Literature Report

Reporter: zhou wei

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Article

Development of High-Specificity Fluorescent Probes to Enable Cannabinoid Type 2 Receptor Studies in Living Cells

Roman C. Sarott, Matthias V. Westphal, Patrick Pfaff, Claudia Korn, David A. Sykes, Thais Gazzi, Benjamin Brennecke, Kenneth Atz, Marie Weise, Yelena Mostinski, Pattarin Hompluem, Eline Koers, Tamara Miljuš, Nicolas J. Roth, Hermon Asmelash, Man C. Vong, Jacopo Piovesan, Wolfgang Guba, Arne C. Rufer, Eric A. Kusznir, Sylwia Huber, Catarina Raposo, Elisabeth A. Zirwes, Anja Osterwald, Anto Pavlovic, Svenja Moes, Jennifer Beck, Irene Benito-Cuesta, Teresa Grande, Samuel Ruiz de Martín Esteban, Alexei Yeliseev, Faye Drawnel, Gabriella Widmer, Daniela Holzer, Tom van der Wel, Harpreet Mandhair, Cheng-Yin Yuan, William R. Drobyski, Yurii Saroz, Natasha Grimsey, Michael Honer, Jürgen Fingerle, Klaus Gawrisch, Julian Romero, Cecilia J. Hillard, Zoltan V. Varga, Mario van der Stelt, Pal Pacher, Jürg Gertsch, Peter J. McCormick, Christoph Ullmer, Sergio Oddi, Mauro Maccarrone, Dmitry B. Veprintsev, Marc Nazaré, Uwe Grether,* and Erick M. Carreira*

作者介绍

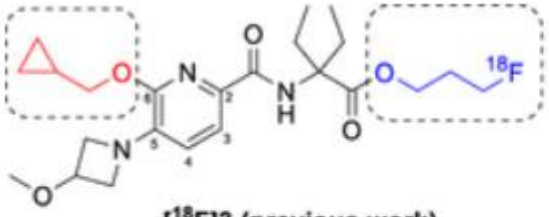
Prof. Dr. Erick M. Carreira

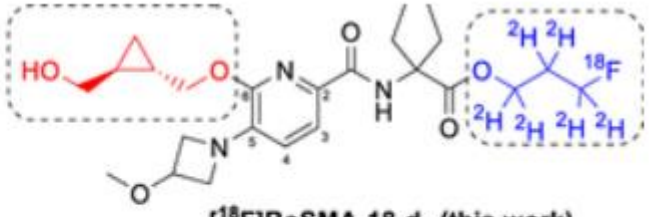
Organic Chemistry Laboratory of the ETH Zurich

asymmetric synthesis of biologically active, stereochemically complex, natural products.

