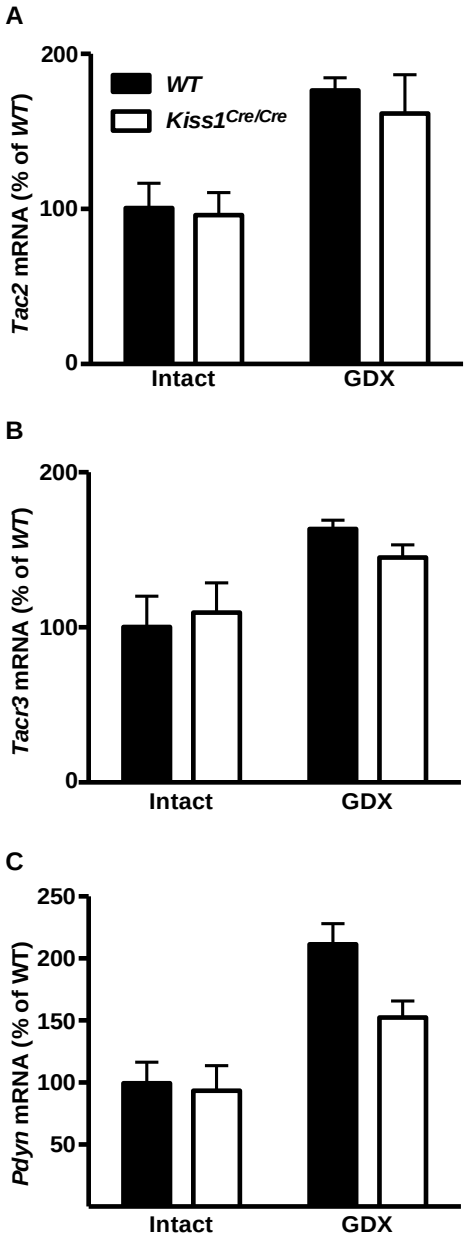

Supplemental Figure 1. Co-neurotransmitter and receptor expression in the MBH of *Kiss1*$^{Cre/Cre}$ male mice. Two-way ANOVA revealed a significant effect of GDX on the expression of **A.** *Tac2* (which encodes NKB, $p < 0.005$), **B.** *Tacr3* (which encodes the NKB receptor, $p < 0.01$), and **C.** *Pdyn* (which encodes dynorphin, $p < 0.001$). There was no effect of genotype and no interaction between treatment and genotype for any of the 3 genes.

Gene – Primer	Oligo Sequence
Tac2-FOR	TTC CAC AGA AAC GTG ACA TGC
Tac2-REV	GGG GGT GTT CTC TTC AAC CAC
Tacr3-FOR	GCC ACA GCC ACT AAG ATT GTC
Tacr3-REV	CGG CCT GGC ATG ACT TTT ATT TT
PDyn-FOR	GAG GTT GCT TTG GAA GAA GGC
PDyn-REV	TTT CCT CTG GGA CGC TGG TAA
Actb-FOR	AGTGTGACGTTGACATCCGTA
Actb-REV	GCCAGAGCAGTAATCTCCTTCT

Supplemental Table 1. Primers for SYBR qRT-PCR.

Cycle	Time	Temperature
1	30 min	50 C
2	10 min	95 C
3	15 - 30 sec	95C
4	1 min	60C
5	Repeat Steps 3-4 for 45 cycles	
6	1 min	95C
7	30 sec	55C
8	55C to 95C temperature ramp with fluorescence read every second	55C-95C
9	30 sec	95C

Supplemental Table 2. qRT-PCR steps. The melting curve in steps 6-9 was only used for SYBR reactions.

Assay	Treatment & Sex	Reportable Range	Intra-assay % CV
LH multiplex	GDX + E females	0.24-30.0 ng/ml	9%
LH multiplex	GDX males	0.24-30.0 ng/ml	7%
FSH multiplex	GDX males	2.4-300.0 ng/ml	4%
Testosterone RIA	Intact males	5.9-1100 ng/dl	4%
LH IRMA	GDX females	0.04-37.4 ng/ml	16%
LH IRMA	Intact females (senktide)	0.04-37.4 ng/ml	7%
Male FSH IRMA	Intact males	3.1-75.0 ng/ml	6%

Supplemental Table 3. Reportable ranges and intra-assay coefficients of variation for hormone assays. % CV = coefficient of variation.

	WT	***Kiss1*$^{Cre/+}$**	***Kiss1*$^{Cre/Cre}$**
***Kiss1* Intact**	11.93 ± 2.48	4.71 ± 0.33	0.75 ± 0.16
***Kiss1* GDX**	100.00 ± 9.56***	43.11 ± 10.47***	5.65 ± 1.01***
LH Intact	0.26 ± 0.17	0.84 ± 0.73	0.39 ± 0.10
LH GDX	3.72 ± 1.28**	2.91 ± 0.75*	1.95 ± 0.39*

Supplemental Table 4. *Kiss1* transcript and LH increase in response to castration. *Kiss1* mRNA levels are expressed as a percent of castrated WT animals. qRT-PCR with Taqman primers/probe data are normalized to *Actb*. $p<0.0001$ for effect of treatment, genotype and interaction between treatment & genotype for *Kiss1* expression. $p<0.0005$ for effect of treatment on LH using two-way ANOVA. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ for comparing intact to GDX animals (with 1-tailed Mann Whitney U-test for LH and t-test for *Kiss1*).