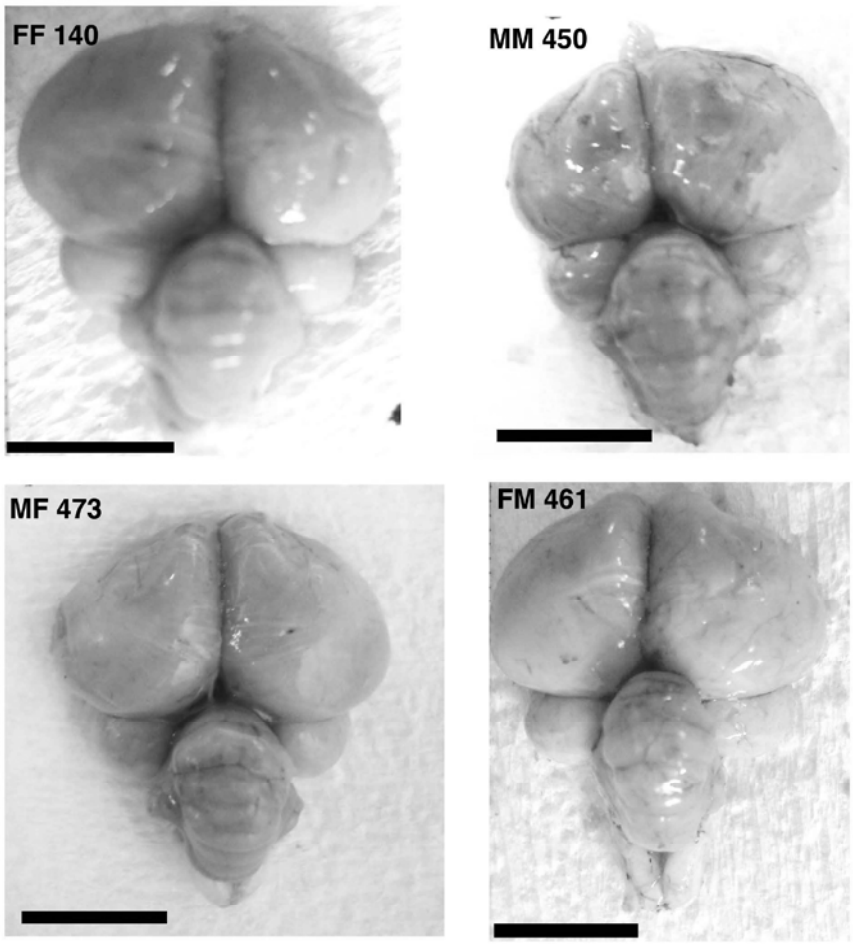
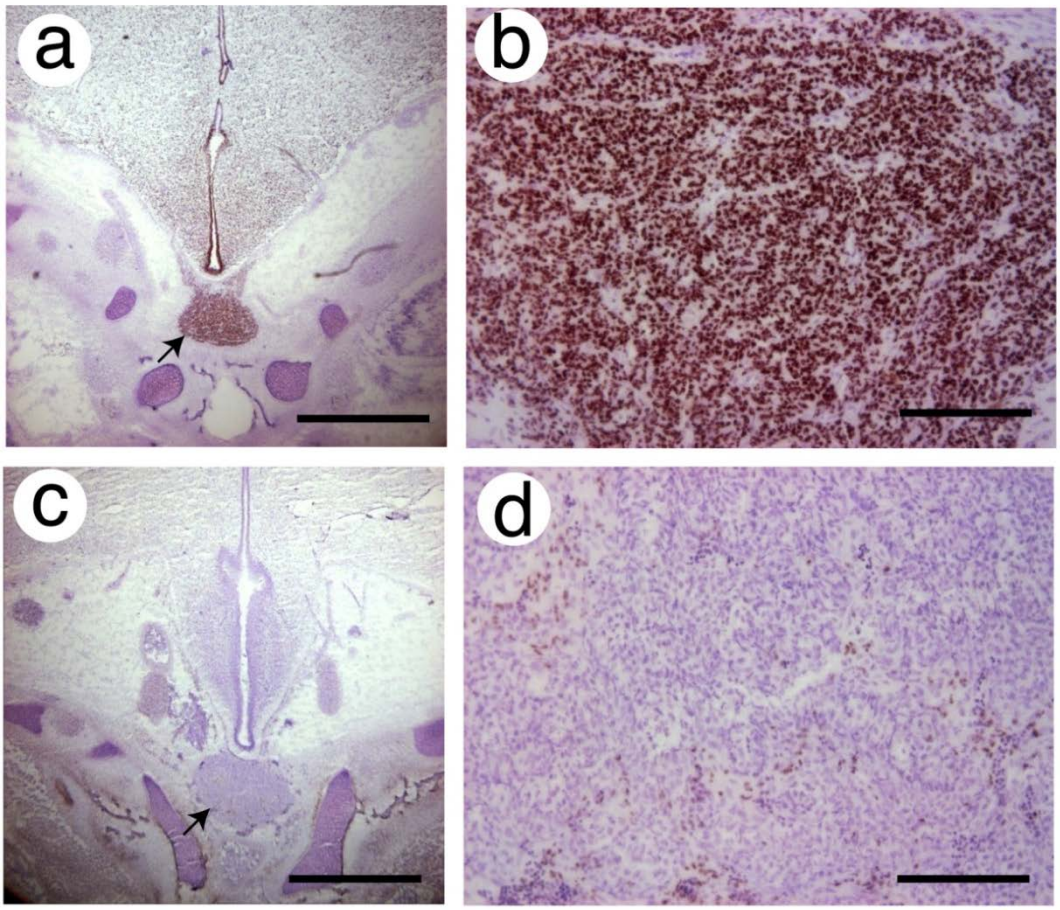
