



Use of the *Caenorhabditis elegans* as an alternative model for evaluating the allergen potential of skin sensitizers

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- Animal testing for the production of cosmetic has been banned;
- ↑ Research about efficient alternative methods;

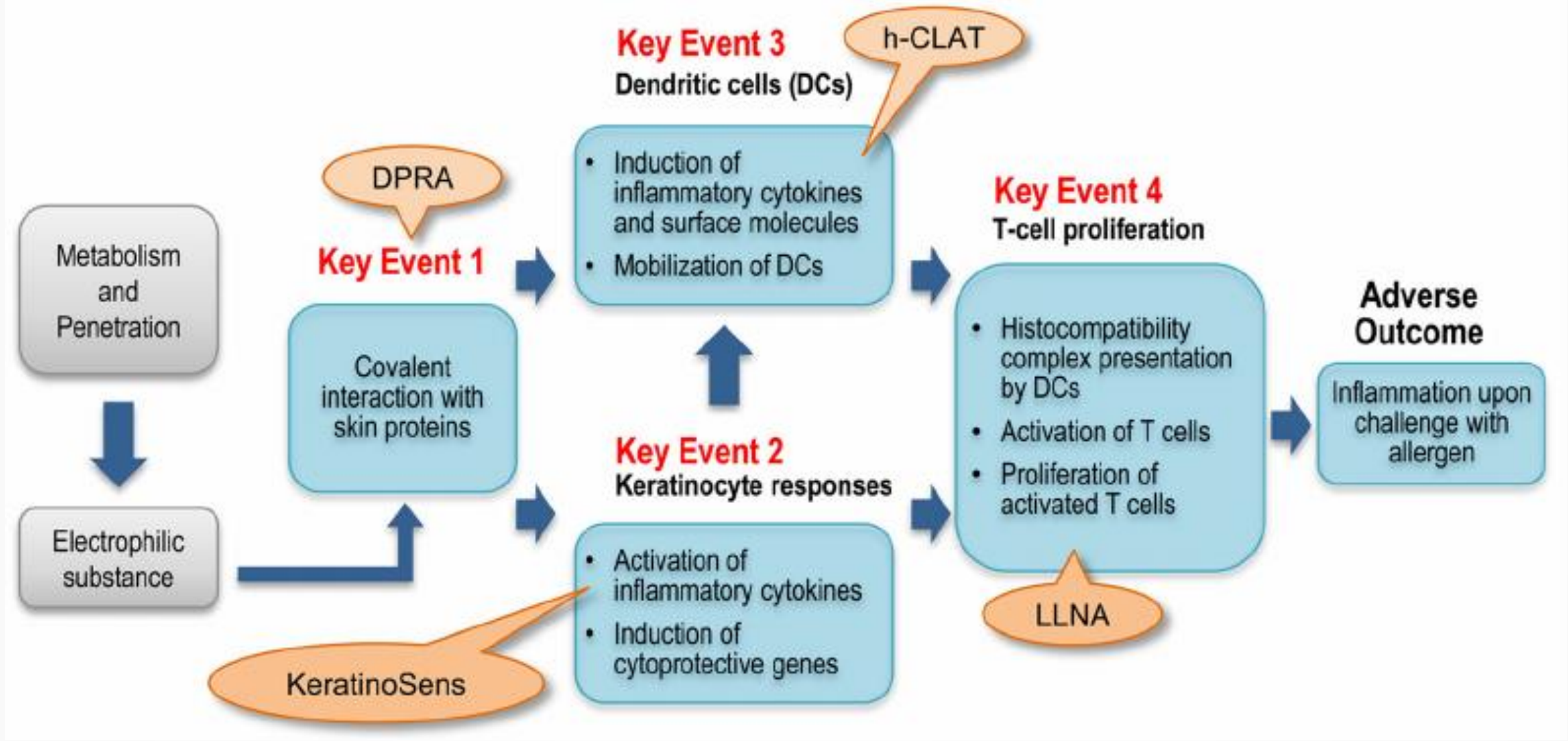


➤ **skin sensitization;**

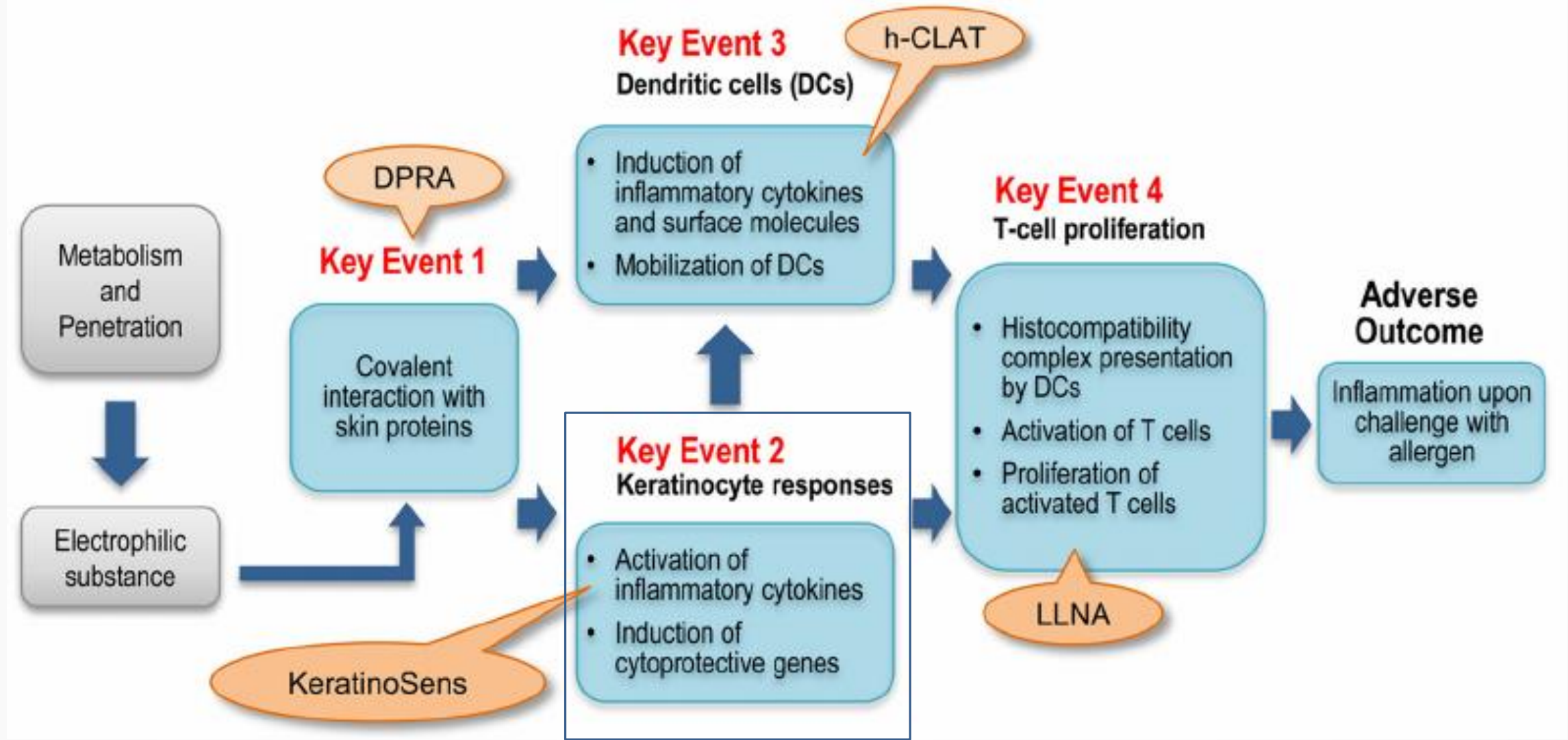
- ACD (allergic contact dermatitis) – type IV hypersensitivity reaction, induced by repeated contact with sensitizers.



