Literature Report 2

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DNA-based fluorescent probes of NOS2 activity in live brains

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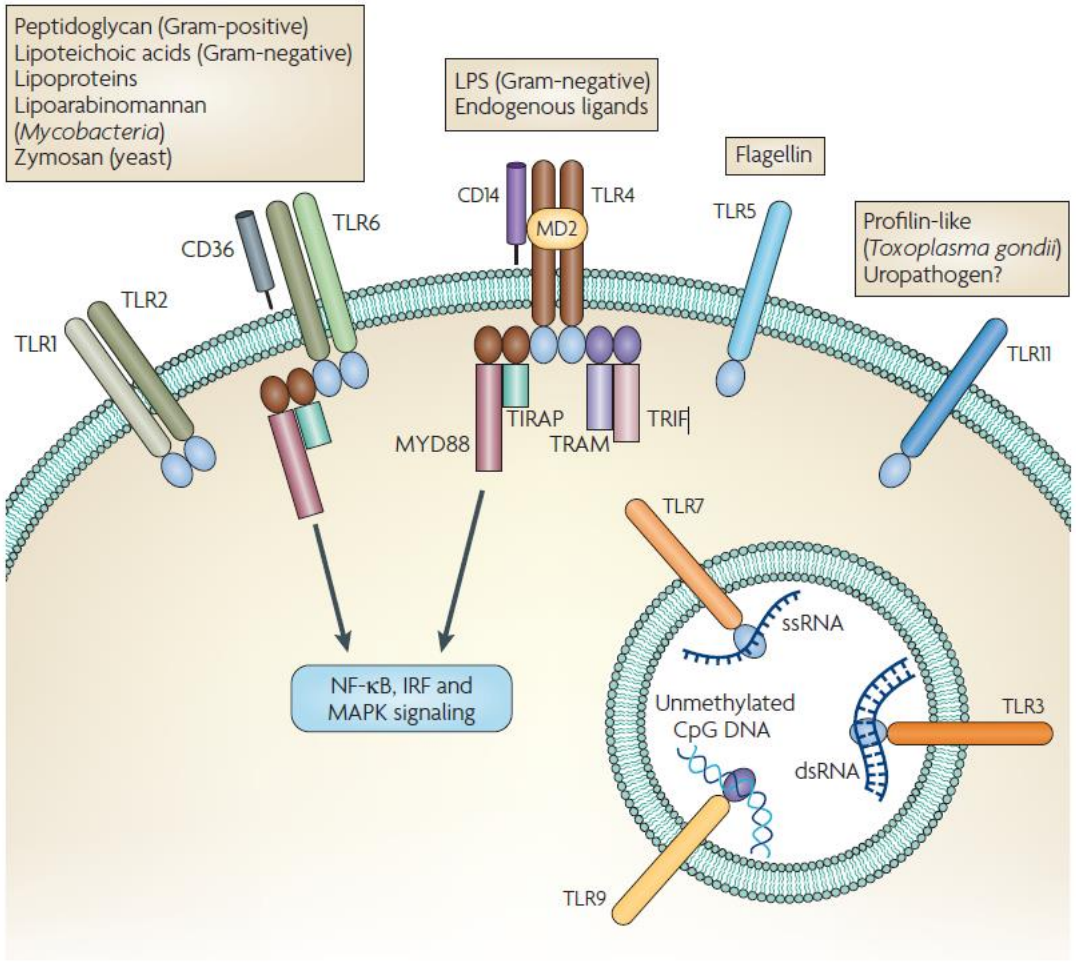
Professor, University of Chicago

Research Interests:

1. Nucleic acid-based Molecular Devices
2. Quantitative Functional Imaging
3. Expanding the technology in living systems
4. Cargo Delivery and Long Duration Live Imaging