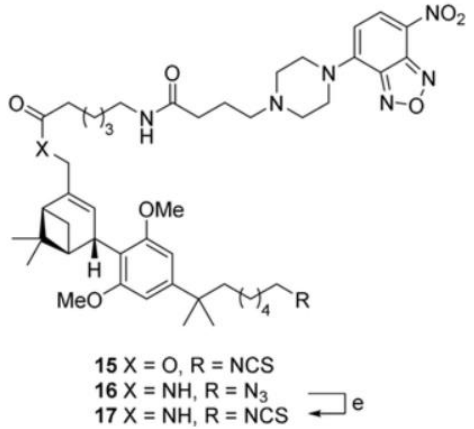
Uwe Grether

Roche Pharma Research & Early Development, Roche Innovation Center Basel



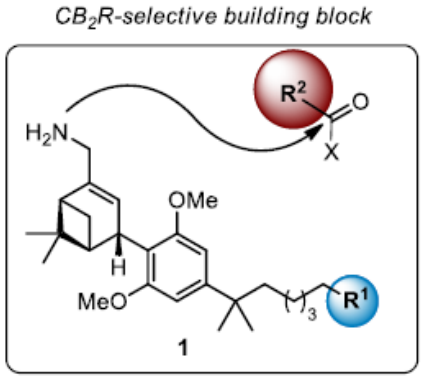


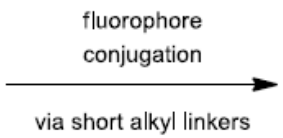
J. Med. Chem-2020-10287

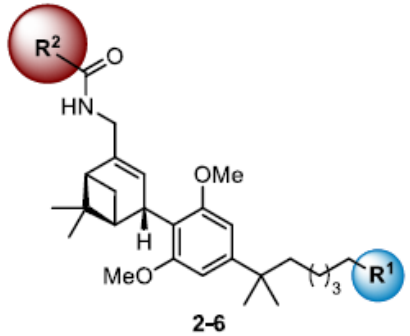


Chem. Eur.J-2020-1380

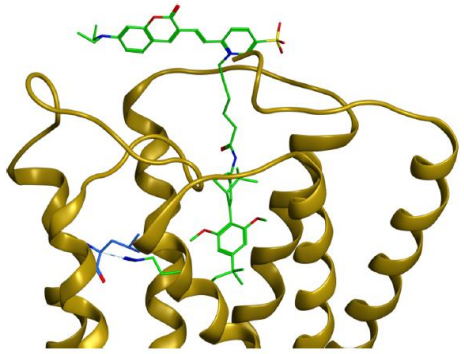
探针设计







- Modularity of design ✓
- Selectivity over CB_1R ✓
- Affinity and specificity for CB_2R ✓
- Tailored photophysical properties ✓
- Applicability across species, techniques and cell types ✓



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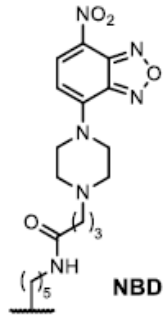

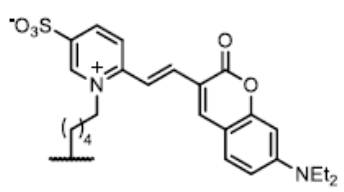

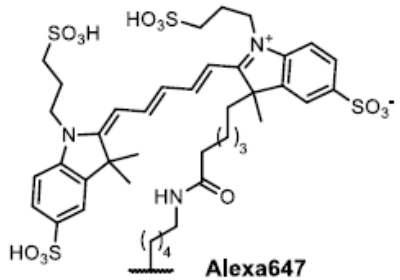

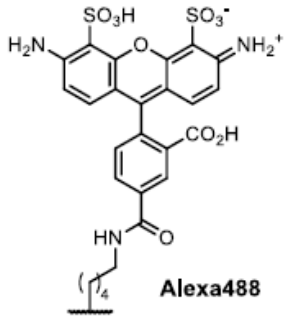

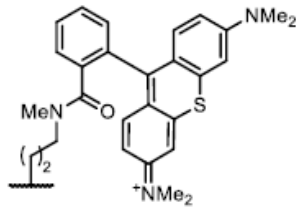

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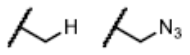


