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Supplementary Information for

Direct evidence that KNDy neurons maintain gonadotropin pulses and folliculogenesis as the GnRH pulse generator

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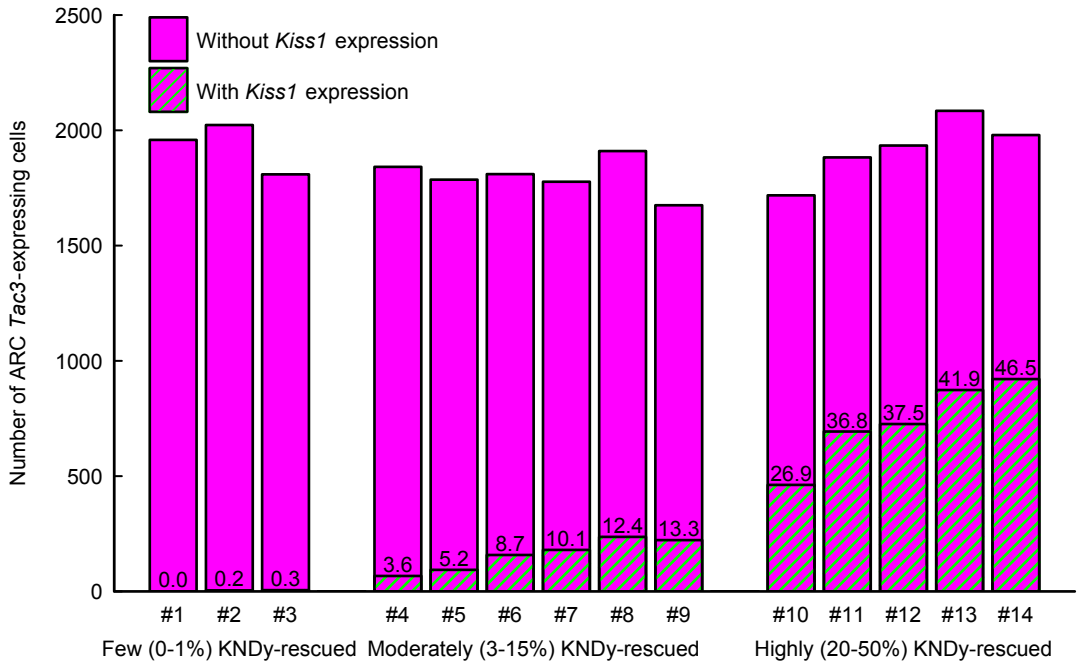

Fig. S1. The number of ARC *Tac3*-expressing cells with or without *Kiss1* expression in individual AAV-*Kiss1*–treated global *Kiss1* KO rats. According to the rescue rates of KNDy neurons by a transfecting CAG-driven *Kiss1* cDNA in *Tac3*-expressing neurons, the AAV-*Kiss1*–treated global *Kiss1* KO rats were divided into the three groups: few (0 to 1%), moderately (3 to 15%), and highly (20 to 50%) KNDy-rescued rats. Numbers in each column indicate the rescue rates of KNDy neurons out of *Tac3*-expressing cells in the ARC in each individual.

