Supporting information

Inhibition of Jagged-specific Notch activation reduces luteal angiogenesis and causes luteal hemorrhaging of hormonally stimulated ovaries

Natalie Kofler[1, 2], L. A. Naiche[3], Lilli D. Zimmerman[4,5], and Jan K. Kitajewski[3, 5,*]

Author affiliations and addresses:
[1] Institute for Sustainability, Energy and the Environment, University of Illinois Urbana-Champaign, Urbana IL 61801
[2] Integrated Program Cellular, Molecular, and Biomedical Studies, Columbia University, New York, NY 10032
[3] Department of Physiology and Biophysics, University of Illinois, Chicago, IL 60612
[4] Weill Cornell Medical College, New York, NY 10065
[5] Department of Obstetrics and Gynecology, Columbia University, New York, NY 10032

*Corresponding Author:

Jan K. Kitajewski
Department of Physiology and Biophysics
University of Illinois Chicago
College of Medicine
835 S. Wolcott Ave. Room 204L
Chicago IL 60612 USA
312-996-7620
kitaj@uic.edu

Supporting Information Table of Contents

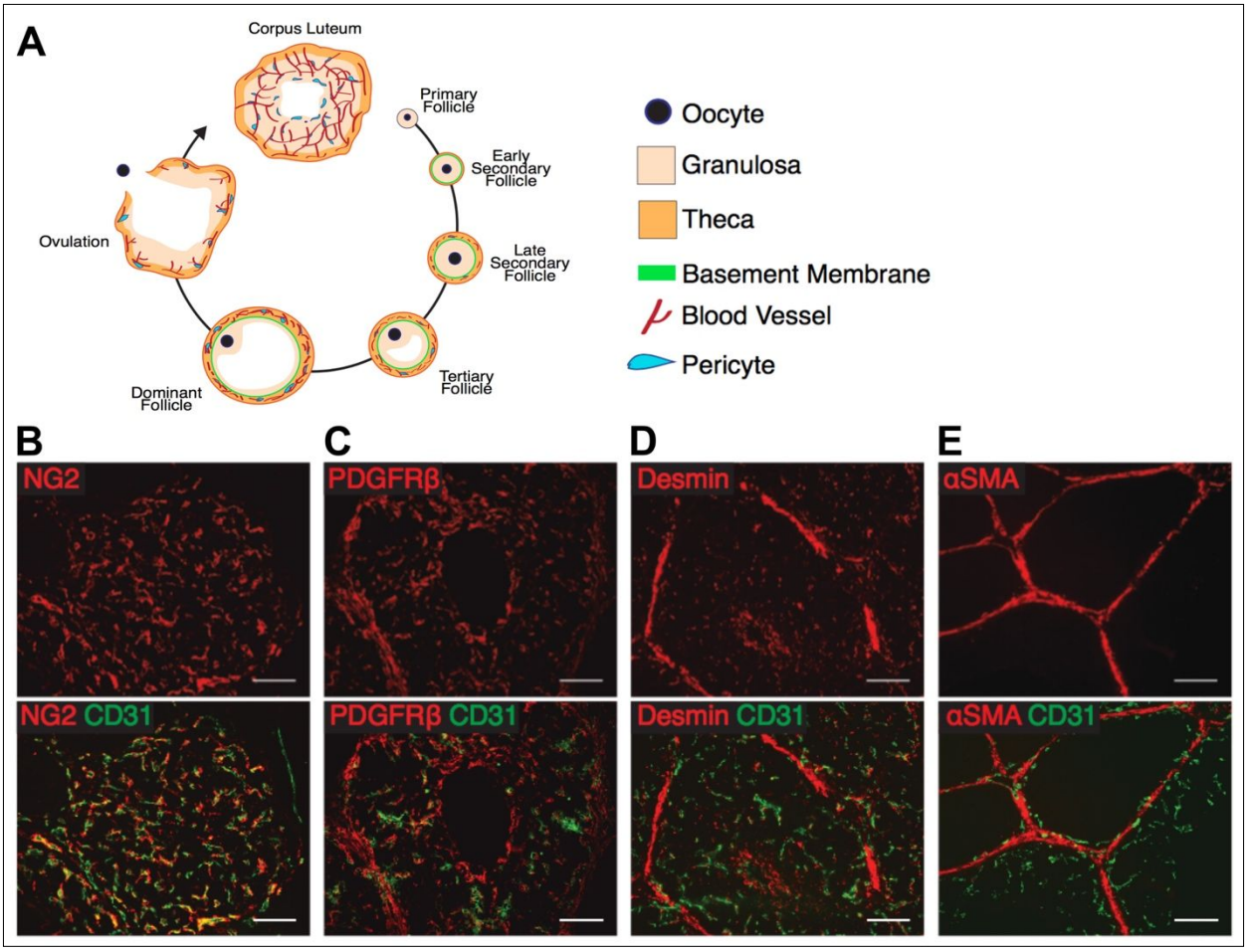

Figure S1. Analysis of mural cell marker expression in the corpus luteum. (A) Schematic showing folliculogenesis, ovulation, and corpus luteum formation in the mouse ovary**. (B-E)** Ovary sections from mice fluorescently stained for mural cell makers NG2, PDGFR- β, desmin, and αSMA. Pericytes lining the CD31-positive capillaries (green) of the corpus luteum are positive for NG2 **(B)**, PDGFR-β **(C)**. **(D)** desmin stains a small number of luteal mural cells but is expressed at higher frequency in the thecal layers. **(E)** αSMA expression is restricted to the mural cells lining the theca blood vessels. Scale bars; 100 μm.

