Supporting information

In vitro characterisation of 6-methyl-3-(2-nitro-1-(thiophen-2-yl)ethyl)-2-phenyl-1*H*-indole (ZCZ011) at the type 1 cannabinoid receptor: allosteric agonist or allosteric modulator?

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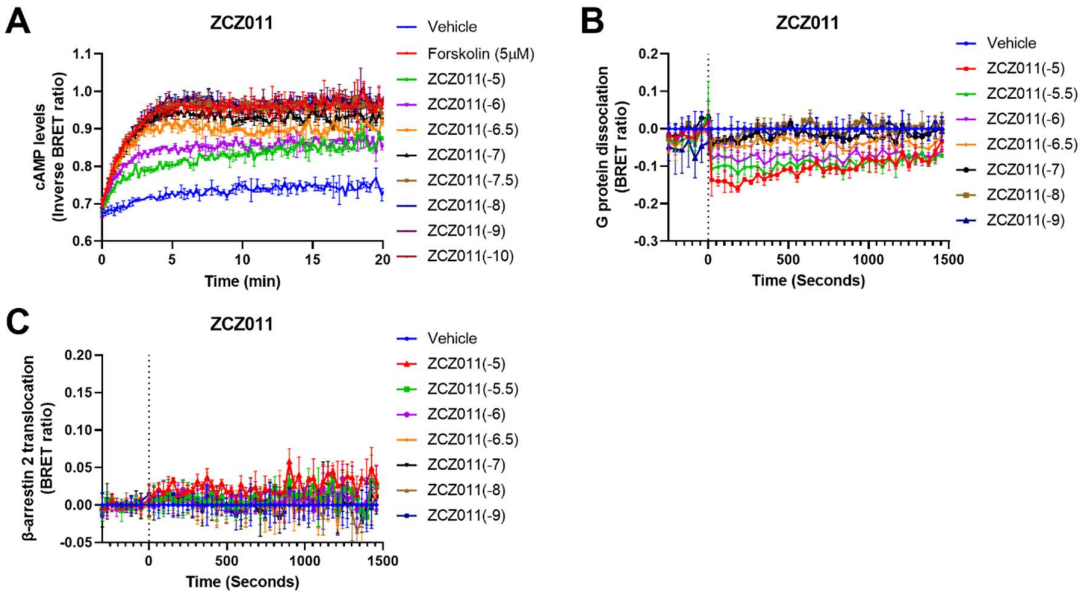

Figure S1: Allosteric agonism by ZCZ011 in HEK293 cells. *(A) inhibition of cAMP stimulated by 5 μM forskolin and a concentration series of ZCZ011, (B) dissociation of $G_{\alpha i3}$ and $G_{\beta\gamma}$ subunits as determined by TRUPATH BRET assay stimulated by ZCZ011, and (C) translocation of β-arrestin-2 by ZCZ011. Data is a representative biological replicate (n=5) and is expressed as mean ± SD of technical duplicates/triplicates.*

