Hennen and Blömeke:

Keratinocytes Improve Prediction of Sensitization Potential and Potency of Chemicals with THP-1 Cells

Supplementary Data

Tab. S1: Compilation of human and murine (LLNA) skin sensitizer categorization, sensitization potency categories and exact values, given as DSA_{05} and EC3 in µg/cm², as well as the assignment to GHS potency subcategories based on a threshold of EC3 = 2%

Chemical	Human		Murine (LLNA)	GHS potency category
	Category[a]	DSA_{05} (µg/cm²)[b]	EC3 (µg/cm²)[c]	
Sensitizers				
oxazolone	NA	NA	0.59	1A
Bandrowski's base	NA	NA	2.5[d]	1A
2,4-dinitro-chlorobenzene	1	3.8	11	1A
acetaminophen	NA	NA	25[e]	#
3-aminophenol	NA	NA	60[f]	1B
cinnamic aldehyde	2	323.8	254	1B
isoeugenol	2	1054	342	1B
citral	3	1078.8	1246	1B
tetramethylthiuram disulfide	3	4610	1396	1B
2-methoxy-4-methylphenol	NA	NA	1450	1B
resorcinol	4	NA	1481	1B
eugenol	3	5926	2743	1B
geraniol	4	3811.5	4474	1B
cinnamic alcohol	3	4454.5	5007	1B
Non-sensitizers				
benzalkonium chloride	5	NA	NC[g]/17	
vanillin	5	NA	NC[h]	
lactic acid	6	NA	NC[h]	
sodium dodecyl sulfate	6	NA	1001	
N,N-diethyl-3-methylbenzamide	NA	NA	>15000[i]	
4-aminoacetanilide	NA	NA	NC[d]	
4-acetamidoacetanilide	NA	NA	NC[d]	
4-amino-2-methyl-acetanilide[j]	NA	NA	NA	
4-amino-3-methyl-acetanilide[j]	NA	NA	NA	
2,5-diacetaminotoluene[j]	NA	NA	NA	

NA, not available

NC, not calculated

drug allergen, not categorized as skin sensitizer

[a] Basketter et al., 2014

[b] data (mean values) obtained from annex II-2 of ICCVAM, 2011

[c] data and classification according annex II-4 of ICCVAM, 2011 except where indicated

[d] Aeby et al., 2009, EC3 value for Bandrowski's base was converted from % to µg/cm² according to ICCVAM, 2011, 4-aminoacetanilide and 4-acetamidoacetanilide were negative in the LLNA when tested up to the solubility limit (approx. 860 and 1100 µg/cm², respectively)

[e] Chipinda et al., 2011, converted from % to µg/cm² according to ICCVAM, 2011

[f] SCCP, 2006, converted from % to µg/cm2 according to ICCVAM, 2011

[g] Basketter et al., 1998

[h] Gerberick et al., 2005

[i] Natsch and Haupt, 2013, and EC3 values converted from % to µg/cm² according to ICCVAM, 2011

[j] categorized as non-sensitizer based on available *in vitro* data (Goebel et al., 2014)

