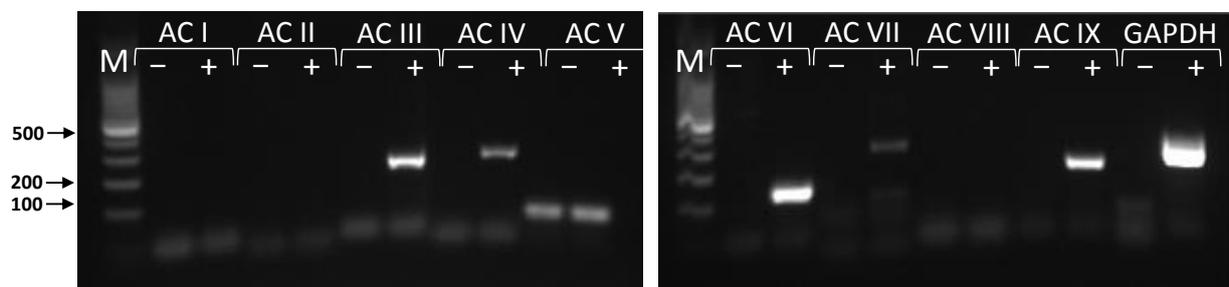


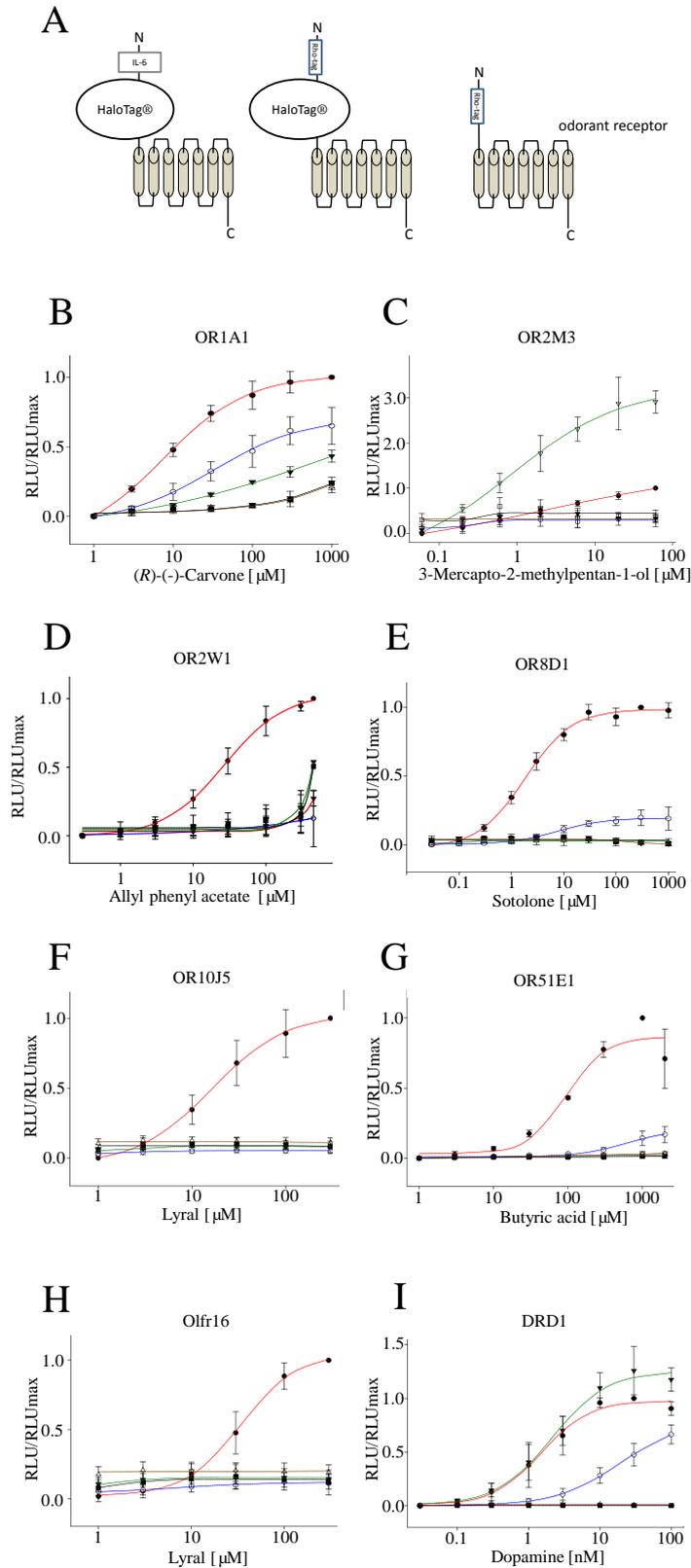
MS#206

**IL-6-HaloTag<sup>®</sup> enables live-cell plasma membrane staining, flow cytometry, functional expression, and de-orphaning of recombinant odorant receptors**

