

Supplementary Materials for

Inhibition of the NLRP3 inflammasome prevents ovarian aging

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Table S1

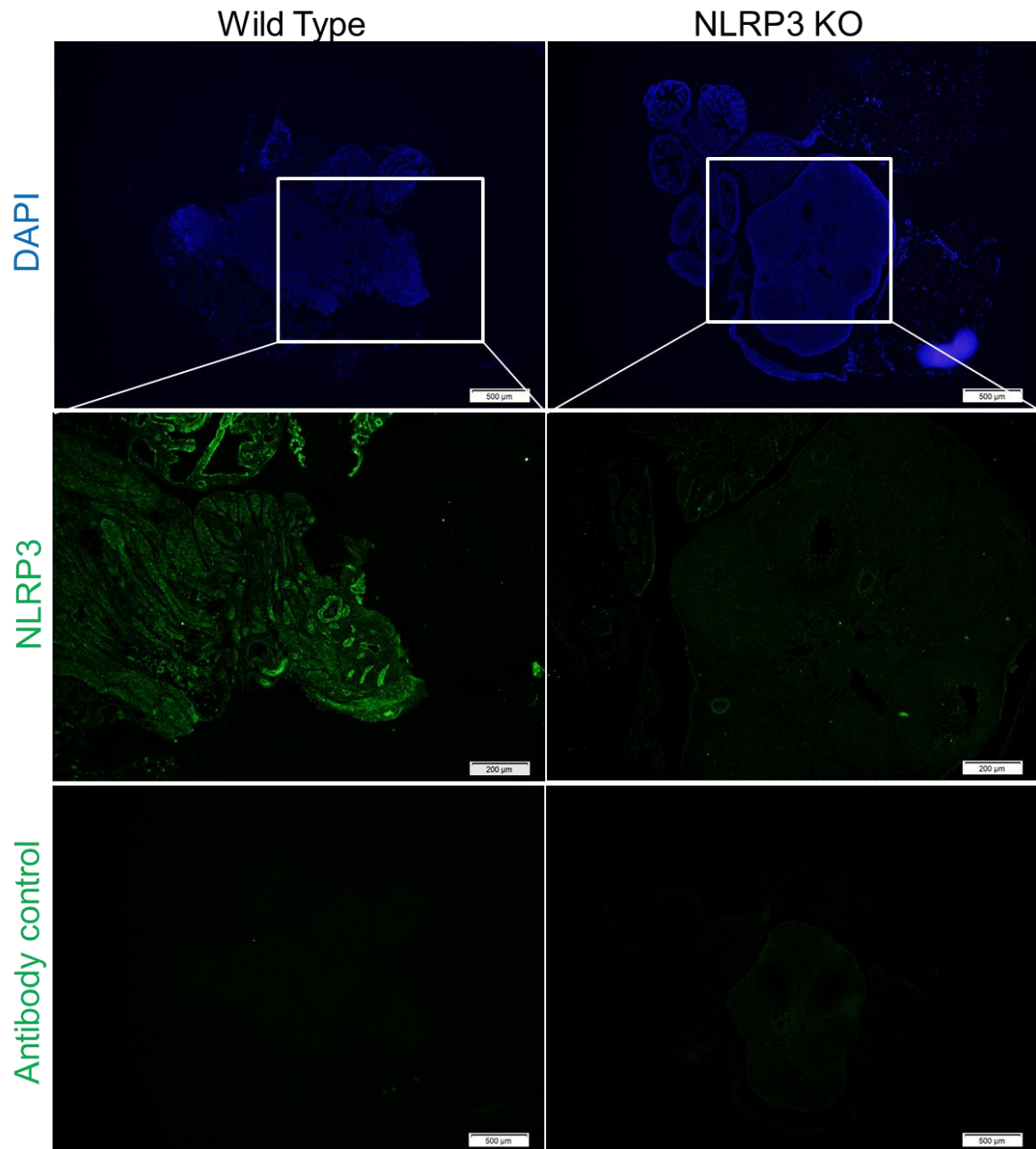
Figs. S1 to S8

Tables

Supplementary Table 1. Characteristic of control and DOR patient groups

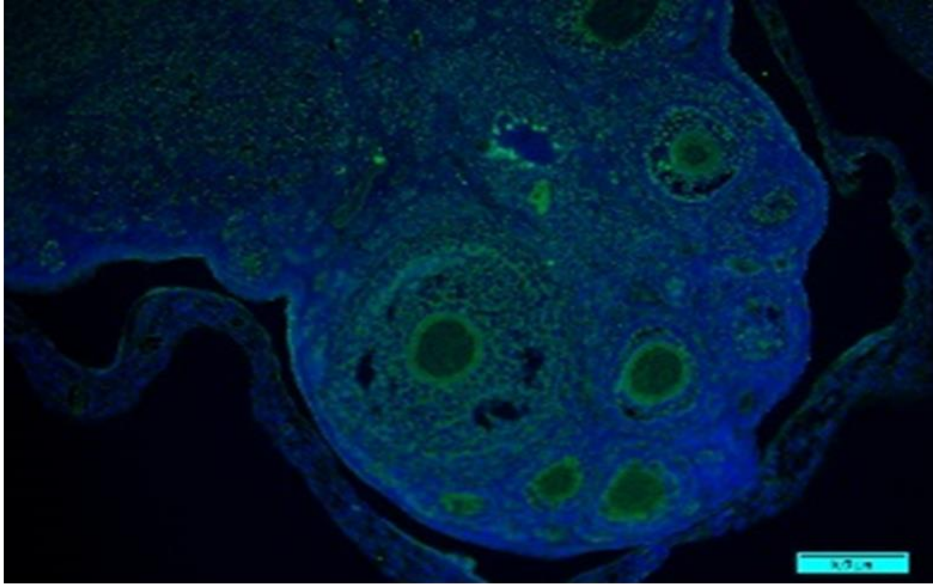
	<i>Control group (20)</i>	<i>DOR groups (20)</i>	<i>P value</i>
<i>Age</i>	31.8±4.1	33.1±3.6	0.293
<i>Alcohol consumption</i>	Sporadic (39%)	Sporadic (42%)	0.841
<i>Tobacco consumption</i>	Sporadic (26%)	Sporadic (23%)	0.097
<i>BMI</i>	24.1±4.4	24.5±3.2	0.744
<i>Overweight, BMI 25.0–29.9 kg/m²</i>	9%	21%	0.276