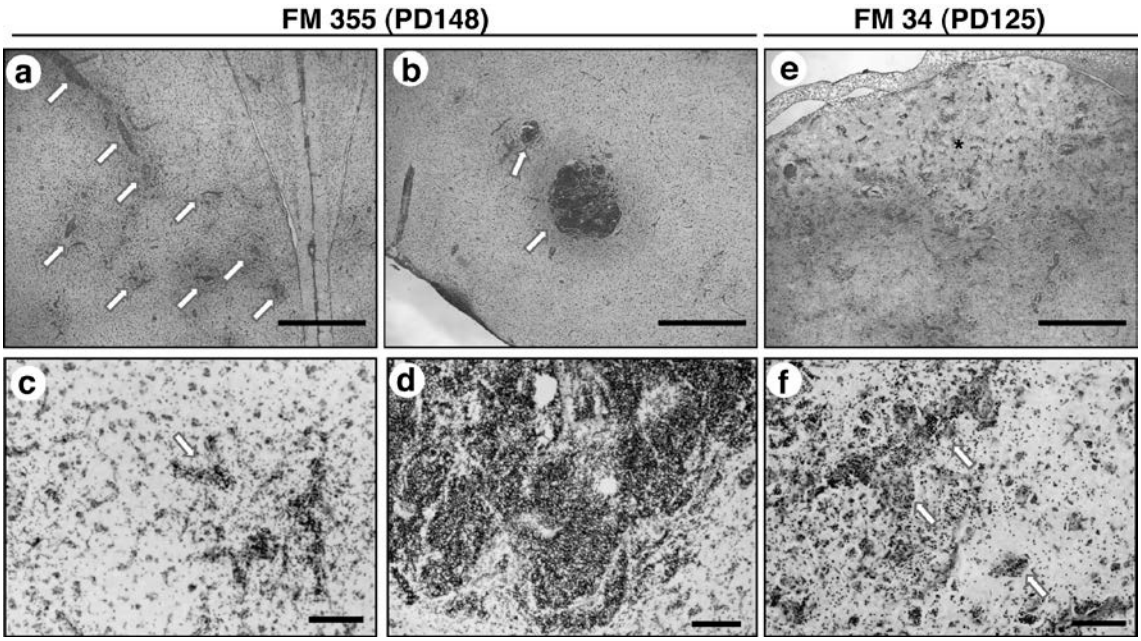


