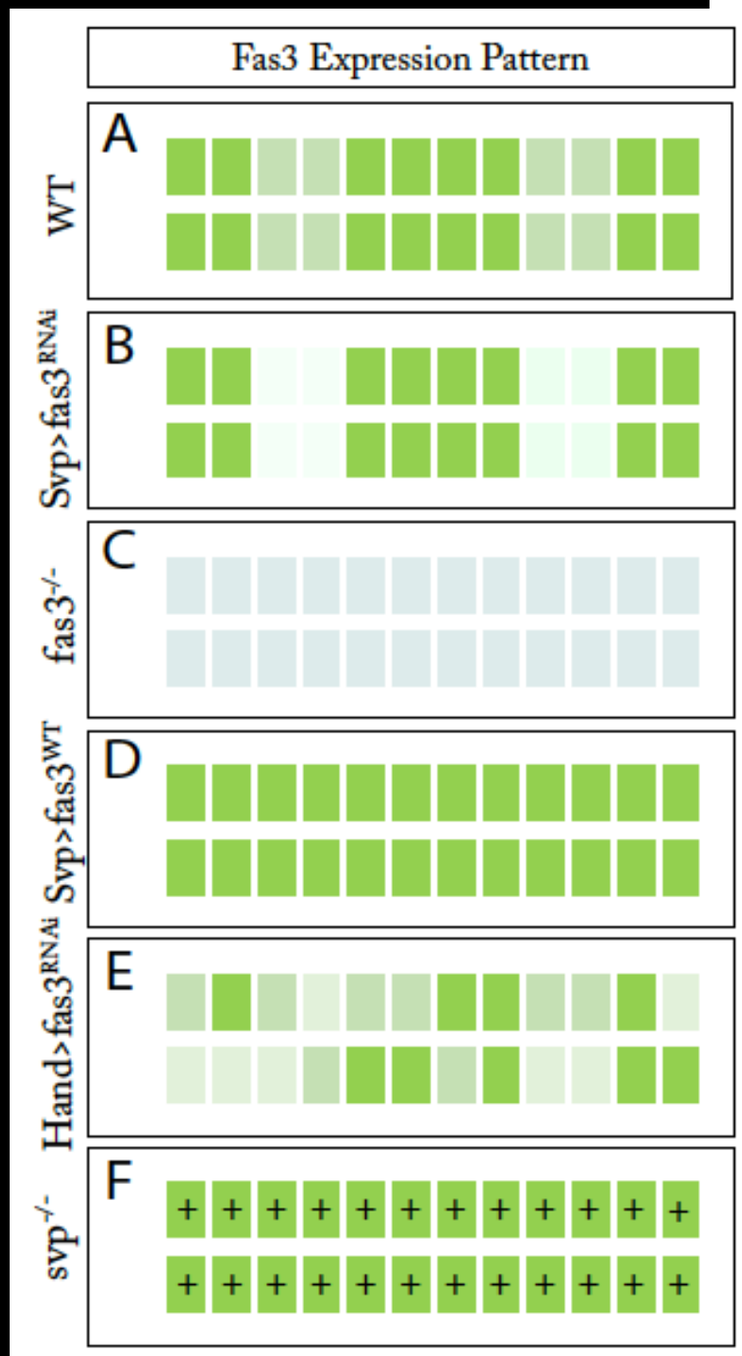
Svp>UAS-moesin-GFP

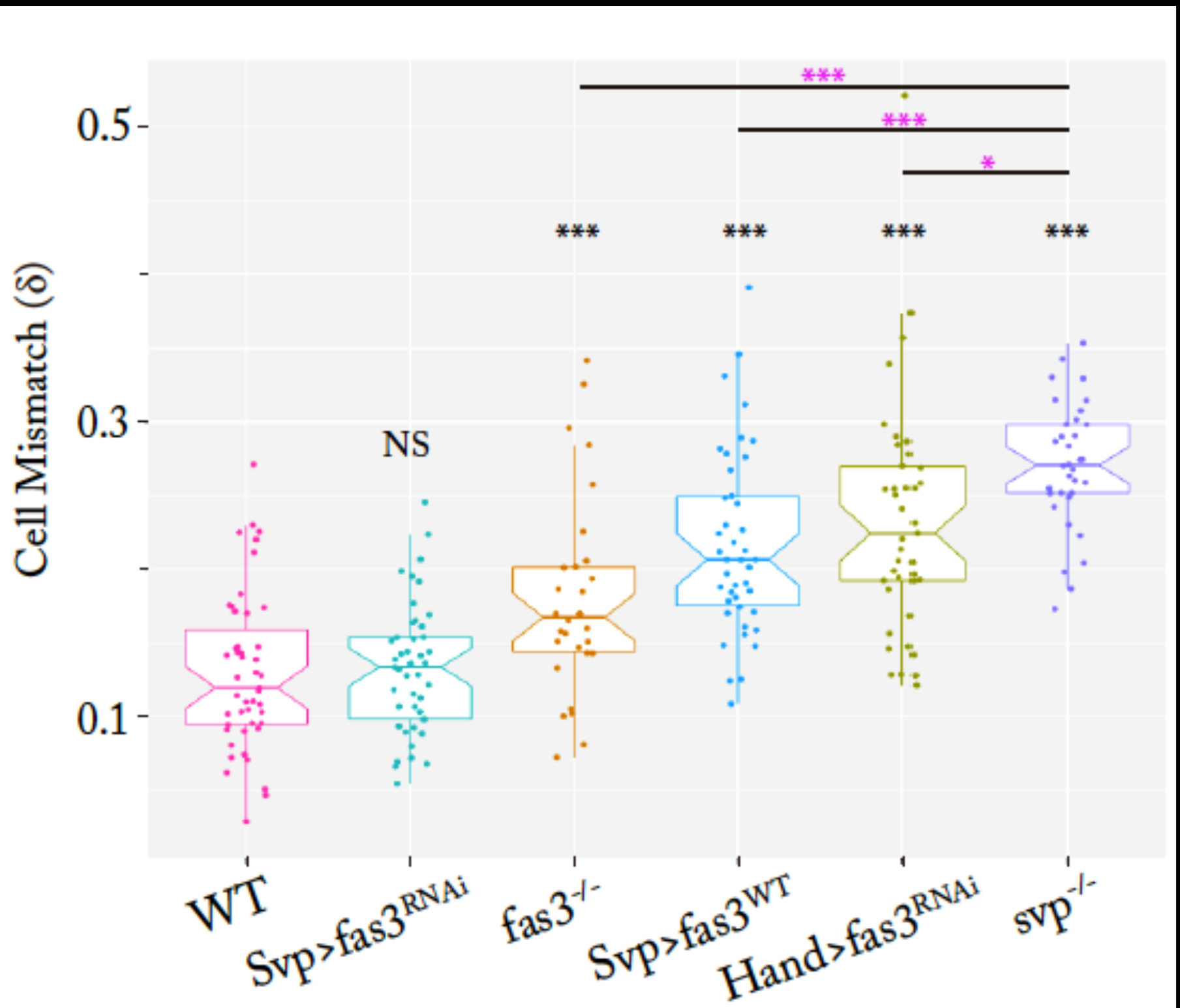
Fas3 up-regulation



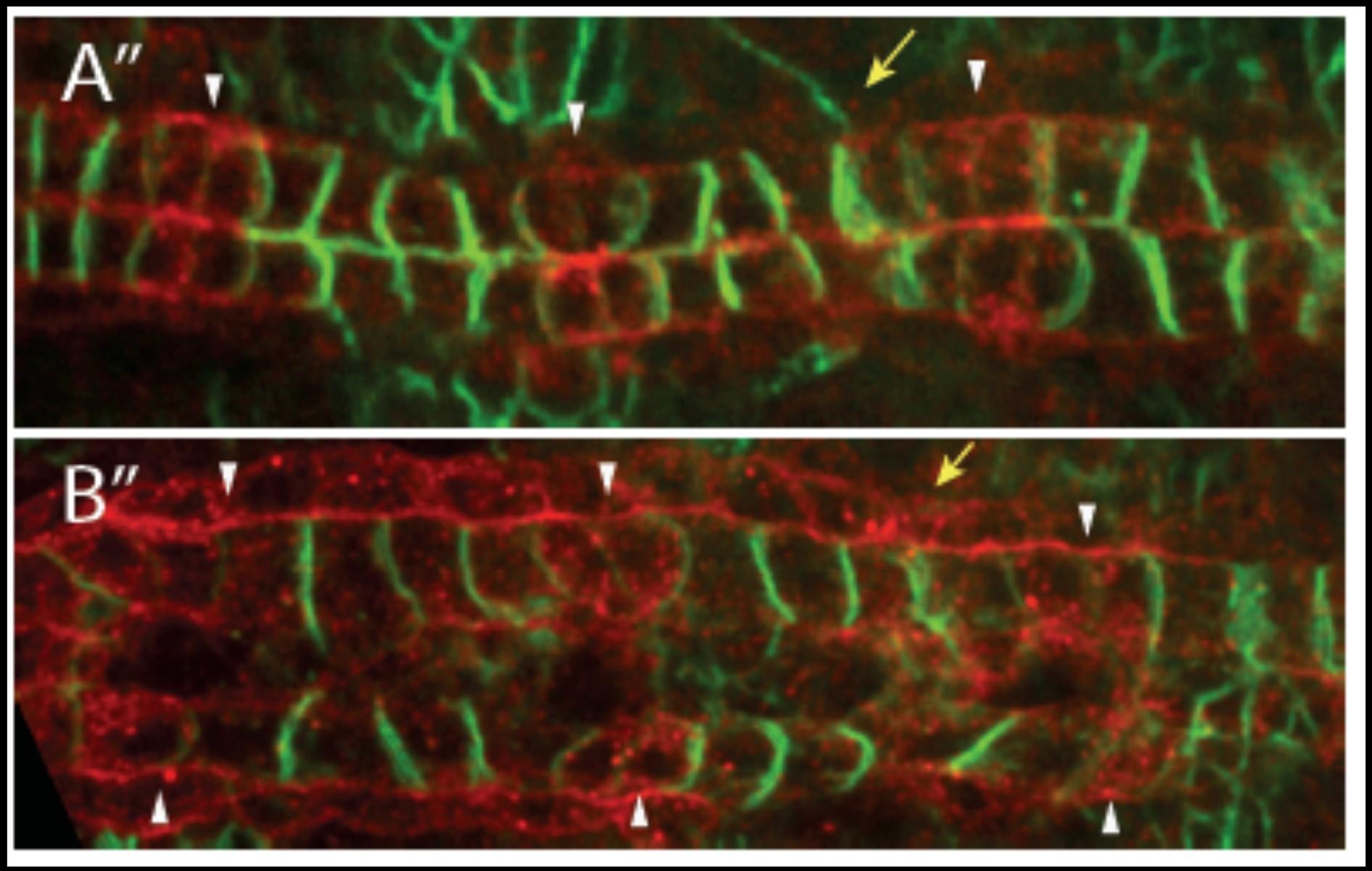
Svp> UAS-moesin-GFP
UAS-Fas3

How does the Fas3 expression pattern affect cell matching?





Ten-m is expressed in a complementary fashion to Fas3



Fas3

MiMIC-Ten-m-GFP

LETTER

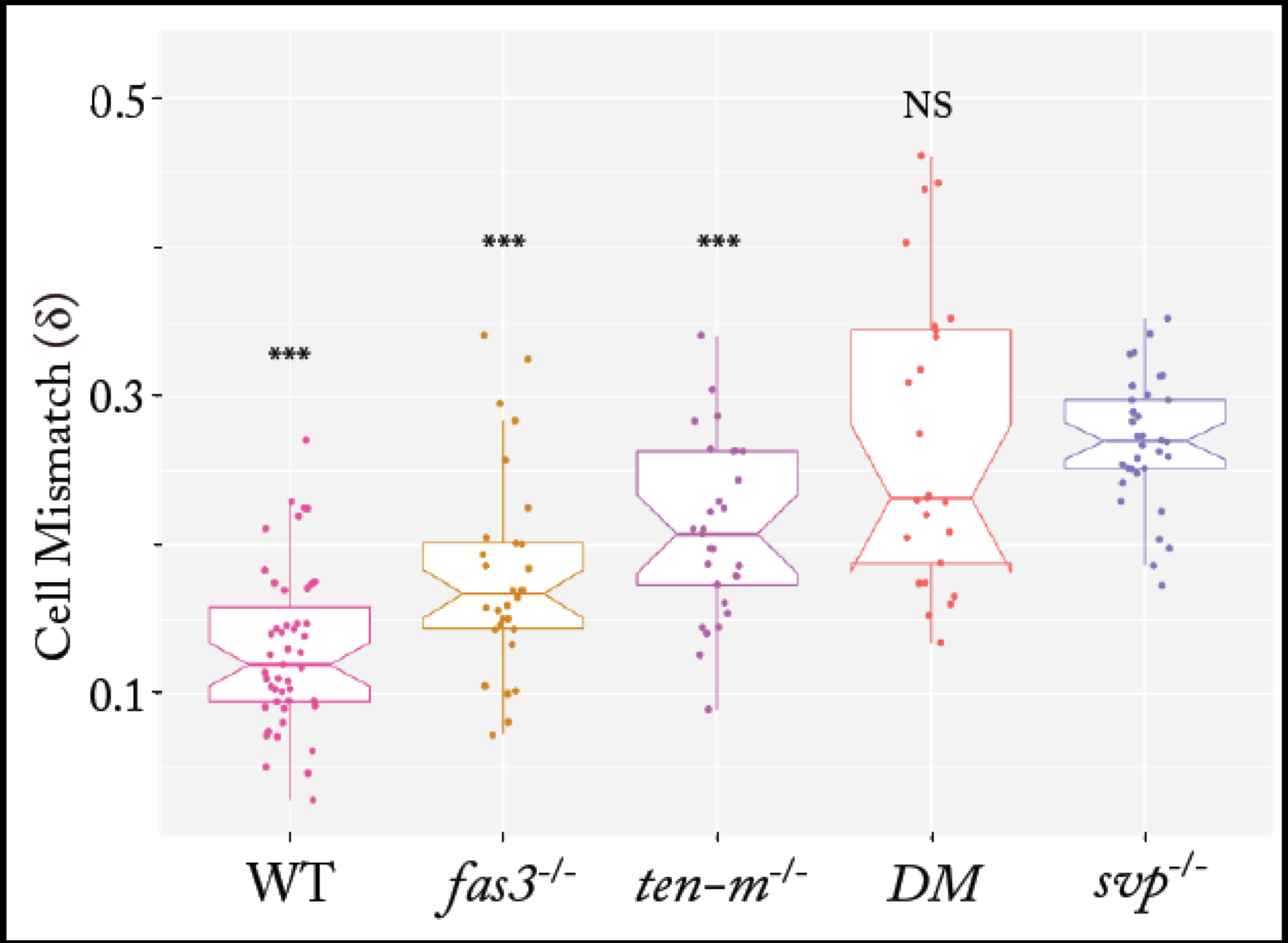
doi:10.1038/nature10923

Trans-synaptic Teneurin signalling in neuromuscular synapse organization and target choice

