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## Quantitative image based analysis of endocrine disruptor effects on mitochondria morphology-function in prostate cancer cells

Aurélie Charazac, Célia Decondé Le Butor, Kévin Giulietti, Jean-Marc A. Lobaccaro, Silvère Baron, Jérôme Gilleron, Patrick Fenichel, Xavier Descombes, Frédéric Bost, Clavel Stéphan, et al.

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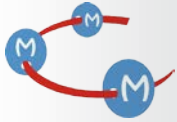
**HAL Id: hal-01548783**

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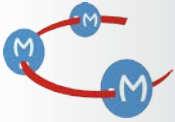
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# Quantitative image based analysis of endocrine disruptor effects on mitochondria morphology-function in prostate cancer cells.



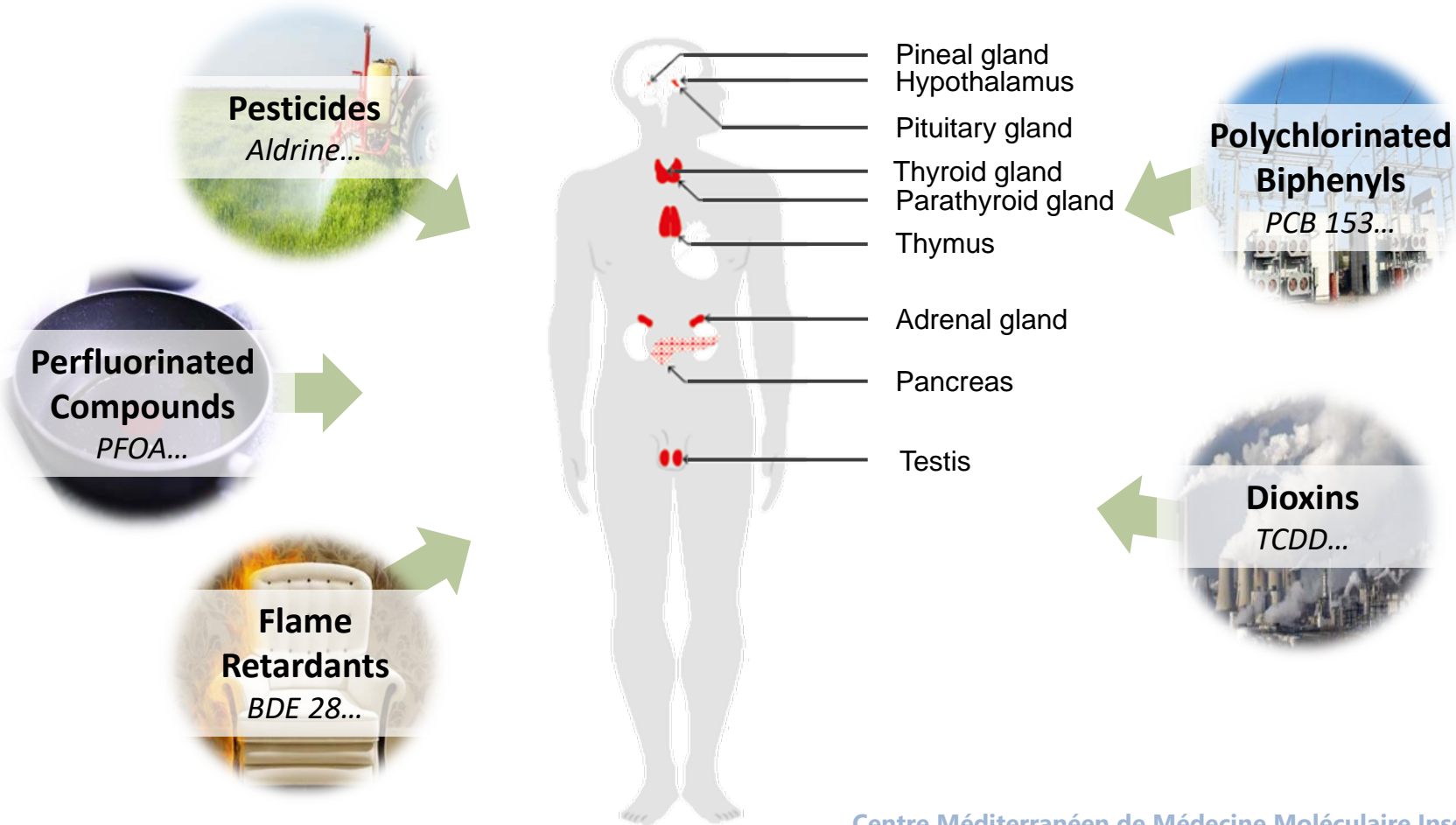
Charazac Aurélie, Decondé le Butor Célia, Giulietti Kévin, Lobaccaro Jean Marc, Baron Silvère, Gilleron Jérôme, Fénichel Patrick, Descombes Xavier, Bost Frédéric, Clavel Stéphan & Chevalier Nicolas.

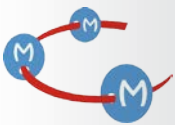


# Endocrine Disruptor Compounds


« An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations. »

World Health Organization





# Major challenge

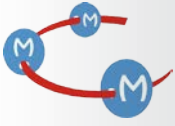
	SIN LIST	DANISH EPA	TEDX	EU REACH SVHC
 Acceptation of WHO definition	✓	—	✗	✓
Based on risk assessment	✗	✗	✗	✗
List updating	✗	—	✓	✓

Adapted from [EndocrineScience.org](http://EndocrineScience.org)




Registration, **E**valuation and **A**utorization of **C**hemicals

↳ Promotes alternative methods for the hazard assessment of substances.