Supplementary Figure S1. Representative dorsal views of adult chimera brains.
Scale bars, 1 cm.



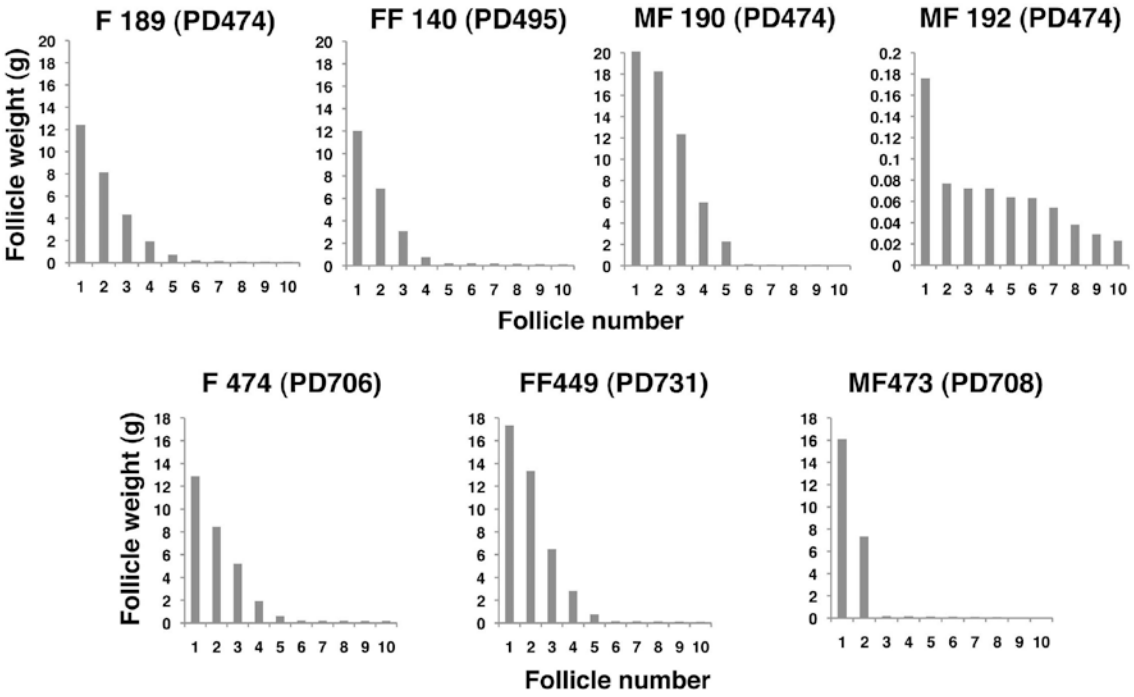
Supplementary Figure S2. Transplantation of the whole brain primordium (WB) between quail and chick embryo revealed graft origin of most of the pituitary tissue.

Grafting was performed as in chicken chimeras, but either with quail donor and chick recipient (**a, b**) or chick donor and quail recipient (**c, d**). At ED12-13 (*n*=2 for each combination), brain was removed and processed for staining with QCPN antibody which could detect quail-specific antigen. High-magnification image of the pituitary (arrows) in **a** or **c** is shown in **b** or **d**, respectively. Brown, QCPN-positive cells. Scale bars in **a, c,** 1 mm; **b, d,** 200 μm.



