Supporting Information

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SI Materials and Methods

Protein Isolation and Immunoblotting. HEK293T cells (ATCC) were maintained in DMEM supplemented with 10% (vol/vol) FBS and 1.5 g/l of sodium bicarbonate (Invitrogen). pcDNA3.1 encoding Kiss1r::myc or nothing was transfected by electroporation into HEK293T cells. After 48 h, proteins were isolated and immunoblotted, as described (1).

Immunofluorescence. The Kiss1r knockout (KO) mice have been described (2). To label sections from Kiss1r WT and KO mice with rabbit anti-GnRH (gonadotropin-releasing hormone) and anti-Kiss1r simultaneously, we used a modified double labeling protocol. Following tissue blocking and permeabilization, sections were incubated with anti-Kiss1r at 1:5,000 overnight at 4 °C, washed with PBS, and incubated with Alexa Fluor 546-conjugated goat anti-rabbit IgG for 1 h at room temperature. Sections were washed with PBS and incubated with 5% (vol/vol) normal rabbit serum in PBS for 1h at room temperature to saturate open binding sites on the first secondary, followed by washing with PBS and incubation with 20 μg/mL goat anti-rabbit Fab fragments (Jackson ImmunoResearch) in PBS for 2 h at room temperature to block any exposed rabbit IgG binding sites. Sections were then washed with PBS and incubated with anti-GnRH at 1:1,000 in PBS overnight at 4 °C. Sections were washed with PBS, incubated with Alexa Fluor 488-conjugated goat anti-rabbit IgG in PBS, washed with PBS again, and mounted. Rabbit anti-adenylyl cyclase III (C-20; Santa Cruz Biotechnology) was used at 1:350. GnRH::GFP tissue was labeled with chicken anti-rootletin (3) at 1:1,000 and anti-Kiss1r at 1:5,000. Secondary antibodies were donkey anti-chicken Cy3 (Jackson ImmunoResearch) and Alexa Fluor 647-conjugated donkey anti-rabbit IgG (Invitrogen).

Quantification of Cilia. In sections from Kiss1r WT and KO mice (n = 3 of each genotype), GnRH neurons were identified and examined for the presence of a Kiss1r-positive cilium. The total number of GnRH neurons with Kiss1r-positive cilia was divided by the total number of GnRH neurons counted and multiplied by 100 to obtain the percentage of GnRH neurons possessing Kiss1r-positive cilia. At least 35 GnRH neurons were counted across sections from each genotype. Sections from Cilia^{GFP} mouse brains were labeled with an antibody against GnRH and images of non-GnRH neurons expressing GFP+ cilia were taken. The non-GnRH+ nuclei in each image were counted and then examined for GFP+ cilia. At least 40 nuclei were counted in each section.

Quantification of GnRH Neurons. For P0 neuron migration analysis, sections for P0 animals were matched to plate 13 of the coronal gestation day (GD) 18 atlas from the Schambra, Lauder and Silver prenatal mouse brain atlas (4). For each animal, four sections containing the paraventricular hypothalamic nucleus (pvh) were analyzed. Reductions in the number of GnRH neurons present in this caudal region at P0 is an indication of aberrant GnRH neuronal migration (5). The number of GFP+ neurons in this particular region was determined by examination under the 20× objective. The numbers counted in the four sections were added together to obtain the number for that animal. The numbers from each animal were then averaged to calculate the mean and SE by genotype.

Real-Time PCR. Total RNA was made from the hypothalami of adult GnRH^{cilia+} and GnRH^{cilia-} mice (n = 4 animals of each genotype) using the RNeasy Miniprep kit (Qiagen), according to manufacturer's instructions. The extracted RNA was treated with DNase using the Ambion DNA-*free* Kit (Ambion) to eliminate genomic DNA contamination. Oligo(dT)$_{12–18}$ primed cDNA was made from purified RNA using Invitrogen SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA). For each sample, 300 ng of RNA was used in the cDNA synthesis reaction. Control "No RT" reactions lacking reverse transcriptase were also performed to test for genomic DNA contamination. cDNA was quantified by qPCR, using the POWER SYBR Green Master Mix (Applied Biosystems) on an ABI Prism 7000 Sequence Detection System (Applied Biosystems). The primers used for β actin were forward 5′-tacagcttcaccaccacagc-3′ and reverse 5′-tctccagggaggaagaggat-3′ to produce a 121-bp product. The primers used for Kiss1r were forward: 5′-ttcaccgcactcctctaccc-3′ and reverse 5′-cacataccagcggtccacac-3′ to produce a 138–bp product. The primers used for GnRH were forward: 5′-cactggtcctatgggttgcg-3′ and reverse: 5′-gctggggttctgccatttga-3′ for a 106-bp product. The efficiency of each primer pair was tested. Reactions were set up in triplicate. Per well, 20-μL reactions consisted of: 10 μL of 2× Power SYBR Green Master Mix, 7.8 μL of DEPC treated DNase, RNase free water (Invitrogen), 0.6 μL of each forward and reverse primer at 10 μM concentration (Integrated DNA Technologies), and 1 μL of diluted cDNA. Data were normalized to mouse β actin expression and was analyzed to determine ΔC_t of Kiss1r and GnRH expression. Cycle threshold was taken at 0.2 ΔRn. Melt curve analysis indicated single products.

