



# From target to preclinical candidate : C3D capabilities and identification of protein-protein interaction inhibitors

Stéphane Giraud, Scientific Director



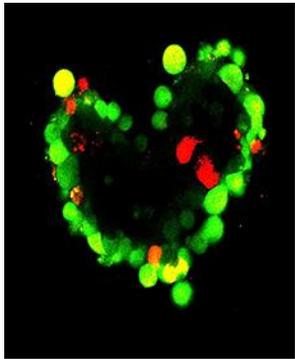
IA-INSERM  
2026-01-23



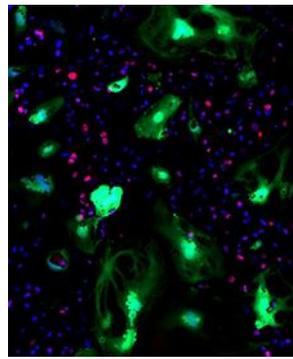
## CRCL in numbers

**22** Research teams

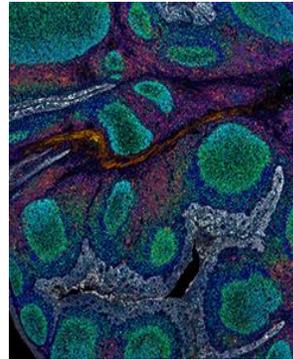
**5** Thematic axes



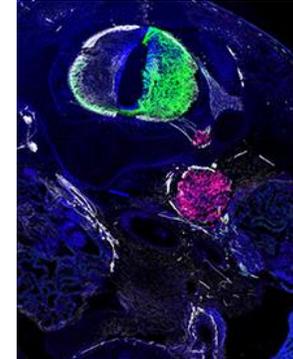
**Tumor initiation  
Prevention**



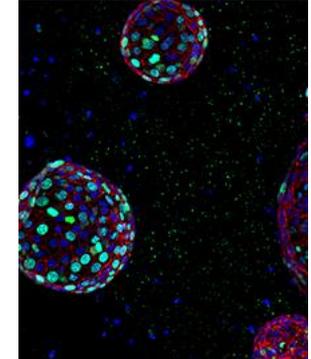
**Resistance  
Therapy**



**Immunology  
Inflammation  
Immunotherapy**



**Microenvironment  
Heterogeneity**



**Oncogenesis  
Tumor progression**

## 12 Core Facilities & Technological platforms



### Customized models

- Sample management
- Sample development
- 3D cell culture
- Microfabrication



### Pathology cytometry

- Cellular imaging
- Pathology
- Flow cytometry
- Immunotherapy



### Molecular analysis

- Cancer genomics
- Transcriptomic
- Bioinformatics

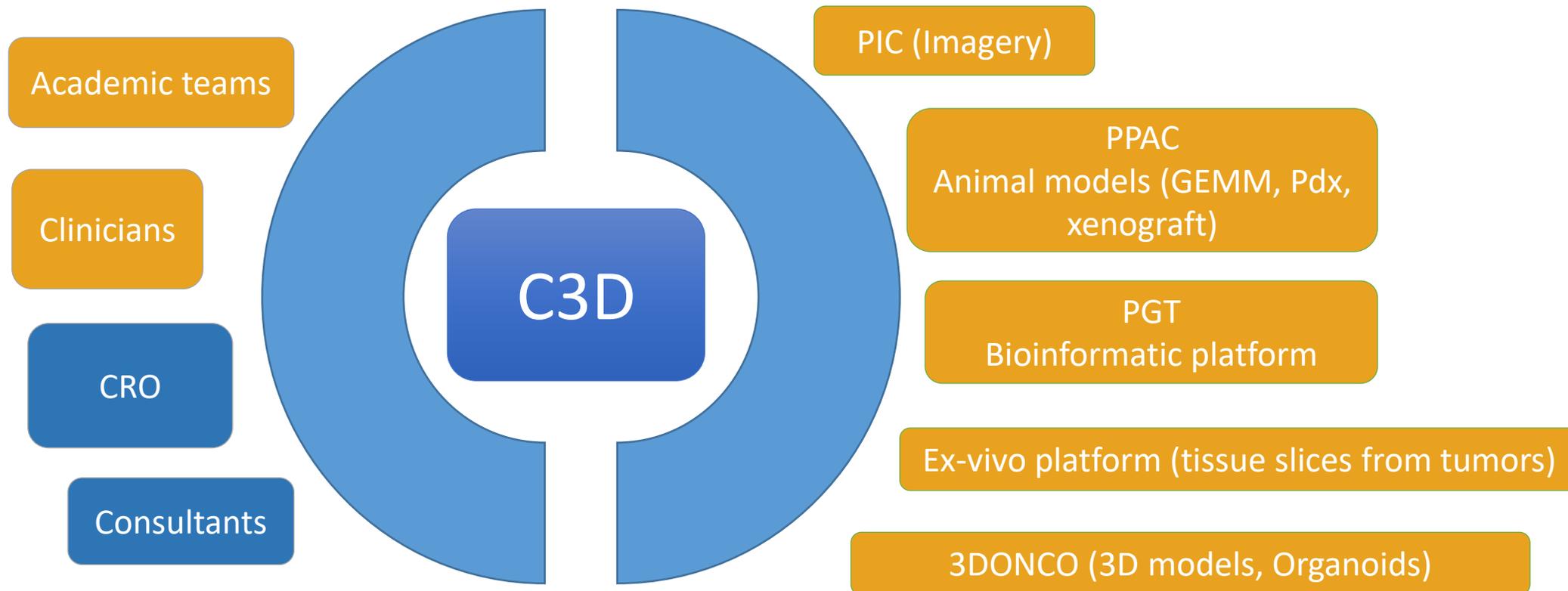


### Drug discovery

- Identification of therapeutic targets
- Identification of new therapies



## Scientific/steering committee



## Valorization committee

# C3D positionning

Years

4 - 6 years



DISCOVERY



DEVELOPMENT



Basic  
Research

Hit to lead

Lead to Preclinical  
candidate

Regulatory  
Preclinical  
development

Clinical trial



C3D

# C3D- Drug Discovery and preclinical activities

Hit to lead

## Compound ID

### Assay development

- Primary screening assay
- Secondary assays

### Screening

- HTS (chemical libraries)
- Drug design; virtual screening

## Compound optimization

- Orthogonal/secondary assays to analyze activity/selectivity/Improvement of ADME properties

Lead to Preclinical candidate

*in vivo* POC

Pharmacology (efficacy)

Early safety & toxicity studies

Early Pharmacokinetics /  
Pharmacodynamics



Drug discovery advising

## Biology

### Biochemical assays

Protein-Protein interaction; Binding assays, Enzyme activity assays

HTRF, BLI, Spectral shift

### Cell based assays

Cell viability assays; apoptose ...

### Phenotypic assays/High Content Screening

### Hybridoma screening (MSD technology)

Activity, PK and Safety optimization based on biology and ADMET

## Chemistry

In collaboration

Synthetic chemistry management  
Structure Activity Relationship study

## Early-ADME

Kinetic solubility (buffer, media)

Passive permeability

Cellular and cardiac toxicity (*in vitro* hERG)

## SBDD

## In silico screening

In collaboration

In house

# C3D – Hit to lead facilities

## Equipment

- Automated liquid handling (robotic platform)
- Multi-technology microplate readers (96 – 384 wells)
- HP D300 dispenser for titration (IC50, Combo))
- Akta Go (Protein purification)
- Dianthus and Octet 96 (molecular interaction)
- Cell culture room

## Libraries

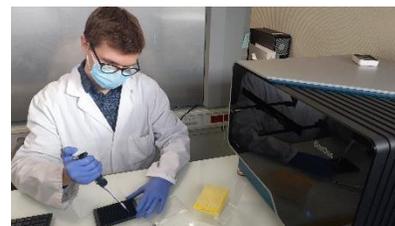
- The Prestwick library, 1200 FDA approved compounds
- A core library of 52 000 « drug like » compounds
- A Protein / protein inhibition library: 13 000 compounds
- Natural like compounds: 1200
- Kinase library (approved or under development drugs): 590 compounds
- FrPPIChem, 10314 compounds, protein-protein interaction inhibition



