

**SoFFœt – Société Française de Fœtopathologie**

**LES MODELES ANIMAUX**

*2 Octobre 2015*

# Le modèle zebrafish appliqué aux ciliopathies rénales

Marion Delous

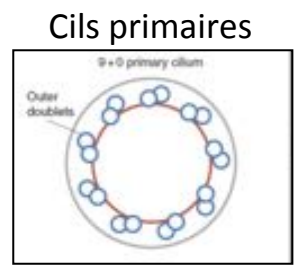
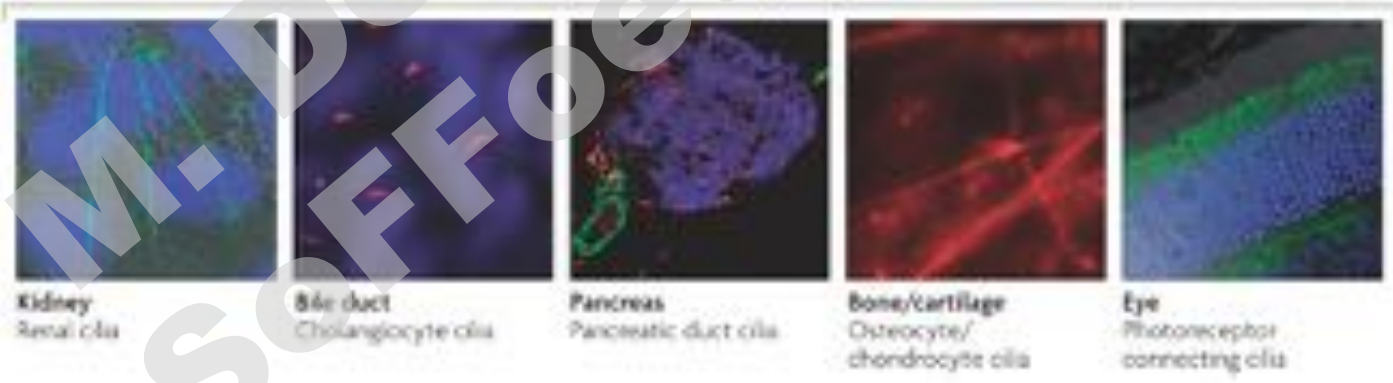
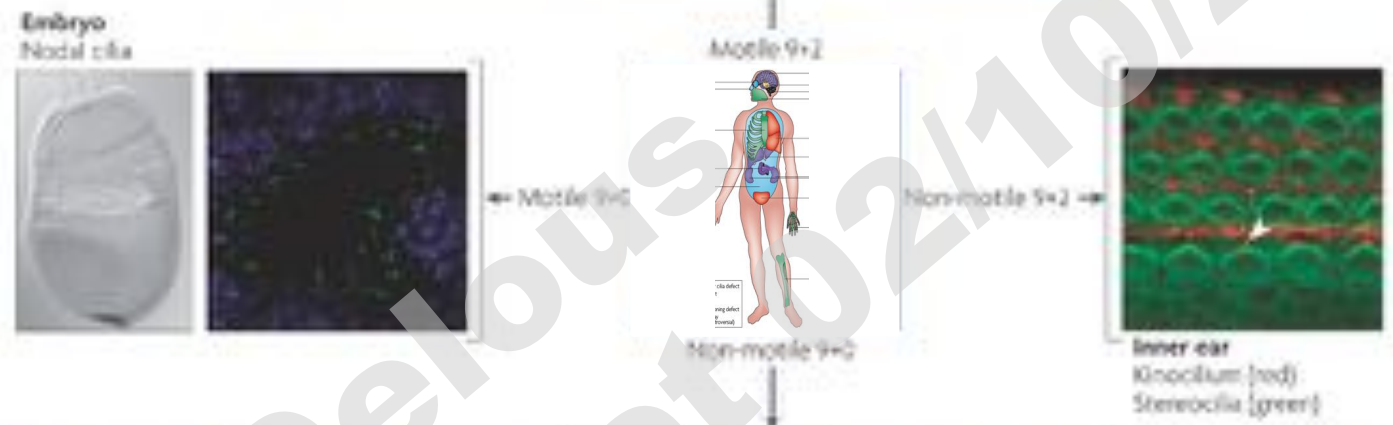
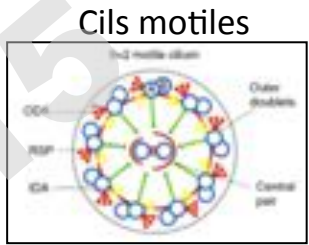
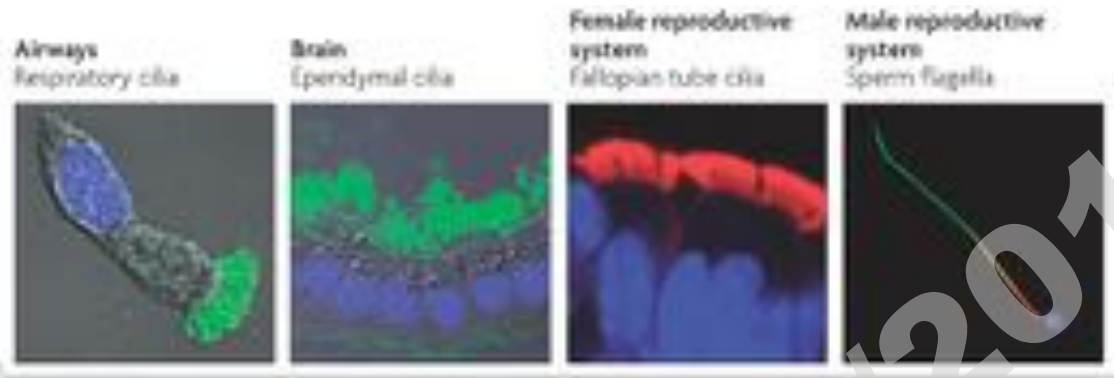
Inserm U1163, Institut Imagine

Laboratoire des Maladies Héréditaires Rénales, Dr. Sophie Saunier

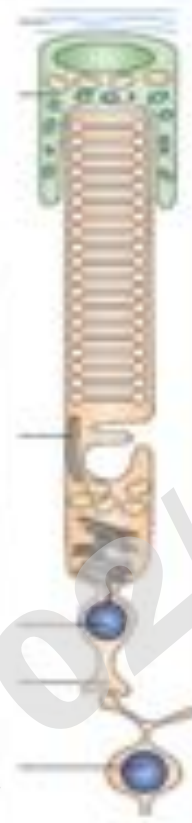
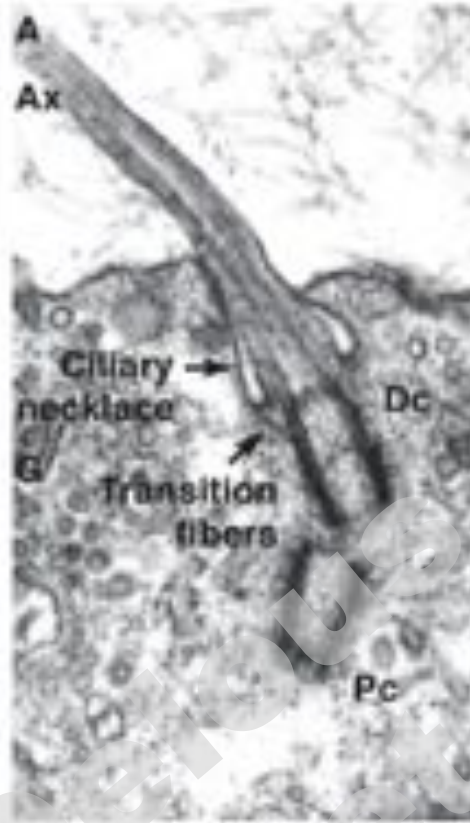
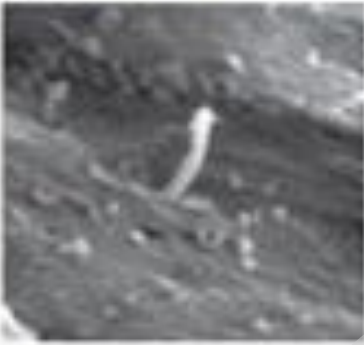
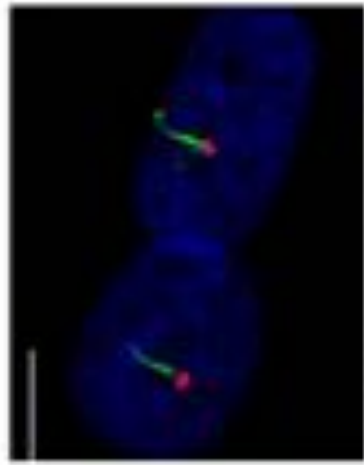
Hôpital Necker, Université Paris Descartes



