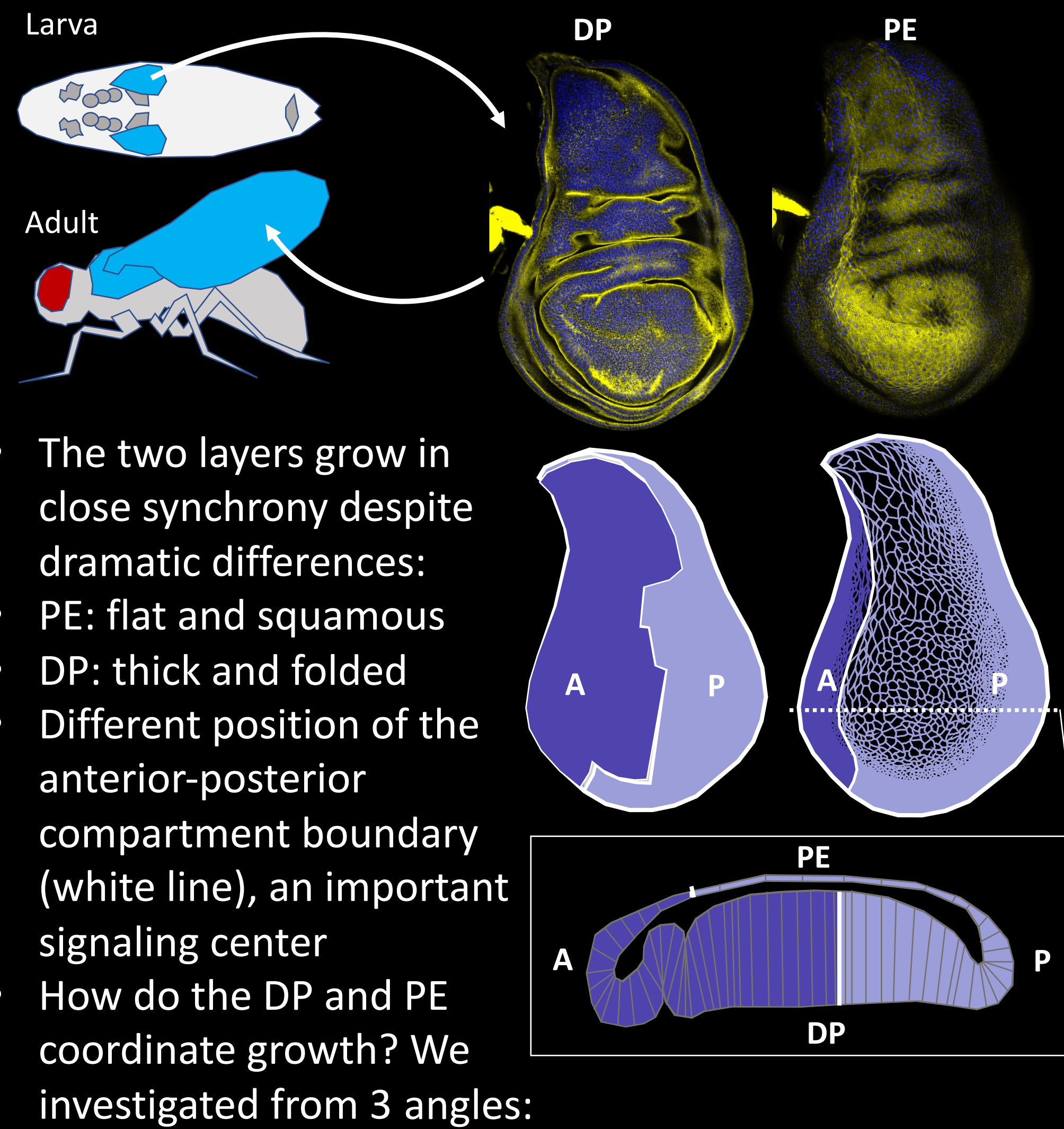


Investigating growth regulation within synchronously developing epithelia

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How do the two layers of the *Drosophila* wing disc grow in synch?

