Lecture 4 - Biased agonism

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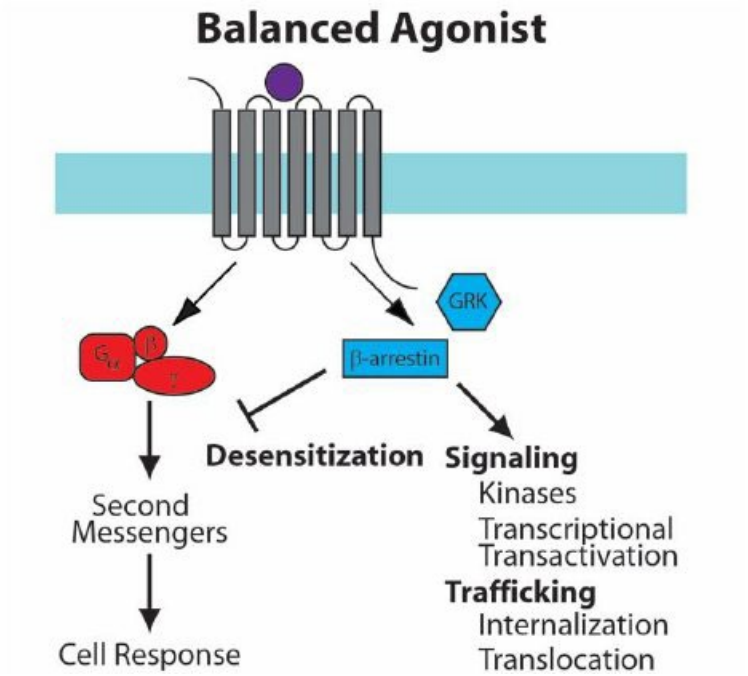
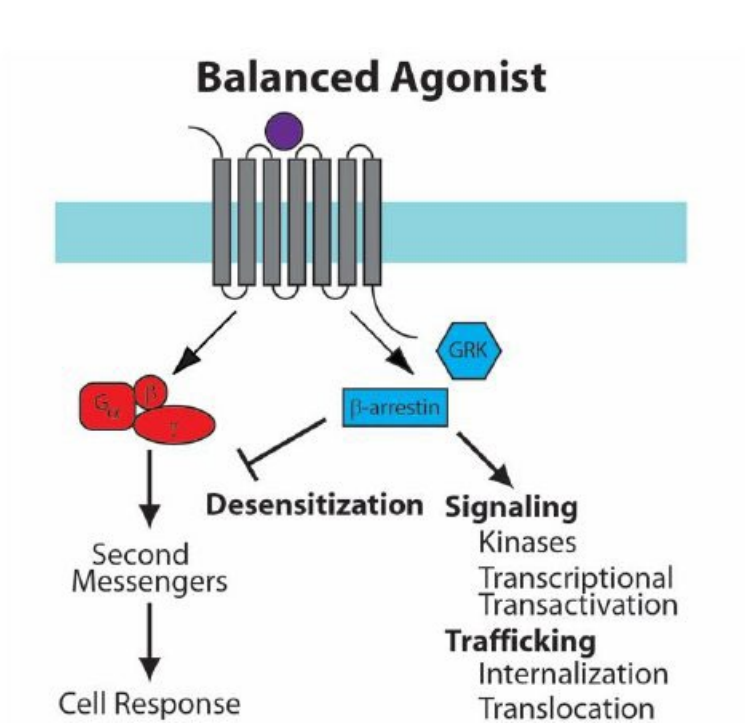
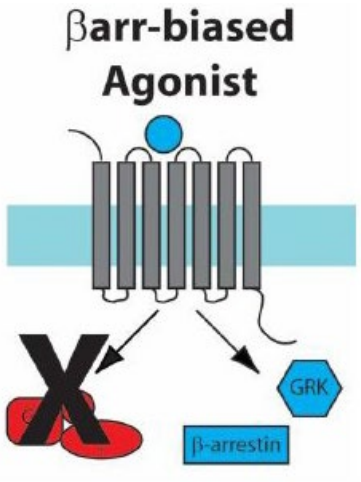
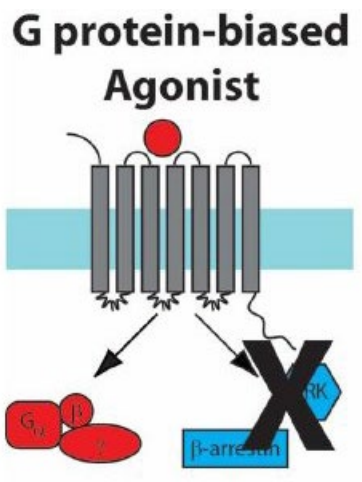


Smith and Rajagopal, *Journal of Biological Chemistry* 2016

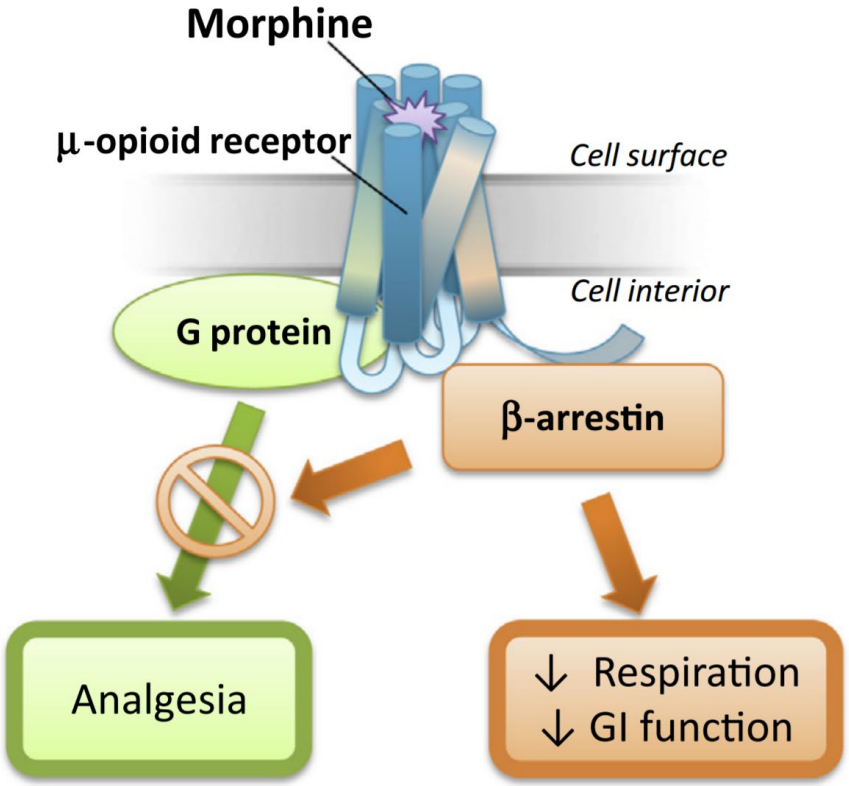






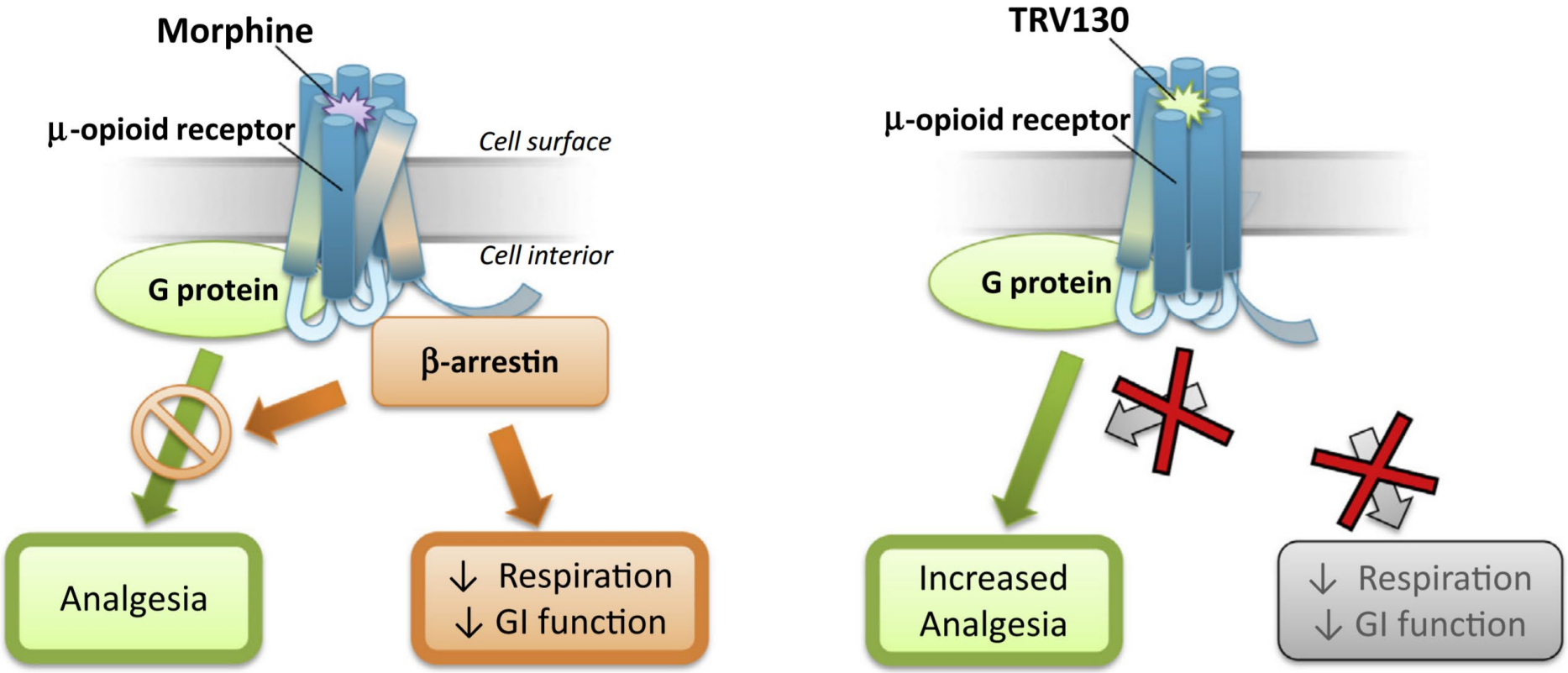
Smith and Rajagopal, *Journal of Biological Chemistry* 2016

What Effects can a Biased Agonist have on Physiology?



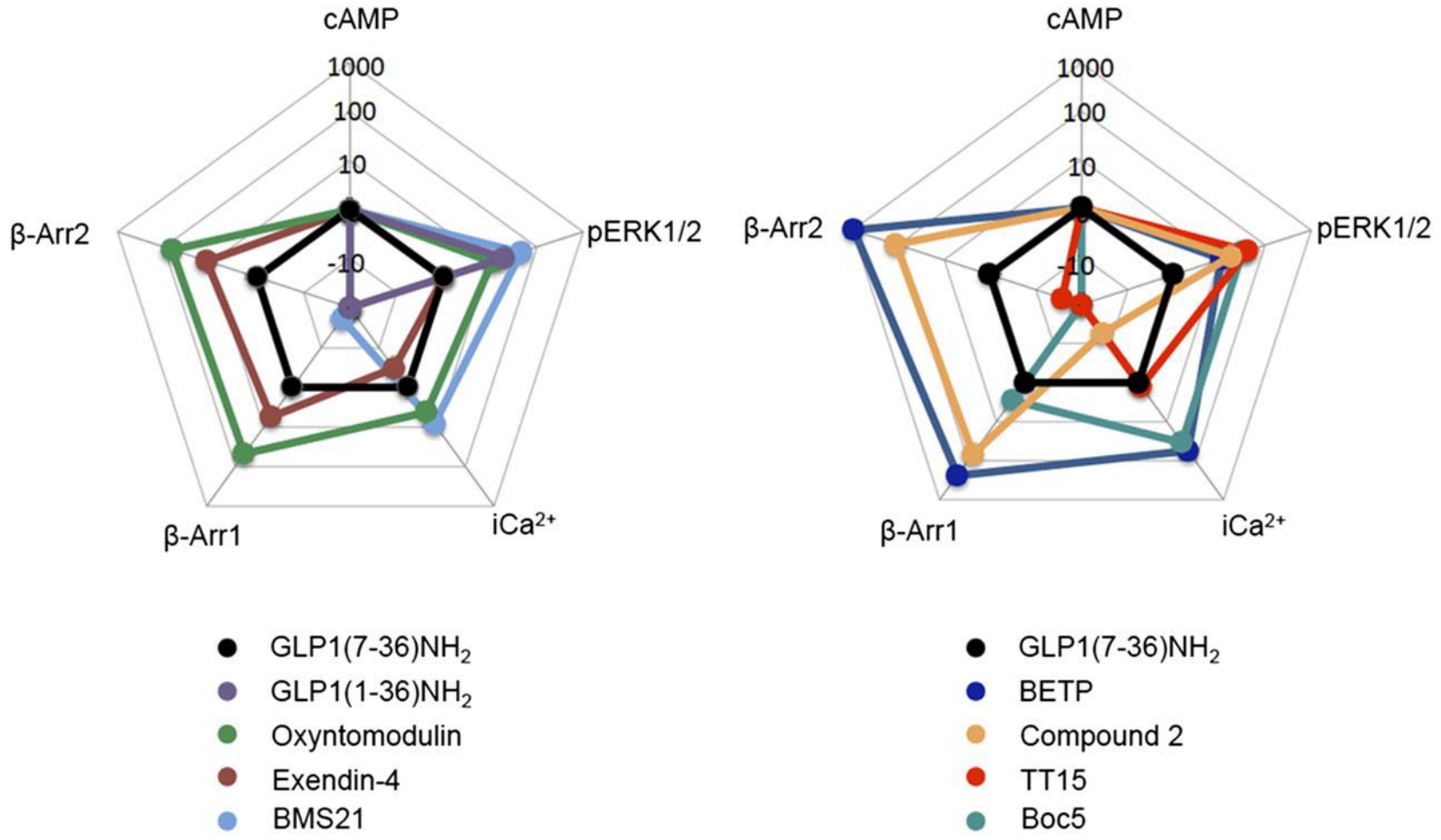
D.G. Soergel et al. / PAIN 155 (2014) 1829–1835

What Effects can a Biased Agonist have on Physiology?



D.G. Soergel et al. / PAIN 155 (2014) 1829–1835

Pluridimensional efficacy and bias at GPCRs



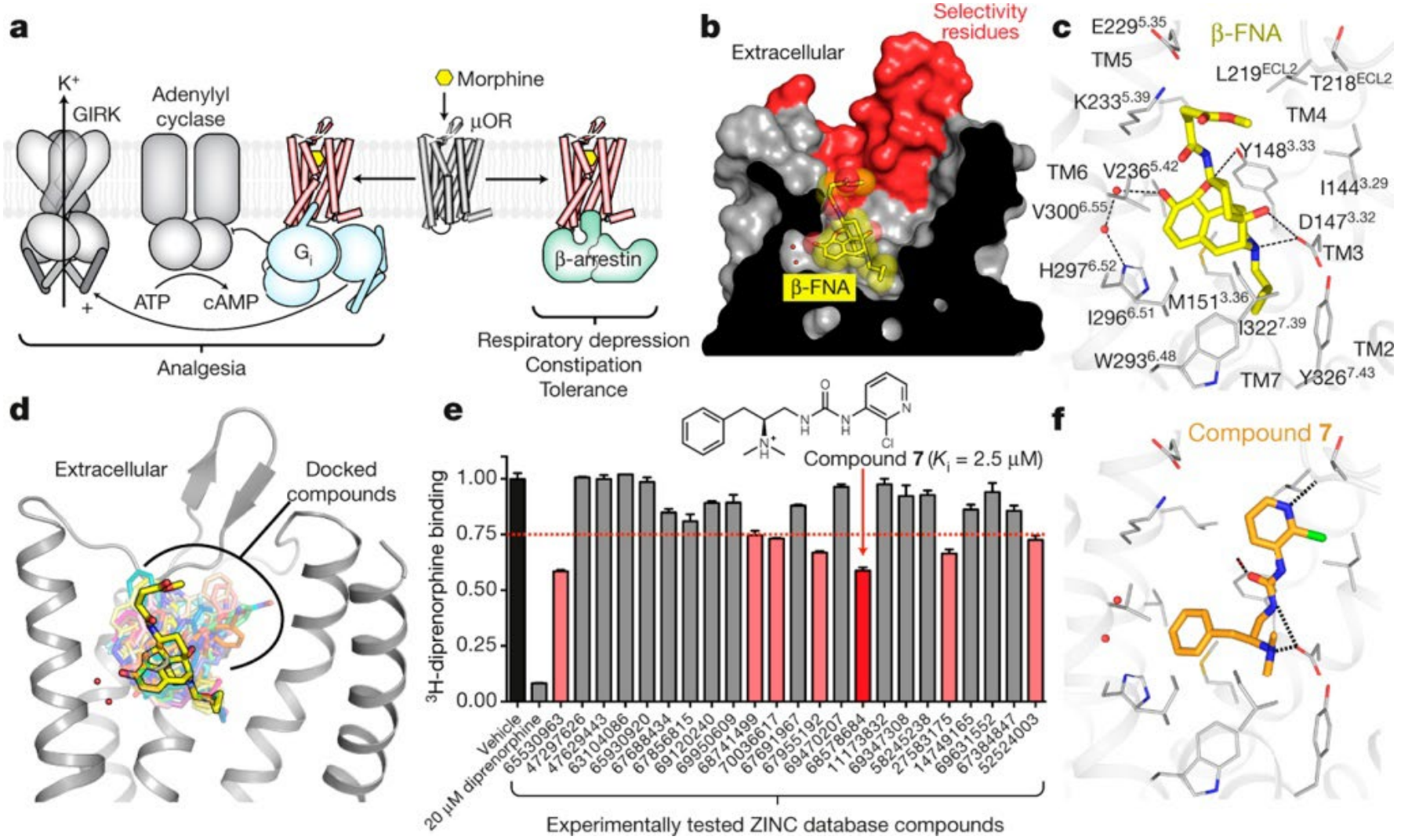
Chris de Graaf et al. Pharmacol Rev 2016;68:954-1013

ASPET | PHARMACOLOGICAL REVIEWS

Structure-based discovery of opioid analgesics with reduced side effects

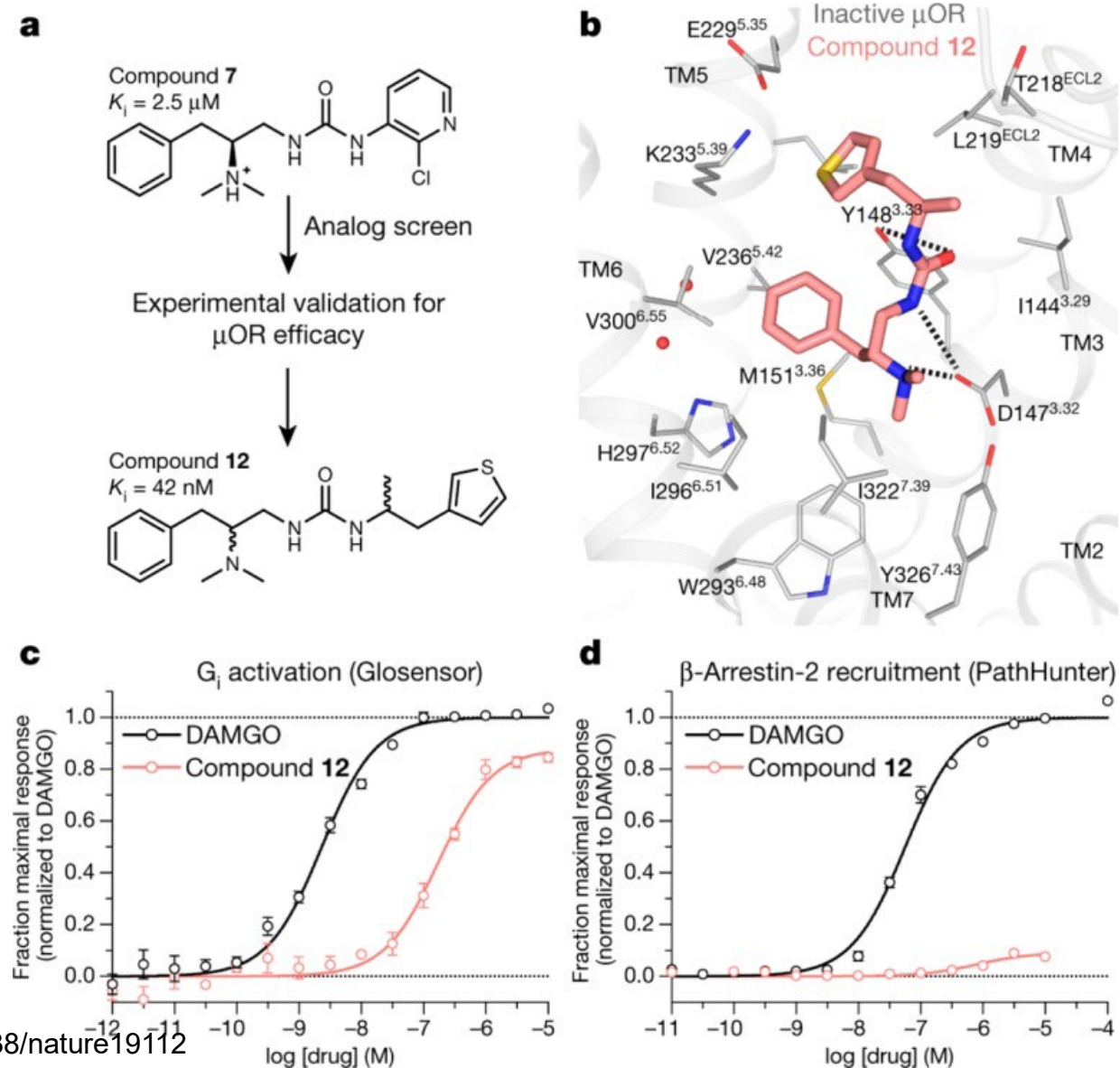
Nature volume 537, pages185–190 (2016)

Structure based ligand discovery for the μOR



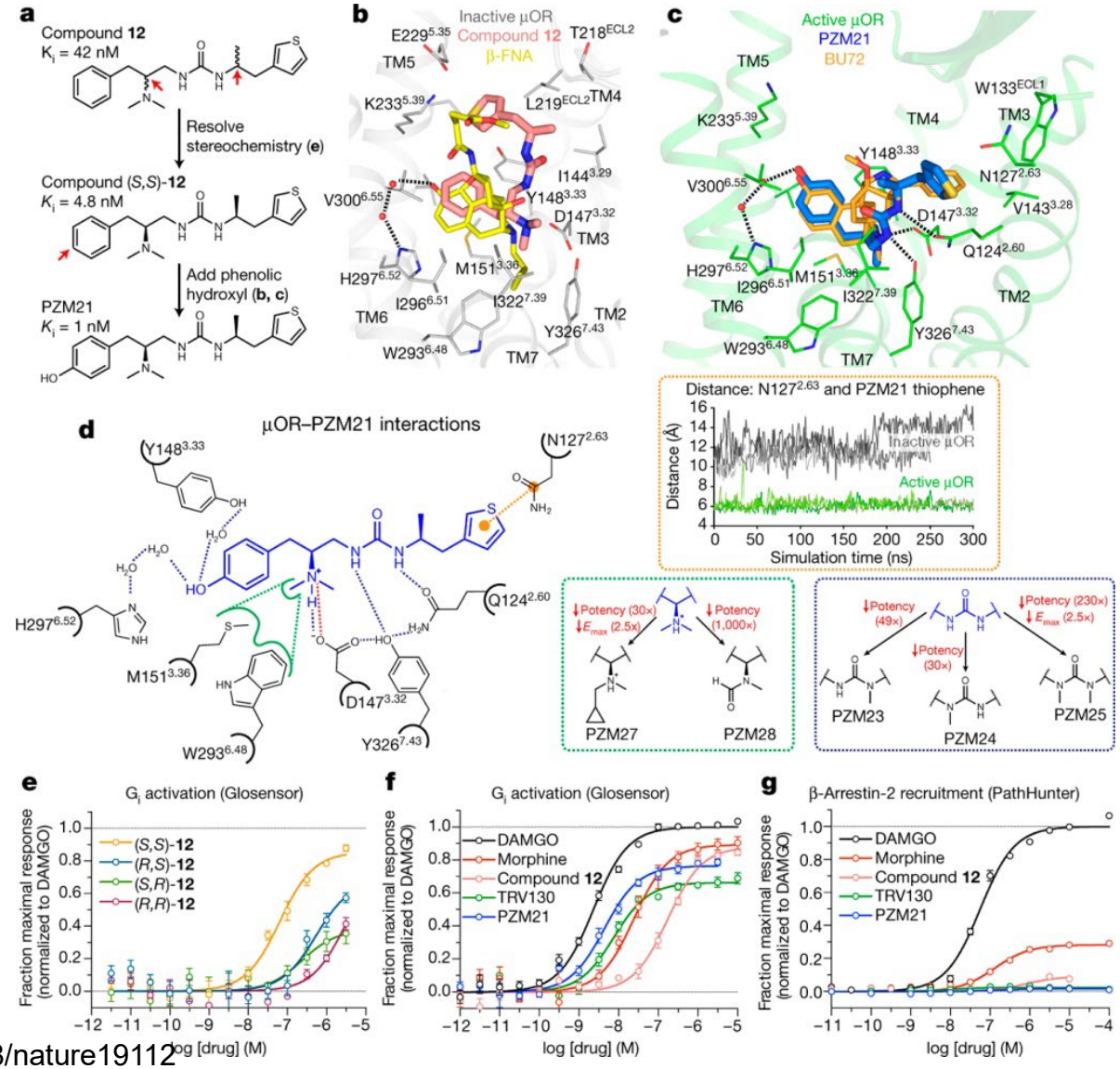
A Manglik *et al. Nature* 1–6 (2016) doi:10.1038/nature19112

Discovery of a novel $G_{i/o}$-biased μOR agonist



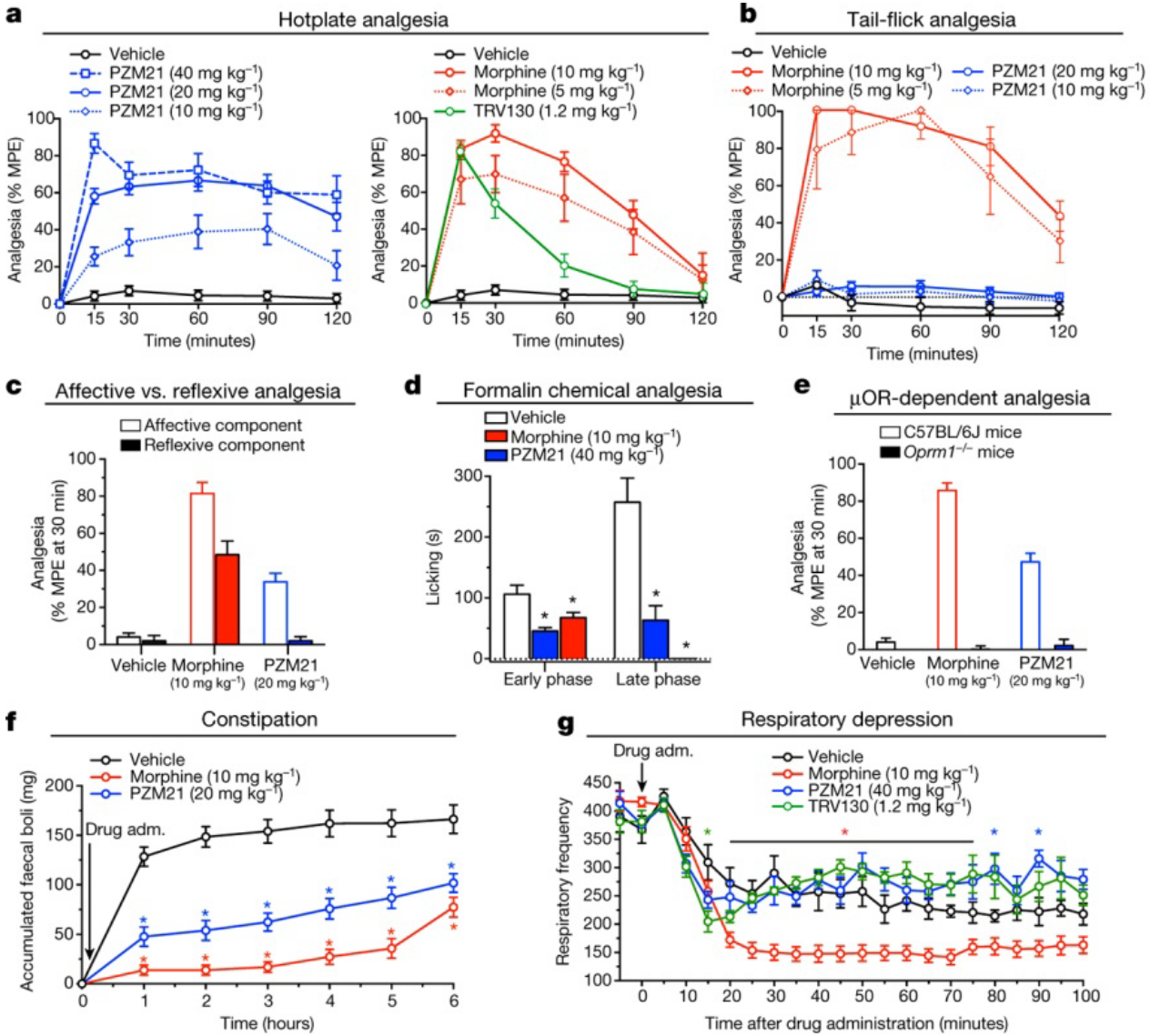
A Manglik *et al. Nature* 1–6 (2016) doi:10.1038/nature19112

Structure-guided optimization towards a potent biased μOR agonist



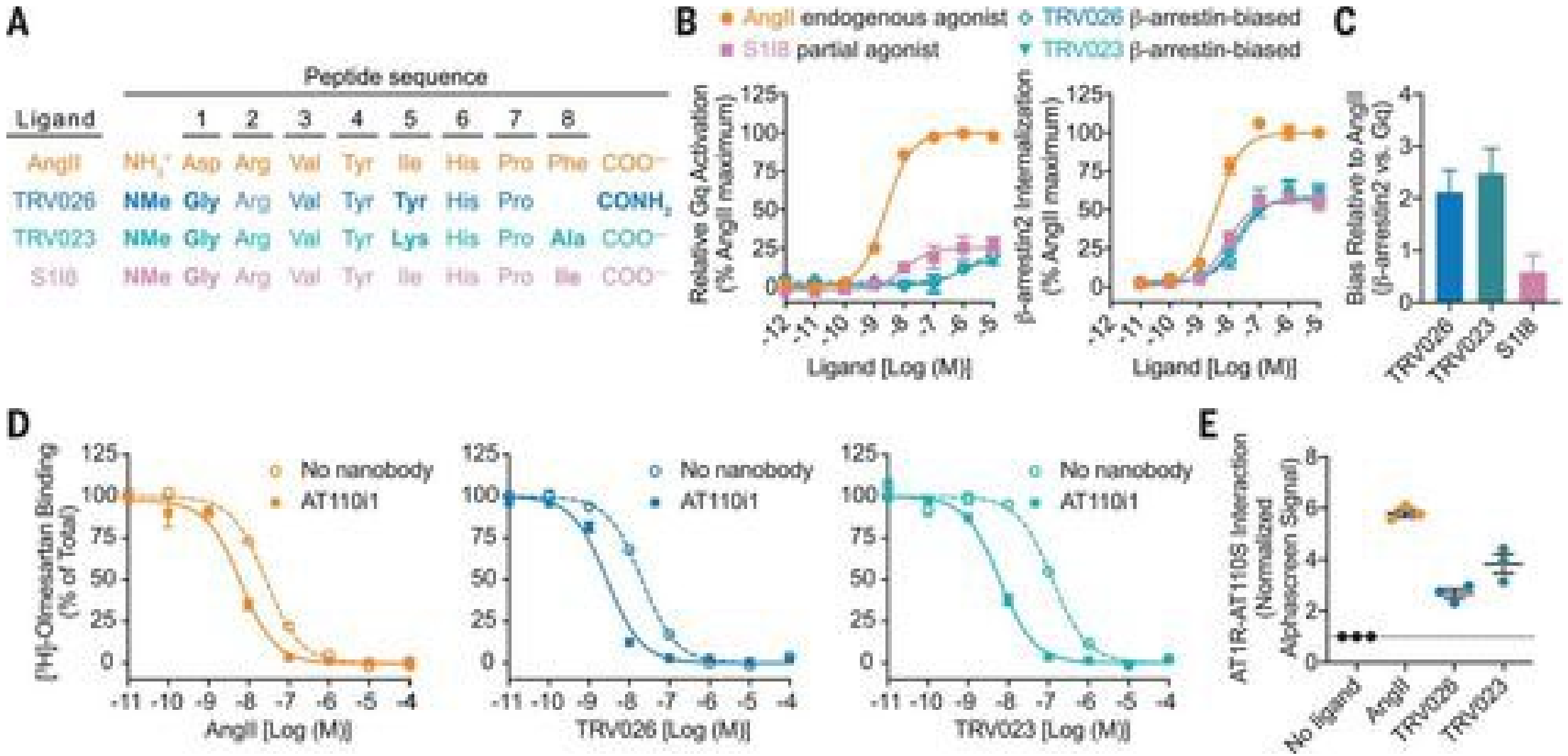
A Manglik *et al. Nature* 1–6 (2016) doi:10.1038/nature19112

