β-arrestin-2-Dependent Mechanism of GPR52 Signalling in Frontal Cortical Neurons

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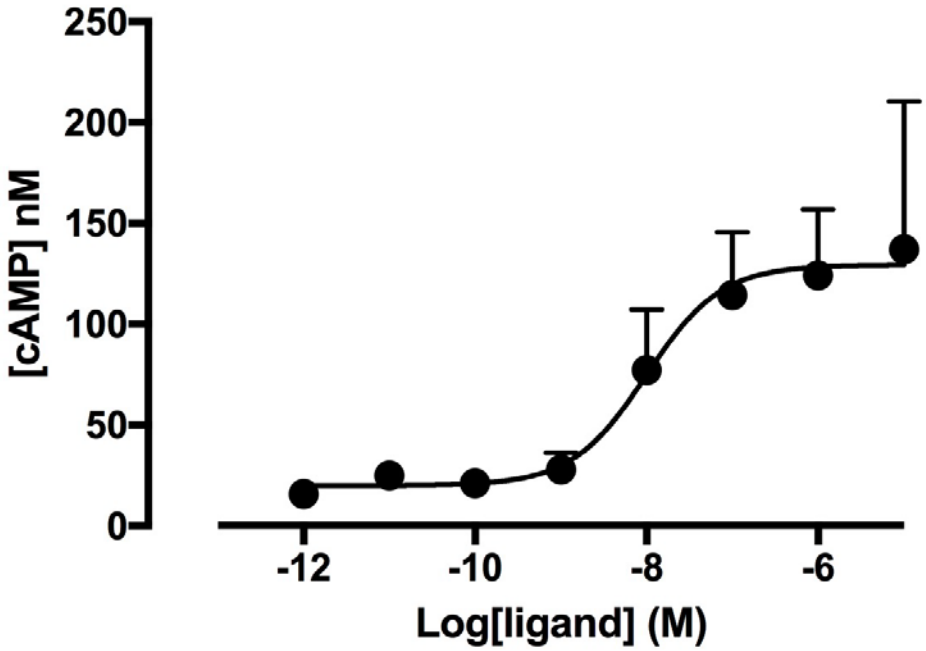
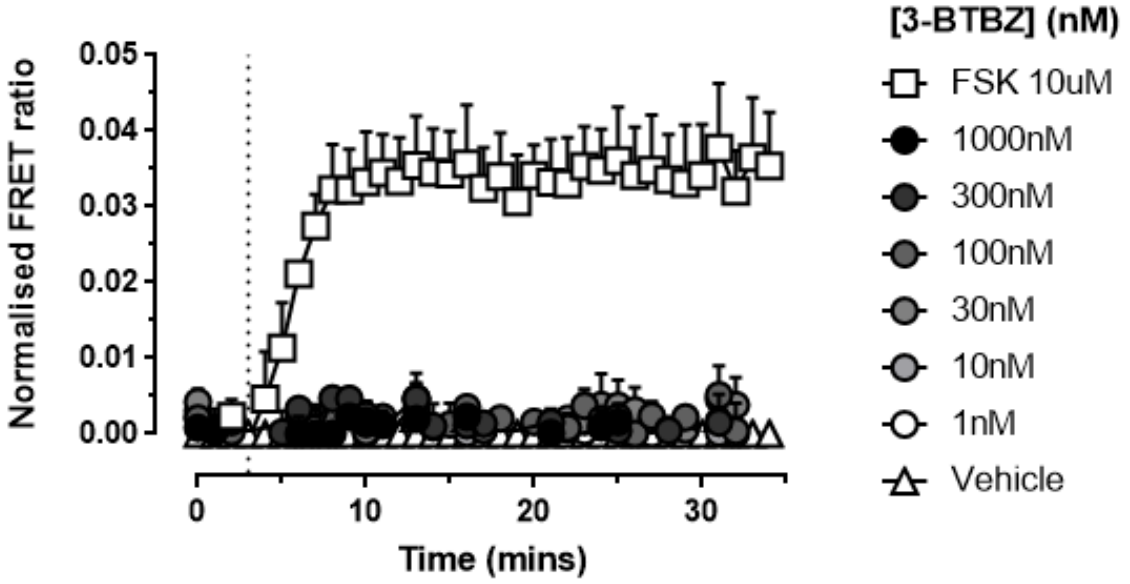
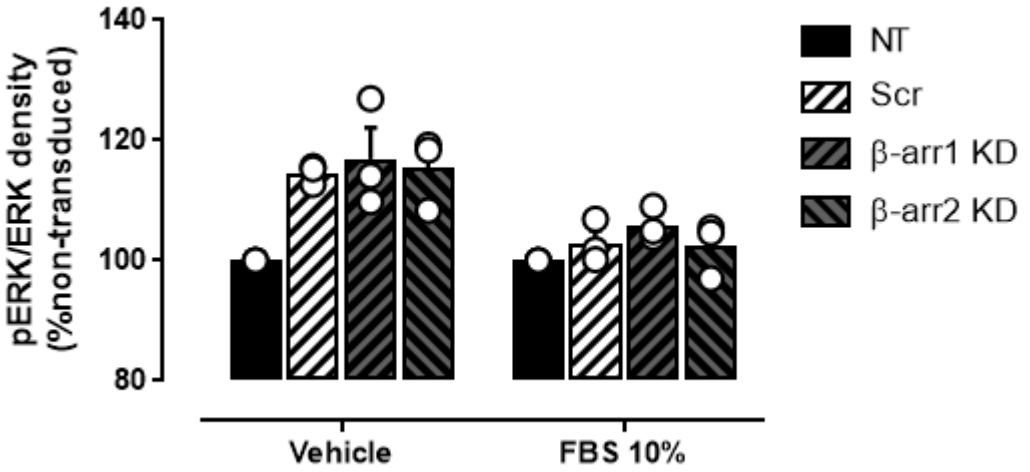