Fos Labeling. Gonad intact adult male GnRH^{cilia+} and GnRH^{cilia-} mice were anesthetized with isoflurane and then injected ICV with either aCSF (vehicle) or aCSF containing 200 pmol KiSS (112–121) Amide. Two hours after injection, mice were transcardially perfused with 4% (wt/vol) paraformaldehyde and brains were collected for immunofluorescence. Sections were labeled with Rabbit anti C-Fos (sc-253; Santa Cruz) and Alexa Fluor 546-conjugated goat anti-rabbit IgG, along with DRAQ-5 as a nuclear marker. Five sections containing GFP+ neurons from each animal were evaluated. The total number of GFP+ neurons with C-Fos positive nuclei was divided by the total number of GFP+ neurons counted in each animal and multiplied by 100 to obtain the percentage of C-Fos–positive GnRH neurons in each animal. At least 50 GFP+ neurons were evaluated per animal.

1. Domire JS, et al. (2011) Dopamine receptor 1 localizes to neuronal cilia in a dynamic process that requires the Bardet-Biedl syndrome proteins. *Cell Mol Life Sci* 68(17): 2951–2960.
2. Kauffman AS, et al. (2007) The kisspeptin receptor GPR54 is required for sexual differentiation of the brain and behavior. *J Neurosci* 27(33):8826–8835.
3. Yang J, et al. (2002) Rootletin, a novel coiled-coil protein, is a structural component of the ciliary rootlet. *J Cell Biol* 159(3):431–440.
4. Schambra UB, Lauder JM, Silver J (1992) *Atlas of the Prenatal Mouse Brain* (Academic Press, San Diego), pp xxix, 327 pp.
5. Heger S, et al. (2003) Overexpression of glutamic acid decarboxylase-67 (GAD-67) in gonadotropin-releasing hormone neurons disrupts migratory fate and female reproductive function in mice. *Endocrinology* 144(6):2566–2579.