PZM21 is an analgesic with reduced on-target liabilities

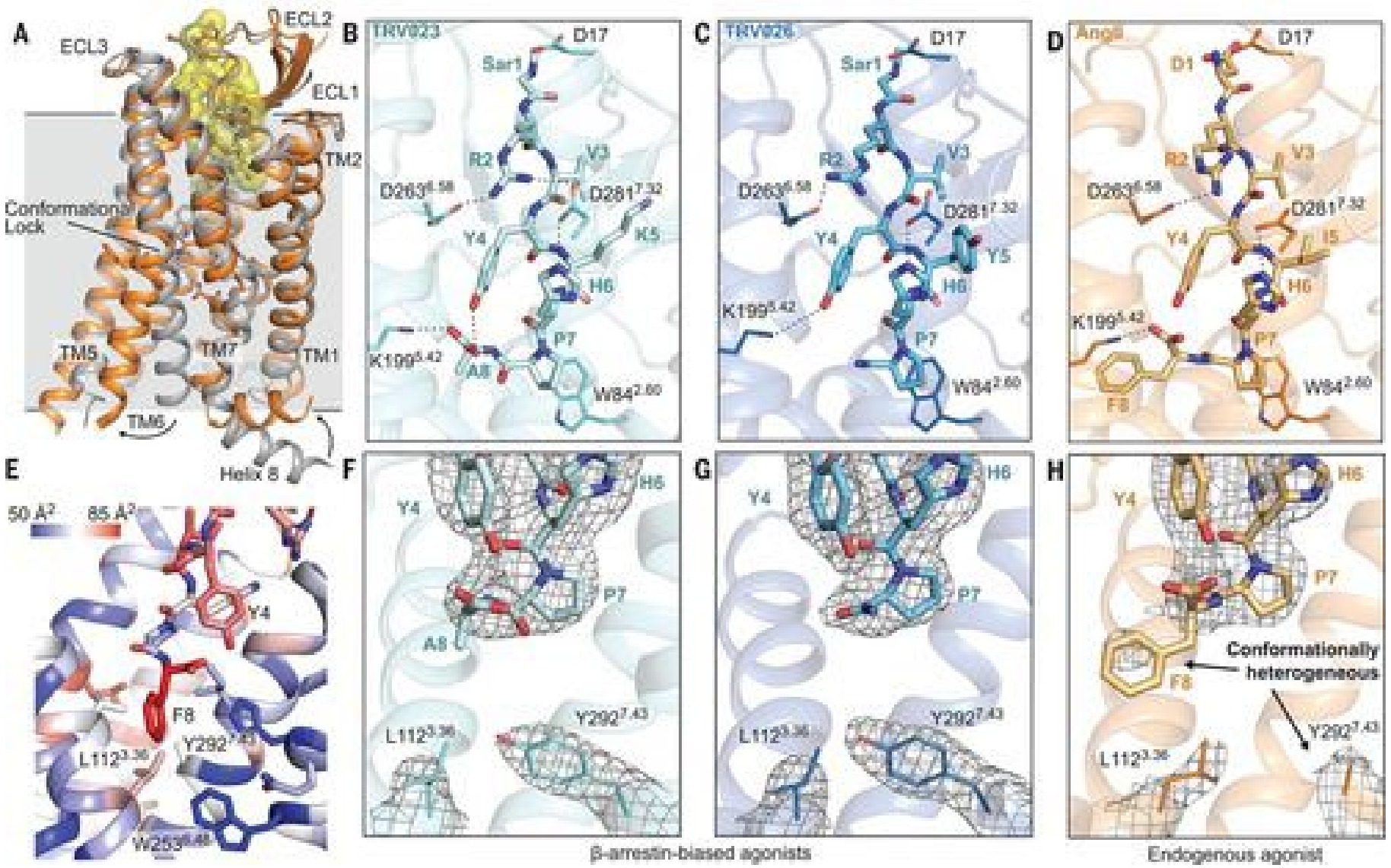


A Manglik *et al. Nature* 1–6 (2016) doi:10.1038/nature19112

Structural Basis of GPCR Biased Agonism

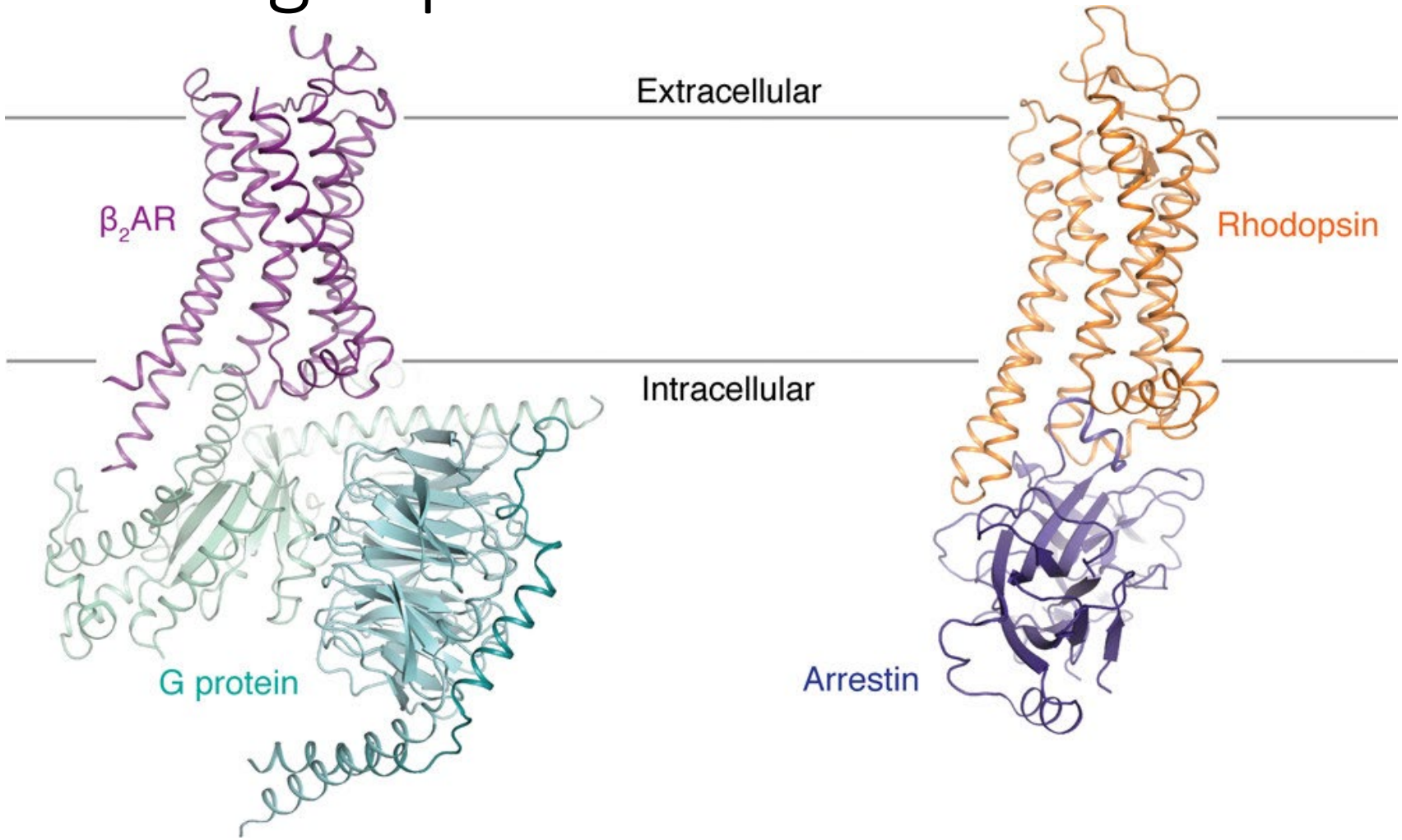


Angiotensin and biased analogs induce structurally distinct active conformations within a GPCR, Volume: 367, Issue: 6480, Pages: 888-892, DOI: (10.1126/science.aay9813)



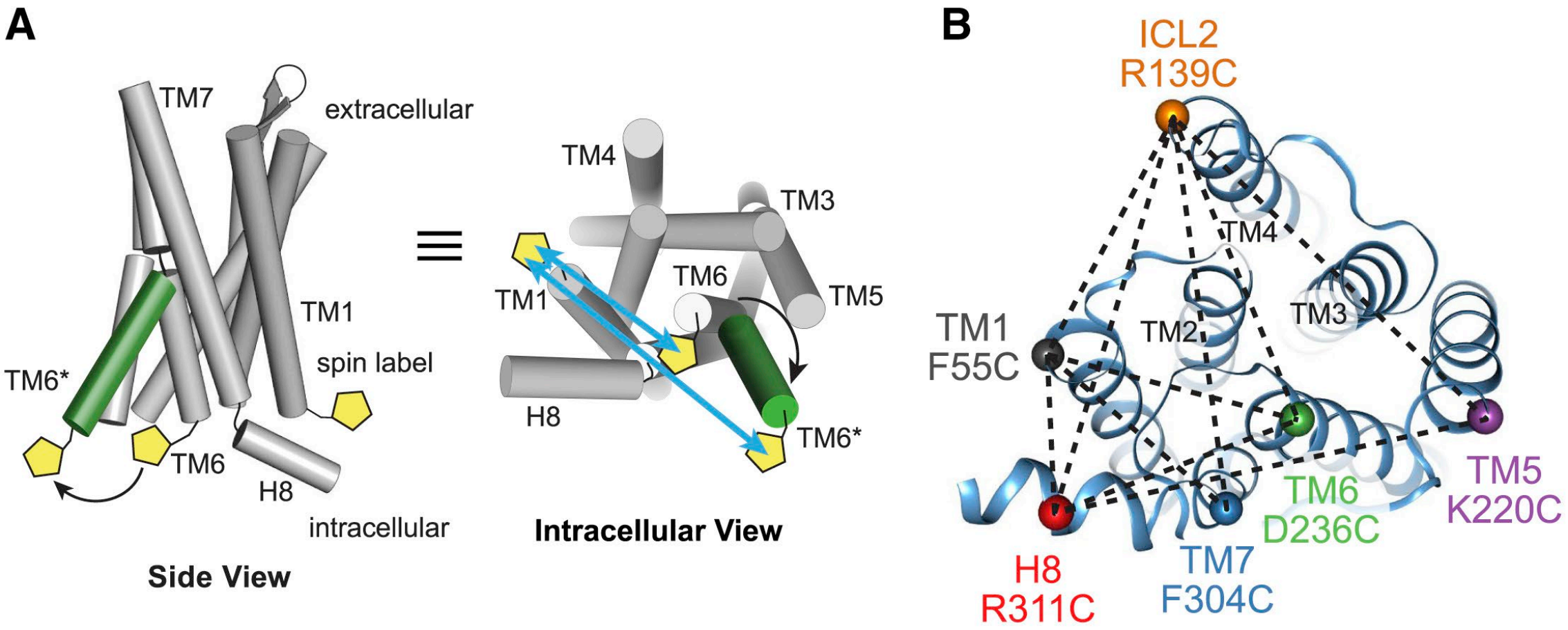
Angiotensin and biased analogs induce structurally distinct active conformations within a GPCR, Volume: 367, Issue: 6480, Pages: 888-892, DOI: (10.1126/science.aay9813)

Similarities in the active GPCR conformation when binding G protein and arrestin



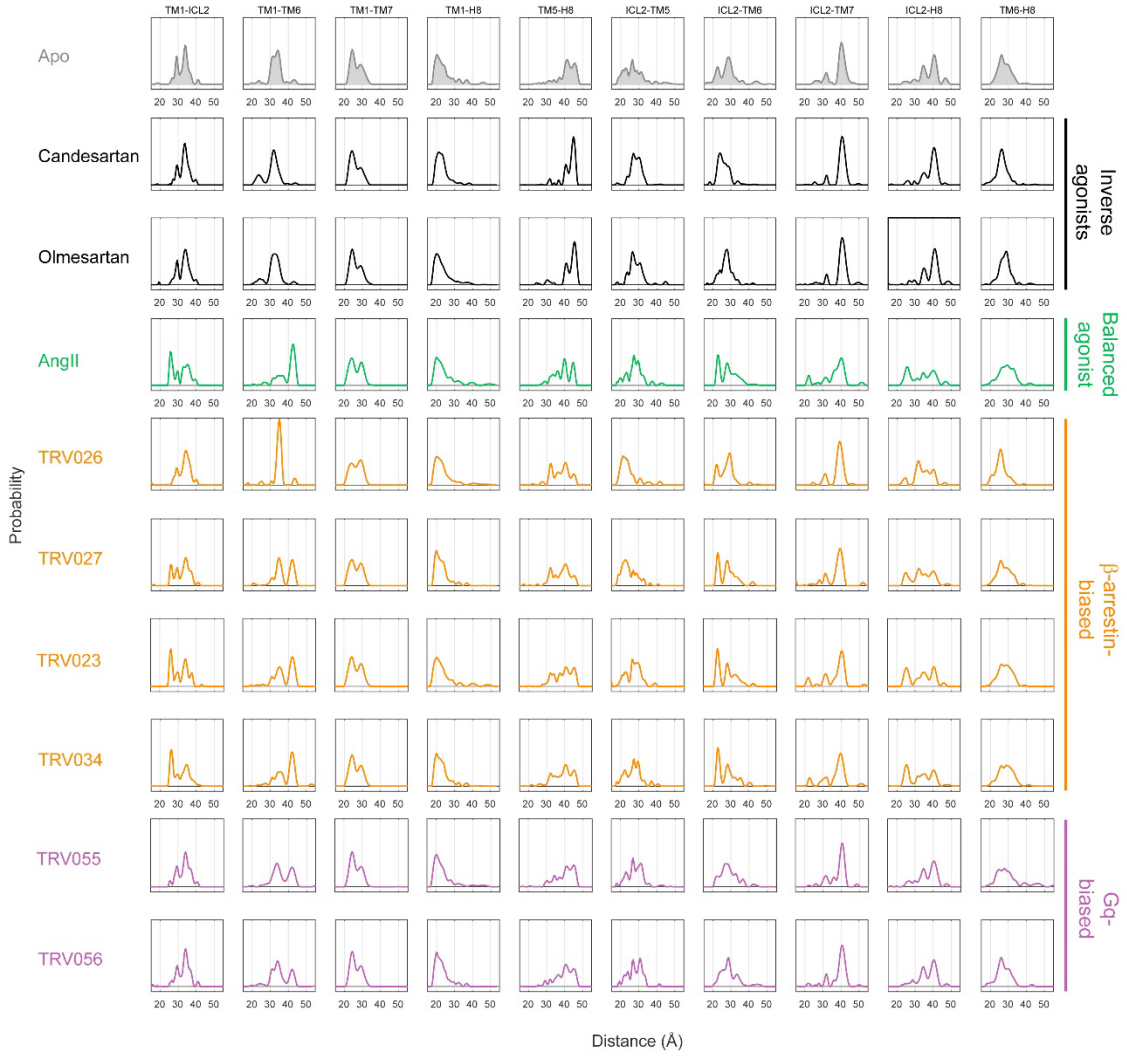
Published in: Naomi R. Latorraca; A. J. Venkatakrishnan; Ron O. Dror; *Chem. Rev.* **2017,** 117, 139-155.
DOI: 10.1021/acs.chemrev.6b00177

DEER spectroscopy to probe the effects of biased ligands at the AT_1R



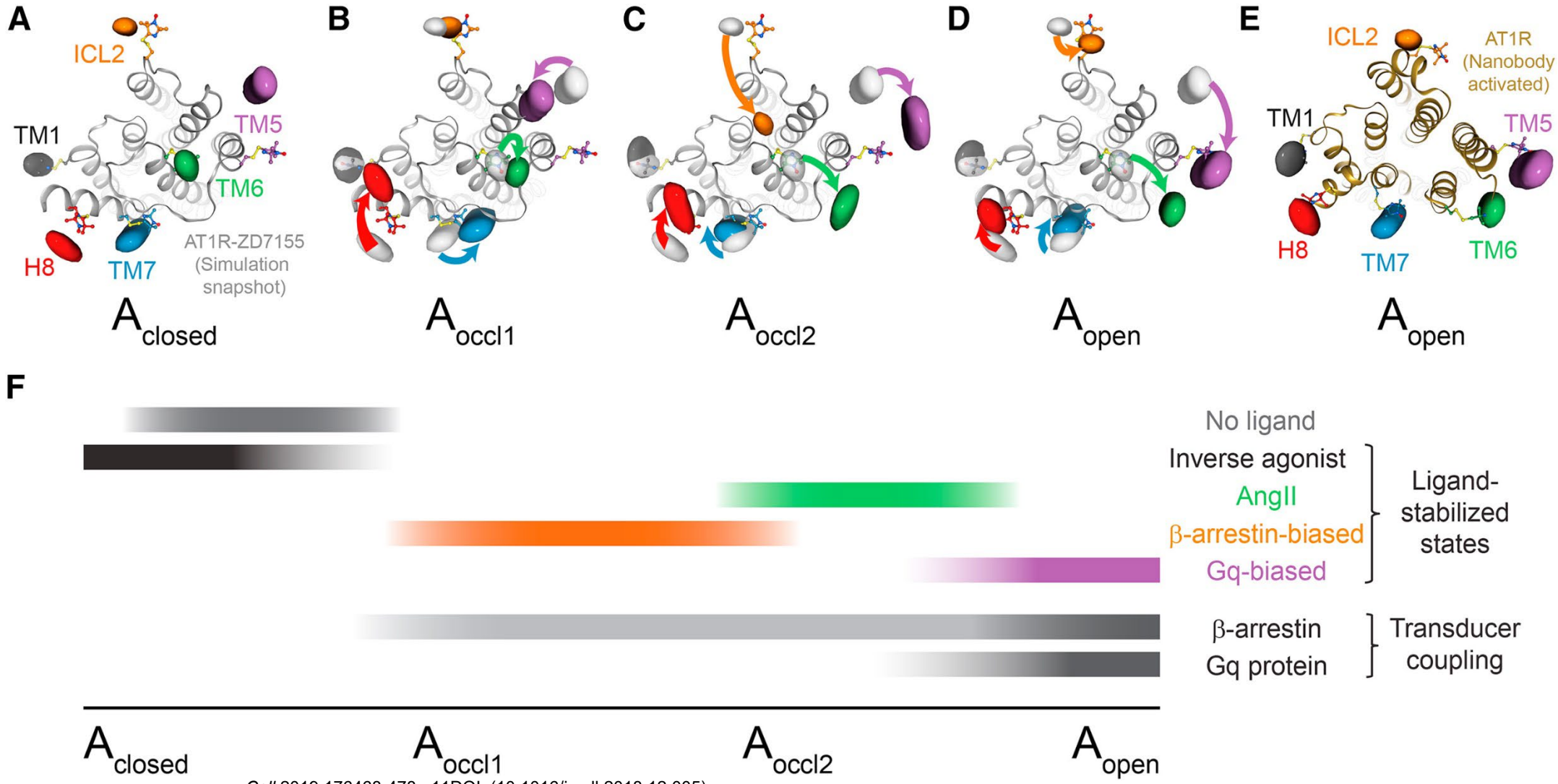
Cell 2019 176468-478.e11DOI: (10.1016/j.cell.2018.12.005)

Data from different pairs for each ligand



Cell 2019 176468-478.e11DOI: (10.1016/j.cell.2018.12.005)

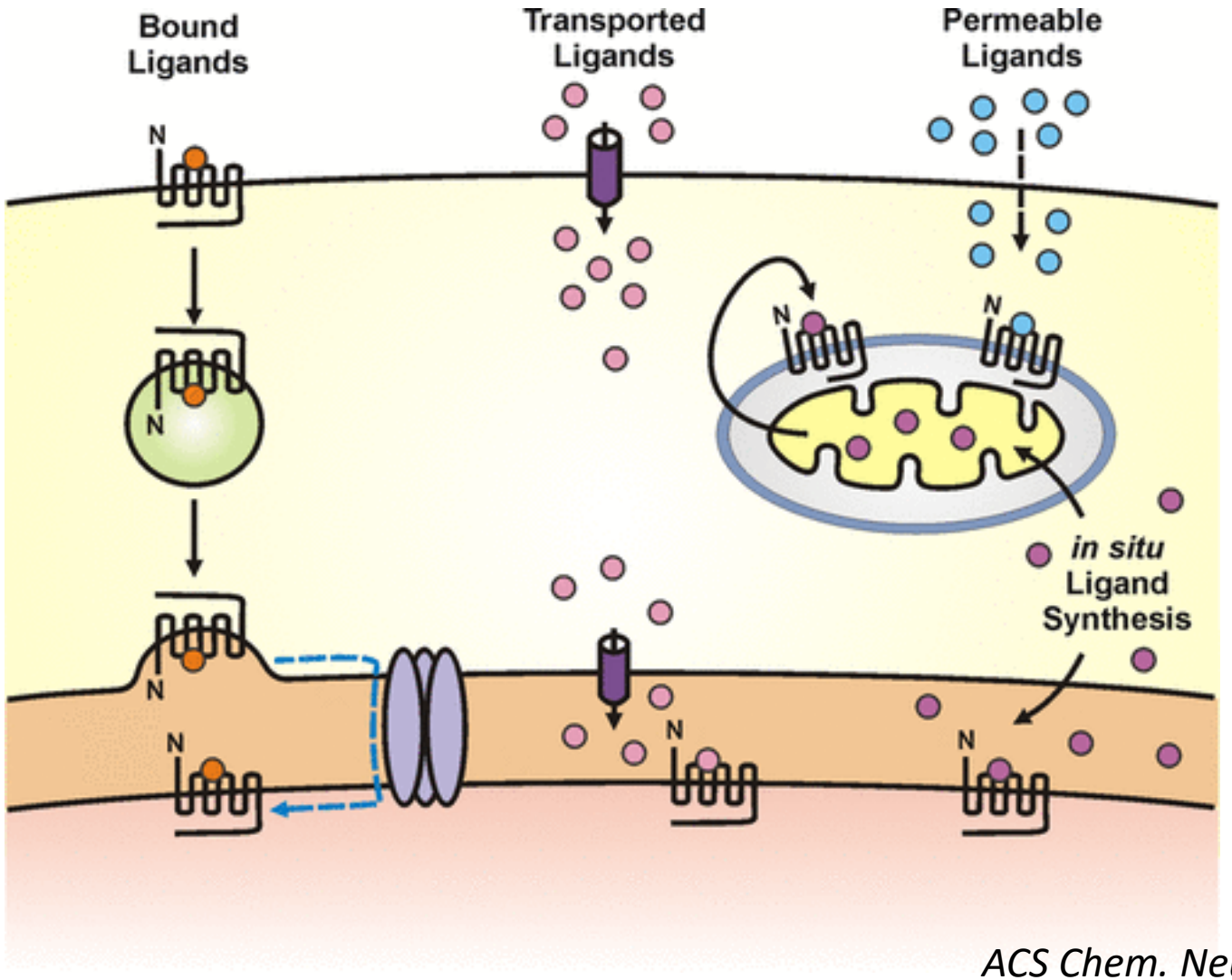
Conformations associated with different ligands



Cell 2019 176468-478.e11DOI: (10.1016/j.cell.2018.12.005)

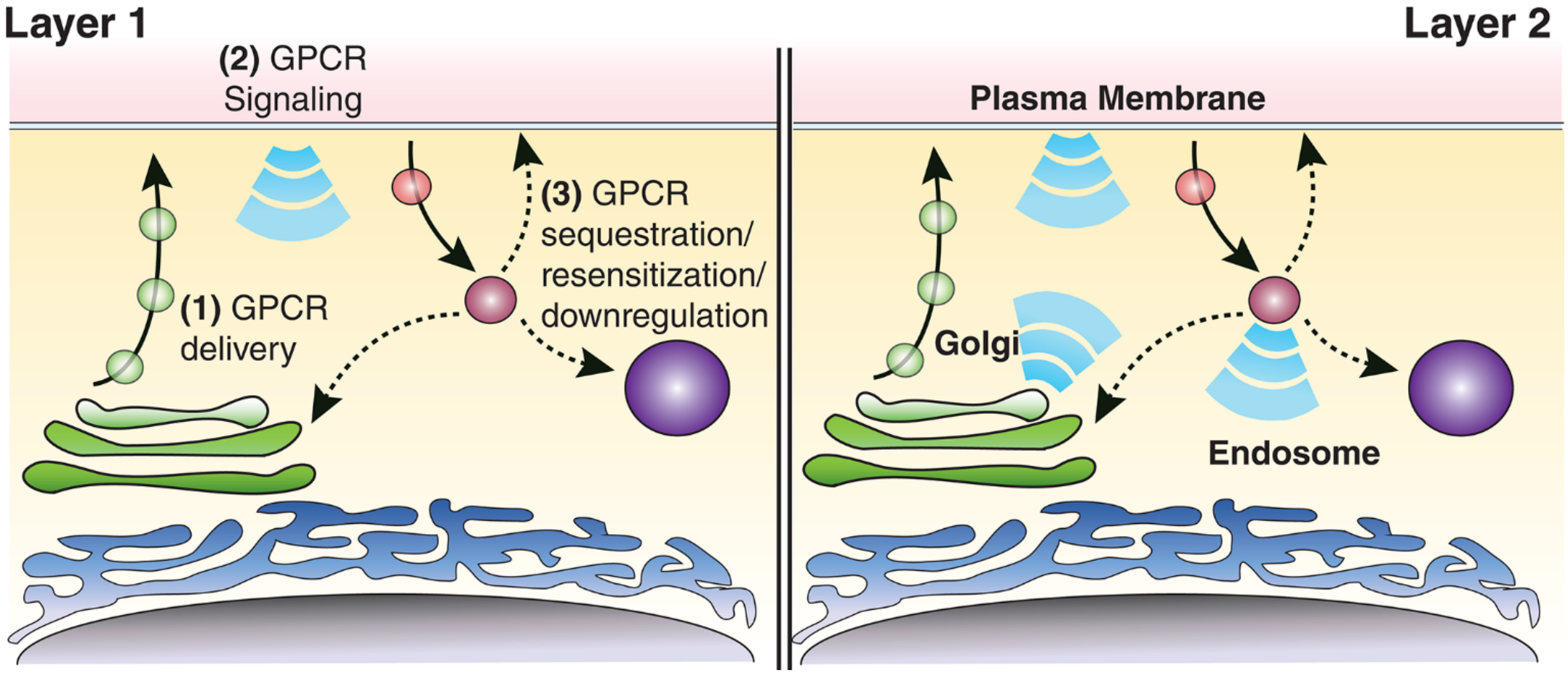
Spatial bias

Different modes of GPCR intracellular signaling

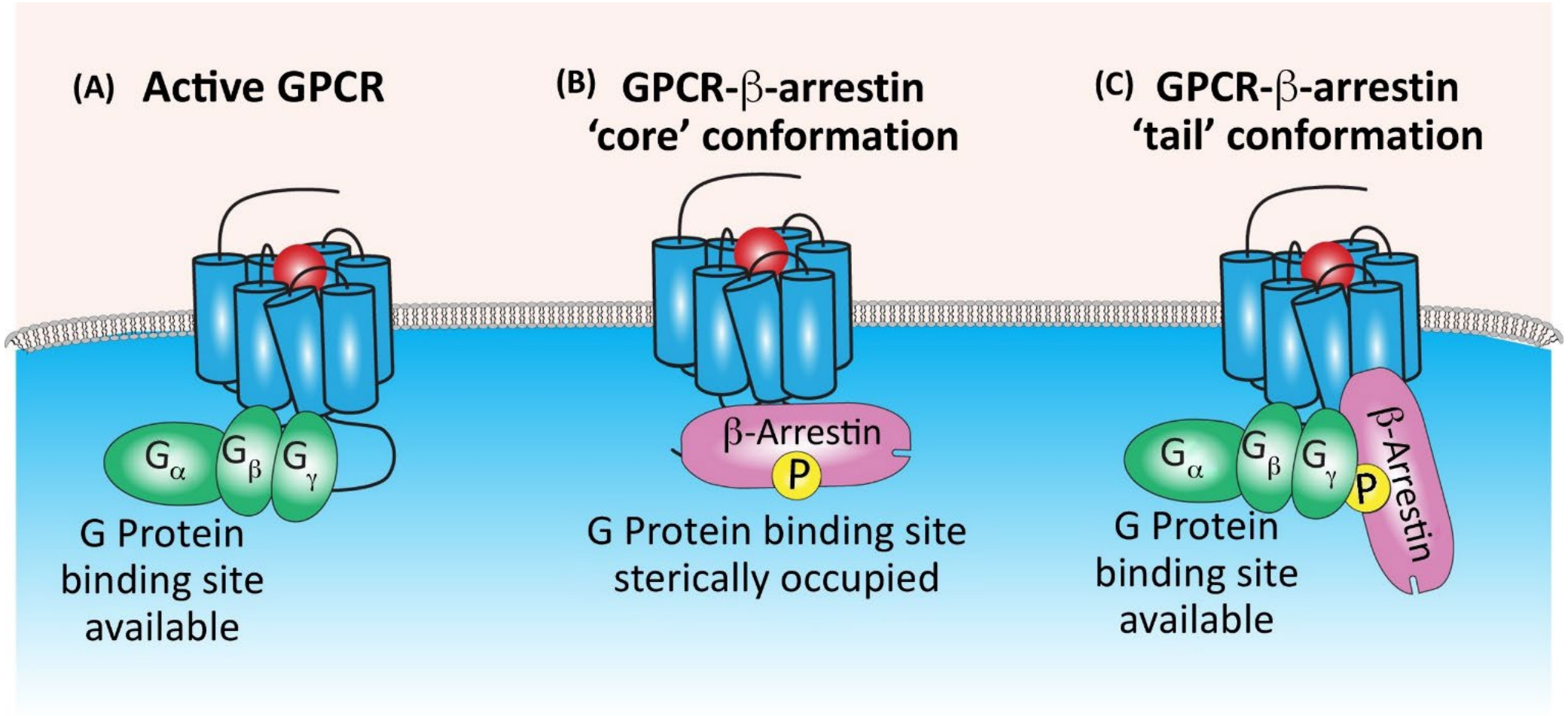


ACS Chem. Neurosci. 2018, 9, 9, 2162–2172

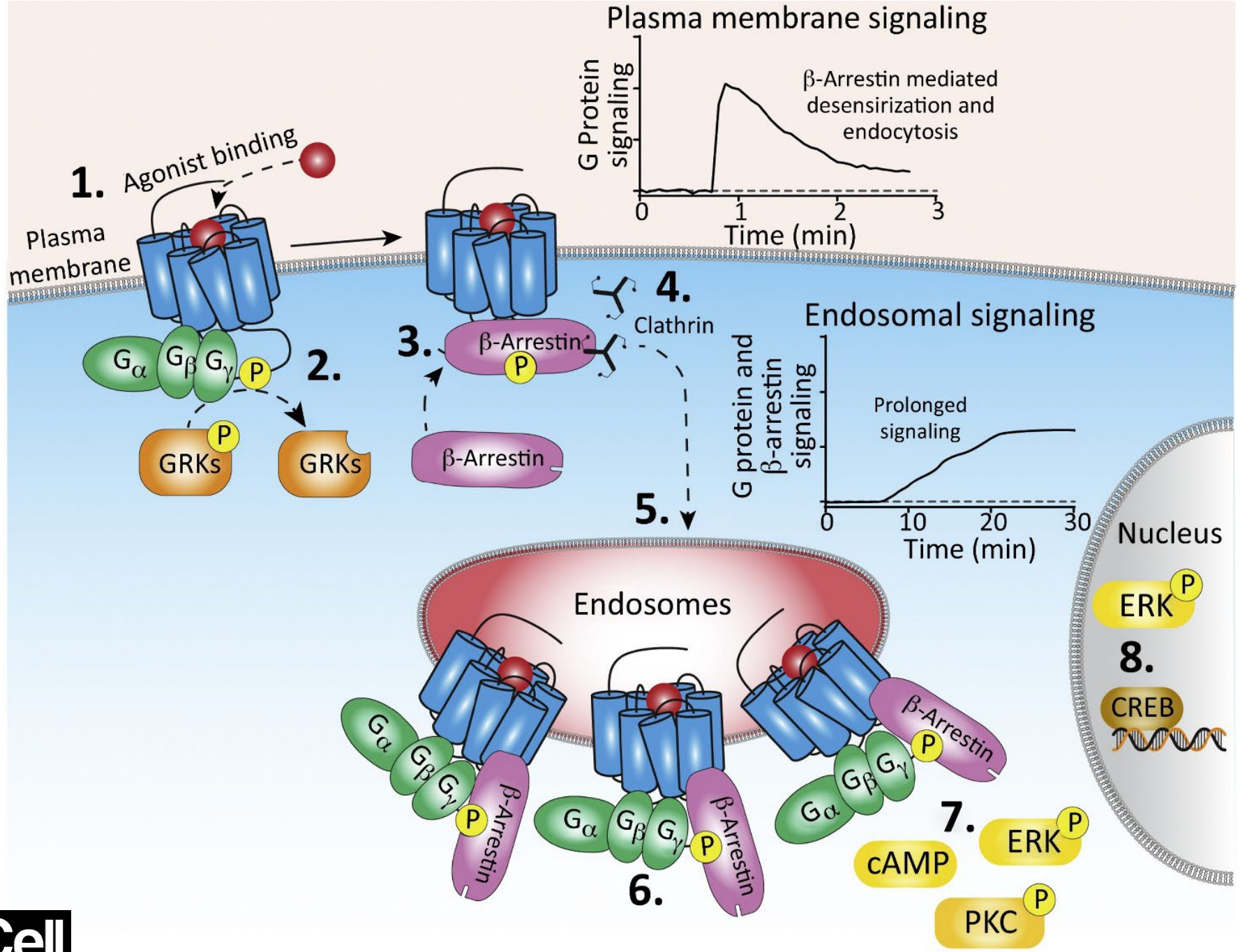
When trafficking and signaling mix: How subcellular location shapes G protein-coupled receptor activation of heterotrimeric G proteins



Traffic, Volume: 20, Issue: 2, Pages: 130-136, First published: 22 December 2018, DOI: (10.1111/tra.12634)



