Quantitative single-cell imaging reveals insulation of morphogenic signal transduction

2014.07.18 | **Adam Coster**
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Dissertation Defense

Today's talk

1. Cell signaling
2. Single-cell image analysis
3. Disentangling **signaling** from **transcriptional** crosstalk in morphogenic pathways
4. Summary

On cell signaling

Some talk-specific definitions

Signaling

The transfer of information from the environment to a cell

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Processing

The transfer of information from one molecular state to another

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The set of protein-protein and protein-chromatin interactions within the nucleus that result in changes to expression

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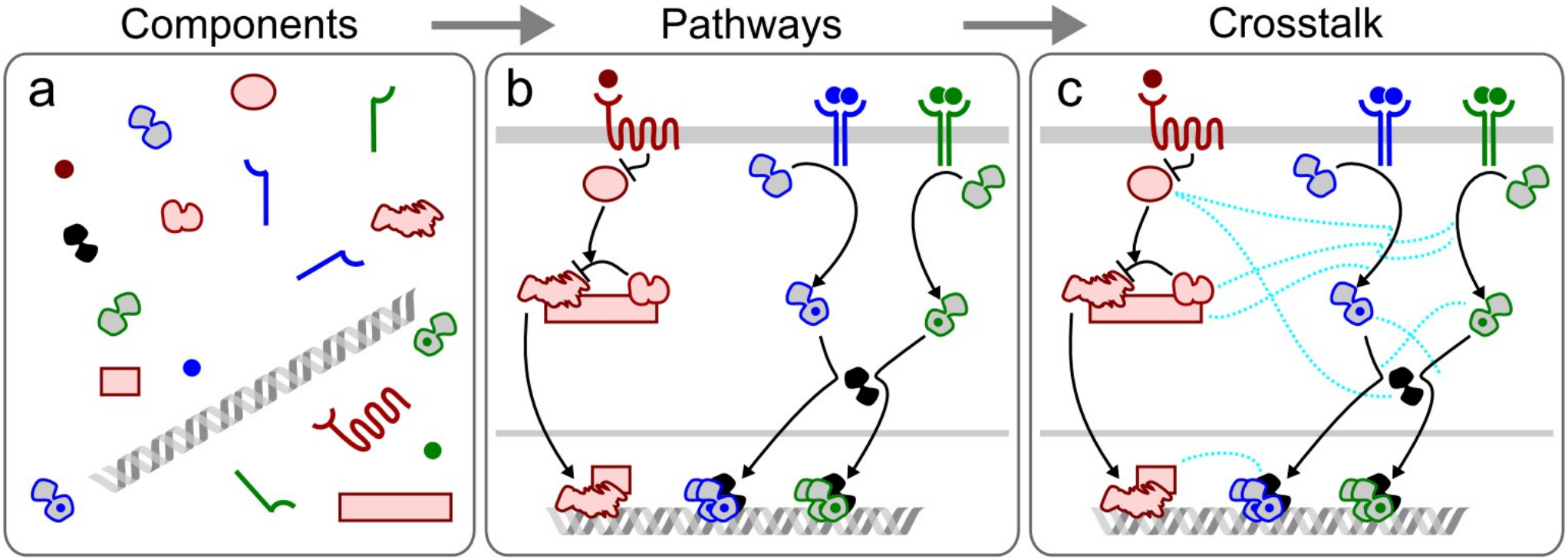
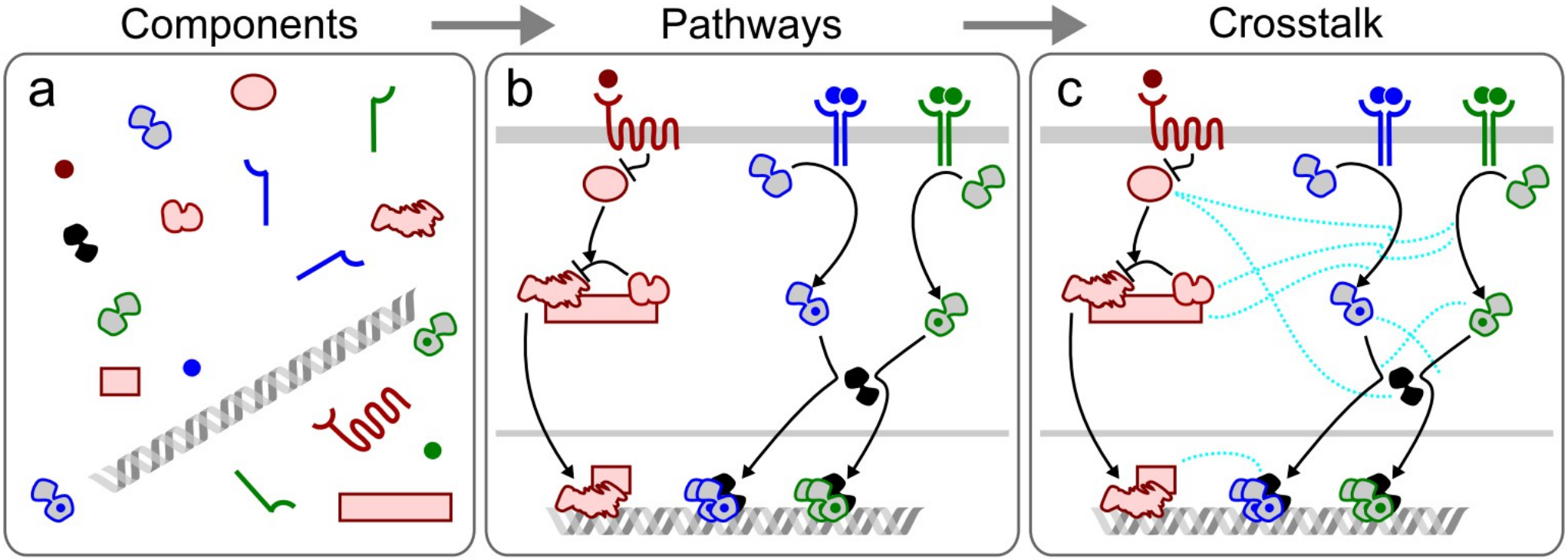
Decision-making
The collapse of all possible outcomes into a subset of outcomes

How we define cell signaling pathways



Dissertation Fig. 1.1

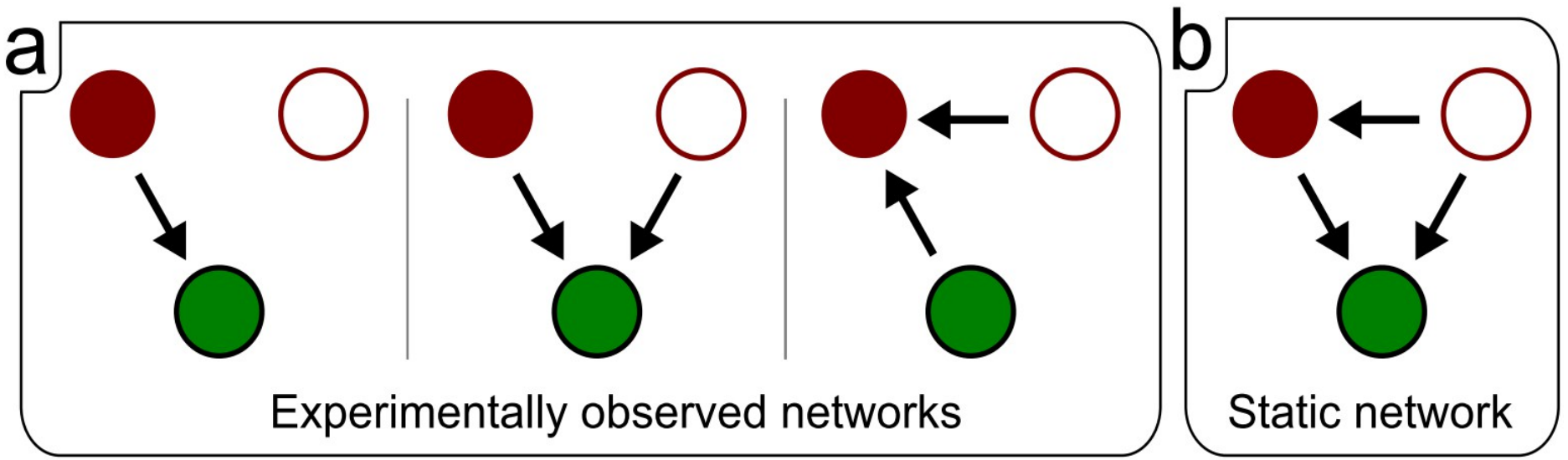
How we define cell signaling pathways



Dissertation Fig. 1.1

- Time often missing;
- Ambiguous arrows;
- Summarizes many experiments.

But! Static signaling networks may not reveal true networks



Dissertation Fig. 1.2

e.g. Polarity in neutrophils,
and the later case study

On single-cell imaging

Why imaging? (besides the beauty?)

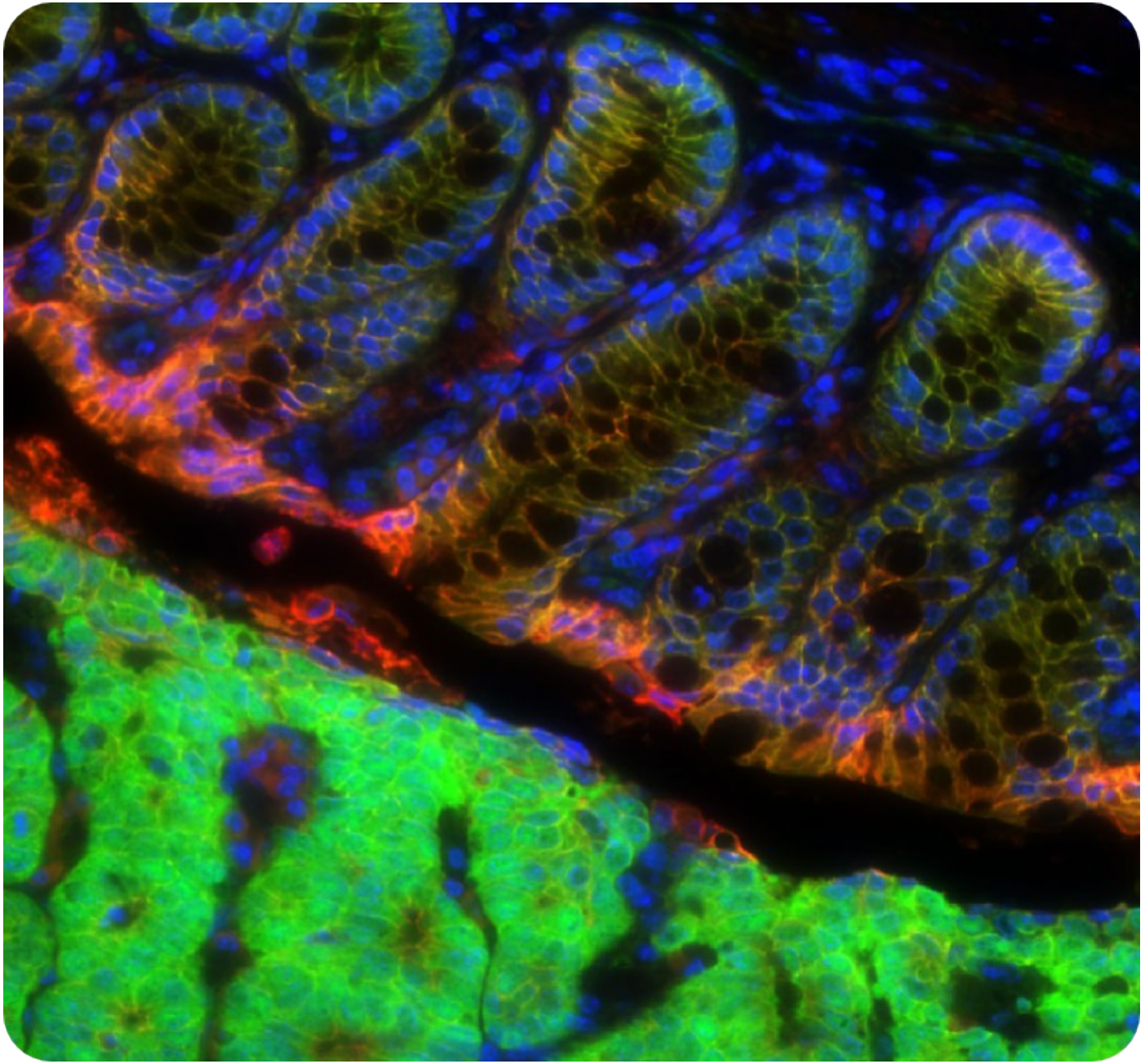


Image courtesy C. Thorne and M. Ramirez (A/W Lab) DNA,β-catenin,E-cadherin

Why imaging?