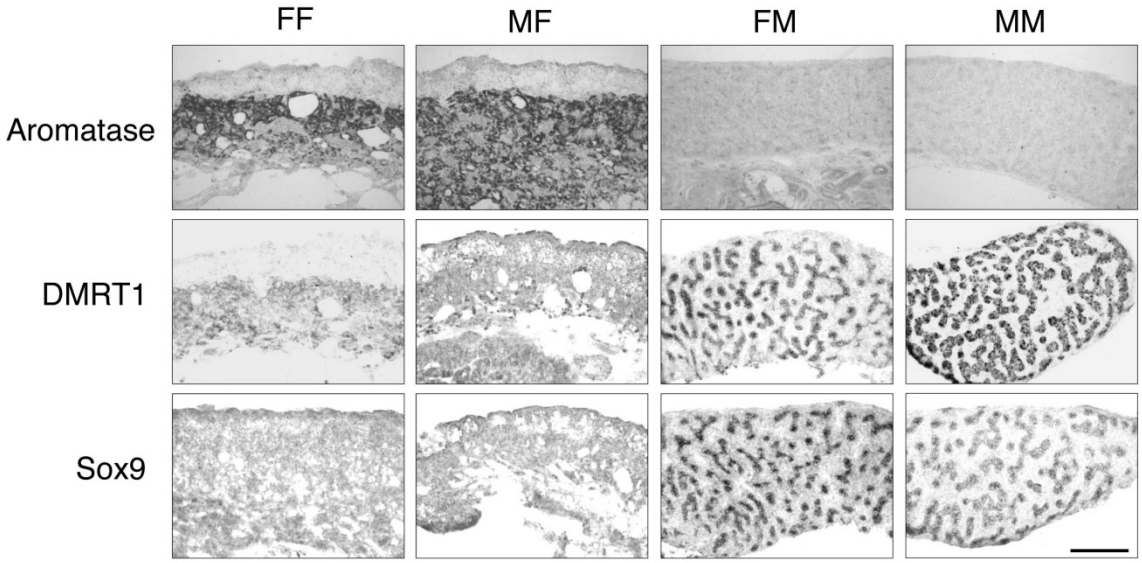
Supplementary Figure S3. Brain sections from FM chimeras untreated with immunosuppressive agent.

a-d, In the grafted tissue of FM 355 at PD148, leucocytes infiltration was frequently observed (**a-c**, white arrows). Tissue was injured in the infiltrated area (**d**). **e**, **f**, Brain tissue was extremely damaged in the FM 34 at PD125 (asterisk in **e**, white arrows in **f**). Nissl staining. Scale bars in **a**, **b**, **e**, 1 mm; **c**, **d**, **f**, 100 μm.



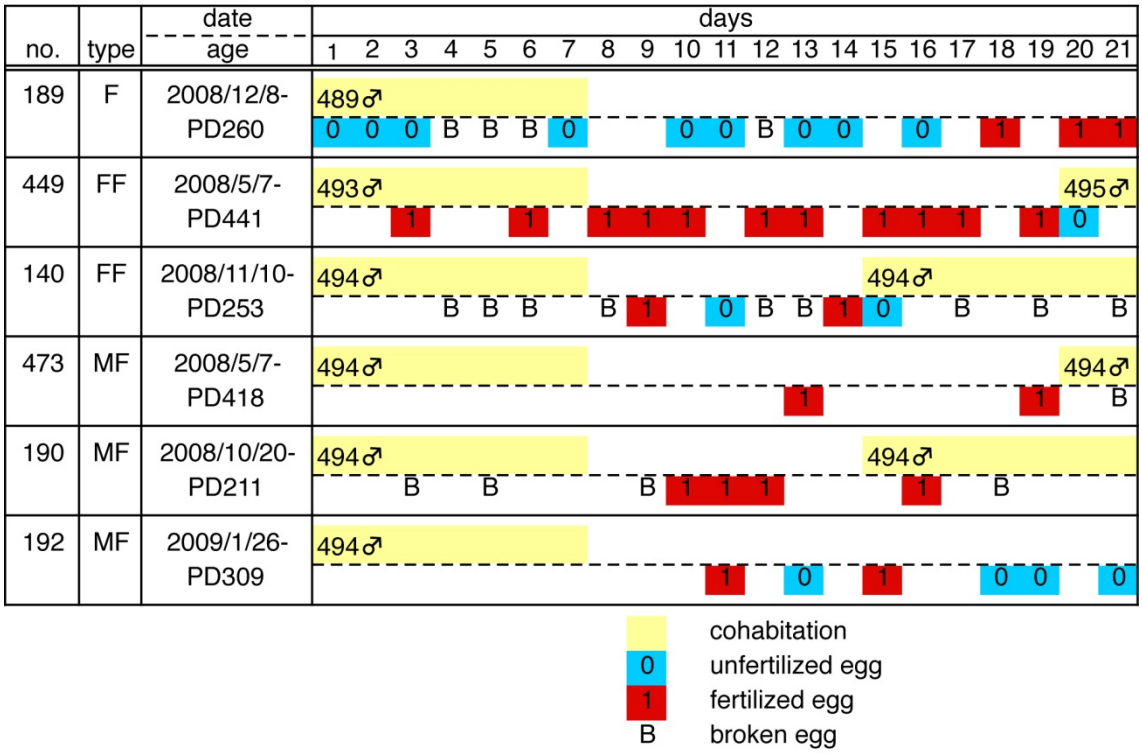
Supplementary Figure S4. Weights of each follicle dissected from the ovary were arranged in descending order.

