

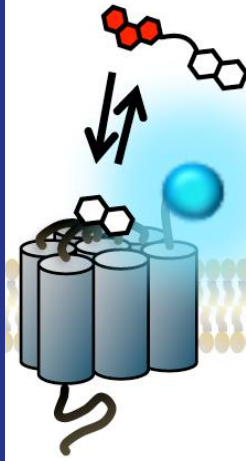
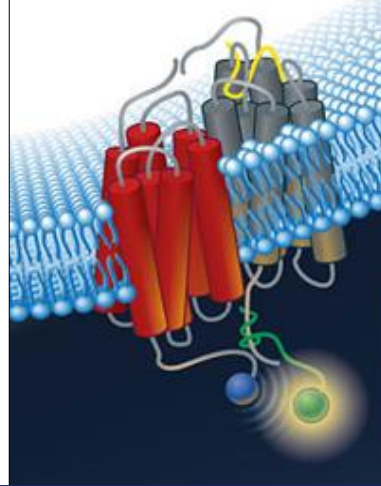
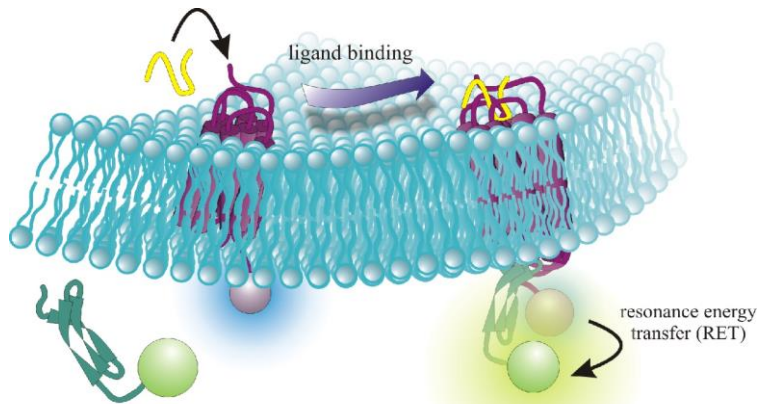
Profiling novel pharmacology of GPCR complexes using Receptor-HIT

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Director Biomedical Innovation,
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Head of Molecular Endocrinology and
Pharmacology,
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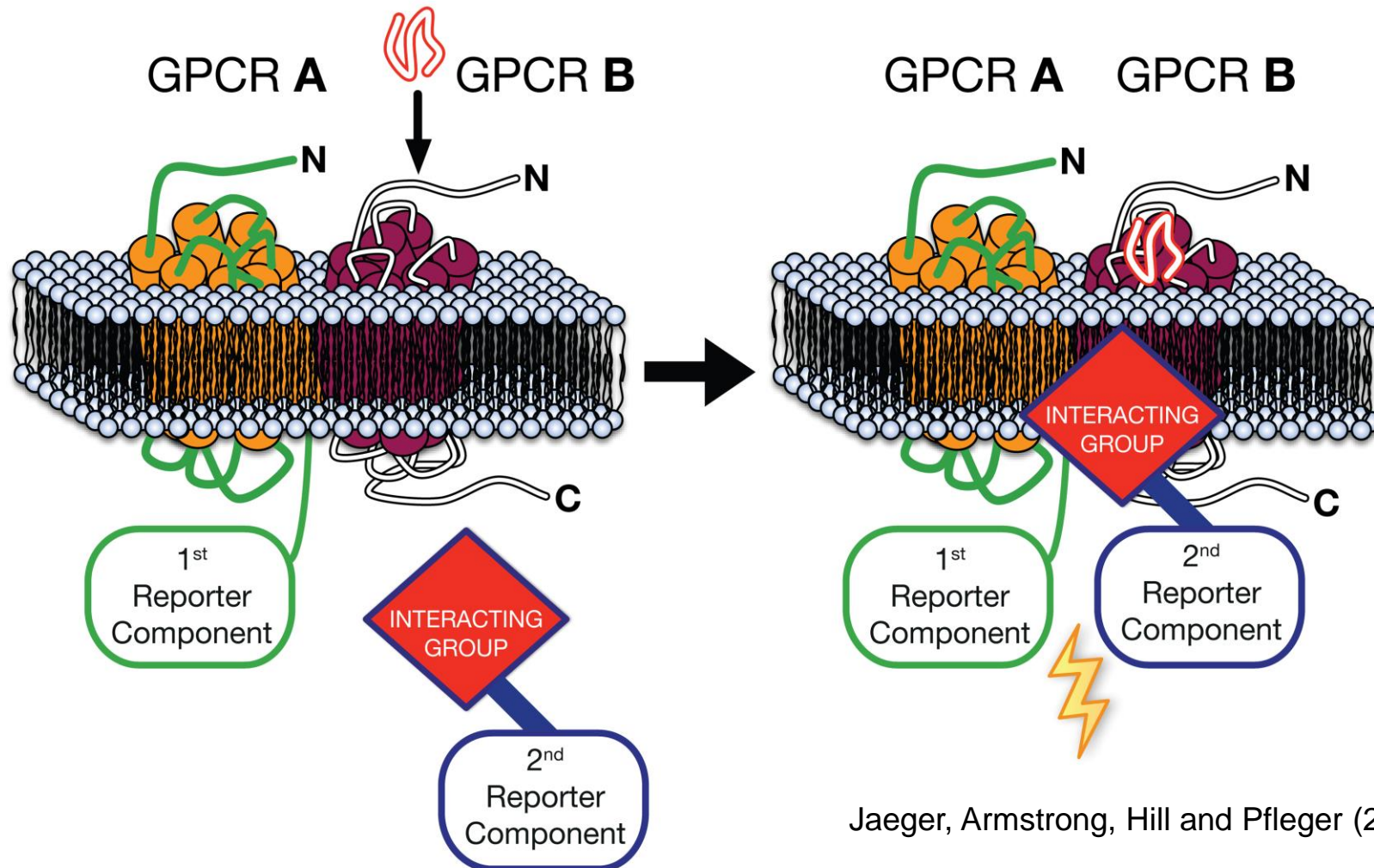
Forward-looking statements



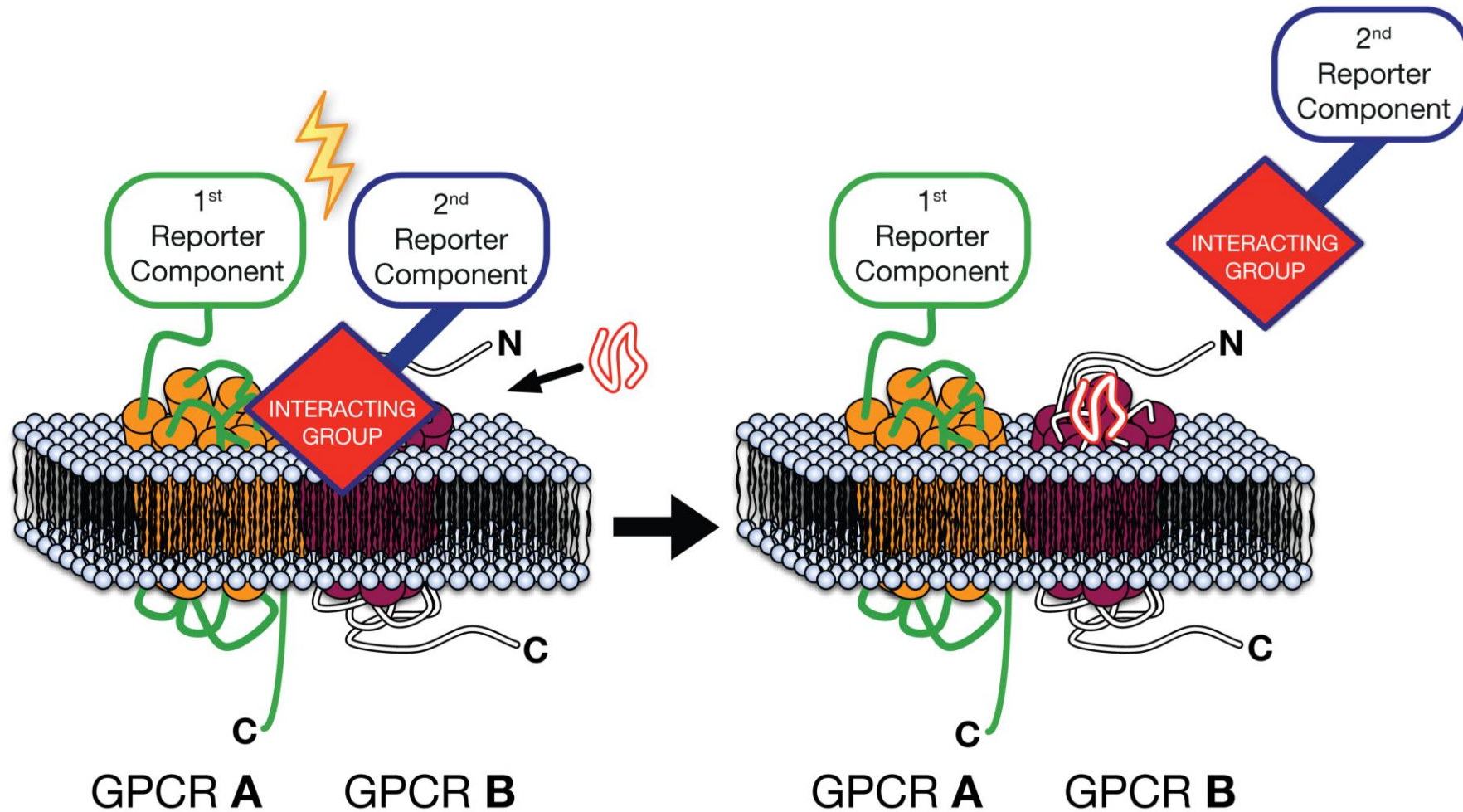
This presentation includes forward-looking statements that are subject to risks and uncertainties. Such statements involve known and unknown risks and important factors that may cause the actual results, performance or achievements of Dimerix to be materially different from the statements in this presentation.

Actual results could differ materially depending on factors such as the availability of resources, the results of clinical studies, the timing and effects of regulatory actions, the strength of competition, the outcome of legal proceedings and the effectiveness of patent protection.