# Major challenge

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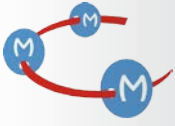


Registration, **E**valuation and **A**utorization of **C**hemicals

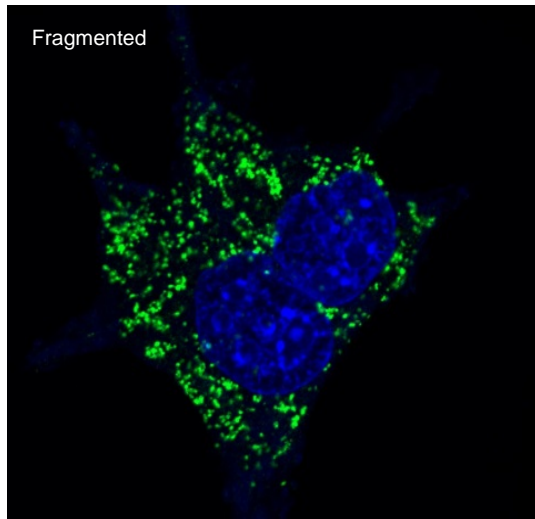
↳ Promotes alternative methods for the hazard assessment of substances.

Urgent need for multiparametric, robust and high throughput cell-based assay :

- To investigate the mechanisms underlying the adverse effects of known EDCs
- To identify new compounds with endocrine-disrupting potential