➤ Adverse Outcome Pathway for skin sensitizers



► Adverse Outcome Pathway for skin sensitizers



► *C.elegans* model

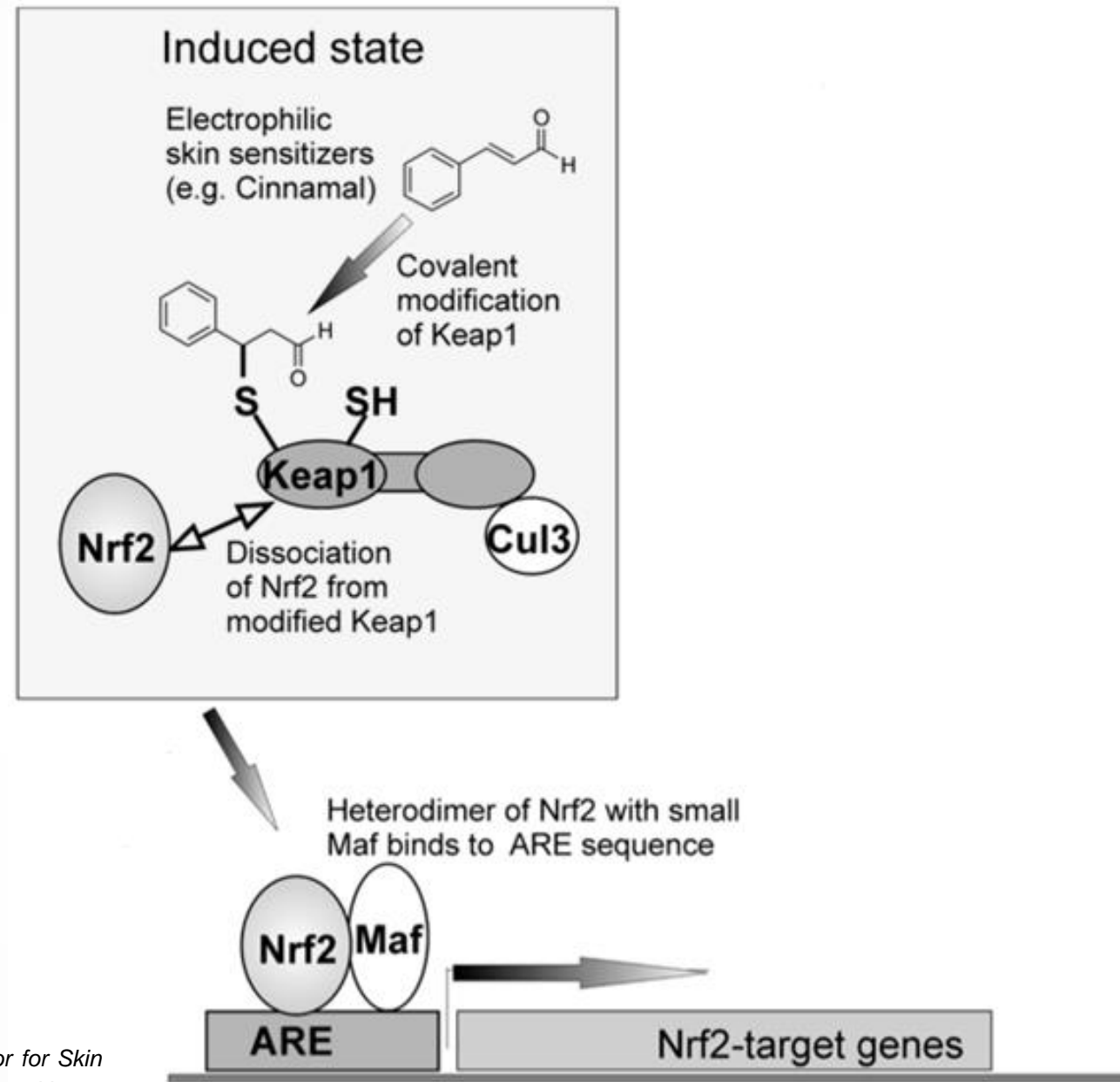
➤ Adverse Outcome Pathway

➤ **Key event 2**



➤ Induction of cytoprotective genes;