# Cellules ciliées chez l'Homme

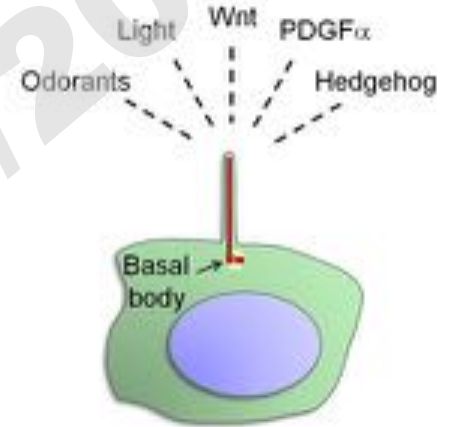


# Cils primaires, antennes de signalisation



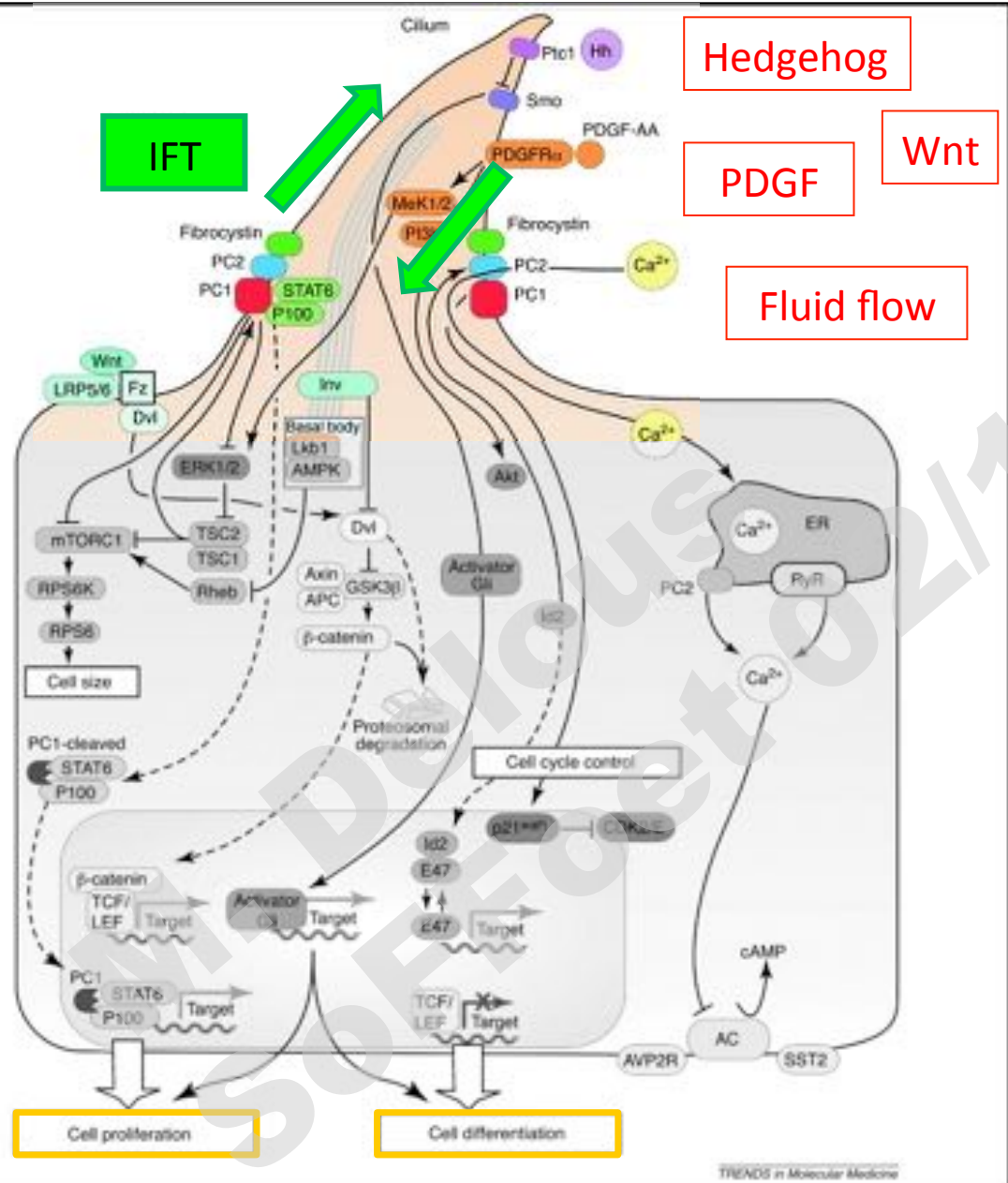
## Fonctions de capteur

- Photoréception
- Réception chimique
- Réception mécanique



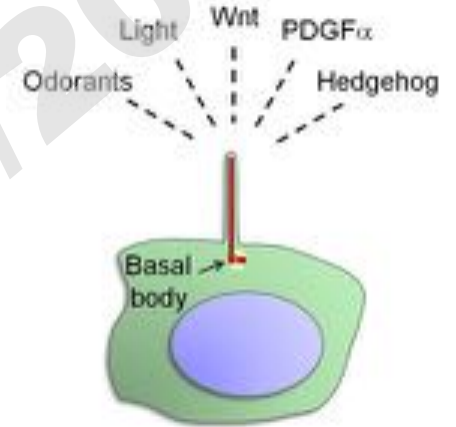
M. D. U. 02/10/2015  
SOFFO

# Cils primaires, antennes de signalisation

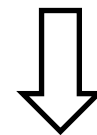


## Fonctions de capteur

- Photoréception
- Réception chimique
- Réception mécanique



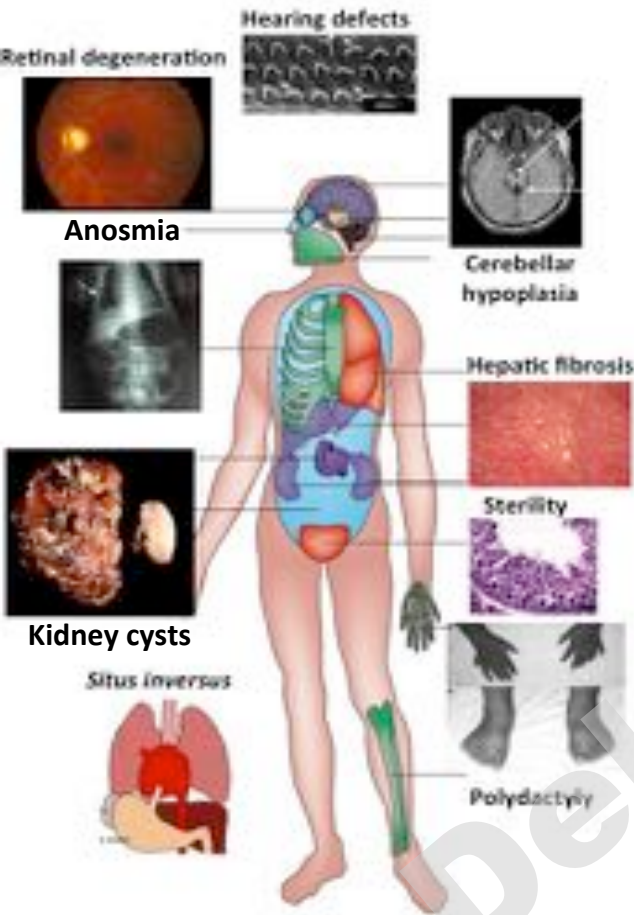
## Fonctions de transduction de signaux



Réponses cellulaires



# Anomalies associées à des défauts ciliaires



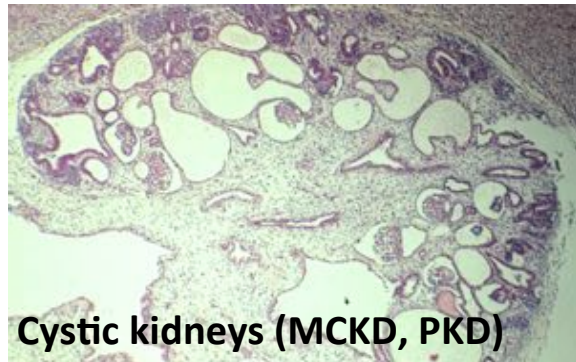
## Ciliopathies

	PKD	Leber amaurosis	Nephronophthisis	Senior Løken	Alstrom	Bardet-Biedl	OFD	JATD	Sensenbrenner	SRP	Joubert	Meckel
Cystic kidneys/ tubulointerstitial nephropathy	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Retinal degeneration		✓	✓	✓	✓	✓	✓	✓			✓	
Liver defects	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
Laterality defects		✓	✓		✓		✓		✓			✓
Polydactyly						✓	✓	✓	✓	✓	✓	✓
Obesity, hypogonadism					✓	✓						
Craniofacial anomalies (palate cleft)							✓		✓	✓		✓
Cerebellar vermis dysgenesis							✓	✓	✓	✓	✓	✓
Neuronal tube defects (encephalocele)											✓	✓
Skeletal dysplasia (Shortening/ bowing of bones )								✓	✓	✓		✓
Ectodermal dysplasia							✓		✓	✓		

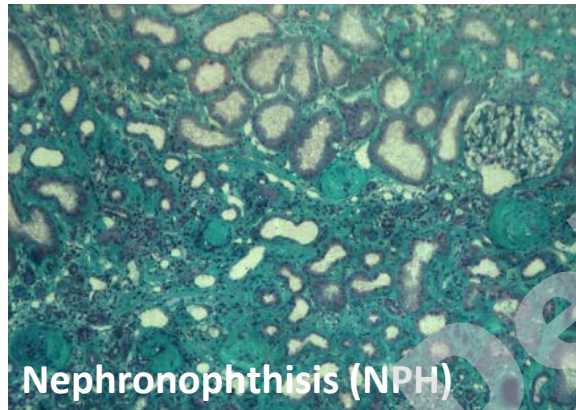
# Anomalies associées à des défauts ciliaires

## Ciliopathies

PKD  
 Leber amaurosis  
 Nephronophthisis  
 Senior Løken  
 Alstrom  
 Bardet-Biedl  
 OFD  
 JATD  
 Sensenbrenner  
 SRP  
 Joubert  
 Meckel



Cystic kidneys (MCKD, PKD)



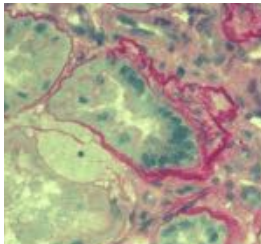
Nephronophthisis (NPH)

	PKD	Leber amaurosis	Nephronophthisis	Senior Løken	Alstrom	Bardet-Biedl	OFD	JATD	Sensenbrenner	SRP	Joubert	Meckel
Cystic kidneys/ tubulointerstitial nephropathy	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Retinal degeneration		✓	✓	✓	✓	✓	✓	✓	✓		✓	
Liver defects	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
Laterality defects			✓	✓		✓		✓		✓		✓
Polydactyly						✓	✓	✓	✓	✓	✓	✓
Obesity, hypogonadism					✓	✓						
Craniofacial anomalies (palate cleft)							✓		✓	✓		✓
Cerebellar vermis dysgenesis							✓	✓	✓	✓	✓	✓
Neuronal tube defects (encephalocele)											✓	✓
Skeletal dysplasia (Shortening/ bowing of bones )								✓	✓	✓		✓
Ectodermal dysplasia							✓		✓	✓		

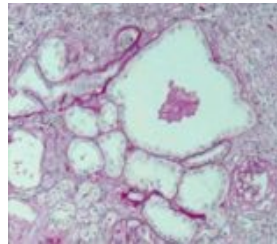
# La néphronophtise

- Néphropathie tubulo-interstitielle
- Polyurie/polydipsie
- IRT au cours de l'enfance/adolescence

- Autosomique récessif
- Hétérogénéité génétique (~18 gènes *NPHP*)

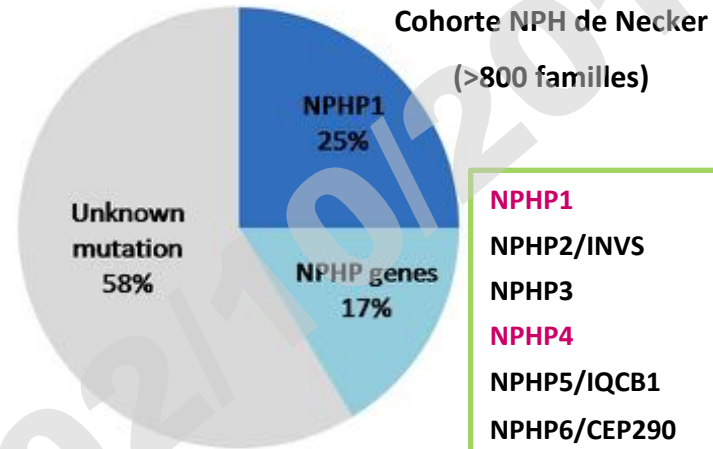
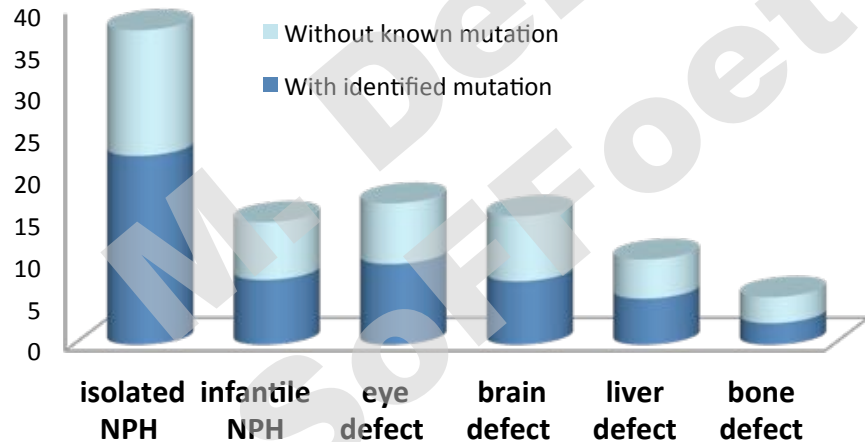


Epaississement de la membrane basale



Fibrose interstitielle et kystes

- Associée à des atteintes extra-rénales (45%)



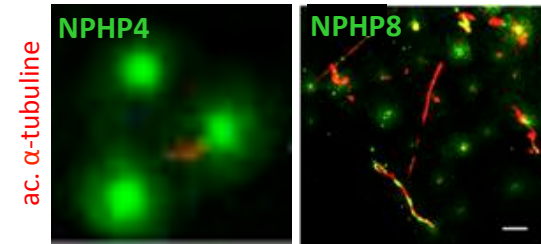
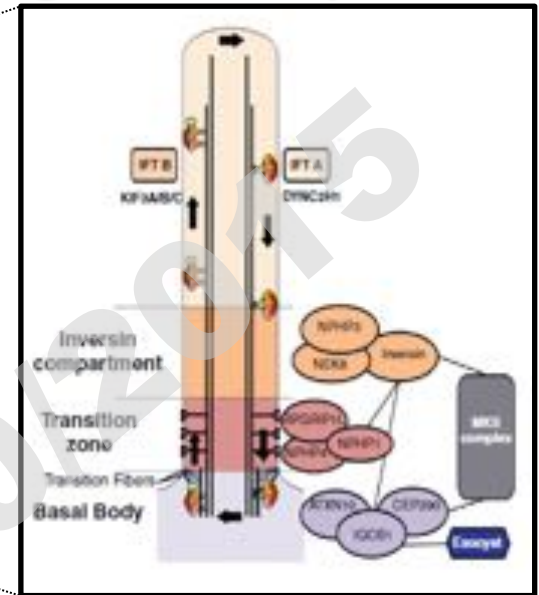
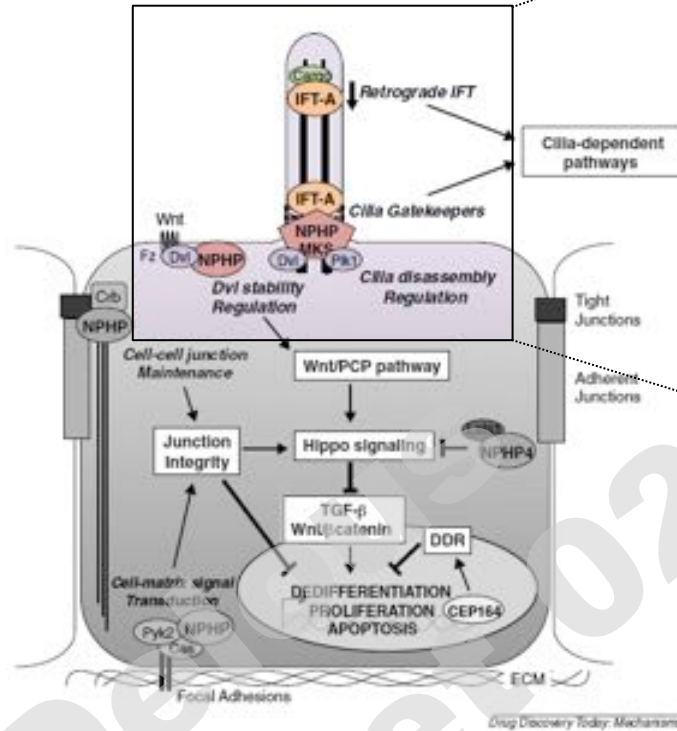
<p><b>NPHP1</b></p> <p>NPHP2/INVS</p> <p>NPHP3</p> <p><b>NPHP4</b></p> <p>NPHP5/IQCB1</p> <p>NPHP6/CEP290</p> <p>NPHP7/GLIS2</p> <p><b>NPHP8/RPGRIP1L</b></p> <p>NPHP9/NEK8</p>	<p>Positional cloning</p> <p>candidat gene approach</p>
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1993 - 2009

<p><b>NPHP10/SDCCAG8</b></p> <p><b>NPHP11/TMEM67</b></p> <p><b>NPHP12/TTC21B/IFT139</b></p> <p><b>NPHP13/WDR19/IFT144</b></p> <p><b>NPHP15/CEP164</b></p> <p><b>IFT140</b></p> <p><b>NPHP16/ANKS6</b></p> <p><b>NPHP17/IFT172</b></p> <p><b>NPHP18/CEP83</b></p>	<p>Next-Generation Sequencing</p>
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2010 - 2014

