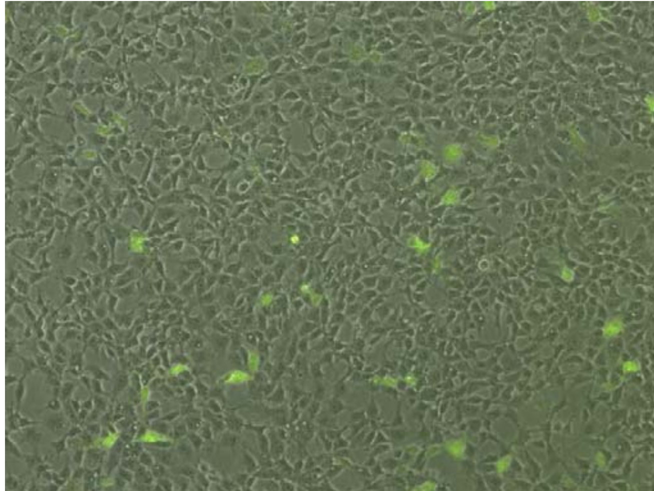
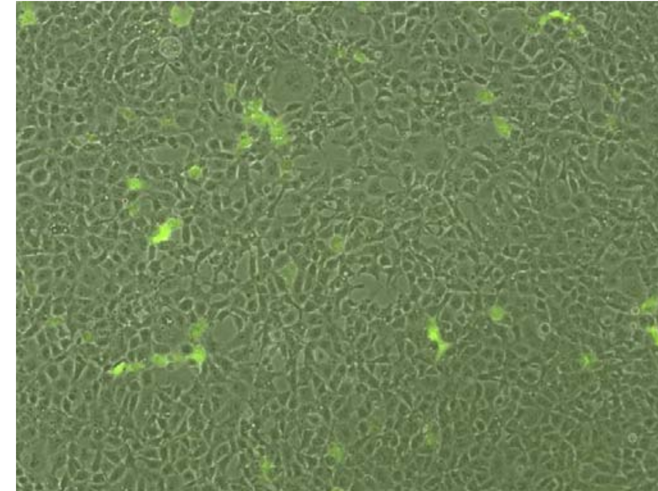
Supplemental figure 1

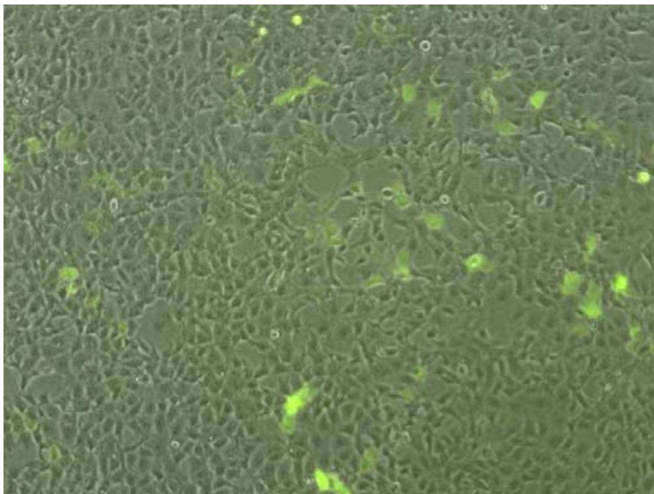
A



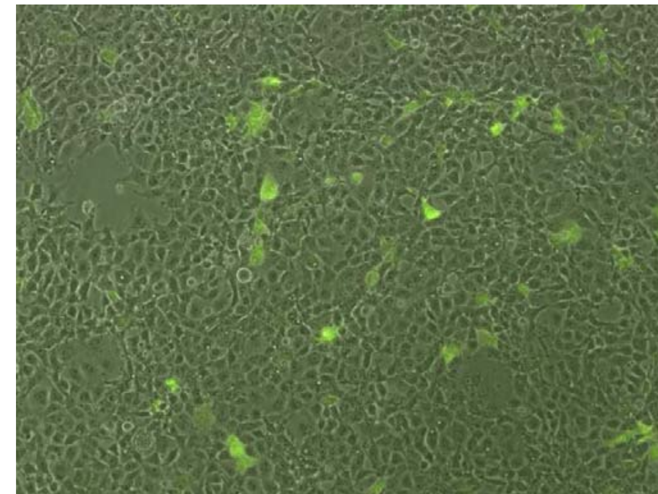
100 ng WT GnRHR + 100 ng GFP (×100)



100 ng pcDNA3 + 100 ng GFP (×100)