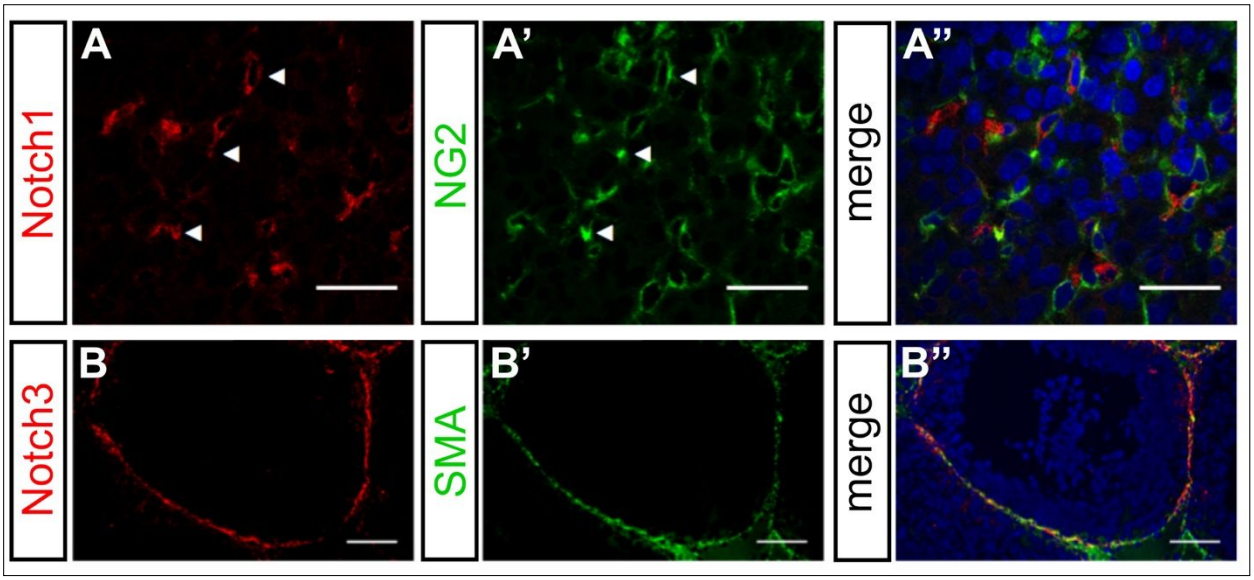

Figure S2. Luteal pericytes express Notch1 but not Notch3. (A&B) Sections of ovaries that had undergone physiologic ovulation stained for Notch1 (red, **A**) and NG2 (green, **A'**) or Notch3 (red, **B**) and αSMA (green, **B'**). A subset of NG2-positive pericytes in the corpus luteum express Notch1 (arrowheads, **A, A'**). Notch3 is expressed in the mural cells lining the theca blood vessels but is not expressed at detectable levels by luteal pericytes. DAPI staining shows nuclei in blue (merged images **A" & B"**). Scale bars; 50 μm (**A**), 100 μm (**B**).