EVO150



Spark



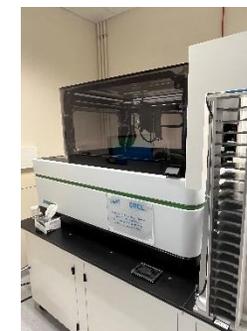
Dianthus



Octet 96



Akta GO



Opera



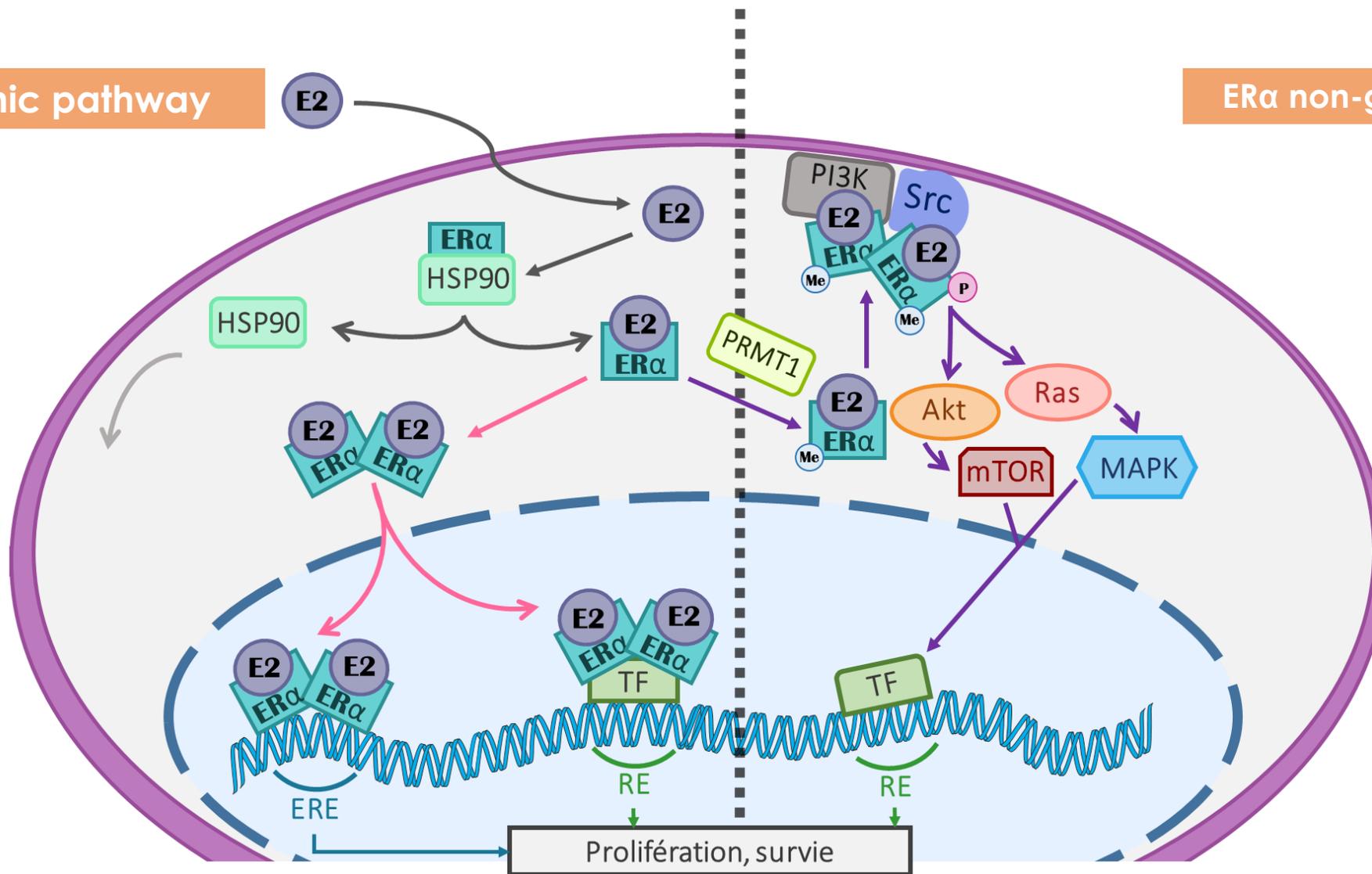
HP D300

# NESTOR New Strategies To Overcome Resistance



ER $\alpha$  genomic pathway

ER $\alpha$  non-genomic pathway



Involved in  
endocrine  
therapy  
resistance

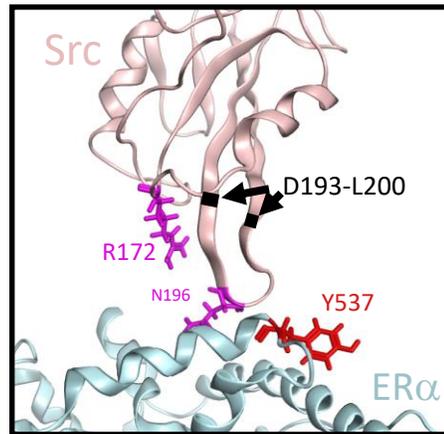
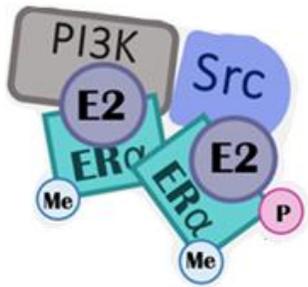
(Migliaccio and al. 1996)  
(Simoncini and al. 2000)  
(Castoria and al. 2001)  
(Song and al. 2002)  
(Le Romancer and al. 2008)

# NESTOR: New Strategies To Overcome Resistance

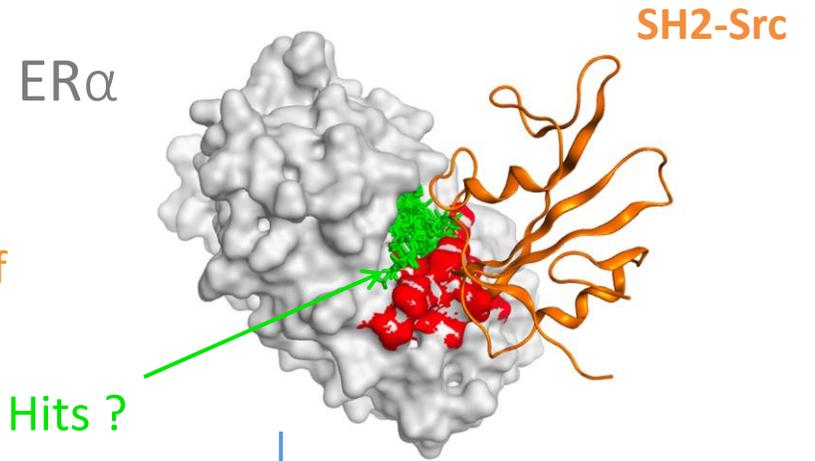


## *In silico* screening – Zinc database

Target



Screening 3M of compounds

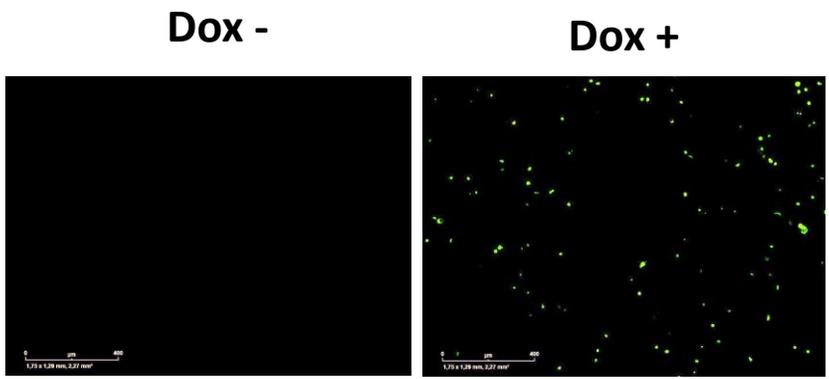
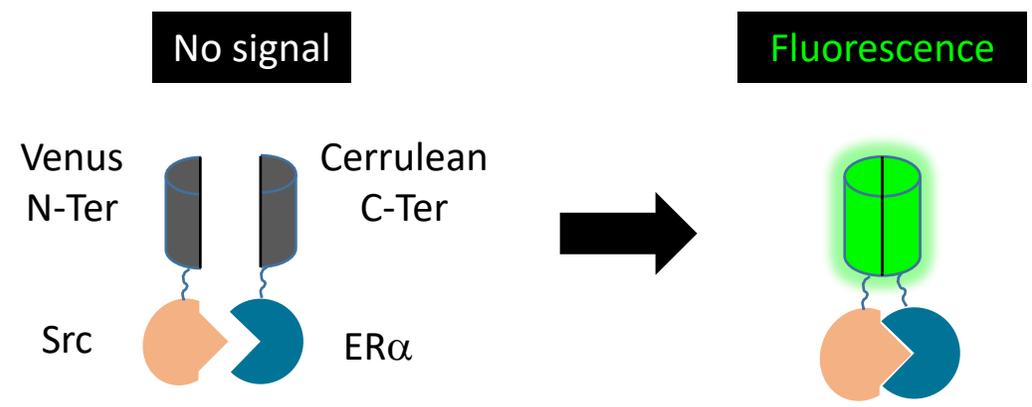


