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# Leveraging signalling dynamics in the small intestine to guide drug scheduling

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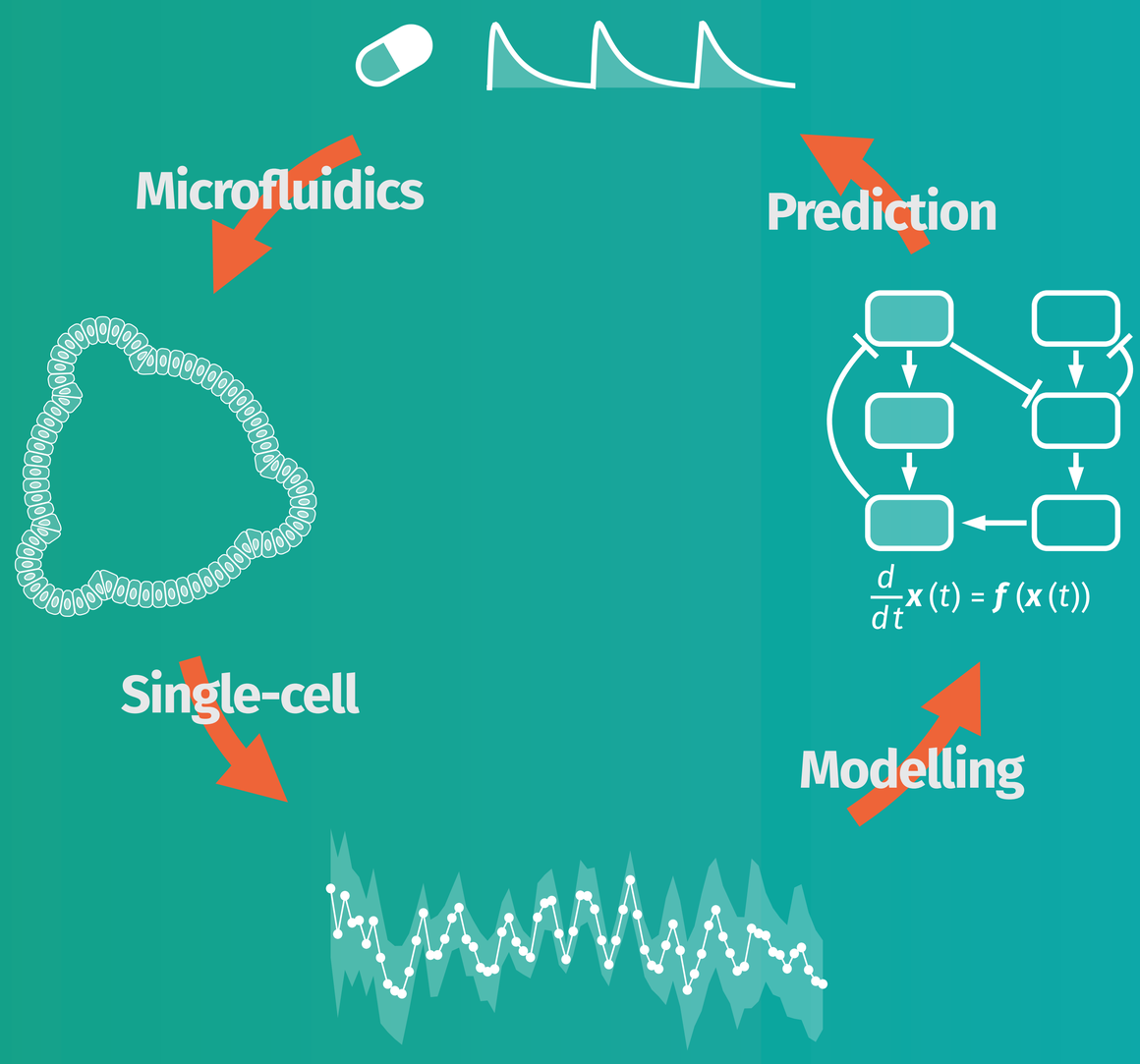



## 1. Plan Of Investigation

a. Study Notch, Wnt, ERK, and p53 ***signalling dynamics*** experimentally and theoretically in mouse organoids sequentially mutated along the ***adenoma-carcinoma*** trajectory[1].

b. Use tumour growth models to ***predict*** small molecule inhibitor ***drug treatments*** to achieve "***tumour remission***" and verify them in mouse tumour organoids.

c. ***Confirm*** effect on ***signalling dynamics*** and the concept of schedule-optimized therapy in ***human intestinal*** and ***CRC organoids***.