- Single-cell and sub-cellular resolution
  (cells are not homogeneous!)
- High data dimensionality
  (can measure 10^2-10^3 single-cell properties)
- High throughput
  (can rapidly obtain data from many perturbations)

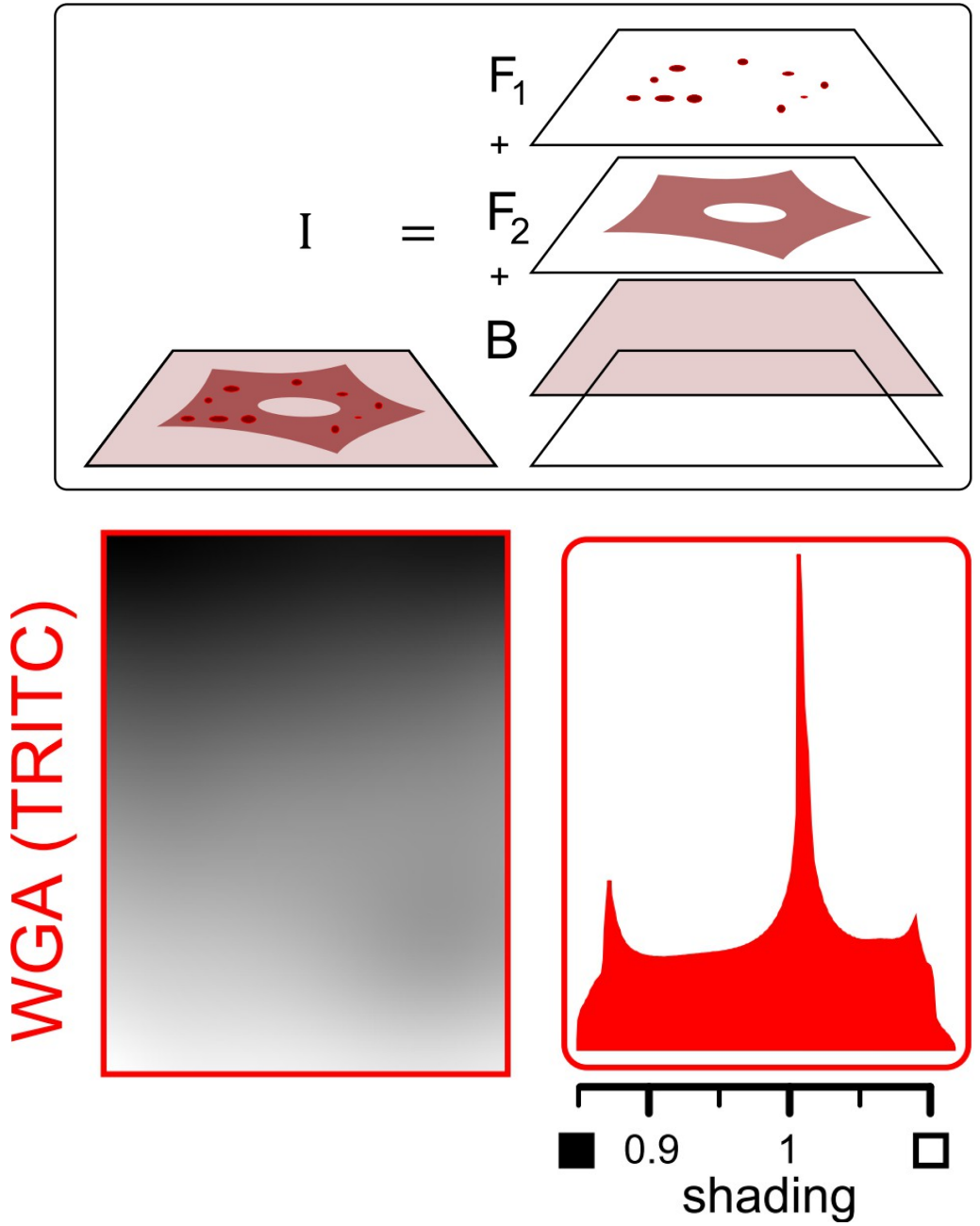
But there are problems...

- Single-cell and sub-cellular resolution
  (artifacts and heterogeneity are hard to interpret)
- High data dimensionality
  (what should we even measure? WHAT DOES IT MEAN?)
- High throughput
  (how do we deal with all the data?)

Mis-interpretation is easy

So how do we extract meaningful, believable data?

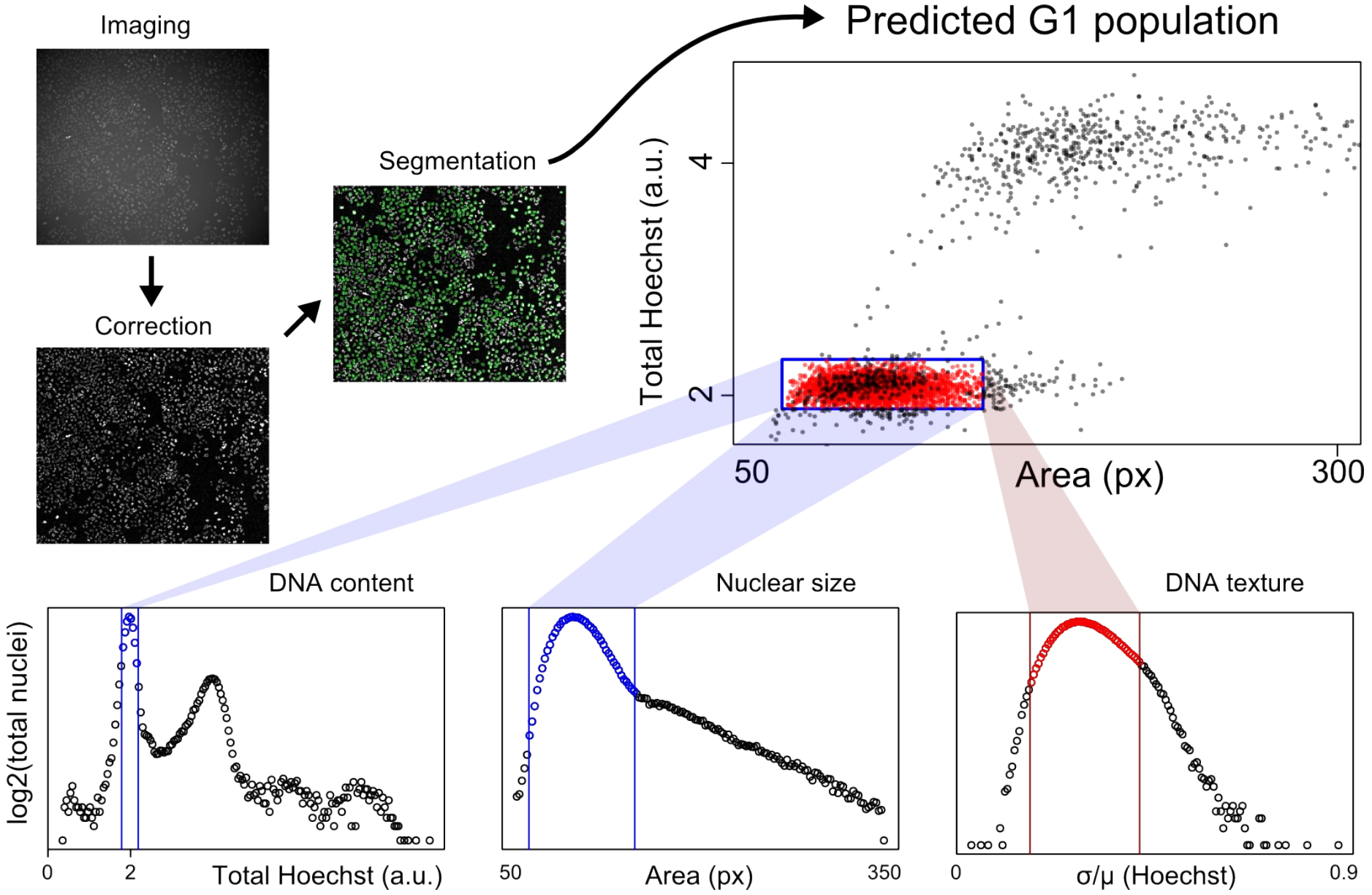
First step: removing non-**F**oreground



Dissertation Figs. 4.1 and 4.4

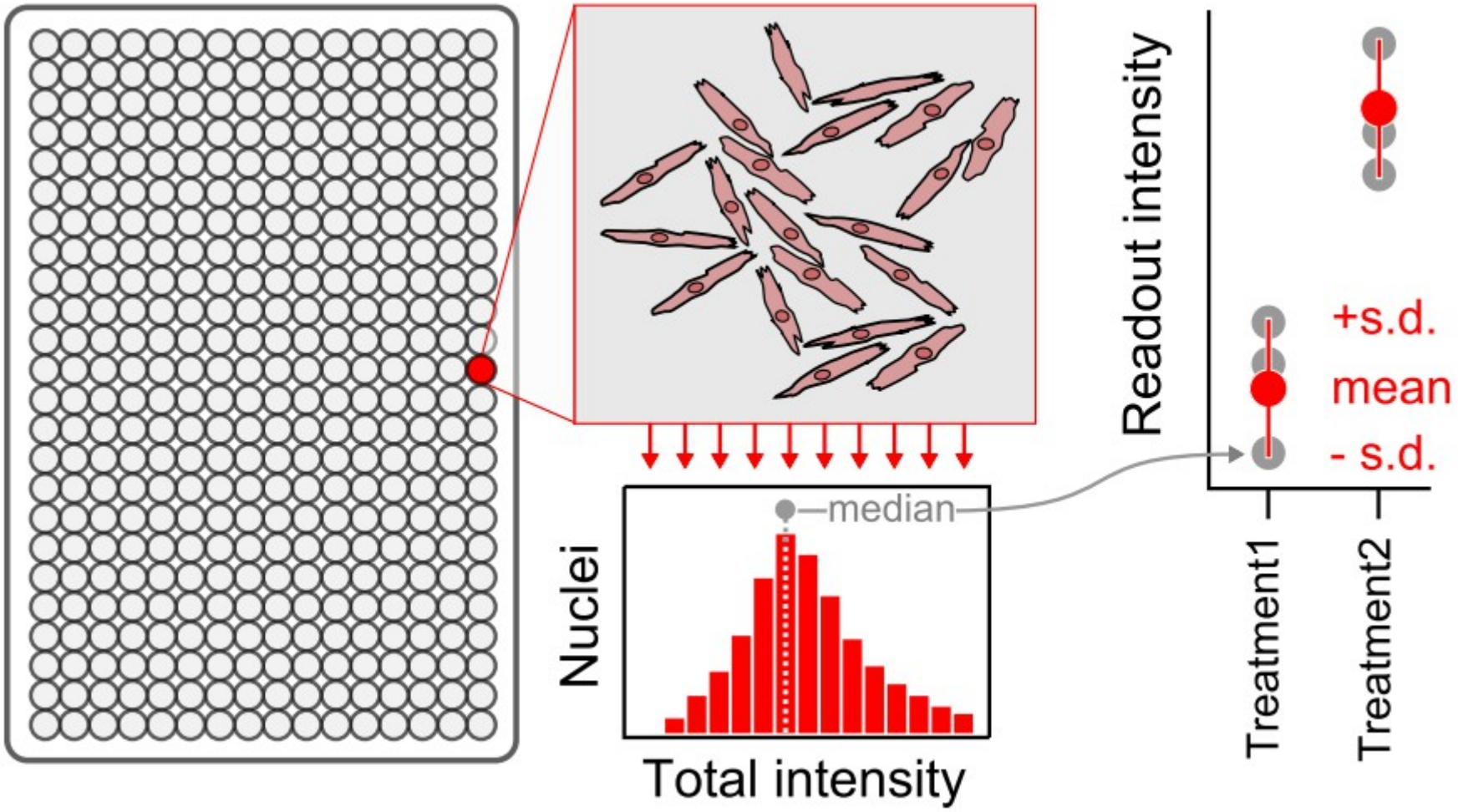
Nature Methods (2014). 11(6), 602.

Second step: finding single cells



(Schematic – Images and plots from different experiments.)