Timothy J. Mosca[1]*, Weizhe Hong[1]*, Vardhan S. Dani[1], Vincenzo Favaloro[1] & Liqun Luo[1]

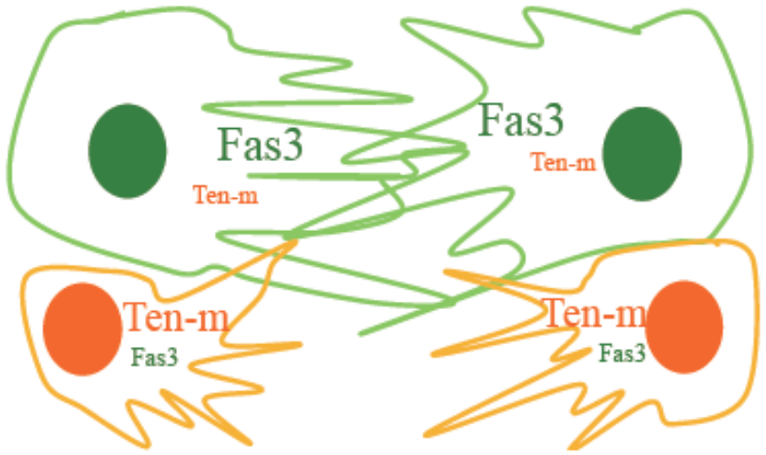
odd oz (*odz*)

Fas3 / Ten-m double mutant has severe matching defects

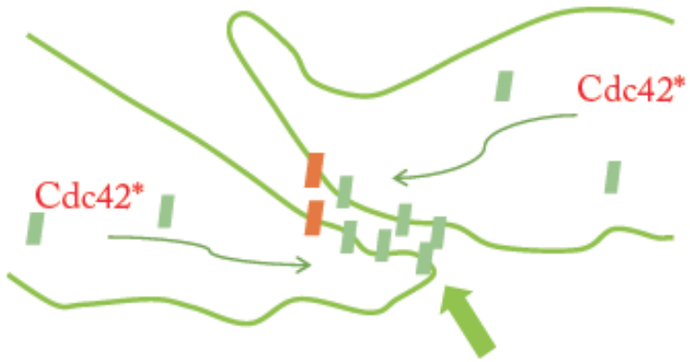


Wild-type Embryos

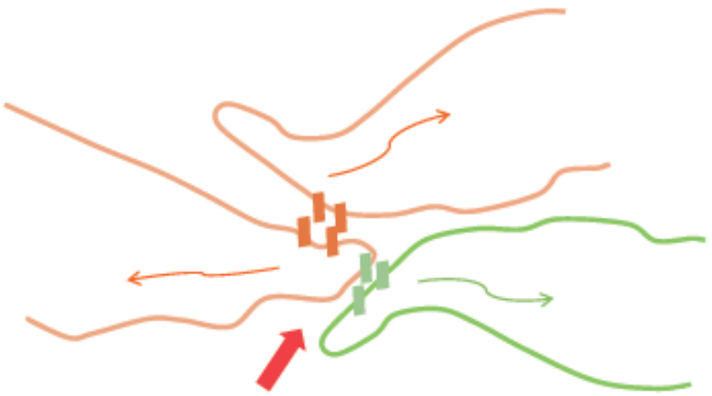
Well-matched Heart



Tin-positive CBs initiate contacts



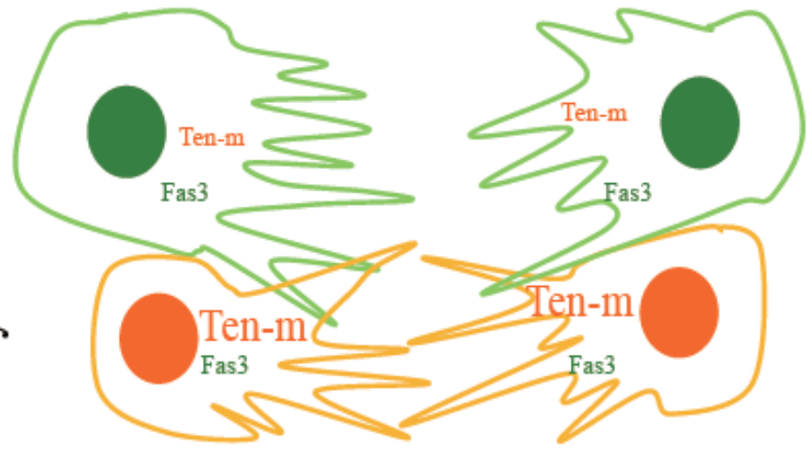
Strong Adhesion
Stable and Extened Filopodia Contacts
(mediated by strong Fas3 expression)



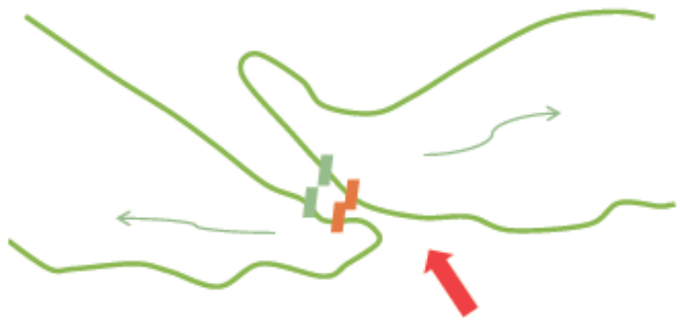
Weak Adhesion
Unstable and Seperated Filopodia Contacts
(potentially caused by binding competition)

Embryos with reduced Fas3 expression in the heart

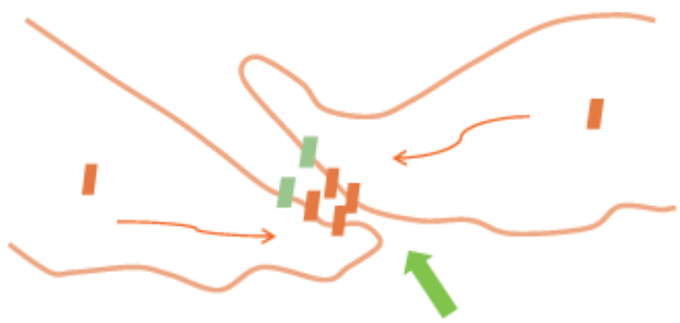
Partly-matched Heart



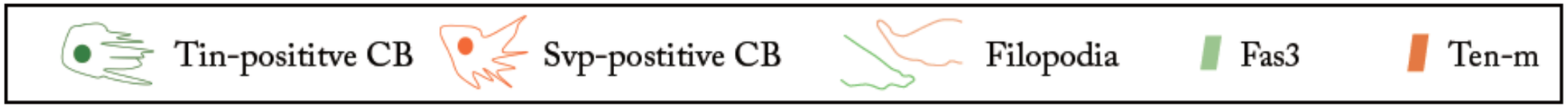
Svp-positive CBs initiate contacts



Weak Adhesion
Unstable and Seperated Filopodia Contacts
(caused by low expression of both Fas3 and Ten-m)



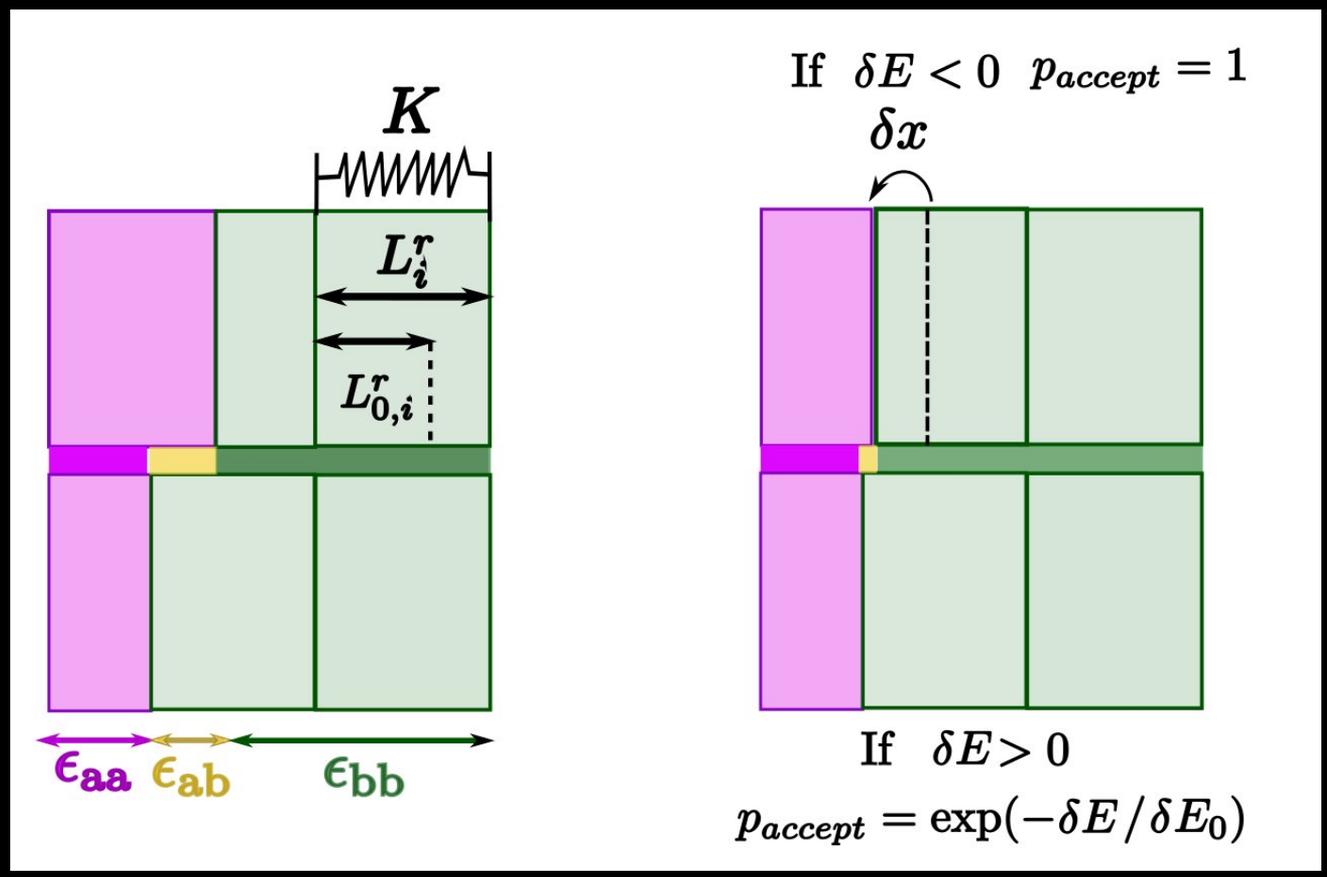
Relatively Stronger Adhesion
Stable and Extened Filopodia Contacts
(mediated by Ten-m when losing Fas3 competition)



Zhang *et al.* Developmental Cell (2018)

Is differential adhesion sufficient to ensure precise cell matching?

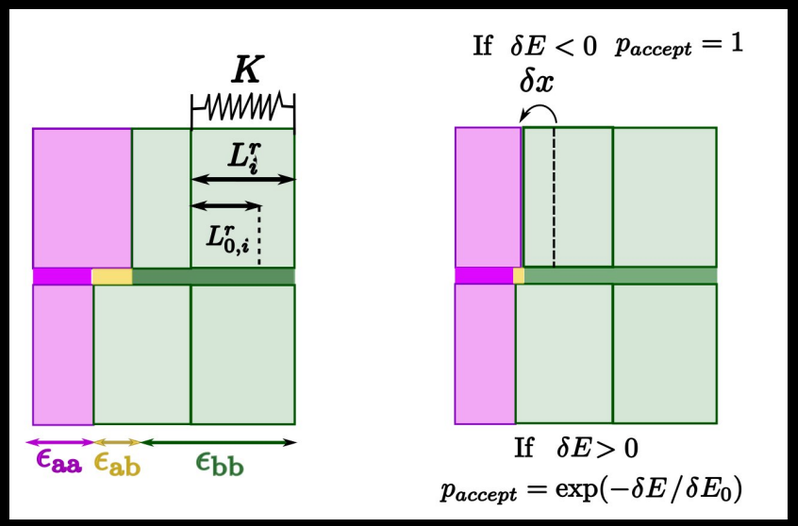
$$E_a = K\left(L_{cell} - L_{0,a}\right)^2 - \left(\epsilon_{aa}.x + \epsilon_{ab}.y\right)$$
$$E_b = K\left(L_{cell} - L_{0,b}\right)^2 - \left(\epsilon_{bb}.x + \epsilon_{ab}.y\right)$$



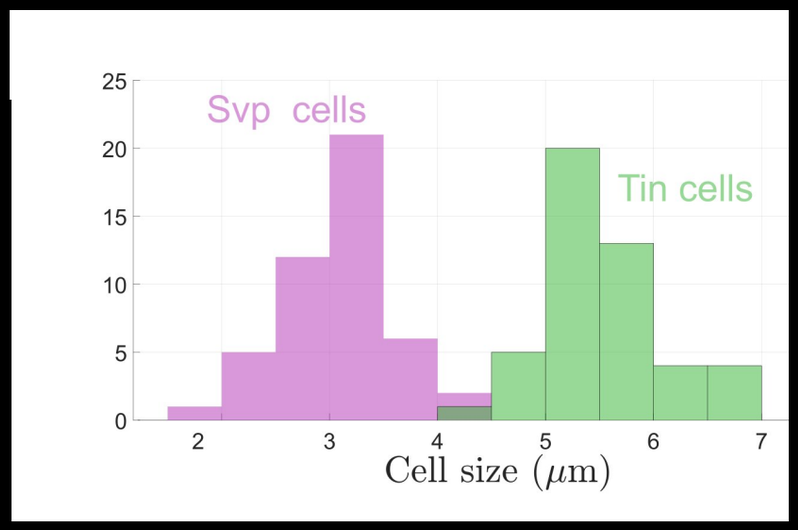
Tlili *et al.* Biorxiv 2019 (653535)

