Quantitative microscopy-based screening method to identify inhibitors of protein-protein interaction

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Cilia, centrosome and pathologies team. B. Delaval

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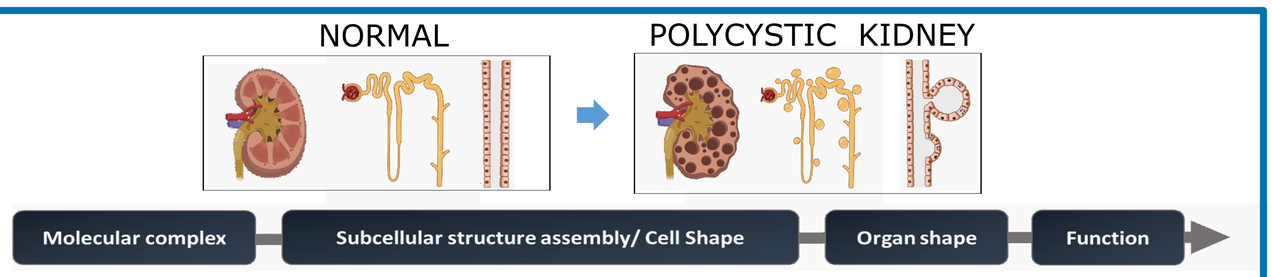
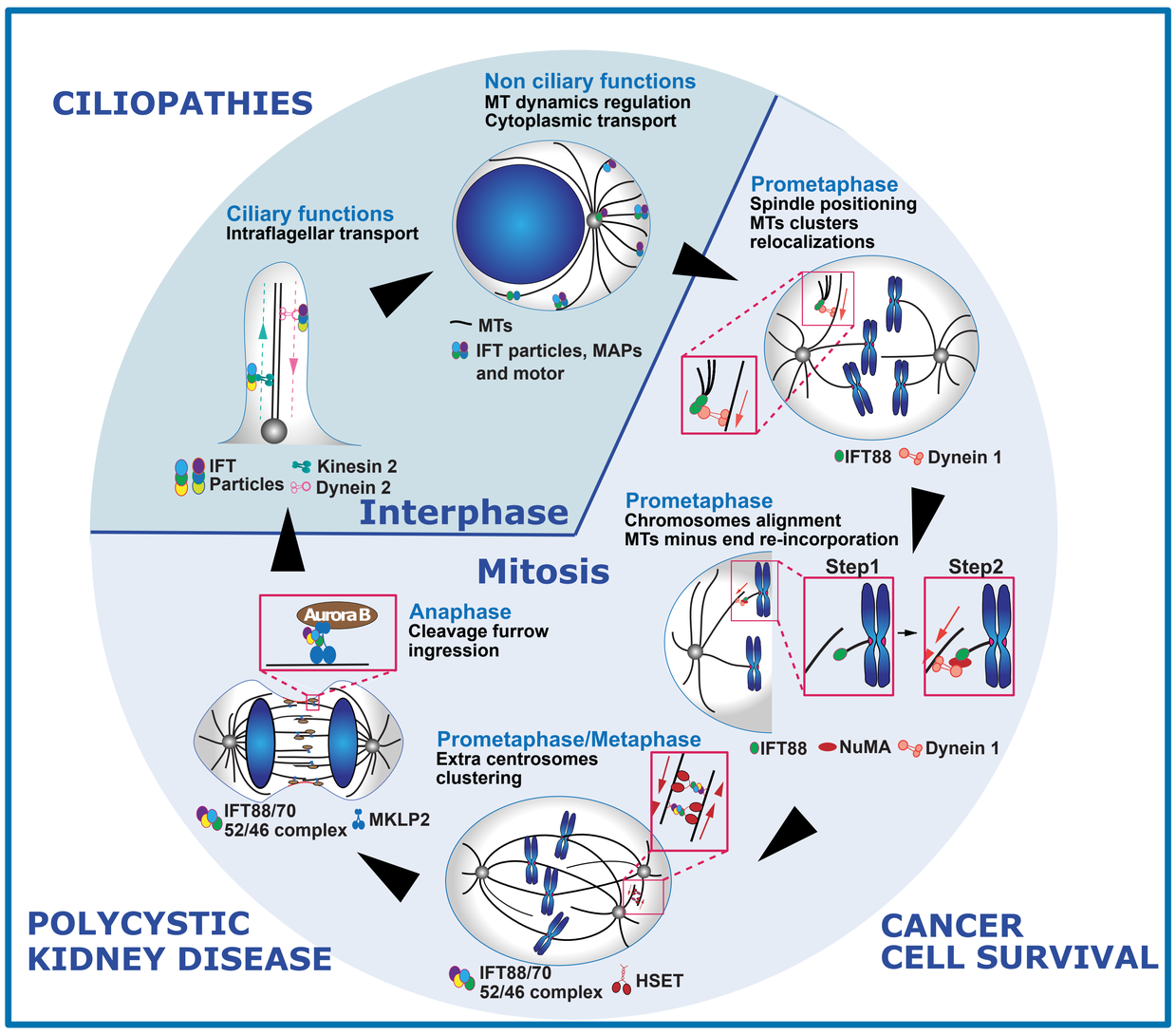
Our team addresses emerging questions in the fields of cell biology and epithelial tissue morphogenesis focusing on the kidney as a model. Indeed, we are interested in understanding how highly dynamic cellular processes such as cell division contribute to 3D epithelial tissue morphogenesis under normal and pathological conditions. At the molecular and cellular scales, we study how intracellular transport complexes (IntraFlagellar Transport proteins, IFT) involved in kidney pathologies and well known for their ciliary function, unexpectedly also control cell division. At the tissue and organ scale, we study the contribution of cell division to kidney tubule morphogenesis under normal and pathological conditions. To tackle these questions we combine cutting-edge cell biology and microscopy approaches on complementary systems of increasing complexity: in vitro reconstituted systems, 2D/3D cell culture and zebrafish as an in vivo model.