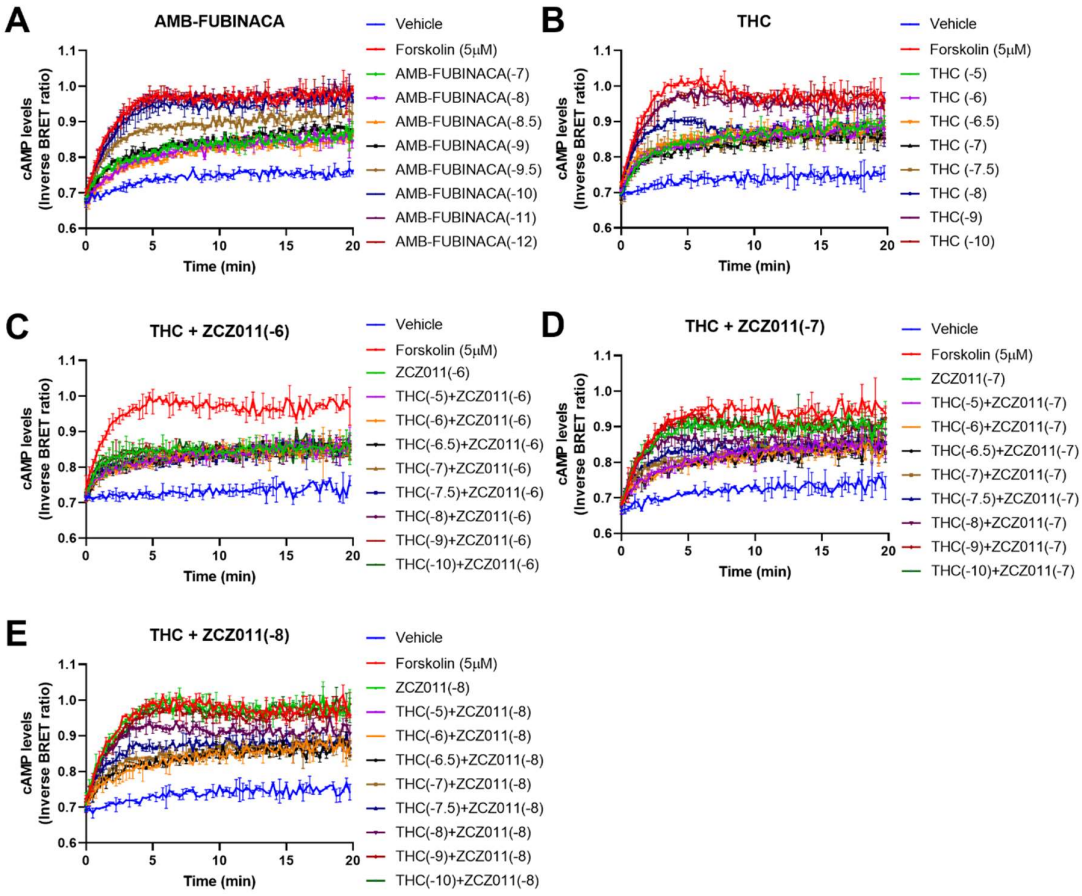

Figure S2: cAMP inhibition of orthosteric agonists alone and in combination with ZCZ011 in HEK293-hCB$_1$ cells*. Real time kinetic traces showing inhibition of cAMP stimulated by 5 μM forskolin and AMB-FUBINACA (A) or THC (B) in the absence of ZCZ011, and THC in the presence of 1 μM (C), 100 nM (D), or 10 nM (E) ZCZ011. Data is a representative biological replicate (n=5) and is expressed as mean ± SD of technical duplicates.*

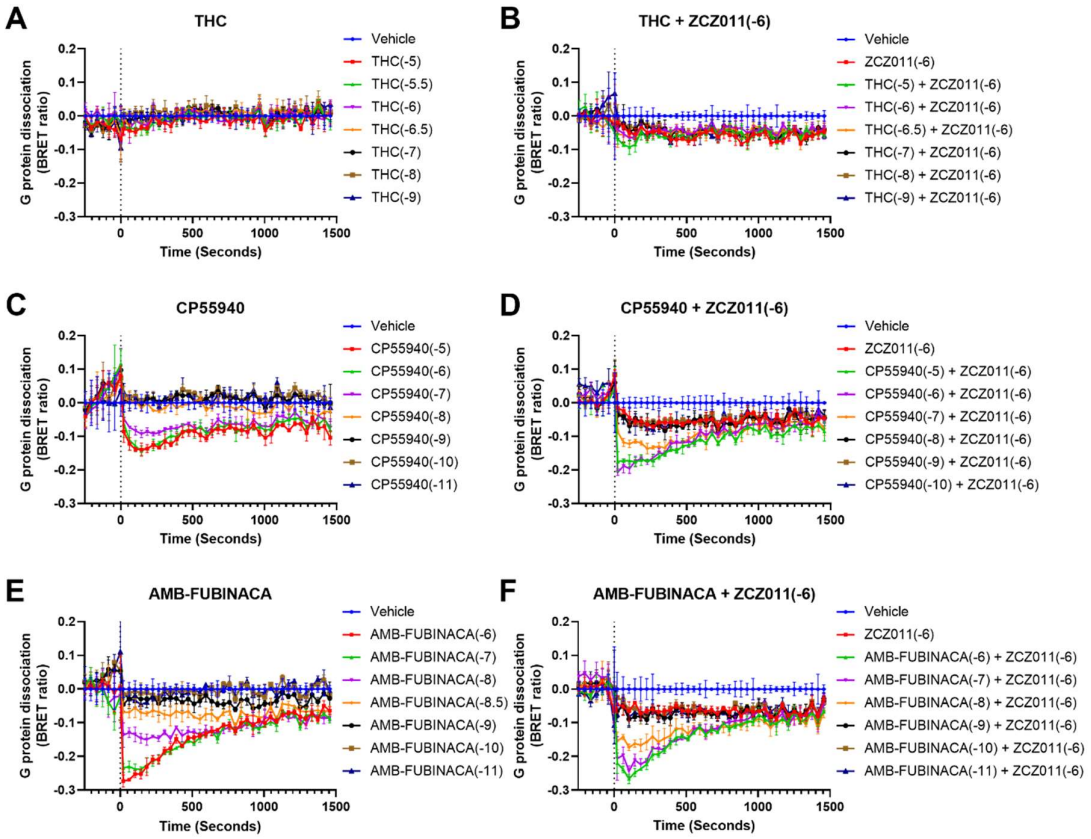

Figure S3: G protein dissociation of orthosteric agonists alone and in combination with ZCZ011 in HEK293-hCB$_1$ cells. *Dissociation of $G_{\alpha i3}$ and $G_{\beta\gamma}$ subunits as determined by TRUPATH BRET assay stimulated with THC, CP55940, and AMB-FUBINACA alone (A, C, E) and in the presence of 1 μM ZCZ011 (B, D, F). Data is a representative biological replicate (n=5) and is expressed as mean ± SD of technical triplicates.*