Cell matching consistent with an equilibrium energy approach

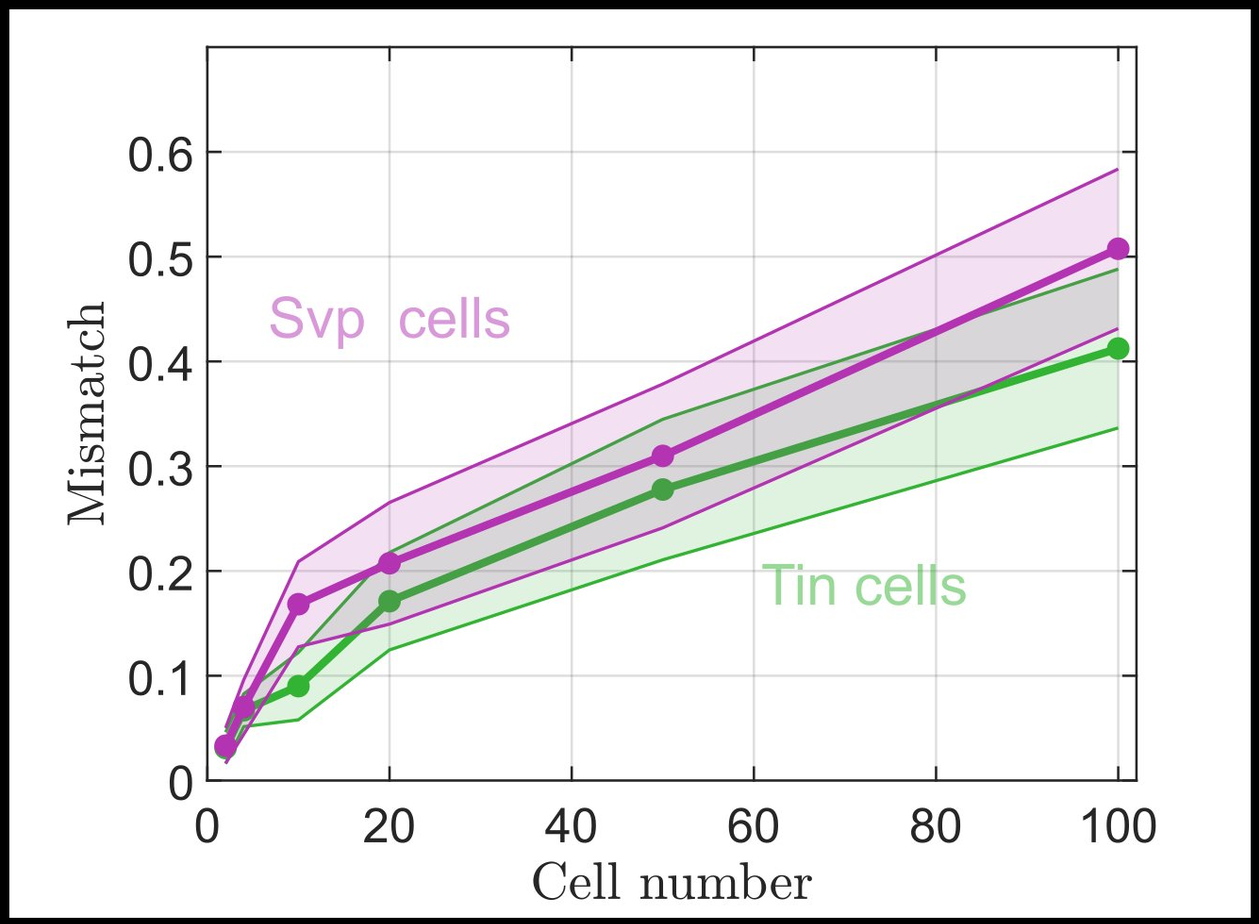
$$E_a = K\left(L_{cell} - L_{0,a}\right)^2 - \left(\epsilon_{aa}.x + \epsilon_{ab}.y\right)$$
$$E_b = K\left(L_{cell} - L_{0,b}\right)^2 - \left(\epsilon_{bb}.x + \epsilon_{ab}.y\right)$$



Input cell sizes



Equilibrate and measure matching



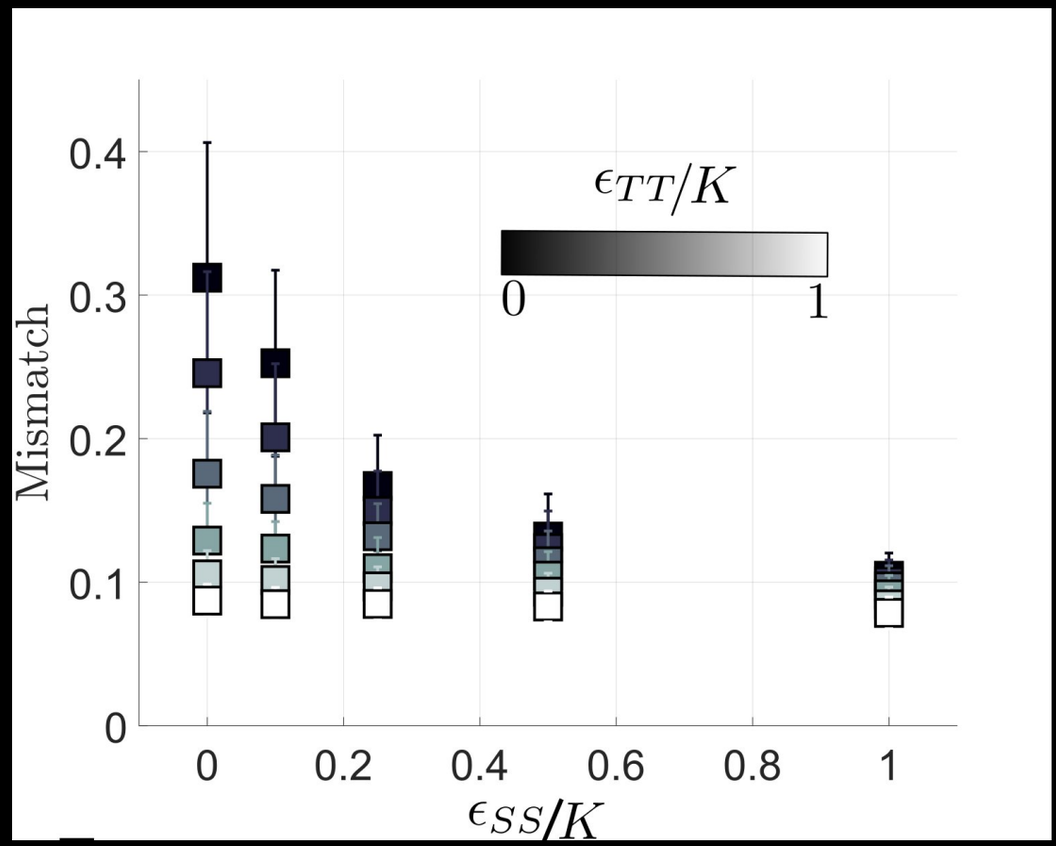
Cell matching consistent with an equilibrium energy approach

$$E_a = K\left(L_{cell} - L_{0,a}\right)^2 - \left(\epsilon_{aa}.x + \epsilon_{ab}.y\right)$$
$$E_b = K\left(L_{cell} - L_{0,b}\right)^2 - \left(\epsilon_{bb}.x + \epsilon_{ab}.y\right)$$

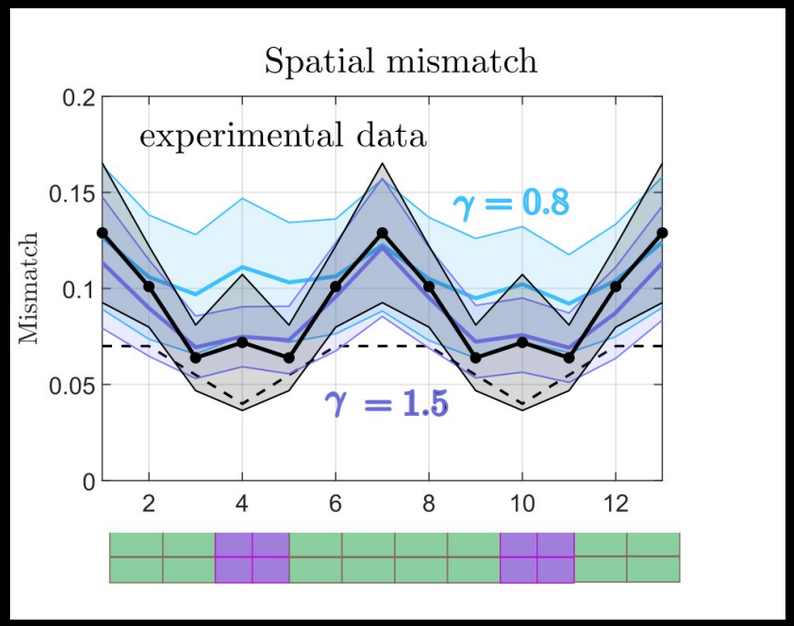
Potts model

Behaviour depends on

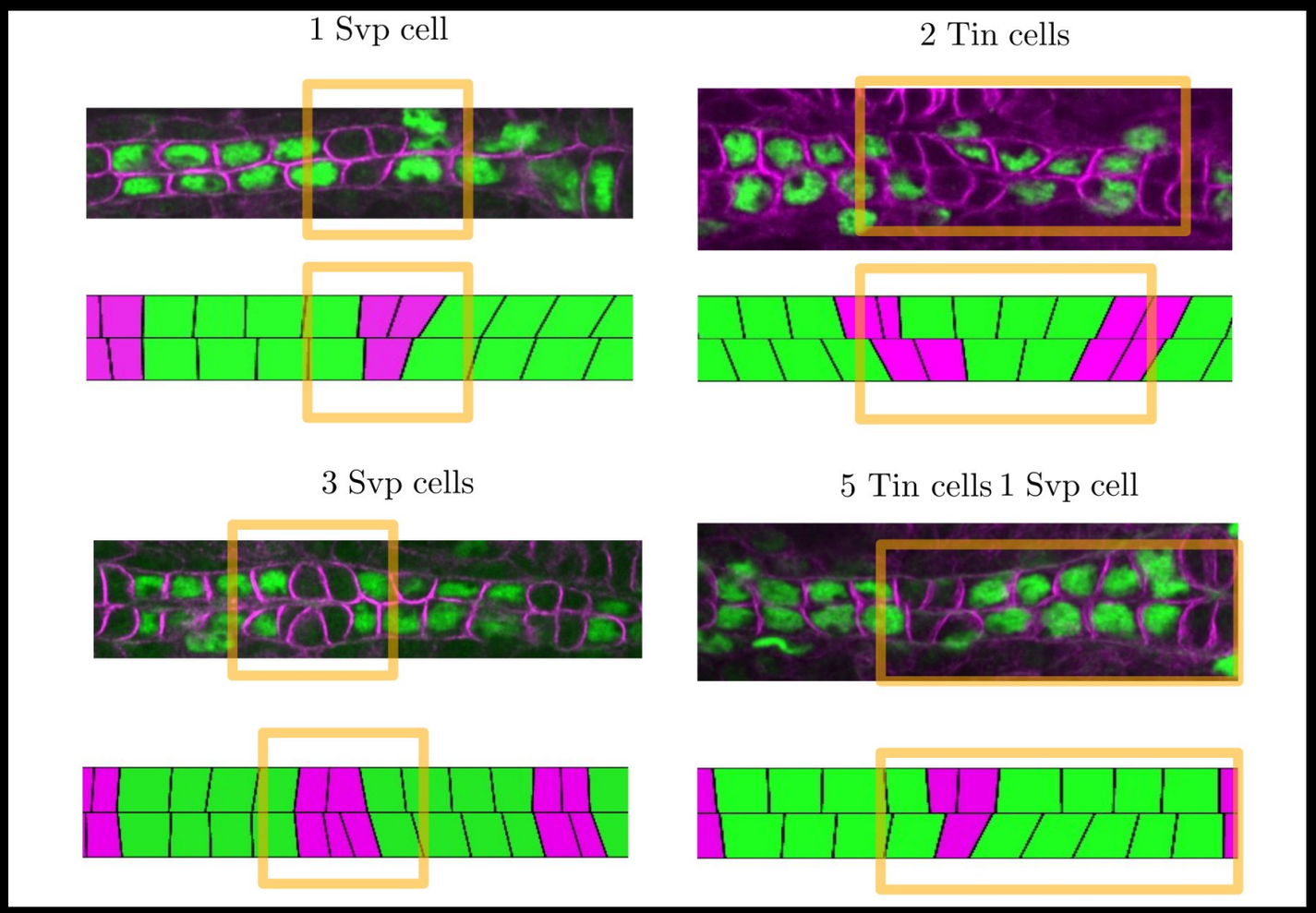
$$\gamma = \frac{(\epsilon_{TT} - \epsilon_{ST}) + (\epsilon_{SS} - \epsilon_{ST})}{K}$$



Mismatch in wild-type and deformed hearts



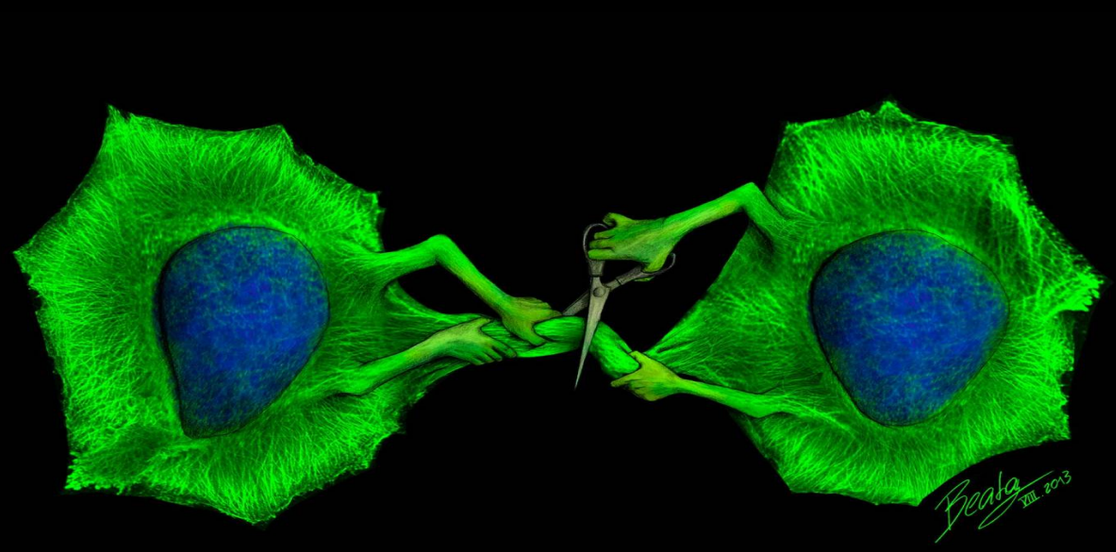
Cell alignment best at boundaries between cell types

