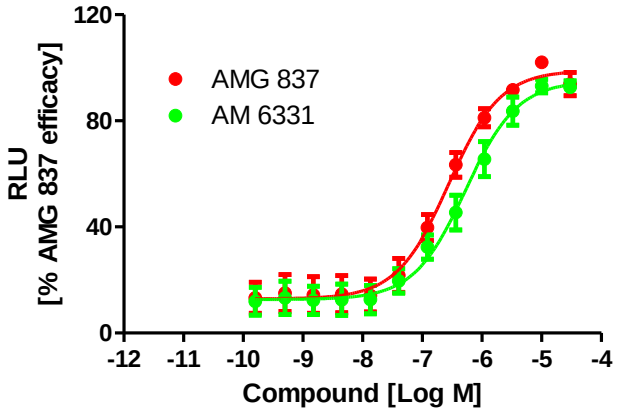
Identification and Pharmacological Characterization of Multiple Allosteric Binding Sites on the FFA1 Receptor

Daniel C.-H. Lin, Qi Guo, Jian Luo, Jane Zhang, Kathy Nguyen, Michael Chen, Thanh Tran, Paul J. Dransfield, Sean P. Brown, Jonathan Houze, Marc Vimolratana, Xian Yun Jiao, Yingcai Wang, Nigel J.M. Birdsall and Gayathri Swaminath

Molecular Pharmacology



Supplemental Fig. 1. Effect of AM 6331 and AMG 837 on FFA1 mediated calcium release in the aequorin assay. Data were combined from four independent experiments performed in duplicate and normalized to percent of AMG 837 $E_{max.}$ Details are as described in "Materials and Methods". The curves are best fits using a simple dose-response equation with a slope factor of 1. The log potency for AMG 837 is reported in Table 1.

Supplemental Figure 1

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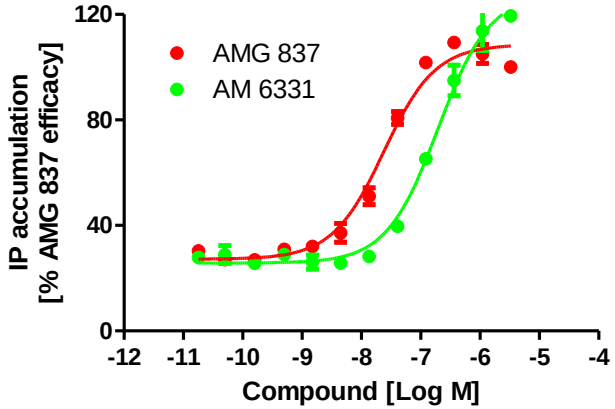

Supplemental Fig. 2. Stimulation of IP accumulation by the partial agonists AMG 837 and AM 6331 on A9 cells expressing the hFFA1 receptor. Data were combined from two or three independent experiments and normalized to percent of AMG 837 E_{max}. Details are as described in "Materials and Methods". The curves are best fits using a simple dose-response equation with a slope factor of 1. The log potency for AMG 837 is reported in Table 1.

Supplemental Figure 2

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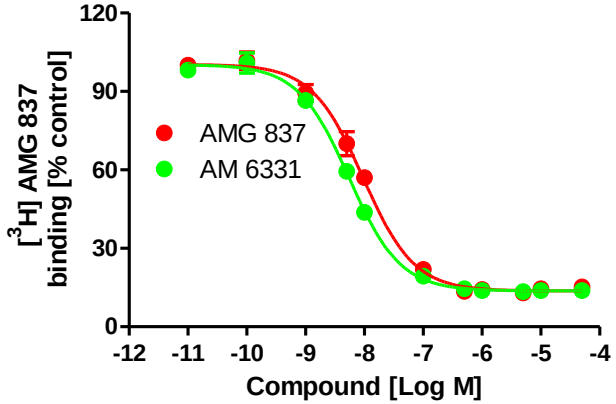

Supplemental Fig. 3. Equilibrium binding studies of the interaction of AM 6331 and AMG 837 with [^{3}H]-AMG 837 on A9 membranes expressing hFFA1 receptor. AM 6331 at high concentrations fully inhibits [^{3}H]-AMG 837 binding. The data are fitted using a single site equation and normalized to percentage of maximal binding. The data represent the mean of three or four independent experiments performed in duplicate. The log affinity of AM 6331 is reported in Table 2.

