Supplemental Material

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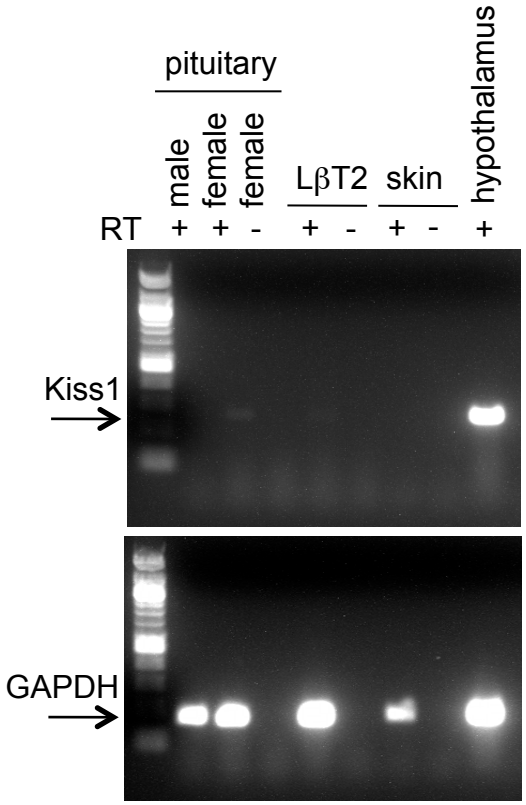
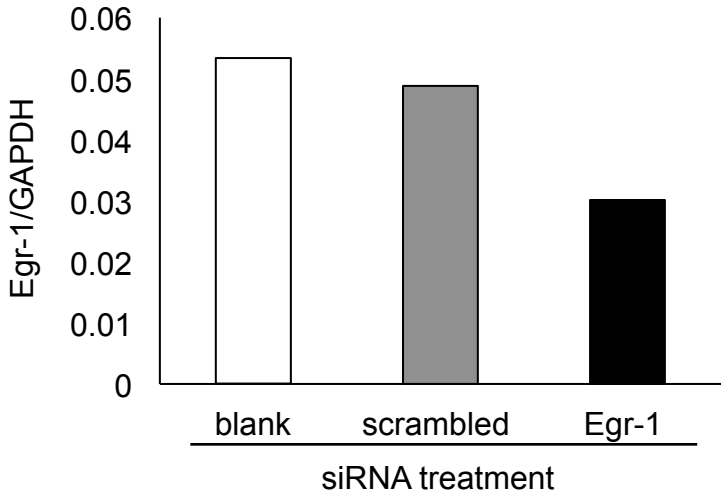
Kisspeptin Regulates Gonadotropin Genes via Immediate Early Gene Induction in Pituitary Gonadotropes