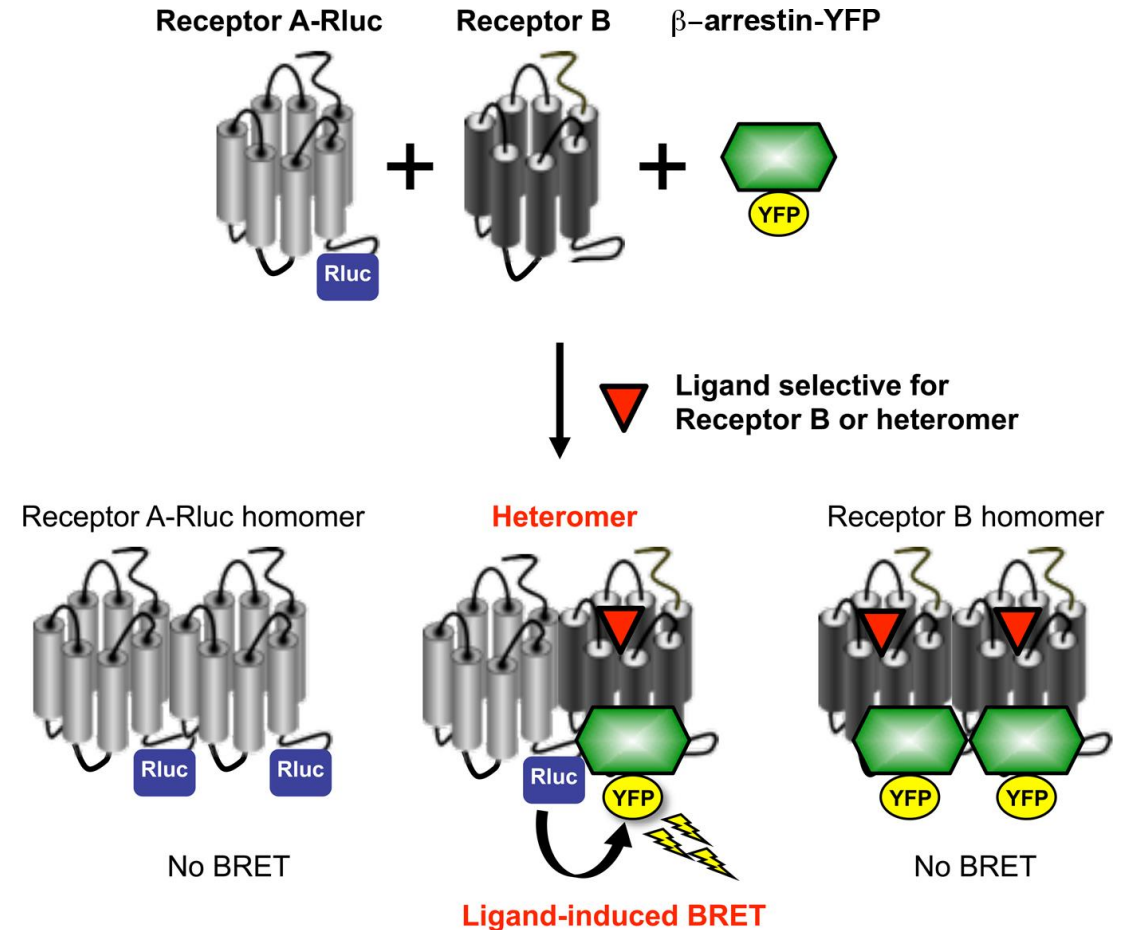
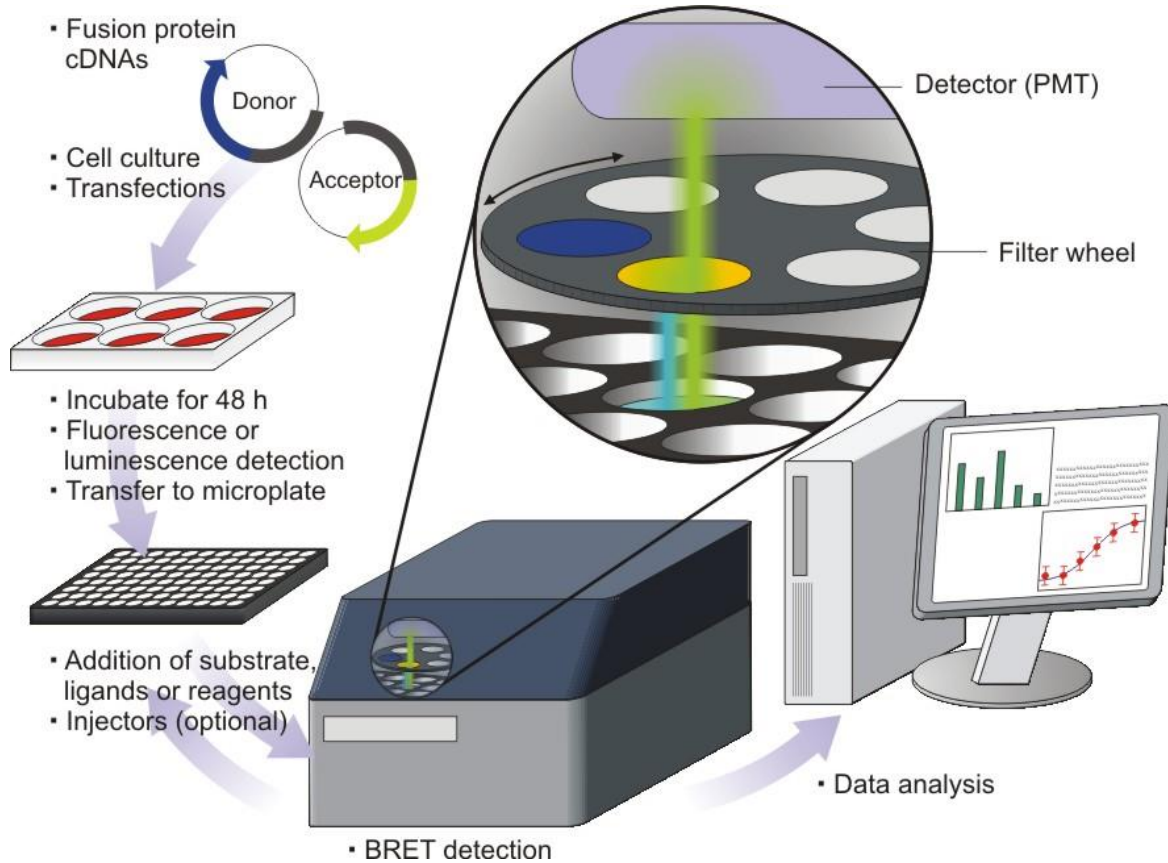
Receptor-HIT: Receptor Heteromer Investigation Technology



Receptor-HIT: Receptor Heteromer Investigation Technology



Receptor-HIT: BRET configuration



Pfleger and Eidne (2006) *Nature Methods* 3:165-174

Pfleger et al. (2006) *Nature Protocols* 1: 337-345

Ayoub and Pfleger (2010) *Current Opinion in Pharmacology* 10:44-52

Our publications describing Receptor-HIT



Original Articles:

See, Seeber et al. (2011) *Assay and Drug Development Technologies* **9**:21-30

Porrello et al. (2011) *Cellular Signalling* **23**:1767-1776

Mustafa, See et al. (2012) *Journal of Biological Chemistry* **287**:12952-12965

Ayoub et al. (2013) *PLoS One* **8**:e64672

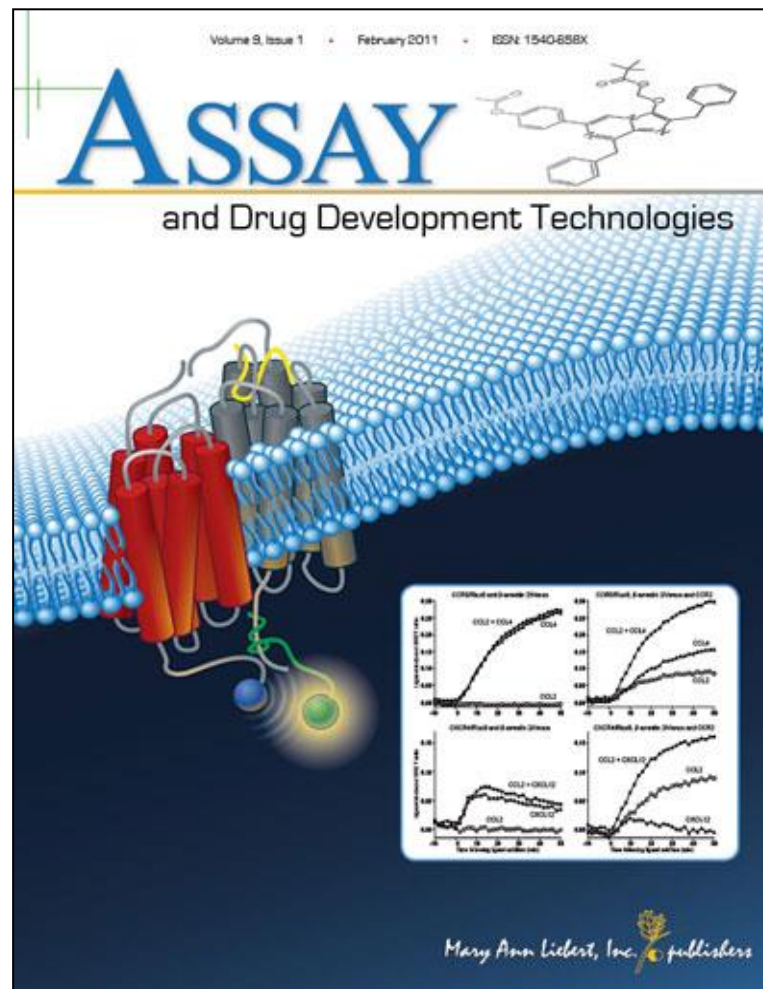
Watts et al. (2013) *British Journal of Pharmacology* **168**:1662-1674

Ayoub, Zhang et al. (2015) *PLoS One* **10**:e0119803

White et al. (2017) *Scientific Reports* **7**:3187

O'Brien et al. (2018) *Biochemical Pharmacology* **158**:232-242

Pickering et al. (2019) *Journal of Clinical Investigation* **129**: 406-421



Reviews/Book Chapters:

Ayoub and Pflieger (2010) *Current Opinion in Pharmacology* **10**:44-52

Mustafa et al. (2010) *Drug Discovery Today: Technologies* **23**:1767-1776

Mustafa and Pflieger (2011) *Journal of Laboratory Automation* **16**:285-291

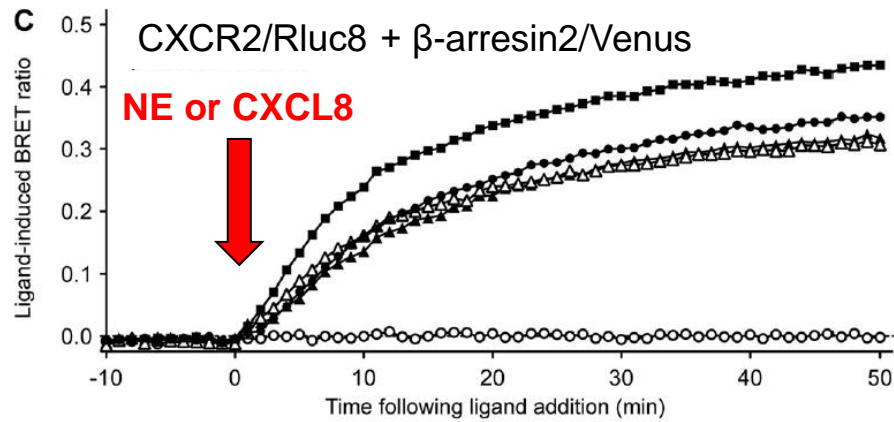
Johnstone and Pflieger (2012) *Frontiers in Endocrinology* **3**:101

Jaeger et al. (2014) *Frontiers in Endocrinology* **5**:26

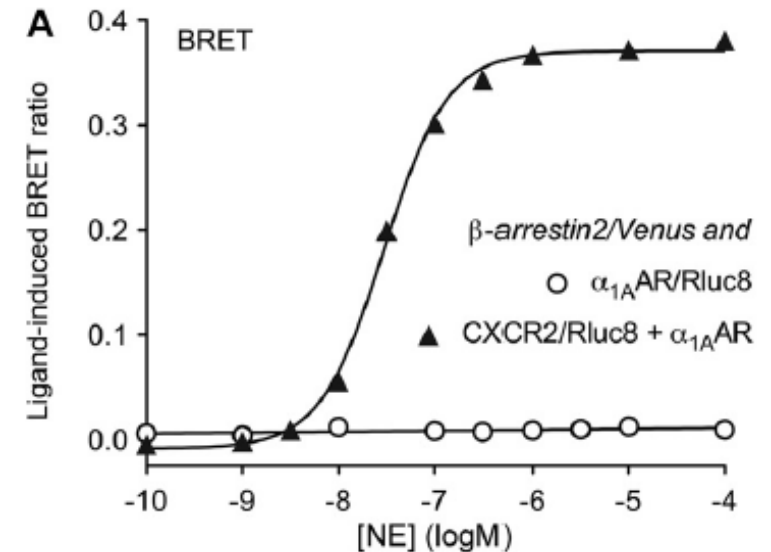
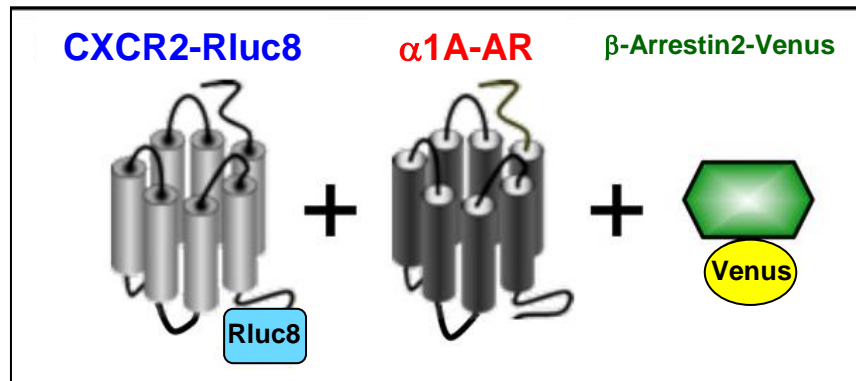
Johnstone and Pflieger (2015) in *G-Protein-Coupled Receptors in Drug Discovery: Methods and Protocols* 191-204

Gomes et al. (2016) *Annual Reviews in Pharmacology and Toxicology* **56**:403-425