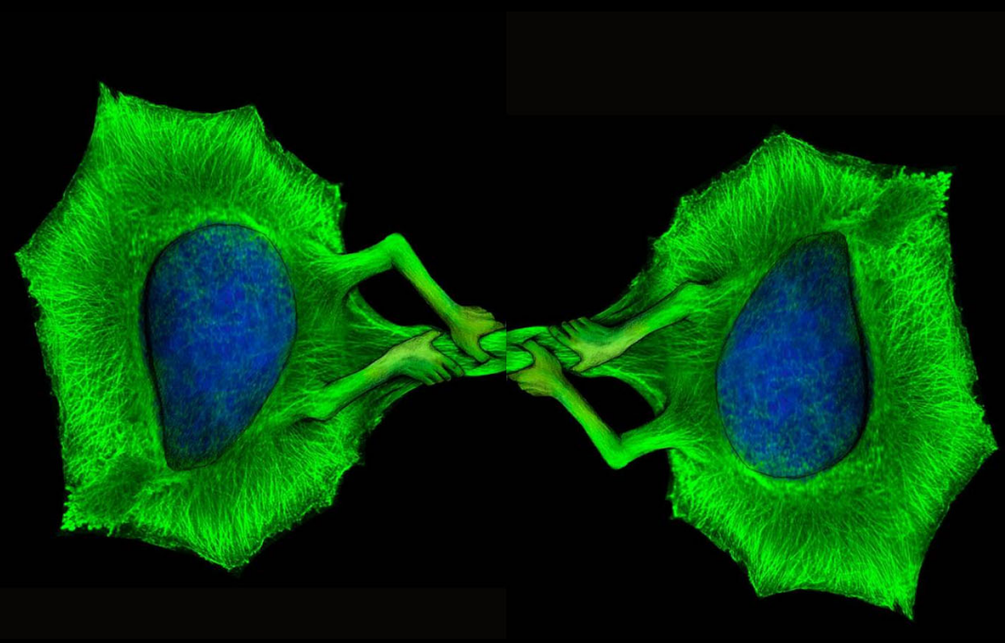


Alignment consistent with observed cases of cardioblast number variation

Tlili *et al.* Biorxiv 2019 (653535)

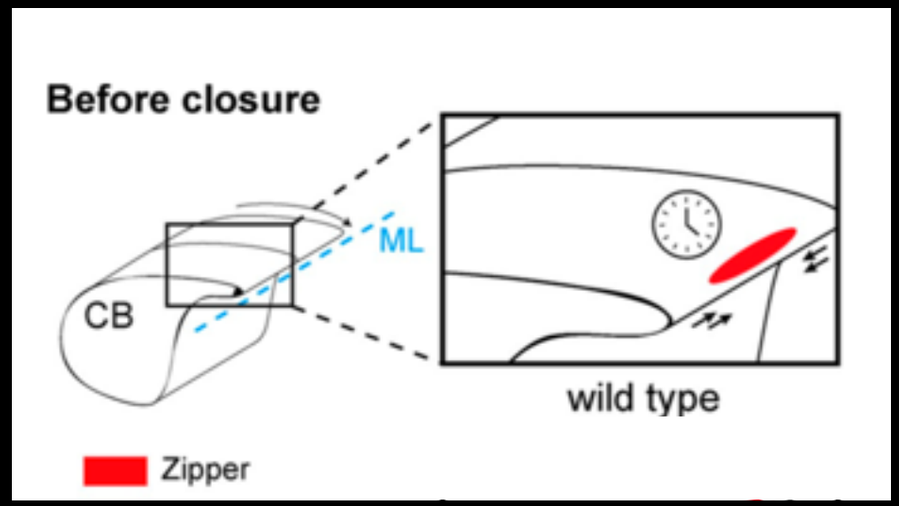
Coupling between waves of Myosin-II and filopodia adhesion regulates cell matching





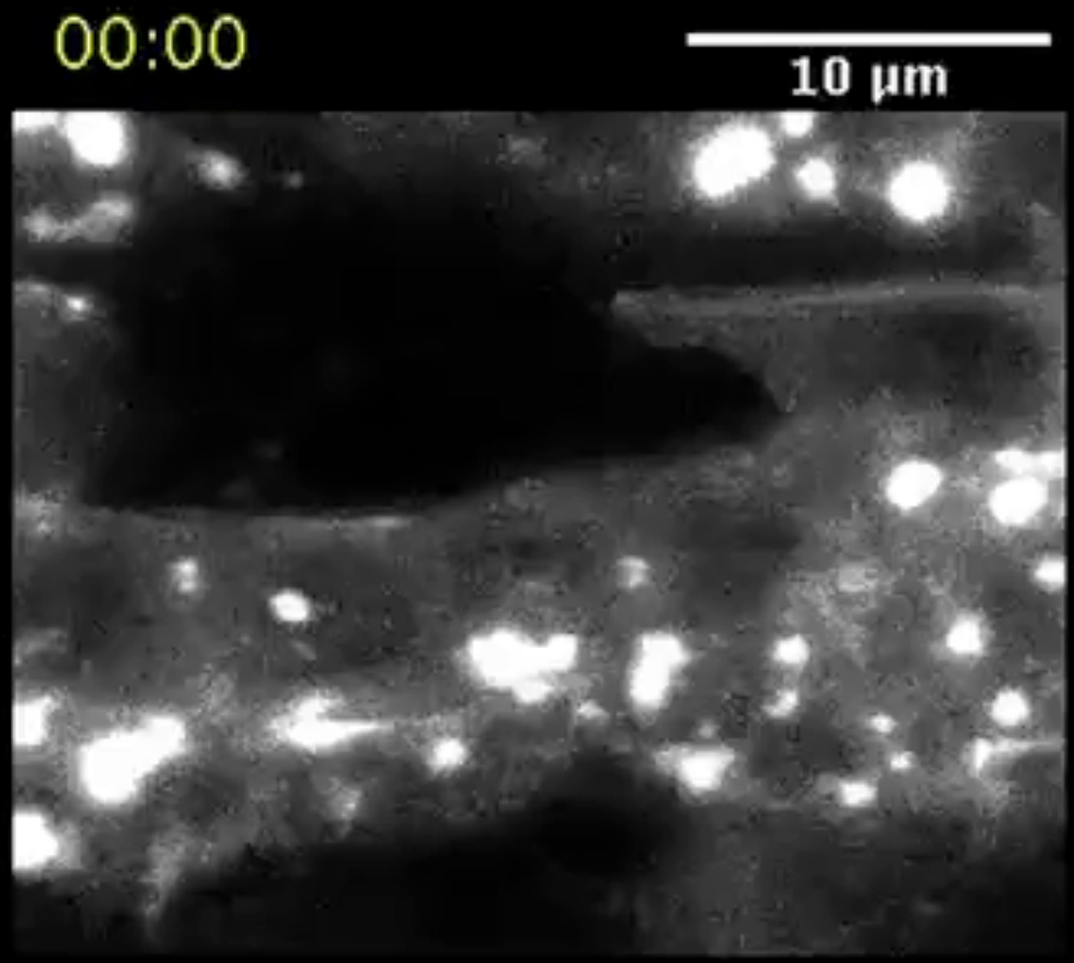
Adopted from http://www.beatascienceart.com/gallery

Known waves of Myosin-II in cardioblasts during matching



Drosophila
Zipper: Myosin II heavy chain
Squash: Myosin II light chain

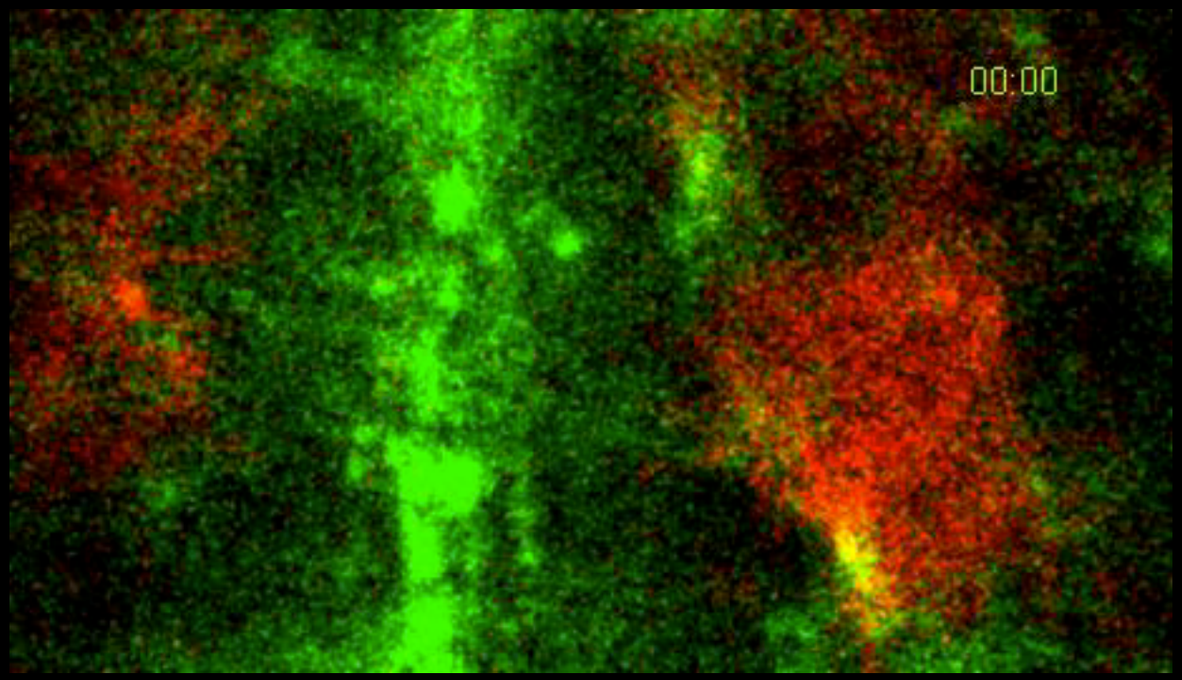
What is the function of this wave?