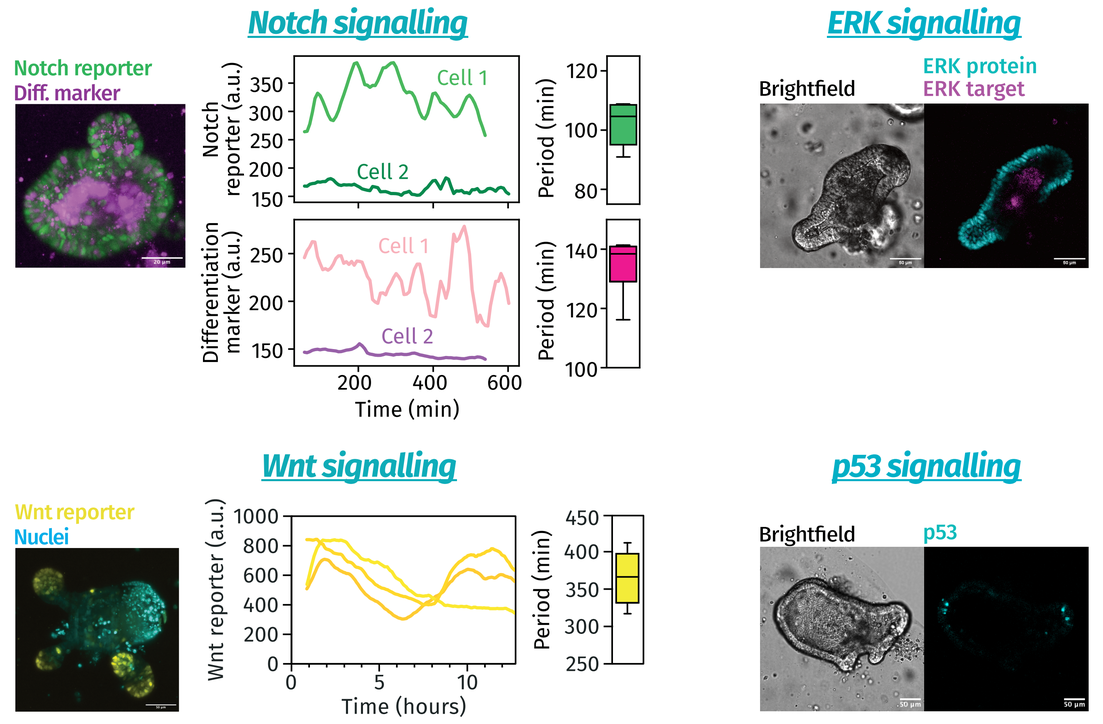
## 2. Dynamic Signalling Reporters

***Approach***

- Mouse intestinal organoids expressing Notch, ERK, Wnt and p53 signalling reporters.
- Live-imaging in inverted light-sheet microscope.
- Single-cell segmentation and tracking in 2D/3D.

***Results***

- **Notch reporter oscillation period: ~1.75 h.**
- **Wnt reporter oscillation period: ~6 h.**