- Animal development requires coordinated epithelial growth
- The fly wing disc consists of two associated epithelia: the disc proper (DP) and peripodial epithelium (PE)

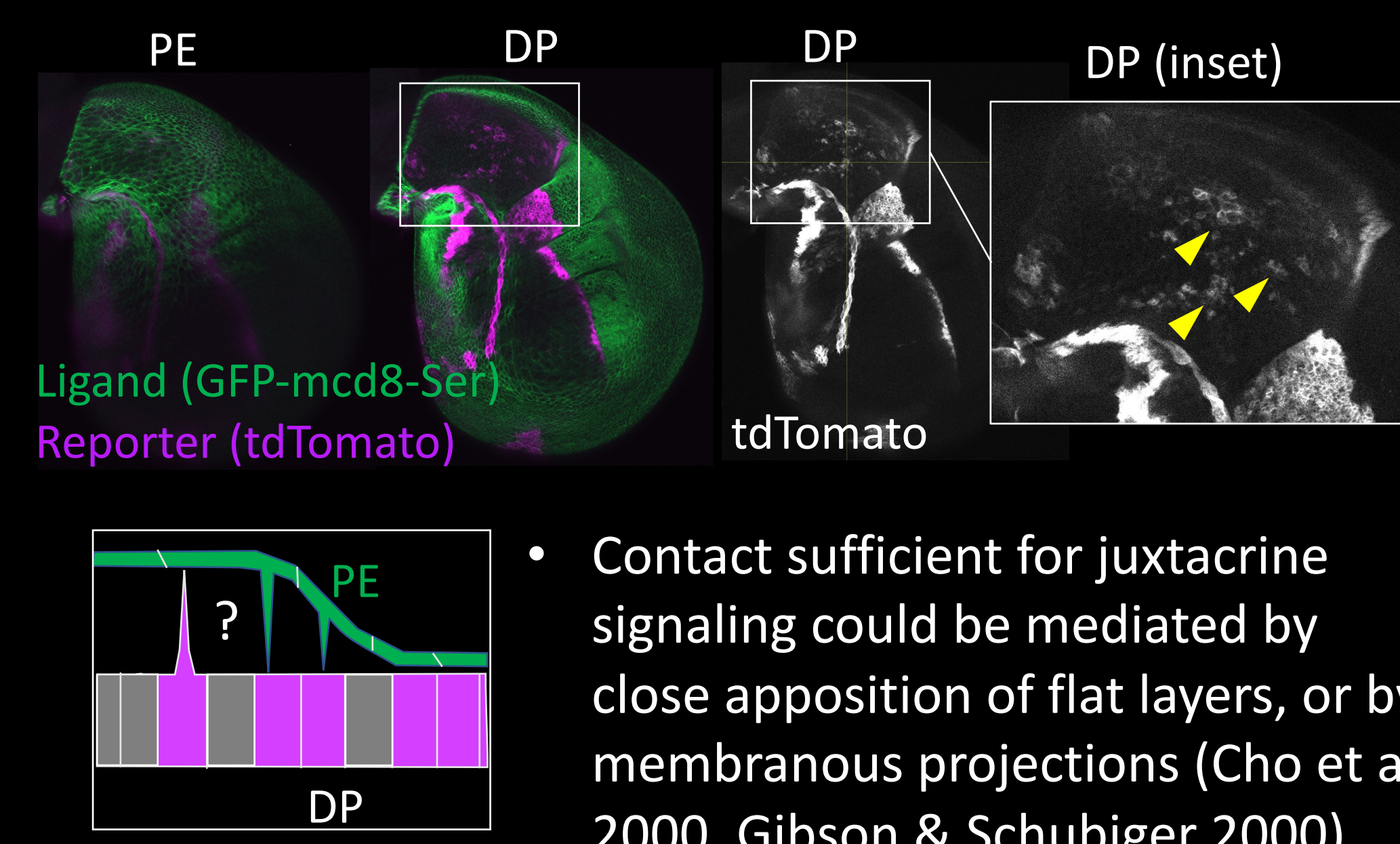


- The two layers grow in close synchrony despite dramatic differences:
 - PE: flat and squamous
 - DP: thick and folded
 - Different position of the anterior-posterior compartment boundary (white line), an important signaling center
- How do the DP and PE coordinate growth? We investigated from 3 angles:

