The intermolecular interactions of the CC-type 1 chemokine receptor (CCR1)

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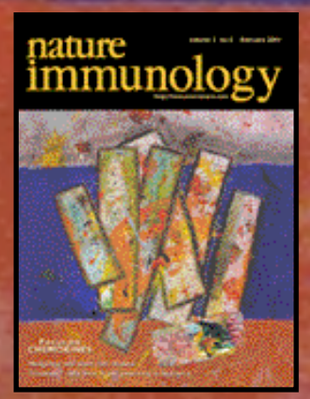
Biomedical Sciences Graduate Program
UC San Diego
September 21, 2012



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Outline

I. Introduction to CCR1

II. CCR1 Constitutive Activity

III. Association with $\beta arrestin_2$

IV. G protein coupling

V. Chemokine receptor homo-/hetero-oligomerization

VI. Additional Activities

VII. Next steps

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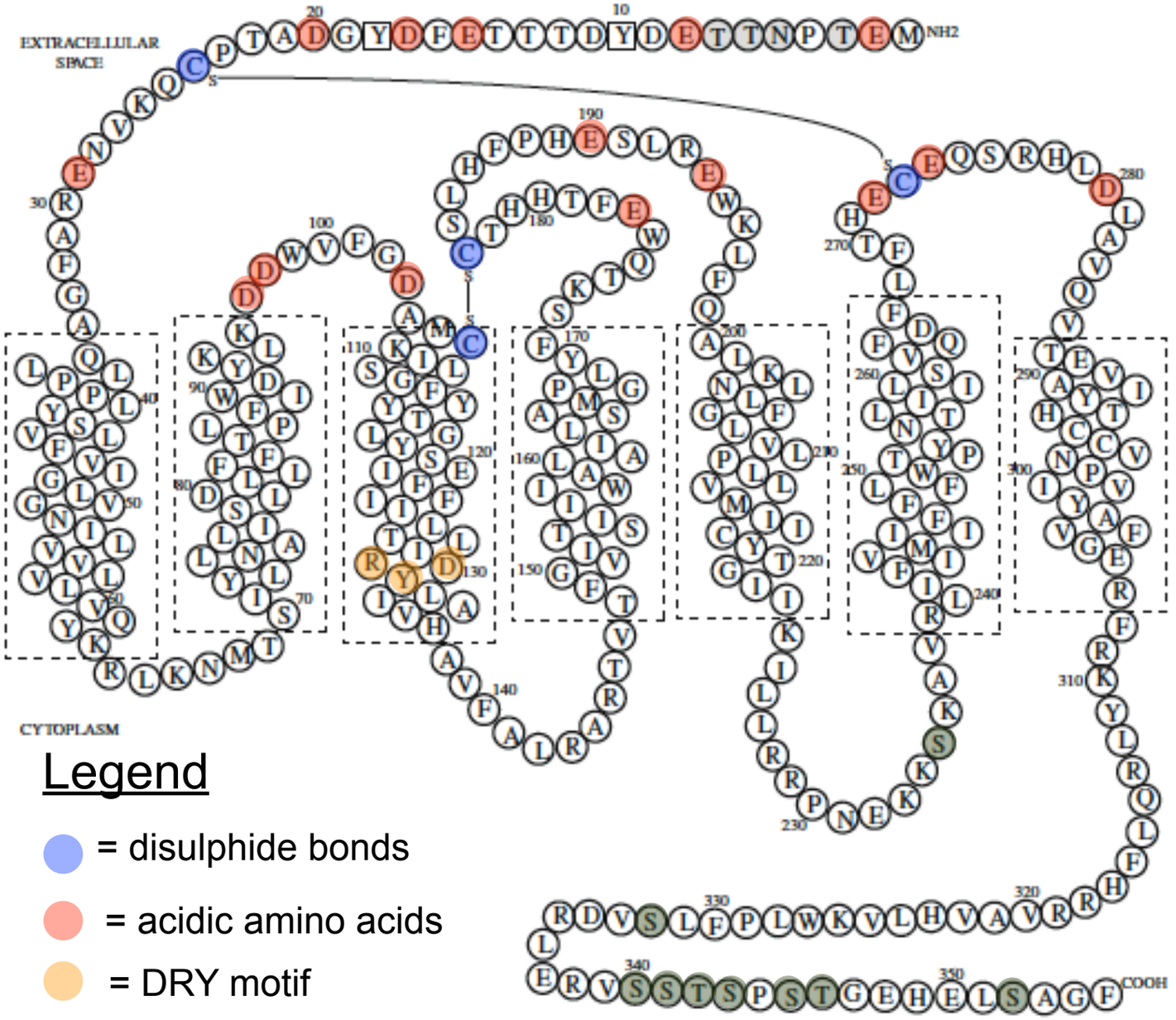
CC-type 1 chemokine receptor (CCR1)

CCR1 Agonists:

- CCL3
- CCL4
- CCL5
- CCL7
- CCL8
- CCL13
- CCL14
- CCL15
- CCL16
- CCL23

Expression:

- monocytes
- eosinophils
- basophils
- NK cells
- activated T cells
- dendritic cells
- neutrophils



Legend

- = disulphide bonds
- = acidic amino acids
- = DRY motif
- = potential phosphorylation sites

Effect of KO:

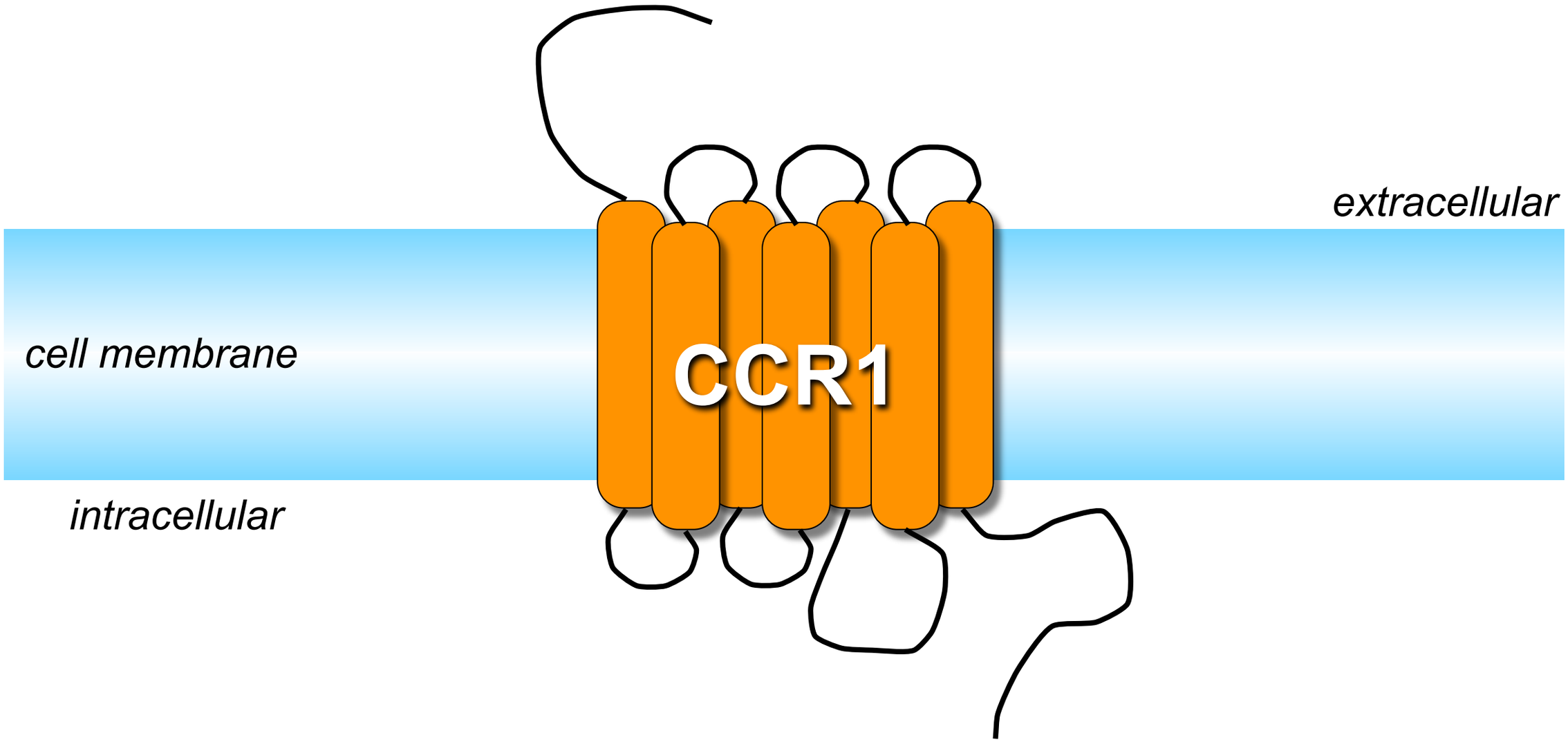
- disordered trafficking & proliferation of myeloid progenitor cells
- reduced chemotaxis of neutrophils
- impaired inflammatory response
- reduced rejection response to tissue allografts
- increased mortality following fungal infection

Disease Implications:

- multiple sclerosis (MS)
- rheumatoid arthritis (RA)
- transplant rejection
- kidney disease
- asthma
- multiple myeloma

Adapted from Allen SJ et al. (2009) J Prot Express & Purif

Intermolecular interactions of chemokine receptors and binding/signaling partners



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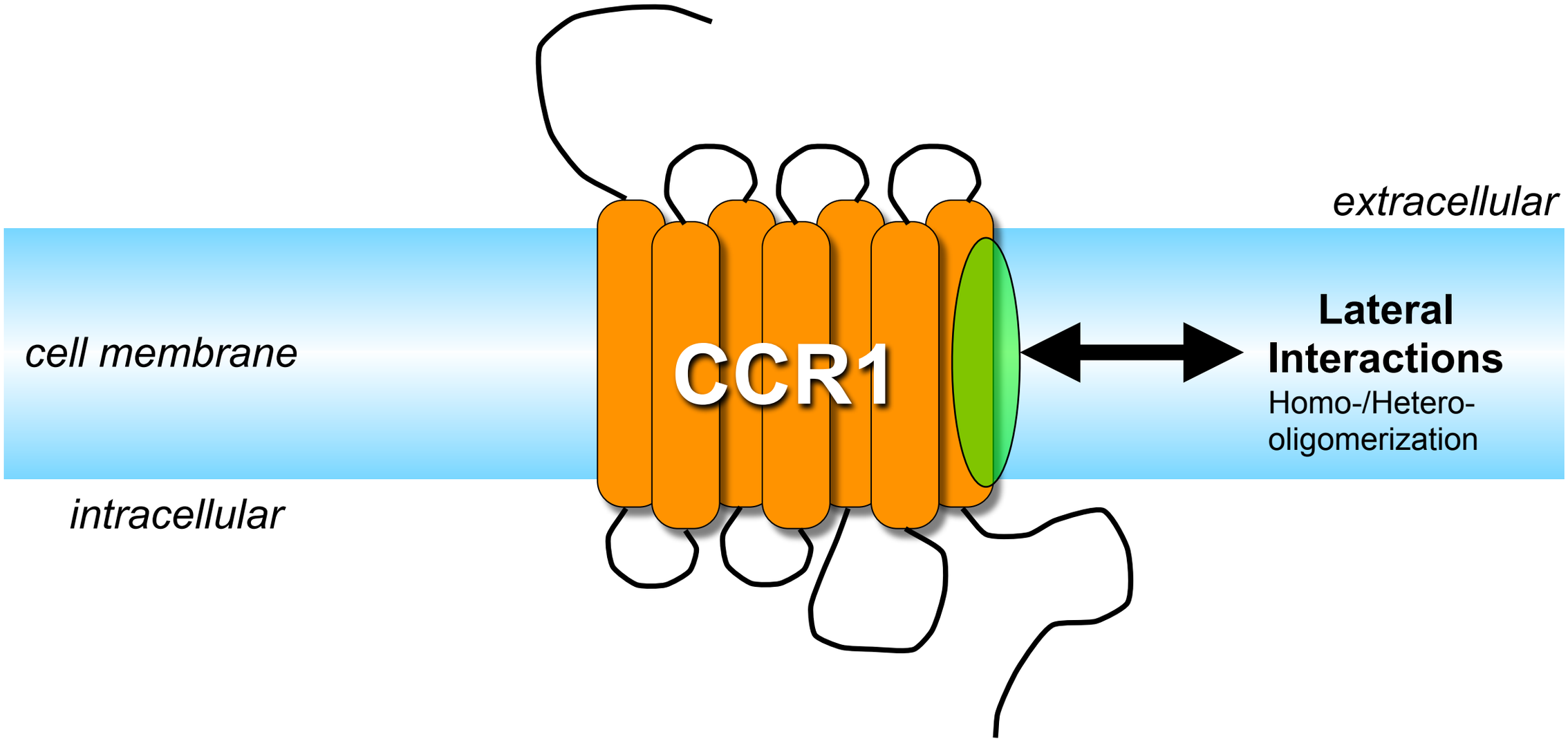

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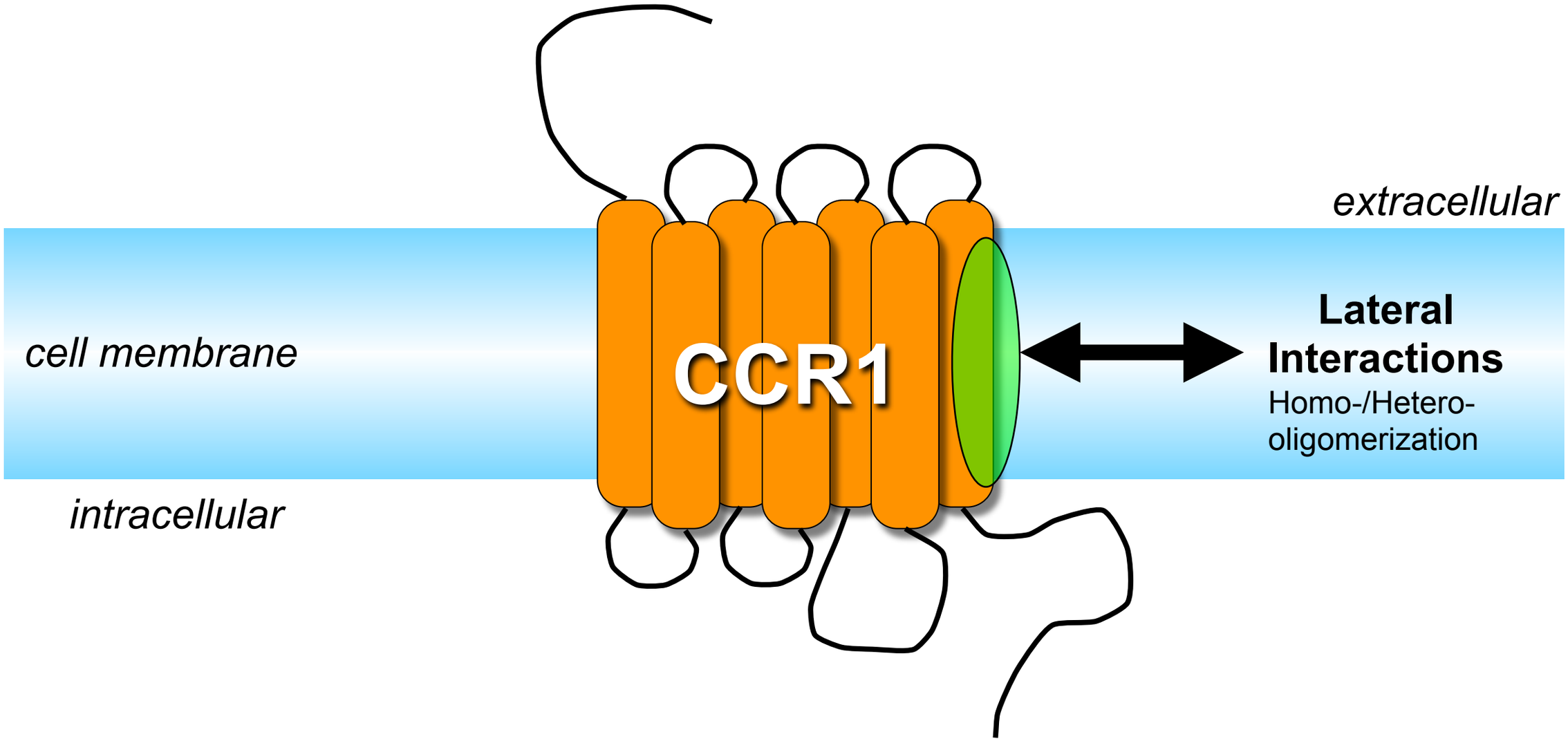

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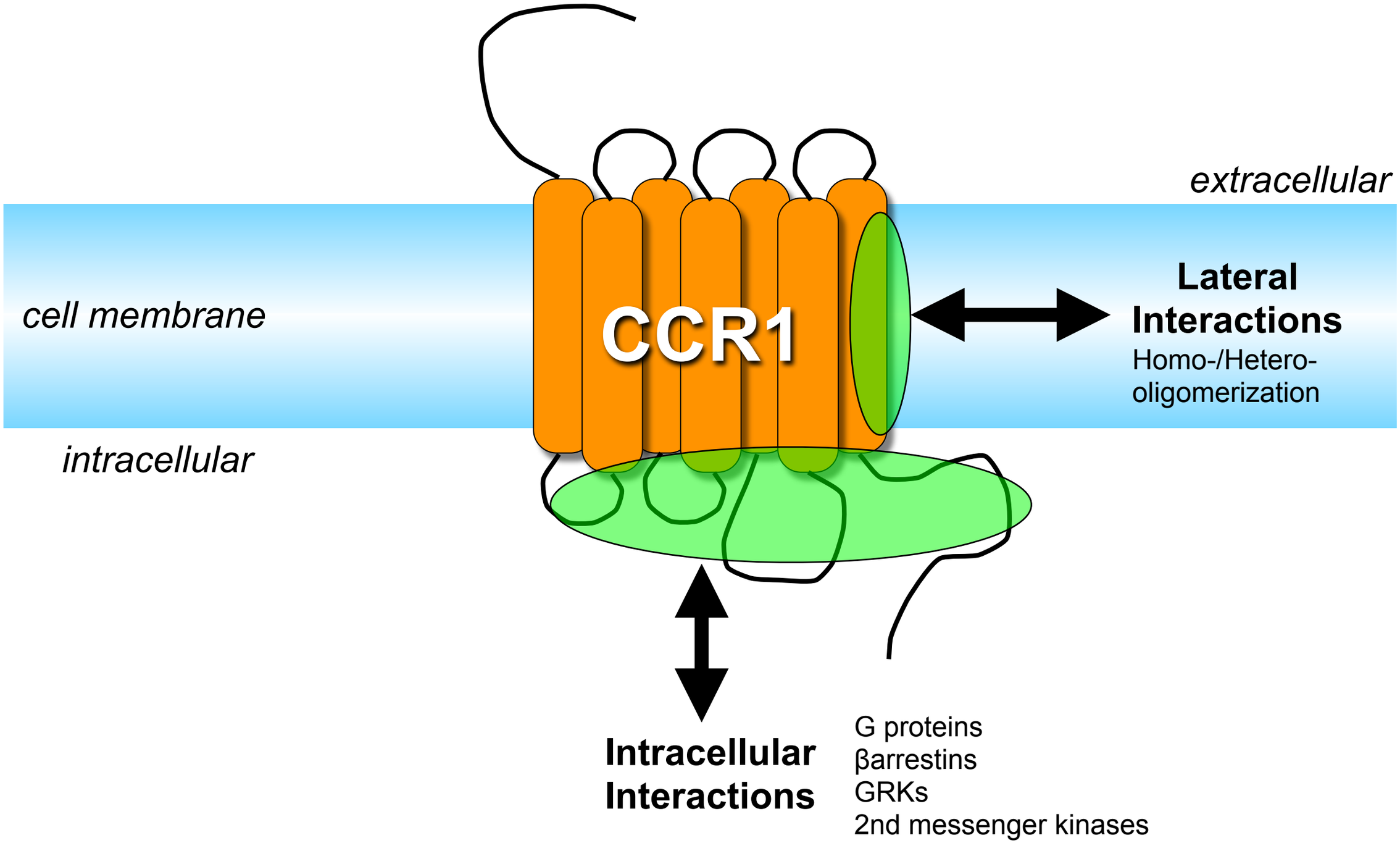
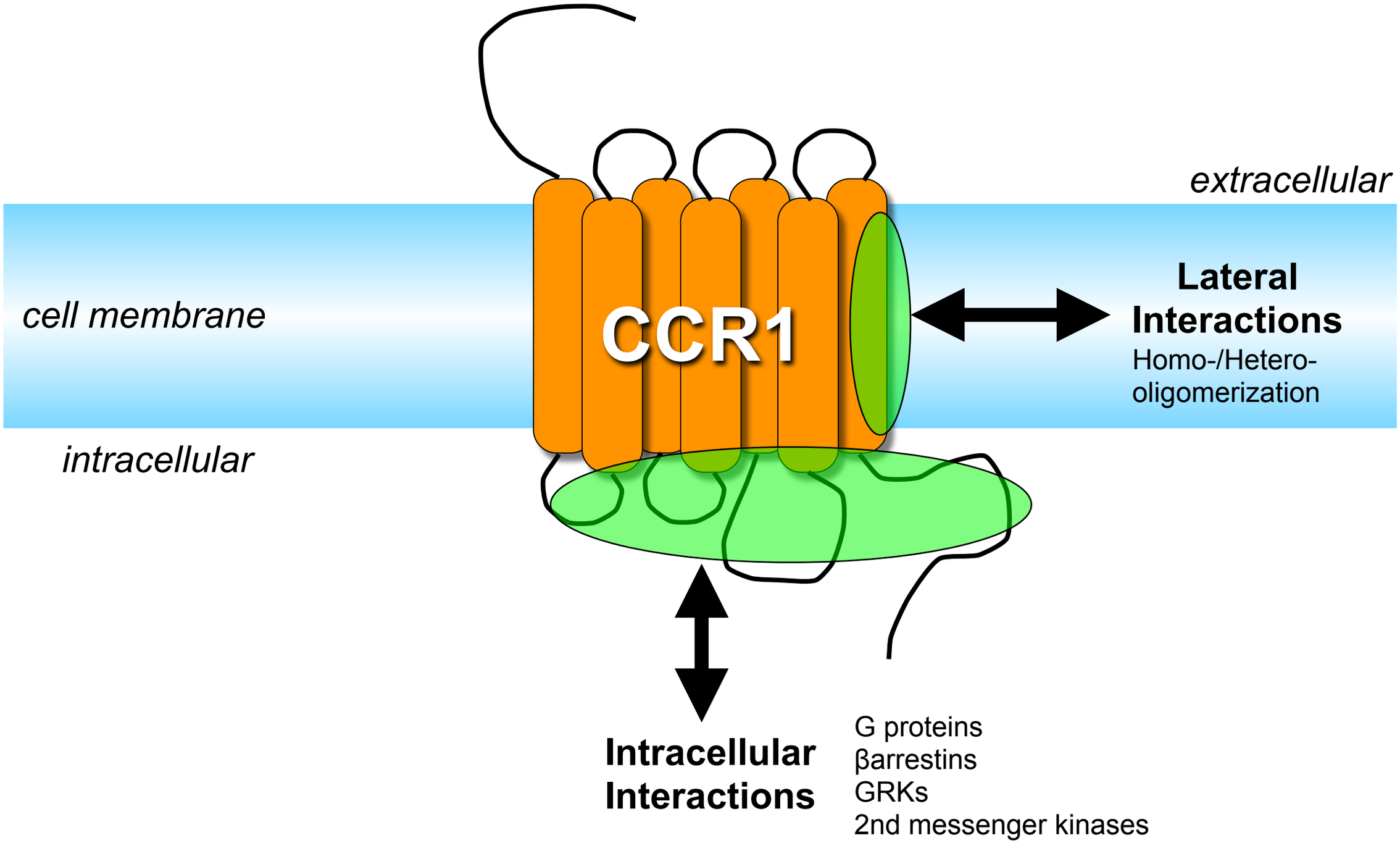


Intermolecular interactions of chemokine receptors and binding/signaling partners



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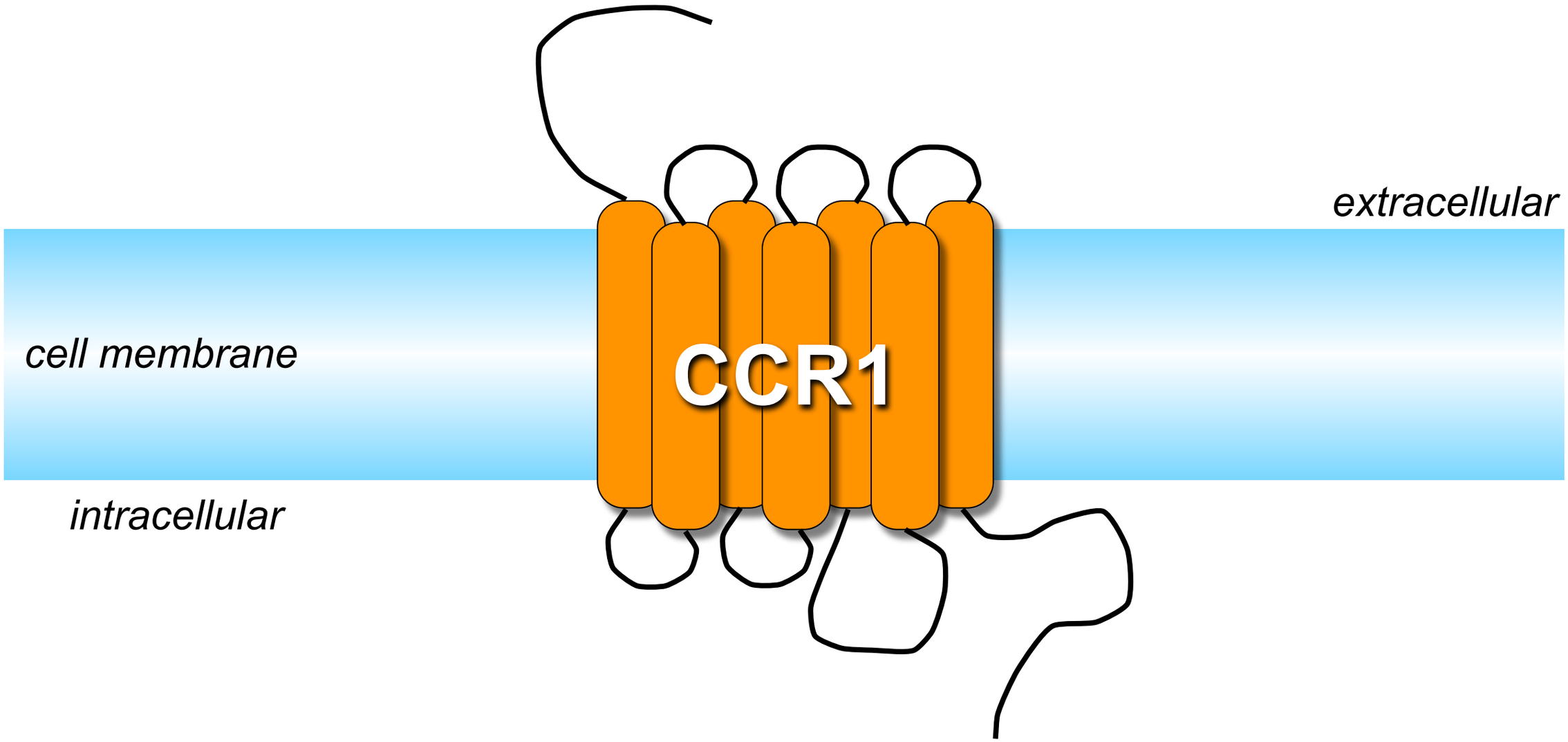

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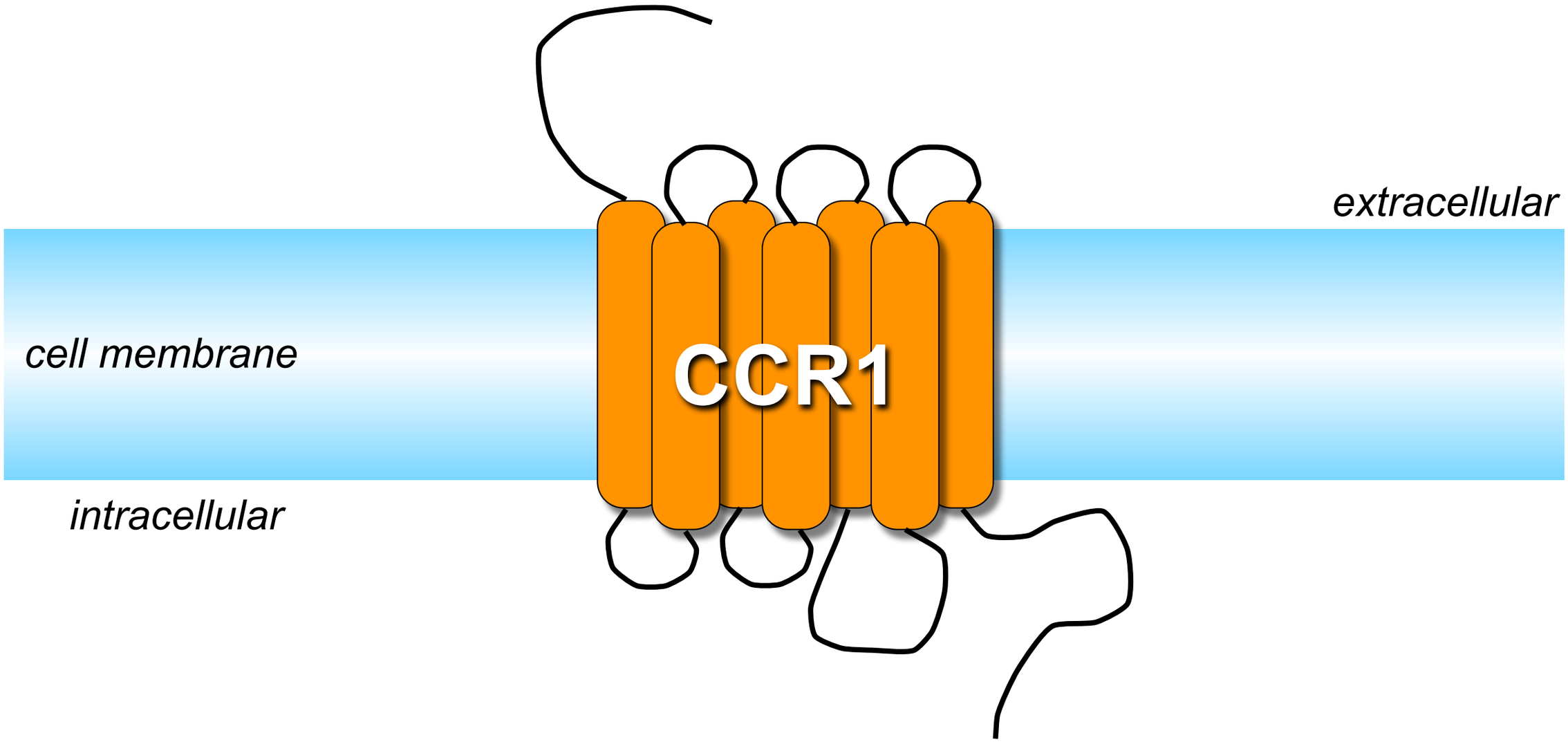


What proteins does CCR1 form intermolecular interactions with?

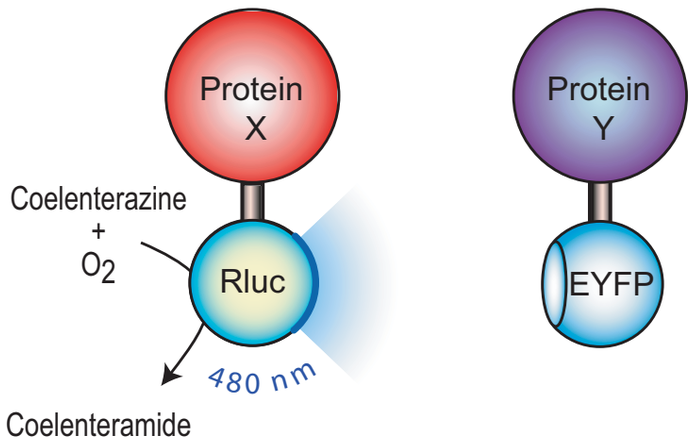
What are the structural domains that modulate these interactions?

How do these interactions affect CCR1 function?