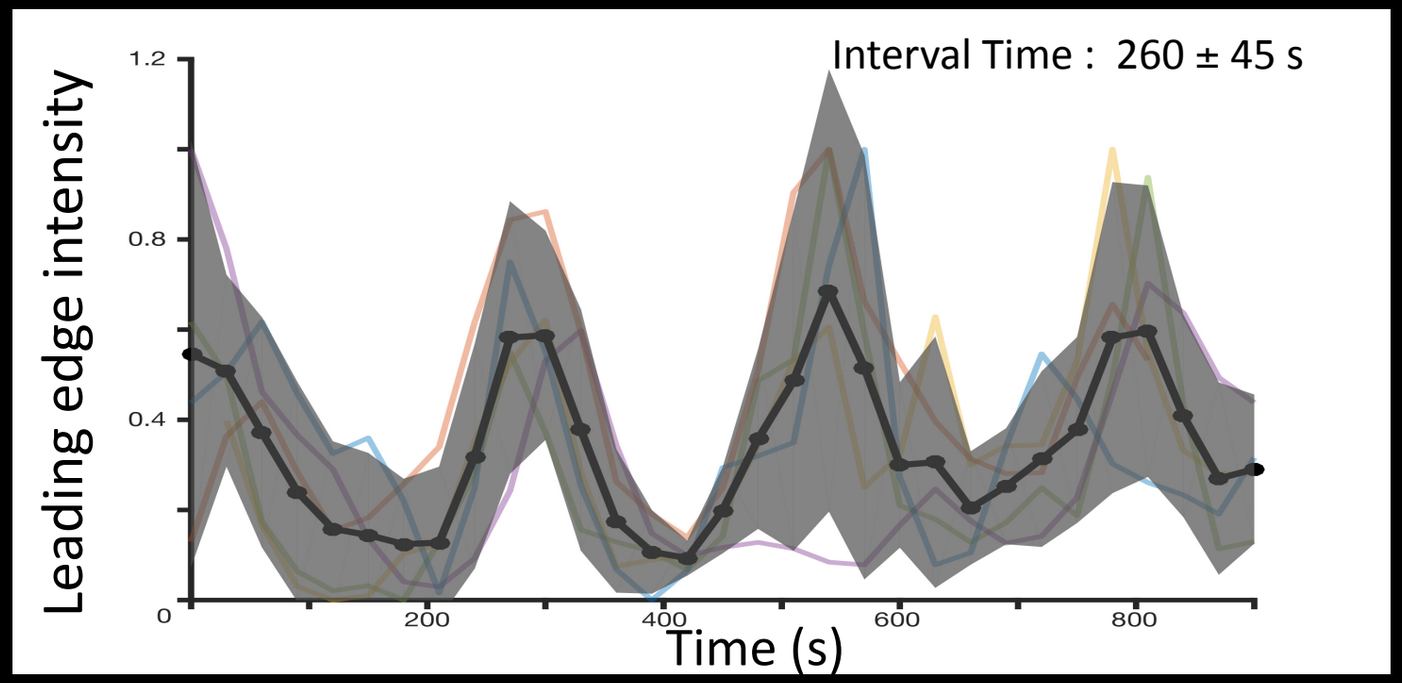
tinCΔ4 > zipper::GFP

Vogler *et al.*, JCB 2014

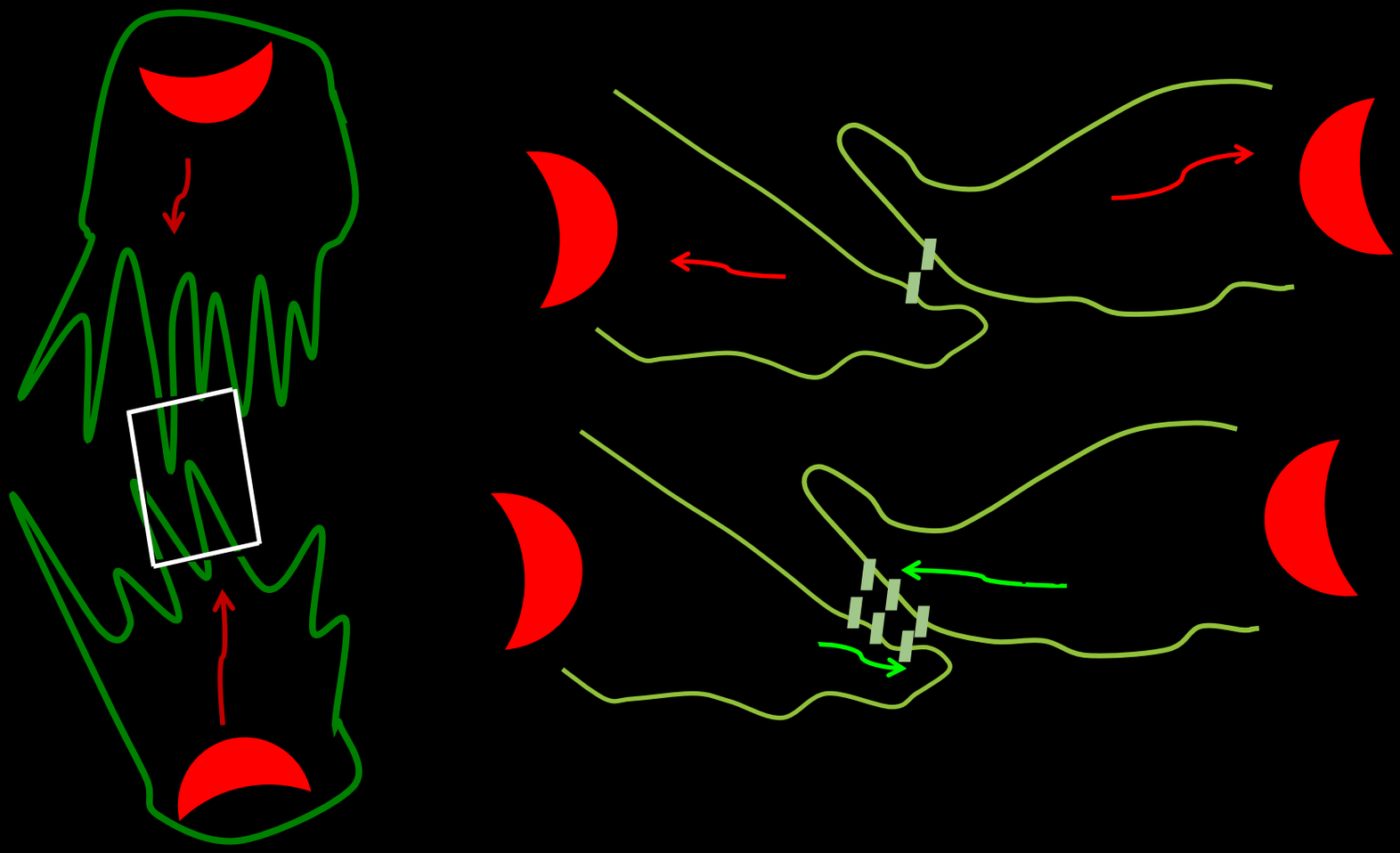
Myosin-II waves have period ~ 4 minutes



Zipper::GFP Hand>Moesin::mCherry



Counterbalance of filopodia adhesion and Myosin II



Weak adhesion

Broken contacts
Retracted filopodia

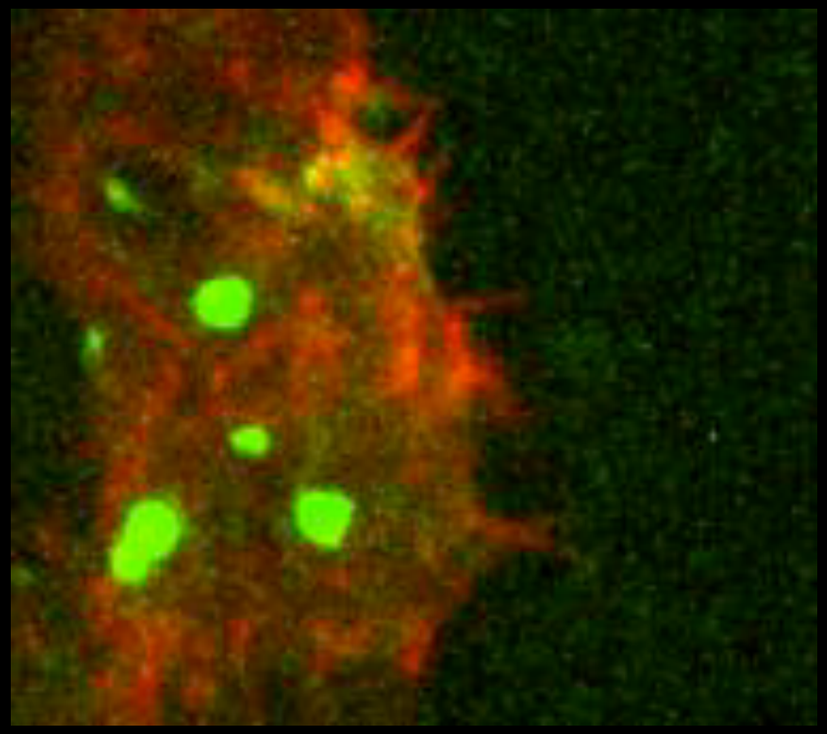
Strong adhesion

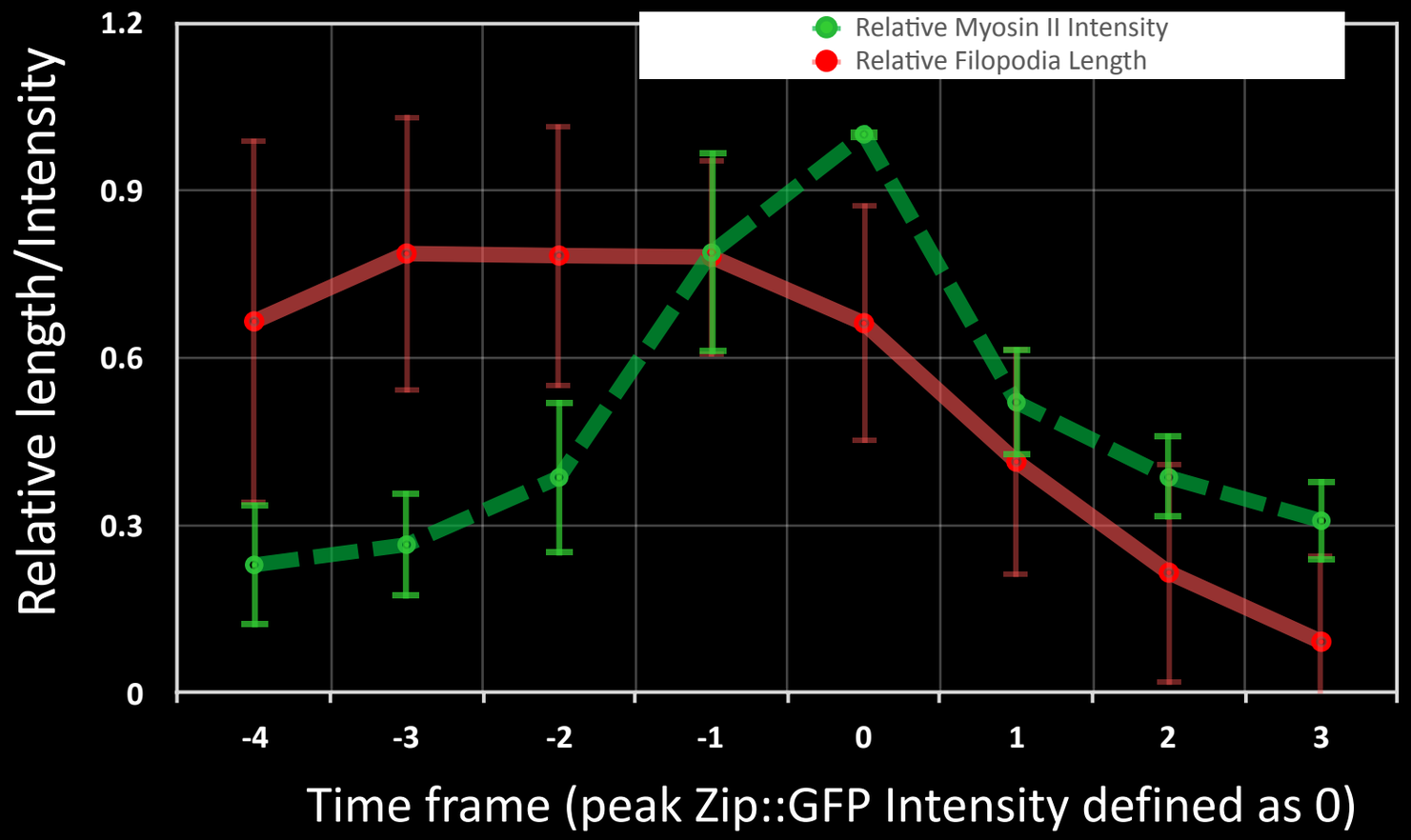
Contacts stabilise
Drives cells matching

Actin-Dependent Stabilisation

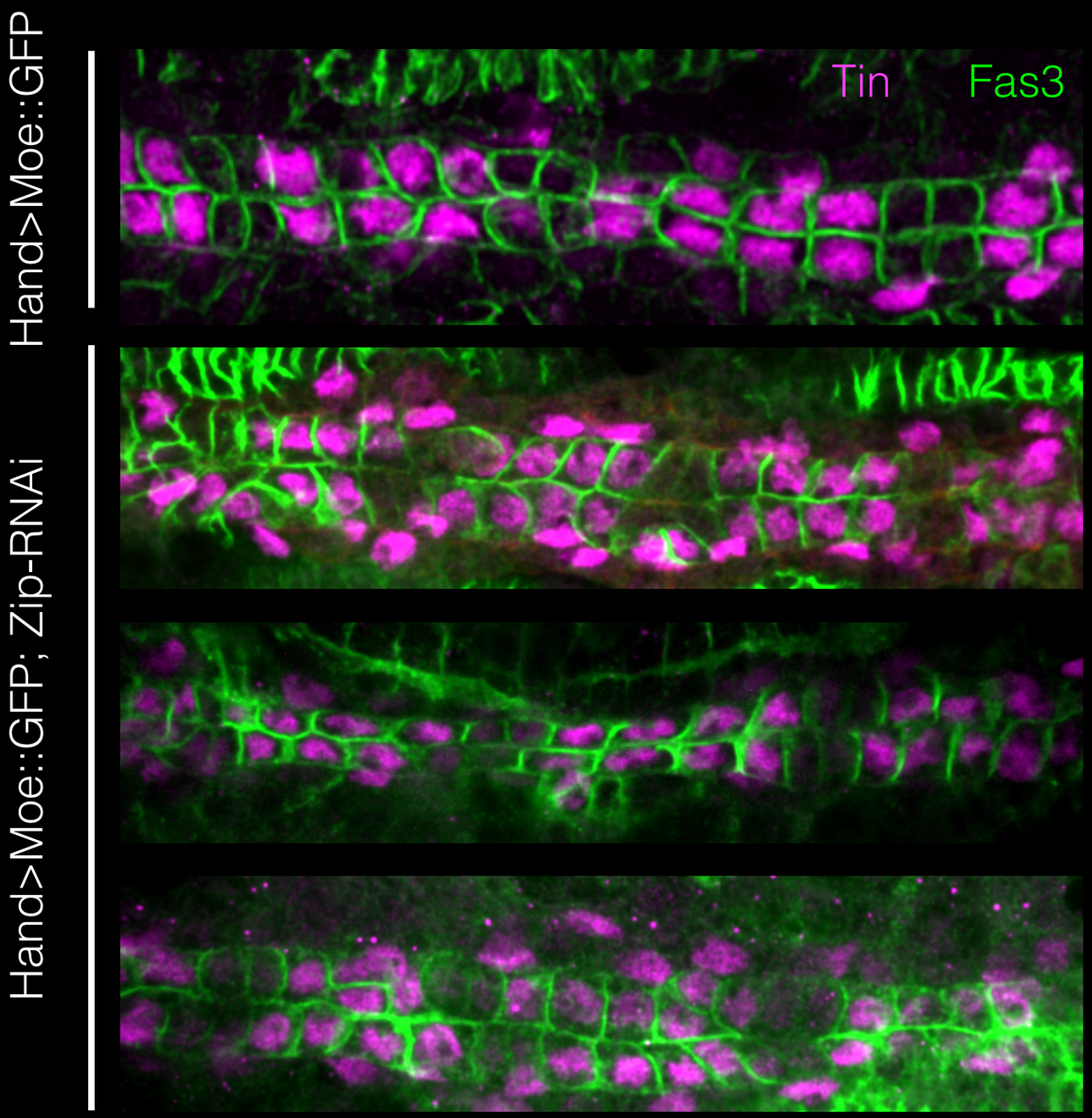
Myosin-II Dependent Contraction

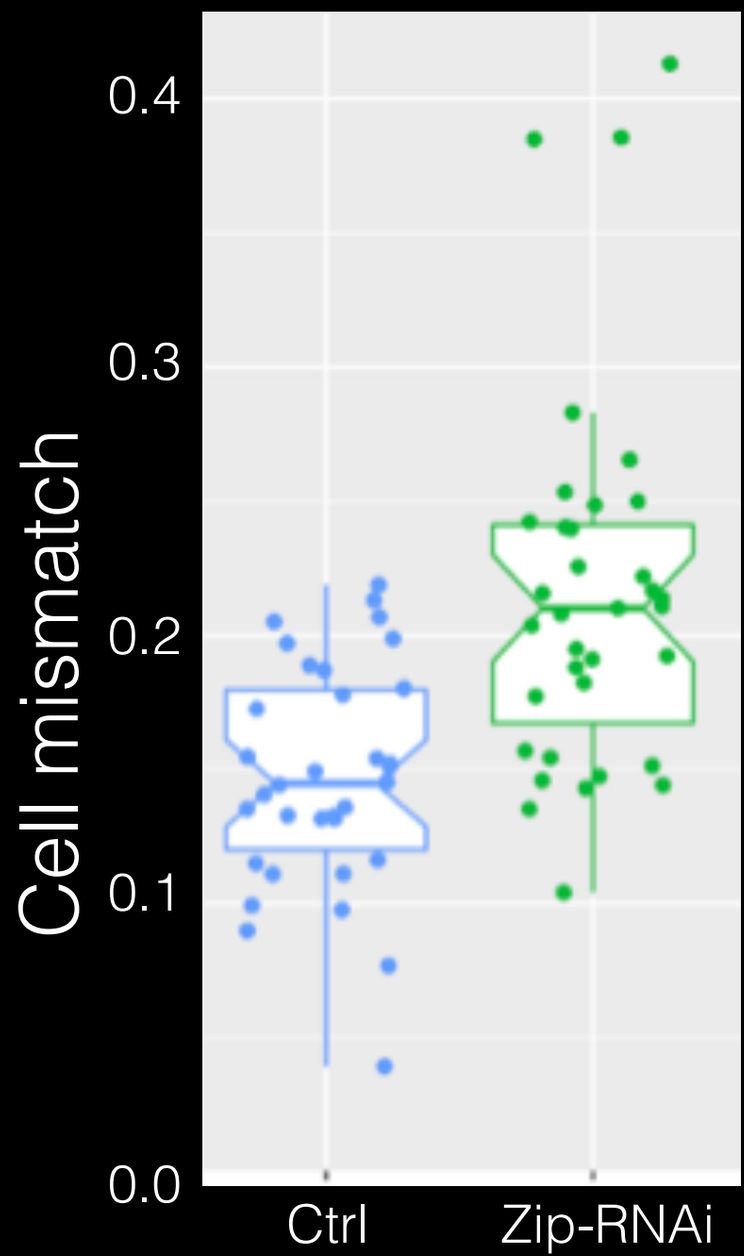
Filopodia retraction correlates with Myosin II appearance at cell leading edge