- juxtacrine signaling between the layers
- role of the morphogen Hh in the PE
- role of the morphogen Dpp (BMP) in the PE

1. Contacts between layers suggest possible juxtacrine signaling

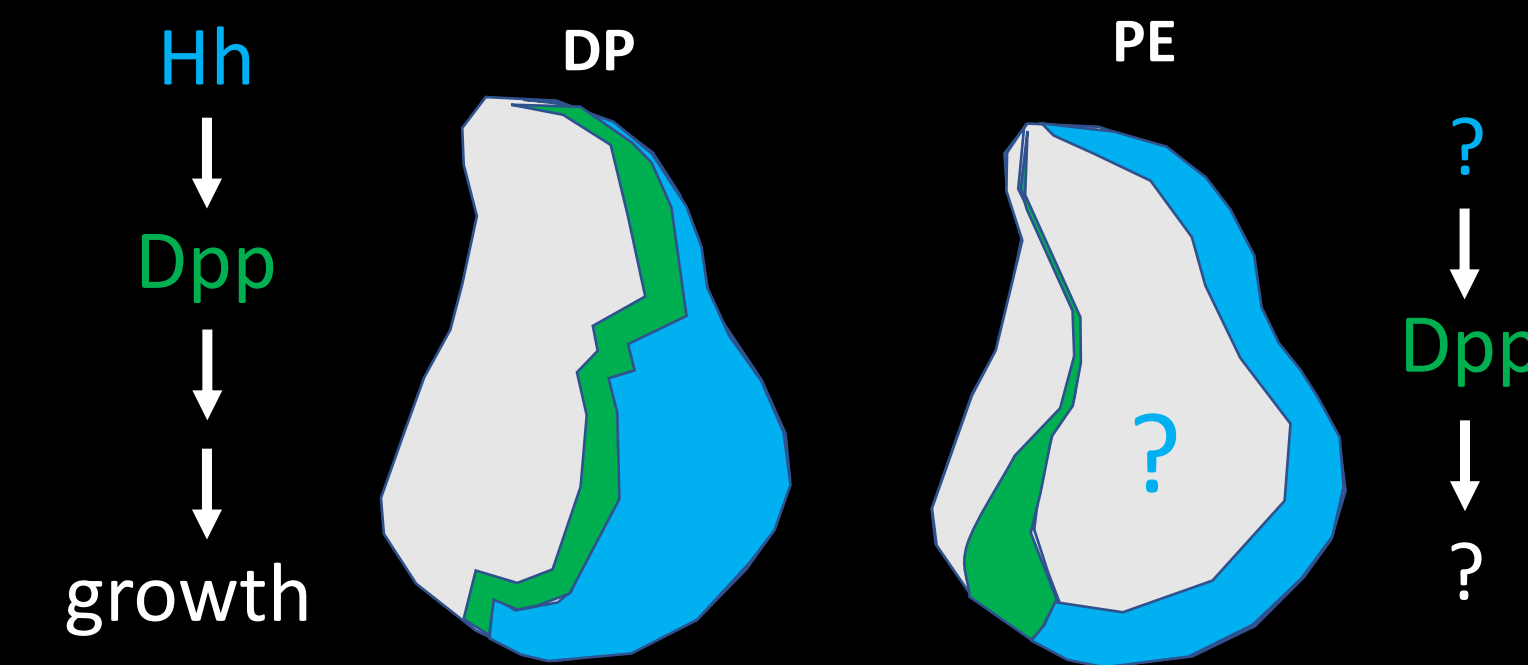
- A synthetic Notch-based system (He et al 2017) detects which cells directly contact each other
- We expressed a juxtacrine ligand (green) in the PE
- Cells which contact ligand-expressing cells express a reporter (magenta)
- Cells in specific regions of the DP (inset, arrowheads) express the reporter in response to PE ligand expression, indicating close contact



