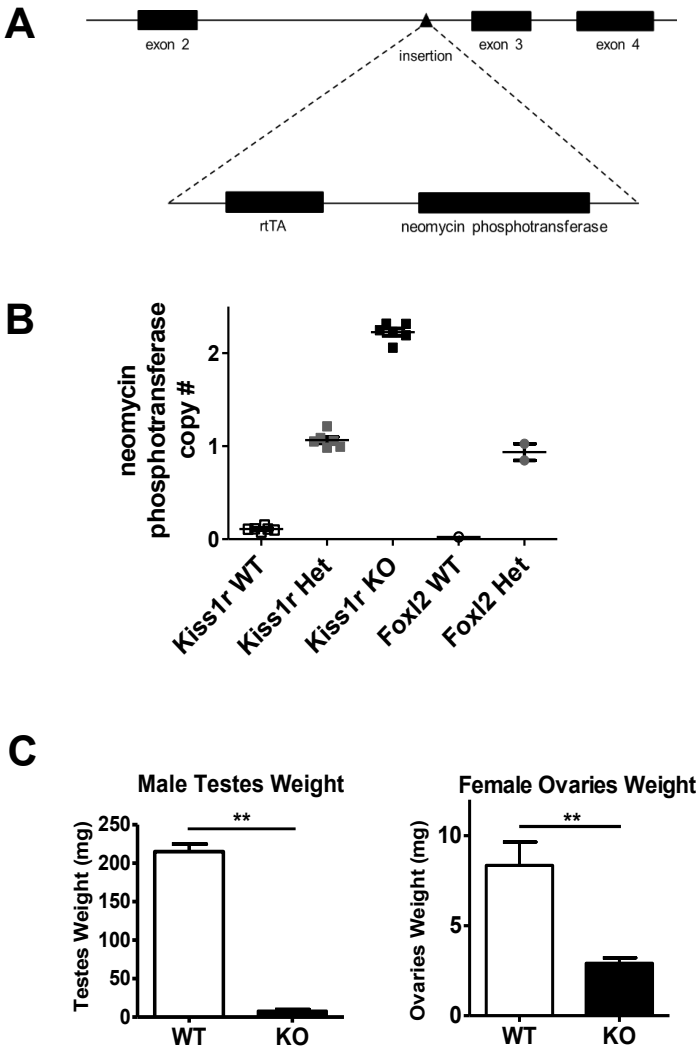
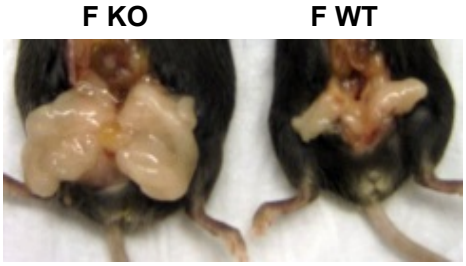
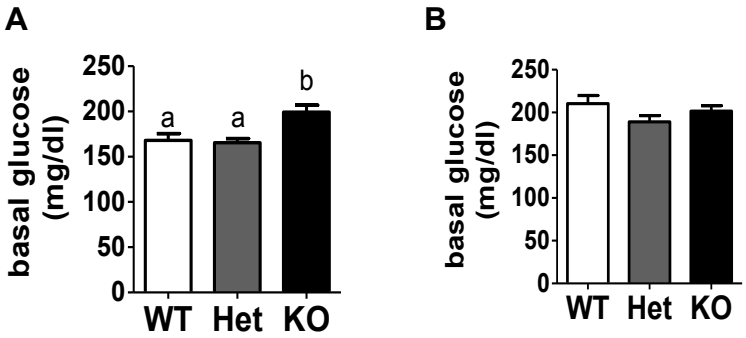
Supplemental Figures



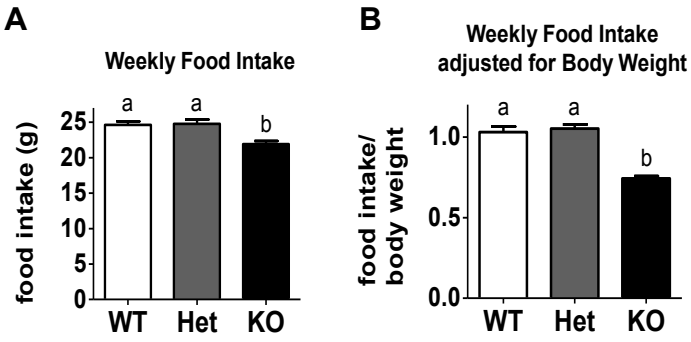
Supplemental Figure S1: (**A**) Schematic diagram of the retroviral insertion in the *Kiss1r* gene, showing insertion between exons 2 and 3. The vector for insertional mutagenesis is described in greater detail in Gragerov A, et al., *PNAS.* 2007;104(36):14406-11. (**B**) Quantitative PCR of genomic DNA, showing that, as expected, *Kiss1r* Hets have one copy of the insertion, *Kiss1r* homozygotes (KOs) have two copies of the insertion, and their *Kiss1r* WT littermates have no copies of the insertion. Data were normalized to a control mouse line (Foxl2 mice) with a known copy number of neomycin phosphotransferase. (**C**) Testes weights from adult *Kiss1R* KO males (19-23 wks old) on a standard chow diet and ovary weights from young adult females at the time of gonadectomy (6-8 wks of age). **p<0.01.