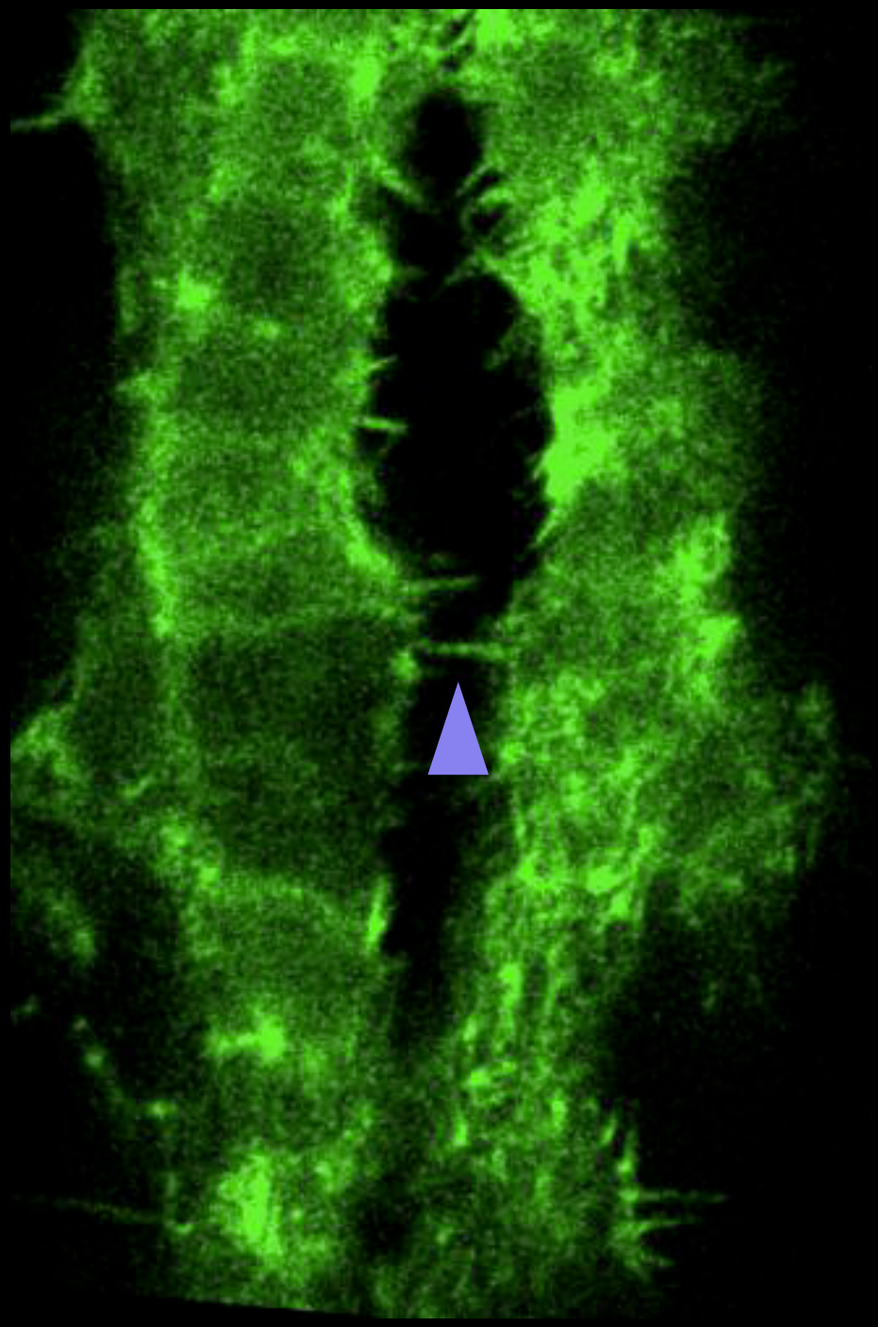
Reduction in Myosin-II results in reduced cell matching