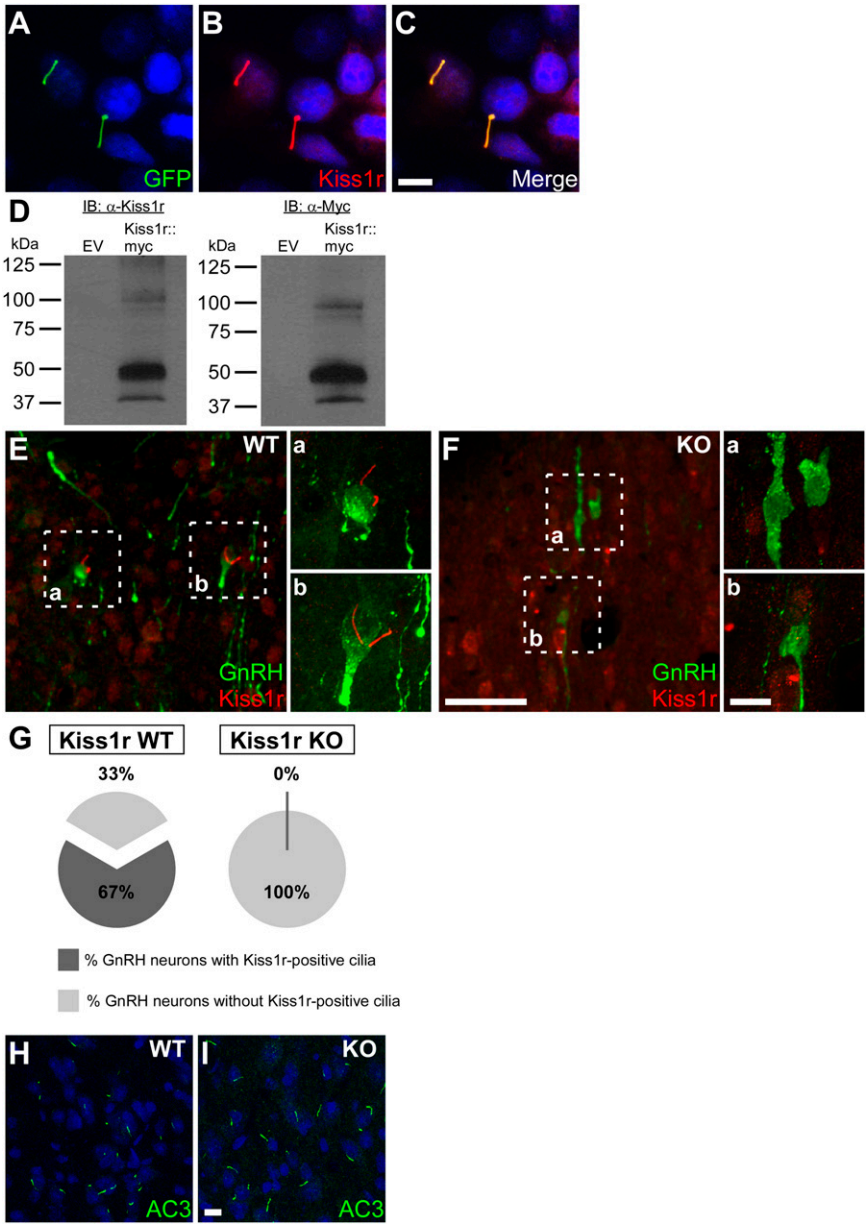


Fig. S1. Kiss1r polyclonal antibody specifically recognizes mouse Kiss1r in immunofluorescent and Western blot analysis. (*A*–*C*) Representative image of transiently transfected IMCD cells expressing Kiss1r fused at the carboxy-terminus to EGFP. (*A*) EGFP fluorescence (GFP; green) shows expression of Kiss1r. (*B*) Kiss1r polyclonal antibody (Kiss1r; red) marks Kiss1r. (*C*) Merged images confirming anti-Kiss1r labels Kiss1r::EGFP. (Scale bar: 10 μm.) (*D*) Extracts from HEK293T cells expressing myc-tagged Kiss1r (Kiss1r::myc) analyzed by Western blotting (IB) with anti-Kiss1r (*Left*) and anti-myc (*Right*). Extracts from HEK293T cells expressing empty vector (EV) are included as a negative control. Note the presence of a band around 43 kDa, which is the predicted molecular weight of Kiss1r::myc, specifically in extracts from cells expressing Kiss1r::myc. (*E* and *F*) Representative images of the medial hypothalamus in adult Kiss1r (*E*) wild-type (WT; *E*) and knockout (KO; *F*) mice ($n = 3$ animals of each genotype) colabeled with anti-GnRH (green) and anti-Kiss1r (red). Corresponding *Insets* (*a* and *b*) show higher magnification images of the boxed regions containing GnRH-positive neurons. Note that GnRH neurons in the WT section possess Kiss1r-positive cilia, whereas GnRH neurons in the KO section do not. (Scale bars: 50 μm in main images and 10 μm in *Insets*.) (*G*) The majority of GnRH neurons in sections from Kiss1r WT mice possessed Kiss1r-positive cilia (24 of 36 GnRH neurons). Kiss1r-positive cilia were not detected (0 of 35 GnRH neurons) in Kiss1r KO sections. (*H* and *I*) Representative images of the medial hypothalamus in adult Kiss1r WT (*H*) and KO (*I*) mice ($n = 3$ animals of each genotype) labeled with anti-AC3 (green). Note the presence of AC3-positive cilia in both genotypes. Nuclei were stained with DRAQ5. (Scale bar: 10 μm.)