https://doi.org/10.14573/altex.1606171s

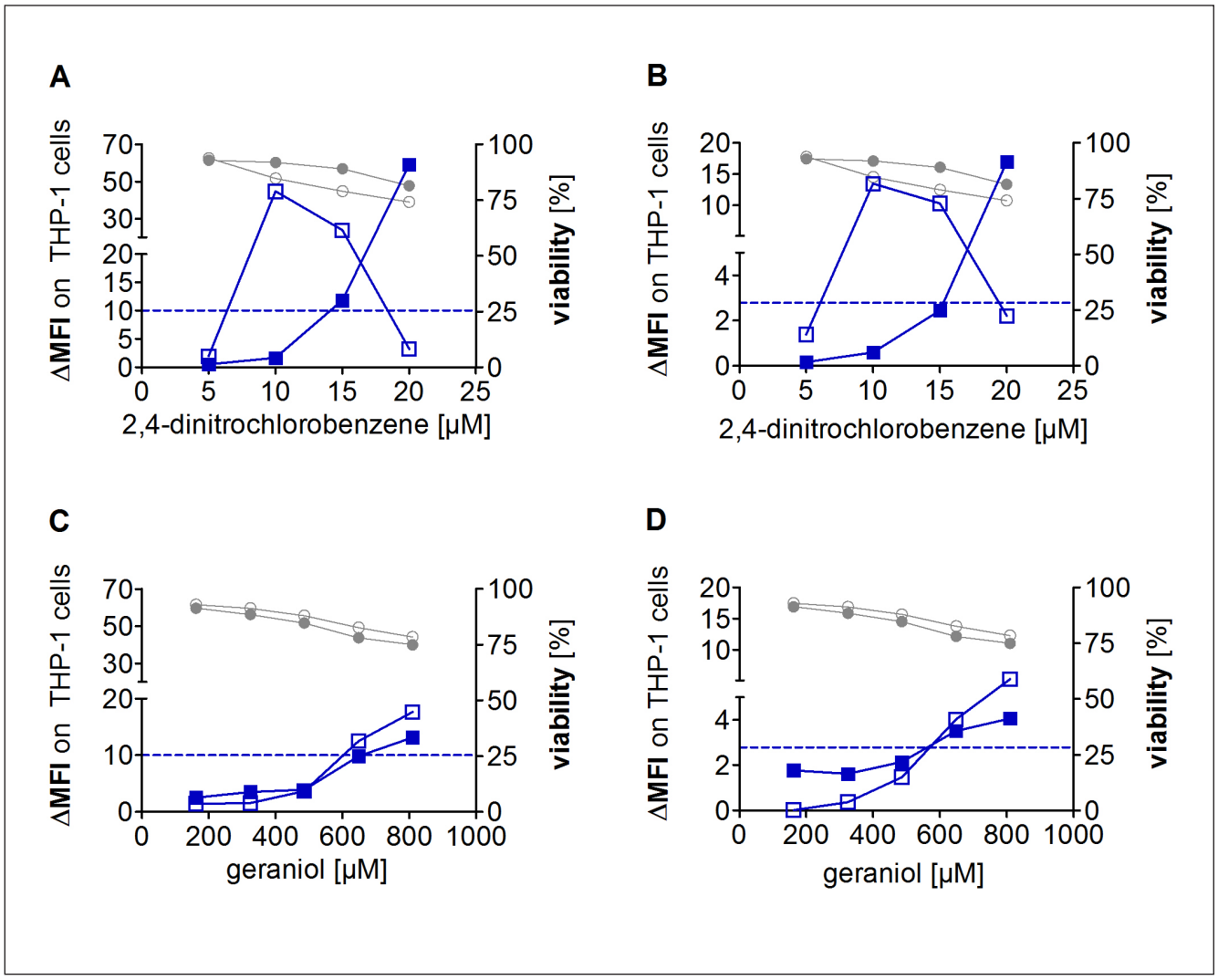


Fig. S1: Comparison of anti-CD86 labeled with FITC and APC

THP-1 cells were treated alone (open symbols) or in coculture with HaCaT cells (filled symbols) with 2,4-dinitrochlorobenzene (A, B) or geraniol (C, D). After 24 h, upregulation of CD86 (blue squares) using either APC-labeled anti-CD86 (A, C) or FITC-labeled anti-CD86 (B, D) on viable THP-1 cells was analyzed via flow cytometry. Percentage of viable THP-1 cells (propidium iodide exclusion) is depicted as grey dots. Horizontal dotted lines represent thresholds of positivity (blue: CD86, at ΔMFI of 10 in panel A, C and at ΔMFI of 2.8 in panel B, D).