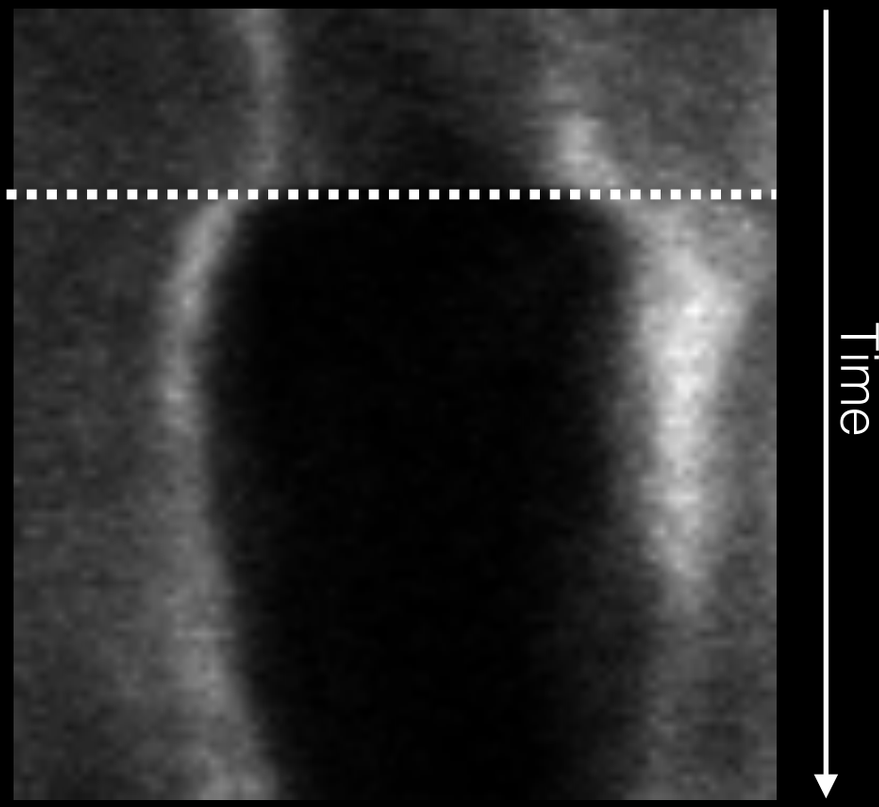




Strong force between filopodia in wildtype hearts

Hand>Moe::GFP

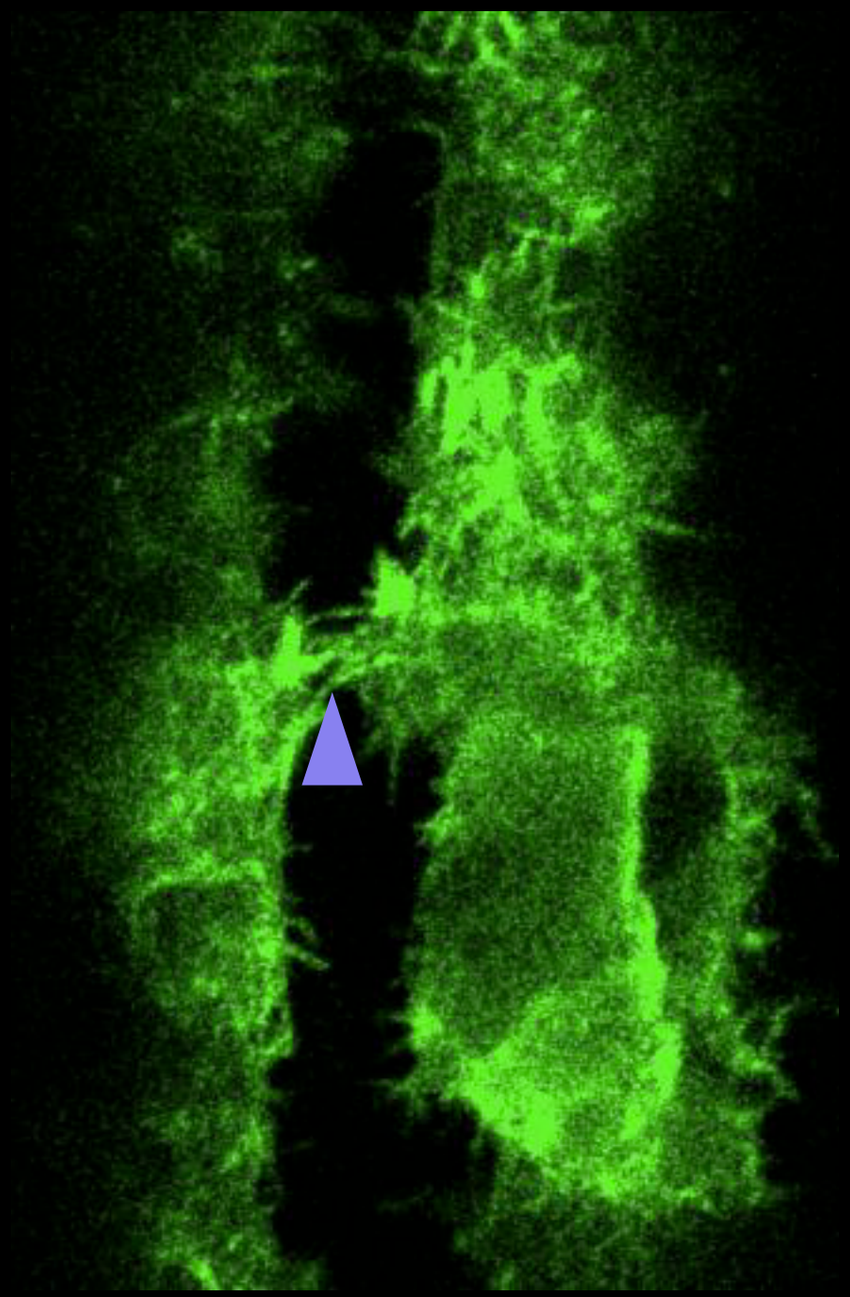


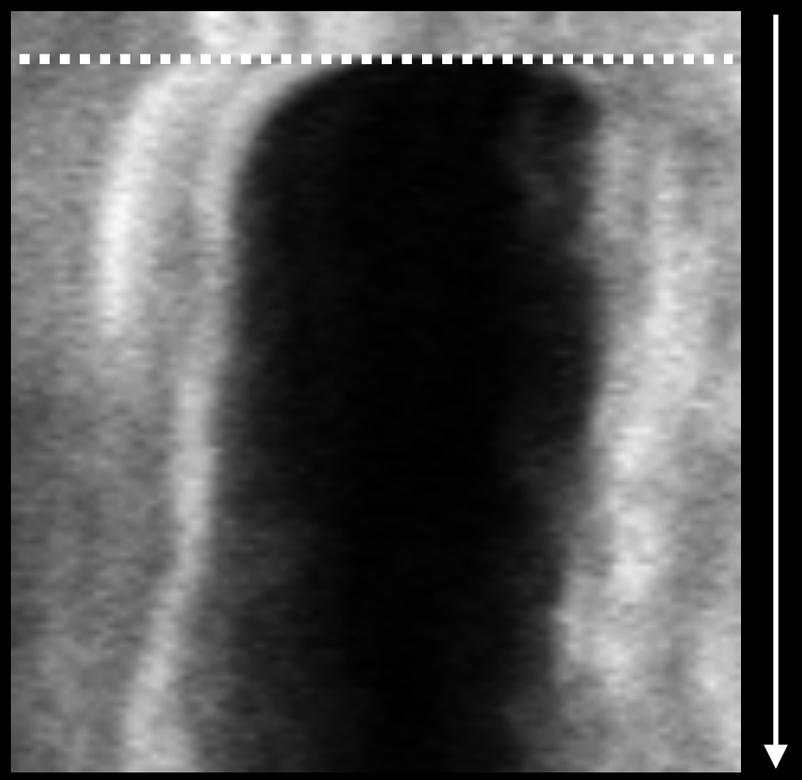


with Y. Toyama

Strong force between filopodia in wildtype hearts

Hand>Moe::GFP

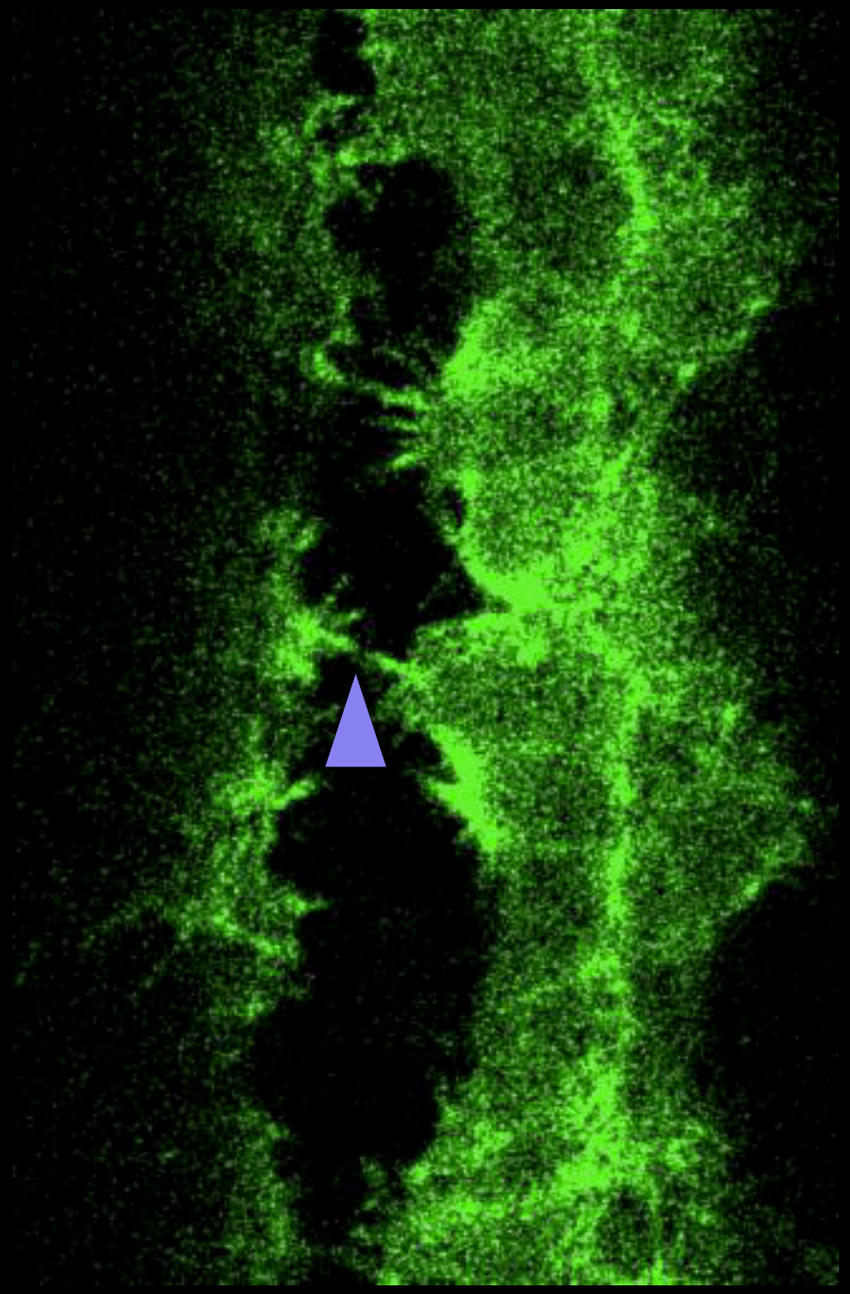


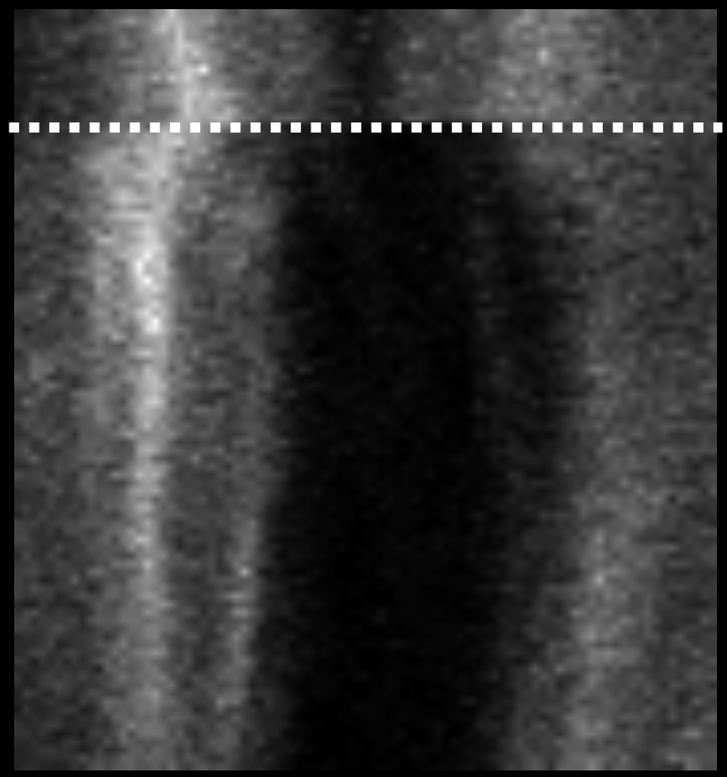


Time

Force between filopodia reduced in Zip knockdown

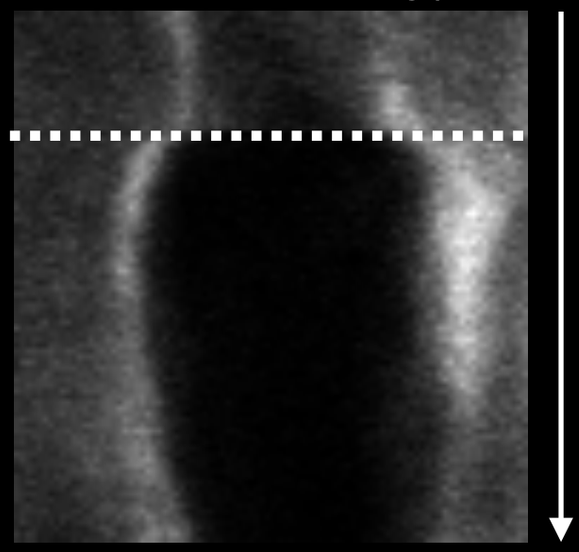
Hand>Moe::GFP; Zip-RNAi





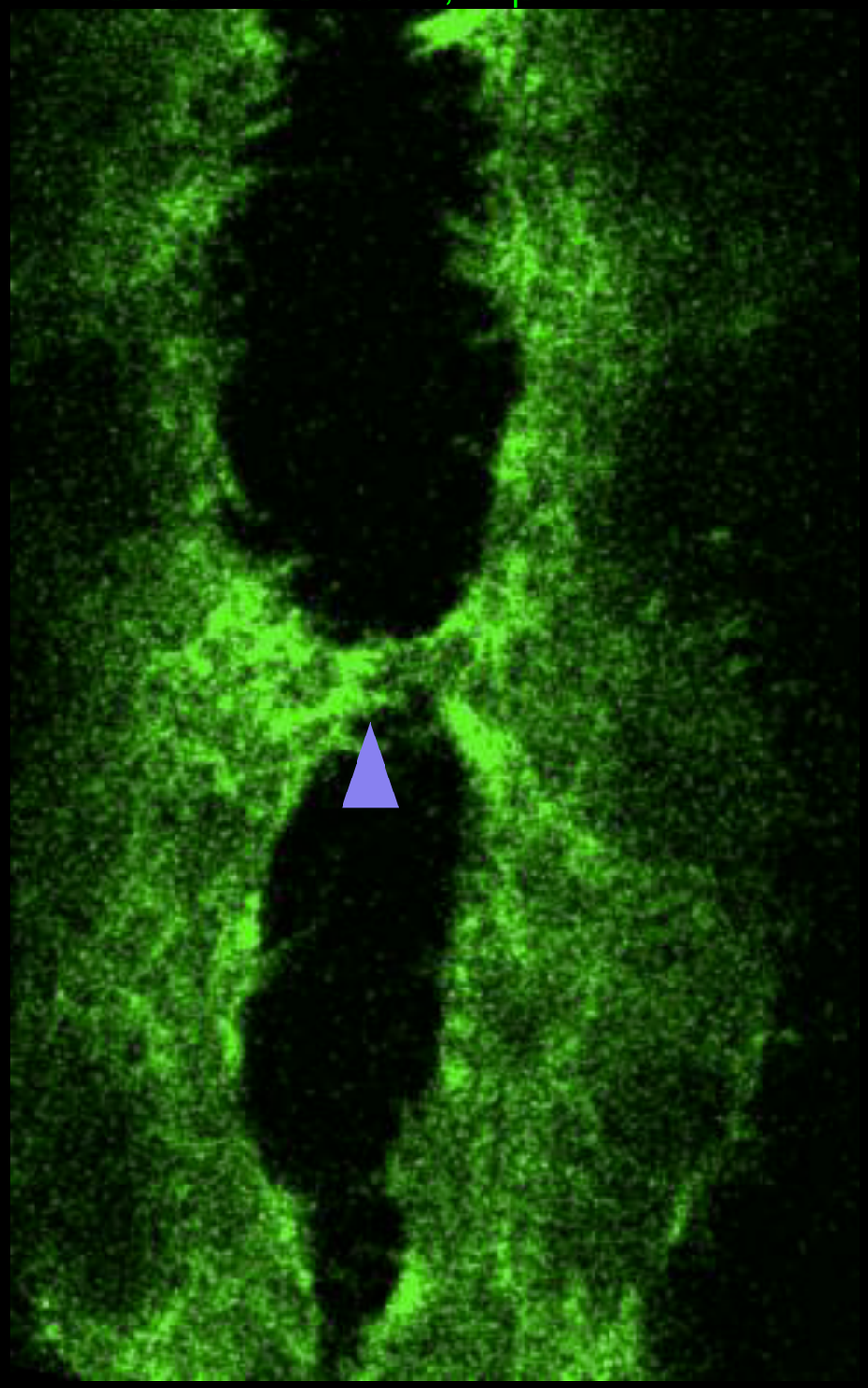
Time

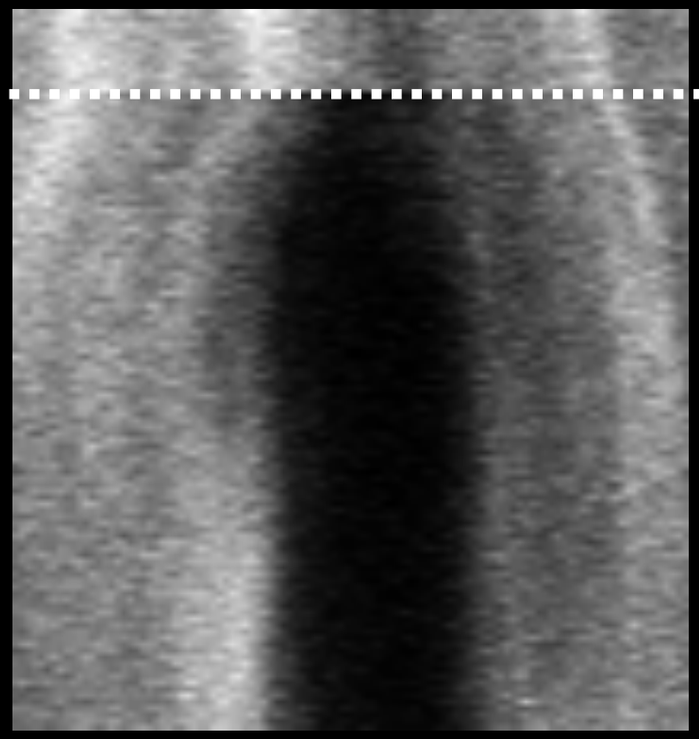
wildtype



Force between filopodia reduced in Zip knockdown

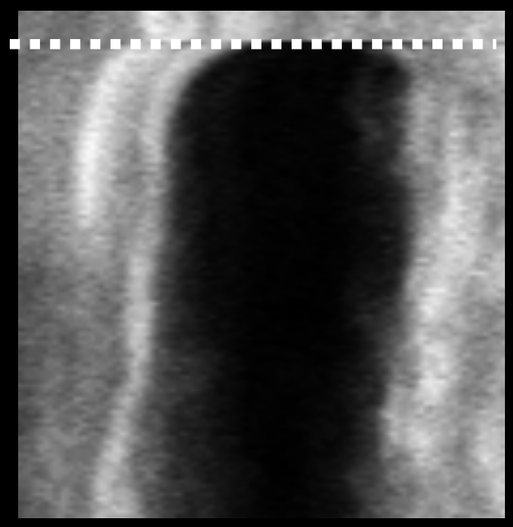
Hand>Moe::GFP; Zip-RNAi



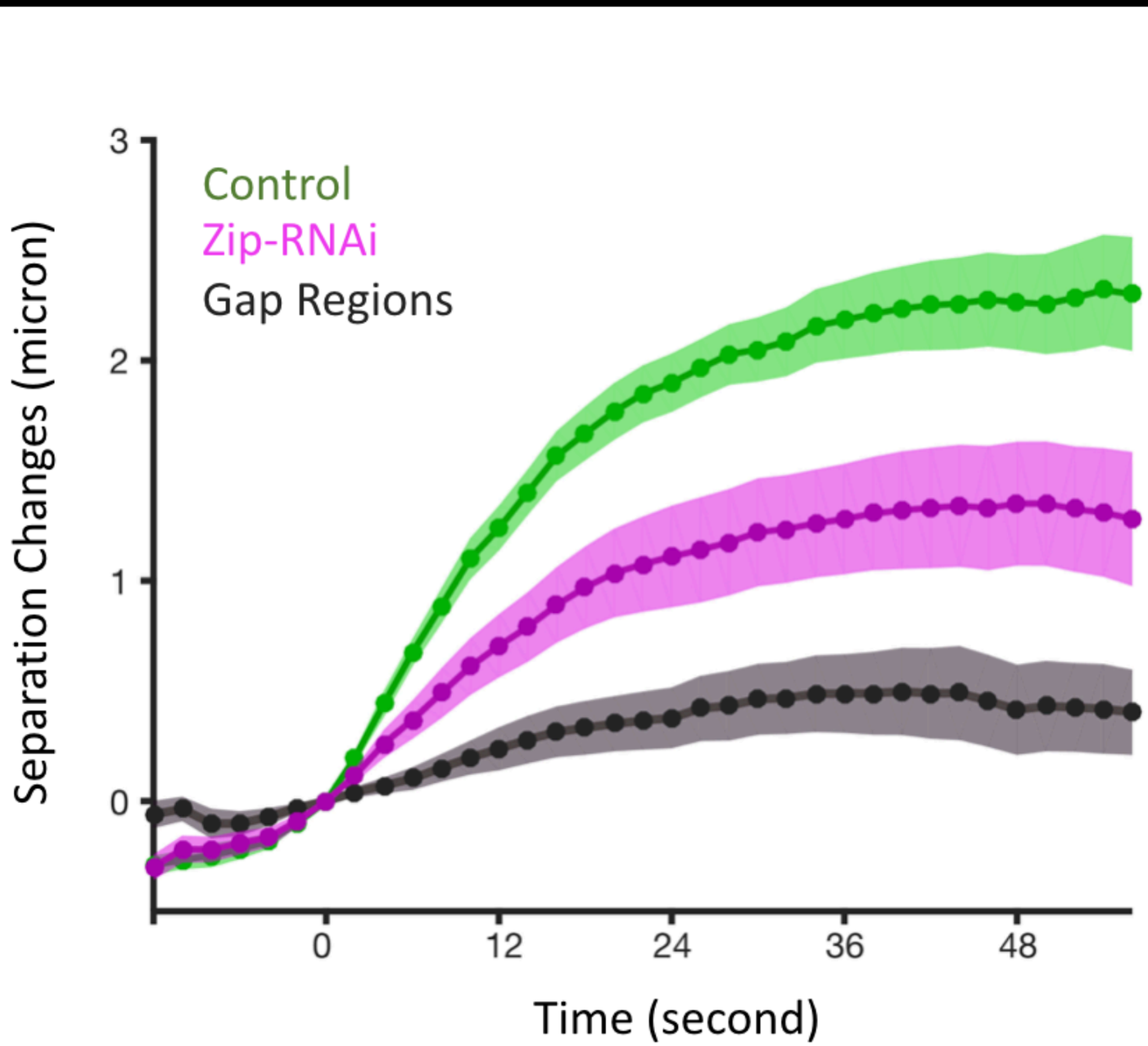


Time

wildtype



Force between filopodia reduced in Zip knockdown



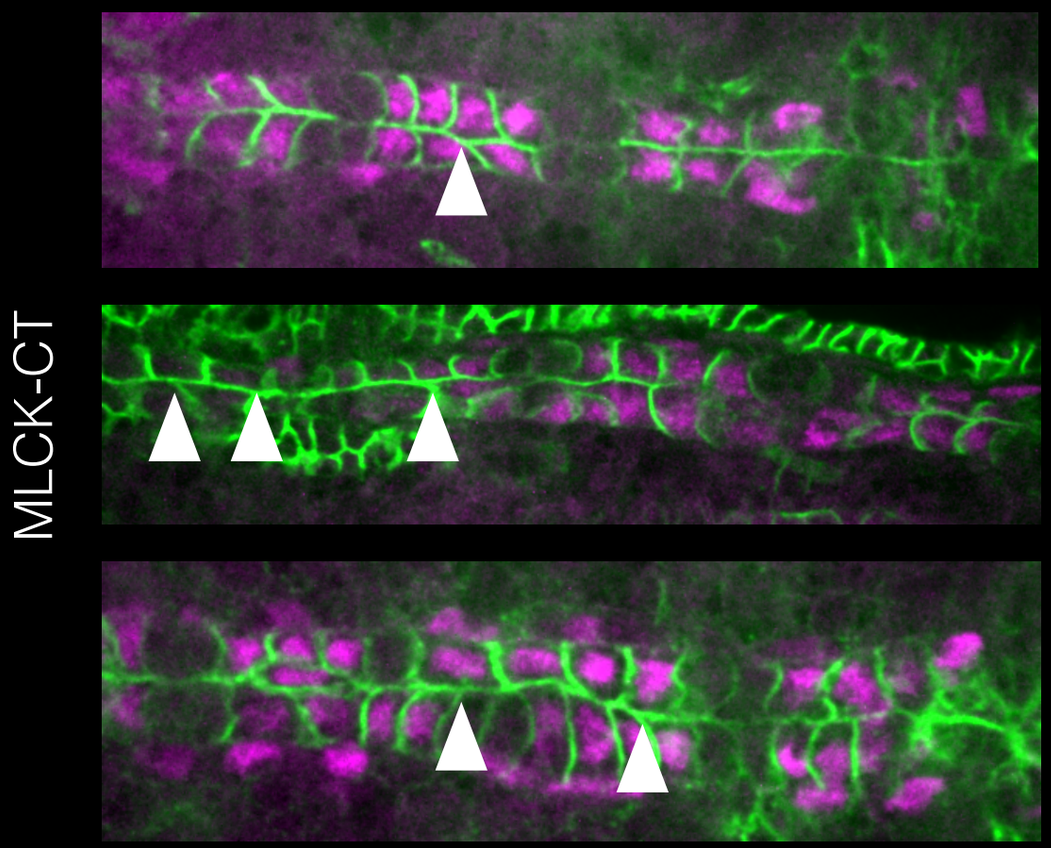
Instantaneous Recoil Velocity

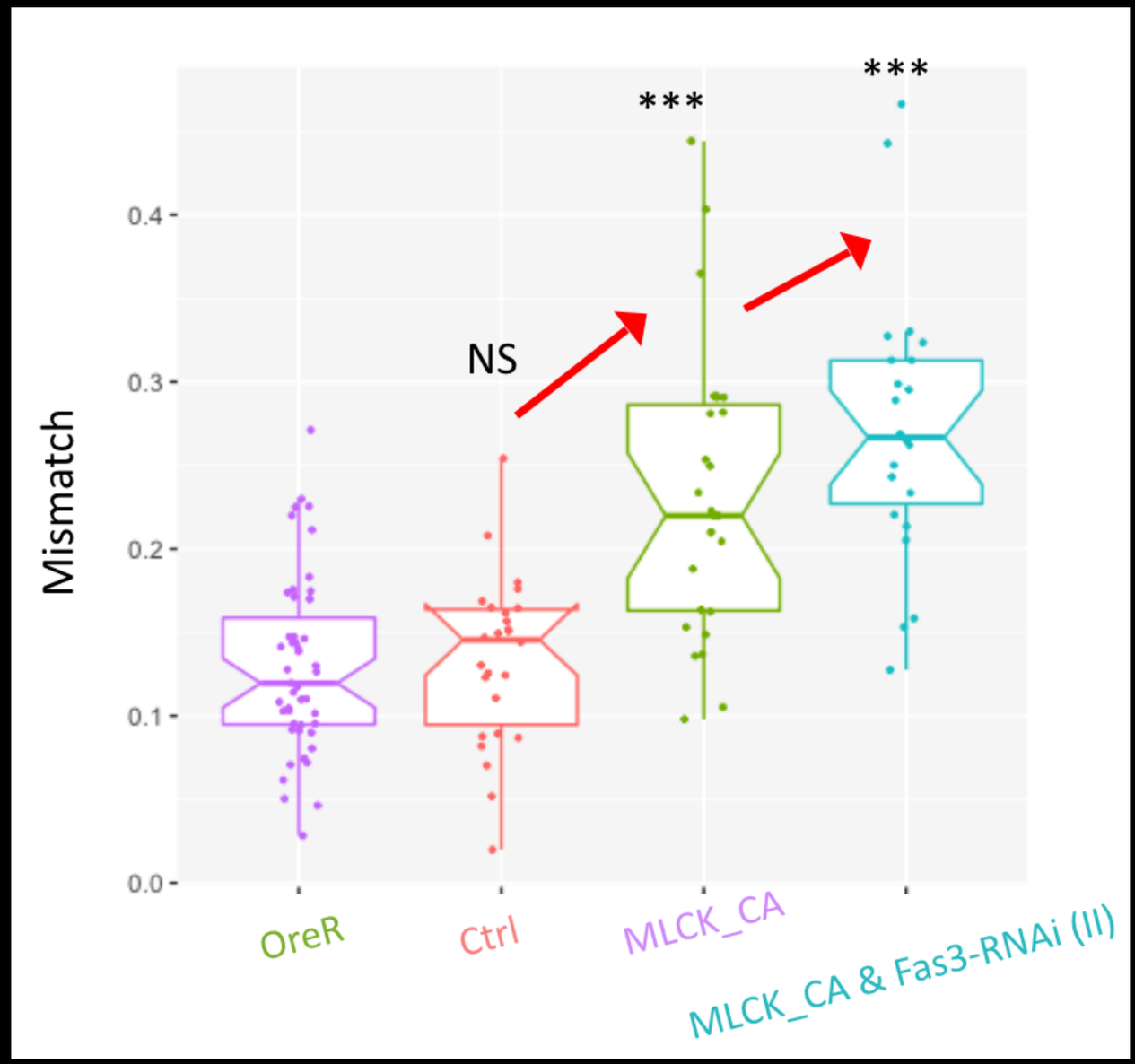