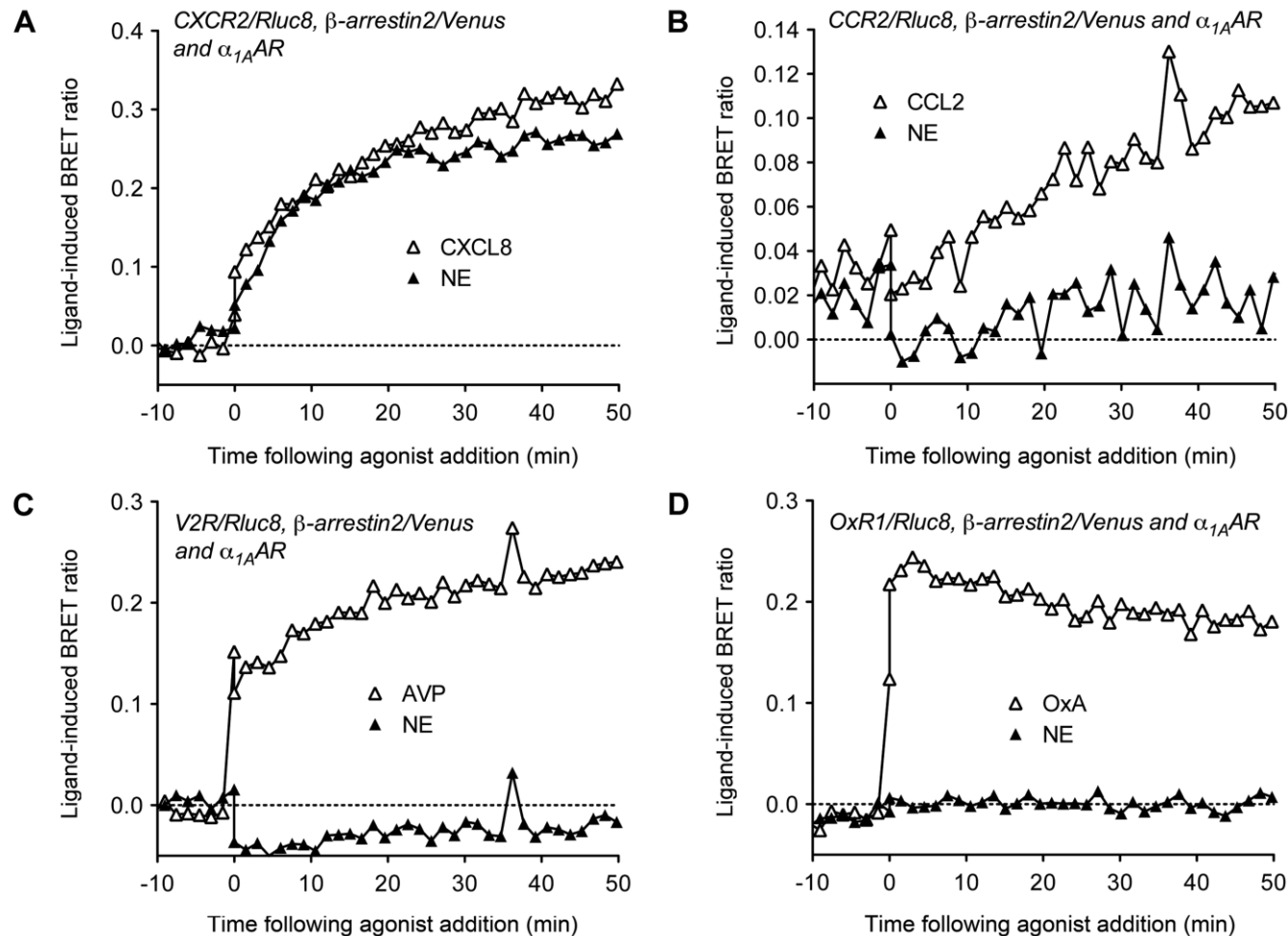
Receptor-HIT with $\alpha_1\alpha$ AR-CXCR2 heteromer



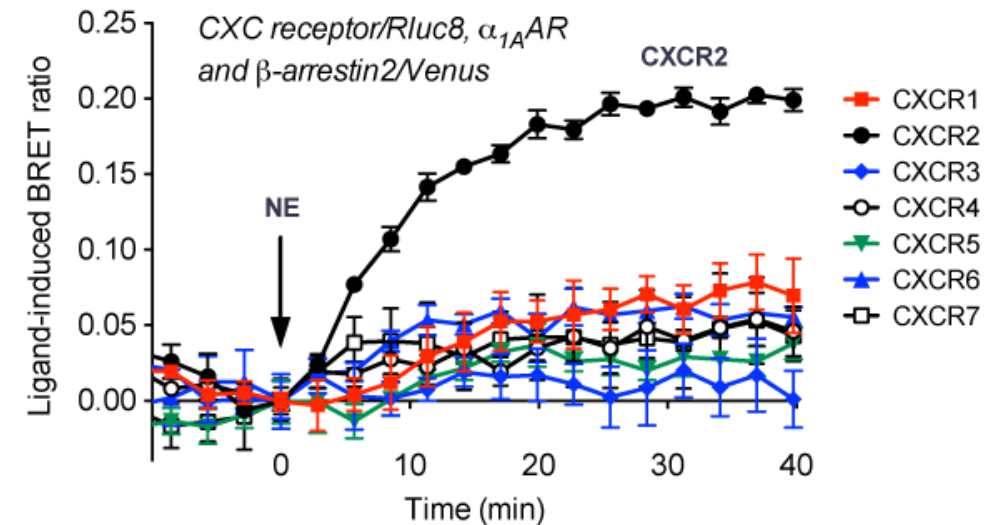
- $\alpha_1\alpha$ AR + NE + CXCL8
- △ $\alpha_1\alpha$ AR + CXCL8
- ▲ $\alpha_1\alpha$ AR + NE
- CXCL8
- NE



Receptor-HIT with α_1 AR-CXCR2 heteromer

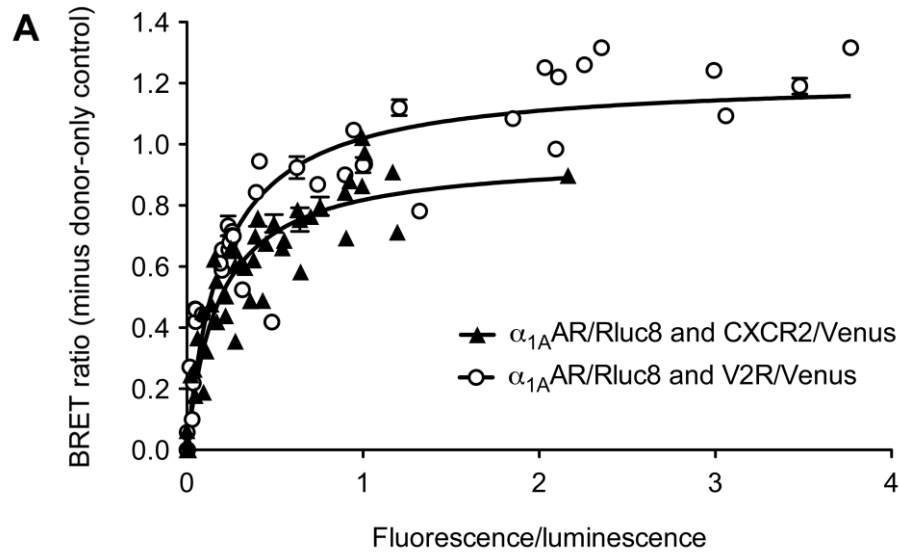


Seeber and Pflieger,
unpublished data:



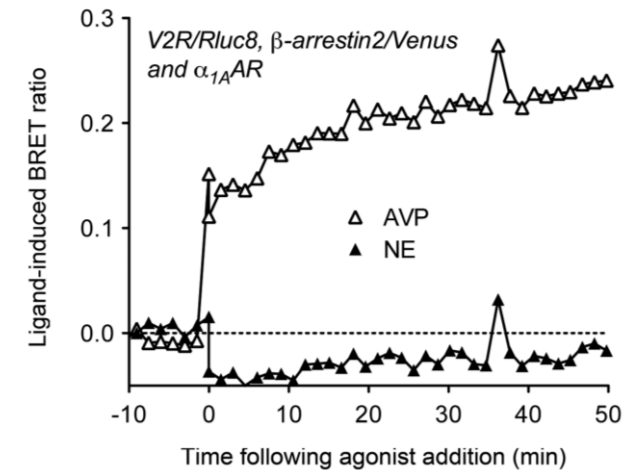
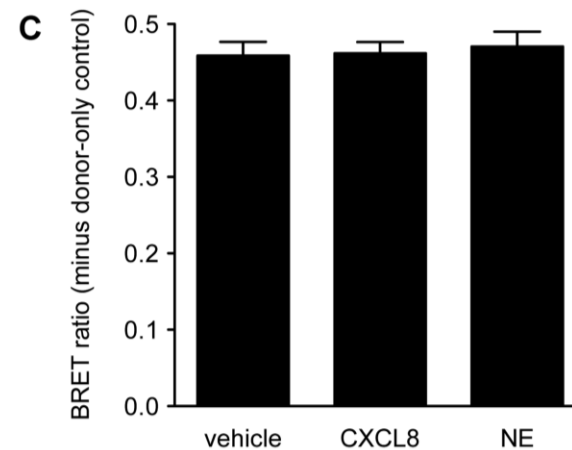
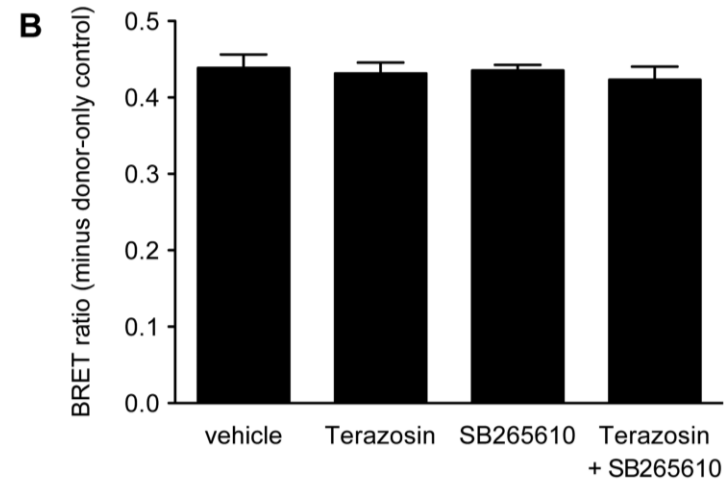
Mustafa, See, Seeber, Armstrong, White, Ventura, Ayoub,
Pflieger *Journal of Biological Chemistry* (2012) **287**:12952-12965

