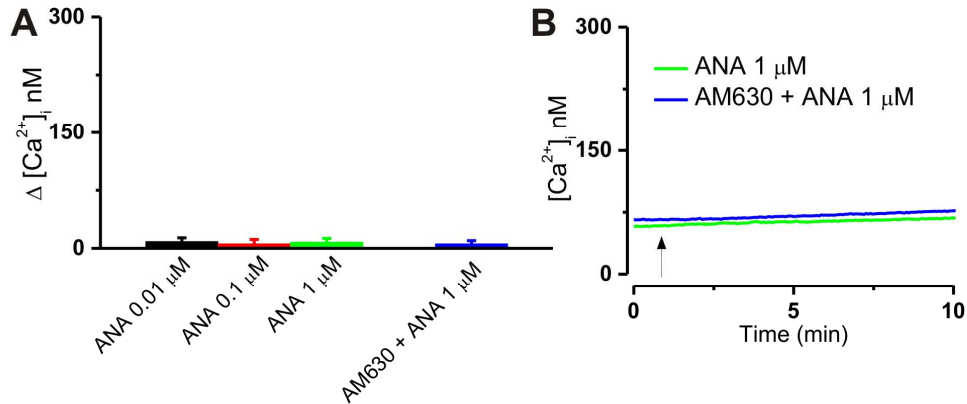


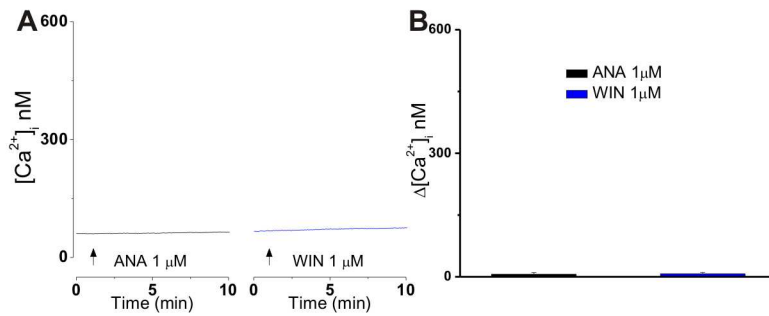
SUPPLEMENTAL DATA

Extracellular administration of anandamide had no effect on $[Ca^{2+}]_i$ of CB_2 -U2OS cells; 0.01, 0.1 and 1 μ M anandamide administered by bath application modified $[Ca^{2+}]_i$ by 9 ± 4.7 nM, $n = 29$ cells; 6 ± 5.5 nM, $n = 41$ and 8 ± 4.9 nM, $n = 37$ respectively; in the presence of 1 μ M AM630, the effect measured 6 ± 3.9 nM, $n = 32$ cells (Supp. Fig. 1)



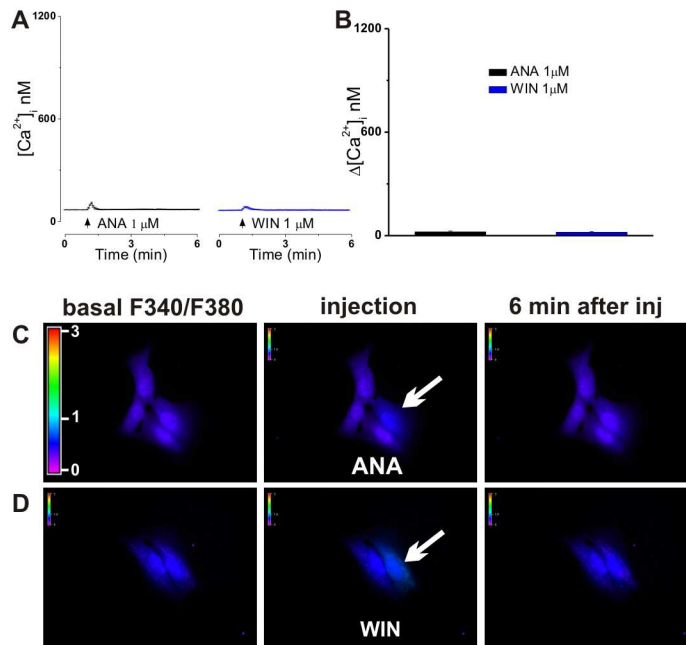
Supplemental Figure 1. CB_2 -transfected U2OS cells do not increase $[Ca^{2+}]_i$ in response to extracellular application of anandamide. **A**, Comparison of the amplitudes of the Ca^{2+} responses produced by extracellular administration of anandamide (ANA, 0.01-1 μ M) or of 1 μ M anandamide in presence of 1 μ M AM630 (CB_2 antagonist) in CB_2 -U2OS cells. **B**, Representative tracings depicting the effect on $[Ca^{2+}]_i$ of 1 μ M anandamide applied by bath in absence or presence of 1 μ M AM630 to CB_2 -expressing U2OS cells.

Extracellular administration of 1 μM anandamide or 1 μM WIN55,212-2 produced small and nonsignificant increases in $[\text{Ca}^{2+}]_i$ in control untransfected U2OS cells, by 6 ± 4.1 nM and 7 ± 3.8 nM, respectively; in each case, $n = 6$ cells (Supp. Fig. 2).



Supplemental Figure 2. Control untransfected U2OS cells do not respond to extracellular administration of anandamide or WIN55,212-2. **A**, Characteristic tracings illustrating nonsignificant Ca^{2+} responses of untransfected U2OS cells to bath-applied anandamide (ANA, 1 μM , left) or WIN55,212-2 (WIN, 1 μM , right). **B**, Comparison of the effects on $[\text{Ca}^{2+}]_i$ produced by extracellular application of anandamide or WIN55,212-2.

Intracellular microinjection of 1 μM anandamide or 1 μM WIN55,212-2 produced small and nonsignificant increases in $[\text{Ca}^{2+}]_i$ in control untransfected U2OS cells, by 21 ± 6 nM ($n = 6$ cells injected) and 19 ± 4 nM ($n = 6$), respectively (Supp. Fig. 3).



Supplemental Figure 3. Control untransfected U2OS cells are not responsive to intracellular administration of anandamide or WIN55,212-2. **A**, Averaged Ca^{2+} responses of untransfected U2OS cells to microinjected anandamide (ANA, 1 μM , left) or WIN55,212-2 (WIN, 1 μM , right). **B**, Comparison of the effects on $[\text{Ca}^{2+}]_i$ produced by intracellular administration of anandamide or WIN55,212-2. **C,D**, Characteristic fluorescence images of Fura-2 AM-loaded CB_2 -U2OS cells before (left), during (middle) and 6 min after (right) intracellular administration of 1 μM anandamide (**C**) or of 1 μM WIN55,212-2 (**D**); arrows indicate the injected cells; the fluorescence scale (0-3) is illustrated in each panel and magnified in the left panel of **C**.