➤ **Keap1-Nrf2-ARE pathway**



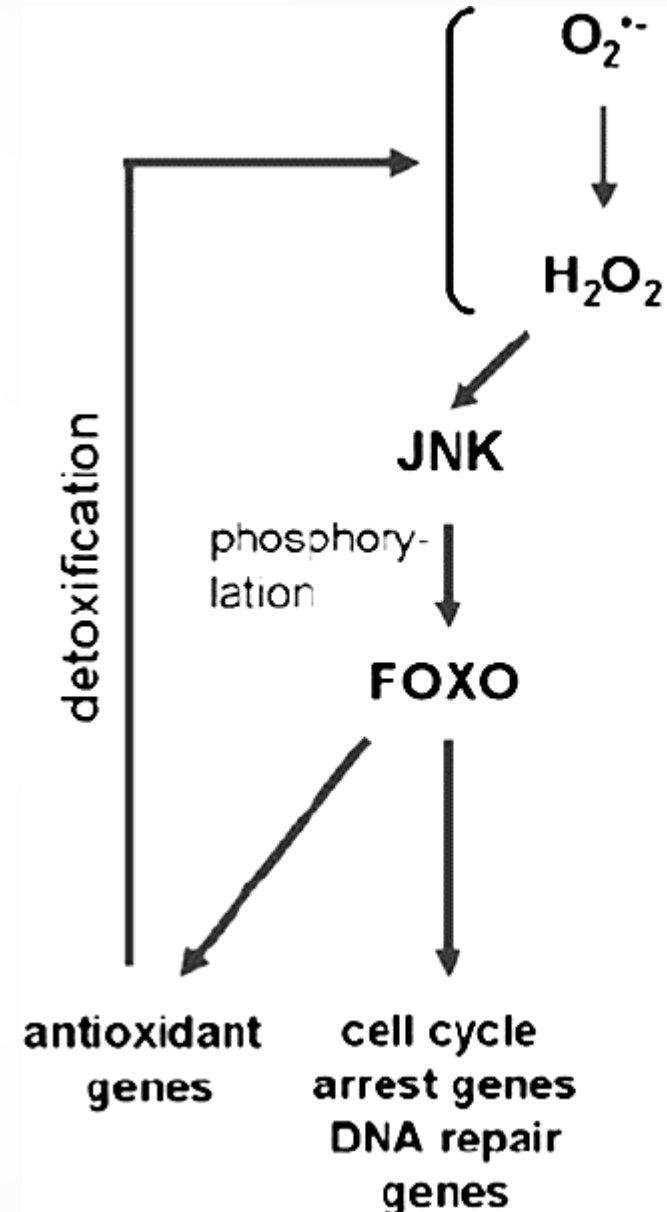
➤ Adverse Outcome Pathway

➤ **Key event 2**

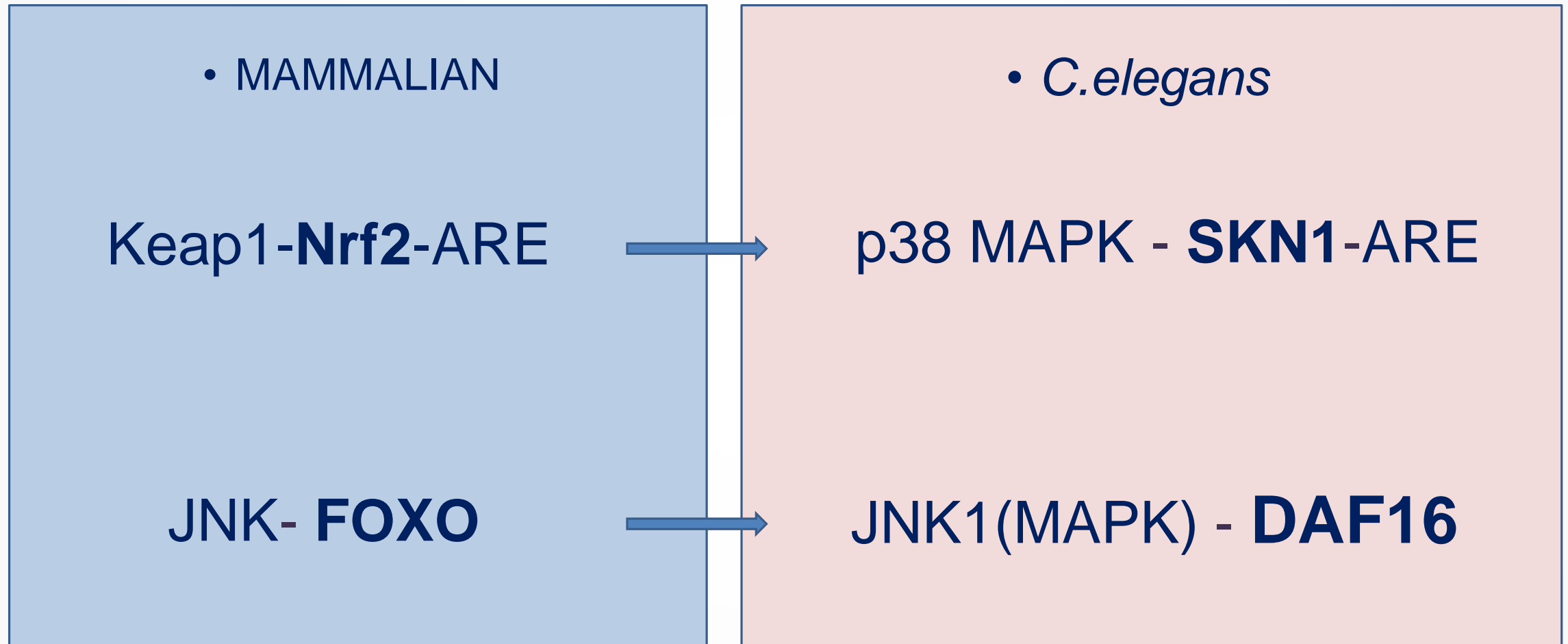


➤ Induction of cytoprotective genes;

➤ **FOXO pathway**

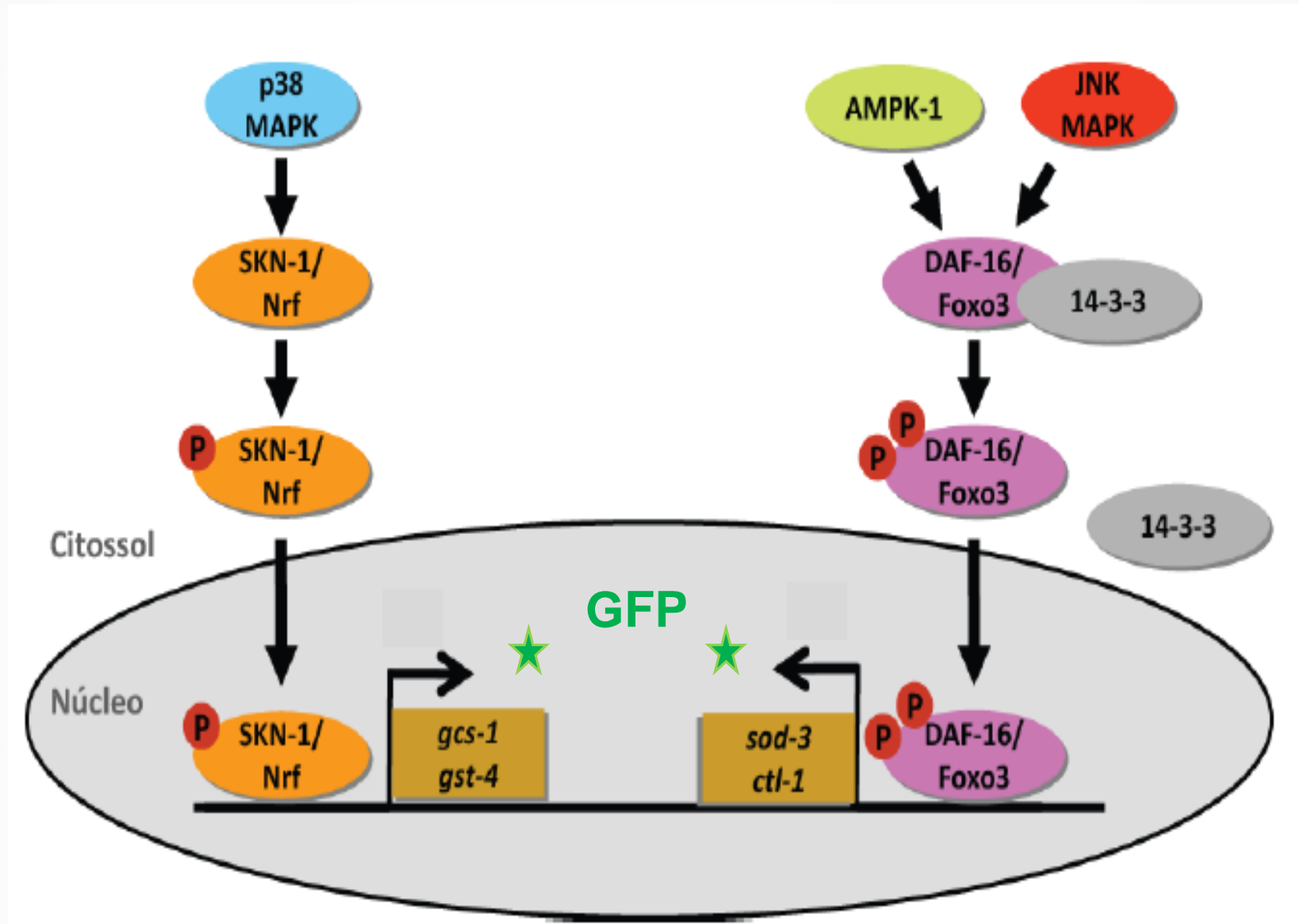


- **ORTHOLOGS:** genes in different species that evolved from a common ancestral gene by speciation. Retain the same function in the course of evolution

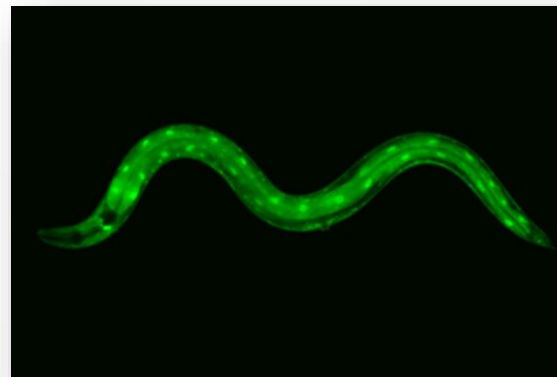


- Both pathways are activated in response to oxidative stress

Genetic modification in *C.elegans*



STRAINS	GENETIC MODIFICATION	ORTHOLOG C.ELEGANS	ORTHOLOG MAMMALIAN	MAMMALIAN SIGNALING PATHWAY
CF1553-<i>psod3::GFP</i>	Addition of GFP molecule in the <i>sod3</i> promoter	DAF-16 – active <i>sod3</i>	FOXO (Forkhead box)	JNK-FOXO
CL2166-<i>pgst4::GFP</i>	Addition of GFP molecule in the <i>gst4</i> promoter	SKN-1 – active <i>gst4</i>	Nrf2 (nuclear factor erythroid 2-related factor 2)	Keap1-Nrf2-ARE
N2 BRISTOL	Wildtype	---	---	---



Cell culture x *C.elegans* models

- Simple Cell Culture model

Very expensive;

Cellular Monolayer;

Contamination;

Loss of phenotypic characteristics;

- *C.elegans* model

Low cost;

Easy manipulation;

Innate immune system;

Collagenous cuticle and 4 layers epidermis;

Sequenced genome in database;