Constitutive complex



Constitutive complex that is not dynamically regulated by ligand

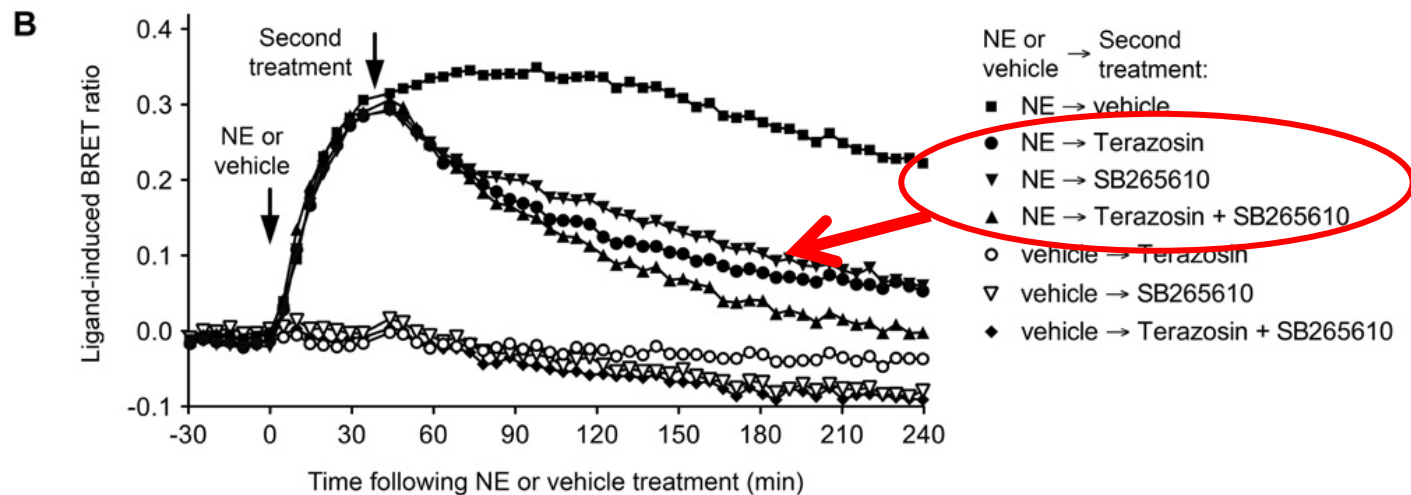
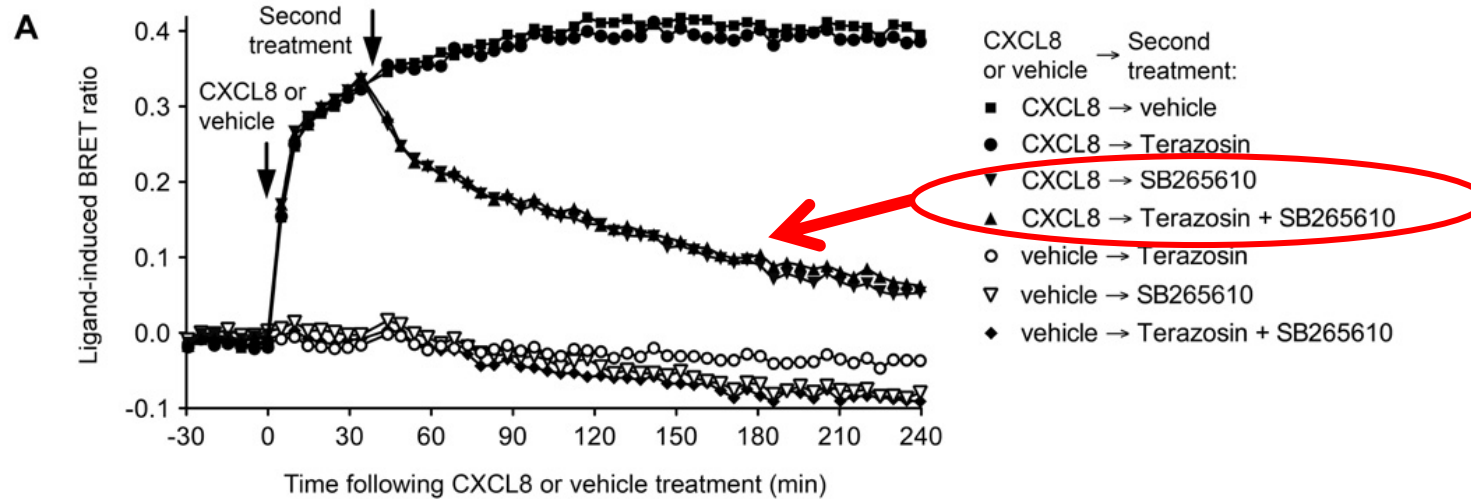
Despite both CXCR2 and V2R appearing to be in close proximity to $\alpha_{1A}AR$, only CXCR2 appears to alter $\alpha_{1A}AR$ pharmacology



Mustafa, See, Seeber, Armstrong, White, Ventura, Ayoub, Pflieger
Journal of Biological Chemistry
(2012) **287**:12952-12965

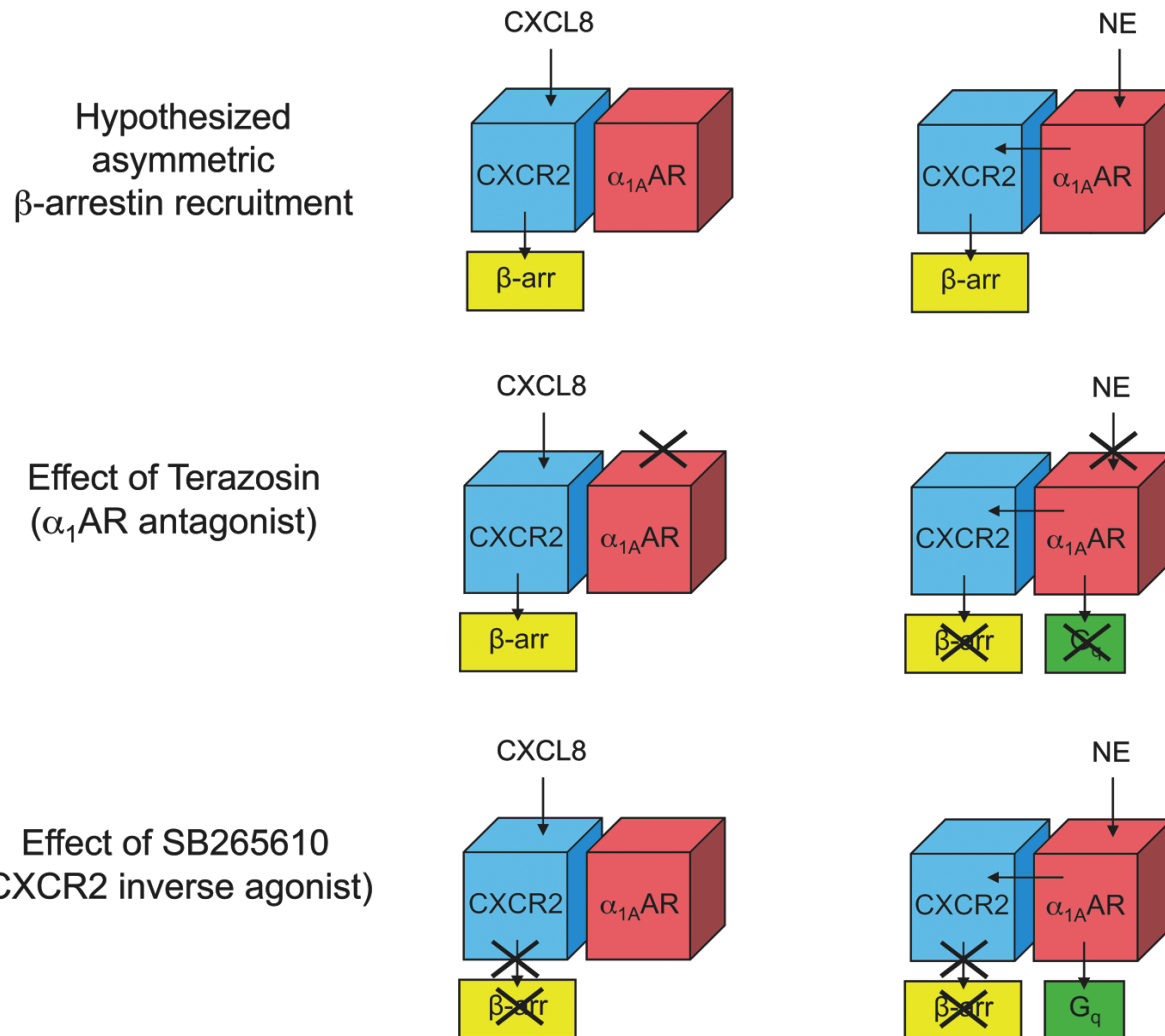
Transactivation mechanism within the α_{1a} AR-CXCR2 heteromer

CXCR2/Rluc8
+ β -arresin2/Venus
+ α_{1a} -AR



Mustafa, See, Seeber,
Armstrong, White, Ventura,
Ayoub, Pflieger *Journal of
Biological Chemistry* (2012)
287:12952-12965

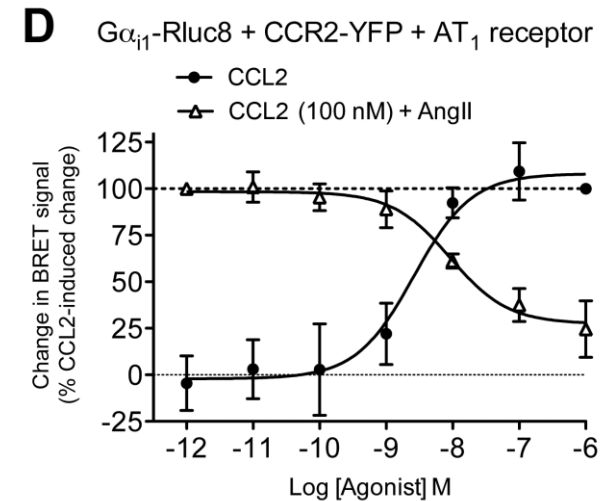
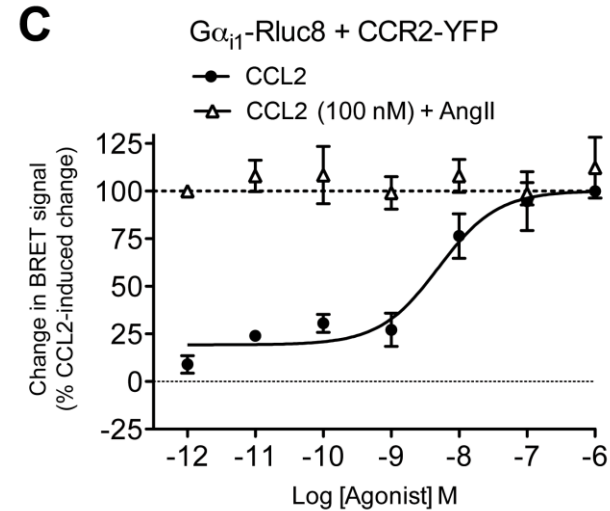
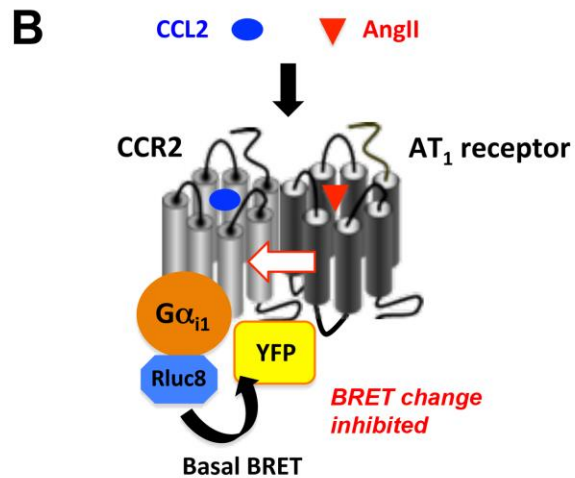
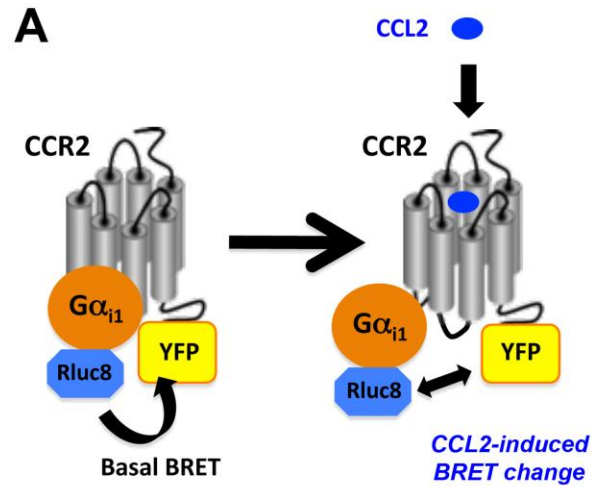
Transactivation mechanism within the α_{1A} AR-CXCR2 heteromer



**Key role of
CXCR2 protomer!**

Mustafa, See, Seeber,
Armstrong, White, Ventura,
Ayoub, Pflieger *Journal of
Biological Chemistry* (2012)
287:12952-12965

