The methodological challenges – what is known about pro-hapten activation of fragrance materials in the in-vivo and in-vitro test methods we use for induction

Part II – in vitro

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In vitro approaches to sensitization

- skin absorption
- protein reactivity
- DC activation potential
- activation of keratinocytes
- T cell response

Fragrance chemicals

total number of fragrance materials listed in the RIFM database:

- 5100 materials

list of fragrance ingredients used in consumer goods (IFRA, 2011):

- 3009 materials

Published: number of fragrance chemicals commonly tested *in vitro* (number of fragrance chemicals/total number of listed chemicals):

- Natsch *et al*., 2013: 39/145
- Urbisch *et al*., 2015: 53/213

- < 2% of fragrance materials tested in validated *in vitro* tests

Fragrance chemicals

number of fragrance chemicals commonly tested *in vitro* (number of fragrance chemicals/total number of listed chemicals):

Natsch *et al*., 2013: 39/145

Urbisch *et al*., 2015: 53/213

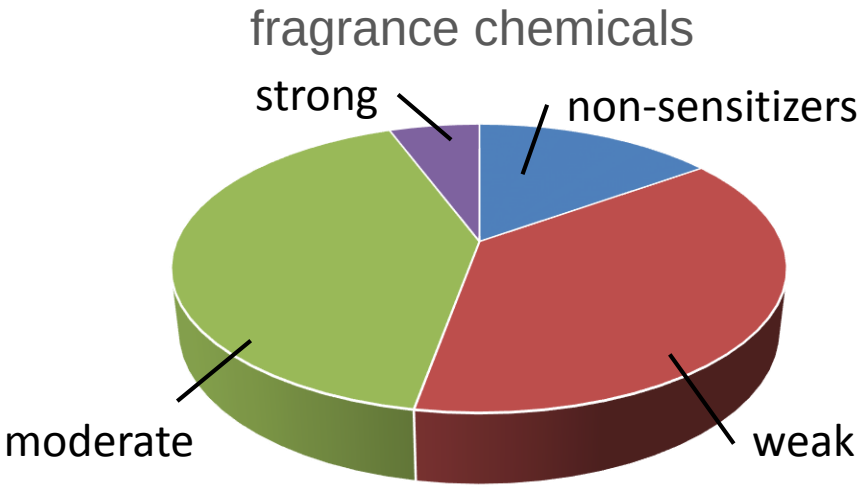
nonsensitizers: 8/53

weak: 20/53

moderate: 22/53

strong: 3/53

extreme: 0/53



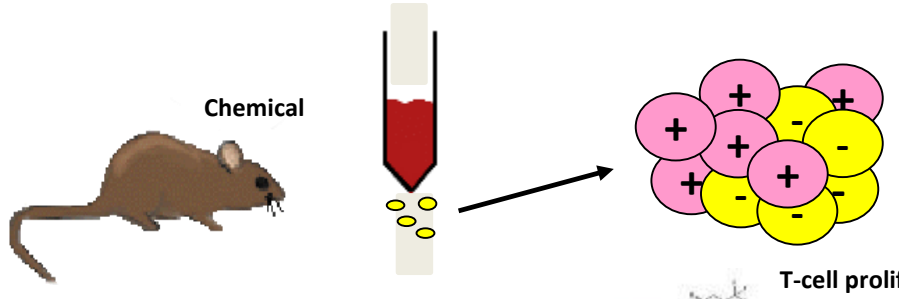
in vitro testing of fragrance chemicals

classification acc. to LLNA	n	positive result (n pos. / n tested)			
		KeratinoSens	hCLAT		DPRA
non-sensitizers	8	5/8	1/7		0/8
weak	20	11/20	14/17		13/20
moderate	22	20/22	15/18		15/20
strong	3	2/3	3/3		2/3