Third step: simplifying the data

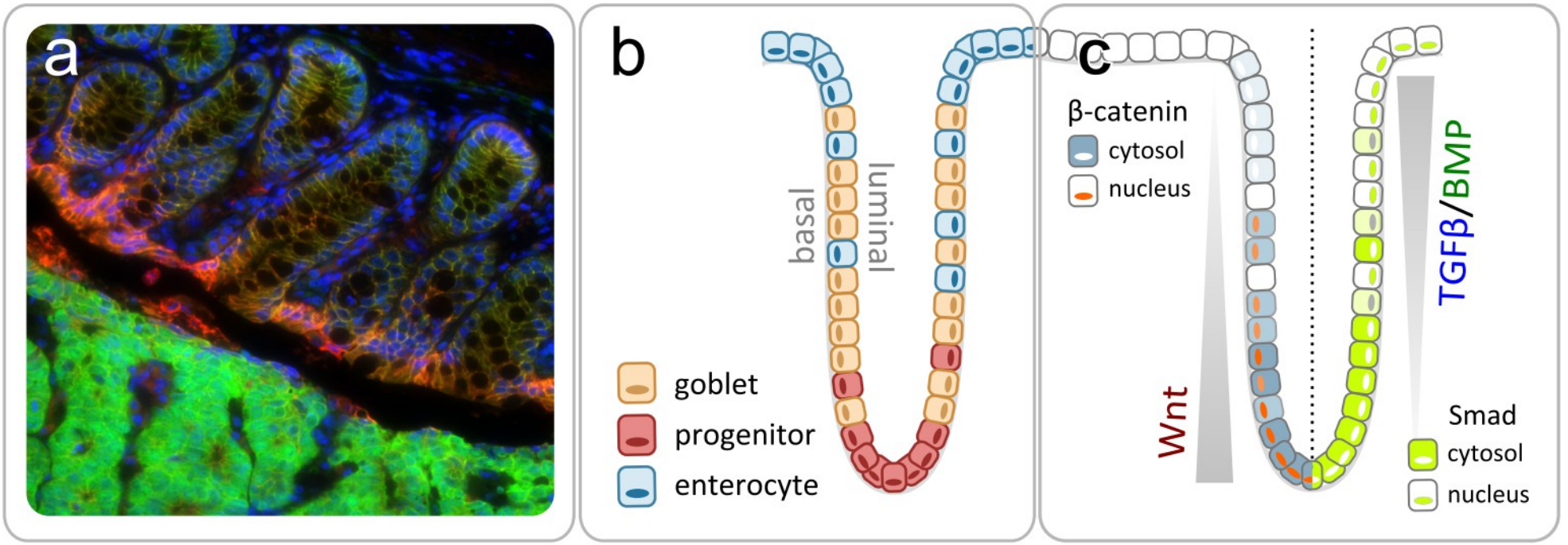


Dissertation Fig. 3.3

Disentangling **signaling**
from **transcriptional** crosstalk
in morphogenic pathways

(a case study of Wnt, TGFB, and BMP)

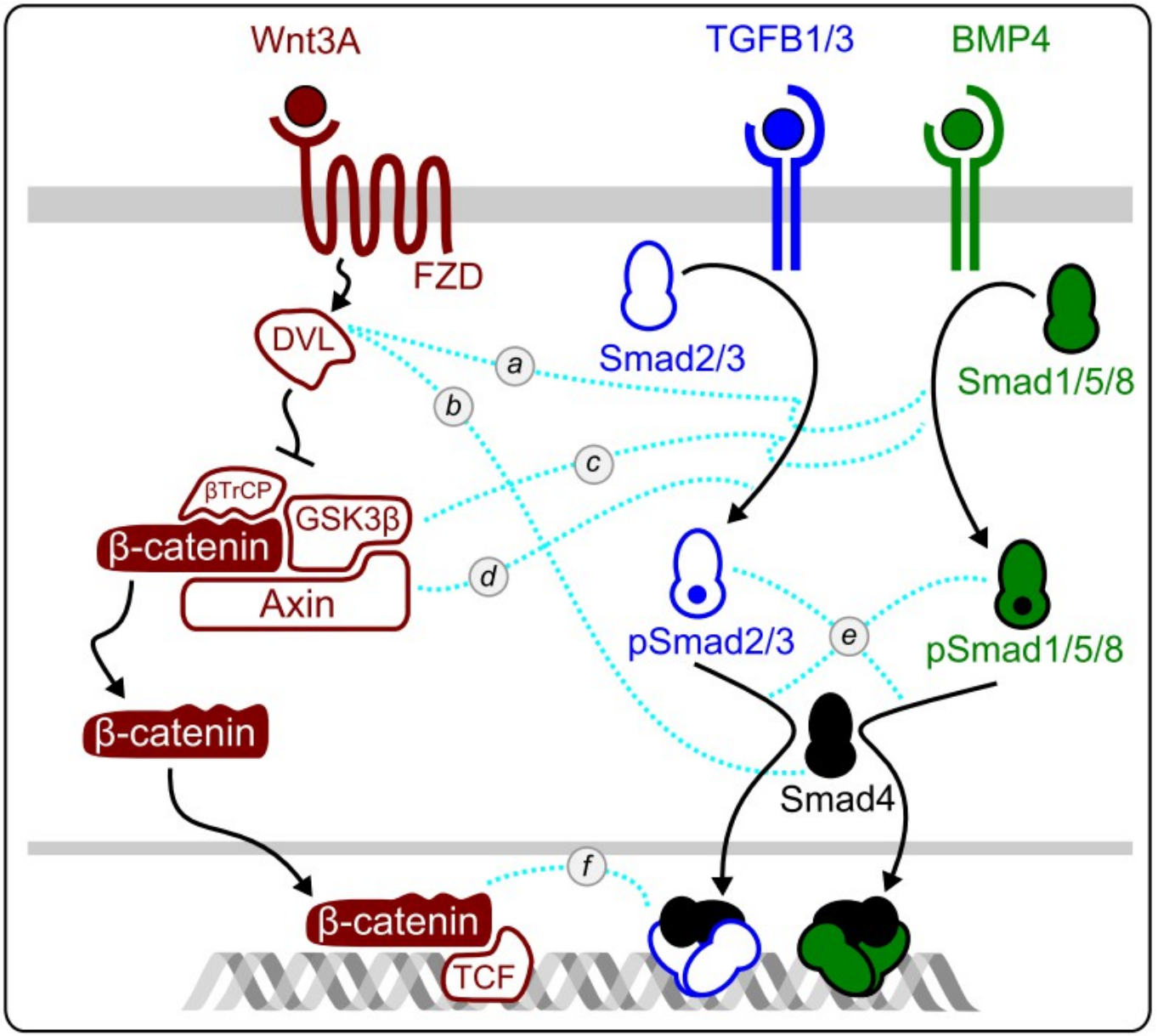
Motivation: **TGFB**, **BMP**, and **Wnt** are essential to tissue homeostasis and misregulated in many diseases



Dissertation Fig. 2.5. Image and cartoon of colonic crypts.

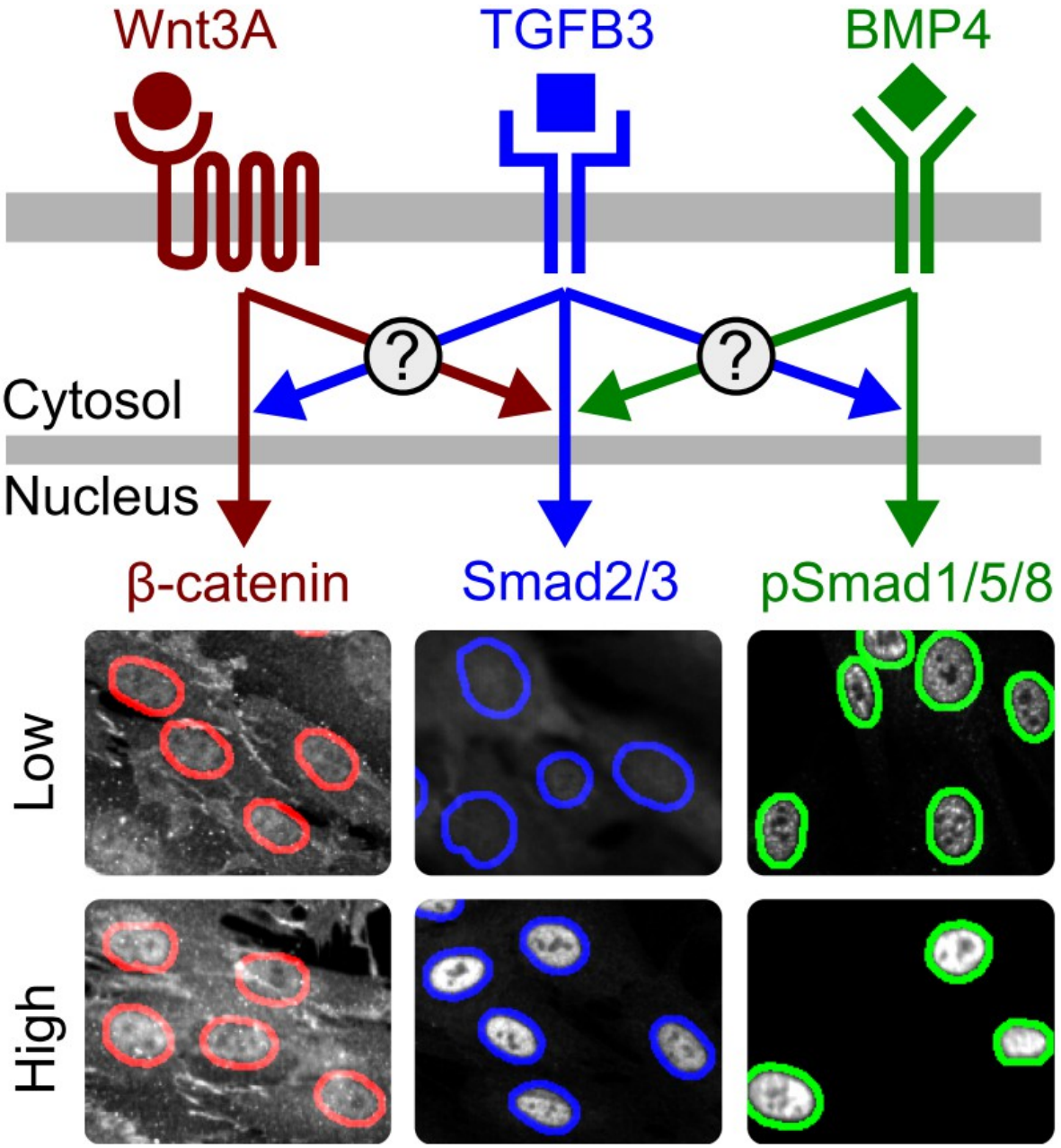
How do cells **integrate** these signals to **make decisions**?

Overview of putative Wnt/TGFB/BMP transduction crosstalk



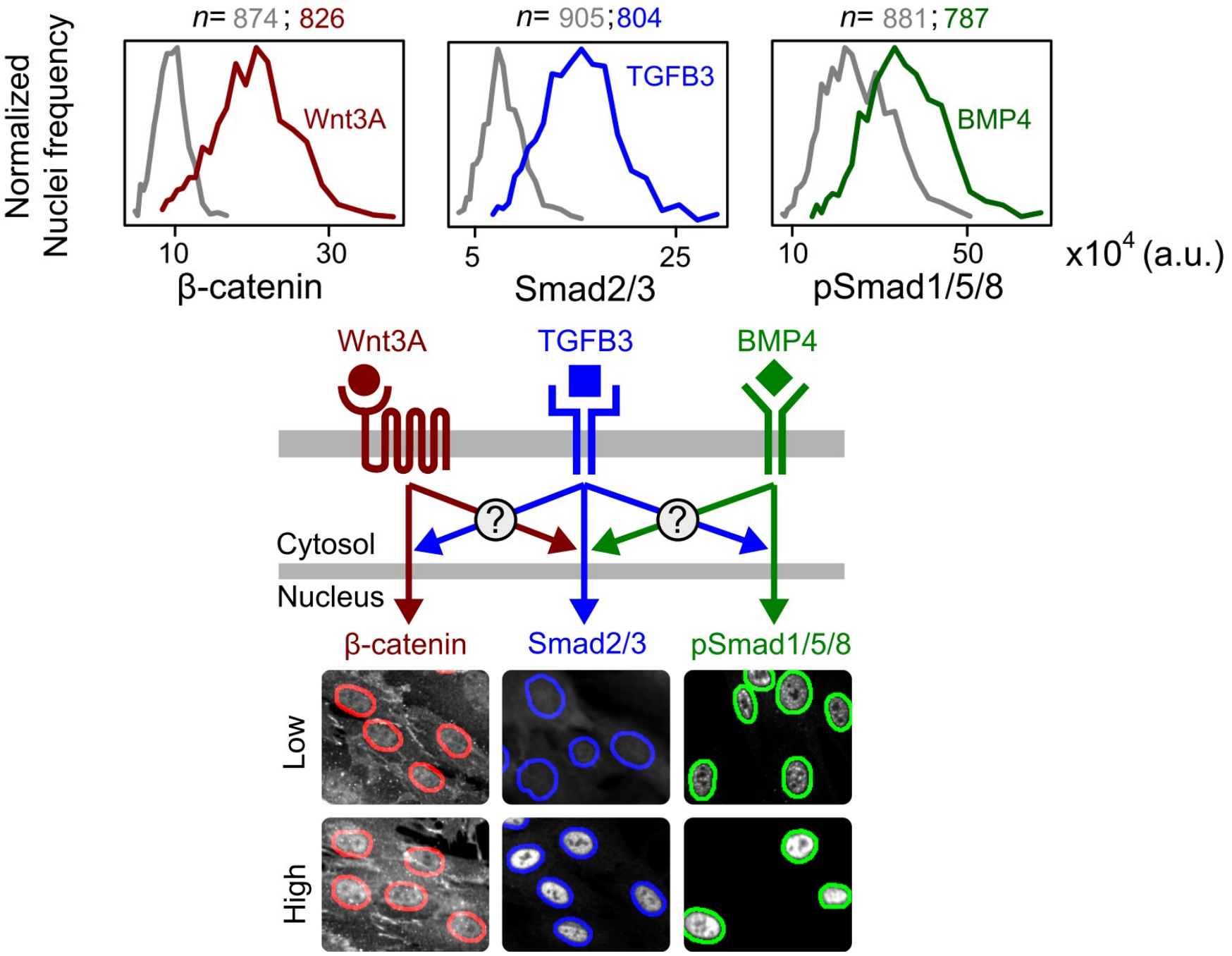
Dissertation Fig. 2.6

Are signals really integrated during transduction?



