DynaScreen: an imaging-based pooled CRISPR screening platform for dynamic signaling phenotypes at single-cell resolution



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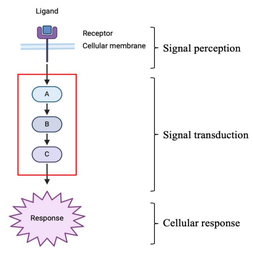
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Why it Matters

The challenge
Importance of dynamics

From extracellular signal to cellular response:

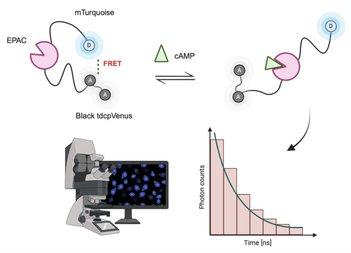


Outcome is defined by intensity and temporal dynamics!

The tools
FRET-FLIM microscopy

FRET-FLIM biosensors reveal spatial and temporal dynamics beyond intensity limits.

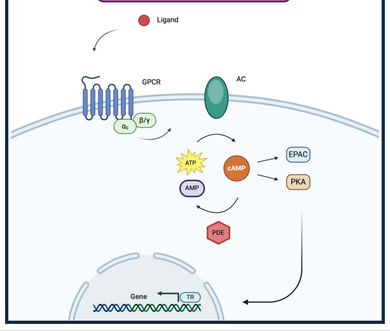
EPAC biosensor:



Our focus
cAMP pathway

One messenger, many responses:

cAMP pathway

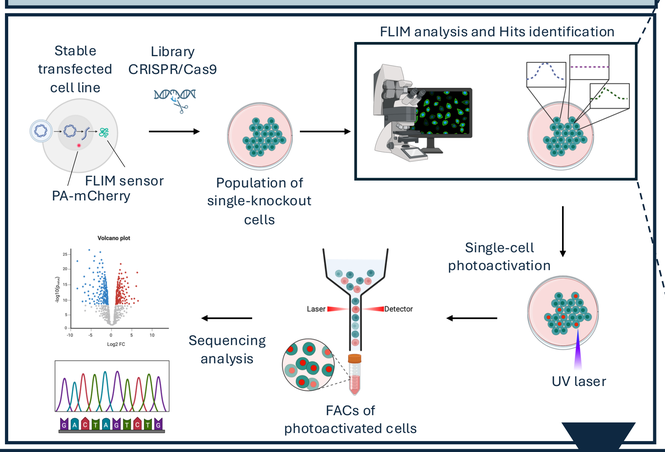


GOAL:

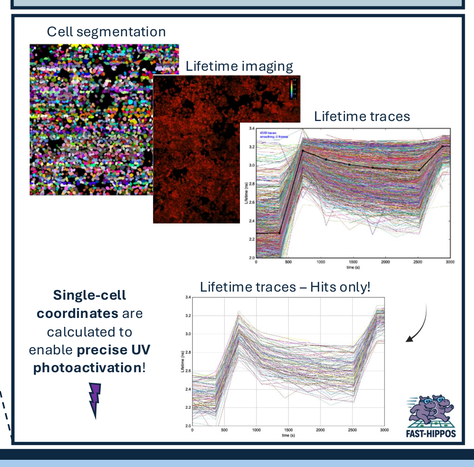
Establish a microscopy-based screening platform for investigating genes involved in shaping the dynamics of cell signaling

DynaScreen

Workflow
How to perform a pooled FLIM screen

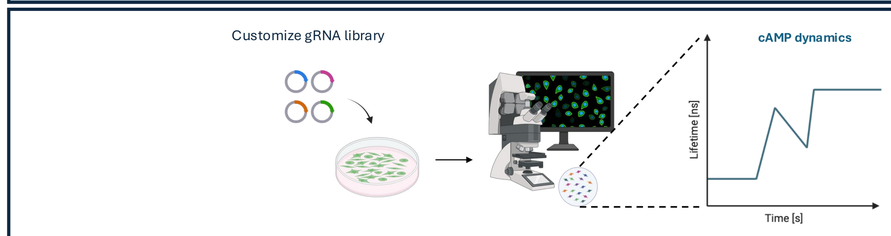


Hits identification
FLIM analysis



Concept Validation

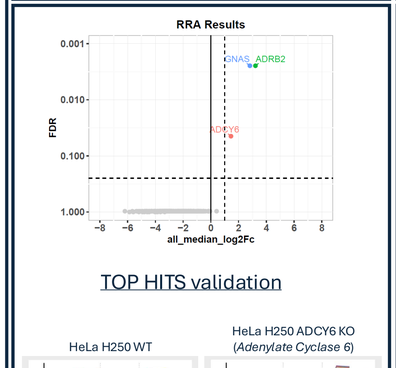
Three successful screens validate the method