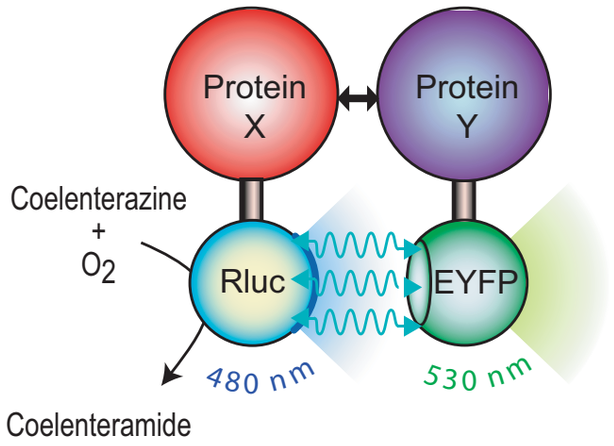
BRET as a tool to measure protein intermolecular interactions

BRET 1

No interaction

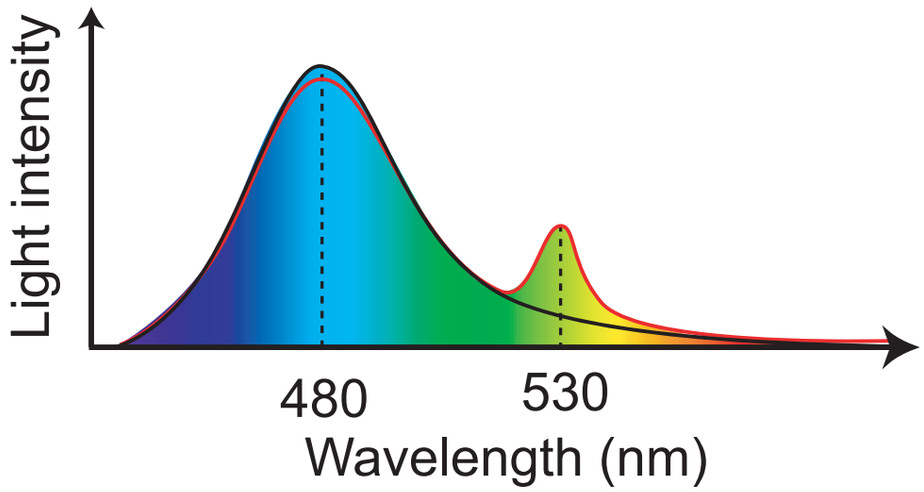


Interaction



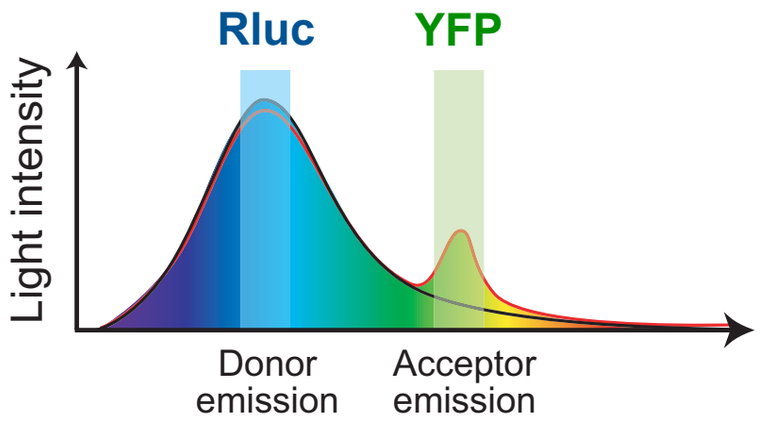
Other compatible acceptors: YFPtopaz, YFPcitrine, YFPVenus, YPet

Spectral properties

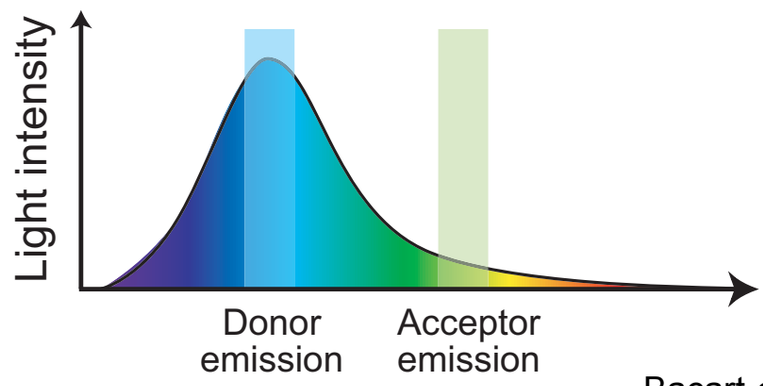


$$BRET_{net} = \left(\frac{\text{long-wavelength emission}}{\text{short-wavelength emission}}\right) - \left(\frac{\text{long-wavelength emission of donor only}}{\text{short-wavelength emission of donor only}}\right)$$

Donor and Acceptor co-expressed



Donor expressed alone



Bacart *et al*. (2008) *Biotechnol. J.*

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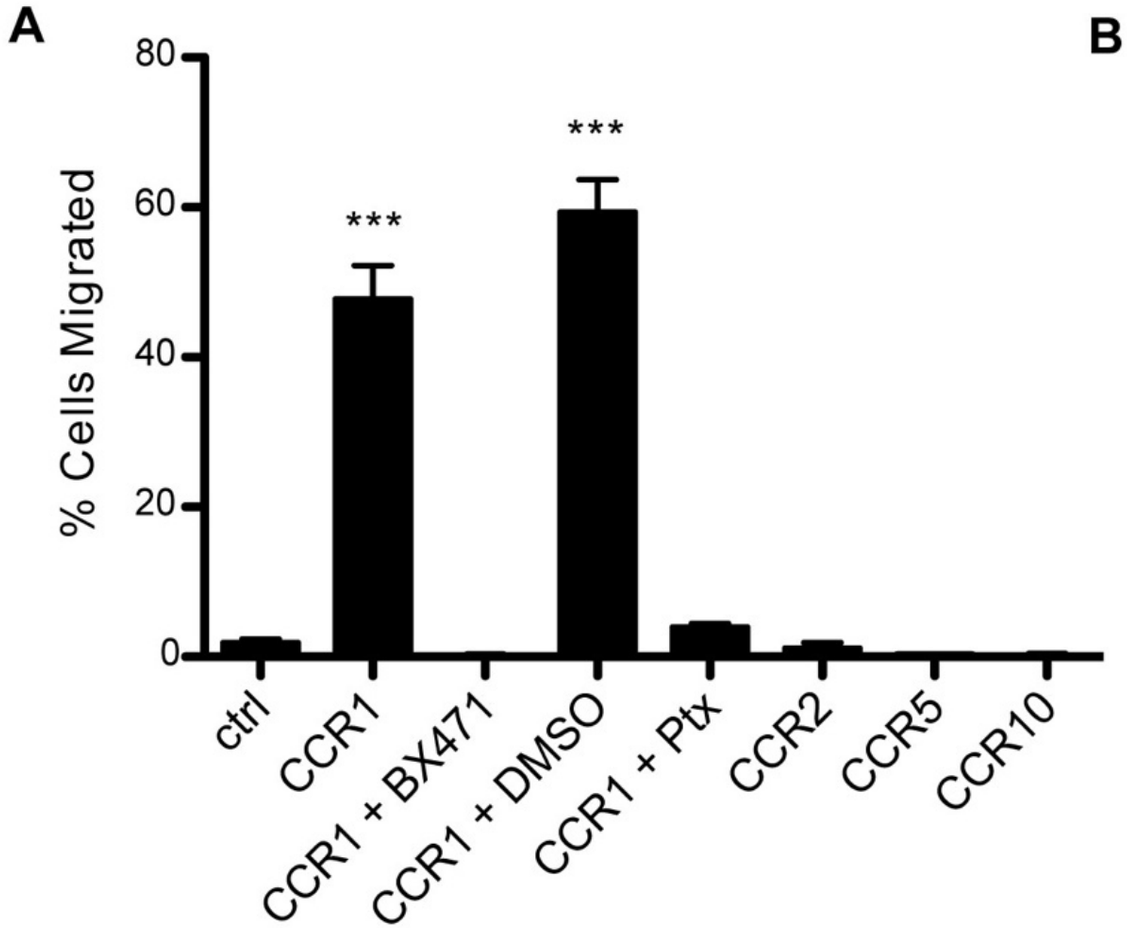
CCR1 expression mediates basal migration

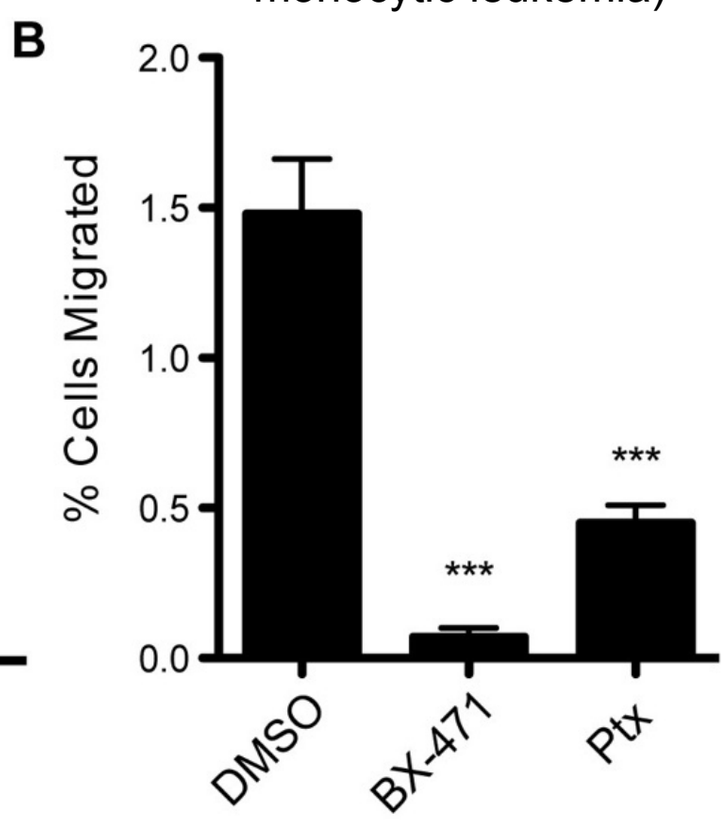
Transient and endogenous expression of CCR1 results in agonist-independent chemotaxis, suggesting that it is constitutively active

Cellular migration (microchemotaxis) assay

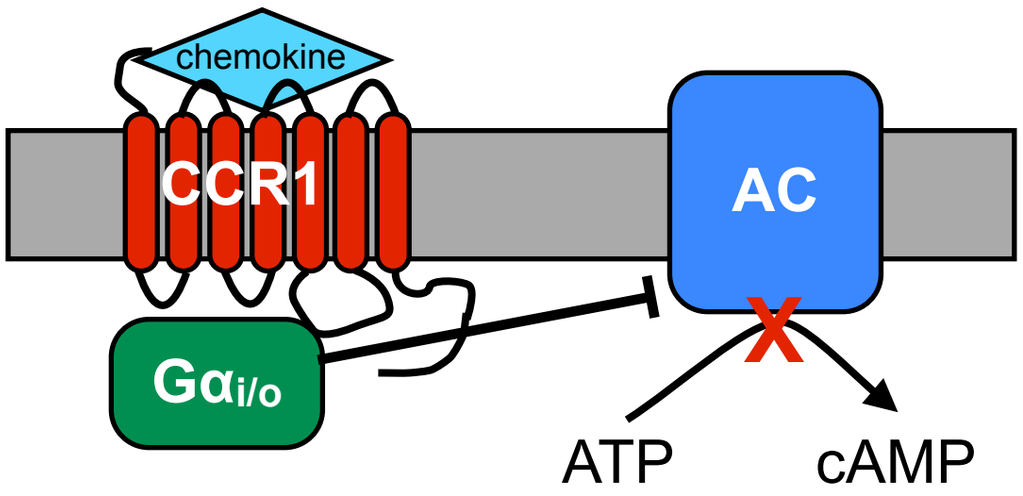
L1.2 (murine pre-B lymphoma)



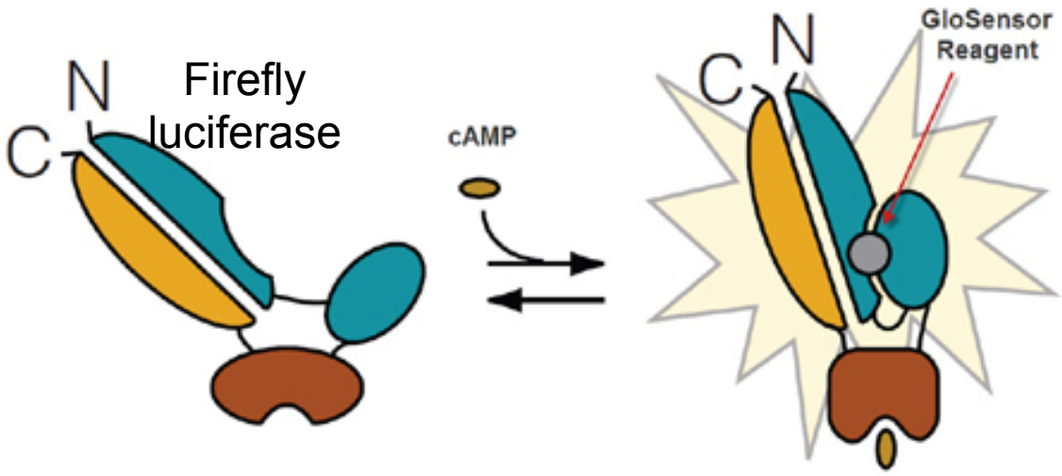
THP-1 (human acute monocytic leukemia)



CCR1 expression reduces forskolin-stimulated cAMP generation

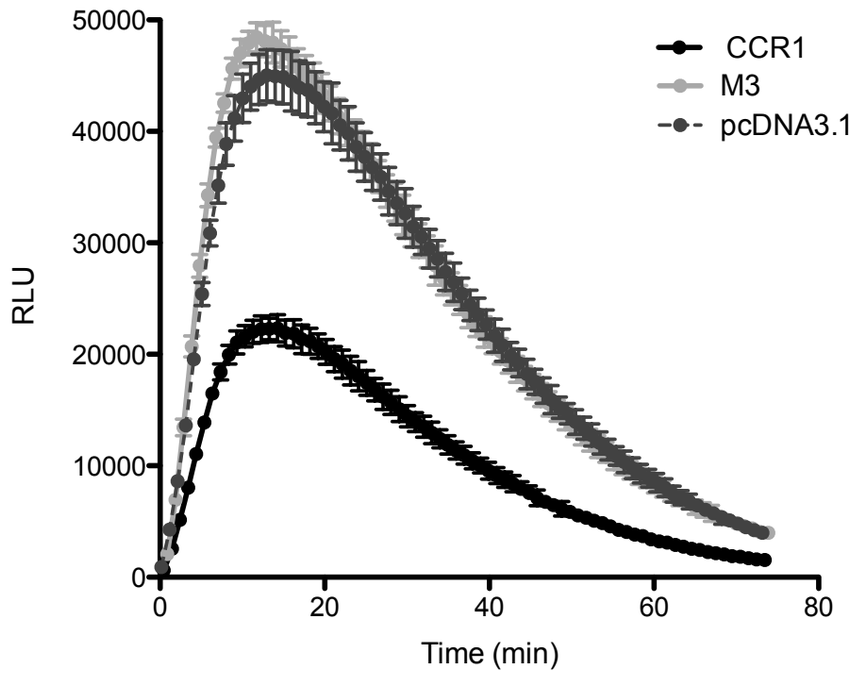


Promega GloSensor cAMP Assay

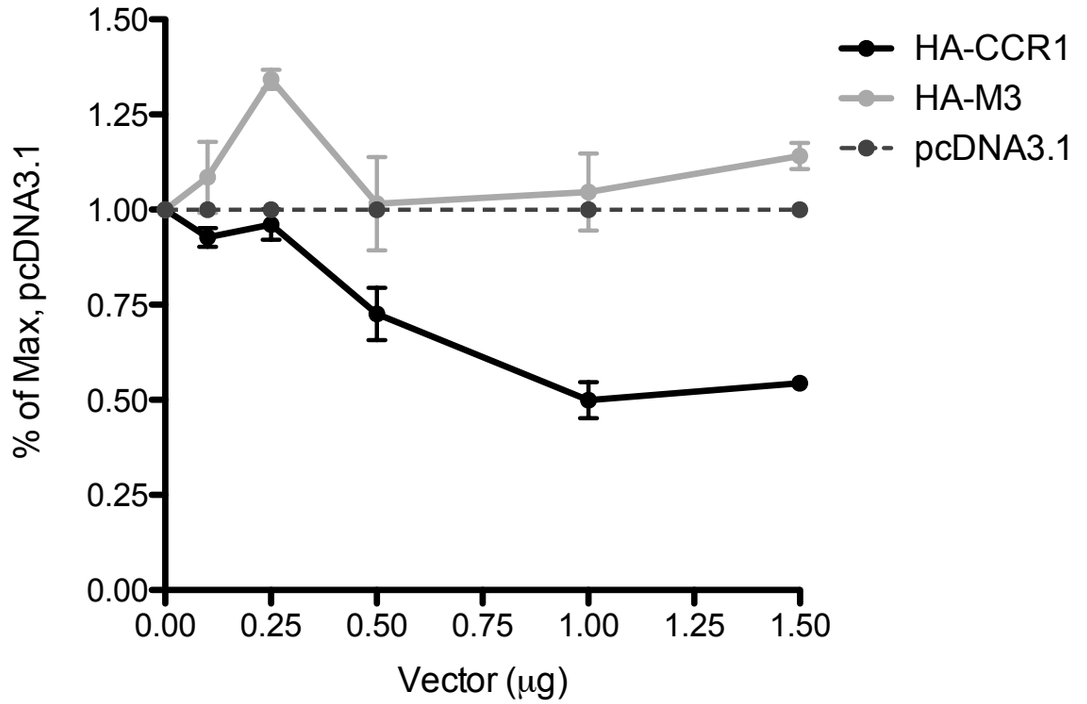


PKA RIIβB cAMP binding domain

Comparison of CCR1 and M3



Gene Dosage Comparison of CCR1 and M3



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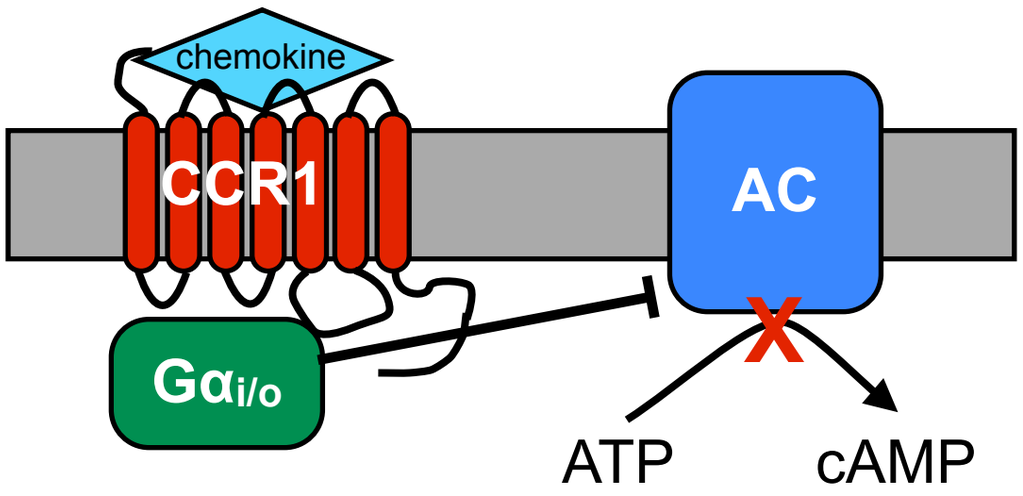

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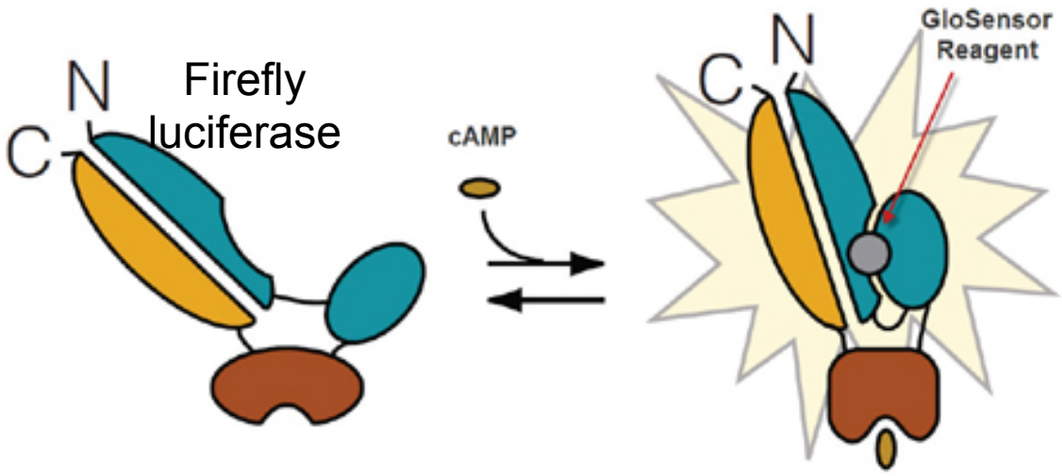

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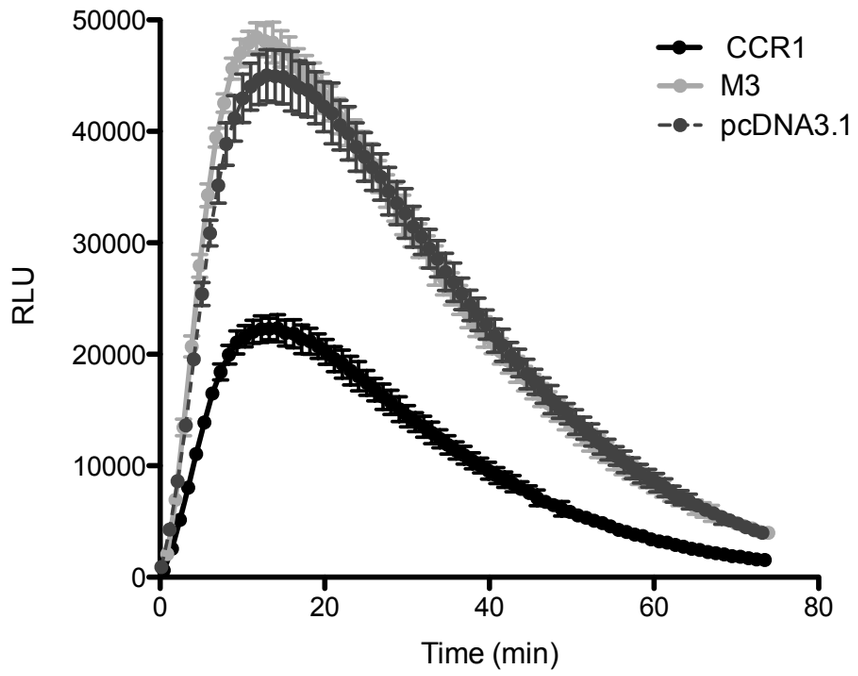

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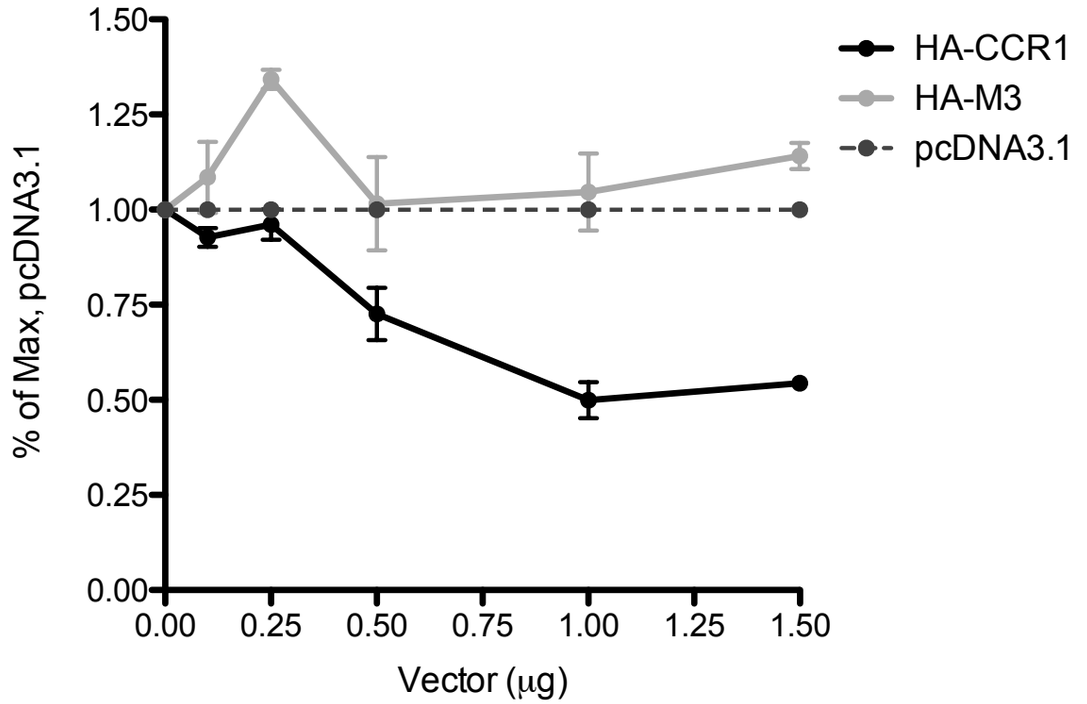
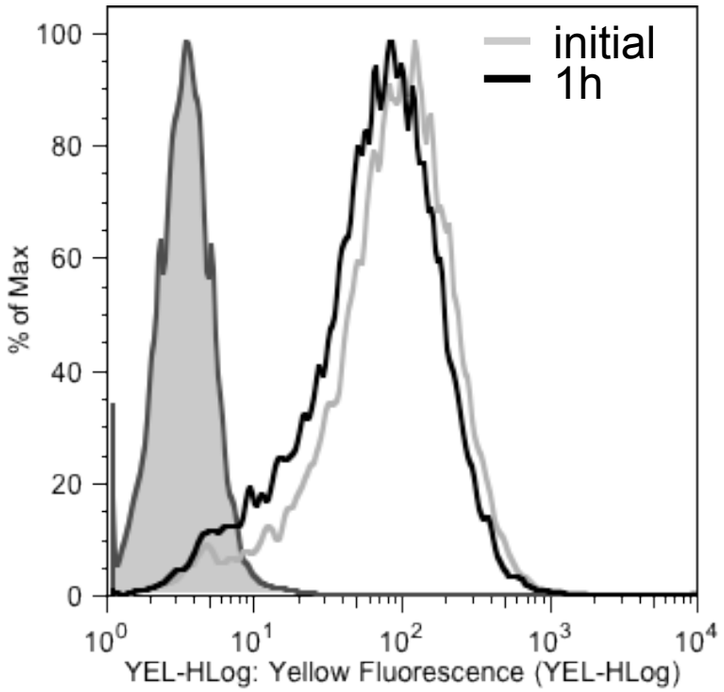


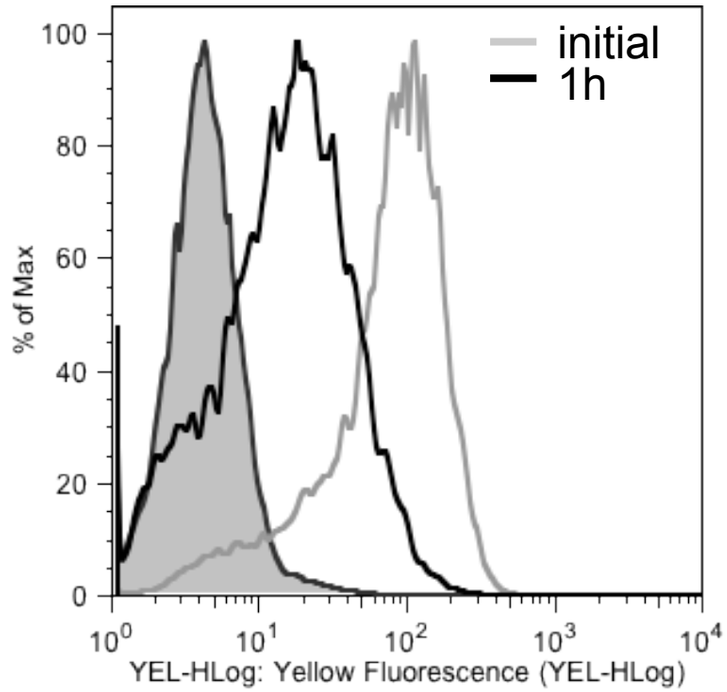
How is the constitutively active CCR1 receptor being regulated?

CCR1 is constitutively internalized

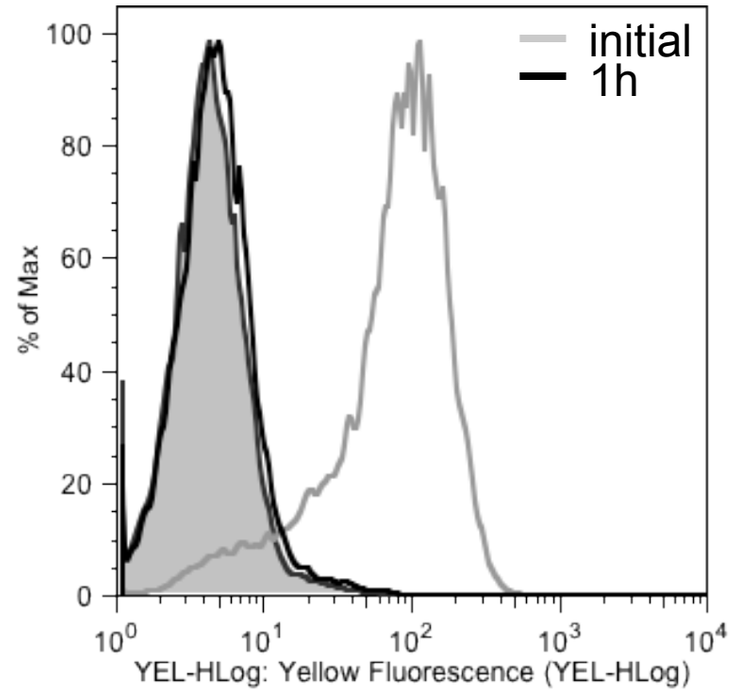
Total Surface Receptor

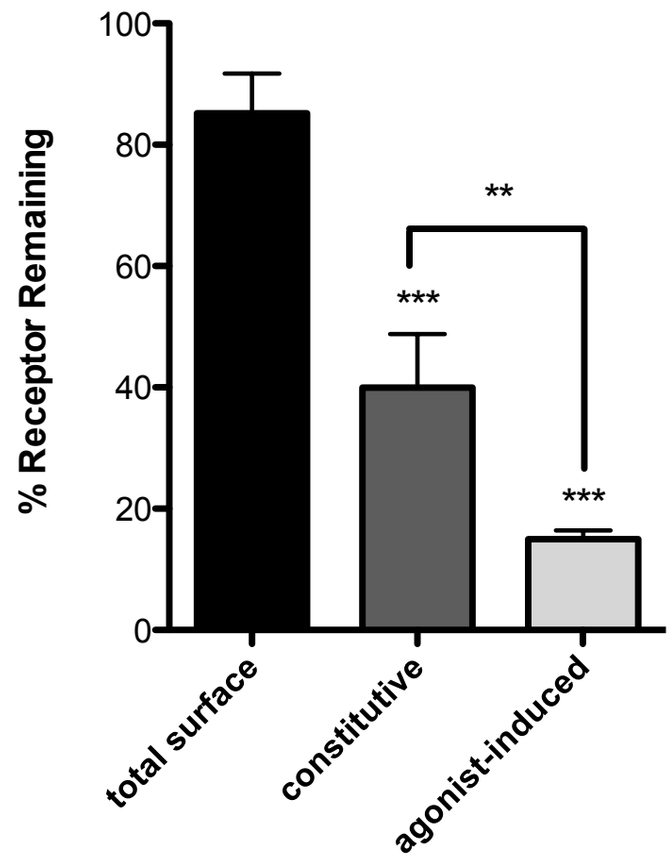


Constitutive Internalization

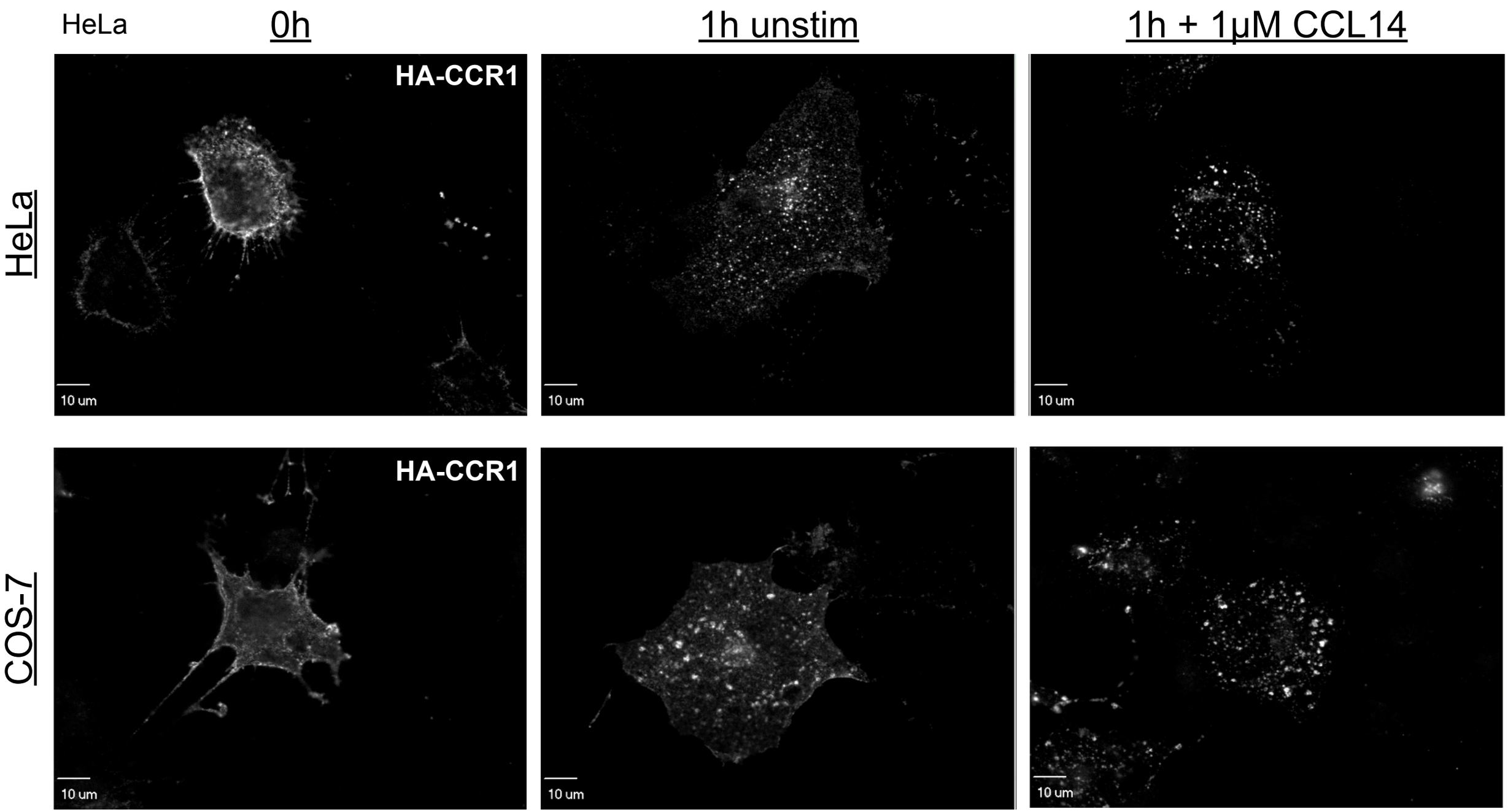


Agonist-induced Internalization





CCR1 is constitutively internalized



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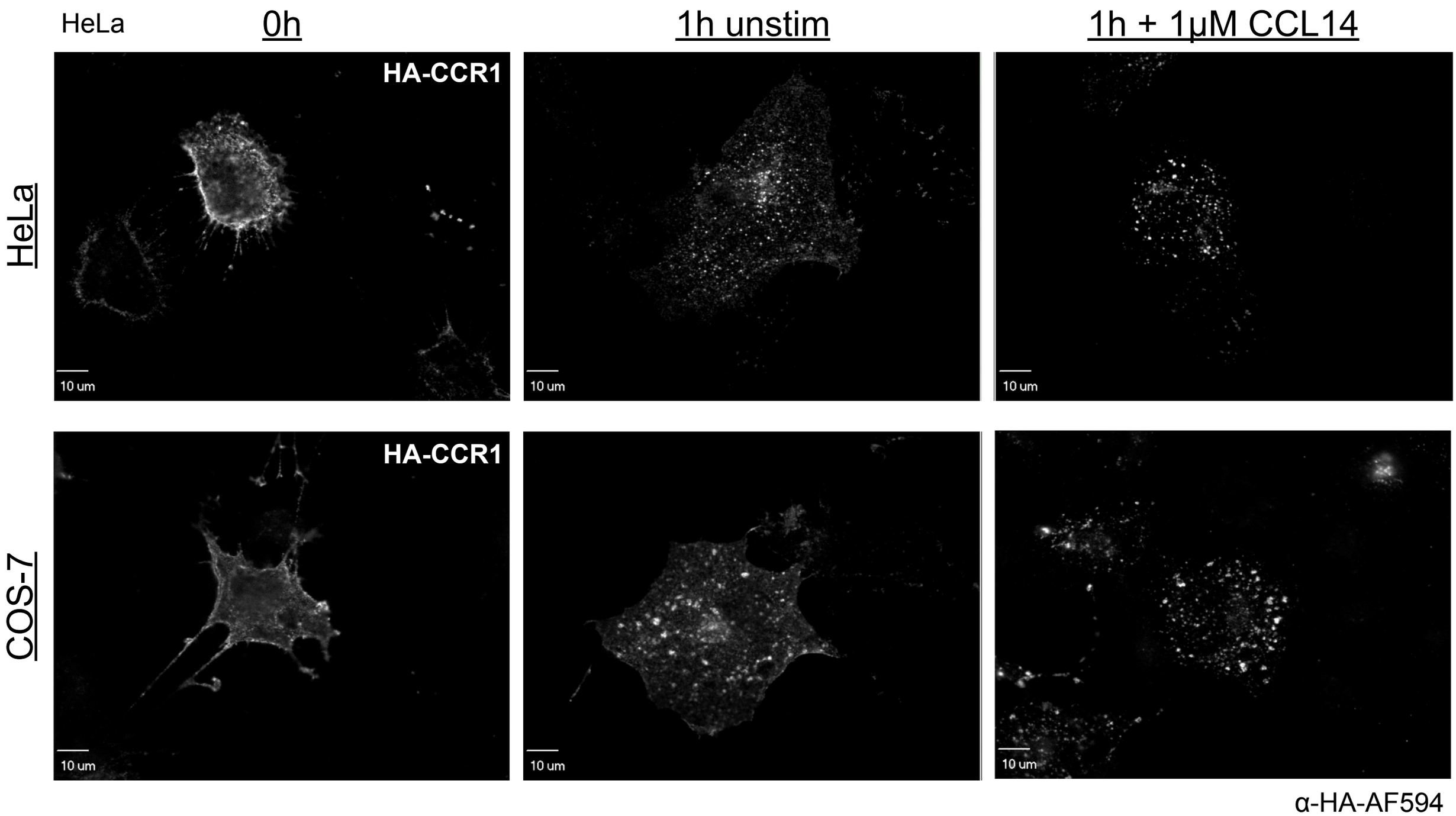


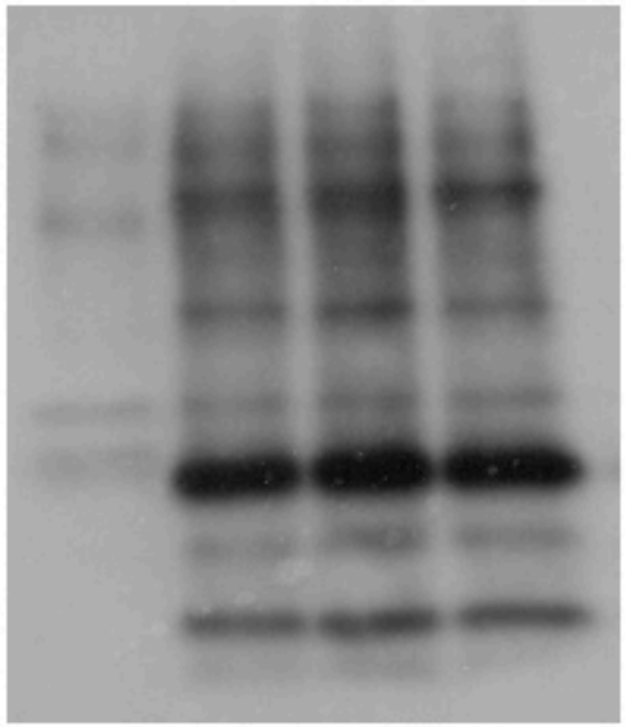
If receptor phosphorylation plays an important role in GPCR regulation, what is the phosphorylation status of CCR1?

