BRET-1 assays for measuring beta-arrestin recruitment as primary screening in G protein coupled receptors in FDSS700

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Innopharma

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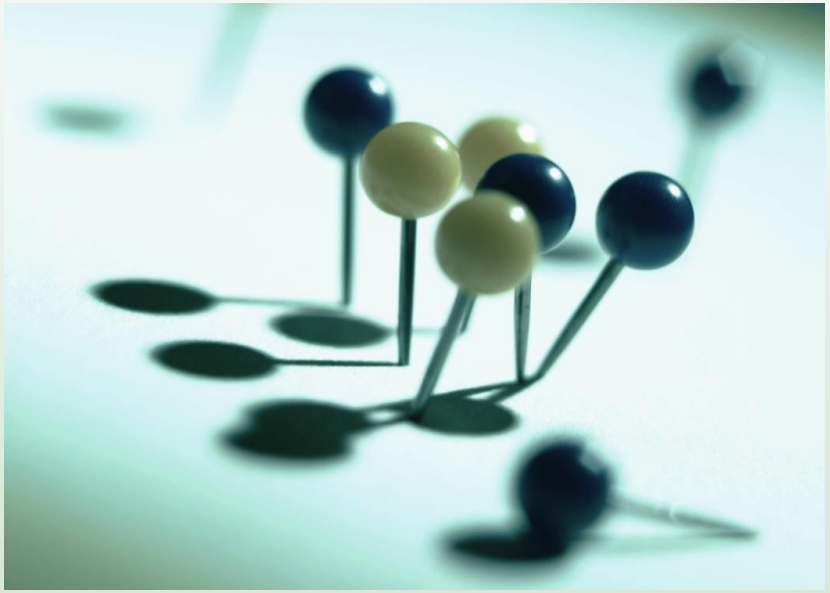
EUROPEAN UNION EUROPEAN REGIONAL DEVELOPMENT FUND

Outline

- What is Innopharma
- GPR120 (FFA4)
- BRET1 screening using FDSS7000
- Summary



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Who we are

BioFarma

GRUPO DE MEDICINA XENÓMICA

Pharmacogenomic Platform Devoted to Knowledge valorisation

- Reference groups with over 15 years experience in genomic medicine and drug discovery. Located at the Research Centre on Molecular Medicine and Chronic Diseases (CIMUS) of the University of Santiago de Compostela (USC).
- Managing a multidisciplinary team of **130 professionals**.
- **Consolidated** knowledge-based **platform**

Experience

- Over 30 Spanish and international **pharmaceutical and biotechnology companies**.
- International **research groups and scientific networks** at the highest level.
- Connected with the best experts in the world on strategic issues.

Collaborations

- Our fundraising average is **2,5 million €/year**.
- We have a **self-funded and validated platform business model.**

Validated model

Surpassing the traditional financial, technical and execution risks associated to Academia

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Objectives

Open innovation and internationalization applied to a pipeline of new drug discovery programs.

Add value to programs devoted to early drug discovery to bridge the gap between basic research in new therapeutic mechanisms and its industrial application**.**

Provide **know how** and **technological support** infrastructure to boost the creation of new knowledge-based companies.

Propose a **sustainability plan** based on open innovation collaborative models.

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Programs Pipeline. Call for proposals

- In its first call for research projects, INNOPHARMA has received 110 EoI, of which 8-10 were initially selected for further development.

- Selected projects were classified in the following categories according to their stage of development in the process of early drug discovery:

Open-lab projects

- go/no go milestones
- initial stage of development
- open innovation
- high scientific value but high risk
- academic approach by and for the industry
- complementarity with companies

Hit-to-candidate projects

Fit into INNOPHARMA capabilities

Preclinical stage projects

Advanced stage of development

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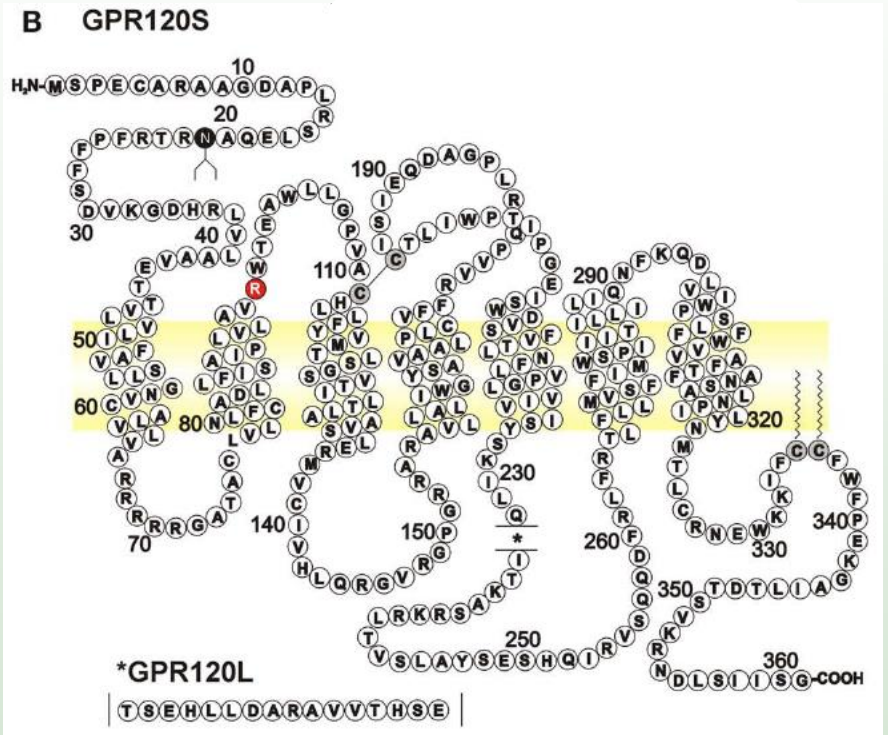
GPR120 (FFA4)