Systemic Model

• **Skin sensitization involves several layers and cells !!!!!**

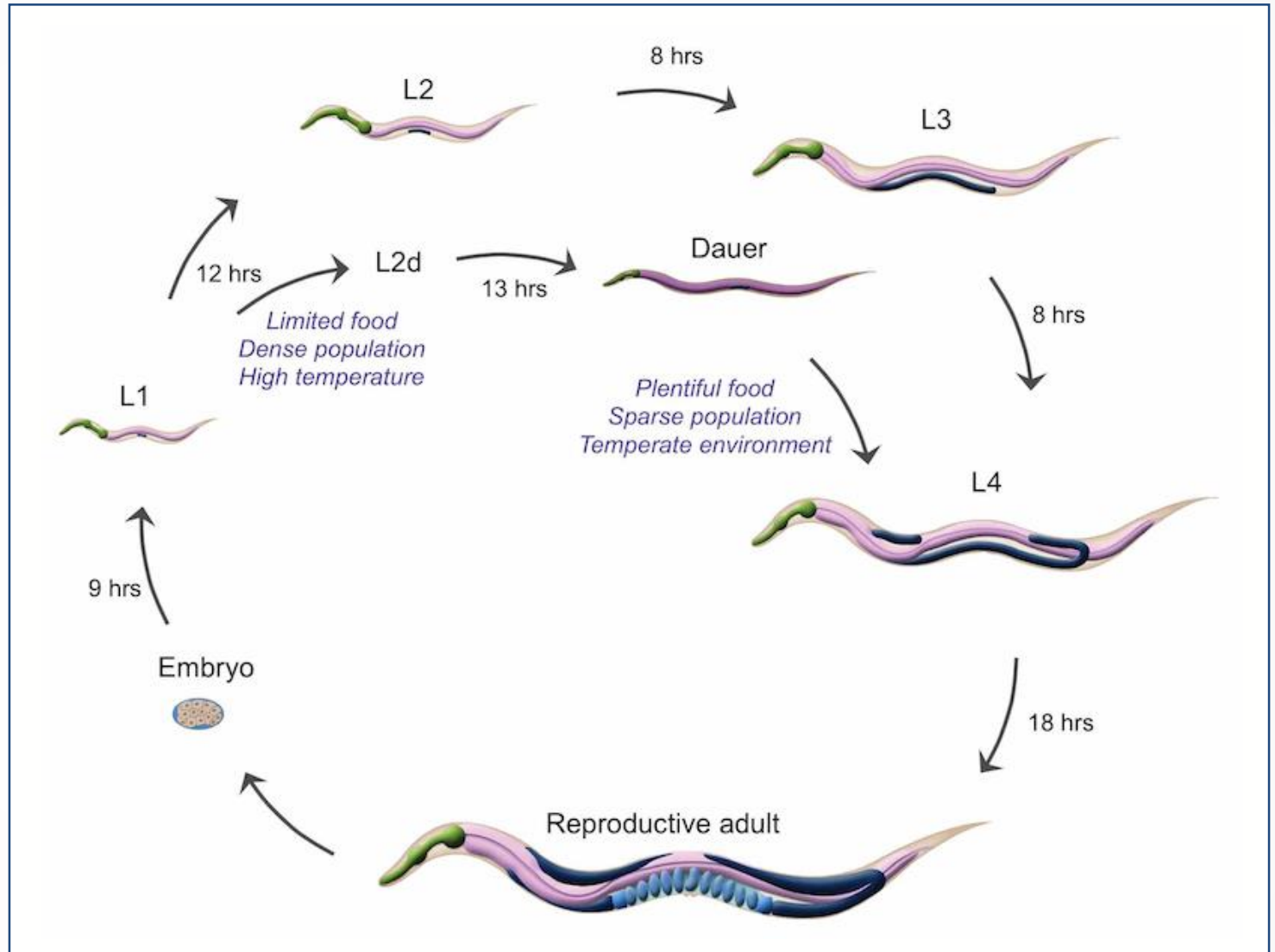
WHY *C.ELEGANS* IS AN ALTERNATIVE MODEL ?

- ▶ **Sentience** is the ability of beings to feel sensations and feelings consciously;
- ▶ Be sentient means being conscious
- ▶ Be sentient is able to be affected positively or negatively

- ▶ ***C.elegans* IS NOT SENTIENCE**

C. elegans model:

- ▶ Nematode – 1mm
- ▶ Feeds *E.coli*
- ▶ Lives in petri dish
- ▶ High reproduction rate (~200 eggs)
- ▶ Lives 28-30 days
- ▶ Transparent (fluorescent)
- ▶ Whole genome was sequenced





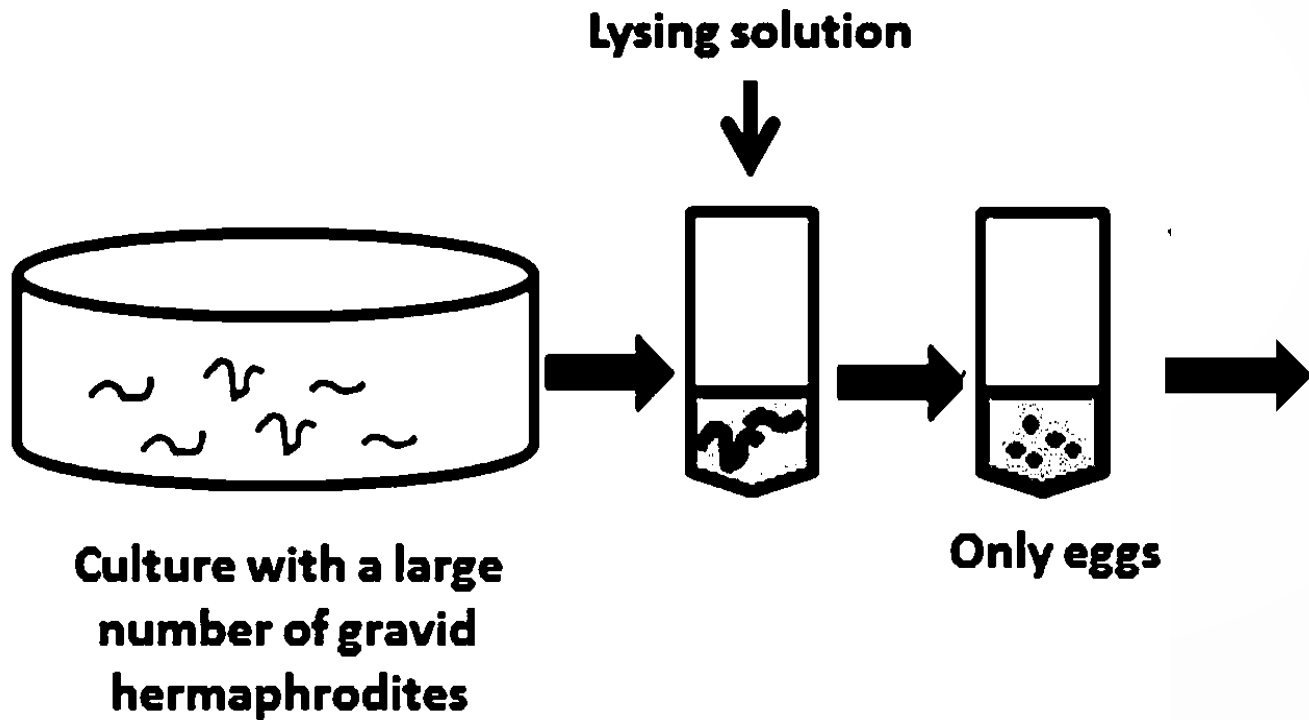
Objective:

- ▶ The present work aims to verify whether the nematode *C. elegans* can help in the evaluation of allergenicity potential.