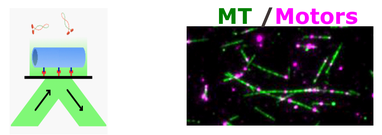
- Define the molecular roles of intracellular transport complexes (IFT proteins)
- Characterize novel cellular functions for IFT proteins using 2D/3D renal cell culture
- Assess the contribution of cell division to epithelial tubule organization in 3D cell culture and in vivo

Introduction: IFTs in cellular functions and diseases

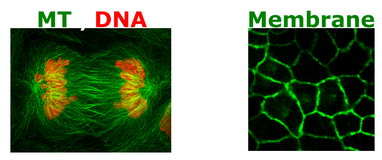


Methods

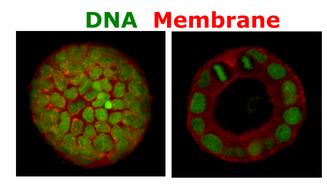
- Single molecule imaging (TIRF) to study molecular interactions and regulations



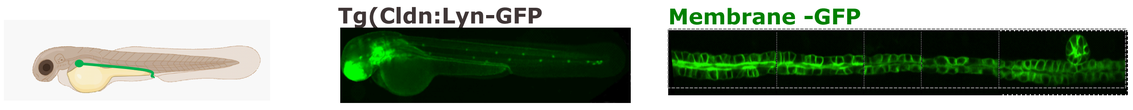
- High resolution (fixed and live) imaging to study fine and dynamic cellular processes at the subcellular and cellular level



-3D MDCK cell culture imaging to study the contribution of cell division perturbations on 3D epithelia

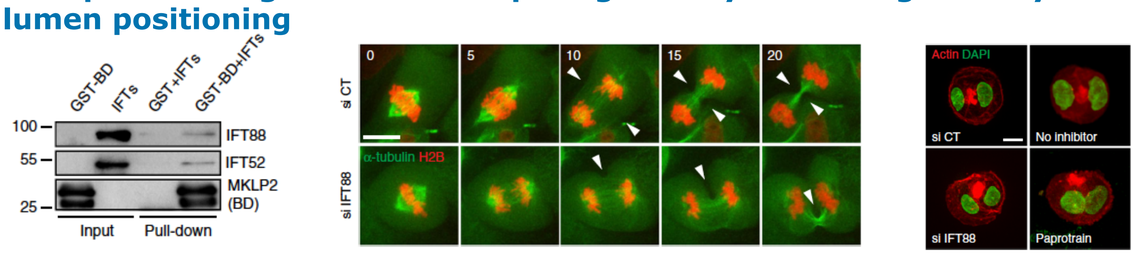


-Zebrafish larvae to study the impact of cell division perturbations on epithelial tubule organization and organ fonction in vivo.