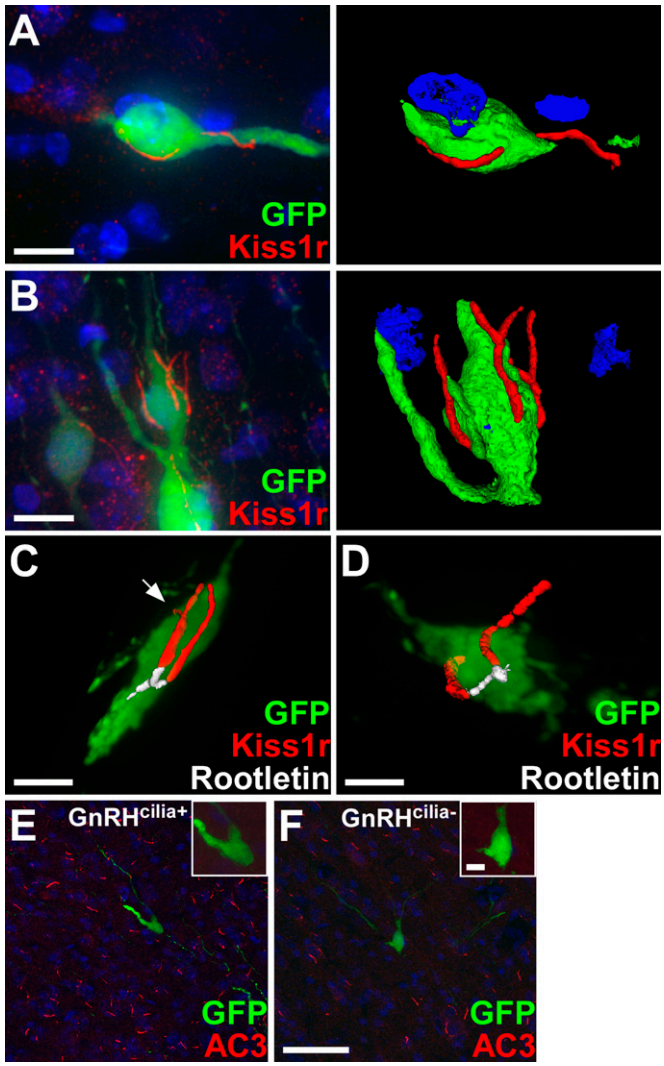


Fig. S2. GnRH neurons project multiple Kiss1r-positive cilia and cilia on non-GnRH neurons are positive for AC3. (*A* and *B*) Representative images of the medial hypothalamus in adult GnRH::GFP mice. GFP fluorescence (green) indicates a GnRH neuron. Labeling for Kiss1r (red) shows the presence of multiple Kiss1r-positive cilia. Two-dimensional projections (*Left*) and corresponding 3D renderings (*Right*) are shown. Nuclei are stained with DRAQ5 (blue). (Scale bar: 10 μm.) (*C* and *D*) Representative images of the medial hypothalamus in adult GnRH::GFP mice. GFP fluorescence (green) indicates a GnRH neuron. Labeling for Kiss1r (red) shows the presence of multiple Kiss1r-positive cilia. Labeling for Rootletin (white) marks the base of each cilium and confirms the cilia are projecting from the same neuron. The cell bodies are displayed as maximum projections and the cilia and rootlets are isosurfaced. Note in *C* the presence of two cilia that project from the cell in parallel and diverge at their tips (indicated by an arrow). (Scale bars: 5 μm.) Representative images of the medial hypothalamus in adult $GnRH^{cilia+}$ (*E*) and $GnRH^{cilia-}$ (*F*) mice. EGFP fluorescence (green) indicates a GnRH neuron. Labeling for AC3 (red) confirms cilia on non-GnRH neurons are positive for AC3 in both $GnRH^{cilia+}$ and $GnRH^{cilia-}$ mice. Note the lack of AC3-positive cilia on GnRH neurons. Nuclei are stained with DRAQ5 (blue). (Scale bars: 50 μm in main images and 5 μm in *Insets*.)