CCR1 is constitutively phosphorylated

^{32}P blot

u.t. | FLAG-CCR1

0 | 0 3 6 min+1uM CCL14



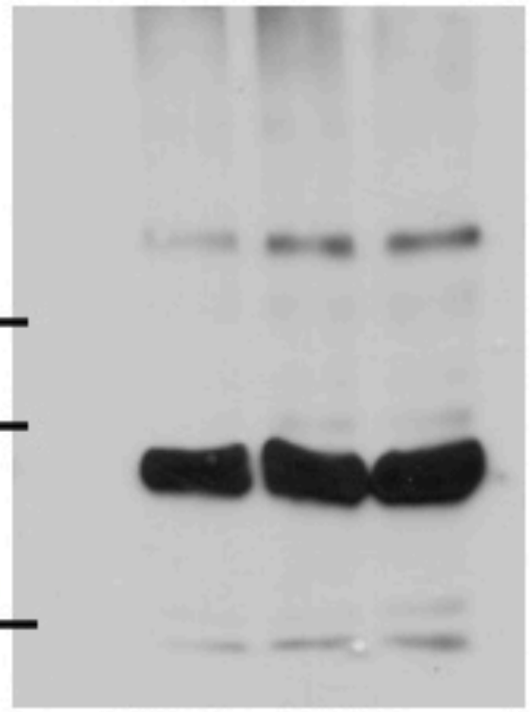
O/N exposure

CCR1

Western blot

u.t. | FLAG-CCR1

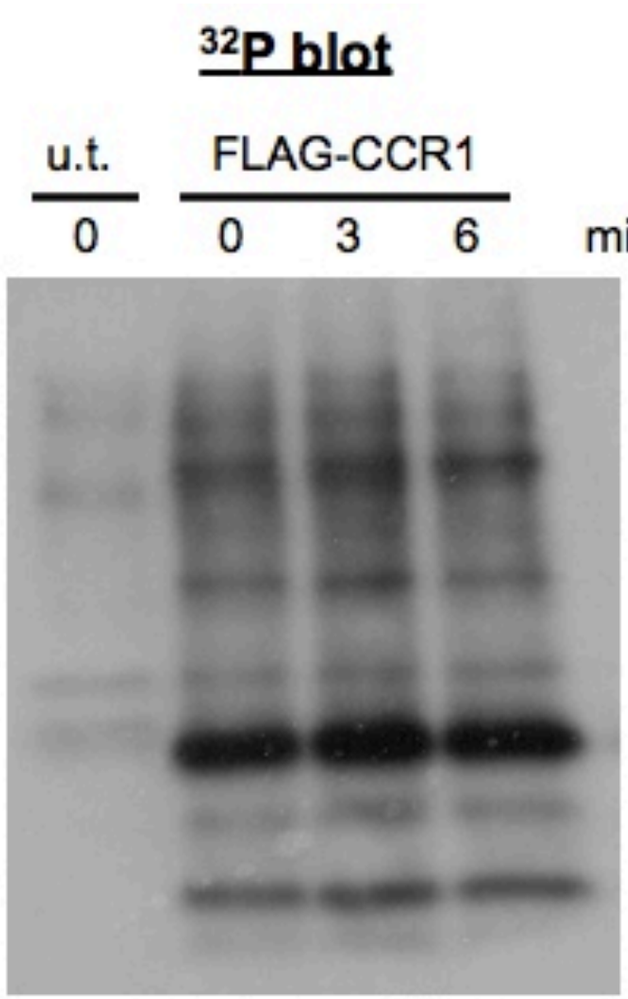
0 | 0 3 6 min+1uM CCL14

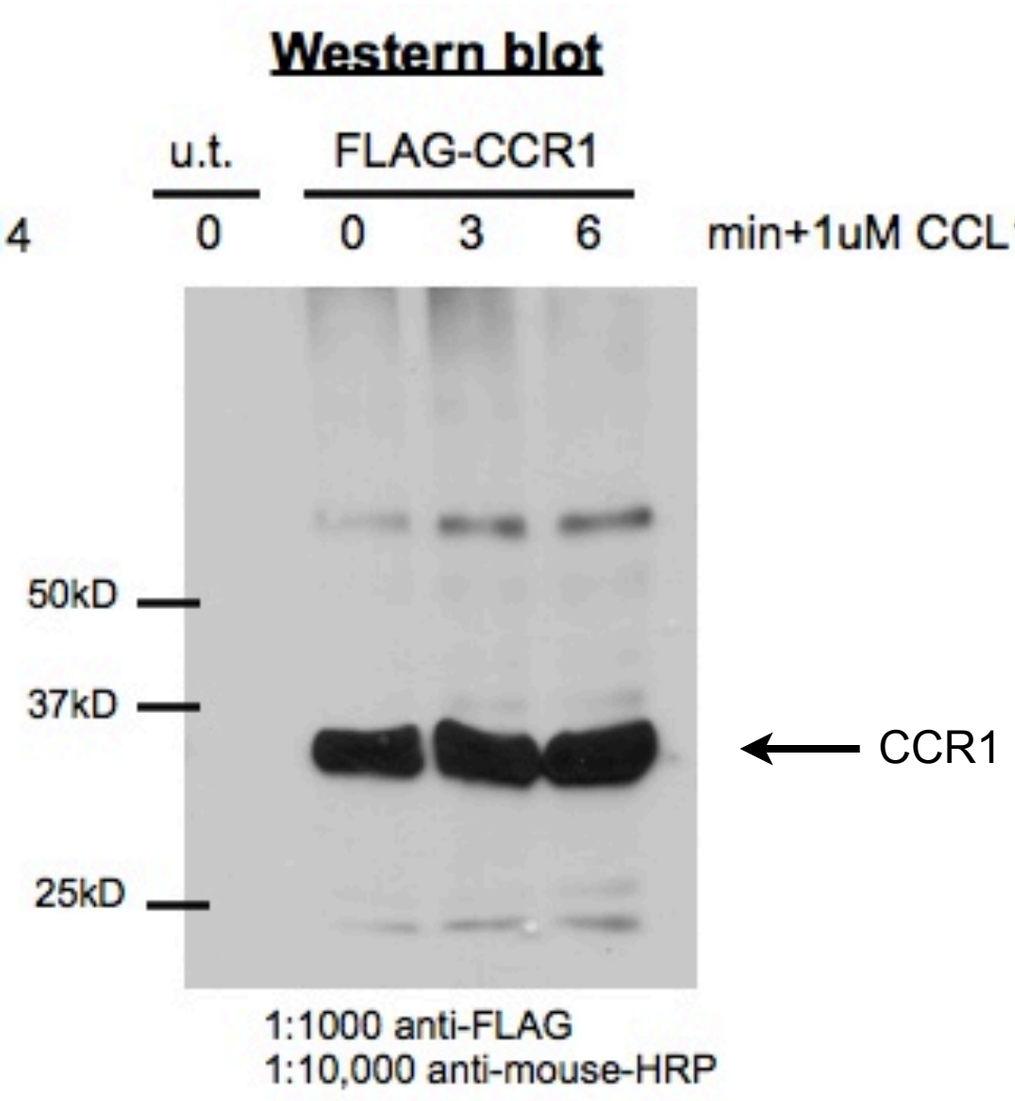


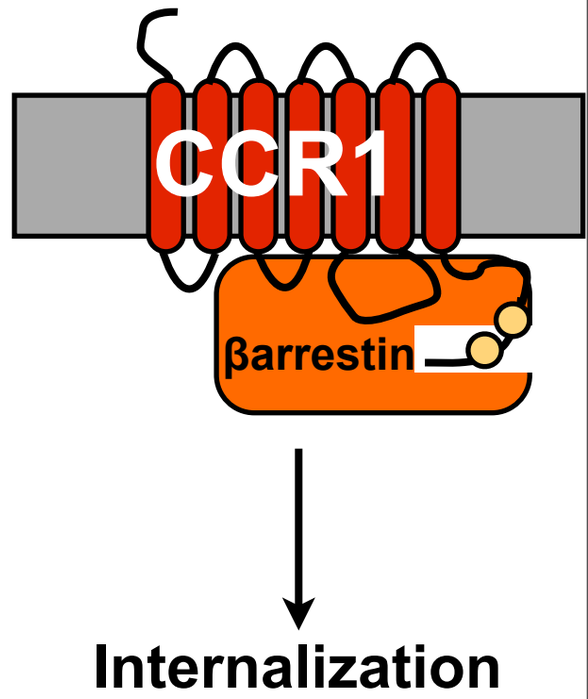
CCR1

1:1000 anti-FLAG
1:10,000 anti-mouse-HRP

CCR1 is constitutively phosphorylated







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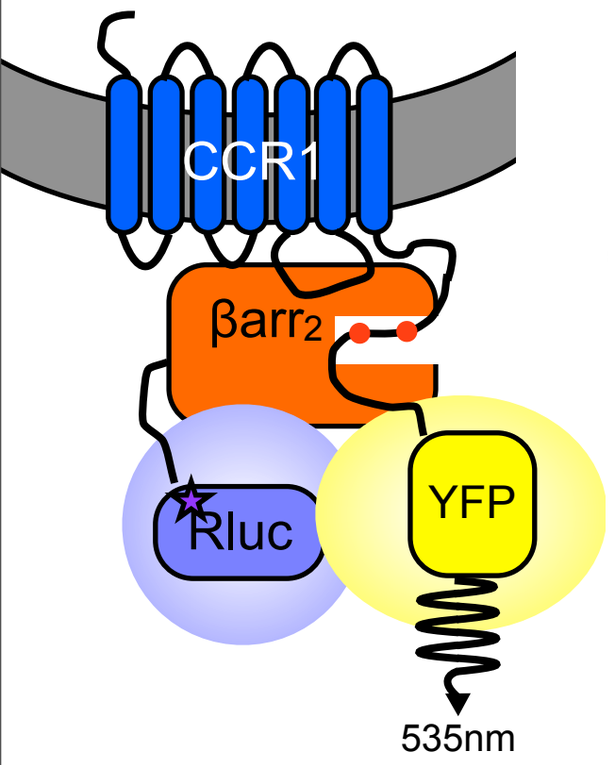
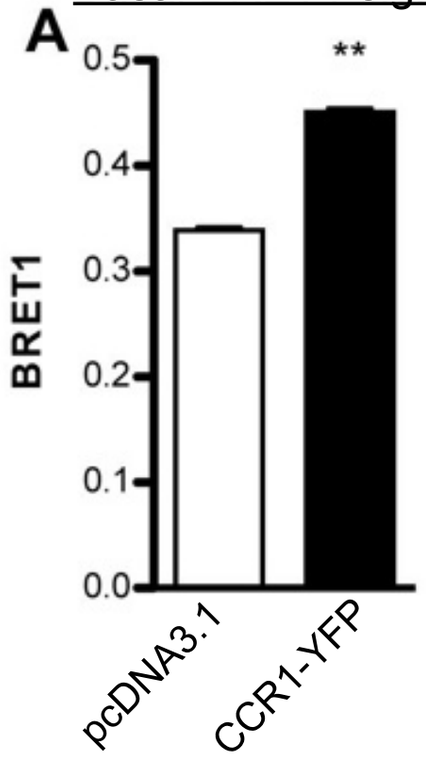
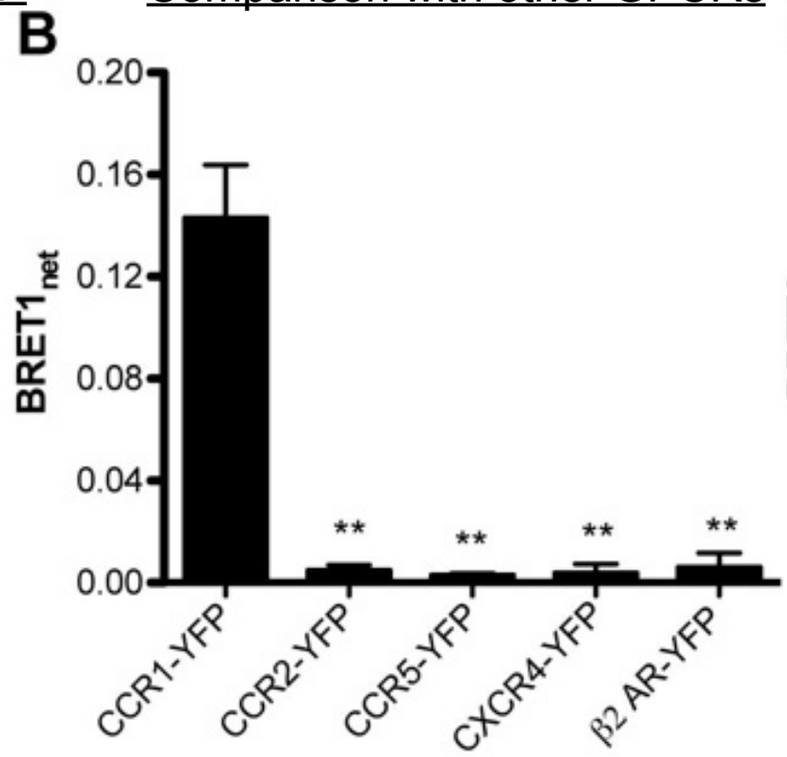
CCR1 is constitutively associated with βarrestin$_2$



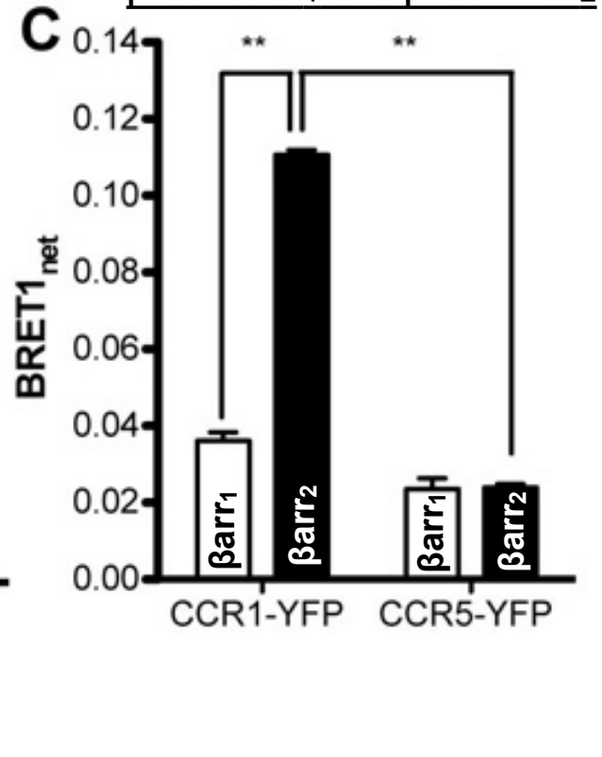
Basal BRET1 signal



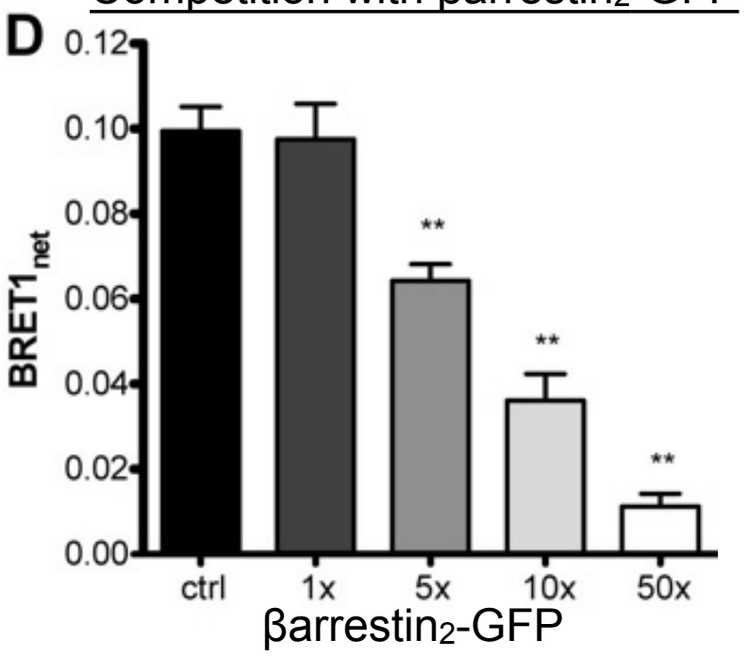
Comparison with other GPCRs



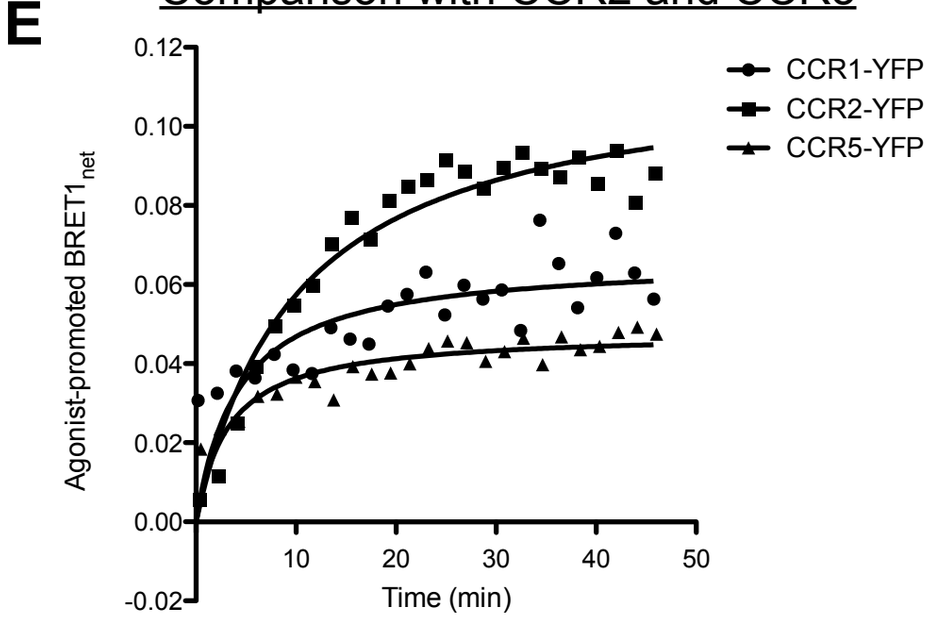
βarrestin$_1$ vs. βarrestin$_2$



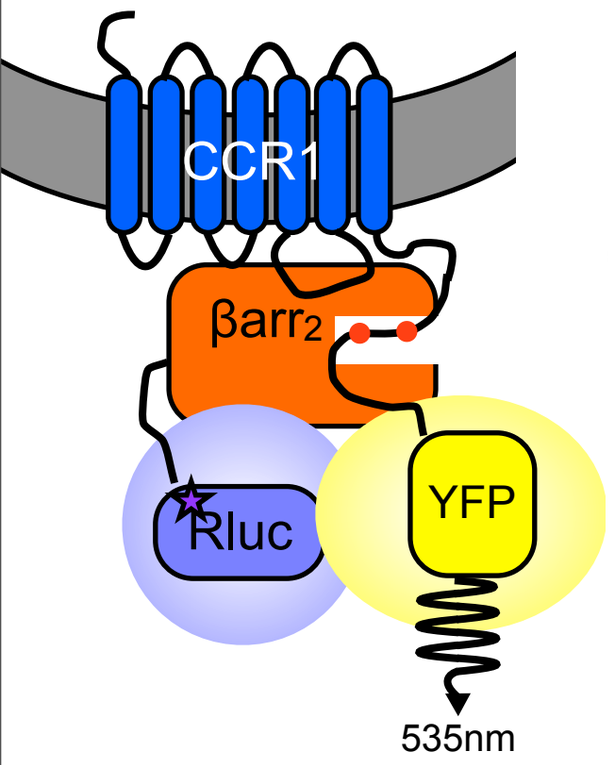
Competition with βarrestin$_2$-GFP



Comparison with CCR2 and CCR5



CCR1 is constitutively associated with βarrestin$_2$



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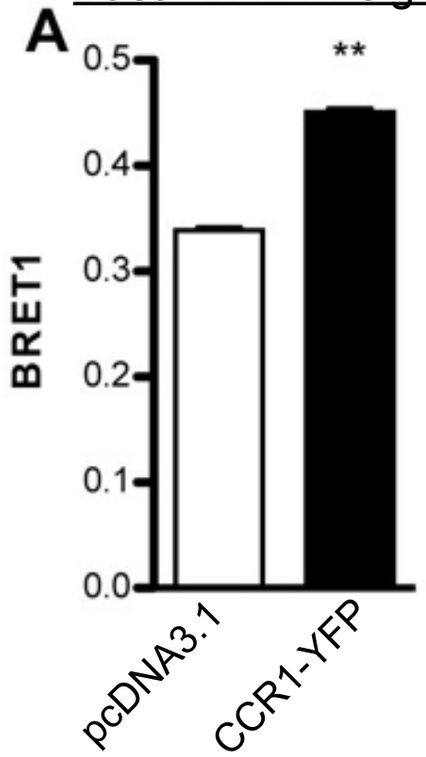

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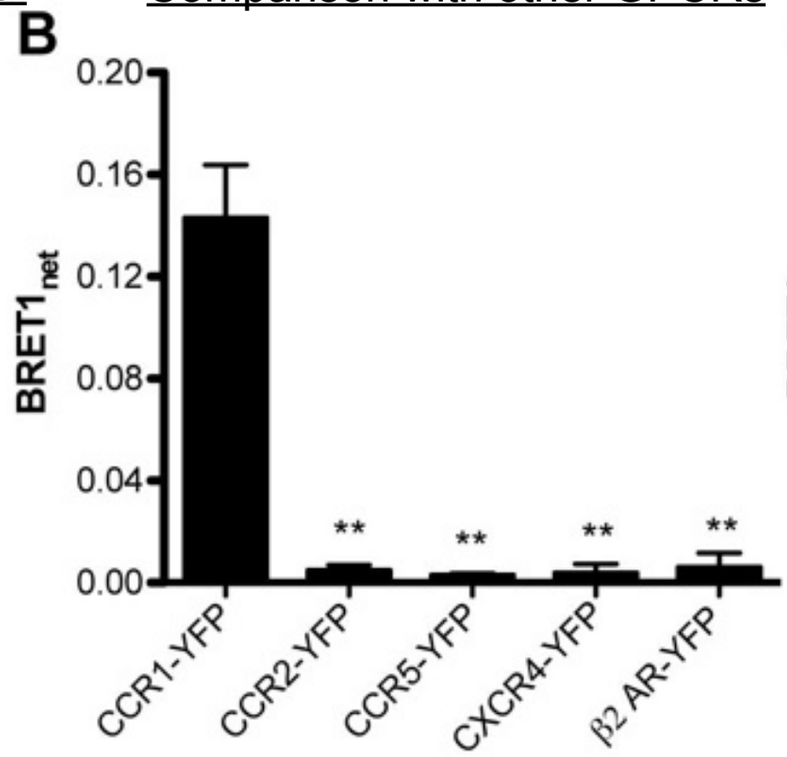
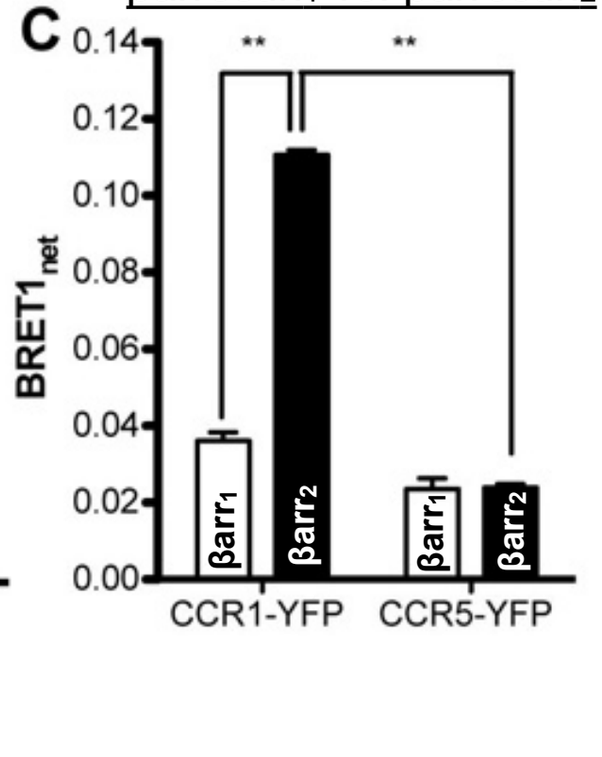


βarrestin$_1$ vs. βarrestin$_2$



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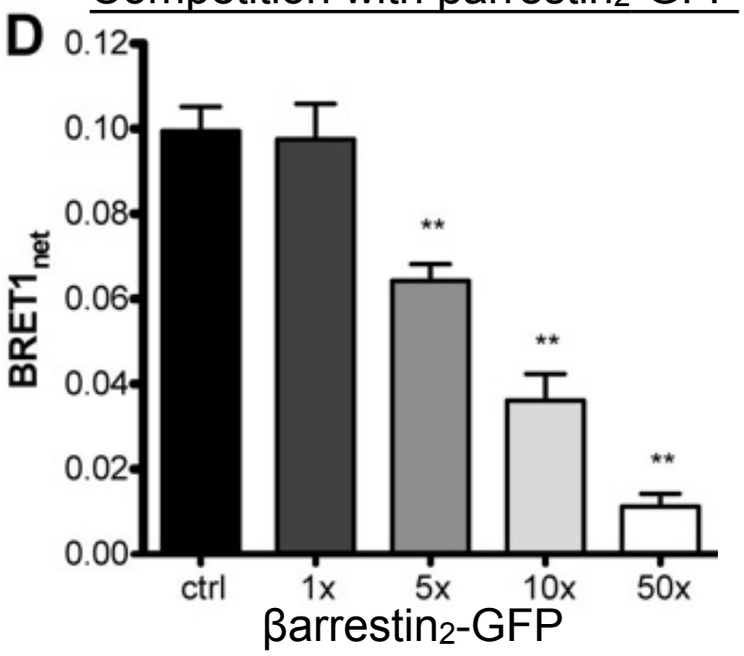
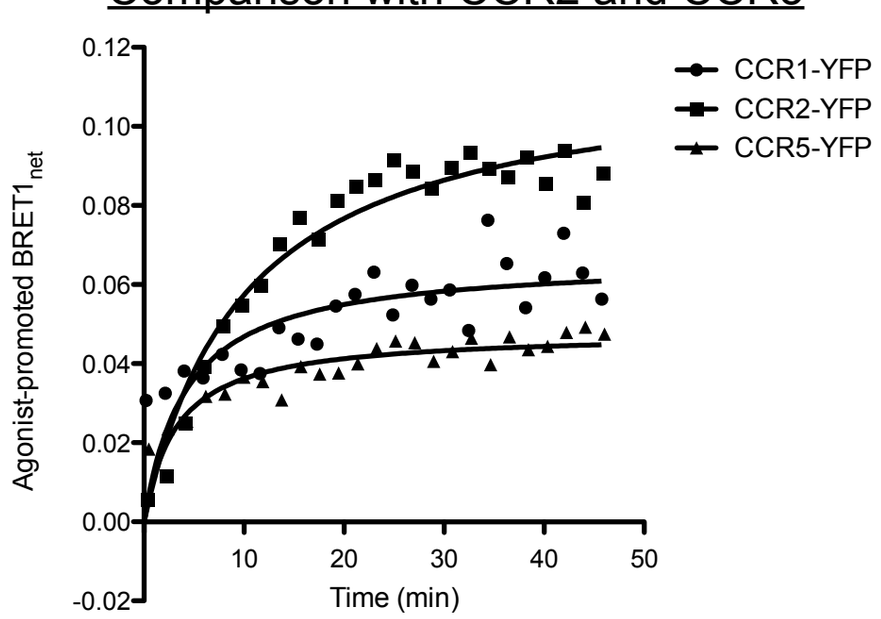
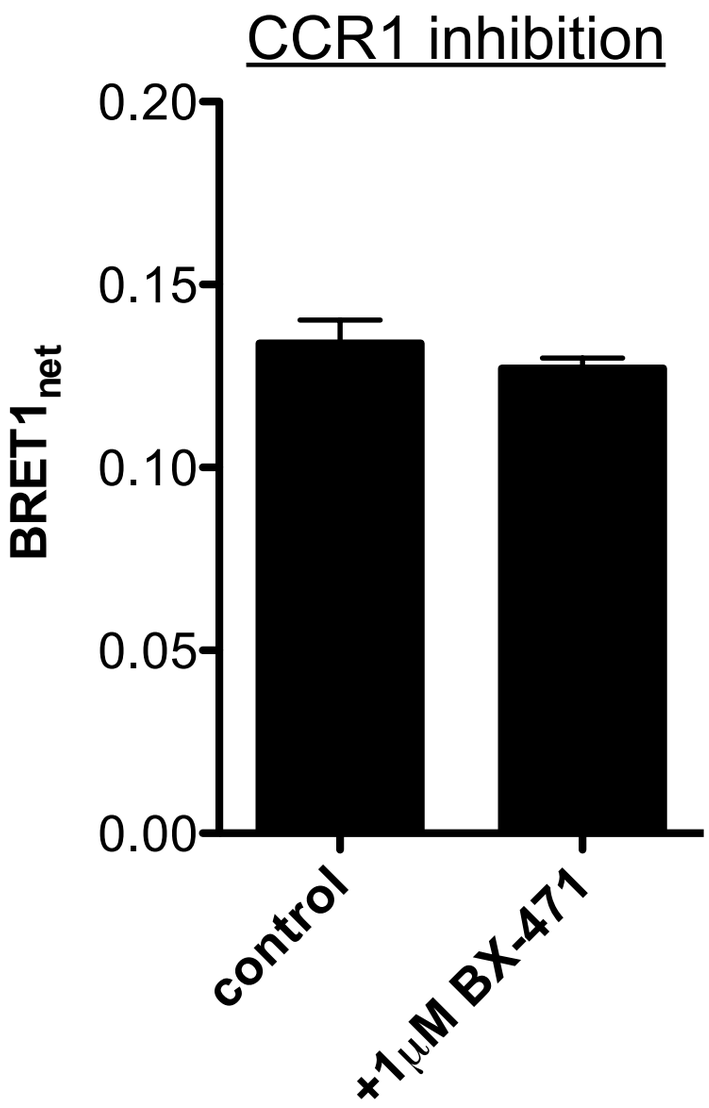


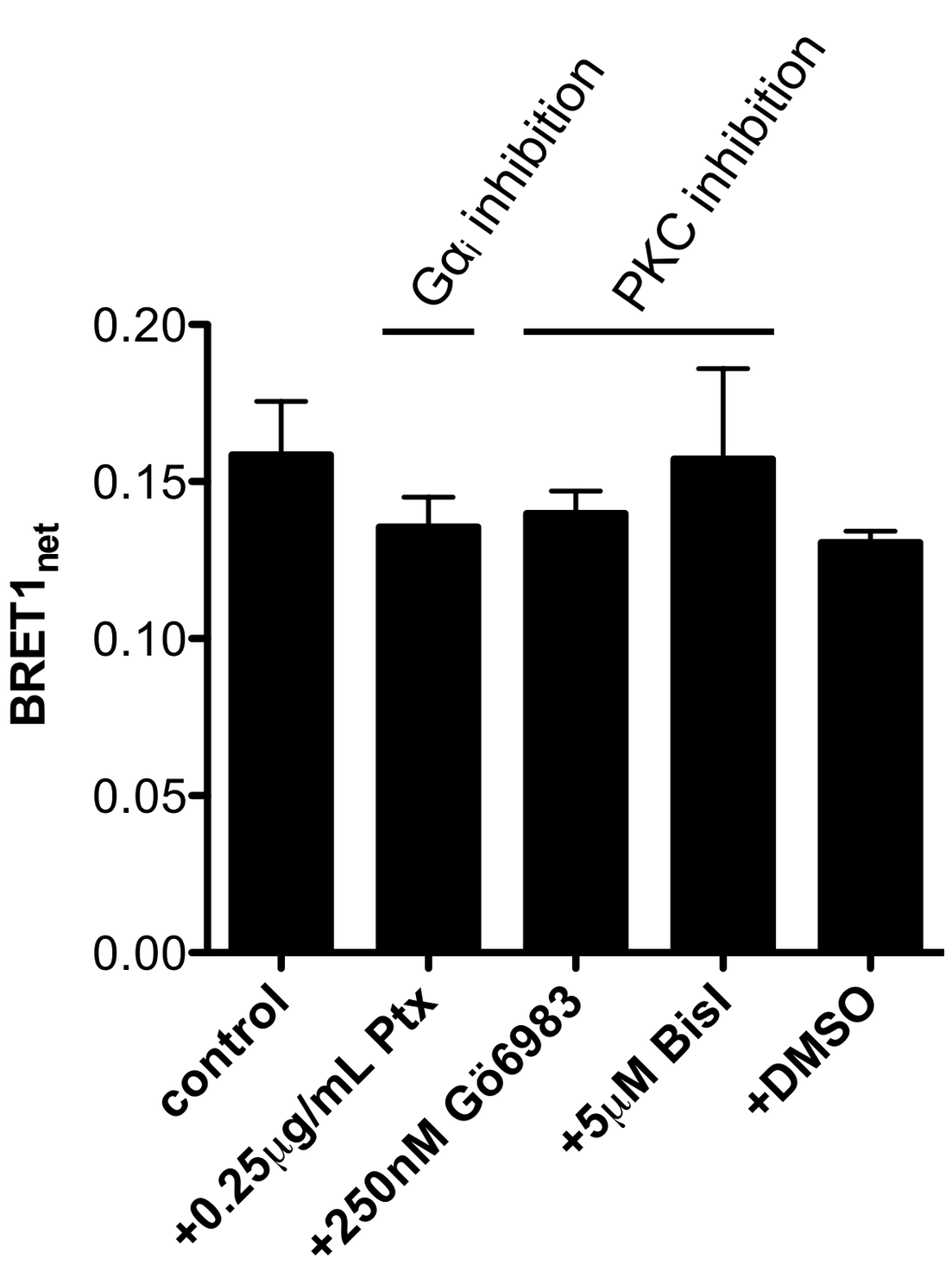
Comparison with CCR2 and CCR5



What role does receptor inhibition and G protein signaling play in this basal association?