In house established alternative models for skin sensitization

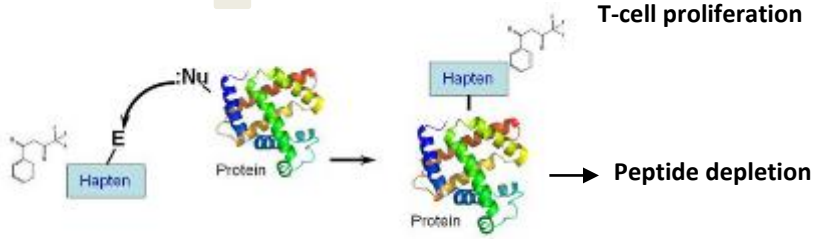
In vivo

e.g. LLNA



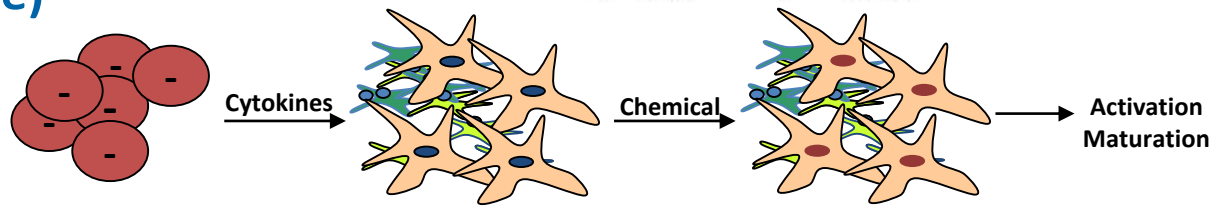
Peptide reactivity

Direct Peptide Reactivity Assay (DPRA)



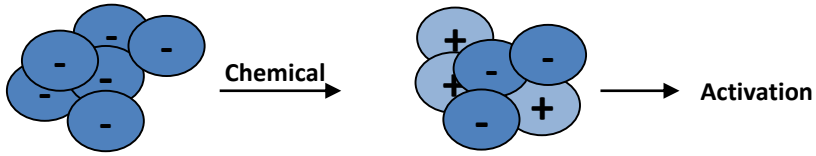
Primary cells (monoculture)

human MoDC



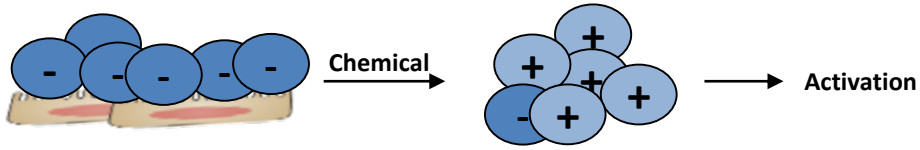
Human cell line (monoculture)

THP-1 (hCLAT)