Receptor-HIT: modulation of CCR2-Gi coupling by AT₁ receptor

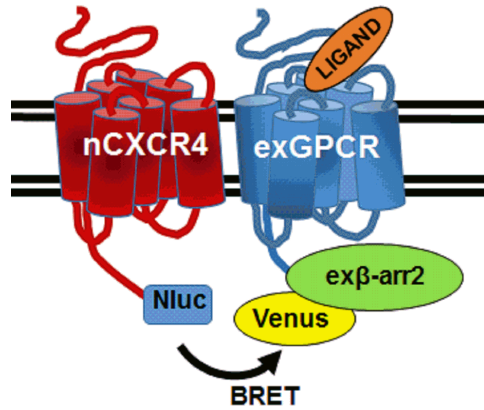



Dimerix

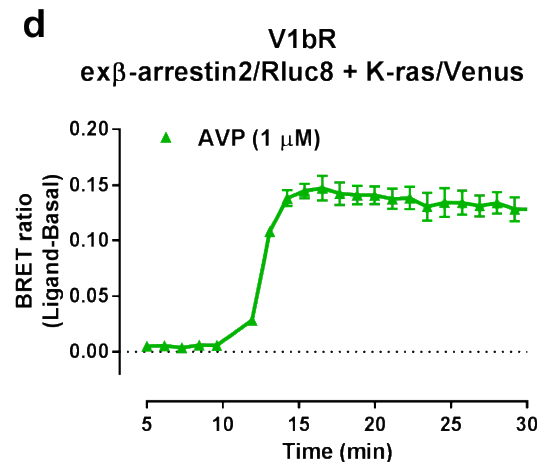
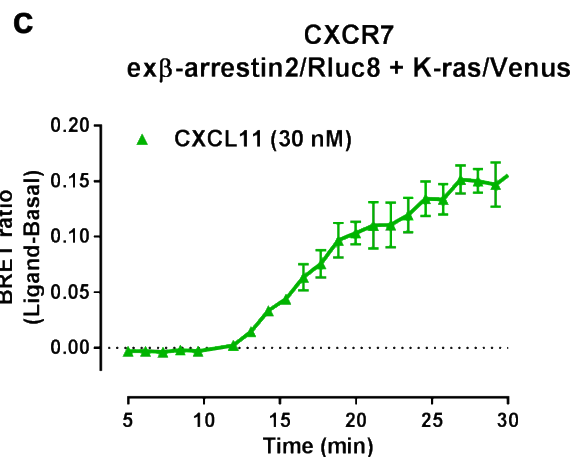
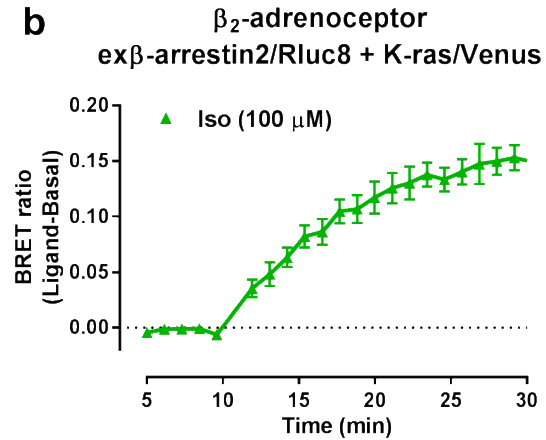
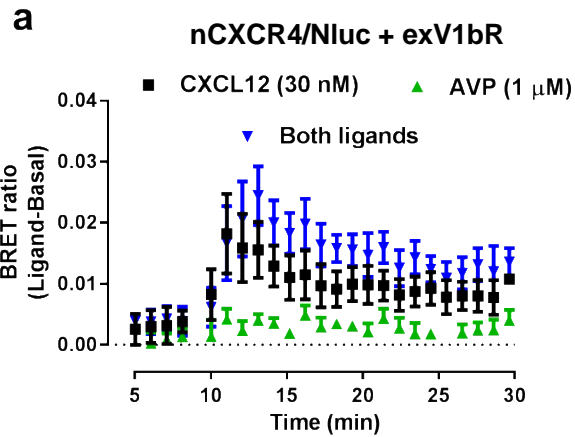
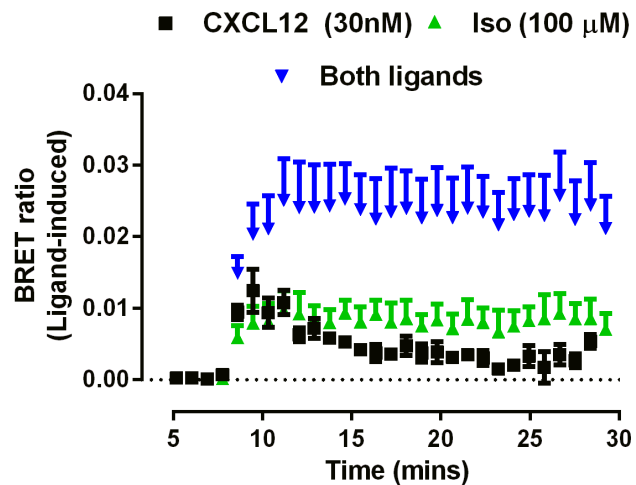
DMX-200

Ayoub, Zhang, Kelly, See,
Johnstone, McCall, Williams,
Kelly, Pflieger (2015) *PLoS One*
10:e0119803

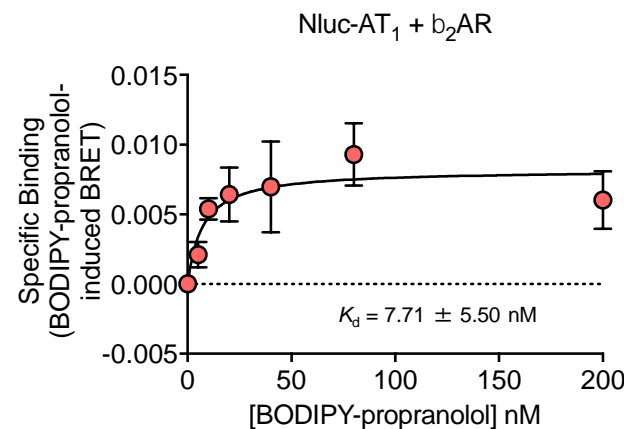
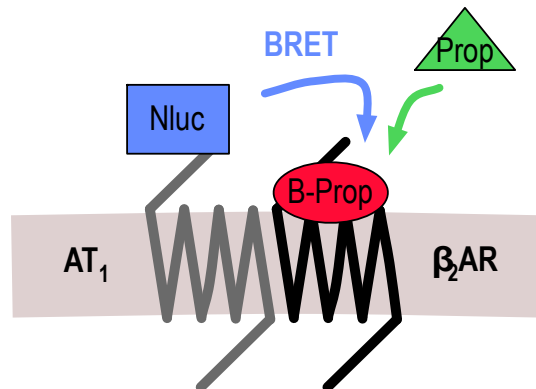
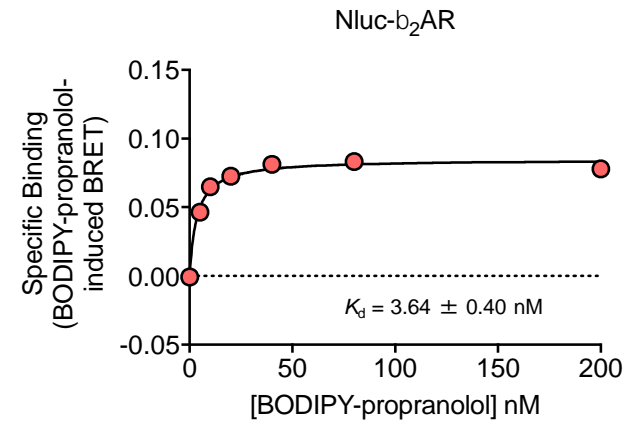
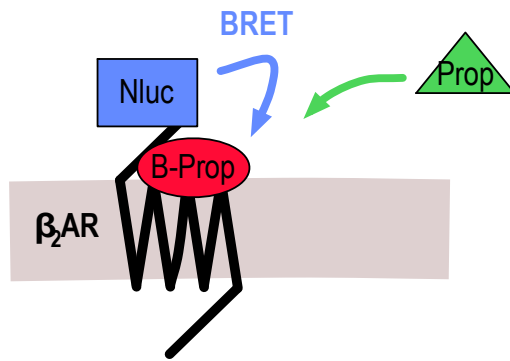
Receptor-HIT with endogenous CXCR4 tagged with Nluc using CRISPR



nCXCR4/Nluc + exβ₂-adrenoceptor



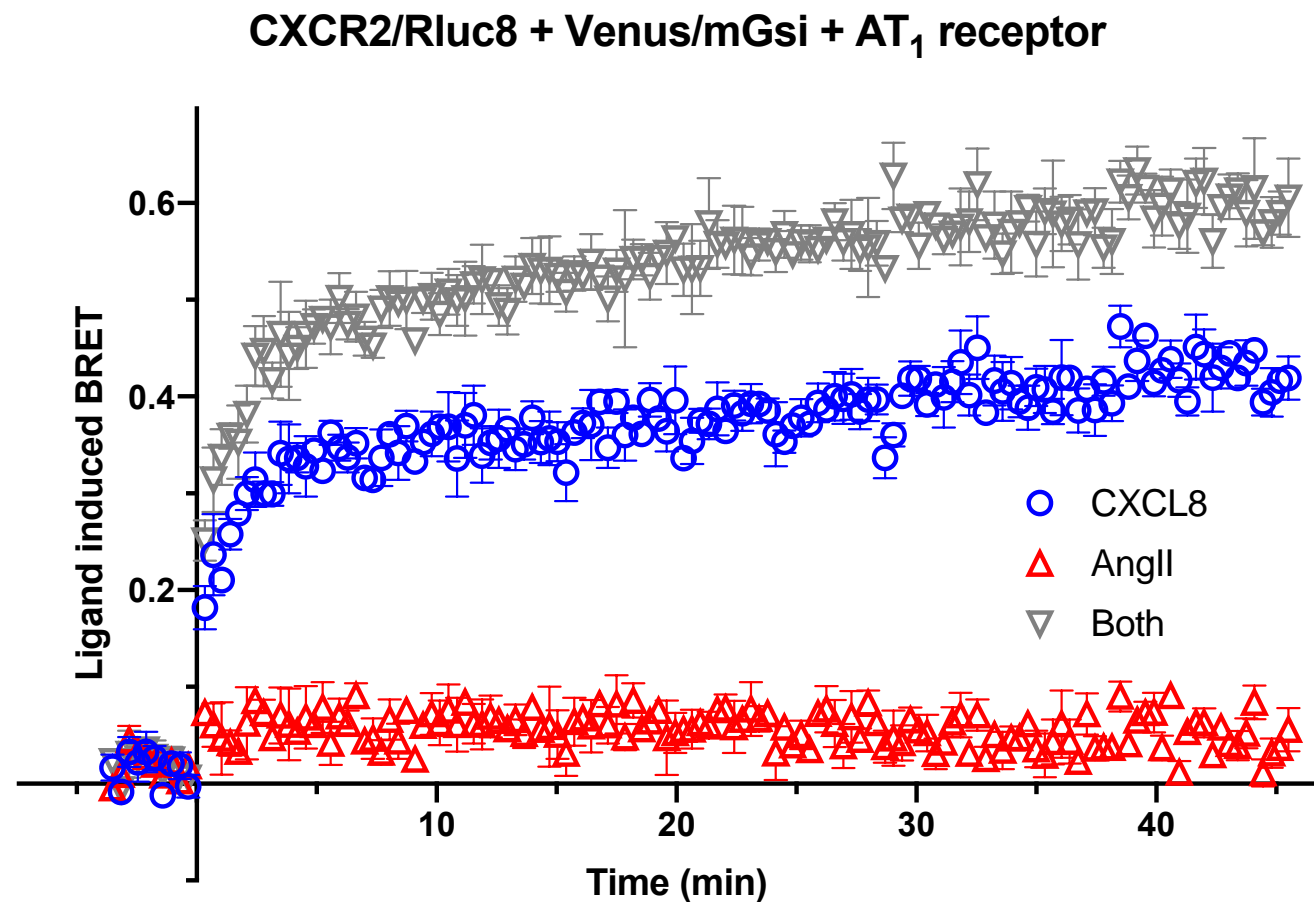
Receptor-HIT ligand binding: AT₁ receptor heteromerisation with β_2 AR



BRET between
BODIPY-
propranolol and
Nluc-AT₁
indicates
heteromerisation
with β_2 AR.