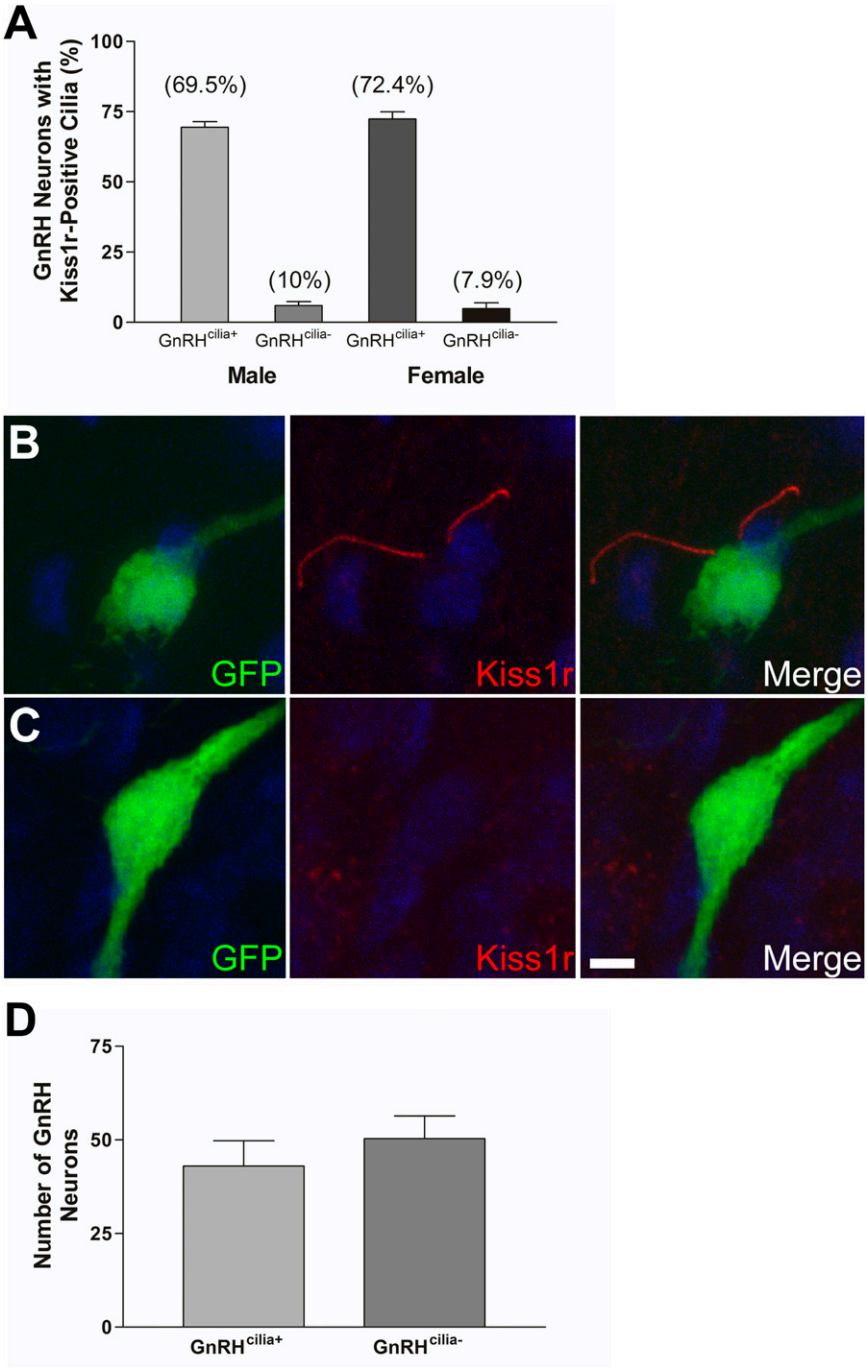


Fig. S3. Loss of Kiss1r-positive cilia primarily occurs prenatally in both male and female GnRH^{cilia-} mice and does not impact GnRH neuronal migration. (*A*) Percentages of GnRH neurons with a Kiss1r-positive cilium in the medial hypothalamus of P0 male and female GnRH^{cilia+} and GnRH^{cilia-} mice ($n = 3$ animals for each sex and genotype). There is no significant difference between the percentage of GnRH neurons with cilia in male and female GnRH^{cilia+} mice or male and female GnRH^{cilia-} mice. (*B* and *C*) Representative images of the medial hypothalamus in adult GnRH^{cilia+} (*B*) and GnRH^{cilia-} (*C*) mice. EGFP fluorescence (green) indicates a GnRH neuron. Labeling for Kiss1r (red) shows the presence of cilia in GnRH^{cilia+} mice (*B*) and the absence of cilia in GnRH^{cilia-} mice (*C*). Nuclei were stained with DRAQ5. (Scale bar: 5 μm.) (*D*) Number of GnRH neurons in the paraventricular hypothalamic nucleus of P0 male GnRH^{cilia+} and GnRH^{cilia-} mice ($n = 3$ animals for each genotype). Note there is no significant difference in the number of GnRH neurons in this region between GnRH^{cilia+} and GnRH^{cilia-} mice. Values are expressed as mean ± SEM.