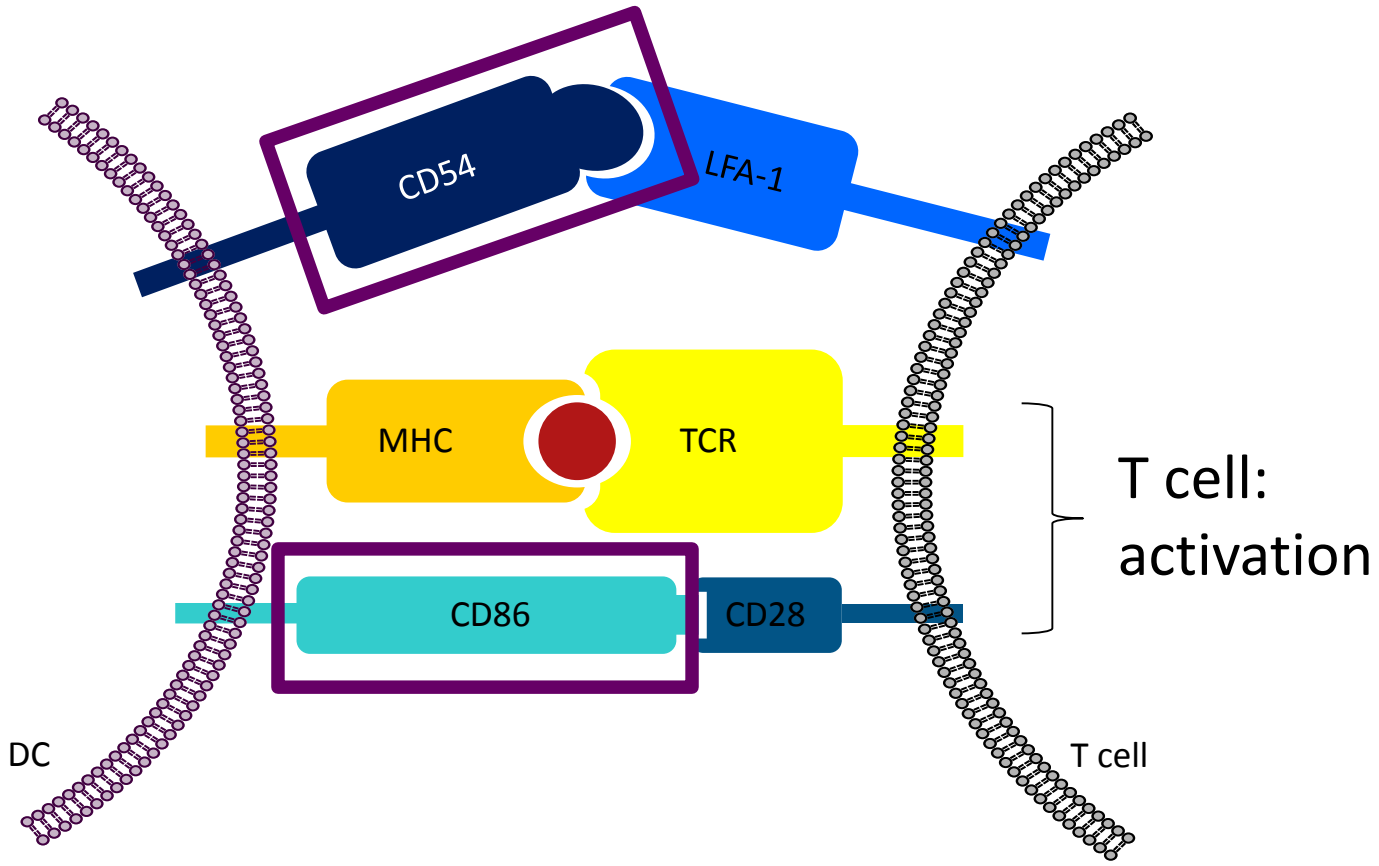


Human cell lines (coculture)

HaCaT + THP-1 = **COCAT**



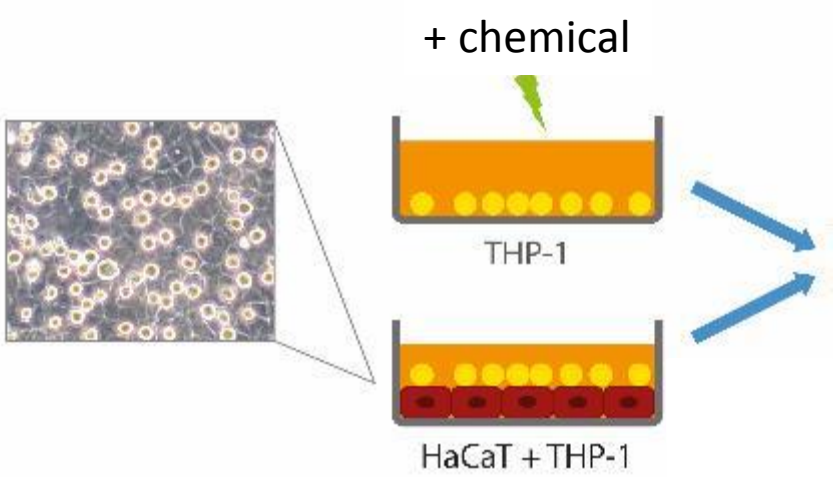
Principle -endpoints



DC activation:
costimulatory molecules and adhesion molecules

→ T cell activation

Model



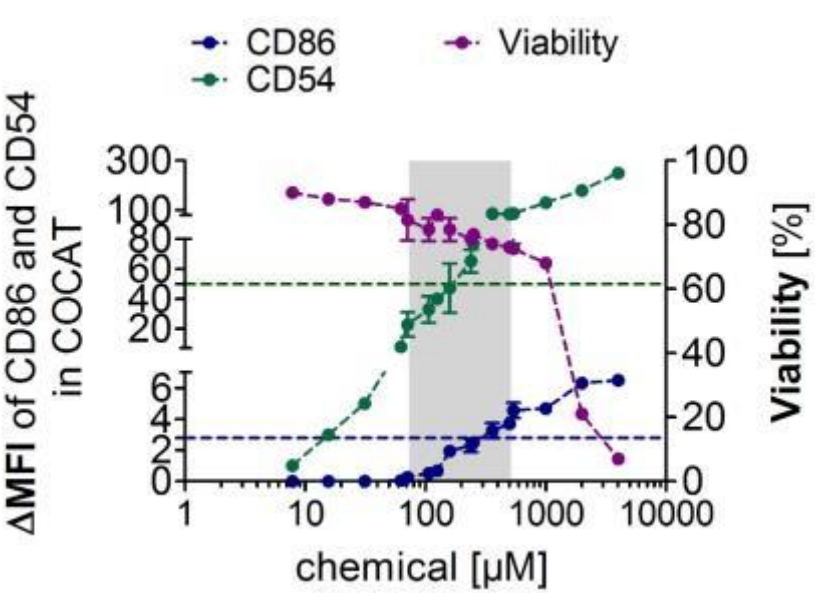
cytokine/chemokine microenvironment
metabolic capacities