University of Glasgow

Prof Graeme Milligan

GPR120 (FFAR4): long chain fatty acid receptor.

Highly expressed in a range of tissues including lung, colon, adipose, brain, taste buds, skeletal muscle, heart and liver.



frontiers in ENDOCRINOLOGY
REVIEW ARTICLE
published: 03 January 2012
doi: 10.3389/fendo.2011.00112

Drug discovery opportunities and challenges at G protein coupled receptors for long chain free fatty acids

Nicholas D. Holliday[1], Sarah-Jane Watson[1] and Alastair J. H. Brown[2]*

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GPR120


Review

Cell

Targeting GPR120 and other fatty acid-sensing GPCRs ameliorates insulin resistance and inflammatory diseases

Saswata Talukdar, Jerrold M Olefsky and Olivia Osborn

GPCR	HFD phenotype
GPR120 KO	Normal chow: GPR120 KO animals were more insulin resistant compared with WT controls. HFD: both WT and GPR120 KO animals were equally insulin resistant. However, upon ω3 supplementation, the WT animals had significantly improved glucose tolerance compared with both WT and KO animals on a HFD. ω3 supplementation was without effect in the GPR120 KO animals.

Humans: GPR120 expression in adipose tissue is significantly higher in obese individuals than in lean controls.

Human genetic study in European populations identified a loss-of-function GPR120 mutation (p.R270H) associated with obesity and insulin resistance.

GPR120, the sensing receptor for long-chain free fatty acids, represents a novel drug target for the treatment of obesity and diabetes.

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GPR120 (FFA4)

Review

Cell

Targeting GPR120 and other fatty acid-sensing GPCRs ameliorates insulin resistance and inflammatory diseases

Saswata Talukdar, Jerrold M Olefsky and Olivia Osborn

Interestingly, we demonstrated that the anti-inflammatory effects of ω3 fatty acids mediated by GPR120 are exclusively dependent on β-arr 2, but independent of Gαq/11, regardless of the fact that GPR120 can be a Gαq/11-coupled receptor in other contexts [18]. These