Neither CCR1 activation, G protein coupling, nor PKC phosphorylation are required for basal association with βarrestin$_2$





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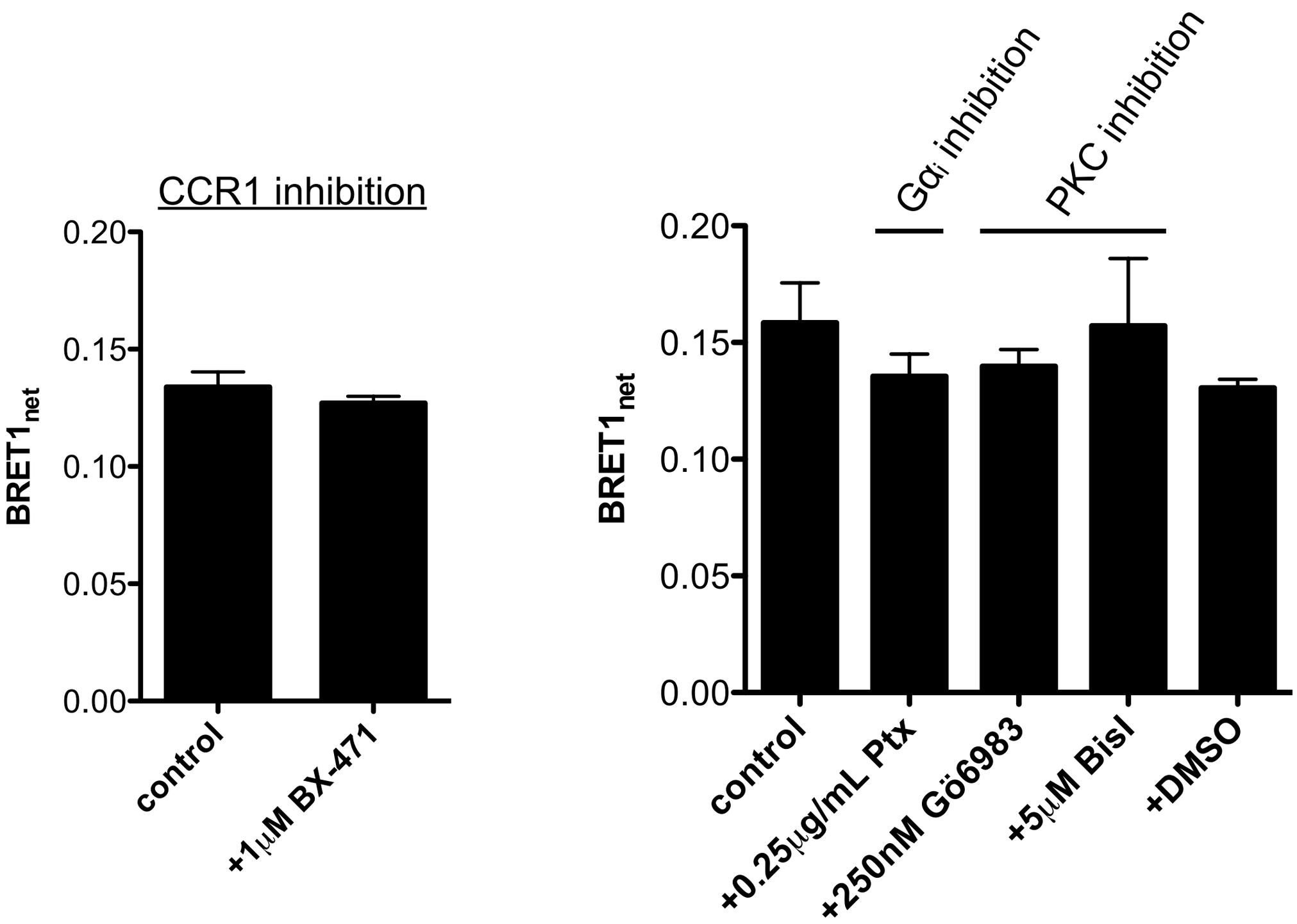
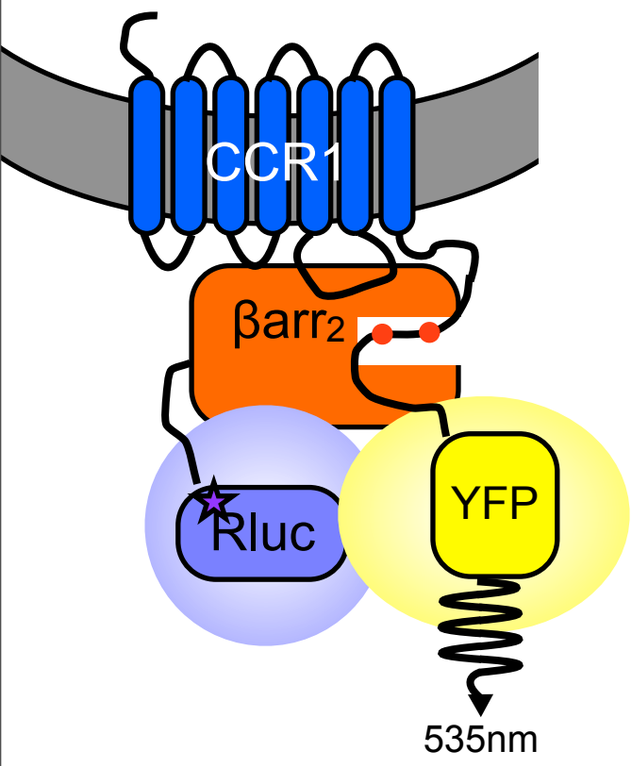
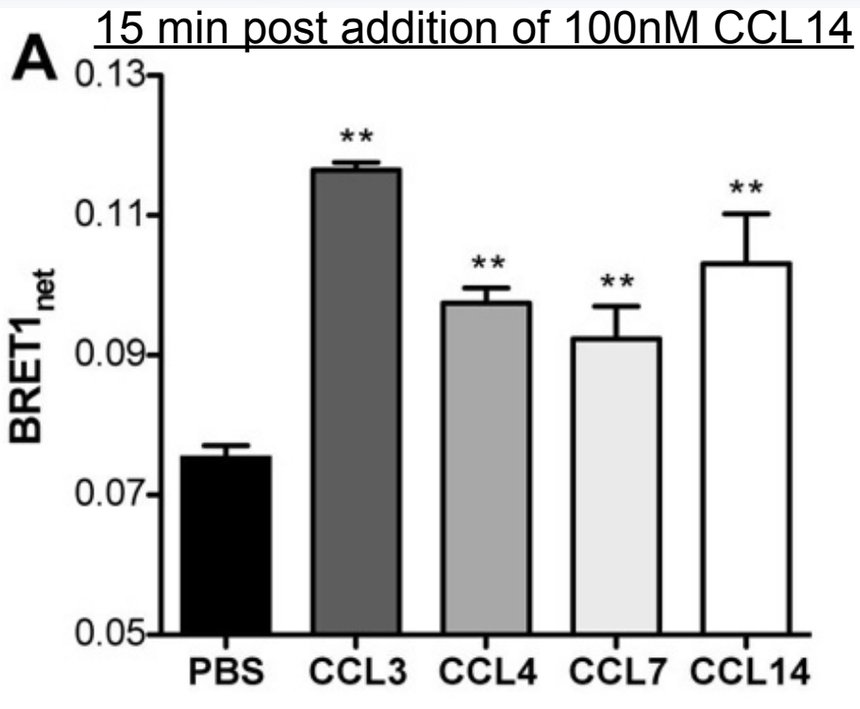
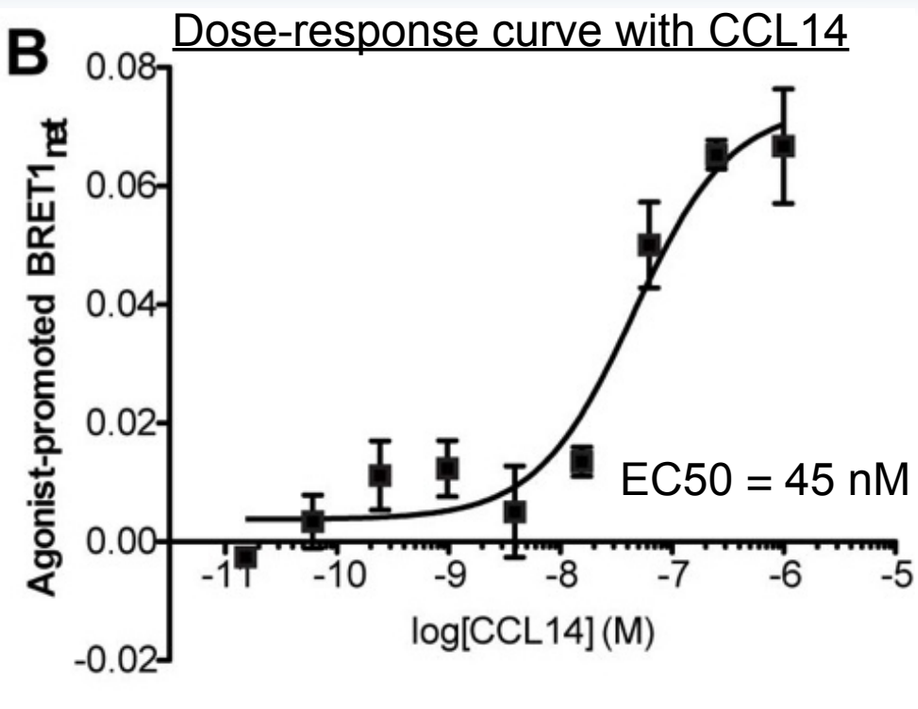


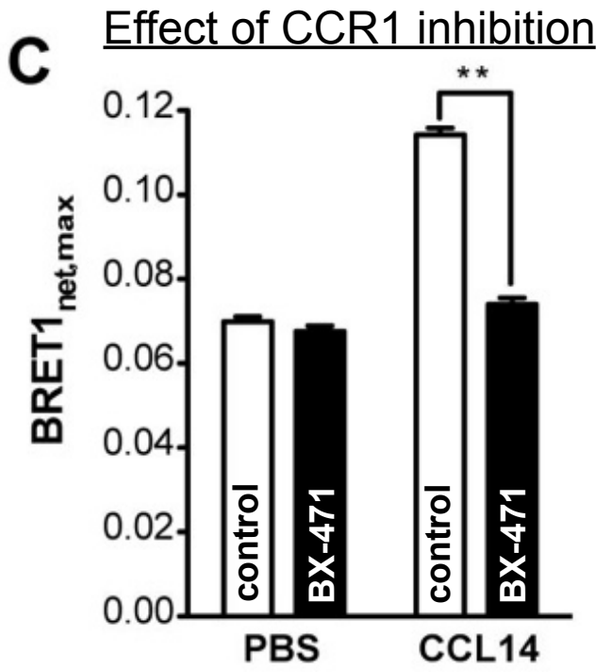
What effect do CCR1 chemokine agonists have if CCR1 is constitutively associated with βarrestin$_2$?

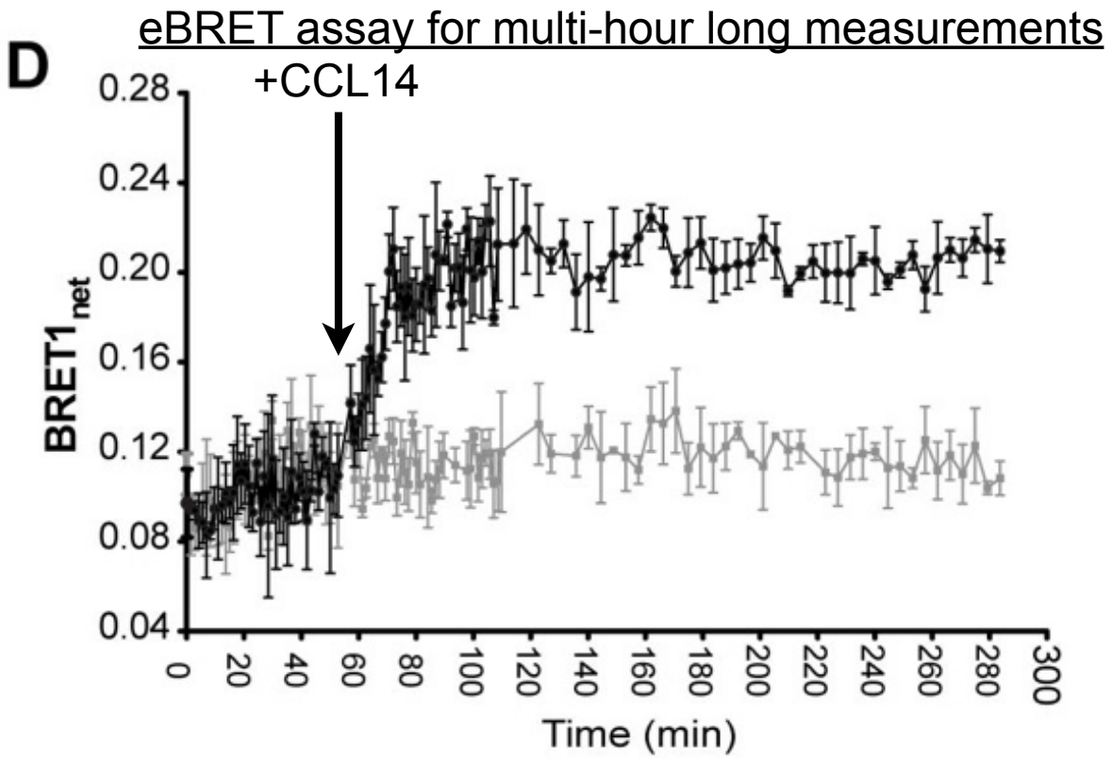
CCR1 agonists increase the BRET signal between CCR1 and βarrestin$_2$











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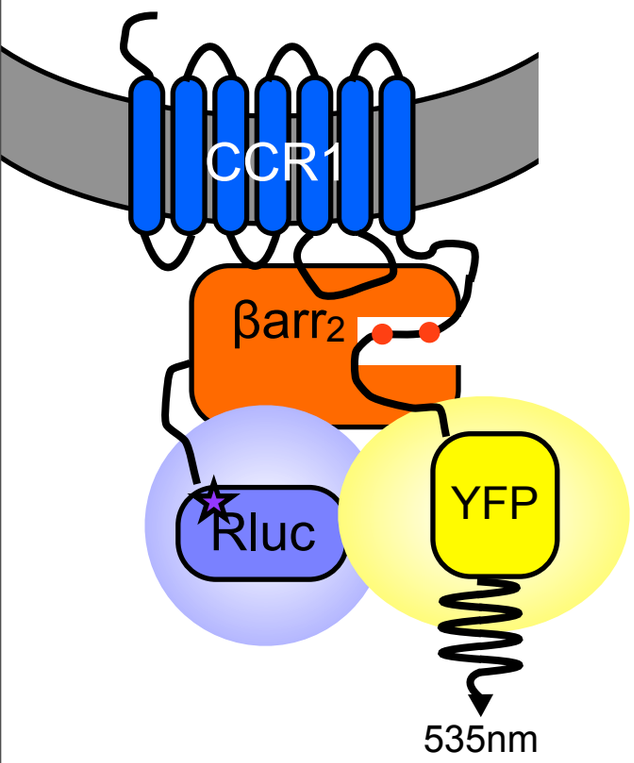
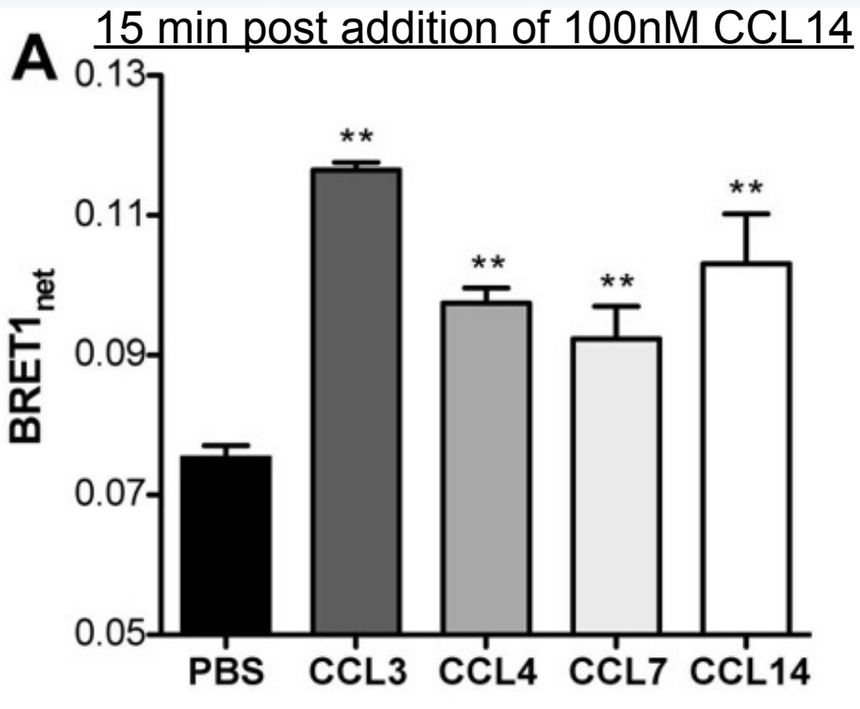
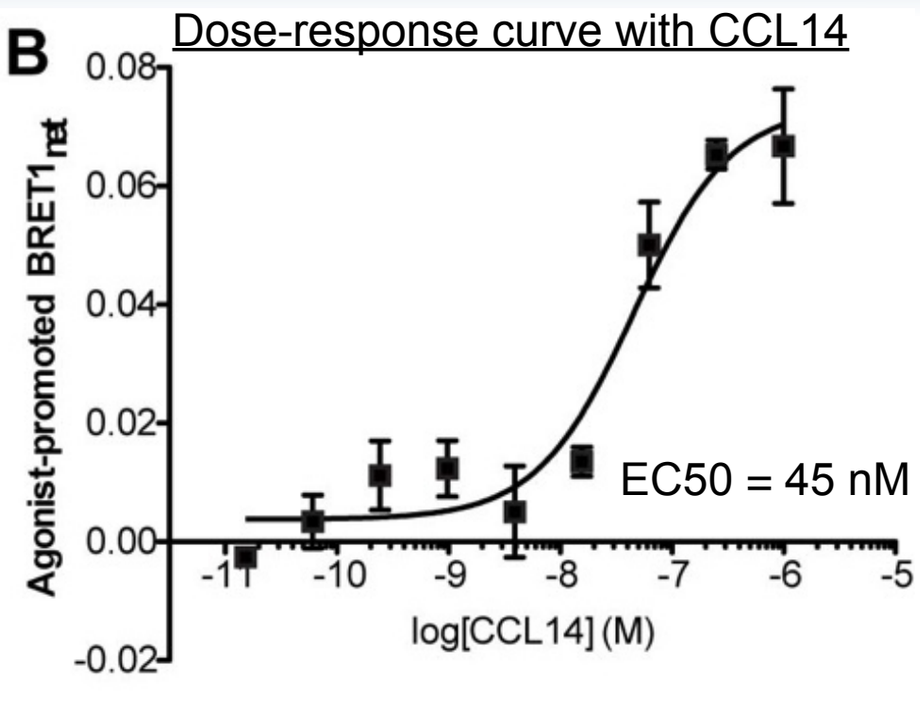
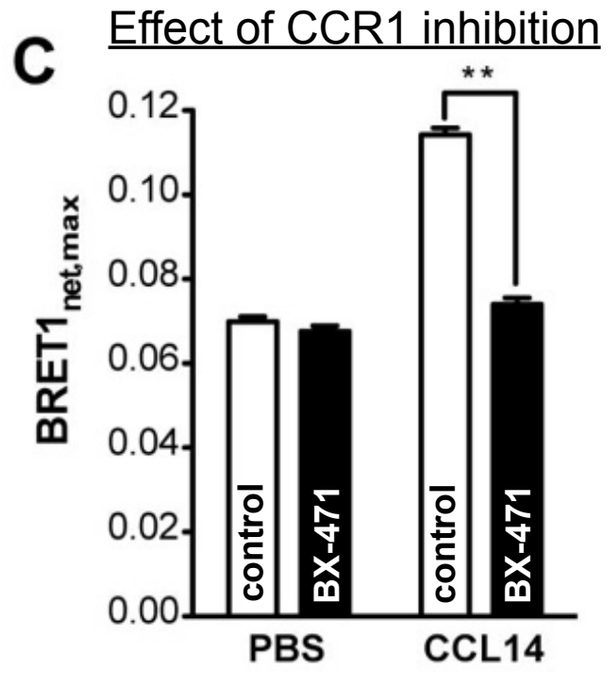
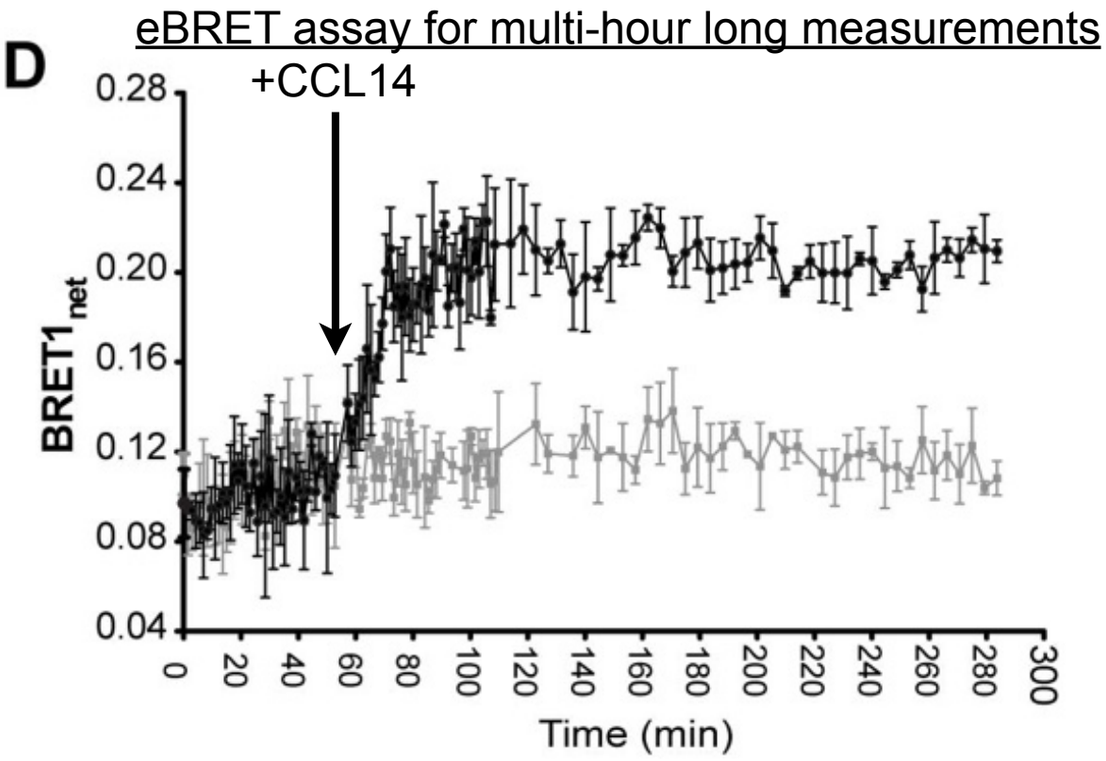






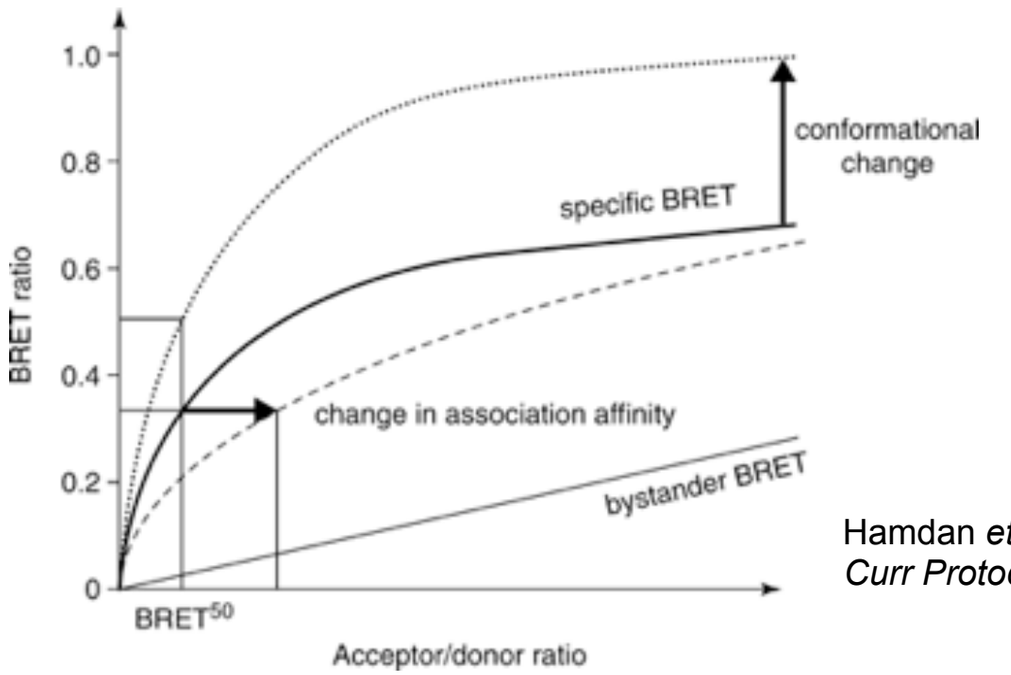




Are chemokine agonists inducing a conformational change in a pre-formed complex, or increasing association affinity of both proteins to one another?

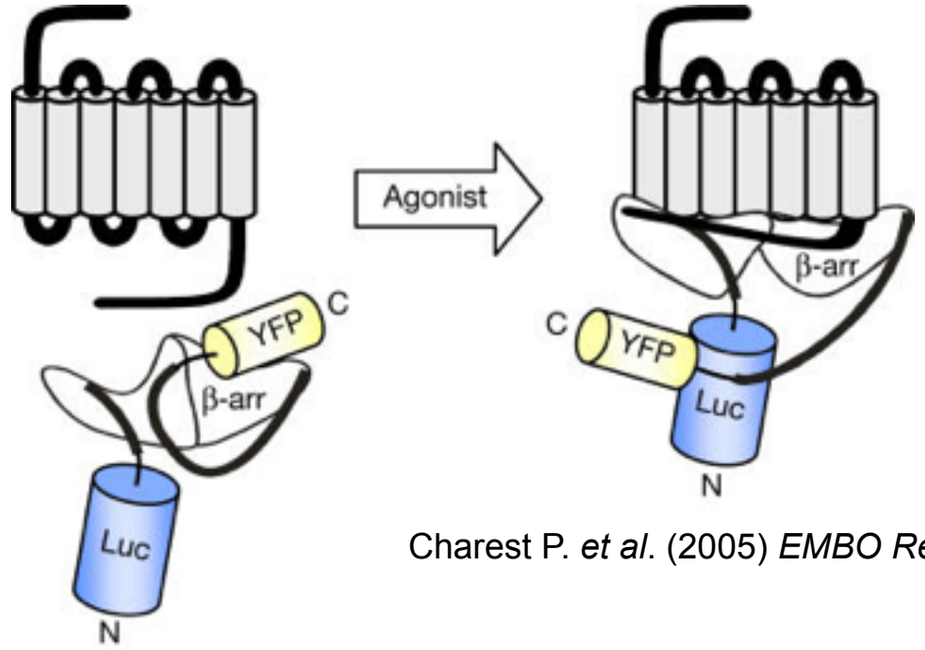
Agonists induce a conformational change within a pre-formed CCR1/βarrestin$_2$ complex

BRET saturation assay



Hamdan *et al.* (2006)
Curr Protoc Neurosci

Double-brilliance βarrestin$_2$ reporter



Charest P. *et al*. (2005) *EMBO Rep*

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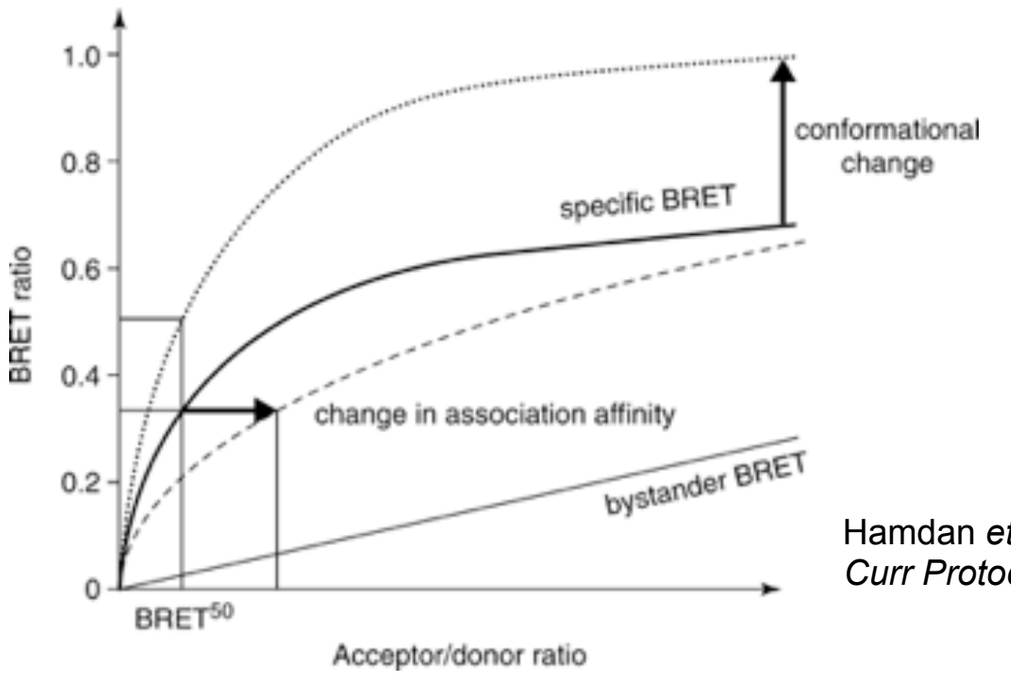

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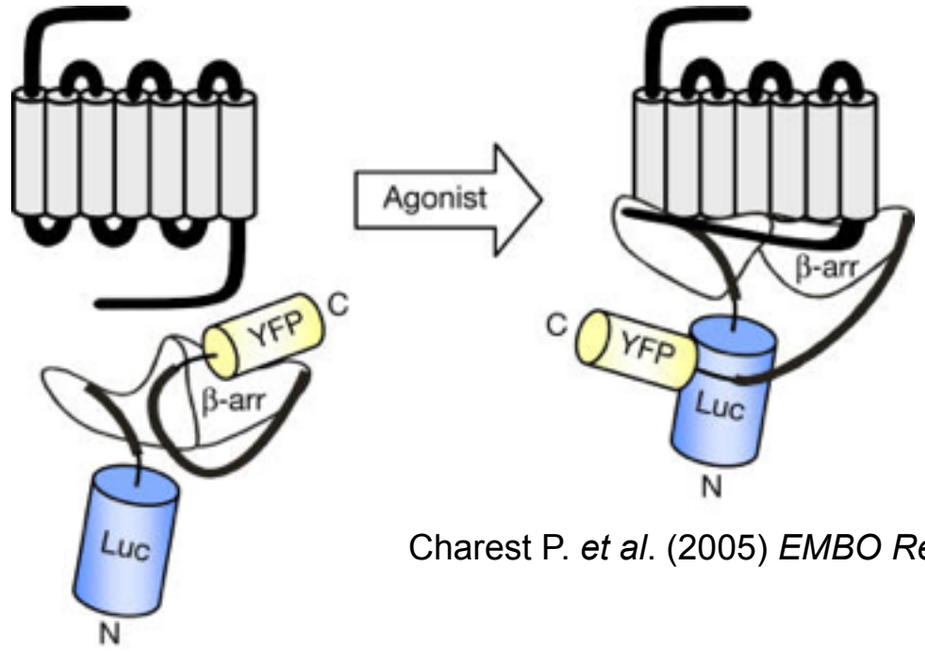
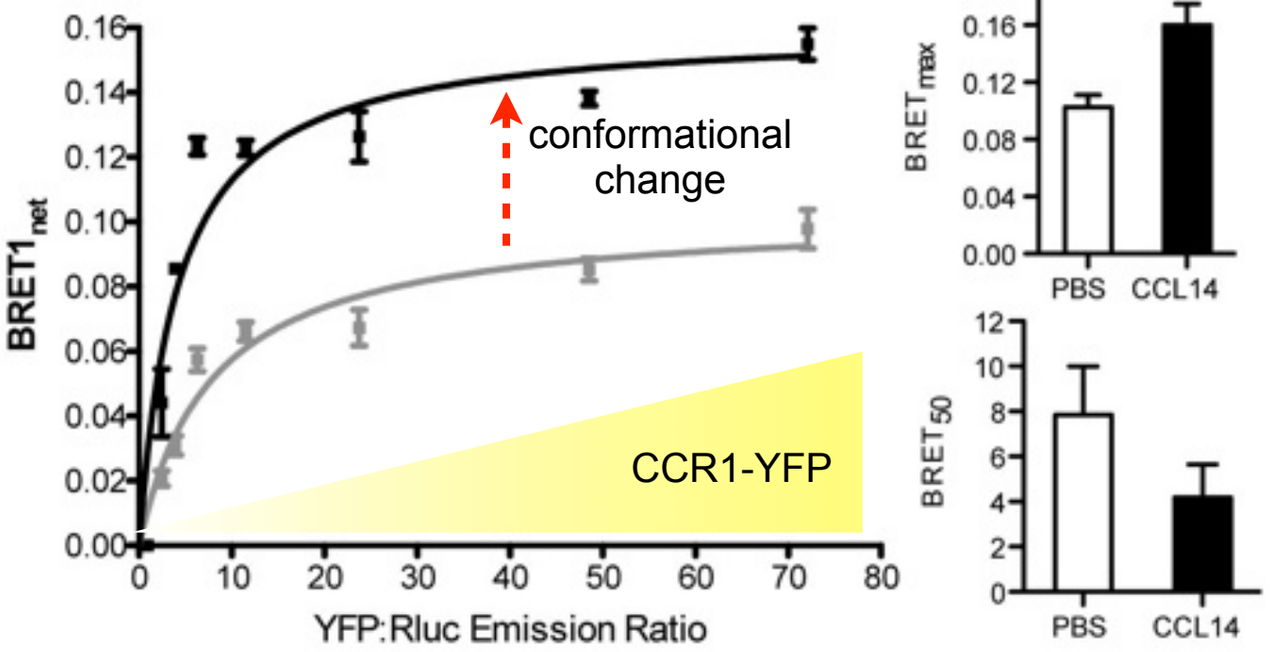