Franziska Noe, Tim Frey, Julia Fiedler, Christiane Geithe, Bettina Nowak, Dietmar Krautwurst

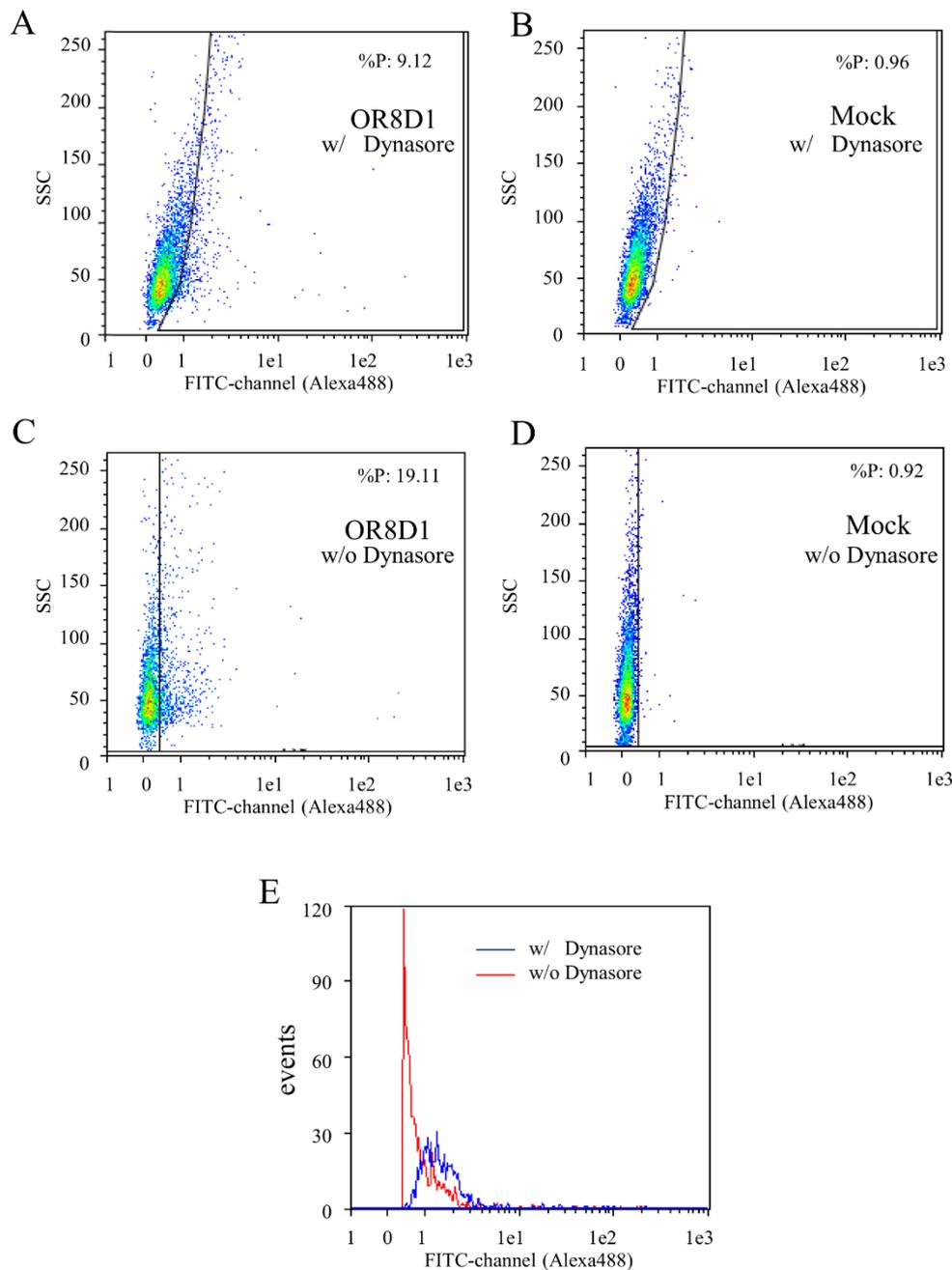


**Figure S1. RT-PCR of adenylyl cyclases in NxG cells.** AC = adenylyl cyclase; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; M = DNA ladder; arrows, DNA sizes in base pairs. -/+ depict the absence or presence of reverse transcriptase. The presence of RNA/cDNA for adenylyl cyclases 3, 4, 6, 7 and 9 was confirmed by sequencing.



