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Developing a high-throughput screening assay to support the drug discovery of γ-aminobutyric acid type A receptor (GABA$_A$R) Positive Allosteric Modulators, for the treatment of Post-Partum Depression.

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Introduction

- Postpartum depression (**PPD**) occurs in around **15%** of mothers.
- PPD can have serious short- and long-term consequences for mother and child, including **psychosis.**
- Current standards of care for PPD are traditional antidepressants and psychotherapy, often with poor outcome[1], while the recently approved drug Brexanolone (allopregnanolone) is effective but causes serious side-effects.
- There is an unmet need for new treatments.

γ-aminobutyric acid type A receptors (GABA$_A$Rs) are implicated in the pathophysiology of PPD

- δ-GABA$_A$Rs have been shown to be more sensitive to the hormonal changes during pregnancy, being downregulated to counteract the enhanced GABA$_A$R activity
- This increased activity may occur due to the increased production of allopregnanolone (an endogenous neurosteroid) during pregnancy.
- There is a rapid decrease in allopregnanolone after birth that is hypothesised to cause an allopregnanolone-withdrawal syndrome that manifests itself as the symptoms of PPD.
- Supported by the recent approval of Brexanolone, a proprietary formulation of allopregnanolone, which is a GABA$_A$R positive allosteric modulator (PAM).

Our hypothesis is that drugs which enhance the function of a specific subtype of GABA$_A$Rs, the extra-synaptic **δ subunit-containing GABA$_A$Rs (δ-GABA$_A$Rs),** will be efficacious in the treatment of PPD but **without the side-effects** that accompany Brexanolone.

To this end, the Medicines Discovery Institute has started the drug discovery project to identify such compounds. A key aspect of that work is to evaluate the functional consequences of such compounds on GABA$_A$Rs.

This work describes the development and characterisation of a fluorescence-based functional assay that will serve as a primary screen for novel compounds.

Aims & Objectives

- Develop a reproducible, reliable in house assay to determine the functional response of δ-GABA$_A$Rs
- Use this assay as a primary screen for novel compounds
- Adapt this assay for use in various GABA$_A$R-expressing cell lines

Materials & Methods

HEK293 *Resting state* — More positive charge outside of cell; Quench; Dye; GABA$_A$R; Cl-; More negative charge inside of cell

Higher extracellular positive charge — Dye is quenched outside the cytoplasmic membrane, in the more positive environment

HEK293 *Depolarized* — More negative charge outside of cell; GABA; Cl-; More positive charge inside of cell