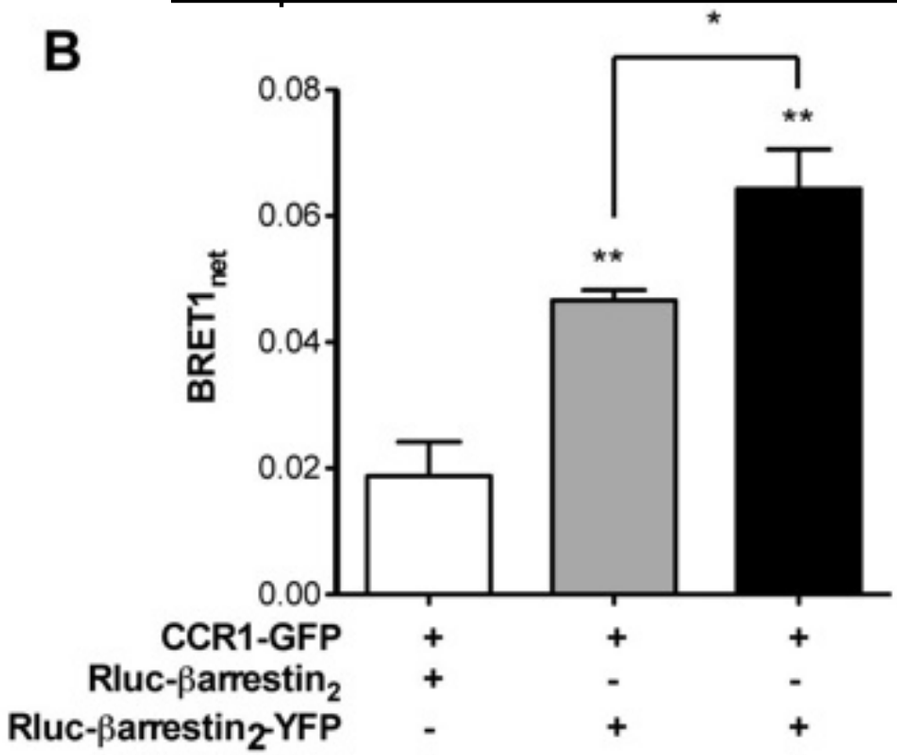
Charest P. *et al*. (2005) *EMBO Rep*

A BRET saturation curve +/- 100nM CCL14

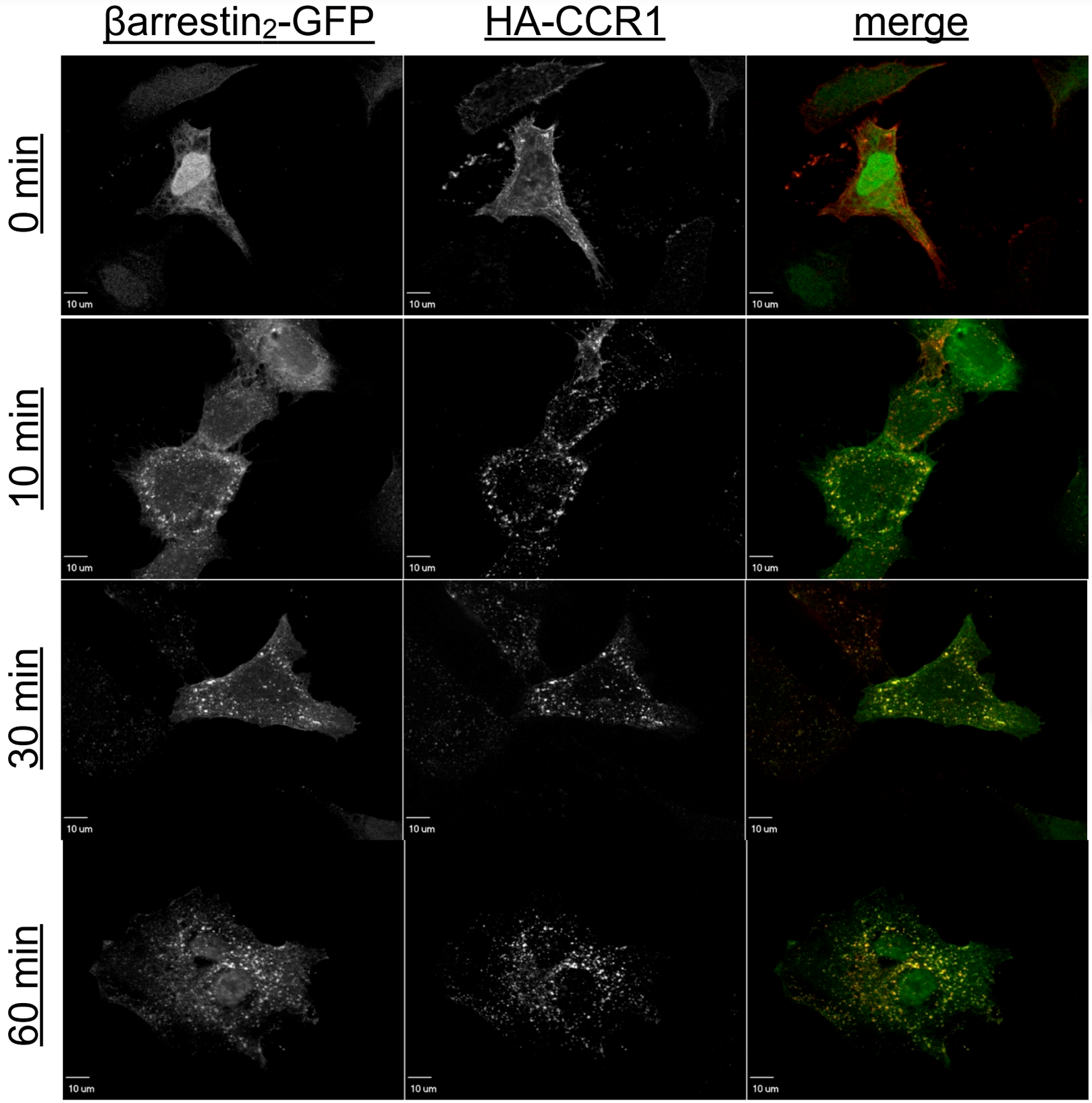


Rluc-βarrestin2-YFP BRET +/- CCL14

B



Agonist-induced co-localization of CCR1 and βarrestin$_2$



30min figure looks kinda orange, maybe replace with "yellower" one?

HeLa

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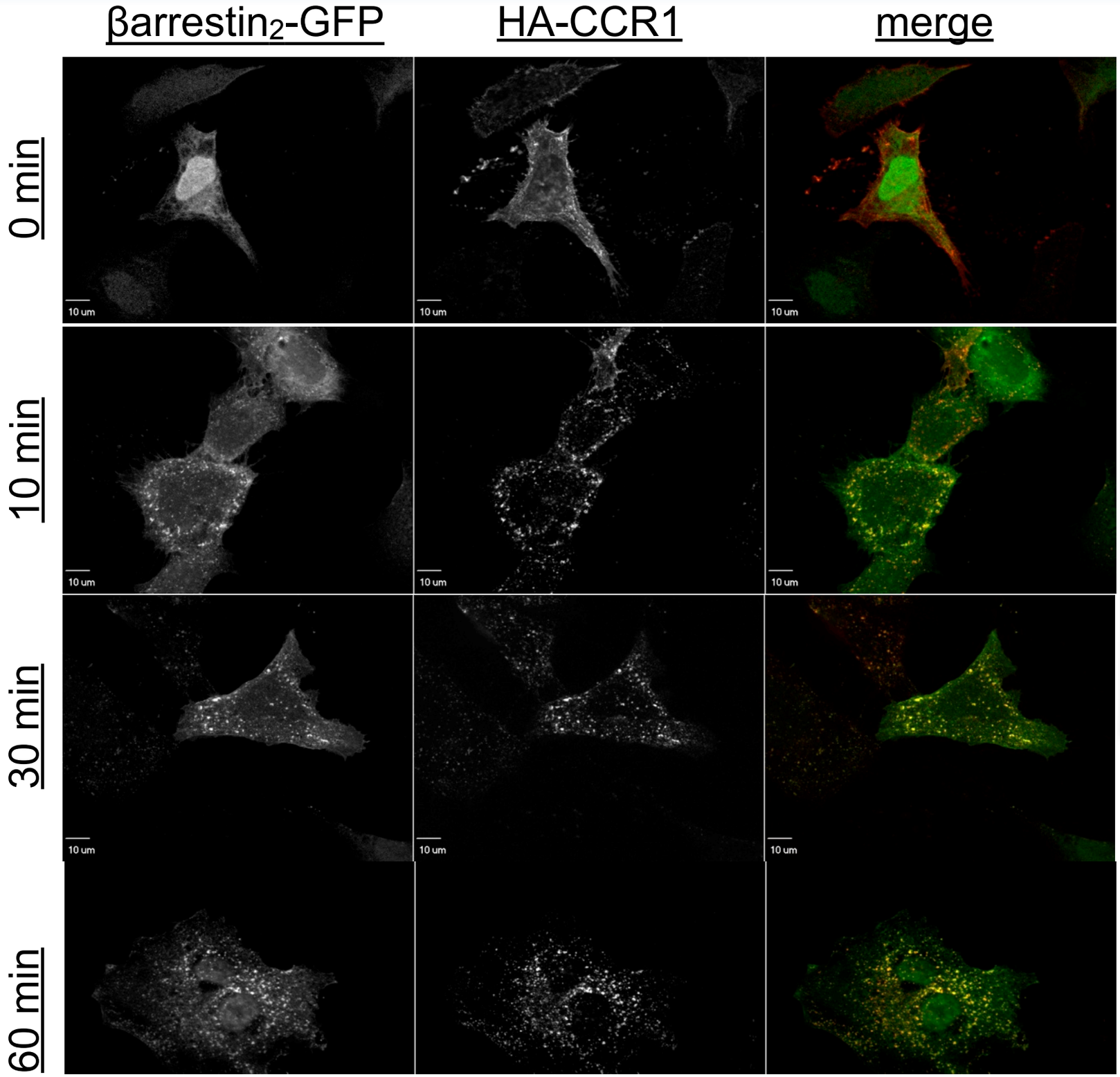
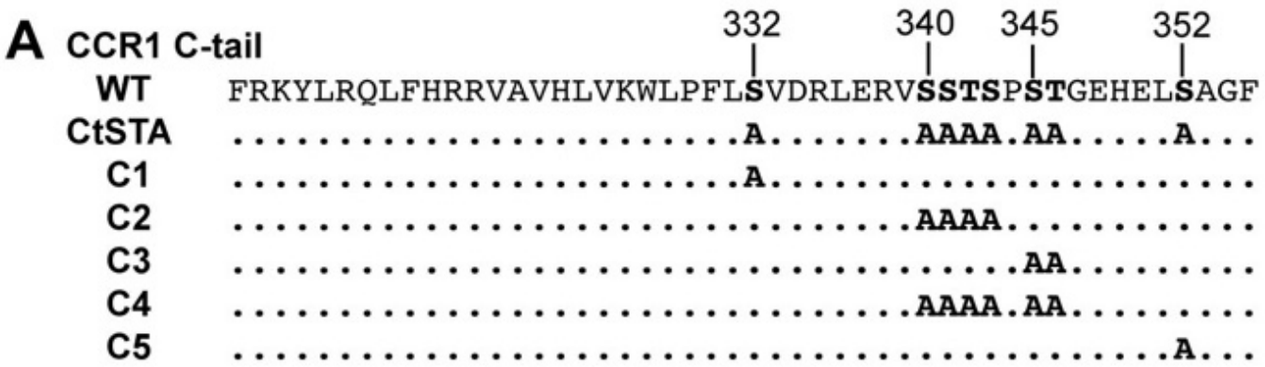


30min figure looks kinda orange, maybe replace with "yellower" one?

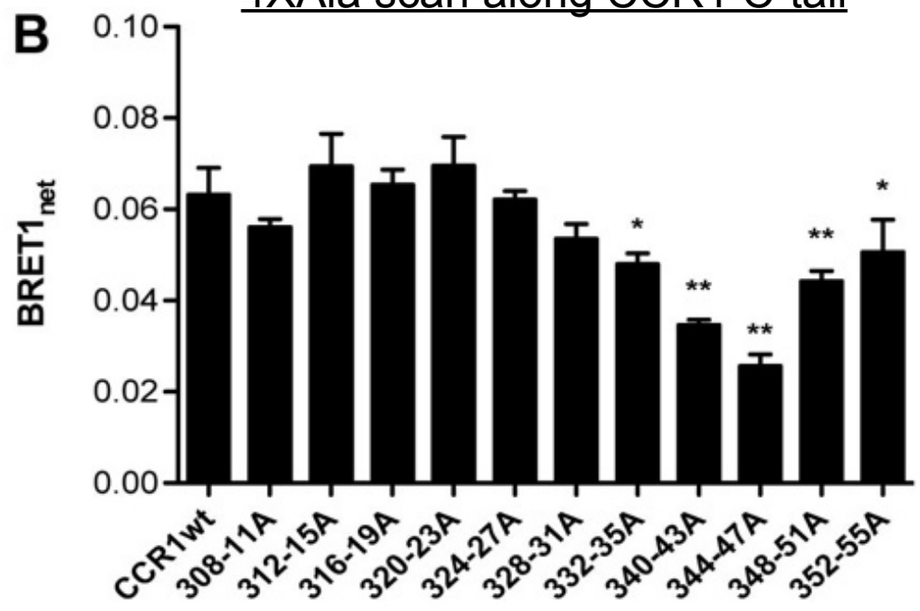
What are the structural domains within CCR1 that mediate this association?

Ser/Thr-rich regions within the distal C-tail are responsible for constitutive interaction with βarrestin$_2$

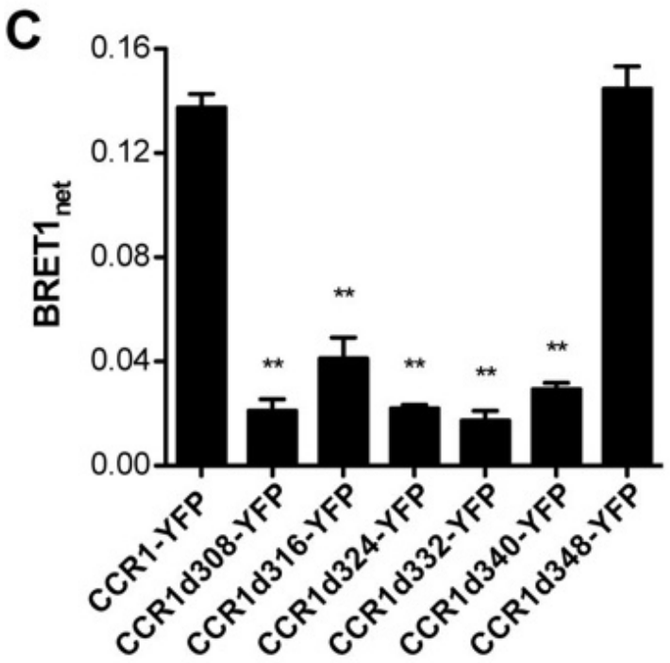
CCR1 C-tail sequence



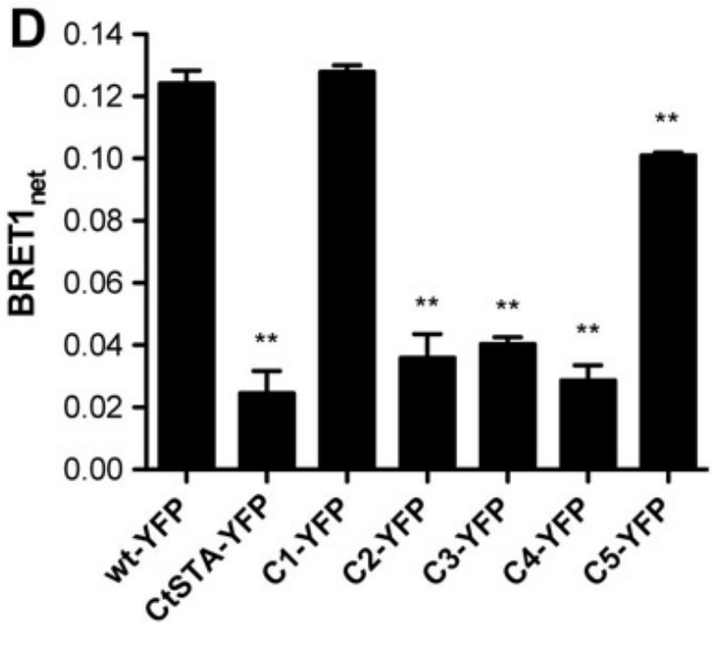
4XAla scan along CCR1 C-tail



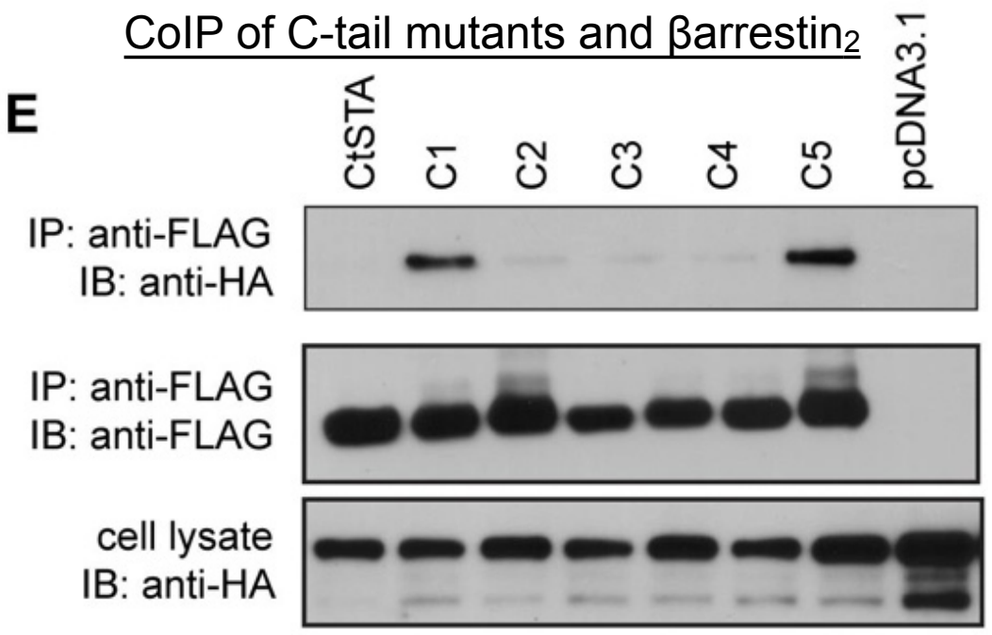
Sequential C-tail deletion



Mutation of Ser/Thr residues in C-tail



CoIP of C-tail mutants and βarrestin$_2$



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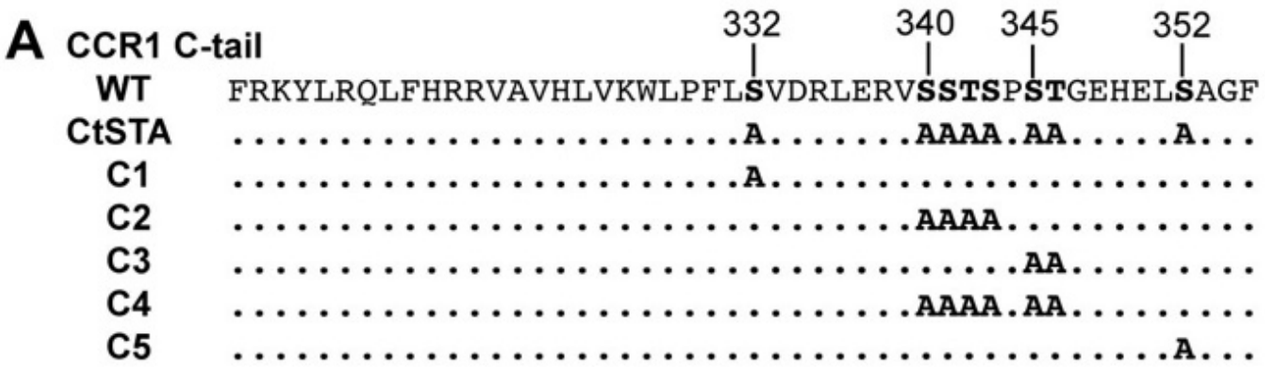
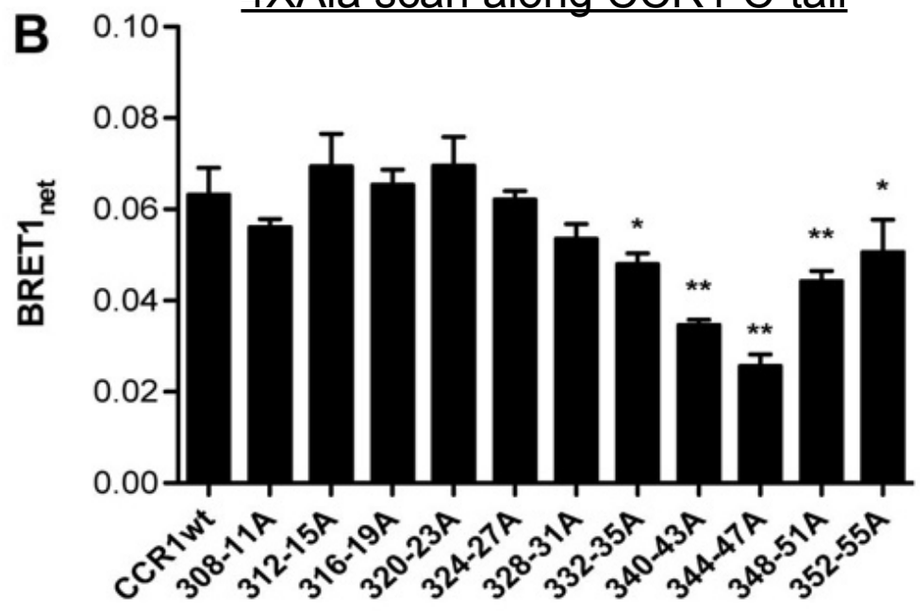


4XAla scan along CCR1 C-tail



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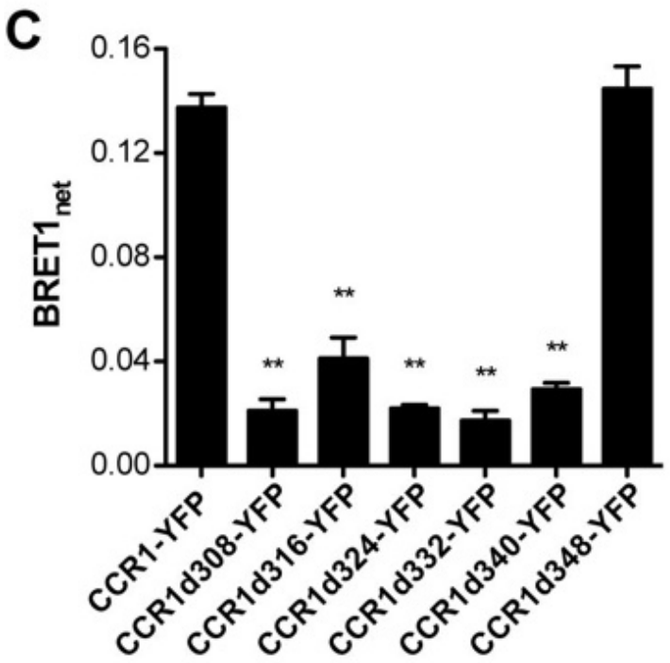

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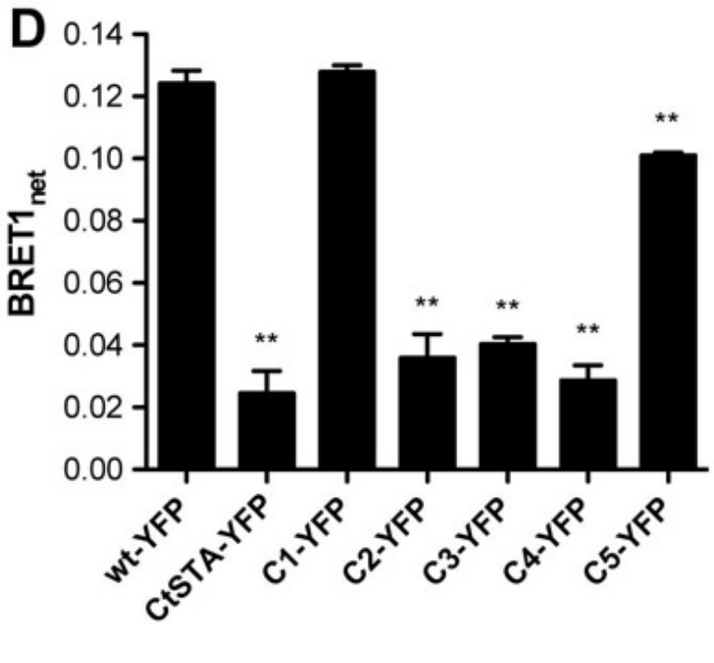

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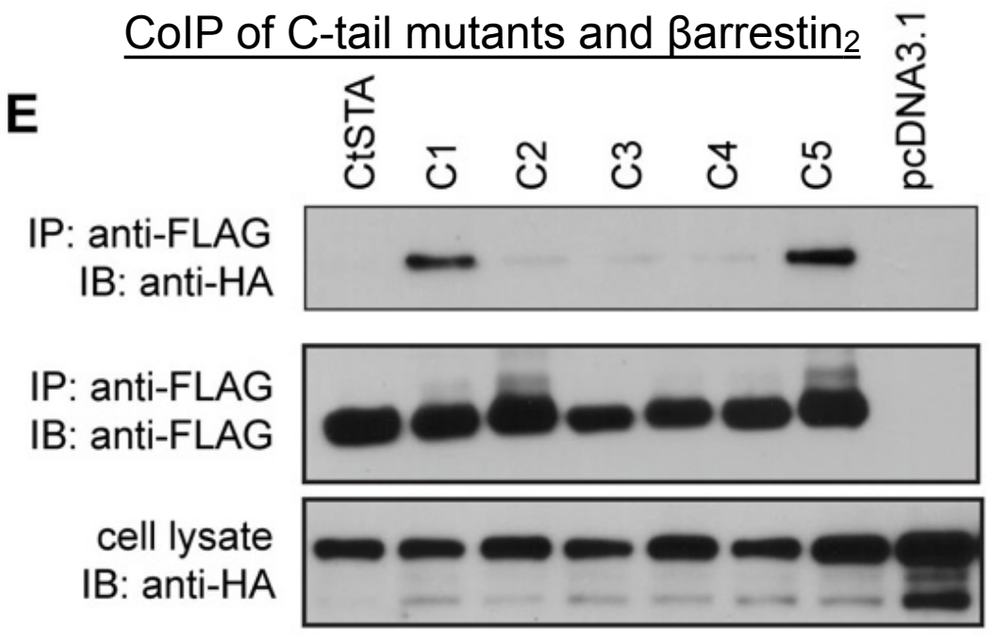
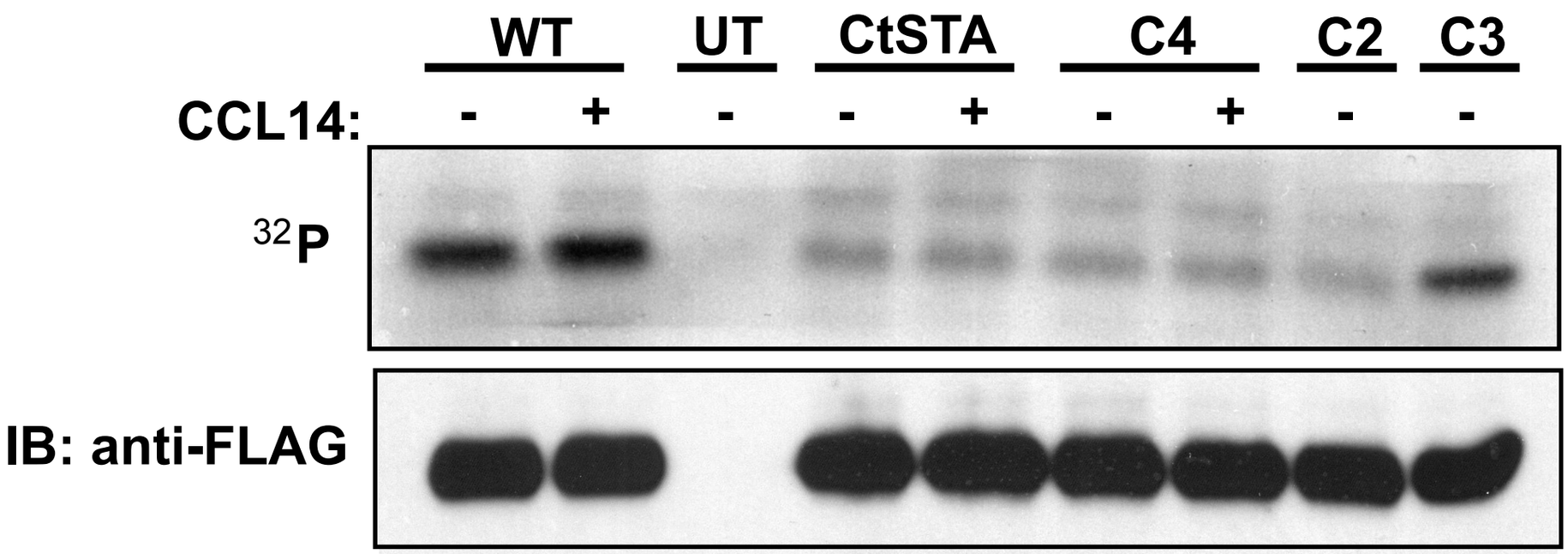


Where is CCR1 being phosphorylated?

CCR1 is basally phosphorylated in its C-terminal tail

A **CCR1 C-tail**

	332	340	345	352
WT	FRKYLRQLFHRRVAVHLVKWLPFL**S**VDRLERV**SSTS**P**ST**GEHEL**S**AGF			
CtSTA	.....**A**.....**AAAA**.**AA**.....**A**...			
C1	.....**A**.....			
C2	.....**AAAA**.....			
C3	.....**AA**.....			
C4	.....**AAAA**.**AA**.....			
C5	.....**A**...			



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C4	.....**AAAA**.**AA**.....			
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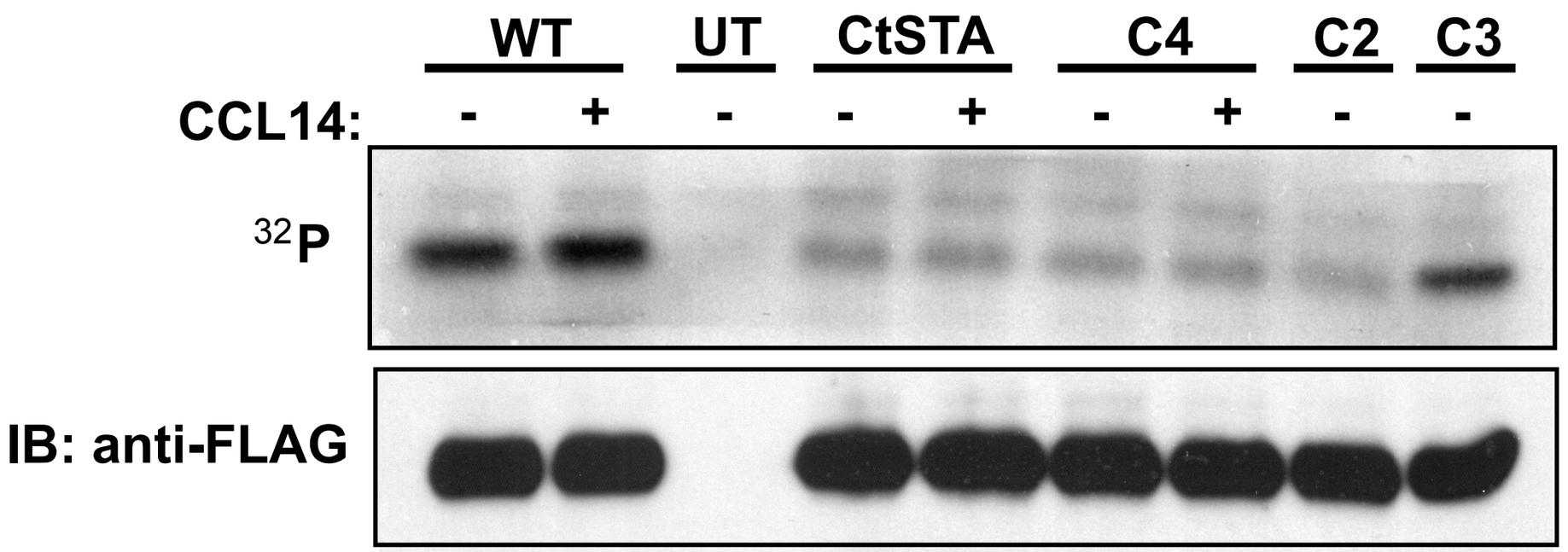
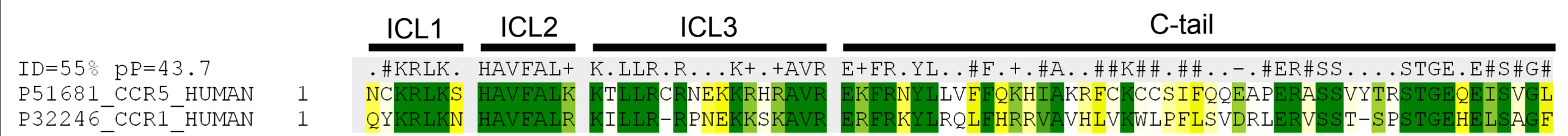
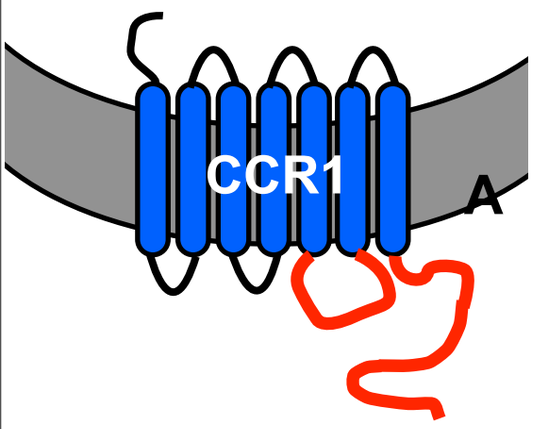


Are these domains sufficient to mediate constitutive association with βarrestin$_2$?