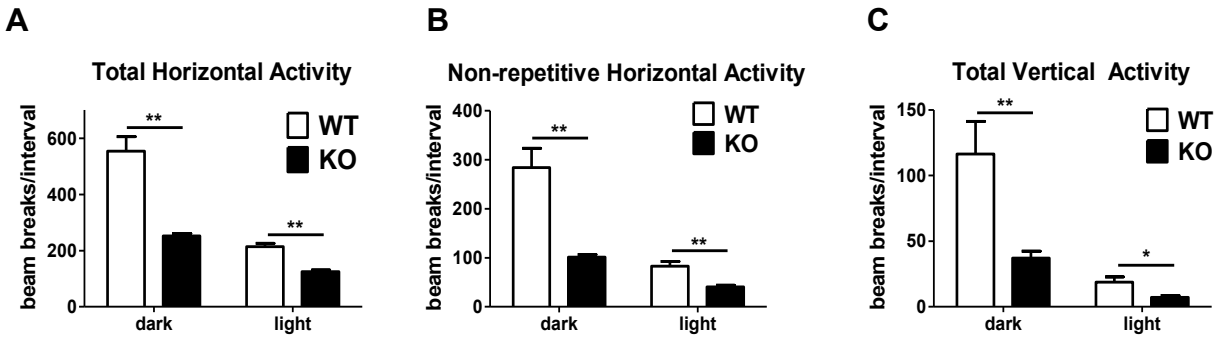
Supplemental Figure S2. Gross appearance of abdominal fat pads in adult *Kiss1r* KO and WT female littermates (i.e., sisters).



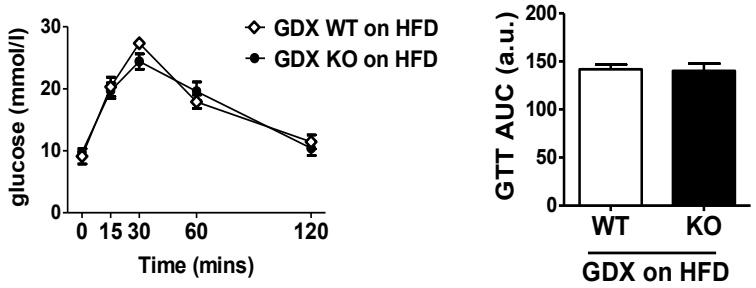
Supplemental Figure S3. Basal glucose levels in semi-fasted adult female (**A**) and male (**B**) *Kiss1r* KO and control littermates on a standard chow diet. Bars labeled with different letters designate significantly different groups ($p<0.05$).



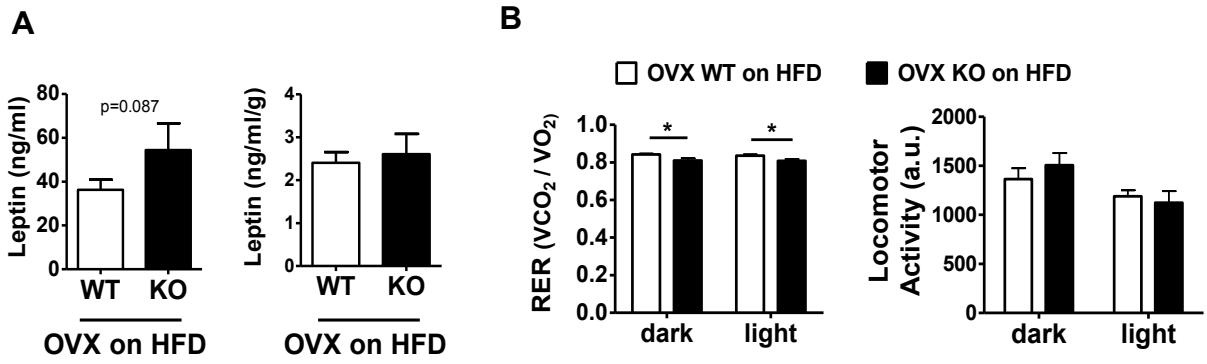
Supplemental Figure S4. (A) Weekly food intake of adult *Kiss1r* KO females on a standard chow diet. (**B**) Weekly food intake of females adjusted for body weight. Bars labeled with different letters designate significantly different groups ($p<0.05$).