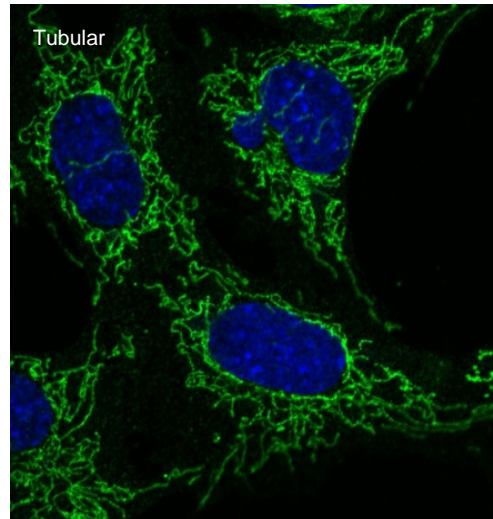


# Mitochondria, a cell sensor



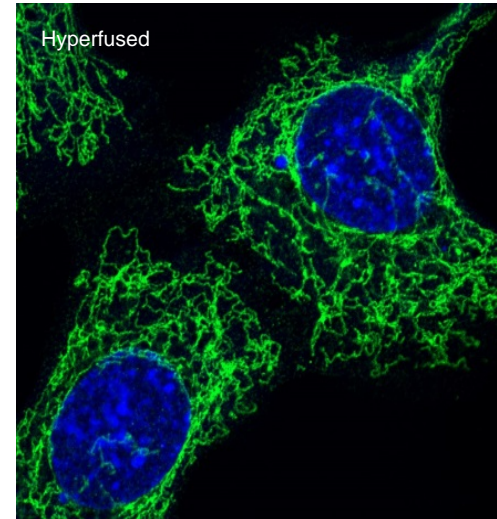
Fragmented

Apoptosis



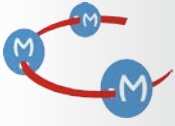
Tubular

Healthy

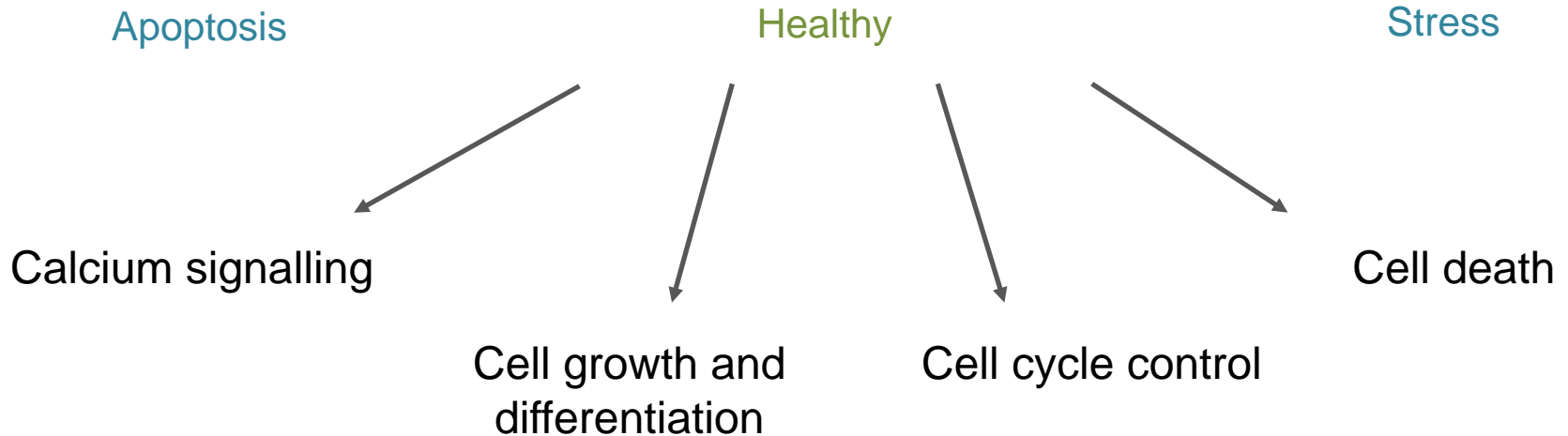
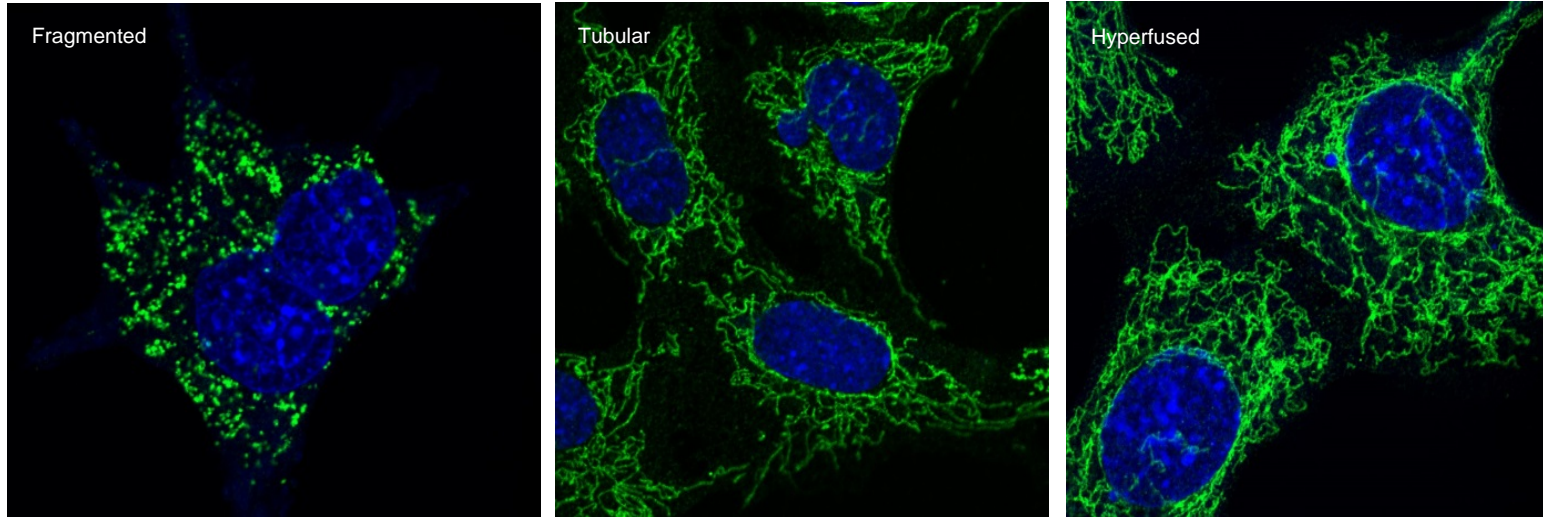


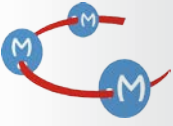
Hyperfused

Stress



# Mitochondria, a cell sensor





# Mitochondria, a cell sensor

## Alteration of **mitochondrial forms and functions** by EDCs ?

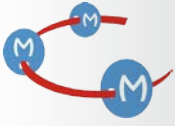
1) Quantitative image based analysis of mitochondrial functions

→ High throughput screening

2) Computational image based analysis of mitochondrial morphology

→ Image analysis and classification

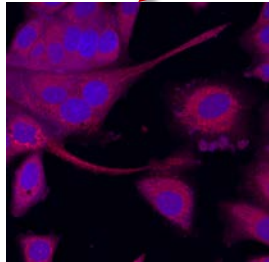
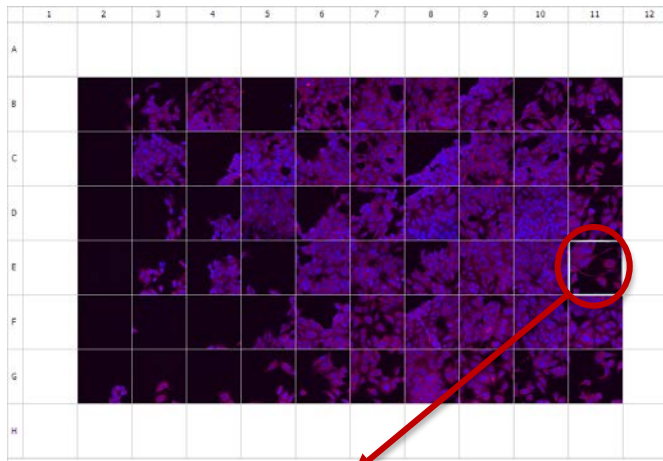




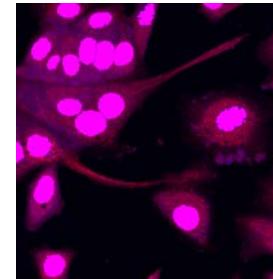
# Multiparametric cell-based assay

## Automated image acquisition

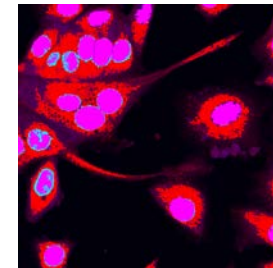
(Nikon A1R-20X)