Background



Nature Reviews Cancer, 2009, 9, 57

清道夫受体（Scavenger receptors）

一类模式识别受体（PRR），存在于吞噬细胞（如小胶质细胞，巨噬细胞和树突细胞）表面，具有结合聚阴离子配体的能力。

模式识别受体TLR（Toll-like receptors, TLR）

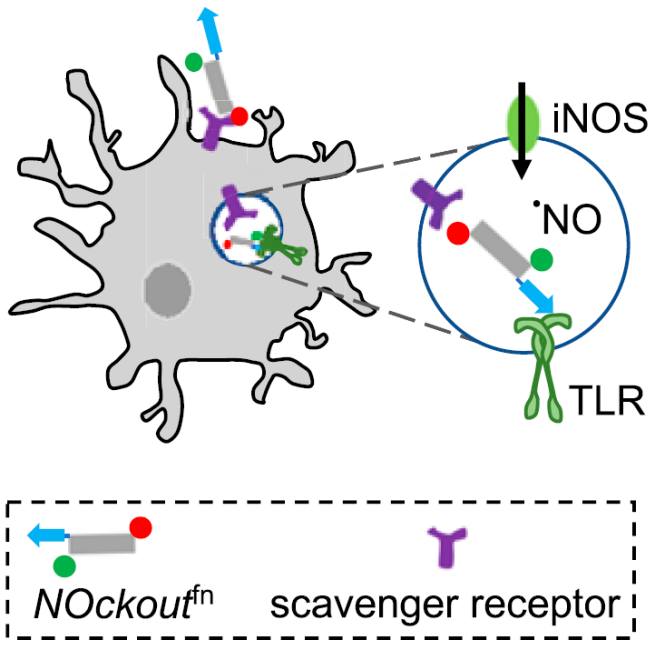
一类模式识别受体（PRR），识别脂质或蛋白质配体的TLR通常位于细胞表面，结合核酸基序的TLR通常位于囊泡上。

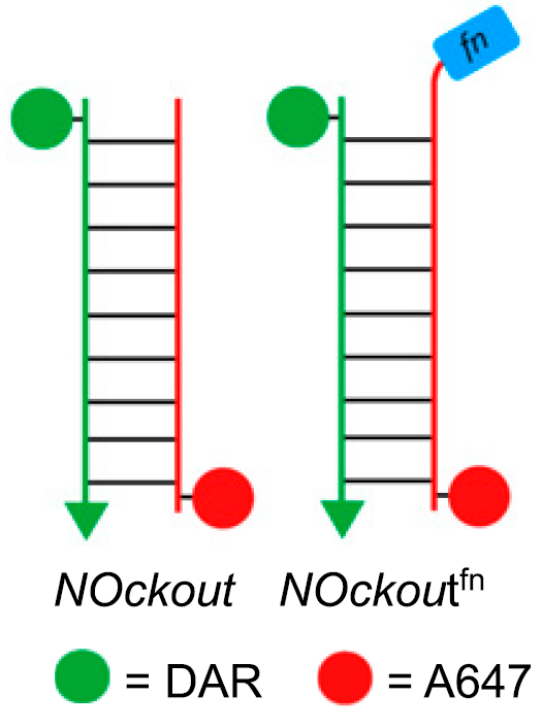
PAMP

被模式识别受体识别的病原体分子中的保守结构基序。TLR识别PAMP后，启动下游的免疫反应，触发NOS2活性，产生NO。

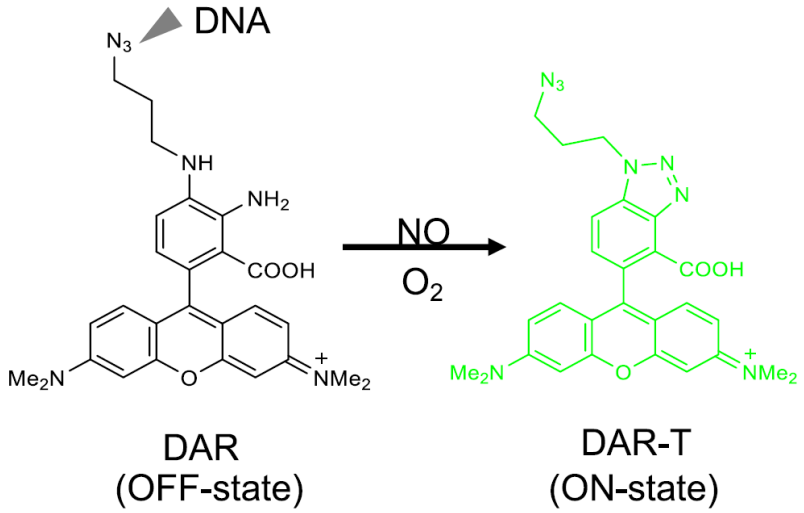
Nat. Rev. Immunol. 2013, 621
The Neuroscience of Dementia, 2020, 2, 57

Probe design





fn : tttccatgacgttcctgacgtt (*NOckout*^{mCpG})
tttccatgagcttcctgagctt (*NOckout*^{mGpC})
tttcgtcgttgtcgttttgtcgtt (*NOckout*^{zCpG})
tttgctgcttgtgcttttgtgctt (*NOckout*^{zGpC})
tttggacggaaagaccccgugg (*NOckout*^{iRNA})
tttggacgggaagaccccgugg (*NOckout*^{RNA})

