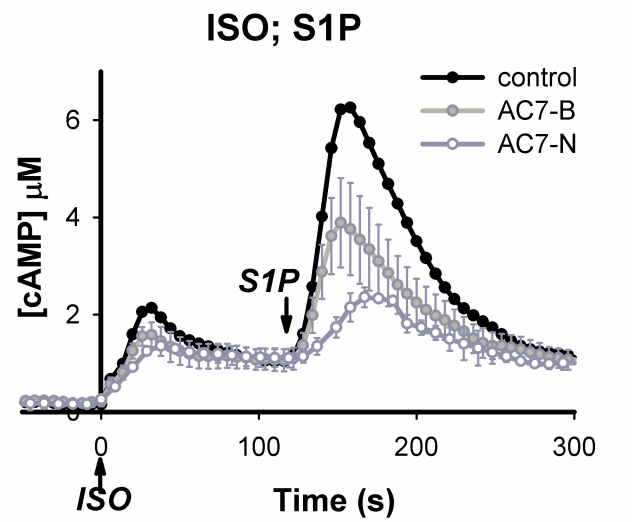
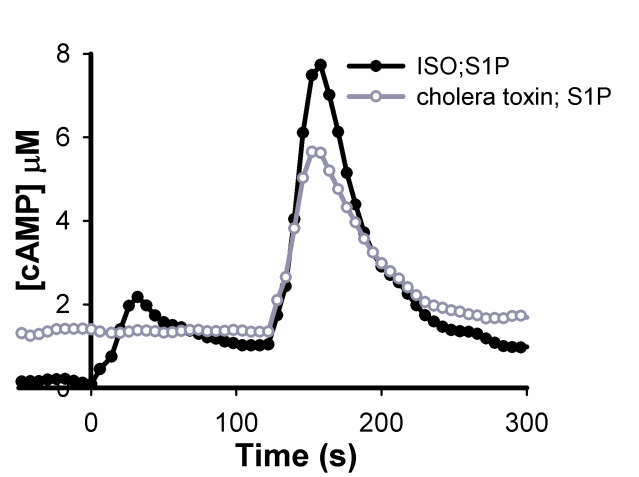
SUPPLEMENTARY FIGURES

Supplementary Figure S1.



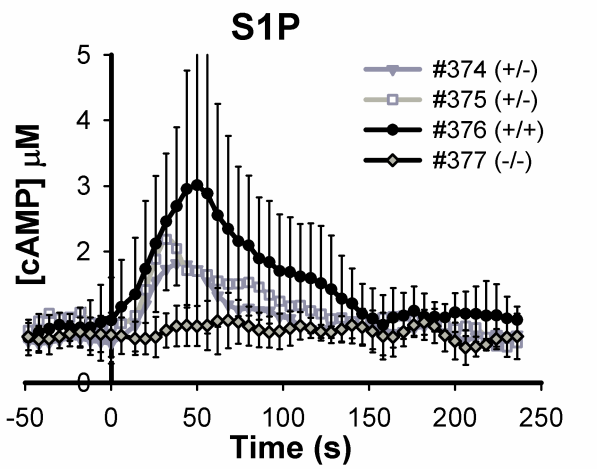
Supplementary Figure S1. Knockdown of AC7 with alternative RNAi sequences produced the same phenotype. Two alternative RNAi sequences were used to knockdown AC7 in RAW cells. AC7-B (5'-TCT CTA TGT GCT GGT GTA T-3') was delivered as shRNA using retrovirus and a stable cell line was established. AC7-N (5'- GAA CTG CGG ACT TAC TTT G-3') was transiently transfected into RAW cells as siRNAs. Knockdown of AC7 mRNA, as determined by qRT-PCR, was 56% and 63% respectively. Cells were stimulated with 16 nM ISO at time 0, followed by addition of 10 nM S1P at 120 seconds as indicated by arrows. Intracellular cAMP was measured over time by BRET using the CAMYEL sensor. Error bars represent the standard deviation of results from 3 independent experiments.

Supplementary Figure S2.