50 selected compounds



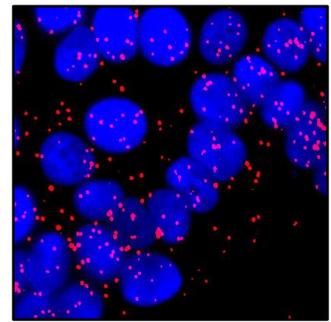
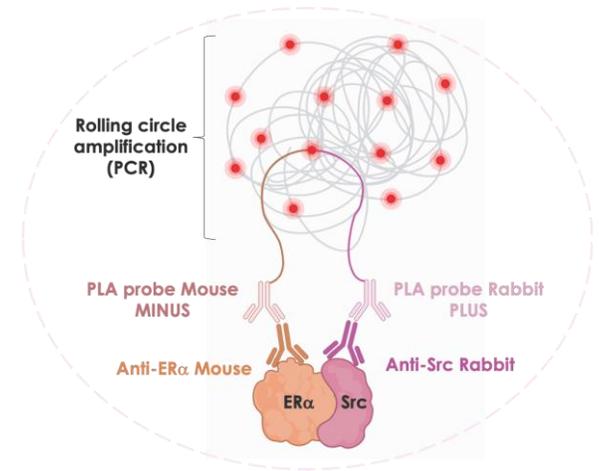
# In cellulo interaction

## Bimolecular Fluorescence Complementation (BiFc)



MCF-7 rTTA

## Proximity Ligation Assay (PLA)



MCF-7



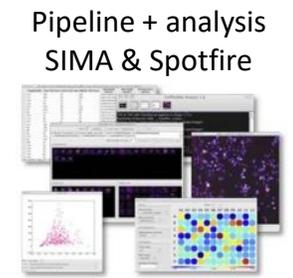
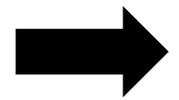
# HTS screening



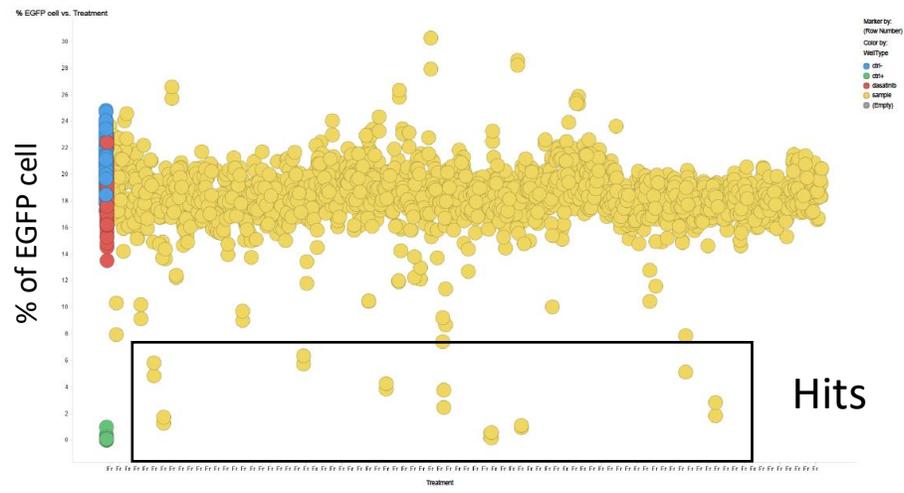
**Fr-PPIChem**  
*Bosc et al, Chem Biol. 2020*



HCS  
Perkin Elmer Opera



Pipeline + analysis  
SIMA & Spotfire



**First selection of 234 hits for validation + hits silico screening**

## Sarcomas in few numbers

→ Rare cancers (~5k cases/y in France), but >10% of **pediatric** tumors

→ 5 Years Survival Rate for STS: <65%

→ WHO recognizes >75 histological subtypes (>150 molecular subtypes) but 20% of sarcomas are still **unclassified** and lack characterization

→ >30% Characterized by a **specific translocation**

## SOFT TISSUE (STS) AND BONE SARCOMAS

**Fibrosarcoma**  
Infantile or Adult  
Fibrous connective tissue

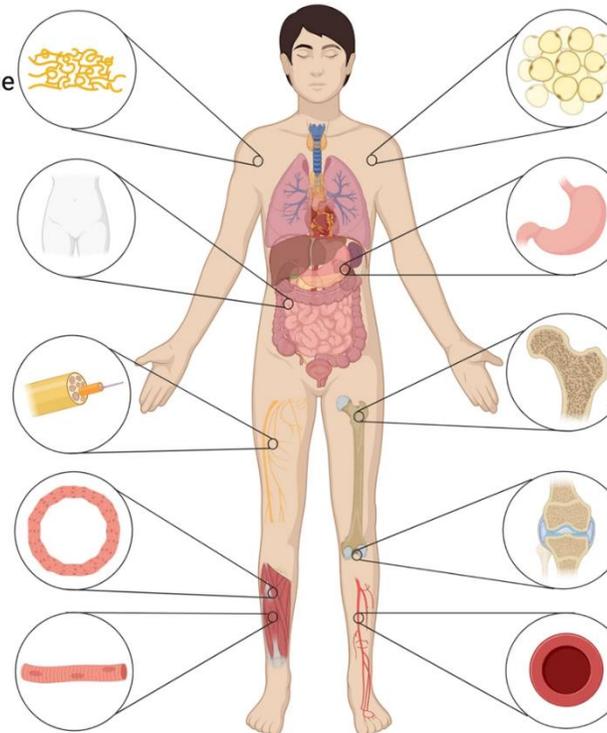
**Synovial sarcoma**  
Connective tissue

**Desmoplastic small round cell tumour**  
Soft tissue of abdomen

**Malignant peripheral nerve sheath tumour**  
Nerves

**Leiomyosarcoma**  
Smooth muscle

**Rhabdomyosarcoma**  
ARMS or ERMS  
Skeletal muscle



**Liposarcoma**  
Myxoid or WD/DDLPS  
Adipose tissue

**Gastrointestinal stromal tumours**  
Gastrointestinal tract

**Osteosarcoma**  
Bone  
**Ewing's sarcoma**  
Bone or STS

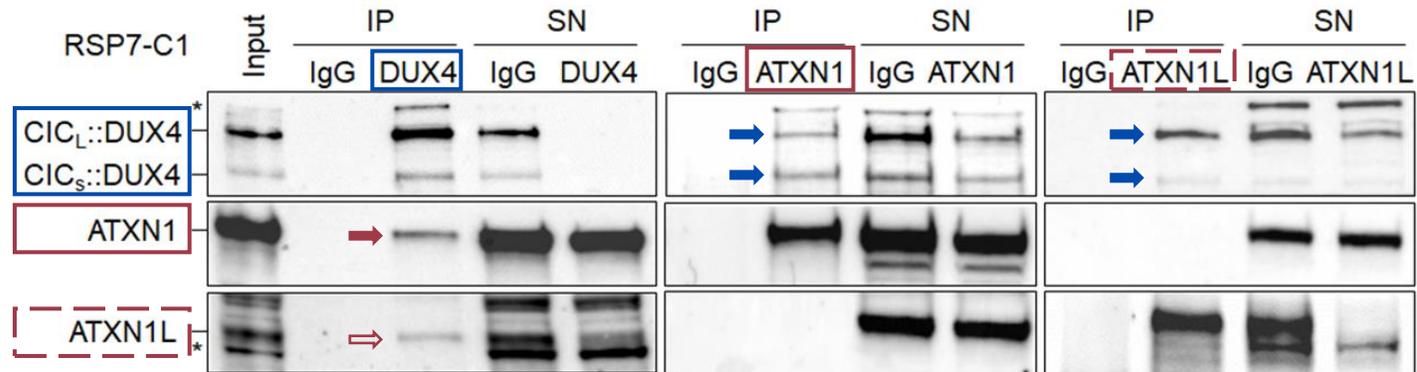
**Chondrosarcoma**  
Myxoid or other  
Cartilage