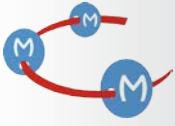
## Image processing



→ 1) **Dapi masking :**  
cell counting



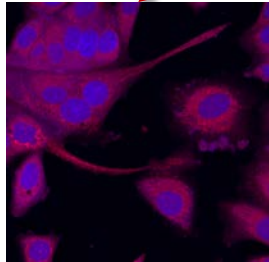
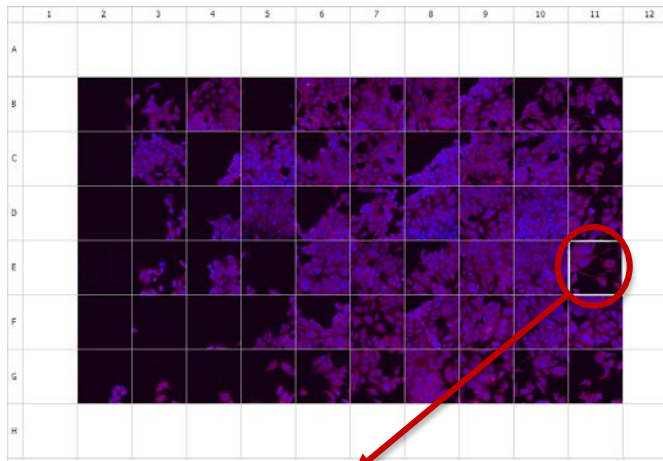
→ 2) **Dye masking :**  
fluorescence intensity



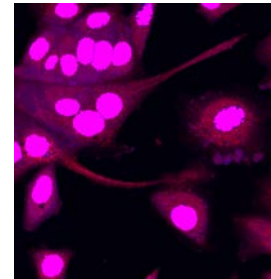
# Multiparametric cell-based assay

## Automated image acquisition

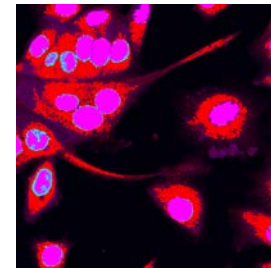
(Nikon A1R-20X)



## Image processing

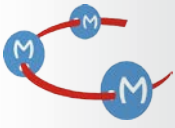


→ 1) **Dapi masking :**  
cell counting



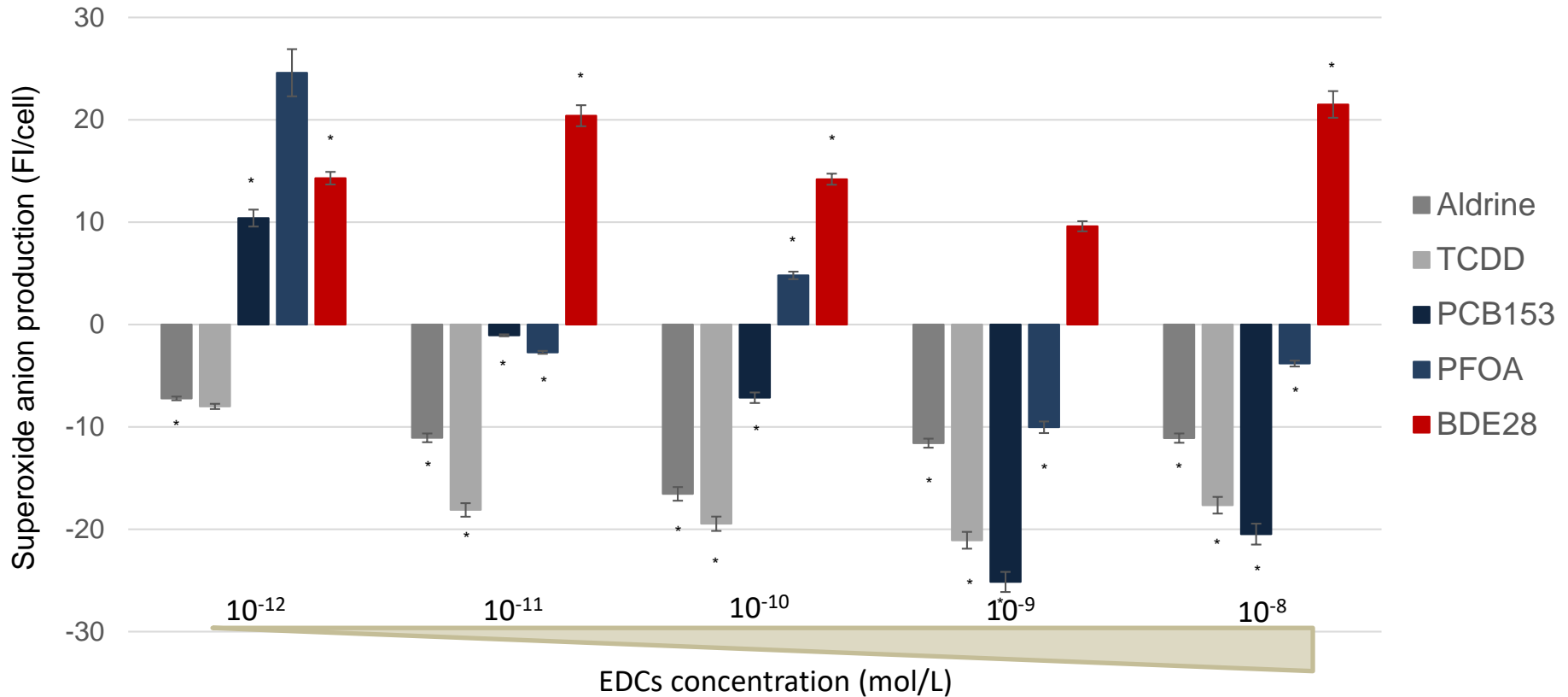
→ 2) **Dye masking :**  
fluorescence intensity