Growth curve

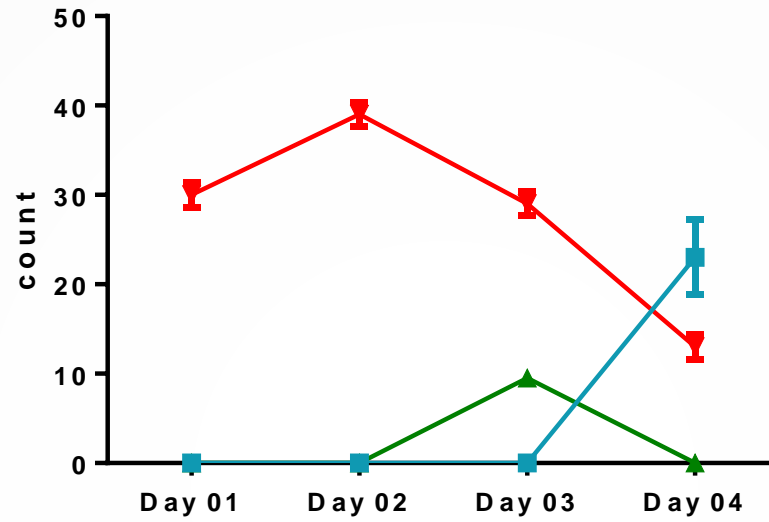
Sodium Hypochlorite 5%
Sodium Hydroxide 2,5M

Counting Worms
- Larvae – Young Adults - Adults

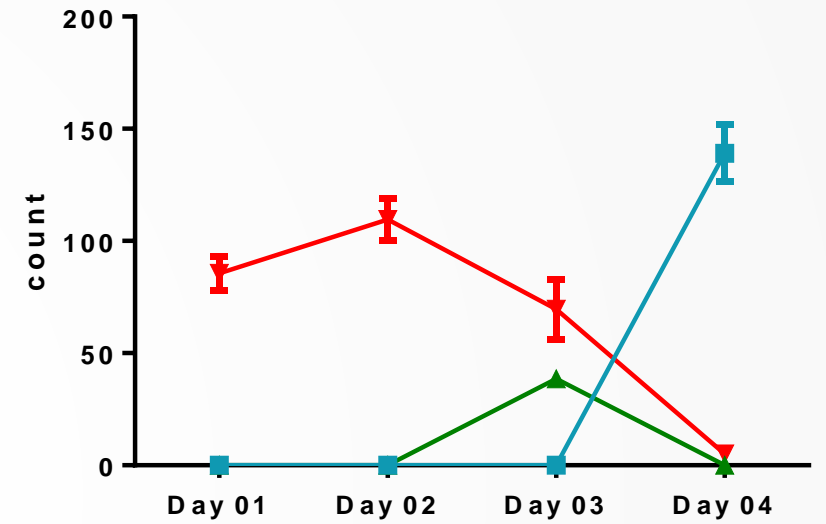


Growth curve

CF1553/DAF-16

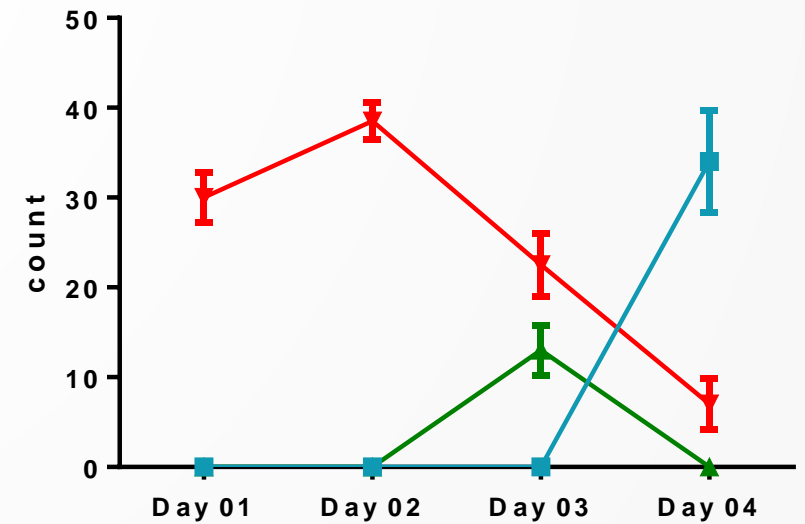


CL2166/SKN-1



- Adults
- ▲ Young Adults
- ▼ Larvae

N2 bristol



Chemicals used in the present study

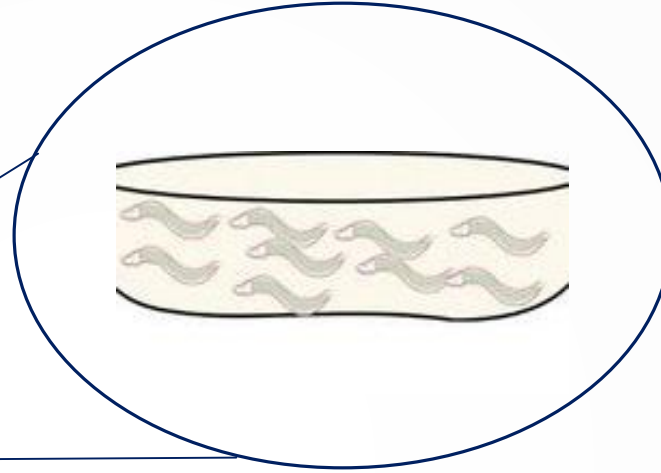
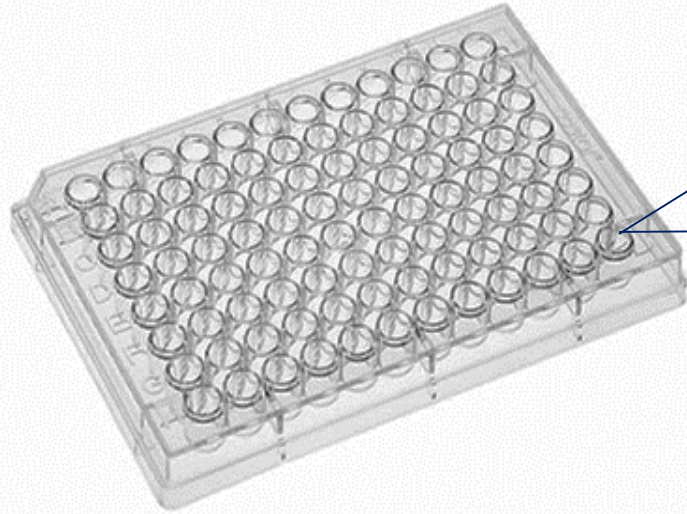
CHEMICAL	Classification LLNA	<i>In vitro</i> classification *	FUNCTION
DMSO (Dimethyl sulfoxide)	Vehicle	Vehicle	Solvent
DNCB (2,4-Dinitrochlorobenzene)	S (extreme)	S	Solvent
PFA (Formaldehyde)	S (strong)	S	Preservative
2-MBT (2-Mercaptobenzothiazole)	S (moderate)	NS	Preservative
EU (Eugenol)	S (weak)	NS	Preservative
PROP (Isopropanol)	NS	NS	Solvent
LPS (Lipopolysaccharide)	Control +	Control +	

*

Parise, C. Sá-Rocha, V.M. De Moraes, J.Z. (2015) Skin sensitizer identification by IL-8 secretion and CD86 expression on THP-1 cells. *Toxicology in vitro*.

LLNA - local lymph node assay, in mices.

