SUPPLEMENTAL MATERIAL

MATERIALS AND METHODS

Brain GnRH immunofluorescence

Serially cut 30 µm thick coronal brain sections were assessed. Sections were processed for immunofluorescence using the free floating technique. Briefly, sections were incubated for 45 min with a 1/3,000 dilution of anti-GnRH monoclonal antibody LHR 13 washed twice with PBS, and incubated for 45 min with a 1/300 dilution of an Alexa 488 anti-mouse Fc.

Anterior pituitary immunohistochemistry

Pituitary sections (4 µm) were incubated for 1 h at room temperature with primary antibodies against LH or FSH (murine, Dako, CA), diluted 1:100. Thoroughly washed sections were then treated for 30 min with a ready-to-use EnVision reaction system (Dako, CA) using the peroxide-sensitive chromogen diaminobenzidine for color development.

Histological assessment of uterus

Uteri were removed, fixed in 4% formaldehyde and embedded in paraffin. Serial ovarian and transversal uterine horn 4-µm thick sections were stained with H&E. Micrographs of ovarian and uterine sections were taken with a DP70 camera.

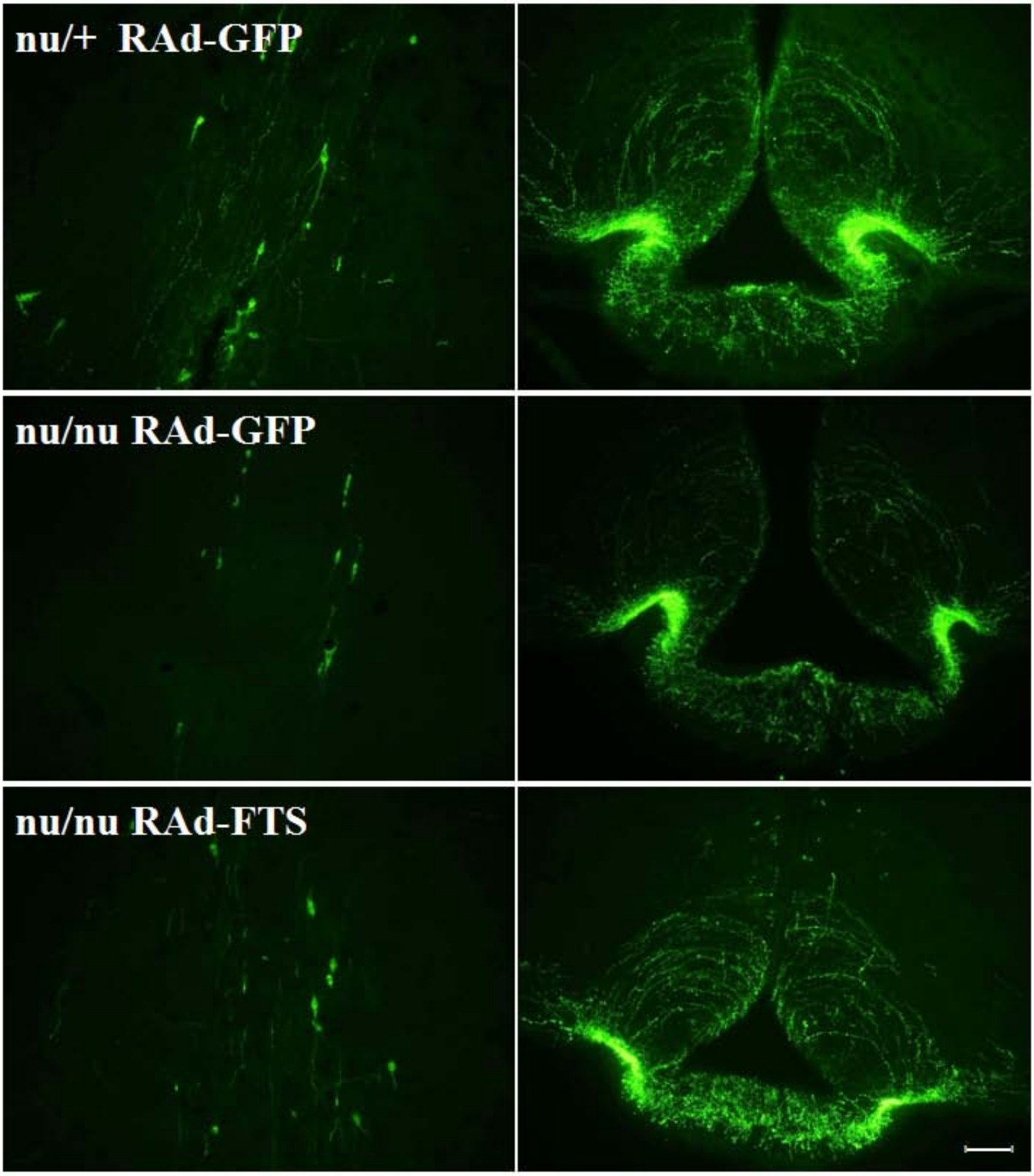
LEGENDS TO SUPPLEMENTAL FIGURES

Supplemental FIG 1. Hypothalamic GnRH neuron populations in control and experimental nude female mice. GnRH perikarya (left panels) and fibers (right panels) in the anterior and mediobasal hypothalamus, respectively, of control hetero and homozygous nude females and in homozygous counterparts submitted to neonatal thymulin gene therapy. Scale bar corresponds to 200 µm.

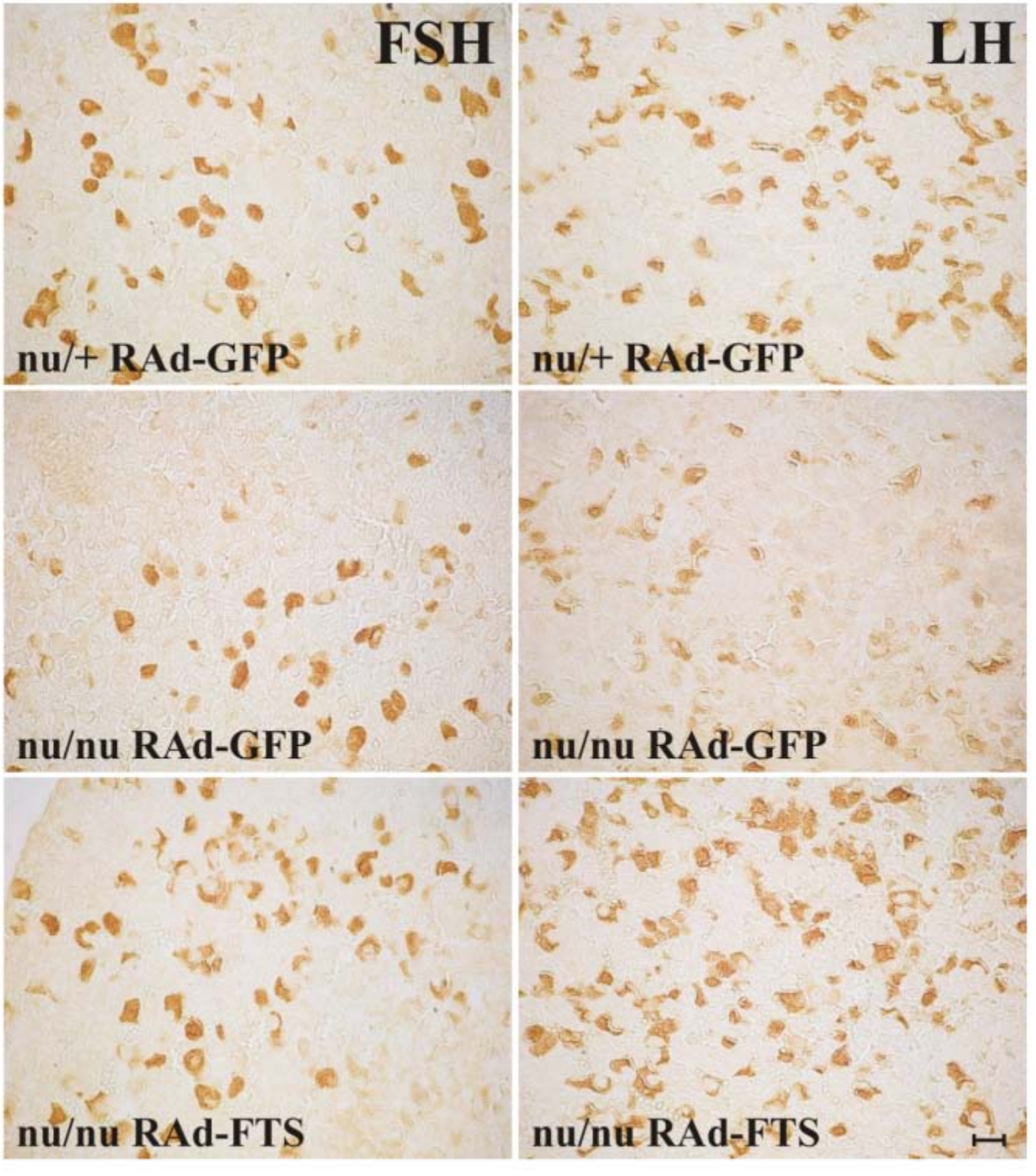
Supplemental FIG 2. Gonadotropic cell populations in the anterior pituitary gland of control and experimental nude female mice. Immunohistochemical labeling of folliculotropic and luteotropic cells in the anterior pituitary of control hetero and homozygous nude females and in homozygous counterparts submitted to neonatal thymulin gene therapy. Scale bar corresponds to 25 µm.