**Angiosarcoma**  
Blood vessels

Damerell, V., et al. *Sig Transduct Target Ther* 6, 246 (2021)

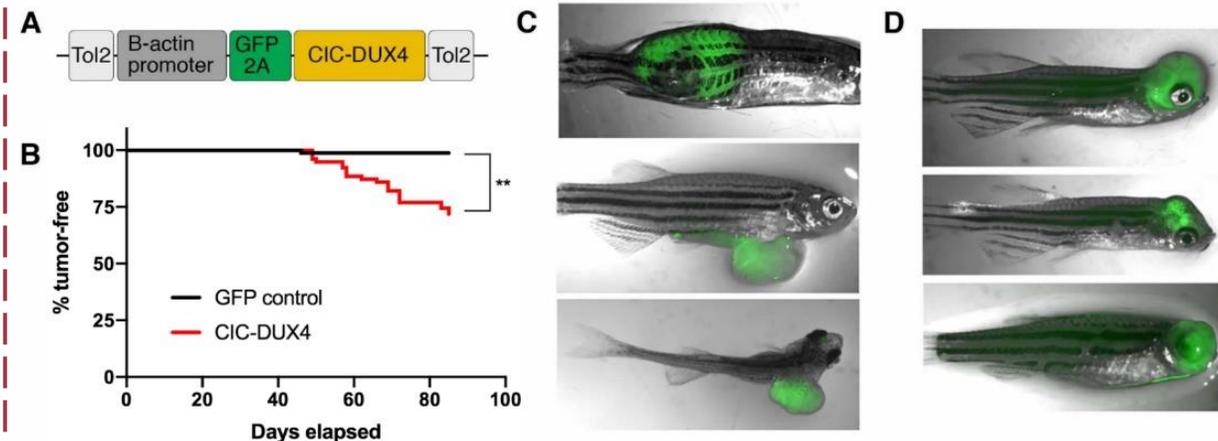
## Franck Tirode's team (CRCL)

- Tumors resistant to chemotherapies
- 5 years overall survival 40%
- Strong association of CIC::DUX4 with ATXN1 and ATXN1L



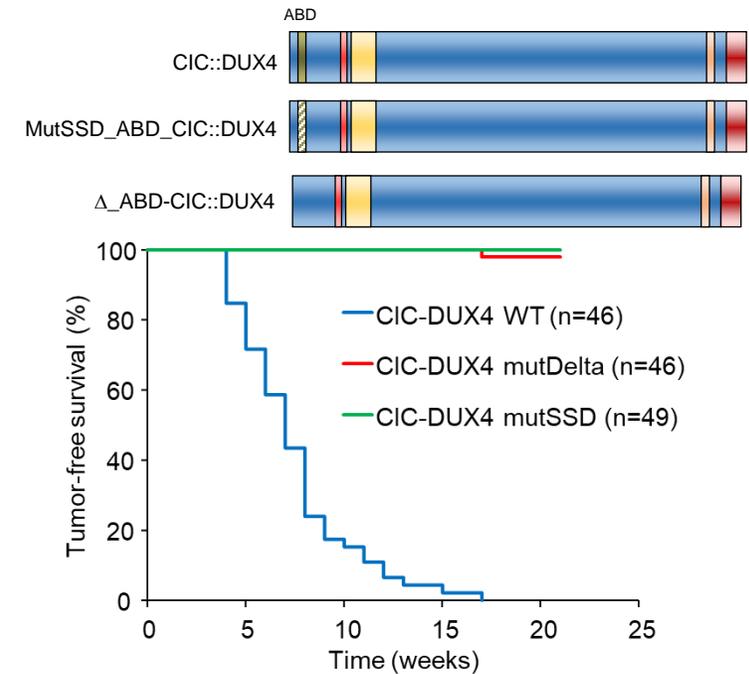
# CIC::DUX4 / ATXN1 function

## CIC::DUX4 zebrafish model



Collaboration with Julien Ablain's team ; CRCL

## ATXN1 Binding domain deletion prevent tumor growth in CIC::DUX zebrafish model

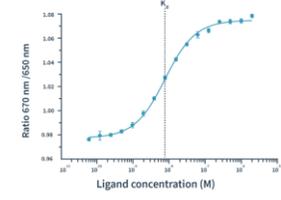
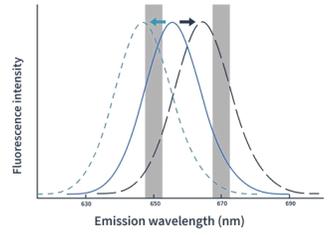
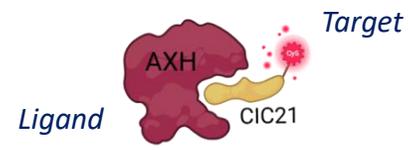




# In vitro interaction

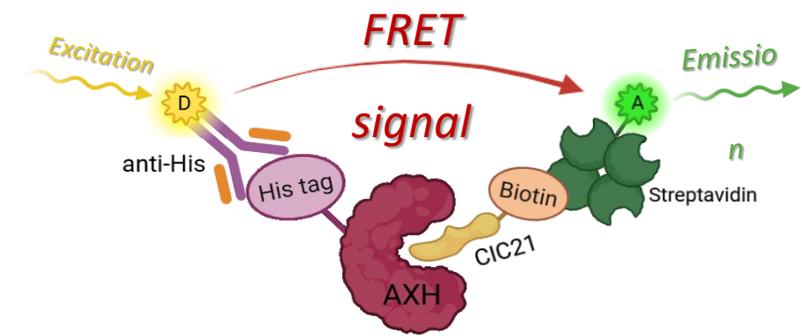
## Biophysical Assay

*Spectral Shift*



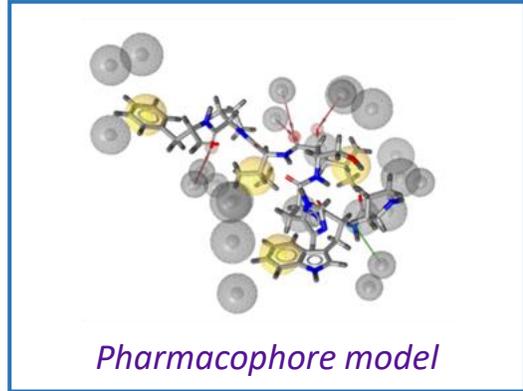
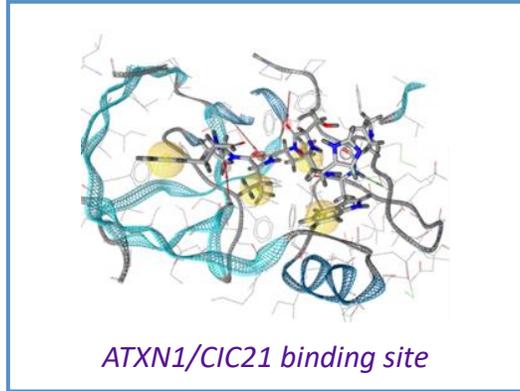
## Biochemical Assay

*HTRF assay*





# *In silico screening*



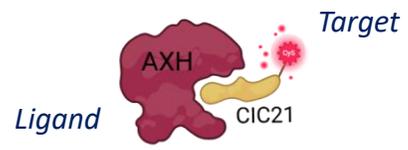
*Designed using LigandScout software  
By Peter Goekjian (ICBMS)  
+C3D platform*