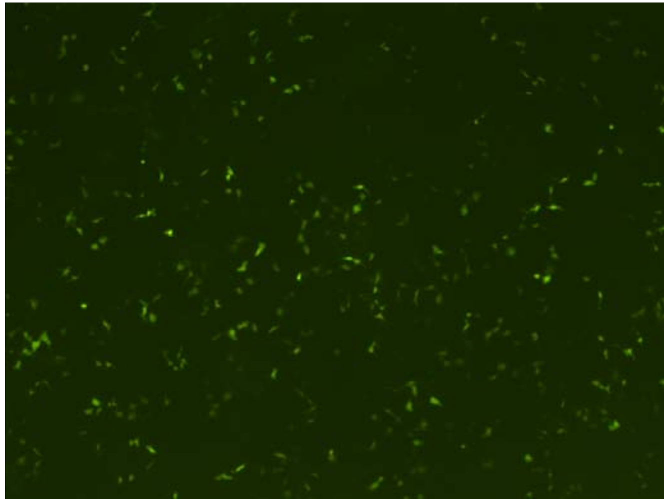


100 ng V134G GnRHR + 100 ng GFP (×100)

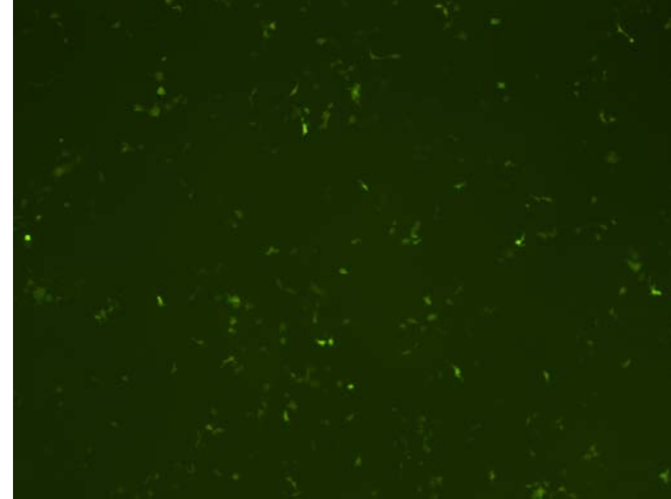


100 ng Y283H GnRHR + 100 ng GFP (×100)

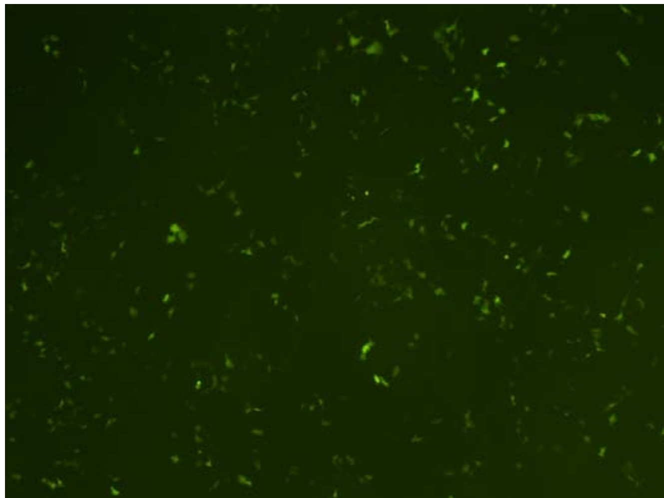
B



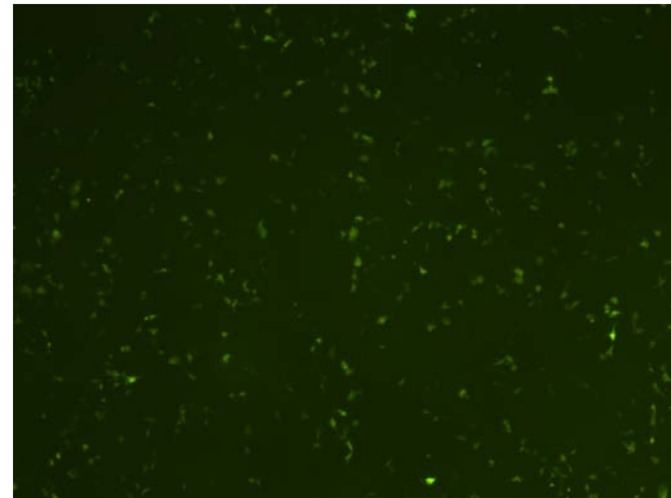
100 ng WT GnRHR + 100 ng GFP (×40)