<i>Obesity BMI 30 kg/m²</i>	9%	21%	0.276
<i>Estradiol (pg/mL)</i>	30.1±2.1	71.3±8.1	0.0001
<i>FSH (IU/mL)</i>	4.2±1.3	19.5±2.7	0.0001
<i>Glucose (mg/dL)</i>	81.3±7.9	88.2±8.1	0.0096
<i>HbA1c (%)</i>	5.3±0.1	5.1±0.4	0.0364
<i>IL-1β (pg/mL)</i>	3.7±1.4	3.1±1.4	0.183
<i>IL-18 (pg/mL)</i>	3.1±2	2.8±1.3	0.577
<i>TNF-α (pg/mL)</i>	14.9±5.8	50.5±8.7	0.0001

20 months

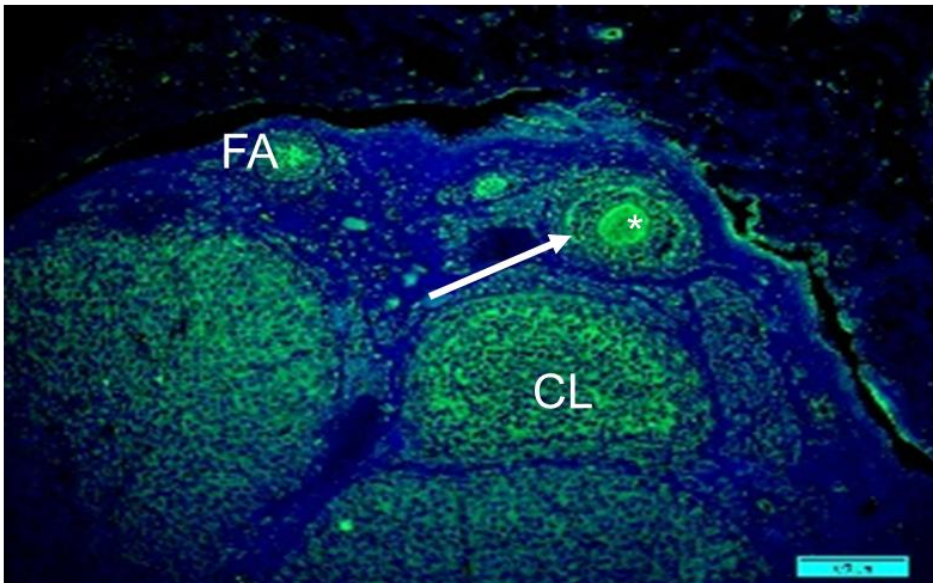


Supplementary Figure 1. Immunofluorescence assay of the NLRP3 antibody. Left, Wild type mouse and right NLRP3 KO mouse. Top: Dapi image, center: NLRP3 image, down: negative control of NLRP3 antibody.