The global conformation of CCR1 is most likely necessary for mediating basal association with βarrestin$_2$





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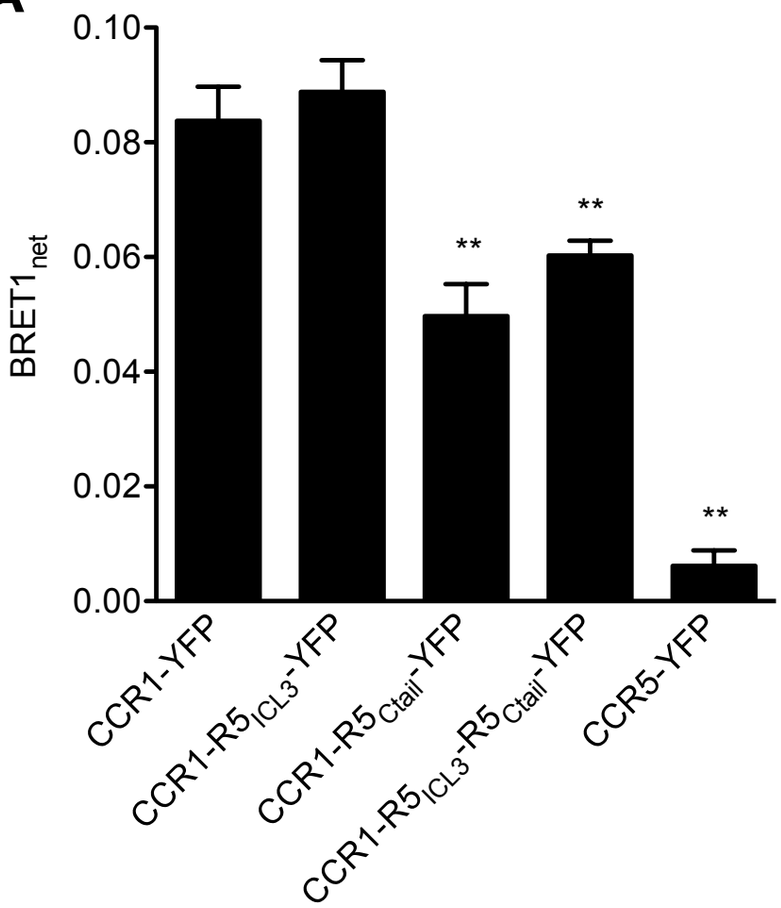

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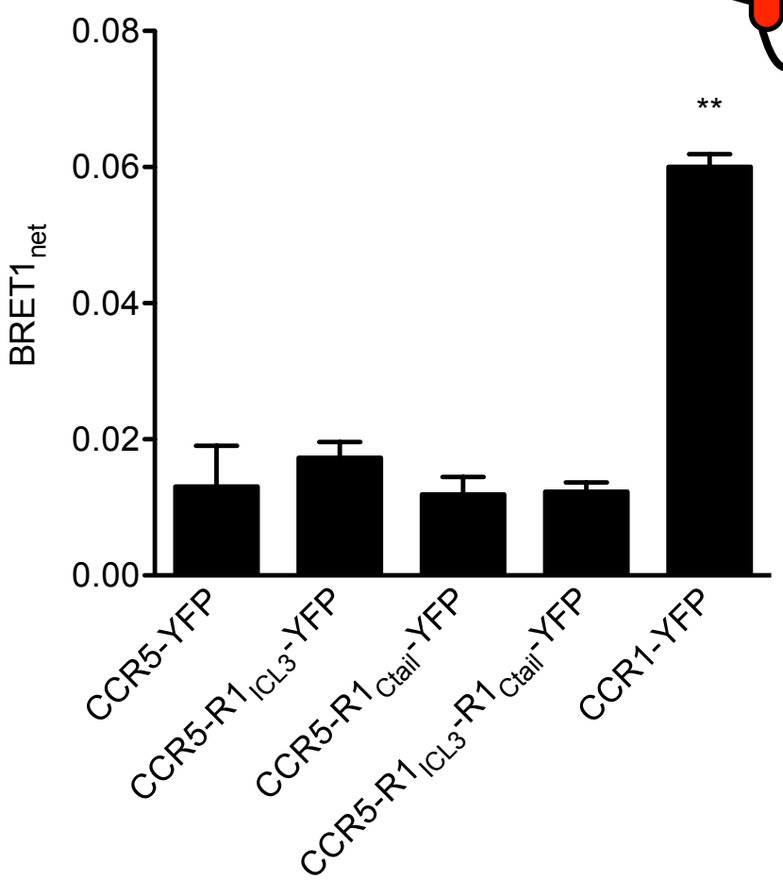

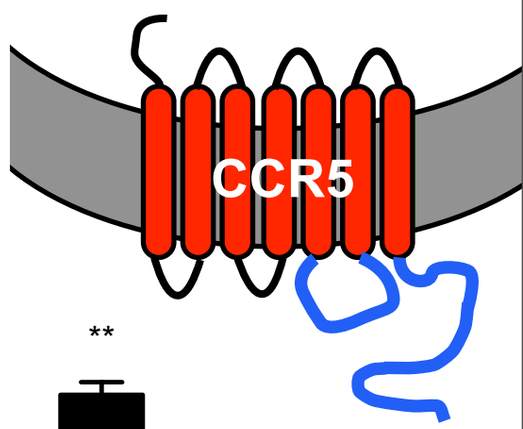

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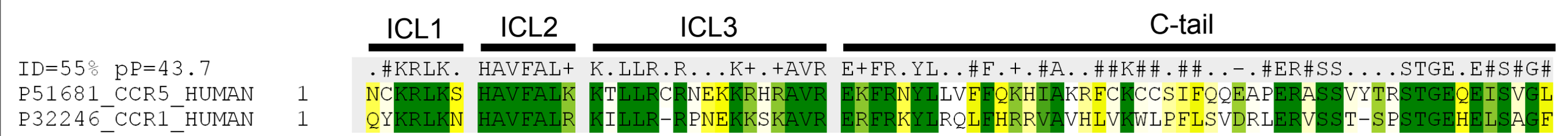

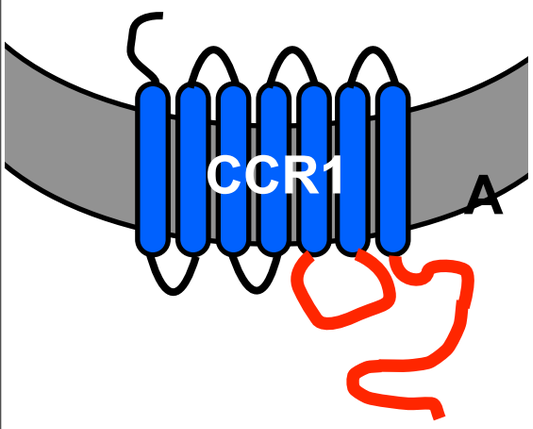

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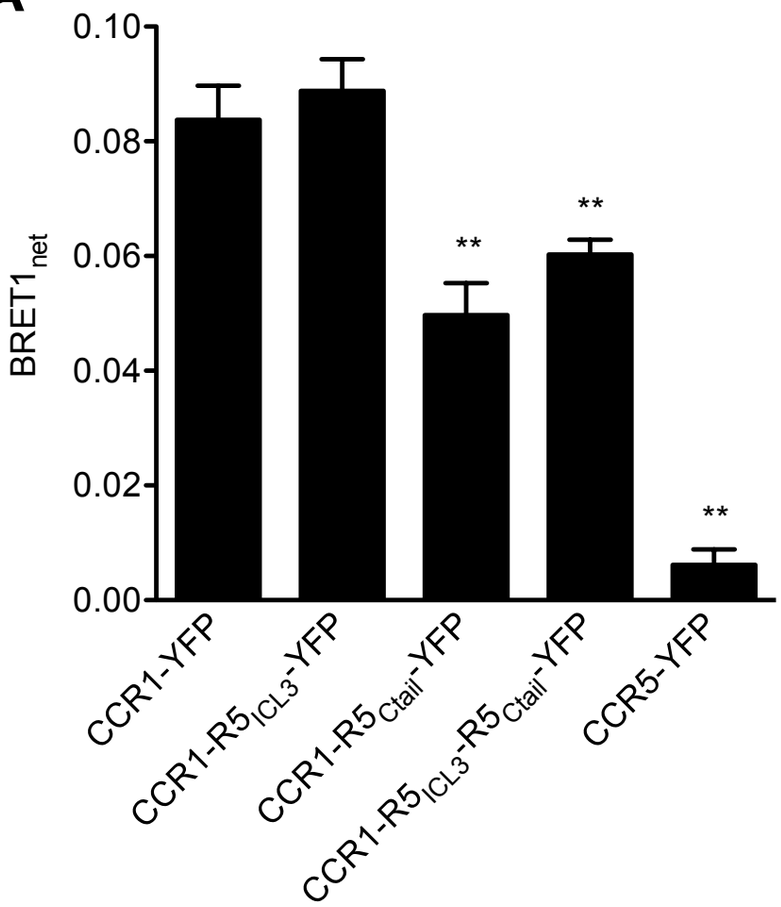

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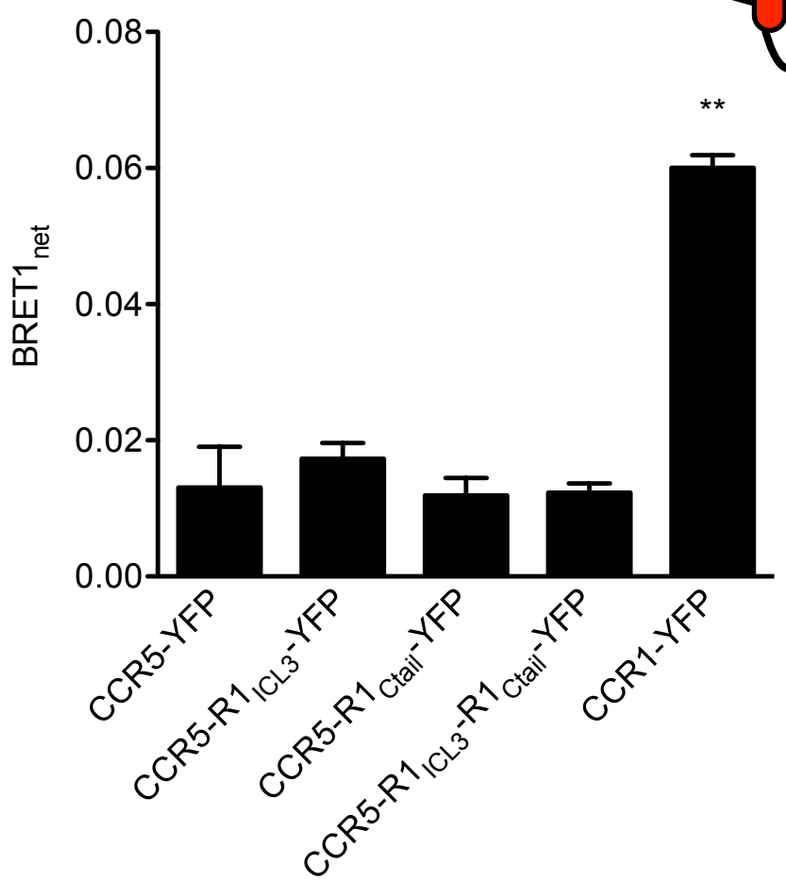
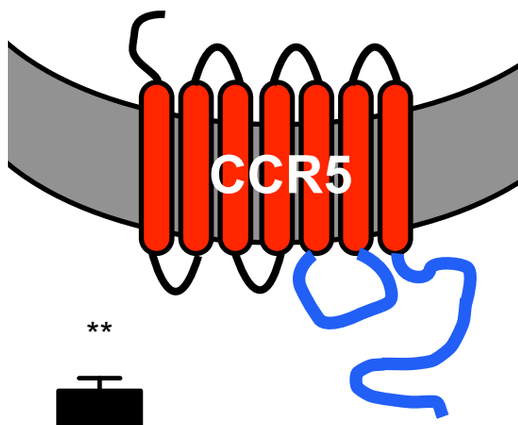
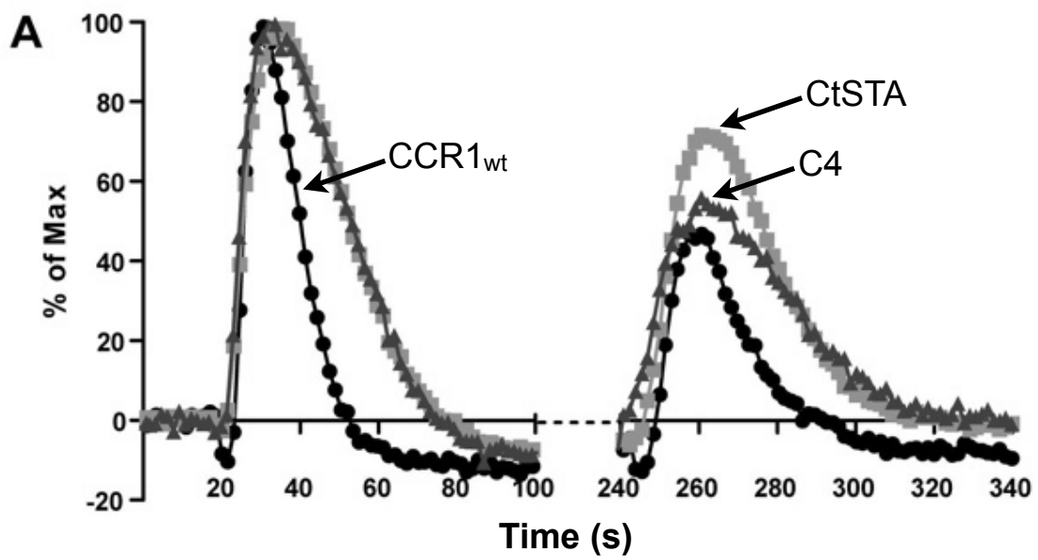




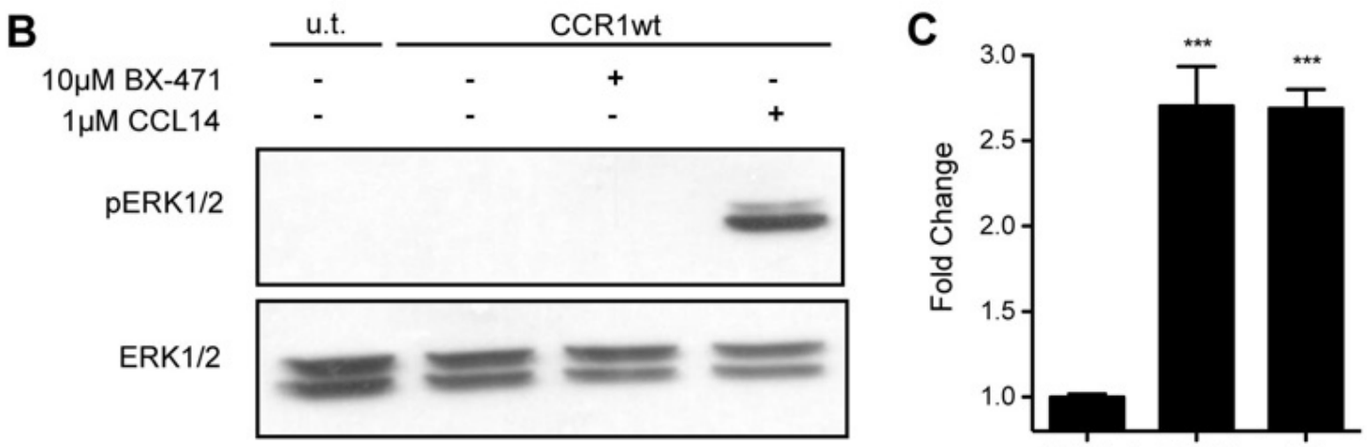
What effect does βarrestin$_2$ have on receptor desensitization and signaling?

CCR1 C-tail mutants with decreased βarrestin$_2$ association exhibit diminished receptor desensitization and internalization

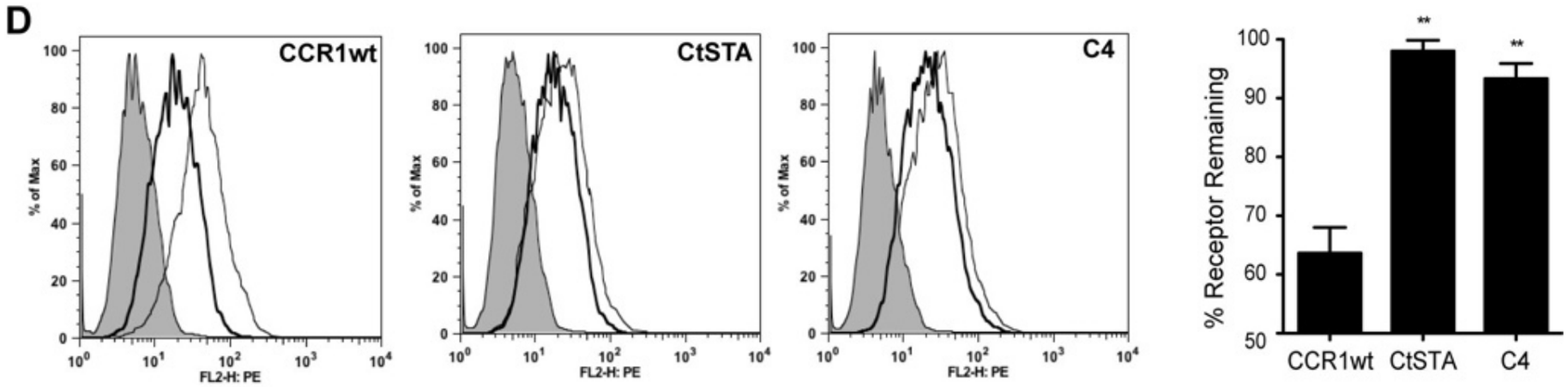
Ca^{2+} mobilization



ERK1/2 phosphorylation



Receptor internalization



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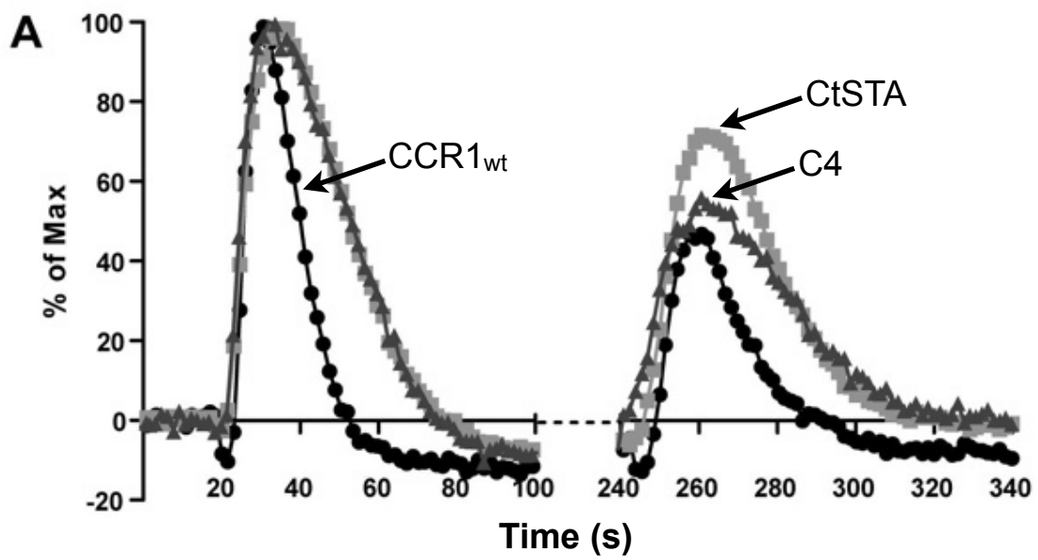

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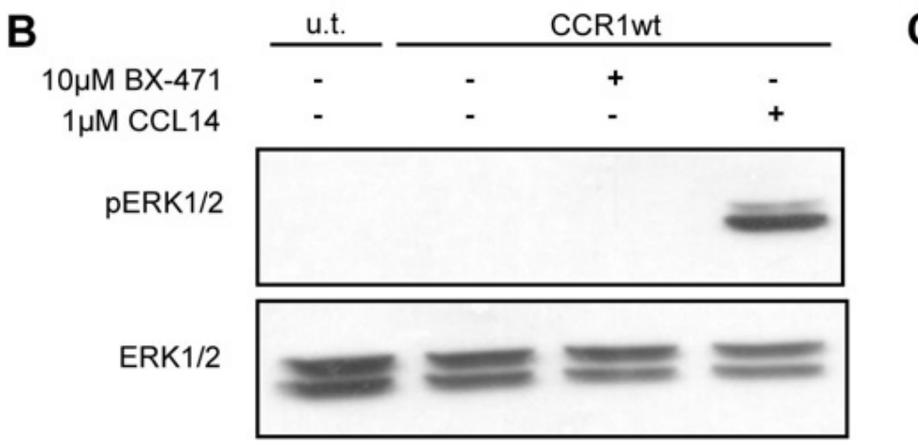
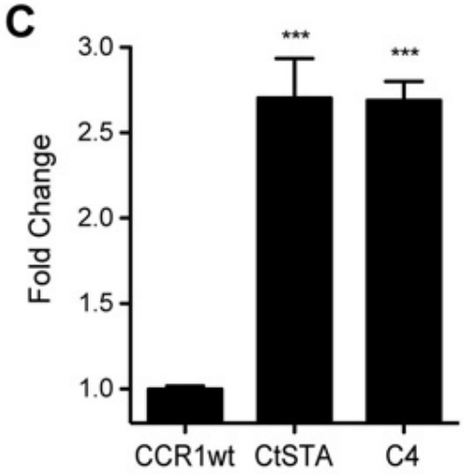
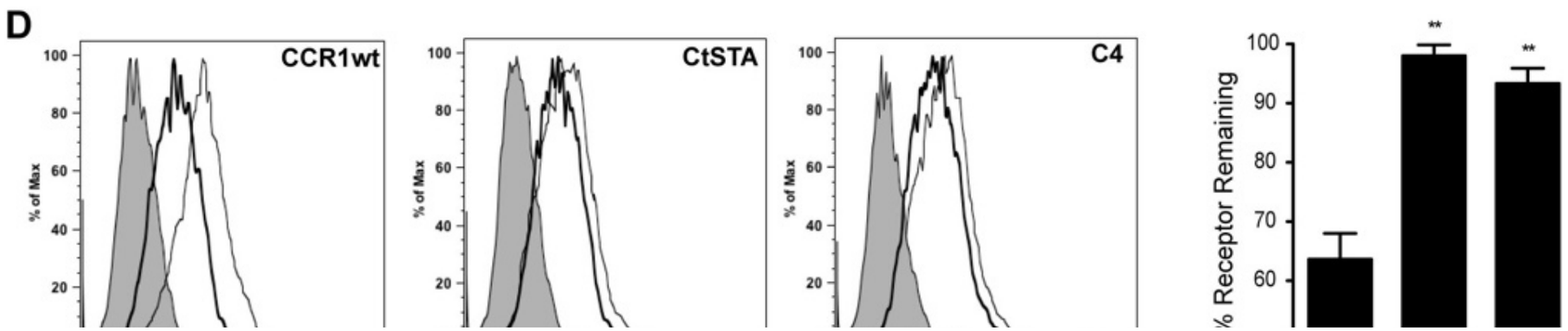


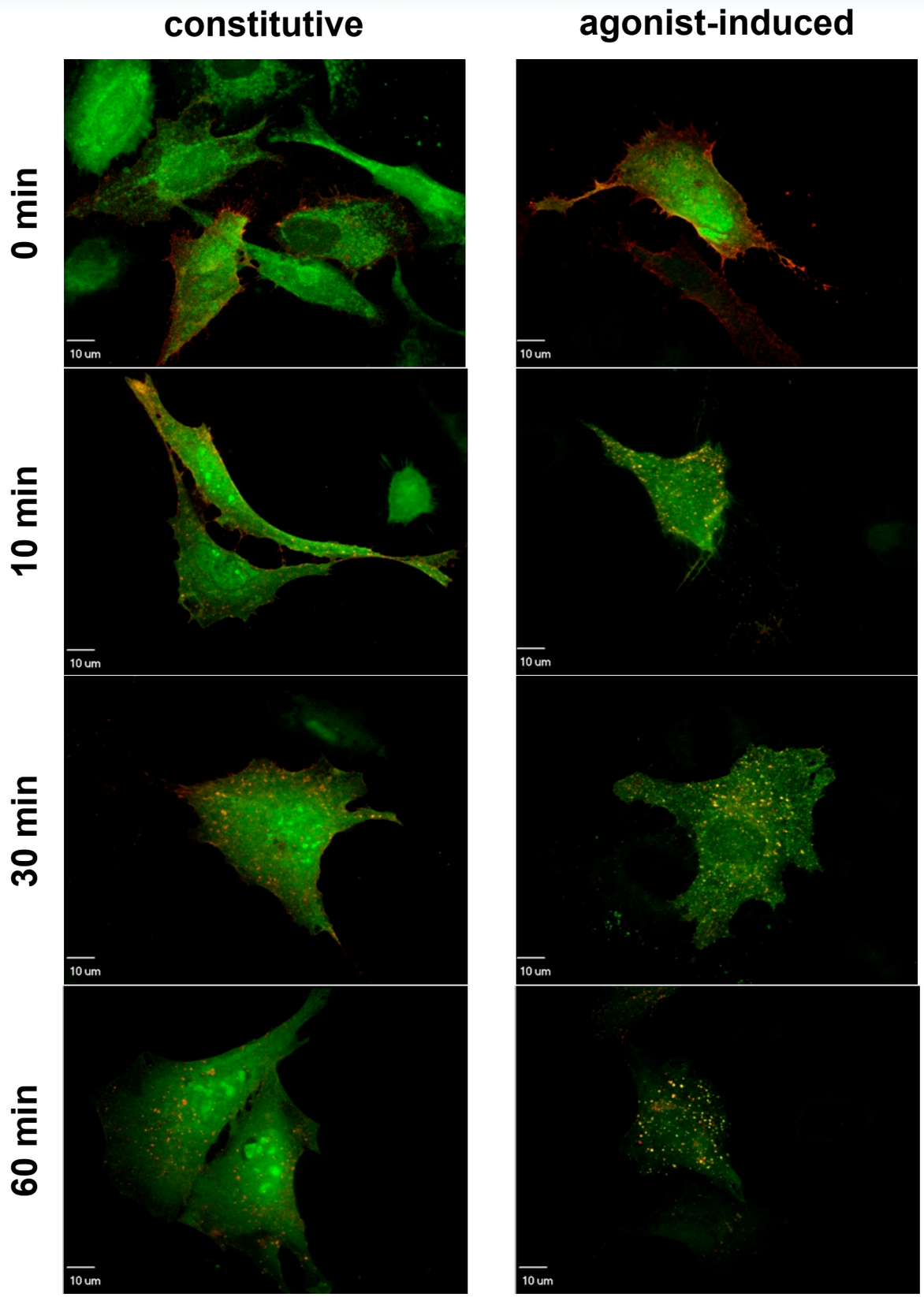


Receptor internalization



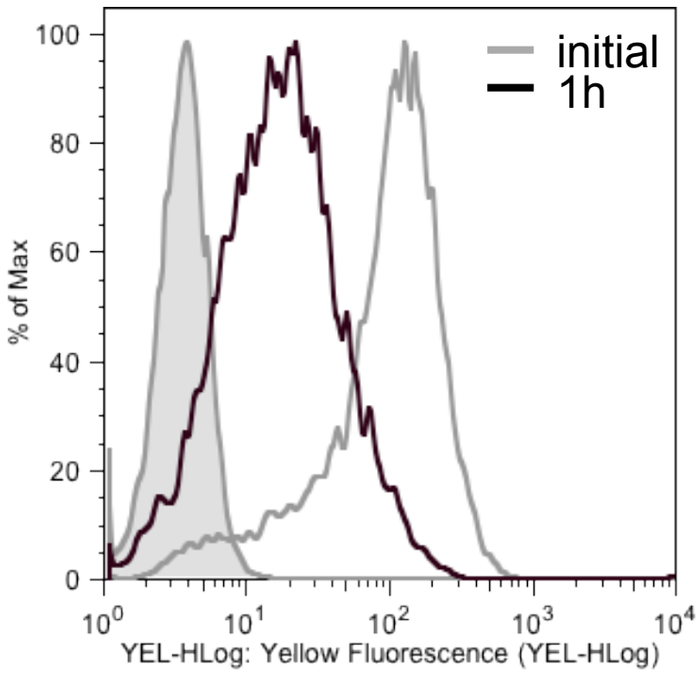
What role does βarrestin$_2$ play in CCR1 constitutive internalization?

The role of βarrestin$_2$ in CCR1 constitutive internalization

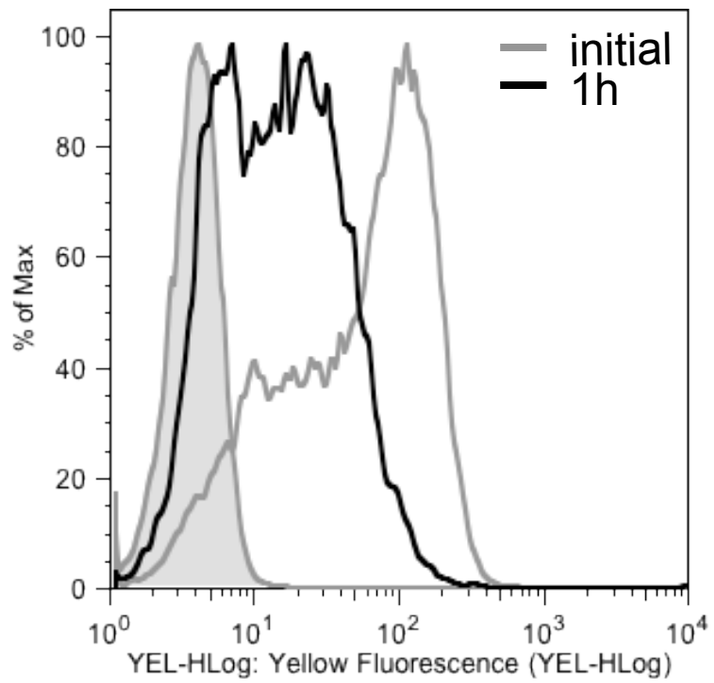


The role of βarrestin$_2$ in CCR1 constitutive internalization

FLAG-CCR1wt/293v



FLAG-CCR1-CtSTA/293v



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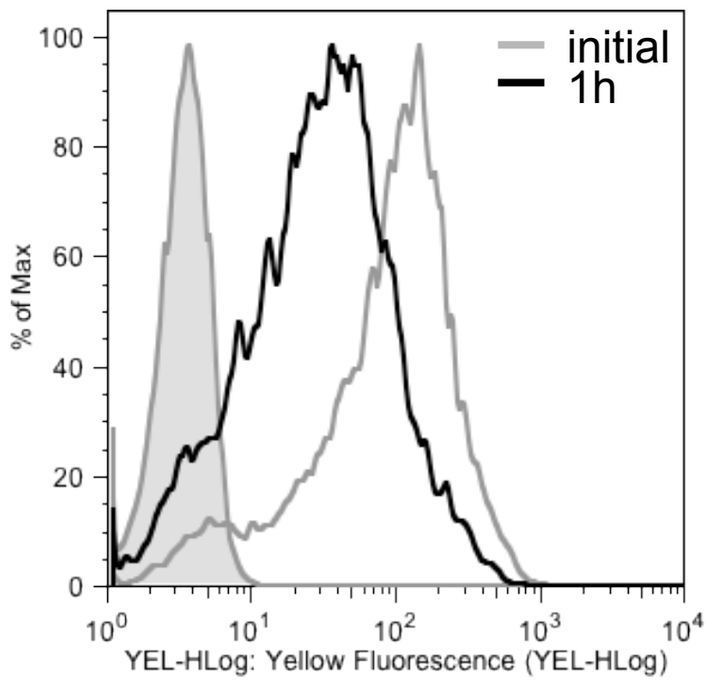