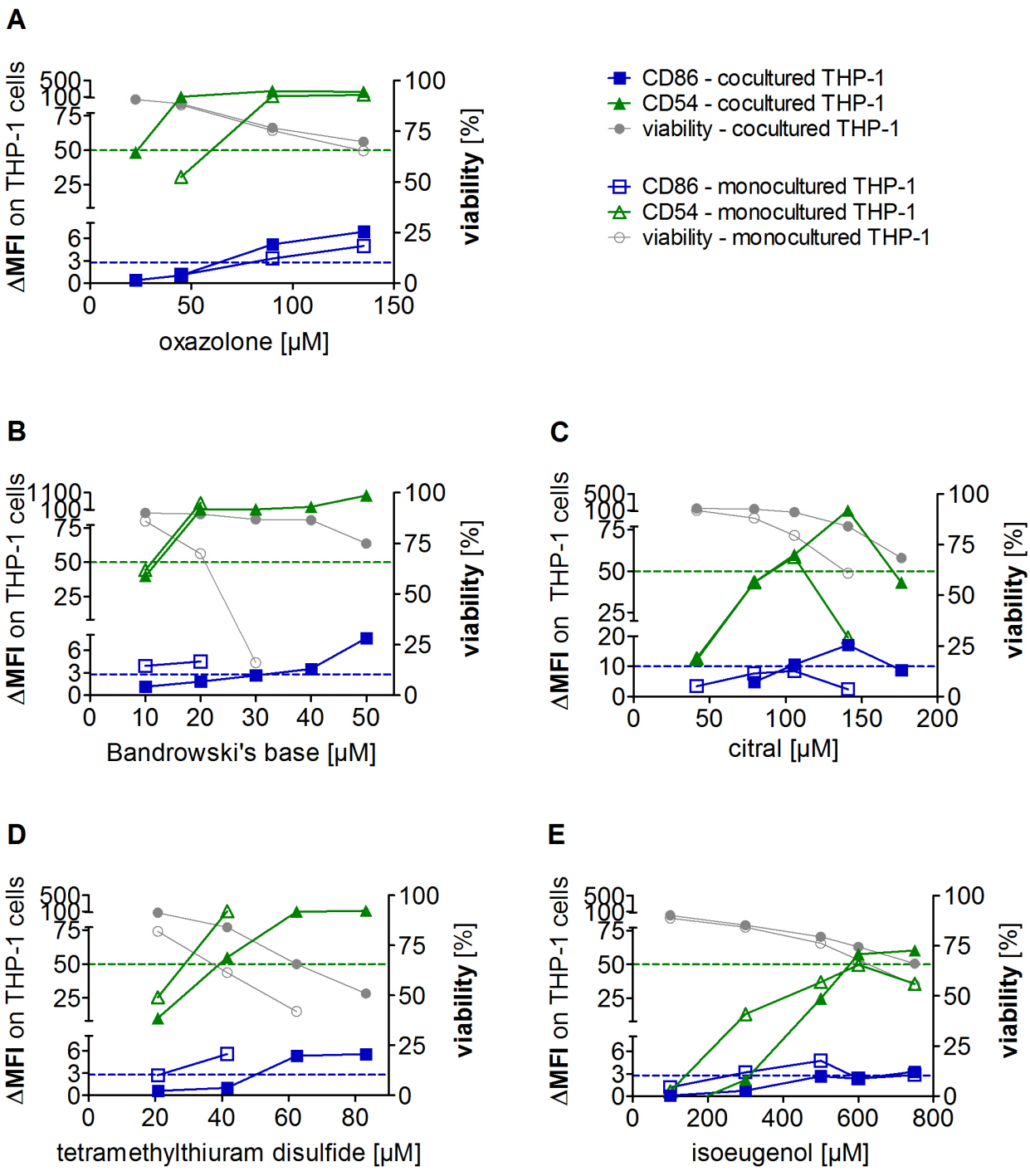
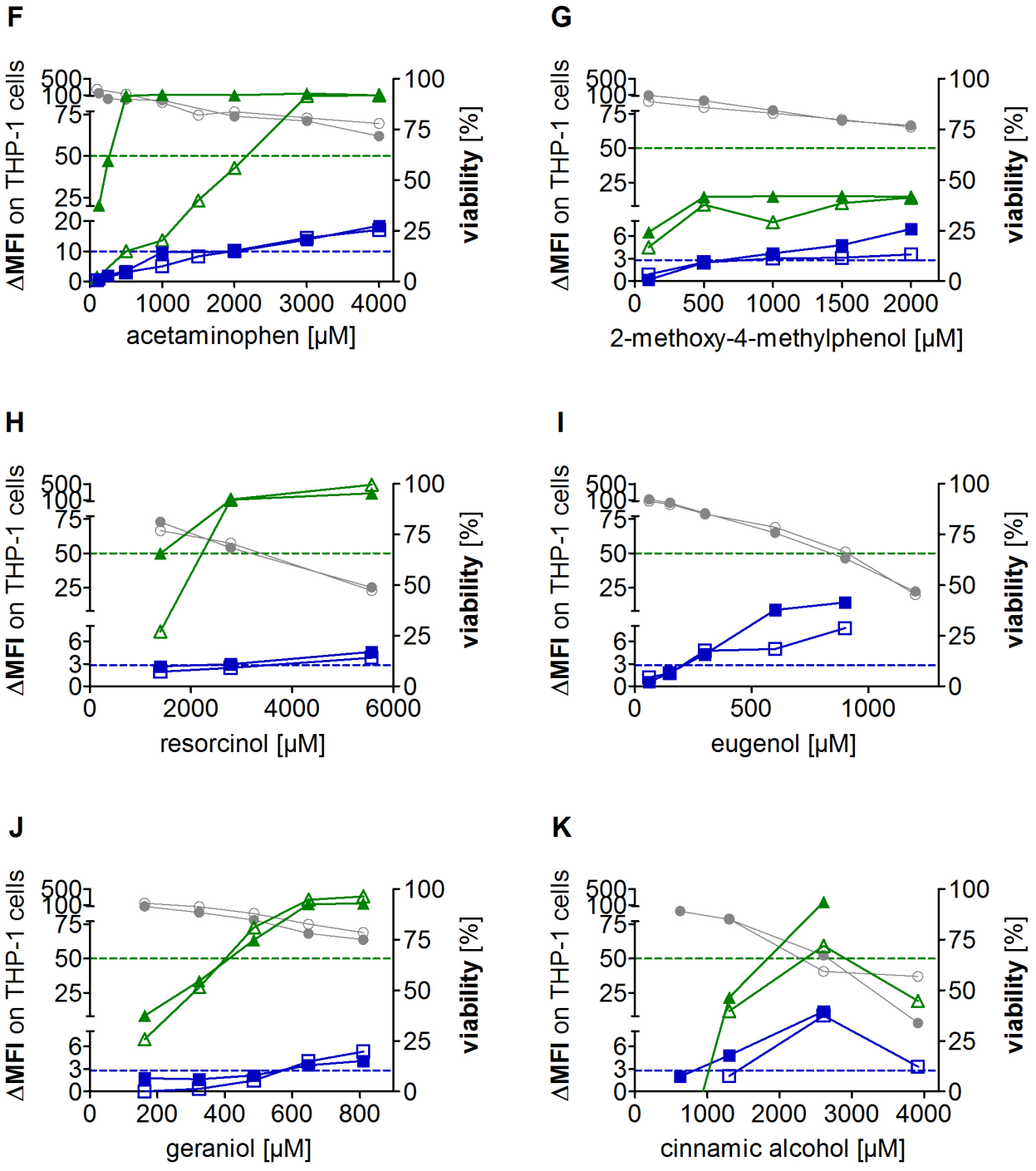


Fig. S2: Impact of HaCaT cells on concentration-dependent upregulation of CD86 and CD54 on THP-1 cells after treatment with sensitizing chemicals

THP-1 cells were treated with the indicated chemicals for 24 h in coculture with HaCaT cells (filled symbols) or alone (open symbols) for 24 h. Expression of CD86 (blue squares) and CD54 (green triangles) on the THP-1 cell surface as well as cell viability (grey dots) were analyzed by flow cytometry. Lines represent thresholds for positivity (blue: ΔMFI of 10 or 2.8 for CD86 and green: ΔMFI of 50 for CD54). Shown are mean of at least 3 independent experiments.


F
ΔMFI on THP-1 cells
viability [%]
acetaminophen [µM]
G
2-methoxy-4-methylphenol [µM]
H
resorcinol [µM]
I
eugenol [µM]
J
geraniol [µM]
K
cinnamic alcohol [µM]

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