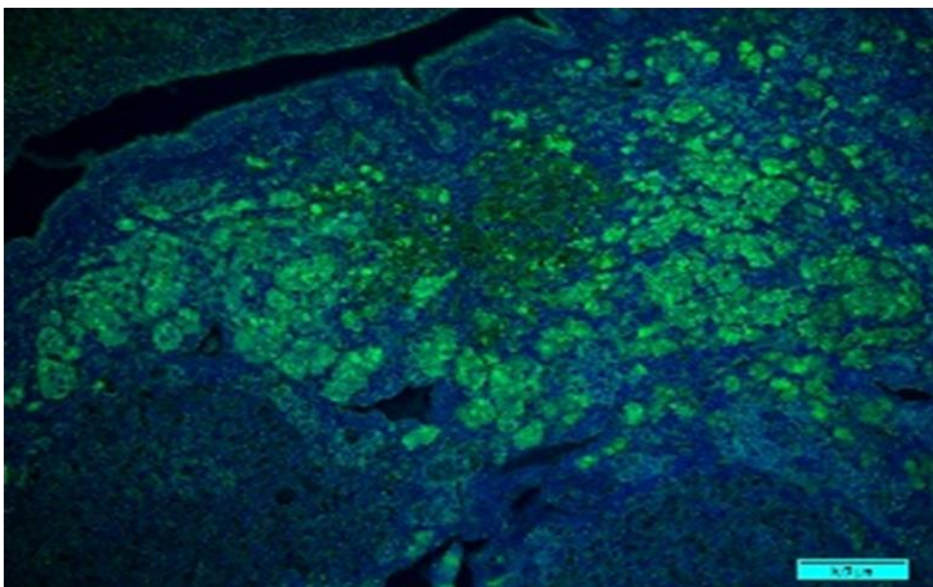
2 months



8 months

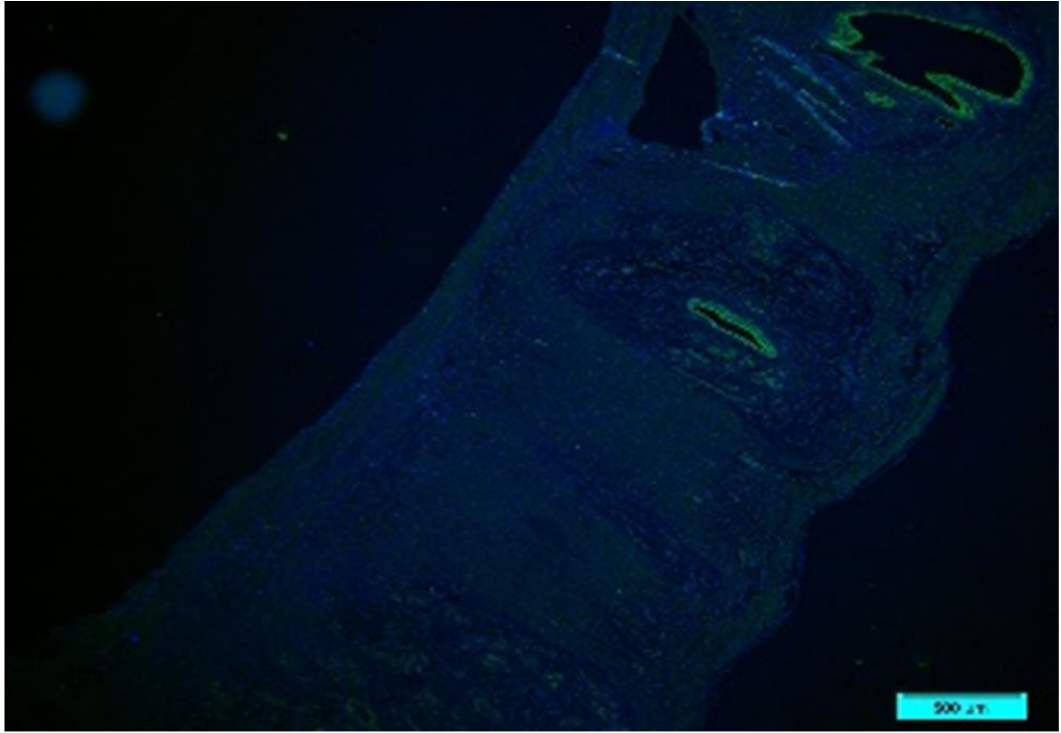


20 months