Determination of Letal Concentration 50% - LC50



50 μ l - M9 medium
50 μ l - Chemicals solution



DEAD WORMS AFTER 24H WERE COUNTED

➔ N=10-12 worms

Letal Concentration 50% - LC50

CHEMICAL	N2	R ² Value	CL2166	R ² Value	CF1553	R ² Value
DMSO <i>(Dimethyl sulfoxide)</i>	1%	0.9645	1%	0.9765	1%	0.9432
DNCB <i>(2,4-Dinitrochlorobenzene)</i>	1.2 mM	0.9984	2.5 mM	0.9652	2.5 mM	0.9652
PFA <i>(Formaldehyde)</i>	20 mM	0,9801	40 mM	0,9797	20 mM	0,9801
2-MBT <i>(2-Mercaptobenzothiazole)</i>	5.0 mM	0.9604	2.5 mM	0.9524	2.5 mM	0.9524
EU <i>(Eugenol)</i>	0.5 mM	0.9829	0.5 mM	0.9829	1.25 mM	0.9829
PROP <i>(Isopropanol)</i>	170 mM	0.9765	170 mM	0.8738	170 mM	0.8738
LPS <i>(Lipopolysaccharide)</i>	1.0 µg/ml	0.9383	0.5 µg/ml	0.9289	1.0 µg/ml	0.9383