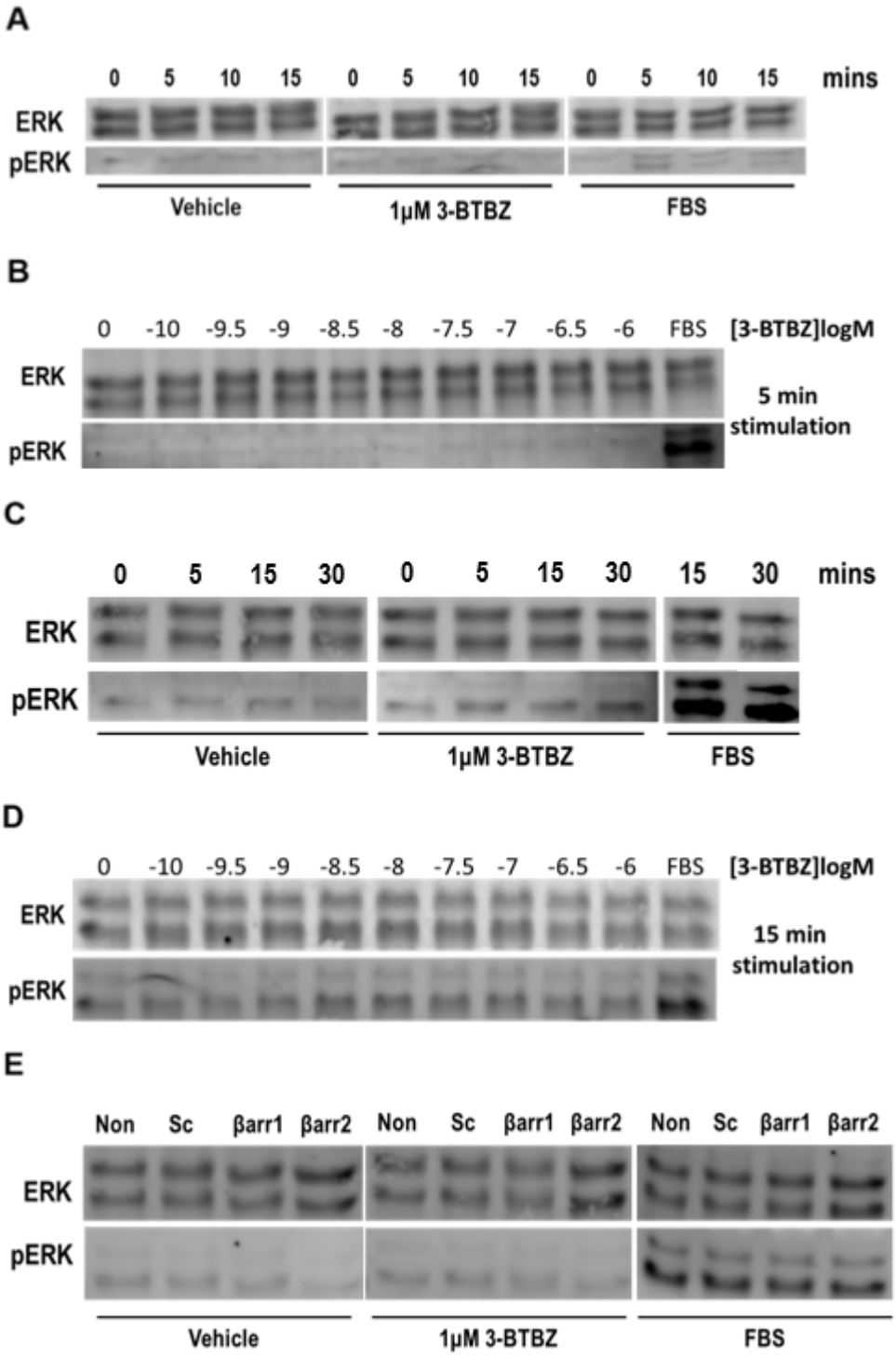
Supplementary Figure 1: 3-BTBZ-induced cAMP signalling in HEK293T cells transiently expressing hGPR52. Concentration-dependence of cAMP production induced by 3-BTBZ using the LANCE Ultra protocol (same protocol used in frontal cortical neurons). Data are presented as mean ± SEM (n=3).