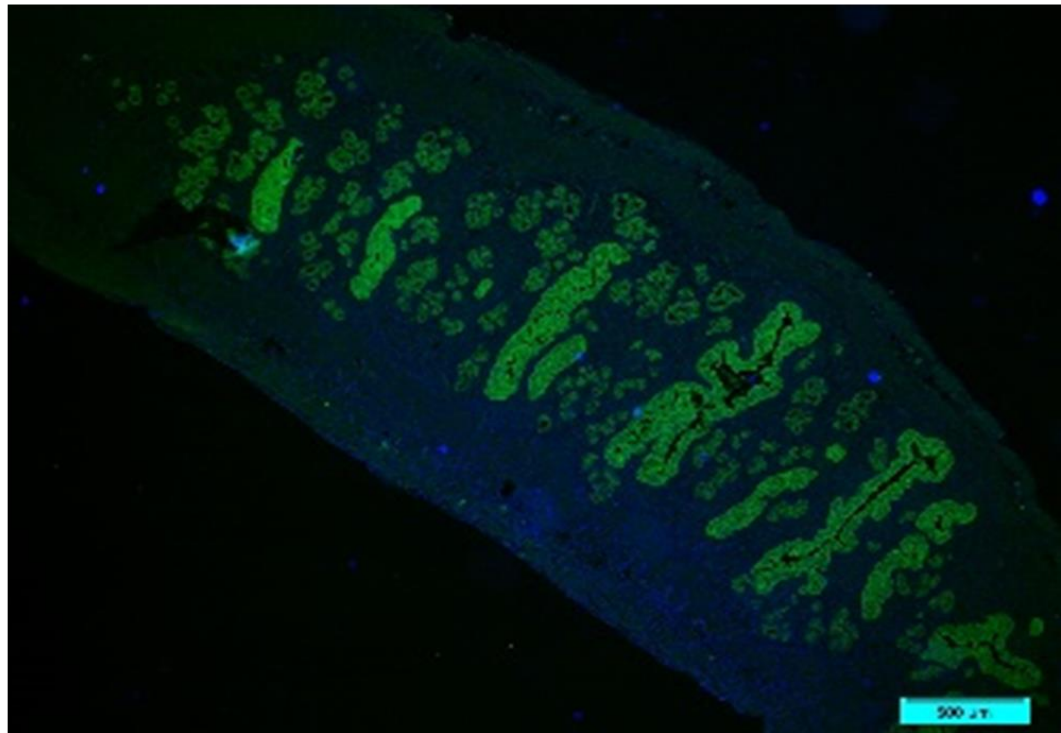


Supplementary Figure 2. Magnification of the panel E, Figure 1; ovary.