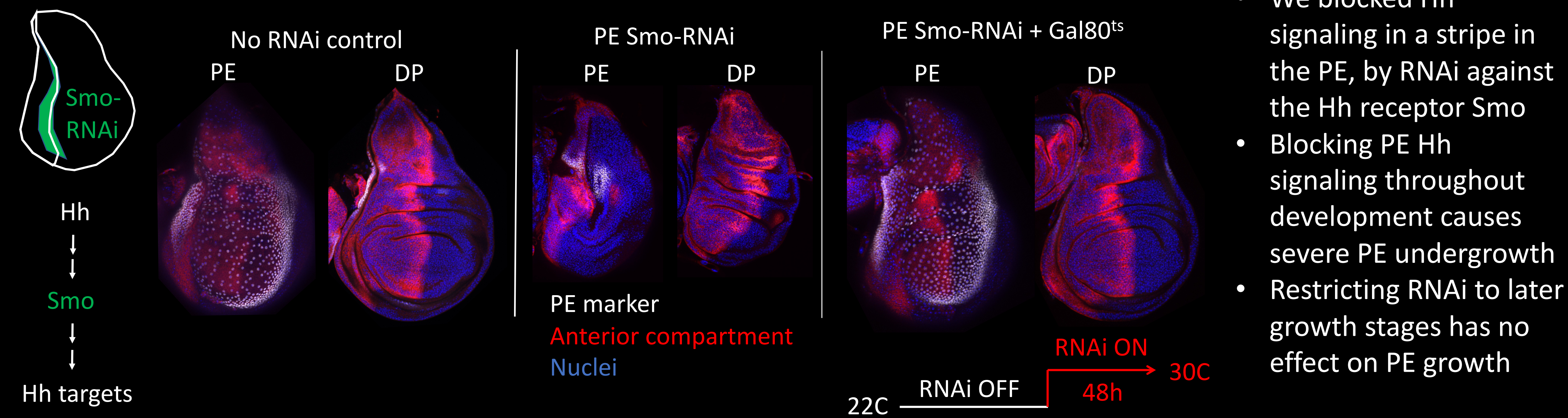
- Contact sufficient for juxtacrine signaling could be mediated by close apposition of flat layers, or by membranous projections (Cho et al 2000, Gibson & Schubiger 2000)

Hh and Dpp (BMP) are conserved morphogens crucial for DP growth, with an unknown role in the PE

- Hh-driven Dpp expression is absolutely critical for growth of the DP
- Hh expression in the PE is low or possibly absent for much of the period of wing disc growth
- Is Hh required for PE expression of Dpp and for PE growth?
- Does Dpp control growth of the PE, as it does in the DP?