Johnstone and Pflieger,
unpublished observations

Receptor-HIT: CXCR2-Gi coupling induced by AT₁ receptor activation with AngII

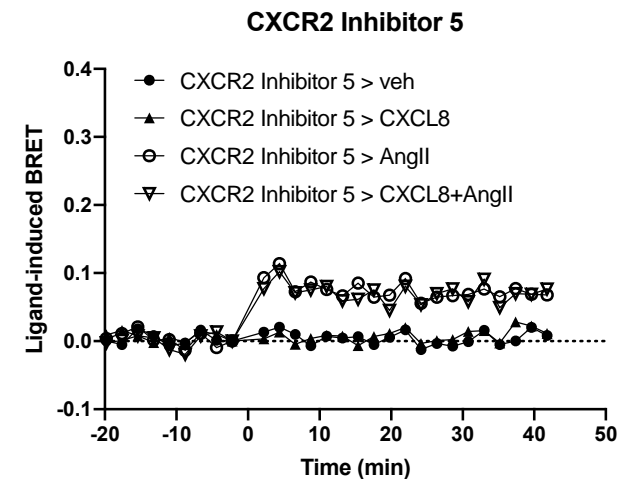
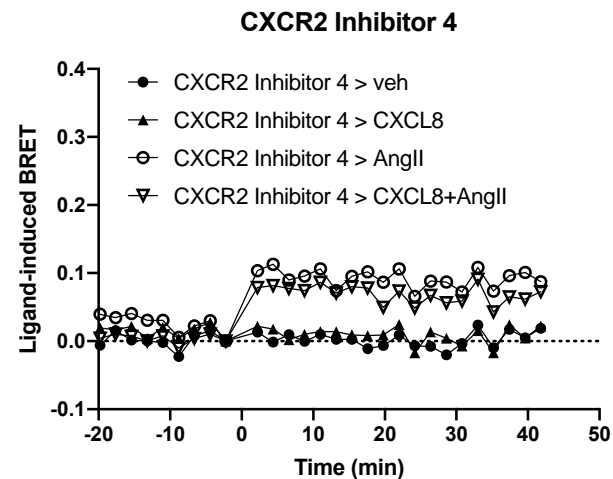
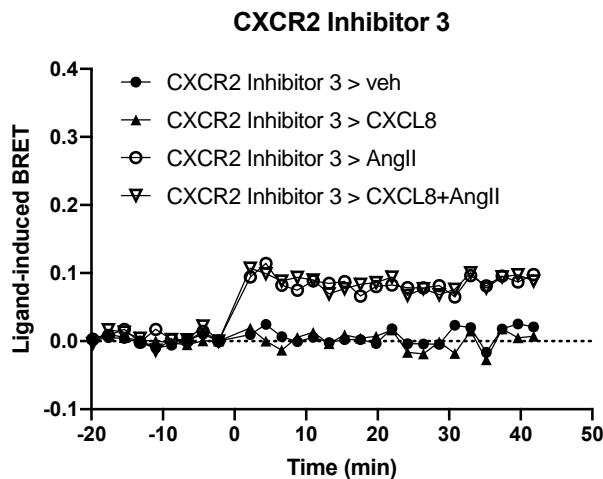
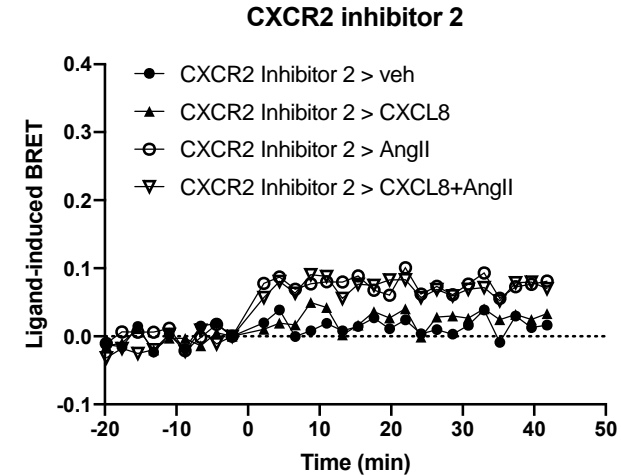
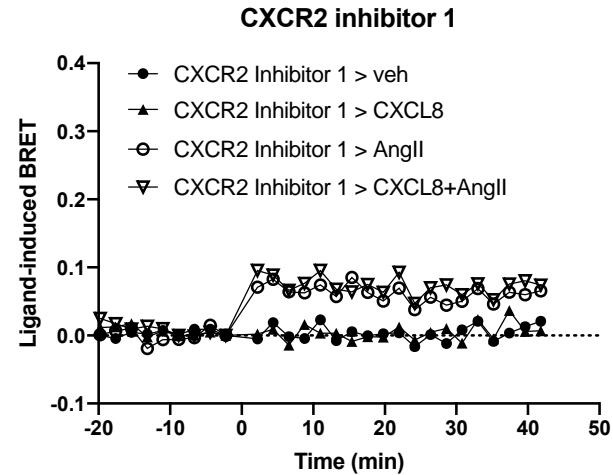
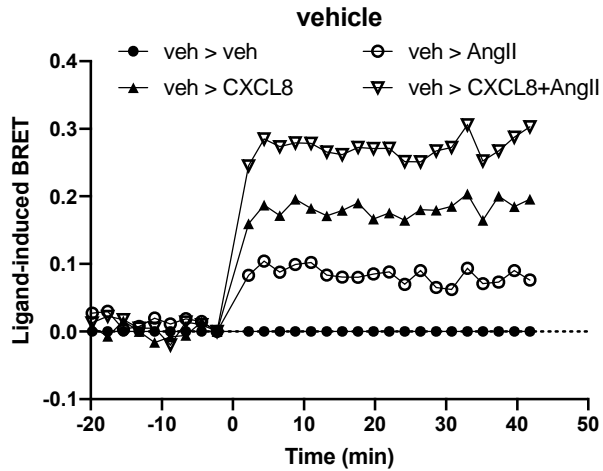


DMX-700

100nM CXCL8
1μM AngII

See, Shepherd and Pflieger unpublished observations

Receptor-HIT: CXCR2 inhibitors acting on CXCR2/Rluc8 + Venus/mGsi + AT₁ receptor



DMX-700

10nM CXCL8
100nM AngII
10µM Inhibitor

See, Shepherd and Pflieger unpublished observations

Receptor-HIT: AT₁R and CXCR2 inhibitors acting on CXCR2/Rluc8 + Venus/mGsi + AT₁ receptor

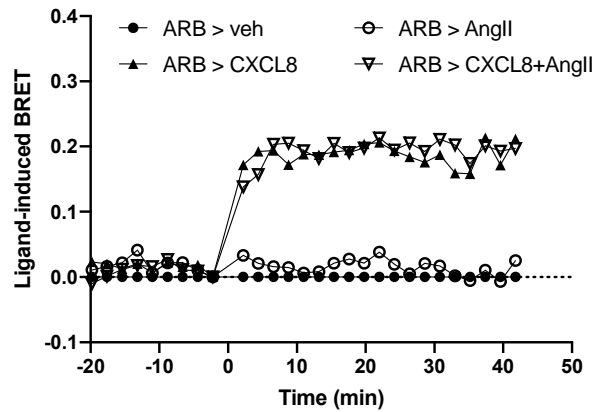


DMX-700

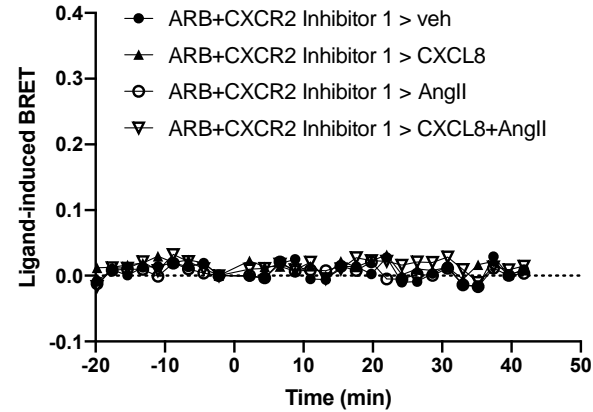
Similar data with
8 different ARBs

See, Shepherd and
Pfleger unpublished
observations

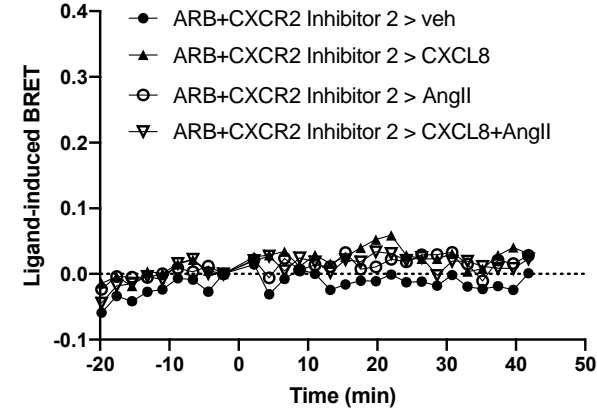
Angiotensin receptor blocker (ARB)