3a 3b

4

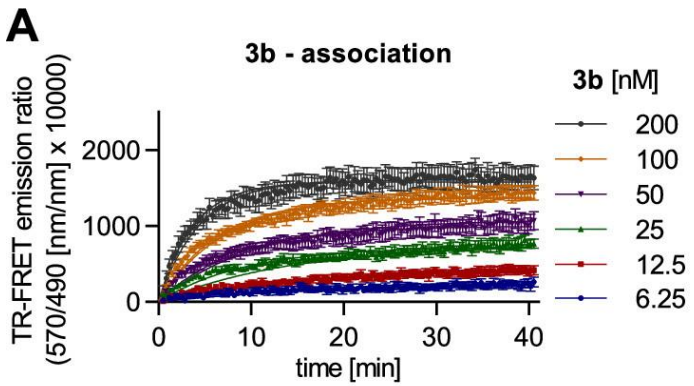
5

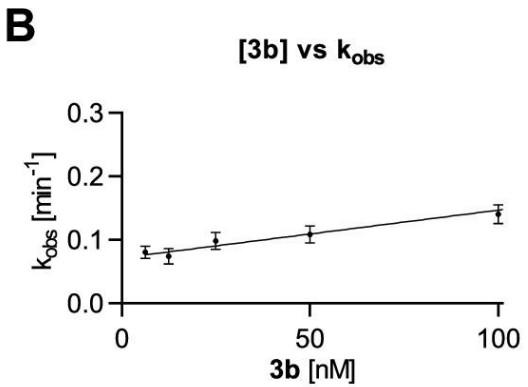
6

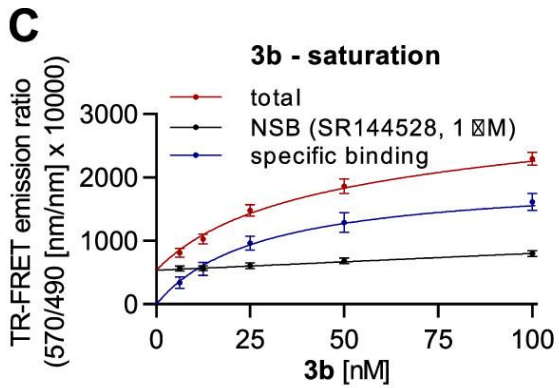
		K_i [nM]				EC_{50} [nM]			
compd	dye	hCB_2R	hCB_1R	mCB_2R	hK_i ratio (CB_1R/CB_2R)	hCB_2R	hCB_1R	mCB_2R	EC_{50} ratio (CB_1R/CB_2R)
2	NBD	4.2	>10 000	n.d.[b]	>2381	n.d.	n.d.	n.d.	n.d.
3a	DY-480XL	99	4031	1986	41	>10 000	>10 000	>10 000	n.d.
3b	DY-480XL	21	2378	1459	113	171 (150)	>10 000	118 (115)	>58
4	Alexa647	2565	>10 000	>10 000	>3.9	25 (109)	2152 (138)	370 (123)	86
5	Alexa488	268	>10 000	1 204	>37	n.d.	n.d.	n.d.	n.d.
6	AttoThio12	4.7	1075	1.1	228	5.6 (74)	>10 000	17 (73)	>1785