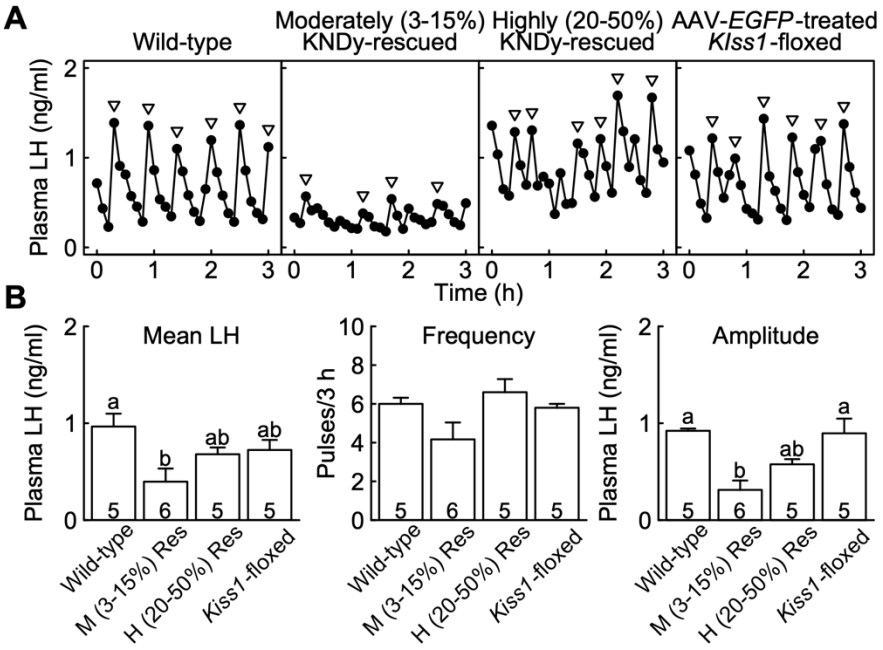

Fig. S2. Pulsatile LH secretion in wild-type and two genetically modified rats. (*A*) Plasma LH profiles in representative wild-type rats, moderately (3 to 15%) and highly (20 to 50%) KNDy-rescued rats, and AAV-*EGFP*–treated *Kiss1*-floxed rats. Arrowheads indicate LH pulses identified with the PULSAR computer program. (*B*) Mean LH concentration and the frequency and amplitude of LH pulses in the wild-type rats, moderately (3 to 15%) and highly (20 to 50%) KNDy-rescued rats, and AAV-*EGFP*–treated *Kiss1*-floxed rats. Values expressed in the bar graphs are mean ± SEM. Numbers in each column indicate the number of animals used. The values with different letters were significantly different from each other ($P < 0.05$) based on one-way ANOVA followed by Tukey's HSD test.

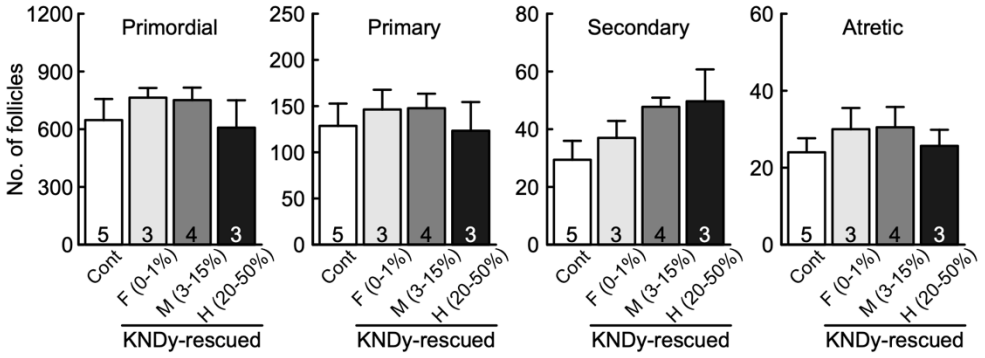

Fig. S3. The number of primordial, primary, secondary, and atretic follicles in the ovary of the KNDy neuron-rescued female rats. Values expressed in the bar graphs are mean ± SEM. Numbers in each column indicate the number of animals used. No significant differences were detected by one-way ANOVA.

