



Supplemental Figure 1. DACT causes dose-dependent inhibition of GnRH-induced intracellular calcium transients in pituitary gonadotrophs. L β T2 cells were exposed to 0, 100, 200, or 300 μ M DACT for 24 hrs and evaluated for GnRH-induced changes in intracellular Ca²⁺ by fluorescence microscopy. Pseudo-colored images of relative Ca²⁺ changes in control (a,b) and DACT-treated (c,d) cells at rest (a,c) and after stimulation with 100 nM GnRH (b,d). A dose-dependent decrease in GnRH-induced Ca²⁺ transients is observed in L β T2 cells exposed to DACT (e), corresponding to decreased peak amplitude (f). Representative images are depicted in a-d. Data in e,f are the means of 3 individual experiments ($n=400 - 600$ cells total). * $P<0.001$. Scale bar = 50 μ m

Methods: L β T2 cells were plated onto multi-well chambered coverglass slides (Nunc) and exposed to 0, 100, 200, or 300 μ M DACT for 24 hrs. Following treatment, cells were loaded with 4 μ M Fluo-4 acetoxymethyl ester (Molecular Probes, Eugene, OR) for 20 min at 37 $^{\circ}$ C and placed for microscopy into Earle's balanced salt solution containing 1.35 mM CaCl₂. Images were acquired every 10 sec for 10 min, with 100 nM GnRH was added after 1 min. Images of Fluo-4 fluorescence were collected at 490 nm excitation/515 nm emission through a 20X NeoFluor air objective using a Zeiss 200M inverted microscope equipped with a DG-4 xenon excitation source (Sutter Instruments, Novato, CA) and ORCA-ER cooled interline charge-coupled device camera (Hamamatsu Photonics, Hamamatsu City, Japan). Data were analyzed as the mean background-subtracted fluorescence intensity of each cell normalized to the intensity of the first image (F/F_0). Both data acquisition and analysis were performed using Slidebook software (v5.0, Intelligent Imaging Innovations, Denver, CO).