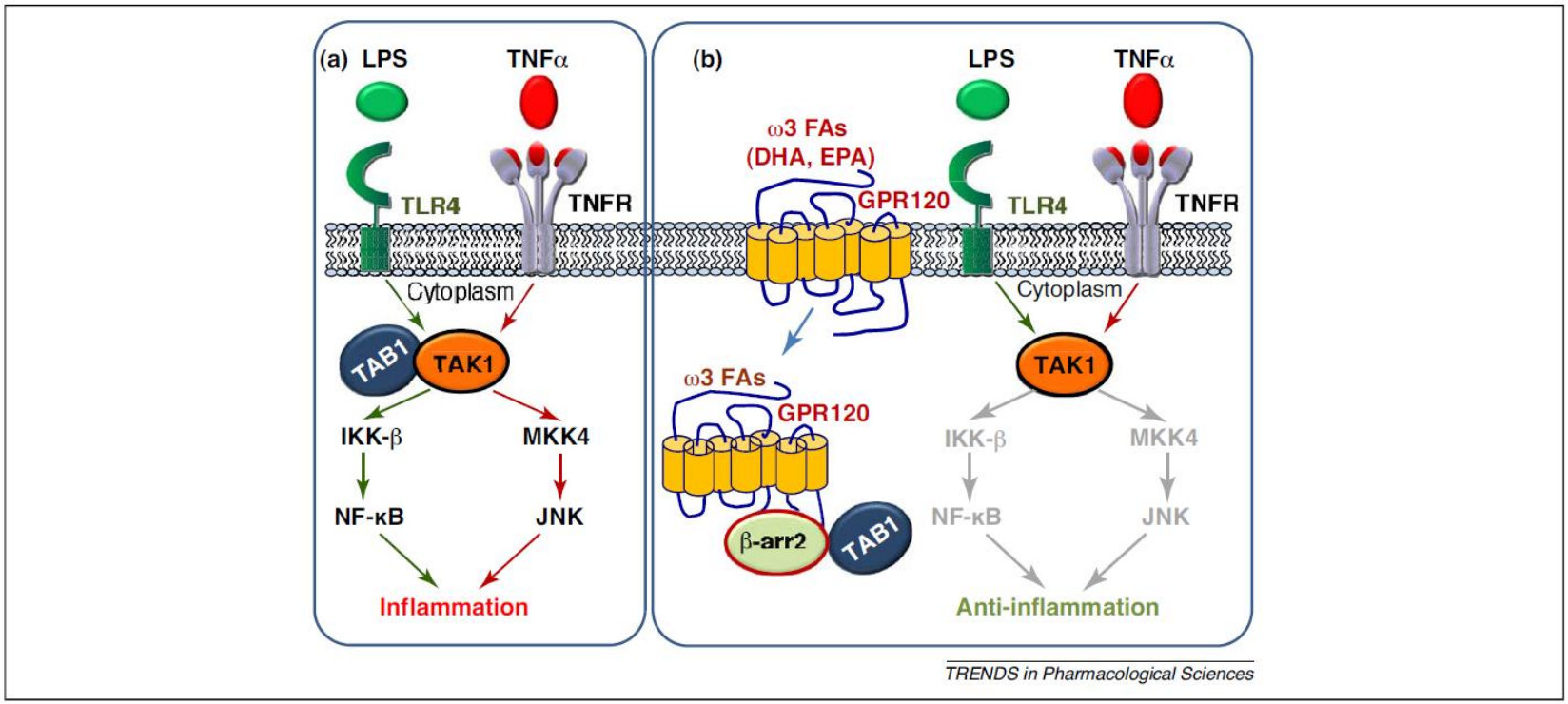

Figure 1. . Mechanism of anti-inflammation upon GPR120 activation by ω3 fatty acids in macrophages. **(a)** Activated toll-like receptor 4 (TLR4) and tumor necrosis factor receptor (TNFR) by lipopolysaccharide (LPS) and tumor necrosis factor-α (TNF-α), respectively, converge on cytoplasmic association of transforming growth factor β (TGF-β) activated kinase 1 (TAK1) with TGF-β activated kinase 1 binding protein 1 (TAB1), mediating proinflammatory cascades by activating NF-κB and c-Jun N-terminal kinase (JNK). **(b)** Activation of GPR120 by ω3 Fas internalizes GPR120 which binds to β-arrestin 2 and sequesters TAB1, inhibiting inflammation.

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GPR120 (FFA4)

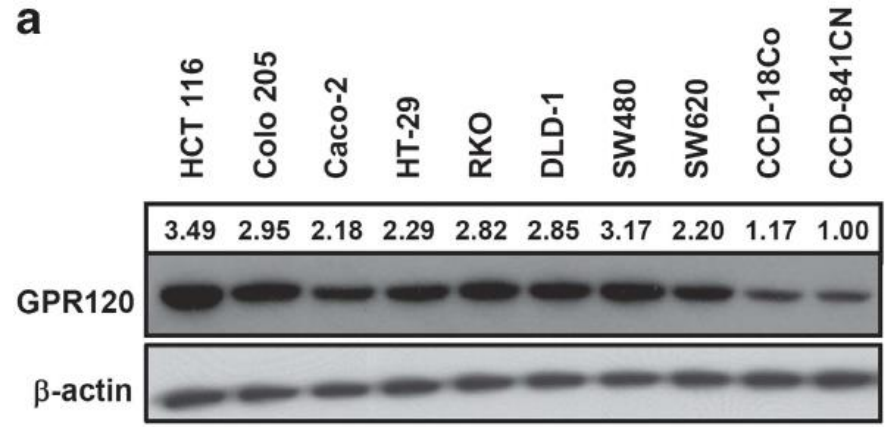
OPEN

Oncogene (2013) **32,** 5541–5550

www.nature.com/onc

ORIGINAL ARTICLE

Identification of G-protein-coupled receptor 120 as a tumor-promoting receptor that induces angiogenesis and migration in human colorectal carcinoma



GPR120 activation:

- promotes angiogenesis in vitro and in vivo
- enhances motility of CRC cells
- Induces EMT