$RV_{Filopodia}$ = 0.15±0.04μm/s

$RV_{Zip\text{-}RNAi}$ = 0.10±0.07μm/s

RV_{Gap} = 0.02±0.03μm/s

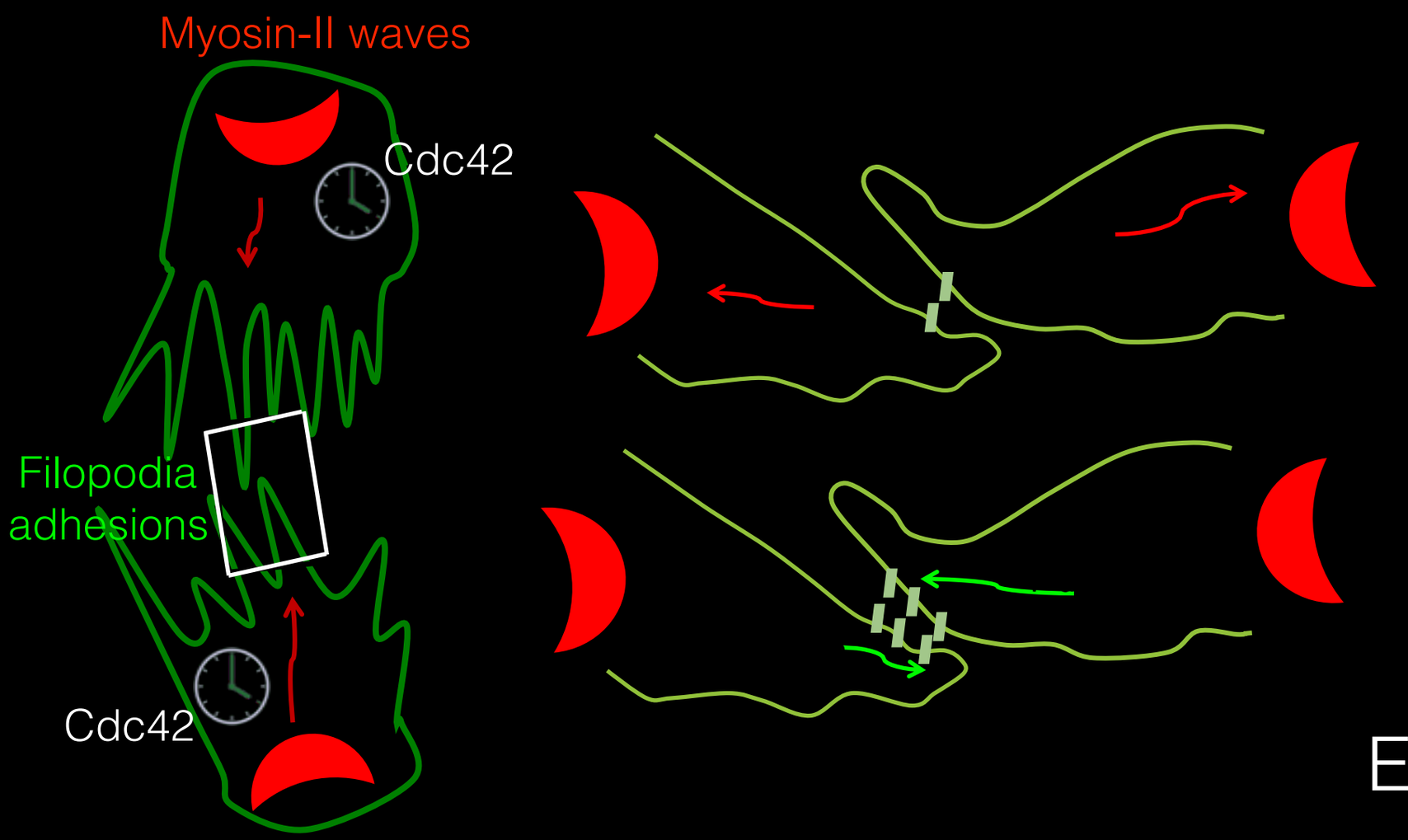
Myosin II over-activation also reduces cell matching





Suggests levels of Myosin are optimised
to enable precise cell matching

Mechanical testing of cellular interactions



Weak adhesion

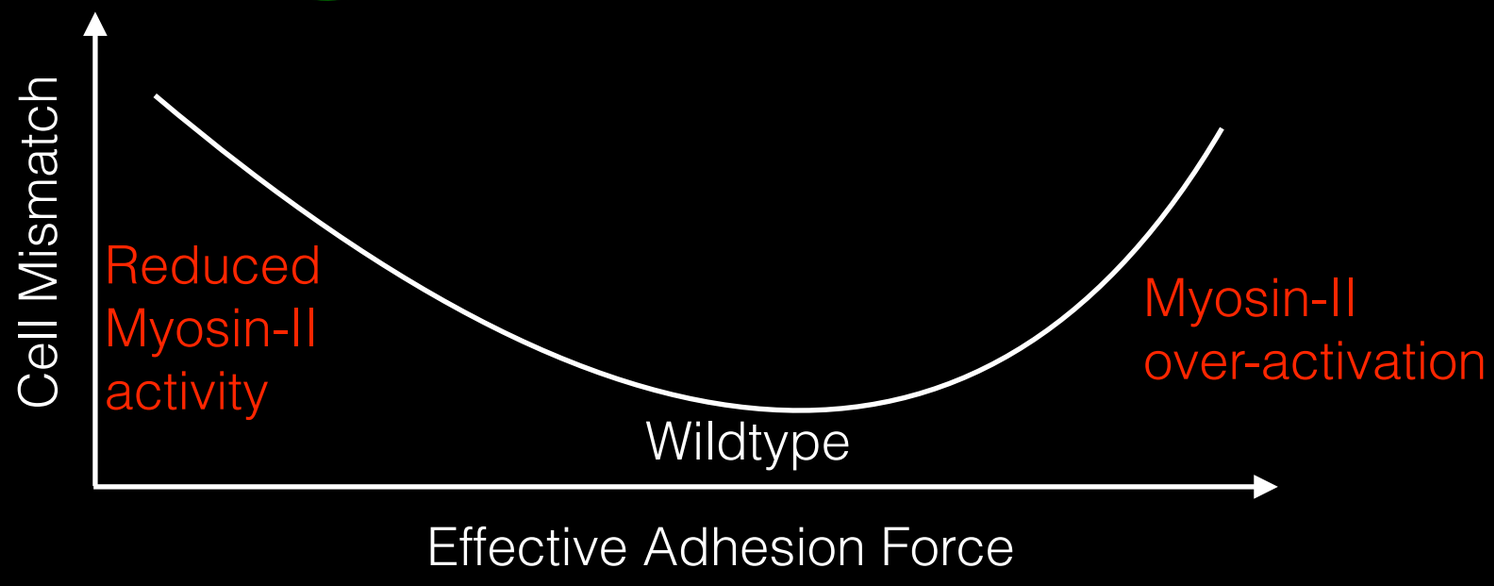
- Broken contacts
- Retracted filopodia

Strong adhesion

- *Some* contacts stabilise
- Drives precise cells matching

Extra strong adhesion

- Contacts *too* stable
- Unable to correct for
. mistakes in matching



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Current lab

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Tricia Loo
Mario Mendieta
Sunandan Dhar
Shaobo Zhang
Prabhat Tiwari
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Former lab members

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