- **Multiparameter :**
  - mitochondrial membrane potential (MitoTracker Red™)
  - superoxide anion production (MitoSox™)
  - mitophagy (Keima probe)

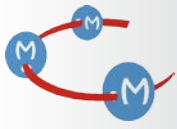


# EDCs affect the mitochondrial function

- ROS production on DU145:

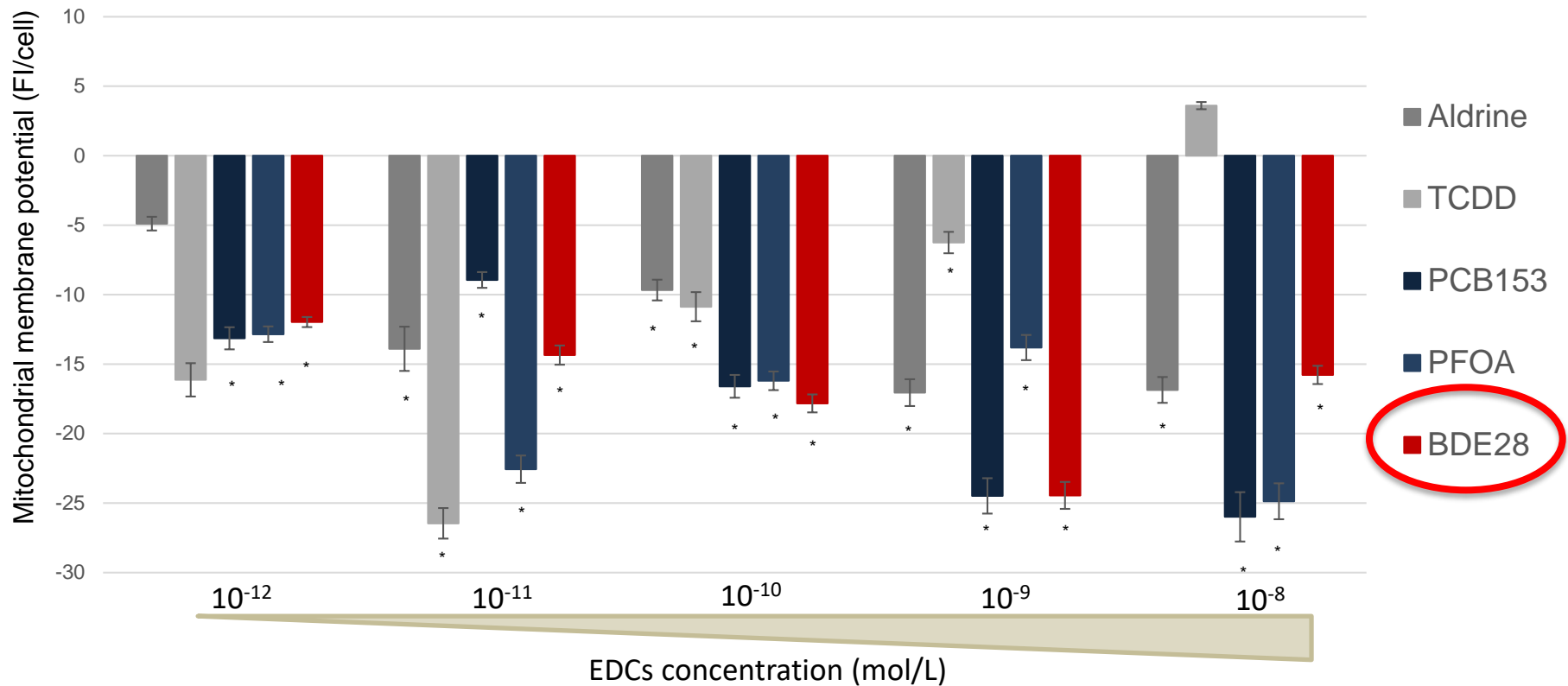


\* significantly different from the control,  $p \leq 0,005$ .