GPR120 functions as tumor-promoting receptor in CRC

We looked for antagonists of GPR120 as putative anticancer drugs

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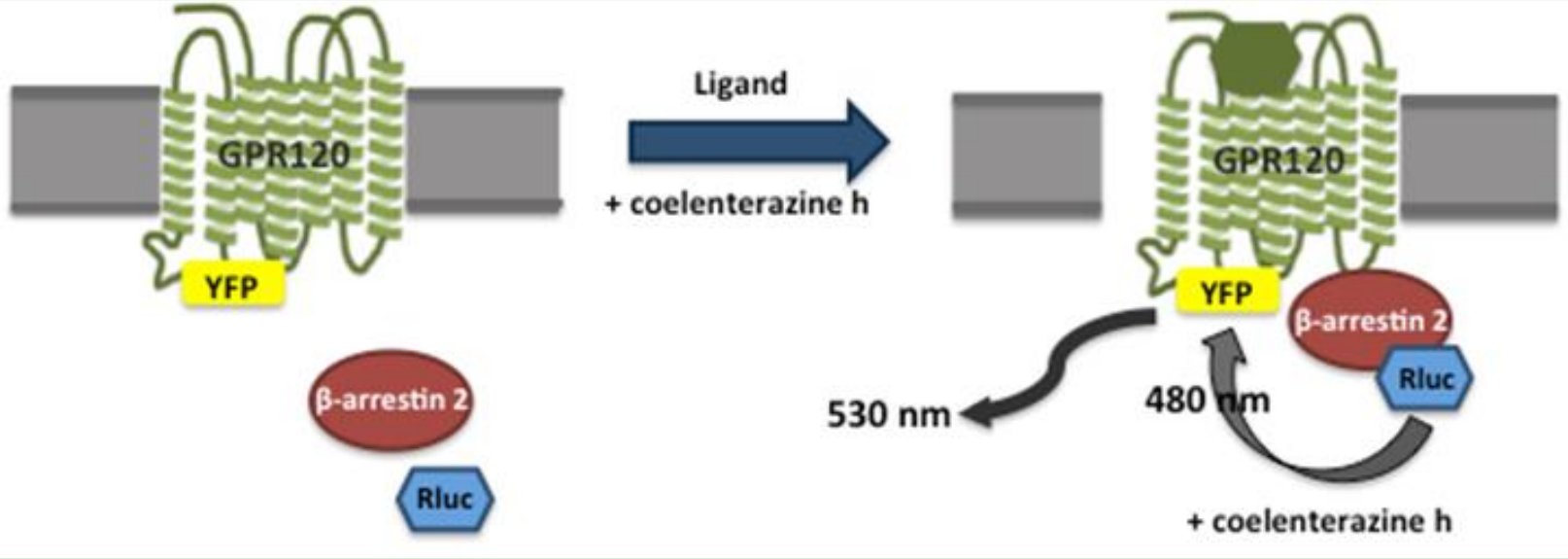
GPR120 (FFA4)

Post deorphanization of GPR120 a number of natural and synthetic ligands have been described, but further ligands with higher potency and selectivity would be valuable.



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GPR120 (FFA4)

Prof Milligan's group at the University of Glasgow developed a cell line constitutively expressing β-arrestin 2- *Renilla* luciferase and able to inducibly express GPR120-eYFP

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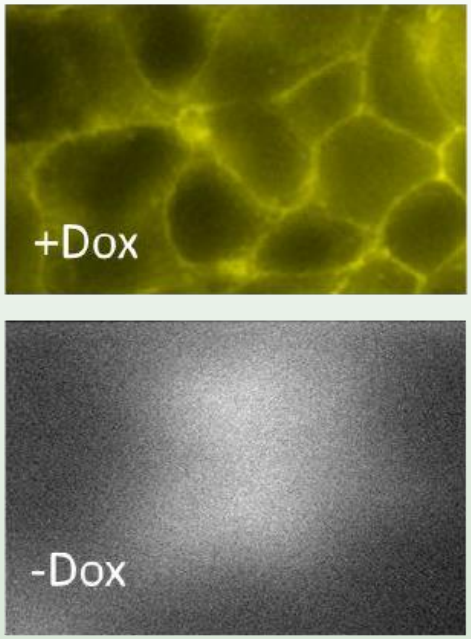