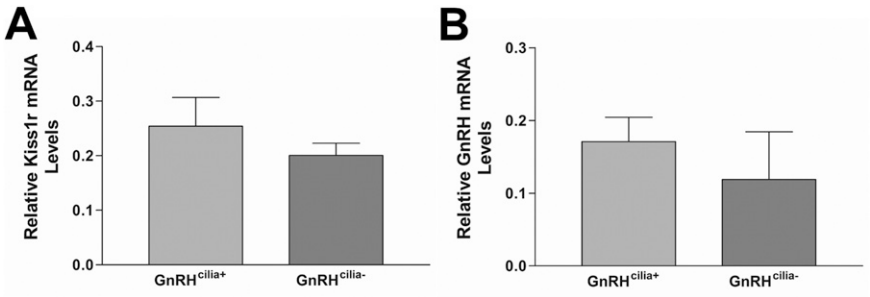


Fig. S4. Kiss1r is expressed at equivalent levels in GnRH^{cilia+} and GnRH^{cilia-} mice. Real-time PCR data representing the relative level of Kiss1r (*A*) and GnRH (*B*) mRNA expression compared with β actin (Kiss1r or GnRH mRNA/β actin mRNA) as determined by quantitative real time PCR using the SYBR green method. Note the relative level of Kiss1r mRNA in the hypothalamus of GnRH^{cilia-} mice is not significantly different from GnRH^{cilia+} mice ($P = 0.384$). RNA was extracted from hypothalamic tissue from adult male GnRH^{cilia+} and GnRH^{cilia-} mice ($n = 4$ animals for each genotype). Values are expressed as mean ± SEM.

