Supplemental Data

Supplemental Fig.1: Western blot of GnRH-induced β-catenin nuclear accumulation in LβT2 cells

A, Time course of GnRH-stimulated nuclear accumulation of β-catenin in LβT2 cells. Cells were treated with 10 nM GnRH for the times indicated. Nuclear extracts were subjected to a quantitative Western blot analysis using a β-catenin-specific antibody. LSD1 (in red) was used as a loading control. **B,** Quantification of Western blot densitometry from three independent experiments, plotted as mean ± SEM. Significance values at each time point in comparison with time 0 (control) obtained by t-test and Bonferroni corrections (0.25h, 0.5h, 1h, 2h, 4h, 6h): $P<.01$, $P<.01$, $P<.01$, $P<.05$, $P<.01$, $P<.01$, respectively.

Supplemental Fig.2: Effects of two JNK inhibitors on GnRH-induced β-catenin nuclear accumulation and FSHβ mRNA expression

A, Cells were serum-starved overnight, pretreated with 20 μM JNK inhibitor III for 45 min, and stimulated with 10 nM GnRH or vehicle for 15 min. Nuclear extracts were subjected to a quantitative Western blot analysis using a β-catenin-specific antibody. LSD1 (in red) was used as a loading control. **B,** Quantification of Western blot densitometry from three independent experiments, plotted as mean ± SEM. Two-way ANOVA n=3; * $P<0.05$. **C,** Cells were serum-starved overnight, pretreated with either 5, 15 or 45 μM SP600125 for 30 min, and stimulated with 10 nM GnRH or vehicle for 15 min. Nuclear extracts were subjected to a quantitative Western blot analysis using a β-catenin-specific antibody. LSD1 (in red) was used as a loading control. **D,** Quantification of Western blot densitometry from three independent experiments, plotted as mean ± SEM. Two-way ANOVA n=3; * $P<0.05$, ** $P<0.01$. **E,** Cells were serum-starved overnight, pretreated with 20 μM JNK inhibitor III for 45 min, and stimulated with slow of

5 nM GnRH for 6 h. Samples were collected 30 min after the last pulse. Relative mRNA copy numbers of FSHβ were determined by quantitative real time-PCR. Two-way ANOVA n=4; ** *P<0.01.*

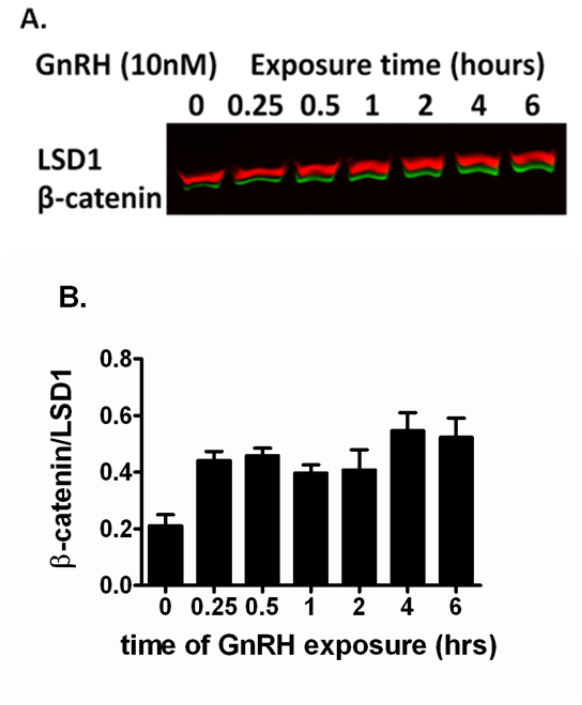
Supplemental Fig.3: Effect of SP600125 on GSK3 phosphorylation in GnRH-stimulated LβT2 cells

A, Cells were serum-starved overnight, pretreated with 40 μM SP600125 for 30 min, and stimulated with 10 nM GnRH or vehicle for 15 min. Whole cell lysates were subjected to a quantitative Western blot analysis using a specific antibody against phospho-GSK3α/β $Ser^{21/9}$. GAPDH (in green) was used as a loading control. **B-C,** Quantification of Western blot densitometry from three independent experiments, plotted as mean ± SEM. The levels of phospho-GSK3α (**B**) or phospho-GSK3β (**C**) were normalized to GAPDH. Two-way ANOVA n=3; * *P<0.05,* ** *P<0.01.*