Supplementary Figure 2: Lack of 3-BTBZ-induced cAMP-EPAC signalling in vector-transfected HEK293T cells expressing the EPAC FRET sensor. 3-BTBZ does not induce cAMP-EPAC signalling in the absence of transfected GPR52 in contrast to the positive control, forskolin. Data are presented as mean values ± SEM (n=3).



Supplementary Figure 3: Effect of viral knockdown of β-arrestin-1 and -2 on baseline and 10% FBS-induced ERK1/2 phosphorylation in primary frontal cortical neurons. There is no effect of viral knockdown of β-arrestin-1 and -2 on baseline- nor 10% FBS-induced ERK1/2 phosphorylation compared to scrambled ($p > 0.05$). Data are presented as normalised individual values representing the mean of triplicates (n=3). NT, non-transduced; Scr, scrambled; KD, knockdown.



Supplementary Figure 4: Representative western blots for ERK1/2 phosphorylation studies. (A) Time-course experiments in hGPR52Nluc-HEK293T cells. (B) Concentration-response experiments in hGPR52Nluc-HEK293T cells. (C) Time-course experiments in frontal cortical neurons. (D) Concentration-response experiments in frontal cortical neurons. (E) Knockdown experiments in frontal cortical neurons. Bands were quantified by densitometry and the pERK/ERK ratio within the same lane was used to quantify ERK1/2 phosphorylation. Non, non-transduced; Sc, scrambled; βarr1, β-arrestin-1 knockdown; βarr2, β-arrestin-2 knockdown.