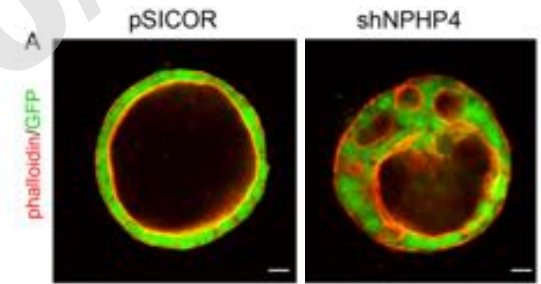
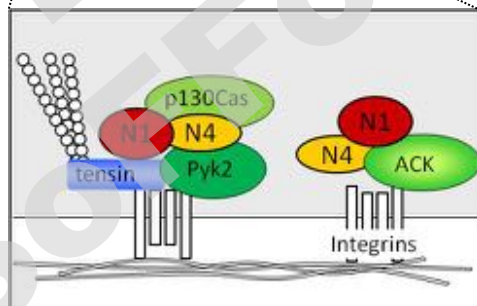
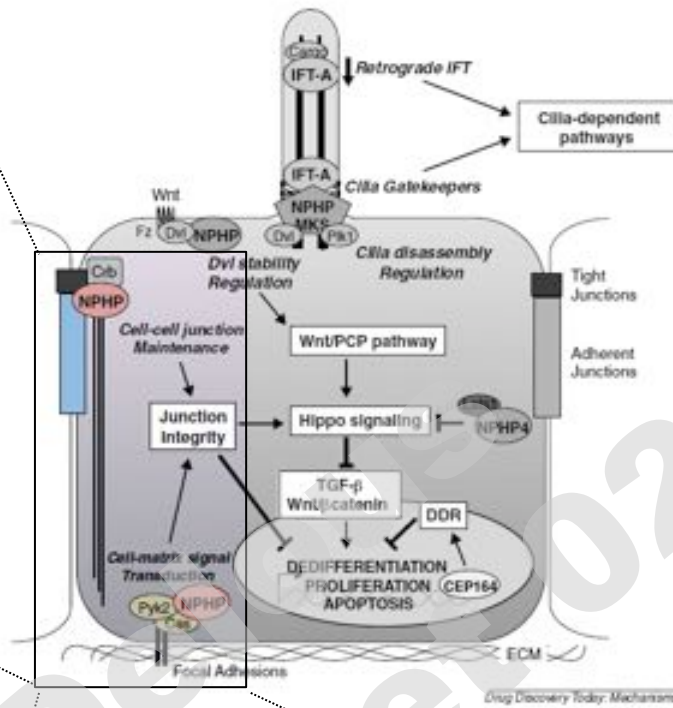
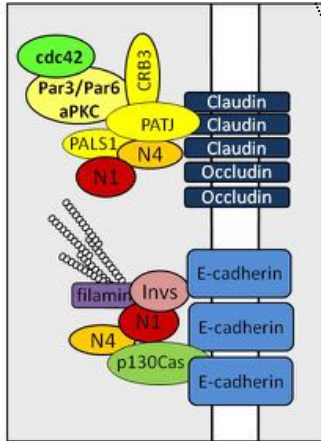
# Protéines NPHP : Rôle dans IFT et "gate keepers"



Cellules MDCK polarisées

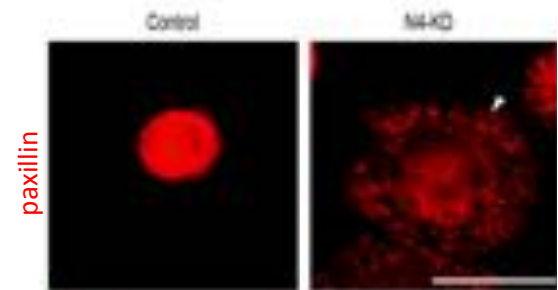


# Protéines NPHP : Rôle dans les jonctions cellulaires



Cellules MDCK en culture 3D

> Polarité apico-basale

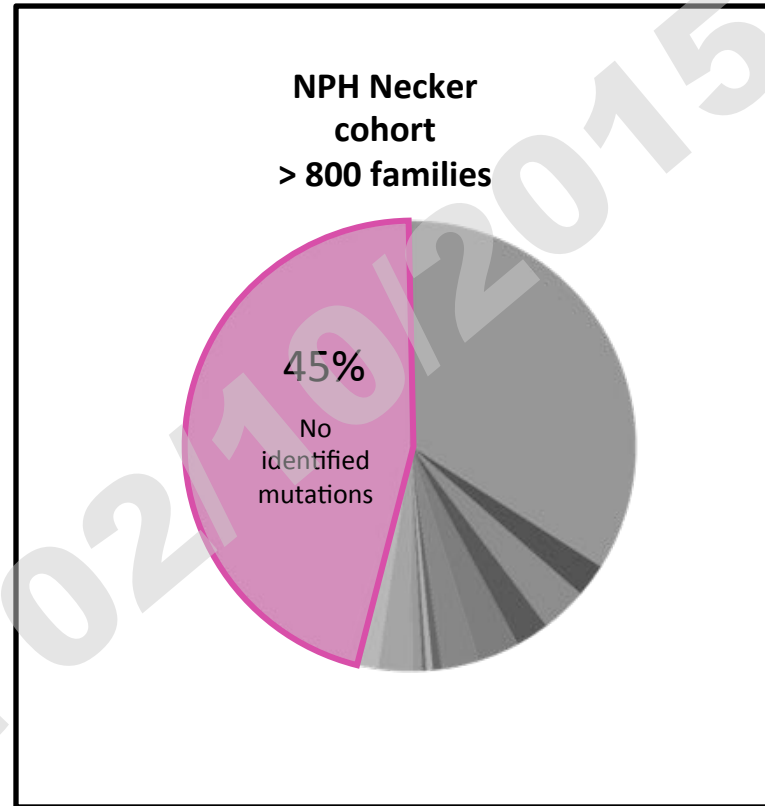
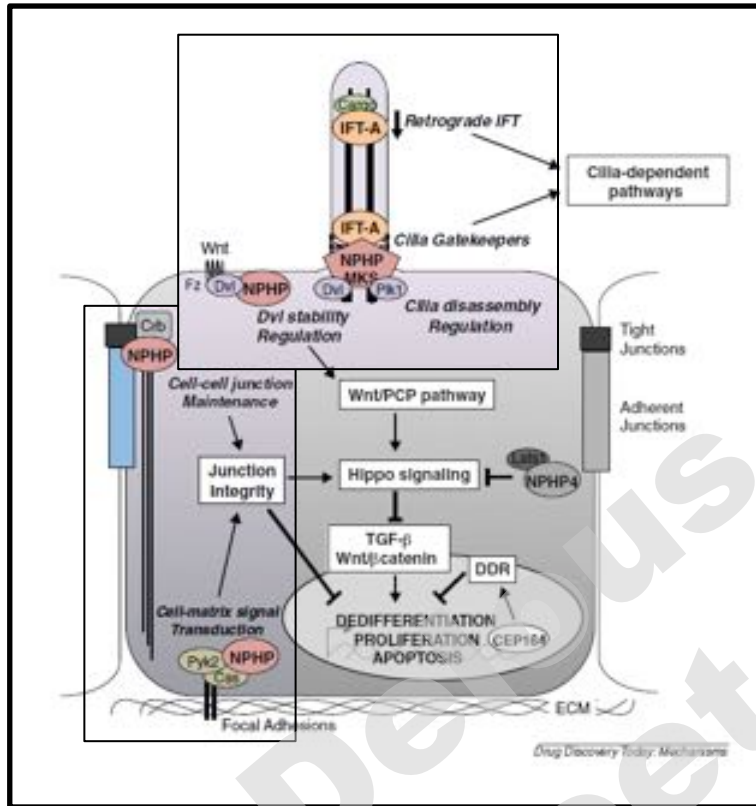


Adhésion sur collagène I

> Adhésion à la matrice



# Approche globale

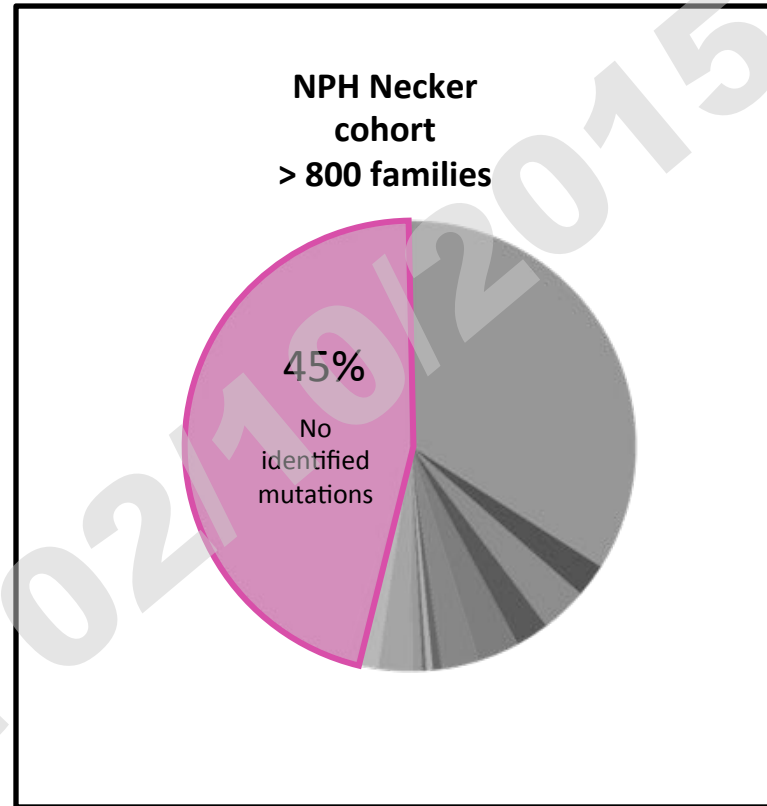
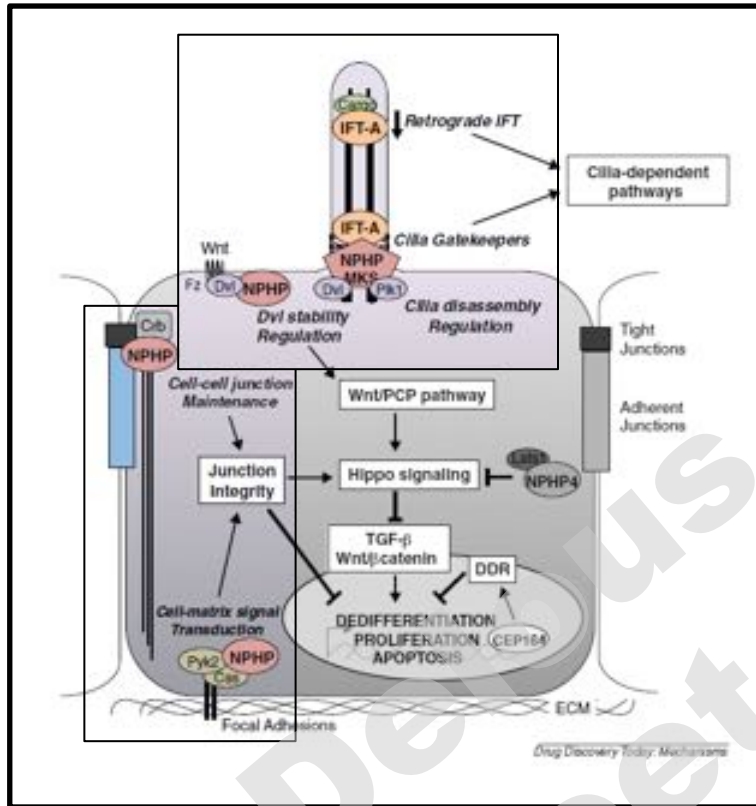


Caractérisation *in vivo* des fonctions des NPHP



Validation et caractérisation des nouveaux gènes candidats

# Approche globale

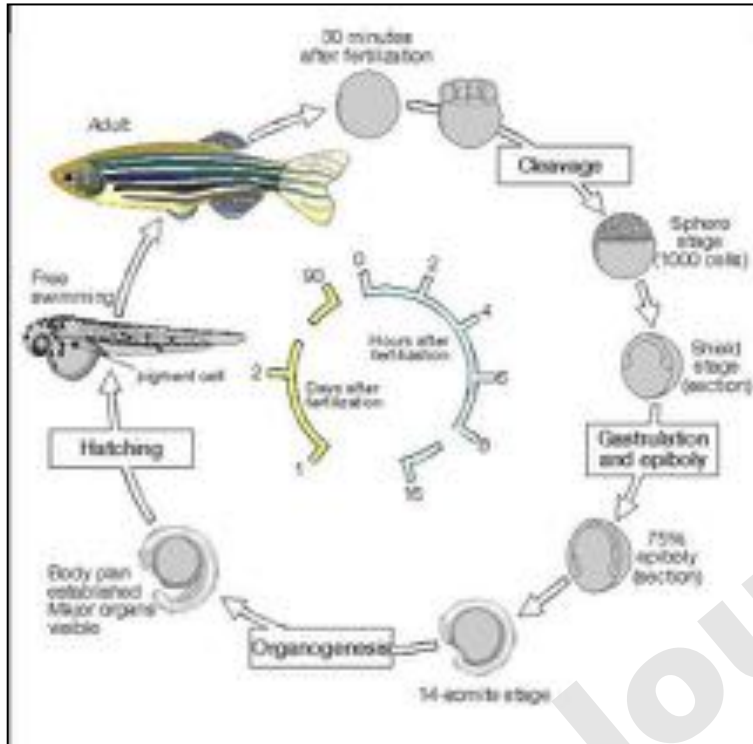


Caractérisation *in vivo* des fonctions des NPHP

Crible de composés chimiques à des fins thérapeutiques

Validation et caractérisation des nouveaux gènes candidats

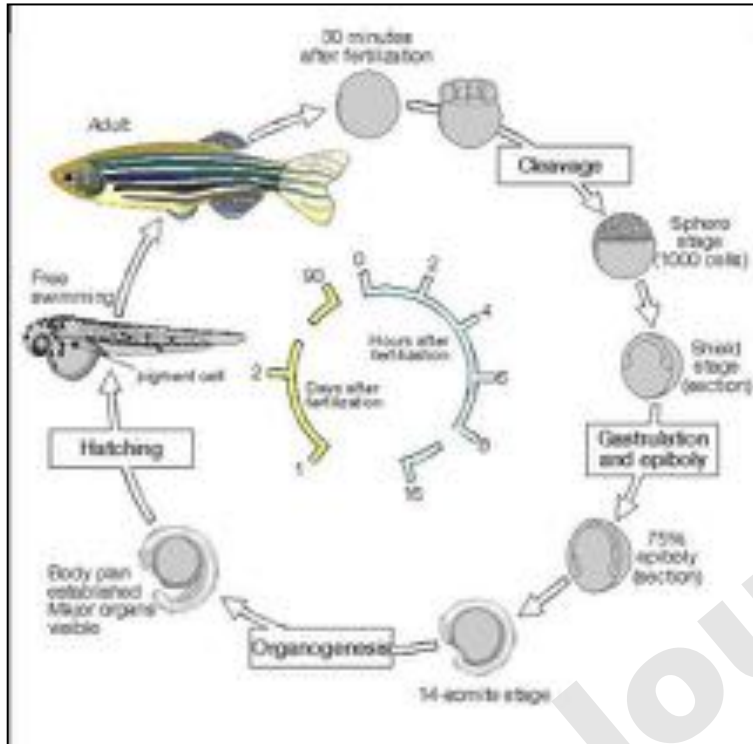
# Le modèle du poisson zèbre



## Avantages du modèle:

- Temps de génération court
- Fécondation externe
- Embryons transparents
- Grandes "portées"
- Animal de petite taille
  