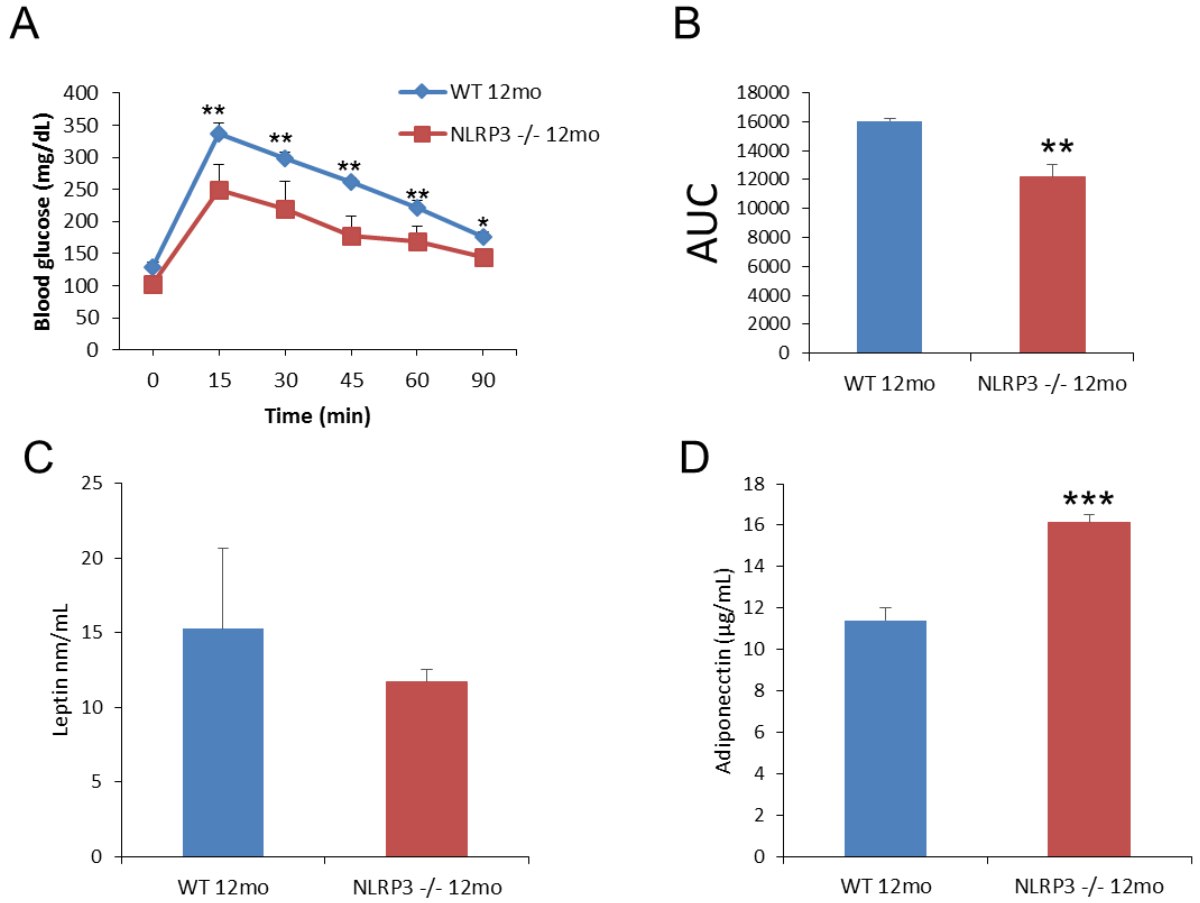
2 months



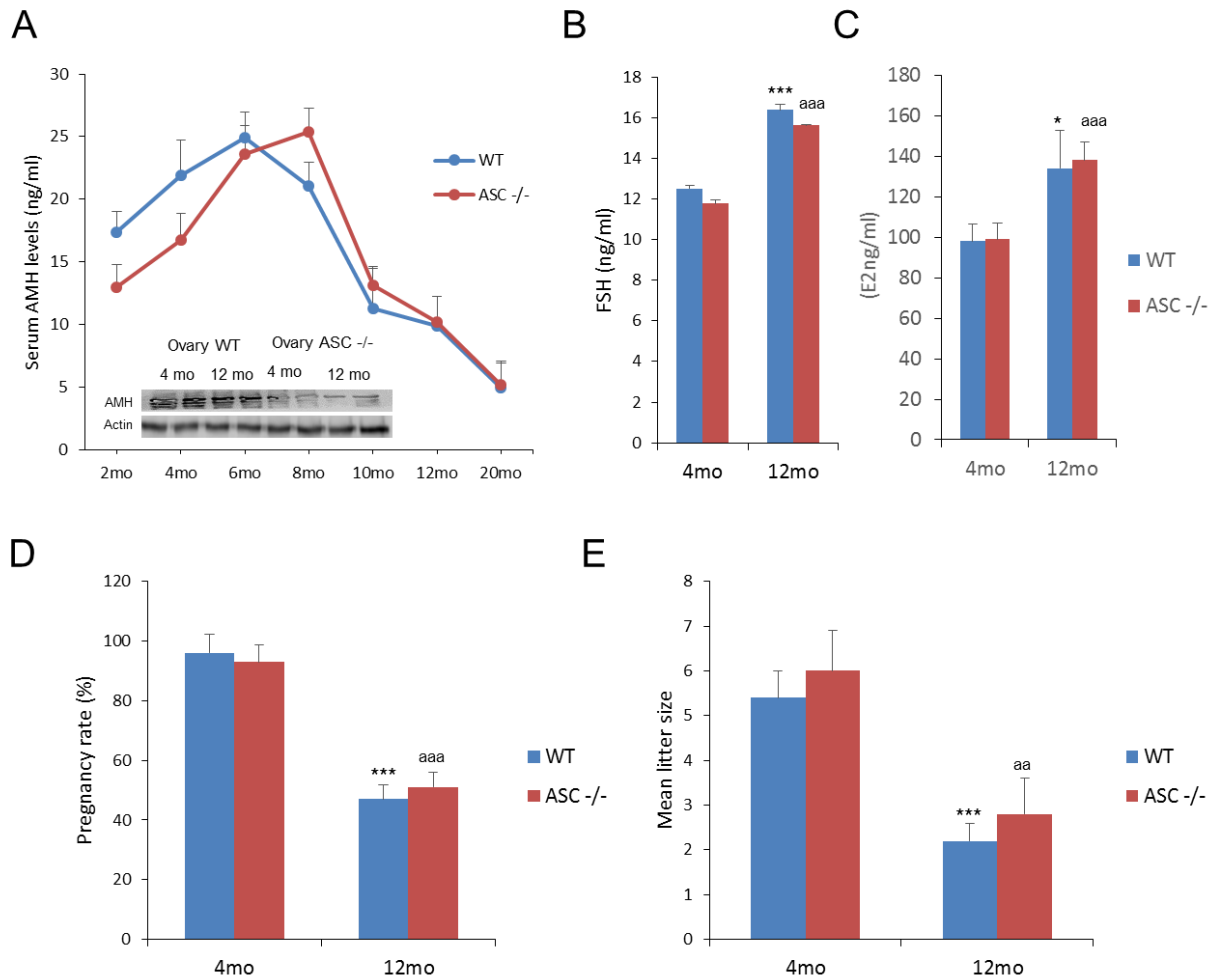
12 months