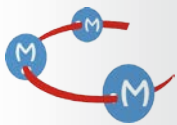


# EDCs affect the mitochondrial function

- Mitochondrial membrane potential on DU145 :

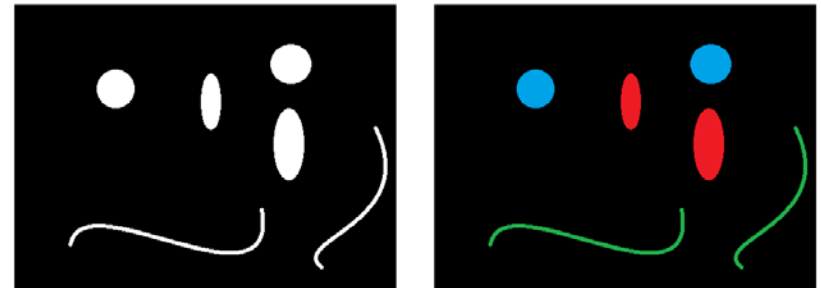
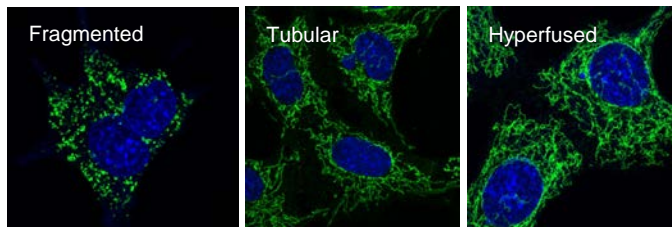


\* significantly different from the control,  $p \leq 0,005$ .

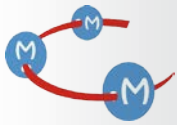


# Forms classification of mitochondrial network

- Collaboration with

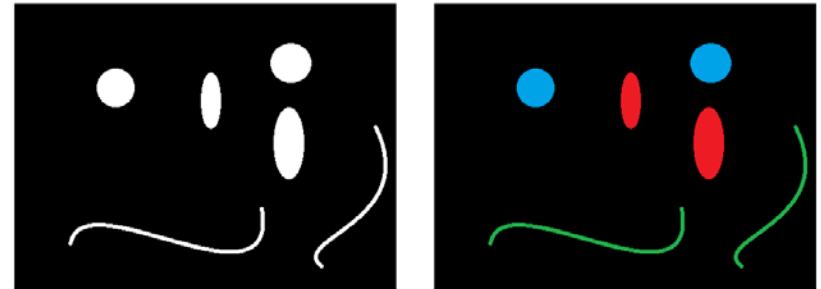
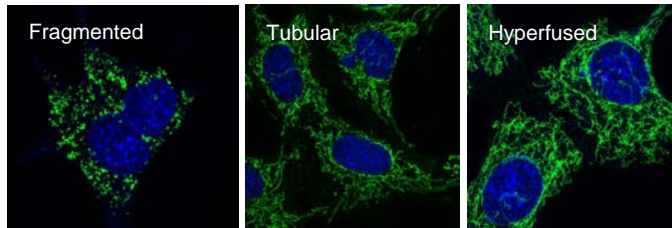


Fragmented, Tubular, Hyperfused



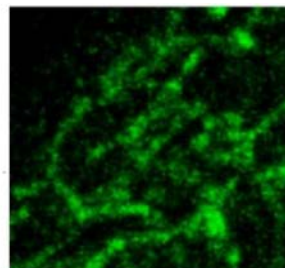
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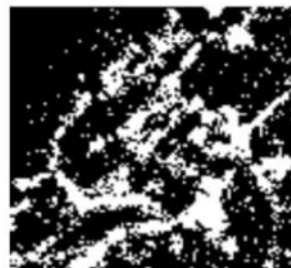


Fragmented, Tubular, Hyperfused

Image

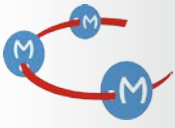


Basic  
thresholding  
method



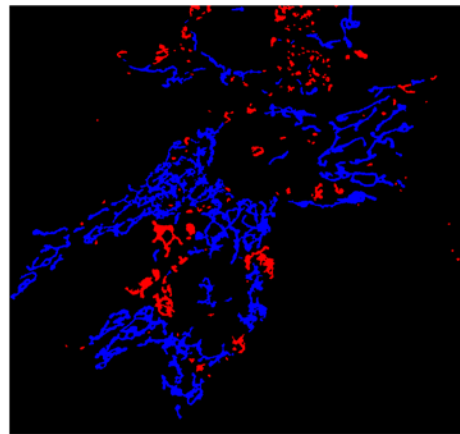
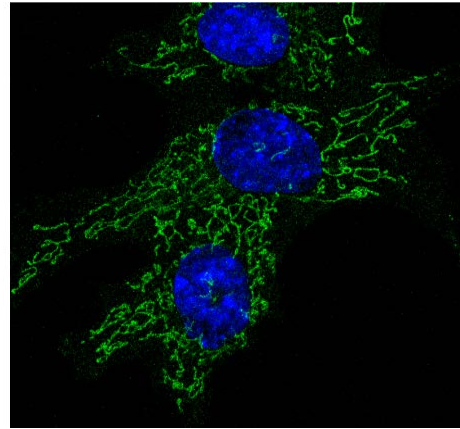
Developed  
method