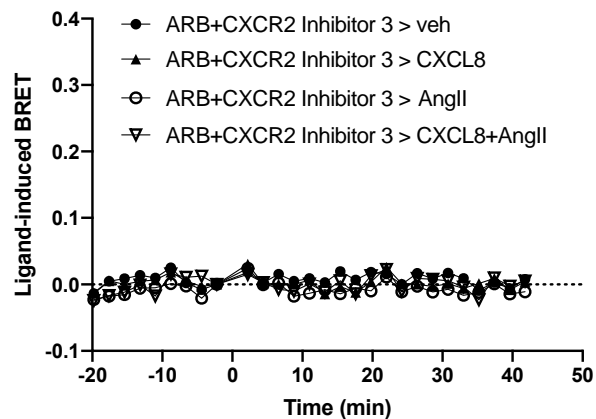
ARB + CXCR2 Inhibitor 1



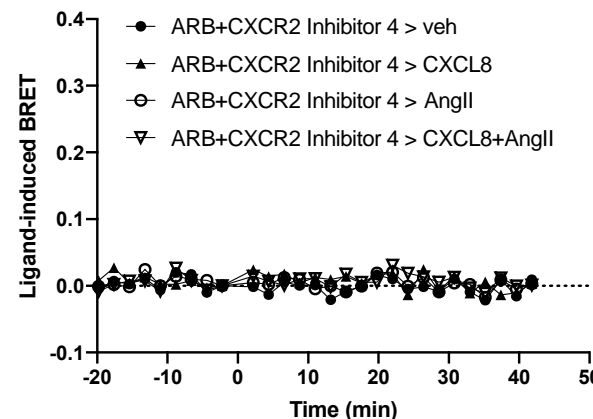
ARB + CXCR2 Inhibitor 2



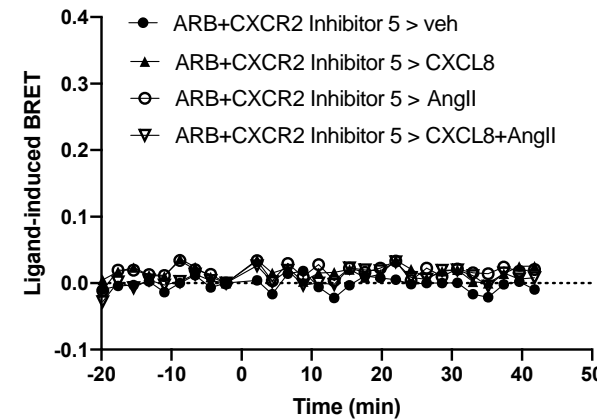
ARB + CXCR2 Inhibitor 3



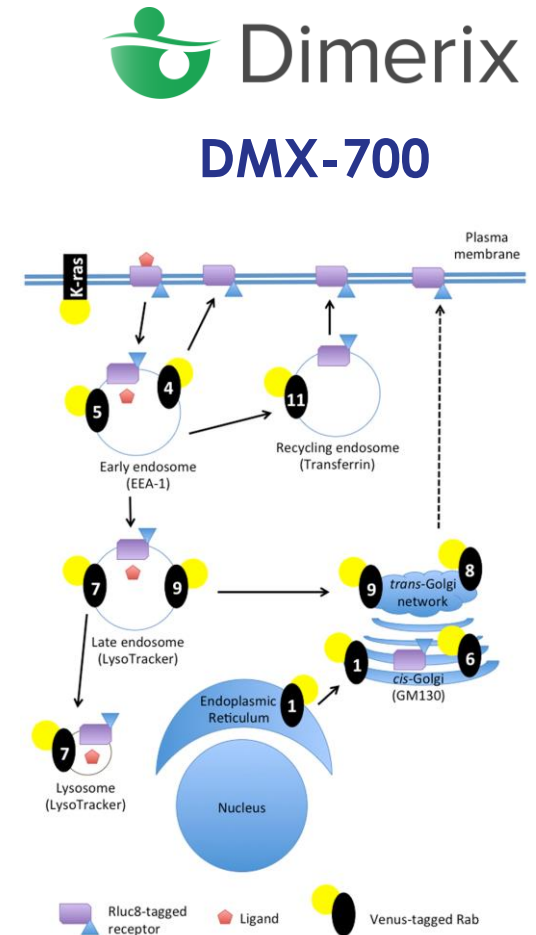
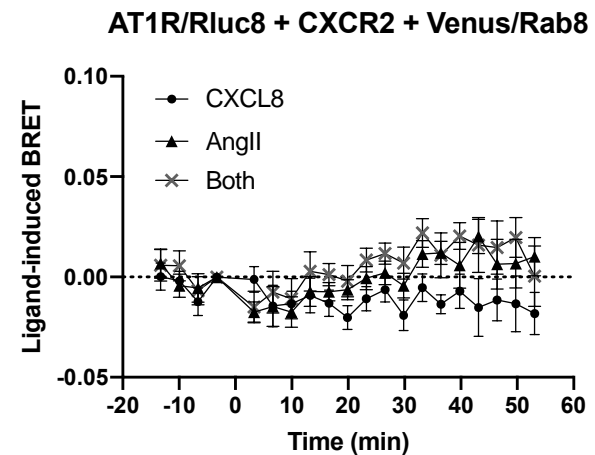
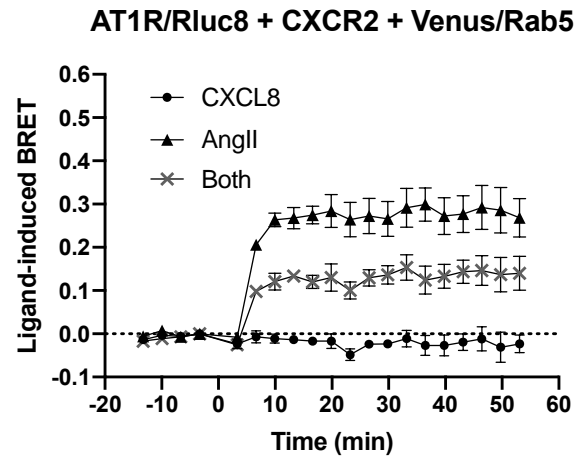
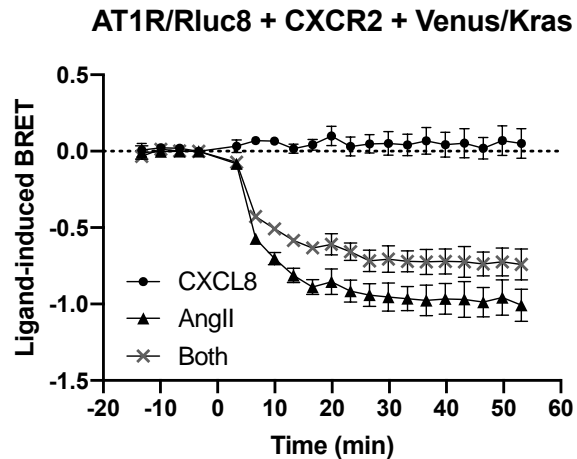
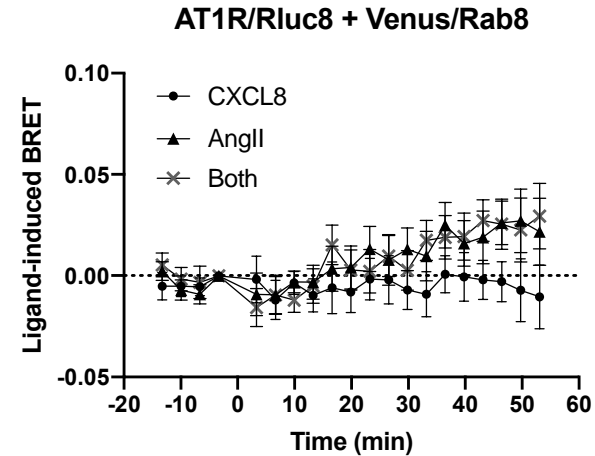
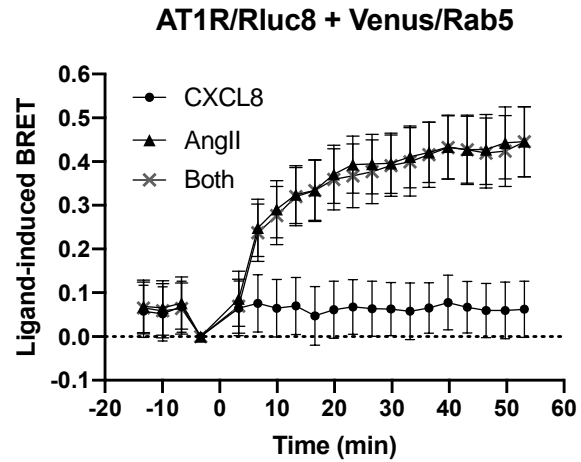
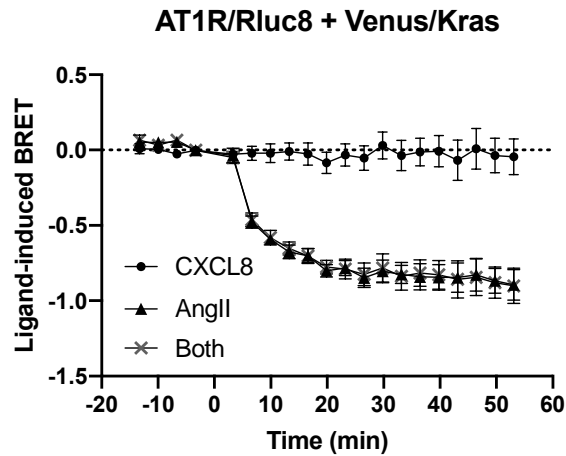
ARB + CXCR2 Inhibitor 4



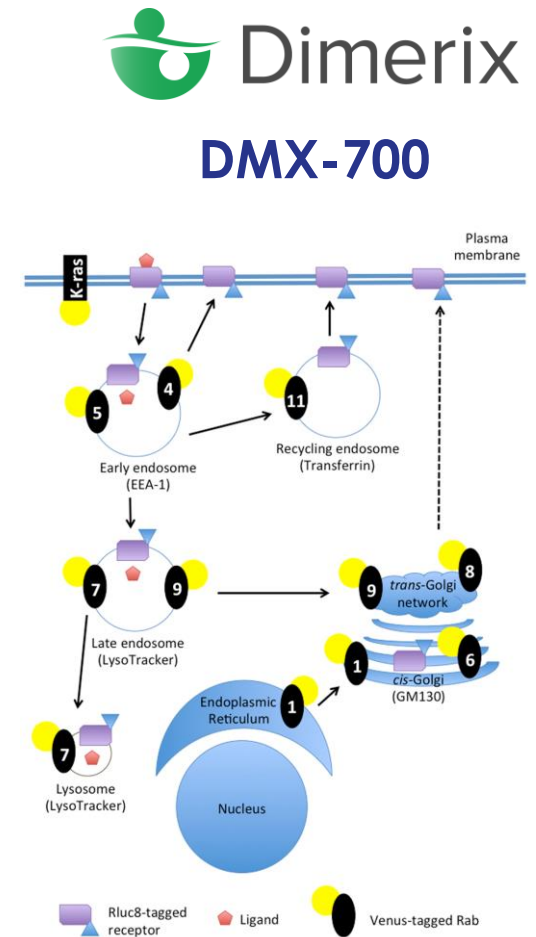
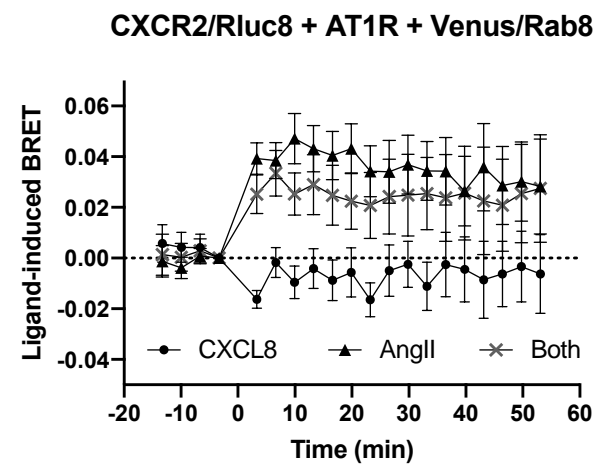
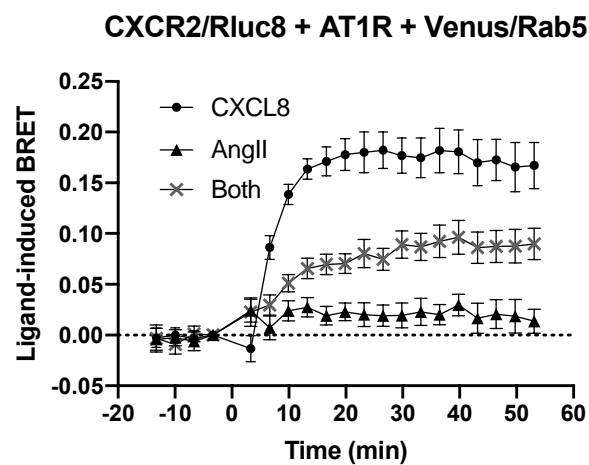
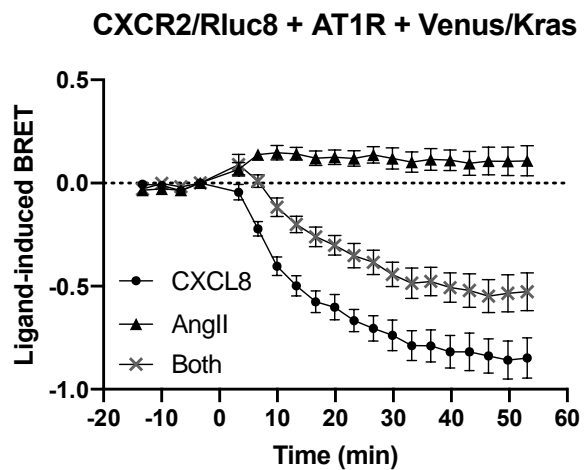
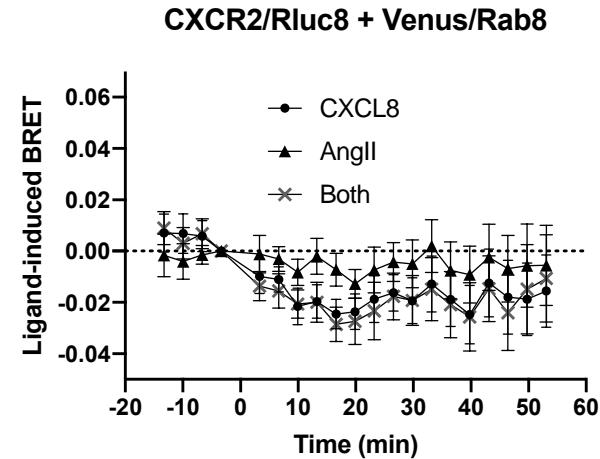
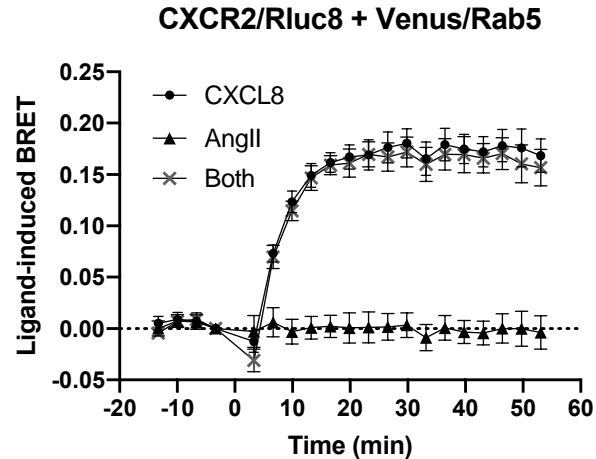
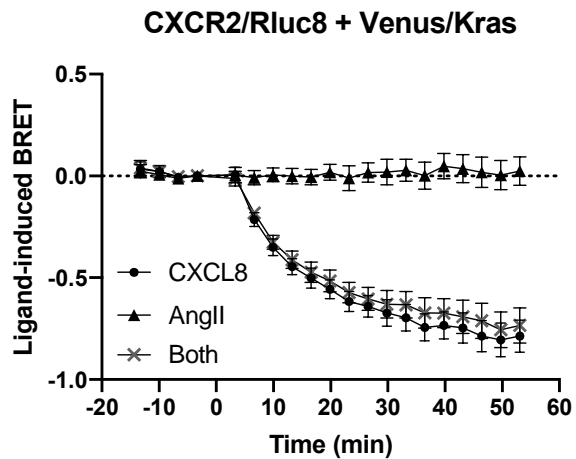
ARB + CXCR2 Inhibitor 5