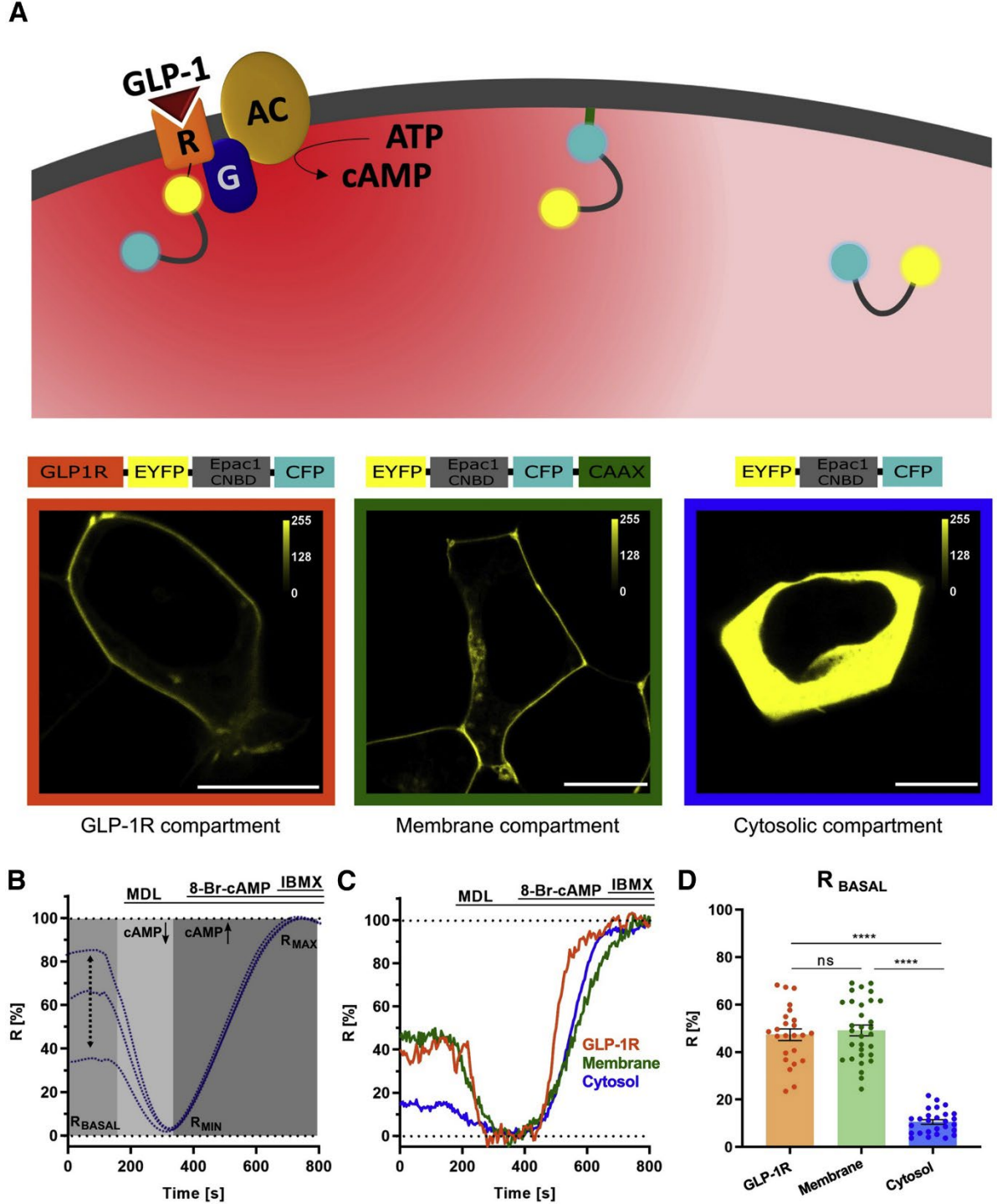
Trends in Pharmacological Sciences

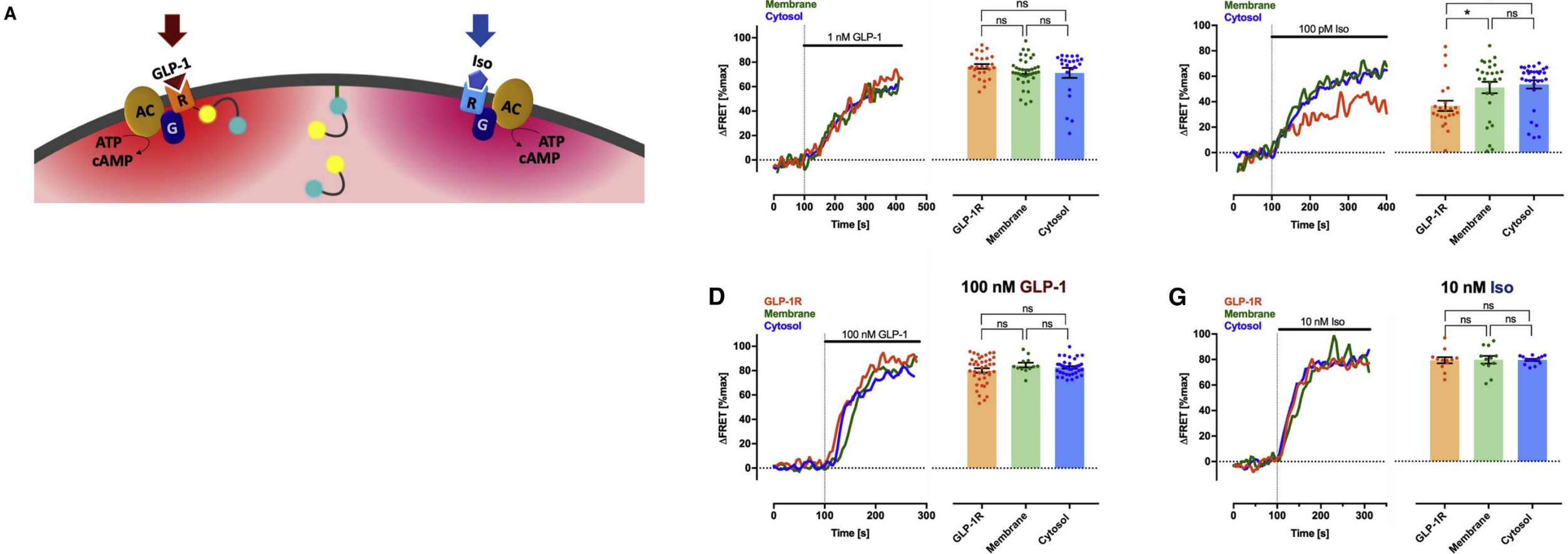


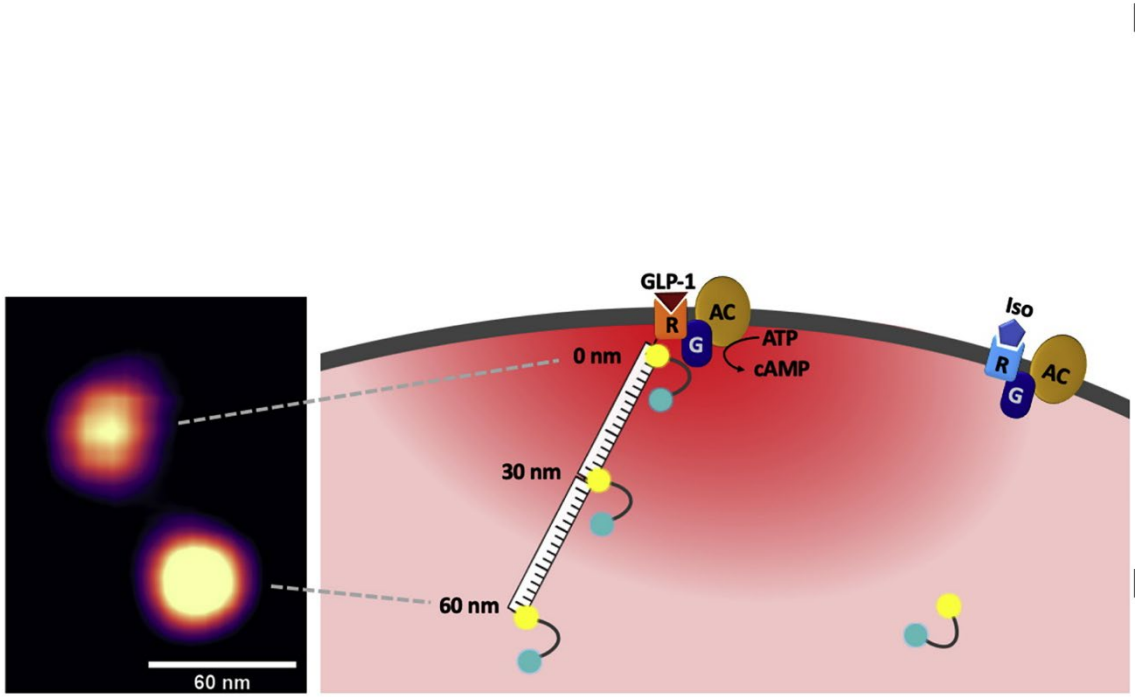
Signaling from microdomains

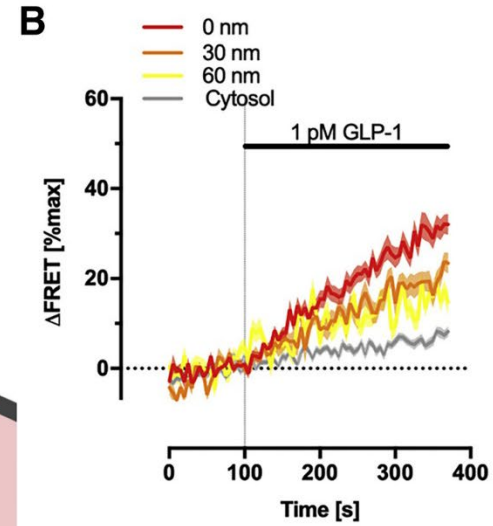
Cell

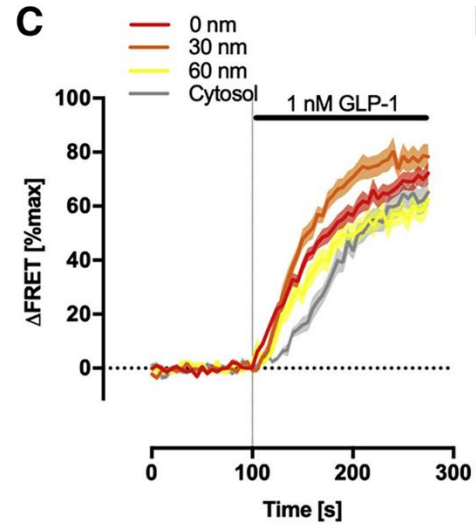
Volume 185, Issue 7, 31 March 2022, Pages 1130-1142.e11

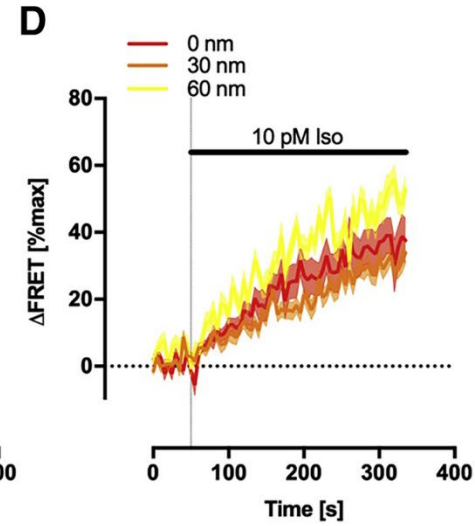

A
GLP-1
R
G
AC
ATP
cAMP
GLP1R
EYFP
Epac1 CNBD
CFP
EYFP
Epac1 CNBD
CFP
CAAX
EYFP
Epac1 CNBD
CFP
255
128
0
GLP-1R compartment
Membrane compartment
Cytosolic compartment
B
MDL
8-Br-cAMP
IBMX
cAMP↓
cAMP↑
R MAX
R BASAL
R MIN
R [%]
Time [s]
C
MDL
8-Br-cAMP
IBMX
GLP-1R
Membrane
Cytosol
R [%]
Time [s]
D
R BASAL
ns
****
****
R [%]
GLP-1R
Membrane
Cytosol

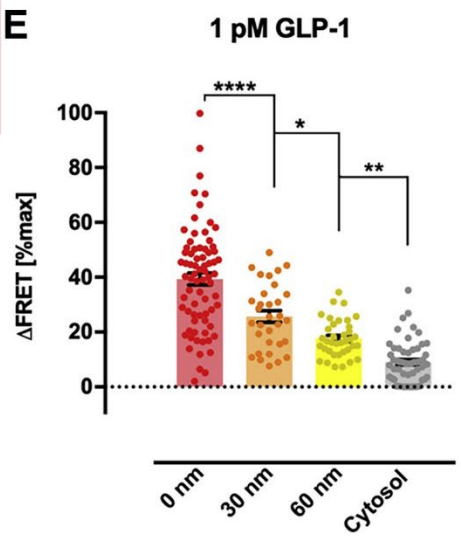

A
GLP-1
R
AC
G
ATP
cAMP
Iso
R
AC
G
ATP
cAMP
B
1 pM GLP-1
GLP-1R
Membrane
Cytosol
1 pM GLP-1
ΔFRET [%max]
Time [s]
****
***
****
GLP-1R
Membrane
Cytosol
C
1 nM GLP-1
GLP-1R
Membrane
Cytosol
1 nM GLP-1
ΔFRET [%max]
Time [s]
ns
ns
ns
GLP-1R
Membrane
Cytosol
D
100 nM GLP-1
GLP-1R
Membrane
Cytosol
100 nM GLP-1
ΔFRET [%max]
Time [s]
ns
ns
ns
GLP-1R
Membrane
Cytosol
E
10 pM Iso
GLP-1R
Membrane
Cytosol
10 pM Iso
ΔFRET [%max]
Time [s]
**
****
****
GLP-1R
Membrane
Cytosol
F
100 pM Iso
GLP-1R
Membrane
Cytosol
100 pM Iso
ΔFRET [%max]
Time [s]
*
*
ns
GLP-1R
Membrane
Cytosol
G
10 nM Iso
GLP-1R
Membrane
Cytosol
10 nM Iso
ΔFRET [%max]
Time [s]
ns
ns
ns
GLP-1R
Membrane
Cytosol

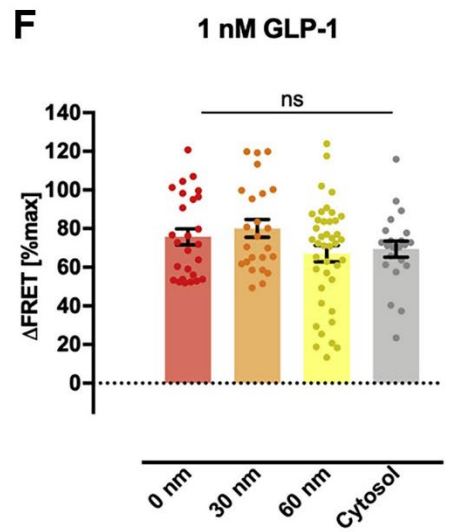

60 nm
GLP-1
R
G
AC
ATP
cAMP
0 nm
30 nm
60 nm
Iso
R
G
AC

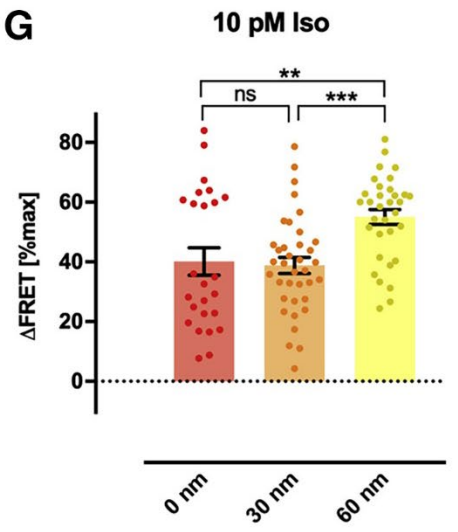

B
0 nm
30 nm
60 nm
Cytosol
1 pM GLP-1
ΔFRET [%max]
Time [s]

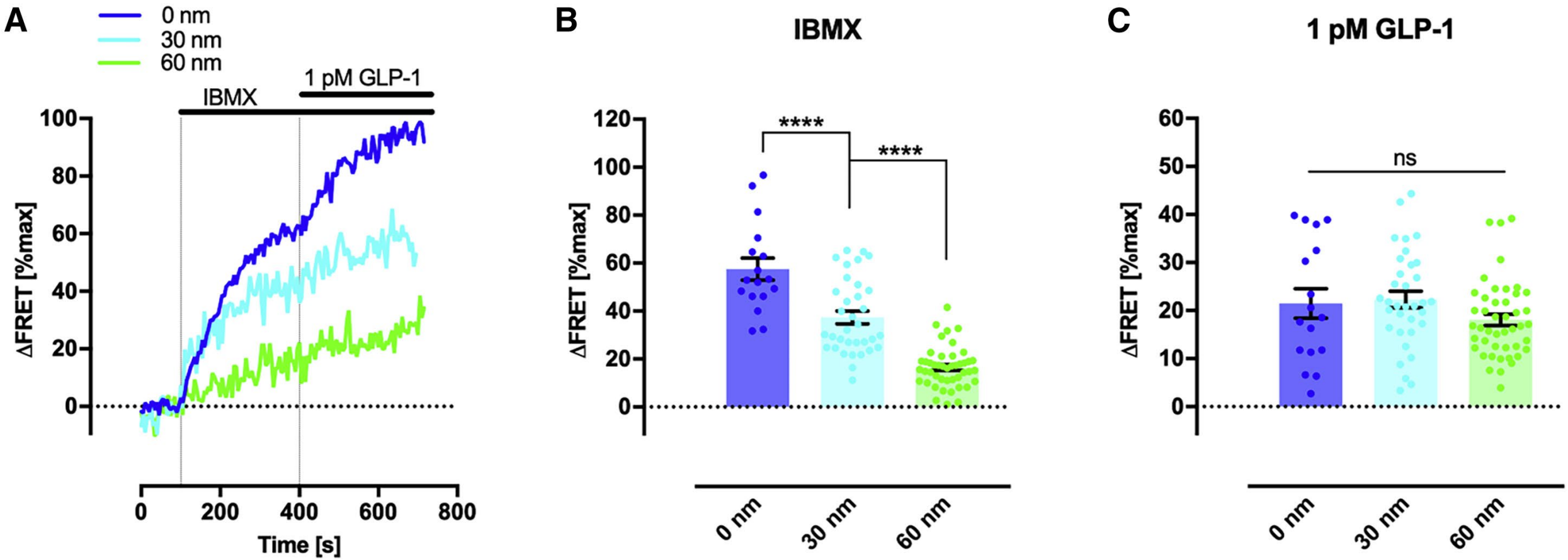

C
0 nm
30 nm
60 nm
Cytosol
1 nM GLP-1
ΔFRET [%max]
Time [s]

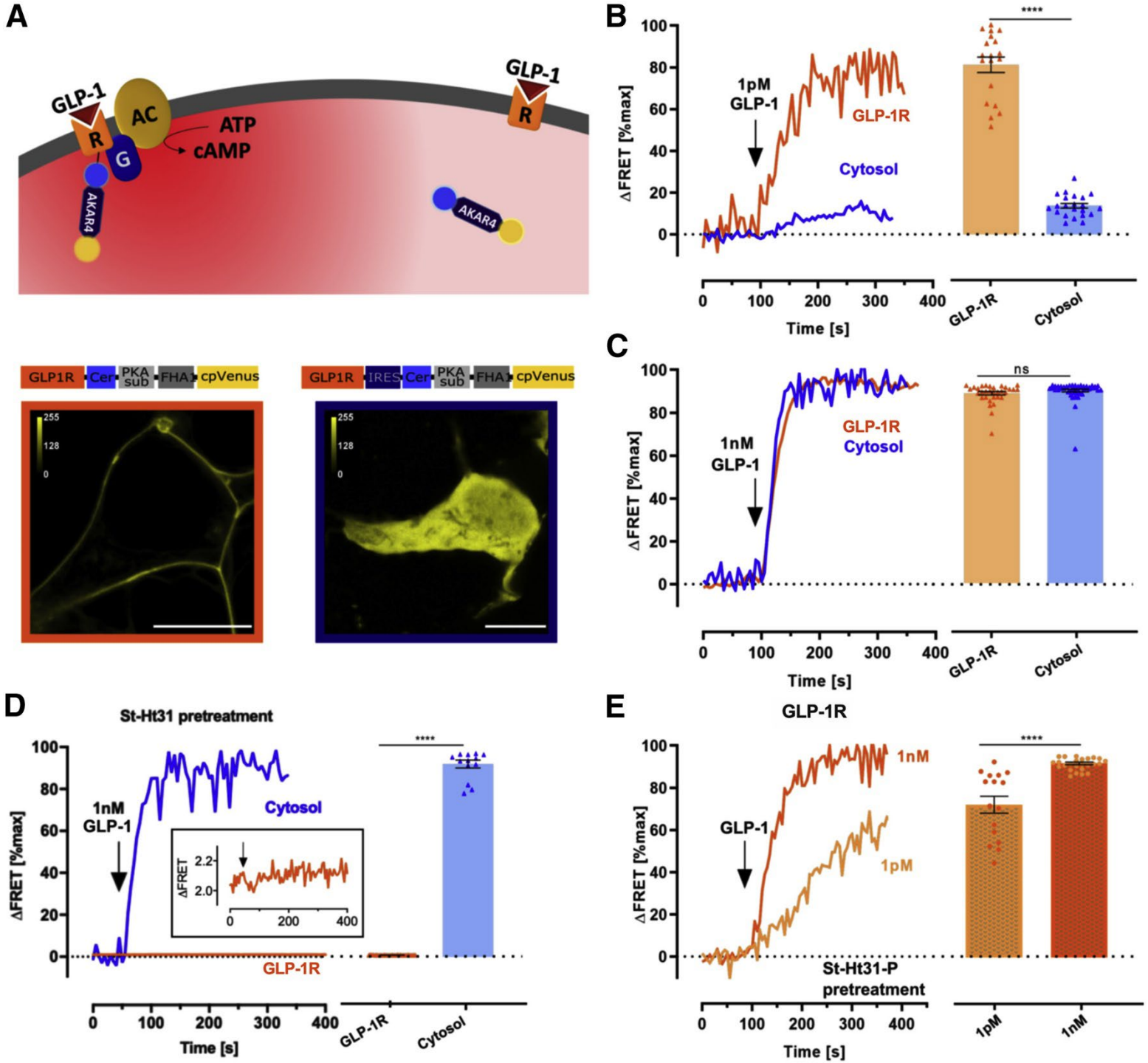

D
0 nm
30 nm
60 nm
10 pM Iso
ΔFRET [%max]
Time [s]

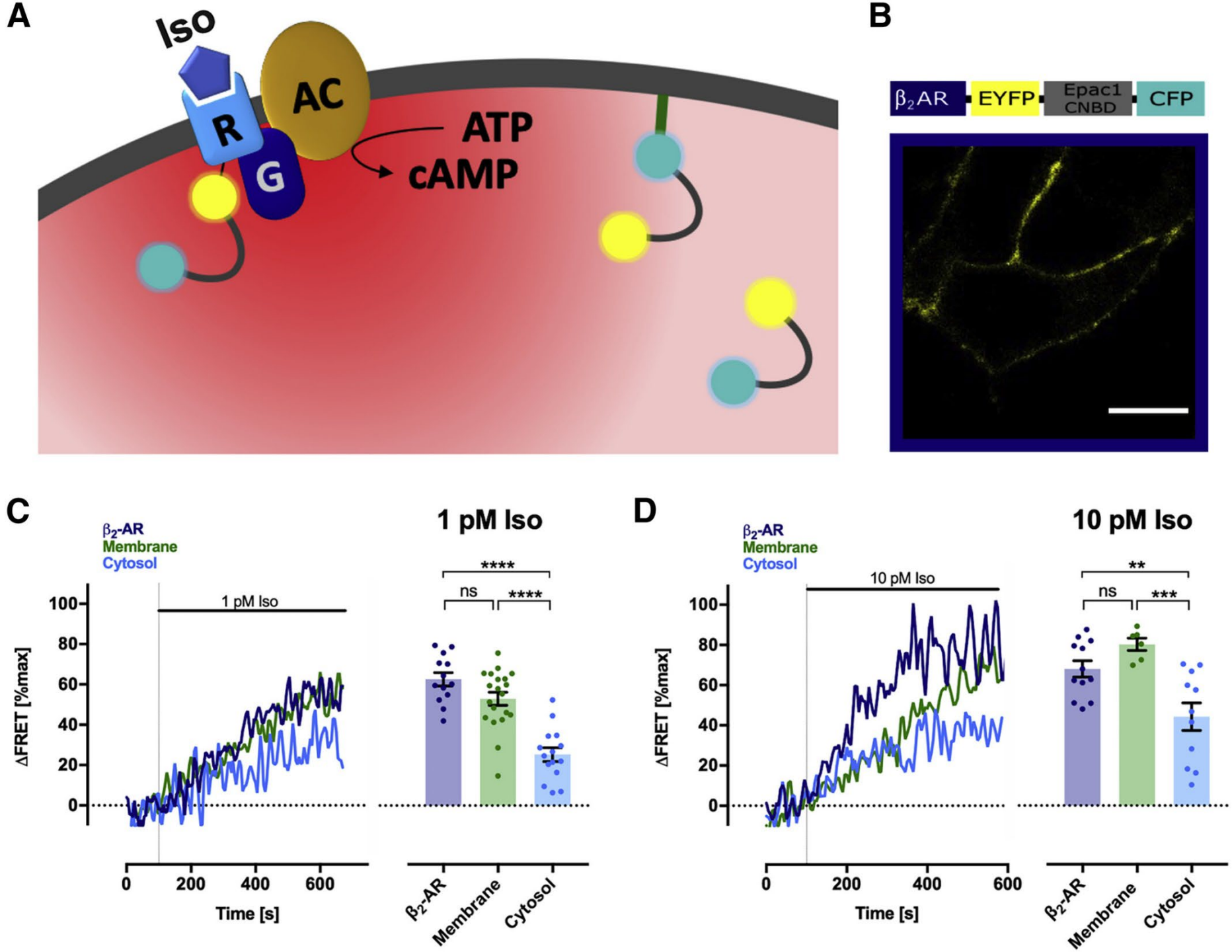

E
1 pM GLP-1
****
*
**
ΔFRET [%max]
0 nm
30 nm
60 nm
Cytosol

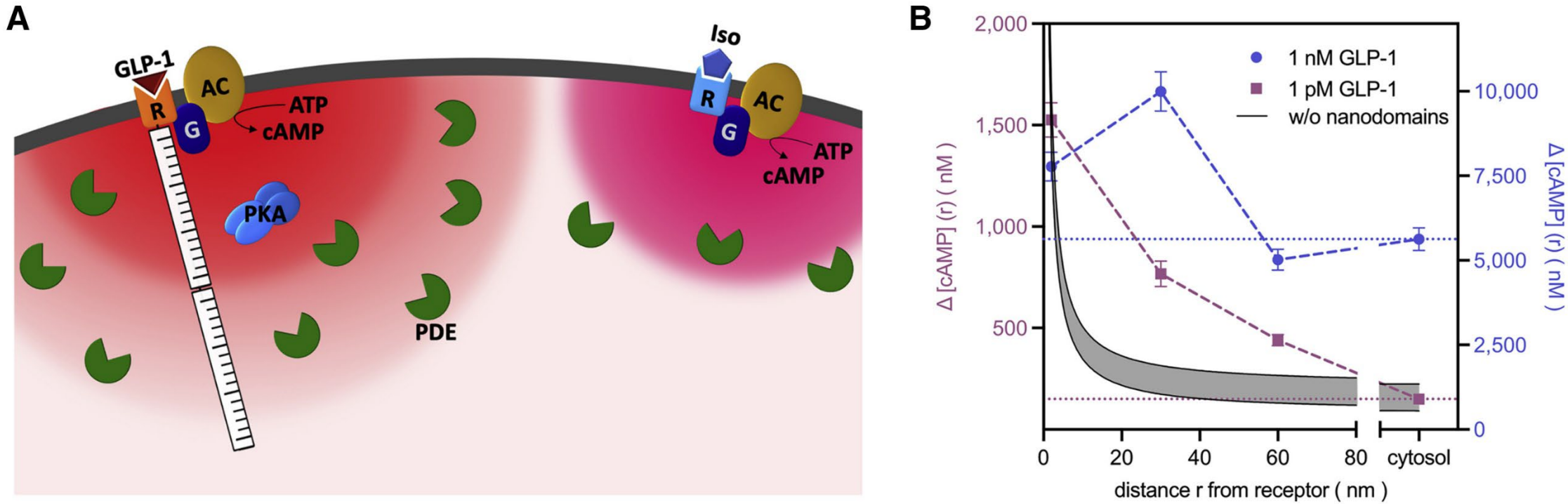

F
1 nM GLP-1
ns
ΔFRET [%max]
0 nm
30 nm
60 nm
Cytosol


G
10 pM Iso
**
ns
***
ΔFRET [%max]
0 nm
30 nm
60 nm


A
0 nm
30 nm
60 nm
IBMX
1 pM GLP-1
ΔFRET [%max]
Time [s]
B
IBMX
****
****
ΔFRET [%max]
0 nm
30 nm
60 nm
C
1 pM GLP-1
ns
ΔFRET [%max]
0 nm
30 nm
60 nm

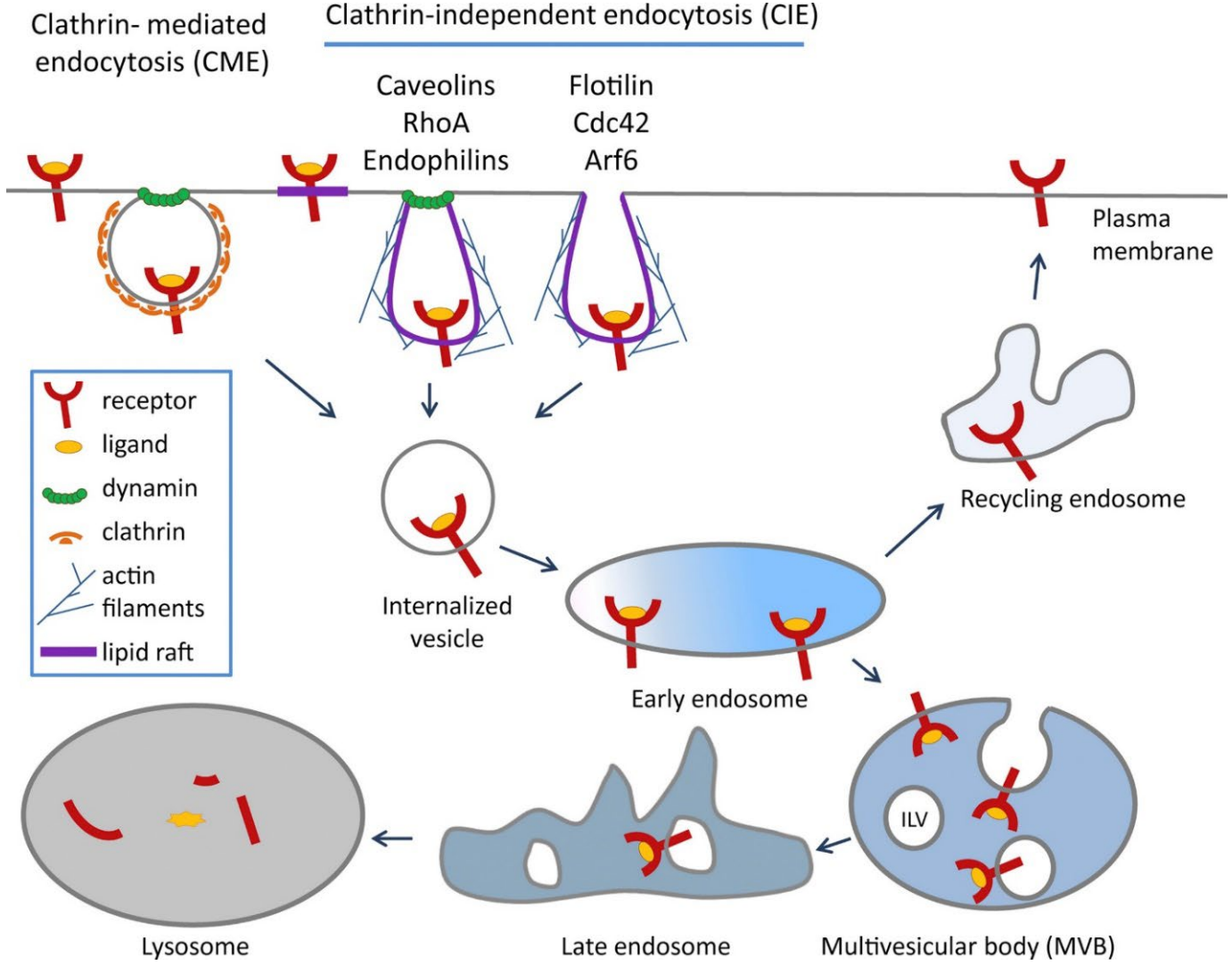

A
GLP-1
R
AC
ATP
cAMP
G
AKAR4
GLP-1
R
AKAR4
GLP1R
Cer
PKA sub
FHA
cpVenus
255
128
0
GLP1R
IRES
Cer
PKA sub
FHA
cpVenus
255
128
0
B
1pM GLP-1
GLP-1R
Cytosol
ΔFRET [%max]
Time [s]
GLP-1R
Cytosol
****
C
1nM GLP-1
GLP-1R
Cytosol
ΔFRET [%max]
Time [s]
GLP-1R
Cytosol
ns
D
St-Ht31 pretreatment
1nM GLP-1
Cytosol
GLP-1R
ΔFRET
ΔFRET [%max]
Time [s]
GLP-1R
Cytosol
****
E
GLP-1R
1nM
1pM
GLP-1
St-Ht31-P pretreatment
ΔFRET [%max]
Time [s]
1pM
1nM
****