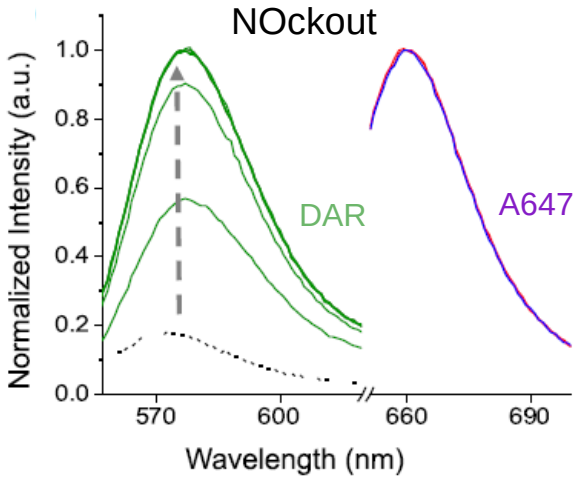


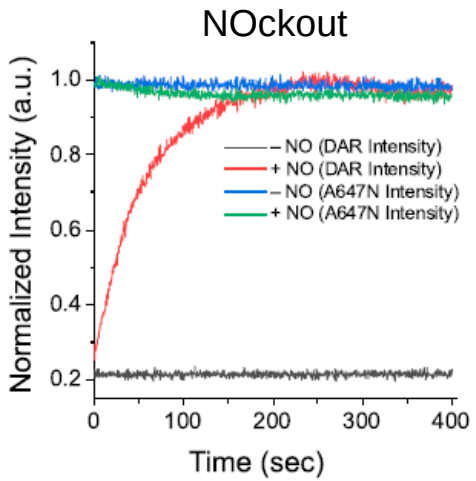
iNOS (NOS2)：一种NO合成酶，主要在免疫细胞中表达

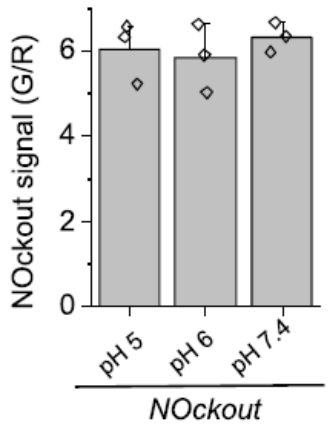
Nockout^{fn} 探针的四个功能区

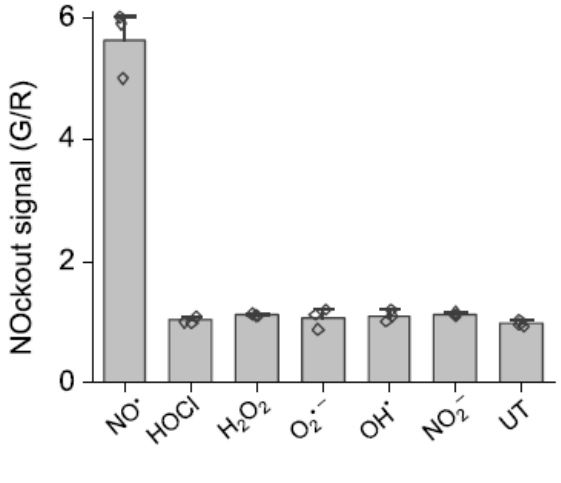
1. DAR：对NO敏感的染料
2. A647：商业化染料ATTO647N，对ROS、RNS化学惰性
3. dsDNA：将探针靶向巨噬细胞
4. fn：免疫原性核苷酸硫代磷酸酯序列mCPG, zCPG, 来自细菌的免疫刺激性核糖体RNA 序列iRNA
   对照序列（非免疫刺激性）mGPC, zGPC, RNA