Dissertation Fig. 3.2

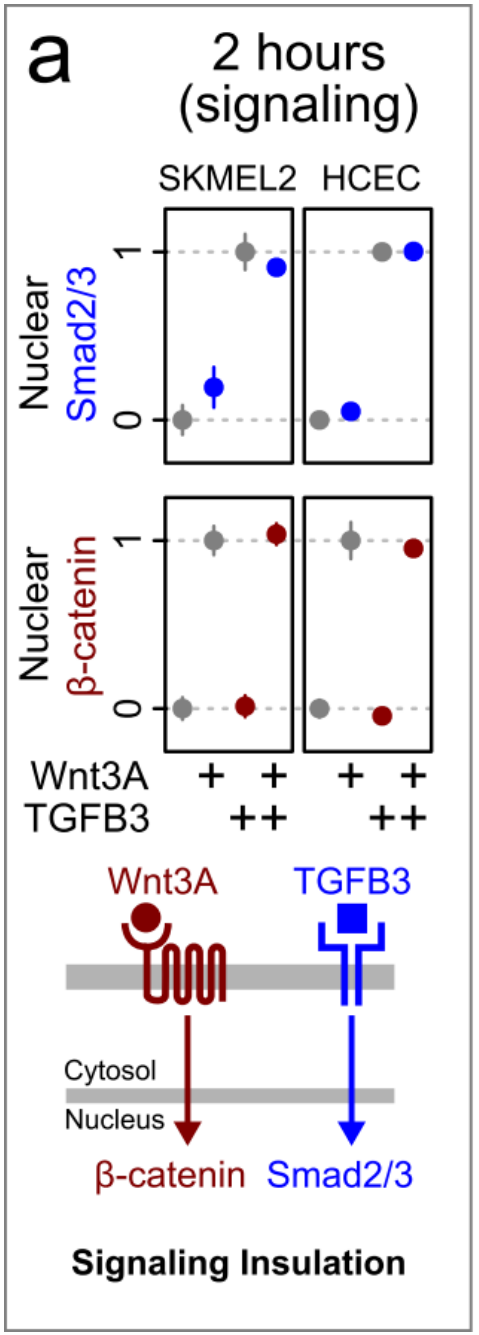
The experiment

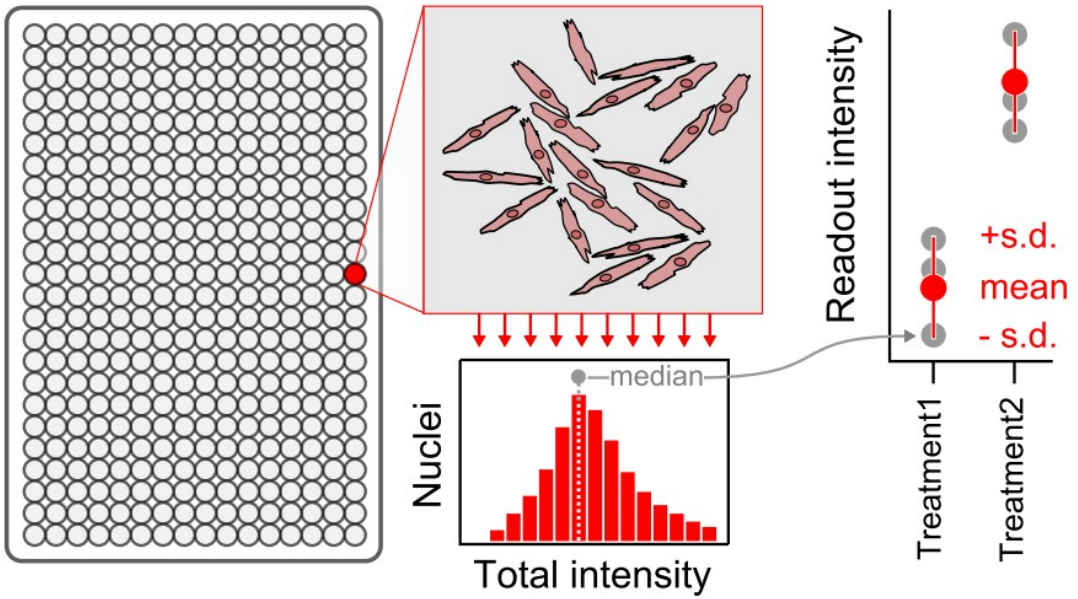


Take a breath...

Things are about to get **REAL**ly data-dense

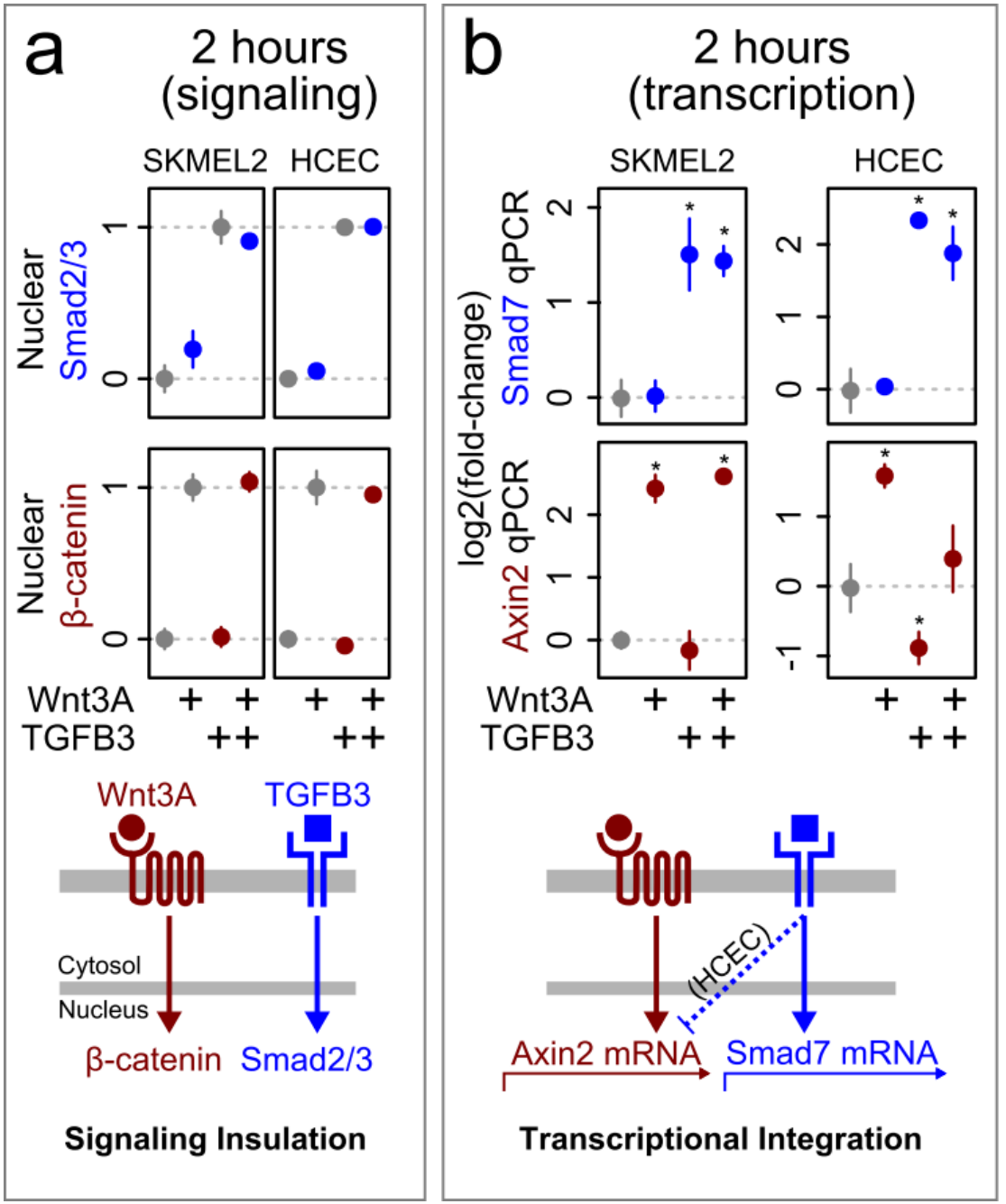
Insulation of Wnt3A/TGFB3 during signal transduction