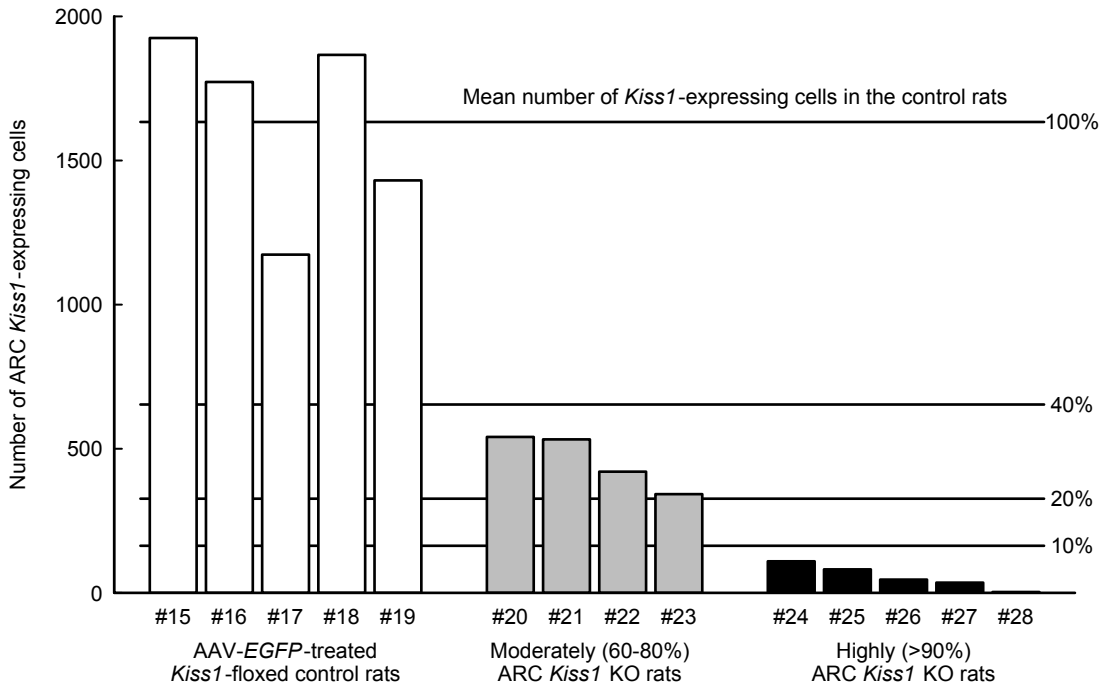

Fig. S4. The number of *Kiss1*-expressing cells in the ARC of individual AAV-*Cre*–treated *Kiss1*-floxed rats and AAV-*EGFP*–treated *Kiss1*-floxed control rats. According to the severity of ARC conditional *Kiss1* KO, AAV-*Cre*–treated *Kiss1*-floxed rats were divided into two groups: moderately (60 to 80%) ARC *Kiss1* KO rats, in which 20 to 40% *Kiss1*-expressing cells remained in the ARC and highly (>90%) ARC *Kiss1* KO rats, in which less than 10% *Kiss1*-expressing cells remained compared to the mean number of *Kiss1*-expressing cells in AAV-*EGFP*–treated *Kiss1*-floxed control rats, respectively.