Supplementary Fig. S5. Locomotor activity measurements of gonad-intact adult *Kiss1r* KO females on a standard chow diet in CLAMS cages. (**A**) Total horizontal activity (total number of X photobeams broken), (**B**) non-repetitive horizontal activity (X photobeams broken excluding repetitive movements such as grooming), and (**C**) total vertical activity (total number of Z photobeams broken, includes behavior such as rearing). *$p<0.05$, **$p<0.01$.



Supplemental Figure S6. Glucose tolerance in gonadectomized (GDX) *Kiss1r* KO males after 6 wks on a HFD, assessed by GTT.



Supplemental Figure S7. Leptin and metabolic measures of ovariectomized (OVX) *Kiss1r* KO females on a high fat diet (HFD). (**A**) Raw leptin values and leptin values corrected for fat mass in OVX females after 10 wks on a HFD. (**B)** Measurements of respiratory exchange ratio (RER) and locomotor activity in OVX *Kiss1r* KO females after 12 wks on a HFD. *p<0.05.

SUPPLEMENTAL METHODS

Animals

The *Kiss1r* KO mouse line, on a C57Bl6 x 129S1/SvImJ background and described previously (1-3), was generated by Omeros Corporation (Seattle, WA) via retroviral mutagenesis (4). Sequencing confirmed that the insertion site was in the *Kiss1r* gene (Supplemental Figure S1A); primers: LM173 5'-CCCACTTCCCACGTCCTCGCTCT-3' and OC3 5'-CAGGAACAGATGGAACAGCTAGAGAACC-3'. Moreover, as expected, *Kiss1r* KO and Het mice were confirmed to have only 2 and 1 copies of the insertion in the genome, respectively, indicating that only the *Kiss1r* gene was impaired (Supplemental Figure S1B). Mirroring previous reports, hypogonadism was confirmed in *Kiss1r* KO mice of both sexes (Supplemental Figure S1-C). Hypogonadal *hpg* (lacking GnRH (5)) mice and controls (WT and HET littermates) were generated by *hpg* Het breeders and are maintained on a mix of their own inbred strain, C57Bl6 x HPG/BmJ (JAX 000804). All *Kiss1r* KO and *hpg* mice were genotyped by PCR of tail DNA. In each experiment, *Kiss1r* KO or *hpg* mice were compared to control littermates (siblings) and thus were on the same genetic background as their controls. Since small strain differences in the body weights of the control animals were observed between *hpg* and *Kiss1r* mice, all experimental animals were compared to control littermates from their own specific mouse strain.

Quantitative PCR (qPCR) of Insertion Copy Number

Quantitative real-time PCR was performed on genomic DNA (gDNA) from tail samples of *Kiss1r* KO, WT, and Het mice to confirm that there was only one copy of the retroviral insertion in the genome (Supplemental Figure S1B). Briefly, DNA was extracted from tails using a DNeasy Blood and Tissue Kit (Qiagen) and real-time quantitative PCR was performed in duplicate using 50 ng of gDNA with iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, Inc.) and the Bio-Rad CFX Connect Real Time System. The primers used to amplify neomycin phosphotransferase were: Neo Fwd 5'-CTTGGGTGGAGAGGCTATTC-3' and Neo Rev 5'-AGGTGAGATGACAGGAGATC-3'. IL2 was used as a gDNA loading control, and the primers were: IL2F 5'-CTAGGCCACAGAATTGAAAGATCT-3' and IL2R 5'-GTAGGTGGAAATTCTAGCATCATCC-3'. Relative differences in gDNA concentration between WT, Het, and KO mice were then calculated using the comparative threshold cycle (C_t) method (6). Data were normalized to a control mouse line (Foxl2 mice) with a known copy number of neomycin phosphotransferase (7).

Weekly growth and feeding analyses

For mice on a standard chow diet, group-housed (2-3 mice of the same sex/cage) *Kiss1r* KO mice and their control littermates were weighed once weekly from 4-18 wks of age (gonad intact experiments; n=16-30/genotype for each sex) or until 21 wks of age (for the OVX female experiments; n=9-14/genotype). Gonad

intact female *hpg* and control mice were weighed every 3 weeks from 3 to 21 wks of age. For weekly feeding measurements in *Kiss1r* KO mice and their control littermates, adult females (15 wks old) on standard chow diet were acclimated to individual housing for 1 wk. Food consumption was then measured weekly for 2 wks (n=16-30/group). To ensure no food was missed, the cage and bedding were checked for spillage or hoarded food. Hourly food intake assessments were also performed in *Kiss1r* KO and WT females housed in CLAMS metabolic cages (n=6/genotype), as described further below.