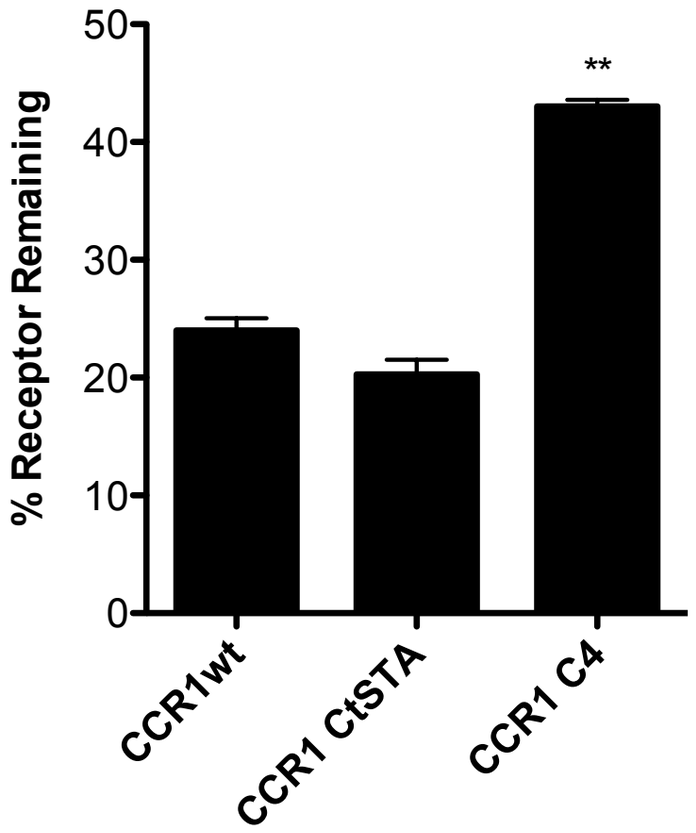

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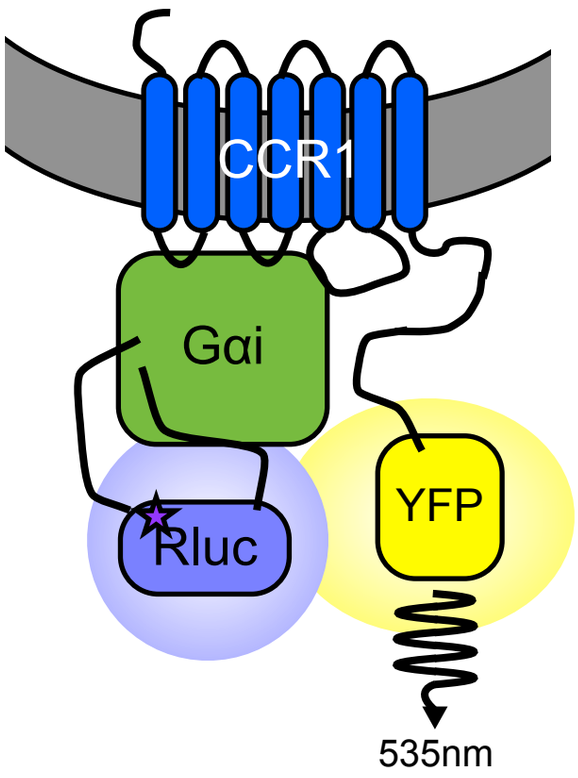
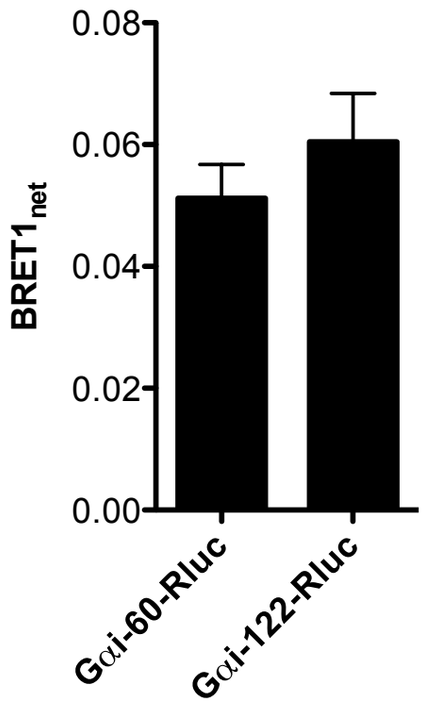
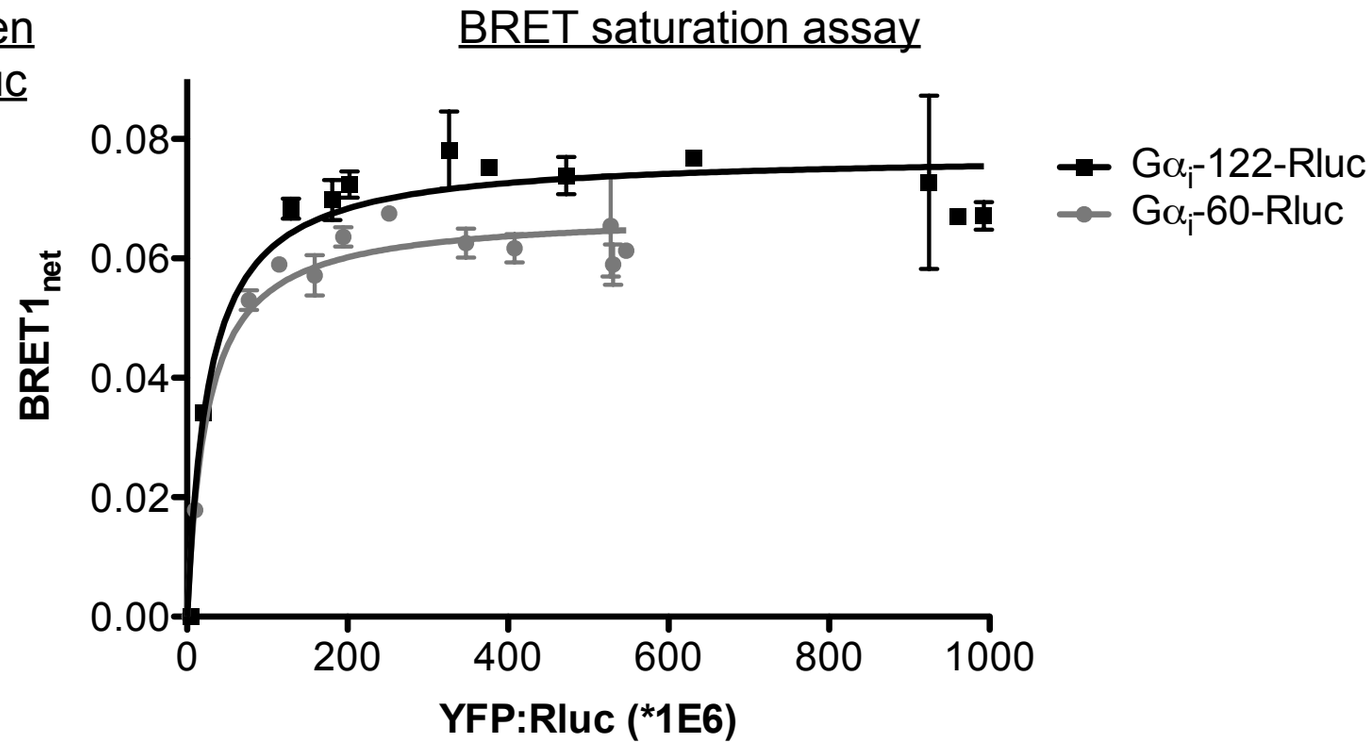
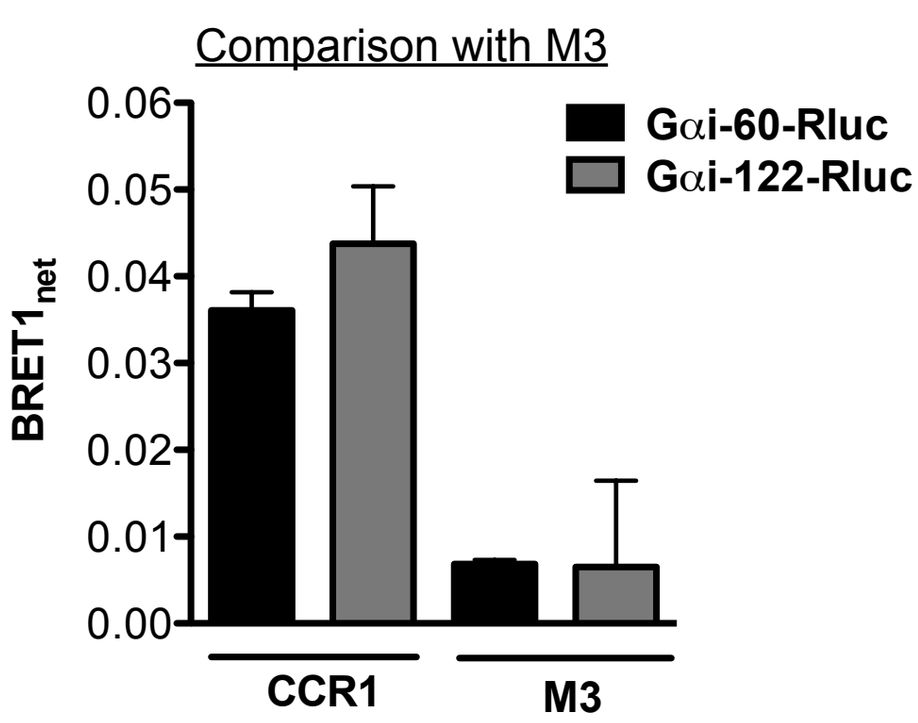
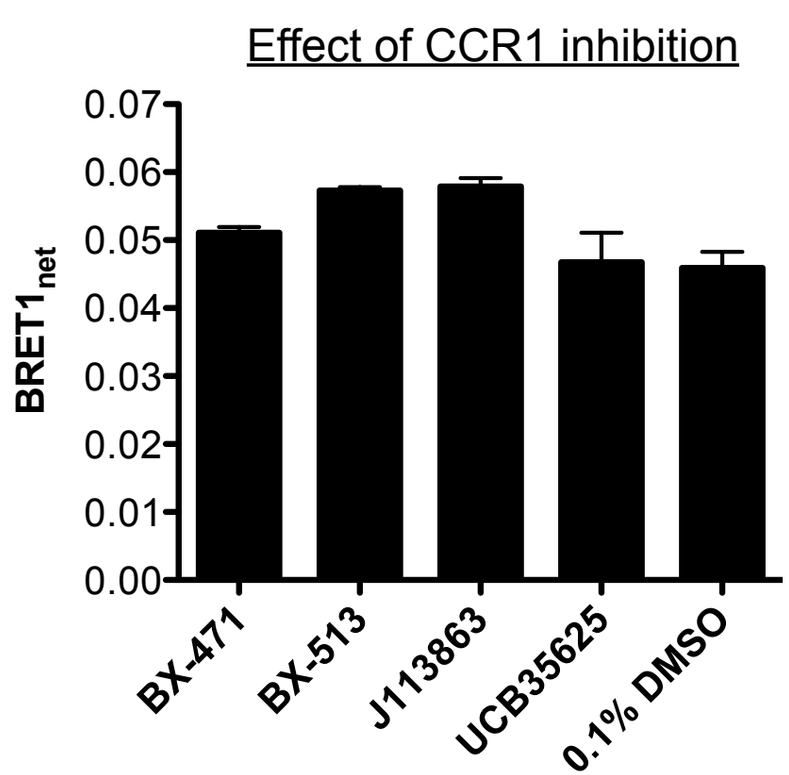
CCR1 is constitutively associated with Gαi



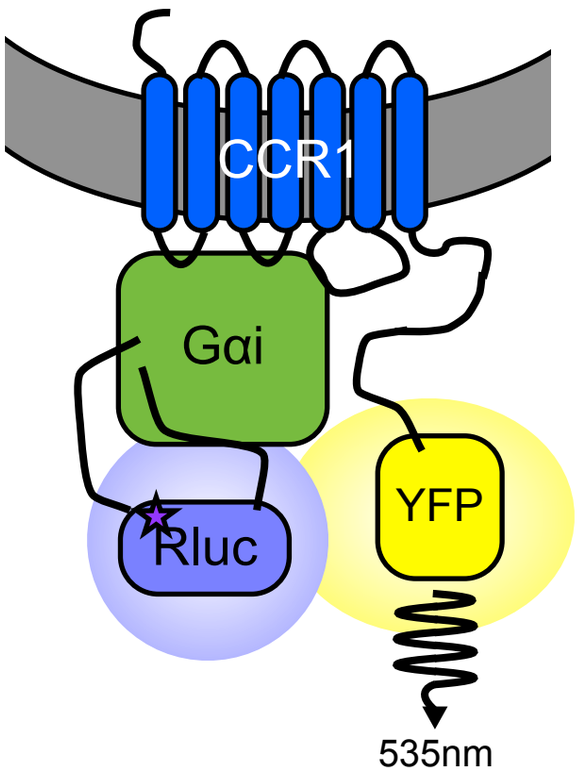


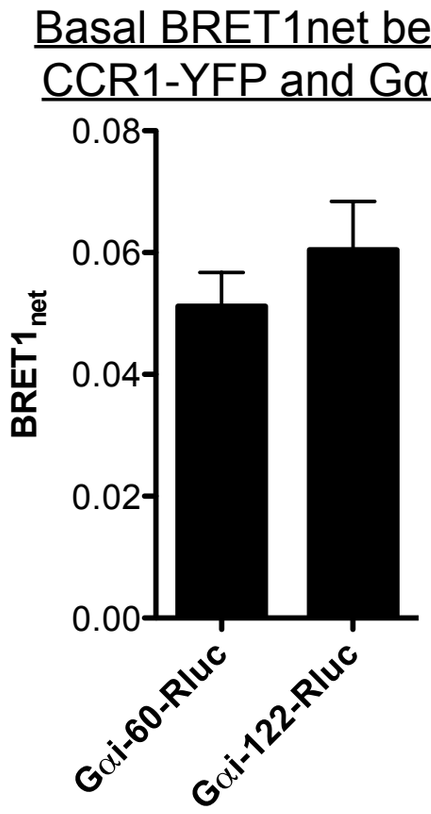


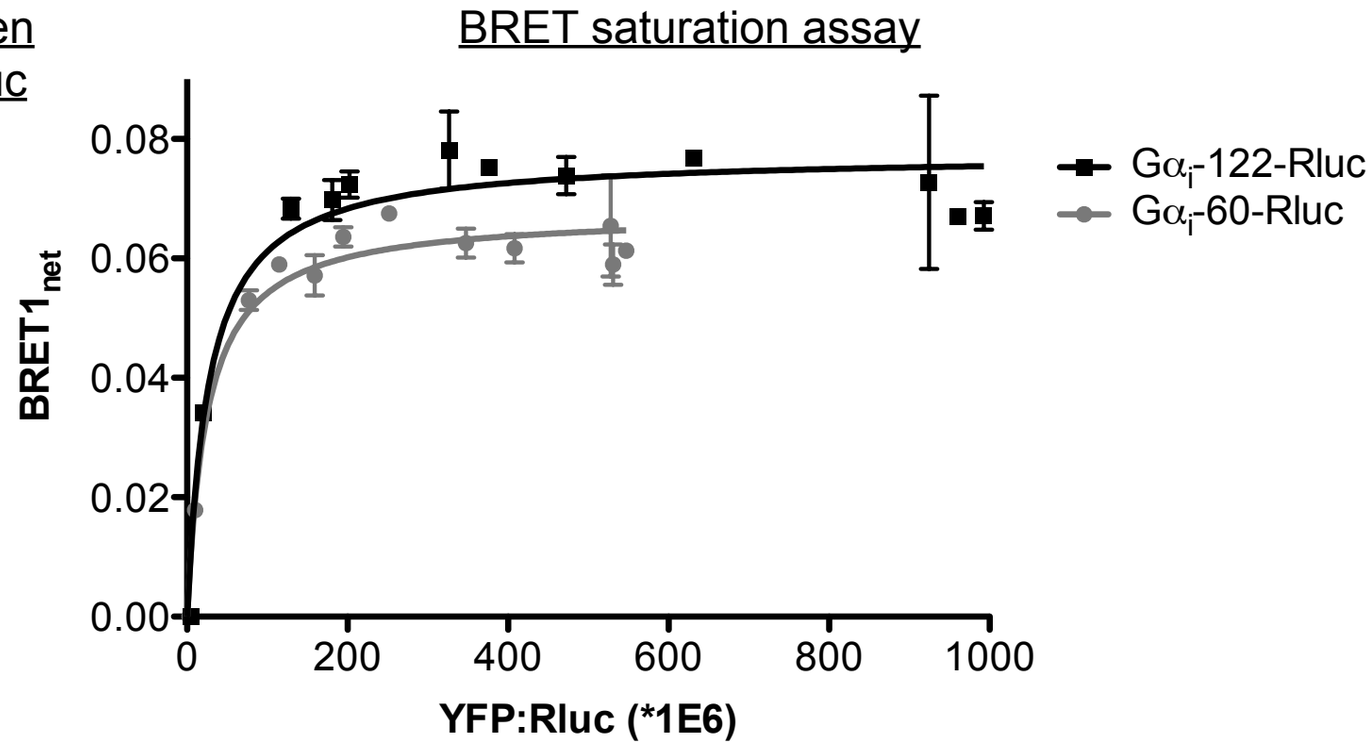


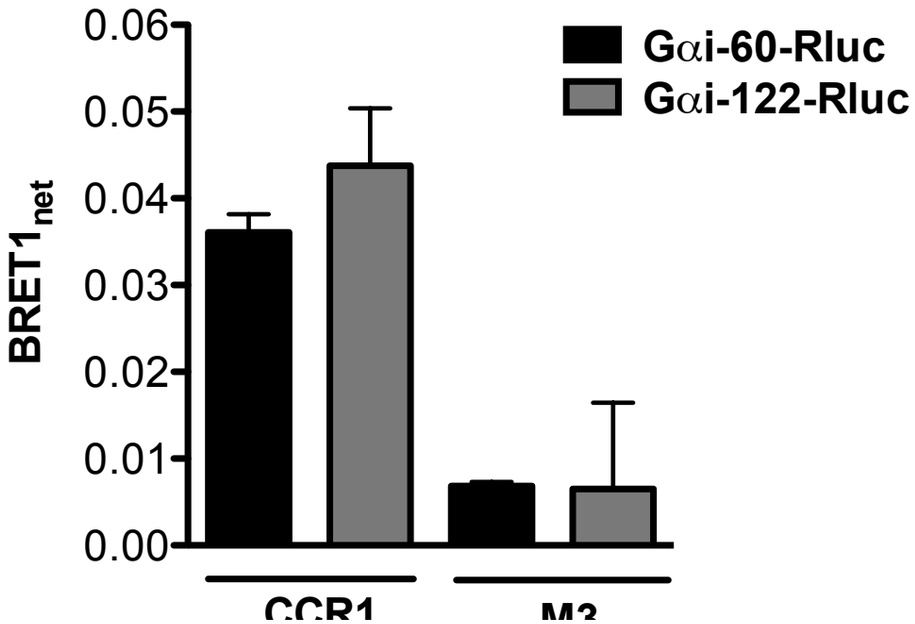


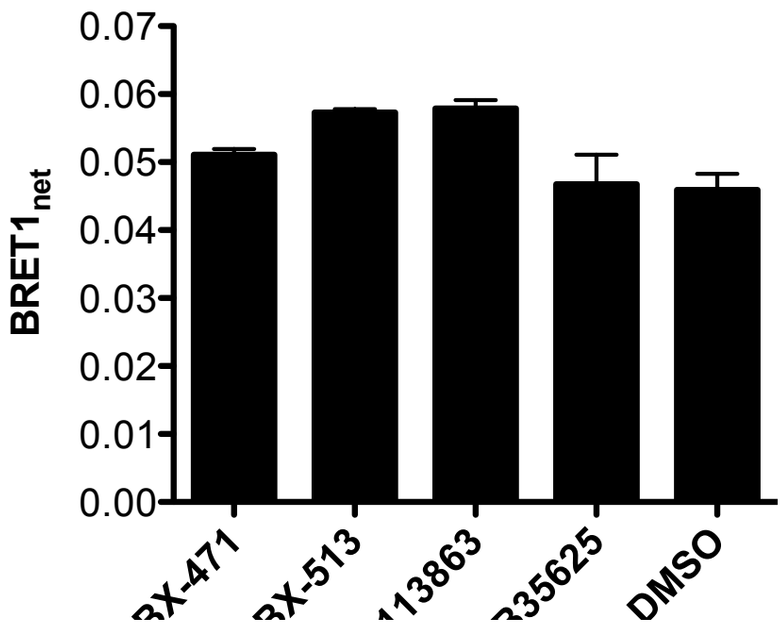
CCR1 is constitutively associated with Gαi





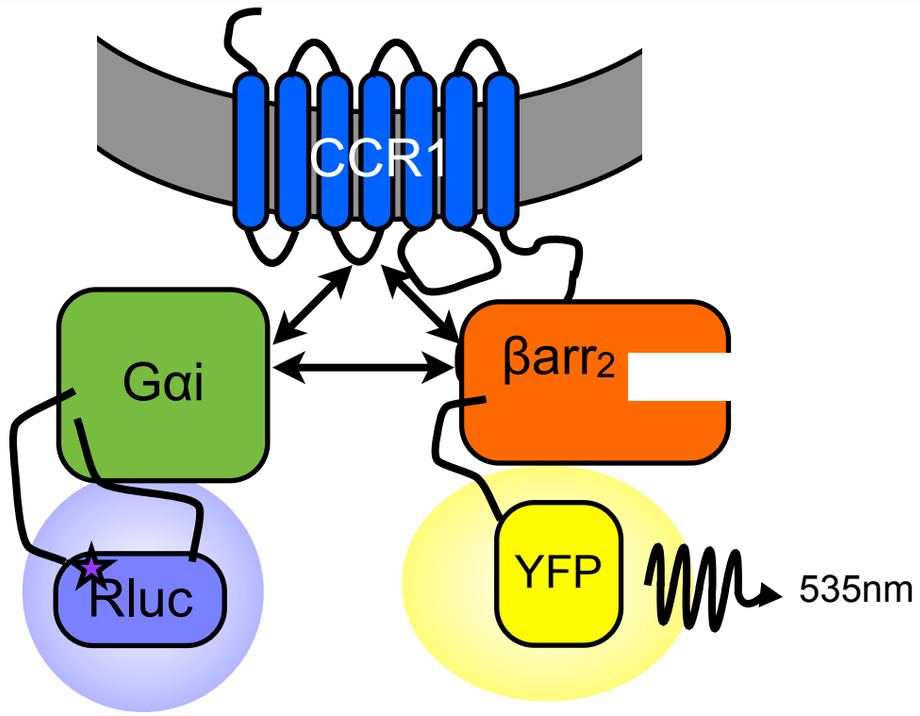




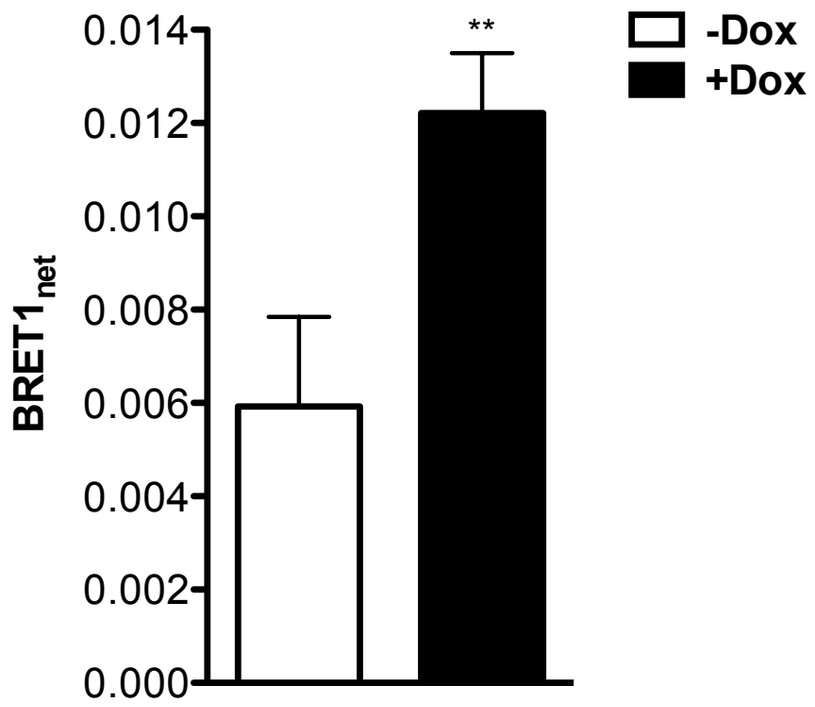


Is CCR1 forming a constitutive multi-protein complex?

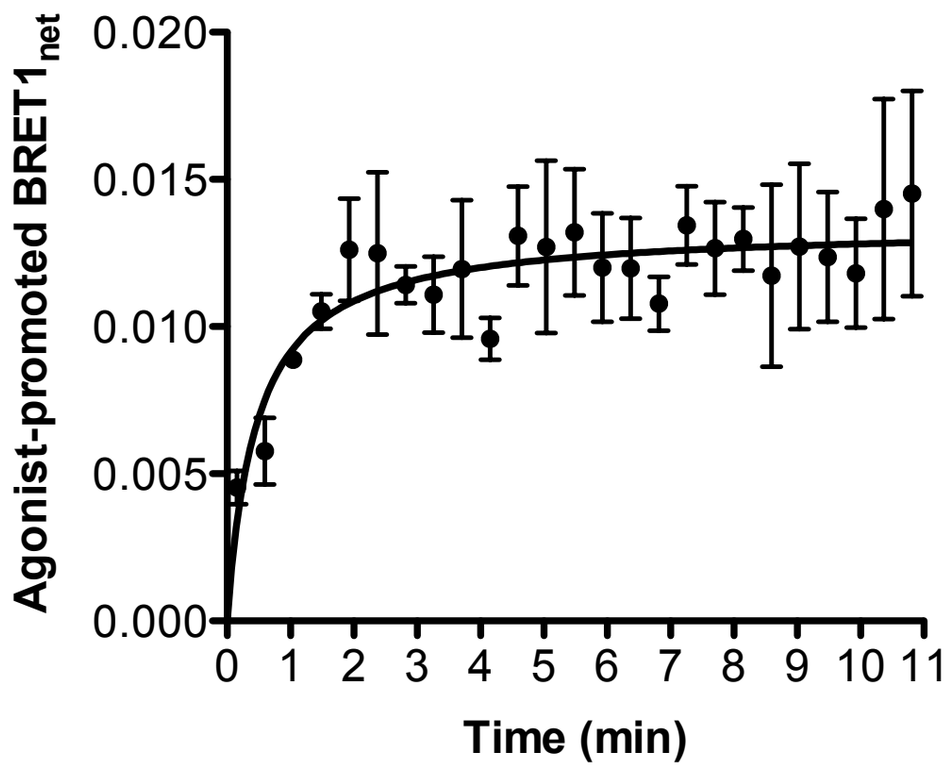
CCR1 expression and activation increases interaction between Gαi and βarrestin$_2$



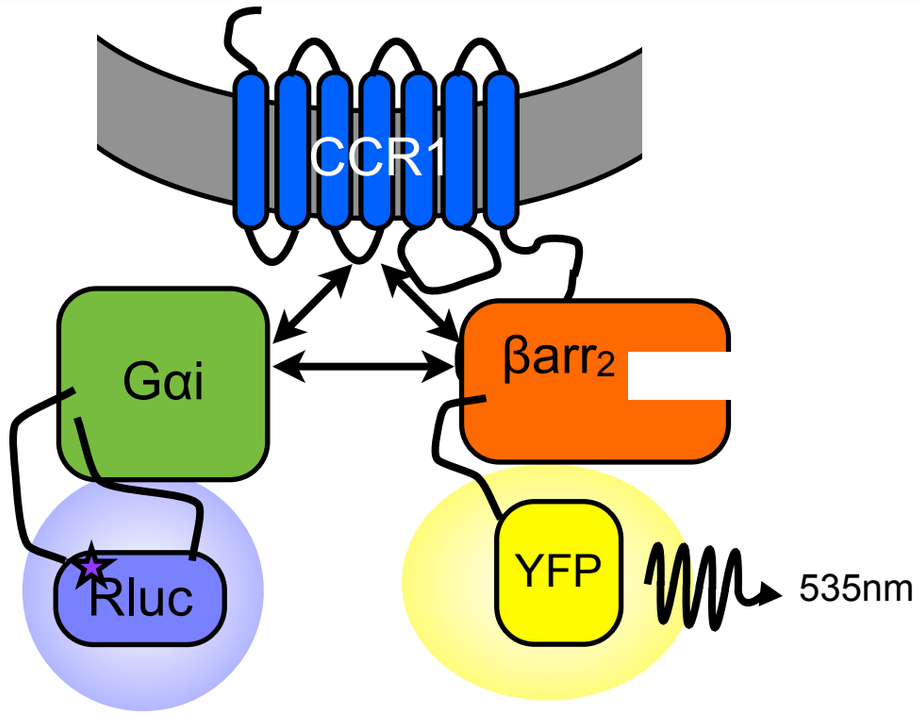
Induction of CCR1 expression



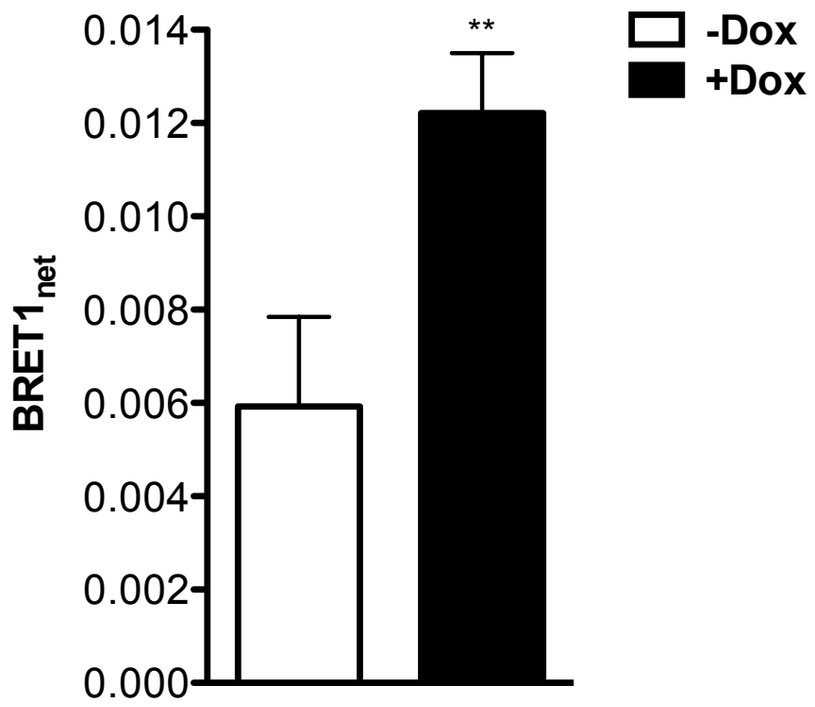
Activation of CCR1 with 1μM CCL14



CCR1 expression and activation increases interaction between Gαi and βarrestin$_2$



Induction of CCR1 expression



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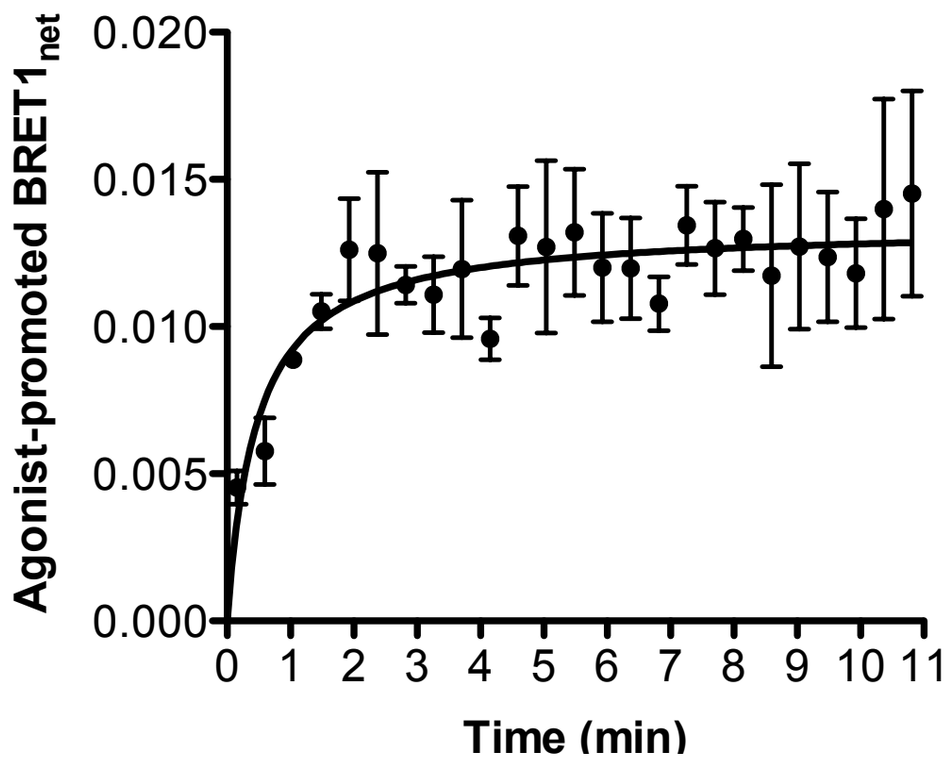

How is CCR1 able to structurally accommodate both G protein and βarrestin$_2$ association?