A
Iso
R
G
AC
ATP
cAMP
B
β2AR
EYFP
Epac1
CNBD
CFP
C
β2-AR
Membrane
Cytosol
1 pM Iso
ΔFRET [%max]
Time [s]
ns
****
D
10 pM Iso
**
***


A
GLP-1
R
G
AC
ATP
cAMP
PKA
PDE
Iso
R
G
AC
ATP
cAMP
B
2,000
1,500
1,000
500
10,000
7,500
5,000
2,500
0
1 nM GLP-1
1 pM GLP-1
w/o nanodomains
Δ [cAMP] (r) (nM)
Δ [cAMP] (r) (nM)
0
20
40
60
80
cytosol
distance r from receptor (nm)

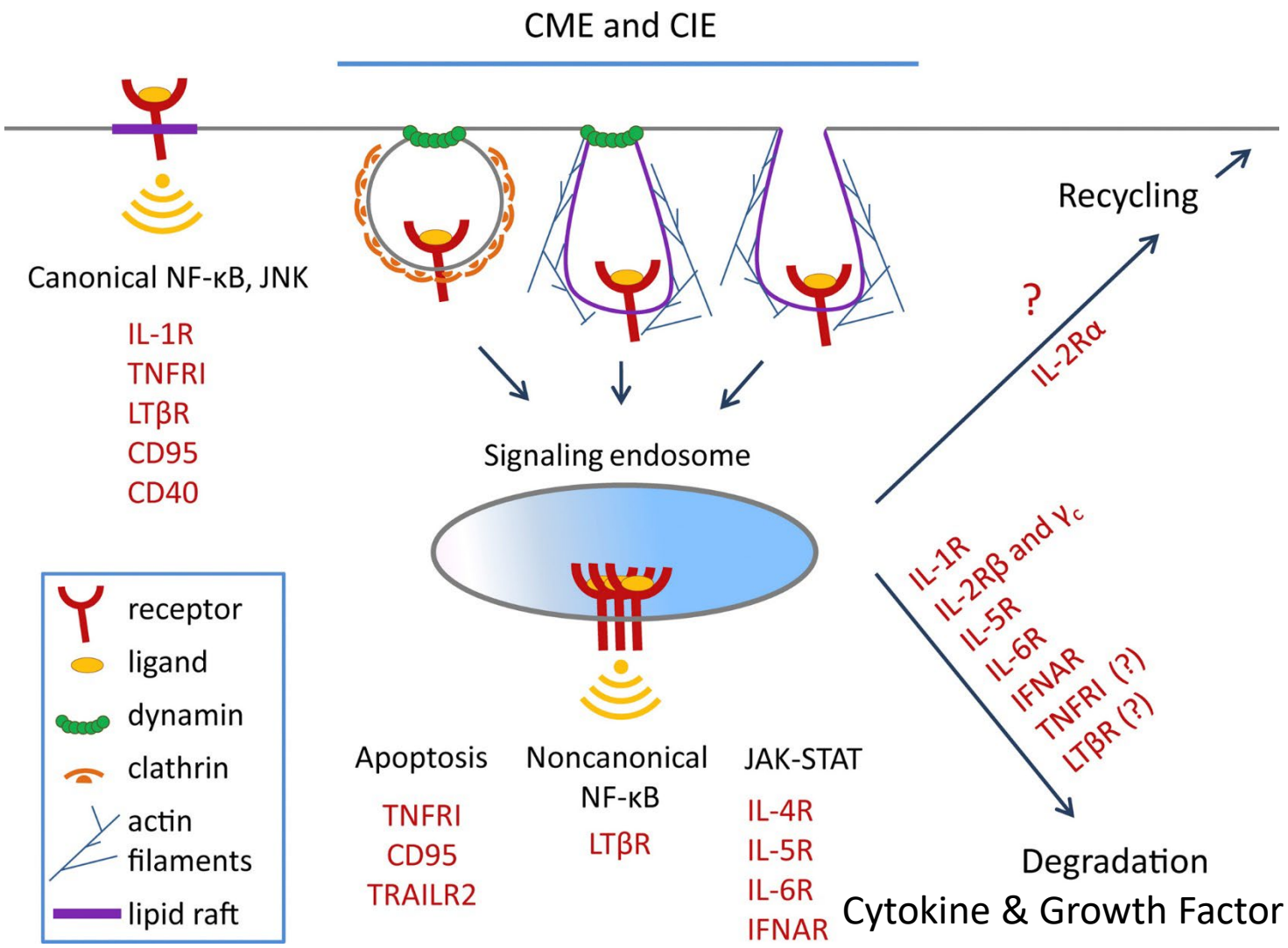
Endosomal signaling by non-GPCRs

Review of cytokine receptor trafficking



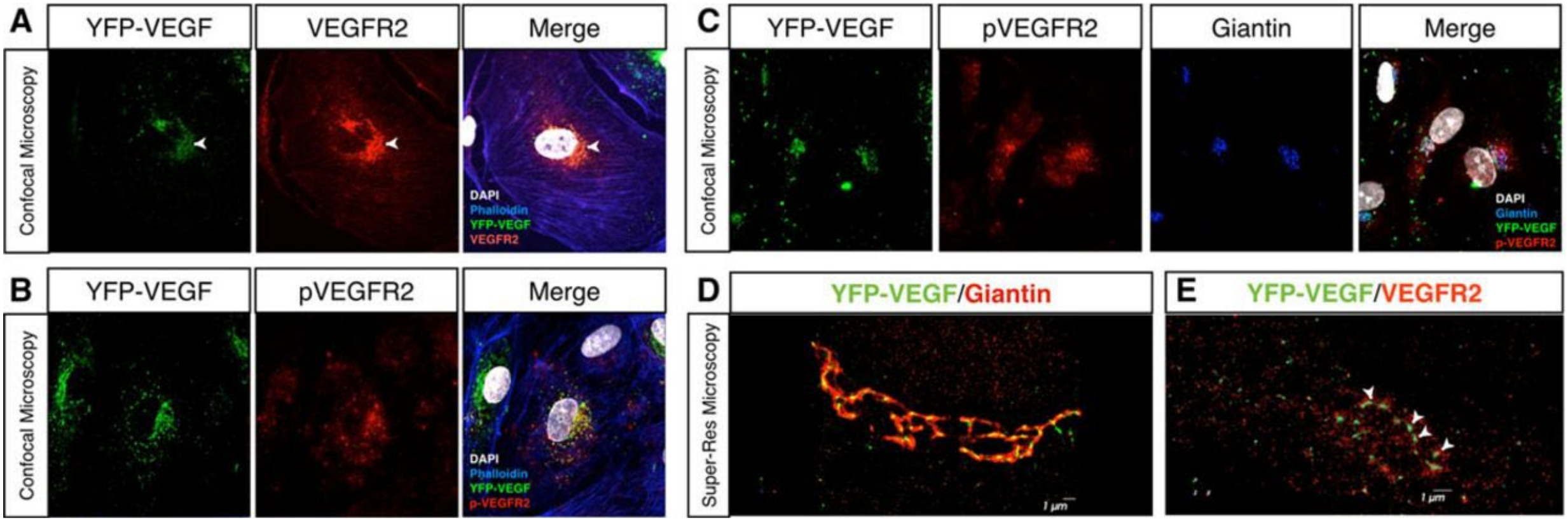
Cytokine & Growth Factor Reviews 32 (2016) 63–73

Endosomal signaling



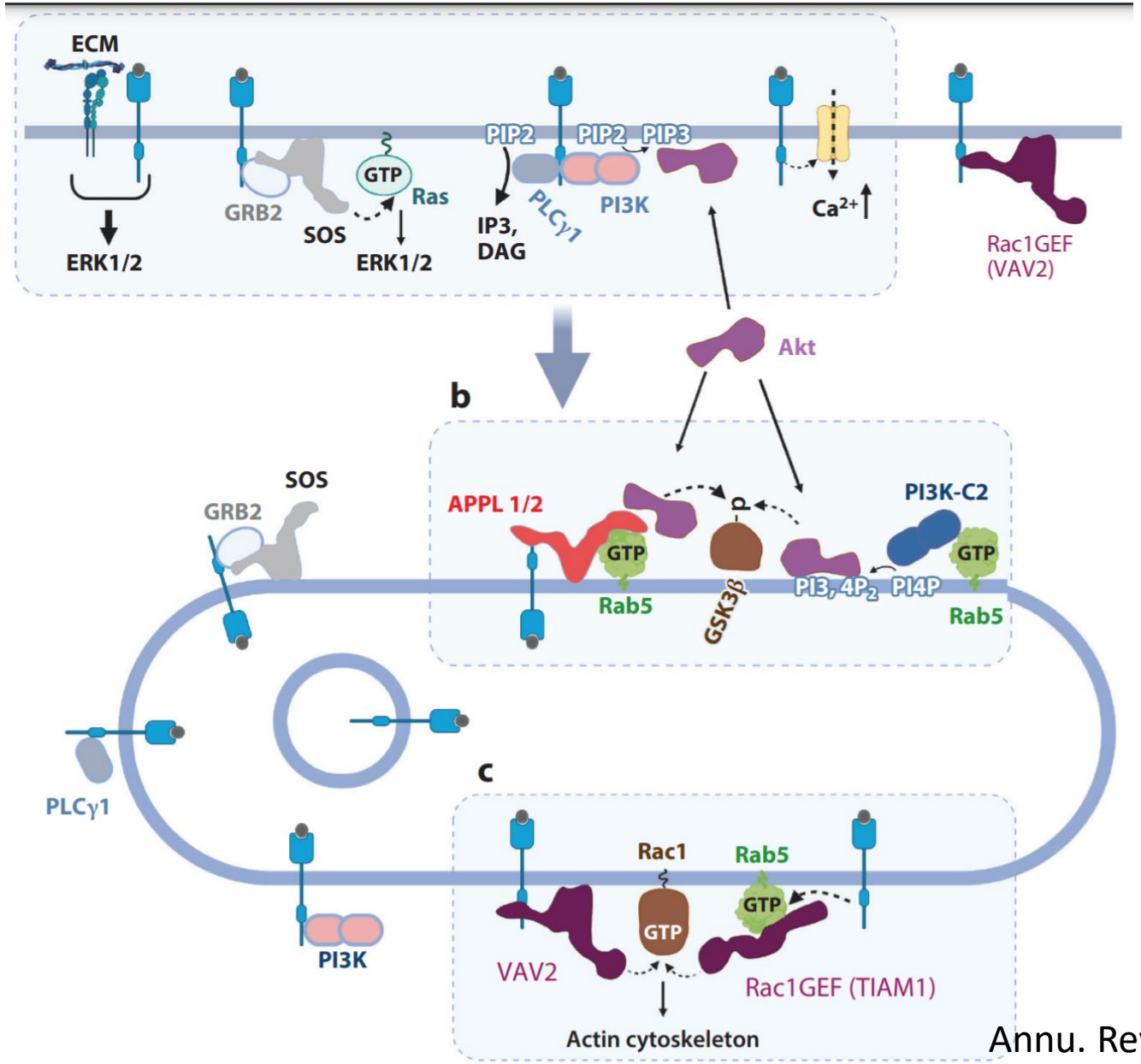
Cytokine & Growth Factor Reviews 32 (2016) 63–73

Intracrine signaling by VEGF



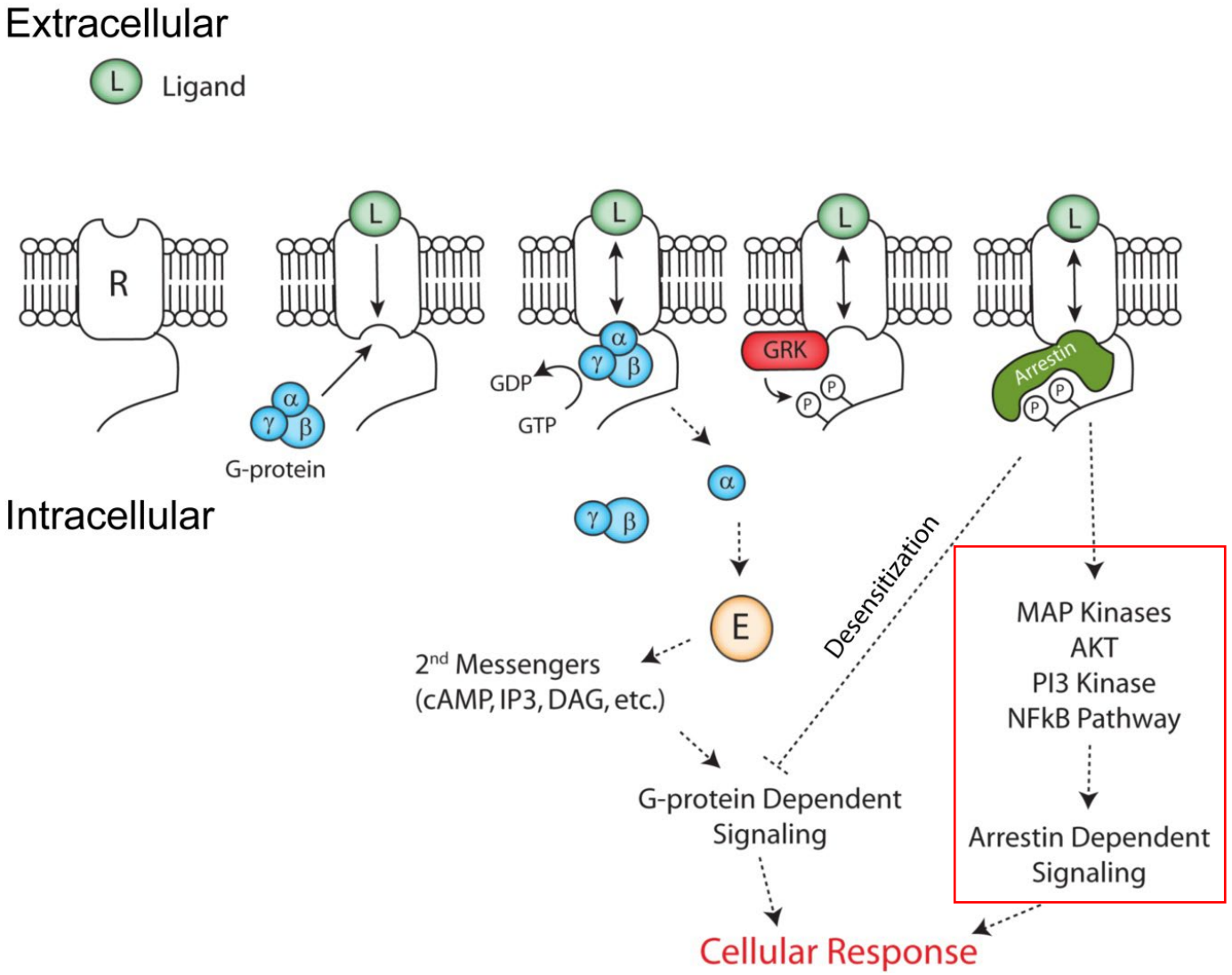
Canonical and Noncanonical Vascular Endothelial Growth Factor Pathways, Volume: 35, Issue: 1, Pages: 30-39, DOI: (10.1161/ATVBAHA.114.303215)

Endocytosis can promote or inhibit specific RTK signaling



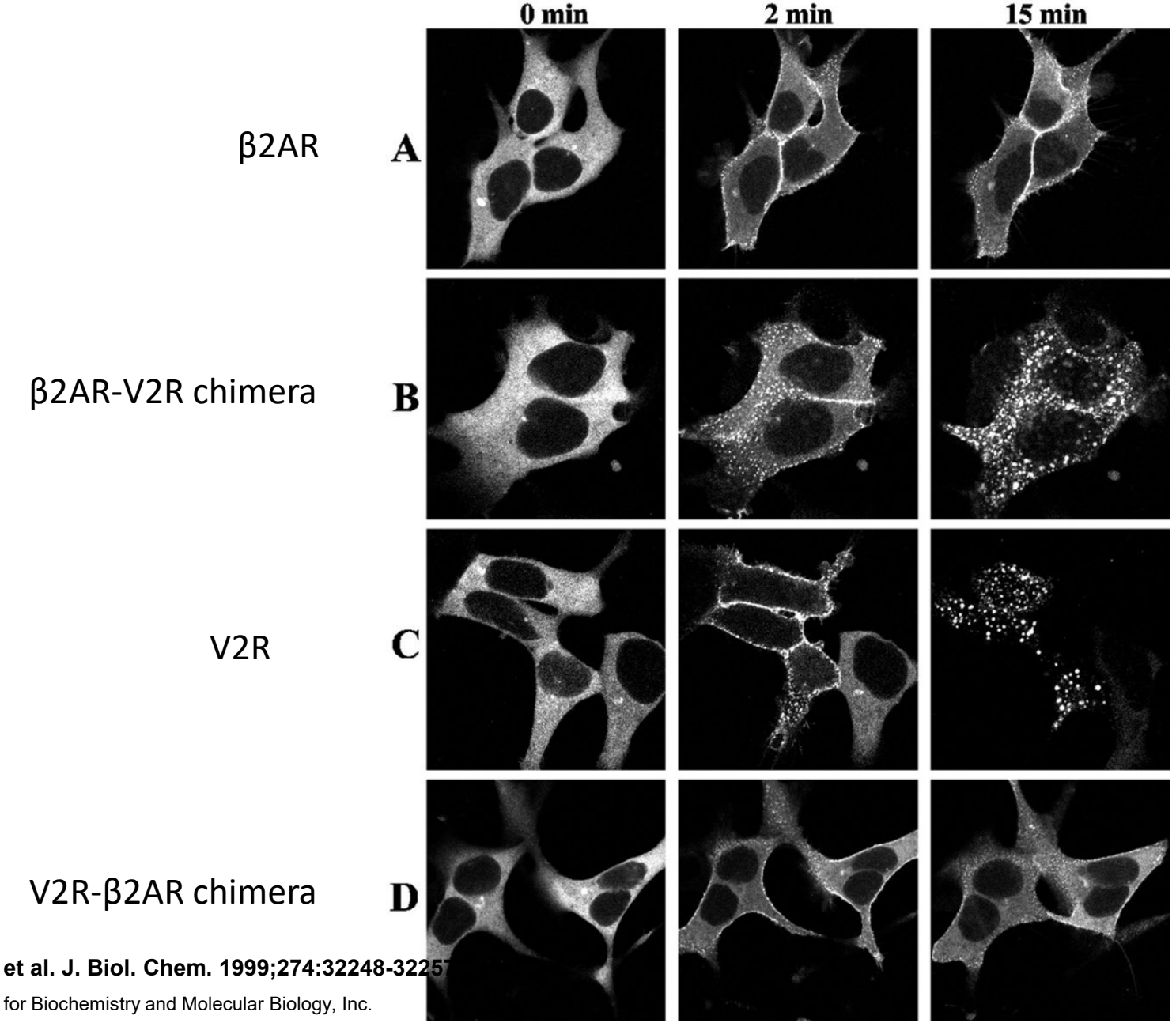
Annu. Rev. Biochem. 2021. 90:5.1–5.29

GPCR Endosomal Signaling



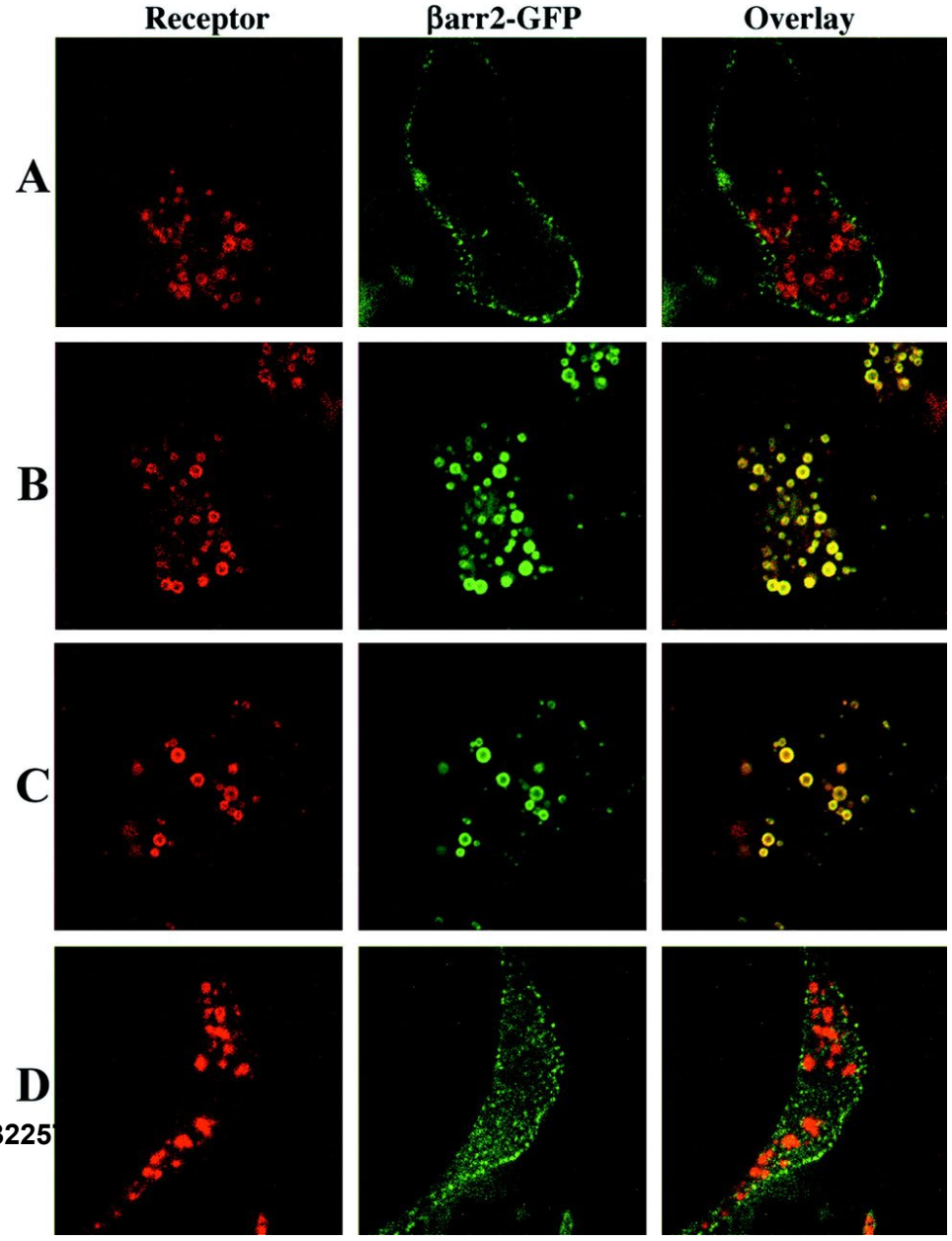
Modified from Dean Staus

Cellular trafficking of βarr2-GFP with the β2AR, V2R , and β2AR-V2R and V2R -β2AR chimeras.



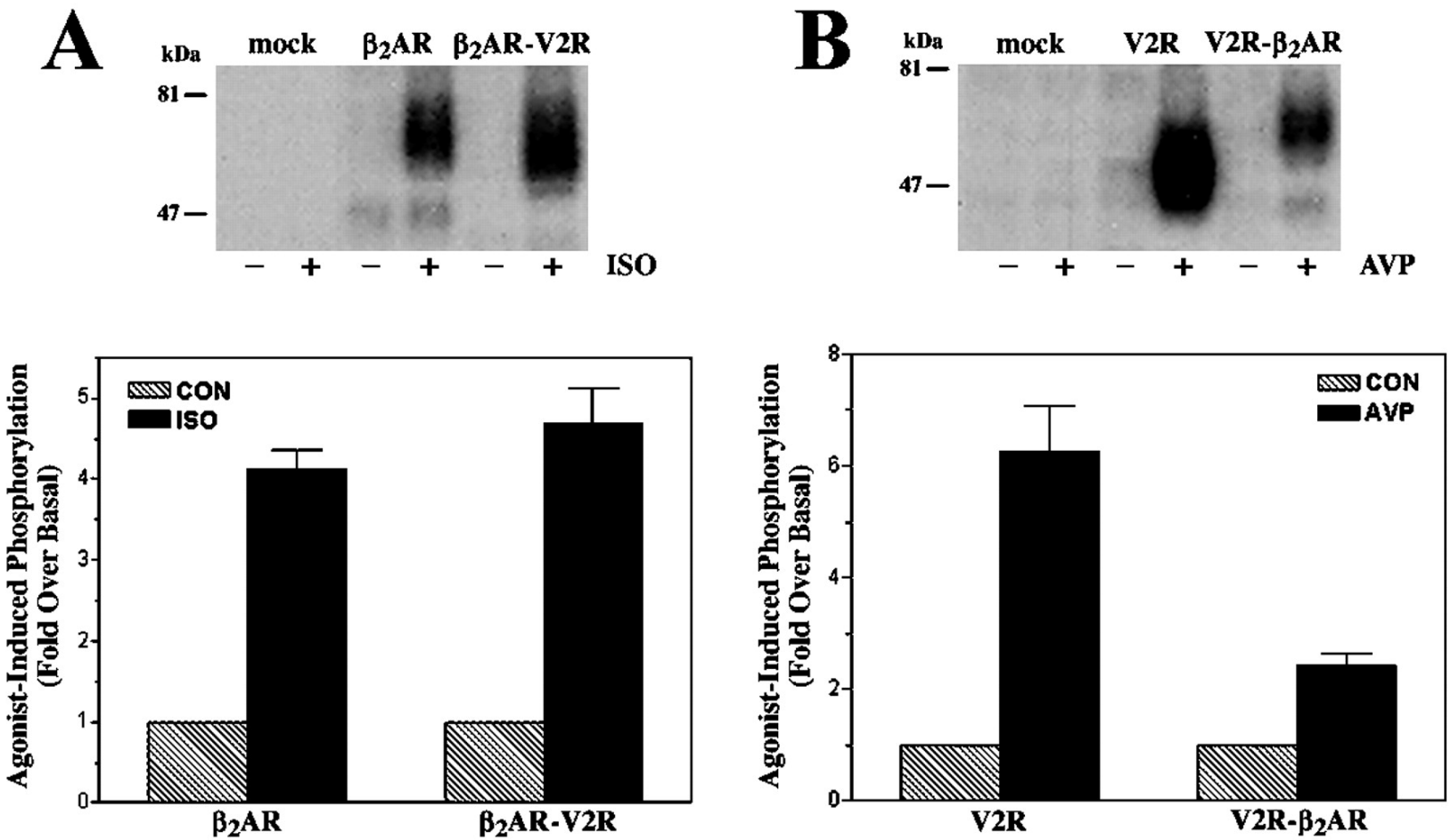
Robert H. Oakley et al. J. Biol. Chem. 1999;274:32248-32257

Colocalization of βarr2-GFP with the internalized β2AR, V2R , and β2AR-V2R and V2R -β2AR chimeras.



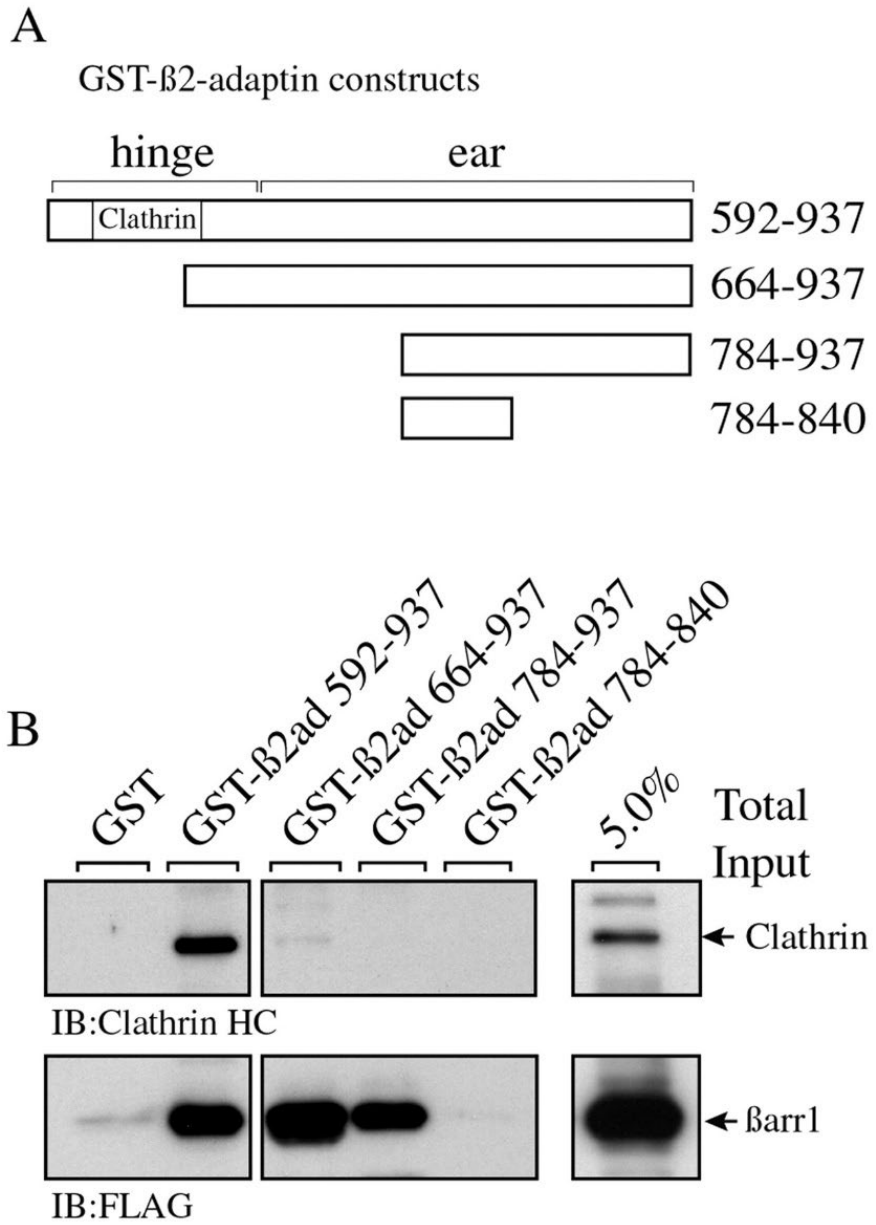
Robert H. Oakley et al. J. Biol. Chem. 1999;274:32248-3225

Phosphorylation of the agonist-activated β2AR, V2R , and β2AR-V2R and V2R -β2AR chimeras.



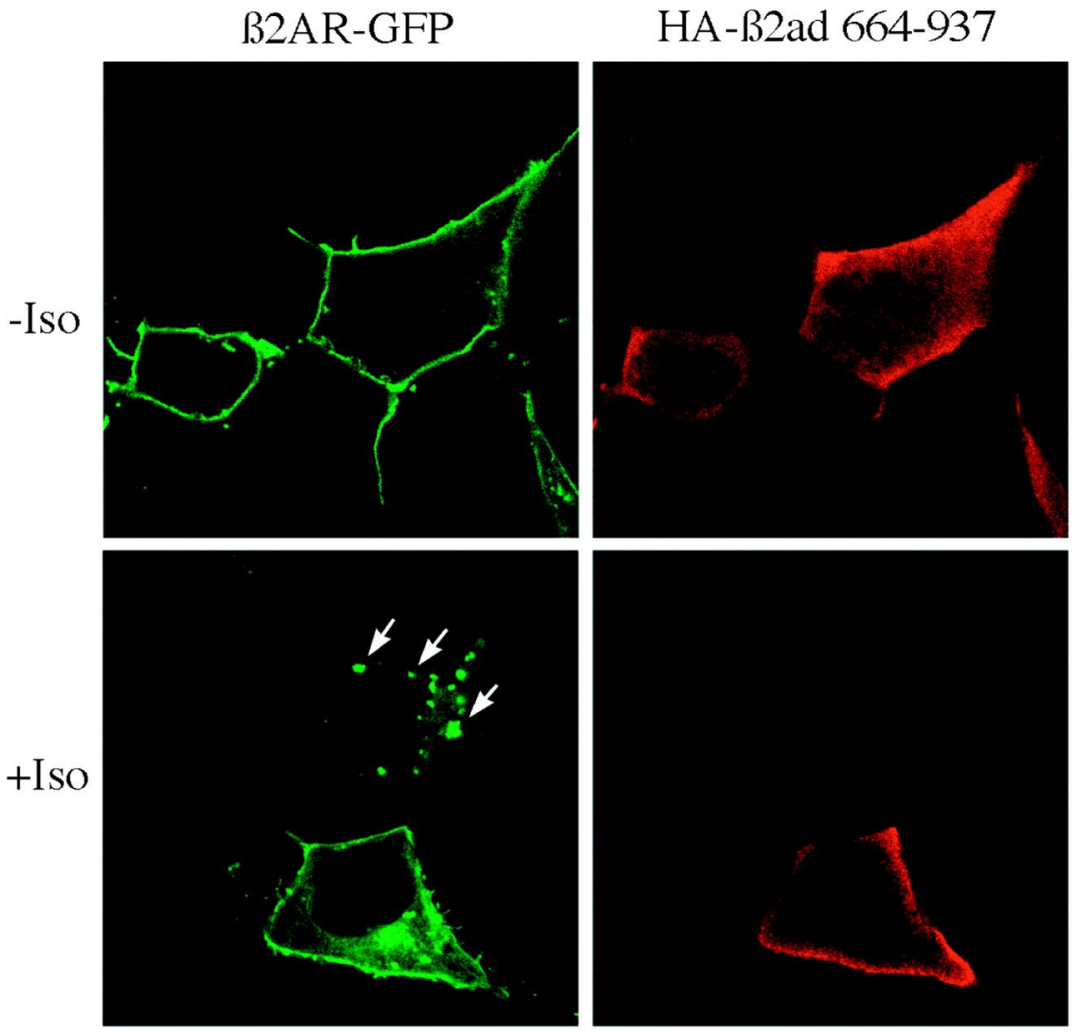
Robert H. Oakley et al. J. Biol. Chem. 1999;274:32248-32257

β-Arrestin binding site is located in the ear domain of β2-adaptin.