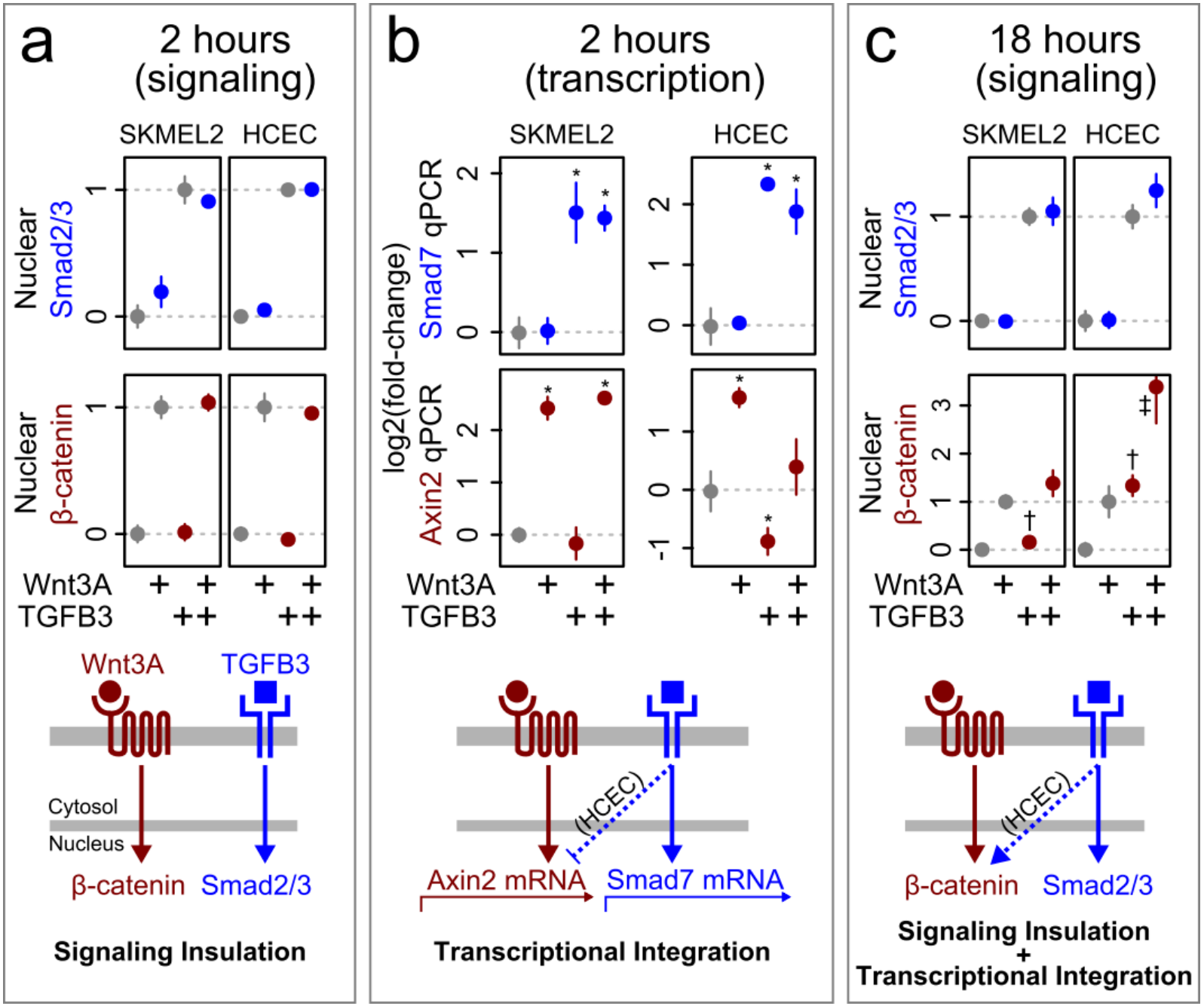
Dissertation Fig. 3.12

Crosstalk of Wnt3A/TGFB3 during transcription



Dissertation Fig. 3.12

Biasing of Wnt3A by long-term TGFB3 treatment

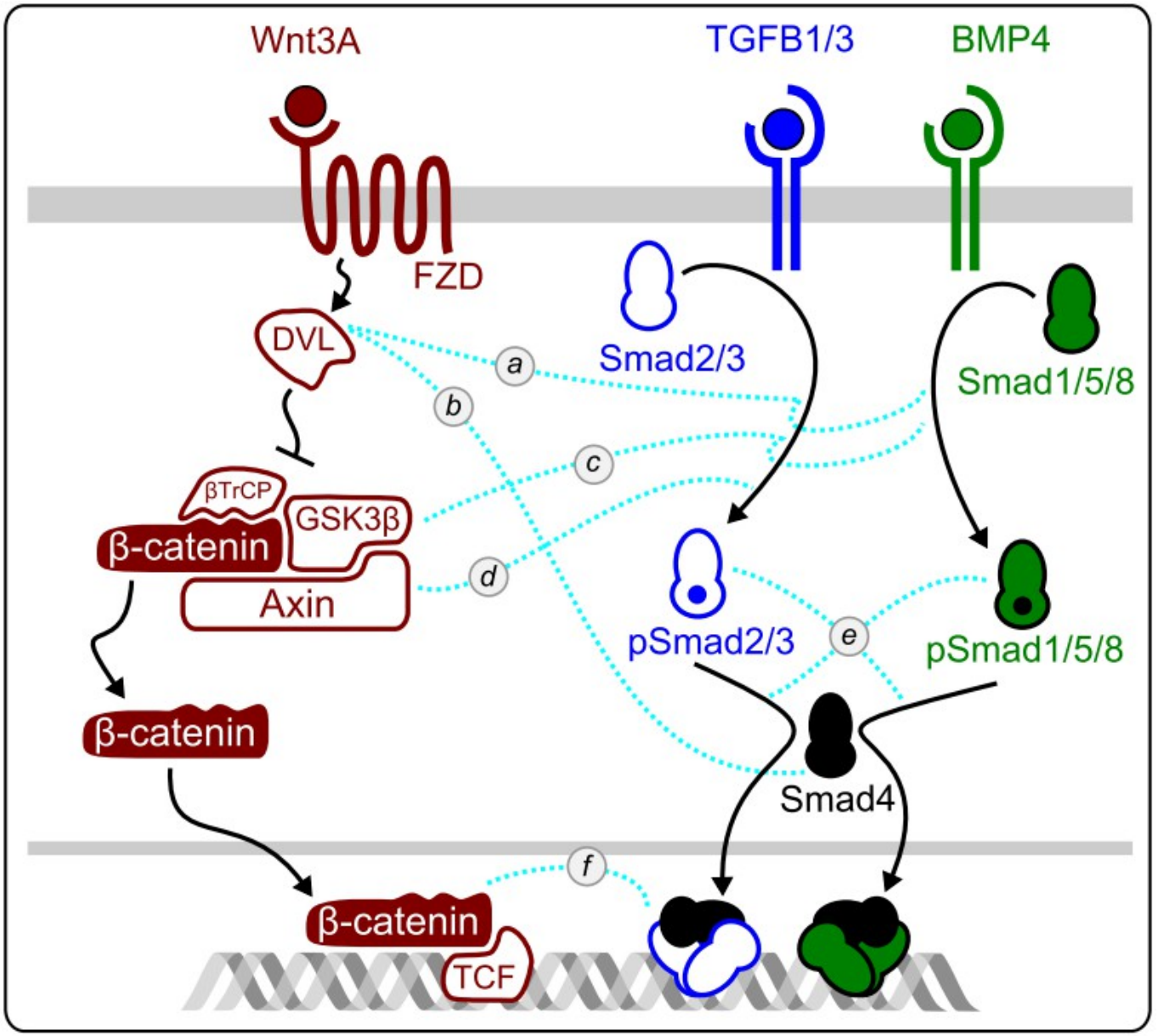


Dissertation Fig. 3.12

Therefore:

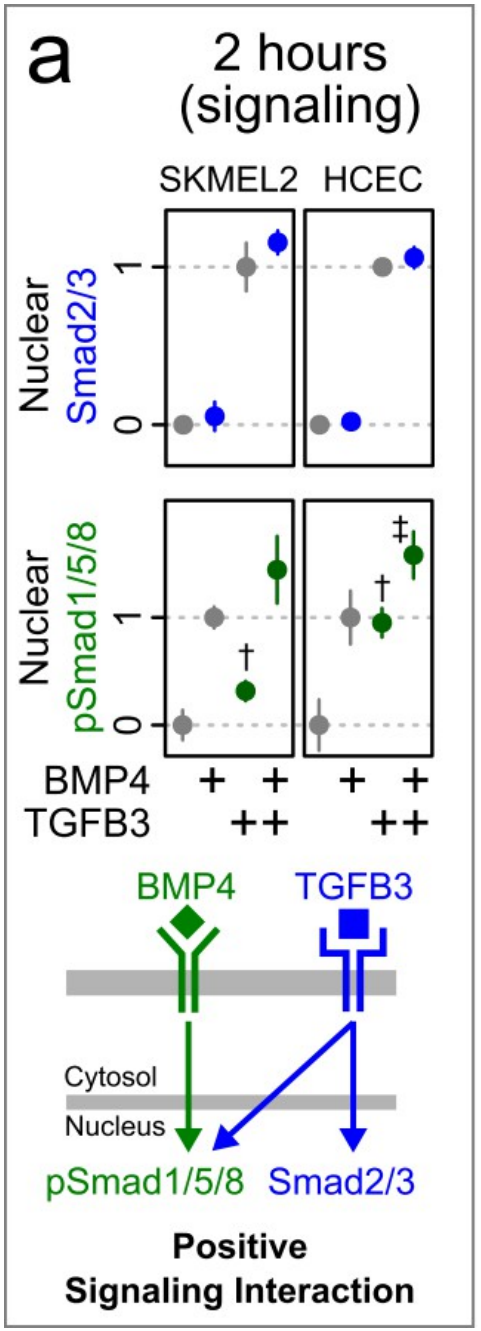
- Wnt/TGFB may be generally insulated during **transduction**
- Wnt/TGFB are idiosyncratically integrated during **translation**
- By conflating transduction/translation we may infer complete idiosyncrasy!

Reminder...



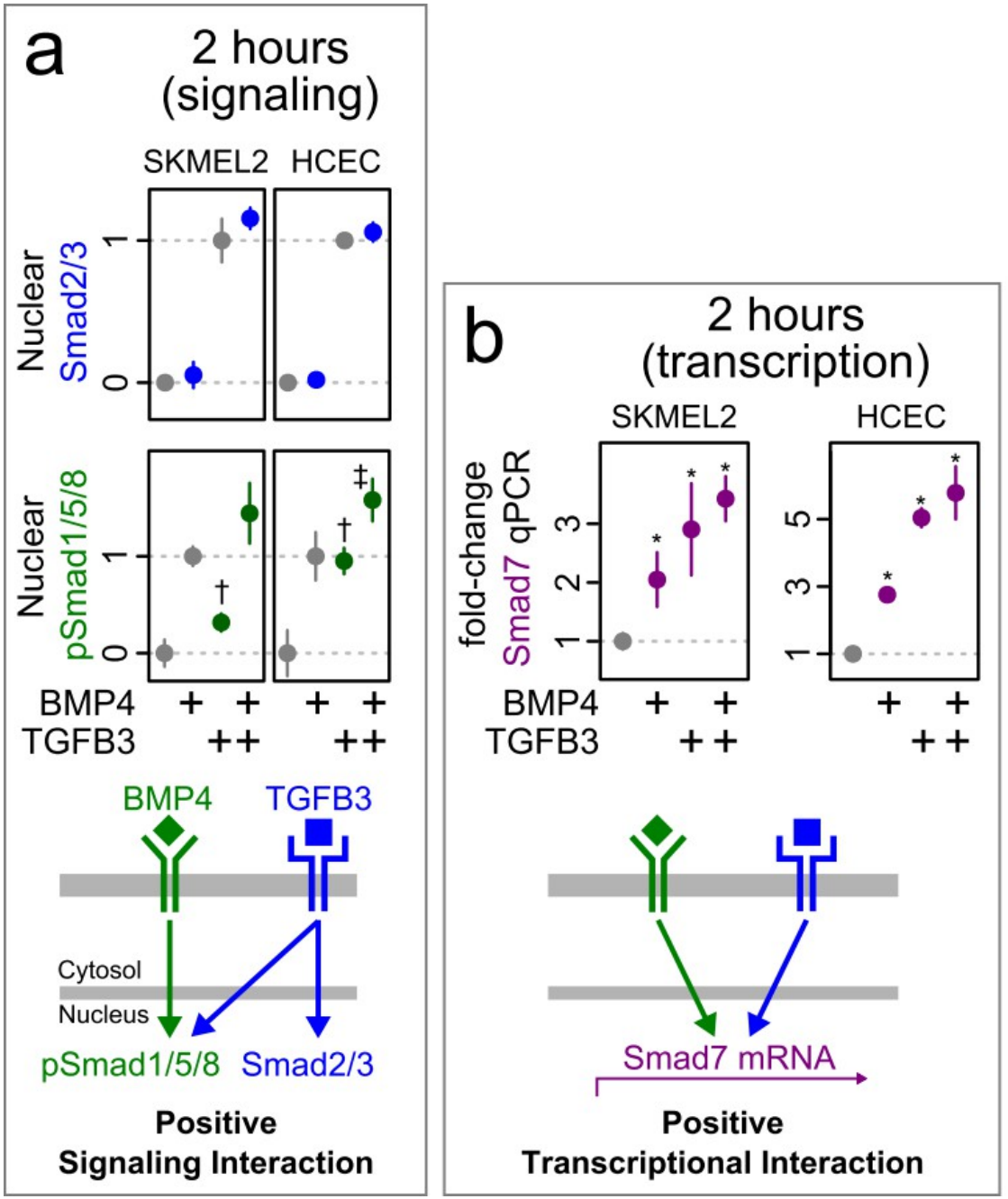
Dissertation Fig. 2.6

Non-negative BMP4/TGFB3 signal integration



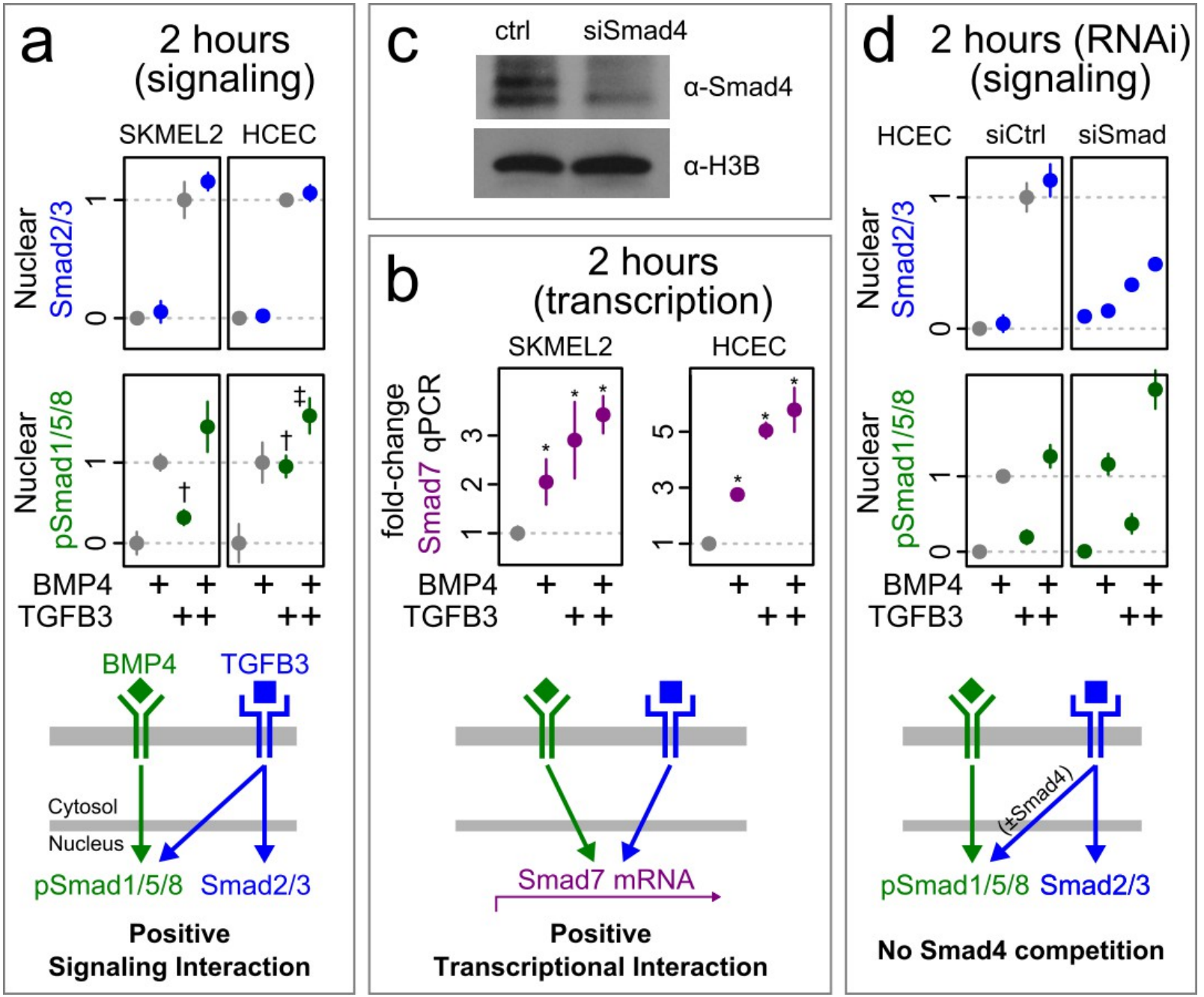
Dissertation Fig. 3.15

Non-negative BMP4/TGFB3 signal integration



Dissertation Fig. 3.15

BMP4/TGFB3 do not compete for Smad4



Dissertation Fig. 3.15

Therefore:

- BMP4/TGFB interact **positively** or **not at all** during **transduction**
- Smad4 levels do not change the interaction;
- BMP4/TGFB do not compete for Smad4;
- As before, long-term idiosyncracies may be due to conflation with **transcription!**

Summary

Single-cell imaging

- Reveals dramatic heterogeneity in single-cell behaviors
- Can be robust, quantitative, and meaningful
- Lacks standard methods and controls
  for interpretation of single-cell features.

Morphogenic signaling insulation

- TGFB/Wnt do not interact during transduction
- TGFB/BMP do not negatively interact during transduction
- Morphogenic transduction interactions may be sparse!