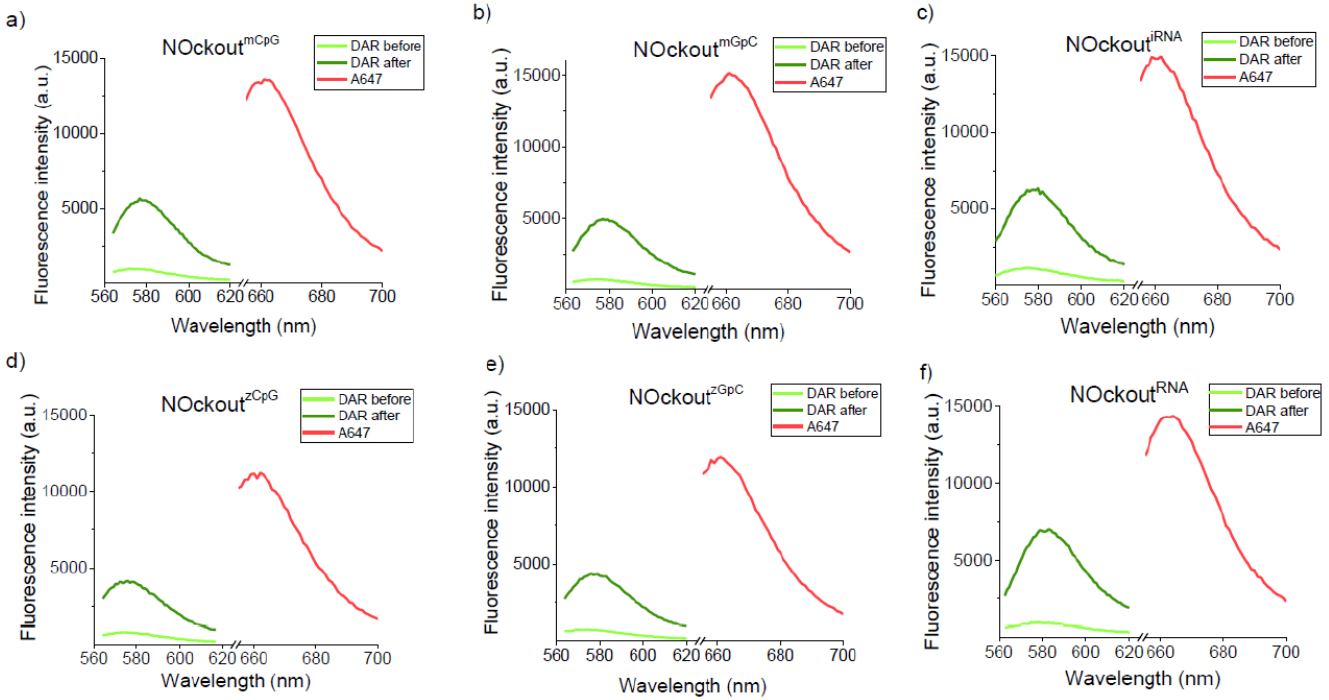
Characterizations



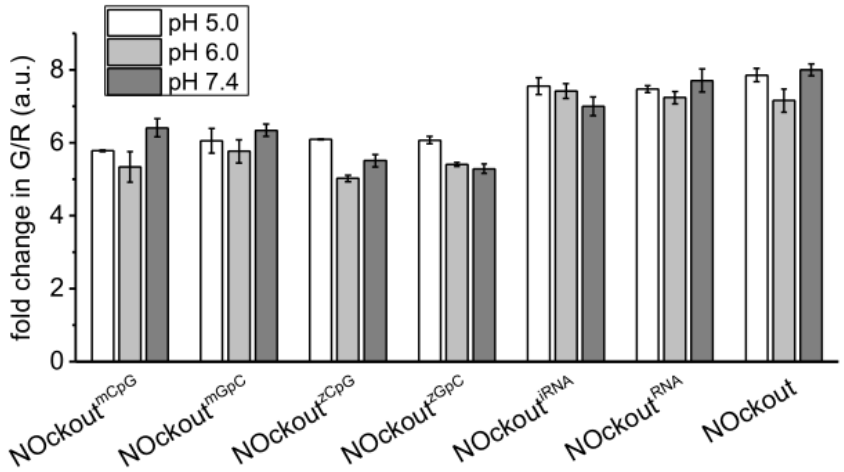




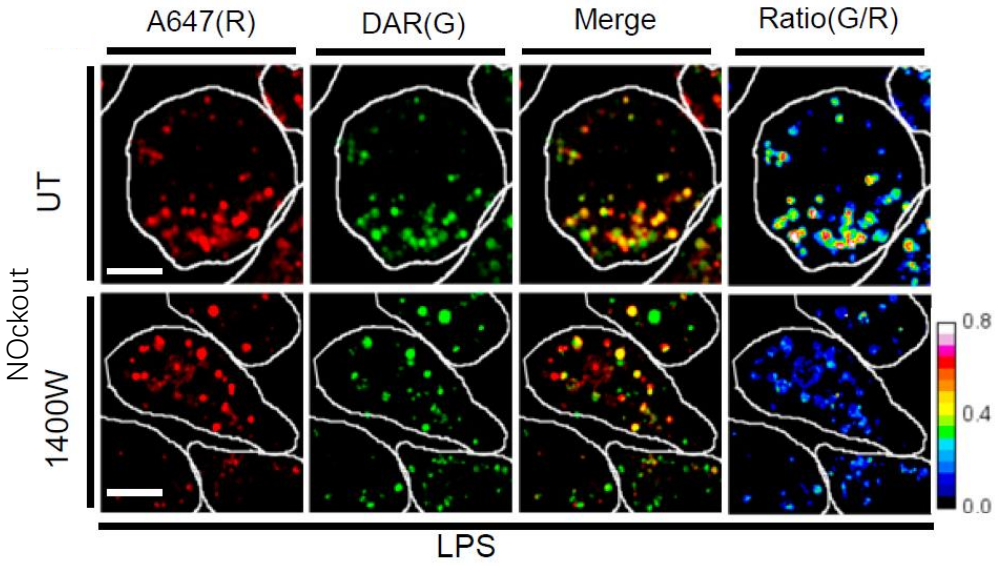


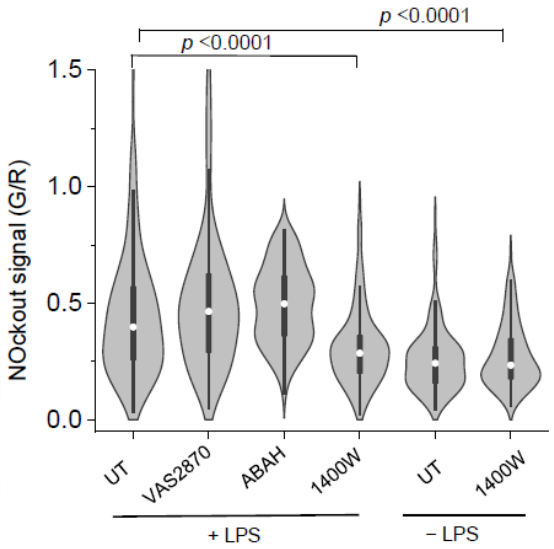


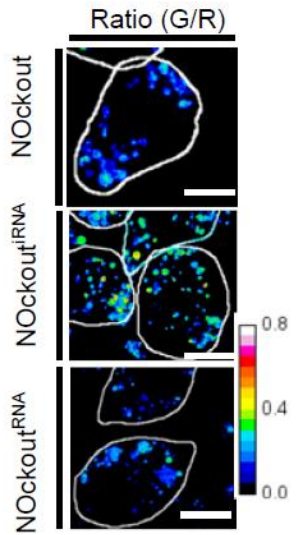
G/R：DAR/A647荧光强度比率

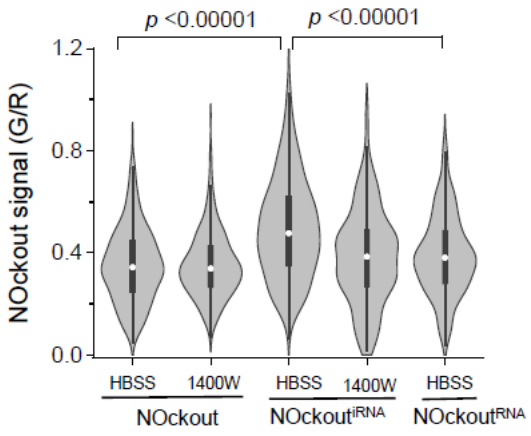


Nockout^{fn} 激活吞噬小体中NOS2的活性