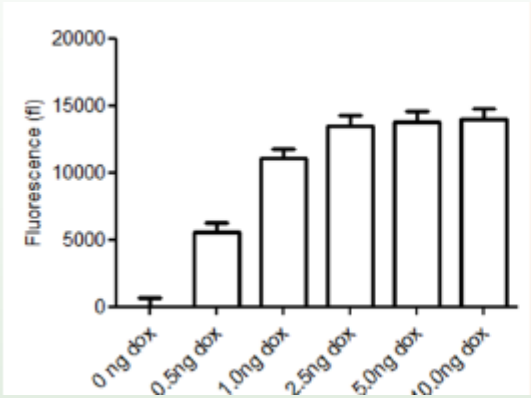

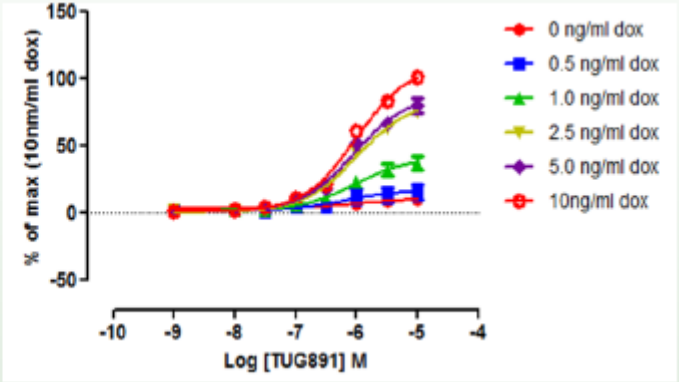

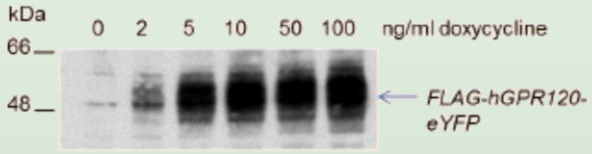

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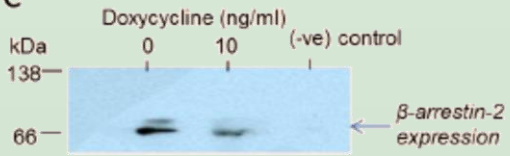

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BRET1 Screening using FDSS7000

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BRET1 Screening using FDSS7000

4880 cpds representative of the chemical diversity of the whole Innopharma library were screened.

FDSS7000 equipped with two 384 heads and two tip racks and automated filter exchanger for measuring BRET1 was employed.

- Flp-In™ T-REx™ 293 cells stably expressing β-arrestin 2 were seeded onto 384-well plates coated with poly-D-lysine and treated with 100 ng/ml doxycycline to induce FFA4 expression.
- After 24 h cells were washed twice with Hank´s balanced salt solution, pH 7.4.
- Test compounds or antagonist TUG1275 at a final concentration 10 µM were added and plate put into FDSS7000 and incubated for 5 min at 37 °C.
- Coelenterazine h was added by FDSS 7000 to a final concentration of 5 µM and cells were incubated for 10 min at 37 °C.
- 10 µM agonist (TUG891) was added by FDSS7000 and luminiscence detected for 10 min.

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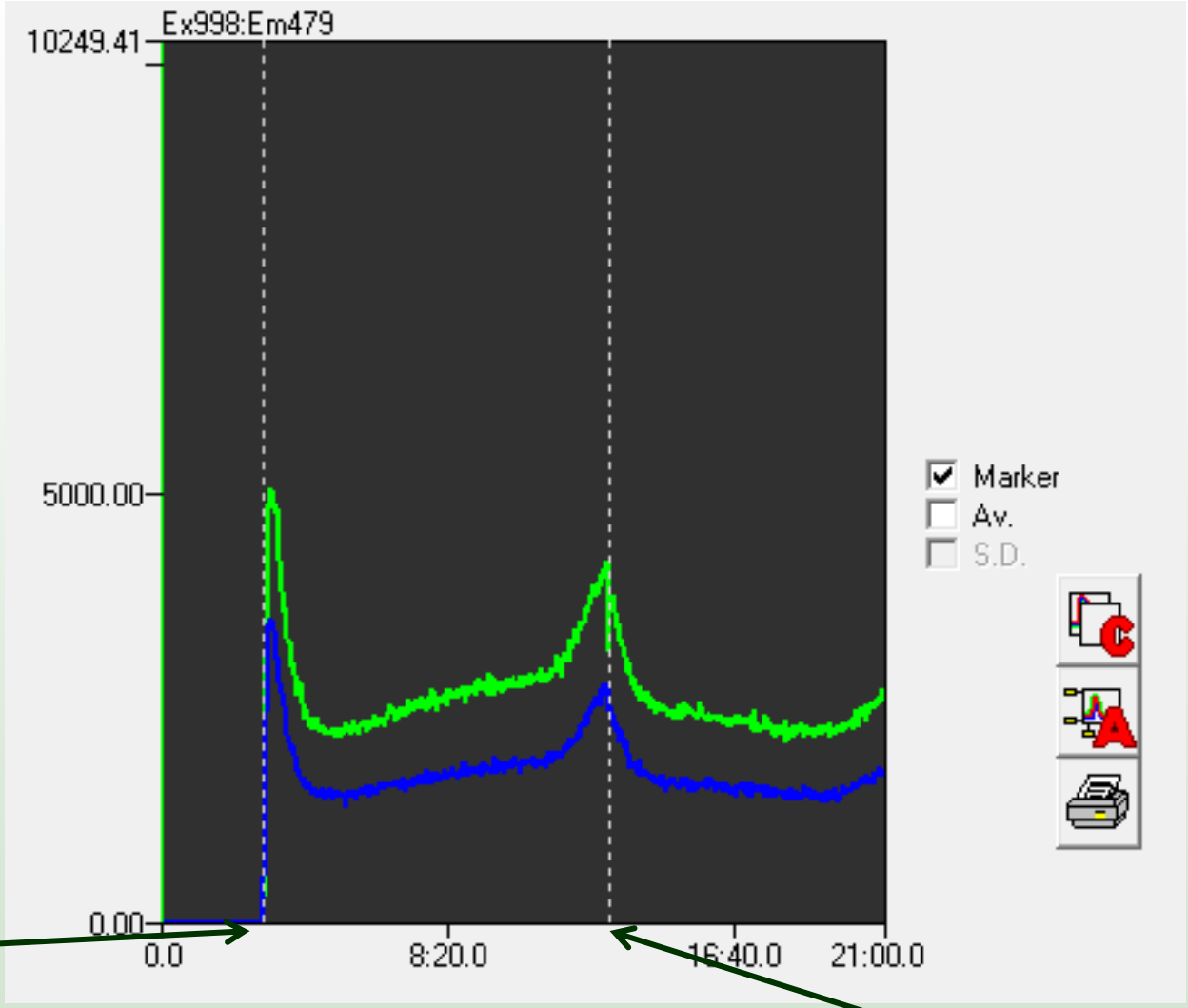
BRET1 Screening using FDSS7000