Supplemental FIG 3. Effect of thymulin gene therapy on uterine morphology in nude mice. H&E stained sections of uteri from nu/+ **(A)**, nu/nu **(C)** mice treated with the control vector (RAd-GFP) and nu/+ **(B)** and nu/nu **(D)** mice submitted to neonatal thymulin gene therapy. Scale bar corresponds to 15 µm.

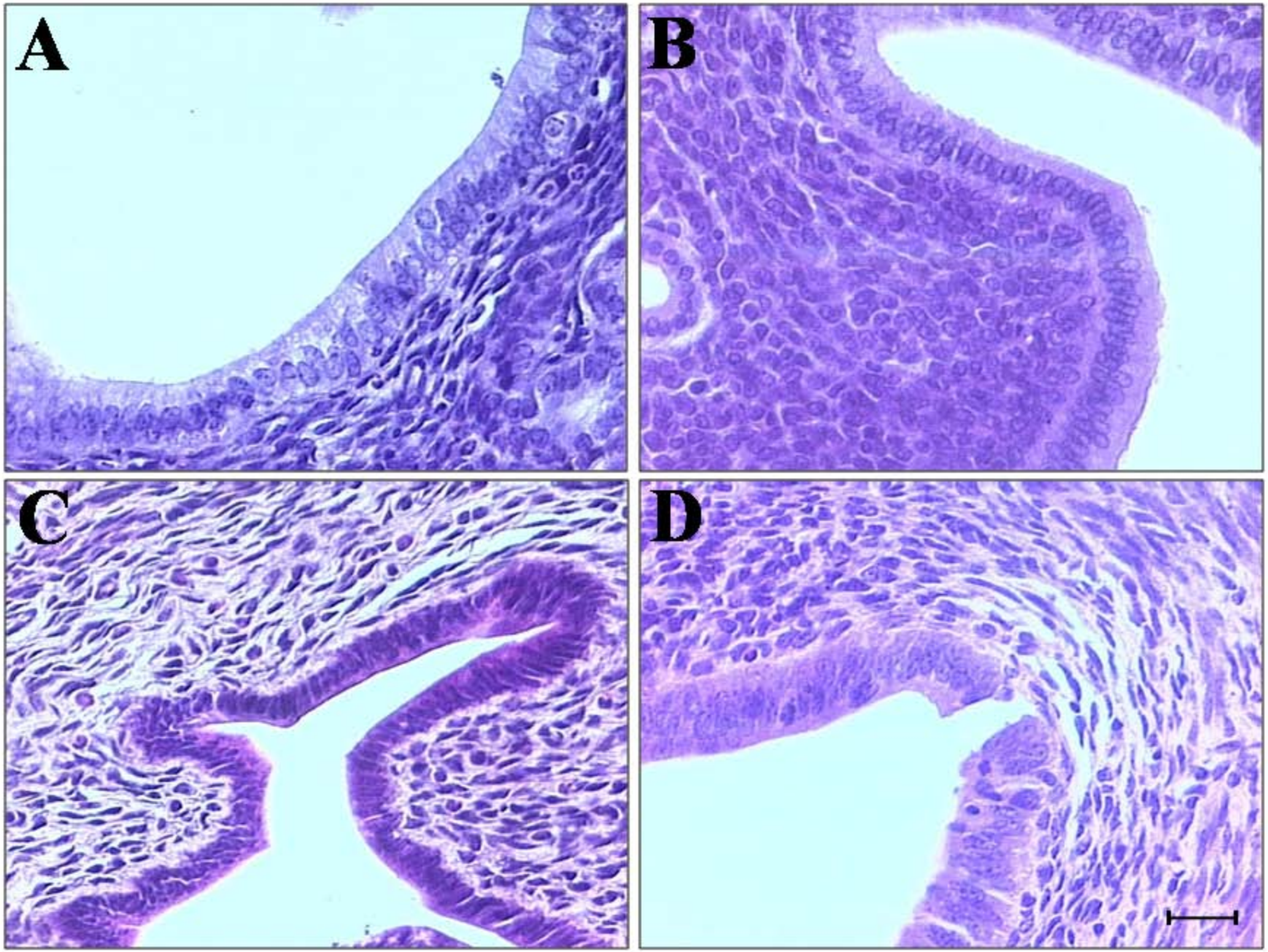
Supplemental FIG 4. Uterine epithelial height in control and experimental nude mice. Epithelial height was assessed in uterine sections from RAd-GFP- and RAd-FTS-treated heterozygous and homozygous female nude mice. Numbers in parentheses above columns represent the N value per group followed by the CV in the format (N;CV). Differences versus corresponding control nu/nu did not reach statistical significance ($p>0.05$).