**Figure S2. Concentration-response relations for selected odorant /OR combinations with three different N-terminal tags in NxG 108CC15 cells.** Schemes of receptor constructs, carrying either IL-6-HaloTag®, Rho-tag-HaloTag®, or just the Rho-tag (A), OR1A1 with (*R*)-(-)-carvone (B), OR2M3 with 3-mercapto-2-methylpentan-1-ol (C), OR2W1 with allyl phenyl acetate (D), OR8D1 with sotolone (E), OR10J5 with lyr al (F), OR51E1 with butyric acid (G), Olfr16 with lyr al (H), and DRD1 with dopamine (I).

Data are given as mean  $\pm$ SD ( $n = 3-5$ ), and were normalized to each receptor's maximum amplitude with the N-terminal tag IL-6-HaloTag®. RLU, relative luminescence units. **Red** colored curves indicate receptors with the N-terminal tag IL-6-HaloTag®; **blue**, with Rho-tag-HaloTag®; **green**, with Rho-tag; **black**, with IL-6-HaloTag® but no OR coding region (Mock); **dark red**, with Rho-tag but no OR coding region (Mock).



**Figure S3.** Flow cytometry analysis of 10,000 NxG 108CC15 cells expressing IL-6-HaloTag®-OR8D1 and labelled with cell membrane impermeant HaloTag® Ligand-Alexa488. **A** and **B.** Cells pretreated with 80  $\mu$ M of Dynasore, a blocker of clathrin-mediated endocytosis. **C** and **D.** Control cells in the absence of Dynasore. **E.** Fluorescence distribution of cells from both experimental conditions.

**Table S1. Investigated compounds.**

IUPAC Name	CAS Number	Trivial Name	Supplier
(R)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one	6485-40-1	(R)-(-)-Carvone	Sigma-Aldrich, Steinheim, Germany
2-Methyl-3-sulfanylpentan-1-ol	227456-27-1	3-Mercapto-2-methylpentan-1-ol	Chemos GmbH, Regenstauf, Germany
Allyl phenyl acetate	1746-13-0	---	Sigma-Aldrich, Steinheim, Germany
4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one	28664-35-9	Sotolone	Sigma-Aldrich, Steinheim, Germany
4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	31906-04-4	Lylal	Sigma-Aldrich, Steinheim, Germany
Butanoic acid	107-92-6	Butyric acid	Sigma-Aldrich, Steinheim, Germany
Dopamin Hydrochlorid	62-31-7		Alfa Aesar, Karlsruhe, Germany

**Table S2. NCBI reference sequences of investigated odorant receptor genes.**

Gene description	Species	NCBI reference sequence (Accession-number)
OR1A1	<i>Homo sapiens</i>	NM_014565.2
OR2M3	<i>Homo sapiens</i>	NM_001004689.1
OR2W1	<i>Homo sapiens</i>	NM_030903.3
OR8D1	<i>Homo sapiens</i>	NM_001002917.1
OR10J5	<i>Homo sapiens</i>	NM_001004469.1
OR51E1	<i>Homo sapiens</i>	NM_152430.3
Olf16	<i>Mus musculus</i>	NM_008763.2

