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Supplemental Data

SUPPLEMENTAL MATERIALS AND METHODS

Tissue composition and ovarian morphometric analysis

Following ovary collection, bodies of six month old +/+ and *tm/tm* mice were collected and scanned using the dual-energy X-ray absorptiometry PIXImus Mouse Densitometer (GE Medical Systems, Madison, WI, USA.) using software version 2.0. Body weight (g), bone mineral density (BMD) (mg/cm^2), bone mineral content (BMC) (mg), bone area (cm^2), lean tissue (g), fat tissue (g), and %fat were determined. Bone density studies for vertebra and femur were also performed.

Ovarian tissue from +/+ and *tm/tm* mice were collected at two and six months, fixed in 10% formalin and paraffin-embedded. Serial ovarian sections (8μm) were mounted on glass microscope slides. Following staining with picric methyl blue, we estimated the number of oocyte-containing primordial, primary and small pre-antral follicles in a stratified randomized sampling of the entire ovary as previously described [1, 2].

REFERENCES USED IN SUPPLEMENTAL DATA

1. Perez GI, Knudson CM, Leykin L, Korsmeyer SJ, Tilly JL. Apoptosis-associated signaling pathways are required for chemotherapy-mediated female germ cell destruction. Nat Med 1997; 3: 1228-1232.

2. Ratts VS, Flaws JA, Kolp R, Sorenson CM, Tilly JL. Ablation of bcl-2 gene expression decreases the numbers of oocytes and primordial follicles established in the post-natal female mouse gonad. Endocrinology 1995; 136: 3665-3668.

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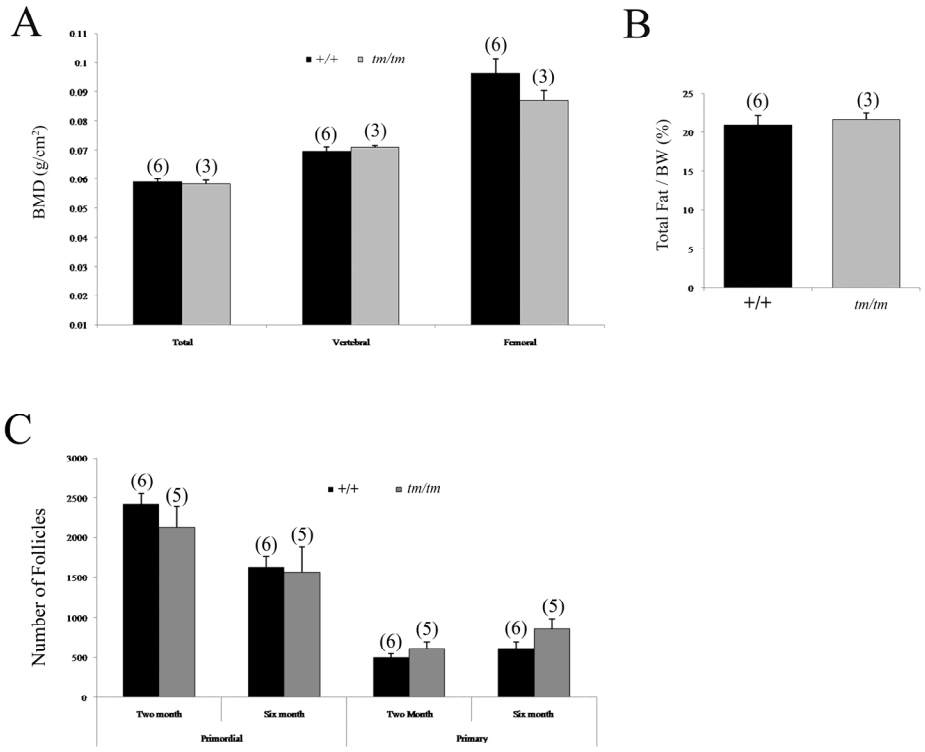
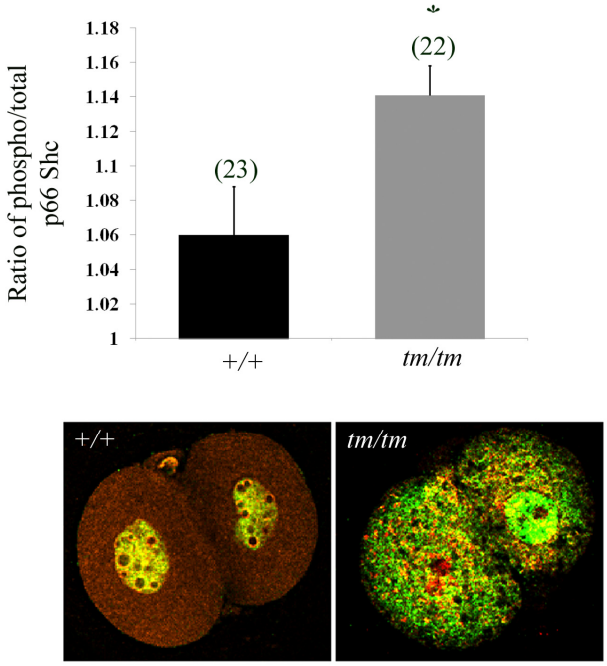
Supplemental Table S1. Sequence of primers used for qRT-PCR.