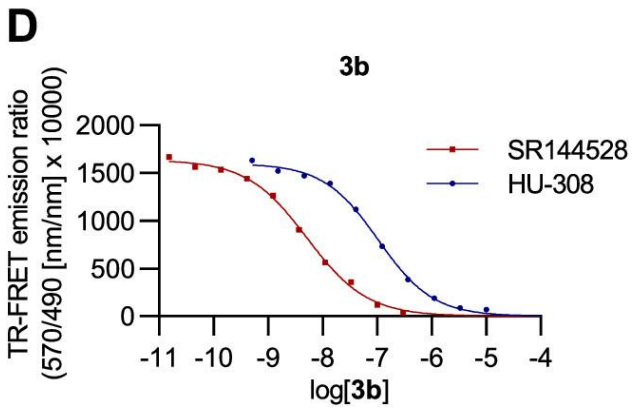
高特异性结合表征

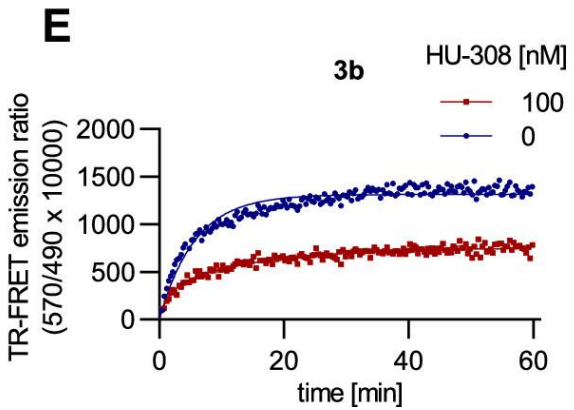
SNAP-Lumi4-Tb
FRET-donor

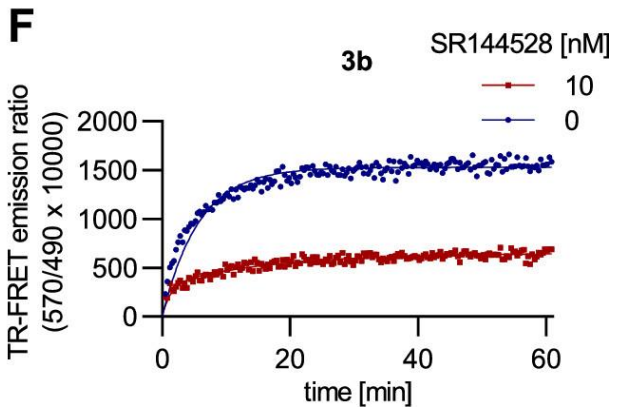






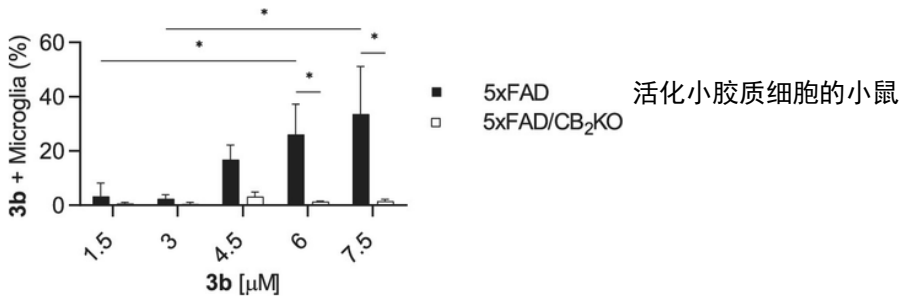




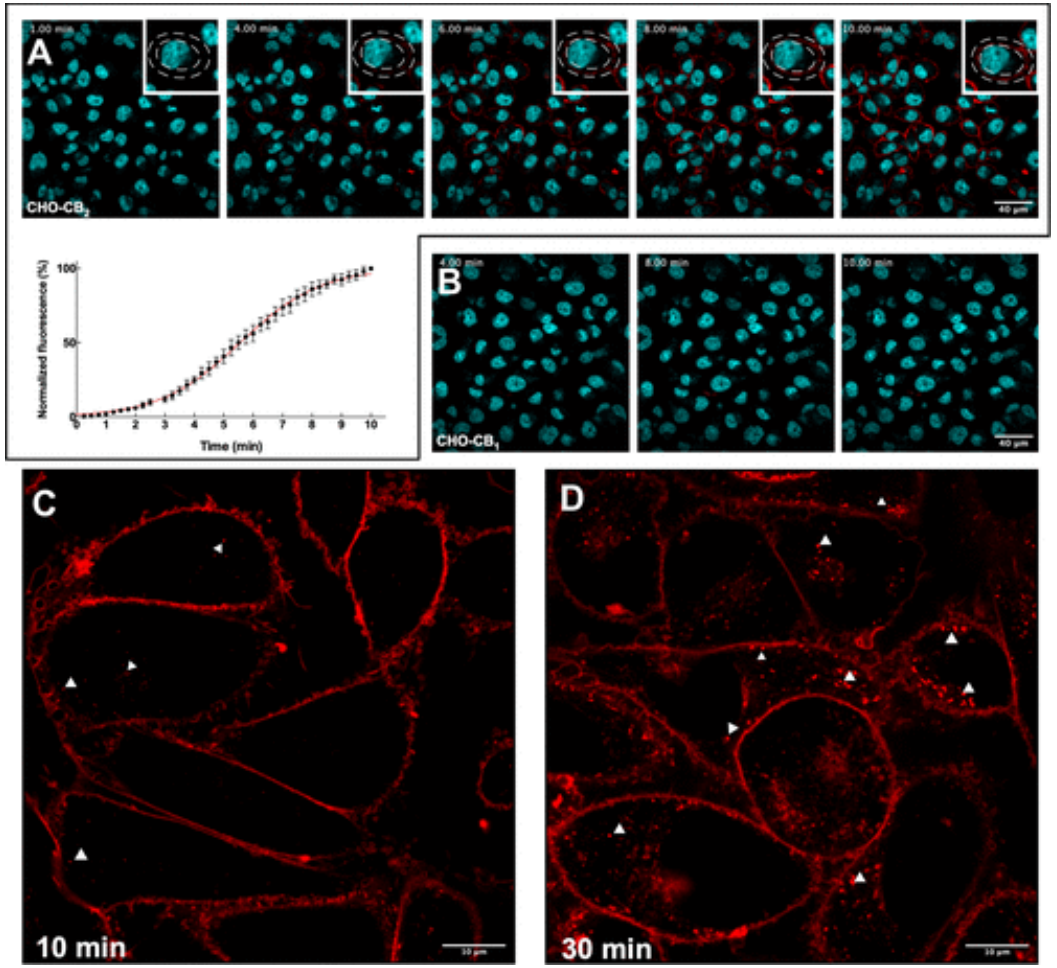


compd	k_{on} [10^6 M^{-1} min^{-1}]	k_{off} [min^{-1}]	kinetic K_d [nM]	saturation K_d [nM]
3b	1.2 ± 0.1	0.04 ± 0.01	35 ± 7	36 ± 4
6	1.9 ± 0.5	0.03 ± 0.05	18 ± 3	13 ± 2

[a]Data are presented as mean ± SEM, N = 3–5.