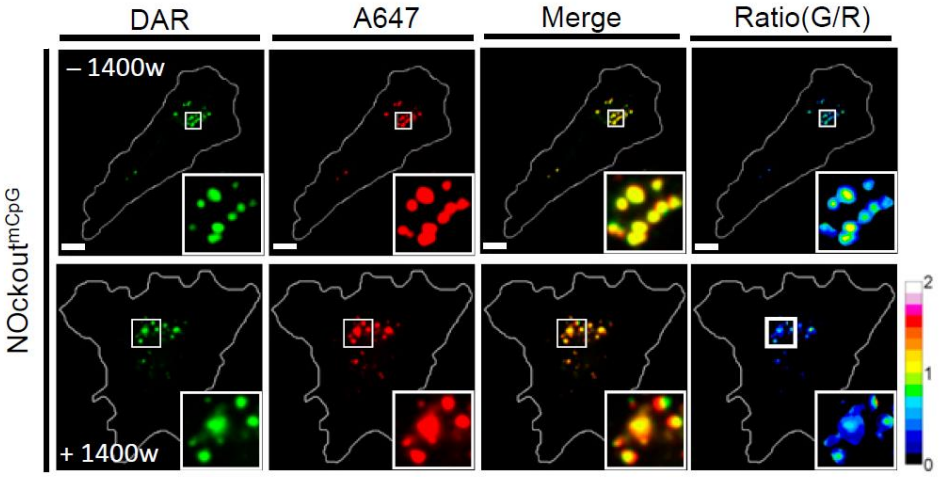


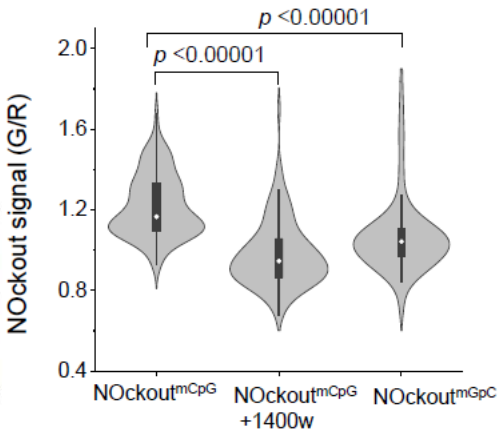


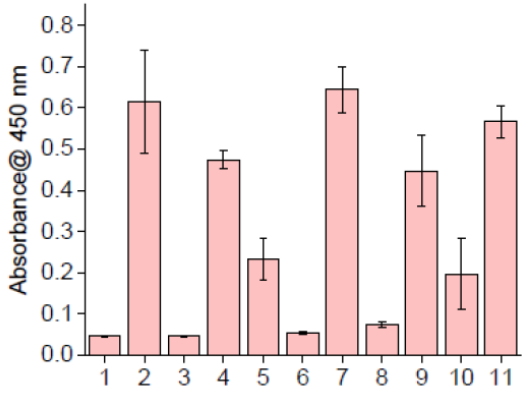




LPS：诱导NOS2，TNF-α表达



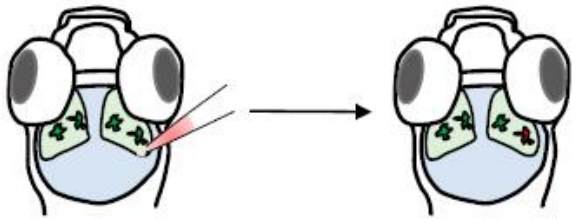


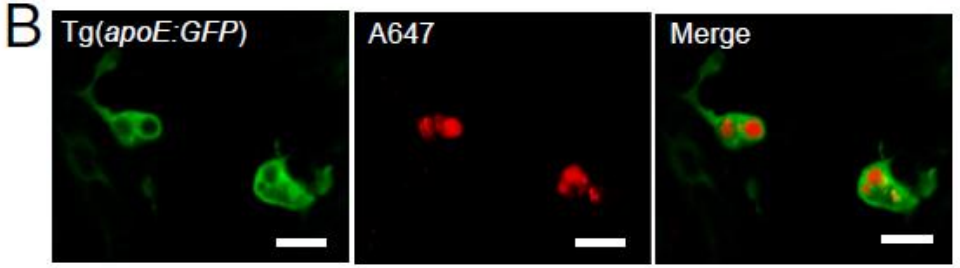


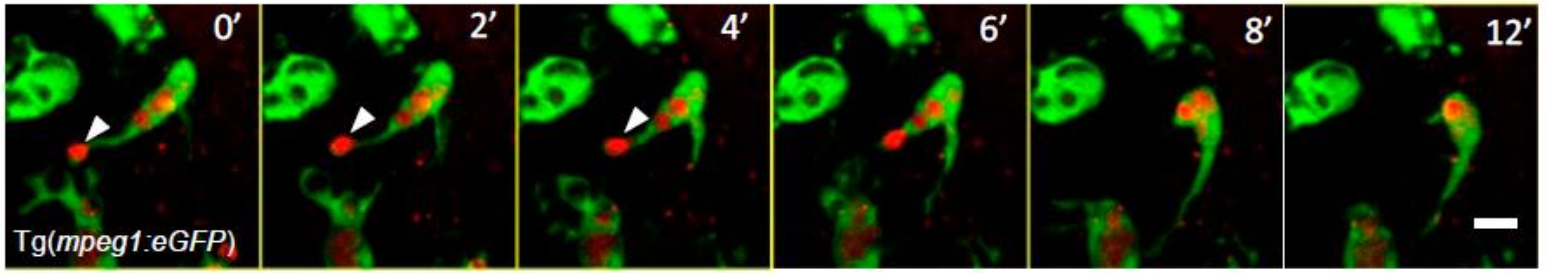
TNF-α quantification

	Sample	TNFα (*pg/ml*)	Std.Dev
1	*Blank*	0.44	0.092
2	*mCpG*	237.29	96.01
3	*mGpC*	1.01	0.07
4	*iRNA*	176.45	17.06
5	*RNA*	72.07	32.22
6	*NOckout*	1.82	0.03
7	*NOckout^{mCpG}*	250.28	112.22
8	*NOckout^{mGpC}*	5.70	3.34
9	*NOckout^{iRNA}*	163.81	68.32
10	*NOckout^{RNA}*	61.93	13.51
11	*LPS (350 ng/ml)*	233.22	32.26