RACIONALE

1

**Most allergenic
potential**

2

**Higher activation
of signaling
pathways**

3

**Increased worms
fluorescence
intensity**

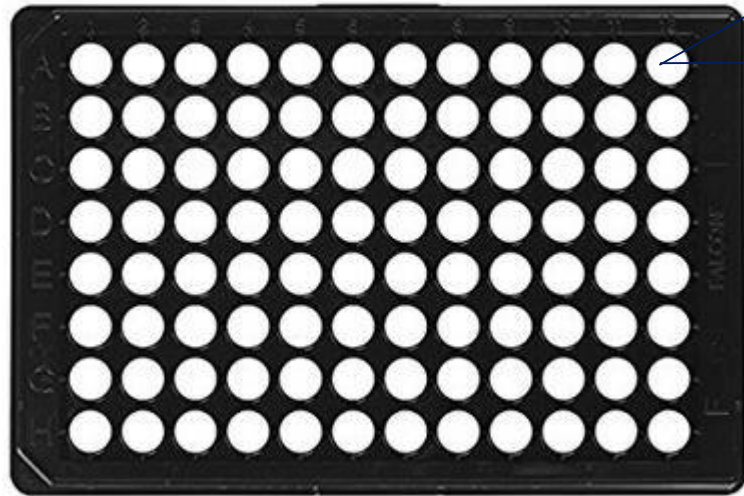


C. elegans strains used:

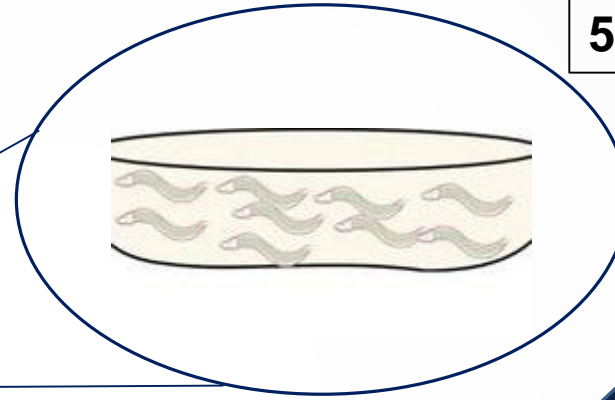
CF1553 – P*sod3*::GFP – DAF16

CL2166 – P*gst4*::GFP – SKN1

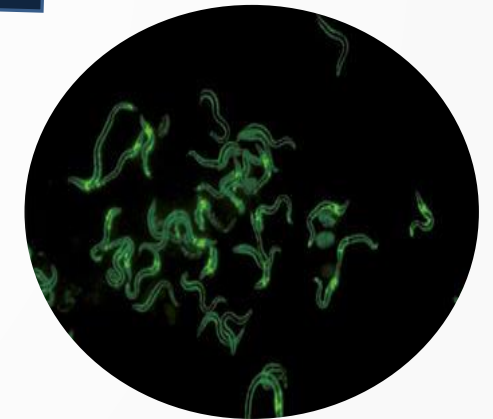
► Selection of the exposure time



4h - short
10h -
moderate
24h - long

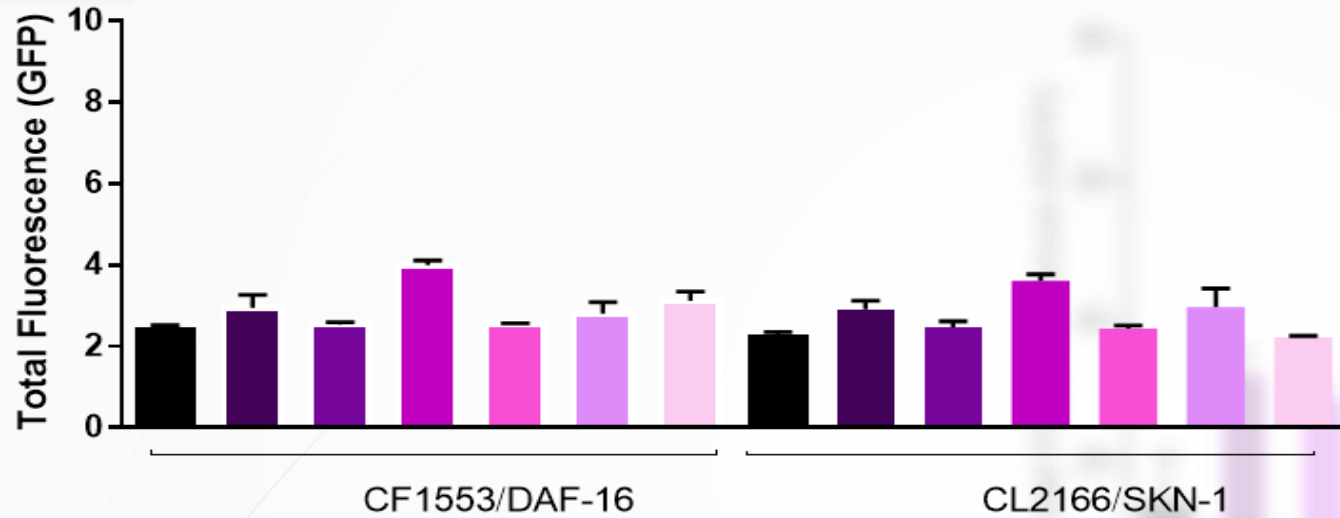


50 μ l - M9 medium
50 μ l - Chemical solution

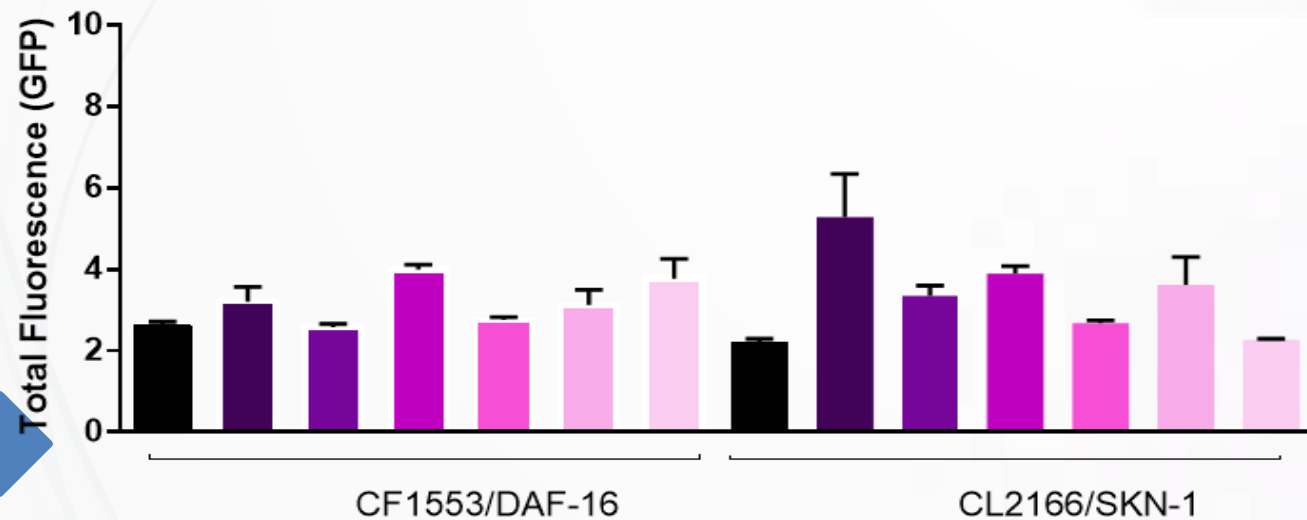


► Selection of the exposure time

4h - SHORT EXPOSURE



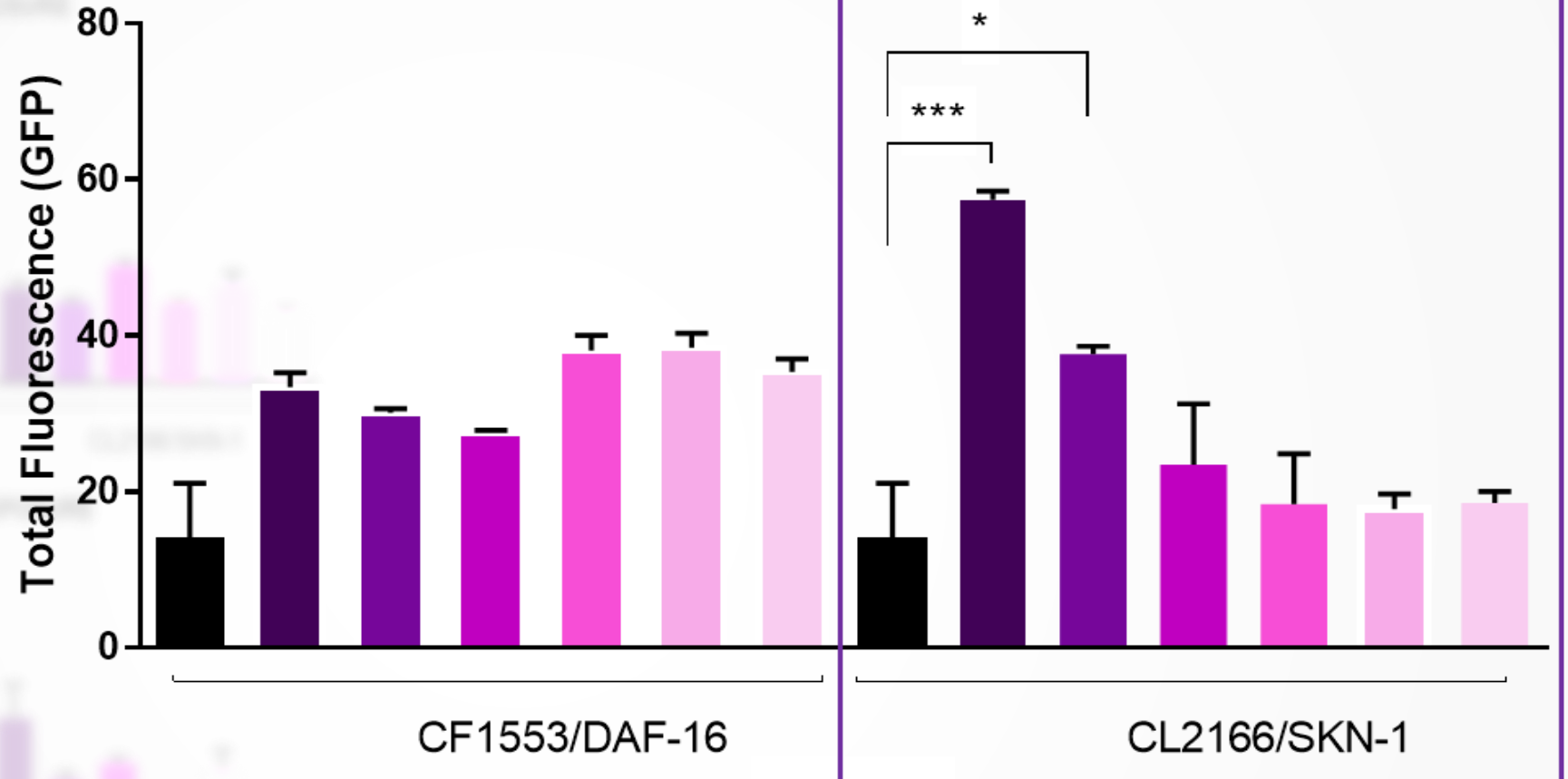
10h - MODERATE EXPOSURE



► N=10-15 worms

► Selection of the exposure time

24h - LONG EXPOSURE



■ DMSO

■ DNCB

■ PFA

■ 2MBT

■ EU

■ PROP

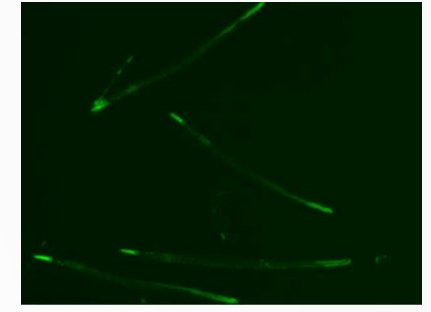
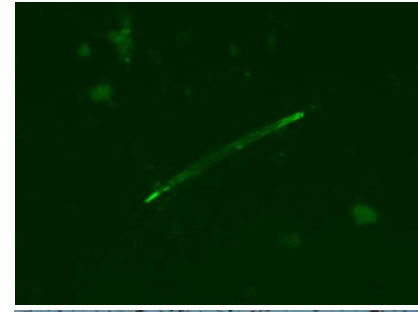
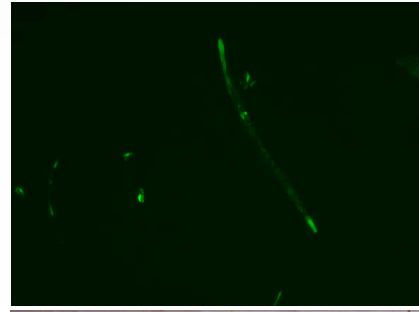
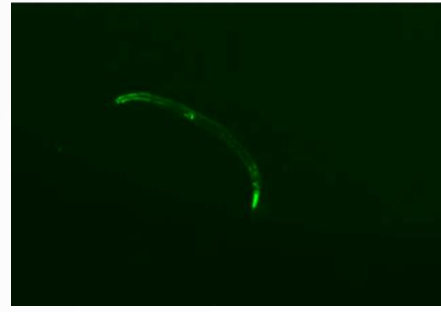
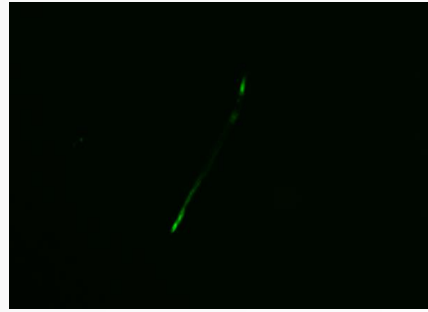
■ LPS

Extreme

No Sensitizer

► N=10-15 worms

► Analysis of the expression of the JNK-DAF16 signaling pathway - CF1553



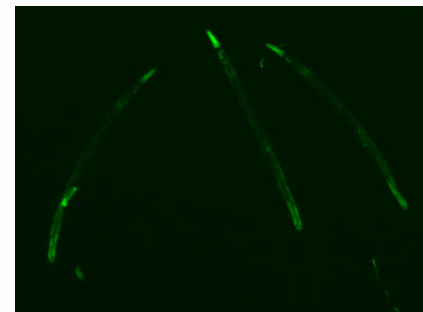
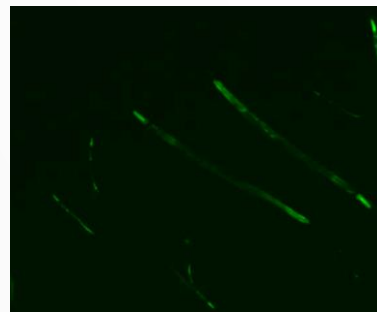
► DMSO