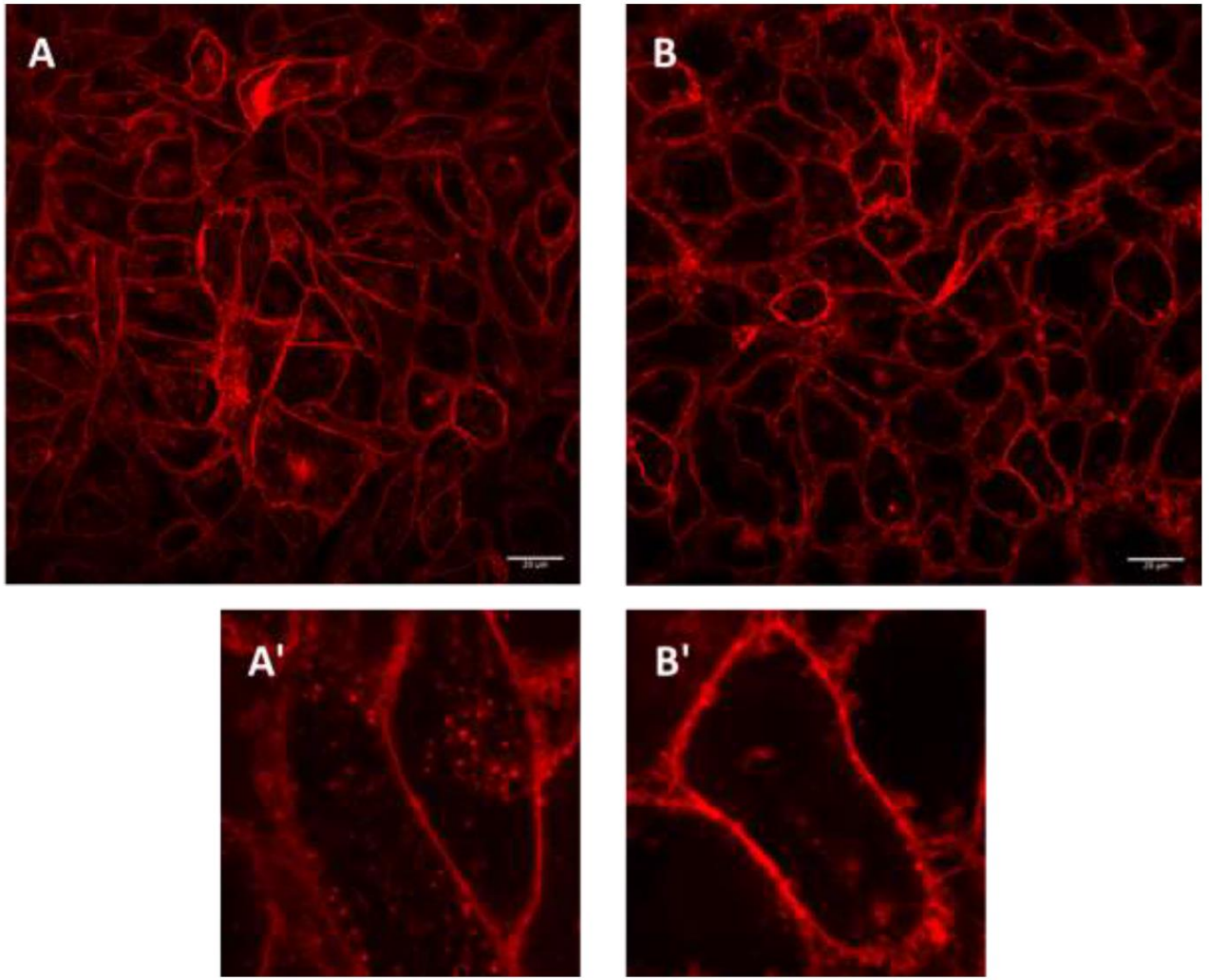


大麻素2型受体实时观察



Confocal fluorescence microscopy with CHO cells. Different frames from time-lapse confocal microscopy of cells co-stained with 3b (red) and Hoechst 33342 (cyan, nucleus counter stain). (A) CHO-hCB2R cells incubated with 0.2 μM 3b at 1, 4, 6, 8, and 10 min; at each time point, a region of interest (white strip-like curve shown in the insets) was drawn around the plasma membrane of cells (N = 6 for each field). The changes in normalized fluorescence intensity were estimated over time (Fiji software), leading to an association curve. The data on the curve represent the mean ± SD of at least three independent experiments. (B) CHO-hCB1R cells incubated with 0.2 μM 3b at 4, 8, and 10 min. See SI Videos 1 and 2 for animated views. Airyscan high-resolution imaging of hCB2R-overexpressing CHO cells incubated for either (C) 10 min or (D) 30 min with 0.2 μM 3b. Cells were optically sectioned using confocal laser-scanning microscopy equipped with an Airyscan detector. (C) In the first 10 min, 3b staining was localized in the plasma membranes of CHO-hCB2R cells. (D) After 30 min, brighter and increased number of vesicles, reminiscent of early endosomes, appeared below the plasma membrane and within the cytosol. Images are representative of three independent experiments.