NOS2 activity detection in living brains

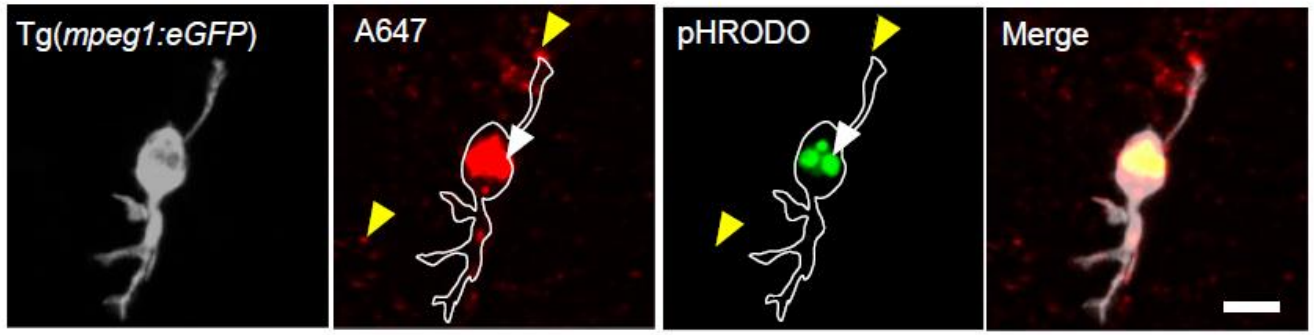


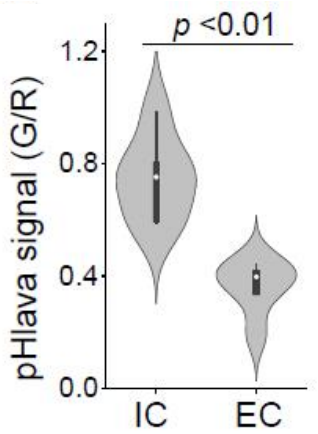


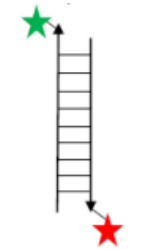


Nockout^{UN}

不含DAR和fn的Nockout探针







★ Phrodo

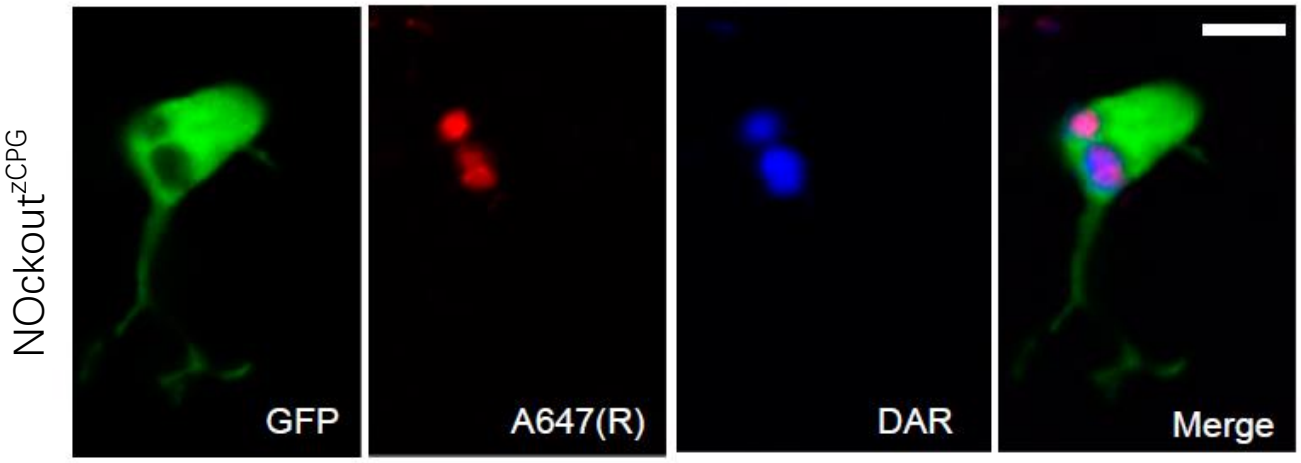
pH敏感的染料
质子化导致高荧光