- 70% des gènes humains ont un orthologue chez le zebrafish

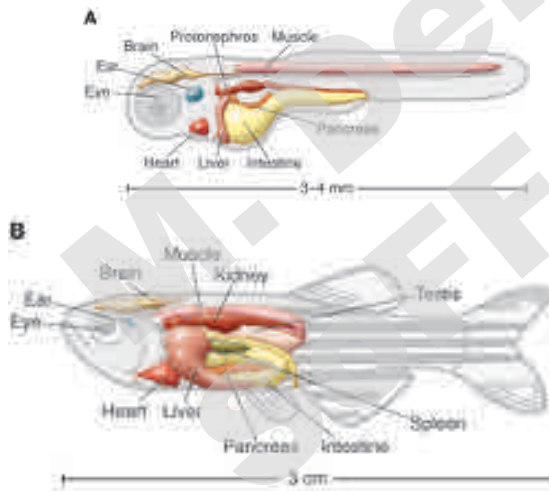
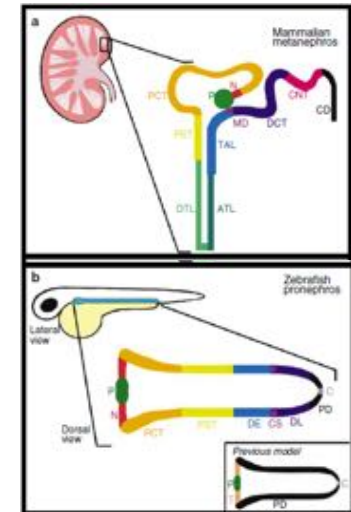
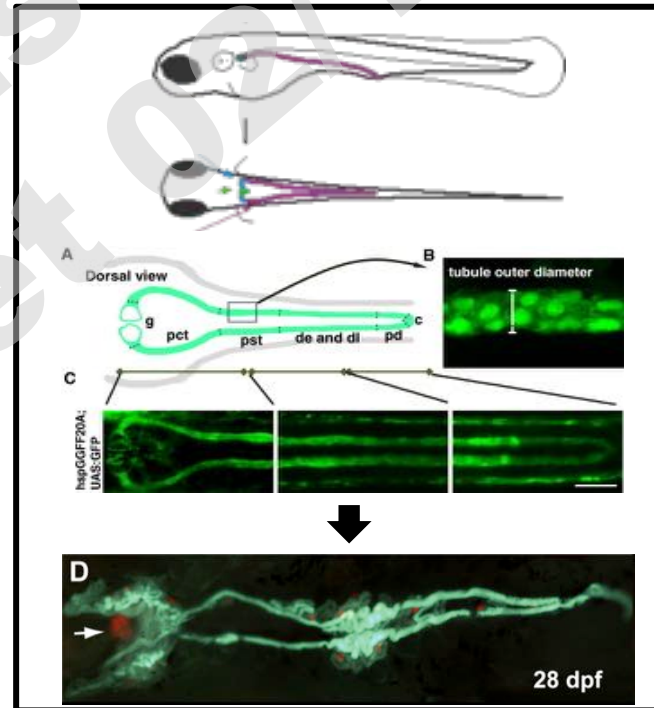
# Le modèle du poisson zèbre



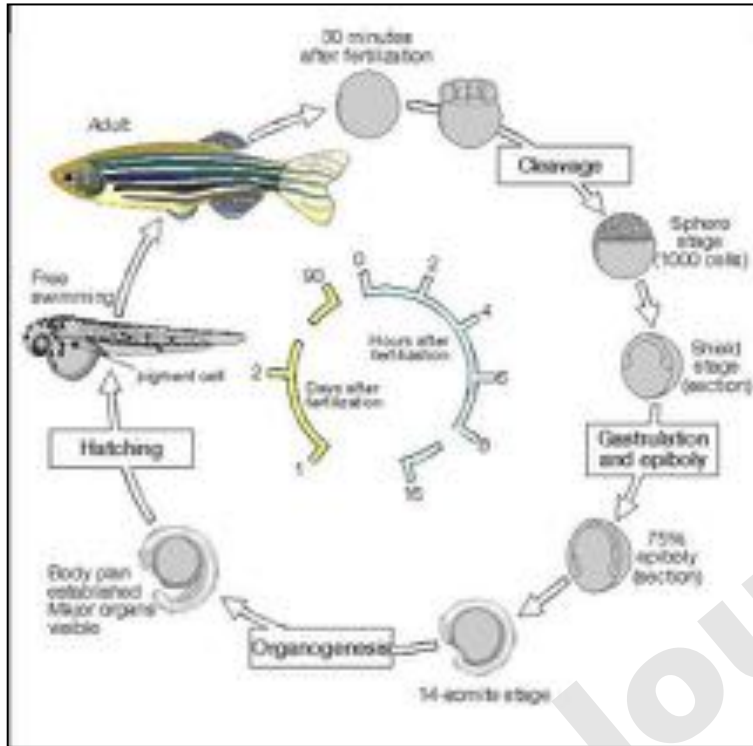
## Avantages du modèle:

- Temps de génération court
- Fécondation externe
- Embryons transparents
- Grandes "portées"
- Animal de petite taille
- 70% des gènes humains ont un orthologue chez le zebrafish

## Pronéphros

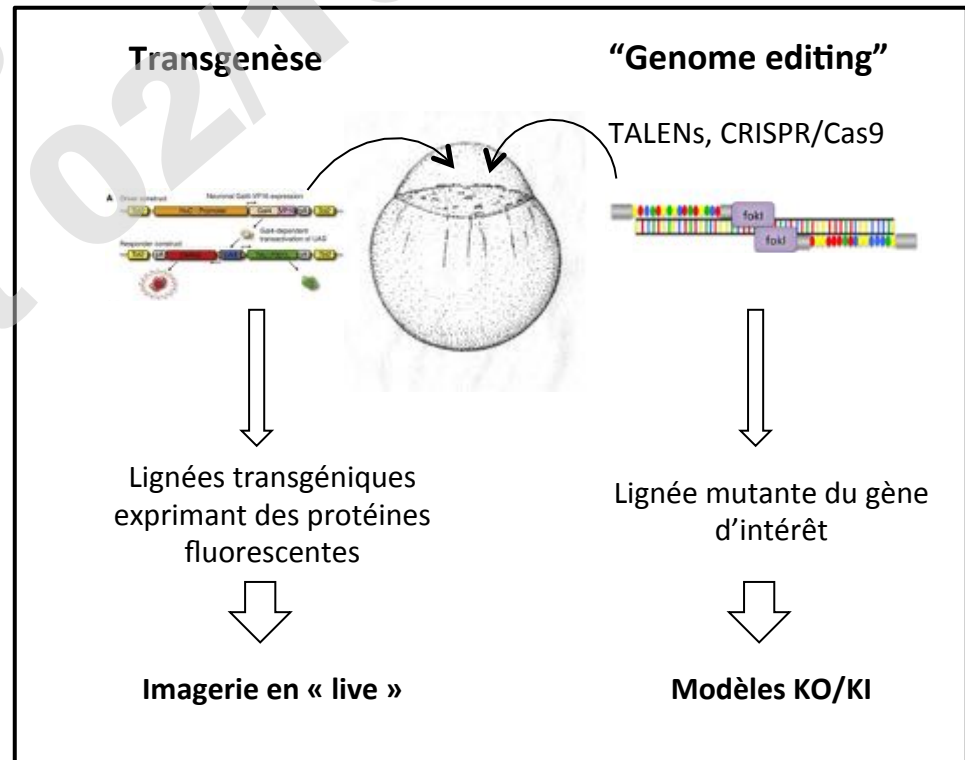
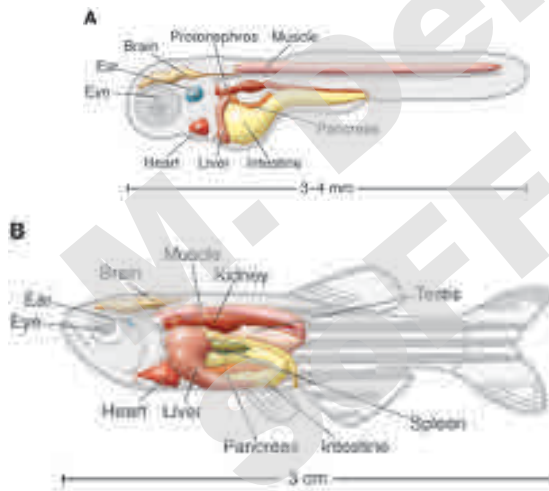


# Le modèle du poisson zèbre



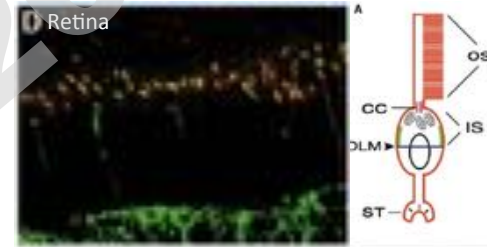
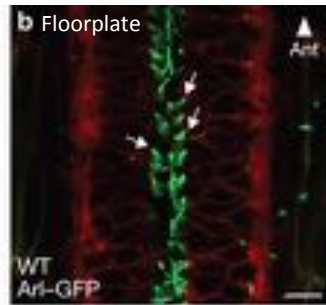
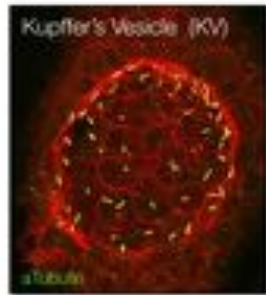
## Avantages du modèle:

- Temps de génération court
- Fécondation externe
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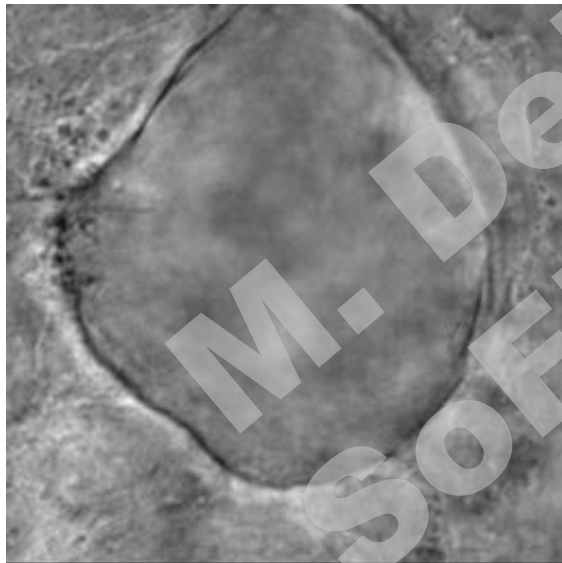




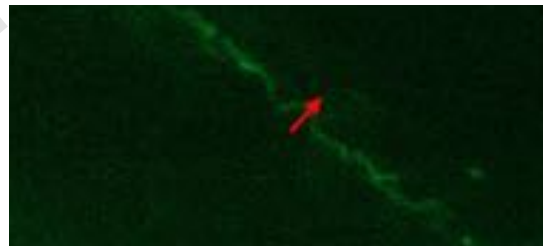
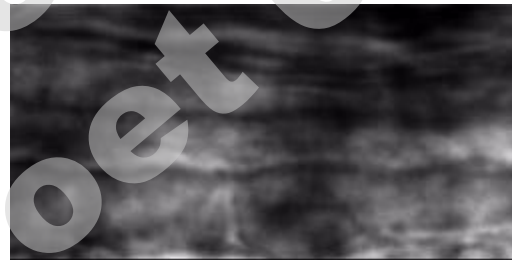
# Le zebrafish, un bon modèle pour les ciliopathies



Vésicule de Kupffer

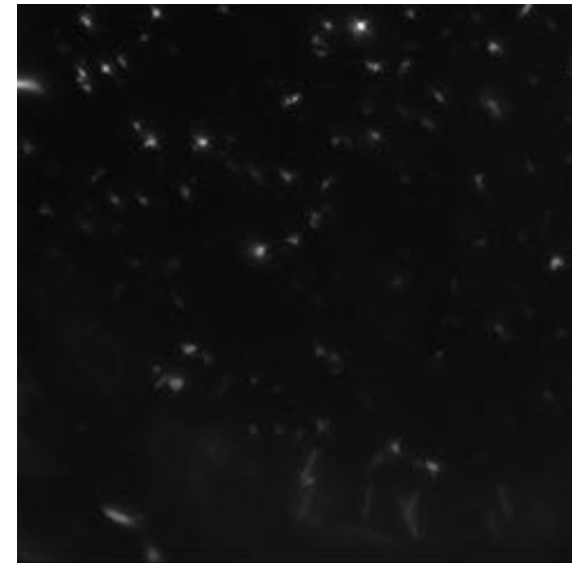


Pronéphros



*Tg(bactin:arl13bGFP)*

Vésicule otique



*Tg(bactin:arl13bGFP)*

# Le zebrafish, un bon modèle pour les ciliopathies

## A genetic screen in zebrafish identifies cilia genes as a principal cause of cystic kidney

Zhaoxia Sun<sup>1,\*</sup>, Adam Amsterdam<sup>1</sup>, Gregory J. Pazour<sup>2</sup>, Douglas G. Cole<sup>3</sup>, Mark S. Miller<sup>3</sup>  
and Nancy Hopkins<sup>1,†</sup>

<sup>1</sup>MIT, Center for Cancer Research and Biology Department, Building E17 Room 340, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

<sup>2</sup>Program in Molecular Medicine, University of Massachusetts Medical School, 373 Plantation Street, Worcester, MA 01605, USA

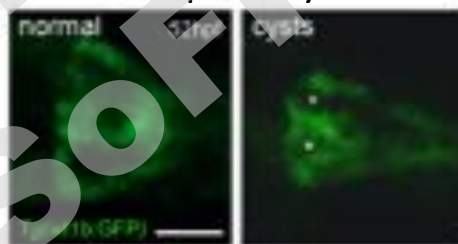
<sup>3</sup>Department of Microbiology, Molecular Biology and Biochemistry (LSS142), University of Idaho, Moscow, ID 83844, USA