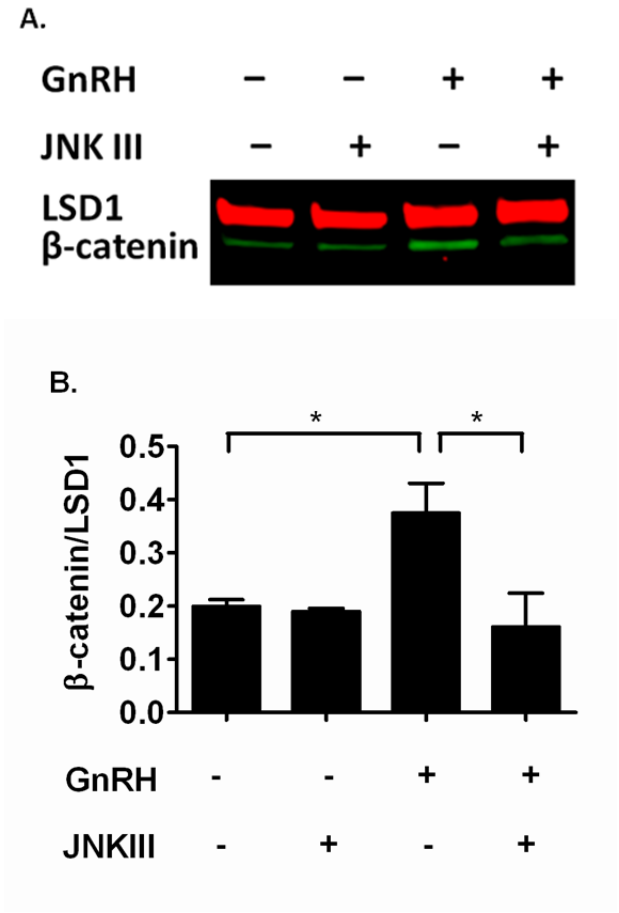
Supplemental Fig.4: Effect of β-catenin gene silencing on GnRH-induced expression of candidate target genes

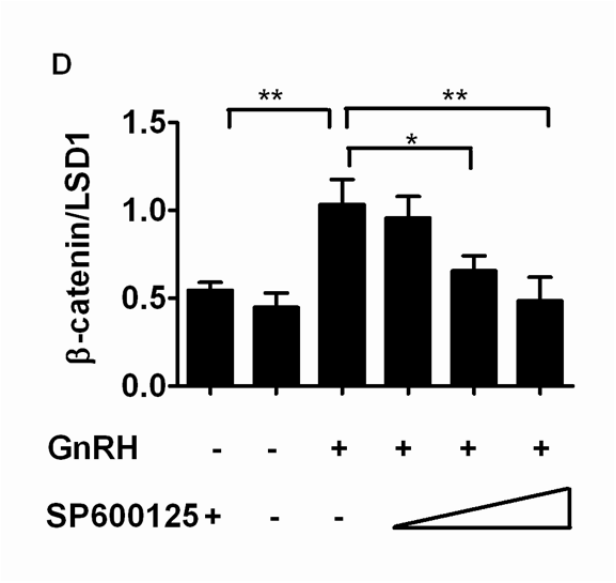
LβT2 cells were transfected with 0.6 μg of either scrambled or β-catenin siRNA for 48 h, serum-starved overnight, and stimulated with 10 nM GnRH or vehicle for 1 h. RNA copy numbers of c-jun, Nur77, Pitx1, Pitx2, β-catenin, and GAPDH were determined by quantitative real time-PCR. Two-way ANOVA n=4; ** *P<0.01*. Primer sequences were as follows: GAPDH/Sense, 5'-TGC GAC TTC AAC AGC AAC TC-3'; GAPDH/Antisense, 5'-CTT GCT CAG TGT CCT TGC TG-3'; β-catenin/Sense, 5'-CAT TAC TAA CTG GGA GCG TG-3'; β-catenin/Antisense, 5'-GAC CCC GTG AGT CTT TAC AG-3'; c-jun/Sense, 5'-TGA AAG CTG TGT CCC CTG TC-3'; c-jun/Antisense, 5'-ATC ACA GCA CAT GCC ACT TC-3'; Nur77/Sense, 5'-GGG TGA CCC CAC TAT TTG TC-3'; Nur77/Antisense, 5'-CGG AAG AGA TCT CGA GTT GG-3'; pitx1/Sense, 5'-GCC AGC AGC AGG AGG AGA A-3'; pitx1/Antisense, 5'-GCA GGC GGA CAG TGG AGA A-3'; pitx2/Sense, 5'-GTG CGC ACT ATG GGA AGG AA-3'; pitx2/Antisense, 5'-CGT CCT CCA ACT GTT GGG AA-3'.