In MF 190 chimera at 15 months, over-developed follicles weighed 20 and 18 g were observed. And in the ovary of MF 192 chimera at same age, only tiny follicles smaller than 0.2 g were observed besides necrotic follicles (not presented in the graph). In MF 473 chimera at about 24 months, smaller number of developing follicles was found as compared with F control and FF chimera of same age.



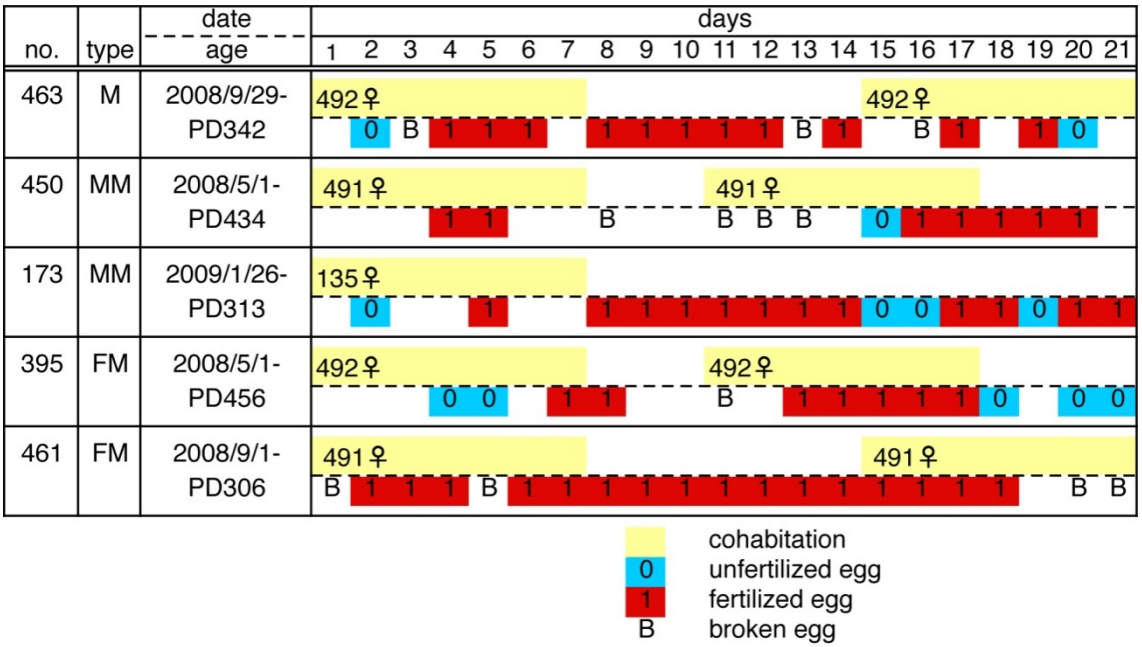
Supplementary Figure S5. Gonads of the chimeras developed normally according to the sex of the body.