Future directions

- Are we missing general features of cell signaling?
- Is **concentration** the appropriate encoding for TGFB/Wnt?
- How common is pathway insulation?

THANKS!

Altschuler & Wu Lab

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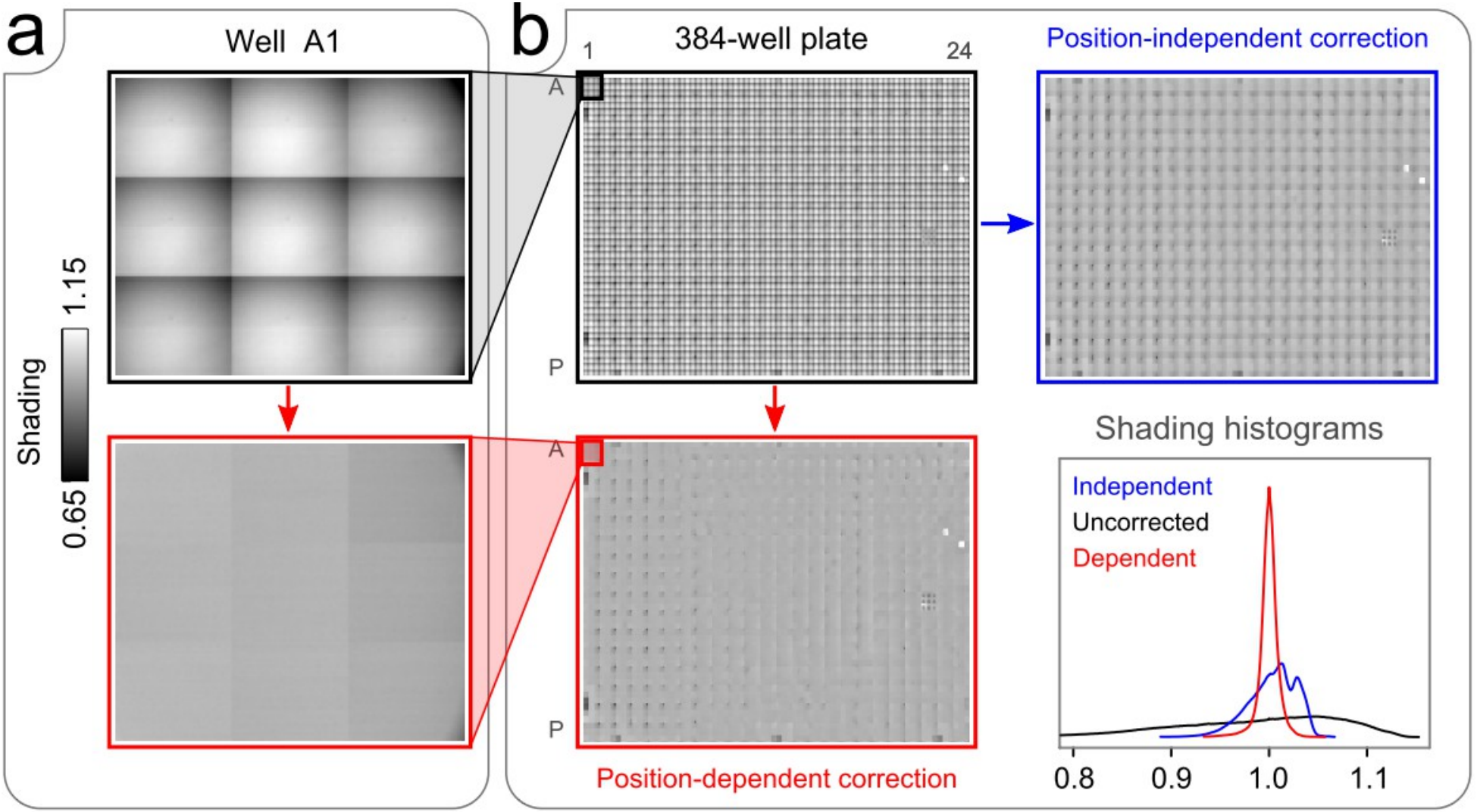
Reagents

Jerry Shay (HCECs)