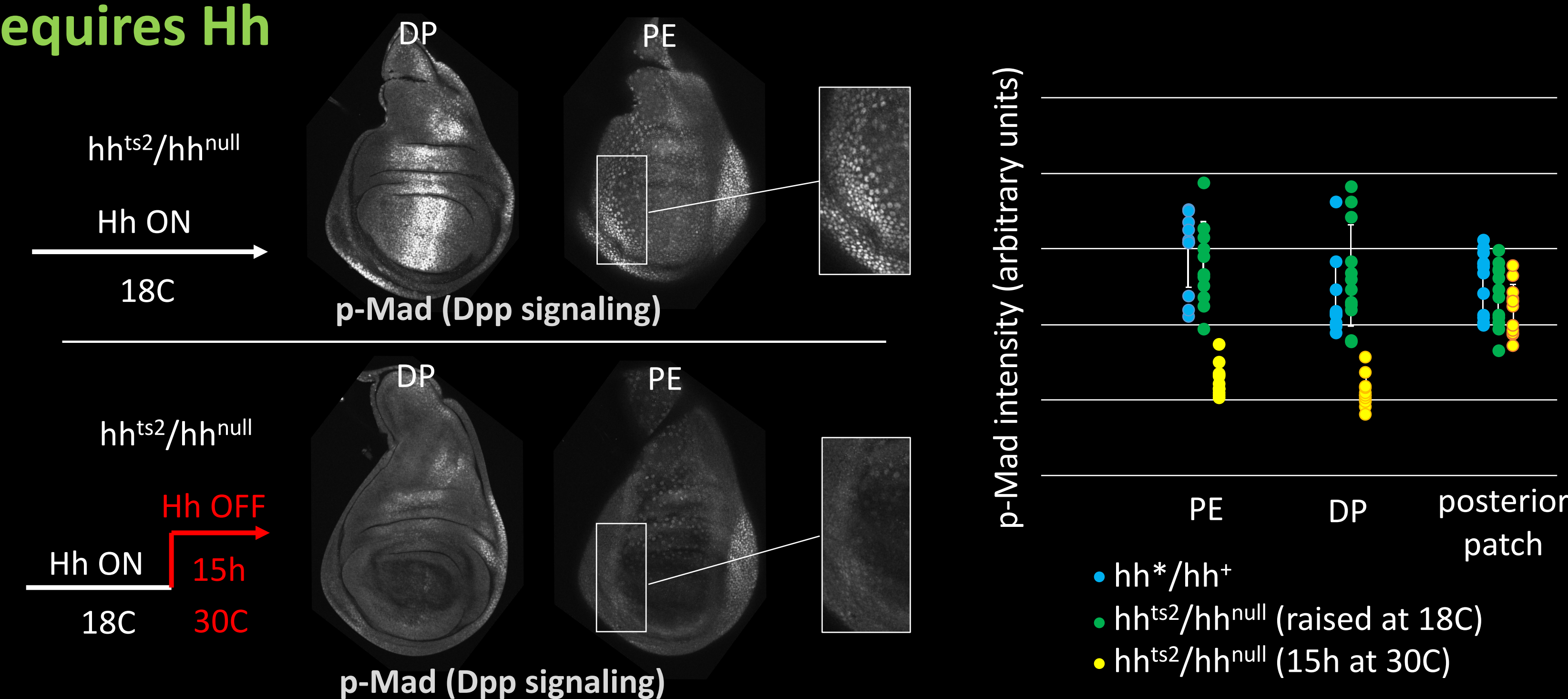
2. Hh is required for PE growth, but not during late stages



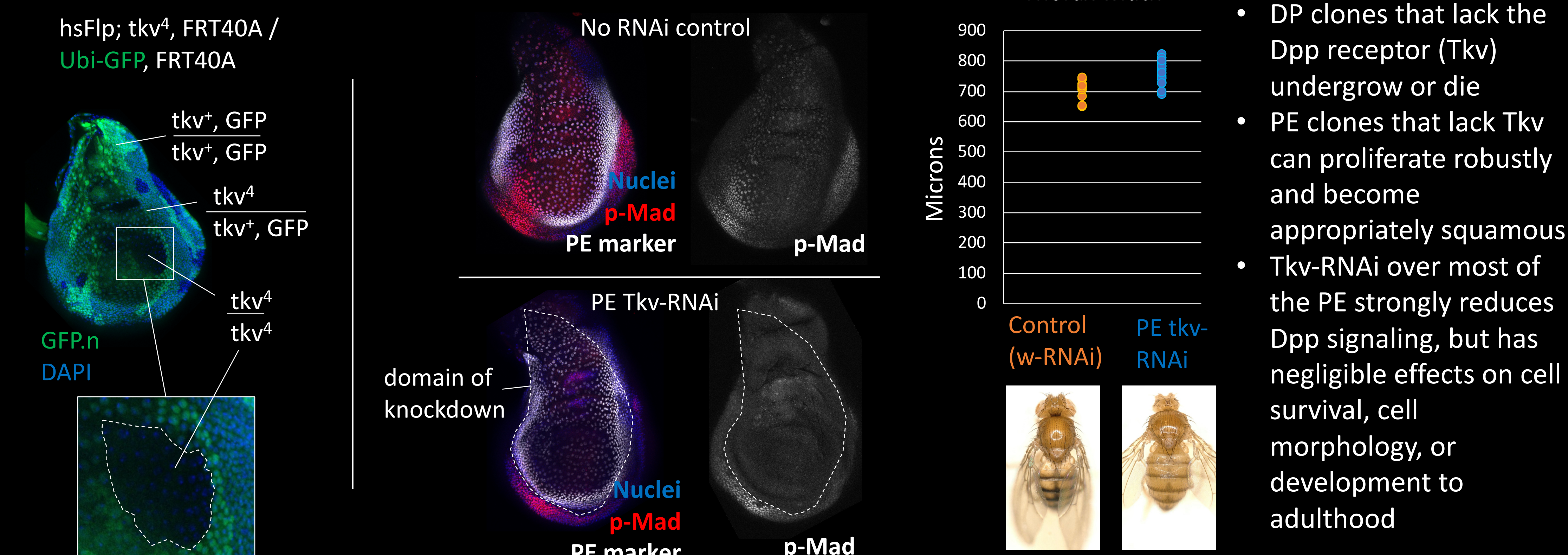
- We blocked Hh signaling in a stripe in the PE, by RNAi against the Hh receptor Smo
- Blocking PE Hh signaling throughout development causes severe PE undergrowth
- Restricting RNAi to later growth stages has no effect on PE growth