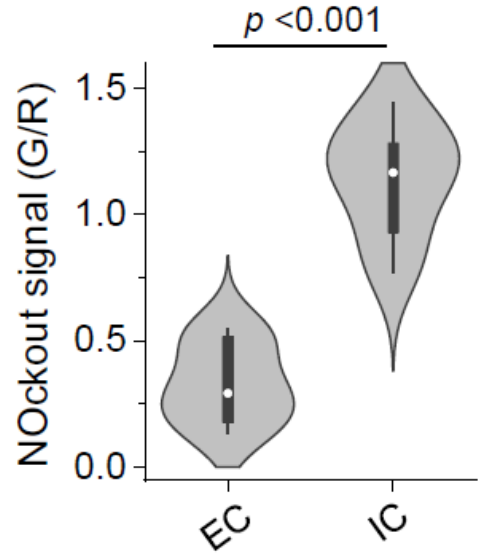
★ A647

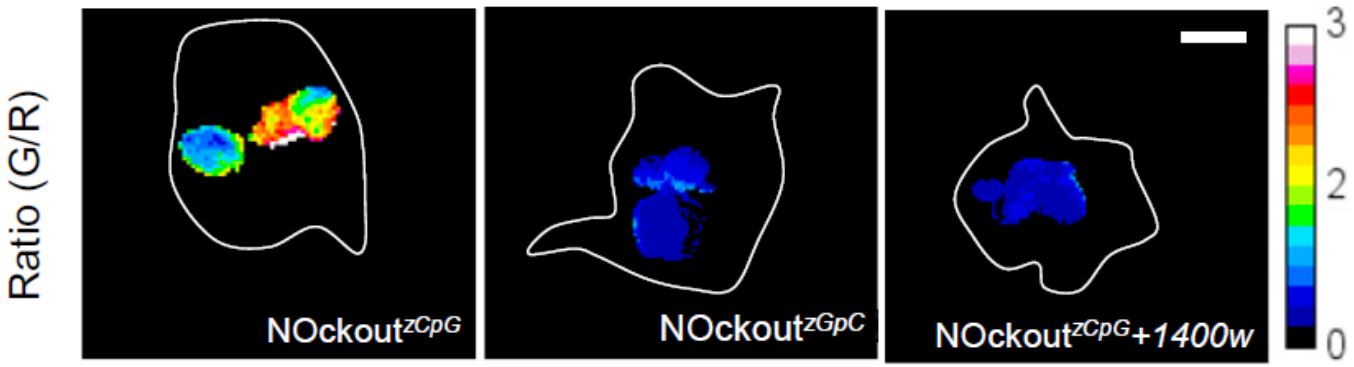
IC：吞噬体

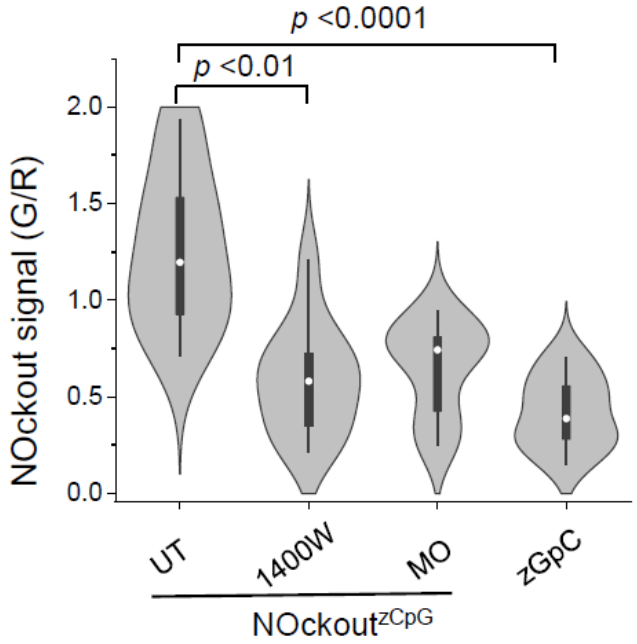
EC：细胞内除吞噬体外的环境

NOS2 activity detection in living brains









探究斑马鱼对细菌性病原体响应的TLR类型

来自金黄色葡萄球菌和大肠杆菌的iRNA激活小鼠的TLR-13产生NO

这种RNA序列与许多感染斑马鱼的天然水生病原体序列相似