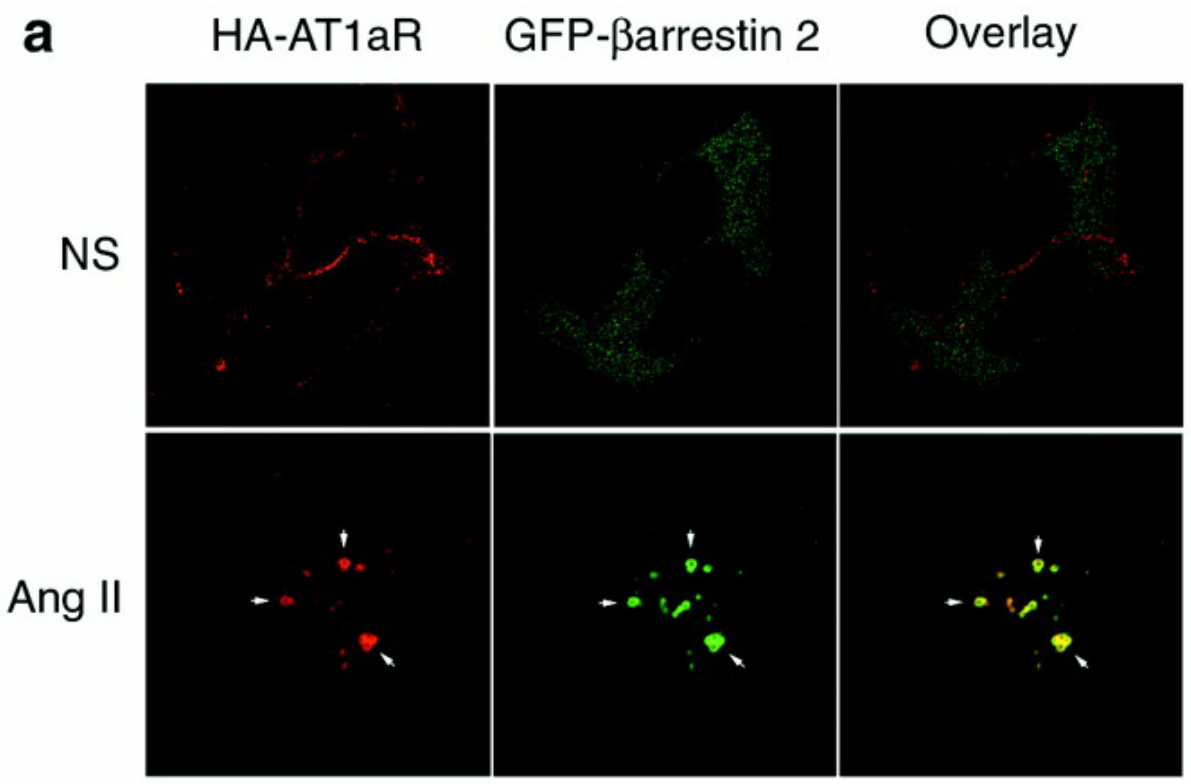
Stéphane A. Laporte et al. J. Biol. Chem. 2002;277:9247-9254

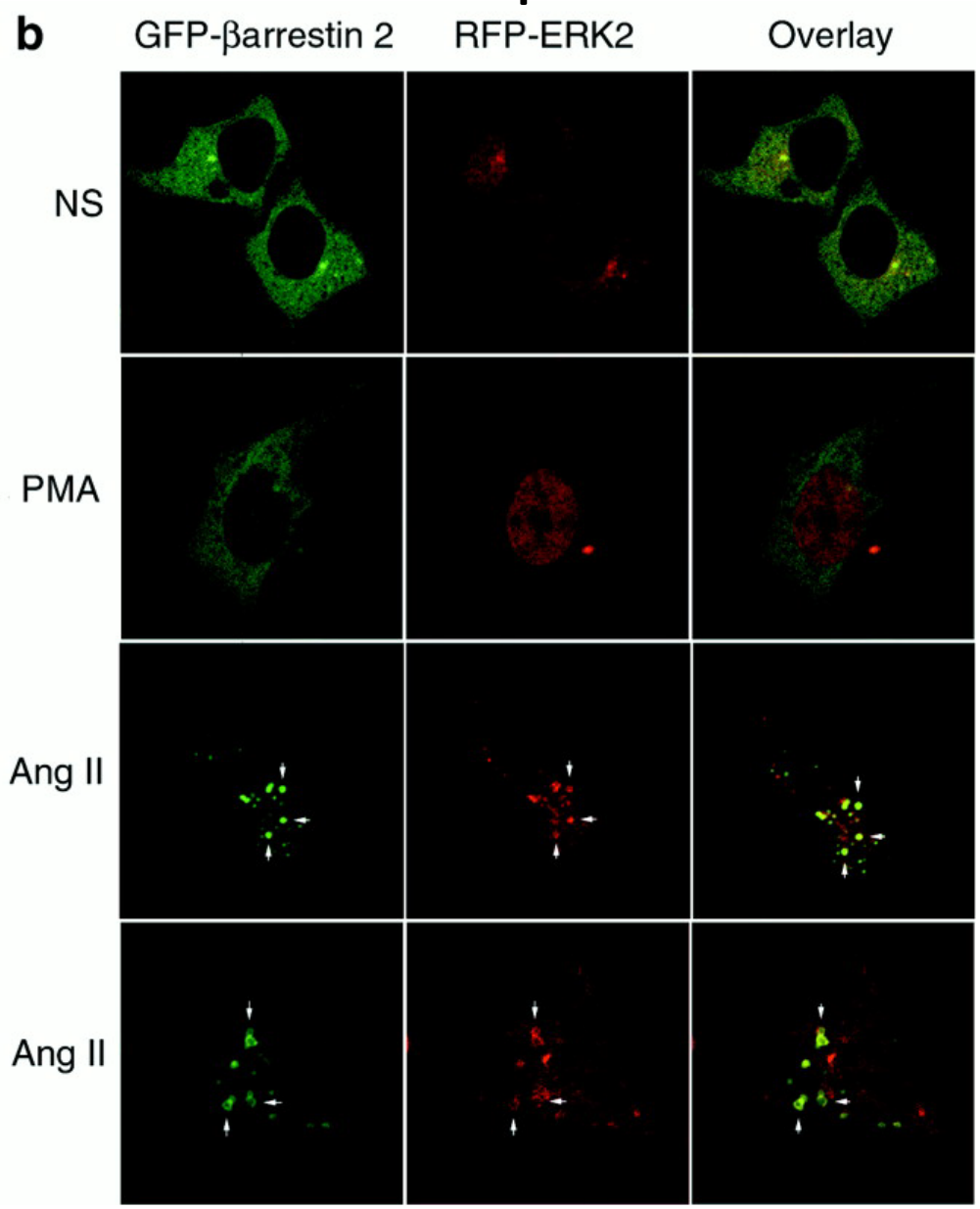
Overexpression β2-adaptin C-terminal subdomain inhibits the agonist-mediated internalization of β2AR.



Stéphane A. Laporte et al. J. Biol. Chem. 2002;277:9247-9254

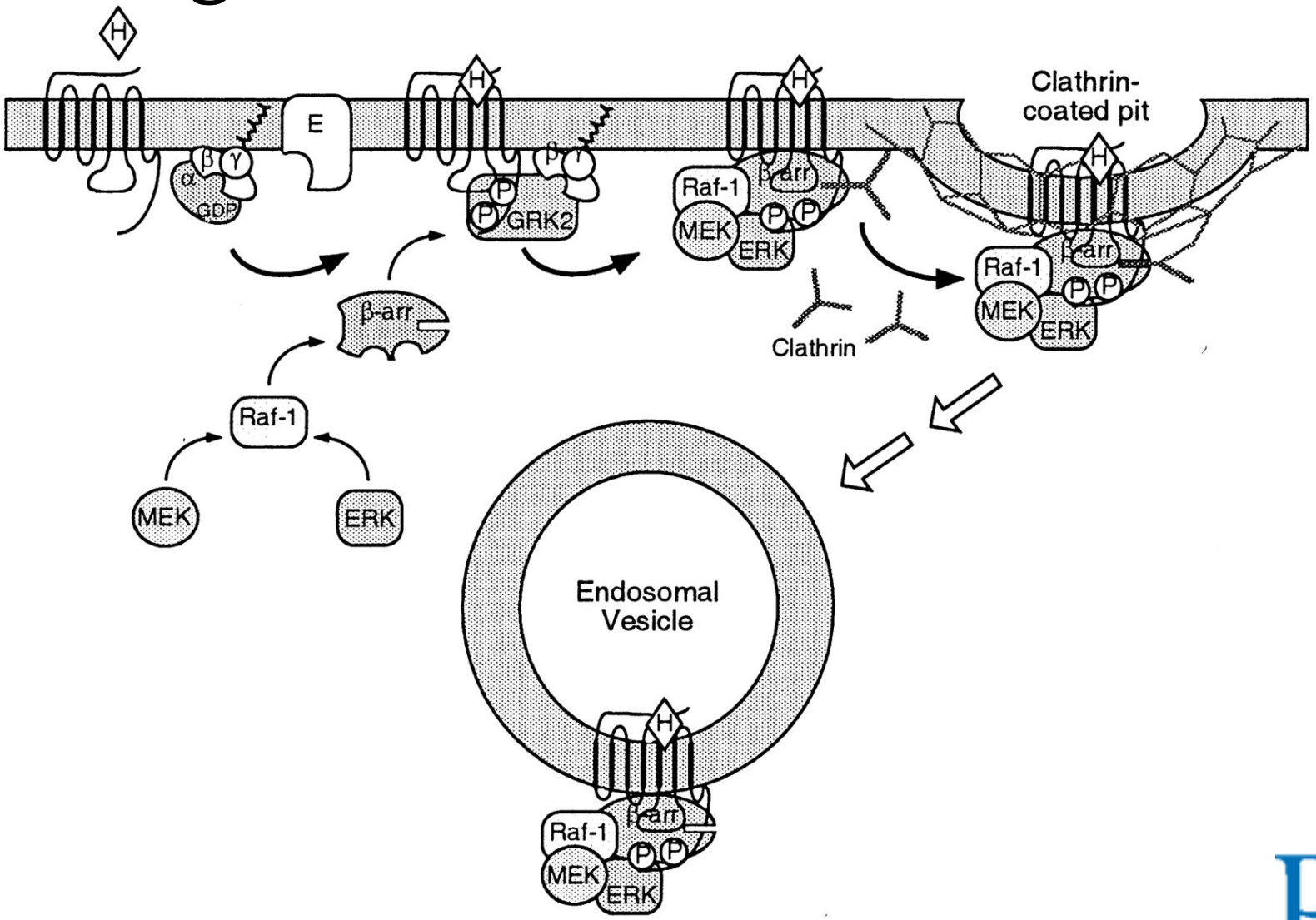
Internalized AT_1R colocalizes with β-arrestin and pERK





Louis M. Luttrell et al. PNAS 2001;98:5:2449-2454

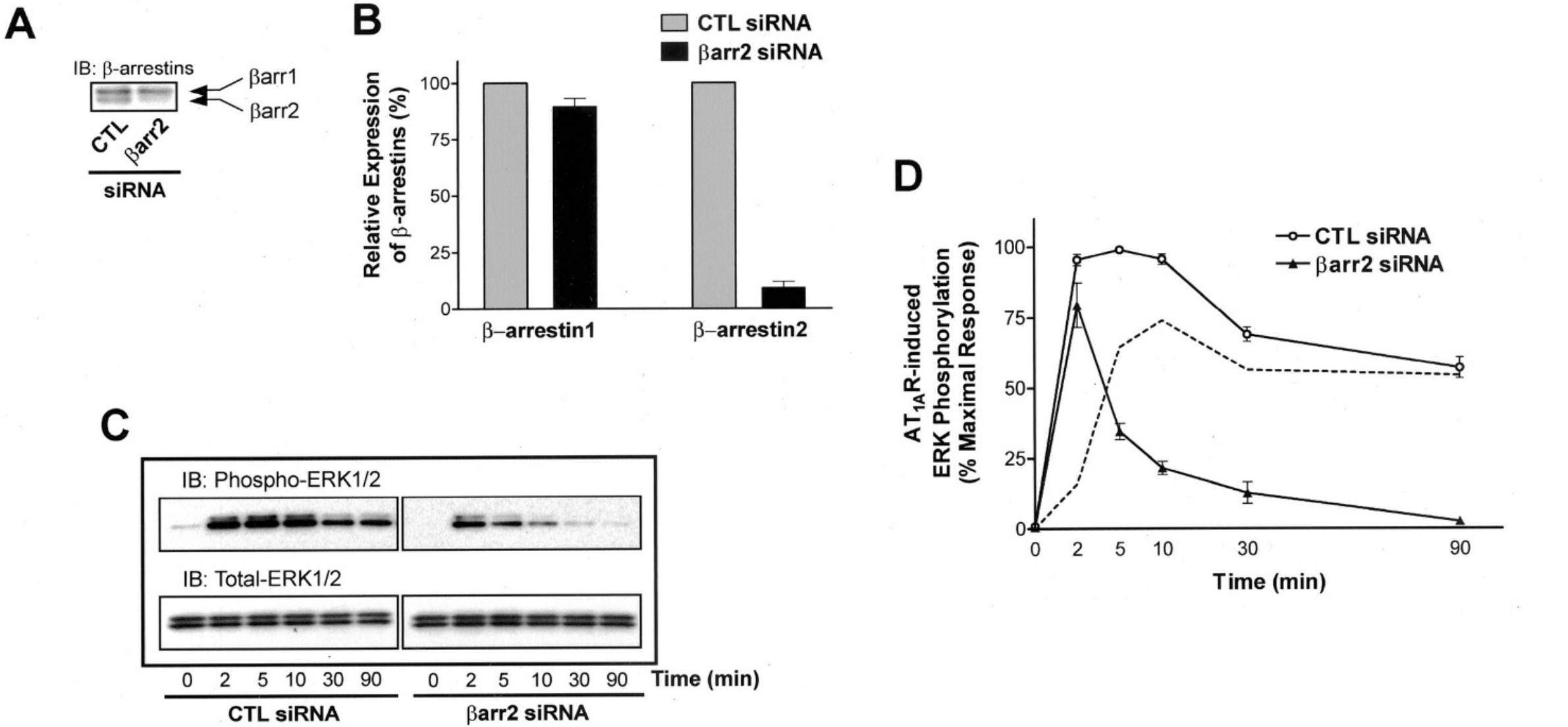
β-arrestin “signalosomes”



Louis M. Luttrell et al. PNAS 2001;98:5:2449-2454

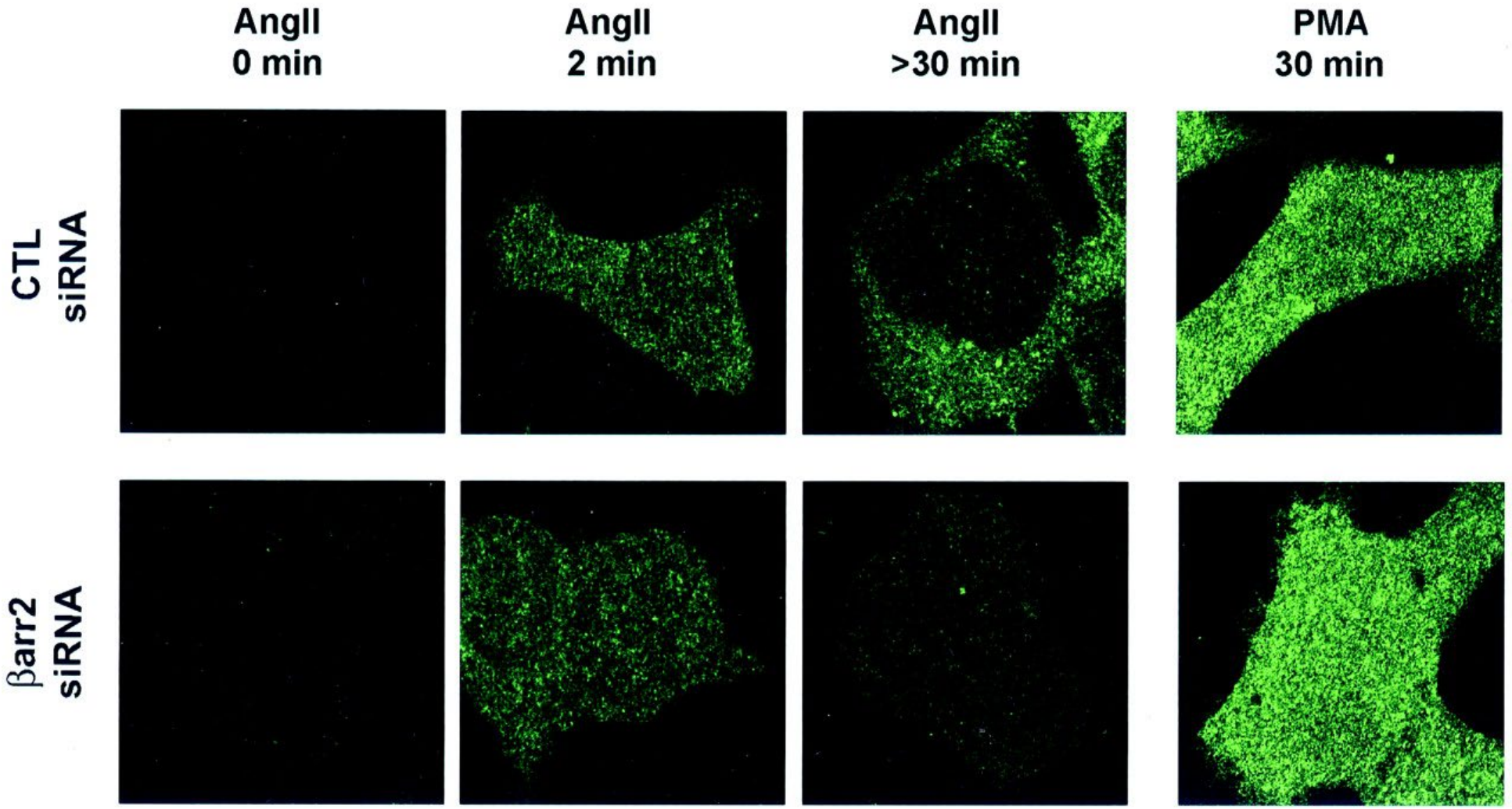
PNAS

Effects of siRNA-suppressed β-arrestin2 (βarr2) expression on the kinetic pattern of ERK1/2 activation following stimulation of the AT1A receptor.



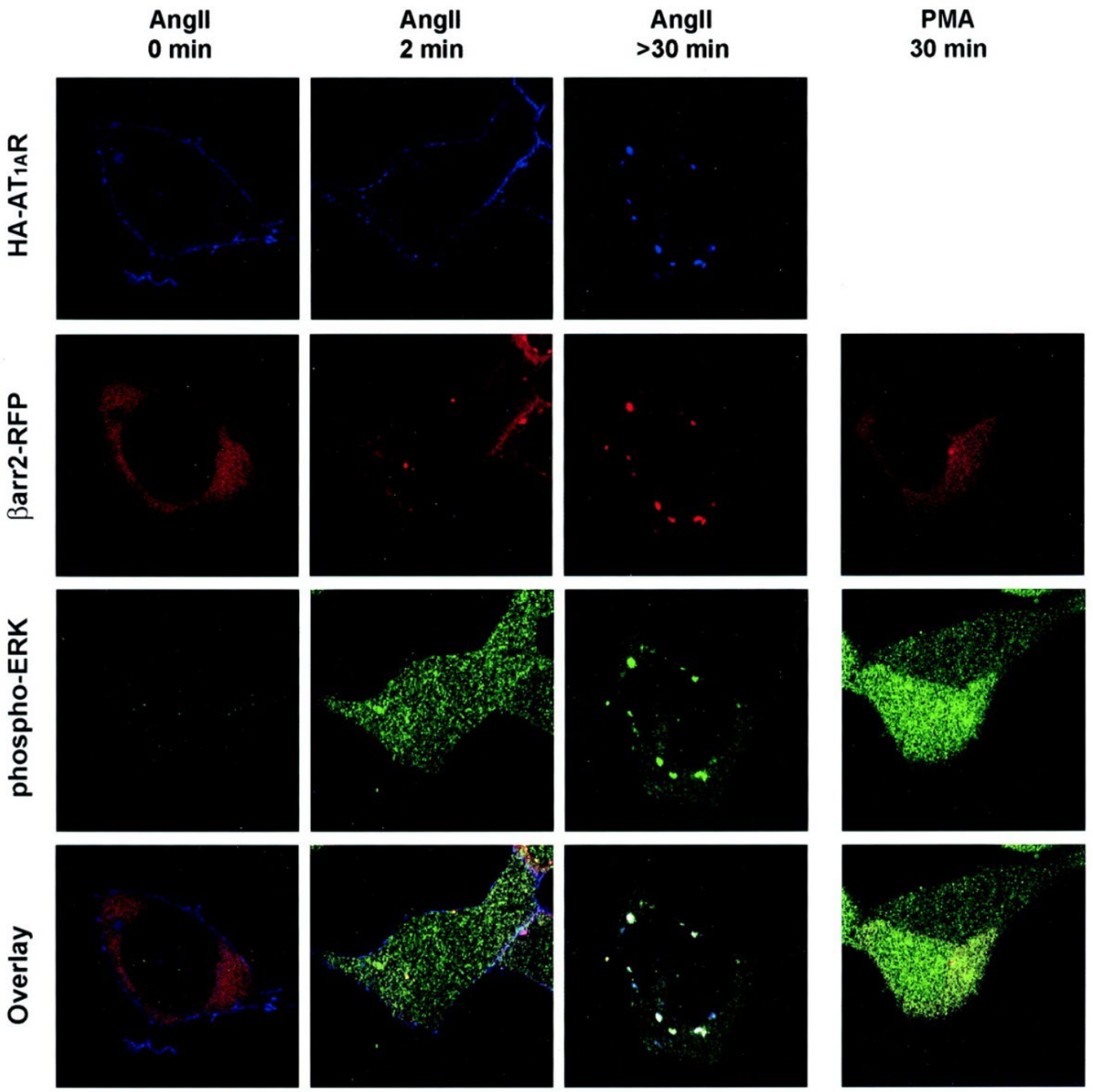
Seungkirl Ahn et al. J. Biol. Chem. 2004;279:35518-35525

Effects of silencing β-arrestin2 (βarr2) expression on the subcellular distribution of phospho-ERK1/2 following different periods of stimulation of the AT1A receptor.



Seungkirl Ahn et al. J. Biol. Chem. 2004;279:35518-35525

Time-dependent subcellular distribution of phospho-ERK1/2 and β-arrestin2 (βarr2)-RFP after stimulation of the AT1A receptor.

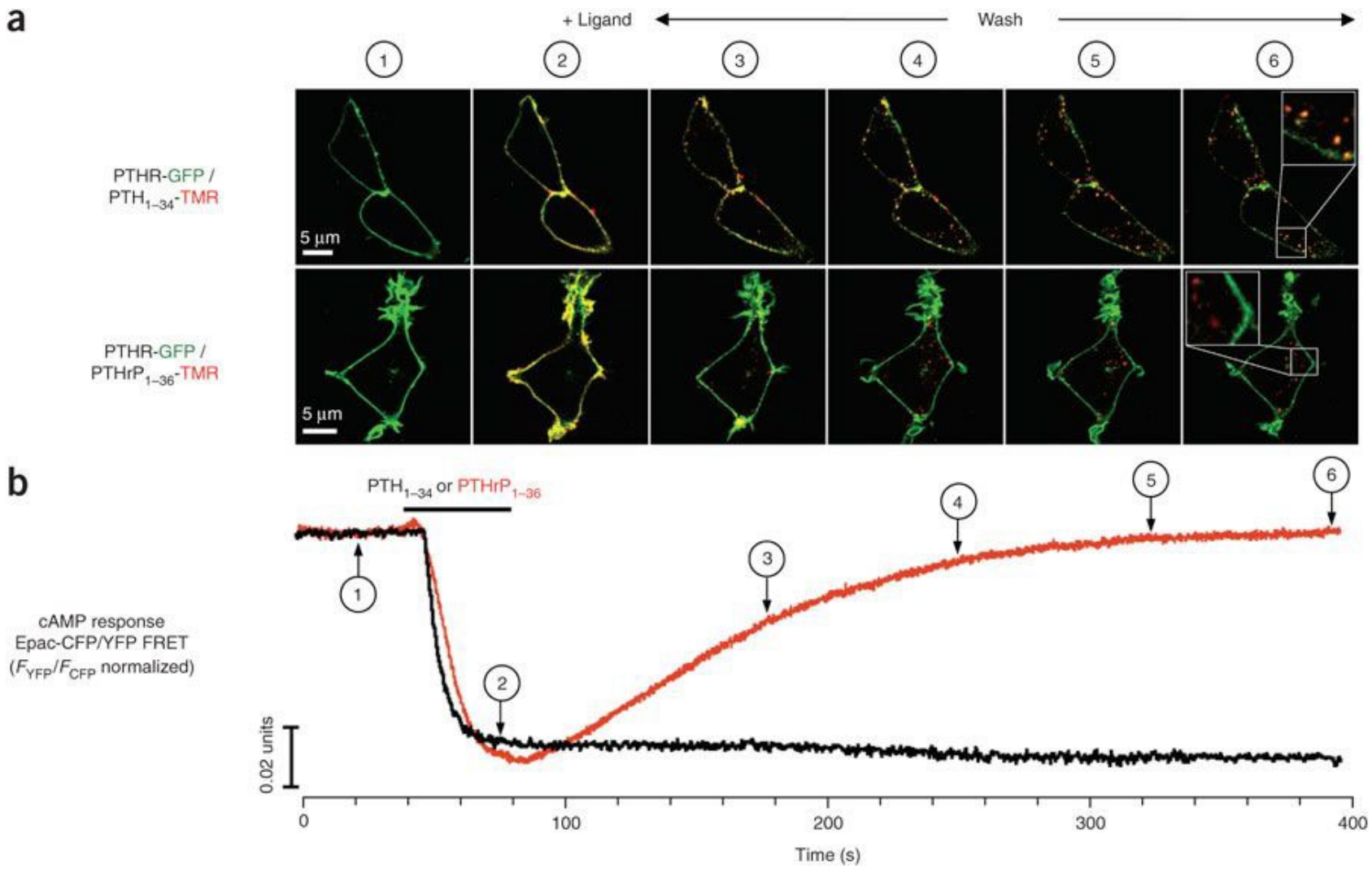


JBC

Seungkirl Ahn et al. J. Biol. Chem. 2004;279:35518-35525
The American Society for Biochemistry and Molecular Biology, Inc.

G protein endosomal signaling

Endosomal cAMP generation

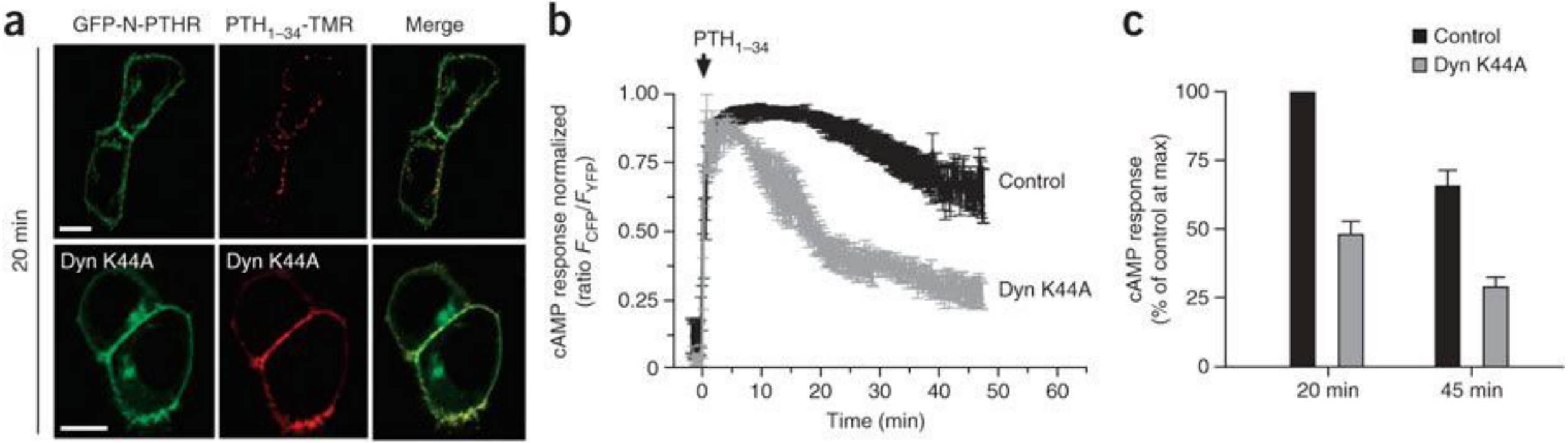


PTHrP1-36
Does not
internalize

PTH1-34
internalizes

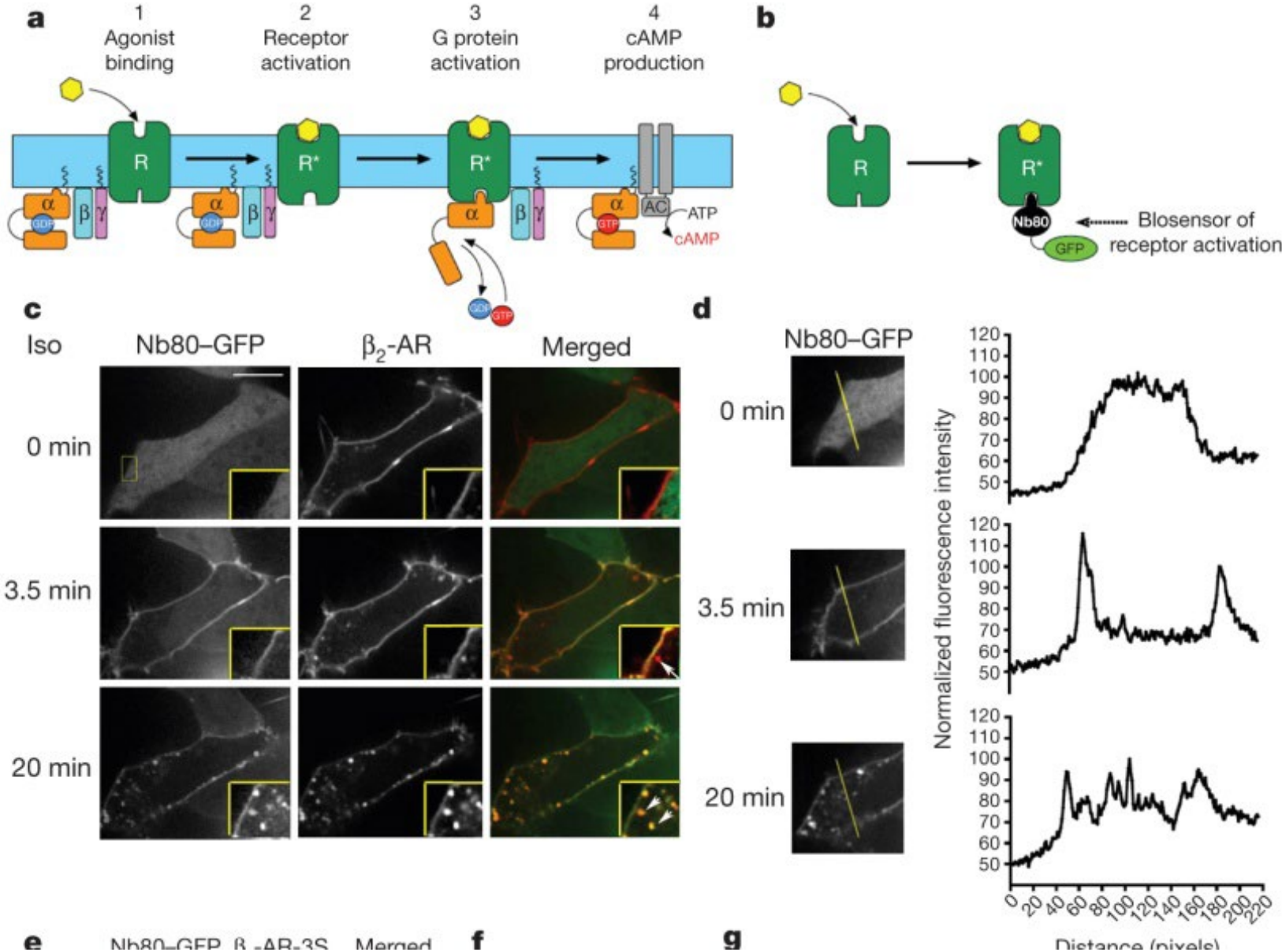
Nat Chem Biol. 2009 Oct; 5(10): 734–742.