*>100millions synthetically accessible structures  
from Enamine library*



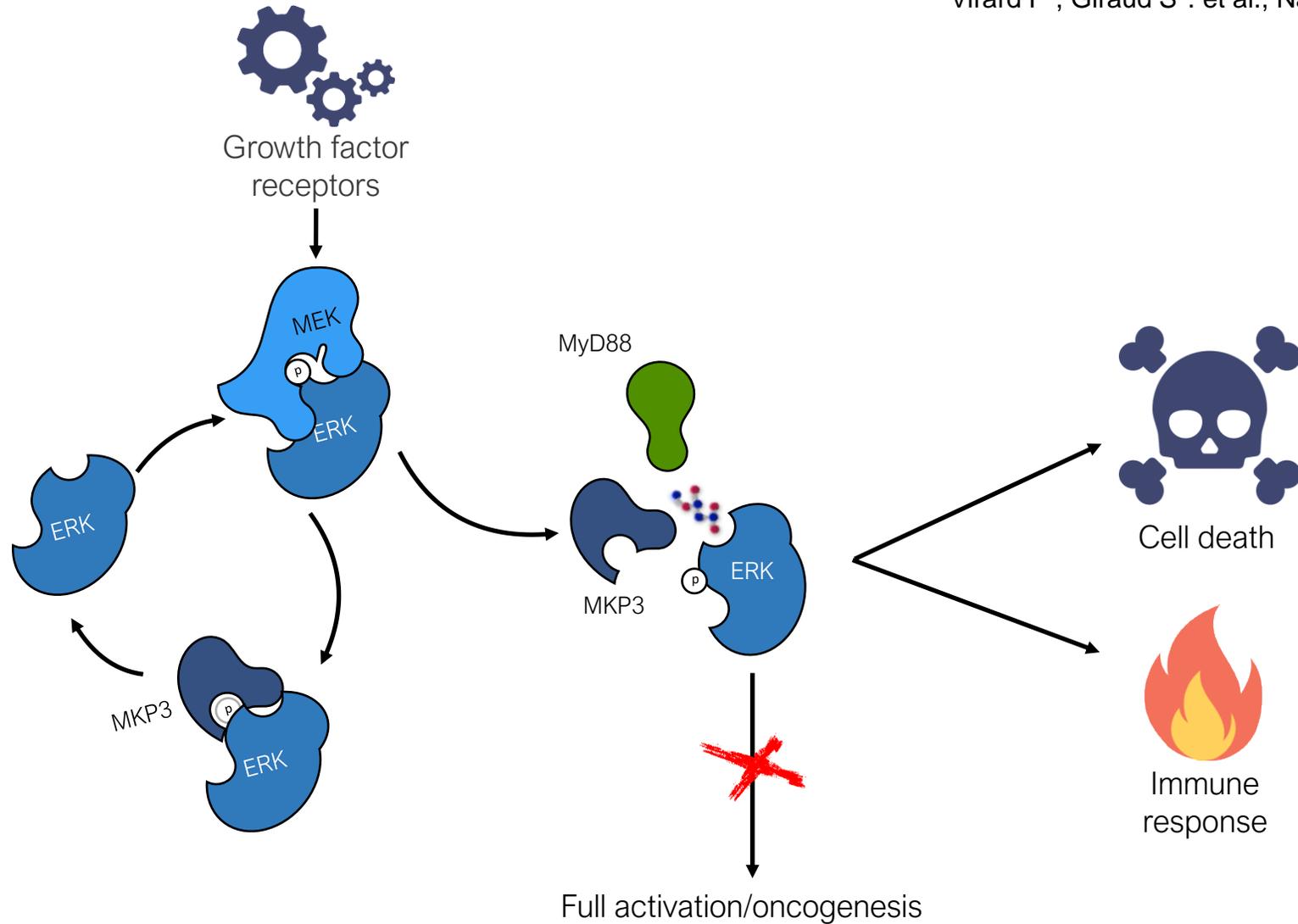
## Ongoing Hit selection

*>900 compounds from ICBMS library (Lyon)*



# Inhibition of ERK-MyD88 Interaction, a Novel Approach to Cancer Treatment

Virard F\*, Giraud S\*. et al., Nature Communications, (2024) 15:7037



# Hit identification

Screening for in vitro protein interaction inhibition:

interaction (ERK-1 and MyD88 peptide) by HTRF: 67 400 compounds



Five chemical series identified



SAR exploration in structural families: selected commercially available compounds



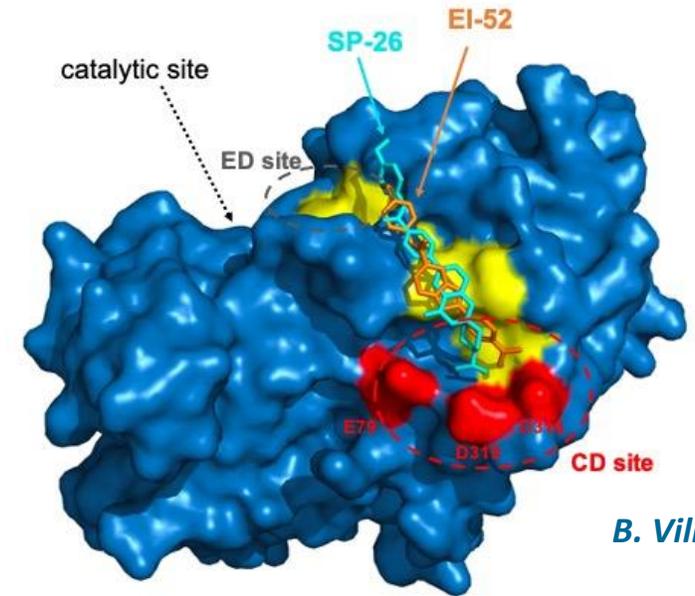
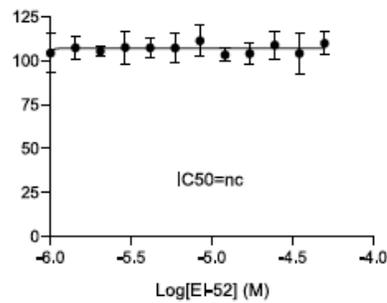
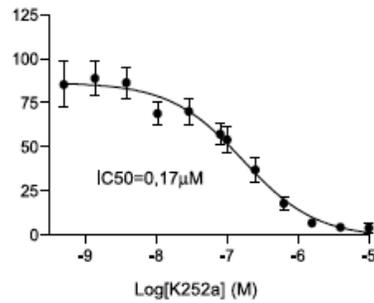
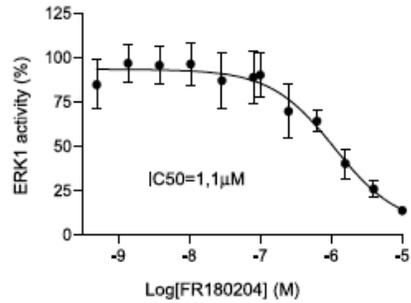
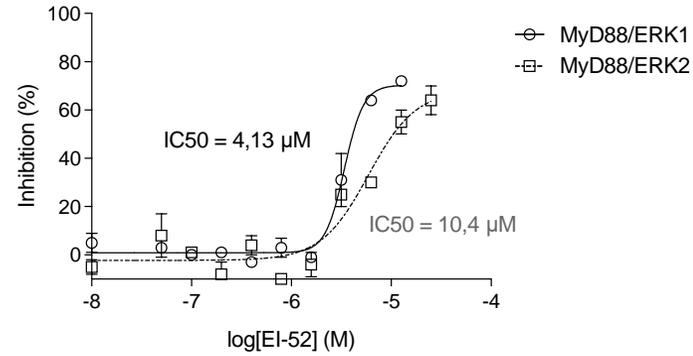
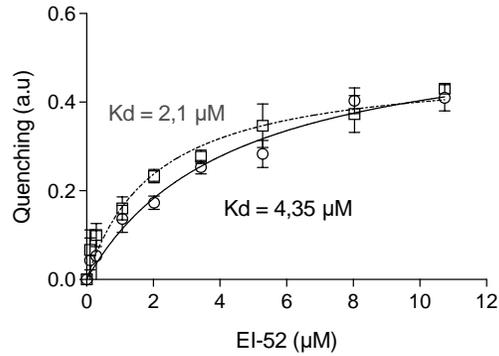
Two families selected