PE Dpp signaling requires Hh

- Inactivating Hh with a temperature-sensitive allele causes loss of Dpp signaling in parts of the PE (inset)
- Hh is required for Dpp expression along the compartment boundary of the PE, like in the DP

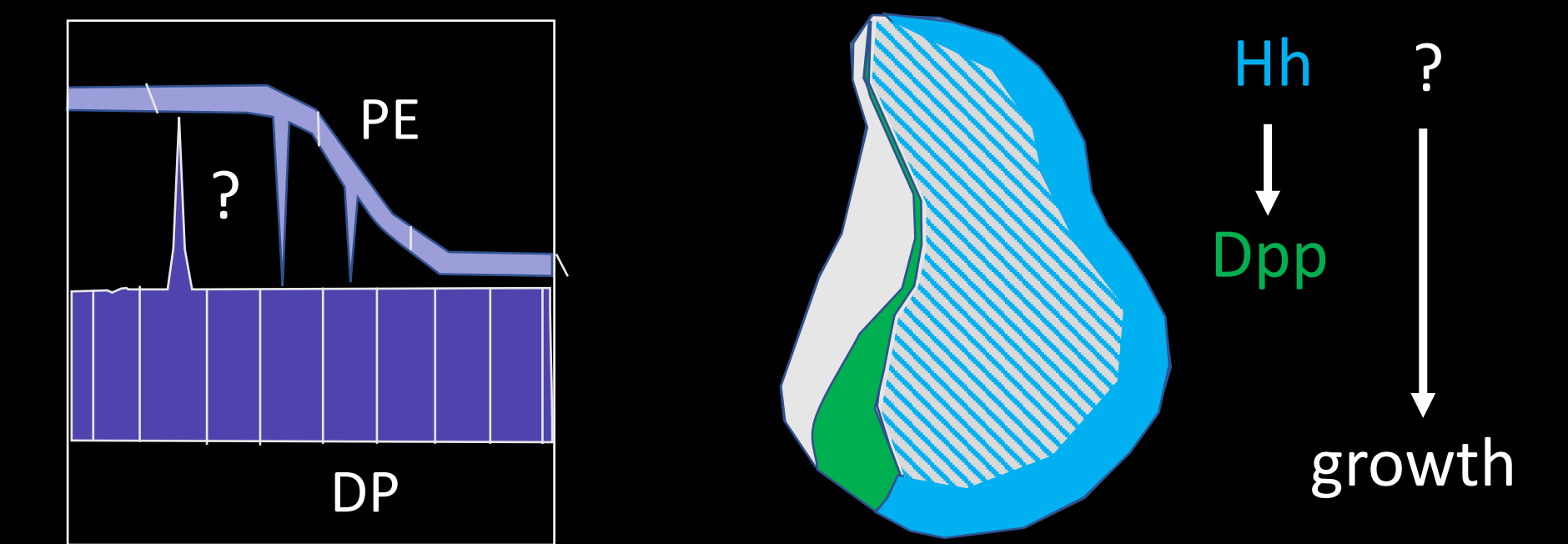


3. Surprisingly, Dpp is dispensable for PE growth



- DP clones that lack the Dpp receptor (Tkv) undergrow or die
- PE clones that lack Tkv can proliferate robustly and become appropriately squamous
- Tkv-RNAi over most of the PE strongly reduces Dpp signaling, but has negligible effects on cell survival, cell morphology, or development to adulthood