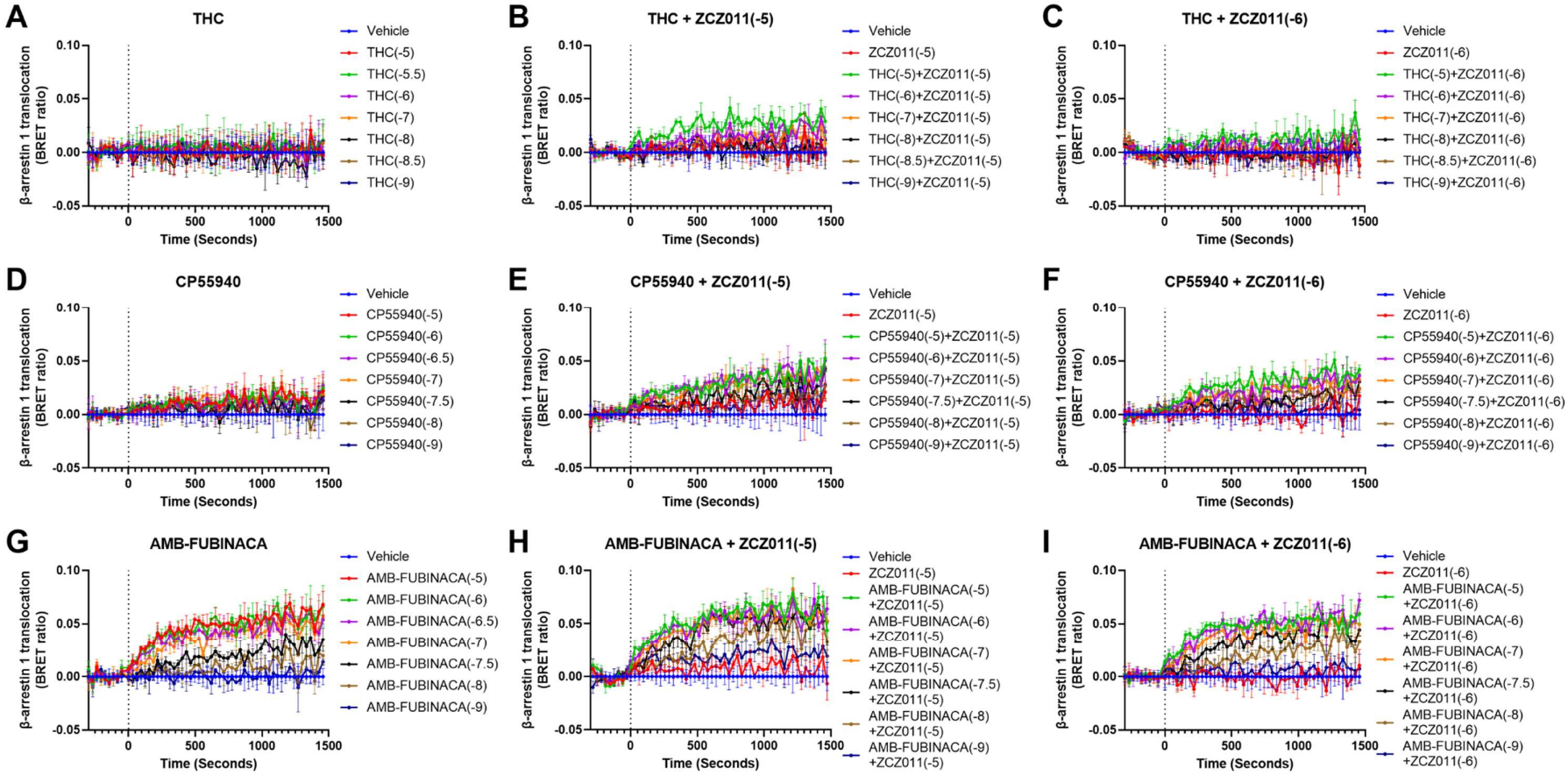

Figure S4: β-arrestin 1 translocation of orthosteric agonists alone and in combination with ZCZ011 in HEK293 cells. *Real-time kinetic traces showing translocation of β-arrestin 1 stimulated by THC, CP55940, and AMB-FUBINACA alone (A, D, G) and in combination with 10 μM (B, E, H) or 1 μM ZCZ011 (C, F, I). Data is a representative biological replicate (n=5) and is expressed as mean ± SD of technical triplicates.*