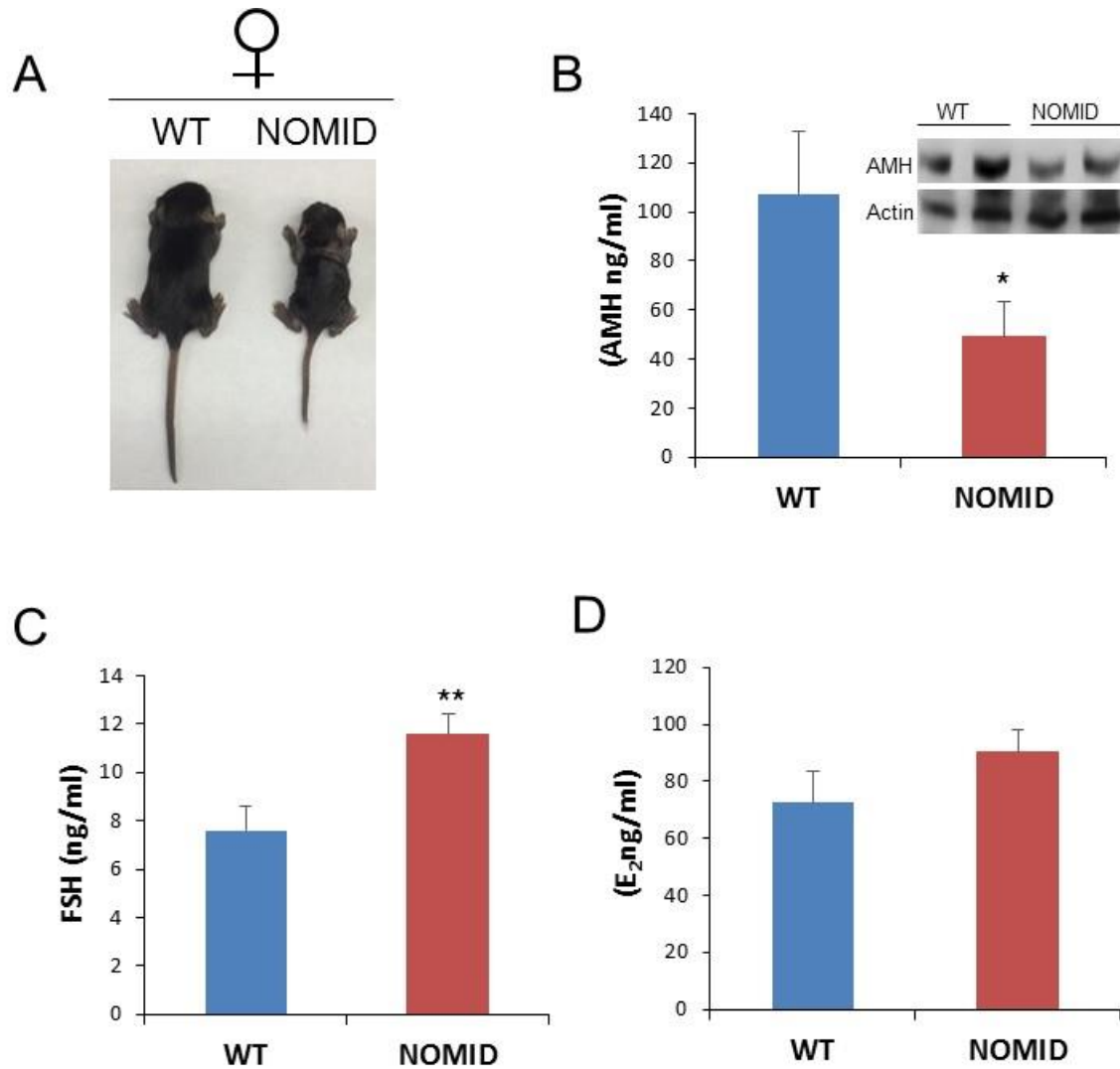
Supplementary Figure 3. Magnification of the panel E, Figure 1; uterus.