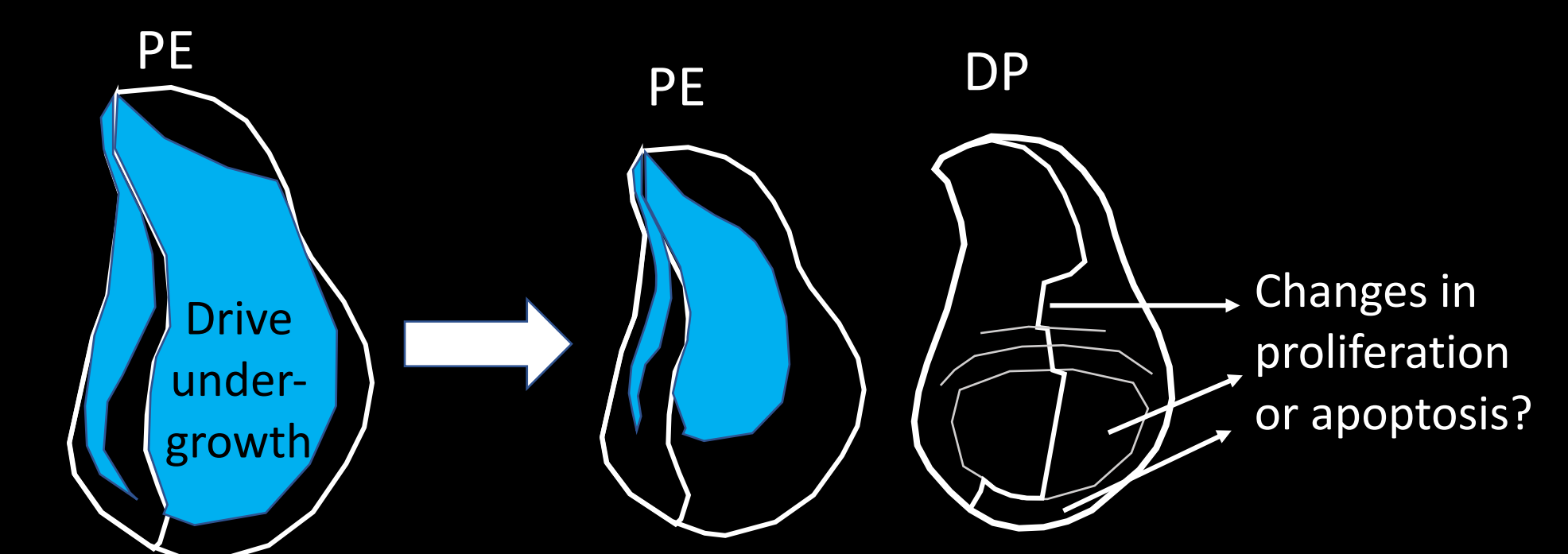
Conclusions



- The PE and DP make contact sufficient for juxtacrine signaling in specific regions
- While Hh was previously stated to be absent from the PE (Pallavi & Shashidhara 2005), it is required for early PE growth and Dpp signaling
- Surprisingly, neither Hh nor Dpp are required for PE growth at late stages

Future Directions

- Identify when Hh is required for PE growth
- Investigate which pathways control PE growth at late stages
- Determine whether endogenous Notch signaling occurs between the DP and the PE
- Directly investigate how each layer responds to changes in growth of the other by layer-specific growth perturbations



Questions? Comments?

- Q&A session: April 30, 2-2:30pm EDT
- Email: sophia_friesen@berkeley.edu

Acknowledgements

Hariharan Lab