2 families on the same target, yielding the same mechanism

On-target MoA confirmed by ERK mutagenesis

# EI-52 binds to ERK and inhibits ERK-MyD88 interaction in vitro



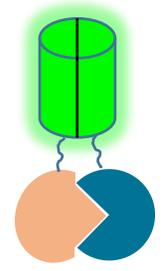
*B. Villoutreix*

Confirmed experimentally by mutagenesis and by NMR and crystallography

# EI-52 inhibit ERK-MyD88 interaction in cells

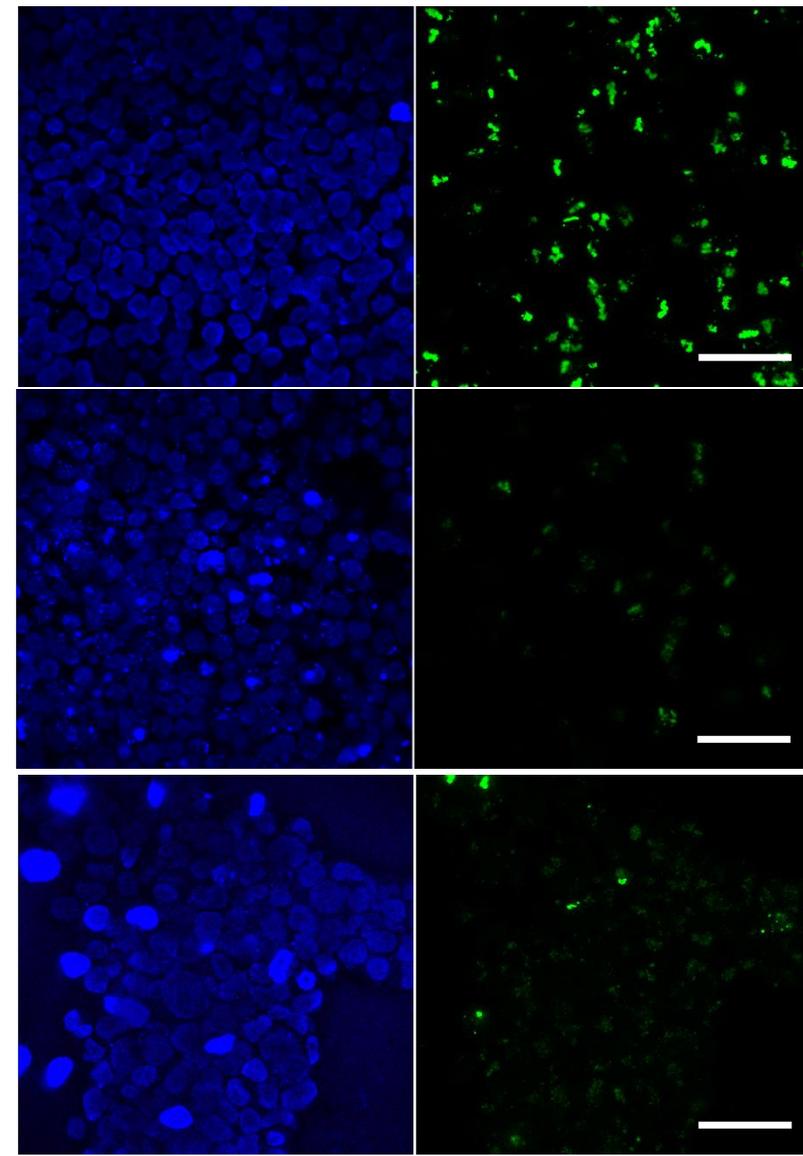


BiFC



Erk

Myd88



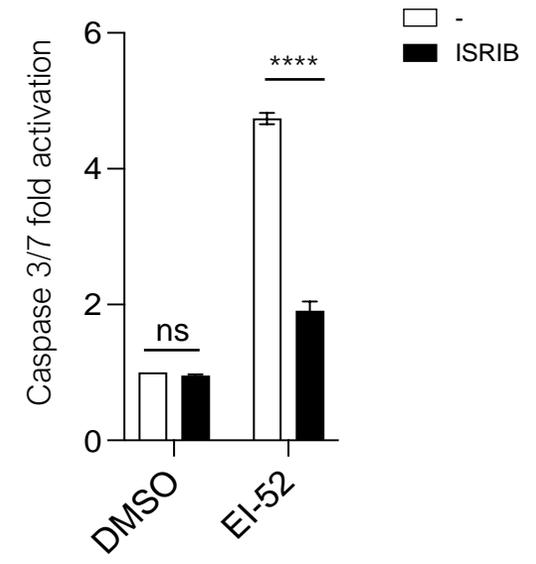
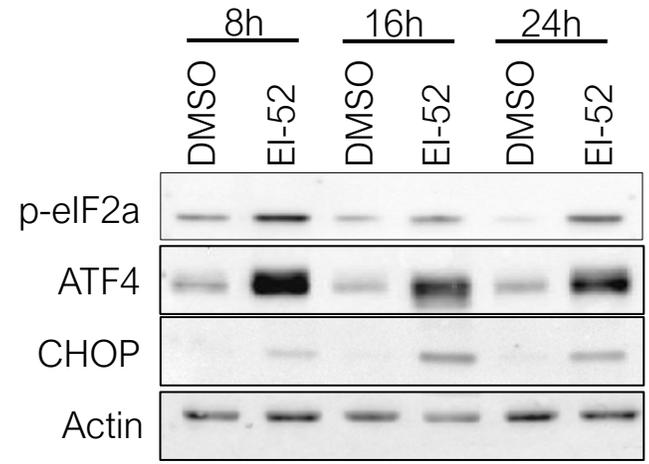
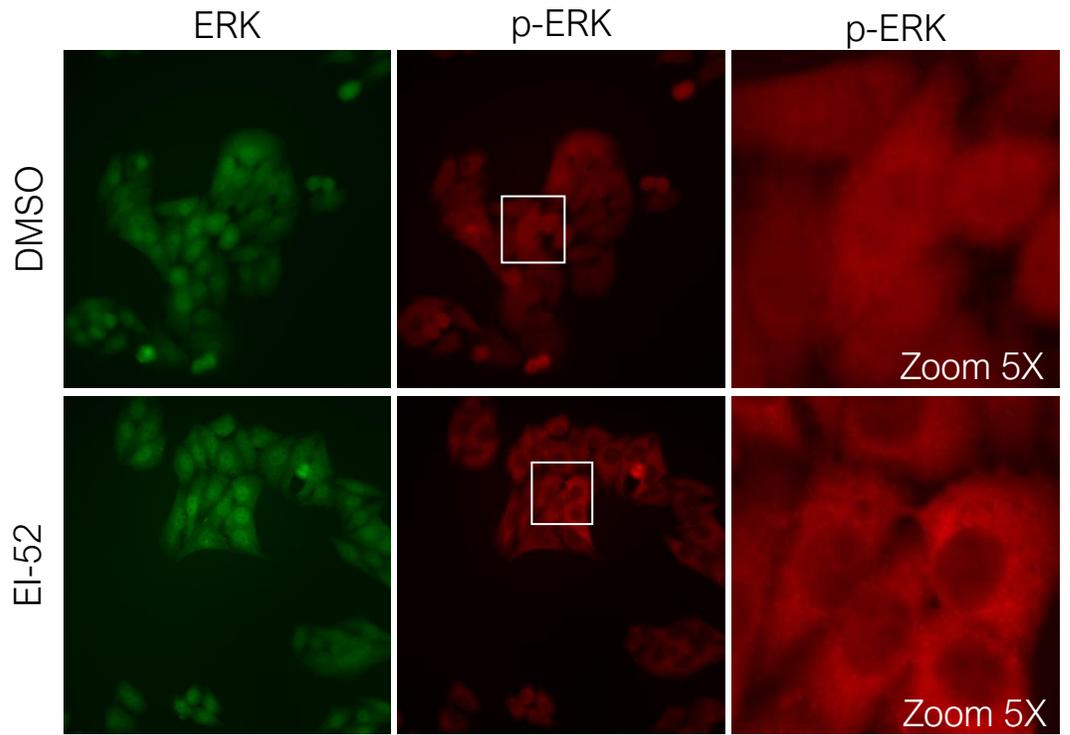
Plix VN ERK + plix CC-Myd88 +  
DMSO