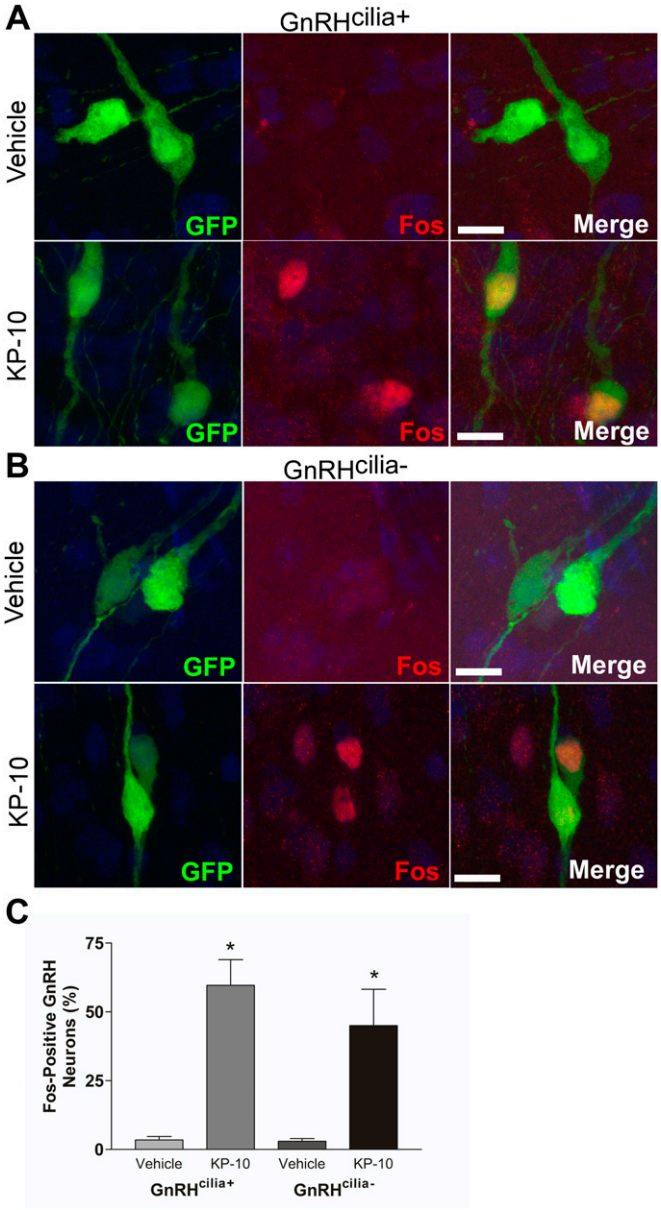


Fig. S5. Intracerebroventricular injection of kisspeptin induces Fos expression in GnRH^{cilia+} and GnRH^{cilia-} mice. GnRH neurons (green) from GnRH^{cilia+} (*A*) and GnRH^{cilia-} (*B*) gonad intact male mice ($n = 3$ animals for each genotype) treated with aCSF (vehicle; *Upper*) show little Fos expression (red). GnRH neurons from GnRH^{cilia+} and GnRH^{cilia-} mice ($n = 3$ animals for each genotype) treated with 200 pmol kisspeptin (KP-10; *Lower*) show induction of Fos expression (red). (*C*) Percentages of GnRH neurons positive for Fos labeling in GnRH^{cilia+} and GnRH^{cilia-} mice in response to aCSF and kisspeptin injection. The percentages of GnRH neurons positive for Fos labeling is low in response to aCSF injection but is significantly increased in response to kisspeptin injection in both GnRH^{cilia+} and GnRH^{cilia-} mice. There was no significant difference in Fos expression between genotypes in response to aCSF or kisspeptin injection. Values are expressed as mean ± SEM *Significantly different from aCSF injection ($P < 0.05$).

Table S1. Quantification of cilia frequency on non-GnRH nuclei

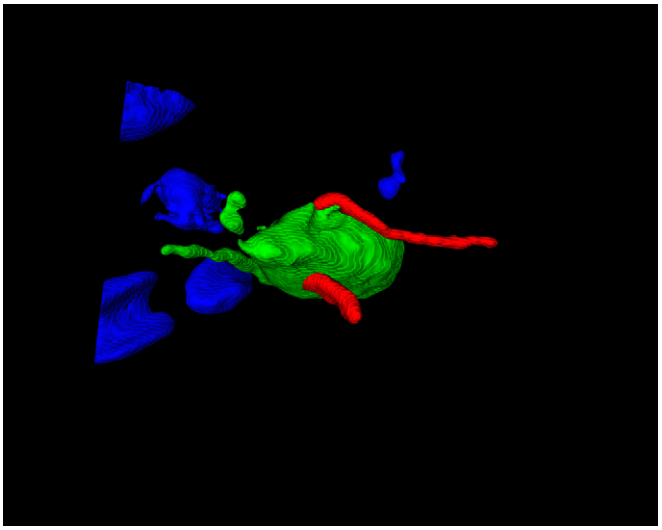
Nuclei	Ciliated nuclei	Multiciliated nuclei
337	240	0

Frequency of cilia and multiple cilia on non-GnRH nuclei in medial hypothalamic sections ($n = 2$) from P60 Cilia^{GFP} mice ($n = 3$).

Table S2. Physiological measures of sexual maturation in GnRH^{cilia+} and GnRH^{cilia-} mice

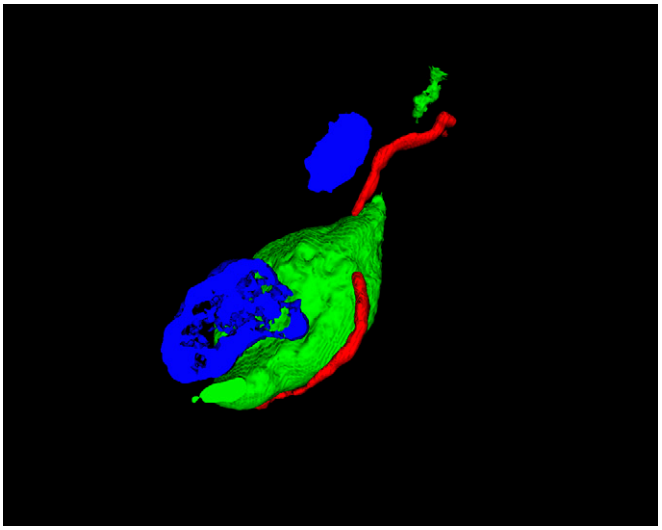
	Male		Female	
	GnRH^{cilia+}	GnRH^{cilia-}	GnRH^{cilia+}	GnRH^{cilia-}
Body weight, g	22.6 ± 0.85	23.8 ± 0.087	19.8 ± 1.03	20.3 ± 0.97
Sex organ weight, mg	153.4 ± 9.1	146.4 ± 9.5	7.6 ± 0.8	7.5 ± 0.7
Time of vaginal opening, day	NA	NA	28.5 ± 0.6	28.0 ± 0.6

Physiological measurements from GnRH^{cilia+} and GnRH^{cilia-} animals ages 2.5–4 mo (n= 8–12). Sex organ refers to both testis for each male and both ovaries for each female. Values are expressed as the mean ± SEM.