\*Present address: Department of Genetics, Yale University School of Medicine, 333 Cedar Street, NSB-393, P.O. Box 208005, New Haven, CT 06520, USA

†Author for correspondence (e-mail: nhopkins@mit.edu)

Genes	Mutants	Phenotypes		Linkage analysis
		Kidney cyst	Curved body	
Group I mutants				
<i>whf1</i>	<i>ht548</i> <i>ht843</i> <i>ht2169</i>	+	-	Golling et al., 2002; Sun and Hopkins, 2001 24/24 mutant; 0/24 wild type for 2188A (Golling et al., 2002)
<i>cod1/cand1</i>	<i>ht2092</i> , <i>ht2188A</i> <i>ht2617</i>	+	-	
Group II mutants				
<i>IFT57</i>	<i>ht3417/carly</i>	-	+	16/16 mutant; 0/46 wild type
<i>IFT81</i>	<i>ht409/larry</i>	-	+	90/90 mutant; 0/36 wild type
<i>IFT172</i>	<i>ht2211/woe</i>	-	+	137/137 mutant; 0/55 wild type
<i>scorpion</i>	<i>ht459/scorpion</i>	+	+	64/64 mutant; 0/102 wild type (Golling et al., 2002)
<i>powin</i>	<i>ht1055B</i>	+	+	45/45 mutant; 0/50 wild type
<i>zeahorse</i>	<i>ht3308/zeahorse</i>	-	+	43/43 mutant; 0/50 wild type
<i>afim</i>	<i>ht3959/afim</i>	-	+	58/58 mutant; 0/87 wild type
<i>pkd2</i>	<i>ht4166</i>	morphant =	-	90/99 mutant; 0/93 wild type
not closed	<i>ht1392/twister</i>	+	+	69/69 mutant; 0/90 wild type
not closed	<i>ht2005/omega</i>	+	+	63/63 mutant; 0/64 wild type

Pronephric cysts



Body curvature

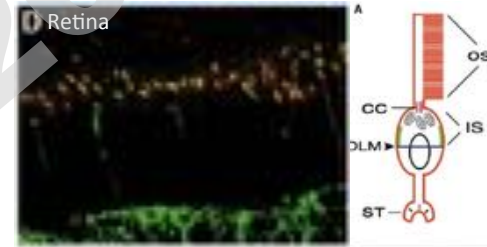
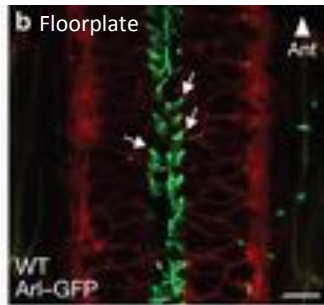
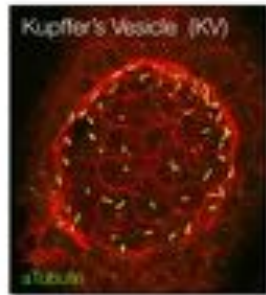


normal



curved

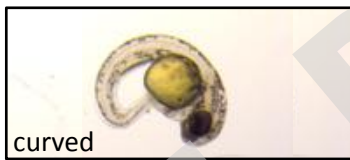
# Le zebrafish, un bon modèle pour les ciliopathies



Dysfunction of cilia



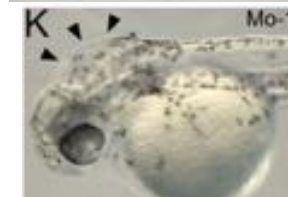
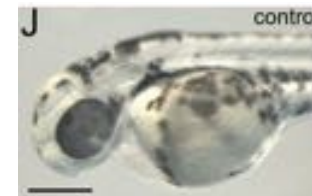
Body curvature



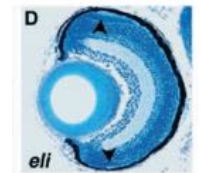
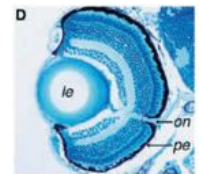
Laterality defects



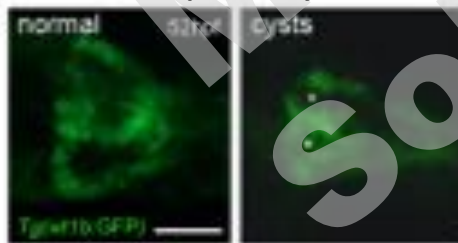
Hydrocephaly



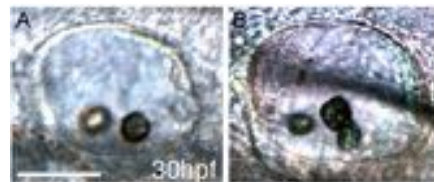
Retinal degeneration



Pronephric cysts



Otolith defects



## Control of the Wnt pathways by nephrocystin-4 is required for morphogenesis of the zebrafish pronephros

Céline Burcklé<sup>1,2,3,4,†</sup>, Helori-Mael Gaudé<sup>1,3,†</sup>, Christine Vesque<sup>2,4</sup>, Flora Silbermann<sup>1,3</sup>, Rémi Salomon<sup>1,3,5</sup>, Cécile Jeanpierre<sup>1,3</sup>, Corinne Antignac<sup>1,3,6</sup>, Sophie Saunier<sup>1,3,\*</sup> and Sylvie Schneider-Maunoury<sup>2,4,\*</sup>‡

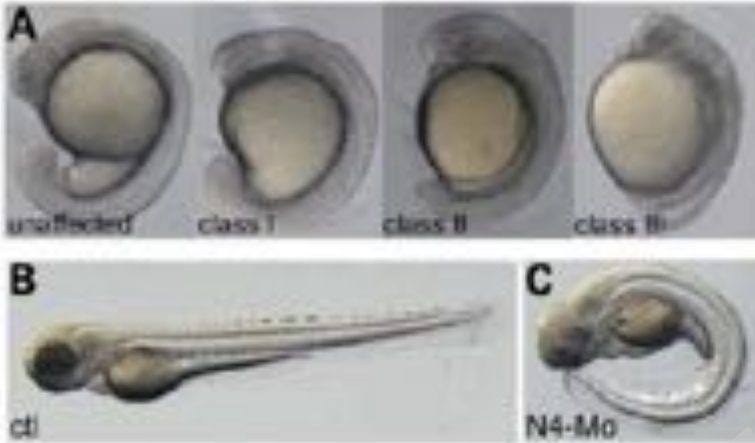
M. Delous  
SoFFoet 02/10/2015



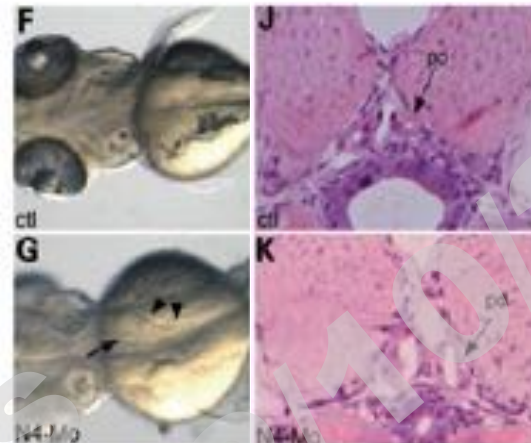
# Caractérisation des fonctions NPHP *in vivo*

## Approche morpholino ciblant *nphp4*

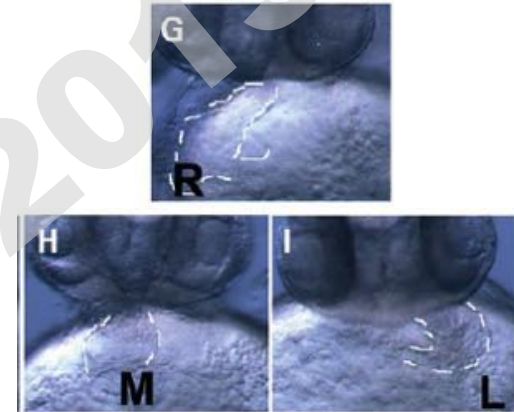
Axe courbé



Kystes pronéphriques

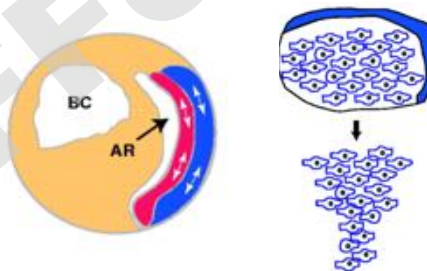


Défauts de latéralité



Défauts de mouvements de convergence-extension

Voie Wnt/Polarité planaire

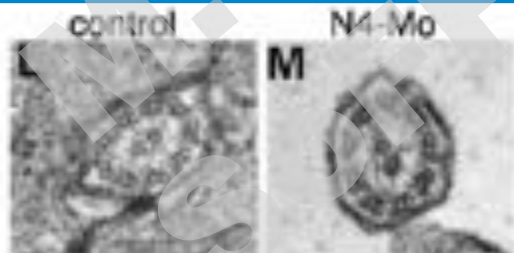
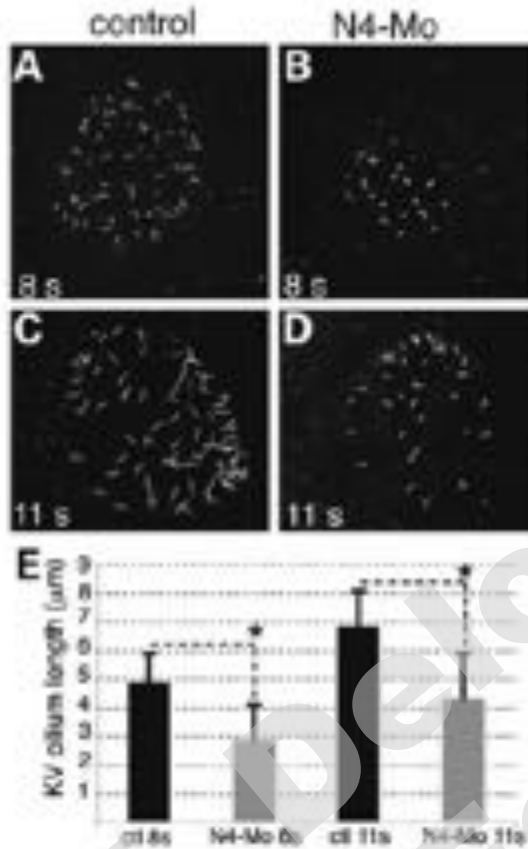


Défauts de ciliogenèse

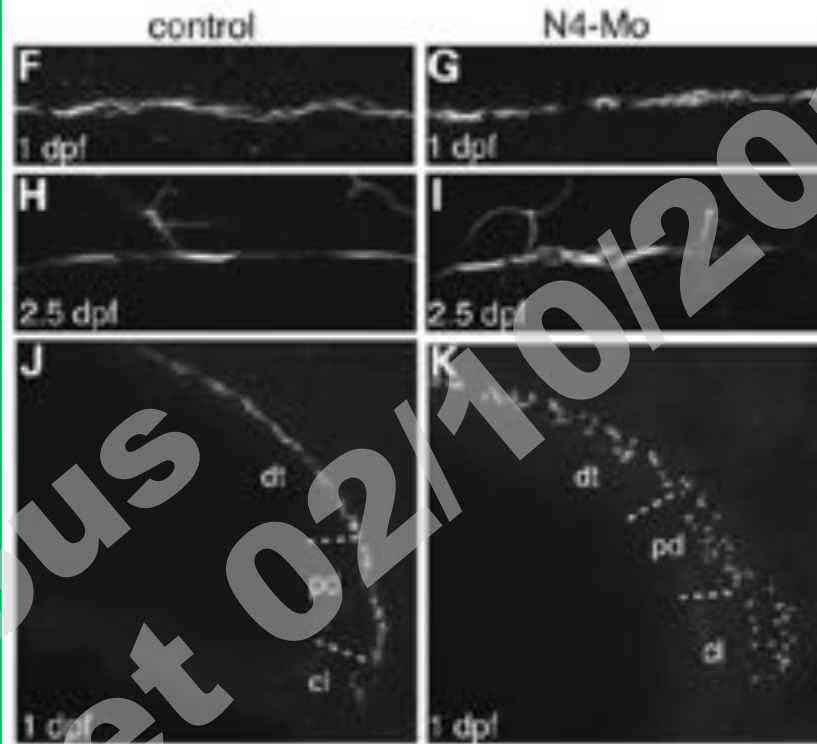


# Caractérisation des fonctions NPHP *in vivo*

## Kupffer's Vesicle

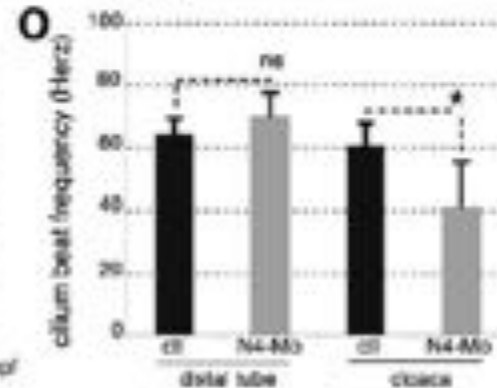
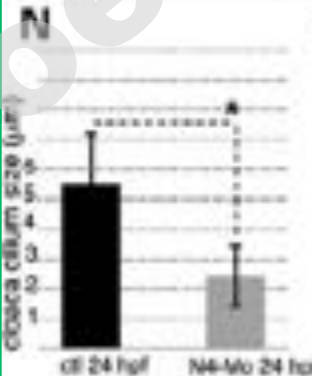