Gonads were removed from the WB chick chimeras at ED17-19 and processed for histology. *In situ* hybridization was performed using *Aromatase*, *DMRT1* or *Sox9* cRNA probes. *Aromatase* expression is known to be restricted to the medullary region of the ovary. DMRT1 and Sox9 serve as markers of sex cords of testis. Scale bar, 200 μm.



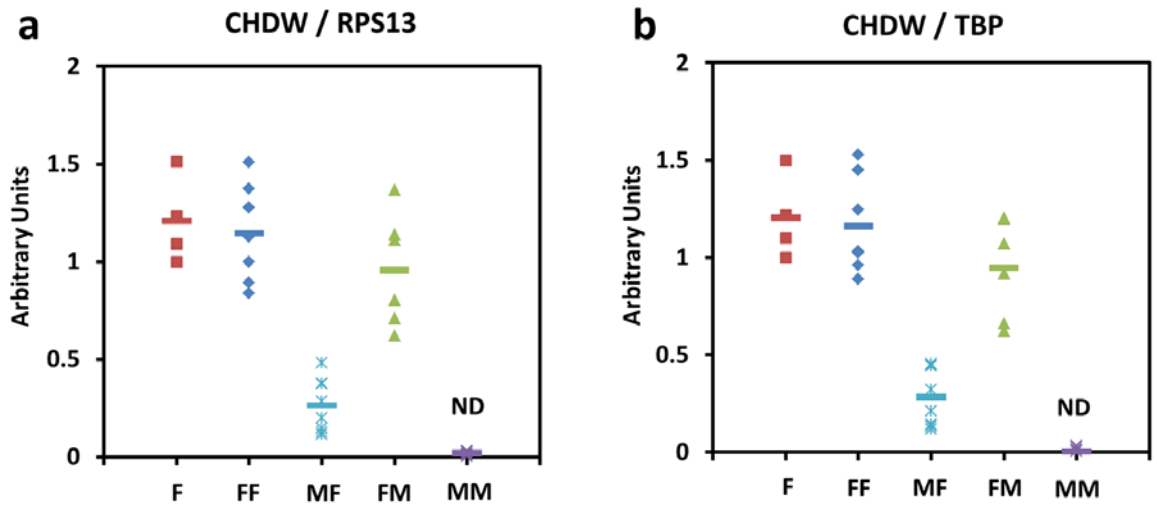
Supplementary Figure S6. MF chimeras were fertile.

F controls, FF and MF chimeras were housed with a cock for 7 days and the eggs were collected and incubated. Fertilized eggs were obtained from each type of birds.



Supplementary Figure S7. FM chimeras were fertile.

M controls, MM and FM chimeras were housed with a hen for 7 days and the eggs were collected and incubated. Fertilized eggs were obtained in every case.



Supplementary Figure S8.
Evaluation of *CHD-W* gene amplification of genomic DNA obtained from brain of chimeras at ED21 using real-time PCR (See Supplementary Methods).

Supplementary Table S1: Details of the statistics.