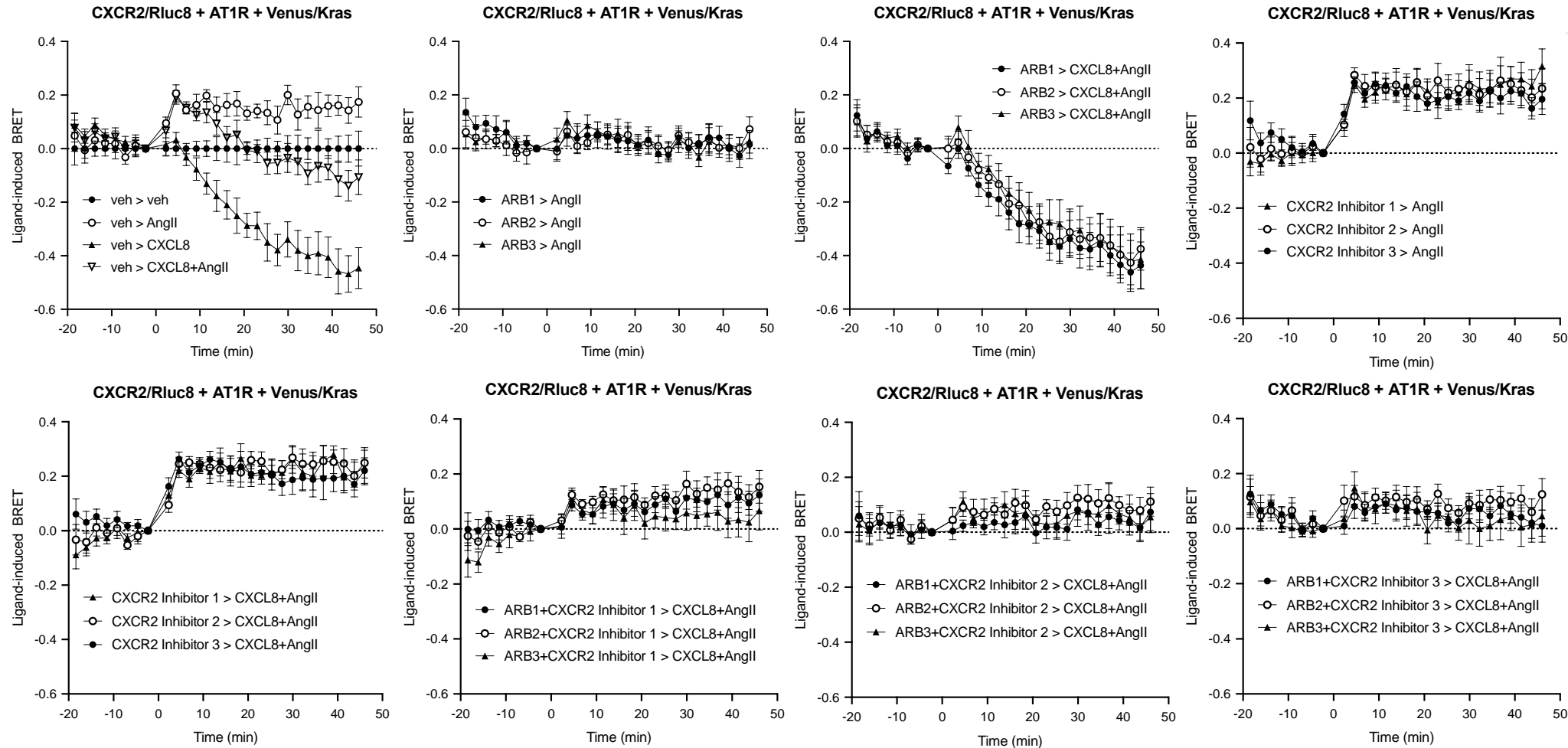
Receptor-HIT localisation: AT₁ receptor/Rluc8 + Venus-tagged localisation marker +/- CXCR2



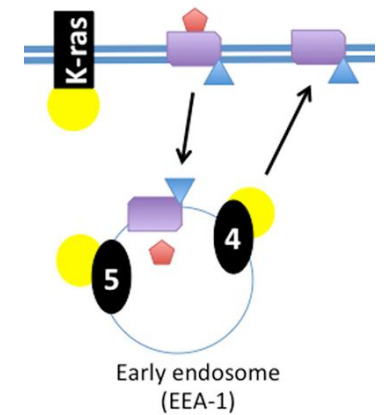
Receptor-HIT localisation: CXCR2/Rluc8 + Venus-tagged localisation marker +/- AT₁ receptor



Receptor-HIT localisation: CXCR2/Rluc8 + Venus/Kras plasma membrane marker + AT₁R

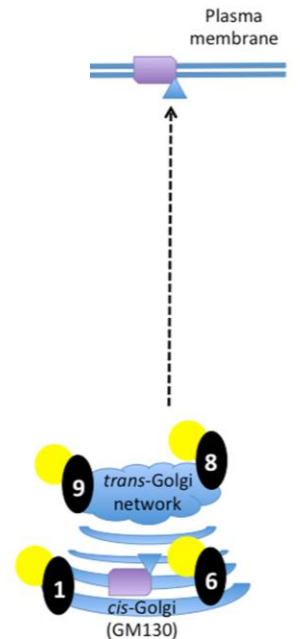



Dimerix
DMX-700

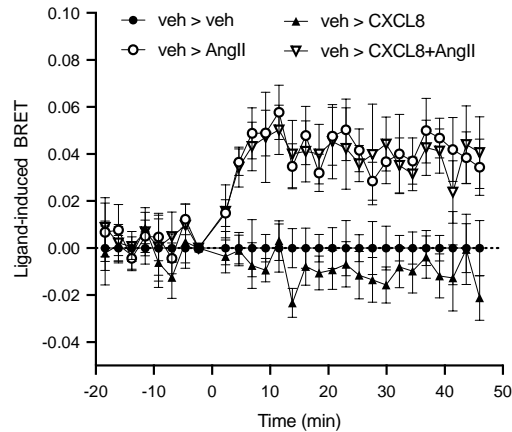


See, Shepherd and Pflieger unpublished observations

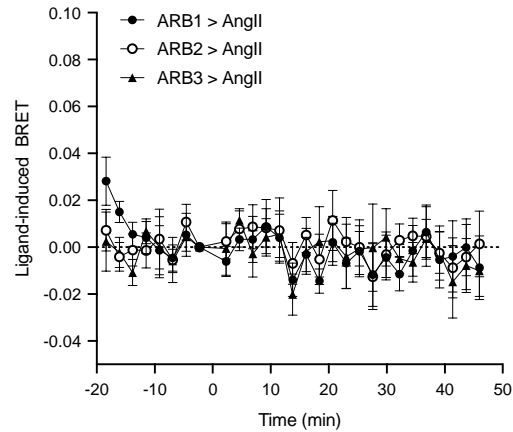
Receptor-HIT localisation: CXCR2/Rluc8 + Venus/Rab8 trafficking to plasma membrane marker + AT₁R



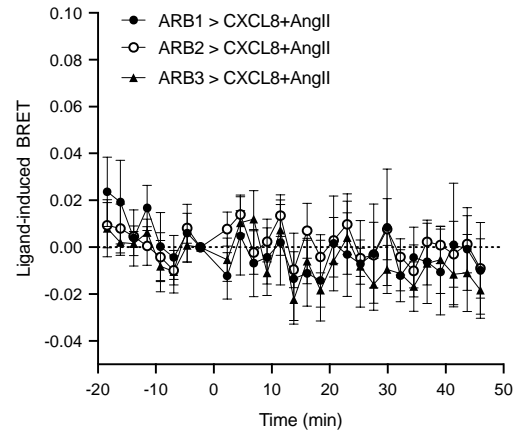
CXCR2/Rluc8 + AT1R + Venus/Rab8



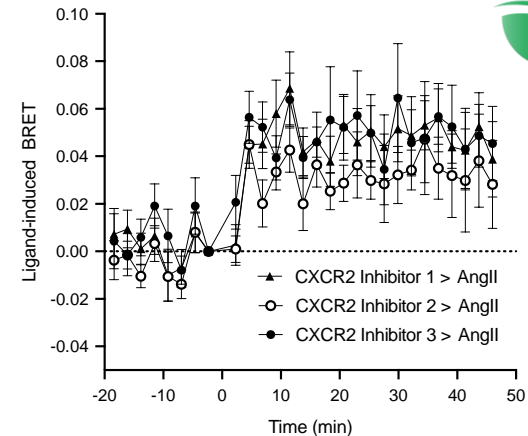
CXCR2/Rluc8 + AT1R + Venus/Rab8



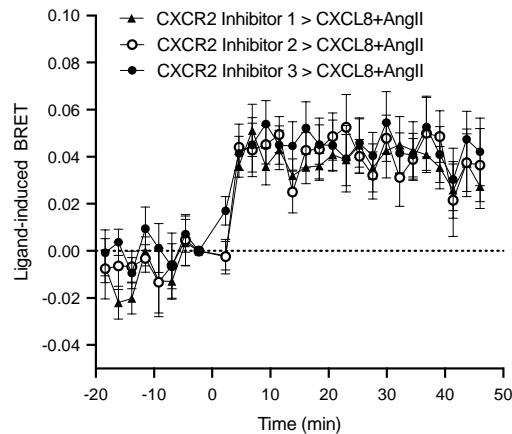
CXCR2/Rluc8 + AT1R + Venus/Rab8



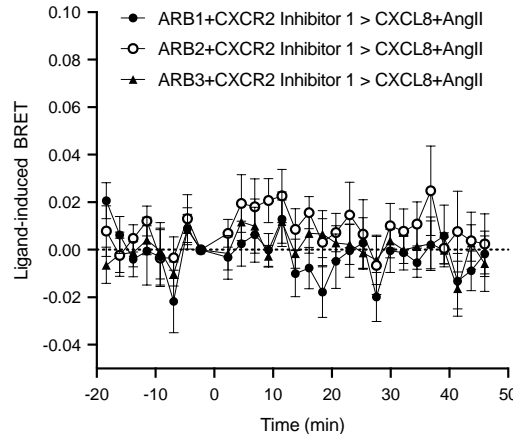
CXCR2/Rluc8 + AT1R + Venus/Rab8



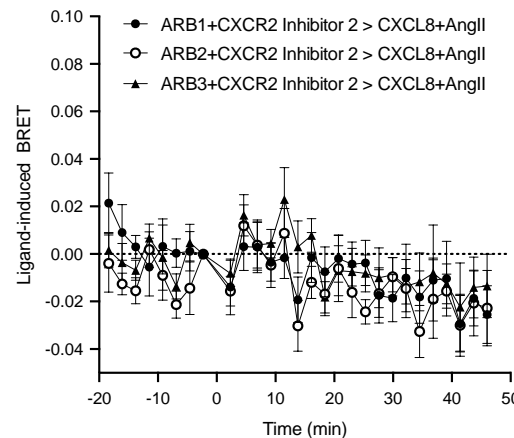
CXCR2/Rluc8 + AT1R + Venus/Rab8



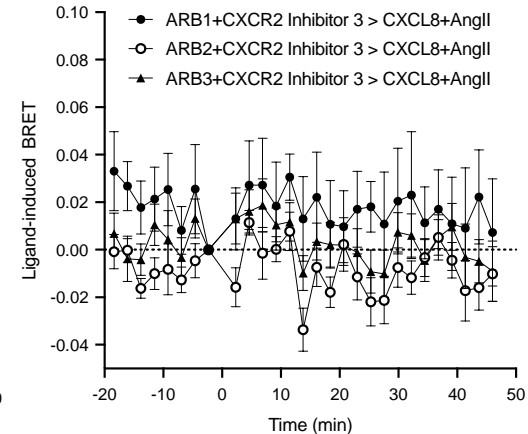
CXCR2/Rluc8 + AT1R + Venus/Rab8



CXCR2/Rluc8 + AT1R + Venus/Rab8



CXCR2/Rluc8 + AT1R + Venus/Rab8



Acknowledgements



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Current: Ethan See Ruth Seeber Rekhati Abhayawardana
Liz Johnstone Carl White Natasha Dale

Selected Recent Past: Mohammed Akli Ayoub Matthew Dalrymple Werner Jaeger
Sanam Mustafa Stephen Armstrong



Academic Collaborators:

Leigh Stoddart, Amanda Wheal, Joëlle Goulding, Stephen Hill and Laura Kilpatrick: Nottingham
Anatoly Tiulpakov and Ivan Dedov: Endocrinology Research Centre, Moscow
Nathan Pavlos, Audrey Chan, Julian Heng and Hannah Vanyai: University of Western Australia



Industry Collaborators:

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