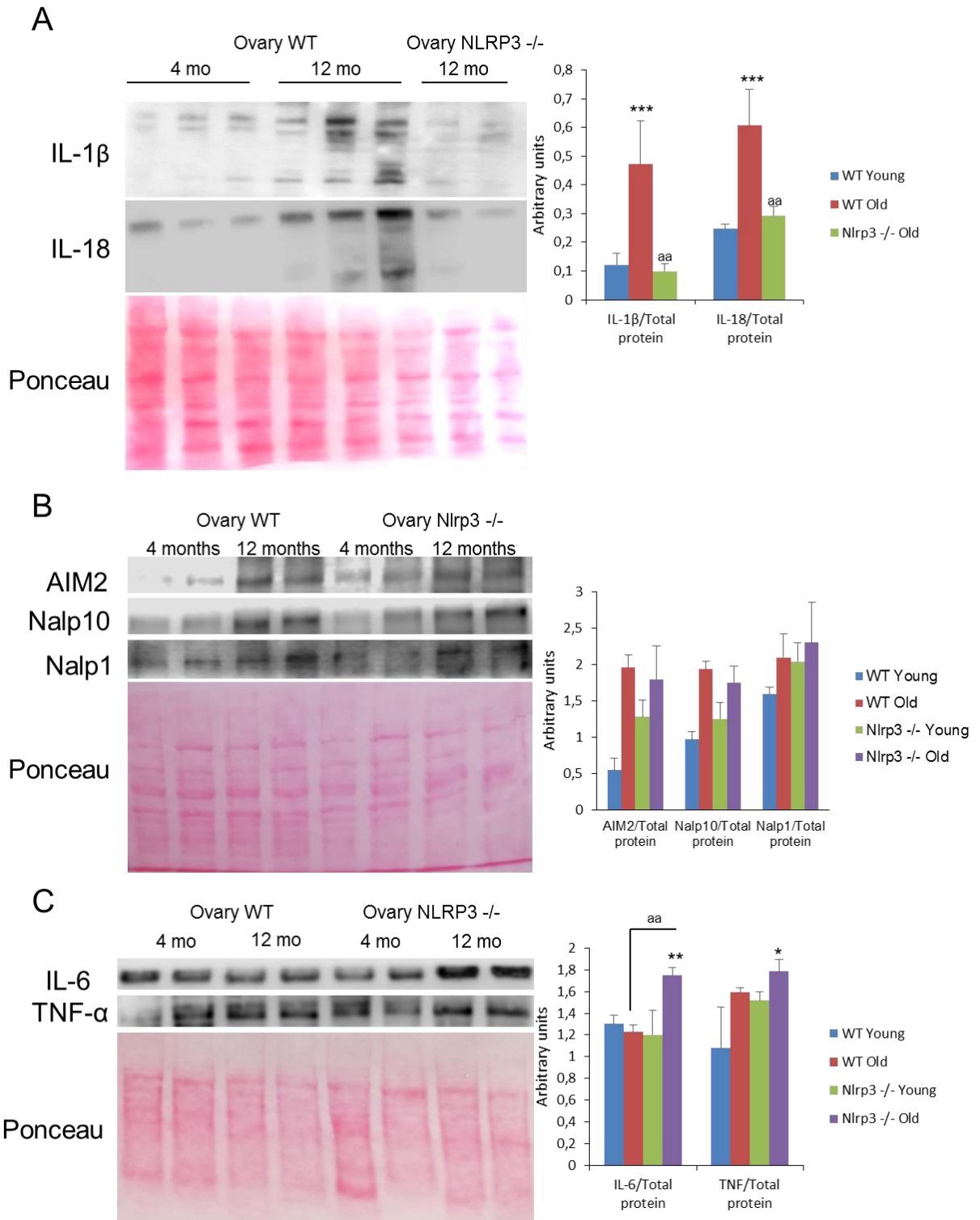
Supplementary Figure 4. Metabolic changes in aged WT and NLRP3^{-/-} mice. (A and B) Oral glucose tolerance test with area under the curve. (C and D) Levels of leptin and adiponectin. Blood samples were collected after overnight fasting. All data are presented as means \pm SD, n = 10 mice; *P < 0.05, **P < 0.005, ***P < 0.001 WT vs NLRP3^{-/-} mice.



Supplementary Figure 5. Fertility profile of ASC^{-/-} mice compared with WT mice. (A) Mean serum AMH levels to evaluate the progression of ovarian reserve during aging in WT mice (blue) compared with ASC^{-/-} mice (red) and ovarian AMH protein level. (B and C) Analysis of serum concentrations of FSH, and E2 as measured by ELISA. Pregnancy rate (D) and mean litter size (E) in aged WT and ASC^{-/-} mice. All data are presented as means \pm SD, n = 10 mice; *P < 0.05, **P < 0.005, ***P < 0.001 4mo vs 12mo WT mice; ^aP < 0.05, ^{aa}P < 0.005, ^{aaa}P < 0.001 4mo vs 12mo ASC^{-/-} mice