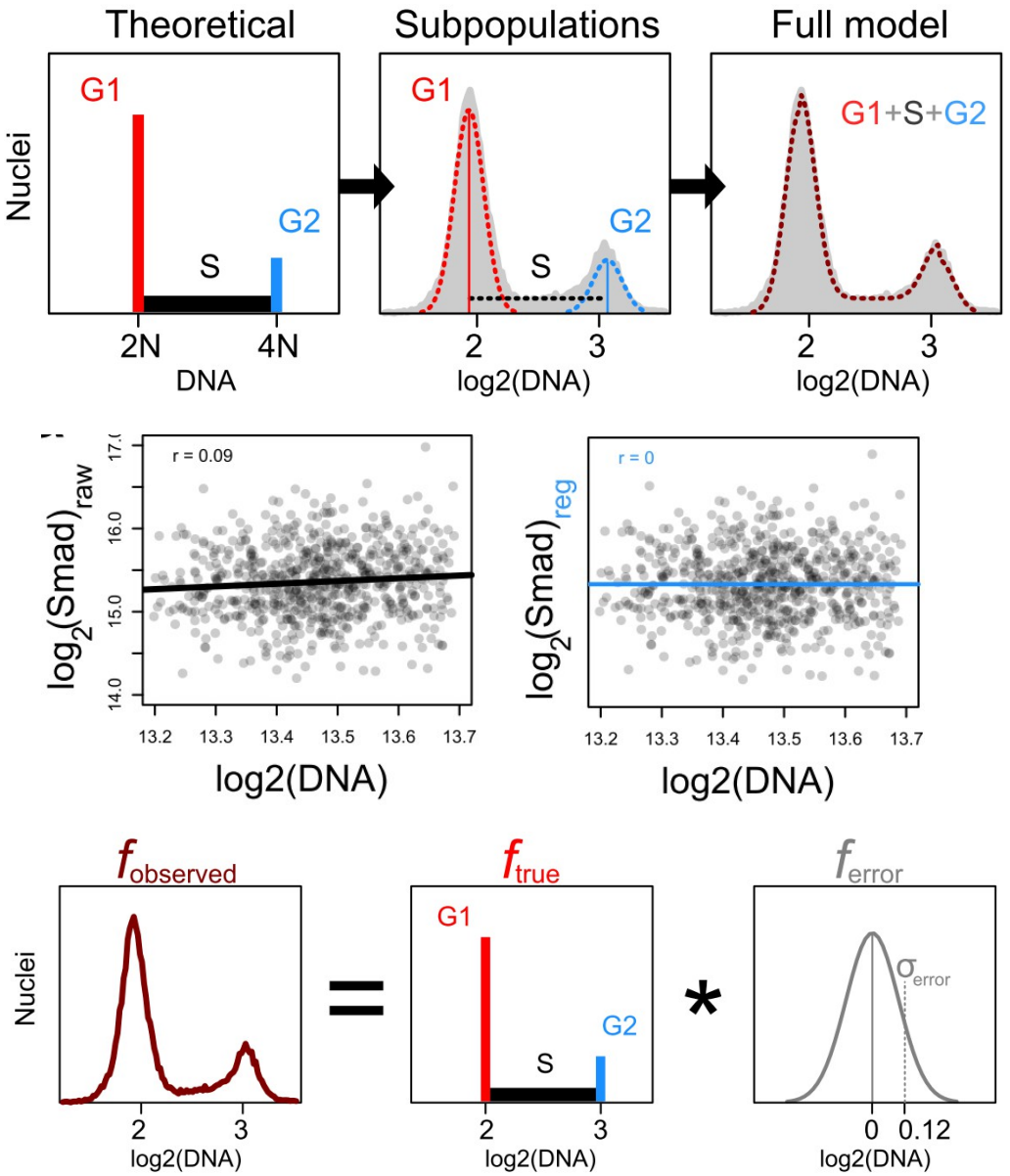
Supplemental Slides

Positional microwell plate image correction



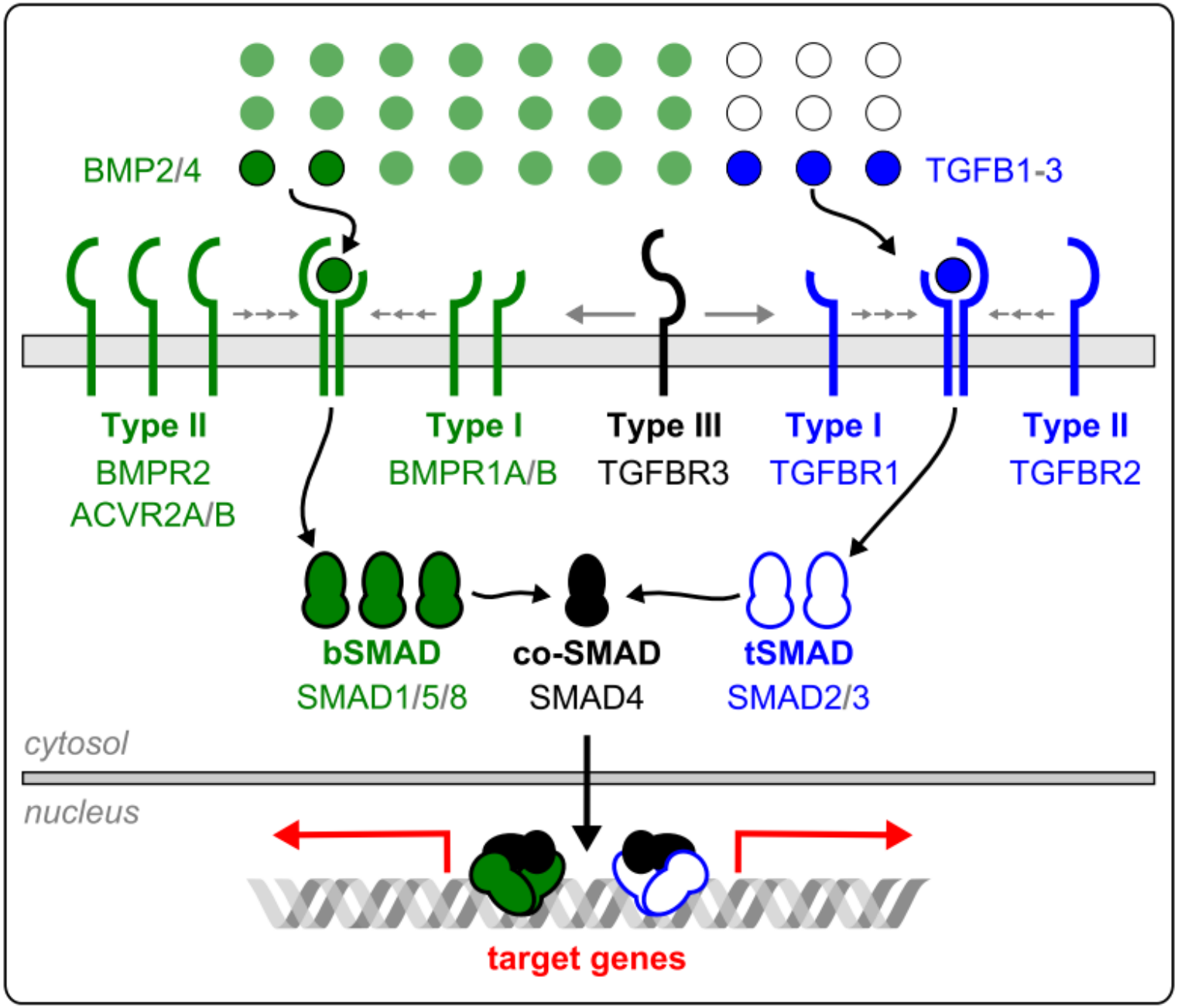
Dissertation Fig. 4.7

Single-cell correction using cell cycle and Hoechst staining



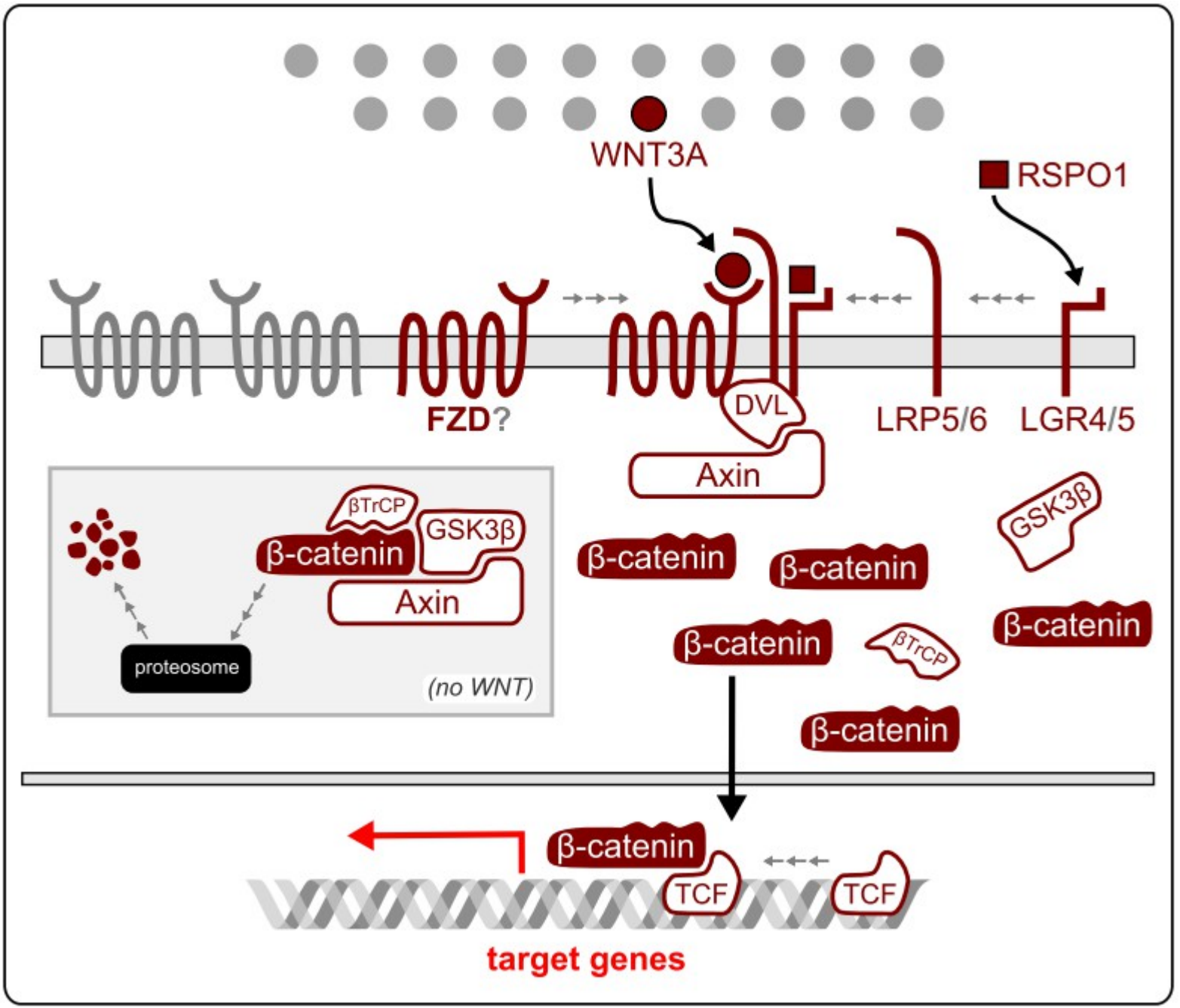
Dissertation Figs. 4.12 and 4.15

TGFB/BMP pathway overview



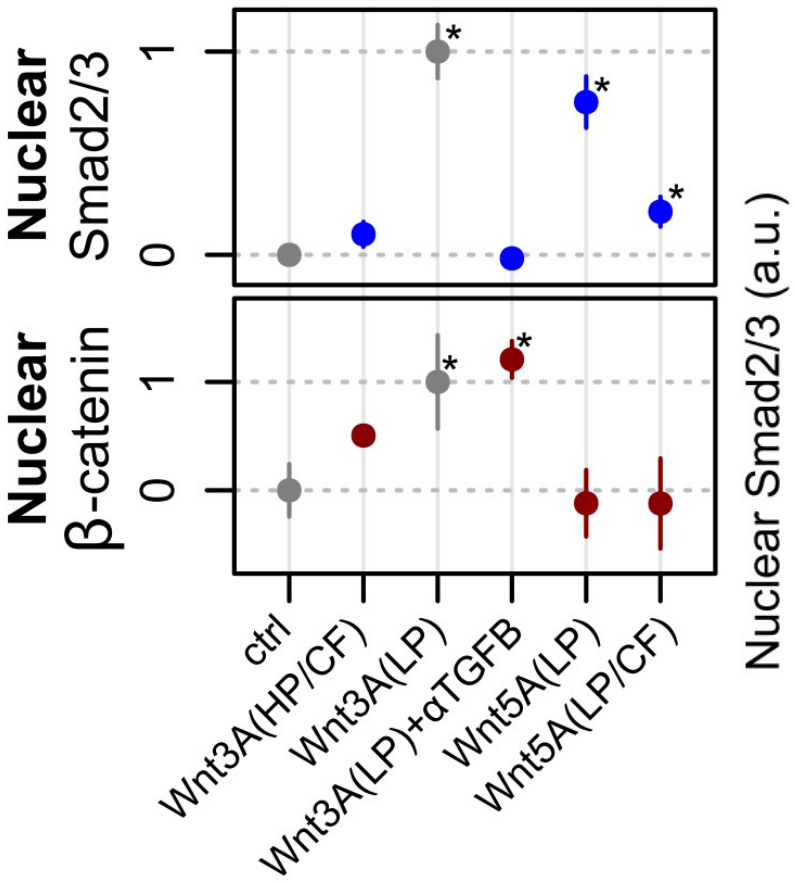
Dissertation Fig. 2.1

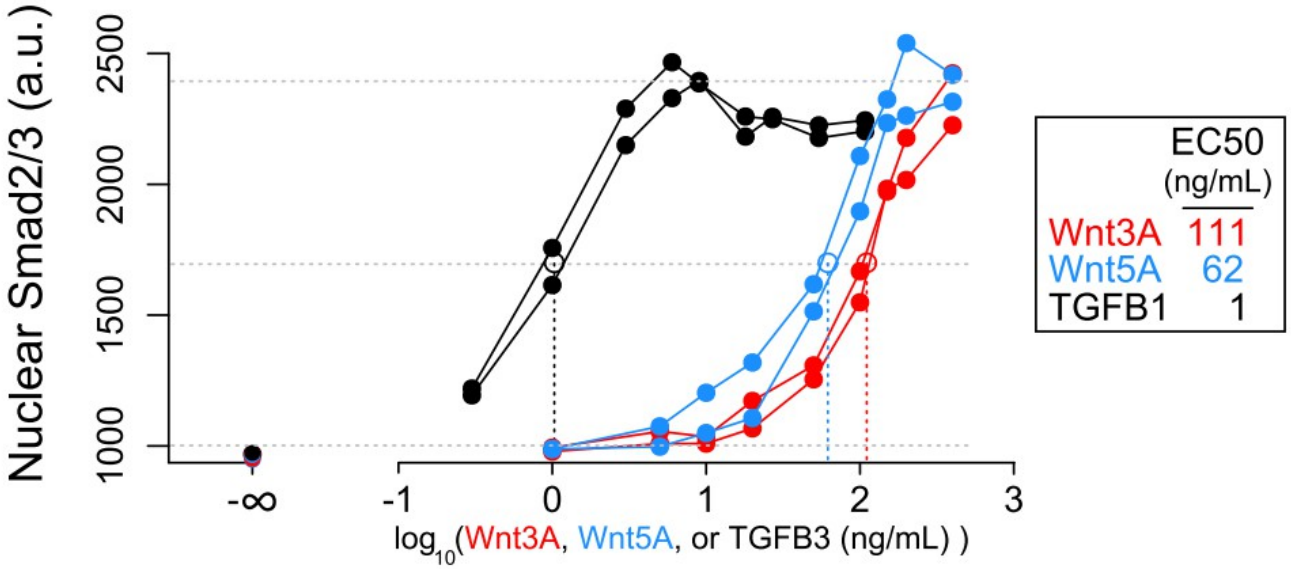
Wnt/B-catenin pathway overview



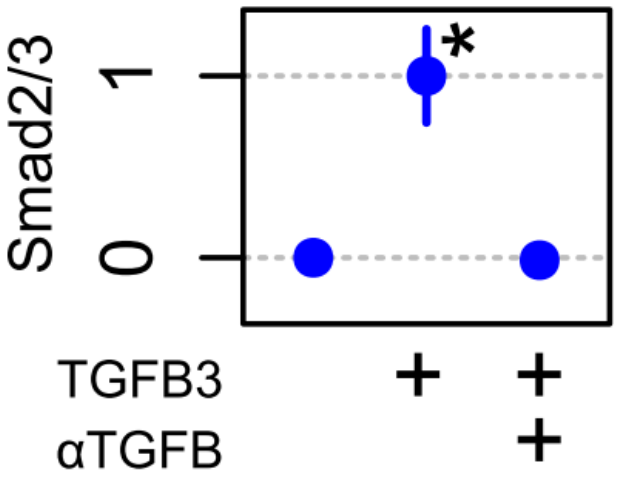
Dissertation Fig. 2.3

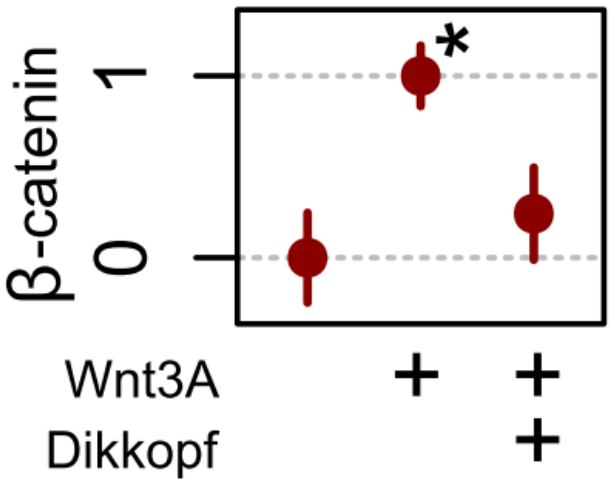
Low-purity Wnts (R&D Biosystems) contain TGFB