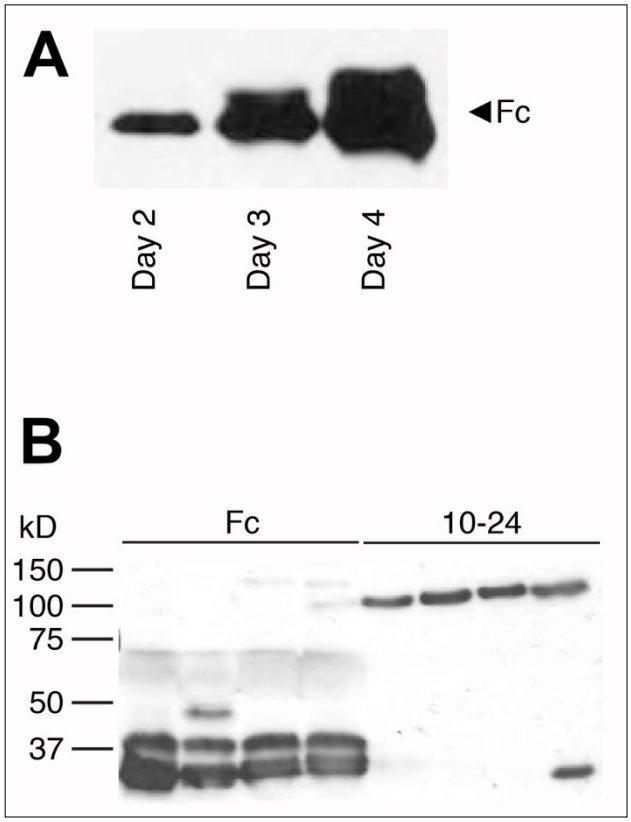

Figure S3. Adenovirus injection on day 1 produces robust levels of expressed proteins in circulating serum throughout luteinization. (A) Circulating serum protein expression can be detected at low levels 24 hours after adenovirus administration, on day 2 of the experiment. Circulating serum expression becomes robust on days 3-4, during luteinization. **(B)** $N1^{10-24}$ decoy and control Fc are robustly expressed in circulating serum on day 4, when ovaries are collected. Each lane represents an individual mouse. anti-Fc antibody used to detect both the Fc protein and the Fc moiety of the $N1^{10-24}$ decoy protein.

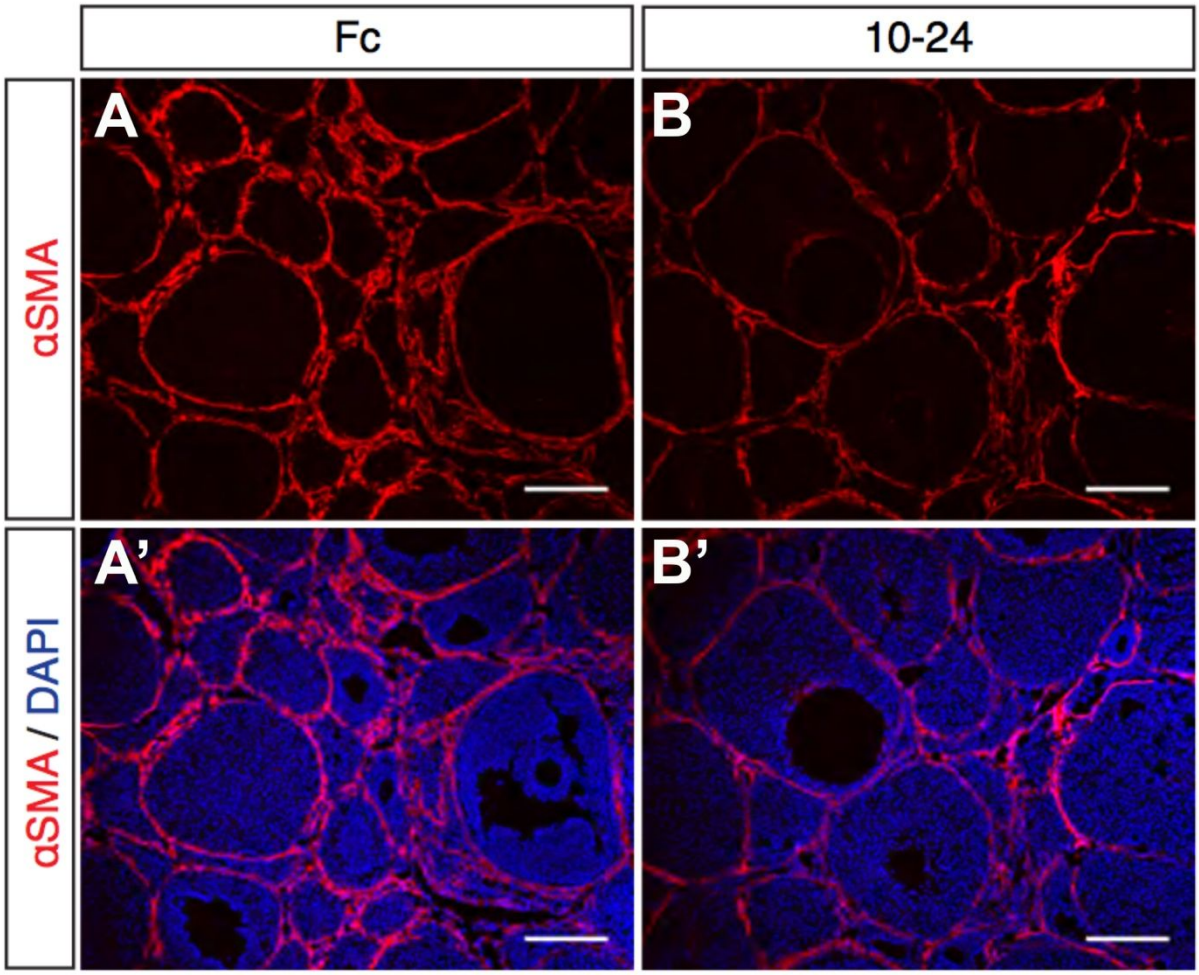

Figure S4. αSMA expression is present in N1$^{10-24}$ decoy-treated hyperstimulated ovaries but may be reduced. (A&B) Ovary sections from human Fc **(A-A')** and N1$^{10-24}$ decoy **(B-B')** treated mice fluorescently stained for αSMA (red) and DAPI (blue). There is mildly reduced fluorescent intensity in the ovarian theca of N1$^{10-24}$ decoy treated female mice as compared to that of the control. Scale bars; 200 μm.