► DNCB

► PFA

► 2-MBT

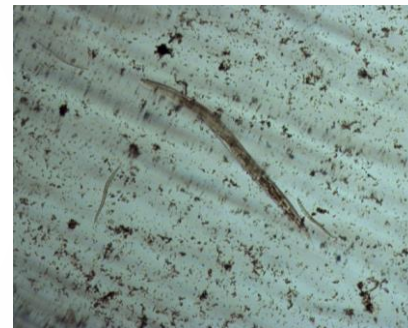
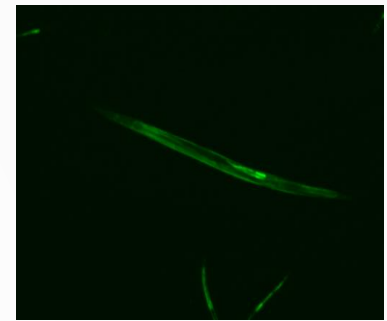
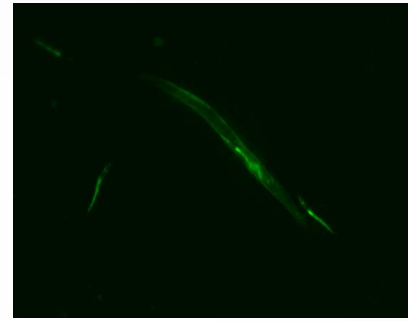
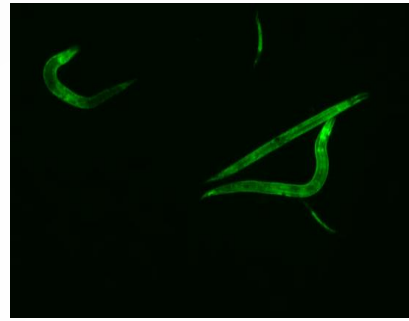
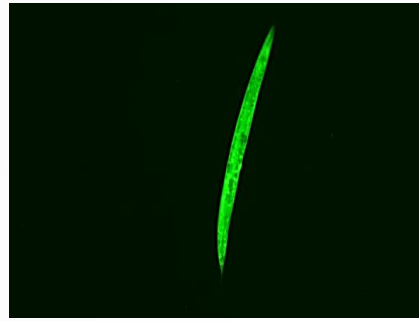
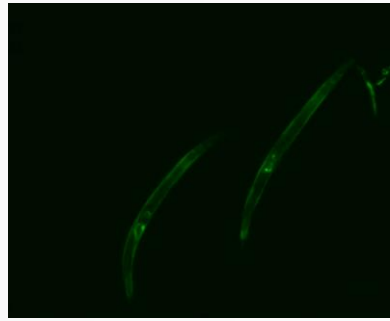
► EU



► PROP

► LPS

► Analysis of the expression of the p38MAPK-SKN-1 signaling pathway - CL2166



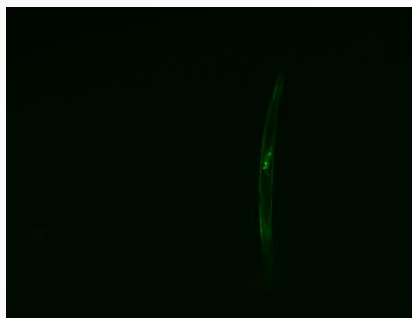
► DMSO

► DNCB

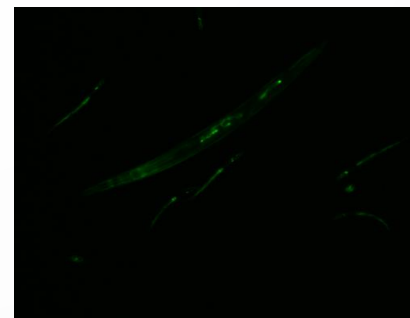
► PFA

► 2-MBT

► EU

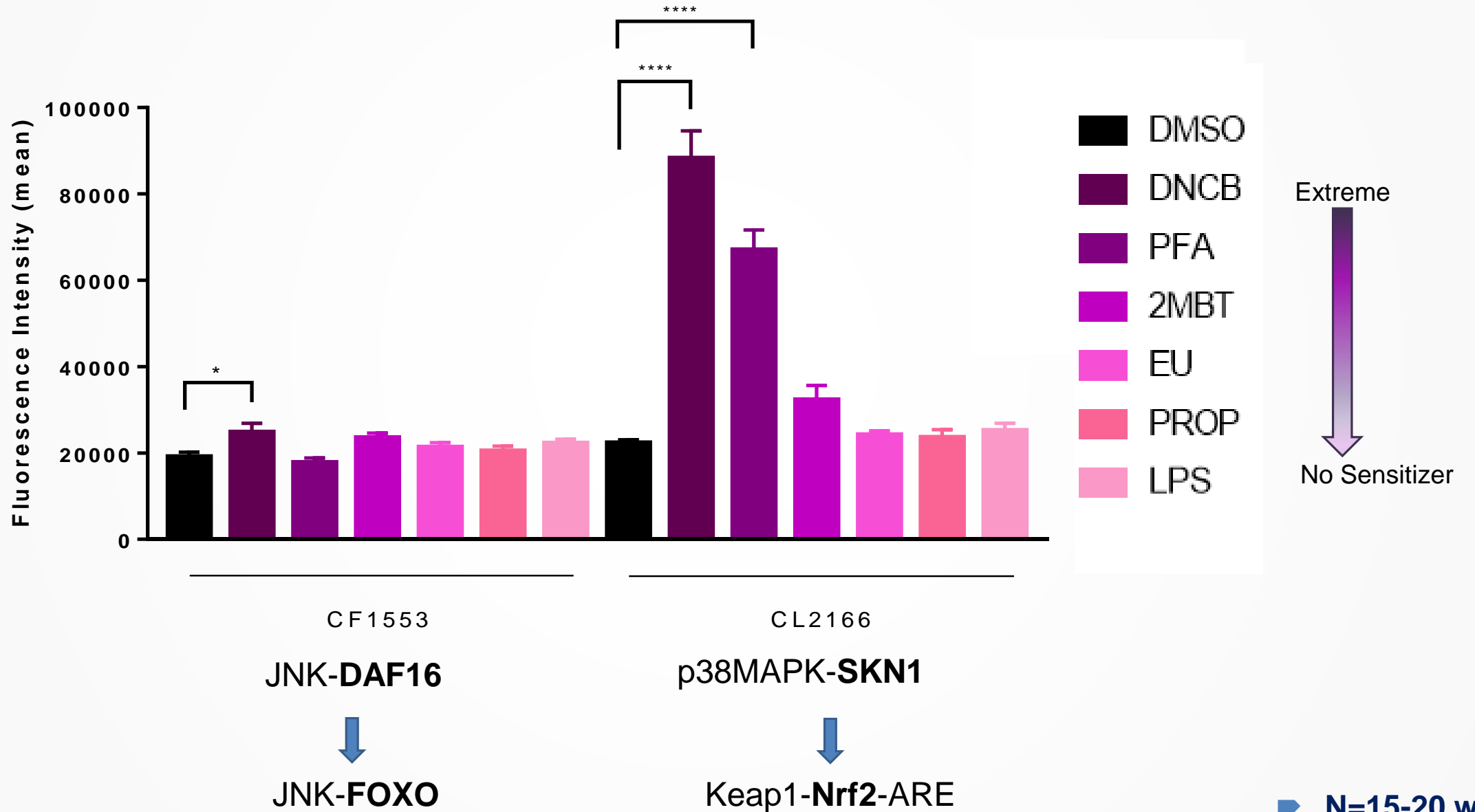


► PROP



► LPS

Fluorescence intensity analysis by the *ImageJ* software



➤ N=15-20 worms

CONCLUSIONS and PROSPECTIVES

- The CL2166 strain, which emit fluorescence when the Keap1-Nrf2-ARE signaling pathway is activated, showed promising potential to predict the allergenicity.
- The CF1553 strain, which emit fluorescence when the JNK-FOXO signaling pathway is activated, was not able to predict the allergen potential of chemicals using the fluorescence emission test;
- These results must be checked by other tests, such as Real Time-PCR, as well as a greater number of chemicals need to be tested to confirm the potential of the approach.

Acknowledgment

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