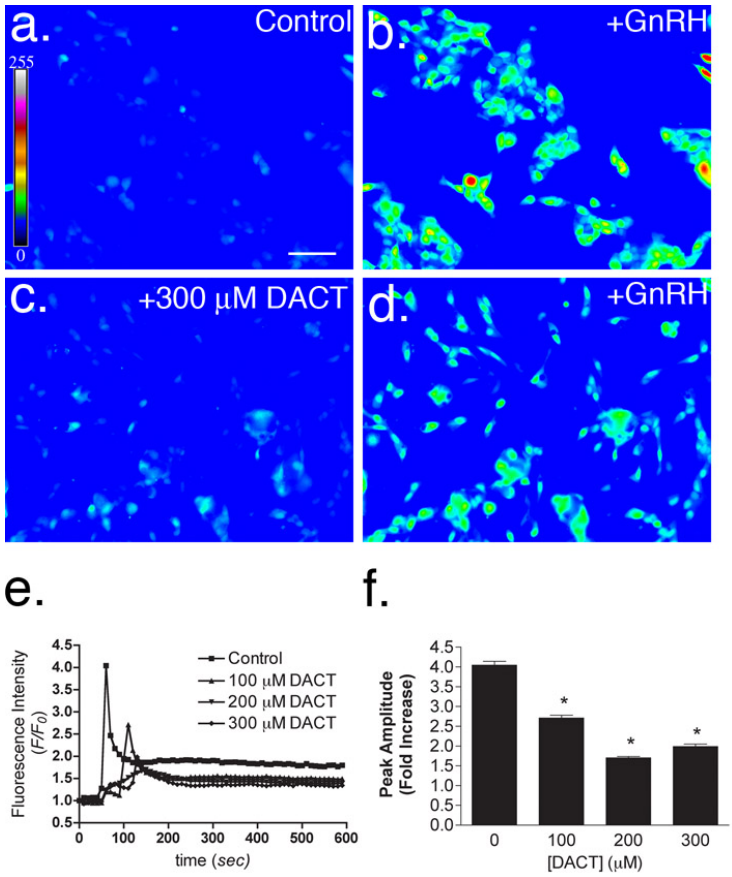

Supplemental Figure 1. DACT causes dose-dependent inhibition of GnRH-induced intracellular calcium transients in pituitary gonadotrophs. LβT2 cells were exposed to 0, 100, 200, or 300 μM DACT for 24 hrs and evaluated for GnRH-induced changes in intracellular Ca^{2+} by fluorescence microscopy. Pseudo-colored images of relative Ca^{2+} 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 Ca^{2+} transients is observed in LβT2 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 μm

Methods: LβT2 cells were plated onto multi-well chambered coverglass slides (Nunc) and exposed to 0, 100, 200, or 300 μM DACT for 24 hrs. Following treatment, cells were loaded with 4 μM Fluo-4 acetoxylmethylesther (Molecular Probes, Eugene, OR) for 20 min at 37 °C and placed for microscopy into Earle's balanced salt solution containing 1.35 mM $CaCl_2$. 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 (Hammamatsu 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).