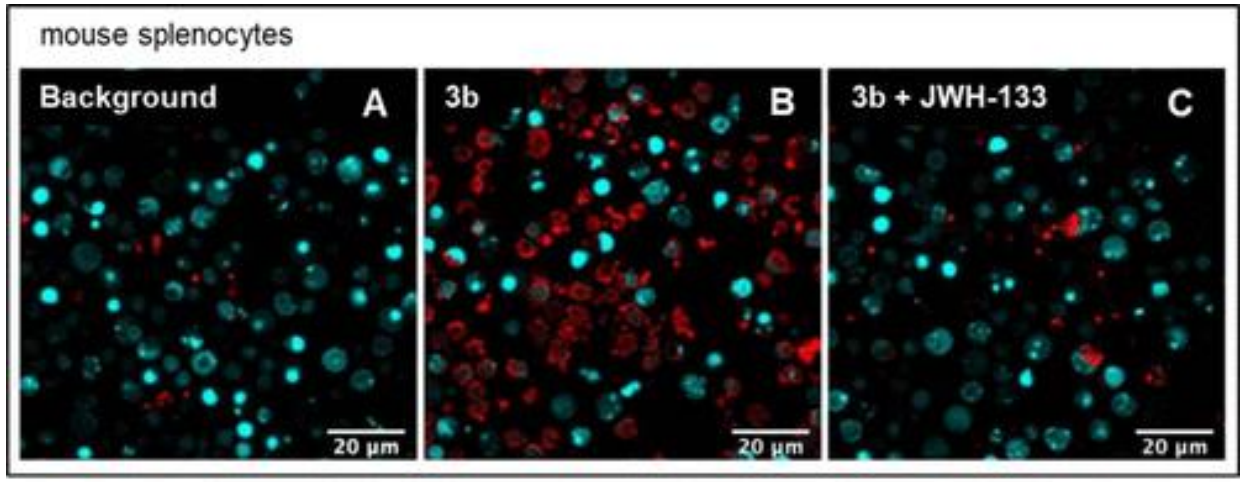
通过限制内吞证实其进入细胞形式



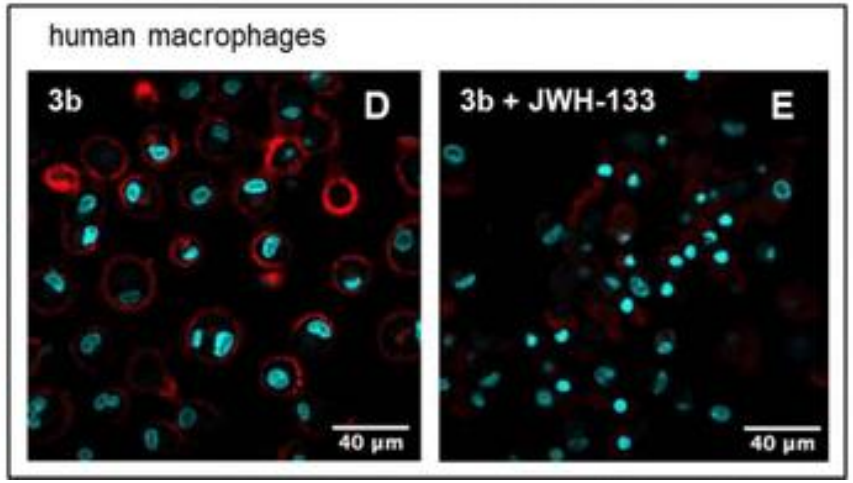
A) Airyscan high-resolution imaging of CHO cells overexpressing hCB2R incubated with 0.2 M **3b**. B) Co-incubation with endocytosis inhibitors reduces level of fluorescent probe internalization. Pictures showed cells incubated for 1 h with **3b** in the absence (A and A') and in the presence (B and B') of endocytosis inhibitors (i.e. 400 mM sucrose and 5 g/mL filipin). In the presence of sucrose and filipin, the reduction of hCB2R receptor endocytosis was almost complete, as shown by the robust decrease in punctate vesicles within the cells (see magnified regions shown in panels A'/B').

原位观察细胞膜大麻素2型受体

小鼠脾细胞



人巨噬细胞



Confocal microscopy with primary cells expressing CB2R endogenously. Confocal microscopy frames that show labeling of CB2R with 3b at 10 min in murine splenocytes and human macrophages. Murine splenocytes incubated for 10 min with (A) vehicle, (B) 0.4 µM 3b alone, or (C) 0.4 µM 3b in the presence of 4 µM known CB2R agonist JWH-133 as competitor. Human macrophages for 10 min with (D) 0.6 µM 3b alone or (E) 0.6 µM 3b in the presence of 4 µM JWH-133. Pretreatment with Hoechst 33342 (cyan) effected nuclear counter-staining.