Plix VN ERK + plix CC-Myd88 +  
EI52-12uM

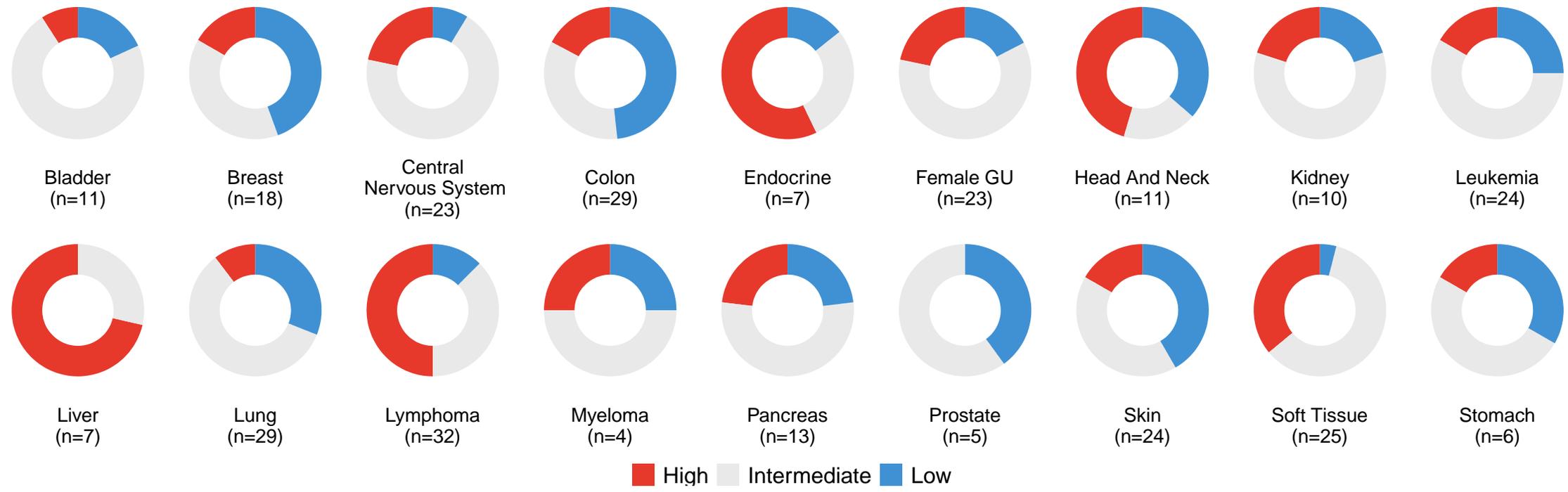
Plix VN ERK + plix CC-Myd88 +  
SP-26-12uM

EI-52 as proof of concept molecule

# EI-52 perturbs ERK localization and induces an integrated stress



# EI-52 is effective in multiple solid and liquid cancers



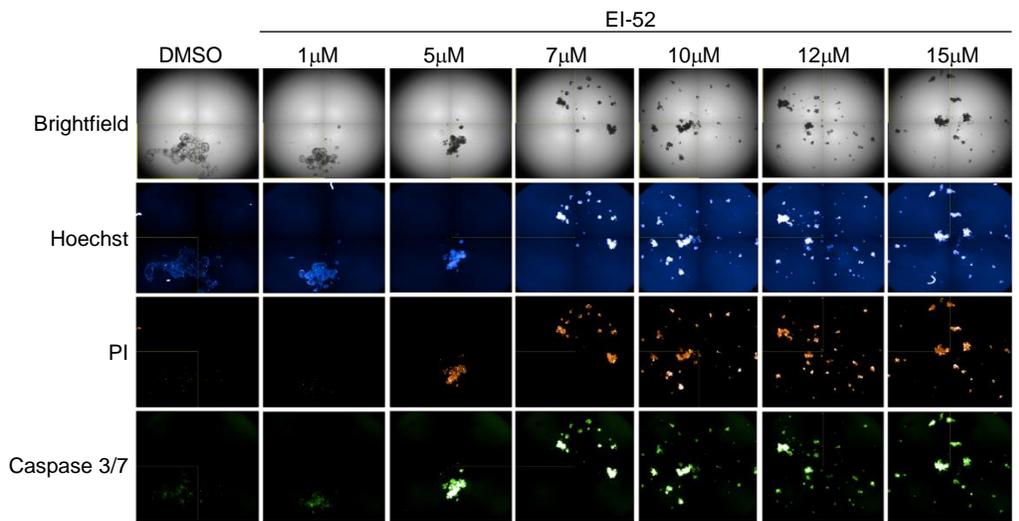
OncoPanel, 301 cell lines, Eurofins



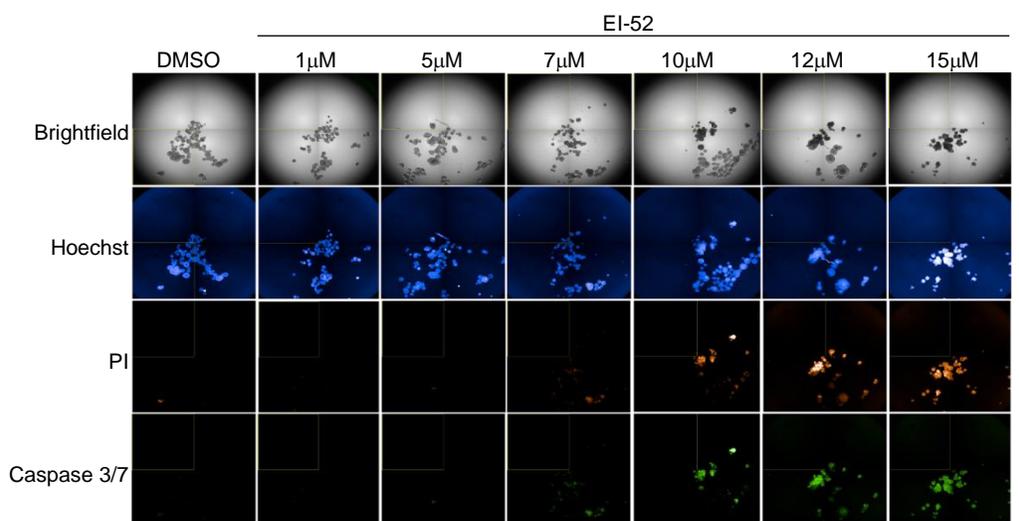
# EI-52 induces cancer cell death in organoid models