Supplemental Figure 3

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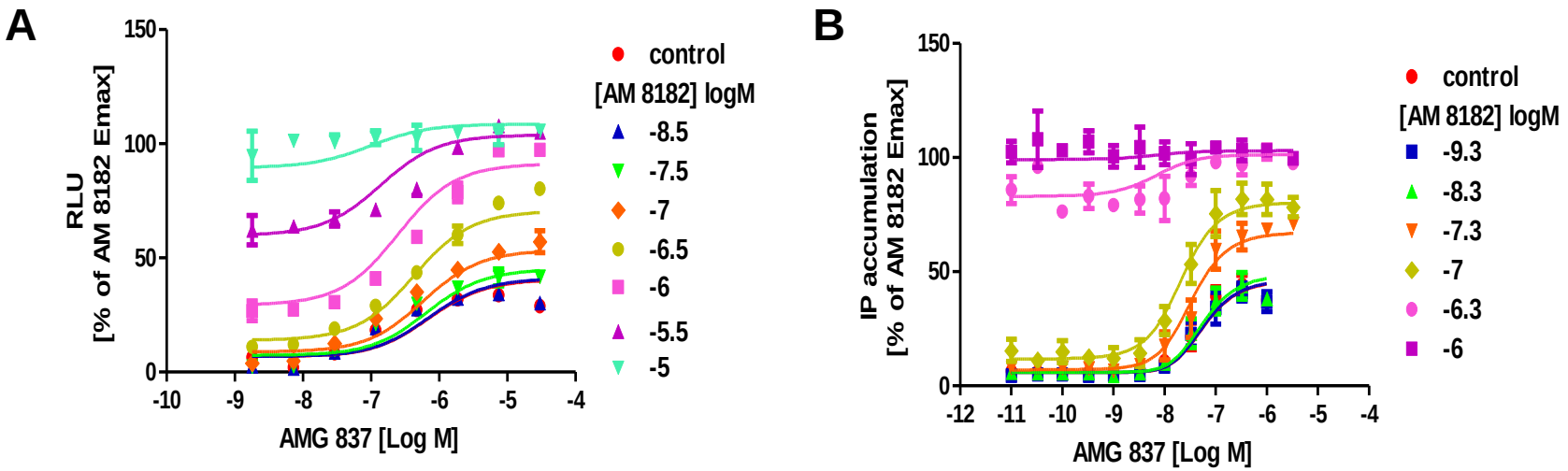

Supplemental Fig. 4. Cooperative interactions between AM 8182 and AMG 837 mediated by FFA1 receptor expressed in CHO cells. These interactions utilized a variation of the protocol used to investigate the allosteric interactions in functional assays. In the experiments described in this figure, the effects of fixed concentrations of AM 8182 on the dose-response curve for AMG 837 were measured. The aequorin assay and IP accumulation assays were used and the results are given in A and B respectively. In both assays the major qualitative effect is to clearly increase the E_{max} of the partial agonist AMG 837 with only small increases in potency – this is what would be expected if AM 8182 were increasing the efficacy of AMG 837. The data were analyzed quantitatively using a minor recasting of the equation used to analyze the data in Figs. 8-11 in order to reflect the slightly different protocol and the way the data are presented although formally the data in this figure could be recast in the format shown in Figs. 8A and B. The curves in A and B represent the best fit to the data using the modified equation for the Operational model of allosteric agonism. The values $\log\tau_B$ (partial agonism of AMG 837) and $\log(\alpha\beta)$ (functional cooperativity) are reported in Table 4. Data points represent the mean ± SEM. obtained from two independent experiments. Data are shown as percent of the response to the highest concentration of AM 8182.

Supplemental Figure 4

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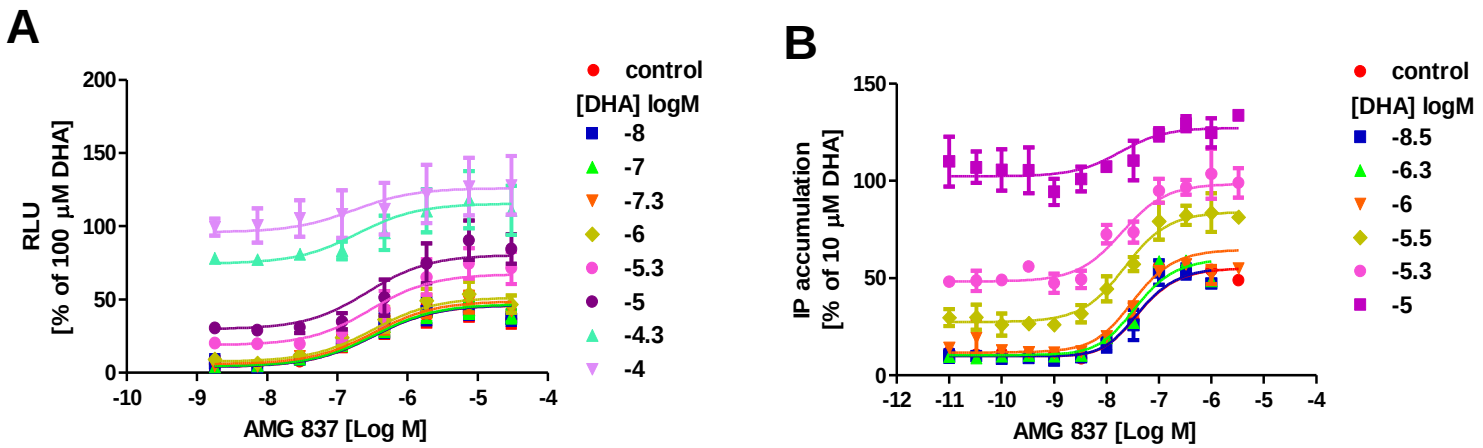

Supplemental Fig .5. Allosteric modulation of AMG 837 activity by DHA in (A) the aequorin assay and (B) the IP accumulation assay. The same protocol and methods of presenting and analyzing the data in Suppl Fig. 4 were used. In both assays the major qualitative effect is to clearly increase the E_{max} of the partial agonist AMG 837 with only small increases in potency, in agreement with DHA increasing the efficacy of AMG 837. The curves in A and B represent the best fit to the data using the modified equation for the Operational model of allosteric agonism. The values $\log\tau_B$ (partial agonism of AMG 837) and $\log(\alpha\beta)$ (functional cooperativity) are reported in Table 4. Data points represent the mean ± S.E.M. obtained from two independent experiments. Data is shown as percent of the response to DHA (10^{-4} M in A and 10^{-5} M in B).

Supplemental Figure 5