## Pronephros



Pronephric tubule

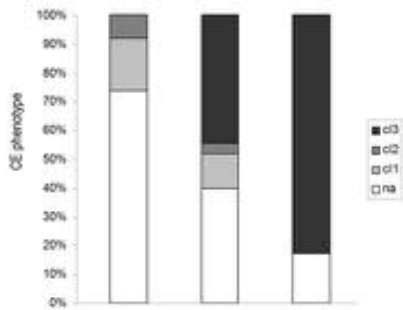
Pronephric duct  
Cloaca



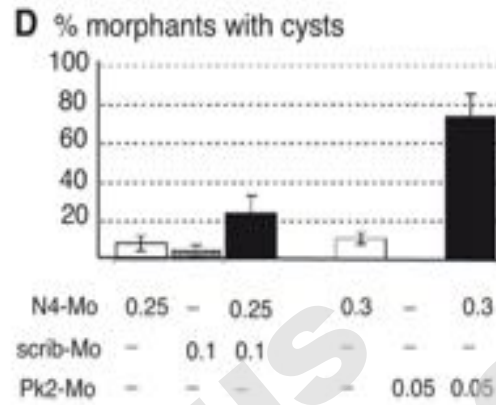
# Caractérisation des fonctions NPHP *in vivo*

---> Nphp4 régule les voies Wnt au cours de la formation du pronéphros

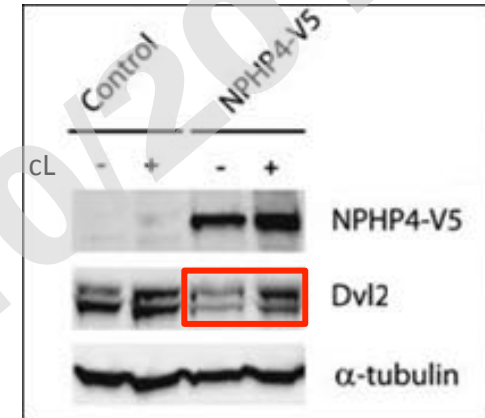
- NPHP4: Activateur de la voie **Wnt/PCP**



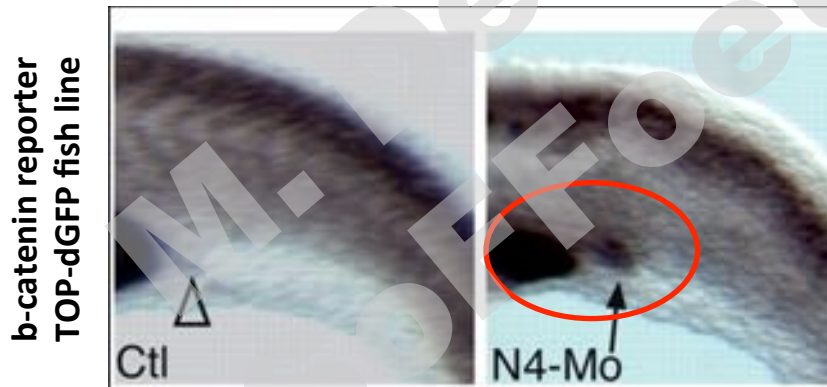
Genetic interaction between *nphp4* and *vangl2*



- NPHP4 régule la dégradation de Dvl par le protéasome

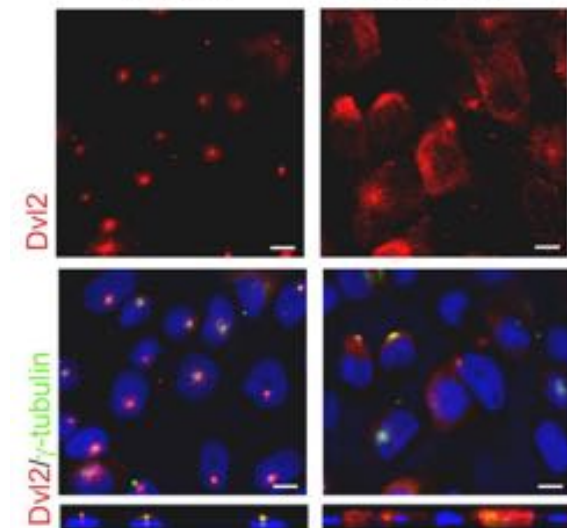


- NPHP4: Répresseur de la voie **Wnt/ $\beta$ -catenin**



b-catenin reporter TOP-dGFP fish line

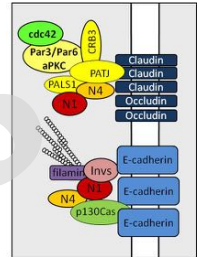
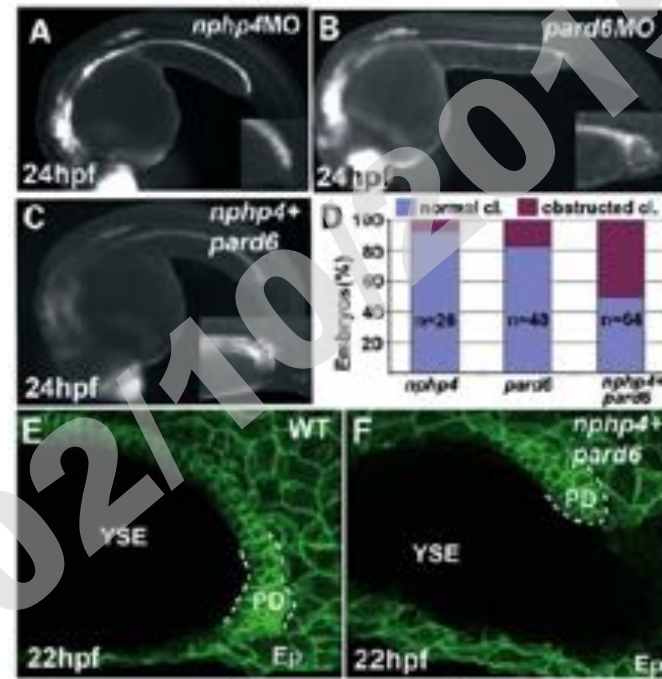
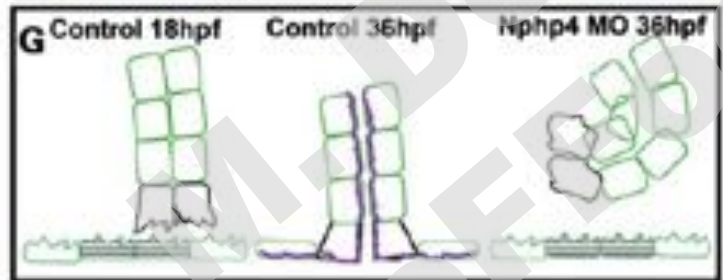
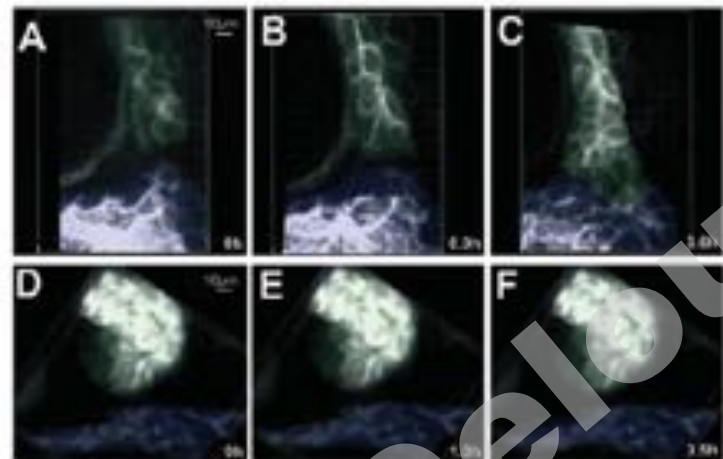
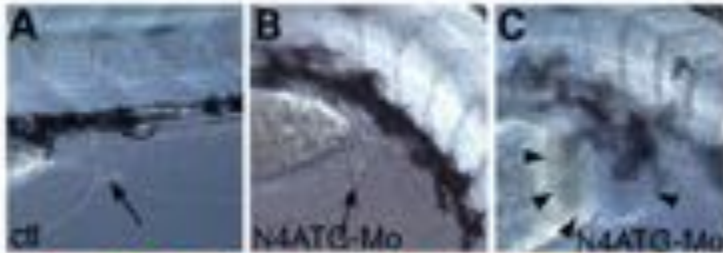
MDCK ctrl MDCK KD-N4



Dvl2  
Dvl2/ $\alpha$ -tubulin

# Caractérisation des fonctions NPHP *in vivo*

Défaut de morphogénèse du cloaque



Défauts de polarité/dynamique du réseau d'actine lors de la migration des cellules ?

# Caractérisation des fonctions NPHP *in vivo*

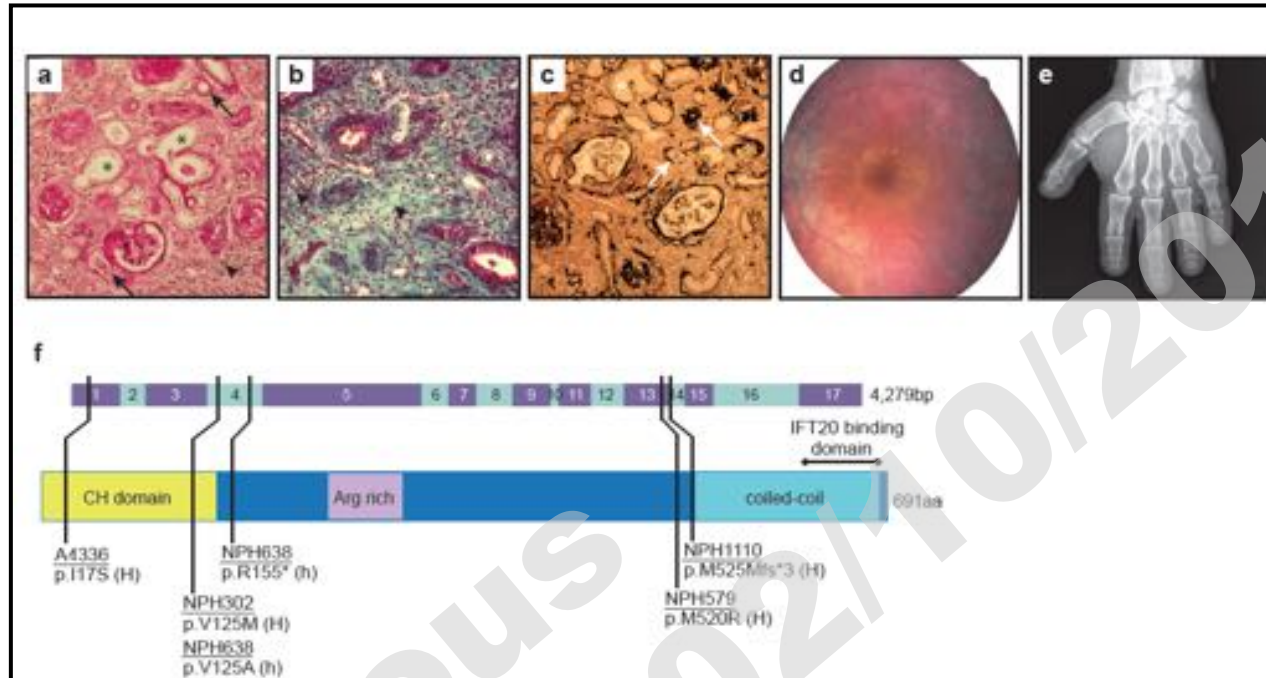
## Mutations in TRAF3IP1/IFT54 reveal a new role for IFT proteins in microtubule stabilization

Albane A. Bizet<sup>1,2</sup>, Anita Becker-Heck<sup>3</sup>, Rebecca Ryan<sup>1,2</sup>, Kristina Weber<sup>4</sup>, Emilie Filhol<sup>1,2</sup>, Pauline Krug<sup>1,2</sup>, Jan Halbritter<sup>5,6</sup>, Marion Delous<sup>1,2</sup>, Marie-Christine Lasbennes<sup>3</sup>, Bolan Linghu<sup>7</sup>, Edward J. Oakeley<sup>3</sup>, Mohammed Zarhrate<sup>2,8</sup>, Patrick Nitschké<sup>2,9</sup>, Meriem Garfa-Traore<sup>10</sup>, Fabrizio Serluca<sup>7</sup>, Fan Yang<sup>7</sup>, Tewis Bouwmeester<sup>3</sup>, Lucile Pinson<sup>11</sup>, Elisabeth Cassuto<sup>12</sup>, Philippe Dubot<sup>13</sup>, Neveen A. Soliman Elshakhs<sup>14</sup>, José A. Sahel<sup>15,16</sup>, Rémi Salomon<sup>1,2,17</sup>, Iain A. Drummond<sup>18,19</sup>, Marie-Claire Gubler<sup>1,2</sup>, Corinne Antignac<sup>1,2,20</sup>, Salahdine Chibout<sup>3</sup>, Joseph D. Szustakowski<sup>6</sup>, Friedhelm Hildebrandt<sup>5</sup>, Esben Lorentzen<sup>4</sup>, Andreas W. Sailer<sup>3</sup>, Alexandre Benmerah<sup>1,2,\*</sup>, Pierre Saint-Mezard<sup>3,\*</sup> & Sophie Saunier<sup>1,2,\*</sup>

M. Delous  
SoFFoet 02/10/2015



# Validation de nouveaux gènes candidats

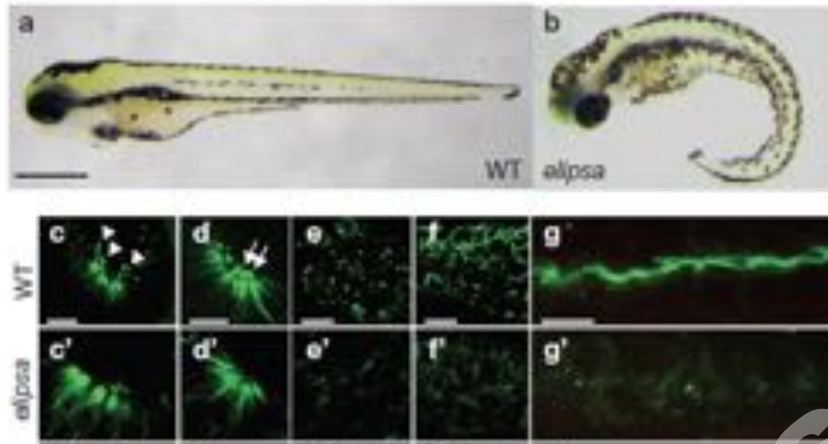


- 8 individuals from 5 unrelated families
  - NPH (ESRD 3-25 yrs) with RP
  - 6/8 with skeletal defects (polydactyly)
- Most patients carried 2 missense mutations, a missense + a truncating mutation or a splice mutation giving rise to RNA decay
  - => Pathogenicity of the missense mutations?

