Supplementary Materials

Involvement of gonadotropin-inhibitory hormone in pubertal disorders induced by thyroid status

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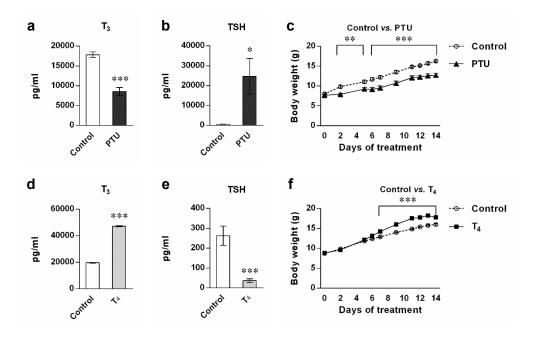
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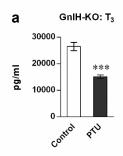
[†]These authors contributed equally to this work.

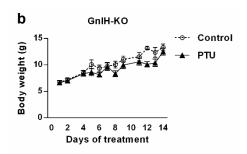
Supplementary Figure 1



Supplementary Figure 1. Successful induction of hypothyroidism and hyperthyroidism in mice. Hypothyroidism (PTU group, Upper panel) or hyperthyroidism (T_4 group, Lower panel) was confirmed in hormonal levels of T_3 (A or D), TSH (B or E) and body weight (C or F). 0.1% PTU or 0.001% T_4 was administered to immature female mice aged 20 days in drinking water for 2 weeks, respectively. Data represent mean \pm SEM (A and B, n = 7 per group; C and F, n = 10 per group, D and E, n = 6 in control and n = 7 in T_4 group). *p < 0.05; **p < 0.01; ***p < 0.001 by unpaired *t* test.

Supplementary Figure 2





Supplementary Figure 2. Induction of hypothyroidism into GnIH-knockout female mice. Effect of PTU administration in GnIH-KO female mice. 0.1% PTU was administered to GnIH-KO female mice aged 20 days in drinking water, then the changes in T_3 levels (A) and body weight (B) were measured. Data represent mean \pm SEM (B, n = 6 in WT and n = 7 in GnIH-KO group; A, n = 5 per group; B, n = 10 in control and n = 16 in PTU group). ***p < 0.001 by unpaired *t* test.

Supplementary Table 1

Target	Sequences	GenBank	Used in
GnIH-F	5'-TGGAAGGACCATAGATGAGAAA-3'	NM_021892.1	qPCR
GnIH-R	5'-GCTGTTGTTCTCCCAAACCT-3'		
Kiss1-F	5'-AGCTGCTGCTTCTCCTCTGT-3'	NM_178260.3	qPCR
Kiss1-R	5'-GCATACCGCGATTCCTTTT-3'		
GnRH-F	5'-AGCACTGGTCCTATGGGTTG-3'	NM_008145.2	qPCR
GnRH-R	5'-CCTGGCTTCCTCTTCAATCA-3'		
GAPDH-F	5'-ACAACTTTGGCATTGTGGAA-3'	NM_008084.2	qPCR
GAPDH-R	5'-GATGCAGGGATGATGTTCTG-3'		
TRα-F	5'-CACTCTTCCTGGAGGTCTTTGA-3'	NM_178060.2	ISH
TRα-R	5'-TCTATATTCTCCCCTTGCTTGG-3'		
TRβ-F	5'-TCCTCAGTGATGCGCTTATG-3'	NM_001113417.1	ISH
TRβ-R	5'-TGTGCATAAGATGGGGTCAG-3'		
- 2207F	5'-CTGAGTGATTCCCCAAGACC-3'	NM_021892.1	ChIP-qPCR
- 2207R	5'-TCTTGCTAGCTTGTATTATTCAGGT-3'		
- 1680F	5'-CTGGTGGAGCAGAAGGGTAG-3'	NM_021892.1	ChIP-qPCR
- 1680R	5'-CGCTCTAAGCAGGTCTCTGG-3'		
- 1257F	5'-TTCTTGGCTACCACAGACCA-3'	NM_021892.1	ChIP-qPCR
- 1257R	5'-CCCTGTTCCGAAGGTGTCTA-3'		
- 1014/999F	5'-GTGTGGAGGTGGATGGATCT-3'	NM_021892.1	ChIP-qPCR
- 1014/999R	5'-TTTTGGAGAGGCTGACTGGT-3'		
- 688F	5'-GAGCAGGAAGGTAGGACGTG-3'	NM_021892.1	ChIP-qPCR
- 688R	5'-GCAGCCTCCTTGTTATTTGC-3'		

Supplementary Table 1. Primers used in this study. Genomic sequence of GnIH promoter region was obtained from chromosome 6:50650672-50654439 bp of mouse genome.