100 ng pcDNA3 + 100 ng GFP (×40)

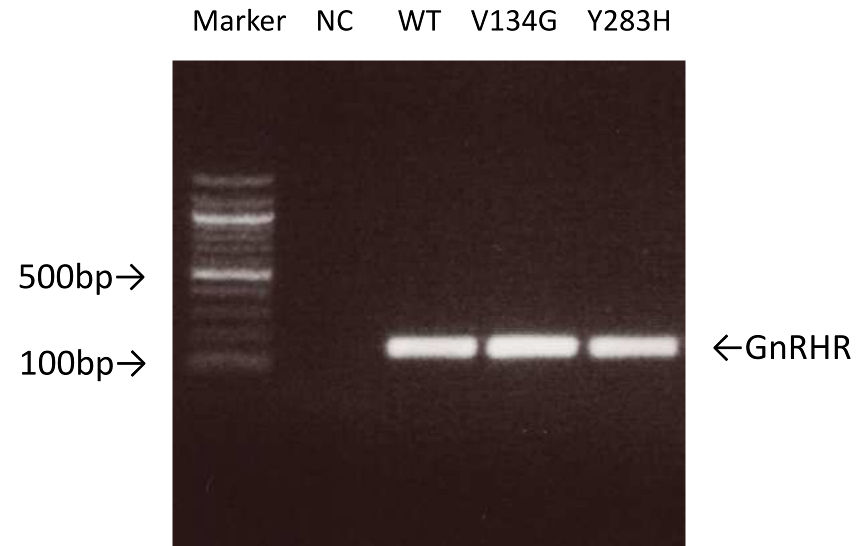


100 ng V134G GnRHR + 100 ng GFP (×40)



100 ng Y283H GnRHR + 100 ng GFP (×40)

C



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Supplemental material

Experiments performed to control transfection efficiency

Methods:

COS-7 cells were grown and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (vol/vol) fetal bovine serum, 100 U/ml of penicillin and 100 µg/ml of streptomycin sulfate in 5% CO2 humidified air at 370C. The cells were transiently transfected using GenePorter transfection reagent (Gelantis, San Diego, CA) in six-well plates with 100 ng/well of WT, V134G, or Y283H GnRHR, or empty vector (pcDNA3) as a negative control, with or without 100 ng/well of an expression vector encoding green fluorescent protein (GFP) (constructed in pcDNA3). The next day, the cells co-transfected with GFP were visualized with fluorescence microscopy.

Forty-eight hours after the cells were transfected with WT, V134G, or Y283H GnRHR, cells were harvested and DNA was extracted using a DNA extraction kit (DNeasy blood and tissue kit, Qiagen). PCR was performed using extracted DNA as template and primers designed based on the GnRHR cDNA sequence. To exclude PCR products amplified from endogenous genomic DNA, the primers were designed to span intron 1 of the *GNRHR* gene (intron size 987 bp). The PCR products were visualized by gel electrophoresis. Parallel inositol phosphate (IP) assays were performed in the cells transfected with WT, V134G, or Y283H GnRHR or empty vector following stimulation with increasing concentrations of GnRH.

Results:

To evaluate transfection efficiency, COS-7 cells transfected with WT, V134G, or Y283H GnRHR were co-transfected with a GFP expression vector. Levels of GFP expression were similar in cells co-transfected with WT, V134G, or Y283H GnRHR, or empty vector (Suppl. Figure), indicating equivalent transfection efficiency in all cells.

Amplified GnRHR PCR products were detected in cells transfected with WT, V134G or Y283H GnRHR, but not in cells transfected with pcDNA3. PCR products were visualized by gel electrophoresis from DNA derived from cells transfected with WT, V134G or Y283H GnRHR (Suppl. Figure) providing further confirmation of equivalent transfection

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effeciency of WT and mutant GnRHR expression vectors. These data exclude variability in transfection efficiency as a factor contributing to the lack of response to GnRH in cells transfected with V134G or Y283H GnRHR.

Figure legend:

Supplemental figure 1: A and B, GFP expression in cells co-transfected with WT, V134G, or Y283H GnRHR visualized with fluorescence microscopy in 100x and 40x magnitude respectively; C, gel electrophoresis showing GnRHR PCR products amplification, using DNA from cells transfected as template. Amplified GnRHR PCR products were detected in cells transfected with WT, V134G or Y283H GnRHR, but not in cells transfected with pcDNA3.

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