Fig. 2	**a**	F vs MF, p<0.0001; FF vs MF, p<0.0001.
	b	F vs MF, p<0.0001; FF vs MF, p=0.0004.
	c	F vs MF, p=0.0002; FF vs MF, p=0.0023.
Fig. 3	**b**	F vs FM, p=0.0074; F vs M, p<0.0001; F vs MM, p=0.0011; FF vs M, p= 0.0280; FF vs MM, p=0.0260; MF vs FM, p=0.0091; MF vs M, p=0.0002; MF vs MM, p=0.0004.
Fig. 4	**a**	4-6: F vs FM, p=0.0006; F vs M, p<0.0001; F vs MM, p<0.0003; FF vs FM, p=0.0197; FF vs M, p<0.0069; FF vs MM, p<0.0239; MF vs FM, p=0.0023; MF vs M, p=0.0001; MF vs MM, p<0.0019. 7-9: F vs FF, p=0.0212; F vs FM, p=0.0003; F vs M, p<0.0001; F vs MM, p=0.0001; MF vs FM, p=0.0221; MF vs M, p=0.0144; MF vs MM, p=0.0182. 10-12: F vs FM, p=0.0016; F vs M, p<0.0001; F vs MM, p=0.0009; MF vs FM, p=0.0235; MF vs M, p<0.0116; MF vs MM, p<0.0241.
	b	0-3: M vs F, p<0.0001; M vs FF, p=0.0099; M vs FM, p=0.0470; M vs MF, p=0.0018; MM vs F, p=0.0088; MM vs MF, p=0.0322. 4-6: M vs F, p<0.0001; M vs FF, p=0.0019; M vs MF, p<0.0001; MM vs F, p=0.0109; MM vs MF, p=0.0245. 7-9: M vs F, p=0.0040; M vs MF, p=0.0229; MM vs MF, p=0.0351; MM vs F, p=0.0160. 13-: M vs F, p<0.0001; M vs FF, p=0.0069; M vs MF, p=0.0008; MM vs F, p=0.0404; FM vs F, p=0.0181.
	e	F vs M, p=0.0002; F vs MM, p=0.0062; FF vs M, p=0.0029; FF vs MM, p=0.0233; MF vs M, p=0.0005; MF vs MM, p=0.0080.
Fig. 6.	**a**	ED21: F vs M, p=0.0031. 13 months: F vs M, p=0.045
	b	FF vs MF, p<0.0001; FF vs MM, p=0.0022, MF vs FM, p=0.0006; FM vs MM p=0.0302

Supplementary Methods

Sexing

As a first-round screening to see the combination of donor and recipient sex, we used multiplex PCR-based detection of the W-chromosome–specific chromohelicase domain (*CHD-W*) gene and the Z-chromosome–specific *CHD-Z* gene in DNA samples obtained from the crest (donor tissue), blood, brain and liver. Sequences of primers were 5'-ctt cca aga atg aga aac tgt gc-3' and 5'-tgt ttt cac ggg gaa tag ttc g-3' for *CHD-W* gene, and 5'-ccc gag gat gag aaa ctg tgc-3' and 5'-cta ctg cgt ctt cct tca ctc-3' for *CHD-Z* gene. To confirm the results from the first-round screening of the brain tissue, we used mRNA *in situ* hybridization of *HINTW*, which is a W chromosome–specific gene (see below).

Alternately, for chimeric embryos used for analysis of steroid contents, *CHD-W* gene amplification was evaluated by real-time PCR (CFX96, Bio-Rad Laboratories). Briefly, following a denaturation step at 98°C for 2 min, PCR was carried out with a thermal protocol consisting of 98°C for 5 s and 55°C for 5 s in 20 μl buffer containing 2×SsoFast EvaGreen Supermix and 0.5 μM of a pair of primers specific to chicken *CHD-W* gene. Genes for chicken ribosomal protein S13 (*RPS13*) and TATA box binding protein (*TBP*) were served as endogenous control. Primers used were as follows: *RPS13*, 5'-atg cca agt tcc gtt tga ttc-3' and 5'-ggg tgg cag tac tct ctt ggt-3'; *TBP*, 5'-tgc aac acc aga ctt gga ctg t-3' and 5'-gca gcc cta caa agc aac tca c-3'. To normalize the data, ΔCT was calculated for each sample by subtracting the CT of *RPS13* from that of *CHD-W*. For relative quantitation, ΔCT of calibrator sample gene was subtracted from ΔCT of each experimental treatment sample to generate the ΔΔCT. The ΔΔCT was then used to calculate the approximate fold difference, $2^{-\Delta\Delta CT}$.