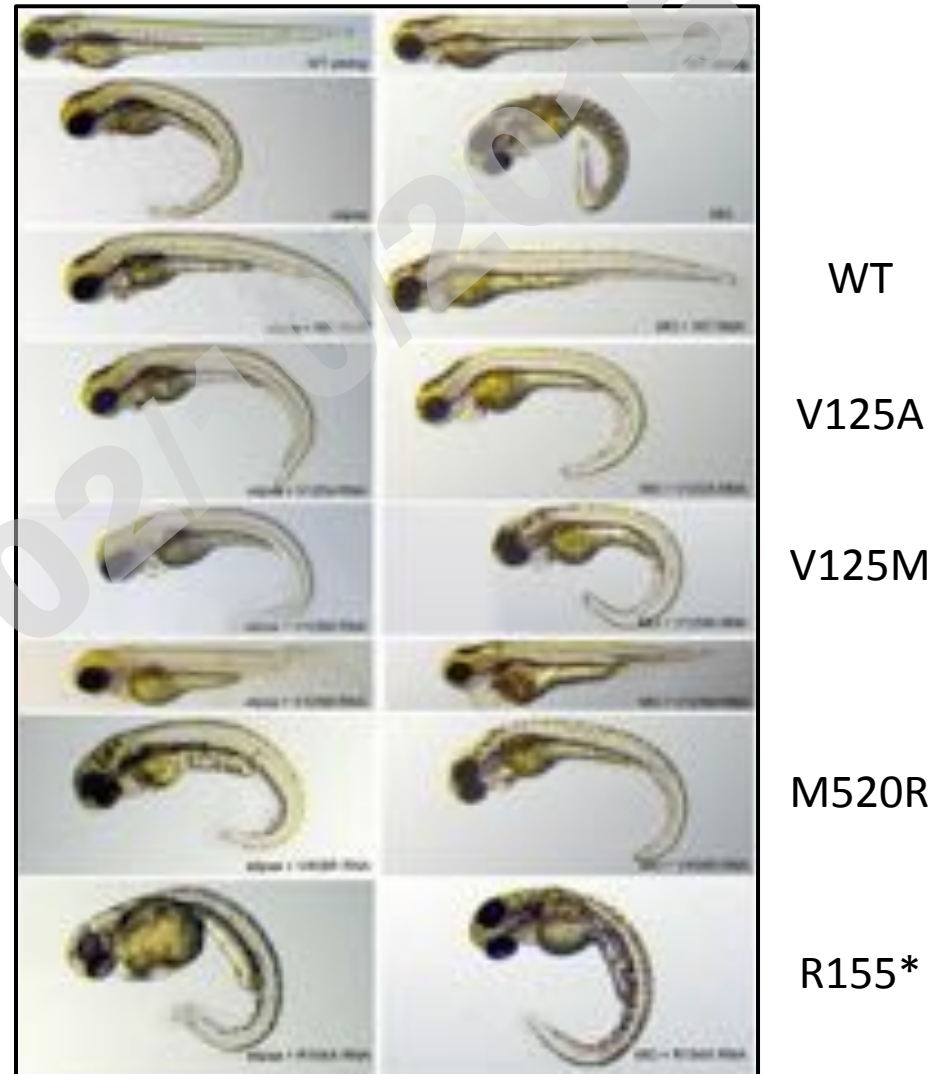


# Validation de nouveaux gènes candidats

Lignée mutante *elipsa/ift54* avec injection ARN *traf3ip* WT ou muté

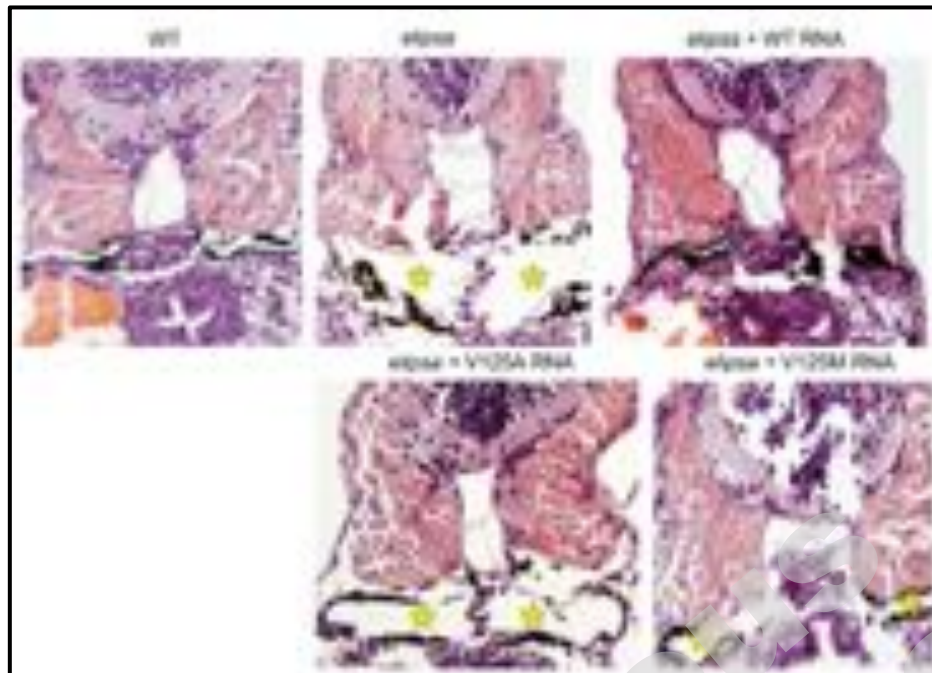


Omori et al., 2008

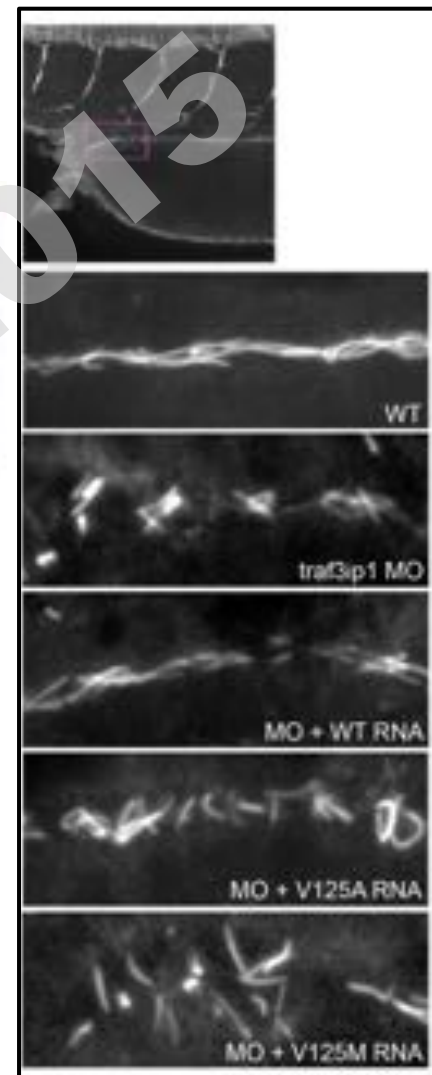


(Bizet et al., 2015)

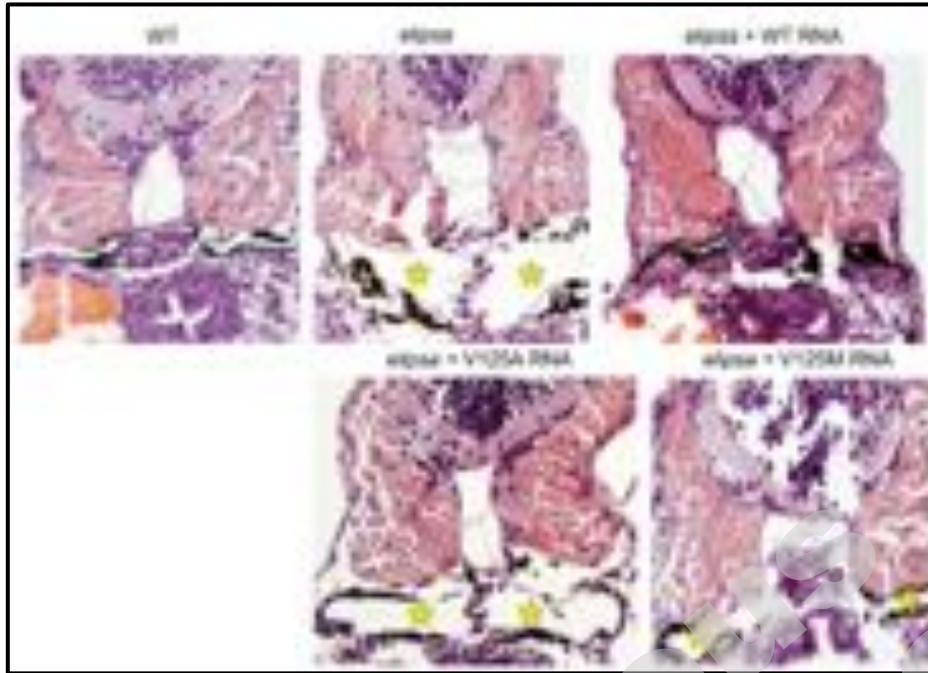
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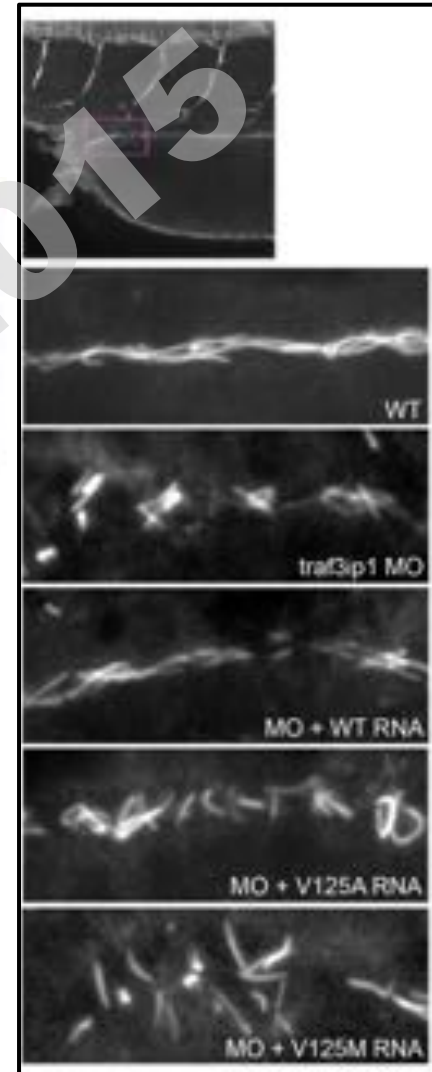
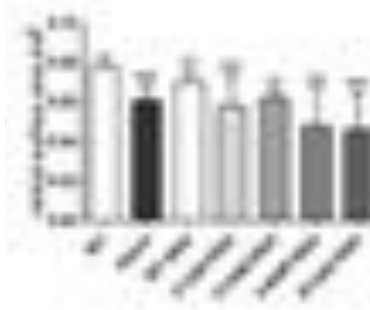
	pronephric cysts (%)
WT	0
<i>elpsa</i>	100
<i>elpsa</i> + WT	15.67
<i>elpsa</i> + V125A	91.33
<i>elpsa</i> + V125M	74.07
<i>elpsa</i> + V459R	83.33
<i>elpsa</i> + R154X	100



# Validation de nouveaux gènes candidats



	pronephric cysts (%)
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<i>elpsa</i> + R154X	100





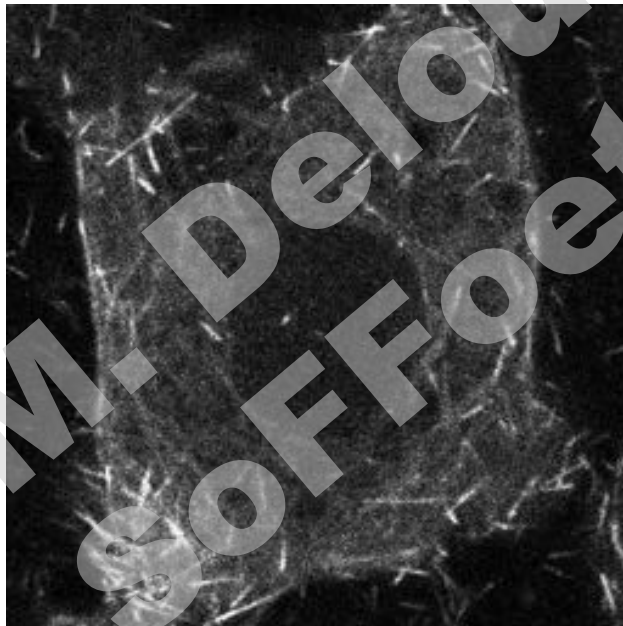
# Validation de nouveaux gènes candidats