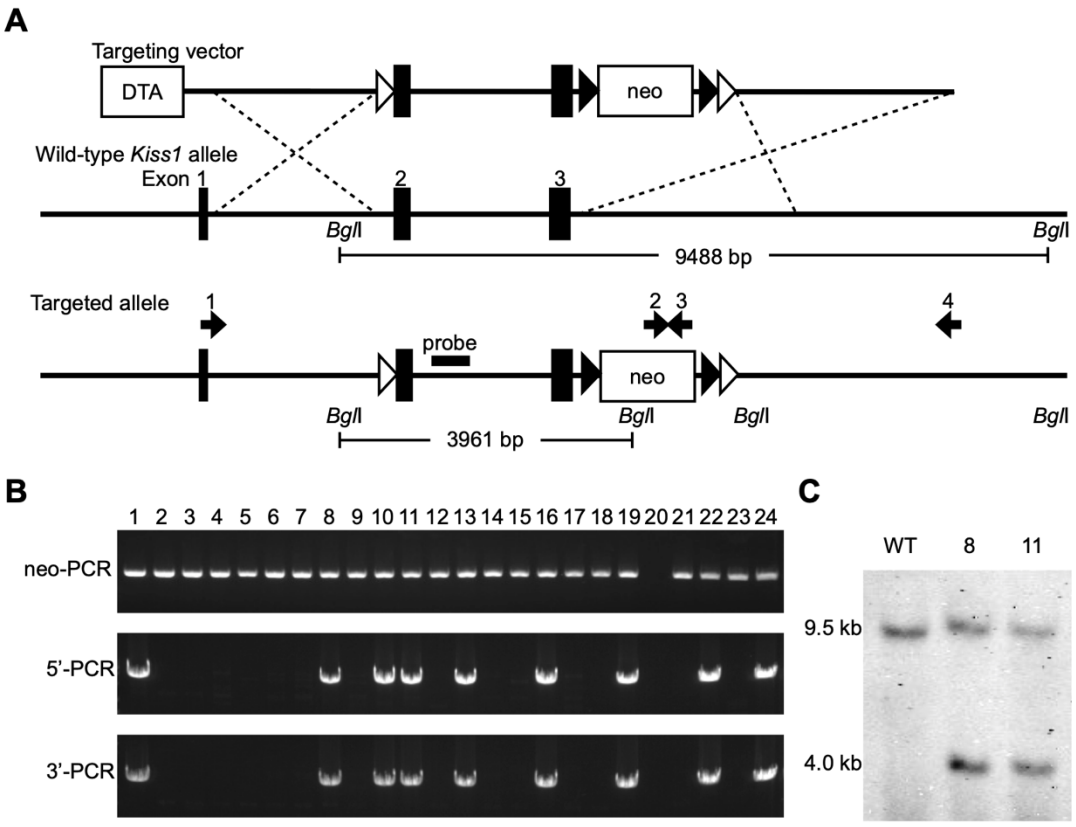

Fig. S5. Targeting of the *Kiss1* locus for the generation of *Kiss1*-floxed allele in rat ES cells. (*A*) Structure of the *Kiss1*-floxed targeting vector (top), the wild-type *Kiss1* allele (middle), and the targeted *Kiss1* allele (bottom), resulting from replacement recombination at the dotted lines. A diphtheria toxin A (DTA) expression cassette in the targeting vector was used for negative selection. (*B*) Screening of ES cells by PCR using primers (solid allows 1, 2, 3, and 4 in the panel *A*) located outside the 5' or 3' end of targeting vector and in the PGK-neo gene. Primer sets of neo-PCR (primers 2 and 3), 5'-PCR (primers 1 and 3), and 3'-PCR (primers 2 and 4) generate 691, 7011, and 4523 bp products, respectively, in the targeted *Kiss1*-floxed ES cells. (*C*) Southern blot analysis of *BglI*-digested DNA using a probe detected 4.0- and 9.5-kb fragments in the targeted *Kiss1* allele and wild-type *Kiss1* allele, respectively.

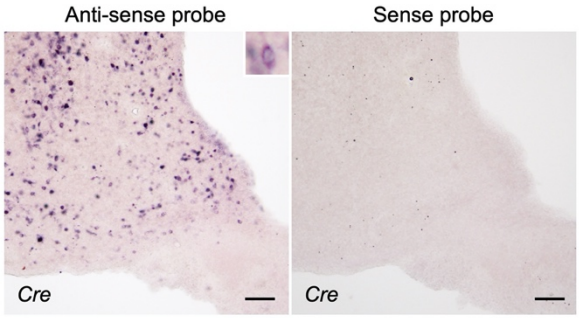

Fig. S6. *Cre*-expressing cells in the ARC of a representative AAV-*Cre*–treated *Kiss1*-floxed rat (left panel). *Inset* shows a representative *Cre*-expressing cell. No signals were found in section incubated with sense probe for *Cre* (right panel). (Scale bars, 100 μm.)

Table S1. Primer sequences for the ES cell selection, probe preparation for Southern blot analysis, genotyping of global *Kiss1* KO and *Kiss1*-floxed rats, and qRT-PCR analyses.

Purpose	Targets	Sequence forward reverse
ES cell selection	neo-PCR	5'-agaggctattcggctatgactg-3' 5'-gcgataccgtaaagcacgag-3'
	5'-PCR	5'-agtgtgctccaactacccaagt-3' 5'-gcgataccgtaaagcacgag-3'
	3'-PCR	5'-agaggctattcggctatgactg-3' 5'-tgtcccagaaggaaaccttg-3'
Southern blotting	DNA probe	5'-gcctgtcaaccctaactcct-3' 5'-gttcaaacccacaccctgac-3'
Genotyping	global *Kiss1* KO rats	5'-ccttgtttggggcttatcct-3' 5'-gatgacggccatgttgttgt-3'
	Kiss1-floxed rats	5'-tcctgcctgaccttaccaac-3' 5'-agtaccgattttggcaccag-3'
qRT-PCR	*Lhb* (NM_012858.2, NM_001033975.1)	5'-atgagttctgcccagtctgc-3' 5'-tggggaaggtcacaggtcat-3'
	Fshb (NM_001007597.2)	5'-agctgttgacttacctggcc-3' 5'-gggtgtttggtctagctggg-3'
	Gnrhr (NM_031038.3)	5'-ccagccttcatgatggtggt-3' 5'-gggatgatgaacaggcagct-3'
	Cyp19a1 (NM_017085.2)	5'-tggatggggattggaagtgc-3' 5'-cttgctgccgaatctggaga-3'
	Cyp17a1 (NM_012753.2)	5'-ggtgggagacatctttgggg-3' 5'-ttctcggatagtggcctcca-3'
	Lhcgr (NM_012978.1)	5'-caccatacccgggaatgctt-3' 5'-ggcctgcaatttggtggaag-3'
	Fshr (NM_199237.1)	5'-gacagagattccgaccgacc-3' 5'-ctggggagattctggaaggc-3'
	Actb (NM_031144.3)	5'-tgtcaccaactgggacgata-3' 5'-ggggtgttgaaggtctcaaa-3'

Gene symbols and protein names are as follows: *Actb,* β-actin; *Cyp17a1*, steroid 17α-hydroxylase; *Cyp19a1*, aromatase; *Fshb,* FSH β-subunit; *Fshr*, FSH receptor; *Gnrhr*, GnRH receptor; *Kiss1,* kisspeptin; *Lhb,* LH β-subunit; and *Lhcgr*, LH/chorionic gonadotropin receptor.