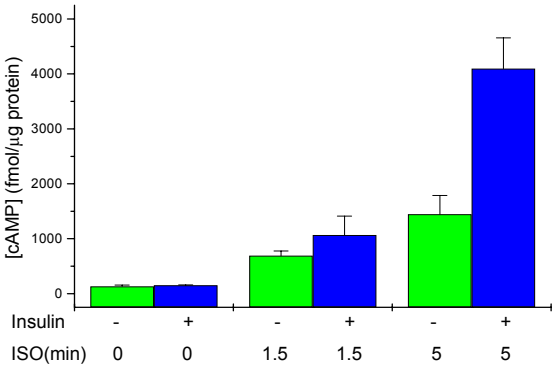
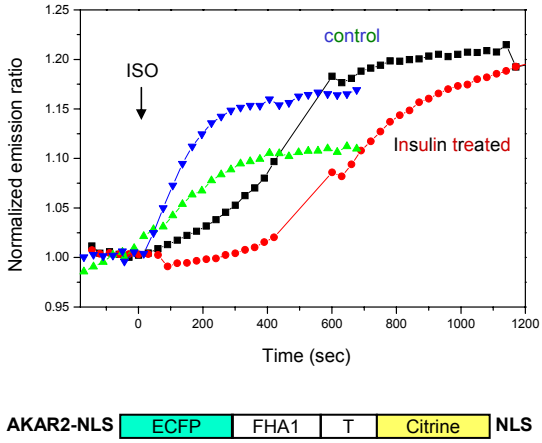
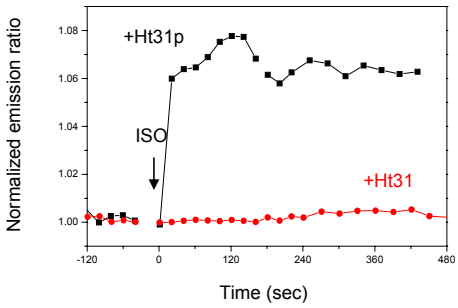


Supplementary Figure 1. Effect of insulin treatment on βAR-associated cAMP generation. Serum-starved 3T3-L1 adipocytes were treated with insulin (17 nM) for 8 hours, washed with PBS, and treated with the β-adrenergic receptor ligand isoproterenol (10 μM) for 1.5 or 5 minutes. Intracellular cAMP was measured by enzyme immunoassay (Amersham Pharmaceutica), per manufacturer's instructions. Data shown are mean ± s.e.m. from three separate experiments.

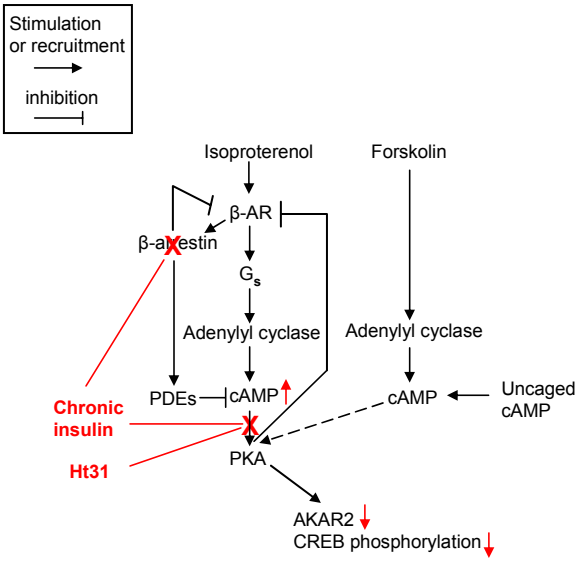
Tsien_Supplementary Figure 1



Supplementary Figure 2. Effect of insulin treatment on PKA activity in the nucleus. Domain structure of AKAR2-NLS shows that the nuclear localization signal (PKKKRKVEDA) is fused to the COOH-terminus of AKAR2. Representative data from cells stimulated with 10 μM isoproterenol is shown (control in blue and green, four experiments; insulin-treated in black and red, three experiments). PKA phosphorylation of AKAR2-NLS in response to isoproterenol (10 μM) is delayed by 5-10 min in insulin-treated cells, whereas inhibition of CREB phosphorylation by chronic insulin treatment persists even 30 minutes after isoproterenol stimulation (data not shown), indicating chronic insulin may have additional effects to disrupt CREB phosphorylation.



Supplementary Figure 3. Effect of the functionally inactive Ht31p. Representative data from cells microinjected with Ht31p (black), an inactive peptide (DLIEEAASRPVDAVPEQVKAAGAY), compared to those microinjected with Ht31 (red). Ht31 or Ht31p peptide was dissolved in microinjection buffer, and mixed with the AKAR2 expression plasmid, yielding an Ht31 concentration of 10 μM prior to injection. Six out of eight cells showed responses in the presence of microinjected Ht31p whereas five out of seven showed no responses in the presence of Ht31 and the other two cells gave slow responses.



Supplementary Figure 4. A model for insulin modulated compartmentation in adipocytes. Stimulation of β-AR leads to activation of a specific pool of PKA localized close to β-ARs, whereas forskolin activated adenylyl cyclases generate bulk cAMP and activate a general pool of PKA. Desensitization involves phosphorylation of β-AR and recruitment of b-arrestin, which further recruits PDE as part of the mechanism for attenuating cAMP signaling. Chronic insulin down-regulates b-arrestin therefore causes supersensitized cAMP production. We propose that chronic insulin, like Ht31, would decouple the preferential linkage between the β-ARs and PKA by affecting their compartmentation, causing delay in AKAR response and attenuated CREB phosphorylation.