Inhibiting endocytosis decreases cAMP



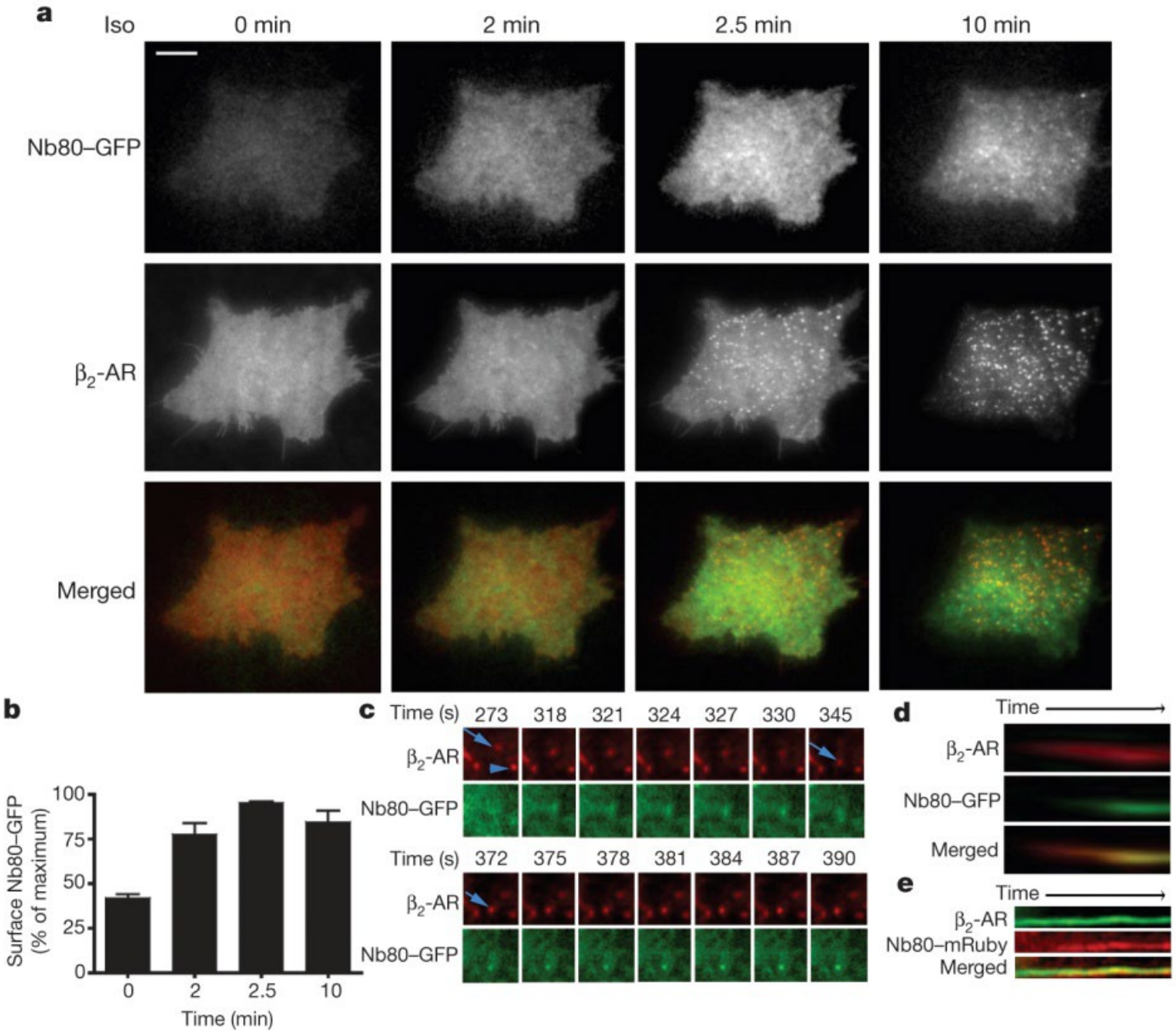
Nat Chem Biol. 2009 Oct; 5(10): 734–742.

Nb80–GFP detects activated β_2-ARs in the plasma membrane and endosomes.



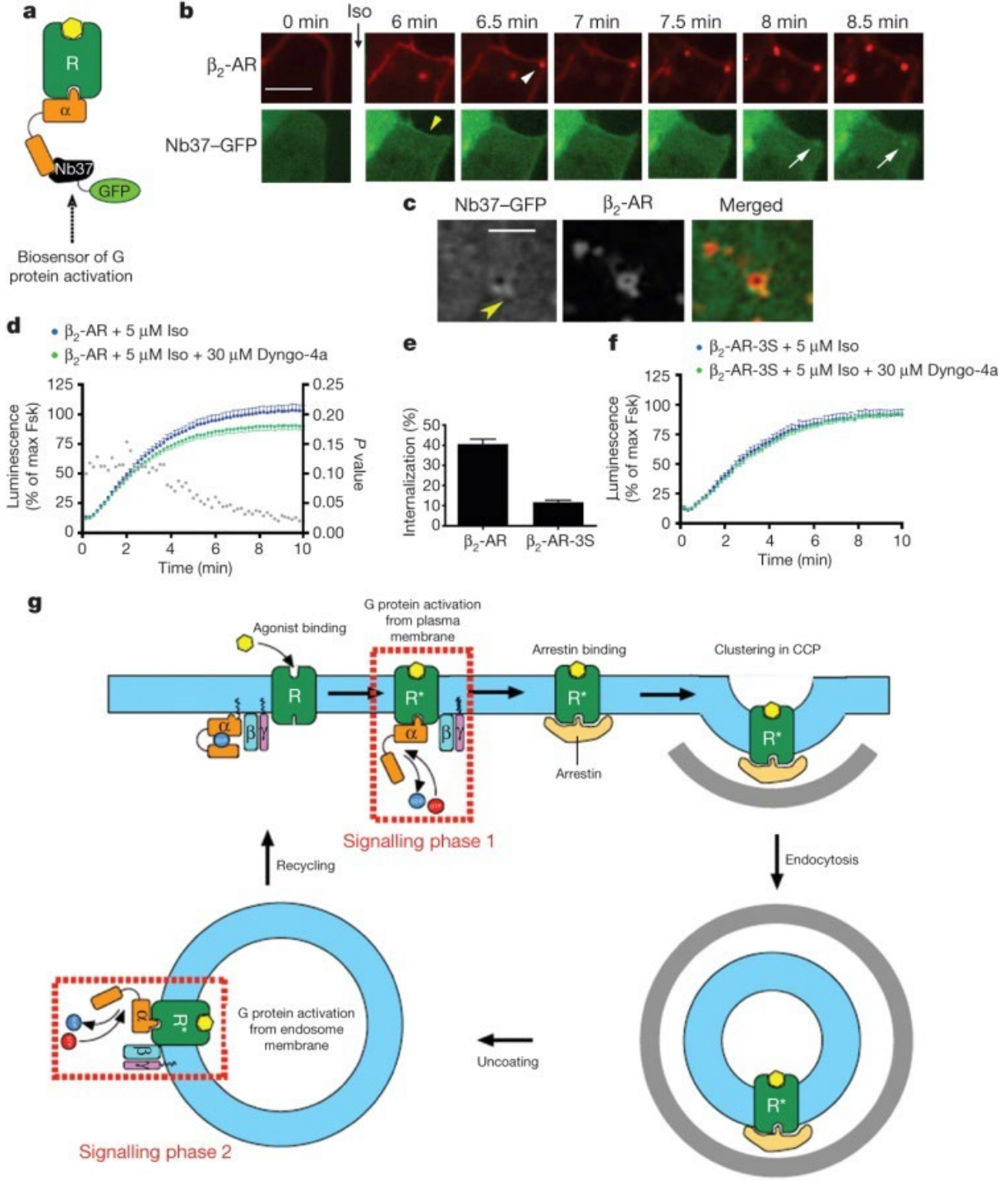
R Irannejad *et al. Nature* **000**, 1-5 (2013) doi:10.1038/nature12000

Nb80–GFP accumulates on β_2-AR-containing endosomes after their formation.



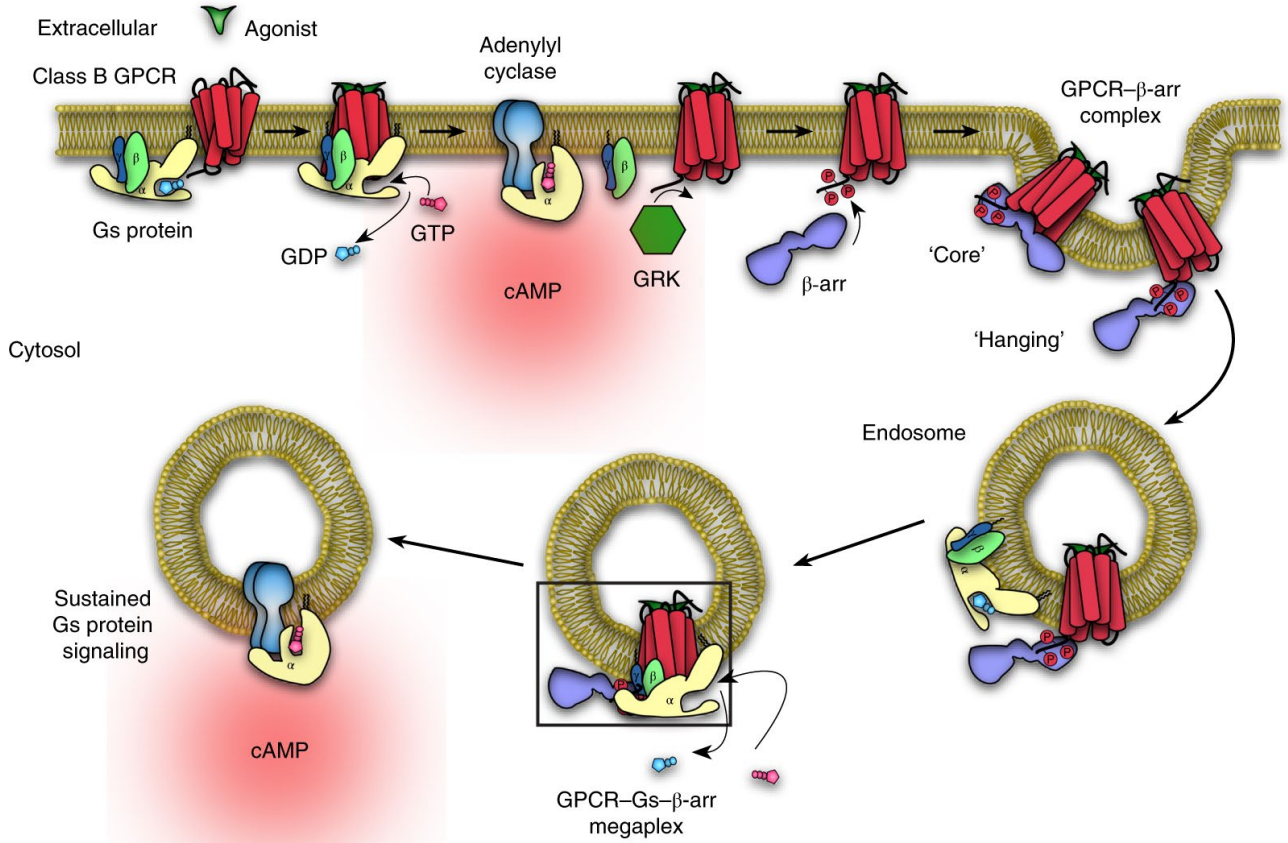
R Irannejad *et al. Nature* **000**, 1-5 (2013) doi:10.1038/nature12000

Internalized β_2-ARs contribute to the acute cAMP response.



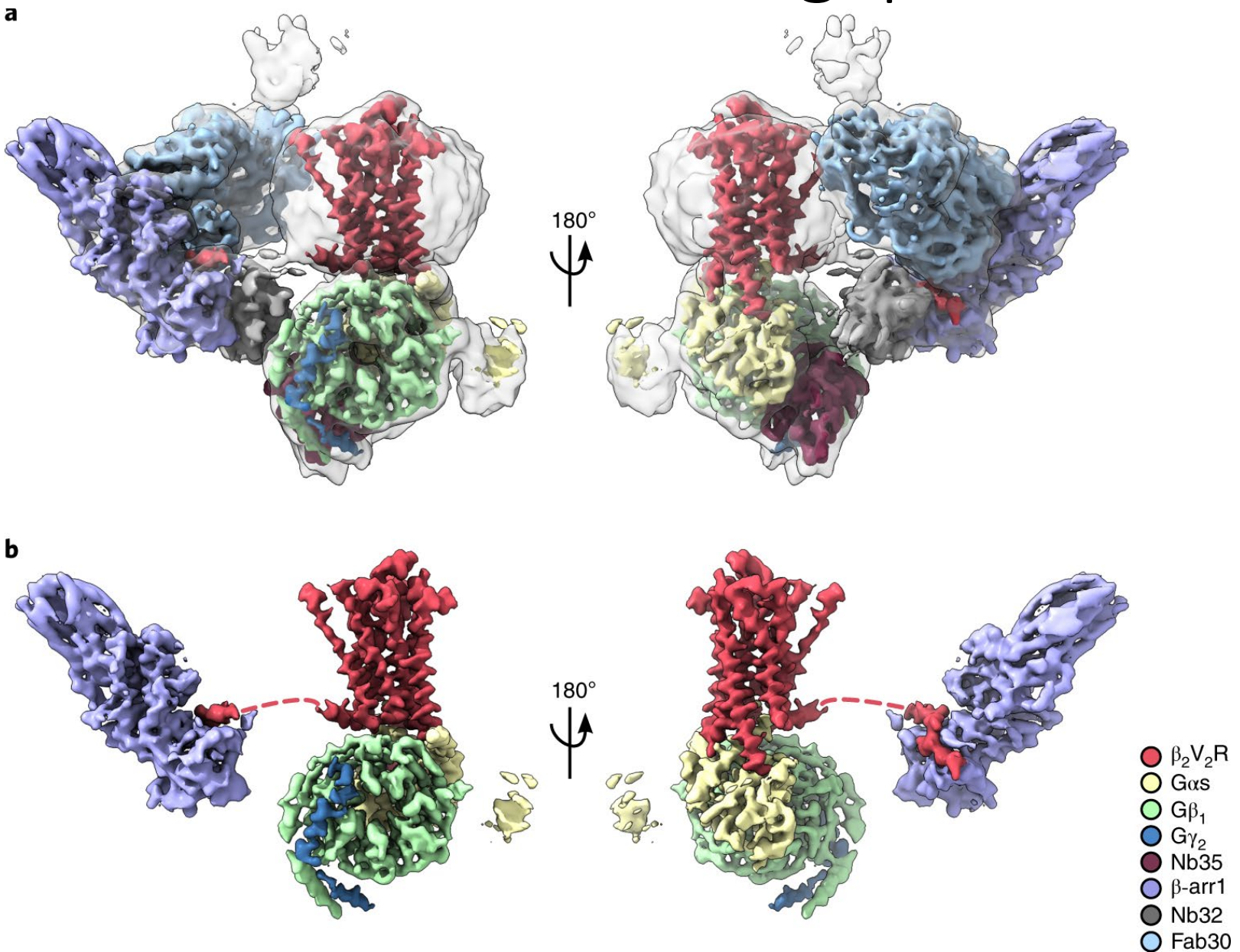
R Irannejad *et al. Nature* **000**, 1-5 (2013) doi:10.1038/nature12000

GPCR Megaplexes as a Source for Endosomal GPCR Signaling?

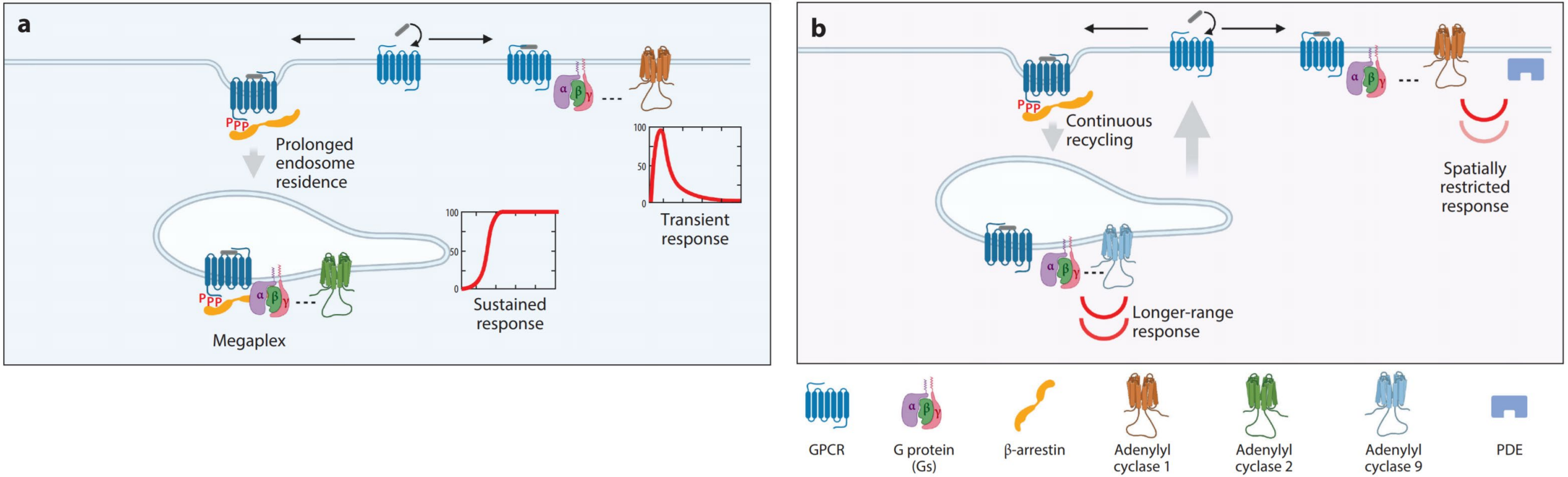


Nature Structural & Molecular Biology volume 26, pages1123–1131(2019)

CryoEM structure of the Megaplex

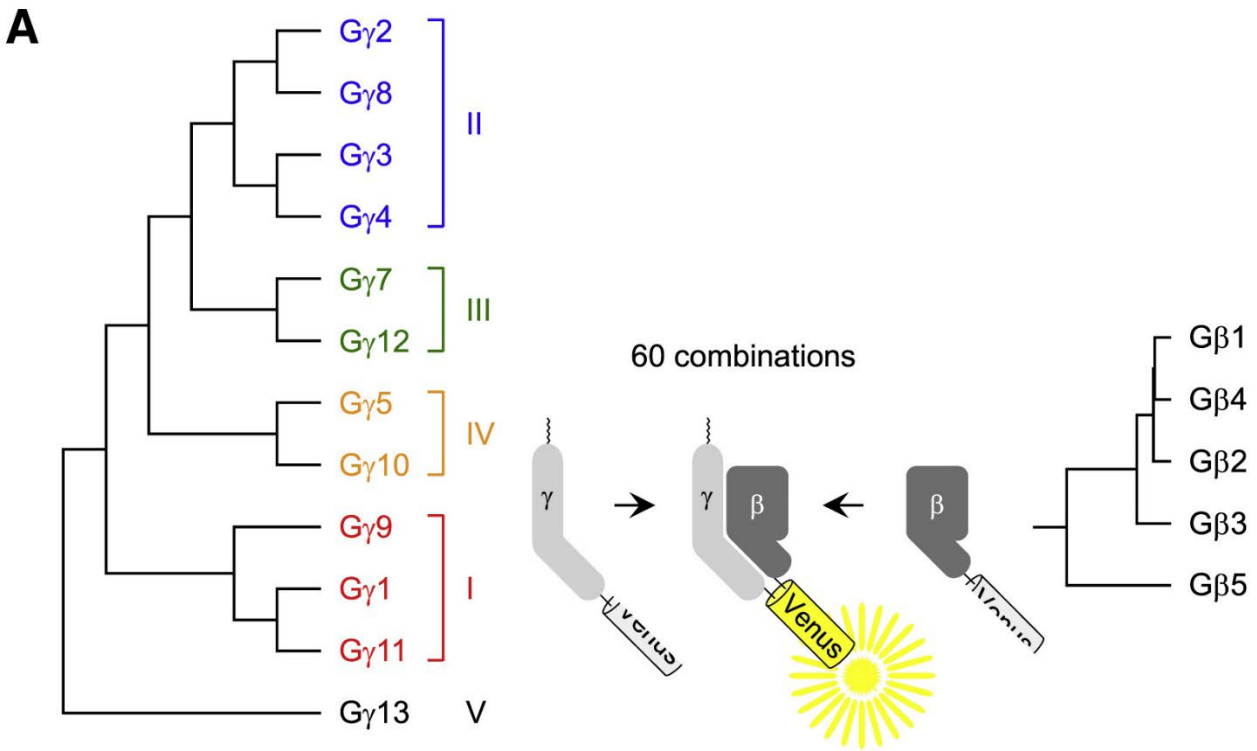


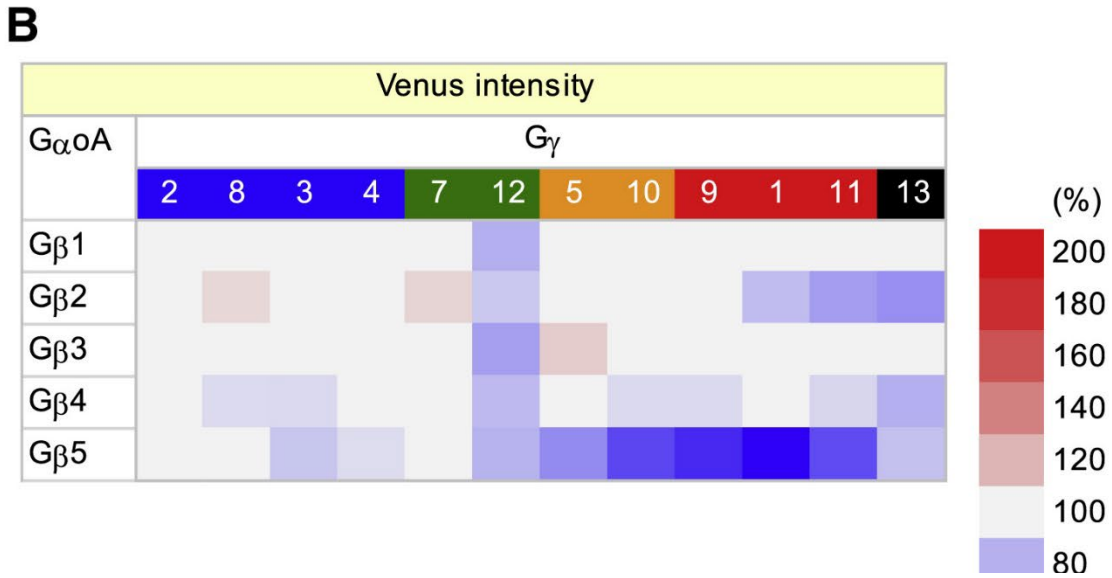
Different spatial and temporal patterns



Annu. Rev. Biochem. 2021. 90:5.1–5.29

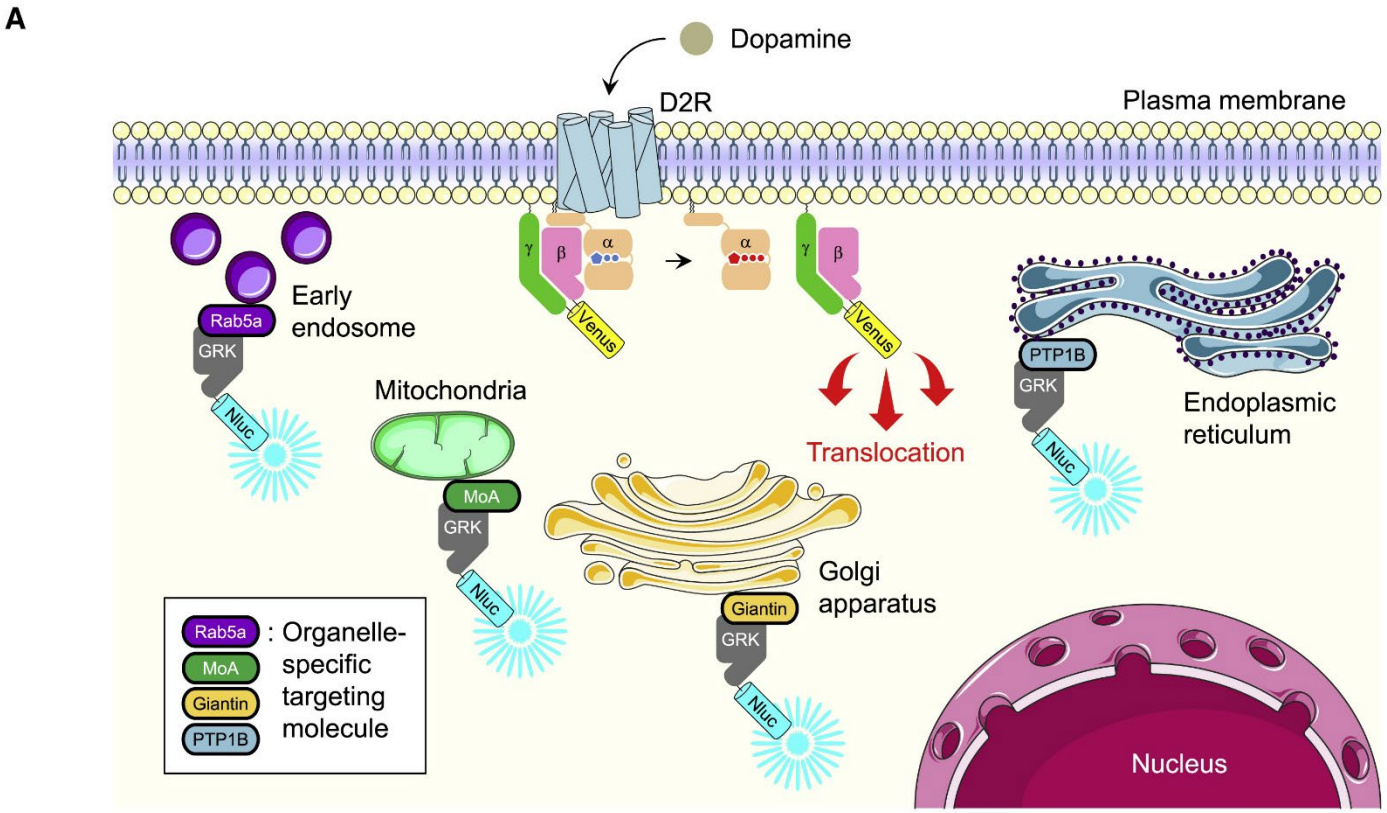
Gβγ: the forgotten signaling molecule

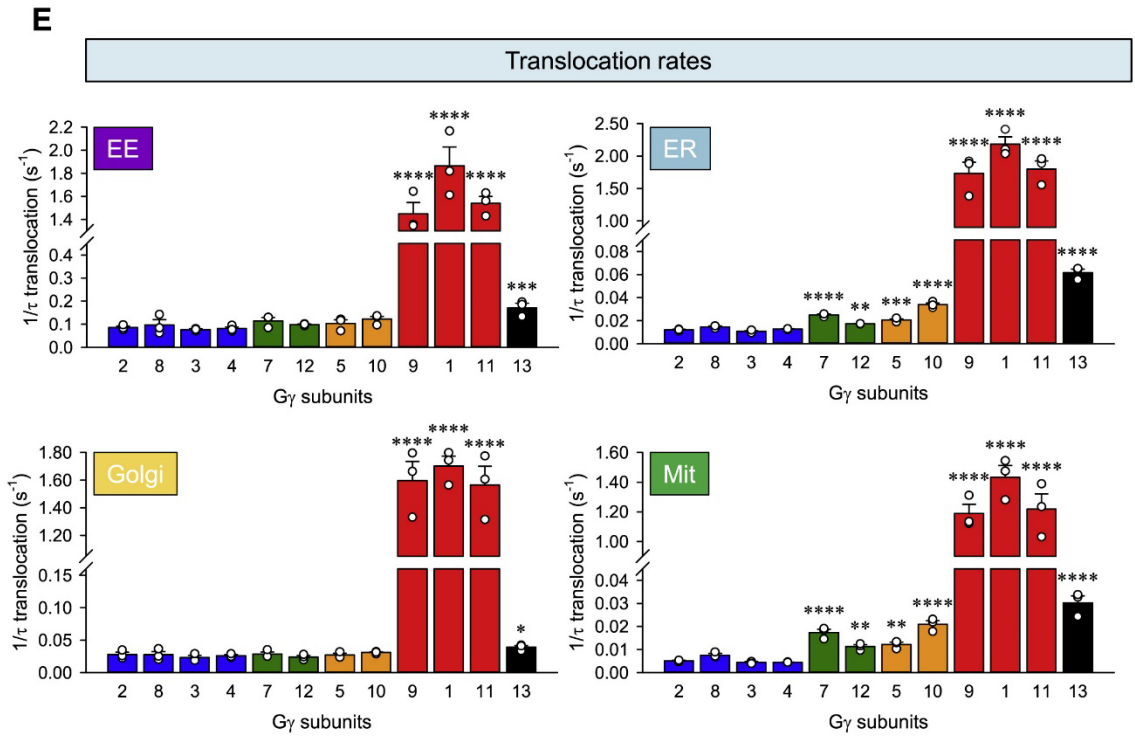




Masuho et al., 2021, *Cell Systems* 12, 1–14

Gβγ can signal at specific subcellular locations



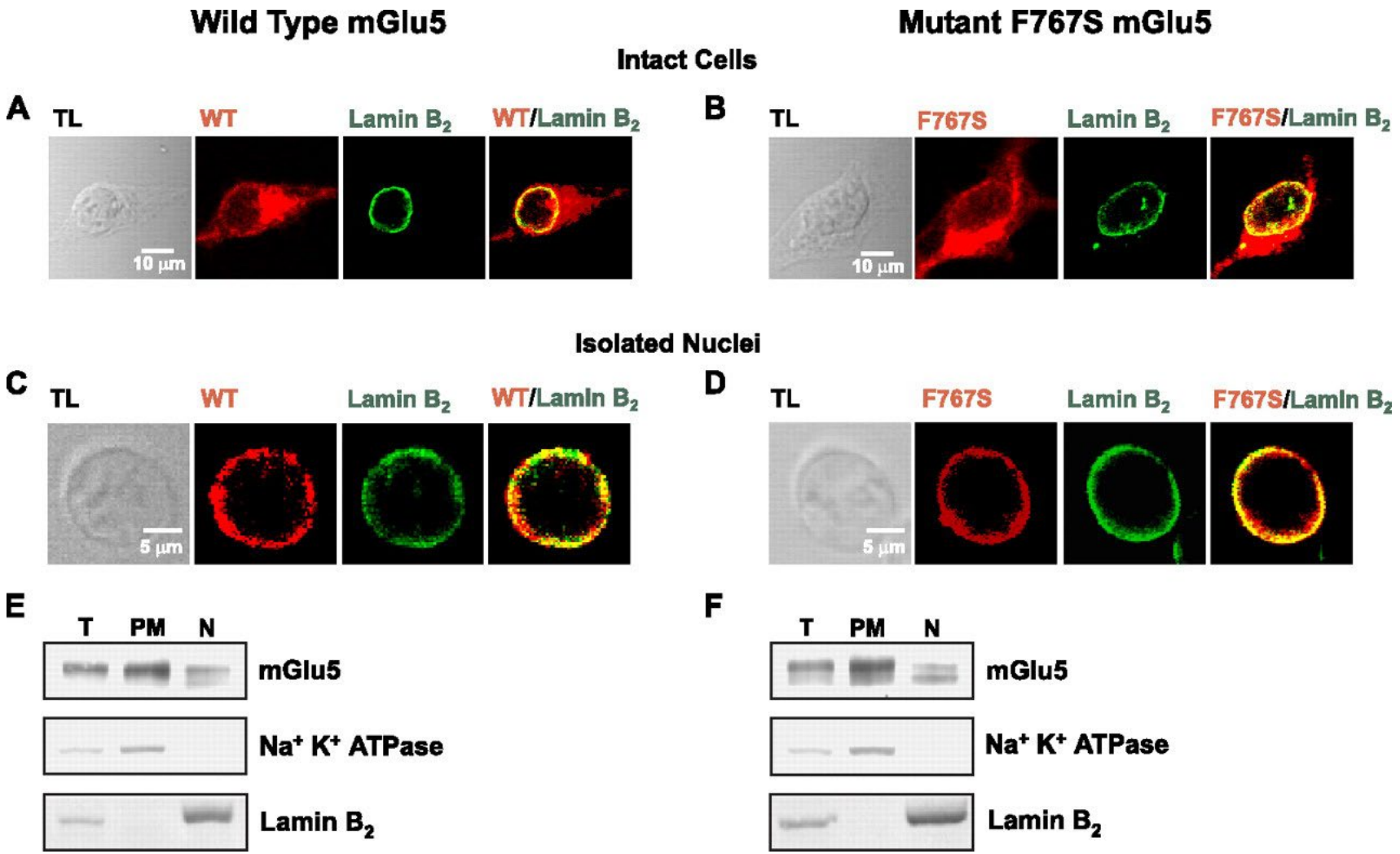


Masuho et al., 2021, *Cell Systems* 12, 1–14

Receptor signaling from other locations

If different ligands promote signaling from different locations, we refer to this as “location bias”

