









Supplementary Fig. 4

### Supplementary Table 1

Concentration-dependence and kinetics of GFP-labeled biosensor translocations in response to MCh in SH-SY5Y cells.

	<b>eGFP-PH</b>	<b>GFP-Tubby</b>	<b>eGFP-C1(2)</b>
<b>pEC<sub>50</sub> (M)</b>	5.19 ± 0.11 (9)	4.53 ± 0.21 (5)*	4.89 ± 0.23 (6)
<b>t<sub>10-90</sub> (sec)</b>	39.2 ± 2.6 (35)	58.8 ± 3.0 (30)***	30.8 ± 1.7 (48)††† ‡

Mean pEC<sub>50</sub> (negative log of the agonist concentration required to produce 50% of the maximal response) and t<sub>10-90</sub> (time ( in sec) between 10 and 90% of peak response to MCh (100 μM)) estimates for eGFP-PH, GFP-Tubby and eGFP-C1(2) translocation in SH-SY5Y cells in response to MCh. Differences between pEC<sub>50</sub> or t<sub>10-90</sub> estimates were determined by one-way ANOVA and Bonferroni's *post-hoc* test, with \* denoting differences between eGFP-PH and GFP-Tubby (\**P*<0.05; \*\*\**P*<0.001), † denoting differences between eGFP-C1(2) and GFP-Tubby (†††*P*<0.001) and ‡ denoting differences between eGFP-C1(2) and eGFP-PH (‡*P*<0.05).

## Supplementary Table 2

eGFP-PH and GFP-Tubby translocation in SH-SY5Y cells in response to MCh (1 mM), in the absence (control) and presence of wortmannin (Wort; 1 or 10  $\mu$ M) or LY294002 (LY; 100  $\mu$ M).

	control	+ 1 $\mu$ M Wort	control	+ 10 $\mu$ M Wort	control	+ 100 $\mu$ M LY
<b>eGFP-PH</b>						
peak	3.38 $\pm$ 0.43 (8)	3.60 $\pm$ 0.40 (8)	3.77 $\pm$ 0.25 (14)	2.97 $\pm$ 0.17 (14)*	3.69 $\pm$ 0.33 (6)	2.46 $\pm$ 0.24 (6)*
plateau	-1.2 $\pm$ 4.9 (8)	4.7 $\pm$ 1.6 (8)	0.9 $\pm$ 2.6 (15)	43.5 $\pm$ 6.4 (15)**	0.4 $\pm$ 3.1 (6)	32.9 $\pm$ 6.7 (6)**
<b>GFP-Tubby</b>						
peak	2.33 $\pm$ 0.27 (9)	2.69 $\pm$ 0.32 (9)	2.97 $\pm$ 0.58 (9)	4.93 $\pm$ 0.67 (9)*	1.98 $\pm$ 0.29 (7)	2.33 $\pm$ 0.22 (7)
plateau	-2.1 $\pm$ 2.8 (8)	-6.0 $\pm$ 3.4 (8)	2.4 $\pm$ 4.9 (9)	78.9 $\pm$ 6.3 (10)***	1.3 $\pm$ 1.9 (7)	62.0 $\pm$ 4.2 (7)***

Data are expressed as means  $\pm$  s.e.m. (number of cells indicated in parentheses) for fold changes in cytosolic fluorescence over basal ( $F/F_0$ ) (for “peak” values) or percent of peak responses remaining 240 sec after peak (for “plateau” values). Differences between control and inhibitor-treated cells were determined by Student’s *t*-test (\* $P$ <0.05; \*\* $P$ <0.01; \*\*\* $P$ <0.001).

### Supplementary Table 3

eGFP-PH and GFP-Tubby translocation in cultured neonatal rat hippocampal neurons in response to MCh (1 mM), in the absence (control) and presence of wortmannin (Wort; 1 or 10  $\mu$ M).

	Control	+ 1 $\mu$ M Wort	+ 10 $\mu$ M Wort
<b>eGFP-PH</b>			
peak	3.33 $\pm$ 0.51 (26)	2.69 $\pm$ 0.60 (12)	2.78 $\pm$ 0.52 (11)
plateau	5.3 $\pm$ 2.8 (26)	5.4 $\pm$ 3.9 (12)	6.2 $\pm$ 3.2 (11)
<b>GFP-Tubby</b>			
peak	1.47 $\pm$ 0.08 (23)	1.29 $\pm$ 0.05 (7)	1.54 $\pm$ 0.21 (11)
plateau	1.4 $\pm$ 4.5 (23)	4.7 $\pm$ 3.6 (6)	35.8 $\pm$ 7.7 (11)***

Data are expressed as means  $\pm$  s.e.m. (number of cells indicated in parentheses) for fold changes in cytosolic fluorescence over basal ( $F/F_0$ ) (for “peak” values) or percent of peak responses remaining 240 sec after peak (for “plateau” values). Differences between control and inhibitor-treated cells were determined by one-way ANOVA and Bonferroni’s *post-hoc* test (\*\*\*) ( $P < 0.001$ ).