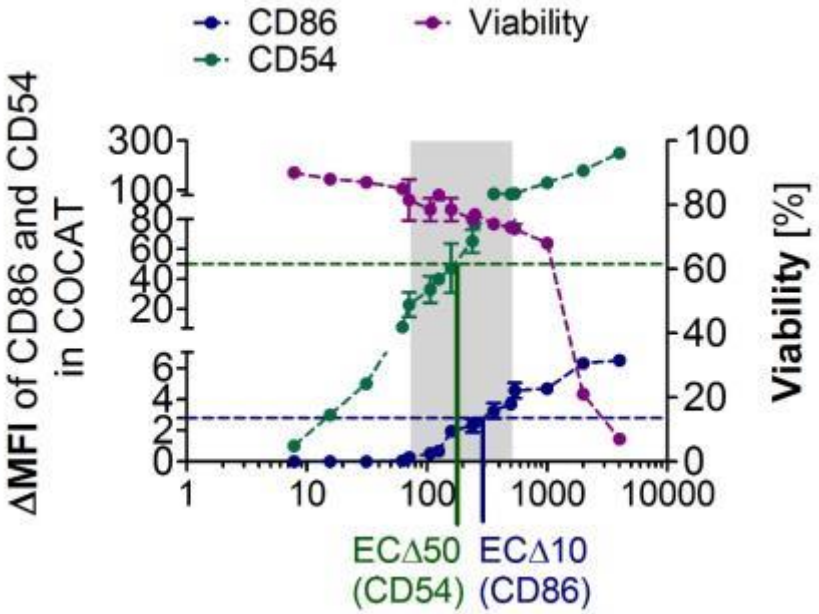
Impact on THP-1 response to chemicals (24h)

→ Upregulation of **CD86** and **CD54** on cell surface of cocultured THP-1 (MoDC)