GABA binds to the receptor. Chloride ions move out of cell. Dye enters cells to more positive environment, binds to cytosolic proteins and increases intracellular fluorescence
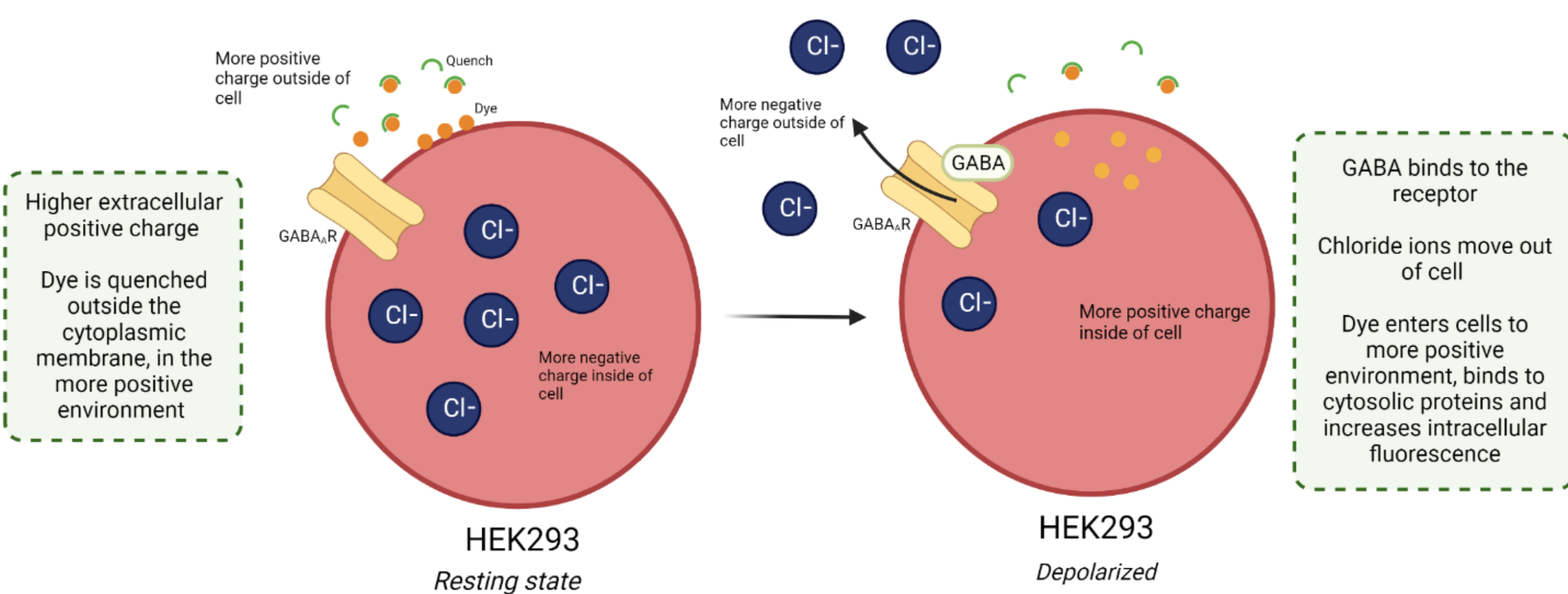
Figure 1. FLIPR Membrane Potential Fluorescence Technology. The fluorescence signal decreases or increases dependent on the polarized state of the membrane. The dye is a lipophilic, anionic, bis-oxonol dye that can partition across the cytoplasmic membrane of live cells. When the cell depolarizes, the dye follows the ions into the cell and binds to cytosolic proteins, increasing the signal intensity. During hyperpolarisation, the dye follows the ions out of the cell, and the signal decreases. These signal changes are detected using the FLIPR (Molecular Devices inc.)[2] Figure generated using BioRender.

Using Fluorescent Imaging Plate Reader (FLIPR) technology, a 384 well in-house assay was developed using Red Membrane Potential Explorer Dye (Molecular Devices Inc) to characterise various compounds against δ-GABA$_A$Rs overexpressing HEK293 cells (α4β3δ and α1β3γ cell lines).

A concentration response series of compounds were added directly to the cells and incubated, prior to addition of GABA at approximately EC$_{30}$, and changes in membrane potential, and therefore receptor activation were measured continuously by fluorescence changes within the FLIPR. This included 4-chloro-N-[2-(2-thienyl)imidazo[1,2-a]pyridine-3-yl benzamide (DS2), a known PAM of δ-GABAARs to validate our assay[3].

The fluorescence readings were normalised by subtraction of baseline fluorescence and negative wells and statistical analysis was conducted using a nonlinear regression model against log[compound], to calculate EC50 values (GraphPad Prism 9.3.1).

Results

The results from known PAMs of δ-GABA$_A$Rs, including DS2, produced data in line with the literature. The average EC50 value for DS2 in the α4β3δ cell line was 480nm and α1β3γ was 1.96uM.