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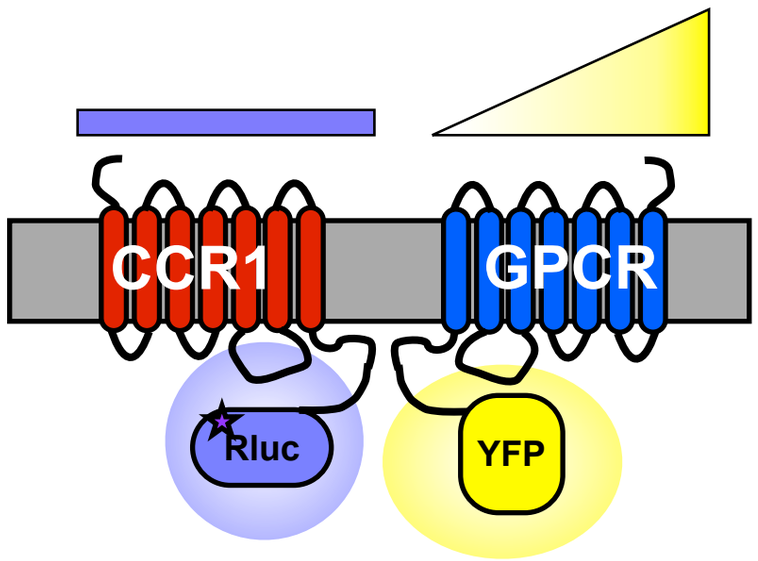
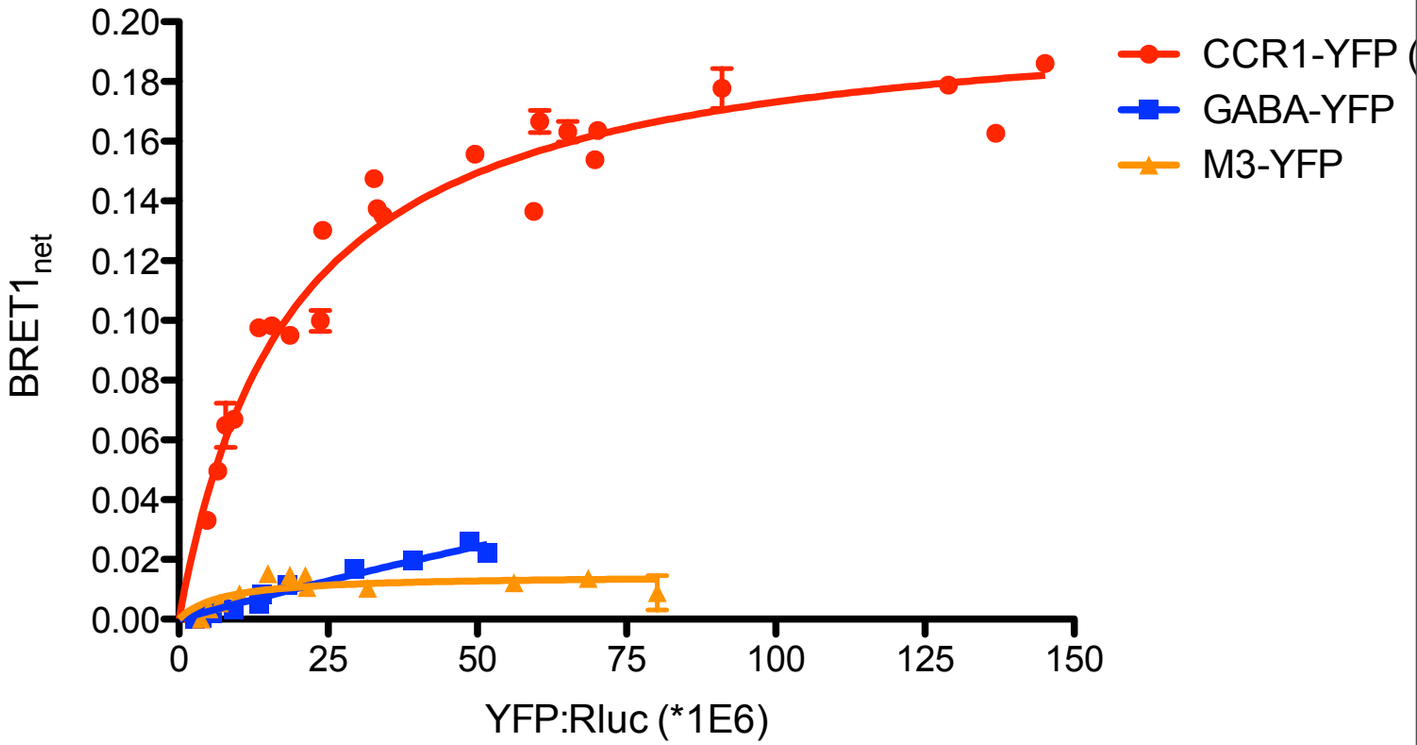
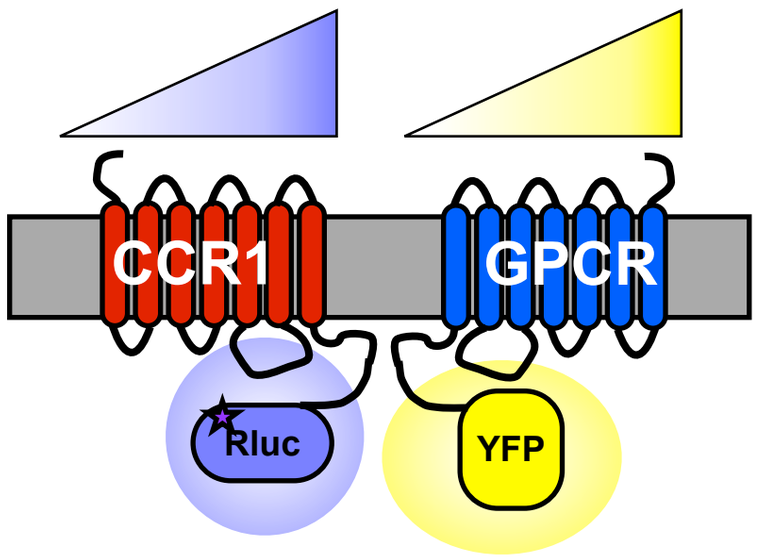
CCR1 forms a constitutive homo-oligomer

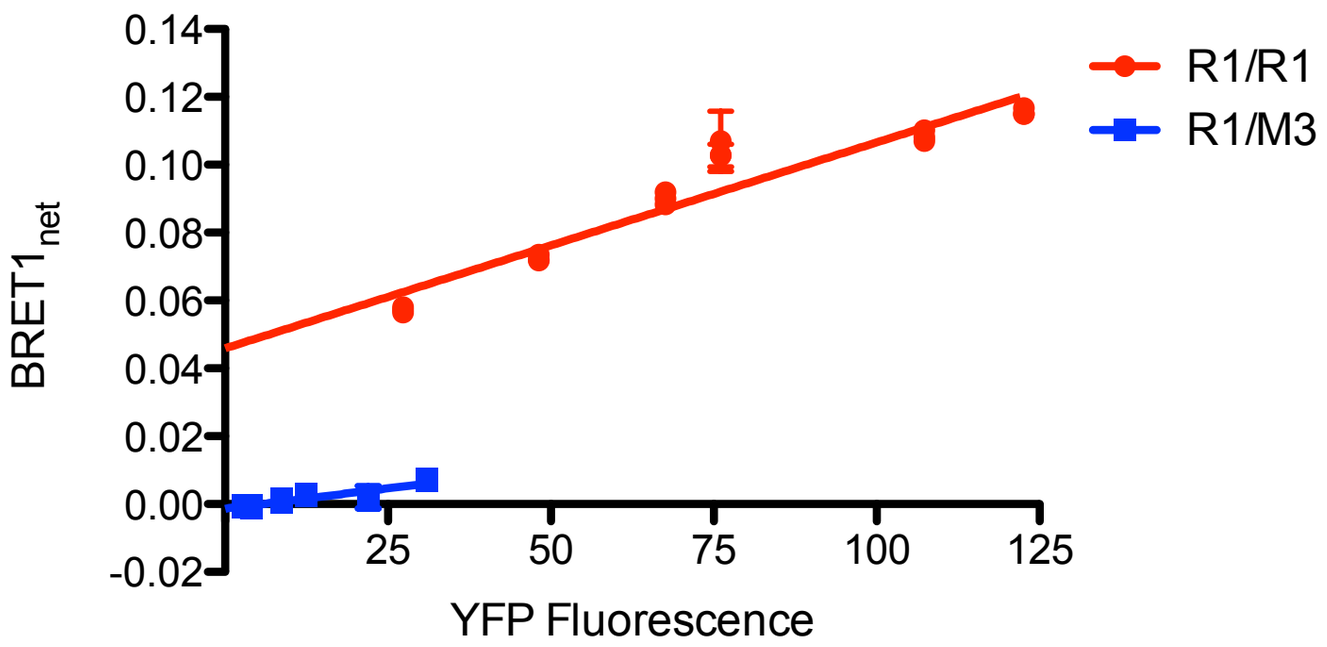
Type I BRET saturation



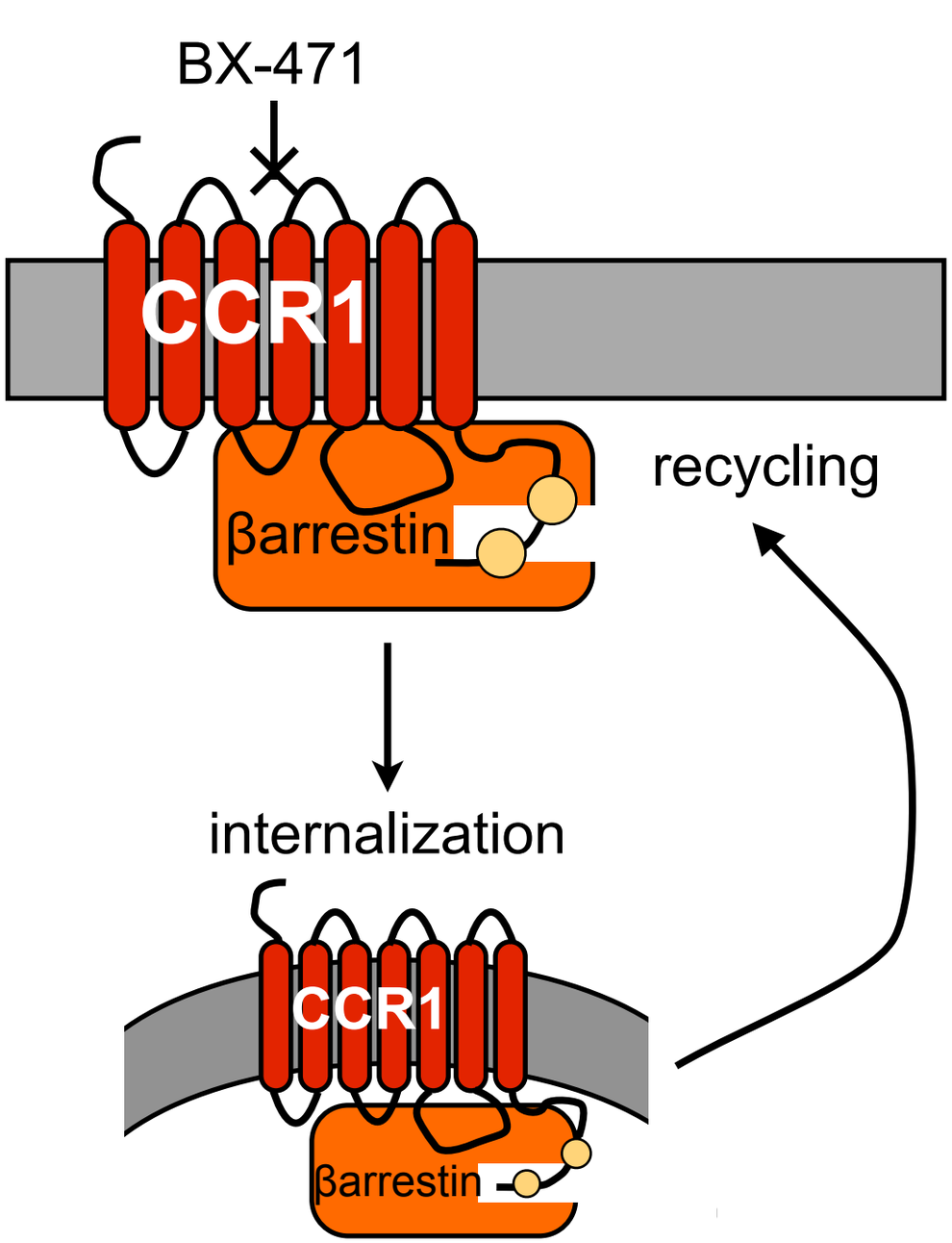


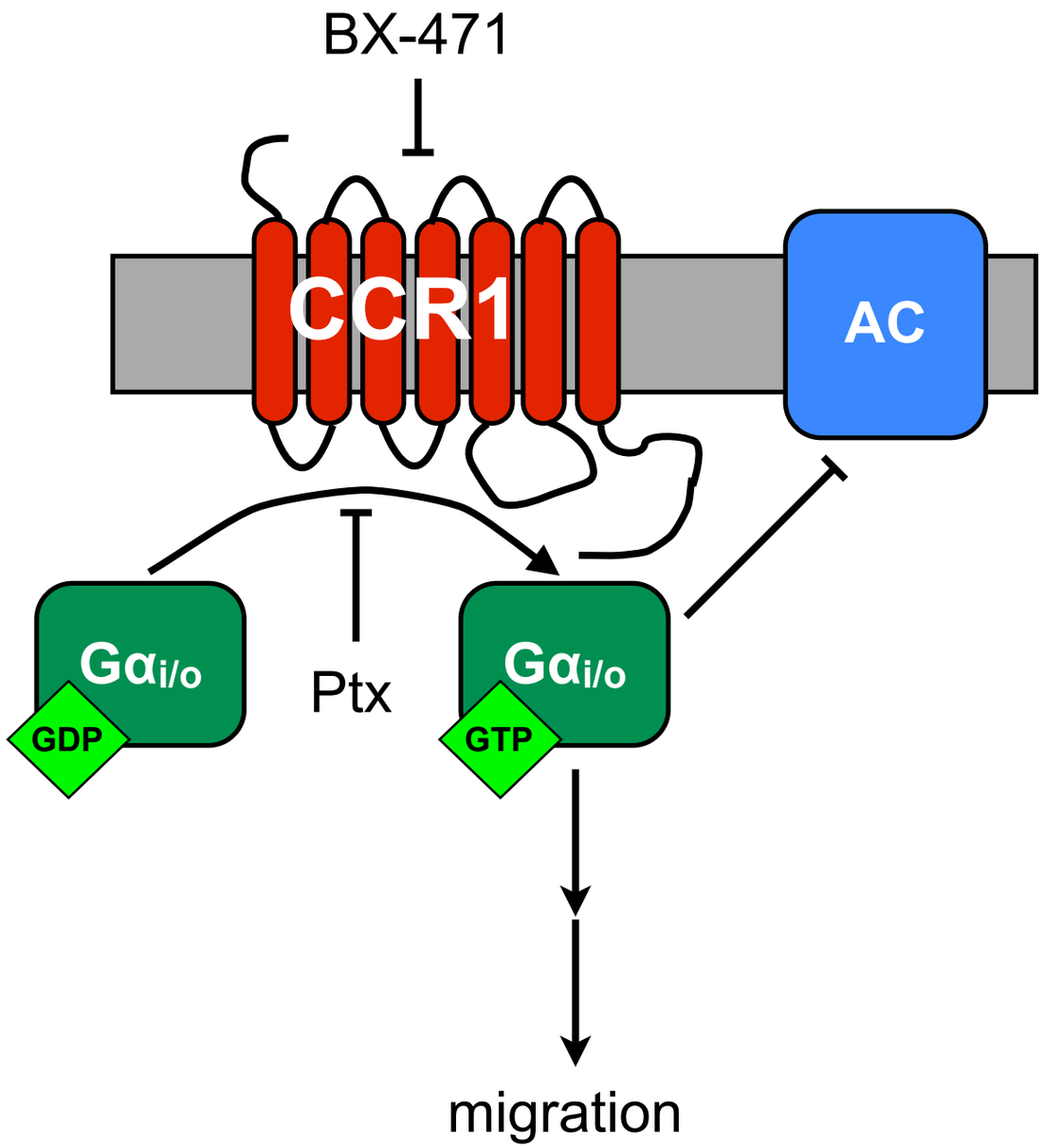
Type II BRET saturation





Conclusion: constitutive activation





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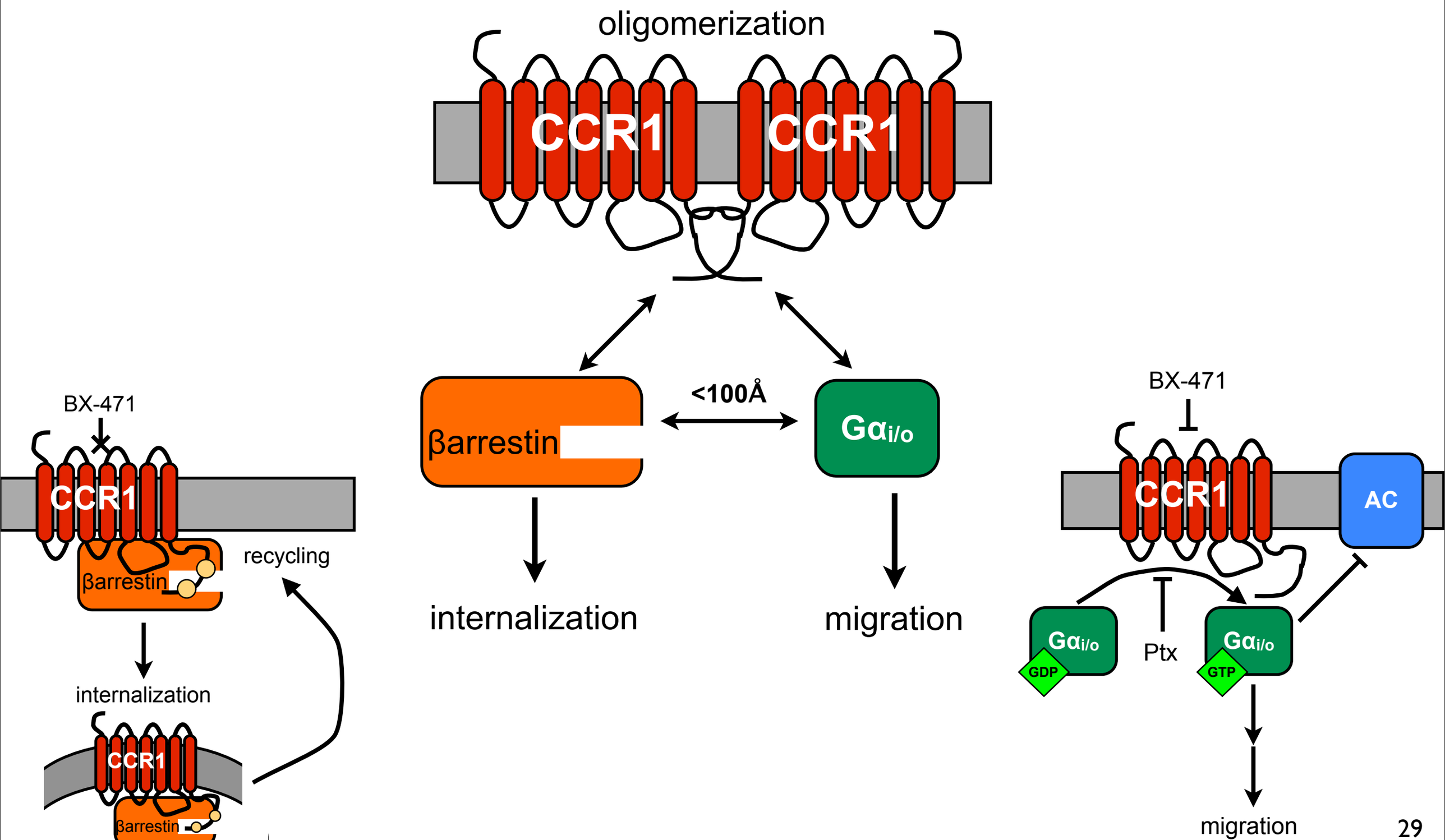
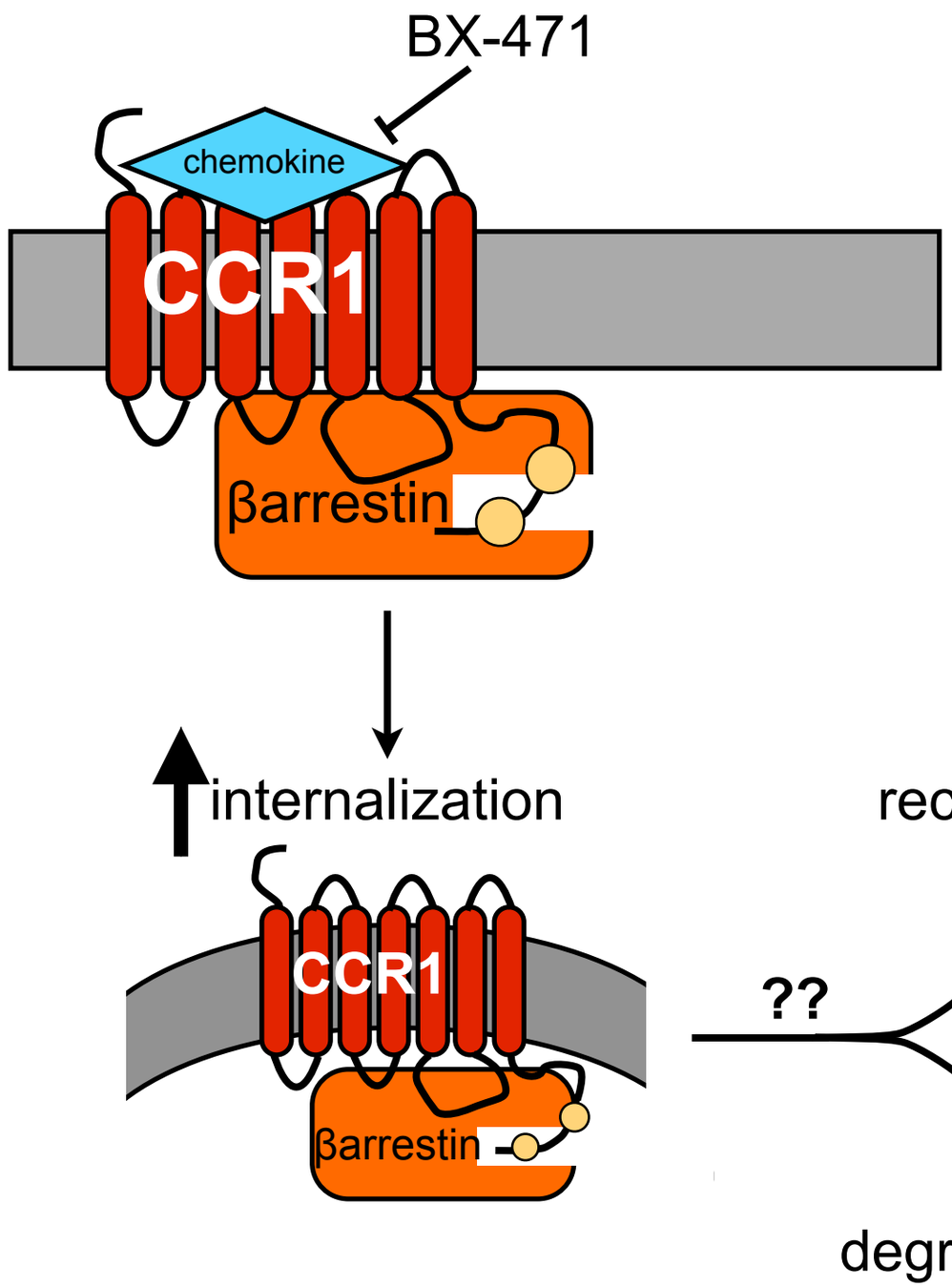
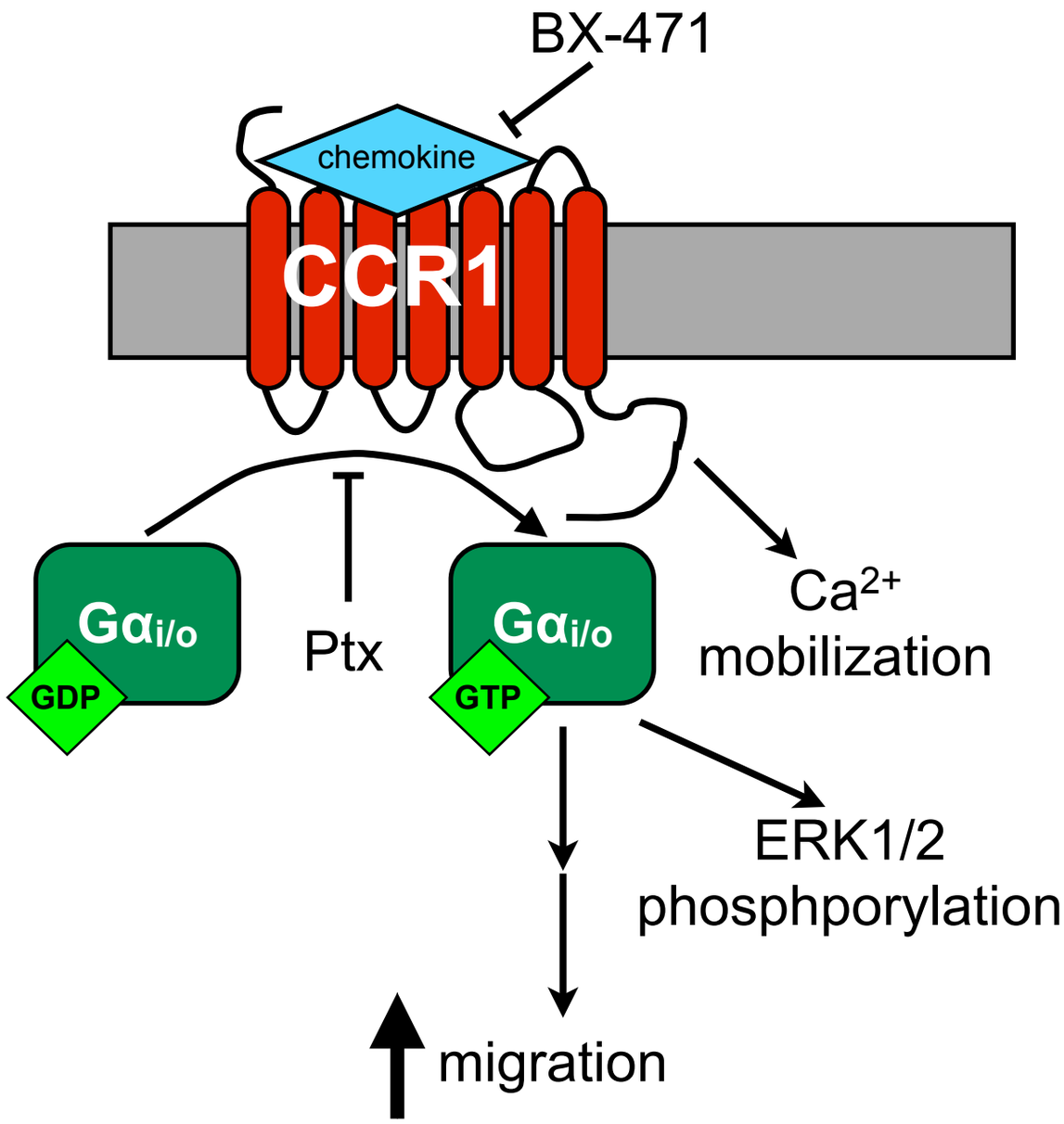


Conclusion: ligand activation





Collaboration with Axikin Pharmaceuticals to measure chemokine receptor heterodimerization

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AXIKIN PHARMACEUTICALS

Motivation

- isolated human tissue samples of patients with an inflammatory condition
- chemokine receptor-specific inhibitors modulated the agonist-induced physiological signal by a non-chemokine GPCR, and *vice versa*

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- BRET type I and type II heterodimerization assays
- co-immunoprecipitation
- co-internalization (via flow cytommetry)
- trans-activation/-inhibition (via Ca^{2+} mobilization)

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Results

- BRET assays indicate specificity of hetero-oligomerization
- receptors have been co-immunoprecipitated from transiently transfected cells
- initial data suggests co-internalization following activation of either receptor
- working with Axikin to optimize trans-activation/-inhibition assay

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Additional Activities: *Academic Connections*

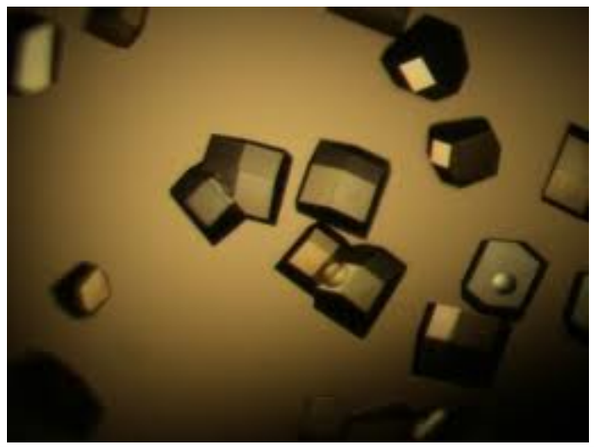
UC San Diego ACADEMIC CONNECTIONS Summer 2011

"It's a GPCR World": Introduction to the Interplay of Biology, Chemistry and Physics.

- introducing fundamental concepts in the fields of biophysics, biochemistry, and cell/ molecular/structural biology by using G-protein coupled receptors (GPCRs) as a model

- understanding the structure, function, and disease relevance of an important family of proteins.

- preparing for collegiate courses in these fields of study.



Additional Activities: *NSF GK12 Socrates Fellowship*

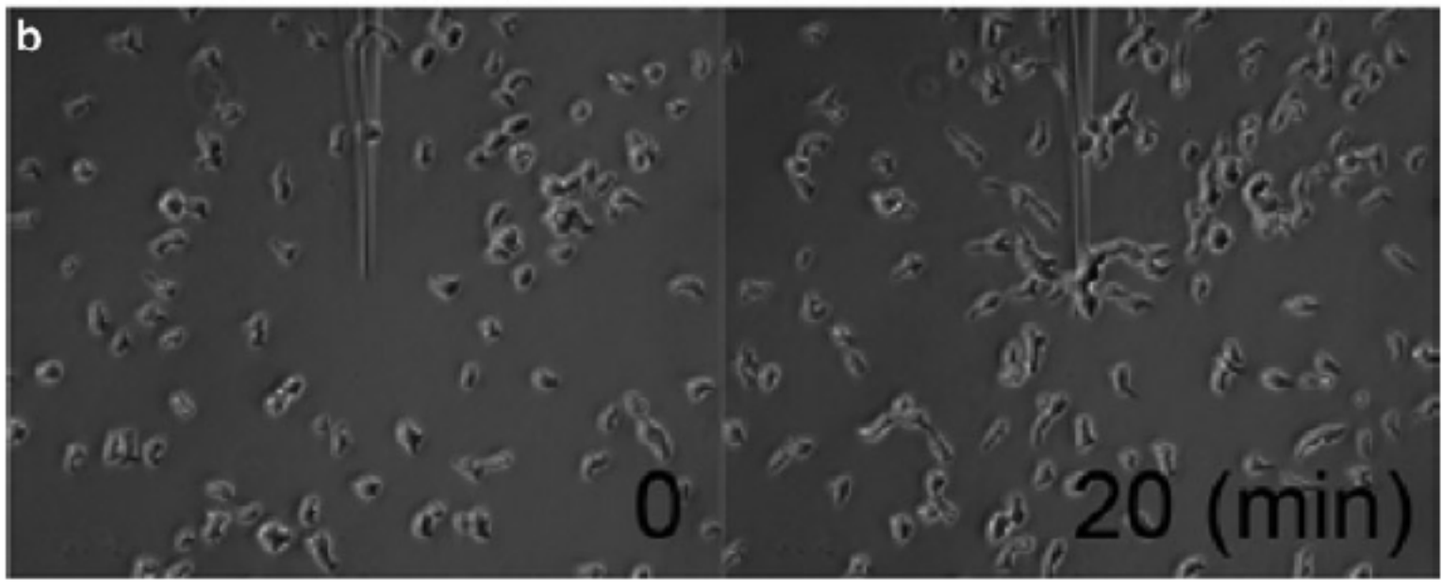
socrates
UCSD ScienceBridge NSF GK-12 Fellowships

NSF Graduate STEM Fellow in
K-12 Education (GK-12) Program

Darci Kimball

<u>Courses in:</u>
Biotechnology
Medical Chemistry
Anatomy & Physiology

Using *Dictyostelium* to understand chemotaxis



Cai *et al.* (2012) *Meth. Mol. Bio.*

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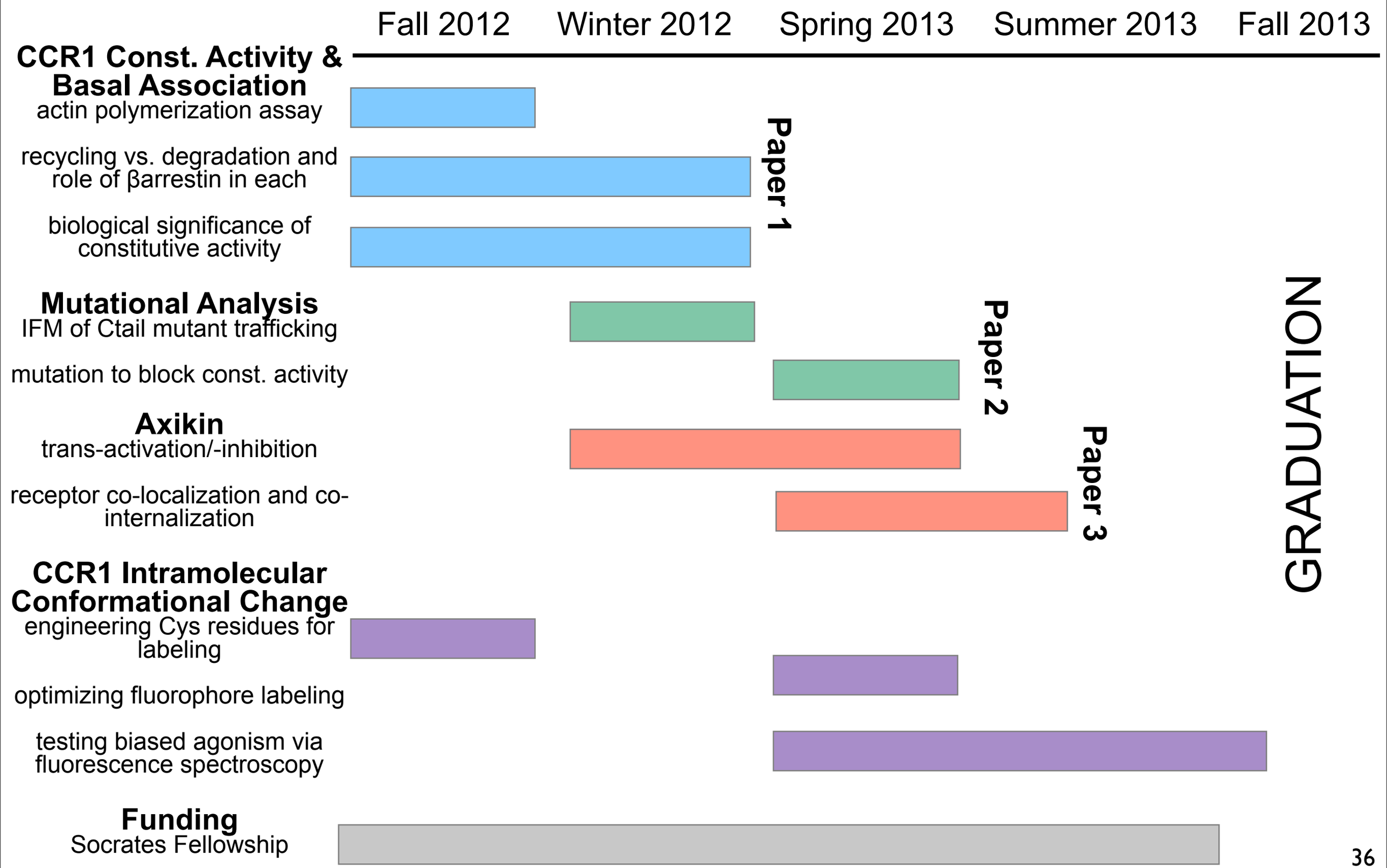
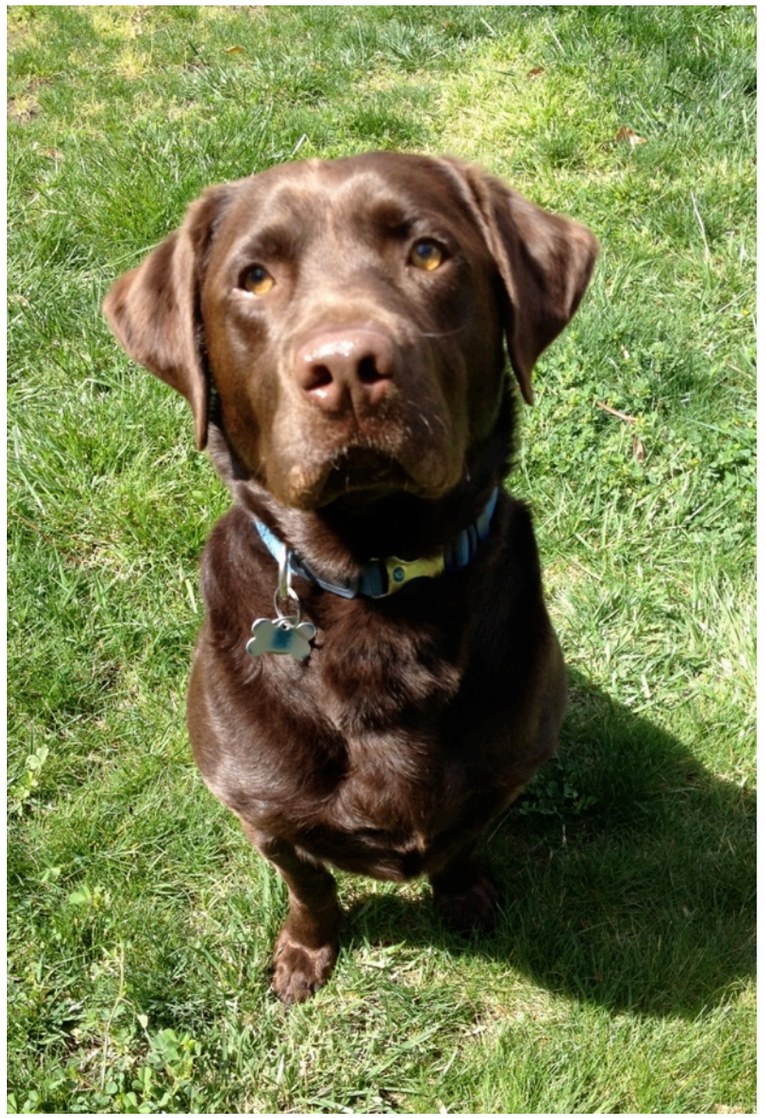

Fall 2012
Winter 2012
Spring 2013
Summer 2013
Fall 2013
CCR1 Const. Activity & Basal Association
actin polymerization assay
recycling vs. degradation and role of βarrestin in each
biological significance of constitutive activity
Paper 1
Mutational Analysis
IFM of Ctail mutant trafficking
mutation to block const. activity
Paper 2
Axikin
trans-activation/-inhibition
receptor co-localization and co-internalization
Paper 3
CCR1 Intramolecular Conformational Change
engineering Cys residues for labeling
optimizing fluorophore labeling
testing biased agonism via fluorescence spectroscopy
Funding
Socrates Fellowship
GRADUATION

Acknowledgements

HANDEL LAB



Collaborators:
JoAnn Trejo (UCSD)
Trejo Lab Members
Michel Bouvier (U Montreal)
Pascale Charest (U Arizona)

Funding:
NSF Graduate Research Fellowship
NIH/UCSD Cellular & Molecular Pharmacology Training Grant
NSF GK12 Socrates Fellowship