Coelenterazine addition

Agonist addition

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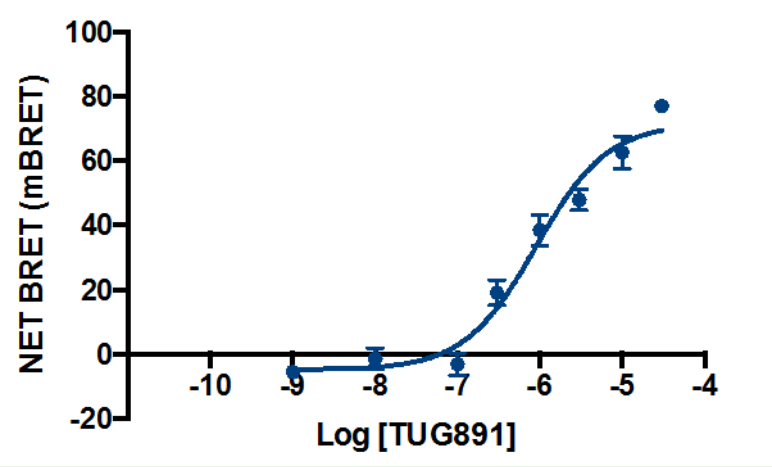
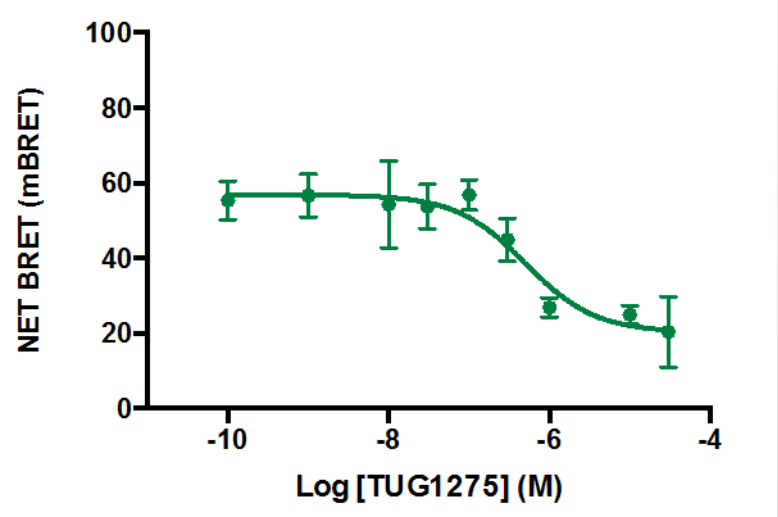
BRET1 Screening using FDSS7000