These values were consistent week on week, which allowed us to confirm the repeatability and transferability of the FLIPR assay. FLIPR data generated is comparable to electrophysiological data, the current gold standard in membrane potential screening[4].
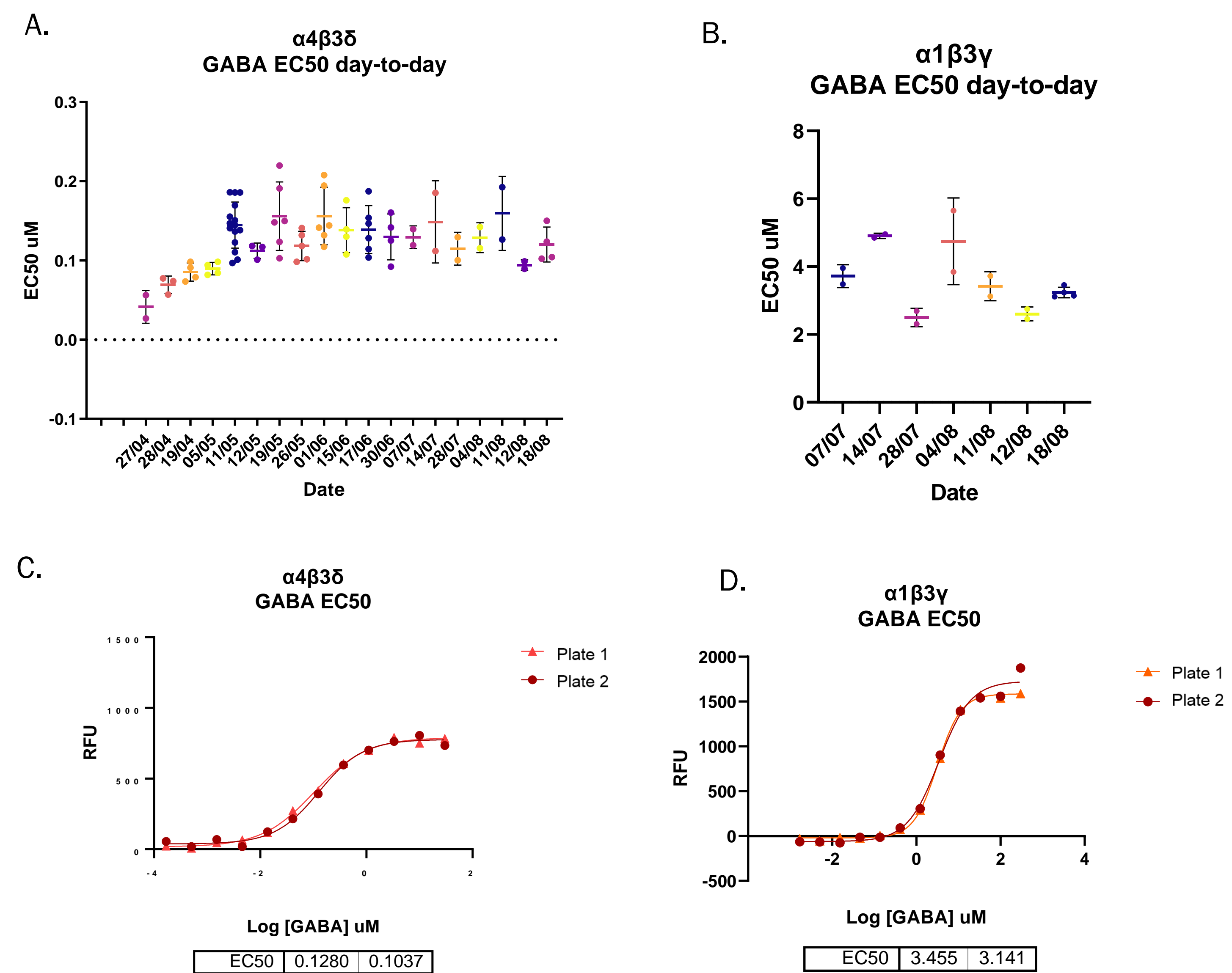