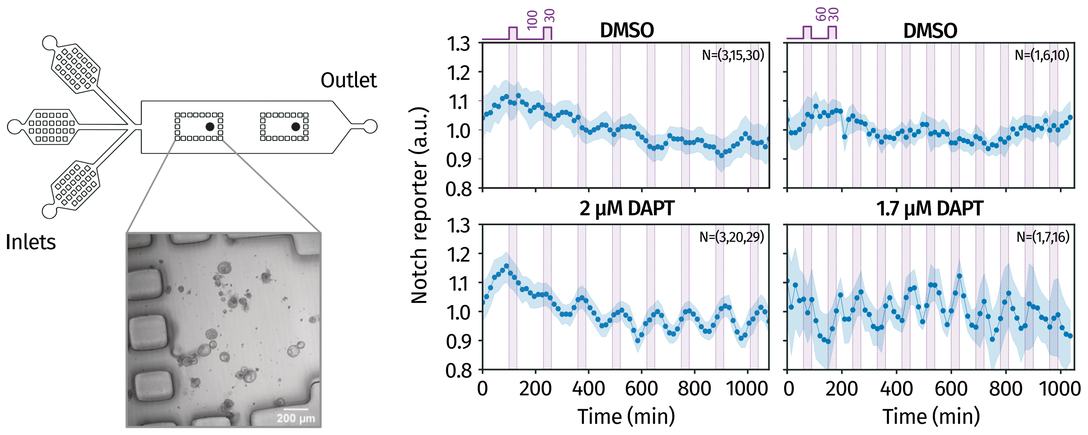

## 3. Notch Microfluidics

***Approach***

- Organoids were cultured in microfluidic chip[2].
- DMSO and Notch signalling inhibitor DAPT were pulsed with periods of 130 and 90 min.

***Results***

- **Notch signalling dynamics synchronise to external pulses.**

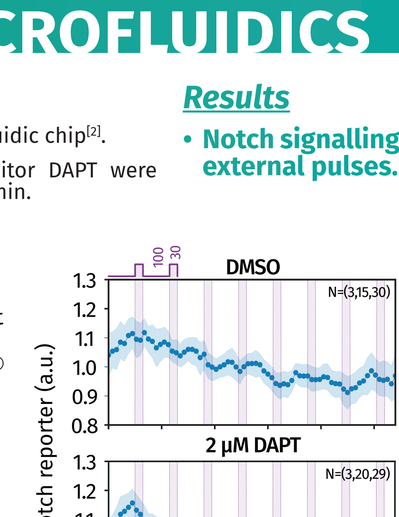



## 4. Using A Mathematical Model To Dissect ERK Signalling Dynamics

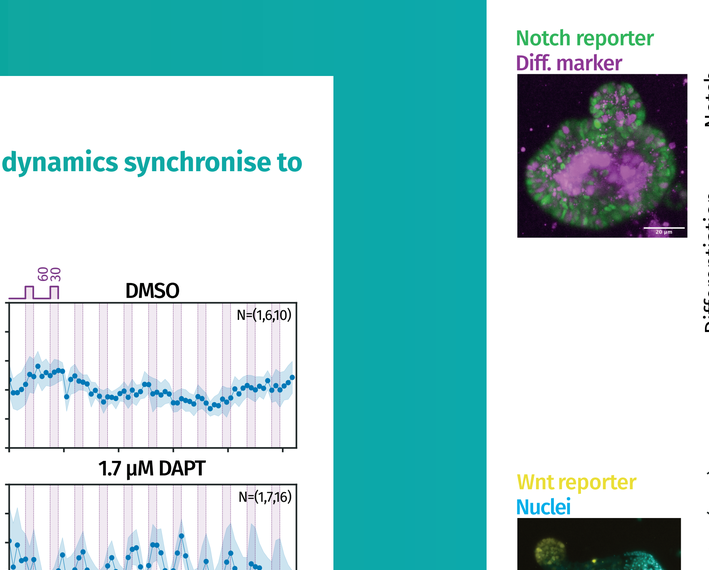
***Approach***

- Implementation of a published ERK signalling model that was informed by single-cell observations in PC-12 cells[3].
- Prediction of phosphorylated ERK activity after sustained or pulsed EGF.
- Transfection of Caco2 cells with EKAREN5[4] FRET sensor.
- Measurement of YFP/CFP ratio in tracked single cells grown in 1 or 10 % FCS after PBS, PMA, and EGF.

***ERK model prediction***




***FRET experiments in Caco2 cells***




***Single-cell quantification***




***Results***

- **Mathematical model predicts dose-dependent single pERK pulse after sustained EGF.**
- **Growing cells in 10 % FCS results in higher baseline ERK activity as compared to 1 % FCS.**
- **Sustained EGF and PMA lead to single ERK pulse qualitatively confirming the model prediction.**
- **After EGF stimulation, ERK returns to baseline faster than after PMA.**

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**References**

[1] J. Drost et al., Nature. 521, 43–47 (2015)
[2] K. F. Sonnen et al., *Cell.* 172, 1079-1090.e12 (2018)
[3] H. Ryu et al., *Mol Syst Biol.* 11, 838 (2015)
[4] B. Ponsioen et al., *Nat Cell Biol.* 23, 377–390 (2021)