**Table S3. Oligonucleotides for molecular cloning of investigated receptors.**

Gene	Oligonucleotide	TM (°C)		Sequence 5' → 3'
hsOR1A1	344	66	fw	<i>CGAT CAATTG ATG AGG GAA AAT AAC CAG TCC TCT ACA CTG GAA TTC ATC C</i>
	cg-196	61	rv	<i>CTGC GCGGCCGC TTA CGA GGA GAT TCT CTT GTT GAA GAG TTT CC</i>
hsOR2M3	jf-071	64	fw	<i>GTCG CAATTG ATG GCA AGG GAG AAT TCG ACC TTC AAC TCC G</i>
	jf-072	63	rv	<i>CTGC GCGGCCGC TCA CTC TCC AGA CTT GCC CTT TCC TAA GAT C</i>
hsOR2W1	dk-807	58	fw	<i>GTGA GAATTC ATG GAC CAA AGC AAT TAT AGT TCT TTA CAT GG</i>
	dk-808	59	rv	<i>GCTAA GCGGCCGC CTA TGA CTT GCA ATT CCT CTT TAT TTT TGT AGA TTT G</i>
hsOR8D1	dk-644	59	fw	<i>CTGT GAATTC ATG ACC ATG GAA AAT TAT TCT ATG GCA GCT C</i>
	dk-645	61	rv	<i>CCTAA GCGGCCGC TCA TTT TCC TAC TAA GAC CTT CCT TAA TGC TTT CTT C</i>
hsOR10J5	567	59	fw	<i>GTGC GAATTC ATG AAG AGA AAG AAC TTC ACA GAA GTG TCA G</i>
	568	60	rv	<i>GCC GCGGCCGC TTA AGA AAT ATT TCT GCC CAC AAC TCT GCA TAG G</i>
hsOR51E1	dk-766	58	fw	<i>GTGA GAATTC ATG ATG GTG GAT CCC AAT GGC AAT G</i>
	dk-767	67	rv	<i>GCTAA GCGGCCGC CTA GGG CTC TGA AGC GTG TGT GGC CAC ATG</i>
mmOlf16	611	62	fw	<i>GTGC GAATTC ATG CAG AGA AAT AAC TTC ACT GAA GTG ATA GAG TTC G</i>
	612	65	rv	<i>CGC GCGGCCGC TTA AGA AGT GTT TCT GCC CAC AGC TCT GCA TAG AGC</i>
DRD1		57	fw	<i>CATC GAATTC ATG AGG ACT CTG AAC ACC TCT GC</i>
		55	rv	<i>GCTA GCGGCCGC TCA GGT TGG GTG CTG ACC G</i>

hs = *Homo sapiens*, mm = *Mus musculus*, TM = melting temperature, fw = forward, rv = reverse

**Table S4. Vector internal oligonucleotides for pFN210A.**

Oligonucleotide	TM (°C)		Sequence 5' → 3'
520	60	fw	<i>GTG GAC ATC GGC CCG GGT C</i>
550	50	rv	<i>CAC AAA TAA AGC ATT TTT TTC ACT GC</i>

**Table S5. Oligonucleotides for RT-PCR of adenylyl cyclases in NxG cells.**

Gene	Oligo-nucleotide	TM (°C)		Sequence 5' → 3'	Amplicon (bp)
ADCY1	cg-144b	61	fw	<i>GTTCTCCGAGTTGGCATCAACGTTG</i>	147
	cg-145a	61	rv	<i>CTCAGTCACCTGAATTCGTCCCTGG</i>	
ADCY2	cg-146a	59	fw	<i>GAAGAAGAAGCTGAATGAAAGGATGATCCAAG</i>	178
	cg-147	58	rv	<i>GGCAGGCACAGGTCACGTCAG</i>	
ADCY3	cg-97a	60	fw	<i>GACTTCTACACTGAGGAGAGCATCAAC</i>	235
	cg-98	60	rv	<i>GCTGCCAGCGCTCCTTGTCTG</i>	
ADCY4	cg-148a	58	fw	<i>CCACTCCACTCCCGGAGAAAAG</i>	250
	cg-149	59	rv	<i>CAGGCTGCGTAGTATTTGAAGGCG</i>	
ADCY5	cg-150	60	fw	<i>CGGTGCTGTCGTTGGGCGC</i>	330
	cg-151	60	rv	<i>CCAGCACCCTGCAATGAGCGC</i>	
ADCY6	cg-152a	59	fw	<i>GCCCACTTCCTGGCCCGG</i>	145
	cg-153	60	rv	<i>GCAGGCACTCCACGCCCTC</i>	
ADCY7	cg-154a	59	fw	<i>CCAAGCCCAAGGGGGACGC</i>	285
	cg-155	60	rv	<i>CAGCAGGGCCTGGTGGAGC</i>	
ADCY8	cg-156	60	fw	<i>CTGCCTCTCACCTGGGCCATC</i>	289
	cg-157	58	rv	<i>CGACAAACCTGGGGAGCACAG</i>	
ADCY9	cg-158a	60	fw	<i>GCACGGCAAAGATCTGGAAGTAGAGAA</i>	212
	cg-159	59	rv	<i>GCTGCATCTTAAAGGGGCGGAATG</i>	
GAPDH	cg-301	60	fw	<i>GATGGGTGTGAACCACGAGAAATATGAC</i>	308
	cg-302	60	rv	<i>CACGGAAGGCCATGCCAGTGAG</i>	

TM = melting temperature, fw = forward, rv = reverse.

Primer matches the sequence of *Mus musculus* and *Rattus norvegicus*.

**Table S6. Numbers from Fig. S3E**

	Events	Membrane Alexa488 (% positive cells)	Membrane Alexa488 mean
OR8D1 w/ Dynasore	678	9.12	2.70
OR8D1 w/o Dynasore	948	19.11	1.61