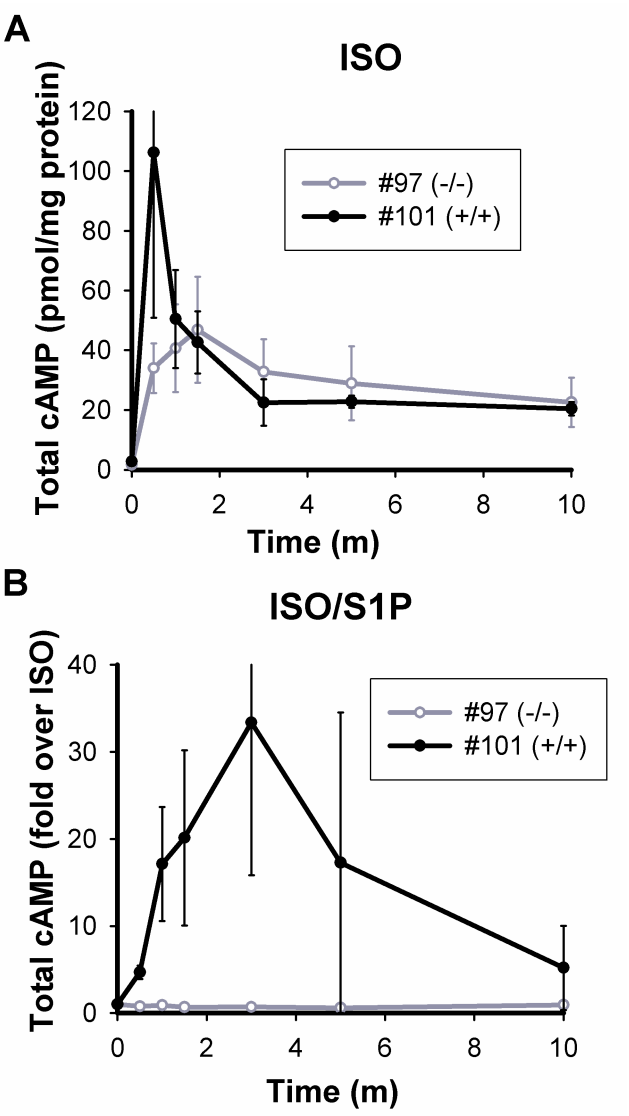
Supplementary Figure S2. Regulation of cAMP by G_{13} does not require activation of G_s-coupled receptors. RAW cells were treated with 1 µg/ml of cholera toxin for 1 hour to activate the G_s protein. Efficacy of the treatment is indicated by an increased concentration of basal cAMP. Addition of 10 nM S1P at 120 seconds produced a large rise of intracellular cAMP concentration and the response is comparable to that induced by S1P following ISO activation of the G_s pathway.

Supplementary Figure S3.



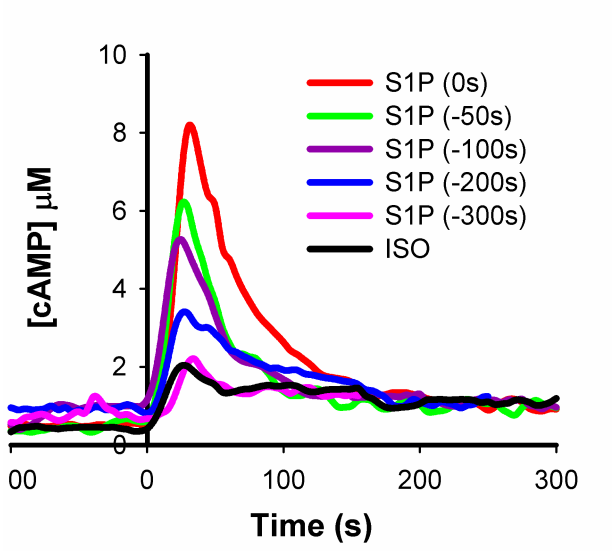
Supplementary Figure S3. S1P elicited a small cAMP rise in BMDM cells. BMDM cells were isolated from 6-week old female mice of the same litter. The genotype of the BMDMs was determined by PCR. Freshly isolated BMDMs were infected with retrovirus carrying the CAMYEL sensor. Intracellular cAMP was measured using the real time BRET assay. Three genotypes of cells were treated with 50 nM S1P at time 0. Wild type BMDM cells produced a small cAMP rise in response to S1P while AC7 deficient BMDMs lacked this response. Error bars represent the standard deviation of results from 3 experiments.

Supplementary Figure S4.



Supplementary Figure S4. The cAMP response measured by EIA in AC7$^{-/-}$ BMDMs is consistent with that measured by CAMYEL. BMDMs were isolated from a 7-week old AC7$^{-/-}$ mouse and its wild type litter mate. At time 0, cells were stimulated with 16 nM ISO (A) or 16 nM ISO together with 4 nM S1P (B). Enhancement with S1P in panel B is expressed as fold stimulation over ISO alone at the matched time point. At the indicated times, reactions were stopped by removal of medium and addition of cell lysis solution; cAMP was determined by enzyme-linked immunoassay (EIA). The results shown are the average of 3 experiments. Error bars represent the standard deviation of results from 3 experiments.

Supplementary Figure S5.



Supplementary Figure S5. Pre-stimulation with S1P transiently enhanced ISO-induced cAMP responses in RAW cells and the S1P effect was lost within 5 minutes. RAW cells carrying CAMYEL sensor were treated with 10 nM S1P at various times as indicated in parenthesis followed by addition of 16 nM ISO at time 0. cAMP responses were measured using BRET assay. These responses were compared to response to ISO alone (black trace). The S1P effect is transient and the transient nature is independent of intracellular cAMP concentration.