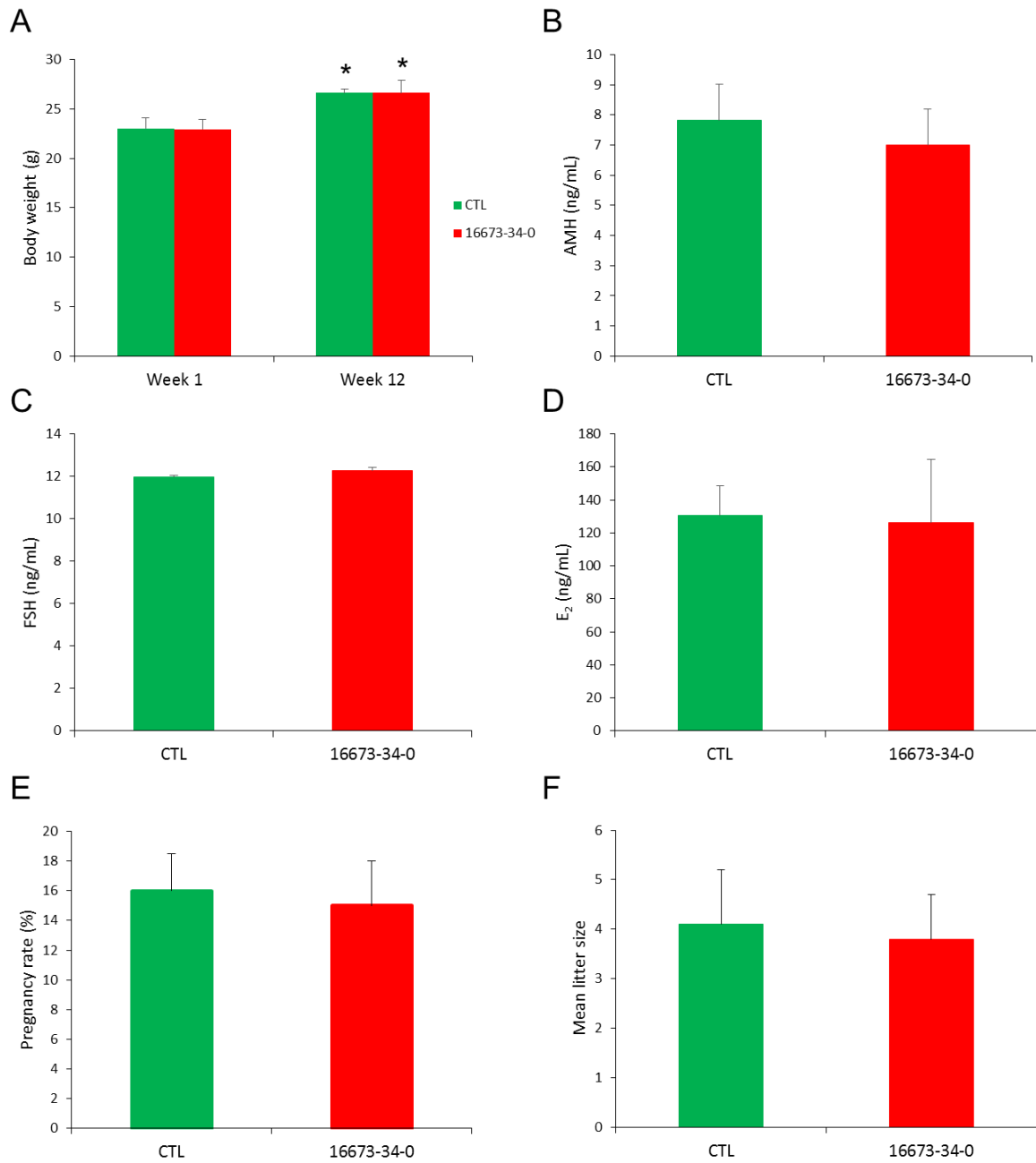


Supplementary Figure 6. We generated *Nlrp3^{fl} (D301N)⁺; LysM-Cre* mice for conditional NLRP3 activation in only myeloid cells. A significant difference of body weight was observed between female WT and NOMID mice (6-7 g for 2-week-old WT mice vs 3-4 g for NOMID mice). (A) Representative photographs of 2-weeks-old NOMID and WT mice. (B) NOMID female mice showed significantly reduced serum levels of AMH by ELISA and ovarian AMH protein levels (top) measured by WB, FSH (C) and E₂ (D) serum levels in female WT compared with NOMID mice. These data would likely be consistent with infertility; however, these mice die at approximately 3-weeks prior to reproductive age. Data are presented as means \pm SD. ** $P < 0.005$, * $P < 0.05$ WT vs NOMID mice. $n=5$ mice per group. Photo credits: Chun Wang, Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO 63110, USA (A).



Supplementary Figure 7. Changes in other inflammasomes during ovary aging. A. Western blot analysis showing reduced levels in the Active forms of IL-1 β and IL-18 in the ovary of aged NLRP3 $-/-$ mice compared with aged WT. B. A. Western blot analysis showing the expression levels of representative inflammasomes AIM2, Nalp10 and Nalp1 in the ovary of aged NLRP3 $-/-$ mice compared with aged WT. C. Western blot

analysis showing the protein expression of senescence markers IL-6 and TNF- α in the ovary of 4mo and 12mo NLRP3 $-/-$ mice compared with WT. n= 4 mice per group and age. All data are presented as means \pm SD; *P < 0.05, **P < 0.005, ***P < 0.001 young vs old $-/-$ mice. ^{aa}P < 0.01, Nlrp3 $-/-$ vs WT mice.



Supplementary Figure 8. CTL (vehicle) and 16673-34-0 treated female mice. (A) Body weights (B-D) Analysis of serum concentrations of AMH, FSH, and E₂ as measured by ELISA. Pregnancy rate (E) and mean litter size (F) in vehicle and 16673-34-0 treated female mice. All data are presented as means ± SD, n= 6 mice; *P < 0.05, 1 week vs 12 weeks.