Hennen et al., 2011, ToxSci

COCAT protocol & prediction model





→ readout: concentration needed for exceeding the thresholds for positivity

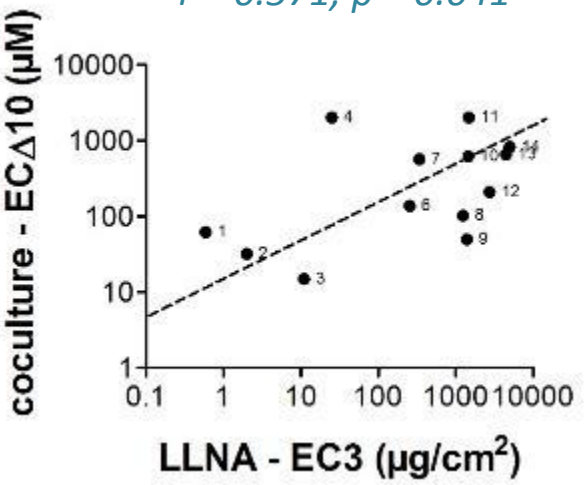
EC∆10 for CD86 and **EC∆50** for CD54

→ **lower value of EC∆10 or EC∆50 is chosen for potency prediction**

Comparison with *in vivo* data: LLNA EC3

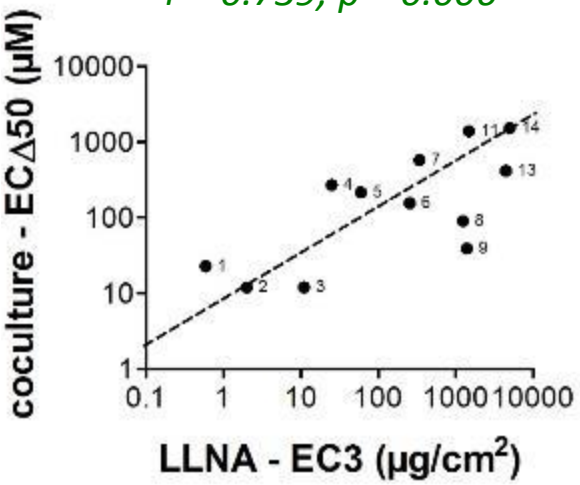
CD86

r = 0.571, p = 0.041

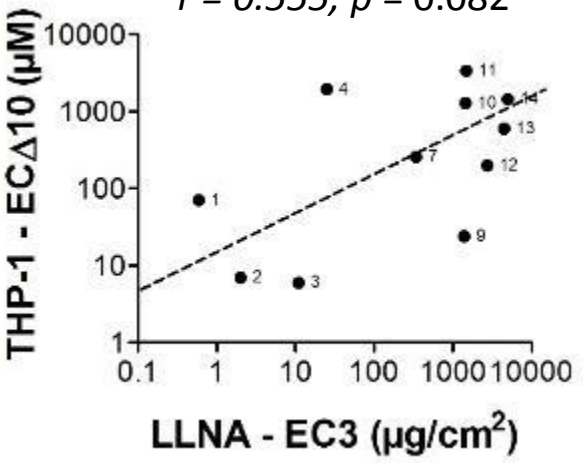


CD54

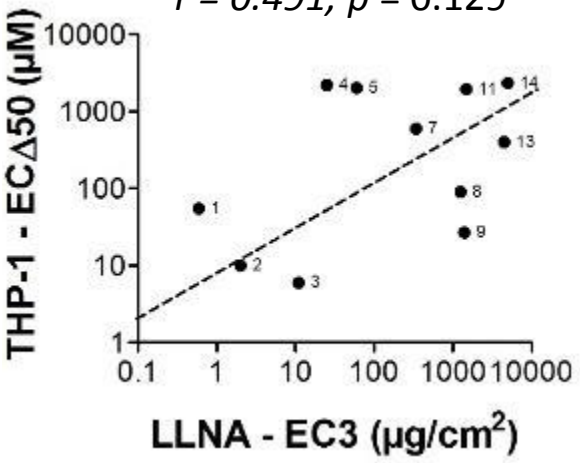
r = 0.739, p = 0.006



r = 0.555, p = 0.082



r = 0.491, p = 0.129



Overall trend: the more potent sensitizer also needs lower concentrations for positivity regarding CD86 and CD54

Significantly improved comparison with EC3

Helpful for quantitative risk assessment

COCAT – Predictive performance

Sensitizers (including 8 pre-/prohaptens):
CD86: 13/14 positive
CD54: 12/13 positive