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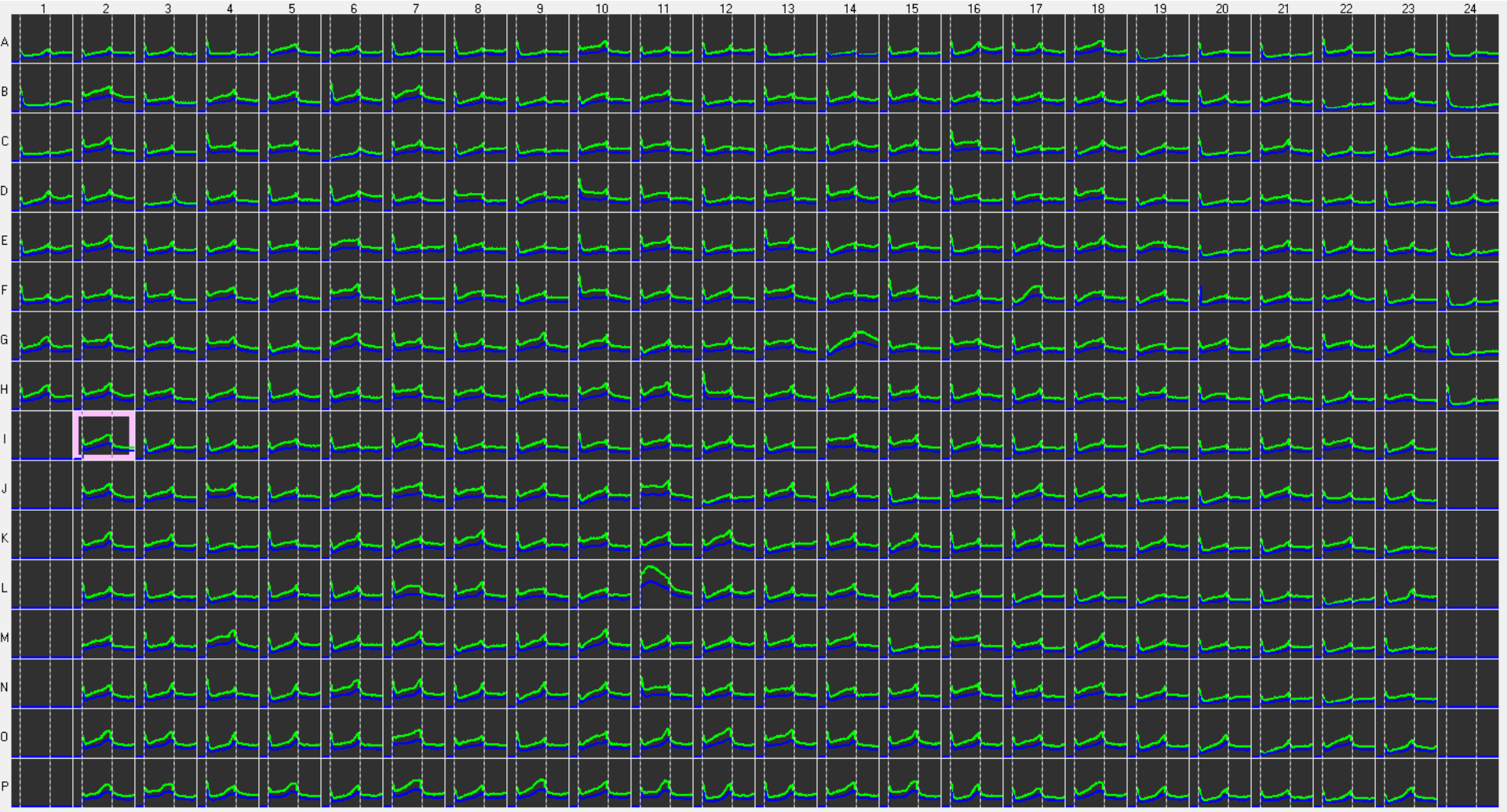
BRET1 Screening using FDSS7000



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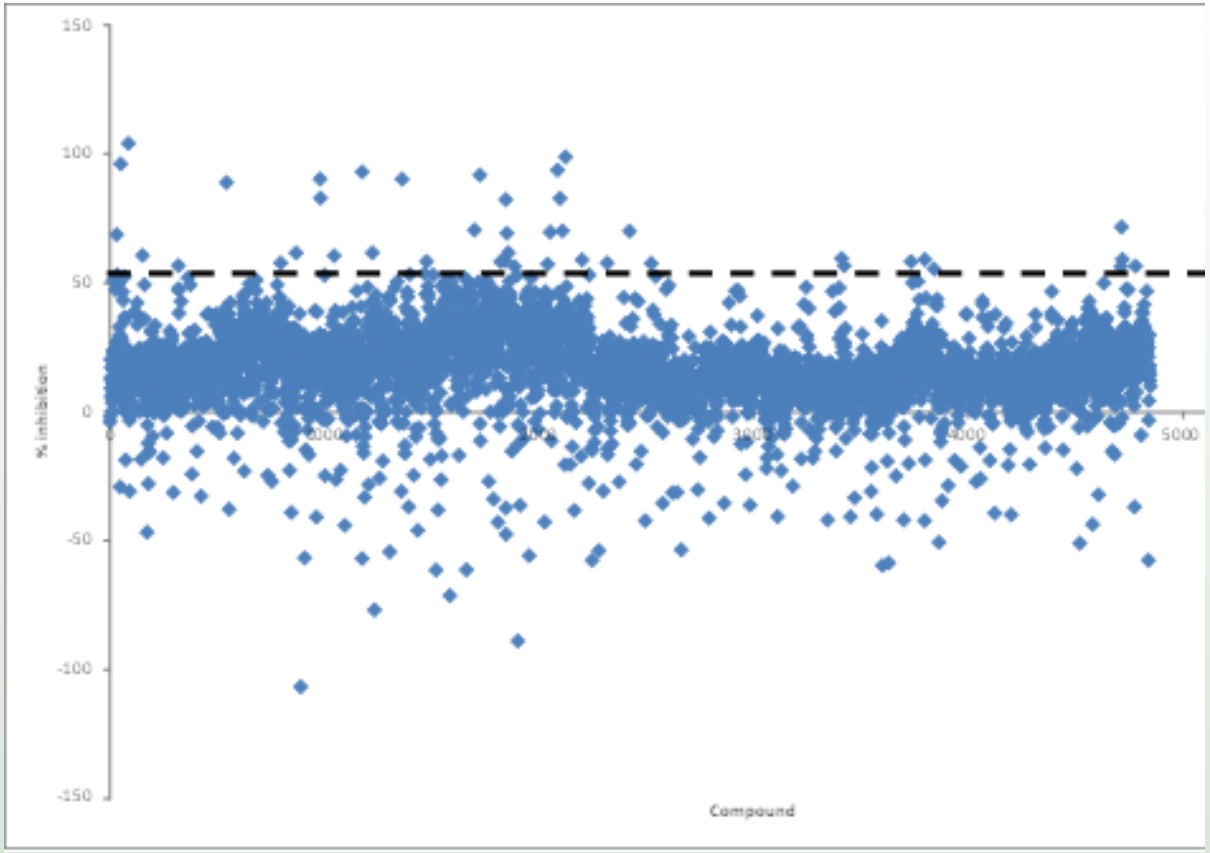
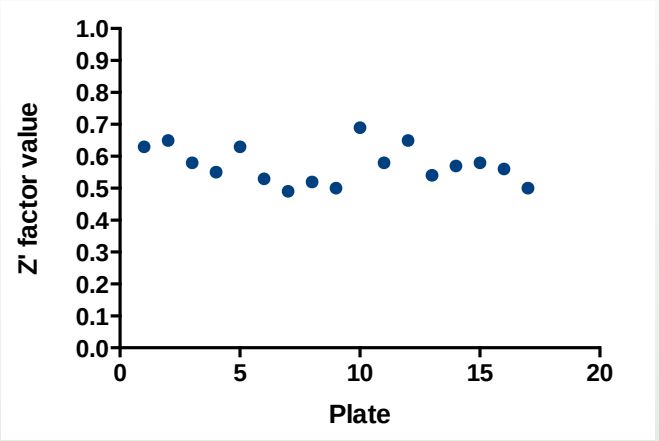
BRET1 Screening using FDSS7000