Patient-derived colon cancer tumoroids

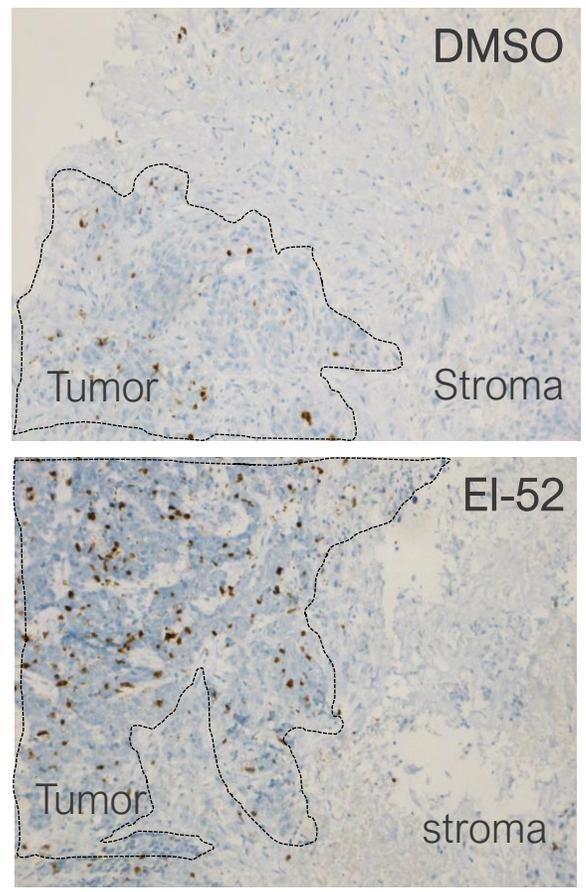


Patient-derived lung cancer tumoroids

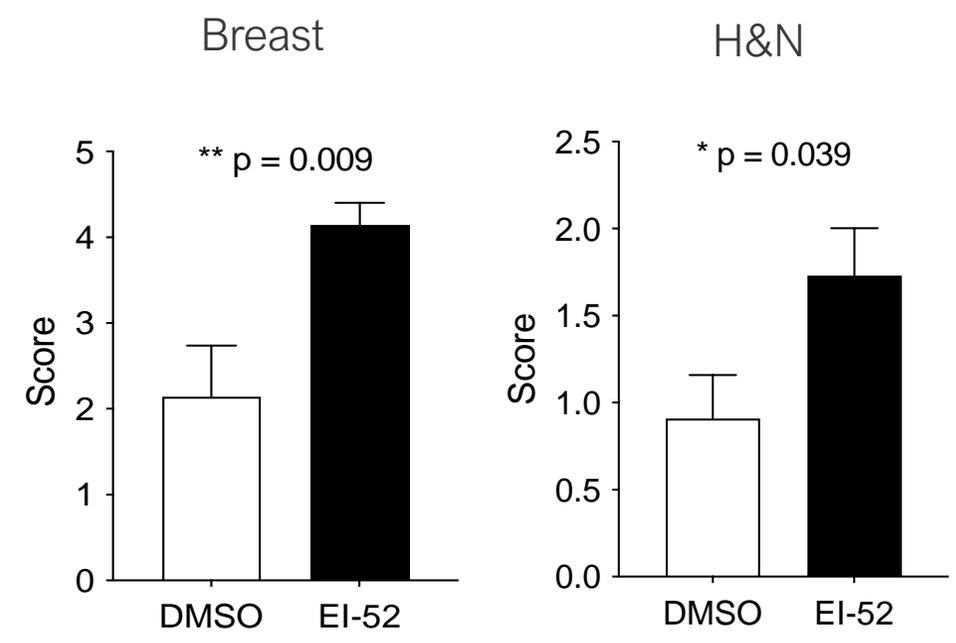


# EI-52 kills cancer cells while sparing stroma in fresh tumor samples

Breast carcinoma

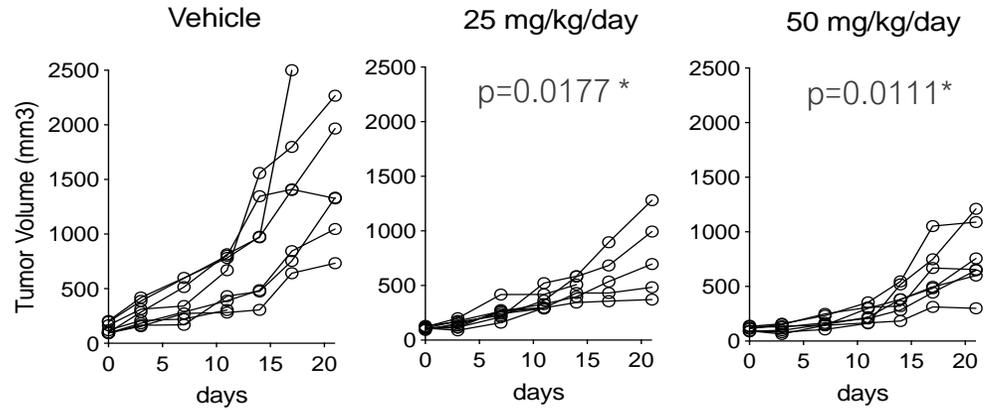


apoptosis (c-PARP)



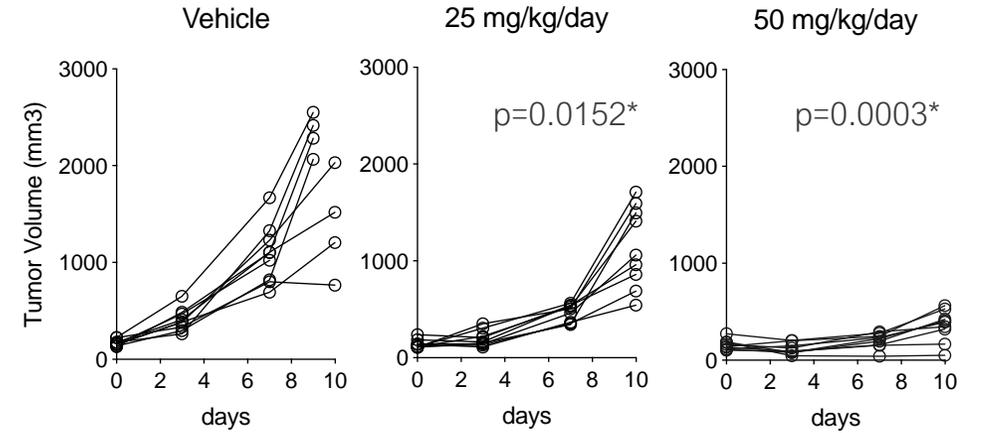
Thick sections from resected breast (n=7) and head & neck (n=11) tumors were treated for 24h with EI-52 or control (DMSO). Histological sections were then stained for cleaved PARP.

## Xenograft mouse model



HCT116 cells in nude mice. Treatments start @ 100 mm<sup>3</sup>

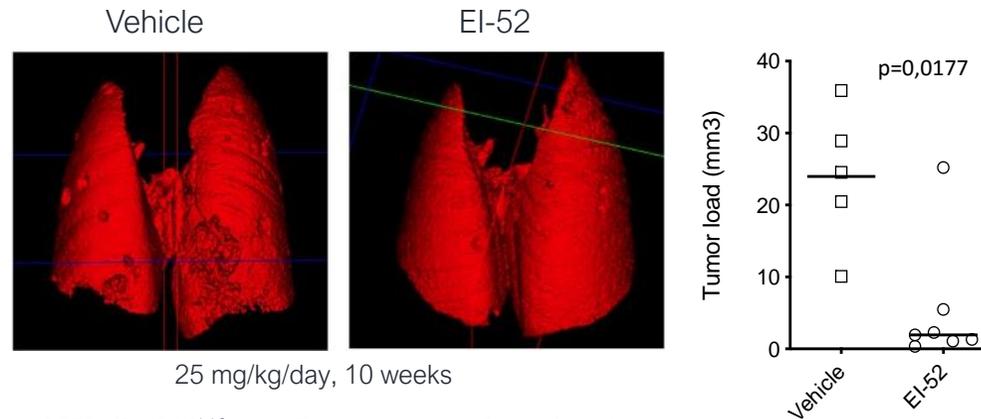
## Syngeneic graft mouse model



LLC cells in C57BL/6 mice. Treatments start @ 100 mm<sup>3</sup>

\* Statistical analysis for the last time point

## Spontaneous mouse model



25 mg/kg/day, 10 weeks

C57BL/6-KRAS<sup>LA2</sup> mice. Treatments start @ age 4 weeks.

- 2 chemical series that target the CD domain of ERK
- Induction of early apoptosis and Immune response
- Induction of immunogenic cell death
- TheraPPI Bioscience created in 2023





# IA and modelling needs

- **Target identification and validation**
- **Protein production and purification**
- **Protein-protein interaction**
- **In silico screening (pharmacophoric, docking)**
- **Drug design**
- **Chemistry and SAR**
- **ADMET (early-ADME, PK, PD, Toxicity)**
- **Pharmacology**



# Acknowledgements

## ERK-MyD88

*T. Renno's team*

*Toufic Renno  
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IGFL

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C. Kundlacz*

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Hôpital Robert Debré, Paris

*B. Villoutreix*



CRCL platforms

*Ex-Vivo  
P-PAC  
Cell Imaging platform*



## ATAXIC

*Franck Tirode's Team*

*TIRODE Franck,  
AMOROSO Enzo,  
BRAHMI Medhi,  
DUC Adeline,  
PERRIN Virginie,  
PISSALOUX Daniel,  
RUBY Samia,  
VANACKER  
Hélène,  
VIGNERON  
Arnaud,  
WAISSI Waisse*

## Nestor

*Team M.LE ROMANCER and O.TREDAN*

*Muriel LE ROMANCER  
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Coralie POULARD  
Sébastien MARTINEZ*