non-sensitizers:
CD86: 0/10 positive
CD54: 1/10 positive

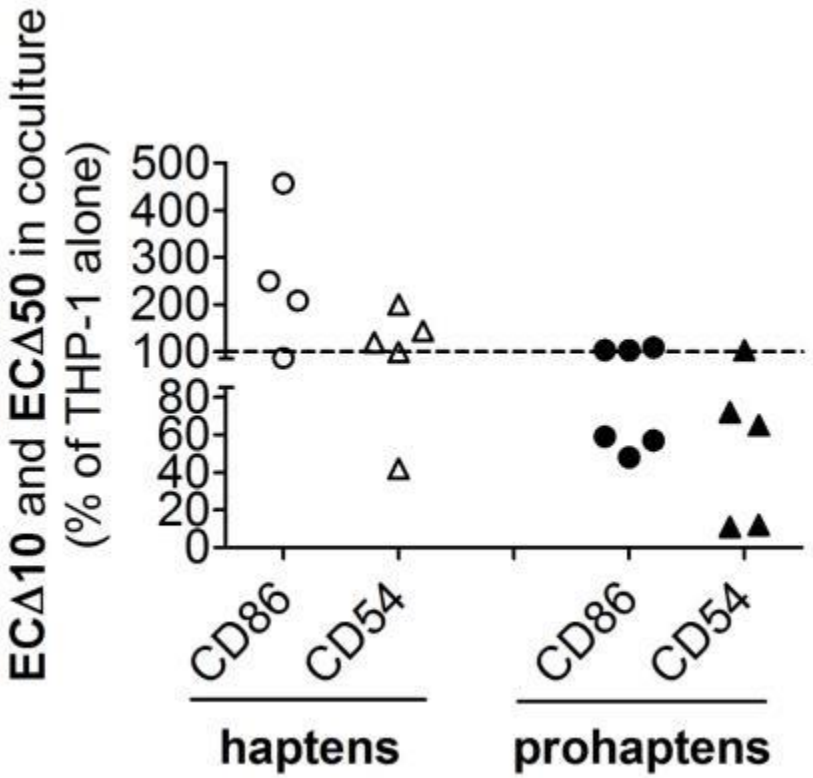
sensitivity:
CD86: 93%
CD54: 92%

specificity:
CD86: 100%
CD54: 90%

accuracy: 96%

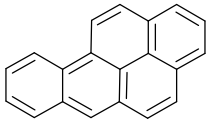
What did we learn?

Concentrations needed for reaching thresholds in COCAT



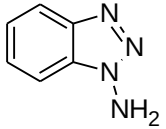
Hennen & Blömeke, in revision

Pro- and pre-haptens – metabolism involved?