Real-time PCR analysis revealed that amplification level of *CHD-W* gene in MF chimera was lower than that of control female (F), FF and FM chimeras. Relative quantification was performed by two internal control genes (see Supplementary Figure 8; **a**, *RPS13*, **b**, *TBP*) giving the same results. All brain samples of chimeras subjected to measurement of neurosteroid contents were validated. Number of analyzed chicks was as follows: FF, 7; MF, 6 FM, 6; MM, 6. Plots in Supplementary Fig. 8 indicate data from individual chicks and the horizontal lines represent group means. ND, non-detectable.

Detection and quantification of quail cells in the quail/chick chimeras

For the analysis of the pituitary origin, we produced the WB chimeras between quail

and chick embryos as above. At ED12-13, the head of the quail-chick brain chimera was embedded in compound and frozen in powdered dry ice. Sections (20 μm thick) were made with a cryostat (CM1900; Leica Microsystems) and mounted on slide glass, dried and stored at -80°C until use. The sections were fixed with 4% paraformaldehyde for 1 h at room temperature. Thereafter, all procedures were done at room temperature unless otherwise noted. The sections were washed for 10 min three times in 0.5% TritonX-100 in PBS (PBST), treated sequentially with PBST containing 0.6% H_2O_2 for 30 min at 37°C, PBST for 10 min, and 3% normal goat serum in PBST for 1 h. To detect quail cells, QCPN antibody (Developmental Studies Hybridoma Bank, University of Iowa; the supernatant of hybridoma cells culture) was applied to the sections for 12 h at 4°C. After washing with PBST, the sections were treated with EnVision+/horseradish peroxidase (DAKO, Glostrup, Denmark) for 1 h. The immunoreactivities were visualized using the diaminobenzidine Liquid System (DAKO).

The sections were counterstained with cresyl violet and were observed under a light microscope (DMRA; Leica) with ×20 objective lens and the RGB color images were directly digitized by a CCD camera (DC350; Leica). Four chick chimeras with whole quail brain and two quail chimeras with whole chick brain were analyzed. We chose 2-6 representative images at 640 × 480 pixels resolution from each chimera. Two types of inverted grayscale images derived from either red channel or all RGB channels of original images were created by Adobe Photoshop CS4 (Adobe Systems Inc., San Jose, CA) and used for analyzing the numbers of QCPN-positive cells (brown color) and all cells (both brown and blue colors), respectively. The number of cells in each image was counted by Automatic Nuclei Counter plug-in (Image-based Tool for Counting Nuclei, ITCN, http://www.bioimage.ucsb.edu/automatic-nuclei-counter-plug-in-for-imagej) for ImageJ (http://rsbweb.nih.gov/ij/). The settings of ITCN were as follows: width 6, minimum distance 3, threshold 1.7. The ratio of the QCPN-positive cells to all cells in each image and the mean of the ratio in each chimera were calculated.

Histology of the gonads

Chicken brain chimeras were constructed as described above, and at ED17-19, the gonadal tissue was dissected and fixed with 4% paraformaldehyde at 4°C for 4 h. It was then cryoprotected, embedded in the compound and frozen in powdered dry ice. Brain and chest tissues were also dissected out from the embryo and used for determination of the sex of the donor and host embryo. Six series of gonadal sections (20 μm thick) were made with a cryostat (CM1900; Leica Microsystems) and mounted on slide glass, dried

and stored at -80°C until use. *In situ* hybridization was performed as described previously[55]. At least two embryos of each combination were examined. Plasmids containing *Aromatase*[60], *DMRT1*[23,61] or *HINTW*[62] cDNA fragments were prepared by polymerase chain reaction (PCR) as described. *Sox 9*[63] plasmid was provided by Y. Kamachi.

Examination of fertility

To check the fertility of the hens, FF and MF chimeras, each bird was housed with a cock on alternate weeks and the eggs were collected daily and incubated, and the presence of an embryo was checked. Cocks were used repeatedly but occasionally changed. To check the fertility of the cocks, MM and FM chimeras, each bird was housed with a hen on alternating weeks, and the eggs were similarly checked. Hens were used repeatedly but occasionally changed.

Supplementary References

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