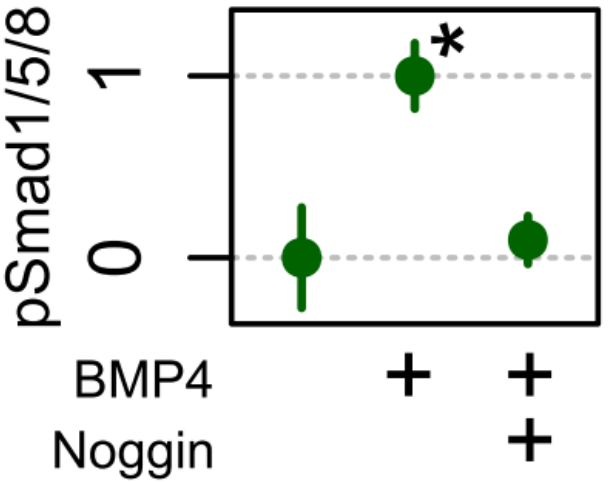




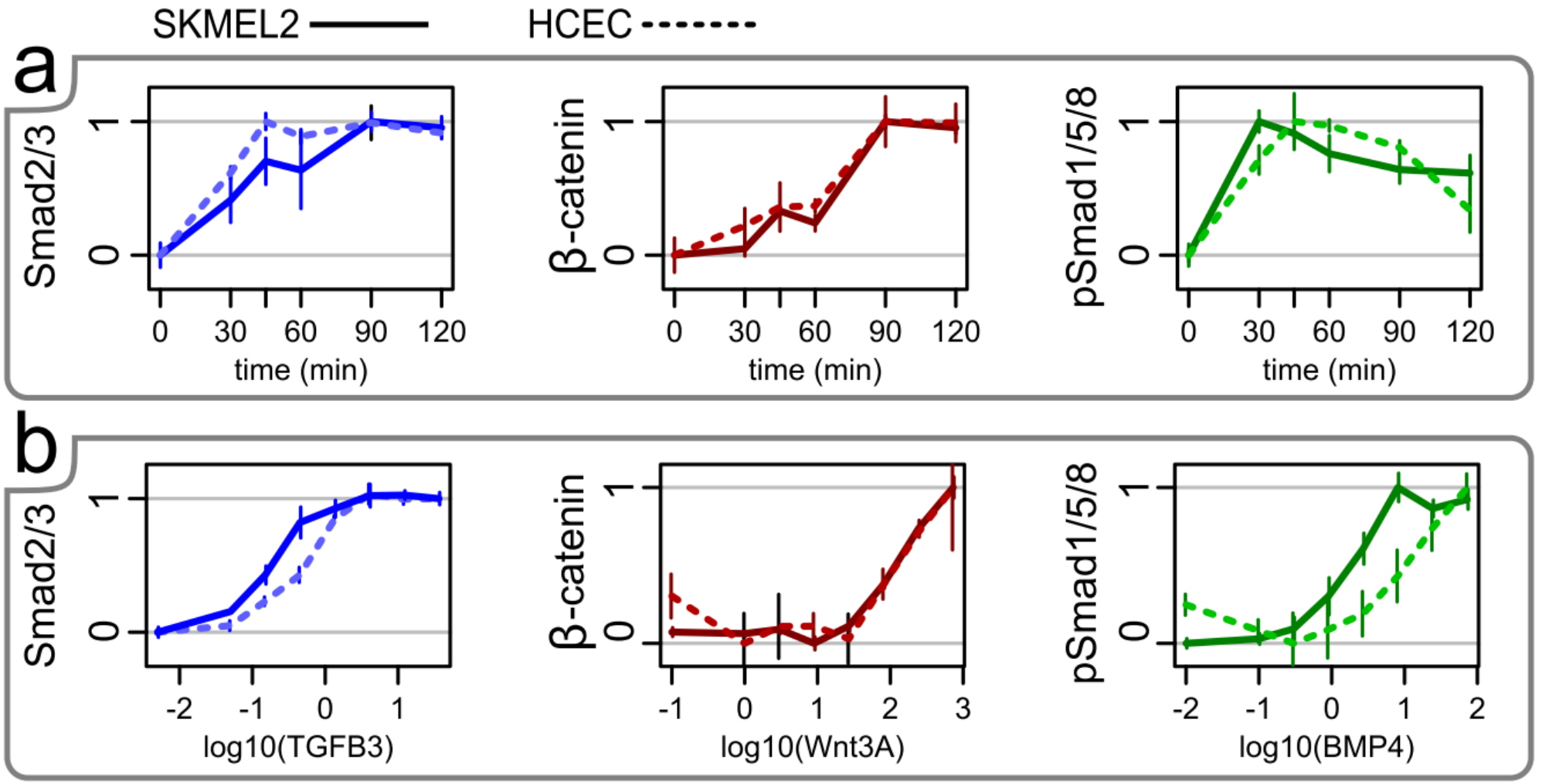
Ligand responses are probably real



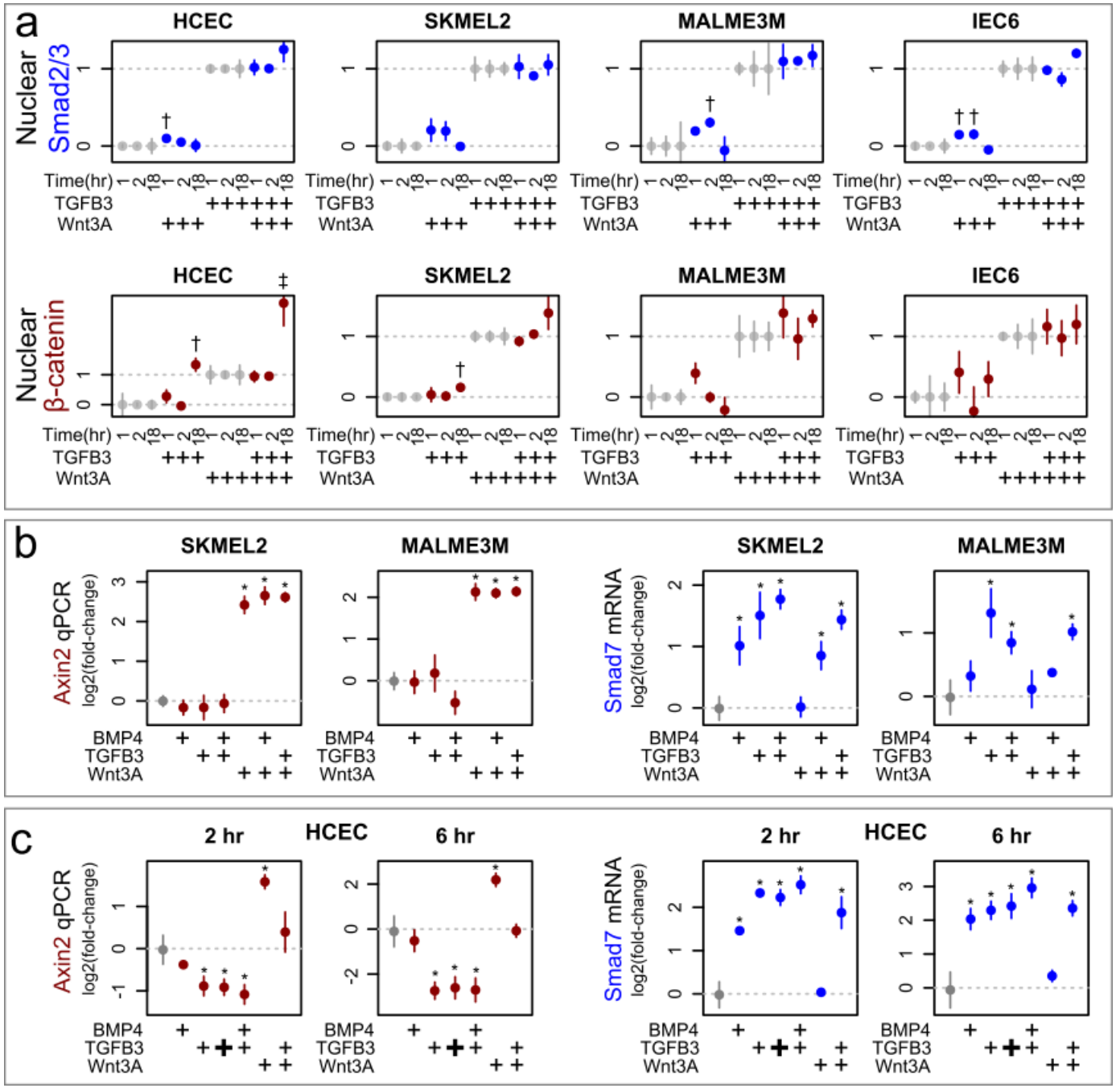




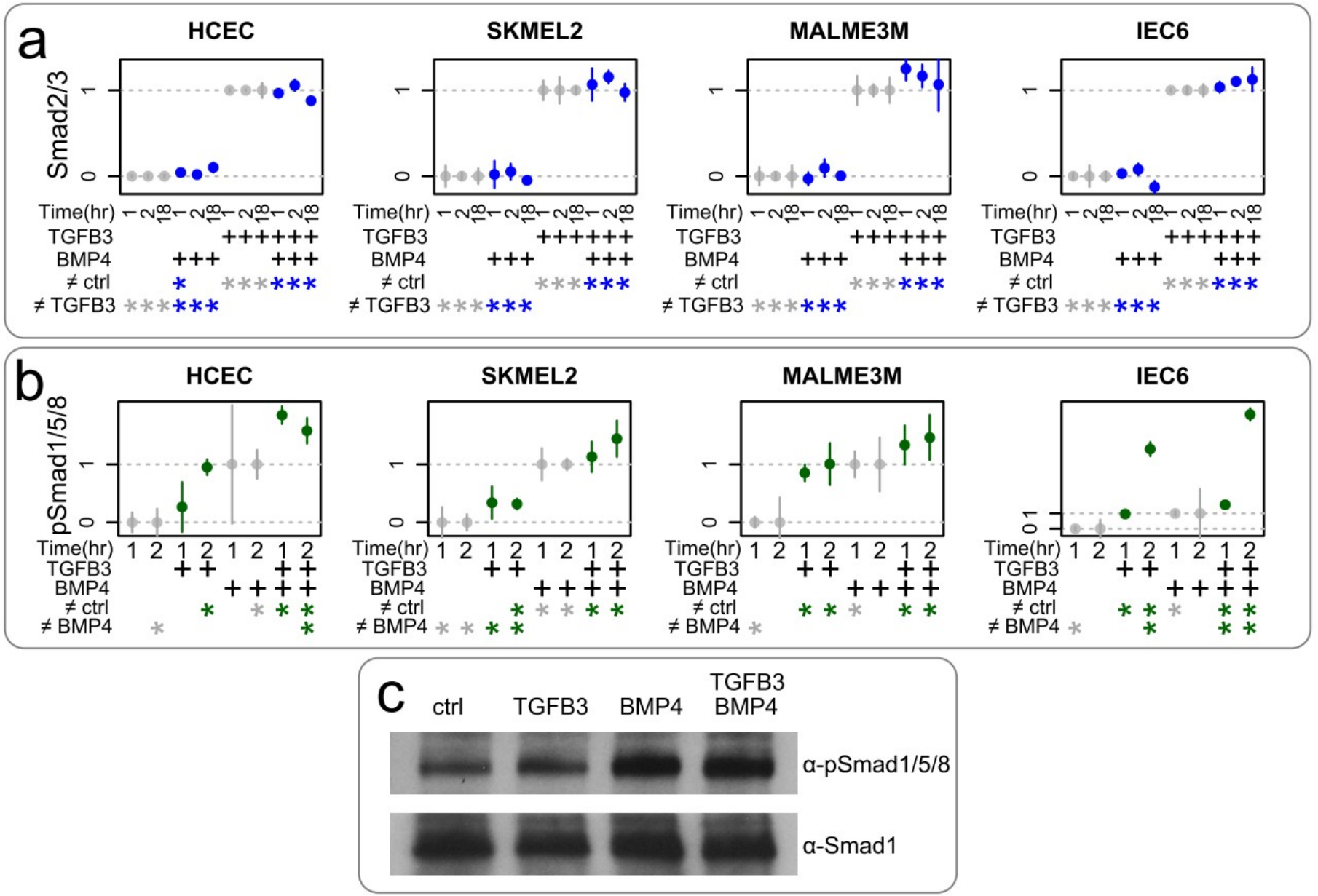
Dose-responses and timecourses



Insulation of TGFB/Wnt (4 cell types)

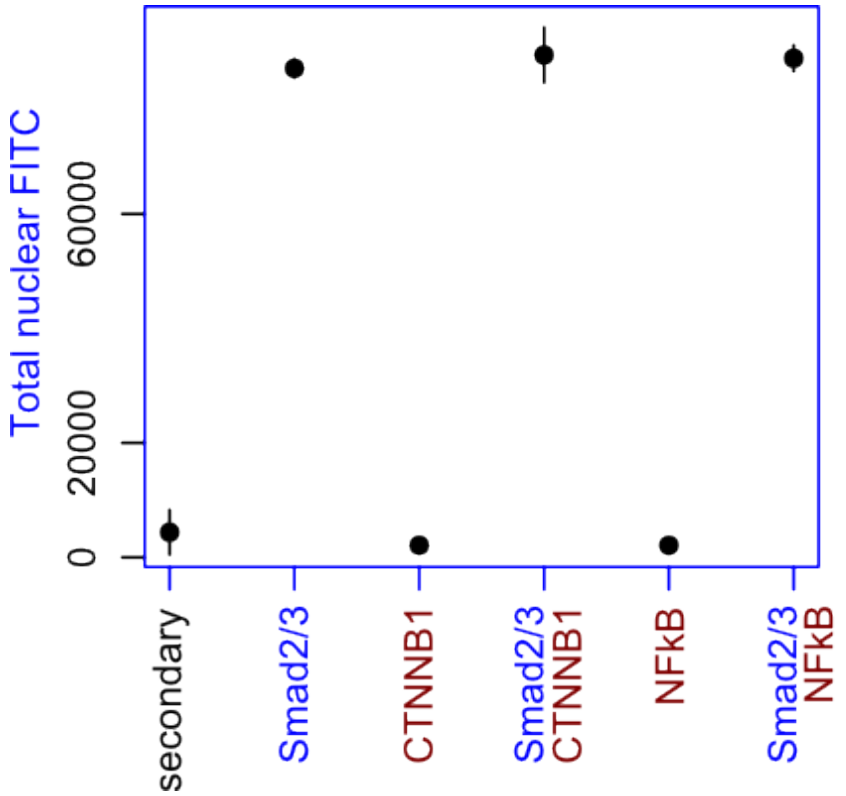


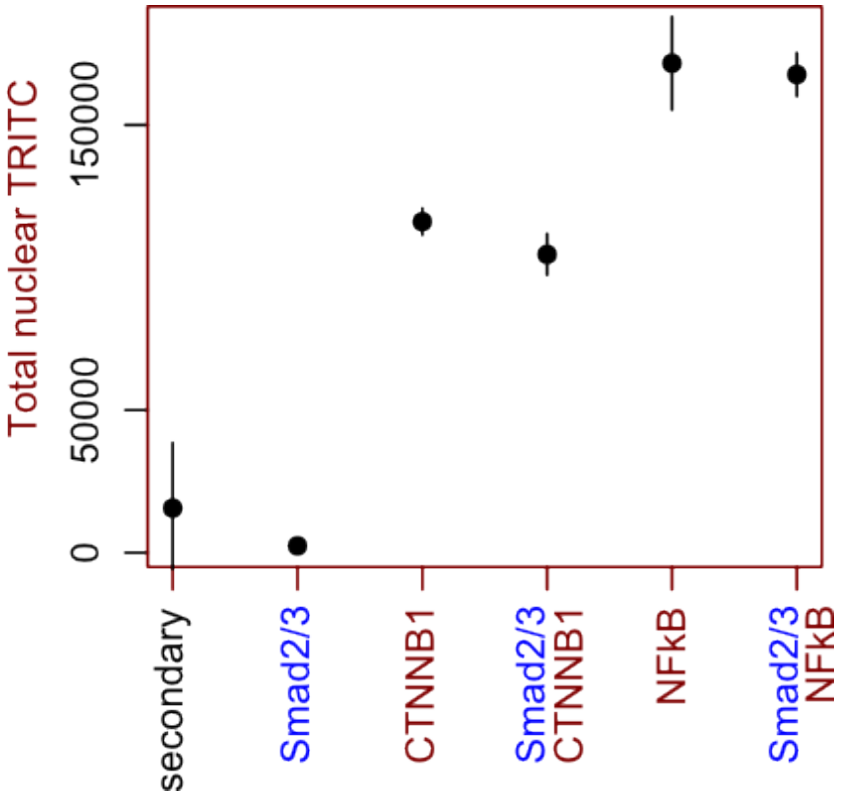
BMP4/TGFB3 do not compete for Smad4



Dissertation Fig. 3.14

Negative control: Primary antibodies are independent





60min treatment, SKMEL2. Points, average total nuclear total over triplicate samples. Colors indicate channel. FITC and TRITC secondary antibodies were used in all cases. Error bars, S.D.

Overview of putative Wnt/TGFB/BMP signaling crosstalk (citations)

a) Warner et al. (2003). FEBS Letters, 539(1-3), 167–173.
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