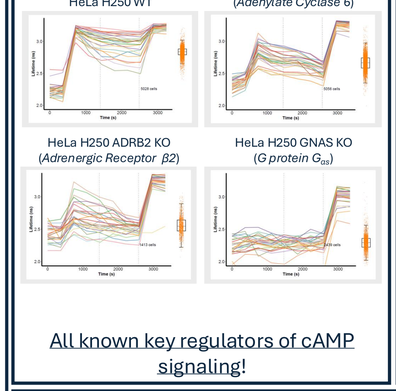
Performed screens:

Customize gRNA library (318 genes) | Customize gRNA library (318 genes) | Customize gRNA library (116 genes)

Low cAMP responder:

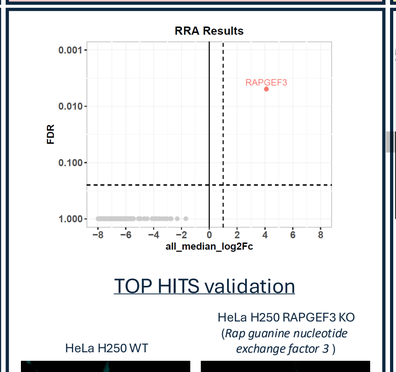


TOP HITS validation

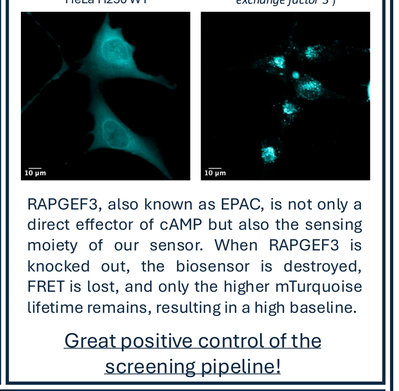


All known key regulators of cAMP signaling!

High cAMP baseline:



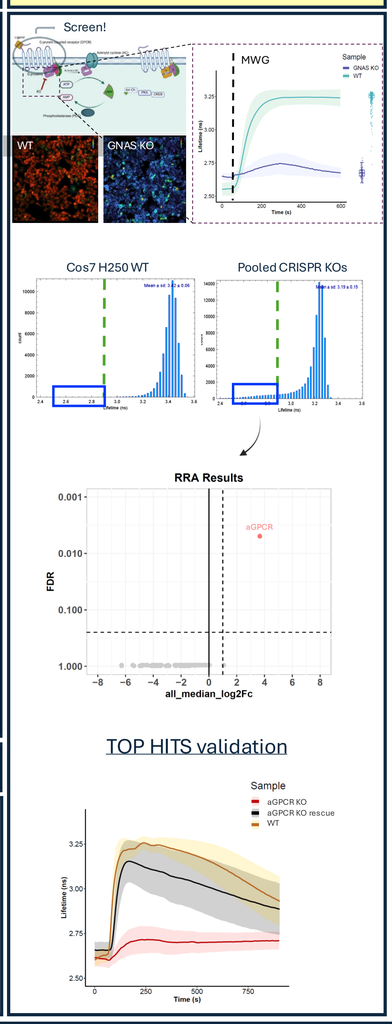
TOP HITS validation



RAPGEF3, also known as EPAC, is not only a direct effector of cAMP but also the sensing moiety of our sensor. When RAPGEF3 is knocked out, FRET is lost, and only the higher mTurquoise lifetime remains, resulting in a high baseline.

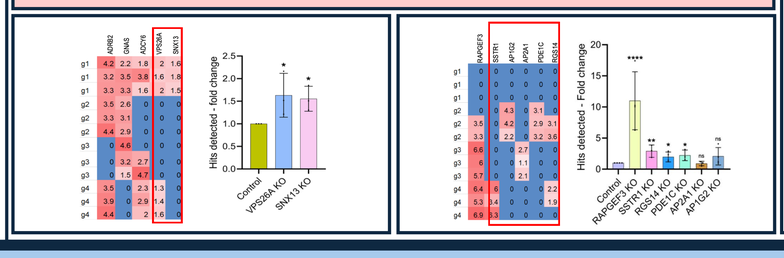
Great positive control of the screening pipeline!

Mechano stimulation:



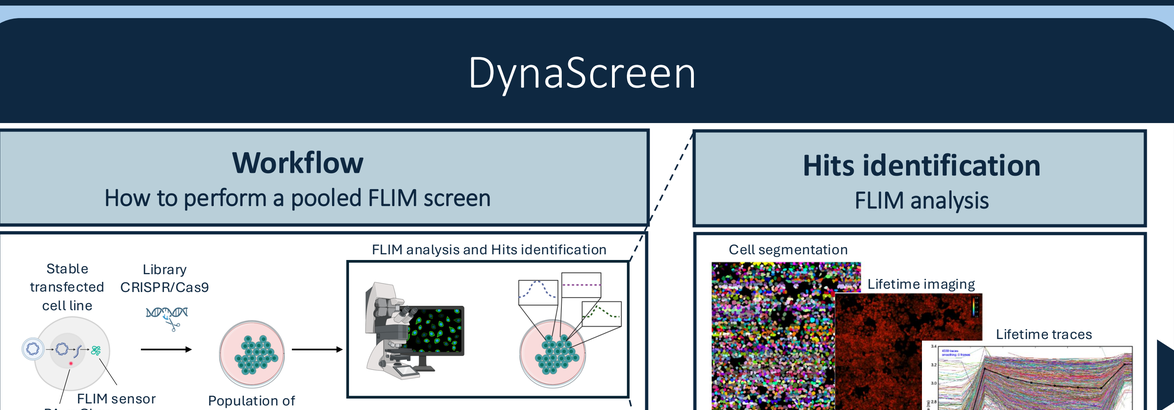
TOP HITS validation

Validation of secondary hits support the sensitivity of the pipeline!



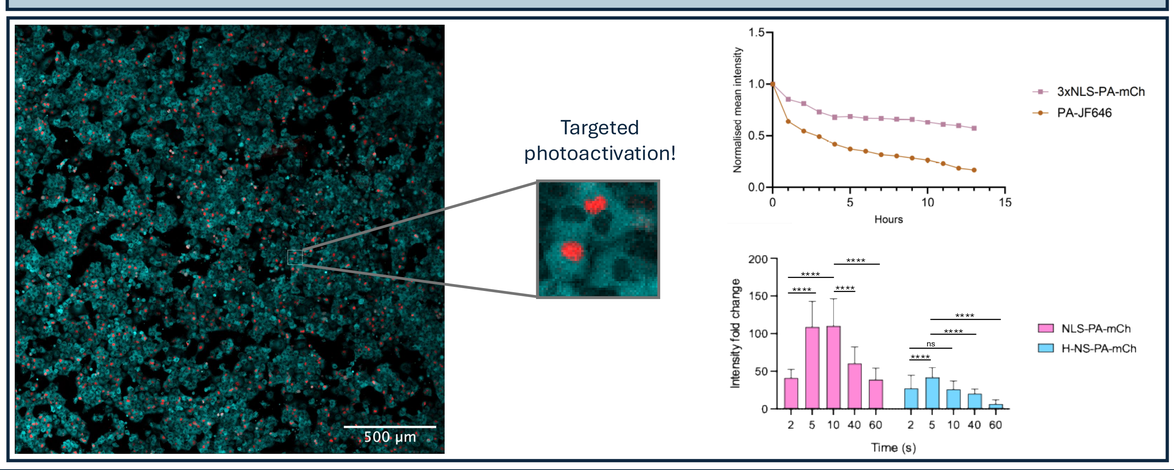
Optimizing the Method

Improved homogeneous cytosolic distribution of Epac based FRET-FLIM biosensor



Novel set of Epac based FRET-FLIM biosensor:
- Fully cytosolic
- High performance
- Real-time cAMP dynamics

Enhanced hits identification using PA-mCherry



Take-home

The proof-of-concept experiments show that the pipeline works well and highlight the power of the approach!

The **pooled genetic FLIM screening approach** allows for:
- **Single-cell resolution** — capturing variability and behaviour across individual cells
- **Scalability** — screening thousands of gene perturbations in parallel
- **Dynamic readouts** — monitoring real-time changes in signalling over time
- **Gene discovery** — identifying novel regulators of signalling pathways
- It's an **adaptable platform for screening** any desired phenotype

What's Next

- **Scale up screening to larger, non-customized libraries**
  - Human kinome (~500 genes)
  - Druggable genome (~3000 genes)
  - Eventually, the whole genome
- **Apply the approach to different cell models to identify novel regulators of cAMP dynamics in relevant biological context** (senescence, IPSCs neuro-differentiation etc.)

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SCAN ME

References

- Klarenbeek B. J, Goedhart J, Van Batenburg A, Groenewald D, Jalink K, '*Fourth-Generation Epac-Based FRET Sensors for cAMP Feature Exceptional Brightness, Photostability and Dynamic Range: Characterization of Dedicated Sensors for FLIM, for Ratiometry and with High Affinity*', PLoS ONE (2015).
- Zanetti G, Klarenbeek J, Jalink K, '*Cytosolic-enhanced dark Epac-based FRET sensors allow for intracellular cAMP detection in live cells via FLIM*', FEBS Lett. (2025).
- Mukherjee S, van Tooren M, Zanetti G, Sfakianakis D, Kemps D, Klarenbeek B. J, Kuiken H. J, Lieftink C, Van den Broek B, Beijersbergen R. L, Jalink K, '*Beyond Static Screens: A High-Throughput Pooled Imaging CRISPR Platform for Dynamic Phenotype Discovery*', BioRxiv (2025).
- Mukherjee S, Zanetti G, Van Tooren M, Van den Broek B, Jalink K, '*Key optimizations for performing an imaging based pooled screen of dynamic phenotypes*', ongoing.