## Données *in vitro*:

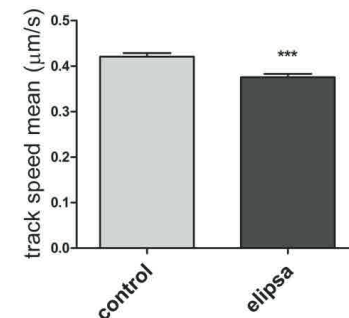
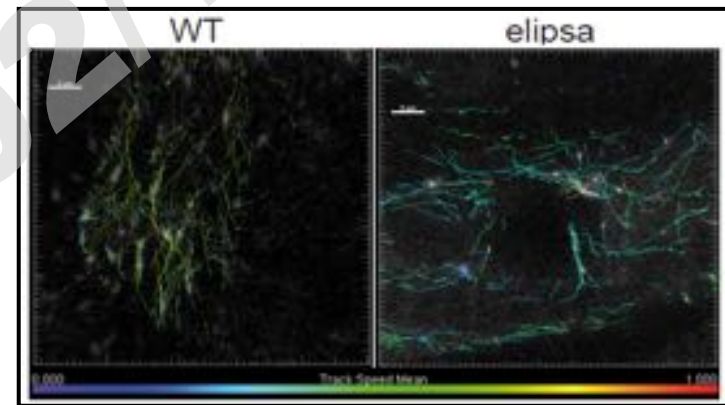
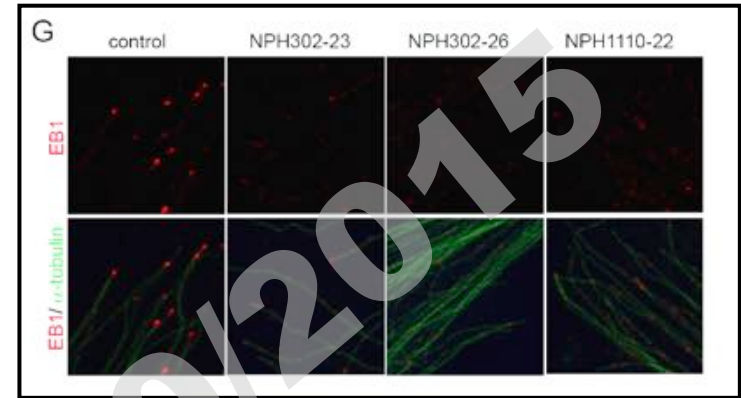
Stabilité accrue des microtubules dans les fibroblastes de patients  
(perte de EB1 aux extrémités des MT,  
résistance des MT au traitement au froid)



Analyse de la dynamique des microtubules *in vivo*



EB3-GFP

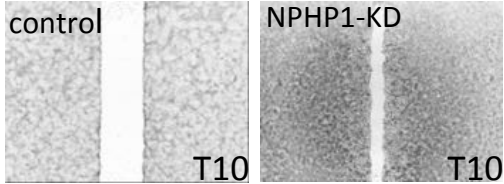


(Bizet et al., 2015)

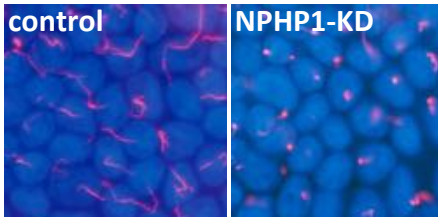
# Crible de molécules chimiques

**Prestwick library**  
(1120 compounds)

## Défauts de migration cellulaire



## Défauts de ciliogenèse



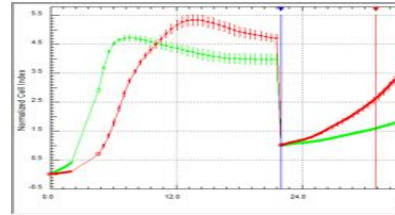
DAPI/ $\alpha$ -tubulin acetylated

**37 hits**



## Tests *in vitro*

xCELLigence technology



Culture 3D



Validation dans le modèle  
zebrafish MO *nphp4*

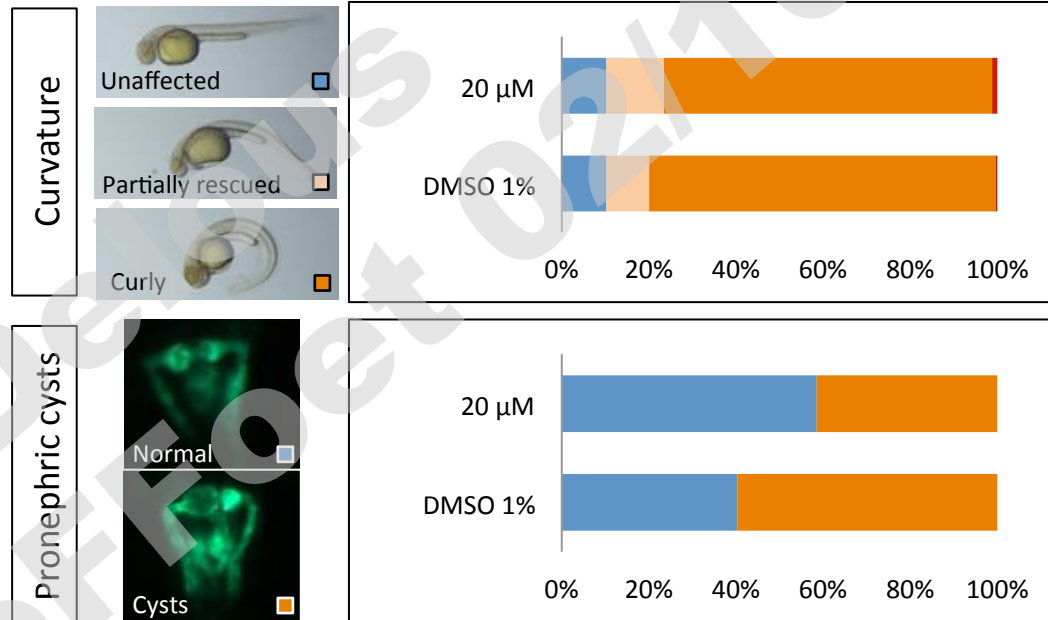
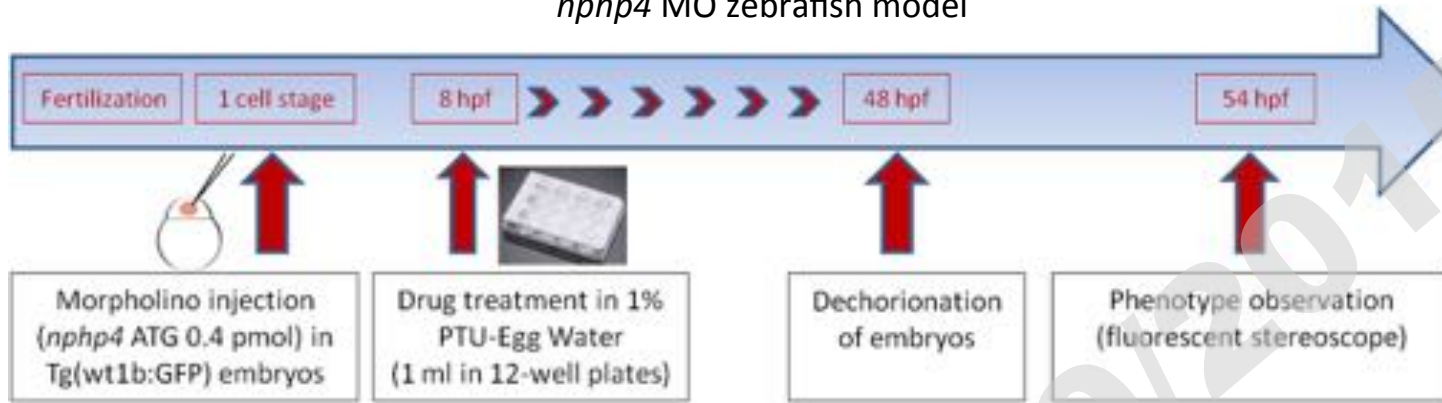


M. Delouis  
SoFFoet 02/10/2015



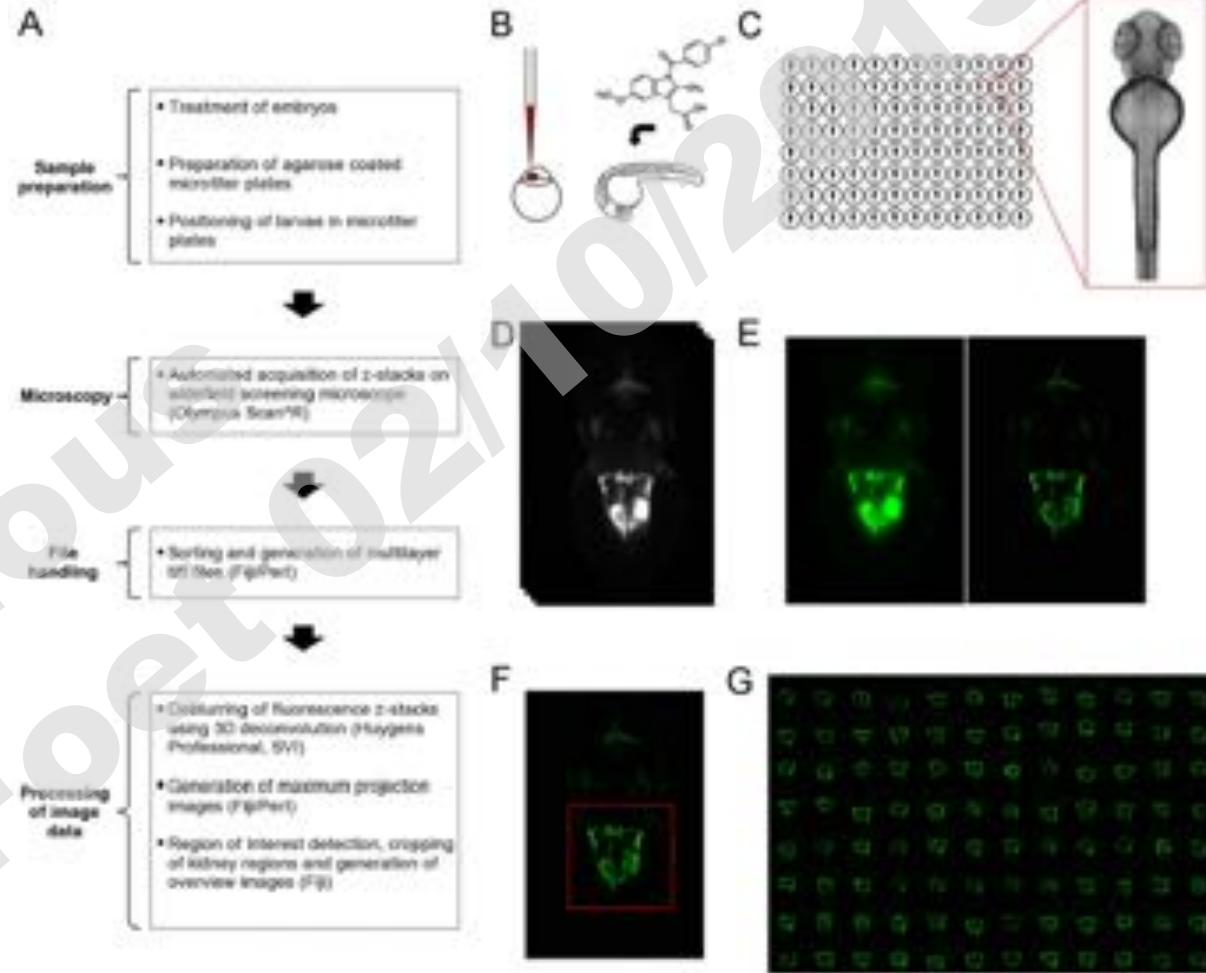
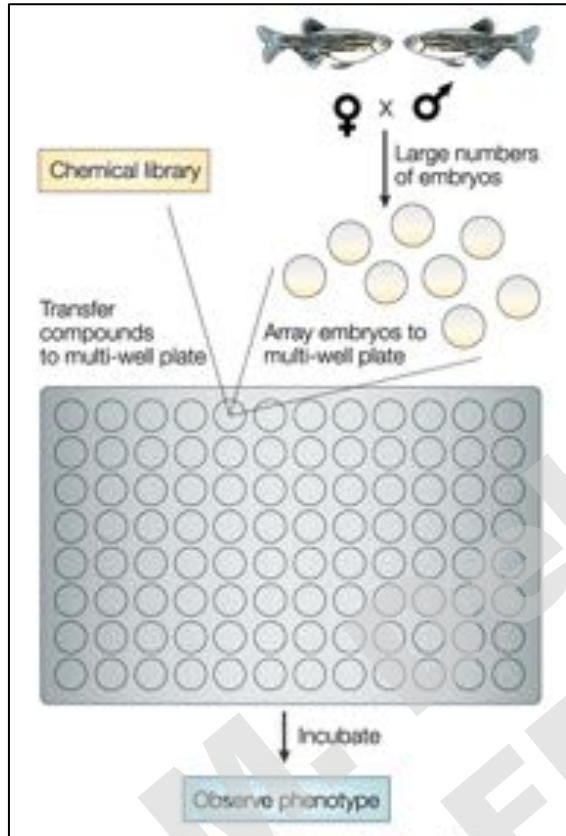
# Crible de molécules chimiques

*nphp4* MO zebrafish model



# Crible de molécules chimiques

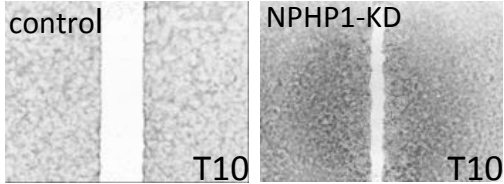
---> Développement d'une méthode semi-automatisée



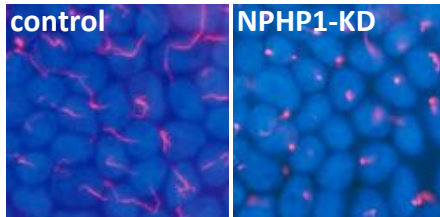
# Crible de molécules chimiques

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## Défauts de migration cellulaire



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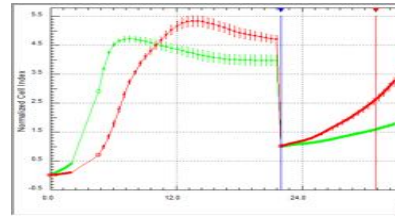
DAPI/ $\alpha$ -tubulin acetylated

**37 hits**



## Tests *in vitro*

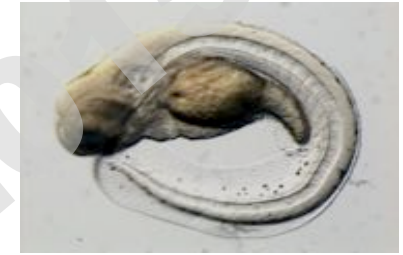
xCELLigence technology



## Culture 3D



Validation dans le modèle  
zebrafish MO *nphp4*



Validation chez la souris  
*Nphp4*<sup>-/-</sup>



- Kystes et fibrose interstitielle après néphrectomie
- Dégénérescence rétinienne
- Infertilité mâle



**Thérapeutique**

# Remerciements

## U1163-Inserm

### S. Saunier

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L. Garnero

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