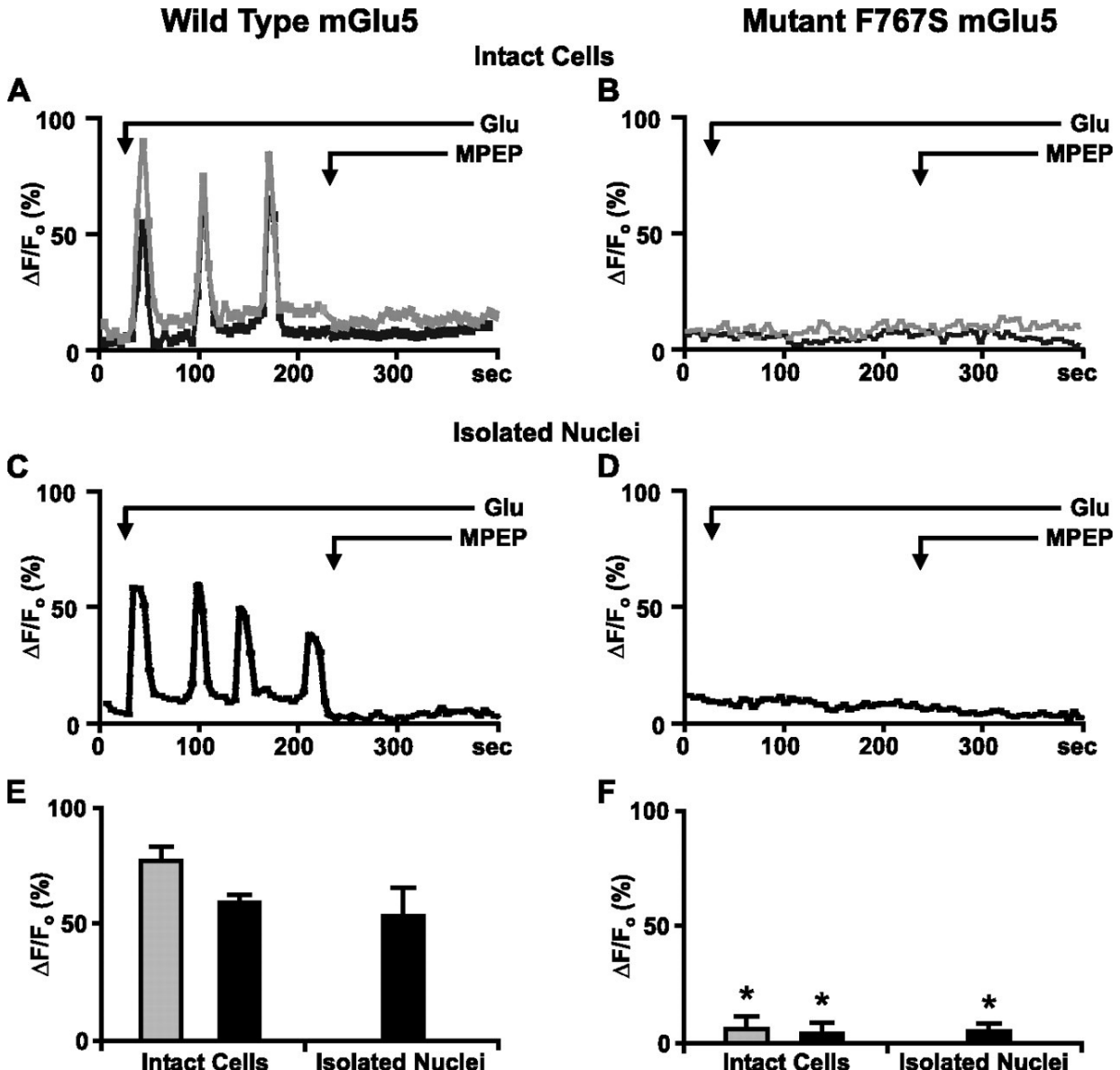
mGlu5 expressed on the nuclear membrane



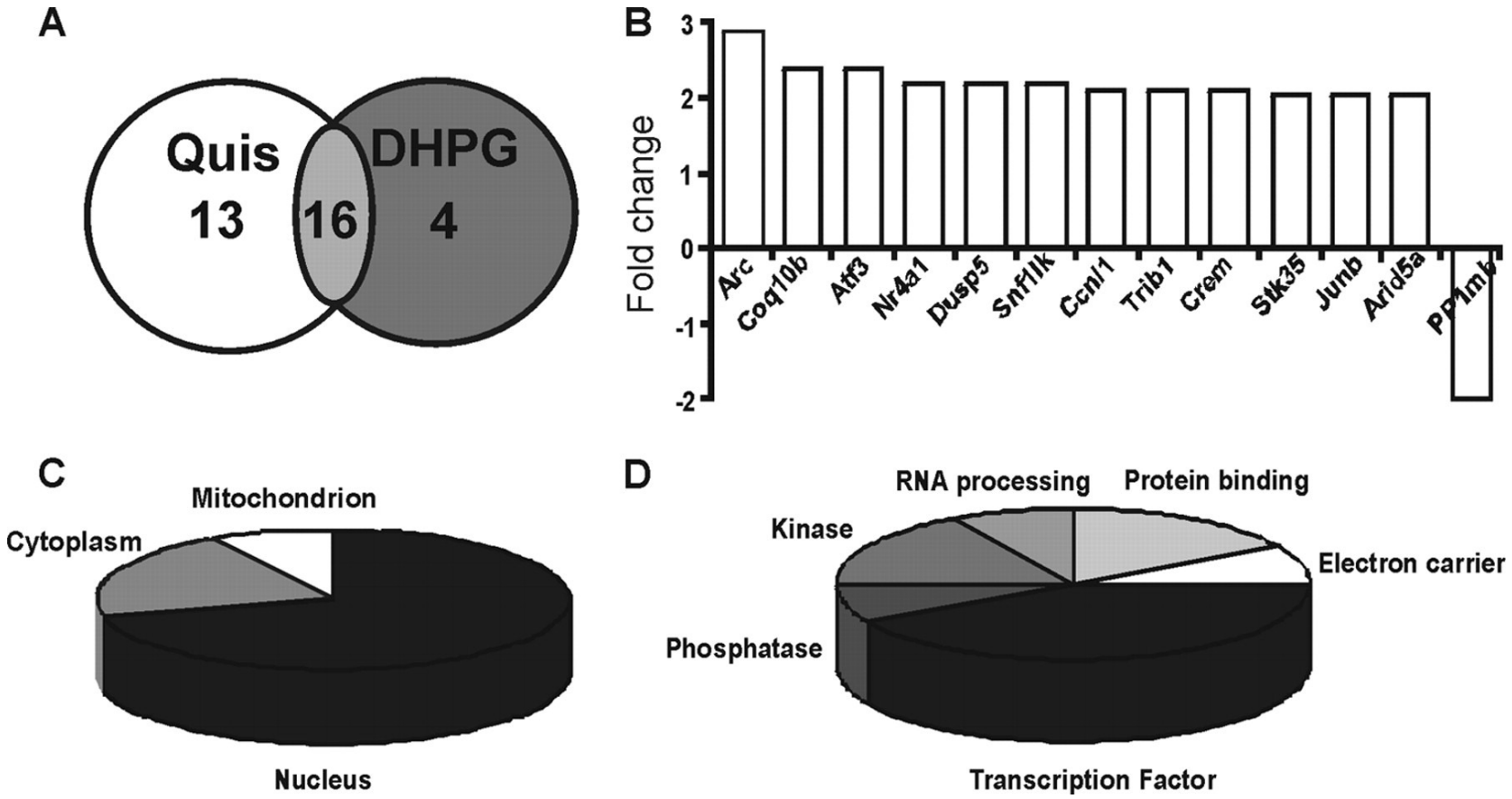
THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 283, NO. 20, pp. 14072–14083,

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 283, NO. 20, pp. 14072–14083,

And couples to nuclear Gq

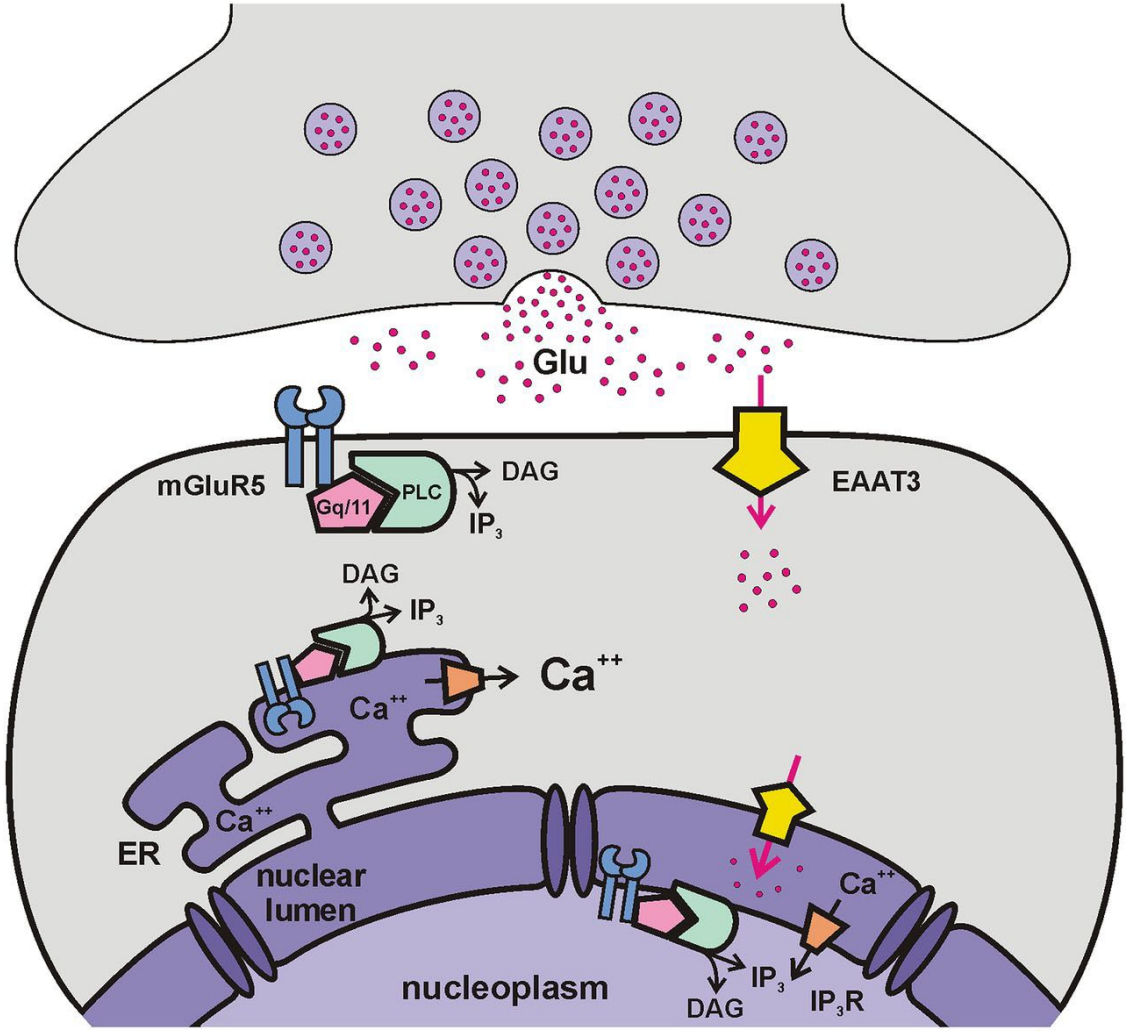


Quis (permeant) vs DHPG (impermeant) – transcriptional changes in neurons



THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 8, pp. 5412–5425, February 17, 2012

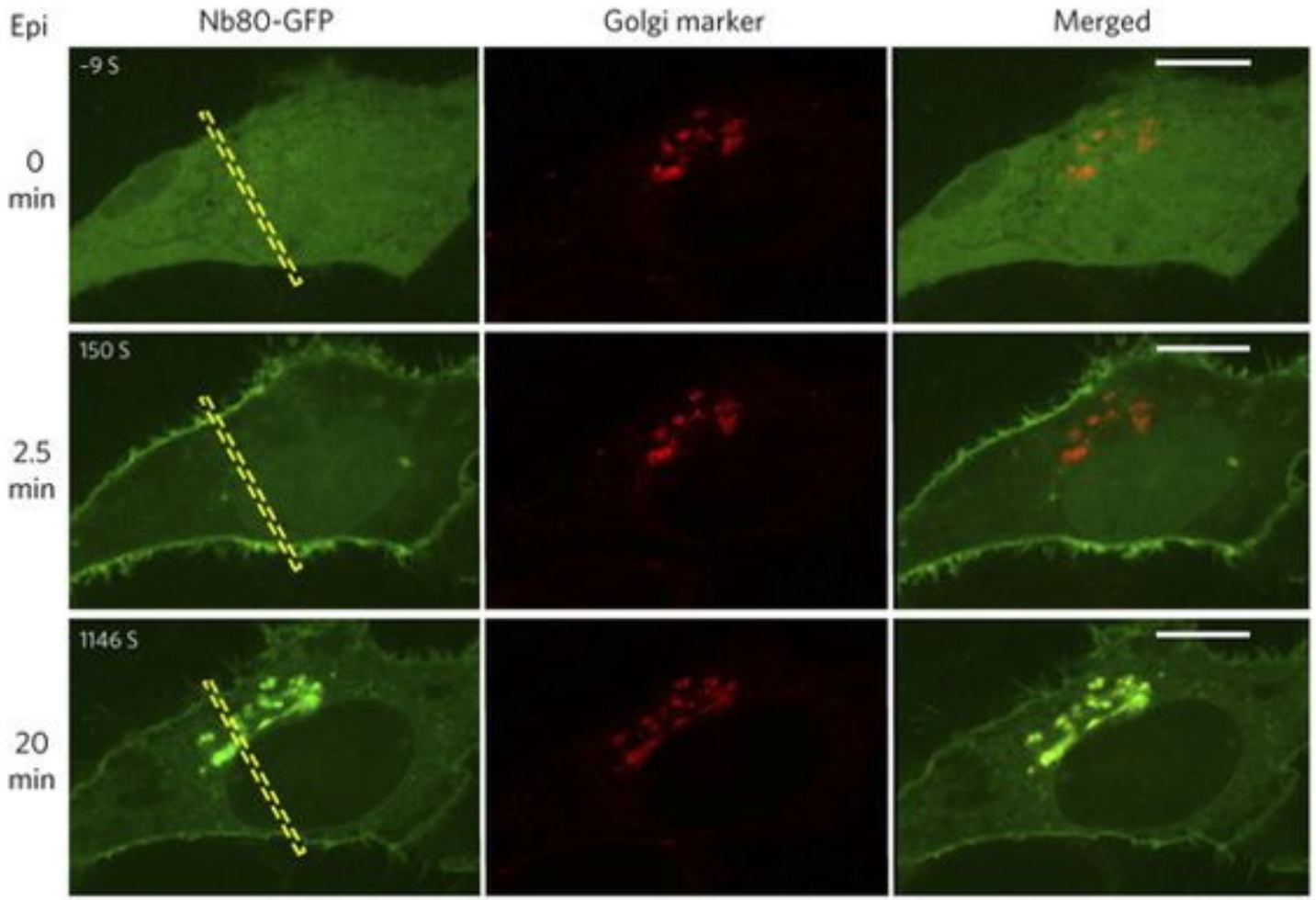
Proposed model of cell surface and intracellular mGlu5 receptor activation by glutamate.



Yuh-Jiin I. Jong et al. Mol Pharmacol 2014;86:774-785

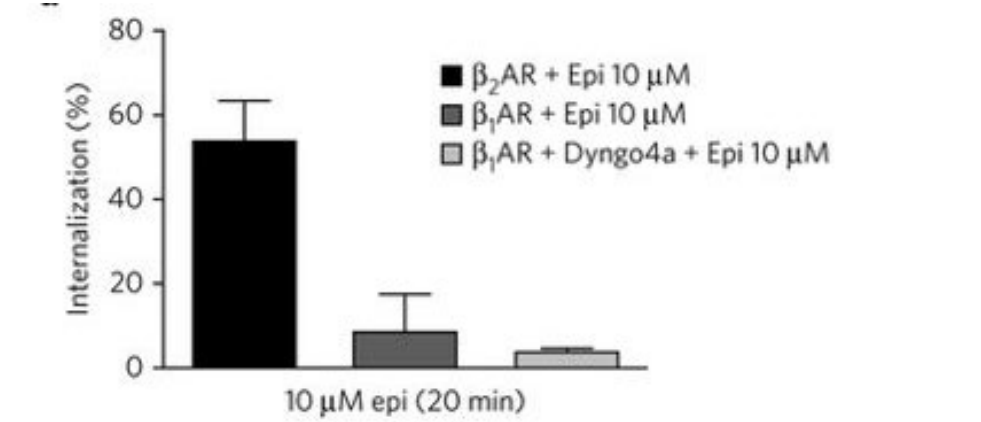
GPCRs in the Golgi

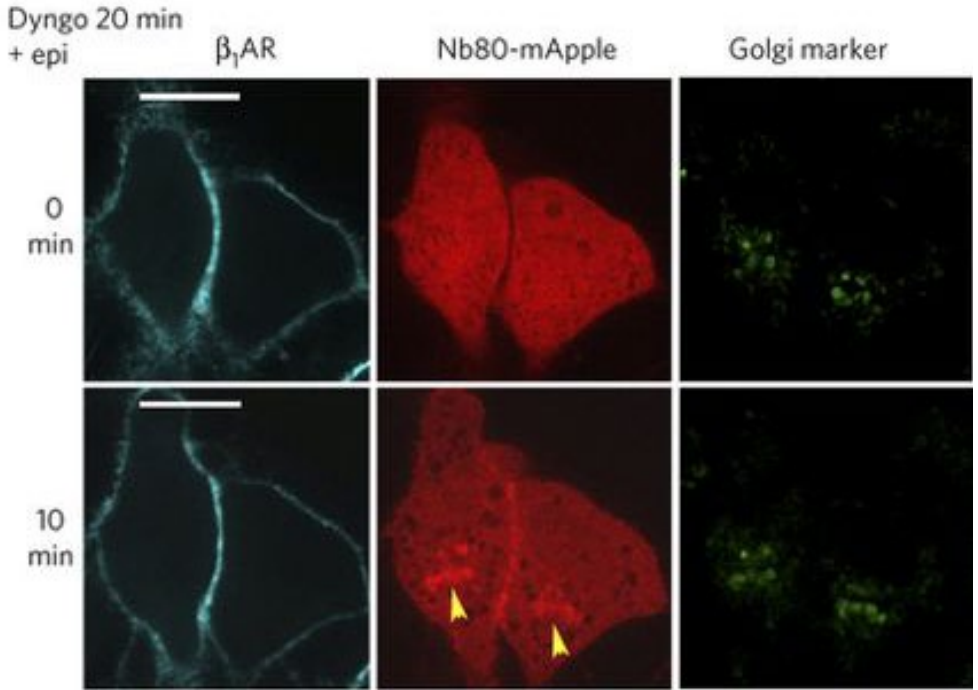
β1AR signaling from Golgi



doi: 10.1038/nchembio.2389

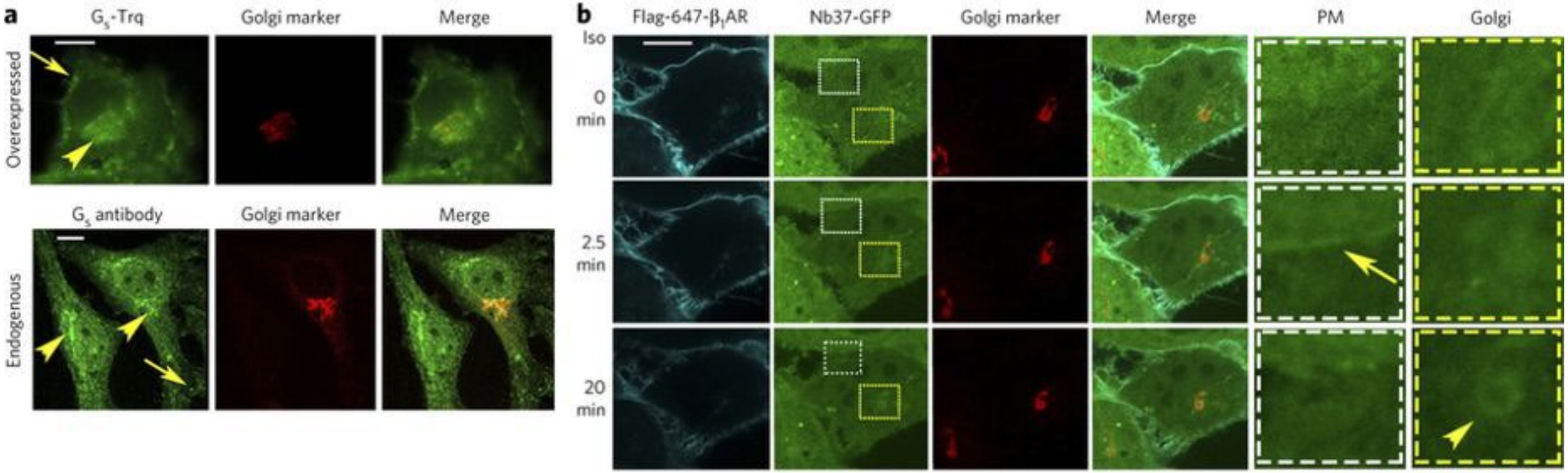
Golgi pool is distinct from endosomal pool





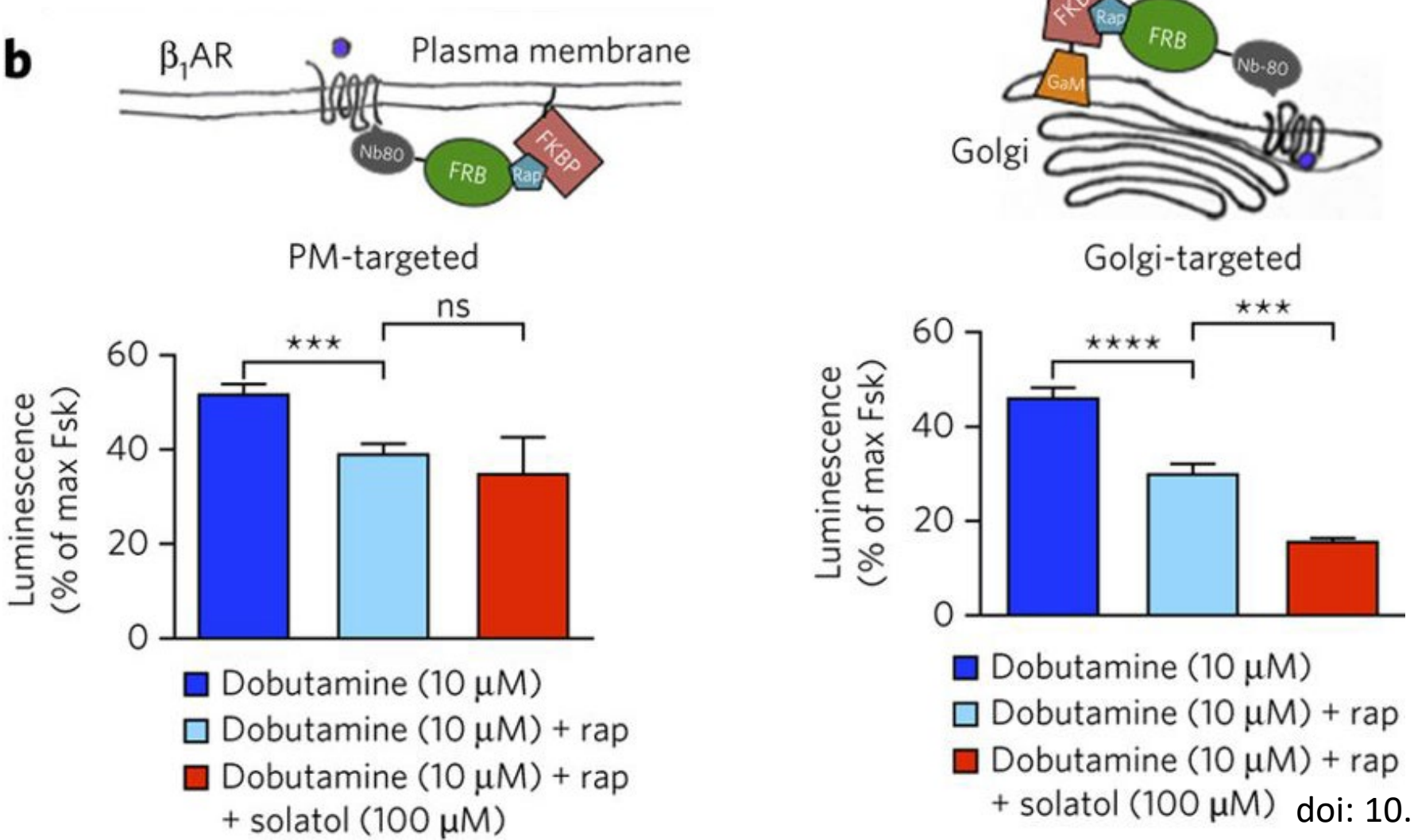
doi: 10.1038/nchembio.2389

Activates Gs at the Golgi



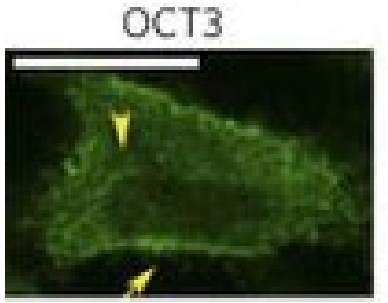
doi: 10.1038/nchembio.2389

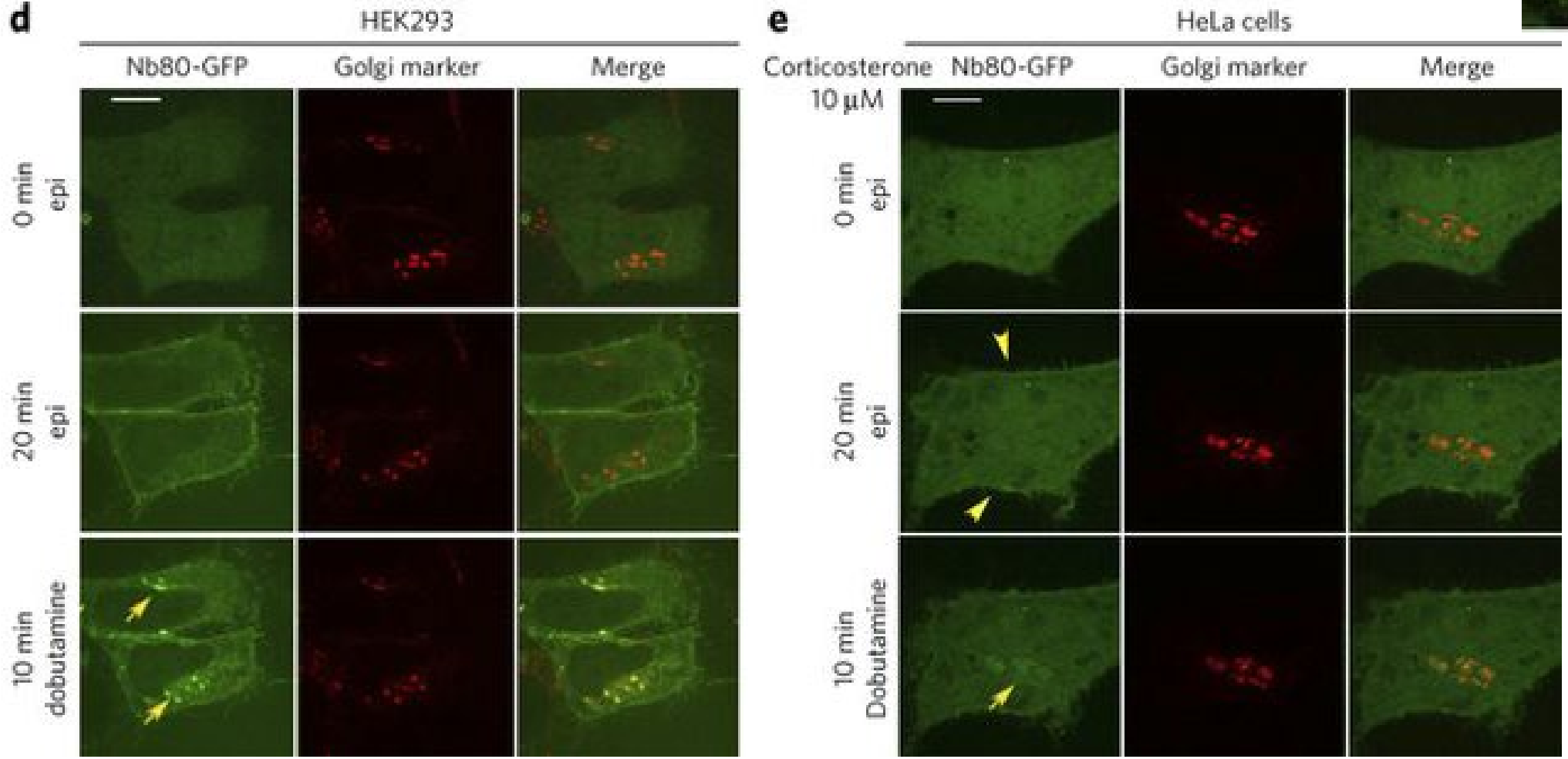
Rapamycin induced recruitment of Nb80 blocks Gs signaling



doi: 10.1038/nchembio.2389

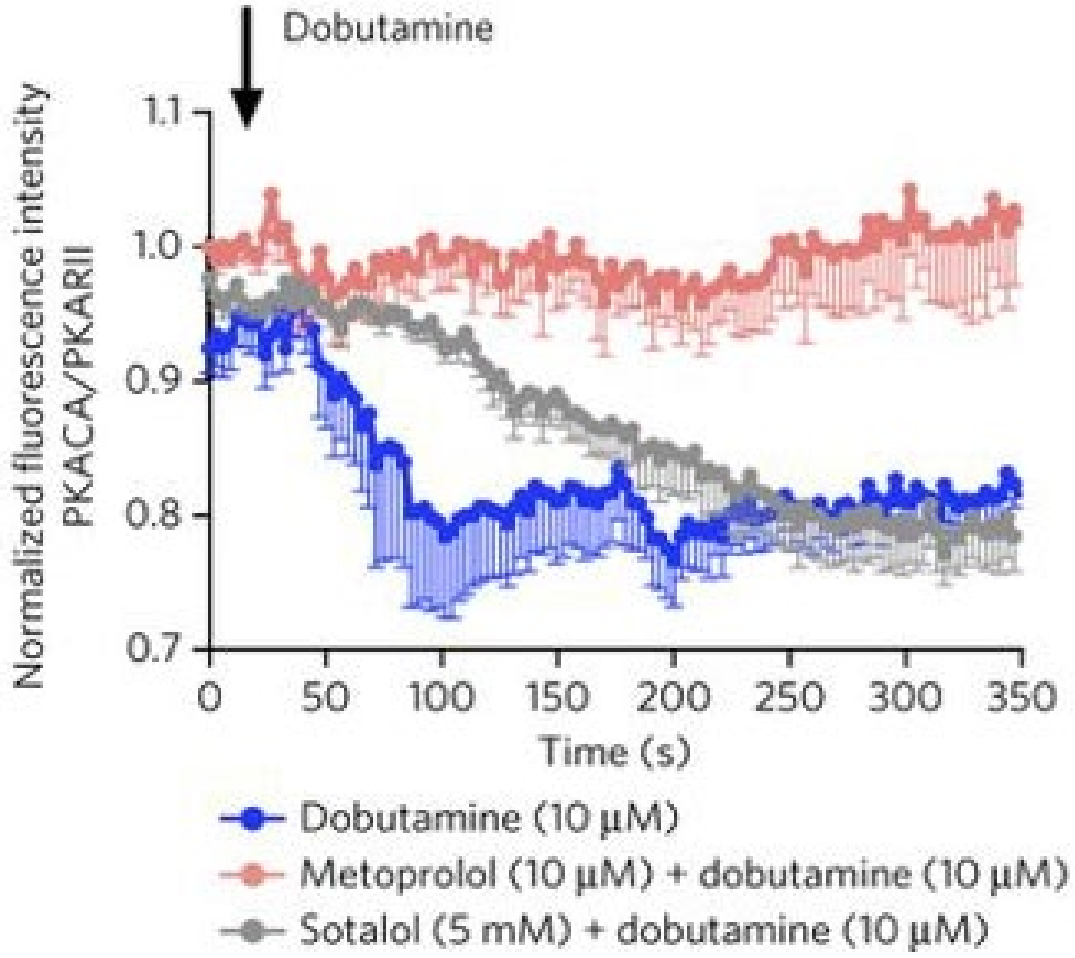
OCT3 transporter needed for impermeant ligands to get to the Golgi