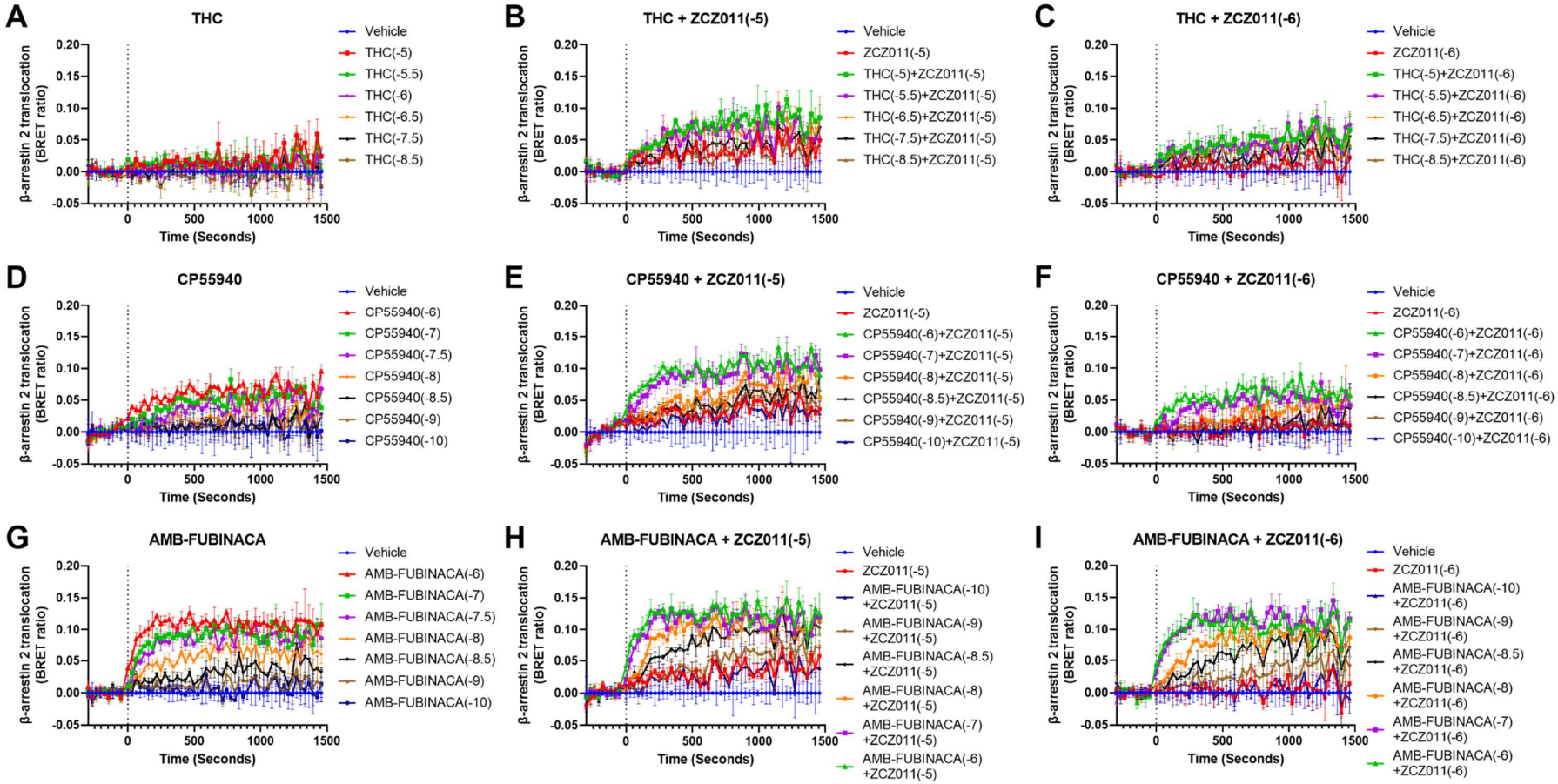

Figure S5: β-arrestin 2 translocation of orthosteric agonists alone and in combination with ZCZ011 in HEK293 cells. *Real-time kinetic traces showing translocation of β-arrestin 2 stimulated by THC, CP55940, and AMB-FUBINACA alone (A, D, G) and in combination with 10 μM (B, E, H) or 1 μM ZCZ011 (C, F, I). Data is a representative biological replicate (n=5) and is expressed as mean ± SD of technical triplicates.*