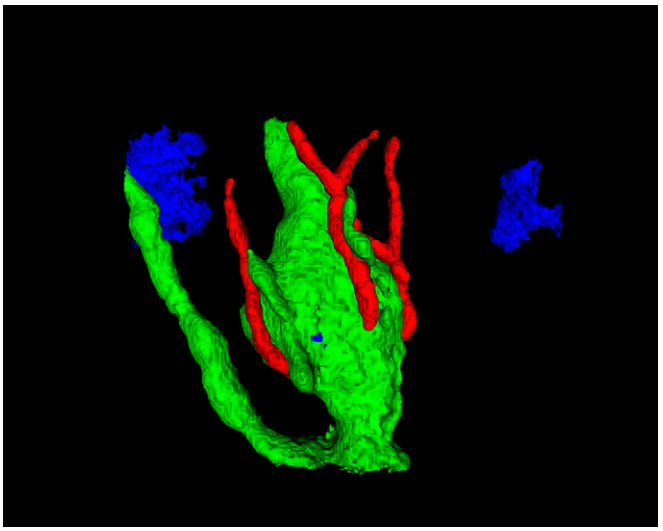
Movie S1. A full rotation (360 degrees) around the vertical axis of the 3D rendering presented in Fig. 1*G*. Movie length is 5 s with 20 frames per s.

Movie S1



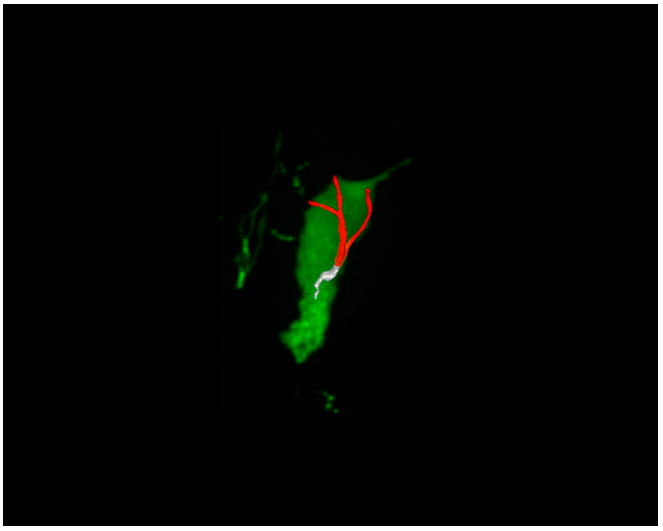
Movie S2. A full rotation (360 degrees) around the horizontal axis of the 3D rendering presented in Fig. S2*A*. Movie length is 5 s with 20 frames per s.

Movie S2



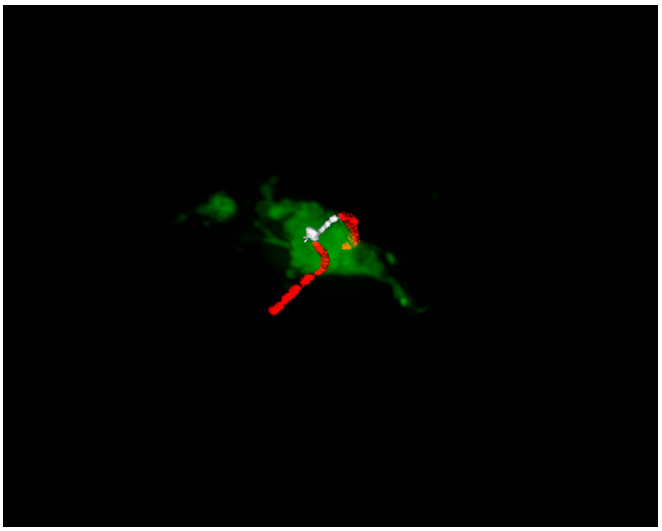
Movie S3. A full rotation (360 degrees) around the vertical axis of the 3D rendering presented in Fig. S2*B*. Movie length is 5 s with 20 frames per s.

Movie S3



Movie S4. A full rotation (360 degrees) around the vertical axis of the 3D rendering presented in Fig. S2*C*. Movie length is 10 s with 20 frames per s.

Movie S4



Movie S5. A full rotation (360 degrees) around the vertical axis of the 3D rendering presented in Fig. S2*D*. Movie length is 10 s with 20 frames per s.

Movie S5