Gene Name	Sequence
Bcl2l10 (Diva)	(F) 5'-tgctctccaaagaccaagac-3' (R) 5'-cctcacttagcagcgaaaag-3'
Bag1	(F) 5'-gctgaatgaagtgcagtgga-3' (R) 5'-gaggagcagggaaattgaca-3'
Hrk	(F) 5'-cgctgggcgacgagctgca-3' (R) 5'-gtcccctctactccacacca-3'
Bok (Mtd)	(F) 5'-cacagacaaggagctggtgg-3' (R) 5'-cggaatacagggacactacc-3'
Bak	(F) 5'-gaaccccaccttcactcctt-3' (R) 5'-ccctcttccccgatacattt-3'
Bax	(F) 5'-tgcagaggatgattgctgac-3' (R) 5'-gatcagctcgggcactttag-3'
Actb	(F) 5'-ccacagctgagagggaaatc-3' (R) 5'-aaggaaggctggaaaagagc-3'

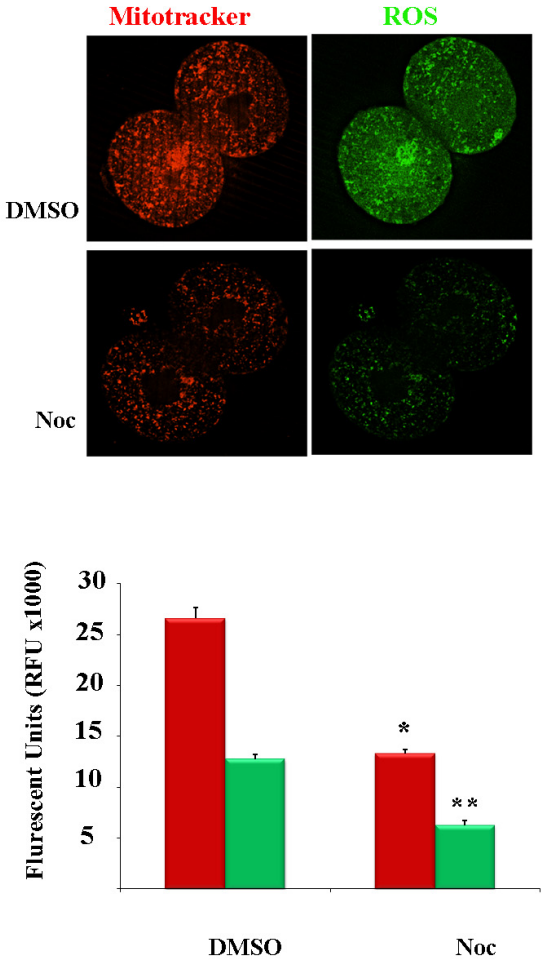
Supplemental Figures



Supplemental Figure S1. Ovarian morphometric analyses in *Nlrp5* mice. (A) Total, vertebral and femoral BMD was calculated from six month old female mice and expressed in g/cm^2. No significant differences were seen in *Nlrp5*$^{tm/tm}$ when compared to +/+. **(B)** Total fat content in six month old *Nlrp5* mice as expressed in percentage. As seen with the BMD, no significant differences were observed in *Nlrp5* deficient females. **(C)** Number of follicles, both primordial and primary were counted for both two and six month old *Nlrp5* females and no significant differences were seen in both cases. Data are shown as mean +/- SEM. Numbers in parenthesis represent the number of mice assessed. (BMD-Bone mass density)



Supplemental Figure S2. Increased phosphorylation of p66SHC also occurs in 2-cell stage embryos. Expression of p66 SHC in 2-cell stage embryos. Relative fluorescent intensity in RFU generated by phopsho p66 (green) to total p66SHC (red) was used to show increased expression of phosphorylated isoform (p=0.019). Numbers in parenthesis represent number of embryos used in experiment and images were taken at 200x.



Supplemental Figure 3. Impact of microtubule disruption on mitochondrial activity.
2-cell wild-type embryos were exposed to vehicle (DMSO; n=26) or nocodazole (n=23) and mitochondrial distribution was subsequently determined with Mitotracker Red. While no obvious change in organelle clustering was detected upon microtubule depolymerisation, Mitotracker intensity reflecting respiring mitochondria (red) was greatly reduced. Decreased activity of mitochondria was also confirmed by diminished reactive oxygen species production (green). Values represent mean fluorescence units ± SEM (* p=0.001; **p<0.001). Images were taken at final magnification of 200x