Figure 2. EC50 values across two different cell lines between assays. The α4β3δ cell line (a) had an average of 136nm, and the α1β3γ (b) had an average of 3.29uM. (c,d) The EC50 values were generated using a nonlinear regression model against log[GABA].
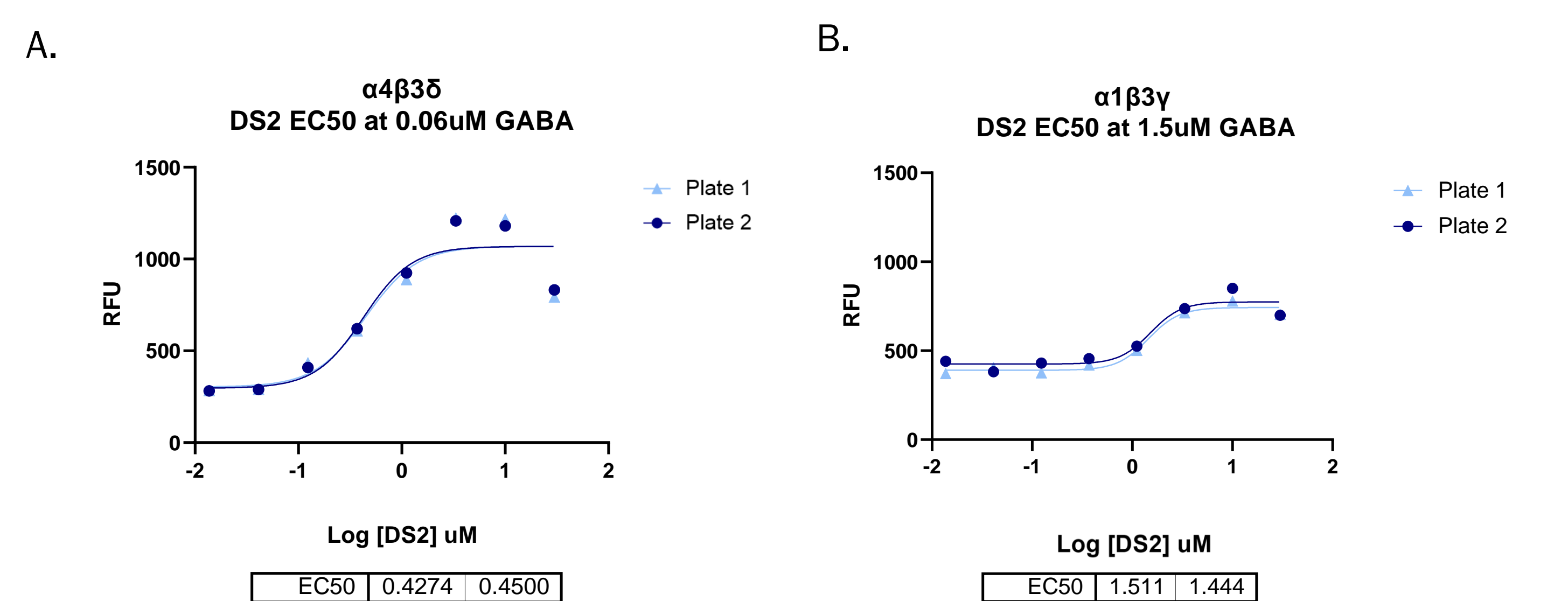

Figure 3. Nonlinear regression model of against log[DS2]. DS2 shows a higher activity in the α4β3δ cell line (a) when compared to the α1β3γ (b).

Conclusions

The results from these experiments support the concept of a δ-GABA$_A$Rs positive allosteric modulator, and this data can be used to generate more specific and efficient compounds to treat PPD. The data generated supports the validity of the FLIPR assay developed in house, which can therefore be used to screen compounds.

Various compounds of interest can therefore be screened through the FLIPR analysis and considered for optimisation and movement to the next stage of the drug discovery process. Alongside the EC50 values, a collective data set including functional affinity, aqueous solubility and cell permeability can be generated.

References

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