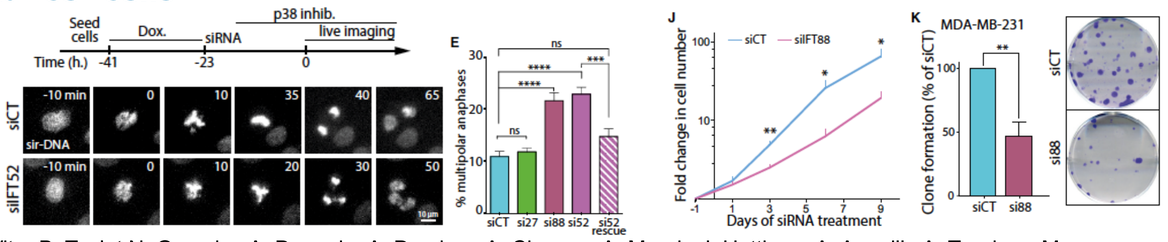
Previous work

IFTs proteins together with Mklp2 regulate cytokinesis geometry and lumen positioning



Taulet N, Vitre B, Anguille C, Douanier A, Rocancourt M, Taschner M, Lorentzen E, Echard A, and Delaval B. **Nature Communications. 2017.**

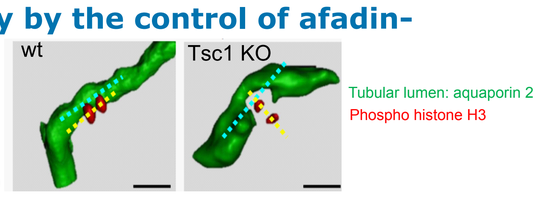
IFTs in association with HSET facilitates extra centrosomes clustering in cancer cells



Vitre B, Taulet N, Guesdon A, Douanier A, Dosdane A, Cisneros A, Maurin J, Hettinger A, Anguille A, Taschner M, Lorentzen E and Delaval B . **EMBO reports. 2020.**

mTOR and S6K1 drive polycystic kidney by the control of afadin-dependant oriented cell division.

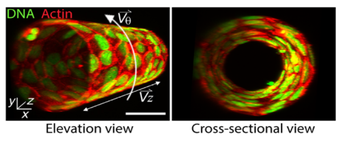
Mouse renal epithelial tubule



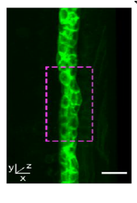
Bonucci M, [...]Delaval B, Piel M, Pende M. **Nat Commun 2020**

The emergence of spontaneous coordinated epithelial rotation on cylindrical curved surfaces.

Bioengineered 3D renal epithelail tubule

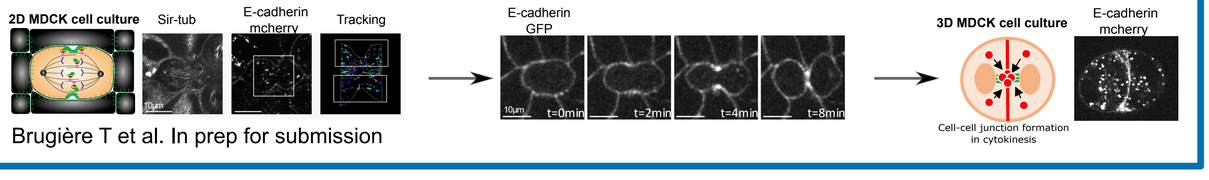


Zebrafish renal epithelial tubule



Glentis A, [...], Douanier A, Delaval B, [...], Ladoux B **Science Advances. 2022**

A Rab7/KIF5B/IFT88 mediated transport of E-cadherin in cytokinesis contributes to junction reformation in 2D and 3D renal cell culture