For experiments with GDX mice on a HFD, *Kiss1r* KO and WT mice of both sexes were weaned onto a standard chow diet and then weight matched at 6-8 wks of age (before obesity onset in the KOs), GDX, and thereafter individually housed. After 1 wk of recovery (designated Week 0 on graphs), all GDX mice were placed on a HFD (23% fat; 46% of total energy from lipids; SF04-027; Specialty Feeds) and body weights were subsequently measured twice weekly for 12 wks (n=5/group).

Body composition analyses

Body composition was determined in adult male and female *Kiss1r* KO and control mice by dual energy x-ray absorptiometry (DEXA). For mice on a standard chow diet, mice (19-21 wks old) were fasted for 4-6 h and then anesthetized (IP injection; ketamine 100mg/kg, xylazine 10mg/kg). Body weight was measured and lean muscle mass and fat mass were determined by scanning with a GE Lunar Pixi Densitometer Machine (n=7-12/group). For GDX mice on a HFD, body composition after 8 wks on HFD (~15-17 wks old) was similarly determined on isoflurane-anesthetized mice by DEXA using a Hologic Discovery A-QDR series bone densitometer (n=5/group).

Hormone assays and glucose tolerance tests

For *Kiss1r* KO and WT mice on a standard chow diet, blood was collected retro-orbitally (18-24 wks old) and the serum stored at -20°C. Serum samples were assayed for leptin using a Mouse/Rat Leptin Quantikine ELISA Kit (#MOB00, R&D Systems; n=4/group), per the manufacturer's instructions. Serum T4 in females was assayed by the National Hormone and Pituitary Program using RIA (Dr. A. Parlow, Harbor UCLA; n=6-10/genotype). For GDX mice on HFD, blood was collected after 9-10 wks of HFD treatment (16-19 wks old), and the plasma stored at -20°C. Plasma leptin (#90030; Crystal Chem Inc.) was measured in accordance with the manufacturer's instructions (n=5/group).

Glucose tolerance tests (GTT) were performed in adult *Kiss1r* mice on a standard chow diet at 18-20 wks of age. Mice of both sexes were fasted for 6 h beforehand with free access to water. Blood glucose was then measured using a handheld glucometer (One Touch UltraMini, LifeScan, Inc.) just before IP glucose injection (time 0; 2g/kg BW in saline) and subsequently at 15, 30, 45, 60, 90 and 120 min post-administration (n=7-15/group). For GDX mice on HFD, GTT was similarly performed after 6 wks on a HFD (13-15 wks old), using 1 g/kg BW glucose in saline (n=5/group).

Metabolic and locomotor analyses

For female *Kiss1r* mice on a standard chow diet, indirect calorimetry was performed at 18-21 wks old using a 12-cage equal flow CLAMS calorimeter system (Columbus Instruments) coupled with photosensors to detect movement. Females were habituated to the metabolic cages (single-housed) for 2 days prior to data acquisition (n=6/genotype). O_2 consumption (VO_2) and CO_2 production (VCO_2) were measured every 12 min/cage. Respiratory exchange ratio (RER) was calculated as the quotient of VCO_2/VO_2. Locomotor activity was measured in 1 minute intervals by photosensors, with the bottom row measuring horizontal movement (total = total number of photobeams broken; ambulatory = number of consecutive beams broken so as to exclude non-ambulatory movements like grooming) and the upper row measuring vertical movement (includes rearing, reaching the drinking tube, walking on top of the food hopper). In addition, feeding was measured in 12 min intervals and calculated for hourly feeding.

For GDX mice on HFD, indirect calorimetry was performed after 12 wks of HFD treatment (19-21 wks old) using a custom system consisting of 8 cages that measure O_2 and CO_2 coupled with infrared sensors to detect locomotion (TSE Systems) (8). HFD *Kiss1r* mice of both sexes were first acclimated for 72 h (n=4/genotype for each sex, single housed) after which VO_2 and VCO_2 were measured for 3 min/cage every 30 min. RER was calculated as the quotient of VCO_2/VO_2. Cage tops were equipped with infrared sensors (TSE InfraMot; TSE Systems) to detect body-heat image and its spatial (3 dimensional) displacement over time to assess general locomotor activity, measured as arbitrary 'InfraMot units'.

Supplemental References

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