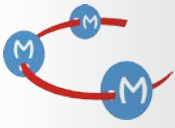




# Exemple of developed method

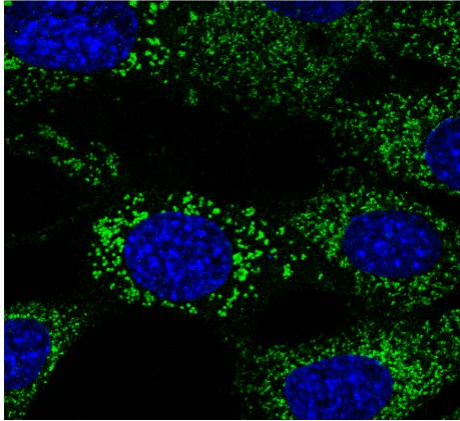
## Tubular



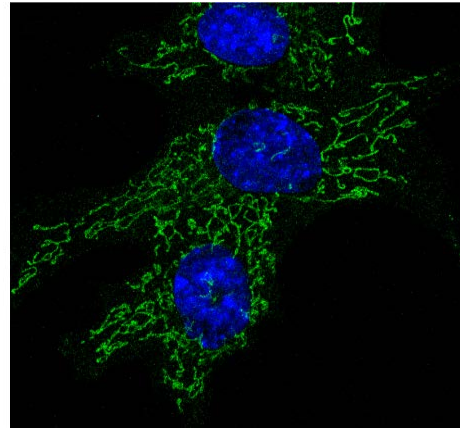


# Exemple of developed method

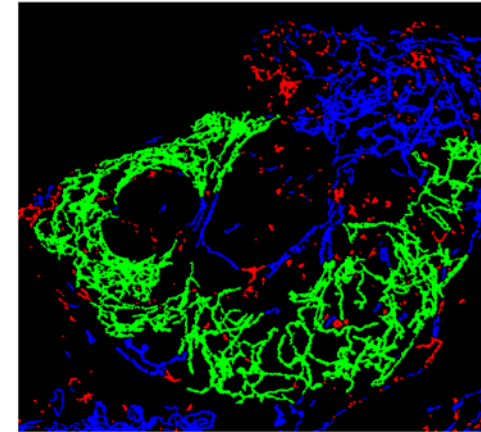
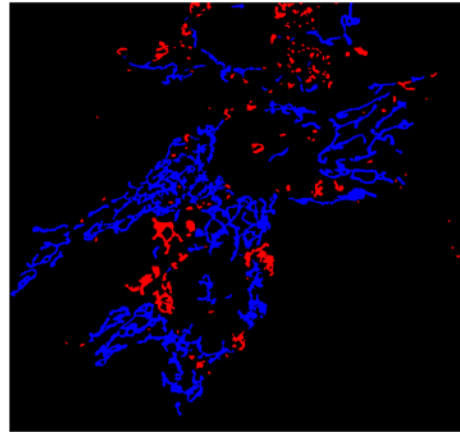
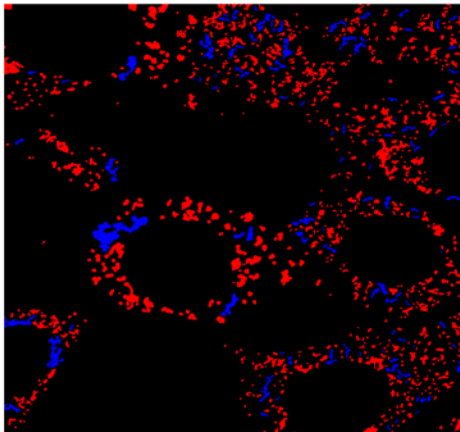
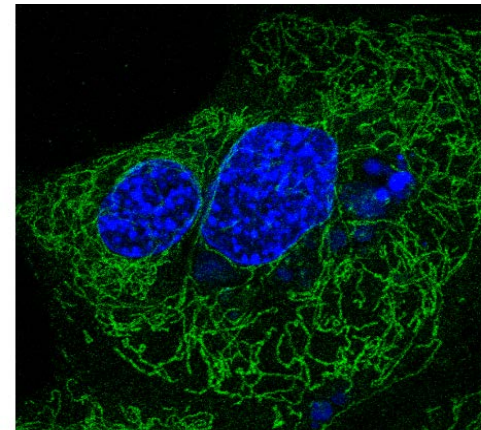
Fragmented  
(Rotenone, 100nM)



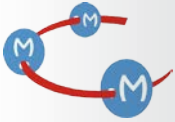
Tubular



Fused  
(Compound C, 20µM)