23 hits were confirmed in an independent assay (hit confirmation rate = 72%)

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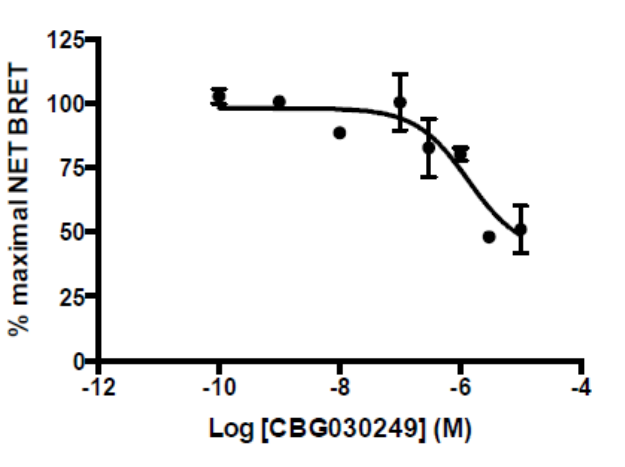
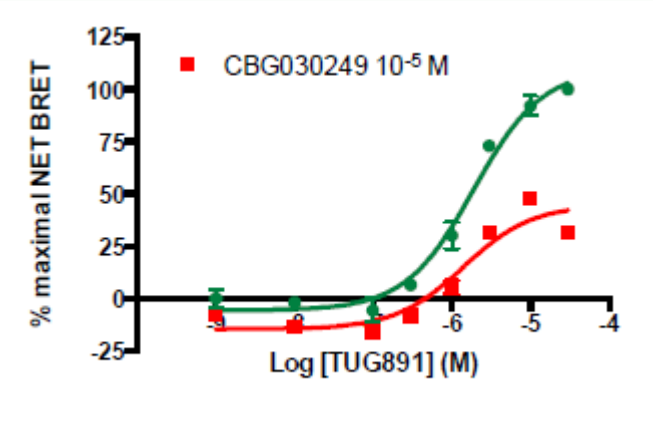
BRET1 Screening using FDSS7000





Concentration-response curves revealed a non-reversible antagonism for some compounds

Further assays are now running with the compounds included in the same cluster of those representative compounds

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Summary

- A BRET1 assay was developed by using a expressing β-arrestin2-Rluc and doxycline-induction of GPR120 .
- A subset of 4880 representative compounds from the Innopharma chemical library was screened by using this methodology.
- Z' values were higher than 0.5 in all the plates screened.
- 23 hits were identified (hit rate= 0.47%; hit confirmation rate=72%).
- Concentration-response curves revealed a non-reversible antagonism of the hits identified.

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Acknowledgements

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

UNIÓN EUROPEA
FONDO EUROPEO DE DESARROLLO REGIONAL
"Una manera de hacer Europa"

University of Glasgow

Prof Graeme Milligan

HAMAMATSU
PHOTON IS OUR BUSINESS

David Castrillo
Jean Marc Dangelo
Cyril Guerinot
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Fundación Pública Galega de Medicina Xenómica

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Funded by:

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