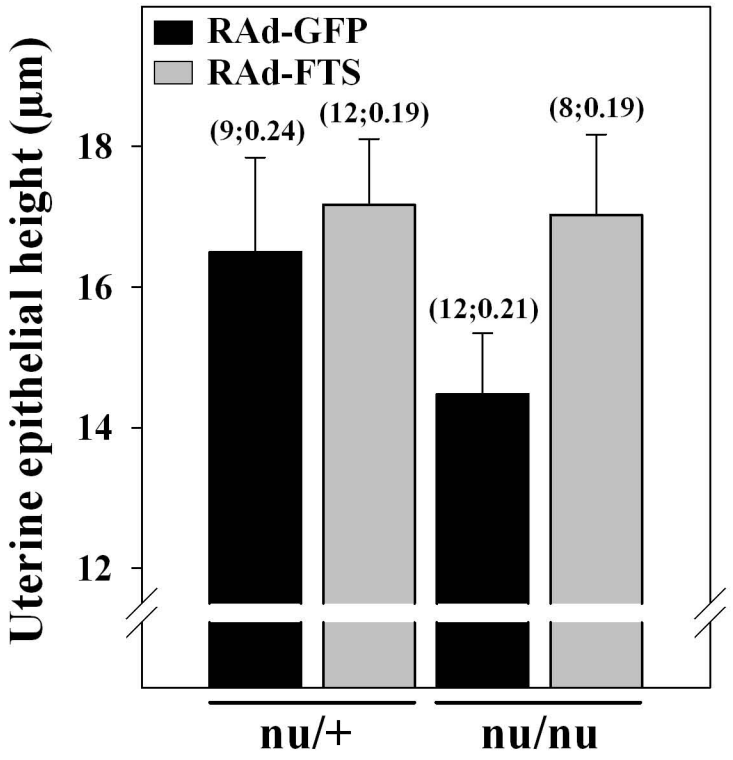
Supplemental FIG 1



Supplemental FIG 2



Supplemental FIG 3



Supplemental FIG 4