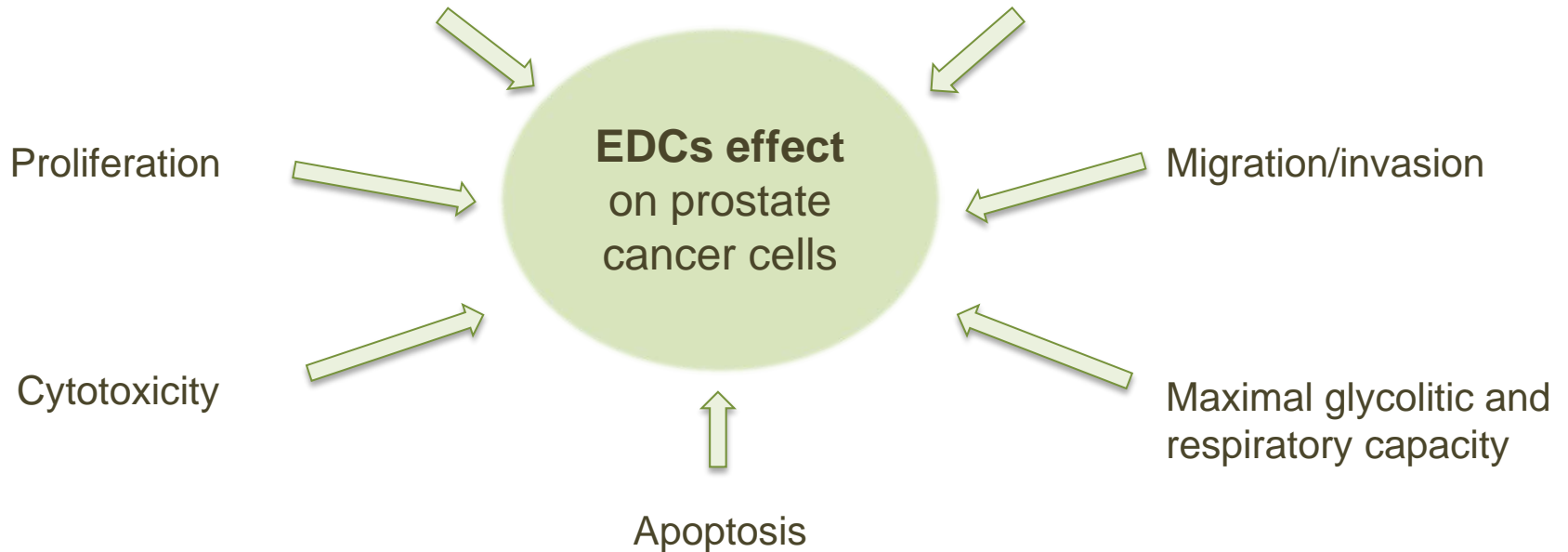


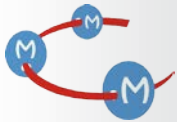
# Final Goal

- Development of a multiparametric high throughput cell based screening :

**Mitochondrial functional parameters**

**Mitochondrial morphological parameters**



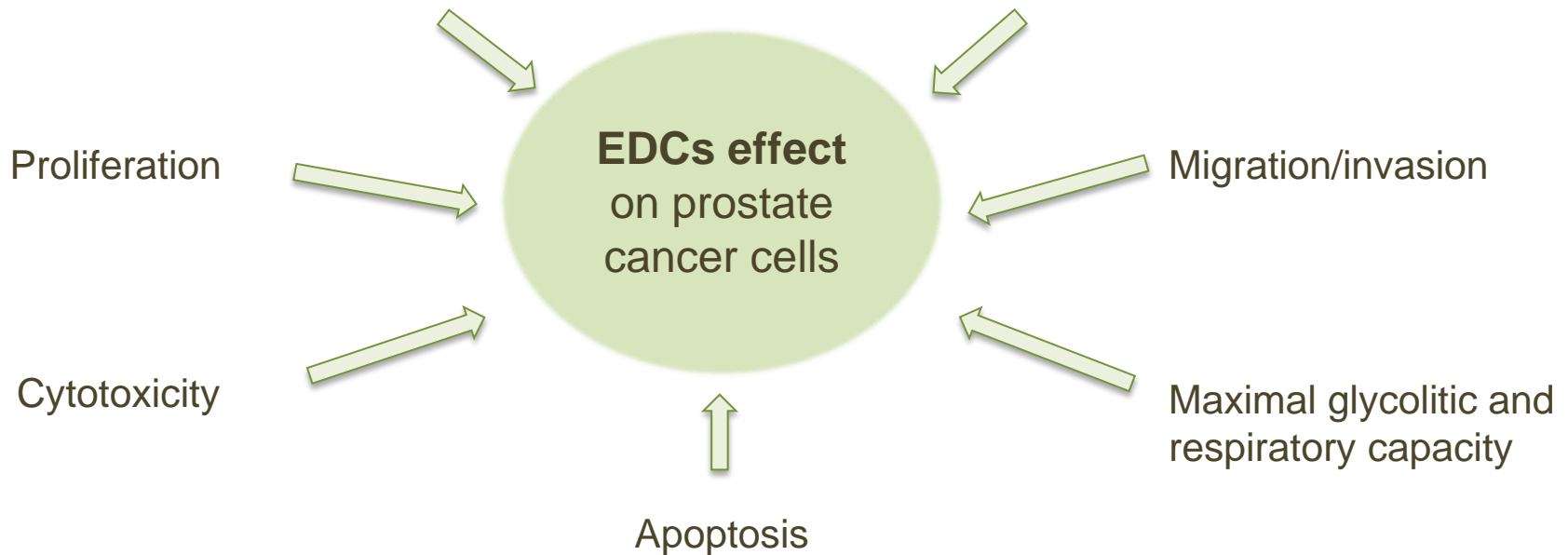


# Final Goal

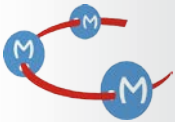
- Development of a multiparametric high throughput cell based screening :

**Mitochondrial functional parameters**

**Mitochondrial morphological parameters**



- ➔ Signature of EDCs based on their effects on cell metabolism
- ➔ New perspective in identification and characterization of EDCs



# Thanks...

## Team 7

Frédéric Bost  
Jean-François Tanti  
Mireille Cormont  
Stephan Clavel  
Célia Deconde Le Butor  
Lisa Kaminski  
Kathiane Laurent  
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Gwenaëlle Bouget  
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Marine Bourcier  
Mounia Tannour-Louet  
Calypso Vacher-Chicane  
Sophie Giorgetti-Péraldi  
Sophia Fazio  
Jean-François Louet  
Stéphanie Torino  
Faustine Pastor