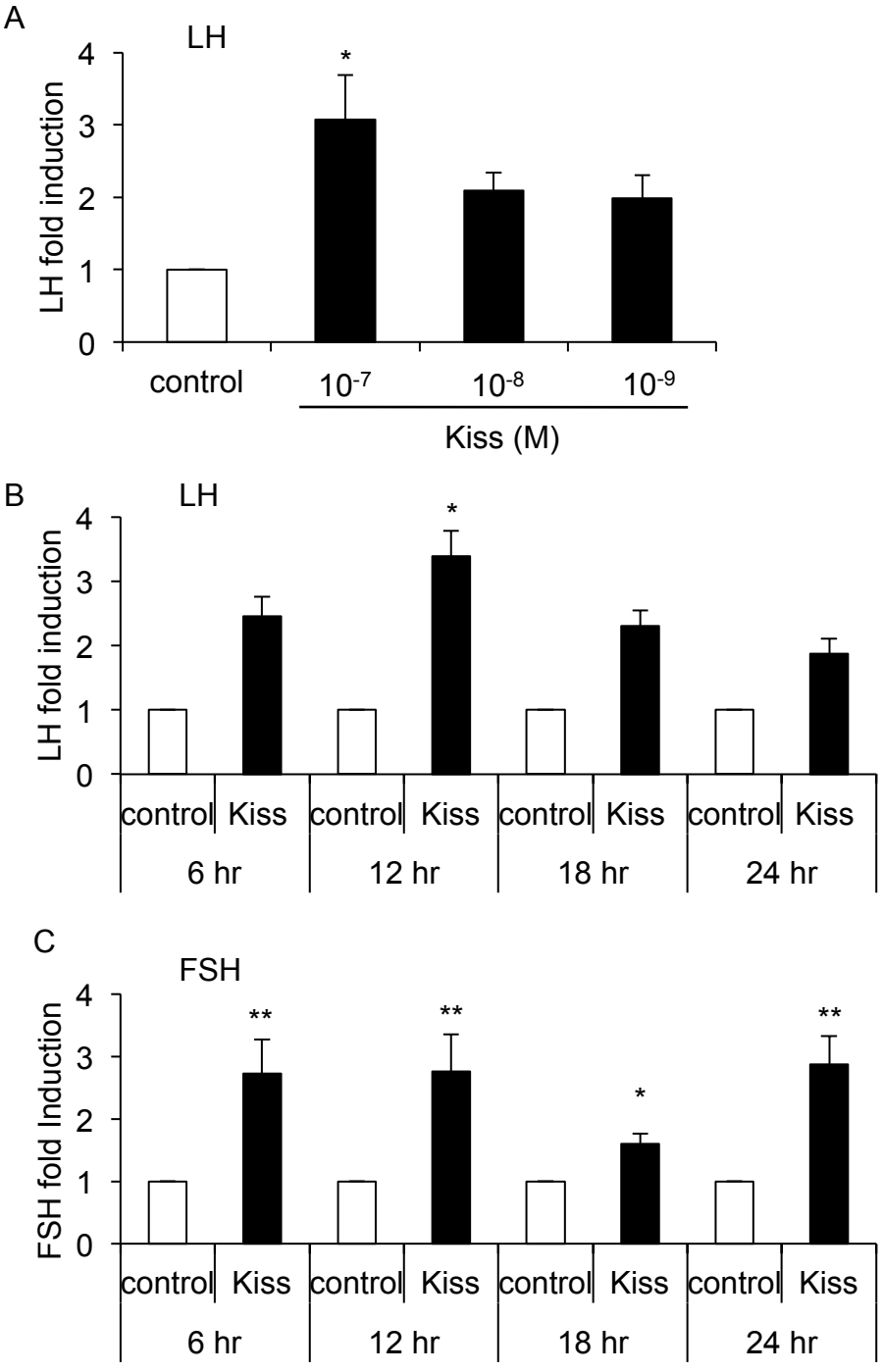
Molecular Endocrinology 2013



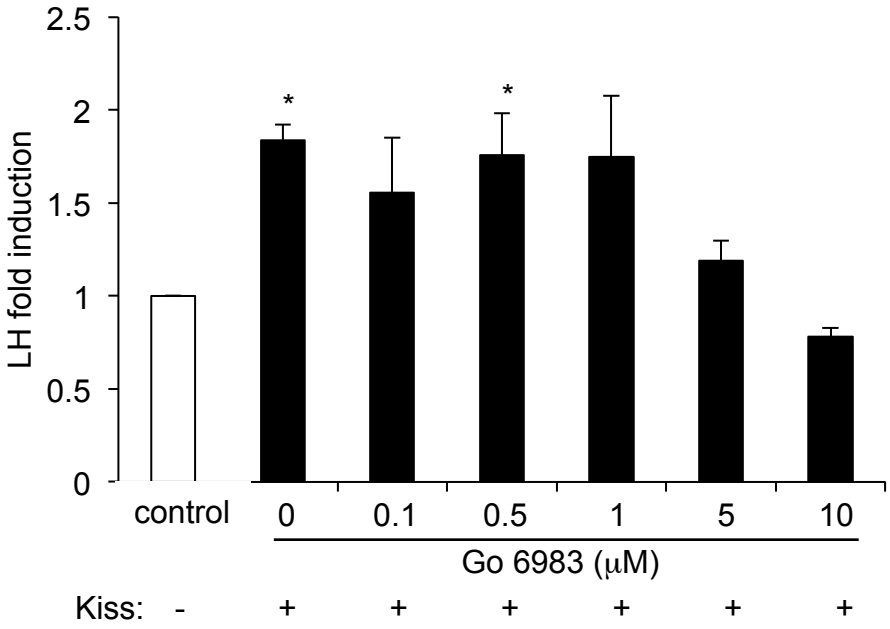
Supplemental Figure 1. Kiss1 is not expressed in mouse pituitary or LβT2 gonadotropes. Pituitaries, hypothalamus, and skin biopsies were harvested from wild-type mice and cDNA was prepared. cDNA was also prepared from LβT2 cells. PCR was performed for Kiss1 and GAPDH as a control.