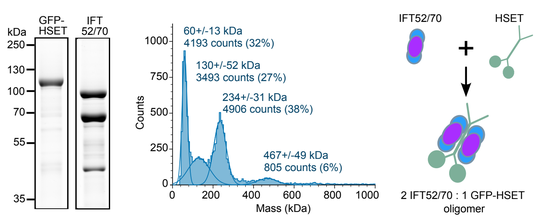


Brugière T et al. In prep for submission

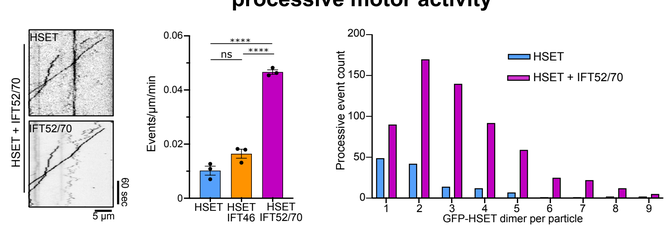
Proposed Master project

HSET/IFT proteins interaction stimulates HSET motor activity in vitro

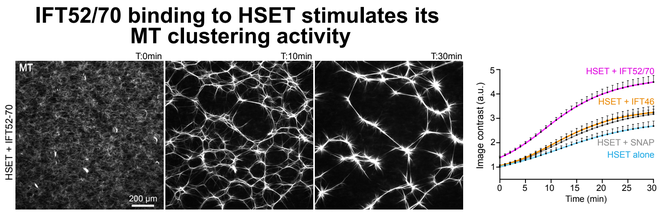
IFT52/70 dimer forms a stable complex with HSET

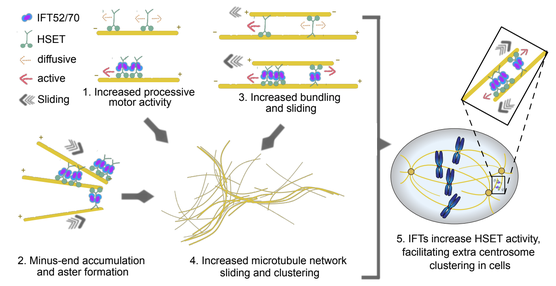


IFT52/70 binding to HSET stimulates its processive motor activity

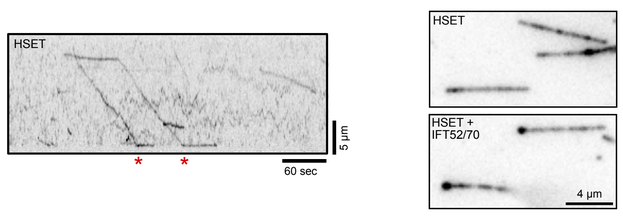


IFT52/70 binding to HSET stimulates its MT clustering activity





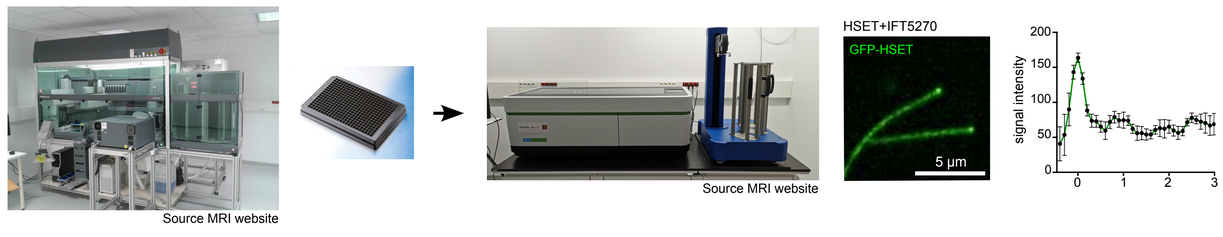
HSET activation by IFT proteins results in MT minus-end accumulation



Identify small molecule inhibitors that prevent HSET /IFT interactions in order to hamper HSET motor activity

Small molecules inhibitor library screening: 10142 compound FrPPIChem

Automated aquisition, opera phenix microscope: MRI screening



Master internship objectives

1. **Optimize sample preparation**
2. **Optimize image acquisition protocol in 384-well plates (with MRI Screening)**
3. **Develop automatic detection of HSET MT minus-end accumulation (with MRI Center for Image Analysis)**

Overall aim

Overall, in the long term, this project aims at identifying inhibitors of HSET /IFT interaction that can be used to reduced HSET activity during extra centrosomes clustering in cancer cells

ZEFIX ZEbraFish and Xenopus Platform

MITI Mission pour les Initiatives Transverses et Interdisciplinaires

MRI Montpellier Ressources Imagerie