Supplemental Figure 1



Supplemental Figure 2




D
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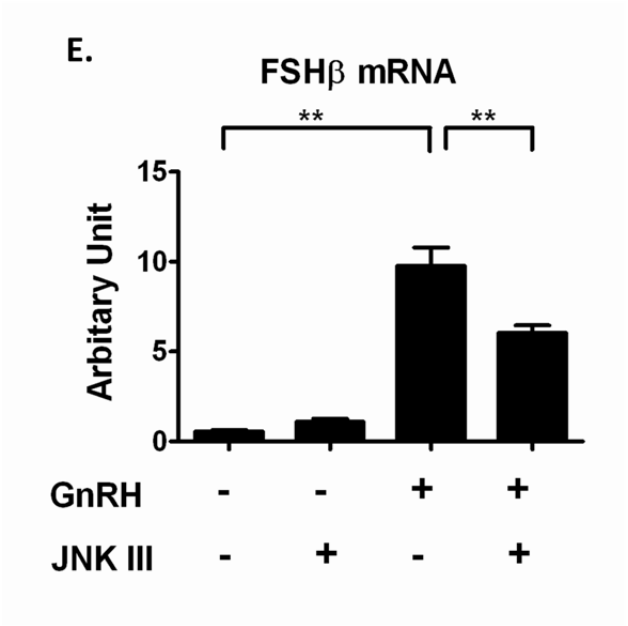
*
1.5
1.0
0.5
0.0
β-catenin/LSD1
GnRH - - + + + +
SP600125 + - -


E.
FSHβ mRNA
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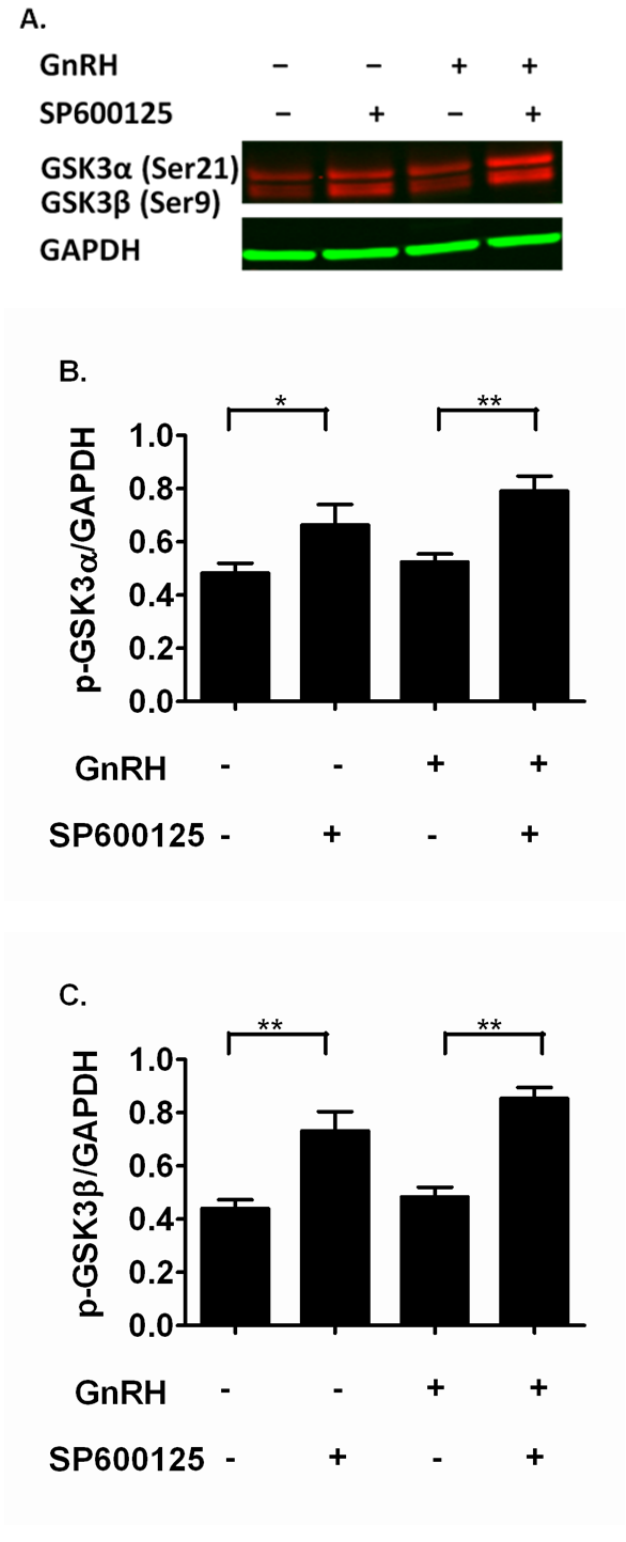
15
10
5
0
Arbitary Unit
GnRH - - + +
JNK III - + - +

Supplemental Figure 3



Supplemental Figure 4