Supplemental Figure 2. Egr-1 expression in LβT2 cells after siRNA transfection. LβT2 cells were transfected with either Egr-1 siRNA, scrambled control, or were left untransfected (blank). cDNA was harvested after 24 h from two independent passages of cells and qPCR was performed for Egr-1 and GAPDH.



Supplemental Figure 3. Dose response and time course for kisspeptin treatment on the LHβ and FHβ promoters. LβT2 cells were transiently transfected with a Kiss1R expression vector and the 1.7 kb LHβ or 1 kb FSHβ luciferase reporter. Cells were treated with vehicle (DMSO for kisspeptin) or kisspeptin at the indicated concentrations and treatment duration, and were subjected to luciferase assay. Data were normalized to vehicle-treated control. A, Cells were treated for 12 h with kisspeptin at the indicated concentrations. * $p<0.05$ by one-way ANOVA. B, Cells were treated with 100 nM kisspeptin for the indicated durations. * $p<0.05$ vs kisspeptin at 6, 18, and 24 hr by one-way ANOVA. C, Cells were treated with 100 nM kisspeptin for the indicated durations. * $p<0.05$ and ** $p<0.01$ vs control by Student's t-test.



Supplemental Figure 4. Dose response for the PKC inhibitor Go 6983. LβT2 cells were transiently transfected with a Kiss1R expression vector and the 1.7 kb LHβ luciferase reporter. Cells were treated for 30 min with Go 6983 or vehicle (DMSO) at the indicated concentrations, after which either vehicle (DMSO) or 100 nM kisspeptin were added for 12 h. Cells were then subjected to luciferase assay. Data were normalized to vehicle-treated control. * $p<.05$ vs control by Student's t-test. The concentration of 5 μM was chosen for all subsequent experiments.

qPCR, GAPDH-F	TGCACCACCAACTGCTTAG
qPCR, GAPDH-R	GGATGCAGGGATGATGTTC
qPCR, Kiss1r-F	GCACCTACTGCAGCGAGGCG
qPCR, Kiss1r-R	CCTTGGTGCGCACTGCTCCG
qPCR, TBP-F	CAAACTCTGACCACTGCACCGTTG
qPCR, TBP-R	GAAGCTGGTGTGGCAGGAGT
qPCR, LHβ-F	CTGTCAACGCAACTCTGG
qPCR, LHβ-R	ACAGGAGGCAAAGCAGC
qPCR, FSHβ-F	GCCGTTTCTGCATAAGC
qPCR, FSHβ-R	CAATCTTACGGTCTCGTATACC
PCR, Kiss1r-F	CTCCTCTACCCGCTGCCCGC
PCR, Kiss1r-R	GCTGAGGCTGACAGCCAGGG
PCR, Kiss1-F	CAAAAGTGAAGCCTGGATCC
PCR, Kiss1-R	GTTGTAGGTGGACAGGTCC
-313 bp hox mut (cFos)	CCTCCCTCCTTTACACAGGATGTCCATCGGA GGACATCTGCGTC
-59 bp hox mut (cFos)	GTGACGTAGGAAGTCCATCCGGCCACAGCGC TTCTATAAAGGC
-91 bp ETS mut (Egr-1)	GTCCTTCCATATTAGGGCTT ACAACTTCCCATATATGGCCATGTACGTCA
-138 bp CRE mut (Egr-1)	GGATGGGAGGGCTTCCACCC ACTCCGGGTCCTCCCGGCCG
-83 bp SRF mut (Egr-1)	GTCCTTCCATATTAGGGCTT CCTGCTTCAAATATATAACCATGTACGTCA
-105 bp SRF mut (Egr-1)	GTCCTTAAATATTAAAGCTT CCTGCTTCCCATATATGGCCATGTACGTCA
-353 bp SRF mut (Egr-1)	CGCCGGAACAGACCTTATTT GGGCAGCGAATTATATAAAGTGGCCCAATA
-368 bp SRF mut (Egr-1)	CGCCGGAACAGAAATTATTT AAGCAGCGCCTTATATGGAGTGGCCCAATA

Supplemental Table 1. 5’ to 3’ oligonucleotide sequences used for qPCR and PCR.