doi: 10.1038/nchembio.2389

Different effects of membrane permeant and impermeant antagonists

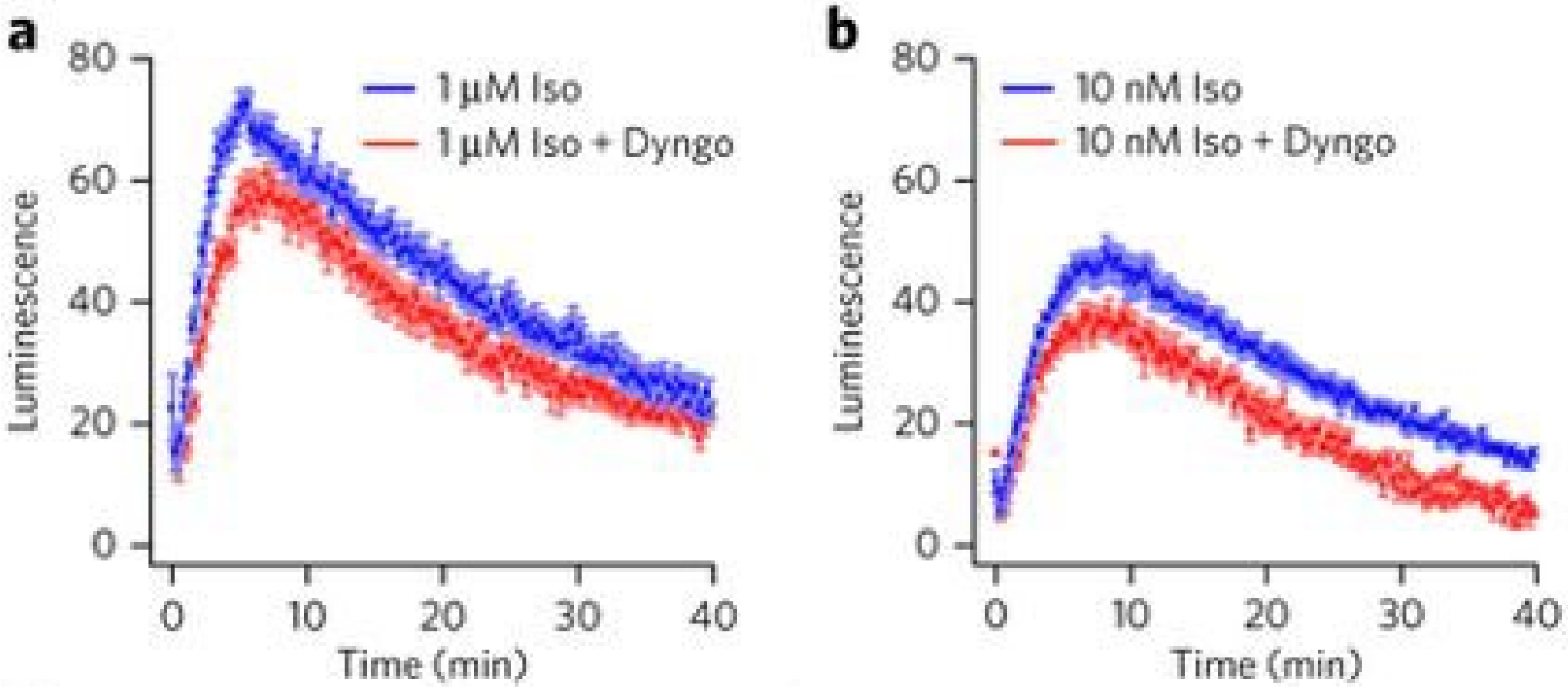


doi: 10.1038/nchembio.2389

Location-encoded signaling

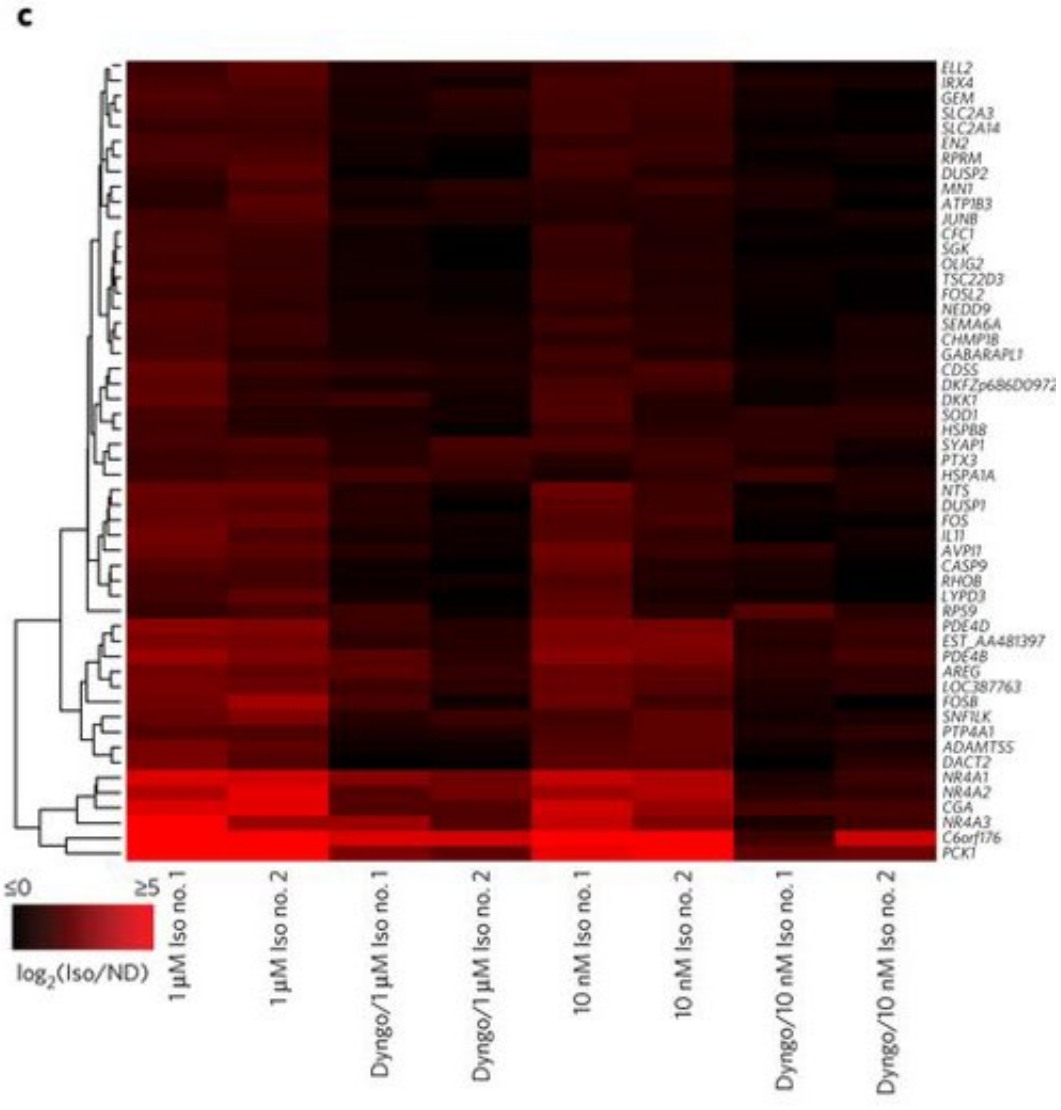
doi : 10.1038/nchembio.1665

β2AR signaling from endosomes

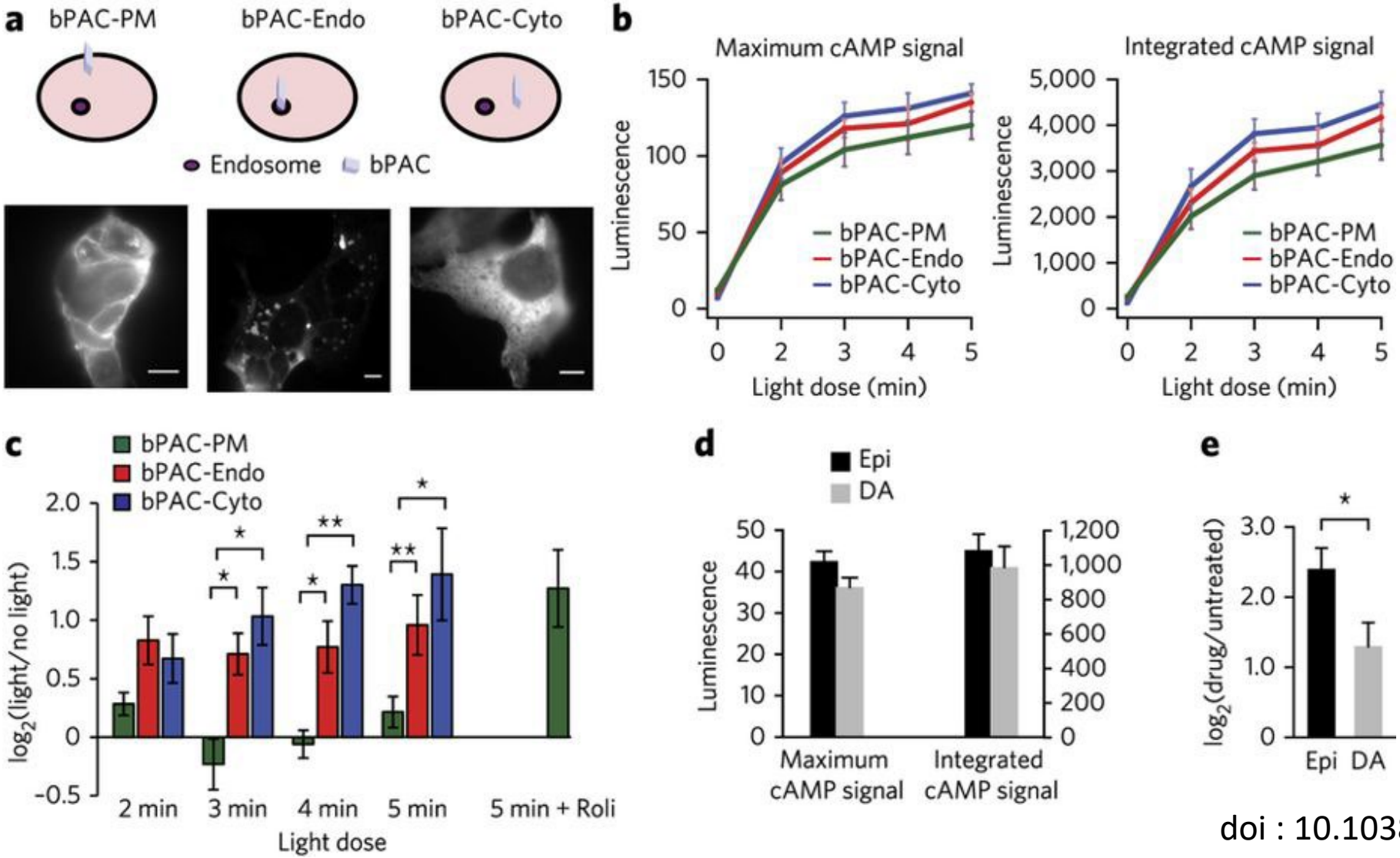


doi : 10.1038/nchembio.1665

Endosomal signaling regulates transcription



Optogenetic activation of cAMP



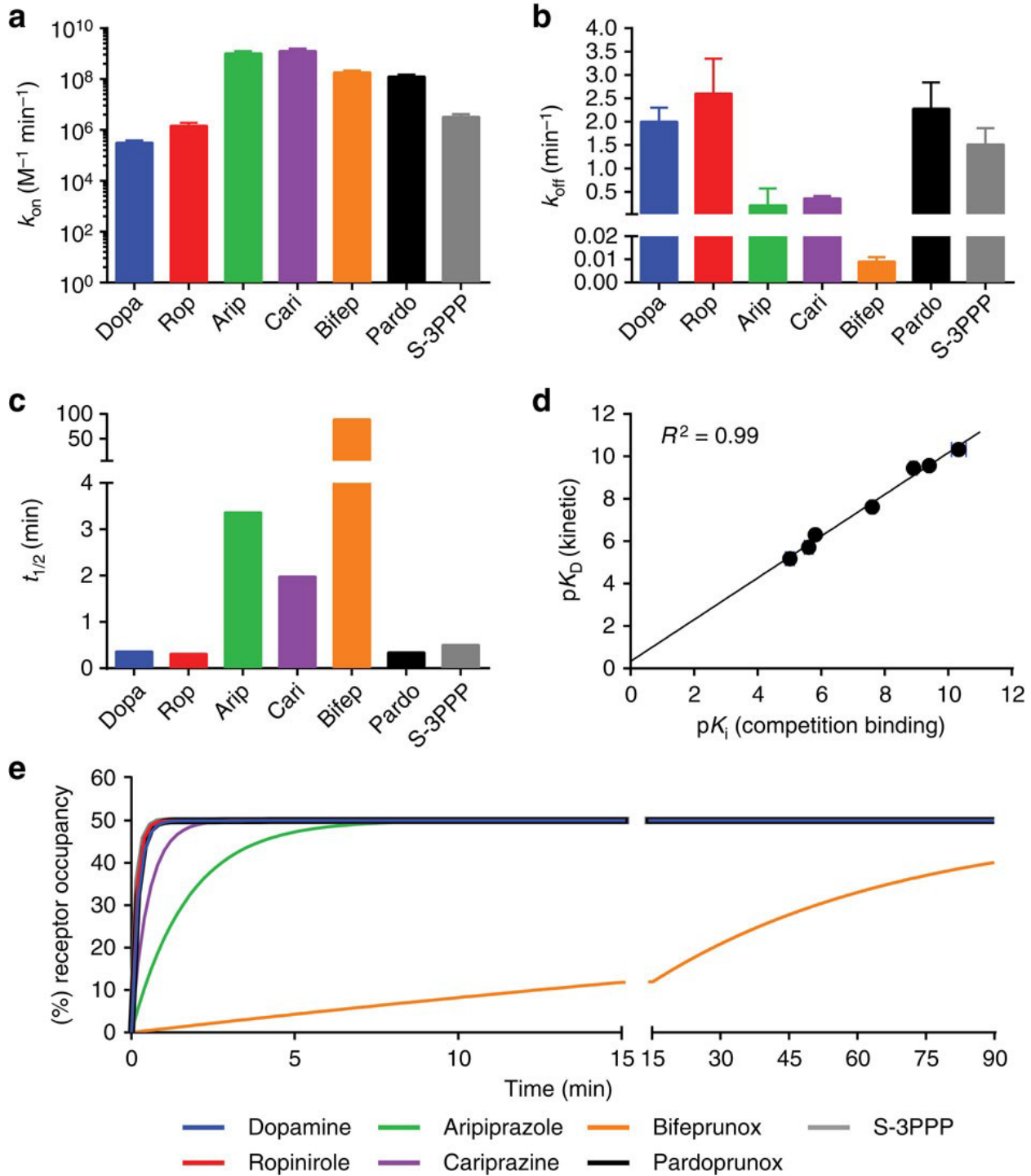
PCK1 expression

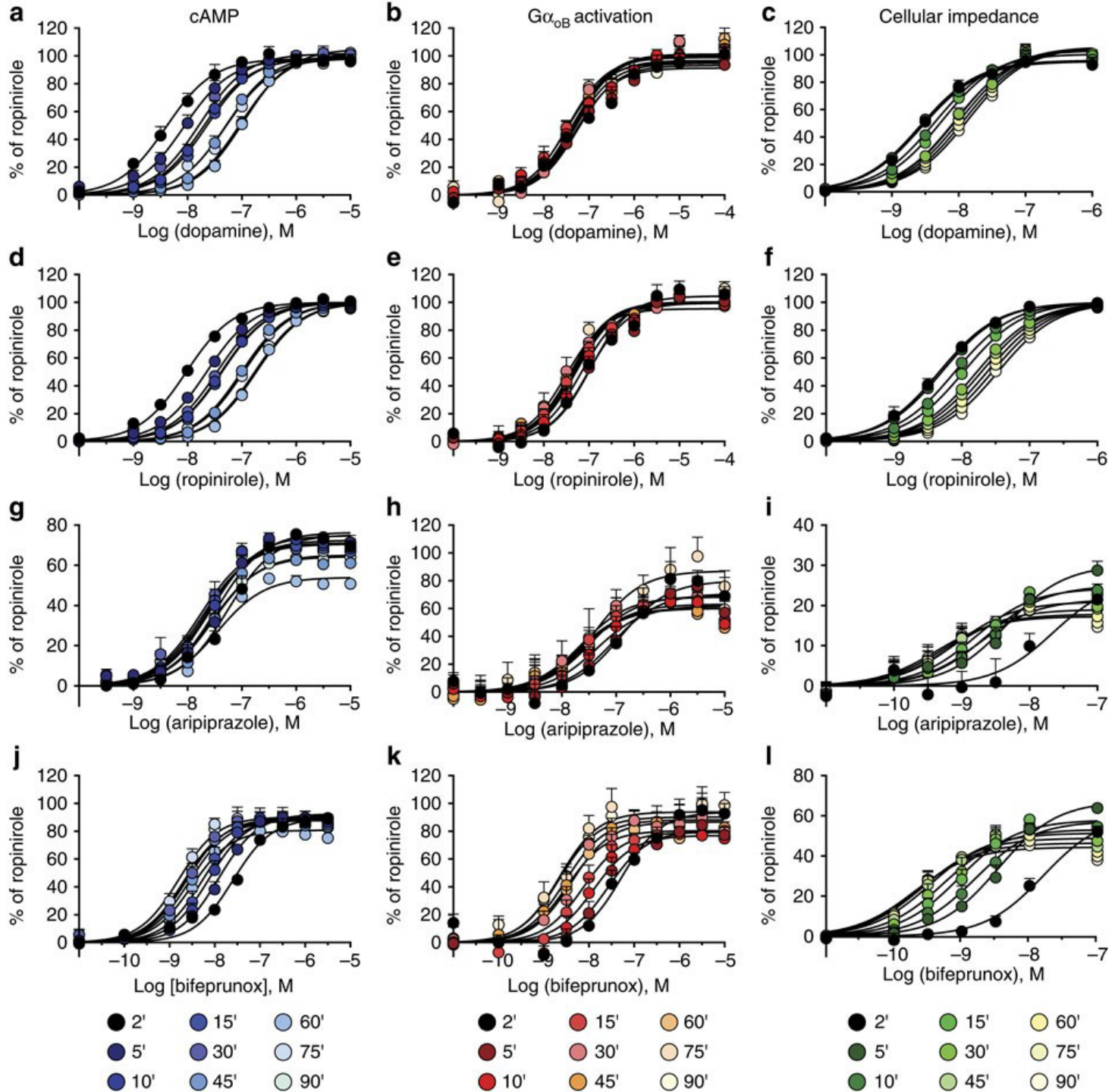
cAMP signal

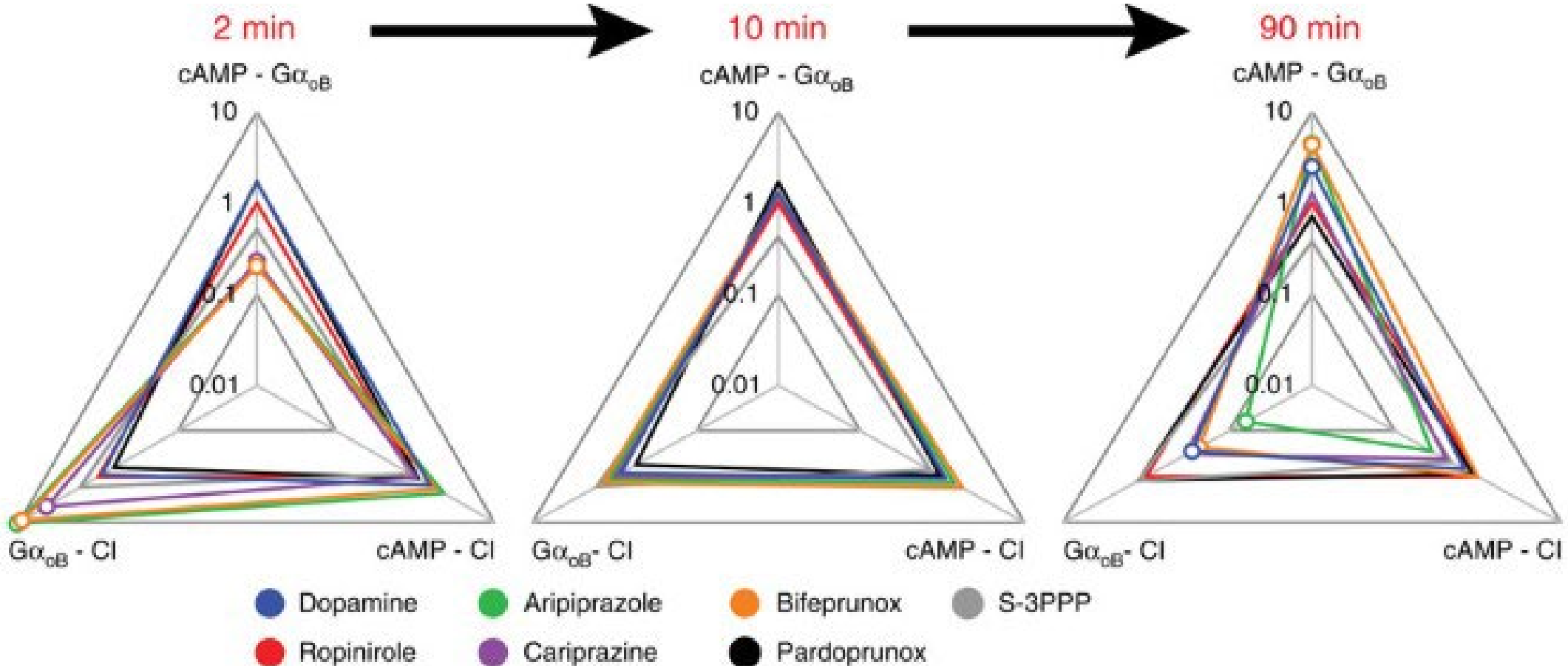
doi : 10.1038/nchembio.1665

Temporal Bias

Nature Communications volume 7, Article number: 10842 (2016)


a
k_{on} (M^{-1} min^{-1})
10^{10}
10^{8}
10^{6}
10^{4}
10^{2}
10^{0}
Dopa
Rop
Arip
Cari
Bifep
Pardo
S-3PPP
b
k_{off} (min^{-1})
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.02
0.01
0.00
Dopa
Rop
Arip
Cari
Bifep
Pardo
S-3PPP
c
$t_{1/2}$ (min)
100
50
4
3
2
1
0
Dopa
Rop
Arip
Cari
Bifep
Pardo
S-3PPP
d
R^2 = 0.99
pK_D (kinetic)
pK_i (competition binding)
0
2
4
6
8
10
12
e
(%) receptor occupancy
60
50
40
30
20
10
0
0
5
10
15
15
30
45
60
75
90
Time (min)
Dopamine
Ropinirole
Aripiprazole
Cariprazine
Bifeprunox
Pardoprunox
S-3PPP

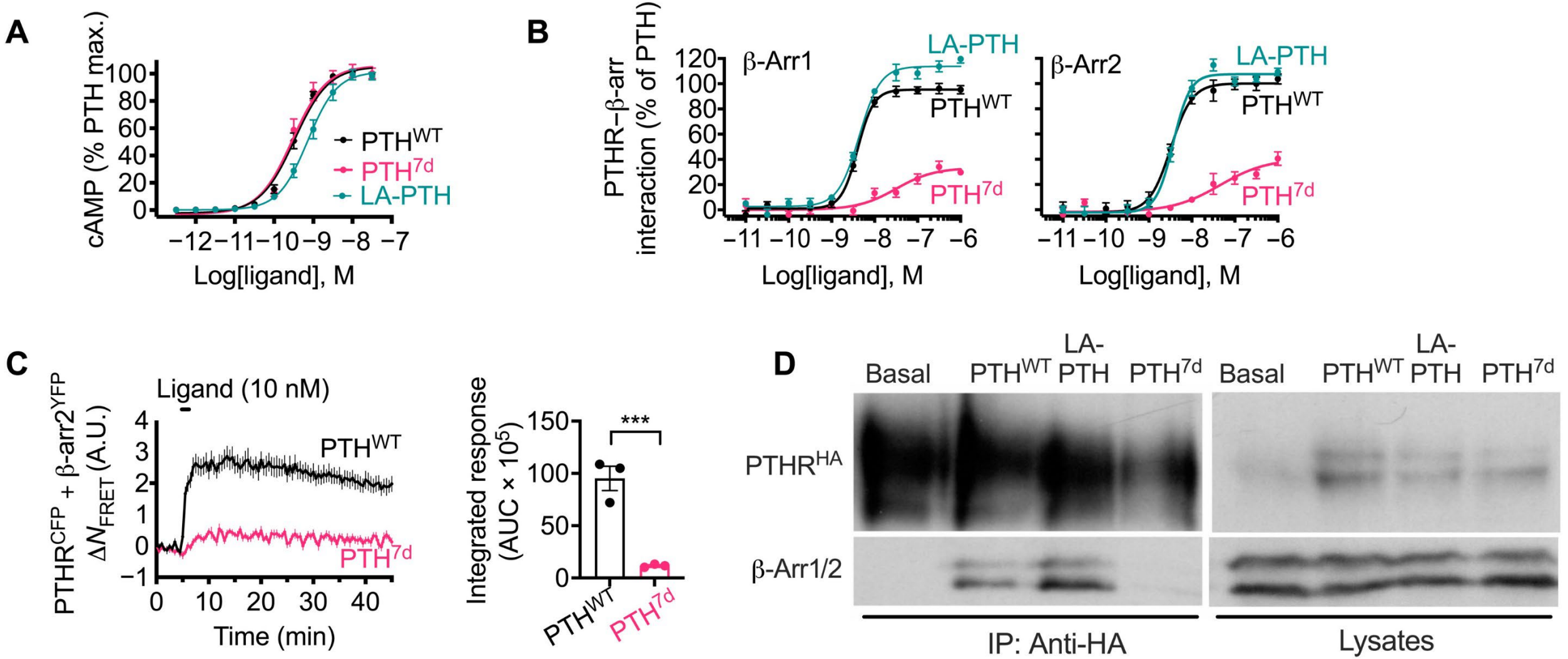

a
cAMP
b
Gαoi activation
c
Cellular impedance
% of ropinirole
Log (dopamine), M
d
e
f
Log (ropinirole), M
g
h
i
Log (aripiprazole), M
j
k
l
Log [bifeprunox], M
Log (bifeprunox), M
2'
5'
10'
15'
30'
45'
60'
75'
90'


2 min
10 min
90 min
cAMP - Gαo1B
10
1
0.1
0.01
Gαo1B - Cl
cAMP - Cl
Dopamine
Ropinirole
Aripiprazole
Cariprazine
Bifeprunox
Pardoprunox
S-3PPP

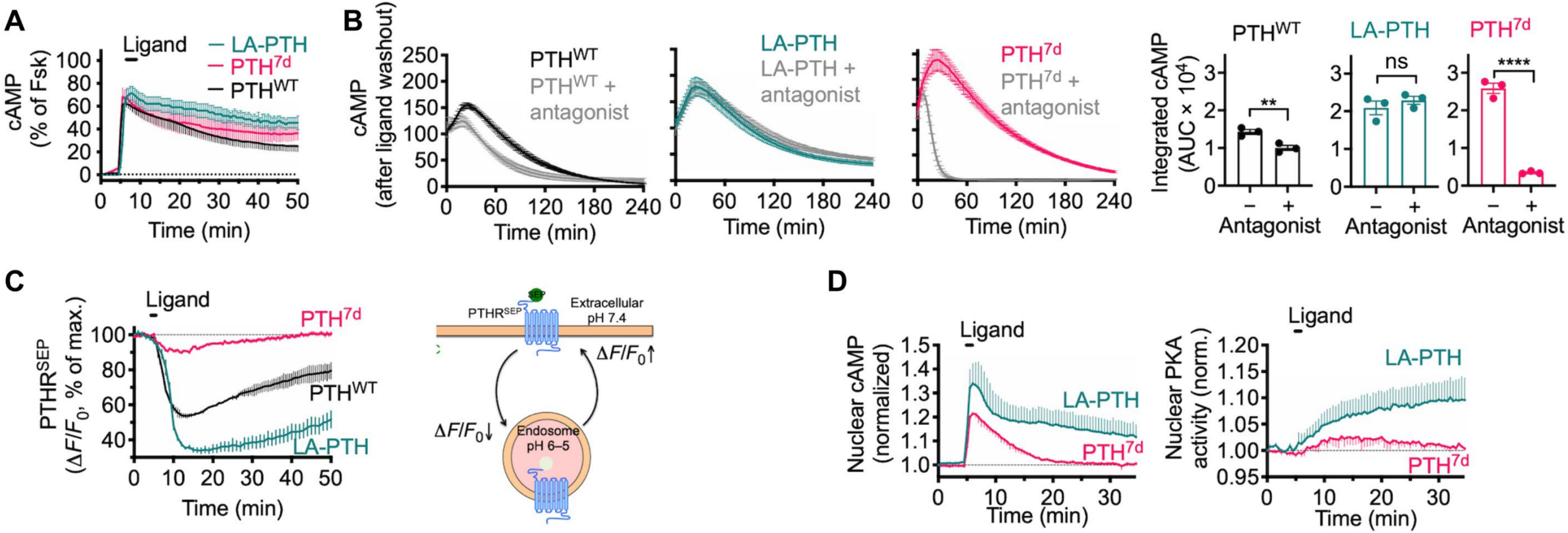
Differentiating the effects of spatial and temporal bias at the PTH1R

SCIENCE SIGNALING
5 Oct 2021
Vol 14, Issue 703
DOI: 10.1126/scisignal.abc5944

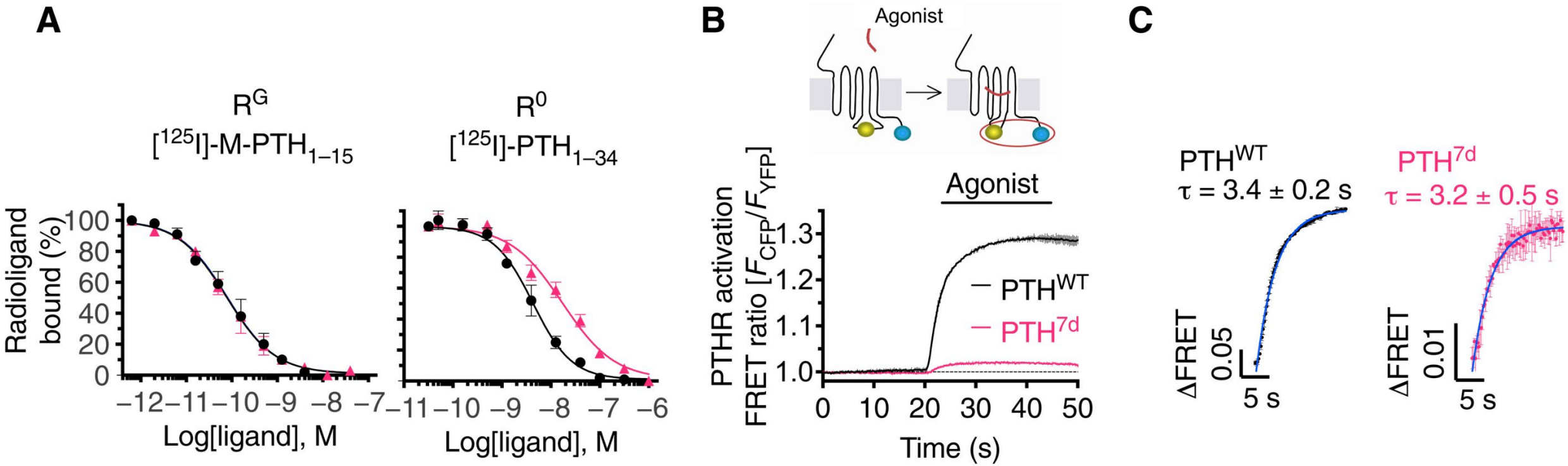
Characterization of a Gs-biased PTH analog generated by amino acid isomerization



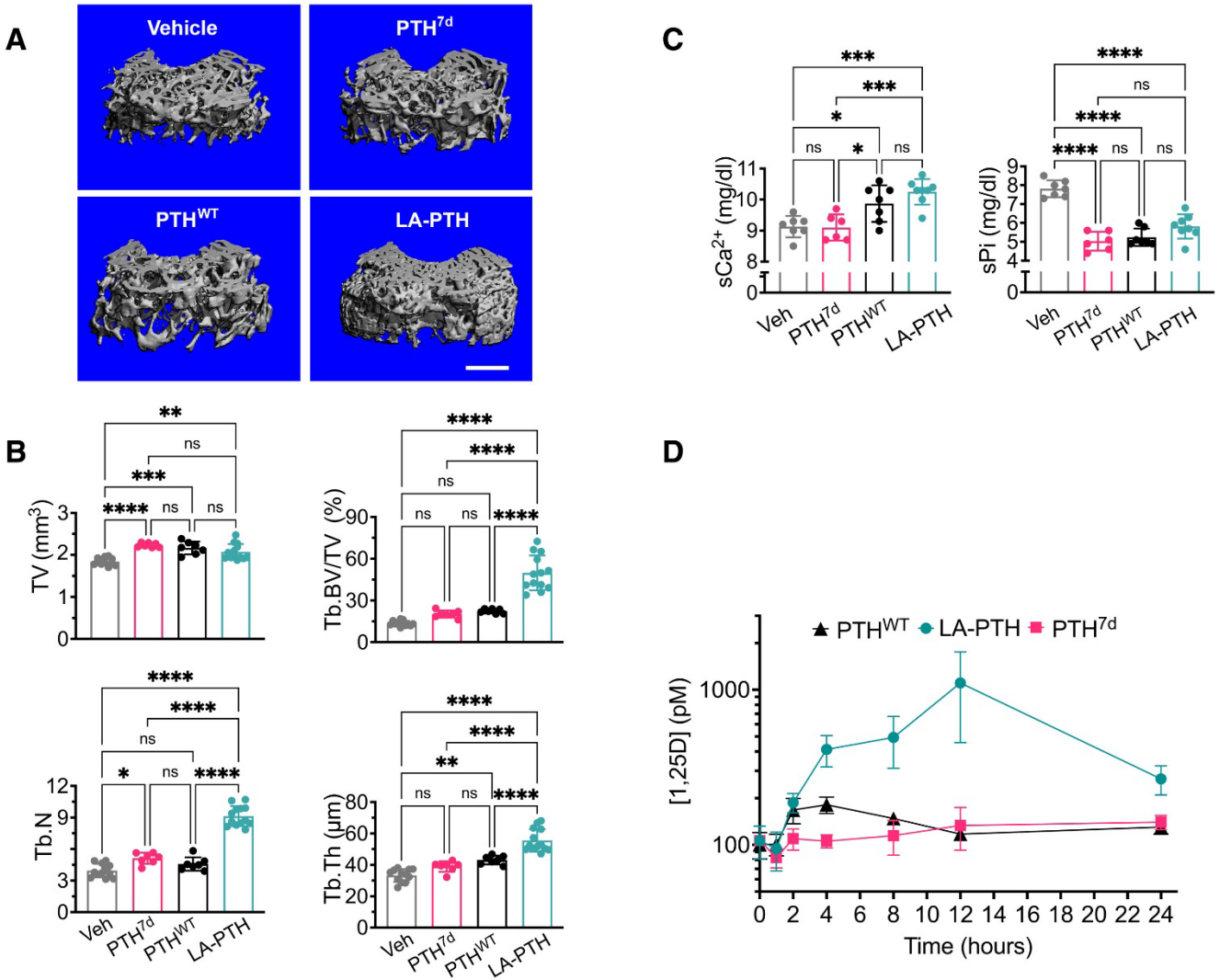
Location bias of PTH7d signaling



Molecular changes induced by PTH7d



Differential pharmacological actions of PTH7d, PTHWT, and LA-PTH in mice



Proposed model for location bias in cAMP and PTHR pharmacology