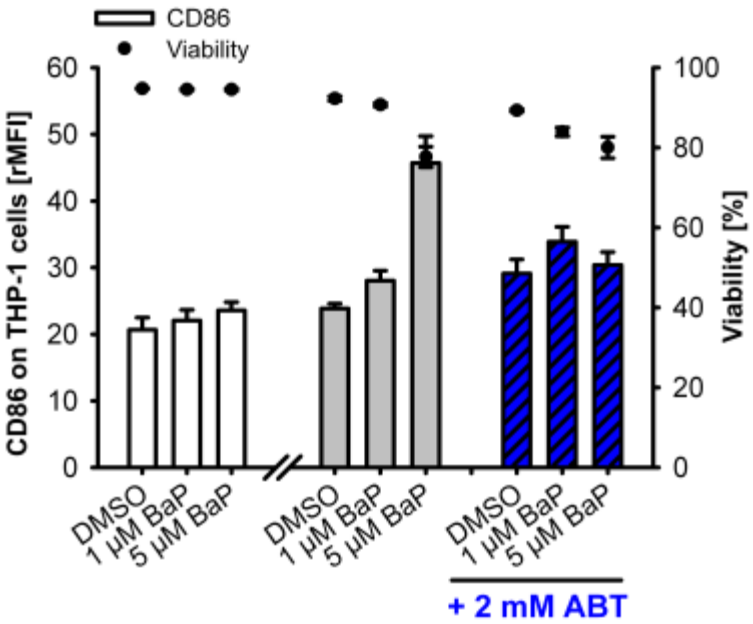


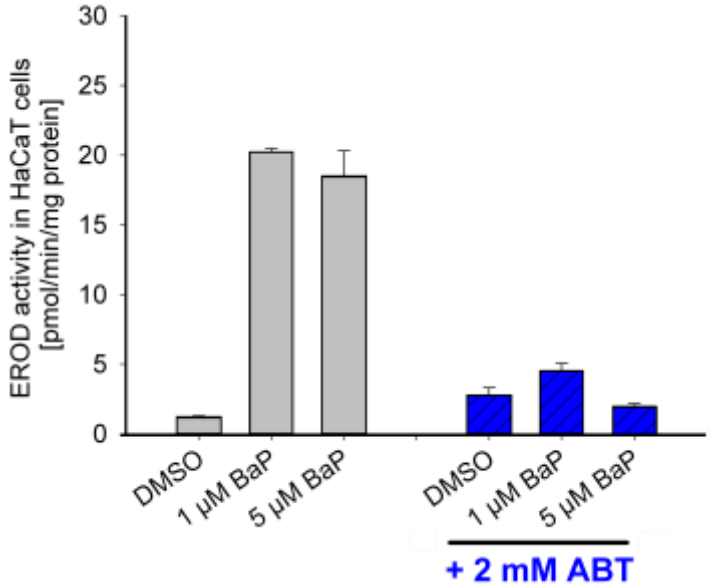
Prohapten B[a]P – Coculture approach

B[a]P



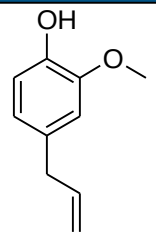
ABT



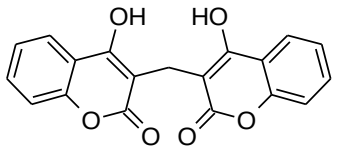


Hennen er al., 2011

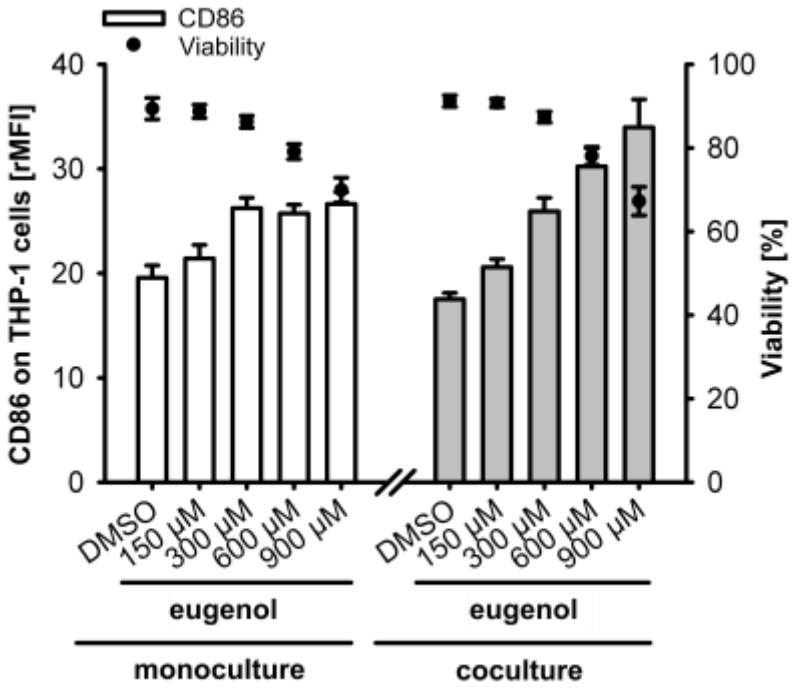
Prohapten eugenol

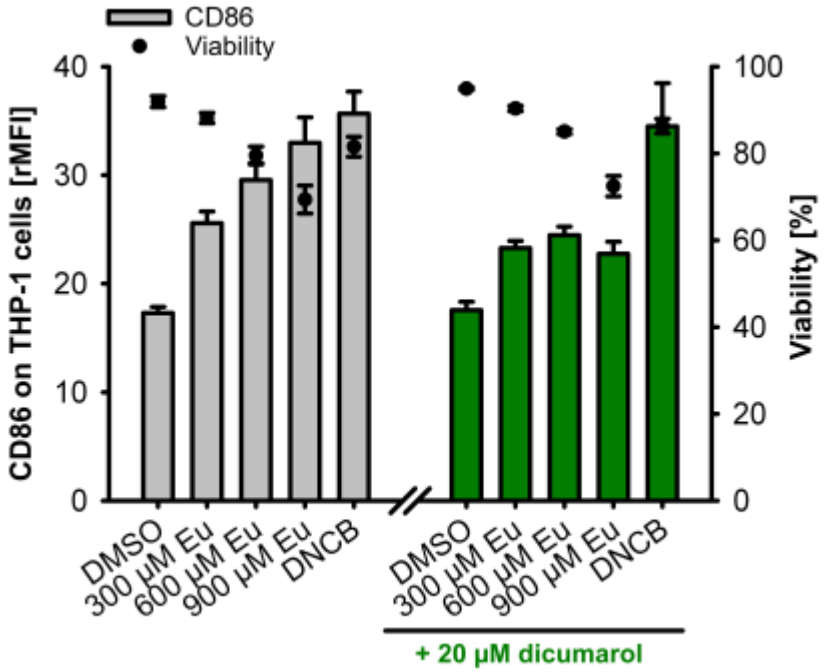


eugenol



dicumarol





Hennen er al., 2011

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Part II – in vitro

- THP-1/HaCaT coculture approach: significantly improved correlation with in vivo EC3 values
- Training set included a set of prohaptens and chemicals that were negative/false positive in other assays
- ....more?